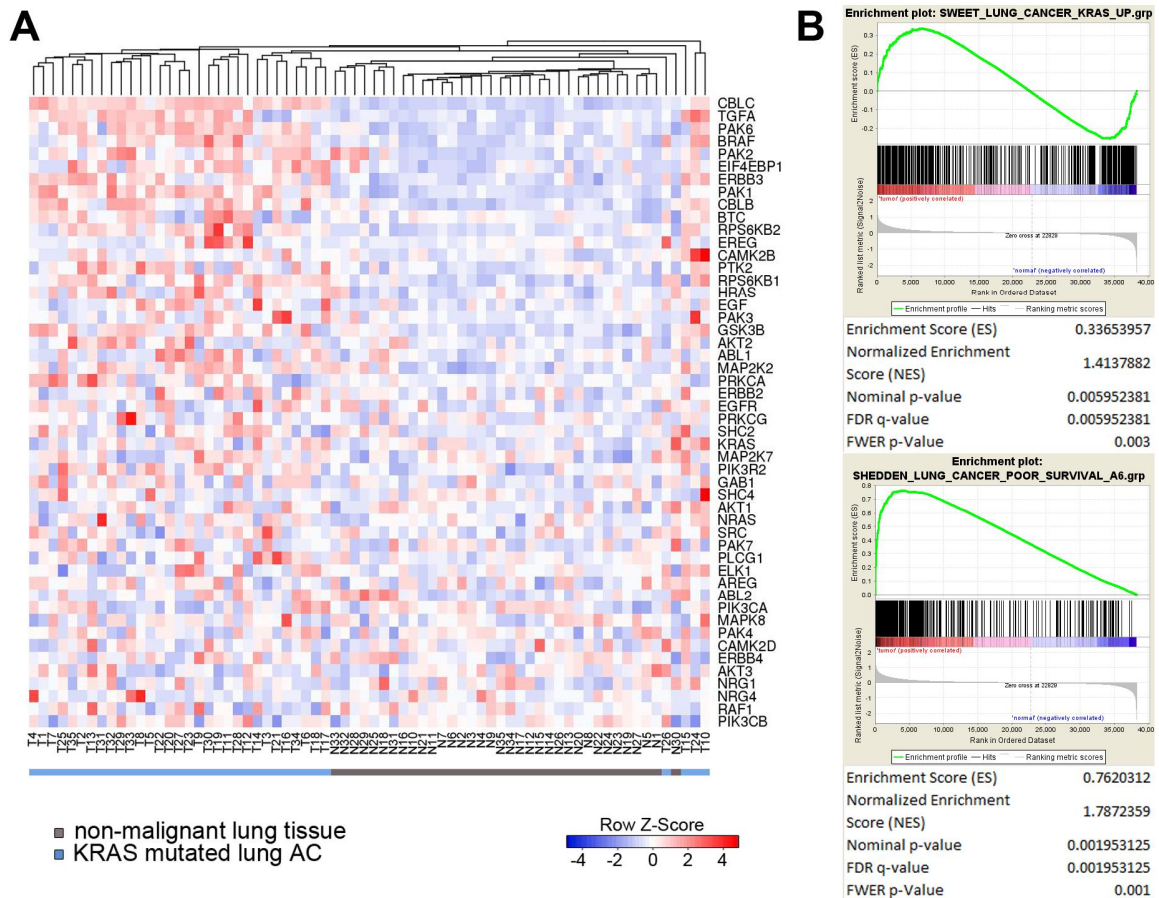
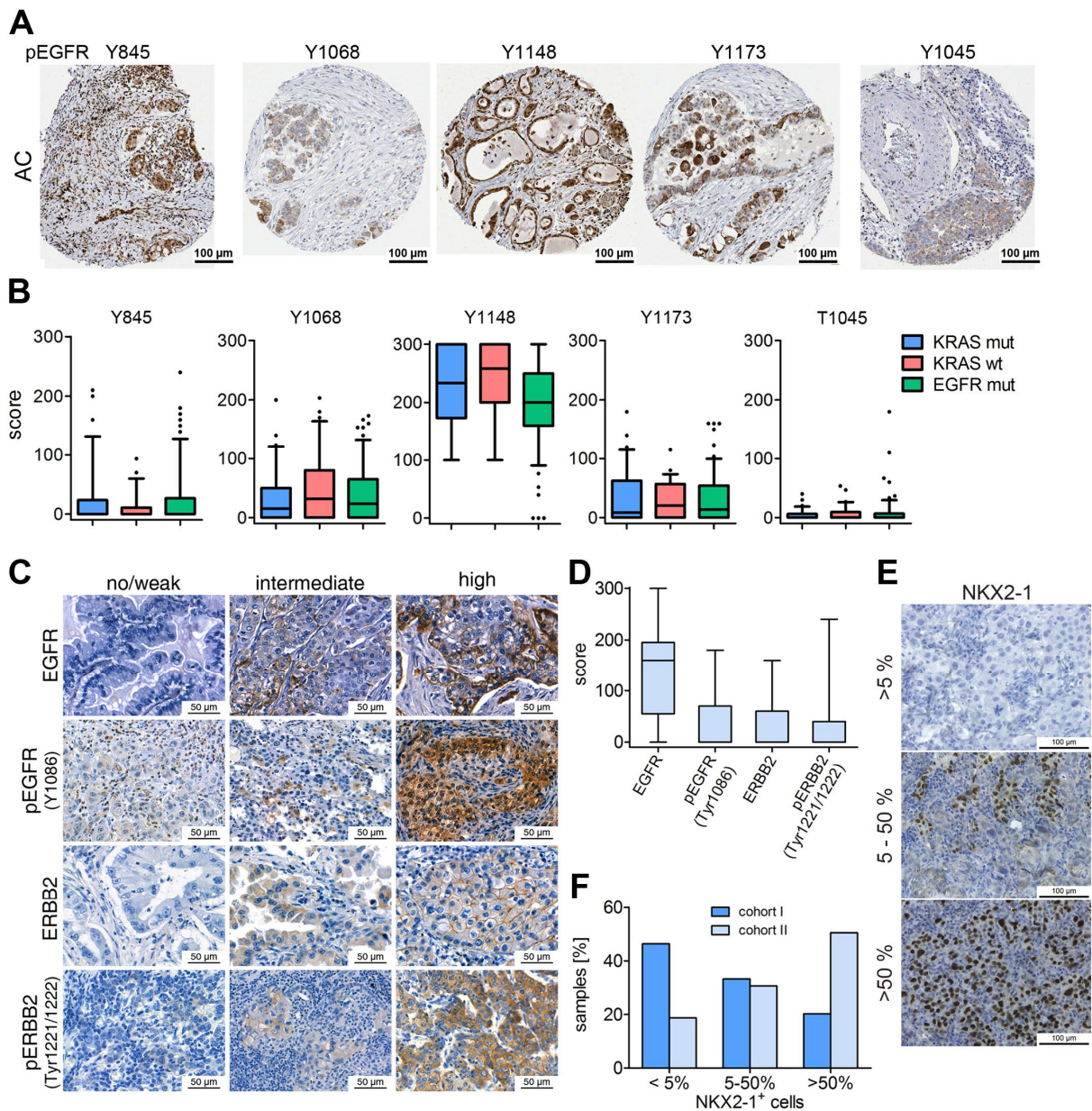


