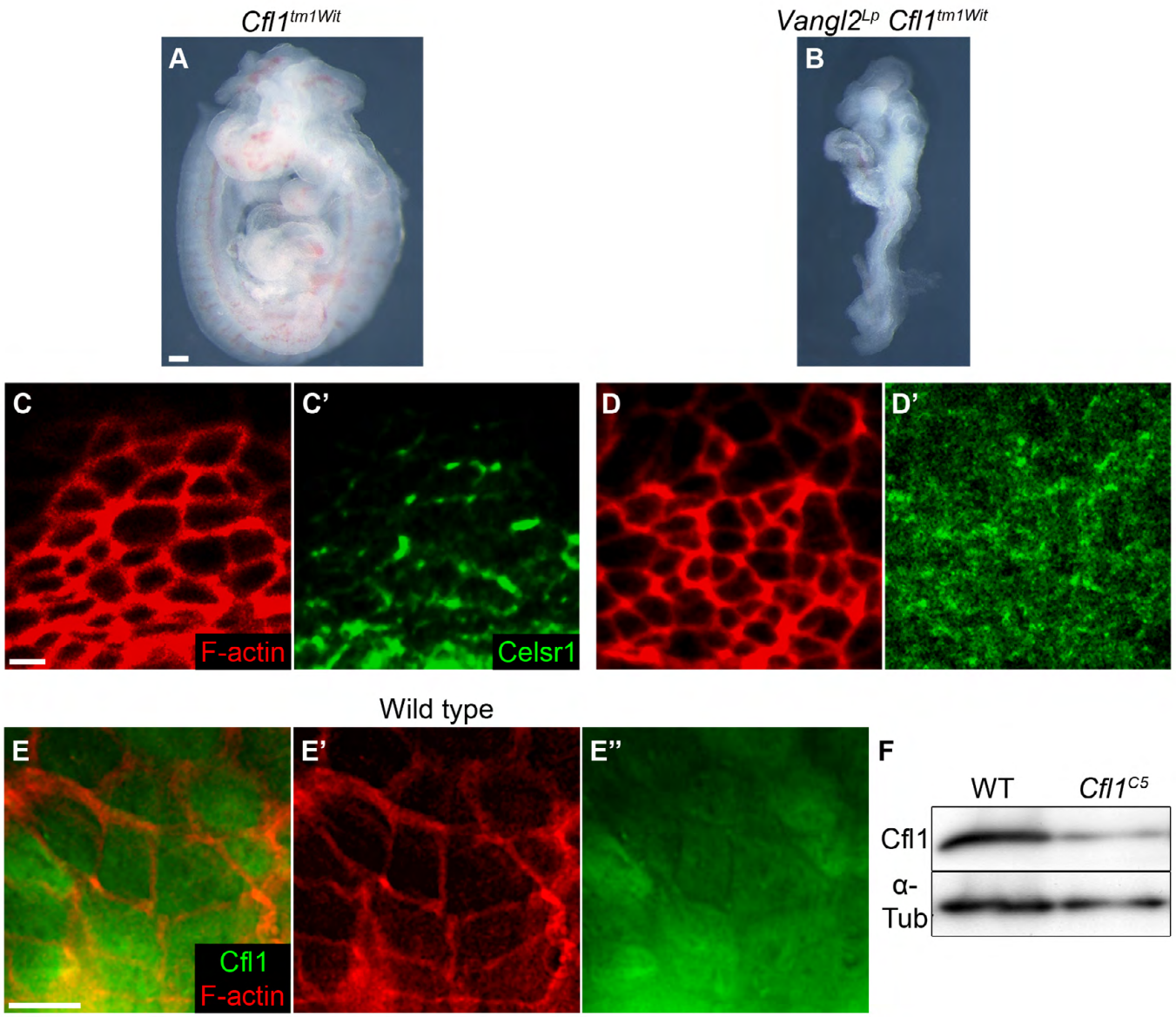

Fig. S1. Planar polarity is strongly disrupted in *Cfl1*^{tm1wit} *Vangl2*^{Lp} double mutants. (**A**) At E9.5, the phenotype of *Cfl1*^{tm1wit} homozygous embryos (homozygous for a targeted null allele) is indistinguishable from that of *Cfl1*^{C5} homozygotes, including exencephaly, a wavy dorsal neural tube and heart defects. (**B**) The *Vangl2*^{Lp} *Cfl1*^{tm1wit} double mutants have the same appearance as *Vangl2*^{Lp} *Cfl1*^{C5} double mutants, with a shorter body axis, heart-looping defects and abnormal somites. (**C**,**D**) As in *Cfl1*^{C5}, Celsr1 protein is localized to the apical membrane in the anterior and posterior faces of node cells in the *Cfl1*^{tm1wit} mutants (C), and this membrane recruitment is lost in the *Vangl2*^{Lp} *Cfl1*^{tm1wit} double mutants (D). (**E-E″**) Cfl1 protein (green) is detected throughout the apical cytoplasm in node cells in wild-type embryos. (**F**) Western blot of whole embryo lysate from a single E9.5 embryo shows a significant decrease in Cfl1 protein in *Cfl1*^{C5} mutants compared with wild-type littermates. Scale bar: 250 µm in A,B; 2.5 µm in C-E.

