The Injured Sciatic Nerve Atlas (iSNAT), Insights into the Cellular and Molecular Basis of Neural Tissue Degeneration and Regeneration

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Abstract

Upon trauma, the adult murine PNS displays a remarkable degree of spontaneous anatomical and functional regeneration. To explore extrinsic mechanisms of neural repair, we carried out single cell analysis of naïve mouse sciatic nerve, peripheral blood mononuclear cells, and crushed sciatic nerves at 1-day, 3-days, and 7-days following injury. During the first week, monocytes and macrophages (Mo/Mac) rapidly accumulate in the injured nerve and undergo extensive metabolic reprogramming. Proinflammatory Mo/Mac in the injured nerve show high glycolytic flux compared to Mo/Mac in blood and dominate the early injury response. They subsequently give way to inflammation resolving Mac, programmed toward oxidative phosphorylation. Nerve crush injury causes partial leakiness of the blood-nerve-barrier, proliferation of endoneurial and perineurial stromal cells, and accumulation of select serum proteins. Micro-dissection of the nerve injury site and distal nerve, followed by single-cell RNA-sequencing, identified distinct immune compartments, triggered by mechanical nerve wounding and Wallerian degeneration, respectively. This finding was independently confirmed with *Sarm1*$^{-/-}$ mice, where Wallerian degeneration is greatly delayed. Experiments with chimeric mice showed that wildtype immune cells readily enter the injury site in *Sarm1-/-* mice, but are sparse in the distal nerve, except for Mo. We used CellChat to explore intercellular communications in the naïve and injured PNS and report on hundreds of ligand-receptor interactions. Our longitudinal analysis represents a new resource for nerve regeneration, reveals location specific immune microenvironments, and reports on large intercellular communication networks. To facilitate mining of scRNAseq datasets, we generated the injured sciatic nerve atlas (iSNAT): https://cdb-rshiny.med.umich.edu/Giger_iSNAT/

57 ## Introduction

58 Axonal injury caused by trauma or metabolic imbalances triggers a biochemical program that results in axon
59 self-destruction, a process known as Wallerian degeneration (WD). In the peripheral nervous system (PNS),
60 WD is associated with nerve fiber disintegration, accumulation of myelin ovoids, reprogramming of Schwann
61 cells (SC) into repair (r)SC, and massive nerve inflammation. Fragmented axons and myelin ovoids are rapidly
62 cleared by rSC and professional phagocytes, including neutrophils and macrophages (Mac) (Jang et al., 2016;
63 Klein and Martini, 2016; Perry and Brown, 1992; Rotshenker, 2011). In the mammalian PNS, timely clearance
64 of fiber debris during WD stands in stark contrast to the central nervous system (CNS), where upon injury,
65 clearance of degenerated axons and myelin is protracted and accompanied by prolonged inflammation
66 (Bastien and Lacroix, 2014; Vargas and Barres, 2007).

67 Successful PNS regeneration depends upon the coordinated action and communication among diverse
68 cell types, including fibroblast-like structural cells, vascular cells, SC, and different types of immune cells
69 (Cattin et al., 2015; Chen et al., 2021; Girouard et al., 2018; Pan et al., 2020; Stratton et al., 2018). Nerve
70 trauma not only results in fiber transection, but also causes necrotic cell death and release of intracellular
71 content at the site of nerve injury. Depending on severity, physical nerve trauma results in vasculature
72 damage, endoneurial bleeding, breakdown of the blood-nerve-barrier (BNB), and tissue hypoxia (Cattin et al.,
73 2015; Rotshenker, 2011). Distal to the injury site, where mechanical damage is not directly experienced,
74 severed axons undergo WD. The vast majority of immune cells in the injured PNS are blood-borne myeloid
75 cells, including neutrophils, monocytes (Mo), and Mac (Kalinski et al., 2020; Ydens et al., 2020). Entry into the
76 injured nerve causes rapid activation and acquisition of specific phenotypes. A comparative analysis between
77 circulating immune cells in peripheral blood, and their descends in the injured nerve, however, has not yet
78 been carried out.

79 It is well established that the immune system plays a key role in the tissue repair response (Bouchery
80 and Harris, 2019). Following PNS injury, the complement system (Ramaglia et al., 2007) and Natural killer cells
81 (NK) promote WD of damaged axons (Davies et al., 2019). Mac and neutrophils phagocytose nerve debris,
82 including degenerating myelin and axon remnants (Chen et al., 2015; Kuhlmann et al., 2002; Lindborg et al.,
83 2017; Rosenberg et al., 2012). Mac protect the injured tissue from secondary necrosis by clearing apoptotic
84 cells through phagocytosis, a process called efferocytosis (Boada-Romero et al., 2020; Greenlee-Wacker,
85 2016; Lantz et al., 2020). In the injured sciatic nerve, efferocytosis readily takes place and is associated with
86 inflammation resolution (Kalinski et al., 2020). The highly dynamic nature of intercellular communications,
87 coupled with spatial differences in the nerve microenvironment, make it difficult to untangle the immune
88 response to nerve trauma from the immune response to WD. While previous studies have employed single-cell
89 RNA sequencing (scRNAseq) to describe naïve or injured PNS tissue (Carr et al., 2019; Kalinski et al., 2020;
90 Wang et al., 2020; Wolbert et al., 2020; Ydens et al., 2020), a longitudinal study to investigate transcriptomic
91 changes and cell-cell communication networks, has not yet been carried out. Moreover, a comparative
92 analysis of cell types and transcriptional states at the nerve injury site versus the distal nerve, does not yet
93 exist.

94 To better understand cellular functions, immune cell trafficking, and intercellular communication, we
95 carried out a longitudinal study using scRNAseq and validated gene expression data by flow cytometry, ELISA,
96 *in situ* hybridization, and immunofluorescence labeling of nerve sections. Peripheral blood mononuclear cells
97 from naïve mice were analyzed by scRNAseq to identify leukocytes that enter the nerve upon injury and to
98 assess their metabolic profile. To distinguish between the immune response to nerve trauma and WD, we
99 employed *Sarm1* null (*Sarm1*$^{-/-}$) mice in which WD is greatly delayed (Osterloh et al., 2012). We show that in
100 injured *Sarm1*$^{-/-}$ mice blood-borne Mac rapidly accumulate at the nerve crush site. However, in the absence of
101 WD, inflammation in the distal nerve is greatly reduced. While monocytes are found in the distal nerve of
102 *Sarm1*$^{-/-}$ mice at 7days following injury, they fail to mature into Mac. In sum, we report temporal changes in the
103 cellular and metabolic landscape of the injured mammalian PNS, identify spatially distinct, yet overlapping
104 immune responses to nerve wounding and WD, and provide insights into WD elicited nerve inflammation.
105

