# Supplementary Information

## *Ex vivo* organotypic culture system of precision-cut slices of human pancreatic ductal adenocarcinoma

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**Running title**: Pancreatic cancer in a dish

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**Key words**: Pancreas, adenocarcinoma, ex vivo, organotypic model, tissue slice technology

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# Supplementary Figure S1

## Outline of the investigations

Preliminary basic optimization steps (data not shown)

| Variables | Entities |
|---|---|
| **1. Culture medium (n=3)** | DMEM + 10% FCS *versus* CMRL1066 + 2.5% human serum AB ☑ |
| | ↓ Use CMRL1066 + 2.5% human serum AB |
| **2. Antioxidant supplement (n=3)** | Pycnogenol and/or N-acetyl cysteine ☑ *versus* No supplementation |
| | ↓ No supplementation with antioxidants |
| **3. Tissue culture insert (n=3)** | Use of insert ☑ (Millicell®, Millipore) *versus* Free-floating tissue slices in culture |
| | ↓ Use insert |
| **4. DPDS supplement (n=2)** | 100 nmol/L ☑ *versus* No supplementation |
| | ↓ Use DPDS |
| | **Culture conditions defined** |

☑ indicates entities the use of which resulted in better tissue survival
☑ indicates entities the use of which did not make any difference in tissue survival

**Characterization of tissue slices (Fig. 2-8)**

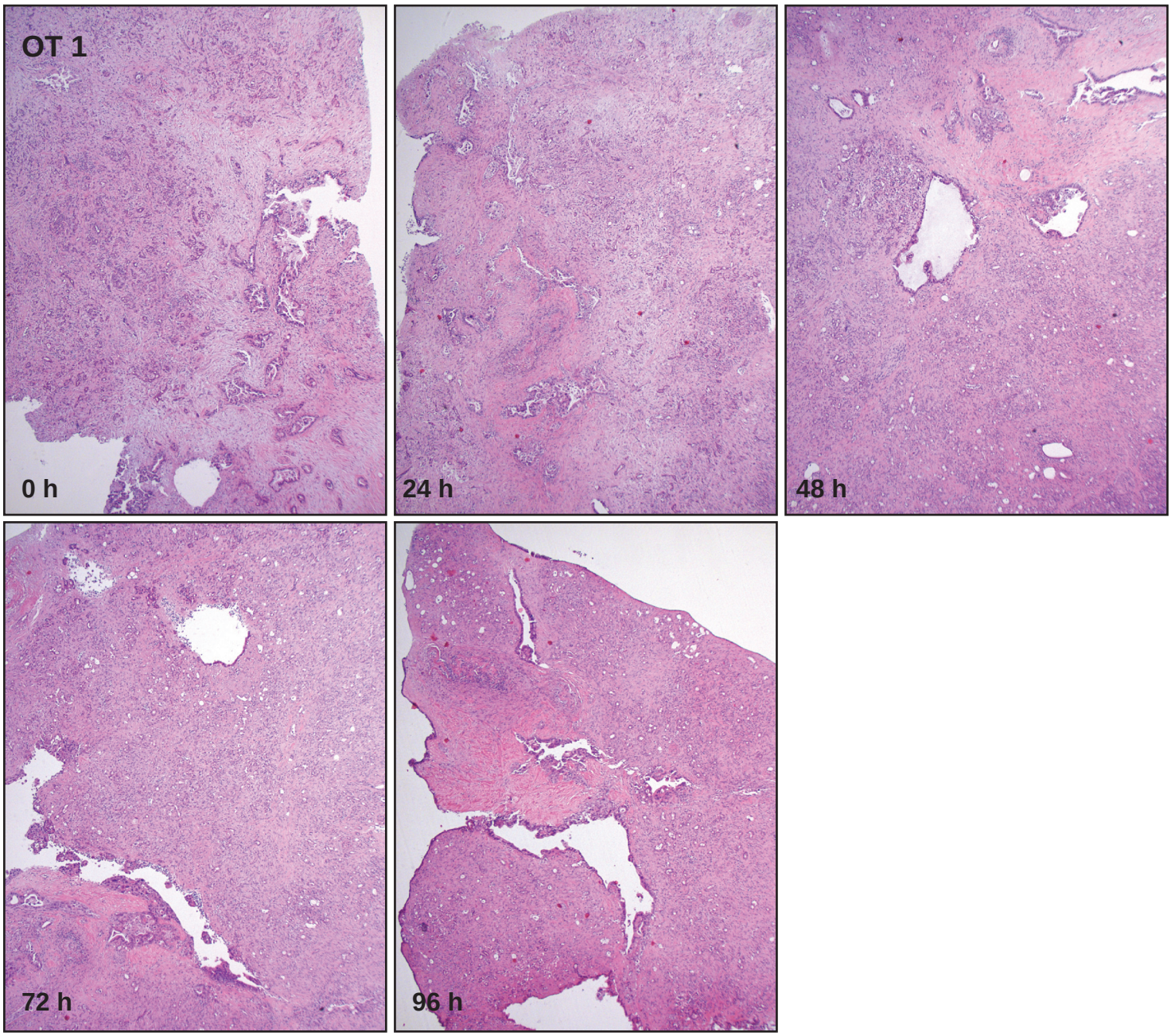
1. Tissue viability: H&E staining, TEM, quantification, n=12
2. Cell outgrowth: H&E staining, IHC, quantification, n=12
3. Proliferative activity: IHC, n=7
4. Immunohistochemical phenotyping:
   - cancer: n=2-6
   - microenvironment: n=2-6
5. Metabolic activity: quantification, n=5

