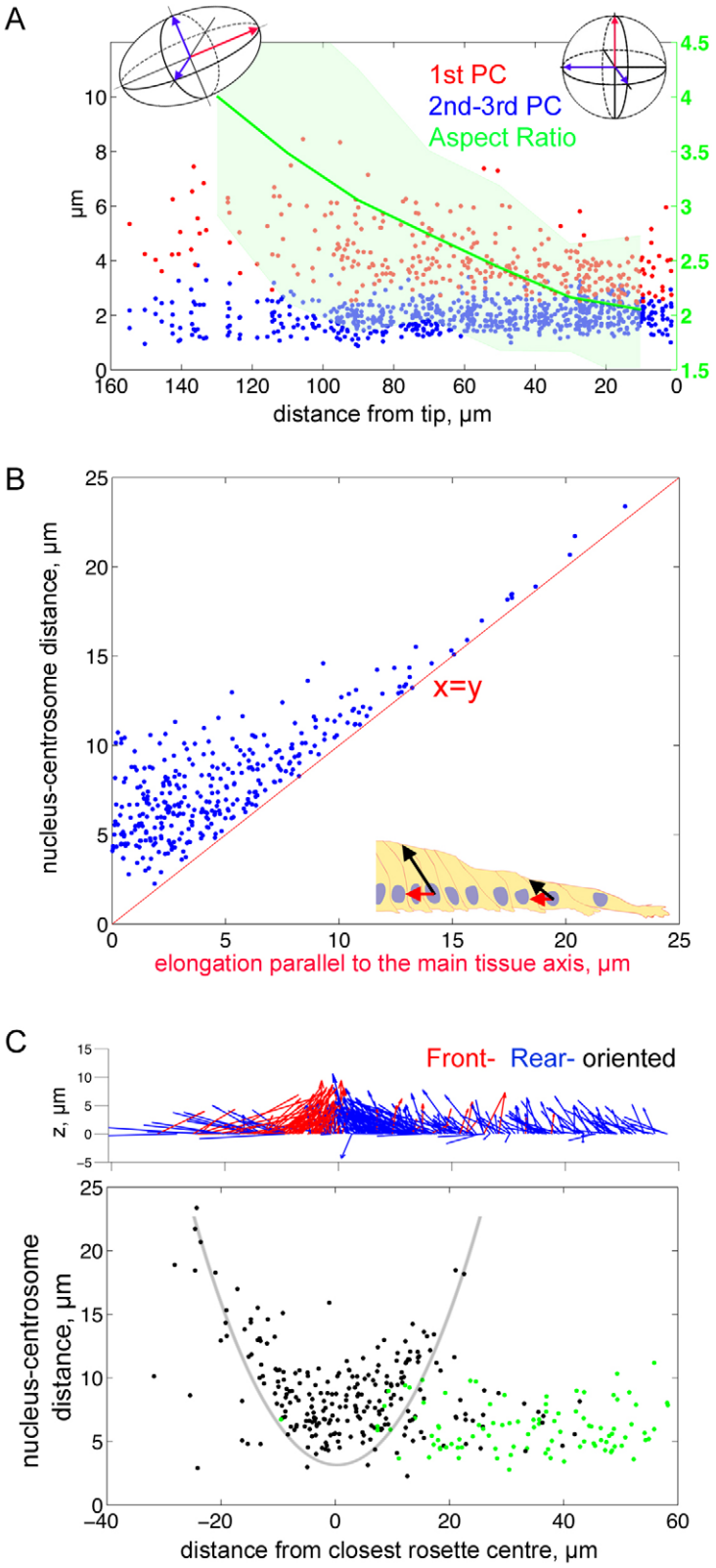


Fig. S1. Cell elongation during the leader to follower transition
(A) Dimensions of individual microtubule networks, defined using principle component analysis, as a function of their distance from the tissue's leading edge. The aspect ratio (mean ratio±sd between the 1st and the 3rd principal components, PC) demonstrates a transition from more spherical to ellipsoidal shapes from front to rear of the tissue. (B) Plot of the nuclear-centrosomal vector length against the length of their x component (along the migration axis) revealing the alignment of the most elongated cells with the migration axis. (C) Plot of nuclear-centrosomal vector length as a function of cell distance to its closest rosette centre, the corresponding mapping of the vectors is shown (top, red front- and blue rear- orientation) showing that cells elongate away from the rosette centres. Data points corresponding to cells of the leading domain (first 30µm) are shown in green, the grey parabola was drawn to highlight the elongation pattern around the rosette centre.