**SUPPLEMENTARY MATERIALS:**

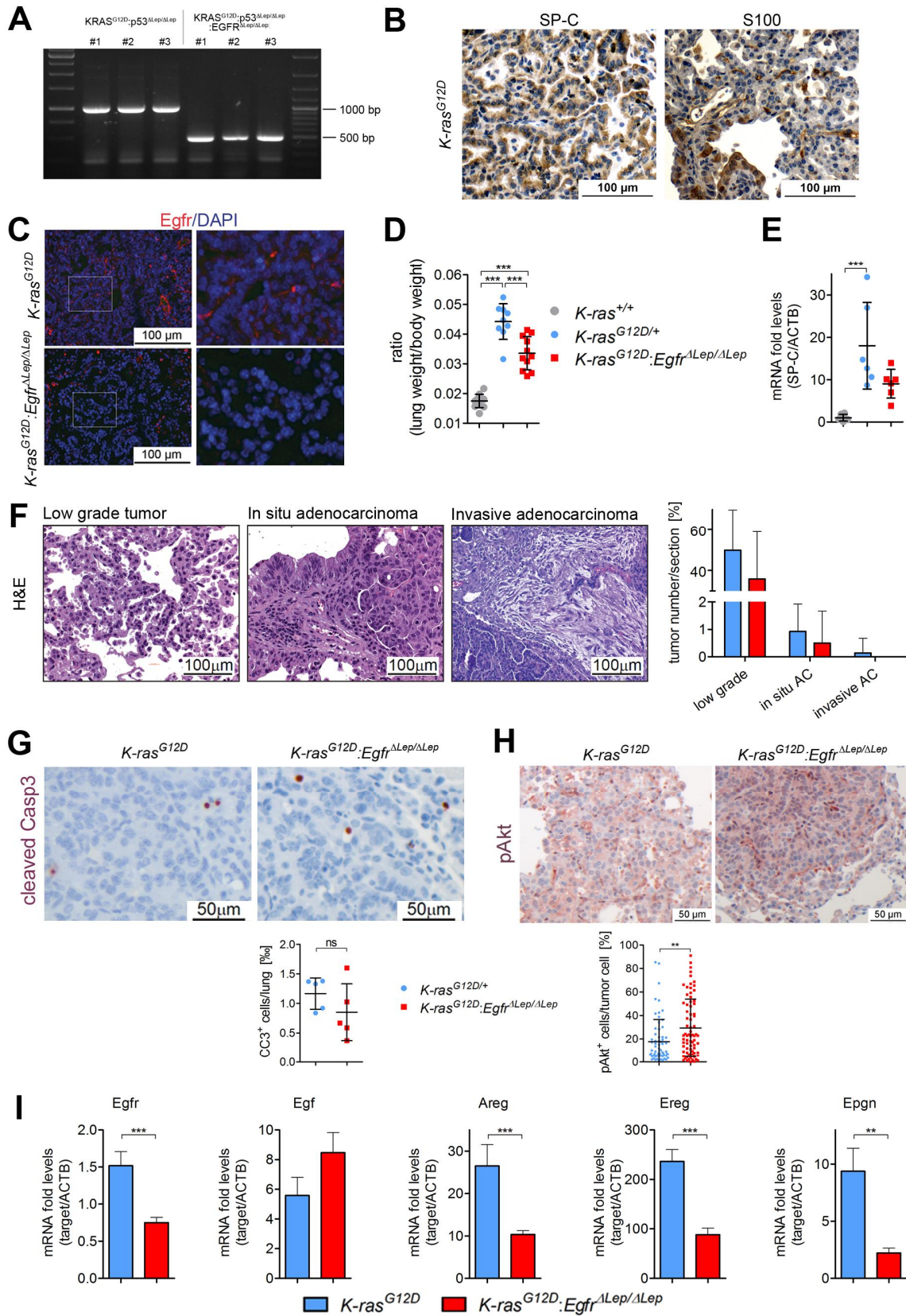


**Fig. S1:** K-RAS mutated lung AC display increased ERBB expression profile. **(A)** Heat map for mRNA expression in K-RAS mutated tumor biopsies (T1-T35) and adjacent non-malignant, healthy lung parenchyma (N1-N35). Displayed are the 50 most differentially regulated genes within the KEGG ERBB signaling pathway and hierarchical clustering was performed using the heatmapper.ca webtool. **(B)** GSEA for indicated gene sets in K-RAS tumors versus healthy normal tissue. Data in **(A)** and **(B)** was retrieved from the Gene Expression Omnibus (GSE75037).

