Supplementary Methods

Bioinformatics analysis

Primary sequencing data produced by RNA-Seq (raw reads) were subjected to quality control (QC). The information of total reads and mapping ratio reads is shown in Table 1. Raw reads were filtered into clean reads using internal software SOAP nuke (version 1.5.2), as follows: Remove reads in which unknown bases (N) are more than 10%; Remove reads with adaptors; Remove low quality reads (we define the low quality read as the percentage ofbase which quality is < 15 and > 50% in a read). QC of alignment was performed to determine if re-sequencing was needed. If the alignment results passed QC, downstream analysis including gene expression, differentially expressed genes, cluster analysis, Gene Ontology (GO) enrichment analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis would be performed.

Functional Enrichment Analysis of DEGs

Functional enrichment analysis was performed through the functional annotation package "cluster Profiler" in R studio software (RStudio, Boston, MA, USA) as we described before [1]. GO and KEGG enrichment analysis were conducted. For each enriched function term, the Q-value of enriched functions and the Q-value by multiple testing corrections were calculated by "cluster Profiler" package in R studio software. The GO functional and KEGG pathway enrichment analysis were performed for DEGs using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) online tools (http://www.geneontology.org and http://www.genome.jp/kegg).

Protein–Protein interaction (PPI) network analysis

The Search Tool for the Retrieval of Interacting Genes (STRING) is used to provide information regarding predicted and experimental interactions of proteins and the prediction method of this database is from neighborhood, gene fusion, co-occurrence, co-expression experiments, databases, and text mining. By setting the Combination score > 0.8 as the reliability threshold value, the web based STRING database (http://string-db.org/) was used to produce PPI predictions after uploading the union gene list to the search bar [2]. Based on the interplayed relationships, a PPI network was established and then visualized using the Cytoscape software [3]. The connectivity

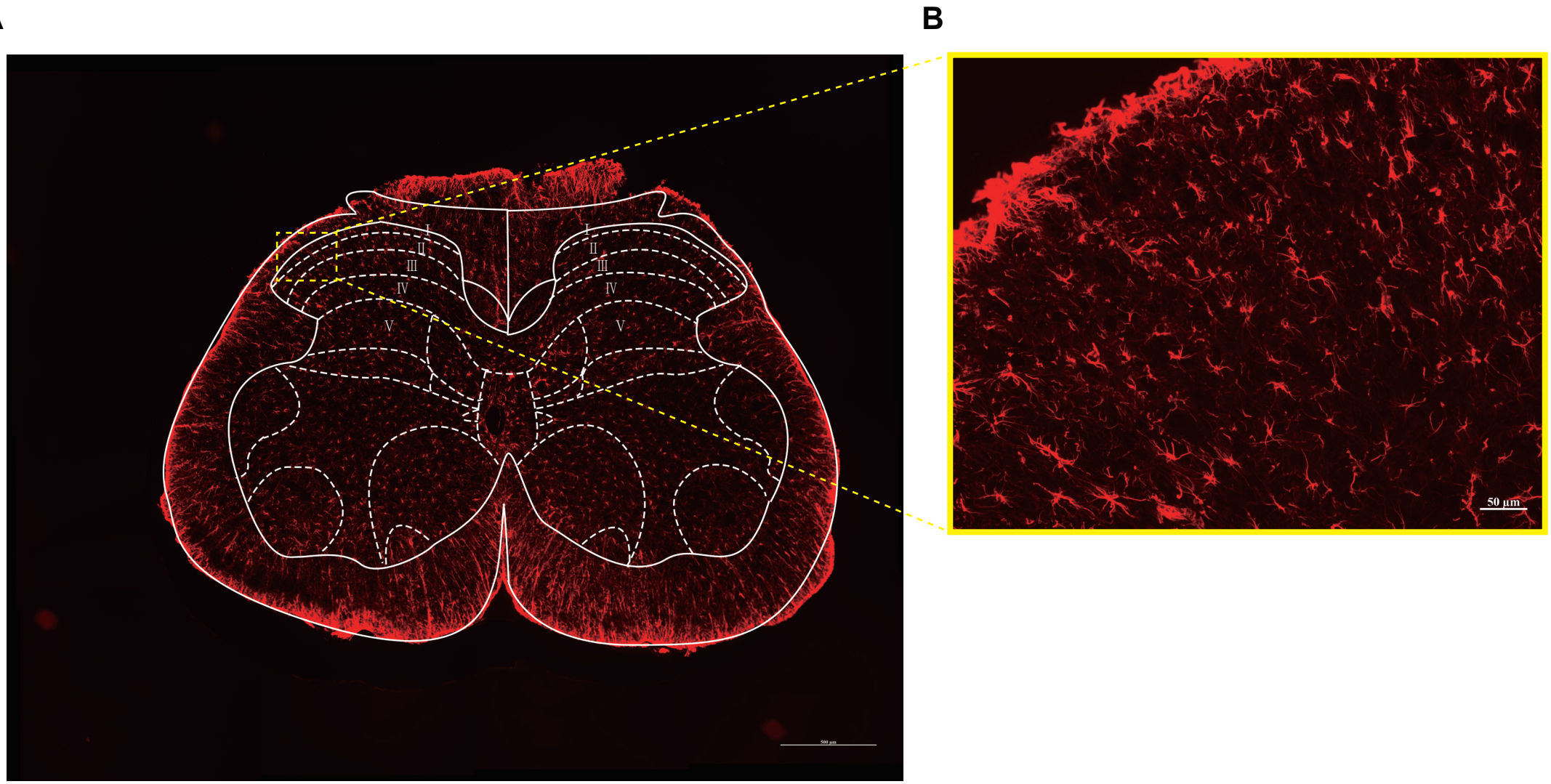
degree of each protein, namely the number of proteins it connected, was calculated to evaluate its importance in this network.

