Table S1. List of shRNAs, target sequences, and protein-level knockdown efficiencies. TRC is an abbreviation for The RNAi Consortium, based at the Broad Institute.

Gene Symbol	Internal shRNA ID	TRC ID	TRC Library Clone Name	Target Sequence	Target Region	Percent Protein Remaining (Mean)	Percent Protein Remaining (Stdev of 2)
AKT1	AKT1_sh1	TRCN0000039794	NM_005163.1-735s1c1	GATCCTCAAGAAGGAAGTCAT	CDS	29.1%	12.0%
AKT1	AKT1_sh4	TRCN0000039797	NM_005163.1-628s1c1	CGCGTGACCATGAACGAGTTT	CDS	57.0%	15.7%
AKT2	AKT2_sh10	TRCN0000000565	NM_001626.x-632s1c1	ACGGGCTAAAGTGACCATGAA	CDS	23.9%	7.3%
AKT2	AKT2_sh12	TRCN0000009819	NM_001626.x-203s1c1	CATGAATGAGGTGTCTGTCAT	5'-UTR	38.9%	12.9%
AKT3	AKT3_sh7	TRCN0000039889	NM_005465.3-1024s1c1	CCAGAGGTGTTAGAAGATAAT	CDS	19.5%	1.1%
AKT3	AKT3_sh9	TRCN0000039891	NM_005465.3-671s1c1	GCAGAGTATTAAAGAACACTA	CDS	23.7%	8.2%
BCAR1	P130CAS_sh3	TRCN0000115984	NM_014567.2-2223s1c1	GCTGAAGCAGTTTGAACGACT	CDS	35.6%	6.2%
BCAR1	P130CAS_sh5	TRCN0000115986	NM_014567.2-1332s1c1	CGTGGTCGACAGTGGTGTGTA	CDS	52.0%	7.6%
CAMK2D	CAMK2D_sh9	TRCN0000196967	NM_001221.2-1917s1c1	GTCACCAACAGTACCCATCAA	CDS	24.9%	2.8%
CAMK2D	CAMK2D_sh4	TRCN0000000474	NM_001221.x-1475s1c1	CAGATGGAGTAAAGGAGTCAA	CDS	31.6%	5.8%
CAMKK2	CAMKK2D_sh9	TRCN0000195473	NM_153499.2-5368s1c1	CCCGATGCTTCTGTTTCATTC	3'-UTR	27.0%	10.4%
CAMKK2	CAMKK2D_sh7	TRCN0000196992	NM_153499.2-4867s1c1	GCAAAGAGGACGCCCATAATT	3'-UTR	32.0%	7.9%
GRB2	GRB_sh3	TRCN0000029371	NM_002086.3-492s1c1	CGGCTTCATTCCCAAGAACTA	CDS	17.4%	8.7%
GRB2	GRB_sh4	TRCN0000029372	NM_002086.3-764s1c1	GATCTACATCTGTCTCCAGAA	CDS	56.9%	10.2%
GSK3A	GSK3-A_sh3	TRCN0000038681	NM_019884.1-1171s1c1	GAGTTCAAGTTCCCTCAGATT	CDS	6.1%	1.1%
GSK3A	GSK3-A_sh4	TRCN0000038682	NM_019884.1-1173s1c1	GTTCAAGTTCCCTCAGATTAA	CDS	6.6%	1.6%
GSK3B	GSK3-B_sh7	TRCN0000040001	NM_002093.2-974s1c1	GCTGAGCTGTTACTAGGACAA	CDS	12.7%	6.4%
GSK3B	GSK3-B_sh12	TRCN0000039565	NM_002093.2-1067s1c1	AGCAAATCAGAGAAATGAAC	CDS	13.7%	5.2%

