

SUPPORTING INFORMATION

Figure S1. Herceptin treatment resulted in increased accumulation of p27^{Kip1} protein. Western blot of total cellular proteins from SK-OV-3 or BT-474 cells treated with 8 µg/ml of Herceptin (Hercep), or untreated control cells (untreat), for the times indicated. Equal amount of proteins were resolved on sodium dodecyl sulfate-polyacrylamide gels and transferred to a 0.2 µm nitrocellulose membrane. The blots were probed with antibodies detecting ErbB2 (A and B) or p27^{Kip1} (C and D) as indicated.

Figure S2. Pathway analysis using the Ingenuity knowledge base in BT-474 cells. (A) Relationships among phosphoproteins identified in the BT-474 cells in the absence of Herceptin. (B) Relationships among phosphoproteins identified in the BT-474 cells following Herceptin treatment for 2 days. The proteins with a grey background were identified in this study, while proteins with a white background are additional participant in the signaling network.

Figure S3. Western blot showing the presence of SFRS10 protein in the anti-phosphotyrosine IP from SK-OV-3 cells. SK-OV-3 cells were grown in the absence of Herceptin and proteins were fractionated from the cell lysate using an anti-phosphotyrosine antibody. Fractionated proteins were resolved on sodium dodecyl sulfate-polyacrylamide gels and transferred to a 0.2 µm nitrocellulose membrane. The blots were probed with antibodies detecting SFRS10 protein.

Figure S4. Cell cycle analysis of SK-OV-3 cells. SK-OV-3 cells (1,500 cells/well, 384-well plate) were transfected with pooled siRNAs for the indicated genes using

lipofectamine-2000. Three days after transfection cells were fixed and DNA was stained with DAPI. Fluorescent intensities of single nuclei were measured using quantitative 3-dimensional microscope and the cell cycle profiles were computed using CYTOSHOP software. Note that the transfection of siRNAs targeting both the SFRS10 and Aurora-B genes increased the number of SK-OV-3 cells in G₂/M phase; however, depletion of only SFRS10 gene reduced the migration of SK-OV-3 cells (Figure 4).

Table S1. Sequence of the siRNAs used to deplete the expression of the indicated genes.

Table S2. Sequences of indicated primers used for SYBR green quantitative PCR analysis following siRNA-mediated suppression of target mRNA expression.

Table S3. Motif analysis of the phosphoproteins identified from the ErbB2-overexpressing cancer cell lines. Analysis was done using the “motif-x” program by selecting tyrosine as the central character and human proteome as the background.

Table S4. A list of proteins, identified in the ErbB2-overexpressing cancer cell lines, which contained the indicated enriched domains.