References

1. Yin C, Hu Q, Liu B, Tai Y, Zheng X, Li Y, et al. Transcriptome profiling of dorsal root ganglia in a rat model of complex regional pain syndrome type-I reveals potential mechanisms involved in pain. J Pain Res. 2019; 12: 1201-16.

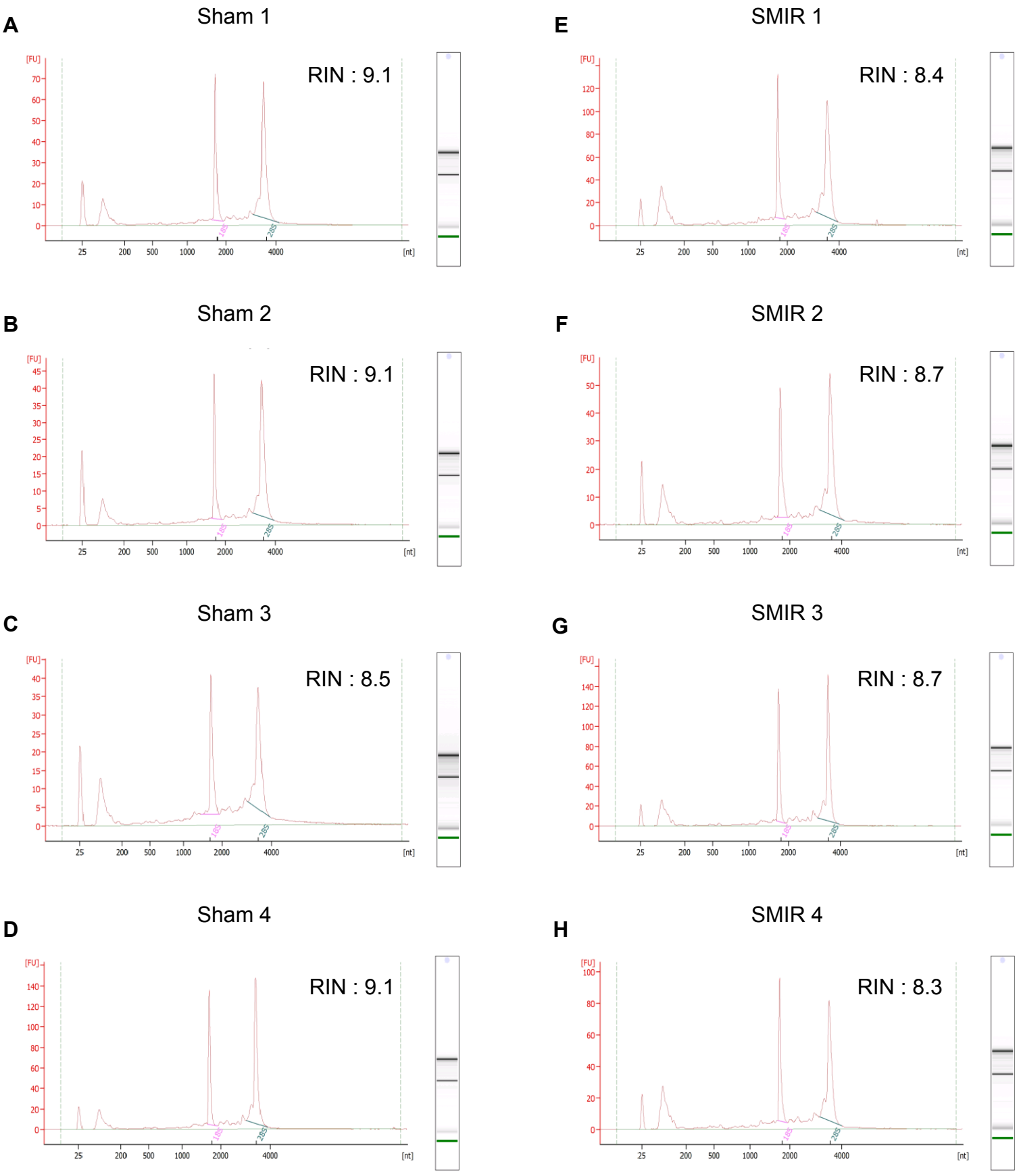
2. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, et al. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res. 2013; 41: D808-15.

3. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003; 13: 2498-504.



Suppl. Fig. 1 Schematic illustration showing the spinal cord dorsal horn laminas captured by immunofluorescence imaging. (A) The picture showing immunostaining of the whole spinal cord section. The proposed laminas I-V were superimposed on the picture. The red color indicates GFAP staining. Scale bar indicates 500 µm. (B) The dotted yellow box indicated the location where we capture the immunofluorescence images and performed the quantifications. Scale bar indicates 50 µm.

Ipsilateral spinal dorsal horn



Suppl. Fig. 2 RNA quality check of loaded RNA samples for RNA-Seq. Images of RNA electrophoresis and deduced RIN were illustrated. (A-D) RNA samples from sham group rats. (E-H) RNA samples from SMIR group rats.