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Supplementary Materials for

Tensile forces drive a reversible fibroblast-to-myofibroblast transition during tissue growth in engineered clefts

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/4/1/eaao4881/DC1)

- movie S1 (.avi format). *Z* stack showing actin (green) and nuclei (blue).
- movie S2 (.avi format). *Z* stack showing nuclei (red) and EdU (green).
- movie S3 (.avi format). *Z* stack showing color-coded FN-FRET signals.
- movie S4 (.avi format). *Z* stack showing actin (green) and α-SMA (red).
- movie S5 (.avi format). *Z* stack showing nuclei (red) and YAP (cyan).
- movie S6 (.avi format). Time lapse recording of a tissue with fluorescently labeled FN.

Supplementary Materials

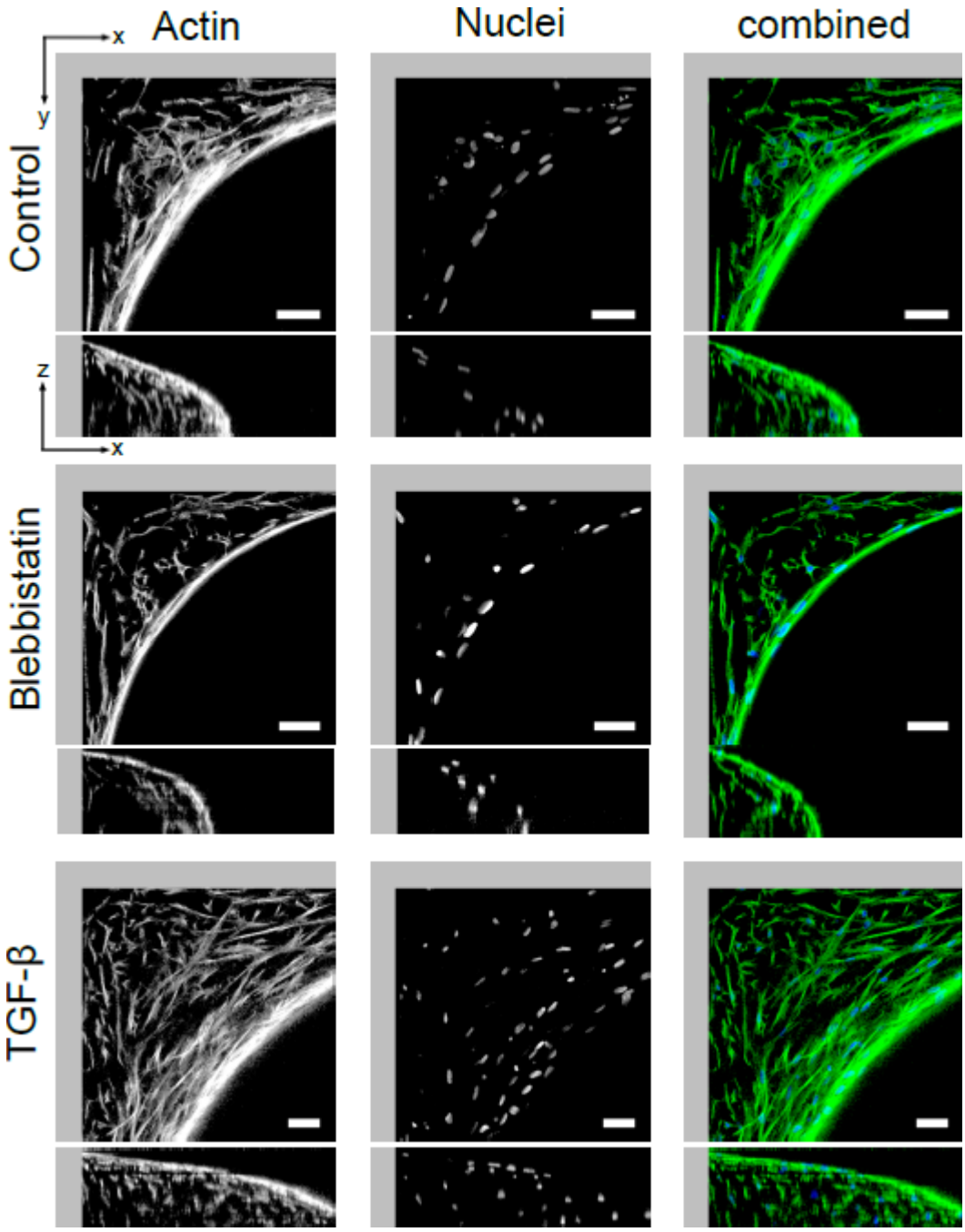


fig. S1. Individual channels of images from Fig. 1, F to H. Single slices through tissues in xy- and xz direction shown as individual channels (left = actin, middle = nuclei, right = overlay) corresponding to the images in Fig. 1F–H, under normal conditions (top), and under conditions of inhibited (middle) and elevated (bottom) cytoskeletal tension in the presence of 10 μM blebbistatin and 1 ng/ml TGF-β, respectively. All scale bars are 50 μm.

