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(54) **Title**: NON-NATURAL SEMAPHORINS 3 AND THEIR MEDICAL USE

(57) **Abstract**: The present invention relates to non-naturally occurring, mutated Semaphorin 3 molecules. Particularly, the invention relates to the mutated Semaphorin 3 or the functional fragment thereof that exhibit improved properties and pharmacologic effects, e.g., in the treatment of angiogenic disease and cancer. In addition, the present invention relates to nucleic acid molecules encoding such polypeptides, and vectors and hosts comprising such nucleic acids. The invention further relates to methods for producing the polypeptides of the invention, and to methods of using them in the treatment of disease, in particular in the medical intervention of angiogenic diseases, tumors and/or cancer.

Non-natural Semaphorins 3 and their medical use

The present invention relates to non-naturally occurring, mutated Semaphorin 3 molecules. Particularly, the invention relates to the mutated Semaphorin 3 or the functional fragment thereof that exhibit improved properties and pharmacologic effects, e.g., in the treatment of angiogenic disease and cancer. In addition, the present invention relates to nucleic acid molecules encoding such polypeptides, and vectors and hosts comprising such nucleic acids. The invention further relates to methods for producing the polypeptides of the invention, and to methods of using them in the treatment of disease, in particular in the medical intervention of angiogenic diseases, tumors and/or cancer.

Cancer development, progression, and metastatization crucially depend on angiogenesis, i.e. the formation of new blood vessels. Yet, due to abnormalities, such as tortuosity, leakiness due to weak cell-to-cell contacts among vascular endothelial cells (ECs) that upholster their walls, and lack of ensheathing mural pericytes, cancer blood vessels are structurally and functionally aberrant (Goel et al., 2011). As a result, vascular permeability is generally elevated in cancer tissues, proteins and fluids accumulate in the extravascular compartment in which interstitial pressure significantly rises, finally impairing the delivery of anti-cancer drugs (Goel et al., 2011). In addition, chronic oxygen shortage up-regulates hepatocyte growth factor/Met tyrosine kinase signaling (Michieli, 2009), which, coupled to the abnormal vascular permeability, strongly favors cancer cell intravasation, dissemination through the bloodstream, and metastatization. Normalization of cancer blood vessel architecture and function could result in a sizeable increase in the effectiveness of standard anti-cancer therapy, that instead can be impaired by improper blood vessel pruning associated with standard anti-angiogenic treatments (Van der Veldt et al., 2012). Remarkably, mounting evidence indicates how the most effective benefits of the vascular normalizing therapy result from the relief in cancers of different hypoxia-driven phenomena, such as a significant increase of cancer stem cells (Conley et al., 2012), the induction of cancer cell de-differentiation (Michieli, 2009), and the stimulation of cancer invasion and metastatization

(Michieli, 2009; Sennino and McDonald, 2012). Thus, to attain the ability of converting aberrant cancer blood vessels in a quasi-normal vascular network, molecules need to be identified that support physiological vascular morphogenesis and are, therefore, of medical use in the treatment, e.g., of disorders wherein aberrant vascular morphogenesis occurs and/or wherein normal vascular morphogenesis is perturbed, like in (solid) cancers.

Semaphorin 3A (also known as Sema3A in mice and SEMA3A in human) is a physiological vascular normalizing molecule. Prior art studies identified molecules that could in principle be pharmacologically exploited for therapies aimed at normalizing the cancer vasculature/abnormal vascular genesis in cancer (Goel et al., 2011). Inhibition of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), may normalize the cancer vasculature. Yet, a major obstacle by this kind of clinical intervention is represented by the fact that these pro-angiogenic factors display a limited temporal efficacy (Goel et al., 2011). Furthermore, vascular networks are under the simultaneous and balanced control of pro-angiogenic and anti-angiogenic factors, the function of both of which is altered in cancer tissues (Maione et al., 2012; Maione et al., 2009).

During embryonic vascular development, ECs generate autocrine chemorepulsive signals of secreted class 3 Semaphorins (also known as Sema3) that, by inhibiting integrins, which are the main class of extracellular matrix (ECM) receptors in multicellular organisms endow the vascular system with the plasticity required for its reshaping (Serini et al., 2003). Different transgenic mouse models of cancer unraveled that during cancer angiogenesis Semaphorin 3A is also expressed in ECs, where it serves as an endogenous inhibitor that is present in pre-malignant lesions, but lost during cancer progression (Maione et al., 2009). Importantly, the lack of Semaphorin 3A in overt cancer lesions clearly correlated with a dramatic increase of integrin activation in ECs (Maione et al., 2009). Reintroducing Semaphorin 3A into cancers by somatic gene transfer restored physiological amounts of active endothelial integrins, finally resulting in reduced blood vessel density, structural and functional vascular normalization, inhibition of cancer growth and metastatization, and significant survival extension (Maione et al., 2012; Maione et al., 2009). Thus, Semaphorin 3A may be a physiological vascular normalizing agent (Serini et al., 2012).

Semaphorin 3A belongs to the Semaphorin (designated as Sema) family whose categorization in seven different classes relies on the similarity of unique domains located at their C-terminus (Tran et al., 2007). Their N-terminus comprise the "sema domain", a seven-blade β-propeller (Gherardi et al., 2004) followed by a Plexin-Semaphorin-integrin (PSI) domain.

Semaphorins are homo-dimeric ligands that signal through Plexins (Kumanogoh and Kikutani, 2013; Tamagnone et al., 1999), a class of sema domain-containing receptors endowed with an extracellular sema domain and a cytosolic GTPase-activating protein (GAP) activity that inhibits R-Ras (Kumanogoh and Kikutani, 2013; Tran et al., 2007) and Rap1 (Bos and Pannekoek, 2012; Wang et al., 2012), two small GTPases known for their ability to promote integrin-mediated cell adhesion to ECM proteins (Kinbara et al., 2003; Shattil et al., 2010). Semaphorin 3A signals through the activation and phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK 1/2) (Kruger et al., 2005). Sema domain homo-dimers of membrane-associated Semaphorins directly bind with high affinity to the sema domains of Plexins. This triggers Plexin dimerization and activation (Janssen et al., 2010; Nogi et al., 2010). In some Semaphorins, like Semaphorin 3A, the receptor complex is formed by neuropilin 1 (Nrp1) in association with type A Plexins (Plexin A) (Tamagnone et al., 1999) representing the ligand binding and the signal transducing subunits (Kumanogoh and Kikutani, 2013). Consistently, downstream to the sema-PSI and immunoglobulin (Ig)-like domains, Semaphorin 3A and other secreted Semaphorins comprise a C-terminal basic aminoacid stretch. While disulphide bound Ig-like domains may physically stabilize sema domain homo-dimerization, the C-terminal basic stretch is required for Semaphorin 3A high affinity binding of the b1 subdomain in the extracellular moiety of Nrp1 (Figure 1) (Kumanogoh and Kikutani, 2013).

Semaphorin 3A comprises multiple furin protease recognition motifs that, once cleaved, can result in the release of this C-terminal portion of the molecule. This leads to an impairment of both Semaphorin 3A binding to Nrp1 as well as stabilization of Semaphorin 3A homo-dimers (Adams et al., 1997; Koppel and Raper, 1998; Parker et al., 2010; Parker et al., 2012). Since Semaphorin 3A does not directly bind Plexin with high affinity (Tamagnone et al., 1999) its furin dependent cleavage and lack of Nrp1-binding was found to result in a dramatic loss of activity in some biological settings, in particular neuron growth cone collapse (Koppel and

Raper, 1998). Notably, furin proteases are widely present in tissue, which provides a built-in regulatory mechanism for Semaphorin 3A. Yet, these proteases may also lead to a short-lived activity of Semaphorin 3A.

The binding of Semaphorin 3A to Nrp1 is responsible for Semaphorin 3A-induced entry of macrophages into avascular cancer areas fostering cancer progression (Casazza et al., 2013). Therefore, the high affinity interaction of wild type Semaphorin 3A with Nrp1 limits its exploitability as an effective anti-cancer drug and even potentially favors cancer progression.

The technical problem underlying the present invention is the provision of means and methods for an improved therapy of angiogenic disorders, tumorous diseases and/or cancer.

The technical problem is solved by provision of the embodiments provided herein below and as characterized in the appended claims.

The present invention relates to a non-naturally occurring/genetically modified/mutated Semaphorin of class 3, particularly of a non-naturally occurring/genetically modified/mutated Semaphorin selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D, most preferably of a non-naturally occurring/genetically modified/mutated Semaphorin 3A.

Accordingly, the present invention relates to a mutated Semaphorin 3 (or a functional fragment thereof functioning as an inhibitor of angiogenesis or a fusion protein/polypeptide comprising said mutated Semaphorin or said functional fragment) that

(a) comprises a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2; or
(b) comprises a hydrophilic amino acid in place of the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2; and
(c) wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D, wherein preferably said Semaphorin 3 is Semaphorin 3A.

Therefore, the present invention in general provides for Semaphorin 3A, 3B, 3C and 3D that are not naturally-occurring and that comprise a hydrophilic amino acid instead of the alanine at position 106 of the exemplified Semaphorin 3A shown in SEQ ID NO: 2.
Herein below corresponding positions for this mutation in other Semaphorin 3 proteins than Semaphorin 3A, namely, Semaphorins 3 B, C and D are exemplified.

Examples of these mutated Semaphorins 3 are Semaphorins comprising said hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2; said hydrophilic amino acid in place of the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6; said hydrophilic amino acid in place of the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or said hydrophilic amino acid in place of the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14.

In other words, the present invention provides for a non-naturally occurring/genetically modified Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and/or Semaphorin 3D , wherein said Semaphorin 3, said functional fragment thereof and/or said fusion protein/polypeptide comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein:

X_1 is K or N;

X_2 is an amino acid selected from the group of W, M and L; and

A_3 is said hydrophilic amino acid that replaces said alanine.

The herein provided consensus motif $CX_1X_2A_3GKD$ is identified for the first time in the present invention.

The present invention also relates to a nucleic acid molecule encoding the non-naturally occurring polypeptides of this invention, i.e. the mutated Semaphorin 3 selected form the group consisting of the mutated Semaphorin 3A, the mutated Semaphorin 3B, the mutated Semaphorin 3C and/or the mutated Semaphorin 3D as characterized and described herein. Also provided are nucleic acid molecules that encode the herein defined functional fragments of the non-naturally occurring Semaphorins 3 as well as nucleic acid molecules encoding fusion proteins/polypeptides comprising the inventive non-naturally occurring Semaphorins 3 or said functional fragments. As laid down herein, the functional fragments as well as the

fusion polypeptides/proteins of this invention retain the surprisingly high inhibition of angiogenesis and/or are capable of surprisingly high vascular normalization of disease tissues (like in cancer tissue and/or tumors). Accordingly, the present invention provides for nucleic acids molecules that encode the polypeptides of this invention. The polypeptides of the invention are mutated Semaphorins 3 or functional fragments thereof as defined herein. The polypeptides of this invention also comprise fusion proteins that comprise a mutated Semaphorin 3 (or a functional fragment of such a mutated Semaphorin 3 as defined herein). The polypeptides of this invention, in particular the fusion proteins as defined herein, function as an inhibitors of angiogenesis and/or as vascular normalizing agents and wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In particular, the present invention relates to nucleic acid molecules, wherein the encoded mutated Semaphorin 3 (or said functional fragment thereof or said fusion polypeptide/protein) comprises an amino acid sequence consensus motif $CX_1X_2A_3GKD$, wherein

X_1 is an amino acid, which is K or N,

X_2 is an amino acid selected from the group of W, M and L

and wherein the alanine (A_3) is replaced by said hydrophilic amino acid, in particular lysine, arginine, asparagine, glutamine, serine, threonine, glutamic acid, aspartic acid or histidine, more preferably lysine or arginine, most preferably, lysine.

The herein provided non-naturally occurring/artificial/mutated Semaphorins (or their herein described functional fragments and/or fusion proteins comprising said non-naturally occurring/artificial/mutated Semaphorins or said non-naturally occurring/artificial/mutated functional fragments of said Semaphorins) have high medical potential. As is illustrated in the appended examples, the medical use of the herein provided inventive molecules is a surprising reduction of cancer progression and metastasis in *in vivo* cancer models. This *in vivo* effect is surprisingly superior over any effect documented or seen with naturally occurring Semaphorins, such as wild type Semaphorin 3A. This surprising effect is

exemplified herein by certain, selected Semaphorins (Semaphorins of the class 3 selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D) comprising an artificial replacement of the (naturally occurring) alanine (A_3) by a hydrophilic amino acid within the herein identified consensus motif $CX_1X_2A_3GKD$ of Semaphorin 3 as defined herein. This replacement in said Semaphorins (or in functional fragments thereof that comprise said motif) results in a surprisingly increased affinity to its Plexin receptor as compared to non-modified, naturally occurring wild type version of said Semaphorins (selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D). In other words, the present invention provides for non-naturally occurring, mutated Semaphorins of class 3 and selected from the group consisting of mutated Semaphorin 3A, mutated Semaphorin 3B, mutated Semaphorin 3C and mutated Semaphorin 3D. Accordingly, the present invention provides for non-naturally occurring, mutated Semaphorins of class 3 that are selected from the group consisting of mutated Semaphorin 3A, mutated Semaphorin 3B, mutated Semaphorin 3C and mutated Semaphorin 3D. The herein described mutated Semaphorins 3 (or the functional fragment of these mutated Semaphorins of this invention as well as fusion proteins comprising the herein described mutated, non-naturally occurring Semaphorins or their functional fragments) bind to Plexin receptor with surprisingly high affinity. The increased binding affinity circumvents the need for involvement of the ambivalent Nrp1 protein. Furthermore, the high affinity binding between the mutated Semaphorin 3 (or the herein described functional fragment thereof or the herein described fusion proteins) and the Plexin receptor effectively triggers its downstream pathway leading to a desired vessel normalization and/or physiological, non-diseased angiogenesis. Without being bound by theory, the mutated Semaphorins 3 of this invention (or the functional fragments or the fusion proteins of this invention comprising the same) enable a high affinity binding between its Plexin receptor independent of the Ig-like domain/basic stretch region which comprises multiple furin protease recognition motifs. In certain embodiments, the invention relates to the mutated Semaphorin 3 or the fragment thereof, wherein the Ig-like domain/basic stretch region is deleted. Due to the lack of the protease (furin) cleavage sites as described herein above and as shown in the appended Figure 2, the retention time of the proteins of the invention can be increased.

As documented in the appended *in vitro* and *in vivo* examples herein below, it was surprisingly found that the replacement of the alanine A_3 by a hydrophilic amino acid in the

consensus motif $CX_1X_2A_3GKD$ of Semaphorin 3 (or functional fragments thereof) as selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D results in increased binding to its Plexin receptor. Thus, the inventive, non-naturally occurring mutated Semaphorins 3 (or the functional fragments thereof or fusion polypeptides/proteins comprising said Semaphorins 3 or their functional fragments) bind with a high affinity to its Plexin receptor independently of Nrp1. It is also documented herein that the replacement of alanine by a hydrophilic amino acid in the consensus motif $CX_1X_2A_3GKD$ of Semaphorin 3 (or in functional fragments of said Semaphorin3 comprising said consensus motif with its mutation/modification) results in a surprisingly increased activation of the Plexin receptors and in a surprisingly increased inhibition of haptotactic migration of human ECs. Plexin receptor binding and activation is a critical step in the control of integrin activation, cell adhesion and migration on ECM proteins. Without being bound by theory, the activation of the Plexin receptors through Semaphorins inactivates integrins and thus impairs the motility of cells within the ECM. Cell migration is crucial for cancer cell progression and metastasis dissemination. Hence, the mutated Semaphorin 3 (and the functional fragments thereof) of this invention can be used to effectively inhibit cancer progression and metastasis. Without being bound by theory, the inventive mutated Semaphorins 3 (and/or the functional fragments thereof and/or fusion proteins/polypeptides comprising said mutated Semaphorins or the herein defined functional fragments) bind with a high affinity to the corresponding Plexin receptor, effectively activate the corresponding downstream pathway and effectively inhibit cell motility. As shown and documented in the examples, the herein defined artificially introduced modification in certain Semaphorins of the class 3 (Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D) lead to surprisingly effective molecules which show even in vivo an increased reduction of cancer progression and/or metastasis. The herein described mutated/non-naturally occurring Semaphorin-like molecules show surprisingly better in vivo and/or in vitro properties when compared to naturally occurring Semaphorins 3. It is documented herein and shown in the appended examples that the replacement of alanine in the consensus motif $CX_1X_2A_3GKD$ of Semaphorins 3 (or in functional fragments thereof or in fusion polypeptides/proteins comprising the herein defined non-naturally occurring Semaphorins 3 or comprising the herein defined functional fragments with said mutated motif) results in an increased reduction of cancer progression and metastasis. The replacement of said alanine (A_3) is preferably a hydrophilic amino acid, most preferably a lysine. This resulting reduction of cancer progression and metastasis in the non-

naturally occurring mutated Semaphorin 3 is surprisingly superior to the effect seen with conventional or naturally occurring Semaphorins, such as wild type Semaphorin 3A. In particular, *in vivo* data presented herein proves a surprisingly increased inhibition of cancer growth and metastasis volume. Two mouse models for human cancer prove the beneficial pharmacologic effect.

Accordingly, as documented in the appended examples and as explained herein, the inventive Semaphorins (and/or the functional fragment thereof and/or the fusion polypeptides/proteins described herein) are superior in the therapy of angiogenic disorders and/or tumorous disease compared to conventional or naturally occurring Semaphorins.

The superior effect of the inventive Semaphorins (or of the functional fragment(s) or fusion polypeptides/proteins comprising the non-naturally occurring Semaphorins of this invention or comprising said functional fragments) is due to the replacement of alanine (A_3) by a hydrophilic amino acid in the consensus motif $CX_1X_2A_3GKD$ like e.g., Semaphorin 3A A106K (mutation of the alanine at position 106 to lysine) in the appended examples (e.g. Semaphorin 3A A106K ΔIg-b as shown in SEQ ID NO: 18 or 20); see appended Figure 3. As is shown in the appended examples, Semaphorin 3 constructs having the same architecture as Semaphorin 3A A106K, with the exception of lacking the point mutation in the conserved motif $CX_1X_2A_3GKD$ (Semaphorin 3A ΔIg-b, e.g. encoding nucleic acid sequences as shown in SEQ ID NO: 43 or 44), see appended Figure 2, failed to show the beneficial pharmacologic effects. Accordingly, the surprising beneficial effect as documented in the present invention can be attributed to the herein described mutation in the $CX_1X_2A_3GKD$ of mouse and/or human Sema3A, Sema3B, Sema3C and Sema3D. Preferably, the present invention relates to human Semaphorins 3 selected form the group consisting of Sema 3A, Sema 3B, Sema 3C and Sema 3D (or functional fragments of these human Semaphorins comprising the herein defined consensus motif) comprising the herein defined mutation in alanine (A_3). Most preferably, the present invention relates to mutated human Sema 3A (or functional fragments of said human Sema 3A) comprising the herein defined motif with the herein described replacement of alanine (A_3) in the sequence motif $CX_1X_2A_3GKD$. Said replacement is a replacement with a hydrophilic amino acid, most preferably a replacement with a lysine (K). The mutated alanine (A_3) is part of a highly conserved sequence motif $CX_1X_2A_3GKD$ that can be found in mouse and human Sema3A, Sema3B, Sema3C and Sema3D; see appended Figure 3. Consistently, equal beneficial effects that are shown in the

appended examples are envisaged and plausible from a replacement of said alanine by a hydrophilic amino acid (like K) in (human) Sema3B, Sema3C and/or Sema3D.

Human and mouse Sema3E, Sema3G and Sema3F have a naturally occurring hydrophilic amino acid where alanine A_3 resides in the consensus sequence $CX_1X_2A_3GK$. Hence, they have a hydrophilic amino acid at the position corresponding to the position 106 of Semaphorin 3A as shown in SEQ ID NO: 2. Both Sema3E and Sema3G comprise a lysine and Sema3F comprise a serine at this position. Yet, Sema 3E and Sema 3F fail to show the inventive properties as demonstrated for the inventive Semaphorins 3 as explained in the following and as documented in the appended examples. The replacement of serine by lysine at the position 107 in Sema3F fails to increase the binding to the Plexin A, B, C, or D receptors. Furthermore, this mutant fails to inhibit EC migration more effectively than its wild type counterpart displaying a serine at the position 107, which is documented herein for the mutated Semaphorin 3 or the functional fragment of this invention, see appended example 2 and Figure 12. Moreover, Fc-tagged Semaphorin 3E and Semaphorin 3F fail to inhibit EC migration as strong as the exemplary mutated Semaphorin 3A comprising the inventive replacement of alanine by a hydrophilic amino acid; see illustrative Figure 13. Consequently, the inventive mutated Semaphorins provided herein, namely mutated Semaphorin 3A, mutated Semaphorin 3B, mutated Semaphorin 3C, and mutated Semaphorin 3D are surprisingly superior inhibitors of EC cell motility compared to Semaphorins 3 comprising a naturally occurring hydrophilic amino acid at the position corresponding to position 106 of Semaphorin 3A as given in SEQ ID NO: 2. The demonstrated strong inhibition of cell motility is an indication that the inventive mutated Semaphorin 3 proteins are superior in the therapy of angiogenic disorders and/or tumorous disease compared to naturally-occurring Semaphorins 3 (see also below).
Accordingly, the present invention does not relate to (human) Sema 3E, Sema 3F and/or Sema 3G.

The replacement of alanine (A_3) by, e.g., lysine in the consensus motif $CX_1X_2A_3GKD$ in Sema 3A/3B/3C or 3D (or in functional fragments thereof) (like, inter alia, in Sema3A the exchange A106K) results in increased inhibition of cancer growth and metastasis formation in two different transgenic mouse models, i.e., spontaneous pancreatic neuroendocrine cancer (RipTag2) and pancreatic ductal adenocarcinoma (PDAC); see example 2. The PDAC mouse

is a model for the frequently and deadly human pancreatic cancer histotype. Most importantly and surprisingly, parenterally delivered Sema3A A106K shows a superior pharmacologic effect in PDAC mice compared to AAV8-wild type Sema3A protein. In detail, Sema3A A106K inhibited cancer growth by 64%, see appended Figure 11A, liver metastasis incidence by 81%, see appended Figure 11B and diminished metastasis volume by 78%. The AAV-8-delivered wild type Sema3A only inhibited cancer growth by 52%, see appended Figure 10A and liver metastasis incidence by 59%. Furthermore, Sema3A A106K reduced vessel area and promoted cancer vessel normalization by enhancing pericyte coverage, increased blood vessel perfusion and inhibited cancer hypoxia in mouse models recapitulating human cancer, see appended Figure 11C.

Accordingly, mutated/non-naturally occurring Semaphorins of the present invention, like Sema3A A106K exert a superior effect in reducing cancer progression and metastasis dissemination compared to conventional, non-modified Semaphorins.

Furthermore, parenterally administered Sema3A A106K extended the survival of RIP-Tag2 mice similarly to adeno-associated virus-8 (AAV8) delivered full length Sema3A. Sema3A A106K i) induced a 67% reduction of cancer volume; ii) efficiently reduced the cancer blood vessel area by 51 %, see appended Figure 9A; iii) favored the normalization of cancer blood vessels in terms of increased pericyte coverage, see appended Figure 9B; iv) enhanced perfusion, see appended Figure 9C and reduced tissue hypoxia, see appended Figure 9D. Thus, the present invention demonstrates its surprising therapeutic effect, which is independent of Nrp1 binding.

Furthermore, the superior effect of the mutated Semaphorin 3 proteins (and/or the functional fragments thereof and/or the fusion proteins/polypeptides comprising said mutated Semaphorins or the therein defined fragments) is also proven in in vitro experiments, see the appended examples. The replacement of alanine (A_3) by a hydrophilic amino acid in the consensus motif $CX_1X_2A_3GKD$ of the Semaphorin 3 or the functional fragment thereof, such as found in the mutated Semaphorin 3A A106K results in a high affinity binding to PlexinA4 compared to wild type Sema3A and Sema3A ΔIg-b, see the appended examples. Furthermore, the mutated Semaphorin 3 or the functional fragment thereof, e.g., Sema3A A106K, shows an increased inhibition of the GTP-loading of Rap1 small GTPase, see appended Figure 8A and an increased phosphorylation of the ERK 1/2 kinase compared to conventional Semaphorins, see appended Figure 8B. Hence, the mutated Semaphorin 3 (or a functional fragment thereof or fusion polypeptides/proteins comprising the same) bind to

Plexin subunits with an exceptionally high affinity and effectively trigger its downstream pathway leading to cancer vessel normalization compared to conventional Semaphorins, like natural human Sema3A.

Furthermore, Semaphorin 3 proteins control the cell motility via integrins, such as the haptotactic migration of ECs towards ECM proteins. Integrin-mediated cell motility on and towards ECM proteins plays crucial roles in several physiological and pathological settings, such as blood vessel formation (angiogenesis) and cancer cell dissemination throughout the body (metastatization) (Desgrosellier and Cheresh, 2010). Accordingly, the migration of cells in response to the conventional or non-naturally occurring Semaphorins 3 or the functional fragments thereof was analyzed. Surprisingly, the replacement of alanine (A_3) by a hydrophilic amino acid in the consensus motif $CX_1X_2A_3GKD$ of the Semaphorin 3 or the functional fragment thereof results in an increased inhibition of the directional migration of human umbilical vein endothelial cells (EC) compared to conventional Semaphorins, see appended Figure 6A-C. Commercial human wild type Semaphorin 3A and mouse Semaphorin 3A ΔIg-b impaired EC motility by only 19-25% (Table 3). Importantly, mutated Semaphorin 3 proteins (and/or the functional fragments thereof and/or the fusion proteins/polypeptides comprising said mutated Semaphorins or the therein defined fragments), such as Sema3A A106K are the most efficient inhibitors of EC motility. In particular, while the maximal (3,5 nM) dose of commercial human SEMA3A WT inhibited EC directional migration by 20%, a 17.5 times lower (0.2 nM) dose of human SEMA3A A106K (e.g., SEQ ID NO: 18) inhibited EC motility by 46% (Table 4).

Accordingly, the mutated Semaphorins 3 of the invention (or the functional fragments thereof and/or the fusion polypeptides/proteins of this invention comprising the mutated, non-naturally occurring Semaphorins or their functional fragments) are superior inhibitors of the motility of cells compared to conventional Semaphorins. The inhibition of cell motility also impairs the metastatic dissemination of cancer cells. Therefore, the inventive, mutated Semaphorins 3 (or the functional fragment thereof and/or the fusion polypeptides/proteins of this invention comprising the mutated, non-naturally occurring Semaphorins or their functional fragments) are superior inhibitors of cancer cell formation and metastatic dissemination compared to conventional Semaphorins.

Accordingly, the experimental data in the appended examples provide for a clear rationale to use the inventive mutated Semaphorins 3 (or functional fragment thereof or fusion

proteins/polypeptides comprising the same) in the improved therapy of angiogenic disorders and/or tumorous disease/cancer.

The present invention has, inter alia, the following advantages over conventional antiangiogenic agents: One advantage of the present invention is the fact that the inventive compounds i.e. the mutated Semaphorins/functional fragments thereof/fusion proteins/polypeptides as described herein bind to the Plexin receptors with a high affinity, but nevertheless circumvent the Nrp-1-dependent Semaphorin induced entry of macrophages into avascular tumor areas that foster cancer progression. As a further advantage, the compounds of this invention effectively trigger Plexin receptor signaling. As a further advantageous property, the inventive compounds activate the Plexin receptor independently from Nrp1. Furthermore, the compounds of the invention inhibit EC migration more effectively compared to conventional Semaphorin 3 proteins. The compounds of this invention are not cleaved by proteases resulting in an increased retention time. As a further advantage, the compounds of this invention (proteins as well as nucleic acid molecules encoding the same) can be delivered parenterally .The compounds of the invention are superior in preventing the formation of new blood vessels, thereby stopping or slowing the growth or spread of tumors. The compounds of the invention are superior in reducing the blood vessel area, normalizing cancer blood vessel, enhancing perfusion of cancer blood vessels and/or reducing tissue hypoxia. The compounds of this invention of the invention can be used as a vascular normalizing agent, i.e., the cancer blood vessels are normalized, the perfusion of cancer blood vessels is enhanced and/or tissue hypoxia is reduced. A further advantage of the invention is that the cancer growth is inhibited more effectively. As a further advantageous property, the proteins and/or nucleic acid molecules of this invention reduce metastasis incidence and diminishes metastasis volume. Consequently, the invention has a superior effect in inhibiting cancer progression and metastasis dissemination.

As used herein the term “mutated Semaphorin 3”, “genetically modified Semaphorin 3”, “non-naturally occurring Semaphorin 3” or “non-natural Semaphorin 3” in accordance with the present invention refers to a mutated form of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C or Semaphorin 3D as defined herein. A mutated Semaphorin 3 differs from a wild type Semaphorin 3 or a functional fragment thereof by at least one mutation that is selected from the group consisting of amino acid substitution(s), addition(s), deletions(s) and

duplication(s). In particular, the mutated form of the Semaphorin 3 comprises a replacement of the alanine by a hydrophilic amino acid at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. In other words, the alanine (A_3) in the consensus motif $CX_1X_2A_3GKD$ (also shown in SEQ ID NO: 73) in the Semaphorin 3 proteins is mutated to a hydrophilic amino acid (table 1). Accordingly, the mutated Semaphorin 3A comprises an amino acid sequence, wherein the alanine at the position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or 4 is replaced by a hydrophilic amino acid. The mutated Semaphorin 3B comprises an amino acid sequence, wherein the alanine at the position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6 or 8 is replaced by a hydrophilic amino acid. The mutated Semaphorin 3C comprises an amino acid sequence, wherein the alanine at the position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10 or 12 is replaced by a hydrophilic amino acid. The mutated Semaphorin 3D comprises an amino acid sequence, wherein the alanine at the position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14 or 16 is replaced by a hydrophilic amino acid. Said hydrophilic amino acid can be, e.g., a lysine, arginine, asparagine, glutamine, serine, threonine, glutamic acid, aspartic acid or histidine, more preferably a lysine or arginine, most preferably a lysine. It is also envisaged herein that the hydrophilic amino acid can be a non-proteinogenic or a non-standard α-amino acid (such as, e.g., ornithine and citrulline). It is shown herein above and in the appended examples that the replacement of the alanine by a hydrophilic amino acid results in an increased inhibition of cancer growth and metastasis formation. In general, the mutation to a hydrophilic amino acid in the Semaphorins 3 results in a high binding affinity to the Plexin receptors. The increased binding of the mutated Semaphorin 3 results in an increased activation of the Plexin receptors and their downstream signaling. Plexin receptors are crucial in the control of integrin activation, cell adhesion and migration on or towards ECM proteins, which are key aspects in cancer cell progression and metastasis. Accordingly, the mutated Semaphorin 3 of the invention can be employed as inhibitor of angiogenesis and as a vascular normalizing agent.

Table 1. The mutated Semaphorin 3 comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein the alanine A_3 is replaced by a hydrophilic amino acid. The amino acids that correspond to X_1 and X_2 are indicated. The positions of the amino acids of the wild type Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D as shown in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14 and 16 are indicated.

C	X_1	X_2	A_3	G	K	D	Semaphorin 3 and SEQ ID NO:
C	K	W	A	G	K	D	Human Semaphorin 3A SEQ ID
103	104	105	106	107	108	109	NO:2
C	N	W	A	G	K	D	Human Semaphorin 3B SEQ ID
102	103	104	105	106	107	108	NO:6
C	K	M	A	G	K	D	Human Semaphorin 3C SEQ ID
101	102	103	104	105	106	107	NO:10
C	K	L	A	G	K	D	Human Semaphorin 3D SEQ ID
117	118	119	120	121	122	123	NO:14
C	K	W	A	G	K	D	Mouse Semaphorin 3A SEQ ID
103	104	105	106	107	108	109	NO:4
C	N	W	A	G	K	D	Mouse Semaphorin 3B SEQ ID
102	103	104	105	106	107	108	NO:8
C	K	M	A	G	K	D	Mouse Semaphorin 3C SEQ ID
101	102	103	104	105	106	107	NO:12
C	K	L	A	G	K	D	Mouse Semaphorin 3D SEQ ID
117	118	119	120	121	122	123	NO:16

In particular, the invention relates to mutated Semaphorin 3 proteins which mean that the mutated Semaphorins are selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D. Likewise, if reference is made to mutated Semaphorin 3 or mutated Semaphorin 3 proteins in the context of the present invention, this is intended to refer to mutated Semaphorin 3A, mutated Semaphorin 3B, mutated Semaphorin 3C and mutated Semaphorin 3D. In most preferred aspects of the invention the mutated Semaphorin 3 is mutated Semaphorin 3A. It is understood herein that the mutated Semaphorin 3 is not

Semaphorin 3E, Semaphorin 3F or Semaphorin 3G. Furthermore, it is herein understood that mutated Semaphorin 3 according to the invention does not include Semaphorin 3B isoform X2, e.g. from Equus przewalskii, or Semaphorin 3B isoform X6, e.g., from Panthera tigris altaica. Such Semaphorins do not comprise the amino acid sequence $CX_1X_2A_3GKD$, wherein the alanine A_3 is replaced by a hydrophilic amino acid. Furthermore, the mutated Semaphorin 3 according to the invention functions as an inhibitor of angiogenesis and/or as a vascular normalizing agent.

The terms "Semaphorin 3A", "Semaphorin 3B", "Semaphorin 3C", and "Semaphorin 3D", "Sema3A", "Sema3B", "Sema3C" and "Sema3D", SEMA3A", "SEMA3B", "SEMA3C" and "SEMA3D" as used herein refer primarily to a protein. "Sema3A", "Sema3B", "Sema3C" and "Sema3D" as defined herein and to be used in accordance with the present invention are preferably human "Sema3A", "Sema3B", "Sema3C" and "Sema3D". "Semaphorin 3A", "Semaphorin 3B", "Semaphorin 3C", and "Semaphorin 3D" as defined herein and to be used in accordance with the present invention are preferably human "Sema3A", "Sema3B", "Sema3C" and "Sema3D". "SEMA3A", "SEMA3B", "SEMA3C" and "SEMA3D" as defined herein and to be used in accordance with the present invention are preferably human "Sema3A", "Sema3B", "Sema3C" and "Sema3D".

Sema3A A106K or Semaphorin 3A A106K is also designated herein and in the appended examples as Fc-tagged Sema3A A106K ΔIg-b.
The amino acid sequences and encoding nucleotide sequences of wild-type Semaphorin 3 are well known in the art. Nucleic acid sequences can be retrieved in public databases like NCBI using the following accession numbers (the following sequences have been retrieved from the NCBI database):

Homo sapiens SEMA3A, >gi|100913215|ref|NM_006080.2| corresponding to SEQ ID NO: 1; Mus musculus Sema3A, >gi| 340523098|ref| NM_009152.4|) corresponding to SEQ ID NO: 3; Homo sapiens SEMA3B, >gi| 586798179|ref| NM_001290060.1| corresponding to SEQ ID NO: 5; Mus musculus Sema3B, >gi| 615276319|ref| NM_001042779.2| corresponding to SEQ ID NO: 7; Homo sapiens SEMA3C >gi| 335057525|ref| NM_006379.3| corresponding to SEQ ID NO: 9; Mus musculus Sema3C, >gi| 118130842|ref| NM_013657.5| corresponding to SEQ ID NO: 11; Homo sapiens SEMA3D, >gi| 41406085|ref| NM_152754.2|

corresponding to SEQ ID NO: 13; or Mus musculus Sema3D >gi| 282847343|ref| NM_028882.4| corresponding to SEQ ID NO: 15.

SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 or SEQ ID NO: 15 encode wild type full length Semaphorin 3 proteins.

Corresponding amino acid sequences can be retrieved in public databases like NCBI. The following sequences have been retrieved from the NCBI database.

Homo sapiens SEMA3A, |ref|NP_006071.1| corresponding to SEQ ID NO: 2;
Mus musculus Sema3A, |ref|NP_033178.2| corresponding to SEQ ID NO: 4;
Homo sapiens SEMA3B, |ref|NP_001276989.1| corresponding to SEQ ID NO: 6;
Mus musculus Sema3B, |ref|NP_001036244.1| corresponding to SEQ ID NO: 8;
Homo sapiens SEMA3C |ref|NP_006370.1| corresponding to SEQ ID NO: 10;
Mus musculus Sema3C, |ref|NP_038685.3| corresponding to SEQ ID NO: 12;
Homo sapiens SEMA3D, |ref|NP_689967.2| corresponding to SEQ ID NO: 14; or
Mus musculus Sema3D, |ref|NP_083158.3|corresponding to SEQ ID NO: 16.

SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16 comprise amino acid sequences of wild type full length Semaphorin 3 proteins.

Amino acid sequences of Semaphorin 3 of the invention can also be obtained from Uniprot, e.g. for mouse and human Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In one embodiment, the invention relates to a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of a mutated Semaphorin 3, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In a further embodiment, the invention relates to a mutated Semaphorin 3, wherein said mutated Semaphorin 3 comprises an amino acid sequence, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In a further embodiment, the invention relates to a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of a mutated Semaphorin 3 or a fragment thereof, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In a further embodiment, the invention relates to the mutated Semaphorin 3 or fragment thereof, wherein said mutated Semaphorin 3 or said fragment thereof comprises an amino acid sequence, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorins of the Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

As explained above, a mutated Semaphorin 3 or a functional fragment thereof comprises a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or comprises a hydrophilic amino acid in place of the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 and wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

As explained above and as illustrated in Table 1, the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 refers to the specific alanine of Semaphorin 3A at position 106 of SEQ ID NO: 2 or the specific alanine amino acid residue in a known wild-type sequence of Semaphorin 3A, 3B, 3C or 3D, preferably Semaphorin 3A, corresponding to said specific alanine of Semaphorin 3A at position 106 of SEQ ID NO: 2. It also means a specific amino acid residue in a known wild-type sequence e.g. Semaphorin 3A, 3B, 3C or 3D that is homologous to said specific alanine at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14. Exemplary homologous amino acids residues can be alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine or tryptophan. Most preferably, the specific amino acid residue is alanine.

The corresponding amino acid residue in other wild-type sequences at the corresponding position can be selected preferably by standard homology screenings or PCR-mediated screening techniques for related sequences as described below. The alanine or the corresponding alanine is replaced by or changed to a hydrophilic amino acid in the mutated Semaphorin 3 according to the invention.

As mentioned and explained herein, the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 refers to the specific alanine amino acid residue in a known wild-type sequence of Semaphorin 3B, 3C or 3D that corresponds to said specific alanine of said Semaphorin 3A at position 106 given in SEQ ID NO: 2. The corresponding alanine is replaced by a hydrophilic amino acid in mutated Semaphorin 3B, 3C or 3D. In other words, the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6, the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14 is replaced by the hydrophilic amino acid.

The alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6 refers to the specific alanine of Semaphorin 3B at position 105 of SEQ ID NO: 6 or the specific alanine amino acid residue in a known wild-type sequence of Semaphorin 3B corresponding to said specific alanine of Semaphorin 3B at position 105 of SEQ ID NO: 6.

The alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10 refers to the specific alanine of Semaphorin 3C at position 104 of SEQ ID NO: 10

or the specific alanine amino acid residue in a known wild-type sequence of Semaphorin 3C corresponding to said specific alanine of Semaphorin 3C at position 104 of SEQ ID NO: 10. The alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14 refers to the specific alanine of Semaphorin 3D at position 120 of SEQ ID NO: 14 or the specific alanine amino acid residue in a known wild-type sequence of Semaphorin 3D corresponding to said specific alanine of Semaphorin 3D at position 120 of SEQ ID NO: 14. As mentioned and detailed herein, the corresponding amino acid residue at the corresponding position can be selected preferably by comparison of homology. Homology among polypeptides or nucleotide sequences is typically inferred from their sequence similarity. Alignments of multiple sequences can herein be used to indicate which regions or specific amino acids of each sequence are homologous. The amino acid sequences of Semaphorin 3A, B, C and D can be used as (a) reference sequences. The homology exist preferably over a stretch of amino acids, e.g. 10, more preferably 20, more preferably 30, more preferably 50, or more preferably 100 amino acid residues, or most preferably the homology exist over the whole amino acid stretch. An illustrative amino acid sequence alignment of exemplary amino acid stretches of Semaphorin 3 proteins is shown in Table 1. The corresponding alanine is most preferably A_3 in the amino acid sequence $CX_1X_2A_3GKD$ comprised in the mutated Semaphorin 3. Thus, the inventive mutation can also be identified with the help of the amino acid sequence $CX_1X_2A_3GKD$.

As described herein, the invention relates to the mutated Semaphorin 3 or the functional fragment thereof that comprises a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or comprises a hydrophilic amino acid in place of the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2
and wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In other words, the mutated Semaphorin 3 or the functional fragment thereof comprises a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or comprises a hydrophilic amino acid in place of the alanine at the position which corresponds in Semaphorin 3 B, C or D by comparison of

homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2; and wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D. In other words, the other Semaphorins are Semaphorin 3B, 3C and 3D.

In other words, the mutated Semaphorin 3 or the functional fragment thereof wherein said Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D and wherein said mutated Semaphorin 3 comprises
a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2;
a hydrophilic amino acid in place of the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6;
a hydrophilic amino acid in place of the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or
a hydrophilic amino acid in place of the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14.
In most preferred embodiments of the invention, the mutated Semaphorin 3 or the functional fragment thereof comprises a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2, and wherein said Semaphorin 3 is Semaphorin 3A. In other words, the present invention relates to a mutated Semaphorin 3 A or a functional fragment thereof, wherein said mutated Semaphorin 3A comprises a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2.

As outlined above and as demonstrated in the appended examples, the replacement of the alanine (A_3) in the amino acid sequence $CX_1X_2A_3GKD$ renders Semaphorin 3A, B, C or D polypeptides to strong angiogenesis inhibitors. Therefore, in the amino acid sequence $CX_1X_2A_3GKD$ comprised in the mutated Semaphorins, the alanine (A_3) is mutated to the hydrophilic amino acid, i.e., the present invention relates to a mutated Semaphorin 3 or a functional fragment thereof wherein said mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D and wherein said mutated Semaphorin 3 or said functional fragment thereof comprises
a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild

type Semaphorin 3A as shown in SEQ ID NO: 2;
a hydrophilic amino acid in place of the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6;
a hydrophilic amino acid in place of the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or
a hydrophilic amino acid in place of the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14; and wherein
said mutated Semaphorin 3 or said functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein

X_1 is an amino acid, which is K or N,

X_2 is an amino acid selected from the group of W, M and L,

and wherein the alanine (A_3) is replaced by said hydrophilic amino acid.

In other words, the present invention relates to a mutated Semaphorin 3 or a functional fragment thereof wherein said mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C, and Semaphorin 3D and
wherein said mutated Semaphorin 3 or said functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein

X_1 is an amino acid, which is K or N,

X_2 is an amino acid selected from the group of W, M and L,

and wherein the alanine (A_3) is replaced by a hydrophilic amino acid; and wherein said mutated Semaphorin 3 or said functional fragment thereof comprises
said hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2;
said hydrophilic amino acid in place of the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6;
said hydrophilic amino acid in place of the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or
said hydrophilic amino acid in place of the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14.

The following description includes all different embodiments. The following relates to the herein provided non-naturally occurring/artificial/mutated Semaphorin 3 proteins or their

herein described functional fragments or the herein described functional sema domains and/or fusion proteins/polypeptides comprising said non-naturally occurring/artificial/mutated Semaphorin 3 proteins or said non-naturally occurring/artificial/mutated functional fragments or said functional sema domains of said Semaphorin 3 proteins that have primarily the activity to function as an inhibitor of angiogenesis. In other words the compounds of the invention, i.e., the mutated Semaphorin 3 proteins/functional fragments thereof/functional sema domains/fusion proteins/polypeptides as described herein have primarily the activity to function as an inhibitor of angiogenesis. In general, an inhibitor of angiogenesis prevents the formation of new blood vessels, thereby stopping or slowing the growth or spread of tumors. As shown herein, the compounds of the invention reduce the blood vessel area, normalize cancer blood vessels, i.e., increase the pericyte coverage, enhance the perfusion of cancer blood vessels and/or reduce the tissue hypoxia. Accordingly, the amino acid sequences and/or nucleic sequences of the present invention relate to a direct and/or an indirect inhibitor of angiogenesis. In general, inhibitors of angiogenesis also bind to receptors on the surface of cells, such as ECs and/or to other proteins in the downstream signaling pathways, blocking their activities. As shown in the appended examples the mutated Semaphorin 3, the functional fragment thereof , the functional sema domain, the fusion protein or the polypeptide of the invention binds to its Plexin receptor, e.g. type A Plexins such as Plexin A4 or Plexin A2, with a high affinity, i.e. displaying dissociation constant K_D in the very-low-nanomolar/sub-nanomolar range. Accordingly, the amino acid sequences and/or nucleic sequences of the present invention inhibit directly and/or indirectly angiogenesis. In particular, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention has an affinity to its Plexin receptor with a dissociation constant K_D lower than 6 nM. In preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention has an affinity to its Plexin receptor with a dissociation constant K_D lower than 4 nM. In even more preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention has an affinity to its Plexin receptor with a dissociation constant K_D lower than 2 nM. In most preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention has an affinity to its Plexin receptor with a dissociation constant K_D lower than 1 nM. The dissociation constant K_D can be measured by standard methods known in the art, such as an assay that is evident from the appended examples. It is documented in the

appended examples, that the mutated Semaphorin 3A binds to Plexin A4 receptor, with a high affinity, i.e. displaying dissociation constant K_D in the very-low-nanomolar/sub-nanomolar range. Furthermore, it is documented that the mutated Semaphorin 3B binds to Plexin A2 receptor, with a high affinity, i.e. displaying dissociation constant K_D in the very-low-nanomolar/sub-nanomolar range.

Further, the mutated Semaphorin 3, the functional fragment thereof, the functional sema domain or the fusion protein/polypeptide of the invention inhibits Rap1 GTP loading (by 65%). Accordingly, the amino acid sequences and/or nucleic sequences of the present invention mediate downstream signaling pathways relevant in angiogenesis. Therefore, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention inhibits directly and/or indirectly angiogenesis, as shown in the appended examples. In particular, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention inhibits Rap1 GTP loading by at least 50%. In preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention inhibits Rap1 GTP loading by at least 55%. In even more preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention inhibits Rap1 GTP loading by at least 55%. In most preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention inhibits Rap1 GTP loading by at least 65%. The inhibition of Rap1 GTP loading can be measured by standard methods known in the art, such as an assay that is evident from the appended examples. Further, the mutated Semaphorin 3, the functional fragment thereof, the functional sema domain or the fusion protein/polypeptide of the invention activates ERK 1/2 phosphorylation (by 3.9 fold). In particular, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention activates ERK 1/2 phosphorylation by at least 2.5 fold. In preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention activates ERK 1/2 phosphorylation by at least 3.0 fold. In even more preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention activates ERK 1/2 phosphorylation by at least 3.5 fold. In most preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention activates ERK 1/2 phosphorylation by at least 4.9

fold. The activation of ERK 1/2 phosphorylation can be measured by standard methods known in the art, such as an assay that is evident from the appended examples.

Further, the mutated Semaphorin 3, the functional fragment thereof, the functional sema domain, the fusion protein or the polypeptide of the invention inhibits the motility of cells, such as ECs (by 46%). Accordingly, the mutated Semaphorin 3 of the invention, the functional fragment thereof and the fusion protein/polypeptide of the invention are (superior) inhibitors of the motility of cells and/or inhibitors of the metastatic dissemination of cancer cells. Thus, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention inhibits metastatic dissemination of cancer cells. In particular, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention inhibits the motility of cells such as endothelial cells by at least 30%. In preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention inhibits the motility of cells such as endothelial cells by at least 35%. In even more preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention inhibits the motility of cells such as endothelial cells by at least 40%. In most preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide of the invention inhibits the motility of cells such as endothelial cells by at least 45%. The motility of cells can be measured by standard methods known in the art, such as an assay that is evident from the appended examples.