**Assessment of effect of oxygen tension (Fig. 9-10)**

1. Hyperoxic (41%) versus normoxic (21%) conditions: n=3
2. Hypoxia assessment *in situ*: n=1-3
3. Histomorphological characterization: n=3
4. Tissue viability: H&E staining, quantification, n=3
5. Cell outgrowth: H&E staining, IHC, quantification, n=3

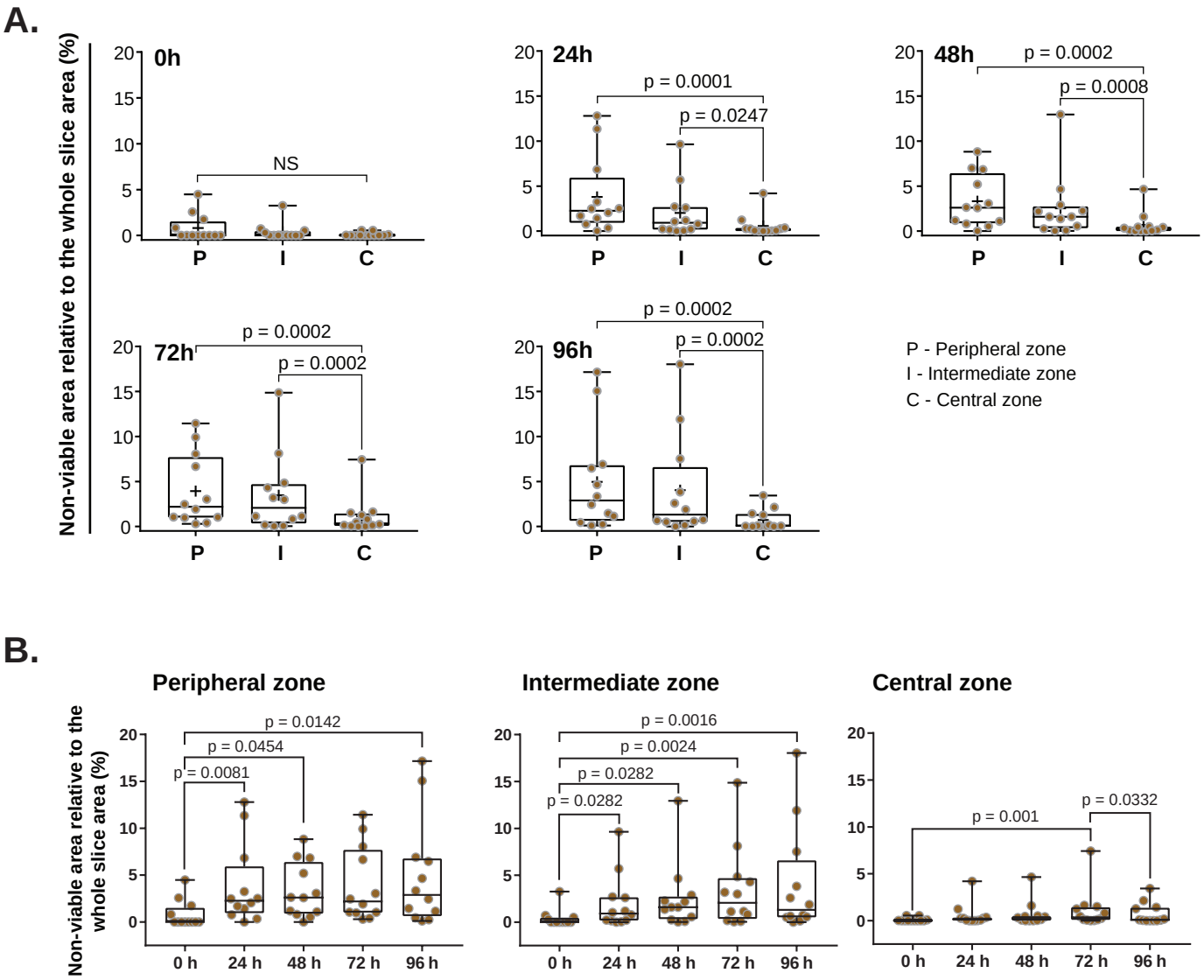
**Supplementary Figure S1.** A flow chart showing experimental progression and different endpoint analyses.

