Tata et al., http://www.jcb.org/cgi/content/full/jcb.201309004/DC1

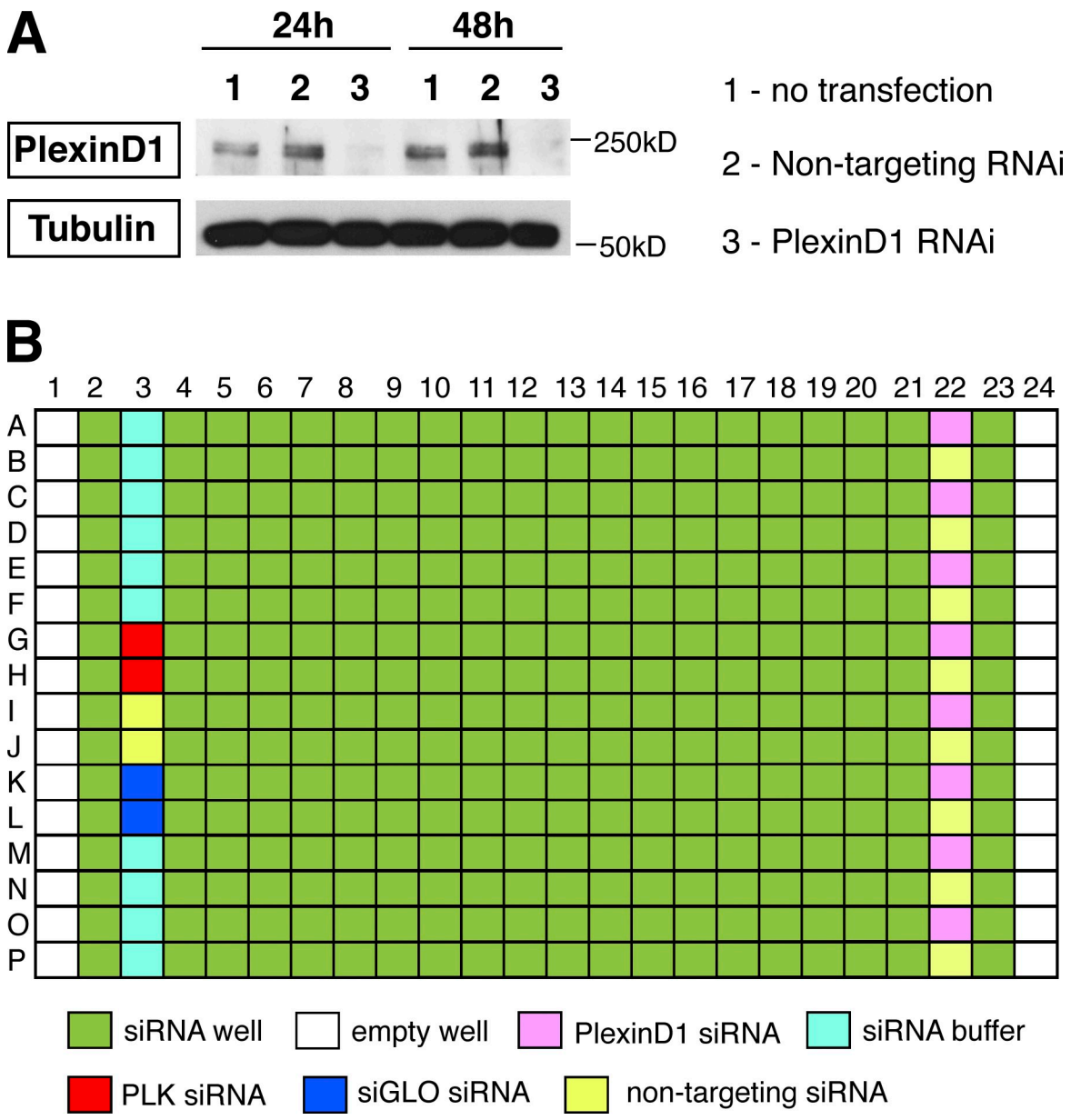


Figure S1. **Endogenous PlexinD1 expression in HUVECs could be down-regulated by specific PlexinD1 siRNA and used as a positive control in the screen.** (A) PlexinD1 siRNA led to a depletion in endogenous PlexinD1 in HUVECs. HUVECs transfected with PlexinD1 siRNA were lysed and analyzed by immunoblotting with PlexinD1 antibody. Endogenous PlexinD1 was efficiently depleted 24 h and 48 h after transfection with PlexinD1 siRNA. Tubulin served as a loading control. (B) Schematic representation of a 384-well plate with all the necessary controls for our screen. PlexinD1 siRNA was used in each plate as a positive control.