In a further embodiment the invention relates to a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of a mutated Semaphorin 3 or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In a further embodiment, the invention relates to the mutated Semaphorin 3 or the functional fragment thereof, wherein said mutated Semaphorin 3 or said functional fragment thereof that

functions as an inhibitor of angiogenesis comprises an amino acid sequence, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In a further embodiment, the invention relates to a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of a genetically modified Semaphorin 3 or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In a further embodiment, the invention relates to the genetically modified Semaphorin 3 or functional fragment thereof, wherein said mutated Semaphorin 3 or said functional fragment thereof that functions as an inhibitor of angiogenesis comprises an amino acid sequence, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In a further embodiment, the herein provided non-naturally occurring/artificial/mutated Semaphorin 3 proteins or their herein described functional fragments and/or fusion proteins/polypeptides comprising said non-naturally occurring/artificial/mutated Semaphorin 3 proteins or said non-naturally occurring/artificial/mutated functional fragments or said functional sema domains of said Semaphorin 3 proteins or the herein provided polypeptides have the activity to function as a vascular normalizing agent. In other words the compounds of the invention, i.e., the mutated Semaphorin 3 proteins/functional fragments

thereof/functional sema domain/fusion polypeptides/proteins as described herein have the activity to function as a vascular normalizing agent, wherein the vascular normalizing agent normalizes cancer blood vessel, i.e., increases the pericyte coverage, enhances the perfusion of cancer blood vessels, reduces tissue hypoxia and/or improves drug delivery to cancer. Accordingly, the amino acid sequences and/or nucleic sequences of the present invention relate to a direct and/or indirect vascular normalizing agent.

Thus, the invention relates to a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of a mutated Semaphorin 3 or a functional fragment thereof that functions as vascular normalizing agent, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

Further, the invention relates to the mutated Semaphorin 3 or the functional fragment thereof, wherein said mutated Semaphorin 3 or said functional fragment thereof that functions as a vascular normalizing agent comprises an amino acid sequence, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

The following description relates to each one of the embodiments of the present invention as described herein above unless explicitly stated otherwise.

Mutated Semaphorin 3 proteins, genetically modified Semaphorin 3 proteins or related polypeptides (functional fragments thereof and/or fusion proteins comprising said mutated Semaphorin 3 proteins or said mutated functional fragments of said Semaphorin 3 proteins having an identity of at least 55 % to the specific Semaphorin 3 proteins provided and defined herein, and the like) have primarily the activity as an angiogenesis inhibitor.

Further, mutated Semaphorin 3 proteins, genetically modified Semaphorin 3 proteins or related polypeptides (functional fragments thereof and/or fusion proteins comprising said mutated Semaphorin 3 proteins or said mutated functional fragments of said Semaphorin 3 proteins having an identity of at least 55 % to the specific Semaphorin 3 proteins provided and defined herein, and the like) have primarily the activity as a vascular normalizing agent.

In preferred aspects, the nucleic acid molecule encoding for the herein provided mutated Semaphorin 3 is preferably at least 50% homologous/identical to the nucleic acid sequence as shown in SEQ ID NO: 1. It is understood that such nucleic acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the nucleic acid sequence encoding the herein provided mutated Semaphorin 3 is at least 52%, 53%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 1, wherein the higher values of sequence identity are preferred.

In certain aspects, the nucleic acid molecule encoding for the herein provided mutated Semaphorin 3 is preferably at least 48% homologous/identical to the nucleic acid sequence as shown in SEQ ID NOs: 5. It is understood that such nucleic acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the nucleic acid sequence encoding the herein provided mutated Semaphorin 3 is at least 50%, 52%, 53%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 5, wherein the higher values of sequence identity are preferred.

In certain aspects, the nucleic acid molecule encoding for the herein provided mutated Semaphorin 3 is preferably at least 55% homologous/identical to the nucleic acid sequence as shown in SEQ ID NO: 9. It is understood that such nucleic acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the nucleic acid sequence encoding the herein provided mutated Semaphorin 3 is at least 57%, 60%, 63%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 9, wherein the higher values of sequence identity are preferred.

In certain aspects, the nucleic acid molecule encoding for the herein provided mutated Semaphorin 3 is preferably at least 45% homologous/identical to the nucleic acid sequence as shown in SEQ ID NO: 13. It is understood that such nucleic acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the nucleic acid sequence encoding the herein provided mutated Semaphorin 3 is at least 48%, 50%, 53%, , 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 13, wherein the higher values of sequence identity are preferred.

In certain aspects, the nucleic acid molecule encoding for the herein provided mutated Semaphorin 3 is preferably at least 55% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 57, 59, 61, 63, 65, 67, 69 or 71. It is understood that such nucleic acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the nucleic acid sequence encoding the herein provided mutated Semaphorin 3 is at least 56%, 58%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 57, 59, 61, 63, 65, 67, 69 or 71, wherein the higher values of sequence identity are preferred. More preferably, the nucleic acid sequence of all aspects encoding the herein provided mutated Semaphorin 3 is at least 60% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: NO: 1, 3, 5, 7, 9, 11, 13, 15, 57, 59, 61, 63, 65, 67, 69 or 71. More preferably, the nucleic acid sequence encoding the herein provided mutated Semaphorin 3 is at least 70% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 57, 59, 61, 63, 65, 67, 69 or 71. Even more preferably, the nucleic acid sequence encoding the herein provided mutated Semaphorin 3 is at least 80% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 57, 59, 61, 63, 65, 67, 69 or 71. Most preferably, the nucleic acid sequence encoding the herein provided mutated Semaphorin 3 is at least 90% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 57, 59, 61, 63, 65, 67, 69 or 71. The above defined orthologous/homologous/identical sequences can also be encompassed in longer or shorter isoforms, spliced variants and fusion transcripts. The term “orthologous protein” or

“orthologous gene” as used herein refers to proteins and genes, respectively, in different species that are similar to each other because they originated from a common ancestor.

Hybridization assays for the characterization of orthologs or other related sequences of known nucleic acid sequences are well known in the art; see e.g. Sambrook, Russell "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Laboratory, N.Y. (2001); Ausubel, "Current Protocols in Molecular Biology", Green Publishing Associates and Wiley Interscience, N.Y. (1989).

The term "hybridization" or "hybridizing", as used herein in connection with nucleic acids, relates to hybridizations under conditions of any degree of stringency. In general, hybridizations of nucleic acids, such as Southern or Northern hybridizations, can be performed under experimental conditions of various degrees of stringency. Usually a nucleic acid immobilized on a solid support such as a membrane is contacted with a liquid containing another, similar nucleic acid (called probe) under suitable buffer and temperature conditions in order to selectively allow the interaction of the probe with the immobilized nucleic acid, wherein the probe has a certain degree of sequence identity to the immobilized nucleic acid to be tested. Commonly the buffer used for the hybridization, in particular the washing steps after hybridization, is standard sodium citrate buffer (SSC; also referred to as saline sodium citrate buffer). A 20-fold concentrated SSC buffer contains 3 M NaCl and 0.3 M sodium citrate, adjusted to pH 7.0 using HCl, and is commercially available, e.g., from Sigma Aldrich. The Na^+ concentration of a corresponding 20-fold SSC buffer is 3.3 M (i.e., 3.3 mol/L), and it is 1.65 M for a 10-fold SSC buffer, 0.825 M for a 5-fold SSC buffer, 0.33 M for a 2-fold SSC buffer, 0.165 M for a 1-fold SSC buffer, and 0.0165 M for a 0.1-fold SSC buffer. Formamide or sodium dodecyl sulfate (SDS) can be added to the SSC buffer to reduce unspecific binding of the probe. The stringency of the hybridization depends on the percentage of the nucleotides G and C present in the sequence of the probe (%G+C) and the hybridization conditions, particularly the temperature, the concentration of Na^+ and the concentration of formamide or SDS (if present). In general, the higher the hybridization temperature and the lower the sodium (Na^+) concentration, the higher will be the stringency. The stringency of the hybridization can thus be controlled by appropriately choosing the temperature for the hybridization, the concentration of the SSC buffer (and thereby the sodium concentration) and optionally the concentration of formamide (or SDS) added to the

SSC buffer. If different concentrations of SSC buffer are used in different steps of the hybridization procedure, the concentrations of sodium and formamide in the most concentrated SSC buffer (which is typically the buffer used for the final washing step) are decisive.

Accordingly, at a given GC-content of the probe and at specific concentrations of sodium and formamide (if present) in the most concentrated buffer used for the washing after hybridization (typically the buffer used for the final washing step), the stringency of the hybridization can be controlled by adjusting the hybridization temperature to a specific number of degrees Celsius (e.g., 25°C or less) below the effective melting temperature (Tm) which can be calculated using the following formula (for DNA-DNA-hybridizations):

$$Tm = 81.5 + 16.6(\log M [Na^+]) + 0.41(\%G+C) - 0.72(\% \text{ formamide})$$

In the above formula, "Tm" is the temperature under which the sequence of the immobilized nucleic acid to be tested needs to match 100% of the sequence of the probe in order for both sequences to hybridize to each other; "log M [Na^+]" is the logarithm to base 10 ($\log_{10}$) of the concentration of sodium (Na^+) in mol/L in the buffer; "%G+C" is the percentage of the nucleotides G and C in the sequence of the probe (GC-content); and "% formamide" is the concentration of formamide in %(volume/volume) in the buffer. As is well known, the length of the probe to be determined constitutes further parameters of the hybridization conditions.

The farther the hybridization temperature is below the Tm, the lower will be the stringency of the hybridization. In particular, for each 1.4°C the hybridization temperature is below the calculated Tm, the hybridization will still occur in the presence of 1% sequence mismatch, i.e., a mismatch of x% of the sequences of the probe and the immobilized nucleic acid to be tested will still lead to hybridization if the hybridization temperature is at least x • 1.4°C below the calculated Tm. For example, if the Tm is calculated to be 90°C and the hybridization experiment is conducted at 55°C (i.e., 35°C below the Tm), nucleic acid sequences matching at least 82.1% of the sequence of the probe will hybridize to the probe (i.e., 35°C/1.4°C = 25.0, meaning that 100% – 25.0% = 75.0%).

As used herein, hybridization under "stringent conditions" preferably means that the hybridization temperature is about 35°C or less below the Tm (calculated using the formula

explained above), which corresponds to a minimum sequence identity of about 75.0% required for hybridization to occur (i.e., 100% – (35°C/1.4°C)%). More preferably, hybridization under stringent conditions means that the hybridization temperature is about 30°C or less below the Tm (corresponding to a minimum sequence identity of about 78.6% required for hybridization), even more preferably, hybridization under stringent conditions means that the hybridization temperature is about 25°C or less below the Tm (corresponding to a minimum sequence identity of about 82.1% required for hybridization), even more preferably, hybridization under stringent conditions means that the hybridization temperature is about 20°C or less below the Tm (corresponding to a minimum sequence identity of about 85.7% required for hybridization), even more preferably about 15°C or less below the Tm (corresponding to a minimum sequence identity of about 89.3% required for hybridization), even more preferably about 10°C or less below the Tm (corresponding to a minimum sequence identity of about 92.9% required for hybridization), even more preferably about 7°C or less below the Tm (corresponding to a minimum sequence identity of about 95.0% required for hybridization), yet even more preferably about 5°C or less below the Tm (corresponding to a minimum sequence identity of about 96.4% required for hybridization), and still more preferably about 3°C or less below the Tm (corresponding to a minimum sequence identity of about 97.9% required for hybridization). Conversely, hybridization under “non-stringent conditions” means that the hybridization temperature is below the above-defined temperature required for stringent hybridization. If not further specified, the conditions are preferably non-stringent. Said hybridization conditions may be established according to conventional protocols described, e.g., in Sambrook (2001) loc. cit.; Ausubel (1989) loc. cit., or Higgins and Hames (Eds.) "Nucleic acid hybridization, a practical approach" IRL Press Oxford, Washington DC, (1985). The setting of conditions is well within the skill of the artisan and can be determined according to protocols described in the art.

In accordance with the present invention, the terms "homology" or "percent homology" or "identical" or "percent identity" or “percentage identity” or “sequence identity” in the context of two or more nucleic acid sequences refers to two or more sequences or subsequences that are the same, or that have a specified percentage of nucleotides that are the same, when compared and aligned for maximum correspondence over the window of comparison (preferably over the full length), or over a designated region (e.g., the functional sema

domain) as measured using a sequence comparison algorithm as known in the art, or by manual alignment and visual inspection. Sequences having, for example, 70% to 90% or greater sequence identity may be considered to be substantially identical. Such a definition also applies to the complement of a test sequence. Preferably the described identity exists over a region that is at least about 15 to about 25 nucleotides in length, more preferably, over a region that is at least about 50 to about 100 nucleotides in length, even more preferably, over a region that is at least about 800 to about 1200 nucleotides in length and most preferably, over the full length. Those having skill in the art will know how to determine percent identity between/among sequences using, for example, algorithms such as those based on CLUSTALW computer program (Thompson Nucl. Acids Res. 2 (1994), 4673-4680) or FASTDB (Brutlag Comp. App. Biosci. 6 (1990), 237-245), as known in the art.

Although the FASTDB algorithm typically does not consider internal non-matching deletions or additions in sequences, i.e., gaps, in its calculation, this can be corrected manually to avoid an overestimation of the % identity. CLUSTALW, however, does take sequence gaps into account in its identity calculations. Also available to those having skill in this art are the BLAST and BLAST 2.0 algorithms (Altschul, (1997) Nucl. Acids Res. 25:3389-3402; Altschul (1993) J. Mol. Evol. 36:290-300; Altschul (1990) J. Mol. Biol. 215:403-410). The BLASTN program for nucleic acid sequences uses as defaults a word length (W) of 11, an expectation (E) of 10, M=5, N=4, and a comparison of both strands. The BLOSUM62 scoring matrix (Henikoff (1989) PNAS 89:10915) uses alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

In order to determine whether a nucleotide in a nucleic acid sequence corresponds to a certain position in the nucleotide sequence of e.g. SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 57, 59, 61, 63, 65, 67, 69 or 71, respectively, the skilled person can use means and methods well-known in the art, e.g., alignments, either manually or by using computer programs such as those mentioned herein. For example, BLAST 2.0, which stands for Basic Local Alignment Search Tool BLAST (Altschul (1997), loc. cit.; Altschul (1993), loc. cit.; Altschul (1990), loc. cit.), can be used to search for local sequence alignments. BLAST, as discussed above, produces alignments of nucleotide sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying similar sequences. The fundamental unit of BLAST algorithm output is the High-

scoring Segment Pair (HSP). An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cut-off score set by the user. The BLAST approach is to look for HSPs between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search. Any database sequence whose match satisfies E is reported in the program output.

Analogous computer techniques using BLAST (Altschul (1997), loc. cit.; Altschul (1993), loc. cit.; Altschul (1990), loc. cit.) are used to search for identical or related molecules in nucleotide databases such as GenBank or EMBL. This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\%\ \text{sequence identity} \times \%\ \text{maximum BLAST score}}{100}$$

and it takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1-2% error; and at 70, the match will be exact. Similar molecules are usually identified by selecting those, which show product scores between 15 and 40, although lower scores may identify related molecules. Another example for a program capable of generating sequence alignments is the CLUSTALW computer program (Thompson (1994) Nucl. Acids Res. 2:4673-4680) or FASTDB (Brutlag (1990) Comp. App. Biosci. 6:237-245), as known in the art.

The explanations and definitions given herein above in respect of “homology/identity of nucleic acid sequences” apply, mutatis mutandis, to “amino acid sequences” of members of the mutated Semaphorin 3 or the functional fragments thereof or the polypeptide, in

particular an amino acid sequence as depicted in SEQ ID NO: 2 (Homo sapiens SEMA3A), SEQ ID NO: 6 (Homo sapiens SEMA3B), SEQ ID NO: 10 (Homo sapiens SEMA3C), SEQ ID NO: 14 (Homo sapiens SEMA3D), SEQ ID NO: 4 (Mus musculus Sema3A), SEQ ID NO: 8 (Mus musculus Sema3B), SEQ ID NO: 12 (Mus musculus Sema3C) and SEQ ID NO: 16 (Mus musculus Sema3D). Exemplary sequences of the Semaphorin 3 proteins comprising a lysine at the position that by comparison of homology corresponds to position 106 of the wild type human Semaphorin 3A as shown in SEQ ID NO: 2 are given in SEQ ID NO: 58, 60, 62, 64, 66, 68, 70 or 72.

The mutated Semaphorin 3 proteins or genetically modified Semaphorin 3 proteins have of at least 55 % homology/identity to a wild type Semaphorin 3 protein/polypeptide having the amino acid sequence as, for example, depicted in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16 and functioning as an inhibitor angiogenesis.

In preferred aspects, the provided mutated Semaphorin 3 of the invention is preferably at least 50% homologous/identical to the amino acid sequence as shown in SEQ ID NO: 2. It is understood that such amino acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the amino acid sequence encoding the herein provided mutated Semaphorin 3 is at least 52%, 53%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the amino acid sequence as shown in any one of SEQ ID NO: 2, wherein the higher values of sequence identity are preferred.

In certain aspects, the herein provided mutated Semaphorin 3 of the invention is preferably at least 48% homologous/identical to the amino acid sequence as shown in SEQ ID NO: 6. It is understood that such amino acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the amino acid sequence encoding the herein provided mutated Semaphorin 3 is at least 50%, 52%, 53%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the amino acid sequence as shown in any one of SEQ ID NO: 6, wherein the higher values of sequence identity are preferred.

In certain aspects, the herein provided mutated Semaphorin 3 of the invention is preferably at least 55% homologous/identical to the amino acid sequence as shown in SEQ ID NO: 10. It is understood that such amino acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the amino acid sequence encoding the herein provided mutated Semaphorin 3 is at least 57%, 60%, 63%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the amino acid sequence as shown in any one of SEQ ID NO: 10, wherein the higher values of sequence identity are preferred.

In certain aspects, the herein provided mutated Semaphorin 3 of the invention is preferably at least 45% homologous/identical to the amino acid sequence as shown in SEQ ID NO: 14. It is understood that such amino acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the amino acid sequence encoding the herein provided mutated Semaphorin 3 is at least 48%, 50%, 53%, , 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the amino acid sequence as shown in any one of SEQ ID NO: 14, wherein the higher values of sequence identity are preferred.

In certain aspects, the provided mutated Semaphorin 3 of the invention is preferably at least 55% homologous/identical to the amino acid sequence as shown in any one of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16.More preferably, the mutated Semaphorin 3 has at least 57%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homology/identity to a wild type Semaphorin 3 protein/polypeptide having the amino acid sequence as, for example, depicted in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14 and 16, respectively, wherein the higher values are preferred. Most preferably, the mutated Semaphorin 3 has at least 99% homology to a wild type Semaphorin 3 protein/polypeptide having the amino acid sequence as, for example, depicted in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16.

The present invention comprises also polypeptides deviating from wild type amino acid sequences described herein above, wherein said deviation may be, for example, the result of amino acid and/or nucleotide substitution(s), deletion(s), addition(s), insertion(s), duplication(s), inversion(s) and/or recombination(s) either alone or in combination. Those deviations may naturally occur or be produced via recombinant DNA techniques well known

in the art; see, for example, the techniques described in Sambrook (Molecular Cloning; A Laboratory Manual, 2nd Edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY (1989)) and Ausubel, "Current Protocols in Molecular Biology", Green Publishing Associates; and Wiley Interscience, N.Y. (1989). The allelic variations may be naturally occurring allelic variants as well as synthetically produced or genetically engineered variants. The polypeptides, peptides or protein fragments encoded by the various derivatives, allelic variants, homologues or analogues of the above-described nucleic acid molecules encoding mutated Semaphorin 3 and/or fragment thereof may share specific common characteristics, such as molecular weight, immunological reactivity, conformation etc., as well as physical properties, such as electrophoretic mobility, chromatographic behavior, sedimentation coefficients, pH optimum, stability, solubility, spectroscopic properties etc.

The terms “complement”, “reverse complement” and “reverse sequence” referred to herein are described in the following example: For sequence 5’AGTGAAGT3’, the complement is 3’TCACTTCA5’, the reverse complement is 3’ACTTCACT5’ and the reverse sequence is 5’TGAAGTGA3’.

The invention relates to the mutated Semaphorin 3 or the functional fragment thereof as defined herein that can be selected from the group of:

(a) a polypeptide that is encoded by a nucleic acid sequence selected from the group of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 9 and SEQ ID NO: 13,
wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding said hydrophilic amino acid,
wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding said hydrophilic amino acid,
wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding said hydrophilic amino acid, and
wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding said hydrophilic amino acid;

(b) a polypeptide having the amino acid sequence selected from the group of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10 and SEQ ID NO: 14, wherein the alanine

residue at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14 is replaced by said hydrophilic amino acid;

(c) a polypeptide that is encoded by a nucleic acid sequence that hybridizes under stringent conditions to the complementary strand of a nucleic acid molecule encoding a polypeptide as defined in (a) or (b);

(d) a polypeptide that functions as an inhibitor of angiogenesis and has at least 55% identity to any one of the polypeptides referred to in (b).

In most preferred embodiments, the invention relates to the mutated Semaphorin 3A or the functional fragment thereof as defined herein that can be selected from the group of:

(a) a polypeptide that is encoded by a nucleic acid sequence as shown in SEQ ID NO: 1
wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding said hydrophilic amino acid,

(b) a polypeptide having the amino acid sequence of SEQ ID NO: 2, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by said hydrophilic amino acid;

(c) a polypeptide that is encoded by a nucleic acid sequence that hybridizes under stringent conditions to the complementary strand of a nucleic acid molecule encoding a polypeptide as defined in (a) or (b);

(d) a polypeptide that functions as an inhibitor of angiogenesis and has at least 50% identity to any one of the polypeptides referred to in (b).

In certain aspects, the mutated Semaphorin 3 or the functional fragment thereof as defined herein can be selected from the group of:

(a) a polypeptide comprising an amino acid sequence encoded by a nucleic acid molecule having the nucleic acid sequence selected from the group of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 9 and SEQ ID NO: 13,
wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding said hydrophilic amino acid,

wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding said hydrophilic amino acid,

wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding said hydrophilic amino acid, and

wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding said hydrophilic amino acid;

(b) a polypeptide having an amino acid sequence selected from the group of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10 and SEQ ID NO: 14, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2, corresponding to position 105 of SEQ ID NO: 6, corresponding to position 104 of SEQ ID NO: 10 or corresponding to position 120 of SEQ ID NO: 14 is replaced by said hydrophilic amino acid;

(c) a polypeptide encoded by a nucleic acid molecule encoding a polypeptide having an amino acid sequence selected from the group of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10 and SEQ ID NO: 14, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2, corresponding to position 105 of SEQ ID NO: 6, corresponding to position 104 of SEQ ID NO: 10 or corresponding to position 120 of SEQ ID NO: 14 is replaced by said hydrophilic amino acid;

(d) a polypeptide encoded by a nucleic acid molecule hybridizing under stringent conditions to the complementary strand of a nucleic acid molecule as defined in (a) or (c);

(e) a polypeptide having at least 55% identity to the polypeptide of any one of (a) to (d) and functioning as an inhibitor of angiogenesis; and

(f) a polypeptide that functions as an inhibitor of angiogenesis comprising an amino acid sequence encoded by a nucleic acid molecule being degenerate as a result of the genetic code to the nucleotide sequence of a nucleic acid molecule as defined in any one of (a), (c) and (d).

In certain aspects, the mutated Semaphorin 3, the functional fragment thereof or the polypeptide comprises the amino acid sequence selected from the group of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 14, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2;

wherein the alanine residue corresponding to position 106 of SEQ ID NO: 4; wherein the alanine residue corresponding to position 105 of SEQ ID NO: 6; wherein the alanine residue corresponding to position 105 of SEQ ID NO: 8; wherein the alanine residue corresponding to position 104 of SEQ ID NO: 10; wherein the alanine residue corresponding to position 104 of SEQ ID NO: 12; wherein the alanine residue corresponding to position 120 of SEQ ID NO: 14; or wherein the alanine residue corresponding to position 120 of SEQ ID NO: 16 is replaced by an hydrophilic amino acid.

In most preferred embodiments the mutated Semaphorin 3A, the functional fragment thereof or the polypeptide comprises the amino acid sequence selected from the group of SEQ ID NO: 2, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 4 is replaced by an hydrophilic amino acid.

In other words, the invention relates to a mutated Semaphorin 3 or a fragment thereof, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2; the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 4; the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6; the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 8; the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 12; the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14; or the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 16 is replaced by a hydrophilic amino acid.

Accordingly, the skilled person understands that in case the inventive mutation is defined herein by a specific position, e.g. the alanine at the position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced, it is clear that also a corresponding amino acid (position) can be meant in other Semaphorin 3 proteins, such as other Semaphorin 3A polypeptides, or Semaphorin 3B, C or D polypeptides, which for instance can be found by comparison of homology. Hence, it is understood herein that for the identification of further wild-type sequences and/or for the detection of the relevant specific amino acid residue corresponding to the alanine on position 106 of wild-type Semaphorin 3A that is mutated according to the invention standard homology screenings (e.g. sequence alignments) or PCR-mediated screening techniques can be employed.

In most preferred embodiments, the invention relates to the mutated Semaphorin 3A or the functional fragment thereof, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid.

SEQ ID NO: 58, 60, 62, 64, 66, 68, 70 or 72 relates to the full length human or mouse mutated Semaphorin 3A, B, C or D, wherein lysine is in place at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. Therefore, comprising a hydrophilic amino acid in place of means that the specific alanine, e.g., corresponding to position 106 shown in SEQ ID NO: 2, that is present in the naturally occurring Semaphorin 3A, B, C or D is mutated or changed to the hydrophilic amino acid, preferably lysine, in the mutated Semaphorin 3A, B, C or D.
An exemplary polypeptide comprising a human mutated Semaphorin 3A has an amino acid sequence as given in SEQ ID NO: 58, wherein a lysine is in place at the position 106.
An exemplary polypeptide comprising a mouse mutated Semaphorin 3A has an amino acid sequence as given in SEQ ID NO: 60, wherein a lysine is in place at the position 106.
An exemplary polypeptide comprising a human mutated Semaphorin 3B has an amino acid sequence as given in SEQ ID NO: 62, wherein a lysine is in place at the position 105.
An exemplary polypeptide comprising a mouse mutated Semaphorin 3B has an amino acid sequence as given in SEQ ID NO: 64, wherein a lysine is in place at the position 105.
An exemplary polypeptide comprising a human mutated Semaphorin 3C has an amino acid sequence as given in SEQ ID NO: 66, wherein a lysine is in place at the position 104.
An exemplary polypeptide comprising a mouse mutated Semaphorin 3C has an amino acid sequence as given in SEQ ID NO: 68, wherein a lysine is in place at the position 104.
An exemplary polypeptide comprising a human mutated Semaphorin 3D has an amino acid sequence as given in SEQ ID NO: 70, wherein a lysine is in place at the position 120.
An exemplary polypeptide comprising a mouse mutated Semaphorin 3D has an amino acid sequence as given in SEQ ID NO: 72, wherein a lysine is in place at the position 120.
Therefore, the invention relates to the mutated Semaphorin 3 or the functional fragment thereof wherein the mutated Semaphorin 3 comprises an amino acid sequence that is selected from the group consisting of SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70 and SEQ ID NO: 72 or a functional fragment thereof. In preferred aspects of the invention, the invention relates to the mutated Semaphorin 3A or the functional fragment thereof wherein the mutated Semaphorin 3A

comprises an amino acid sequence that is SEQ ID NO: 58 or SEQ ID NO: 60. In preferred aspects, the functional fragment is the sema domain as detailed herein below.

Further, the invention relates to a nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof. The nucleic acid molecule of the invention can be selected from the group of:

(a) a nucleic acid molecule selected from the group of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 9 and SEQ ID NO: 13,
wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding said hydrophilic amino acid,
wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding said hydrophilic amino acid,
wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding said hydrophilic amino acid, and
wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding said hydrophilic amino acid;

(b) a nucleic acid molecule encoding a polypeptide selected from the group of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10 and SEQ ID NO: 14, wherein the alanine residue at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14 is replaced by said hydrophilic amino acid;

(c) a nucleic acid molecule hybridizing under stringent conditions to the complementary strand of a nucleic acid molecule as defined in (a) or (b);

(d) a nucleic acid molecule encoding a polypeptide that functions as an inhibitor of angiogenesis and has at least 55% identity to any one of the polypeptides referred to in (b); and

(e) a nucleic acid molecule being degenerate as a result of the genetic code to the nucleotide sequence of a nucleic acid molecule as defined in any one of (a) to (d), wherein the degenerate nucleic acid molecule encodes a polypeptide that functions as an inhibitor of angiogenesis.

In certain aspects, the encoded mutated Semaphorin 3 or the functional fragment thereof as defined herein can be selected from the group of:

(a) a nucleic acid molecule comprising a nucleic acid molecule having a DNA sequence selected from the group of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 9 and SEQ ID NO: 13,
wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding said hydrophilic amino acid,
wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding said hydrophilic amino acid,
wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding said hydrophilic amino acid, and
wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding said hydrophilic amino acid;

(b) a nucleic acid molecule comprising a nucleic acid molecule encoding a polypeptide having the amino acid sequence selected from the group of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10 and SEQ ID NO: 14, wherein the alanine residue at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14 is replaced by said hydrophilic amino acid;

(c) a nucleic acid molecule hybridizing under stringent conditions to the complementary strand of a nucleic acid molecule as defined in (a) or (b);

(d) a nucleic acid molecule comprising a nucleic acid molecule encoding a polypeptide that functions as an inhibitor of angiogenesis and has at least 55% identity to any one of the polypeptides referred to in (b); and

(e) a nucleic acid molecule being degenerate as a result of the genetic code to the nucleotide sequence of a nucleic acid molecule as defined in any one of (a) to (d), wherein the degenerate nucleic acid molecule encodes a polypeptide that functions as an inhibitor of angiogenesis.

In most preferred embodiments, the encoded mutated Semaphorin 3A or the functional fragment thereof as defined herein can be selected from the group of:

(a) a nucleic acid molecule as shown in SEQ ID NO: 1, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding said hydrophilic amino acid,

(b) a nucleic acid molecule encoding a polypeptide as shown in SEQ ID NO: 2, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by said hydrophilic amino acid;

(c) a nucleic acid molecule hybridizing under stringent conditions to the complementary strand of a nucleic acid molecule as defined in (a) or (b);

(d) a nucleic acid molecule encoding a polypeptide that functions as an inhibitor of angiogenesis and has at least 50% identity to any one of the polypeptides referred to in (b); and

(e) a nucleic acid molecule being degenerate as a result of the genetic code to the nucleotide sequence of a nucleic acid molecule as defined in any one of (a) to (d), wherein the degenerate nucleic acid molecule encodes a polypeptide that functions as an inhibitor of angiogenesis.

In certain aspects, the encoded mutated Semaphorin 3 or the functional fragment thereof as defined herein can be selected from the group of:

a nucleic acid molecule comprising a nucleic acid sequence as defined in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11,SEQ ID NO: 13 and SEQ ID NO: 15,

wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding said hydrophilic amino acid,

wherein the nucleotides GCT at position 965 to 967 of SEQ ID NO: 3 are replaced by nucleotides encoding said hydrophilic amino acid,

wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding said hydrophilic amino acid,

wherein the nucleotides GCT at position 712 to 714 of SEQ ID NO: 7 are replaced by nucleotides encoding said hydrophilic amino acid,

wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding said hydrophilic amino acid,

wherein the nucleotides GCT at position 498 to 500 of SEQ ID NO: 11 are replaced by nucleotides encoding said hydrophilic amino acid,

wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding said hydrophilic amino acid, or

wherein the nucleotides GCT at position 904 to 906 of SEQ ID NO: 15 are replaced by nucleotides encoding said hydrophilic amino acid.

SEQ ID NO: 57, 59, 61, 63, 65, 67, 69 or 71 relates to a nucleic acid sequence encoding the full length human or mouse mutated Semaphorin 3A, B, C or D, wherein lysine is in place at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2.

An exemplary nucleic acid molecule encoding the mutated human Semaphorin 3A comprises a nucleic acid sequence as defined in SEQ ID NO: 57, wherein the nucleotides at position 631 to 633 of SEQ ID NO: 57 encode for the amino acid lysine.

An exemplary nucleic acid molecule encoding the mutated mouse Semaphorin 3A comprises a nucleic acid sequence as defined in SEQ ID NO: 59, wherein the nucleotides at position 965 to 967 of SEQ ID NO: 59 encode for the amino acid lysine.

An exemplary nucleic acid molecule encoding the mutated human Semaphorin 3B comprises a nucleic acid sequence as defined in SEQ ID NO: 61, wherein the nucleotides at position 559 to 561 of SEQ ID NO: 61 encode for the amino acid lysine.

An exemplary nucleic acid molecule encoding the mutated mouse Semaphorin 3B comprises a nucleic acid sequence as defined in SEQ ID NO: 63, wherein the nucleotides at position 712 to 714 of SEQ ID NO: 63 encode for the amino acid lysine.

An exemplary nucleic acid molecule encoding the mutated human Semaphorin 3C comprises a nucleic acid sequence as defined in SEQ ID NO: 65, wherein the nucleotides at position 872 to 874 of SEQ ID NO: 65 encode for the amino acid lysine.

An exemplary nucleic acid molecule encoding the mutated mouse Semaphorin 3C comprises a nucleic acid sequence as defined in SEQ ID NO: 67, wherein the nucleotides at position 498 to 500 of SEQ ID NO: 67 encode for the amino acid lysine.

An exemplary nucleic acid molecule encoding the mutated human Semaphorin 3D comprises a nucleic acid sequence as defined in SEQ ID NO: 69, wherein the nucleotides at position 398 to 400 of SEQ ID NO: 69 encode for the amino acid lysine.

An exemplary nucleic acid molecule encoding the mutated mouse Semaphorin 3D comprises a nucleic acid sequence as defined in SEQ ID NO: 71, wherein the nucleotides at position 904 to 906 of SEQ ID NO: 71 encode for the amino acid lysine.

The nucleic acid given in SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69 or SEQ ID NO: 71 encodes full length mutated Semaphorin 3 proteins. Therefore, the invention relates to the mutated Semaphorin 3 or the functional fragment thereof wherein the mutated Semaphorin 3 is encoded by a nucleic acid molecule comprising the nucleic acid selected from the group consisting of SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69 and SEQ ID NO: 71. In preferred aspects of the invention, the mutated Semaphorin 3 or the functional fragment thereof is mutated Semaphorin 3A or the functional fragment thereof, wherein the mutated Semaphorin 3A is encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO: 57 or SEQ ID NO: 59. In preferred aspects, the functional fragment is the sema domain as detailed herein below.

In certain aspects, the encoded mutated Semaphorin 3 or the functional fragment thereof as defined herein can be selected from the group of:

> a nucleic acid molecule comprising a nucleic acid sequence as defined in SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 9 and SEQ ID NO: 13,
> wherein the codon at nucleotide position 631 to 633 of SEQ ID NO: 1 is replaced by a codon encoding said hydrophilic amino acid,
> wherein the codon at nucleotide position 559 to 561 of SEQ ID NO: 5 is replaced by a codon encoding said hydrophilic amino acid,
> wherein the codon at nucleotide position 872 to 874 of SEQ ID NO: 9 is replaced by a codon encoding said hydrophilic amino acid, and
> wherein the codon at nucleotide position 398 to 400 of SEQ ID NO: 13 is replaced by a codon encoding said hydrophilic amino acid;

A codon encoding a hydrophilic amino acid means in accordance with the present invention, a codon, which according to the standard genetic code (as illustrated, inter alia, in Stryer (1995), "Biochemistry", Freeman and Company, ISBN 0-7167-2009-4) codes for a

"hydrophilic amino acid". In certain aspects, K is encoded by a codon coding for K. In particular preferred aspects, K is encoded by the codon AAG or AAA. The degeneracy of the genetic code permits the same amino acid sequence to be encoded and translated in many different ways. For example, leucine, serine and arginine are each encoded by six different codons, while valine, proline, threonine, alanine and glycine are each encoded by four different codons. However, the frequency of use of such synonymous codons varies from genome to genome among eukaryotes and prokaryotes. For example, synonymous codon-choice patterns among mammals are very similar, while evolutionarily distant organisms such as yeast (S. cerevisiae), bacteria (such as E. coli) and insects (such as D. melanogaster) reveal a clearly different pattern of genomic codon use frequencies. Therefore, codon optimized genes can be used in the present invention. The design of codon optimized genes should take into account a variety of factors, including the frequency of codon usage in an organism, nearest neighbor frequencies, RNA stability, the potential for secondary structure formation, the route of synthesis and the intended future DNA manipulations of that gene.
It is contemplated herein that codon optimized nucleic acid sequences can be employed. Such codon optimized genes (SEQ ID NOs: 17, 19, 43 and 44) were used in the appended examples.

As was shown herein, the replacement of alanine by a hydrophilic amino acid in the consensus motif $CX_1X_2A_3GKD$ results in beneficial pharmacologic effects.
Therefore, the amino acid sequences of the present invention relates to the mutated Semaphorin 3 or the functional fragment thereof, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein

X_1 is an amino acid, which is K or N,

X_2 is an amino acid selected from the group of W, M and L

and wherein the alanine (A_3) is replaced by said hydrophilic amino acid.
In other words, the present invention relates to the mutated Semaphorin 3 or the functional fragment thereof, wherein said mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C, and Semaphorin 3D, wherein preferably said mutated Semaphorin is Semaphorin 3A, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein

X_1 is an amino acid, which is K or N,

X_2 is an amino acid selected from the group of W, M and L,

and wherein the alanine (A_3) is replaced by said hydrophilic amino acid.

It is herein understood that A_3 refers to the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2; to the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6, to the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10, or to the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14. "A_3" usually refers to the specific alanine; however, "A_3" can also refer to an amino acid residue that is homologous to alanine, such as valine, isoleucine, leucine, methionine, phenylalanine, tyrosine or tryptophan. Most preferably, "A_3" is alanine. Furthermore, the amino acid residues defined by "C", "X_1", "X_2", "G", "K" and "D" can also refer to amino acid residues that are homologous to said respective defined amino acid residues as long as the mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3 A, B, C and D. According to the invention the mutated Semaphorin 3 is not Semaphorin 3E, F or G. In preferred aspects of the invention, "X_1" is not isoleucine or valine.

Said hydrophilic amino acid is selected from the group consisting of lysine, arginine, asparagine, glutamine, serine, threonine, glutamic acid, aspartic acid and histidine. More preferably, said hydrophilic amino acid is lysine or arginine and most preferably, said hydrophilic amino acid is lysine.

The term "hydrophilic amino acid" preferably means an amino acid selected from the group consisting of N, Q, S, T, E, D, K, R and H. According to the standard three letter amino acid code and single letter code arginine can be abbreviated (Arg) or (R). Lysine can be abbreviated (Lys) or (K). Aspartic acid can be abbreviated (Asp) or (D). Glutamic acid can be abbreviated (Glu) or (E). Glutamine can be abbreviated (Gln) or (Q). Asparagine can be abbreviated (Asn) or (N). Histidine can be abbreviated (His) or (H). Serine can be abbreviated (Ser) or (S). Threonine can be abbreviated (Thr) or (T). N, Q, S and T are hydrophilic uncharged amino acids and E, D, K, R and H are hydrophilic charged amino acids. It is also envisaged herein that said hydrophilic amino acid can be a non-proteinogenic and/or non-standard α-amino acid (such as, e.g., ornithine and citrulline).

The present invention relates to the amino acid sequences comprising the mutated Semaphorin 3 or the functional fragment thereof, wherein said hydrophilic amino acid is

selected from the group of K, R, N, Q, S, T, E, D, and H. In preferred aspects, the present invention relates to the amino acid sequences comprising the mutated Semaphorin 3 or the functional fragment thereof, wherein said hydrophilic amino acid is selected from the group of K, R, E, D, and H. In even more preferred aspects, the present invention relates to the amino acid sequences comprising the mutated Semaphorin 3 or the functional fragment thereof, wherein said hydrophilic amino acid is K or R. In most preferred aspects, the present invention relates to the amino acid sequences comprising the mutated Semaphorin 3 or the functional fragment thereof, wherein said hydrophilic amino acid is K.

In certain aspects, the present invention relates to the amino acid sequences comprising the mutated Semaphorin 3 or the functional fragment thereof, wherein the alanine (A_3) in the amino acid sequence motif $CX_1X_2A_3GKD$ is replaced by said hydrophilic amino acid selected from the group of K, R, N, Q, S, T, E, D, and H. In certain aspects, the present invention relates to the mutated Semaphorin 3 or the functional fragment thereof, wherein the alanine (A_3) in the amino acid sequence motif $CX_1X_2A_3GKD$ is replaced by said hydrophilic amino acid selected from the group of K, R, E, D, and H. In preferred aspects, the present invention relates to the mutated Semaphorin 3 or the functional fragment thereof, wherein the alanine (A_3) in the amino acid sequence motif $CX_1X_2A_3GKD$ is replaced by said hydrophilic amino acid is K or R. In particularly preferred aspects the present invention relates to the mutated Semaphorin 3 or the functional fragment thereof, wherein the alanine (A_3) in the amino acid sequence motif $CX_1X_2A_3GKD$ is replaced by said hydrophilic amino acid is K.

Further, the invention relates to the polypeptides comprising the mutated Semaphorin 3 or the functional fragment thereof, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises said hydrophilic amino acid at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14. In certain aspects the polypeptides of the present invention comprises at least one additional mutation selected from the group consisting of amino acid substitution(s), addition(s), deletions(s), inversion(s) and duplication(s). Most preferred, the invention relates to the polypeptide comprising the mutated Semaphorin 3A or the functional fragment thereof, wherein said mutated Semaphorin 3A or said functional fragment thereof comprises said hydrophilic amino acid at position 106 of SEQ ID NO: 2 and comprises at least one additional mutation selected from the group consisting of amino acid substitution(s),

addition(s), deletions(s), inversion(s) and duplication(s).

In other words, the amino acid sequences of the present invention relates to the mutated Semaphorin 3 or the functional fragment thereof, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises said hydrophilic amino acid at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14 and comprises at least one additional mutation selected from the group consisting of amino acid substitution(s), addition(s), deletions(s), inversion(s) and duplication(s).

In certain aspects, the amino acid sequences of the present invention relates to the mutated Semaphorin 3 or the functional fragment thereof, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises said hydrophilic amino acid at position 106 of SEQ ID NO: 2; at position 106 of SEQ ID NO: 4; at position 105 of SEQ ID NO: 6; at position 105 of SEQ ID NO: 8; at position 104 of SEQ ID NO: 10; at position 104 of SEQ ID NO: 12; at position 120 of SEQ ID NO: 14; or at position 120 of SEQ ID NO: 16 acid and comprises at least one additional mutation selected from the group consisting of amino acid substitution(s), addition(s), deletions(s), inversion(s) and duplication(s).

The following relates to mutated Semaphorin 3 proteins that are encompassed in the fusion proteins/polypeptides. The mutated Semaphorin 3 protein encompassed in the fusion protein/polypeptide can also be a functional fragment of the mutated Semaphorin 3 protein. In other words, the following relates to the herein provided functional fragments of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins or the herein provided functional fragments of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins that are comprised in the fusion protein/polypeptide:

In most preferred embodiments, the herein provided inventive functional fragment of the mutated Semaphorin 3 comprises a functional sema domain, wherein the sema domain comprises the hydrophilic amino acid at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 and wherein the sema domain has the properties of mutated Semaphorin 3A, B, C or D. The term "sema domain" refers to a structural domain of Semaphorin 3 proteins. In general,

Semaphorins comprise a sema domain fold [NCBI Position-Specific Scoring Matrix (PSSM)-ID: 214747; Conserved Domain Database (CDD): smart00630 and cl15693], which is described in prior art as an about 500 amino acid long variation of the so-called β propeller topology (Chen et al., 2011; Gherardi et al., 2004). More generally, β-propeller proteins are a wide family of disc-like structures generated by circularly arranged structural modules, also known as blades, around a central channel. Each blade is a four-stranded antiparallel β sheet. The strands are designated as strand A to D starting from the N-terminal end of each (Chen et al., 2011; Gherardi et al., 2004). The inner strand of each blade (A) lines the channel at the center of the propeller, with strands B and C of the same repeat radiating outward, and strand D of the next repeat forming the outer edge of the blade. The fact that the inner strand of each sheet (strand A) runs parallel to the central axis whereas the outer one (strand D) runs perpendicular (twisting each β sheet to look like a propeller blade) combine to give the domain its propeller-like appearance. In general, the sema domain as described in prior art is a seven blade β propeller and is the largest known variant of the β propeller fold. The large size of the sema domain results from the presence of additional secondary structure elements inserted in several blades and giving rise to several long loops, mostly on the top face of the β propeller, that were termed as extrusions by Love and colleagues, when they described the first Semaphorin crystal structure (Love et al., 2003). The sema domain displays two extrusions named extrusion 1 (between blade 1 and blade 2) and extrusion 2 (inside blade 5). The sema domain uses a 'loop and hook' system to close the circle between the first and the last blades. The fold is stabilized by inter-sheet hydrophobic contacts and, in most structures, by a ‘velcro’-type ring closure in which the N-terminal β strand closes the circle by providing the outermost strand (D) of the seventh (C-terminal) blade. The β propeller is further stabilized by an extension of the N terminus, providing an additional, fifth β strand on the outer edge of blade 6. For example in Semaphorin 3A: i) blade 1 has an additional strand and a helix; ii) blade 4 has an extra helix; blade 5 has the largest insertion composed of three helices and two strands (Antipenko et al., 2003). The sema domain may be characterized by a conserved set of cysteine residues, which form four disulphide bonds to stabilize the structure: Cys 103 – Cys 114, Cys 132 – Cys 144, Cys 269 – Cys 381, and Cys 293 – Cys 341. Hence, a sema domain according to the invention can comprise at least one or all of Cys 103 – Cys 114, Cys 132 – Cys 144, Cys 269 – Cys 381, and Cys 293 – Cys 341.

In Semaphorins the C-terminal β strand of blade 7 of the sema domain leads directly into an about 50 amino acid long Plexin Semaphorin Integrin (PSI) domain (NCBI PSSM-ID: 214655; smart00423), which nestles against the side of blade 6 of the sema domain β propeller (Love et al., 2003). The PSI is a domain formed by a two-stranded antiparallel β-sheet, with two flanking short α helices, connected by three disulfide bridges forming the inner domain core. This repeat motif is found in semaphorins, in several different extracellular receptors, including plexins, and in the β subunit of αβ integrin heterodimers (Xiong et al., 2004). A key difference between the plexin, semaphorin, and integrin PSI domains is a distinctively shorter interstrand AB loop in plexins and semaphorins. The overall structures of the Plexin-, Semaphorin-, and Integrin-PSI domains are different in the C-terminal half of the domain, suggesting how the function of this portion of PSI is defined by its specific structural context.

The heterodimer interface between the sema domain of a Semaphorin and the sema domain of a Plexin receptor can involve three motifs/sequences/consensus motifs included in the sema domain of Semaphorins. As described herein above, the sema domain of the Semaphorins comprises within its structural fold two extrusions named extrusion 1 (between blade 1 and blade 2) and extrusion 2 (inside blade 5). The three consensus motifs/sequences/motifs that are included in the interface between the Semaphorin and the Plexin receptor are localized in the Semaphorin 3A, B, C or D in: i) extrusion 1 herein referred as motif-1 (e.g. SEMA3A amino acids 104-113 of SEQ ID NO: 2 corresponding to SEQ ID NO: 25; SEMA3B amino acids 103-112 of SEQ ID NO: 6 corresponding to SEQ ID NO: 28; SEMA3C amino acids 102-111 of SEQ ID NO: 10 corresponding to SEQ ID NO: 31; SEMA3D amino acids 118-127 of SEQ ID NO:14 corresponding to SEQ ID NO: 34); ii) blade 3 herein referred as motif-2 (e.g. SEMA3A amino acids 214-221 of SEQ ID NO: 2 corresponding to SEQ ID NO: 26; SEMA3B amino acids 213-220 of SEQ ID NO: 6 corresponding to SEQ ID NO: 29; SEMA3C amino acids 211-218 of SEQ ID NO: 10 corresponding to SEQ ID NO: 32; SEMA3D amino acids 231-238 of SEQ ID NO:14 corresponding to SEQ ID NO: 35); and iii) blade 4 herein referred as motif-3 (e.g. SEMA3A amino acids 274-287 of SEQ ID NO: 2 corresponding to SEQ ID NO: 27; SEMA3B amino acids 274-287 of SEQ ID NO: 6 corresponding to SEQ ID NO: 30; SEMA3C amino acids 271-284 of SEQ ID NO: 10 corresponding to SEQ ID NO: 33; SEMA3D amino acids 291-

304 of SEQ ID NO:14 corresponding to SEQ ID NO: 36). Accordingly, the sema domain of the invention can comprise motif-1, motif-2 and/or motif-3.

