

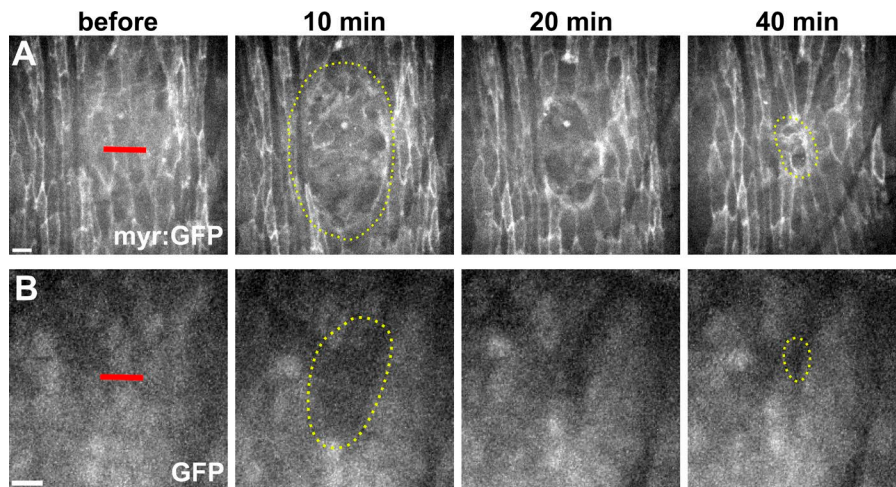
Hunter et al., <http://www.jcb.org/cgi/content/full/jcb.201501076/DC1>

Figure S1. **Membrane and cytoplasmic dynamics during wound repair.** (A and B) Epidermal cells in embryos expressing myristoylated:GFP (myr:GFP; A) or cytoplasmic GFP (B) driven by *tubulin-Gal4*. Time after wounding is shown. Red lines indicate wound sites. Yellow dotted lines outline the wounds. Anterior left, dorsal up. Bars, 5  $\mu$ m.

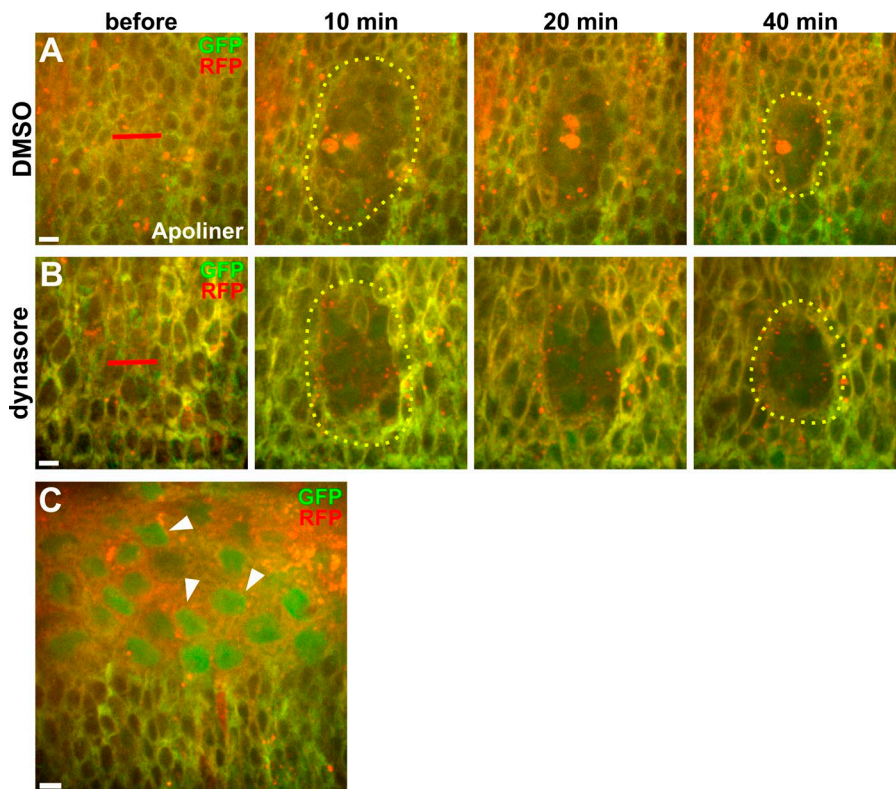


Figure S2. **Dynasore treatment does not induce apoptosis.** (A and B) Epidermal cells expressing Apoliner, a caspase biosensor, in DMSO (A) or dynasore-injected (B) embryos. No nuclear GFP signal (green) is observed. Time after wounding is shown. Red lines indicate wound sites. Yellow dotted lines outline the wounds. (C) Amnioserosa cells expressing Apoliner in an embryo undergoing dorsal closure. Arrowheads indicate the presence of apoptotic cells displaying nuclear GFP. Anterior left, dorsal up. Bars, 5  $\mu$ m.

