INVENTORY OF SUPPLEMENTAL INFORMATION

Figure S5, related to Figure 6. Senescence markers reappear upon partial recovery from pancreatitis-induced injury.

Figure S6, related to Figure 7. Inhibition of the inflammatory response by Sulindac reverts tissue damage and delays progression of mPanIN lesions.

Figure S7, related to Figure 8. Lack of P16INK4a immunostaining in human low-grade PanINs of patients not treated with anti-inflammatory drugs.

SUPPLEMENTAL TABLE

Table S1, related to Figure 8. Clinical histories of chronic pancreatitis patients whose biopsies have been characterized in this study.

SUPPLEMENTAL INFORMATION

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Mouse treatments

Doxycycline (2 mg/ml, Sigma) was provided in the drinking water as a sucrose (5% w/v) solution to pregnant mothers (from the time of conception) and to their offspring until the time required to allow expression of the Cre recombinase. Pancreatitis was induced by daily (5 days per week) intraperitoneal injections of caerulein (125 μg/Kg, Sigma). Sulindac (0.18 mg/ml, Sigma) was provided in the drinking water for the indicated time.

Senescence-associated β-galactosidase staining

SA-β-Gal staining was performed essentially as described (Collado et al., 2005) on 10 μm thick cryosections using a commercial kit (Cell Signaling Technology), following the manufacturer's instructions.

Laser capture microdissection

Cells were obtained from pancreas cryosections (10 μm) stained with X-Gal to identify K-Ras^{G12V} expressing cells. Slides were counterstained with NFR (Nuclear Fast Red) for 1 minute, as previously described (Guerra et al., 2003). After dehydratation 7,000 to 10,000 blue cells (K-Ras^{G12V} expressing cells) and white cells (K-Ras^{G12V} negative cells) were isolated by laser capture microdissection using a PALM microbeam Zeiss Axio Observer (Carl Zeiss).

PCR analysis

Cells were obtained from pancreas cryosections (10 μm) stained with X-Gal to identify

K-Ras^{G12V} expressing cells. Slides were counterstained with NFR (Nuclear Fast Red) for 1 minute, as previously described (Guerra et al., 2003). After dehydration by two successive washes in 70% and in 98% ethanol, 7,000 to 10,000 blue cells (K-Ras^{G12V} expressing cells) and white cells (K-Ras^{G12V} negative cells) were isolated by laser capture microdissection using a PALM microbeam Zeiss Axio Observer (Carl Zeiss). The cap containing captured cells was mix with NID buffer (50mM KCl, 10mM TrisHCl pH 7.5, 2mM $MgCl_2$, 0,45% NP40, 0,45% Tween 20) overnight at 55ºC. Samples were then incubated in a boiling water bath for 10 minutes to inactivate the proteinase K. After cooling, samples were subjected to PCR in a total volume of 25µl. The reaction mixture consisted of 1mM primers, 2mM Mg, and 0.5 UI Taq polimerase. Cycling conditions for p16Ink4a: 94ºC for 5 minutes, and 30 cycles: 94ºC 30 seconds, 59ºC 30 seconds, 72ºC 50 seconds, and extension at 72ºC for 10 minutes. The primers used for detection of exon 2 and 3 deletion of p16Ink4a were: INK4aΔF: '5-TTGGGAGGCACACTTTCTTGG-3' and INK4aΔR: '5-ACGTGTATGCCACCCTGACC-3', that give a 280 bp fragment for the deleted allele. The primers used for detection of presence 3' loxP site inserted in Csp451 site, 3´ of third exon were: INK4aloxF: '5-CCTGACTATGGTAGTAAAGTGG-3' and INK4aloxR: '5-ACGTGTATGCCACCCTGACC-3'. PCR amplification with the INK4aloxF and INK4aloxR give a product of 390 bp and 290 bp for the floxed and wild type alleles, respectively. Cycling conditions for Trp53: 94ºC for 2 minutes, and 30 cycles: 94ºC 30 seconds, 58ºC 30 seconds, 72ºC 50 seconds, and extension at 72ºC for 5 minutes. We detected Trp53$^{F2\text{-}10}$ allele by PCR amplification of the loxP site in intron 1, yielding products of 370 bp and 288 bp for the floxed and wild type alleles, respectively. Detection of the Trp53$^{\Delta 2\text{-}10}$ allele yielded a 612 bp fragment. The primers used for detections of loxP site in intron 1 were: 1F: '5-

CACAAAAACAGGTTAAACCCAG-3' and 1R '5-AGCACATAGGAGGCAGAGAC-3'. The primers used for detection of the $Trp53^{\Delta 2\text{-}10}$ allele were: 1F: '5-CACAAAAACAGGTTAAACCCAG-3' and 10R: '5-GAAGACAGAAAAGGGGAGGG-3'.

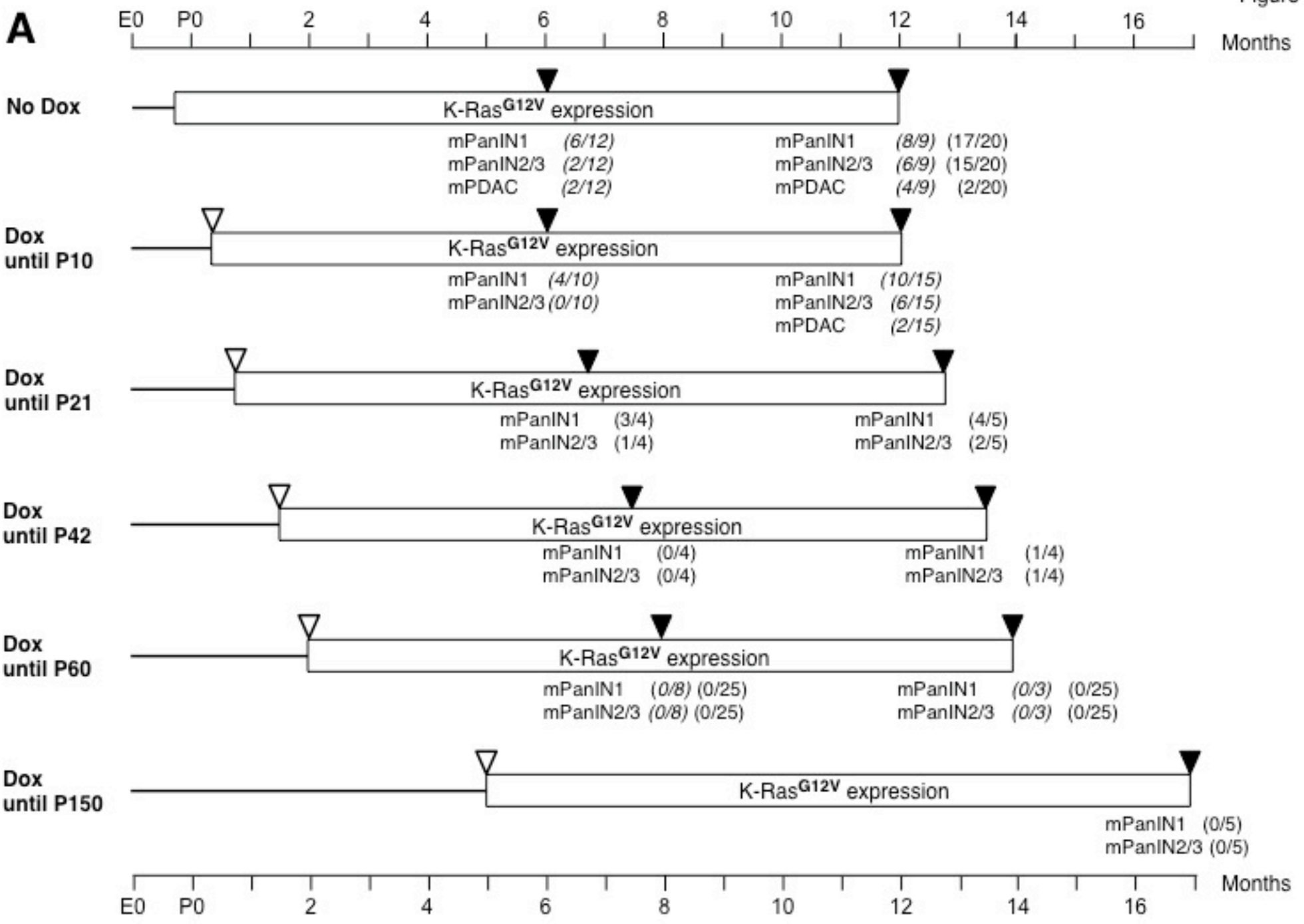
Human Samples

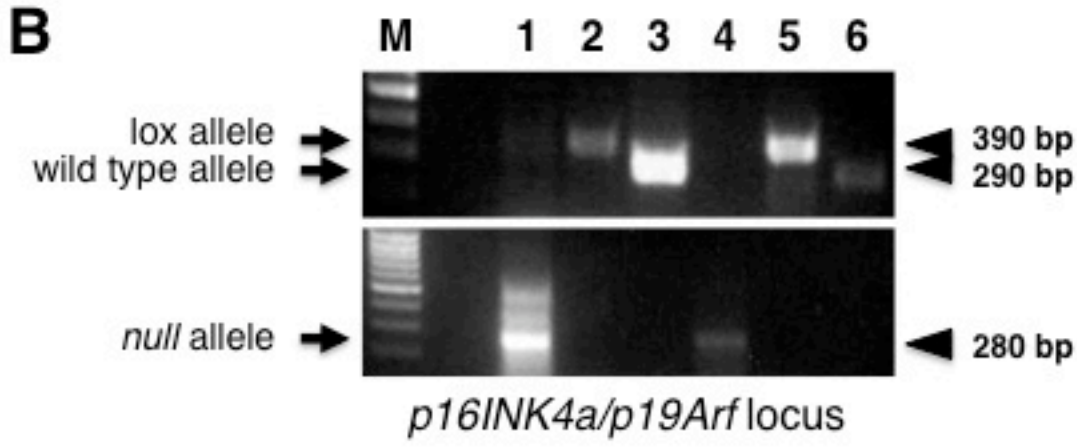
Medical records regarding preoperative treatment with anti-inflammatory drugs were retrieved for patients with chronic pancreatitis. The mean age in the adenocarcinoma group was 69 years (range 35-88), 22 men and 14 women; and mean age in the chronic pancreatitis group was 63 years (range 25-79), 6 men and 5 female. Four patients with chronic pancreatitis received anti-inflammatory drugs preoperatively. Two patients were treated with 30 mg prednisolone/4 months, with a suspicion diagnosis of autoimmune pancreatitis; and 2 patients received NSAIDs (non-steroidal anti-inflammatory drugs, regimen and duration unknown). H&E-stained slides from 47 pancreas surgical specimens (36 from ductal adenocarcinoma and 11 from chronic pancreatitis) were screened by light microscopy for the presence of low-grade PanINs (1A and 1B) according to the criteria proposed by Hruban et al (Hruban et al., 2001). A median of 10 slides containing pancreatic tissue were available from the chronic pancreatitis group and a median of 14 slides from the adenocarcinoma group. Close sections for each of the samples were evaluated for the presence of senescence by immunolabelling with P16INK4a and Ki67 antibodies.

SUPPLEMENTAL REFERENCE

Hruban, R.H., Adsay, N.V., Albores-Saavedra, J., Compton, C., Garrett, E.S., Goodman, S.N., Kern, S.E., Klimstra, D.S., Klöppel, G., Longnecker, D.S., Lüttges, J., and Offerhaus, G.J. (2001). Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions. Am J Surg Pathol. *25*, 579-586.

Figure S1


A
E0
P0
2
4
6
8
10
12
14
16
Months
No Dox
K-Ras^{G12V} expression
mPanIN1 (6/12)
mPanIN2/3 (2/12)
mPDAC (2/12)
mPanIN1 (8/9) (17/20)
mPanIN2/3 (6/9) (15/20)
mPDAC (4/9) (2/20)
Dox until P10
K-Ras^{G12V} expression
mPanIN1 (4/10)
mPanIN2/3 (0/10)
mPanIN1 (10/15)
mPanIN2/3 (6/15)
mPDAC (2/15)
Dox until P21
K-Ras^{G12V} expression
mPanIN1 (3/4)
mPanIN2/3 (1/4)
mPanIN1 (4/5)
mPanIN2/3 (2/5)
Dox until P42
K-Ras^{G12V} expression
mPanIN1 (0/4)
mPanIN2/3 (0/4)
mPanIN1 (1/4)
mPanIN2/3 (1/4)
Dox until P60
K-Ras^{G12V} expression
mPanIN1 (0/8) (0/25)
mPanIN2/3 (0/8) (0/25)
mPanIN1 (0/3) (0/25)
mPanIN2/3 (0/3) (0/25)
Dox until P150
K-Ras^{G12V} expression
mPanIN1 (0/5)
mPanIN2/3 (0/5)
Months
E0
P0
2
4
6
8
10
12
14
16


B
M 1 2 3 4 5 6
lox allele
wild type allele
390 bp
290 bp
null allele
280 bp
p16INK4a/p19Arf locus

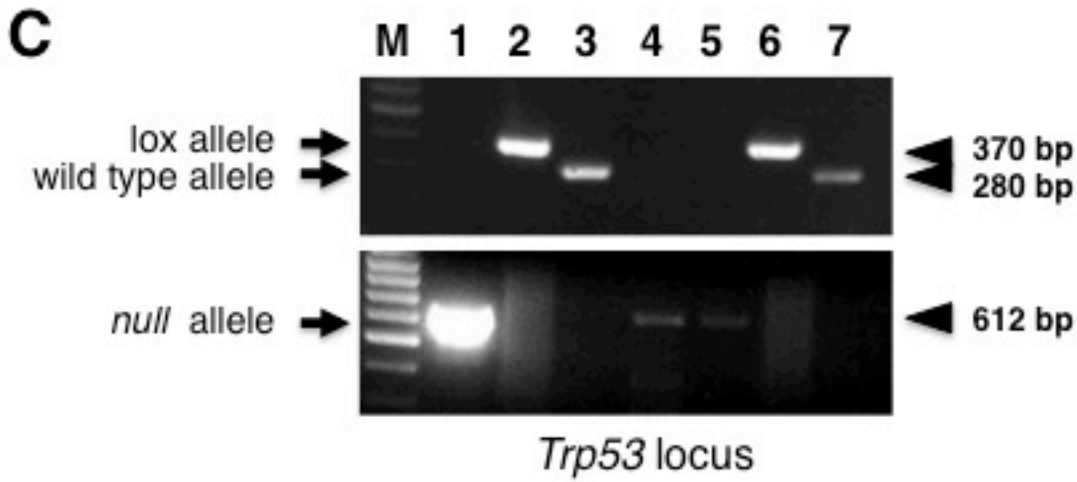

C
M 1 2 3 4 5 6 7
lox allele
wild type allele
370 bp
280 bp
null allele
612 bp
Trp53 locus

Figure S1 related to Figure 1: Adult acinar cells are resistant to multiple oncogenic insults.

(A) Induction of mPanINs and mPDAC depends on the time of expression of a resident K-*Ras* oncogene. The left column indicates the time of exposure of K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre mice to doxycycline (Dox) to prevent K-*Ras*^{G12V} expression. Doxycycline treatment is represented by a thin line. Open arrowheads indicated the end of doxycycline treatment. K-*Ras*^{G12V} expression in indicated by a solid box. Solid arrowheads indicate the time when pancreata was analyzed (six and twelve months after initiation of K-*Ras*^{G12V} expression). Numbers in parenthesis indicate the number of mice positive for low-grade mPanIN1, high-grade mPanIN2/3 and mPDAC versus total number of mice analyzed at the indicated times. Numbers in italics correspond to mice described in Guerra et al., (2007). Numbers in regular typeset indicate mice analyzed in this study. E0 indicates conception and P0 indicates birth.

(B, C). Excision of the conditional *p16Ink4a*/*p19Arf*^{lox} and *Trp53*^{lox} alleles in K-*Ras*^{G12V}-expressing acinar cells.

PCR analysis of DNA extracted from areas of X-Gal positive acinar cells isolated with the help of a laser-capture microscope (see Supplemental Experimental Procedures).

(**B**) Analysis of *p16Ink4a*/*p19Arf* alleles from DNA samples obtained from:

Lane 1: mPanIN cells of K-*Ras*$^{+/G12V}$;p16*Ink4a*/p19*Arf*$^{lox/lox}$;*Elas*-tTA/*tetO*-Cre mice.

Lane 2: Tail tissue of K-*Ras*$^{+/G12V}$;p16*Ink4a*/p19*Arf*$^{lox/lox}$;*Elas*-tTA/*tetO*-Cre mice.

Lane 3: Tail tissue of K-*Ras*$^{+/G12V}$;p16*Ink4a*/p19*Arf*$^{+/+}$;*Elas*-tTA/*tetO*-Cre mice.

Lane 4: X-Gal positive normal acinar cells from K-*Ras*$^{+/G12V}$;p16*Ink4a*/p19*Arf*$^{lox/lox}$;*Elas*-tTA/*tetO*-Cre mice.

Lane 5: X-Gal negative normal acinar cells from K-*Ras*$^{+/G12V}$;p16*Ink4a*/p19*Arf*$^{lox/lox}$;*Elas*-tTA/*tetO*-Cre mice.

Lane 6: X-Gal positive normal acinar cells from K-*Ras*$^{+/G12V}$;p16*Ink4a*/p19*Arf*$^{+/+}$;*Elas*-tTA/*tetO*-Cre mice.

(**C**) Analysis of *Trp53* alleles from DNA samples obtained from:

Lane 1: mPanIN cells of K-*Ras*$^{+/G12V}$;*Trp53*$^{lox/lox}$;*Elas*-tTA/*tetO*-Cre mice.

Lane 2: Tail tissue of K-*Ras*$^{+/G12V}$;*Trp53*;*Elas*-tTA/*tetO*-Cre mice.

Lane 3: Tail tissue of K-*Ras*$^{+/G12V}$;*Trp53*$^{+/+}$;*Elas*-tTA/*tetO*-Cre mice.

Lane 4: mPanIN cells of K-*Ras*$^{+/G12V}$;*Trp53*$^{lox/lox}$;*Elas*-tTA/*tetO*-Cre mice.