106 ## Results

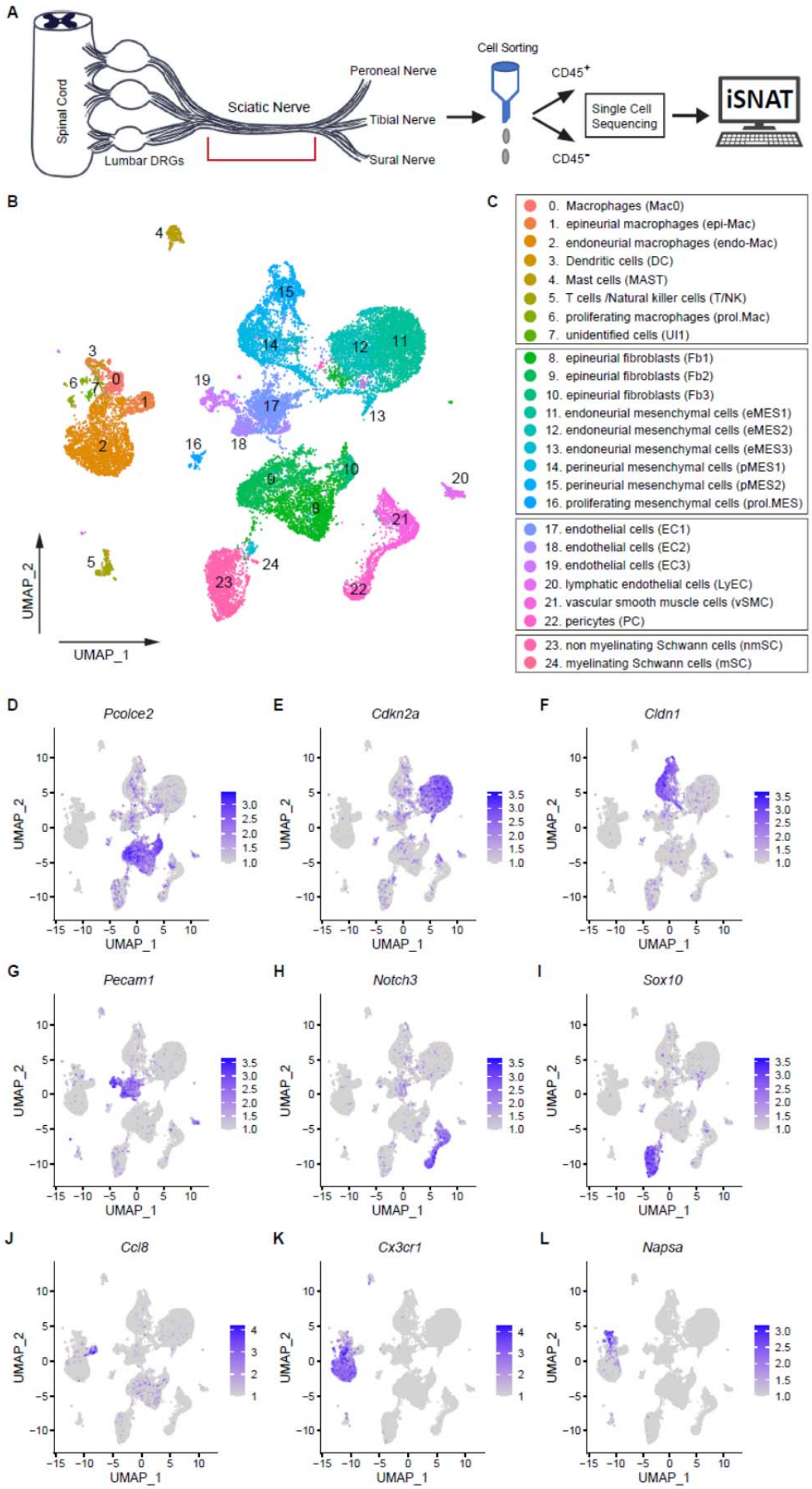
107 **The cellular and molecular landscape of naïve sciatic nerve**

108 To gain insights into extrinsic mechanisms associated with neural degeneration and regeneration, we carried
109 out a longitudinal analysis of injured mouse sciatic nerve tissue using single cell transcriptomics. We first
110 captured CD45$^+$ immune cells from sciatic nerve trunk, and in parallel, CD45$^-$ non-immune cells for scRNAseq
111 analysis (**Fig. 1A**). The resulting datasets were combined, high-quality cells identified, and subjected to
112 dimensional reduction using the top 30 principal components (**Table. S1**). Uniform manifold approximation and
113 projection (UMAP) was used for visualization of cell clusters and clusters determined using the Louvain
114 algorithm with a resolution of 0.5, revealing 24 clusters (**Fig. 1B**). Marker gene expression analysis was used
115 for cell type identification (**Fig. 1C**). Stromal cells, often referred as structural cells, are abundant in the naïve
116 nerve. They include epineurial fibroblasts (Fb1-Fb3, clusters 7-9), identified by their strong expression of
117 *Pcolce2*/procollagen C-endopeptidase enhance 2 (**Fig. 1D**), endoneurial mesenchymal cells (eMES1-eMES3,
118 clusters 10-12), expressing the tumor suppressor *Cdkn2a*/cyclin-dependent kinase inhibitor 2A (**Fig. 1E**), and
119 perineurial (p)MES in clusters 13 and 14, expressing the tight junction molecule *Cldn1*/claudin 1 (**Fig. 1F**).

120 The nerve vasculature is comprised of three clusters with endothelial cells (EC1-EC3, clusters 16-18),
121 strongly expressing *Pecam1/*CD31 (**Fig. 1G**). EC express high levels of adherent junction (*Cldn5*/claudin 5),
122 tight junction (*Tjp1*), and junctional adhesion molecules (*Jam2*), key components of the BNB (**Fig. 1, Suppl.**
123 **1A**) (Maiuolo et al., 2019). Clusters 20 and 21 are connected and harbor *Notch3*$^+$ cells (**Fig. 1H**) that can be
124 further subdivided into vascular smooth muscle cells (vSMC, *Acta2*$^+$) and pericytes (PC, *Pdgfrb*$^+$) (**Fig. 1,**
125 **Suppl. 1A**). A small island (cluster 19) with lymphatic endothelial cells (LyEC, *Prox1*) was detected (**Fig. 1,**
126 **Suppl. 1A**). Schwann cells (SC, *Sox10*) are mainly comprised of non-myelinating (nm)SC (cluster 22) and few
127 myelinating (m)SC (cluster 23) (**Fig. 1I**) (Gerber et al., 2021). The low number of mSC indicates that many are
128 lost during the cell isolation process from naïve nerve (**Fig. 1, Suppl. 1A**).

129 Mac (clusters 0, 1, and 2) are the most abundant immune cell type in the naïve nerve, representing
130 75% of all immune cells. Smaller immune cell clusters with dendritic cells (DC, 3%), mast cells (Mast, 6%), T
131 cells (TC) and Natural killer cells (NK) 5% are detected (**Fig. 1B, Suppl. 1B**). Less than 1% of immune cells
132 express markers for granulocytes (GC, *Cxcr2*/C-X-C motif chemokine receptor 2), monocytes (Mo,
133 *Chil3*/chitinase-like protein 3), or B cells (BC, *Cd79a/*B cell antigen receptor-associated protein), indicating
134 these cell types are sparse in the naïve PNS of healthy mice. Tissue resident Mac in the naïve PNS strongly
135 express the scavenger receptor SCARI1, encoded by *Cd163*. A small group of *Fn1*(fibronectin) expressing
136 Mac (cluster 0) was detected and these may represent leukocytes that recently entered the nerve (**Fig. 1,**
137 **Suppl. 2A, 2B**). Previous scRNA-seq studies employed cell sorting to isolate Mac from naïve PNS, using
138 CD45$^+$CD64$^+$F4/80$^+$ (Ydens et al., 2020) or CD45^{int}CD64$^+$ sorting (Wang et al., 2020), and reported
139 transcriptionally distinct epineurial (epi) Mac and endoneurial (endo) Mac. When compared to our dataset, we
140 find that cells in cluster 1 express *Fcna*/Ficolin-A and *Ccl8/*monocyte chemotactic protein 2, and thus,
141 represent epiMac (**Fig. 1J** and **Fig. 1, Suppl. 2C**). The expression of *Ccl8* by epiMac was validated by *in situ*
142 hybridization using RNAscope. *Ccl8* labeling was highest in Mac located in the epineurium (**Fig. 1, Suppl.**