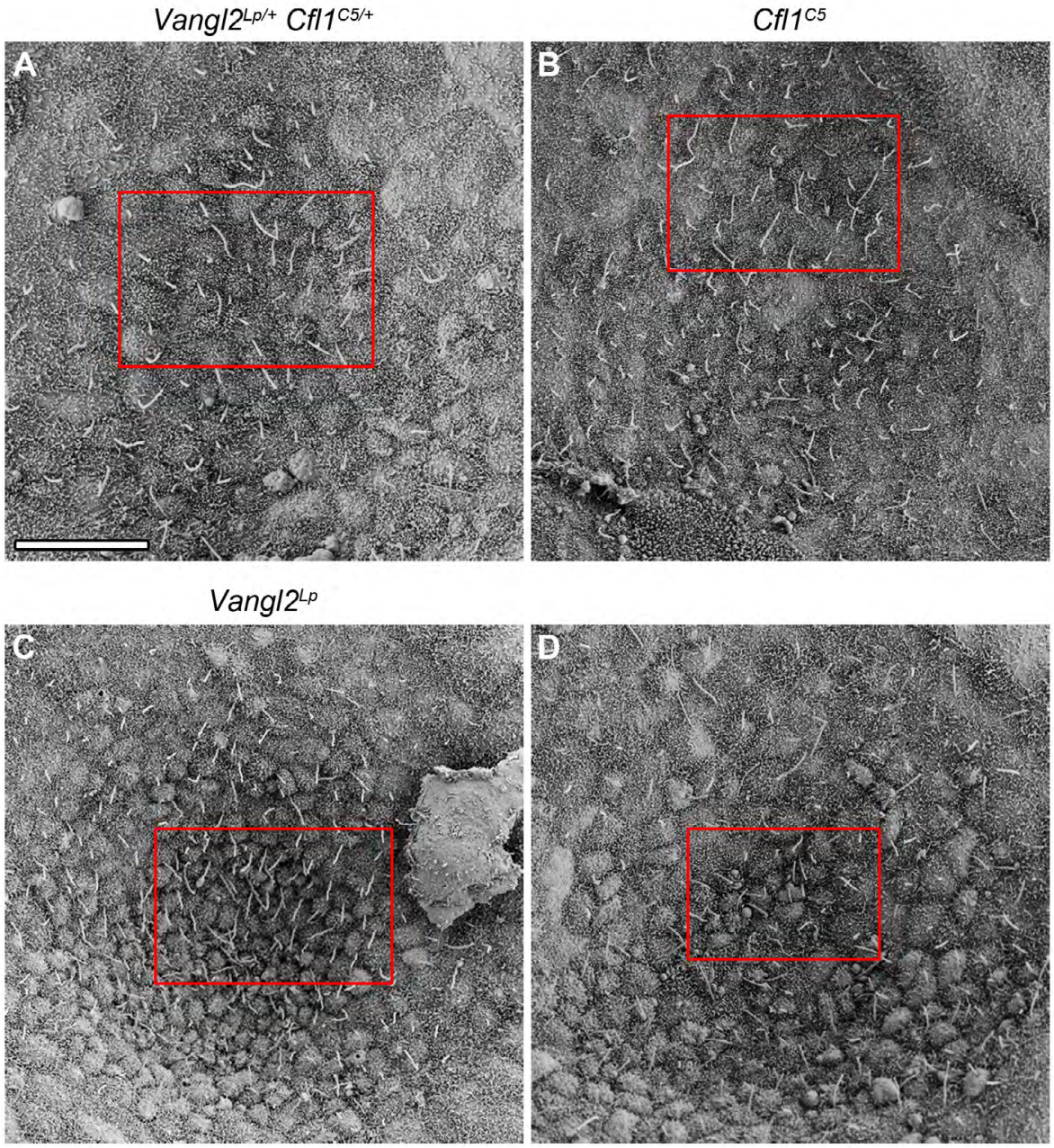

Fig. S2. Nodal cilia are not properly polarized in *Vangl2^{Lp} Cfl1^{C5}* double mutants. (**A-D**) Low magnification scanning electron microscope images of nodal cilia from wild-type (A), *Cfl1^{C5}* (B), *Vangl2^{Lp}* (C) and *Vangl2^{Lp} Cfl1^{C5}* (D) embryos show a disorganization of cilia polarity in the double mutants. These images come from the same embryos as shown in Fig. 4; the high magnification region is boxed in red. Scale bar: 10 µm.

