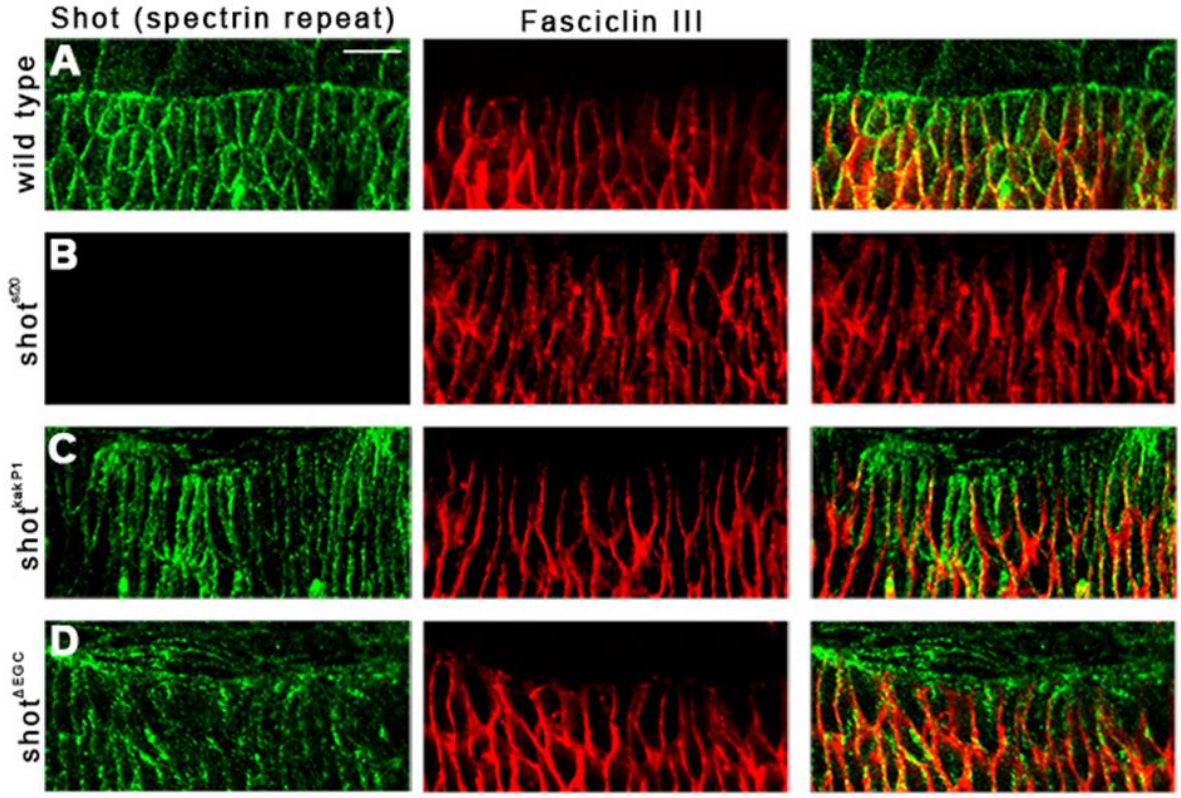


Figure S1. Expression of endogenous Shot in different *shot* mutant DME cells.

(A-D) Immunofluorescence staining of wild-type (A), *shot*^{sf20} (B), *shot*$^{\Delta EGC}$ (C), and *shot*^{kakP1} (D) mutant DME. Shot antibody raised against spectrin repeats recognizes truncated Shot proteins expressed in *shot*$^{\Delta EGC}$ and *shot*^{kakP1} mutant embryos (green). No Shot expression is detectable in *shot*^{sf20} null mutants. FasciclinIII staining highlights outline of the epithelial cells (red).