The amino acid sequence (of extrusion 1/motif-1) as illustrated in SEQ ID NO: 25, 28, 31 or 34 (corresponding to amino acid sequences of Semaphorin A, B, C or D ,respectively) corresponds to the amino acid sequence $CX_1X_2A_3GKD$ (wherein X_1 is K or N; X_2 is an amino acid selected from the group of W, M and L), wherein SEQ ID NOs: 25, 28, 31 or 34 lacks the N-terminal cysteine (C) and comprises further amino acids at the C-terminus. The amino acid sequence (of extrusion 1/motif-1) as illustrated in SEQ ID NO: 25, 28, 31 or 34 is comprised in the herein described mutated Semaphorin 3, the functional fragment thereof of the invention or in the functional sema domain according to the invention, wherein the alanine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by the hydrophilic amino acid. Thus, the functional fragment of the mutated Semaphorin 3 comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 25, 28, 31 and 34, wherein the alanine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by the hydrophilic amino acid. In preferred embodiments, the functional fragment of the mutated Semaphorin 3A comprises the amino acid sequence as shown in SEQ ID NO: 25, wherein the alanine corresponding to the position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by the hydrophilic amino acid.

The amino acid sequence as illustrated in SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55 or SEQ ID NO: 56 (corresponding to amino acid sequences of mutated Semaphorin 3 A, B, C or D ,respectively) corresponds to SEQ ID NO: 25, 28, 31 or 34 (motif-1), respectively, with the difference that the amino acid sequence as shown in SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55 or SEQ ID NO: 56 has a lysine in place at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. The amino acid sequence as shown in SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55 or SEQ ID NO: 56 is herein referred as “motif-1*”. The herein described mutated Semaphorin 3, the functional fragment thereof or the functional sema domain of the invention comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55 and SEQ ID NO: 56. In most preferred embodiments, the

functional fragment of the inventive mutated Semaphorin 3A comprises the amino acid sequence as shown in SEQ ID NO: 53.

In summary, the herein described functional fragment of the non-naturally occurring/artificial/mutated Semaphorin 3 comprises:

- the amino acid sequence $CX_1X_2A_3GKD$, wherein X_1 is an amino acid, which is K or N, X_2 is an amino acid selected from the group of W, M and L and wherein the alanine (A_3) is replaced by the hydrophilic amino acid;
- the amino acid sequence selected from the group consisting of SEQ ID NO: 25, 28, 31 and 34, wherein the alanine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by the hydrophilic amino acid; or
- the amino acid sequence SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55 or SEQ ID NO: 56,

and wherein the functional fragment has the properties/characteristics of mutated Semaphorin 3A, B, C or D and has not the properties/characteristics of Semaphorin 3E, F or G.

The functional fragment of the mutated Semaphorin 3 can comprise further to the amino acid sequence of motif 1* (SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55 or SEQ ID NO: 56) the amino sequence of motif-2 (SEQ ID NO: 26, SEQ ID NO: 29, SEQ ID NO: 32 or SEQ ID NO: 35) and/or the amino sequence of motif-3 (SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NO: 33 or SEQ ID NO: 36). Accordingly, the mutated Semaphorin 3 or the inventive functional fragment of the mutated Semaphorin 3 comprises further to the amino acid sequence $CX_1X_2A_3GKD$ one or more of the following amino acid sequences as defined in any one of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 35 or SEQ ID NO: 36, wherein X_1 is an amino acid, which is K or N, X_2 is an amino acid selected from the group of W, M and L and wherein the alanine (A_3) is replaced by the hydrophilic amino acid, and wherein the functional fragment should have the characteristics of mutated Semaphorin 3A, B, C or D. It is herein understood that the functional fragment of the mutated Semaphorin 3 of this invention should not have the characteristics of Semaphorin 3E, F or G. In preferred embodiments, the functional fragment of the mutated Semaphorin 3A comprises further to the amino acid sequence $CX_1X_2A_3GKD$ one or more of the amino acid sequences as defined in SEQ ID NO: 26 or

SEQ ID NO: 27, wherein X_1 is K, X_2 is W and wherein the alanine (A_3) is replaced by the hydrophilic amino acid, and wherein the functional fragment should have the characteristics of mutated Semaphorin 3A.

The PSI domain stabilizes the structural conformation and/or the structural integrity of the functional sema domain, the functional fragment of the mutated Semaphorin 3 or the fusion protein/polypeptide. The functional fragment of the mutated Semaphorin 3 can comprise fragments of the PSI domain so long as the fragment of the PSI domain has the function to stabilize the mutated Semaphorin 3 or the functional fragment thereof or the fusion protein/ polypeptide, more preferably, the functional sema domain. The PSI domain of Semaphorins 3A, B, C or D shares conserved amino acid sequences illustrated in the consensus motifs/sequences/motifs SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48, respectively. Accordingly, the PSI domain of the invention comprises one or more of the following sequences SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48. An exemplary amino acid sequence of the PSI domain of human Semaphorin 3A spans from amino acid residues 517 to 567 of SEQ ID NO: 2. As shown in the appended examples and thus more preferably, an exemplary amino acid sequence of a shorter PSI domain, which lacks furin protease cleavage sites, spans from amino acid residues 517 to 548 of SEQ ID NO: 2. Exemplary PSI domains are given in the following: an exemplary amino acid sequence of the PSI domain of human Semaphorin 3A spans from amino acid residues 517 to 548 of SEQ ID NO: 2, an exemplary amino acid sequence of the PSI domain of human Semaphorin 3B spans from amino acid residues 516 to 547 of SEQ ID NO: 6, an exemplary amino acid sequence of the PSI domain of human Semaphorin 3C spans from amino acid residues 514 to 545 of SEQ ID NO: 10, and an exemplary amino acid sequence of the PSI domain of human Semaphorin 3D spans from amino acid residues 534 to 565) of SEQ ID NO: 14.

Further, the herein provided mutated Semaphorin 3 or the functional fragment of the mutated Semaphorin 3 can comprise further to the amino acid sequence $CX_1X_2A_3GKD$ one or more of the following amino acid sequence(s) as defined in any one of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 35,SEQ ID NO: 36, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48, wherein X_1 is an amino acid, which is K or N, X_2 is an amino acid selected from the group of W, M and L and wherein the alanine (A_3) is replaced by the hydrophilic amino acid, and

wherein the functional fragment should have the characteristics of mutated Semaphorin 3A, B, C or D. It is understood herein that the functional fragment of the mutated Semaphorin 3 of this invention should not have the characteristics of Semaphorin 3E, F or G. In preferred embodiments, the mutated Semaphorin 3 or the functional fragment of the mutated Semaphorin 3A comprises further to the amino acid sequence $CX_1X_2A_3GKD$ one or more of the following amino acid sequences as defined in any one of SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 45, wherein X_1 is K, X_2 is W and wherein the alanine (A_3) is replaced by the hydrophilic amino acid, and wherein the functional fragment should have the characteristics of mutated Semaphorin 3A. It is herein understood that the herein above given amino acid sequences, inter alia, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55 or SEQ ID NO: 56, can be in the context of the amino acid sequence of naturally occurring Semaphorin 3 proteins. It is also herein envisaged that these amino acid sequences can be linked together with artificial amino acid linkers, e.g., serine-glycine linkers. The length of the functional fragments of the mutated Semaphorin 3 proteins is not limited as long as the functional fragments according to the invention exhibit the function e.g., as an angiogenesis inhibitor and/or as a vascular normalizing agent, as described above for the mutated Semaphorin 3, the functional fragment thereof, the fusion protein comprising the functional fragment or the functional sema domain. It is envisaged herein that such functional fragments can have a length of e.g., 10, 20, 30, 40, 50, 60, 80, 100, 200, 250, 300, 400, 500 or 600 amino acids. Preferably, such fragments have a length of about 400 to 500 amino acids. Preferably, such fragments have a length of about 300 to 400 amino acids. Preferably, such fragments have a length of about 100 to 300 amino acids. It is herein envisaged that that the amino acid sequence of the herein provided functional fragments of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins can be truncated at the N-terminus, the C-terminus and/or in the body of the amino acid sequence. It is herein envisaged that, e.g., 10, 20, 30, 40, or 50 amino acids can be deleted. These deletions/modifications do not depart from the scope of the invention as long as the functional fragment has the characteristics of mutated Semaphorin 3A, B, C or D. Further, these deletions/modifications are not limited as long as the herein provided functional fragment or the fusion protein/polypeptide comprising the herein provided functional fragment, which has the hydrophilic amino acid in place of the alanine at the position which corresponds by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2, has the function/activity as defined herein above, e.g., as an angiogenesis inhibitor and/or as a vascular normalizing agent, of the

mutated Semaphorin 3, the functional fragment thereof, the fusion protein comprising the functional fragment or the functional sema domain.

In most preferred embodiments, the functional fragment of the mutated Semaphorin 3 according to the invention comprises the functional sema domain, wherein said sema domain comprises the hydrophilic amino acid at the position that by comparison of homology corresponds to the position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. In other words, the mutated Semaphorin 3 according to the invention may comprise the functional sema domain, wherein said sema domain comprises the hydrophilic amino acid at the position that by comparison of homology corresponds to the position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. The functional sema domain of the invention comprises the amino acid sequence as described in motif-1* or comprises the amino acid sequence $CX_1X_2A_3GKD$, wherein X_1 is an amino acid, which is K or N, X_2 is an amino acid selected from the group of W, M and L and wherein the alanine (A_3) is replaced by the hydrophilic amino acid, and wherein said sema domain should have the characteristics of mutated Semaphorin 3A, B, C or D. It is herein understood that the herein described functional sema domain of the mutated Semaphorin 3 should not have the characteristics of Semaphorin 3E, F or G. Furthermore, the functional sema domain of the invention can comprise further to the amino acid sequence of motif 1* (SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55 and SEQ ID NO: 56) the amino sequence of motif-2 (SEQ ID NO: 26, SEQ ID NO: 29, SEQ ID NO: 32 or SEQ ID NO: 35) and/or the amino sequence of motif-3 (SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NO: 33, SEQ ID NO: 36, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48. Furthermore, the functional sema domain can comprise at least one additional mutation selected from the group consisting of amino acid substitution(s), addition(s), deletions(s), inversion(s) and duplication(s). Further, the functional sema domain can comprise additional amino acid deletion(s). It is envisaged herein that that the amino acid sequence of the functional sema domain can be truncated at the N-terminus, the C-terminus and/or in the body of the amino acid sequence. It is envisaged herein that, e.g., 10, 20, 30, 40, 50 or 100 amino acids can be deleted. These deletions/modifications do not depart from the scope of the invention as long as the functional sema domain has the characteristics of the mutated Semaphorin 3A, B, C or D as defined herein above. Further, these deletions/modifications are not limited as long as the herein provided functional sema domain or the herein provided fusion protein comprising

said sema domain, which has the hydrophilic amino acid in place of the alanine at the position which corresponds by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2, exhibits the function/activity as defined herein above, e.g., as an angiogenesis inhibitor and/or as a vascular normalizing agent, of the mutated Semaphorin 3 or the functional fragment thereof or of the fusion protein comprising the functional fragment or the functional sema domain. It is herein understood that the functional sema domain interacts with another functional sema domain of Semaphorins and the Plexin receptor. This can occur by means of different surface exposed areas of the sema domain. Without being bound by theory a sema domain displays at least two distinct areas on its surface. The first are area supports its binding to another sema domain of a Semaphorin and the second area is involved in its binding to the Plexin receptor.

An exemplary nucleic acid molecule encoding the functional sema domain or the functional fragment of the mutated Semaphorin 3 of the present invention can comprise:
the nucleotides from 601 to 1206 of SEQ ID NO: 1, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding the hydrophilic amino acid;
the nucleotides from 529 to 1137 of SEQ ID NO: 5, wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding the hydrophilic amino acid;
the nucleotides from 842 to 1444 of SEQ ID NO: 9, wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding the hydrophilic amino acid; or
the nucleotides from 368 to 982 of SEQ ID NO: 13 wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding the hydrophilic amino acid.
SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 9 or SEQ ID NO: 13 is the full-length nucleic acid sequence encoding wild type human Semaphorin 3A, B, C or D, respectively.

In preferred embodiments, the nucleic acid molecule encoding the functional sema domain or the functional fragment of the mutated Semaphorin 3A of the present invention comprises the nucleotides from 601 to 1206 of SEQ ID NO: 1, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding the hydrophilic amino acid.

Further, an exemplary nucleic acid molecule encoding the functional sema domain or the functional fragment of the mutated Semaphorin 3 of the present invention can comprise the nucleotides from 601 to 1206 of SEQ ID NO: 57; the nucleotides from 529 to 1137 of SEQ ID NO: 61; the nucleotides from 842 to 1444 of SEQ ID NO: 65; or the nucleotides from 368 to 982 of SEQ ID NO: 69. SEQ ID NO: 57, 61, 65 or 65 comprises a nucleic acid sequence encoding the mutated Semaphorin 3A, B, C or D, respectively, wherein lysine is in place at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. In preferred embodiments, the nucleic acid molecule encoding the functional sema domain or the functional fragment of the mutated Semaphorin 3A of the present invention comprises the nucleotides from 601 to 1206 of SEQ ID NO: 57.

Further, an exemplary polypeptide comprises the functional fragment of the mutated Semaphorin 3, wherein the functional fragment comprises or is the functional sema domain of the mutated Semaphorin 3 of the present invention as shown in:
In other words, the mutated Semaphorin 3 or the functional fragment thereof comprises or is the functional sema domain of the mutated Semaphorin 3 of the present invention as shown in:
SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by the hydrophilic amino acid;
SEQ ID NO: 22, wherein the alanine residue corresponding to position 105 of SEQ ID NO: 6 is replaced by the hydrophilic amino acid;
SEQ ID NO: 23 wherein the alanine residue corresponding to position 104 of SEQ ID NO: 10 is replaced by the hydrophilic amino acid; or
SEQ ID NO: 24, wherein the alanine residue corresponding to position 120 of SEQ ID NO: 14 is replaced by the hydrophilic amino acid.
In preferred embodiments, the amino acid sequence of the functional sema domain or the functional fragment of the mutated Semaphorin 3A of the present invention comprises an amino acid sequence as shown in SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by the hydrophilic amino acid.

SEQ ID NO: 49, 50, 51 or 52 comprises an amino acid sequence of an exemplary functional sema domain or an exemplary functional fragment of the mutated Semaphorin 3A, B, C or D,

respectively, wherein the alanine is replaced by a lysine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. Thus, an exemplary amino acid sequence of the functional sema domain or the functional fragment of the mutated Semaphorin 3 of the present invention can comprise the amino acid sequence that is selected from the group consisting of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51 and SEQ ID NO: 52. In preferred embodiments, the amino acid sequence of the functional sema domain or the functional fragment of the mutated Semaphorin 3A of the present invention comprises the amino acid sequence as shown in SEQ ID NO: 49. Fragments of the functional sema domain are also envisaged herein. For example, the sema domain can also comprise shortened versions of the herein defined exemplary sema domains.

The following relates to the most preferred embodiment of the invention, the fusion protein/polypeptide. In most preferred embodiments, the polypeptide of the invention is the fusion protein. The fusion protein comprises the non-naturally occurring/artificial/mutated Semaphorin 3 protein, the non-naturally occurring/artificial/mutated functional fragment of the Semaphorin 3, the non-naturally occurring/artificial/mutated functional sema domain of the Semaphorin 3, the stabilizer domain and/or the dimerization domain. Any one of the herein above defined functional fragments of the mutated Semaphorin 3 can be comprised in the fusion protein, wherein the functional fragments have the characteristics/properties of mutated Semaphorin 3A, B, C or D and not of Semaphorin 3E, F or G. In other words, the fusion protein may comprise the mutated Semaphorin 3 or the functional fragment thereof according to the invention.

Accordingly, the fusion protein of the invention comprises the mutated Semaphorin 3 or the functional fragment thereof, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 25, 28, 31 and 34, wherein the alanine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by the hydrophilic amino acid. Optionally, the fusion protein of the invention comprises the mutated Semaphorin 3 or the functional fragment thereof, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises the amino acid sequence $CX_1X_2A_3GKD$, wherein X_1 is an amino acid, which is K or N, X_2 is an amino acid selected

from the group of W, M and L and wherein the alanine (A_3) is replaced by the hydrophilic amino acid. Optionally, the fusion protein of the invention comprises the mutated Semaphorin 3 or the functional fragment thereof, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55 and SEQ ID NO: 56.

In preferred embodiments, the fusion protein of the invention comprises the amino acid sequence as shown in SEQ ID NO: 25, wherein the alanine corresponding to the position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by the hydrophilic amino acid. Optionally, in preferred embodiments, the inventive fusion protein comprises the amino acid sequence as shown in SEQ ID NO: 53.

Further, the inventive fusion protein comprises the mutated Semaphorin 3 or the functional fragment thereof, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises further to the amino acid sequence $CX_1X_2A_3GKD$ one or more of the following amino acid sequences as defined in any one of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 35 or SEQ ID NO: 36, wherein X_1 is an amino acid, which is K or N, X_2 is an amino acid selected from the group of W, M and L and wherein the alanine (A_3) is replaced by the hydrophilic amino acid, and wherein the functional fragment should have the characteristics of mutated Semaphorin 3A, B, C or D. It is herein understood that the functional fragment of the mutated Semaphorin 3 of this invention should not have the characteristics of Semaphorin 3E, F or G. In preferred embodiments, the fusion protein of the invention comprises the mutated Semaphorin 3A or the functional fragment thereof, wherein the mutated Semaphorin 3A or the functional fragment thereof comprises further to the amino acid sequence $CX_1X_2A_3GKD$ one or more of the following amino acid sequences as defined in SEQ ID NO: 26 or SEQ ID NO: 27, wherein X_1 is K, X_2 is W and wherein the alanine (A_3) is replaced by the hydrophilic amino acid, and wherein the functional fragment should have the characteristics of mutated Semaphorin 3A.

Further, the fusion protein of the invention comprises the mutated Semaphorin 3 or the functional fragment thereof, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises further to the amino acid sequence $CX_1X_2A_3GKD$ one or more of the

following amino acid sequence(s) as defined in any one of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 35,SEQ ID NO: 36, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 and SEQ ID NO: 48, wherein X_1 is an amino acid, which is K or N, X_2 is an amino acid selected from the group of W, M and L and wherein the alanine (A_3) is replaced by the hydrophilic amino acid, and wherein the functional fragment should have the characteristics of Semaphorin mutated 3A, B, C or D. It is herein understood that the functional fragment of the mutated Semaphorin 3 of this invention should not have the characteristics of Semaphorin 3E, F or G. In preferred embodiments, the fusion protein of the invention comprises the mutated Semaphorin 3A or the functional fragment thereof, wherein the mutated Semaphorin 3A or the functional fragment thereof comprises further to the amino acid sequence $CX_1X_2A_3GKD$ one or more of the following amino acid sequences as defined in any one of SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 45, wherein X_1 is K, X_2 is W and wherein the alanine (A_3) is replaced by the hydrophilic amino acid, and wherein the functional fragment should have the characteristics of mutated Semaphorin 3A.

In most preferred embodiments, the invention relates to the fusion protein comprising the functional sema domain, wherein within the functional sema domain of the mutated Semaphorin 3 the alanine corresponding to position 106 of the wild type Semaphorin 3A of SEQ ID NO: 2 is replaced by the hydrophilic amino acid or wherein the alanine corresponding to said alanine 106 in Semaphorin 3B, 3C or 3D is replaced by the hydrophilic amino acid. In other words, the fusion protein of the invention comprises the functional fragment of the mutated Semaphorin 3, wherein said functional fragment comprises the functional sema domain, wherein the sema domain comprises the hydrophilic amino acid at the position that by comparison of homology corresponds to the position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. In other words, the fusion protein of the invention comprises the functional sema domain, wherein the sema domain comprises the hydrophilic amino acid at the position that by comparison of homology corresponds to the position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2.

The functional sema domain comprised in the fusion protein of the invention comprises the amino acid sequence as shown in motif-1* (SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55 and SEQ ID NO: 56) or the functional sema domain comprised in the fusion protein of the invention comprises the amino acid sequence $CX_1X_2A_3GKD$, wherein X_1 is an amino acid,

which is K or N, X_2 is an amino acid selected from the group of W, M and L and wherein the alanine (A_3) is replaced by the hydrophilic amino acid, and wherein the functional sema domain should have the characteristics of Semaphorin 3A, B, C or D. It is herein understood that the functional sema domain of the mutated Semaphorin 3 comprised in the fusion protein of this invention should not have the characteristics of Semaphorin 3E, F or G, but should have the characteristics of mutated Semaphorin 3A, B, C or D. Further, the fusion protein comprising the inventive functional sema domain can comprise further to the amino acid sequence of motif 1* (SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55 and SEQ ID NO: 56) the amino sequence of motif-2 (SEQ ID NO: 26, SEQ ID NO: 29, SEQ ID NO: 32 or SEQ ID NO: 35) and/or the amino sequence of motif-3 (SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NO: 33 or SEQ ID NO: 36). Furthermore, the functional sema domain or the functional fragment of the mutated Semaphorin 3 comprised in the fusion protein can comprise at least one additional mutation selected from the group consisting of amino acid substitution(s), addition(s), deletions(s), inversion(s) and duplication(s). It is herein envisaged that that the amino acid sequence of the functional sema domain or the functional fragment of the mutated Semaphorin 3 comprised in the fusion protein can be truncated at the N-terminus, the C-terminus and/or in the body of the amino acid sequence. It is envisaged herein that, e.g., 10, 20, 30, 40, 50 or 100 amino acids can be deleted. These deletions/modifications do not depart from the scope of the invention as long as the functional fragment or the functional sema domain has the characteristics of mutated Semaphorin 3A, B, C or D. Further, these deletions/modifications are not limited as long as the fusion protein comprising the functional sema domain or the functional fragment of the mutated Semaphorin 3 exhibits the herein above defined function/activity of the fusion protein/polypeptide comprising said non-naturally occurring/artificial/mutated Semaphorin 3 proteins or said non-naturally occurring/artificial/mutated functional fragments or functional sema domains of said Semaphorin 3 proteins, e.g., as an angiogenesis inhibitor and/or as a vascular normalizing agent. The fusion protein can also comprise a short isoform of the mutated Semaphorin 3.

An exemplary nucleic acid molecule encoding the functional sema domain or the functional fragment of the mutated Semaphorin 3 that can be comprised in the fusion protein is given in the following:

the nucleotides from 601 to 1206 of SEQ ID NO: 1, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding a hydrophilic amino acid; the nucleotides from 529 to 1137 of SEQ ID NO: 5, wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding a hydrophilic amino acid; the nucleotides from 842 to 1444 of SEQ ID NO: 9, wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding a hydrophilic amino acid; or the nucleotides from 368 to 982 of SEQ ID NO: 13 wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding a hydrophilic amino acid.

In preferred embodiments, the fusion protein comprises the functional sema domain of the present invention, wherein the sema domain is encoded by the nucleic acid molecule that comprises the nucleotides from 601 to 1206 of SEQ ID NO: 1, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding the hydrophilic amino acid.

An exemplary nucleic acid molecule encoding the functional sema domain or the functional fragment of the mutated Semaphorin 3 comprised in the fusion protein can comprise the nucleotides from 601 to 1206 of SEQ ID NO: 57; the nucleotides from 529 to 1137 of SEQ ID NO: 61; the nucleotides from 842 to 1444 of SEQ ID NO: 65; or the nucleotides from 368 to 982 of SEQ ID NO: 69. SEQ ID NO: 57, 61, 65 or 65 comprises a nucleic acid sequence encoding the full length mutated Semaphorin 3A, B, C or D, respectively, wherein lysine is in place at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. In preferred embodiments, the nucleic acid molecule encoding the functional sema domain of the mutated Semaphorin 3A comprised in the fusion protein comprises the nucleotides from 601 to 1206 of SEQ ID NO: 57.

Further, an exemplary fusion protein/polypeptide can comprise the functional fragment of the mutated Semaphorin 3, wherein the functional fragment comprises or is the functional sema domain of mutated Semaphorin 3 as defined in:

SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;

SEQ ID NO: 22, wherein the alanine residue corresponding to position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;
SEQ ID NO: 23 wherein the alanine residue corresponding to position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or
SEQ ID NO: 24, wherein the alanine residue corresponding to position 120 of SEQ ID NO: 14 is replaced by a hydrophilic amino acid.
In preferred embodiments, the amino acid sequence of the functional sema domain of the mutated Semaphorin 3A comprised in the fusion protein comprises an amino acid sequence as shown in SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid.

SEQ ID NO: 49, 50, 51 and 52 comprise amino acid sequences of exemplary functional sema domains or functional fragments of the mutated Semaphorin 3A, B, C and D, respectively, that can be comprised in the fusion protein, wherein the alanine is replaced by a lysine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. Thus, an exemplary fusion protein can comprise the functional fragment of the mutated Semaphorin 3, wherein the functional fragment comprises or is the sema domain selected from the group consisting of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51 and SEQ ID NO: 52. In preferred embodiments, the amino acid sequence of the functional sema domain of the mutated Semaphorin 3A comprised in the fusion protein comprises the amino acid sequence as shown in SEQ ID NO: 49.

In preferred embodiments, the fusion protein comprises further to the mutated Semaphorin 3 or the functional fragment thereof a stabilizer domain. Said stabilizer domain stabilizes the structural conformation and/or the structural integrity of the non-naturally occurring/artificial/mutated Semaphorin 3 protein, the herein provided functional fragment of the non-naturally occurring/artificial/mutated Semaphorin 3 protein, or the herein provided functional sema domain. As defined herein above, such a stabilizer domain can be the PSI domain or fragments thereof. Therefore, the fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof can be stabilized by the PSI domain. The stabilizer domain can be the PSI domain or a fragment thereof, wherein said PSI domain can comprise one of the following consensus sequence motifs SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48. An exemplary amino acid sequence of the PSI domain of

human Semaphorin 3A spans from amino acid residues 517 to 567 of SEQ ID NO: 2. As shown in the appended examples and thus more preferably, an exemplary amino acid sequence of the PSI domain spans from amino acid residues 517 to 548 of SEQ ID NO: 2. Exemplary PSI domains that can be comprised in the fusion protein are given in the following: an exemplary amino acid sequence of the PSI domain of human Semaphorin 3A spans from amino acid residues 517 to 548 of SEQ ID NO: 2, an exemplary amino acid sequence of the PSI domain of human Semaphorin 3B spans from amino acid residues 516 to 547 of SEQ ID NO: 6, an exemplary amino acid sequence of the PSI domain of human Semaphorin 3C spans from amino acid residues 514 to 545 of SEQ ID NO: 10, and an exemplary amino acid sequence of the PSI domain of human Semaphorin 3D spans from amino acid residues 534 to 565 of SEQ ID NO: 14.

In preferred embodiments, the fusion protein can comprise the mutated Semaphorin 3 or the functional fragment thereof, wherein the functional fragment comprises the sema domain and the PSI domain. Therefore, an exemplary fusion protein can comprise the mutated Semaphorin 3 or the functional fragment thereof, wherein the functional fragment thereof comprise an amino acid sequence:

spanning from amino acid residues 1 to 548 of SEQ ID NO: 2, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by the hydrophilic amino acid;

spanning from amino acid residues 1 to 547 of SEQ ID NO: 6, wherein the alanine residue at position 105 of SEQ ID NO: 6 is replaced by the hydrophilic amino acid;

spanning from amino acid residues 1 to 565 of SEQ ID NO: 10, wherein the alanine residue at position 104 of SEQ ID NO: 10 is replaced by the hydrophilic amino acid; or

spanning from amino acid residues 1 to 545 of SEQ ID NO: 14, wherein the alanine residue at position 120 of SEQ ID NO: 14 is replaced by the hydrophilic amino acid. In preferred embodiments, the functional fragment of the mutated Semaphorin 3A comprised in the fusion protein has a polypeptide spanning from amino acid residues 1 to 548 of SEQ ID NO: 2, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by the hydrophilic amino acid.

In other words, the fusion protein can comprise the mutated Semaphorin 3 comprising the sema domain and the PSI domain. Therefore, an exemplary fusion protein can comprise the mutated Semaphorin 3 comprising an amino acid sequence:

spanning from amino acid residues 1 to 548 of SEQ ID NO: 2, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by the hydrophilic amino acid;
spanning from amino acid residues 1 to 547 of SEQ ID NO: 6, wherein the alanine residue at position 105 of SEQ ID NO: 6 is replaced by the hydrophilic amino acid;
spanning from amino acid residues 1 to 565 of SEQ ID NO: 10, wherein the alanine residue at position 104 of SEQ ID NO: 10 is replaced by the hydrophilic amino acid; or
spanning from amino acid residues 1 to 545 of SEQ ID NO: 14, wherein the alanine residue at position 120 of SEQ ID NO: 14 is replaced by the hydrophilic amino acid.

As indicated in the appended examples and as explained above, dimerization of the mutated Semaphorin 3 proteins increases the inhibiting effect of EC migration. Therefore, the mutated Semaphorin 3 proteins of the invention are preferably in the form of the dimer. A functional sema domain of the mutated Semaphorin 3 proteins may be responsible for the dimerization and the binding to the Plexin receptors. The binding of the Semaphorin 3 to its Plexin receptor leads to an activation of the cytoplasmic region of the Plexin receptor, which results in active downstream signaling. Without being bound by theory, the Plexin receptors are activated by the Semaphorin induced dimerization. Therefore, in most preferred embodiments of the invention, the herein provided mutated Semaphorin 3 or the functional sema domain or functional fragment of the mutated Semaphorin 3 is in the form of a dimer with another herein provided mutated Semaphorin 3 or the functional sema domain or functional fragment of the mutated Semaphorin 3. The dimerization of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins or the herein provided functional fragments of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins that are comprised in the fusion protein can be induced by said functional fragments, e.g. by the functional sema domain itself and/or can be induced/promoted by a dimerization domain.

In most preferred embodiments, the fusion protein comprises a dimerization domain further to the mutated Semaphorin 3, the functional fragment of the mutated Semaphorin 3 and/or the stabilizer domain. In other words, the fusion protein comprises further to the mutated Semaphorin 3 or to the functional fragment thereof a stabilizer domain stabilizing the structural integrity of the molecule and/or a dimerization domain inducing homo- or hetero-dimers. In other words, the fusion protein can comprise the stabilizer domain and/or the dimerization domain. The “dimerization domain” refers to a domain that induce/promote

spatial proximity of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins, the herein provided functional fragments of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins, or the herein provided functional sema domains. It is herein understood that a "dimer" is an oligomer consisting of two structural similar monomers joined by bonds that can be either weak or strong, i.e., intermolecular or covalent. The two monomers that form the dimer can be comprised in the same fusion protein or in two fusion proteins. The dimerization domain can be any dimerization domain so long as the two dimerization domains have with each other a dissociation constant K_D in the range of 10^{-5} M to 10^{-6} M. The binding affinity of two sema domains of Semaphorin 3 was found to be in the range of 10^{-5} to 10^{-6} M (Antipenko et al., 2003). The dimerization domain can be selected from the group of a C-terminal IgG constant domain, DARPin and leucine zipper. In a preferred embodiment, the dimerization domain is an IgG constant domain. In an even more preferred embodiment, the dimerization domain is an IgG1 or IgG3 domain. In an even more preferred embodiment, the dimerization domain is an IgG1. Such exemplary amino acid and encoding nucleic acid sequences of human IgG1 are given in SEQ ID NOs: 37, 38 and 41. In a most preferred embodiment, the constant fragment of the IgG1 domain is used as a dimerization domain comprising the amino acid sequence spanning from the position 104 to 330. Such an exemplary amino acid sequence is depicted in SEQ ID NO: 41. The mouse IgG1 constant fragment can also be used corresponding amino acid and nucleic acid sequences are depicted in SEQ ID NOs: 39, 40 and 42. The affinity strength with which, for example, leucine-zippers and/or constant domains, like immunoglobulin CH3 or Fc fragments, hetero- and homo-dimerize is estimated to be at a dissociation constant K_D in the range of ~ 10^{-5} to 10^{-6} M. The dissociation constant of a dimer of sema domains was estimated to be in the range of 10^{-5} to 10^{-6} M (Antipenko et al., 2003). In general, the K_Ds referred to herein (i) apply to, (ii) are at or (iii) are to be measured at a temperature of 4 to 38 °C, preferably 4 to 20 °C (for example 10°C) or 20 to 38 °C (for example 30°C), and/or a pH of 4,5 to 8 (for example a pH of 7). As shown in the appended examples, the dimerization domains, e.g. the IgG1 domain, can be stabilized by disulphide bridges. The two monomers of the dimerization domains, e.g. the IgG1 domains, can be stabilized by disulphide bridges within the dimer.

In most preferred embodiments, the non-naturally occurring/artificial/mutated Semaphorin 3 proteins or the functional fragments thereof or the fusion proteins comprising said

Semaphorin 3 protein(s) or said functional fragment(s) thereof form homo- or hetero-dimers with each other. The term "homo-dimer" means that two identical monomers are in the form of a dimer. The term "hetero-dimer" means that two different monomers are in the form of a dimer.

In certain aspects, the two monomers of the dimer can be comprised in one fusion protein. It is envisaged herein that two of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins, two of the herein provided functional fragments of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins or two of the herein provided functional sema domains can be comprised in one fusion protein. In further aspects, a wild type protein together with the non-naturally occurring/artificial/mutated Semaphorin 3 protein, the herein provided functional fragment of the non-naturally occurring/artificial/mutated Semaphorin 3 protein, or the herein provided functional sema domain can also form the dimer. Thus, a wild type Semaphorin 3 or the fragment thereof can be comprised together with the non-naturally occurring/artificial/mutated Semaphorin 3 protein, the herein provided functional fragment of the non-naturally occurring/artificial/mutated Semaphorin 3 protein or the herein provided functional sema domain in one fusion protein, wherein the fusion protein has the characteristics of mutated Semaphorin 3A, B, C or D. It is understood herein, that the term "first polypeptide" refers to the first monomer in the dimer. The term "second polypeptide" refers to the second monomer in the dimer. In certain aspects, the fusion protein can comprise two non-naturally occurring/artificial/mutated Semaphorin 3 proteins, two of the herein provided functional fragments of the non-naturally occurring/artificial/mutated Semaphorin 3 protein(s), or two of the herein provided functional sema domains, two stabilizer domains and/or one or two dimerization domain(s). The fusion protein can comprise mutated Semaphorin 3, the functional fragment thereof, the functional sema domains and/or the herein described polypeptides of the invention in any combination, wherein the fusion protein has the characteristics of mutated Semaphorin 3A, B, C or D. In the following aspects, such combinations are exemplified:

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3 or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type

Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid and a second polypeptide comprising a Semaphorin 3 or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein said Semaphorin 3 proteins are selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3 or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid and a second polypeptide comprising a mutated Semaphorin 3 or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 proteins are selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3A or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid and a second polypeptide comprising a Semaphorin 3 or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein said Semaphorin 3 protein is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3A or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid and a second polypeptide comprising a mutated Semaphorin 3A or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position

106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid.

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3A or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid and a second polypeptide comprising a mutated Semaphorin 3B or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6 is replaced by a hydrophilic amino acid.

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3A or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid and a second polypeptide comprising a mutated Semaphorin 3C or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10 is replaced by a hydrophilic amino acid.

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3A or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid and a second polypeptide comprising a mutated Semaphorin 3D or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14 is replaced by a hydrophilic amino acid.

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3B or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 105 of the wild type

Semaphorin 3B as shown in SEQ ID NO: 6 is replaced by a hydrophilic amino acid and a second polypeptide comprising a Semaphorin 3 or a functional fragment thereof, wherein said Semaphorin 3 protein is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3B or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6 is replaced by a hydrophilic amino acid and a second polypeptide comprising a mutated Semaphorin 3B or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6 is replaced by a hydrophilic amino acid.

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3B or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6 is replaced by a hydrophilic amino acid and a second polypeptide comprising a mutated Semaphorin 3C or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10 is replaced by a hydrophilic amino acid.

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3B or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6 is replaced by a hydrophilic amino acid and a second polypeptide comprising a mutated Semaphorin 3D or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14 is replaced by a hydrophilic amino acid.

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3C or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10 is replaced by a hydrophilic amino acid and a second polypeptide comprising a Semaphorin 3 or a functional fragment thereof, wherein said Semaphorin 3 protein is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3C or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10 is replaced by a hydrophilic amino acid and a second polypeptide comprising a mutated Semaphorin 3C or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10 is replaced by a hydrophilic amino acid.

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3C or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10 is replaced by a hydrophilic amino acid and a second polypeptide comprising a mutated Semaphorin 3D or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14 is replaced by a hydrophilic amino acid.

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3D or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14 is replaced by a hydrophilic amino acid and a second polypeptide comprising a Semaphorin 3 or a functional fragment thereof, wherein said Semaphorin 3 protein is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In certain aspects, the fusion protein of the invention comprises a first polypeptide comprising a mutated Semaphorin 3D or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14 is replaced by a hydrophilic amino acid and a second polypeptide comprising a mutated Semaphorin 3D or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14 is replaced by a hydrophilic amino acid

It is also envisaged herein that the fusion protein of the mutated Semaphorin 3 comprises the following domains:
(i) a sema domain;
(ii) a PSI domain; and
(iii) a C-terminal IgG constant domain fused to the C-terminus of the PSI domain,
and is further characterized in that the alanine (A_3) residue comprised in the motif $CX_1X_2A_3GKD$ of the Semaphorin 3 is mutated to lysine, wherein the Semaphorin 3 is selected from the group consisting of Semaphorin 3A, B, C and D.

The fusion protein comprises the functional sema domain, wherein said sema domain comprises a hydrophilic amino acid in place of the alanine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 and a dimerization domain and/or a stabilizer domain.
In other words, the fusion protein comprises the functional sema domain, wherein said sema domain is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D, wherein said sema domain comprises
a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2;
a hydrophilic amino acid in place of the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6;
a hydrophilic amino acid in place of the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or
a hydrophilic amino acid in place of the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14;

and wherein said fusion protein further comprises a dimerization domain and/or a stabilizer domain.

Most preferably, the fusion protein comprises a functional sema domain, wherein within said functional sema domain the alanine corresponding to position 106 of the wild type Semaphorin 3A of SEQ ID NO: 2 is replaced by a hydrophilic amino acid or wherein the alanine corresponding to said alanine 106 in Semaphorin 3B, 3C or 3D is replaced by a hydrophilic amino acid and a dimerization domain, wherein the dimerization domain is IgG1 and/or a stabilizer domain, wherein the stabilizer domain is the PSI domain. In most preferred aspects, the fusion protein comprises the functional sema domain of Semaphorin 3A, wherein said sema domain comprises a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 and wherein said fusion protein further comprises a dimerization domain and/or a stabilizer domain.

In most preferred embodiments of the invention, the fusion proteins/polypeptides of the invention can comprise the functional sema domain that is fused to the PSI domain (as shown in the appended examples). The resulting sema-PSI domain is fused to the dimerization domain, e.g., the constant fragment of the IgG1 domain. Furthermore, such fusion proteins lack the Nrp1 binding and/or the furin cleavable Ig-like (amino acids spanning from 580-670 of SEQ ID NO: 2)/basic region (amino acids spanning from 715-771 of SEQ ID NO: 2). Such fusion proteins are herein most preferred embodiments and exemplary nucleic acid molecules of such a encoded fusion protein can comprise a nucleic acid sequence having:

a nucleic acid sequence spanning from nucleotides 316 to 1959 of SEQ ID NO: 1 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding the hydrophilic amino acid;

a nucleic acid sequence spanning from nucleotides 247 to 1887 of SEQ ID NO: 5 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding the hydrophilic amino acid;

a nucleic acid sequence spanning from nucleotides 563 to 2197 of SEQ ID NO: 9 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding the hydrophilic amino acid; or

a nucleic acid sequence spanning from nucleotides 41 to 1735 of SEQ ID NO: 13 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding the hydrophilic amino acid.

In most preferred embodiments, the fusion protein of the mutated Semaphorin 3A is encoded by a nucleic acid sequence spanning from nucleotides 316 to 1959 of SEQ ID NO: 1 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding the hydrophilic amino acid.

It is contemplated herein that codon optimized nucleic acid sequences can be employed as shown in the appended examples (SEQ ID NOs: 17, 19, 43 and 44).

In most preferred embodiments, the fusion protein comprises a sema domain, a stabilizer domain and a dimerization domain. The functional sema domain is fused to the stabilizer domain, e.g., the PSI domain. The resulting sema-PSI domain is fused to the dimerization domain, e.g., the constant fragment of the IgG1 domain as shown in SEQ ID NO: 38 or 41. Such an exemplary fusion protein comprises a sema domain, a PSI and a dimerization domain, wherein the fusion protein comprises an amino acid sequence:

spanning from amino acid residues 1 to 548 of SEQ ID NO: 2 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by the hydrophilic amino acid;

spanning from amino acid residues 1 to 547 of SEQ ID NO: 6 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 105 of SEQ ID NO: 6 is replaced by the hydrophilic amino acid;

spanning from amino acid residues 1 to 565 of SEQ ID NO: 10 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 104 of SEQ ID NO: 10 is replaced by the hydrophilic amino acid; or

spanning from amino acid residues 1 to 545 of SEQ ID NO: 14 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 120 of SEQ ID NO: 14 is replaced by the hydrophilic amino acid.

In most preferred embodiments, the fusion protein of the mutated Semaphorin 3A comprises a polypeptide: spanning from amino acid residues 1 to 548 of SEQ ID NO: 2 and an amino

acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by the hydrophilic amino acid

An exemplary fusion protein comprising the functional sema domain of mutated Semaphorin 3A, the PSI domain, the IgG1 domain is shown in SEQ ID NO: 18 or 20. An exemplary fusion protein comprising the functional sema domain of mutated Semaphorin 3B, mutated Semaphorin 3C, or mutated Semaphorin 3D and the PSI domain, the IgG1 domain is shown in SEQ ID NO: 76, 78 or 79, respectively. SEQ ID NO: 18 shows a fusion protein comprising the functional sema domain of the human mutated Semaphorin 3A, wherein the alanine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO:2 is replaced by lysine, the PSI domain and the human constant fragment of IgG1. The corresponding nucleic acid sequence encoding the fusion protein comprising the functional sema domain of the human mutated Semaphorin 3A, wherein the alanine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO:2 is replaced by lysine, the PSI domain and the human constant fragment of IgG1is given in SEQ ID NO: 17. SEQ ID NOs: 74 and 75 show an amino acid sequence and the encoding nucleic acid sequence of an exemplary fusion protein of Semaphorin 3B without the inventive mutation.
SEQ ID NO: 20 shows a fusion protein comprising the functional sema domain of mouse mutated Semaphorin 3A, wherein the alanine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO:2 is replaced by lysine, the PSI domain and the human constant fragment of IgG1. The corresponding nucleic acid sequence encoding the fusion protein comprising the functional sema domain of the mouse mutated Semaphorin 3A, wherein the alanine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO:2 is replaced by lysine, the PSI domain and the mouse constant fragment of IgG1is given in SEQ ID NO: 19.

The fusion protein as described herein can be a heterologous protein, wherein the non-naturally occurring/artificial/mutated Semaphorin 3 protein, the non-naturally occurring/artificial/mutated functional fragment of the Semaphorin 3 or the non-naturally occurring/artificial/mutated functional sema domain of the Semaphorin 3, the dimerization domain and/or the stabilization domain are from different sources, e.g. from different species.

It is understood herein that the non-naturally occurring/artificial/mutated Semaphorin 3 protein, the non-naturally occurring/artificial/mutated functional fragment of the Semaphorin 3, the non-naturally occurring/artificial/mutated functional sema domain of the Semaphorin 3, the dimerization domain and/or the stabilization domain can be linked/fused together as found in natural occurring Semaphorin 3 proteins, wherein the character of the mutated Semaphorin 3A, B, C or D is maintained. Further, the non-naturally occurring/artificial/mutated Semaphorin 3 protein, the non-naturally occurring/artificial/mutated functional fragment of the Semaphorin 3, the non-naturally occurring/artificial/mutated functional sema domain of the Semaphorin 3, the dimerization domain and/or the stabilization domain can be linked/fused together as is not found in nature. The non-naturally occurring/artificial/mutated Semaphorin 3 protein, the non-naturally occurring/artificial/mutated functional fragment of the Semaphorin 3, the non-naturally occurring/artificial/mutated functional sema domain of the Semaphorin 3, the dimerization domain and/or the stabilization domain can be conjugated/linked together via amino acid linkers, e.g., serine-glycine linkers. Such linkers are known in the art and can be for example short peptide sequences that occur between protein domains. The linkers are often composed of flexible residues like glycine and serine so that the adjacent protein domains are free to move relative to one another. Longer linkers are used when it is necessary to ensure that two adjacent domains do not sterically interfere with one another. Non-peptide bonds are also envisaged herein. Such non-peptide bonds may include disulfide bonds, e.g. between Cys side chains, thioether bonds or non-peptide covalent bonds induced by chemical cross-linkers, such as disuccinimidyl suberate (DSS) or sulfosuccinimidyl 4-[p-maleimidophenyl] butyrate (Sulfo-SMPB), metal-chelating/complexing groups, as well as non-covalent protein-protein interactions. These are merely embodiments of the present invention and it is evident for the skilled artisan that modifications can easily be made within the fusion proteins and used without deferring from the gist of the present invention.

It is also envisaged herein that the stability of the mutated Semaphorin 3 or the functional fragment thereof can be optimized by adding immunoglobulin-like domains and to simultaneously enhance pharmacokinetic properties like prolonged half-life in serum and protection from proteolytic digestion by proteases. Moreover, stability of the formats can be enhanced by optimizing the production. Since linker sequences which are utilized to covalently join domains often leads to aggregates, production lines have been established that

first produce two or three polypeptides that can be easily reassembled in order to generate a functional drug. Such techniques utilize directed disulphide-bridges or crosslinking reagents to covalently join two different polypeptides. Other techniques make use of hetero- or homo-dimerization domains like leucine-zipper domains, Fc-domains and others like knob into hole technologies (see, for example, WO 2007/062466).

The present invention also relates to a vector comprising the nucleic acid sequence(s) of the present invention.

Many suitable vectors are known to those skilled in molecular biology, the choice of which would depend on the function desired and include plasmids, cosmids, viruses, bacteriophages and other vectors used conventionally in genetic engineering. Methods which are well known to those skilled in the art can be used to construct various plasmids and vectors; see, for example, the techniques described in Sambrook et al. (loc cit.) and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1989), (1994). Alternatively, the polynucleotides and vectors of the invention can be reconstituted into liposomes for delivery to target cells. Relevant sequences can be transferred into expression vectors where expression of a particular polypeptide is required. Typical cloning vectors include pBluescript SK, pGEM, pUC9, pBR322 and pGBT9. Typical expression vectors include pTRE, pCAL-n-EK, pESP-1, pOP13CAT.

