

Supplementary Figure 1. Effect of MMC on proliferation of HPPL. HPPL was plated on 6 well dishes coated with laminin at a density of 5×10^4 cells/well 1 day before the addition of MMC. After incubation of culture in the presence of MMC for 6 hours, medium containing MMC was replaced with fresh medium. At 0, 1 and 2 days, culture was treated with trypsin and then the number of cell from each well was counted.

Supplementary Figure 2. Time course of tubular morphogenesis between 14 and 23 hours after the overlay. X-Y images of vertical X-Z sections in Fig. 3B at the level of top and bottom of the cell layers are shown in panels 2. Boxes in X-Y images in low magnification (panels 1) indicate that the right side of the tubular structure was focused in Fig. 3B.

Supplementary Figure 3. Uptake of rhodamine 123 by HPPL in sandwich culture. Rhodamine 123 (Rho123) was added to culture at 4 days after the overlay. After 1 hour of incubation at 37°C, images were taken on a confocal microscope. Rho123 was accumulated in cytosol but not in the apical luminal space.

Supplementary Figure 4. Analysis for apoptosis in perinatal liver. Frozen sections of E18, P5, and P10 livers were incubated with anti-EpCAM, a cholangiocyte marker, and anti-cleaved Caspase 3. Arrows indicate apoptotic cholangiocytes, whereas arrowheads indicate apoptotic cells other than cholangiocytes.

Supplementary Movies

At 2 hours after the overlay, we started taking images at 16 different places along the Z-axis at a selected area. We selected the third and seventh X-Y planes and arranged the images into movies. Movie 1 is from X-Y images at the Z7 plane. This movie shows HPPL in the original monolayer. In addition to abundant cellular movements, some cells disappeared from this movie. Movie 2 is from X-Y images at the Z3 plane. This movie shows a X-Y plane above the original monolayer where no cells were detected at the initial time point. Later, the cells that disappeared from movie 1 appeared in this movie, indicating these cells moved up along the Z-axis.

