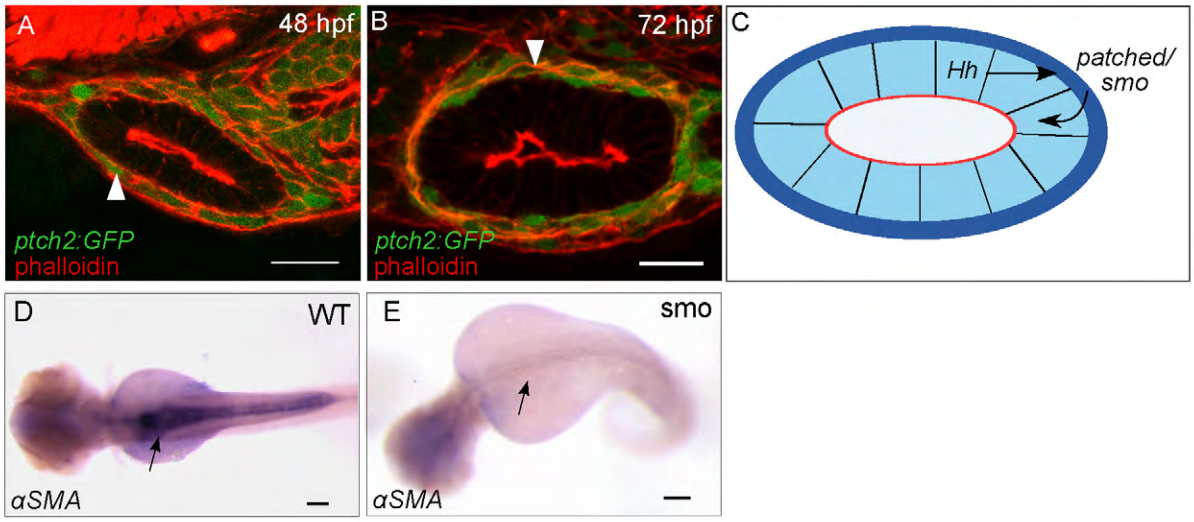
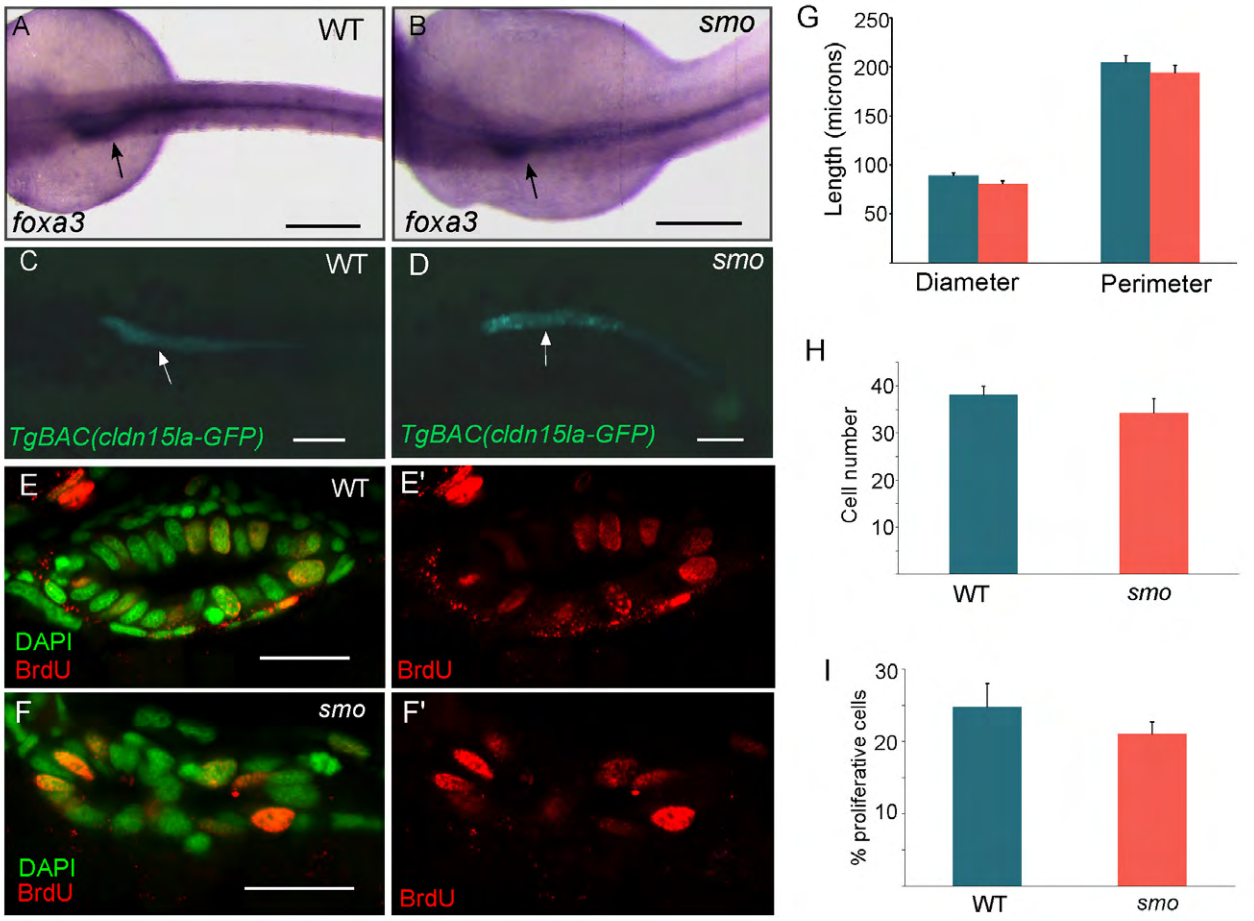
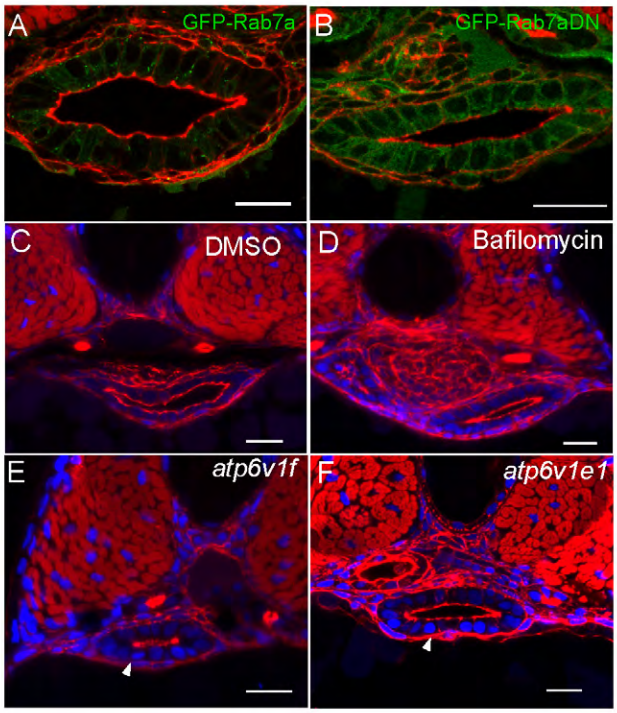


