Supporting Information

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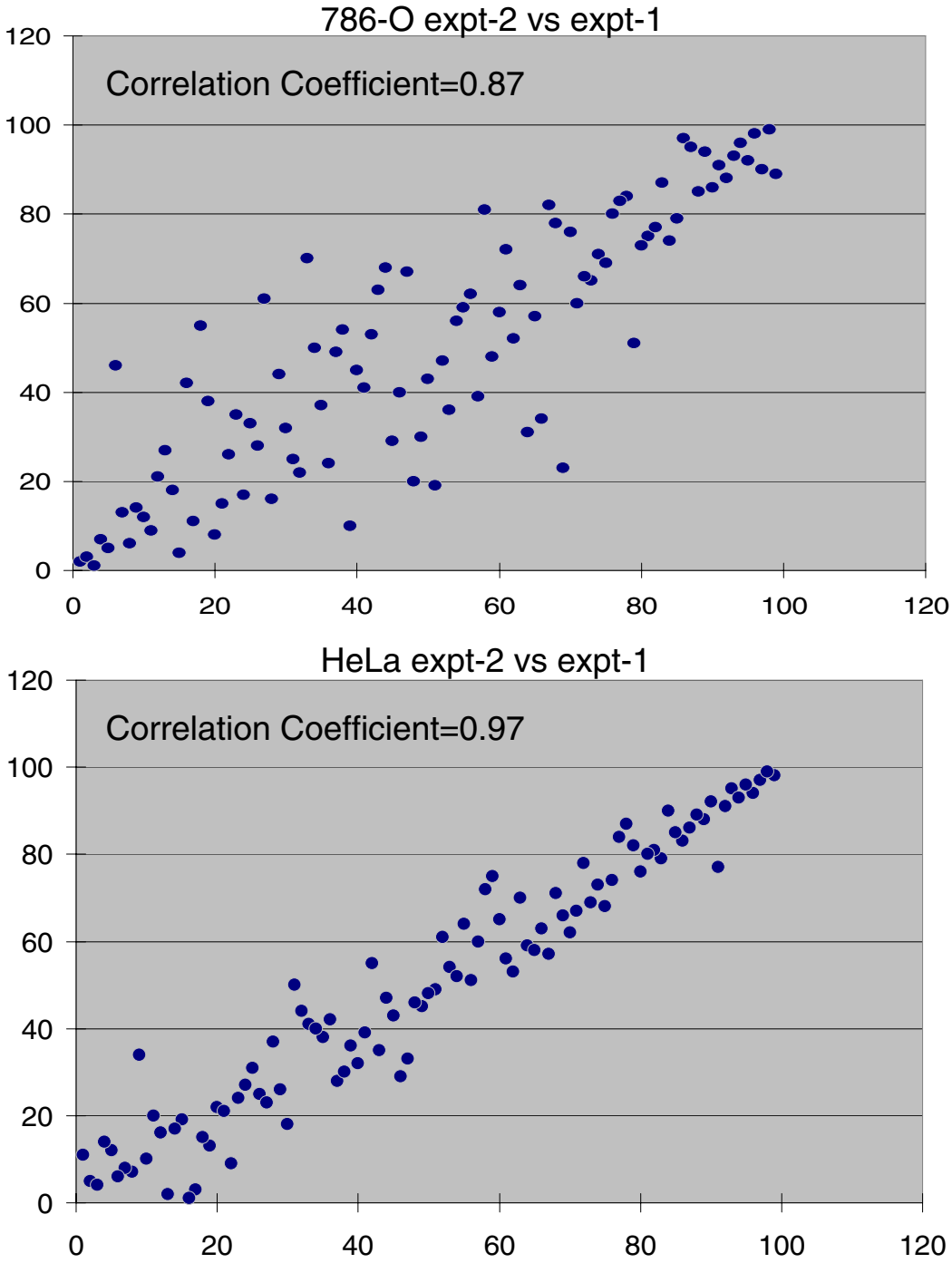

Fig. S1. Reproducibility of proliferation and viability screen. Rank order values were derived from percent reduction in proliferation and viability after normalization for HeLa and 786-O cells, which followed the transduction of 100 lentiviral shRNA expression vectors targeting 80 unique kinases plus controls. Data were plotted from two independent 786-O experiments are compared on the *x*- and *y* axis with a correlation coefficient of 0.87, and two independent HeLa experiments with a correlation coefficient of 0.97, demonstrating reproducibility between replicate screens.