143 **2G,G'**). Cells in cluster 2 preferentially express *Cx3cr1, Trem2, Lilra5*, and the small peptide transporter
144 *Slc15a2* and represent endoMac (**Fig. 1K** and **Fig. 1 Suppl. 2D-2F**). Longitudinal sciatic nerve sections of
145 *Cx3cr1-GFP* reporter mice confirmed labeling of endoMac (**Fig. 1, Suppl 2H,H')**. Mac are educated by the
146 local nerve microenvironment and acquire niche specific phenotypes. In the naïve PNS, epiMac (*Lyve1*^{hi},
147 *Cx3cr1*^{lo}) are located in the heavily vascularized epineurium and resemble perivascular Mac (Chakarov et al.,
148 2019), while endoMac exhibit neural niche gene signatures reminiscent of Mac in the enteric plexus (De
149 Schepper et al., 2019) and microglia (Wang et al., 2020). There are few DC in the naïve nerve, they function as
150 professional antigen presenting cells and can be identified by their strong expression of *Napsa/*aspartic
151 peptidase (**Fig. 1L**). Mast cells in the naïve nerve are readily identified by their preferential expression of
152 *Fcer1a* (the immunoglobulin epsilon receptor for IgE) and *Cpa3* (carboxypeptidase A) (**Fig. 1, Suppl 1B**).
153


A
Spinal Cord
Lumbar DRGs
Sciatic Nerve
Peroneal Nerve
Tibial Nerve
Sural Nerve
Cell Sorting
CD45+
CD45-
Single Cell Sequencing
iSNAT
B
UMAP_1
UMAP_2
C
0. Macrophages (Mac0)
1. epineurial macrophages (epi-Mac)
2. endoneurial macrophages (endo-Mac)
3. Dendritic cells (DC)
4. Mast cells (MAST)
5. T cells /Natural killer cells (T/NK)
6. proliferating macrophages (prol.Mac)
7. unidentified cells (UI1)
8. epineurial fibroblasts (Fb1)
9. epineurial fibroblasts (Fb2)
10. epineurial fibroblasts (Fb3)
11. endoneurial mesenchymal cells (eMES1)
12. endoneurial mesenchymal cells (eMES2)
13. endoneurial mesenchymal cells (eMES3)
14. perineurial mesenchymal cells (pMES1)
15. perineurial mesenchymal cells (pMES2)
16. proliferating mesenchymal cells (prol.MES)
17. endothelial cells (EC1)
18. endothelial cells (EC2)
19. endothelial cells (EC3)
20. lymphatic endothelial cells (LyEC)
21. vascular smooth muscle cells (vSMC)
22. pericytes (PC)
23. non myelinating Schwann cells (nmSC)
24. myelinating Schwann cells (mSC)
D Pcolce2
E Cdkn2a
F Cldn1
G Pecam1
H Notch3
I Sox10
J Ccl8
K Cx3cr1
L Napsa

155 **Figure 1**

156 **The cellular and molecular landscape of naïve mouse sciatic nerve**

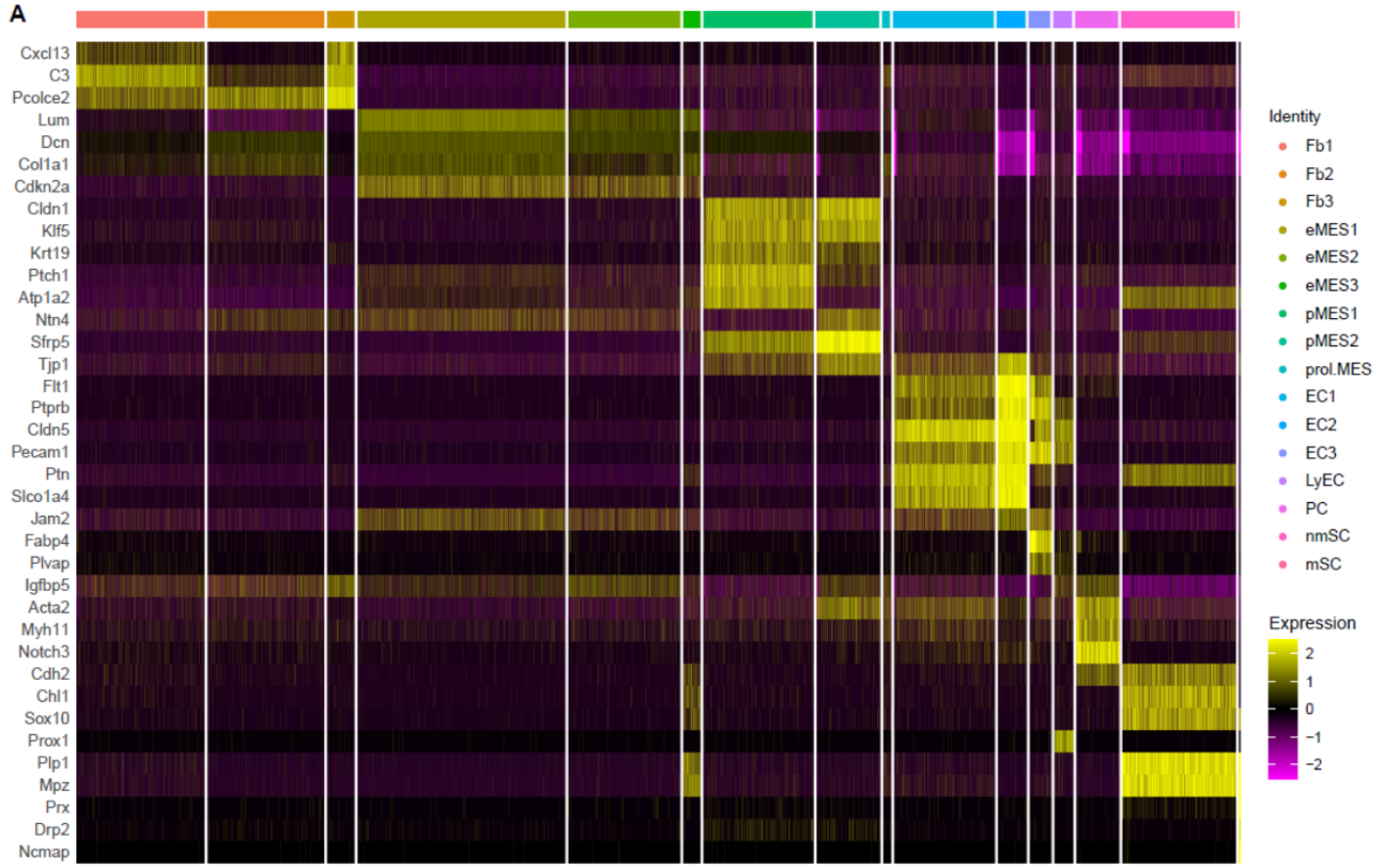
157 **(A)** Workflow for peripheral nervous tissue analysis. Cartoon of a mouse lumbar spinal cord with dorsal root
158 ganglia (DRGs), sciatic nerve trunk and major branches. The sciatic nerve trunk is marked with a red bracket
159 and was harvested for further analysis. Immune cells were captured using anti-CD45 magnetic beads. The flow
160 through containing non-immune ($CD45^-$) cells collected. In separate scRNAseq runs, $CD45^+$ and $CD45^-$ single-
161 cell transcriptomes were determined. A total of 21,973 high-quality transcriptomes, including 4,539 $CD45^+$ cells
162 and 17,434 $CD45^-$ cells were generated and used for downstream analysis. **(B)** UMAP embedding of naïve
163 sciatic nerve cells. Unsupervised Seurat-based clustering identified 24 clusters. **(C)** List of cell types identified
164 in the naïve sciatic nerve, grouped into immune cells (clusters 0-7), structural/stromal cells (clusters 8-16), cells
165 associated with the nerve vasculature (clusters 17-22), and Schwann cells (clusters 23 and 24). **(D-L)** Feature
166 plots of canonical markers used to identify major cell types, including epineurial fibroblasts
167 (*Pcolce2/*procollagen C-endopeptidase enhancer 2), endoneurial MES (*Cdkn2a/*cyclin dependent kinase
168 inhibitor 2A), perineurial MES (*Cldn1/*claudin-1), endothelial cells (*Pecam1*/CD31), vascular smooth muscle
169 cells and pericytes (*Notch3/*notch receptor 3), Schwann cells (*Sox10/*SRY-box transcription factor 10),
170 epineurial Mac (*Ccl8/*C-C motif chemokine ligand 8), endoneurial Mac (*Cx3cr1/*C-X3-C motif chemokine
171 receptor 1), and dendritic cells (*Napsa/*napsin A aspartic peptidase). Expression levels are color coded and
172 calibrated to average gene expression.

