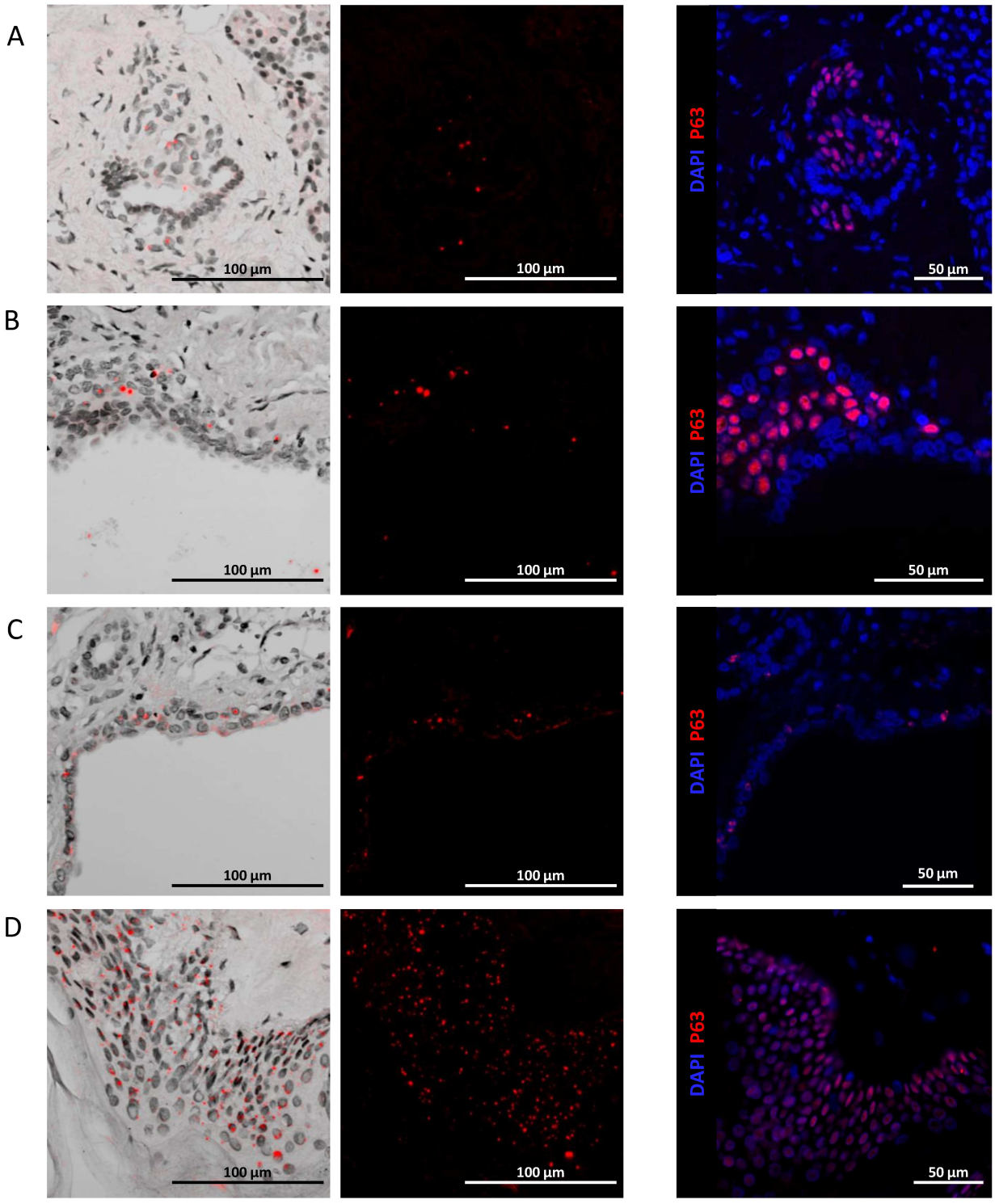
A
100 µm
100 µm
DAPI P63
50 µm
B
100 µm
100 µm
DAPI P63
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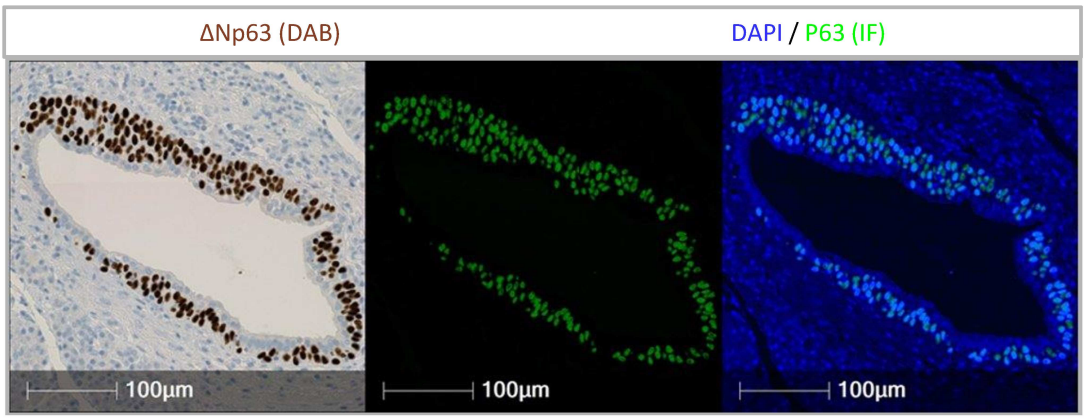
