## Supplementary Materials and Methods

### Transgenic mouse studies and treatments

All procedure described were approved by the animal welfare committee of the University of Louvain Medical School (ethic approval number: 2017/UCL/MD/020) and the ethical committee of the Spanish National Cancer Research Center (CNIO). *LSL-Kras$^{G12D}$* and *Elastase-CreER* (*ElaC$^{ER}$*) mice have been described previously (1,2); these mice were maintained in a CD1-enriched background. Adult *ElaC$^{ER}$ Kras$^{G12D/+}$* (*ElaC$^{ER}$ K$^{G12D/+}$)* mice of six- to eight-week-old were treated simultaneously by gavage with tamoxifen (30 mg/ml in corn oil) and subcutaneous injection with 4-hydroxytamoxifen (0.3 mg/ml in corn oil), every other day for five days, followed by two days of recovery (Supplementary Table S2). *Elastase-tTA/TetO-Cre/Kras$^{LSLG12Vgeo/+}$* (*ElaTK$^{G12V/+}$*), *Elastase-tTA/TetO-Cre/Kras$^{LSLG12Vgeo/+}$/c-Raf$^{lox/lox}$* (*ElaTK$^{G12V/+}$c-Raf$^{lox/lox}$*), *Elastase-tTA/TetO-Cre/Kras$^{LSLG12Vgeo/+}$/p53$^{lox/lox}$* (*ElaTK$^{G12V/+}$p53$^{lox/lox}$*), *Elastase-tTA/TetO-Cre/Kras$^{LSLG12Vgeo/+}$/p53$^{lox/lox}$/c-Raf$^{lox/lox}$* (*ElaTK$^{G12V/+}$p53$^{lox/lox}$c-Raf$^{lox/lox}$*) were previously described (3). In these Tet-Off strains, Cre is expressed in acinar cells in the absence of doxycycline from the embryonic day 16.5. Cre expression was prevented by doxycycline (2 mg/ml) (Sigma Aldrich) provided in the drinking water as a sucrose (5% w/v) solution to pregnant mothers from the time of conception and to their descendants until eight to twelve weeks after birth; at this time, *Kras$^{G12V}$* was turned on in acinar cells by removing doxycycline for four weeks.

Cerulein (125 µg/kg) (As-24252, AnaSpec; C9026-1MG, Sigma Aldrich) were injected intraperitoneally at different time points. To induce acute pancreatitis, mice were treated with cerulein (7 injections/day) every other day for three or five days followed by one or two days of recovery, respectively (acute setting). The three-day protocol was mainly used for RNA-Seq and related RTqPCR experiments to detect early

molecular events occurring in acinar cells; this protocol was also used to determine the early changes in KRAS expression pattern using our citrine models. The five-day protocol was used for immunostaining/labeling and histological analysis as the morphological consequences were more visible at this time point. To induce chronic pancreatitis, mice were first treated with cerulein in an acute setting, and then received one daily cerulein injection, for two-to-six weeks, followed by two days of recovery at the end of cerulein injections (chronic setting). *ElaTK*$^{G12V/+}$ and *ElaTK*$^{G12V/+}$*c-Raf*$^{lox/lox}$ mice were injected with cerulein for five days (acute setting) and three months (chronic setting) and sacrificed after two days and eight months of recovery, respectively. For *ElaTK*$^{G12V/+}$*p53*$^{lox/lox}$ and *ElaTK*$^{G12V/+}$*p53*$^{lox/lox}$*c-Raf*$^{lox/lox}$ mice, daily cerulein treatment started at 3 months of age until humane end point. Pancreata were dissected for RNA/protein extraction or to perform histological analysis and immunolabeling (see below). Detailed description of treatment protocols and mice ages and genotypes used in the present study can be found in Supplementary Table S2.

