Cell Reports, Volume *40*

Supplemental information

Sensory neurons display cell-type-specific vulnerability to loss of neuron-glia interactions

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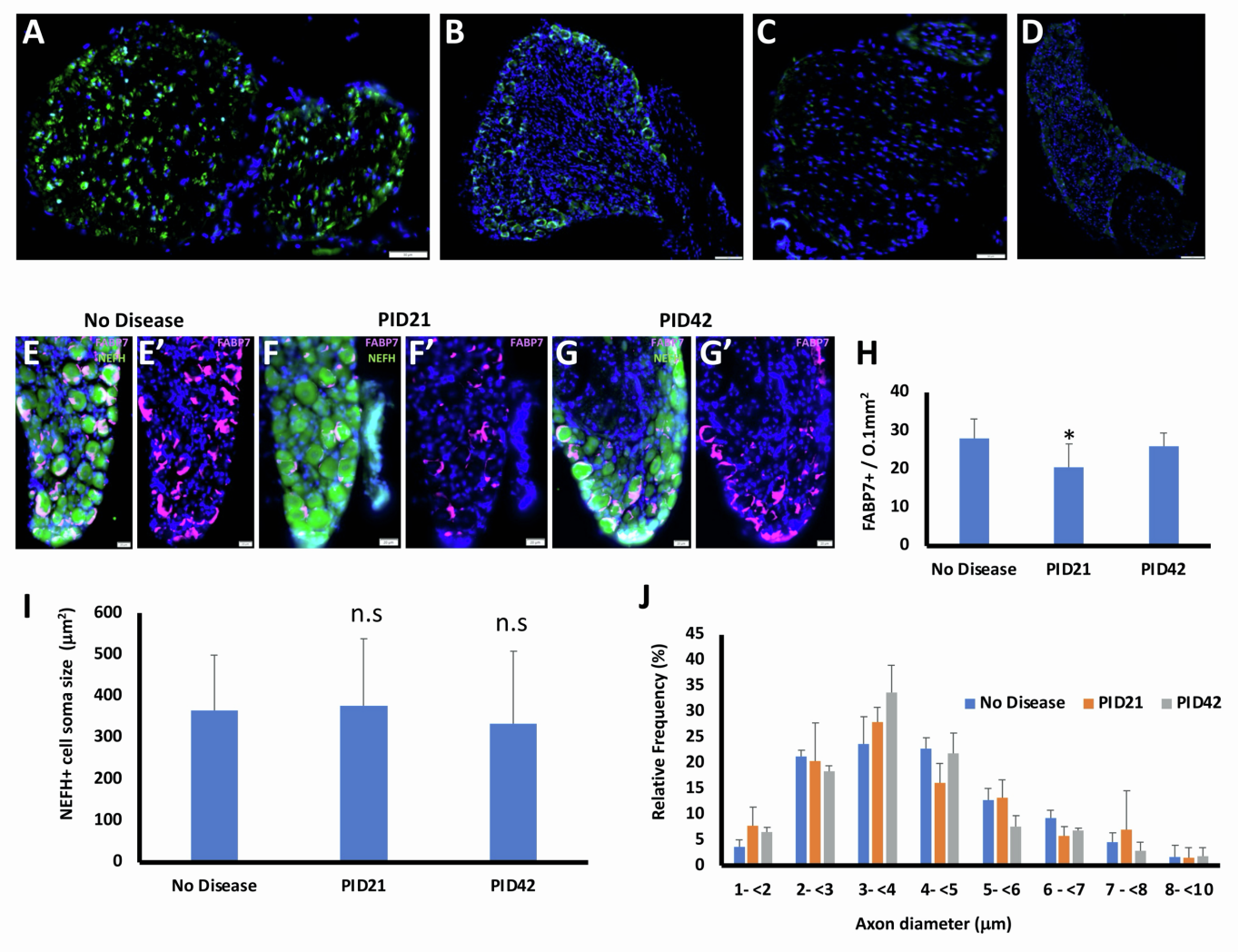

