METHODS

Plasmid construction and cloning.

We used 2 monomeric bright fluorescent proteins (FP) of similar size that encode for a the mGreenLantern (https://doi.org/10.1073/pnas.2000942117) and mScarlet (PMID: 27869816 DOI: 10.1038/nmeth.4074) and fused in frame with the core histone H2B in the amino terminus for nuclear localization of the FPs. Both fusions were synthetically constructed.

-For the mGreenLantern was constructed first by generating a synthetic cDNA optimized to the Human codon usage. The rat amino acid sequence H2B/Histone H2B type 1- C/E/G (accession #NP_001100822) was fused in frame to mGreenLantern with a linker of 8 amino acids (PPAGSPPA) between H2B and mGreenLantern. The fusion protein was cloned into pAAV-CAG-GFP (Addgene #37825) by substituting the GFP with H2B-mGreenLantern using restriction enzymes BamHI and XhoI.

-For the mScarlet the human H2B clustered histone 11 (H2BC11) (accession #NM_021058) was fused in frame without a linker in between and cloned into the pAAV-CAG-tdTomato (Addgene #59462) using the sites KpnI and EcoRI at the 5 and 3 prime end respectively.

Viral production.

rAAV2-retro-H2B-mGreenLantern was produced at the University of Miami viral core facility at the MIami Project to Cure Paralysis, titer = $1.4x10^{13}$ particles/mL. Virus was concentrated and resuspended in sterile HBSS and used without further dilution. The rAAV2-retro-H2B-mScarlet was made by the University of North Carolina Viral Vector Core, titer + $8.7x10^{12}$ particles/mL

Spinal cord surgery.

All animal procedures were approved by the University' Institutional Animal Care and Use Committee and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Adult female C57BL/6 mice (6-8 weeks old, 20–22 g) were used for these experiments. One microliter of rAAV2- retro particles was injected into the spinal cord with a Hamilton syringe driven by a Stoelting QSI pump (catalog #53311) and guided by a micromanipulator (pumping rate: 0.04 µL/min). AAV viral particles were injected at C4-C5, T10, L1, L4 vertebrae, unilateral injections at the same location, 0.35 mm lateral to the midline, and to depths of 0.6 and 0.8 mm.

Spinal cord crush injury.

Adult female C57BL/6 mice (6-8 weeks old, 18–22 g) were anesthetized by ketamine/xylazine. A laminectomy of vertebra T10-12 was performed using a fine pair of forceps and care was taken not to damage the dura. For each crush mouse, one of the two pairs of forceps were used to laterally compress the spinal cord to the corresponding thickness (0.15 and 0.4 mm, depending on the pair of forceps) and thereby establishing two groups with varying injury severity: the 0.15mm and 0.4mm injury groups. Carefully compress the spinal cord with forceps until the spacers connect. Hold in place for 15

sec with 2 times to avoiding bias. A mild injury control group received identical treatment, including exposure, laminectomy, and placement of the forceps around the spinal cord, but no crush injury was performed

Tissue clearing and imaging.

After 2 wk expression, euthanized with approved procedures. Animals underwent transcardial perfusion with 0.9% saline and 4% paraformaldehyde solutions in 1×-PBS (15710, Electron Microscopy Sciences). Whole brains and spinal cords were dissected and fixed overnight in 4% paraformaldehyde at 4°C and washed three times in PBS pH 7.4, followed by storage in PBS. The dura was carefully and completely removed as residual dura can trap bubbles that prevent effective light-sheet microscopy. The brains and spinal cords were cleared using a modified version of the 3DISCO (PMID: 26023683 PMCID: PMC4444235 DOI: 10.1523/ENEURO.0001-15.2015 and PMID: 30341180 PMCID: PMC6284107 DOI: 10.1523/JNEUROSCI.1196-18.2018) Samples were incubated on a shaker at room temperature in 50, 80, and 100% peroxide-free tetrahydrofuran (THF; Sigma-Aldrich, 401757) for 12 hr each. Peroxides were removed from THF by passing 100% THF through a chromatography column filled with basic activated aluminum oxide (Sigma- Aldrich, 199443) as previously described (7, 8). The next day, samples were transferred to BABB solution (1:2 ratio of benzyl alcohol, Sigma-Aldrich, 305197; and benzyl benzoate, Sigma-Aldrich, B6630) for at least 3 hr. After clearing, samples were imaged within 48 hr by light-sheet microscopy (Ultramicroscope, LaVision BioTec). The ultramicroscope uses a fluorescence macro zoom microscope (Olympus MVX10) with a 2× Plan Apochromatic zoom objective (NA 0.50). Image analysis and 3D reconstructions were performed using Imaris v9.5 software (Bitplane) after removing autofluorescence using the Imaris Background Subtraction function with the default filter width so that only broad intensity variations were eliminated.