RESEARCH ARTICLE

Figures and figure supplements

Damage-induced basal epithelial cell migration modulates the spatial organization of redox signaling and sensory neuron regeneration

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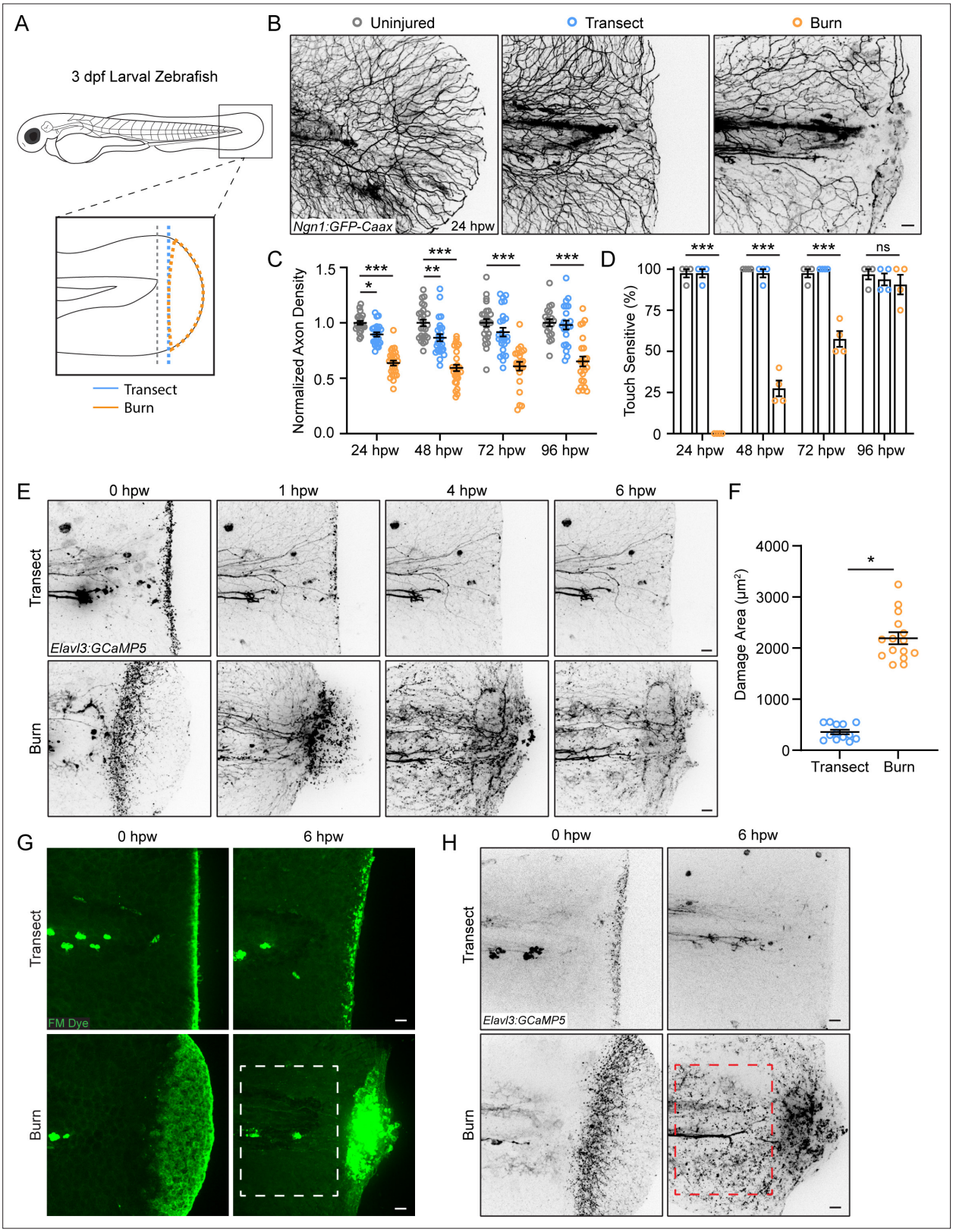

Figure 1. Peripheral sensory axons have impaired regeneration after burn injury. (**A**) Schematic of larval zebrafish injury. Gray dashed line denotes area used to measure axon density to the right of the notochord. (**B**) Confocal max-projected images of sensory axons in uninjured, transected, and burned *Tg(Ngn1:GFP-Caax)* caudal fins 24 hr post-wound (hpw). (**C**) Quantification of axon density for uninjured, transected, and burned larvae in the wound area 24–96 hpw. N>20 larvae per condition from four replicates. (**D**) Quantification of sensory perception for uninjured, transected, and burned

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larvae 24–96 hpw. N>32 larvae per condition from four replicates. (**E**) Confocal time-series images of axonal damage, indicated by calcium-positive punctae (black dots), in *Tg(Elavl3:GCaMP5)* larvae following either transection or burn injury. Each series follows one representative larva over 6 hpw. (**F**) Quantification of axon damage area in transected and burned larvae 6 hpw. N>12 larvae per condition from two replicates. (**G**) Images of larvae either transected or burned in the presence of FM 1–43 dye. White dashed box denotes area of uninjured tissue in which axonal damage appears in H. (**H**) Images show axonal damage following transection or burn injury. Red dashed box corresponds to the tissue region highlighted in G. In all cases, scale bars = 20 μm. *p<0.05, **p<0.01, ***p<0.001, ns = not significant.

