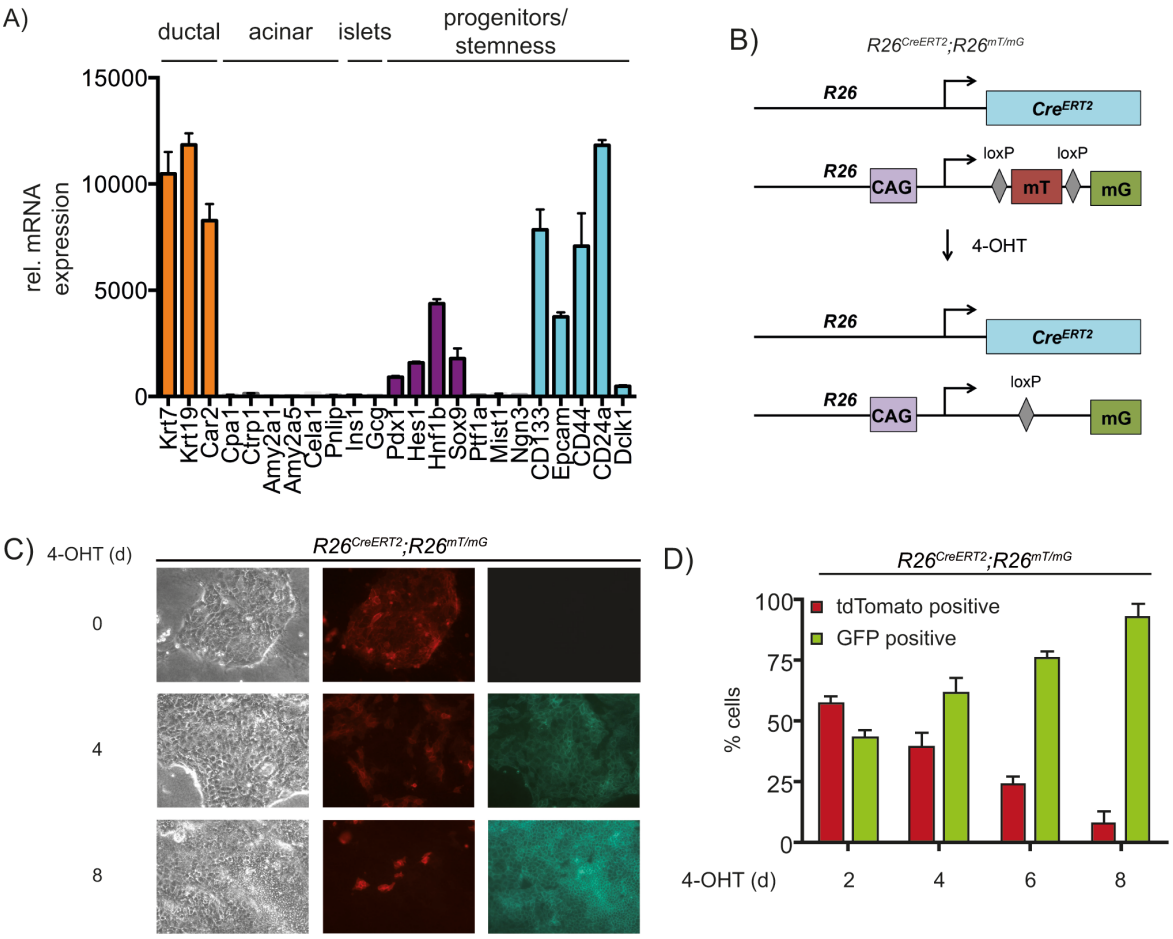
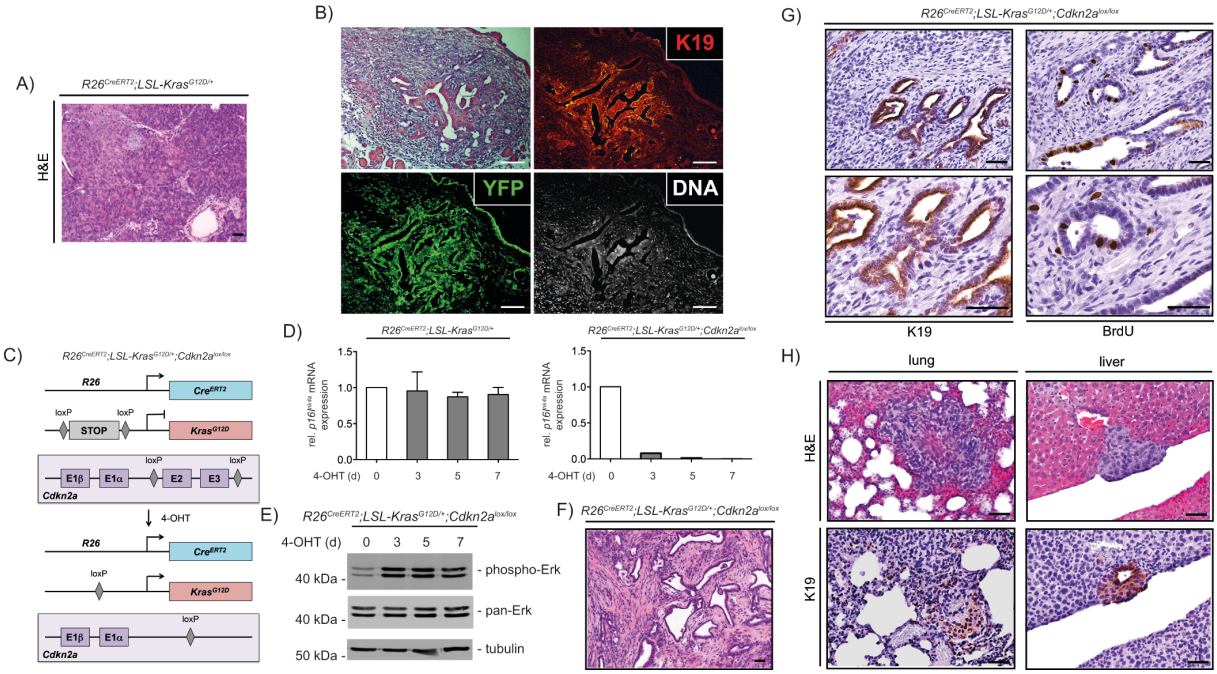