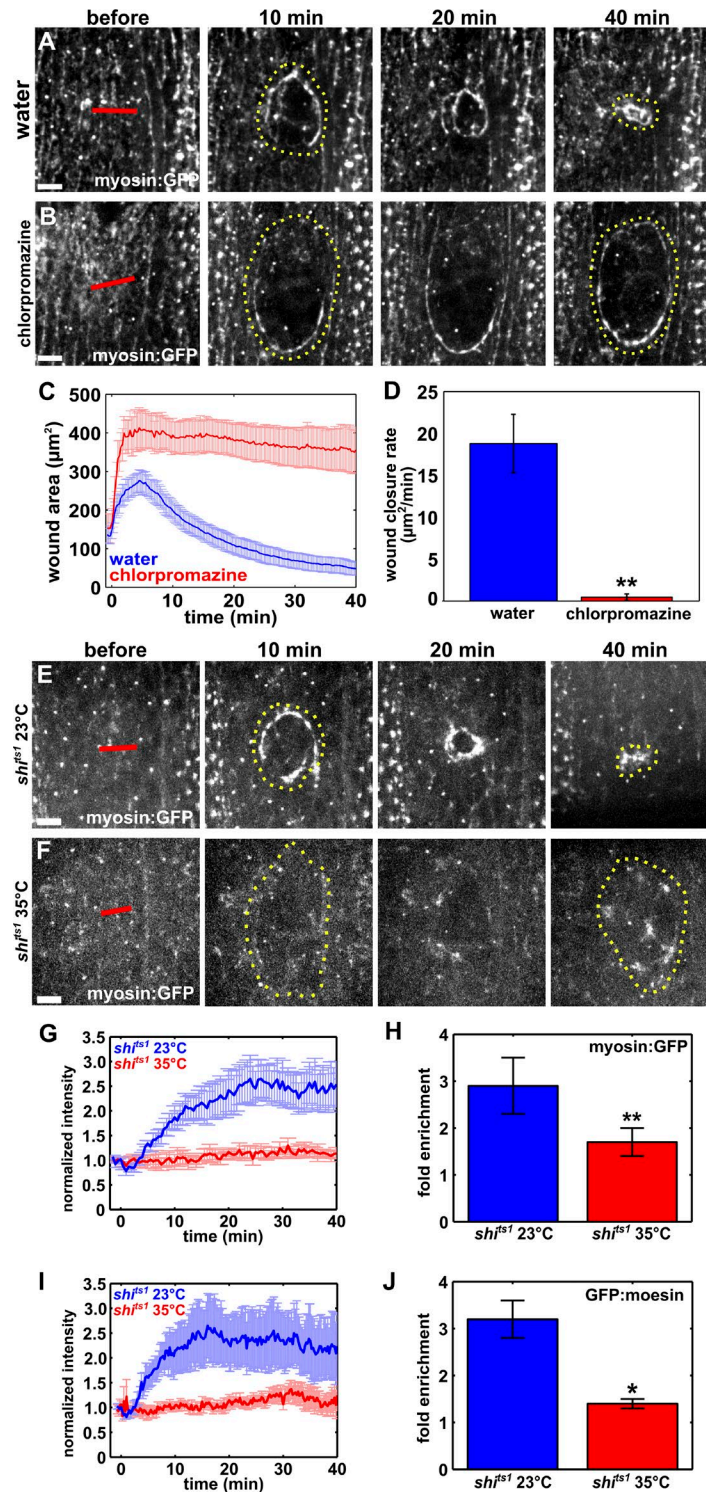


Figure S3. **Clathrin-mediated endocytosis is required for efficient wound repair and actomyosin accumulation at the wound margin is impaired in *shi<sup>ts1</sup>* embryos at the restrictive temperature.** (A and B) Epidermal cells in embryos expressing myosin:GFP, injected with water (A) or chlorpromazine (B), an inhibitor of clathrin-mediated endocytosis, before wounding. (C and D) Mean wound area over time (C) and closure rate for the fast phase of wound repair (D) for water (blue;  $n = 6$ ) and chlorpromazine-injected (red;  $n = 6$ ) embryos. (E and F) Epidermal cells in *shi<sup>ts1</sup>* embryos at the permissive temperature (E) or the restrictive temperature (F) expressing myosin:GFP. (A, B, E, and F) Time after wounding is shown. Red lines indicate wound sites. Yellow dotted lines outline the wounds. Anterior left, dorsal up. Bars, 5  $\mu\text{m}$ . (G and I) myosin:GFP (G) and GFP:moesin (I) fluorescence at the wound margin over time in *shi<sup>ts1</sup>* embryos at the permissive temperature (blue;  $n = 8$  in G and  $n = 6$  in I) or the restrictive temperature (red;  $n = 5$  in G and  $n = 6$  in I). (H and J) Maximum fold enrichment at the wound margin of myosin:GFP (H) or GFP:moesin (J). Error bars, SEM; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

