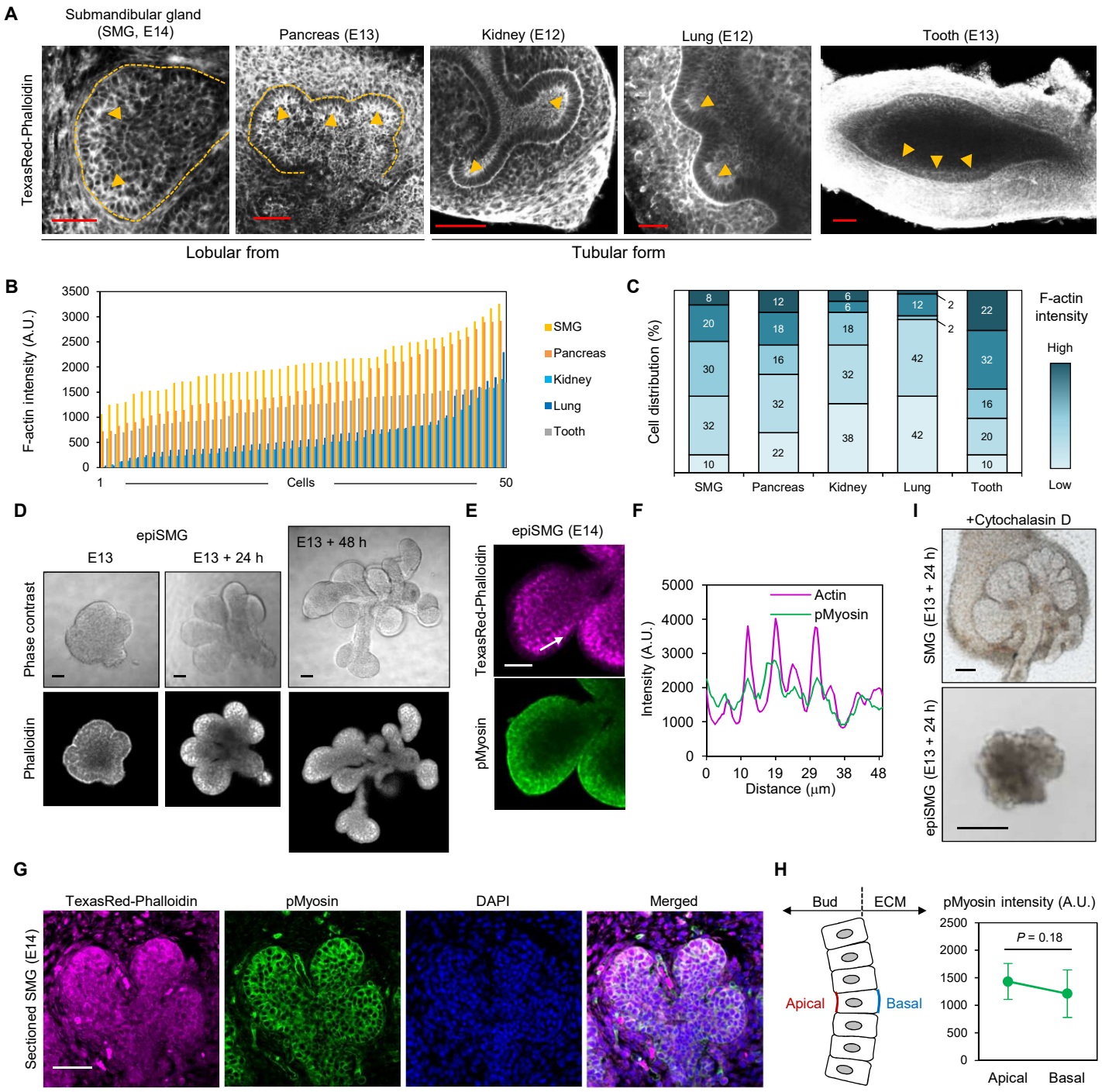


Fig. S1. Characteristic F-actin patterns in diverse epithelial organs. (**A**) F-actin (TexasRed-phalloidin) images of explant cultures of epithelial organs. Yellow dashed lines present the outline of epithelial buds. Yellow arrowheads present regions of high actin signals. (**B**) F-actin intensity of single cells of epithelial buds. (**C**) Percentage of epithelial cells in the different F-actin intensity. n = 50 cells per group. (**D**) Phase contrast (upper) and TexasRed-phalloidin (lower) images of developing epiSMG cultures for 48 h. (**E**) TexasRed-phalloidin and immunolabelled phosphorylated myosin (pMyosin) images of epiSMG culture. The white arrow indicates the region use for the intensity plot (panel **F**). (**F**) Intensity plot of F-actin and phosphorylated myosin signals. (**G**) The images of TexasRed-phalloidin (magenta), pMyosin (green) and DAPI (blue) of sectioned SMG cultures (4 μm thickness). (**H**) Intensity of pMyosin signals in apical and basal part of peripheral epithelial cells in panel **G** (n = 20 cells). Paired t-test. Mean ± STD. (**I**) Morphological changes in mouse embryonic SMG (upper) and epiSMG (lower) cultures upon 100 nM cytochalasin D. Scale bars: 50 μm.