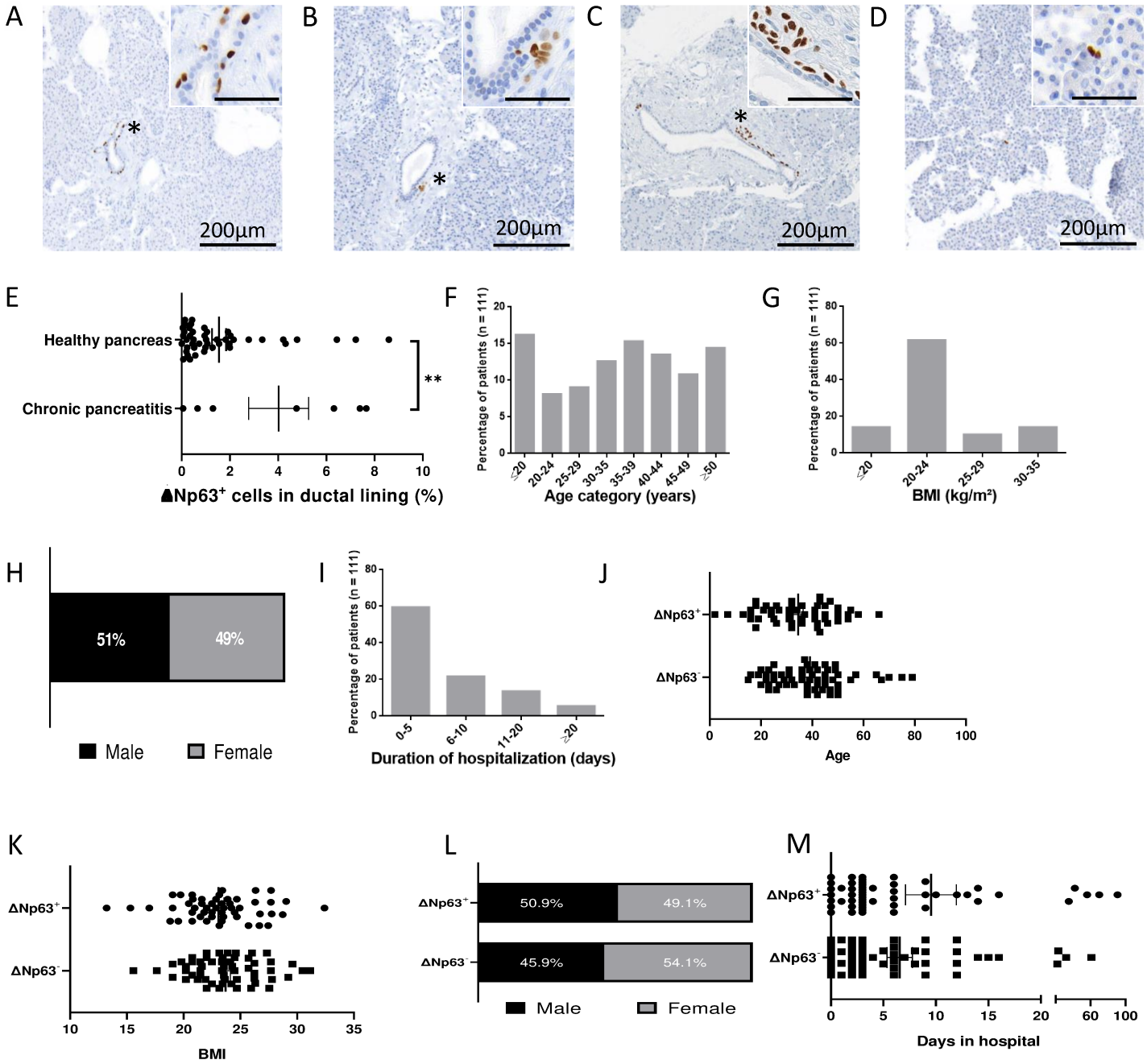
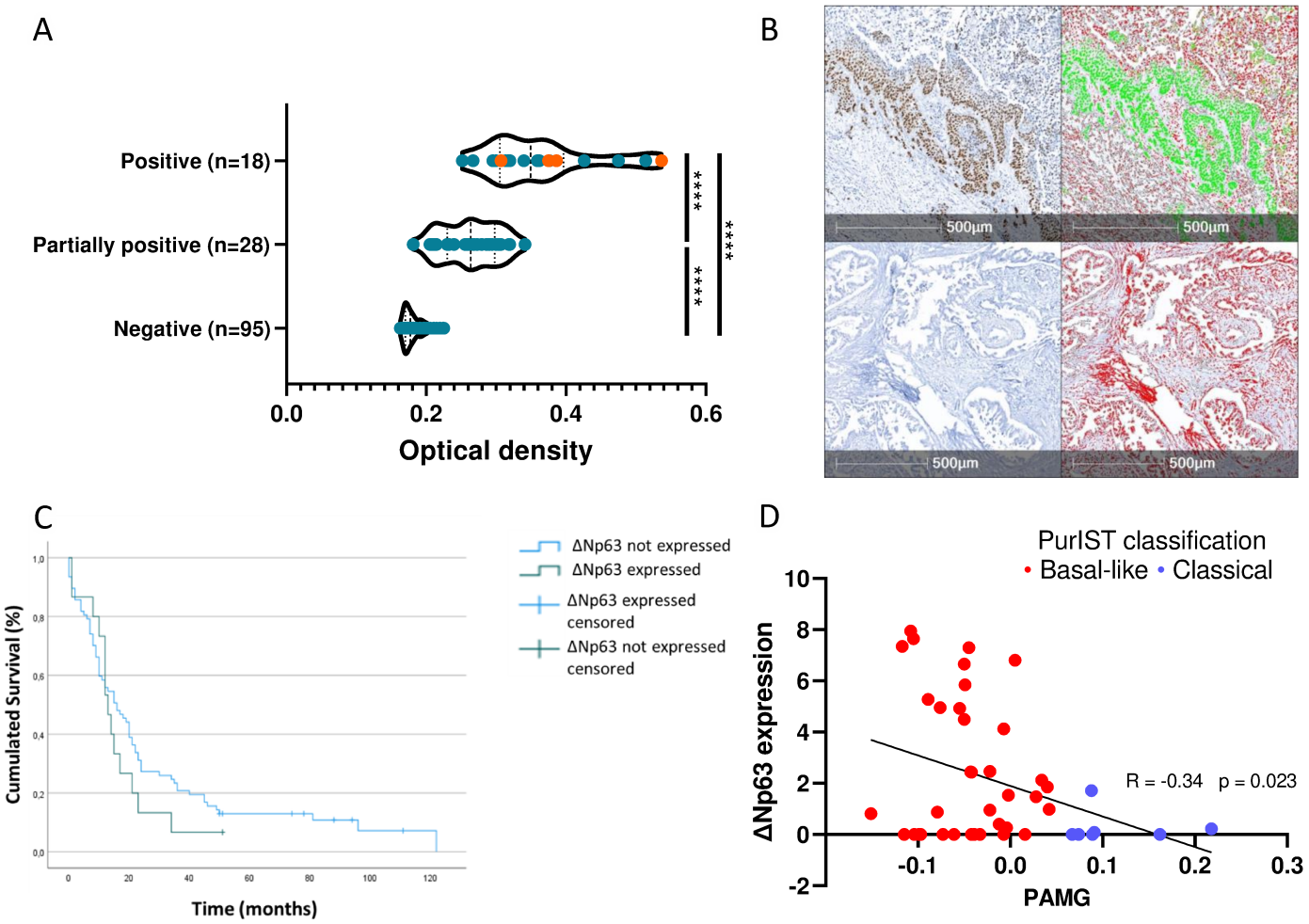
C
100 µm
100 µm
DAPI P63
50 µm
D
100 µm
100 µm
DAPI P63
50 µm


