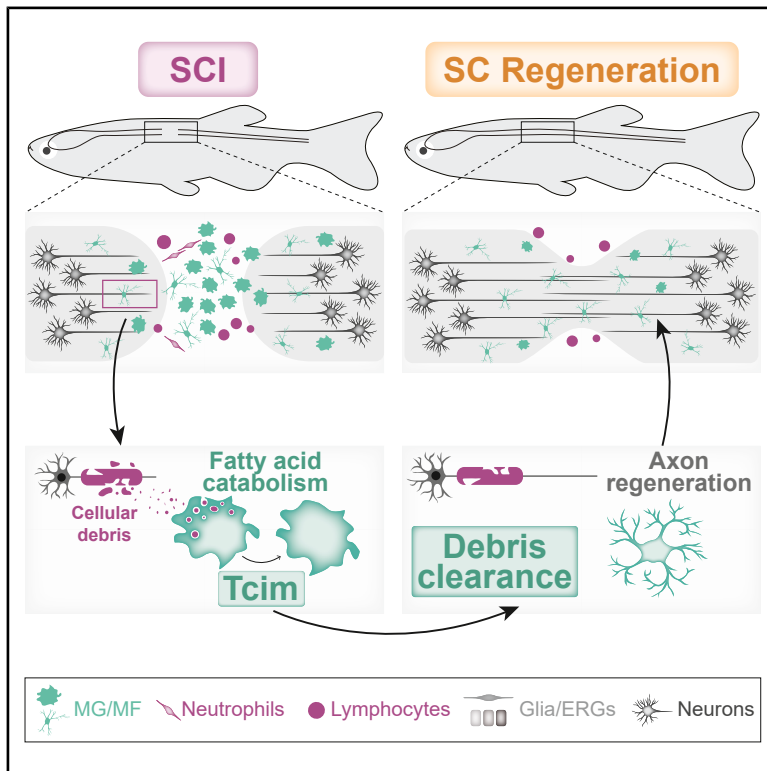


## Elevated phagocytic capacity directs innate spinal cord repair

### Graphical abstract



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### In brief

Klatt Shaw et al. report that transient activation of zebrafish macrophages directs spontaneous regeneration after spinal cord injury. By comparing regenerative and non-regenerative vertebrates, they identify *Tcim* as a central regulator of lipid metabolism and phagocytosis following injury. *Tcim* expression enhances lipid and debris clearance in zebrafish and mouse macrophages.

### Highlights

- Lipid-rich debris are efficiently cleared in zebrafish after spinal cord injury
- A transient microglia/macrophage response is required for spinal cord repair
- *tcim* loss impairs lipid processing, phagocytosis and functional repair
- Expression of TCIM in zebrafish or mouse macrophages accelerates debris and lipid clearance



## Article

# Elevated phagocytic capacity directs innate spinal cord repair

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## SUMMARY

Immune cells elicit a continuum of transcriptional states after spinal cord injury (SCI). In mammals, inefficient debris clearance and chronic inflammation impede recovery and overshadow pro-regenerative immune functions. We found that zebrafish SCI elicits transient immune activation and efficient debris clearance. Transcriptomics and genetic ablation showed zebrafish macrophages are highly phagocytic and required for regeneration. Comparisons between zebrafish and mammalian macrophages identified *transcription and immune response regulator (tcim)* as an immune-enriched regenerative gene. Deletion of zebrafish *tcim* impairs phagocytosis and regeneration and activates a pro-inflammatory signature in leukocytes. *Tcim* expression in zebrafish and mouse macrophages establishes its conserved roles by promoting lipid metabolism and the reprogramming of myeloid precursors into activated phagocytes. This study underscores a requirement for elevated phagocytic capacity to achieve innate spinal cord repair and identifies a gene with conserved function in mammalian cells.

## INTRODUCTION

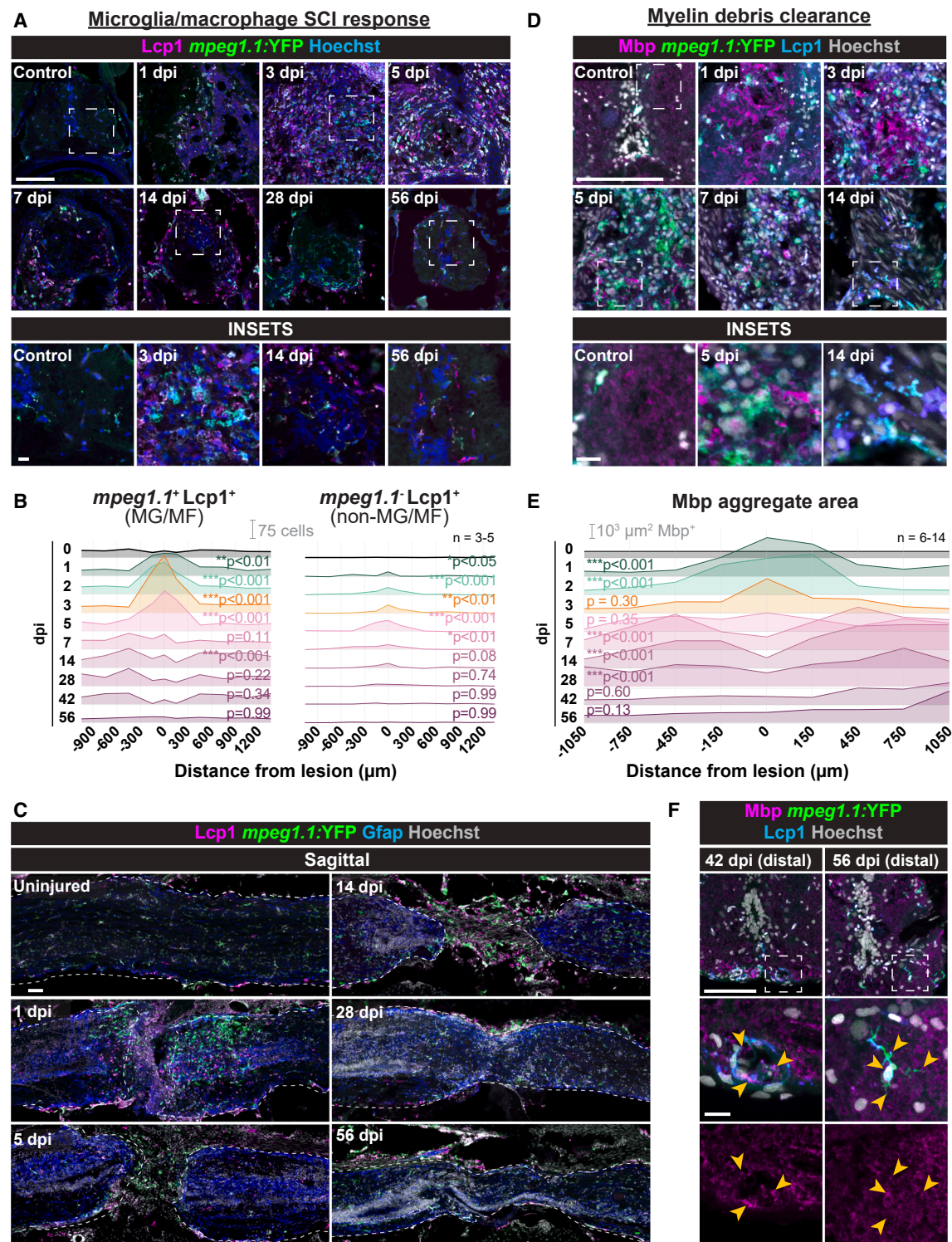
Spinal cord injury (SCI) elicits multicellular and systemic complications that cause permanent neurological deficits in mammals.<sup>1–3</sup> As the first line of defense against neural damage, immune cells are activated as early as hours after injury and persist years after human SCI.<sup>4–6</sup> Immune activation in the central nervous system (CNS) engages tissue-resident microglia and blood-derived leukocytes. Immediately following SCI, neutrophils and lesion-adjacent microglia phagocytose cellular debris and recruit infiltrating immune cells into the spinal cord (SC).<sup>7–10</sup> Distinct populations of innate and adaptive immune cells, which do not cross the blood SC barrier under homeostatic conditions, are sequentially recruited to SC lesions.<sup>9,11–13</sup> Yet, the timeline, composition, and activation profiles of immune cells are infinitely diverse depending on the type, severity, and location of injury.<sup>14–17</sup> Thus, immune cells adopt a range of transcriptional states and functional outputs after mammalian SCI.

Immune cell activation plays dual roles that are challenging to decrypt after mammalian SCI. Consistent with the emergence of an exaggerated inflammatory response that impedes mammalian SC repair, treatment with anti-inflammatory drugs or broad depletion of microglia/macrophages improves functional recovery

after mouse SCI.<sup>18</sup> Sustained, chronic inflammation is a chief contributor to secondary neurotoxicity, glial cell reactivity, and the recruitment of scar-depositing fibroblasts into the lesion.<sup>19</sup> Recently developed tools that enabled targeted microglia/macrophage ablation in acute SCI revealed a requirement for early immune activation in mice.<sup>15,20–22</sup> The advent of single-cell transcriptomics provided molecular evidence that microglia/macrophages are highly plastic cells that exist in a spectrum of context-dependent activation states.<sup>17,23</sup> However, correlations between specific immune profiles and their corresponding phenotypic outputs remain to be drawn. The heterogeneity and complexity of immune responses following mammalian SCI present confounding predicaments to deciphering pro-regenerative immune mechanisms in mammals.

Adult zebrafish present a valuable model to untangle pro-regenerative immune functions in vertebrates. Like mammals, the zebrafish immune system comprises evolutionarily conserved innate myeloid and adaptive lymphocyte branches, in addition to CNS-resident microglia.<sup>24,25</sup> Yet, unlike mammals, adult zebrafish are capable of functional recovery after complete SC transection.<sup>26–29</sup> Studies of immune cell SCI responses in zebrafish have been limited to distinct immune subsets and/or to larval stages.<sup>30–32</sup> Establishing an immune requirement for larval SC repair, blocking





**Figure 1. Microglia/macrophage response is transient after SC regeneration**

(A and B) Immunostaining of cross sections of the pan-leukocyte marker L-plastin (Lcp1) and the microglia/macrophage marker *Tg(mpeg1.1:YFP)*. (B) Ridgeline plots of microglia/macrophages (MG/MF, *mpeg1.1*:YFP<sup>+</sup>Lcp1<sup>+</sup>) or non-MG/MF (*mpeg1.1*:YFP<sup>-</sup>Lcp1<sup>+</sup>). Distance from lesion (μm) (legend continued on next page)



immune activation in 3-day old zebrafish larvae reduces functional and anatomical regeneration.<sup>30,31</sup> However, developmental migration of microglia into the zebrafish CNS extends past 15 days post-fertilization,<sup>25</sup> while mature zebrafish lymphocytes do not emerge until 1 month post-fertilization.<sup>33,34</sup> In adult zebrafish, regulatory T cells were shown to be required for SC repair.<sup>32</sup> Yet, a comprehensive resource of leukocyte composition and activation profiles after SCI is required to decipher pro-regenerative immune cell states and mechanisms in adult zebrafish.

Limited phagocytic capacity and inefficient debris clearance are associated with mammalian SCI and inhibit SC repair.<sup>35</sup> The extracellular milieu of the lesioned cord is flooded with debris-derived, lipid-containing molecules that are potent inhibitors of axon regeneration.<sup>36</sup> Molecules released by degenerating oligodendrocytes, including neurite outgrowth inhibitors (Nogo) and myelin-associated glycoproteins (MAG), cause growth cone collapse and neurite retraction.<sup>37,38</sup> Inefficient processing of lipid-rich debris transforms phagocytes into lipid-laden foam cells that persist for months to years in human SC lesions.<sup>5,39,40</sup> Hyper-inflammatory, foamy macrophages contribute to chronic inflammation and limit regenerative capacity.<sup>8,41–44</sup> We posit that highly regenerative vertebrates like zebrafish elicit potent phagocytic properties that enable efficient debris clearing after injury. While devising strategies to enhance phagocytic capacity is a prerequisite to improving recovery after SCI, deciphering if and how phagocytic capacity differs between zebrafish and mammals requires cross-species comparisons and mechanistic investigation.

This study examines pro-regenerative immune responses to SCI in adult zebrafish. We report transient and reversible immune cell activation predominantly composed of infiltrating and tissue-resident macrophages. Genetic macrophage depletion during acute and sub-acute SCI impairs functional and anatomical regeneration. Cross-species comparisons between zebrafish and mammalian macrophages identified *transcription and immune response regulator* (*tcim*) as a macrophage-enriched zebrafish gene. Genetic deletion of zebrafish *tcim* impairs regeneration and causes excessive accumulation of lipid droplets and reduced phagocytic capacity. Conversely, genetic overexpression of *TCIM* accelerates debris clearance and regeneration by reprogramming myeloid precursors into activated phagocytes. This study elucidates fundamental differences in the cellular and molecular profiles of immune cells between regenerative and non-regenerative vertebrates in response to SCI and suggests a requirement for elevated phagocytic capacity to achieve innate SC repair.

## RESULTS

### An acute, transient immune response is activated after SCI in adult zebrafish

We first sought to explore the composition of leukocytes responding to SCI. Consistent with prior findings in larval zebrafish

and mice,<sup>4,6,30</sup> neutrophils were the first leukocytes to respond to SCI, peaking at 12 h post-injury (Figures S1A and S1B), while T cells showed maximum infiltration at 14 days post-infection (dpi) (Figures S1C and S1D). However, only 10.6% of leukocytes were neutrophils at 1 dpi and 14.5% of leukocytes were T cells at 14 dpi (Figure S1E), indicating that neutrophils and T cells comprise small fractions of Lcp1<sup>+</sup> leukocytes after zebrafish SCI. We next analyzed SC sections from *Tg(mpeg1.1:YFP)* fish to assess infiltrating and tissue-resident macrophages between 1 and 56 dpi.<sup>45</sup> Prior to injury, the number of *mpeg1.1*<sup>+</sup> leukocytes averaged 22.3 ± 3.3 per section (Figures 1A–1C). Following SCI, tissue-resident and infiltrating macrophages showed a 12-fold increase from 0 to 3 dpi (Figures 1A–1C). Macrophage numbers returned to pre-injury levels at 28 dpi (Figures 1A–1C). *mpeg1.1*<sup>+</sup> Lcp1<sup>+</sup> leukocytes were present in smaller numbers at the lesion site and rarely infiltrated beyond 300 μm into the rostral or caudal SC parenchyma (Figure 1B). Thus, while activated leukocytes persist into the chronic injury phase in mammalian tissues after SCI,<sup>46,47</sup> immune responses to SCI are transient in zebrafish. These studies showed that infiltrating and tissue-resident macrophages are the predominant leukocyte populations that respond to SCI in adult zebrafish and revealed a correlation between acute phagocyte activation and SC regeneration.

### Myelin debris is efficiently cleared after zebrafish SCI

To assess the extent of debris clearance following zebrafish SCI, we performed myelin basic protein (Mbp) staining on SC sections from *Tg(mpeg1.1:YFP)* fish. Mbp, which surrounded myelinated axons in uninjured SC sections, formed debris aggregates within the lesion site after SCI (Figure 1D). We found minimal Mbp<sup>+</sup> aggregation in uninjured SC sections. Following SCI, Mbp<sup>+</sup> aggregation increased 8.1- and 6.9-fold at 1 and 3 dpi, respectively (Figures 1D and 1E). By 5 dpi, Mbp<sup>+</sup> aggregation within the lesion site was statistically comparable to controls (Figure 1E). Clearance of Mbp<sup>+</sup> punctae within the SC parenchyma extended until 28 dpi (Figure 1E), and bright Mbp<sup>+</sup> punctae co-localized with *mpeg1.1*<sup>+</sup> cells in the distal SC as late as 56 dpi (Figure 1F). These data suggested that microglia participate in myelin remodeling weeks to months after SCI, supporting previous studies reporting microglia activation in lesion remote zones at 14 dpi.<sup>48</sup> However, efficient myelin debris clearance within the lesion site is a unique feature that correlates with elevated regenerative capacity after zebrafish SCI.