Figure. S1, related to figure 1: Ablation of satellite glial cells. (A-D) Eight-week-old Plp-CreERt;ROSA26-EYFP mice and Cre negative ROSA26-EYFP mice (used as control) were injected with tamoxifen, and the sciatic nerve and the L3-L5 DRGs were dissected. (A) In the sciatic nerve of the Plp-CreERt;ROSA26-EYFP mice, the expression of EYFP was detected by anti GFP antibody. (B) The expression of EYFP in the DRG of the Plp-CreERt;ROSA26-EYFP mice. (C) The expression of EYFP was not detected in the sciatic nerve of the ROSA26-EYFP mice. (D) The expression of EYFP was not detected in the DRG of the ROSA26-EYFP mice. n=3 for the Cre positive and the Cre negative mice. (E-G). Eight-week-old Plp-CreERt;ROSA26-eGFP-DTA mice and Cre negative ROSA26-eGFP-DTA mice (used as control) were injected with tamoxifen, and L3-L5 DRGs were dissected for IHC. The DRGs were labeled at No disease (E), at PID21 (F) and PID42 (G). (H) Statistically significant reduction in the density of FABP7 labelled cells at PID21. (I) No change in NEFH positive cell soma size. (J). No effect on axonal diameter

distribution in the sciatic nerve. For panels E-I n=3 for no disease, n=3 for PID21, and 3 for PID42. For panel I, n=3 for no disease, n=4 for PID21, and 3 for PID42.