E
ΔNp63 (DAB)
DAPI / P63 (IF)
100µm
100µm
100µm

S Figure 1: RNA detection of ΔNp63 using BaseScope RNA *in situ* hybridization. (A-C) RNA detection in healthy donor pancreas (A), chronic pancreatitis (B) and normal tissue adjacent to PDAC area (C), with the corresponding P63 staining below. RNA is visualized as red dots. (D) RNA detection in positive control tissue (human skin). (E) Validation of P63 antibody in immunofluorescence (IF) on the right with ΔNp63 antibody staining in immunohistochemistry (IHC) on the left.

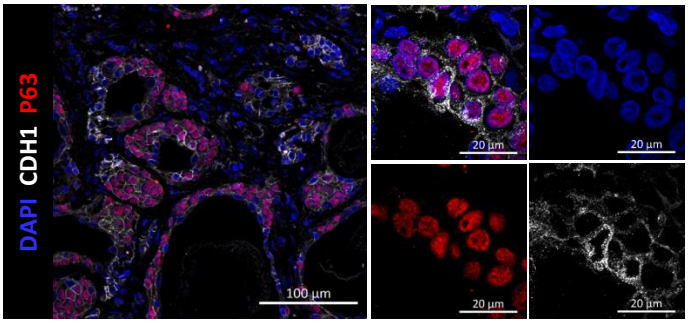


S Figure 2: (A-D) Location of ΔNp63⁺ cells throughout a healthy pancreas: (A) Cells can be located basally within a duct, (B) they can form a small cluster near a duct or (C) there can be both groups and single cells combined. (D) ΔNp63⁺ cells can rarely be found within acinar tissue. Scale bars indicate 200µm, scale bars on insets are 50 µm. (E) Percentage of cells within the ductal lining in normal pancreas (n=46) and chronic pancreatitis (n=7) (**p=0,044) Characteristics of all normal human pancreas donors with ΔNp63 detected in a section (n=53): (F) Age, (G) Gender, (H) Days spent in the intensive care unit, (I). Characteristics of all human pancreas donors with ΔNp63 detected in a section (n=53) and without ΔNp63 detected in a section (n=61). (J) Age, (K) Gender, (L) Days spent in the intensive care unit and (M) BMI.

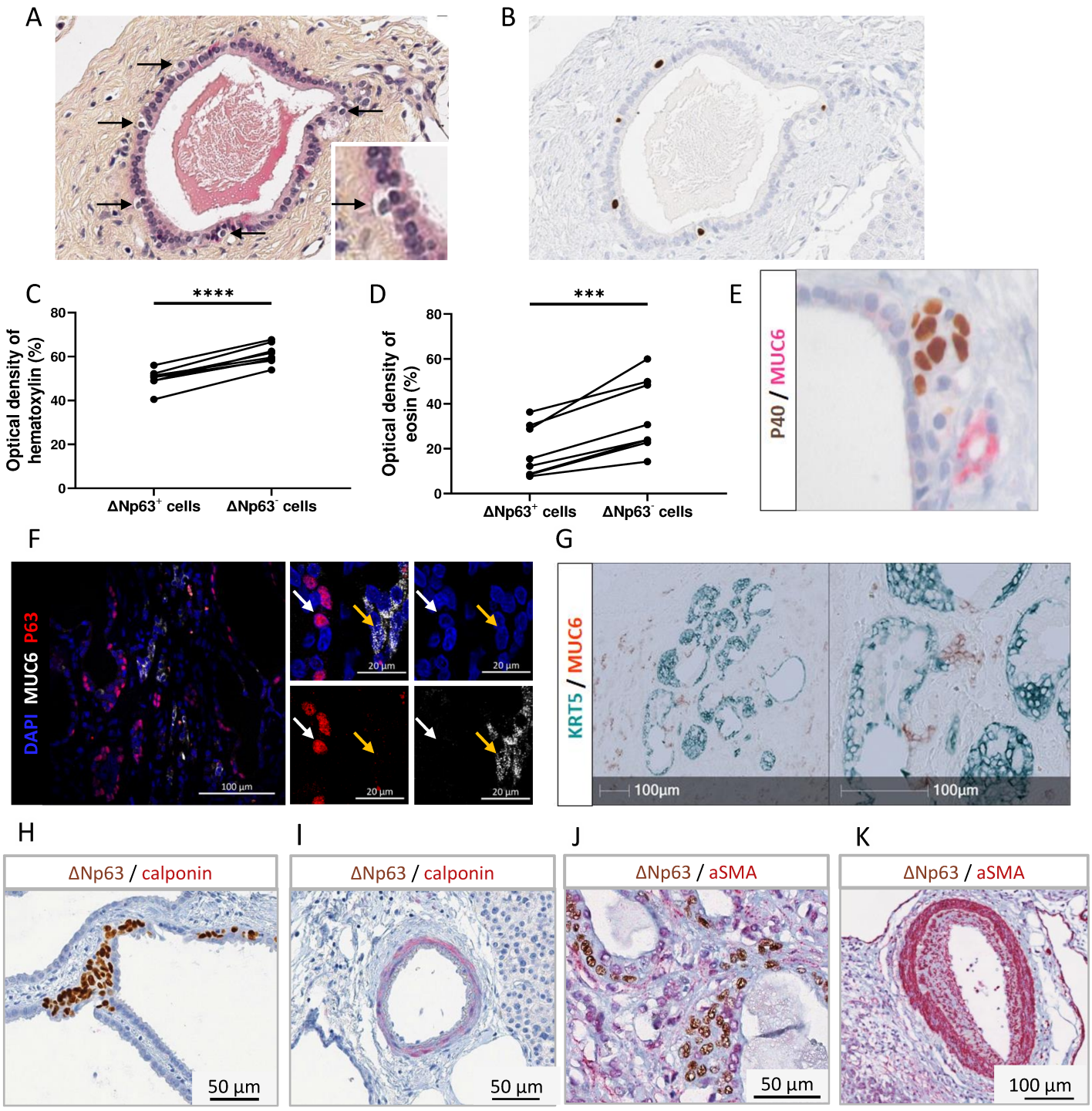


S Figure 3: ΔNp63 expression in pancreatic ductal adenocarcinoma samples. (A-B) Quantification of ΔNp63 expression displayed as optical density for four different groups of tumours (n=141), lacking ΔNp63 (negative), only in a few cells (partially positive) and samples that express ΔNp63 (positive). Four adenosquamous samples, which all fall in the positive group, are indicated in orange. Mean ± SEM is shown. ****p<0,001 (B) Visualization of the quantification through image analysis (HALO). IHC stainings are quantified in red (haematoxylin) and green (ΔNp63). (C) Cumulated survival in patients with PDAC with and without ΔNp63 expression (n=92 with data available). (D) Negative correlation between ΔNp63 and the PAMG score in PDAC cell lines (n=44).

