Functional Regeneration of the Sensory Root via Axonal Invasion

Graphical Abstract

Highlights

- Visualization of regenerating nerves in zebrafish model of avulsion-like injuries
- Regenerating DRG sensory axons do not invade back into the spinal cord
- Stabilization of invasion components induces regeneration
- Regenerated axons restore neural circuits and behavior by 1–2 days after injury

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In Brief
Dorsal root ganglion (DRG) sensory axons are unable to regenerate into the spinal cord after injury. Nichols and Smith demonstrate in zebrafish that injured DRG axons do not initiate actin-based invasion components during re-entry into the spinal cord. Pharmacological and cell-autonomous genetic manipulations that promote actin-mediated cell invasion to restore sensory behavior.

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Functional Regeneration of the Sensory Root via Axonal Invasion

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SUMMARY
Regeneration following spinal root avulsion is broadly unsuccessful despite the regenerative capacity of other PNS-located nerves. By combining focal laser lesioning to model root avulsion in zebrafish, time-lapse imaging, and transgenesis, we identify that regenerating DRG neurons fail to recapitulate developmental paradigms of actin-based invasion after injury. We demonstrate that inducing actin reorganization into invasive components via pharmacological and genetic approaches in the regenerating axon can rescue sensory axon spinal cord entry. Cell-autonomous induction of invasion components using constitutively active Src induces DRG axon regeneration, suggesting an intrinsic mechanism can be activated to drive regeneration. Furthermore, analyses of neuronal activity and animal behavior show restoration of sensory circuit activity and behavior upon stimulating axons to re-enter the spinal cord via invasion. Altogether, our data identify induction of invasive components as sufficient for functional sensory root regeneration after injury.

INTRODUCTION
The peripheral nervous system (PNS) can regenerate following injury (Ertürk et al., 2007; Gribble et al., 2018; Rosenberg et al., 2012, 2014). One exception is the dorsal root following avulsion injuries in which the peripheral nerve root is torn from the CNS (Hoeber et al., 2017; Di Maio et al., 2011; Ramón-Cueto and Nieto-Sampedro, 1994). In humans, these injuries occur in adulthood following severe trauma or in neonates at birth. The latter type, obstetrical brachial plexus injury (OBPI), occurs in 1 in 3,000 live human births, leaving patients with permanent sensorimotor defects (Thatte and Mehta, 2011). Across phylogeny, root avulsions do not fully recover because PNS-located sensory axons in the dorsal root ganglion (DRG) cannot re-enter the spinal cord. Attempts at aiding DRG axon re-entry into the CNS have been successful in the laboratory: implantation of stem cells or glia, addition of ectopic growth factors to the dorsal root, inhibition of the glial scar, and peripheral nerve injury (Hellal et al., 2011; Hoeber et al., 2017; Neumann and Woollf, 1999). However, each of these approaches faces important drawbacks for clinical use.

Here, we explore the relationship between regenerating DRG axons following OBPI-like injuries and developmental paradigms that drive pioneer axon dorsal root entry zone (DREZ) entry in larval zebrafish. We show that regenerating axons do not form invasive actin concentrates to re-enter the spinal cord. However, stabilization of invasion components with both pharmacological and cell-autonomous interventions promotes DRG axon spinal entry after avulsion. Promotion of sensory regeneration via cell invasion also rescues animal function at the circuit and behavioral levels. Altogether, our data identify cell invasion as a mechanism of regeneration following neural injury.

RESULTS
Sensory Root Regeneration Fails Because Axons Are Unable to Invade the Spinal Cord

The sensory root does not regenerate following avulsion injuries (Figure 1A; Hoeber et al., 2017; Di Maio et al., 2011; Ramón-Cueto and Nieto-Sampedro, 1994). However, attempted regeneration by DRG axons has not been imaged in totality, limiting our understanding of mechanisms underlying failed regeneration. To provide mechanistic insight into this process, we used a recently developed zebrafish model for avulsion-like injuries (Green et al., 2019). We used focal laser-pulse lesioning (Ablate) to axotomize single DRG axons in the PNS at 3 days post-fertilization (dpf) (Green et al., 2019; Figure 1B). This laser specifically targets select diffraction-limited regions with scalable laser pulse energies to minimize damage to surrounding tissue (Green et al., 2019). A sensory root injury at this zebrafish age corresponds with OBPI cases in human development, namely, the onset of myelination and the expansion of nerve roots (Green et al., 2019).

To visualize actin in the DRG, we expressed Lifeact-GFP in DRG cells using sox10 promoter elements with Tg(sox10:gal4); Tg(uas:lifeact-gfp) animals (Helker et al., 2013; Hines et al., 2015). After performing a single axotomy in each animal, we imaged actin dynamics in regenerating neurons every 5 min for 24 h. Within 1 h after injury, the lesioned axon retracts to the DRG soma (Figure 1C; Video S1). This retraction is reminiscent of injured spinal axons (Bradke et al., 2012; Kerschensteiner et al., 2005; Windle, 1980). The DRG axon then re-extends dorsally toward the DREZ. After contacting the DREZ, the regenerated axon again retracts back to the soma (Figures 1C and 1D; Video S1). One day after injury, the soma lacks a dorsal axon...
while maintaining a peripheral axon. These axon-less cell somas do not undergo apoptosis through 4 days post-injury.

In development, DRG pioneer axons enter the spinal cord by concentrating growth cone actin to form invadopodia (Nichols and Smith, 2019). These invadopodia are required to cross the spinal cord boundary, or glia limitans. A glial scar forms at the glia limitans following avulsion, so we hypothesized that DRG regeneration fails because the regenerating axon does not form invadopodia (Hellal et al., 2011; Hoeber et al., 2017; Mokalled et al., 2016). To test this, we traced Lifeact-GFP intensity in the re-extending growth cone in our videos and observed short but sharp spikes in Lifeact-GFP intensity at the DREZ (Figure 1E). Each regenerating axon initiated multiple (3.2 ± 0.2) actin peaks, but these spikes were transient; that is, they were only present for 26.31 ± 2.68 min (n = 6 DRG). Compared with developmental invasion in which actin concentrates persist for
~40 min, regenerating axons form short-lived actin invasion formations.

