Table S1. mRNA amounts injected per blastomere

mRNA amounts injected per blastomere:	
KD-PDGFR	100pg
mbGFP	150pg
mbRFP	100pg
KD-Pak1	300pg
CA-Pak1	40pg
eB1-mCherry	200pg
lf-PDGF-A	400pg
mPDGFdn	200pg

Table S2. Morpholino antisense oligonucleotides

Target	Sequence	Injection per blastomere
ephrinB1[1]	5' GGAGCCCTTCCATCCGCACAGGTGG 3'	20ng
ephrinB2[1]	5' ACACCGAGTCCCCGCTCAGTGCCAT 3'	20ng
xFN1[2]	5' CGCTCTGGAGACTATAAAAGCCAAT 3'	18ng
xFN2[2]	5' CGCATTTTTCAAACGCTCTGAAGAC 3'	18ng
xSyn4.1[3]	5' GCACAAACAGCAGGGTCGGACTCAT 3'	18ng
xSyn4.2[3]	5' CTAAAAGCAGCAGGAGGCGATTCAT 3'	18ng
xIntβ1[4]	5' GTGAATACTGGATAATGGGCCATCT 3'	20ng
xC-cadh.[5]	5' CCACCGTCCCGAACAGAAGCCTCAT 3'	20ng

All morpholinos have been characterized previously: [1]Rohani et al. (2011, 2014); Wen and Winklbauer (2017); [2]Davidson et al. (2006); [3]Munoz et al. (2006); Matthews et al. (2008); Ohkawara et al. (2011); Zhang et al. (2016); [4]Morita et al. 2012; [5]Ninomiya et al. (2012).

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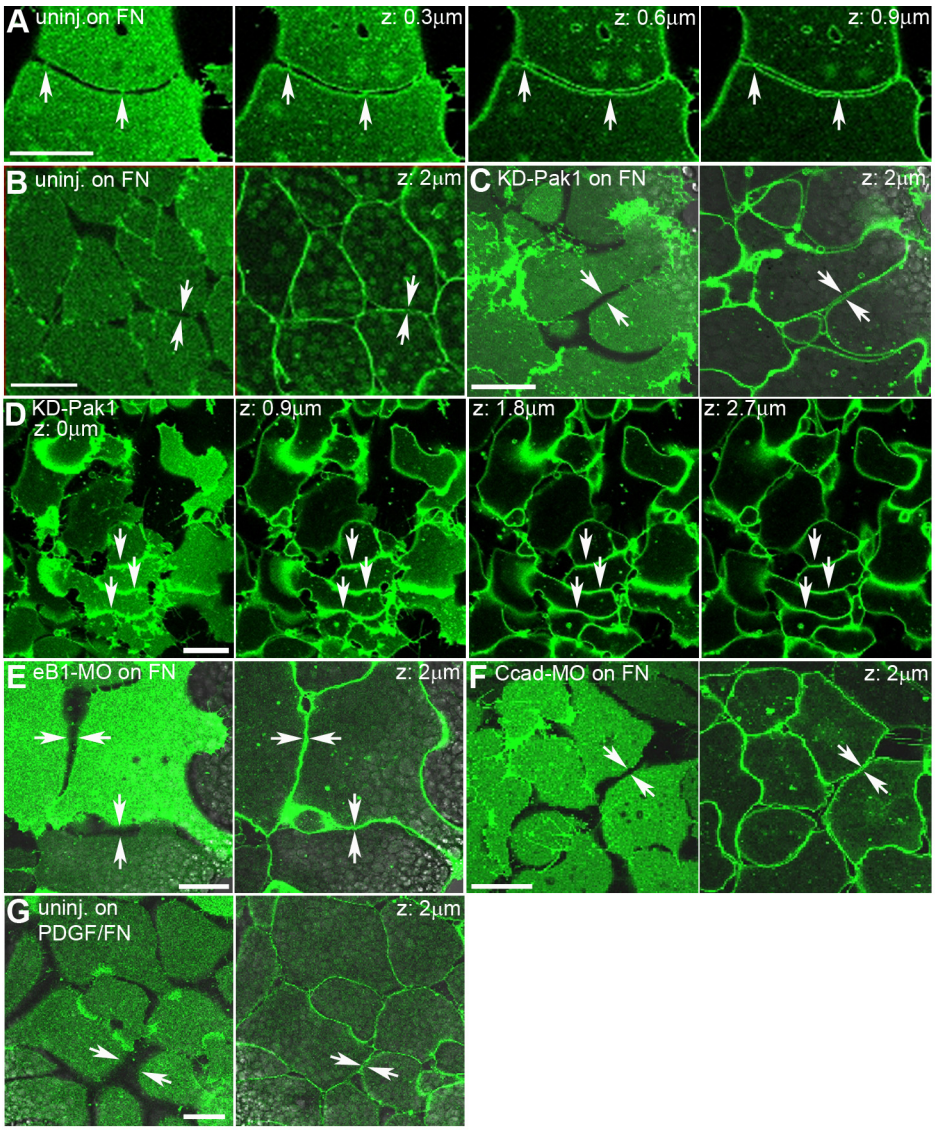

