Supplement information

Supplemental Table 1

Primers used for real-time PCR analysis.

Supplemental Figure S1

GSK3i treatment impairs acinar to duct formation in response to TGF-α. Representative brightfield images (left panels) and Hoechst 33324 (right panels) fluorescence images from 6-day cultures treated as indicated. Conditions were the same as those described in Figure 2A. Note the intact nuclei indicating that the cells are not apoptotic. Bars, 200 μm.

Supplemental Figure S2

GSK-3β deletion does not alter the subcellular localization of β-catenin or result in increased expression of β-catenin target genes. (A) Pancreatic tissues from mice of the indicated genotypes treated with saline or 2 and 7 days post caerulein treatment were stained for β-catenin expression. Nuclei were counter-stained with Hoechst (blue). Scale bar, 20 μm. (B) CCND1 (left) and Myc (right) gene expression were measured in pancreas samples prepared from the indicated genotypes and treatments using the Qiagen RT^2 PCR array kit. Data were analyzed and expressed as mean ± SEM. n = 5.

Supplemental Figure S3

GSK-3β deletion impairs ADM and PanIN lesion formation in response to acute inflammation. (A) H&E (left panel) and immunofluorescence staining of amylase and

CK19 (right panel) from KO mice treated with saline or 2 and 7 days post caerulein injection. Nuclei were counter-stained with Hoechst (blue). (B) Pancreatic tissues (normal acinar areas and regions with ADM and PanINs) from mice of desired genotypes were stained with Alcian Blue after saline treatment or 2 and 7 days post caerulein injection. Scale bar, 200 μm. (C) H&E stained tissue sections from KRas or RKO mice 2 and 7 days post caerulein injection were evaluated and quantitatively analyzed for areas with abnormal structures including ADM area (red) and PanIN lesions (blue) and expressed as percentage to total pancreas. (D) Quantification of the relative level of CK19 staining as determined by fluorescence signal from KRas or RKO mice 2 and 7 days post caerulein injection. Data were analyzed and expressed as mean ± SEM. *P<0.05 RKO versus KRas mice. n = 6.

Supplemental Figure S4

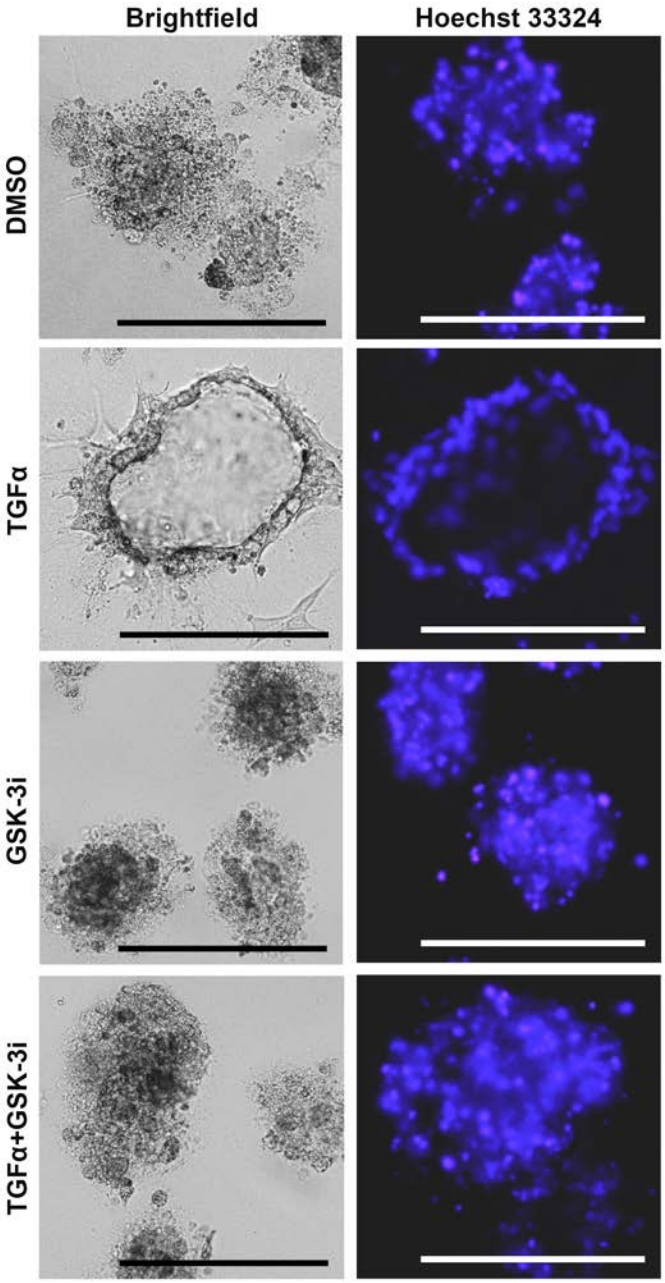
GSK-3β deletion decreases cell proliferation. (A) Triple-labeling of pancreatic sections from WT and KRas mice using α–smooth muscle actin (αSMA) (green), α–amylase (red) and CK19 (magenta). Nuclei were counterstained with Hoechst (blue). Note the lack of αSMA staining around the normal pancreatic duct but around the blood vessel in the WT sample. In contrast, αSMA staining is associated with the PanIN and metaplastic ductal cells. (B) Pancreatic sections from WT, KRas, KO and RKO mice from day 2-cearulein-treated mice were stained with αSMA (magenta), Ki-67 (red), CK19 (green) and nuclei (blue). (C) EdU (green) staining was performed using Click-iT® EdU Alexa Fluor® 488 Imaging Kit in pancreatic sections from KRas and RKO mice 2 or 7 days post caerulein injection. (D) Quantification of the number of EdU positive cells

