Supplementary Material - Supplementary Figures

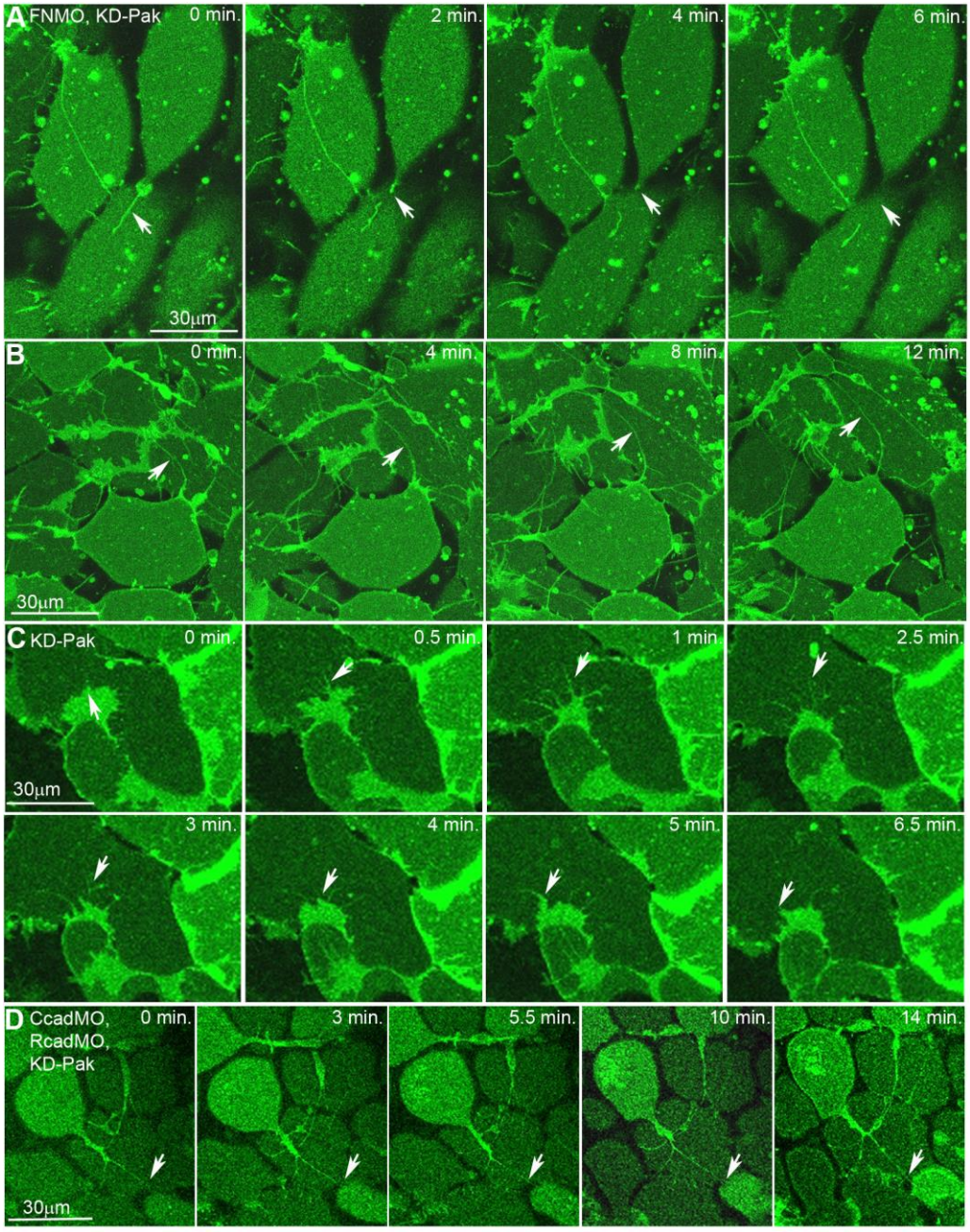

Figure S1. Fibronectin and cadherin requirements for lamellipodia retraction. Time lapse recordings of kinase-dead Pak1/membrane-GFP expressing LEM explants with (A,B) (n=7; 2 experiments) or without FN-MO injection (C) (n=5; 2 experiments), or with coinjection of C-cad-MO and R-cad-MO (D) (n=10; 2 experiments). Arrows, retracting or breaking retraction fibers.