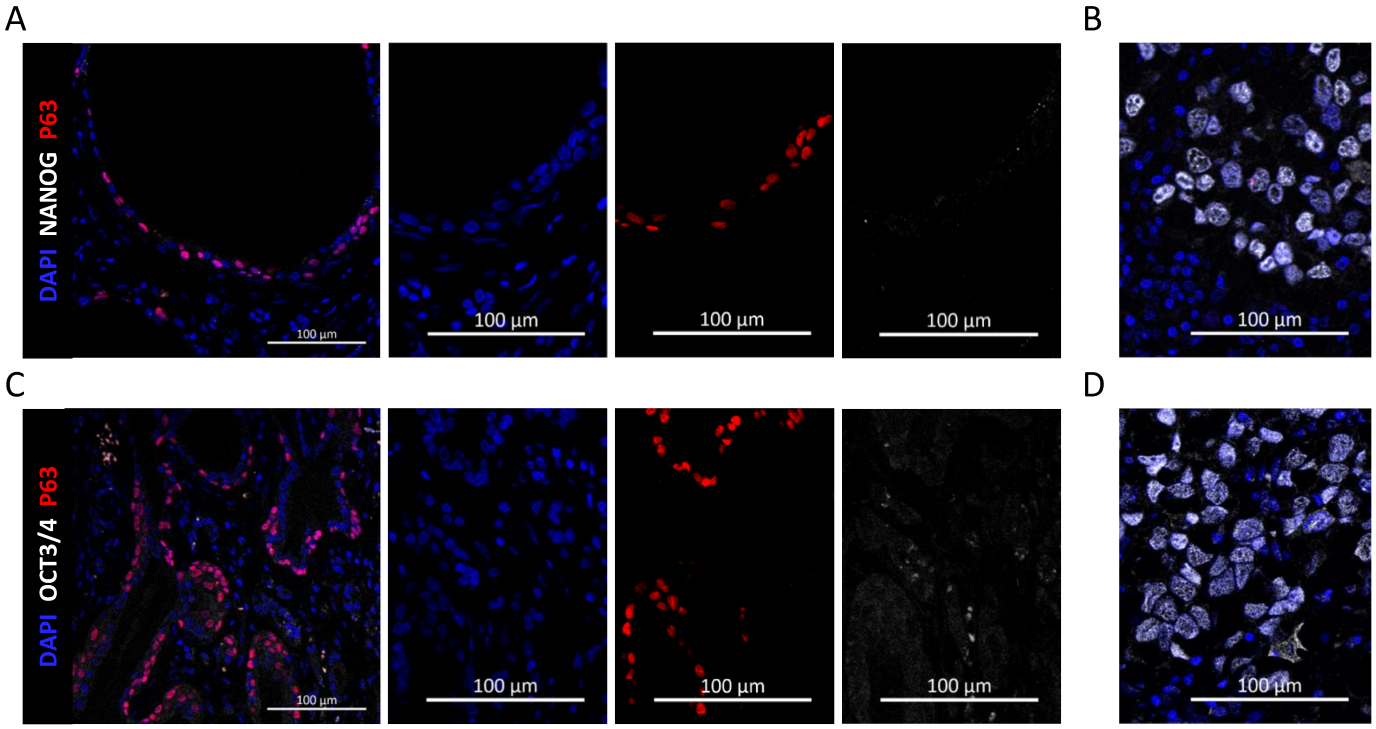
A



S Figure 4: **ΔNp63⁺ cells are epithelial cells.** IF for E-cadherin (white) and P63 (red).

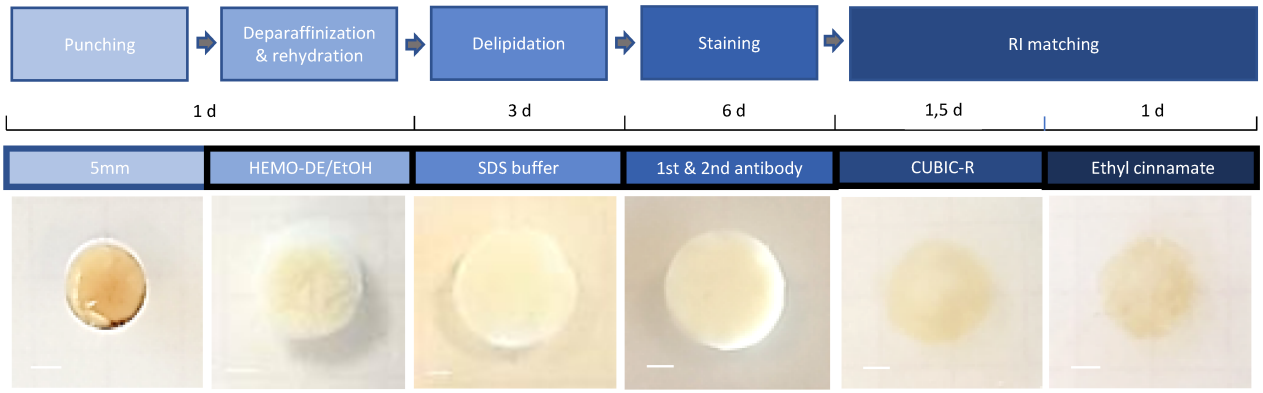


S Figure 5: (A) HES staining of a duct in a human healthy pancreas. Inset on the right bottom shows magnification of one ΔNp63⁺ cell. Black arrows point to ΔNp63⁺ cells. (B) Consecutive section of A showing IHC staining for ΔNp63. Quantification of the (C) haematoxylin and (D) eosin positivity in ΔNp63 cells compared to ductal cells (n=8). One line indicates one slide that was analysed for both ΔNp63⁺ and ΔNp63⁻ cells. (***p< 0,001; ****p< 0,0001). (E) IHC for ΔNp63 (brown) and MUC6 (red). (F) IF for P63 (red) and MUC6 (white). White arrow indicates ΔNp63⁺ cell, the orange arrow indicates a MUC6⁺ cell. (G) IHC staining for KRT5 (green) and MUC6 (red). (H) IHC for ΔNp63 (brown) and calponin (red) in a duct positive for ΔNp63. (I) shows positive control for calponin in the wall of a blood vessel. (J) IHC staining for ΔNp63 (brown) and aSMA (red) in a duct positive for ΔNp63. (K) shows positive control for aSMA.



S Figure 6: **ΔNp63⁺ cells do not express typical pluripotent stem cell markers.** (A) IF for P63 (red) and NANOG (white). (B) Positive expression of NANOG is shown in a seminoma in panel. (C) IF for P63 (red) and OCT4 (white). (D) Positive expression of OCT4 is shown in a seminoma in panel.

