SUPPLEMENTAL MATERIAL INFORMATION

Legends to Supplemental Figures

Supplemental Figure 1. Expression of p110 isoforms in pancreatic cancer cell lines. (*A, B*) Analysis of p110 isoforms and its regulatory subunit expression by western-blot in 5 human pancreatic cancer cell lines and 7 primary murine $Kras^{G12D}$ -driven PanINs or PDAC cell lines with and without $p53^{R172H}$ expression as described in D. (*C*) Regenotyping of mouse cell lines from GEMMs by PCR (Hingorani et al. 2005). (*D*) Origin of the murine cell lines.

Supplemental Figure 2. Validation of Cre-induced recombination of *Pik3ca* gene in pancreata only. (*A*) Schematic representation of conditional *Pik3ca* gene targeting: the genetically modified allele is represented before ($p110\alpha^{lox}$) and after Cre recombination ($p110\alpha^{\Delta DFG}$) (Graupera et al. 2008). DFG is a conserved motif in the activation loop of the p110α kinase domain critical for the activity. The gene targeting strategy used is different from a traditional conditional knock-out strategy. Instead, it is deleting two exons in the catalytic domain of *Pik3ca* in the 3' part of the gene, allowing expression of a truncated inactive p110α after recombination (Graupera et al. 2008). Red lines correspond to the pair of genotyping primers (pre-cre primers): FE1 - GGATGCGGTCTTTATTGTC; FE4 - TGGCATGCTGCCGAATTG; annealing temperature: 65°C; wild type (+)-640bp, lox-708bp. Blue lines corresponds to post-cre primers used to verify the presence of recombined allele ΔDGF: ma9 - ACACACTGCATCAATGGC; a5 = GCTGCCGAATTGCTAGGTAAGC; annealing temperature: 65°C; recombined ΔDGF-544 bp, wild type (+) or unrecombined lox- >10kpb amplicon. (*B*) Genotyping of tail samples. Detection of LSL-Kras allele; detection of Pdx1-Cre transgene, as described (Hingorani et al. 2003); detection of *pik3ca* alleles (FE1/FE4 primers). (*C*) Validation of specific cre recombination of *pik3ca* gene in the pancreas by PCR on genomic DNA. Samples from liver were used as a negative control. As expected, the recombined allele $p110\alpha^{\Delta DFG}$ is observed in cre expressing tissue only

(pancreas): full recombination in KC;p110$\alpha^{lox/lox}$ and partial recombination in KC;p110$\alpha^{+/lox}$ is demonstrated by respectively the absence of the lox allele or presence of the WT allele. (*D*) WB using indicated antibodies on 2 month-old pancreatic lysates from indicated genotypes (left panel). Relative quantification is shown in the right panel (N=9 for each genotype). Isoform-specificity of antibody was previously shown in (Guillermet-Guibert et al. 2008). Full recombination of lox allele was verified for all samples as shown for the samples presented here (lower panel).

Supplemental Figure 3. p110α inactivation with and without Kras activation does not lead to Langerhans islet defects or to mouse lethality, but blocks Kras induced pancreatic carcinogenesis. (*A*) Hematoxylin and eosin staining of pancreas sections from 6 month-old p110$\alpha^{lox/lox}$ and C;p110$\alpha^{lox/lox}$ mice. Scale=200µm. (*B*) IHC analysis of insulin, glucagon and somatostatin in 6 months-old p110$\alpha^{lox/lox}$ and C;p110$\alpha^{lox/lox}$ mice. Quantification of the percentage of glucagon and somatostatin positive cells is shown in the lower panel (quantification was performed on 5 Langherhans islets per mice, N=4 mice for each genotype, Student's t-test). Considering the crucial role of p110α in glucose metabolism and insulin receptor adapters IRS1/2-mediated signalling (Foukas et al. 2006; Vanhaesebroeck et al. 2010), we confirmed that the cell repartition of the endocrine compartment was unchanged after inactivation of p110α in the pancreas. In particular, in contrast with IRS2 knock-out, glucagon-positive cells were present (Kubota et al. 2000). Scale=100µm. (*C*) Homozygous inactivation of p110α in the pancreas did not significantly affect circulating levels of glucose or insulin of young randomly fed mice (N>11; 7-week old; ns, Mann-Whitney test) nor body weight (not shown). (*D*) Quantification of ADMs and PanINs in KC;p110$\alpha^{+/lox}$ (N=12) and KC;p110$\alpha^{lox/lox}$ mice (N=14; 6 month old; *p<0.05, Mann-Whitney test). The percentage of ADM or PanIN1 surface over total pancreatic surface was quantified for each mouse. Each point represents a single mouse, and horizontal bars represent mean percentage for each group. *P* values are < 0.05 Mann-Whitney test. (*E*) Hematoxylin and eosin and IHC staining of recombined KC;p110$\alpha^{lox/lox}$ pancreas sections from 6 month-old KC;p110$\alpha^{lox/lox}$ mice harboring low grade PanINs lesions. These lesions are devoid of

surrounding fibrous reaction (arrow heads). Scale=200µm (H&E); 100 µm (IHC stainings). (*F*) Recombination of *pik3ca* locus was verified in these PanIN lesions after microdissection of paraffin embedded 4µm sections (hematoxylin counterstain) by analyzing the disappearance of the lox allele by nested PCR after DNA extraction; T corresponds to tail DNA from KC;p110α$^{lox/lox}$. Genotyping CRE PCR was used as a control for DNA extraction. First sample was dissected twice with similar results.

Supplemental Figure 4. p110α activity is crucial for induction and maintenance of pancreatic ADM/PanIN lesions induced by inflammation in the context of mutated Kras. Representative IF or IHC of pancreata of indicated genotype, 1 day (A, B, C) or 5 days (B, C) after the last caerulein or vehicle injection using indicated antibodies (N=3 for each group). For quantification, each point represents the mean of 5 fields per mouse (N=3, magnification x20, ** p<0.01 Student's t-test). Black arrowheads show positively stained-cells. (*A*) Scale=10µm. (*B*) Ki67=Scale=100µm (*C*) Scale= 90µm. (*D*) Scale=500-90µm. (*E*) Schematic representation of the role of p110α in inflammation-induced pancreatic injury and regeneration in the presence or absence of oncogenic Kras.