per field. Data were analyzed and expressed as mean ± SEM. *P<0.05 RKO versus KRas mice. n = 6.

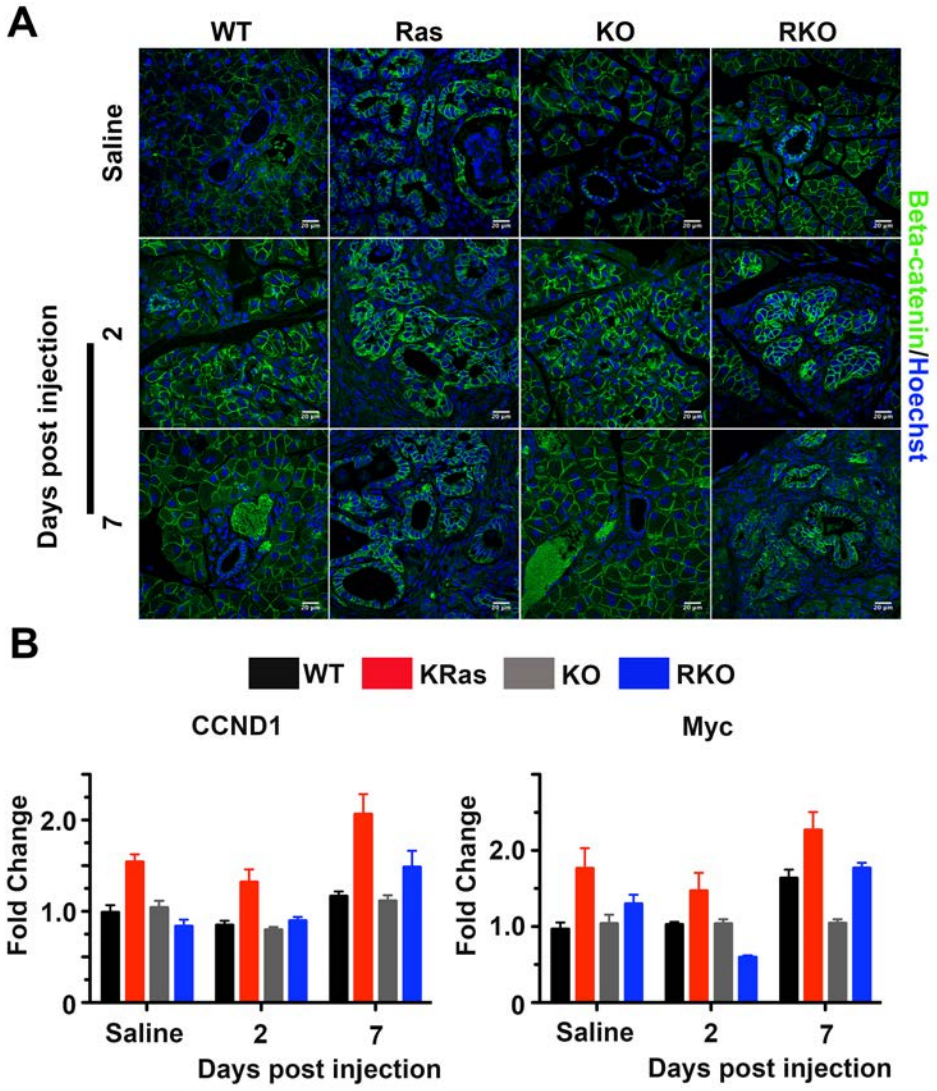
Supplemental Figure S5

GSK-3β deletion reduces the levels of pS6 in caerulein-treated mice and AR42J cells. (A) Quantification of the co-localization of pS6 with CK19 percentage versus total CK19 area. Data were analyzed and expressed as mean ± SEM. *P<0.05 RKO versus KRas mice. n = 6. (B) The average signal intensity of phosphorylated $S6^{235/236}$ and phosphorylated $S6^{240/244}$ from Figure 6B were quantified and expressed as mean ± SEM. n=6. *P<0.05 GSK-3i versus DMSO. (C) The average signal intensity of GSK-3β, phosphorylated $S6^{235/236}$, phosphorylated $S6^{240/244}$ from Figure 6C were quantified and expressed as mean ± SEM. n=6. *P<0.05 shGSK-3β versus stable control.