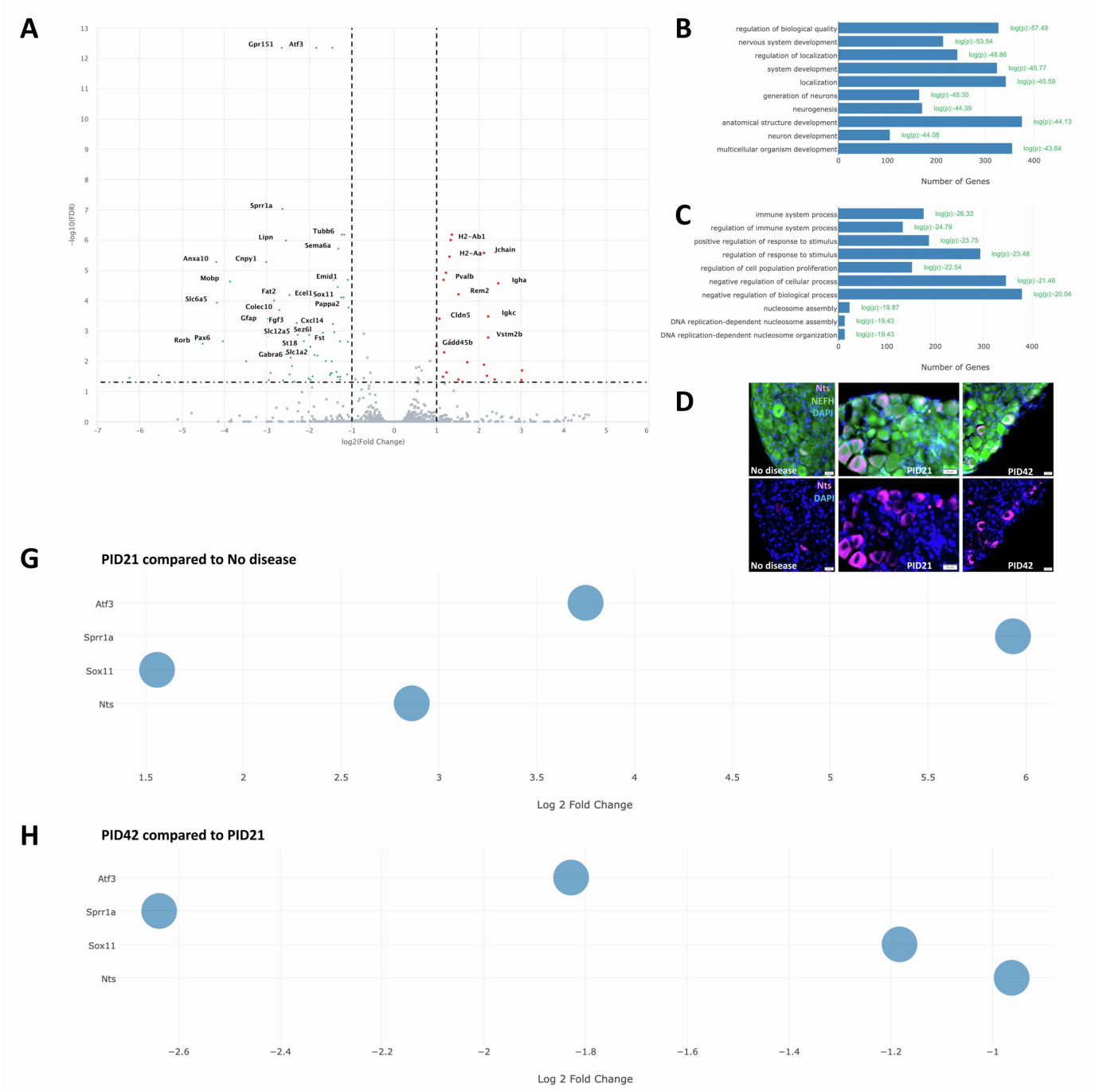

Figure. S2, related to figure 2: The expression of Regeneration Associated Genes in the DRG at PID42. Eight-week-old Plp-CreERt;ROSA26-eGFP-DTA mice and Cre negative ROSA26-eGFP-DTA mice (used as control) were injected with tamoxifen, and L3-L5 DRGs were dissected for bulk RNA-seq at PID21 and PID42. (A) Volcano plot of the Differentially Expressed Genes (DEG) in the DRG at PID42 compared to PID21. Green- downregulated genes, red-

upregulated genes. (B) Biological processes analysis of downregulated genes. (C) Biological processes analysis of upregulated genes. (D) Verification of NTS upregulation upon disease by IHC. (G) The expression of *Atf3*, *Sprr1a*, *Sox11* and *Nts* is upregulated at PID21 compared to no disease. (H) The expression of *Atf3*, *Sprr1a*, *Sox11* and *Nts* is downregulated at PID42 compared to PID21. n = 2 biologically independent experiments. (FDR ≤ 0.05, fold-change ≥ 2). For panel D, n=3 for no disease, n=3 for PID21, and 3 for PID42.