Supplemental Figure 5. Genetic inactivation of p110α blocks ADM induction *ex vivo*. Phase contrast images of KC, KC;p110α$^{+/lox}$ and KC;p110α$^{lox/lox}$ acinar cells 6 days after isolation and treatment with TGF- α (50 ng/ml). Quantification of the percentage of ductal structures after 6 days in culture (*ex vivo* acinar cultures from independent mice; N=2 mice in triplicate; * p<0.05 , **p<0.01, Student's t-test). Scale=50 µm.

Supplemental Figure 6. p110α regulates actin cytoskeleton during inflammation-induced ADM and PanIN formation. (*A*) Lower magnification (x63) of confocal microscopic images of actin staining on paraffin-embedded pancreata in indicated conditions (N=3 per genotype). Positive controls for actin staining unchanged by pancreatic-specific p110α inactivation are Langerhans islets (not shown). Scale=10µm. (*B*) F-actin network in A66 or vehicle treated transdifferentiated acinar cells *in vitro*.

Representative signal intensity scans across cell is shown. Scale=10µm. (*C*) WB in KC;p110α$^{+/lox}$ versus KC;p110α$^{lox/lox}$ mice and sacrificed 1 day after last caerulein or PBS injection. Quantification of all samples (N>2).

Supplemental Figure 7. p110α is a critical signalling hub in pancreatic preneoplastic lesion initiation. (*A*) Ras *in vivo* activity from pancreatic lysates of KC;p110α$^{+/lox}$ compared to KC;p110α$^{lox/lox}$ mice (N=3), sacrificed 1 day after last caerulein injection. Relative quantification of all mice analyzed is shown below (* p<0.05, Student's t-test). Positive and negative controls correspond respectively to GTP and GDP loading of Raf1-RBD (not shown). (*B*) IHC with anti-NF-κB/p65 antibodies in indicated conditions (8h after caerulein treatment). Scale=10µm. For quantification, each point represents the mean of 5 fields per mouse (N≥3, **p<0.01, Student's t-test). (*C*) Quantification of pSTAT3-positive nuclei in indicated conditions. For quantification, each point represents the mean of 5 fields per mouse (N=3, **p<0.01, Student's t-test, versus KC). (*D*) Relative quantification of pSTAT3 and EGFR in all mice analyzed by western-blot is shown (N≥3; * p<0.05, Student's t-test).

Supplemental Figure 8. Full p110α inactivation in the KPC model results in preneoplastic lesions with decreased activation of key signalling pathways. (A) Genotyping for recombined *Pik3ca* allele in PanIN lesions of KPC;p110α$^{lox/lox}$ mice. (B) Phenotypic characterization and analysis of signalling pathways activated in these lesions.

Supplemental Figure 9. p110α specific inhibitor A66 decreases Akt/mTOR downstream signalling pathway in murine embryonic fibroblasts. After 24 h serum starvation, MEF cells with indicated stable transfections were pre-treated with p110α specific inhibitor A66 (10µM) or vehicle during 1h and stimulated with 2%FBS during 10 min. Western-blot analysis was performed using indicated antibodies.

DETAILED EXPERIMENTAL PROCEDURES

Plasmatic dosage of insulin. Plasmatic levels of insulin in 7 week-old mice was measured using ELISA assay (Mercodia).

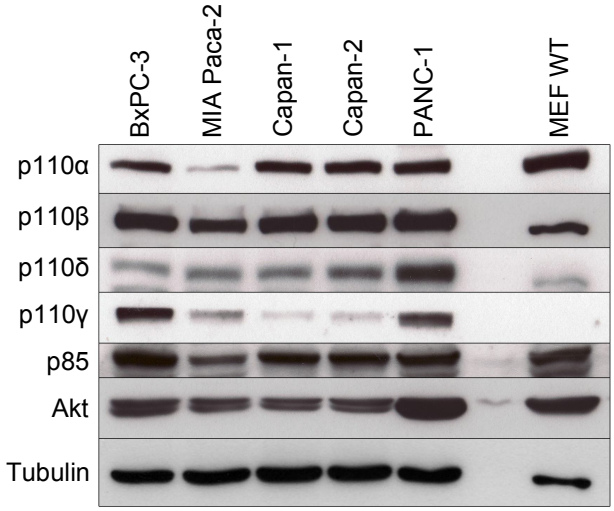
DNA extraction and PCR conditions for microdissected samples. DNA was extracted from hematoxylin stained 4µm FFPE sections; DNA extraction from Arcturus caps and PCR was performed using Sigma Redextract NAmp. After first amplication using FE1/FE4 primers, a nested PCR was used using the following primers: nested FE1=TTGTCTCTTCTGTCCGATGT; nested FE4=TTGCTAGGTAAGCCTTGTAAC. 2 rounds of CRE PCR is used as a control for DNA extraction.