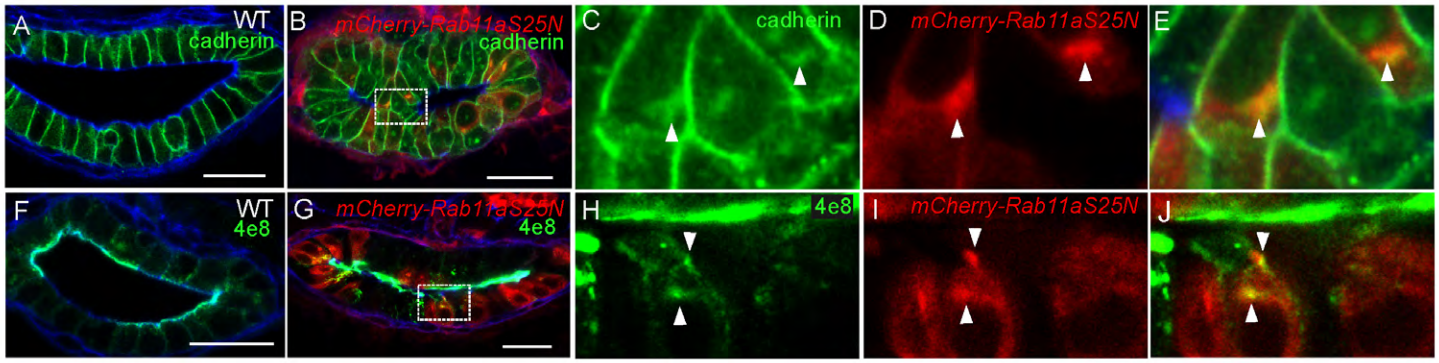
Supplementary material Fig. S1. *smoothened* signaling acts in the surrounding mesenchyme. (A,B) Confocal cross section of the transcriptional reporter *Tg(GBS-ptch2:EGFP)umz23*. Phalloidin (red), DAPI (blue). Scale bar: 20 µm. (C) *Hh* is expressed in the epithelium and binds to *ptch* in the mesenchyme to activate *smo* mediated downstream transcription. Thus, *smo* regulates the epithelium through epithelial-mesenchymal signaling pathways. (D,E) Dorsal view of an *in situ* hybridization showing αSMA expression in WT and *smo* mutant embryos at 72 hpf. Arrows point to smooth muscle. Scale bar: 100 µm.



