

Figure EV1. *Nras* mutations of murine cancer cell lines.

A Representative Sanger sequencing traces of *Nras* codons 60–63 of some mouse cancer cell lines employed in these studies showing *Nras* mutations (red font, black arrows). Shown is one representative of three traces.

B Weekly monitored primary tumor volume of C57BL/6, BALB/c, and NOD/SCID host mice after s.c. delivery of 0.5×10^6 mouse or 10^6 human tumor cells (*n* for each group is given in Fig 1E, table).

C-E mRNA and protein of mouse and human tumor cell lines harboring wild-type (WT) and mutant *NRAS* and *KRAS* alleles were examined by qPCR (C, $n = 3$) and immunoblotting (D, E; shown are one representative of three experiments).

Data information: Cell lines are described in the text. All data are presented as mean \pm SEM. *P*: probability by two-way ANOVA (B) and one-way ANOVA (C).

(B, D, E): *NRAS*-mutant cell lines are in red font. (C): Genes overexpressed specifically by *NRAS*-mutant cell lines are in bold font.

Source data are available online for this figure.

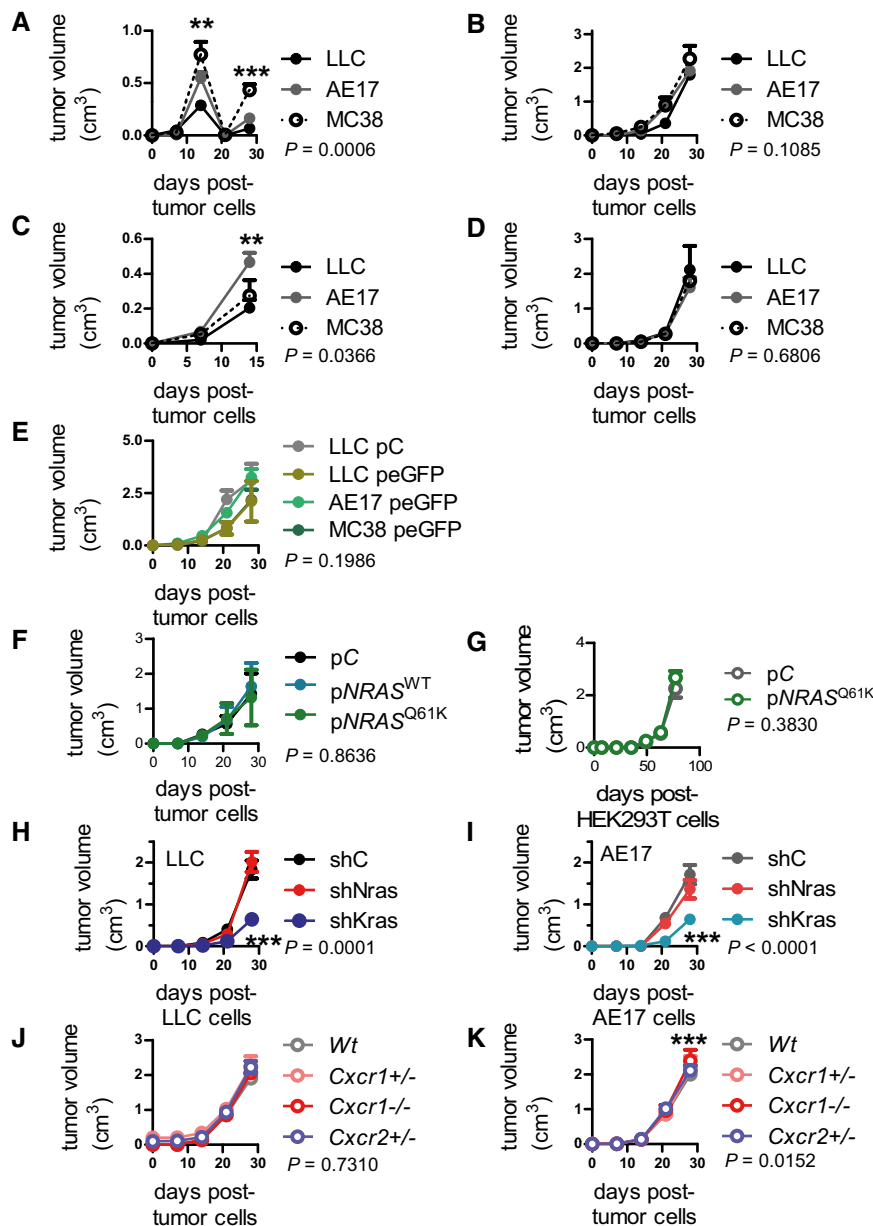


Figure EV2. Primary tumor volume of experiments from Figs 2–4 and 7.

A Primary tumor volume of experiment from Fig 2A ($n = 3$ mice/cell line).