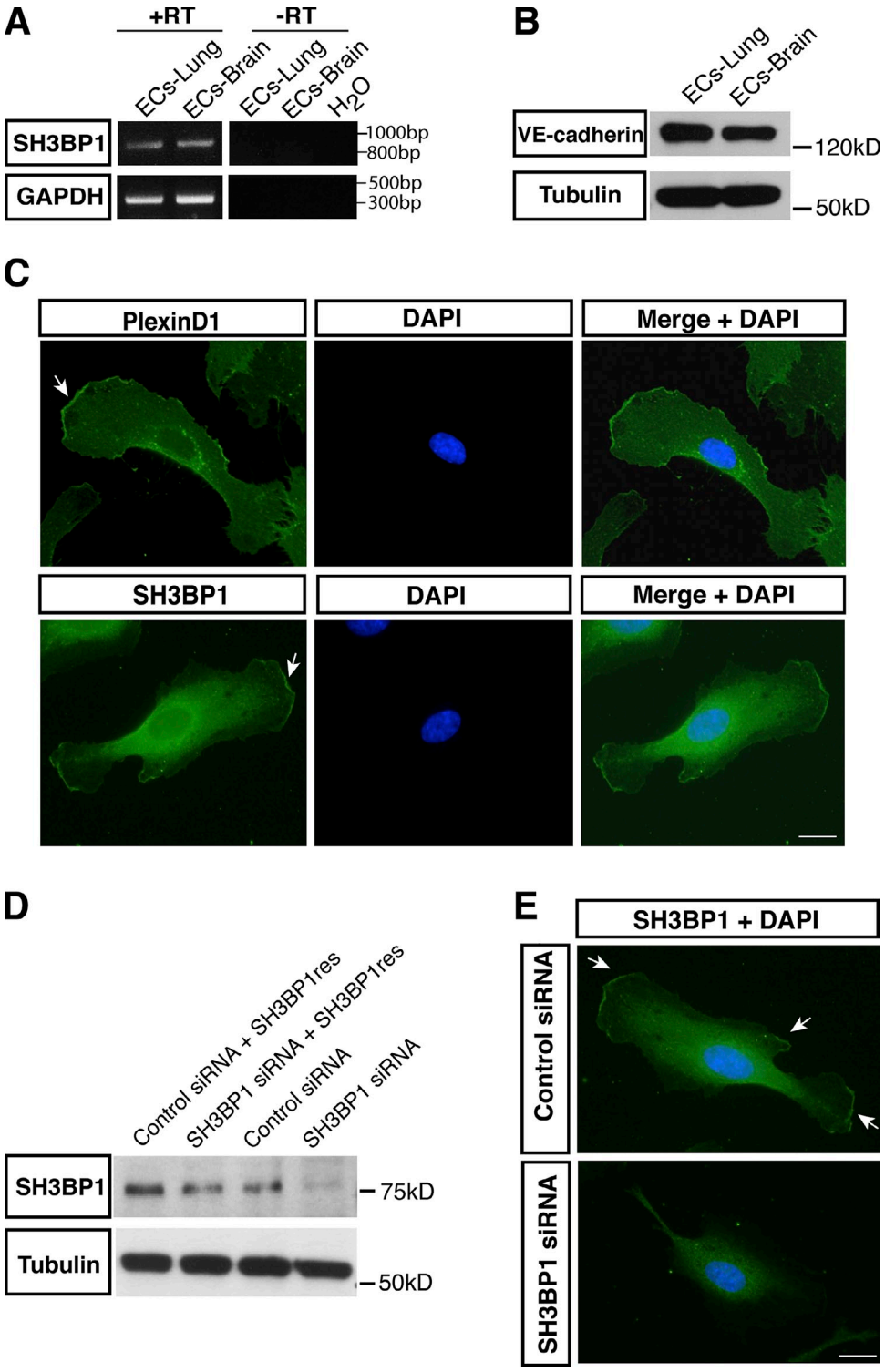


Figure S2. **Expression of SH3BP1 and specificity of SH3BP1 siRNA.** (A) SH3BP1 was expressed in lung and brain ECs isolated from E18.5 mouse embryos. RT-PCR with SH3BP1 and GAPDH primers is shown. (B) Cells used for RT-PCR were indeed ECs. Western blotting with endothelial marker VE-cadherin is shown. Tubulin served as a loading control. (C) SH3BP1 was expressed in HUVECs in a similar pattern as PlexinD1. Immunohistochemistry with SH3BP1 and PlexinD1 antibodies is shown. Arrows indicate the localization of SH3BP1 and PlexinD1 in the leading edge of the cell. Bar, 10 µm. (D) Specific SH3BP1 siRNA down-regulated SH3BP1 protein. HUVECs were transfected with SH3BP1 siRNA alone or together with an siRNA-resistant rescue construct, lysed, and analyzed by immunoblotting with SH3BP1 antibody. Endogenous SH3BP1 was efficiently depleted in SH3BP1 siRNA–transfected cells, but depletion of SH3BP1 could be restored by an siRNA-resistant rescue construct. Tubulin served as a loading control. (E) SH3BP1 siRNA down-regulated SH3BP1 in the leading edge of the cell. Cells were transfected with control or SH3BP1 siRNA and immunostained with SH3BP1 antibody. SH3BP1 was depleted in SH3BP1 siRNA–transfected cells in