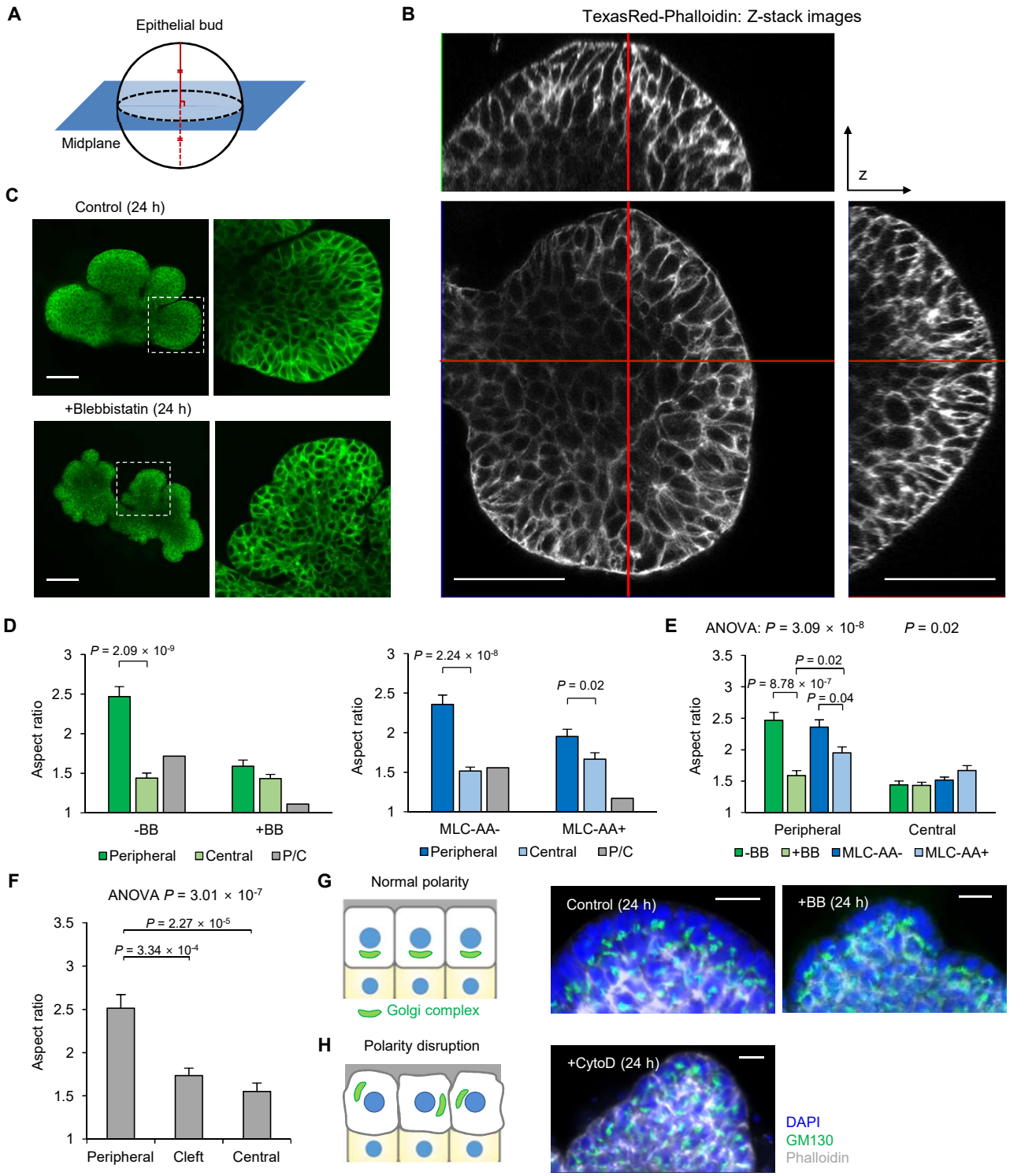


Fig. S2. Changes in diverse cell parameters upon perturbation of actomyosin contractility (**A**) Schematic representation of the optically sectioned imaging plane for single cell anlaysis. (**B**) Z-stack images of TexasRed-phalloidin in epiSMG culture (E13 + 24 h). 1 µm section, z: 71.00 µm. (**C**) Immunolabelled images of E-cadherin (green) in E13 eSMG culture (left) and blebbistatin-treated (right) cultures. White dashed regions indicate the enlarged area. (**D and E**) Aspect ratio of cells in peripheral (P) and central (C) region of epithelial buds. Mean ± sem. n = 30 cells (-BB and +BB) and 34 cells (MLC-AA- and MLC-AA+). (**F**) Aspect ratio of cells in different regions of epithelial buds. Mean ± sem. n = 20 cells per group. (**G**) Apically polarized location of the Golgi complex (immunolabelled by GM130) in control and blebbistatin-treated eSMG cultures (E13). (**H**) Disrupted polarization of the Golgi complex in eSMG cultures treated with low-concentration cytochalasin D. Scale bars: 50 µm (**B**, **C**), 20 µm (**G**, **H**)

