# SUPPLEMENTARY MATERIALS

Supplemental Table S1.

| Antigen | Concentration/Dilution | Company& Cat. No |
|---|---|---|
| DAPI | 1:5000 | Thermo Fisher Scientific D1306 |
| Phalloidin | 1:100 | Thermo Fisher Scientific R415 |
| K19 | 1:100 | Developmental Studies Hybridoma Bank Troma III |
| E-cadherin | 1:100 | Cell Signaling 3195S |
| ZO-1 | 1:100 | Thermo Fisher Scientific 61-7300 |
| Apical sodium bile salt transporter (ASBT) | 1:50 | Abcam ab203205 |
| Sambucus nigra lectin (SNA) | 20 µg/ml | Vector Laboratories B-1305 |
| Soybean agglutinin lectin (SBA) | 20 µg/ml | Vector Laboratories B-1075 |
| Acetylated α-tubulin | 1:100 | Thermo Fisher Scientific |

| | | |
|---|---|---|
| | | 32-2700 |
| Ki67 | 1:100 | Abcam<br>ab16667 |
| EpCAM | 1:100 | Abcam<br>ab237384 |

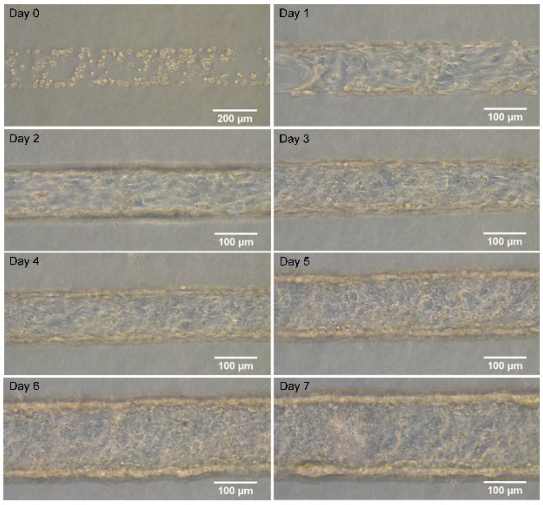



Figure S1. Representative bright field images of the channel lined by a layer of mouse cholangiocytes (cell line), imaged daily over 7 days after seeding. Scale bar, 100 μm.

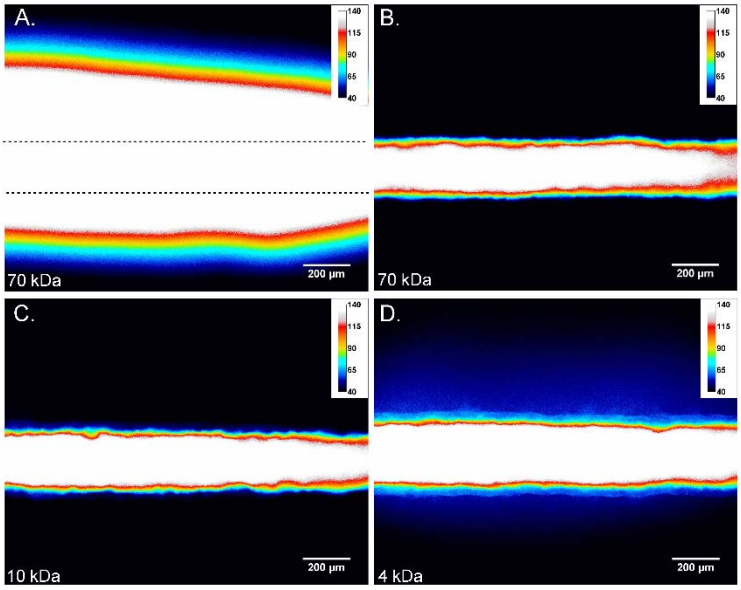



Figure S2. Representative pseudo color images of FITC-Dextran in the devices. A. Diffusion of 70 kDa FITC-Dextran into collagen gels without cholangiocytes in the channel, with image taken after 2 min. Scale bar, 200 μm. B-D. Representative pseudo color images of 70 kDa, 10 kDa and 4 kDa FITC-Dextran in a cell-lined channel after 10 min. Scale bar, 200 μm.

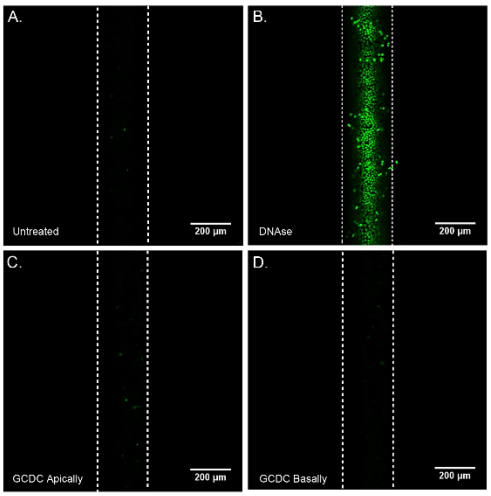



Figure S3. Representative TUNEL staining of GCDC-treated cholangiocytes in the devices. A. Untreated cholangiocytes as a negative control. B. Cholangiocytes treated with DNAse (3 U/ml) as a positive control. C. Cholangiocytes treated with 1 mM GCDC from the apical side for 1 h. D. Cholangiocytes treated with 1 mM GCDC from the basal side for 1 h.

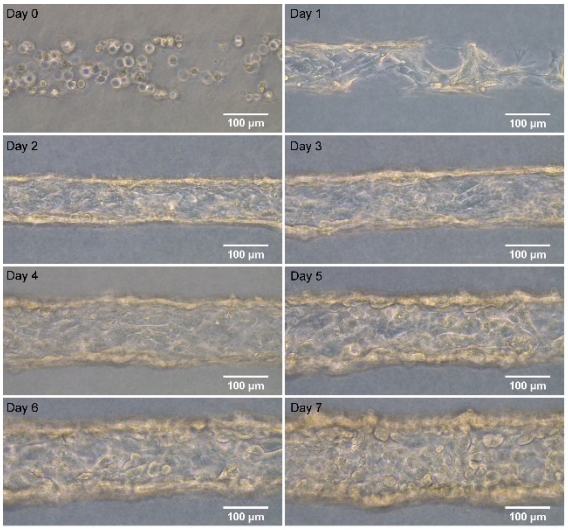



Figure S4. Representative bright field images of a channel lined by a layer of primary mouse cholangiocytes, imaged daily over 7 days after seeding. Scale bar, 100 μm.

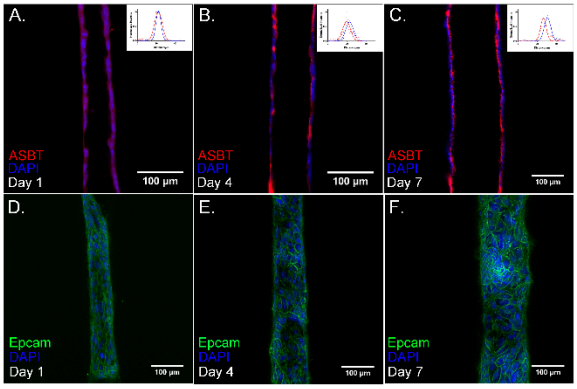



Figure S5. Immunofluorescence images across the middle (A, B, C) and bottom (D, E, F) of channels lined with primary cells and stained with antibodies (shown in red or green) against (A, B, C) ASBT or (D, E, F) EpCAM, with DAPI nuclear staining (blue), imaged at days 1, 4, and 7 after seeding. Inserts in A, B, C show the distribution of ASBT and nucleus intensity across the monolayer. Images are representative of 3 independent experiments.