the leading edge of the cells. Some level of cytoplasmic expression was still observed. Control cells showed unchanged localization of SH3BP1 protein. Arrows indicate unchanged localization of SH3BP1. Bar, 10 µm.

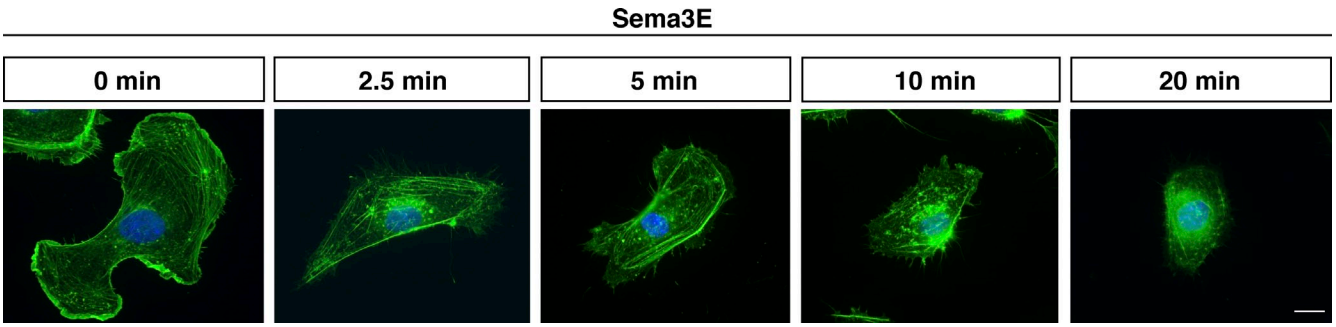


Figure S3. **Sema3E-regulated cytoskeleton changes.** HUVECs were treated with Sema3E at 0, 2.5, 5, 10, and 20 min, and stained with phalloidin to label actin cytoskeleton. Noticeable changes were already observed at 2.5 and 5 min after ligand treatment. Strong actin changes were present at later time points of Sema3E treatment. Bar, 10 µm.

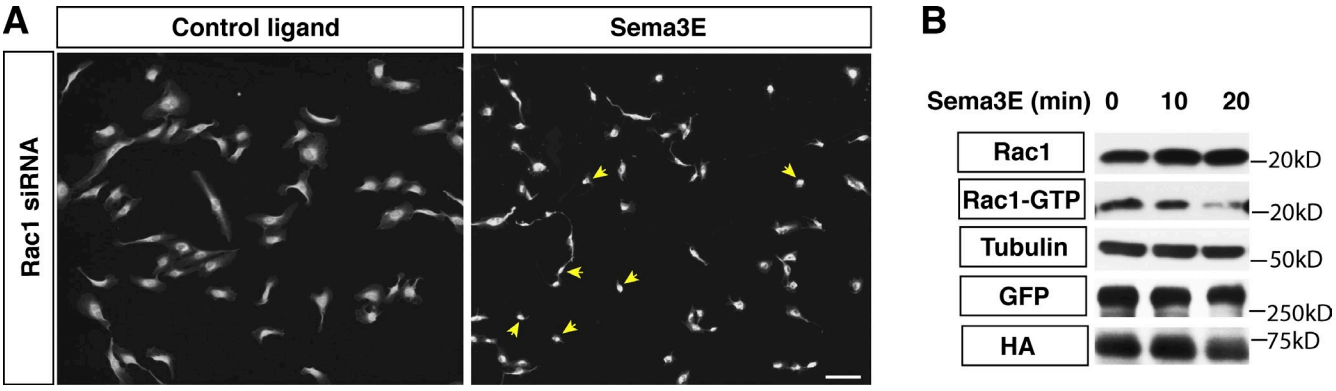


Figure S4. **Rac1 is regulated by Sema3E.** (A) Depletion of Rac1 led to a hypercollapse of HUVECs upon Sema3E treatment. Automated imaging of Rac1 siRNA–transfected cells treated with Sema3E or control ligand is shown. Arrows indicate hypercollapsed cells. Bar, 100 µm. (B) Rac1 activity in HEK293T cells. HEK293T cells were overexpressed with PlexinD1-GFP and SH3BP1-HA constructs. After 48 h, cells were treated with Sema3E for 0, 10, and 20 min, and Rac1 activity pull-down was performed. Rac1-GTP is down-regulated after 20 min of Sema3E treatment. Rac1-GTP, total Rac1, GFP, HA, and Tubulin blots are shown.