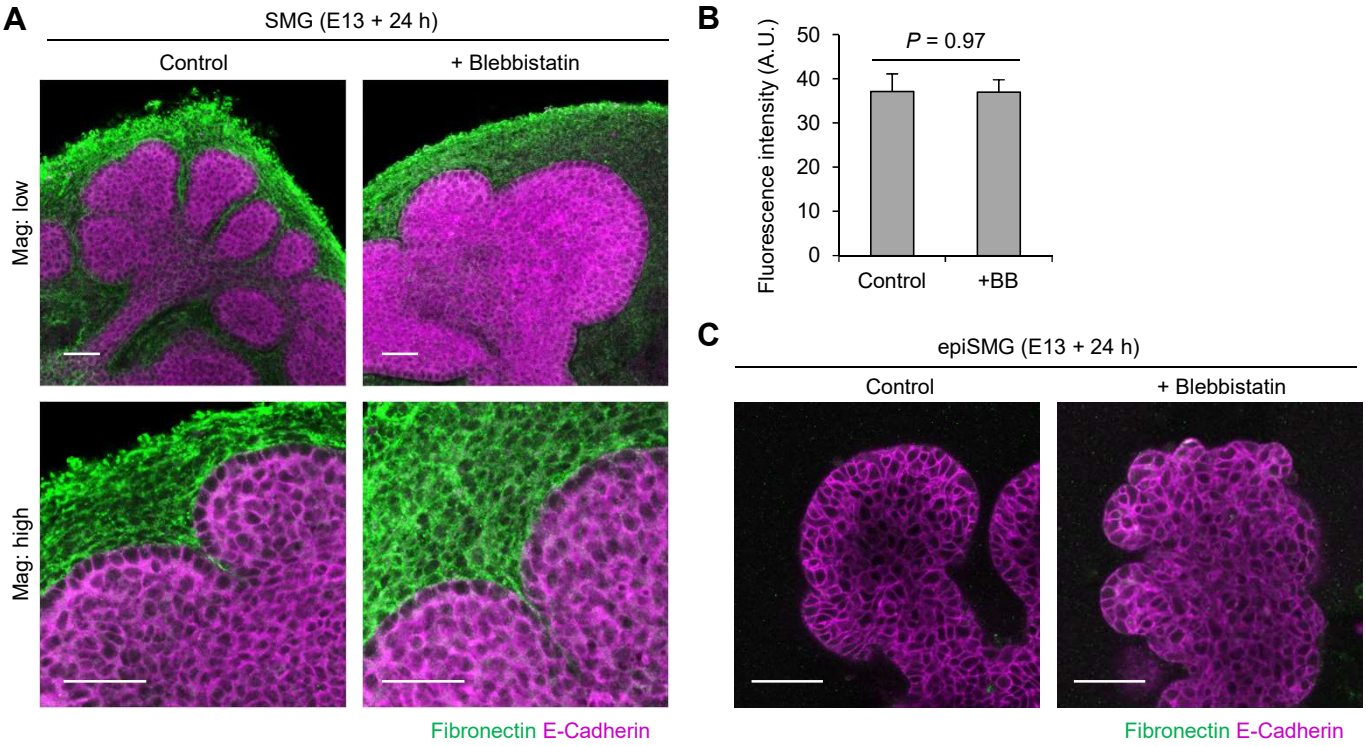


Fig. S3. **Fibronectin patterns of SMG upon blebbistatin treatment.** (**A**) Immunolabeled signals of fibronectin (green) and E-cadherin (magenta) of developing SMG cultures. (**B**) Quantified results of fluorescence signals of fibronectin of developing SMG cultures. Mean ± sem. n = 12 regions per group. (**C**) Immunolabeled signals of fibronectin (green) and E-cadherin (magenta) of developing epiSMG cultures. Scale bars: 50 µm.