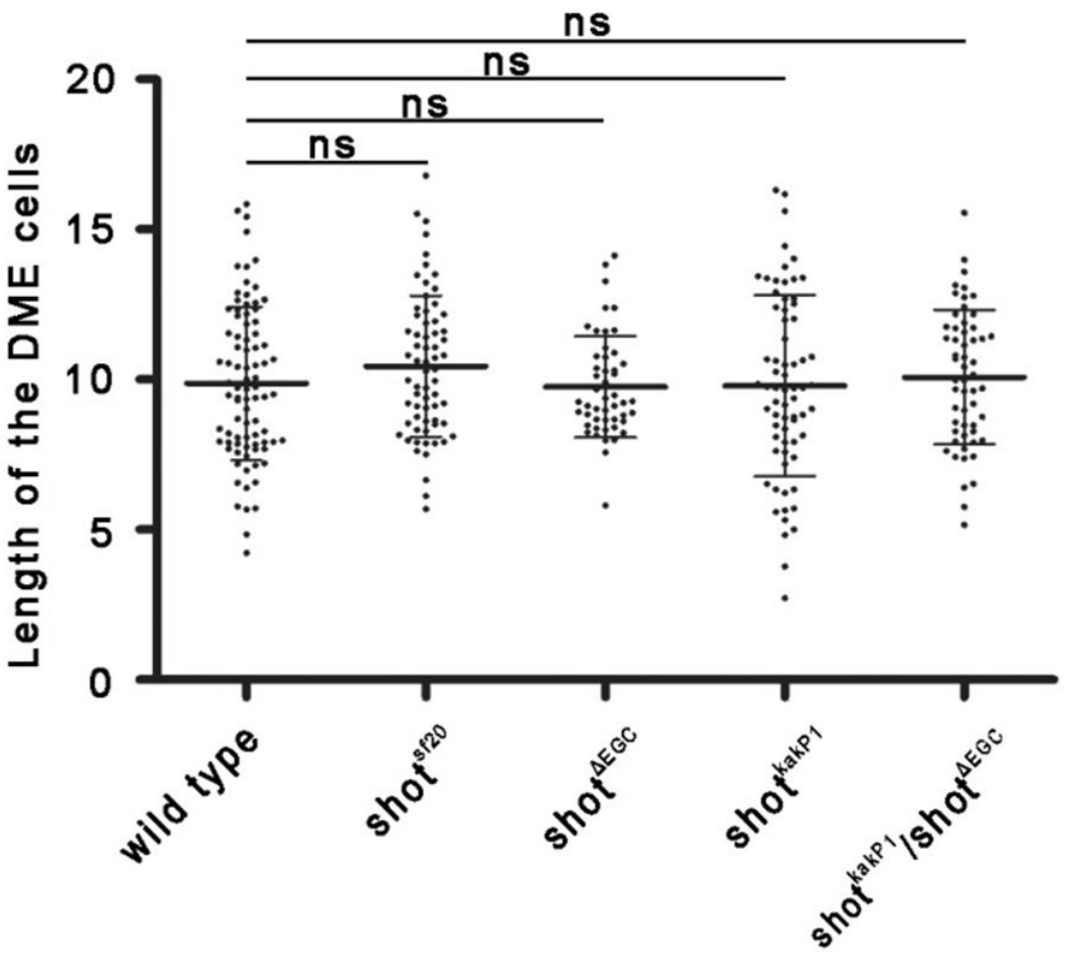


Figure S2. Elongation of the DME cells. Length of DME cells is shown. In *shot* mutants, DME cells elongate normally. Mean±s.d. is shown, T-test, ns – not significant.

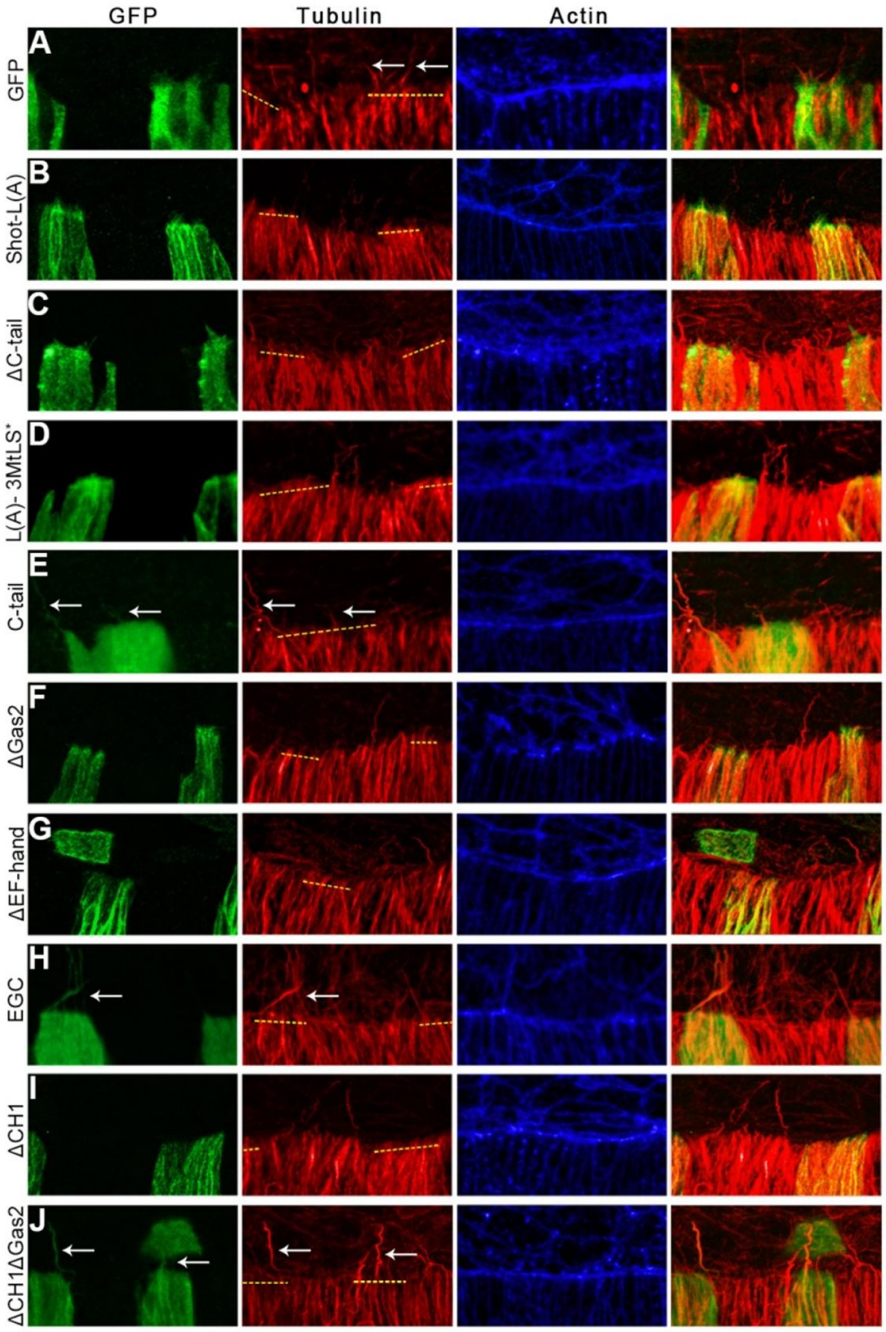


Figure S3. Actin- and MT-binding activities of Shot are simultaneously required for proper MT organization in DME cells.