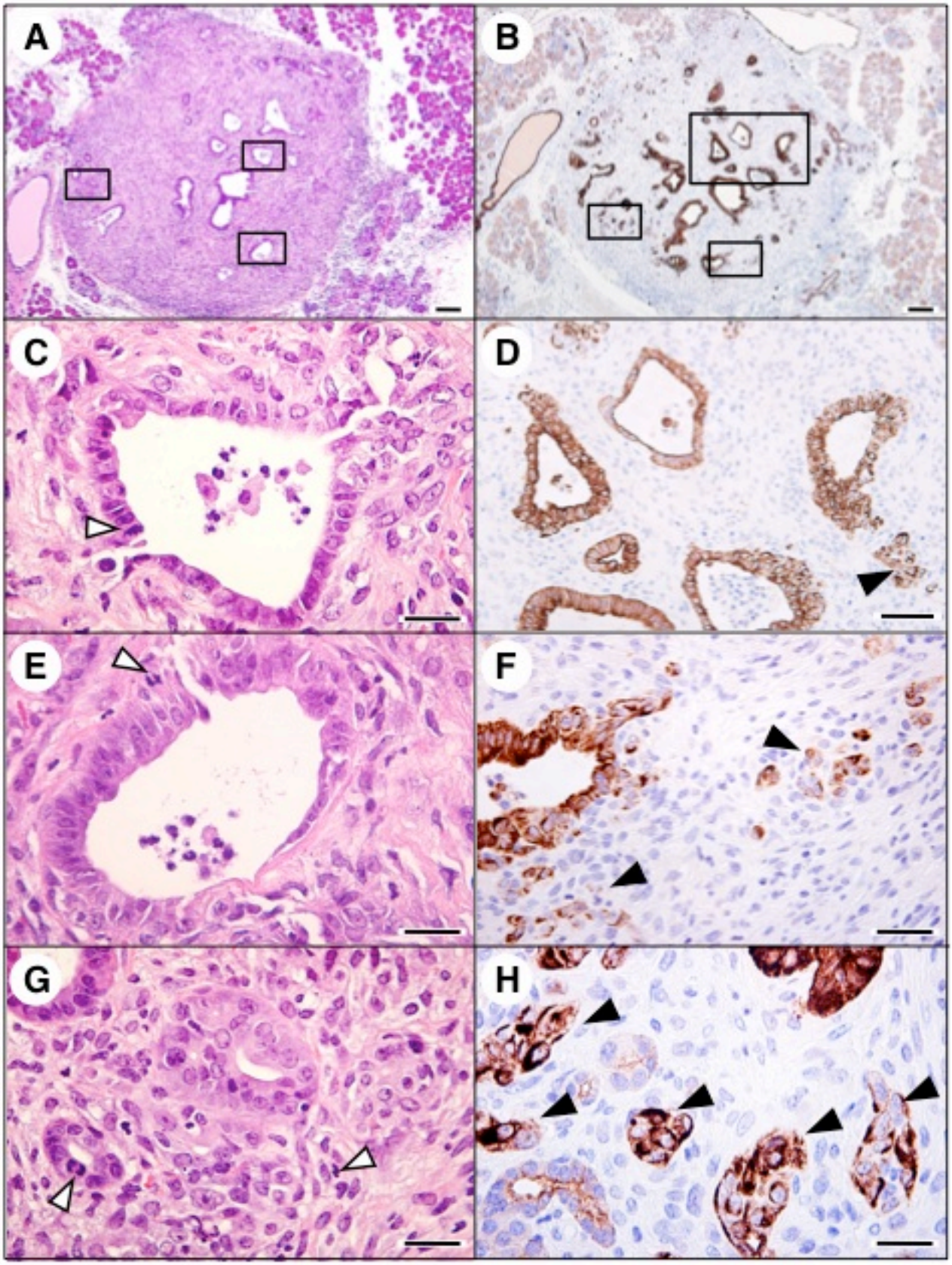
Lane 5: X-Gal positive normal acinar cells from K-*Ras*$^{+/G12V}$;*Trp53*$^{lox/lox}$;*Elas*-tTA/*tetO*-Cre mice.

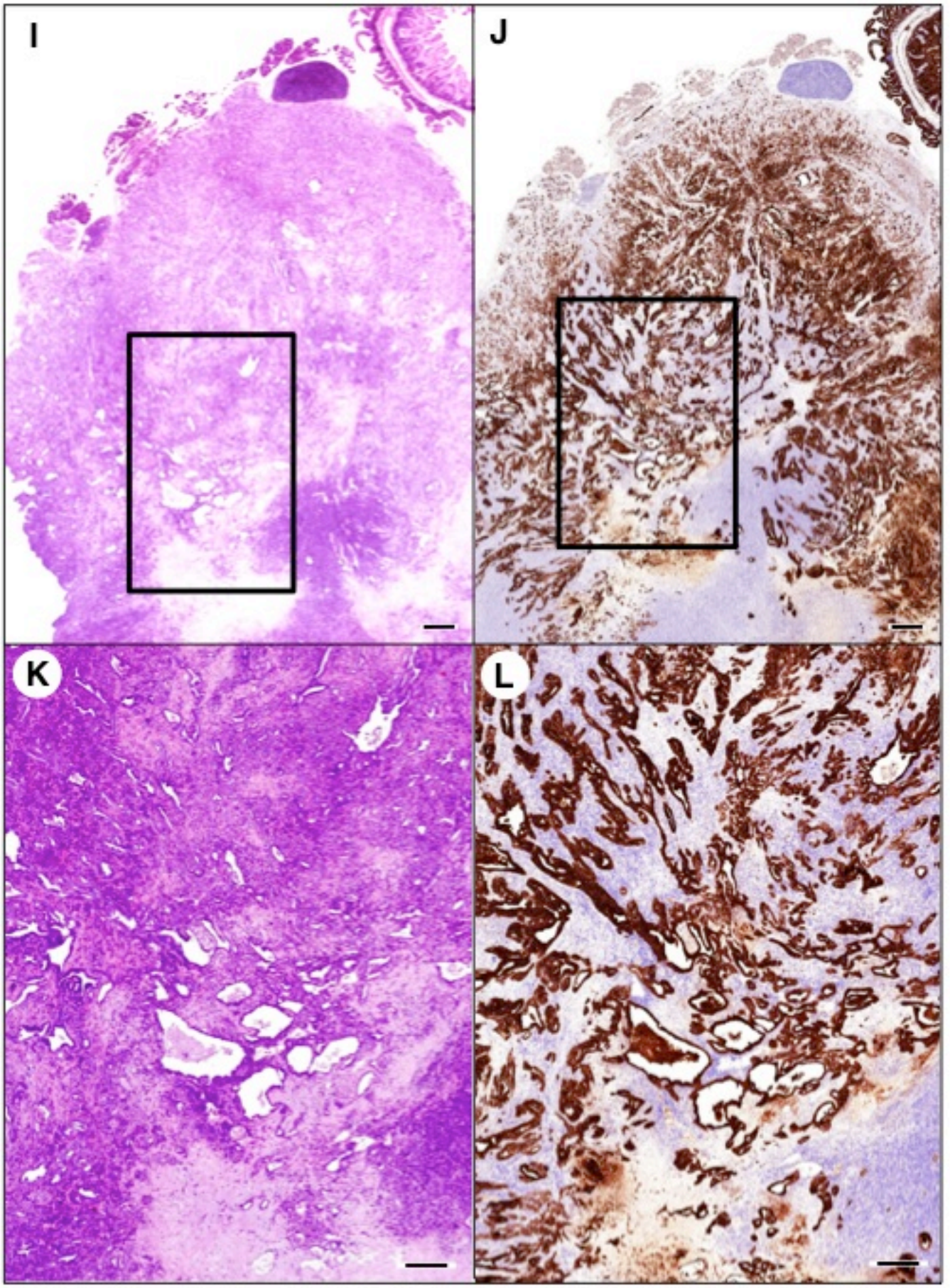
Lane 6: X-Gal negative normal acinar cells from K-*Ras*$^{+/G12V}$;*Trp53*$^{lox/lox}$;*Elas*-tTA/*tetO*-Cre mice.

Lane 7: X-Gal positive normal acinar cells from K-*Ras*$^{+/G12V}$;*Trp53*$^{+/+}$;*Elas*-tTA/*tetO*-Cre mice.

The migration of the amplified DNA bands corresponding to wild type, lox and *null* alleles is indicated by arrows. The size of the DNA bands is indicated by arrowheads.

Lane M indicates molecular weight markers.


A
B
C
D
E
F
G
H


I
J
K
L

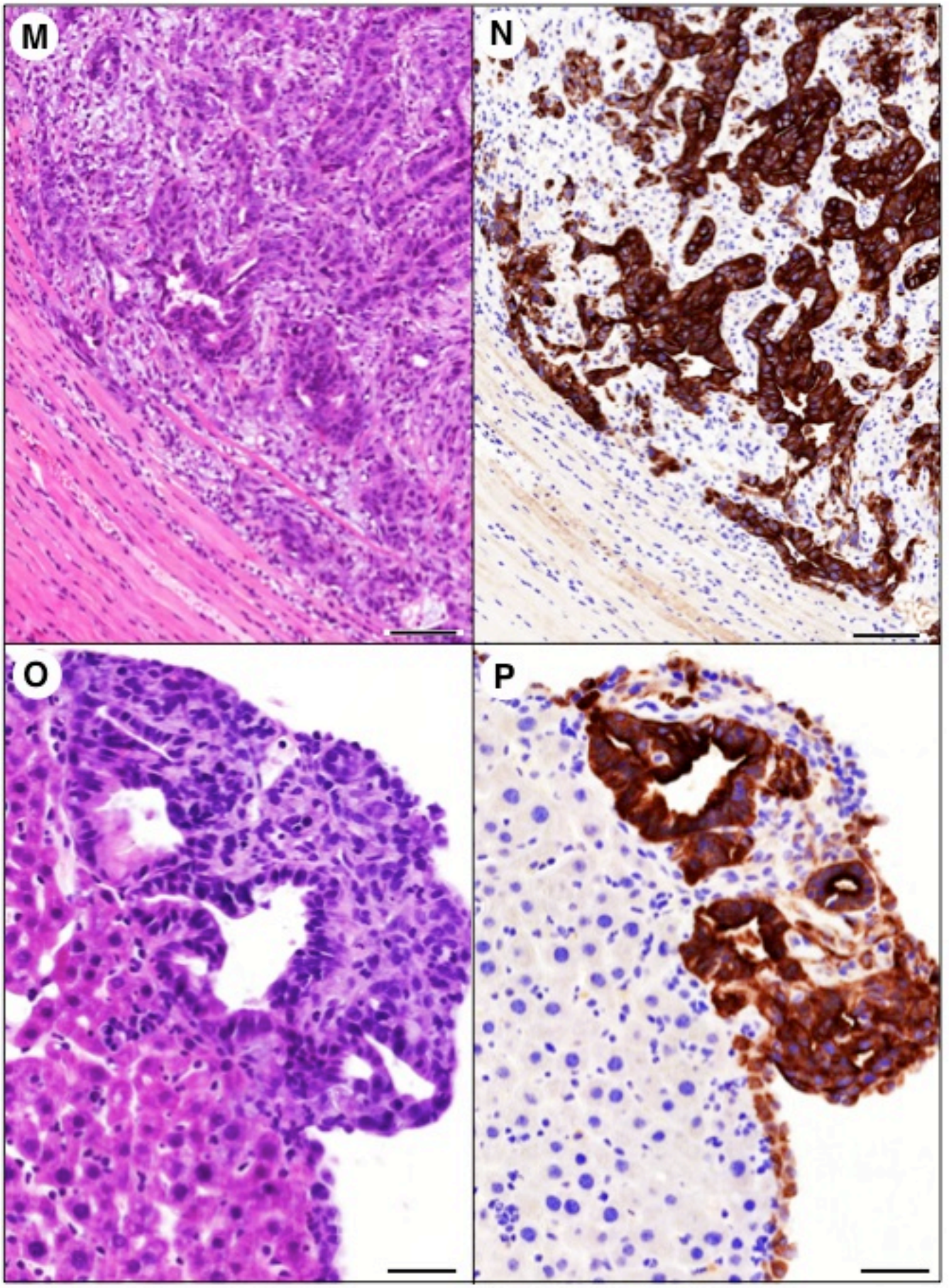

M
N
O
P

Figure S2, related to Figure 2. Loss of *p16Ink4a/p19Arf* locus cooperates with a resident K-Ras^{G12V} oncogene to induce metastatic mPDAC in adult mice.

(**A,B**) H&E and Cytokeratin 19 stained consecutive paraffin sections showing a panoramic view of a moderately differentiated mPDAC present in a six month old K-*Ras*$^{+/G12V}$;*p16Ink4a/p19Arf*$^{lox/lox}$;*Elas*-tTA/*tetO*-Cre adult mouse exposed to doxycycline until P60 and treated with caerulein for three months, from P90 until the animal was sacrificed (P180). Boxes indicate amplified areas depicted below.

(**C,E,G**) H&E stained paraffin sections of the tumoral area marked in **A**. Mitotic figures are indicated by open arrowheads.

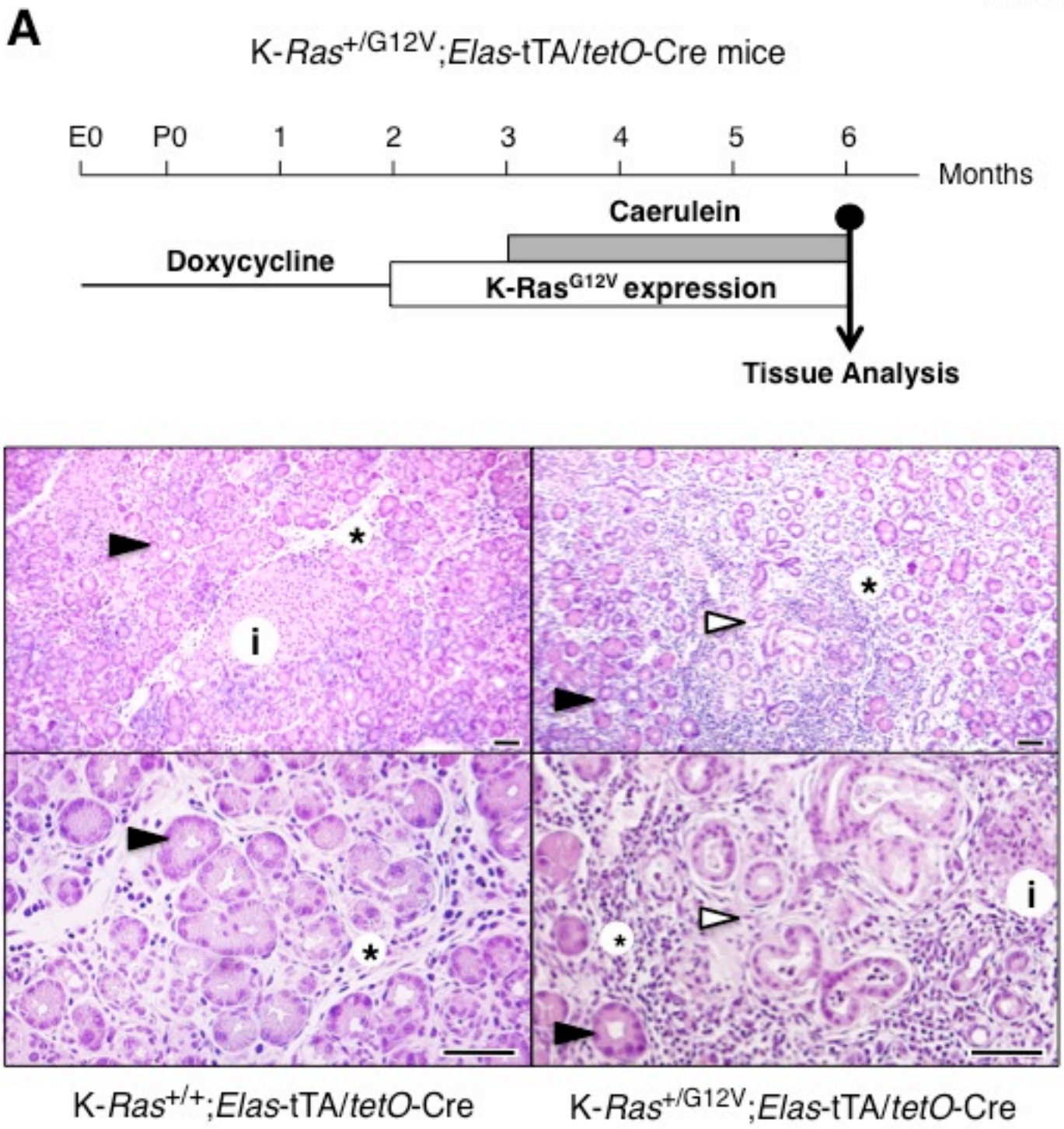
(**D,F,H**) Cytokeratin 19 staining of the areas marked in **B** by open rectangles. Solid arrowheads indicate infiltrating glands.

(**I,J**) H&E and Cytokeratin 19 stained consecutive paraffin sections showing panoramic views of a metastatic mPDAC present in an eleven month old K-*Ras*$^{+/G12V}$;*p16Ink4a/p19Arf*$^{lox/lox}$;*Elas*-tTA/*tetO*-Cre adult mouse exposed to doxycycline until P60 and treated with caerulein. Boxes indicate amplified areas depicted in **K** and **L**, respectively.

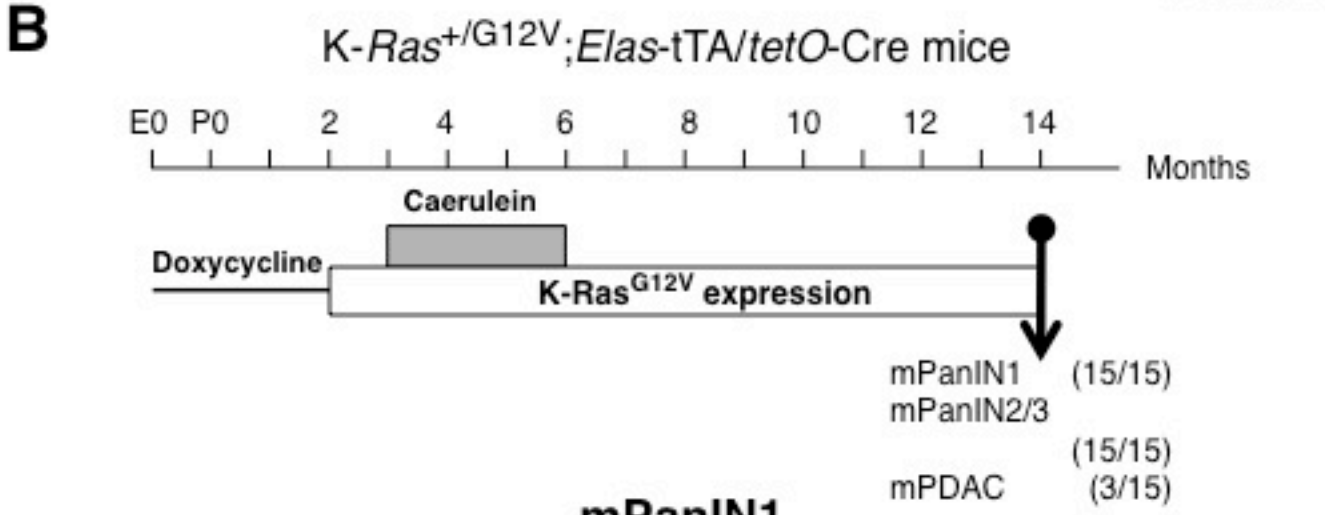
(**M,N**) H&E and Cytokeratin 19 stained consecutive paraffin sections showing a metastasis in the diaphragm derived from the mPDAC shown in I,J.

(**O,P**) H&E and Cytokeratin 19 stained consecutive paraffin sections showing a metastasis in liver derived from the mPDAC shown in I,J.

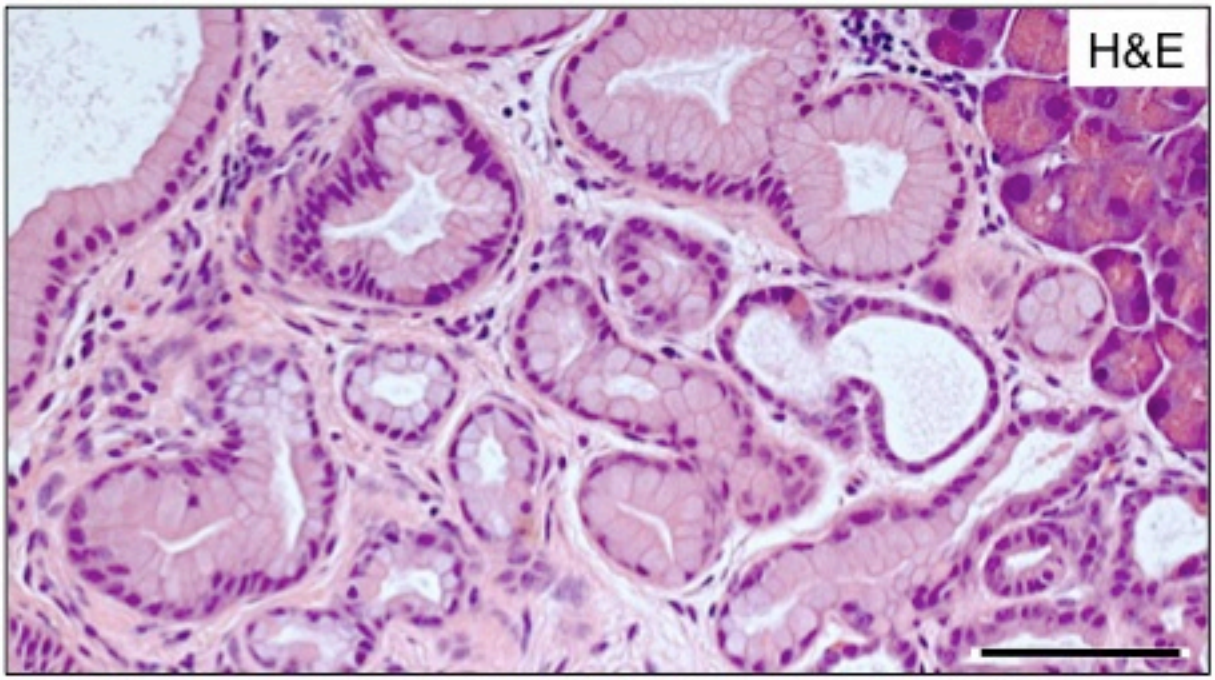
Scale bar represents 20 μm in **C, E, G, H**; 50 μm in **D, F, O, P**; 100 μm in **A, B, M, N**; 250 μm in **K, L**; 500 μm in **I, J** .


