

Supplementary Table 1: Metastasis-Free Survival analysis for all RhoGTPases

Gene SYMBOL	Probeset	P value Log-rank (Mantel-Cox) Test	P value Gehan-Breslow-Wilcoxon Test	Hazard Ratio	95% CI of Ratio
<i>CDC42</i>	208727_s_at	0.8081	0.9764	0.9106	0.4276 - 1.939
<i>CDC42</i>	208728_s_at	0.6567	0.1615	1.192	0.5502 - 2.580
<i>CHP</i>	207993_s_at	0.3119	0.1848	0.676	0.3165 - 1.444
<i>CHP</i>	214665_s_at	0.7463	0.7183	0.8826	0.4142 - 1.881
<i>RAC1</i>	208640_at	0.0954	0.3208	0.4919	0.2137 - 1.132
<i>RAC1</i>	208641_s_at	0.3706	0.3232	0.7075	0.3318 - 1.509
<i>RAC2</i>	207419_s_at	0.7566	0.5622	0.8871	0.4159 - 1.892
<i>RAC2</i>	213603_s_at	0.6124	0.5837	1.218	0.5683 - 2.610
<i>RHOA</i>	200059_s_at	0.0927	0.1359	0.5182	0.2408 - 1.115
<i>RHOB</i>	212099_at	0.1949	0.0768	1.65	0.7738 - 3.520
<i>RHOBTB2</i>	209441_at	0.384	0.6064	0.7133	0.3334 - 1.526
<i>RHOBTB3</i>	202975_s_at	0.6161	0.5987	1.214	0.5687 - 2.592
<i>RHOC</i>	200885_at	0.4278	0.5255	0.7349	0.3432 - 1.574
<i>RHOD</i>	31846_at	0.0027	0.0009	3.284	1.510 - 7.146
<i>RHOD</i>	209885_at	0.0155	0.0119	2.595	1.199 - 5.614
<i>RHOF</i>	219045_at	0.9772	0.4852	1.011	0.4626 - 2.212
<i>RHOF</i>	219154_at	0.6061	0.6074	1.22	0.5730 - 2.597
<i>RHOG</i>	203175_at	0.0735	0.1341	2	0.9362 - 4.273
<i>RHOH</i>	204951_at	0.3978	0.1829	1.389	0.6485 - 2.975
<i>RHOQ</i>	212117_at	0.2256	0.1064	0.6241	0.2912 - 1.338
<i>RHOQ</i>	212119_at	0.1861	0.1167	0.6	0.2813 - 1.279
<i>RHOQ</i>	212120_at	0.4146	0.2748	0.7281	0.3396 - 1.561
<i>RHOT1</i>	218323_at	0.7125	0.7317	0.8675	0.4073 - 1.848
<i>RHOT1</i>	222148_s_at	0.3784	0.3782	1.405	0.6592 - 2.996
<i>RHOT2</i>	221789_x_at	0.4544	0.4544	1.336	0.6252 - 2.857
<i>RHOT2</i>	222131_x_at	0.79	0.6073	1.108	0.5204 - 2.359
<i>RHOT2</i>	65770_at	0.4482	0.2491	1.34	0.6291 - 2.853
<i>RND1</i>	210056_at	0.012	0.0029	2.644	1.239 - 5.643
<i>RND2</i>	213467_at	0.6613	0.4314	1.185	0.5550 - 2.529
<i>RND3</i>	212724_at	0.8221	0.5979	0.9134	0.4147 - 2.012

The mRNA of each Rho-family GTPase was analysed for the power to predict metastasis in MSKCC breast cancer data set