### *Ex vivo* culture of dissociated mouse pancreas

Cultures of dissociated mouse pancreata that mimic ADM were isolated as previously described (4). Briefly, pancreata were cut into small pieces and digested with collagenase P (0.35 mg/ml in Hank's balanced salt solution (HBSS) buffer). Collagenase incubation was performed for 15 minutes at 37°C with 180 rotations per minutes (rpm). The cell suspension was washed three times with HBSS/5% FBS and filtered through 500 µm and 100 µm strain filters. Finally, cells were dropped gently on a cushion of 30% FBS and centrifuged at 1000 rpm, for 2 minutes. The day of isolation (day 0) is equivalent to normal pancreas. Metaplasia was induced by maintaining acinar cells in 3D suspension culture, for three days, in Advanced RPMI supplemented

with 5% FBS, 1% penicillin-streptomycin and 0.1 mg/ml soybean trypsin inhibitor, at 37°C and 5% $CO_2$. Cell Line Authentication is not relevant for the present study.

**Pharmacological EGFR inhibition**

Freshly isolated human and murine acinar cells were treated with Gefitinib (4765S, CST) or Erlotinib (5083S, CST) at a concentration of 5 µM or 10 µM, respectively. Treatment of cells with Gefitinib and Erlotinib did not result in significant cellular toxicity as evidenced by Trypan blue dye staining and cell counting (data not shown). Control cells were treated with vehicle DMSO at 0.05% or 0.1%. Culture medium and inhibitors were renewed every 24 hours. After three days, DMSO-treated and Gefitinib- and Erlotinib-treated cells were lysed to extract proteins for western blot analysis.

**Western blotting**

Tissues and cultured cells were lysed in 50 mM Tris-base-HCl, 150 mM NaCl and 1% NP40 buffer. Protease (11836153001, Sigma Aldrich) and phosphatase (4906837001, Sigma Aldrich) inhibitors were added just before lysis. Tissues were homogenized using Dounce homogenizer and cells were lysed by vortexing multiple times and pipetting. Samples were maintained on ice during the procedure. Cell debris were pelleted by centrifugation (14000 g, 10 minutes, at 4°C). Human pancreas and lung protein extracts were purchased from Zyagen (HT-313 and HT-601). Proteins were quantified using a Bradford assay. Samples containing 40 to 80 µg of total proteins were separated on 7.5% to 12.5% SDS polyacrylamide gels. PVDF membranes (ISEQ00010, Millipore) were blocked with a solution of 5% low-fat milk diluted in Tris-buffered saline (TBS)/ 0.1% Tween-20. Membranes were incubated overnight with primary antibodies at 4°C. Incubation conditions and antibody dilutions are given in

Supplementary Table S3. Next day, membranes were washed with TBS/0.1% Tween-20 and incubated with secondary antibodies for 1 hour at room temperature (RT). After incubation, membranes were washed again and revealed using chemiluminescence (kits 34577 and 34094, Thermo Fisher). Pictures were taken with a Fusion Solo S machine (Vilber). Densitometric analysis was performed using the Image Studio Lite 5.2 software (Li-Cor). HSC70 and Ponceau S staining were used as loading controls. For the quantification of experiences with cerulein treatments, Ponceau S staining was used since expression of standard loading controls, including β-Actin, GAPDH, Tubulin and HSC70, was modified by cerulein. Membranes were stained with Ponceau S immediately after transfer and then scanned. The entire track of each sample was quantified and the obtained densitometric value was used for normalization of target protein expression.

**Fluorescence-activated cell sorting (FACS)**

Pancreata were digested using collagenase P (0.6 mg/ml), as previously described (5). Briefly, an EGTA-buffered solution was injected in the main duct for optimization of pancreas dissociation. Pancreata were digested in $Ca^{2+}$ buffer for 15-20 minutes, at 37°C. After washing, the cells were filtered through a 35 µm cell strainer and sorted by FACS (FACSAria™ III cell sorter, BD Biosciences).