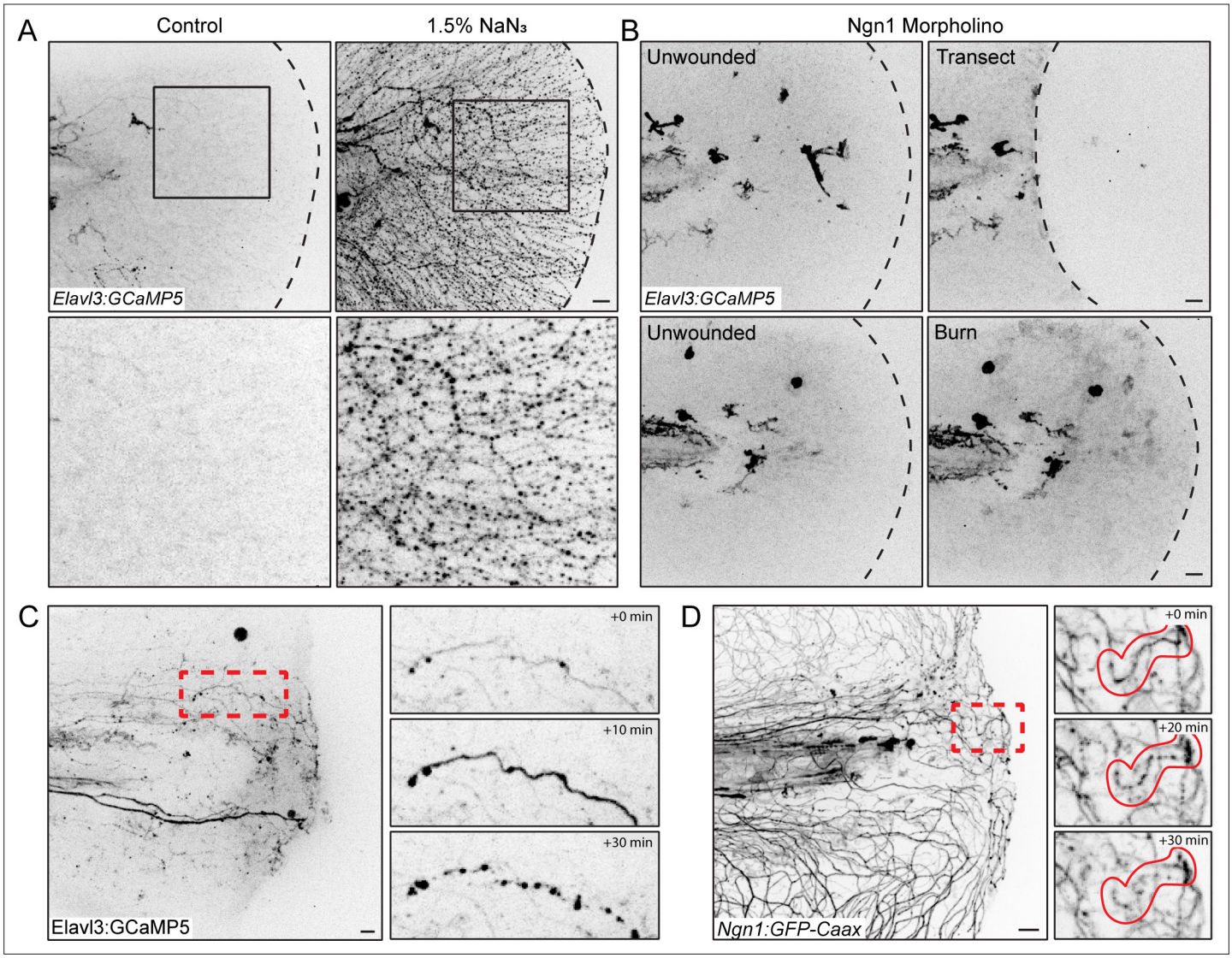

Figure 1—figure supplement 1. Elavl3-GCaMP5 transgenic fish show sensory axon damage. (**A**) Confocal max-projected images of axon damage in *Tg(Elavl3:GCaMP5)* larval zebrafish caudal fins either untreated or 30 min post-treatment with the neurotoxin sodium azide (NaN_3, 1.5% final concentration). Sensory neuron damage is indicated by calcium-positive axon fragments (black dots). Dashed black lines denote the fin edge. Black boxes highlight area of inset, shown below. (**B**) Confocal max-projected images of *Tg(Elavl3:GCaMP5)* larvae injected with Ngn1 morpholino both before and 5 min after the indicated injury. (**C**) Confocal max-projected images of *Tg(Elavl3:GCaMP5)* larvae taken from a time series. Red dashed box denotes inset area shown on right of a sensory axon fragmenting over a period of 30 min. (**D**) Confocal max-projected images of *Tg(Ngn1:GFP-Caax)* larvae taken from a time series. Red dashed box denotes inset area shown on right of a sensory axon fragmenting over a period of 30 min. In all cases, scale bar = 20 μm.