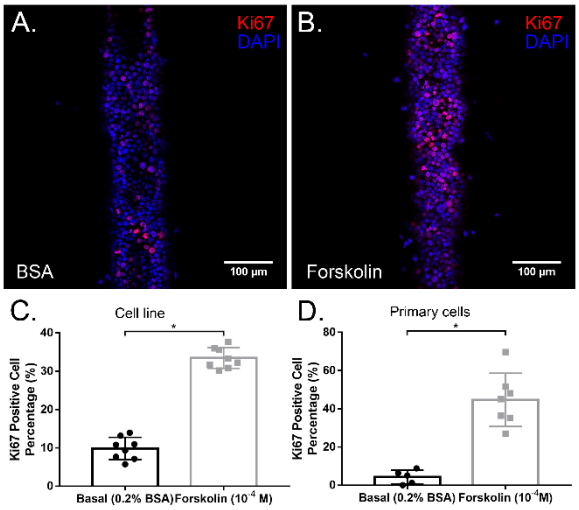
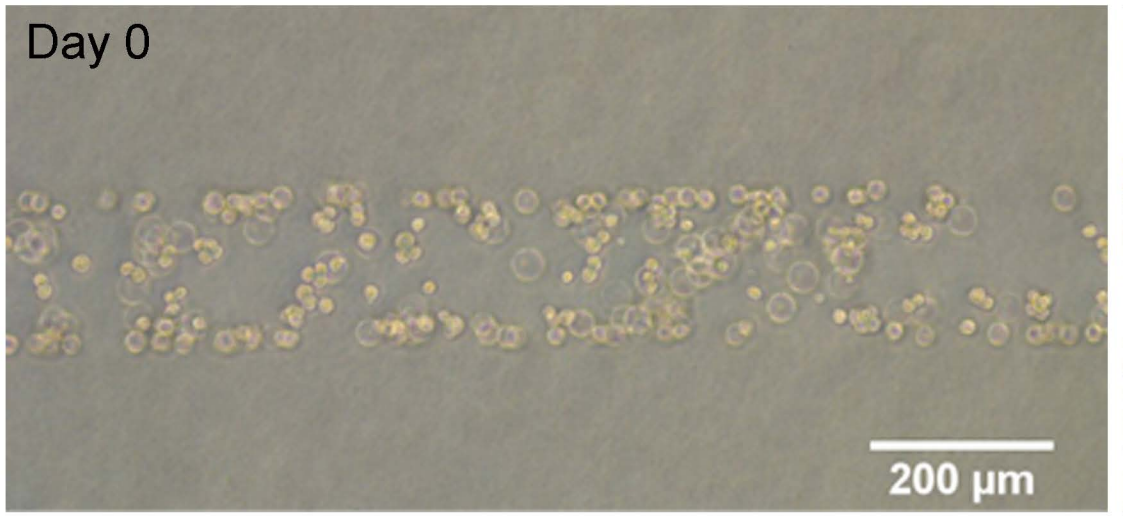


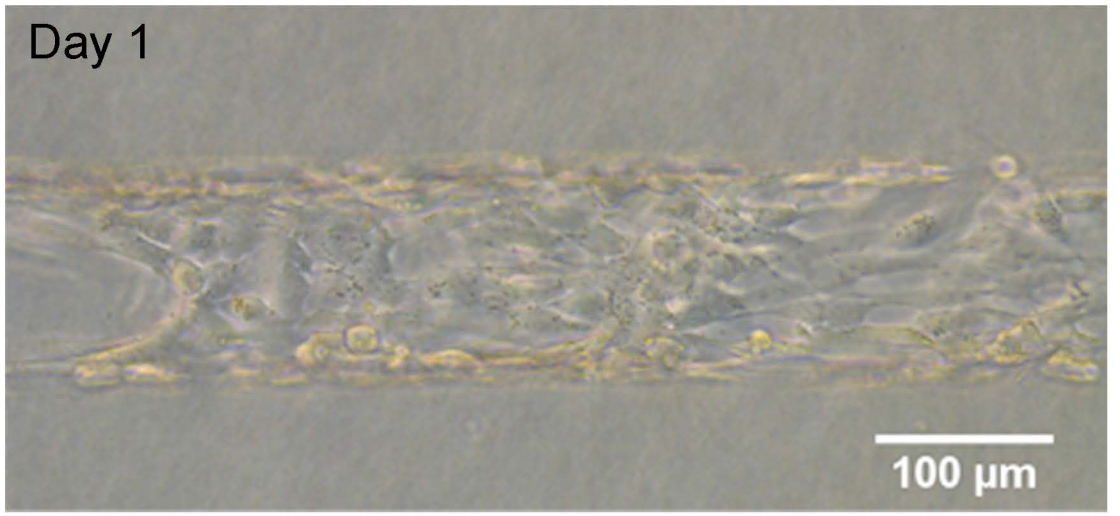

Figure S6. Forskolin promotes cholangiocyte proliferation. A. Ki67 staining of cholangiocytes (cell line) in the device treated with 0.2% BSA. B. Ki67 staining of cholangiocytes (cell line) in the device treated with 0.1 mM forskolin. C. Quantification of the percentage of Ki67 positive cholangiocytes treated with forskolin compared to those treated with 0.2% BSA; n=8. D. Quantification of the percentage of Ki67 positive cholangiocytes (primary cells) treated with forskolin compared to those treated with 0.2% BSA; n=5-7. All data are presented as mean ± SD, *P<0.05.

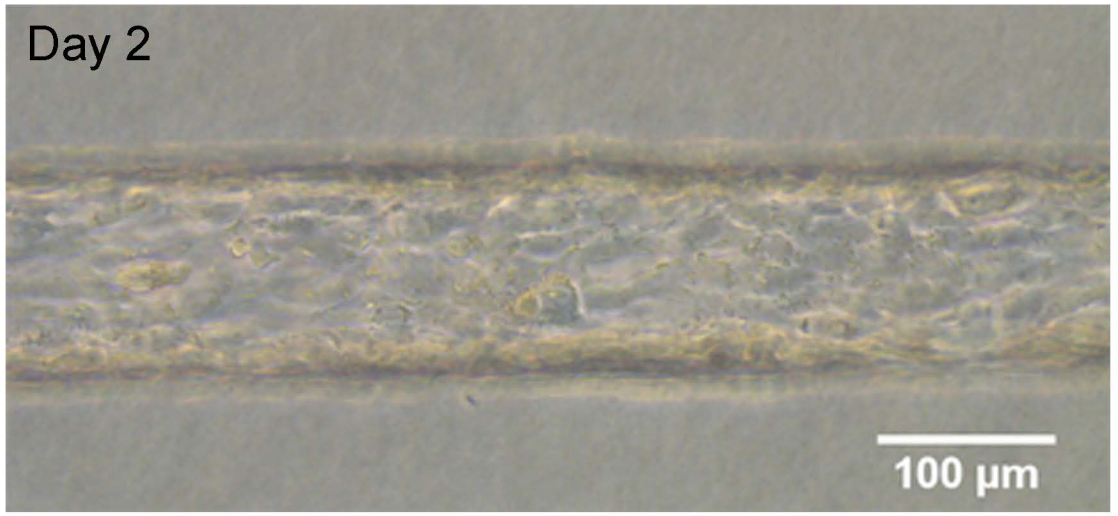
Movie S1. Representative time lapse imaging of primary cholangiocyte-lined channels perfused with FITC-dextran (4 kDa) for 10 min.