(A-I) Immunofluorescence staining of *shot*^{sf20} mutant embryos expressing various GFP tagged *shot* rescue constructs in four-cell-wide *en*-Gal4-stripes. Tubulin staining labels the MT network (red); actin is labeled with phalloidin (blue); GFP labels Shot protein isoforms (green). Yellow dotted lines on the tubulin staining indicate the four-cell-wide *en*-Gal4-stripes along the epithelial front. Scale bar represents 5 µm. Rescue is indicated by the absence of long, bent MTs protruding from the epithelial cells expressing the rescue construct.

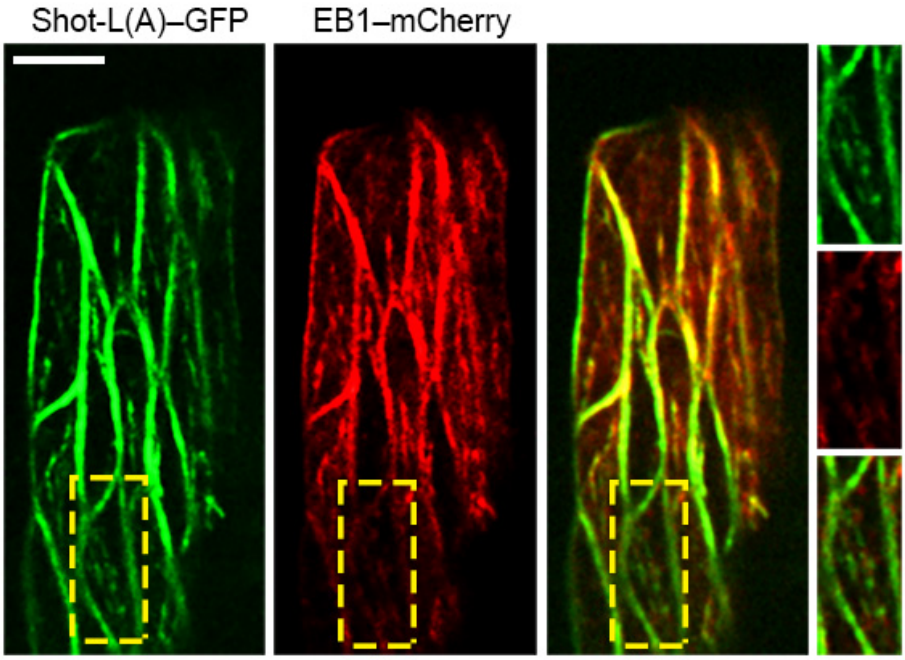


Figure S4. Images show four-cell-wide *en*-Gal4-stripe of the epithel in an embryo co-expressing Shot-L(A):GFP and EB1:mCherry. Shot-L(A):GFP does not co-localize with EB1.

Supplementary Table 1

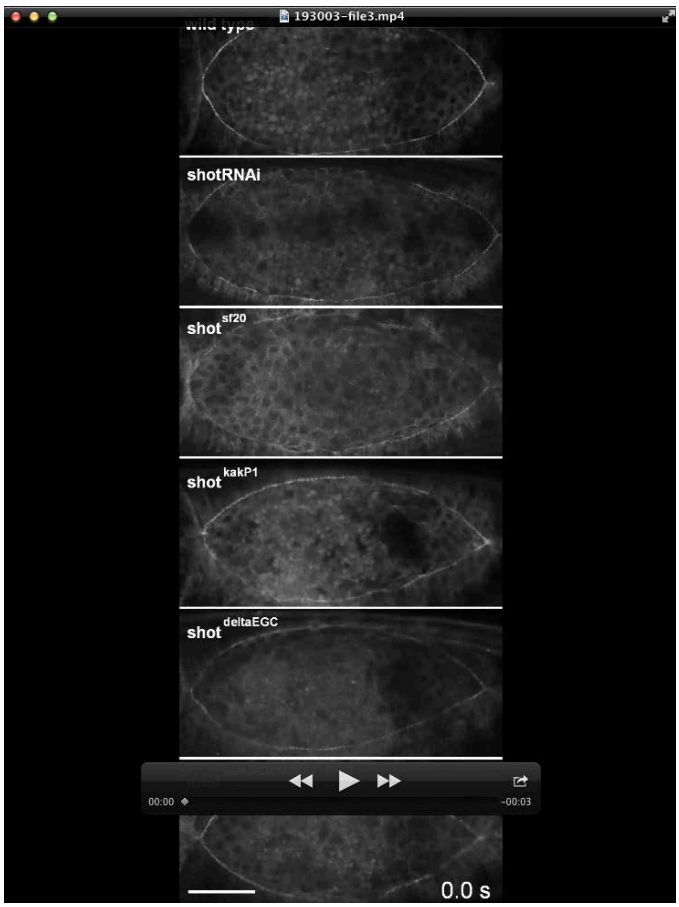
Gene	forward	reverse
Msps	ACGGAACGCGAAATAACC	AACGGTTGTCGTGGTCAG
clip190	GGGTGTTGTCCTTGACGA	GGGTGTTGTCCTTGACGA
EB1	GATCGCCACGTGATTAAATAG	ACGAGTCTGTGAACCTTTCAA
Apc	TTTCGTCGTGGAGATTGG	ATGCAGAAGCGGAAGTCA
Apc2	AGAGACGATCCGCTCAGAC	TCCCTTCTCGGAGATTGAG
shot	AACCCACAGCTCCCAGAT	TGGCAACAAAGCTGATCC
Glued	TTGCCCAACATCTTCTGG	GTGGACGTGTTGCCTGAT
Dhc64C	CAAGTTGCGACACCTTCTG	GTGGCTTGGATTACCGAAC
Klp10A	GGAATCGACTGGCTTTCC	GGGCAAGTGCATCACAGT
Klp59C	GAATGCTGACCACATCCTG	TGCGTGGTAAGGAACTGC
CG13366	ATCCAGCACTTCGCTCAG	ACTTGCAAACCCAGCTGAC
katanin60	CTCGTTGGTGTTCGATGTG	CAAGGCCATTCAGAGAACC
Lis-1	TCTGGGCTCAAATGGCTA	TCGTTATGCTGGCACGAT
Patronin	ATTTCCGGGAGATTTGACAC	GATAGGTGAGATTGCCGCT
Spastin	TCCTCTGTGCACAAACAGAAC	CATTCAACCGTTGGAGA
chb	TCCACGCTAAGGACATGG	CTGCGGCTGAGTGTGATAG
ssp2	TGAACGGATGAACAACTCG	TCCGCTGTAGTAGCACTCG