# Supplementary Figure S2



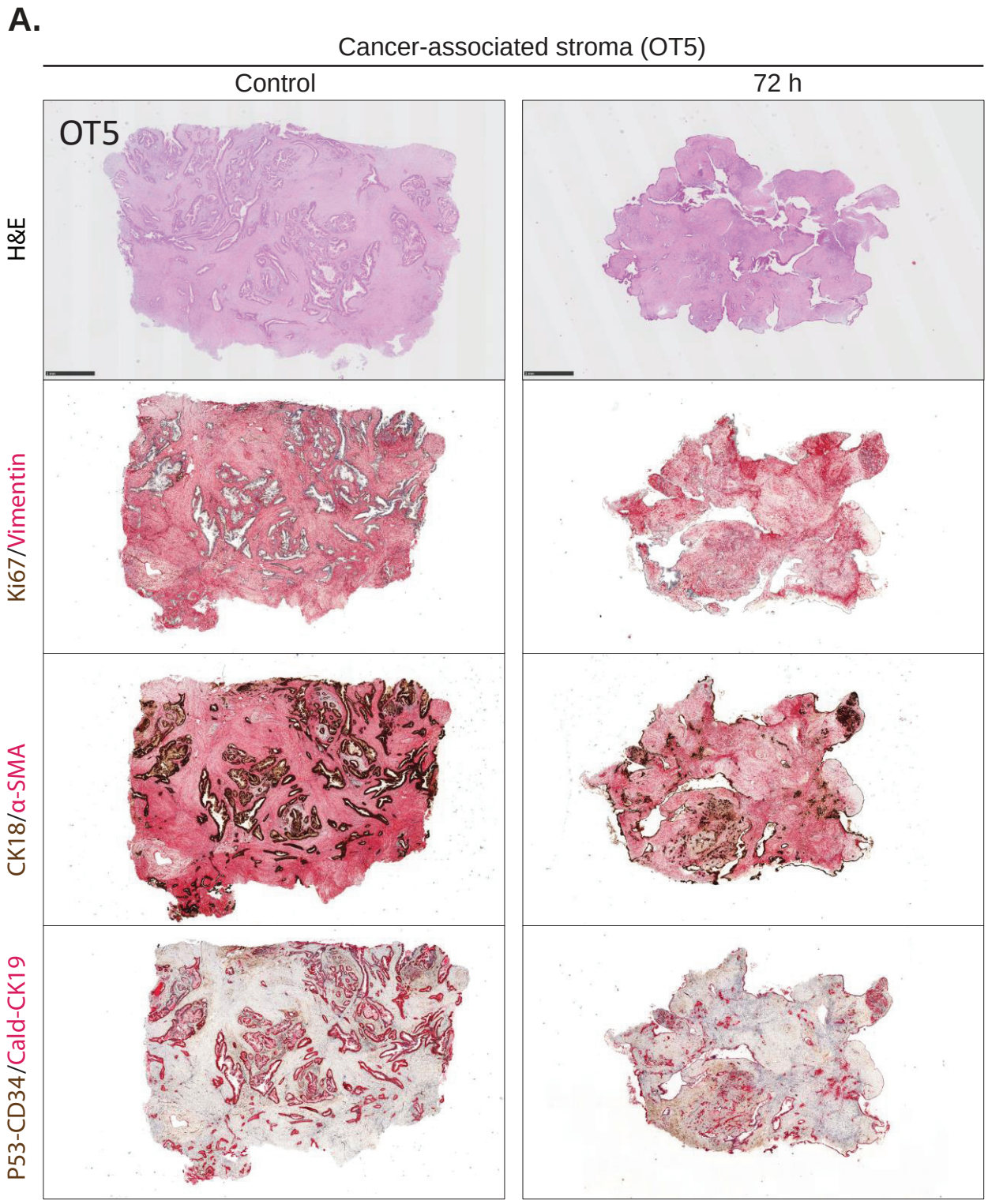

**Supplementary Figure S2**. Low magnification (4X) images of H&E-stained control (0 h) and cultured (24h -96 h) tissue slices.