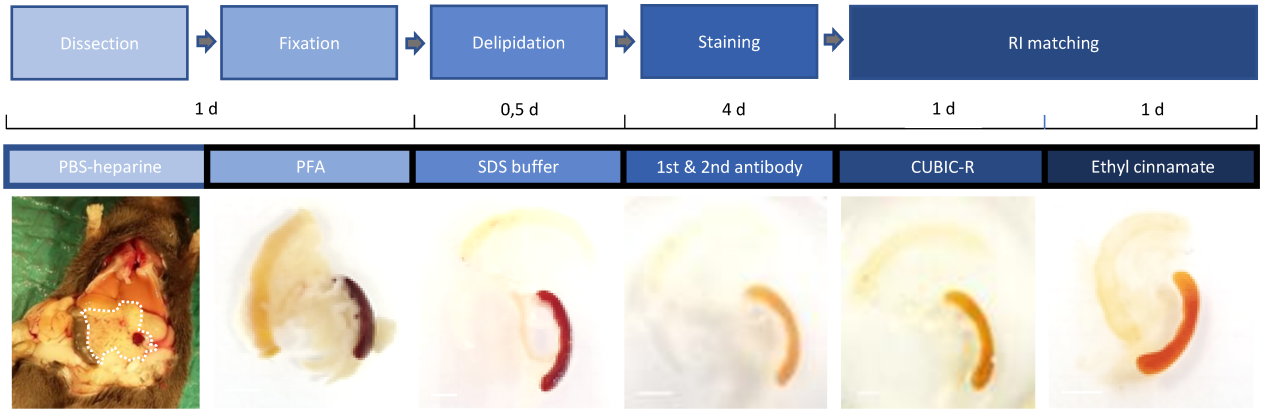
A



B

Protocol	Delipidation	RI matching	Duration	High resolution imaging
Modified IDISCO[19]	dichloromethane/methanol	dibenzyl ether (DBE)	20 days	confocal microscopy
FLIP-IT	SDS buffer	CUBIC-R/Ethyl Cinnamate(Eci)	13 days	Lightsheet miscroscopy