HRAS	HRAS_sh9	TRCN0000040088	NM_005343.1-108s1c1	AGAGGATTCCTACCGGAAGCA	CDS	41.8%	8.0%
HRAS	HRAS_sh12	TRCN0000010358	NM_005343.x-108s1c1	AGAGGATTCCTACCGGAAGCA	CDS	82.2%	9.8%
KRAS	KRAS_sh3	TRCN0000040150	NM_004985.3-570s1c1	CTCAGGACTTAGCAAGAAGTT	CDS	27.6%	6.8%
KRAS	KRAS_sh5	TRCN0000040152	NM_004985.3-492s1c1	AGGACTCTGAAGATGTACCTA	CDS	28.7%	1.7%
MAP2K1	MAP2K1_sh1	TRCN0000002329	NM_002755.x-455s1c1	GCTTCTATGGTGCGTTCTACA	CDS	40.1%	4.6%
MAP2K1	MAP2K1_sh4	TRCN0000002330	NM_002755.x-1163s1c1	CTGATGCTGAGGAAGTGGATT	CDS	57.2%	5.6%
MAP2K2	MAP2K2_sh4	TRCN0000007008	NM_030662.2-1218s1c1	CTGGACTATATTGTGAACGAG	CDS	41.1%	3.9%
MAP2K2	MAP2K2_sh5	TRCN0000195037	NM_030662.2-1277s1c1	CTTCCAGGAGTTTGTCAATAA	CDS	43.2%	2.5%
MAPK1	MAPK1_sh2	TRCN0000010040	NM_138957.x-381s1c1	CAAAGTTCGAGTAGCTATCAA	CDS	8.2%	5.8%
MAPK1	MAPK1_sh5	TRCN0000010050	NM_138957.x-657s1c1	TATCCATTCAGCTAACGTTCT	CDS	12.4%	3.4%
MAPK3	MAPK3_sh1	TRCN0000006150	NM_002746.1-876s1c1	CCTGAATTGTATCATCAACAT	CDS	1.7%	1.6%
MAPK3	MAPK3_sh4	TRCN0000010998	NM_002746.1-480s1c1	GCAGCTGAGCAATGACCATAT	CDS	6.8%	2.6%
PAK1	PAK1_sh8	TRCN0000199394	NM_002576.3-1655s1c1	CCAGAGGTTGTGACACGAAAG	CDS	4.1%	0.7%
PAK1	PAK1_sh7	TRCN0000195500	NM_002576.3-456s1c1	CCCTAAACCATGGTTCTAAAC	CDS	8.3%	1.9%
PDPK1	PDPK1_sh4d	TRCN0000196933	NM_002613.3-676s1c1	GATGAGACCTGTACCCGATTT	CDS	5.4%	1.7%
PDPK1	PDPK1_sh10	TRCN0000039782	NM_002613.1-1553s1c1	CAAAGTTCTGAAAGGTGAAAT	CDS	7.3%	1.1%
PIK3CA	PIK3CA_sh6	TRCN0000010406	NM_006218.x-79s1c1	GAATGTTTACTACCAAATGGA	CDS	4.6%	1.0%
PIK3CA	PIK3CA_sh5	TRCN0000039603	NM_006218.1-3251s1c1	GATTCCACACTGCACTGTTAA	3'-UTR	13.6%	8.1%
PIK3CB	PIK3CB_sh3	TRCN0000194877	NM_006219.1-2802s1c1	CCACATTGACTTTGGACATAT	CDS	10.7%	3.7%
PIK3CB	PIK3CB_sh16	TRCN0000009860	NM_006219.x-2586s1c1	CATTCAGCTGAACAGTAGCAA	CDS	13.9%	0.4%
PLCG1	PLCG1_sh3	TRCN0000006979	NM_002660.2-3562s1c1	CCAGATCAGTAACCCTGAATT	CDS	13.3%	2.8%