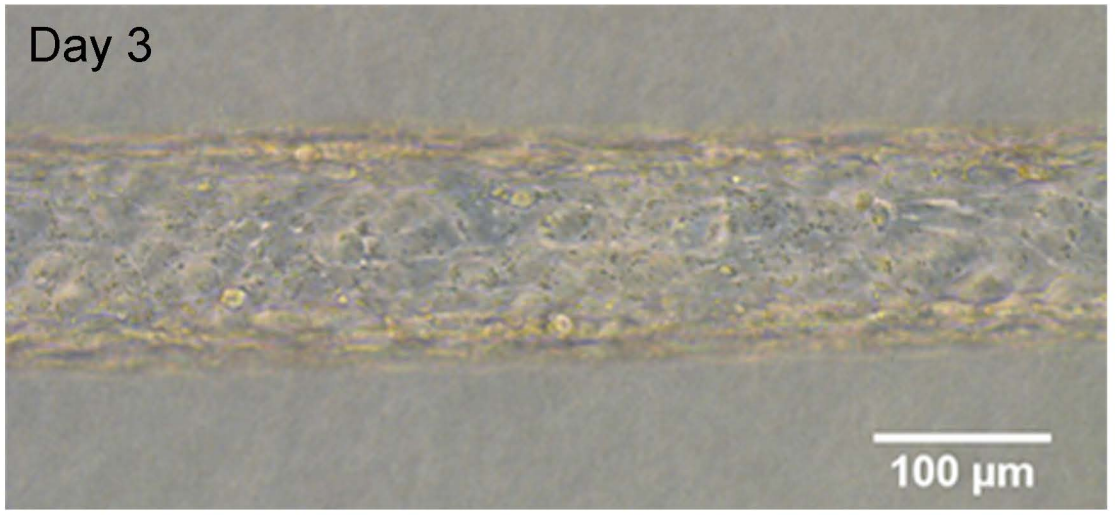
Movie S2. Representative time lapse imaging of fluorescent microbeads flowing through the cholangiocyte channel at 0.02 dyne/cm$^2$.

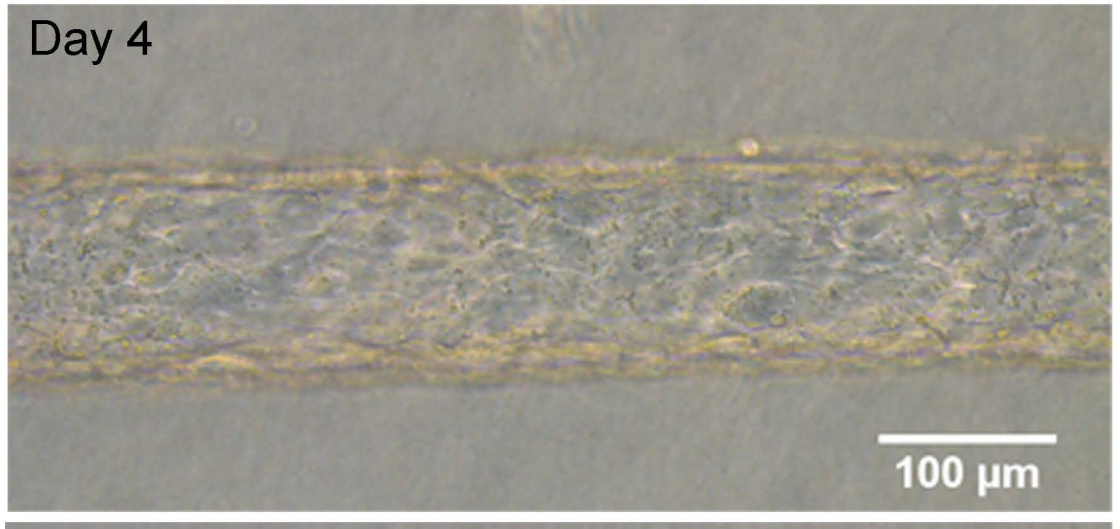
Movie S3. Representative time lapse imaging of calcium signaling of cholangiocytes in the channel while stimulated with luminal flow (3.9 dyne/cm$^2$).