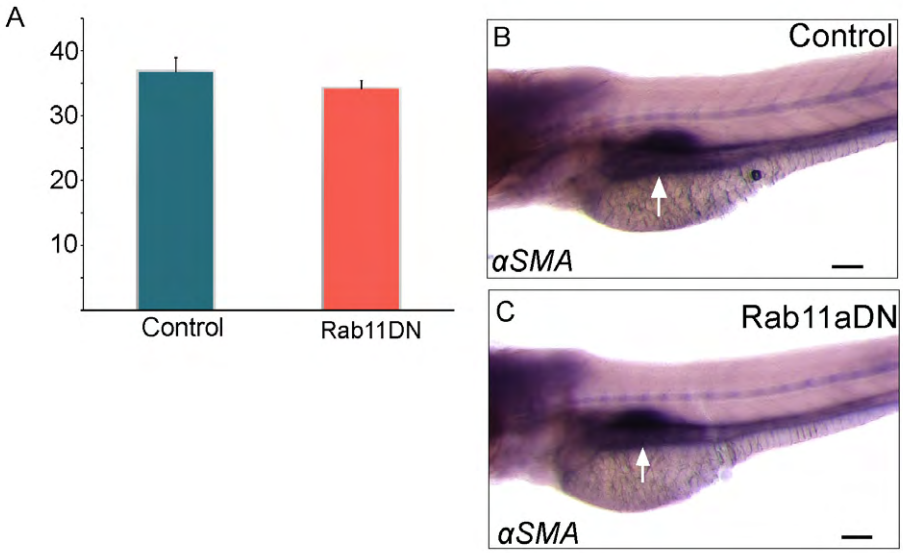
Supplementary material Fig. S2. Gut tube shape and total cell number are similar in wildtype and mutant embryos. (A,B) *In situ* hybridization of WT and *smo* mutant embryos expressing *foxa3* at 30 hpf. Scale bar: 200 µm. (C-D) WT and *smo* mutant embryo expressing *TgBAC(cldn15la-GFP)* at 48 hpf. Arrows indicate intestine. (E-F′) Cross section of WT and *smo* mutant guts at 72 hpf stained for BrdU to label proliferating cells. Scale bars: 20 µm. (G) Quantification of the diameter and perimeter of WT and *smo* mutant guts from transverse cross sections. Wt n=14, mutant n=18, diameter P>0.18, perimeter P>0.48. (H) Quantification of total cell number in WT and mutant guts. WT n=14, mutant n=18, P>0.30 (I) Quantification of the percent of BrdU positive cells in WT and *smo* mutant guts. WT n=14, mutant n=19, P>0.32.



Supplementary material Fig. S3. The degradation pathway is not involved in lumen formation. (A,B) Confocal cross sections from *Tg(hsp70l:GFP-Rab7)* and *Tg(hsp70l:GFP-Rab7DN)* embryos. Phalloidin (red). (C,D) Confocal cross sections from DMSO and bafilomycin treated embryos. (E,F) Confocal cross sections from *atp6* ^{v1f} *and atp3*^{v1e} mutant embryos. Phalloidin (red). Scale bars: 20 µm.