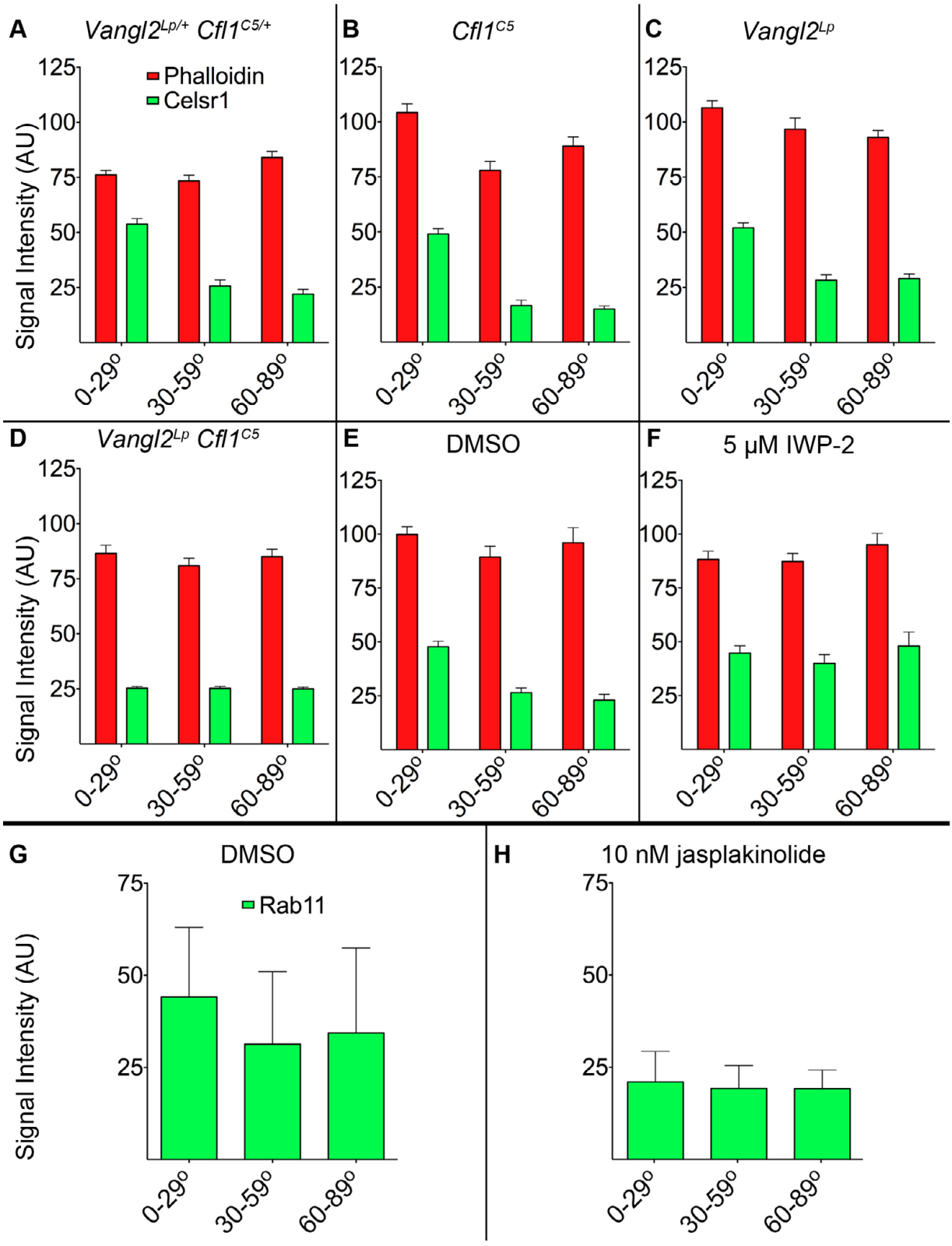

Fig. S3. Actin dynamics are required for the membrane association of Celsr1 and Rab11. (**A-F**) Raw data for phalloidin and Celsr1 signal intensities from Figs 5 and 6; these data have not been normalized. (**G**) Rab11 signal intensity at the apical membrane, normalized to the phalloidin intensity at the given angles. Although the mean signal intensity for Rab11 along the horizontal lines is greater than both 30-59º and 60-89º, the difference is not statistically significant. (**H**) The membrane association of Rab11 is lost after treatment with 10 nM jasplakinolide. These data have also been normalized to the phalloidin intensity at the membrane.

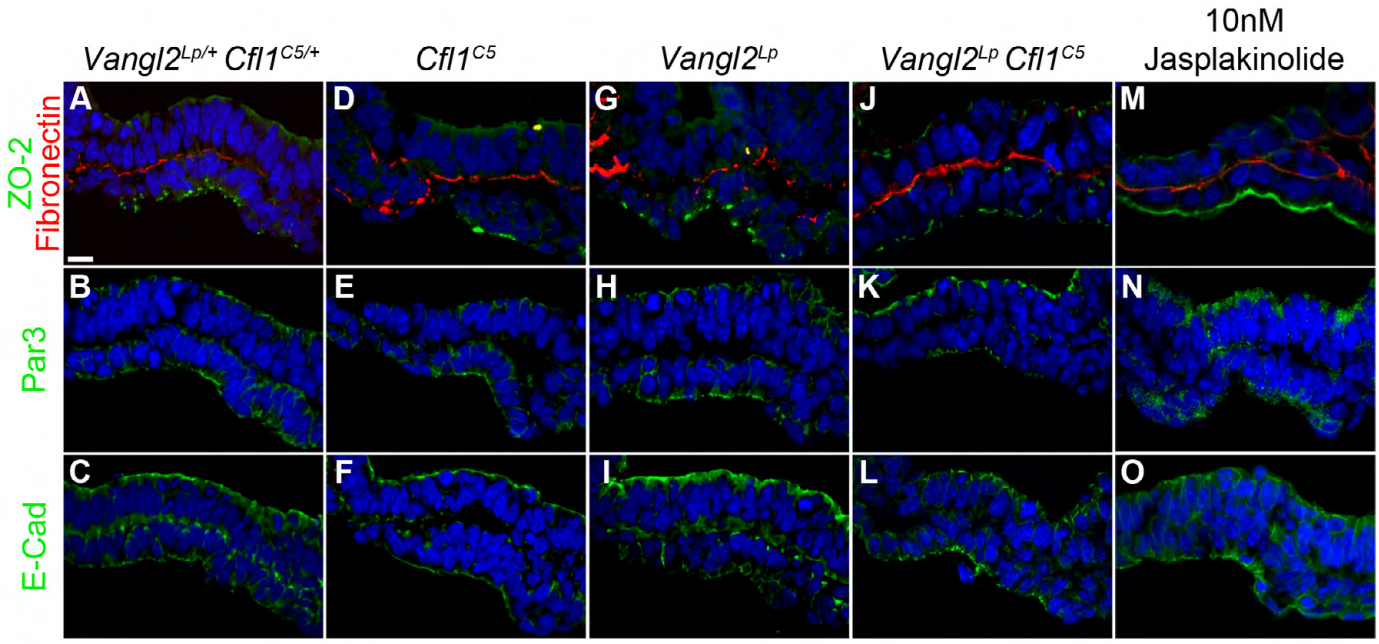

Fig. S4. Apical-basal polarity is normal in *Vangl2*^{Lp} *Cfl1*^{C5} mutant or jasplakinolide-treated nodes. (**A-O**) For all images, the ventral node is the lower epithelium, and the apical membrane faces the bottom of the image. The apical markers ZO-2 (A,D,G,J,M), Par3 (B,E,H,K,N) and E-cadherin (C,F,I,L,O) are localized correctly to the apical membrane of the node. The basal lamina component fibronectin (red) (A,D,G,J,M) is localized correctly to the basal side of node cells in sections of wild type (A-C), *Cfl1*^{C5} (D-F) and *Vangl2*^{Lp} (G-I) single mutants, as well as the compound mutants *Vangl2*^{Lp} *Cfl1*^{C5} (J-L). Apical-basal polarity is not disrupted in wild-type embryos after 14-18 hours of culture in 10 nM jasplakinolide (M-O). Scale bar: 10 µm.