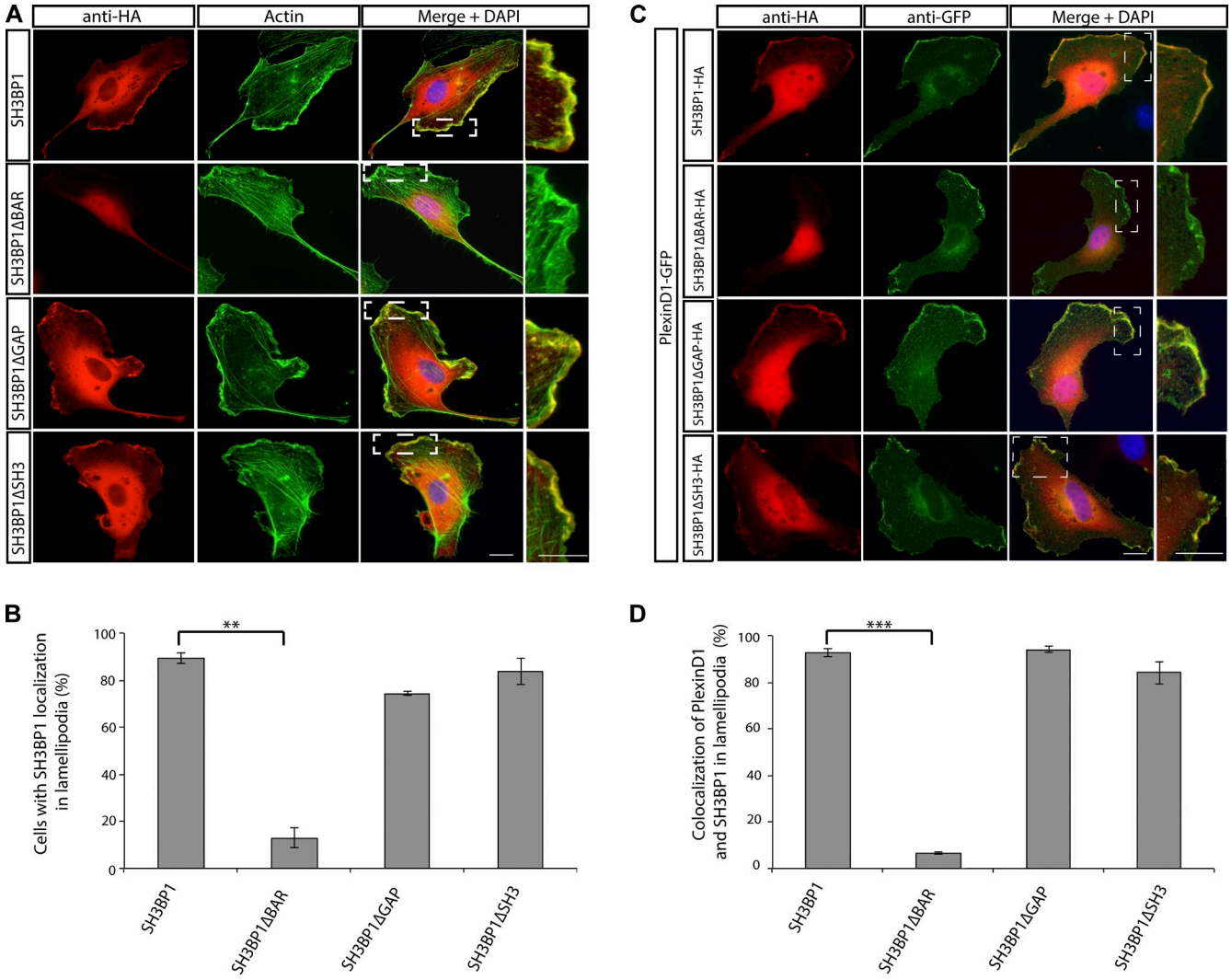
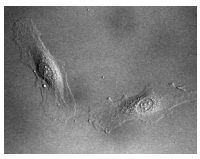