Development • Supplementary information

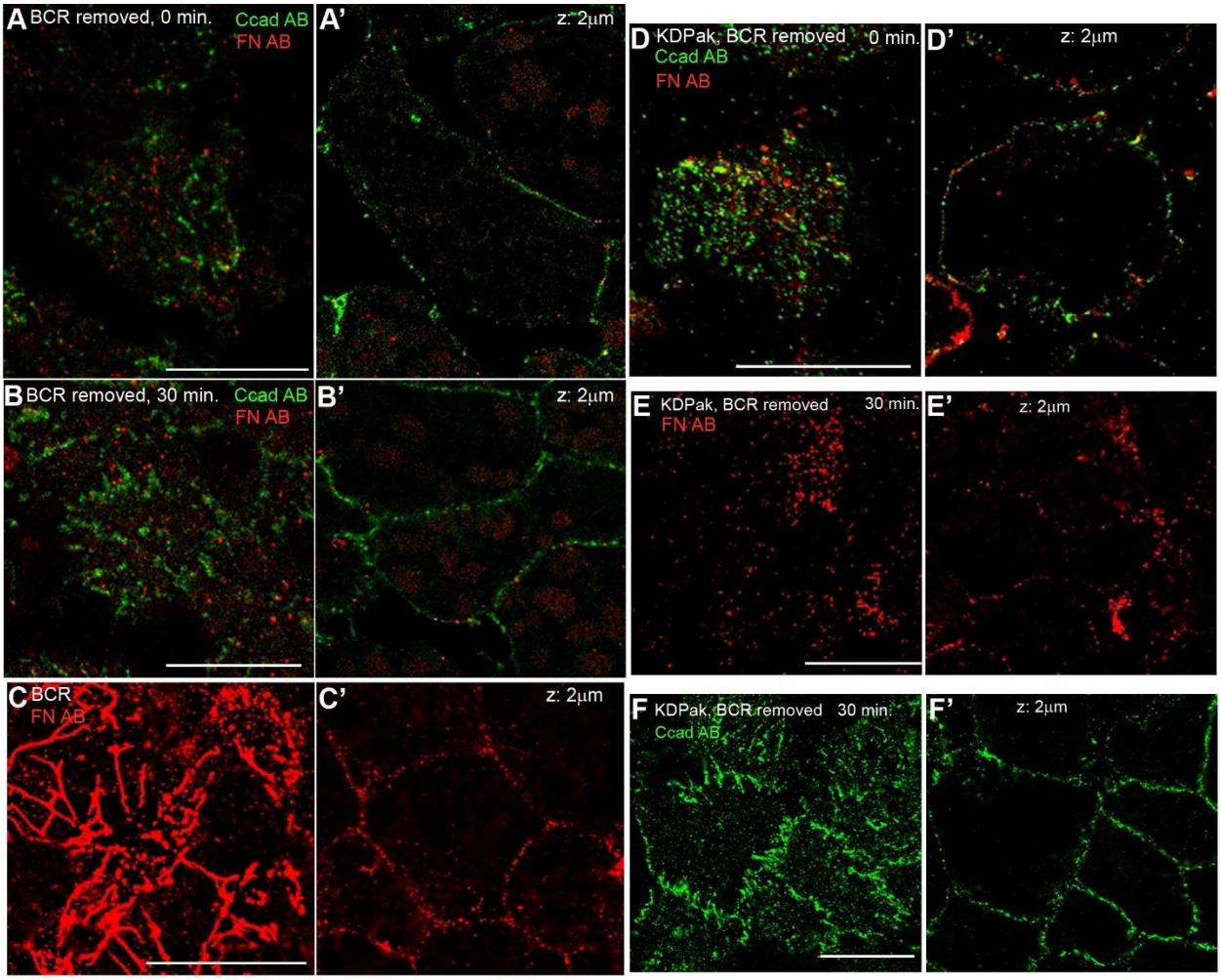

Figure S2. csFN and C-cadherin on the surface of cells. (A,D) BCR-facing side of untreated (n=28; 2 experiments) or kinase-dead Pak1 expressing LEM (n=17) stained immediately after BCR removal for FN (red) and C-cadherin (green), viewed at surface (A,D) and deep in tissue (A',D'). (B,B') Same as (A,A') but fixed and stained 30 minutes after removal of BCR (n=14). (E-F') same as (B,B') but with kinase-dead Pak1 expressing LEM stained for FN (E,E') (n=14) and C-cadherin (F,F') (n=15). (C,C') FN staining shows fibrils on the BCR cell surface (C) and csFN puncta between cells deep within the BCR (C') (n=11). Bars, 30 µm.