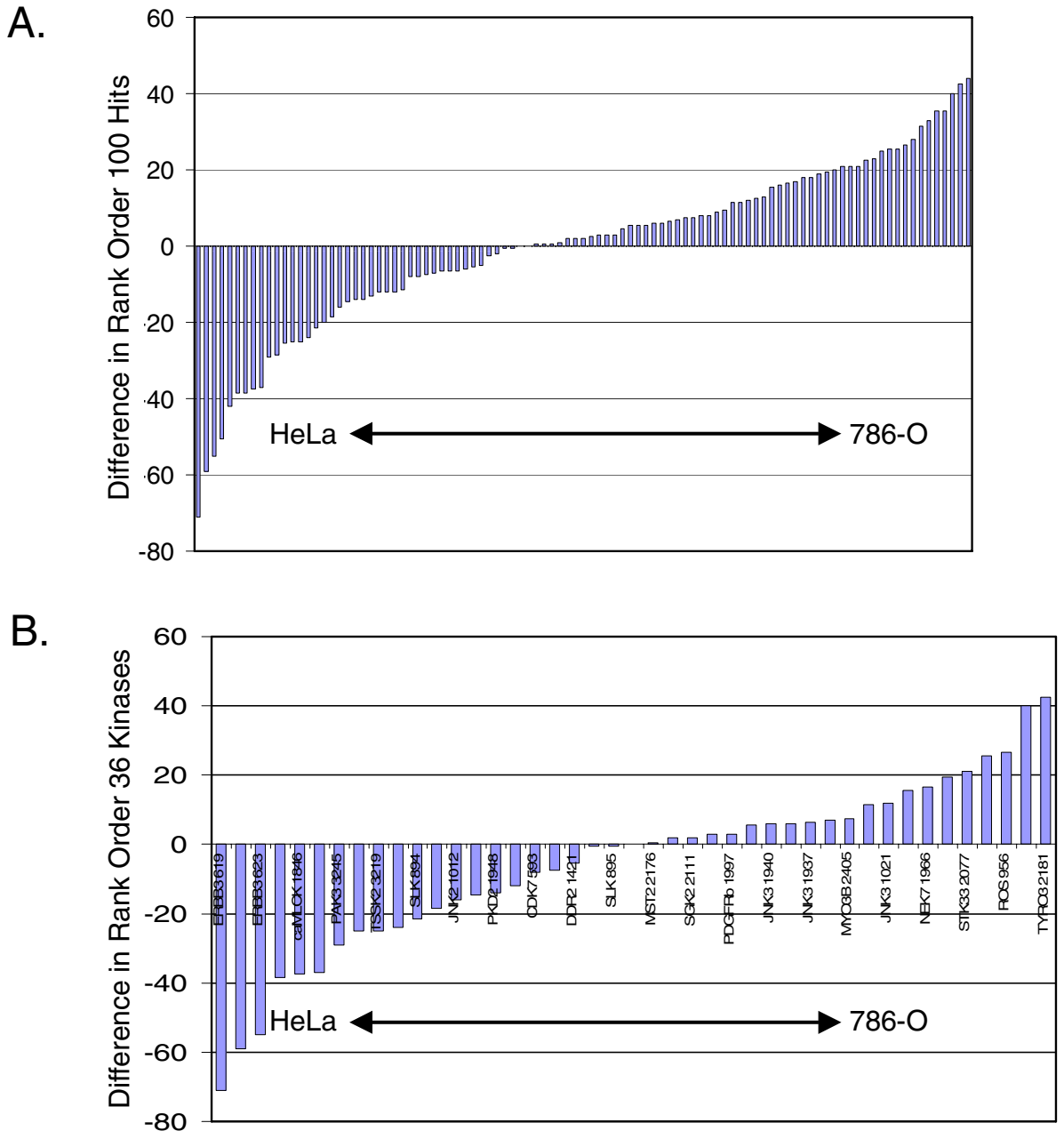

Fig. S2. Differential kinase requirements in HeLa and 786-O cells. Difference in rank order was derived from percent reduction in proliferation and viability after normalization for HeLa cells and 786-O cells transduced with (*A*) 100 lentivirus shRNA expression vectors targeting 80 unique kinases plus controls or (*B*) with the subset containing 173 lentivirus shRNA expression vectors targeting 36 kinases.