A
K-Ras+/G12V;Elas-tTA/tetO-Cre mice
E0
P0
1
2
3
4
5
6
Months
Caerulein
Doxycycline
K-RasG12V expression
Tissue Analysis
i
*
K-Ras+/+;Elas-tTA/tetO-Cre
K-Ras+/G12V;Elas-tTA/tetO-Cre

B



mPanIN1



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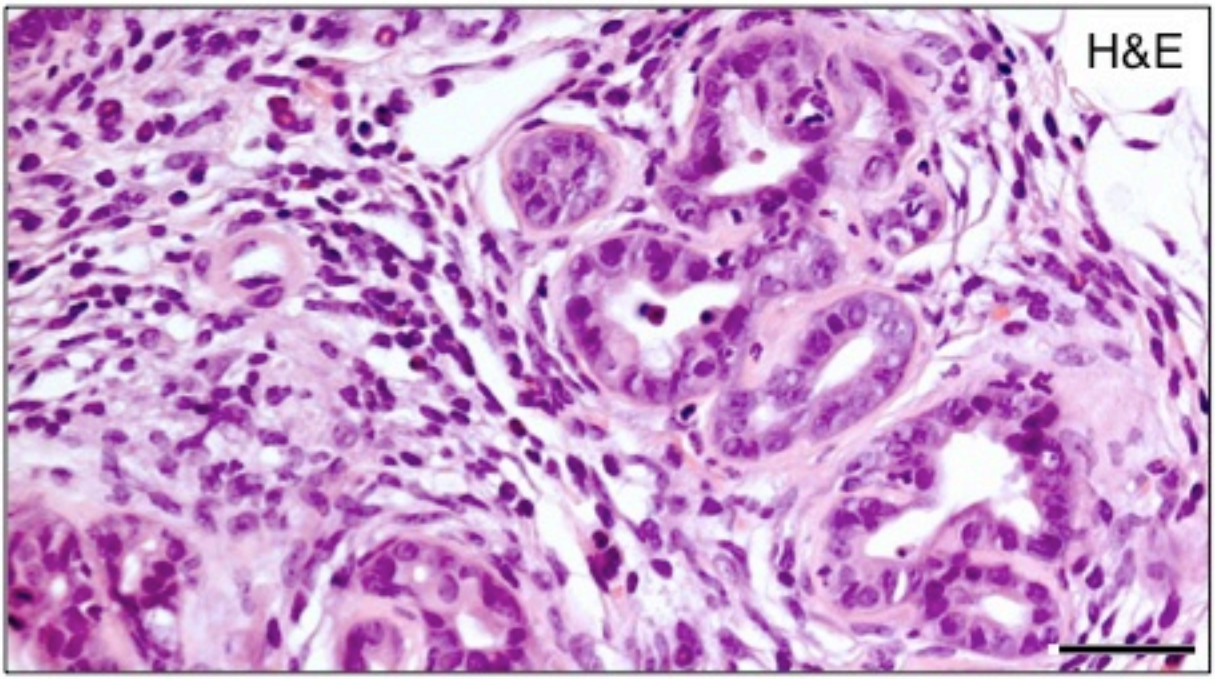
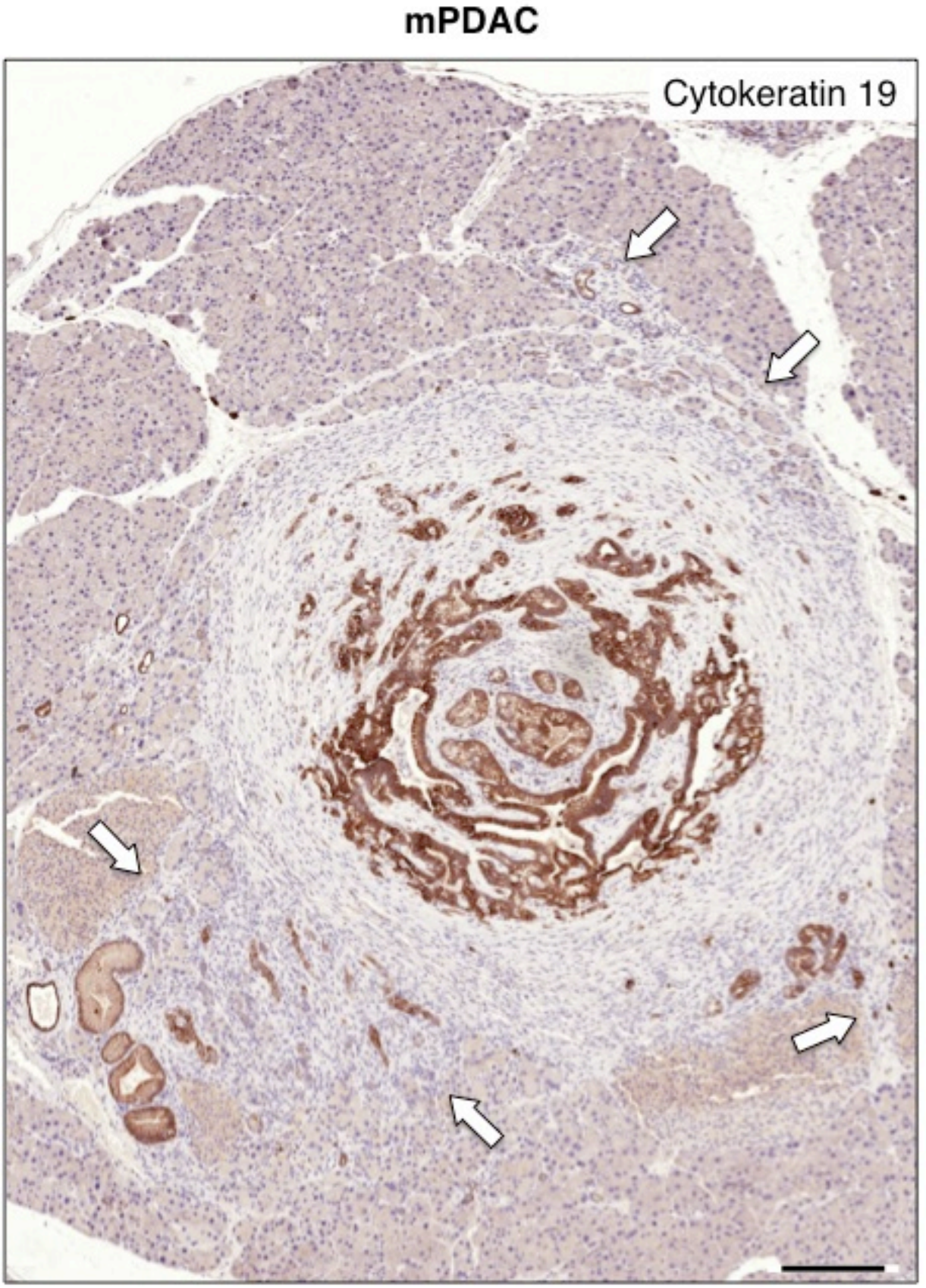


C



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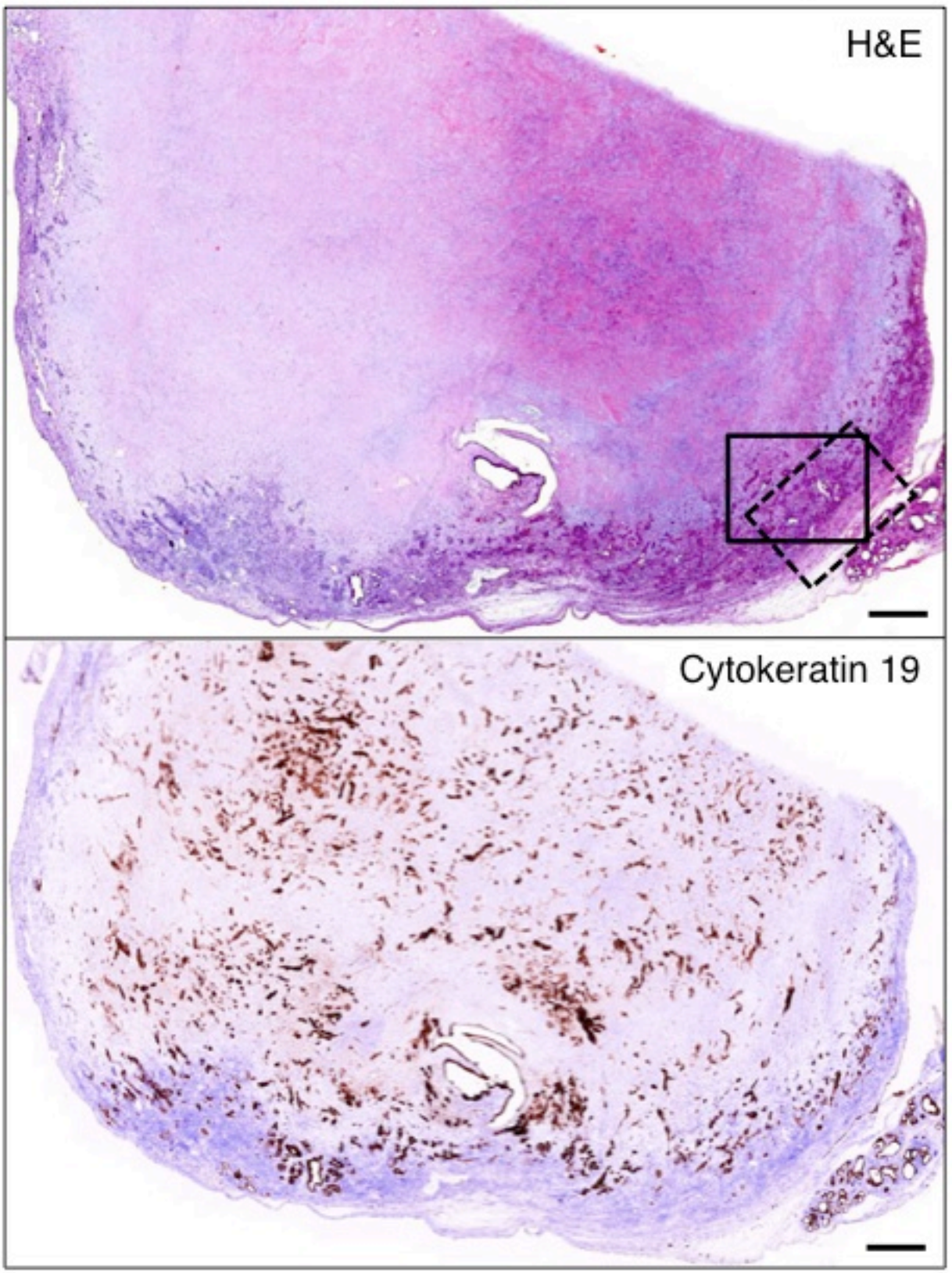

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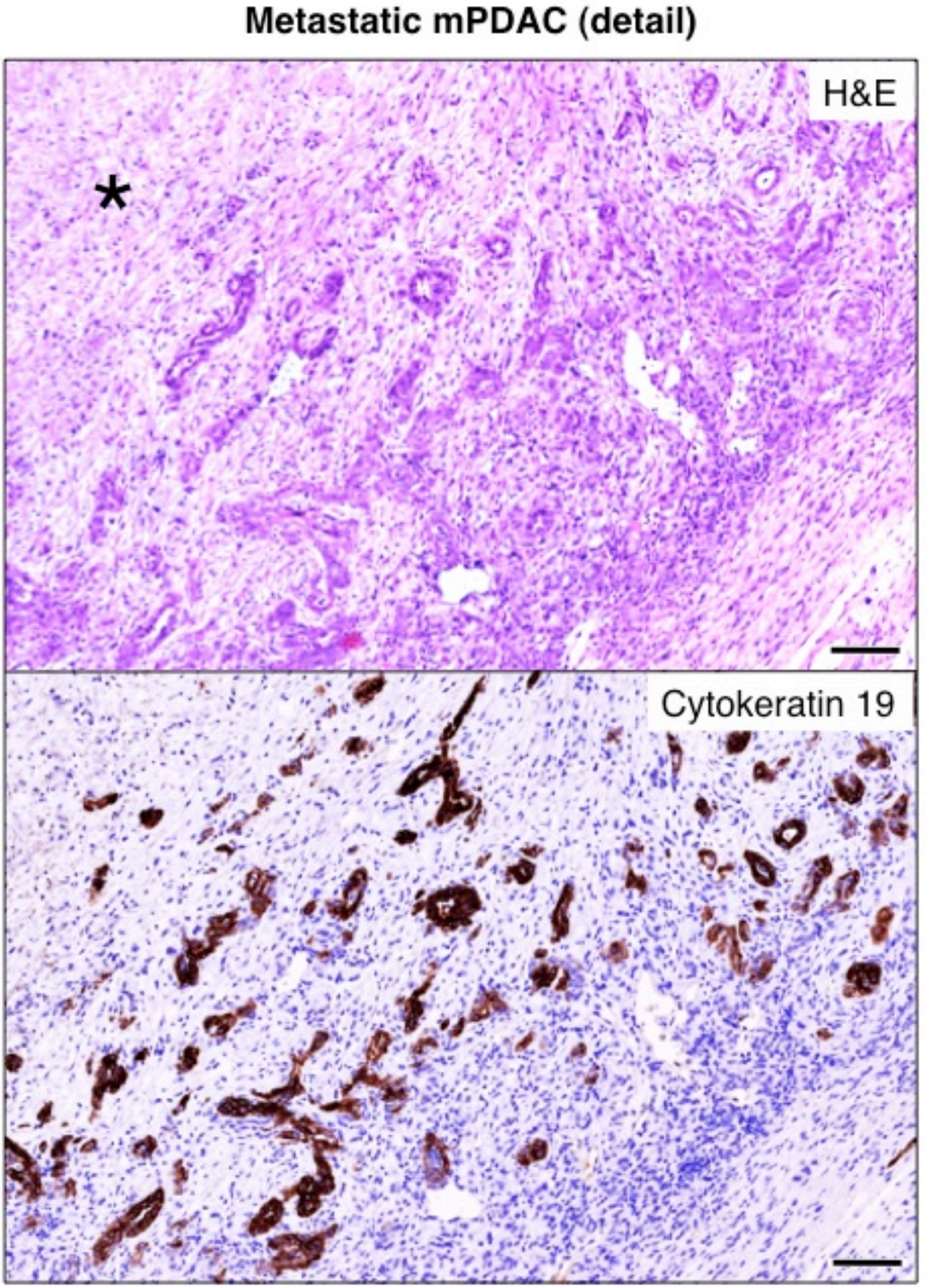
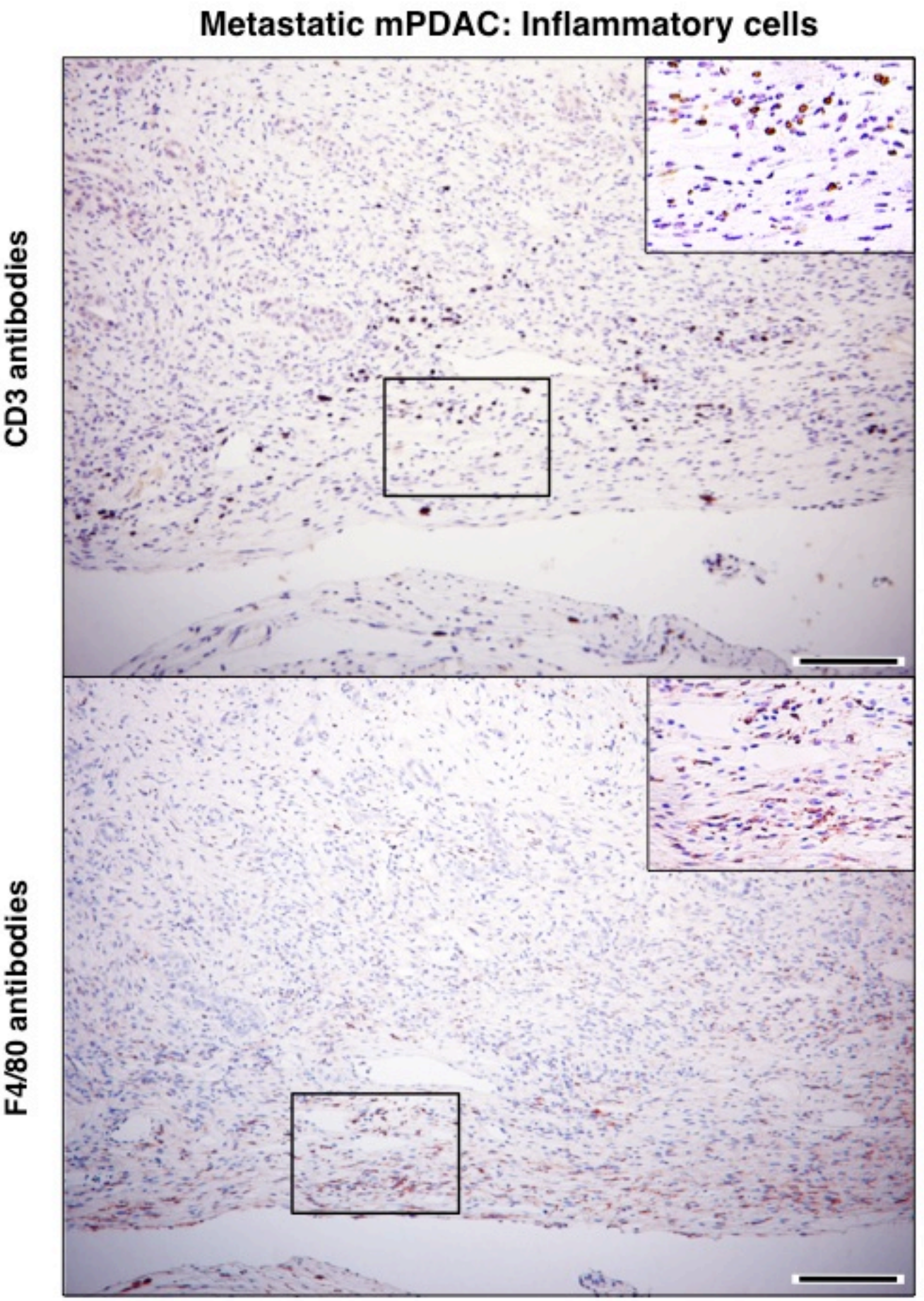
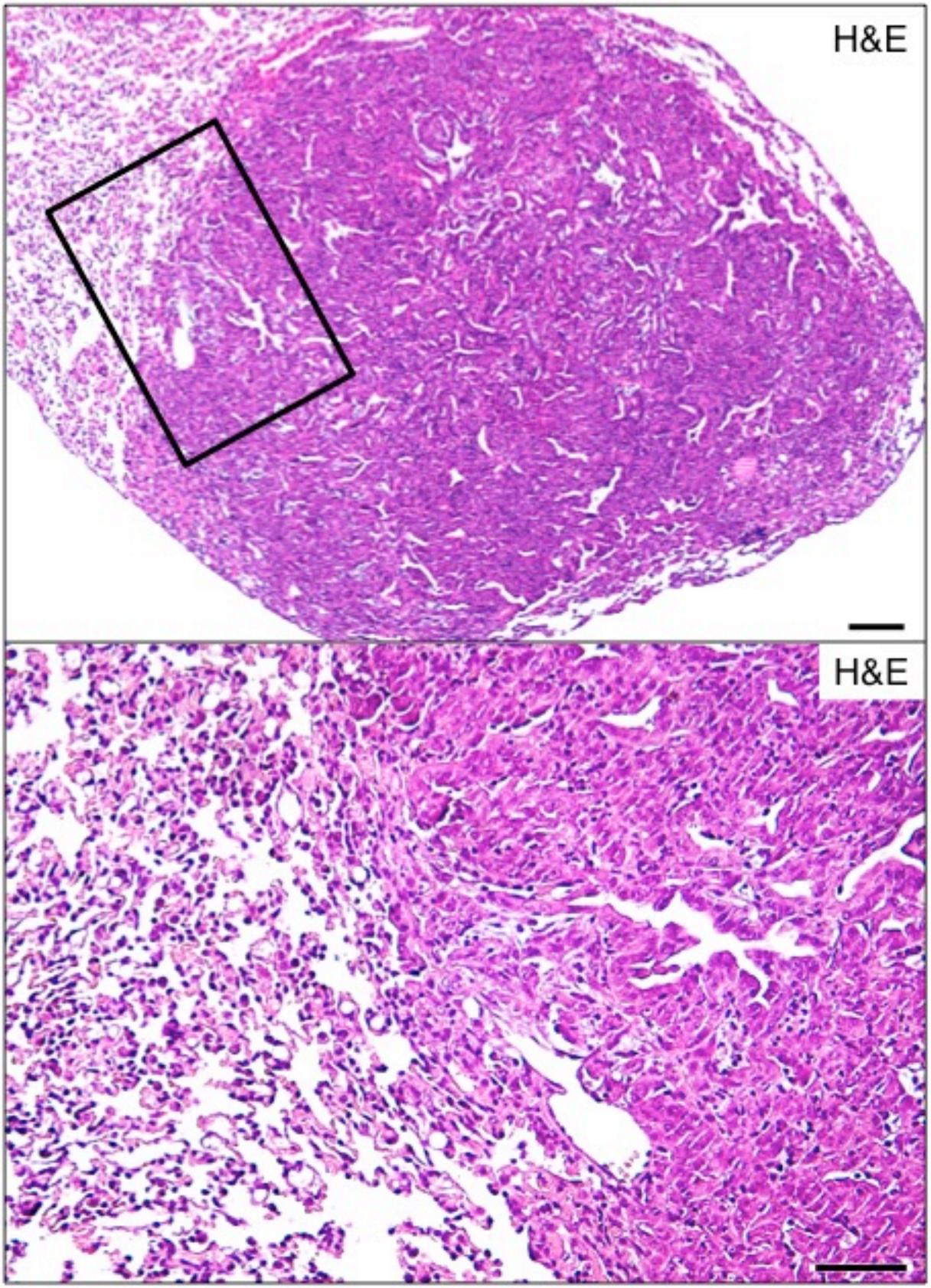