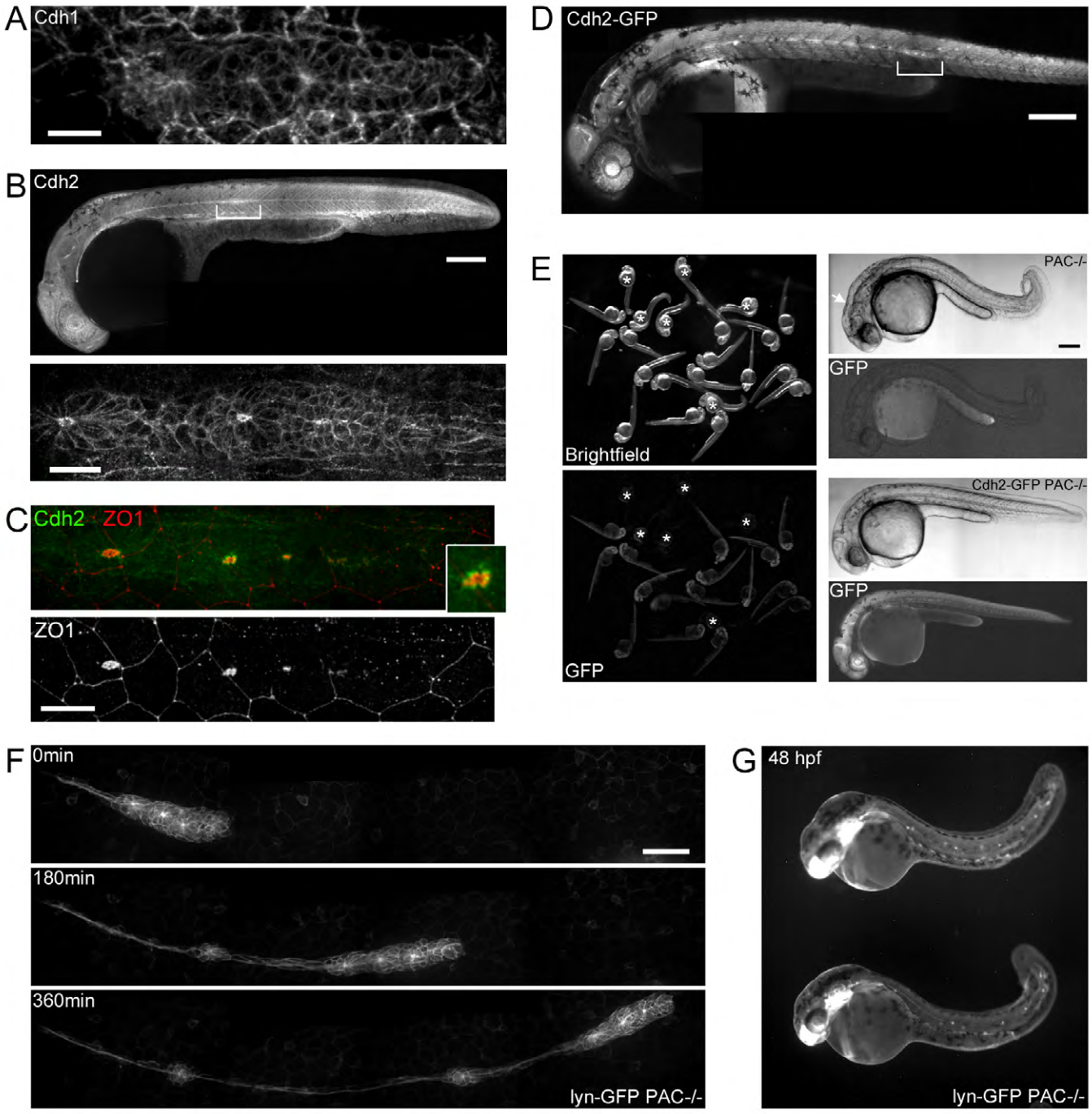


Fig. S2. Generation of live adherens junction reporter lines
(A) Immunohistochemistry of Cdh1 in the pLLP. (B) Immunohistochemistry of Cdh2 in a 30hpf embryo and in the pLLP (lower panel). (C) Double antibody staining for Cdh2 and ZO1 in the pLLP. The twice-enlarged view of a rosette highlights the broader localisation of Cdh2 around ZO1. (D) Whole embryo overview of the cadherin2:Cadherin2-GFP BAC that recapitulates endogenous Cdh2 expression. Brackets highlight the position of the pLLP. (E) Rescue of the parachute mutant (cdh2^{tm101b}-/-, PAC-/-) by cadherin2:Cadherin2-GFP transgenics. Left panels show a clutch of embryos from an incross of