For RNA extraction, YFP-positive acinar cells were sorted from *ElaC$^{ER;YFP}$* and *ElaC$^{ER}$K$^{G12D/+;YFP}$* mice treated or not with cerulein for three days. A *wild-type* (*WT)* mouse was used as negative control, to determine the level of auto-fluorescence.

For quantification of Cit-K- and Cit-K$^{G12D}$-positive acinar cells, cell suspensions from dissociated pancreata of *Kras$^{Cit/Cit}$* and *ElaC$^{ER}$ K$^{Cit-G12D/+}$* mice, treated or not with cerulein for three days, were stained with Peanut agglutinin (PNA) coupled to Alexa

Fluor 647 (10 µg/ml) (L32460, Thermo Fisher). Cells were incubated with PNA for 15 minutes at 4°C, with gentle rotation. PNA specifically recognizes acinar cells (6). After a single wash, cells were filtered through a 35 µm cell strainer. Cells stained with PNA from *WT* mice were used as a negative control to determine the level of auto-fluorescence. The percentage of Cit-K- and Cit-$K^{G12D}$-positive acinar cells was then determined in the total PNA-positive acinar population.

**RNA-Sequencing (RNA-SEQ) and data analysis**

RNA was extracted from FACS-sorted acinar cells using a column-based protocol (AM1912, Thermo Fisher). The RNA Integrity Number (RIN) was measured using Agilent 2100 Bio-analyzer (Agilent). Samples with a RIN ≥ 7 were selected for RNA-SEQ experiments, which were performed by the Novogene Company. Firstly, Epicentre Ribo-zeroTM rRNA Removal Kit was used to remove ribosomal RNA from samples (Epicentre). Sequencing libraries were generated using the rRNA-depleted RNA by NEBNext®UltraTM Directional RNA Library Prep Kit for Illumina® (NEB) following manufacturer's recommendations. Briefly, fragmentation was carried out using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer (5X). First strand cDNA was synthesized using random hexamer primer and M-MuLV Reverse Transcriptase (RNase H minus). Second strand cDNA synthesis was subsequently performed using DNA polymerase I and RNase H. In the reaction buffer, dNTP with dUTP replacing dTTP were used. Then NEBNext adaptors with hairpin loop structure were ligated to the adenylated 3' ends of DNA fragments. The cDNA fragments of preferentially 150~200 bp in length were purified using AMPure XP system (Beckman Coulter). Library preparations were sequenced on an Illumina Hiseq platform and paired-end reads were generated.

For data analysis, clean data (clean reads) were obtained by removing reads containing adapter, reads containing poly-N, and low-quality reads from raw data. All the downstream analyses were based on clean data of high quality. Reference genome and gene model annotation files were directly downloaded from genome website. Index of the reference genome was built using Bowtie v2.0.6 and paired-end clean reads were aligned to the reference genome using TopHat v2.0.9. Then, Cuffdiff (v2.1.1) was used to calculate fragments per kilo-base of exon per million fragments mapped (FPKMs) of coding genes in each sample (7). KOBAS software was used to test the statistical enrichment of differentially expressed genes in KEGG pathways. RNA-SEQ data can be downloaded using the following accession numbers: GSE163039, GSE163254 and GSE163263.

### *In silico* analysis of single-cell and single-nucleus RNA-SEQ data on human pancreas

Single-cell RNA-SEQ (scRNA-SEQ) data of human pancreas from Segerstolpe et al. 2016 (8), was downloaded from ArrayExpress (EBI) with accession number E-MTAB-5061. The following patients were analyzed: AZ (acinar cells, n=4), HP1502401 (acinar cells, n=20), HP1504901 (acinar cells, n=3), HP1506401 (acinar cells, n=80), HP1507101 (acinar cells, n=3) and HP1509101 (acinar cells, n=2). Given the low number of cells in most patients, we further processed data from HP1506401 (acinar cells, n=80), which contains the highest number of cells to allow reliable study of acinar cell subpopulations. Filtering out unexpressed genes left 17568 detected genes in the dataset. Data were normalized and $\log_2$-transformed, and highly variable genes (HVGs) were detected using scran v1.18.1 R package (9). These were used for computing top principal components using scater v1.18.3 R package (10), and a k-