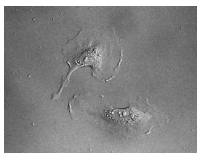
Figure S5. **SH3BP1ΔBAR did not colocalize with PlexinD1 in the lamellipodia.** (A) Localization of SH3BP1 constructs in lamellipodia. HUVECs were transfected with SH3BP1 constructs and stained with HA antibody and phalloidin. SH3BP1, SH3BP1ΔGAP, and SH3BP1ΔSH3 were present in cell lamellipodia, whereas SH3BP1ΔBAR localization was seen mostly in the cytoplasm. Boxed regions are enlarged on the right. Bar, 10 µm. (B) Quantification of the results presented in A. $n = 3$; **, $P < 0.001$. Error bars indicate SEM. (C) Colocalization of SH3BP1 constructs with PlexinD1 at the leading edge of the cell. HUVECs were cotransfected with PlexinD1 and SH3BP1 constructs and stained with GFP and HA antibodies. SH3BP1ΔBAR did not colocalize with PlexinD1 at the leading edge of the cell. Boxed regions are enlarged on the right. Bar, 10 µm. (D) Quantification of the results presented in C. $n = 3$; ***, $P < 0.0001$. Error bars indicate SEM.

Table S1. **Exemplar genes demonstrating Sema3E-induced hypercollapse following gene down-regulation**

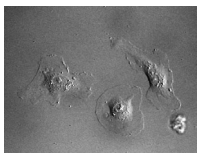
Gene symbol	GenBank accession number	Gene name
CNKSR1	NM_006314	Connector Enhancer of kinase suppressor of Ras 1
COPB2	NM_004766	Coatomer Protein Complex, Subunit β2
STYK1	NM_018423	Serine/threonine/tyrosine kinase 1
CARD14	NM_024110	Caspase recruitment domain family, member 14
AKAP11	NM_016248	A kinase (PRKA) anchor protein 11
ALK	NM_004304	Anaplastic lymphoma receptor tyrosine kinase
BUB1B	NM_001211	BUB1 mitotic checkpoint serine/threonine kinase B
BLNK	NM_013314	B-cell linker
AVPR1B	NM_000707	Arginine vasopressin receptor 1B
ILK	NM_004517	Integrin-linked kinase
RAC1	NM_018890	Ras-related C3 botulinum toxin substrate 1
PRPS2	NM_002765	Phosphoribosyl pyrophosphate synthetase 2
KIT	NM_000222	V-kit Hardy-Zuckerman 4 teline sarcoma viral oncogene homolog
PRKCN	NM_005813	Protein kinase D3
PDGFRB	NM_002609	Platelet-derived growth factor receptor, β polypeptide
RIOK3	NM_003831	RIO kinase 3
RPS6KA3	NM_004586	Ribosomal protein S6 kinase 90kD polypeptide
TYK2	NM_003331	Tyrosine kinase 2
TLK1	NM_012290	Tousled-like kinase 1
TGFBR2	NM_003242	Transforming growth factor, beta receptor II



Video 1. **Control siRNA-transfected cells underwent morphological changes and exhibited cell collapse in response to Sema3E treatment.** Control siRNA–transfected HUVECs were analyzed after addition of Sema3E ligand with a microscope (VivaView FL LCV-110; Olympus). Images were taken every 10 s for 20 min (frame rate of replay: 7 frames per second).



Video 2. **PlexinD1 siRNA-transfected cells did not display any morphological changes after Sema3E treatment.** PlexinD1 siRNA–transfected HUVECs were analyzed after the addition of Sema3E ligand with a microscope (VivaView FL LCV-110; Olympus). Images were taken every 10 s for 20 min (frame rate of replay: 7 frames per second).



Video 3. **SH3BP1 siRNA-transfected cells did not display any morphological changes after Sema3E treatment.** SH3BP1 siRNA–transfected HUVECs were analyzed after the addition of Sema3E ligand with a microscope (VivaView FL LCV-110; Olympus). Images were taken every 10 s for 20 min (frame rate of replay: 7 frames per second).

Table S2 gives a list of genes identified in the secondary screen.

A custom MATLAB algorithm is available for download as a TXT file. This program processes the images after they have been segmented by CellProfiler, analyzes the protrusions of each cell by shrinking the cells and comparing with the original one, measures object properties, and merges sections belonging to the same cell back together.

