

Supplementary Data

CRISPR/Cas9 edited RAS & MEK mutant cells acquire BRAF and MEK inhibitor resistance with MEK1 Q56P restoring sensitivity to MEK/BRAF inhibitor combo and KRAS G13D gaining sensitivity to immunotherapy

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Supplementary Figure Legends

Supplementary Figure S1. CRISPR/Cas9 Target-site cDNA sequencing of A375 melanoma models. **A.** Exon structure of unprocessed (top) and processed (bottom) NRAS mRNA. Red arrows indicate the location of primers used for PCR amplification and Sanger sequencing of NRAS cDNA, green arrow indicates location of the translation start codon. **B.** Sanger sequencing chromatogram of NRAS Q61K knock-in target site cDNA expressing heterozygous NRAS c.184 C>A point mutation. **C.** Exon structure of unprocessed (top) and processed (bottom) KRAS mRNA. Red arrows indicate the location of primers used for PCR amplification and Sanger sequencing of KRAS cDNA, green arrow indicates location of the translation start codon. **D.** Sanger sequencing chromatogram of KRAS G13D knock-in target site cDNA expressing heterozygous KRAS c.41 G>A point mutation. **E.** Exon structure of unprocessed (top) and processed (bottom) MEK1 mRNA. Red arrows indicate the location of primers used for PCR amplification and Sanger sequencing of MEK1 cDNA, green arrow indicates location of the translation start codon. **F.** Sanger sequencing chromatogram of MEK1 Q56P knock-in target site cDNA expressing homozygous MEK1 c.170 A>G point mutation. **G.** Sequence of primer pairs used for amplification and Sanger sequencing of NRAS, KRAS, and MEK1 CRISPR/Cas9 target sites.

Supplementary Figure S2. Drug resistance and growth rates of A375 melanoma models in 2D tissue culture. **A.** Survival rates by CellTiter-Glo viability assay of A375 WT, NRAS Q61K, KRAS G13D, and MEK1 Q56P melanoma models at 1 nM, 10 nM, and 100 nM dabrafenib. Statistical analysis was performed by two-way ANOVA with multiple comparisons, n=3 for each condition. **B.** Cell survival rates as in (A) at 10 nM, 100 nM, and 1 µM vemurafenib. **C.** Survival rates by CellTiter-Glo viability assay of A375 WT and MEK1 Q56P melanoma models at 1 nM, 4 nM, and 10 nM trametinib. Statistical analysis was performed by two-way ANOVA with multiple comparisons, n=3 for each condition. **D.** Cell survival rates as in (C) at 10 nM, 100 nM, and 1 µM binimetinib. **E.** Growth rates of A375 WT, NRAS Q61K, KRAS G13D, and MEK1 Q56P melanoma models in 2D tissue culture, as measured by confluence.

Supplementary Figure S3. Genomic loci screened for off-target Cas9 cutting in A375 NRAS Q61K. **A.** Off-target cutting screen for NRAS knock-in sgRNA1 with off-target sequence, genomic