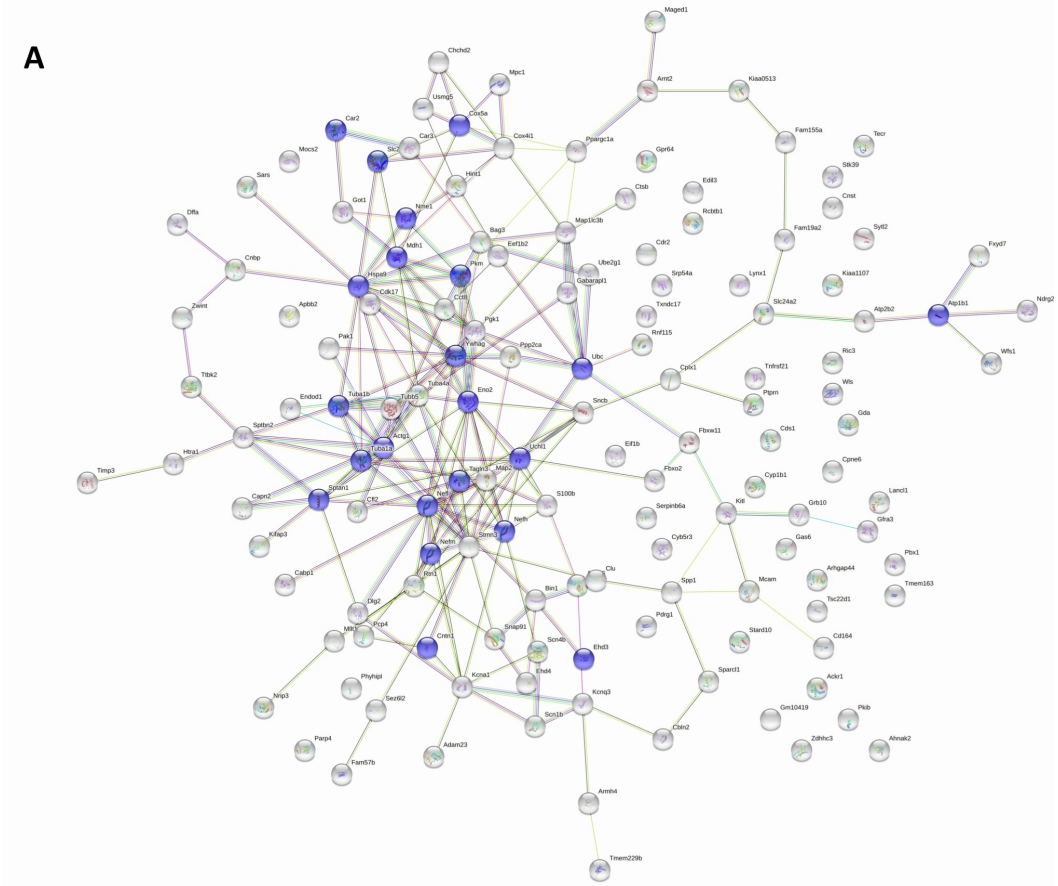
A
Maged1
Chchd2
Mpc1
Amt2
Kiaa0513
Usmp5
Cox5a
Car2
Car3
Cox4i1
Psmgc1a
Fam155a
Tecr
Mocs2
Slc2
Gpr64
Stk39
Sars
Hint1
Edf3
Ctsb
Cnst
Got1
Nme1
Rcbtb1
Dffa
Map1lc3b
Fam19a2
Syt12
Mdh1
Bag3
Eef1b2
Cdr2
Fxyd7
Cnbp
Pkm
Ube2g1
Srp54a
Lynx1
Kiaa1107
Hspa9
Gabarapl1
Cdk17
Ccl8
Slc24a2
Atp2b2
Atp1b1
Ndrg2
Zwint
Apbb2
Pgk1
Txndc17
Rnf115
Pak1
Ywhag
Ppp2ca
Ubc
Rtc3
Wfs1
Cplx1
Tnfrsf21
Tribk2
Ptprn
Wls
Endod1
Tuba1b
Tubb5
Tuba4a
Eno2
Sncb
Gda
Sptbn2
Actg1
Fbxw11
Cds1
Htra1
Tuba1a
Uchl1
Eif1b
Timp3
Tagln3
Msi2
Fbxo2
Cpne6
Spdin1
Ckb
S100b
Cyp1b1
Lancl1
Capn2
Ckb
Serpinb6
Kit
Grb10
Gfra3
Klhl3
Nefm
Cyb5r3
Gas6
Nefh
Arhgap44
Ptbx1
Cabp1
Rtn1
Clu
Spp1
Mcam
Tmem163
Bin1
Tsc22d1
Dlg2
Pdrg1
Prcp
Mt3
Snap91
Stard10
Cd164
Cnn1
Scn4b
Ehd3
Ehd4
Sparcl1
Ackr1
Phyhipl
Kcna1
Kcnq3
Nrip3
Sez6l2
Scn1b
Gm10419
Pkib
Cbln2
Parp4
Adam23
Zdhhc3
Ahnak2
Fam57b
Armh4
Tmem229b
B
Klf6
Bits
C
Atf3
D
Sox11
E
Atf3
F
Jun
G
intracellular part
log(p)-36.08
intracellular
log(p)-36.08
cell part
log(p)-34.85
cell
log(p)-34.79
cytoplasm
log(p)-29.33
cytoplasmic part
log(p)-24.47
organelle
log(p)-21.82
intracellular organelle
log(p)-21.70
nucleus
log(p)-19.72
membrane-bounded organelle
log(p)-19.39
Number of Genes
H
neuron part
log(p)-25.92
cytoplasm
log(p)-25.79
neuron projection
log(p)-22.31
cell projection
log(p)-21.29
axon
log(p)-20.75
neuronal cell body
log(p)-20.64
plasma membrane bounded cell projection
log(p)-20.23
cell body
log(p)-20.06
synapse
log(p)-19.73
somatodendritic compartment
log(p)-19.70
Number of Genes