Antibodies and conditions

Protein targeted	Origin	Targeted species	Company	Dilution	Fixation/ ıtigen retrieval	Use
Amylase	Rabbit	Human, mouse, rat	Sigma A8273	1/1000	PFA 3,7 %	IF
CAII	Rabbit	Human, rat	Gift, Fanjul M	1/100	PFA 3,7 %	IF
CK19	Hybridome TR3	rat	Gift, Gigoux V	1/2	PFA 3,7 %	IF
Trypsin	Sheep	NA	Gift, Fanjul M	1/100	PFA 3,7 %	IF
Phalloïdin-TRITC	NA	NA	SigmaP1951	1/500	PFA 3,7 %	IF
Ab II anti-Rabbit	Goat	Rabbit	Lifetechnologies	1/1000	PFA 3,7 %	IF
Ab II anti-Rat FITC	Mouse	Rat	Interchim UPB91360	1/1000	PFA 3,7 %	IF
Ab II anti-sheep HRP	Rabbit	Sheep	DAKO P0163	1/1000	PFA 3,7 %	IF
pSer473 Akt	Rabbit	Human, mouse, rat	Cell Signaling Technologies, XP #4060	1/20 1/1000	Formalin, Citrate	IHC WB
pAkt Substrate	Rabbit	All	Cell Signaling Technologies, #9611	1/1000	Formalin, Citrate	IHC
pThr202/Tyr204 ERK1/2	Rabbit	Human, mouse, rat	Cell Signaling Technologies, #4370	1/100	Formalin, Citrate	IHC
Ki67	Rabbit	Human	Epitomics, #4203-1	1/400	Formalin, Citrate	IHC
CK19	Rabbit	Mouse	Epitomics, #3863-S	1/1000	Formalin, Citrate	IHC
β-actin	Mouse	All	Sigma (AC-74)	1/100 1/1000	Formalin, Citrate	IF WB
Amylase	Rabbit		Sigma	1/3000	Formalin, Citrate	IF
IL6	Rabbit		Abcam #6672	1/250	Formalin, Citrate	IHC
αSMA	Mouse	Mouse, rat, rabbit, human, pig, sheep	Abcam, ab7817	1/250	Formalin, Citrate	IHC
p65	Rabbit	Human, mouse, rat, monkey,dog	Cell Signaling Technologies, #8542	1/200	Formalin, Citrate	IHC
insulin	Guinea pig	Mouse, rat, bovine	BioGenex, AR029-5R/PU029-UP	none	Formalin, Citrate	IHC
glucagon	Mouse	Human, mouse, rabbit, rat	Sigma, G 2654	1/5000	Formalin, Citrate	IHC
somatostatin	Rabbit		Dako, A0566	1/300	Formalin,	IHC

					Citrate	
pTyr705 STAT3	Rabbit	Human, mouse, rat	Cell Signaling Technologies, #9145	1/100 1/1000	Formalin, EDTA	IHC, WB
EGFR	Rabbit	Human, mouse, rat	Epitomics, #1902-1	1/100 1/1000	Formalin, Citrate	IHC WB
CD45	Rat	Mouse	R&D Systems, MAB114	1/5000	Formalin, Citrate	IHC
B220	Rat	Mouse	R&D Systems, MAB1217	1/1000	Formalin, Citrate	IHC
LY6G	Rat	Mouse	Abcam, ab25377	1/3000	Formalin, Citrate	IHC
CD3	Rat	Mouse	R&D Systems, MAB4841	1/5000	Formalin, Citrate	IHC
CD68	Rat	Mouse	ABD serotech, MCA 1957	1/100	Formalin, Citrate	IHC
F4/80	Rat	Mouse	Abcam, ab6640	1/5000	Formalin, Citrate	IHC
Pdx1	Rabbit	Mouse, Rat, Cow, Dog, Human	Abcam	Ab47267	Formalin, Citrate	
Ab II anti-rabbit	Rabbit	Rabbit	Cell Signaling Technologies, #8114	-	Formalin	IHC
p85	Rabbit	Rabbit, human, mouse, monkey	Millipore/ Upstate, 06-497	1/2000		WB
pThr246 PRAS40	Rabbit	Human, mouse, rat, monkey	Cell Signaling Technologies, #2997	1/1000		WB
pSer240/244 S6	Rabbit	Human, mouse, rat, monkey	Cell Signaling Technologies, XP #5364	1/1000		WB
p110α	Mouse	Human, rat	BD Bioscience 611398	1/500		WB
p110β	Rabbit	Human, mouse, rat	Santa Cruz, SC-602	1/1000		WB
p110γ	Rabbit		In house kind gift from E. Hirsch	1/2 in HBSS		WB
p110δ	Rabbit	Human, mouse, rat	Santa Cruz, SC-7176	1/1000		WB

A



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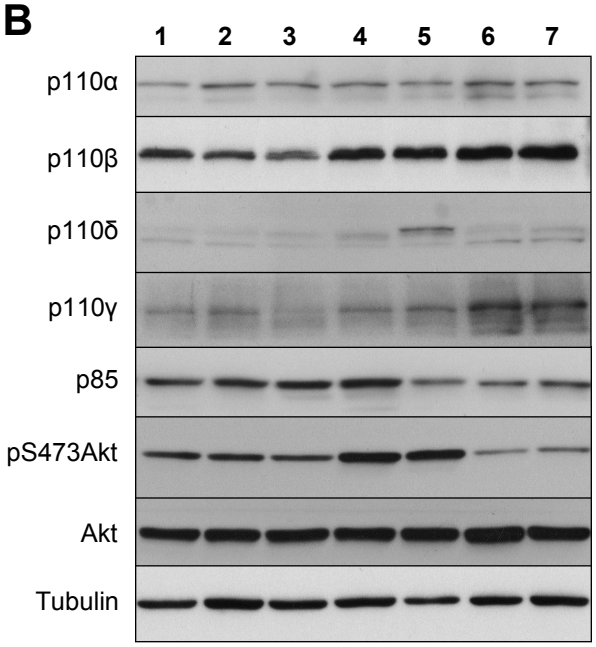