### *mpeg1.1*<sup>+</sup> macrophages/microglia are required for SC regeneration

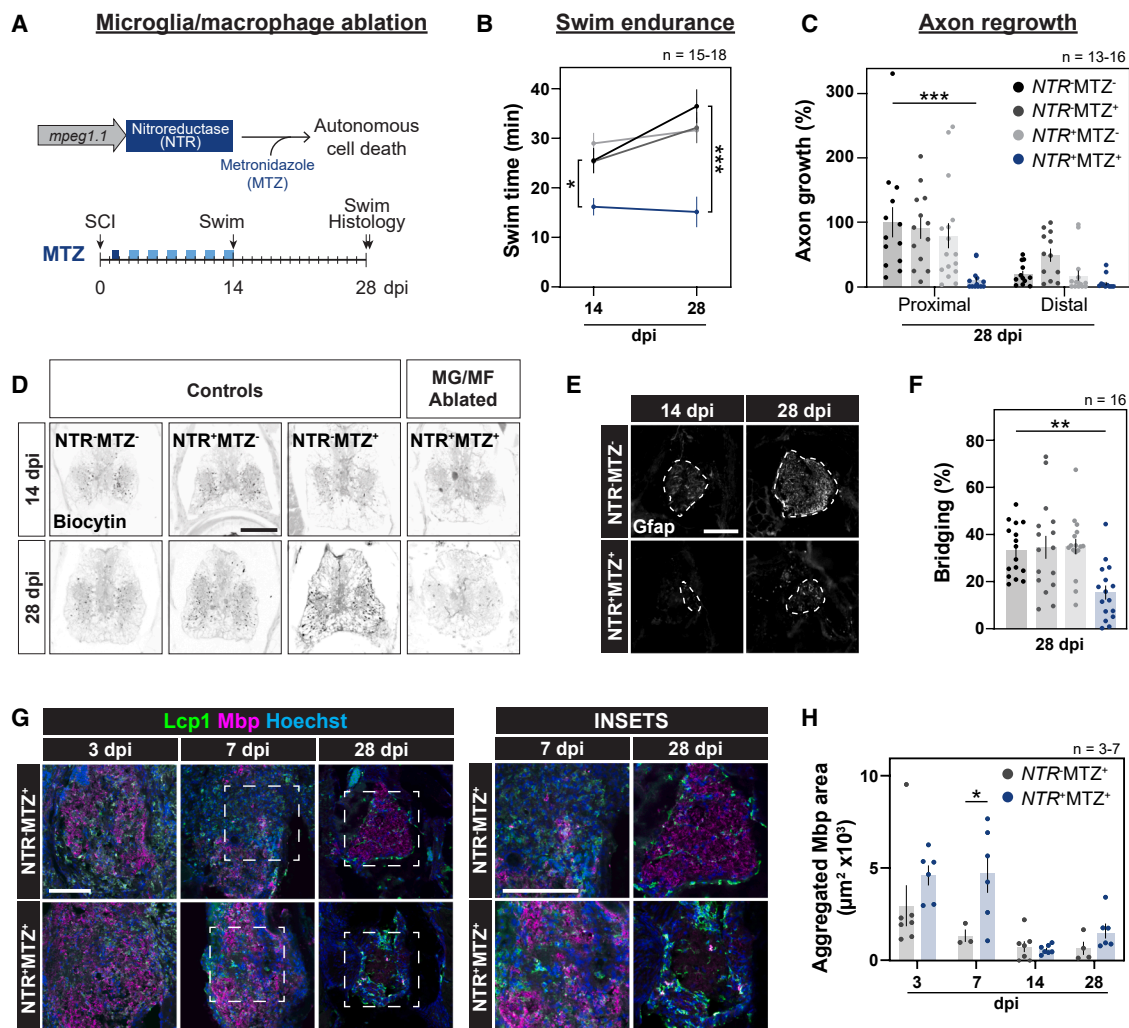
To examine whether transient phagocyte activation is required for SC regeneration, we depleted *mpeg1.1*<sup>+</sup> cells using *Tg(mpeg1.1:NTR-IRES-EGFP)* fish.<sup>49</sup> With this inducible genetic ablation system, *mpeg1.1*-driven expression of the nitroreductase (NTR) enzyme catalyzes metronidazole (MTZ) prodrug into a

from the lesion is indicated in μm (–Rostral, +Caudal). two-way ANOVA (Dunnett's): \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001. Comparisons are to uninjured controls.

(C) Sagittal sections of Lcp1 staining in *Tg(mpeg1.1:YFP)* zebrafish. Gfap staining (pan-glia) is used to delineate SCs.

(D–F) Cross sections of Mbp and Lcp1 staining in *Tg(mpeg1.1:YFP)* zebrafish. One-way ANOVA (Dunnett's): \*\*\**p* < 0.0001. In (F), Mbp<sup>+</sup> punctae that are engulfed (arrowheads). Scale bars in insets and in (F), 100 μm. All other scale bars, 100 μm. See also Figure S1.





**Figure 2. Microglia/macrophage response is required for SC regeneration**

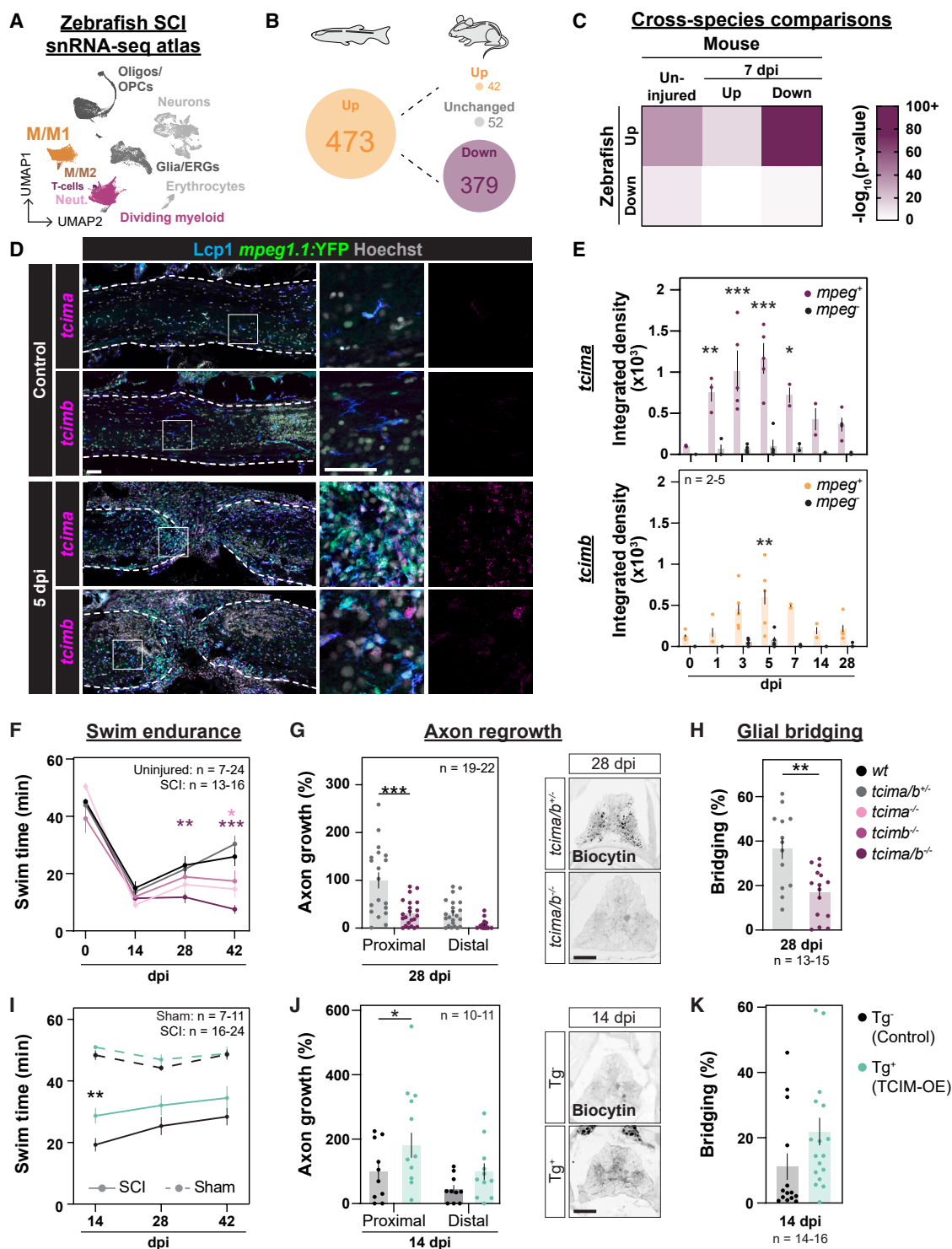
(A) *Tg(mpeg1.1:NTR-IRES-EGFP)* fish were treated with Metronidazole (MTZ) to ablate *mpeg1.1*<sup>+</sup> cells after SCI. (B) Swim endurance of microglia/macrophage-depleted fish. two-way ANOVA (Sidak's): \**p* < 0.05, \*\*\**p* < 0.001. All samples were compared to time-matched *NTR-MTZ*<sup>-/-</sup>. (C–F) Anatomical regeneration of microglia/macrophage-depleted fish. In (D), representative proximal caudal (–300 to –450 μm) images of anterograde axon labeling are shown. In (E), representative images of the center of the Gfap<sup>+</sup> glial bridge are shown. The cross-section of the glial bridge is outlined. One-way ANOVA (Sidak's): \*\**p* < 0.01, \*\*\**p* < 0.001. Comparisons are to *NTR-MTZ*<sup>-/-</sup>. (G and H) Cross sections of the lesion site of Mbp and Lcp1 staining in *Tg(mpeg1.1:NTR-IRES-EGFP)* zebrafish. two-way ANOVA (Sidak's): \**p* < 0.05. Error bars represent SEM. All scale bars, 100 μm. See also Figure S1.

cytotoxic product, resulting in cell-autonomous ablation of *mpeg1.1*<sup>+</sup> cells.<sup>50</sup> Because macrophages undergo rapid replacement after depletion, continuous MTZ pulses were required to sustain *mpeg1.1*<sup>+</sup> cell depletion (Figure 2A). This pulsed MTZ treatment decreased Lcp1<sup>+</sup> cells by 74.4% at 3 dpi (Figure S1F). Macrophage-depleted animals exhibited a decrease in swim endurance at 14 and 28 dpi compared to controls (Figure 2B). Using anterograde axon tracing and Gfap immunostaining,<sup>27</sup> proximal axon regrowth and glial bridging across the lesion were significantly reduced in macrophage-ablated animals compared to control cohorts at 28 dpi (Figures 2C–2F). Although MTZ removal at 14 dpi was sufficient for *mpeg1.1*<sup>+</sup> cells to repopulate SC tis-

ues, delayed macrophage emergence was not sufficient to direct regeneration between 14 and 28 dpi (Figures 1C–1F). Depletion of *mpeg1.1*<sup>+</sup> cells also abrogated the clearance of Mbp<sup>+</sup> debris within the lesion site at 7 dpi (Figures 1G and 1H). These results indicated *mpeg1.1*<sup>+</sup> phagocytes are acutely required for SC regeneration and debris clearance in adult zebrafish.

### Comparative transcriptomics identifies a unique transcriptional signature in zebrafish macrophages/microglia

We postulated that zebrafish macrophages elicit a unique transcriptional signature that directs elevated phagocytic



**Figure 3. *tcim* is enriched in microglia/macrophages and required for SC regeneration**

(A–C) A pipeline was used to identify zebrafish-enriched immune genes after SCI to compare zebrafish<sup>51</sup> and mouse<sup>52</sup> MG/MF at 7 dpi. In (C), hypergeometric scoring was used to compare the overlap between up- and downregulated genes in mouse and fish after SCI.

(D and E) HCR *in situ* hybridization of *tcima* or *tcimb* and Lcp1 staining on *Tg(mpeg1.1:YFP)* sagittal SC sections. Cross sections were used for quantifications in (E). two-way ANOVA (Sidak's): \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

(F) Swim endurance following *tcim* loss-of-function. One-way ANOVA (Sidak's): \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Comparisons are to time point-matched wild-type siblings.

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capacity and pro-regenerative functions. To test this hypothesis, we analyzed the profiles of zebrafish and mouse immune cells after SCI using previously generated single-cell RNA-sequencing datasets (Figure 3A).<sup>51,52</sup> Two zebrafish leukocyte clusters expressed canonical microglia/macrophage markers (Figure S2A). We identified 473 macrophage-enriched and injury-induced zebrafish genes (Table S1). Out of the 473 M/M1-enriched zebrafish genes, 379 genes were downregulated in mouse microglia at the same time point (Figure 3B). Hypergeometric distribution showed that zebrafish microglia/macrophage populations are transcriptionally similar to mouse microglia prior to injury ( $p = 2.03 \times 10^{-23}$ ) (Figure 3C). However, at 7 dpi, genes upregulated in zebrafish macrophages/microglia more closely matched genes that are downregulated in mouse microglia at 7 dpi ( $p = 3.91 \times 10^{-262}$ ) (Figure 3C). Among the 379 genes upregulated in fish and downregulated in mouse microglia, 72 were identified as transcriptional modulators in the DAVID gene ontology database (Table S1). Postulating that transcriptional regulators are likely to drive these large transcriptional changes during regeneration, we clustered these 72 transcriptional regulators into a gene regulatory network (Figure S2B). Within this gene regulatory network, several transcription factors that have been characterized in regeneration appear as hubs, including *smad3a*,<sup>53</sup> *gsk3b*,<sup>54</sup> and *stat3*.<sup>55</sup> We also identified transcriptional modulators within this network that were not previously implicated in regeneration, including *elf1* and *tcima*. These comparative studies identified genes that are preferentially enriched in zebrafish microglia/macrophages and likely to be associated with increased regenerative capacities in zebrafish.

### ***tcim* expression is upregulated in zebrafish macrophages after SCI**

Because *transcription and immune response regulator* (*tcim*) had been identified in a previous transcriptomic analysis,<sup>27</sup> we sought to understand its functional role in microglia/macrophages during SC regeneration. The zebrafish genome harbors the gene paralogs *tcima* and *tcimb*. *tcim* genes regulate cell proliferation and inflammation.<sup>56–62</sup> However, the *in vivo* functions of *Tcim* during regeneration or within the CNS are unknown. At 7 dpi, zebrafish *tcima* expression is increased in microglia/macrophages, while murine *Tcim* is downregulated in microglia (Figure S2C).<sup>51,52</sup> To confirm these observations, we performed HCR *in situ* hybridization for *tcim* in zebrafish before and after SCI. Concomitant with the emergence of microglia/macrophages after SCI, *tcima* showed minimal baseline expression in uninjured SC tissues but was upregulated in *mpeg1.1<sup>+</sup>Lcp1<sup>+</sup>* cells at 5 dpi (Figures 3D and 3E). As zebrafish paralogs have been shown to play redundant functions upon genetic perturbation,<sup>63</sup> we also examined the

expression of *tcimb* after SCI. *tcimb* transcripts were expressed in a subset of *mpeg1.1<sup>+</sup>Lcp1<sup>+</sup>* cells and in *mpeg1.1<sup>+</sup>Lcp1<sup>+</sup>* cells after SCI (Figures 3D and 3E). We confirmed that *Tcim* is expressed in CD68<sup>+</sup> microglia in mouse SC tissues but is downregulated upon SCI (Figure S2D). These studies showed that zebrafish *tcim* expression is enriched in zebrafish microglia/macrophages after SCI.

### ***tcim* loss of function impairs SC regeneration**

To determine the role of *tcim* during SC regeneration, we generated CRISPR-Cas9-mediated gene deletions of zebrafish *tcima* and *tcimb* (Figures S2E and S2F). Single and double *tcima<sup>-/-</sup>tcimb<sup>-/-</sup>* zebrafish (referred to hereafter as *tcima/b* mutants or *tcima/b<sup>-/-</sup>* fish) are adult viable and show normal swim endurance prior to injury (Figure 3F). After SCI, swim endurance was reduced by 48.6% in *tcima/b* mutants compared to wild-type siblings at 28 and 42 dpi (Figure 3F). At 42 dpi, *tcima* loss-of-function elicited an intermediate phenotype compared to *tcima/b* mutants, suggesting a compensatory role for *tcimb* in *tcima* mutants (Figure 3F). We therefore used *tcima/b* mutants for the remaining loss-of-function studies. As *tcima/b* heterozygous and wild-type siblings showed comparable swim endurance (Figure 3F), *tcima/b* heterozygous animals were used as controls. By anterograde axon tracing, *tcima/b* mutants exhibited a 69.0% reduction in proximal axon regrowth at 28 dpi (Figure 3G). Glial bridging also decreased by 53.9% in *tcima/b* mutants relative to controls (Figure 3H). These studies indicated *tcima/b* are required for functional recovery and anatomical regeneration in adult zebrafish.

### **Human TCIM enhances SC regeneration**

The amino acid composition of zebrafish *Tcima* is 56% identical to human TCIM (Figure S2G). To examine regenerative functions of TCIM, we expressed human TCIM under control of a heat-inducible promoter to generate *Tg(hsp70l:hTCIM-2A-EGFP)* fish and assessed the outcomes of TCIM expression on functional and anatomical SC repair (Figures S2H–S2J).<sup>64</sup> Swim endurance was comparable between sham-injured TCIM-expressing and wild-type siblings, indicating TCIM expression did not alter swim capacity in the absence of SCI (Figure 3I). However, at 14 dpi, swim endurance of TCIM-expressing fish was significantly increased compared to controls, and comparable to the performance of control fish at 42 dpi (Figure 3I). TCIM-expressing fish displayed a 114.5% increase in proximal axon regrowth, while glial bridging was not significantly increased compared to controls at 14 dpi (Figures 3J and 3K). These results indicated that expression of human TCIM is sufficient to accelerate axon regrowth and functional recovery after SCI.