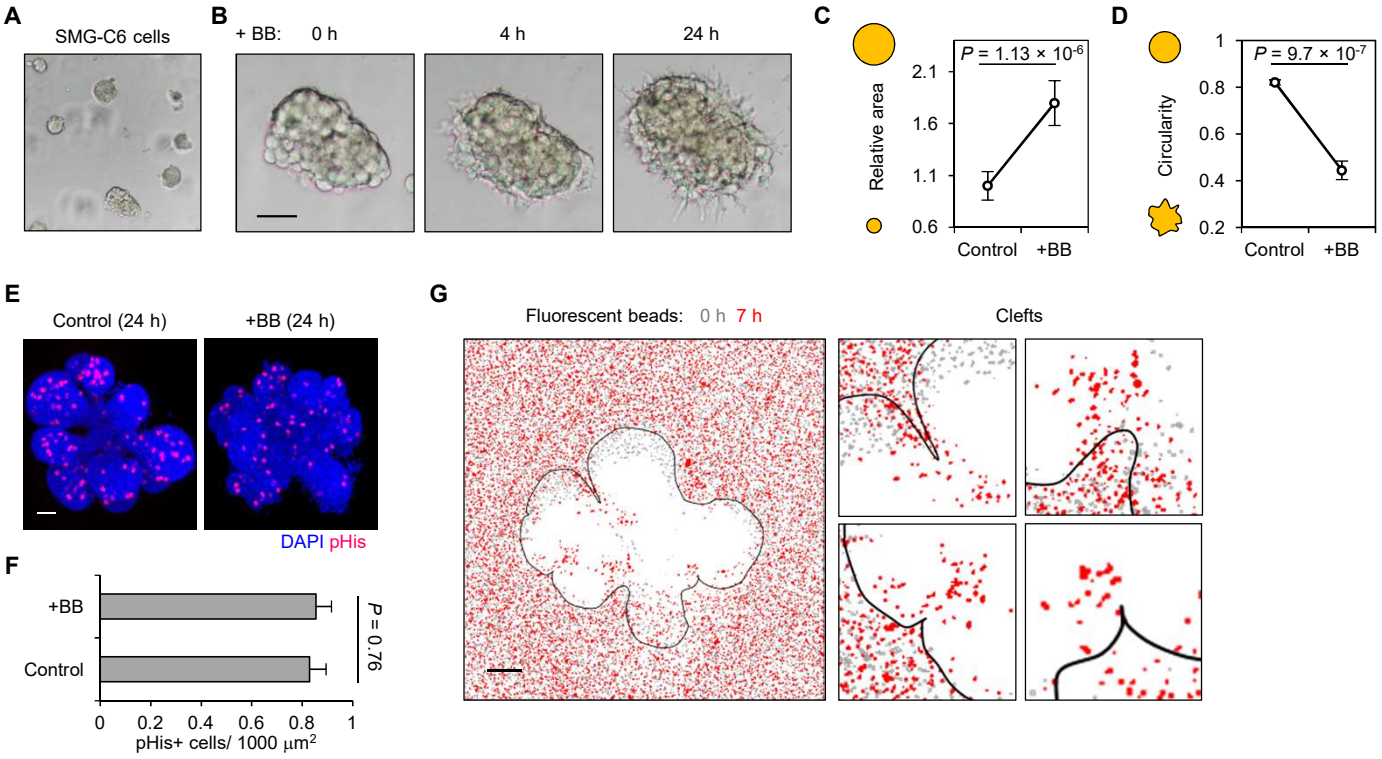


Fig. S4. The mechanism of force transmission in branching morphogenesis (**A**) Phase contrast image of salisphere culture using the SMG-C6 cell line (rat SMG acinar cell line). (**B**) Time-dependent morphological changes of salisphere cultures. (**C** and **D**) Quantified value of the relative area (**C**) and circularity (**D**) of salisphere cultures. Mean ± sem. n = 12 cultures per group. (**E**) Immunolabelled phosphorylated histone H3 signals (pHis, red) in developing eSMG cultures (E 13) without (Control) or with blebbistatin treatment (+BB). (**F**) Quantified pHis+ cell density (per 1,000 μm^3) in developing eSMG cultures (E 13). Mean ± sem. n = 5 cultures per group. (**G**) The location changes in fluorescent beads around developing epiSMG culture. Right subpanels show enlarged pericleft regions. Scale bars: 50 μm.