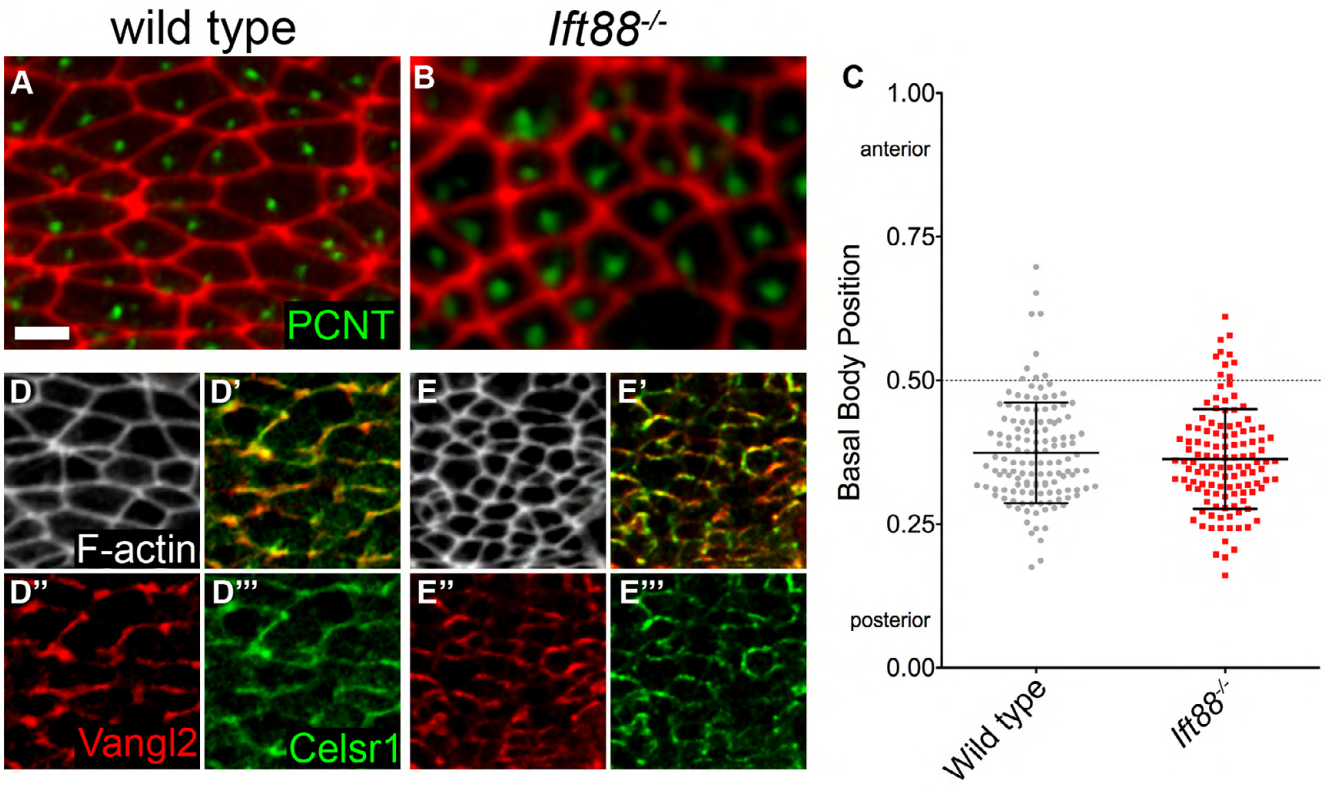

Fig. S5. Planar cell polarity is normal in the node of *Ift88*$^{-/-}$ mutant embryos. (**A-C**) Like wild-type embryos, pericentrin (green) is posteriorly polarized in the nodes of *Ift88*$^{-/-}$ mutants at E8.0. Phalloidin staining (red) outlines the cells. (**D**,**E**) Recruitment of Vangl2 and Celsr1 to the anterior and posterior faces of node cells is also normal in *Ift88*$^{-/-}$ mutants. Scale bar: 5 µm.