Supplementary Table 2: Univariate and Multivariate analysis for survival

Factor	Comparison	MFS Univariate			MFS Multivariate	
		P Log-rank	p G-B-W	HR (95%CI)	p*	HR (95%CI)
Rnd1 level	L+M vs H	0.005	0.0016	3.069 (1.403---6.712)	0.018	4.533(1.299- --15.848)
Age (years)	≤50 vs >50	0.4321	0.6547	0.7344(0.34- --1.586)	0.2	0.578(0.250- --1.337)
Size (cm)	≤2 vs >2	0.1891	0.217	2.095(0.6948- --6.315)	0.39	1.511(0.592- --3.851)
Lymph node	Neg vs pos	0.9621	0.7835	1.02(0.4558- --2.281)	0.59	0.797(0.345- --1.839)
ER status	neg vs pos	0.1801	0.0611	1.699(0.7827- --3.686)	0.72	1.291(0.323- --5.171)
PgR status	neg vs pos	0.506	0.1788	1.293(0.6065- --2.756)	0.56	0.699(0.210- --2.327)
70gene signature	good vs poor	0.1326	0.0466	0.5576(0.2605- --1.194)	0.7	0.803(0.268- --2.407)

ER: Estrogen Receptor, G---B---W: Gehan---Breslow---Wilcoxon, H:high, HR: Hazard Ratio, L:low, M:medium, MFS: Metastasis---free Survival, PgR: Progesterone Receptor. * Cox proportional hazards model

Supplementary Table 3: list of genes up- or down-regulated in RND1 knocked down cells

Up-regulated Genes

	gene symbol	gene product	fold change (control vs sh#1)	fold change (control vs sh#2)
EMT related	<i>CDC42EP3</i>	CDC42 effector protein (Rho GTPase binding) 3	1.63729	2.02938
	<i>SIPA1L2</i>	signal-induced proliferation-associated 1 like 2	2.12248	2.13322
	<i>TGFBR3</i>	transforming growth factor, beta receptor III	1.7973	1.77181
	<i>ITGA10</i>	integrin, alpha 10	2.4247	2.9642
	<i>S100A4</i>	S100 calcium binding protein A4 (S100A4), transcript variant 2	2.2911	1.72438
	<i>FBLN1</i>	fibulin 1, transcript variant D	1.74791	1.83227
	<i>PAPLN</i>	papilin, proteoglycan-like sulfated glycoprotein	1.79059	1.81581
	<i>STMN1</i>	stathmin 1, destabilizing microtubules.	1.43284	2.38549
	<i>GJC2</i>	gap junction protein, alpha 12, 47kda	1.55218	2.00241
	<i>PHACTR3</i>	phosphatase and actin regulator 3	2.83352	1.69246
	<i>COL4A1</i>	collagen, type IV, alpha 1	1.50137	1.47775
Mitotic Regulators	<i>CCNB2</i>	cyclin B2	1.59929	2.88619
	<i>CDC25C</i>	cell division cycle 25 homolog C (S. pombe)	1.65158	3.16087
	<i>WEE1</i>	WEE1 homolog (S. pombe)	1.87385	3.18037
	<i>BIRC3</i>	baculoviral IAP repeat-containing 3	1.92229	2.27714
	<i>CENPF</i>	centromere protein f, 350/400ka (mitosin)	1.64802	3.16346