1. Transcription factors			
Specific			
Gene Symbol	Hit	Genbank accession number	Gene name
LITAF	2/4	NM_004862	lipopolysaccharide-induced TNF factor
TRIM29	2/4	NM_012101	tripartite motif-containing 29
ZNF228	2/4	NM_013380	zinc finger protein 228
ZNF444	2/4	NM_018337	zinc finger protein 444
FOXA1	2/4	NM_004496	forkhead box A1
HEY1	2/4	NM_012258	hairy/enhancer-of-split related with YRPW motif 1
NFIC	2/4	NM_005597	nuclear factor I/C (CCAAT-binding transcription factor)
TRIM8	2/4	NM_030912	tripartite motif-containing 8
IRF3	3/4	NM_001571	interferon regulatory factor 3
KLF13	3/4	NM_015995	Kruppel-like factor 13
NKX3	3/4	NM_006167	NK3 transcription factor related, locus 1 (Drosophila)
DMRT2	3/4	NM_181872	doublesex and mab-3 related transcription factor 2
HES4	3/4	NM_021170	hairy and enhancer of split 4 (Drosophila)
CREG1	4/4	NM_003851	cellular repressor of E1A-stimulated genes 1
Non-Specific			
SET	2/4	NM_003011	SET translocation (myeloid leukemia-associated)
SETMAR	2/4	NM_006515	SET domain and mariner transposase fusion gene
UHRF1	2/4	NM_013282	ubiquitin-like, containing PHD and RING finger domains, 1
EP300	2/4	NM_001429	E1A binding protein p300

GTF2H4	2/4	NM_001517	general transcription factor IIH, polypeptide 4, 52kDa
MBD3	2/4	NM_003926	methyl-CpG binding domain protein 3
PCAF	3/4	NM_003884	p300/CBP-associated factor
MIZF	4/4	NM_015517	MBD2-interacting zinc finger
Histone			
HIST1H1C	2/4	NM_005319	histone cluster 1, H1c
CHAF1B	3/4	NM_005441	chromatin assembly factor 1, subunit B (p60)
HIST2H2BE	3/4	NM_003528	histone cluster 2, H2be
2. Translation Factors			
Specific			
Gene Symbol	Hit	Genbank accession number	Gene name
SNRPN	2/4	NM_003093	small nuclear ribonucleoprotein polypeptide C
TCEFG1	2/4	NM_006706	transcription elongation regulator 1
GRSF1	2/4	NM_002092	G-rich RNA sequence binding factor 1
HNRPF	3/4	NM_004966	heterogeneous nuclear ribonucleoprotein F
GEMIN4	3/4	NM_015721	gem (nuclear organelle) associated protein 4
LSM5	4/4	NM_012322	LSM5 homolog, U6 small nuclear RNA associated (S. cerevisiae)
Non-specific			
EIF3S1	2/4	NM_003758	eukaryotic translation initiation factor 3, subunit 1 alpha, 35kDa
GTF2H4	2/4	NM_001517	general transcription factor IIH, polypeptide 4, 52kDa

MRPS33	2/4	NM_016071	mitochondrial ribosomal protein S33
EIF1AY	4/4	NM_004681	eukaryotic translation initiation factor 1A, Y-linked
3. Endocytosis/ Exocytosis/ Vesicle/ Protein Trafficking			
Gene Symbol	Hit	Genbank accession number	Gene name
ARC	3/4	NM_015193	activity-regulated cytoskeleton-associated protein
NECAP1	3/4	NM_015509	NECAP endocytosis associated 1
RAB3-GAP150	2/4	NM_012414	RAB3 GTPase activating protein subunit 2 (non-catalytic)
STXBP6	3/4	NM_014178	syntaxin binding protein 6 (amisyn)
SLC30A3	2/4	NM_003459	solute carrier family 30 (zinc transporter), member 3
TBL1X	2/4	NM_005647	transducin (beta)-like 1X-linked
SNX1	2/4	NM_003099	sorting nexin 1
GOLPH3L	3/4	NM_018178	golgi phosphoprotein 3-like
KIF20A	3/4	NM_005733	kinesin family member 20A
4. Cytoskeletal Proteins and Regulatory Proteins			
Cytoskeletal Components			
Gene Symbol	Hit	Genbank accession number	Gene name
TUBB4	3/4	NM_006087	tubulin, beta 4
KIF11	4/4	NM_004523	kinesin family member 11
Regulators of Cytoskeletal Components			
BAIAP2L1	2/4	NM_018842	BAI1-associated protein 2-like 1
MAPK8IP3	2/4	NM_015133	mitogen-activated protein kinase 8 interacting protein 3

