**Self-organogenesis from 2D micropatterns to 3D biomimetic biliary trees**

Supplementary information

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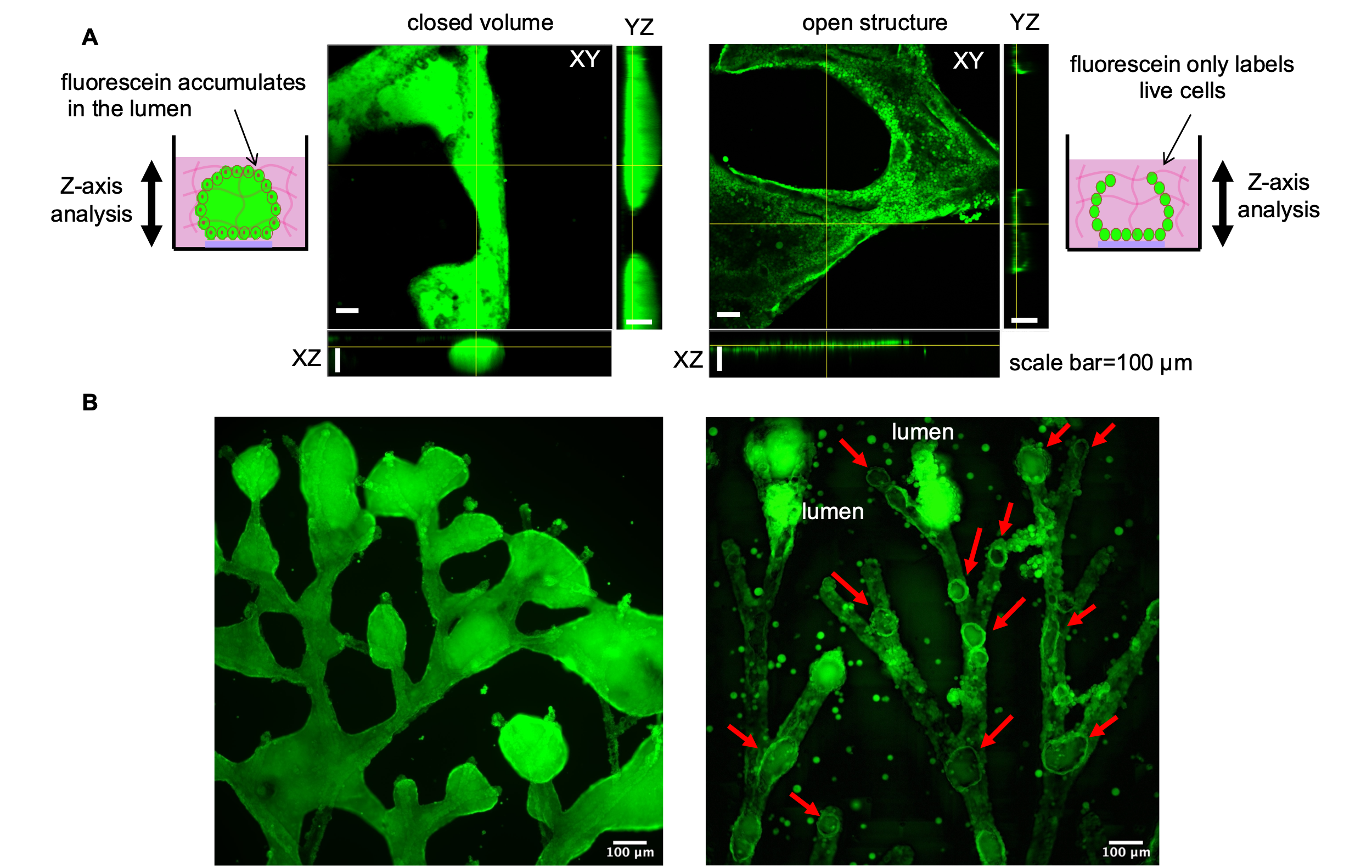
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**Supplementary Figures**