Table S1. Sequences of primers used to generate templates for *in vitro* transcription in the RNAi experiments.

Supplementary Table 2

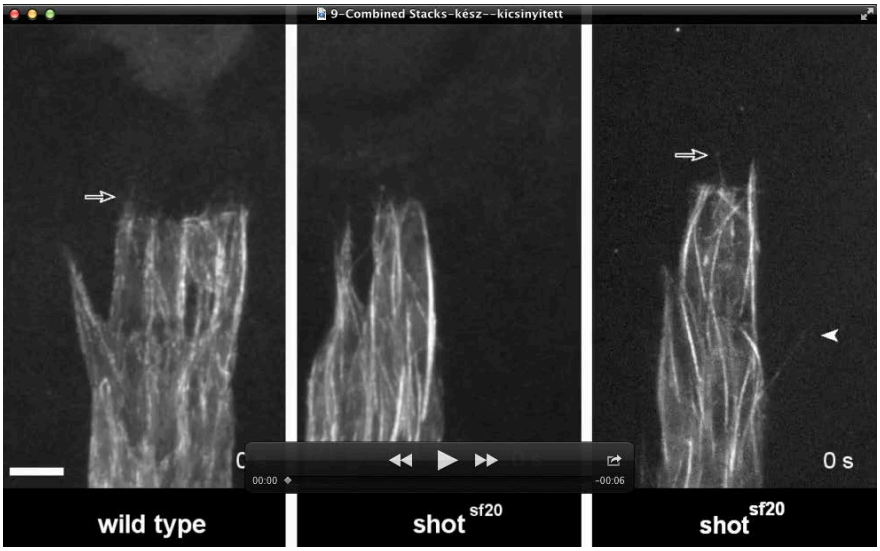
target 1	ccaagatacttgtttaaatcccc
target 2	cccagatactgtgcatcgtttgc

Table S2. Guide RNA recognition sites in the *shot* locus.