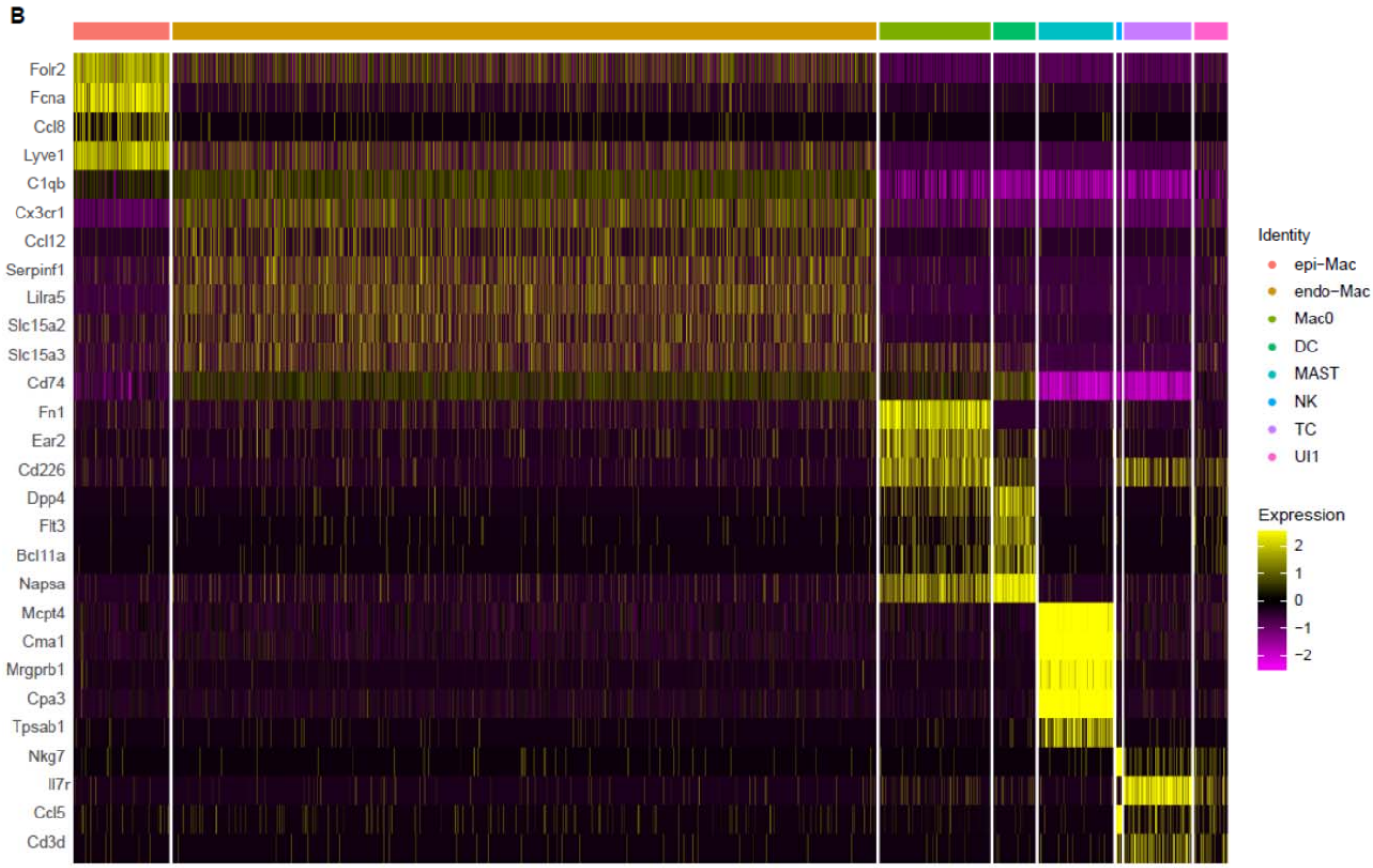
	total number of cells sequenced (analyzed)	n of independent runs (n of technical replicates)	median UMI counts per cell	median genes per Cell	number of reads	sequencing saturation
PBMC (blood)	37,292 (24,842)	1 (1)	4,724	1,315	1.09B	49.60%
naive SN (anti-CD45(+))	5,121 (4,539)	1 (1)	6,217	2,159	0.38B	82.70%
naive SN (anti-CD45(-))	18,001 (17,434)	1 (1)	5,585	2,099	1.45B	83%
1d injured SN	32,907 (29,070)	2 (5)	4,677	1,887	2.1B	78.31%
3d injured SN	28,370 (24,672)	4 (6)	6,577	2,364	2.7B	78.40%
7d injured SN	36,508 (32,967)	3 (8)	7,868	2,568	2.8B	78.50%
3d injured SN (injury site)	8,349 (6,971)	1 (2)	7,703	2,549	0.77B	78.50%
3d injured SN (distal nerve)	9,055 (7,370)	1 (2)	8,784	2,734	1.1B	83.50%