F


Metastatic mPDAC: Inflammatory cells
CD3 antibodies
F4/80 antibodies

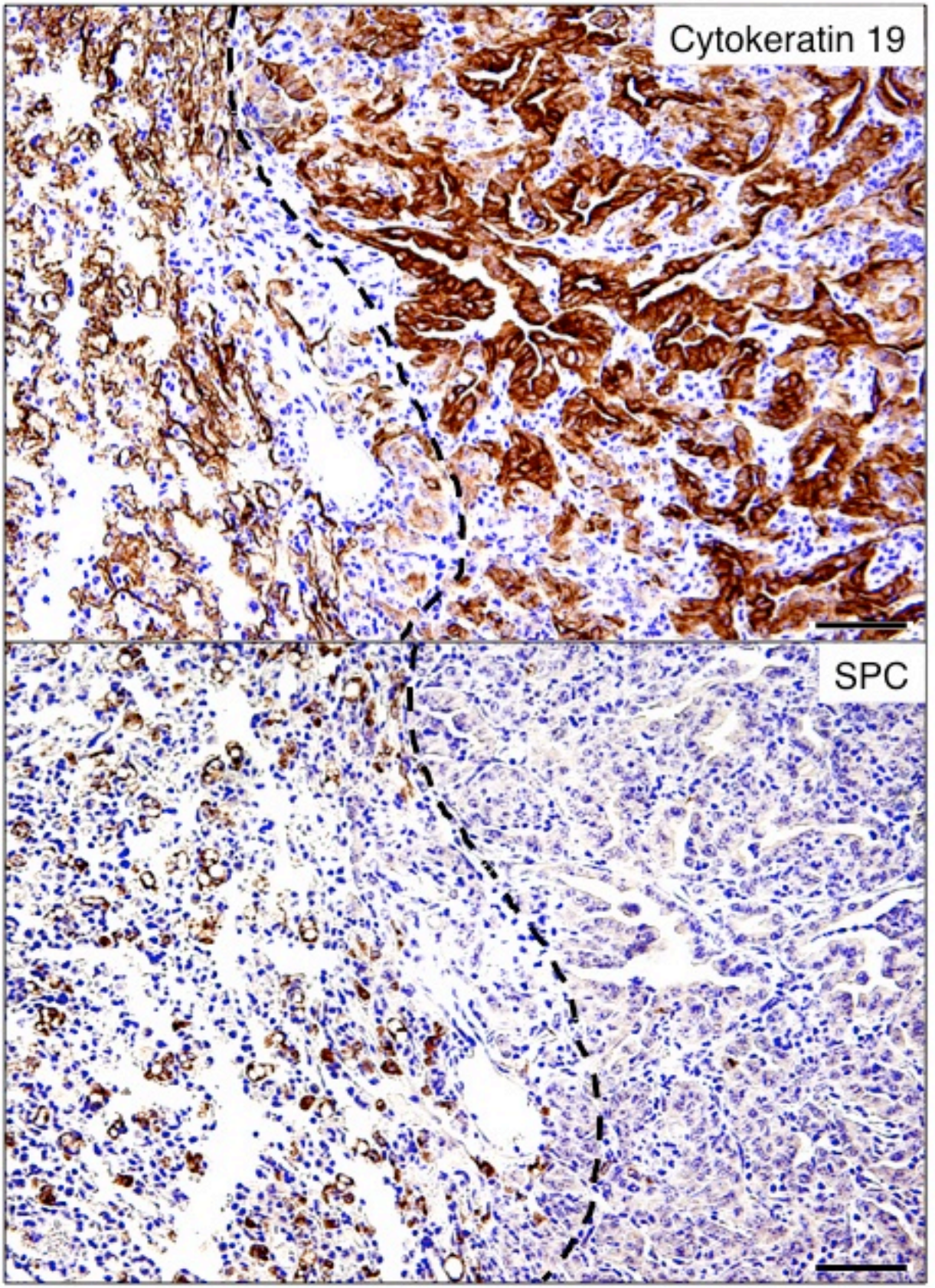
G

Lung Metastasis



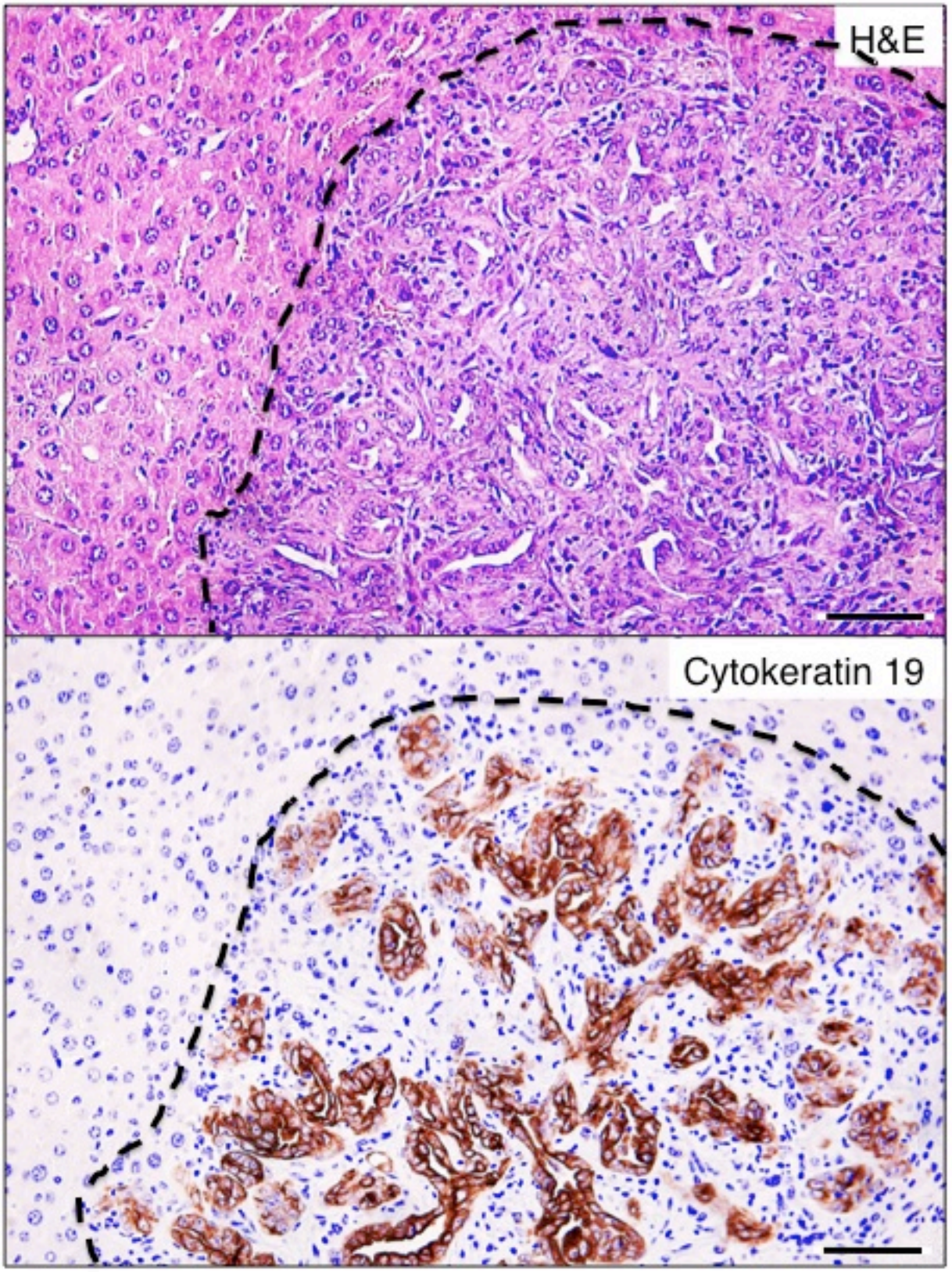
H

Lung Metastasis (detail)



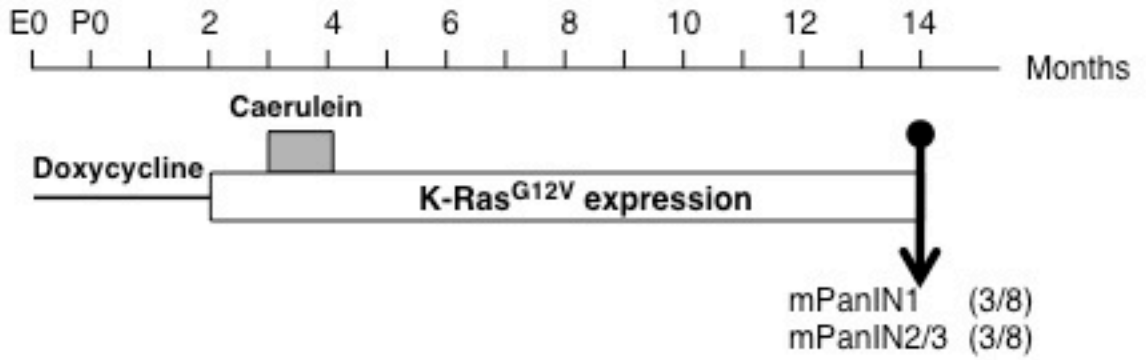
I

Liver Metastasis



J

K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre mice



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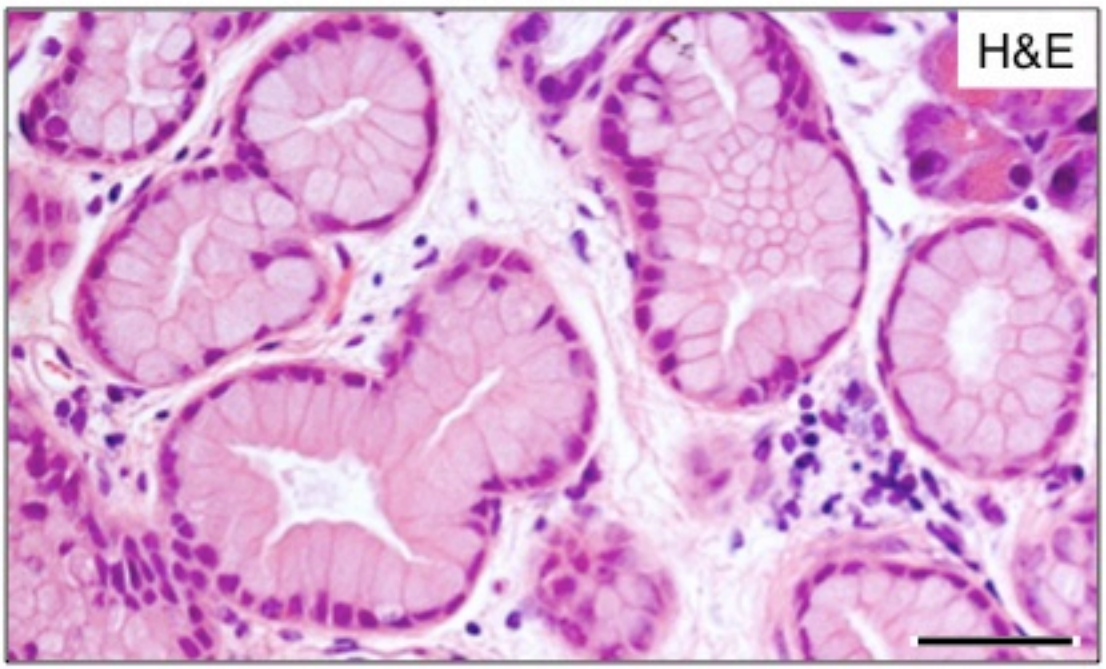

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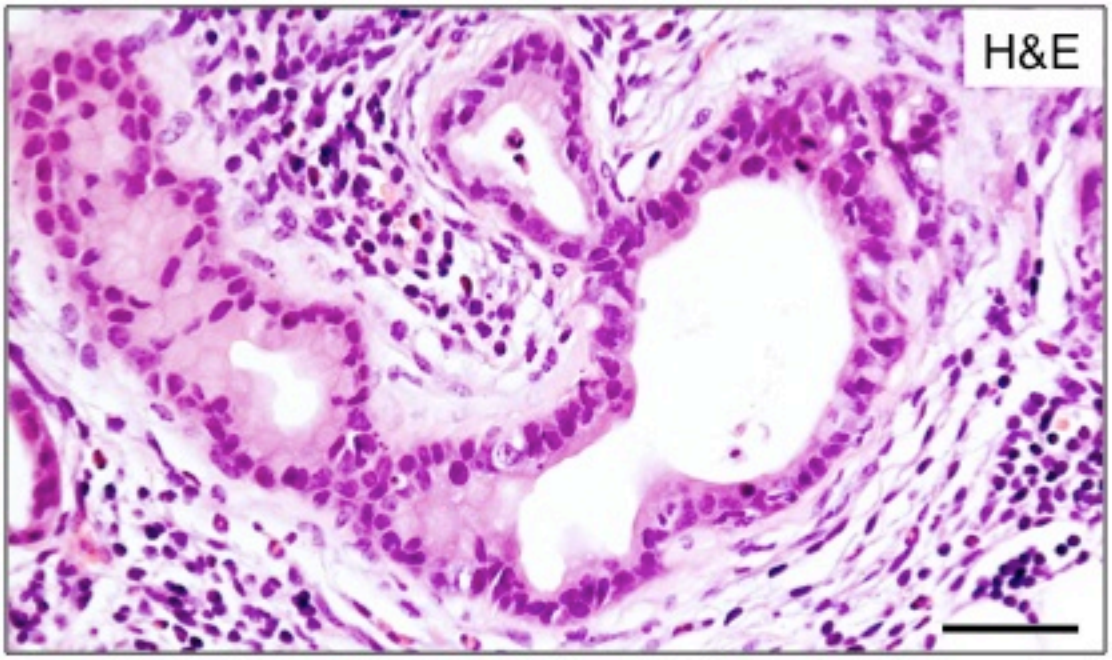

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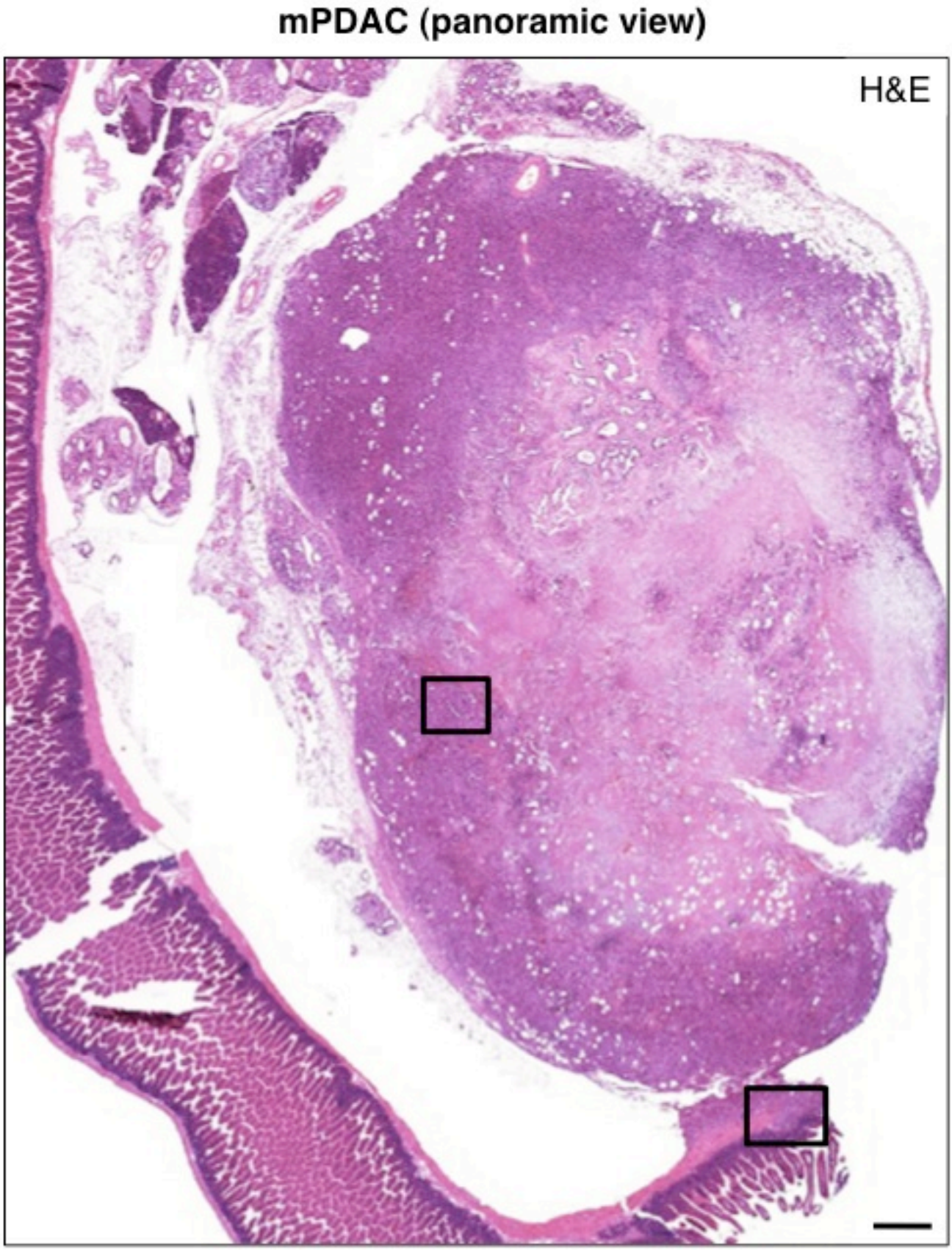