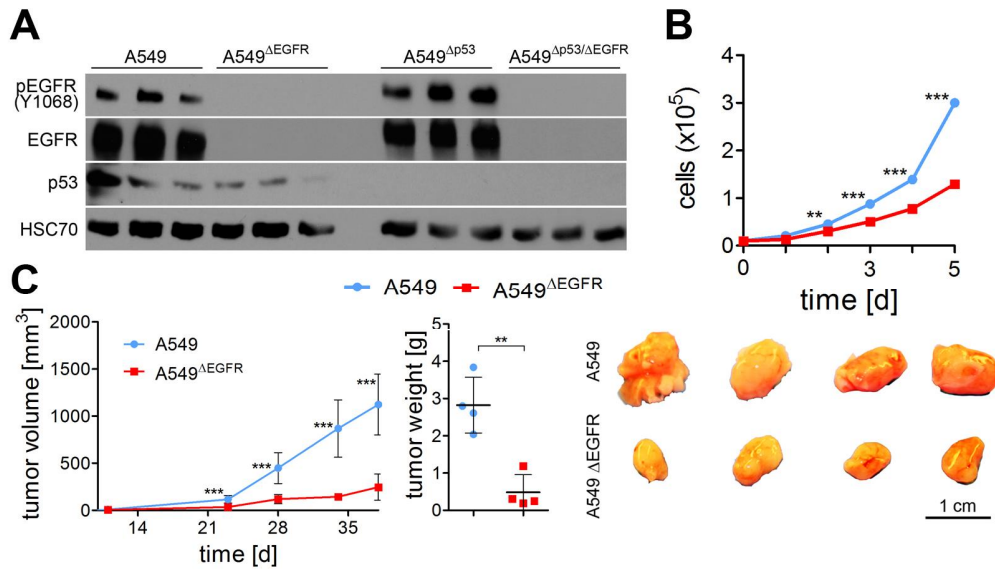


**Fig. S2: K-RAS mutated lung AC exhibit activated EGFR.** (A) Low magnification of representative immunohistological stainings for indicated phosphorylation sites in human lung AC samples harboring *K-RAS* mutations. (B) Comparison of scores for indicated EGFR phosphorylation sites in tumor cells of human lung AC harboring mutated *K-RAS* (n=31-35), wildtype (wt) *K-RAS* (n=31-32) or mutated *EGFR* (n=65). (C) Representative

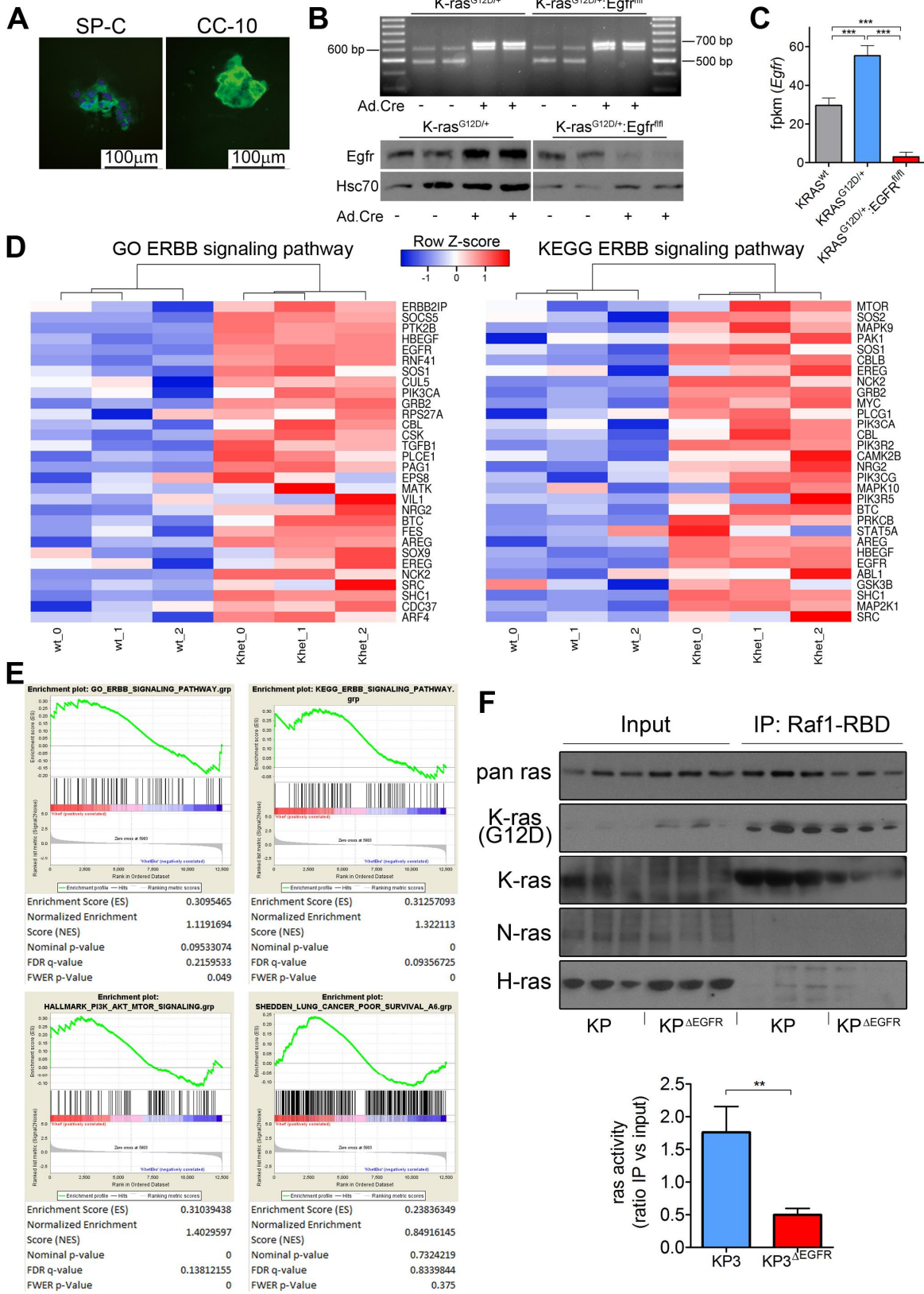
immunohistochemical stainings for total and (phospho-) EGFR and ERBB2 with non/weak, intermediate and strong staining intensity in human K-RAS mutated lung AC and **(D)** boxplot (min to max) of scoring values. n×35 per group. **(E)** Representative pictures of NKX2-1 staining in *K-RAS* mutated human lung AC tissue samples showing tumors with <5 %, 5-50% and >50% of NKX2-1 positive tumor cells. **(F)** Distribution of NKX2-1 positive tumor cells in the two different cohorts of *K-RAS* mutated lung AC samples used for analysis of EGFR expression/activation.



