Piezo acts as a molecular brake on wound closure to ensure effective inflammation and maintenance of epithelial integrity

Highlights

- Loss of Piezo accelerates wound closure and weakens inflammation in vivo
- Loss of Piezo enhances wound edge cell intercalation and myosin cable heterogeneity
- Loss of Piezo reduces epidermal Ca^{++} levels and damage-induced ROS production
- Loss of Piezo compromises post-wounding epithelial barrier function and survival

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In brief

Zechini et al. have shown that the mechanosensitive channel Piezo delays wound closure by limiting myosin cable heterogeneity and wound edge cell intercalation, while also ensuring a sustained inflammatory response. Piezo is essential for efficient restoration of epithelial barrier function and to ensure post-wound survival in vivo.
Piezo acts as a molecular brake on wound closure to ensure effective inflammation and maintenance of epithelial integrity

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SUMMARY

Wound healing entails a fine balance between re-epithelialization and inflammation1,2 so that the risk of infection is minimized, tissue architecture is restored without scarring, and the epithelium regains its ability to withstand mechanical forces. How the two events are orchestrated in vivo remains poorly understood, largely due to the experimental challenges of simultaneously addressing mechanical and molecular aspects of the damage response. Here, exploiting Drosophila’s genetic tractability and live imaging potential, we uncover a dual role for Piezo—a mechanosensitive channel involved in calcium influx3—during re-epithelialization and inflammation following injury in vivo. We show that loss of Piezo leads to faster wound closure due to increased wound edge intercalation and exacerbated myosin cable heterogeneity. Moreover, we show that loss of Piezo leads to impaired inflammation due to lower epidermal calcium levels and, subsequently, insufficient damage-induced ROS production. Despite initially appearing beneficial, loss of Piezo is severely detrimental to the long-term effectiveness of repair. In fact, wounds inflicted on Piezo knockout embryos become a permanent point of weakness within the epithelium, leading to impaired barrier function and reduced ability of wounded embryos to survive. In summary, our study uncovers a role for Piezo in regulating epithelial cell dynamics and immune cell responsiveness during damage repair in vivo. We propose a model whereby Piezo acts as molecular brake during wound healing, slowing down closure to ensure activation of sustained inflammation and re-establishment of a fully functional epithelial barrier.

RESULTS AND DISCUSSION

Loss of Piezo accelerates epithelial wound closure

To create reproducible wounds on the ventral epithelium of Drosophila embryos, we used a well-established laser ablation assay3 followed by confocal live imaging to monitor wound closure. Analysis of wounds inflicted to stage 15 control and Piezo knockout (Piezo−/−) embryos labeled with the GFP protein trap for the septate junction component neuroglian (Nrg) revealed that loss of Piezo strikingly accelerates wound closure (Figure 1A). Measuring wound size throughout repair clearly showed that, despite a comparable initial area (Figure 1B), wounds generated in Piezo−/− embryos close significantly faster than those in control embryos (Figure 1C). Accordingly, a reduced T50% (time required to reach 50% of the maximal size) and an increased wound closure rate (area of wound closed over time) is observed in Piezo−/− when compared to control embryos (Figure 1C). Accordingly, a reduced T50% (time required to reach 50% of the maximal size) and an increased wound closure rate (area of wound closed over time) is observed in Piezo−/− when compared to control embryos (Figures 1D and 1E). We then asked within which tissue Piezo exerts its wound closure-regulating function. Since Piezo is involved in sensing changes in mechanical forces,4,5 and wounding represents a dramatic mechanical input, we hypothesized that Piezo regulates wound closure by acting within the epidermis. To understand whether Piezo is expressed within this tissue, we performed immunostaining of embryos carrying a MiMIC-based GFP protein trap6 construct within the Piezo locus, which revealed Piezo’s prominent expression at the plasma membrane of ventral epidermal cells (Figure S1A). Using the GAL4/UAS system,7 we then specifically suppressed Piezo expression within the epidermis (PiezoRNAi), which resulted in a significant reduction in Piezo expression (Figures S1B and S1C) and a comparable acceleration of wound closure (Figure 1F). Again, despite comparable initial areas (Figure 1G), wounds made to PiezoRNAi embryos showed reduced T50% and faster wound closure rate (Figures 1I and 1J). Taken together, these data demonstrate that Piezo is expressed in the Drosophila embryonic epidermis and that its loss within this tissue accelerates wound closure, suggesting a model whereby epithelial Piezo acts cell-autonomously as a molecular brake to slow down re-epithelialization. Lastly, we asked whether loss of Piezo impacts other aspects of epithelial homeostasis and dynamics, including maintenance of cell numbers across the embryonic epidermis and effectiveness of dorsal closure—a morphogenetic episode that has been shown to parallel several aspects of wound repair.2,8 As shown in Figures S1D–S1G, we
found that loss of Piezo does not lead to any significant difference in epidermal cell number, nor to an impairment of dorsal closure.