Preferably said vector is a gene targeting vector and/or a gene transfer vector. Gene therapy, which is based on introducing therapeutic genes (for example for vaccination) into cells by *ex vivo* or *in vivo* techniques, is one of the most important applications of gene transfer. Suitable vectors, vector systems and methods for *in vitro* or *in vivo* gene therapy are described in the literature and are known to the person skilled in the art; see, e.g., Giordano, Nature Medicine 2 (1996), 534-539; Schaper, Circ. Res. 79 (1996), 911-919; Anderson, Science 256 (1992), 808-813, Isner, Lancet 348 (1996), 370-374; Muhlhauser, Circ. Res. 77 (1995), 1077-1086; Wang, Nature Medicine 2 (1996), 714-716; WO 94/29469; WO 97/00957, Schaper, Current Opinion in Biotechnology 7 (1996), 635-640 or Verma, Nature 389 (1997), 239-242 and references cited therein. In certain aspects, the vector is an adeno-associated-virus (AAV) vector. In particular aspects, the AAV virus is an AAV8 and thus the vector is an AAV8 vector. AAV vectors are attractive for gene therapy. The AAV system has several advantages

including long-term gene expression, the inability to autonomously replicate without a helper virus, transduction of dividing and nondividing cells, and the lack of pathogenicity from wild-type infections. It is envisaged herein that AAV serotypes display different organ tropism. Accordingly, different AAV serotypes can be employed to target the proteins/polypeptides of the invention to cancers of different organs. It is envisaged herein that different AAV vectors can be employed in gene therapy according to standard protocols (Grieger et al., 2012 and Asokan et al., 2012).

The nucleic acid molecules of the invention and vectors as described herein above may be designed for direct introduction or for introduction via liposomes, or viral vectors (e.g. adenoviral, retroviral) into the cell. Additionally, baculoviral systems or systems based on vaccinia virus or Semliki Forest Virus can be used as eukaryotic expression system for the nucleic acid molecules of the invention. In addition to recombinant production, fragments of the protein, the fusion protein or antigenic fragments of the invention may be produced by direct peptide synthesis using solid-phase techniques (cf Stewart et al. (1969) Solid Phase Peptide Synthesis, WH Freeman Co, San Francisco; Merrifield, J. Am. Chem. Soc. 85 (1963), 2149-2154). *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City CA) in accordance with the instructions provided by the manufacturer. Various fragments may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

In certain aspects, the vector comprises a nucleic acid sequence which is a regulatory sequence operably linked to said nucleic acid sequence defined herein.

The term "regulatory sequence" refers to DNA sequences, which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, control sequences generally include promoter, ribosomal binding site, and terminators. In eukaryotes general control sequences include promoters, terminators and, in some instances, enhancers, transactivators or transcription factors. The term "control sequence" is intended to include, at a minimum, all components the presence of which are necessary for expression, and may also include additional advantageous components.

The term "operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. In case the control sequence is a promoter, it is obvious for a skilled person that double-stranded nucleic acid is preferably used.

The recited vector can also be an expression vector. An "expression vector" is a construct that can be used to transform a selected host and provides for expression of a coding sequence in the selected host. Expression vectors can for instance be cloning vectors, binary vectors or integrating vectors. Expression comprises transcription of the nucleic acid molecule preferably into a translatable mRNA. Regulatory elements ensuring expression in prokaryotes and/or eukaryotic cells are well known to those skilled in the art. In the case of eukaryotic cells they comprise normal promoters ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Possible regulatory elements permitting expression in prokaryotic host cells comprise, e.g., the P_L, *lac*, *trp* or *tac* promoter in *E. coli*, and examples of regulatory elements permitting expression in eukaryotic host cells are the *AOX1* or *GAL1* promoter in yeast or the CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells.

Beside elements, which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. Furthermore, depending on the expression system used leader sequences capable of directing the polypeptide to a cellular compartment or secreting it into the medium may be added to the coding sequence of the recited nucleic acid sequence and are well known in the art. The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein, or a portion thereof, into the periplasmic space or extracellular medium. Accordingly, such a leader sequence can also be a signal peptide. Thus, the mutated Semaphorin 3 polypeptides can comprise a signal peptide. The signal peptide is a short stretch of amino acids usually present at the N-terminus of proteins that are destined towards the secretory pathway. Such proteins

include those that reside either inside certain organelles, like the endoplasmic reticulum, golgi or endosomes, or are secreted from the cell. The signal peptide is cleaved off and active polypeptides usually do not comprise signal peptides. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product; see supra. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (Invitrogen), pEF-DHFR, pEF-ADA or pEF-neo (Mack et al. PNAS (1995) 92, 7021-7025 and Raum et al. Cancer Immunol Immunother (2001) 50(3), 141-150) or pSPORT1 (GIBCO BRL).

Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming of transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and as desired, the collection and purification of the polypeptide of the invention may follow; see, e.g., the appended examples.

The present invention relates to a host transformed with a vector of the present invention or to a host comprising the nucleic acid molecule of this invention. Said host may be any prokaryotic or eukaryotic cell. Suitable prokaryotic/bacterial cells are those generally used for cloning like E. coli or Bacillus subtilis. Said eukaryotic host may be a mammalian cell.
In a preferred embodiment said mammalian cell is a neuronal cell and/or a cultured cell like, inter alia, a HEK 293 (human embryonic kidney) cell, a CHO, HeLa, NIH3T3, BHK or a PC12 cell. In a particularly preferred embodiment, the HEK293 cell line expresses stably the Epstein-Barr virus nuclear antigen-1 (HEK293-EBNA1, or 293E). In general, this is the most commonly used cell line for large-scale transfection (CSH Protocols; 2008; doi:10.1101/pdb.prot4976).

It is particularly envisaged that the recited host may be a mammalian cell. Particularly preferred host cells comprise HEK cells, HEK293E cells, HEK293 cell line stably expressing the Epstein-Barr virus nuclear antigen-1 (HEK293-EBNA1, or 293E).

The term "cell" or "mammalian cell" as used in this context may also comprise a plurality of cells as well as cells comprised in a tissue. The cell to be used in the screening or validation method may be obtained from samples from a (transgenic) non-human animal or human suffering from a disease, e.g. angiogenic disease, cancer or a disease associated with Semaphorin dependent Plexin receptor activation. The cell (e.g. a tumor cell and the like) may also be obtained or derived from patient samples (e.g. biopsies), in particular a biopsy/biopsies from a patient/subject suffering from a disease as defined herein above or below. Accordingly, the cell may be a human cell. Again, such a cell to be used in the present screening or validation methods may be comprised in a tissue or tissue sample, like in a sample biopsy. The invention also provides for a host transformed or transfected with a vector of the invention. Said host may be produced by introducing the above described vector of the invention or the above described nucleic acid molecule of the invention into the host. The presence of at least one vector or at least one nucleic acid molecule in the host may mediate the expression of a gene encoding the above described mutated Semaphorin 3 or the fragment thereof. The described nucleic acid molecule or vector of the invention, which is introduced in the host, may either integrate into the genome of the host or it may be maintained extrachromosomally. The host can be any prokaryote or eukaryotic cell.

An alternative expression system is the insect system or the insect cell expression system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The coding sequence of a recited nucleic acid molecule may be cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of said coding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses are then used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which the protein of the invention is expressed (Smith, J. Virol. 46 (1983), 584; Engelhard, Proc. Nat. Acad. Sci. USA 91 (1994), 3224-3227). In certain aspects, the pFBDM vector can be used for expression. The insertion into the MultiBac baculoviral DNA is mediated via the Tn7 transposition sequence upon transformation in DH10 MultiBac E. coli cells (Berger et al., 2004; Fitzgerald et al., 2006). Virus amplification and expression can be performed in Sf21 (Spodoptera frugiperda) (Gibco, Invitrogen) and/or High Five (Trichoplusia ni) (Gibco, Invitrogen) cell.

Additional regulatory elements may include transcriptional as well as translational enhancers. Advantageously, the above-described vectors of the invention comprise a selectable and/or scorable marker.

Selectable marker genes useful for the selection of transformed cells and, e.g., plant tissue and plants are well known to those skilled in the art and comprise, for example, antimetabolite resistance as the basis of selection for dhfr, which confers resistance to methotrexate (Reiss, Plant Physiol. (Life Sci. Adv.) 13 (1994), 143-149); npt, which confers resistance to the aminoglycosides neomycin, kanamycin and paromycin (Herrera-Estrella, EMBO J. 2 (1983), 987-995) and hygro, which confers resistance to hygromycin (Marsh, Gene 32 (1984), 481-485). Additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman, Proc. Natl. Acad. Sci. USA 85 (1988), 8047); mannose-6-phosphate isomerase which allows cells to utilize mannose (WO 94/20627) and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue, 1987, In: Current Communications in Molecular Biology, Cold Spring Harbor Laboratory ed.) or deaminase from Aspergillus terreus which confers resistance to Blasticidin S (Tamura, Biosci. Biotechnol. Biochem. 59 (1995), 2336-2338).

Useful scorable markers are also known to those skilled in the art and are commercially available. Advantageously, said marker is a gene encoding luciferase (Giacomin, Pl. Sci. 116 (1996), 59-72; Scikantha, J. Bact. 178 (1996), 121), green fluorescent protein (Gerdes, FEBS Lett. 389 (1996), 44-47) or ß-glucuronidase (Jefferson, EMBO J. 6 (1987), 3901-3907). This embodiment is particularly useful for simple and rapid screening of cells, tissues and organisms containing a recited vector.

As described above, the recited nucleic acid molecule can be used alone or as part of a vector to express the polypeptide of the invention in cells, for, e.g., purification but also for gene therapy purposes. The nucleic acid molecules or vectors containing the DNA sequence(s) encoding any one of the above described polypeptide of the invention is introduced into the cells which in turn produce the polypeptide of interest. Gene therapy, which is based on

introducing therapeutic genes into cells by ex vivo or in vivo techniques, is one of the most important applications of gene transfer. Suitable vectors, methods or gene-delivery systems for in-vitro or in-vivo gene therapy are described in the literature and are known to the person skilled in the art; see, e.g., Giordano, Nature Medicine 2 (1996), 534-539; Schaper, Circ. Res. 79 (1996), 911-919; Anderson, Science 256 (1992), 808-813; Verma, Nature 389 (1994), 239; Isner, Lancet 348 (1996), 370-374; Muhlhauser, Circ. Res. 77 (1995), 1077-1086; Onodera, Blood 91 (1998), 30-36; Verma, Gene Ther. 5 (1998), 692-699; Nabel, Ann. N.Y. Acad. Sci. 811 (1997), 289-292; Verzeletti, Hum. Gene Ther. 9 (1998), 2243-51; Wang, Nature Medicine 2 (1996), 714-716; WO 94/29469; WO 97/00957, US 5,580,859; US 5,589,466; or Schaper, Current Opinion in Biotechnology 7 (1996), 635-640. The recited nucleic acid molecules and vectors may be designed for direct introduction or for introduction via liposomes, or viral vectors (e.g., adenoviral, retroviral) into the cell. Preferably, said cell is a germ line cell, embryonic cell, or egg cell or derived there from, most preferably said cell is a stem cell. An example for an embryonic stem cell can be, inter alia, a stem cell as described in Nagy, Proc. Natl. Acad. Sci. USA 90 (1993), 8424-8428.

The term "prokaryote" is meant to include all bacteria, which can be transformed or transfected with DNA or RNA molecules for the expression of a protein of the invention. Prokaryotic hosts may include gram negative as well as gram positive bacteria such as, for example, E. coli, S. typhimurium, Serratia marcescens and Bacillus subtilis. The term "eukaryotic" is meant to include yeast, higher plant, insect and preferably mammalian cells. Depending upon the host employed in a recombinant production procedure, the protein encoded by the polynucleotide of the present invention may be glycosylated or may be non-glycosylated. Especially preferred is the use of a plasmid or a virus containing the coding sequence of the polypeptide of the invention and genetically fused thereto to a Protein A tag. An above described polynucleotide can be used to transform or transfect the host using any of the techniques commonly known to those of ordinary skill in the art. Furthermore, methods for preparing fused, operably linked genes and expressing them in, e.g., mammalian cells and bacteria are well-known in the art (Sambrook, loc cit.).

Herein provided is also a process for the production of a polypeptide to be used in accordance with the present invention, said process comprising culturing/raising the host of the invention

under conditions allowing the expression of the polypeptide of the invention and optionally recovering/isolating the produced polypeptide from the culture.

The transformed hosts can be grown in fermenters and cultured according to techniques known in the art to achieve optimal cell growth. The polypeptide of the invention can then be isolated from the growth medium, cellular lysates, or cellular membrane fractions. The isolation and purification of the, e.g., microbially expressed polypeptides of the invention may be by any conventional means such as, for example, preparative chromatographic separations and immunological separations such as those involving the use of monoclonal or polyclonal antibodies directed, e.g., against a tag of the polypeptide of the invention or as described in the appended examples.

The conditions for the culturing of a host, which allow the expression, are known in the art to depend on the host system and the expression system/vector used in such process. The parameters to be modified in order to achieve conditions allowing the expression of a recombinant polypeptide are known in the art. Thus, suitable conditions can be determined by the person skilled in the art in the absence of further inventive input.

Once expressed, the polypeptide of the invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like; see, Scopes, "Protein Purification", Springer-Verlag, N.Y. (1982). Substantially pure polypeptides of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity are most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptide of the invention may then be used therapeutically (including extracorporeally) or in developing and performing assay procedures. Furthermore, examples for methods for the recovery of the polypeptide of the invention from a culture are described in the appended examples.

As detailed herein, the present invention also relates to an antibody specifically binding to the mutated Semaphorin 3, the functional fragment thereof or to the inventive fusion protein comprising said mutated Semaphorin 3 or the functional fragment thereof. In particular, herein provided is an antibody specifically binding to the mutated Semaphorin 3 or the functional fragment thereof, wherein said antibody specifically binds to an epitope

comprising the hydrophilic amino acid which replaces the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorins 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein the said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D. Accordingly, the invention relates to an antibody specifically binding to the mutated Semaphorin 3 or the functional fragment thereof, wherein said antibody specifically binds to an epitope comprising a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2; a hydrophilic amino acid in place of the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6; a hydrophilic amino acid in place of the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or a hydrophilic amino acid in place of the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14, and wherein said Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

The term “antibody”, in accordance with the present invention, comprises polyclonal and monoclonal antibodies as well as derivatives or fragments thereof which still retain the binding specificity. Techniques for the production of antibodies are well known in the art and described, e.g. in Harlow and Lane "Antibodies, A Laboratory Manual", Cold Spring Harbor Laboratory Press, 1988 and Harlow and Lane “Using Antibodies: A Laboratory Manual” Cold Spring Harbor Laboratory Press, 1999. The term “antibody” in accordance with the invention also includes embodiments such as chimeric, single chain and humanized antibodies, as well as antibody fragments, like, inter alia, Fab fragments, fusion proteins consisting of Eph receptors, ephrin or phosphatase extracellular domains and Fc. Antibody fragments or derivatives further comprise $F(ab')_2$, Fv fragments, scFvs, single domain V_H or V-like domains, such as VhH or V-NAR-domains, as well as multimeric formats such as minibodies, diabodies, tribodies, tetrabodies or chemically conjugated Fab’-multimers; see, for example, Harlow and Lane (1988) and (1999), Altshuler (2010) Biochemistry (Moscow) 75, 1584-605 or Holliger (2005) Nature Biotechnology 23, 1126-36. Various procedures are known in the art and may be used for the production of such antibodies and/or fragments. Thus, the (antibody) derivatives can be produced by peptidomimetics. Further, techniques described for the production of single chain antibodies (see, inter alia, US Patent 4,946,778)

can be adapted to produce single chain antibodies specific for polypeptide(s) and fusion proteins of this invention. Also, transgenic animals may be used to express humanized antibodies specific for polypeptides and fusion proteins of this invention. Most preferably, the antibody of this invention is a monoclonal antibody. For the preparation of monoclonal antibodies, any technique, which provides antibodies produced by continuous cell line cultures, can be used. Examples for such techniques include the original hybridoma technique (Köhler and Milstein (1975) Nature 256, 495) as further developed by the art, the trioma technique, the human B-cell hybridoma technique (Kozbor (1983) Immunology Today 4, 72) and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al. (1985) Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., 77). The term antibody also relates to humanized antibodies. "Humanized" forms of non-human (e.g. murine or rabbit) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Often, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibody may comprise residues, which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody may also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see: Jones Nature 321 (1986), 522-525; Reichmann Nature 332 (1998), 323-327 and Presta Curr Op Struct Biol 2 (1992), 593-596.

A popular method for humanization of antibodies involves CDR grafting, where a functional antigen-binding site from a non-human ‘donor’ antibody is grafted onto a human ‘acceptor’ antibody. CDR grafting methods are known in the art and described, for example, in US 5,225,539, US 5,693,761 and US 6,407,213. Another related method is the production of

humanized antibodies from transgenic animals that are genetically engineered to contain one or more humanized immunoglobulin loci which are capable of undergoing gene rearrangement and gene conversion (see, for example, US 7,129,084). Further methods for designing and producing humanized antibodies are described in US 07/290,975, US 07/310,252 and US 2003/0229208 or by Queen PNAS (1989), 10029-10033.

Surface plasmon resonance as employed in the BIAcore system can be used to increase the efficiency like efficiency and/or selectivity of phage antibodies which bind to an epitope of a polypeptide of the invention (Schier (1996) Human Antibodies Hybridomas 7, 97; Malmborg (1995) J. Immunol. Methods 183, 7). It is also envisaged in the context of this invention that the term "antibody" comprises antibody constructs, which may be expressed in cells, e.g. antibody constructs which may be transfected and/or transduced via, amongst others, viruses or plasmid vectors. The antibody described in the context of the invention is capable to specifically bind/interact with an epitope of the mentioned polypeptide or the mutated Semaphorin 3 as defined herein. The term "specifically binding/interacting with" as used in accordance with the present invention means that the antibody does not or essentially does not cross-react with an epitope of similar structure. Cross-reactivity of a panel of antibodies under investigation may be tested, for example, by assessing binding of said panel of antibodies under conventional conditions to the epitope of interest as well as to a number of more or less (structurally and/or functionally) closely related epitopes. Only those antibodies that bind to the epitope of interest in its relevant context (e.g. a specific motif in the structure of mutated Semaphorin 3 or the functional fragment thereof) but do not or do not essentially bind to any of the other epitopes are considered specific for the epitope of interest and thus to be antibodies in accordance with this invention. Corresponding methods are described e.g. in Harlow and Lane, 1988 and 1999, loc cit. The antibody specifically binds to/interacts with conformational or continuous epitopes, which are unique for the mentioned polypeptide, preferably mutated Semaphorin 3. A conformational or discontinuous epitope is characterized for polypeptide antigens by the presence of two or more discrete amino acid residues which are separated in the primary sequence, but come together on the surface of the molecule when the polypeptide folds into the native protein/antigen (Sela (1969) Science 166, 1365; Laver (1990) Cell 61, 553). The two or more discrete amino acid residues contributing to the epitope are present on separate sections of one or more polypeptide chain(s). These residues come together on the surface of the molecule when the polypeptide chain(s) fold(s) into a three-dimensional structure to constitute the epitope. In contrast, a

continuous or linear epitope consists of two or more discrete amino acid residues, which are present in a single linear segment of a polypeptide chain.

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see Morimoto et al (1992) Journal of Biochemical and Biophysical Methods 24:107-117; and Brennan et al (1985) Science 229:81). Antibody fragments can also be produced directly by recombinant host cells and the antibody phage libraries discussed above. Fab′-SH fragments can be directly recovered from E. coli and chemically coupled to form F(ab′)2 fragments (Carter et al (1992) Bio/Technology 10:163-167). According to another approach, F(ab′)2 fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Pat. No. 5,571,894; and U.S. Pat. No. 5,587,458. The antibody fragment may also be a “linear antibody”, e.g., as described in U.S. Pat. No. 5,641,870. Such linear antibody fragments may be monospecific or bispecific.

Bispecific antibodies with binding specificities for at least two different epitopes (Millstein et al (1983), Nature 305:537-539) may bind to two different epitopes of the mutated Semaphorin 3. Techniques for generating bispecific antibodies from antibody fragments have also been described, such as using chemical linkage wherein intact antibodies are proteolytically cleaved to generate F(ab′)2 fragments (Brennan et al (1985) Science 229:81). Fab′-SH fragments can be recovered from E. coli and chemically coupled to form bispecific antibodies (Shalaby et al (1992) J. Exp. Med. 175:217-225. The “diabody” technology provides an alternative method for making bispecific antibody fragments (Hollinger et al (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448).

A “Fab fragment” generally is comprised of one light chain and the C_H1 and variable regions of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule. An "Fc" region generally contains two heavy chain fragments comprising the C_H2 and C_H3 domains of an antibody. The two heavy chain fragments are held together by two or more disulfide bonds and by hydrophobic interactions of the C_H3 domains. A "Fab' fragment" generally contains one light chain and a portion of one heavy

chain that contains the V_H domain and the C_H1 domain and also the region between the C_H1 and C_H2 domains, such that an interchain disulfide bond can be formed between the two heavy chains of two Fab' fragments to form a $F(ab')_2$ molecule. A "$F(ab')_2$ fragment" generally contains two light chains and two heavy chains containing a portion of the constant region between the C_H1 and C_H2 domains, such that an interchain disulfide bond is formed between the two heavy chains. A $F(ab')_2$ fragment thus is composed of two Fab' fragments that are held together by a disulfide bond between the two heavy chains. The "Fv region" generally comprises the variable regions from both the heavy and light chains, but lacks the constant regions. Antibodies with more than two valencies are contemplated. Multivalent, “Octopus” antibodies with three or more antigen binding sites and two or more variable domains can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody (US 2002/0004586; WO01/77342). For example, trispecific antibodies can be prepared (Tutt et al (1991) J. Immunol. 147:60.).

Binding molecules/antibodies/antigen-binding fragments provided herein are preferably in the IgG1 or IgG3 framework, more preferred human IgG1 or IgG3 framework. Binding molecules, antibodies or antigen-binding fragments of these antibodies in the IgG1 or IgG3, preferably human IgG1 or IgG3, framework, are particularly preferred for use in vaccine therapy.

The following exemplary assay can be used to determine that a candidate antibody is indeed specifically binding to the mutated Semaphorin 3 or a functional fragment thereof in accordance with the present invention.

Antibodies that selectively and specifically bind to the epitope comprising the hydrophilic amino acid at the position that by comparison of homology corresponds to position 106 of Semaphorin 3A as shown in SEQ ID NO:2 can be identified by, e.g., capture ELISA assays. In order to perform such an assay, Corning 96 Well Clear Polystyrene High Bind Stripwell Microplate (product #2592) are coated overnight with increasing amounts (0.02 nM to 3.5 nM) of the affinity purified mutated Semaphorin 3 or (the functional fragment thereof) or the purified wild type Semaphorin 3 (or the functional fragment thereof) in 0.05 M Na_2CO_3 (pH 9.6) at 4 °C. Subsequently, the reaction is blocked with PBS containing 0.05% Tween-20 (PBS-T) with 5% BSA for 2 hours at room temperature. Solutions containing equal amounts

of generated antibodies are captured by overnight incubation at 4 °C. Unbound material is removed by extensive washing with PBS-T. The binding of anti- mutated-Semaphorin 3 antibodies is detected by incubating wells with appropriate horseradish peroxidase-conjugated secondary antibodies in PBS-T containing 1% BSA for 1 h at 4 °C. Following further washing, mutated Semaphorin 3-bound (or functional fragment thereof-bound) or wild type Semaphorin 3-bound (or functional fragment thereof-bound) anti-mutated-Semaphorin 3 antibodies are detected by a chromogenic reaction with ortho-phenylenediamine. Antibodies that specifically bind to the mutated Semaphorin 3 (or the functional fragment thereof), but not to the wild type Semaphorin 3 (or the functional fragment thereof) are selected.

In a further embodiment, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide according to the invention can be used as a medicament, i.e. the mutated Semaphorin 3 or the functional fragment thereof provided and described herein are for use in medicine. The terms "medicament" and "pharmaceutical composition" are used interchangeably herein. Accordingly, definitions and explanations provided herein in relation to "pharmaceutical compositions", apply, mutatis mutandis, to the term "medicament".

The term "treatment of a disorder or disease" as used herein, such as "treatment of cancer", is well known in the art. "Treatment of a disorder or disease" implies that a disorder or disease is suspected or has been diagnosed in a patient/subject. A patient/subject suspected of suffering from a disorder or disease typically shows specific clinical and/or pathological symptoms which a skilled person can attribute to a specific pathological condition (i.e., diagnose a disorder or disease).

The "treatment" of a disorder or disease may, for example, lead to a halt in the progression of the disorder or disease (e.g., no deterioration of symptoms) or a delay in the progression of the disorder or disease (in case the halt in progression is of a transient nature only). The "treatment" of a disorder or disease may also lead to a partial response (e.g., amelioration of symptoms) or complete response (e.g., disappearance of symptoms) of the subject/patient suffering from the disorder or disease. Accordingly, the "treatment" of a disorder or disease may also refer to an amelioration of the disorder or disease, which may, e.g., lead to a halt in

the progression of the disorder or disease or a delay in the progression of the disorder or disease. Such a partial or complete response may be followed by a relapse. It is to be understood that a subject/patient may experience a broad range of responses to a treatment (e.g., the exemplary responses as described herein above). The treatment of a disorder or disease may, inter alia, comprise curative treatment (preferably leading to a complete response and eventually to healing of the disorder or disease) and palliative treatment (including symptomatic relief). Thus, the term "treatment" means obtaining a desired pharmacological and/or physiological effect. The effect may also be prophylactic in terms of completely or partially preventing a disease/medical condition/disorder or symptom thereof and/or may be therapeutic in terms of partially or completely curing a disease/medical condition/disorder and/or adverse effect attributed to the disease/medical condition/disorder. The term "prevention of a disorder or disease" as used herein, such as "prevention of cancer", is also well known in the art. For example, a patient/subject suspected of being prone to suffer from a disorder or disease as defined herein may, in particular, benefit from a prevention of the disorder or disease. The subject/patient may have a susceptibility or predisposition for a disorder or disease, including but not limited to hereditary predisposition. Such a predisposition can be determined by standard assays, using, for example, genetic markers or phenotypic indicators. It is to be understood that a disorder or disease to be prevented in accordance with the present invention has not been diagnosed or cannot be diagnosed in the patient/subject (for example, the patient/subject does not show any clinical or pathological symptoms). Thus, the term "prevention" comprises the use of compounds of the present invention before any clinical and/or pathological symptoms are diagnosed or determined or can be diagnosed or determined by the attending physician.

The present invention provides a pharmaceutical composition comprising the nucleic acid molecule of the invention, the mutated Semaphorin 3 or the functional fragment thereof of the invention, the fusion protein or the polypeptide of the invention. The pharmaceutical composition can comprise a pharmaceutical excipient. It is understood herein that said pharmaceutical excipient can comprise or is a pharmaceutical carrier, vehicle and/or diluent. In one specific embodiment, said pharmaceutical carrier is a virus. In a preferred embodiment said virus is an adeno-associated-virus (AAV), wherein the adeno-associated-virus is AAV8. AAV can be for instance employed in gene therapy. Thus, in one aspect, the invention provides a nucleotide sequence which contains elements of an adenovirus genome as well as

a mutated Semaphorin 3 or fragments thereof that is under the control of a eukaryotic transcriptional promoter. This nucleic acid sequence can function as a vector allowing expression of the aforementioned heterologous gene when the vector is introduced in a cell of an individual.

Further, the invention provides the pharmaceutical composition for use as a medicament. Further, the invention provides the pharmaceutical composition for use in the treatment of an angiogenic disorder, cancer, tumor, tumorous disease, vascular retinopathy, blood-brain barrier permeability alterations, neuroinflammatory disorders, inflammatory disorders, osteoporosis, psoriasis, obesity Mycobacterial infections, and/or granulomas. Further the invention provides the pharmaceutical composition for use of the treatment of tumor, wherein the tumor is a solid tumor. In particular, the invention provides the pharmaceutical composition for use in the treatment of tumor, wherein the tumor is a pancreatic tumor. Further, the invention provides the pharmaceutical composition for use in the treatment of cancer, wherein the cancer is selected from the group consisting of pancreatic cancer, cervical cancer, breast cancer, colon cancer, melanoma, prostate cancer, bladder cancer and tongue cancer. In particular, the invention provides the pharmaceutical composition for use in the treatment of pancreatic cancer. Further, the invention provides the pharmaceutical composition, wherein vascular normalization, reduction of tumor growth, reduction of metastatization or survival extension is involved. Further, the invention provides the pharmaceutical composition, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide, which is to be administered in combination with a with an anti-proliferative drug, an anticancer drug, a cytostatic drug, a cytotoxic drug and/or radiotherapy. In particular preferred aspects, the pharmaceutical composition, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide is to be administered parenterally.

Furthermore, the invention provides the pharmaceutical composition, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide or the encoding nucleic acid molecule for use in the treatment of a tumor or cancer for inhibiting cancer growth, reducing liver metastasis or metastasis volume, reducing vessel area and/or

promoting cancer vessel normalization by enhancing pericyte coverage, and/or increasing blood vessel perfusion and inhibiting cancer hypoxia.

The pharmaceutical composition, the nucleic acid molecule, the vector, said mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide can be used in combination with other therapeutic agents. When a compound of the invention is used in combination with a second therapeutic agent active against the same disease, the dose of each compound may differ from that when the compound is used alone. The combination of a compound of the present invention with a second therapeutic agent may comprise the administration of the second therapeutic agent with the compound of the invention. Such an administration may comprise simultaneous/concomitant administration. However, also sequential/separate administration is envisaged, as also explained below.

Preferably, the second therapeutic agent to be administered in combination with the compounds of this invention is an anticancer drug. The anticancer drug to be administered in combination with the pharmaceutical composition, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide according to the present invention may be: a tumor angiogenesis inhibitor (for example, a protease inhibitor, an epidermal growth factor receptor kinase inhibitor, or a vascular endothelial growth factor receptor kinase inhibitor); a cytotoxic drug (for example, an antimetabolite, such as purine and pyrimidine analogue antimetabolites); an antimitotic agent (for example, a microtubule stabilizing drug or an antimitotic alkaloid); a platinum coordination complex; an anti-tumor antibiotic; an alkylating agent (for example, a nitrogen mustard or a nitrosourea); an endocrine agent (for example, an adrenocorticosteroid, an androgen, an anti-androgen, an estrogen, an anti-estrogen, an aromatase inhibitor, a gonadotropin-releasing hormone agonist, or a somatostatin analogue); or a compound that targets an enzyme or receptor that is overexpressed and/or otherwise involved in a specific metabolic pathway that is misregulated in the tumor cell (for example, ATP and GTP phosphodiesterase inhibitors, histone deacetylase inhibitors, protein kinase inhibitors (such as serine, threonine and tyrosine kinase inhibitors (for example, Abelson protein tyrosine kinase)) and the various growth factors, their receptors and corresponding kinase inhibitors (such as epidermal growth factor receptor kinase inhibitors, vascular endothelial growth factor receptor kinase inhibitors, fibroblast growth factor inhibitors, insulin-like growth factor

receptor inhibitors and platelet-derived growth factor receptor kinase inhibitors)); methionine, aminopeptidase inhibitors, proteasome inhibitors, cyclooxygenase inhibitors (for example, cyclooxygenase-1 or cyclooxygenase-2 inhibitors) and topoisomerase inhibitors (for example, topoisomerase I inhibitors or topoisomerase II inhibitors).

An alkylating agent which can be used as an anticancer drug in combination with the pharmaceutical composition, the nucleic acid, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide of the present invention may be, for example, a nitrogen mustard (such as cyclophosphamide, mechlorethamine (chlormethine), uramustine, melphalan, chlorambucil, ifosfamide, bendamustine, or trofosfamide), a nitrosourea (such as carmustine, streptozocin, fotemustine, lomustine, nimustine, prednimustine, ranimustine, or semustine), an alkyl sulfonate (such as busulfan, mannosulfan, or treosulfan), an aziridine (such as hexamethylmelamine (altretamine), triethylenemelamine, ThioTEPA (N,N'N'-triethylenethiophosphoramide), carboquone, or triaziquone), a hydrazine (such as procarbazine), a triazene (such as dacarbazine), or an imidazotetrazines (such as temozolomide).

A platinum coordination complex which can be used as an anticancer drug in combination with the pharmaceutical composition, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide of the present invention may be, for example, cisplatin, carboplatin, nedaplatin, oxaliplatin, satraplatin, or triplatin tetranitrate.

A cytotoxic drug which can be used as an anticancer drug in combination with the pharmaceutical composition, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide of the present invention may be, for example, an antimetabolite, including folic acid analogue antimetabolites (such as aminopterin, methotrexate, pemetrexed, or raltitrexed), purine analogue antimetabolites (such as cladribine, clofarabine, fludarabine, 6-mercaptopurine (including its prodrug form azathioprine), pentostatin, or 6-thioguanine), and pyrimidine analogue antimetabolites (such as cytarabine, decitabine, 5-fluorouracil (including its prodrug forms capecitabine and tegafur), floxuridine, gemcitabine, enocitabine, or sapacitabine).

An antimitotic agent which can be used as an anticancer drug in combination with the pharmaceutical composition, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide of the present invention may be, for example, a taxane (such as docetaxel, larotaxel, ortataxel, paclitaxel/taxol, or tesetaxel), a Vinca alkaloid (such as vinblastine, vincristine, vinflunine, vindesine, or vinorelbine), an epothilone (such as epothilone A, epothilone B, epothilone C, epothilone D, epothilone E, or epothilone F) or an epothilone B analogue (such as ixabepilone/azaepothilone B).

An anti-tumor antibiotic which can be used as an anticancer drug in combination with the pharmaceutical composition, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide of the present invention may be, for example, an anthracycline (such as aclarubicin, daunorubicin, doxorubicin, epirubicin, idarubicin, amrubicin, pirarubicin, valrubicin, or zorubicin), an anthracenedione (such as mitoxantrone, or pixantrone) or an anti-tumor antibiotic isolated from Streptomyces (such as actinomycin (including actinomycin D), bleomycin, mitomycin (including mitomycin C), or plicamycin).

A tyrosine kinase inhibitor which can be used as an anticancer drug in combination with the pharmaceutical composition, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide of the present invention may be, for example, axitinib, bosutinib, cediranib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, lestaurtinib, nilotinib, semaxanib, sorafenib, sunitinib, or vandetanib.

A topoisomerase-inhibitor which can be used as an anticancer drug in combination with the pharmaceutical composition, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide of the present invention may be, for example, a topoisomerase I inhibitor (such as irinotecan, topotecan, camptothecin, belotecan, rubitecan, or lamellarin D) or a topoisomerase II inhibitor (such as amsacrine, etoposide, etoposide phosphate, teniposide, or doxorubicin).

Further anticancer drugs may be used in combination with the pharmaceutical composition, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment

thereof and/or the fusion protein/polypeptide of the present invention. The anticancer drugs may comprise biological or chemical molecules, like TNF-related apoptosis-inducing ligand (TRAIL), tamoxifen, amsacrine, bexarotene, estramustine, irofulven, trabectedin, cetuximab, panitumumab, tositumomab, alemtuzumab, bevacizumab, edrecolomab, gemtuzumab, trastuzumab, pertuzumab, alvocidib, seliciclib, aminolevulinic acid, methyl aminolevulinate, efaproxiral, porfimer sodium, talaporfin, temoporfin, verteporfin, alitretinoin, tretinoin, anagrelide, arsenic trioxide, atrasentan, bortezomib, carmofur, celecoxib, demecolcine, elesclomol, elsamitrucin, etoglucid, lonidamine, lucanthone, masoprocol, mitobronitol, mitoguazone, mitotane, oblimersen, omacetaxine, sitimagene, ceradenovec, tegafur, testolactone, tiazofurine, tipifarnib, and vorinostat.

Also biological drugs, like antibodies, antibody fragments, antibody constructs (for example, single-chain constructs), and/or modified antibodies (like CDR-grafted antibodies, humanized antibodies, "full humanized" antibodies, etc.) directed against cancer or tumor markers/factors/cytokines involved in proliferative diseases can be employed in co-therapy approaches with the pharmaceutical composition, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide of the invention. Examples of such biological molecules are anti-HER2 antibodies (e.g. trastuzumab, Herceptin®), anti-CD20 antibodies (e.g. Rituximab, Rituxan®, MabThera®, Reditux®), anti-CD19/CD3 constructs (see, e.g., EP-B1 1071752) and anti-TNF antibodies (see, e.g., Taylor PC. Antibody therapy for rheumatoid arthritis. Curr Opin Pharmacol. 2003. 3(3):323-328). Further antibodies, antibody fragments, antibody constructs and/or modified antibodies to be used in co-therapy approaches with the pharmaceutical composition, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the polypeptide of the invention can be found in Taylor PC. Curr Opin Pharmacol. 2003. 3(3):323-328; Roxana A. Maedica. 2006. 1(1):63-65.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation. The individual components of such combinations may be administered either sequentially or simultaneously/concomitantly in separate or combined pharmaceutical formulations by any convenient route. When administration is sequential, either the pharmaceutical composition, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide of the

present invention or the second therapeutic agent may be administered first. When administration is simultaneous, the combination may be administered either in the same or different pharmaceutical composition. When combined in the same formulation it will be appreciated that the two compounds must be stable and compatible with each other and the other components of the formulation. When formulated separately, they may be provided in any convenient formulation.

The pharmaceutical composition, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide can also be administered in combination with physical therapy, such as radiotherapy. Radiotherapy may commence before, after, or simultaneously with administration of the compounds of the invention. For example, radiotherapy may commence 1-10 minutes, 1-10 hours or 24-72 hours after administration of the compounds. Yet, these time frames are not to be construed as limiting. The subject is exposed to radiation, preferably gamma radiation, whereby the radiation may be provided in a single dose or in multiple doses that are administered over several hours, days and/or weeks. Gamma radiation may be delivered according to standard radiotherapeutic protocols using standard dosages and regimens.

The present invention thus relates to a nucleic acid molecule, a mutated Semaphorin 3, a functional fragment thereof, a fusion protein or a polypeptide and/or a pharmaceutically acceptable salt, solvate, or prodrug thereof, or a pharmaceutical composition comprising any of the aforementioned entities in combination with a pharmaceutically acceptable excipient, for use in the treatment or prevention of cancer, in particular the treatment or prevention of pancreatic cancer, wherein the compound or the pharmaceutical composition is to be administered in combination with an anticancer drug and/or in combination with radiotherapy.

In other words, the invention provides the nucleic acid molecule of the invention, the vector of the invention and/or the mutated Semaphorin 3 or the functional fragment thereof, the fusion protein of the invention or the polypeptide of the invention for use as a medicament. Further the invention provides the nucleic acid molecule of the invention, the vector of the invention and/or the mutated Semaphorin 3 or the functional fragment thereof of the invention or the fusion protein/polypeptide of the invention for use in the treatment of an

angiogenic disorder, cancer, tumorous disease, vascular retinopathy, blood-brain barrier permeability alterations, neuroinflammatory disorders, osteoporosis, obesity Mycobacterial infections, and/or granulomas. Further the invention provides the nucleic acid molecule of the invention, the vector of the invention and/or the mutated Semaphorin 3 or the functional fragment thereof of the invention or the fusion protein/polypeptide of the invention for use in the treatment of tumor, wherein the tumor is a solid tumor. Further the invention provides the nucleic acid molecule of the invention, the vector of the invention and/or the mutated Semaphorin 3 or the functional fragment thereof of the invention or the fusion protein/polypeptide of the invention for use in the treatment of tumor selected from the group consisting of pancreatic tumor, cervical cancer, breast cancer, colon cancer, melanoma, prostate cancer, bladder cancer and tongue cancer. In particular the invention provides the nucleic acid molecule of the invention, the vector of the invention and/or the mutated Semaphorin 3 or the functional fragment thereof of the invention or the fusion protein/polypeptide of the invention for use in the treatment of pancreatic cancer. Further the invention provides the nucleic acid molecule of the invention, the vector of the invention and/or the mutated Semaphorin 3 or the functional fragment thereof of the invention or the fusion protein/polypeptide of the invention, wherein vascular normalization, reduction of tumor growth, reduction of metastatization or survival extension is involved.

The term "Angiogenesis" means that a vascular EC germinates from a pre-existing vessel and a capillary vessel is formed in a way that goes into a tissue. A formative process is the digestion of the vascular basement membrane by a protease, the migration/growth of a vascular EC, and the lumen formation. "Angiogenic disorder" is a vascular disease such as arterial sclerosis, hypertonia, angina pectoris, obstructive arteriosclerosis, myocardial infarction, cerebral infarction, diabetic angiopathy or vascular malformation; inflammatory disease such as hepatitis, pneumonitis, glomerular nephritis, thyroiditis, osteitis, arthromeningitis, osteoclasia, chondrolysis, rheumatism, bronchial asthma, sarcoidosis, Crow-Fukase syndrome, pannus, allergic oedema, ulcers, hydroperitoneum, peritoneal sclerosis or tissular conglutination; entoptic neovascular disease such as diabetic retinopathy, occlusion of retinal vein or aging macular degeneration; reproductive system disease such as uterus dysfunction, placental dysfunction, ovarian hyperergasia or follicle cyst; central nervous system disease such as retinosis, cerebral apoplexy, vascular dementia or Alzheimer

disease; cancer such as solid cancer, angiomatous, hemangioendothelioma, sarcomas, Kaposi's sarcoma or hematopoietic organic ulcer.

“Cancer", in accordance with the present invention, refers to a class of diseases or disorders characterized by uncontrolled division of cells and the ability of these to spread, either by direct growth into adjacent tissue through invasion, or by implantation into distant sites by metastasis, where cancer cells are transported through the bloodstream or lymphatic system. The tumorous disease can be any form of a cancer, a tumor or is chosen from pancreas cancer, breast cancer, epithelial cancer, hepatocellular carcinoma, cholangiocellular cancer, stomach cancer, colon cancer, prostate cancer, bladder cancer, tongue cancer, head and neck cancer, skin cancer (melanoma), a cancer of the urogenital tract, e.g., ovarian cancer, endometrial cancer, cervix cancer, and kidney cancer; lung cancer, gastric cancer, a cancer of the small intestine, liver cancer, gall bladder cancer, a cancer of the bile duct, esophagus cancer, a cancer of the salivary glands or a cancer of the thyroid gland.

The angiogenesis inhibitor can be used as a preventive or therapeutic agent for a disease whose condition can become serious by angiogenesis in the above diseases. The disease on which the angiogenesis inhibitor has effect is vascular disease, inflammatory disease, entoptic neovascular disease, reproductive system disease, central nervous system disease, cancer or the like. A form of formulation of a vector of this invention (a gene therapy agent) can be one of different forms according to the above each form of administration. For example, when it is an injection comprising DNA of this invention which is an active ingredient, the injection can be prepared by a usual method. A base ingredient for a gene therapy agent is not especially restricted as long as it is a base ingredient usually used for an injection. It is, for example, distilled water, sodium chloride, a salt solution such as mixture of sodium chloride and mineral salts, a solution such as mannitol, lactose, dextran or glucose, an amino acid solution such as glycine or arginine, or mixture of an organic acid solution or a salt solution and a glucose solution. An injection can be prepared with an auxiliary such as an osmotic adjustment agent, pH adjustment agent, plant oil such as sesame oil or soybean oil, surfactant such as lecithin or nonionic surfactant or the like according to a usual method as a solution, suspension or dispersion. The injection as above can be a preparation dissolved in use by manipulation such as disintegration or lyophilization.

Further, the invention provides the use of the mutated Semaphorin 3 or the functional fragment thereof, of the nucleic acid molecule, of the fusion protein, of the polypeptide or of the host according to the invention.

Further, the invention provides a method of treatment for angiogenic disorder and/or tumorous disease and/or cancer comprising the step of administering to a subject in need of such treatment a pharmaceutical active amount of the nucleic acid molecule of the present invention, or the mutated Semaphorin 3 or the functional fragment thereof according to the present invention, the fusion protein, the polypeptide of the invention, the pharmaceutical composition of the present invention or as produced by the method as described herein.

The subject or patient to be treated in accordance with the invention may be an animal (e.g., a non-human animal), a vertebrate animal, a mammal, a rodent (e.g., a guinea pig, a hamster, a rat, a mouse), a murine (e.g., a mouse), a canine (e.g., a dog), a feline (e.g., a cat), a porcine (e.g., a pig), an equine (e.g., a horse), a primate, a simian (e.g., a monkey or ape), a monkey (e.g., a marmoset, a baboon), an ape (e.g., a gorilla, chimpanzee, orang-utan, gibbon), or a human. In the context of this invention, it is particularly envisaged that animals are to be treated which are economically, agronomically or scientifically important. Scientifically important organisms include, but are not limited to, mice, rats, and rabbits. Lower organisms such as, e.g., fruit flies like *Drosophila melanogaster* and nematodes like *Caenorhabditis elegans* may also be used in scientific approaches. Non-limiting examples of agronomically important animals are sheep, cattle and pigs, while, for example, cats and dogs may be considered as economically important animals. Preferably, the subject/patient is a mammal; more preferably, the subject/patient is a human or a non-human mammal (such as, e.g., a guinea pig, a hamster, a rat, a mouse, a rabbit, a dog, a cat, a horse, a monkey, an ape, a marmoset, a baboon, a gorilla, a chimpanzee, an orang-utan, a gibbon, a sheep, cattle, or a pig); most preferably, the subject/patient is a human.

The pharmaceutical effective amount can be higher than 10 mg/kg of body weight. Further, the pharmaceutical effective amount can be lower than 0.5 mg/kg of body weight. In particular preferred aspects, the invention provides a method of treatment according to the invention, wherein the pharmaceutical effective amount is the range of 0.5 to 10 mg/kg of body weight.

It is envisaged herein that the content of DNA of a preparation is different depending on a disease of therapeutic purpose, administration site, number of doses, desired duration of therapy, an age or body weight of a patient or the like and can be suitably adjusted. It is usually about 0.01 - 2000 mg and preferably 0.1 - 100 mg of DNA encoding a protein of this invention for a patient (the body weight is 60 kg).

The pharmaceutical composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient, the site of delivery of the pharmaceutical composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" of the pharmaceutical composition for purposes herein is thus determined by such considerations.

The skilled person knows that the effective amount of pharmaceutical composition administered to an individual will, inter alia, depend on the nature of the compound.

The administration of the herein provided compositions may, inter alia, comprise an administration twice daily, every day, every other day, every third day, every fourth day, every fifth day, once a week, once every second week, once every third week, once every month, etc.

For example, if said compound is a (poly)peptide or protein the total pharmaceutically effective amount of pharmaceutical composition administered parenterally per dose will be in the range of about 1 μg protein /kg/day to 15 mg protein /kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg protein/kg/day, and most preferably for humans between about 0.01 and 1 mg protein /kg/day. If given continuously, the pharmaceutical composition is typically administered at a dose rate of about 1 μg/kg/hour to about 50 μg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect. The particular amounts may be determined by conventional tests which are well known to the person skilled in the art.

Pharmaceutical compositions of the invention may be administered parenterally, orally, rectally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch) or bucally. In particular preferred embodiments, the pharmaceutical composition is administered parenterally.

Pharmaceutical compositions of the invention preferably comprise a pharmaceutically acceptable carrier. By "pharmaceutically acceptable carrier" is meant a virus, a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intratracheal, intranasal, intrasternal, subcutaneous and intraarticular injection and infusion.

The pharmaceutical composition is also suitably administered by sustained release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., Biopolymers 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (R. Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and R. Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., Id.) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained release pharmaceutical composition also include liposomally entrapped compound. Liposomes containing the pharmaceutical composition are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal therapy.

For parenteral administration, the pharmaceutical composition is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-

toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation.

Generally, the formulations are prepared by contacting the components of the pharmaceutical composition uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes. The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) (poly)peptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The components of the pharmaceutical composition to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic components of the pharmaceutical composition generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The components of the pharmaceutical composition ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous solution, and the resulting

mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized compound(s) using bacteriostatic Water-for-Injection.

The invention further relates to kit comprising the mutated Semaphorin 3 or the functional fragment thereof, the fusion protein, the nucleic acid molecule, the antibody and/or the pharmaceutical composition as defined herein. Such a kit can be used, for example, in the treatment of cancer, tumor and/or tumorous disease, wherein said tumor cancer, tumorous disease is a solid tumor, in particular a pancreatic tumor/cancer.

The term "nucleic acid molecule" in accordance with the present invention comprises coding and, wherever applicable, non-coding sequences (like promoters, enhancers etc.).

