## **Supporting Information**

Allegra Mascaro et al. 10.1073/pnas.1219256110

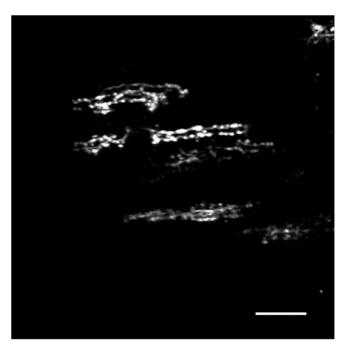


Fig. S1. Climbing fibers (CFs) GFP labeling induced by viral injection. Representative two-photon fluorescence (TPF) transversal view (maximum intensity z-projection of 20 images acquired from 30 to 70 μm deep below the pial surface) of sparsely GFP-labeled CFs in the cerebellar molecular layer. (Scale bar, 20 μm.)

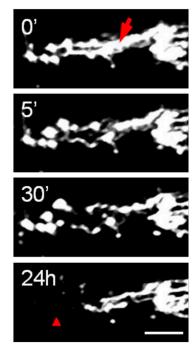
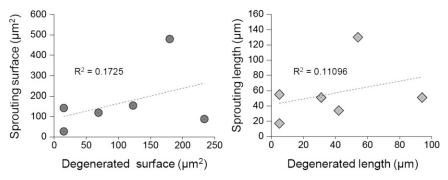


Fig. S2. Short-term effects of laser nanosurgery on GFP-labeled CF. Time lapse of maximum intensity z-projections (60  $\mu$ m thick) of an irradiated CF. Red arrowhead highlights the degeneration of the CF distal portion. (Scale bar, 10  $\mu$ m.)



**Fig. S3.** Correlation between degeneration and sprouting. The two graphs correlate the degenerated and sprouting extent in the laser axotomized CFs. Surface values on the *Left* refer to the area included in a segmented line around the degenerated distal region or around the sprouted branches. The length in the graph on the *Right* is the summed values of the length of single branches involved in degeneration or sprouting for each CF.

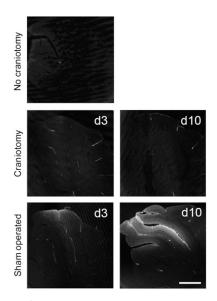
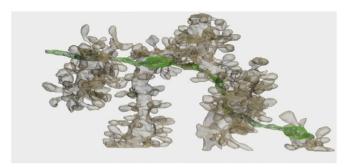


Fig. S4. Application of the optical window does not significantly induce microglial activation. The microglia was revealed by anti-CD11b immunostaining (mouse monoclonal; Serotec) in cerebellar coronal slices. Images in the second row are obtained 3 d and 10 d after application of the optical window. Microglia activation is comparable with that observed in naïve mice (*Upper* image). The last row shows progressively increased glial activation measured 3 d and 10 d after a sham craniotomy. In this case, we applied a downward pressure on the brain surface with the tweezers tip. (Scale bar, 750 μm.)



Movie S1. Rotation of the 3D reconstruction shown in Fig. 4B, Center.

## Movie S1