**Loss of Piezo locally increases tissue fluidity and exacerbates myosin cable heterogeneity**

Which epithelial properties, affected by the loss of Piezo, could account for faster re-epithelialization? Like different morphogenetic processes, wound healing requires a certain degree of epithelial mechanical adaptation.\(^2,9\) Specifically, for wounds to close effectively, epithelial cells in direct contact with the damaged area need to be able to slide past their neighbors—progressively withdrawing from the wound edge—in a process termed “wound edge intercalation.”\(^10\) Therefore, we asked whether loss of Piezo increases epithelial cells’ ability to intercalate. Tracking of individual wound edge-facing epithelial cells revealed that, despite occurring in both genotypes (Figure 2A), intercalation is more frequent and faster in Piezo\(^{-/-}\) embryos (Figures 2B and 2C). We also asked whether loss of Piezo affects the spatial distribution of intercalation events. Epidermal cells display an anisotropic morphology, with an extended margin along the embryonic dorso-ventral (D-V) axis, and a narrow margin along the embryonic antero-posterior (A-P) axis. Cells located along the A-P axis face the wound by their longest sides, while those along the D-V axis face it by their shortest sides (Figure 2D). In order to intercalate, the former need to re-establish cell-cell contacts on a longer stretch of plasma membrane compared to the latter, which should render intercalation along the A-P axis a far greater challenge. Accordingly, in control embryos, the vast majority of wound-edge intercalation events occur along the D-V axis. However, in Piezo\(^{-/-}\) embryos, a larger proportion of intercalation events occur along the embryonic A-P axis—and independently of their relative position to the wound (Figure 2E). These results indicate that loss of Piezo facilitates the transition from a solid-like (jammed) to a more fluid-like (un-jammed) epithelium upon wounding. We wondered whether such fluidization affects the entire epithelium or is instead localized to the wound-surrounding epithelial cells. To address this question, we tracked individual epithelial cells two rows away from the damaged area and found no increase in the rate of intercalation (Figure S2A)—suggesting that loss of Piezo triggers a regionalized, rather than a tissue-wide, increase in fluidity. Further supporting this notion, we found no difference in overall epithelial tension—as measured by cortical myosin levels—in unchallenged epithelia (Figure S2B). Wound-facing epithelial cells respond quickly to tissue damage by mobilizing their cytoskeletal components to assemble an actomyosin cable, a supercellular structure that provides the force to coordinate cell contraction—ensuring a seamless closure\(^1\)—while also conferring an elliptic shape to the healing wound. While control wounds remain elliptic as they close, Piezo\(^{-/-}\) wounds gradually lose symmetry (Figure 2F). We asked whether such irregularity could be linked to a dysfunctional actomyosin cable assembly. To address this, we monitored myosin dynamics at the wound edge during re-epithelialization, which showed that a myosin-rich cable is swiftly assembled around the wound in both genotypes (Figure 2G) with no measurable difference in myosin enrichment (Figures S2C and S2D). However, while myosin remains confined within the wound outline in control embryos, its distribution along the wound in Piezo\(^{-/-}\) embryos appeared more disorganized, with myosin clusters (arrows) forming as the wound closes. Intriguingly, myosin heterogeneity has been previously reported to facilitate wound closure,\(^5,11\) which prompted us to ask whether loss of Piezo exacerbates this heterogeneity, contributing to a faster re-epithelialization. To quantify myosin heterogeneity around the wound edge, we measured its intensity along eight equally long segments and normalized the averaged intensity values. Strikingly, we found that myosin heterogeneity around the wound perimeter is greatly enhanced in Piezo\(^{-/-}\) embryos (Figures 2H, S2E, and S2G). Altogether, our data show that loss of Piezo accelerates re-epithelialization by locally increasing tissue fluidity around the wound edge—leading to more frequent, faster, and more widespread intercalation events—and by exacerbating myosin cable heterogeneity.