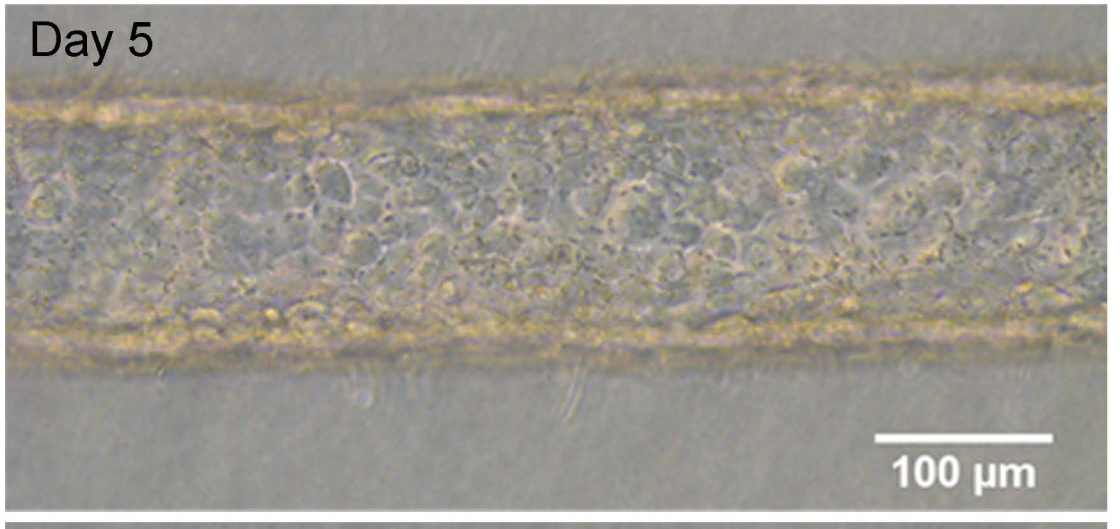

Day 0
200 µm

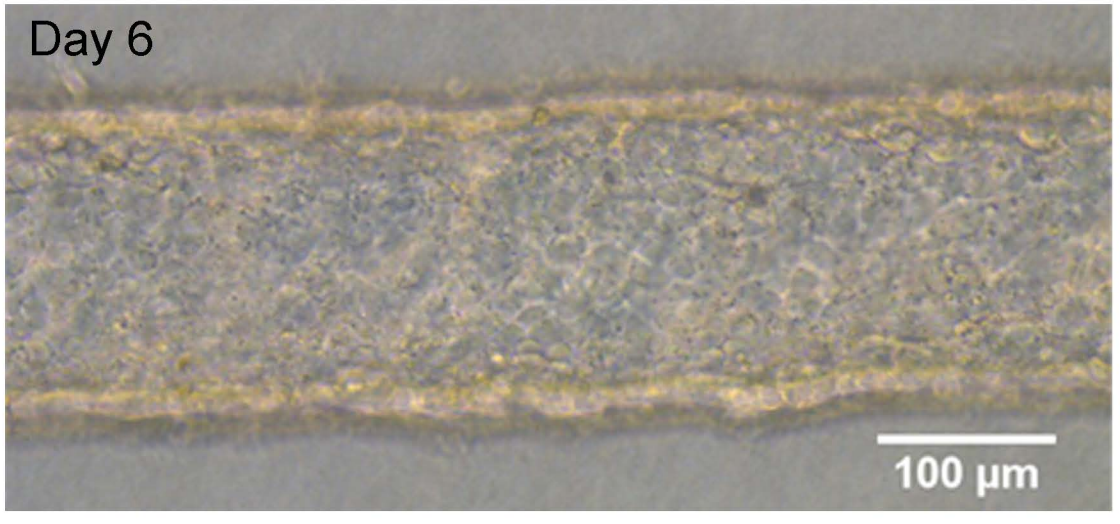

Day 1
100 µm


Day 2
100 µm

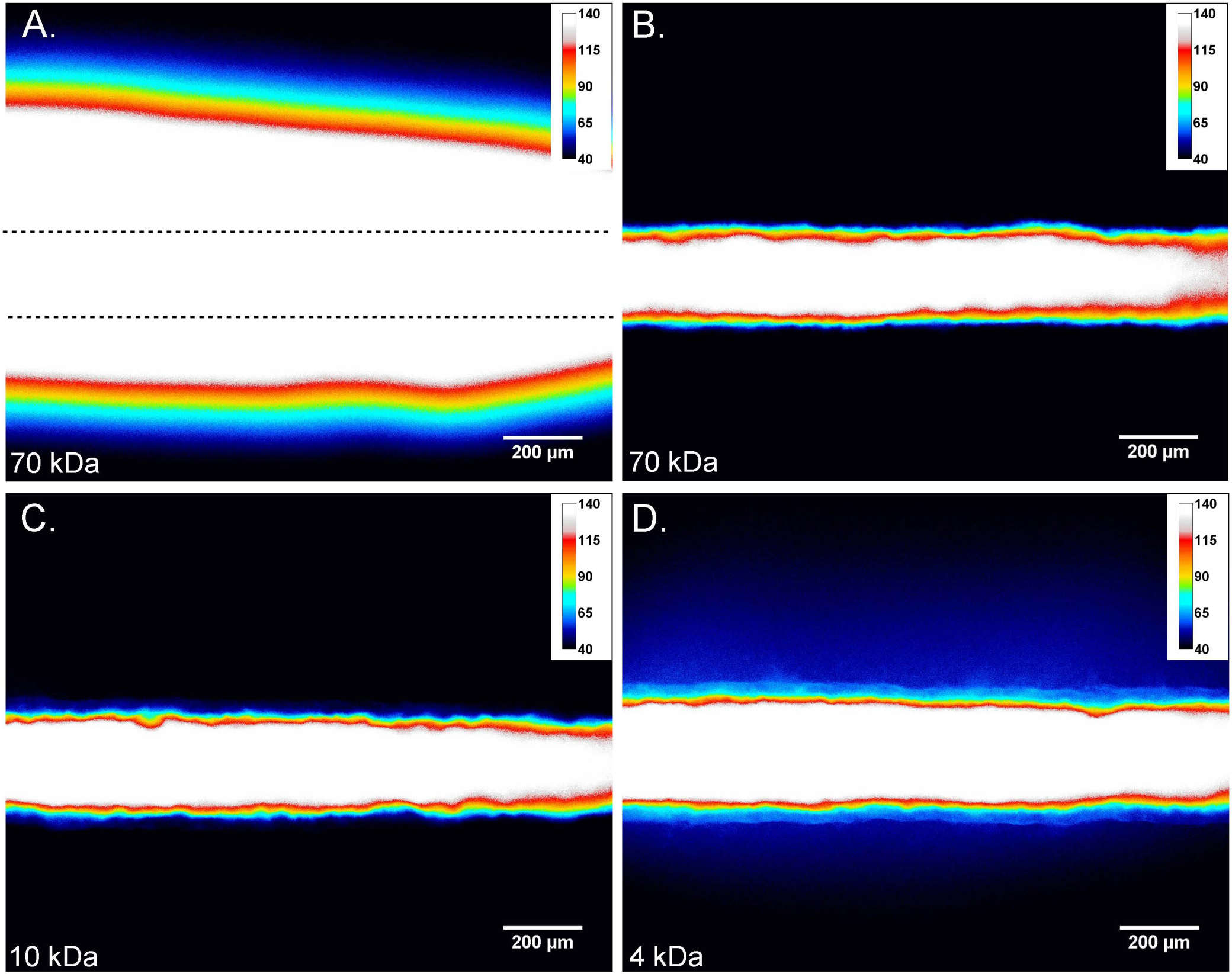

Day 3
100 µm

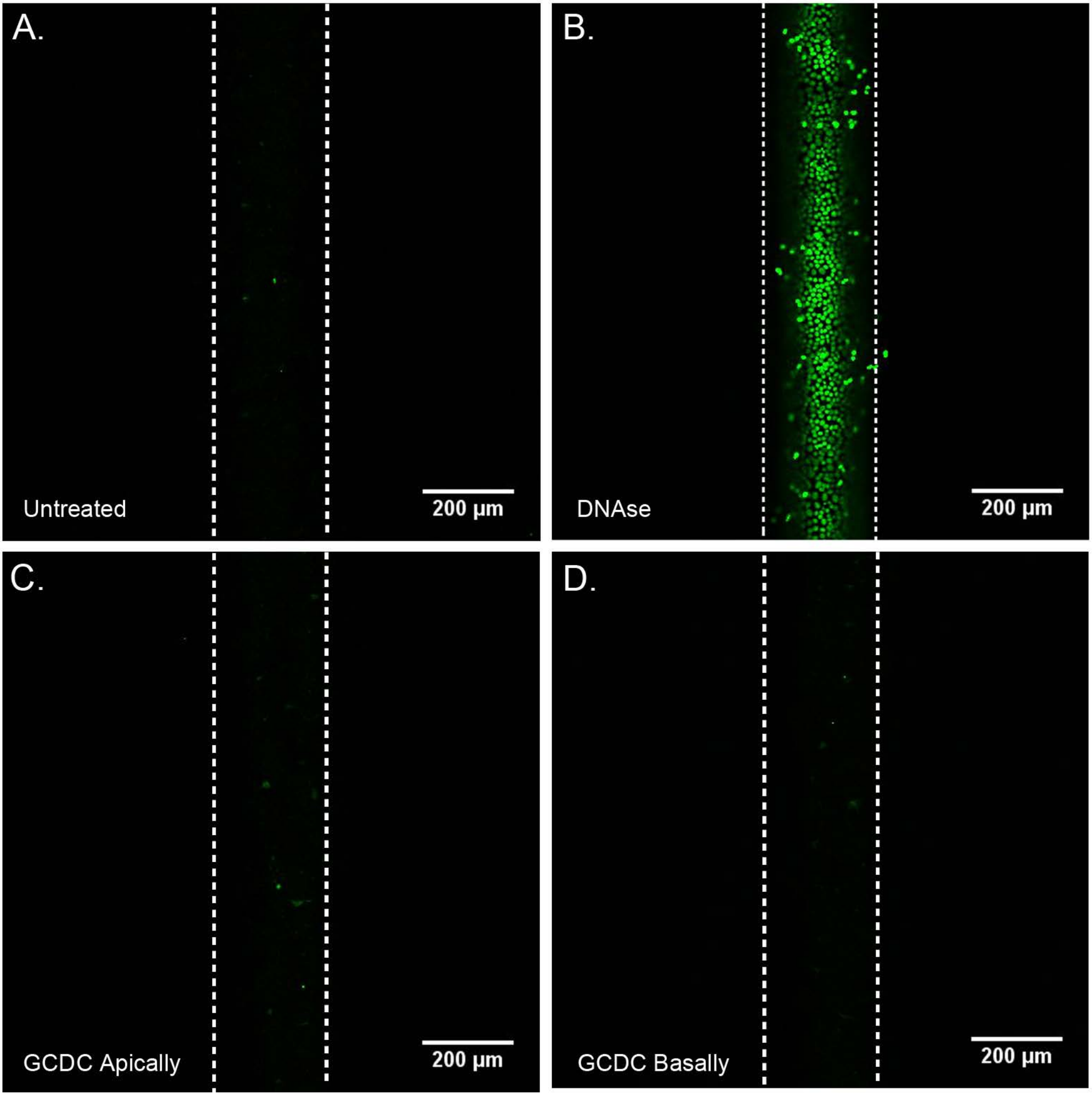

Day 4
100 µm

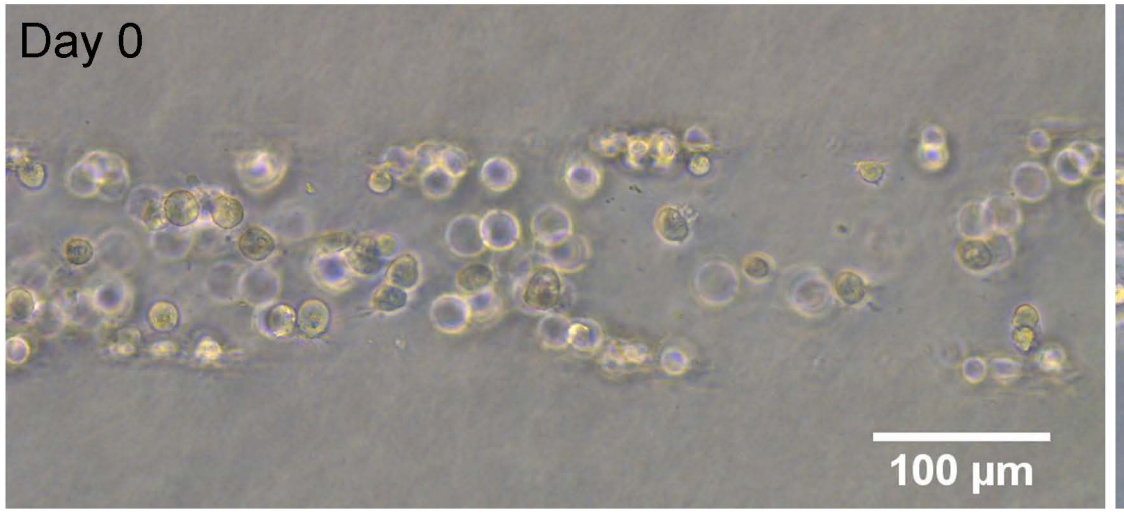

Day 5
100 µm