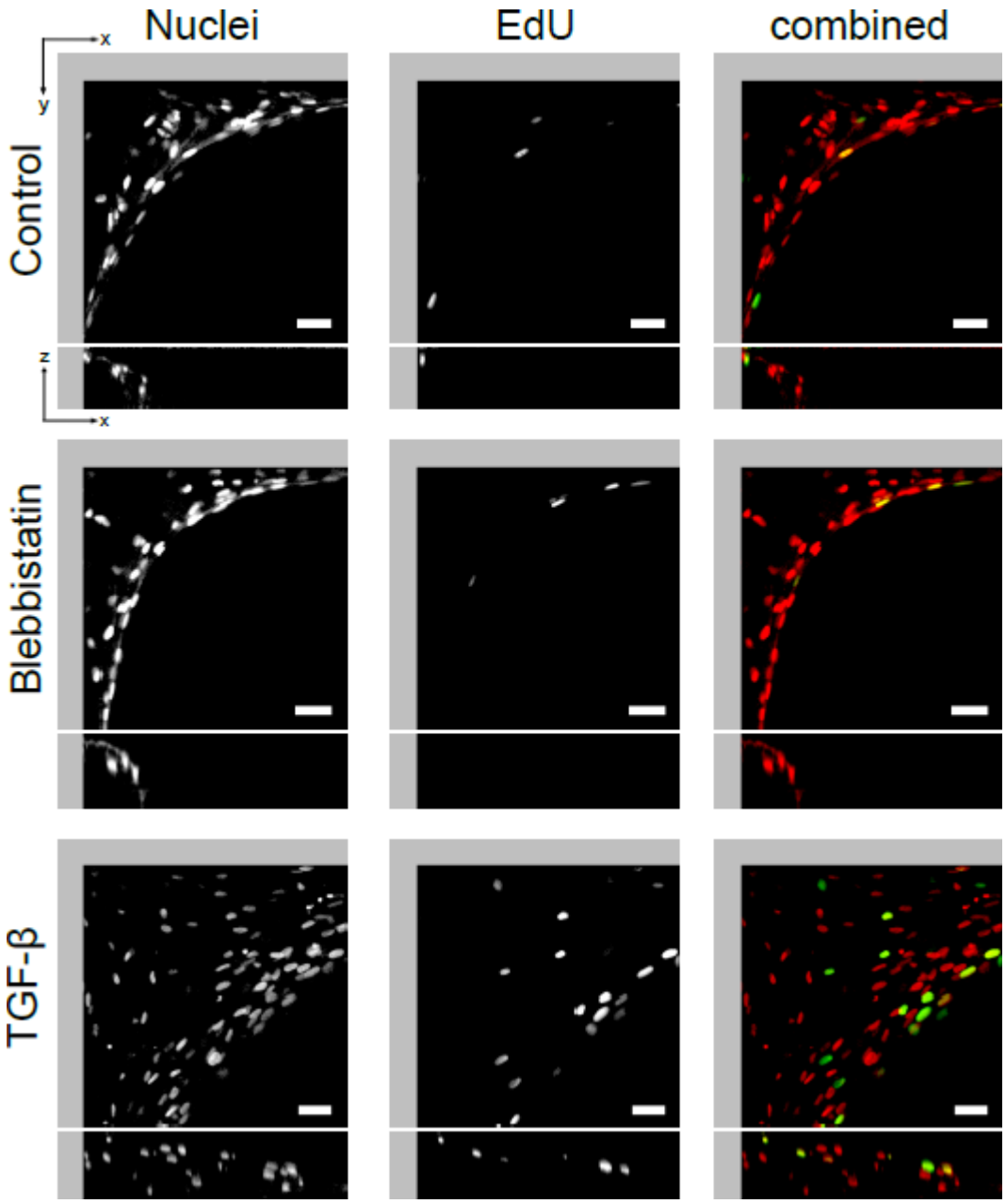


fig. S2. Individual channels of images from Fig. 2, A to C. Single slices through tissues in xy- and xz direction shown as individual channels (left = nuclei, middle = EdU, right = combined) corresponding to the images in Fig. 2A–C, under normal conditions (top), and under conditions of inhibited (middle) and elevated (bottom) cytoskeletal tension in the presence of 10 μM blebbistatin and 1 ng/ml TGF-β, respectively. All scale bars are 50 μm.