Supplementary Figure 1

Plate cells



-1 day

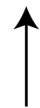


0day

Count cells



1day



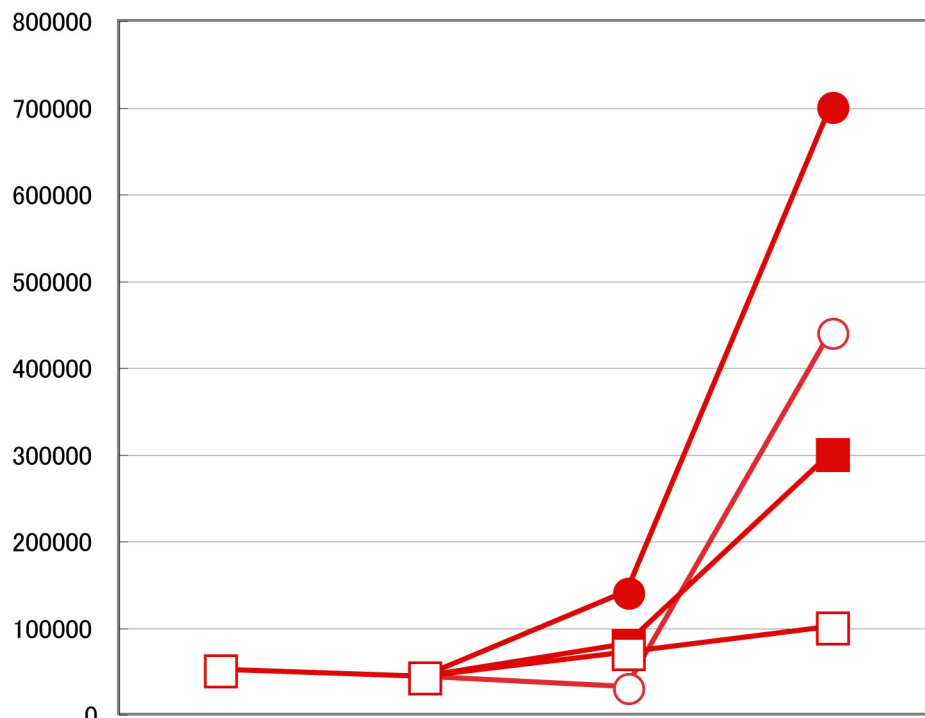
2day

Culture with MMC



6 hours

Number of cells



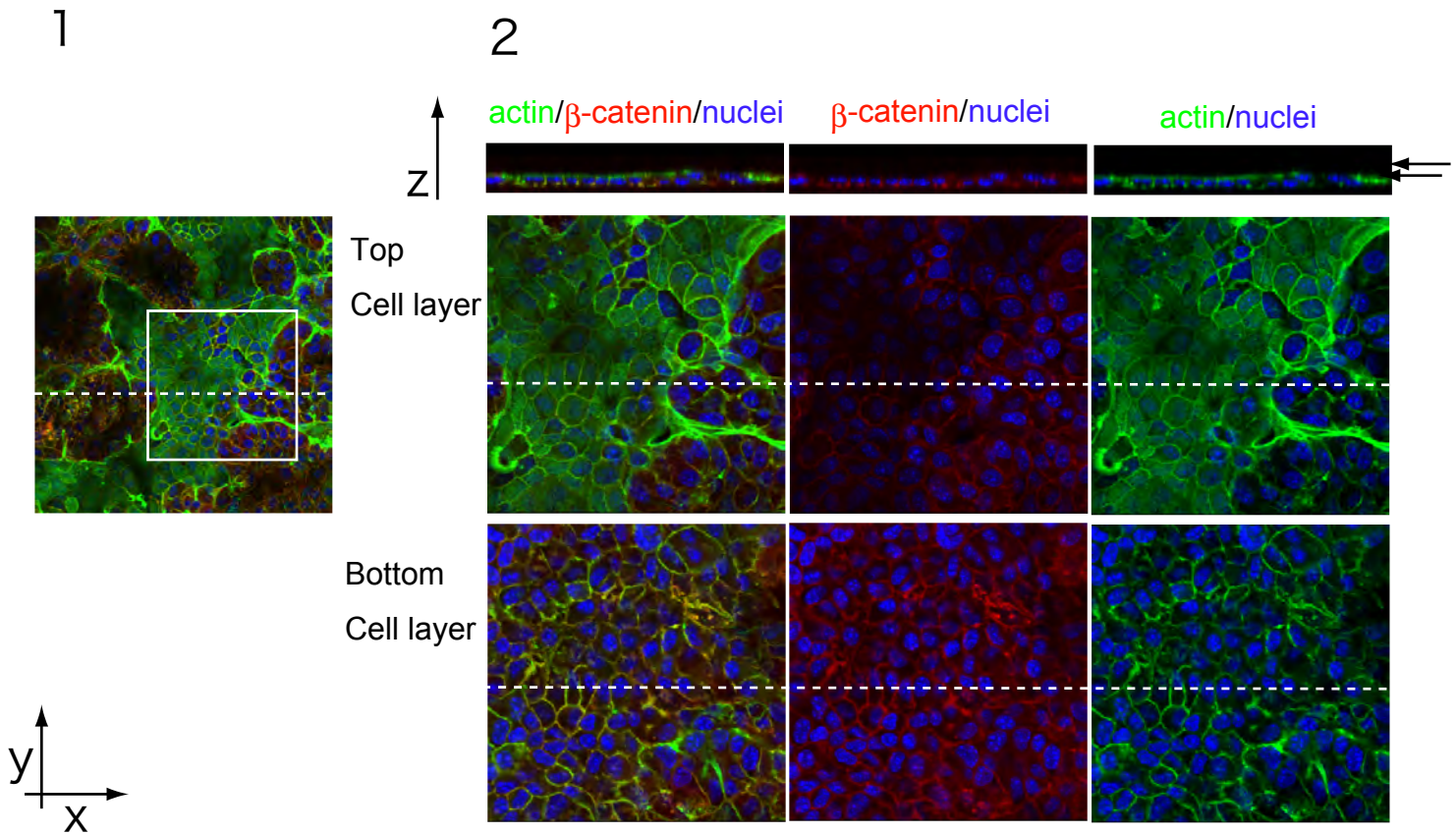
- Control
- 0.5 $\mu\text{g/ml}$ MMC
- 1 $\mu\text{g/ml}$ MMC
- 2 $\mu\text{g/ml}$ MMC