cadherin2:Cadherin2-GFP;cdh2^{tm101b}-/-. Non-transgenic embryos (*) show characteristic mutant phenotype illustrated in the top right panels (curled tail and brain defects, white arrow) whereas transgenic siblings develop normally (bottom right) and survive. Scale bars embryo=200µm, pLLP=20µm. (F) Images from a time-lapse movie of a cldnb:lyn-GFP;cdh2^{tm101b}-/- showing the migration of the pLLP at 30hpf and the formation and deposition of neuromasts in the absence of Cdh2. Scale bar=50µm.(G) 48hpf cldnb:lyn-GFP;cdh2^{tm101b}-/- embryos depicting the posterior lateral line neuromast pattern after the pLLP has reached the tail.

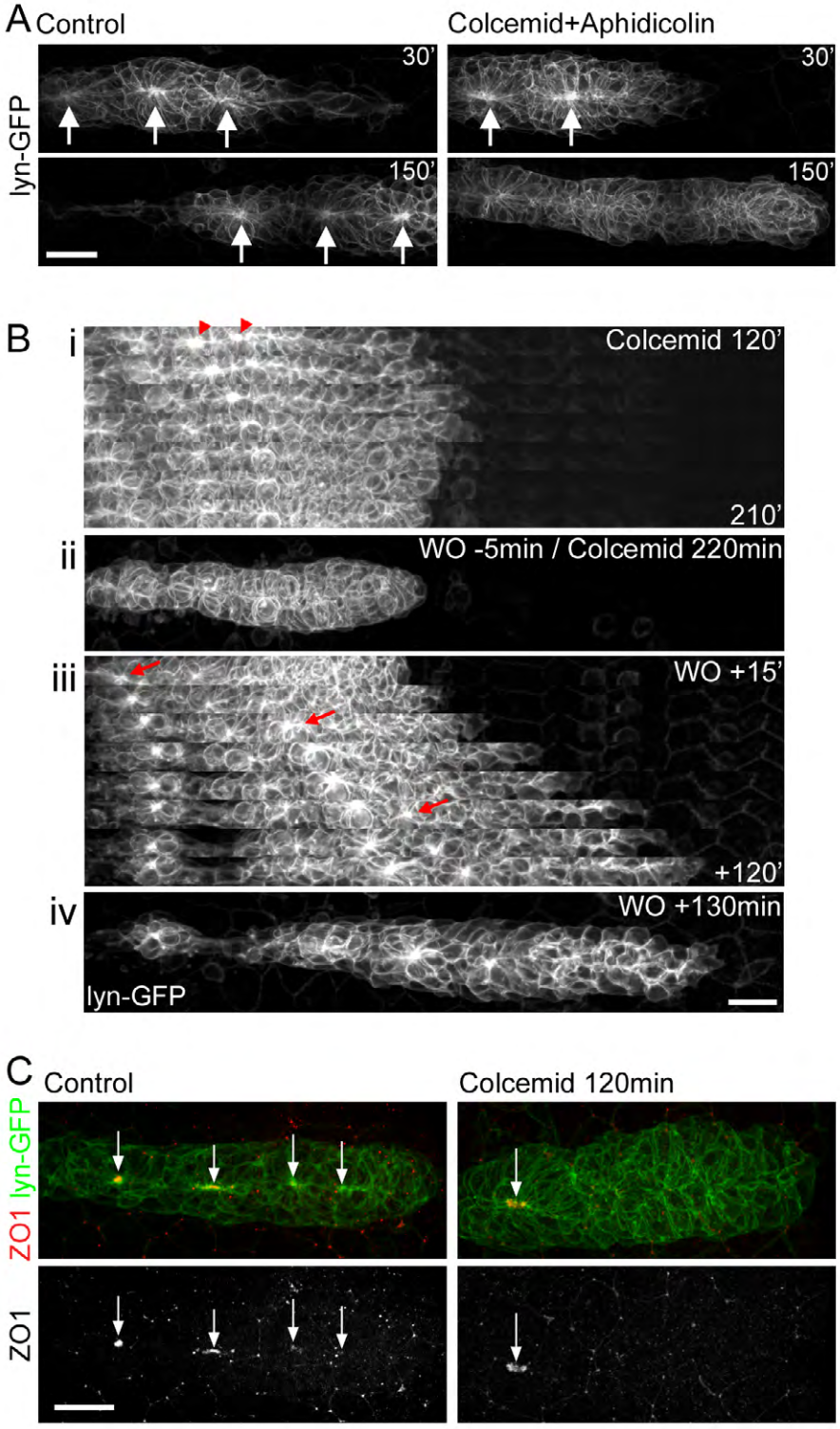
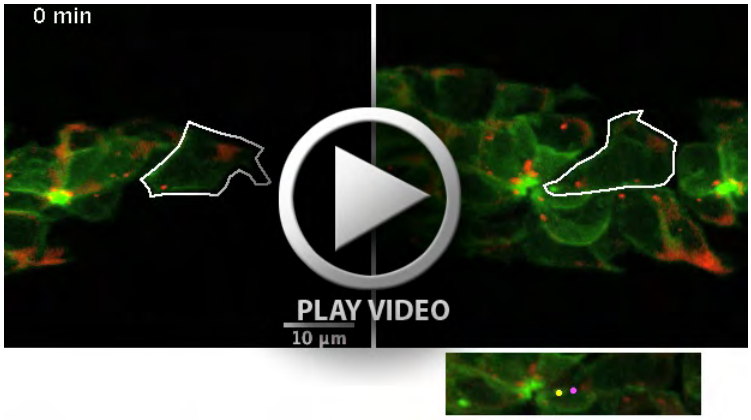


