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Supplemental information

Rho activation drives luminal collapse and eversion in epithelial acini

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Supplementary Figures

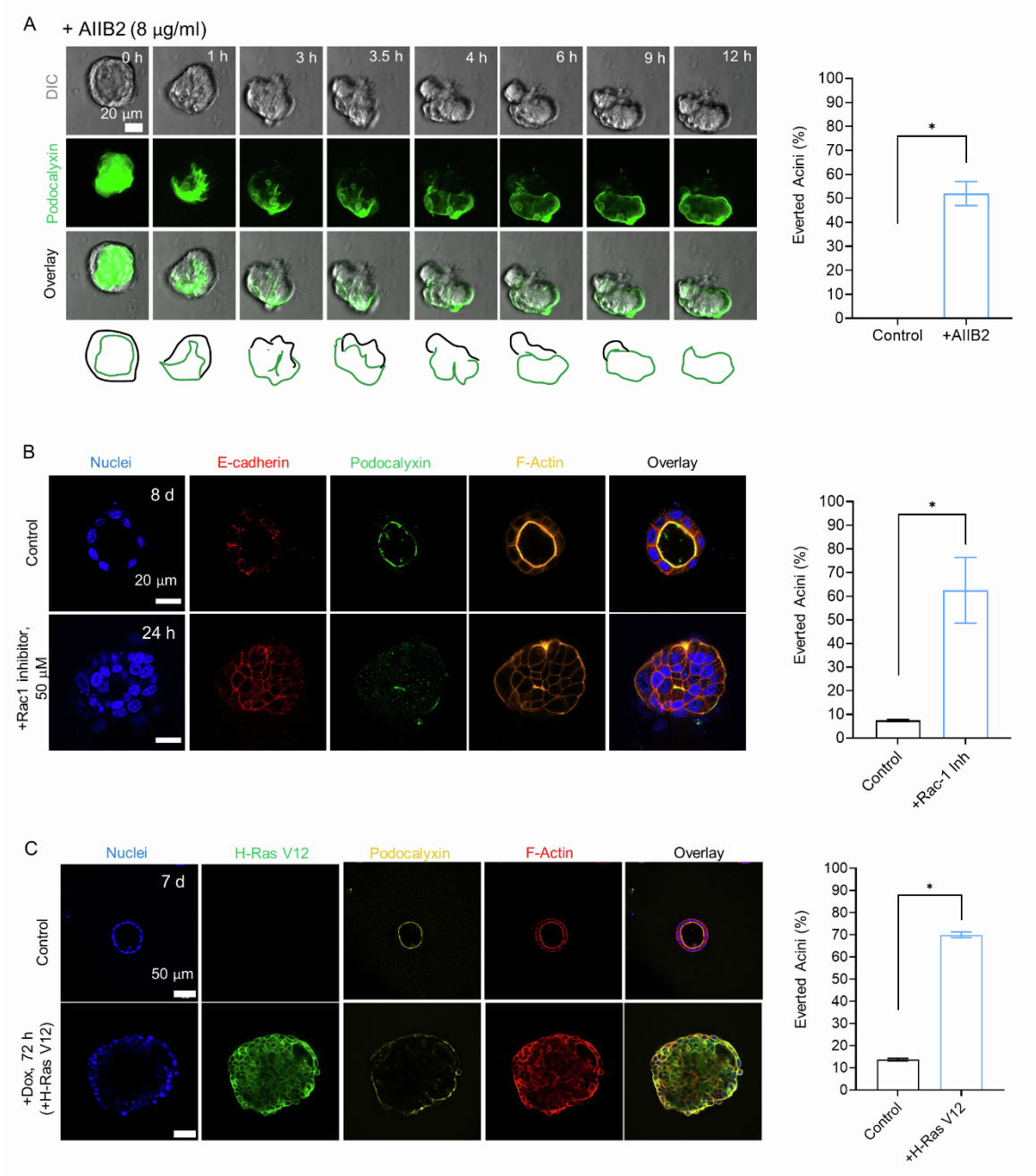

Fig. S1. (A) Time-lapse differential interference contrast (DIC) microscopy images and fluorescent GFP-podocalyxin images (GFP) of a 7-day old acinus treated with AIIB2 (β-1 integrin function blocking antibody). 8 µg/ml AIIB2 was added at T = 0 h (Movie 11). About 50% of inhibitor-treated acini everted over 12 hours. Data is representative of total of 65 control acini and 132 AIIB2 treated acini from 3 independent experiments. Error bars are SEM, *p<0.05 by Student's T test. Scale bar: 20µm (B) Percentage of everted MDCK II acini with and without Rac1 inhibitor (Rac1 inh, 50µM) treatment on day 7 of acinar

morphogenesis, for 24 hours. About 60% of inhibitor-treated acini everted over 24 hours. Data is representative of a total of 218 acini (untreated control) and 250 acini (Rac1 inh, 24h) over 2 independent experiments (*$p < 0.05$, Student t-test). Scale bar: 20μm (C) MDCK cells expressing doxycycline-inducible GFP H-Ras V12 were grown in Matrigel for 7 days. Doxycycline was added to the 7-day old acinus at T=0 to induce GFP H-Ras V12 expression, for 72 hours. About 70% of doxycycline induced acini everted after 72 hours. Data is representative of 60 acini per condition from 3 independent experiments. Error bars are SEM, *$p<0.05$ by Student's T test. Scale bar: 50μm