**Loss of Piezo reduces epithelial calcium levels, affects damage-induced ROS production, and weakens the inflammatory response**

Piezo is a non-selective cation channel\(^3\) mainly involved in calcium influx. Since calcium is a conserved early damage signal—spreading as an instantaneous wave across epithelia in response to wounds—we asked whether loss of Piezo affects calcium dynamics and/or levels within epidermal cells. To achieve this, we expressed two independent calcium reporters, R-Geco and GCaMP3, within the epithelium of control and Piezo\(^{-/-}\) embryos. Visual analysis indicated that Piezo is not required for the generation of a damage-induced calcium wave (Figures 3A and S3A). However, quantitative measurements revealed a dramatic reduction in basal calcium levels across unwounded Piezo\(^{-/-}\) epithelia (Figures 3B and S2B), which, in turn, resulted in a significant decrease in damage-induced calcium peak levels across wounded epithelia (Figures 3C and S3C). Therefore, while Piezo does not mediate damage-induced calcium influx and spread, it plays a role in maintaining homeostatic epithelial calcium levels. Despite no significant difference in the normalized peak calcium intensity (Figures 3D and S3D), we hypothesized that the overall lower peak calcium levels across the epidermis may hinder the activation of downstream damage responses. A conserved

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**Figure 1. Loss of Piezo accelerates epithelial wound closure**

(A and F) Confocal time-lapse of stage 15 embryos expressing Nrg to mark epidermis (green). Asterisk: wound. Time-points refer to minutes post-wounding. Dashed line: wound outline.

(B and G) Initial wound area.

(C and H) Closure progression from wounds’ maximal extension.

(D and I) Time required for wounds to reach 50% of maximal size.

(E and J) Rate of wound closure. Scale bars, 20 \(\mu\)m; error bars: SEM.

Related to Figure S1. See Videos S1 and S2.
Figure 2. Loss of Piezo increases tissue fluidity and exacerbates myosin heterogeneity at the wound edge

(A) Examples of wound edge intercalation. Nrg (gray): epithelium; cyan: intercalating cells; yellow/magenta: neighboring cells.

(B) Percentage of intercalating cells.

(C) Time required for intercalation.

(D) Schematic representation of a stage 15 embryo with A-P/D-V axes indicated. Left: lateral view, ventral side highlighted by magenta box. Middle/right: ventral view of an unwounded/wounded (black ellipse) embryo. Relative position of epidermal cells facing the wound: cyan: cells along the D-V axis; yellow: cells along the A-P axis.

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Piezo—located in the ventral embryonic epidermis—and assessed the ability of severed a single denticle belt (a myosin-rich pattern present on ing wound repair. To test this hypothesis, we made wounds that cule that delays damage repair? One explanation might be that entuitive: why would evolution favor the expression of a mole-
and reduces post-wounding survival

Loss of Piezo compromises epithelial barrier function and reduces post-wounding survival

The negative regulation of Piezo on wound closure seems coun-terintuitive: why would evolution favor the expression of a mole-
cule that delays damage repair? One explanation might be that slower closure is necessary to maintain epithelial patte-rning during wound repair. To test this hypothesis, we made wounds that severed a single denticle belt (a myosin-rich pattern present on the ventral embryonic epidermis) and assessed the ability of Piezo−/− embryos to reconstitute it during re-epithelialization. Denticle alignment post-repair was indistinguishable between control and Piezo−/− embryos (Figure 4A), indicating that loss of Piezo does not compromise the ability of closing wounds to maintain tissue geometry. Another plausible explanation for the requirement of a molecular brake during wound healing is that, despite being initially beneficial, a rapid re-epithelialization may be detrimental in the long-term. To explore this possibility, we performed long-term live imaging to study the evolution of wounded tissues in control and Piezo−/− embryos as they pro-ceeded through development. This revealed that, beginning at 4–6 hours post-wounding, damaged Piezo−/− epithelia degenerate into a large epidermal gap (Figures 4B and S4A)—a pheno-menon rarely seen in control animals. Furthermore, while control embryos tend to develop small and rapidly resolving melanotic plugging—scab-like structures typically observed during the late larval damage response (Piezo−/− embryos failed to resolve and became accompanied by a persistent and progressively large melanotic plug at the original wound site (Figures 4B, 4C, and S4A). Accordingly, while wounded control embryos develop into larvae that are indistin-
guishable from unwounded animals, a large proportion of wounded Piezo−/− embryos develop into larvae exhibiting epithelial discontinuity, a prominent melanotic plug, and a failure to assemble the cuticle layer at the original wound site (Figure 4D).