Development • Supplementary information

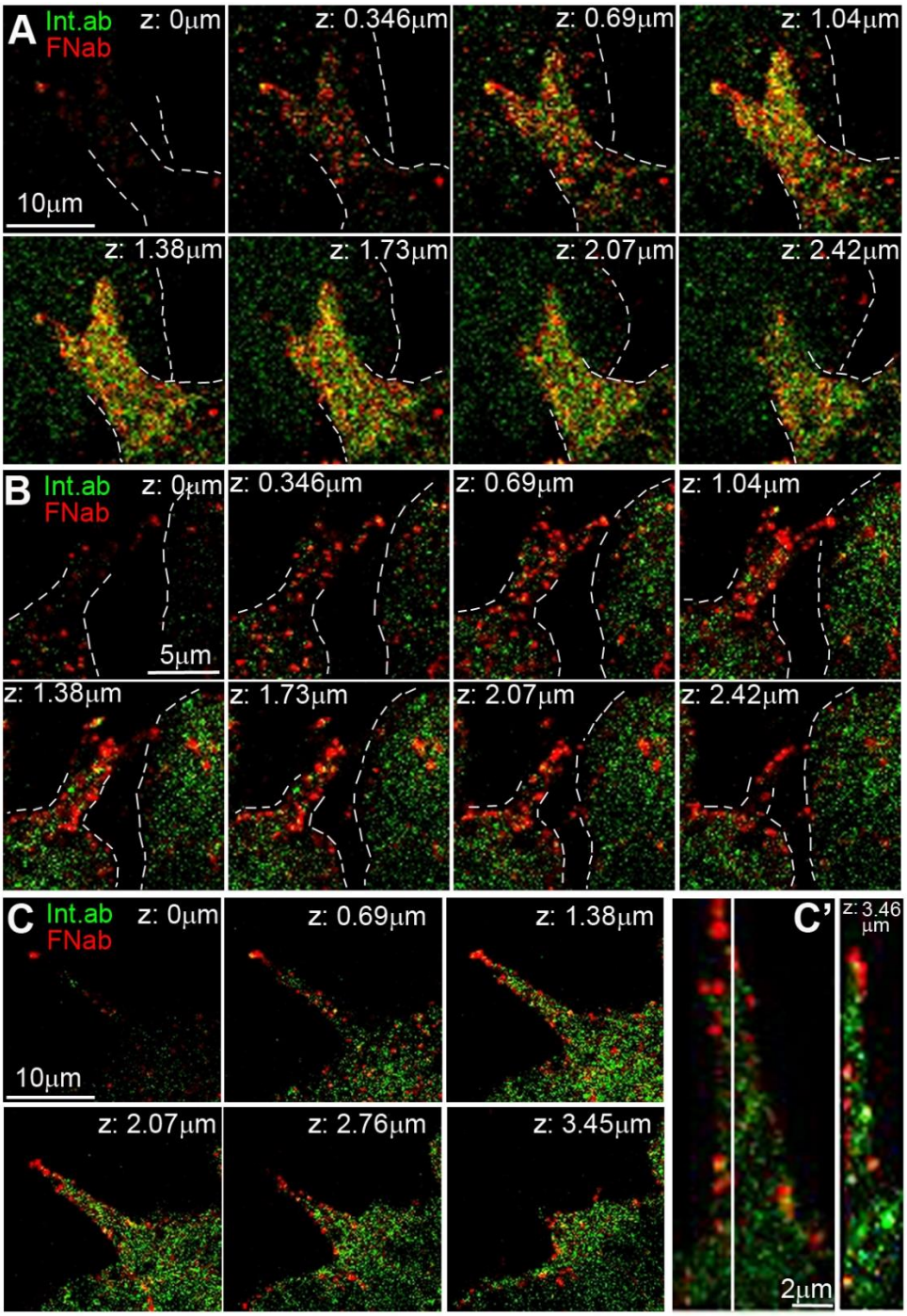

Figure S3. csFN puncta and integrinβ1 on LEM cell protrusions. (A) LEM protrusion on LEM cell surface (see Figure 4F) viewed at different z-planes from free surface to substratum surface. (B,C) LEM cell protrusions over gaps between cells viewed at different z-planes show csFN puncta on all surfaces. (C') z-plane projection. Red, csFN puncta; green, integrinβ1 puncta. (n=22). Dashed lines outline cell bodies.