To understand the relationship between growth cone and F-actin, we quantified the area and average Lifeact-GFP intensity of the growth cone at each point in its navigation and generated scatterplots (Figures 1F and 1G). In these graphs, each point represents a single time point. A small portion of time points had a small growth cone but a large Lifeact-GFP signal, similar to invasive structures in development (Nichols and Smith, 2019). Comparison of these time points with Figure 1E revealed that these time points were the identified actin concentrates. We next observed a group of time points for which the growth cones were slightly smaller and displayed less Lifeact-GFP. These were the time points temporally adjacent to actin concentrates, which we termed actin transitions. The remaining time points displayed dispersed actin (a larger growth cone area and lower Lifeact-GFP). This analysis indicates that the growth cone dynamically adjusts its actin organization. These cytoskeletal states include the initiation of actin rearrangements consistent with invasion, but these structures fail to stabilize.

Pharmacological Stabilization of Invasion Rescues Axon Entry after Injury

If DRG regeneration fails because axons do not form invasion components, then stabilization of invasion components should promote axonal spinal re-entry. We tested this by treating avulsed animals with the microtubule stabilizer paclitaxel (Taxol), which stabilizes invasion components in DRG pioneer axons and pro-invasive cells (Nichols and Smith, 2019; Quintavalle et al., 2011). We visualized sensory neurons by expressing GFP under the ngn1 promoter, Tg(ngn1:gfp), and the glia limitans by expressing mCherry with ggap promoter elements, Tg(gfap:nsfb-mcherry) (McGraw et al., 2008; Smith et al., 2016). We performed avulsion-like injuries at 3 dpf, immediately treated with Taxol, and time lapse imaged for 24 hours post-injury (hpi). 67% of injured Taxol-treated axons did not retract a second time and instead formed anterior and posterior projections (n = 6 DRG; Figures 1H–1K). To ask whether they re-entered the spinal cord, we digitally rotated these images 90° and detected a GFP+ axonal puncta inside of the mCherry+ glia limitans (Figure S1A). 0% of DMSO-treated control axons re-entered the spinal cord and instead exhibited no dorsal axon (n = 6 DRG; Figures 1H–1K). We considered the possibility that Taxol allows for spinal re-entry by preventing the initial retraction of the axon but ruled this out because our time-lapse videos still demonstrated Taxol-treated axons retracting to the DRG before regenerating dorsally (Figures S1B–S1E). It is also possible that Taxol-mediated regeneration is specific to a 3 dpf injury, not older ages. To test this, we repeated this experiment in 5 dpf animals. In this experiment, 57% of Taxol-treated axons regenerated into the spinal cord, comparable with regeneration in 3 dpf animals. This supports the conclusion that Taxol-mediated regeneration could extend to later ages (n = 7 Taxol, 7 DMSO; Figures 1H, 1I, 1K, and S1F).

Our data demonstrate that the axon will retract toward the DRG after a failed attempt at re-entry. This suggests that there may be a window for Taxol treatment to successfully promote regeneration. To test this, we created avulsion-like injuries at 3 dpf and applied treatment of Taxol at intervals of 4 h after injury. We then imaged these animals at 48 hpi to assay for regeneration. Similar levels of regeneration can be achieved with Taxol treatment starting from 0–8 hpi (n = 5 DRG per treatment per time; Figures 1L and S1G). Rates of successful regeneration begin to steadily decrease starting at 12 hpi treatments and falling to 33% success by 16 hpi (n = 6 DRG per treatment per time). All attempts at regeneration with Taxol treatment after 20 hpi were unsuccessful (n = 6 DRG per treatment per time). These measures correspond with the approximate time of axon retraction in Figure 1D, indicating that the second axonal retraction rapidly decreases the probability of re-entry. Altogether, these data suggest that treatment between 0 and 16 hpi is required for successful Taxol-induced regeneration.

Taxol Stabilizes Invasion Components to Drive Spinal Cord Re-entry following Injury

To test potential mechanisms for Taxol-mediated regeneration, we visualized actin in regenerating axons using Tg(sox10:lifeact-gfp) animals and subjected them to our avulsion model. We considered that Taxol promotes regeneration by initiating invasion components but ruled this out, because Lifeact-GFP peaks initiate indistinguishably in DMSO and Taxol-treated animals (n = 6 DRG per treatment; Figures 2A and 2B; Video S2). When we compared the time after injury that these spikes initiated and the total number of spikes between treatments, we also did not detect differences (Figures S2A and S2B). We next considered that invasion of these regenerating neurons was dependent on stabilization of actin-based invasion components by measuring the duration of Lifeact-GFP peaks (Figure 2F). Actin-based invasion components in DMSO-treated animals formed for 40.59 ± 3.71 min (n = 17 concentrates) compared to Taxol-treated animals that stabilized for 110.7 ± 14.96 min (n = 20 concentrates), consistent with regeneration dependent on invasion stabilization. We also repeated our analysis of growth cone size and Lifeact-GFP intensity. Taxol-treated growth cones navigated with actin concentrates and actin transitions more than DMSO-treated growth cones (p < 0.0001; Figures 2G, 2H, S2C, and S2D). The simplest explanation for these data is that Taxol promotes invasion of regenerating sensory axons via stabilization of actin-based invasion.

Invasion relies on coordination of Src-driven actin invasive structures and matrix metalloproteinases (MMPs) (Clancy et al., 2015; Santiago-Medina et al., 2015; Sedgwick et al., 2015). If Taxol is driving regeneration via stabilization of invasive structures, then inhibition of these other components should abolish this regeneration. To test this, we co-treated avulsed animals with GM6001 and Taxol, as well as SU6656 and Taxol. GM6001 is a pan-inhibitor of MMPs, and SU6656 inhibits Src family kinases. After injury, these axons retracted, re-extended, and then retracted a second time (n = 6 for GM6001, 4 for SU6656; Figures 2C and 2D; Video S3). As such, none of the co-treated axons re-entered the spinal cord (Figure 2E). Like DMSO-treated axons, cotreated axons did not form robust actin concentrates but rather transient actin accumulations (n = 20 GM6001 concentrates, 14 SU6656 concentrates; p < 0.0001, one-way ANOVA; Figure 2F). We also compared the number of actin accumulations (p = 0.8341, one-way ANOVA) and the
time of initiation (p = 0.0892, one-way ANOVA) but did not observe differences between treatments (Figures S2A and S2B). These observations were supported by measurements of the growth cone size and Lifeact-GFP intensity at individual time points (p < 0.0001, two-way ANOVA; Figures 2H, S2D, and S2E). Altogether, these data support the hypothesis that Taxol stabilizes invasive components in regenerating axons, whereas inhibition of molecules necessary for invasion counteracts Taxol’s pro-regenerative effects.