This epidermal breach represents a clear compromise in epithelial barrier function, as evidenced by the dramatic increase in leakage of a cell-impermeable dye in wounded Piezo−/− em-

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constantly subjected to injuries throughout every organism’s life; being able to repair them is an evolutionarily widespread prerogative. It is only natural, given their life-threatening potential, to assume that wounds must be closed within the shortest time-frame possible. Our work, however, clearly demonstrates that—in vivo—wounds are dealt with using a “slow and steady wins the race” approach.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

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**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2022.06.041.

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**AUTHOR CONTRIBUTIONS**

Conceptualization: L.Z., C.A., A.S., and W.W.; Methodology: L.Z., C.A., and A.S.; Formal Analysis: L.Z., C.A., and A.S.; Investigation: L.Z., C.A., and A.S.; Writing – Original Draft: L.Z., C.A., A.S., and W.W.; Writing – Review & Editing - L.Z., C.A., A.S., and W.W.; Visualization: C.A.; Funding Acquisition: C.A. and W.W.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

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**REFERENCES**

1. Razzell, W., Evans, I.R., Martin, P., and Wood, W. (2013). Calcium flashes orchestrate the wound inflammatory response through DUOX activation and hydrogen peroxide release. Curr. Biol. 23, 424–429. https://doi.org/10.1016/j.cub.2013.01.058.
2. Wood, W., Jacinto, A., Grose, R., Woolner, S., Gale, J., Wilson, C., and Martin, P. (2002). Wound healing recapitulates morphogenesis in Drosophila embryos. Nat. Cell Biol. 4, 907–912. https://doi.org/10.1038/ncb875.
3. Coste, B., Mathur, J., Schmidt, M., Earley, T.J., Ranade, S., Petrus, M.J., Dubin, A.E., and Patapoutian, A. (2010). Piezo1 and Piezo2 Are Essential Components of Distinct Mechanically Activated Cation Channels. Science 330, 55–60. https://doi.org/10.1126/science.1193270.
4. Eisenhoffer, G.T., Loftus, P.D., Yoshigi, M., Otsuna, H., Chien, C.B., Morcos, P.A., and Rosenblatt, J. (2012). Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia. Nature 484, 548–549. https://doi.org/10.1038/nature10999.
5. Gudipaty, S.A., Lindblom, J., Loftus, P.D., Redd, M.J., Edes, K., Davey, C.F., Krishnegowda, V., and Rosenblatt, J. (2017). Mechanical stretch
triggers rapid epithelial cell division through Piezo1. Nature 543, 118–121. https://doi.org/10.1038/nature21407.

6. Nagarkar-Jaiswal, S., DeLuca, S.Z., Lee, P.T., Lin, W.W., Pan, H., Zuo, Z., Lv, J., Spradling, A.C., and Bellen, H.J. (2015). A genetic toolkit for tagging intronic MiMIC containing genes. Elife 4, https://doi.org/10.7554/eLife.08469.

7. Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118, 401–415. https://doi.org/10.1242/dev.118.2.401.

8. Razzell, W., Wood, W., and Martin, P. (2014). Recapitulation of morphogenetic cell shape changes enables wound re-epithelialisation. Development 141, 1814–1820. https://doi.org/10.1242/dev.107045.

9. Jain, A., Ulman, V., Mukherjee, A., Prakash, M., Cuenca, M.B., Pimpale, L.G., Haase, R., Panfilio, K.A., Jug, F., Grill, S.W., Tomancak, P., and Pavlopoulos, A. (2020). Regionalized tissue fluidization is required for epithelial gap closure during insect gastrulation. Nat. Commun. 11, 5604. https://doi.org/10.1038/s41467-020-19356-x.

10. Tetley, R.J., Staddon, M.F., Heller, D., Hoppe, A., Banerjee, S., and Mao, Y. (2019). Tissue Fluidity Promotes Epithelial Wound Healing. Nat. Phys. 15, 1195–1203. https://doi.org/10.1038/s41567-019-0618-1.

11. Zulueta-Coarasa, T., and Fernandez-Gonzalez, R. (2018). Dynamic force patterns promote collective cell movements during embryonic wound repair. Nat. Phys. 14, 750–758. https://doi.org/10.1038/s41567-018-0111-2.

12. He, L., Si, G., Huang, J., Samuel, A.D.T., and Perrimon, N. (2018). Mechanical regulation of stem-cell differentiation by the stretch-activated Piezo channel. Nature 555, 103–106. https://doi.org/10.1038/nature25744.

