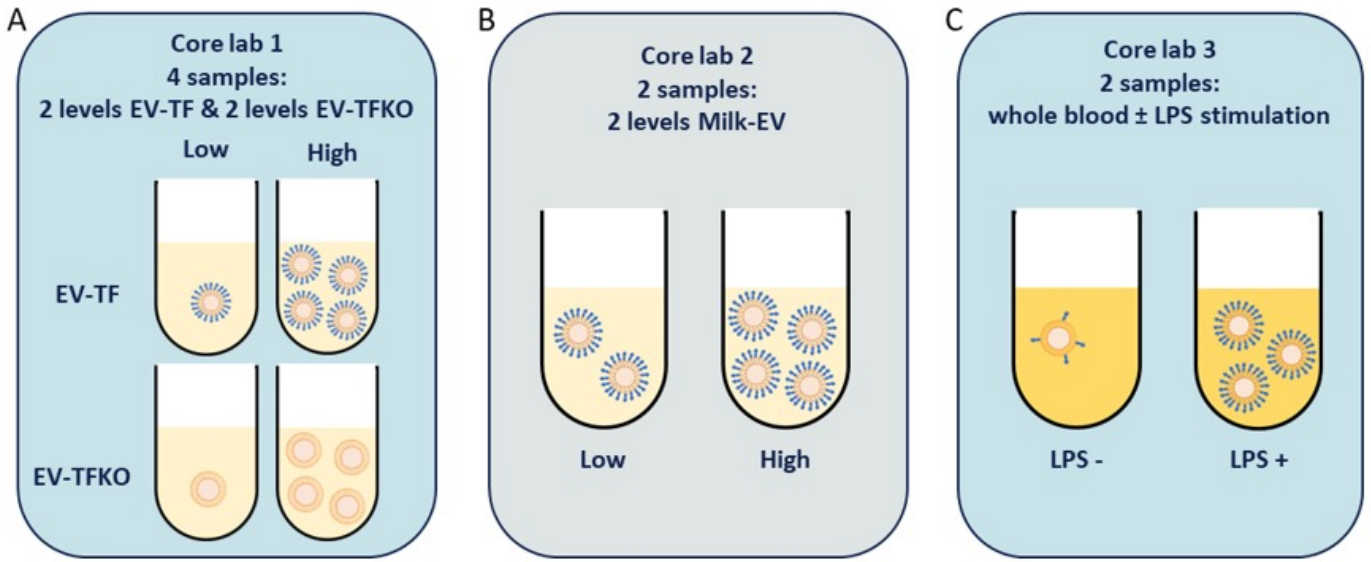
Supplemental Figure 1: **Samples used for the study** (A) Core lab 1: EV-free plasma supplemented with EV-TF from the parental HAP-1 cell line or EV-TFKO from the HAP-1 knockout TF cell line. (B) Core lab 2: EV-free plasma supplemented with EVs derived from human milk at 2 distinct concentrations. (C) Core lab 3: Platelet-depleted plasma prepared from whole blood with or without LPS stimulation.

Supplemental Figure 2: **Analysis of the tissue factor calibrant**Tissue factor (TF) calibrant and Innovin (recombinant tissue factor) were detected by western blotting. The position of tissue factor is shown (rTF). The large band marked with an arrowhead is bovine serum albumin, which is used as a carrier.

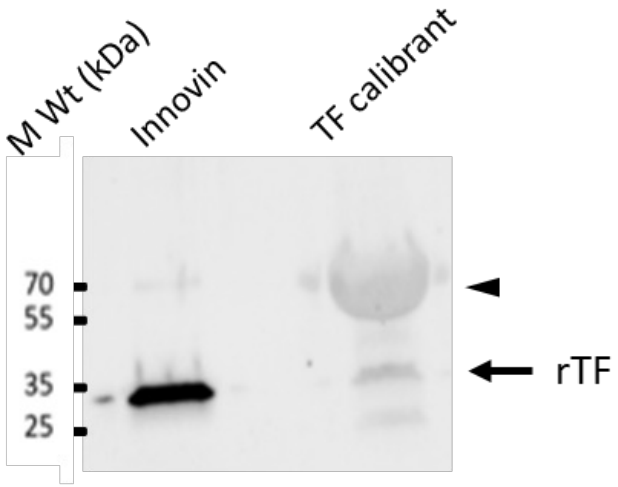
Supplemental Figure 3: **Characterization of HAP-1 cell lines and EV-TFKO** (A) Relative expression of TF mRNA by RT-qPCR in the 2 cell lines HAP-1 wild-type and HAP-1 TFKO. (B) TF protein expression of HAP-1 wild-type and HAP-1 TFKO cell lines by western blotting. (C) TF protein expression in EV derived from wild-type or TFKO HAP-1 cells by western blotting. (D) TF activity of HAP-1 wild-type and HAP-1 TFKO cell lines measured using a FXa generation assay.