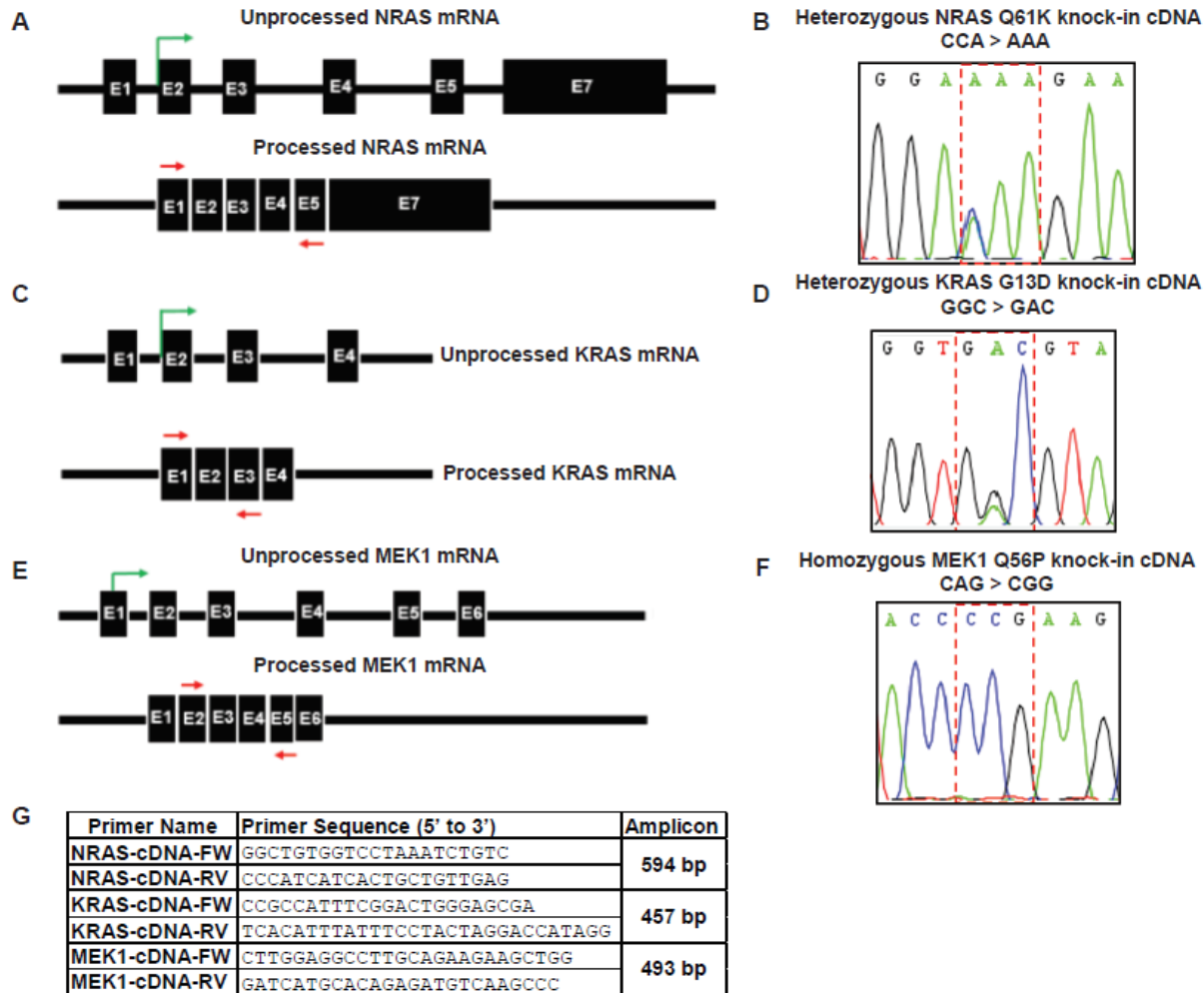
coordinates, number of mismatches from the target sequence, and sequence of primers used for amplification and Sanger sequencing of each off-target locus. Off-target sites were selected based on number of mismatches relative to the target sequence and by proximity to known coding regions. No indels were detected. **B.** The same as in (A) for NRAS knock-in sgRNA2. No indels were detected.

Supplementary Figure S4. Genomic loci screened for off-target Cas9 cutting in A375 KRAS G13D. A. Off-target cutting screen for KRAS knock-in sgRNA1 with off-target sequence, genomic coordinates, number of mismatches from the target sequence, and sequence of primers used for amplification and Sanger sequencing of each off-target locus. Off-target sites were selected based on number of mismatches relative to the target sequence and by proximity to known coding regions. No indels were detected. **B.** The same as in (A) for KRAS knock-in sgRNA2. No indels were detected.