Figure S1. Cell contacts in aggregates. (A) Z-stack of an apposed cell pair on FN. Tiny processes (white arrows) bridge narrow gaps between cells at different levels above the substratum. (B) In aggregates cells are separated by small gaps (white arrow pairs) where attached to the FN substratum, but are more tightly packed above the substratum level. (C,D) In KD-Pak1 expressing aggregates cells are less densely packed at the FN substratum, with irregular gaps between cells, but tightly packed above. Underlapping cells are closely attached interiorly (D). (E–G) Cells are loosely packed in ephrinB1-MO aggregates (E) and C-cad-MO aggregates (F) on FN and in untreated aggregates on PDGF/FN substratum (G). Bars are 30 µm.

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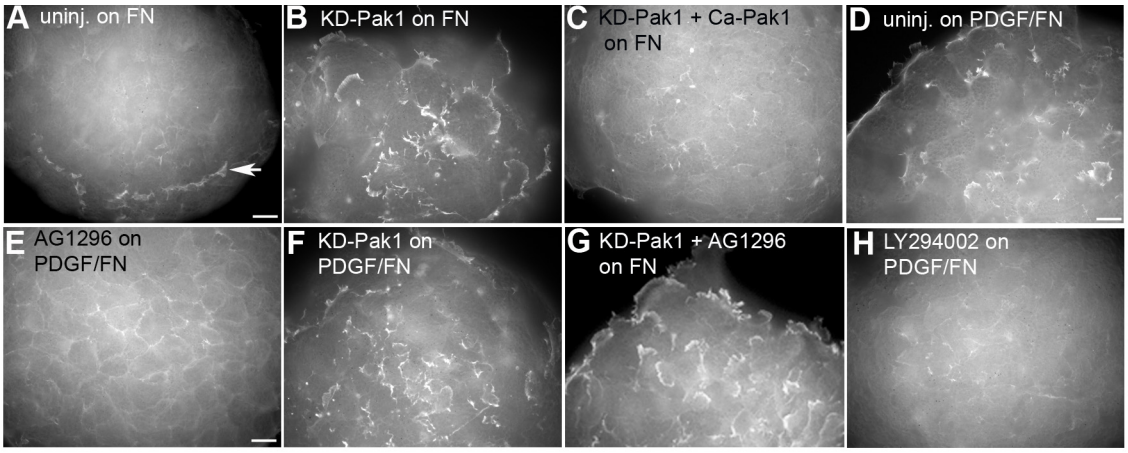

Figure S2. Lamelliform protrusions in fixed aggregates visualized by phalloidin-fluorescein staining of F-actin. In untreated aggregates on FN (A) Cells only show lamellipodia at the margin (white arrow). Sub-marginal lamellipodia were induced by KD-Pak1 (B), and the effect was reversed by co-expression of constitutively active CA-Pak1(C). Aggregates on PDGF-FN (D) show submarginal protrusions which were suppressed by the PDGFR inhibitor AG1296 (E) and the PI3K inhibitor LY294002 (H). DN-Pak1 augments lamellipodia formation on PDGF-FN (F) and rescues submarginal lamellipodia suppressed by AG 1296 (G). Bars are 30 μm.