The terms “polypeptide”, “(poly)peptide”, “peptide” and “protein” are used herein interchangeably and refer to a polymer of two or more amino acids linked via amide bonds that are formed between an amino group of one amino acid and a carboxyl group of another amino acid. The amino acids comprised in the peptide or protein, which are also referred to as amino acid residues, may be selected from the 20 standard proteinogenic α-amino acids (i.e., Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val) but also from non-proteinogenic and/or non-standard α-amino acids (such as, e.g., ornithine, citrulline, homolysine, pyrrolysine, or 4-hydroxyproline) as well as β-amino acids (e.g., β-alanine), γ-amino acids and δ-amino acids. Preferably, the amino acid residues comprised in the peptide or protein are selected from α-amino acids, more preferably from the 20 standard proteinogenic α-amino acids (which can be present as the L-isomer or the D-isomer, and are preferably all present as the L-isomer). The peptide or protein may be unmodified or may be modified, e.g., at its N-terminus, at its C-terminus and/or at a functional group in the side chain of any of its amino acid residues (particularly at the side chain functional group of one or more Lys, His, Ser, Thr, Tyr, Cys, Asp, Glu, and/or Arg residues). Such modifications may include, e.g., the attachment of any of the protecting groups described for the corresponding functional groups in: Wuts PG & Greene TW, Greene’s protective groups in organic synthesis, John Wiley & Sons, 2006. Such modifications may also include the covalent attachment of one or more polyethylene glycol (PEG) chains (forming a PEGylated peptide or protein), the glycosylation and/or the acylation with one or more fatty acids (e.g., one or more C_{8-30} alkanoic or alkenoic acids;

forming a fatty acid acylated peptide or protein). The amino acid residues comprised in the peptide or protein may, e.g., be present as a linear molecular chain (forming a linear peptide or protein) or may form one or more rings (corresponding to a cyclic peptide or protein). The peptide or protein may also form oligomers consisting of two or more identical or different molecules. As used herein, the term "domain" relates to any region/part of an amino acid sequence that is capable of autonomously adopting a specific structure and/or function. In the context of the present invention, accordingly, a "domain" may represent a functional domain or a structural domain.

The term “consensus sequence” or “consensus sequence motif” is the calculated order of most frequent residues, either nucleotide or amino acid, found at each position in a sequence alignment. It represents the results of a multiple sequence alignments in which related sequences are compared to each other and similar sequence motifs are calculated.

As used herein, the terms “comprising” and “including” or grammatical variants thereof are to be taken as specifying the stated features, integers, steps or components but do not preclude the addition of one or more additional features, integers, steps, components or groups thereof. This term encompasses the terms “consisting of” and “consisting essentially of.”

Thus, the terms “comprising”/“including”/”having” mean that any further component (or likewise features, integers, steps and the like) can/may be present.

The term “consisting of” means that no further component (or likewise features, integers, steps and the like) is present.

The term “consisting essentially of” or grammatical variants thereof when used herein are to be taken as specifying the stated features, integers, steps or components but do not preclude the addition of one or more additional features, integers, steps, components or groups thereof but only if the additional features, integers, steps, components or groups thereof do not materially alter the basic and novel characteristics of the claimed composition, device or method.

Thus, the term “consisting essentially of” means those specific further components (or likewise features, integers, steps and the like) can be present, namely those not materially affecting the essential characteristics of the composition, device or method. In other words, the term "consisting essentially of" (which can be interchangeably used herein with the term "comprising substantially"), allows the presence of other components in the composition, device or method in addition to the mandatory components (or likewise features, integers, steps and the like), provided that the essential characteristics of the device or method are not materially affected by the presence of other components.

The term “method” refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, biological and biophysical arts.

The term "about" preferably refers to ±10% of the indicated numerical value, more preferably to ±5% of the indicated numerical value, and in particular to the exact numerical value indicated.

As used herein, the term “about” refers to ±10% of the indicated numerical value, and in particular to ±5% of the indicated numerical value. Whenever the term “about” is used, a specific reference to the exact numerical value indicated is also included. If the term “about” is used in connection with a parameter that is quantified in integers, such as the number of nucleotides in a given nucleic acid, the numbers corresponding to ±10% or ±5% of the indicated numerical value are to be rounded to the nearest integer. For example, the expression “about 25 nucleotides” refers to the range of 23 to 28 nucleotides, in particular the range of 24 to 26 nucleotides, and preferably refers to the specific value of 25 nucleotides.

The present invention is further described by reference to the following non-limiting figures and examples. Unless otherwise indicated, established methods of recombinant gene technology were used as described, for example, in Sambrook, Russell "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Laboratory, N.Y. (2001)) which is incorporated herein by reference in its entirety.

As used herein, the term "isolated" refers to a composition that has been removed from its in-vivo location. Preferably the isolated compositions or compounds of the present invention are substantially free from other substances (e.g., other proteins or other compounds) that are present in their in-vivo location (i.e. purified or semi-purified compositions or compounds.)

The given definitions and explanations are also applicable to these items and apply mutatis mutandis. In accordance with the above, the present invention relates to the following items in certain embodiments.

1. A nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of a mutated Semaphorin 3 or a functional fragment thereof that functions as an inhibitor of angiogenesis, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

2. The nucleic acid molecule of item 1, wherein said mutated Semaphorin 3 or said functional fragment thereof functions as an inhibitor of angiogenesis.

3. The nucleic acid molecule of item 1 or 2, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein
   X_1 is K or N,
   X_2 is an amino acid selected from the group of W, M and L
   and wherein the alanine (A_3) is replaced by said hydrophilic amino acid.

4. The nucleic acid molecule of any one of items 1 to 3, which is selected from the group of:
   (a) a nucleic acid molecule selected from the group of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 9 and SEQ ID NO: 13,
   wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding said hydrophilic amino acid,

wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding said hydrophilic amino acid,
wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding said hydrophilic amino acid, and
wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding said hydrophilic amino acid;

(b) a nucleic acid molecule encoding a polypeptide selected from the group of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10 and SEQ ID NO: 14, wherein the alanine residue at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14 is replaced by said hydrophilic amino acid;

(c) a nucleic acid molecule hybridizing under stringent conditions to the complementary strand of a nucleic acid molecule as defined in (a) or (b);

(d) a nucleic acid molecule encoding a polypeptide that functions as an inhibitor of angiogenesis and has at least 55% identity to any one of the polypeptides referred to in (b); and

(e) a nucleic acid molecule being degenerate as a result of the genetic code to the nucleotide sequence of a nucleic acid molecule as defined in any one of (a) to (d), wherein the degenerate nucleic acid molecule encodes a polypeptide that functions as an inhibitor of angiogenesis.

5. The nucleic acid molecule of any one of items 1 to 4, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises said hydrophilic amino acid at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14.

6. The nucleic acid molecule of any one of items 1 to 5, wherein said mutated Semaphorin 3 or functional fragment thereof comprises at least one additional mutation selected from the group consisting of amino acid substitution(s), addition(s), deletions(s) and duplication(s).

7. The nucleic acid molecule of any one of items 1 to 6, wherein said hydrophilic amino acid is selected from the group of K, R, N, Q, S,T, E, D, and H.

8. The nucleic acid molecule of item 7, wherein said hydrophilic amino acid is selected from the group of K, R, E, D, and H.

9. The nucleic acid molecule of item 7, wherein said hydrophilic amino acid residue is K or R.

10. The nucleic acid molecule of item 7, wherein said hydrophilic amino acid residue is K.

11. The nucleic acid molecule of any one of items 1 to 10, wherein K is encoded by a codon AAG or AAA.

12. The nucleic acid molecule of any one of items 1 to 11, wherein said mutated Semaphorin 3 or said functional fragment thereof is a mutated human or mouse Semaphorin 3 or a functional fragment thereof.

13. The nucleic acid molecule of any one of items 1 to 12, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises one or more of the following sequence(s) as defined in any one of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 and SEQ ID NO: 48.

14. The nucleic acid molecule of any one of items 1 to 13, wherein said nucleic acid molecule comprises:
    (a) the nucleotides from 601 to 1206 of SEQ ID NO: 1, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding a hydrophilic amino acid;
    (b) the nucleotides from 529 to 1137 of SEQ ID NO: 5, wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding a hydrophilic amino acid;
    (c) the nucleotides from 842 to 1444 of SEQ ID NO: 9, wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding a hydrophilic amino acid; or

(d) the nucleotides from 368 to 982 of SEQ ID NO: 13 wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding a hydrophilic amino acid.

15. The nucleic acid molecule of any one of items 1 to 14, wherein said nucleic acid molecule comprises the nucleotides from 601 to 1206 of SEQ ID NO: 57; the nucleotides from 529 to 1137 of SEQ ID NO: 61; the nucleotides from 842 to 1444 of SEQ ID NO: 65; or the nucleotides from 368 to 982 of SEQ ID NO: 69.

16. The nucleic acid molecule of any one of items 1 to 15, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises an amino acid sequence as shown in:

(a) SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;
(b) SEQ ID NO: 22, wherein the alanine residue corresponding to position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;
(c) SEQ ID NO: 23 wherein the alanine residue corresponding to position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or
(d) SEQ ID NO: 24, wherein the alanine residue corresponding to position 120 of SEQ ID NO: 14 is replaced by a hydrophilic amino acid.

17. The nucleic acid molecule of any one of items 1 to 16, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises an amino acid sequence that is selected from the group of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51 and SEQ ID NO: 52.

18. The nucleic acid molecule of any one of items 1 to 17, wherein said polypeptide is a fusion protein.

19. The nucleic acid molecule of item 18, wherein said polypeptide comprises said mutated Semaphorin 3 or said functional fragment thereof, a stabilizer domain and/or a dimerization domain.

20. The nucleic acid molecule of item 18 or 19, wherein said stabilizer domain is a Plexin Semaphorin Integrin (PSI) domain, wherein said PSI domain comprises one or more of the following sequences SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48.

21. The nucleic acid molecule of any one of items 18 to 20, wherein said dimerization domain has a dissociation constant K_D in the range of 10^{-5} M to 10^{-6} M with another such dimerization domain.

22. The nucleic acid molecule of any one of items 18 to 21, wherein said dimerization domain is selected from the group of a C-terminal IgG constant domain, DARPin and leucine zipper.

23. The nucleic acid molecule of item 22, wherein the IgG constant domain is IgG1 or IgG3.

24. The nucleic acid molecule of any one of items 1 to 23, wherein said nucleic acid molecule comprises a nucleic acid sequence having:

(a) a nucleic acid sequence spanning from nucleotides 316 to 1959 of SEQ ID NO: 1 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding a hydrophilic amino acid;

(b) a nucleic acid sequence spanning from nucleotides 247 to 1887 of SEQ ID NO: 5 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding a hydrophilic amino acid;

(c) a nucleic acid sequence spanning from nucleotides 563 to 2197 of SEQ ID NO: 9 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding a hydrophilic amino acid; or

(d) a nucleic acid sequence spanning from nucleotides 41 to 1735 of SEQ ID NO: 13 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID

NO: 37, wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding a hydrophilic amino acid.

25. The nucleic acid molecule of any one of items 1 to 24, wherein said polypeptide comprises an amino acid sequence:
    (a) spanning from amino acid residues 1 to 548 of SEQ ID NO: 2 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;
    (b) spanning from amino acid residues 1 to 547 of SEQ ID NO: 6 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;
    (c) spanning from amino acid residues 1 to 565 of SEQ ID NO: 10 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or
    (d) spanning from amino acid residues 1 to 545 of SEQ ID NO: 14 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 120 of SEQ ID NO: 14 is replaced by said hydrophilic amino acid.

26. A vector comprising the nucleic acid molecule of any one of items 1 to 25.

27. The vector of item 26, wherein said vector is a gene targeting vector or a gene transfer vector.

28. The vector of item 26 or 27, wherein said vector is an adeno-associated-virus (AAV) vector.

29. The vector of any one of items 26 to 28, wherein the adeno-associated-virus is AAV8 vector.

30. A host transformed with a vector of any one of items 26 to 29 or comprising the nucleic acid molecule of item 1 to 22.

31. The host of item 30 which is a mammalian cell.

32. The host of item 30 or 31, wherein the mammalian cell is a HEK cell.

33. The host of any one of items 30 to 32, wherein the HEK cell is a HEK293-EBNA1 or a HEK293E cell.

34. A method for producing said polypeptide, said mutated Semaphorin 3 or said functional fragment thereof encoded by the nucleic acid molecule of any one of items 1 to 25 said method comprising culturing/raising the host of any one of items 30 to 33 and optionally isolating the produced polypeptide.

35. A polypeptide which is encoded by the nucleic acid molecule of any one of items 1 to 25.

36. A mutated Semaphorin 3 or a functional fragment thereof, wherein said mutated Semaphorin 3 or said functional fragment thereof that functions as an inhibitor of angiogenesis comprises an amino acid sequence, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

37. The mutated Semaphorin 3 or the functional fragment thereof according to item 36, wherein said mutated Semaphorin 3 or said functional fragment thereof functions as an inhibitor of angiogenesis.

38. The mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 37, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein

X_1 is K or N,

X_2 is an amino acid selected from the group of W, M and L

and wherein the alanine (A_3) is replaced by said hydrophilic amino acid.

39. The mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 38, wherein said mutated Semaphorin 3 is selected from the group of:

(a) a polypeptide encoded by a nucleic acid molecule selected from the group of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 9 and SEQ ID NO: 13,
wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding said hydrophilic amino acid,
wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding said hydrophilic amino acid,
wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding said hydrophilic amino acid, and
wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding said hydrophilic amino acid;

(b) a polypeptide selected from the group of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10 and SEQ ID NO: 14, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2, corresponding to position 105 of SEQ ID NO: 6, corresponding to position 104 of SEQ ID NO: 10 or corresponding to position 120 of SEQ ID NO: 14 is replaced by said hydrophilic amino acid;

(c) a polypeptide encoded by a nucleic acid molecule hybridizing under stringent conditions to the complementary strand of a nucleic acid molecule as defined in (a) or (c);

(d) a polypeptide having at least 55% identity to the polypeptide of any one of (a) to (d) and functioning as an inhibitor of angiogenesis; and

(e) a polypeptide that functions as an inhibitor of angiogenesis comprising an amino acid sequence encoded by a nucleic acid molecule being degenerate as a result of the genetic code to the nucleotide sequence of a nucleic acid molecule as defined in any one of (a), (c) and (d).

40. The mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 39, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises said hydrophilic amino acid at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14.

41. The mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 40, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises at least one additional mutation selected from the group consisting of amino acid substitution(s), addition(s), deletions(s) and duplication(s).

42. The mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 41, wherein said hydrophilic amino acid is selected from the group of K, R, N, Q, S,T, E, D, and H.

43. The mutated Semaphorin 3 or the functional fragment thereof according to item 42, wherein said hydrophilic amino acid is selected from the group of K, R, E, D, and H.

44. The mutated Semaphorin 3 or the functional fragment thereof according to item 42, wherein said hydrophilic amino acid is K or R.

45. The mutated Semaphorin 3 or the functional fragment thereof according to item 42, wherein said hydrophilic amino acid is K.

46. The mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 45, wherein said mutated Semaphorin 3 or said functional fragment thereof is a mutated human or mouse Semaphorin 3 or a functional fragment thereof.

47. The mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 46, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises one or more of the following sequence(s) as defined in any one of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 35,SEQ ID NO: 36, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 and SEQ ID NO: 48.

48. The mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 47, wherein said mutated Semaphorin 3 or said functional fragment thereof is encoded by a nucleic acid molecule comprising:

(a) the nucleotides from 601 to 1206 of SEQ ID NO: 1, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding a hydrophilic amino acid;

(b) the nucleotides from 529 to 1137 of SEQ ID NO: 5, wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding a hydrophilic amino acid;

(c) the nucleotides from 842 to 1444 of SEQ ID NO: 9, wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding a hydrophilic amino acid; or

(d) the nucleotides from 368 to 982 of SEQ ID NO: 13, wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding a hydrophilic amino acid.

49. The mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 48, wherein said mutated Semaphorin 3 or said functional fragment thereof is encoded by a nucleic acid molecule comprising the nucleotides from 601 to 1206 of SEQ ID NO: 57; the nucleotides from 529 to 1137 of SEQ ID NO: 61; the nucleotides from 842 to 1444 of SEQ ID NO: 65; or the nucleotides from 368 to 982 of SEQ ID NO: 69.

50. The mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 49, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises an amino acid sequence as shown in:

(a) SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;

(b) SEQ ID NO: 22, wherein the alanine residue corresponding to position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;

(c) SEQ ID NO: 23 wherein the alanine residue corresponding to position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or

(d) SEQ ID NO: 24, wherein the alanine residue corresponding to position 120 of SEQ ID NO: 14 is replaced by a hydrophilic amino acid.

51. The mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 50, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises an amino acid sequence that is selected from the group of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51 and SEQ ID NO: 52.

52. A polypeptide, wherein the polypeptide comprises the mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 51.

53. The polypeptide according to item 52, wherein said polypeptide is a fusion protein.

54. The polypeptide of item 52 or 53, wherein said polypeptide comprises said mutated Semaphorin 3 or said functional fragment thereof, a stabilizer domain and/or a dimerization domain.

55. The polypeptide according to any one of items 52 to 54, wherein said stabilizer domain is a Plexin Semaphorin Integrin (PSI) domain, wherein said PSI domain comprises one or more of the following sequences SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48.

56. The polypeptide according to any one of items 52 to 55, wherein said dimerization domain has a dissociation constant K_D in the range of 10^{-5} M to 10^{-6} M with another such dimerization domain.

57. The polypeptide according to any one of items 52 to 56, wherein said dimerization domain is selected from the group of a C-terminal IgG constant domain, DARPin and leucine zippers.

58. The polypeptide according to item 57, wherein the IgG constant domain is IgG1 or IgG3.

59. The polypeptide according to any one of items 52 to 58, wherein said polypeptide is encoded by a nucleic acid molecule comprising a nucleic acid sequence having:

(a) a nucleic acid sequence spanning from nucleotides 316 to 1959 of SEQ ID NO: 1 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding a hydrophilic amino acid;

(b) a nucleic acid sequence spanning from nucleotides 247 to 1887 of SEQ ID NO: 5 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding a hydrophilic amino acid;

(c) a nucleic acid sequence spanning from nucleotides 563 to 2197 of SEQ ID NO: 9 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding a hydrophilic amino acid; or

(d) a nucleic acid sequence spanning from nucleotides 41 to 1735 of SEQ ID NO: 13 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding a hydrophilic amino acid.

60. The polypeptide according to any one of items 52 to 59, wherein said polypeptide comprises an amino acid sequence:

(a) spanning from amino acid residues 1 to 548 of SEQ ID NO: 2 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;

(b) spanning from amino acid residues 1 to 547 of SEQ ID NO: 6 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;

(c) spanning from amino acid residues 1 to 565 of SEQ ID NO: 10 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or

(d) spanning from amino acid residues 1 to 545 of SEQ ID NO: 14 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 120 of SEQ ID NO: 14 is replaced by said hydrophilic amino acid.

61. An antibody specifically binding to the mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 51, wherein said antibody specifically binds to an epitope comprising the hydrophilic amino acid which replaces the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

62. A pharmaceutical composition comprising the nucleic acid molecule of any one of items 1 to 25 or the mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 51 or the polypeptide according to any one of items 52 to 60 optionally comprising a pharmaceutical excipient.

63. The pharmaceutical composition of item 62, wherein the composition comprises the mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 51 or the polypeptide according to any one of items of 52 to 60 optionally comprising a pharmaceutical excipient.

64. The pharmaceutical composition of item 62 or 63, wherein the pharmaceutical excipient is a pharmaceutical carrier, which is a virus.

65. The pharmaceutical composition of any one of items 62 to 64, wherein said virus is an adeno-associated-virus (AAV).

66. The pharmaceutical composition of any one of items 62 to 65, wherein the adeno-associated-virus is AAV8.

67. The nucleic acid molecule of any one of items 1 to 25, the vector of any one of items 26 or 29, the mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 51, the polypeptide according to any one of items of 52 to 60 or the pharmaceutical composition of any one of items 62 to 66 for use as a medicament.

68. The nucleic acid molecule of any one of items 1 to 25, the vector of any one of items 26 or 29, the mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 51, the polypeptide according to any one of items of 52 to 60 or the pharmaceutical composition of any one of items 62 to 66 for use in the treatment of an angiogenic disorder, cancer, tumor, tumorous disease, vascular retinopathies, blood-brain barrier permeability alterations, neuroinflammatory disorder, osteoporosis, obesity, mycobacterial infection, and/or granuloma.

69. The nucleic acid molecule of any one of items 1 to 25 for use according to item 68, the vector of any one of items 26 or 29 for use according to item 68, the mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 51 for use according to item 68, the polypeptide of any one of items 52 to 60 for use according to item 68 or the pharmaceutical composition of any one of items 62 to 66 for use according to item 68, wherein the tumor is a solid tumor.

70. The nucleic acid molecule of any one of items 1 to 25 for use according to item 68 or 69, the vector of any one of items 26 or 29 for use according to item 68 or 69, the mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 51 for use according to item 68 or 69, the polypeptide of any one of items 52 to 60 for use according to item 68 or 69 or the pharmaceutical composition of any one of items 62 to 66 for use according to item 68 or 69, wherein the tumor is pancreatic tumor.

71. The nucleic acid molecule of any one of items 1 to 25 for use according to any one of items 68 to 70, the vector of any one of items 26 or 29 for use according to any one of items 68 to 70, the mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 51 for use according to any one of items 68 to 70, the polypeptide of any one of items 52 to 60 for use according to any one of items 68 to 70 or the pharmaceutical composition of any one of items 62 to 66 for use according to any one of items 68 to 70, wherein the cancer is selected from the group consisting of pancreatic cancer, cervical cancer, breast cancer, colon cancer, melanoma, prostate cancer, bladder cancer and tongue cancer.

72. The nucleic acid molecule of any one of items 1 to 25 for use according to any one of items 68 to 71, the vector of any one of items 26 or 29 for use according to any one of items 68 to 71, the mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 51 for use according to any one of items 68 to 71, the polypeptide of any one of items 52 to 60 for use according to any one of items 68 to 71 or the pharmaceutical composition of any one of items 62 to 66 for use according to any one of items 68 to 71, wherein vascular normalization, reduction of tumor growth, reduction of metastatization or survival extension is involved.

73. The nucleic acid molecule of any one of items 1 to 25 for use according to any one of items 68 to 72, the vector of any one of items 26 or 29 for use according to item 68, the mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 51 for use according to any one of items 68 to 72, the polypeptide of any one of items 52 to 60 for use according to any one of items 68 to 72 or the pharmaceutical composition of any one of items 62 to 66 for use according to any one of items 68 to 72, wherein said nucleic acid molecule, said vector, said mutated Semaphorin 3, said functional fragment thereof, said polypeptide or said pharmaceutical composition is to be administered in combination with a with an anti-proliferative drug, an anticancer drug, a cytostatic drug, a cytotoxic drug and/or radiotherapy.

74. The nucleic acid molecule of any one of items 1 to 25 for use according to any one of items 68 to 72, the vector of any one of items 26 or 29 for use according to item 68, the mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 51 for use according to any one of items 68 to 72, the polypeptide of any one of items 52 to 60 for use according to any one of items 68 to 72 or the pharmaceutical composition of any one of items 62 to 66 for use according to any one of items 68 to 72, wherein said nucleic acid molecule, said vector, said mutated Semaphorin 3, said functional fragment thereof, said polypeptide or said pharmaceutical composition is to be administered parenterally.

75. Use of the mutated Semaphorin 3 or the functional fragment thereof as encoded by the nucleic acid molecule of any one of items 1 to 25 or of the mutated Semaphorin 3 or the

functional fragment thereof according to any one of items 36 to 51 or of the polypeptide according to any one of items 52 to 60 or of the host of any one of items 30 to 33.

76. A method of treatment for angiogenic disorder, tumorous disease and/or cancer comprising the step of administering to a subject in need of such treatment a pharmaceutical effective amount of the nucleic acid molecule of any one of items 1 to 23, or the mutated Semaphorin 3 or the functional fragment thereof according to any one of items 36 to 51, the polypeptide according to any one of items 52 to 60, the pharmaceutical composition of any one of items 62 to 68 or as produced by the method of item 34.

77. The method of treatment according to item 76, wherein the subject is a human.

78. The method of treatment according to item 76 or 77, wherein the pharmaceutical effective amount is the range of 0.5 to 10 mg/kg of body weight.

The present invention is further described by reference to the following non-limiting figures and examples.

Unless otherwise indicated, established methods of recombinant gene technology were used as described, for example, in Sambrook, Russell "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Laboratory, N.Y. (2001)) which is incorporated herein by reference in its entirety.

The Figures show:

Figure 1. The Sema3A-Nrp1-PlexinA4 signaling complex. Sema3A is characterized by the presence of a sema domain at its NH_2-terminal end, followed by a PSI domain, an Ig-like domain, and an additional short C-terminal basic stretch (b). The extracellular moiety of Nrp1 contains two repeated complement-binding domains (a1–a2 domains), two coagulation factor-like domains (b1–b2 domains), and a juxtamembrane meprin/A5/mu-phosphatase (MAM; c) homology domain. Plexin A4 is characterized by an extracellular sema domain, followed by multiple PSI and integrin-plexin-transcription factor (IPT) domains, while

intracellularly displays a half-split GAP domain. The C-terminal basic stretch of Sema3A binds with high affinity (*black border double arrow*) the b1 domain of Nrp1, which acts as a co-receptor that keeps dimeric Sema3A close to Plexin A4. Without being bound by theory, Sema3A is thought to drive Plexin A4 dimerization and activation *via* a very low affinity sema domain - sema domain interaction (*grey border double arrows*), which results in the inhibition of R-Ras and Rap1 GTP-loading and in the phosphorylation of ERK 1/2.

Figure 2. Schematic representation of Sema3A protein constructs. Representatives of Sema3A mutants are depicted: (A) Sema3A domain comprising the A106K mutation with or without an additional PSI domain or a domain that can stabilize the structure of the functional sema domain (stabilizer domain); (B) Sema3A A106K ΔIg-b; (C) Sema3A ΔIg-b, and (D) full length SEMA3A WT (R&D Systems).

Figure 3. Alignment of amino acidic sequences of mouse Sema3A, Sema3B, Sema3C, Sema3D, Sema3E, Sema3F and Sema3G. The protein sequences of mouse Sema3A, Sema3B, Sema3C, Sema3D, Sema3E, Sema3F and Sema3G and human SEMA3A, SEMA3B, SEMA3C and SEMA3D are depicted in the single letter amino acid code. (A) The $CX_1X_2A_3GKD$ peptide motif is highlighted (bold). (B) Three consensus sequence motifs are highlighted in bold that are predicted to allow for a strong interaction of the sema domain with the sema domain of Plexins. (C) One consensus sequence motifs is highlighted in bold in the PSI domain. The conservation is indicated with ClustalW consensus symbols: an asterisk (*) indicates positions which have a single, fully conserved residue; a colon (:) indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix; a period (.) indicates conservation between groups of weakly similar properties - scoring =< 0.5 in the Gonnet PAM 250 matrix.

Figure 4. Analysis of Semaphorin 3 and mutated Semaphorin 3 proteins binding to type A Plexin and Nrp1 receptors. (A) cDNAs of alkaline phosphatase (AP) and full-length Sema3A WT, mutant Sema3A ΔIg-b or mutant Sema3A A106K ΔIg-b were fused to generate the corresponding AP-conjugated Sema3A ligands. In an in situ binding assay, COS-7 cells were transfected with different candidate receptors. Ligand binding to cells expressing specific receptors was revealed by phosphatase substrate nitroblue tetrazolium. (B-D) Binding curves (B, D) and Scatchard analysis (C) of Plexin A4 (B, C) or Nrp1 (D)

binding at different concentrations of ligands (B, C, Sema3A A106K ΔIg-b; D, Sema3A WT) was independently quantified by spectrometry of chromogenic conversion of AP substrate p-nitrophenyl phosphate. (E). Estimated affinities of Sema3A WT, Sema3A ΔIg-b, and Sema3A A106K ΔIg-b for Plexin A4 receptor. Ligand binding curves of AP-conjugated Sema3A WT, mutant Sema3A ΔIg-b or mutant Sema3A A106K ΔIg-b to COS-7 cells expressing Plexin A4 receptor was independently quantified by spectrometry of chromogenic conversion of AP substrate p-nitrophenyl phosphate. The binding of Sema3A WT, mutant Sema3A ΔIg-b or mutant Sema3A A106K ΔIg-b to Plexin A4 respectively display an estimated K_d of 7 nM, 200 nM, and 0.7 nM. (F). In an in situ binding assay, COS-7 cells were transfected with different type A Plexin receptors or green fluorescent protein (Mock), for control purposes. Binding of human Fc-tagged SEMA3B ΔIg-b, SEMA3B A105K ΔIg-b, and SEMA3A A106K ΔIg-b to cells expressing different type A Plexin receptors was revealed in immunocytochemistry by means of an alkaline phosphatase (AP) conjugated goat anti-human IgG Fc secondary antibody. A combination of nitro-blue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3'-indolyphosphate p-toluidine salt (BCIP) was employed as AP substrate to yield an insoluble black-purple-colored product at locations where AP-conjugated goat anti-human IgG Fc secondary antibody was bound to human Fc tagged recombinant SEMA3 proteins.

Figure 5. Purification of Fc-tagged Sema3A ΔIg-b and Sema3A A106K ΔIg-b recombinant proteins. Proteins were produced in human HEK 293 cells, purified from supernatant on protein A-Sepharose, eluted and analyzed by NuPAGE 4-12% gel under non-reducing or reducing conditions. Lanes: 1) Mol. weight markers; 2) Fc-tagged Sema3A ΔIg-b cell culture medium; 3) Unbound protein A; 4) Fc-tagged Sema3A ΔIg-b Fraction 1; 5) Fc-tagged Sema3A ΔIg-b Fraction 2; 6) Fc-tagged Sema3A ΔIg-b Fraction 3; 7) Fc-tagged Sema3A A106K ΔIg-b cell culture medium; 8) Unbound protein A; 9) Fc-tagged Sema3A A106K ΔIg-b Fraction 1; 10) Fc-tagged Sema3A A106K ΔIg-b Fraction 2; 11) Fc-tagged Sema3A A106K ΔIg-b Fraction 3; 12) Fc-tagged Sema3A ΔIg-b, pool Fractions 1, 2 and 3; 13) Fc-tagged Sema3A ΔIg-b, pool Fractions 1, 2 and 3 (reduced); 14) Fc-tagged Sema3A A106K ΔIg-b, pool Fractions 1, 2 and 3; 15) Fc-tagged Sema3A A106K ΔIg-b, pool Fractions 1, 2 and 3 (reduced). In non-reducing conditions, both Fc-tagged Sema3A ΔIg-b and Fc-tagged Sema3A A106K ΔIg-b appear as a ~200 kDa dimer without degradation products.

Figure 6. Fc-tagged Sema3A A106K ΔIg-b is more effective than commercially available Fc-tagged SEMA3A WT in inhibiting EC directional migration. Directional EC migration towards type I collagen (1 μg/ml) was analyzed by real time analysis. (A-C) The migration of ECs was tracked over a 4 hour-long period in CIM-Plates 16 of an xCELLigence system platform either in the absence (Control, *black solid line*) or the presence of 0,2 nM (A), 0,9 nM (B), and 3,5 nM (C) Fc-tagged SEMA3A WT (*grey solid line*) or Fc-tagged Sema3A ΔIg-b (*black dotted line*) or Fc-tagged Sema3A A106K ΔIg-b (*black dashed line*). Each curve is the average of four technical replicates ± SD. Statistical analysis: results were analyzed by a two-tailed heteroscedastic Student's t-test; * Fc-tagged Sema3A A106K ΔIg-b vs. Fc-tagged SEMA3A WT; # Fc-tagged Sema3A A106K ΔIg-b vs. Fc-tagged Sema3A ΔIg-b; *, # $p<0.05$, **, ## $p<0.01$, ***,### $p<0.001$. (D, E) Control silenced (D) or Plexin A4 silenced (E) ECs were left to migrate over a 2 hours and half-long period either in the absence (Control, *black solid line*) or the presence of 1,8 nM Fc-tagged Sema3A A106K ΔIg-b (*black dashed line*); *, $p<0.05$; **, $p<0.01$; *** $p<0.001$.

Figure 7. Human Fc-tagged SEMA3A A106K ΔIg-b protein is more effective than commercially available Fc-tagged SEMA3A WT in inhibiting EC directional migration. Directional EC migration towards type I collagen (1 μg/ml) was analyzed by real time analysis. The migration of ECs was tracked over a 4 hour long period in CIM-Plates 16 of an xCELLigence system platform either in the absence (Control, *black solid line*) or the presence of: (A) equimolar (0,9 nM) amounts of commercial Fc-tagged SEMA3A WT (*grey solid line*) or Fc-tagged human SEMA3A A106K ΔIg-b (*black dashed line*); (B) 3,5 nM commercial Fc-tagged SEMA3A WT (*grey solid line*) or 0,2 nM Fc-tagged human SEMA3A A106K ΔIg-b (*black dashed line*). Each curve is the average of four technical replicates ± SD. Statistical analysis: results were analyzed by a two-tailed heteroscedastic Student's t-test; * Fc-tagged SEMA3A A106K ΔIg-b vs. Fc-tagged SEMA3A WT; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Figure 8. Fc-tagged Sema3A A106K ΔIg-b protein is much more effective than commercially available Fc-tagged SEMA3A WT in eliciting biochemical signaling in ECs. (A) Pull-down assay of active Rap1 GTP in ECs that were treated or not for 1 minute with 0,02 nM Fc-tagged SEMA3A WT, Sema3A ΔIg-b or Sema3A A106K ΔIg-b. Total Rap1, detected in the input fractions, was used to calculate the normalized optical density

(N.O.D.) of active Rap1. (**B**) Western blot analysis of activated phospho-ERK1/2 in ECs that were treated or not for 15 minutes with 0,2 nM Fc-tagged SEMA3A WT or Sema3A ΔIg-b or Sema3A A106K ΔIg-b. Western blot analysis of total ERK1/2 was used to calculate the N.O.D. of active ERK 1/2. A representative of three independent assays with similar results is shown. Bands were quantified and N.O.D.s were calculated relative to control (Values are means ± SD; n = 3 separate assays). Statistical analysis: results were analyzed by a two-tailed heteroscedastic Student's t-test; * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Figure 9. Sema3A A106K ΔIg-b impairs angiogenesis and normalizes the cancer vasculature of RIP-Tag2 mice. Immunofluorescence analysis of control and Sema3A A106K ΔIg-b-treated cancer tissues were stained as indicated. Confocal images are representative of 7 mice per group and of five fields per cancers. (**A**) An anti-Meca32 Ab (*green*) was employed to stain vascular ECs. Sema3A A106K ΔIg-b significantly reduced blood vessel area by 51%. (**B**) An anti-α-smooth muscle actin (ASMA) was used to detect pericytes (*red*) in combination with an anti-Meca32 (*green*). Sema3A A106K ΔIg-b strongly enhanced blood vessel pericytes. (**C**) Control and Sema3A A106K ΔIg-b-treated mice were heart-perfused with FITC-lectin (*green*) and cancer tissues were then stained with an anti-Meca32 Ab (*red*). While control cancers display a poorly perfused vasculature, Sema3A A106K ΔIg-b enhanced the perfusion of cancer blood vessels. (**D**) Sema3A A106K ΔIg-b efficiently inhibited cancer hypoxia that was detected by injecting mice before sacrifice with pimonidazole (Maione et al 2012).

Figure 10. AAV8-full length SEMA3A WT inhibits cancer growth and metastasis formation in a PDAC mouse model. Full length SEMA3A WT was transduced in the pancreas of PDAC mice by AAV-8-mediated somatic gene transfer, as previously described (Maione et. al 2009, 2012). AAV8-LacZ was transduced as a control. After three weeks of SEMA3A WT gene therapy mice were sacrificed and analyzed. AAV8-SEMA3A WT inhibited cancer volume by 52% (**A**) and reduced liver metastasis incidence by 59% (**B**). Number of mice per group (n=10). Statistical analysis: Mann-Whitney *U* test was used, **$p<0.01$.

Figure 11. Sema3A A106K ΔIg-b inhibits cancer growth, impairs metastasis formation and increases blood vessel coverage by pericytes in a PDAC mouse model. (A, B)

Cancer-bearing PDAC mice were treated with 3 mg/kg (i.p.) of Sema3A A106K ΔIg-b for three weeks. Sema3A A106K ΔIg-b shrinks cancer volume by 64% (**A**) and inhibits liver metastasis incidence by 81% (**B**); number of mice per group (n=10); statistical analysis: Mann-Whitney *U* test was used, **p<0.01, ***p<0.001. (**C**) Confocal images of immunofluorescence analysis of Sema3A A106K ΔIg-b-treated and control cancer tissues stained with an anti-NG2 (*red*, to label pericytes), and an anti-Meca32 Ab (*green*). Sema3A A106K ΔIg-b strongly enhanced vessel pericyte, compared to controls. Images a representative of 7 mice per group and of five fields per cancer.

Figure 12. Sema3F A106K fails to increase the inhibition of endothelial cell migration. Directional EC migration towards type I collagen (1 μg/ml) was analyzed by real time analysis. The migration of ECs was tracked over a 4 hour-long period in CIM-Plates 16 of an xCELLigence system platform either in the absence (Control, *black solid line*) or the presence of 3,5 nM Fc-tagged Sema3F ΔIg-b (*black dotted line*) or Fc-tagged Sema3F S107K ΔIg-b (*dashed*). Each curve is the average of four technical replicates ± SD. Statistical analysis: results were analyzed by a two-tailed heteroscedastic Student's t-test; * Fc-tagged Sema3F ΔIg-b vs. Control; *, p<0.05; **, p<0.01; *** p<0.001.

Figure 13. Fc-tagged Sema3A A106K ΔIg-b is more effective than commercially available Fc-tagged SEMA3E and SEMA3F in inhibiting EC directional migration. Directional EC migration towards type I collagen (1 μg/ml) was analyzed by real time analysis. (A-B) The migration of ECs was tracked over a 4 hour-long period in CIM-Plates 16 of an xCELLigence system platform either in the absence (Control, *black solid line*) or the presence of equimolar amounts of Fc-tagged Sema3A A106K ΔIg-b (A-B, *black dashed line*) or commercially available Fc-tagged SEMA3E WT (*black dotted line*) or commercially available Fc-tagged Sema3F WT (*grey solid line*). Each curve is the average of four technical replicates ± SD. Statistical analysis: results were analyzed by a two-tailed heteroscedastic Student's t-test; * Fc-tagged Sema3A A106K ΔIg-b vs. Fc-tagged SEMA3E WT or Fc-tagged Sema3F WT; *, p<0.05, **, p<0.01, ***, p<0.001.

The following non-binding Examples illustrate the invention.

Example 1 - Sema3A A106K ΔIg-b is a specific binder, powerful activator of PlexinA4 and inhibitor of haptotactic migration of ECs

Materials and methods

Cell-binding assays and Scatchard analysis.

In situ binding assays were performed with a modification of the protocol described previously (Tamagnone et al 1999). In particular, COS7 cells transfected with cDNA constructs expressing different semaphorin receptors were seeded in wells of 48-well cluster dishes. They were then incubated for 1 hour at 37°C with complete medium containing recombinant semaphorin molecules fused with secreted placental Alkaline Phosphatase (e.g. Sema3A-AP). After five washes, cells were fixed, heated for 10 min at 65°C to inactivate endogenous phosphatases, and incubated with NBT–BCIP (nitro blue tetrazolium–5-bromo-4-chloro-3-indolyl-phosphate) AP substrate (Promega, Catalog # S3771) for in situ cell staining. For a quantitative assessment of ligand binding, receptor-expressing cells were incubated with increasing concentrations of AP-conjugated ligands (with predetermined specific activity/μg); cell-bound AP activity was eventually revealed by incubation with the chromogenic soluble substrate p-nitrophenylphosphate (Sigma-Aldrich, Catalog # P7998) and measured by a multi-well spectrophotometer (absorbance at 405 nm). Scatchard plot analysis was performed using Prism 6 (GraphPad Software Inc.).

Haptotactic endothelial cell migration assays

Real-time directional migration of human umbilical vein ECs was monitored by means of xCELLigence (Acea Biosciences Inc.), an electrical impedance-based system in which microelectronic sensor arrays are integrated microplate wells. The impedance-based xCELLigence system is based on the Real-Time Cell Analyzer (RTCA) instrument. The core of the instrument is the E-plate. E-Plates 16 are single-use plates and each individual well on an E-Plate 16 has incorporated a sensor electrode array that allows cells in the well to be constantly monitored. Set up the experiment file using the RTCA Software 1.2.: this software converts impedance values to obtain parameters such as: cell index (CI), average values, maximum and minimum values, standard deviation (SD), half maximum effect of concentration (EC50), half maximum inhibition of concentration (IC50). Data expressed in CI units can then be exported for any type of mathematical and statistical analyses.

In this assay, we monitored the directional migration of ECs in real-time by using the CIM-Plate 16 of the xCELLigence RTCA DP instrument. The bottom side of the upper chamber (the side facing the lower chamber) of the CIM-Plate was coated with 30 µl of type I collagen (1 µg/ml) for 30 minutes inside the tissue culture hood. Each lower chamber well was first filled with 160 µl of serum-free M199 medium (containing or not Sema3A) and then assembled to the upper chamber. The assembled plate was incubated at 37°C for one hour to equilibrate (30µl of serum free media was added in each well of the upper chamber). ECs were detached and resuspended to a final concentration of 30000 cells/100 µl. The BLANCK step was started to measure the background impedance of cell culture medium, which was then used as reference impedance for calculating CI values. 100 µl of cell suspension (30,000 cells) were then added to each well of the upper chamber. The CIM-Plate 16 was placed in the RTCA DP Instrument equilibrated in a CO_2 incubator. ECs migration was continuously monitored using the RTCA DP Instrument.

For statistical evaluation, results were analyzed by a two-tailed heteroscedastic Student's *t*-test. The average, standard deviation and p value have been calculated on the CI data exported from RTCA instrument for the technical replicates of each experimental condition in the time. Migration data are represented as a percentage considering the control samples as 100%.

Rap1-GTP pull-down assay

ECs were first starved for 3 hours and then treated or not with 0.02nM of SEMA3A, for 1 minute at 37°C. Active Rap1-GTP was then pulled-down on a glutathione-S-transferase-fusion protein of the Rap1-binding domain (RBD) of human Ral guanine nucleotide dissociation stimulator (RalGDS) isolated by means of a glutathione agarose resin. We proceeded with the active Rap1 Pull-down assay according to the manufacturer's guideline and detection kit (Thermo Scientific, Product Cat. #16120). Total Rap1, detected in the input fractions, was used to calculate the normalized optical density (N.O.D.) of active Rap1-GTP. A representative of three independent pull-down assays with similar results is shown. Bands were quantified and N.O.D.s were calculated relative to control (Values are means ± SD; n = 3 separate assays). For statistical evaluation, results were analyzed by a two-tailed heteroscedastic Student's *t*-test.

ERK 1/2 phosphorylation

ECs were first starved for 3 hours and then treated or not with SEMA3A [0,225 nM] for 15' at 37°C. We lysed cells and proceeded to protein analysis through WB with anti-phopsho ERK 1/2 [Mouse anti-phospho-p44/42 MAPK (ERK1/2; Thr202/Tyr204) (clone E10); dilution 1:1000; Cell Signaling Technology, Product Cat #9106] and anti-Tot ERK [Rabbit anti-p44/42 MAPK (ERK1/2) (clone 137F5); dilution 1:1000; Cell Signaling Technology, Product Cat #4695] antibodies. The total protein amount was determined using the bicinchoninic acid (BCA) protein assay reagent (Thermo Scientific). Equivalent amounts of protein separated by SDS–PAGE with precast Bolt 4-12% Bis-Tris gel (Invitrogen). Proteins were then transferred to a Trans-Blot Turbo™ Mini Nitrocellulose Transfer (Biorad), probed with either goat anti-rabbit IgG (H+L) secondary antibody, HRP conjugate (Invitrogen, Catalog#: 65-6120) or goat anti-mouse IgG (H+L) secondary antibody, HRP conjugate, (Jackson ImmunoResearch Inc. Catalog#: 11-035-062) and detected by enhanced chemiluminescence technique (Western Lightning Plus-ECL, Enhanced Chemiluminescence Substrate; Perkin Elmer, Catalog# NEL105001EA). Total ERK1/2, detected in the input fractions, was used to calculate the N.O.D. of phospho-ERK1/2. A representative of three independent pull-down assays with similar results is shown. Bands were quantified and N.O.D.s were calculated relative to control (Values are means ± SD; n = 3 separate assays). For statistical evaluation, results were analyzed by a two-tailed heteroscedastic Student's *t*-test.

Human and mouse Semaphorin 3 proteins are conserved proteins. Human Semaphorin 3A as shown in SEQ ID NO: 1 is at least 52% homologous/identical to human Semaphorin 3B as shown in SEQ ID NO: 5. Further, human Semaphorin 3A as shown in SEQ ID NO: 1 is at least 45% homologous/identical to human Semaphorin 3C as shown in SEQ ID NO: 9. Further, human Semaphorin 3A as shown in SEQ ID NO: 1 is at least 53% homologous/identical to human Semaphorin 3D as shown in SEQ ID NO: 13. Further, mouse Semaphorin 3A as shown in SEQ ID NO: 3 is at least 53% homologous/identical to human or mouse Semaphorin 3A as shown in SEQ ID NO: 13 or SEQ ID NO: 15. Further, mouse Semaphorin 3A as shown in SEQ ID NO: 3 is at least 45% homologous/identical to mouse Semaphorin 3C as shown in SEQ ID NO: 11.

Results

We developed a strategy in order to provide a novel, easy to purify and parenterally deliverable mutant synthetic protein derived from Sema3A WT. This mutant should comprise the following features: i) is unable to bind to Nrp1; ii) lacks any furin protease cleavage site; iii) is stably dimeric; and iv) is able to bind to Plexins with high affinity. Therefore, a novel recombinant mouse Sema3A mutant protein was generated. This exemplary mutant Sema3A was designed in the format of a fusion protein. This mutant Semaphorin 3A comprises the features summarized in table 2 and is designated Sema3A A106K ΔIg-b (Table 2 and Figure 2). Exemplary, amino acid sequences are illustrated in SEQ ID NO: 18 or 20. Furthermore, corresponding fusion proteins of mutated Semaphorin 3B, C and D were also generated. These mutants are herein designated Sema3B A105K-Fc, Sema3C A104K-Fc and Sema3D A120K-Fc (shown in SEQ ID NO 76, 78 or 79, respectively). A mutant of Semaphorin 3A retaining the alanine at position 106 but comprising the same architecture as Sema3A A106K ΔIg-b is designated Sema3A ΔIg-b, the nucleic acid sequence of such a molecule is given in SEQ ID NO: 43 or 44 (human and mouse Sema3A ΔIg-b, respectively).

Table 2. Molecular features of the novel Sema3A A106K ΔIg-b

Aim	Wild type Sema3A modifications
To abolish mutant Sema3A **binding to Nrp1**	**Deletion of the basic stretch**
To abolish **furin cleavage** of mutant Sema3A	**Deletion of the Ig-like domain and basic stretch**
To render mutant Sema3A **stably dimeric**	**Fusion of the remaining sema-PSI domain with IgG1 Fc**
To allow **high affinity binding** of mutant Sema3A **to PlexinA4**	**Mutation of Ala 106 into Lys**

The Sema3A A106K ΔIg-b mutant is characterized by the following features and advantages:

1) the Nrp1 binding and the furin cleavable Ig-like/basic region of Sema3A WT (e.g., amino acids 549-772) is deleted;
2) the remaining Sema-PSI domain region (e.g., amino acids 1-548) of the mutant mouse Sema3A is fused with its C-terminus to the IgG1 constant fragment (e.g., from mouse) (Fc, formed by hinge, CH2, and CH3 domains) to induce dimerization. The IgG1 Fc allowed easy and large scale purification on Sepharose protein A of the mutant Sema3A protein;

3) the Ala_{106} residue of Sema3A WT was substituted with a Lys (A106K) endowing the mutant form a high (K_d = 0.7 nM) affinity to type A plexin PlexinA4.