Development • Supplementary information

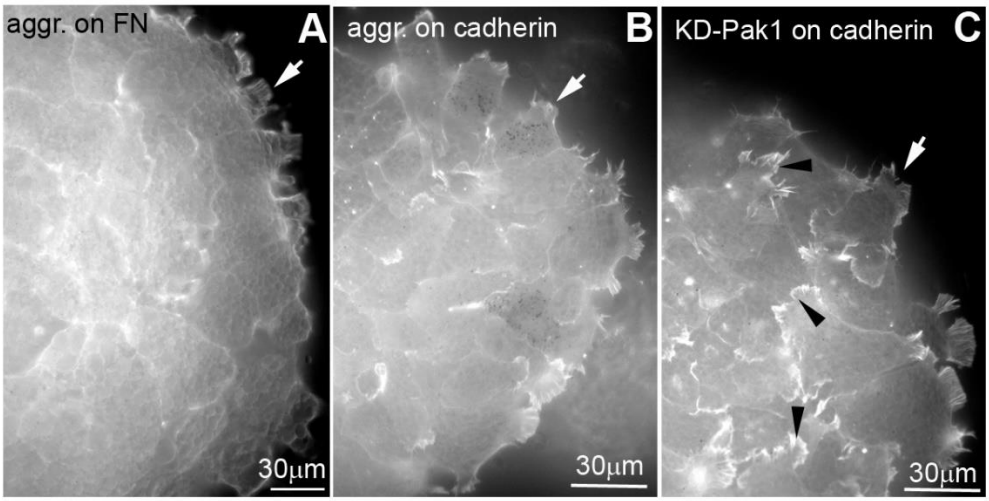

Figure S4. LEM cells can form lamellipodia on C-cadherin substratum. (A,B) F-actin staining with fluorescent phalloidin reveals that LEM explants on substratum coated with bovine serum FN (A) (n=8) or with the extracellular domain of C-cadherin (B) (n=2) form lamellipodia at free margin (arrows). (C) When expressing kinase-dead Pak1, cells form submarginal lamellipodia in addition (arrowheads) (n=3).