	<i>CCNF</i>	cyclin f	1.49932	2.42448
	<i>DPYD</i>	dihydropyrimidine dehydrogenase, a pyrimidine catabolic enzyme	1.87989	2.33798
	<i>SIPA1L2</i>	signal-induced proliferation-associated 1 like 2	2.12248	2.13322
Angiogenesis	<i>ANGPT1</i>	angiopoietin 1	1.67361	1.52754
	<i>PLAT</i>	plasminogen activator, tissue, transcript variant 1	1.67983	1.50575
	<i>VASN</i>	vasorin	2.02635	2.15671
Negative Growth Regulators	<i>CDKN3</i>	cyclin-dependent kinase inhibitor 3, prevent the activation of CDK2 kinase	1.36071	2.91339
	<i>LRIG1</i>	leucine-rich repeats and immunoglobulin-like domains 1	1.57403	1.82242
	<i>FOXA2</i>	forkhead box A2, transcript variant 1	2.09283	2.05538
	<i>BTG2</i>	B-cell translocation gene 2, anti-proliferative	1.58888	1.88369
	<i>PPARG</i>	peroxisome proliferator-activated receptor gamma, transcript variant 2	1.59622	1.93806
	<i>RARRES3</i>	retinoic acid receptor responder (tazarotene induced) 3	1.66466	3.14628
	<i>MT1G</i>	metallothionein 1G	1.6696	1.74126
	<i>MT1X</i>	metallothionein 1X	1.74118	2.62623
	<i>GBP2</i>	guanylate binding protein 2, interferon-inducible	1.69411	1.79886
	<i>IFIT2</i>	interferon-induced protein with tetratricopeptide repeats 2	1.8635	2.7175
	<i>PTEN</i>	phosphatase and tensin homolog	1.66368	1.63782
	<i>CRYAB</i>	crystallin, alpha B, a P53 target required for apoptosis	2.01403	1.7476
	<i>SSBP2</i>	single-stranded DNA binding protein 2	1.93056	2.04682
Signaling	<i>PELI1</i>	pellino homolog 1 (Drosophila)	1.62076	1.82792
	<i>ABR</i>	active BCR-related gene	1.5895	1.58884
	<i>CHN1</i>	chimerin (chimaerin) 1, transcript variant 1	1.72916	2.56126
	<i>CHN2</i>	chimerin (chimaerin) 2, transcript variant 2	2.04196	1.78951
	<i>PHACTR3</i>	phosphatase and actin regulator 3, transcript variant 3	2.83352	1.69246
	<i>RHOBTB3</i>	Rho-related BTB domain containing 3	2.59618	2.38417
	<i>CDC42EP3</i>	cdc42 effector protein (rho gtpase binding) 3	1.63729	2.02938
	<i>IL7R</i>	interleukin 7 receptor	1.66687	2.11533
	<i>IL11RA</i>	interleukin 11 receptor, alpha	1.46776	1.41203

Down-regulated genes

Cell Junctions	<i>CDH1</i>	cadherin 1, type 1, E-cadherin (epithelial)	-1.83709	-3.72623
	<i>CDH3</i>	cadherin 3, type 1, P-cadherin (placental)	-1.5567	-2.7827
	<i>DSC2</i>	desmocollin 2, transcript variant Dsc2b	-2.13311	-1.50835
	<i>CLDN1</i>	claudin 1	-1.7962	-1.706
	<i>CRB3</i>	crumbs homolog 3 (Drosophila), transcript variant 3	-1.80476	-3.25495