Supplemental Figure 4: **Variability of high and low milk-EV triplicate measurement by antigenic assays** Each dot represents one value of the triplicate obtain for high and low milk-EV samples for each antigen assay.

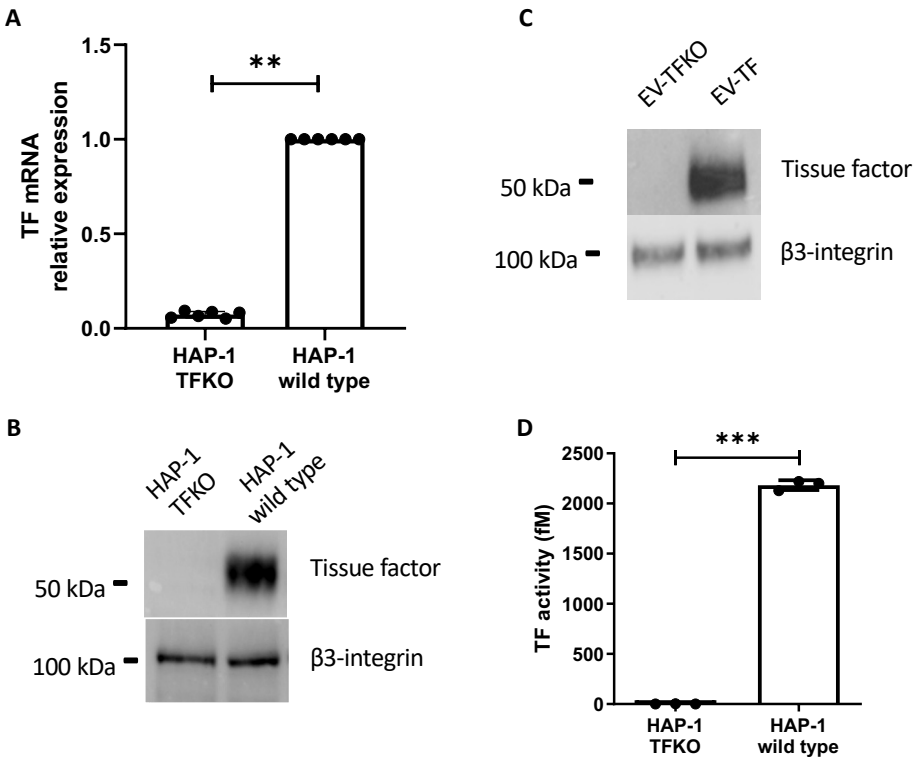
Supplemental figure 1



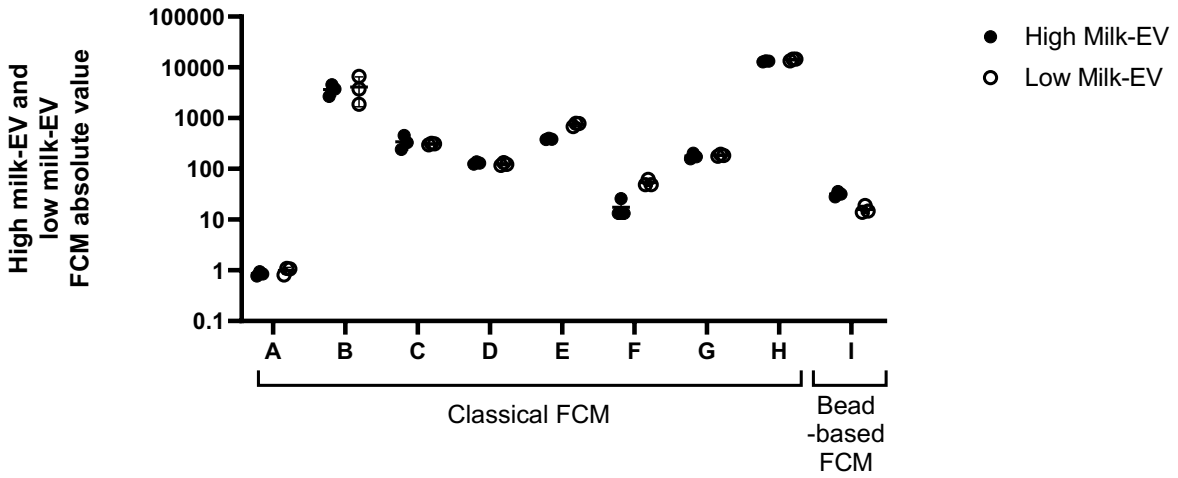
Supplemental figure 2



Supplemental figure 3



Supplemental figure 4



Supplemental Table 1: Characteristics of the functional assays

Assay N°	Assay subtype	Sample Volume (µL)	Purification EV	Blocking well	mAb Anti-TF reference	Control IgG	Coagulation factors	$CaCl_2$ (mM)	Substrate	Assay type	Analysis order	Reference
1	FXa generation	200	20,000 g for 15 min *2	mAb αTF, HTF-1, 10µg/mL	BD Biosciences Cat no: 550252	IgG1κ, MOPC-21, BD Biosciences, cat no: 554121) 10µg/mL	FX: 300nM FVIIa: 5nM	10	FXa substrate S-2765TM (Chromogenix)	Kinetic	A and B	/
2	FXa generation	100	20,000 g for 15 min *2	mAb αTF, HTF-1, 7.8µg/mL	BD Biosciences Cat no: 550252	IgG from mouse serum, Sigma Aldrich I5381	FX: 73.2nM FVIIa: 2.4nM	5	Pefachrome FXa 8595, 0.67 mmol/L	Endpoint	A and B	(Hisada & Mackman, 2019)
3	FXa generation	300	24,000 g for 60 min *2	mAb αTF, B4C9/SBTF1, 10µg/mL	Purified Mouse Anti-Human CD142, BioCytex-STAGO	IgG1, a-DNP 2H11–2H12, 10 µg/mL	FX: 190nM FVII: 10nM	5	CBS 31.39, STAGO	Kinetic	B and A	Adapted from (Vallier et al., 2019)