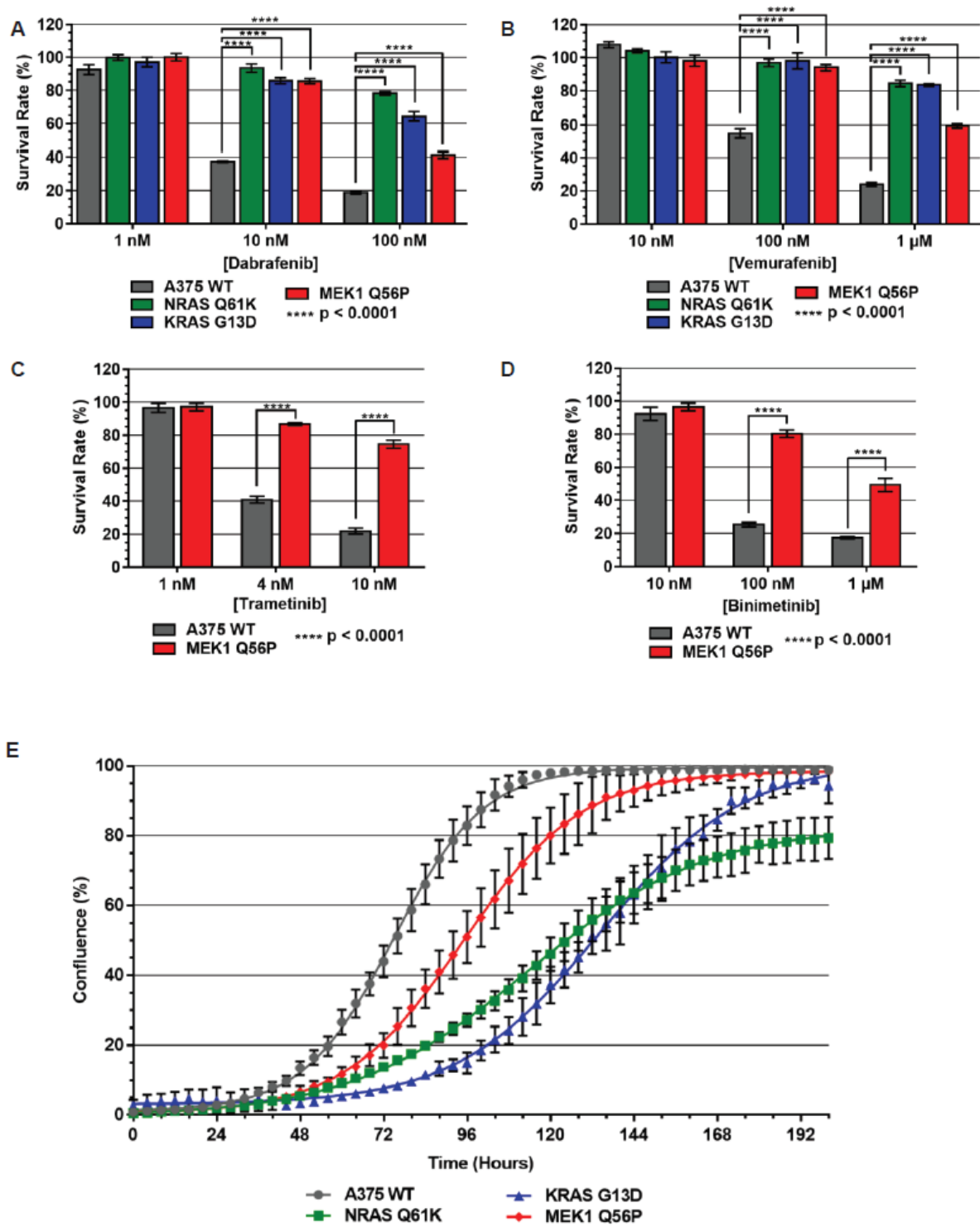
Supplementary Figure S5. Genomic loci screened for off-target Cas9 cutting in A375 MEK1 Q56P. A. Off-target cutting screen for MEK1 knock-in sgRNA1 with off-target sequence, genomic coordinates, number of mismatches from the target sequence, and sequence of primers used for amplification and Sanger sequencing of each off-target locus. Off-target sites were selected based on number of mismatches relative to the target sequence. No indels were detected. **B.** The same as in (A) for MEK1 knock-in sgRNA2. No indels were detected.