Supplemental figure 1 *Characterization of PDECs.*

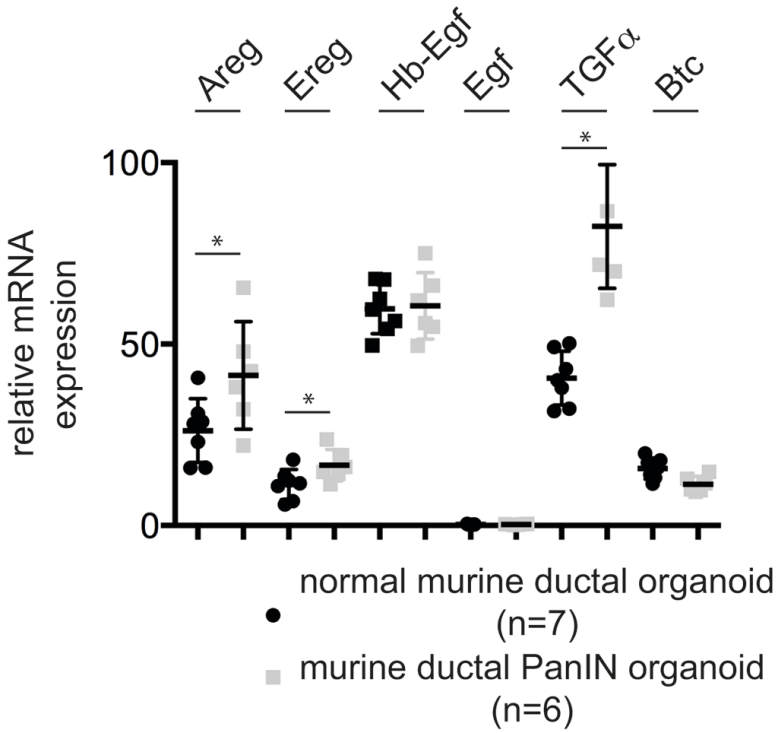
A) mRNA expression of ductal, acinar, islets and progenitor/stemness marker genes in PDECs. Shown is the mean and standard error of the mean (S.E.M) of mRNA expression of microarrays of four different PDEC preparations. mRNA was isolated after three passages. B) Genetic strategy to quantify tamoxifen-induced recombination events with a double fluorescent floxed tdTomato-EGFP reporter line (*R26*$^{mT/mG}$). Mouse lines are described in the supplementary material and methods section. C) PDECs from *R26*^{CreERT2};*R26*$^{mT/mG}$ mice were treated with 4-OHT (200 nM) over time. EGFP and tdTomato labeling were identified by fluorescence microscopy. D) Quantification of recombination efficiency in PDECs from *R26*^{CreERT2};*R26*$^{mT/mG}$ mice, treated over time with 4-OHT (200 nM). Shown is the mean and standard error of the mean (S.E.M) (n=5 fields counted, magnification 200x).