4	CY-QUANT MV-TF activity (RUO)	500	24,000 g for 60 min *2	mAb αTF, B4C9/SBTF1	Purified Mouse Anti-Human CD142, BioCytex-STAGO	IgG1, CeLLine, BioCytex	FX: 2200nM FVII: 0.31nM	10	Chromogenic anti-FXa 02.44, STAGO	Kinetic	B and A	Adapted from (Vallier et al., 2019)
5	CY-QUANT MV-TF activity (IMS)	100	IMS beads, 30 min	mAb αTF, B4C9/SBTF1	Purified Mouse Anti-Human CD142, BioCytex-STAGO	IgG1, CeLLine, BioCytex	FX: 2200nM FVII: 0.31nM	10	Chromogenic anti-FXa 02.44, STAGO	Kinetic	B and A	Adapted from (Franco et al., 2020)
6	FXa generation	500	20,000 g for 30 min 4°C *3	mAb αTF, HTF1, 200µg/mL	BD Biosciences Cat no: 550252	No	FX: 300nM FVIIa: 10nM	10	PN.A.PEP 1065, Cryopep	End point	B and A	Adapted from (Khorana et al., 2008)
7	FXa generation	200	18,000 g for 20 min *2	mAb αTF, HTF1	BD Biosciences Cat no: 550252	IgG from mouse serum, Sigma Aldrich I5381	FX: 73.2nM FVIIa: 2.4nM	10	Chromogenix S2765, Diapharma	End point	A and B	(Hisada & Mackman, 2019)
8	Zymuphen MP-TF	20	Microplate coated with anti-TF antibody	No	n.a.	No	Unknow	Yes	Factor Xa specific chromogenic substrate (CS 11(65))	Kinetic	/	According to the manufacturer's instructions