Development • Supplementary information

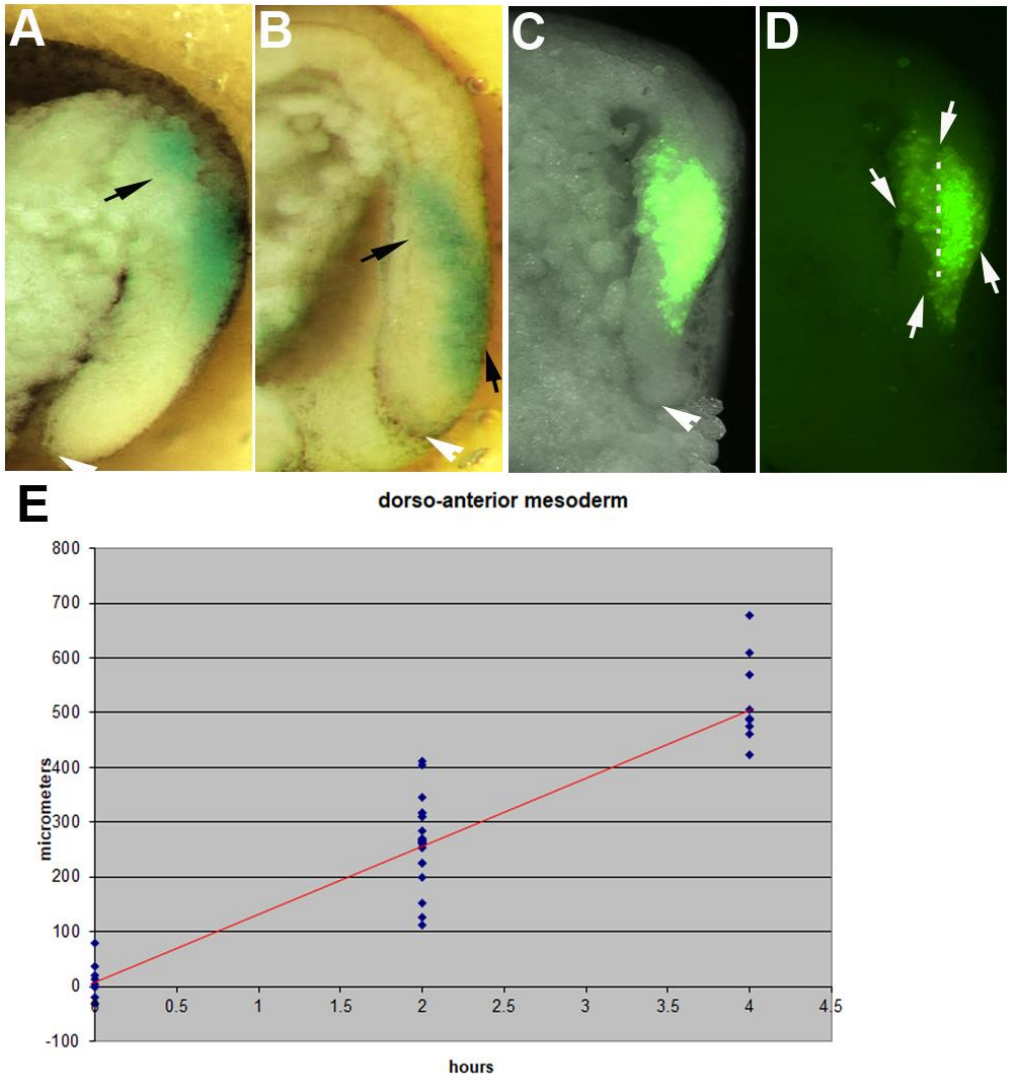

Figure S5. Comparison of LEM and chordamesoderm movements in the gastrula. (A,B) LEM (A) or chordamesoderm (B) were in vivo labeled in register with the overlying BCR by inserting a crystal of nile blue sulfate in the mid-early gastrula. Embryos were fixed after two hours and cut in half mid-sagittally to view the vital stain in mesoderm and BCR (black arrows). (C,D) A plug of BCR and adjacent underlying chordamesoderm was transplanted homotopically from a fluorescein-dextran injected into a non-labeled gastrula. Embryos were fixed after 2 hours and visualized under the fluorescence microscope with (C) or without (D) additional indirect illumination. Dashed line indicates BCR-chordamesoderm boundary. White arrowheads, blastopore. (E) Distances between centers of labeled spots in BCR and LEM were measured for each embryo in specimens fixed after 0, 2 and 4 hours. An average velocity of LEM advance of 2.1 µm/min was calculated from the data (red line).