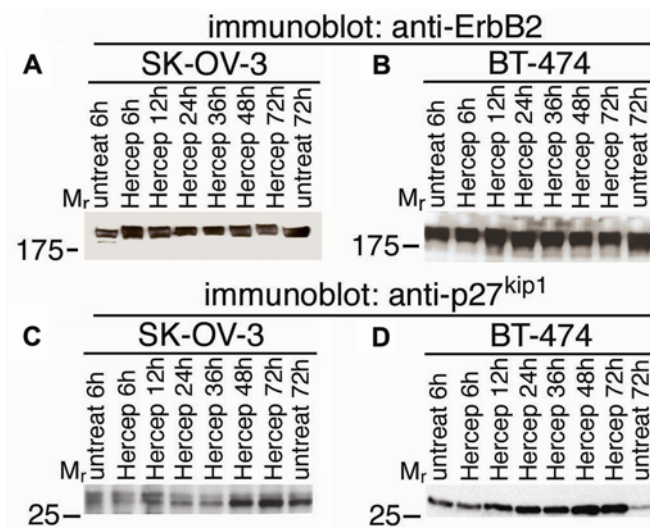


FIGURE S1

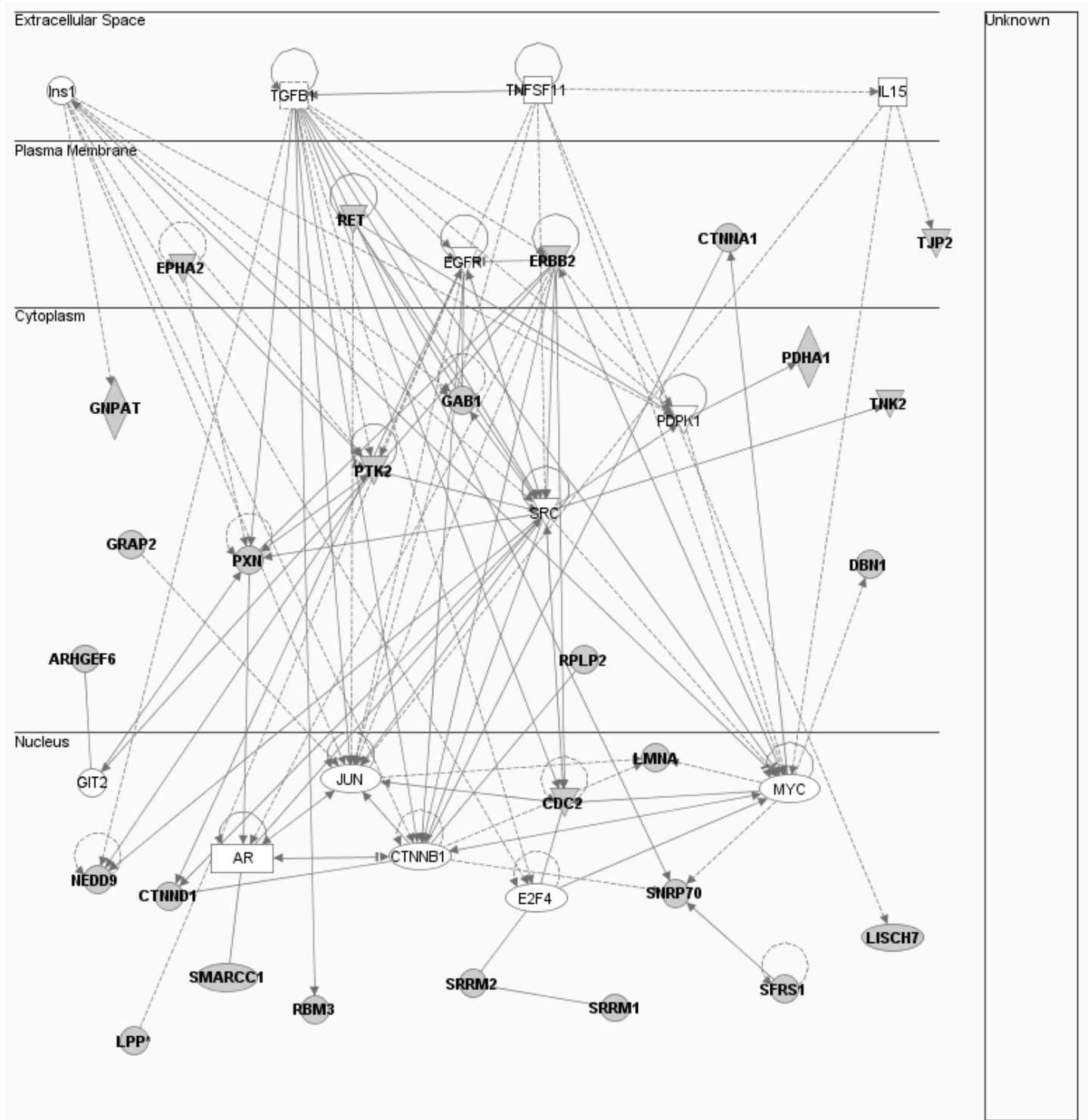


FIGURE S2A

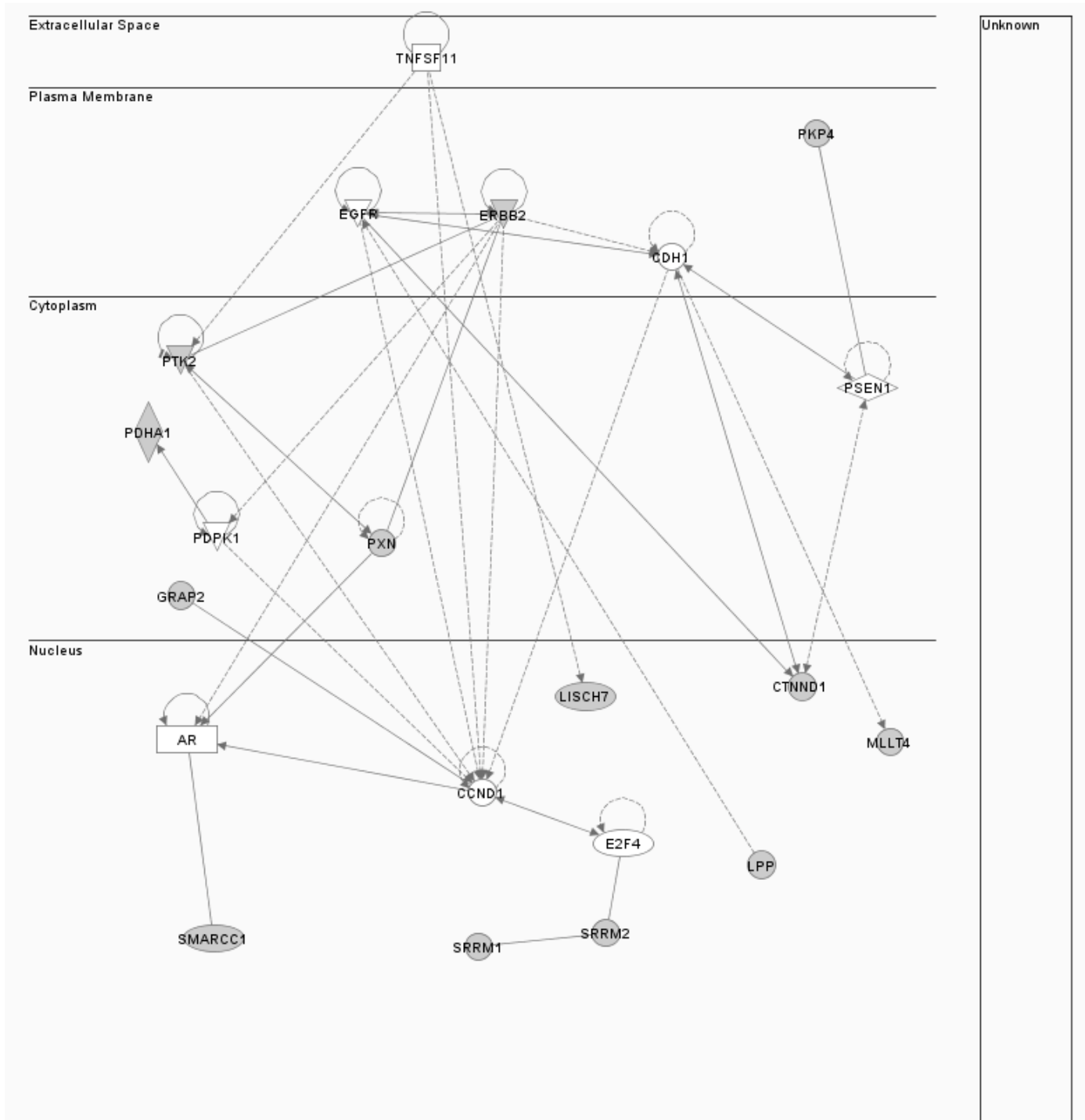


FIGURE S2B

Immunoblot: anti-SFRS10
SK-OV-3, pY-IP
37kDa —
26kDa —



FIGURE S3

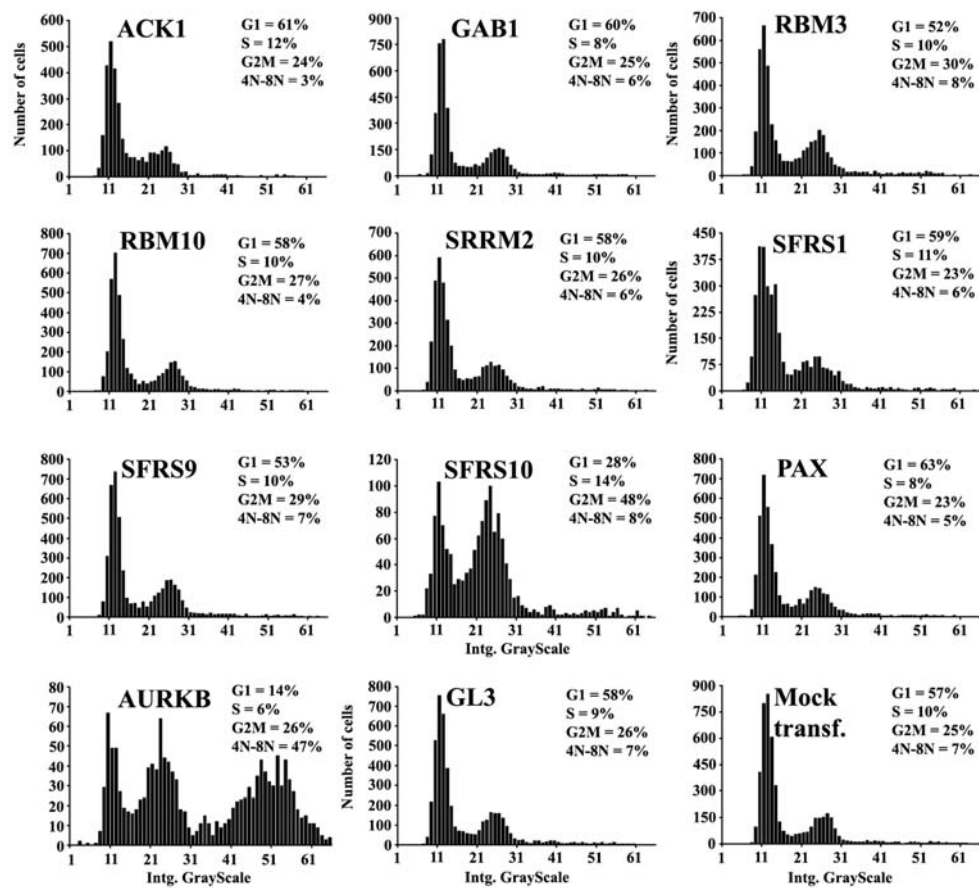


FIGURE S4

Protein/Gene	siRNA name	siRNA Sequence