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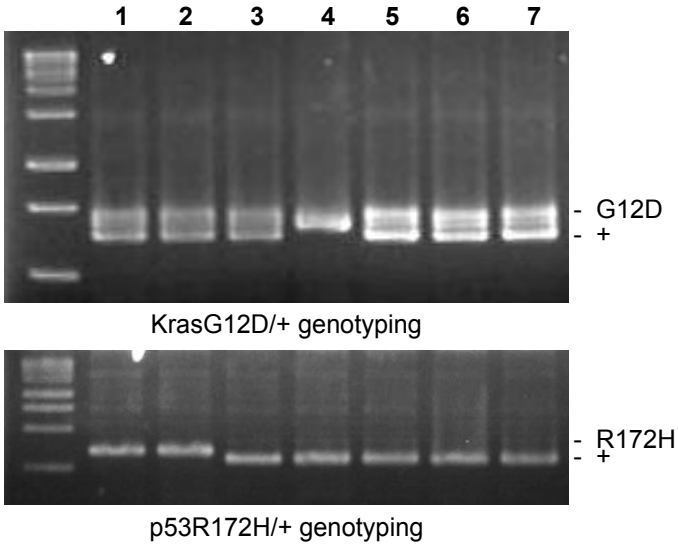
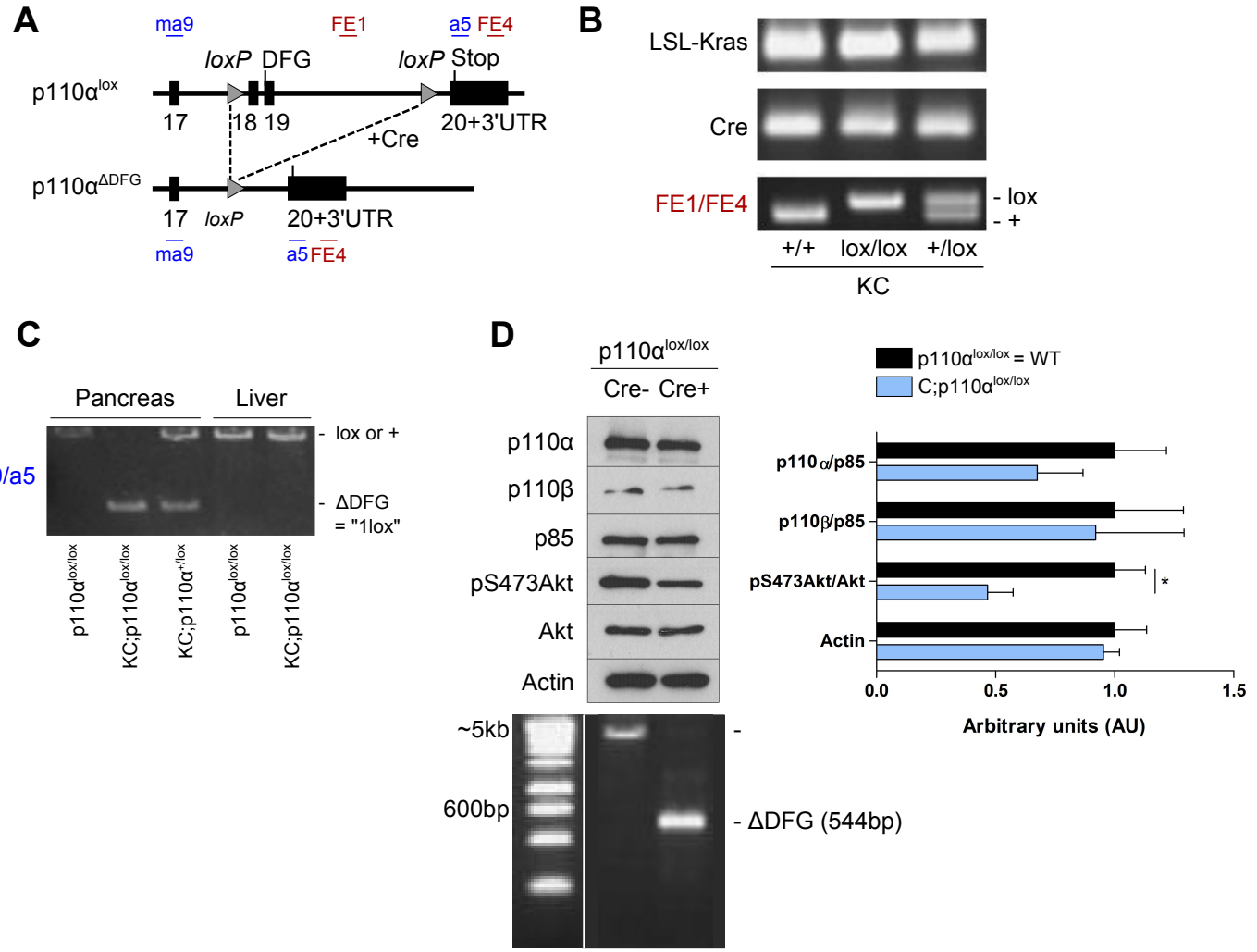