173

174 **Table. S1**

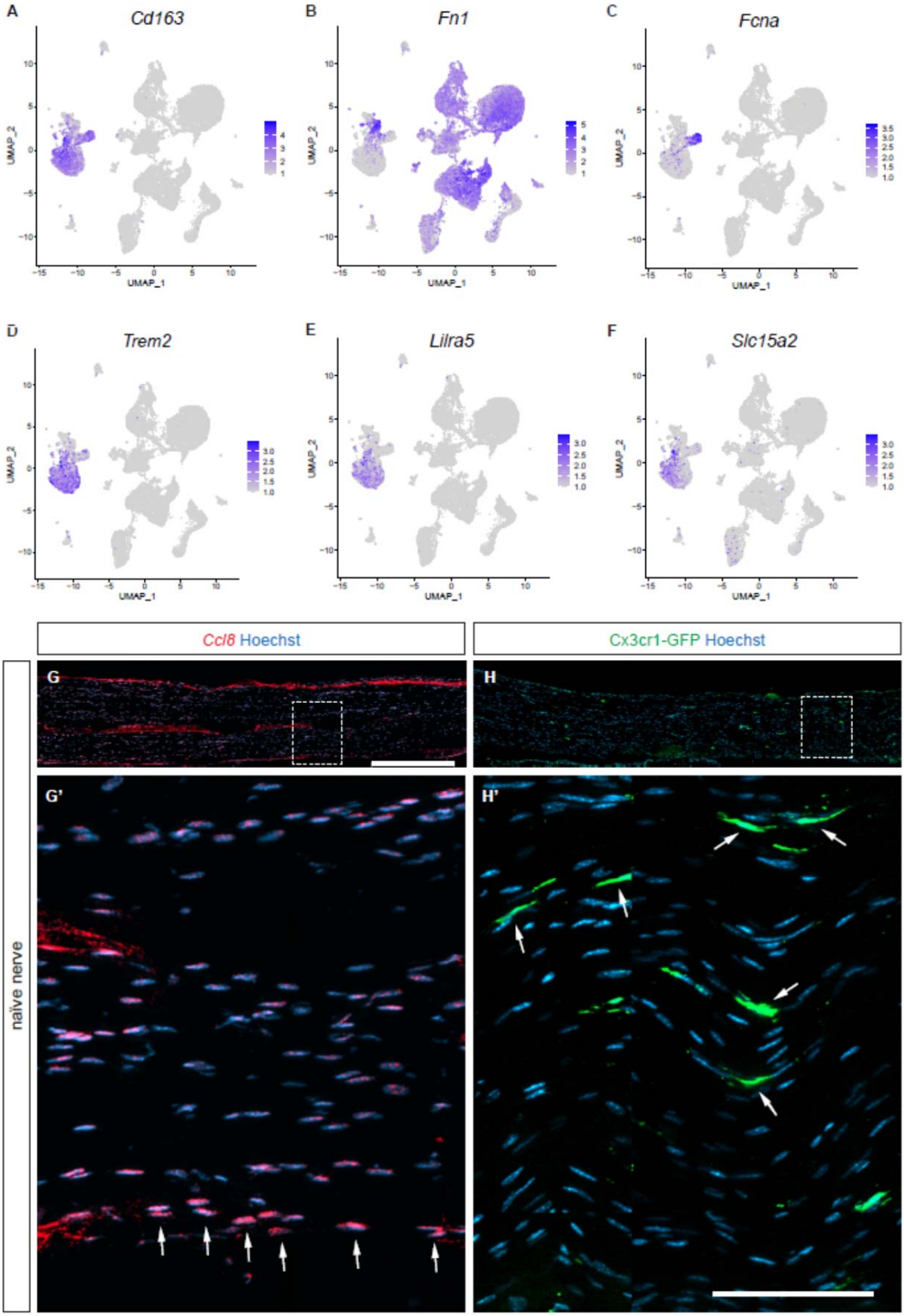
175 Summary of scRNAseq datasets generated and analyzed in this study. A total of 147,865 high-quality single
176 cell transcriptomes were generated from naïve mouse sciatic nerve, injured sciatic nerve, and peripheral blood
177 mononuclear cells (PBMC). Some of the 3-day (3d) injured nerves were divided into injury site and distal nerve
178 and sequenced separately. The number of independent runs, biological replicates, is shown and defined as
179 cells sequenced from different cohorts of mice. The number of technical replicates is shown in parenthesis and
180 indicates how many times cells from the same cohort of mice was sequenced.


A
Cxcl13
C3
Pcolce2
Lum
Dcn
Col1a1
Cdkn2a
Cldn1
Klf5
Krt19
Ptch1
Atp1a2
Ntn4
Sfrp5
Tjp1
Flt1
Ptprb
Cldn5
Pecam1
Ptn
Slco1a4
Jam2
Fabp4
Plvap
Igfbp5
Acta2
Myh11
Notch3
Cdh2
Chl1
Sox10
Prox1
Plp1
Mpz
Prx
Drp2
Ncmap
Identity
Fb1
Fb2
Fb3
eMES1
eMES2
eMES3
pMES1
pMES2
prol.MES
EC1
EC2
EC3
LyEC
PC
nmSC
mSC
Expression
2
1
0
−1
−2


B
Folr2
Fcna
Ccl8
Lyve1
C1qb
Cx3cr1
Ccl12
Serpinf1
Lilra5
Slc15a2
Slc15a3
Cd74
Fn1
Ear2
Cd226
Dpp4
Flt3
Bcl11a
Napsa
Mcpt4
Cma1
Mrgprb1
Cpa3
Tpsab1
Nkg7
Il7r
Ccl5
Cd3d
Identity
epi−Mac
endo−Mac
Mac0
DC
MAST
NK
TC
UI1
Expression
2
1
0
−1
−2

183 **Figure 1, Suppl. 1**

184 Heatmaps of top cluster enriched gene products in the naïve mouse sciatic nerve trunk for **(A)** non-immune
185 cells, and **(B)** immune cells. Expression levels are calibrated to median gene expression. Abbreviations: Fb,
186 fibroblasts; eMES, endoneurial mesenchymal cells; pMES, perineurial mesenchymal cells; prol.MES,
187 proliferating mesenchymal cells; EC, endothelial cells; LyEC, lymphatic endothelial cells; PC, pericytes; nmSC,
188 non-myelinating Schwann cells; mSC, myelinating Schwann cells; epiMac, epineurial macrophages; endoMac,
189 endoneurial macrophages; DC, dendritic cells; MAST, mast cells; NK, natural killer cells; TC, T cells; UI,
190 unidentified cells.


A
Cd163
B
Fn1
C
Fcna
D
Trem2
E
Lilra5
F
Slc15a2
UMAP_1
UMAP_2
Ccl8 Hoechst
Cx3cr1-GFP Hoechst
G
H
G'
H'
naïve nerve

191

192 **Figure 1, Suppl. 2**

193 **(A-F)** Feature plots of naïve sciatic nerve. (**A**) *Cd163* is expressed by epi-Mac and endo-Mac. (**B**) A small
194 subpopulation of Mac, expressing very high levels of *Fn1* (fibronectin), is observed in the naïve nerve. In
195 addition, *Fn1* is expressed by most non-immune cells. (**C**) *Fcna* (Ficolin A) is strongly and selectively
196 expressed by epi-Mac. (**D-F**) Show expression of the endo-Mac markers *Trem2* (triggering receptor expressed
197 on myeloid cells 2), *Lilra5* (leukocyte immunoglobulin like receptor A5), and *Slc15a1* (solute carrier family 15
198 member 1). Expression levels are projected onto the UMAP with a minimum expression cutoff of 1. **(G,G')**
199 Longitudinal sections of naïve mouse sciatic nerve. *In situ* hybridization of *Ccl8* with RNAscope revealed
200 preferential staining of epi-Mac, labeled with arrows. (**H,H'**) Longitudinal sciatic nerve section of *Cx3cr1-GFP*
201 reporter mice revealed preferential labeling of endo-Mac. Scale bar (**G,H**), 500 μm; (**G',H'**),100 μm.
202