Supplementary Figure S6. Cas9/CMV plasmid sequence integration screening of A375 NRAS Q61K, KRAS G13D, and MEK1 Q56P melanoma models. A. Cas9/CMV plasmid sequence integration screening of A375 NRAS Q61K. **B.** Cas9/CMV plasmid sequence integration screening of A375 KRAS G13D. **C.** Cas9/CMV plasmid sequence integration screening of A375 MEK1 Q56P. **D.** Primer pairs used in Cas9/CMV plasmid integration screening.

Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3

A

Target	Coordinates	Strand	MM	Target Sequence	PAM	Gene	Primer Sequences (5' to 3')	Result
NRAS sgRNA1 Target	chr1:114714381-114714403	-	0	TAGGGCCA[GGTTATAATTGG]	TGG	NRAS	TGCAIGTTTGTCTTCTGAGA ATCTTTTCCATTACATACTGAAAGATG	N/A
NRAS sgRNA1 OT1	chr19:39083209-39083231	+	3	TACGGGA[GGTTATAATTGG]	GGG	PAPL	CCCAACACCTTGTATCACAATAGC CACATAGGCTTCTACTGATTGIGG	No Indel
NRAS sgRNA1 OT2	chr13:74391887-74391909	-	3	TAGTGTCA[GAATTATAATTGG]	AGG	RPL21P108	GGTGTGGACATTATAAGCTAAACATGTAA CCAGGCACAGAGTACTTGTACGGGAAGTGT	No Indel
NRAS sgRNA1 OT3	chrX:107907663-107907685	-	3	GAGGGACA[GGTGATAATTGG]	AGG	MID2	CAGAGACACAGACATTGAGAAATGAGGAGA GGAAGAGATGTCTCTTCATTGTGCTTTGG	No Indel
NRAS sgRNA1 OT4	chr1:176489495-176489517	+	4	TGGTGACA[CGTTATAATTGG]	TAG	PAPPA2	GGATTTTATGCTAGAGAGGAGCTTCC GAGATTCAGCATCTCTGCTTGCCATA	No Indel
NRAS sgRNA1 OT5	chr2:232785583-232785605	+	4	TAGTCCA[CGTTATAATTGG]	GGG	GIGYF2	GGGTTTGATCCACTTCCAGACTCAC GAGATTCAGCATCTCTGCTTGCCATA	No Indel
NRAS sgRNA1 OT6	chr2:195608816-195608838	+	4	TGCAGCCA[GGCTATAATTGG]	CAG	SLC39A10	GTGTCATGAGGGCTAAGTGCAGTAA CCAGCCGTGGGAGGTTATTTCAGAC	No Indel