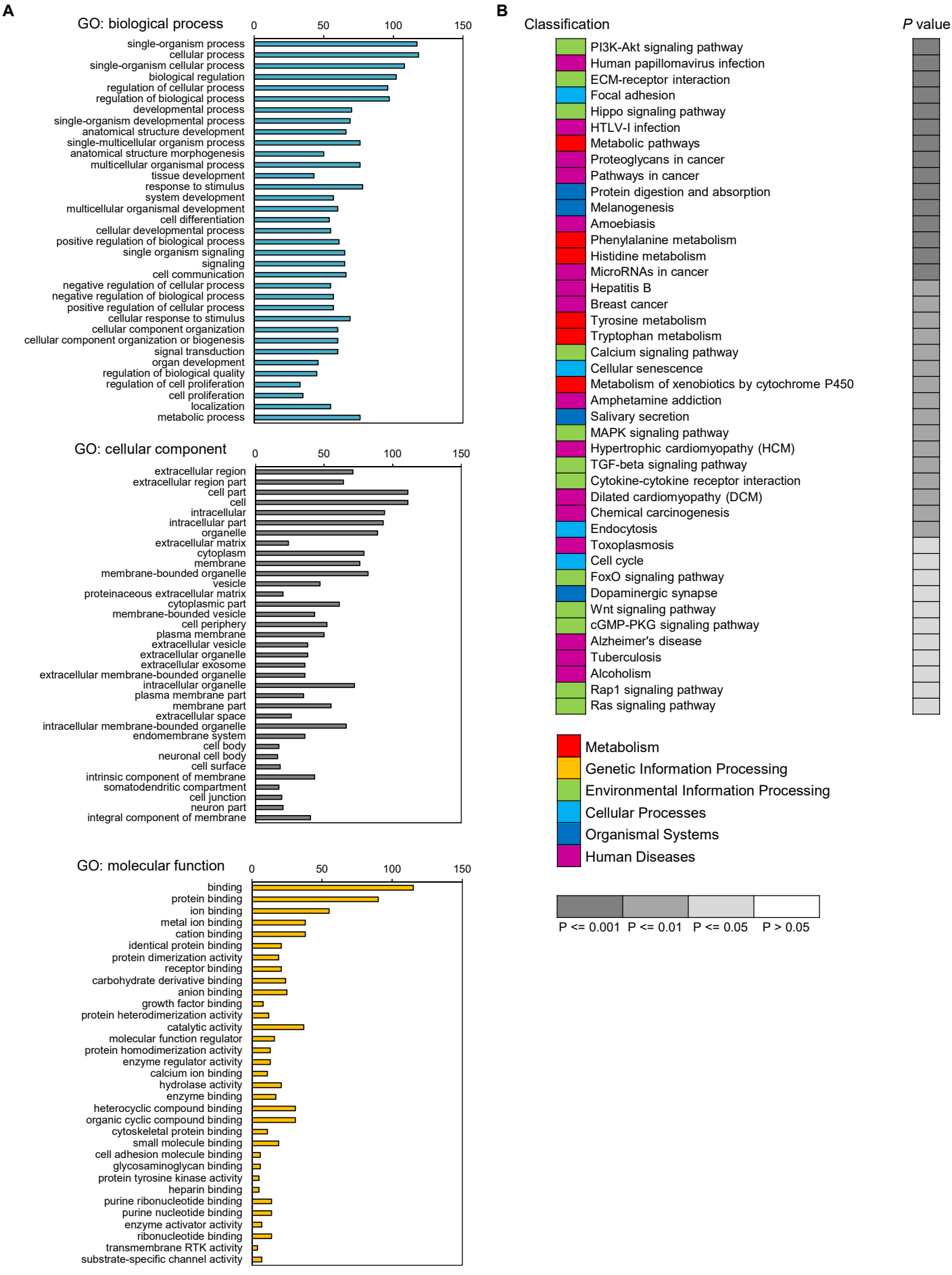


Fig. S5. RNA-seq data analysis (**A**) The top 35 gene ontology annotations with high significance (Control versus Blebbistatin, $P < 0.005$). The horizontal axis indicates DEG count. (**B**) KEGG enrichment map score (based on P value). The lists are arranged by descending significance (ascending P values). The data can be accessed at NCBI GEO accession number GSE137928.