# Supplementary Figure S3




**Supplementary Figure S3.** (**A**) Quantification of non-viable tissue areas at different time points and for the three tissue zones. At culture initiation (0 h), there were no significant differences in the extent of non-viable tissue areas ($p > 0.05$). At later time points (24 h – 96 h), non-viable tissue area was significantly higher in the periphery and the intermediate zones than in the central zone (n=12; Friedman test followed by two-stage linear step-up multiple comparison procedure according to Benjamini, Krieger and Yekutieli; $p < 0.05$). (**B**) Temporal changes in tissue viability within a defined zone. In all zones, significant changes in tissue viability were observed compared to the control at 0 h (similar statistical analysis as above; $p < 0.05$). Following damage within the first 24 h of culture, no further increase in non-viable tissue area could be observed, except for the central zone in which tissue viability was significantly lower at 72 h.

# Supplementary Figure S4

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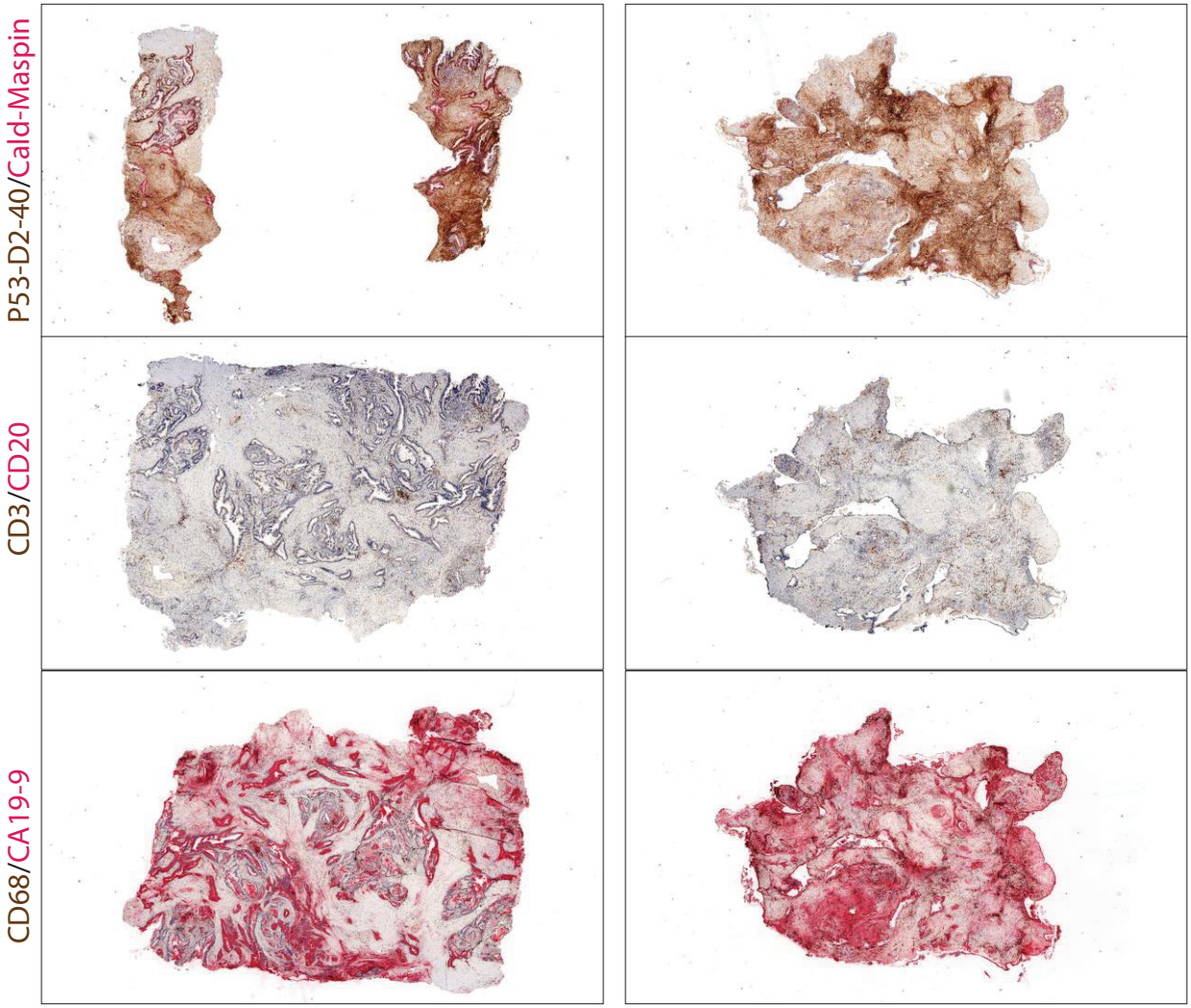
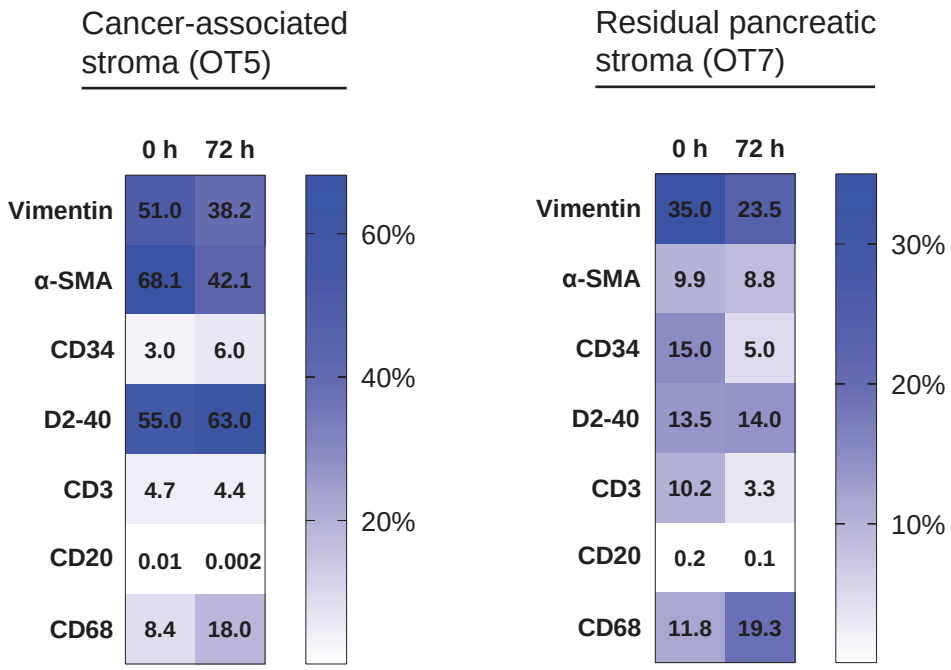
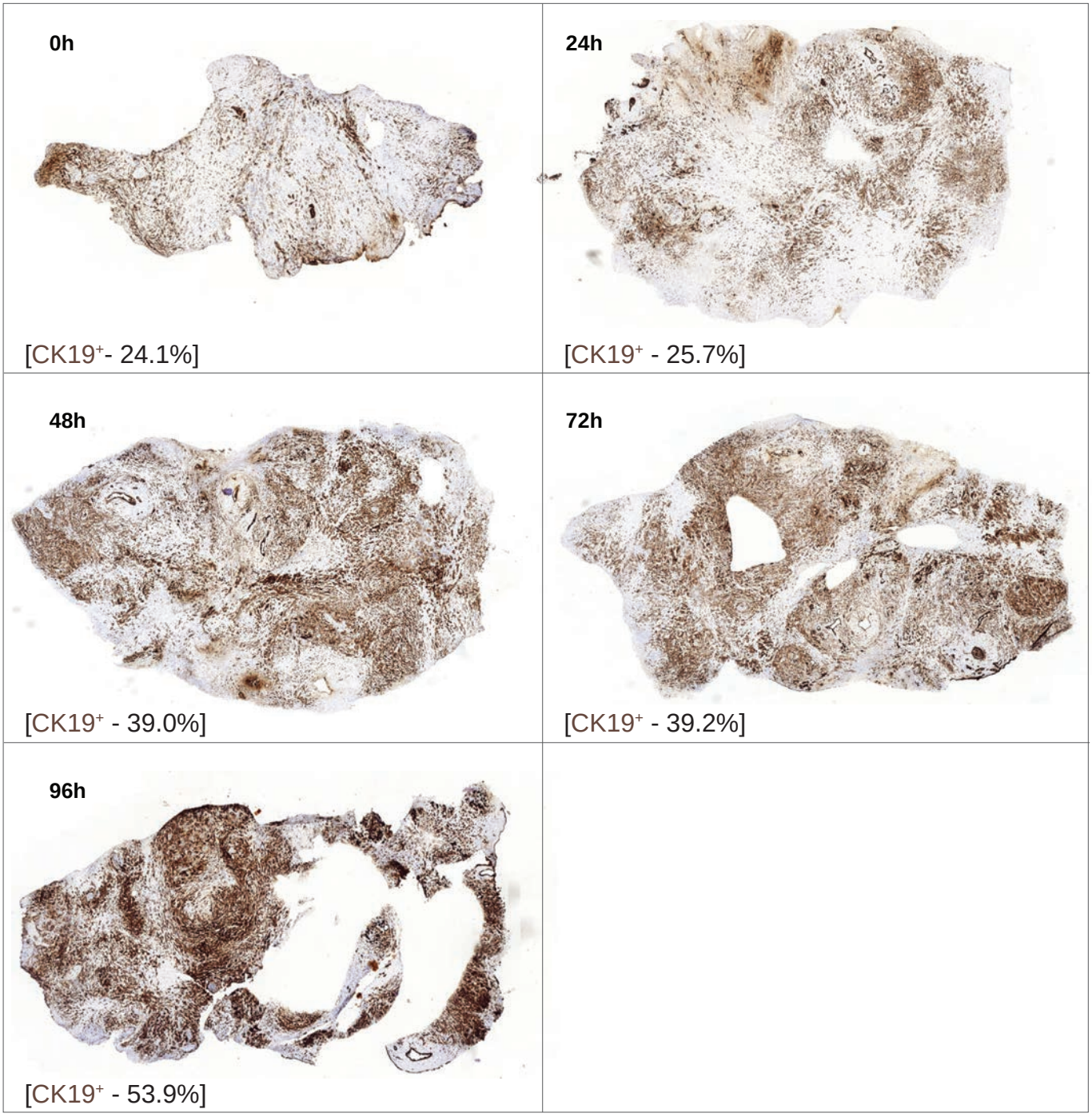