B

Target	Coordinates	Strand	MM	Target Sequence	PAM	Gene	Primer Sequences (5' to 3')	Result
NRAS sgRNA2 Target	chr1:114713566-114713588	-	0	ACATAAGT[GAAGCTTGCTAC]	TGG	NRAS	TGCAIGTTTGTCTTCTGAGA ATCTTTTCCATTACATACTGAAAGATG	N/A
NRAS sgRNA2 OT1	chr5:141428584-141428606	-	3	TCTTCAGT[GAAGCTTGCTAC]	CAG	PCDHGB8P	GCCACACAGAAAGGAACAGGAAT GGTTGCTTTCACCTTGGTCTCTGT	No Indel
NRAS sgRNA2 OT2	chr6:6363617-6363639	-	3	AAACAAGT[GCAGCTTGCTAC]	CAG	LY86-AS1	GCAAGGCTGTTGACAAAAGTCTCTG GCCTGAGAATACTCTTGTCTCTCATCTT	No Indel
NRAS sgRNA2 OT3	chr3:60951631-60951653	+	3	AAAGAAGT[GCAGCTTGCTAC]	CAG	FHIT	ACAATAAGGCAGCCCATGACCAA CCCATCTAGATCATCTCTCTTAAATACT	No Indel
NRAS sgRNA2 OT4	chr4:186040203-186040225	+	3	ACAGTAGT[TAAGCTTGCTAC]	CAG	RNU4-64P	GTCTGTGCAACGCGGAGCCATTGAT CCCATATAAGCTCTGGAGTATTAAGGAC	No Indel
NRAS sgRNA2 OT5	chr4:24464902-24464924	-	4	GCAGAGGG[GAAGCTTGCTAC]	TGG	AC092846.1	GGATTCCTTGAGTCCAGGCGTTCAAGAC CCCATATAAGCTCTGGAGTATTAAGGAC	No Indel
NRAS sgRNA2 OT6	chr2:113212262-113212284	+	4	CTGTCACT[GAAGCTTGCTAC]	CAG	PAX8-AS1	GCAATTAACTCTTCTGGGTCTCTCA CTCCAGTTTCAGGAGCCGAGCTCTCT	No Indel

Supplementary Figure S4

A

Target	Coordinates	Strand	MM	Target Sequence	PAM	Gene	Primer Sequences (5' to 3')	Result
KRAS sgRNA1 Target	chr12:25245605-25245627	-	0	GCATTTT [CTTAAGCGTGA]	TGG	KRAS	CCCATGACACAATCCAGCTT CTGCTGCTGGTCTTTACTTTGG	N/A
KRAS sgRNA1 OT1	chr11:119058359-119058381	+	3	GCATTTTC [CTTAAGCTGGA]	TGG	HYOU1	GTGCTGTAAATCCAGCTACTCAGA GACCACTCTCCACCTAAGCATCAGAAA	No Indel
KRAS sgRNA1 OT2	chr8:108920528-108920550	+	3	GCATCTTT [CTTAAGCATGA]	GGG	RP11-1084E5.1	GCTCCAGATCACCGCTCTTCTCTAC GTCACTTCATGCTATGAGAAGTTCTGCA	No Indel
KRAS sgRNA1 OT3	chr9:5939470-5939492	+	4	GAATCTTT [CTTAATCATGA]	TGG	KIAA2026	GTTATCATTTCTTACAATATCTTCAGTTA ATCACTGAAATCATAATACCATGCCATC	No Indel
KRAS sgRNA1 OT4	chrX:120553913-120553935	+	4	ACATTTCT [TTTAAGCGTAGA]	GGG	CUL4B	GCAACATAGCAAGACCCCATATCTC GAGCTCTGAAGCCACAGATTTTAAATTC	No Indel
KRAS sgRNA1 OT5	chr16:87963594-87963616	+	4	GGACTTTT [CTTAAGGCTGA]	TGG	BANP	GACTGGCCGCTCTGCATGGATCTGAT GTCAAGCCAGGCTTCTGTATTTAACAAT	No Indel
KRAS sgRNA1 OT6	chr15:60162524-60162546	-	4	GCATAATT [CTTAAGCATGGA]	AGG	RP11-82L7.4	GCTGCAGTTGGATTGGATCGGACTT GTGCAGAGTGCCAGCAAGCTGGTTT	No Indel