203 To independently validate naïve nerve scRNAseq datasets, we examined the presence of some of the
204 corresponding proteins in naïve sciatic nerve lysates by ELISA. We used an ELISA kit that allows simultaneous
205 profiling of 111 extracellular proteins, including cytokines, chemokines, growth factors, proteases, and protease
206 inhibitors (**Fig. 1, Suppl. 3A**). Proteins abundantly detected include, FGF1 (*Fgf1*), IGF binding protein 6
207 (*Igfbp6*), the anti-angiogenic factor endostatin (*Col18a1*), interleukin 33 (*Il33*), coagulation factor III (*F3*),
208 cystatin C (*Cst3*), retinoic acid binding protein 4 (*Rbp4*), matrix metallopeptidase 2 (*Mmp2*), intercellular
209 adhesion molecule 1 (*Icam1*), fetuin A/alpha 2-HS glycoprotein (*Ahsg*), resistin/adipose tissue-specific
210 secretory factor (*Retn*), low-density lipoprotein receptor (*Ldlr*), serpin F1/neurotrophic protein (*Serpinf1*), and
211 adiponectin (*Adipoq*), a regulator of fat metabolism (**Fig. 1, Suppl. 3B**). For many of these proteins, the
212 corresponding transcripts are present in our scRNAseq data (**Fig. 1, Suppl. 3C**). Gene products abundantly
213 detected by ELISA, but not by scRNAseq, include fetuin A, resistin, and adiponectin (**Fig. 1, Suppl. 3C**).
214 Resistin and adiponectin are both secreted by adipocytes, and fetuin A is secreted by hepatocytes. All three
215 proteins are known serum components, and thus, may have entered the nerve via the circulatory system.
216 Indeed, ELISA for serum proteins from naïve mice, revealed high levels of fetuin A, resistin, and adiponectin
217 (**Fig. 1, Suppl. 3D**). Importantly, some of the most abundant serum proteins, including E-selectin (*Sele*),
218 IGFBPs (*Igfbp1, 2, 3* and *5*), proprotein convertase 9 (*Pcsk1*), soluble C1qR1 (*Cd93)*, angiopoietin-2 (*Angpt2*),
219 C-C motif chemokine ligand 21 (*Ccl21*), and CRP/C-reactive protein (*Crp*) are not present in nerve lysates,
220 providing confidence that mice were perfused properly, and nerve samples not contaminated with serum (**Fig.
221 1, Suppl. 3E**). These experiments also identify serum proteins that can or cannot cross the intact BNB.
222 Collectively, our analysis establishes a baseline of the cellular landscape and molecular milieu of naïve PNS.
223 These data will be used as reference to determine how a crush injury alters the nerve microenvironment.