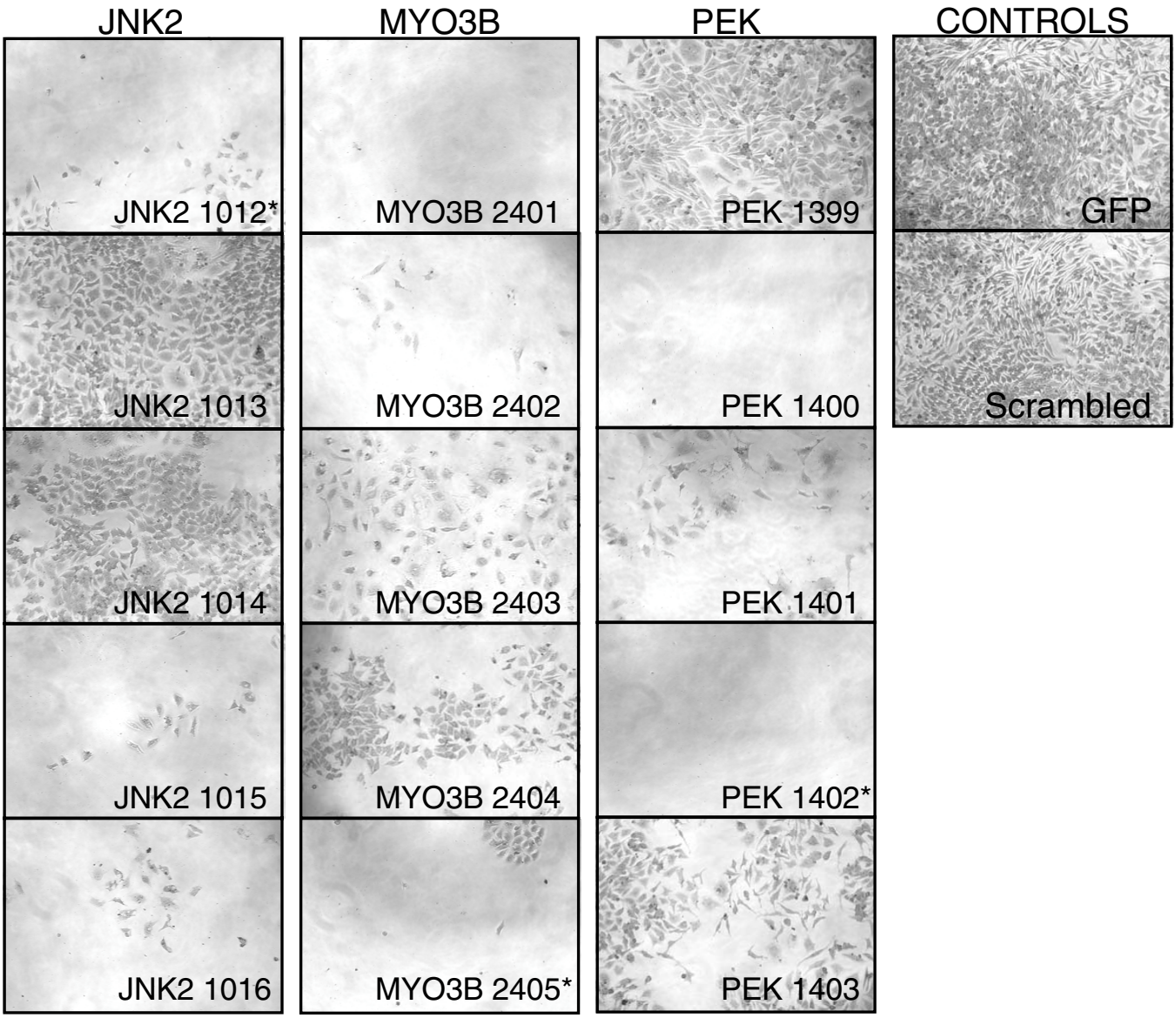

Fig. S3. Viral transduction of additional shRNAs targeting the same kinase. Photomicrographs of crystal violet stained HeLa cells 6 days after viral transduction of 5 hairpins each for *JNK2*, *MYO3B*, and *PEK* genes demonstrating decreased numbers of cells when infected with hairpins that scored as effective killers. The control infections include a GFP-containing lentiviral vector and a scrambled hairpin in the lentiviral vector that do not inhibit cell growth.