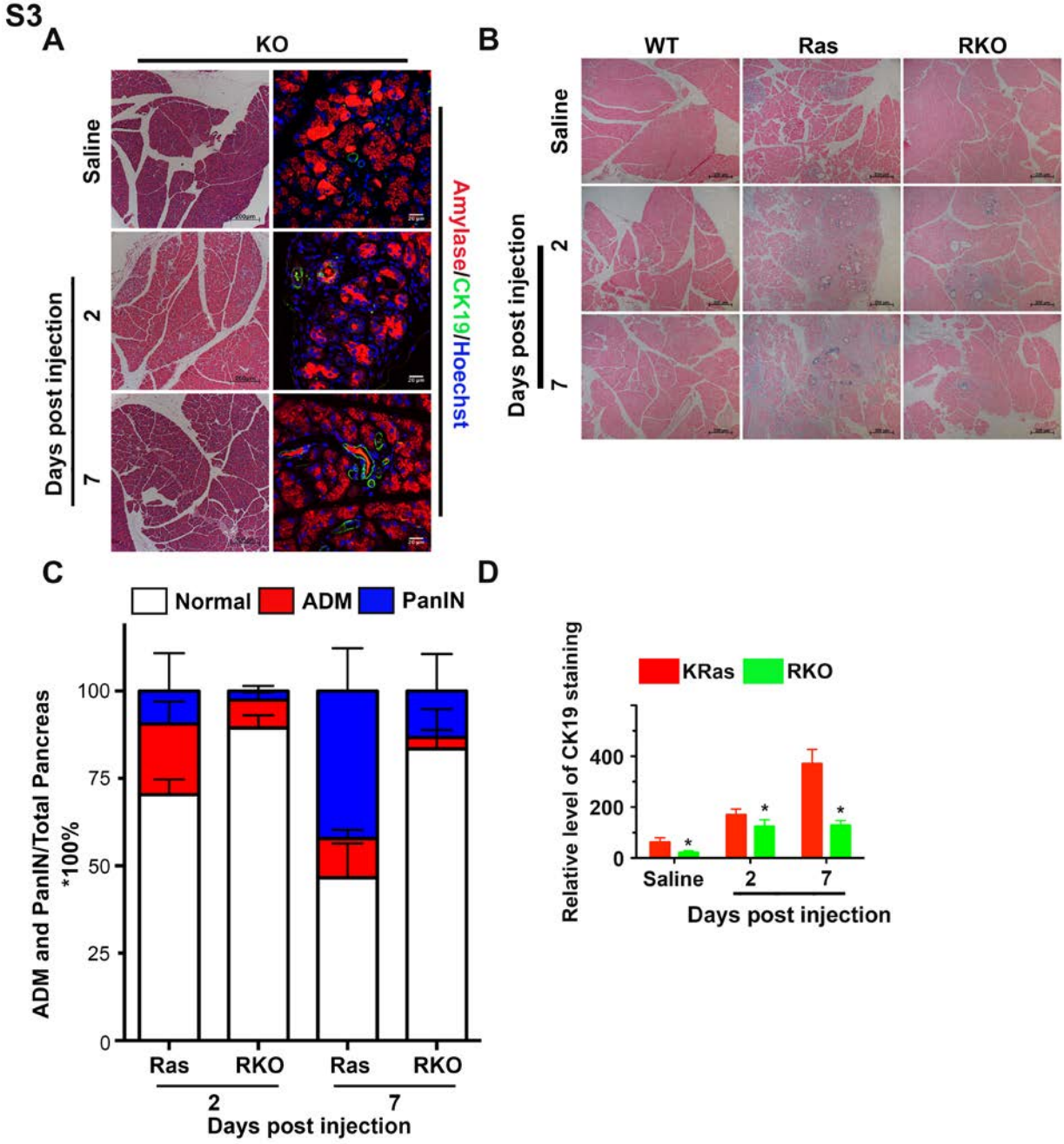
S1



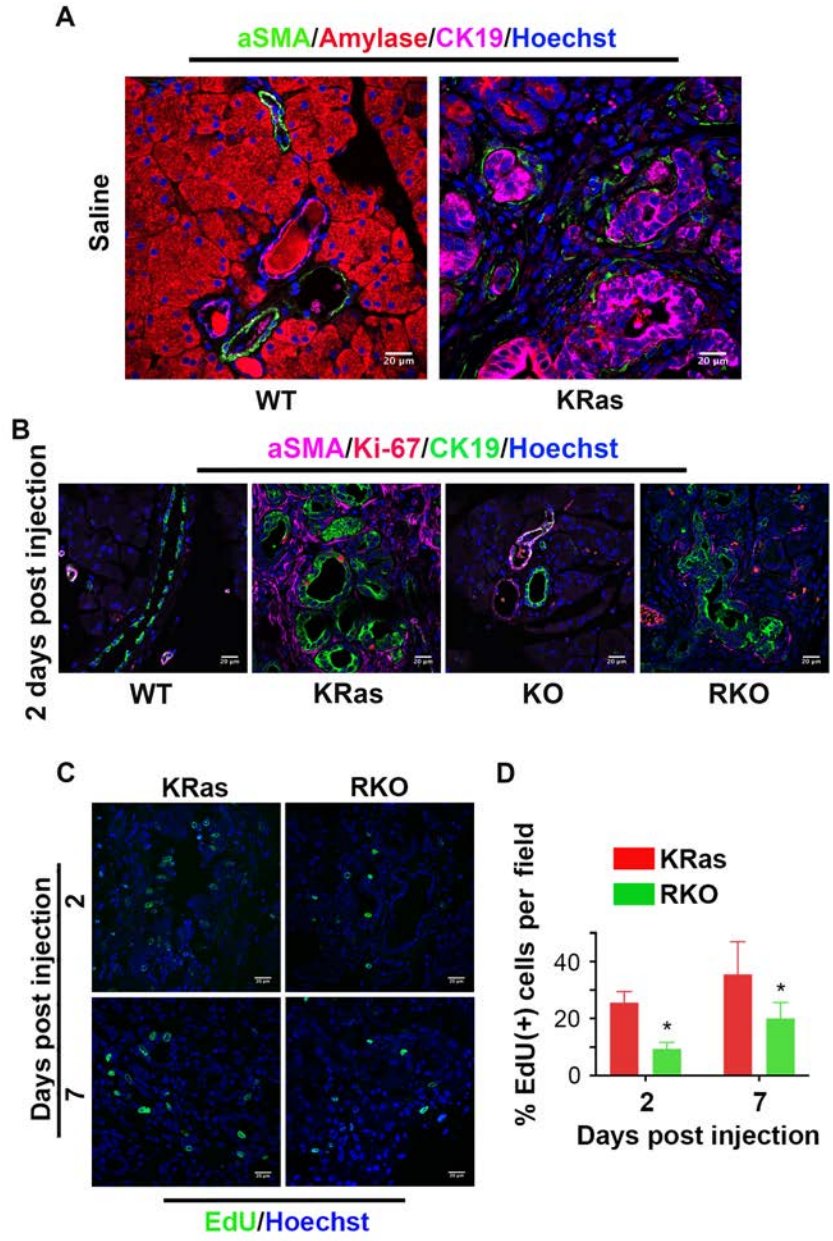
S2


A
WT
Ras
KO
RKO
Saline
2
7
Days post injection
Beta-catenin/Hoechst
B
WT
KRas
KO
RKO
CCND1
Myc
Fold Change
2.0
1.0
0
Saline
2
7
Days post injection