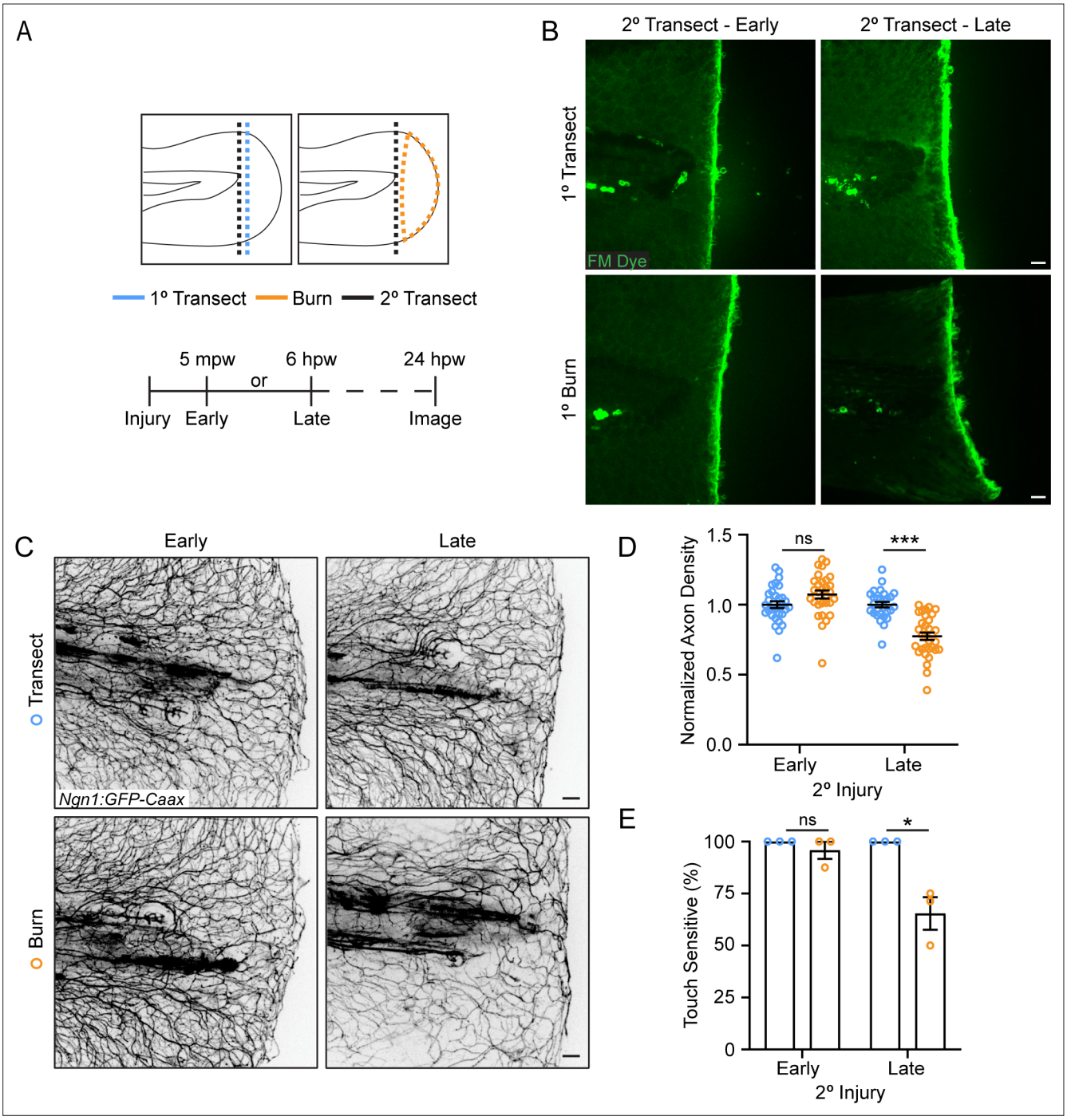

Figure 2. The burn wound microenvironment contributes to impaired sensory axon regeneration. (**A**) Schematic of two-wound experiment design. (**B**) Confocal max-projected images of FM dye staining following secondary transection in the two-wound experiment at 5 min post-wound (mpw) and 6 hr post-wound (hpw). (**C**) Images of sensory axons in larvae subjected to an initial transection or burn injury followed by subsequent transection either early (5 mpw) or late (6 hpw). (**D**) Quantification of axon density in wounded tissue 24 hpw from larvae wounded as in B. N>28 larvae per condition from three replicates. (**E**) Quantification of sensory perception in wounded tissue 24 hpw from larvae wounded as in B. N = 24 larvae each from three replicates. In all cases, scale bars = 20 μm. *p<0.05, ***p<0.001, ns = not significant.

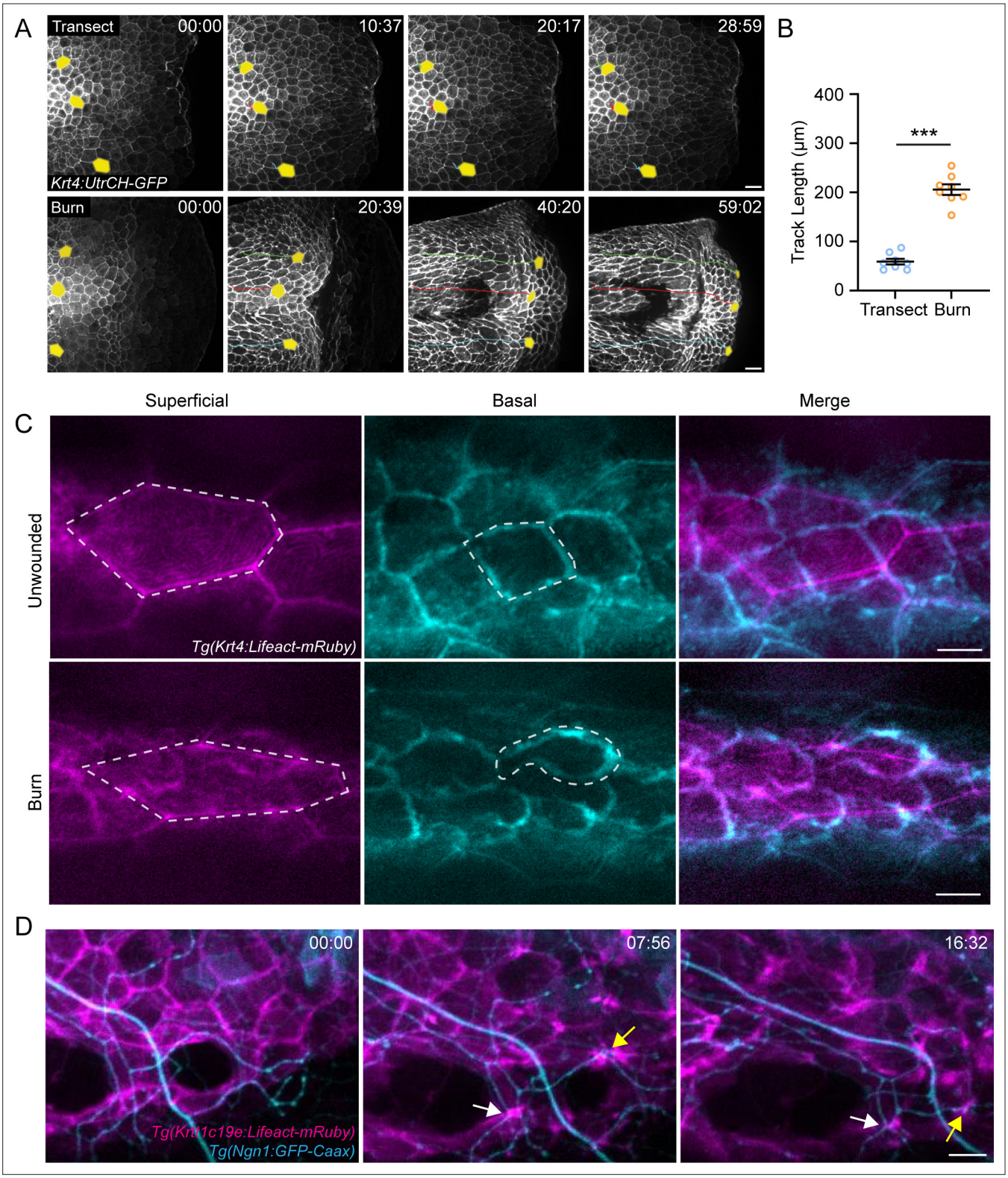

Figure 3. Burn injury induces coordinated keratinocyte and sensory axon movement. (**A**) Confocal max-projected time-series images of *Tg(Krt4:UtrCH-GFP)* larvae after either transection or burn injury. Yellow pseudocolored cells and colored tracks highlight keratinocyte displacement. Scale bar = 20 μm. (**B**) Quantification of keratinocyte movement distance over 1 hr post-wound (hpw). N = 8 larvae each collected from three replicates. (**C**) Confocal max-projected images of superficial and basal keratinocytes in *Tg(Krt4:Lifeact-mRuby)* labeled larvae. Left, superficial keratinocytes. Middle, basal keratinocytes. Right, merge. Superficial and basal cell images were taken from the same z-stack and pseudocolored to match the appropriate cell