To dissect the mechanism of Taxol-mediated regeneration, we considered the possibility that Taxol could have dynamic effects on the growth cone independent of actin organizations. To do this, we measured the velocity of the growth cone during actin concentrates identified earlier. In DMSO axons, the axon navigated at $-0.0758 \pm 0.103 \text{ mm/min}$, indicating slight retreats from the DREZ. In Taxol-treated axons, the opposite occurred with the axons extending at $0.142 \pm 0.44 \text{ mm/min}$ (p = 0.0037, Tukey’s honest significant difference [HSD]; Figure S2F). This suggests that Taxol confers a pro-growth state. However, the same effects on axon velocity were observed in cotreatment axons (GM6001, 0.0226 ± 0.037 mm/min; SU6656, 0.066 ± 0.051 mm/min) axons, despite lack of re-entry (p > 0.3213, Tukey’s HSD; Figure S2F). The simplest explanation for these data is that permissive growth cone extension by Taxol treatment is not sufficient for regeneration.

**Stabilization of Invasion Machinery Cell-Autonomously Promotes DRG Regeneration**

Previous studies have identified Taxol as a negative regulator of glial scar formation after dorsal hemisection (Hellal et al., 2011). This raises the possibility that induction of invasion components in regenerating axons could be secondary to glial scar inhibition. We sought to test this by inducing invasion cell-autonomously. Src activation has been shown to enhance cell invasion by
stabilizing actin-rich invasion (Murphy and Courtneidge, 2011), so we hypothesized that cell-specific expression of constitutively active Src (CA-Src) in DRG cells would drive invasion in regenerating growth cones. Replacing an inhibitory tyrosine phosphorylation site with a phenylalanine (Y528F) in Src encodes CA-Src (Seiler et al., 2012). We expressed CA-Src fused to mCherry in DRG cells to drive expression under the sox10 promoter. We also visualized Lifeact-GFP in DRG cells, created avulsion-like injuries, and imaged actin (n = 6 CA-Src, 6 Src; Figures 3A and 3B; Video S4). Src-mCherry overexpression was used as a control. CA-Src-expressing cells had actin peaks that stabilized for 78.95 ± 9.11 min (n = 19 concentrates). In controls, Lifeact-GFP peaks were present for 33.94 ± 2.86 min (n = 17 concentrates; Figure 3D). However, we observed no difference in number of peaks or time of peak initiation (Figures S3A and S3B). Likewise, CA-Src-expressing axons navigated more with actin concentrates and actin transitions compared with axons expressing Src (Figures 3F, S3D, and S3E). These data indicate that expression of CA-Src in regenerating axons is sufficient to restore actin-based invasion components in regenerating axons. To assay for re-entry, we stained 24 hpi animals for GFAP, a marker for the glial limitans. Using surface intensity plots to mark the edge of the glial limitans and the Lifeact-GFP + axons, we detected that 71.4% of axons recrossed the glial limitans (Figures 3E and S3C). This is in contrast to Src-expressing axons, in which 0% re-entered the spinal cord (Figures 3A and 3E). To test the potential of CA-Src to promote regeneration in additional cell types, we expressed CA-Src under a gfap promoter and assayed for axonal regeneration. In these animals, 0% of axons regenerated into the spinal cord at 24 hpi (n = 7 animals; Figures 3E and S3F).

Our data thus far indicate two distinct mechanisms can promote actin-based invasion axonal regeneration: microtubule stabilization and Src activation. To test potential overlap in these pathways, we treated CA-Src axons with Taxol and assayed regeneration (Figure 3C). We detected no difference in actin dynamics compared with CA-Src expression alone (n = 5 DRG; Figures 3D, 3F, S3A, S3B, and S3G). However, axon entry

Figure 3. Induction of Invasion Components in DRG Neurons Promotes Regeneration

(A–C) Z-projection time-lapse images of avulsed DRG in Tg(sox10:gal4); Tg(uas:lifeact-gfp) animals expressing uas:src-mcherry (A), uas:CA-src-mcherry (B), or uas:CA-src-mcherry and treated with Taxol (C). Graphs show Lifeact-GFP intensity tracings throughout regeneration. The green arrow denotes a stable invasive structure. Red arrows and brackets represent peaks of Lifeact-GFP.

(D) Actin concentrate duration in Src DRG (n = 6), CA-Src DRG (n = 6), and CA-Src+Taxol DRG (n = 5).

(E) Regeneration outcomes in DRG expressing sox10:Src, sox10:CA-Src, or sox10:CA-Src+Taxol or radial glial expressing gfap:CA-Src.

(F) Percentage of time points the regenerating growth cone navigated using each actin organization. Tukey’s HSD was used in (D) and two-way ANOVA was used in (F). Scale bar, 10 μm.
measurements revealed that 100% of Taxol-treated CA-Src axons re-entered the spinal cord (n = 5 DRG; Figures 3E and S3H). These data suggest that Taxol and CA-Src redundantly promote invasive actin formations, a common determinant of DRG regeneration.

**DRG Axon Regeneration after Injury Restores Sensory Circuitry and Behavior**

To first determine whether invasion-mediated regeneration restores sensory function, we revisited our videos of regeneration with Taxol. All regenerated neurons formed anterior and posterior projections that traveled to their proper dorsal location by 24 hpi (Figure S4A). Furthermore, we tested whether spinal synaptic connections are restored following regeneration. To do this, we expressed GFP-tagged Synaptophysin (Syn-GFP) in DRG neurons in Tg(sox10:syn-gfp) animals, created an avulsion-like injury, treated with Taxol, and time lapse imaged. Soon after re-entry, Syn-GFP puncta were deposited along the regenerating axon. By 7 h post-entry, the synaptic puncta topographically resembled synapses preinjury (Figure 4A). These data indicate that regenerating sensory axons could still rapidly synapse with spinal cord neurons.

To test the function of the sensory circuit, we assayed for regeneration, activation of DRG and spinal neurons, and sensory-mediated behavior in the same animals (Figure 4B). To visualize regeneration, we used Tg(ngn1:gfp) animals and created eight consecutive avulsion-like injuries at 3 dpf. Avulsed animals were treated with Taxol or DMSO to dissect the effects of failed regeneration.
regeneration on behavior and neuronal activity. Non-avulsed animals were also used as controls. As a behavioral assay, we exposed animals to 4°C, which causes firing of DRG and spinal neurons during a hypothermic shivering behavior (Fosque et al., 2015). We gauged neuronal activation using MAP mapping at 24 hpi to visualize phosphorylated Erk (pErk) (Randlett et al., 2019).