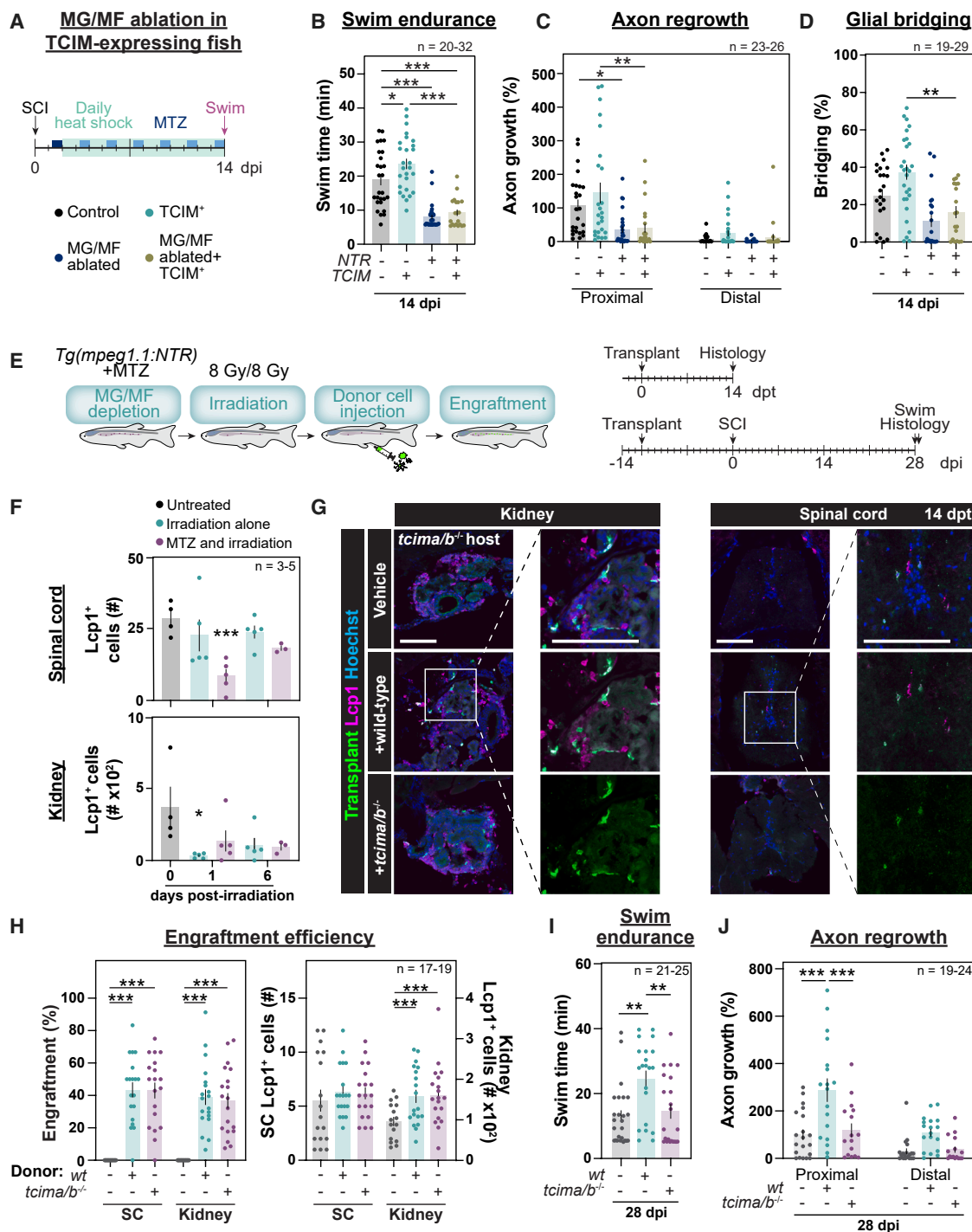
(G and H) Anatomical regeneration of *tcima/b<sup>-/-</sup>* and control fish. One-way ANOVA (Sidak's, axon regrowth): \*\*\* $p < 0.001$ . Unpaired *t* test (glial bridging): \*\* $p < 0.01$ .

(I) Swim endurance in *Tg(hsp70l:hTCIM-2A-EGFP)* TCIM-expressing transgene-positive (*Tg<sup>+</sup>*) and control transgene-negative (*Tg<sup>-</sup>*) siblings. Dotted lines indicate sham-injured controls. One-way ANOVA (Sidak's): \*\* $p < 0.01$ . Comparisons are to time-matched transgene-negative controls.

(J and K) Anatomical regeneration in TCIM-expressing fish and control siblings. One-way ANOVA (Sidak's, axon regrowth assay): \* $p < 0.05$ . Unpaired *t* test (glial bridging). Comparisons are to transgene-negative controls.

Error bars represent SEM. Scale bars in (D), 50  $\mu$ m. All other scale bars, 100  $\mu$ m. See also Figure S2 and Table S1.





**Figure 4. Tcim is acting within immune cells to drive SC regeneration**

(A) Microglia/macrophage depletion via *Tg(mpeg1.1:NTR-IRES-EGFP)* was combined with expression of human TCIM via *Tg(hsp70l:hTCIM-2A-EGFP)*. (B) Swim endurance in *Tg(mpeg1.1:NTR-IRES-EGFP);Tg(hsp70l:hTCIM-2A-EGFP)* and control fish at 14 dpi. two-way ANOVA (Tukey's): \* $p < 0.05$ , \*\*\* $p < 0.001$ . (C and D) Anatomical recovery in *Tg(mpeg1.1:NTR-IRES-EGFP);Tg(hsp70l:hTCIM-2A-EGFP)* and control fish. two-way ANOVA (Tukey's): \* $p < 0.05$ , \*\* $p < 0.01$ . (E) Experimental paradigm to generate immune chimeras via whole kidney marrow replacement. For histology in Figures 4G and 4H, animals were collected at 14 dpt (top). For regeneration assays in Figures 4I and 4J, SCI was performed at 14 dpt and animals were collected at 28 dpt (42 dpt, bottom). (F) Quantifications of Lcp1 staining in irradiated and microglia/macrophage-depleted animals. two-way ANOVA (Dunnett's): \* $p < 0.05$ , \*\*\* $p < 0.001$ . Comparisons are to untreated controls.

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### Tcim is required in the immune compartment to drive SC regeneration

To determine if accelerated regeneration in TCIM-expressing animals requires microglia/macrophages, we crossed *Tg(hsp70l: hTCIM-2A-EGFP)* animals into the *Tg(mpeg1.1:NTR-IRES-EGFP)* background (Figure 4A). Similar to microglia/macrophage depletion in wild-type fish, microglia/macrophage depletion impaired functional and anatomical regeneration metrics in TCIM-expressing fish (Figures 4B–4D). These results indicated that human TCIM enhances SC repair via a microglia/macrophage-dependent mechanism.

To determine the cell-specific requirement for *tcima/b*, we combined hematopoietic transplantation protocols<sup>65</sup> with microglia/macrophage ablation,<sup>21,66</sup> which allowed us to differentially control the genotypes of immune cells (Figure 4E). Following depletion of peripheral hematopoietic cells and microglia/macrophages (Figure 4F), transgenically labeled dissociated marrow cells were injected into immunocompromised recipients. To assess the transplantation efficiency of donor cells in *tcima/b* mutant hosts, we quantified the proportions of Transplant<sup>+</sup>Lcp1<sup>+</sup> cells in uninjured hosts in the kidney marrow and SC. Unlike mammalian bone marrow, the zebrafish hematopoietic niche is located in the kidney.<sup>34</sup> At 14 days post-transplantation (dpt), wild-type and *tcima/b* mutant donor cells accounted for >35% and >40% of Lcp1<sup>+</sup> cells in the kidney and SC, respectively (Figures 4G and 4H). These results indicated successful transplantation of wild-type and *tcima/b*<sup>−/−</sup> cells and allowed us to selectively modify immune cell genotypes during SC regeneration. Compared to either vehicle injections or *tcima/b*<sup>−/−</sup> hematopoietic transplantation, wild-type kidney transplants enhanced swim endurance by 87.6% and proximal axon regrowth by 149.4% in *tcima/b*<sup>−/−</sup> hosts (Figures 4I and 4J). Underscoring an immune requirement for Tcim, transplanting wild-type leukocytes is sufficient to rescue the regeneration defects in *tcima/b* mutants.

### tcim loss of function impairs immune cell clearance following SCI

To determine immune cell composition upon *tcima/b* loss of function, we performed histology for microglia/macrophages and pan-leukocytes in *tcima/b* mutants at 5 and 14 dpi. The numbers of lesion-associated or parenchymal *mpeg1.1*<sup>+</sup>Lcp1<sup>+</sup> leukocytes were not changed in *tcima/b* mutants compared to controls at either 5 or 14 dpi (Figures 5A and 5B). However, at 5 dpi, the numbers of lesion-associated *mpeg1.1*<sup>+</sup>Lcp1<sup>+</sup> leukocytes increased 4.8-fold in *tcima/b* mutants compared to controls (Figures 5A and 5B). While *mpeg1.1*<sup>+</sup>Lcp1<sup>+</sup> leukocytes were cleared from the lesion sites of control animals at 14 dpi, the numbers of parenchymal *mpeg1.1*<sup>+</sup>Lcp1<sup>+</sup> leukocytes increased 81.2-fold in *tcima/b* mutants (Figures 5A and 5B). To explore the identity of aberrant *mpeg1.1*<sup>+</sup>Lcp1<sup>+</sup> leukocytes in *tcima/b* mutants, we performed HCR *in situ* hybridization for

*mpx*<sup>+</sup> neutrophils and *lck*<sup>+</sup> T cells in *tcima/b* mutants and control siblings. Neutrophil numbers did not change between experimental groups (Figures S3A and S3B), but a trending expansion of *lck*<sup>+</sup> T cells was observed in the lesion and parenchyma in *tcima/b* mutants at 14 dpi (Figures S3C and S3D). These data suggested that the changes in leukocyte composition upon *tcim* deletion are driven in part by lymphocytes. These results demonstrated aberrant immune cell composition and chronic immune activation in *tcima/b* mutants after SCI.

To survey the molecular mechanisms that underlie impaired immune composition in *tcima/b* mutants, we performed bulk RNA-sequencing on *tcima/b* mutant and control siblings at 5 dpi. We found 159 genes upregulated and 108 genes downregulated in *tcima/b* mutants compared to controls (Figure 5C; Table S2). Genes upregulated in *tcima/b* mutants included pro-inflammatory genes, such as *il1b*, *nfk1*, and *tnfa* (Figure 5C; Table S2). Conversely, genes downregulated in *tcima/b* mutants were enriched for genes related to immune homeostasis and lipid processing (Table S2). Specifically, *smad2*, an important regulator of immune suppression,<sup>67</sup> and the lipid processing genes *apolipoprotein bb.1* (*apobb.1*) and *acyl-CoA oxidase 1* (*acox1*)<sup>68,69</sup> were markedly downregulated in *tcima/b* mutants (Figure 5C). Using hypergeometric scoring, *tcim* mutants showed increased transcriptional similarity with non-regenerative mouse microglia<sup>52</sup> (upregulated, *p* = 0.019 and downregulated, *p* = 0.0002) (Figure S3E; Table S2). Thus, a pro-inflammatory signature is activated in *tcima/b* mutants much like the signature found in mouse microglia after SCI, suggesting that *tcim* regulates pro-regenerative immune cell activation and function following SCI.

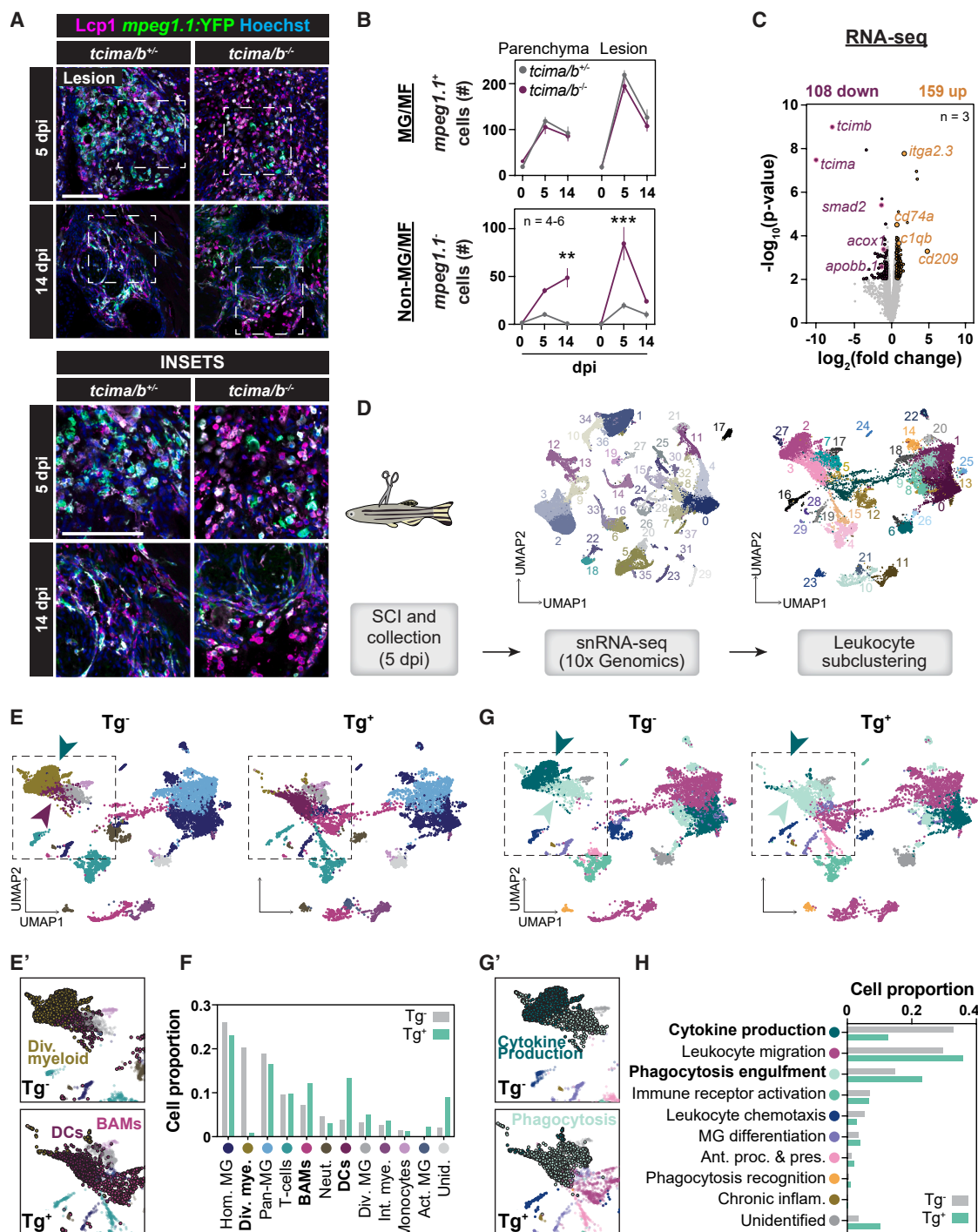
### TCIM expression reprograms myeloid precursors after SCI

To elucidate the molecular mechanisms by which TCIM enhances SC regeneration, we performed single nuclear RNA-sequencing (snRNA-seq) on TCIM-expressing SC tissues (Figure 5D; Figures S4A and S4B). Cell identities were determined using our previously developed hypergeometric scoring tool that optimized cell identification for non-mammalian vertebrates (Figures S4C–S4E).<sup>51</sup> *tcima* was expressed in leukocytes, while *tcimb* showed lower expression in leukocytes and non-immune cells, including the endothelium (Figure S4F). We noted that leukocyte proportions were not altered in the complete dataset upon TCIM expression (Figure S4E). However, by sub-clustering leukocytes into 30 distinct clusters (Figure S4G), cluster 2 was predominantly composed of wild-type cells, whereas clusters 3, 5, 6, and 29 were more prominent upon TCIM expression (Figure S4H). To determine TCIM-induced cell identities, we performed hypergeometric scoring against a leukocyte marker database (Figure S4I; Table S3).<sup>51,52</sup> This cell type classification identified 11 immune subtypes (Figures 5E and 5F, S5A). *tcima* was strongly enriched in microglia, while

(G and H) GFP and Lcp1 staining following hematopoietic transplantation. Scale bars, 100  $\mu$ m. One-way ANOVA (Sidak's); \*\*\**p* < 0.0001. Comparisons are to vehicle-injected controls.

(I) Swim endurance in transplanted *tcima/b*<sup>−/−</sup> mutant hosts. One-way ANOVA (Sidak's); \*\**p* < 0.01. Comparisons are to wild-type donors.

(J) Anatomical recovery of transplanted *tcima/b*<sup>−/−</sup> mutant hosts was measured by anterograde axon labeling relative to wild-type donors. One-way ANOVA (Sidak's); \*\*\**p* < 0.001. Error bars represent SEM.



**Figure 5. *tcim* loss generates non-regenerative leukocytes, while TCIM reprograms myeloid precursors into phagocytes to enhance SC repair**

(A and B) Cross sections of Lcp1 staining on *Tg(mpeg1.1:YFP);tcim/b<sup>−/−</sup>* and *Tg(mpeg1.1:YFP);tcim/b<sup>+/−</sup>* fish. Scale bars in (A), 100  $\mu$ m. Parenchyma indicates 150  $\mu$ m caudal to the lesion core. two-way ANOVA (Sidak's): \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Comparisons are to time-matched *Tg(mpeg1.1:YFP);tcim/b<sup>+/−</sup>* siblings. Error bars represent SEM.

(C) Bulk RNA-seq of *tcim/b<sup>−/−</sup>* and control SCs at 5 dpi.

(D) snRNA-seq was performed on TCIM-expressing and control SC tissues at 5 dpi. Leukocytes were subclustered for further analysis.

(E) UMAP representation of leukocyte subclusters in TCIM-expressing and control fish. Subclusters that changed in proportion upon TCIM expression are indicated in (E').

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*tcimb* showed lower expression in a subset of leukocytes (Figure S5A). The proportion of dividing myeloid cells was reduced 19.2% in TCIM-expressing tissues compared to controls, whereas two populations of mononuclear phagocytes, border associated macrophages (BAMs)<sup>70</sup> and dendritic cells,<sup>71</sup> were 1.69- and 3.41-fold increased upon TCIM expression (Figures 5E and 5F). These results suggested TCIM expression reprograms immature myeloid precursors into differentiated, phagocytic myeloid cells after SCI.