D

Murine cell lines	1	2	3	4	5	6	7
KrasG12D/+	+	+	+	+	+	+	+
p53R172H/+	+	+					
PDAC	+	+			+	+	+
PanIN			+	+			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ma9
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a5 FE4
loxP DFG
loxP Stop
p110α^lox
17
18 19
20+3'UTR
+Cre
p110α^ΔDFG
17
loxP
20+3'UTR
ma9
a5 FE4
B
LSL-Kras
Cre
FE1/FE4
- lox
- +
+/+
lox/lox
+/lox
KC
C
Pancreas
Liver
ma9/a5
- lox or +
- ΔDFG
= "1lox"
p110α^lox/lox
KC;p110α^lox/lox
KC;p110α^+/lox
p110α^lox/lox
KC;p110α^lox/lox
D
p110α^lox/lox
Cre- Cre+
p110α
p110β
p85
pS473Akt
Akt
Actin
~5kb
600bp
-
- ΔDFG (544bp)
p110α^lox/lox = WT
C;p110α^lox/lox
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Actin
*
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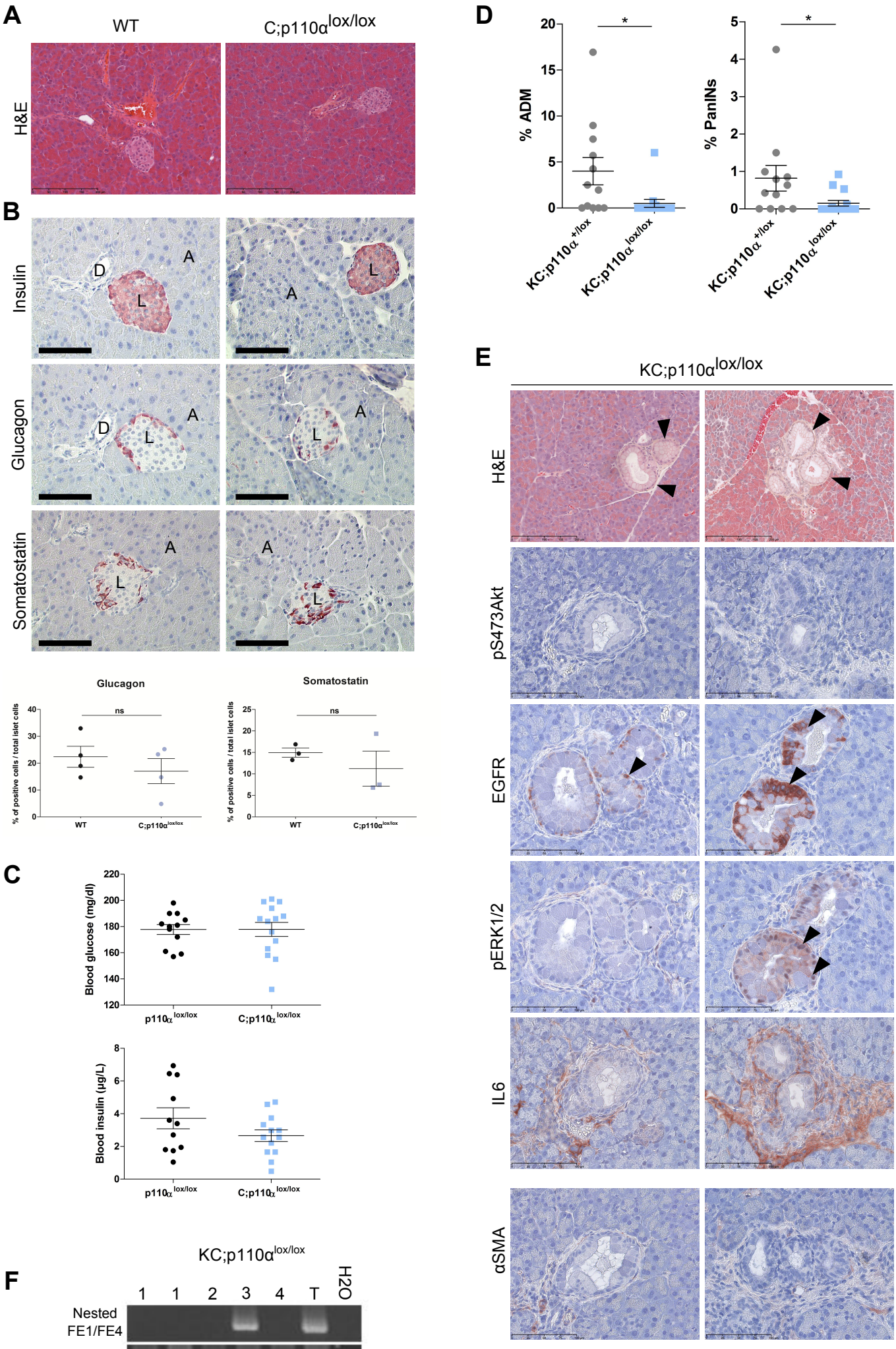
0.5
1.0
1.5
Arbitrary units (AU)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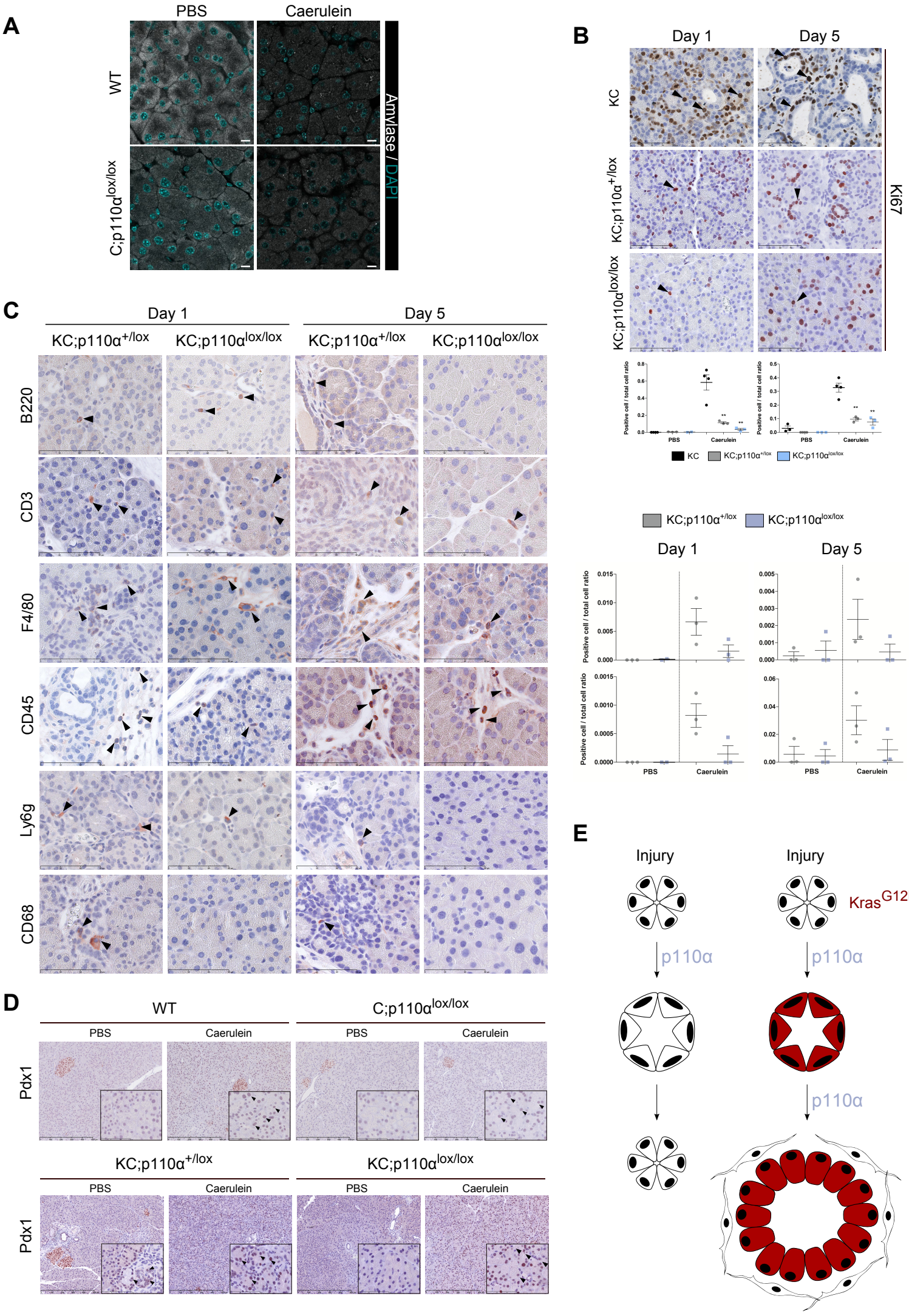
WT
C;p110α lox/lox
H&E
B
Insulin
Glucagon
Somatostatin
D
L
A
Glucagon
Somatostatin
ns
% of positive cells / total islet cells
WT
C;p110α lox/lox
C
Blood glucose (mg/dl)
Blood insulin (µg/L)
p110α lox/lox
C;p110α lox/lox
D
% ADM
% PanINs
*
KC;p110α +/lox
KC;p110α lox/lox
E
KC;p110α lox/lox
H&E
pS473Akt
EGFR
pERK1/2
IL6
αSMA
F
KC;p110α lox/lox
1 1 2 3 4 T H2O
Nested FE1/FE4
Cre

