

# APPENDIX

Research Article

## ***NRAS* destines tumor cells to the lungs**

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## TABLE OF CONTENTS

	Pages
<b>APPENDIX SUPPLEMENTARY TABLES</b>	
<b>Appendix Table S1</b> - Top 20 of 107 transcripts overrepresented more than two-fold in LLC cells stably expressing anti- <i>Nras</i> shRNA compared with control cells expressing random shRNA	2
<b>Appendix Table S2</b> - Top 20 of 154 transcripts underrepresented more than two-fold in LLC cells stably expressing anti- <i>Nras</i> shRNA compared with control cells expressing random shRNA	3
<b>Appendix Table S3</b> - Top 20 of 1110 transcripts overrepresented more than two-fold in AE17 cells stably expressing anti- <i>Nras</i> shRNA compared with control cells expressing random shRNA	4
<b>Appendix Table S4</b> - Top 20 of 901 transcripts underrepresented more than two-fold in AE17 cells stably expressing anti- <i>Nras</i> shRNA compared with control cells expressing random shRNA	5
<b>Appendix Table S5</b> - PCR primers used for these studies	6-7
<b>Appendix Table S6</b> - Antibodies used for these studies	8

**Appendix Table S1 - Top 20 of 107 transcripts overrepresented more than two-fold in LLC cells stably expressing anti-*Nras* shRNA compared with control cells expressing random shRNA**

Gene symbol	Gene name	$\Delta$ GE <sup>a</sup>
<i>Itgb7</i>	integrin beta 7	+4.57
<i>Cd68</i>	CD68 antigen	+3.85
<i>Rnu3a</i>	U3A small nuclear RNA	+3.72
<i>Tuba1b</i>	tubulin, alpha 1B	+3.21
<i>Acta2</i>	actin, alpha 2, smooth muscle, aorta	+2.92
<i>Pfkl</i>	phosphofructokinase, liver, B-type	+2.88
<i>Nptx1</i>	neuronal pentraxin 1	+2.87
<i>Ndrg1</i>	N-myc downstream regulated gene 1	+2.80
<i>Hist1h2bk</i>	histone cluster 1, H2bk	+2.66
<i>Acy3</i>	aspartoacylase (aminoacylase) 3	+2.65
<i>Glt8d2</i>	glycosyltransferase 8 domain containing 2	+2.58
<i>Snora73a</i>	small nucleolar RNA, H/ACA box 73a	+2.57
<i>Snora44</i>	small nucleolar RNA, H/ACA box 44	+2.53
<i>Tppp3</i>	tubulin polymerization-promoting protein family member 3	+2.53
<i>Krt18</i>	keratin 18	+2.53
<i>Pgam1</i>	phosphoglycerate mutase 1	+2.48
<i>Fam83d</i>	family with sequence similarity 83, member D	+2.47
<i>Hist1h2bn l j f</i>	histone cluster 1, H2bn   l   j   f	+2.46
<i>Tigit</i>	T cell immunoreceptor with Ig and ITIM domains	+2.41
<i>Lpin1</i>	lipin 1	+2.41

<sup>a</sup>  $\Delta$ GE, difference in gene expression between LLC cells stably expressing anti-*Nras* shRNA compared with control cells expressing random shRNA.

**Appendix Table S2 - Top 20 of 154 transcripts underrepresented more than two-fold in LLC cells stably expressing anti-*Nras* shRNA compared with control cells expressing random shRNA**

Gene symbol	Gene name	$\Delta$ GE <sup>a</sup>
<i>Cxcl5</i>	chemokine (C-X-C motif) ligand 5	-16.30
<i>Ranbp3l</i>	RAN binding protein 3-like	-7.71
<i>Phxr4</i>	per-hexamer repeat gene 4	-4.55
<i>Mir763</i>	microRNA 763	-3.91
<i>5730469M10Rik</i>	RIKEN cDNA 5730469M10 gene	-3.81
<i>Slc7a11</i>	solute carrier family 7, member 11	-3.62
<i>Gm20199</i>	predicted gene, 20199	-3.60
<i>Ccl2</i>	chemokine (C-C motif) ligand 2	-3.59
<i>Ccl7</i>	chemokine (C-C motif) ligand 7	-3.55
<i>Saa3</i>	serum amyloid A 3	-3.43
<i>Gm7265</i>	predicted gene 7265	-3.39
<i>C030013D06Rik</i>	RIKEN cDNA C030013D06 gene	-3.35
<i>Fgf7</i>	fibroblast growth factor 7	-3.32
<i>Gm4787</i>	predicted gene 4787	-3.29
<i>Mir3094</i>	microRNA 3094	-3.29
<i>Zfp932</i>	zinc finger protein 932	-3.27
<i>9930017N22Rik L</i>	RIKEN cDNA 9930017N22 gene	-3.21
<i>OC100503407</i>	uncharacterized LOC100503407	
<i>Gm19270</i>	predicted gene, 19270	-3.07
<i>Fabp4</i>	fatty acid binding protein 4, adipocyte	-3.01
<i>Igfbp7</i>	insulin-like growth factor binding protein 7	-2.96

<sup>a</sup>  $\Delta$ GE, difference in gene expression between LLC cells stably expressing anti-*Nras* shRNA compared with control cells expressing random shRNA. *Cxcl5* was studied further.