13. Galko, M.J., and Krasnow, M.A. (2004). Cellular and genetic analysis of wound healing in Drosophila larvae. PLoS Biol. 2, E239. https://doi.org/10.1371/journal.pbio.0020239.

14. Kim, S.E., Coste, B., Chadha, A., Cook, B., and Patapoutian, A. (2012). The role of Drosophila Piezo in mechanical nociception. Nature 483, 209–212. https://doi.org/10.1038/nature10801.

15. Evans, I.R., Hu, N., Skaer, H., and Wood, W. (2010). Interdependence of macrophage migration and ventral nerve cord development in Drosophila embryos. Development 137, 1625–1633. https://doi.org/10.1242/dev.046797.

16. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., and Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019.

17. Meijering, E., Dzyubachyk, O., and Smal, I. (2012). Methods for Cell and Particle Tracking. Method Enzymol 504, 183–200. https://doi.org/10.1016/B978-0-12-391857-4.00009-4.

Figure 4. Loss of Piezo compromises epithelial barrier function after wounding
(A) Confocal images of embryos expressing Sqh-GFP (gray) to label a ventral denticle belt (pseudo-colored in magenta), severed by wounding (+1 min). Recovery of tissue architecture is shown at 1 h and 2 h post-wounding.
(B) Piezo-/- embryo-to-L1 larva imaged ~7 h post-wounding. A gap in the epidermis (Nrg, green) is highlighted by the dashed square; a melanotic plug is shown in brightfield. Dashed magenta line: epithelial gap outline.
(C) Embryo-to-L1 larvae imaged by brightfield to highlight the original site of wounding (black rectangles), with a persisting melanotic plug observed in Piezo-/- embryos.
(D) Piezo-/- L1 larva imaged ~24 h post-wounding. A large melanotic plug (brightfield) is present at the wound site, accompanied by a compromised underlying epithelium (Nrg, green) and defective cuticle (autofluorescence, magenta).
(E) Permeability of control and Piezo-/- embryos prior to and upon wounding.
(F) Survival rate of unwounded and wounded control and Piezo-/- embryos.
(G) Correlation analysis of melanotic plug persistency and lethality.
(H) Survival rate of unwounded and wounded control and PiezoRNAi embryos.
Scale bars: (A): 20 μm; (D) (E): 50 μm. Error bars: SEM.
Related to Figure S4.
## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| α-GFP               | Abcam  | Cat# ab13970; RRID: AB_300798 |
| α-chicken AF488     | Invitrogen | Cat# A-11039; RRID: AB_142924 |
| **Chemicals, peptides, and recombinant proteins** | | |
| Voltalef oil        | VWR    | Cat# 24627.188 |
| Vectashield         | Vector labs | Cat# H-1000 RRID: AB_2336789 |
| Heptane             | Sigma  | Cat# 246654 |
| Formaldehyde 16%    | MP Bio | Cat# 199983 |
| Methanol            | Sigma  | Cat# 34860 |
| DMSO                | Sigma  | Cat# 34873 |
| Amplex™ UltraRed Reagent | Invitrogen | Cat# A36006 |
| Methylene Blue      | Sigma  | Cat# MB-1 |
| Diethyl Ether       | Fisher scientific | Cat# 10696442 |
| Femtotips           | Eppendorf | Cat# 930000035 |
| **Experimental models: Organisms/strains** | | |
| D. melanogaster: Piezo⁺:w⁺[ ]; PBac[w⁺[mC]=RB5.WH5|Piezo[KO] | Bloomington Drosophila Stock Center | RRID: BDSC_58770; FlyBase: FBT0147345 |
| D. melanogaster: Nrg:w⁺[1118] P[w⁺[mC]=PTT- GA|Nrg[000305] | Bloomington Drosophila Stock Center | RRID: BDSC_6844; FlyBase: FBT0002785 |
| D. melanogaster: 69B-GL4:w⁺[ ]; P[w⁺[mW,hs]=GawB]69B | Bloomington Drosophila Stock Center | RRID: BDSC_1774; FlyBase: FBT0002093 |
| D. melanogaster: LexA|min;[sc[ ]; v[1] sever[21]; P[y⁺[t7.7] v[1]+[t1.8]=TRIP-HMS05768] attP40 | Bloomington Drosophila Stock Center | RRID: BDSC_67942; FlyBase: FBT018675 |
| D. melanogaster: LexA|min;[sc[ ]; v[1] sever[21]; P[y⁺[t7.7] v[1]+[t1.8]=TRIP-HMS05775]attP2 | Bloomington Drosophila Stock Center | RRID: BDSC_67946; FlyBase: FBT0186764 |
| D. melanogaster: Piezo|MIMIC|y[1] w[67c23]; Mpi7; PT-GFSTF.0|Piezo[004189-GFSTF.0] | Bloomington Drosophila Stock Center | RRID: BDSC_60209; FlyBase: FBT017858 |
| D. melanogaster: PMCA|min;[sc[ ]; v[1]; P[y⁺[t7.7] v[1]+[t1.8]=TRIP-JF01145]attP2 | Bloomington Drosophila Stock Center | RRID: BDSC_31572; FlyBase: FBT0130608 |
| D. melanogaster: UAS-R-Geco:w⁺[ ]; PBac[y⁺[mDint2] w⁺[mC]=20XUAS-IVS-NES-RGECO1a-p10]VK00005/M66, Tb[1] | Bloomington Drosophila Stock Center | RRID: BDSC_63794; FlyBase: FBT0180190 |
| D. melanogaster: Srp-MoeCherry:w⁺[1118]; P[w⁺[mC]=spHemo-Moe.3XmCherry]3 | Bloomington Drosophila Stock Center | RRID: BDSC_78362; FlyBase: FBT0197718 |
| D. melanogaster: Sqh-GFP:w⁺[1118]; P[w⁺[mC]=sqh-GFP.RLC]3 | Bloomington Drosophila Stock Center | RRID: BDSC_57145; FlyBase: FBT0150058 |
| **Software and algorithms** | | |
| GraphPad Prism V9.2.0 | GraphPad Software | [https://www.graphpad.com/scientific-software/prism/](https://www.graphpad.com/scientific-software/prism/)
| ImageJ/Fiji V2.3.0/1.53f | National Institute of Health | [https://imagej.nih.gov/ij/](https://imagej.nih.gov/ij/)
| Zen Black | Zeiss | [https://www.zeiss.com/microscopy/int/products/microscope-software/zen.html](https://www.zeiss.com/microscopy/int/products/microscope-software/zen.html)