**Fig. S3: Genetic EGFR ablation in K-RAS mutated lung AC reduces tumor growth. (A)** Genotyping of cell lines isolated from lungs following death of respective mice used for survival analysis. The ~ 1 kb band shows wildtype EGFR, whereas the ~ 500 bp band indicates the recombined EGFR allele. **(B)** Representative pictures of stainings for SP-C and S100 in tumors of K-ras<sup>G12D</sup> mice and **(C)** representative picture of immunofluorescence staining for Egfr including higher magnification of indicated area (right panel) in tumors of K-ras<sup>G12D</sup> and K-ras<sup>G12D</sup>:Egfr<sup>ΔLep/ΔLep</sup> mice. (n=5 per group). **(D)** Ratios of lung weight over body weight of tumor free wildtype, K-ras<sup>G12D</sup> and K-ras<sup>G12D</sup>:Egfr<sup>ΔLep/ΔLep</sup> mice, 10 weeks post inhalation with Ad.Cre. (n=9-11 mice per group). Graph represent mean ± s.d., \*\*\*P<0.001. **(E)** Relative *SP-C* mRNA expression levels in total lung lysates of tumor free wildtype, K-ras<sup>G12D</sup> and K-ras<sup>G12D</sup>:Egfr<sup>ΔLep/ΔLep</sup> mice, 10 weeks post tumor initiation, normalized to *Actb* mRNA expression. Graph represent mean ± s.d., \*\*\*P<0.001. **(F)** Representative pictures of low grade, *In situ* AC and invasive AC as classified by board certified pathologists, and tumor numbers of respective tumors in lungs of K-ras<sup>G12D</sup> and K-ras<sup>G12D</sup>:Egfr<sup>ΔLep/ΔLep</sup> mice. n=14 for K-ras<sup>G12D</sup> and n=18 for K-ras<sup>G12D</sup>:Egfr<sup>ΔLep/ΔLep</sup>. **(G), (H)** Immunohistochemistry for cleaved Caspase 3 and pAkt in tumors of indicated mice, 10 weeks post Ad.Cre inhalation. Graphs represent mean ± s.d. of the percentage of Caspase 3 positive cells in whole lung sections and pAkt positive tumor cells in at least 8 individual tumors per mouse as evaluated using TissueGnostics software. (n×5 mice per group). \*\*P<0.01 **(I)** Relative mRNA expression of *Egfr* and Egfr ligands in lung lysates of K-ras<sup>G12D</sup> and K-ras<sup>G12D</sup>:Egfr<sup>ΔLep/ΔLep</sup> mice, 10 weeks post Ad.Cre inhalation, normalized to *Actb* mRNA expression (n×6). Bars represent mean values + s.d. \*\*P<0.01, \*\*\*P<0.001.