-1 0 1 2

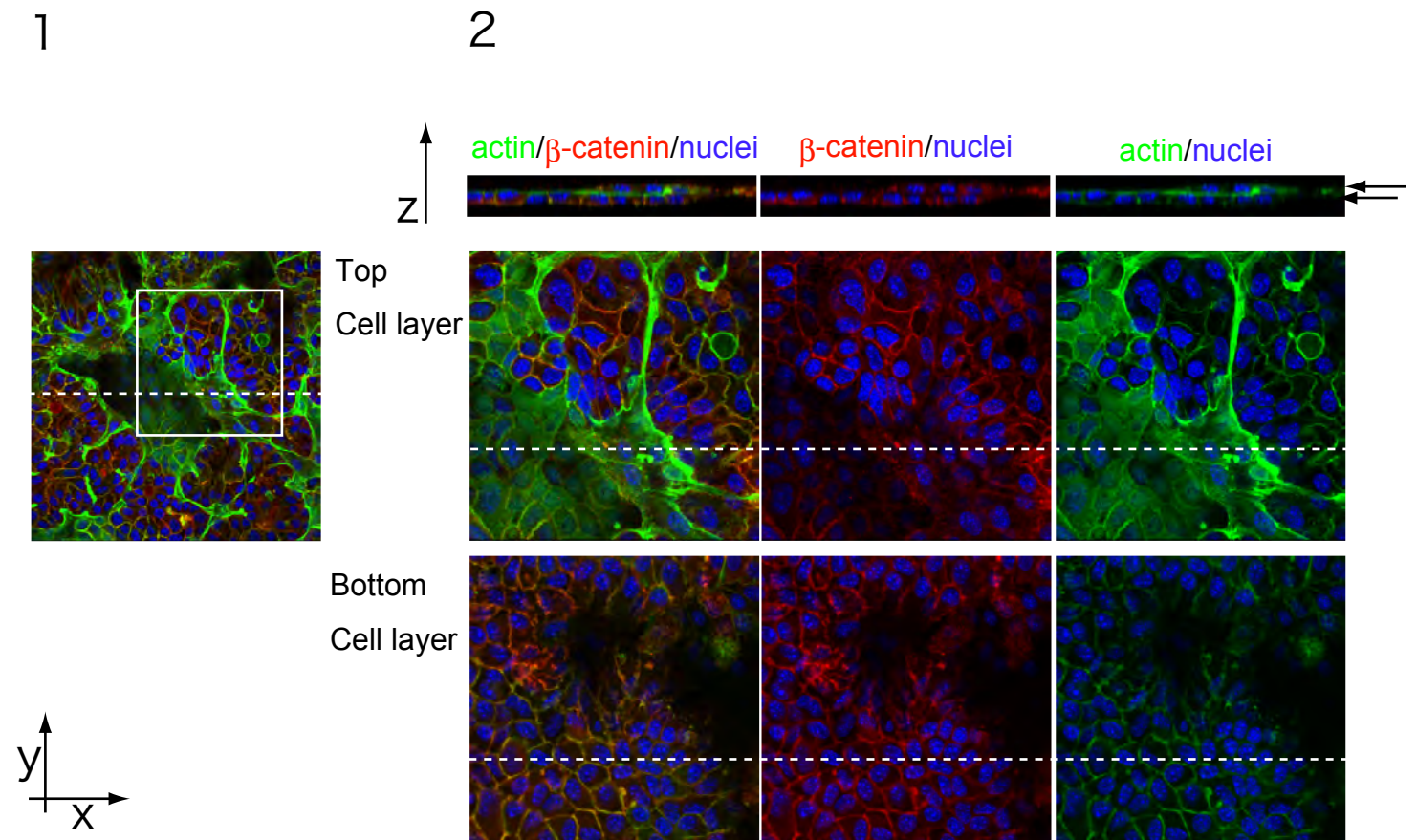
Incubation (days)