PLCG1	PLCG1_sh2	TRCN0000006978	NM_002660.2-335s1c1	GCCATTGACATTCGTGAAATT	CDS	21.5%	2.9%
PPP1CA	PPP1CA_sh4	TRCN0000002454	NM_002708.x-1030s1c1	CCGCAATTCCGCCAAAGCCAA	CDS	291.2%	109.9%
PPP1CA	PPP1CA_sh5	TRCN0000002452	NM_002708.x-1082s1c1	AGATGATGGATTGATTGTACA	3'-UTR	376.9%	152.4%
PPP2CA	PPP2CA_sh4	TRCN0000002483	NM_002715.x-1036s1c1	TGGAACTTGACGATACTCTAA	CDS	36.9%	2.6%
PPP2CA	PPP2CA_sh5	TRCN0000002486	NM_002715.x-1684s1c1	CCCATGTTGTTCTTTGTTATT	3'-UTR	37.6%	6.3%
PRKCA	PKC-A_sh3	TRCN0000001692	NM_002737.x-921s1c1	CATGGAACTCAGGCAGAAATT	CDS	20.1%	9.3%
PRKCA	PKC-A_sh8	TRCN0000196909	NM_002737.2-1280s1c1	GCTGTACTTCGTCATGGAATA	CDS	20.4%	1.6%
PRKCB1	PKC-B_sh5	TRCN0000195381	NM_002738.5-745s1c1	CCTGTCAGATCCCTACGTAAA	CDS	27.1%	7.5%
PRKCB1	PKC-B_sh2	TRCN0000003117	NM_002738.x-847s1c1	GCTGAAAGAATCGGACAAAGA	CDS	27.6%	1.9%
PRKCD	PKC-D_sh6	TRCN0000010193	NM_006254.x-1385s1c1	GGCCGCTTTGAACTCTACCGT	CDS	14.5%	18.2%
PRKCD	PKC-D_sh8	TRCN0000010203	NM_006254.x-826s1c1	GCAGGGATTAAAGTGTGAAGA	CDS	27.3%	12.7%
PRKCE	PKC-E_sh1	TRCN0000000844	NM_005400.x-1288s1c1	GCAGAACTCAAGGGCAAAGAT	CDS	26.4%	7.4%
PRKCE	PKC-E_sh5	TRCN0000000847	NM_005400.x-591s1c1	TGTCATAGGAAAGCAGGGATA	CDS	17.7%	3.3%
PTEN	PTEN_sh4	TRCN0000002748	NM_000314.x-1956s1c1	CGTGCAGATAATGACAAGGAA	CDS	8.9%	2.8%
PTEN	PTEN_sh1	TRCN0000002746	NM_000314.x-1320s1c1	CCACAGCTAGAACTTATCAAA	CDS	22.8%	10.1%
PTK2	FAK_sh16	TRCN0000121319	NM_005607.3-1043s1c1	GCCCAGAAGAAGGAATCAGTT	CDS	6.4%	1.4%
PTK2	FAK_sh21	TRCN0000196310	NM_005607.3-817s1c1	GATGTTGGTTTAAAGCGATTT	CDS	6.7%	5.7%
PTPN1	PTPN1_sh1	TRCN0000002779	NM_002827.x-400s1c1	GAAGCCCAAAGGAGTTACATT	CDS	13.6%	4.6%
PTPN1	PTPN1_sh5	TRCN0000002777	NM_002827.x-3232s1c1	GCTGCTCTGCTATATGCCTTA	3'-UTR	35.0%	5.2%
PTPN11	PTPN11_sh4	TRCN0000005003	NM_002834.3-1570s1c1	CGCTAAGAGAACTTAAACTTT	CDS	10.8%	3.1%
PTPN11	PTPN11_sh1	TRCN0000005002	NM_002834.3-2452s1c1	GCAGTTAAATTGTGCGCTGTA	3'-UTR	28.4%	10.6%

PTPN6	PTPN6_sh3	TRCN0000006887	NM_002831.3-1572s1c1	CGACATGCTCATGGAGAACAT	CDS	10.5%	2.0%
PTPN6	PTPN6_sh2	TRCN0000006886	NM_002831.3-1326s1c1	GCATGACACAACCGAATACAA	CDS	16.4%	5.3%
RAF-A	A-RAF_sh1	TRCN0000000571	NM_001654.x-390s1c1	GACTCATCAAGGGACGAAAGA	CDS	19.7%	8.2%
RAF-A	A-RAF_sh4	TRCN0000000568	NM_001654.x-939s1c1	GTAGAGGAGGTAGTGATGGAA	CDS	22.5%	6.8%
RAF-B	B-RAF_sh1	TRCN0000006292	NM_004333.2-1538s1c1	CAGCAGTTACAAGCCTTCAAA	CDS	22.6%	3.8%
RAF-B	B-RAF_sh4	TRCN0000006291	NM_004333.2-2267s1c1	GCTGGTTTCCAAACAGAGGAT	CDS	25.0%	7.4%
RAF-C	C-RAF_sh5	TRCN0000001068	NM_002880.x-1529s1c1	GAGACATGAAATCCAACAATA	CDS	16.8%	4.8%
RAF-C	C-RAF_sh3	TRCN0000001066	NM_002880.x-1236s1c1	CGGAGATGTTGCAGTAAAGAT	CDS	28.3%	11.3%
ROCK2	ROCK2_4	TRCN0000000980	NM_004850.x-4337s1c1	CGTTGCCATATTAAGTGTCAT	CDS	19.3%	11.5%
ROCK2	ROCK2_6	TRCN0000194836	NM_004850.3-5578s1c1	CCTTGATGTCTGTCTATTATT	3’-UTR	30.8%	4.7%
RPS6KA1	p90RSK_sh4	TRCN0000001385	NM_002953.x-564s1c1	GCTCTATCTCATTCTGGACTT	CDS	16.2%	4.5%
RPS6KA1	p90RSK_sh1	TRCN0000001384	NM_002953.x-2576s1c1	AGCGATTCACTGTATAAACTT	3’-UTR	23.4%	11.9%
SHC1	SHC1_52_sh2	TRCN0000040209	NM_003029.3-555s1c1	CCACATGCAATCTATCTCATT	CDS	60.5%	14.6%
SHC1	SHC1_52_sh3	TRCN0000040210	NM_003029.3-198s1c1	CCACGGGAGCTTTGTCAATAA	CDS	74.2%	2.7%
SRC	SRC_sh1	TRCN0000038150	NM_198291.1-648s1c1	GACAGACCTGTCCTTCAAGAA	CDS	5.9%	1.7%
SRC	SRC_sh2	TRCN0000038153	NM_198291.1-675s1c1	GCGGCTCCAGATTGTCAACAA	CDS	19.6%	3.0%