Baer_Supplemental Fig4



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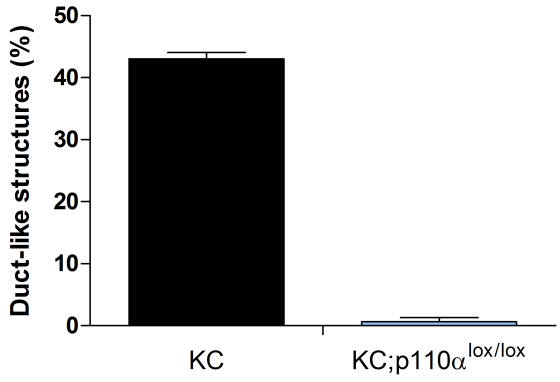

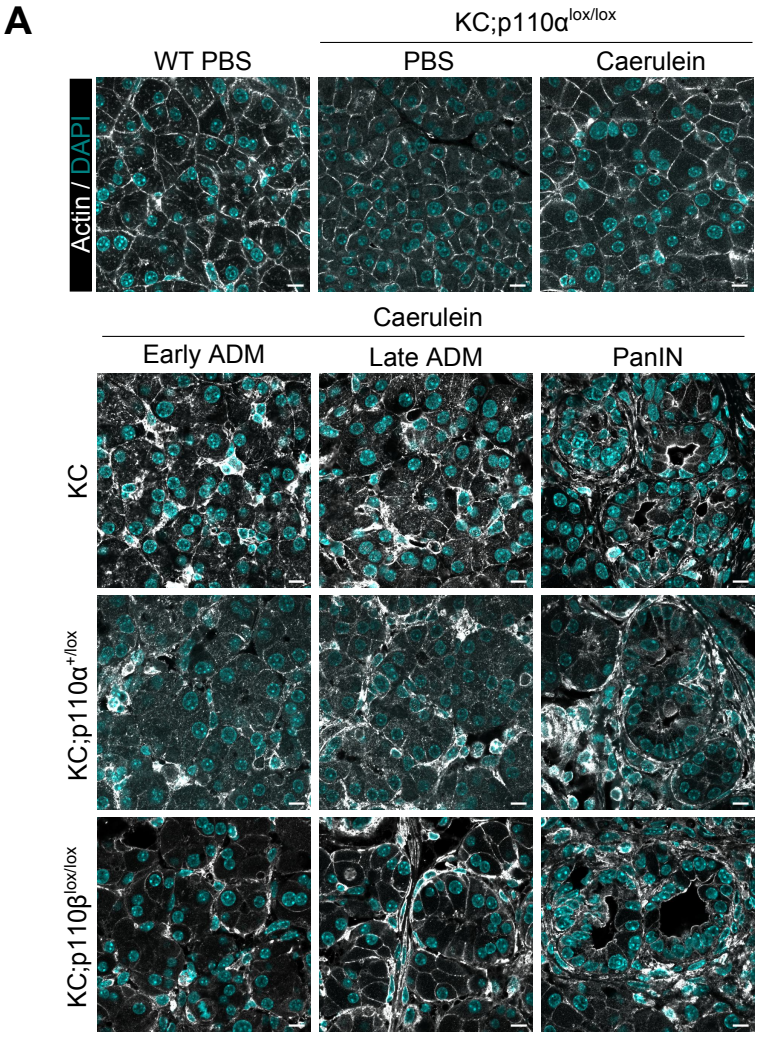
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WT PBS
PBS
Caerulein
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PanIN
KC
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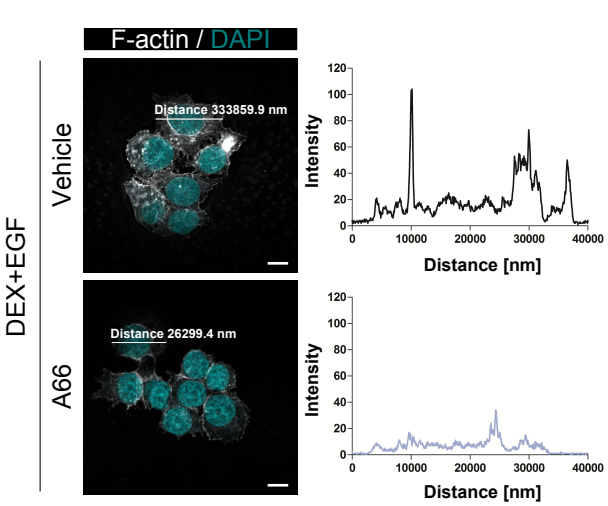
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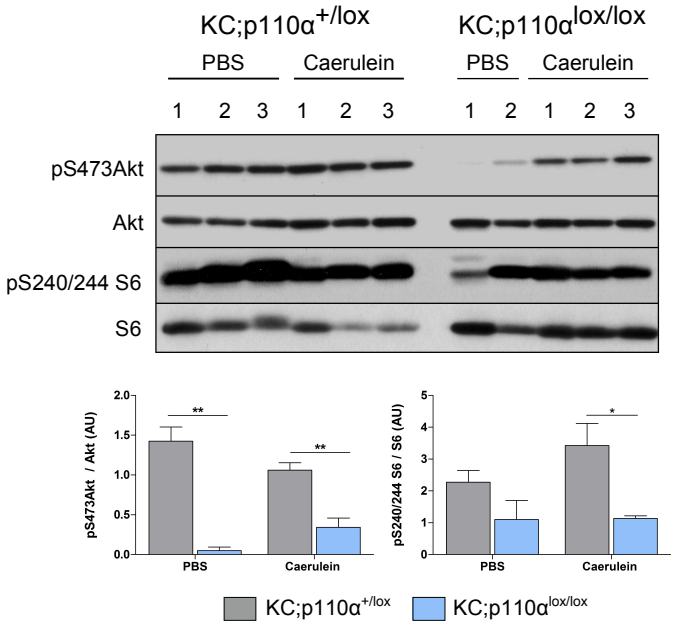
F-actin / DAPI
DEX+EGF
Vehicle
A66
Distance 333859.9 nm
Distance 26299.4 nm
Intensity
Distance [nm]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KC;p110α lox/lox
PBS
Caerulein
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1 2 1 2 3
pS473Akt
Akt
pS240/244 S6
S6
pS473Akt / Akt (AU)
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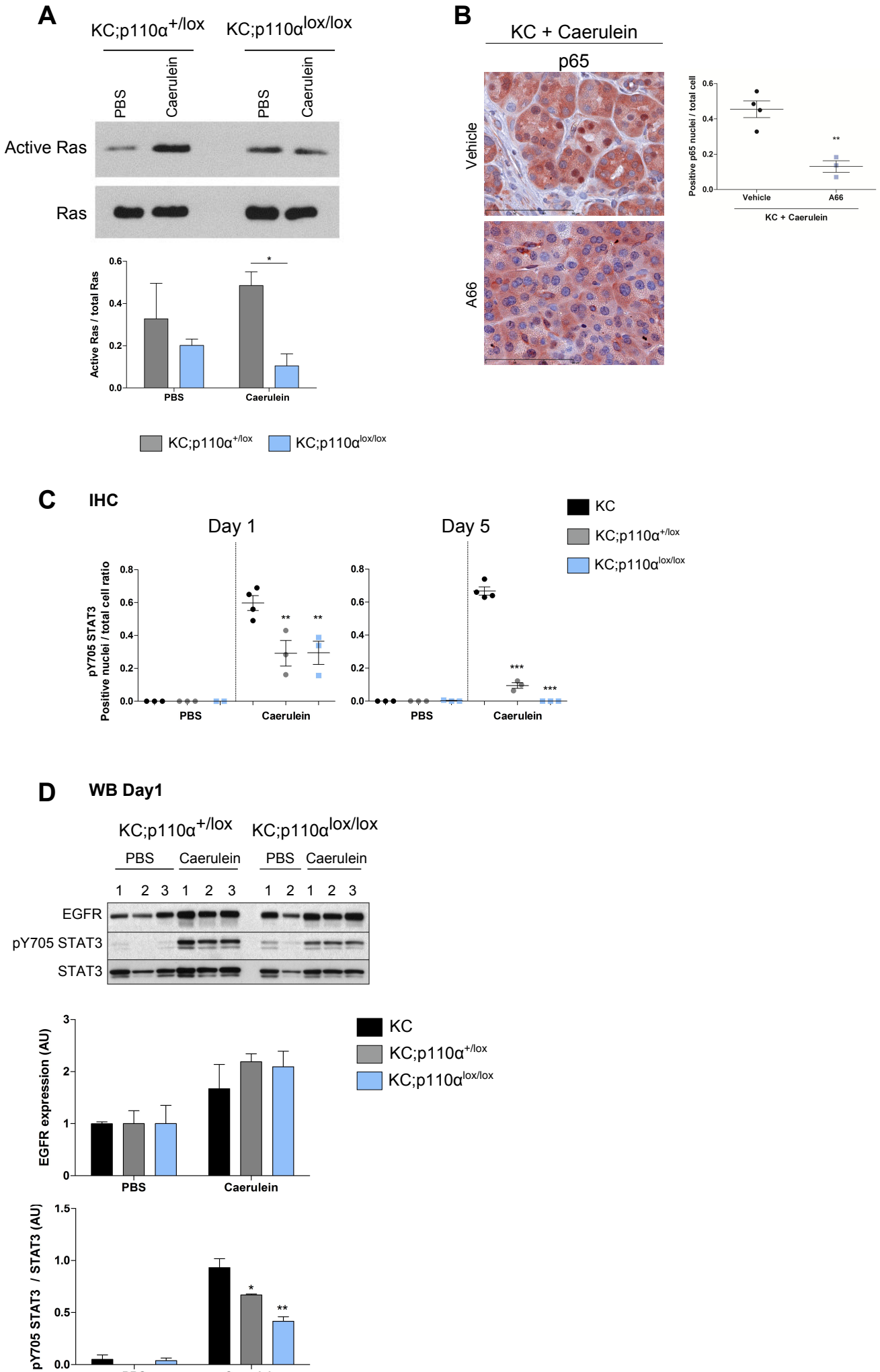