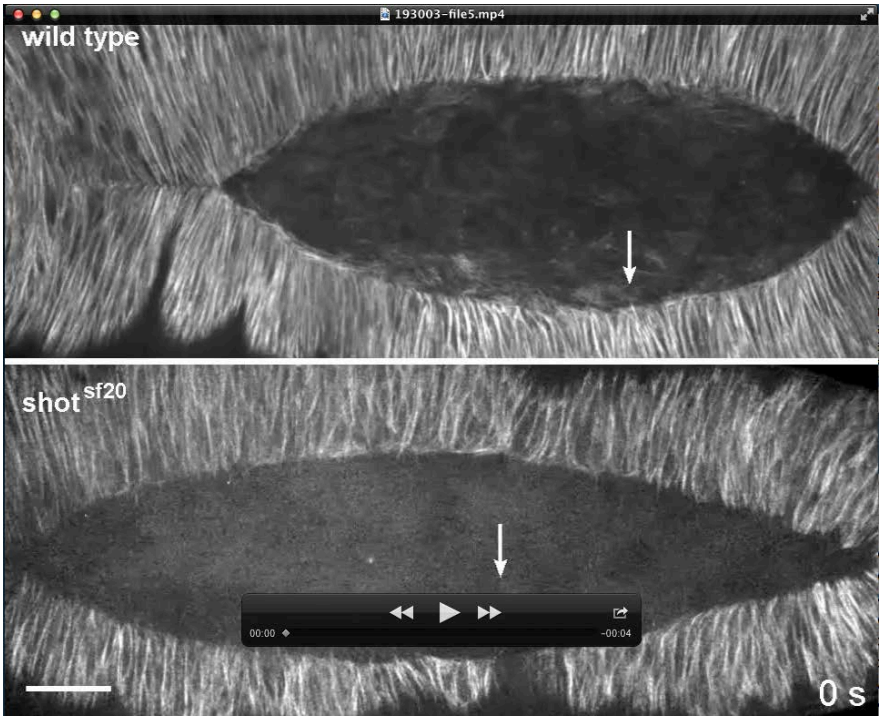


Supplementary movie 01

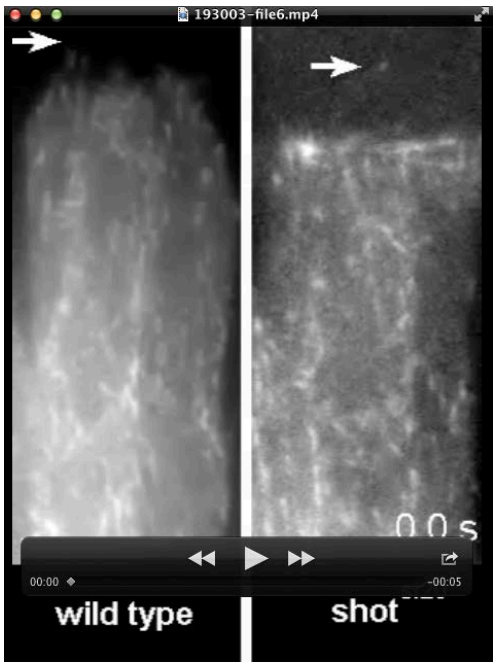
Movies show the convergence and zippering of the two opposite epithelial cell sheets. The first cell row of the epithelial sheets is highlighted by EGFP. Wild-type, shot RNAi and shot mutant embryos are shown. Convergence happens similar to the wild type but zippering is delayed.



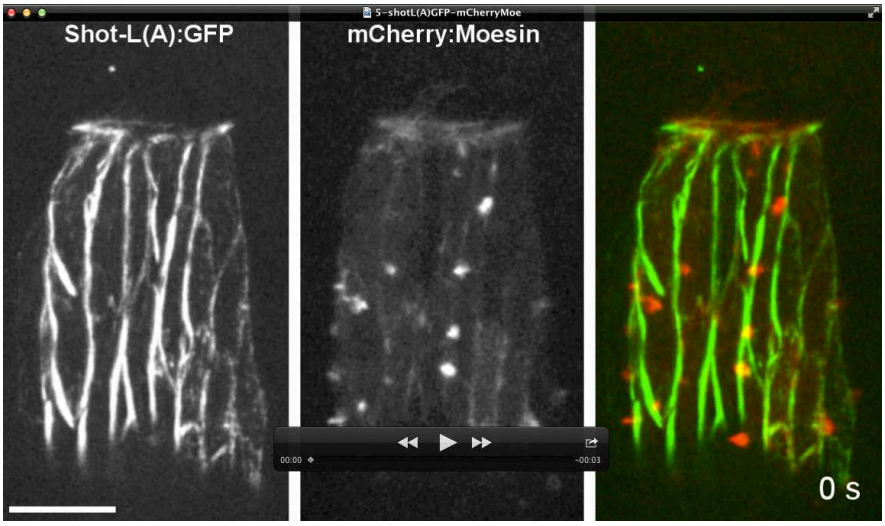
Supplementary movie 02
Epithelial cells expressing Tubulin:EGFP are shown in a wild-type and in shot null mutant embryos. Empty arrows indicate MTs growing into protrusions, white arrows indicate bending MTs, and arrowhead indicates a MT protruding at the lateral surface of a mutant epithelial cell.



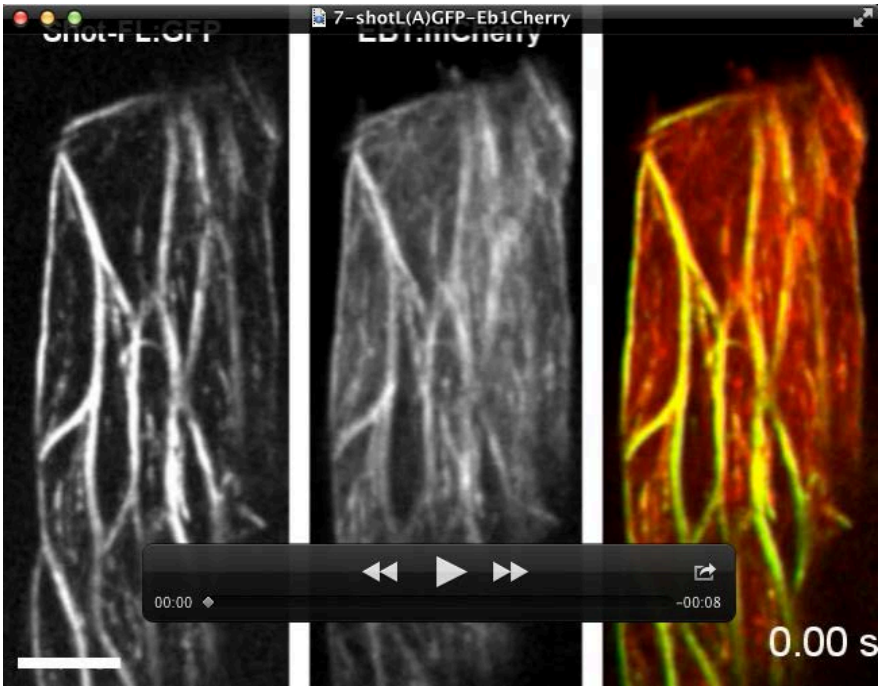
Supplementary movie 03
Epithelial cells expressing Tubulin:EGFP are shown in a wild-type and in a shot null mutant embryo. White arrows indicate MTs growing into protrusions. MTs are abnormally long and curled at the leading edge of *shot* null mutant DME cells. The emergence of abnormal MTs extends throughout the entire leading front of the dorsal epithelium.