Epithelial Adhesion to BM	<i>TUBB3</i>	tubulin, beta 3	-1.93787	-2.59339	
	<i>LAD1</i>	laminin, an anchoring filament of basement membranes	-1.75324	-1.84379	
	<i>TUBA4A</i>	tubulin, alpha4a	-1.9954	-1.74174	
	<i>TUBB2A</i>	tubulin, beta2a	-1.41566	-1.62096	
	<i>ITGA2</i>	integrin, alpha 2	-1.64961	-1.5971	
	<i>TUBB6</i>	tubulin, beta6	-1.57568	-1.49258	
	<i>COL17A1</i>	collagen, type XVII, alpha 1, the transmembrane component of cutaneous basal membrane zone	-1.23374	-1.46532	
	<i>LAMB3</i>	laminin beta3	-1.64008	-1.43765	
	Epithelial Cytoskeleton	<i>KRT6B</i>	keratin 6B	-3.21289	-2.81739
		<i>KRT34</i>	keratin 34	-2.62775	-2.61525
<i>KRT14</i>		keratin 14 (epidermolysis bullosa simplex, Dowling-Meara, Koebner)	-3.77633	-2.24538	
<i>TPM4</i>		tropomyosin 4	-1.78543	-1.97184	
<i>KRT81</i>		keratin 81	-1.8799	-1.91926	
<i>KRT17</i>		keratin 17	-1.79489	-1.7026	
<i>KRT16</i>		keratin 16 (focal non-epidermolytic palmoplantar keratoderma)	-1.92604	-1.69184	
<i>KRT6C</i>		keratin 6C	-2.15716	-1.61961	
<i>KRT6A</i>		keratin 6A	-1.71376	-1.54036	
Metastasis suppressor		<i>S100A14</i>	s100 calcium binding protein a14	-1.82409	-2.76174
	<i>S100A3</i>	s100 calcium binding protein a3	-1.873	-2.21364	
	<i>S100A2</i>	S100 calcium binding protein A2	-1.95481	-1.84239	
Growth Factors/ Cytokines	<i>HBEGF</i>	heparin-binding EGF-like growth factor	-2.3136	-3.64493	
	<i>IGFL1</i>	IGF-like family member 1	-1.99918	-2.5052	
	<i>CYR61</i>	cysteine-rich, angiogenic inducer, 61	-1.83103	-2.18025	
	<i>FST</i>	follicle-stimulating hormone, transcript variant FST317	-1.89926	-1.90283	
Signaling	<i>ROS1</i>	c-ros oncogene 1, receptor tyrosine kinase	-1.55888	-1.85886	
	<i>LPAR1</i>	lysophosphatidic acid receptor 1	-1.65656	-1.38664	
	<i>TICAM1</i>	toll-like receptor adaptor molecule 1, activates nuclear factor kappa-B	-1.70659	-1.45353	
	<i>NRAS</i>	neuroblastoma RAS viral (v-ras) oncogene homolog	-2.5358	-1.86564	
	<i>RAP1B</i>	member of RAS oncogene family	-1.41695	-1.78678	
	<i>PAK6</i>	p21-activated kinase 6	-1.54128	-2.09613	
	<i>MAPK13</i>	mitogen-activated protein kinase 13	-2.00943	-2.22	
	<i>NET1</i>	neuroepithelial cell transforming gene 1, RhoGEF	-1.8819	-1.67742	
	<i>RTKN</i>	rhotekin	-1.33548	-1.52309	
	<i>DUSP14</i>	dual specificity phosphatase 14	-1.82221	-1.56145	
	<i>YWHAG</i>	14-3-3GAMMA	-1.79748	-1.5077	
	Other category	<i>CD24</i>	cd24 antigen (small cell lung carcinoma cluster 4 antigen)	-1.97114	-1.98974
		<i>ANGPTL4</i>	angiopoietin-like 4	-1.94266	-1.79609
		<i>MYO5C</i>	myosin VC	-1.97763	-1.50163

Rnd1 knockdown MCF10A and control cells (3 days of knockdown) were subjected to RNA extraction and microarray (Human HT-12 Expression BeadChip) analysis.

Supplementary Table 4: Cases positive for RND1 deletion by FISH related to Fig.8a and Supplementary Fig.8a

Patient ID	Del/total	% of Deletion	Tumor	TNM	Grade	ER	PR	HER2
1	63/110	57	IDC	T2 N1	3	NEG	NEG	POS 2+
2	90/120	73	IDC	T2 N0	3	NEG	NEG	NEG
3	22/108	20	other	T3 N0	3	NEG	NEG	POS 2+
4	44/130	34	IDC	T2 N1	3	POS	POS	POS 3+
5	16/80	20	IDC	T2 N2	3	POS	NEG	POS 2+
6	17/80	21	IDC	T1c N1	3	NEG	NEG	POS 2+
7	59/139	42	ILC	T1b N0	2	POS	POS	NEG
8	25/100	25	ILC	T2 N0	2	POS	NEG	NEG
9	18/84	21	IDC	T1c N1	3	NEG	NEG	POS 3+
10	18/82	22	IDC	T2 N1	2	NEG	NEG	POS 2+
11	65/261	25	IDC	T2 N1	2	POS	POS	POS 2+
12	36/100	36	IDC	T1b N0	2	POS	POS	POS 2+
13	20/100	20	IDC	T2 N0	2	POS	POS	POS 2+
14	36/100	36	IDC	T2 N3	2	POS	POS	NEG
15	28/139	20	IDC	T2 N1	3	POS	POS	POS 2+
16	26/100	26	IDC	T1b N0	2	POS	POS	POS 2+
17	56/125	45	ILC	T2 N1	2	POS	POS	NEG
18	64/128	50	other	T2 Nx	3	NEG	NEG	NEG
19	26/89	29	IDC	T2 N0	2	POS	POS	POS 3+