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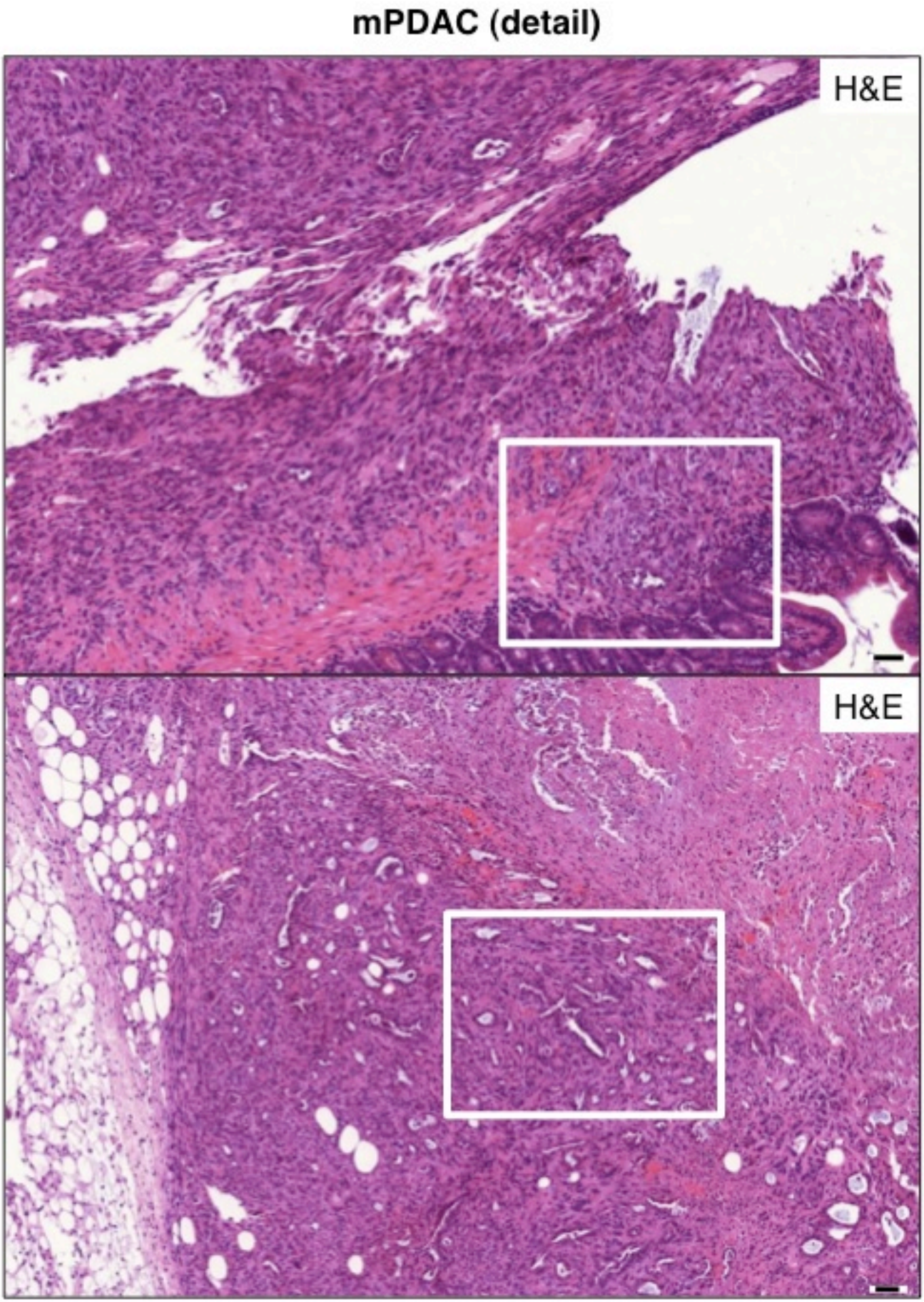
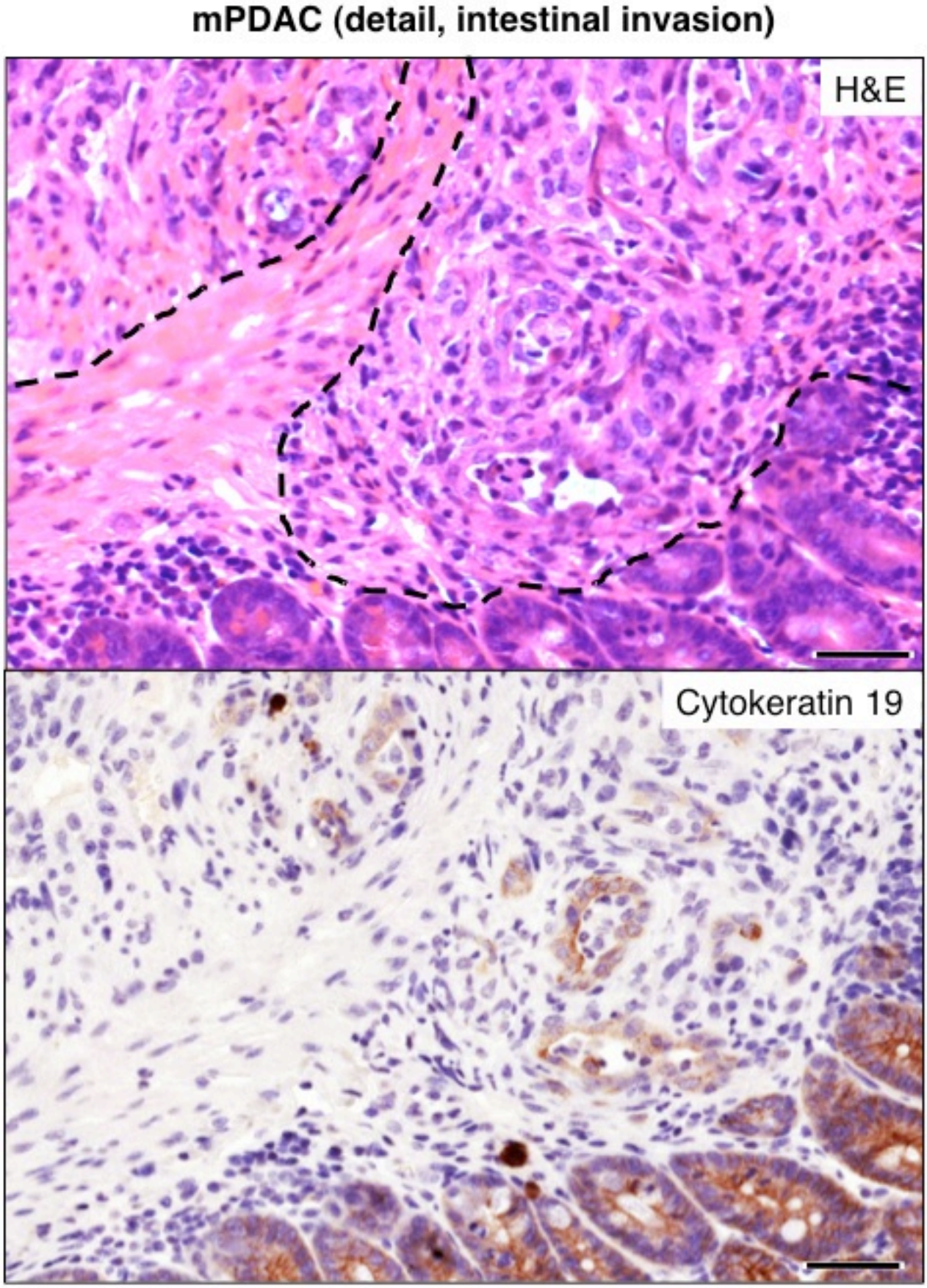
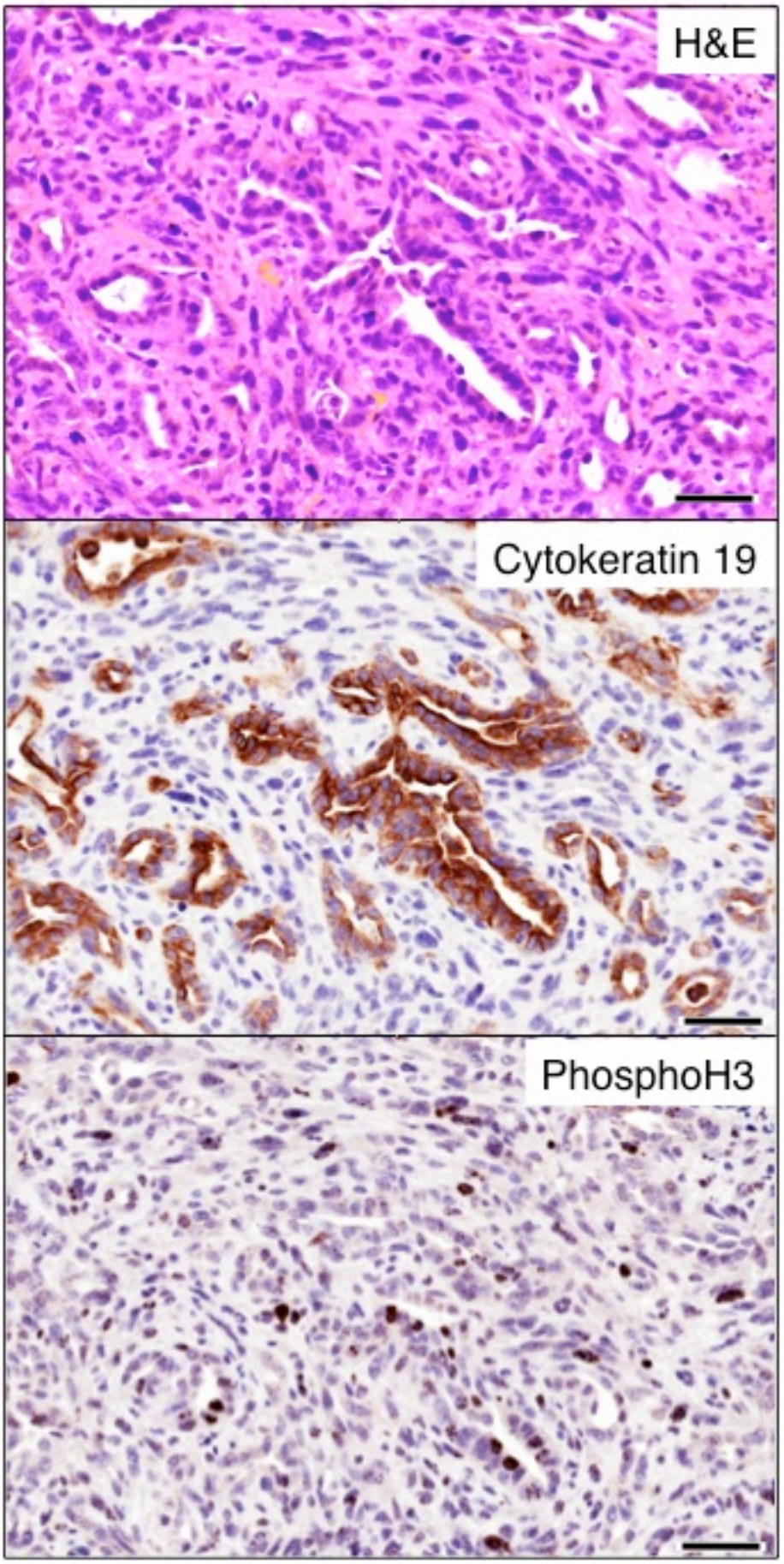


M



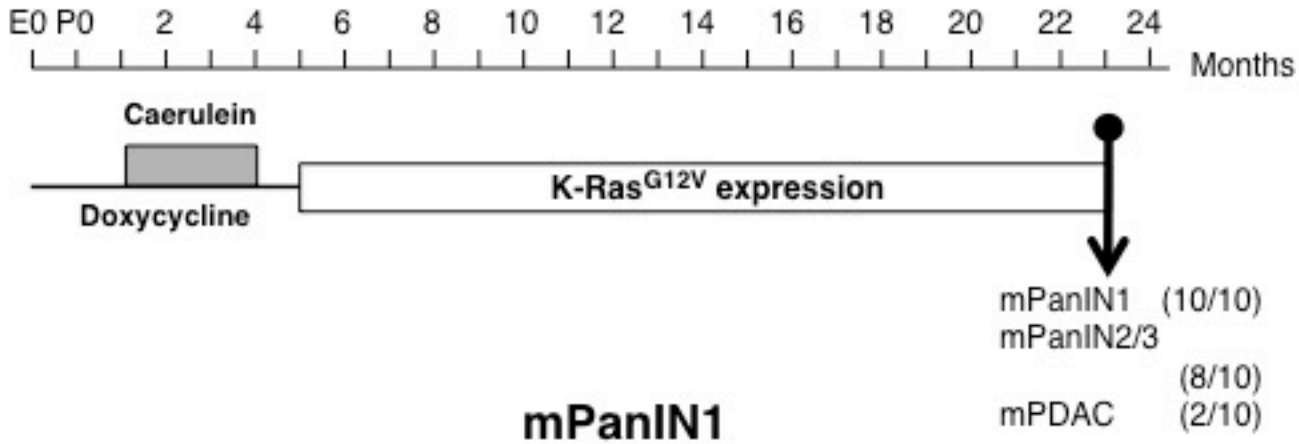
N

mPDAC (proliferative properties)



O

K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre mice



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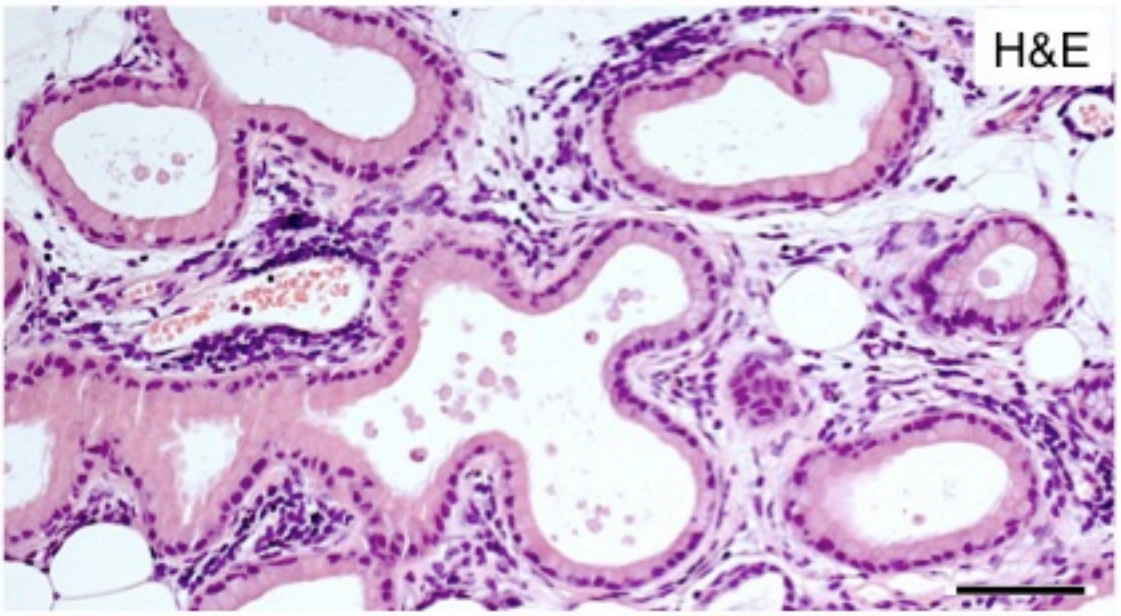