**Supplementary Figure 1**

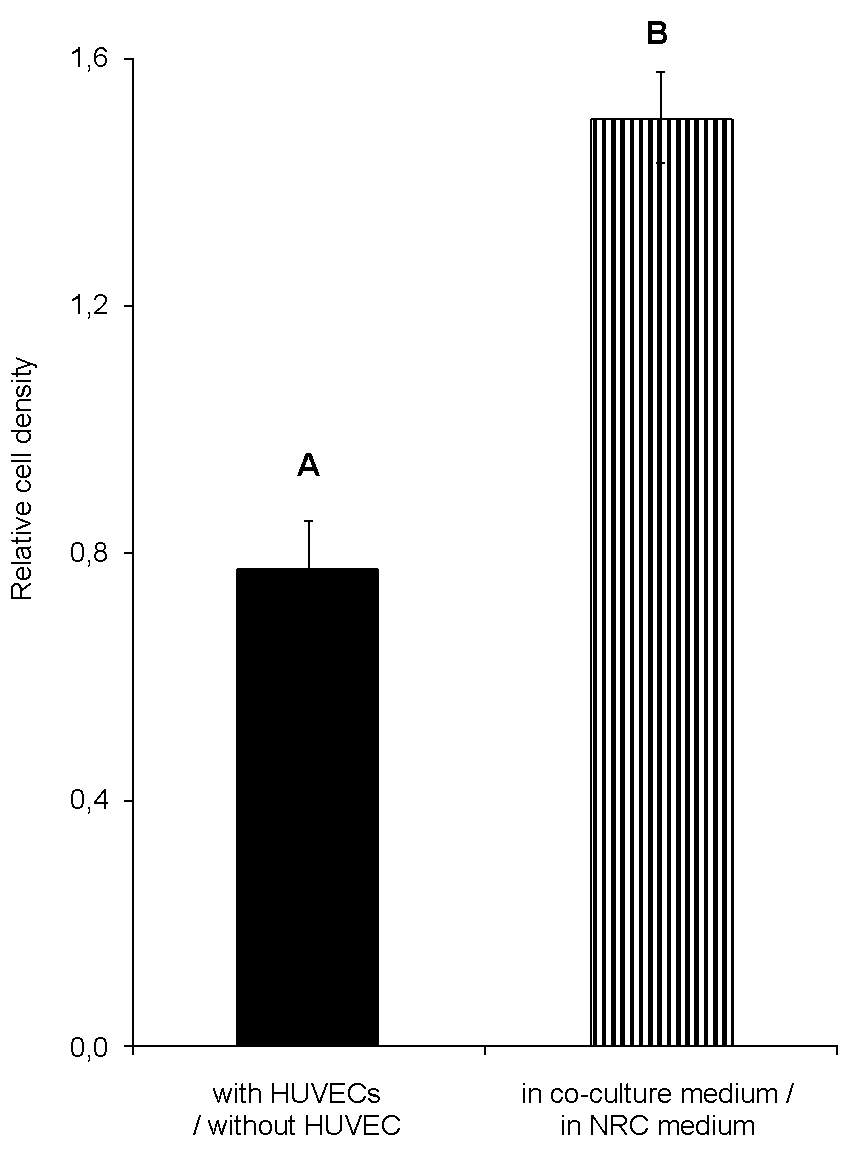


**Supplementary Fig. 1. Characterization of biliary trees**

(A) Orthogonal views of biliary tree branches after a fluorescein secretion test. Images show a closed volume secreting fluorescein (left) *vs* an open structure (incomplete organogenesis) labeling only live cells (right). These were for systematic analysis of lumen occurrence and geometrical characterization. (B) Fluorescence microscopy images of a biliary tree formed in a co-culture of NRCs/HUVECs at a 10:1 ratio revealing a continuous luminal network secreting fluorescein (left) *vs* a biliary tree formed in a monoculture of NRCs (right) showing a discontinuous luminal network (red arrows).