IDC: Invasive ductal carcinoma, ILC: Invasive lobular carcinoma, TNM classification: Primary tumor (T), T1: Tumor 2.0 cm or less in greatest dimension, (T1a: 0.1 cm < T < 0.5 cm, T1b: 0.5 cm < T < 1.0 cm, T1c: 1 cm < T < 2.0 cm), T2: Tumor more than 2.0 cm but not more than 5.0 cm, T3: Tumor more than 5.0 cm, Regional lymph nodes (N), NX: Regional lymph nodes cannot be assessed (e.g., previously removed), N0: No regional lymph node metastasis, N1: Metastasis to movable ipsilateral axillary lymph node(s), N2: Metastasis to ipsilateral axillary lymph node(s) fixed to each other or to other structure, N3: Metastasis to ipsilateral internal mammary lymph node(s).

Supplementary Table 5: Solid sequencing results for each mutation of RND1 related to Fig.8

pool	Protein Mut	aCtiDoSn Mutatio	Codon	Genomic Coordinates	Mutation Type	Position in hg19	Ref	Var	Reads1	Reads2	VarFreq (%)	Pvalue
Pool---2	p.G70R	c.208G>C	GGA	chr12:49255900 --- 49258566	Missense	49258566	C	G	16568	269	1.6	3.8784E--61
Pool---4	p.E98D	c.294G>C	GAG	chr12:49255816 --- 49255818	Missense	49255816	C	G	8464	219	2.52	2.0376E--55
Pool---1	p.F180C	c.539T>G	TTT	chr12:49251938 --- 49251940	Missense	49251939	A	C	23240	1798	7.18	0
Pool---4	p.M185V	c.553A>G	ATG	chr12:49251923 --- 49251925	Missense	49251925	T	C	16621	1288	7.19	0

Supplementary Table 6: Antibody list used in this study

Antibodies	Company	Catalogue No	Clone No	Dilution Western Blot	IF
Rac1	Upstate	05---389	23A8	1/1000	
RhoGDI	Santa Cruz	SC360		1/4000	
Cyclin D1 (A---12)	Santa Cruz	SC8396		1/1000	
Cyclin A (H432)	Santa Cruz	SC751		1/1000	