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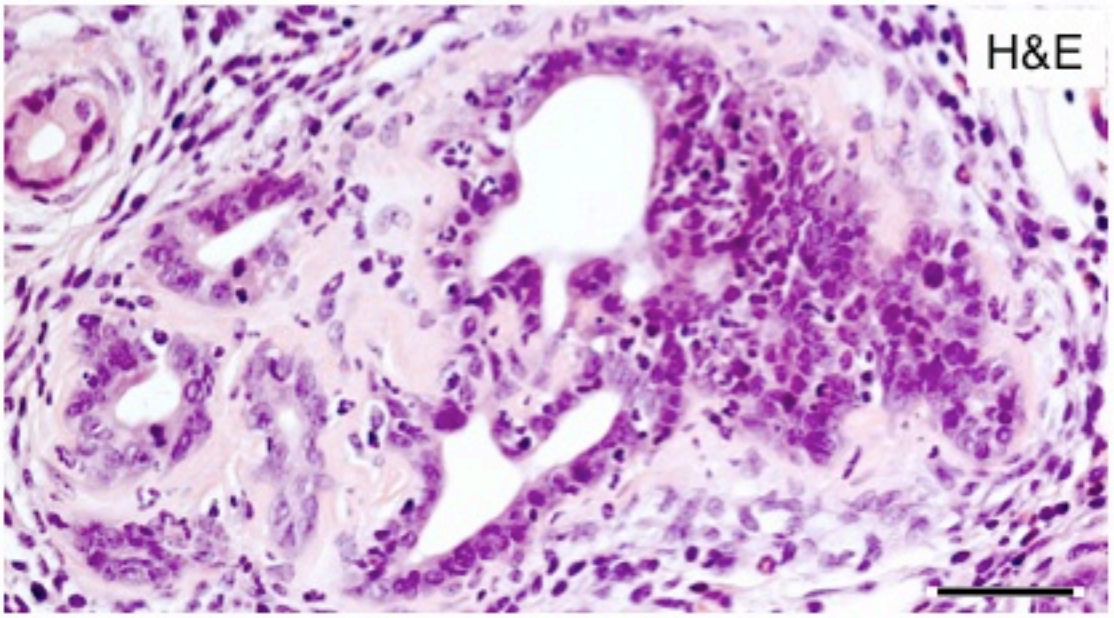
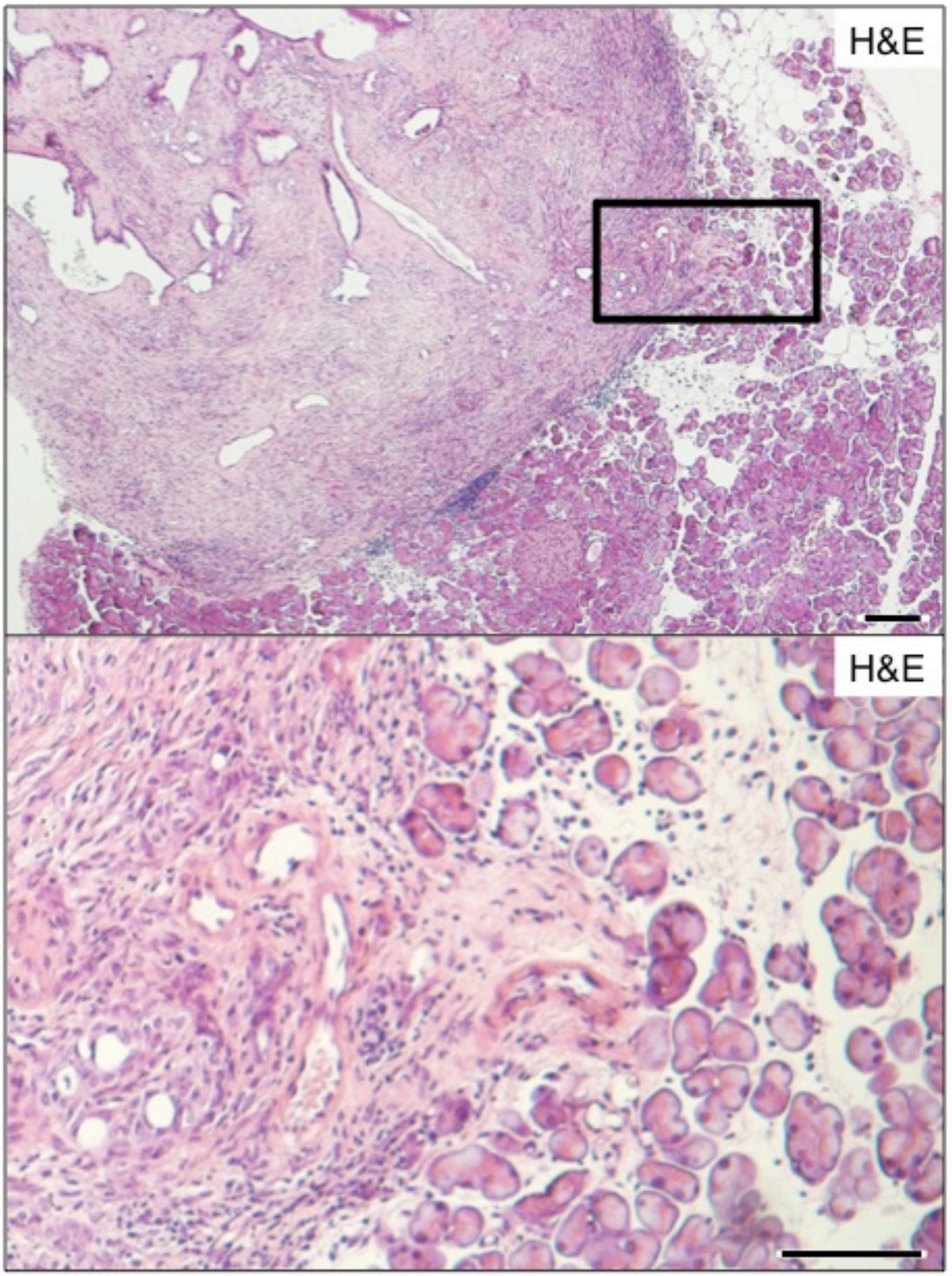
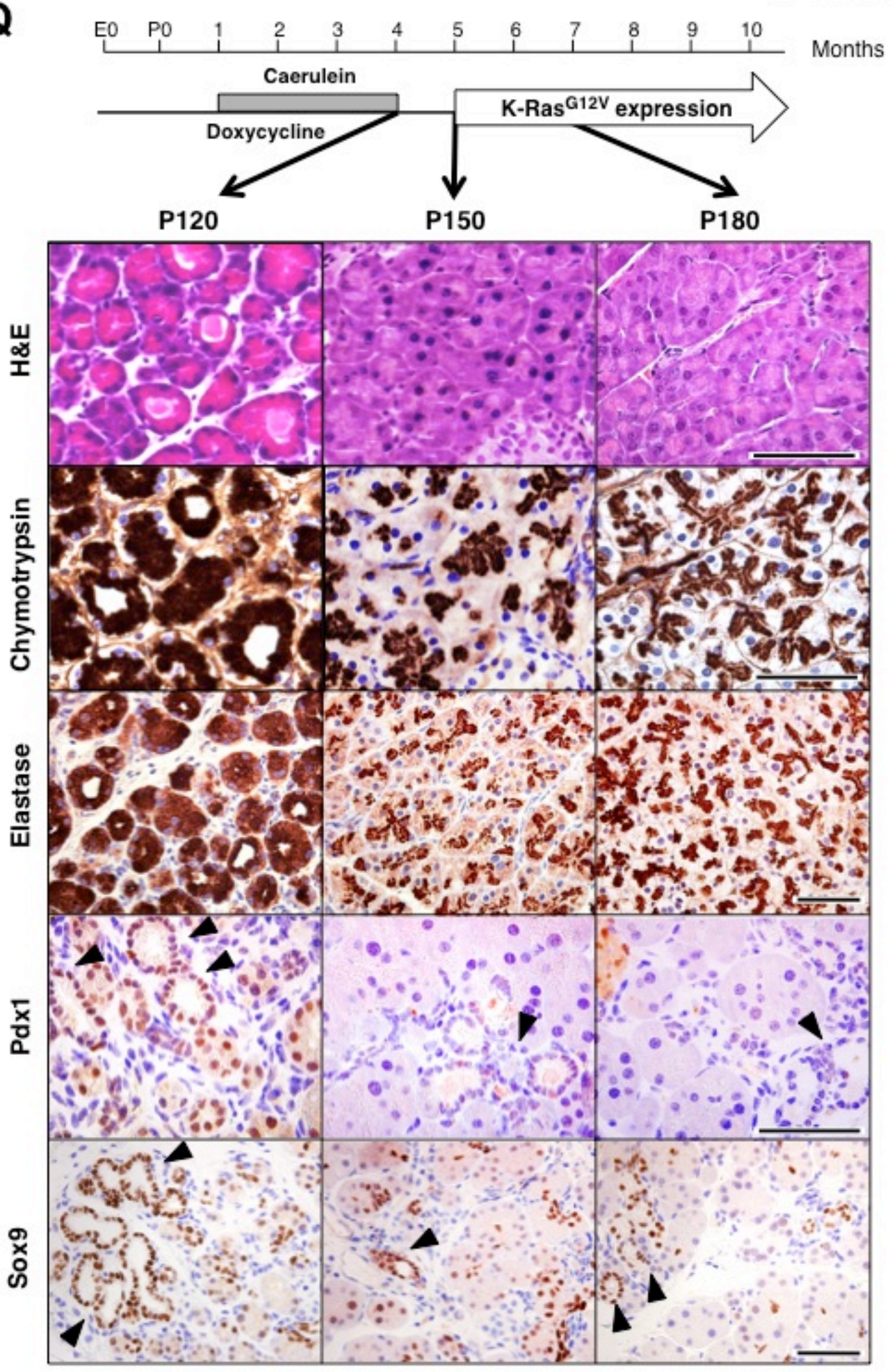


P

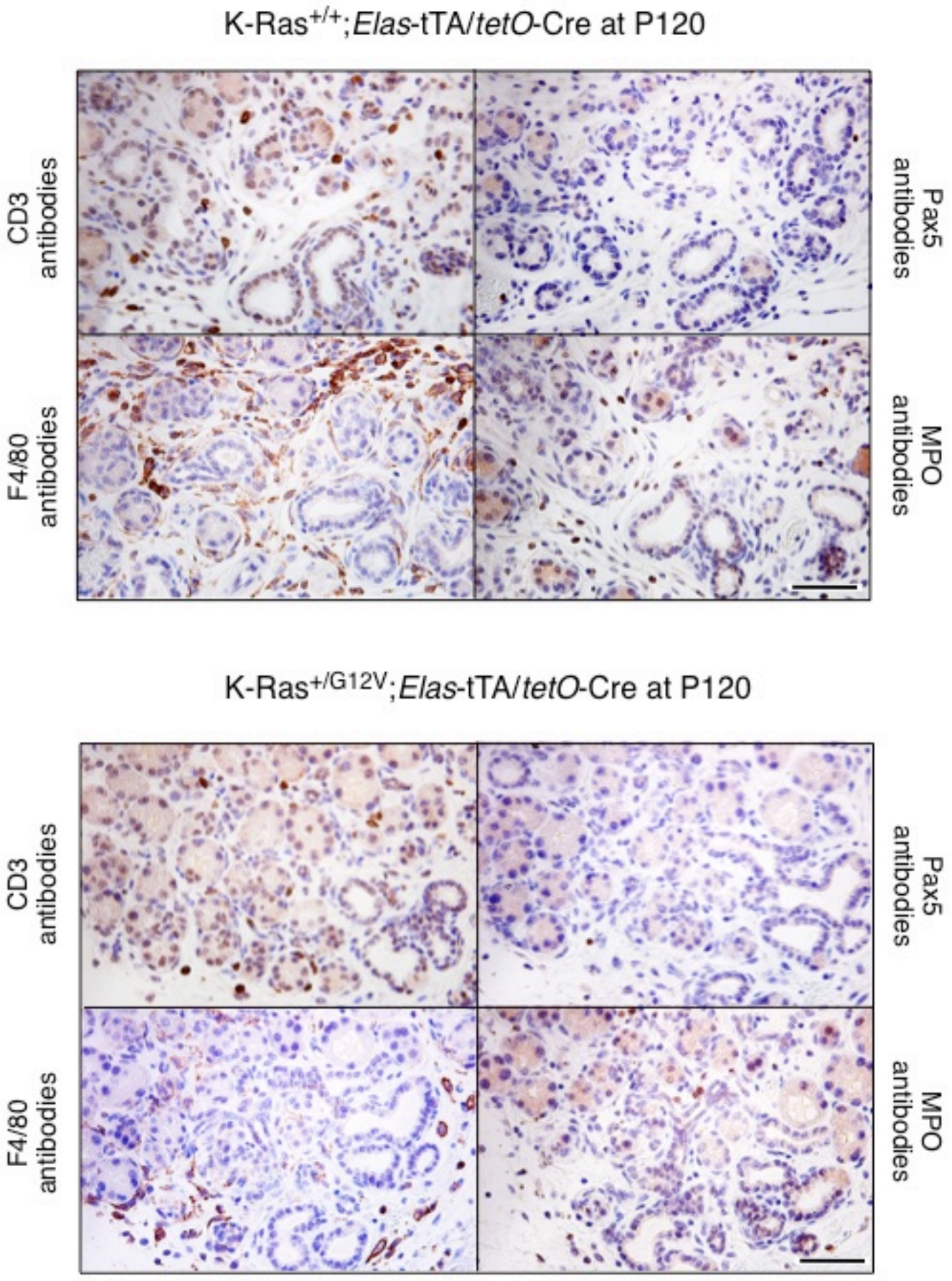
mPDAC



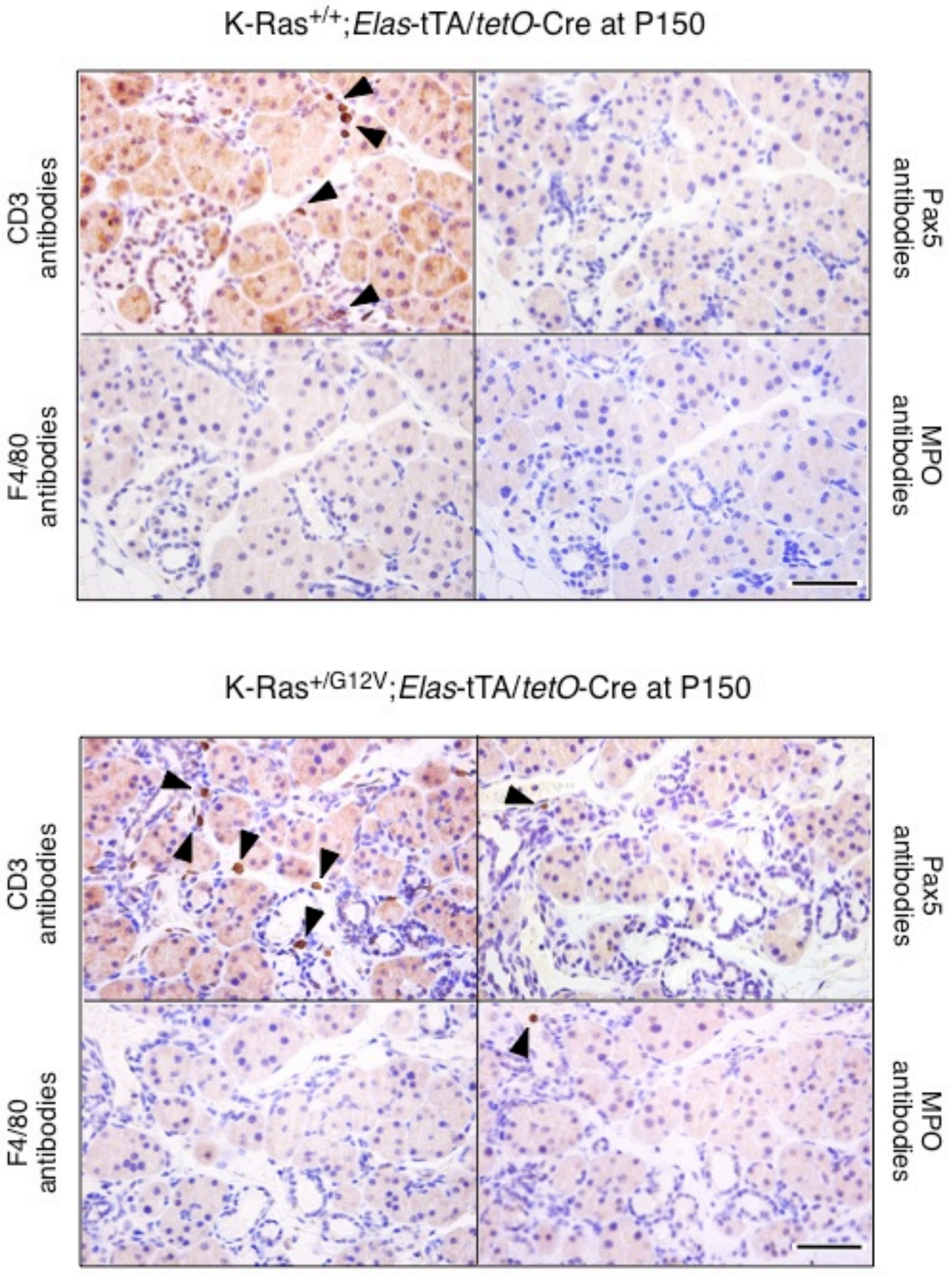
Q


E0
P0
1
2
3
4
5
6
7
8
9
10
Months
Caerulein
Doxycycline
K-Ras^{G12V} expression
P120
P150
P180
H&E
Chymotrypsin
Elastase
Pdx1
Sox9

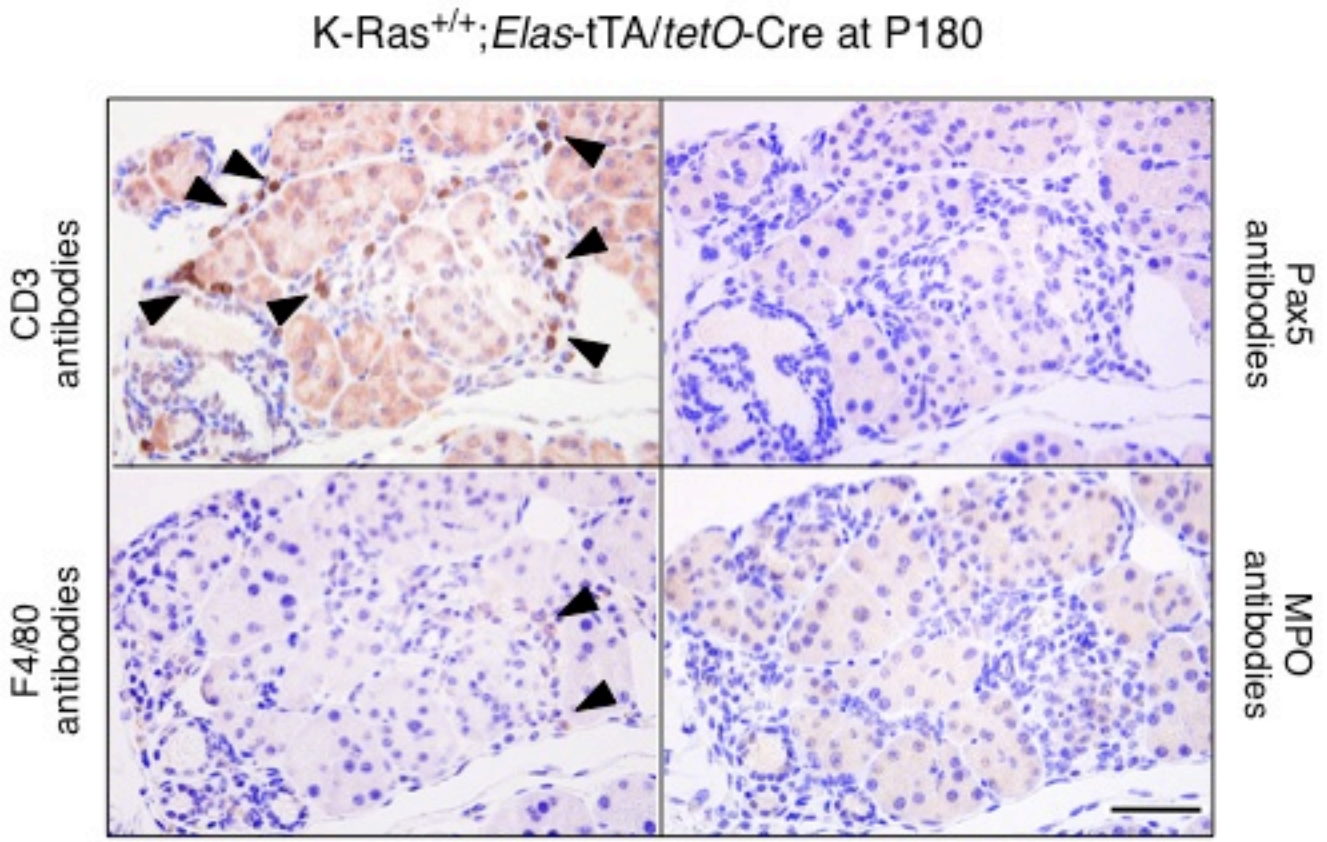
R


K-Ras+/+;Elas-tTA/tetO-Cre at P120
CD3 antibodies
Pax5 antibodies
F4/80 antibodies
MPO antibodies
K-Ras+/G12V;Elas-tTA/tetO-Cre at P120
CD3 antibodies
Pax5 antibodies
F4/80 antibodies
MPO antibodies

S


K-Ras+/+;Elas-tTA/tetO-Cre at P150
CD3 antibodies
Pax5 antibodies
F4/80 antibodies
MPO antibodies
K-Ras+/G12V;Elas-tTA/tetO-Cre at P150
CD3 antibodies
Pax5 antibodies
F4/80 antibodies
MPO antibodies

T


K-Ras+/+;Elas-tTA/tetO-Cre at P180
CD3 antibodies
Pax5 antibodies
F4/80 antibodies
MPO antibodies

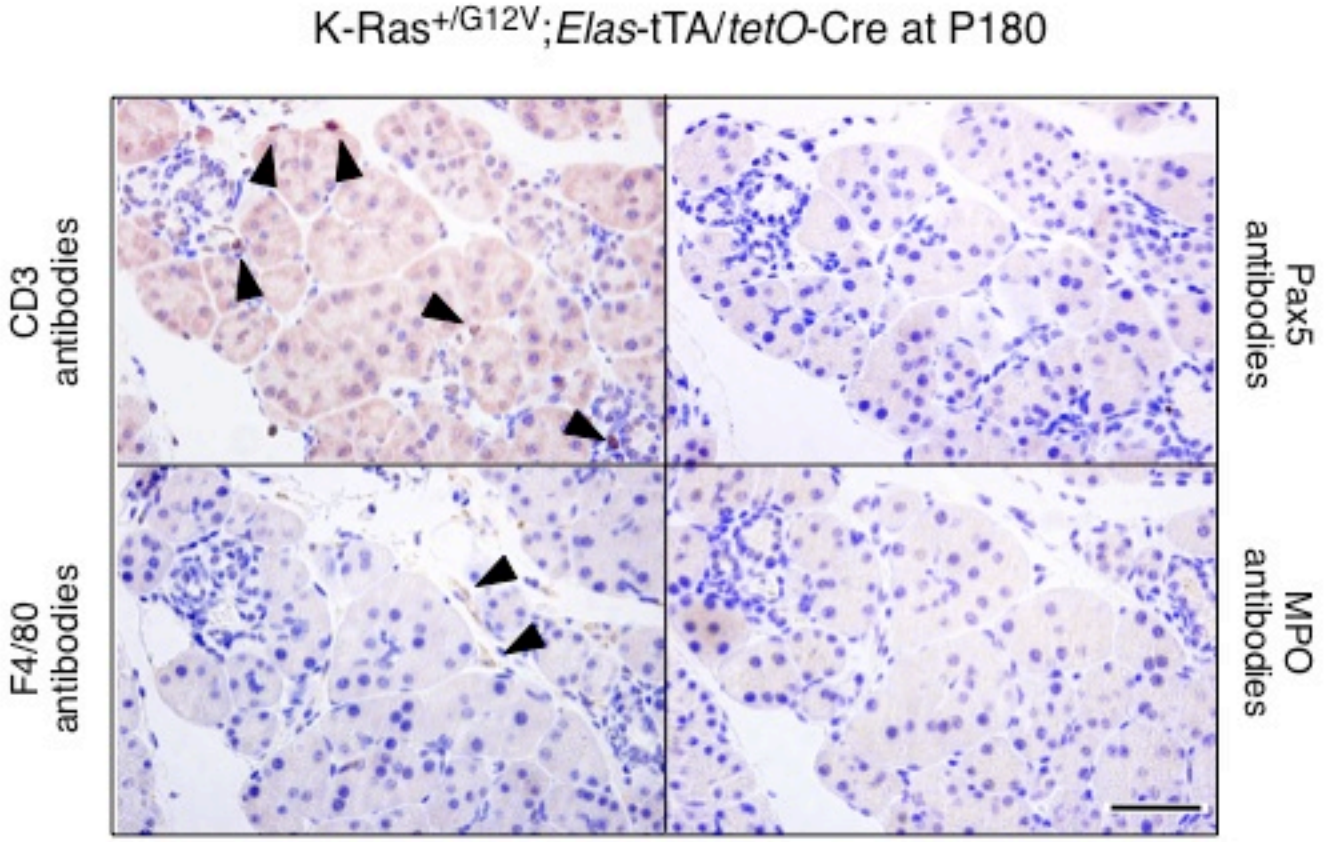

K-Ras+/G12V;Elas-tTA/tetO-Cre at P180
CD3 antibodies
Pax5 antibodies
F4/80 antibodies
MPO antibodies

Figure S3, related to Figure 3. Episodic pancreatitis cooperates with a resident K-Ras^{G12V} oncogene to induce mPanINs and mPDAC.