### TCIM expression alters myeloid gene expression toward lipid metabolism and debris clearance

To explore the biological processes regulated by TCIM, we assembled a list of genes related to “immune” or “leukocyte” GO terms (Table S3). We performed a hypergeometric probability test to identify the GO term that is most associated with each cluster (Figure S5B). The proportions of cytokine-producing leukocytes decreased by 61.3% upon TCIM expression (Figures 5G and 5H). Conversely, the proportions of leukocytes that were enriched for “phagocytosis engulfment” genes increased by 55.3% in TCIM-expressing fish compared to controls (Figures 5G and 5H). We also found “phagocytosis engulfment” gene expression was specifically enriched in TCIM-expressing leukocytes (Figure S5C). Confirming that similar gene expression changes were detectable in the absence of *tcim*, phagocytic genes *psma3* and *rac1a* were reduced in *tcim* mutants (Figures S5D–S5I). On the other hand, expansion in *psma3* and *rac1a* were present in TCIM-expressing fish compared to controls (Figures S5D–S5I). We identified the top markers for the dividing myeloid (cluster 2), BAM (clusters 5, 10, and 29), and dendritic (cluster 3) cell clusters for unbiased GO analysis. Consistent with their dendritic cell identity, cluster 3 cells were enriched for genes involved in antigen processing (Table S4). Top markers of the BAM clusters were implicated in vascular processes (Table S4). Notably, genes related to phagocytic cup formation were elevated, while negative regulators of lipid metabolism were depleted in TCIM-expressing macrophages (Table S4). These results indicated human TCIM reprograms myeloid cells to enhance phagocytic capacity.

### Tcim directs debris engulfment and lipid processing during SC regeneration

To determine if phagocytosis of cellular debris is altered in *tcima/b* mutants, we first measured lesion-associated myelin debris. At 14 dpi, myelin engulfment decreased 1.9-fold in *tcima/b* mutants compared to controls (Figures S6A and S6B). To confirm these results, an *in vivo* phagocytosis assay was performed at 3 dpi (Figure 6A). Relative to control leukocytes, *tcima/b*<sup>−/−</sup> leukocytes engulfed significantly less phagocytic cargo (Figures 6B–6D). To examine whether lipid processing is defective in *tcima/*

*b* mutant leukocytes, we stained neutral lipids using boron dipyrromethene (BODIPY) dye. In control animals, lipid droplets were readily seen in amoeboid Lcp1<sup>+</sup> cells at 5 dpi but were drastically reduced at 14 dpi, indicating efficient lipid processing and clearance of foamy leukocytes under control conditions (Figure 6E). Conversely, the engulfment of BODIPY<sup>+</sup> droplets was reduced by 48.4% in *tcima/b* mutants at 5 dpi (Figures 6E–6G). At 14 dpi, *tcima/b* mutants showed persistent lipid droplet-containing Lcp1<sup>+</sup> cells, in addition to large BODIPY<sup>+</sup> liposomes in the extracellular space (Figures 6E–6G). These studies indicated reduced phagocytic capacity and compromised clearance of intracellular lipids in *tcima/b* mutants.

### Tcim interacts with proteins necessary for lipid binding and degradation

To determine putative binding partners of zebrafish Tcima, we immunoprecipitated Tcima with protein lysates collected from either whole zebrafish larvae at 2 days post-fertilization or adult SC tissues at 3 dpi (Figures 6H and S6C). Trapped ion mobility (timsTOF) mass spectrometry of immunoprecipitated protein complexes identified 322 putative Tcima-binding partners, in addition to Tcima (Figures 6I and S6D; Table S5). Our Tcima interactome enriched for genes implicated in cytoskeletal organization, fatty acid degradation, the complement pathway, and phago/endocytosis (phagosome) (Figures 6J and S6E; Table S5). We confirmed direct interactions between Tcima and myelin-binding protein (Mbpa), complement opsonins (C3a.4, anaphylatoxin fragment only), and an enzyme that catalyzes fatty acid degradation (Hadhb) (Figures S6F–S6H). These results suggested that Tcima is interacting with both phagolysosomal and mitochondrial proteins during SC regeneration.

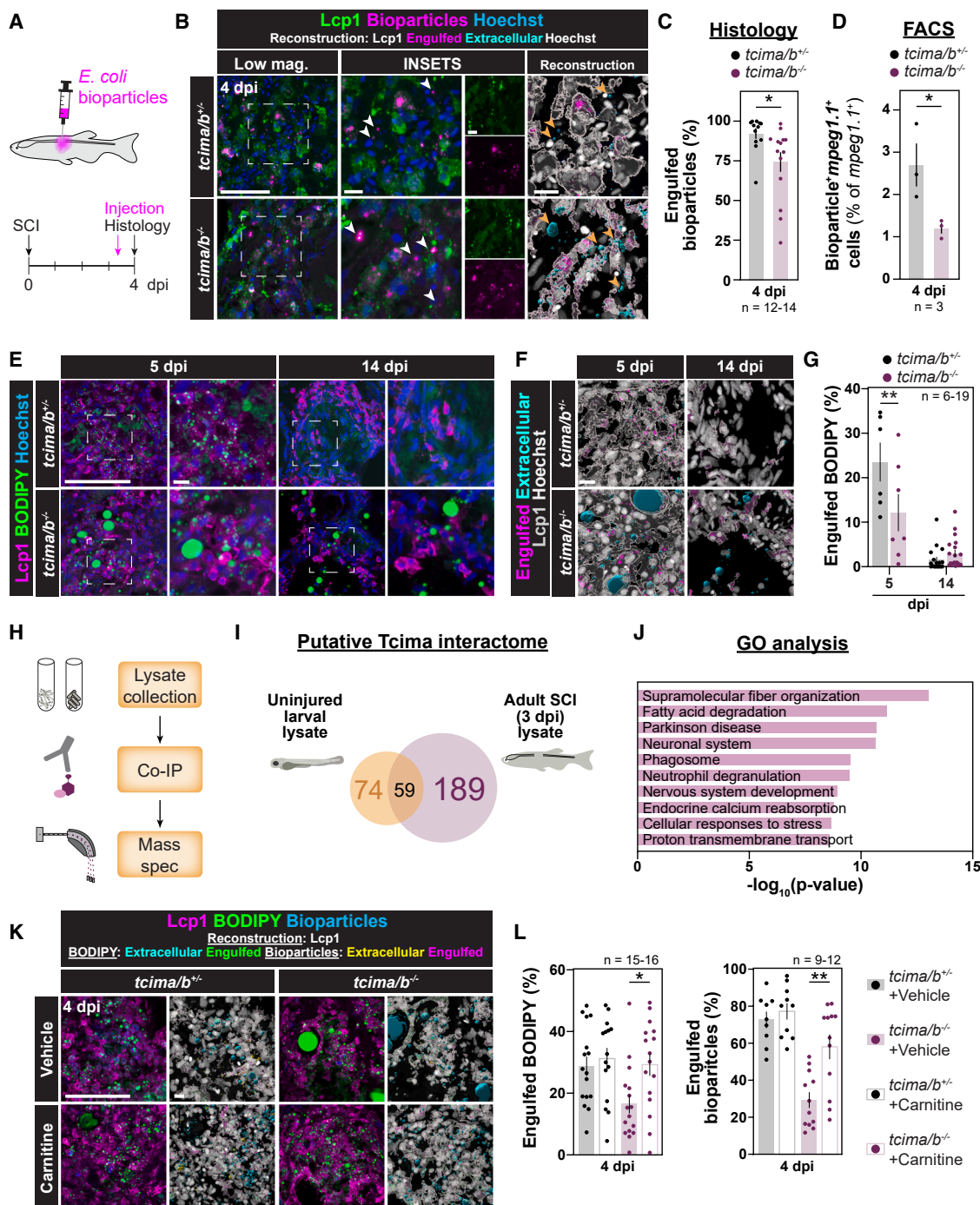
### Stimulating lipid breakdown rescues debris and lipid processing in tcim mutants

To test if mitochondrial lipid breakdown is downstream of Tcim, we stimulated lipid catabolism by injecting L-carnitine<sup>72–75</sup> into the lesion site in *tcima/b* mutant and control siblings at 3 dpi. At 4 dpi in control animals, total BODIPY<sup>+</sup> area decreased by 50.6% following L-carnitine injection compared to vehicle-injected controls (Figures 6K and 6L). Whereas vehicle-treated *tcima/b* mutants accumulated large extracellular lipid deposits, L-carnitine treatment normalized lipid distribution in *tcima/b* mutants (Figures 6K and 6L). In an *in vivo* phagocytosis assay, phagocytic clearance was 2-fold improved in L-carnitine-injected *tcima/b* mutants relative to vehicle treatment (Figures 6K and 6L). These results indicated that stimulating fatty acid oxidation is sufficient to rescue phagocytosis and lipid processing defects in *tcima/b* mutants. These data suggested mitochondrial lipid catabolism is downstream of Tcim.

(F) Proportions of the leukocyte subclusters shown in (E). Leukocyte subtypes that changed upon TCIM expression are shown in bold. Homeostatic microglia (Hom. MG), dividing myeloid (Div. mye.), border-associated macrophages (BAMs), neutrophils (Neut.), dendritic cells (DCs), dividing microglia (Div. MG), interferon myeloid (Int. mye.), activated microglia (Act. MG), Unidentified (Unid.).

(G) UMAPs for leukocytes colored based on the most enriched immune-related GO term within the top marker genes from each subcluster. GO term subclusters that change in proportion upon TCIM expression are indicated in (G').

(H) Leukocyte proportions based on GO term subclustering shown in (G). Leukocyte subtypes that change between TCIM-expressing and control fish are shown in bold. See also Figures S3, S4, and S5 and Tables S2–S4.



**Figure 6. Tcim directs the phagocytic capacity of microglia/macrophages during SC regeneration**

(A) Schematic of *in vivo* phagocytosis assays in *tcima/b<sup>-/-</sup>* animals and control siblings.  
(B and C) Histology of bioparticle-injected *tcima/b<sup>-/-</sup>* and control fish. Imaris was used to partition bioparticles into Extracellular (cyan) or Engulfed (magenta) channels based on Lcp1 surfaces (gray). Unpaired *t* test (Welch's): \**p* < 0.05.  
(D) FACS of a phagocytosis assay in *Tg(mpeg1.1:YFP);tcima/b<sup>-/-</sup>* and *Tg(mpeg1.1:YFP);tcima/b<sup>+/+</sup>* fish. Unpaired *t* test: \**p* < 0.05.  
(E–G) BODIPY and Lcp1 staining in *tcima/b<sup>-/-</sup>* animals and control siblings. In (F), Imaris reconstructions. One-way ANOVA (Sidak's): \*\**p* < 0.01. (H) Recombinant GST-Tcim was used for immunoprecipitation (coIP) with whole larvae or injured SC protein lysates. CoIP was followed by timsTOF mass spectrometry.

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### Tcim expression enhances the phagocytic capacity of macrophages across species

We next determined if TCIM expression was sufficient to enhance myelin debris clearance in TCIM-expressing fish. A decrease in bright Mbp<sup>+</sup> punctae was observed as early as 3 dpi in TCIM-expressing fish compared to controls (Figures S7A and S7B). To test whether TCIM accelerates SC repair by promoting phagocytic clearance, we performed an *in vivo* phagocytosis assay in TCIM-expressing fish after SCI. Compared to control siblings, TCIM expression reduced total bioparticle volume by 64.1% at 4 dpi (Figures 7A and 7B). This enhanced phagocytic capacity correlated with increased lipid clearance, as TCIM expression enhanced lipid engulfment by 54.6% at 3 dpi, compared to control siblings (Figures 7C and 7D). These data suggest that human TCIM enhances SC regeneration by accelerating the engulfment and clearance of lipid-rich debris following SCI.

To test the conservation of *Tcim* gene function in a mammalian system, we treated mouse Raw 264.7 macrophages with pHrodo-conjugated bioparticles, which will only fluoresce in acidic cellular environments, such as the lysosome. Indicating increased bioparticle processing upon *Tcim* expression, engulfed pHrodo<sup>+</sup> was 2.7-fold higher in cells transfected with *Tcima*-EGFP compared to control cells (Figures 7E and 7F). To assess lipid uptake and lipid metabolism, we imaged and chased Bodipy labeling upon *Tcim* expression (Figures 7G and 7H). By quantifying Bodipy puncta, we found lipid uptake was significantly increased in *Tcim*-expressing cells compared to controls, particularly at t0. To selectively assess the rates of lipid metabolism in this experiment, we normalized BODIPY levels for each group to t0. This analysis confirmed that *Tcim*-expressing macrophages cleared BODIPY-labeled lipids more efficiently than control cells. Supporting a model in which *Tcim*-mediated lipid clearance requires carnitine-mediated transport of fatty acids into the mitochondria, inhibiting carnitine synthesis with mildronate was sufficient to block the *Tcim*-driven increase in lipid clearance. These data suggested that expression of *Tcim* is sufficient to promote lipid uptake, lipid breakdown, and the phagocytic capacity of mouse macrophages and support a conserved phagocytic role for *Tcim* in zebrafish and mammals.

### DISCUSSION

This study suggests that phagocytic competence is one of the factors that regulates differential regenerative capacity between mammals and zebrafish after spinal cord injury. By elucidating the cellular landscape and molecular identities of immune cells after SCI, we found that *Tcim* reprograms dividing myeloid cells into phagocytic macrophages to support efficient debris clearance and innate SC repair.

Zebrafish elicit pro-regenerative neuronal and glial cell responses that direct SC repair.<sup>76</sup> Our comparative studies are consistent with zebrafish macrophages eliciting superior phago-

cytic capacity, which enables debris clearance prior to neuronal or glial repair. Zebrafish possess myelin-associated proteins such as NogoA and chondroitin sulfate proteoglycans (CSPGs),<sup>77,78</sup> which play complex inhibitory roles after mammalian SCI.<sup>79,80</sup> While the inhibitory effects of NogoA are thought to be dampened in fish compared to mammals,<sup>77</sup> regeneration is transiently improved in zebrafish treated with the bacterial enzyme chondroitinase ABC (ChABC) within 7 days of injury.<sup>78</sup> Our studies indicate that within the lesion site, lipid-rich debris are cleared prior to axon regrowth and that manipulations that delay sub-acute debris clearance impair regeneration. Moreover, our comparative studies showed regulators of extracellular matrix (ECM) breakdown were preferentially upregulated in zebrafish leukocytes (Table S1). We propose that enhanced ECM breakdown and efficient phagocytosis by fish leukocytes underscore elevated regenerative capacity of zebrafish. We expect future comparisons and zebrafish studies will contribute new avenues to fine-tune immune SCI responses for neural repair.

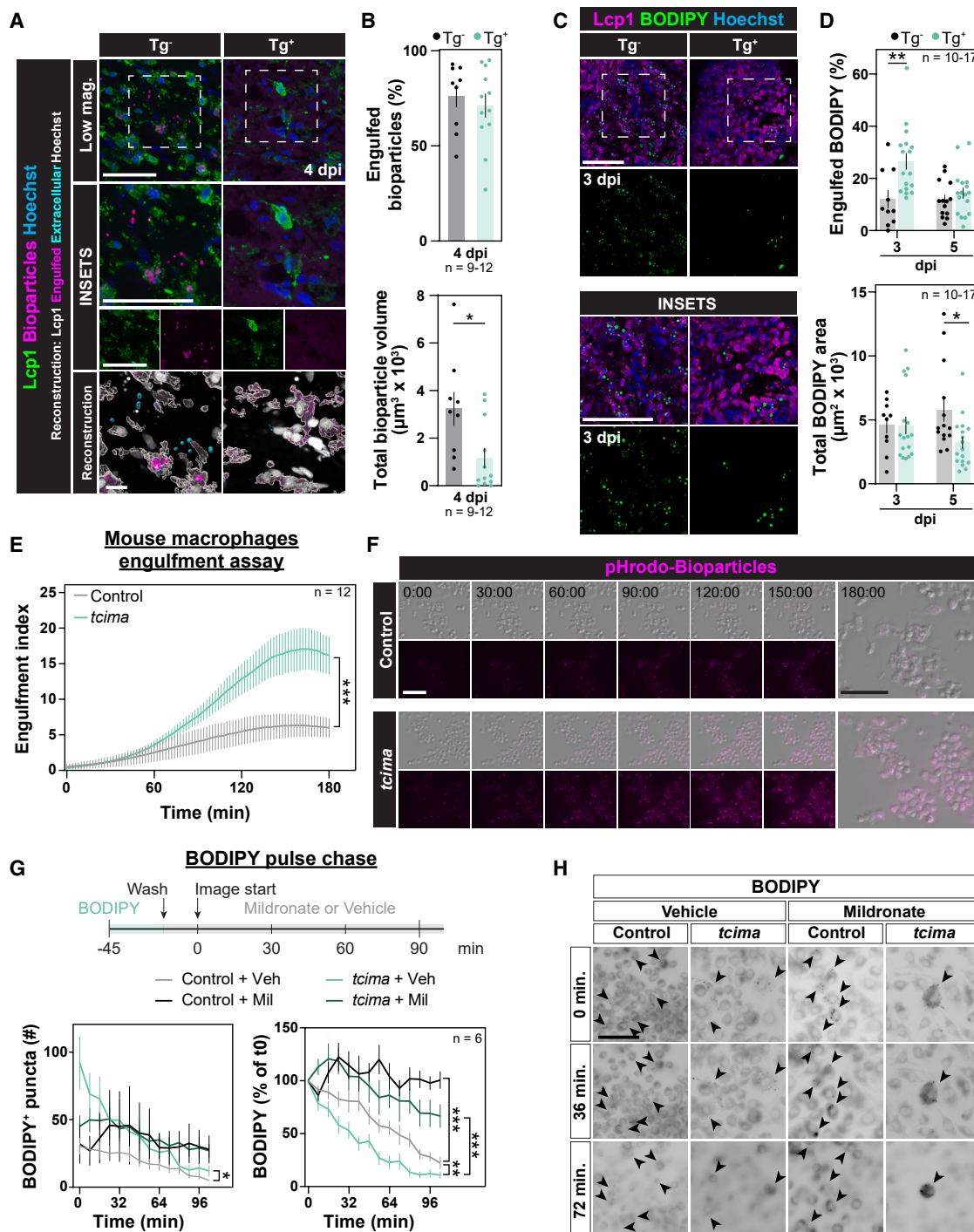
The metabolic state of myeloid cells influences their cellular identity.<sup>81–84</sup> Our results support a model whereby zebrafish macrophages modulate their metabolic state and phagocytic output to support SC repair. Biomolecule catabolism is necessary for monocytes and myeloid precursors to differentiate into macrophages,<sup>85,86</sup> while adopting a phagocytic phenotype is sufficient to shift macrophages toward a reactive phenotype during muscle regeneration.<sup>87</sup> Lipid processing is a chief component of biomolecule catabolism. Lipid-laden myeloid cells that fail to process and recycle lipids transition into a hyper-inflammatory, foamy phenotype.<sup>42</sup> On the other hand, lipid breakdown and fatty acid oxidation enhance phagocytic capacity and promote a neuroprotective phenotype in microglia in mouse models of neurodegeneration.<sup>88</sup> While limited lipid processing and foamy macrophages persist months to years after mammalian SCI,<sup>5,40</sup> we found that lipid-rich *mpeg1.1*<sup>+</sup> cells are transient and disappear by 14 dpi in zebrafish. We propose that efficient lipid processing stimulates a feedforward loop that enhances phagocytic capacity in zebrafish macrophages.