P53-D2-40/Cald-Maspin
CD3/CD20
CD68/CA19-9

**B.**


Cancer-associated stroma (OT5)
0 h
72 h
Vimentin 51.0 38.2
α-SMA 68.1 42.1
CD34 3.0 6.0
D2-40 55.0 63.0
CD3 4.7 4.4
CD20 0.01 0.002
CD68 8.4 18.0
60%
40%
20%
Residual pancreatic stroma (OT7)
0 h
72 h
Vimentin 35.0 23.5
α-SMA 9.9 8.8
CD34 15.0 5.0
D2-40 13.5 14.0
CD3 10.2 3.3
CD20 0.2 0.1
CD68 11.8 19.3
30%
20%
10%

**Supplementary Figure S4.** (**A**) Photomicrographs of control (0 h) and cultured (72 h) organotypic slices from a well differentiated tumor (OT5) stained with immunohistochemical markers for the characterization of stroma (vimentin, αSMA, CD34, D2-40) and immune cells (CD3, CD20 and CD68). (**B**) Quantification of stroma and immune markers, expressed as their extent (%) in the whole tissue area.

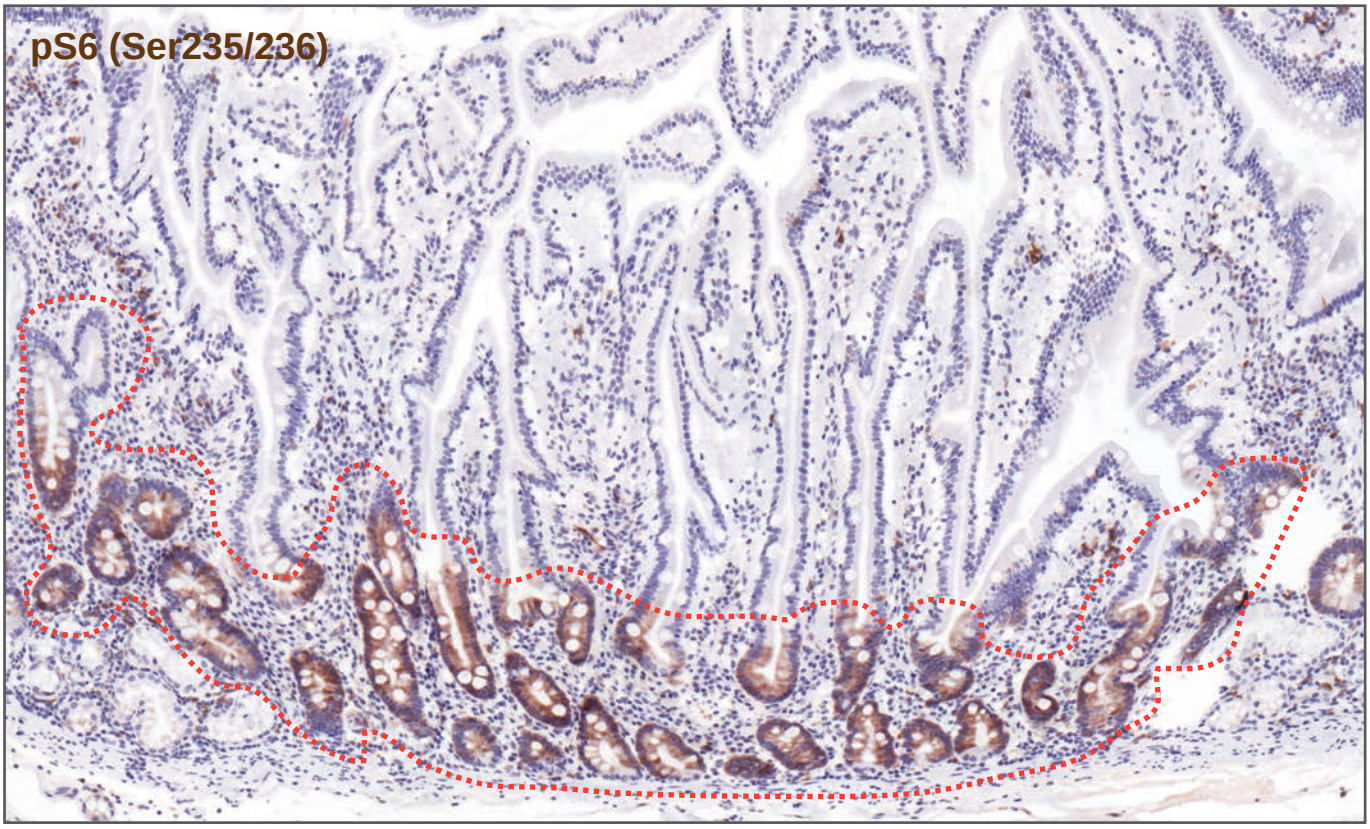
# Supplementary Figure S5



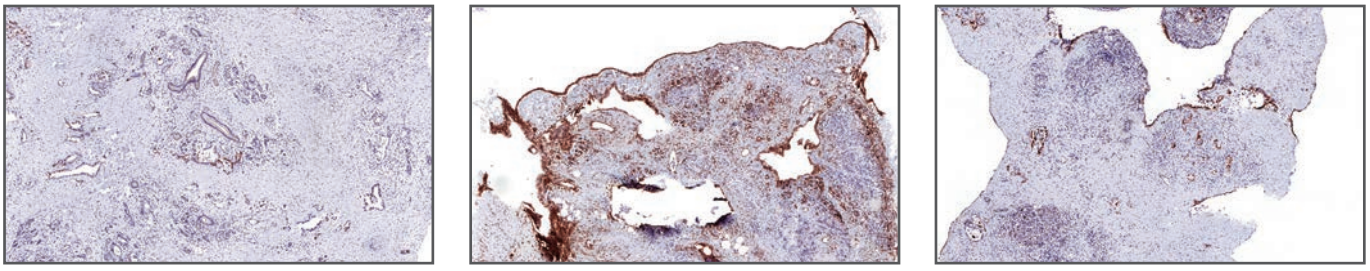

**Supplementary Figure S5.** Immunohistochemical staining for CK19 (OT2) at all time points, showing a progressive increase in the number of cancer cells within the tissue slice. CK19 positivity indicates area occupancy of cancerous cells within the tissue slices.