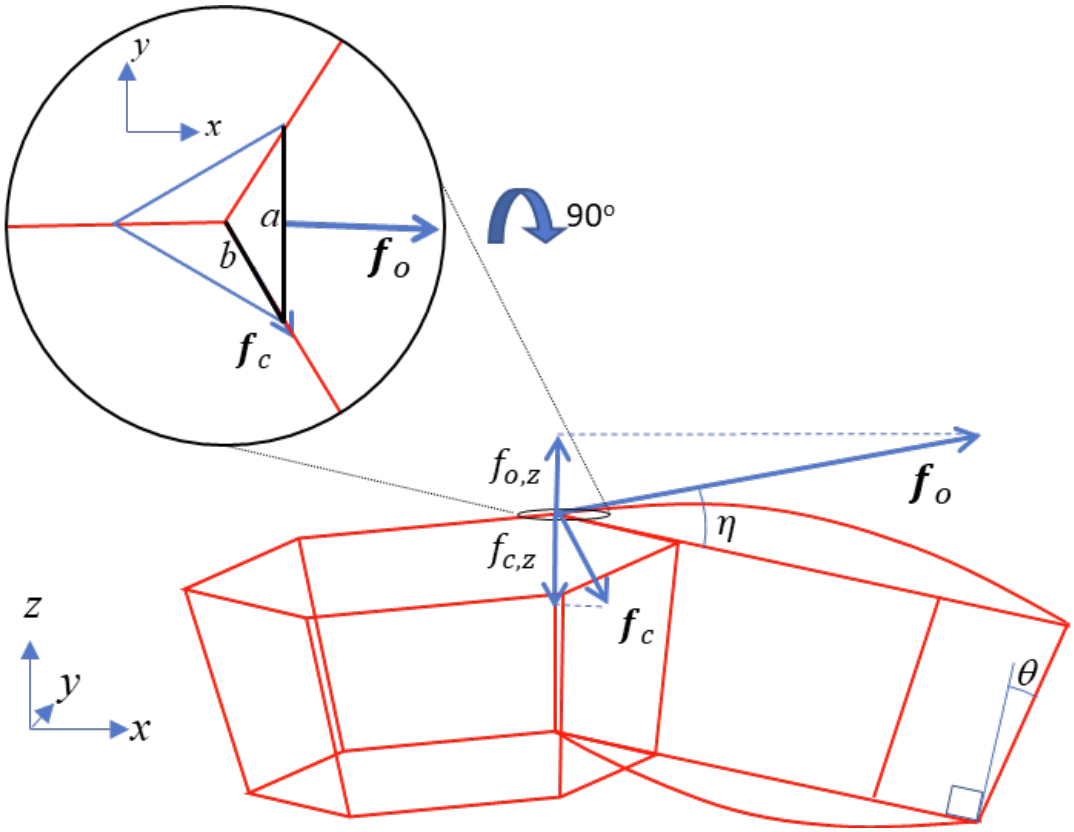

Figure S2 – Force balance at the basal junction between three identical adjacent hexagonal cells. Consider the forces on a small equilateral triangular prism enclosing the junction point. Due to the surface tension, τ_o, each basal cell surface exerts a force $\boldsymbol{f}_o$ directed tangentially to the surface at the junction point. In the frame of reference shown, the z-component in the limit of small a is $f_{o,z} = a\tau_o \sin(\eta - \theta) + O(a^2)$, where η is the contact angle and θ is the acinus angular change from the cell center to the junction point. Pressure forces are also $O(a^2)$. The z-component for the lateral-face surface tension, τ_c, along the segment b is $f_{c,z} = -\frac{a}{\sqrt{3}}\tau_c$, noting $b = \frac{a}{\sqrt{3}}$. Dividing by a and taking $a \to 0$ yields the force balance at the three-cell junction, $\frac{\tau_c}{\sqrt{3}} = \tau_o \sin(\eta - \theta)$. An identical derivation on the apical surface with surface tension, τ_i, yields $\frac{\tau_c}{\sqrt{3}} = \tau_i \sin(\eta + \theta)$.