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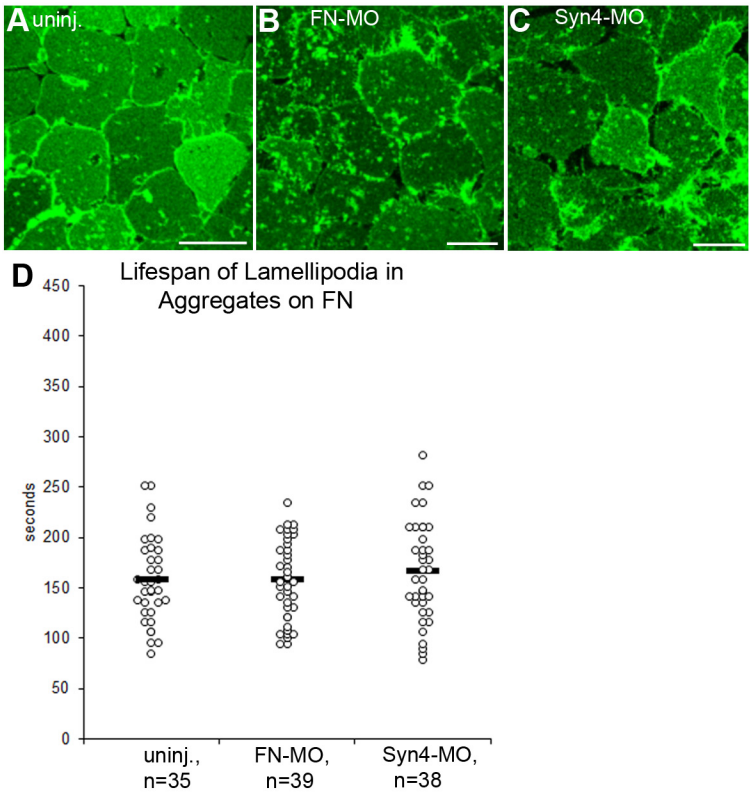

Figure S3. Knockdown of csFN or syndecan-4 has no effect on submarginal lamellipodia. (A) uninjected, (B) FN-MO injected and (C) Syn-4-MO injected explants on FN substratum, labeled with membrane-GFP. (D) Lifespan of lamellipodia.

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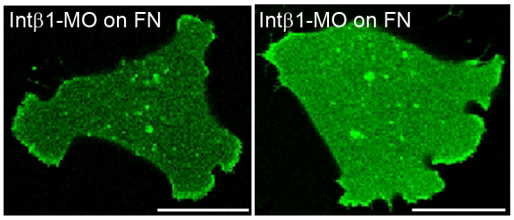

Figure S4. Single cells injected with integrin-MO on FN substratum. Bars are 30 μm.