Our data support a model whereby *Tcim* directs lipid processing and debris clearance to promote SC repair. Consistent with disrupted lipid homeostasis and impaired phagocytosis, lipid droplets and foam cells persist for weeks in *tcima/b* mutant SC tissues. Conversely, TCIM expression is sufficient to enhance phagocytosis and carnitine-mediated lipid processing in zebrafish and mouse macrophages. Since lipid and biomolecule breakdown shifts myeloid cells toward differentiation or activation,<sup>85,87</sup> it is plausible that TCIM alters immune cell composition by enhancing leukocytic lipid catabolism. However, noting that phagocytosis could also be indirectly enhanced by improving lipid metabolism and energy supply in macrophages, it is equally possible that the phagocytic effects of *Tcim* are secondary or independent of its role in lipid metabolism. *Tcima* interacts with proteins in both the phagolysosomal and mitochondrial fatty acid oxidation pathways including the fatty acid beta-oxidation

(I and J) Putative *Tcima* interacting proteins (I) were analyzed for GO term enrichment (J).

(K and L) Histology of carnitine- or vehicle-injected *tcima/b*<sup>-/-</sup> and control fish. All animals were subjected to a phagocytosis assay and stained with BODIPY and Lcp1. two-way ANOVA (Tukey's): \**p* < 0.05, \*\**p* < 0.01. Error bars represent SEM. Scale bars in insets and Imaris reconstructions, 10 μm. All other scale bars, 100 μm. See also Figure S6 and Table S5





**Figure 7. Expression of human TCIM enhances the phagocytic capacity of macrophages during SC regeneration**

(A and B) Phagocytosis assay in TCIM-expressing and control fish. Unpaired *t* test (Welch's): \**p* < 0.05.

(C and D) BODIPY and Lcp1 staining in TCIM-expressing and control fish.

One-way ANOVA (Sidak's): \**p* < 0.05, \*\**p* < 0.01. Comparisons are between TCIM-expressing fish and their time-matched control groups.

(E and F) Engulfment assay for cultured Raw 264.7 mouse macrophages. The engulfment index indicates the pHrodo<sup>+</sup> area divided by the total cell area. An unpaired *t* test was performed comparing the area under the curve: \*\*\**p* < 0.001.

(G and H) BODIPY pulse-chase in cultured Raw 264.7 mouse macrophages. Vehicle control (Veh) and mildronate-treated (Mil). The numbers of Bodipy puncta (left graph) and the proportions of Bodipy signals normalized to T0 (right graph) are shown. An unpaired *t* test was performed comparing the area under the curve: \*\*\**p* < 0.001. Error bars represent SEM. Scale bars in Imaris reconstruction, 10  $\mu$ m. All other scale bars, 100  $\mu$ m. See also Figure S7.



enzyme hydroxyacyl-CoA dehydrogenase (Hadhb). Lysosomes and mitochondria communicate via lipid chaperones and enzymes to increase lipid metabolism under cellular stress.<sup>89</sup> More work is needed to test whether *Tcim* facilitates crosstalk between these organelles to enhance lipid breakdown and boost phagocytic flux.

Our findings suggest TCIM function is conserved across vertebrates but that its expression is not. Supporting a conserved role for *tcim* in larval and adult zebrafish, *tcim* is also upregulated in larval zebrafish SCI.<sup>31</sup> Murine *Tcim* is expressed by microglia and macrophages in uninjured SCs and at 1 dpi but is downregulated at 7 dpi,<sup>52,90</sup> whereas human TCIM is not detectable in homeostatic microglia or macrophages.<sup>91</sup> Though microglial *Tcim* expression is also unchanged in a mouse hemisection injury model,<sup>92</sup> we cannot rule out that the species-specific gene expression differences we report in this study may be due to differences in injury models (full transection in zebrafish versus crush injuries in mouse). Nevertheless, we show that human TCIM accelerates regeneration in zebrafish and that overexpression of zebrafish *Tcim* in mouse macrophages enhances phagocytic capacity and lipid clearance, providing a proof-of-principle study for *Tcim*-based gene therapies to promote debris clearance following mammalian SCI.

Our findings suggest phagocytic debris clearance as a key target to promote neural regeneration. Debris accumulation and phagocytosis impact functional outcomes in models of neural injury,<sup>93–95</sup> multiple sclerosis,<sup>96,97</sup> and neurodegeneration.<sup>98–100</sup> We propose that zebrafish present a valuable model and screening platform to devise pro-regenerative immune cell interventions for neural repair.

## LIMITATIONS OF THIS STUDY

While our study identifies *tcim* as a major determinant of debris clearance and lipid metabolism after spinal cord injury, questions remain for future investigation. It is unclear if *Tcim* enhances phagocytosis directly or if phagocytosis is accelerated due to indirect effects on lipid metabolism. Additionally, more work is needed to identify the precise molecular mechanism whereby *Tcim* boosts lipid metabolism. Further, we cannot rule out if differences in injury models between mouse and zebrafish are partially responsible for the gene expression differences in these two species. An additional consideration is that molecular or genetic tools are underdeveloped in zebrafish to effectively delineate between SC-resident microglia and peripheral macrophages. Finally, the SC environment is complex, with many cell types and molecules burdening regeneration. While this study focused on debris phagocytosis and lipid breakdown by leukocytes, more work is needed to determine other immune and non-immune mechanisms dictating differential regenerative capacity across species.

## RESOURCE AVAILABILITY

### Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Dr. Mayssa Mokalled ([mmokalled@wustl.edu](mailto:mmokalled@wustl.edu)).

## Materials availability

Animals and DNA plasmids generated in this study will be made available on request from the lead contact without restriction.

## Data and code availability

- The data underlying histology in [Figures 1–7](#) and [S1–S7](#) are available from the corresponding author upon request. Transcriptomic and proteomic data underlying [Figures 3](#) and [5](#), and [S2–S5](#) are available through the NCBI Gene Expression Omnibus: GSE268474, GSE268473, GSE235395, and GSE162610.
- Previously unpublished code used for data analysis has been deposited on Github (<https://doi.org/10.5281/zenodo.11585743>).
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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## AUTHOR CONTRIBUTIONS

Conceptualization, D.K.S. and M.H.M.; resources, D.K.S., V.M.S., A.R.M., L.Z., D.P., R.M., A.S., A.J.D., K.S., and B.U.; data curation, D.K.S. and V.M.S.; formal analysis, D.K.S.; investigation, D.K.S. and V.M.S.; visualization, D.K.S.; methodology, D.K.S., V.M.S., D.P., C.H., A.N.J., and M.H.M.; writing – original draft, D.K.S. and M.H.M.; writing – review and editing, D.K.S. and M.H.M.; funding acquisition, D.K.S. and M.H.M.; supervision, C.G.G., A.N.J., and M.H.M.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

## STAR★METHODS

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

- **KEY RESOURCES TABLE**
- **EXPERIMENTAL MODEL DETAILS**
  - Zebrafish care and husbandry
  - Mouse care
  - Culturing of raw 264.7 macrophages
  - Culturing of HEK293 cells
- **METHOD DETAILS**
  - Generation of new zebrafish lines
  - Zebrafish procedures
  - Zebrafish histology
  - Mouse histology
  - Fluorescence-activated cell sorting (FACS)
  - Bulk RNA-sequencing

- Single nuclear RNA-sequencing
- Co-immunoprecipitation of tcima
- Raw 264.7 macrophage live imaging
- Cloning expression plasmids for cell culture
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

### SUPPLEMENTAL INFORMATION

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## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE                                        | SOURCE                                                                                                                                  | IDENTIFIER                              |
|------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|
| <b>Antibodies</b>                                          |                                                                                                                                         |                                         |
| Chicken anti-GFP                                           | AVES Labs                                                                                                                               | Cat# 1020; RRID: AB_10000240            |
| Mouse anti-Gfap                                            | ZIRC                                                                                                                                    | Cat# Zrf1; RRID: AB_10013806            |
| Rabbit anti-Lcp1                                           | Michael Redd                                                                                                                            | N/A                                     |
| Rabbit anti-Mbp                                            | Appel Lab, CU Anschutz                                                                                                                  | N/A                                     |
| Rat anti-CD68 FA-11                                        | Bio-rad                                                                                                                                 | Cat# MCA1957T; RRID: AB_2074849         |
| Mouse anti-Flag M2                                         | Sigma Aldrich                                                                                                                           | Cat# F1804; RRID: AB_262044             |
| Mouse anti-HA                                              | Cell Signaling Technology                                                                                                               | Cat# 3724; RRID: AB_1549585             |
| Goat anti-chicken 488                                      | Jackson Immuno                                                                                                                          | Cat# 115-605-003; RRID: AB_2338902      |
| Goat anti-mouse 594                                        | Jackson Immuno                                                                                                                          | Cat# 115-585-003; RRID: AB_2338871      |
| Goat anti-mouse 647                                        | Jackson Immuno                                                                                                                          | Cat# 115-605-003; RRID: AB_2338902      |
| Goat anti-rabbit 594                                       | Jackson Immuno                                                                                                                          | Cat# 111-585-003; RRID: AB_2338059      |
| Goat anti-rabbit 674                                       | Jackson Immuno                                                                                                                          | Cat# 111-605-003; RRID: AB_2338072      |
| <b>Chemicals, peptides, and recombinant proteins</b>       |                                                                                                                                         |                                         |
| Synaine (Tricaine methylsulfonate)                         | Fisher Scientific                                                                                                                       | Cat# 15070063                           |
| Buprenorphine                                              | Par Pharmaceutical Chestnut Ridge                                                                                                       | Cas# 53152-21-9                         |
| Penicillin-streptomycin                                    | ThermoFisher                                                                                                                            | Cat# 15070063                           |
| L-Glutamine                                                | Invitrogen                                                                                                                              | Cat # 25030081                          |
| Cas9                                                       | PNA BIO                                                                                                                                 | Cat# CP01                               |
| Metronidazole                                              | Sigma Aldrich                                                                                                                           | Cat# M1547-25G                          |
| L-carnitine                                                | Sigma Aldrich                                                                                                                           | Cat# 8400920025                         |
| Alexa Fluor 594 conjugate                                  | ThermoFisher                                                                                                                            | Cat# E23370                             |
| Gelfoam Gelatin Sponge                                     | Pfizer                                                                                                                                  | Cat# 09-0315-08                         |
| Biocytin, saturated solution                               | Sigma Aldrich                                                                                                                           | Cat# B4261                              |
| AlexaFluor-594-conjugated Streptavidin                     | Molecular Probes                                                                                                                        | Cat# S-11227                            |
| Escherichia coli Bioparticles Alexa Fluor 594 conjugate    | ThermoFisher                                                                                                                            | Cat# E23370                             |
| pHrodo Red E. coli Bioparticles Conjugate for Phagocytosis | ThermoFisher                                                                                                                            | Cat# P35361                             |
| Fluoromount-G                                              | SouthernBiotech                                                                                                                         | Cat# 0100-01                            |
| BODIPY 493/503                                             | Fisher Scientific                                                                                                                       | Cat# NC1707119                          |
| Sodium pentobarbital                                       | VPL                                                                                                                                     | Cat# 9373                               |
| Mildronate (Meldonium dihydrate)                           | Millipore Sigma                                                                                                                         | Cat# M5199                              |
| <b>Critical commercial assays</b>                          |                                                                                                                                         |                                         |
| Hybridization Chain Reaction (HCR) RNA <i>in situ</i> Kit  | Molecular Instruments                                                                                                                   | N/A                                     |
| LIVE/DEAD cell stain                                       | ThermoFisher                                                                                                                            | Cat# L34973                             |
| SMARTer Ultra Low RNA kit for Illumina Sequencing          | Takara-Clontech                                                                                                                         | Cat# 634936                             |
| Chromium Next GEM Single Cell 3' Kit v3.1                  | Chromium                                                                                                                                | Cat# PN-1000268                         |
| Chromium Next GEM Chip G Single Cell Kit                   | Chromium                                                                                                                                | Cat# PN-1000120                         |
| Dual Index Kit TT Set A                                    | Chromium                                                                                                                                | Cat# PN-1000215                         |
| Immobilon Western HRP Substrate                            | Millipore Sigma                                                                                                                         | Cat# WBKLS0500                          |
| pENTR/D-TOPO cloning kit                                   | ThermoFisher                                                                                                                            | Cat# K240020                            |
| <b>Deposited data</b>                                      |                                                                                                                                         |                                         |
| <i>tcima/b</i> mutant bulk RNA-sequencing                  | <a href="https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE268474">https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE268474</a> | NCBI Gene Expression Omnibus: GSE268474 |

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**Continued**

| REAGENT or RESOURCE                                                            | SOURCE                                                                                                                                    | IDENTIFIER                              |
|--------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|
| TCIM-expressing snRNA-sequencing                                               | <a href="https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE268473">https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE268473</a>   | NCBI Gene Expression Omnibus: GSE268473 |
| Wild-type single-cell analysis of innate spinal cord regeneration in zebrafish | <a href="https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM7501733">https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM7501733</a> | NCBI Gene Expression Omnibus: GSE235395 |
| Single cell analysis of the injured mouse spinal cord                          | <a href="https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE162610">https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE162610</a>   | NCBI Gene Expression Omnibus: GSE162610 |

**Experimental models: Cell lines**

|                       |      |               |
|-----------------------|------|---------------|
| Raw 264.7 macrophages | ATCC | Cat# TIB-71   |
| HEK 293T cells        | ATCC | Cat# CRL-1573 |

**Experimental models: Organisms/strains**

|                                                      |                       |                 |
|------------------------------------------------------|-----------------------|-----------------|
| Tubingen zebrafish                                   | ZIRC                  | Tu              |
| Ekkwill zebrafish                                    | Ekkwill Breeders (FL) | EKW             |
| AB zebrafish                                         |                       | AB              |
| <i>Tg(mpeg1.1:YFP)<sup>w200</sup></i>                | Monk Lab, OHSU        | w200            |
| <i>Tg(lyz:EGFP)<sup>nz117</sup></i>                  | Roh-Johnson Lab, Utah | nz117           |
| <i>Tg(lck:lck-EGFP)<sup>cz2</sup></i>                | Tanner Lab, NIH       | cz2             |
| <i>Tg(mpeg1.1:NTR-IRES-EGFP-CAAX)<sup>co57</sup></i> | Appel Lab             | co57            |
| <i>Tg(act2b:membrane-Citrine)<sup>hm30</sup></i>     | Tsai Lab              | hm30            |
| <i>tcima<sup>stl661</sup></i>                        | This study            | stl661          |
| <i>tcimb<sup>stl662</sup></i>                        | This study            | stl662          |
| <i>Tg(hsp70L:hTCIM-2A-EGFP)<sup>stl682</sup></i>     | This study            | stl682          |
| C57BL/6 mice                                         | JAX                   | IMSR_JAX:000664 |

**Oligonucleotides**

|                                                   |            |     |
|---------------------------------------------------|------------|-----|
| Primers for genotyping and cloning (See Table S7) | This study | N/A |
| HCR probe oligo pools                             | This study | N/A |

**Recombinant DNA**

|                                           |               |                 |
|-------------------------------------------|---------------|-----------------|
| <i>pCS2-hsp70L:hTCIM-2A-EGFP</i>          | This study    | N/A             |
| <i>pGEX-4T-1</i>                          | Sigma Aldrich | Cat# 28-9546-49 |
| <i>pDest-CMV-3xHA-Tcima-EGFP</i>          | This study    | N/A             |
| <i>pDest-CMV-3xHA-gateway-EGFP</i>        | This study    | N/A             |
| <i>pDest-CMV-3xFLAG-gateway-EGFP</i>      | Addgene       | Cat# 122845     |
| <i>pDest-CMV-3xFLAG-acadvl-EGFP</i>       | This study    | N/A             |
| <i>pDest-CMV-3xFLAG-apoa4b-EGFP</i>       | This study    | N/A             |
| <i>pDest-CMV-3xFLAG-c3a.4_anatox-EGFP</i> | This study    | N/A             |
| <i>pDest-CMV-3xFLAG-cpt2-EGFP</i>         | This study    | N/A             |
| <i>pDest-CMV-3xFLAG-crata-EGFP</i>        | This study    | N/A             |
| <i>pDest-CMV-3xFLAG-habp2-EGFP</i>        | This study    | N/A             |
| <i>pDest-CMV-3xFLAG-hadhaa-EGFP</i>       | This study    | N/A             |
| <i>pDest-CMV-3xFLAG-hadhb-EGFP</i>        | This study    | N/A             |
| <i>pDest-CMV-3xFLAG-mbpa-EGFP</i>         | This study    | N/A             |
| <i>pDest-CMV-3xFLAG-tcima-EGFP</i>        | This study    | N/A             |
| <i>pDest-CMV-3xFLAG-trem2-EGFP</i>        | This study    | N/A             |
| <i>p3E-2A-mCherryCAAX</i>                 | Addgene       | Cat# 195971     |
| <i>pDest-3xHA-mCherry-EGFP</i>            | This study    | N/A             |