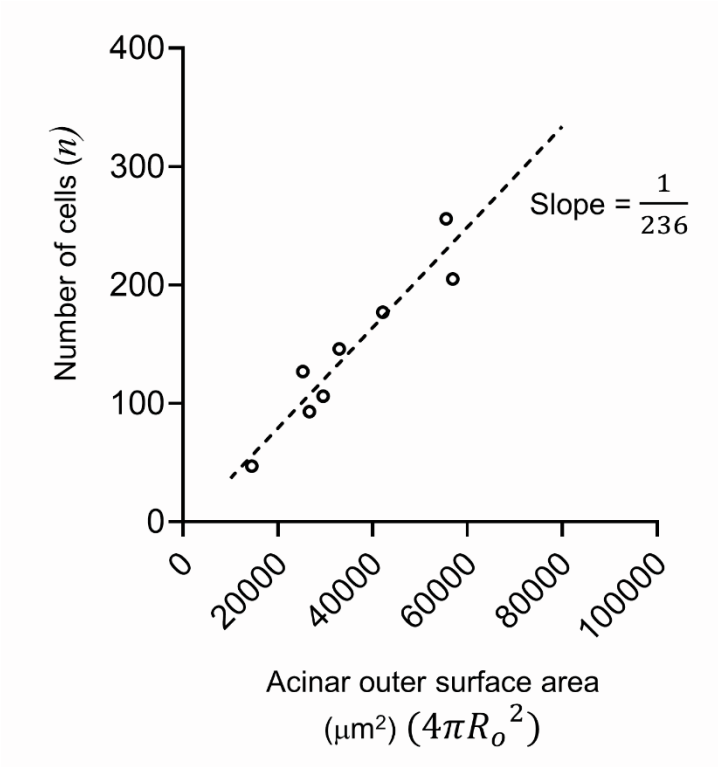

Fig. S3. Experimentally determined relationship between number of cells in acini (n) and acinar outer radii (R_o). Data shows n is directly proportional to ${R_o}^2$. The fitted line was obtained from measured values of n and outer surface areas ($4\pi {R_o}^2$) of 8 acini with a single lumen.

Movie Legends

Movie 1. Live imaging of MDCK acinus expressing GFP-podocalyxin formed over 10 days of three-dimensional culture. At time T = 0 h, the acinus was treated with Rho activator II. Scale bar, 20 μm.

Movie 2. Live imaging of MDCK acinus expressing GFP-podocalyxin formed over 10 days of three-dimensional culture and treated at time T = 0 h with Rho activator II. Scale bar, 20 μm.

Movie 3. Live imaging of MDCK acinus expressing GFP-podocalyxin formed over 10 days of three-dimensional culture and treated at time T = 0 h with Rho activator II. Scale bar, 20 μm.

Movie 4. Live imaging of MDCK acinus expressing GFP-podocalyxin after 10 days of three-dimensional culture and treated at time T = 0 h with DMSO as vehicle control. Scale bar, 20 μm.

Movie 5. Live imaging of MDCK acinus expressing GFP-H2B, emerald-occludin after 10 days of three-dimensional culture and treated at time T=0h with 1 μg/ml Rho Activator II. Scale bar, 25 μm.

Movie 6. 3D confocal scan of a single MDCK acinus expressing GFP-podocalyxin, treated with 1 μg/ml Rho Activator II for 12 h.

Movie 7. Live imaging of a single acinus expressing mCherry-KASH1 and GFP-podocalyxin. Cells were cultured for 10 days in matrigel, then treated at T = 0 h with doxycycline to induce the expression of mCherry-KASH1. Scale bars, 20 μm.

Movie 8. Live imaging of a single acinus expressing mCherry-KASH1 and GFP-podocalyxin. Cells were cultured for 10 days in matrigel, then treated at T = 0 h with doxycycline to induce the expression of mCherry-KASH1. Scale bar, 20 μm.

Movie 9. Live imaging of a single acinus expressing mCherry-KASH1 and GFP-podocalyxin. Cells were cultured for 10 days in matrigel, then treated at T = 0 h with doxycycline to induce the expression of mCherry-KASH1. Scale bar, 20 μm.

Movie 10. Live imaging of a single acinus expressing mCherry-KASHI1ΔPPPL and GFP-podocalyxin. Cells were cultured for 10 days in matrigel, then treated at T = 0 h with doxycycline to induce the expression of mCherry- KASH1ΔPPPL. Scale bar, 20 μm.

Movie 11. Live imaging of MDCK acinus expressing GFP-podocalyxin, after 7 days of three-dimensional culture and treated at time T=0 h with 8 μg/ml β-1 integrin function blocking antibody (AIIB2). Scale bar, 20 μm.

Movie 12. Live imaging of a single acinus assembled by 344SQ lung cancer cells treated with 1 μg/ml of Rho activator II at 0 h. Scale bar is 50 μm.

Movie 13. Live imaging of MDCK acinus expressing GFP-podocalyxin, after 7 days of three-dimensional culture where the acinus was laser ablated at T=0 h. Scale bar, 25 μm.

Movie 14. Live imaging of MDCK acinus expressing GFP-podocalyxin, after 7 days of three-dimensional culture where the acinus was laser ablated at T=0 h. Scale bar, 25 μm.