

Table 1. siRNA and shRNA sequences and annotations

siRNA Sequences and Annotation

<u>Annotation</u>	<u>Accession</u>	<u>Target start</u>	<u>19-mer sequence</u>	<u>Alternative name</u>	<u>Sequence</u>	<u>Possible Off-target Genes</u>
PGL2 (luciferase)	CONTROL		CGTACGCGGAATACT TCGA		AACGTACGCGGAAT ACTTCGATT	none detected
SCRAMBLE	CONTROL		GCTCAGTACGATCGA ATCA		AAGCTCAGTACGATC GAATCA TT	none detected
H.s. mitogen-activated protein kinase kinase 7 (MAP3K7), mRNA	NM_003188	527	TATTGTAGAGCTTCGG CAG	TAK1	AATATTGTAGAGCTT CGGCAGTT	OAZ (NM_015069)
H.s PTK2 protein tyrosine kinase 2 (PTK2), mRNA	NM_153831	1851	AGATTTGTACACAGG GACA	FAK	AAAGATTTGTACACA GGGACATT	CTEN(NM_032865)
MAPK9: mitogen-activated protein kinase 9	NM_002752	722	TTCCAAGGCACTGAC CATA	JNK2	AATTCCAAGGCACTG ACCATATT	LRP6(NM_002336), MRF2 (XM_084482), C14orf93 (NM_021944), ESR1(NM_000125)
H.s. lymphocyte-specific protein tyrosine kinase (LCK), mRNA	NM_005356	1308	CAAGTCAGATGTGTG GTCT		AACAAGTCAGATGTG TGGTCTTT	MET (NM_000245), ITK (NM_005546), P101-PI3K (NM_014308), LYN (NM_002350), PCDHGC5 (NM_018929)
H.s v-abl Abelson murine leukemia viral oncogene homolog 2 (arg, Abelson-related gene) (ABL2), transcri	NM_005158	905	TGAAGGAAATCAAGC ATCC	ARG	AATGAAGGAAATCAA GCATCCTT	RNPC6 (NM_153020), TRPC7 (NM_020389), LY6G5B (NM_021221), TMC2 (NM_080751), LSR7(NM_018559)
H.s. protein kinase C, zeta (PRKCZ), mRNA	NM_002744	265	GAAGGCCTCATCATT CATG		AAGAAGGCCTCATC ATTCATGTT	hypothetical protein BC009489 (NM_138396), MYO1E (NM_004998), MLL5 (NM_018682)
H.s. mitogen-activated protein kinase kinase kinase 4 (MAP4K4), mRNA	NM_004834	347	GATGACCAACTCTGG CTTG		AAGATGACCAACTCT GGCTTGTT	hypothetical protein DKFZp434I2117 (NM_031478), Hypothetical protein LOC348761
MAP3K1: mitogen-activated protein kinase kinase kinase 1	XM_042066	2451	CTGTTAGAAATGCTGA GTG	MEKK1	AACTGTTAGAAATGC TGAGTGTT	HSA9761(NM_014473)
H.s. v-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (SRC), mRNA	NM_005417	990	GAAGAAGCTGAGGCA TGAG		AAGAAGAAGCTGAG GCATGAGTT	ADCY4 (NM_139247), KIAA1922 protein(XM_057040), hypothetical protein MGC15631(NM_032753), FANCC (NM_000136), CICE (Hs.301002), KIAA0841protein (XM_049237),TGFB1(NM_000358), LOC339385(XM_290863), ERBB3 (NM_001982), LOC286167 (XM_212210), PHKB (NM_000293)
H.s mRNA for protein kinase C delta-type, complete cds	NM_006254	652	GAAATGCATCGACAA GATC		AAGAAATGCATCGAC AAGATCTT	MOCS1(NM_005942)
H.s protein kinase, cAMP-dependent, catalytic, alpha (PRKACA), mRNA	NM_002730	428	CTTATACATGGTCATG GAG		AACTTATACATGGTC ATGGAGTT	FLJ25818 (NM_173503), PRKACB (NM_002731), ESD (XM_166266)
H.s. mitogen-activated protein kinase kinase kinase 1 (MAP4K1), mRNA	NM_007181	312	ACTCTGGATCTGCAT GGAA	HPK1	AAACTCTGGATCTGC ATGGAATT	SR-BP1 (NM_005866), KCNA2 (NM_004974)
H.s diacylglycerol kinase, iota (DGKI), mRNA	NM_004717	1890	TAACTACTTCAGCCTT GGA		AATAACTACTTCAGC CTTGATT	FLJ12076 (NM_025187), RYK (NM_002958), DGKZ (NM_003646)
Homo sapiens phosphoinositide-3-kinase, regulatory subunit, polypeptide 3 (p55, gamma) (PIK3R3), mRNA	NM_003629	1577	GCAAGAAAGGATGCT ATGC		AAGCAAGAAAGGAT GCTATGCTT	none detected