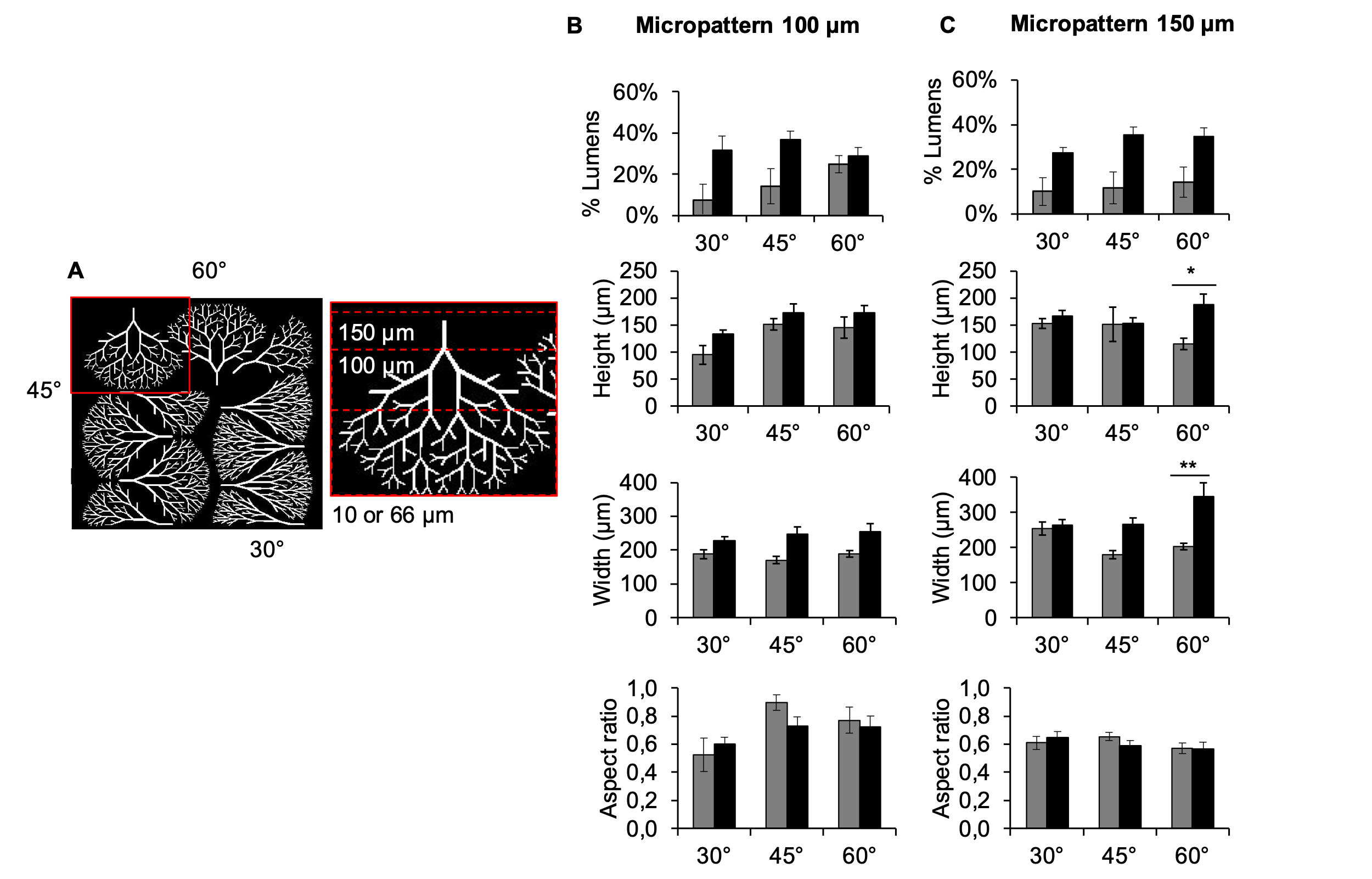
**Supplementary video 1. Kinetics of fluorescein secretion in a bile duct network.** Fluorescein accumulation in a tubular network formed in a 10:1 NRC/HUVEC co-culture in co-culture medium at day 10 post-HUVEC seeding with superimposition of DIC and fluorescence images. Images were captured every minute over one hour. Scale bar=100 µm.

**Supplementary Figure 2**



**Supplementary Fig. 2. NRC proliferation in the different culture conditions.** Bar graphs showing relative cell density of (A) NRCs with or without HUVECs in the co-culture medium (N=6), calculated from densities at day 1 post-NRC seeding and (B) NRCs in the co-culture medium *vs* in NRC medium (N=2), calculated from densities at day 4 and day 7 post-NRC seeding.