We first measured regeneration and pErk levels in each of treatment group both ipsilateral and contralateral to the injuries. Measurements of pErk intensity of the spinal cord were elevated in uninjured animals, consistent with circuit activity. Intensity of pErk in Taxol-treated animals was also elevated compared with DMSO-treated animals but less than uninjured animals, consistent with a partially functioning sensory circuit (n = 8 animals per treatment; Figures 4C and 4D). We next compared the number of regenerated axons to the pErk intensity in spinal circuitry. Our data identify a strong positive correlation between the number of regenerated DRG and the number of spinal pErk ($r^2 = 0.6665$, $p = 0.0014$, n = 8 animals; Figure 4E).

We considered the possibility that differences in pErk intensities could result from the injuries. To test this, we repeated the experiment and assayed for pErk levels in each treatment with exposure to 23°C. We did not detect differences in spinal pErk between treatments following exposure to 23°C (n = 5 animals per treatment; Figures S4B and S4C). Collectively, these data are consistent with the conclusion that regeneration of the sensory root partially restores circuit function.

We next measured behavioral responses to 4°C in the same animals to gauge sensory-mediated behavior. To quantify behavioral response, we measured the number of hypothermic shivers and the percentage of time spent shivering. These measurements were then used to classify behavioral responses into four categories: typical (3+ shivers and greater than 12% shivering), stunted (1–3 shivers but 7%–12%, moderate (1–3 shivers and 1%–7%), or absent (no shivering). All DMSO-treated animals displayed absent behavioral response, whereas 60% of unlesioned animals displayed typical behavior. Taxol-treated animals displayed 37.5% moderate responses. A smaller portion exhibited stunted behavior (n = 8 animals per treatment; Figures 4F and 4D). Partial recovery is consistent when comparing the number of shivers and the percentage of time spent shivering individually (Figures 4H and 4I). Taxol-treated animals displayed partial recovery, but not as robust as unlesioned animals. We observed a positive correlation between percentage of time shaking and the number of regenerated axons in Taxol-treated animals ($r^2 = 0.6463$, $p = 0.0162$; Figure 4J). Similarly, pErk intensity in spinal circuitry was strongly correlated with shivering ($r^2 = 0.9196$, $p = 0.0002$; Figure 4K). Collectively, these data support the hypothesis that regeneration partially rescues sensory circuits and behavior by 24 hpi.

We next considered the possibility that recovery of sensory circuitry could continue past 24 hpi. To test this, we repeated the preceding experiment and assayed for behavior at 48 hpi. These measurements revealed similar phenotypes between unlesioned and Taxol-treated animals, indicating enhanced recovery at 48 hpi (n = 5 animals per treatment; Figures 4G and S4D). In contrast, behavioral recovery in DMSO-treated animals was unilateral and exaggerated to the uninjured side of the animal (Figure S4D). Nonetheless, the level of recovery of DMSO-treated animals remained less than that of Taxol-treated animals and non-injured animals. When we quantified behavioral dynamics, we did not detect differences in the number of shivers (p = 0.8031, one-way ANOVA) or the percentage of time shaking (p = 0.0783, one-way ANOVA) between unlesioned and Taxol-treated animals (Figures S4E and S4F). These data support the conclusion that invasion machinery can regenerate DRG axons and restore sensory circuitry and behavior.

**DISCUSSION**

Many have proposed that the homeostatic state of mature neurons results in a fundamentally different intracellular milieu from developing neurons preventing regeneration (He and Jin, 2016; Di Maio et al., 2011; Perry et al., 2018). However, this explanation is likely insufficient. Our data suggest that invasive machinery is present in re-extending DRG axons, but these components cannot coordinate without pharmacological or molecular aid to allow the axon to extend past inhibitory cues at the injury site. Although our data indicate that avulsion injuries are an exception, zebrafish display enhanced regenerative capabilities, including spinal cord regeneration. In larval zebrafish, after complete spinal transection, functional recovery can be detected by 3 dpi, with complete recovery by 9 dpi (Briona and Dorsky, 2014). Alternatively, our data demonstrate behavioral recovery within 1–2 dpi in larval zebrafish after 8 consecutive avulsion-like injuries, a short timescale compared with other types of zebrafish regeneration.

Taxol has previously been identified as an enhancer of neuronal regeneration (Erturk et al., 2007; Hellal et al., 2011; Hsu et al., 2017; Sengottuvel et al., 2011). Previous studies have identified it as a negative regulator of glial scar extracellular matrix (ECM) deposition following dorsal hemisection (Hellal et al., 2011). The corresponding decrease in ECM also correlated with axon regeneration, but these studies did not interrogate potential effects of Taxol on axonal invasion machinery. In the study above, cell-autonomous activation of invasion through expression of CA-Src is sufficient for sensory axon regeneration.

Mechanistically, these data also present a paradox: Taxol, a microtubule stabilizer, produces stabilized actin invasion. A molecular cascade connecting stable microtubules and actin nucleation could be responsible, because previous studies have shown a correlation between microtubule stability and Arp2/3 activity via HDAC6 and cortactin, a regulator of invadopodia (Shi et al., 2019). Alternatively, the microtubule plus-end protein EB1 could form a complex with actin regulators Src and Cortactin (Biosse Duplan et al., 2014). Altogether, the data presented here suggest an additional avenue for axon regeneration across restrictive boundaries by promoting cell invasion machinery to overcome physical limits to axonal regrowth.

**STAR METHODS**

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Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2019.12.008.

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AUTHOR CONTRIBUTIONS

E.L.N. performed the experiments with advice from C.J.S., designed the experiments and wrote the paper with input from C.J.S., and conceived the idea to use Taxol for regeneration. C.J.S. funded the project.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Biosse Duplan, M., Zalli, D., Stephens, S., Zenger, S., Neff, L., Oelkers, J.M., Lai, F.P.L., Horne, W., Rottner, K., and Baron, R. (2014). Microtubule dynamic instability controls podosome patterning in osteoclasts through EB1, cortactin, and Src: Mol. Cell. Biol. 34, 16–29.

Bradke, F., Fawcett, J.W., and Spira, M.E. (2012). Assembly of a new growth cone after axotomy: the precursor to axon regeneration. Nat. Rev. Neurosci. 13, 183–193.

Brida, L.K., and Dorsky, R.I. (2014). Radial glial progenitors repair the zebrafish spinal cord following transection. Exp. Neuro. 256, 81–92.