Supplementary Fig. 2

A. 14 hours after the overlay



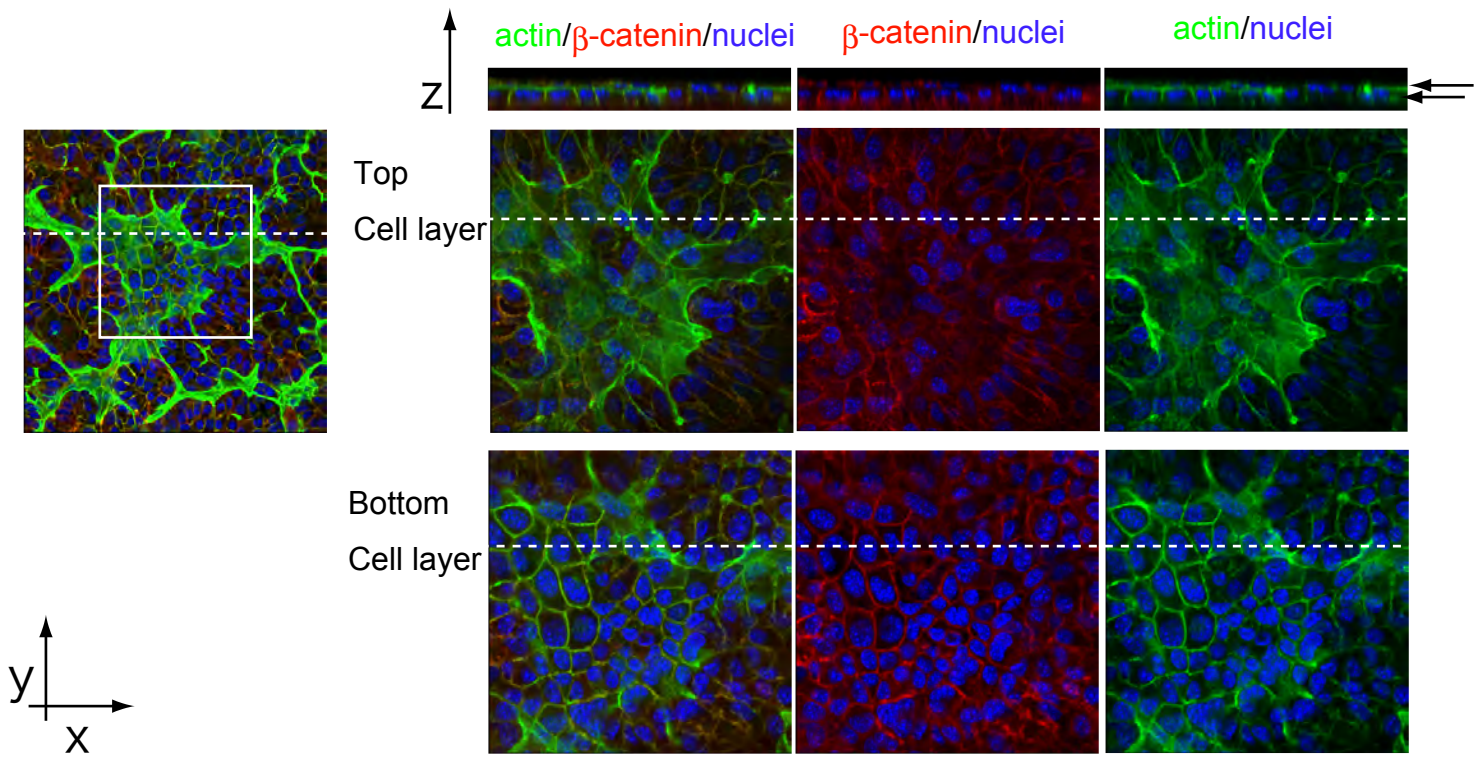
B. 17 hours after the overlay



C. 19 hours after the overlay