# Supplementary Figure S6

## A.



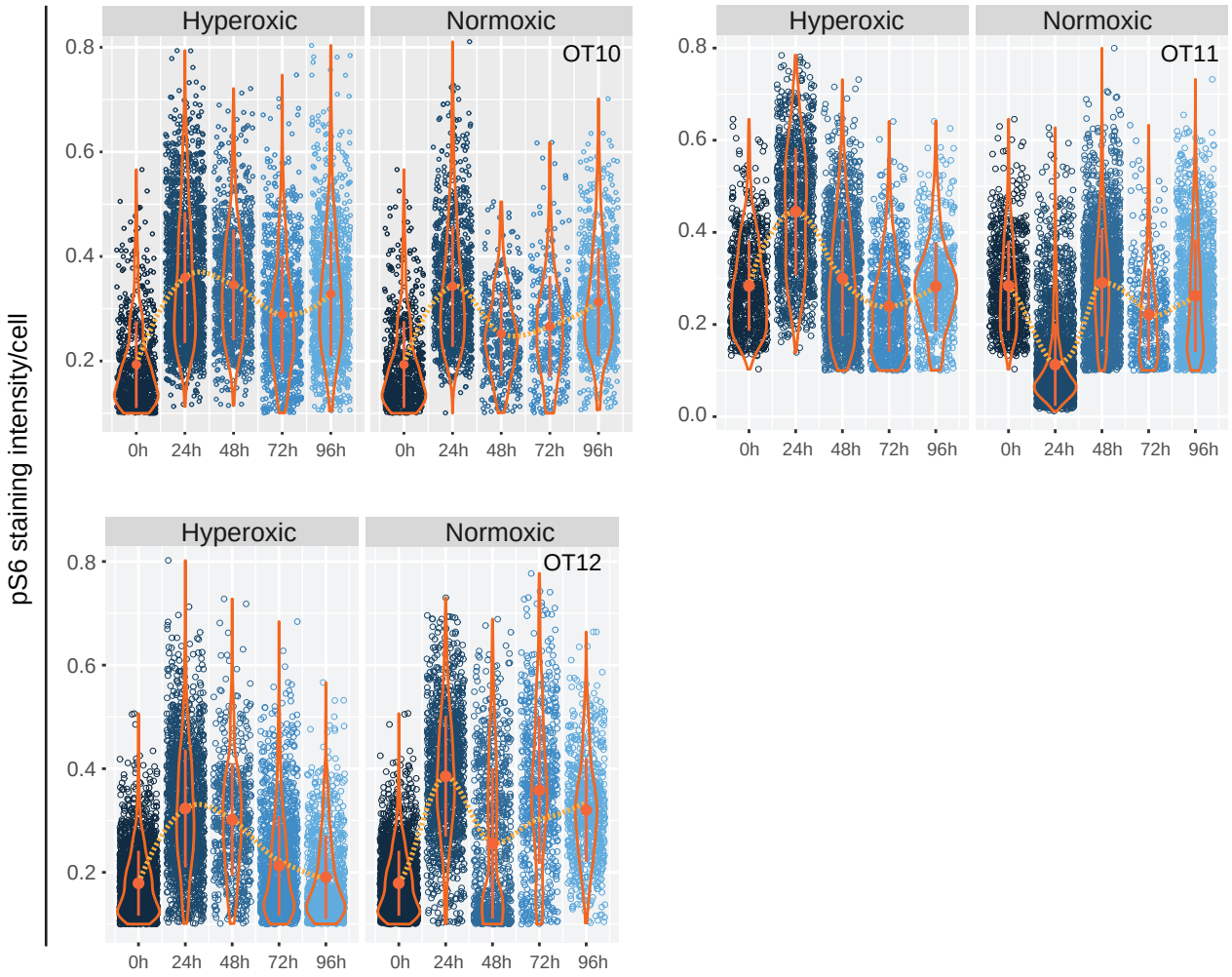

## B.



Control tissue at acquisition | Control tissue after 96 h culture | Rapamycin treatment - 72 h

**Supplementary Figure S6.** (**A**) Positive immunohistochemical staining for pS6 in duodenal crypt cells was used as a positive control. (**B**) pS6 staining in cultured tissue slice either untreated or subjected to rapamycin (50 nM) treatment for 72 h. Matched control tissue at acquisition was used to assess the pS6 positivity prior to culture.

# Supplementary Figure S7




**Supplementary Figure S7.** Temporal changes in pS6 staining intensity of cancerous cells in patient-matched tissue slices under hyperoxic or normoxic conditions (n=3). The dotted lines show the conditional mean smooth curve fitted to the data points.

**Supplementary Table 1:** Antibody list for immunohistochemical analysis.

| Antibody | Clone name | Dilution | Product code |
|---|---|---|---|
| Actin (smooth muscle) | 1A4 | 1:500 | M0851 |
| CA19-9 | CA241:5:1:4 | 1:400 | NCL-L-CA19-9 |
| CAIX | TH22 | 1:25 | NCL-L-CAIX |