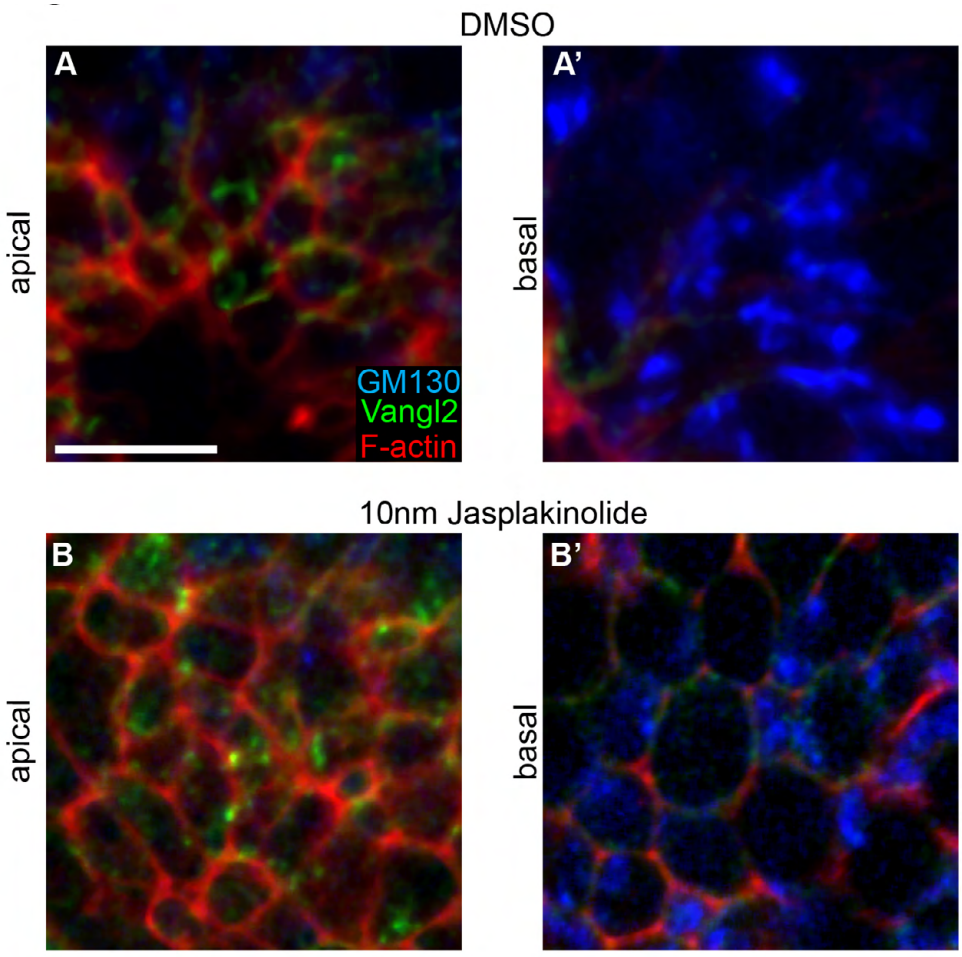

Fig. S6. Vangl2 accumulates in apical vesicles when remodeling of the actin cytoskeleton is blocked with jasplakinolide. (**A**) Like Celsr1, Vangl2 (green) is planar polarized in the apical membrane of wild-type node cells, as seen in a 3 µm projection from a *z*-stack of the apical domain of cells from the node of a wild-type embryo cultured in DMSO for 14-18 hours. (**A′**) In the same sample, GM130 (blue), a marker for the cis-Golgi network, is 4.5 µm below (basal to) the region where Celsr1 is enriched. (**B**) After treatment with 10 nM jasplakinolide, Vangl2 is found in cytoplasmic puncta enriched in the apical domain. (**B′**) These cytoplasmic puncta are apical to the Golgi, as GM130 is found 3.75 µm below the apical surface in this embryo. There is increased phalloidin staining along the basolateral membrane after treatment with 10 nM jasplakinolide. Scale bar: 10 µm.