Mutated and wild-type Semaphorin 3 A, B, C, D, E, and F were produced using standard protein purification. In particular, human and mouse Fc-tagged Sema3A A106K ΔIg-b (given in SEQ ID NO: 18 or 20, respectively) as well as mouse Fc-tagged Sema3A ΔIg-b, were produced using routine protein purification by a service. SEMA3A WT full-length was purchased from R&D Systems Inc., Minneapolis, MN; catalog # 1250-S3-025. The Sema3A A106K ΔIg-b coding cDNA designated as SEQ ID NO:19 was generated by synthetic gene design (GeneArt® Gene Synthesis, Life-Technologies / Thermo Fisher Scientific Inc.).
The Sema3A A106K ΔIg-b coding cDNA (SEQ ID NO:19) was then subcloned in the pUPE expression vector. A transfection grade preparation of pUPE expression vector carrying the Sema3A A106K ΔIg-b coding cDNA (Sema3A A106K ΔIg-b pUPE) was then transiently transfected into suspension growing HEK293 cell line stably expressing the Epstein-Barr virus nuclear antigen-1 (HEK293-EBNA1, or 293E), *i.e.* the cell line that is most commonly employed for large-scale transfections. After one week the medium of Sema3A A106K ΔIg-b pUPE HEK293E suspension cultures was harvested, by centrifugation. The fusion proteins were bound batch-wise to protein A-Sepharose. The beads were collected and transferred into a gravity-flow column. Specifically bound proteins were removed by washing the column with phosphate buffered saline (PBS). The bound fusion proteins were eluted using 20 mM citrate, 150 mM NaCl pH 2.7 and 0.9 ml fractions were collected in Eppendorf tubes containing 0.1 ml 1M KH2PO4/K2HPO4 pH 8.0 for neutralization.

To pinpoint the function of the A106K mutation in Sema3A, a Sema3A ΔIg-b recombinant protein was generated. Sema3A ΔIg-b lacked the Ig-like/basic region but preserved the wild type residue Ala_{106} (Figures 2). The biochemical and biological activities of both Sema3A ΔIg-b and Sema3A A106K ΔIg-b mutants were compared with that of the commercially available full-length SEMA3A WT (from R&D Systems Inc., Minneapolis, MN; catalog # 1250-S3-025).

A ligand-receptor in situ binding assay (Flanagan et al., 2000; Tamagnone et al., 1999) confirmed that alkaline phosphatase (AP)-conjugated Sema3A WT was interacting with Nrp1

in COS cells. However, neither Sema3A ΔIg-b nor Sema3A A106K ΔIg-b mutants interacted with Nrp1 (Figure 4A).

The Semaphorin proteins are ligands that signal through Plexins (Kumanogoh and Kikutani, 2013; Tamagnone et al., 1999). Therefore, the ability of these constructs to directly bind to Plexin family members was screened. Importantly, Sema3A A106K ΔIg-b bound to PlexinA4 with high affinity compared to Sema3A WT or the Sema3A ΔIg-b mutant (Figure 4 A and B). The binding affinity of Sema3A A106K ΔIg-b to PlexinA4 was increased to a K_d of 0,7 nM, as evaluated by Scatchard plot analysis (Figure 4C). In comparison, the K_d of Sema3A WT binding to Nrp1 was 1,1 nM (Figure 4D), which is consistent with previous data (Takahashi et al., 1999). Furthermore, no other type-A Plexin displayed detectable binding to Sema3A A106K ΔIg-b (Figure 4A). Accordingly, the binding of Sema3A A106K ΔIg-b was highly specific for PlexinA4. Finally, ligand binding assays allowed to estimate the affinity range of Sema3A WT, Sema3A ΔIg-b, and Sema3A A106K ΔIg-b for Plexin A4 receptor and to reveal how the binding of Sema3A WT, mutant Sema3A ΔIg-b or mutant Sema3A A106K ΔIg-b to Plexin A4 respectively displays an estimated Kd of 7 nM, 200 nM, and 0.7 nM (Figure 4E). Furthermore, the binding affinity of mutated Semaphorin 3B to Plexins was analyzed (Figure 4F). For example, illustrative Sema3B A105K-Fc demonstrated binding to Plexin A2 with a high binding affinity. Semaphorin 3B that has the same construct design as Sema3B A105K-Fc, but lacking the alanine to lysine mutation at position 105 (SEMA3B ΔIg-b), showed no increased binding in comparison to the mutated version. As described herein above, the strong interaction of the Semaphorin 3 proteins with its Plexin receptor is important for receptor activation and Semaphorin 3 downstream signaling. Therefore, these data provide evidence that mutated Semaphorin 3A, B, C and D can be employed in the improved anti-angiogenesis and/or anti-vasculogenesis therapy. The further analysis in in vitro as well as in vivo systems is herein exemplified on mutated Semaphorin 3A.

In order to assess the haptotactic migration of human umbilical vein endothelial cells (HUVECs or ECs) towards ECM proteins, we generated and purified Fc-tagged Sema3A ΔIg-b and Sema3A A106K ΔIg-b proteins (Figures 2, 3, 5). Equimolar amounts of Fc-tagged Sema3A ΔIg-b, Sema3A A106K ΔIg-b proteins and SEMA3A WT were compared in inhibiting the haptotactic migration of ECs towards ECM proteins, such as type I Collagen in a xCELLigence system platform (Acea Biosciences Inc., San Diego, CA) (Figure 6). Fc-

tagged Sema3A A106K ΔIg-b was always much more effective than Fc-tagged Sema3A ΔIg-b or commercial Fc-tagged SEMA3A WT in inhibiting EC directional migration over a wide range of concentrations (0,2 – 3,5 nM) (Figure 6A-C). As detailed in Table 3, while mouse Fc-tagged Sema3A A106K ΔIg-b inhibited EC directional migration by 36-45%, commercial human Fc-tagged SEMA3A WT and mouse Fc-tagged Sema3A A106K ΔIg-b impaired EC motility by only 23-25% and 19-24%, respectively. Furthermore, gene silencing experiments of PlexinA4 increased the migration of ECs in response to Sema3A A106K ΔIg-b to control levels. Accordingly, the inhibitory activity on EC haptotaxis of Fc-tagged Sema3A A106K ΔIg-b is dependent on PlexinA4 (Figure 6D, E), which is consistent with its type A Plexin receptor binding profile (Figure 4A).

Table 3. Additional quantitative details concerning the ability of Fc-tagged SEMA3A WT, mouse Fc-tagged Sema3A ΔIg-b, and mouse Fc-tagged Sema3A A106K ΔIg-b in inhibiting EC directional migration. Raw numeric data corresponding to the experimental time point 3 hours and 45 minutes of the representative directional migration experiment depicted in Figure 6 are reported here.

Time point: 3h:45m	Relative migration	p value Sema3A A106K ΔIg-b vs. SEMA3A WT	p value Sema3A A106K ΔIg-b vs. Sema3A ΔIg-b
Control	100 ± 0,01		
SEMA3A WT 0,2 nM	75 ± 0,01		
Sema3A ΔIg-b 0,2nM	77 ± 0,01		
Sema3A A106K ΔIg-b 0,2 nM	60 ± 0,001	0,0009	0,0007
SEMA3A WT 0,9 nM	77 ± 0,004		
Sema3A ΔIg-b 0,9nM	76 ± 0,06		
Sema3A A106K ΔIg-b 0,9 nM	64 ± 0,02	0,001	0,001
SEMA3A WT 3,5 nM	77 ± 0,02		
Sema3A ΔIg-b 3,5nM	81 ± 0,007		
Sema3A A106K ΔIg-b 3,5 nM	55 ± 0,01	0,003	0,0001

Table 4. Additional quantitative details concerning the ability of Fc-tagged SEMA3A WT and human Fc-tagged SEMA3A A106K ΔIg-b in inhibiting EC directional migration. Raw numeric data corresponding to the experimental time point 3 hours and 36 minutes of the representative directional migration experiment depicted in Figure 7 are reported here.

Time point: 3h:36m	Relative migration	p value SEMA3A A106K ΔIg-b vs. SEMA3A WT
Control	100 ± 0,01	
SEMA3A WT 3,5 nM	80 ± 0,01	
SEMA3A A106K ΔIg-b 0,2 nM	64 ± 0,01	0,00009

We generated and tested a human Fc-tagged SEMA3A A106K ΔIg-b, whose biological activity was essentially superimposable to that of its murine counterpart (Figure 7). As detailed in Table 4, while the maximal (3,5 nM) dose of commercial human Fc-tagged SEMA3A WT inhibited EC directional migration by 20%, a 17.5 times lower (0.2 nM) dose of human Fc-tagged SEMA3A A106K ΔIg-b inhibited EC motility by 46%. Furthermore, dimerization of SEMA3A A106K induced by the Fc-tag further increased the inhibition of EC directional migration (data not shown).

Sema3F possess a polar amino acid, i.e. serine (Ser_{107}) at the position that corresponds by comparison of homology to the position 106 of Sema3A. This is in contrast to Sema3A, Sema3B, Sema3C and Sema3D, which comprise a hydrophobic alanine at the corresponding position. Therefore, for control purposes, we investigated whether mutating the polar Ser_{107} into a positively charged Lys (Sema3F S107K) results in a significant increase of Sema3F affinity for Plexin(s) and an ability to inhibit EC migration towards ECM proteins. By means of ligand-receptor in situ binding assays in COS cells, we found how AP-conjugated Sema3F ΔIg-b and Sema3F S107K ΔIg-b mutants failed to bind at high affinity to any type of A, B, C, or D Plexin family members. Accordingly, we observed how the ability of inhibiting EC migration towards ECM proteins of equimolar (3.5 nM) amounts of Sema3F ΔIg-b and Sema3F S107K ΔIg-b was largely superimposable (Figure 12). Similarly, SEMA3A A106K ΔIg-b showed an increased inhibition of EC directional migration compared to Fc-tagged SEMA 3E (R&D Systems) or Sema 3F (R&D Systems) (Figure 13). The EC migration assay was performed with equimolar amounts of protein, respectively. Therefore, without being

bound by theory, we concluded that the synthetic introduction of positively charged amino acids into Sema3 proteins is more likely increasing their affinity for Plexin receptor(s) and chemorepulsive activity, if the substituted amino acid is hydrophobic, such as in the case of Sema3A A106. In other words, the herein described artificial introduction of the hydrophilic amino acid in the context of Sema 3 A, B, C or D increases the anti-angiogenic and/or vasculogenic properties.

In order to test the effect of the Sema3A constructs on downstream signaling, pull-down assays of Sema3A binding partners were performed. The Fc-tagged Sema3A A106K ΔIg-b was significantly more powerful than Fc-tagged Sema3A ΔIg-b and commercial Fc-tagged SEMA3A WT in inhibiting the GTP-loading of Rap1 small GTPase (Figure 8A) as well as in triggering the phosphorylation of ERK 1/2 kinase (Figure 8B). In particular, commercial Fc-tagged SEMA3A WT, Fc-tagged Sema3A ΔIg-b, and Fc-tagged Sema3A A106K ΔIg-b respectively: i) inhibited Rap1 GTP loading by 42%, 44%, and 65%; ii) activated ERK 1/2 phosphorylation by 1.95 fold, 2.3 fold, and 3.9 fold.

Therefore, it could be concluded that the novel Fc-tagged Sema3A A106K ΔIg-b mutant i.) bound specifically with a high affinity to the receptor PlexinA4; ii.) was a much more powerful activator of Plexin signaling; iii.) was a much more powerful inhibitor of EC function; iv.) did not interact with Nrp1; and v.) could not be cleaved.

This renders Sema3A A106K ΔIg-b as a specific binder, powerful activator of PlexinA4 and inhibitor of haptotactic migration of ECs

Example 2 - Sema3A A106K ΔIg-b effectively inhibits cancer growth and metastatization in pancreas cancer

Considering the present *in vitro* data and the pre-clinical experience with the mouse model of spontaneous pancreatic neuroendocrine cancer (RIP-Tag2) (Maione et al., 2012; Maione et al., 2009), the ability of a wide range [0,5 - 5 mg/kg/mouse, delivered by means of either osmotic mini-pumps or intra-peritoneal (i.p.) for 4 weeks] of Fc-tagged Sema3A ΔIg-b and Sema3A A106K ΔIg-b in halting cancer progression and extending RIP-Tag2 mice survival was assessed at least up to 16 weeks of age, as we previously observed by means of Adeno-Associated Virus (AAV)-8-mediated gene transfer (Maione et al., 2012; Maione et al., 2009). We found that no dose of Sema3A ΔIg-b delivered by any therapeutic method was able to extend RIP-Tag2 mice survival as AAV-8 full length Sema3A did (Maione et al., 2012;

Maione et al., 2009). On the contrary, Sema3A A106K ΔIg-b displayed a pro-survival activity similar to AAV-8 full length Sema3A, thus indicating how the increased Plexin A4-binding activity endowed Sema3A A106K ΔIg-b with a powerful Nrp1-independent anti-cancer effect. In particular, the delivery of 3 mg/kg of Sema3A A106K ΔIg-b by i.p. injection three times a week was the most effective and non-toxic therapeutic regimen to impair cancer progression, normalize the cancer vasculature, and extend RIP-Tag2 mice survival. In fact, compared to saline-treated controls, one month of treatment of RIP-Tag2 with Sema3A A106K ΔIg-b (3mg/kg, i.p, three times a week): i) induced a 67% reduction of cancer volume; ii) efficiently reduced the cancer blood vessel area by 51 % (Figure 9A); iii) favored the normalization of cancer blood vessels in terms of increased pericyte coverage (Figure 9B); iv) enhanced perfusion (Figure 9C) and reduced tissue hypoxia (Figure 9D).

Furthermore, the effect of Sema3A A106K ΔIg-b was analyzed in a mouse model of a significantly more frequent and deadly human pancreatic cancer histotype, namely a syngeneic K-Ras^{G12D};Ink4a/$Arf^{-/-}$;$p53^{R172H}$ orthotopic mouse model of pancreatic ductal adenocarcinoma (PDAC). The full length SEMA3A WT was delivered in the orthotopic Ras^{G12D}; Ink4a/$Arf^{-/-}$; $p53^{R172H}$ PDAC mouse model by means AAV-8-mediated gene transfer (Maione et al., 2009). The treatment with full length SEMA3A WT resulted in an inhibition of both cancer growth (by 52%) and liver metastasis (by 59%) (Figure 10A, B) after 3 weeks. Stemming from the previous promising data, we then treated cancer-bearing PDAC mice with purified Sema3A A106K ΔIg-b mutant protein (3mg/kg, i.p., three times a week) and assessed its pharmacological effect on cancer growth and metastasis formation. Sema3A A106K ΔIg-b protein strongly inhibited cancer growth (by 64%) (Figure 11A), reduced the incidence of liver metastasis by 81% (Figure 11B) and diminished metastasis volume by 78%. Most importantly and surprisingly, Sema3A A106K ΔIg-b exerted a stronger effect in reducing cancer progression and metastasis dissemination compared with Sema3A full length in PDAC mice. Consistent with the treatment of RIP-tag2 mice, Sema3A A106K ΔIg-b reduced vessel area (data not shown) and promoted cancer vessel normalization also in the PDAC mouse model by enhancing pericyte coverage (Figure 11C), increasing blood vessel perfusion and inhibiting cancer hypoxia.

All references cited herein are fully incorporated by reference. Having now fully described the invention, it will be understood by a person skilled in the art that the invention may be practiced within a wide and equivalent range of conditions, parameters and the like, without affecting the spirit or scope of the invention or any embodiment thereof.

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Claims

1. A mutated Semaphorin 3 or a functional fragment thereof wherein said mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C, and Semaphorin 3D and wherein said mutated Semaphorin 3 or said functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein

   X_1 is an amino acid, which is K or N,

   X_2 is an amino acid selected from the group of W, M and L,

   and wherein the alanine (A_3) is replaced by a hydrophilic amino acid.

2. The mutated Semaphorin 3 or the functional fragment thereof of claim 1, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises

   said hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2;

   said hydrophilic amino acid in place of the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6;

   said hydrophilic amino acid in place of the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or

   said hydrophilic amino acid in place of the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14.

3. The mutated Semaphorin 3 or the functional fragment thereof of claim 1 or 2, wherein said Semaphorin 3 is selected from the group of:

   (a) a polypeptide that is encoded by a nucleic acid sequence selected from the group of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 9 and SEQ ID NO: 13, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding said hydrophilic amino acid,

   wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding said hydrophilic amino acid,

wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding said hydrophilic amino acid, and
wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding said hydrophilic amino acid;

(b) a polypeptide having the amino acid sequence selected from the group of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10 and SEQ ID NO: 14, wherein the alanine residue at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14 is replaced by said hydrophilic amino acid;

(c) a polypeptide that is encoded by a nucleic acid sequence that hybridizes under stringent conditions to the complementary strand of a nucleic acid molecule encoding a polypeptide as defined in (a) or (b);

(d) a polypeptide that functions as an inhibitor of angiogenesis and has at least 55% identity to any one of the polypeptides referred to in (b).

4. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 3, wherein said Semaphorin 3 or said functional fragment thereof comprises a hydrophilic amino acid

(a) at position 106 of SEQ ID NO: 2;

(b) at position 105 of SEQ ID NO: 6;

(c) at position 104 of SEQ ID NO: 10; or

(d) at position 120 of SEQ ID NO: 14.

5. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 4, wherein said Semaphorin 3 or said functional fragment thereof comprises a functional sema domain and comprises at least one additional mutation selected from the group consisting of amino acid substitution(s), addition(s), deletions(s) and duplication(s).

6. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 5, wherein said hydrophilic amino acid is selected from the group of K, R, N, Q, S,T, E, D, and H.

7. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 6, wherein said hydrophilic amino acid is K or R.

8. The mutated Semaphorin 3 or the functional fragment thereof of claim 6, wherein said hydrophilic amino acid replacing said alanine is K.

9. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 8, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises one or more of the following sequence(s) as defined in any one of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48.

10. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 9, wherein a nucleic acid molecule encoding said Semaphorin 3 or said functional fragment thereof comprises:
    (a) the nucleotides from 601 to 1206 of SEQ ID NO: 1 and wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding a hydrophilic amino acid;
    (b) the nucleotides from 529 to 1137 of SEQ ID NO: 5 and wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding a hydrophilic amino acid;
    (c) the nucleotides from 842 to 1444 of SEQ ID NO: 9 and wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding a hydrophilic amino acid; or
    (d) the nucleotides from 368 to 982 of SEQ ID NO: 13 and wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding a hydrophilic amino acid.

11. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 10, wherein said Semaphorin 3 or said functional fragment thereof comprises said functional sema domain, wherein said sema domain is selected from an amino acid sequence as shown in:

(a) SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;

(b) SEQ ID NO: 22, wherein the alanine residue corresponding to position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;

(c) SEQ ID NO: 23, wherein the alanine residue corresponding to position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or

(d) SEQ ID NO: 24, wherein the alanine residue corresponding to position 120 of SEQ ID NO: 14 is replaced by a hydrophilic amino acid.

12. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 11, wherein the mutated Semaphorin 3 comprises an amino acid sequence that is selected from the group consisting of SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70 and SEQ ID NO: 72.

13. A fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof that functions as an inhibitor of angiogenesis as defined in any one of claims 1 to 12.

14. The fusion protein of claim 13, wherein said functional sema domain comprises or is a polypeptide as defined in:

(a) SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;

(b) SEQ ID NO: 22, wherein the alanine residue corresponding to position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;

(c) SEQ ID NO: 23, wherein the alanine residue corresponding to position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or

(d) SEQ ID NO: 24, wherein the alanine residue corresponding to position 120 of SEQ ID NO: 14 is replaced by a hydrophilic amino acid.

15. The fusion protein of claim 13 or 14, wherein said fusion protein comprises said mutated Semaphorin 3 or said functional fragment thereof as defined in any one of claims 1 to 12, wherein said fusion protein further comprises:

(a) a stabilizer domain; and/or

(b) a dimerization domain.

16. The fusion protein of claim 15, wherein

(a) said stabilizer domain is a Plexin Semaphorin Integrin (PSI) domain, wherein said PSI domain comprises one or more of the following sequences SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48; and/or

(b) said dimerization domain has a dissociation constant K_D in the range of 10^{-5} M to 10^{-6} M with another such dimerization domain and/or wherein said dimerization domain is selected from the group of a C-terminal IgG constant domain, DARPin and leucine zipper.

17. The fusion protein of claim 16, wherein the IgG constant domain is IgG1 or IgG3.

18. The fusion protein of any one of claims 13 to 17, wherein said fusion protein is encoded by a nucleic acid molecule comprising a nucleic acid sequence having:

a nucleic acid sequence spanning from nucleotides 316 to 1959 of SEQ ID NO: 1 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding a hydrophilic amino acid;

a nucleic acid sequence spanning from nucleotides 247 to 1887 of SEQ ID NO: 5 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding a hydrophilic amino acid;

a nucleic acid sequence spanning from nucleotides 563 to 2197 of SEQ ID NO: 9 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding a hydrophilic amino acid; or

a nucleic acid sequence spanning from nucleotides 41 to 1735 of SEQ ID NO: 13 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding a hydrophilic amino acid.

19. The fusion protein of any one of claims 13 to 18, wherein said fusion protein comprises an amino acid sequence:

spanning from amino acid residues 1 to 548 of SEQ ID NO: 2 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;

spanning from amino acid residues 1 to 547 of SEQ ID NO: 6 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;

spanning from amino acid residues 1 to 565 of SEQ ID NO: 10 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or

spanning from amino acid residues 1 to 545 of SEQ ID NO: 14 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 120 of SEQ ID NO: 14 is replaced by said hydrophilic amino acid.

20. The fusion protein of any one of claims 13 to 19, wherein the fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 18, SEQ ID NO: 76, SEQ ID NO: 78 and SEQ ID NO: 79.

21. An antibody specifically binding to the mutated Semaphorin 3 or the functional fragment thereof as defined in any one of claims 1 to 12 or to the fusion protein as defined in any one of claims 13 to 20.

22. A nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 12 or the fusion protein of any one of claims 13 to 20.

23. A vector comprising the nucleic acid molecule of claim 22.

24. The vector of claim 23, wherein said vector is a gene targeting vector or a gene transfer vector.

25. The vector of claim 23 or 24, wherein said vector is an adeno-associated-virus (AAV) vector.

26. The vector of any one of claims 23 to 25, wherein the adeno-associated-virus is AAV8 vector.

27. A host transformed with a vector of any one of claims 23 to 26 or comprising the nucleic acid molecule of claim 22.

28. The host of claim 27 which is a mammalian cell.

29. The host of claim 27 or 28, wherein the mammalian cell is a HEK cell.

30. The host of any one of claims 27 to 29, wherein the HEK cell is a HEK293-EBNA1 or a HEK293E cell.

31. A method for producing the mutated Semaphorin 3, the functional fragment thereof or the fusion protein encoded by the nucleic acid molecule of claim 22, wherein the method comprises culturing/raising the host of any one of claims 27 to 30 and optionally isolating the produced polypeptide.

32. A polypeptide which is encoded by the nucleic acid molecule of claim 22 and/or which is produced by the method of claim 31.

33. A pharmaceutical composition comprising
    (a) the mutated Semaphorin 3 or the functional fragment thereof according to any one of claims 1 to 12;
    (b) the fusion protein of any one of claims 13 to 20; and/or
    (c) the nucleic acid molecule of claim 22;
    optionally comprising a pharmaceutical excipient.

34. The pharmaceutical composition of claim 33, wherein the pharmaceutical excipient is a pharmaceutical carrier.

35. The pharmaceutical composition of claim 34, wherein the pharmaceutical carrier is a virus.

36. The pharmaceutical composition of claim 35, wherein said virus is an adeno-associated-virus (AAV).

37. The pharmaceutical composition of claim 35 or 36, wherein the adeno-associated-virus is AAV8.

38. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 12, the fusion protein of any one of claims 13 to 20, the nucleic acid molecule of claim 22, the pharmaceutical composition of any one of claims 33 to 37, and/or the polypeptide as produced by claim 31 for use as a medicament.

39. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 12, the fusion protein of any one of claims 13 to 20, the nucleic acid molecule of claim 22, the pharmaceutical composition of any one of claims 33 to 37, and/or the polypeptide as produced by claim 31 for use in the treatment of a cancer, tumor, tumorous disease, angiogenic disorder, vascular retinopathy, blood-brain barrier permeability alterations, neuroinflammatory disorder, osteoporosis, obesity, mycobacterial infection, and/or granuloma.

40. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 12, the fusion protein of any one of claims 13 to 20, the nucleic acid molecule of claim 22, the pharmaceutical composition of any one of claims 33 to 37, and/or the polypeptide as produced by claim 31 for use in the treatment of cancer, tumor and/or tumorous disease, wherein said tumor, cancer, and/or tumorous disease is a solid tumor.

41. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 12 for use according to claim 39 or 40, the fusion protein of any one of claims 13 to 20 for use according to claim 39 or 40, the nucleic acid molecule of claim 22 for use

according to claim 39 or 40, the pharmaceutical composition of any one of claims 33 to 37 for use according to claim 39 or 40, and/or the polypeptide as produced by claim 31 for use according to claim 39 or 40, wherein the cancer is selected from the group consisting of pancreatic cancer, cervical cancer, breast cancer, colon cancer, melanoma, prostate cancer, bladder cancer and tongue cancer.

42. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 12 for use according to any one of claims 39 to 41, the fusion protein of any one of claims 13 to 20 for use according to any one of claims 39 to 41, the nucleic acid molecule of claim 22 for use according to any one of claims 39 to 41, the pharmaceutical composition of any one of claims 33 to 37 for use according to any one of claims 39 to 41, and/or the polypeptide as produced by claim 31 for use according to any one of claims 39 to 41, wherein said tumor, cancer, or tumorous disease is a pancreatic tumor/cancer.

43. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 12 for use according to any one of claims 39 to 42, the fusion protein of any one of claims 13 to 20 for use according to any one of claims 39 to 42, the nucleic acid molecule of claim 22 for use according to any one of claims 39 to 42, the pharmaceutical composition of any one of claims 33 to 37 for use according to any one of claims 39 to 42, and/or the polypeptide as produced by claim 31 for use according to any one of claims 39 to 42, wherein vascular normalization, reduction of tumor growth, reduction of metastatization and/or survival extension is involved.

44. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 12 for use according to any one of claims 39 to 43, the fusion protein of any one of claims 13 to 20 for use according to any one of claims 39 to 43, the nucleic acid molecule of claim 22 for use according to any one of claims 39 to 43, the pharmaceutical composition of any one of claims 33 to 37 for use according to any one of claims 39 to 43, and/or the polypeptide as produced by claim 31 for use according to any one of claims 39 to 43, wherein the mutated Semaphorin 3 or the functional fragment thereof, the fusion protein, the nucleic acid molecule, the pharmaceutical composition and/or the polypeptide is to be administered in

combination with an anti-proliferative drug, an anticancer drug, a cytostatic drug, a cytotoxic drug and/or radiotherapy.

45. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 12 for use according to any one of claims 39 to 44, the fusion protein of any one of claims 13 to 20 for use according to any one of claims 39 to 44, the nucleic acid molecule of claim 22 for use according to any one of claims 39 to 44, the pharmaceutical composition of any one of claims 33 to 37 for use according to any one of claims 39 to 44, and/or the polypeptide as produced by claim 31 for use according to any one of claims 39 to 44, wherein the mutated Semaphorin 3 or the functional fragment thereof, the fusion protein, the nucleic acid molecule, the pharmaceutical composition and/or the polypeptide is to be administered parenterally.

46. Use of the mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 12, the fusion protein of any one of claims 13 to 20, the antibody of claim 21, the nucleic acid molecule of claim 22, the vector of any one of claims 23 to 26, the host of any one of claims 27 to 30, the polypeptide as produced by claim 31, and/or the pharmaceutical composition of any one of claims 33 to 37 as a medicament.

47. A method of treatment for angiogenic disorder, tumorous disease and/or cancer comprising the step of administering to a subject in need of such treatment a pharmaceutical effective amount of the mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 12, the fusion protein of any one of claims 13 to 20, the nucleic acid molecule of claim 22, the vector of any one of claims 23 to 26, the polypeptide as produced by claim 31, and/or the pharmaceutical composition of any one of claims 33 to 37.

48. The method of treatment according to claim 47, wherein the subject is a human.

49. The method of treatment according to claim 47 or 48, wherein the pharmaceutical effective amount is the range of 0.5 to 10 mg/kg of body weight.

Figure 1.

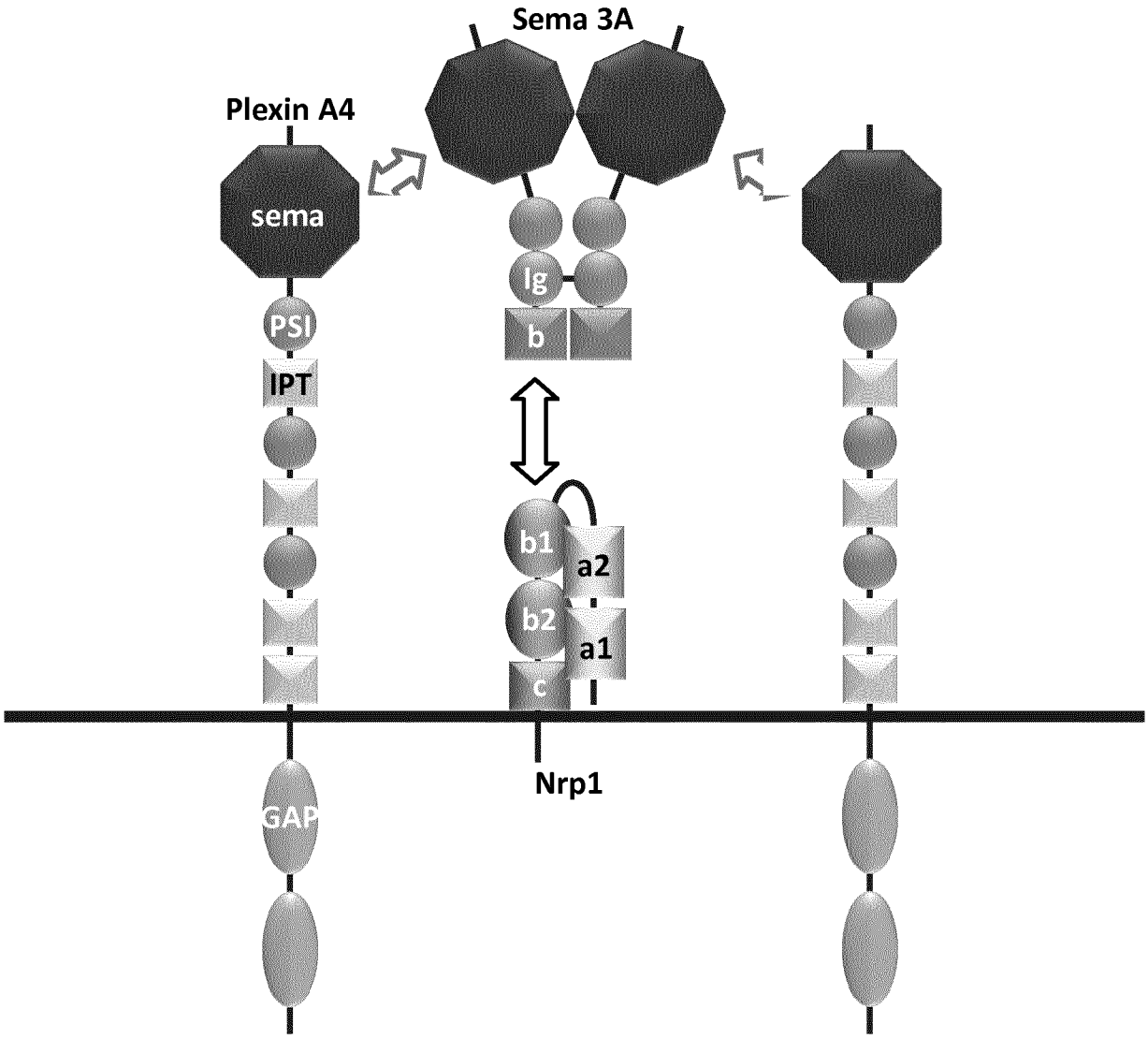

Figure 2.

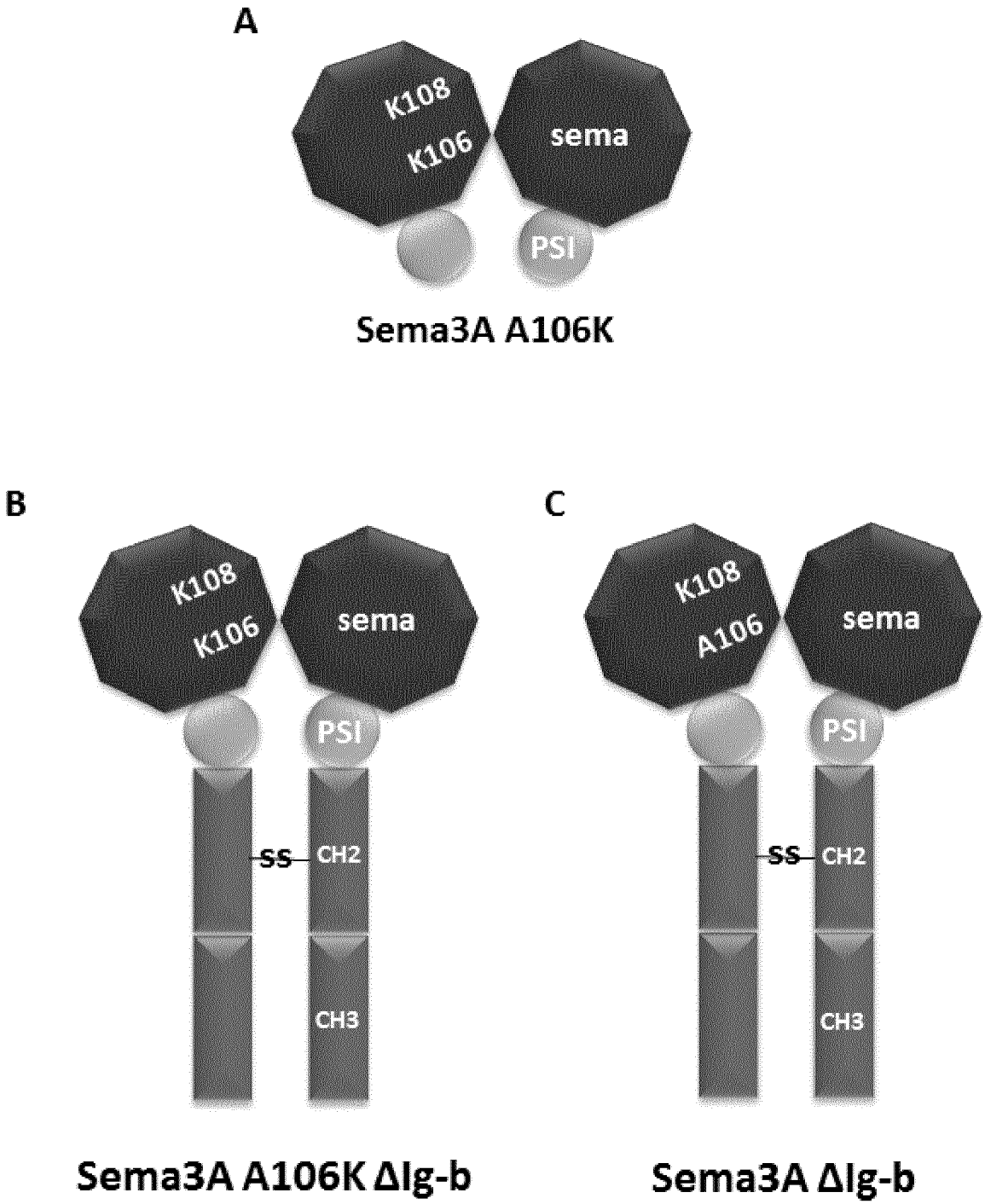
A
K108
K106
sema
PSI
Sema3A A106K
B
K108
K106
sema
PSI
SS
CH2
CH3
Sema3A A106K ΔIg-b
C
K108
A106
sema
PSI
SS
CH2
CH3
Sema3A ΔIg-b

Figure 2 (cont. 1).

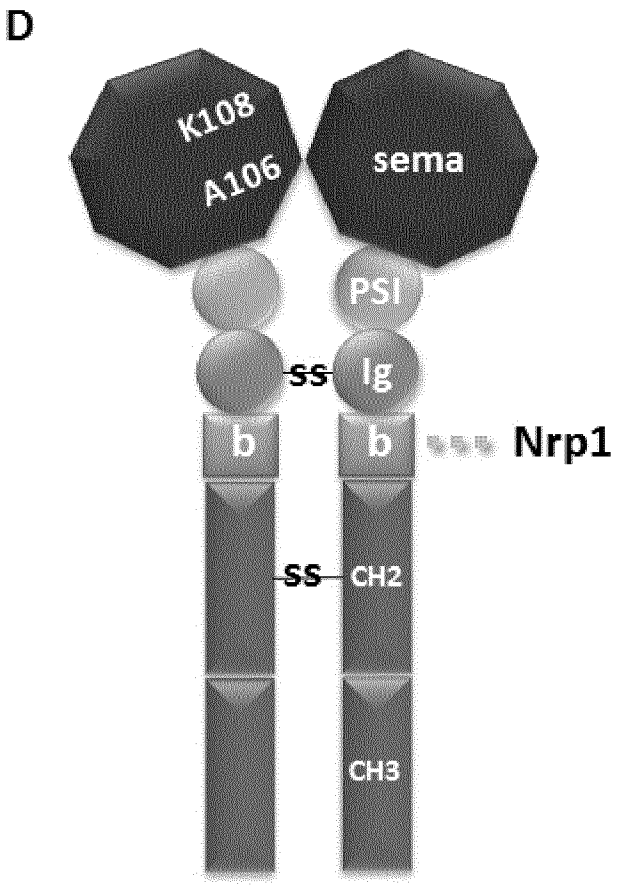

Figure 3.

A

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Sema3A ---------------MGWFTGIACLFWGVLLTARANYANGKNNVPRLKLSYKEMLESNNV 45
SEMA3A ---------------MGWLTRIVCLFWGVLLTARANYQNGKNNVPRLKLSYKEMLESNNV 45
Sema3D MNVTKDENPRSRSQDLHLFHAWMMLIMTVLFLPVTETS--KQNIPRLKLTYKDLLLSNTC 58
SEMA3D MNANKDERLKARSQDFHLFPALMMLSMTMLFLPVTGTL--KQNIPRLKLTYKDLLLSNSC 58
Sema3B ---------------MGRAEAAAMIPG-LALLWVAGLGDTAPNLPRLRLSFQELQARHGV 44
SEMA3B ---------------MGRAGAAAVIPG-LALLWAVGLGSAAPSPPRLRLSFQELQAWHGL 44
Sema3E --------------MAPAGHILTLLLWGHLLELWTPGHSANPSYPRLRLSHKELLELNRT 46
SEMA3E --------------MASAGHIITLLLWGYLLELWTGGHTADTTHPRLRLSHKELLNLNRT 46
Sema3G --------------MDPSAWAICCLLGSLLFHVGIPSPGPSPSVPRLRLSYRDLLSTNRS 46
SEMA3G --------------MAPSAWAICWLLGGLLLHGGSSGPSPGPSVPRLRLSYRDLLSANRS 46
Sema3C ----------------MAFRAICVLVGVFICSICVRGS--SQPQARVYLTFDELRETKTS 42
SEMA3C ----------------MAFRTICVLVGVFICSICVKGS--SQPQARVYLTFDELRETKTS 42
Sema3F -------------MLVTAFILWASLLTGAWPATPIQDQ--LPATPRVRLSFKELKATGTA 45
SEMA3F -------------MLVAGLLLWASLLTGAWPSFPTQDH--LPATPRVRLSFKELKATGTA 45
                              :                  .*: *:. ::

Sema3A ITFNGLANSSSYHTFLLDEERSRLYVGAKDHIFSFNLVNI-KDFQKIVWPVSYTRRDE**CK** 104
SEMA3A ITFNGLANSSSYHTFLLDEERSRLYVGAKDHIFSFDLVNI-KDFQKIVWPVSYTRRDE**CK** 104
Sema3D IPFLGSSEGLDFQTLLLDEERGILLLGAKDHVFLLSLVDLNKNFKKIYWPAAKERVEL**CK** 118
SEMA3D IPFLGSSEGLDFQTLLLDEERGRLLLGAKDHIFLLSLVDLNKNFKKIYWPAAKERVEL**CK** 118
Sema3B RTFR-LERTCCYEALLVDEERGRLFVGAENHVASLSLDNISKRAKKLAWPAPVEWREE**CN** 103
SEMA3B QTFS-LERTCCYQALLVDEERGRLFVGAENHVASLNLDNISKRAKKLAWPAPVEWREE**CN** 103
Sema3E SIFQSPLGFLDLHTMLLDEYQERLFVGGRDLVYSLNLERVSDGYREIYWPSTAVKVEE**CI** 106
SEMA3E SIFHSPFGFLDLHTMLLDEYQERLFVGGRDLVYSLSLERISDGYKEIHWPSTALKMEE**CI** 106
Sema3G AIFLGPRGSLDLQVMYLDEYRDRLFLGSRDALYSLRLDQAWPDPREVLWLPQPGQKVE**CV** 106
SEMA3G AIFLGPQGSLNLQAMYLDEYRDRLFLGGLDALYSLRLDQAWPDPREVLWPPQPGQREE**CV** 106
Sema3C EYFSLSHQQLDYRILLMDEDQDRIYVGSKDHILSLNINNISQEPLSVFWPASTIKVEE**CK** 102
SEMA3C EYFSLSHHPLDYRILLMDEDQDRIYVGSKDHILSLNINNISQEALSVFWPASTIKVEE**CK** 102
Sema3F HFFNFLLNTTDYRILLKDEDHDRMYVGSKDYVLSLDLHDINREPLIIHWAASPQRIEE**CI** 105
SEMA3F HFFNFLLNTTDYRILLKDEDHDRMYVGSKDYVLSLDLHDINREPLIIHWAASPQRIEE**CV** 105
         *          . :  ** :  : :*. : :  : :          : *          *

Sema3A **WAG**KD**D**ILKECANFIKVLEAYNQTHLYACGTGAFHPICTYIEVGHHPEDNIFKLQDSHFEN 164
SEMA3A **WAGKD**ILKECANFIKVLKAYNQTHLYACGTGAFHPICTYIEIGHHPEDNIFKLENSHFEN 164
Sema3D **LAGKD**ANAECANFIRVLQPYNKTHVYVCGTGAFHPLCGYIDLGANKEELIFKLDTHNLES 178
SEMA3D **LAGKD**ANTECANFIRVLQPYNKTHIYVCGTGAFHPICGYIDLGVYKEDIIFKLDTHNLES 178
Sema3B **WAGKD**IGTECMNFVKLLHTYNHTHLLACGTGAFHPTCAFVEVGHRLEEPMLQLDRRKLED 163
SEMA3B **WAGKD**IGTECMNFVKLLHAYNRTHLLACGTGAFHPTCAFVEVGHRAEEPVLRLDPGRIED 163
Sema3E **MKGKD**-ANECANYIRVLHHYNRTHLLTCATGAFDPHCAFIRVGHHSEEPLFHLESHRSER 165
SEMA3E **MKGKD**-AGECANYVRVLHHYNRTHLLTCGTGAFDPVCAFIRVGYHLEDPLFHLESPRSER 165
Sema3G **RKGKD**PLTECANFVRVLQPHNRTHLLACGTGAFQPICTFITVGHRG-EHVLRLDASSVEN 165
SEMA3G **RKGRD**PLTECANFVRVLQPHNRTHLLACGTGAFQPTCALITVGHRG-EHVLHLEPGSVES 165
Sema3C **MAGKD**PTHGCGNFVRVIQTFNRTHLYVCGSGAFSPVCTYLNRGRRSEDQVFMIDS-KCES 161
SEMA3C **MAGKD**PTHGCGNFVRVIQTFNRTHLYVCGSGAFSPVCTYLNRGRRSEDQVFMIDS-KCES 161
Sema3F **LSGKD**GNGECGNFVRLIQPWNRTHLYVCGTGAYNPMCTYVNRGRRAQDYIFYLEPEKLES 165
SEMA3F **LSGKD**VNGECGNFVRLIQPWNRTHLYVCGTGAYNPMCTYVNRGRRAQATPWTQTQAVRGR 165
         *:*    * *::::::.  *:**: .*.:**: * *  :  *
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Figure 3 (cont. 1).

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Sema3A GRGKSPYDPKLLTASLLIDGELYSGTAADFMGRDFAIFRTLG---HHHPIRTEQHDSRWL 221
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Sema3D GRLKCPFDPQQPFASVMTDEHLYSGTASDFLGKDTAFTRSLGLMQDHHSIRTDISEHHWL 238
SEMA3D GRLKCPFDPQQPFASVMTDEYLYSGTASDFLGKDTAFTRSLGPTHDHHYIRTDISEHYWL 238
Sema3B GKGKTPYDPRHRAASVLVGEELYSGVTADLMGRDFTIFRSLG---QNPSLRTEPHDSRWL 220
SEMA3B GKGKSPYDPRHRAASVLVGEELYSGVAADLMGRDFTIFRSLG---QRPSLRTEPHDSRWL 220
Sema3E GRGRCPFDPNSSFVSTLVGNELFAGLYSDYWGRDSAIFRSMG---KLGHIRTEHDDERLL 222
SEMA3E GRGRCPFDPSSSFISTLIGSELFAGLYSDYWSRDAAIFRSMG---RLAHIRTEHDDERLL 222
Sema3G GRGRCPHEPSRPFASTFVGGELYTGLTADFLGREAMIFRSGG---PRPALRSDSD-QSLL 221
SEMA3G GRGRCPHEPSRPFASTFIDGELYTGLTADFLGREAMIFRSGG---PRPALRSDSD-QSLL 221
Sema3C GKGRCSFNPNVNTVSVMINEELFSGMYIDFMGTDAAIFRSLT---KRNAVRTDQHNSKWL 218
SEMA3C GKGRCSFNPNVNTVSVMINEELFSGMYIDFMGTDAAIFRSLT---KRNAVRTDQHNSKWL 218
Sema3F GKGKCPYDPKLDTASALINEELYAGVYIDFMGTDAAIFRTLG---KQTAMRTDQYNSRWL 222
SEMA3F GSRATDGALRPMPTAPRQDYIFYLEPERLESGKGKCPYDPKL---DTASALINEELYAGV 222
       *            :   .  ::       .      .           :     :

Sema3A NDPRFISAHLIPESDNPEDDKVYFFFRENAIDGE-HSGKATHARIGQICKNDFGGHRSLV 280
SEMA3A NDPKFISAHLISESDNPEDDKVYFFFRENAIDGE-HSGKATHARIGQICKNDFGGHRSLV 280
Sema3D NGAKFIGTFPIPDTYNPDDDKIYFFFRESSQEGS-TSDRSILSRVGRVCKNDVGGQRSLI 297
SEMA3D NGAKFIGTFFIPDTYNPDDDKIYFFFRESSQEGS-TSDKTILSRVGRVCKNDVGGQRSLI 297
Sema3B NEPKFVKVFWIPESENPDDDKIYFFFRESAVEAAPAMGRMSVSRVGQICRNDLGGQRSLV 280
SEMA3B NEPKFVKVFWIPESENPDDDKIYFFFRETAVEAAPALGRLSVSRVGQICRNDVGGQRSLV 280
Sema3E KEPKFVGSYMIPDNEDRDDNKMYFFFTEKALEAENN-AHTIYTRVGRLCVNDMGGQRILV 281
SEMA3E KEPKFVGSYMIPDNEDRDDNKVYFFFTEKALEAENN-AHAIYTRVGRLCVNDVGGQRILV 281
Sema3G HEPRFVMAARIPDNSDRDDDKVYFFFSETVPSPDGGPGHVTISRVGRVCVNDAGGQRVLV 281
SEMA3G HDPRFVMAARIPENSDQDNDKVYFFFSETVPSPDGGSNHVTVSRVGRVCVNDAGGQRVLV 281
Sema3C SEPMFVDAHVIPDGTDPNDAKVYFFFKERLTDNN-RSTKQIHSMIARICPNDTGGQRSLV 277
SEMA3C SEPMFVDAHVIPDGTDPNDAKVYFFFKEKLTDNN-RSTKQIHSMIARICPNDTGGLRSLV 277
Sema3F NDPSFIHAELIPDSAERNDDKLYFFFRERSAEA--PQNPAVYARIGRICLNDDGGHCCLV 280
SEMA3F YIDFMGTDAAIFRTLGKQTAMRTDQYNSRWLND--PSFIHAELIPDSAERNDDKLYFFFR 280
           :     *      :       : .   .                  **      :

Sema3A NKWTTFLKARLICSVPGPNGIDTHFDELQDVFLMNSKDPKNPIVYGVFTTSSNIFKGSAV 340
SEMA3A NKWTTFLKARLICSVPGPNGIDTHFDELQDVFLMNFKDPKNPVVYGVFTTSSNIFKGSAV 340
Sema3D NKWTTFLKARLICSIPGSDGADTHFDELQDIYLLPTRDERNPVVYGVFTTTSSIFKGSAV 357
SEMA3D NKWTTFLKARLICSIPGSDGADTYFDELQDIYLLPTRDERNPVVYGVFTTTSSIFKGSAV 357
Sema3B NKWTTFLKARLVCSVPGVEG-DTHFDQLQDVFLLSSRDRQTPLLYAVFSTSSGVFQGSAV 339
SEMA3B NKWTTFLKARLVCSVPGVEG-DTHFDQLQDVFLLSSRDHRTPLLYAVFSTSSSIFQGSAV 339
Sema3E NKWSTFLKARLVCSVPGMNGIDTYFDELEDVFLLPTRDPKNPVIFGLFNTTSNIFRGHAV 341
SEMA3E NKWSTFLKARLVCSVPGMNGIDTYFDELEDVFLLPTRDHKNPVIFGLFNTTSNIFRGHAI 341
Sema3G NKWSTFLKARLVCSVPGPGGAETHFDQLEDVFLLWPKAGKSLEVYALFSTVSAVFQGFAV 341
SEMA3G NKWSTFLKARLVCSVPGPGGAETHFDQLEDVFLLWPKAGKSLEVYALFSTVSAVFQGFAV 341
Sema3C NKWTTFLKARLVCSVTDEDGPETHFDELEDVFLLETDNPRTTLVYGIFTTSSSVFKGSAV 337
SEMA3C NKWTTFLKARLVCSVTDEDGPETHFDELEDVFLLETDNPRTTLVYGIFTTSSSVFKGSAV 337
Sema3F NKWSTFLKARLVCSVPGEDGIETHFDELQDVFVQQTQDVRNPVIYAVFTSSGSVFRGSAV 340
SEMA3F ERSAEAPQSPAVYARIGRICLNDDGGHCCLVNKWSTFLKARLVCSVPGEDGIETHFDELQ 340
       :: :   ::  : :  .    :   ..   :                       . .
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Figure 3 (cont. 2).