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layer. Dashed lines outline one individual keratinocyte. Scale bar = 10 μm. (**D**) Confocal max-projected time-series images of sensory axons and basal keratinocytes in dual-labeled *Tg(Krt4:Lifeact-mRuby); Tg(Ngn1:GFP-Caax)* larvae unwounded or after burn. Arrows highlight coincident movement between keratinocytes and associated sensory axons. Unless otherwise stated, scale bar = 20 μm. ***p<0.001.

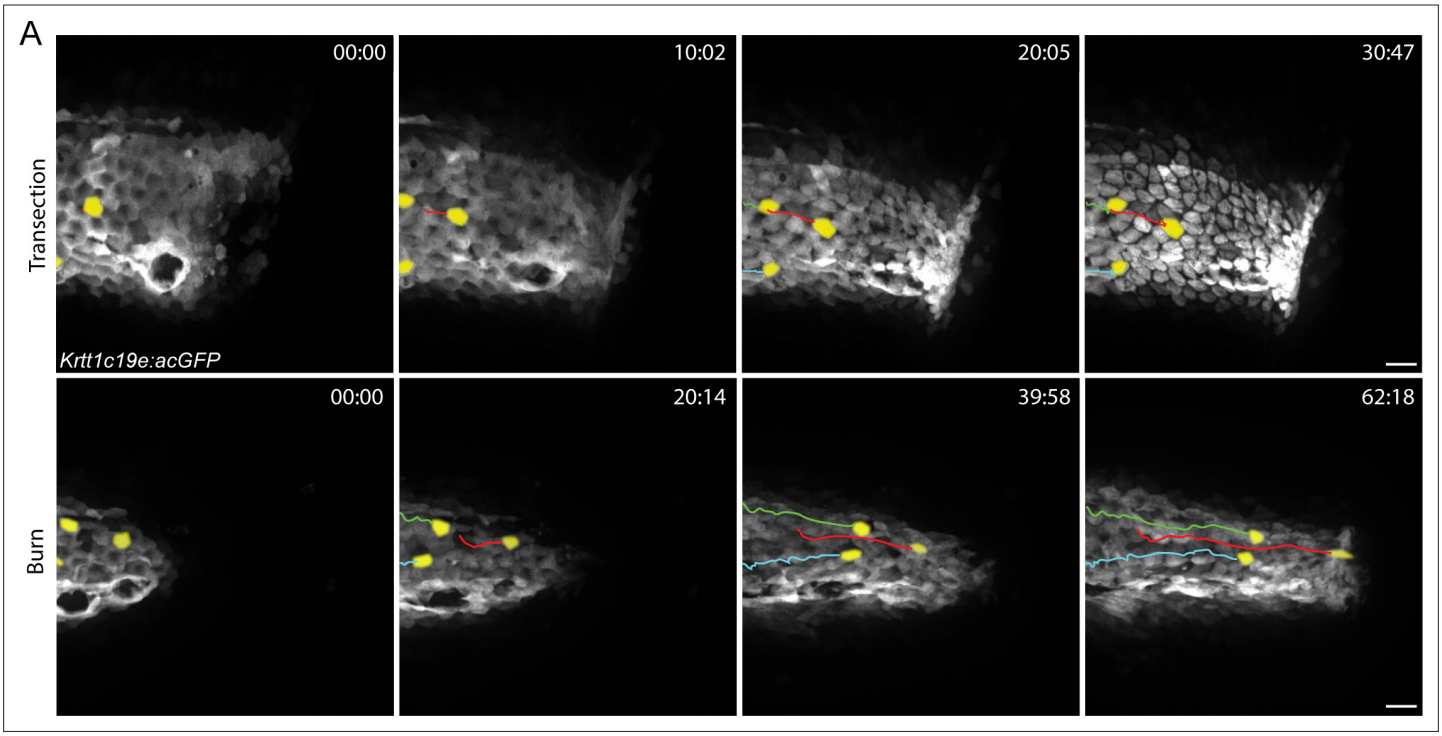

Figure 3—figure supplement 1. Basal keratinocyte migration in response to injury. (**A**) Confocal time series of basal keratinocyte, *Tg(Krtt1c19e:acGFP)*, movement after the indicated injury. Yellow pseudocolored cells highlight keratinocyte displacement. Scale bars = 20 μm.

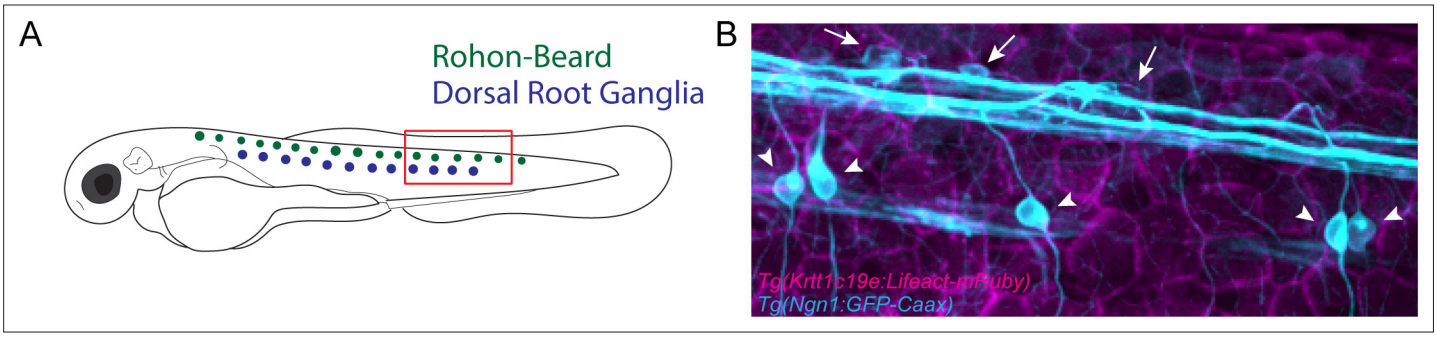

Figure 3—figure supplement 2. Sensory axon cell bodies are not displaced following burn injury. (**A**) Schematic of Rohon-Beard (RB) (green) and dorsal root ganglia (DRG) (blue) soma localization in 3 days post-fertilization (dpf) zebrafish. Red box denotes area in which the image shown in B was acquired. (**B**) Representative confocal max-projected image of intact RB and DRG somas 24 hr post-wound (hpw) in a *Tg(Ngn1:GFP-Caax); Tg(Krtt1c19e:Lifeact-mRuby)* dual-labeled larva. Arrows denote RB somas, while arrowheads indicate DRG somas. Soma position was unchanged compared to pre-wounding.