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, William Wood (w.wood@ed.ac.uk).

Materials availability
- This study did not generate new unique reagents.
- Fly strains used in this study are available from the lead contact upon request.

Data and code availability
- All data reported in this paper will be shared by the lead contact upon request.
- This study did not generate original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Drosophila strains and husbandry
All Drosophila strains were raised at 25°C on standard cornmeal-agar food at 50%–60% relative humidity in a 12:12 h light:dark cycle. All genotypes used in this study are listed in Methods S1B.

METHOD DETAILS

Drosophila embryo injection and wounding
Embryos were collected from apple juice agar plates from overnight laying cages maintained at 25°C. Embryos were collected in cell strainers, dechorionated in bleach for 90 s and washed repeatedly with distilled water. Stage 15 embryos (stage 14 to visualize dorsal closure) were manually selected and mounted ventral side up on a glass slide with double-sided sticky tape (dorsal side up to visualize dorsal closure and mounted on glass bottom dishes to image late embryo/L1 larvae), embedded in VOLTALEF oil15, and covered with No 1.0 coverslip (SLS). For injection, embryos were dehydrated for 15 minutes on silica beads. Injection was performed using Femtotip II (Eppendorf) on a Femtojet Injectman Rig (Eppendorf). Epithelial wounds were generated using laser ablation (nitrogen-pumped micropoint ablation laser tuned to 435 nm, Andor Technologies) as previously described2.

Imaging, image processing, and analysis
Confocal imaging was performed on a Zeiss LSM880 laser scanning confocal microscope equipped with a 40x/1.3 oil immersion objective. GFP and mCherry were excited at 488 and 561 nm respectively; the cuticle autofluorescence was obtained by exciting at 405 nm. Images were imported into Fiji16 and processed as required. To aid late embryo/L1 live imaging, animals were sedated by exposure to Diethyl Ether vapors.

Wound closure
To monitor wound closure, 11 μm deep Z-stacks were acquired every 30 seconds, and the wound area measured at regular intervals (5 frames= 2.5 minutes) using the Fiji Freehand selections tool. Wound area values were then exported to excel to calculate \( T_{50\%} \) and rate of wound closure. \( T_{50\%} \) values were calculated performing a polynomial interpolation on wound area values (\( R^2_{\text{Control}}= 0.986 \pm 0.008, R^2_{\text{Piezo-/-}} = 0.982 \pm 0.016, \text{Mean} \pm \text{S.D.} \)). Rate of wound closure indicate the μm² of wound area that are lost per minute as the wound closes and reaches 75% of closure.