Figure S3, related to figure 4. The transcriptional response of neurons to demyelination. (A) Network analysis of genes induced in neurons only upon demyelination, generated by STRING (Szklarczyk et al., 2015). The most significantly enriched cellular component was myelin sheath, with false discovery rate of 2.02e-16. Purple nodes denoted “myelin sheath component” proteins and first shell of interactors; white nodes denote second shell of interactors. (B-F) Gene set motif enrichment analysis. The genes induced only by demyelination were enriched for (B) Motif MA1517.1.KLF6 (Relative enrichment ratio 2.13, *p*- value 3.92E-03, *q*-value 2.55E-02) and (C) Motif MA0605.1.Atf3 (Relative enrichment ratio 1.2, *p*- value 6.57E-03, *q*-value 2.85E-02). The genes induced by only by SpNT were enriched for (D) Motif MA0869.2.Sox11 (Relative enrichment ratio 1.49, *p*- value 9.88E-03, *q*-value 1.43E-02), (E) Motif MA1988.1.Atf3 (Relative enrichment ratio 1.73, *p*- value 2.04E-05, *q*-value 6.64E-05) and (F) Motif MA0489.2.Jun (Relative enrichment ratio 1.88, *p*- value 1.99E-04, *q*-value 4.32E-04) as well as motifs (B) (Relative enrichment ratio 1.35, *p*- value 1.39E-06, *q*-value 6.04E-06) and (C) (Relative enrichment ratio 1.18, *p*- value 7.60E-04, *q*-value 1.23E-03). The genes induced by both, SpNT and demyelination were enriched for motif (C), (Relative enrichment ratio 1.92, *p*- value 1.07E-03, *q*-value 6.94E-03). (G). Cellular component analysis of the genes induced only by SpNT. (H). Cellular component analysis of the genes induced by both demyelination and SpNT.