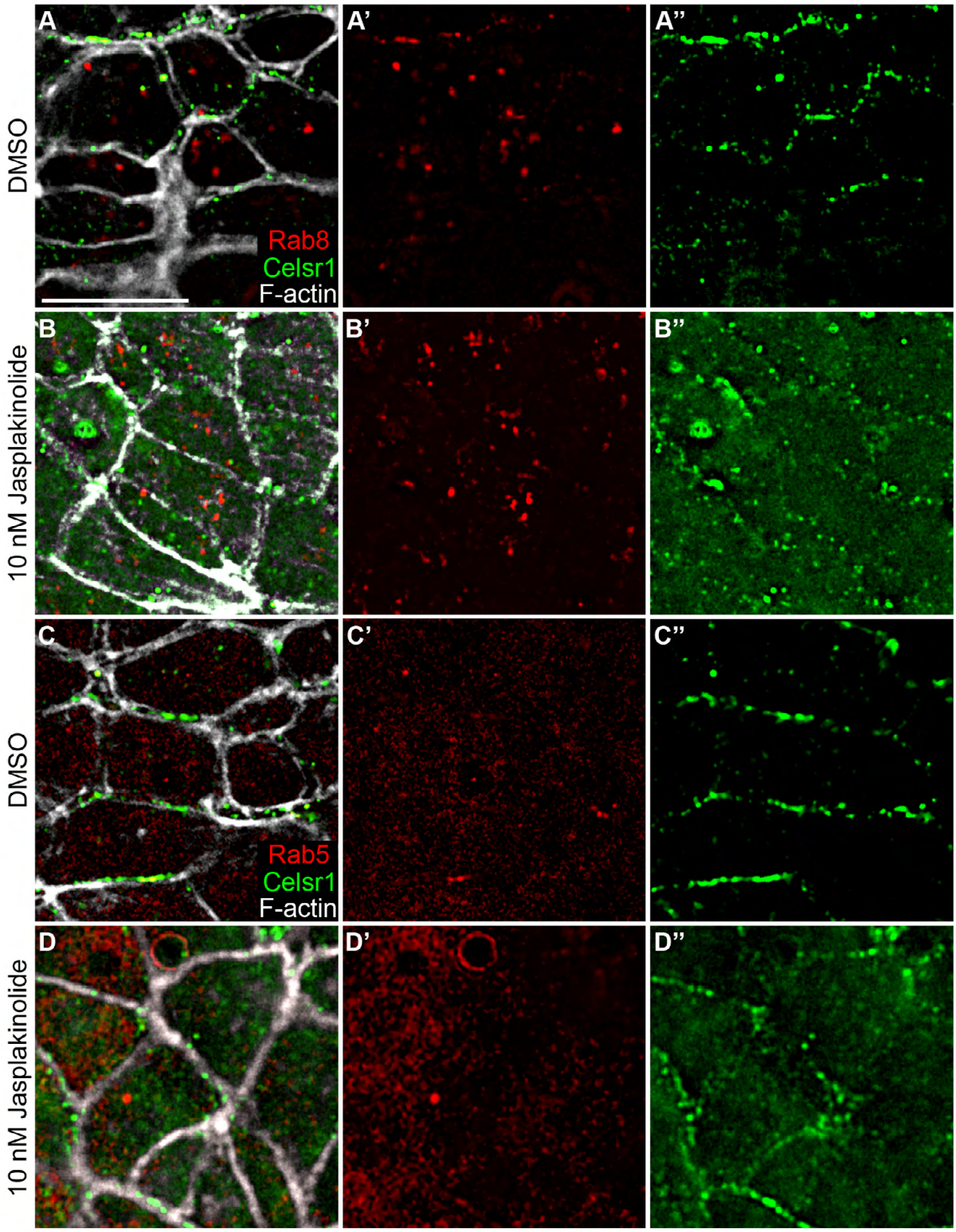

Fig. S7. Cytoplasmic vesicles containing PCP proteins do not colocalize with Rab8 or Rab5. (**A-A″**,**C-C″**) Wild-type embryos cultured for 14 hours in 0.1% DMSO have Celsr1 (green) enrichment in the apical membrane along the anterior/posterior faces of node cells. There is no colocalization between Celsr1 and Rab8 (A-A″) or Rab5 (C-C″) in these DMSO controls. (**B-B″**,**D-D″**) Treatment with 10 nM jasplakinolide inhibits the membrane association of Celsr1, which is now localized in cytoplasmic puncta. These cytoplasmic vesicles containing Celsr1 do not co-stain for Rab8 (B-B″) or Rab5 (D-D″). F-actin (white) is stained with phalloidin. Scale bar: 10 μm.