Table S2. List of shRNA pool components. To produce pools of shRNA expression vectors, equal volumes of titer-normalized viral stocks of component shRNAs were mixed. The total viral titer of each shRNA pool thus matched the viral titer of the component shRNAs.

shRNA Pools Targeting Kinases	Component shRNAs
AKT pool:	AKT1_sh1
	AKT2_sh10
	AKT3_sh7
Cytoskeleton pool:	FAK_sh21
	P130CAS_sh5
GSK3 pool:	GSK3-A_sh3
	GSK3-B_sh12
MAP2K pool:	MAP2K1_sh1
	MAP2K2_sh4
MAPK pool:	MAPK1_sh2
	MAPK3_sh4
PI3K pool:	PIK3CA_sh6
	PIK3CB_sh3
PKC pool:	PKC-A_sh8
	PKC-B_sh5
	PKC-D_sh6
	PKC-E_sh5
RAF pool:	A-RAF_sh4
	B-RAF_sh1
	C-RAF_sh5
RAS pool:	HRAS_sh9
	KRAS_sh5

shRNA Pools Targeting Phosphatases	Component shRNAs
PPCA pool:	PPP1CA_sh4
	PPP2CA_sh5
PTPN pool:	PTPN1_sh1
	PTPN11_sh1
	PTPN6_sh3
All phosphatases pool:	PPP1CA_sh4
	PPP2CA_sh5
	PTPN1_sh1
	PTPN11_sh1
	PTPN6_sh3
	PTEN_sh4

Table S3. List of antibodies used with vendor information.

Primary Antibodies

Target Protein	Phosphorylation Site	Supplier	Catalog Number
Akt1/2/3	Ser^{473}	Cell Signaling Technologies	CST4058
Calmodulin	Ser^{81}	Santa Cruz	sc17019R
c-Cbl	Tyr^{774}	Cell Signaling Technologies	CST3555
c-Jun	Ser^{63}	Cell Signaling Technologies	CST9261
CREB	Ser^{133}	Cell Signaling Technologies	CST9198
EGFR	Tyr^{845}	Cell Signaling Technologies	CST2231
ERK1/2	(1: Thr^{202}, Tyr^{204}) / (2: Thr^{185}, Tyr^{187})	Cell Signaling Technologies	CST4377
FGFR1	Tyr^{653}, Tyr^{654}	Cell Signaling Technologies	CST3471
GSK-3α/β	(α: Ser^{21}) / (β: Ser^{9})	Cell Signaling Technologies	CST9327
IGF-1R	Tyr^{1135}, Tyr^{1136}	Cell Signaling Technologies	CST3024
MEK1/2	Ser^{217}/Ser^{221}	Cell Signaling Technologies	CST9154
MARCKS	Ser^{152}, Ser^{156}	Cell Signaling Technologies	CST2741
c-Met	Tyr^{1349}	Cell Signaling Technologies	CST3133
NF-κB	Ser^{536}	Cell Signaling Technologies	CST3033
NTRK2	Tyr^{706}, Tyr^{707}	Cell Signaling Technologies	CST4621
p90RSK	Ser^{380}	Cell Signaling Technologies	CST9341
Paxillin	Tyr^{118}	Cell Signaling Technologies	CST2541
PDGFRβ	Tyr^{771}	Cell Signaling Technologies	CST3173
PKCβ	Ser^{660}	Cell Signaling Technologies	CST9371
PKCδ	Ser^{643}	Cell Signaling Technologies	CST9376
c-Raf	Ser^{289}, Ser^{296}, Ser^{301}	Cell Signaling Technologies	CST9431
Ribosomal Protein S6	Ser^{235}, Ser^{236}	Cell Signaling Technologies	CST2211
Ribosomal Protein S6	Ser^{240}, Ser^{244}	Cell Signaling Technologies	CST2215
RSK3	Thr^{356}, Ser^{360}	Cell Signaling Technologies	CST9348
Shc	Tyr^{317}	Cell Signaling Technologies	CST2431
STAT1	Tyr^{701}	Cell Signaling Technologies	CST9167
STAT3	Tyr^{705}	Cell Signaling Technologies	CST9145
β-Actin	Not Applicable, Loading Control	Sigma	A1978

Secondary Antibodies

Target Protein	Emission Wavelength	Supplier	Catalog Number
IRDye Goat anti-Mouse	800 nm	LI-COR	926-32210
IRDye Goat anti-Rabbit	680 nm	LI-COR	926-32221