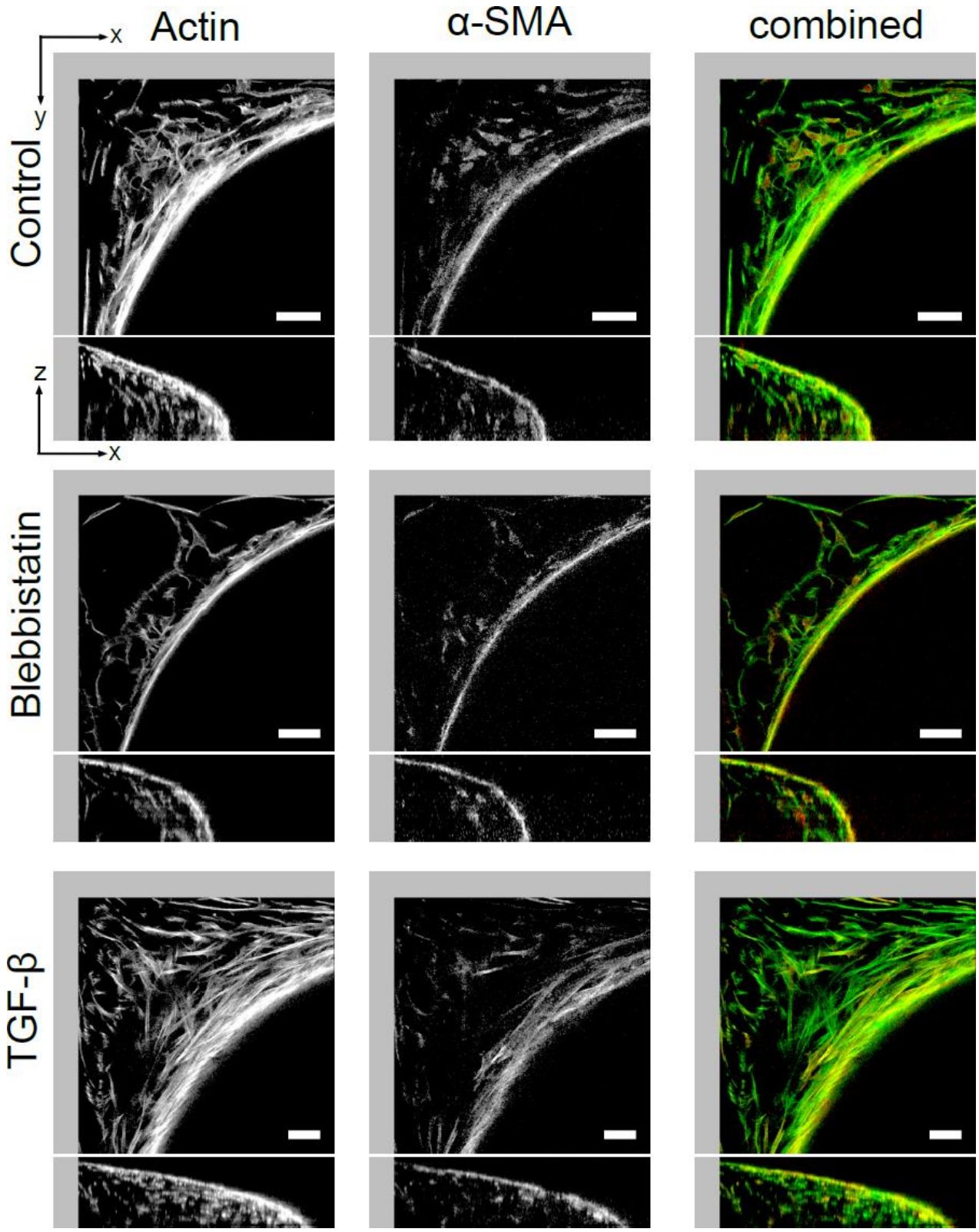


fig. S3. Individual channels of images from Fig. 4, A to C. Single slices through tissues in xy- and xz direction shown as individual channels (left = actin, middle = αSMA, right = combined) corresponding to the images in Fig.4A–C, under normal conditions (top), and under conditions of inhibited (middle) and elevated (bottom) cytoskeletal tension in the presence of 10 μM blebbistatin and 1 ng/ml TGF-β, respectively. All scale bars are 50 μm.