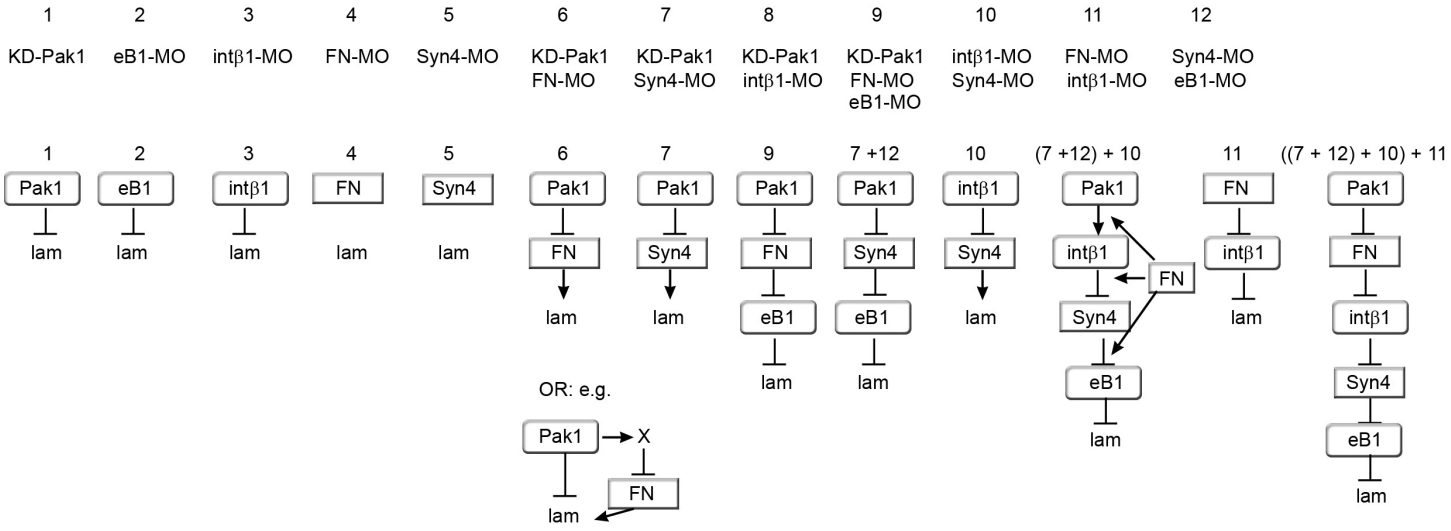

Figure S5. Step-by-step construction of the epistatic interaction module for contact inhibition of lamellipodia. Top row, relevant experimental treatments of LEM explants on FN substratum, numbered for identification. Below, epistatic relationships deduced from respective treatments. Inhibition of Pak1, ephrinB1 (eB1) and integrin β1 (intβ1) relieve contact inhibition, indicating that these factors promote lamellipodia collapse (inhibitory arrows) when active (round cartouches) (1-3). Inhibition of fibronectin (FN) and syndecan-4 (syn4) alone have no effect, suggesting that they are not active (rectangular cartouches) in normal explants (4,5). Inhibition of these factors reverses the effects of Pak1 inhibition, placing them downstream of Pak1 (6,7). Alternatively, they could act in parallel pathways, but since they interact with Pak1, this would require additional factors (e.g. X) and additional interactions (e.g. 6, bottom). In the absence of any evidence for such additional components, we always assume in the following the simplest possible interactions. eB1 reverses the effects of FN or syn4 downstream of Pak1, placing it downstream of these factors (9, 7+12), and syn4 inhibition reverses the effect of intβ1 inhibition, placing it downstream (10). Combining (7+12) and (10) orders Pak1, intβ1, syn4, and eB1, and since (11) indicates that intβ1 acts downstream of FN, the final sequence of components is derived (last column). That all interactions are inhibitory is contingent in the sense that the list of participating components is not necessarily complete. With additional components, further inhibitory, or activating interactions could become introduced.