Clancy, J.W., Sedgwick, A., Rosse, C., Muralidharan-Chari, V., Rapsos, G., Method, M., Chavrier, P., and D’Souza-Schorey, C. (2015). Regulated delivery of molecular cargo to invasive tumor-derived microvesicles. Nat. Commun. 6, 6919.

Di Maio, A., Skuba, A., Himes, B.T., Bhagat, S.L., Hyun, J.K., Tessler, A., Bishop, D., and Son, Y.J. (2011). In Vivo Imaging of Dorsal Root Regeneration: Rapid Immobilization and Presynaptic Differentiation at the CNS/PNS Border. J. Neurosci 31, 4569–4582.

Don, E.K., Formella, I., Badrock, A.P., Hall, T.E., Morsch, M., Hortie, E., Hogan, A., Chow, S., Gwee, S.S.L., Stoddart, J.J., et al. (2017). A Tol2 Gateway- Compatible Toolbox for the Study of the Nervous System and Neurodegenerative Disease. Zebrafish 14, 69–72.

Ertürk, A., Helal, F., Enes, J., and Bradke, F. (2007). Disorganized microtubules underlie the formation of retraction bulbs and the failure of axonal regeneration. J. Neurosci. 27, 9169–9180.

Fosque, B.F., Sun, Y., Dana, H., Yang, C.-T., Ohyama, T., Tadross, M.R., Patel, R., Zlatic, M., Kim, D.S., Ahrens, M.B., et al. (2015). Neural circuits. Labeling of active neural circuits in vivo with designed calcium integrators. Science 347, 755–760.

Green, L.A., Nebiolo, J.C., and Smith, C.J. (2019). Microglia exit the CNS in spinal root avulsion. PLoS Biol. 17, e3000159.

Gribble, K.D., Walker, L.J., Saint-Amant, L., Kuwada, J.Y., and Granato, M. (2018). The synaptic receptor Lp4 promotes peripheral nerve regeneration. Nat. Commun. 9, 2389.

He, Z., and Jin, Y. (2016). Intrinsic Control of Axon Regeneration. Neuron 90, 437–451.

Heap, L.A., Goh, C.C., Kassahn, K.S., and Scott, E.K. (2013). Cerebellar Output in Zebrafish: An Analysis of Spatial Pattern and Topography in Eurydendroid Cell Projections. Front. Neural Circuits 7, 53.

Heier, C.S.M., Schuermann, A., Karpanen, T., Zeuschner, D., Belting, H.-G., Affolter, M., Schulte-Merker, S., and Herzog, W. (2013). The zebrafish common cardinal veins develop by a novel mechanism: lumen ensheathment. Development 140, 2776–2786.

Helial, F., Hurtado, A., Ruschel, J., Flynn, K.C., Laskowski, C.J., Umlauf, M., Kaptein, L.C., Striks, D., Lemmon, V., Bixby, J., et al. (2011). Microtubule stabilization reduces scarring and causes axon regeneration after spinal cord injury. Science 331, 928–931.

Hines, J.H., Ravaneili, A.M., Schwindt, R., Scott, E.K., and Appel, B. (2015). Neuronal activity biases axon selection for myelination in vivo. Nat. Neurosci. 18, 683–689.

Hoeber, J., König, N., Trolle, C., Leholm, E., Zhou, C., Panikratova, S., Åkeson, E., Fredriksson, R., Aldskogius, H., and Kozlova, E.N. (2017). A Combinatorial Approach to Induce Sensory Axon Regeneration into the Dorsal Root Avulsed Spinal Cord. Stem Cells Dev. 26, 1065–1077.

Hsu, S.-T., Yao, C.-H., Hsu, Y.-M., Lin, J.-H., Chen, Y.-H., and Chen, Y.-S. (2017). Effects of Taxol on Regeneration in a Rat Sciatic Nerve Transection Model. Sci. Rep. 7, 42280.

Johnson, K., Barragan, J., Bashiruddin, S., Smith, C.J., Tyrell, C., Parsons, M.J., Doris, R., Kucenas, S., Downes, G.B., Velez, C.M., et al. (2016). Gfap-positive radial glial cells are an essential progenitor population for later-born neurons and glia in the zebrafish spinal cord. Glia 64, 1170–1189.

Kerschensteiner, M., Schwab, M.E., Lichtman, J.W., and Misgelid, T. (2005). In vivo imaging of axonal degeneration and regeneration in the injured spinal cord. Nat. Med. 11, 572–577.

Kwan, K.M., Fujimoto, E., Grabher, C., Mangum, B.D., Hardy, M.E., Campbell, D.S., Parant, J.M., Yost, H.J., Kanki, J.P., and Chien, C.-B. (2007). The Tol2kit: a multisite gateway-based construction kit for Tol2 transposon transgenesis constructs. Dev. Dyn. 236, 3088–3099.

McGraw, H.F., Neciporuk, A., and Raible, D.W. (2008). Zebrafish dorsal root ganglia neural precursor cells adopt a glial fate in the absence of neurogenin1. J. Neurosci. 28, 12558–12569.

Mokalled, M.H., Patra, C., Dickson, A.L., Endo, T., Stainer, D.Y.R., and Poss, K.D. (2016). Injury-induced ctgfa directs glial bridging and spinal cord regeneration in zebrafish. Science 354, 630–634.

Murphy, D.A., and Courtneidge, S.A. (2011). The ‘ins’ and ‘outs’ of podosomes and invadopodia: characteristics, formation and function. Nat. Rev. Mol. Cell Biol. 12, 413–426.

Neumann, S., and Wollf, C.J. (1999). Regeneration of dorsal column fibers into and beyond the lesion site following adult spinal cord injury. Neuron 23, 83–91.
Nichols, E.L., and Smith, C.J. (2019). Pioneer axons employ Cajal’s battering ram to enter the spinal cord. Nat. Commun. 10, 562.

Perry, R.B., Hezroni, H., Goldrich, M.J., and Ullitsky, I. (2018). Regulation of Neuroregeneration by Long Noncoding RNAs. Mol. Cell 72, 553–567.

Quintavalle, M., Elia, L., Price, J.H., Heynen-Genel, S., and Courtneidge, S.A. (2011). A cell-based high-content screening assay reveals activators and inhibitors of cancer cell invasion. Sci. Signal. 4, ra49.

Ramón-Cueto, A., and Nieto-Sampedro, M. (1994). Regeneration into the spinal cord of transected dorsal root axons is promoted by ensheathing glia transplants. Exp. Neurol. 127, 232–244.