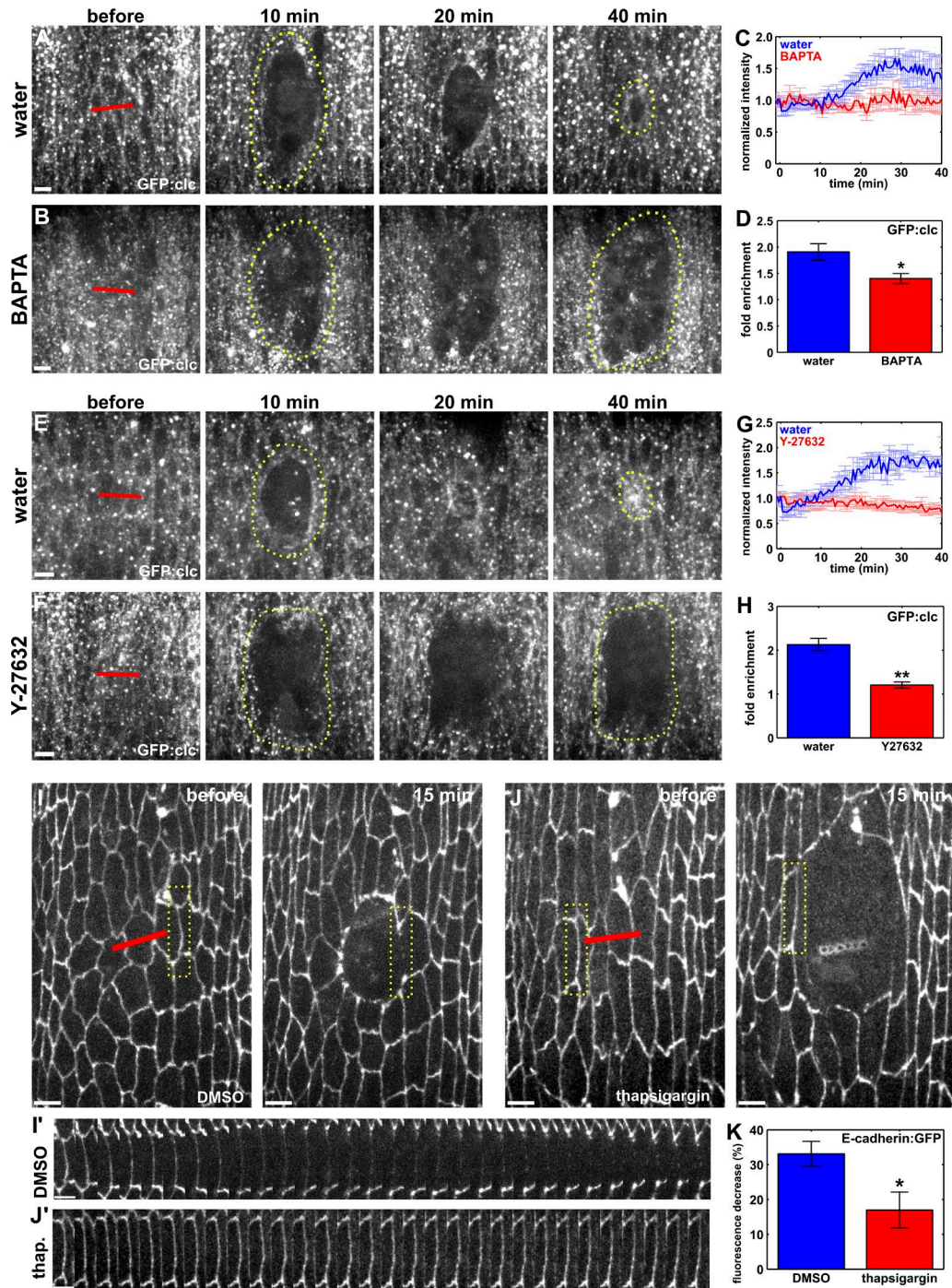


Figure S4. **Calcium and actomyosin contractility are required for the recruitment of clathrin to the wound margin, and calcium is also necessary for E-cadherin redistribution and wound closure.** (A, B, E, and F) Epidermal cells in embryos expressing GFP:clc, injected with water (A and E), BAPTA (B), or Y-27632 (F). Time after wounding is shown. Red line indicates wound site. Yellow dotted line outlines the wound. Anterior left, dorsal up. Bars, 5  $\mu$ m. (C and G) GFP:clc fluorescence at the wound margin over time, in water (blue;  $n = 7$  in C and  $n = 4$  in G), BAPTA (red;  $n = 7$  in C), or Y-27632 (red;  $n = 4$  in G)-injected embryos. (D and H) Maximum fold enrichment of GFP:clc at the wound margin in BAPTA-treated (D) or Y-27632-treated (H) embryos. (I and J) Epidermal cells expressing E-cadherin:GFP in a DMSO (I) or thapsigargin (J)-injected embryo. Time after wounding is shown. Red lines indicate wound sites. Yellow boxes outline the interfaces used to generate the kymographs in I' and J'. Anterior left, dorsal up. Bars, 5  $\mu$ m. (I' and J') Kymographs showing the redistribution of E-cadherin from the interfaces indicated in I and J. Anterior left, dorsal up. Bars, 30 s. (K) Percentage