Supplemental figure 2 *PDECs are susceptible to Kras^{G12D}-driven transformation.*

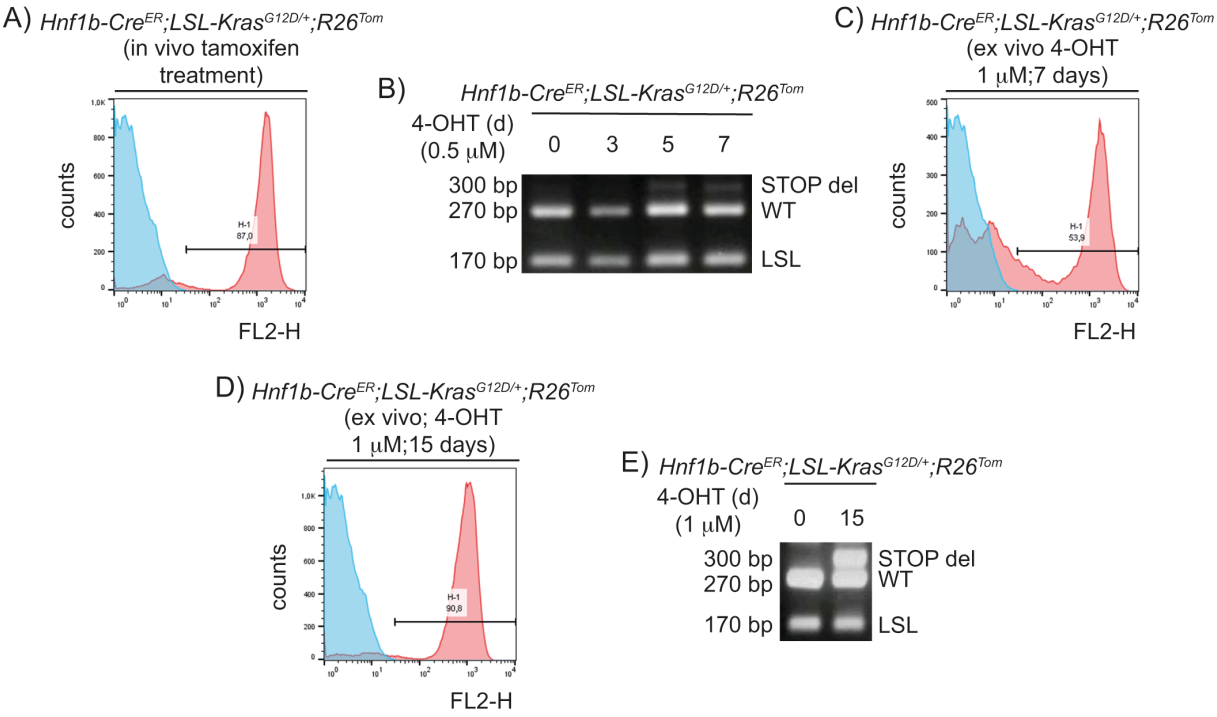
A) PDECs from *R26^{CreERT2};LSL-Kras$^{G12D/+}$* mice were treated with 4-OHT (200 nM) for 6 days *ex vivo*. Afterwards 150.000 cells were orthotopically transplanted into the pancreas of NOD-SCID-Il2rγ$^{-/-}$ (NSG) mice (n=3). After 51 days, no PDAC was detected in NSG mice orthotopically transplanted with *ex vivo* 4-OHT treated *R26^{CreERT2};LSL-Kras$^{G12D/+}$* PDECs. Transplantation was performed at passage four. Representative H&E staining of the pancreas is shown. Scale bar: 50 μm. B) Panel shows representative staining of pancreatic lesions derived from *ex vivo* recombined *LSL-Kras$^{G12D/+}$;R26^{YFP}* PDECs 21 days after orthotopic transplantation into *Ncr* nude mice (n=3). Recombination *ex vivo* was induced by a lentiviral self-deactivating Cre-recombinase followed by FACS sorting of YFP positive PDECs. 7.5x10^5 cells were orthotopically transplanted. H&E staining illustrates PanIN-like/ductal lesion. K19 (red) immunofluorescence (IF) staining (anti-K19, ((TROMAIII), Developmental Studies Hybridoma Bank, Iowa City, IA, USA) is associated with tubular structures. Nuclear counterstain was performed using DAPI (white). YFP (green) indicates donor-origin of PanIN-like lesion. Scale bar: 100 μM. C) Genetic strategy to induce the expression of *Kras^{G12D}* and delete *Cdkn2a* in PDECs. Mouse lines are described in the supplementary material and methods section. D) PDECs from *R26^{CreERT2};LSL-Kras$^{G12D/+}$* or *R26^{CreERT2};LSL-Kras$^{G12D/+}$;Cdkn2a$^{lox/lox}$* mice were treated with 4-OHT (200 nM) over time.