**Software and algorithms**

|                                    |        |                                                                                       |
|------------------------------------|--------|---------------------------------------------------------------------------------------|
| Fiji                               | N/A    | <a href="https://imagej.net/software/fiji/">https://imagej.net/software/fiji/</a>     |
| DAVID Functional Annotation Tool   | Online | <a href="https://david.ncifcrf.gov/tools.jsp">https://david.ncifcrf.gov/tools.jsp</a> |
| STRING Protein-protein interaction | Online | <a href="https://string-db.org/">https://string-db.org/</a>                           |

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**Continued**

| REAGENT or RESOURCE                        | SOURCE               | IDENTIFIER                                                                                                  |
|--------------------------------------------|----------------------|-------------------------------------------------------------------------------------------------------------|
| Metascape gene ontology                    | Online               | <a href="https://metascape.org/">https://metascape.org/</a>                                                 |
| Seurat (v4.1.1) package with R (v4.2.1)    | R Core Team          | N/A                                                                                                         |
| Amigo gene ontology database               | Online               | <a href="http://amigo.geneontology.org/amigo/">http://amigo.geneontology.org/amigo/</a>                     |
| ITCN: Image based Tool for counting nuclei | Online               | <a href="https://imagej.nih.gov/ij/plugins/itcn.htm">https://imagej.nih.gov/ij/plugins/itcn.htm</a>         |
| Imaris                                     | WUCCI                | N/A                                                                                                         |
| GraphPad Prism                             | N/A                  | <a href="https://www.graphpad.com/">https://www.graphpad.com/</a>                                           |
| Adobe Illustrator                          | Adobe Creative Cloud | <a href="https://www.adobe.com/creativecloud.html">https://www.adobe.com/creativecloud.html</a>             |
| a Plasmid Editor (aPE)                     | Online               | <a href="https://jorgensen.biology.utah.edu/wayned/aPe/">https://jorgensen.biology.utah.edu/wayned/aPe/</a> |

**Other**

|                                               |                 |                    |
|-----------------------------------------------|-----------------|--------------------|
| MEGAscript <sup>TM</sup> T7 transcription Kit | Invitrogen      | Cat# AM1354        |
| RNA clean & concentrator-25                   | Zymo Research   | Cat# R1017         |
| Hamilton gastight syringe                     | Hamilton        | Cat# 80008         |
| 5L swim tunnel device                         | Loligo          | Cat# SW100605L     |
| 100 $\mu$ m cell strainer                     | MIDSCI          | Cat# ASCCELLSTAIN2 |
| 30 G insulin syringe                          | BD Biosciences  | Cat# 328431        |
| Glutathione Sepharose 4B beads                | GE Healthcare   | Cat# 17-0756-01    |
| Poly-Prep Chromatography column               | Bio-rad         | Cat# 731-1550      |
| Lipofectamine-2000 Reagent                    | Invitrogen      | Cat# 15338100      |
| Lipofectamine-LTX Reagent with PLUS Reagent   | Invitrogen      | Cat# 15338100      |
| EZview Anti-HA Affinity Gel                   | Millipore Sigma | Cat# E6779         |
| Anti-Flag M2 Affinity Gel                     | Millipore Sigma | Cat# A2220         |
| Gateway LR Clonase II Plus                    | ThermoFisher    | Cat# 12538120      |
| NEBuilder HiFi DNA Assembly Master Mix        | NEB             | Cat# E2621L        |

## EXPERIMENTAL MODEL DETAILS

### Zebrafish care and husbandry

All zebrafish animal procedures were approved by the Washington University School of Medicine Institutional Animal Use and Care Committee (IACUC). Adult zebrafish of the Tubingen, AB, and Ekkwel strains were maintained at the Washington University Zebrafish Core Facility. All experiments were performed in compliance with institutional animal protocols. Animal breeding, lineages, and husbandry was cataloged and tracked using a comprehensive application for zebrafish husbandry management.<sup>101</sup> Male and female animals ~6 months of age were used. Experimental fish and control clutchmate siblings of similar size and equal sex distribution were used for all experiments. Spinal cord transection surgeries and regeneration analyses were completed in a blinded manner, and two to three independent experiments were performed using different clutches of animals. Previously published zebrafish lines used in the study are as follows: *Tg(mpeg1.1:YFP)*<sup>w200,102</sup> *Tg(lyz:EGFP)*<sup>nz117,103</sup> *Tg(lck:lck-EGFP)*<sup>cz2,104</sup> *Tg(mpeg1.1:NTR-IRES-EGFP-CAAX)*<sup>co57,49</sup> and *Tg(act2b:membrane-Citrine)*<sup>hm30,105</sup>. Newly constructed strains are described below.

### Mouse care

All experimental animal procedures were approved by the Texas A&M University Institutional Animal Use and Care Committee (IACUC). Male C57BL/6 mice were purchased from JAX (strain 000664). Mice were housed as described previously.<sup>106</sup> Mice were randomly assigned to SCI or sham-injury groups and underwent procedures at 76 days old. For SCI, mice were anesthetized by 3% induction and maintained on 1.5% isoflurane inhalation. The surgical site at thoracic T8 was shaved and sterilized with isopropyl alcohol. A partial dorsal laminectomy was performed at T8 to expose the SC. SCI was performed using the NYU-MASCIS weight-drop impactor at 50 kdyn with 2 s dwell time. Following SCI, back muscles were sutured, and skin was closed with surgical glue. Sham mice received only laminectomy. Following surgery, all mice received saline solution and 0.05 mg/kg of buprenorphine (Par Pharmaceutical Chestnut Ridge) daily for 3 days and 5 mg/kg penicillin (Bayer healthcare) daily for 7 days. Mice were monitored with manual bladder expression twice daily.

### Culturing of raw 264.7 macrophages

Raw 264.7 macrophages were acquired from the American Type Culture Collection (ATCC Lot # 70046149). Cells used for all experiments were of a low passage number (<20 passages). Raw 264.7 macrophages were maintained at 37°C and 5% CO<sub>2</sub> during culture. Raw 264.7 cells were cultured in DMEM/F-12 (Invitrogen cat# 11330032) supplemented with 10% fetal bovine serum (Sigma cat# F2442), 1 × L-Glutamine (Invitrogen cat# 25030081), and 500 U/mL of penicillin-streptomycin (ThermoFisher cat# 15070063). To passage, cells were treated with Trypsin EDTA (0.25%) (Invitrogen cat# 25200056) for 5 min at 37°C and then lifted off the plastic dish using Corning Falcon Cell Scrapers (ThermoFisher cat# 353085).

### Culturing of HEK293 cells

HEK-293 cells were acquired from ATCC. HEK-293 cells were maintained at 37°C and 5% CO<sub>2</sub> during culture. HEK-293 cells were cultured in DMEM/F-12 (Invitrogen cat# 11330032) supplemented with 10% fetal bovine serum (Sigma cat# F2442), 1 × L-Glutamine (Invitrogen cat# 25030081), and 500 U/mL of penicillin-streptomycin (ThermoFisher cat# 15070063). To passage, cells were treated with Trypsin EDTA (0.25%) (Invitrogen cat# 25200056) for 2 min at 37°C, resuspended in DMEM, and centrifuged at 500 g.

## METHOD DETAILS

### Generation of new zebrafish lines

**Generation of *tcima* and *tcimb* mutant zebrafish.** sgRNA was prepared by *in vitro* transcription of double-stranded deoxyoligonucleotide templates as described.<sup>107</sup> gRNA target sequences are as follows: *tcima* (GGCATGAAATCTAACTAAC and GGACAAGGCATAATGTTTAC) and *tcimb* (GGAACGGTTAGTGAACGCC and GGACAAATTCATGCTGATTA). sgRNA was transcribed from the annealed double-stranded oligonucleotide template using MEGAshortscript T7 transcription Kit (Invitrogen) according to the manufacturer's instructions. Transcribed sgRNA was purified using RNA clean & concentrator-25 (ZYMO Research) and recovered in distilled water. For microinjection, 25–30 ng/μL sgRNA was gently mixed with 0.5 μg/μL Cas9 (PNA BIO cat# CP01), and 0.5 μL 0.25% phenol red solution and diluted with RNase-free water to a final volume of 4 μL. Prior to microinjection, the RNP complex solution was incubated at 37°C for 5 min and then placed at room temperature. ~1 nL of 5 μM sgRNA:Cas9 RNP complex was injected into the cytoplasm of one-cell stage embryos.

The following primers were used for genotyping: *tcima*\_common\_F: ggccaccagaatttctaaaga, *tcima*\_wt\_R: ctccctcggttctatgtctc, and *tcima*\_mutant\_R: tgacaaagtcagtcatttaagaca, *tcimb*\_common\_F: ggcgacatcagtcggtattt, *tcimb*\_wt\_R: aacgccatgtctttctacgtg, *tcimb*\_mutant\_R: aagttcatgtctgctgcaga. The full name of the *tcim* mutant lines are *tcima*<sup>stl661</sup> and *tcimb*<sup>stl662</sup>.

**Generation of *Tg(hsp70L:hTCIM-2A-EGFP)* zebrafish.** The following forward primers were used to amplify human TCIM (hTCIM) cDNA: Clal forward primer 5'-gatcATCGATcgccaccatgaaagcaaagcgaagcc-3' and XhoI reverse primer 5'- gatcCTCGAGgtgaacttgatggaat-3' where the uppercase sequences indicate restriction enzyme sites. The genomic fragments were subcloned into Clal/XhoI-digested pCS2-hsp70L:2A-EGFP plasmid. Clones were co-injected into one-cell stage wild-type embryos with I-SceI. Five founders were isolated and propagated for this transgene. The highest expressing line was selected for our studies. The full name of this line is *Tg(hsp70L:hTCIM-2A-EGFP)*<sup>stl682</sup>. Animals were analyzed as hemizygotes.

### Zebrafish procedures

**Spinal cord transections and treatments.** Zebrafish were anesthetized in 0.2 g/L of MS-222 (Syncline, Fisher Scientific cat# 15070063) buffered to pH 7.0. Fine scissors were used to make a small incision that transects the spinal cord 4 mm caudal to the brainstem region. Complete transection was visually confirmed at the time of surgery. Injured animals were also assessed at 2 and 3 dpi to confirm loss of swim capacity post-surgery.

**Heat shock treatments.** Zebrafish were maintained in a circulating system retrofitted with a controlled heater (Tecniplast). Water temperature was increased to 37°C for 1 h per day and gradually returned to 28°C to induce transgene expression.

**Drug treatments.** For metronidazole (MTZ) treatments, MTZ (Sigma cat# M1547-25G) was dissolved directly into fish water to a concentration of either 5 mM (1 dpi treatments), 1 mM (pulsed treatments), or 10 mM (transplant experiments). Fish were placed in individual treatment beakers. 5 mM treatments lasted 18 h, while 1 mM or 10 mM treatments were for 24 h, in the dark. MTZ treatments were pulsed every other day intermingled with fresh fish water treatments. In the case of combining MTZ treatment with daily heat shocks, MTZ treatments were performed for 18 h in between daily heat shocks at standard water temperature (28°C). The dosing and timing of drug treatments were optimized prior to performing experiments, and effective depletion was confirmed using histology for Lcp1. In our hands, we did not observe EGFP expression from the IRES-EGFP cassette present in *Tg(mpeg1.1:NTR-IRES-EGFP)* fish.

For L-carnitine treatments, 100 mg/mL (2×) stock of L-carnitine (Sigma cat# 8400920025) was made in sterile water. On the day of injection, this stock was diluted to 50 mg/mL with 1 mg/mL *Escherichia coli* bioparticles Alexa Fluor 594 conjugate (ThermoFisher cat# E23370) as described in the phagocytosis assay section. Fish were placed upright in a slotted cellulose sponge and 5 μL of working solution was injected directly into the lesion site at 3 dpi with a Hamilton gastight syringe (cat# 80008). Dosing was determined based on previous administration of L-carnitine in zebrafish.<sup>108</sup>

**Swim endurance assays.** Zebrafish were exercised in groups of 8–15 in a 5L swim tunnel device (Loligo, cat# SW100605L, 120V/60Hz). After 15 min of acclimation (5 min with no flow, 5 min with 9 cm/s flow, then 5 min with 10 cm/s flow) inside the enclosed tunnel,

water current velocity was increased every two minutes and fish swam against the current until they reached exhaustion as described previously.<sup>109</sup> Exhausted animals were removed from the chamber without disturbing the remaining fish. Swim times at exhaustion were recorded.

**Anatomical regeneration assays.** Anterograde axon tracing was performed on adult fish. Fish were anesthetized using MS-222 and fine scissors were used to transect the cord 4 mm rostral to the lesion site. Biocytin-soaked Gelfoam Gelatin Sponge was applied at the new injury site (Gelfoam, Pfizer, cat# 09-0315-08; Biocytin, saturated solution, Sigma, cat# B4261). Fish were euthanized 4 h post-treatment and biocytin was histologically detected using AlexaFluor-594-conjugated Streptavidin (Molecular Probes, cat# S-11227).

**Zebrafish phagocytosis assays.** For phagocytosis assays, 5  $\mu$ L of 1 mg/mL *Escherichia coli* Bioparticles Alexa Fluor 594 conjugate (ThermoFisher cat# E23370) were injected directly below the sealed wound at 3 dpi using a Hamilton syringe while fish were anesthetized. After 10 s, fish were returned to fresh fish water. Fish were collected at 16 h after bioparticle injection for histology.

**Transplantation of whole kidney marrow.** For transplantation experiments, *Tg(mpeg1.1:NTR-IRES-EGFP)* fish in the *tcima/b*<sup>−/−</sup> background were treated for 24 h with 10 mM MTZ as described in the drug treatment section. Adult zebrafish were irradiated with 8 Gy radiation in the xStrahl Small Animal Radiation Research Platform (SARRP) in the Radiology Department at Washington University for two consecutive days. Individual fish were placed in 5 cm petri dishes in fresh fish water on the radiator platform in a single layer for irradiation. After each radiation dose, individual fish were recovered in beakers in 100–150 mL of fresh system water. The day after the second radiation dose, fish received dissociated marrow cells or vehicle.

To collect cells for transplantation, kidney tissues were dissected from *Tg(actb2:membrane-Citrine)*<sup>hm30</sup> donor fish (in either a wild-type or *tcima/b*<sup>−/−</sup> background) and placed in chilled HBSS+H (HBSS, 1% HEPES). Kidneys were treated with 0.05% Trypsin for 5 min at room temperature, and then washed with HBSS+H. Cells were dissociated with 30 brisk triturations in DMEM+20% FBS, placed through a 100  $\mu$ m cell strainer (MIDSCI cat# ASCCELLSTAIN2), and resuspended in HBSS+2% FBS. Phenol red was added (1:1000) in cell suspensions or in vehicle for injections. For each experiment, 250,000–350,000 donor cells or the same volume of empty HBSS+2% FBS vehicle were intraperitoneally (IP) injected using a 30 G insulin syringe (BD Biosciences cat# 328431). Transplanted cells were allowed 2 weeks to engraft prior to SCI.

## Zebrafish histology

Zebrafish were anesthetized in 0.2 g/L of MS-222 buffered to pH 7.0. Trunk tissues were dissected on ice and fixed overnight at 4°C in 4% paraformaldehyde. Samples were washed in PBS 3  $\times$  40 min at room temperature and incubated overnight in 30% sucrose at 4°C prior to embedding in OCT. Sixteen  $\mu$ m cross or 20  $\mu$ m longitudinal cryosections of paraformaldehyde-fixed SC tissues were used. Serial sections were collected –150  $\mu$ m apart for ~3 mm rostral and caudal to the transection site.

For immunohistochemistry, tissue sections were circumscribed with a hydrophobic barrier pen and rehydrated in PBT (0.1% Tween 20 in PBS). After 2  $\times$  5 min washes in PBT, sections were treated with blocking agent (5% goat serum in PBT) for 1 h at room temperature. Sections were incubated overnight with indicated primary antibodies diluted in blocking agent, washed in PBT, and treated for 1 h in secondary antibodies diluted in blocking agent. Following washes, sections were incubated in 1  $\mu$ g/mL of Hoechst, washed in PBS, and mounted in Fluoromount-G (SouthernBiotech cat# 0100-01) mounting media. Primary antibodies used in these zebrafish studies were chicken anti-GFP (AVES, cat# 1020, 1:1000), mouse anti-Gfap (ZIRC, cat# Zrf1, 1:1000), rabbit anti-Lcp1 (a kind gift from Michael Redd, 1:10000), and rabbit anti-Mbp (a kind gift from Bruce Appel, 1:1000). Secondary antibodies (Jackson Immuno, 1:250) used in this study were Alexa Fluor 488, Alexa Fluor 594, and Alexa Fluor 647 goat anti-rabbit, anti-mouse, or anti-chicken antibodies.

BODIPY 493/503 (Fisher Scientific cat# NC1707119) was reconstituted as a 1 mg/mL stock, which was diluted 1:250 in PBT at the time of use, and sections were incubated for 30 min in diluted BODIPY prior to several washes with PBT.