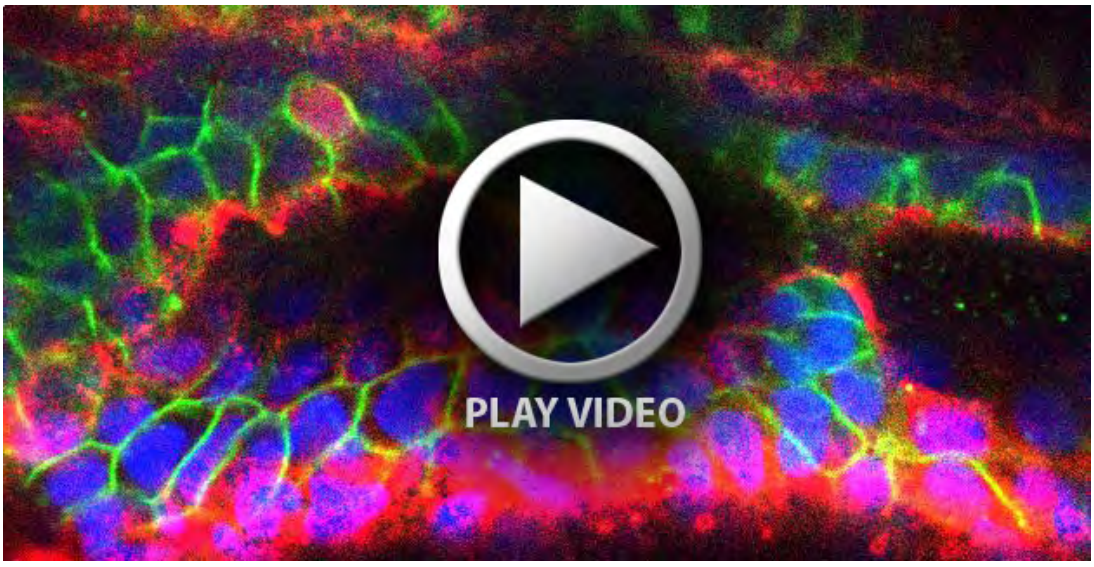
Supplementary material Fig. S4. Apical and basolateral proteins colocalize with Rab11aDN compartments. (A-E) Confocal cross section of a WT and Rab11aDN embryos at 72 hpf stained for cadherin. Arrowheads point to Rab11DN and cadherin co-localization in internal compartments. (F-J) Confocal cross section of a WT and Rab11aDN embryos at 72 hpf stained for 4e8. Arrowheads point to Rab11DN and 4e8 co-localization in internal compartments Scale bars: 20 µm



Supplementary material Fig. S5. Cell number and mesenchymal differentiation is not impaired in Rab11DN embryos. (A) Quantification of total cell number in the gut in WT and Rab11aDN embryos. WT *n*=13, DN *n*=11, *P*>0.32. (B,C) Lateral view of an *in situ* hybridization showing αSMA expression in WT and Rab11aDN embryos at 72 hpf. Arrow points to smooth muscle. Scale bar: 100 µm. (D-E") Confocal section of WT and Rab11DN embryo stained for Myh11. Arrowhead points to Mhy11 in the mesenchyme. Asterisk indicates non-specific epithelial staining as observed previously (Wallace et al., 2005). Scale bar: 20 µm.

Supplementary material Movie 1. Selective Plane Illumination Microscopy of a *TgBAC(cldn15la-GFP)* embryo from 48-72 hpf. The images from top to bottom represent different z-planes of a single embryo.

Supplementary material Movie 2. Selective Plane Illumination Microscopy of a *TgBAC(cldn15la-GFP)* embryo from 56-70 hpf. Small lumens open and fuse along the length of the gut, resulting in two large luminal compartments separated by a cellular bridge. However, this movie ends prior to complete lumen resolution.



Supplementary material Movie 3. Confocal stack of a fixed whole mount *Tg(hsp:GFP-podlx)* (red) embryo stained with cadherin (green) and DAPI (blue).