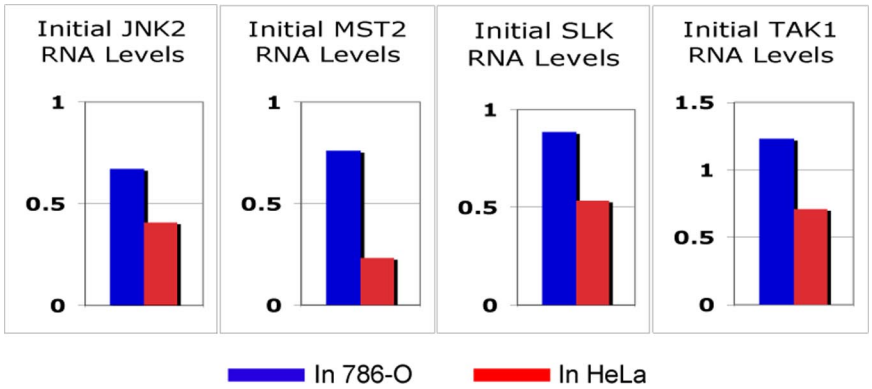

Fig. S4. Target mRNA levels in HeLa and 786-O cells. Panomics QuantiGene assays were used to quantify starting mRNA levels in HeLa and 786-O cells. JNK2, MST2, SLK, and TAK1 mRNA levels were measured and normalized to GAPDH.

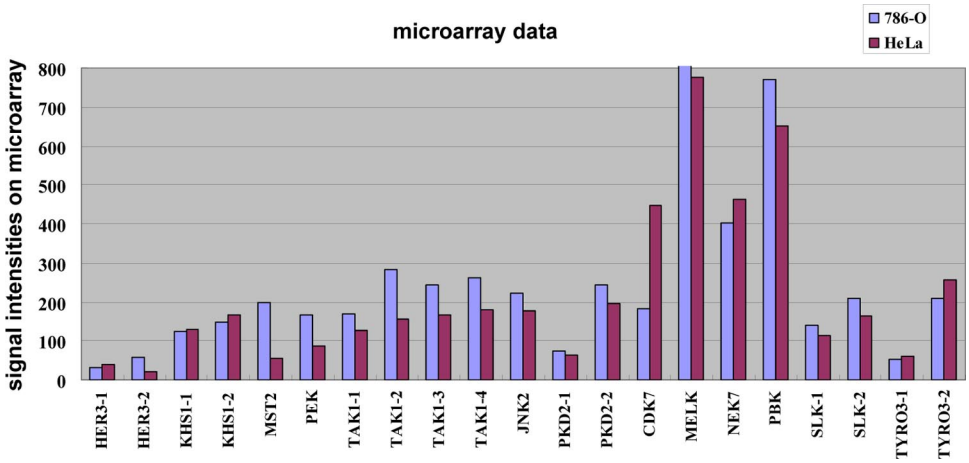

Fig. S5. RNA expression levels for kinases are similar between HeLa and 786-O cells. Microarray data showing gene expression patterns for 13 kinases in 786-O cells (in blue) and HeLa cells (in red) (http://wombat.gnf.org/index.html). For the majority of kinases, the RNA expression levels are slightly higher in 786-O cells levels, and for many, closely follow patterns shown for HeLa cells.