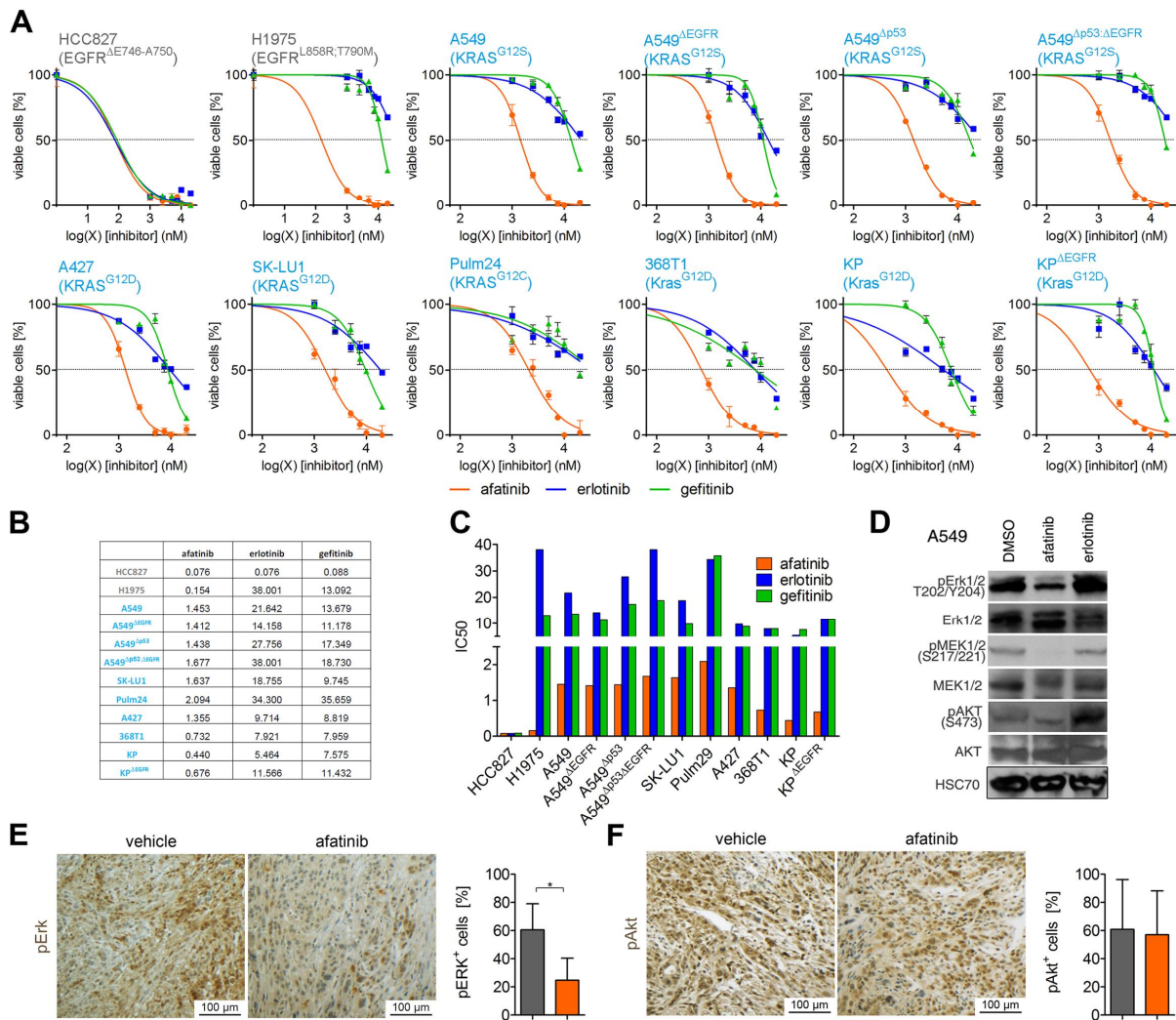


**Fig. S4: Genetic EGFR ablation in K-RAS mutated lung AC cells reduces tumor growth. (A)** Western blot of A549, A549<sup>ΔEGFR</sup>, A549<sup>Δp53</sup>, A549<sup>Δp53/ΔEGFR</sup> cells probing for indicated proteins and **(B)** cell counts at indicated time points of A549 (blue line) and A549<sup>ΔEGFR</sup> (red line) following standard cultivation *in vitro* (n=3 clones per group). **(C)** Mean tumor volumes of xenografted A549 and A549<sup>ΔEGFR</sup> cells monitored over the course of the experiment and endpoint tumor weight ± s.d. are indicated (n=4 per group). Picture of tumors after finalizing the experiment is shown. **(B), (C)** Unpaired two-tailed t-test for individual time points and tumor weight. \*\*p<0.01, \*\*\*p<0.001.

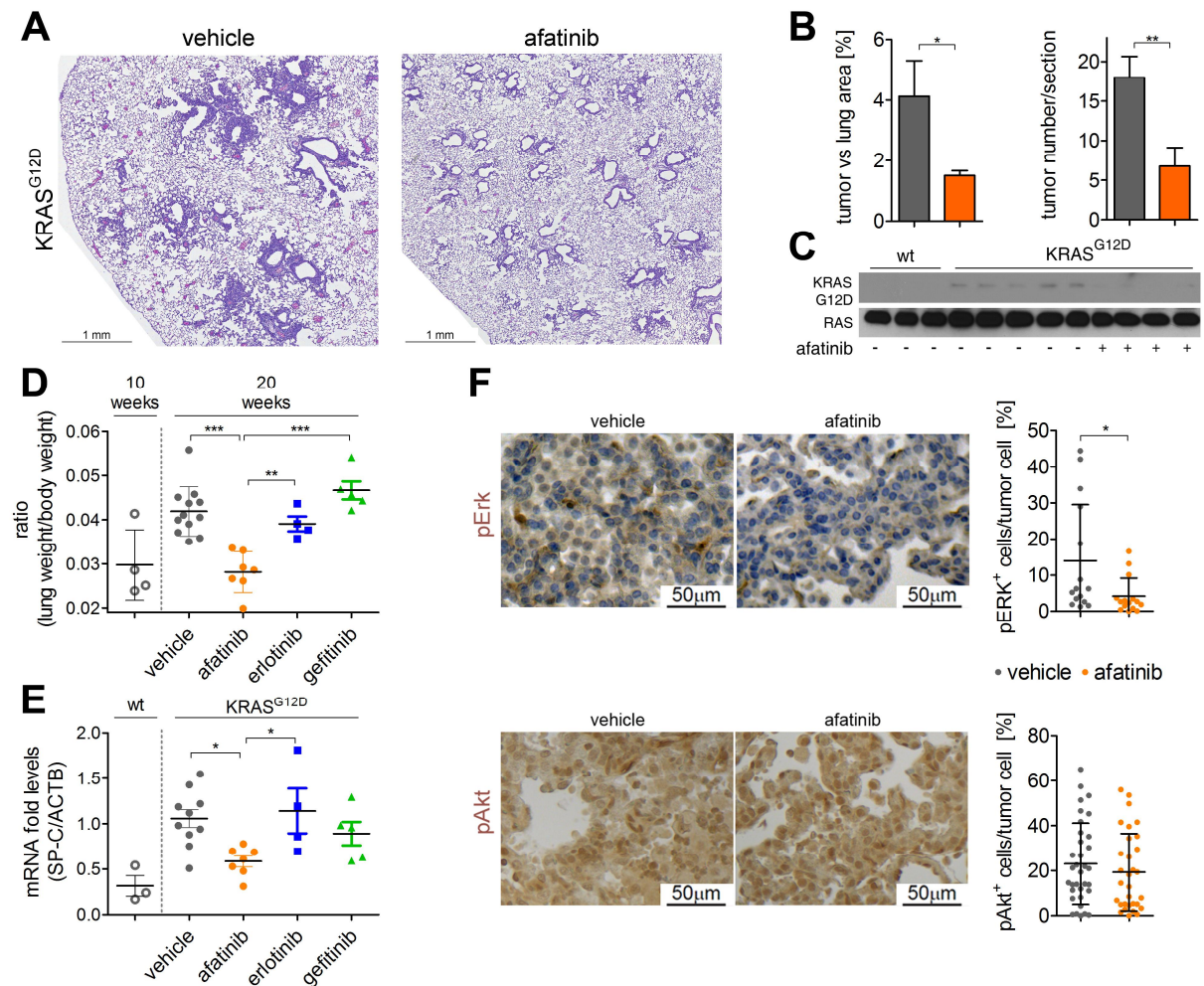


**Fig. S5: Inhibition of EGFR signaling downregulates mutated K-RAS activity. (A)** Immunofluorescence staining of primary lung cell isolates for SP-C and CC-10 expression. **(B)** PCR analysis for recombination of the KRAS<sup>G12D</sup> allele (~500 kb: LSL-G12D, ~620 kb: wildtype, ~650 kb: recombined G12D) and Western blot probing for EGFR and HSC70 expression in primary pneumocytes isolated from K-ras<sup>G12D/+</sup> and K-ras<sup>G12D</sup>:Egfr<sup>fl/fl</sup> mice. Cells were transduced with 250 MOI Ad.Cre 2 days before cell lysis. **(C)** *Egfr* mRNA expression in primary pneumocyte isolates of wildtype, K-ras<sup>G12D/+</sup> and K-ras<sup>G12D</sup>:Egfr<sup>fl/fl</sup> mice 5 days post Ad.Cre transduction. (n=3), one way ANOVA, \*\*\*p<0.001. **(D)** Heat map illustrating expression of top 30 induced genes in the GO and KEGG ERBB signaling pathway, comparing Ad.Cre transduced pneumocytes isolated from wildtype (wt\_0-2) and K-ras<sup>G12D/+</sup> (Khet\_0-2) mice. **(E)** GSEA for depicted gene sets, comparing RNAseq data of transduced pneumocytes isolated from K-ras<sup>G12D/+</sup> (Khet) and K-ras<sup>G12D</sup>:Egfr<sup>fl/fl</sup> (KhetEko) mice. **(F)** Western blot analysis of KP and KP<sup>ΔEGFR</sup> cell lysates before and after pulldown with Raf1-RBD and probing for indicated proteins. Due to the similar molecular weight of ras isoforms, each protein was probed on an individual membrane. Blot represents ratio of densitometric values for the Ras signal in pulldowns over input. (n=3), \*\*p<0.01