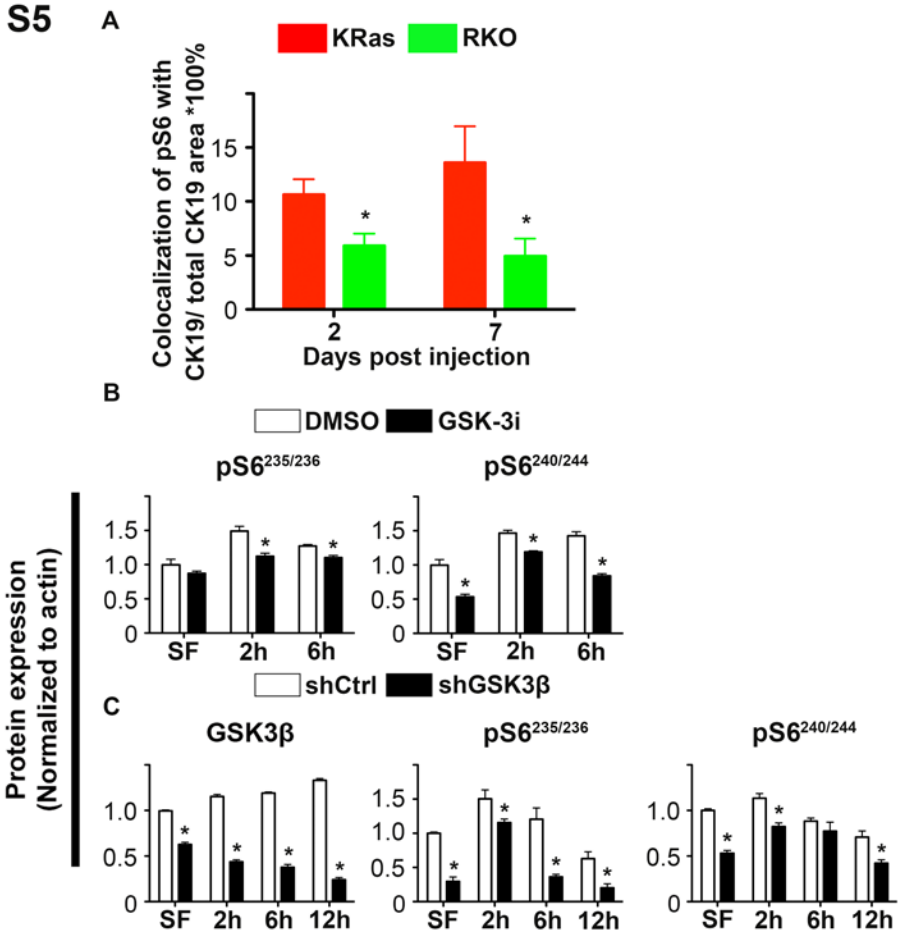
S3


A
KO
Saline
Days post injection
2
7
Amylase/CK19/Hoechst
B
WT
Ras
RKO
Saline
Days post injection
2
7
C
Normal
ADM
PanIN
ADM and PanIN/Total Pancreas *100%
100
75
50
25
0
Ras
RKO
Ras
RKO
2
7
Days post injection
D
KRas
RKO
Relative level of CK19 staining
400
200
0
*
*
*
Saline
2
7
Days post injection

S4


A
aSMA/Amylase/CK19/Hoechst
Saline
20 μm
WT
KRas
B
aSMA/Ki-67/CK19/Hoechst
2 days post injection
WT
KRas
KO
RKO
C
KRas
RKO
Days post injection
2
7
EdU/Hoechst
D
KRas
RKO
% EdU(+) cells per field
40
20
0
*
*
2
7
Days post injection

S5


A
KRas
RKO
Colocalization of pS6 with CK19/ total CK19 area *100%
15
10
5
0
2
7
Days post injection
B
DMSO
GSK-3i
pS6235/236
pS6240/244
Protein expression (Normalized to actin)
1.5
1.0
0.5
0
SF
2h
6h
shCtrl
shGSK3β
C
GSK3β
pS6235/236
pS6240/244
12h