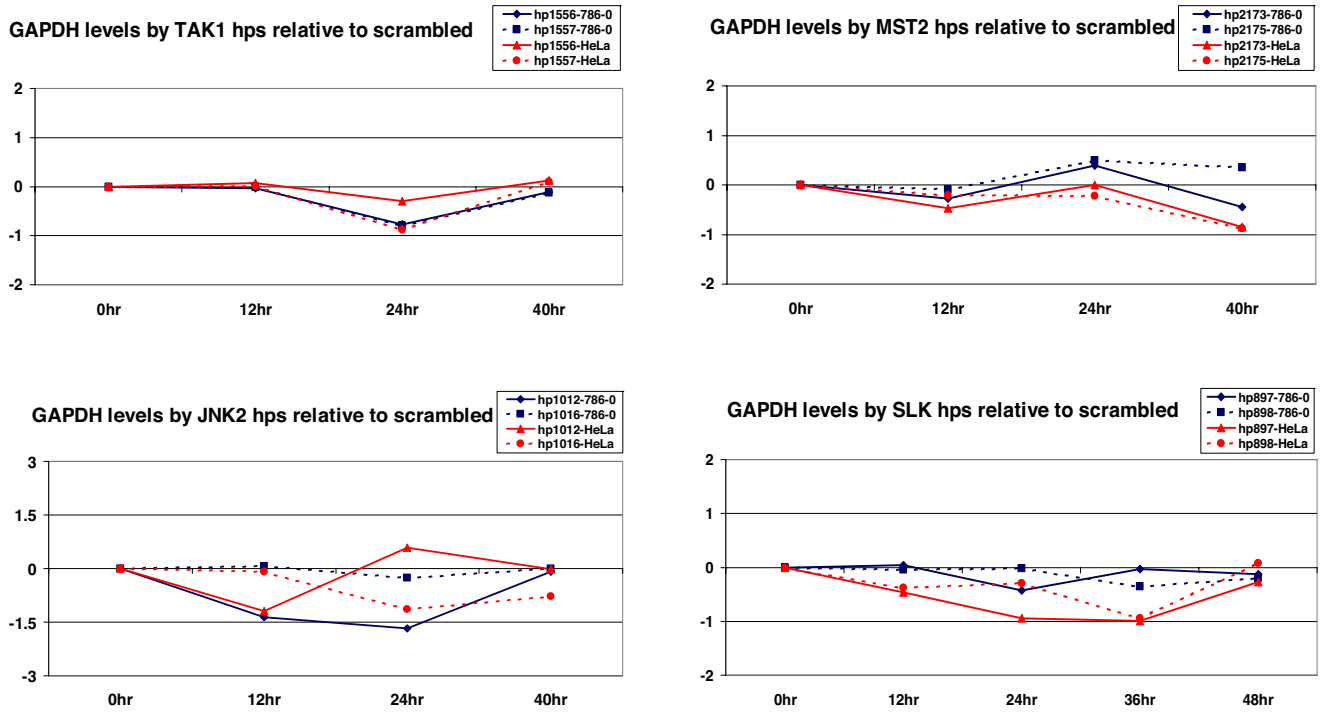

Fig. S6. GAPDH mRNA levels remain constant over time after essential kinase down regulation. Both HeLa and 786-O cells were transduced with 2 shRNA-containing viruses each for TAK1, MST2, JNK2 and SLK genes. As determined by Panomics QuantiGene assay, GAPDH mRNA levels do not change significantly during infection, arguing that the specific decay of kinase mRNA levels is due its targeting hairpin and is not a consequence of the impending cell death.

