

Fig. S1. Circularity ratio and controls for RNAi assays. (A-C) Circularity ratio for shg^{k03401} , $ed^{M/Z}$ (A); $Cdc42^4/Cdc42^6$ (B); and zip^1 (C) (shg^{k03401} n=6; $ed^{M/Z}$ n=5; $Cdc42^4/Cdc42^6$ n=5; zip^1 n=4; wt, n=10; results are given as means±s.e.m.). The circularity ratio was measured in mutant embryos and compared to wildtype embryos. A perfect circle has a value of 1. (A) In shg^{k03401} and $ed^{M/Z}$, where the actin cable is discontinuous, wounds are jagged and have irregular shape, the circularity ratio is lower compared to wildtype embryos. As the wound fronts become closer and zippering events more frequent, the circularity ratio fluctuates. (B) In contrast, $Cdc42^4/Cdc42^6$ embryos display a high circularity ratio, correlating with the rounded shape of wounds observed in this mutant due to the tension provided by the actin cable. (C) The circularity ratio of zip^1 mutant is higher than wildtype embryos, although the wounds edges are jagged and the actin cable disrupted. This is likely the result of zip^1 mutant epithelium cells having a smaller apical surface than wildtype and being more likely to grab neighboring protrusions in areas where the wound is least circular. Moreover, the circularity ratio fluctuates, correlating with the zippering events that mediate wound closure in this mutant. (**D-E**) RNAi assays controls. (D) Control assays for $Cdc42^{RNAi}$. Wildtype embryos were injected with Cdc42 dsRNA and compared with $Cdc42^4/Cdc42^6$ mutant embryos. Quantification of the wound area over time is shown (Zip^1 , n=4; Zip^1 control), and compared to zip^1 mutant embryos. Quantification of the wound area over time is shown (Zip^1 , n=4; Zip^1 control), n=4; results are given as means \pm s.e.m.).



Movie 1. Dynamics of cytoskeleton and adherens junctions components in epithelial wound repair. Dual fluorescence 4D video showing the wound repair response in stage 15 embryo expressing markers for (A) actin and nuclei (sGMCA; His2Av-mRFP), (B) actin and myosin (sChMCA, sqh-GFP), and (C) actin and E-cadherin (sChMCA, DE-cadherin). (A) Actin-rich protrusions are observed a few minutes post-wounding followed by the actin cable. In later stages of the healing process, protrusions from opposite edges contact each other and close the wound. Images were captured at 30 seconds intervals for 22 minutes follow by a minute interval for 57 minutes. (B) Actin and myosin co-localize at the leading edge. Note that myosin II is excluded from the actin rich protrusions. (C) DE-Cadherin accumulates at the cell junctions along the leading edge and co-localizes with the actin purse string. Images were captured every minute for 21 minutes followed by 5 minutes intervals until closure (B, C). Videos are displayed at 3 frames/second. Scale bars: 20 µm.



Movie 2. An actomyosin cable and actin rich protrusions are required for epithelial wound repair. 4D video showing the wound repair process in stage 15 wildtype (A), zip^{1} (B), shg^{k03401} (C), $ed^{M/Z}$ (D), and $Cdc42^{4}/Cdc42^{6}$ (E) embryos expressing an actin marker (sGMCA). (B) zip^{1} homozygous mutant embryos fail to assemble a continuous actin cable at the wound edge. Healing is mediated by actin rich protrusion mediated contraction and knitting between neighboring cells. (C) shg^{k03401} and $ed^{M/Z}$ homozygous mutants assemble a discontinuous actin cable. In these embryos wound closure is mediated by local contraction of actin patches and by zippering of actin rich protrusions. (E) In $Cdc42^{4}/Cdc42^{6}$ mutant embryos a contractile actin cable is assembled at the wound edge. Nevertheless, these embryos fail to completely close the wound due to the lack of actin rich protrusions. Images were captured every minute for 21 minutes followed by 5 minute intervals until closure. Scale bars: 20 µm.



Movie 3. Dynamic changes in the organization of plasma membrane at wounds. 4D single fluorescence video of stage 15 embryos expressing (A, B) PH-PLC-GFP and (C) Venus-GAP43. Highly dynamic protrusions are observed probing the wound area and reaching out to neighboring cells. Images were captured every minute for 21 minutes followed by 5 minute intervals. Videos are displayed at 3 frames/second. Scale bar: 20 µm.