TBL1X	2/4	NM_005647	transducin (beta)-like 1X-linked
MSN	3/4	NM_002444	moesin
KIF20A	3/4	NM_005733	kinesin family member 20A
5. Cell Division			
Cycle Regulators			
Gene Symbol	Hit	Genbank accession number	Gene name
GPC1	2/4	NM_002081	glypican 1
HUS1B	2/4	NM_148959	HUS1 checkpoint homolog b (S. pombe)
CAPRIN1	3/4	NM_005898	cell cycle associated protein 1
CCNE1	3/4	NM_001238	cyclin E1
SFRP1	3/4	NM_003012	secreted frizzled-related protein 1
CCND1	4/4	NM_053056	cyclin D1
KIF11	4/4	NM_004523	kinesin family member 11
DNA Regulators			
CNAP1	2/4	NM_014865	non-SMC condensin I complex, subunit D2
SET	2/4	NM_003011	SET translocation (myeloid leukemia-associated)
RAD21	2/4	NM_006265	RAD21 homolog (S. pombe)
RBMS2	2/4	NM_002898	RNA binding motif, single stranded interacting protein 2
RRM1	3/4	NM_001033	ribonucleotide reductase M1 polypeptide
ESPL1	3/4	NM_012291	extra spindle pole bodies homolog 1 (S. cerevisiae)
SMC4L1	3/4	NM_005496	structural maintenance of chromosomes 4

NIN	4/4	NM_016350	ninein (GSK3B interacting protein)
POLA2	4/4	NM_002689	polymerase (DNA directed), alpha 2 (70kD subunit)
MYBL2	4/4	NM_002466	v-myb myeloblastosis viral oncogene homolog (avian)-like 2
INCENP	4/4	NM_020238	inner centromere protein antigens 135/155kDa
TOP2A	4/4	NM_001067	topoisomerase (DNA) II alpha 170kDa
6. Transmembrane Proteins			
Known Receptors			
Gene Symbol	Hit	Genbank accession number	Gene name
ADCY7	2/4	NM_001114	adenylate cyclase 7
THRB	2/4	NM_000461	thyroid hormone receptor, beta (erythroblastic leukemia viral (v-erb-a) oncogene homolog 2, avian)
FZD9	2/4	NM_003508	frizzled homolog 9 (Drosophila)
FFAR1	3/4	NM_005303	free fatty acid receptor 1
EPOR	4/4	NM_000121	erythropoietin receptor
PLEXIND1	4/4	NM_015103	plexin D1
Other Transmembrane Proteins			
CAV3	2/4	NM_001234	caveolin 3
GJA3	2/4	NM_021954, NM_029726	gap junction protein, alpha 3, 46kDa
LMNB2	2/4	NM_032737	lamin B2
MUCDHL	2/4	NM_017717	mucin-like protocadherin
XPNPEP2	2/4	NM_003399	X-prolyl aminopeptidase (aminopeptidase P) 2, membrane-bound

POM121	3/4	NM_172020	POM121 membrane glycoprotein (rat)
SLC13A4	3/4	NM_012450	solute carrier family 13 (sodium/sulfate symporters), member 4
GPR27	4/4	NM_018971	G protein-coupled receptor 27
Ion Channels			
CLIC2	2/4	NM_001289	chloride intracellular channel 2
CLCA4	2/4	NM_012128	chloride channel, calcium activated, family member 4
KCND1	2/4	NM_004979	potassium voltage-gated channel, Shal-related subfamily, member 1
KCNK9	2/4	NM_016601	potassium channel, subfamily K, member 9
KCNA5	3/4	NM_002234	potassium voltage-gated channel, shaker-related subfamily, member 5
Ligands/ Secreted Proteins			
GDNF	2/4	NM_000514, NM_015802	glial cell derived neurotrophic factor
RBP4	3/4	NM_006744	retinol binding protein 4, plasma
SERPINC1	3/4	NM_000488	serpin peptidase inhibitor, clade C (antithrombin), member 1
SFRP1	3/4	NM_003012	secreted frizzled-related protein 1
7. Second Messenger Associated Proteins			
Ca2+ Related			
Gene Symbol	Hit	Genbank accession number	Gene name

CLCA4	2/4	NM_012128	chloride channel, calcium activated, family member 4
STRN3	2/4	NM_014574	striatin, calmodulin binding protein 3
VSNL1	2/4	NM_003385	visinin-like 1
FFAR1	3/4	NM_005303	free fatty acid receptor 1
CAMK2N1	3/4	NM_018584	calcium/calmodulin-dependent protein kinase II inhibitor 1
cAMP Related			
ADCY7	2/4	NM_001114	adenylate cyclase 7
EP300	2/4	NM_001429	E1A binding protein p300
8. Signaling Molecules			
Small GTPases/ Regulatory Proteins			
Gene Symbol	Hit	Genbank accession number	Gene name
RAB3GAP2	2/4	NM_012414	RAB3 GTPase activating protein subunit 2 (non-catalytic)
SH3BP1	2/4	NM_018957	SH3-domain binding protein 1
RAP2A	3/4	NM_021033	RAP2A, member of RAS oncogene family
KIF20A	3/4	NM_005733	kinesin family member 20A
RACGAP1	4/4	NM_013277	Rac GTPase activating protein 1
Signaling Kinases/ Phosphatases			
DGKZ	2/4	NM_003646	diacylglycerol kinase, zeta 104kDa
IRAK1	2/4	NM_001569	interleukin-1 receptor-associated kinase 1
MAPKAPK5	2/4	NM_003668	mitogen-activated protein kinase-activated protein kinase 5