Development • Supplementary information

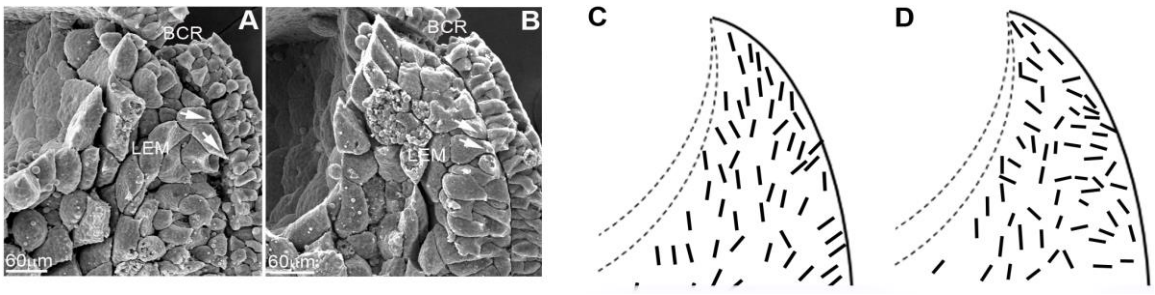

Figure S6. Counter examples to shingle arrangement. (A,B) Two cases were found among 31 scanning electron microscope specimens that showed LEM cells inclined vegetally instead of animally at the BCR-apposed surface in sagittal fractures. (C,D) Cell long axes in the LEM as seen in scanning electron micrographs in the majority of cases (samples from 3 embryos combined) (C) and from the two exceptional cases found with counter-shingle arrangement, combined (D).