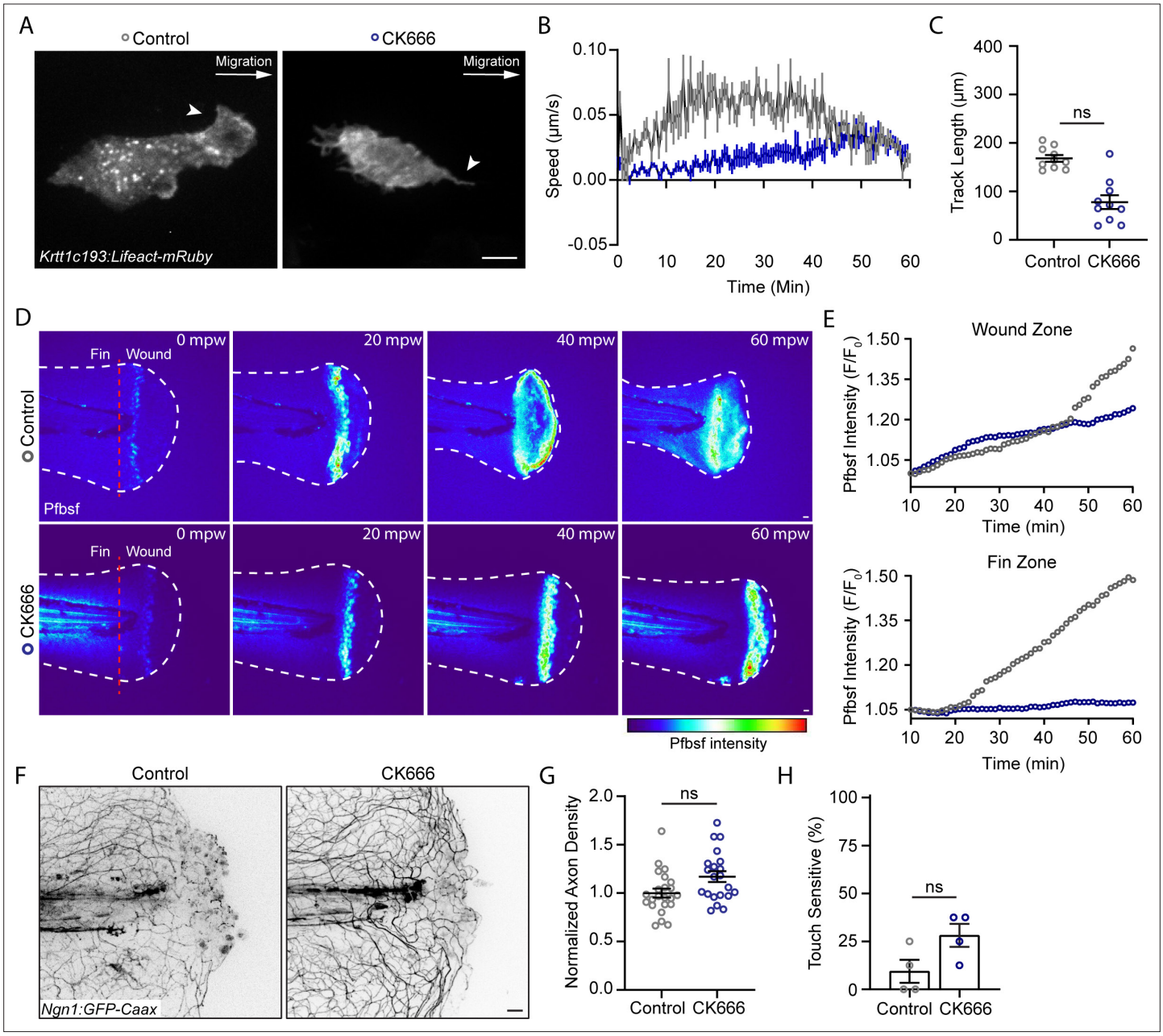

Figure 4. The Arp 2/3 inhibitor CK666 impairs early keratinocyte movement and alters the spatial distribution of reactive oxygen species signaling. (**A**) Confocal max-projected images of control or CK666-treated transiently injected *Tg(Krtt1c19e:Lifeact-mRuby)* larvae. Arrows point to lamellipodia in the control larva, and lack of lamellipodia in the CK666-treated larva. Scale bar = 10 μm. (**B**) Plot of keratinocyte speed over 1 hr post-wound (hpw) as treated in A. N = 10 larvae each collected from three replicates. (**C**) Plot of keratinocyte distance moved over 1 hpw as treated in A. N = 10 larvae each collected from three replicates. (**D**) Confocal sum-projected time-series images of hydrogen peroxide level (pentafluorobenzenesulfonyl fluorescein [Pfbsf] intensity) in 1 larva over 1 hpw in the indicated treatment. (**E**) Quantification of Pfbsf intensity in the wound or fin area of the represented larva after burn injury as treated in D over 1 hpw. N = 1 representative larva per condition. (**F**) Confocal max-projected images of sensory axons 24 hpw in larvae wounded in control medium or CK666. (**G**) Quantification of axon density 24 hpw in larvae treated as in J. N>22 larvae per condition from four replicates. (**H**) Quantification of sensory perception 24 hpw in larvae treated as in J. N = 32 larvae per condition from four replicates. Unless otherwise specified, scale bars = 20 μm. ns = not significant.