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KC;p110αlox/lox
PBS
Caerulein
PBS
Caerulein
Active Ras
Ras
Active Ras / total Ras
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0.4
0.2
0.0
*
PBS
Caerulein
KC;p110α+/lox
KC;p110αlox/lox
B
KC + Caerulein
p65
Vehicle
A66
Positive p65 nuclei / total cell
0.6
0.4
0.2
0.0
**
Vehicle
A66
KC + Caerulein
C
IHC
Day 1
Day 5
KC
KC;p110α+/lox
KC;p110αlox/lox
pY705 STAT3
Positive nuclei / total cell ratio
0.8
0.6
0.4
0.2
0.0
**
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***
***
PBS
Caerulein
PBS
Caerulein
D
WB Day1
KC;p110α+/lox
KC;p110αlox/lox
PBS
Caerulein
PBS
Caerulein
1 2 3 1 2 3 1 2 1 2 3
EGFR
pY705 STAT3
STAT3
KC
KC;p110α+/lox
KC;p110αlox/lox
EGFR expression (AU)
3
2
1
0
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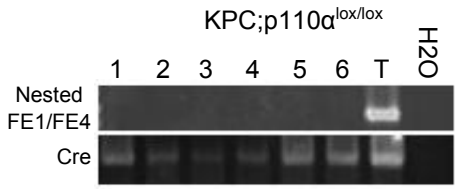
Caerulein
pY705 STAT3 / STAT3 (AU)
1.5
1.0
0.5
0.0
*
**
PBS
Caerulein