B

Target	Coordinates	Strand	MM	Target Sequence	PAM	Gene	Primer Sequences (5' to 3')	Result
KRAS sgRNA2 Target	chr12:25245015-25245037	-	0	GTATTTC [GAGTTTCGTGAG]	AGG	KRAS	CCCATGACACAATCCAGCTT CTGCTGCTGGTCTTTACTTTGG	N/A
KRAS sgRNA2 OT1	chr18:73343401-73343423	+	4	GCTTTTCA [GACGTTCTGAG]	AGG	CTD-2354A18.1	GCCAGCTGGTCTTGACCTCTGA CCTGTAOCTCACTGATCACTTTTGGC	No Indel
KRAS sgRNA2 OT2	chr5:126961339-126961361	+	4	AAATTTCA [GAGAACTGTGAG]	AGG	3-Mar	GCAAGCACAACACAGGTCCTTCTAC GCATACTGTGGCATTATCTCTGCCCTT	No Indel
KRAS sgRNA2 OT3	chr3:102032478-102032500	+	4	GAACTTCT [GAGTTTAGTGAG]	TGG	RP11-221J22.2	TGTGAAGTGCTTACTGAACATCTTTGTGG CCACAAATGTTATTTTGTCTGAGGC	No Indel
KRAS sgRNA2 OT4	chr15:41054090-41054112	-	4	GTTTGA [GAGTTTGTGAG]	AGG	INO80	CACCCCTATTAGTGATAAAATGTCCTAA GAGGTCTTCTTTACTTACCTTGTCAGA	No Indel
KRAS sgRNA2 OT5	chr6:144838615-144838637	-	4	GIGTATA [GAGTTTGTGAG]	GGG	UTRN	CAGCTCATCTCCAGGCAATATTGAGA GTAACATCTGCACTCCCTATATGATGG	No Indel
KRAS sgRNA2 OT6	chr16:3756542-3756564	-	4	CCATTTC [GGGTTTGTGAG]	TGG	CREBBP	GACTTGCAATGAGTGTGCTTCTCTA GGCACATGGACATTACAGGCTG	No Indel

Supplementary Figure S5

A

Target	Coordinates	Strand	MM	Target Sequence	PAM	Gene	Primer Sequences (5' to 3')	Result
MEK1 sgRNA1 Target	chr15:66434832-66434854	+	0	CATGTTGG [TGATAGTCATCC]	CGG	MEK1	GAGGATTTCTACTGTTGTGATTACAG GGTATCCTGGCATTCTCTCCACAG	N/A
MEK1 sgRNA1 OT1	chr1:10853864-10853886	-	3	AATGATGG [TGACAGTCATCC]	TGG	HSPE1P24	ACCACCCACCAACCATTTGCTAC TGGTGATGTGGATGTTGATCAGGATAGT	No Indel
MEK1 sgRNA1 OT2	chr22:29816431-29816453	+	3	CAAGTTGG [AGTTAGTCATCC]	AGG	ASCC2	CAAATGGTTGCCAGAGTCTGTG ATGCTCGAACTACGCACTCAC	No Indel
MEK1 sgRNA1 OT3	chr2:86435176-86435198	-	3	CATGTTT [TGACAGTCATCC]	AGG	KDM3A	CTGAGGGAGGAGAATTGGTTG TCTATGGCTGATTCACACTACAG	No Indel
MEK1 sgRNA1 OT4	chr20:4745349-4745371	-	3	AATGTTGG [TGTCAGTCATCC]	TGG	PRNT	GGTGATCCCTTTGCCCATTTGT GGAGCCATAAGCTCTCAAGTCTGT	No Indel
MEK1 sgRNA1 OT5	chr11:108532803-108532825	-	4	CAAGTATG [AGATAGTCATCC]	AGG	EXPH5	CTCTTGAAGCAAGGCTAGCCA CTTGCAATCCAGGGCTGTACTC	No Indel
MEK1 sgRNA1 OT6	chr19:5663907-5663929	+	4	ACTCTTGG [TGAAAGTCATCC]	TGG	SAFB	CGAGAGAGATGGCAGGTGCATT GGTCCCTGTGGTCAAAAGTCGTGG	No Indel
MEK1 sgRNA1 OT7	chr3:185058989-185059011	-	4	CTTTTGA [TCATAGTCATCC]	TGG	VPS8	GTGGGCAGATCACTTGAAGCTAG GCCACGTTGTGGGATTCAGGAT	No Indel
MEK1 sgRNA1 OT8	chr8:137426391-137426413	-	4	GATATGG [TGACAGTCATCC]	AGG	ZYXP1	GCATGGTGGCAGGCGACTATAA GCCTGTGGAAAGTGAAGTCGGAAA	No Indel
MEK1 sgRNA1 OT9	chr15:29974505-29974527	-	4	CATTTCT [TAATAGTCATCC]	CGG	TJP1	GGGCAACACAGTGGGACTTCATC CCTTGCTTTGAGTTTATCTGCTCCTCT	No Indel
MEK1 sgRNA1 OT10	chr3:112605446-112605468	+	4	CATGATGA [CGGTAGTCATCC]	TGG	CCDC80	GAGGTTTCAATAAATACTCATGAATAGG CCCAATGTGGTCCATGGTATGTG	No Indel