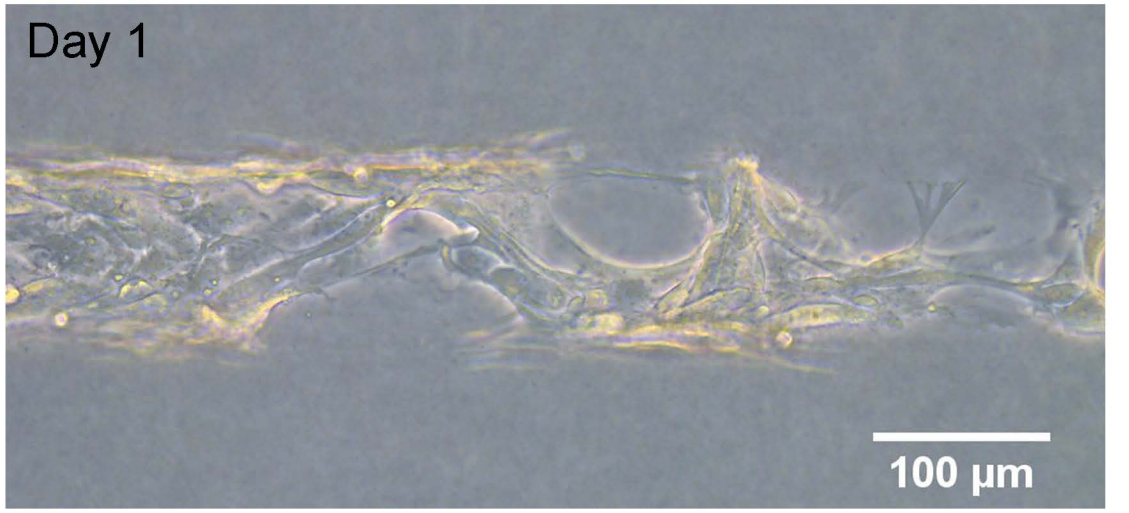

Day 6
100 µm

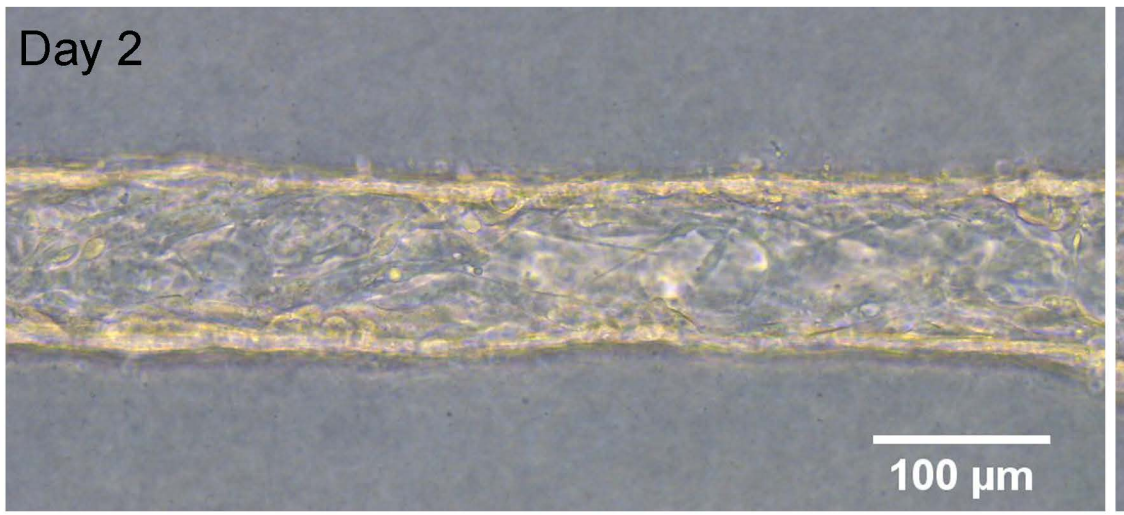

Day 7
100 µm

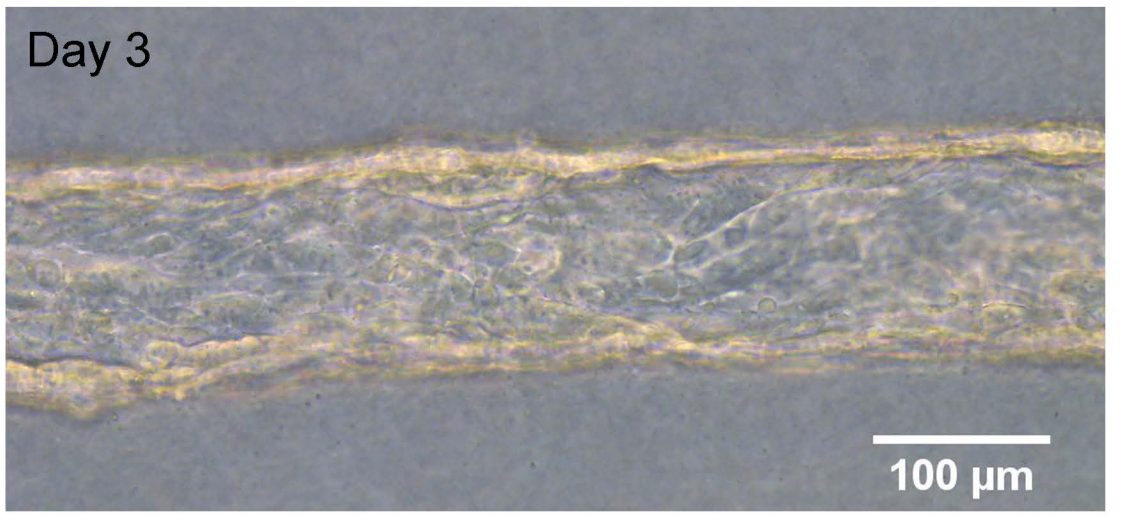

A.
140
115
90
65
40
70 kDa
200 µm
B.
140
115
90
65
40
70 kDa
200 µm
C.
140
115
90
65
40
10 kDa
200 µm
D.
140
115
90
65
40
4 kDa
200 µm