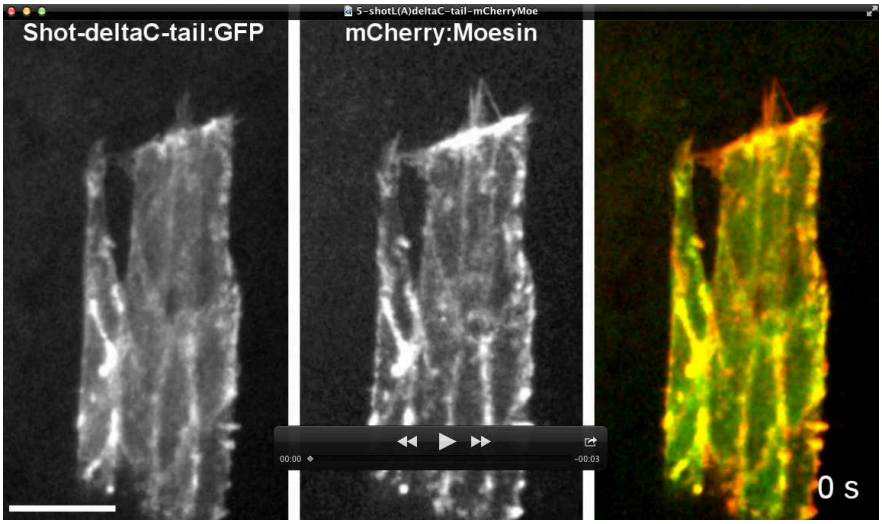
Supplementary movie 04
Movies show DME cells of a wild type and a shot mutant embryo expressing EB1-GFP to highlight growing MT plus ends. Most of the EB1-GFP comets move parallel to the long axis of DME cells. White arrows indicate MTs growing into protrusions.



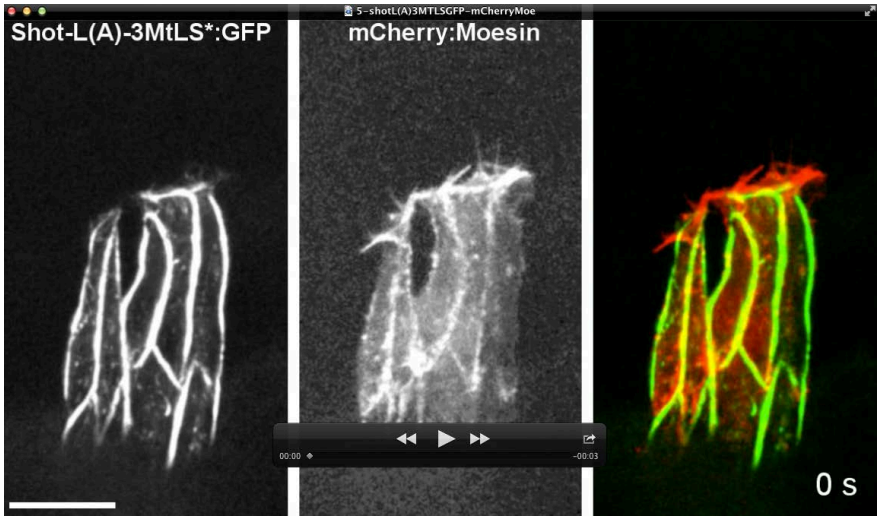
Supplementary movie 05
Movie shows DME cells of a wild type embryo co-expressing Shot-L(A):GFP and mCherry:Moesin. Scale bar represents 10 µm.



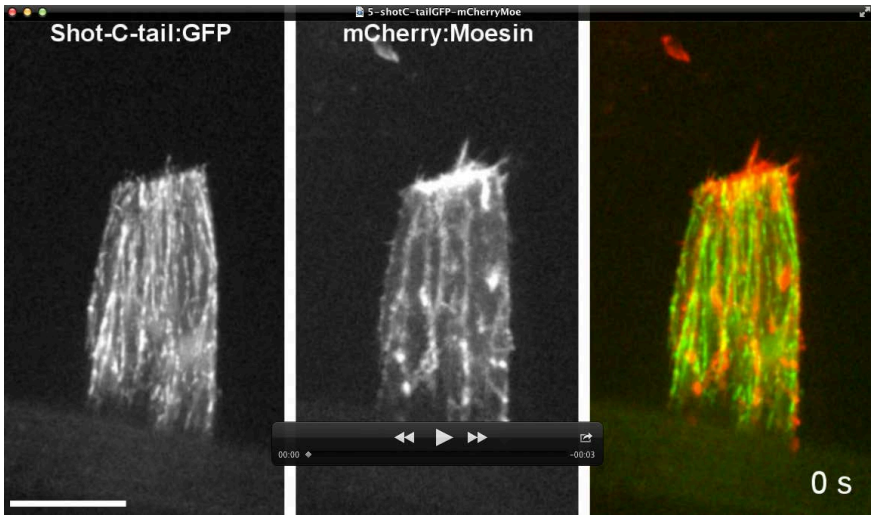
Supplementary movie 06
Movie shows DME cells of a wild type embryo co-expressing Shot-L(A):GFP and EB1:mCherry. Scale bar represents 5 µm.