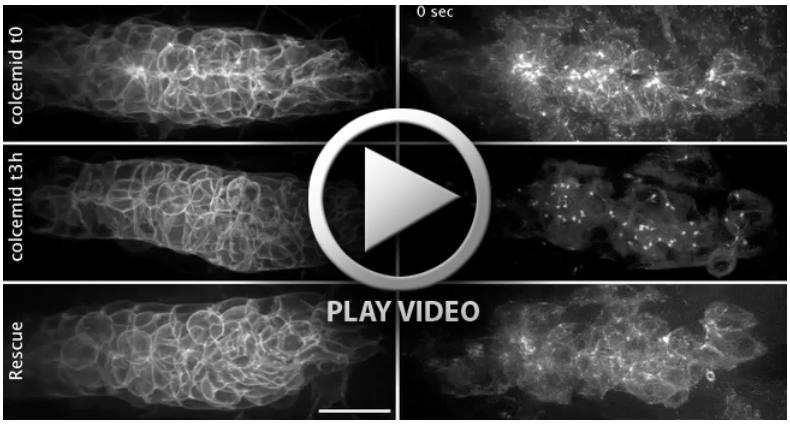
Fig. S3. Reversible loss of rosette constrictions upon microtubule depolymerisation

(A) Time-lapse images of a control and a pLLP treated with colcemid and aphidicolin, which prevents cell entry into S phase, illustrating rosette disassembly even in absence of mitosis. Arrows indicate rosette constrictions. (B) (i) Concatenated time-lapse images at 15-minute intervals of a cldnb:lyn-GFP pLLP treated with colcemid showing loss of rosettes (red arrowheads). (ii) Image of the same pLLP just before washout and inactivation of the drug (WO). (iii) Concatenated time-lapse images of the pLLP after washout, appearing rosettes are highlighted with red arrows. (iv) Image of the pLLP after washout showing normal tissue organisation. (C) Immunohistochemistry for the tight junction protein ZO1 in a control and a 2 hour-colcemid treated pLLP showing the loss of its apical concentration in a front to back manner after microtubule depolymerisation. Arrows point at apical ZO1 concentrations. Scale bars=20μm.