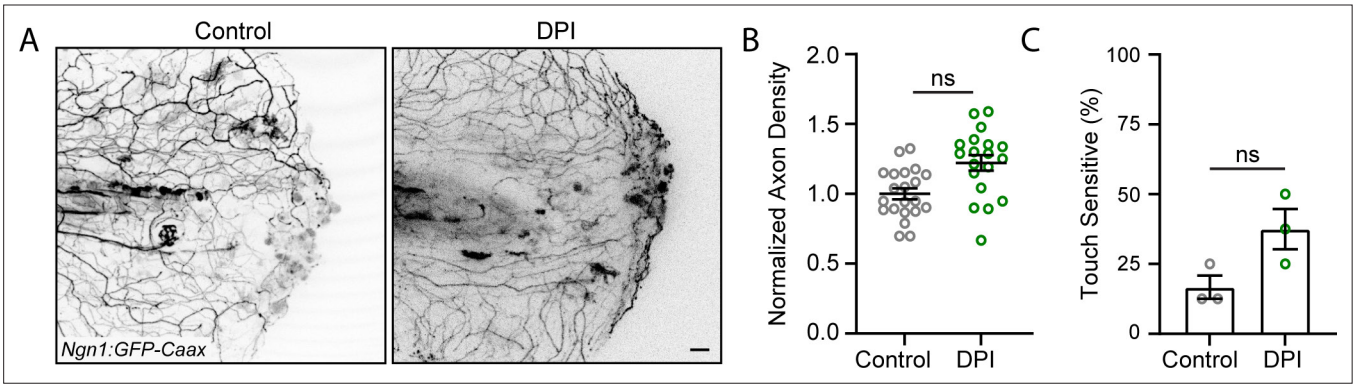

Figure 4—figure supplement 1. Early reactive oxygen species (ROS) inhibition is not sufficient to improve axon regeneration. (**A**) Confocal max-projected images of sensory axons treated with diphenyleneiodonium (DPI). (**B**) Quantification of axon density 24 hr post-wound (hpw). N>19 larvae per condition from three replicates. (**C**) Quantification of sensory perception 24 hpw. N = 24 larvae each from three replicates. Scale bar = 20 μm. ns = not significant.