(A-I) Results derived from mice exposed to caerulein for three months (P90 to P180)

(A) Three month long episodic pancreatitis induces low and high grade mPanINs

Top: Diagram indicates the periods of doxycycline (thin line) and caerulein (thick gray line) treatment. Temporal expression of the K-*Ras*^{G12V} oncogene (thick solid line) is indicated. Mice were sacrificed at the end of the caerulein treatment.

Bottom: H&E stained paraffin sections of pancreata obtained from (**left panels**) K-*Ras*$^{+/+}$;*Elas*-tTA/*tetO*-Cre and (**right panels**) K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre mice.

Upper left panel shows acinar atrophy with distended lumen and loss of apical granulations (solid arrowhead) as well as increased fibrosis with sparse inflammatory infiltrates (asterisk). (i) indicates an islet.

Lower left panel shows a magnification of the upper panel to illustrate the acinar atrophy (solid arrowhead). Inflammatory infiltrates are indicated by an asterisk.

Upper right panel depicts areas with significant more severe acinar atrophy (solid arrowhead) and fibrosis with inflammatory infiltrates (asterisk) than in K-*Ras*$^{+/+}$;*Elas*-tTA/*tetO*-Cre mice. Open arrowhead points to mPanIN lesions surrounded by large numbers of inflammatory infiltrates.

Lower right panel shows a magnification of the upper panel to illustrate acinar atrophy with distended lumen and loss of apical granulations (solid arrowhead). Inflammatory infiltrates are indicated by an asterisk. Open arrowhead points to a mPanIN lesion. (i) indicates an islet.

Scale bar represents 50 μm.

(B) Three month long episodic pancreatitis induces low and high grade mPanINs as well as mPDAC twelve months after turning on K-Ras^{G12V} expression.

Top: K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre mice exposed to doxycycline (thin line) from conception (E0) to P60 and treated with caerulein (gray box) from P90 to P180 (3 months) were analyzed after twelve months of continuous K-Ras^{G12V} expression (14 months of age).

Middle: H&E staining of representative low-grade mPanINs. Scale bar, 50 μm.

Bottom: H&E staining of representative high-grade mPanINs. Scale bar 25 μm.

(C) Representative small mPDAC.

The tumor was stained with antibodies raised against Cytokeratin 19 to illustrate the ductal-like epithelial nature of the tumor cells. Tumor invasion into the parenchyma is indicated by arrows. Scale bar, 200 μm.

(D) Representative large metastatic mPDAC.

H&E **(Top)** and Cytokeratin 19 **(Bottom)** stained sections. Rectangles indicate areas shown in detailed in **(E)** and **(F).** Scale bar 500 μm.

(E) Detailed region of the above tumor indicated by the solid line rectangle in (D).

This region depicts a necrotic area and sparse tumor cells embedded into the stroma. Asterisk indicates the necrotic area. Scale 100 μm.

(F) Detailed region of the above tumor indicated by the dotted line rectangle in (D).

Inflammatory infiltrates of adjacent sections using antibodies against T lymphocytes (CD3 antibodies) and macrophages (F4/80 antibodies). Insets corresponding to the areas indicated by solid line rectangles show detailed views to better illustrate the presence of the immune cells. Scale bars represent 100 μm.

(G) Lung metastasis.

Top: H&E staining of a lung metastasis

Bottom: Detailed picture of the area indicated above by the solid line rectangle.

Scale bar, 100 μm.

(H) Lung metastasis.

Serial sections of the area shown above were stained with antibodies against Cytokeratin 19 to identify the ductal-like cells and against surfactant protein C (SPC) to identify lung tissue (type II bronchioalveolar cells).

(I) Liver metastasis.

H&E (**top**) and Cytokeratin 19 (**bottom**) staining. Metastatic cells are indicated by dotted lines. Scale bar, 50 μm.

(J-N) Results derived from mice exposed to caerulein for one month (P90 to P120)

(J) One month long episodic pancreatitis also induces low and high grade mPanINs.

Top: K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre mice exposed to doxycycline (thin line) from conception (E0) to P60 and treated with caerulein (gray box) from P90 to P120 (1 month) were analyzed after twelve months of continuous K-Ras^{G12V} expression (14 months of age).

Middle: H&E staining of representative low grade mPanINs. Scale bar, 25 μm.

Bottom: H&E staining of representative high grade mPanINs. Scale bar, 25 μm.

(K) One month long episodic pancreatitis also induces mPDAC.

H&E staining of an invasive mPDAC present in a 2 year old mouse shown at low magnification to illustrate invasion of adjacent intestinal tissue. Rectangles correspond to amplified areas shown in **L**. Scale bar 500 μm.

(L) One month long episodic pancreatitis also induces mPDAC.

Detailed areas indicated above by solid line rectangles. White line rectangles indicate areas shown at higher magnification in (**M**). Scale bar 500 μm.

(M) Detailed picture of tumor cells invading the adjacent intestinal tissue.

H&E staining (**top**) and Cytokeratin 19 staining (**bottom**) of the areas indicated in (**L**) by the white line rectangles. Scale bar, 50 μm.

(N) Proliferative properties of tumors cells of the tumor described in K-M

Serial sections depicting tumor glands are stained with H&E (**top**), with cytokeratin 19 antibodies to illustrate their ductal-like epithelial nature (**middle**) and with anti Phospho-Histone H3 (PhosphoH3) to illustrate their high proliferative index (**bottom**). Scale bar, 50 μm.

(O-T) Results derived from mice exposed to caerulein for three months (P30 to P120) prior to K-Ras^{G12V} expression.

(O) The occurrence of episodic pancreatitis prior to K-Ras^{G12V} expression also induces mPanIN and mPDAC development.

Top: K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre mice exposed to doxycycline (thin line) from conception (E0) to P150 and treated with caerulein (gray box) from P30 to P120 (3 months) were analyzed after eighteen months of continuous K-Ras^{G12V} expression (23 months of age).

Middle: H&E staining of representative low grade mPanINs. Scale bar, 50 μm.

Bottom: H&E staining of representative high grade mPanINs. Scale bar, 50 μm.

(P) mPDAC induced by to K-Ras^{G12V} expression following episodic pancreatitis.

Top: H&E staining of a representative mPDAC examined after 18 months of oncogene expression. Solid line rectangle indicates area magnified below. Scale bar, 100 μm.

Bottom: Detailed area indicated above showing invasion of the adjacent parenchyma. Scale bar, 50 μm.

(Q) Characterization of acinar cells immediately after caerulein treatment (P120) as well as before (P150) and after (P180) K-Ras^{G12V} oncogene expression.

Top: K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre mice exposed to doxycycline (thin line) from conception (E0) to P150 and treated with caerulein (gray box) from P30 to P120 were analyzed at P120 (immediately after caerulein treatment), P150 (immediately after cessation of doxycycline exposure) and P180 [one month after turning on K-*Ras*^{G12V} oncogene expression (open box)].

H&E: H&E staining of pancreata analyzed at the indicated times. Note that the atrophy displayed at P120, immediately after caerulein treatment, has disappeared in samples obtained at P150 and P180.

Chymotrypsin: Chymotrypsin staining of consecutive sections. Note that immediately after caerulein treatment (P120) chymotrypsin is expressed in the entire cytoplasm as well as in the interstitial space. At later times (P150 and P180), chymotrypsin expression is restricted to the apical side of the acini.

Elastase: Elastase staining of pancreata analyzed at the indicated times. Note that the pattern of expression of elastase is similar to that of chymotrypsin.

Pdx1: Pdx1 expression in pancreata analyzed at the indicated times. Note that Pdx1 is highly expressed in acinar cells and metaplasias at P120 (arrowheads), immediately after caerulein treatment. No significant levels of expression in acinar cells were observed at P150 or P180. Few positive cells remain in metaplasias at P150 or P180 (arrowheads). Islet at P180 serves as positive control.

Sox9: Sox9 expression in pancreata analyzed at the indicated times. Note high levels of nuclear staining of acinar, centroacinar and ductal cells as well as in metaplasias at P120, immediately after caerulein treatment. At P150, there is a decrease in the number of positive acinar cells. At P180, Sox9 staining is found only in centroacinar and ductal cells as well as in metaplasias. Metaplasias are indicated by arrowheads.

(R) Inflammatory infiltrates after caerulein treatment (P120)

K-*Ras*$^{+/+}$;*Elas*-tTA/*tetO*-Cre (**top**) and K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre (**bottom**) mice exposed to doxycycline from conception (E0) to P120 and treated with caerulein from P30 to P120. Mice were analyzed at P120 immediately after cessation of caerulein exposure. Immunostaining of inflammatory cells using antibodies against T lymphocytes (CD3 antibodies), B lymphocytes (Pax5 antibodies), macrophages (F4/80 antibodies) and neutrophils (MPO antibodies).

Scale bars represent 50 μm.

(S) Inflammatory infiltrates one month after the end of caerulein treatment (P150)

K-*Ras*$^{+/+}$;*Elas*-tTA/*tetO*-Cre (**top**) and K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre (**bottom**) mice exposed to doxycycline from conception (E0) to P150 and treated with caerulein

from P30 to P120. Mice were analyzed at P150 immediately after cessation of doxycycline exposure. Immunostaining of inflammatory cells using antibodies against T lymphocytes (CD3 antibodies), B lymphocytes (Pax5 antibodies), macrophages (F4/80 antibodies) and neutrophils (MPO antibodies). Inflammatory cells are indicated by arrowheads. Please note that most cells are T lymphocytes.

Scale bars represent 50 μm.

(T) Inflammatory infiltrates one month after turning on K-Ras^{G12V} expression (P180).

K-*Ras*$^{+/+}$;*Elas*-tTA/*tetO*-Cre (**top**) and K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre (**bottom**) mice exposed to doxycycline from conception (E0) to P150 and treated with caerulein from P30 to P120. Mice were analyzed at P180, one month after turning on K-*Ras*^{G12V} oncogene expression. Immunostaining of inflammatory cells using antibodies against T lymphocytes (CD3 antibodies), B lymphocytes (Pax5 antibodies), macrophages (F4/80 antibodies) and neutrophils (MPO antibodies). Inflammatory cells are indicated by arrowheads. Please note that most cells are T lymphocytes.

Scale bars represent 50 μm.

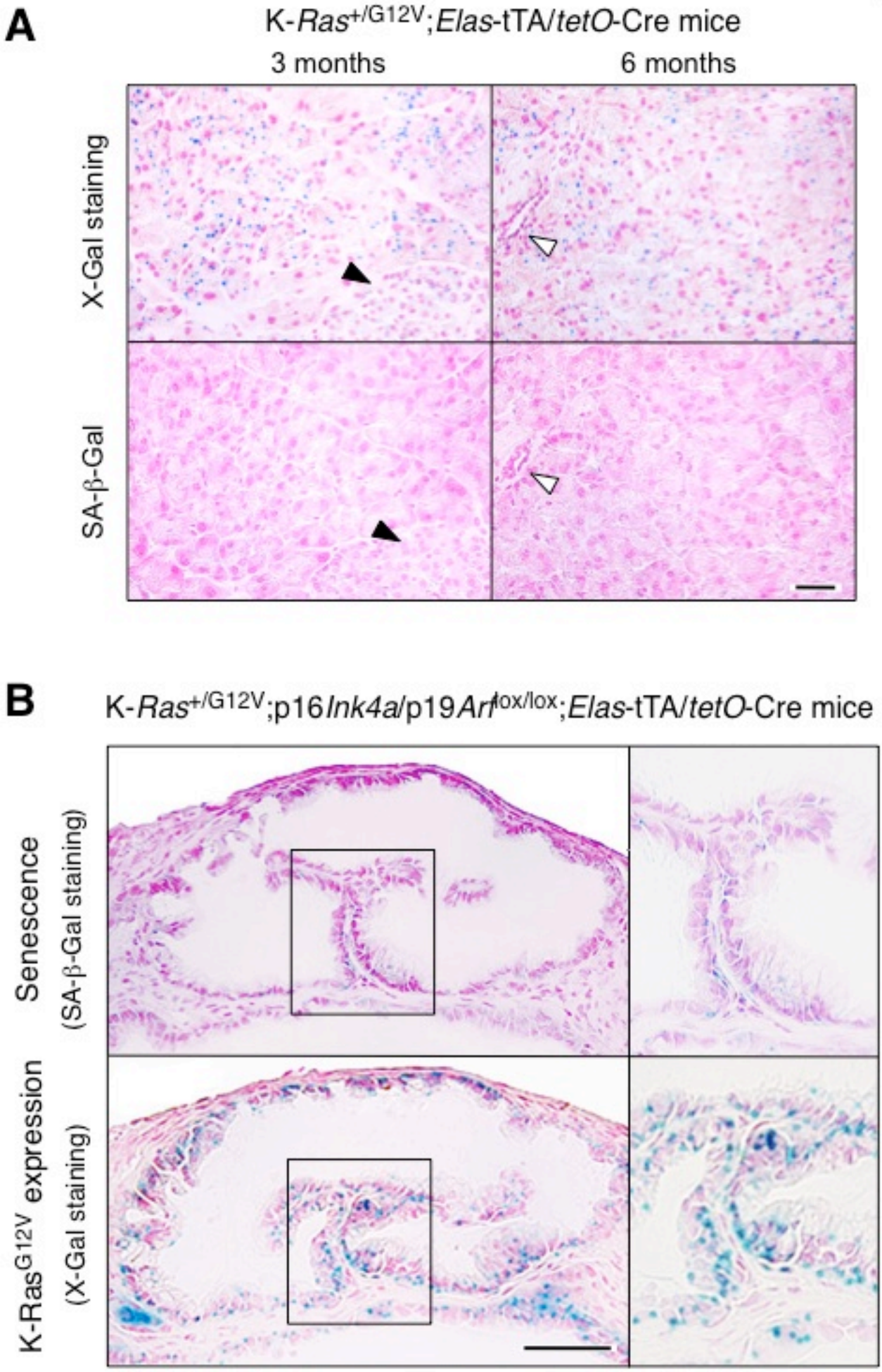

A
K-Ras+/G12V;Elas-tTA/tetO-Cre mice
3 months
6 months
X-Gal staining
SA-β-Gal
B
K-Ras+/G12V;p16Ink4a/p19Arflox/lox;Elas-tTA/tetO-Cre mice
Senescence
(SA-β-Gal staining)
K-RasG12V expression
(X-Gal staining)

Figure S4, related to Figure 5. Role of K-*Ras*^{G12V} and p16*Ink4a*/p19*Arf* in the induction of senescence.

(A) Expression of a resident K-*Ras*^{G12V} oncogene in adult acinar cells is not sufficient to induce OIS.

Upper panels: Analysis of β-galactosidase activity (X-Gal staining) in pancreata of (**Upper left panel**) 3 and (**Upper right panel**) 6 month old K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre mice exposed to doxycycline until P60 to achieve expression of K-*Ras*^{G12V} in the adult pancreas (blue dots). These mice did not develop any obvious histological alterations in their pancreata.

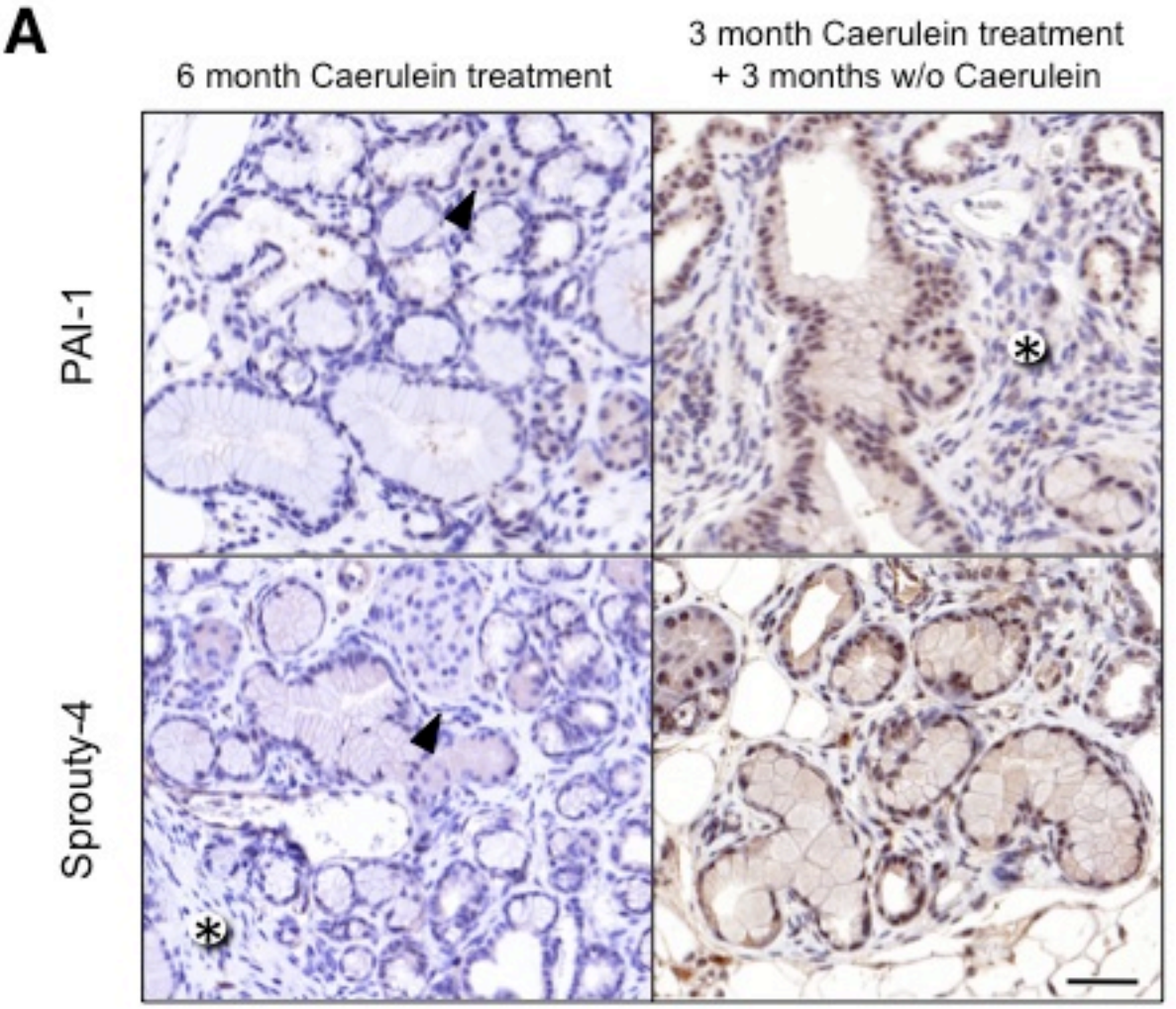
Lower panels: Lack of senescence associated β-galactosidase (SA-β-Gal) staining of consecutive cryosections. An islet is indicated by solid arrowheads. A normal duct is indicated by open arrowheads. Scale bar represents 50 μm.