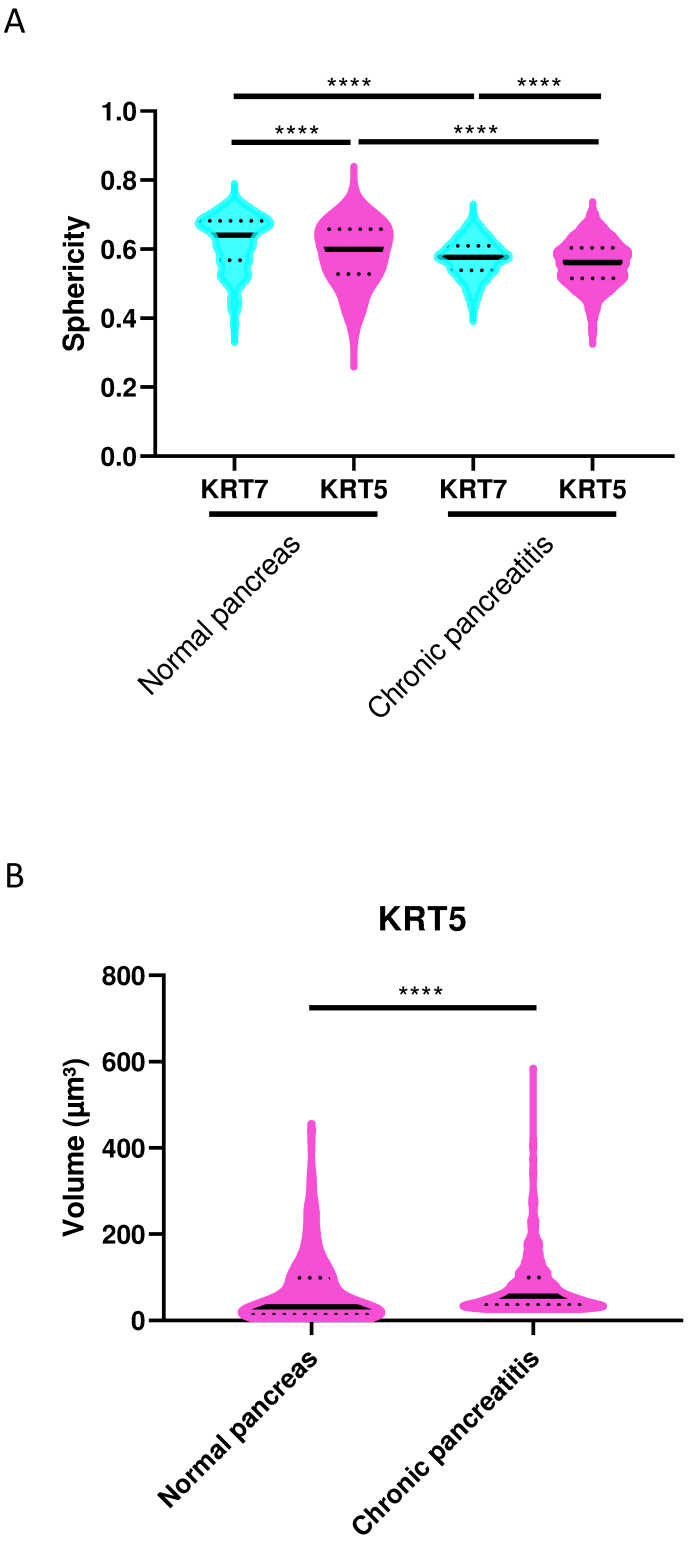
C



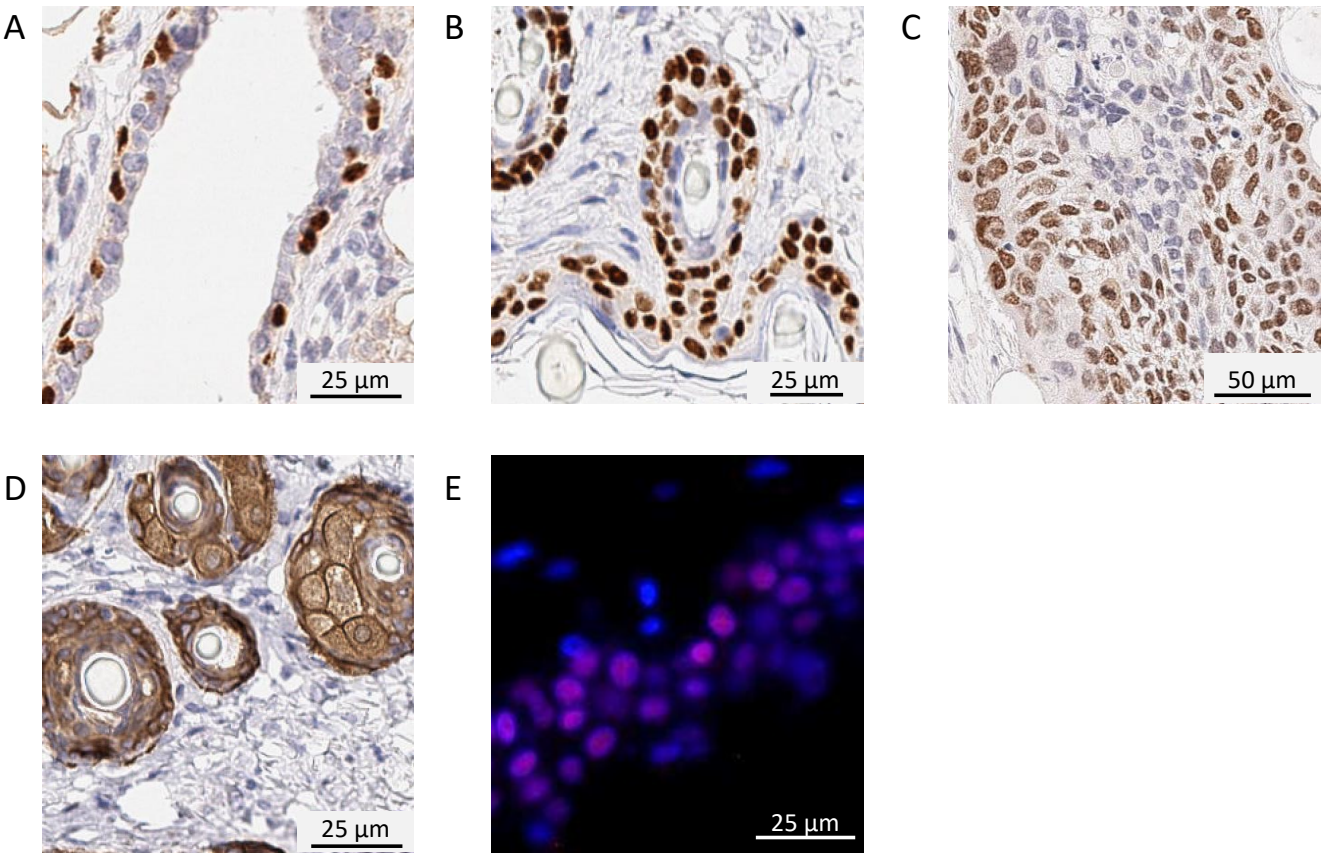
D

Protocol	Delipidation	RI matching	Duration	High resolution imaging
FLASH[35]	SDS/boric acid	Methyl Salicylate	7 days	confocal microscopy
FLIP-IT	SDS buffer	CUBIC-R/Ethyl Cinnamate(Eci)	8 days	Lightsheet miscroscopy

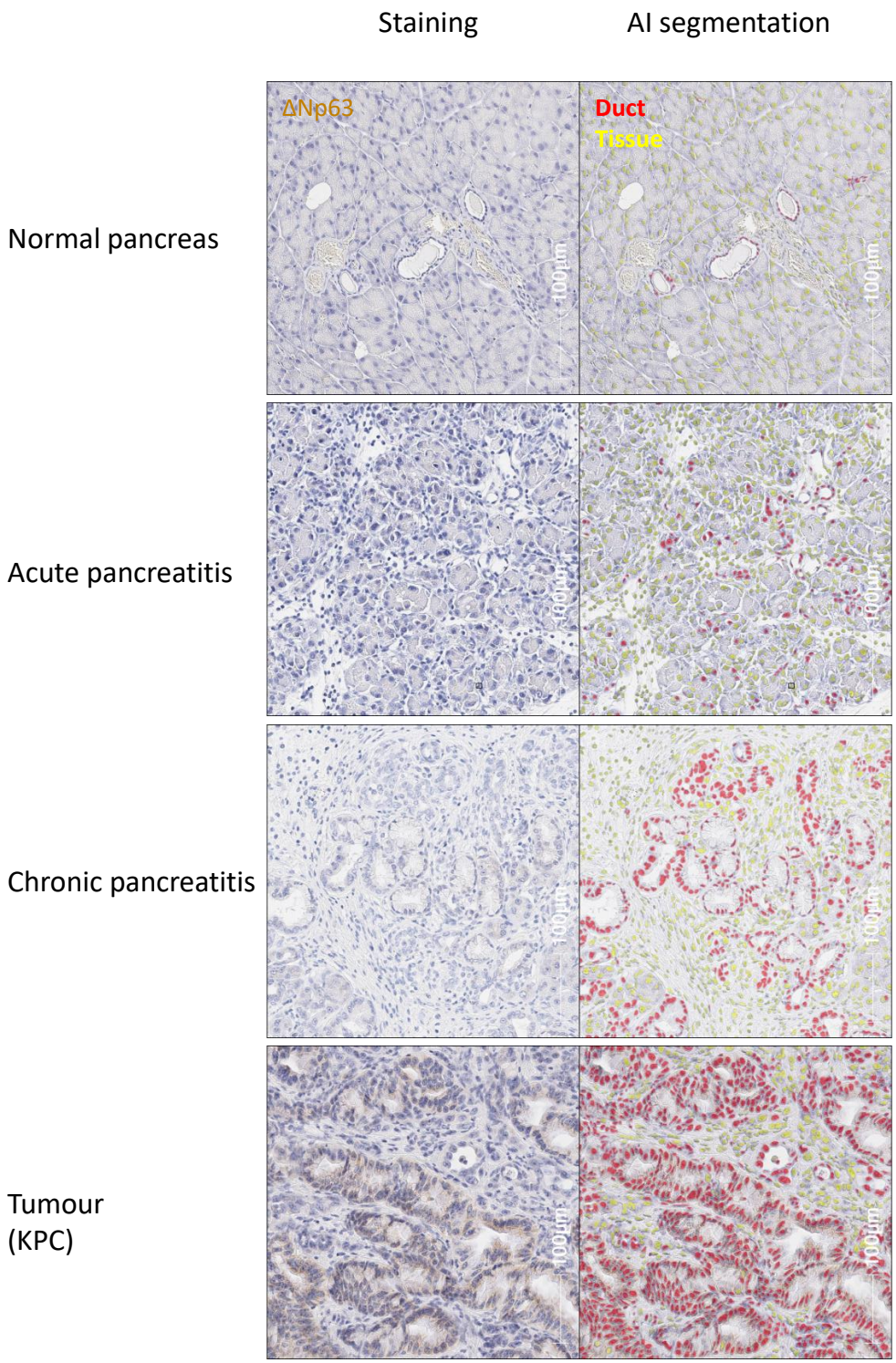
S Figure 7: FLIP-IT overview of human and mouse sample processing. (A) FLIP-IT protocol steps in archival FFPE human samples and representative pictures of the samples. Scale bars correspond to 2mm. (B) Table comparing key protocol steps for 3D human pancreas sample processing workflow. (C) FLIP-IT in fresh PFA-fixed mouse samples and representative pictures of the samples. Scale bars correspond to 5mm. (D) Table comparing key protocol steps for whole mouse pancreas sample processing workflow.