MINK1	2/4	NM_015716	misshapen-like kinase 1 (zebrafish)
AKAP10	3/4	NM_007202	A kinase (PRKA) anchor protein 10
Other Signaling Molecules			
HBP17	2/4	NM_005130	fibroblast growth factor binding protein 1
PROSAPIP2	2/4	XM_375469	TBK1 binding protein 1
CCL4	2/4	NM_002984	chemokine (C-C motif) ligand 4
SRGAP2	2/4	NM_015326, XM_059095	SLIT-ROBO Rho GTPase activating protein 2
GADD45A	3/4	NM_001924	growth arrest and DNA-damage-inducible, alpha
9. Apoptosis			
Pro-Apoptotic			
Gene Symbol	Hit	Genbank accession number	Gene name
NCR1	3/4	NM_004829	natural cytotoxicity triggering receptor 1
CASP8AP2	4/4	NM_012115	CASP8 associated protein 2
Anti-Apoptotic			
BRE	2/4	NM_004899	brain and reproductive organ-expressed (TNFRSF1A modulator)
BIRC5	2/4	NM_001168	baculoviral IAP repeat-containing 5 (survivin)
SIVA1	3/4	NM_006427	SIVA1, apoptosis-inducing factor
10. Immune System			

Gene Symbol	Hit	Genbank accession number	Gene name
DEFB1	2/4	NM_005218	defensin, beta 1
11. Basic Cell Biological Function			
Gene Symbol	Hit	Genbank accession number	Gene name
ALDH18A1	2/4	NM_002860	aldehyde dehydrogenase 18 family, member A1
ARSA	2/4	NM_000487	arylsulfatase A
CAD	2/4	NM_004341	carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase
CYP11B1	2/4	NM_000497	cytochrome P450, family 11, subfamily B, polypeptide 1
GALNAC4S-6ST	2/4	NM_014863	B cell RAG associated protein
PAPSS1	2/4	NM_005443	3'-phosphoadenosine 5'-phosphosulfate synthase 1
POLG	2/4	NM_002693	polymerase (DNA directed), gamma
RBP3	2/4	NM_006744	retinol binding protein 4, plasma
ACOX1	2/4	NM_004035	acyl-Coenzyme A oxidase 1, palmitoyl
MGAT1	2/4	NM_002406	mannosyl (alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase
FTL	3/4	NM_000146	ferritin, light polypeptide
CPS1	3/4	NM_001875	carbamoyl-phosphate synthetase 1, mitochondrial
12. Not Otherwise Classified			
Gene Symbol	Hit	Genbank accession number	Gene name
PHEMX	2/4	NM_005705	tetraspanin 32
PRKDC	2/4	NM_006904	protein kinase, DNA-activated, catalytic polypeptide

NEUGRIN	2/4	NM_016645	neugrin, neurite outgrowth associated
PRG-3	3/4	NM_017753	plasticity related gene 3
13. No Functional Data			
Gene Symbol	Hit	Genbank accession number	Gene name
CPorf98	2/4	NM_152572	chromosome 9 open reading frame 98
PID1	2/4	NM_017933	phosphotyrosine interaction domain containing 1
PTCD3	2/4	NM_017952	Pentatricopeptide repeat domain 3
ZNF646	2/4	NM_014699	zinc finger protein 646
LY6H	2/4	NM_002347	lymphocyte antigen 6 complex, locus H
MAPBPIP	2/4	NM_014017	mitogen-activated protein-binding protein-interacting protein
RAB3IL1	2/4	NM_013401	RAB3A interacting protein (rabin3)-like 1
PDE6H	2/4	NM_006205	phosphodiesterase 6H, cGMP-specific, cone, gamma
ANKIB1	2/4	XM_377955	ankyrin repeat and IBR domain containing 1
ZUBR1	2/4	NM_020765	zinc finger, UBR1 type 1
SCML1	2/4	NM_006746	sex comb on midleg-like 1 (Drosophila)
IK	3/4	NM_006083	IK cytokine, down-regulator of HLA II
SPATA3	3/4	NM_139073	spermatogenesis associated 3
C20orf4	3/4	NM_015511	chromosome 20 open reading frame 4
NMB	3/4	NM_021077	neuromedin B
LRRC20	4/4	NM_018205	leucine rich repeat containing 20
C16orf28	4/4	NM_023076	chromosome 16 open reading frame 28