Development • Supplementary information

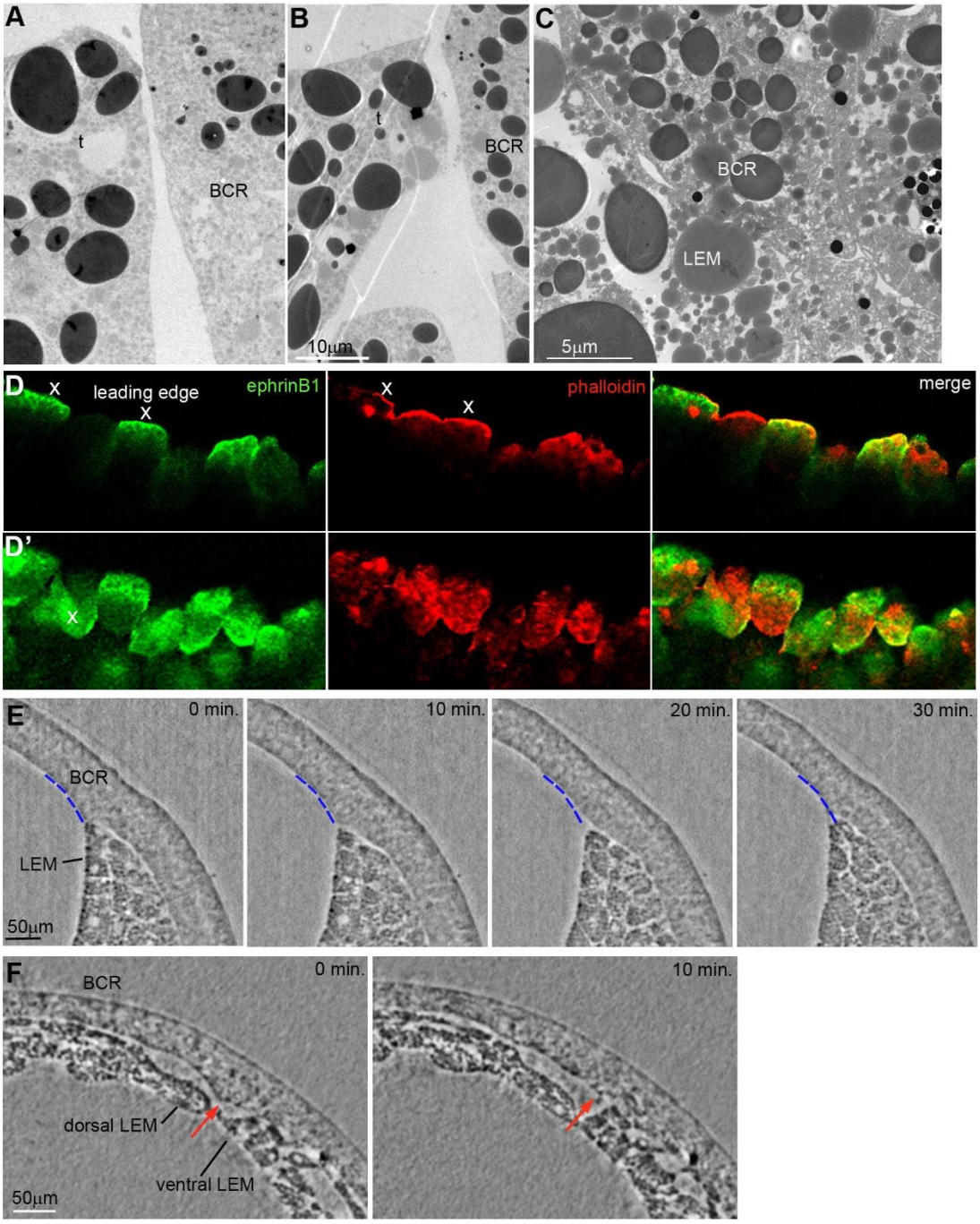

Figure S7. Tip cell characteristics. (A,B) Tip cells (t) can contact the BCR not only through close contacts, as shown in Figure 7G,H, but also through wide contacts, similarly to the contact types seen in LEM lamellipodia-LEM cell surface interactions (n=12). (C) Intimate contact between LEM and BCR cells behind tip during attachment phase (n=24). (D,D') Staining the margin of fixed and excised mid-gastrula LEM with antibody (green) reveals that LEM cells express

Development • Supplementary information

ephrinB1, but co-staining for F-actin with fluorescent phalloidin (red) and focussing at different planes (D,D') shows that ephrinB1 is alternatingly enriched at the very leading edge of the tip cells (x) and behind in the cell body (x). (E,F) TXPCμT analysis of BCR cusp retraction. (E) Middle gastrula stage. BCR surface up to cusp apex before transient tip cell detachment (blue dashed line) is indicated at later time points to show degree of cusp retraction. (F) Late gastrula. Black arrows, dorsal and ventral tip cells of LEM meet and detach from BCR cusp (position indicated by red arrow). Retraction by 15 μm (E) or 25 μm (F) takes at most 10 min, i.e. retraction velocities are larger than 1.5 – 2.5 μm/min.