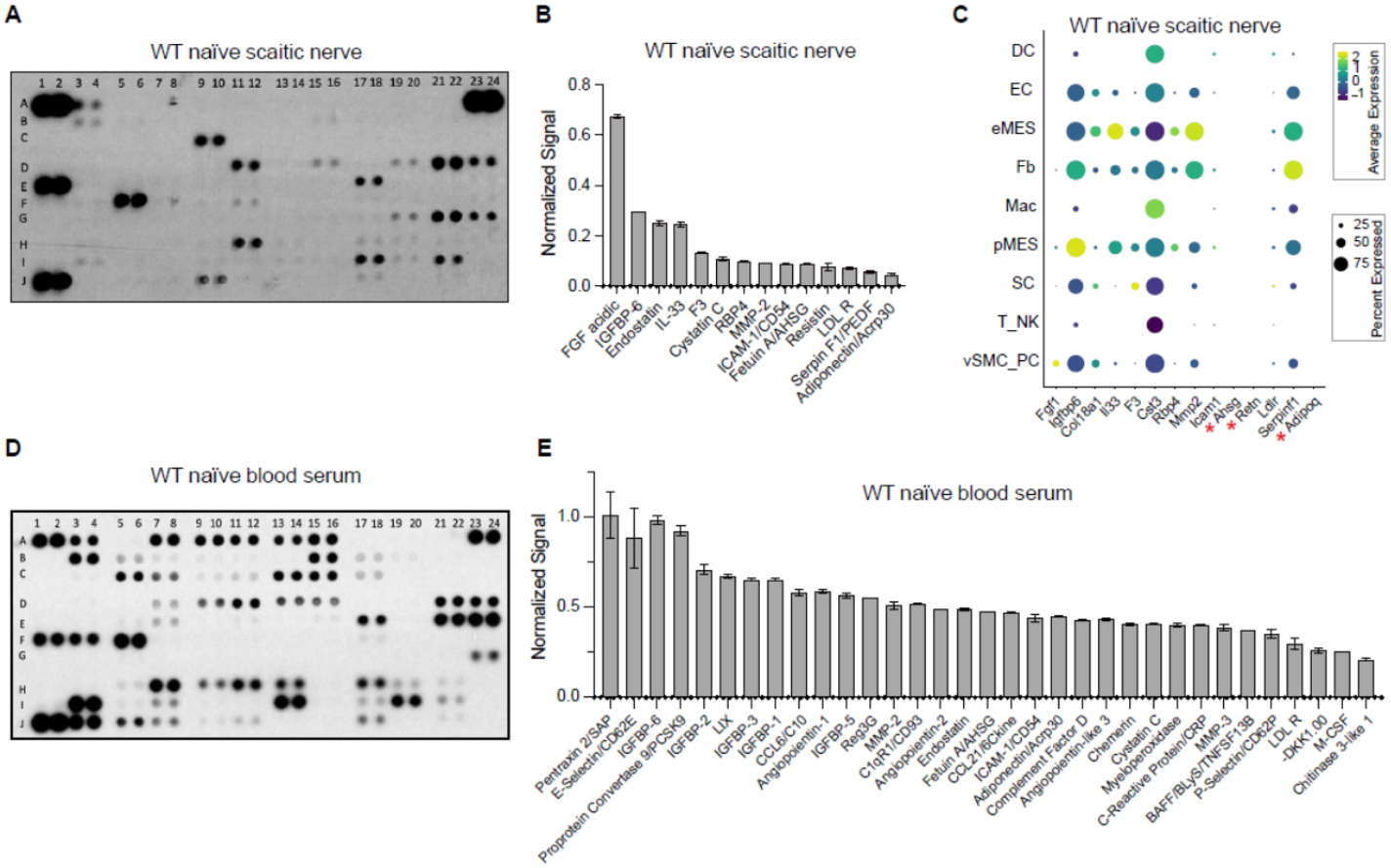


Figure 1, Suppl. 3

(A) ELISA membrane probed with naïve sciatic nerve lysate. **(B)** List of top proteins detected. For quantification, ELISA signals were normalized to reference spots shown at coordinates (A1,A2), (A23,A24), and (J1,J2). **(C)** Dotplot analysis of the corresponding gene products of naïve sciatic nerve cells, as identified by scRNAseq. Relative expression levels, normalized to average gene expression (color coded) are shown. For each cell cluster, the percentile of cells expressing a specific gene product is indicated by the dot size. **(D)** ELISA membrane probed with serum from naïve mice. (**E**) List of top serum proteins detected. For quantification, ELISA signals were normalized to reference spots shown at coordinates (A1,A2), (A23,A24), and (J1,J2). Number of biological replicates, n =1. Coordinates of proteins that can be detected by the ELISA, (A1, A2) Reference spots, (A3, A4) Adiponectin [*Adipoq*], (A5, A6) Amphiregulin [*Areg*], (A7, A8) Angiopoientin-1 [*Angpt1*], (A9, A10) Angiopoientin-2 [*Angpt2*], (A11, A12) Angiopoientin-like 3 [*Angptl3*], (A13, A14) BAFF [*Tnfrsf13b*], (A15, A16) C1qR1 [*Cd93*], (A17, A18) CCL2 [*Ccl2*], (A19, A20) CCL3 [*Ccl3*], (A21, A22) CCL5 [*Ccl5*], (A23, A24) Reference spots, (B3, B4) CCL6 [*Ccl6*], (B5, B6) CCL11 [*Ccl11*], (B7, B8) CCL12 [*Ccl12*], (B9, B10) CCL17 [*Ccl17*], (B11, B12) CCL19 [*Ccl19*], (B13, B14) CCL20 [*Ccl20*], (B15, B16) CCL21 [*Ccl21*], (B17, B18) CCL22 [*Ccl22*], (B19, B20) CD14 [*Cd14*], (B21, B22) CD40 [*Cd40*], (C3, C4) CD160 [*Cd160*], (C5, C6) Chemerin [*Rarres2*], (C7, C8) Chitinase 3-like 1 [*Chil3*], (C9, C10) Coagulation Factor III [*F3*], (C11, C12) Complement Component C5 [*C5*], (C13, C14) Complement Factor D [*Cfd*], (C15, C16) C-Reactive Protein [*Crp*], (C17, C18) CX3CL1 [*Cx3cl1*], (C19, C20) CXCL1 [*Cxcl1*], (C21, C22) CXCL2 [*Cxcl2*], (D1, D2) CXCL9 [*Cxcl9*], (D3, D4) CXCL10 [*Cxcl10*], (D5, D6) CXCL11 [*Cxcl11*], (D7, D8) CXCL13 [*Cxcl13*], (D9, D10) CXCL16

244 [*Cxcl16*], (D11, D12) Cystatin C [*Cst3*], (D13, D14) DKK-1 [*Dkk1*], (D15, D16) DPPIV [*Dpp4*], (D17, D18) EGF
245 [*Egf*], (D19, D20) Endoglin [*Eng*], (D21, D22) Endostatin [*Col18a1*], (D23, D24) Fetuin A [*Ahsg*], (E1, E2) FGF
246 acidic [*Fgf1*], (E3, E4) FGF-21 [*Fgf21*], (E5, E6) Flt-3 Ligand [*Flt3l*], (E7, E8) Gas 6 [*Gas6*], (E9, E10) G-CSF
247 [*Csf3*], (E11, E12) GDF-15 [*Gdf15*], (E13, E14) GM-CSF [*Csf2*], (E15, E16) HGF [*Hgf*], (E17, E18) ICAM-1
248 [*Icam1*], (E19, E20) IFN-gamma [*Ifng*], (E21, E22) IGFBP-1 [*Igfbp1*], (E23, E24) IGFBP-2 [*Igfbp2*], (F1, F2)
249 IGFBP-3 [*Igfbp3*], (F3, F4) IGFBP-5 [*Igfbp5*], (F5, F6) IGFBP-6 [*Igfbp6*], (F7, F8) IL-1alpha [*Il1a*], (F9, F10) IL-
250 1Beta [*Il1b*], (F11, F12) IL-1ra [*Il1rn*], (F13, F14) IL-2 [*Il2*], (F15, F16) IL-3 [*Il3*], (F17, F18) IL-4 [*Il4*], (F19, F20)
251 IL-5 [*Il5*], (F21, F22) IL-6 [*Il6*], (F23, F24) IL-7 [*Il7*], (G1, G2) IL-10 [*Il10*], (G3, G4) IL-11 [*Il11*], (G5, G6) IL-12
252 p40 [*Il12*], (G7, G8) IL-13 [*Il13*], (G9, G10) IL-15 [*Il15*], (G11, G12) IL-17A [*Il17a*], (G13, G14) IL-22 [*Il22*], (G15,
253 G16) IL-23 [*Il23*], (G17, G18) IL-27 p28 [*Il27*], (G19, G20) IL-28 [*Ifnl3*], (G21, G22) IL-33 [*Il33*], (G23, G24) LDL
254 R [*Ldlr*], (H1, H2) Leptin [*Lep*], (H3, H4) LIF [*Lif*], (H5, H6) Lipocalin-2 [*Lcn2*], (H7, H8) LIX [*Cxcl5*], (H9, H10)
255 M-CSF [*Csf1*], (H11, H12) MMP-2 [*Mmp2*], (H13, H14) MMP-3 [*Mmp3*], (H15, H16) MMP-9 [*Mmp9*], (H17,
256 H18) Myeloperoxidase [*Mpo*], (H19, H20) Osteopontin [*Spp1*], (H21, H22) Osteoprotegerin [*Tnfrsf11b*], (H23,
257 H24) PD-ECGF [*Tymp*], (I1, I2) PDGF-BB [*Pdgfb*], (I3, I4) Pentraxin 2 [*Nptx2*], (I5, I6) Pentraxin 3 [*Ptx3*], (I7,
258 I8) Periostin [*Postn*], (I9, I10) Pref-1 [*Dlk1*], (I11, I12) Proliferin [*Prl2c2*], (I13, I14) Proprotein Convertase 9
259 [*Pcsk9*], (I15, I16) RAGE [*Ager*], (I17, I18) RBP4 [*Rbp4*], (I19, I20) Reg3G [*Reg3g*], (I21, I22) Resistin [*Retn*],
260 (J1, J2) Reference spots, (J3, J4) E-Selectin [*Sele*], (J5, J6) P-Selectin [*Selp*], (J7, J8) Serpin E1 [*Serpine1*],
261 (J9, J10) Serpin F1 [*Serpinf1*], (J11, J12) Thrombopoietin [*Thpo*], (J13, J14) TIM-1 [*Havcr1*], (J15, J16) TNF-
262 alpha [*Tnf*], (J17, J18) VCAM-1 [*Vcam1*], (J19, J20) VEGF [*Vegf*], (J21, J22) WISP-1 [*Ccn4*], (J23, J24)
263 negative control.