9	Zymuphen MP-TF	20	Microplate coated with anti-TF antibody	No	n.a.	No	Unknow	Yes	Factor Xa specific chromogenic substrate (CS 11(65))	Kinetic	/	According to the manufacturer's instructions
10	FXa generation	40	No	No	n.a.	No	Unknow	8	S2765	Parallel line model	/	/
11	FXa generation	Pellets from 300µL samples in 80µL and from 500µL samples in 140µL then 50µL used	16,000 g for 30 min *2	No	n.a.	No	FX: 150nM FVIIa: 5nM	5	Chromogenic substrate BIOPHEN CS-11(65), Hyphen-BioMed, 0,67mM	Absorption 405nm	/	Adapted from (Beckmann et al., 2022)
12	FXa generation	50	No	No	n.a.	No	FX: 73nM FVIIa: 2.4nM	5	Chromogenic CS-011(32) substrate	Absorption 410nm	/	Adapted from (Featherby et al., 2019)
13	Actichrome	All the pellet in 30µL and 25µL used. New freezing step	20,000 g for 70 min *2	No	n.a.	No	FX: 7nM FVIIa: 3.5nM	Yes	SPECTROZYME® FXa 5µM	Kinetic	/	According to the manufacturer's instructions
14	Actichrome	25	No	No	n.a.	No	FX: 7nM FVIIa: 3.5nM	Yes	SPECTROZYME® FXa 5µM	Kinetic	/	According to the manufacturer's instructions
15	Thrombin generation	300	20,000 g for 30 min	Human F VIIa Inactivated, Enzyme Research Laboratories, cat no: HFVIIai, 0.12µg/mL	n.a.	No	FX: 18.8nM FVIIa: 34pM FII: 130nM FV/Va: 4.8µg/mL	8.3	Pefachrome® FXa 8595 5-Diagnostics AG n°085-27	End point	A and B	(Østerud et al., 2022)
16	Thrombin generation CAT	EV Pellet resuspended in 200µL of EV-free plasma (2,500 g 15min twice and 20000 g 1h) and 20µL used for the assay	20,000 g for 60 min *2	mAb αTF, HTF-1, 7.84µg/mL	BD Biosciences Cat no: 550252	No	Standard plasma	Yes	7-amino-4methylcoumarin	Lag Time	B and A	(Kristensen & Nybo, 2023)
17	Thrombin generation	20	No	No	n.a.	No	Plasma Barium Sulfate Eluate	5	Chromogenic CS-01(81) substrate	Absorption 410nm	/	Adapted from (Ettelaie et al., 2008)
18	Clotting assay	90	No	mAb αTF, HTF-1, 30µg/ml	eBio-sciences Cat no : 17101152	No	n.a.	14	DO 405	½ Vmax (s)	B and A	(Berckmans et al., 2011)

Legend:

In the column intitled analysis order, the letter “A” means the conversion of raw data to U/mL and “B” means the subtraction of the value obtained using the anti-TF antibody from the value obtained using the control antibody.

Zymuphen manufacturer’s instruction: https://www.coachrom.com/fileadmin/docs/hbm/en/521196.pdf

Actichrome manufacturer’s instruction: https://search.cosmobio.co.jp/cosmo_search_p/search_gate2/docs/BDX_/846.20180622.pdf

	Laboratory	A	B	C	D	E	F	G	H	I
Sample preparation	**Sample centrifugation**	No	No	No	20,000 g for 30 min *2	No	No	No	No	3,000 g 15 min and 10,000 g 15 min pellet discarded
	Sample dilution	1/100	1/20	1/10	1/40	No	1/9	1/5	No	No
	Sample washing steps	No	No	No	No	No	No	No	No	Twice with PBS
Sample staining	**Sample volume**	10 µL	10 µL	10 µL	5 µL	50 µl	20 µL	12.5 µL	50 µl	All
	αTF antibody (clone, labelling, company, cat no, concentration/volume)	mAb H-9, FITC, Santa Cruz, cat no: sc-374441	mAb NY2, PE, BioLegend, cat no: 365203; 2.5 µg/mL	mAb HTF1, PE, Miltenyi, cat no: 130-098-742; 10 µL	mAb VD8, FITC, BioMedica Diagnostics; cat no: 4508CJ	mAb HTF-1, BV421, Becton Dickinson, cat no:744003; 2 µg/ml	mAb IIID8, unconjugated, Sekisui Diagnostics; cat no: 4509; 4 µL	mAb HTF-1, PE, Becton Dickinson, cat no:BD550312	mAb VD8, FITC, American Diagnostica cat no: 4508CJ; 5 µL	mAb HTF1, FITC, Miltenyi; cat no: 130-122-211;
	Other antibodies/reagents	mAb αCD31, PE-Cy7, BioLegend, cat no: 303118; mAb αCD63, APC, BioLegend, cat no: 353018; mAb αCD9, PE (BioLegend, cat no: 312106	No	No	Calcein violet AM	Calcein AM, FITC, Life Technologies, cat no: C3100MP, 100 µM	AnnexinV, FITC, eBiosciences, cat no: MDS500FI; mAb αmouse IgG, Alexa fluor 647	No	No	No
	Buffer (volume and type)	/	180 µL DPBS	/	45 µL 0.1 µm filtered PBS-/-	200 µL 0.1 µm-filtered PBS+ 15 µM PPACK	140 µL Annexin V buffer	300 µL FACS buffer	300 µL 0.22 µm-filtered PBS	/
Staining controls	**Buffer-only**	0.22 µm-filtered PBS	PBS	Yes	0.1 µm-filtered PBS	0.1 µm-filtered PBS+PPACK	0.22 µm-filtered Annexin V buffer	Yes	0.22 µm-filtered PBS	No
	Buffer with reagents w/o sample	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes
	Buffer with sample w/o reagents (unstained control)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
	Isotype controls or Fluorescence minus one	No	No	mAb αmouse IgG1 clone IS5-21F5, PE, Miltenyi, cat no: 130-113-200	Isotype-matched control antibodies (Miltenyi Biotec)	FMO	mAb αmouse IgG1, clone MOPC-21, FITC, BioLegend, cat no: 400107; mAb goat αmouse IgG1, Alexa Fluor 647,	mAb αMouse IgG, clone MOPC-31C, PE, Becton Dickinson, no cat: BD550617	No	2 control beads unconjugated