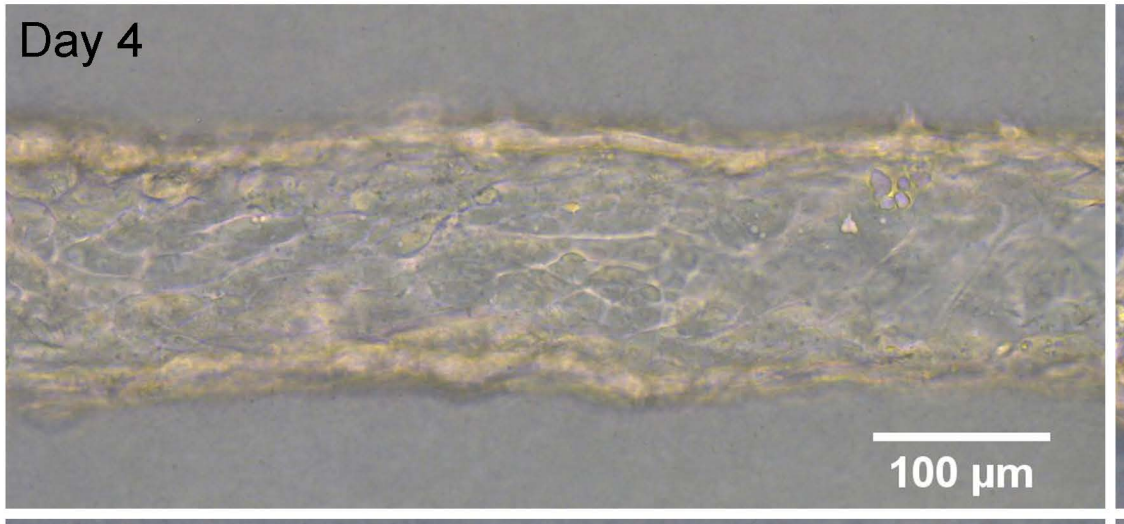

A.
Untreated
200 µm
B.
DNAse
200 µm
C.
GCDC Apically
200 µm
D.
GCDC Basally
200 µm

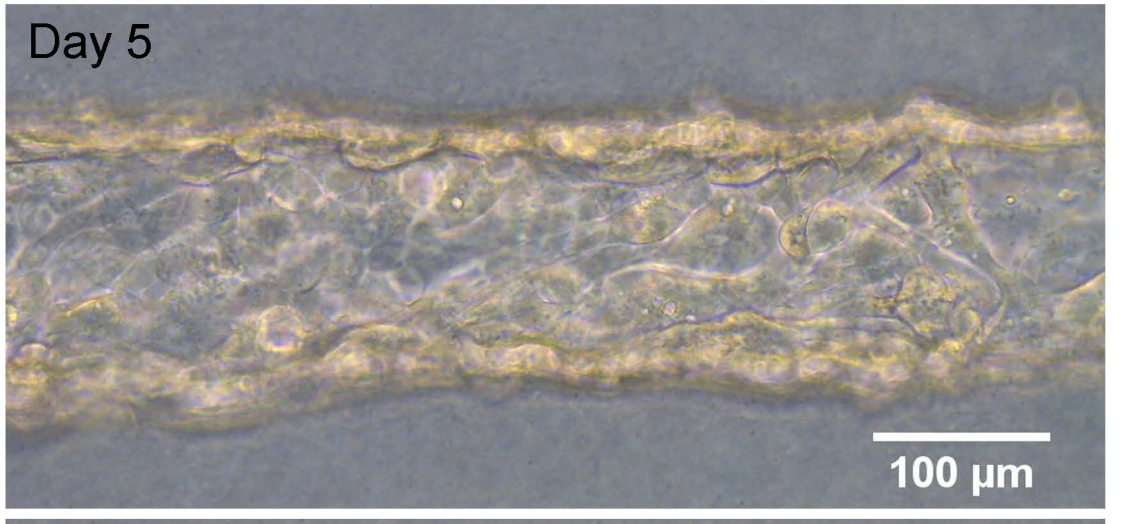

Day 0
100 µm

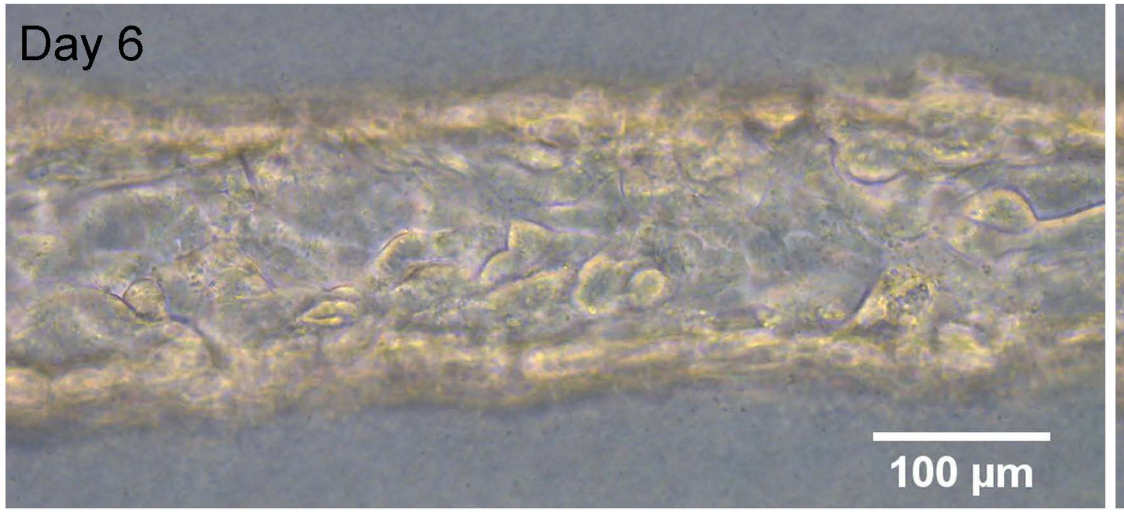

Day 1
100 µm

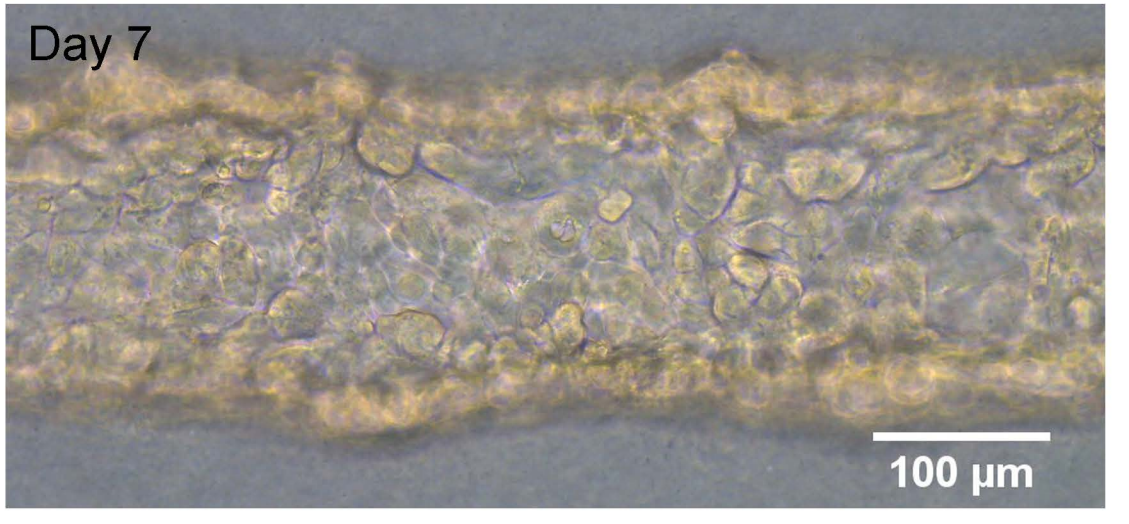

Day 2
100 µm

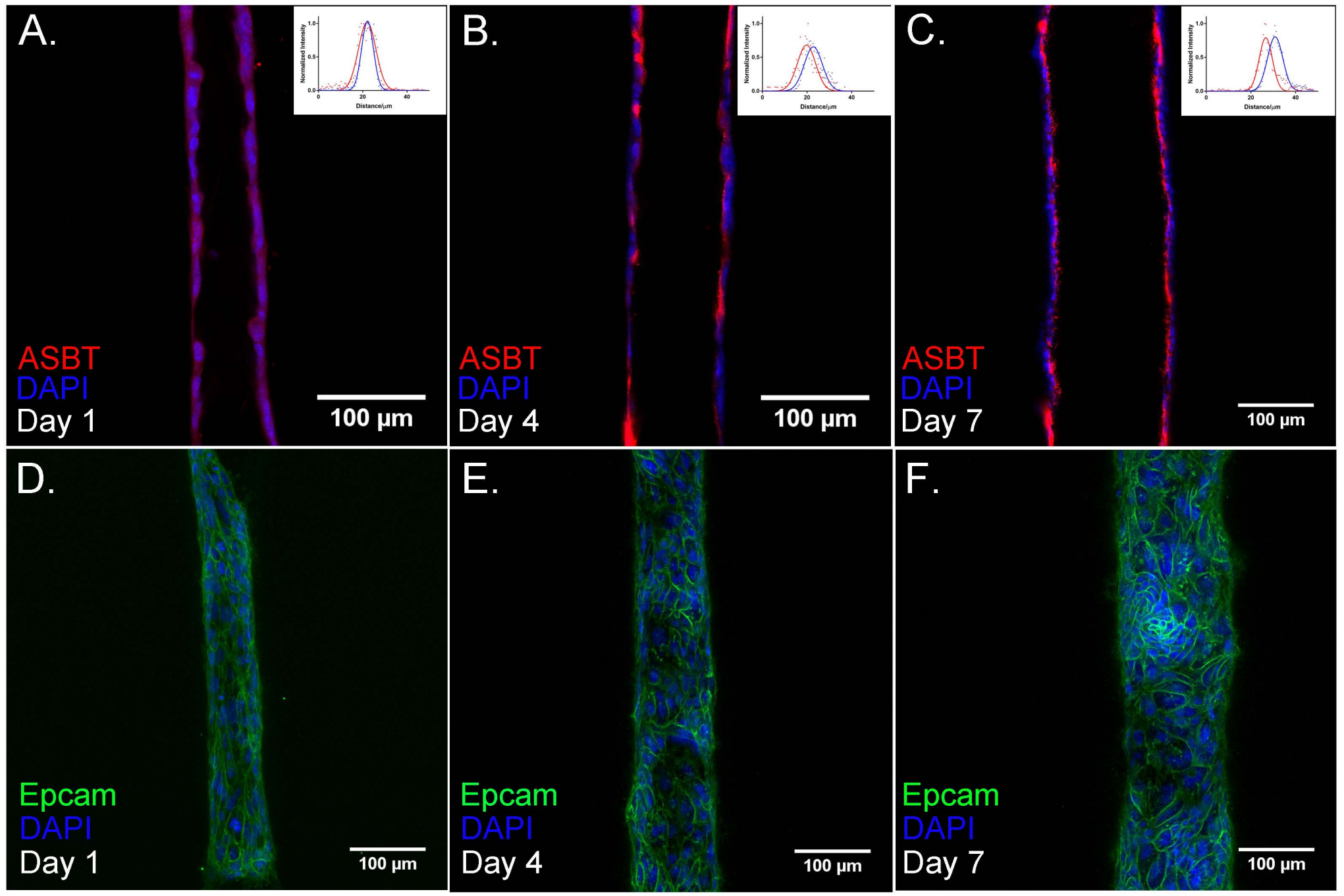

Day 3