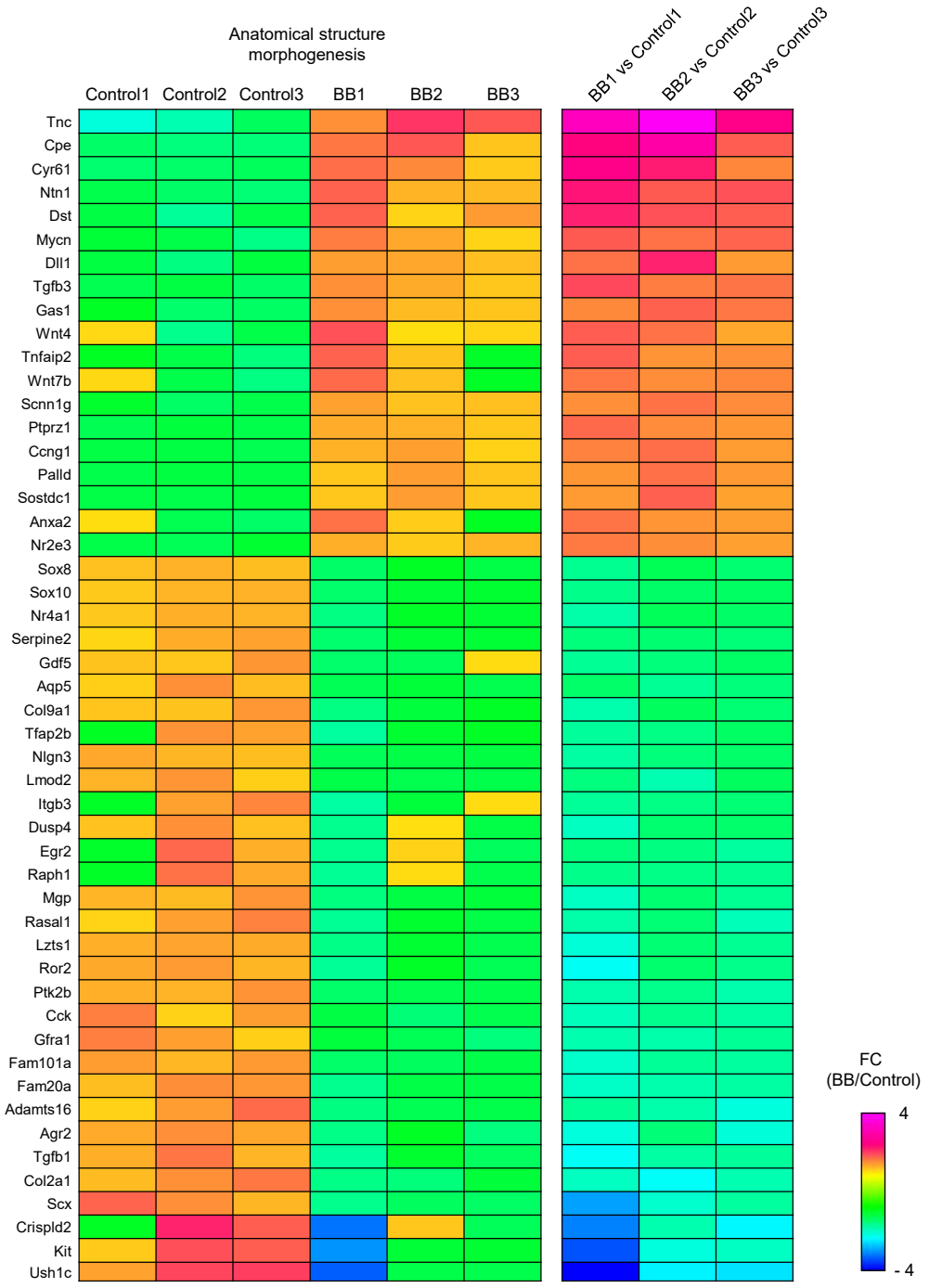


Fig. S6. Heatmap showing total DEGs in the anatomical structure morphogenesis annotation. DEGs are arranged by descending fold-change (FC).

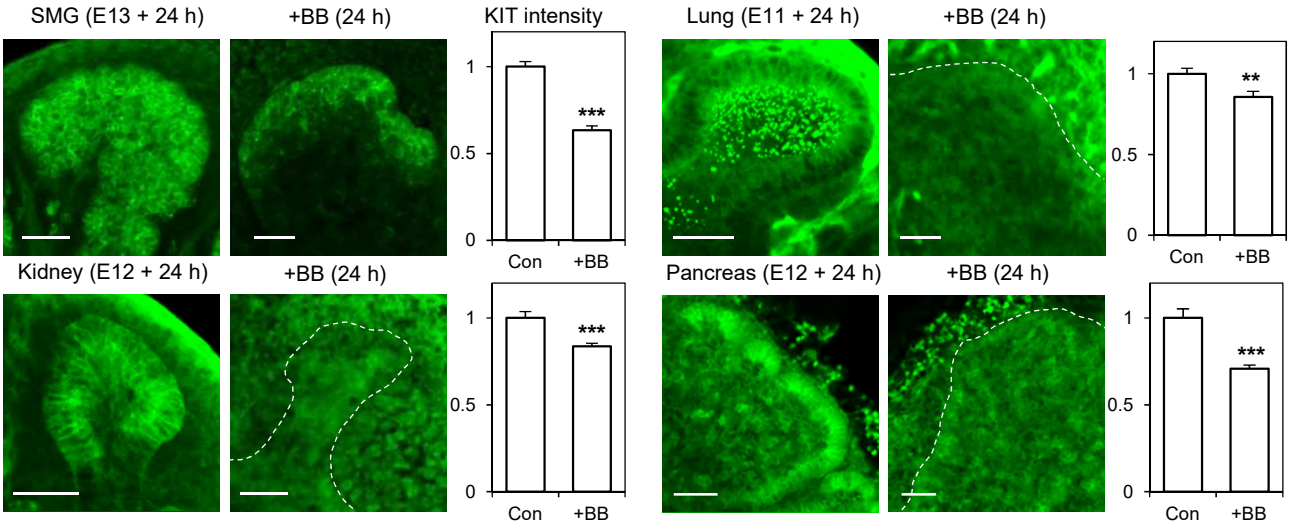


Fig. S7. Immunolabelled images of KIT expression in epithelial organs (images) and quantified intensity of KIT signals (bar graphs). Mean ± sem. n = 15 buds per group. White dashed regions represent the outline of epithelial region. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