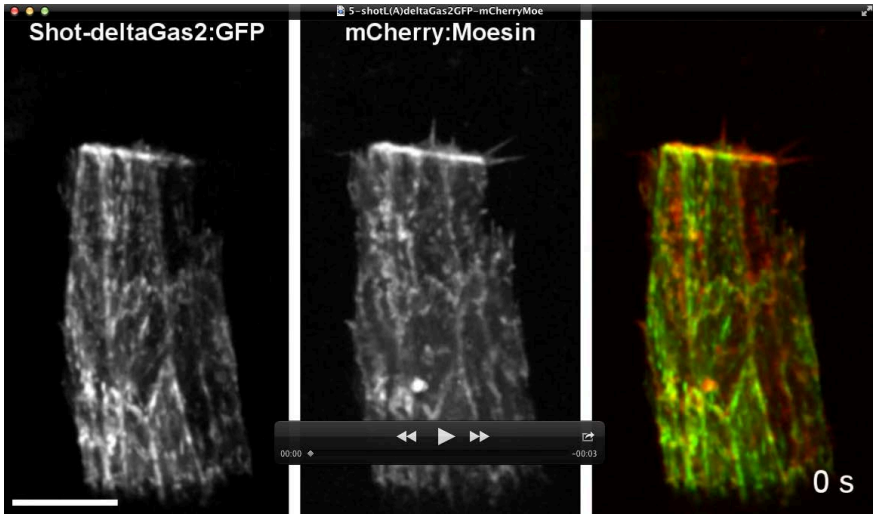
Supplementary movie 07
Movie shows DME cells of a wild type embryo co-expressing Shot-ΔC-tail:GFP and mCherry:Moesin. Scale bar represents 10 µm.



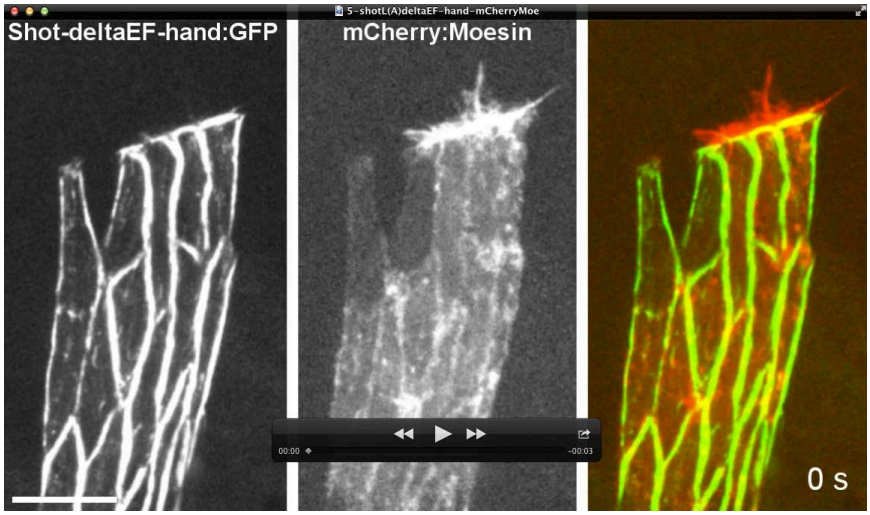
Supplementary movie 08
Movie shows DME cells of a wild type embryo co-expressing Shot-L(A)-3MtLS*:GFP and mCherry:Moesin. Scale bar represents 10 µm.



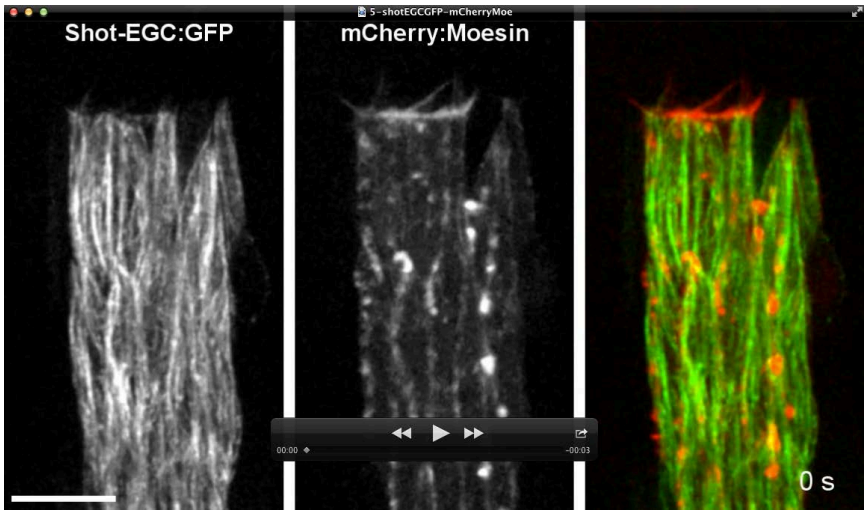
Supplementary movie 09
Movie shows DME cells of a wild type embryo co-expressing Shot-C-tail:GFP and mCherry:Moesin. Scale bar represents 10 µm.