S Figure 8: KRT5⁺ cell sphericity and volume changes in chronic pancreatitis compared to normal human pancreas. (A) Sphericity quantification of KRT5⁺ and KRT7⁺ cells in both normal human pancreas and chronic pancreatitis. (B) Volume quantification of KRT5⁺ cells in both normal human pancreas and chronic pancreatitis. (****p< 0,0001; n=1; >250 cells counted per group.)

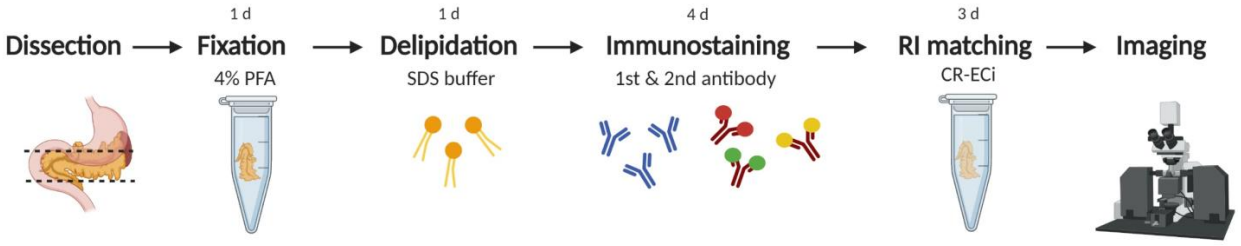


S Figure 9: The ΔNp63 and KRT14 antibodies showed strong positivity in positive control mouse tissues. (A) ΔNp63 IHC staining in a healthy mouse mammary gland, staining the myo-epithelial cells. (B) ΔNp63 IHC staining in healthy mouse skin, staining nuclei in the epidermis. (C) ΔNp63 IHC staining in a human adenosquamous tumour. (D) KRT14 IHC staining in the hair follicles of a human skin section. (E) P63 IF staining of nuclei of the basal cells in the epidermis of human skin.

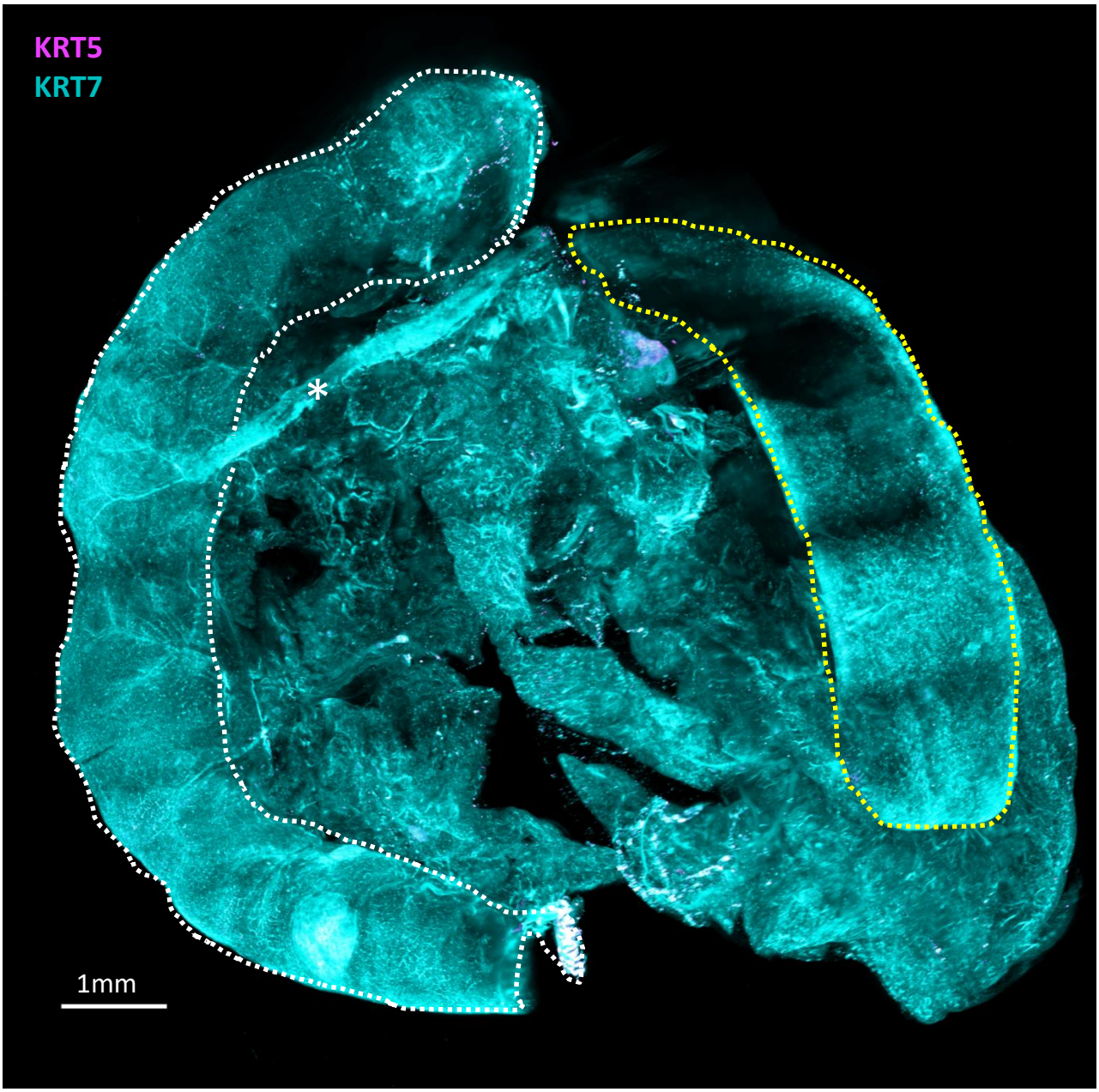


S Figure 10: ΔNp63⁺ are not found in healthy or diseased murine pancreas. Representative images of normal mouse pancreas, acute pancreatitis, chronic pancreatitis and KPC tumour model do not show staining for ΔNp63. AI segmentation shows ductal (red) and tissue cells (yellow). None of the segmented cells shows ΔNp63 positivity. n>71