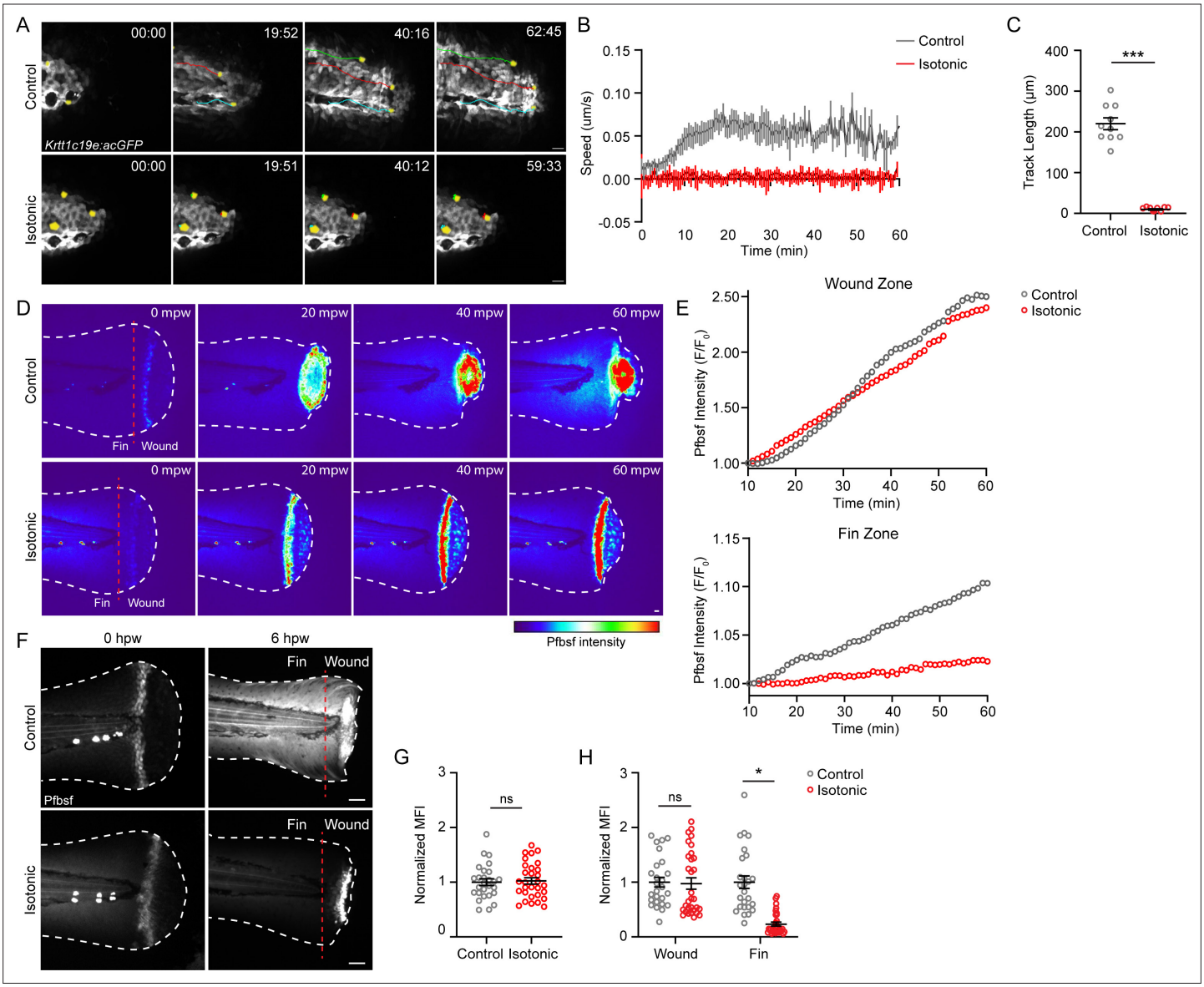

Figure 5. Treatment with isotonic solution inhibits keratinocyte migration and dampens reactive oxygen species (ROS) signaling. (**A**) Confocal time-series images of basal keratinocyte movement in *Tg(Krtt1c19e:acGFP)* larvae over 1 hr post-wound (hpw) after burn injury in the indicated treatment. (**B**) Plot of basal keratinocyte average speed over 1 hpw treated as in A. N = 10 larvae per condition collected from three replicates. (**C**) Distance of keratinocyte movement over 1 hpw treated as in A. N = 10 larvae per condition collected from three replicates. (**D**) Confocal sum-projected, heat-mapped time-series images of hydrogen peroxide level (pentafluorobenzenesulfonyl fluorescein [Pfbsf] intensity) over 1 hpw as treated in A. (**E**) Quantification of Pfbsf intensity in the wound or fin area of the represented larva after burn injury as treated in D over 1 hpw. N = 1 representative larva per condition. (**F**) Confocal sum-projected images of Pfbsf intensity in the fin and wound zone either 0 or 6 hr following burn injury. Dashed red line denotes the boundary between the wound area and distal fin tissue. Scale bar = 50 μm. (**G**) Quantification of mean Pfbsf fluorescence intensity (MFI) immediately (0 hpw) after burn injury normalized to the control condition. N>27 larvae per condition from three replicates. (**H**) Quantification of MFI 6 hpw in the indicated region of the fin normalized to the control condition. N>26 larvae per condition from three replicates. Unless otherwise indicated, scale bars = 20 μM. *p<0.05, ***p<0.001, ns = not significant.

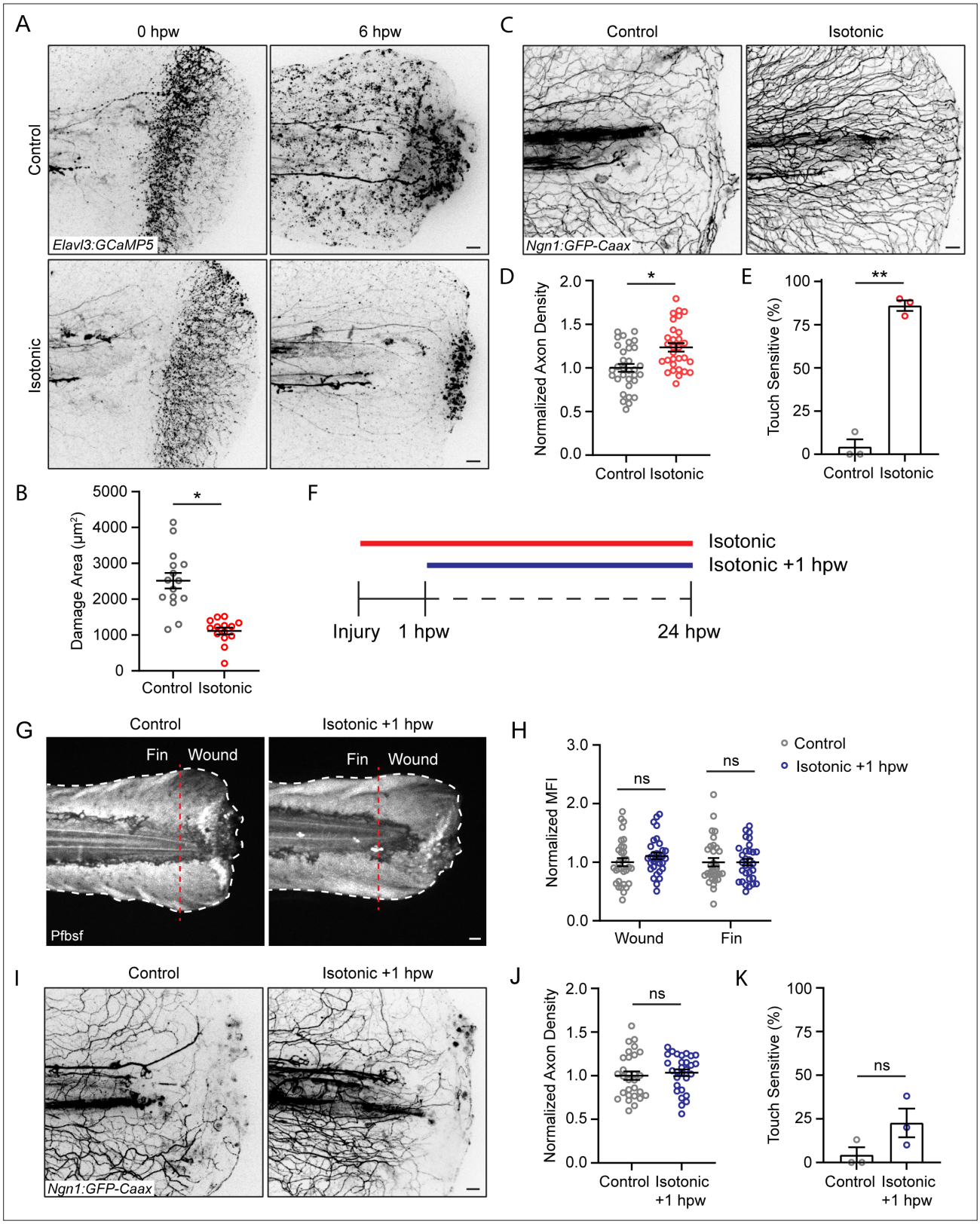

Figure 6. Isotonic treatment improves axon regeneration. (**A**) Confocal max-projected images of axon damage in control or isotonic-treated *Tg(Elavl3:GCaMP5)* larvae 0 or 6 hr post-wound (hpw). (**B**) Quantification of axon damage in control and isotonic-treated burned fins at 6 hpw as treated in A. N = 16 larvae per condition from three replicates. (**C**) Confocal max-projected images of sensory axons in larvae 24 hpw as treated in A. (**D**) Quantification of axon density 24 hpw in larvae treated as depicted in C. N>30 larvae per condition from three replicates. (**E**) Quantification of

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sensory perception 24 hpw in larvae treated as in C. N = 24 larvae each from three replicates. (**F**) Schematic illustrating the different isotonic treatment paradigms that are being compared. (**G**) Confocal sum-projected images of pentafluorobenzenesulfonyl fluorescein [Pfbsf] intensity in control and isotonic +1 hpw treated burned larvae. Dashed red line denotes the boundary between the wound area and distal fin tissue. White dashed line denotes the fin. (**H**) Quantification of mean Pfbsf fluorescence intensity (MFI) 6 hpw in the indicated region of the fin normalized to the control condition. N = 31 larvae per condition from three replicates. (**I**) Confocal max-projected images of sensory axons 24 hpw in burned control or isotonic-treated larvae starting 1 hpw. (**J**) Quantification of axon density 24 hpw in larvae treated as in D. N = 29 larvae per condition from three replicates. (**K**) Quantification of sensory perception 24 hpw in larvae treated as in D. N = 24 larvae per condition from three replicates. Unless otherwise indicated, scale bars = 20 μm. *p<0.05, **p<0.01, ns = not significant.