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Sema3D CVYSMADIRAVFNGPYAHKESADHRWVQYDGRIPYPRPGTCPSKTYDP---LIKSTRDFP 414
SEMA3D CVYSMADIRAVFNGPYAHKESADHRWVQYDGRIPYPRPGTCPSKTYDP---LIKSTRDFP 414
Sema3B CVYSMNDVRRAFLGPFAHKEGPTHQWVSYQGRVPYPRPGMCPSKTFG----TFSSTKDFP 395
SEMA3B CVYSMNDVRRAFLGPFAHKEGPMHQWVSYQGRVPYPRPGMCPSKTFG----TFSSTKDFP 395
Sema3E CVYHMSSIREAFNGPYAHKEGPEYHWSLYEGKVPYPRPGSCASKVNG--GK-YGTTKDYP 398
SEMA3E CVYHMSSIRAAFNGPYAHKEGPEYHWSVYEGKVPYPRPGSCASKVNG--GR-YGTTKDYP 398
Sema3G CVYHMVDIWEVFNGPFAHRDGPQHQWGPYGGKVPFPRPGVCPSKMTAQPGRPFGSTKDYP 401
SEMA3G CVYHMADIWEVFNGPFAHRDGPQHQWGPYGGKVPFPRPGVCPSKMTAQPGRPFGSTKDYP 401
Sema3C CVYHLSDIQTVFNGPFAHKEGPNHQLISYQGRIPYPRPGTCPGGAFTP---NMRTTKDFP 394
SEMA3C CVYHLSDIQTVFNGPFAHKEGPNHQLISYQGRIPYPRPGTCPGGAFTP---NMRTTKEFP 394
Sema3F CVYSMADIRMVFNGPFAHKEGPNYQWMPFSGKMPYPRPGTCPGGTFTP---SMKSTKDYP 397
SEMA3F DVFVQQTQDVRNPVIYAVFTSSGSVFRGSAVCVYSMADIRMVFNGPFA---HKEGPNYQW 397
        ::            :*   ..          :                    ..

Sema3A DDVITFARSHPAMYNPVFPINNRPIMIKTDVNYQFTQIVVDRVDAEDGQYDVMFIGTDVG 456
SEMA3A DDVITFARSHPAMYNPVFPMNNRPIVIKTDVNYQFTQIVVDRVDAEDGQYDVMFIGTDVG 456
Sema3D DDVISFIRRHPVMYKSVYPVAGAPTFKRINVDYRLTQIVVDHVVAEDGQYDVMFLGTDIG 474
SEMA3D DDVISFIKRHSVMYKSVYPVAGGPTFKRINVDYRLTQIVVDHVIAEDGQYDVMFLGTDIG 474
Sema3B DDVIQFARNHPLMYNPVLPMGGRPLFLQVGAGYTFTQIAADRVAAADGHYDVLFIGTDVG 455
SEMA3B DDVIQFARNHPLMYNSVLPTGGRPLFLQVGANYTFTQIAADRVAAADGHYDVLFIGTDVG 455
Sema3E DDAIRFARMHPLMYQPIKPVHKKPILVKTDGKYNLRQLAVDRVEAEDGQYDVLFIGTDTG 458
SEMA3E DDAIRFARSHPLMYQAIKPAHKKPILVKTDGKYNLKQIAVDRVEAEDGQYDVLFIGTDNG 458
Sema3G DEVLQFVRDHPLMFQPVRPRRGRPVLVKTHLAQRLRQIVVDRVEAEDGTYDVIFLGTDSG 461
SEMA3G DEVLQFARAHPLMFWPVRPRHGRPVLVKTHLAQQLHQIVVDRVEAEDGTYDVIFLGTDSG 461
Sema3C DDVVTFIRNHPLMYNSIYPIHRRPLIVRIGTDYKYTKIAVDRVNAADGRYHVLFLGTDRG 454
SEMA3C DDVVTFIRNHPLMYNSIYPIHKRPLIVRIGTDYKYTKIAVDRVNAADGRYHVLFLGTDRG 454
Sema3F DEVINFMRTHPLMYQAVYPLQRRPLVVRTGAPYRLTTVAVDQVDAADGRYEVLFLGTDRG 457
SEMA3F MPFSGKMPYPRPGTCPGGTFTPSMKSTKDYPDEVINFMRSHPLMYQAVYPLQRRPLVVRT 457
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Sema3A TVLKVVSVPKETWHDLEEVLLEEMTVFREPTTISAMELSTKQQQLYIGSTAGVAQLPLHR 516
SEMA3A TVLKVVSIPKETWYDLEEVLLEEMTVFREPTAISAMELSTKQQQLYIGSTAGVAQLPLHR 516
Sema3D TVLKVVSISKEKWN-MEEVVLEELQVFKHPTAILNMELSLKQQQLYVGSWDGLVQLSLHR 533
SEMA3D TVLKVVSISKEKWN-MEEVVLEELQIFKHSSIILNMELSLKQQQLYIGSRDGLVQLSLHR 533
Sema3B TVLKVISVPKGSRPNSEGLLLEELQVFEDSAAITSMQISSKRQQLYIASRSAVAQIALHR 515
SEMA3B TVLKVISVPKGSRPSAEGLLLEELHVFEDSAAVTSMQISSKRHQLYVASRSAVAQIALHR 515
Sema3E IVLKVITIYNQETEWMEEVILEELQIFKDPAPIISMEISSKRQQLYIGSASAVAQVRFHH 518
SEMA3E IVLKVITIYNQEMESMEEVILEELQIFKDPVPIISMEISSKRQQLYIGSASAVAQVRFHH 518
Sema3G SVLKVIALQGGGLTEPEEVVLEELQVFKVPTPITEMEISVKRQTLYVGSPLGVARLQLHQ 521
SEMA3G SVLKVIALQAGGSAEPEEVVLEELQVFKVPTPITEMEISVKRQMLYVGSRLGVAQLRLHQ 521
Sema3C TVQKVVVLPTNSSA-SGELILEELEVFKNHVPITTMKISSKKQQLYVSSNEGVSQVSLHR 513
SEMA3C TVQKVVVLPTNNSV-SGELILEELEVFKNHAPITTMKISSKKQQLYVSSNEGVSQVSLHR 513
Sema3F TVQKVIVLPKDDQE-VEELMLEEVEVFKEPAPVKTMTISSKRQQLYVASAVGVTHLSLHR 516
SEMA3F GAPYRLTTIAVDQV-DAADGRYEVLFLGTDRGTVQKVIVLPKDDQELEELMLEEVEVFKD 516
        .   :               *: .:          :   :.   : .        ::
```

Figure 3 (cont. 3).

```
Sema3A CDIYGKACAECCLARDPYCAWDGSSCSRYFPT---AKRRTRRQDIRNGDPLTHCSDLQHH 573
SEMA3A CDIYGKACAECCLARDPYCAWDGSACSRYFPT---AKRRTRRQDIRNGDPLTHCSDLHHD 573
Sema3D CDTYGKACADCCLARDPYCAWDGNACSRYAPT---SKRRARRQDVKYGDPITQCWDIEDS 590
SEMA3D CDTYGKACADCCLARDPYCAWDGNACSRYAPT---SKRRARRQDVKYGDPITQCWDIEDS 590
Sema3B CTALGRACAECCLARDPYCAWDGSACTRFQPT---AKRRFRRQDIRNGDPSTLCS----G 568
SEMA3B CAAHGRVCTECCLARDPYCAWDGVACTRFQPS---AKRRFRRQDVRNGDPSTLCS----G 568
Sema3E CDMYGSACADCCLARDPYCAWDGISCSRYYPTGAHAKRRFRRQDVRHGNAAQQCFGQQFV 578
SEMA3E CDMYGSACADCCLARDPYCAWDGISCSRYYPTGTHAKRRFRRQDVRHGNAAQQCFGQQFV 578
Sema3G CETYGSACAECCLARDPYCAWDGTACARYRPS--SGKRRFRRQDIRHGNPAVQCLGQGQS 579
SEMA3G CETYGTACAECCLARDPYCAWDGASCTHYRPS--LGKRRFRRQDIRHGNPALQCLGQSQE 579
Sema3C CHIYGTACADCCLARDPYCAWDGHSCSRFYPT---GKRRSRRQDVRHGNPLTQCRG--FN 568
SEMA3C CHIYGTACADCCLARDPYCAWDGHSCSRFYPT---GKRRSRRQDVRHGNPLTQCRG--FN 568
Sema3F CQAYGAACADCCLARDPYCAWDGQACSRYTAS---SKRRSRRQDVRHGNPIRQCRG--FN 571
SEMA3F PAPVKTMTISSKRQQLYVASAVGVTHLSLHRC---QAYGAACADCCLARDPYCAWD--GQ 571
                ..   :   .:  * :                  *   .     .

Sema3A DNHHGPSLEERIIYGVENSSTFLECSPKSQRALVYWQFQRRNEDRKEEIRMGDHIIRTEQ 633
SEMA3A NHHGHSPEERIIYGVENSSTFLECSPKSQRALVYWQFQRRNEERKEEIRVDDHIIRTDQG 633
Sema3D ISH-ETADEKVIFGIEFNSTFLECIPKSQQASVEWYIQRSGDEHREELKPDERIIKTDYG 649
SEMA3D ISH-ETADEKVIFGIEFNSTFLECIPKSQQATIKWYIQRSGDEHREELKPDERIIKTEYG 649
Sema3B DSSHSVLLEKKVLGVESGSAFLECEPRSLQAHVQWTFQGAGEAAHTQVLAEERVERTARG 628
SEMA3B DSSRPALLEHKVFGVEGSSAFLECEPRSLQARVEWTFQRAGVTAHTQVLAEERTERTARG 628
Sema3E GDALDRTEERLAYGIESNSTLLECTPRSLQAKVIWFVQKGRDVRKEEVKTDDRVVKMDLG 638
SEMA3E GDALDKTEEHLAYGIENNSTLLECTPRSLQAKVIWFVQKGRETRKEEVKTDDRVVKMDLG 638
Sema3G QNKAASGLMTRVFGTEHNSTFLECLPKSPQAAVRWFLQRPGDKGTDQVKTDERVVQTAQG 639
SEMA3G EEAVGLVAATMVYGTEHNSTFLECLPKSPQAAVRWLLQRPGDEGPDQVKTDERVLHTERG 639
Sema3C LKAYRNAAEIVQYGVRNNSTFLECAPKSPQASIKWLLQKDK-DRRKEVKLNERIIATSQG 627
SEMA3C LKAYRNAAEIVQYGVKNNTTFLECAPKSPQASIKWLLQKDK-DRRKEVKLNERIIATSQG 627
Sema3F SNANKNAVESVQYGVAGSAAFLECQPRSPQATVKWLFQRDPSDRRREIRAEDRFLRTEQG 631
SEMA3F ACSRYTASSKRRSRRQDVRHGNPIRQCRGFNSNANKNAVESVQYGVAGSAAFLECQPRSP 631


Sema3A GLLLRSLQKKDSGNYLCHAVEHGFMQTLLKVTLEVIDTEHLEELLHKDDDGD--GSKIKE 691
SEMA3A LLLRSLQQKDSGNYLCHAVEHGFIQTLLKVTLEVIDTEHLEELLHKDDDGDG--SKTKEM 691
Sema3D LLIRSLQKKDSGMYYCKAQEHTFIHTIVKLTLNVIENEQMENTQRAEYQEG--------Q 701
SEMA3D LLIRSLQKKDSGMYYCKAQEHTFIHTIVKLTLNVIENEQMENTQRAEHEEG--------K 701
Sema3B LLLRGLRRQDSGVYLCVAVEQGFSQPLRRLVLHVLSAAQAERLARAEEAAA--------- 679
SEMA3B LLLRRLRRRDSGVYLCAAVEQGFTQPLRRLSLHVLSATQAERLARAEEAAP--------- 679
Sema3E LLFLRVRKSDAGTYFCQTVEHNFVHTVRKITLEVVEEHKVEGMFHKDHEEERHHKMPCPP 698
SEMA3E LLFLRLHKSDAGTYFCQTVEHSFVHTVRKITLEVVEEEKVEDMFNKDDEEDRHHRMPCPA 698
Sema3G LLFRRLSRHDAGNYTCTTLEHGFSQTVVRFALEVIAAVQLDSLFLRESRLE----EPSAW 695
SEMA3G LLFRRLSRFDAGTYTCTTLEHGFSQTVVRLALVVIVASQLDNLFPPEPKPE----EPPAR 695
Sema3C LLIRSVQDSDQGLYHCIATENSFKQTIAKINFKVLDSEMV--AVVTDKWSPWT------- 678
SEMA3C LLIRSVQGSDQGLYHCIATENSFKQTIAKINFKVLDSEMV--AVVTDKWSPWT------- 678
Sema3F LLLRALQLGDRGLYSCTATENNFKHIVTRVQLHVLGRDAVHAALFPPLAVSVP------- 684
SEMA3F QATVKWLFQRDPGDRRREIRAEDRFLRTEQGLLLRALQLSDRGLYSCTATENN------- 684
```

Figure 3 (cont. 4).

```
Sema3A MSSSMTPSQKVWYRDFMQLINHP---NLNTMDEFCEQVWKR--------DRKQRRQRPGH 740
SEMA3A SNSMTPSQKVWYRDFMQLINHPN---LNTMDEFCEQVWKRD--------RKQRRQRPGHT 740
Sema3D VKDLLAESRLRYKDYIQILSSP----NFSLDQYCEQMWYKE--------KRRQRNK---- 745
SEMA3D VKDLLAESRLRYKDYIQILSSP----NFSLDQYCEQMWHRE--------KRRQRNK---- 745
Sema3B --PAPPGPKLWYRDFLQLVEPGGGGGANSLRMCRPQPGHHS--------VAADSRRK--- 726
SEMA3B --AAPPGPKLWYRDFLQLVEPGGGGSANSLRMCRPQPALQS--------LPLESRRK--- 726
Sema3E LSGMSQGTKPWYKEFLQLIGYSN---FQRVEEYCEKVWCTD--------KKRKKLKM--- 744
SEMA3E QSSISQGAKPWYKEFLQLIGYSN---FQRVEEYCEKVWCTD--------RKRKKLKM--- 744
Sema3G GSLASASPKTWYKDILQLTGFAN---LPRVDEYCERVWCRGVGERSGSFRGKGKQAK--- 749
SEMA3G GGLASTPPKAWYKDILQLIGFAN---LPRVDEYCERVWCRGTTECSGCFRSRSRGKQ--- 749
Sema3C WAGSVRALPFHPKDILGAFSHSE---MQLINQYCKDTRQQQ--------QLGEEPQK--- 724
SEMA3C WASSVRALPFHPKDIMGAFSHSE---MQMINQYCKDTRQQH--------QQGDESQK--- 724
Sema3F PPPGTGPPTPPYQELAQLLAQPE---VGLIHQYCQGYWRHV--------PPRPREAP--- 730
SEMA3F FKHVVTRVQLHVLGRDAVHAALF---PPLSMSAPPPPGAGP--------PTPPYQEL--- 730


Sema3A SQGSSNKWKHMQESKKGRNRRTHEFERAPRSV------------------------ 772
SEMA3A PGNSNKWKHLQENKKGRNRRTHEFERAPRSV------------------------ 771
Sema3D --GSPKWKHMQEMKKKRNRRHHRDLDELQRSVAT---------------------- 777
SEMA3D --GGPKWKHMQEMKKKRNRRHHRDLDELPRAVAT---------------------- 777
Sema3B --GRNRRMHVSELRAERGPRSAAHW-------------------------------- 749
SEMA3B --GRNRRTHAPEPRAERGPRSATHW-------------------------------- 749
Sema3E --SPSKWKYANPQEKRLRSKAEHFRLPRHTLLS----------------------- 775
SEMA3E --SPSKWKYANPQEKKLRSKPEHYRLPRHTLDS----------------------- 775
Sema3G --GKSWAGLELGKKMKSRVLAEHNRTPREVEAT---------------------- 780
SEMA3G --ARGKSWAGLELGKKMKSRVHAEHNRTPREVEAT--------------------- 782
Sema3C --MRGDYGKLKALINSRKSRNRRNQLPES---------------------------- 751
SEMA3C --MRGDYGKLKALINSRKSRNRRNQLPES---------------------------- 751
Sema3F --GALRPPELQDQKKPRNRRHHPPDT------------------------------- 754
SEMA3F --AQLLAQPEVGLIHQYCQGYWRHVPPSPREAPGAPRSPEPQDQKKPRNRRHHPPDT 785
```

Figure 3 (cont. 5).

B

```
SEMA3A -------------MGWLTRIVCLFWGVLLTARANYQNGKNNVPRLKLSYKEMLESNNVIT 47
SEMA3B -------------MGRAG-AAAVIPGLALLWAVGLGSAAPSPPRLRLSFQELQAWHGLQT 46
SEMA3D MNANKDERLKARSQDFHLFPALMMLSMTMLFLPVTGTLKQNIPRLKLTYKDLLLSNSCIP 60
SEMA3C ---------------MAFRTICVLVGVFICSICVKGSSQPQ-ARVYLTFDELRETKTSEY 44
                              :: .: :       .   . .*: *::.::   :

SEMA3A FNGLANSSSYHTFLLDEERSRLYVGAKDHIFSFDLVNI-KDFQKIVWPVSYTRRDECKWA 106
SEMA3B FS-LERTCCYQALLVDEERGRLFVGAENHVASLNLDNISKRAKKLAWPAPVEWREECNWA 105
SEMA3D FLGSSEGLDFQTLLLDEERGRLLLGAKDHIFLLSLVDLNKNFKKIYWPAAKERVELCKLA 120
SEMA3C FSLSHHPLDYRILLMDEDQDRIYVGSKDHILSLNINNISQEALSVFWPASTIKVEECKMA 104
       *    .   :: :*:**::.*: :*:::*:  :.: :: :   .: **..    : *: *

SEMA3A GKDILKECANFIKVLKAYNQTHLYACGTGAFHPICTYIEIGHHPEDNIFKLENSHFENGR 166
SEMA3B GKDIGTECMNFVKLLHAYNRTHLLACGTGAFHPTCAFVEVGHRAEEPVLRLDPGRIEDGK 165
SEMA3D GKDANTECANFIRVLQPYNKTHIYVCGTGAFHPICGYIDLGVYKEDIIFKLDTHNLESGR 180
SEMA3C GKDPTHGCGNFVRVIQTFNRTHLYVCGSGAFSPVCTYLNRGRRSEDQVFMIDS-KCESGK 163
       ***    * **::::::.:*:**: .**:*** * * ::: *   *: :: ::  . *.*:

SEMA3A GKSPYDPKLLTASLLIDGELYSGTAADFMGRDFAIFRTLG---HHHPIRTEQHDSRWLND 223
SEMA3B GKSPYDPRHRAASVLVGEELYSGVAADLMGRDFTIFRSLG---QRPSLRTEPHDSRWLNE 222
SEMA3D LKCPFDPQQPFASVMTDEYLYSGTASDFLGKDTAFTRSLGPTHDHHYIRTDISEHYWLNG 240
SEMA3C GRCSFNPNVNTVSVMINEELFSGMYIDFMGTDAAIFRSLT---KRNAVRTDQHNSKWLSE 220
        :..::*.   .*:: .  *:**   *::* * :: *:*    .:  :**:  :  **.

SEMA3A PKFISAHLISESDNPEDDKVYFFFRENAIDGEHS-GKATHARIGQICKNDFGGHRSLVNK 282
SEMA3B PKFVKVFWIPESENPDDDKIYFFFRETAVEAAPALGRLSVSRVGQICRNDVGGQRSLVNK 282
SEMA3D AKFIGTFFIPDTYNPDDDKIYFFFRESSQEGSTS-DKTILSRVGRVCKNDVGGQRSLINK 299
SEMA3C PMFVDAHVIPDGTDPNDAKVYFFFKEKLTDNNRS-TKQIHSMIARICPNDTGGLRSLVNK 279
       . *: .. *.:  :*:* *:****:*.  :   :  :   : :.::* ** ** ***:**

SEMA3A WTTFLKARLICSVPGPNGIDTHFDELQDVFLMNFKDPKNPVVYGVFTTSSNIFKGSAVCM 342
SEMA3B WTTFLKARLVCSVPGVEG-DTHFDQLQDVFLLSSRDHRTPLLYAVFSTSSSIFQGSAVCV 341
SEMA3D WTTFLKARLICSIPGSDGADTYFDELQDIYLLPTRDERNPVVYGVFTTTSSIFKGSAVCV 359
SEMA3C WTTFLKARLVCSVTDEDGPETHFDELEDVFLLETDNPRTTLVYGIFTTSSSVFKGSAVCV 339
       *********:**:.. :* :*:**:*:*::*:   : :..::*.:*:*:*.:*:*****:

SEMA3A YSMSDVRRVFLGPYAHRDGPNYQWVPYQGRVPYPRPGTCPSKTFG-GFDSTKDLPDDVIT 401
SEMA3B YSMNDVRRAFLGPFAHKEGPMHQWVSYQGRVPYPRPGMCPSKTFG-TFSSTKDFPDDVIQ 400
SEMA3D YSMADIRAVFNGPYAHKESADHRWVQYDGRIPYPRPGTCPSKTYDPLIKSTRDFPDDVIS 419
SEMA3C YHLSDIQTVFNGPFAHKEGPNHQLISYQGRIPYPRPGTCPGGAFTPNMRTTKEFPDDVVT 399
       * : *:: .* **:**::.. :: : *:**:****** **. ::   : :*:::****:

SEMA3A FARSHPAMYNPVFPMNNRPIVIKTDVNYQFTQIVVDRVDAEDGQYDVMFIGTDVGTVLKV 461
SEMA3B FARNHPLMYNSVLPTGGRPLFLQVGANYTFTQIAADRVAAADGHYDVLFIGTDVGTVLKV 460
SEMA3D FIKRHSVMYKSVYPVAGGPTFKRINVDYRLTQIVVDHVIAEDGQYDVMFLGTDIGTVLKV 479
SEMA3C FIRNHPLMYNSIYPIHKRPLIVRIGTDYKYTKIAVDRVNAADGRYHVLFLGTDRGTVQKV 459
       * : *. **:.: *    * . : ..:*  *:*..*:* * **:*.*:*:*** *** **

SEMA3A VSIPKETWYDLEEVLLEEMTVFREPTAISAMELSTKQQQLYIGSTAGVAQLPLHRCDIYG 521
SEMA3B ISVPKGSRPSAEGLLLEELHVFEDSAAVTSMQISSKRHQLYVASRSAVAQIALHRCAAHG 520
SEMA3D VSISKEKWN-MEEVVLEELQIFKHSSIILNMELSLKQQQLYIGSRDGLVQLSLHRCDTYG 538
SEMA3C VVLP-TNNSVSGELILEELEVFKNHAPITTMKISSKKQQLYVSSNEGVSQVSLHRCHIYG 518
       : :.  .      ::***: :*.. : :  *::* *::***:.*  .: *:.****  :*
```

Figure 3 (cont. 6).

```
SEMA3A KACAECCLARDPYCAWDGSACSRYFPTAKRRTRRQDIRNGDPLTHCSDLHHDNHHGHSPE 581
SEMA3B RVCTECCLARDPYCAWDGVACTRFQPSAKRRFRRQDVRNGDPSTLCS----GDSSRPALL 576
SEMA3D KACADCCLARDPYCAWDGNACSRYAPTSKRRARRQDVKYGDPITQCWD-IEDSISHETAD 597
SEMA3C TACADCCLARDPYCAWDGHSCSRFYPTGKRRSRRQDVRHGNPLTQCRG--FNLKAYRNAA 576
        .*::************** :*:*: *:.*** ****:: *:* * *     .

SEMA3A ERIIYGVENSSTFLECSPKSQRALVYWQFQRRNEERKEEIRVDDHIIRTDQGLLLRSLQQ 641
SEMA3B EHKVFGVEGSSAFLECEPRSLQARVEWTFQRAGVTAHTQVLAEERTERTARGLLLRRLRR 636
SEMA3D EKVIFGIEFNSTFLECIPKSQQATIKWYIQRSGDEHREELKPDERIIKTEYGLLIRSLQK 657
SEMA3C EIVQYGVKNNTTFLECAPKSPQASIKWLLQK-DKDRRKEVKLNERIIATSQGLLIRSVQG 635
       *   :*:: .::**** *:* :* : * :*: .   : ::  :::   *  ***:* ::

SEMA3A KDSGNYLCHAVEHGFIQTLLKVTLEVIDTEHLEELLHKDDDGDGSKTKEMSNSMTPSQKV 701
SEMA3B RDSGVYLCAAVEQGFTQPLRRLSLHVLSATQAERLAR---------AEEAAPAAPPGPKL 687
SEMA3D KDSGMYYCKAQEHTFIHTIVKLTLNVIENEQMENTQR------AEHEEGKVKDLLAESRL 711
SEMA3C SDQGLYHCIATENSFKQTIAKINFKVLDSEMVAVVTD-------KWSPWTWASSVRALPF 688
        *.* * * * *: * :.: ::.:.*:.                          .

SEMA3A WYRDFMQLINHPNLNTMDEFCEQVWKRDRKQRRQRPGHTPGNSNKWKHLQENKKGRNRRT 761
SEMA3B WYRDFLQLVEPGGGGSAN---------SLRMCRPQPALQS------LPLESRRKGRNRRT 732
SEMA3D RYKDYIQILSSPN-FSLDQYCEQMWHREKRRQRNKGGPKW------KHMQEMKKKRNRRH 764
SEMA3C HPKDIMGAFSHSEMQMINQYCK-----DTRQQHQQGDESQKMRGDYGKLKALINSRKSRN 743
         :* :  ..       :         . :  : :             ::   : *: *

SEMA3A HE----FERAPRSV--- 771
SEMA3B HAPEPRAERGPRSATHW 749
SEMA3D HR---DLDELPRAVAT- 777
SEMA3C RR-----NQLPES---- 751
       :      :. *.:
```

C