cMyc (N262)	Santa Cruz	SC764			1/1000	
Erk2 (c---14)	Santa Cruz	SC154			1/1000	
FAK (H1)	Santa Cruz	SC1688			1/1000	
Rho	Santa Cruz	SC179			1/1000	
H---Ras	Santa Cruz	SC520			1/1000	
p53	Santa Cruz	SC263			1/1000	
p21	Santa Cruz	SC6246			1/1000	
AKT	Santa Cruz	SC1618			1/1000	
RAF	Santa Cruz	SC133			1/1000	
RasGAP (B4F8)	Santa Cruz	SC63			1/1000	
Rap1GAP (H93)	Santa Cruz	SC28189			1/1000	
VSVG	sigma	V5507		P5D4	1/1000	
phospho Rb	cell signaling		9308		1/1000	
phospho H3	cell signaling		9701		1/1000	
phospho H2AX	cell signaling		2577		1/1000	
phospho CHK2	cell signaling		2661		1/1000	
phospho ERK	cell signaling		9101		1/2000	
phospho AKT	cell signaling		9277		1/1000	
phospho RAF	cell signaling		9427		1/1000	
phospho MEK	cell signaling		9121		1/1000	
Histone H3	cell signaling		9715		1/1000	
MEK	cell signaling		9122		1/1000	
HER2	cell signaling		2242		1/1000	
phospho HER2	cell signaling	2247/2249			1/1000	
MET	cell signaling		3148		1/1000	
phospho MET	cell signaling		3126		1/1000	
Rap1A/B	cell signaling		2399		1/1000	
SUZ12	cell signaling		3737		1/1000	
cleaved caspase---3	cell signaling		9661		1/1000	
Bmi1	cell signaling		5856		1/1000	1/100
Ras	BD biosciences		6100001		18 1/5000	
Rap1	BD biosciences		610196		3 1/1000	
E---cadherin	BD biosciences		610181		36 1/1000	
alpha catenin	BD biosciences		610193		5 1/1000	1/200
beta catenin	BD biosciences		610153		14 1/1000	
beta catenin	Zymed/Invitrogen	71-2700			1/1000	
p120 catenin	BD biosciences		610133		98 1/1000	1/100
fibronectin	BD biosciences		610078		10 1/1000	
N---cadherin	BD biosciences		610920		32 1/1000	
RhoGDI	BD biosciences		610255		16 1/1000	
CDC42	BD biosciences		610928		44 1/1000	
EZH2	BD biosciences		612666		11 1/3000	
p27	BD biosciences		610241		57 1/1000	
Semaphorin 4D	BD biosciences		610670		30 1/2000	
GM130	BD biosciences		610822		35 1/1000	
PlexinB1	R&D	MAB10402		19A.7.1	1/1000	1/100
K---Ras	Abnova	H00003845-M02		4F3	1/1000	
Rb	Pharminen	554136		G3-245	1/1000	
Plexin A1	Millipore	MAB10419		14A.1.2	1/1000	1/100
Myc Tag	MSKCC	9E10 MSKCC			1/1000	
Ki---67	Novocastra	NCL-Ki67-MM1		MM1	1/1000	
HA tag	Covance (Babco)	MMS-101R		16B12	1/3000	1/100
c---MYC (9E10)	Santa Cruz	SC40	9E10		1/1000	
ZO---1	Zymed/Invitrogen	40-2300				1/100
Vimentin	Pharminen		550513 RV202		1/3000	1/100
Actin beta	sigma	A5316	AC---74		1/10000	1/100
Tubulin alpha	sigma	T9026	DM1A		1/10000	

Supplementary Table 7: Q-PCR Primer list

Gene name	Applied Biosystem ID	Species
ARHE (RhoE)	Hs00170603_m1	homo sapiens
CCNB2	Hs00270424_m1	homo sapiens
CDC2	Hs00938777_m1	homo sapiens
CDCA1(NUF2)	Hs00230097_m1	homo sapiens
CDH1 (E---Cadherin)	Hs00170423_m1	homo sapiens
CDKN1A (p21)	Hs00355782_m1	homo sapiens
Dusp 3	Hs00187940_m1	homo sapiens
Dusp1	Hs00610256_g1	homo sapiens
FOXC2	Hs00270951_s1	homo sapiens
GAPDH	Hs99999905_m1	homo sapiens
GAPDH	PART NUMBER 4352662	mus musculus
GATA3	Hs00231122_m1	homo sapiens

MMP14	Hs01037009_g1	homo sapiens
MMP2	Hs01548733_m1	homo sapiens
MMP3	Hs00968308_m1	homo sapiens
Nf1	Hs00169714_m1	homo sapiens
PLK1	Hs00983227_m1	homo sapiens
RhoD	Hs00205854_m1	homo sapiens
RND1	Hs01109198_m1	homo sapiens
RND1	Mm0130221_m1	mus musculus
RND1	Mm00553835_m1	mus musculus
SNAIL1	Hs00195591_m1	homo sapiens
SNAIL2	Hs00950344_m1	homo sapiens
Spread1	Hs00544790_m1	homo sapiens
Spread2	Hs00986220_m1	homo sapiens
Spry2	Hs00183386_m1	homo sapiens
Spry3	Hs01921738_s1	homo sapiens
Spry4	Hs00540086_m1	homo sapiens
TGFB1	Hs99999918_m1	homo sapiens
TWIST1	Hs00361186_m1	homo sapiens
Zeb1	Hs01566407_m1	homo sapiens
Zeb2	Hs00207691_m1	homo sapiens