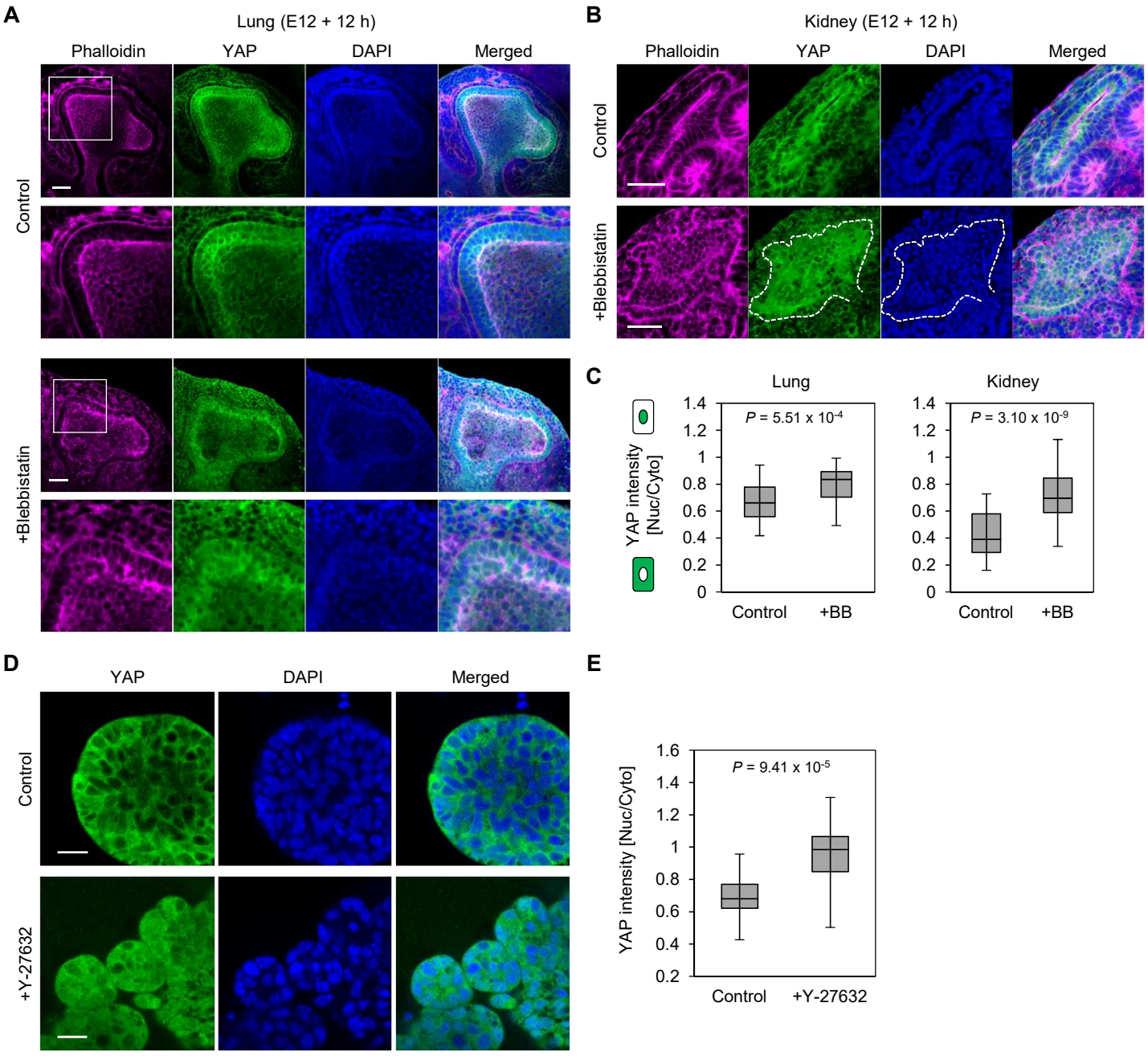


Fig. S8. YAP localization changes upon the perturbation of actomyosin contractility. (**A** and **B**) Immunolabelled images of phalloidin (magenta), YAP (green), DAPI (blue) in lung (**A**) and kidney (**B**) explant cultures in the control and blebbistatin-treated groups. (**C**) Relative intensity of nuclear and cytoplasmic YAP signals in control (n = 35 cells) and blebbistatin (n = 35 cells)-treated lung and kidney cultures. Median ± max/min. (**D**) Immunolabelled images of YAP (green) patterns and DAPI (blue) signals in control and Y-27632-treated E13 SMG cultures (24 h). (**E**) Relative intensity of nuclear and cytoplasmic YAP signals in control (n = 20 cells) and Y-27632 (n = 20 cells)-treated SMG cultures. Median ± max/min. Scale bars: 50 µm (**A**, **B**), 20 µm (**D**).

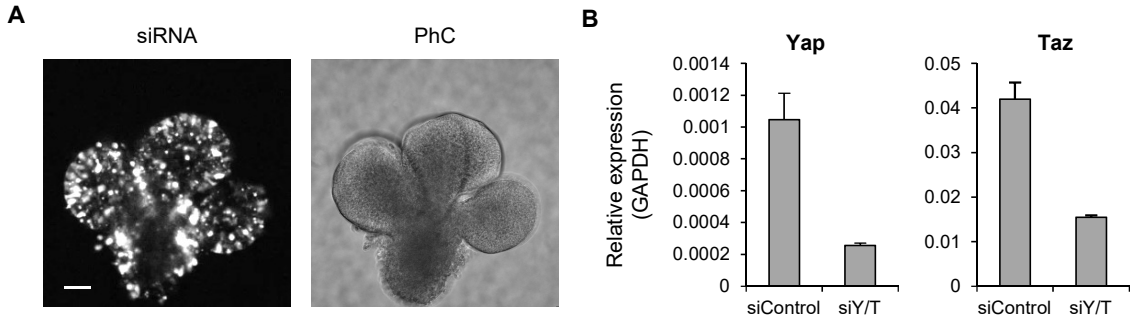
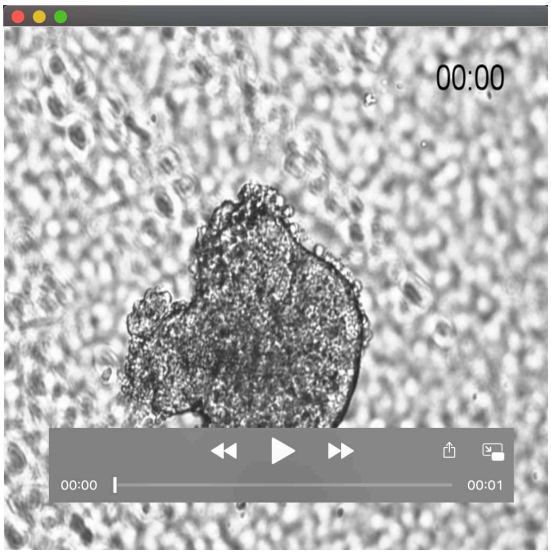
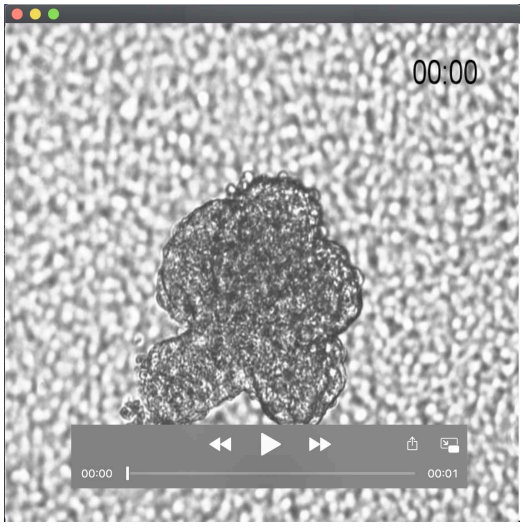