A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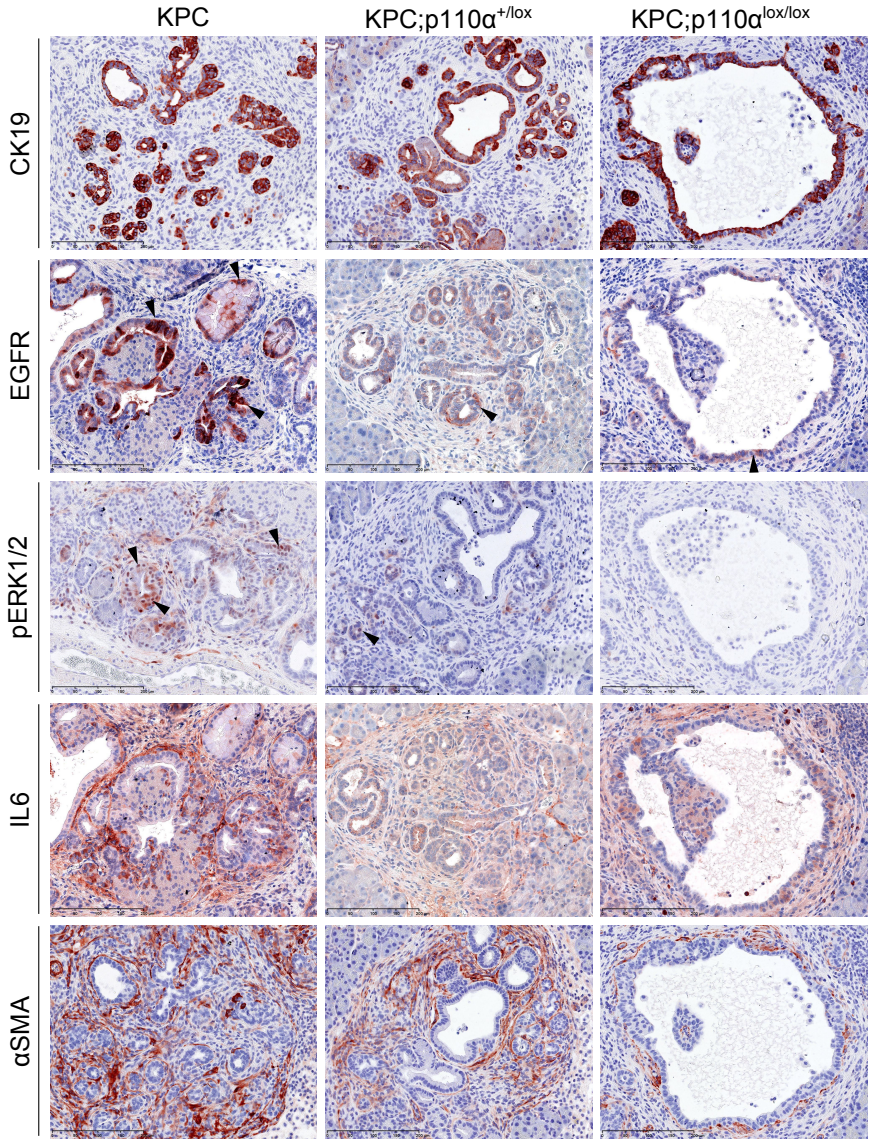
2
3
4
5
6
T
H2O
Nested FE1/FE4
Cre

B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KPC;p110α lox/lox
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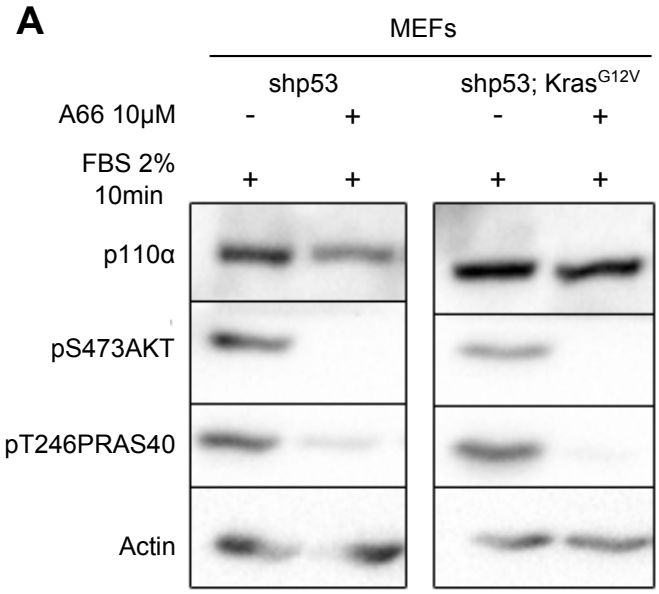
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shp53; Kras^{G12V}
A66 10μM
- + - +
FBS 2%
10min
+ + + +
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pT246PRAS40
Actin