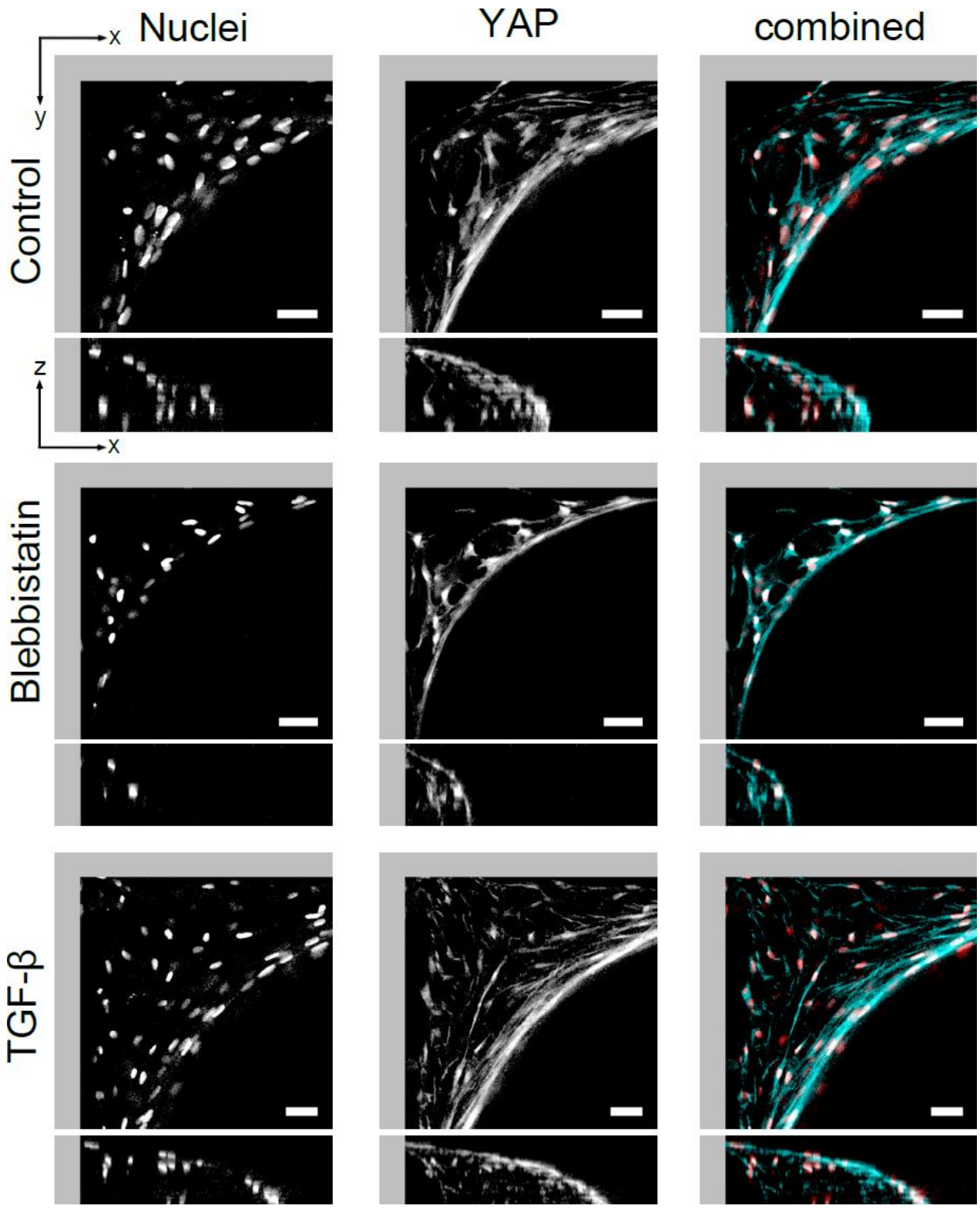
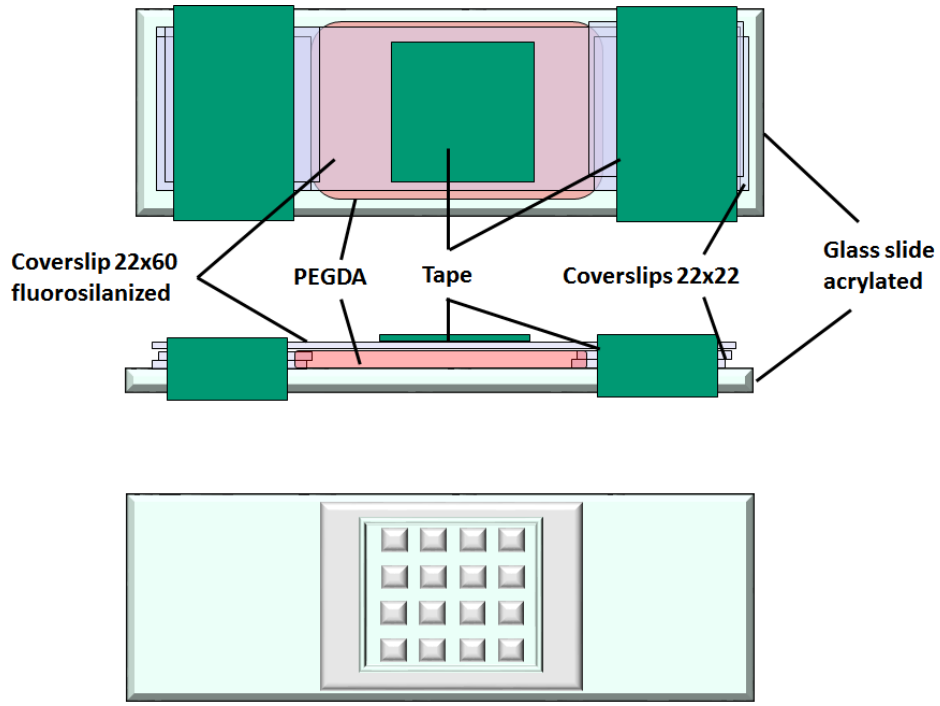