of decrease in E-cadherin:GFP fluorescence at wound margin interfaces 15 min after wounding in DMSO ( $n = 26$  interfaces) and thapsigargin ( $n = 30$  interfaces)-injected embryos. Error bars, SEM; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

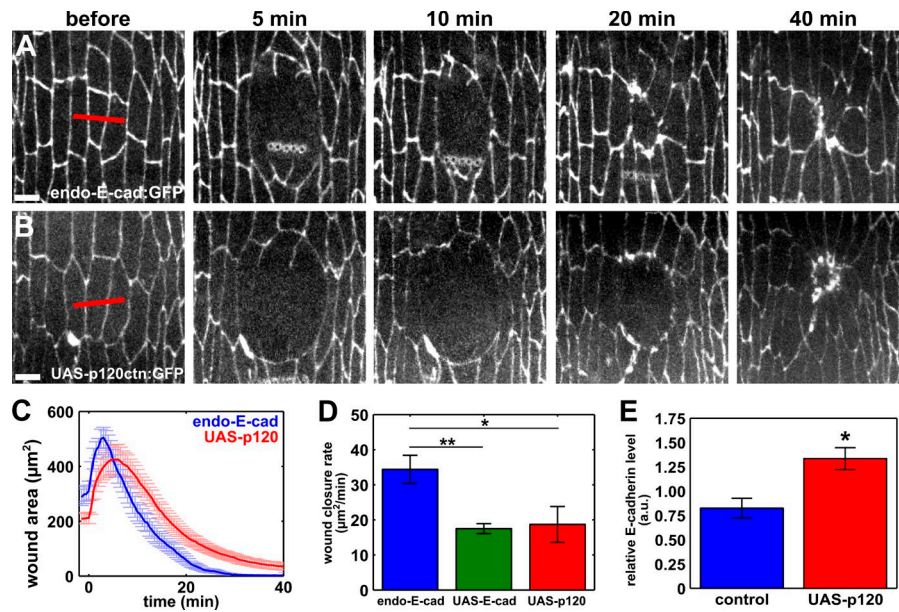
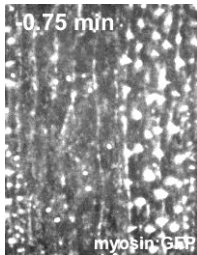
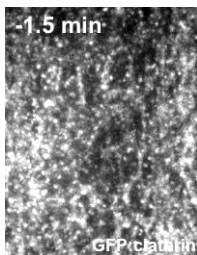


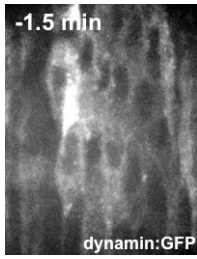
Figure S5. **Overexpression of p120-catenin causes a delay in wound closure.** (A and B) Epidermal cells in embryos expressing E-cadherin at wild-type levels (A) or overexpressing UAS-p120-catenin:GFP (B). Time after wounding is shown. Red lines indicate wound sites. Anterior left, dorsal up. Bars, 5 μm. (C) Wound area over time for control embryos (blue;  $n = 7$ ) or embryos overexpressing p120-catenin (red;  $n = 8$ ). (D) Mean wound closure rate for the fast phase of wound repair. (E) Relative junctional E-cadherin level with respect to the counterstain (Dlg) in stage 14–15 embryos overexpressing UAS-p120-catenin:GFP driven by *tubulin-Gal4* ( $n = 4$ ) or control embryos from *yellow white* males crossed to *tubulin-Gal4* females ( $n = 3$ ). Error bars, SEM; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .



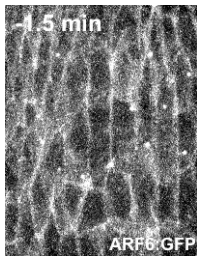
Video 1. **Myosin accumulates at the wound margin.** Epidermal cells expressing myosin:GFP. Images were acquired by time-lapse confocal microscopy using a spinning disk confocal microscope (Revolution XD; Andor Technology). A stack was acquired every 15 s for 41 min. Time after wounding is shown. Anterior left, dorsal up.



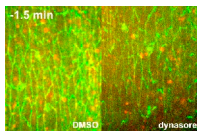
Video 2. **Clathrin accumulates at the wound margin.** Epidermal cells expressing GFP:clc. Images were acquired by time-lapse confocal microscopy using a spinning disk confocal microscope (Revolution XD; Andor Technology). A stack was acquired every 30 s for 41.5 min. Time after wounding is shown. Anterior left, dorsal up.



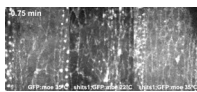
Video 3. **Dynamain accumulates at the wound margin.** Epidermal cells expressing dynamin:GFP. Images were acquired by time-lapse confocal microscopy using a spinning disk confocal microscope (Revolution XD; Andor Technology). A stack was acquired every 30 s for 53.5 min. Time after wounding is shown. Anterior left, dorsal up.



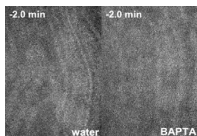
Video 4. **ARF6 accumulates at the wound margin.** Epidermal cells expressing ARF6:GFP. Images were acquired by time-lapse confocal microscopy using a spinning disk confocal microscope (Revolution XD; Andor Technology). A stack was acquired every 30 s for 41.5 min. Time after wounding is shown. Anterior left, dorsal up.



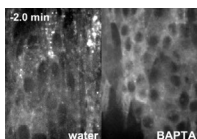
Video 5. **Dynasore treatment impairs wound closure.** Epidermal cells expressing ubi-E-cadherin:GFP (green) and myosin:mCherry (red) in a control embryo injected with 50% DMSO (left) or 50 mM dynasore (right). Images were acquired by time-lapse confocal microscopy using a spinning disk confocal microscope (Revolution XD; Andor Technology). A stack was acquired every 30 s for 44 min. Time after wounding is shown. Anterior left, dorsal up.



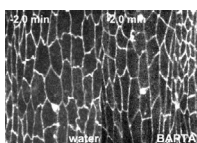
Video 6. **Delayed wound closure in *shits1* embryos heated to the restrictive temperature.** Epidermal cells expressing GFP:moesin in a wild-type embryo heated to the restrictive temperature (35°C; left), a *shits1* embryo imaged at the permissive temperature (23°C; middle), or a *shits1* embryo heated to the restrictive temperature (35°C; right). Images were acquired by time-lapse confocal microscopy using a spinning disk confocal microscope (Revolution XD; Andor Technology). A stack was acquired every 15 s for 42.5 min. Time after wounding is shown. Anterior left, dorsal up.



Video 7. **BAPTA injection prevents extracellular calcium release upon wounding.** Epidermal cells expressing GCaMP3, a fluorescent calcium biosensor, in embryos injected with water (left) or 50 mM BAPTA (right). Images were acquired by time-lapse confocal microscopy using a spinning disk confocal microscope (Revolution XD; Andor Technology). A stack was acquired every 30 s for 41.5 min. Time after wounding is shown. Anterior left, dorsal up.



Video 8. **Calcium is required for polarized accumulation of dynamain at the wound margin.** Epidermal cells in embryos expressing dynamin:GFP, injected with water (left) or 50 mM BAPTA (right). Images were acquired by time-lapse confocal microscopy using a spinning disk confocal microscope (Revolution XD; Andor Technology). A stack was acquired every 30 s for 51.5 min. Time after wounding is shown. Anterior left, dorsal up.



Video 9. **Calcium is required for E-cadherin removal from the wound margin.** Epidermal cells in embryos expressing endo-E-cadherin:GFP, injected with water (left) or 50 mM BAPTA (right). Images were acquired by time-lapse confocal microscopy using a spinning disk confocal microscope (Revolution XD; Andor Technology). A stack was acquired every 30 s for 42 min. Time after wounding is shown. Anterior left, dorsal up.