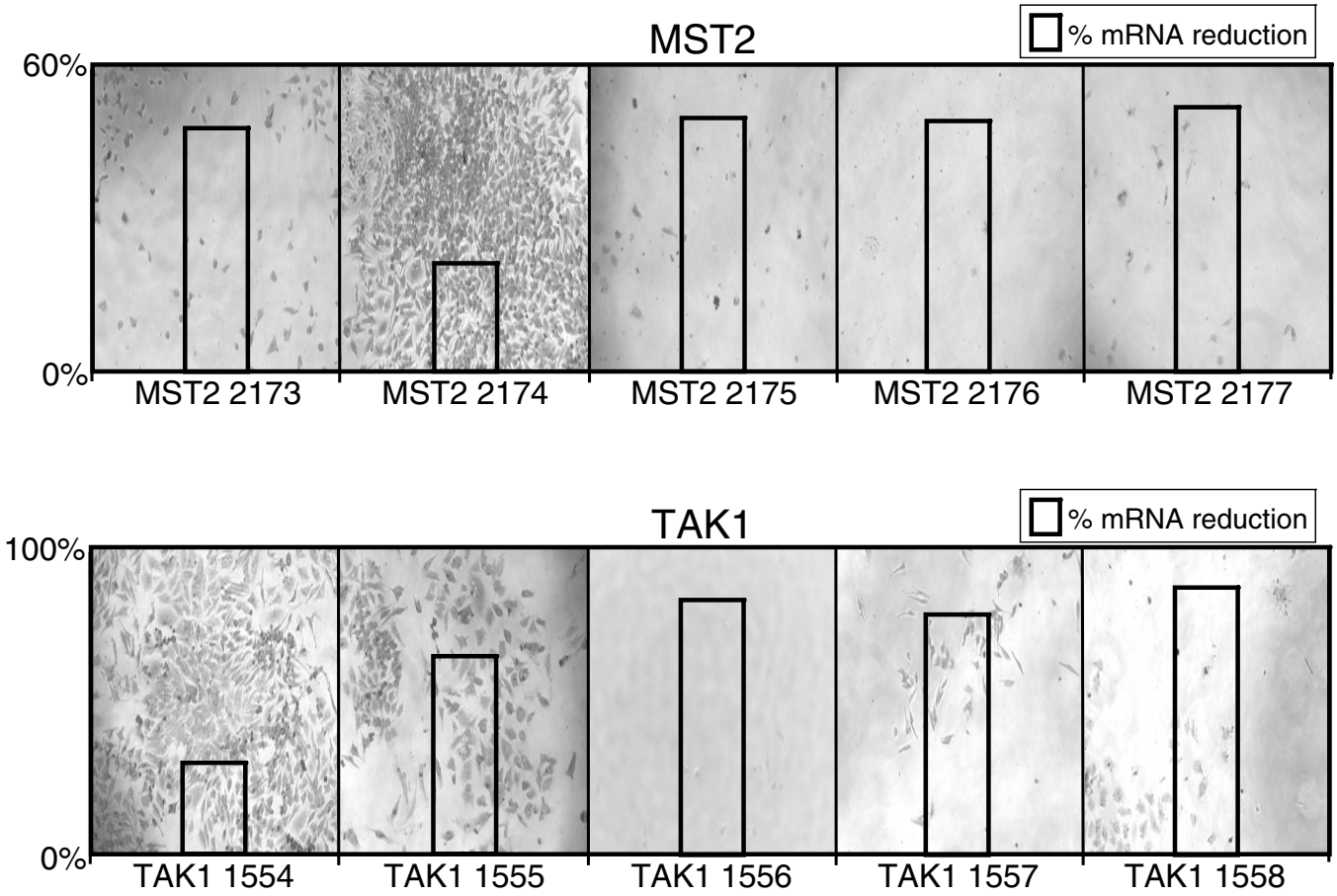

Fig. S7. Correlation between target knockdown and cell phenotype. Cells were infected with lentiviruses expressing one of five different shRNAs targeting either MST2 or TAK1. Target mRNA knockdown was determined by Panomics QuantiGene assay, and cell phenotype assessed by microscopy of crystal violet stained cells. The bar graph demonstrates mRNA knockdown levels in HeLa cells assayed 24 h post infection. Photomicrographs were taken 6 days after viral infection.