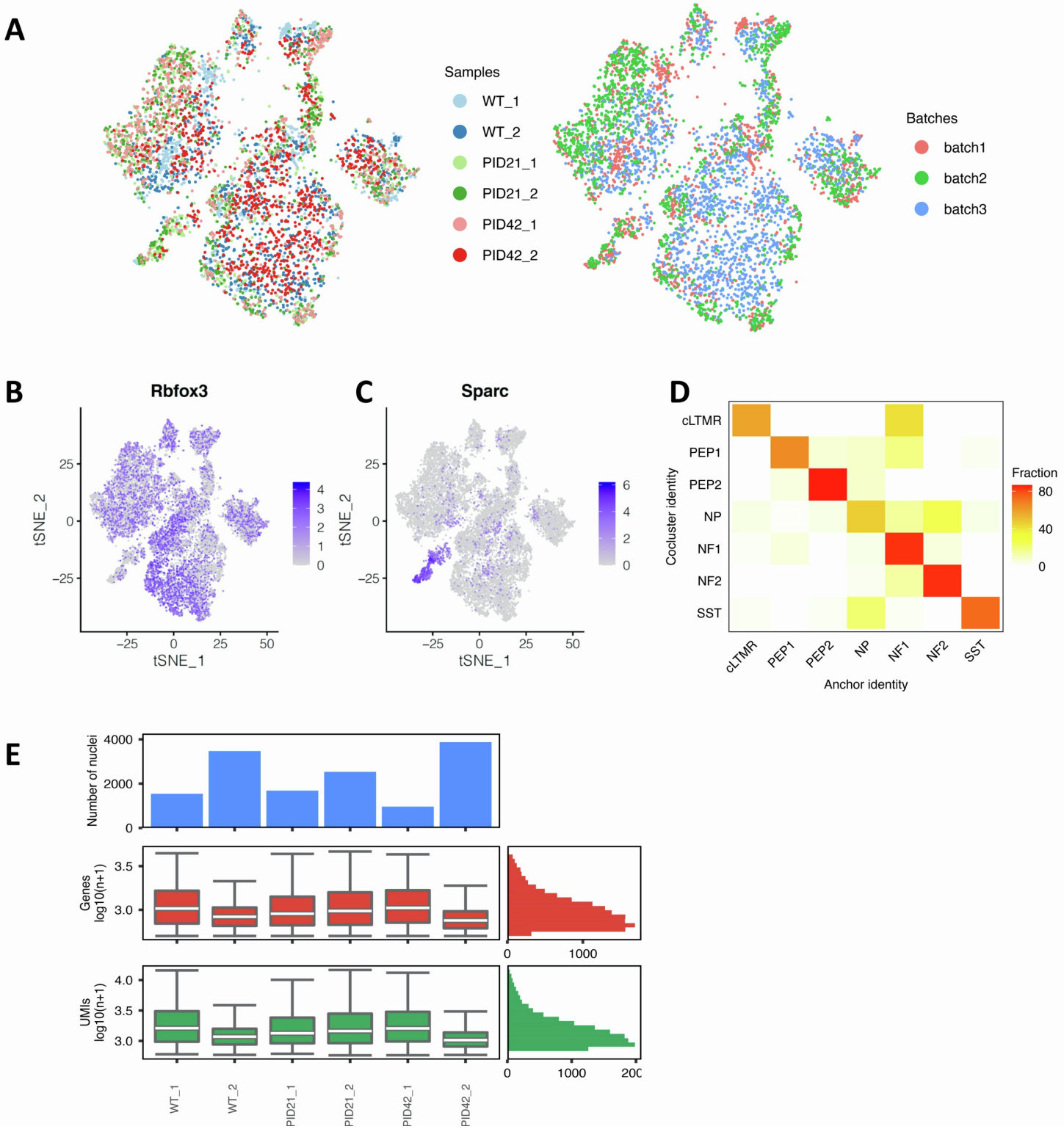
A
Samples
WT_1
WT_2
PID21_1
PID21_2
PID42_1
PID42_2
Batches
batch1
batch2
batch3
B
Rbfox3
tSNE_2
tSNE_1
C
Sparc
D
Cocluster identity
Anchor identity
cLTMR
PEP1
PEP2
NP
NF1
NF2
SST
Fraction
E
Number of nuclei
Genes log10(n+1)
UMIs log10(n+1)
WT_1
WT_2
PID21_1
PID21_2
PID42_1
PID42_2

Figure S4: snRNA-seq metrics for all sequencing samples. (A) tSNE plot of the nuclei derived from three biological replicates of the experimental conditions. Nuclei are colored by their biological replicate. Each cluster has a mix of nuclei from each replicate, suggesting there are minimal if any batch effects between replicates. (B and C) tSNE plots of the neuronal cluster expressing *Rbfox3*, and non-neuronal clusters expressing *Sparc*. Nuclei are colored by their log2 expression of the neuronal marker gene *Rbfox3* (B) and non-neuronal marker gene, *Sparc* (C). (D) Overlap of cell type classifications of injured nuclei between coclustering with Spinal Nerve Transection (from Figure 4A) and anchoring to the uninjured clusters (See methods). The fraction of injured nuclei within the initial cell type assignment that is assigned to each cell type after anchoring is displayed. (E). Number of nuclei from each library with > 500 unique genes (top box). Box plots display number of genes per nucleus (log10 transformed, middle row), and unique molecular identifiers (UMI) per nucleus (log10 transformed, bottom row). Boxes indicate quartiles and whiskers are 1.5-times the interquartile range (Q1-Q3). The median is a white line inside each box. The distribution is aggregated across all samples and displayed on the horizontal histogram.