							Thermo Fisher Scientific, cat no: A-21235			
Sample controls	**EV sample serial dilution**	Yes	Yes	No	Yes	Yes	No	No	No	No
	Detergent-treated EV samples	No	No	No	1mg/mL Saponin	0.1% Triton-x 100, 5 min, R.T.	1% Triton-x 100, 5 min, R.T.	No	No	No
Instrument configuration	**Instrument manufacturer**	Cytek Biosciences	Apogee Flow Systems	Beckman Coulter	Miltenyi Biotec	Beckman Coulter	Beckman Coulter	Beckman Coulter	Becton Dickinson	Miltenyi
	Instrument model	Aurora spectral flow cytometer	A60-Micro Plus	CytoFLEX LX	MACSQuant® Analyzer 16	Gallios	CytoFLEX	CytoFLEX LX	FACS Lyric	MACSQuant Analyzer-10
	Compensation description	Not required	Not required	Not required	Not required	Not required	FITC-APC 0% and APC-FITC 10%	Not required	Not required	Not required
Instrument calibration & data acquisition	**Trigger channel(s) and threshold(s)**	405 nm laser; Threshold=1000 AU	Threshold=2300 AU on SSC	405 nm laser; Threshold=1000 AU on VSSC	Threshold=0.71 AU on SSC	405 nm laser; wide angle on FSC, Threshold=1 on SSC-A	VSSC	VSSC	VSSC	n.a.
	Flow rate	15 µL/min	1.5 µL/min	10µl/min	20µl/min	10µl/min	10µl/min	10µl/min	12µl/min	High flow rate
	Quantification – **Volumetric**	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	n.a.
	Quantification – **Bead-mediated**	No	No	No	No	Trucount Beads	No	No	No	n.a.
	Fluorescence Calibration	SpectroFlo QC Beads	Quantum TM R-PE beads	No	MACSQuant Calibration Beads	Flow-check Pro Fluorospheres	CytoFLEX daily QC fluorospheres	Yes	Cytometer Setup & Tracking Beads	???
	Light Scatter Calibration	ApogeeMix beads (0.180, 0.240, 0.300, 0.590, 0.880 and 1.300 µm) and Thermofisher	Rosetta Calibration beads	Polystyrene beads (0.25, 0.58, 0.79 and 1.34µm)	MACSQuant Calibration Beads	Megamix-FSC Plus beads (0.5, 0.9, 3µm)	Mix 1:1 of Megamix-FSC & SSC Plus beads	Megamix-FSC Plus beads (0.5, 0.9, 3µm)	Megamix-Plus SSC beads (0.16, 0.20, 0.24, and 0.5 µm)	n.a.

		Scientific NIST Traceable PS Beads (0.080 µm)								
	EV gate definition by size	0.080 - 1.300 µm	No	0.25 - 1.34 µm	0.1 - 0.9 µm	0.5 - 3µm	0.1 - 0.9 µm	0.5- 1 µm	0.20 - 0.5 µm	No
	EV gate definition by fluorescence	No	Comparing CD142-PE positive events from 488nm-Orange channel between non-stained and stained samples	No	No	No	No	No	No	Gate on capture beads and then on CD142 positive EV