means clustering was performed using bluster v1.0 .0 R package. Markers for each cluster were identified by performing pairwise t-tests using the findMarkers function of scran R package. MAPK signaling pathway genes (KEGG) found to be significantly and differentially expressed in our bulk RNA-SEQ from the citrine model, and showing a significant difference (FDR < 0.05) between the two clusters (*Kras*$^{High}$ and *Kras*$^{Low}$) identified in the scRNA-SEQ, are shown on the heatmap.

Single-nucleus RNA-SEQ (snRNA-SEQ) data of human pancreas from Tosti et al. 2020 (11) was downloaded from http://singlecell.charite.de/pancreas. The following patients were analyzed: TUM25 (acinar nuclei, n = 6464), TUM13 (acinar nuclei, n = 3495) and TUMC1 (acinar nuclei, n = 6850). The differences in acinar and MAPK gene expression were reproducible in all patients. TUM25 was selected to illustrate the obtained data. Genes with very low expression levels (less than three reads) were removed from the analysis, giving a total number of 2536 detected genes in the TUM25 sample. Data processing was performed as described above to compare the “Acinar-i” and “Acinar-s” populations defined by the authors. MAPK signaling pathway genes (KEGG) found to be significantly and differentially expressed in our bulk RNA-SEQ from the citrine model and showing a significant difference (FDR < 0.05) between the “Acinar-i” and “Acinar-s” populations are shown on the heatmap.

**Reverse transcription (RT) quantitative PCR**

RT was performed on 150-250 ng of total RNA using the M-MLV reverse transcriptase (M1705, Promega). Quantitative PCR was carried out in a final volume of 10 µl (1 µl neosynthetized cDNA, 2 µl primers 10 µM, 5 µl Sybergreen KAPA mix 2X (KK4601, Sigma Aldrich), 2 µl nuclease-free water) using the CFX96 Real-Time System thermocycler (C1000, Biorad). The expression of target genes was normalized to the

*Rpl04* and *Gapdh* housekeeping genes. Relative expression was calculated using the ΔΔCt method. Primers used in the study are listed in Supplementary Table S4.

**KRAS activation assay**

Dissected pancreata were homogenized on ice using Dounce homogenizer. After centrifugation (14000 g, 5 minutes, 4°C), 2 to 3 mg of total proteins in a final volume of 1 ml were used for the assay. Agarose beads coated with RAF1 RAS-binding domain (RBD) (ab211176, Abcam) were added into protein lysate (40 µl beads/1ml lysate) to selectively pull-down RAS-GTP, with gentle rotation for 1 hour, at 4°C. After washing, beads were resuspended in Laemmli buffer 2X (1:1 ratio) and boiled 5 minutes at 100°C. Then beads were pelleted by rapid centrifugation, and supernatant was loaded on 12.5% SDS polyacrylamide gel for western blot analysis. KRAS-GTP was detected using a home-made KRAS antibody.

**Subcellular protein fractionation**

Subcellular protein fractionation was performed according to manufacturer's instructions (87790, Thermo Fisher). Dissected pancreata were snap-frozen in liquid nitrogen and grinded to obtain tissue powder. For 50 mg tissue, we added 500 µl of cytosolic extraction buffer (CEB) with protease inhibitors and incubated 15 minutes at 4°C, with rotation. After centrifugation (500 g, 5 minutes, 4°C), a supernatant containing the cytosolic proteins was obtained and 325 µl of membrane extraction buffer (MEB) with proteases inhibitors was added to the remaining pellet. Incubation with MEB lasted 10-15 minutes at 4°C, with rotation; supernatant containing solubilized membrane protein was obtained following centrifugation (3000 g, 5 minutes, 4°C).