Randlett, O., Wee, C.L., Naumann, E.A., Nnaemeka, O., Schoppik, D., Fitzgerald, J.E., Portugues, R., Lacoste, A.M.B., Riegler, C., Engert, F., and Schier, A.F. (2015). Whole-brain activity mapping onto a zebrafish brain atlas. Nat. Methods 12, 1039–1046.

Rosenberg, A.F., Wolman, M.A., Franzini-Armstrong, C., and Granato, M. (2012). In vivo nerve-macrophage interactions following peripheral nerve injury. J. Neurosci. 32, 3898–3909.

Rosenberg, A.F., Isaacman-Beck, J., Franzini-Armstrong, C., and Granato, M. (2014). Schwann cells and deleted in colorectal carcinoma direct regenerating motor axons towards their original path. J. Neurosci. 34, 14688–14681.

Santiago-Medina, M., Gregus, K.A., Nichol, R.H., O’Toole, S.M., and Gomez, T.M. (2015). Regulation of ECM degradation and axon guidance by growth cone invadosomes. Development 142, 486–496.

Sedgwick, A.E., Clancy, J.W., Olivia Balmert, M., and D’Souza-Schorey, C. (2015). Extracellular microvesicles and invadopodia mediate non-overlapping modes of tumor cell invasion. Sci. Rep. 5, 14748.

Seiler, C., Davuluri, G., Abrams, J., Byfield, F.J., Janney, P.A., and Pack, M. (2012). Smooth muscle tension induces invasive remodeling of the zebrafish intestine. PLoS Biol. 10, e1001386.

Sengottuvel, V., Leibinger, M., Pfreimer, M., Andreadaki, A., and Fischer, D. (2011). Taxol facilitates axon regeneration in the mature CNS. J. Neurosci. 31, 2688–2699.

Shi, P., Wang, Y., Huang, Y., Zhang, C., Li, Y., Liu, Y., Li, T., Wang, W., Liang, X., and Wu, C. (2019). Arp2/3-branched actin regulates microtubule acetylation levels and affects mitochondrial distribution. J. Cell Sci. 132, jcs226506.

Smith, C.J., Johnson, K., Welsh, T.G., Barresi, M.J.F., and Kucenas, S. (2016). Radial glia inhibit peripheral glial infiltration into the spinal cord at motor exit point transition zones. Glia 64, 1138–1153.

Thatte, M.R., and Mehta, R. (2011). Obstetric brachial plexus injury. Indian J. Plast. Surg. 44, 380–389.

Windle, W.F. (1980). Inhibition of regeneration of severed axons in the spinal cord. Exp. Neurol. 69, 209–211.
## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Zrf-1 (anti-GFAP, mouse monoclonal) | ZIRC | RRID: AB_10013806 |
| Phospho-p44/42 MAPK (anti-ERK1/2 Thr202/204, rabbit monoclonal) | Cell Signaling | Cat #: 9101S; RRID: AB_331772 |
| Alexa Fluor 647 goat anti-mouse | Thermo Fisher | Cat #: A-21235; RRID: AB_2535806 |
| Alexa Fluor 647 goat anti-rabbit | Thermo Fisher | Cat #: A-20991; RRID: AB_2535814 |
| **Bacterial and Virus Strains** |        |            |
| Mix & Go Competent Cells | Zymo Research | Cat #: T3007 |
| **Chemicals, Peptides, and Recombinant Proteins** |        |            |
| Paclitaxel | Acros | Cat #: 328420050; CAS: 33069-62-4 |
| GM6001 | Santa Cruz Biotechnology | Cat #: sc-203979; CAS: 142880-36-2 |
| SU66566 | Santa Cruz Biotechnology | Cat #: sc-203286; CAS: 330161-87-0 |
| LR Clonase II Plus | Thermo Fisher | Cat #: 12538120 |
| **Experimental Models: Organisms/Strains** |        |            |
| AB | ZIRC | Wildtype zebrafish strain |
| Zebrafish: Tg(ngn1:gfp) | McGraw et al., 2008 | ZFIN: ZDB-ALT-090806-1 |
| Zebrafish: Tg(sox10:gal4) | Hines et al., 2015 | ZFIN: ZDB-FISH-150901-5454 |
| Zebrafish: Tg(uas:lifeact-gfp) | Helker et al., 2013 | ZFIN: ZDB-FISH-150901-1674 |
| Zebrafish: Tg(gfap:nsfb-mcherry) | Johnson et al., 2016 | ZFIN: ZDB-ALT-1606630-2 |
| Zebrafish: Tg(uas:syn-gfp) | Heap et al., 2013 | ZFIN: ZBD-ALT-130702-2 |
| **Recombinant DNA** |        |            |
| pS5-E-UAS | Kwan et al., 2007 | http://tol2kit.genetics.utah.edu/index.php/Main_Page |
| pS5-gfap | Don et al., 2017 |            |
| pME-Src | Seiler et al., 2012 |            |
| pME-CA-Src | Seiler et al., 2012 |            |
| p3E-mCherry-pA | Kwan et al., 2007 | http://tol2kit.genetics.utah.edu/index.php/Main_Page |
| pDestTol2CG2 | Kwan et al., 2007 | http://tol2kit.genetics.utah.edu/index.php/Main_Page |
| psELN05 (pTo2-uas:CA-Src-mCherry, cmc12:gf) | This paper | N/A |
| psELN06 (pTo2-uas:Src-mCherry, cmc12:gf) | This paper | N/A |
| psELN10 (pTo2-gfap:CA-Src-mCherry, cmc12:gf) | This paper | N/A |
| **Software and Algorithms** |        |            |
| ImageJ | NIH | RRID: SCR_003070; https://imagej.nih.gov/ij/ |
| MTrackJ | Image Science | https://imagescience.org/meijering/software/mtrackj/ |
| Slidebook | 3i | RRID: SCR_014300; https://www.intelligent-imaging.com/slidebook |
| Prism | GraphPad | RRID: SCR_002798; https://www.graphpad.com/ |

## LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Cody J. Smith (csmith67@nd.edu). All materials generated in this study are available upon request.
**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Zebrafish**
The University of Notre Dame Institutional Animal Care and Use Committee approved all animal studies. Zebrafish used in this study were: Tg(sox10:gal4) (Hines et al., 2015); Tg(uas:lifeact-gfp) (Helker et al., 2013), Tg(ngn1:gfp) (McGraw et al., 2008), and Tg(gfap:nsfb-mcherry) (Smith et al., 2016). Stable, transgenic lines were used in all experiments. Embryos were produced by pairwise matings and raised in darkness in egg water at 28 °C. Zebrafish from 2-5 dpf were used for experiments. Healthy immune status zebrafish were utilized for experiments. Experiments utilized zebrafish embryos before sex is determined. Animals were subjected to one avulsion per animal with the exception of the behavioral experiments in Figure 4. All embryos were drug/test naive before the desired experiment.