| Caldesmon | h-CD | 1:300 | M3557 |
| CD20 | L26 | 1:1000 | M0755 |
| CD3 | LN10 | 1:100 | NCL-L-CD3-565 |
| CD34 | QBEnd/10 | 1:50 | M7165 |
| CD68 | PG-M1 | 1:100 | M0876 |
| CK18 | DC-10 | 1:50 | M7010 |
| CK19 | b170 | 1:100 | NCL-CK19 |
| D2-40 | D2-40 | 1:50 | M3619 |
| Ki-67 | MIB-1 | 1:100 | M7240 |
| Maspin | G167-70 | 1:300 | BDP 554292 |
| p53 | DO-7 | 1:300 | NCL-L-p53-DO7 |
| SMAD4 | B-8 | 1:300 | sc-7966 |
| pS6 ribosomal protein | | 1:100 | 2211S |
| Trypsin | Not available | 1:100000 | MAB1482 |
| Vimentin | V9 | 1:1500 | M0725 |

Antibody suppliers:

BDP: BD Pharmingen - BD Biosciences, Franklin Lakes, New Jersey, United States.
M: Dako - Agilent: Dako, Glostrup, Denmark.
MAB: Millipore – Merck, Billerica, Massachusetts, United States.
NCL: Novocastra Leica Biosystems Ltd, Newcastle Upon Tyne, United Kingdom.
sc: Santa Cruz Biotechnology, Inc., Dallas, United States.
pS6 – Cell Signaling Technology, Leiden, Netherlands.

All listed antibodies were stained using a Leica BOND III automated immunostainer, with exception of CAIX and pS6 that were stained in a Ventana Benchmark Ultra immunostainer.