B

Target	Coordinates	Strand	MM	Target Sequence	PAM	Gene	Primer Sequences (5' to 3')	Result
MEK1 sgRNA2 Target	chr15:66435621-66435643	-	0	AAAATTCA [AACAGCACGGCT]	GGG	MEK1	GAGGATTTCTACTGTTGTGATTACAG GGTATCCTGGCATTCTCTCCACAG	N/A
MEK1 sgRNA2 OT1	chr9:128918779-128918801	-	4	AAAAATTA [GCCAGCACGGCT]	GGG	LRRC8A	AATGCAGTGGCTCGATCTC CCTAGCTTCATGCACATCCT	No Indel
MEK1 sgRNA2 OT2	chr10:126245050-126245072	+	3	CAAAATGCA [AACAGCAAGGCT]	GGG	RP11-81B10.2	GGGACAGGATGGGGGACTAGAAA GTGGCCCTTTCCAAAGCACAGACTG	No Indel
MEK1 sgRNA2 OT3	chr18:51488559-51488581	-	3	AAAAATTA [AACAGCATGGCT]	GGG	RP11-267C16.1	CTCCCAAGTAGCTGAGACTAAG CGATCATGAAGGGTGTGTGT	No Indel
MEK1 sgRNA2 OT4	chr7:67719250-67719272	-	4	TAAAATTA [AACATCACGGCT]	GGG	RP4-736H5.3	GGCTGAGCCTCCCGAATAGCTGG GATGAGGCCAGCTAGGTTACAG	No Indel
MEK1 sgRNA2 OT5	chr7:52788178-52788200	-	4	AAGAAATGA [AACAGGACGGCT]	GGG	N/A	CGTAAGTGGGACTACAGGGGTGTACC CTGGACCCCCAACCCAATACACAA	No Indel
MEK1 sgRNA2 OT6	chr2:74963783-74963805	-	4	AAAATATA [ACAAGCACGGCT]	GGG	POLE4	CACATCATCTCAGCCTCTCAGGG GGGAGTGTCTGGGAGAGGGGAATGA	No Indel
MEK1 sgRNA2 OT7	chr15:26017137-26017159	+	4	AAACACA [AGCAACACGGCT]	GGG	RP11-1084I9.1	GCCCAGGTGGTTTACAAGGACAT CTGAATGTGTTCCGGTGTAGTGGG	No Indel
MEK1 sgRNA2 OT8	chr5:157400207-157400229	+	4	CAAAAACA [AACAGCAAGGCT]	TGG	ADAM19	CCTGTATGCACAGGAAAAGGCACAGC CTTCTTTTAAATCAGCTATATCTCCCTGC	No Indel
MEK1 sgRNA2 OT9	chr4:155183086-155183108	-	4	AAACTAG [AACAGCTCGGCT]	GGG	AC097467.2	CTCACTGCRAACTCCGCTCTCTGG GTTTAGCAAGTAGGCACCGCACAG	No Indel
MEK1 sgRNA2 OT10	chr17:2524519-2524541	-	4	AACATGCA [ATCAGCTCGGCT]	GGG	METTL16	GGTTTCAGTGAGCCGAGATT AAAGACTCAGGAGAGCTCTAGG	No Indel

Supplementary Figure S6