**Appendix Table S3 - Top 20 of 1110 transcripts overrepresented more than two-fold in AE17 cells stably expressing anti-*Nras* shRNA compared with control cells expressing random shRNA**

Gene symbol	Gene name	$\Delta$ GE <sup>a</sup>
<i>2310002L13Rik</i>	RIKEN cDNA 2310002L13 gene	+17.92
<i>Lox</i>	lysyl oxidase	+17.47
<i>Hist1h2bk</i>	histone cluster 1, H2bk	+16.50
<i>Ccl5</i>	chemokine (C-C motif) ligand 5	+15.32
<i>Pcsk9</i>	proprotein convertase subtilisin/kexin type 9	+15.09
<i>Cdh2</i>	cadherin 2	+14.65
<i>Lipg</i>	lipase, endothelial	+14.27
<i>Btg3</i>	B cell translocation gene 3	+13.48
<i>Efna5</i>	ephrin A5	+13.06
<i>Manea</i>	mannosidase, endo-alpha	+12.50
<i>Zfx4</i>	zinc finger homeodomain 4	+12.41
<i>F11r</i>	F11 receptor	+12.08
<i>Nfib</i>	nuclear factor I/B	+11.65
<i>Sdc3</i>	syndecan 3	+11.50
<i>Gpr126</i>	G protein-coupled receptor 126	+11.49
<i>Dnmbp</i>	dynamin binding protein	+11.14
<i>Colec12</i>	collectin sub-family member 12	+11.12
<i>Fn1</i>	fibronectin 1	+10.95
<i>Tnc</i>	tenascin C	+10.81
<i>Nrn1</i>	neuritin 1	+10.61

<sup>a</sup>  $\Delta$ GE, difference in gene expression between AE17 cells stably expressing anti-*Nras* shRNA compared with control cells expressing random shRNA.

**Appendix Table S4 - Top 20 of 901 transcripts underrepresented more than two-fold in AE17 cells stably expressing anti-*Nras* shRNA compared with control cells expressing random shRNA**

Gene symbol	Gene name	$\Delta$ GE <sup>a</sup>
<i>Ppbbp</i>	pro-platelet basic protein	-38.83
<i>C1qtnf3</i>	C1q and tumor necrosis factor related protein 3	-18.08
<i>Ptn</i>	pleiotrophin	-13.59
<i>A430107O13Rik</i>	RIKEN cDNA A430107O13 gene	-12.36
<i>Cdk15</i>	cyclin-dependent kinase 15	-12.26
<i>Timp4</i>	tissue inhibitor of metalloproteinase 4	-12.01
<i>Bmp5</i>	bone morphogenetic protein 5	-11.84
<i>Adm</i>	adrenomedullin	-10.72
<i>Prph</i>	peripherin	-10.58
<i>Wfdc15a</i>	WAP four-disulfide core domain 15A	-10.55
<i>Gm364</i>	predicted gene 364	-10.49
<i>Gm5077</i>	predicted gene 5077	-10.17
<i>Rhox2g</i>	reproductive homeobox 2G	-9.70
<i>Ogn</i>	osteoglycin	-9.29
<i>Nxf3</i>	nuclear RNA export factor 3	-9.19
<i>Rcn3</i>	reticulocalbin 3, EF-hand calcium binding domain	-9.13
<i>Dock10</i>	dedicator of cytokinesis 10	-9.11
<i>Dkk2</i>	dickkopf homolog 2 ( <i>Xenopus laevis</i> )	-9.07
<i>Pla2g4a</i>	phospholipase A2, group IVA	-8.89
<i>Actc1</i>	actin, alpha, cardiac muscle 1	-8.83

<sup>a</sup>  $\Delta$ GE, difference in gene expression between AE17 cells stably expressing anti-*Nras* shRNA compared with control cells expressing random shRNA. *Ppbbp* was studied further.