Epithelial cell number
Embryonic epithelial cell number was calculated by manually counting the number of cells within three equally-sized squares placed on comparable positions across different embryos.

Continued

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Illustrator         | Adobe  | https://www.adobe.com/uk/products/illustrator.html |
| Photoshop           | Adobe  | https://www.adobe.com/uk/products/photoshop.html |
**Epithelial cells intercalation**
Individual epithelial cells were tracked using the MTrackJ Fiji plugin from t= 5 minutes post-wound (earliest time at which the wound edge is clearly identifiable) to t= 90 minutes. Intercalating cells were defined as those that lose contact with the initially neighboring cells. Intercalation time corresponds to the time (in minutes) required for individual epithelial cells to fully move past neighboring cells.

**Wound ellipticity**
Wound ellipticity at given time-points (5-, 15-, 30-, 45-, 60- and 75-minutes post-wounding), was defined by the ratio between the wound area and the area of the smallest ellipse (defined by the Fiji Elliptical selection tool) able to contain the wound. Consequently, an ellipticity of 1 indicates a perfectly elliptic wound, lower values indicate a progressively more irregular shape.

**Myosin wound-edge measurements**
Absolute wound edge myosin fluorescence intensity values were obtained using the Fiji freehand Line tool. To measure wound edge myosin heterogeneity, each wound was divided into 8 equally-long segments (1-8), and the averaged myosin intensity along each segment was normalized to the averaged wound edge myosin intensity; normalized values were then ranked (lowest to highest) and plotted as heatmaps. To obtain the myosin heterogeneity score, we compared the SD of the wound edge myosin distribution across the 8 segments. To visually represent wound edge myosin heterogeneity, myosin intensity was plotted along a set length of wound edge, and the lowest and the highest myosin intensity values then connected by differentially-colored rectangles; progressively taller rectangles correspond to a more widely-spread myosin intensity values.

**Tissue tension measurement**
Cortical myosin was used as epithelial tension readout. Average cortical myosin fluorescence intensity values were obtained using the Fiji freehand Line by outlining ten non-neighboring epithelial cells within individual embryos.

**Calcium signal**
To measure R-Geco calcium signal, 14 µm deep Z-stacks were acquired every 5 seconds. Regions of interest (ROIs) were manually selected on average intensity projections, and average pixel intensity values (basal and peak) of the ROI obtained using the time series V3.0 Fiji plugin. To measure GCaMP3 calcium signal, 4 µm deep Z-stacks were acquired every 5 seconds, and ROIs manually selected on average intensity projections. Basal calcium intensity was obtained from the pre-wound frame, peak calcium intensity was obtained from the brightest time-frame. Peak ΔF/F0 was calculated as difference of the average peak fluorescence intensity and the basal intensity (Fpeak-Fbasal), divided by the basal intensity (Fbasal) values.

**Damage-induced ROS production**
To monitor ROS levels upon wounding, 5 µm deep Z-stacks were acquired every 30 seconds and ROIs manually selected on average intensity projections. For each time-point, the ROI absolute fluorescence intensity (ROIAbsolute) was calculated (ROIMean * ROI Area) and normalized against the corrected background fluorescence intensity [BackgroundCorrected= (BackgroundMean * Background Area) – ROIAbsolute].

**Macrophage recruitment to wound**
Individual macrophages were tracked throughout closure using the MTrackJ Fiji plugin, and resulting tracks analyzed using the Chemotaxis tool (ibidi GmbH) Fiji plugin to obtain individual macrophages speed values.

**Embryo permeability test**
Stage 15 embryos were dechorionated as described above and then devitellinized in Heptane for 15 minutes. Embryos were mounted ventral side up on a glass slide in oil, wounded as described above, kept at 25° for 4 hours, collected from the wounding slides and then submerged in a Methylene Blue solution (25 mM in distilled water) for 1 hour. Embryos were then extensively washed in distilled water and imaged on an EVOS XL Core microscope equipped with 40X objective.

**Embryo survival after wound and larval imaging**
Stage 15 embryos mounted and subjected to either wound or mock wound were allowed to recover for 1 h at 18°C. Embryos were then detached from the microscope slide, placed on apple plates, kept at 25° and monitored for the following 10 days to record the adult emergence rate. Alternatively, collected embryos were individually placed on apple plates to monitor their development in the following 24 hours. L1 larvae were snap frozen in dry ice before microscopic observation.