```
SEMA3A: 527-539
SEMA3B: 526-538
SEMA3D: 544-556
SEMA3C: 524-536
```

```
SEMA3A KACAE**CCLARDPYCAWDG**SACSRYFPTAKRRTRRQDIRNGDPLTHCSDLHHDNHHGHSPE 581
SEMA3B RVCTE**CCLARDPYCAWDG**VACTRFQPSAKRRFRRQDVRNGDPSTLCS----GDSSRPALL 576
SEMA3D KACAD**CCLARDPYCAWDG**NACSRYAPTSKRRARRQDVKYGDPITQCWD-IEDSISHETAD 597
SEMA3C TACAD**CCLARDPYCAWDG**HSCSRFYPTGKRRSRRQDVRHGNPLTQCRG--FNLKAYRNAA 576
```

Figure 4.

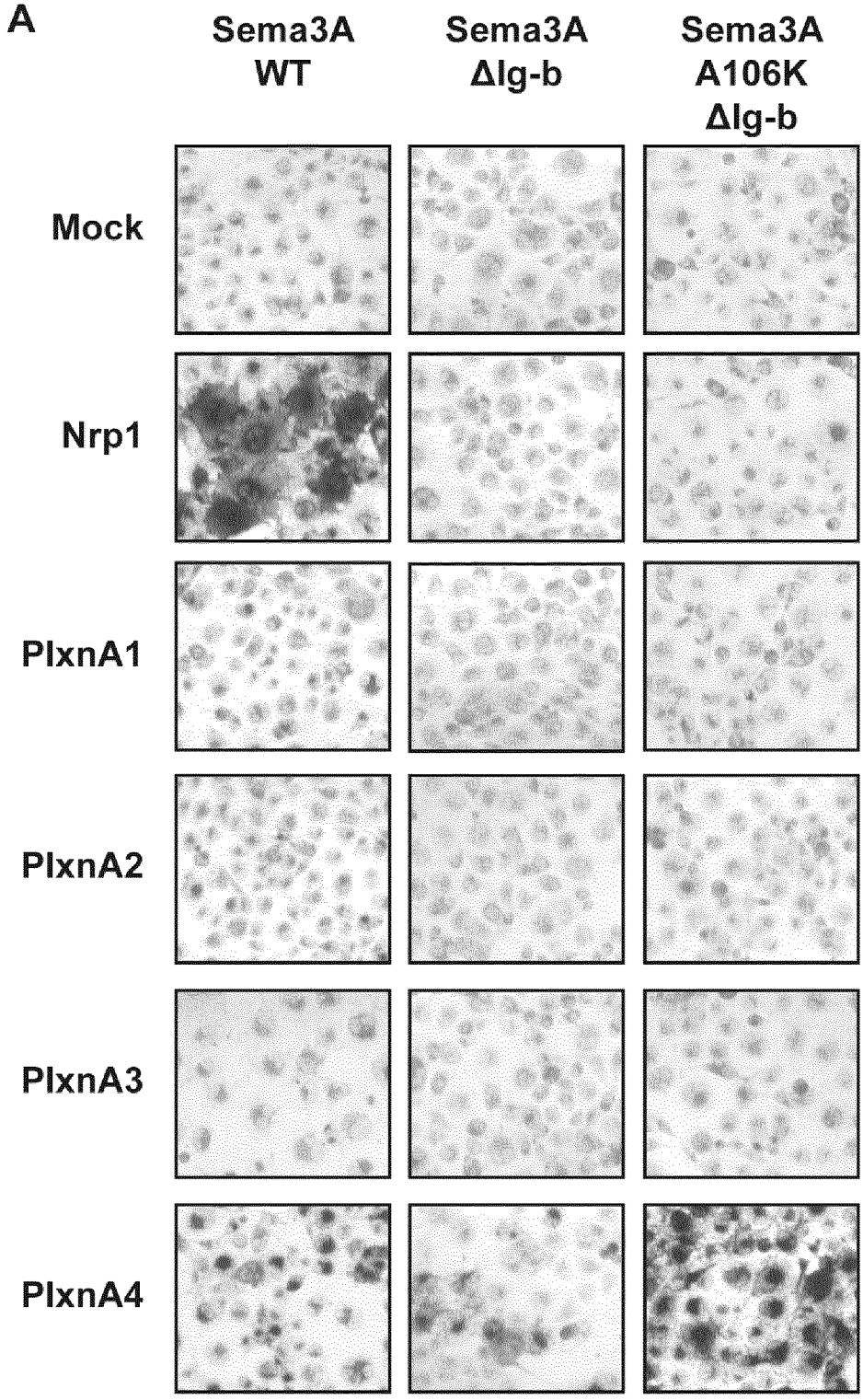
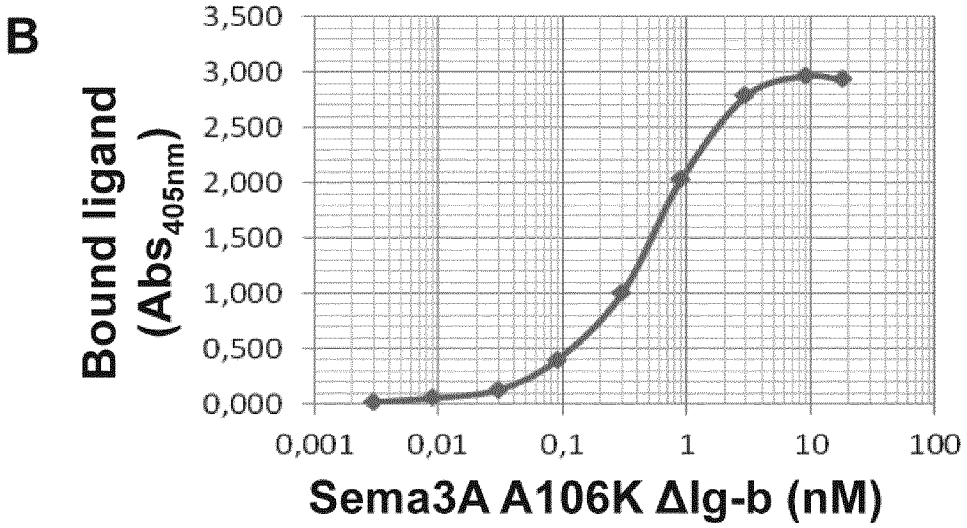
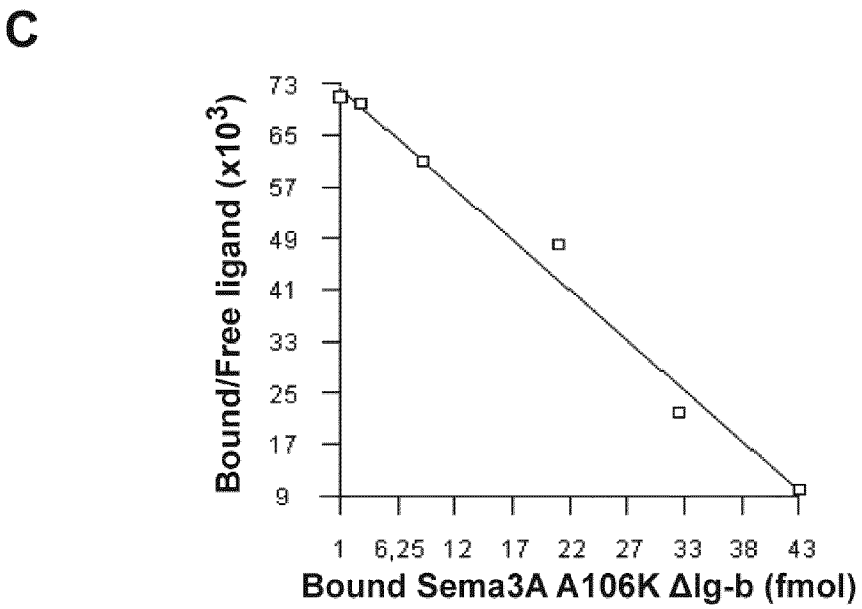

A
Sema3A WT
Sema3A Δlg-b
Sema3A A106K Δlg-b
Mock
Nrp1
PlxnA1
PlxnA2
PlxnA3
PlxnA4

Figure 4 (cont. 1).





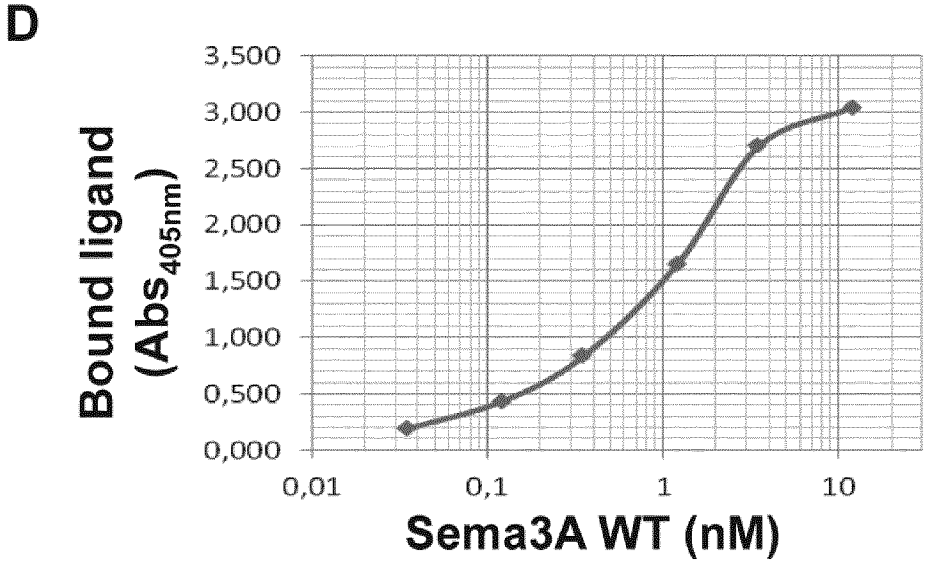
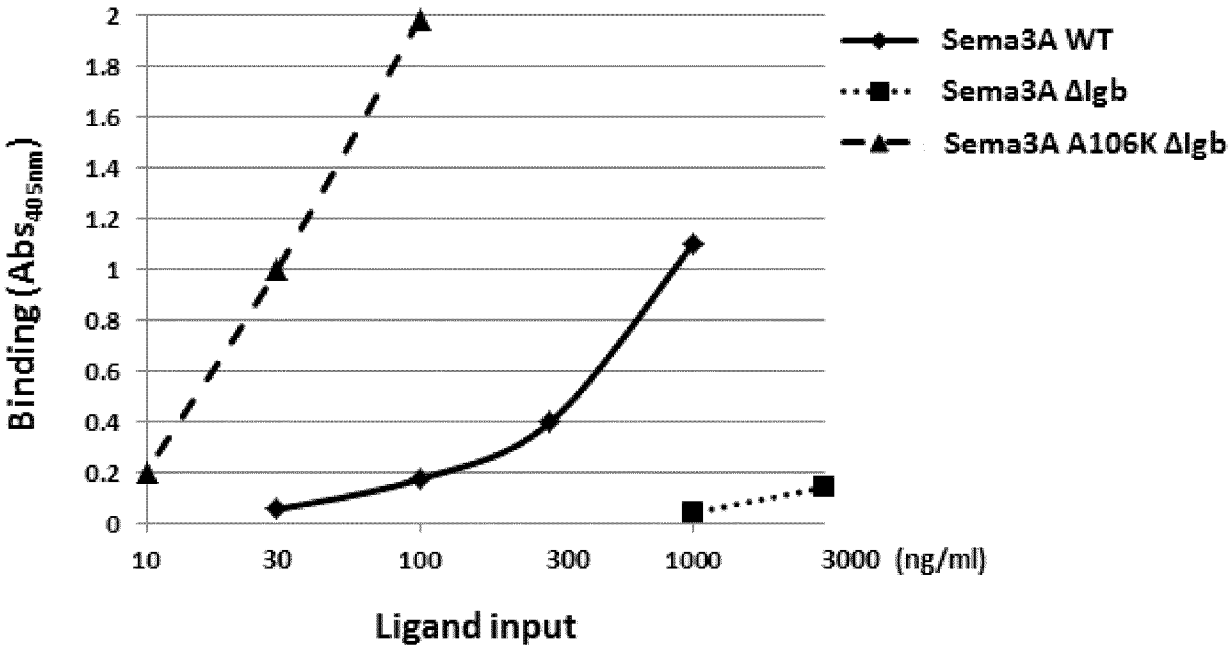


Figure 4 (cont. 2).

E



Estimated affinity:

Sema3A A106K Δlgb:	Kd ≈ 0.7 nM
Sema3A WT:	Kd ≈ 7 nM
Sema3A Δlgb:	Kd ≈ 200 nM

Figure 4 (cont. 3).

F

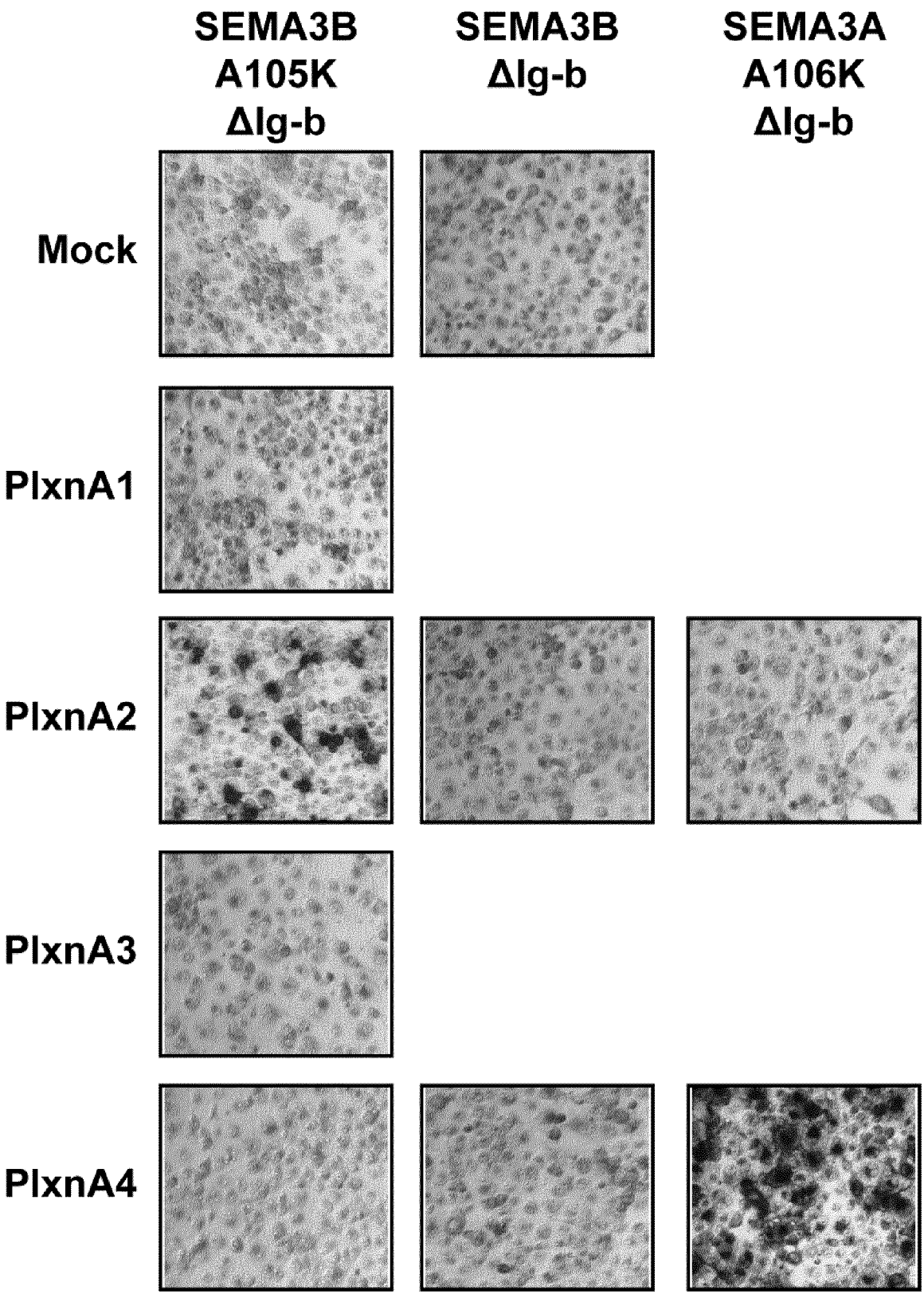


Figure 5.

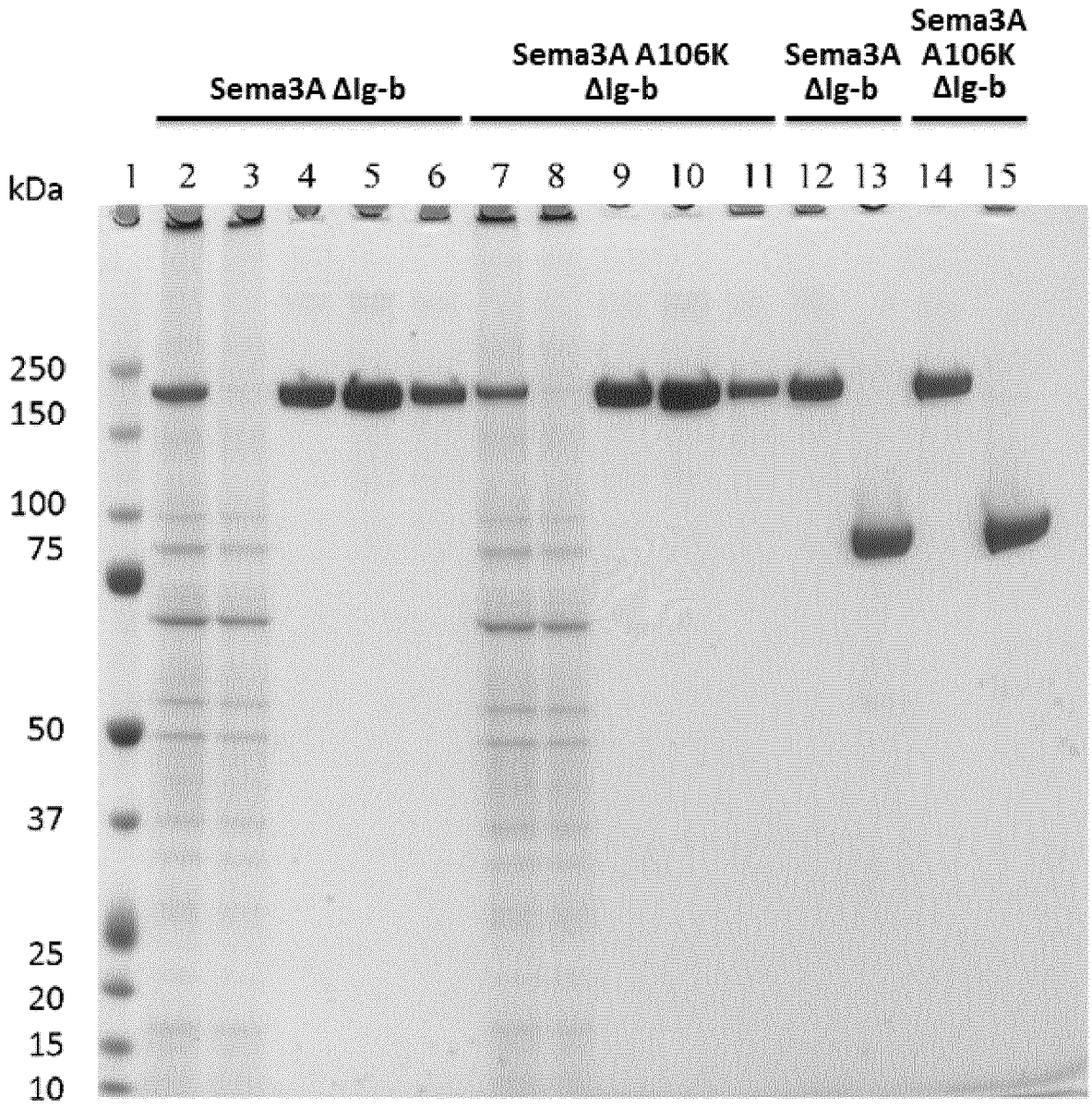

Sema3A Δlg-b
Sema3A A106K Δlg-b
Sema3A Δlg-b
Sema3A A106K Δlg-b
kDa
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
250
150
100
75
50
37
25
20
15
10

Figure 6.

A

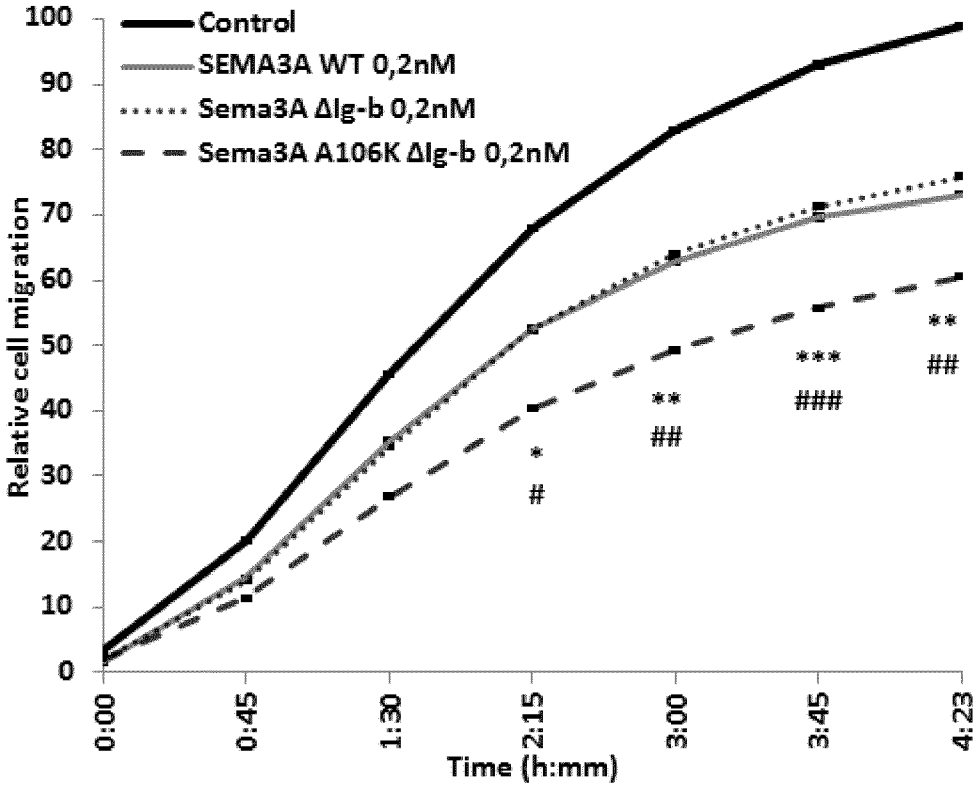

Control
SEMA3A WT 0,2nM
Sema3A Δlg-b 0,2nM
Sema3A A106K Δlg-b 0,2nM
Relative cell migration
Time (h:mm)
0:00
0:45
1:30
2:15
3:00
3:45
4:23
0
10
20
30
40
50
60
70
80
90
100
*
#
**
##
***
###
**
##

Figure 6 (cont. 1).

B

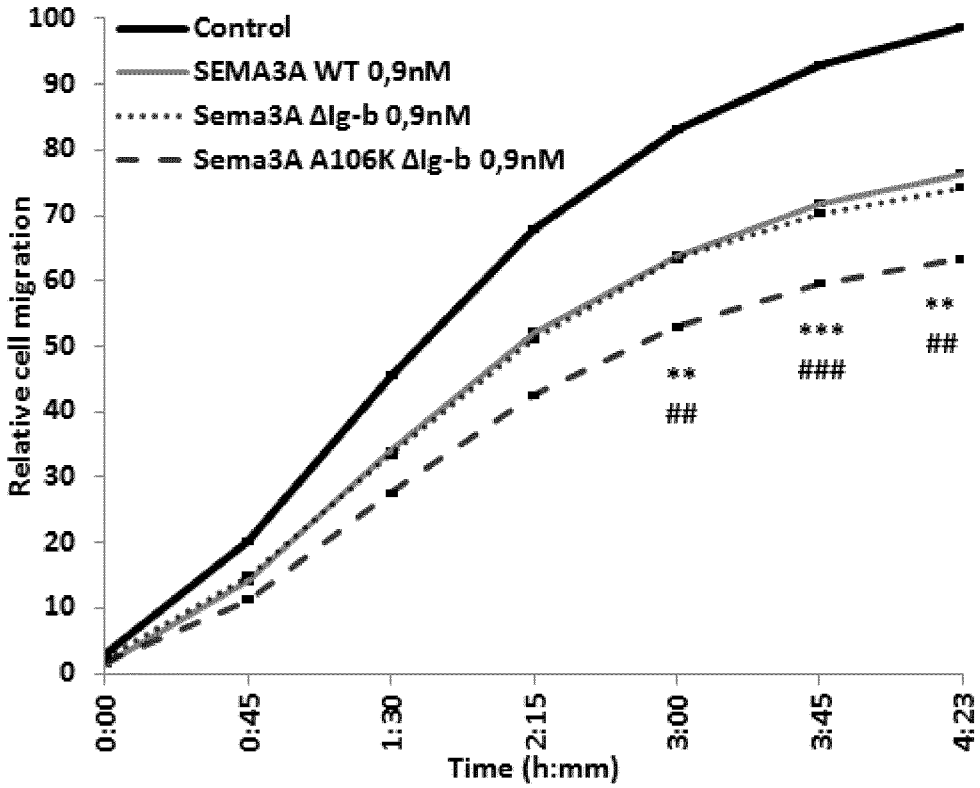


Figure 6 (cont. 2).

C

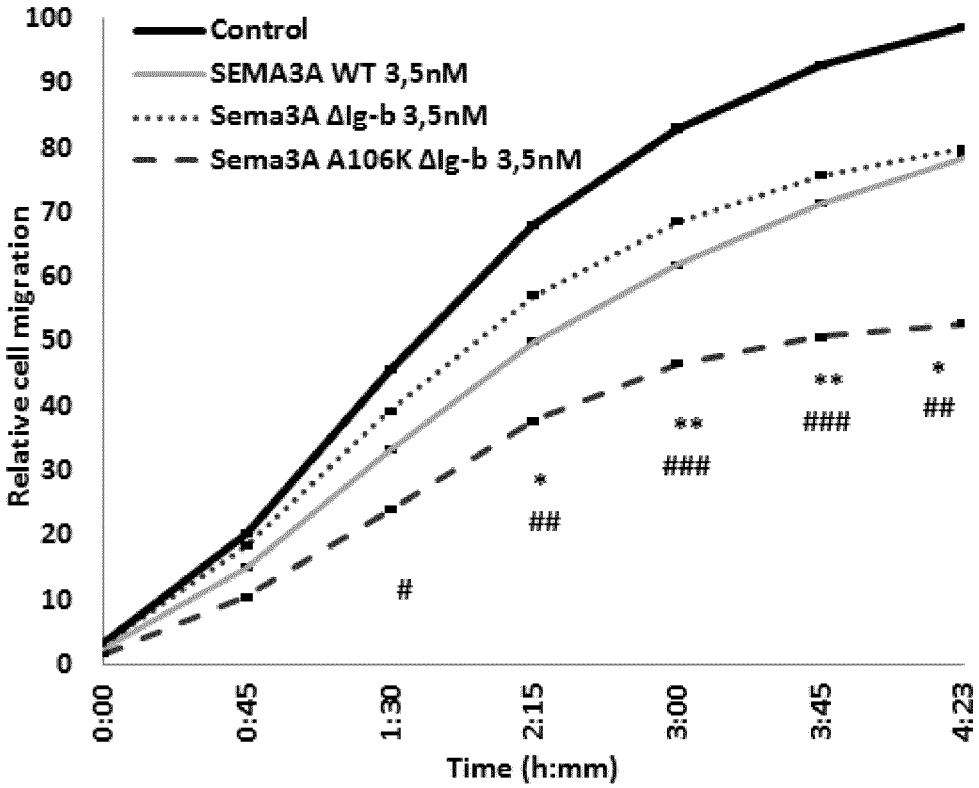


Figure 6 (cont. 3).

D

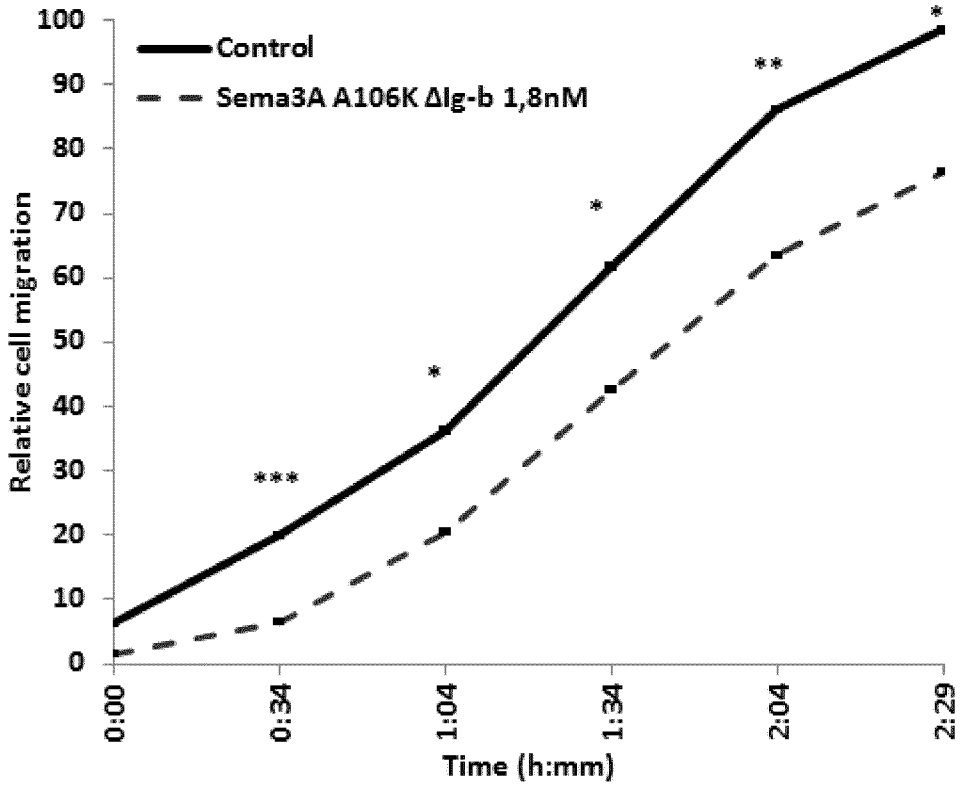


Figure 6 (cont. 4).

E

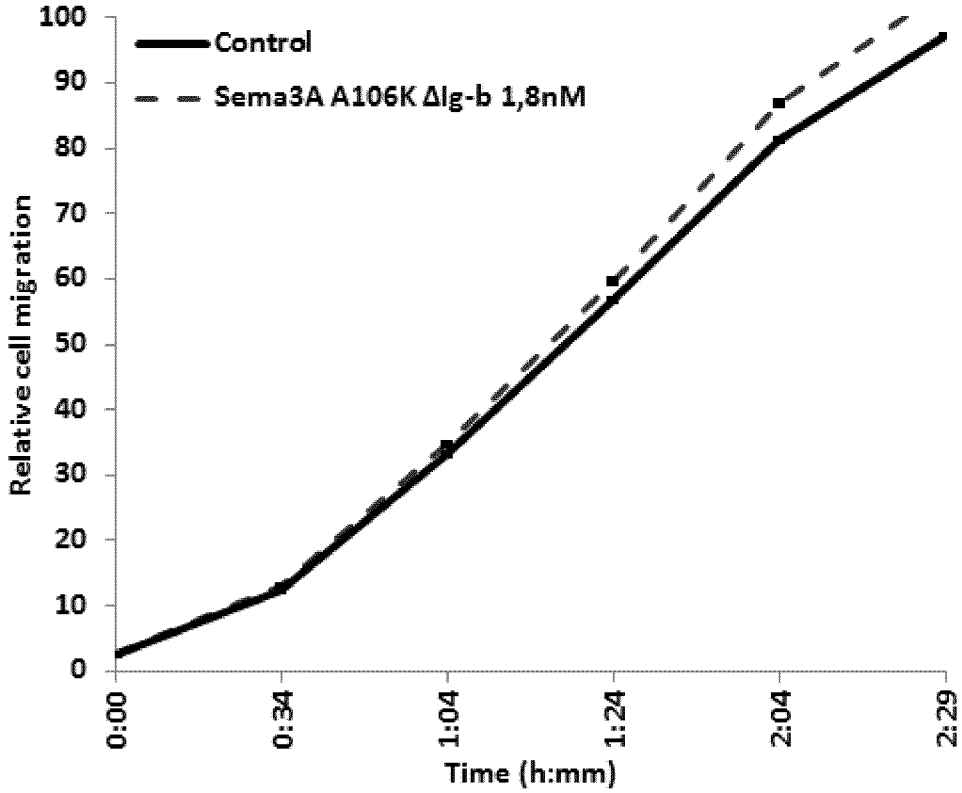


Figure 7.

A

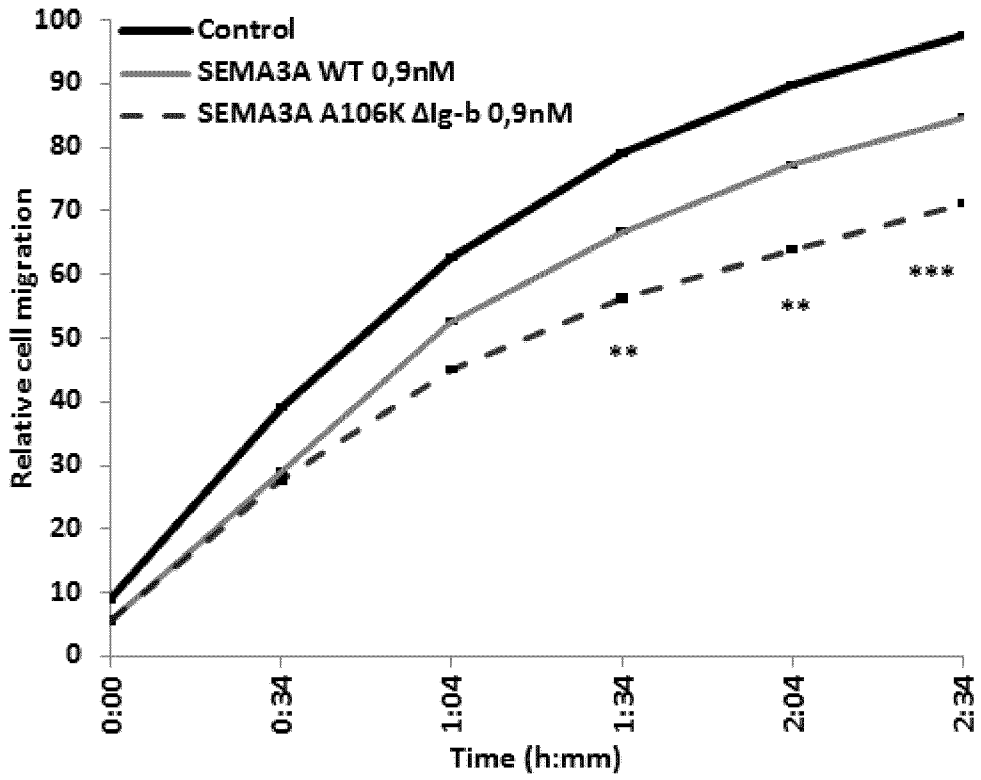

Control
SEMA3A WT 0,9nM
SEMA3A A106K ΔIg-b 0,9nM
Relative cell migration
100
90
80
70
60
50
40
30
20
10
0
0:00
0:34
1:04
1:34
2:04
2:34
Time (h:mm)
**
**
***

Figure 7 (cont. 1).

B

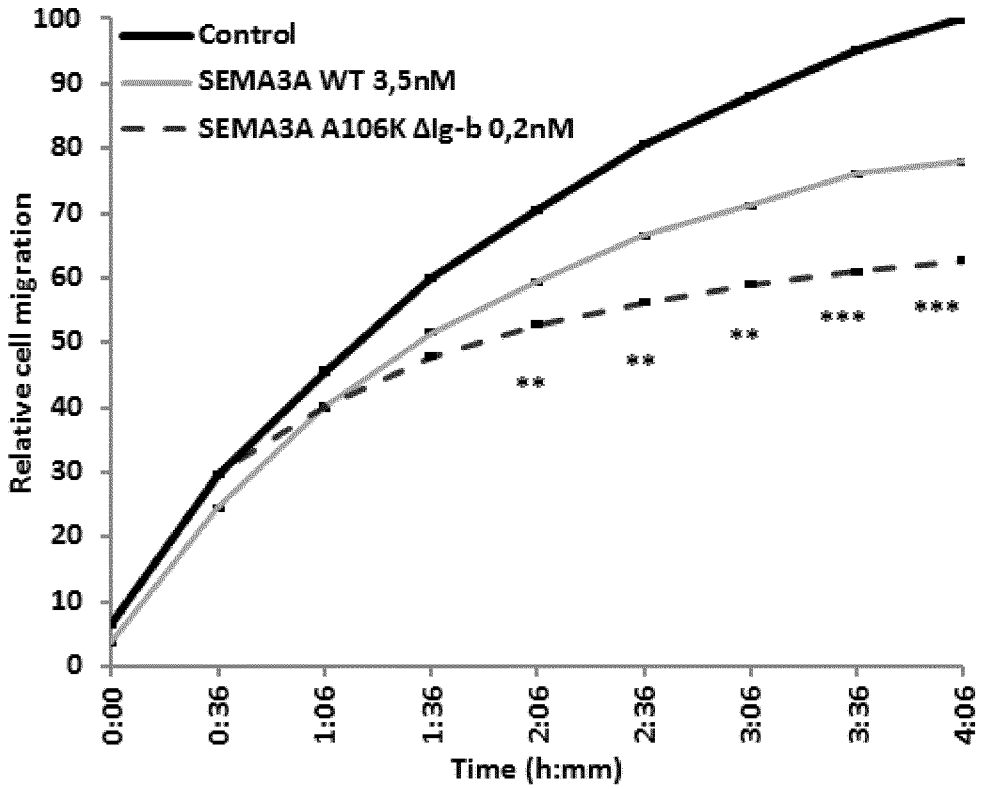


Figure 8.

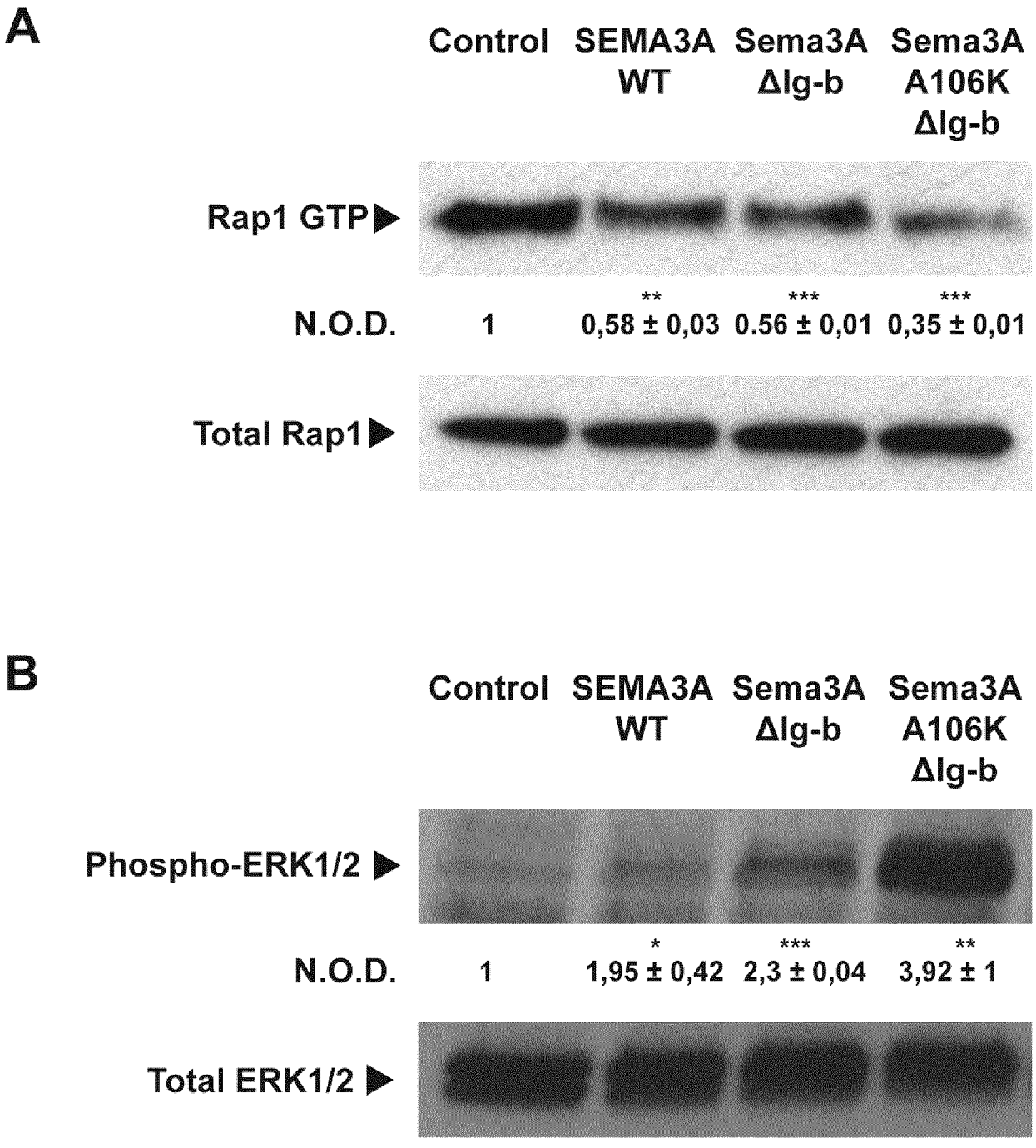

A
Control
SEMA3A WT
Sema3A Δlg-b
Sema3A A106K Δlg-b
Rap1 GTP
N.O.D.
1
** 0,58 ± 0,03
*** 0.56 ± 0,01
*** 0,35 ± 0,01
Total Rap1
B
Control
SEMA3A WT
Sema3A Δlg-b
Sema3A A106K Δlg-b
Phospho-ERK1/2
N.O.D.
1
* 1,95 ± 0,42
*** 2,3 ± 0,04
** 3,92 ± 1
Total ERK1/2

Figure 9.

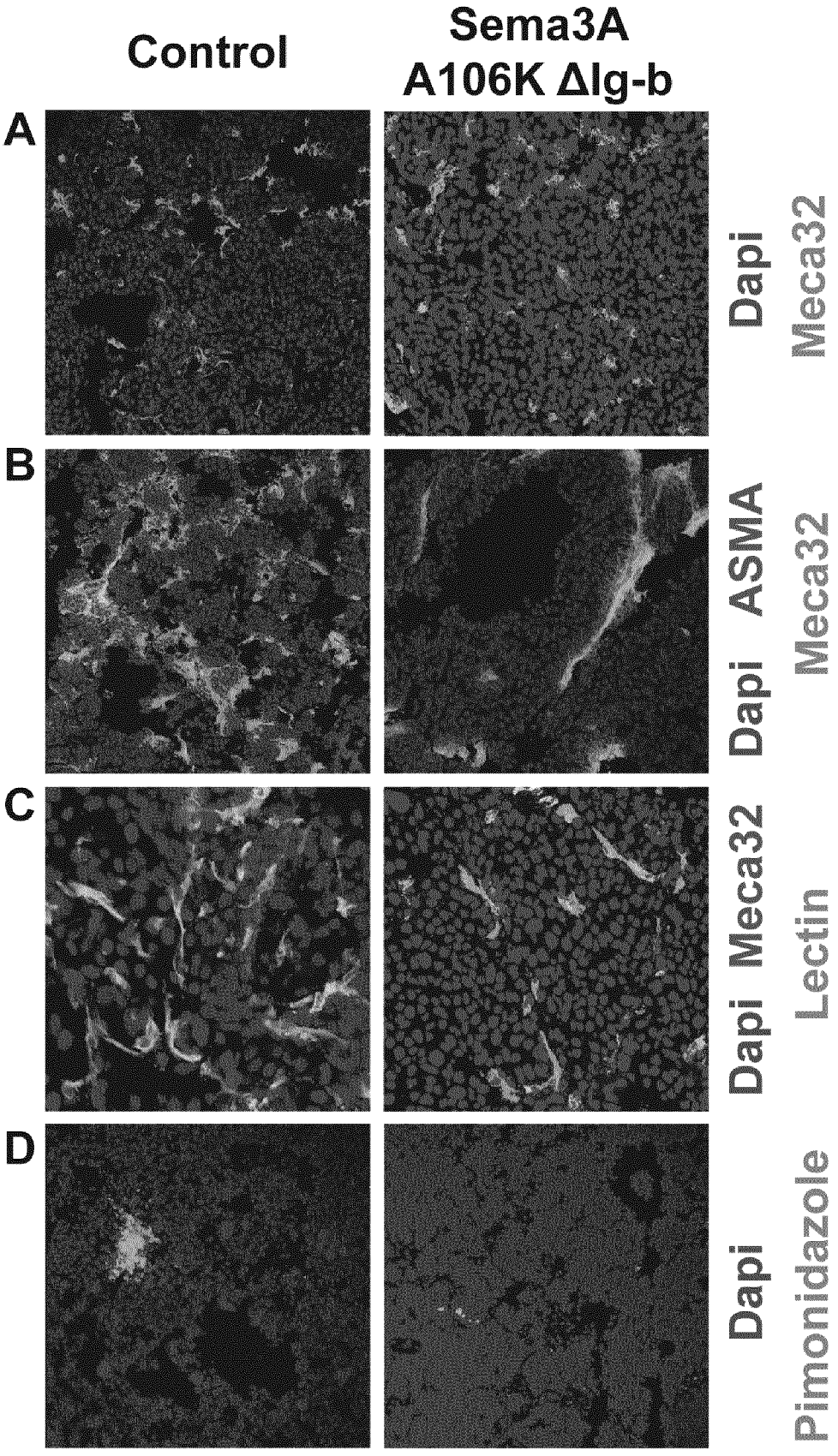

Control
Sema3A
A106K Δlg-b
A
B
C
D
Dapi
Meca32
Dapi ASMA
Meca32
Dapi Meca32
Lectin
Dapi
Pimonidazole

Figure 10.

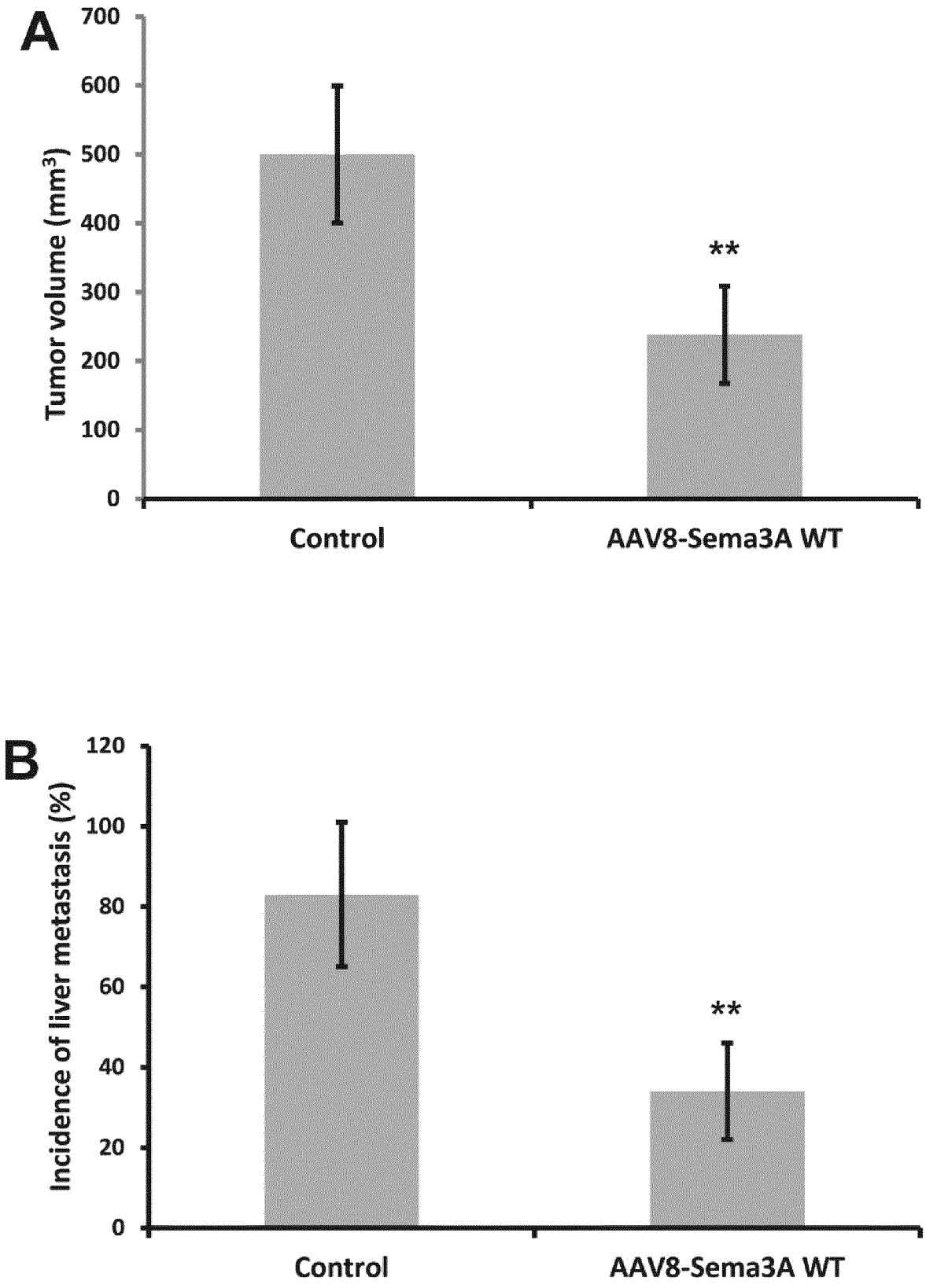

A
Tumor volume (mm3)
700
600
500
400
300
200
100
0
**
Control
AAV8-Sema3A WT
B
Incidence of liver metastasis (%)
120
100
80
60
40
20
0
**
Control
AAV8-Sema3A WT

Figure 11.

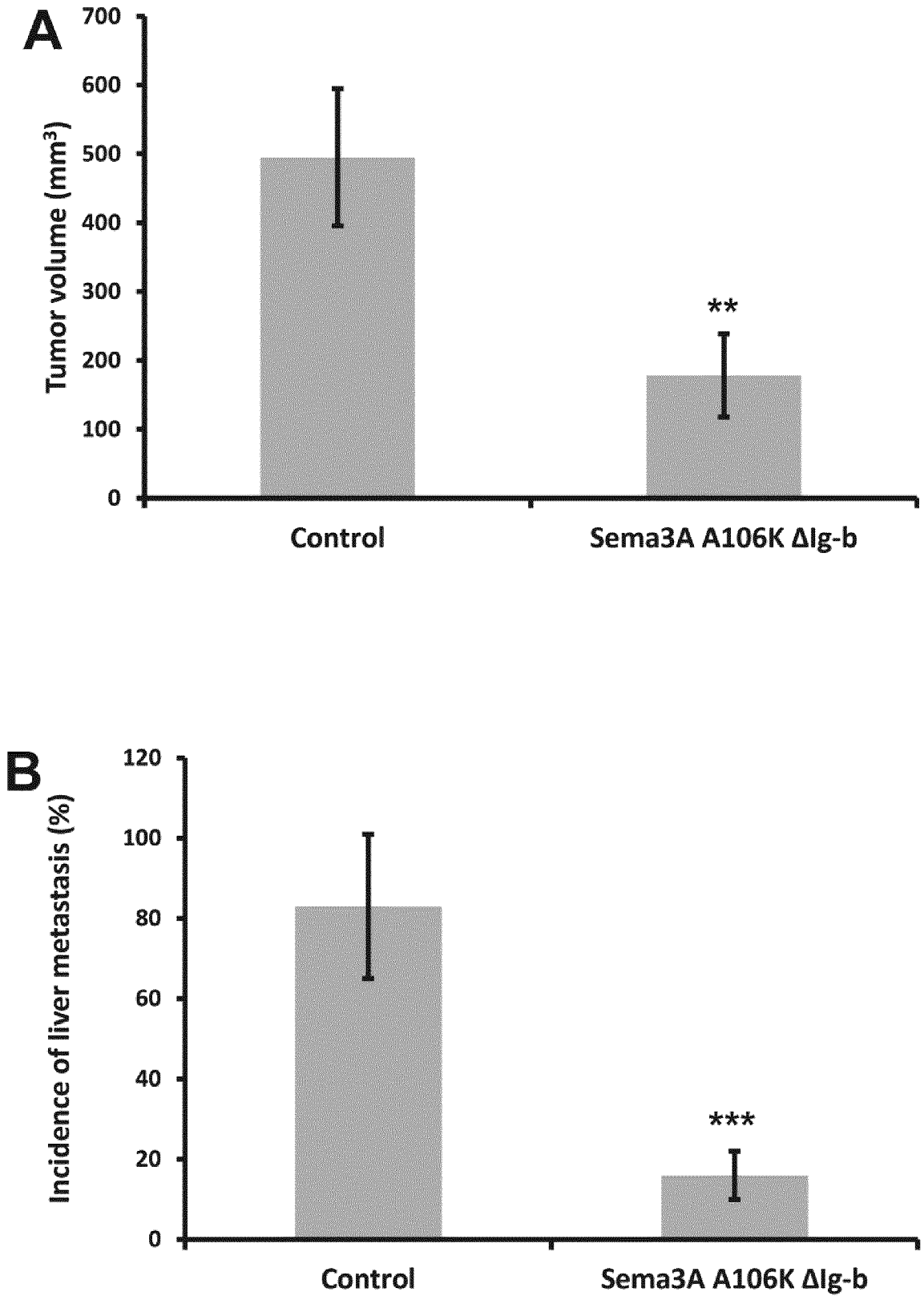

A
Tumor volume (mm³)
700
600
500
400
300
200
100
0
**
Control
Sema3A A106K ΔIg-b
B
Incidence of liver metastasis (%)
120
100
80
60
40
20
0
***
Control
Sema3A A106K ΔIg-b

Figure 11. (cont. 1)

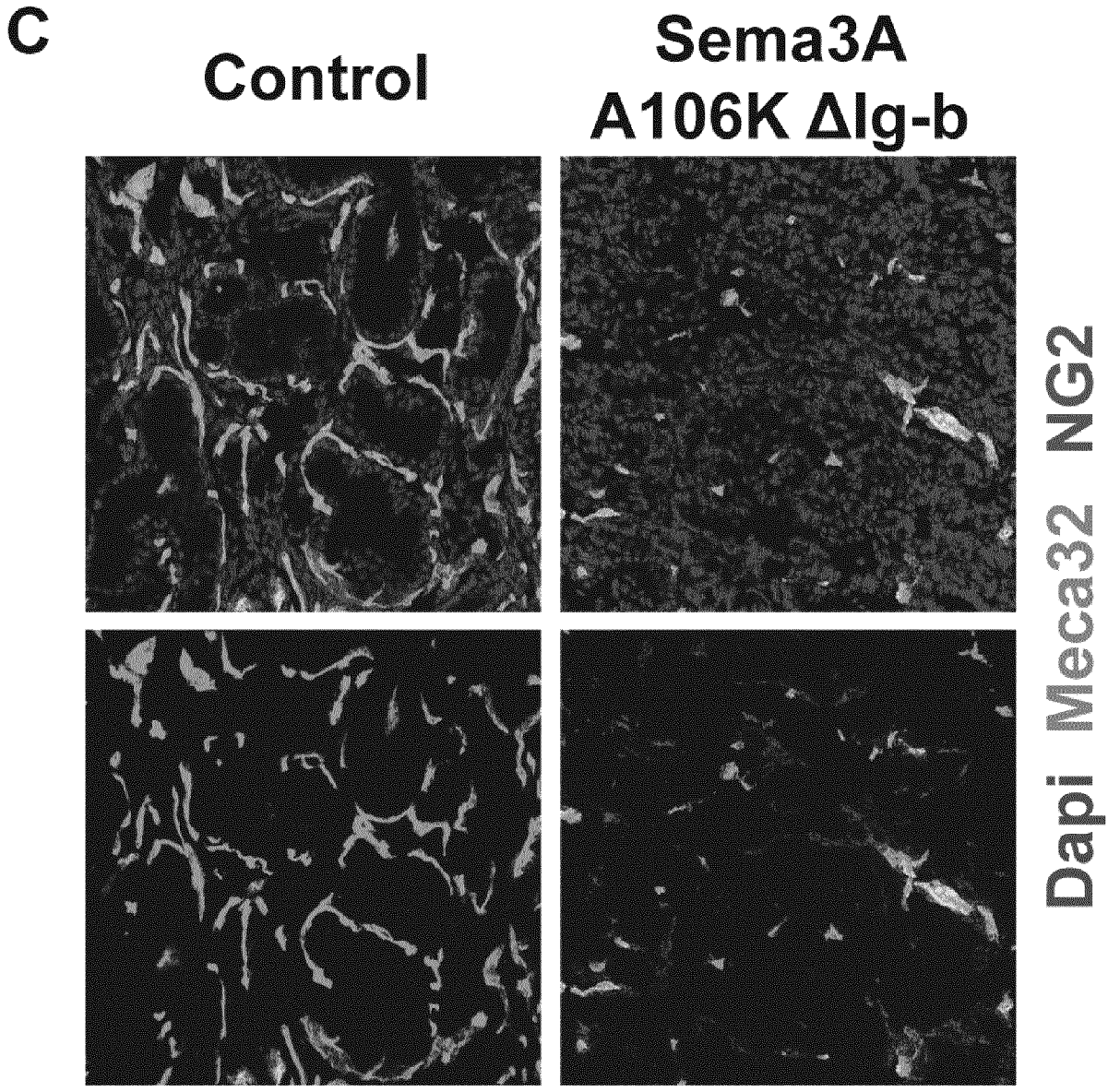


Figure 12.

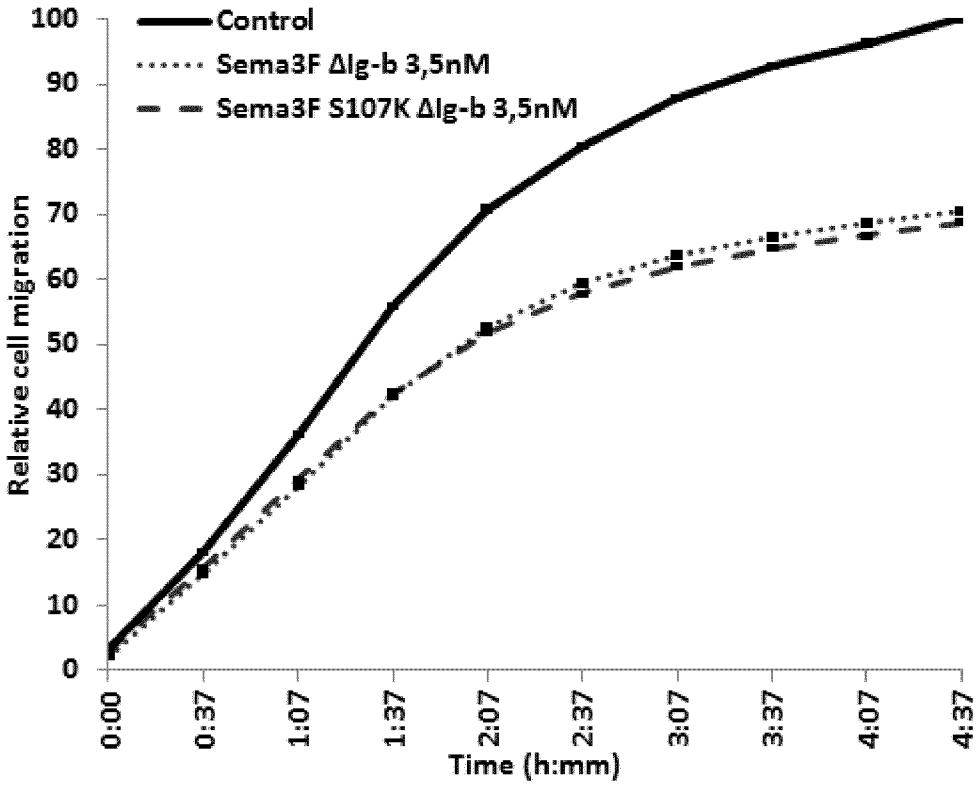

Control
Sema3F Δlg-b 3,5nM
Sema3F S107K Δlg-b 3,5nM
Relative cell migration
100
90
80
70
60
50
40
30
20
10
0
0:00
0:37
1:07
1:37
2:07
2:37
3:07
3:37
4:07
4:37
Time (h:mm)

Figure 13.

A

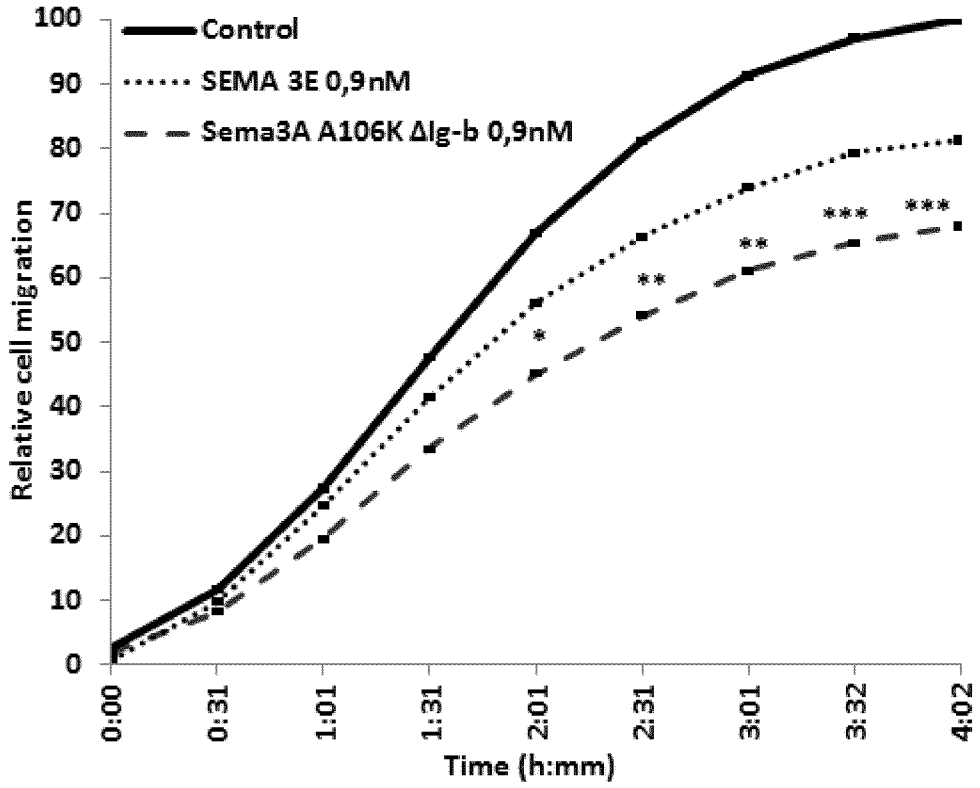
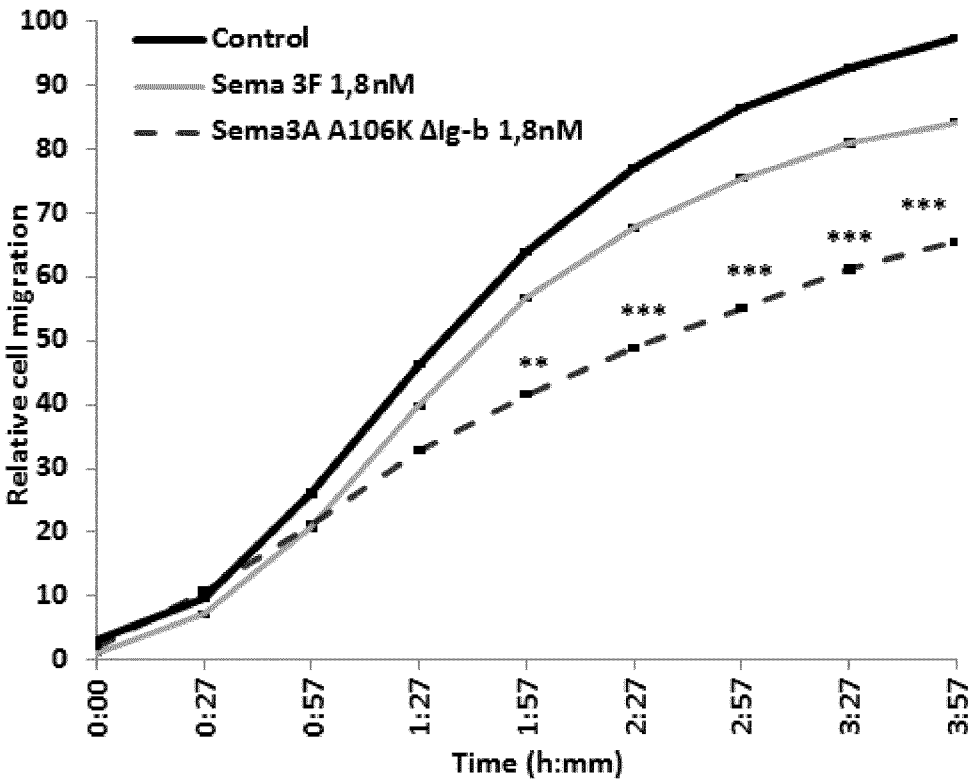

Control
SEMA 3E 0,9nM
Sema3A A106K Δlg-b 0,9nM
Relative cell migration
Time (h:mm)
0
10
20
30
40
50
60
70
80
90
100
0:00
0:31
1:01
1:31
2:01
2:31
3:01
3:32
4:02
*
**
**
***
***

Figure 13. (cont. 1)

B



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/053750

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/47 A61K38/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE BioProject [Online] 14 July 2014 (2014-07-14), "PREDICTED: semaphorin-3B isoform X2 [Equus przewalskii]", XP002744032, retrieved from NCBI accession no. XP_008528038 Database accession no. XP_008528038 the whole document 88.1% sequence identity with SEQ ID NO: 6 ----- -/--	1-49

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
9 June 2016	20/06/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sprinks, Matthew

1

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE BioProject [Online] 7 March 2014 (2014-03-07), "PREDICTED: semaphorin-3B isoform X6 [Panthera tigris altaica]", XP002744033, retrieved from NCBI accession no. XP_007088627 Database accession no. XP_007088627 the whole document 85.5% sequence identity with SEQ ID NO: 6 -----	1-49
A	DATABASE BioProject [Online] 6 December 2014 (2014-12-06), "PREDICTED: semaphorin-3F [Haliaeetus leucocephalus]", XP002744034, retrieved from NCBI accession no. XP_010571212 Database accession no. XP_010571212 the whole document 56.5% sequence identity with SEQ ID NO: 10 -----	1-49
A	WO 95/07706 A1 (UNIV CALIFORNIA [US]) 23 March 1995 (1995-03-23) the whole document -----	1-49

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/053750

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