GAPDH and E-CAD were used as loading controls and markers of purity for cytosolic and membrane fractions, respectively.

## Supplementary References


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| Antibody | Reference | Dilution | Antigen retrieval | Application |
|---|---|---|---|---|
| GFP | Ab6556, Abcam | 1/250 | Tris-EDTA (pH=9), PTM | IF/IHC |
| P-ERK1/2$^{T202/Y204}$ | 9101S, CST | 1/200 | Tris-EDTA (pH=9)/Citrate (pH=6), PTM | IF/IHC |
| P-EGFR$^{Y1068}$ | Ab5644, Abcam | 1/200 | Citrate (pH=6), CC | IHC |
| proSP-C | AB3786, Millipore | 1/1000 | Tris-EDTA (pH=9), PTM | IF |
| Podoplanin (PDPN) | DSHB 8.1.1, DSHB | 1/64 | Tris-EDTA (pH=9), PTM | IF |
| α-SMA | M0851, Dako | 1/1000 | Tris-EDTA (pH=9), PTM | IF |
| Amylase (G-10) | Sc-46657, SCB | 1/500 | Tris-EDTA (pH=9), PTM | IF |
| E-Cadherin | 610182, BD lab | 1/1000 | Tris-EDTA (pH=9), PTM | IF |
| P-STAT3$^{Y705}$ | 9131S, CST | 1/1000 | Tris-EDTA (pH=9), PTM | IF |
| SOX9 | AB5535, Millipore | 1/1000 | Tris-EDTA (pH=9), PTM | IF |
| Insulin | A0564, Dako | 1/500 | Tris-EDTA (pH=9), PTM | IF |
| AF488 Donkey anti-Goat | A11055, TF | 1/1000 | | IF |
| AF594 Donkey anti-Mouse | A21203, TF | 1/1000 | | IF |

| | | | | |
|---|---|---|---|---|
| AF647 Donkey anti-Rabbit | A31573, TF | 1/1000 | | IF |
| AF488-Plus Donkey anti-Goat | A32814, TF | 1/1000 | | IF |
| AF594 Donkey anti-Syrian Hamster | A21113, TF | 1/1000 | | IF |
| AF594 Donkey anti-Rabbit | A21207, Invitrogen | 1/1000 | | IF |
| AF594 Goat anti-Guinea Pig | A11076, TF | 1/1000 | | IF |
| Donkey anti-Goat Biotinylated | A16009, TF | 1/1000 | | IHC |
| Goat anti-Rabbit HRP | 31460, TF | 1/1000 | | IHC |

**Supplementary Table S1. Antibodies used for immunolabeling of tissue sections.** IF, immunofluorescence; IHC, immunohistochemistry; CC, pressure cooker; PTM, PT-module; CST, Cell Signaling Technology; SCB, Santa-Cruz Biotechnology; DSHB, Developmental Studies Hybridoma Bank; TF, ThermoFisher.