**METHOD DETAILS**

**In Vivo Imaging**
3-amino-benzoic acid ester (Tricaine) was used to anesthetize animals for imaging. After anesthetization, they were covered in 0.8% low-melting point agarose in glass-bottomed 35 mm Petri dishes and mounted on their right side. Images were captured using a spinning disk confocal microscope custom build by 3i technology© with: Zeiss Axio Observer Z1 Advanced Mariana Microscope, X-cite 120LED White Light LED System, filter cubes for GFP and mRFP, a motorized X,Y stage, piezo Z stage, 20X Air (0.50 NA), 63X (1.5NA), 40X (1.1NA) objectives, CSU-W1 T2 Spinning Disk Confocal Head (50 uM) with 1X camera adaptor, and an iXon3 1Kx1K EMCCD camera, dichroic mirrors for 446, 515, 561, 405, 488, 561, 640 excitation, laser stack with 405 nm, 445 nm, 488 nm, 561 nm and 637 nm with laser stack FiberSwitcher, photomanipulation from vector© high speed point scanner ablations at diffraction limited capacity, Ablate!TM© Photoablation System (532 nm pulsed laser, pulse energy 60J @ 200 HZ), Time-lapse images were collected every 5 min for 24 hr. Images were processed using Slidebook software and ImageJ. Only brightness and contrast were adjusted for all images.

**Laser-Induced Spinal Avulsion**
Animals were first mounted in 0.8% agarose as described above. One avulsion was made per animal except in Figure 4. Injuries were consistently made in the trunk of the animal at DRG 10–14. The specific site of laser injury was chosen by following dorsal projections from the DRG into the spinal cord through the z-plane. An area of clear axon fluorescence in the PNS was marked and brought into focus. The image window was then adjusted 1um laterally out of the z-plane to avoid spinal injury, and a double-click on the axon using a 4 μm selection tool created the injury. All laser parameters used are relative to our confocal microscope: laser power (1), raster block size (1), double-click rectangle size (8), double-click repetitions (4).

**Chemical Treatments**
The chemical treatments used in this study are taxol (Acros), GM6001 (Santa Cruz Biotechnology), and SU6656 (Santa Cruz Biotechnology). Stock solutions of these reagents were stored at −20 °C. The taxol stock solution was stored at 2.2 mM, the GM6001 stock solution was stored at 1.0 mM, and the SU6656 stock was stored at 375μM - all in DMSO. Treated animals were exposed to the chemical directly following injury (expect in Figure 2F where animals were exposed at varying time-points after injury) at concentrations of 22 μM for taxol, 1 μM for GM6001, and 3μM diluted in egg water (Nichols and Smith, 2019). DMSO-treated animals were exposed to 1% DMSO in egg water.

**Immunohistochemistry**
The primary antibodies used in this study were Zrf-1 (1:100, mouse, ZIRC) and phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (1:500, rabbit, Cell Signaling). Alexa Fluor 647 goat anti-mouse (1:600, Thermo Fisher) and Alexa Fluor 647 goat anti-rabbit (1:600, Thermo Fisher) were used as secondary antibodies. Fish were stained in 4% PFA in PBST (PBS, 0.1% Triton X-100) for 1 hr followed by 4°C overnight. The samples were then washed in acetone for 10 min at −20°C. Fish were exposed to three washes of PBSTx for 5 min each at 25°C followed by 1 hr of 5% goat serum in PBSTx at 25°C. The fish were then incubated with the primary antibody in PBSTx and 5% goat serum for 1 hr at 25°C followed by 4°C overnight. The following day, fish were washed three times with PBSTx for 30 min and once in PBSTx for 1 hr, all at 25°C. The fish were then incubated with the secondary antibody in PBSTx and 5% goat serum at 25°C for 1 hr followed by 4°C overnight. The next day, the samples were washed three times in PBSTx for 30 min at 25°C. Stained fish were then stored in PBS and 50% glycerol at 4°C until imaging. Stained fish were mounted and imaged using the same techniques described above.

**Molecular Biology**
Creation of psELN05 (uas:ca-src-mcherry + clmc2:gfps), psELN06 (uas:src-mcherry + clmc2:gfps), and psELN10 (gfap:ca-src-mcherry) were generated using the LR Clonease II Plus (ThermoFisher) using zebrafish compatible Tol2 constructs. To express the UAS promoter, p5e-UAS and p5e-gfap vectors were used (Don et al., 2017), pMe-Src and pMe-CASrc vectors were used to
express the two Src forms (Seiler et al., 2012) and were fused to mCherry using the p3e-mCherryP4 construct. Multisite gateway recombination of these constructs was accomplished using pDestTol2CG2 (Kwan et al., 2007). The pMe-Src and pMe-CASrc constructs were generous gifts of Christoph Seiler. A uas: syn-gfp plasmid was also used to generate animals for the synapse analysis (Hines et al., 2015).

Expression of Plasmid Constructs
To express the uas:src-mcherry, uas:ca-src-mcherry, gfap:ca-src-mcherry, uas: syn-gfp constructs, the DNA was diluted in water to a 75 ng/µl working solution. For injections, the working solution was diluted further to 12.5 ng/µl in water with 25 ng/µl of pCS2FA-transposase RNA in water. This solution was injected into single-cell embryos. For uas: src-mcherry and uas: ca-src-mcherry injections, Tg(sox10:gal4); Tg(uas: lifeac-gfp) animals were used. For gfap: ca-src-mcherry injections, Tg(ngn1: gfp) animals were used. For uas: syn-gfp injections, Tg(sox10:gal4) animals were used. At 3dpf, injected animals were assayed for transgenic background fluorescent expression. One DRG per animal was imaged to confirm mCherry expression and then subjected to avulsion-like injury. Time-lapse imaging was then taken of avulsed DRG. Following imaging, fish were immediately fixed and stained for GFAP to visualize the glia limitans.