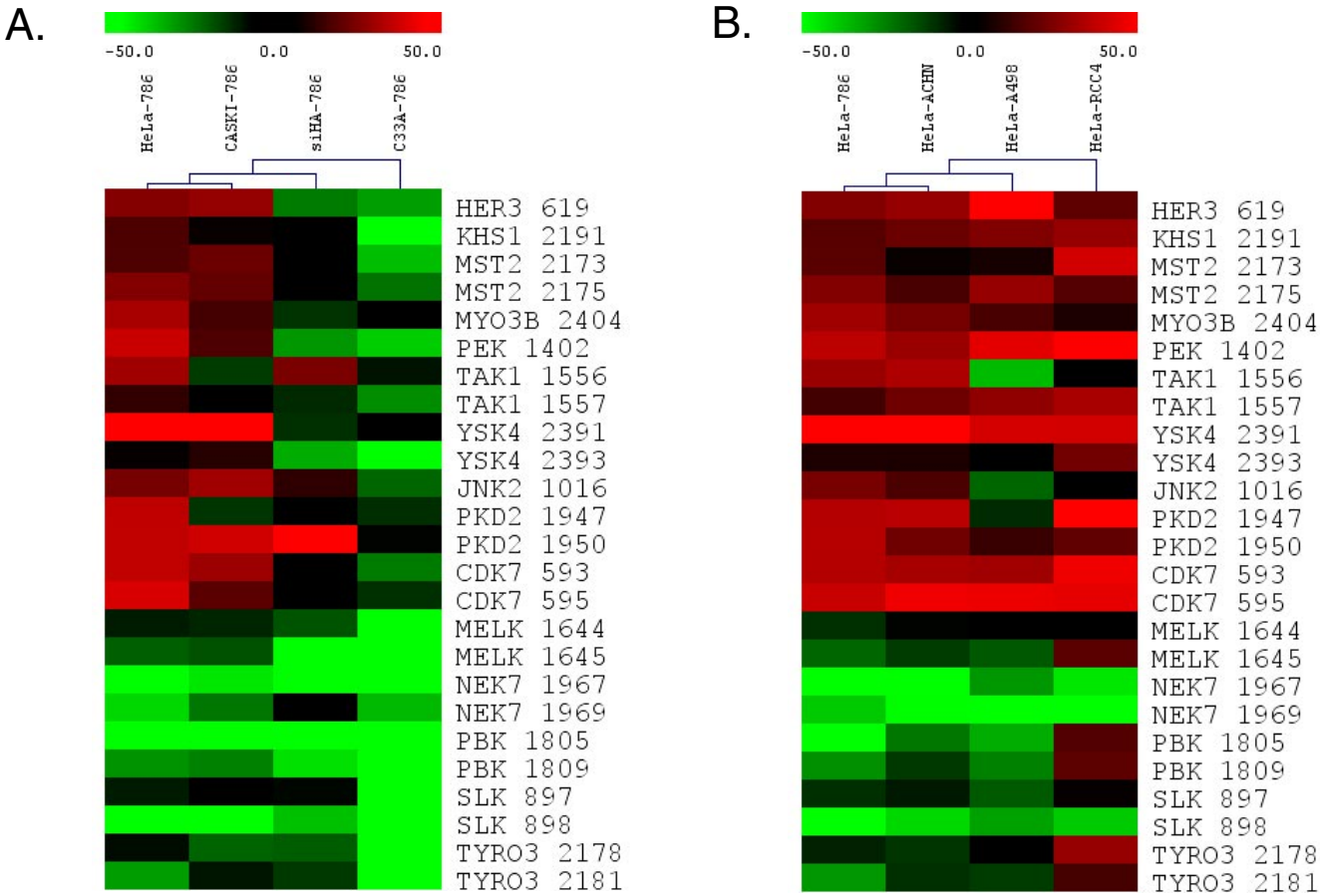

Fig. S8. Similar kinase requirements shown in additional cervical and renal cell lines. Four cervical cancer cell lines, including HeLa, CaSki, SiHa, and C33A; and four renal cancer cell lines, including 786-O, ACHN, A498, and RCC4 were transduced with 25 shRNA-expressing viruses targeting 15 genes previously identified as preferential killers in HeLa (group 1 shRNAs) or 786-O (group 2 shRNAs) cells. Cells were grown in the presence of puromycin. Alamar Blue measurements were taken 6 days after transduction, and difference in percent reduction in viability was calculated after normalization (with a scrambled shRNA as nonkilling control) between pairs of cervical and renal cell lines. Color scale ranges represent changes in viability between four cervical cancer cell lines and 786-O (Panel A), or between HeLa and four renal cancer cell lines (Panel B). Red corresponds to more growth inhibition for cervical cell lines. Green corresponds to more growth inhibition for renal cell lines. Columns display different cell lines tested. Rows display shRNAs from groups 1 and 2. Data were clustered by an Euclidean distance algorithm.

Table S1. Results of replicate essential kinase screens performed in 786-O and HeLa cells

Gene TRCN	786-O expt-1	786-O expt-2	HeLa expt-1	HeLa expt-2
AAK1 1945	42	53	67	57
ADCK4 7332	64	31	40	32
ALK 788	23	35	70	62
ALK4 1810	12	21	11	20
AMPKa1 859	15	4	19	13
BRD2 6312	89	94	63	70
BTK 360	9	14	23	24
BUBR1 462	29	44	2	5
CaMK1G 1452	61	72	44	47
CAMK2A 10286	79	51	49	45
CamK4 580	73	65	51	49
CAMKK1 1982	24	17	50	48
caMLCK 1846	37	49	79	82
CDK10 1821	30	32	25	31
CDK10 1822	52	47	31	50
CDK11 3141	86	97	89	88
CDK3 482	38	54	86	83
CDK4 365	60	58	53	54
CDK6 487	32	22	1	11
CDK7 593	74	71	77	84
CDK9 495	11	9	8	7
CDK9 498	4	7	7	8
CK1e 1834	6	46	24	27
CK1e 1835	8	6	12	16
CK1e 603	85	79	93	95
CK2a2 611	46	40	46	29
CLIK1L 2296	26	28	71	67
CLK3 745	47	67	52	61
DDR2 1421	93	93	99	98
EEF2K 6222	10	12	64	59
EPHB1 821	55	59	83	79
EPHB4 1775	70	76	73	69
FER 2347	59	48	26	25
FGFR3 373	72	66	27	23
FYN 3098	83	87	85	85
GFP	1	2	13	2
HER3 619	22	26	96	94
HER3 623	25	33	91	77
HER4 1410	5	5	10	10
HIPK2 3201	34	50	37	28
HUNK 2271	54	56	48	46
IRR 3189	62	52	9	34
JNK2 1012	56	62	72	78
JNK3 1018	2	3	17	3
JNK3 1021	21	15	6	6
JNK3 1937	90	86	82	81
JNK3 1940	96	98	90	92
KHS1 2187	81	75	60	65
LATS2 883	35	37	35	38
LKB1 409	27	61	20	22
MAP2K1 2328	36	24	28	37
MAPAPK3 6155	31	25	22	9
MELK 1644	98	99	84	90
MET 396	20	8	5	12
MISR2 1957	67	82	36	42
MST2 2176	99	89	94	93
MYO3B 2405	92	88	78	87
MYT1 6236	78	84	58	72
NEK7 1966	58	81	54	52
NLK 2068	95	92	87	86
PACGFP	3	1	16	1
PAK3 3244	80	73	88	89
PAK3 3245	40	45	75	68
PBK 1809	68	78	56	51