(B) Lack of senescence in mPanIN lesions of mice lacking p16*Ink4a*/p19*Arf*.

Upper panel: A representative low-grade mPanIN lesion of K-*Ras*$^{+/G12V}$;p16*Ink4a*/p19*Arf*$^{lox/lox}$;*Elas*-tTA/*tetO*-Cre mice not exposed to doxycycline is negative for Senescence associated β-galactosidase (SA-β-Gal).

Lower panel: However, an adjacent section is positive for K-*Ras*^{G12V} expression, as determined by X-Gal staining that detects the surrogate bacterial β-galactosidase marker.

Images depicted at the right correspond to amplified areas indicated by the solid line rectangles. Scale bar represents 50 μm.


A
6 month Caerulein treatment
3 month Caerulein treatment
+ 3 months w/o Caerulein
PAI-1
Sprouty-4

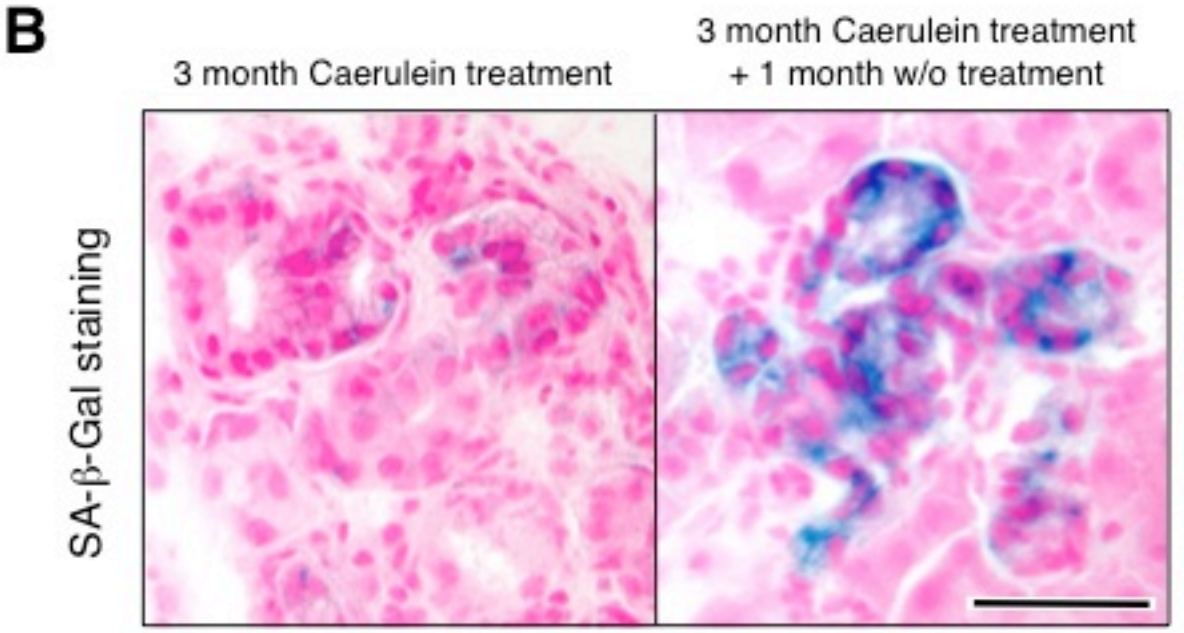

B
3 month Caerulein treatment
3 month Caerulein treatment
+ 1 month w/o treatment
SA-β-Gal staining

Figure S5, related to Figure 6. Senescence markers reappear upon partial recovery from pancreatitis-induced injury.

(A) Senescent low-grade mPanINs reappear upon partial recovery from pancreatitis injury. mPanINs present in pancreata of K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre mice not exposed to doxycycline and treated with caerulein (**left panels**) for six months (P30 to P210) or (**right panels**) for three months (P30 to P120) and allowed to recover from three additional months (P120 to P210). Sections were stained with (**upper panels**) anti PAI-1 and (**lower panels**) anti Sprouty-4 antibodies. Islets are indicated by solid arrowheads. Stroma is indicated by an asterisk.

Scale bar represents 50 μm.

(B) Senescence cells reappeared in low-grade mPanIN1 lesions shortly after cessation of caerulein exposure.

Left panel: Low-grade mPanIN1 lesions found in pancreata of K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre mice at the end of caerulein treatment are negative for SA-β-Gal staining.

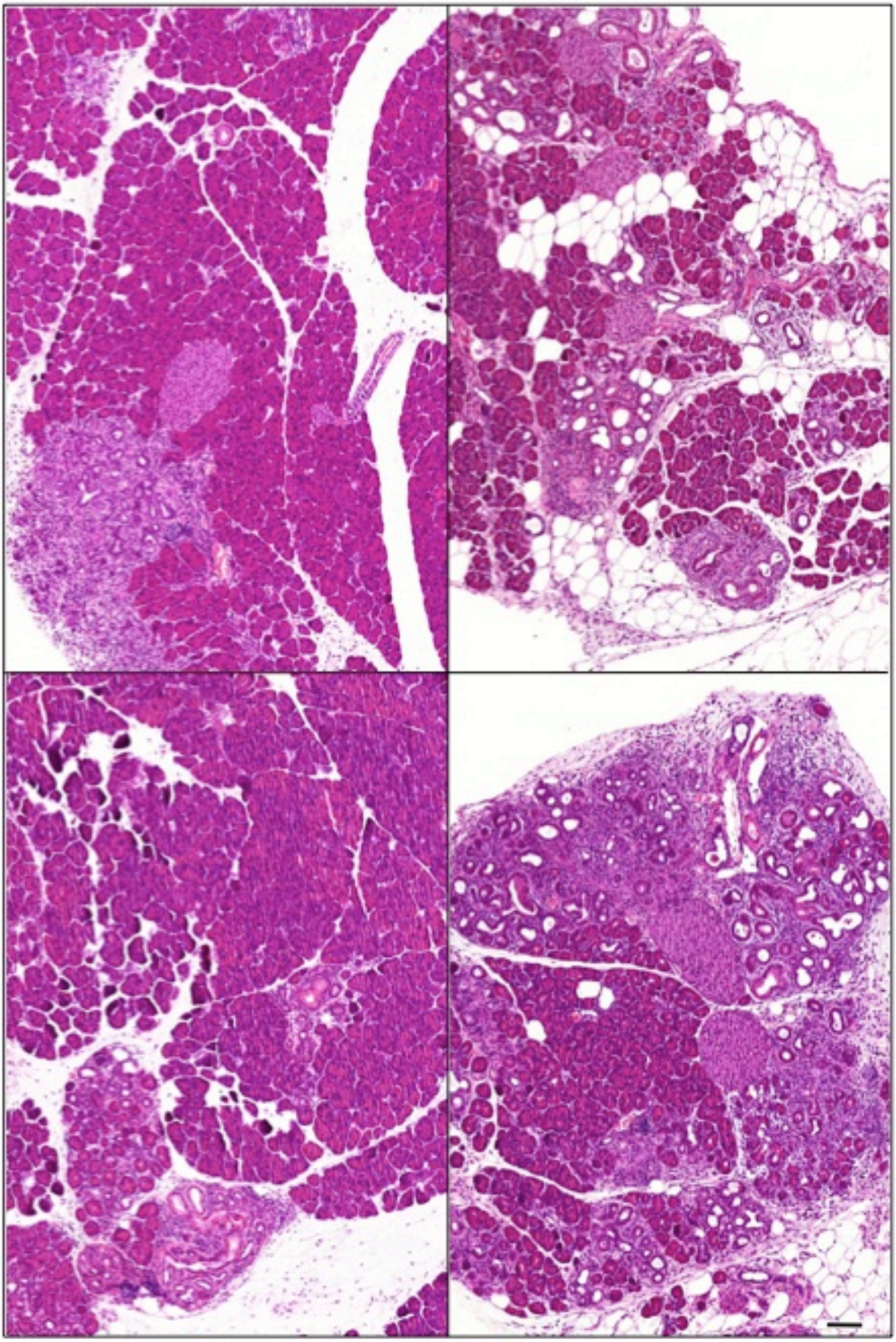
Right panel: Low-grade mPanIN1 present in pancreata of K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre mice one month after caerulein treatment are positive for SA-β-Gal staining.

Scale bar represents 50 μm.

A

K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre mice

No Sulindac treatment



B

K-*Ras*$^{+/G12V}$;*Elas*-tTA/*tetO*-Cre mice

Sulindac treatment

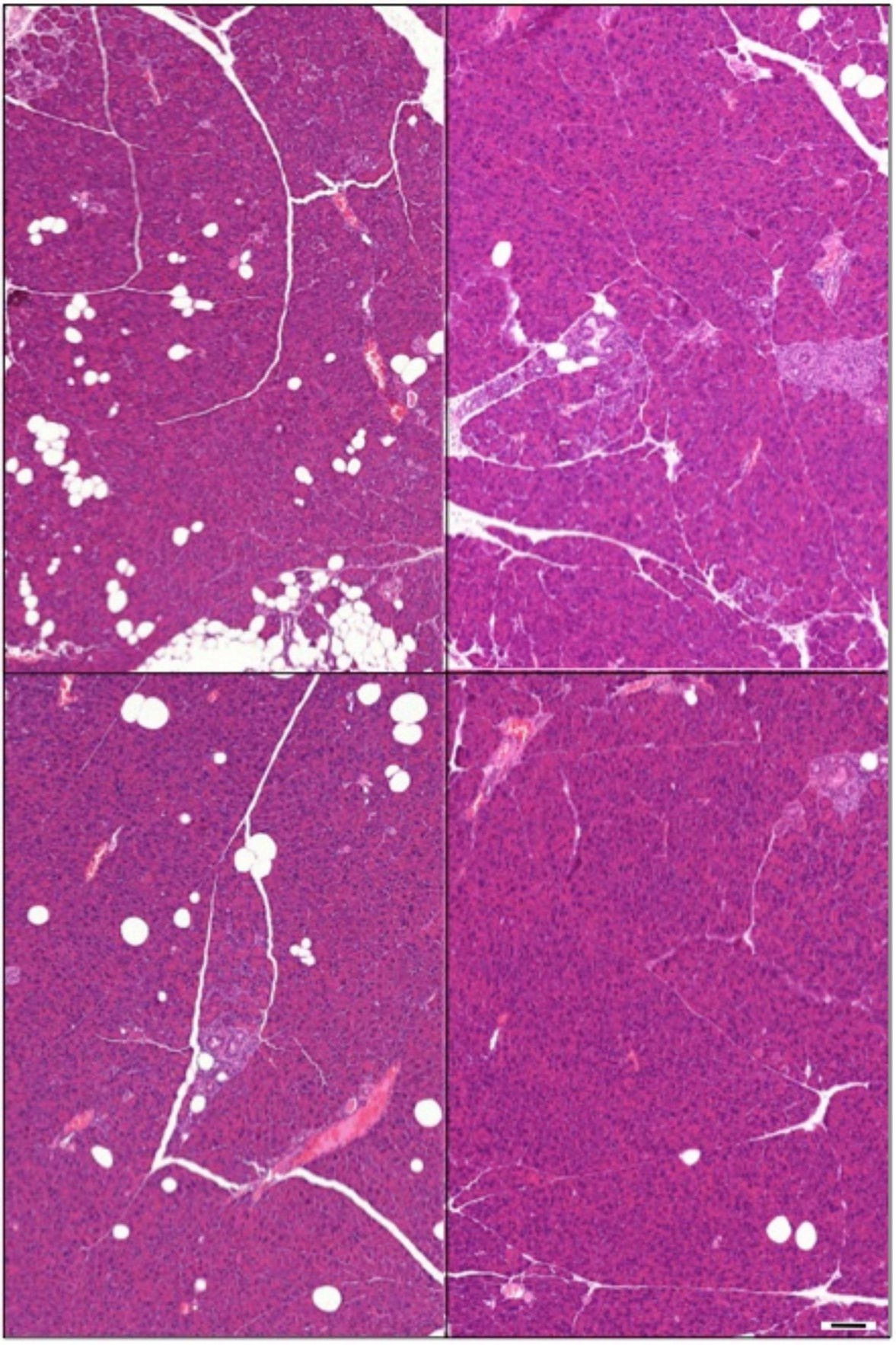


Figure S6, related to Figure 7. Inhibition of the inflammatory response by Sulindac reverts tissue damage and delays progression of mPanIN lesions.

(A) H&E stained paraffin sections showing panoramic view of pancreata of four representative K-*Ras*$^{+/G12V}$; *Elas*-tTA/*tetO*-Cre mice exposed to doxycycline until P60 and treated with caerulein form three months (P90 to P180).

(**B**) H&E stained paraffin sections showing panoramic view of pancreata of four representative K-*Ras*$^{+/G12V}$; *Elas*-tTA/*tetO*-Cre mice exposed to doxycycline until P60 and treated with caerulein form three months (P90 to P180) and treated with Sulindac for three additional months (P180 to P270).

Note that the pancreata of the four untreated mice displayed in (**A**) have high levels of oedema, fibrosis, parenchyma atrophy and abundant large mPanIN lesions. In contrast, the four mice that underwent Sulindac treatment shown in (**B**) have a well-preserved parenchyma and contained few small mPanINs.

Scale bar represents 100 μm.

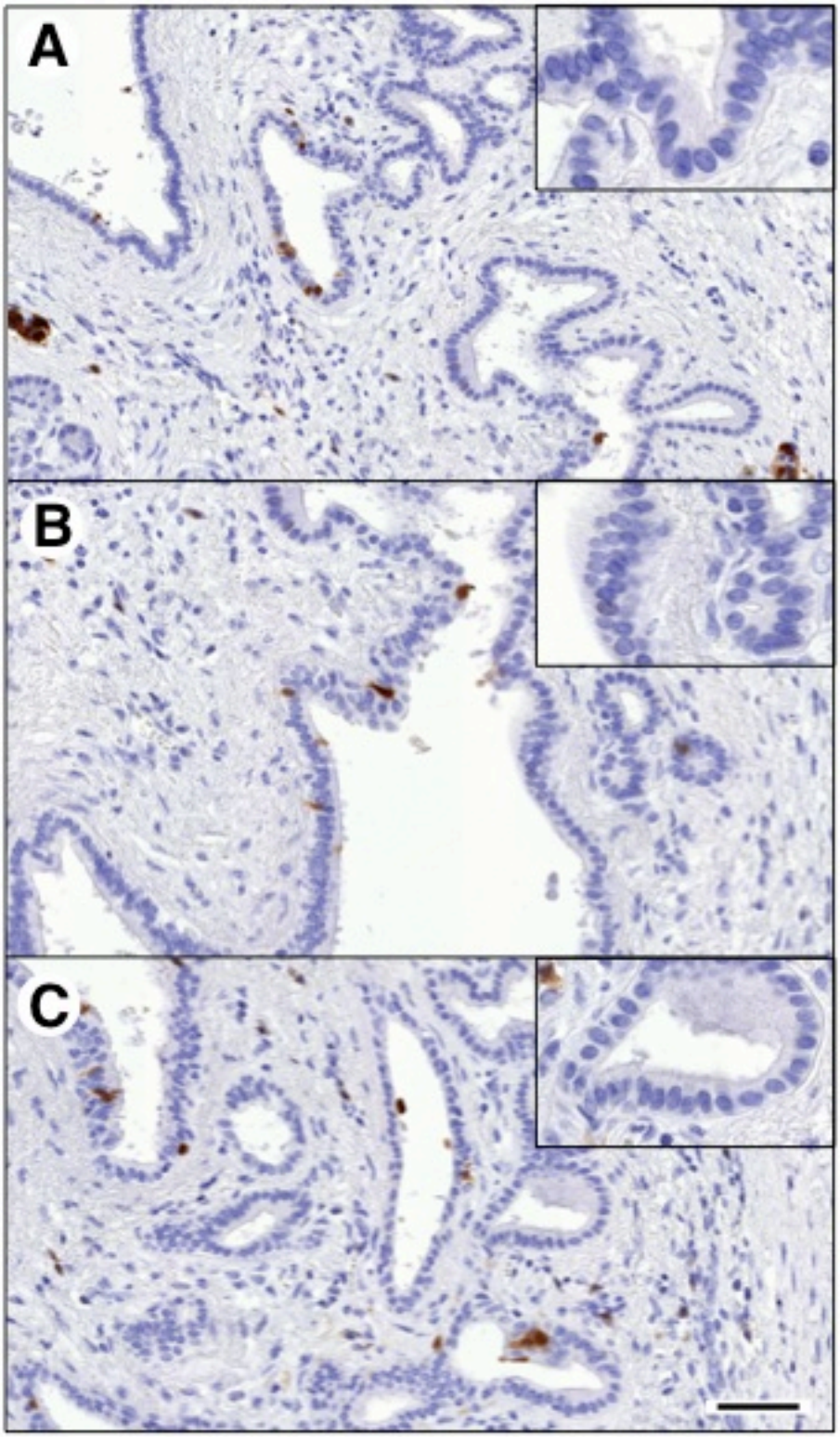

A
B
C

Figure S7, related to Figure 8. Lack of P16INK4a immunostaining in human low-grade PanINs of patients not treated with anti-inflammatory drugs.

(A-C) Representative sections displaying low-grade PanIN1s negative for P16INK4a staining present in biopsies obtained from three different patients not-treated with anti-inflammatory drugs.

Insets show amplified images.

Scale bar represents 50 μm.

Table S1, related to Figure 8. Clinical histories of chronic pancreatitis patients whose biopsies have been characterized in this study.

NO HISTORY OF NSAIDs / STEROID TREATMENT

Gender/Age	Presentation	Imaging	Evolution
Female 41	Severe gastric pain and chronic pancreatitis.	Calcification of pancreas with stones throughout main and side ducts branches of pancreas	Initial treatment – sphinterectomy Pancreatic insufficiency and mild elevated HbA1C
Female 35	Abdominal pain and pancreatitis. Found to have gallstones and underwent cholecystectomy	No structuring of the ducts but calcification in head of pancreas. Dilated common bile duct	Despite cholecystectomy presented with recurrent pain, managed conservatively (pancreatic rest). Abnormal liver function test. Failed ERCP (Endoscopic retrograde cholangiopancreatography)
Male 30	Chronic pancreatitis treated conservatively	Pseudocyst with marked inflammatory changes around body and tail of pancreas	On follow up – development of pseudocyst non amenable to percutaneous drainage
Male 60	Epigastric pain. No history of alcohol. Treated with laparoscopic appendicectomy.	Calcification head of pancreas Pseudocyst Inflammatory mass head of pancreas	Due to ongoing pain imaging (CT) suggested calcification tail of pancreas and pancreatitis. Laparoscopic cholecystectomy. Pseudocyst and inflammatory mass head of pancreas
Female 51	Follow up for renal cyst. Abdominal distension. No exocrine dysfunction	Severe changes of chronic pancreatitis. Dilated pancreatic duct. Multiple stones. Tight stricture head of pancreas	Failed ERCP. No amenable for pancreatic endotherapy

HISTORY OF NSAIDs / STEROID TREATMENT

Gender/Age	Presentation	Imaging	Evolution
Male 79	History of mild alcohol intake. Abnormal function liver tests.	CT – mass in head of pancreas (HoP)	Treated initially with indomethacin 50mg/twice daily/7 days. Symptoms no resolved. Because mass HoP surgical resection.
Male 46	History of Diabetes type 2. Presented with epigastric pain. Serum bilirubin and serum ALT elevated	Diffuse enlargement of the pancreas with structuring of common bile duct.	Treated with 30mg prednisolone/4 months because suspicious of autoimmune / sclerosing pancreatitis
Female 52	Weight loss and abdominal pain. History of alcohol intake and recurrent pancreatitis.	Calcification throughout pancreas. Inflammatory mass	Diclofenac 150mg/day for 4 weeks. Symptoms not resolved. Abnormal pancreatic function
Female 54	Biliary colic pain but no jaundice. Mildly raised bilirubin and antinuclear antibodies (1:40). No medical history known. No other associated autoimmune type diseases	Pancreas enlargement but no pancreas mass irregularity of biliary tree but no strictures	30 mg prednisolone / 4 months.