**Drosophila embryo fixation and immunostaining**
Dechorionated embryos were fixed in 1:1 4% PFA:heptane mixture for 30 minutes at room temperature, washed with PBS-Tx-BSA and incubated in primary antibodies at 4°C overnight. Embryos were incubated with secondary antibodies for 1 hour at room temperature. Washed embryos were then mounted in Vectashield mounting medium. Primary antibodies: α-GFP (1:500, Abcam Ab13970), Secondary antibodies: α-chicken AF488 (1:200 Invitrogen A11039).
QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis
All datasets underwent Shapiro-Wilk normality tests to ensure that the appropriate statistical tests were performed. Two-tailed unpaired t-tests and Mann-Whitney tests were then performed on normally-distributed and non-normally-distributed data respectively. Datasets with more than two groups were compared using ANOVA tests; for data with comparable variances (F-tested) Tukey’s or Sidak’s multiple comparisons were performed, as recommended by the GraphPad Prism V8.4.1 software. All graphs show mean ± SEM. Statistical details can be found in Method S1A.
Supplemental Information

Piezo acts as a molecular brake on wound closure to ensure effective inflammation and maintenance of epithelial integrity

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Figure S1. Piezo epidermal expression, Piezo RNAi effectiveness, epidermal cell density and dorsal closure. Related to figure 1. A. Representative immunofluorescent staining of stage 15 PiezoRNAi embryo. Scale bar: 20μm, inset scale bar: 10 μm. B. Representative confocal images of stage 15 PiezoRNAi embryo showing the RNAi efficiency. Scale bar: 20μm. C. Quantification of the fluorescence intensity as in B. D. Quantification of epidermal cell density in control and PiezoRNAi stage 15 embryos. E. Representative confocal images of the dorsal epidermis of stage 14 and 15 control and PiezoRNAi embryos showing the completion of dorsal closure. Scale bar: 20μm. F. Quantification of dorsal closure rate in control and PiezoRNAi embryos. G. Confocal images of ventral and dorsal epithelia of the same PiezoRNAi embryo, showing that no changes to sealed dorsal hole (white rectangles) occurs following wounding on the ventral epidermis (asterisk: wound). Scale bar: 20μm.
Figure S2. Loss of Piezo increases localised tissue fluidity and exacerbates wound edge myosin heterogeneity. Related to figure 2. A. Quantification of intercalations two rows from the wound edge. B. Quantification of cortical myosin fluorescence intensity in unchallenged epidermis. C. Quantification of wound edge myosin fluorescence intensity at different percentages of closure. D. Representative images of wound edge myosin fluorescence intensity at different percentages of closure. Scale bar: 20μm. E. Heatmaps of wound edge myosin heterogeneity at different percentages of closure. F. Myosin heterogeneity score at different percentages of wound closure. G. Representative fluorescence plots showing myosin heterogeneity at different percentages of wound closure. Rectangles connect the lowest to the highest values. Control: black; Piezo⁻/⁻: magenta.
**Figure S3. Effects of loss of Piezo on calcium and damage-induced ROS production.** Related to figure 3. A. Confocal images of basal (pre-wounding) and peak (post-wounding) calcium flux across the ventral epithelium of stage 15 embryos expressing the GCaMP3 calcium reporter. Asterisks: wound. Scale bar: 20μm. B. Quantification of basal (pre-wounding) calcium levels. C. Quantification of peak (post-wounding) calcium levels. D. Quantification of normalized peak (post-wounding) calcium levels. E. Normalised Amplex™ UltraRed intensity fluorescence prior to- and post-wounding. F. Confocal images of unwounded (-30 s) and wounded (+240 s) Piezo<sup>−/−</sup>; PMCA<sup>IR</sup> and Piezo<sup>−/−</sup>; Control<sup>IR</sup> embryos expressing an epithelial marker (Nrg, green) and injected with Amplex™ UltraRed (magenta); asterisk: wound.
Figure S4. Wound healing progression upon loss of Piezo. Related to figure 4. A. Piezo<sup>-/-</sup> embryo-to-L1 larva imaged prior to wounding (Pre-wound), immediately after wounding (Post-wound), and at regular 1 hour intervals up to 7 hours post-wounding. The damaged area is indicated by the dashed square and shown in greater details within the insets. A gap in the epidermis (Nrg, green) and a melanotic plug (BF) are highlighted by the dashed white outline. Asterisks: wound. Scale bar: 20μm.