fig. S4. Individual channels of images from Fig. 4, G to I. Single slices through tissues in xy- and xz direction shown as individual channels (left = nuclei, middle = YAP, right = combined) corresponding to the images in Fig.4G–I, under normal conditions (top), and under conditions of inhibited (middle) and elevated (bottom) cytoskeletal tension in the presence of 10 μM blebbistatin and 1 ng/ml TGF-β, respectively. All scale bars are 50 μm.



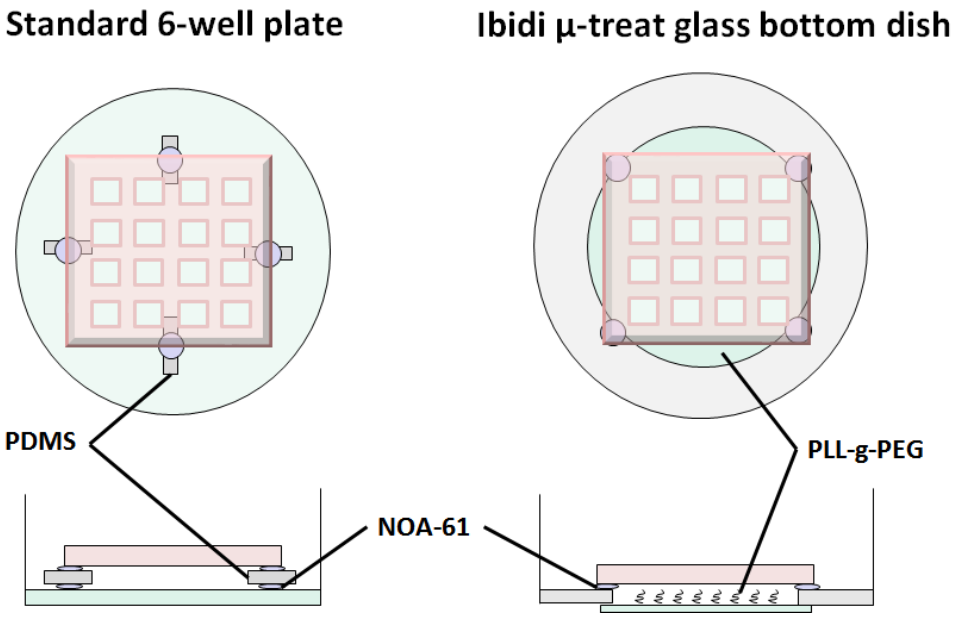


fig. S5. Illustration of the scaffold fabrication method. Top: schematic of the master fabrication, with PEG-DA photopolymer (red) between an acrylated glass slide (light blue) and a fluorosilanized coverslip (blue) fixed together by tape (green). Middle: The final polymerized master after removing the coverslips. Bottom left: functionalized PDMS scaffold attached to a standard 6-well plate. Bottom right: functionalized PDMS scaffold attached to a passivated glass bottom dish.