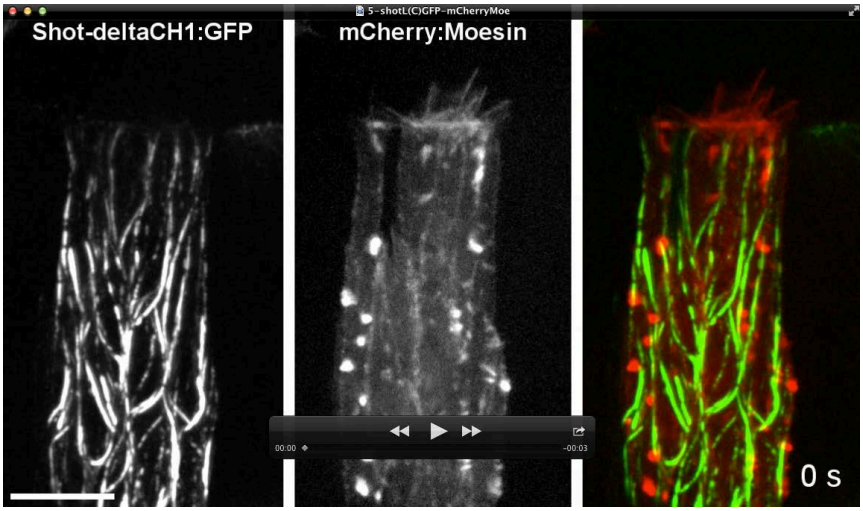
Supplementary movie 10
Movie shows DME cells of a wild type embryo co-expressing Shot-ΔGas2:GFP and mCherry:Moesin. Scale bar represents 10 µm.



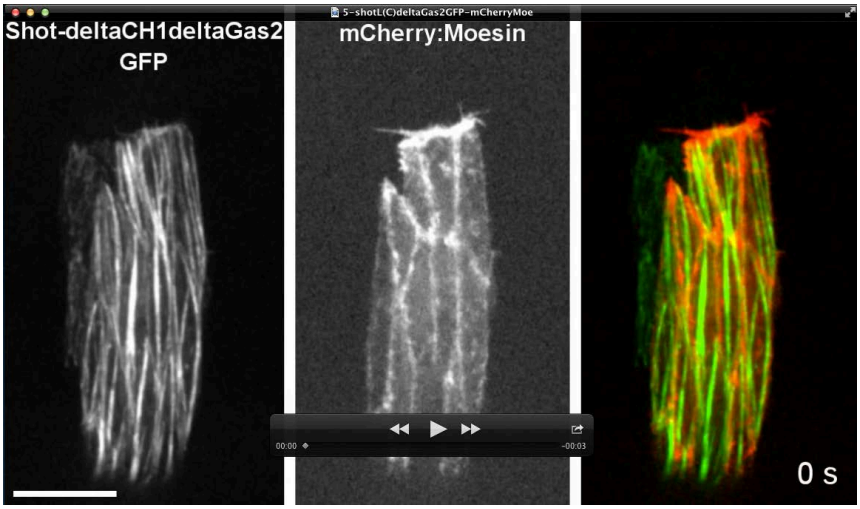
Supplementary movie 11
Movie shows DME cells of a wild type embryo co-expressing Shot-ΔEF-hand:GFP and mCherry:Moesin. Scale bar represents 10 µm.



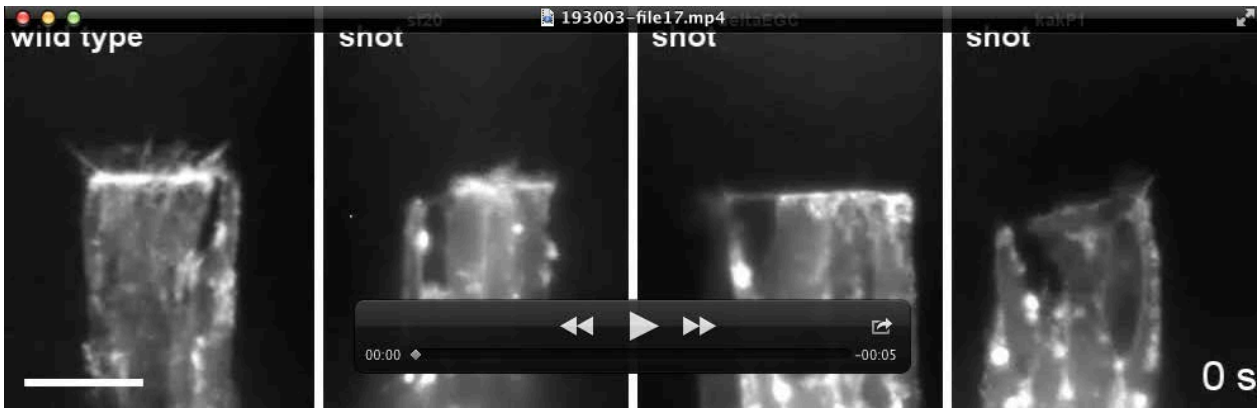
Supplementary movie 12
Movie shows DME cells of a wild type embryo co-expressing Shot-EGC:GFP and mCherry:Moesin. Scale bar represents 10 µm.



Supplementary movie 13
Movie shows DME cells of a wild type embryo co-expressing Shot-ΔCH1:GFP and mCherry:Moesin. Scale bar represents 10 µm.



Supplementary movie 14
Movie shows DME cells of a wild type embryo co-expressing Shot-ΔCH1ΔGas2:GFP and mCherry:Moesin. Scale bar represents 10 µm.



Supplementary movie 15
Movies show DME cells expressing actin:EGFP. Protrusion formation is reduced in the shot mutant DME cells. White arrows indicate extensions faintly decorated with actin:EGFP.