| Genotype | Age | Tam protocol | Doxy protocol | Acute cerulein protocol | Chronic cerulein protocol |
|---|---|---|---|---|---|
| *ElaC$^{ER}$ K$^{G12D/+}$*<br><br>*ElaC$^{ER}$ K$^{Cit-G12D/+}$* | Start Tam at 6 to 8 weeks of age (to induce *Kras$^{G12D}$*) | Duration: 5 days<br><br>Inject/week: day 1, 3 and 5 (×1/day)<br><br>End: 1-week recovery before acute cerulein, or 4-month recovery before sacrifice | - | Duration: 3 to 5 days<br><br>Inject/week: day 1 and 3 or day 1, 3 and 5 (×7/day)<br><br>End: 1-day or 2-day recovery before sacrifice, or 2-day recovery before chronic cerulein | Duration: 3 weeks<br><br>Inject/week: day 1, 2, 3, 4 and 5 (×1/day)<br><br>End: 2-day recovery before sacrifice |
| *ElaTK$^{G12V/+}$*<br><br>*ElaTK$^{G12V/+}$c-Raf$^{lox/lox}$* | Start Doxy at conception of the embryo (to prevent *Kras$^{G12V}$* induction) | - | Duration: Doxy until 8 weeks of age<br><br>Inject/week: day 1, 2, 3, 4, 5, 6 and 7 (drinking water)<br><br>End: 4-week recovery before acute cerulein | Duration: 5 days<br><br>Inject/week: day 1, 3 and 5 (×7/day)<br><br>End: 2-day recovery before sacrifice, or 2-day recovery before chronic cerulein | Duration: 12 weeks<br><br>Inject/week: day 1, 2, 3, 4 and 5 (×1/day)<br><br>End: 8-month recovery before sacrifice |
| *ElaTK$^{G12V/+}$p53$^{lox/lox}$*<br><br>*ElaTK$^{G12V/+}$p53$^{lox/lox}$c-Raf$^{lox/lox}$* | Start Doxy at conception of the embryo (to prevent *Kras$^{G12V}$* induction) | - | Duration: Doxy until 12 weeks of age<br><br>Inject/week: day 1, 2, 3, 4, 5, 6 and 7 (drinking water)<br><br>End: 4-week recovery before chronic cerulein | - | Duration: until humane endpoint<br><br>Inject/week: day 1, 2, 3, 4 and 5 (×1/day)<br><br>End: until humane endpoint |
| *PdxC$^{re}$ K$^{Cit-G12D/+}$*<br><br>*Kras$^{Cit/Cit}$* | Start cerulein at 6 to 8 weeks of age | - | - | Duration: 5 days<br><br>Inject/week: day 1, 3 and 5 (×7/day)<br><br>End: 2-day recovery before | Duration: 2 to 6 weeks<br><br>Inject/week: day 1, 2, 3, 4 and 5 (×1/day) |

| | | | | | |
|---|---|---|---|---|---|
| | | | | sacrifice, or 2-day recovery before chronic cerulein | End: 2-day recovery before sacrifice |
| *PgkC$^{re}$ K$^{Cit\text{-}G12D/+}$* | Sacrificed at 8 weeks of age (*Cit-Kras$^{G12D}$* was spontaneously induced organism-wide) | - | - | - | - |

**Supplementary Table S2. Conditions and treatments for mice models used in the present study.** Tam, tamoxifen; Dox, doxycycline; Inject, injections.

| Antibody | Reference | Dilution | Ab incubation conditions |
|---|---|---|---|
| B-RAF | 14814S, CST | 1/1000 | 5% BSA, overnight, 4°C |
| C-RAF | 53745S, CST | 1/2000 | 5% BSA, overnight, 4°C |
| P-C-RAF$^{S338}$ | 9427S, CST | 1/1000 | 5% BSA, overnight, 4°C |
| RAS$^{G12D}$ | 14429S, CST | 1/1000 | 5% Milk, overnight, 4°C |
| MEK1/2 | 9122S, CST | 1/1000 | 5% BSA, overnight, 4°C |
| P-MEK1/2$^{S217/221}$ | 9121S, CST | 1/1000 | 5% BSA, overnight, 4°C |
| ERK1/2 | 9102S, CST | 1/2000 | 5% BSA, overnight, 4°C |
| P-ERK1/2$^{T202/Y204}$ | 9101S, CST | 1/2000 | 5% BSA, overnight, 4°C |
| SOS1 | 5890S, CST | 1/1000 | 5% BSA, overnight, 4°C |
| SOS2 (B-6) | Sc-393667, SCB | 1/200 | 5% Milk, overnight, 4°C |
| EGFR (A-10) | Sc-373746, SCB | 1/500 | 5% Milk, overnight, 4°C |
| P-EGFR$^{Y1068}$ | 2234S, CST | 1/1000 | 5% BSA, overnight, 4°C |
| GRB2 | 3972S, CST | 1/1000 | 5% BSA, overnight, 4°C |
| HSC70 | Sc-7298, SCB | 1/5000 | 5% Milk, overnight, 4°C |
| p110-α | 4249S, CST | 1/1000 | 5% BSA, overnight, 4°C |
| p110-β | Sc-602, CST | 1/500 | 5% BSA, overnight, 4°C |
| p85 | 06-195, Millipore | 1/1000 | 5% BSA, overnight, 4°C |
| AKT | 9272S, CST | 1/2000 | 5% BSA, overnight, 4°C |
| P-AKT$^{S473}$ | 4060S, CST | 1/1000 | 5% BSA, overnight, 4°C |
| P-AKT$^{T308}$ | 2965S, CST | 1/1000 | 5% BSA, overnight, 4°C |
| KRAS | home-made | 1/500 | 5% Milk, overnight, 4°C |
| KRAS | WH0003845M1, SA | 1/500 | 5% Milk, overnight, 4°C |
| KSR1 | 4640S, CST | 1/1000 | 5% BSA, overnight, 4°C |