B Primary tumor volume of experiment from Fig 2B ($n = 5$ mice/cell line).

C Primary tumor volume of experiment from Fig 2C ($n = 5$ mice/cell line).

D Primary tumor volume of experiment from Fig 2D ($n = 4$ mice/cell line).

E Primary tumor volume of experiment from Fig 3A ($n = 4$ mice/cell line).

F Primary tumor volume of experiment from Fig 3D ($n = 8$ mice/cell line).

G Primary tumor volume of experiment from Fig 3E ($n = 7-8/\text{cell line}$).

H, I Primary tumor volume of experiments from Fig 4C (*n* is given in Fig 4C, tables).

J, K Primary tumor volume of experiments from Fig 7A (*n* is given in Fig 7A, tables).

Data information: Cell lines are described in the text.

All data are presented as mean \pm SEM. *P*: probability by two-way ANOVA. ** and ***: $P < 0.01$ and $P < 0.001$ compared to controls at the time point indicated by Bonferroni post-test.

A Mus musculus *Nras* (GenBank Accession ID: NM_010937.2)

1	gggactgggg	cgccctgggc	gcctagtgat	tacgtagcgg	gtggggccgg	aagtgccgct
61	ccctggcggg	ggctgttcat	ggcggtttcg	gggtctccaa	cagcttctca	ggttgaagtc
121	caaaagcctc	ccgaggcggg	gtctgcggag	tttgaggttt	ttgctgggtg	gaaatgactg
181	agtacaaact	ggtggtggtt	ggagcagggt	gtgttgggaa	aagcgccttg	acgatccagc
241	taatccagaa	ccactttgtg	gatgaatatg	atcccaccat	agaggattct	taccgaaagc
301	aagtgggtgat	tgatggtgag	acctgcctgc	tggacatact	ggacacagct	ggacaagagg
361	agtacagtgc	catgagagac	cagtacatga	ggacaggcga	agggttcctc	tgtgtatttg
421	ccatcaataa	tagcaaatca	tttgcagata	ttaacctcta	caggagagca	attaagcgtg
481	tgaagatttc	tgatgatgtc	cccatggtgc	tggtaggcaa	caagtgtgac	ttgccaa caa
541	ggacagttga	cacaaa gcaa	gccacgaac	tggccaagag	ttacggaatt	ccattcattg
601	agacctcagc	caagaccoga	cagggtgtgg	aggatgcctt	ttacacactg	gtaagggaga
661	tacgccagta	ccgaatgaaa	aagctcaaca	gcagtgacga	tggcactcaa	ggttgatg
721	ggctgcctcg	tgtgctgatg	tag taagaca	ctttgaaagt	tctgtcatca	gaaaagagcc
781	actttgaagc	tgcactgatg	ccctggttct	gacatccctg	gaggagacct	gttcctgctg
...						
1321	taaatccaac	attcacaaca	aagtgccttt	ttcctaaaag	tgctctgtag	gtccattac
1381	agtttgtaat	tggaa tagat	gtgtca agaa	ccattgtata	ggaaagtgac	tctgagccat
1441	ctaccttga	gggaaagggtg	tatgtacctg	atggcagatg	ctttgtgtat	gcacatgaag
...						
2701	tacaggcaca	ttccaccaca	cacacctgac	tgagctcggt	cctaattgagt	tttcattaag
2761	caaattccc	atcaccttga	aactaa tcag	aagggggaag	aaacatttgc	tatgctcctg
2821	agtgctaaca	ctgggatcat	tcacatgggg	tttgcattcc	taggcaact	aaactgctgc
...						

B Mus musculus *Kras* (GenBank Accession ID: NM_021284.6)

1	aggcggcggc	cgcgcgcgct	gaggcggcag	cgctgtggcg	gcggctgaga	cggcagggga
61	aggcggcggc	ggctcggccc	ggagtcccgc	tcccgcgcca	tttcggaccc	ggagcgagcg
121	cggcgcgggc	ctgaaggcgg	cggcgggagc	ctgaggcgcg	gcggctccgc	ggcgcgagga
181	gaggcctgct	gaaaatgact	gagtataaac	ttgtggtggt	tggagctggt	ggcgtaggca
241	agagcgctt	gacgatacag	ctaattcaga	atcactttgt	ggatgagtat	gacctacga
301	tagaggactc	ctacaggaaa	caagtagtaa	ttgatggaga	aacctgtctc	ttggatattc
361	tgcacacagc	aggtcaagag	gagtacagtg	caatgaggga	ccagtacatg	agaactgggg
421	agggtcttct	ttgtgtattt	gccataaata	atactaaatc	atttgaagat	attcaccatt
481	atagagaaca	aattaaaaga	gtaaaggact	ctgaagatgt	gcctatggtc	ctggtaggga
541	ataagtgtga	tttgccttct	agaacagtag	acacgaaaca	ggctcaggag	ttagcaagga
601	gttacgggat	tccgttcatt	gagacctcag	caaagacaag	acagggtggt	gacgatgcct
661	tctatacatt	agtcggagaa	attcgaaaac	ataaagaaaa	gatgagcaaa	gatgggaaga
721	agaagaagaa	gaagtcaagg	acaagggtga	cagttatgtg	atacttttgt	actctttctt
...						
1321	tattttttct	agaaaagggg	aaaaatggaa	aaaaaaaggc	aatggaaaat	gttgaaatcc
1381	attcagtttc	catgttagct	aaattactgt	aagattccta	taatagcttt	tcctggtaag
1441	gcagaccag	tatgaaatag	taataaccat	ttgggtata	ttacatgct	actaaatctt
...						