PIK3CD: phosphoinositide-3-kinase, catalytic, delta polypeptide	NM_005026	2268	ACTGAAGGCCCTGAA TGAC		AAACTGAAGGCCCT GAATGACTT	STK10 (NM_005990), PRDX5(NM_012094), PLCB2 (NM_004573), ANKRD5 (NM_022096), ALDOB (NM_000035), PFKFB1(NM_002625)
Homo sapiens mitogen-activated protein kinase kinase kinase 2 (MAP3K2), mRNA	NM_006609	1449	TTAAAAGCATATGGCG CTC	MEKK2	AATTTAAAAGCATATG GCGCTCTT	none detected
H.s. v-raf-1 murine leukemia viral oncogene homolog 1 (RAF1),	NM_002880	475	ACTGATGCTGCGTCTT TGA		AAACTGATGCTGCGT CTTTGATT	ANLN (NM_018685)

shRNA Sequences and Annotations

Annotation	Accession	Sense Oligo	Antisense Oligo	Possible Off-target Genes
CD29	NM_002211	aagCTTTCCCGGTAGAAAGTCGGGACAAAT TcaagagaAATTTGTCCCGACTTTCTACcttctc gag	CtcgagaaaGGTAGAAAGTCGGGACAAATTc CCCgACTTTCTACCGGGAAAGcct	none detected
TERE1	NM_013319	agctttcccTGTGATTTGGCAGTCAGGGTAttcaa gagaTACCCTGACTGCCAAATCACAtttttc	TcgagaaaaTGTGATTTGGCAGTCAGGGT AtctctgaaTACCCTGACTGCCAAATCAC gggaa	ELD/OSA1(NM_020732), DMBT1 (NM_004406),
BLK	NM_001715	agctttcccCAACATGAAGGTGGCCATTAAttcaa gagaTTAATGGCCACCTTCATGTTGtttttc	TcgagaaaaCAACATGAAGGTGGCCATTA AtctctgaaTTAATGGCCACCTTCATGTTG gggaa	PPP1R13B(NM_015316), ADAMTS6 (NM_014273), HYOU1 (NM_006389), SLC38A2(NM_018976), BA11(NM_001702), STAU (NM_004602), ZDHHC5(XM_290511), LOC283270 (XM_210956), KIAA0540 (XM_291064), FPRL1 (NM_001462)

Sequences for small inhibitory RNAs (siRNAs) and short-hairpin RNAs (shRNAs) used in this study are shown, along with targeted genes and corresponding accession numbers. All siRNAs utilized, with the exception of those targeting MAP3K7 and PTK2, demonstrated knockdown of signal induced by at least one AP-1 modulator (see Fig. 3A) and were assumed to be functional. The siRNAs corresponding to MAP3K7 and PTK2 have previously been shown to abrogate tumor necrosis factor (TNF) α -induced NF κ B signaling and enhance TRAIL-mediated apoptosis, respectively (P. Aza-Blanc, personal communication). These activities are consistent with reported loss-of-function phenotypes associated with these molecules (1, 2) and thus suggest that these siRNAs are also functional. Finally, mRNAs that are potentially affected by off-target si/shRNA activities were determined by Smith–Waterman similarity searches against a Unigene database (Uniq-1-2EST Human v.160). Genes (as annotated by LOCUSLINK) with 10 or more contiguous base-pairs of identity to the targeting (si/shRNA) sequence are shown.

1. Funakoshi-Tago, M., Sonoda, Y., Tanaka, S., Hashimoto, K., Tago, K., Tominaga, S. & Kasahara, T. (2003) *J. Biol. Chem.* **278**, 29359–29365.
2. Sanna, M. G., da Silva Correia, J., Ducrey, O., Lee, J., Nomoto, K., Schrantz, N., Deveraux, Q. L. & Ulevitch, R. J. (2002) *Mol. Cell. Biol.* **22**, 1754–1766.