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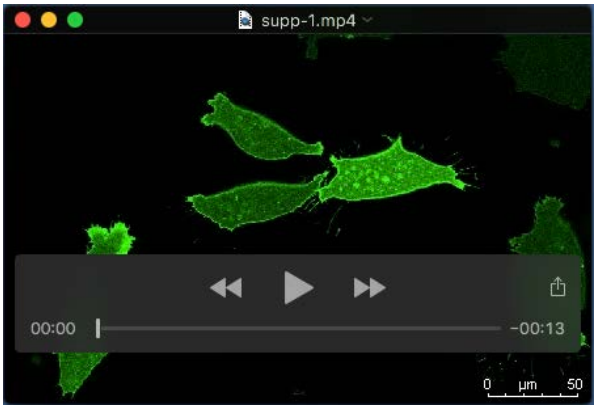
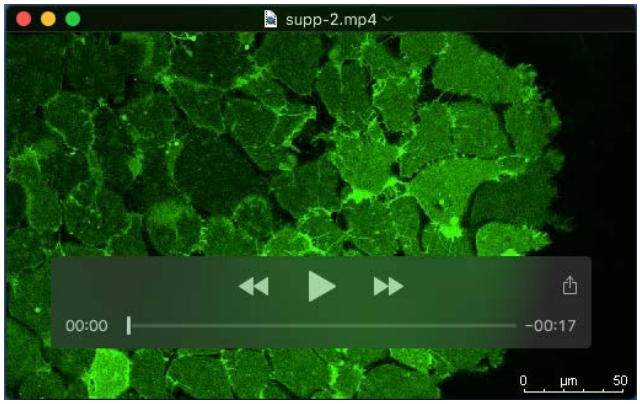
Movies



Movie 1. Protrusion formation and behavior of single mesoderm cells on FN. The confocal time lapse recording shows dissociated mesoderm cells expressing mb-GFP migrating on a FN coated dish. Cells extend lamelliform protrusions, which collapse immediately after contact with another cell.



Movie 2. Protrusive activity in mesoderm aggregates on FN. In this confocal time lapse recording of an mb-GFP expressing mesoderm aggregate on FN substratum, small short-lived protrusions form which collapse immediately after contact with adjacent cells. On the free margin (right side) cells extend long-lived lamellipodia.

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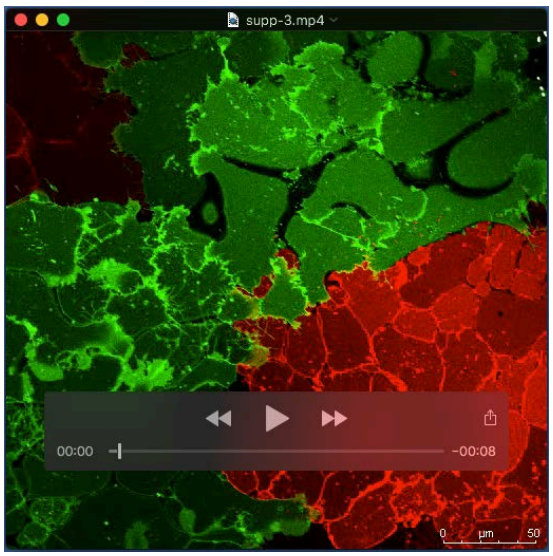
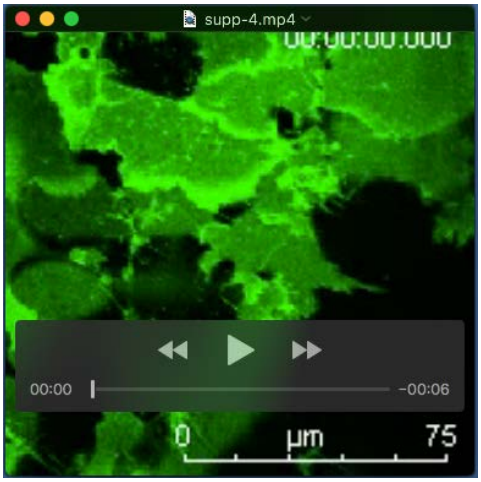


Movie 3. In kinase-dead Pak1 expressing aggregates cells overcome contact inhibition of lamellipodia. A time lapse recording of a KD-Pak1 expressing aggregate (green, mb-GFP) is shown side by side with an untreated aggregate (red, mb-RFP) on a FN coated dish. The KD-Pak1 expressing cells show an increased number of large protrusions with an increased survival time. Untreated cells in the aggregate are more densely packed and have only small short lived protrusions.