Gene TRCN	786-O expt-1	786-O expt-2	HeLa expt-1	HeLa expt-2
PCTAIRE1 10258	16	42	33	41
PDGFRa 1422	51	19	3	4
PDGFRb 1997	76	80	76	74
PDHK2 2316	50	43	55	64
PEK 1402	18	55	95	96
PITSLRE 6207	57	39	74	73
PITSLRE 6210	63	64	65	58
PKD2 1948	7	13	30	18
PKD2 1949	88	85	81	80
PKD3 1412	65	57	69	66
PKG1 997	53	36	61	56
PKN1 1485	94	96	68	71
PLK1 6247	33	70	45	43
RNASEL 924	69	23	62	53
ROS 956	87	95	66	63
RSK3 1384	48	20	15	19
SGK2 2111	97	90	92	91
SgK495 1817	19	38	59	75
SLK 894	14	18	39	36
SLK 895	49	30	41	39
SRPK2 6274	13	27	14	17
STK33 2077	82	77	57	60
SURTK106 1742	44	68	32	44
TAK1 1557	75	69	97	97
TLK1 7056	17	11	38	30
TNK1 743	39	10	18	15
TRAD 1428	41	41	21	21
TRRAP 5365	66	34	29	26
TSSK2 3219	43	63	80	76
TYRO3 2181	91	91	42	55
ULK4 2202	84	74	43	35
VACAMKL 10253	28	16	4	14
VRK3 912	71	60	47	33
YSK4 2393	77	83	98	99
ZC1/HGK 1830	45	29	34	40

Rank order values were derived from percent reduction in proliferation and viability after normalization to GFP-expressing lentiviral vector for 786-O and HeLa cells, which followed the transduction of 100 lentiviral shRNA expression vectors targeting 80 unique kinases plus controls. A low rank value represents a lesser amount of growth inhibition and a high rank value represents a greater amount of growth inhibition.

Table S2. Select list of essential kinases.

AAK1	JNK2	PEK
ALK	JNK3	PKD2
caMLCK	KHS1	PKN1
CDK3	LATS2	ROS1
CDK4	MELK	SGK2
CDK7	MST2	SLK
CDK11	MYO3B	STK33
DDR2	NEK7	TAK
EPHB1	NLK	TSSK2
EPHB4	PAK3	TYRO3
ERBB3	PBK	ULK4
FYN	PDGFRB	YSK4

Initially one hundred hairpins targeting 80 unique kinases plus controls were scored as ″hits″ by inhibiting growth by 50% or more in HeLa or 293T cells as compared with the median proliferation value of all shRNAs. Listed are the names of 36 out of 80 kinases used to transduce HeLa and 786-O cells.

Table S3. Viral transduction of additional shRNAs targeting the same kinase

Gene symbol	shRNA ID no.	HeLa > 786-O	786-O > HeLa
CDK7	593* 595	x	
ERBB3	619* 623*	x	
JNK2	1012* 1015 1016	x	
KHS1	2187* 2191	x	
MELK	1644* 1645		x
MST2	2173 2175 2176*	x	
MYO3B	2401 2402 2403 2404 2405*	x	
NEK7	1966* 1967 1969		x
PBK	1805 1809*		x
PEK	1400 1401 1402* 1403	x	
PKD2	1947 1949* 1950	x	
SLK	894* 897 898		x
TAK1	1556 1557*	x	
TYRO3	2178 2181*		x
YSK4	2391 2393*	x	

Selected genes were brought forward for more detailed studies; specifically 4 or 5 hairpins each for 36 genes were retested for their ability to inhibit cell growth. Summary table identifying all hairpins for a given gene that inhibited growth by 50% or more in HeLa cells. Reduction in viability induced by each hairpin calculated relative to an shRNA targeting scrambled as a baseline control. The asterisk shows original scoring shRNA.