Activated p21cdc42Hs kinase	ACK1a	GCAGUCAGAUCCUGCAUAA
	ACK1b	GCGAGCACUACCUCAGAAU
Gab1	GAB1a	GAGGCAUGUAUCUAUUAGU
	GAB1b	GCUAGACACUAUUCAGAU
RNA binding motif protein 3	RBM3a	GGAGGAAAUUACAGAGACA
	RBM3b	GGACGUUCCAGAGACUAUA
RNA binding protein 10	RBM10a	GCAAGACCAUCA AUGUUGA
	RBM10a	GCCAGUCGUAUACCAUCAU
RNA binding protein	SRRM2a	GCGGUCUCGAAGUACAACA
	SRRM2b	GCAGCCCUUAUGAAGACAA
Splicing factor 2	SFRS1a	GCGACAUCGACCUCAAGAA
	SFRS1b	GGAUAAACACUAAGUUUAGA
Arginine/serine-rich 9	SFRS9a	GCCUCCUACAAGAAGAUUCU
	SFRS9b	GAGGAUGCUAUUUUAUGGAA
Arginine/serine-rich 10	SFRS10a	GCGUAGGAUCAGAGUUGAU
	SFRS10b	GGCGUAGGAUCAGAGUUGA
Paxillin	PXNa	GCAGCAACCUUUCUGAACU
	PXNb	GCAAGGACUACUUCGACAU