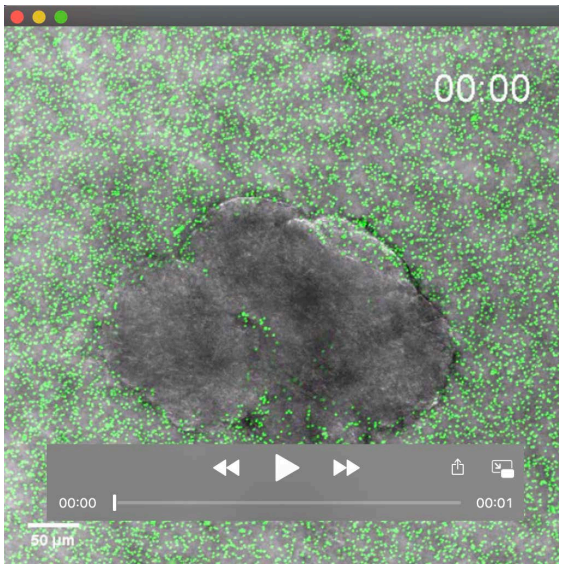
Fig. S9. Regulation of YAP/TAZ expression in epiSMG cultures. (**A**) The expression pattern of siRNA conjugated with fluorescent dye in E13 eSMG cultures. The transfected cultures were incubated for 24 h. PhC: phase contrast. (**B**) Nested qPCR results of the changes in Yap and Taz expression in eSMG cultures cotransfected with siYAP and siTAZ (siY/T). Mean ± sem. n = 3. Scale bar: 50 μm.



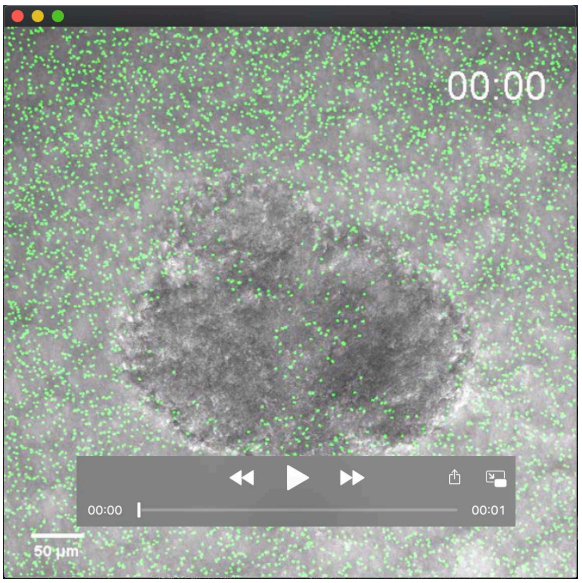
Movie 1. Branching morphogenesis of eSMG culture. Time-lapse phase contrast images of the initial branching process of eSMG cultures (E13). The images were acquired for 16 h with 10-min intervals.



Movie 2. Branching morphogenesis of eSMG culture treated with blebbistatin. Time-lapse phase contrast images of the initial branching process of eSMG cultures (E13) treated with 50 μM blebbistatin. The images were acquired for 16 h with 10-min intervals.



Movie 3. 3-D traction force analysis (Control). Time-lapse images of the initial branching process of eSMG cultures (E13). The isolated epithelial rudiments were embedded in Matrigel-DMEM/F-12 mixture containing 5% fluorescent microbeads (2.0 µm diameter). The images were acquired for 7 h with 10-min intervals.



Movie 4. 3-D traction force analysis (+Blebbistatin). Time-lapse images of the initial branching process of eSMG cultures (E13) treated with 50 µM blebbistatin. The isolated epithelial rudiments were embedded in Matrigel-DMEM/F-12 mixture containing 5% fluorescent microbeads (2.0 µm diameter). The images were acquired for 7 h with 10-min intervals.

Table S1. Lists of all DEGs along with their FC values. Differentially expressed transcripts are satisfied with |fold change|≥1.5 and independent t-test raw p <0.05.

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