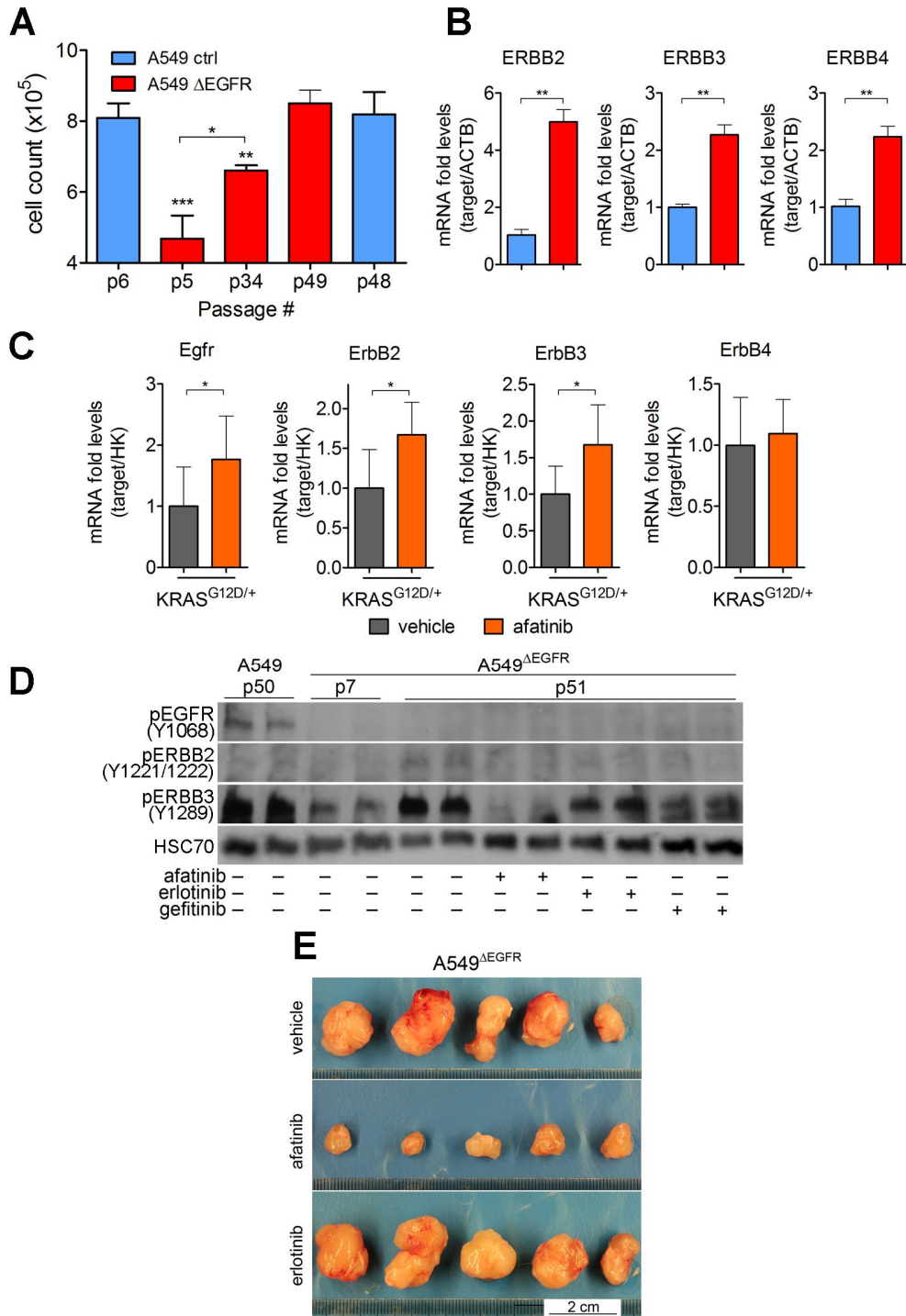


**Fig. S6: Afatinib reduces growth of K-RAS mutant lung AC in vitro.** (A) Cell viability of lung cancer cell lines harboring mutations in the *EGFR* or *K-RAS* gene as indicated, following 3 day treatment of escalating doses afatinib, erlotinib or gefitinib, as determined by MTT assay. (B), (C) IC50 values as determined in (A) are displayed. (D) Western blot analysis probing for indicated (phospho) proteins in cell lysates of A549 cells after 24h treatment with 1  $\mu$ M afatinib and erlotinib, respectively. (E) Representative pictures of pErk and (F) pAkt staining of 368T1 cell line derived grafts upon vehicle and afatinib treatment in tumors harvested at the end of the experiment. Graphs illustrate the quantitation of positive cells for the respective staining as determined using TissueGnostics software (n=5).



**Fig. S7: Afatinib reduces K-RAS mediated tumorigenesis in vivo.** (A) Representative pictures of H&E stained lung sections of  $Kras^{G12D/+}$  mice 10 weeks post Ad.Cre inhalation and treatment over the last 9 weeks with vehicle or afatinib (5 mg/kg body weight, 5 times per week via oral gavage). (B) Graphs represent mean of ratios + s.d. of tumor area versus total lung area and mean of tumor numbers + s.d. per section of lung of mice. Unpaired two-tailed t-test for individual time points and tumor weight. \* $p < 0.05$ , \*\* $p < 0.01$ . (C) Western blot of lysates of lung derived from mice treated as in (A), using antibody specific for mutant K-RAS<sup>G12D</sup> as a measurement for quantitation of tumor cells and for total RAS as control. (A)-(C)  $n = 5$  for vehicle group and  $n = 4$  for afatinib group. (D) Ratios of lung weight over body weight of K-ras<sup>G12D</sup> mice 10 weeks post

inhalation with Ad.Cre and after 20 weeks post inhalation and treatment with vehicle, afatinib, erlotinib or gefitinib for the last 10 weeks of the experiment (5 mg/kg body weight, 5 times per week via oral gavage, n=4-11 mice per group). Graph represent mean  $\pm$  s.d., \*\*p<0.01, \*\*\*P<0.001. **(E)** Relative *SP-C* mRNA expression levels in total lung lysates of tumor free wildtype and K-ras<sup>G12D</sup> mice treated as in **(D)**, normalized to *Actb* mRNA expression. Graph represent mean  $\pm$  s.d., \*p<0.05. **(F)** Representative pictures of pErk and pAkt staining of lung tumors 20 weeks post Ad.Cre induction and 10 weeks of vehicle versus afatinib treatment. Tumor cells positive for respective stainings in at least 3 tumors per mouse were quantitated using TissueGnostic software, and blot indicates mean  $\pm$  s.d. of the percentage of positive tumor cells (n=4 mice per group).