p16^{Ink4a} mRNA expression was determined by qPCR using *cyclophilin A* mRNA expression as reference. Shown is the mean and standard error of the mean (S.E.M) (n=3 for *R26*^{CreERT2}*;LSL-Kras*$^{G12D/+}$ PDECs and n=1 for *R26*^{CreERT2}*;LSL-Kras*$^{G12D/+}$*;Cdkn2a*$^{lox/lox}$ PDECs). Primer sequences are described in the supplementary material and methods section. E) PDECs from *R26*^{CreERT2}*;LSL-Kras*$^{G12D/+}$*;Cdkn2a*$^{lox/lox}$ mice were treated with 4-OHT (200 nM) as indicated. Phosphorylation of ERK and expression of pan-ERK was detected in western blots (α-tubulin: loading control). F) PDECs from *R26*^{CreERT2}*;LSL-Kras*$^{G12D/+}$*;Cdkn2a*$^{lox/lox}$ mice were treated with 4-OHT (200 nM) for 6 days *ex vivo*. Afterwards 150.000 cells were orthotopically transplanted into the pancreas of NSG mice (n=3). All orthotopically transplanted *R26*^{CreERT2}*;LSL-Kras*$^{G12D/+}$*;Cdkn2a*$^{lox/lox}$ PDECs developed PDAC after 51 to 86 days. Transplantation was performed at passage four. Representative H&E staining of the pancreas is shown. Scale bar: 50 μm. G) K19 (anti-K19) immunohistochemistry and BrdU incorporation (anti-BrdU ((MCA2060), AbD Serotec, Düsseldorf, Germany)) of PDACs formed from *ex vivo* 4-OHT treated *R26*^{CreERT2}*;LSL-Kras*$^{G12D/+}$*;Cdkn2a*$^{lox/lox}$ PDECs. Antibodies conjugated to biotin (Vector Laboratories, Peterborough, UK) were used as secondary antibodies. Peroxidase conjugated streptavidin was used with 3,3'-diaminobenzidine tetrahydrochloride (Vectastain® elite ABC Kit and DAB Peroxidase Substrat Kit, Vector Laboratories) as chromogen for detection. Sections were counterstained with haematoxylin. All scale bars: 50 μm. H) H&E staining (see supplementary material and methods) and K19 immunohistochemistry of micrometastases in lung and liver. All scale bars: 50 μm.



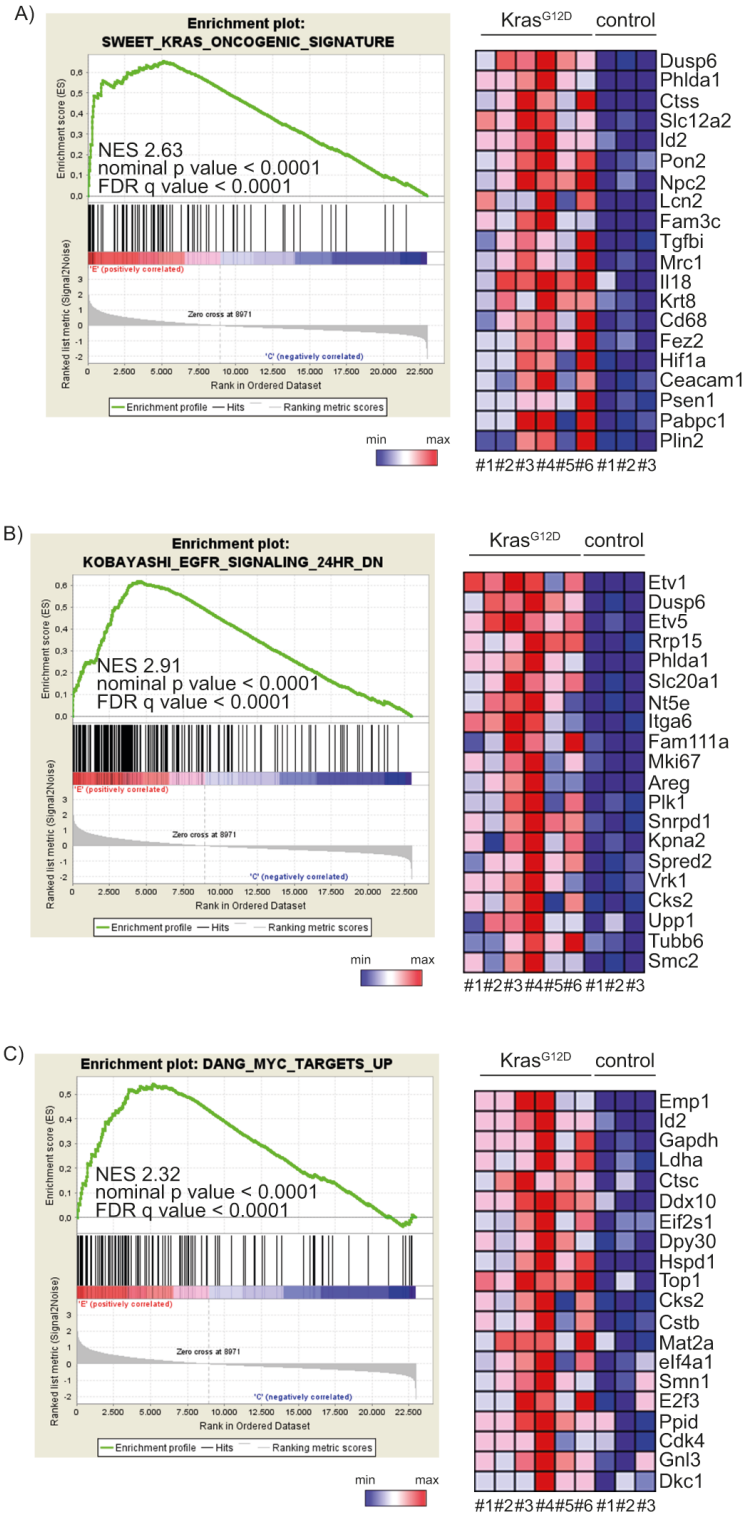
Supplemental figure 3 *EGFR ligands are induced in a murine organoid PanIN model.*