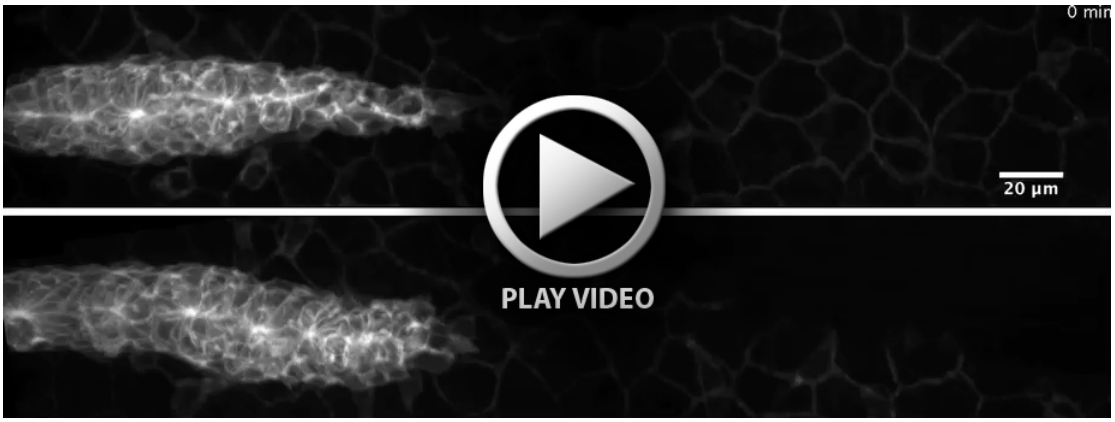
Movie 1
2-photon time-lapse of a BAC cdh2:Cdh2-GFP expressing pLLP showing the appearance of apical clusters behind the leading edge and their coalescence into organised apical poles of rosette cells. The pLLP position has been stabilised in the time-lapse to ease visualisation of the process.



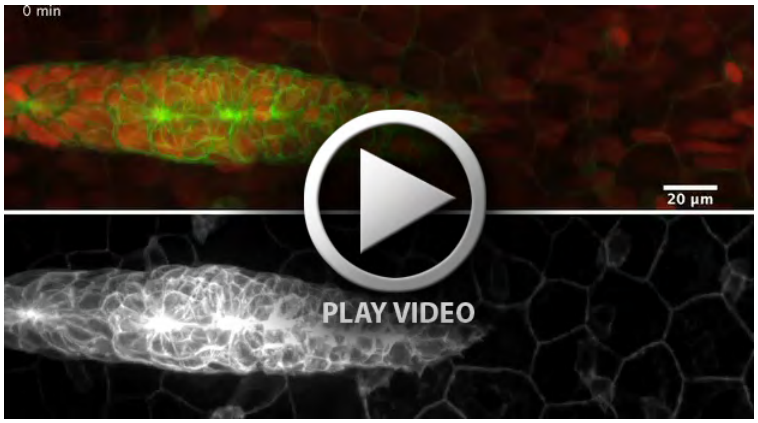
Movie 2
Time-lapse of Cdh2-GFP (green), centrin-tdTomato (red) expressing cells transplanted into unlabelled hosts showing a backward oriented cell of the front of the pLLP (left) and a cell shifting polarity to a front orientation (right). In the left panel, the front cell does not acquire a detectable stable apical Cdh2-GFP cluster and its centrosome remains constantly localised towards the back of the cell. In the right panel, as a new Cdh2-GFP cluster is formed at the cell front in contact with a forming rosette, the centrosome reorients towards it. Inset shows tracks of the position of the Cdh2-GFP cluster (yellow dot) and of the centrosome (pink dot) revealing that the centrosome follows the Cdh2-GFP cluster. Cells are outlined in the first snapshot and their positions have been stabilised in the time-lapse to ease visualisation of the process.



Movie 3
Time-lapse movie showing Eb3-tdTomato labelling of microtubule growing tips (right) in a lyn-GFP pLLP (left) before colcemid treatment (top). After 3 hours of treatment (middle), comets are no longer detectable but reappear upon colcemid washout and UV inactivation (bottom). The decrease in signal to noise is due to fluorophore bleaching.



Movie 4
Colcemid treatment of two lyn-GFP expressing pLLP imaged simultaneously. In the lower panel, the drug is UV inactivated when indicated inducing the rapid recovery of the pLLP organisation.



Movie 5
Lyn-GFP (green) and nls-tdTomato (red) expressing pLLP treated with 25mM EGTA. Lower panel shows the lyn-GFP channel with enhanced contrast to demonstrate that periderm integrity is preserved during this time range.