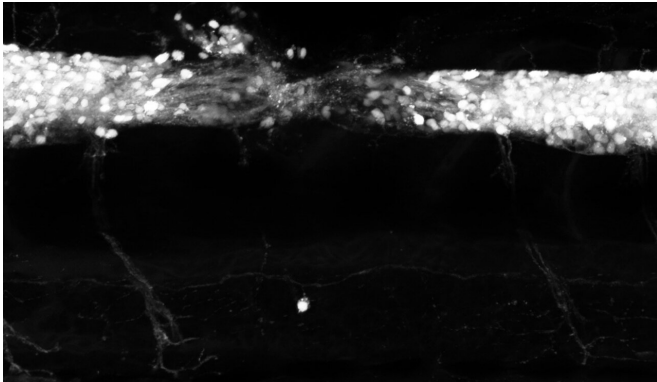


# New, rapid CRISPR/Cas9 method identifies key genes in zebrafish spinal cord regeneration

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A re-connected spinal cord in a 5-day-old zebrafish larva. Nerve cells (white band) in the spinal cord reconnect over the injury site (lighter area in the middle). Credit: Keatinge M et al., 2021, PLOS Genetics

A new, rapid screening approach uses CRISPR/Cas9 technology to identify immune system-related genes that play a crucial role in repairing zebrafish spinal cord injuries. Marcus Keatinge and Themistoklis Tsarouchas of the University of Edinburgh, U.K., and colleagues present these findings in the open-access journal *PLOS Genetics*.

In humans and other mammals, severed spinal-cord nerve connections do not heal, so a spinal cord injury may lead to permanent paralysis. In contrast, zebrafish are capable of recovering from spinal cord injury in a process that involves inflammation controlled by macrophages—a type of immune system cell. However, the precise process by which macrophages aid spinal cord regeneration in zebrafish remains mysterious.

To help clarify this process, Keatinge, Tsarouchas and colleagues developed a new method for

rapidly identifying macrophage-related genes that are involved in zebrafish spinal cord regeneration. The strategy employs CRISPR/Cas9 technology, which enables researchers to target and disrupt [specific genes](#), thereby revealing their function. Molecules known as synthetic RNA Oligo CRISPR guide RNAs (sCrRNAs) enable this gene-specific targeting.

The researchers applied the new method to study spinal cord regeneration in larval zebrafish. Key to the method was a prescreening step in which they tested over 350 sCrRNAs that [target genes](#) already known to potentially play an important role in inflammation-related spinal cord regeneration. Introducing these sCrRNAs to the zebrafish enabled identification 10 genes that, when disrupted, impaired recovery from spinal cord injury.

Further analysis narrowed the list to four genes that appear to be crucial for repair of severed spinal nerve connections, validating the novel method. One gene in particular, *tgfb1*, appears to play an essential signaling role in controlling inflammation during the recovery process.

The new method and findings could help deepen understanding of spinal cord regeneration in zebrafish. The researchers also say the method could be adapted to screen for genes that play important roles in other biological processes, as well.

The authors add, "Zebrafish can fully regenerate their spinal cords after injury. Using a new and very rapid screening platform, we discover [genes](#) of the immune system that are essential for [regeneration](#). We envision our findings to lead to new insights into the inability of mammals to regenerate and our versatile screening platform to be adapted to other

disease or [injury](#) models in [zebrafish](#)."

**More information:** Keatinge M, Tsarouchas TM, Munir T, Porter NJ, Larraz J, Gianni D, et al. (2021) CRISPR gRNA phenotypic screening in zebrafish reveals pro-regenerative genes in spinal cord injury. *PLoS Genet* 17(4): e1009515. [doi.org/10.1371/journal.pgen.1009515](https://doi.org/10.1371/journal.pgen.1009515)

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