**Fig. S8:** ERBB family members mediate resistance to EGFR inhibition, which can be blocked by afatinib. **(A)** Cell counts of A549 and A549<sup>ΔEGFR</sup> cells of different passages after clonal expansion, after seeding equal cell numbers and 3 days incubation. **(B)** Relative mRNA

expression levels of ERBB family members in A549 and A549<sup>ΔEGFR</sup> cells, normalized to *ACTB* expression. n=3 per group. (C) Relative mRNA expression in lung lysates of KRAS<sup>G12D</sup> mice, 20 weeks after tumor induction and ten weeks after start of vehicle or afatinib treatment (5 mg/kg body weight, 5 times per week via oral gavage, n=8 per group). (D) Western blot analysis of A549 cells and A549<sup>ΔEGFR</sup> cells in low passage (p7) and higher passage (p51), untreated and treated with 1 μM afatinib, erlotinib or gefitinib for 24 h. (E) Photographs of A549<sup>ΔEGFR</sup> xenografts in mice receiving indicated treatments harvested at the end of experiment. (A) - (C) Unpaired two-tailed t-test for individual time points and tumor weight. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

> Genes up-regulated in primary lung alveolar cells upon KRAS G12D transduction						
F630028O10Rik	Isg15	En1	Tgfb1	Kcnd1	Plcb2	Cp
Mir223	Ubn2	Ccm2l	Fcer1g	Txk	Snph	Lce1f
Tgtp2	mmu-mir-7670	Mcpt8	Saa3	Trpc6	Mmp12	Gm13086
Gm8730	Fcgr2b	Pdgfra	Nfam1	Rgs4	Parp1	Gata4
MIAT_exon1	Cdc136	mmu-mir-7025	Lrm2	Ugt1a1	mmu-mir-6904	Rinl
MIAT_exon5_1	Gm26220	Tnfaipl8l2	Rgs20	Ugt1a10	Il16	Xirp2
MIAT_exon5_2	Ifi204	Rhox5	Dnajc6	Ugt1a2	Fam13a	Bcl3
MIAT_exon5_3	Cbs	Gm16241	Pcdh10	Ugt1a5	Abcb1a	Rep15
Miat	Hormad2	Gm20400	Vwc2	Ugt1a6a	Ribc1	Bcl2a1b
Arg1	Fcgr1	Card11	Oas1b	Ugt1a6b	Ubash3b	Mthfs
Wfdc17	Aqp4	Mmp9	Car12	Ugt1a7c	Il18	Stc1
Ccr2	Cd300lf	Pyhin1	Csf2rb	Ugt1a8	Gucy2g	Atp6v0d2
Hnf4a	Sifn10-ps	Lama1	mmu-mir-7676-2	Ugt1a9	Lgals3bp	2610018G03Rik
Gm20746	Gm12505	Oas3	Gm2302	4930431P03Rik	Gm13431	Slc7a2
Gm26445	Ccr1	Gme	Sash3	Gm17173	Cpa4	Laptm5
Abca8a	Snx20	C1qb	Tnni1	Ppp1r3a	Sv2a	Gfra2
Cacng8	Fcrls	Trem12	Selp1g	Gabra3	Arhgap6	Adams14
Gm23450	Samsn1	Gda	Gap43	Wt1	Cpxm1	Trem2
Gm25043	Gm14165	Gad1	Il10ra	Klra4	Cebpd	Stxbp3b
Oas2	Gm9285	Glip1r	Chi3l3	Cd38	Gm22413	C3ar1
Onecut3	Mir1894	Ifi44	Rph3a	B3gnt8	Gria3	B430306N03Rik
Irg1	Ppp1r10	Fcgr3	Igtp	Plek	Inpp4b	Cd28
Ccl4	Soga3	Trim30a	Irgm2	Cyp7b1	Clec4e	Cmb1
Ccl6	Rgs5	Cd86	Sp5	Iglon5	Oas1a	Fcgr4
Ifi205	Tnfsf8	Tlr8	Cd200r3	Sp1	Sprr2h	Pde1a
S100a9	Xaf1	Sifn5	Pamr1	C330013J21Rik	Ano3	Dll4
Ngp	Ddx60	Nptx1	Serpina3n	Elavl2	Vdr	A230050P20Rik
Atp1a3	A1662270	Adap2	Ptafr	Vmn1r-ps128	Atp8b4	Lrrc2
Sifn8	Traf3ip3	Gm17384	Clec7a	Paqr5	Gm13105	Clec4d
Ednra	Srgn	Aqp9	Gata5	Dock10	Aldh1b1	Gm26848
Tifab	Cd2	Mnda	Gm15280	Cnr2	Gm15287	Slc27a3
Tnfsf11	Ifit1	Klra2	Gbp7	Pex5l	Gpr64	Rspo3
Syt9	Msr1	Rtp4	Icosl	Vstm2a	Mir31	Neto1
Bin2	Ptpn7	Dusp15	Il21r	Fam84a	Il6	Trac
Ccl3	Ano4	Ltf	Trim15	Nlrp3	Wnt2	Traj32
Il33	Ndst3	BC028471	Rac2	A330021E22Rik	9330132A10Rik	Traj33
Pax5	Cd52	Gm20488	Asic3	Hs3st1	Dok6	Gm12177
Lcp2	C5ar1	Medag	Pla2g7	Ivi	Lifr	Nhs
Steap4	Oasl2	Crabp2	Aim2	B3gnt7	C2	Adams9
Ccr5	Ifi203	Gp49a	Myo1g	Pri2c2	Cfb	Siglec1
Ednrb	Mndal	Lilrb4	Stra6	Lrrc3b	Gm20547	Serpina3g
Sifn2	Dlx4	Cd200r1	Serpina1a	Mcm7	Ccdc88b	Serpina3h
S100a8	Apol9b	Gbp6	Ptprc	Mir25	Gli2	Serpina3i
Csf2	Ilgam	F13a1	Il24	Mir93	Osmr	Espn
Gm1840	Ncf4	A8124611	Fam78b	Calcb	Stat2	Gabre
Star	Lrmp	Slit3	Igfbp3	Socs3	I8300120A16Rik	Mir224
Dock3	Apol9a	Il1a	Olr1	1200007C13Rik	Ifit3	Kif17
Gm22238	Tfr2	Cd53	Plk1s1	Nckap1l	Ilgb2	Sh2d5
Oasl1	RP24-312B12.1	Gm4070	Gm11747	Syt13	Gabrq	Gm14418
Gm11425	Clec5a	RP24-196H4.1	Sim2	Csn3	Neu3	Mrc1
Arrb2	Kcnt1	Tmem74b	Ccbe1	Mfap2	Ifitm3	Gm16564
mmu-mir-7115	Plat	Cxcl1	Cyth4	Klhl1	Susd1	Rpgr
Pri2c5	Gbp5	AW011738	Pilra	Gm13293	Hey1	Srpx
Serpina10	Spry3	Adam22	Cd5	Tmem179	Fermt3	Akap12
Serpina2	Cd33	Tlr13	4833427G06Rik	Arl11	Pcdh11x	Spock3
Wnt4	C1qa	C1qc	Gpr88	Ncf2	Trim66	Ptprn
Ifi2712a	Irf7	Lgi2	Kcnk12	Coro1a	Arsi	Cyp11b1
Gbp3	Gm9625	C1qtnf1	Il1rn	Rpp25	Vcam1	Abca1
Ntrk2	Ahgap30	Pf4	Lpxn	Igkc	Phf11d	Stat1
Zic1	Ly86	Emr1	SMAD5-AS1_2	Igkj4	Cd14	Galnt6
Btk	Pilrb2	Gas1	Smad5	St8sia4	Col5a3	Alox5
Wdfy4	Pnma2	Ptger2	Nfkbi2	Gm13227	1810011H11Rik	Casp4
Alox5ap	Spp1	Themis2	Mmp8	Gm13230	Pabpc4l	Atp2b4
Scn5a	Cd9	Ccdc158	Clec4n	Pld4	Rplp0-ps1	mmu-mir-6903
Gm12840	Naip5	F830016B08Rik	Npl	Mettl20	Ank1	Angptl7
Cd7	Mx2	Dnmt3l	H19	Abca9	Tyrobp	Sncap
Gbp4	C4b	Scin	Mir675	Nrros	Rnf213	Hoxc6
Gbp8	Casp1	Tnfaipl6	Trim30d	Kcnq5	Angptl4	Gbp2
Gbp9	Zbp1	Gm13295	Gm8995	Cfn	Cd1d1	Adams4