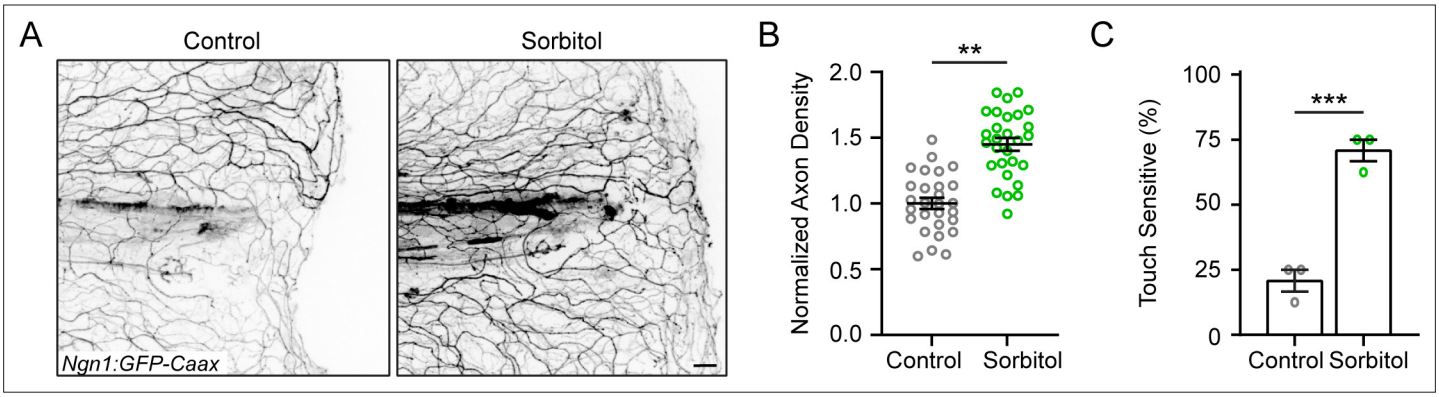
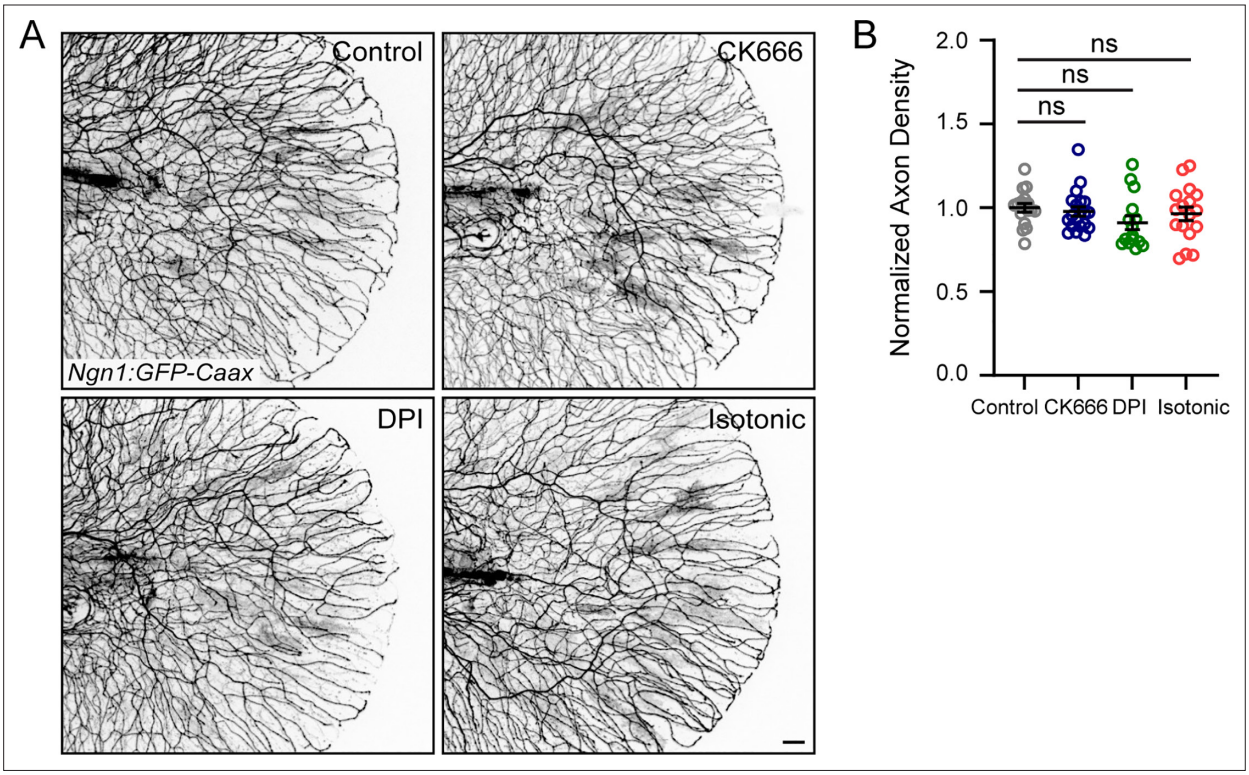


Figure 6—figure supplement 1. Keratinocyte movement after injury and effect of D-Sorbitol on sensory axon regeneration. (**A**) Representative max-projected confocal images of sensory axons in control and isotonic D-Sorbitol-treated larvae 24 hr post-wound (hpw). (**B**) Quantification of axon density in wounded tissue 24 hpw. N = 28 larvae each from four replicates. (**C**) Quantification of sensory perception 24 hpw. N = 24 larvae each collected from four replicates. Scale bar = 20 μm. **p<0.01, ***p<0.001,.



Appendix 1—figure 1. Drug treatments do not affect axon density in unwounded larvae. (**A**) Representative max-projected confocal images of sensory axons in unwounded, drug-treated 4 dpf larvae. (**B**) Quantification of axon density in larvae treated as stated in E. N>15 larvae each from 3 replicates. Scale bar = 20 μm. ns = not significant.