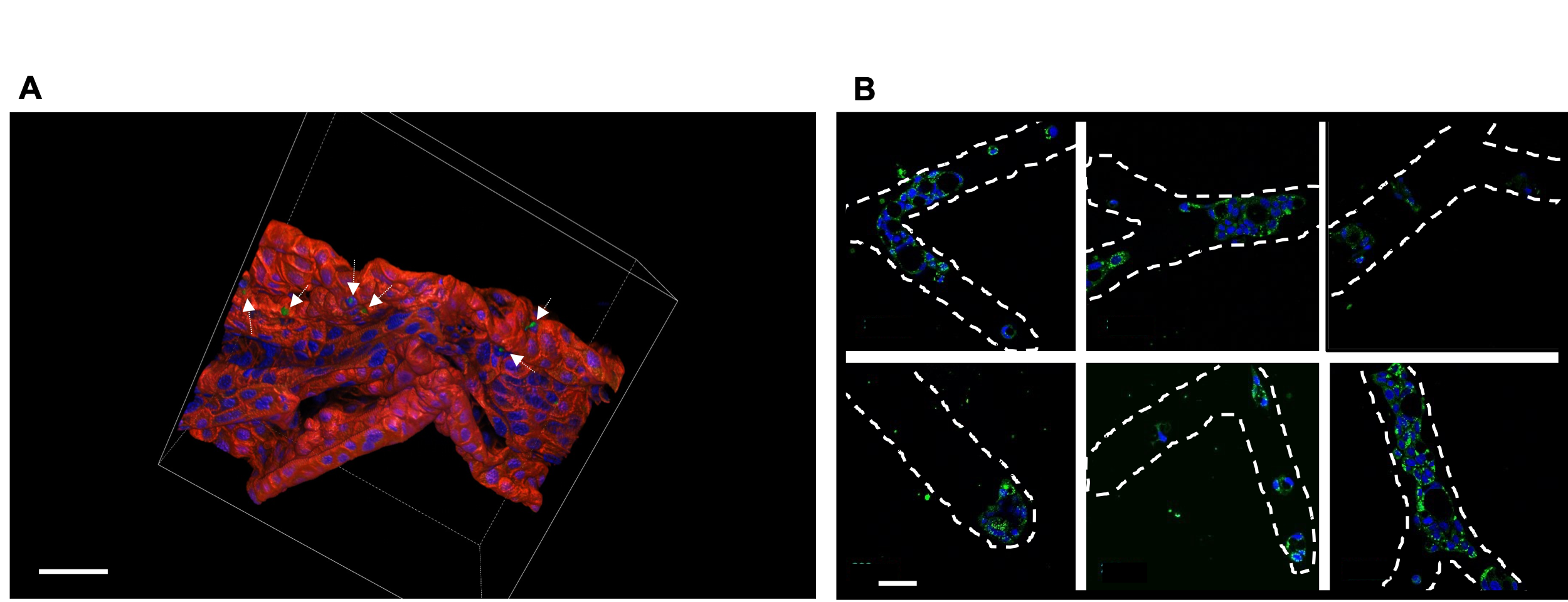
**Supplementary Figure 3**



**Supplementary Fig. 3. Characterization of lumen occurrence and tube geometry as a function of micropattern configuration.** Confocal pictures of fluorescein-stained tubular networks at day 10 post-HUVEC seeding in NRC/HUVEC 10:1 co-culture and in a NRC monoculture were analyzed. Bar graphs of height, width, and aspect ratio of luminal structures formed on (B) 100 µm-micropattern widths (mean ± SEM), (C) 150 µm-micropattern widths (mean ± SEM), height 150 µm 60°: p= 0.0337<0.05\*, width 150 µm 60°: p= 0.0011<0.01\*\*.

**Supplementary video 2. A biliary tree detached from a 60° angle-micropattern and secreting fluorescein in its luminal network.** One image is taken every minute during 30 min to observe the filling of the lumen with fluorescein. Scale bar=1 mm.

**Supplementary Figure 4**



**Supplementary Fig. 4. Localization of HUVECs on the detached trees and on micropatterns after tree detachment.** Biliary networks from NRC/HUVEC 10:1 co-culture (>10 days old) were detached and micropatterns and trees were fixed and probed for nuclei. Confocal pictures showing the positioning of HUVECs-GFP on (A) the detached tree, white arrows (scale bar= 50 µm) and on (B) the micropattern (scale bar=200 µm).

**Supplementary Table 1**

|  |  |  |  |
| --- | --- | --- | --- |
| Antibody | Catalog number | Company | Dilution |
| CK7 | Sc-23876 | Santa Cruz Biotechnology | 1:100 |
| CK19 | Sc-374192 | Santa Cruz Biotechnology | 1:100 |
| Epcam | Sc-66020 | Santa Cruz Biotechnology | 1:10 |
| Osteopontin | Ab-63856 | Abcam | 1:80 |
| PKCζ | Sc-216 | Santa Cruz Biotechnology | 1:100 |
| Plakoglobin | 13-8500 | Invitrogen | 1:100 |
| ZO-1 |  | In house made | undiluted |
| Acetylated α tubulin | 5335 | Cell Signaling | 1:10 |
| Goat anti mouse IgG (H+L) plus 647 | A32728 | Invitrogen | 1:400 |
| Goat anti-rabbit IgG (H+L) 488 | A11034 | Invitrogen | 1:400 |
| Goat anti-rabbit IgG (H+L) 568 | A11011 | Invitrogen | 1:400 |
| Goat anti-rat IgG (H+L) 488 | A11006 | Invitrogen | 1:400 |
| Goat anti-rabbit IgG (H+L) plus 647 | A32733 | Invitrogen | 1:400 |
| Goat anti-mouse IgG1 (H+L) 568 | A21124 | Invitrogen | 1:400 |
| Goat anti-mouse IgG2a (H+L) 633 | A21136 | Invitrogen | 1:400 |
| Goat anti-mouse IgG2b (H+L) 568 | A21144 | Invitrogen | 1:400 |
| Goat anti-mouse IgG1 (H+L) 488 | A32723 | Invitrogen | 1:400 |

**Supplementary Table 1. Antibody list.** Information regarding the primary and secondary antibodies used in the immunofluorescence assays is listed.

**Supplementary Material and Methods**

**3D reconstruction analysis.** Using the z-stacks taken on the Nikon Eclipse TE-2000-E

confocal microscope and the Nikon NIS-elements software, a 3D reconstruction of the tubular structures was generated.