1

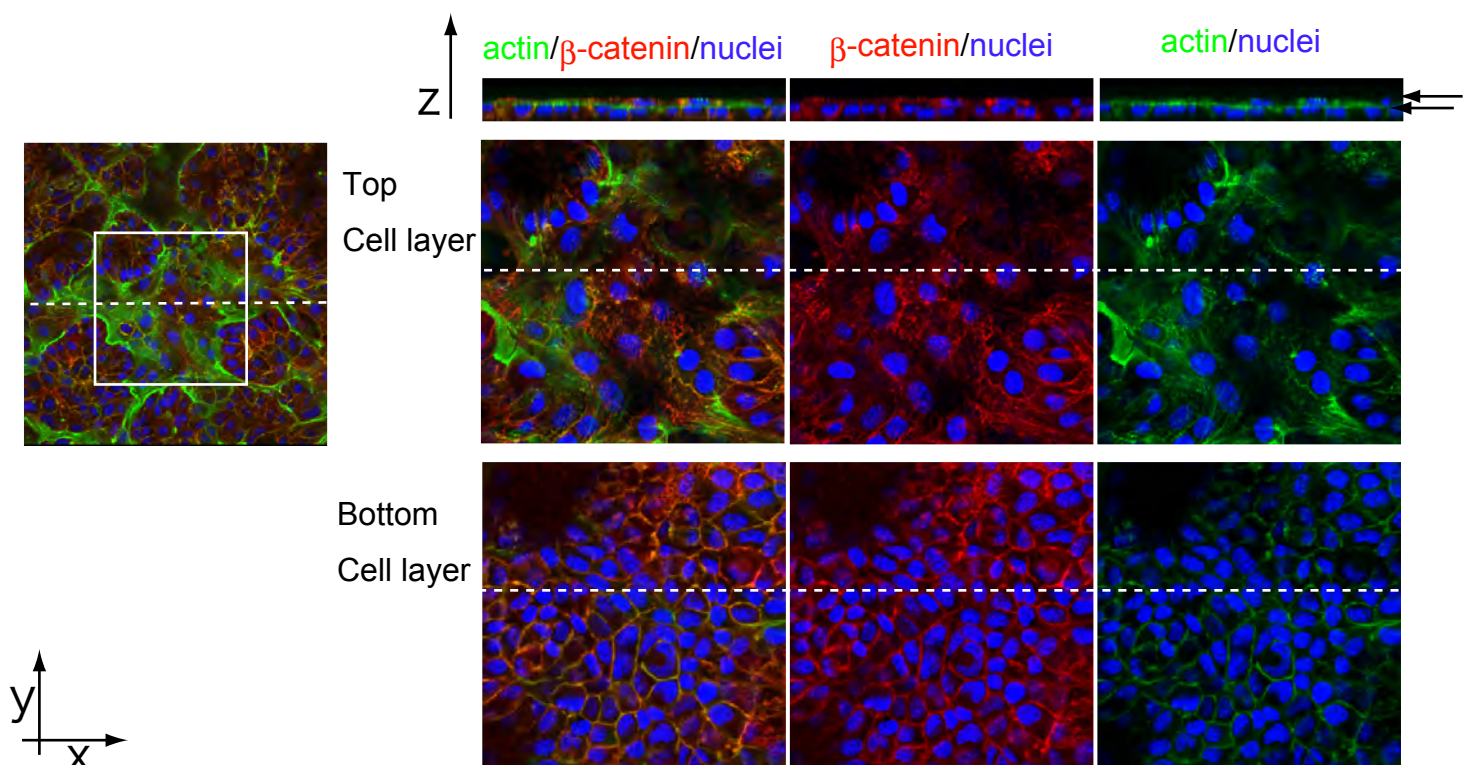
2



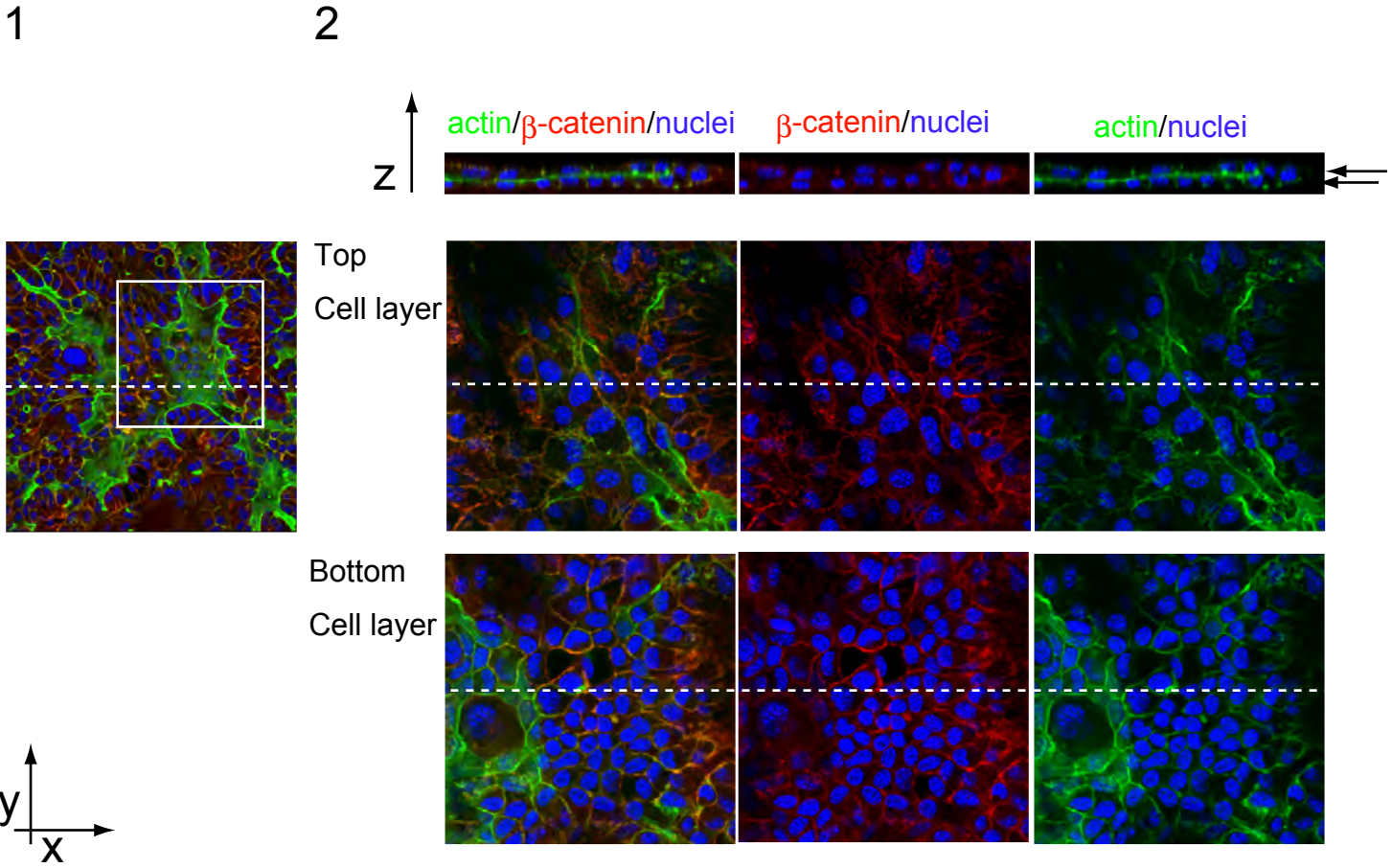
D. 21 hours after the overlay

1

2

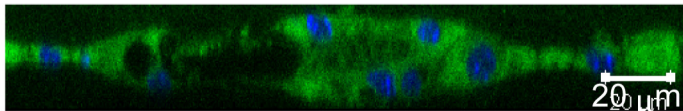