**Appendix Table S5 - PCR primers used for these studies**

Method <sup>a</sup>	Primer	Sequence	Amplicon (bp)
Seq	KrasF	CCATTTTCGGACCCGGAG	904
	KrasR	CTTTAGTCTCTTCCACAGGCA	
	NrasF	GCGCCTAGTGATTACGTAGC	905
	NrasR	TGAAGAGGTCTCAGGTTAGATGG	
	Hras1F	CGGAGCTCATGCGCGG	881, 734
	Hras1R	TCCGGGCACCTCCATGTCCT	
	Hras1F2	ACACAGCAGGTCAAGAAGAG	577, 430
	Hras1R	TCCGGGCACCTCCATGTCCT	
	mEgfrF1	GCCTGATAACTGGACTGACCT	F1/R1 1673; F2R2
	mEgfrR1	AGAATCAACTCTCGGAACCTTTGG	
	mEgfrF2	CTCCTCTTCTTCCCGCACTG	2261; F2/R 2073;
	mEgfrR2	AGAATCTGAGACCTCTGGCTG	
	mEgfrR	GCATAGGTGGCAGACATTATTGG	1S1/R1 1174;
	mEgfr1S1	ACAACTGCATCCAGTGTGCC	1S2/R1 672;
	mEgfr1S2	GGCCATCAAGGAGTTAAGAG	1S1/R2 1759
	mEgfr2S1	AGAGAATCCCTTTGGAGAACC	1S1/R 1571;
	mEgfr2S2	CCACCACTCATGCTGTACAACC	2S2/R2 1256;
	mEgfr2S3	GTCGTTGGCCTGAACATCAC	2S2/R 1068;
	Pik3caF1	ATTCTGACTCCATAAGGCGG	2S3/R2 731; 2S3/R
	Pik3caR1	GAACCAATCAAACCTCCAACCTC	543
	Pik3caF2	GCTGAACCCTATTGGTGTACTG	1493
	Pik3caR2	GCTCAAGTCCTAATGTTGTTCCCT	
	BrafF	CGCTGTCTTCGGAATAACCA	1551
	BrafR	AATTCTTTCCATCATGCCTGACC	
	BrafF1	GGCGGGTTCCAGAGGTG	2066
	BrafR1	CACTCCACCGAGATTTCACTG	
	Trp53F	GTAGCTTCAGTTCATTGGGA	1354, 1450
	Trp53R	GAAGTCATAAGACAGCAAGGA	
	Stk11F	GGGACGAGGACAAAGAGTGG	1183, 1374
	Stk11R	CTGCCAGAGATCCTGATGAC	
qPCR	NrasF	CCACCATAGAGGATTCTTACCGA	149
	NrasR	GGCAAATACACAGAGGAACCC	
	Cxcl5F	TTGGGCAGTGACAAAAAGAA	100
	Cxcl5R	TTCAGTGGGGTCAGAGTCCT	
	PbpF	GTACAGGCCAGGAGTTCACT	106
	PbpR	GACGATTCTCTTGACGCCAG	
	Cxcr1F	TCTACGTGCTGAACCTAGCC	117
	Cxcr1R	GTGAGACCATCTTGACACAGG	
	Cxcr2F	TTCTGCTACGGGTTCACACT	128
	Cxcr2R	AGAACCAGGTTGTAGGGCAG	
	GusbF	TTACTTTAAGACGCTGATCACC	165
	GusbR	ACCTCCAAATGCCCATAGTC	

GapdhF	TGTGTCCGTCGTGGATCTGA	150
GapdhR	TTGCTGTTGAAGTCGCAGGAG	
HprtF	AGTCCCAGCGTCGTGATTAG	88
HprtR	TTTCCAAATCCTCGGCATAATGA	
NRASF	TCCAGCTAATCCAGAACCACT	148
NRASR	TGGTCTCTCATGGCACTGTA	
CXCL6F	CGAACCCTCTCTTGACCACT	149
CXCL6R	CACAGCAGAGACAGGACCAG	
CXCL7F	CGAAAGGCAAAGAGGAAAGTC	120
CXCL7R	CTTTCCCGATCACTTCCAAA	
CXCL8F	GCAGAGGGTTGTGGAGAAGTT	150
CXCL8R	ACCCTACAACAGACCCACAC	
GUSBF	CTACTTGAAGATGGTGATCGCTC	118
GUSBR	ACAGATCACATCCACATACGG	

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<sup>a</sup>Application: Seq, sequencing; qPCR, quantitative (real-time) PCR.

**Appendix Table S6 - Antibodies used for these studies**

Method <sup>a</sup>	Target	Provider <sup>b</sup>	Catalog #	Dilution	Conjugate <sup>c</sup>
IF	CXCR1	Abcam	Ab10400	1:20	-
	CXCR2		Ab14935	1:100	-
	GFP	Santa Cruz	sc-8334	1:200	-
	CD34	eBioscience	11-0341	1:200	FITC
IH	PCNA	Abcam	ab2426	1:2000	-
FC	CD11b	eBioscience	12-0112	0.1 µg / 10 <sup>6</sup> cells	PE
	Ly6C		45-5932-82		PerCP- Cy5.5
	Gr1		25-5931-82		PE-Cy7
WIB	NRAS	Santa Cruz	sc-31	1:500	-
	β-actin		sc-47778	1:500	-
	Goat anti-rabbit & anti-mouse IgG	Southern Biotech	4030-05 & 1030-05	1:8000	HRP
IF	Donkey anti-rabbit & anti-mouse IgG	Molecular Probes	A21206	1:1000	AF488
			A21202		AF488
			A10042		AF568
			A10037		AF568
			A31573		AF647
			A31571		AF647

<sup>a</sup> Application: IF, immunofluorescence; WIB, Western immunoblotting; IH, immunohistochemistry; FC, flow cytometry.

<sup>b</sup> Providers: Abcam, Cambridge, UK; eBioscience, San Diego, CA; Santa Cruz Biotechnology, Dallas, TX; Southern Biotech, Birmingham, AL; Molecular Probes, Eugene, OR.

<sup>c</sup> Conjugates: FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin-chlorophyll protein; HRP, horse radish peroxidase; AF, Alexa Fluor.