100 µm

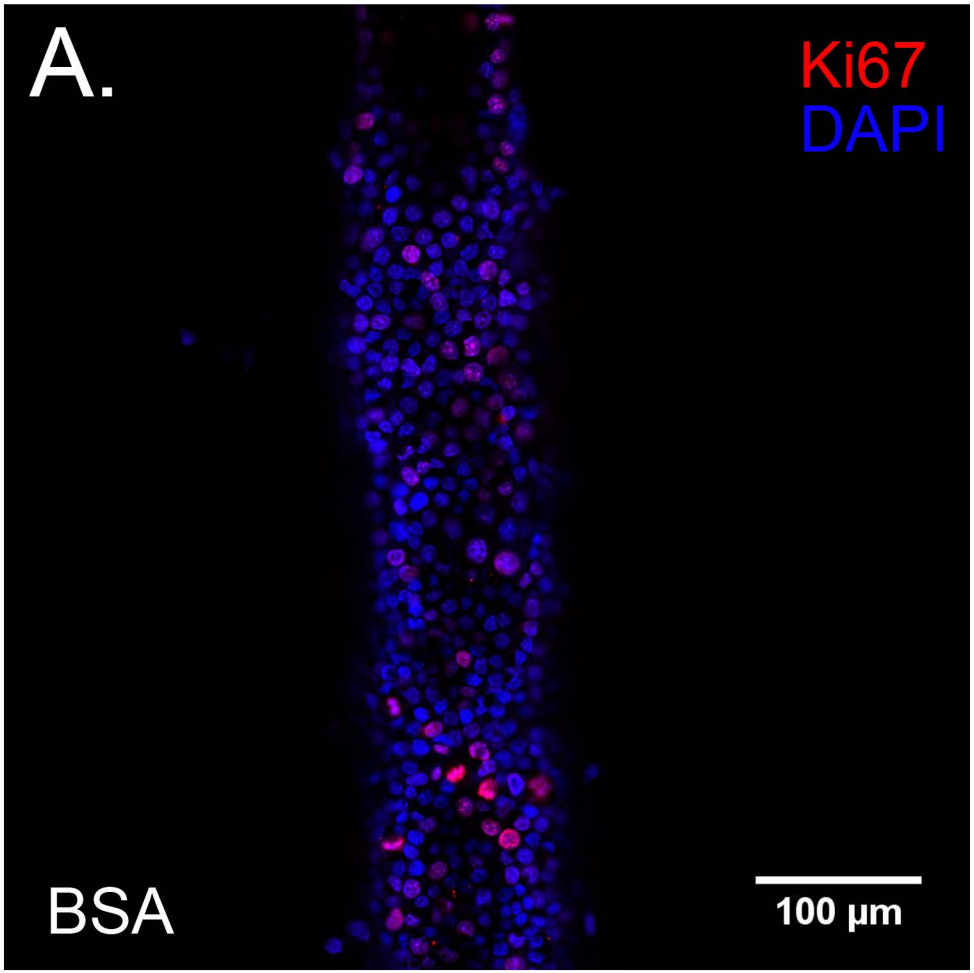

Day 4
100 µm

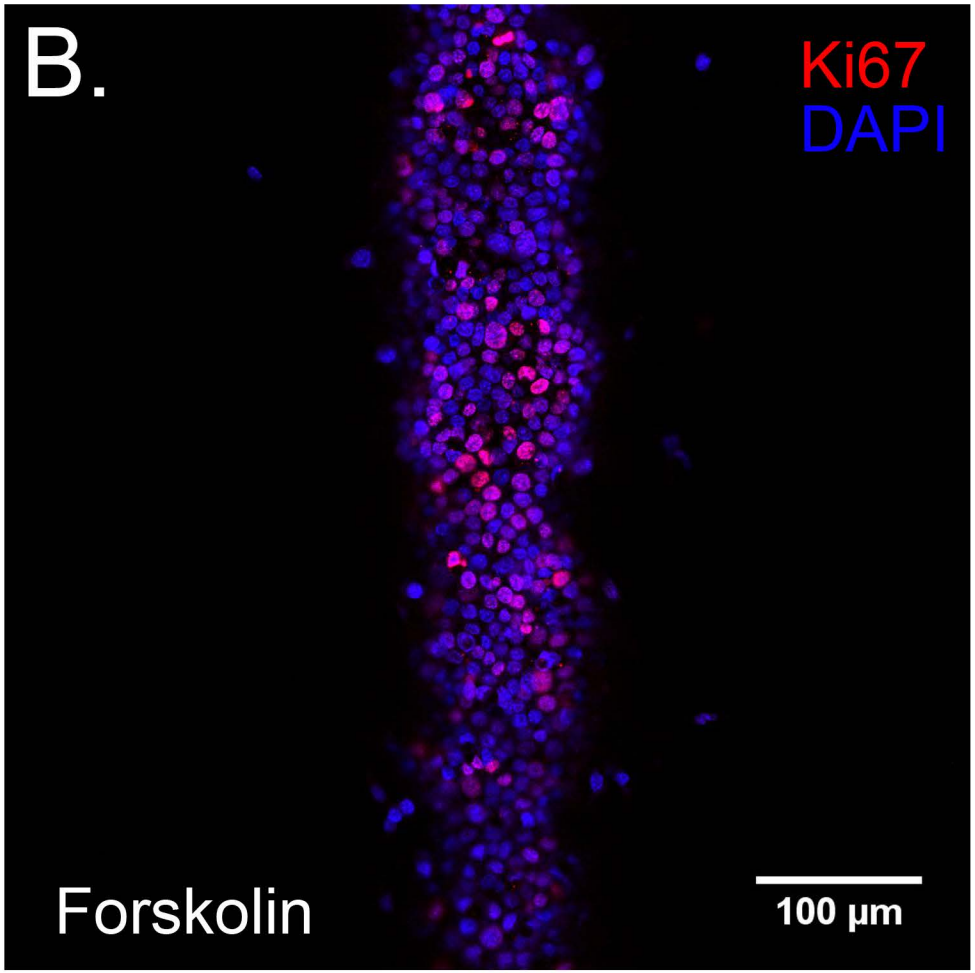

Day 5
100 µm

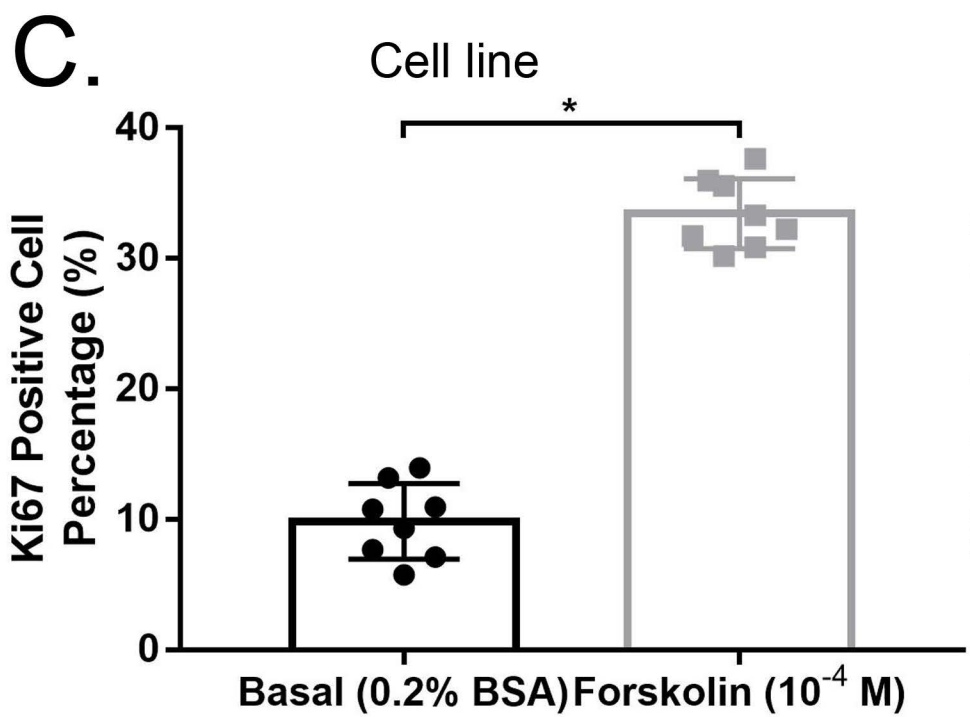

Day 6
100 µm

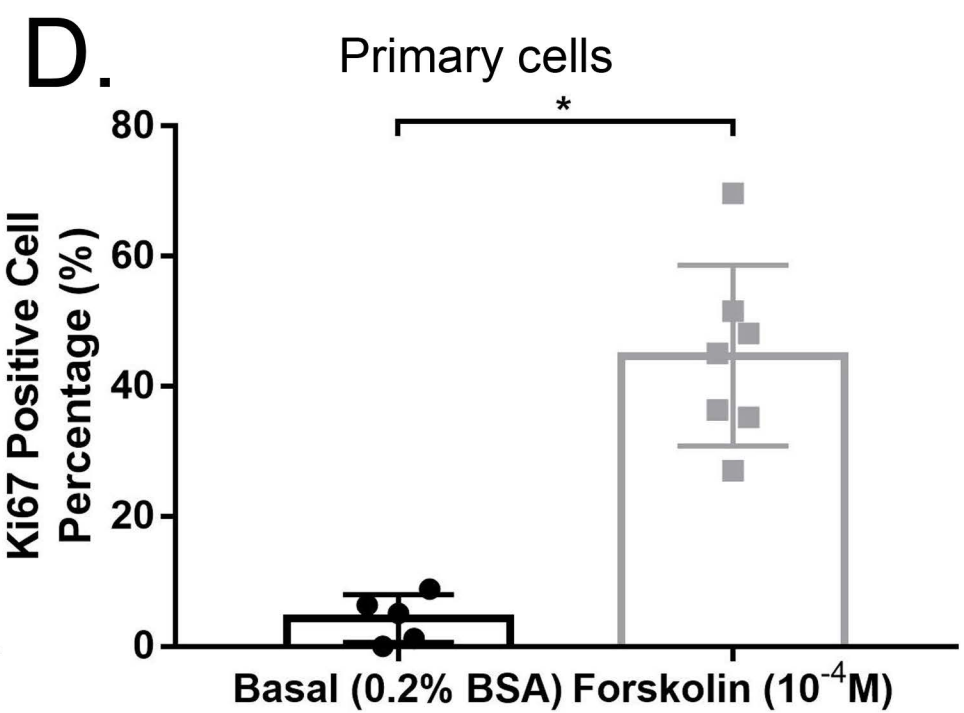

Day 7
100 µm


A.
ASBT
DAPI
Day 1
100 μm
Normalized Intensity
Distance/μm
B.
ASBT
DAPI
Day 4
100 μm
C.
ASBT
DAPI
Day 7
100 μm
D.
Epcam
DAPI
Day 1
100 μm
E.
Epcam
DAPI
Day 4
100 μm
F.
Epcam
DAPI
Day 7
100 μm


A.
Ki67
DAPI
BSA
100 µm


B.
Ki67
DAPI
Forskolin
100 µm


C.
Cell line
*
Ki67 Positive Cell Percentage (%)
0
10
20
30
40
Basal (0.2% BSA)
Forskolin (10-4 M)


D.
Primary cells
*
Ki67 Positive Cell Percentage (%)
0
20
40
60
80
Basal (0.2% BSA)
Forskolin (10-4M)