Supplementary Table 1: Alveolar\_KRAS\_up gene set

<b>conditional gene</b>		
<i>K-ras</i>	primer #1 (5'-3')	GTCTTTCCCCAGCACAGTGC
	primer #2 (5'-3')	CTCTTGCCTACGCCACCAGCTC
	primer #3 (5'-3')	AGCTAGCCACCATGGCTTGAGTAAGTCTGCA
<i>Egfr</i>	primer #1 (5'-3')	AAGTTTAAGAAACCCCGCTCTACT
	primer #2 (5'-3')	GCCTGTGTCCGGGTCTCGTCG
	primer #3 (5'-3')	CAACCAGTGACCTAGCCTGG
<i>p53</i>	primer #1 (5'-3')	CACAAAAACAGGTTAAACCCAG
	primer #2 (5'-3')	AGCACATAGGAGGCAGAGAC

**Supplementary Table 2:** List of genotyping primers

Target	Sequence fwd primer(5'-3')	Sequence fwd primer(5'-3')
human EGFR	GTGAACCCCGAGGGCAAATA	ATTCCGTTACACACTTTGCGG
human ERBB2	TCCTCCTCGCCCTCTTGC	AGTTCAGGTTTCCCTGCAC
human ERBB3	GGGACCGAGATGCTGAGATA	GCCCAAAGCAGTGACCATTA
human ERBB4	CGGGCCATTCCACTTTACCA	GAGCTTGATTGGGTGCTGTG
human 28S	CAGTTCTCTTGGGAATCCAG	TTCAGCAAAGGAGTCAATCCAC
human ACTB	GCACAGAGCCTCGCCTTTGCC	CATGCCACCATCACGCCCTGG
mouse Egfr	ACACTGCTGGTGTGCTGAC	CCCAAGGACCACTTCACAGT
mouse ErbB2	GTCGCAACTTCATGTCGGTA	GTGATCATCATGGAGCTGGC
mouse ErbB3	GGTACTGGTTGTCAGCATCG	GTGCTGGGTTTCCTTCTCAG
mouse ErbB4	GGGATCTGAGACTTGCCAAA	CAAGGCTCGGTACTGCTGTT
mouse Egf	CACCAATGCTGGTGATTG	ACTGTCAGCCAGGTCCCTCTC
mouse Ereg	GATTCTCCTGGGATGCATGA	CACCGAGAAAGAAGGATGGA
mouse Epgn	GCTTCAGCTCATGGTGGAAT	CACAGCACAGCAGAGCAACT
mouse Tgfa	TGTGGCCCTGGCTGTCCTCA	GCGCTGGGCTTCTCATGTCT
mouse Areg	TTGTCCTCAGCTAGGCAATG	ATCATCCTCGCAGCTATTGG
mouse SP-C	TCCTGATGGAGAGTCCACCG	ATCACCACGACAACGAGGAC
mouse Actb	GCTCATAGCTCTTCTCCAGGG	CCTGAACCCTAAGGCCAACCG
mouse 28S	ATACCGGCACGAGACCGATAGTCA	GCGGACCCACCCGTTTACCTC

**Supplementary Table 3: List of primers for qPCR analysis**

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