Development • Supplementary information

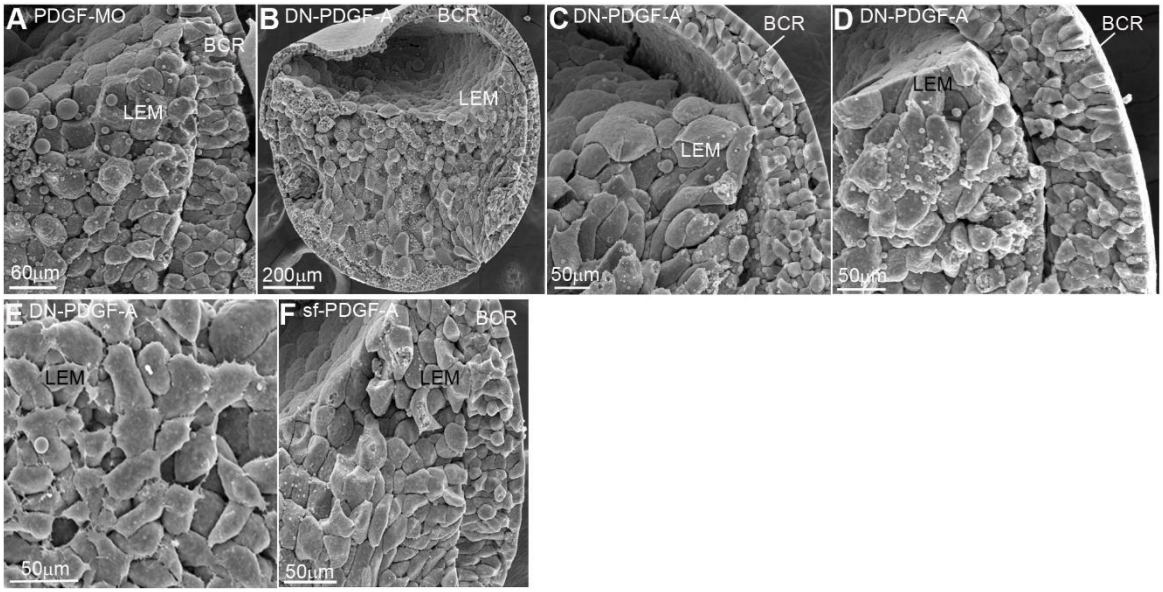

Figure S8. LEM phenotypes after interference with PDGF-A signaling. (A) Knock-down of PDGF-A with MO in the BCR (n=8; 2 experiments). (B-E) Expression of dominant-negative PDGF-A in the BCR. In 10 out of 11 cases, no BCR cusp was present at the position of the LEM tip (n=21; 4 experiments). (F) Overexpression of sf-PDGF-A (n=12; 2 experiments). Sagittal fracturs (A-D,F) and view from the BCR side (E) are shown.

Development • Supplementary information

Supplementary Material - Supplementary Table

Table S1. Extent of LEM-BCR contacts

A. Scanning electron micrographs (n = 31 embryos)

	front of LEM			rear of LEM			total		
contact	close	mixed	separate	close	mixed	separate	close	mixed	separate
fraction	**0.35**	**0.13**	**0.52**	**0.61**	**0.13**	**0.26**	**0.26**	**0.55**	**0.19**
expected							0.21	0.65	0.14

B. TXPCµT movie (n = 27 frames from 3 different planes of same embryo)

	front of LEM			rear of LEM			total		
contact	close	mixed	separate	close	mixed	separate	close	mixed	separate
fraction	**0.44**	**0.26**	**0.30**	**0.41**	**0.41**	**0.19**	**0.33**	**0.48**	**0.19**
episodes	2.3	1.3	1.7	1.7	2.0	1.7	1.3	2.0	1.7
duration (min)	15.3	16.0	14.1	19.3	16.4	8.9	21.0	19.3	9.0

C. Spatial and temporal distribution of contacts

Semi-quantitative description: close contact = 1; separate = 0; mixed = 0.5

	time	0	10	20	30	40	50	60	70	80
front of LEM	plane 312	0	0.5	0	0	1	0	0.5	0.5	1
	350	0	1	1	1	0.5	0.5	0.5	1	1
	380	1	0	0	0	1	1	0.5	1	1
rear of LEM	312	0	1	1	1	1	0	0.5	1	0.5
	350	0.5	0.5	0.5	0.5	0.5	0	1	1	1
	380	0.5	0.5	0	0.5	1	0	0.5	1	1
average front		0.4	0.4	0.3	0.6	0.7	0.5	0.7	0.9	
average rear		0.5	0.6	0.6	0.8	0.4	0.3	0.9	0.9	
ratio front/rear		0.8	0.7	0.5	0.8	1.8	1.7	0.8	1.0	

(A-C) BCR contacts of front region of LEM (large cells behind tip cell, usually 1-3 cells) and rear region (small cells behind front region) were scored as close (all cells in contact with BCR), separate (none in contact with BCR), and mixed. (A) Scanning electron micrographs (good spatial resolution, no time dimension) and (B,C) a TXPCµT movie (temporal resolution 10 minutes, spatial resolution ca. 1 µm) were used to score contacts. In (A,B) fraction of respective contact types are indicated in bold. In (A) the score for the whole LEM (front plus rear region) are also indicated and compared to the fractions expected from the combinations of the separately scored regions. In (B) successive frames in a given plane showing the same score (0, 1 or 0.5) were counted as “episodes” and averaged over the three planes, and from the total filming time of 80 minutes, the duration of episodes was estimated. In (C) values of 1, 0.5 and 0 were assigned to close, mixed and separate contacts, respectively, determined at 9 time points and at 3 different planes, to calculate averages for front and rear regions for pairs of consecutive frames.

Development • Supplementary information