Movie 4. Expression of kinase-dead Pak1 in aggregates leads to the formation of chains of underlapping cells. The time lapse recording shows cells underlapping each other, with the lamellipodia pointing in the same direction.

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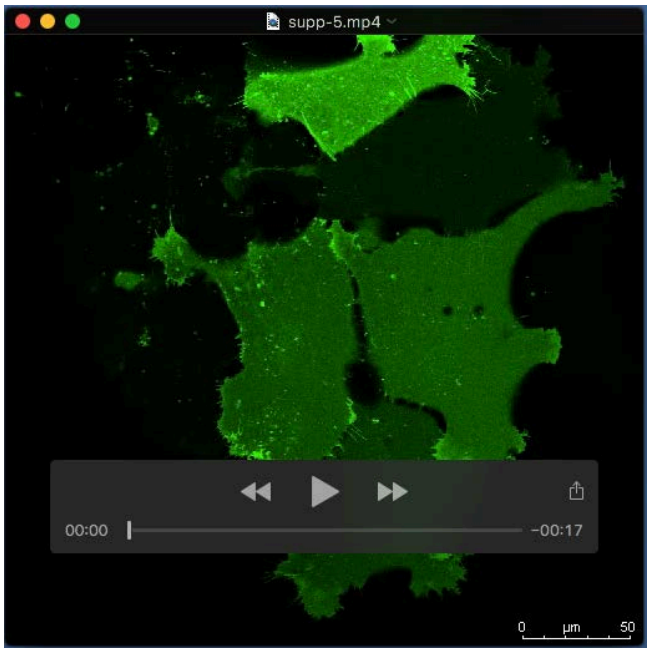
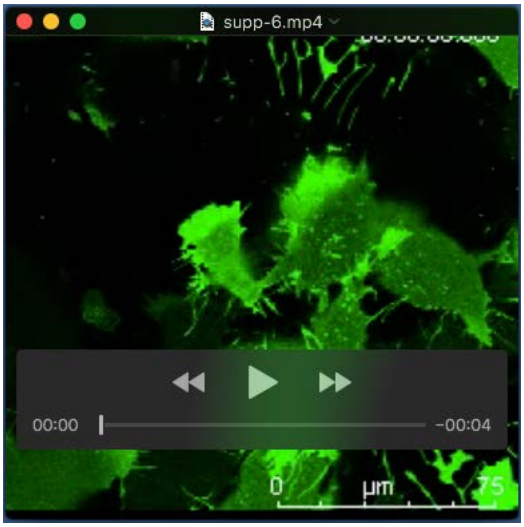


Movie 5. Knockdown of ephrinB1 stimulates sub-marginal protrusion formation. Time lapse recording shows an aggregate injected with ephrinB1-MO. Cells underlap each other with large smooth-rimmed lamellipodia.



Movie 6. Knockdown of ephrinB1 leads to formation of shingle arrangement. The time lapse recording shows cells underlapping each other in series, with the lamellipodia pointing in the same direction.