Published RNA-Seq data [13] of normal ductal organoids (n=7) and organoids formed from isolated ductal cells of $Kras^{G12D}$ mice (PanIN organoids) at PanIN stages (n=6) were accessed and analyzed for the expression of EGFR ligands. Unpaired Student`s t-test * $p<0.05$.



Supplemental figure 4 *Ductal origin of isolated PDEC: linage tracing using Hnf1β-Cre^{ER} mice.*

A) Tamoxifen (Sigma, dissolved in Corn Oil) (100 µg/g body weight) was injected i.p. in *Hnf1β-Cre^{ER};LSL-Kras$^{G12D/+}$;R26^{Tom}* mice once at week five and once at week six. Two weeks after the last tamoxifen injection, PDECs were isolated and propagated. After 3 passages PDECs were FACS analysed. Minimum 50.000 events were counted and gated for living cells. Percentage of red fluorescent cells are indicated as population H-1. The blue graph represents negative control PDECs without fluorescence signal. B) Genotyping PCR of the *Kras* locus of the indicated PDECs treated with 4-OHT (500 nM) over time. WT: wild type allele; LSL: *Lox-Stop-Lox* allele; STOP del: recombined LSL-allele. Primer sequences are depicted in the supplementary material and methods section. C) PDECs of untreated *Hnf1β-Cre^{ER};LSL-Kras$^{G12D/+}$;R26^{Tom}* mice were isolated and treated *ex vivo* with 1µM 4-OHT over 7 days. Afterwards, FACS analysis was determined as described in A). D) PDECs of untreated *Hnf1β-Cre^{ER};LSL-Kras$^{G12D/+}$;R26^{Tom}* mice were isolated and treated *ex vivo* with 1 µM 4-OHT over 15 days. Afterwards, FACS analysis was determined as described in A). E) Genotyping PCR of the *Kras* locus in the indicated PDECs treated with 4-OHT (1µM) over 15 days. WT: wild type allele; LSL: *Lox-Stop-Lox* allele; STOP del: recombined LSL-allele.



Supplemental figure 5 *Kras, EGFR, and MYC signatures in Kras^{G12D}-driven carcinogenesis in vivo.*

Recently published microarray expression data [38] of control (n=3) and *Pdx1-Cre;LSL-Kras$^{G12D/+}$* mice (n=6; aged between 10 and 20 weeks) generated from duct and duct-like cells were analyzed by GSEA. Enrichment plots of A) a Kras signature and corresponding

heatmap (top 20 genes) B) an EGFR signature and corresponding heatmap (top 20 EGFR controlled genes induced by $Kras^{G12D}$) and C) a MYC signature (top 20 MYC controlled genes induced by $Kras^{G12D}$). NES: normalized enrichment score; FDR: false discovery rate. The nominal p value is indicated. See supplementary material and methods for description of the GSEA.