| | | | |
|---|---|---|---|
| GEF-H1 | 4076S, CST | 1/1000 | 5% BSA, overnight, 4°C |
| PP2Aa | 2041T, CST | 1/1000 | 5% BSA, overnight, 4°C |
| PP2Ab | 2290T, CST | 1/1000 | 5% BSA, overnight, 4°C |
| PP2Ac | 2259T, CST | 1/1000 | 5% BSA, overnight, 4°C |
| SOX9 | AB5535, Millipore | 1/1000 | 5% Milk, overnight, 4°C |
| GFP | Ab6556, Abcam | 1/1000 | 5% Milk, overnight, 4°C |
| E-Cadherin | 610182, BD lab | 1/1000 | 5% Milk, overnight, 4°C |
| GAPDH | Sc-32233, SCB | 1/1000 | 5% Milk, overnight, 4°C |
| Anti-mouse-HRP | 7076S, CST | 1/5000 | 5% Milk, 1h, RT |
| Anti-Rabbit-HRP | ADI-SAB-300-J, Enzo | 1/5000 | 5% Milk, 1h, RT |
| Anti-Goat-HRP | Sc-2020, SCB | 1/2000 | 5% Milk, 1h, RT |

**Supplementary Table S3. Antibodies used in western blot experiments.** Ab, Antibody; CST, Cell Signaling Technology; SCB, Santa-Cruz Biotechnology; SA, Sigma Aldrich.

| Gene | Forward (5’-3’) | Reverse (5’-3’) | Species |
|---|---|---|---|
| *Egfr* | GGGATTGGCCTATTCATGCG | TCCTCAAGTGGGCTTGGTTT | Mouse |
| *Sos1* | TTCGATTCTGACCACTCGGC | GGATGTGGGTTGCCTAGGAG | Mouse |
| *Sos2* | TTAGCAGAGGAGCAAGCGTT | TCACTGCATGCCTTCAACTTT | Mouse |
| *Kras* | CCTACGATAGAGGACTCCTAC | CATGTACTGGTCCCTCATTGC | Mouse |
| *Raf1* | GGCGCTAGTGCAGCGAT | GAGCCATCAAACACCGCATC | Mouse |
| *Cit-K* | ACT CTC GGC ATG GAC GAG | G GAC ATC ACA TAT AAC TG | Mouse |
| *Rpl04* | CGCAACATCCCTGGTATTACT | TGTGCATGGGCAGGTTATAGT | Mouse |
| *Gapdh* | GGTCCTCAGTGTAGCCCAAG | AATGTGTCCGTCGTGGATCT | Mouse |

**Supplementary Table S4. PCR primers used in the present study.**