TABLE S1

Primer name	Protein/Gene	Primer Sequence
ACK1_1F	Activated p21cdc42Hs kinase	CGACCTGGTCAAGATCGG
ACK1_1R		CGATGTTTCCTGCATGACG
ACK1_2F	Activated p21cdc42Hs kinase	GGAAATGTTACCTACGGC
ACK1_2R		GCTCCCCCTCCTTGTC
GAB1_1F	Gab1	TCCTCCAACACCTGGTAATA
GAB1_1R		GGATACTACACGTTTCCACAG
GAB1_F2	Gab1	AAGCAGATGGAGAACTCTATG
GAB1_R2		TGATGTCTGTCCCAAGGTT
RBM3_1F	RNA binding motif protein 3	CAGTCGACCTGGAGGG
RBM3_1R		TCCTGAGTAGCGGTCATAAC
RBM3_2F	RNA binding motif protein 3	GGGCTATGGGAGTGGCAG
RBM3_2R		CCATTATAGTCTCTGGAACGTCC
RBM10_1F	RNA binding protein 10	CCACCACTCACTATCGACG
RBM10_1R		GCCTTCATTGGAGGCC
RBM10_2F	RNA binding protein 10	TCTACCAACAATCAGCCGAG
RBM10_2R		CAGCGGGTGACATGATG
SRRM2_1F	RNA binding protein	CTAAGGACAAAAAGCGAAAG
SRRM2_1R		GTTGTATGAGTTTTAGCTGCAG
SRRM2_F2	RNA binding protein	CACCAGTTGGCAGAATTAAATGAG
SRRM2_R2		CGAACAAGGCTGTAAGGTTTGG
SRRM2_F3	RNA binding protein	TTGGCAGAATTAAATGAGAAG
SRRM2_R3		CCGAACAAGGCTGTAAGG
SFRS1_1F	Splicing factor 2	AAGGACATTGAGGACGTGTTCTAC
SFRS1_1R		TCCTCGAACTCAACGAAGGC
SFRS1_F2	Splicing factor 2	GGAGTTTGTACGGAAGAAG
SFRS1_R2		ACTTTAACCCGGATGTAGG
SFRS9_1F	Arginine/serine-rich 9	GAGGACCCCGAGATG
SFRS9_1R		CGACCTCCATAAGTCCTGG
SFRS9_F2	Arginine/serine-rich 9	GCTTCGTGTGGAGTTCCC
SFRS9_R2		CAAGAACTCGGAAATCAGATC
SR10_1F	Arginine/serine-rich 10	CAAGGAAGCTAAAGAACGTG
SR10_1R		GGTCTTTTTTGTATAGAGAAATCAAC
SR10_2F	Arginine/serine-rich 10	AGCTAAAGAACGTGCCAATG
SR10_2R		CGTATGTGGTCTTTTTTGTATAGAG
PXN_1F	Paxillin	GTTGGCTCTCCGTGCTCC
PXN_1R		CGTTCAGTTCCAGCAGCAG
PXN_2F	Paxillin	GAAGGGTTCCACGAGAAG
PXN_2R		GGCACACAAAGCACTCAG

TABLE S2

Motif	Motif Score	Foreground Matches	Foreground Size	Background Matches	Background Size	Fold Increase
.....YD.PP..	27.35	15	1944	58	322611	42.92
..R...Y...R.R	15.25	10	1768	95	304918	18.15
.....YG..R..	9.45	21	1758	989	304823	3.68
.....Y...P..	6.89	161	1929	17635	322553	1.53
.....DY.....	5.12	133	1737	15746	303834	1.48

TABLE S3

Motif sequence	Protein name	Symbol	Entrez ID
FQQQMIYDSPPSR	Gab1	GAB1	2549
RHVSISYDIPPTP	Gab1	GAB1	2549
SNHHAVYDVPPSV	Crk-associated substrate p130Cas	BCAR1	9564
PGPQDIYDVPPVR	Crk-associated substrate p130Cas	BCAR1	9564
PPAEDVYDVPPPA	Crk-associated substrate p130Cas	BCAR1	9564
PPAPDLYDVPPGL	Crk-associated substrate p130Cas	BCAR1	9564
QYGQEVYDTPPMA	Crk-associated substrate p130Cas	BCAR1	9564
DPLLEVYDVPPSV	Crk-associated substrate p130Cas	BCAR1	9564
LLREETYDVPPAF	Crk-associated substrate p130Cas	BCAR1	9564
LPPLPLYDQPPSS	KIAA 0769 protein	SH3MD3	9873
LRPEGVYDIPPTC	Cas-L	NEDD9	4739
RYQKDVYDIPPSH	Cas-L	NEDD9	4739
GLREKDYDFPPPM	Cas-L	NEDD9	4739
HTTQGVYDIPPS	Cas-L	NEDD9	4739
IKPQGVYDIPPTK	Cas-L	NEDD9	4739
GPRSPSYGRSRSR	arginine/serine-rich 1	SFRS1	6426
NSRSRSYSPRRSR	arginine/serine-rich 1	SFRS1	6426
RSRGRRYSRSRSR	arginine/serine-rich 7	SFRS7	6432
PERSTSYGYSRSR	arginine/serine-rich 9	SFRS9	8683
GYRRPYFRGRNR	TRAP150	THRAP3	9967
PERLKHYDDHRHR	Ribonuclease III, RNase III, p241	RNASE3L	29102
PSRHRSYERSRER	Ribonuclease III, RNase III, p241	RNASE3L	29102
QSRKRPYEENRGR	E1B-55kDa-associated protein 5	E1B-AP5	11100
GGRSRAYMPPRSR	LISCH protein	LISCH7	51599
RSRDPHYDDFRSR	LISCH protein	LISCH7	51599

TABLE S4