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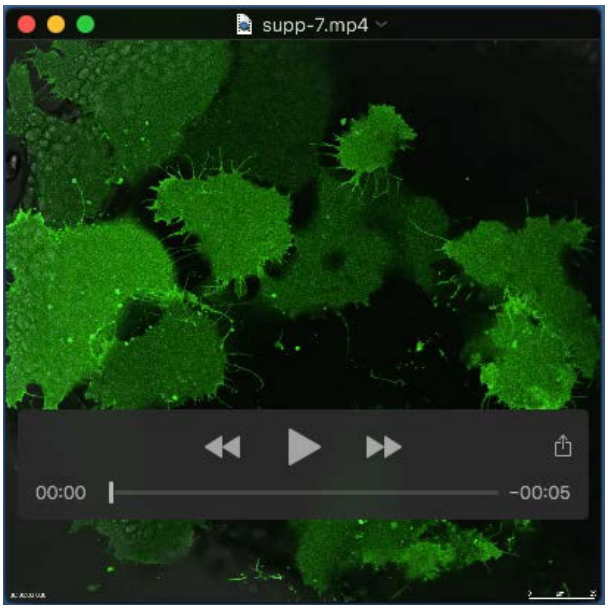
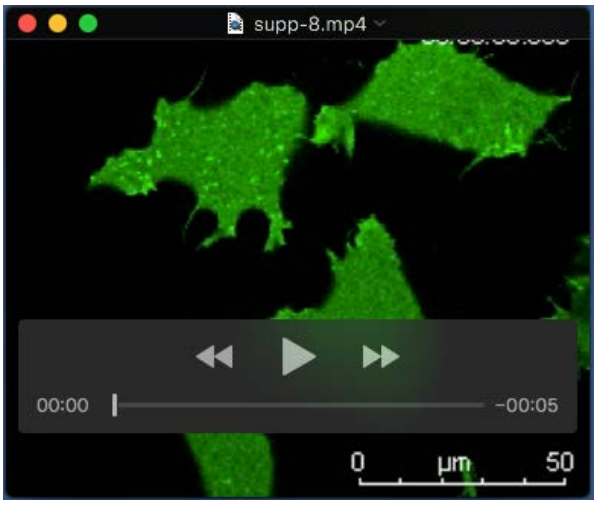


Movie 7. Injection of FN-MO together with KD-Pak1 mRNA leads to rapid collapse of lamellipodia. In this time lapse recording cells in the aggregate form large lamellipodia which rapidly switch to filopodia extension, and retreat, leaving retraction fibers behind.



Movie 8. Protrusion formation and behavior of single mesoderm cells on FN-PDGF. In the time lapse recording single cells are seen to migrate on an FN-PDGF coated dish. They form large lamellipodia and often underlap each other when they meet..

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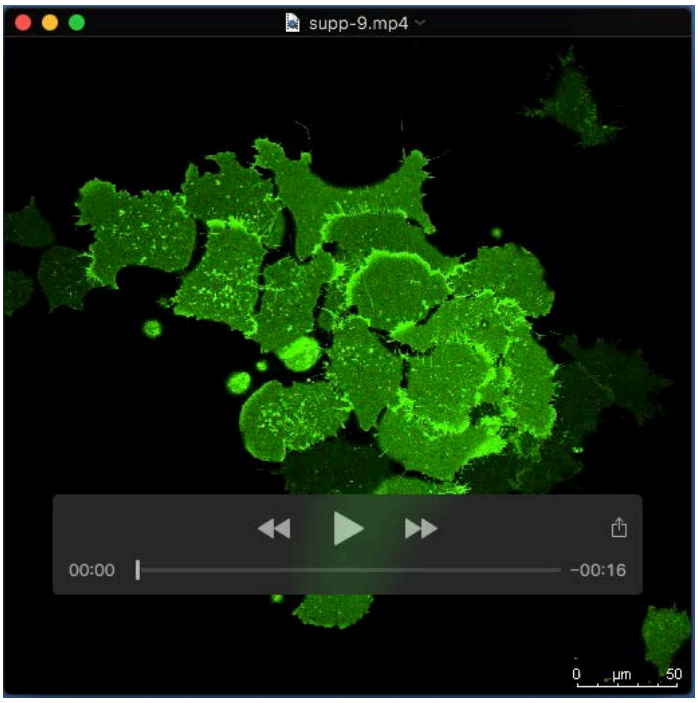

Movie 9. PDGF signaling promotes sub-marginal protrusion formation in LEM aggregates. The time lapse recording shows mesoderm aggregate on FN-PDGF substratum, where cells underlap each other with large, smooth lamellipodia and can form shingle arrangements.

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