A

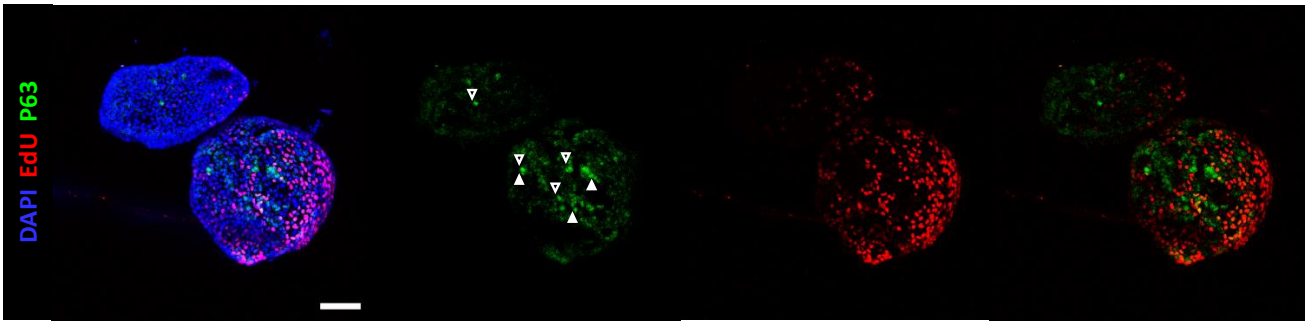

Dissection
1 d
Fixation
4% PFA
1 d
Delipidation
SDS buffer
4 d
Immunostaining
1st & 2nd antibody
3 d
RI matching
CR-ECi
Imaging

B

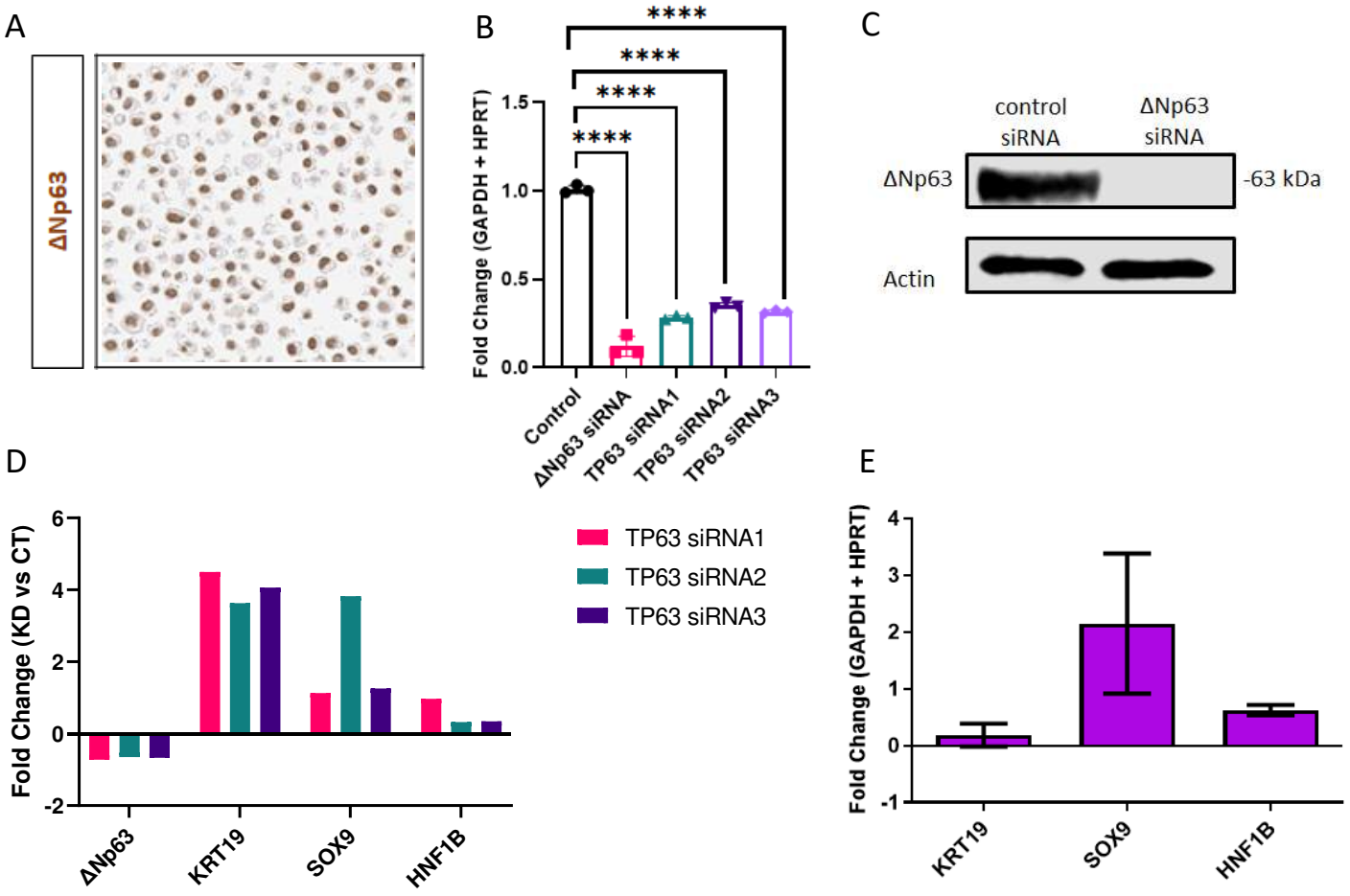

KRT5
KRT7
*
1mm

S Figure 11: FLIP-IT applied to whole mouse pancreas and attached duodenum and spleen. (A) Processing protocol of fresh mouse samples. (B) Overview 3D rendering of normal mouse pancreas stained for KRT5 (pink) and KRT7 (cyan). No KRT5^{+} were seen in the mouse pancreas. Some pink color is present in areas showing nonspecific staining (confirmed at higher magnification). Asterisk shows large duct. White dotted line shows duodenum. Yellow dotted line shows spleen. Objective 5x, zoom 0.36 . Scale bar corresponds to 1mm. n=3

A



S Figure 12: IF staining for ΔNp63 (green) and EdU (red). Nuclei are stained blue (DAPI). White arrowheads indicate $p63^+$ EdU^+ cells, whereas non-filled arrowheads indicate $P63^+$, EdU^- cells. Scale bar indicates 100 µm.



S Figure 13: Confirmation and validation of (ΔN)P63 knockdown. (A) IHC staining for ΔNp63 in HPDE cells. (B) Validation of other *TP63* siRNA's (****p< 0,0001). (C) Western blot for ΔNp63 and β-actin. (D) qRT-PCR for *ΔNp63, KRT19, SOX9* and *HNF1B*. (E) qRT-PCR analysis for *Krt19, Sox9* and *Hnf1b* in organoids derived from medium sized ducts.