Behavioral Assay
One day after injury, individual zebrafish were removed from their 23°C husbandry temperature, immersed in 4°C and imaged with a Zeiss Axiozoom microscope and a monochrome camera controlled by Zen software. Movies of the 20 s immediately following exposure to 4°C or 23°C water were taken at 20 frames per second. These videos were compiled and analyzed for shivering behaviors. Shivering was defined as bilateral movement of the larval tail that did not result in the animal’s forward locomotion. All analysis was completed by a researcher blind to the experimental paradigm the animal was exposed to. Quantified numbers and percent of time shivering was classified into four categories of behavioral responses: typical (3+ shivers and greater than 12% shivering), stunted (7%–12% but 1-3 shivers), moderate (1-3 shivers and 1%–7%), or absent (no shivering).

QUANTIFICATION AND STATISTICAL ANALYSIS
Slidebook software was used to generate composite z-images and y-orthogonal images. The mean and SEM are shown for all representations of error. Values for n reflect the number of DRG quantified unless otherwise noted. Intensity quantifications were completed using ImageJ and cell tracing through time-lapse movies was performed using the MTrackJ plugin in ImageJ. GraphPad Prism software was used to generate all graphs in this study and for all statistical analyses. Additional methods to determine if the data met assumptions of the statistical test were not used. Statistical tests, sample size and definition of precision measurements for each experiment can be found in the main text and figure legends for all experiments.

DATA AND CODE AVAILABILITY
All data generated in this study are included in the article. No code was used or generated in this study.
Supplemental Information

Functional Regeneration of the Sensory Root via Axonal Invasion

Evan L. Nichols and Cody J. Smith
Figure S1. Taxol promotes regeneration as the axon re-extends, related to Figure 1. (A,F). Y-orthogonal images of the animals from Figure 1I treated with taxol at 3dpf (A) and 5dpf (F). White arrows denote GFP puncta within glia limitans. Graphs show representative tracings of GFP (regenerated axon) and mCherry (glia limitans) from the y-orthogonal images. Green arrows denote regenerated axon and red arrows denote glia limitans. Asterisk denotes neuron in the spinal cord. (B). Z-projection time-lapse images of Tg(ngn1:gfp); Tg(gfap:nsfb-mcherry) animals at 0 and 12hpi treated with DMSO or taxol. White and green arrows denote regenerating growth cone. (C). Y-orthogonal images of animals from (B) treated with taxol. Graphs show representative tracings of GFP (axon) and mCherry (glia limitans) from y-orthogonal images. Green arrows denote axon and red arrows denote the glia limitans. (D). Length of re-extending axons treated with DMSO and taxol after injury. (E). Percent of axons treated with DMSO and taxol that retract to the DRG after injury. (n=6 DRG per treatment) (G). Left: Z-projection images of Tg(ngn1:gfp); Tg(gfap:nsfb-mcherry) animals at 48hpi and treated with DMSO or taxol at 4, 8, 12, 16, 20, and 24hpi. Green arrows denote the regenerated axon. Right: Y-orthogonal images of animals treated with taxol. Graphs show representative tracings of GFP (axon) and mCherry (glia limitans) from the y-orthogonal images. Green and white arrows denote axon and red arrows denote the glia limitans. Scale bar is 1 µm.
Figure S2. Taxol treatment favors actin organization into concentrates, related to Figure 2. (A-B). Graphs of the number of actin concentrates (A) and time of actin concentrate initiation (B) DMSO (n=7), taxol (n=6), taxol+GM6001 (n=6), and taxol+SU6656 (n=4) treated animals. (C-E). Representative scatterplots comparing growth cone area and average Lifeact-GFP intensity at each time point during regeneration in an axon treated with DMSO (C), taxol+GM6001 (D), and taxol+SU6656 (E). Each time point is represented by a point. Pink points denote actin concentrates. Blue points denote actin transitions. Green points denote dispersed actin. (F). Graph of average growth cone velocity during actin concentrate formation. DMSO-treated axons display negative velocities in the presence of actin concentrates, while taxol and cotreated axons do not. (A,B,F) use Tukey’s HSD.
Figure S3. CA-Src expression in DRG cells favors actin organization into concentrates, related to Figure 3. (A-B). Graphs of number of actin concentrates (A), and time of actin concentrate initiation (B) in DRG expressing Src, CA-Src, and CA-Src+taxol. CA-Src and CA-Src+taxol axons demonstrate an increased duration of actin concentrates. (n=6 Src and CA-Src, n=5 CA-Src+taxol) (C,H). Y-orthogonal image of the animals in Figure 3B (C) and Figure 3C (H) stained for GFAP. White arrow denotes GFP+ puncta within the glia limitans. Graphs show representative tracings of regenerated axon (green) and glia limitans (red) from the y-orthogonal image. The regenerated axon is inside the spinal boundary. Green arrow denotes regenerated axon and red arrow denote the glia limitans. (D,E,G). Representative scatterplots comparing growth cone area and average Lifeact-GFP intensity at each time point during regeneration in an axon expressing Src (D), CA-SRC (E), and CA-Src+taxol (G). Each time point is represented by a point. Pink points denote actin concentrates. Blue points denote actin transitions. Green points denote dispersed actin. (F). Z-projection images of a Tg(ngn1:gfp); gfap:-CA-src-mcherry animal before injury and 24hpi. At 24hpi, the animal was fixed and stained for GFAP. At 24hpi, the neuron has retracted its axon consistent with failed regeneration. (A,B) use Tukey’s HSD. Scale bar is 10 µm.
Figure S4. Dynamics of circuit and behavioral functioning following multiple avulsions, related to Figure 4. (A). Z-projection time-lapse images of a Tg(ngn1:gfp); Tg(gfap:nsfb-mcherry) animal treated with taxol following avulsion-like injury showing atypical DRG spinal entry and navigation of the axon to the DLF. White and green arrows denote regenerating growth cone (B). Z-projection images of Tg(ngn1:gfp) animals stained for p-Erk after exposure to 25°C and without avulsions, 8 consecutive avulsions and DMSO treatment, or 8 consecutive avulsions and taxol treatment. (C). Average intensity of p-Erk in the spinal cord of after exposure to 25°C. (n=5 animals per treatment) (D). Time-lapse mages of animals without avulsions, 8 consecutive avulsions and DMSO treatment, or 8 consecutive avulsions and taxol treatment at 1 dpi and 2 dpi. Black arrows denote shivering behavior. (E-F). Percent of time in shaking behavior (E) and number of shaking behaviors (F) at 2 dpi. (n=5 animals per treatment) (A,C,E-F) use a one-way ANOVA. Scale bar is 1 µm in (B) and 0.1 mm in (D).