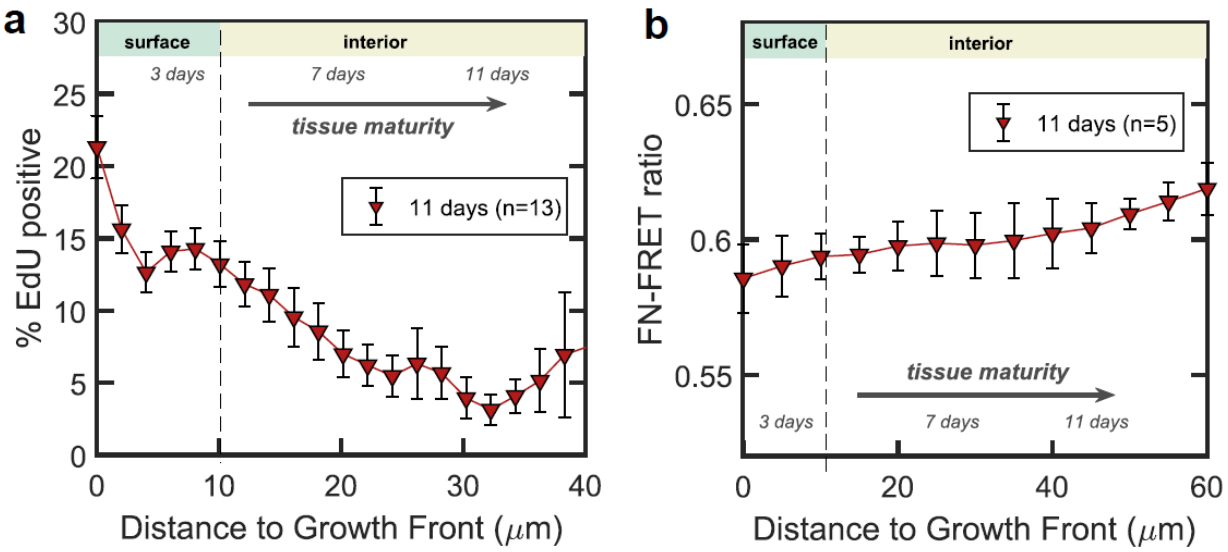


fig. S6. Gradients of cell phenotype and FN stretch after 11 days. (**a**) Percentage of EdU positive volume compared to total nuclear volume as a function of tissue depth and age. (**b**) FN-FRET ratio as a function of distance to the growth front or, equivalently, tissue maturity. Tissues were cultured for 11 days. Error bars indicate standard error of the mean.

Supplementary Movies

movie S1. *Z* stack showing actin (green) and nuclei (blue). This movie shows a fly-through from top to bottom through a 19-day old microtissue grown under control conditions, with actin (green) and nuclei (blue). The growth front is to the bottom right. Scale bar 50 µm.

movie S2. *Z* stack showing nuclei (red) and EdU (green). This movie shows a fly-through from top to bottom through a 19-day old microtissue grown under control conditions, with nuclei (red) and EdU-positive nuclei (green). The growth front is to the bottom right. Scale bar 50 µm.

movie S3. *Z* stack showing color-coded FN-FRET signals. This movie shows a fly-through from top to bottom through a 19-day old microtissue grown under control conditions, with the FRET intensity ratio color-coded. The growth front is to the bottom right. Scale bar 50 µm.

movie S4. *Z* stack showing actin (green) and α-SMA (red). This movie shows a fly-through from top to bottom through a 19-day old microtissue grown under control conditions, with actin (green) and α-SMA (red). The growth front is to the bottom right. Scale bar 50 µm.

movie S5. *Z* stack showing nuclei (red) and YAP (cyan). This movie shows a fly-through from top to bottom through a 19-day old microtissue grown under control conditions, with nuclei (red) and YAP (cyan). The growth front is to the bottom right. Scale bar 50 µm.

movie S6. Time lapse recording of a tissue with fluorescently labeled FN. This movie shows a 3-hour time lapse of the Fibronectin extracellular matrix at a z-depth of 40 µm in a microtissue grown for 20 days under control conditions. Fibronectin was added 24h prior to imaging. The growth front is to the bottom right. Scale bar 50 µm.