Hybridization Chain Reaction (HCR) RNA *in situ* hybridization protocol was adapted from Molecular Instruments (<https://www.molecularinstruments.com/hcr-ihc-protocols>).<sup>110</sup> HCR RNA probes used in this study (*tcima*, *tcimb*, *psma3*, and *rac1a*) were designed using a custom python script and ordered as 50 pmol opools (IDT). Oligo sequences are provided in Table S6. Briefly, tissue sections were hydrated in PBS, dehydrated into 100% ethanol, then rehydrated stepwise into PBT (0.1% Tween 20 in PBS). Sections were boiled in citrate buffer (10mM Citric Acid, 0.05% Tween 20, pH 6.0) for 10 min, allowed to cool to room temperature, and then washed thoroughly in PBT. For blocking, sections were incubated in prewarmed hybridization buffer (Molecular Instruments) for 1 h at 37°C. For hybridization, sections were incubated in prewarmed DNA probe sets diluted to 0.015 pmol/ $\mu$ L in hybridization buffer for 48 h at 37°C. Washes were performed in wash buffer (Molecular Instruments) at 37°C followed by 5 $\times$  SSCT (3M NaCl, 0.3 M Sodium Citrate, 0.1% Tween 20, pH 7.0) at room temperature. For pre-amplification, sections were incubated in amplification buffer (Molecular Instruments) for 1 h at room temperature. Prior to amplification, h1 and h2 hairpins were snap-cooled in individual tubes by heating to 95°C and allowing tubes to return to ambient temperature slowly. For amplification, snap-cooled hairpins were mixed and diluted 1:50 in Amplification buffer overnight at room temperature in the dark. Samples were washed in 5 $\times$  SSCT, 5 $\times$  SSC, and PBT at room temperature before proceeding with immunohistochemistry.

Tissue sections were imaged using a Zeiss AxioScan (Axio Scan.Z1, Objective: Plan-Apochromat 20 $\times$ /0.8 M77, Imaging device: OrcaFlash) widefield microscope for axon regrowth and glial bridging. A Zeiss LSM 800 confocal microscope (Axio Imager.Z2, Objective: Plan-Apochromat 20 $\times$ /0.8 M27 or Plan Apochromat 40 $\times$ /1.4/ $\infty$ /0.17, Detector: Multialkali-PMT) was used for all other fixed immunofluorescence. Zen software was used to acquire all fixed histology images. To image the lesion site, the imaging window



was set immediately dorsal to the vertebral column. For immune histology, parenchymal sections 150  $\mu$ m rostral (unless otherwise indicated) to the lesion were imaged.

### Mouse histology

For histology, mice were anesthetized with intraperitoneal injection of 100  $\mu$ L of Sodium pentobarbital (VPL cat# 9373) before being euthanized by perfusion with 4% paraformaldehyde in 1  $\times$  PBS. SCs were dissected, incubated overnight in 15% and 30% sucrose, and embedded in OCT. Embedded samples were sectioned longitudinally to a thickness of 25  $\mu$ m. Histology of mouse sections was completed as described above in the immunohistochemistry and HCR staining paragraphs. Oligo sequences for the *Tcim* HCR probe are listed in Table S6. The Rat anti-CD68 FA-11 primary antibody (Bio-rad cat# MCA1957T) was used at 1:500.

### Fluorescence-activated cell sorting (FACS)

Four mm SC tissue sections, including the lesion site plus additional rostral and caudal tissue proximal to the lesion, were collected from adult injured zebrafish or control uninjured siblings. Three biological replicates were used, and each replicate represents a pool of 4–5 SCs. Tissues were dissociated using 0.05% Trypsin for 5 min at room temperature and subjected to 3 washes with Hanks' Balanced Salt Solution (HBSS, Gibco). Cell supernatants were triturated in DMEM media with 20% fetal bovine serum using a 1000  $\mu$ L pipette and applied to a 100  $\mu$ m cell strainer (MidSci, cat# 100ICS). Dissociated cells were pelleted at 500 g for 5 min, resuspended in HBSS with 2% FBS, and sorted using a MoFlo Cell sorter machine. FACS experiments were performed at the Siteman Cancer Center flow cytometry core. Wild-type or *Tg(mpeg1.1:YFP)* animals were used to set up YFP FACS gates. For the AlexaFluor-conjugated bioparticle phagocytosis assays, wild-type animals were injected and used to set gates for Alexa Fluor 594. LIVE/DEAD cell stain (ThermoFisher cat# L34973) was used to label live cells. Sorted cells were collected into serum-supplemented DMEM for RNA extraction.

### Bulk RNA-sequencing

**RNA collection.** Total RNA was prepared using NucleoSpin® RNA Plus XS (Clontech, cat# 740990). RNA was prepared from 4 mm SC sections at 5 dpi in *tcima/b<sup>-/-</sup>* and wild-type controls for bulk RNA sequencing. 9–10 SCs were pooled for each replicate and 3 biological replicates were used. RNA sequencing was performed at the Genome Technology Access Center at Washington University. Total RNA integrity was determined using Agilent Bioanalyzer or 4200 TapeStation. Library preparation was performed with 10 ng of total RNA with a Bioanalyzer RIN score greater than 8.0. ds-cDNA was prepared using the SMARTer Ultra Low RNA kit for Illumina Sequencing (Takara-Clontech cat# 634936) per manufacturer's protocol. cDNA was fragmented using a Covaris E220 sonicator using peak incident power 18, duty factor 20%, cycles per burst 50 for 120 s cDNA was blunt ended, had an A base added to the 3' ends, and had Illumina sequencing adapters ligated to the ends. Ligated fragments were then amplified for 12–15 cycles using primers incorporating unique dual index tags. Fragments were sequenced on an Illumina NovaSeq-6000 using paired end reads extending 150 bases. All Metadata, raw data, and processed data associated with bulk RNA-sequencing can be found in the NCBI Gene Expression Omnibus: GSE268474).

**Gene regulatory network analysis.** Transcriptional modulators were identified using the DAVID Functional Annotation Tool (<https://david.ncifcrf.gov/tools.jsp>). Genes within the terms “transcription factor,” “transcriptional modulation” or “DNA binding” were included. To identify potential interactions between transcriptional modulators, the STRING tool for functional protein association networks was used (<https://string-db.org/>) with *Danio rerio* as the input species.

**Gene ontology analysis.** Gene ontology (GO) analysis was performed using Metascape (<https://metascape.org/>). Default parameters were used to cluster overlapping GO terms (minimum overlap of 3 genes, *p*-value cutoff of 0.01, minimum gene enrichment of 1.5). Gene sets selected for analysis were GO Biological Processes and KEGG Pathways. GO term titles were shortened in some cases for brevity in figures. The full list of term titles and hierarchical GO terms can be found in supplemental tables.

### Single nuclear RNA-sequencing

**Identification of pro-regenerative immune genes.** A snRNA-seq atlas of the regenerating zebrafish SC was used.<sup>51</sup> To identify top markers of microglia/macrophages, we filtered for genes that were expressed in >10% of the cells identified in the microglia/macrophage 1 cluster, enriched with a  $\log_2$ (fold enrichment) > 0.25 compared to all non-microglia/macrophage cells, and possessed a *p*-value of <0.01 with the Wilcoxon Rank-Sum test. Genes upregulated (>0.25  $\log_2$ (fold change)) at 7 dpi compared to uninjured were selected. To identify injury-induced mouse microglia marker genes, a single cell RNA-sequencing atlas of mouse SCI was used.<sup>52</sup> Selected top markers showed >10% cell expression in microglia,  $\log_2$ (fold enrichment) > 0.25 compared to all other cell clusters, and  $\log_2$ (fold change) > 0.25 after SCI (either up or down). Code for this analysis has been deposited on Github (Seurat\_Species\_Converter and DE\_Marker\_Scoring, DOI: <https://doi.org/10.5281/zenodo.11585743>).

**Isolation of spinal cord nuclei for RNA sequencing.** Nuclei were isolated as previously described.<sup>51</sup> Three mm SC tissue, flanking the lesion sites were collected from 35 to 39 adult zebrafish at 5 dpi from heat shocked (at 2, 3, and 4 dpi) *Tg(hsp70:hTCIM-2A-EGFP)* and wild-type siblings. For tissue lysis, SC tissues were homogenized at a low setting for 15 s. Density gradient separation using sucrose solution was used to sediment nuclei from the supernatant. Final nuclear lysates were resuspended using 100  $\mu$ L of

resuspension solution (1× PBS, 2% BSA, 0.2U/μL RNase inhibitor; New England Biolabs, cat# M0314S). Hoechst staining was performed to assess the quality of isolated nuclei based on their morphology. Samples in which more than 70% of the nuclei were scored as ‘healthy’ were submitted for snRNA-seq.

**Single nuclear RNA sequencing (snRNA-seq).** For snRNA-seq, 30 μL of isolated nuclei at a concentration of ~1000 nuclei/μL was submitted to Genome Technology Access Center at McDonnell Genome Institute of Washington University. Two biological replicates of each genotype were used. cDNA was prepared after GEM generation and barcoding, followed by the GEM-RT reaction and bead clean-up steps. cDNA was amplified for 11–13 cycles and purified using SPRIselect beads. Purified cDNA samples were run on a Bioanalyzer to determine the cDNA concentration. GEX libraries were prepared as recommended by the 10× Genomics Chromium Single Cell 3′ Reagent Kits User Guide (v3.1 Chemistry Dual Index) with appropriate modifications to the PCR cycles based on the calculated cDNA concentration. For sample preparation on the 10× Genomics platform, the Chromium Next GEM Single Cell 3′ Kit v3.1, 16 rxns (PN-1000268), Chromium Next GEM Chip G Single Cell Kit, 48 rxns (PN-1000120), and Dual Index Kit TT Set A, 96 rxns (PN-1000215) were used. The concentration of each library was accurately determined through qPCR utilizing the KAPA library Quantification Kit according to the manufacturer’s protocol (KAPA Biosystems/Roche cat# KK4824) to produce cluster counts appropriate for the Illumina NovaSeq6000 instrument. Normalized libraries were sequenced on a NovaSeq6000 S4 Flow Cell using the XP workflow and a 50 × 10 × 16 × 150 sequencing recipe according to manufacturer protocol. A median sequencing depth of 50,000 reads/cell was targeted for each Gene Expression Library. All metadata, raw data, and processed data associated with snRNA-sequencing from this study can be found in the NCBI Gene Expression Omnibus: GSE268473).

**Aligning snRNA-seq reads.** After sequencing, the Illumina output was processed using the Cell Ranger (v6.0.0) recommended pipeline to generate gene-barcode count matrices. A custom reference genome was generated with the “cellranger mkref” command using the fasta file of zebrafish reference genome GRCz11 constructed from the Ensemble genome build ([https://useast.ensembl.org/Danio\\_reio/Info/Index](https://useast.ensembl.org/Danio_reio/Info/Index)) and the sorted Gene Transfer Format file (v4.3.2) from the improved zebrafish transcriptome annotation.<sup>111</sup> Base call files for each sample from Illumina were demultiplexed into FASTQ reads. Then, the “cellranger count” pipeline was used to align sequencing reads in FASTQ files to the custom reference genome. Both exon and intron sequences were aligned. The filtered gene-barcode count matrices generated by “cellranger count” was used for downstream analysis.

**Integrated analysis of snRNA-seq data.** All the datasets were combined and analyzed using Seurat (v4.1.1) package with R (v4.2.1) (R Core Team, 2018<sup>112</sup>). Each sample count matrix was filtered for genes that were expressed in at least 3 cells and cells expressing at least 200 genes, followed by cell quality assessment using commonly used QC matrixes.<sup>113</sup> Cells having a unique number of genes between 200 and 4000 and a mitochondrial gene percentage <5 were used for downstream processing. Each dataset was independently normalized and scaled using the “SCTransform” function, which is an improved method for normalization, that performs a variance-stabilizing transformation using negative binomial regression.<sup>114</sup> Standard integration workflow of Seurat was used to identify shared sources of variation across experiments as well as mutual nearest neighbors.<sup>115,116</sup> Integration features were selected based on the top 4000 highly variable features using “SelectIntegrationFeatures” function (nfeatures = 4000), which was used as input for the “anchor.features” argument of the “FindIntegrationAnchors” function. Principal Component Analysis (PCA) analysis was performed on the 4000 variable features and the top 50 principal components selected based on the elbow plot heuristic, which measures the contribution of variation in each component. These 50 principal components were used in “FindNeighbors” and “FindClusters” functions to perform graph-based clustering on a shared nearest neighbor graph.<sup>117,118</sup> Louvain algorithm was used for modularity optimization in clustering the cells using “FindClusters” function. The resolution parameter (res = 0.5) that determines the granularity of the clustering was selected by visually inspecting clusters with resolutions ranging 0.1–2.0 as well as cluster-tree graphs.<sup>119</sup> Uniform Manifold Approximation and Reduction (UMAP) was used for non-linear dimensional reduction of the first 50 principal components and visualize the data using “RunUMAP” function.<sup>120</sup> Data were graphed using different plot functions, such as “DimPlot”, “VlnPlot”, “FeaturePlot”, “Dotplot” and “DoHeatmap”, to view the cell cluster identity and marker gene expression. Cell proportion data were extracted using “table” and “prop.table” functions. Differential gene expression for individual cluster was identified using Wilcoxon rank-sum test in the “FindAllMarkers” function. Marker genes detected in at least 25% of the clustered cells and with a log<sub>2</sub>(fold change) threshold of 0.25 were selected, unless mentioned otherwise. Only positive markers were reported.

**Subset analysis of leukocyte clusters.** Leukocytes identified from the complete dataset were subclustered using the “subset” function for subcluster analysis. The subset was normalized and scaled using “SCTransform” function with glmGamPoi method.<sup>121</sup> Fifty principal components were used, and the resolution parameter was set to 0.7. Further downstream analysis was done as described above for the integrated analysis.

**Cluster identification using differentially expressed markers.** Cell identities were determined as previously described.<sup>51</sup> Briefly, a “Vertebrate CNS Markers” database of previously published cell markers of vertebrate brain and/or SC tissues was compiled.<sup>31,52,122–135</sup> For each cell cluster, every marker gene identified a top differentially expressed (DE) marker of that cluster was cross-referenced with our compiled VNM database using our “DEMarkerScoring” algorithm. For every matching marker gene, one point was given to the respective cluster under the column name with the matching cell identity. Iteration over every marker gene was performed to generate a scoring matrix with varying points for each cluster against the different cell identities compiled in the VNM database. The “phyper” function in R was then used to calculate binomial probabilities using hypergeometric distribution for the total score obtained by each cluster against each cell identity in the database. The -log<sub>10</sub> of probability values were then calculated. The resulting values were scaled to 0–100 and plotted as a heatmap using GraphPad Prism. Each cluster was given an identity based on the maximum -log<sub>10</sub> P score obtained in the heatmap. The top DE markers of clusters with ambiguous scores were

manually searched in the literature to confirm cluster identity. “Renameldents” function was used to assign identity to each cluster. For each cluster, cell identities were confirmed by surveying canonical marker expression. A similar method was used for subclustering leukocytes, but an altered “Leukocyte markers” database that contained leukocyte identities in the VNM database was used. A full list of “Leukocyte markers” are detailed in Table S4.

**Gene ontology-based cell clustering.** The GO term database Amigo (<http://amigo.geneontology.org/amigo/>) was used. To identify immune-related biological processes, the Amigo Drilldown Viewer was first used to view GO term hierarchy. All terms located directly in the layer under “immune system process” were selected. Terms with 15–400 genes that aligned to the *Danio rerio* genome were used. Second, the keyword search terms “immune” and “leukocyte” were used to identify more specific immune-related GO terms. These GO terms were also filtered by selecting terms with 50–400 genes that aligned to the *Danio rerio* genome. Following filtering, prospective gene lists were analyzed by Metascape. Any gene list that did not return the GO term in question within the top three results was removed from the analysis. Only GO terms that were the most statistically significant among the leukocyte subclusters are shown. The full list of genes with this “Leukocyte GO term” database is in Table S5.

### Co-immunoprecipitation of *tcima*

**Generation of recombinant GST-Tcima.** The *tcima* ORF was cloned into the BamHI/EcoRI sites in the pGEX-4T-1 backbone (Sigma cat# 28-9546-49). The zebrafish *tcima* ORF was PCR amplified from cDNA generated from 5 dpi wildtype SC using the following primers: *tcima\_ORF\_F* (gacGGATCCATGTCGAGCGACATGTACTC) and *tcima\_ORF\_R* (gacGAATTCGCGCAGCTTCAGAAAGTTTC) where the underlined sequences indicate restriction enzyme sites. Once sequence confirmed, the resulting plasmid was transformed into BL21(DE3) bacteria. Bacterial cultures were grown until the OD600 reached 0.6–0.8. Expression was then induced with 1 mL of 100 mM IPTG for 5 h. Pelleted bacteria were resuspended in PBS and sonicated under the following conditions: 15 s on, 45 s off at 45% input for a total of 6:15 min. The suspension was centrifuged for 30 min at 12,000 g at 4°C. Glutathione Sepharose 4B beads (GE healthcare cat# 17-0756-01) were washed with 10 mL PBS then centrifuged at 1000 g at 4°C for 1 min for a total of three times. Supernatant was then incubated with pre-washed beads for 30 min at 4°C. Beads were washed again with 10 mL PBS then centrifuged at 1000 g at 4°C for 1 min for 3 times. The slurry was then placed in a BioRad Poly-Prep Chromatography column (cat# 731–1550) and eluted in 2 mL of elution buffer (50 mM Tris-HCl pH 8.0, 10 mM glutathione). Dialysis was performed in dialysis tubing submersed in dialysis buffer (5 mM HEPES pH 7.6, 1 mM DTT, 0.2 mM PMSF, 1 mM EDTA, 10% glycerol) with gentle stirring at 4°C overnight. Samples were aliquoted and stored at –80°C.