Figure EV3. Target specificity of shRNAs against murine *Nras* and *Kras* transcripts.

Partial *Mus musculus* *Nras* (A) and *Kras* (B) transcripts with start (green boxes) and stop (red boxes) codons, showing the target sequences of the anti-*Nras* (red font) and anti-*Kras* (blue font) shRNA pools employed in the present study. Note that there is no sequence homology between *Nras*- and *Kras*-specific target sequences and that no *Nras*-specific target sequence is present in the *Kras* transcript and *vice versa*.

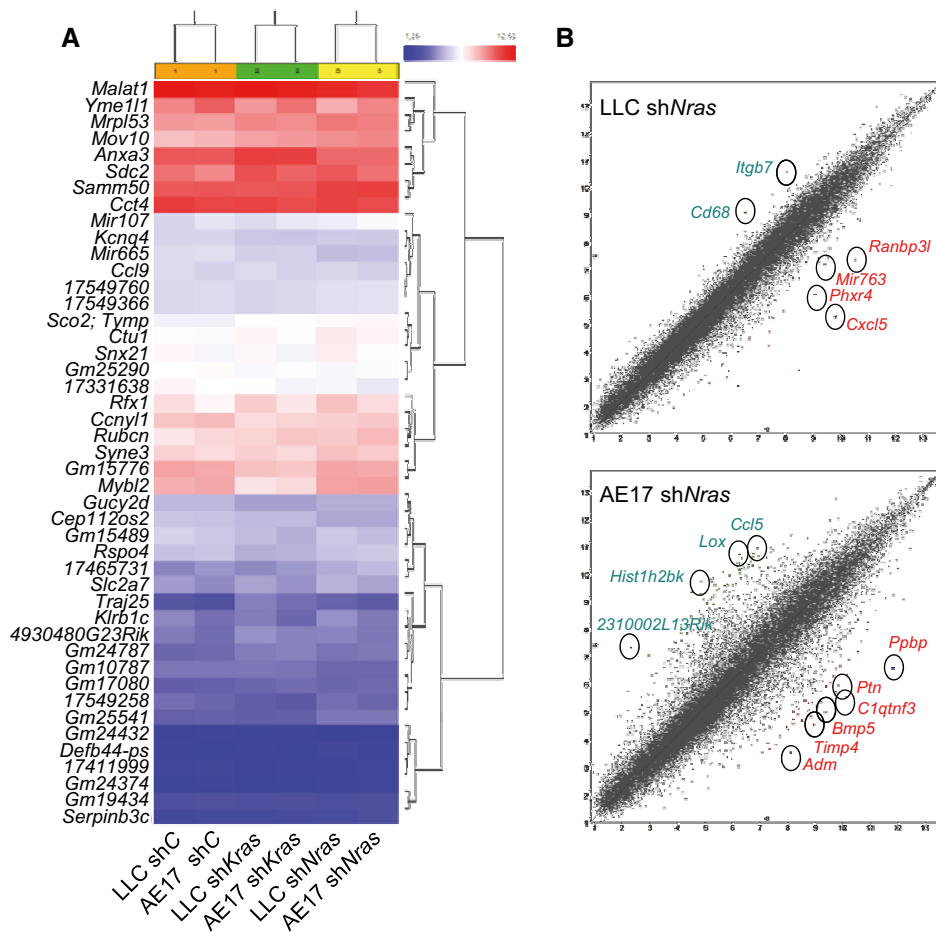


Figure EV4. Microarray comparing differential gene expression (Δ GE) of *Nras*^{Q61H}-mutant mouse tumor cells (LLC and AE17) expressing control (shC), anti-Kras (shKras), or anti-Nras (shNras) shRNAs.

- A Hierarchical clustering using paired ANOVA cutoff probability value of $P < 0.001$ identifies 45 differentially expressed genes and clusters cells by silenced gene and not by cell line.
- B Volcano plots of changes in gene expression by cell line showing the top altered genes. Transcripts downregulated by shNras are shown in red font and transcripts upregulated in green font. The distance from diagonal lines represent probability value.