E. 23 hours after the overlay



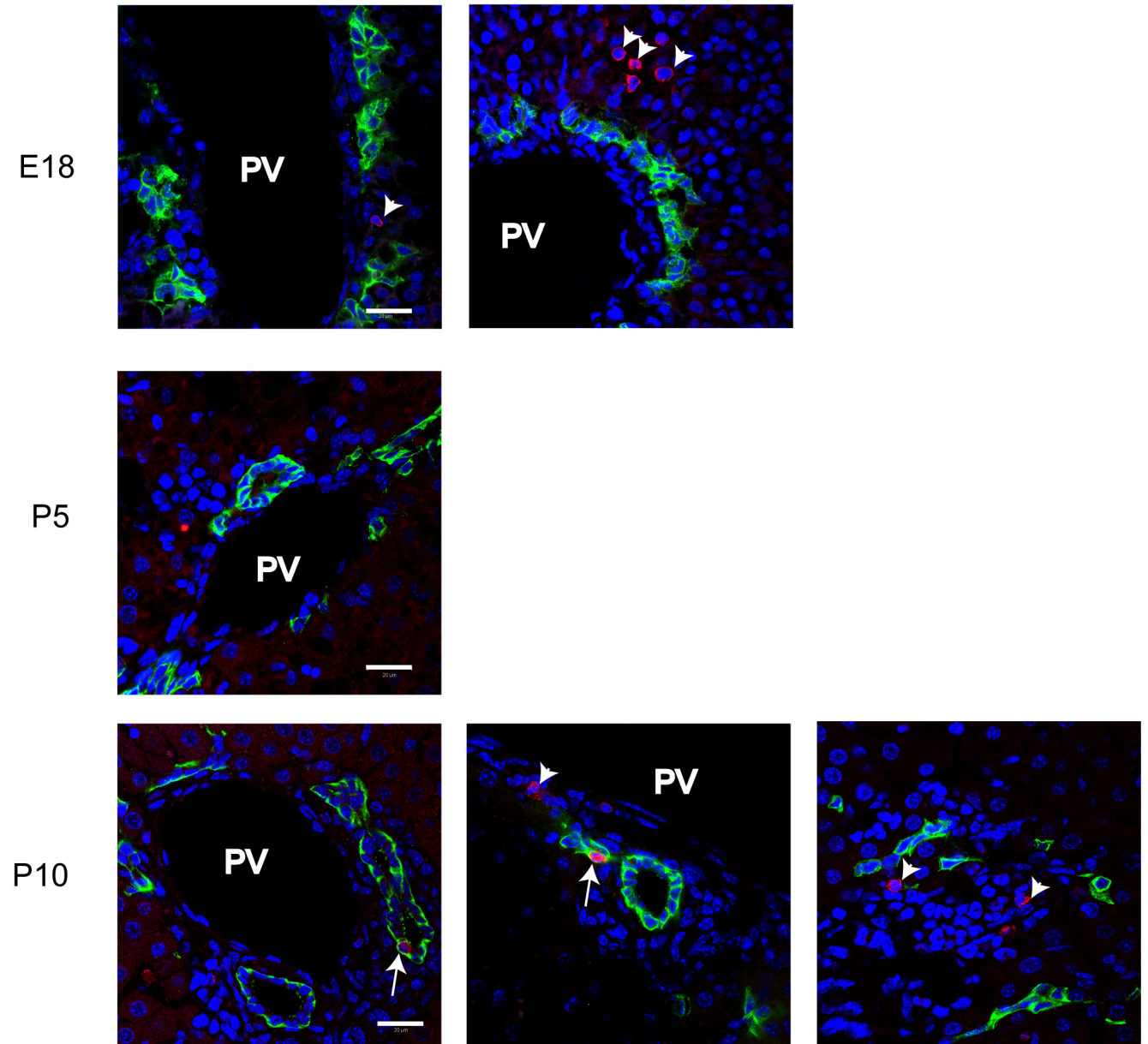
Supplementary Figure 3

Rho123/Nuclei



Supplementary Figure 4

EpCAM/Cleaved Caspase3/Nuclei



EpCAM+ cells: cholangiocytes

Cleaved Caspase 3+ cell: apoptotic cells

← EpCAM+ Cleaved Caspase 3+ cells

◀ EpCAM- Cleaved Caspase 3+ cells