**Generation of protein lysate.** For protein lysates, 100 embryos at 2 days post-fertilization (dpf) or 50 SCs at 3 dpi were used. Adult injured SCs were collected as described above. Samples were added to 4°C lysis buffer (50 mM Tris-HCl pH 7.5, 125 mM NaCl, 1.5 mM MgCl, 1 mM EDTA, 5% glycerol, 0.4% NP-40, 0.1% Tween 20, 200 mM PMSF, and PI cocktail) and homogenized with a pestle. A second volume of lysis buffer was added and mixed by pipetting up and down. Samples were centrifuged at 16,000 g for 15 min at 4°C. The supernatant was transferred to a new pre-chilled microfuge tube. 1/10 volume of NP-40 was added and the sample was mixed by vortexing. Samples were then centrifuged at 16,000 g for 15 min at 4°C. The supernatant was transferred to a new pre-chilled microfuge tube and 4.25  $\mu$ L of 100% glycerol was added per 10  $\mu$ L of supernatant. Protein concentration was measured using Qubit and stored at –80°C. The final volume of each lysate was 1 mL.

**Immunoprecipitation.** For immunoprecipitation, 50  $\mu$ L (per sample) of Glutathione Sepharose 4B beads were washed with 10 mL PBS then centrifuged at 1000 g at 4°C for 1 min for a total of three times. GST-Tcima or GST alone were incubated with pre-washed GST-beads for 2 h at 4°C with gentle rocking. Samples were centrifuged at 1000 g for 3 min at 4°C. The supernatant was removed, and beads were washed with 10 mL PBS+1% Triton X-100 then centrifuged at 1000 g at 4°C for 1 min for 3 times. The supernatant was removed and 100  $\mu$ L of protein lysate was added. Samples were incubated 16 h at 4°C with gentle rocking. Samples were then centrifuged at 100 g for 3 min at 4°C. Samples were washed three times for 5 min each at 4°C with fresh wash buffer (5 mM HEPES pH 7.5, 1 mM DTT, 0.2 mM PMSF, 1 mM EDTA, and 10% glycerol). Samples were resuspended in wash buffer and analyzed on the time-TOF Pro ion mobility mass spectrophotometer interfaced to a nanoElute LC system the Washington University Proteomics Shared Resource. Peptides with >5 spectral counts in the GST-Tcima samples and 0 spectral counts in GST-only samples were considered putative Tcima binding partners.

**Pairwise co-immunoprecipitations.** For transfections of HEK293 cells, cells were washed with pre-warmed DPBS (Invitrogen cat# 14190136), and the media was replaced with pre-warmed DMEM with no serum or supplements. Cells were returned to 37°C and 5% CO<sub>2</sub> until transfection. Lipofectamine-2000 Reagent (Invitrogen cat# 15338100) was used for all HEK-293 transfections. Cells were seeded at a density of 100,000 cells per well of a 6-well plate and transfected with 4.4  $\mu$ g of total plasmid DNA. For co-immunoprecipitations, 400 ng of pDest-CMV-3xHA-Tcima-EGFP and 4  $\mu$ g of candidate gene plasmid were transfected. DNA was diluted in DMEM per manufacturer’s specifications. Lipofectamine-2000 was diluted in DMEM separately and incubated for 5 min at room temperature according to manufacturer’s specifications. The DNA and Lipofectamine mixtures were combined and incubated at room temperature for 20 min prior to transfection. Cells were incubated for 3 h in the transfection mixture at 37°C with 5% CO<sub>2</sub>, washed with DBPS, and placed in complete growth media (DMEM + FBS + L-Glutamine + pen/strep) for 48 h prior to cell collection.

For collection of cell lysates, growth media was removed, cells were washed with DBPS, and 200  $\mu$ L of lysis buffer (20 mM HEPES (pH 7.5), 100 mM KCl, 5% glycerol, 2.5 mM EDTA, 1% NP-40, and 1 $\times$  protease inhibitor) was added per well. Cells were incubated

in lysis buffer on ice for 30 min, cells were lysed by pipetting up and down, and samples were incubated rotating end-over-end at 4°C for 30 min. Lysates were centrifuged at max speed for 5 min at 4°C and the supernatant was placed in a prechilled tube, flash frozen in liquid nitrogen, and stored at –80°C until use.

For immunoprecipitation, 20  $\mu$ L per sample of chilled antibody-conjugated affinity gel (either EZview Anti-HA Affinity Gel, Millipore Sigma cat# E6779 or Anti-Flag M2 Affinity Gel, Millipore Sigma cat# A2220) was added to a prechilled microfuge tube. Affinity gel was washed with cold PBS, centrifuged at 500 g at 4°C for 1 min, and then the supernatant was removed for a total of two times. Affinity gel was then washed in cold wash buffer (20 mM HEPES (pH 7.9), 10 mM KCl, 3 mM MgCl<sub>2</sub>, 250 mM NaCl, 0.1% Triton X-100, 1 $\times$  protease inhibitor). Cell lysates were added to 20  $\mu$ L of washed affinity gel and incubated at 4°C rotating end-over-end overnight. Gel/lysate slurries were washed with cold wash buffer, centrifuged 500g at 4°C for 1 min, and then the supernatant was removed for a total of 5 times. On the final wash, the entire supernatant was removed without disturbing the affinity gel. Samples were resuspended in 20  $\mu$ L of Laemmli sample buffer (Bio-rad cat# 1610747) with 1 $\times$  2-mercaptoethanol and incubated at 95°C for 10 min. The supernatants were then run on an SDS-PAGE gel and transferred onto a Immobilon PVDF membrane (Millipore Sigma cat# IPVH304F0).

Immunoblotting. For blotting, the membrane was washed in TBST (2 mM Tris-HCl, 15 mM NaCl, 0.1% Tween 20, pH 8.0). After incubation in blocking agent (5% BSA in TBST) at room temperature for 1 h, the membrane was probed with either Mouse anti-Flag M2 (Sigma cat# F1804) or Mouse anti-HA (Cell Signaling Technology cat# 3724) primary antibodies diluted 1:1000 in blocking agent overnight at 4°C. After 6  $\times$  10 min washes in TBST at room temperature, the membrane was incubated at room temperature for 1 h in Mouse- or Rabbit-conjugated HRP secondary antibodies diluted 1:1000 in blocking agent. Following 3  $\times$  10 min washes in TBST, the membrane was probed with Immobilon Western HRP Substrate (Millipore Sigma cat# WBKLS0500) according to the manufacturer's specifications. Blot images were taken using the Bio-rad ChemiDoc imager using the Chemiluminescence tray. For western blot heatmaps, the intensity of the band center of mass was normalized to the blot background intensity and the intensity of the negative control band. Raw images of blots are shown in supplemental figures and the brightness and contrast of blot images were adjusted linearly to aid in viewing.

### Raw 264.7 macrophage live imaging

*Transfection of Raw 264.7 macrophages.* On the day of transfection, cells were washed with pre-warmed DPBS (Invitrogen cat# 14190136), and the media was replaced with pre-warmed OptiMEM reduced serum media (Invitrogen cat# 31985070). Cells were returned to 37°C and 5% CO<sub>2</sub> until transfection. Lipofectamine-LTX Reagent with PLUS Reagent (Invitrogen cat# 15338100) was used for all Raw 264.7 transfections. The following amounts of DNA were used for Raw 264.7 transfections: 8-well chamber microscope slide (CELLTREAT cat# 50-114-9054, 500 ng of DNA), 12-well plate (1  $\mu$ g of DNA), or glass-bottom 96-well plate (150 ng of DNA). DNA was mixed with PLUS Reagent and diluted in OptiMEM media according to manufacturer's specifications. Lipofectamine-LTX was diluted in OptiMEM media separately and incubated for 5 min at room temperature. The DNA/PLUS Reagent/OptiMEM and Lipofectamine/OptiMEM mixtures were combined and incubated at room temperature for 30 min prior to transfection. Cells were incubated for 16 h in the transfection mixture at 37°C with 5% CO<sub>2</sub> prior to downstream experiments.

*Raw 264.7 engulfment assay.* For the Bioparticle engulfment assay, Raw 264.7 macrophages were plated in a 12-well plate at the density of 100,000 cells per well. Cells were transfected as described above with pDest-CMV-3xHA-Tcma-EGFP or empty pDest-CMV-3xHA-gateway-EGFP as a control. On the day of the assay and 16 h after transfection, cells were washed in DPBS and the media was replaced with FluoroBrite DMEM (ThermoFisher cat# A189671) supplemented with 10% FBS. pHrodo Red E. coli Bioparticles Conjugate for Phagocytosis (ThermoFisher cat# P35361) were resuspended with DEPC water to a concentration of 1 mg/mL prior to the start of the assay. At the start of the assay, pHrodo-conjugated Bioparticles were added to the 12-well plate in a 1:100 dilution. Cells were imaged on a Zeiss Cell Discoverer 7 microscope with a 20 $\times$  objective in the Washington University Center for Cellular Imaging. Widefield images of 568 fluorescence and Oblique transmitted light were acquired every 2 min for a total of 3 h. Cells were incubated at 37°C and 5% CO<sub>2</sub> during imaging. To quantify the pHrodo<sup>+</sup> area and the total cell area, a custom Fiji script was used to threshold images and quantify the total thresholded areas. The pHrodo<sup>+</sup> area was divided by the total cell area to yield the engulfment index. Quantifications were performed blind to condition. The experiment was performed on two separate passages of cells in independent replicates.

*Raw 264.7 BODIPY pulse-chase.* Raw 264.7 macrophages were plated in a glass-bottom 96-well plate at the density of 5,000 cells per well. Cells were transfected as described above, and transfection media was replaced with fresh culture media 16 h after transfection. On the day of the assay, which was 48 h after transfection, cells were washed with PBS and the media was placed with fresh culture media with serum that contained 1  $\mu$ g/mL BODIPY. Cells were incubated at 37°C and 5% CO<sub>2</sub> for 30 min, washed with PBS three times, and the media was replaced with FluoroBrite DMEM (ThermoFisher cat# A189671) supplemented with 10% FBS. Imaging media contained either vehicle (DEPC water) or 10 mM mildronate (Meldonium dihydrate, Millipore Sigma cat# M5199). Cells were imaged using the widefield setting on a Zeiss LSM 980 microscope with a 20 $\times$  objective in the Notre Dame Integrated Imaging Facility. Widefield images of 488 fluorescence and transmitted light were acquired every 3 min for a total of 1.5 h. Cells were incubated at 37°C and 5% CO<sub>2</sub> during imaging. To quantify the BODIPY<sup>+</sup> area, a custom Fiji script was used to threshold images and quantify the total thresholded areas. These values were normalized to the BODIPY<sup>+</sup> area present at t = 0. To quantify BODIPY, a manual count was used due to membrane-stained BODIPY interfering with the lipid droplet signal. The Fiji plug-in Cell Counter was used.



### Cloning expression plasmids for cell culture

**Cloning of 3xFlag- and 3xHA-tagged ColP baits and preys.** Cloning of the pDest-CMV-3xHA-Tcima-EGFP bait plasmid is described below. Cloning pDest-CMV-3xFlag-Prey-EGFP plasmids were performed similarly. Briefly, coding sequences were PCR amplified from zebrafish cDNA using gene-specific primers and cloned into the pENTR vector using the pENTR/D-TOPO cloning kit (ThermoFisher cat# K240020) according to the manufacturer's specifications. Gene-specific primers for pENTR plasmids are listed in Table S6. To generate the final expression vectors, a Gateway LR Clonase II Plus (ThermoFisher cat# 12538120) reaction was used to insert coding sequences in-frame in between the 3xFlag tag and the GFP coding sequence present in the pDest-CMV-3xFlag-gateway-EGFP vector.

**Cloning 3xHA-tagged Tcima.** The 3xFlag sequence in pDest-CMV-3xFLAG-gateway-EGFP (Addgene cat# 122845) was replaced in-frame by a 3xHA tag sequence using the KpnI and HindIII restriction enzyme sites and T4 ligation (NEB cat# M0202S) to generate pDest-CMV-3xHA-gateway-EGFP. The *tcima* coding sequence was PCR amplified from zebrafish cDNA using gene-specific primers (pENTR\_*tcima*\_F: CACCATGTCGAGCGACATGTACTC and pENTR:*tcima*\_R: GCGCAGCTTCAGAAAGTTTCGC) and cloned into the pENTR vector using the pENTR/D-TOPO cloning kit (ThermoFisher cat# K240020) according to the manufacturer's specifications. To generate the final expression vector, a Gateway LR Clonase II Plus (ThermoFisher cat# 12538120) reaction was used to insert the *tcima* coding sequence in-frame in between the 3xHA tag and the GFP coding sequence present in the pDest-CMV-3xHA-gateway-EGFP vector to yield pDest-CMV-3xHA-Tcima-EGFP.

**Cloning 3xHA-mCherry-EGFP control plasmid.** The mCherry coding sequence was PCR amplified from p3E-2A-mCherry-CAAX (Addgene cat# 195971) using the primers pDest-mCherry\_F: tgacgtccagattacgtaagatggtgagcaagggcg and pDest-mCherry\_R: cctgccctgtcaccatctcctgtacagctcgcc. The pDest-CMV-3xHA-gateway-EGFP plasmid was cut with XhoI and HindIII and mCherry was inserted using the NEBuilder HiFi DNA Assembly Master Mix (NEB cat# E2621L) according to the manufacturer's specifications. The sequences of all plasmids were confirmed by whole plasmid sequencing (Azenta) prior to use.

### QUANTIFICATION AND STATISTICAL ANALYSIS

All procedures and quantifications were performed blind to condition. Glial bridging, the cross-sectional Gfap<sup>+</sup> area at the lesion core was normalized to the cross-sectional area of the intact spinal cord rostral to the lesion. Cell counting was performed using a customized Fiji script (adapting ITCN: Image based Tool for counting nuclei, <https://imagej.nih.gov/ij/plugins/itcn.html>). This customized Fiji script incorporated user-defined inputs to define channels (including Hoechst) and to outline SC perimeters. To quantify nuclei, the following parameters were set in ITCN counter: width, 15; minimal distance, 7.5; threshold, 0.4. For each staining, thresholds were user-defined. Raw counts from Fiji were processed using a customized R Studio script. Absolute cell numbers are provided.

**Mbp aggregation.** A pixel intensity threshold value was determined for each experimental replicate based on an intensity threshold that included <1% of the whole imaged area in uninjured sections. This was to exclude quantification of myelinated axons, which had much lower signal intensity than Mbp aggregates that were present in injured fish. This threshold value was then used to identify the size of the imaged area that contained Mbp staining that was brighter than the set threshold. This quantified area was then labeled "Mbp<sup>+</sup> Aggregate" area.

**Anterograde axon labeling (axon regrowth).** To calculate axon regrowth, biocytin labeled axons were quantified using the threshold and particle analysis tool in Fiji. Multiple sections for each fish at each level were quantified: (1) caudal-proximal, five sections taken 150, 300, 450, 600, and 750  $\mu$ m caudal to the lesion core, (2) caudal-distal, five sections taken 900, 1050, 1200, 1350, and 1500  $\mu$ m caudal to the lesion core, (3) rostral, two sections taken 300 and 450  $\mu$ m rostral to the lesion core. The number of biocytin<sup>+</sup> particles in each region of the spinal cord were averaged for each individual fish. The caudal-proximal and caudal-distal values were divided by the rostral value to give the axon growth index. This value was then normalized to the average caudal-proximal value in sibling controls to normalize for variability in labeling across experiments. To calculate glial bridging, the cross-sectional Gfap<sup>+</sup> area of the center of the lesion was normalized the cross-sectional area of the spinal cord 1 mm rostral to the lesion site.

**Volumetric quantifications of phagocytic cargo (Mbp, bioparticles, and Bodipy).** Imaris was used. The Lcp1 channel was used to generate leukocyte surfaces. Any Lcp1<sup>+</sup> surfaces <1000 pixels were removed to minimize background and bleed through of exceptionally strong puncta. The Lcp1<sup>+</sup> surfaces were used to mask signal internal (engulfed) or external (extracellular) to leukocytes. Phagocytic cargo surfaces for each mask were then created using a single consistent threshold value across the entire experiment. Phagocytic cargo surfaces of <10 pixels were excluded to minimize background. The total volume of each mask was then calculated. The percent engulfment was calculated by dividing the internal engulfed volume by the total cargo volume for the image.

**Hypergeometric distribution.** To calculate the hypergeometric *p*-value between gene list overlap, the "calculate\_hypergeometric\_p\_value" function was used in RStudio. The size of the zebrafish genome was used for the total population size (26,206 genes). The number of total genes within each gene list and the number of genes that overlap between lists was taken into account with this function.

All sample sizes (n) are indicated for the number of animals used in each experiment, with specific sample sizes per each time point, genotype, and/or treatment indicated in figure legends. In cases where animals were lost due to death, the smallest sample size is indicated in the legend. The exact sample sizes are indicated in [Table S7](#).

Statistical differences between the same group across independent replicates were ruled out prior to merging data from independent replicates. In all graphs, dots indicate single animals and mean  $\pm$  SEM are plotted. GraphPad Prism software was used to perform all statistical analyses. Unpaired Student's *t* test (with Welch's correction, whenever appropriate) or Mann Whitney test were used for comparing two groups. One-way or two-way ANOVA with appropriate corrections for multiple comparisons were used for comparing three or more groups. Specific statistical tests used are indicated in figure legends:  $p > 0.05$ ;  $*p < 0.05$ ,  $**p < 0.01$ , and  $***p < 0.001$ .