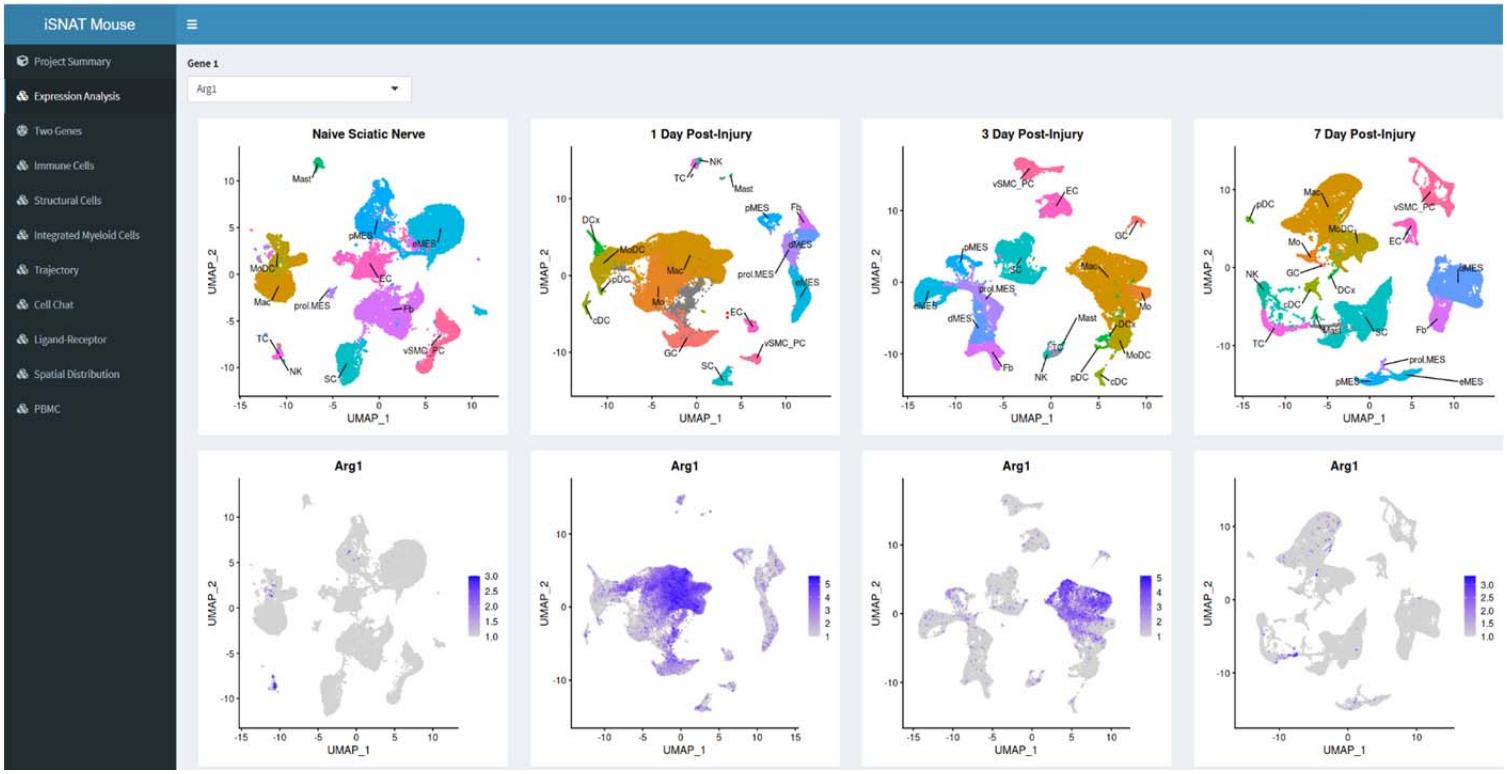
264

265 **The injured sciatic nerve atlas (iSNAT)**

266 To describe the dynamic nature of the cellular and molecular landscape of injured PNS tissue, we subjected
267 adult mice to sciatic nerve crush injury (SNC) and carried out scRNAseq at 1-day post-SNC (1dpc), 3dpc, and
268 7dpc (**Fig. 2A)**. **Table S1** shows the number of cells analyzed at each time point. The longitudinal study
269 revealed marker genes that allow reliable identification of major cell types in the naïve and injured PNS (**Fig. 2,**
270 **Suppl. 1A-D**). SNC triggers massive infiltration of blood-borne immune cells (Kalinski et al., 2020; Ydens et
271 al., 2020). In the 1dpc UMAP plot we identified granulocytes (GC, *Cxcr2*) in cluster 11 (Fig. 2D), Mo (*Ly6c2/*
272 lymphocyte antigen 6C2 and *Chil3/*Ym1) in cluster 0 (**Fig. 2F, Fig. 2, Suppl. 1B**), and five Mac subpopulations
273 (Mac-I to Mac-V) in clusters 1-5, expressing *Adgre1*(F4/80) (**Fig. 2G**). GC are primarily composed of
274 neutrophils (*Retnlg/*resistin-like gamma*, Grina/*NMDA receptor associated protein 1), intermingled with a
275 smaller population of eosinophils (*Siglecf*) (**Fig. 2E**). Mo that recently entered the nerve can be identified by
276 high levels of *Cd177* (data not shown) a known surface molecule that interacts with the EC adhesion molecule
277 *Pecam1*/CD31 and promotes transmigration. Mo strongly express *Ly6c2*, suggesting they represent Ly6C^{hi}
278 proinflammatory cells. In the UMAP plot of 1dpc nerves, cluster 0 (Mo) has a volcano-like shape connected at
279 the top to cluster 1, harboring Mac-I (*Ccr2^{hi}, Ly6c2^{low}, Cx3cr1^{-}*), indicating these are maturing monocyte-derived
280 Mac (**Fig. 2B**). Four smaller Mac clusters include, Mac-II (*C1qa*, complement factor C1q), Mac-III

281 (*Ltc4s*/leukotriene C4 synthase, *Mmp19*/matrix metallopeptidase 19), Mac-IV expressing MHCII genes (*H2-Aa,*
282 *H2-Eb1, H2-Ab1, Cd74*), and Mac-V (*Il1rn*/IL1 receptor antagonist, *Cd36/*ECM receptor*,* coagulation factors *F7*
283 and *F10*), suggesting roles in opsonization, matrix remodeling, antigen presentation, and stopping of bleeding
284 (**Fig. 2B**, and data not shown). Cluster 6 harbors monocyte-derived dendritic cells (MoDC: *Cd209a*/DC-SIGN,
285 *H2-Aa*/MHC class II antigen A, alpha), professional antigen presenting cells (**Fig. 2H, 2I**). A small island
286 (cluster 13) harbors a mixture of TC and NK (**Fig. 2B**). The 1dpc dataset contains five clusters with structural
287 cells (Fb, differentiating (d)MES, eMES, and pMES), including a small number of proliferating (*Mki67*$^+$/Ki67)
288 cells, vasculature cells, EC (cluster 20), vSMC and PC (cluster 21) (**Fig. 2B and Fig. 2, Suppl. 2B**).
289 In the UMAP plot of 3dpc nerve (**Fig. 2J**), far fewer GC (*Cxcr2*) (**Fig. 2L**) and Mo (*Ly6c2*) (**Fig. 2M**) are
290 present when compared to 1dpc nerve. *Adgre1*$^+$ Mac are the most dominant immune cell type at 3dpc,
291 comprised of five subpopulations, (clusters 1-5) designated Mac1-Mac4, and prol. Mac (**Fig. 2O**). *Ccr2* labels
292 most Mac and DC, except for Mac4 and a subset of Mac1 cells (**Fig. 2N**). As discussed below, there is no one-
293 to-one match of Mac subclusters identified in the 1dpc and 3dpc nerve, likely owing to their high degree of
294 transcriptional plasticity. To emphasize this observation, we labeled Mac in the 1dpc nerve as MacI-MacV and
295 Mac in the 3dpc nerve as Mac1-Mac4 and prol.Mac (**Fig. 2C and 2K**). MoDC (*Cd209a*) are readily detected in
296 the 3dpc nerve (**Fig. 2P**). Compared to the 1dpc nerve, the fraction of MHCII$^+$ (*H2-Aa*) cells is increased,
297 including four subpopulations of dendritic cells (MoDC, cDC, DCx, and pDC, see below for details), as well as
298 subpopulations of Mac (**Fig. 2Q**). Structural cells begin to proliferate heavily at 3dpc, suggesting that a nerve
299 crush injury causes substantial damage to the epineurium, perineurium, and endoneurium (**Fig. 2, Suppl. 2C**).
300 SNC is known to inflict vascular damage and breach in the BNB. This is underscored by the strong
301 proliferative response of vascular cells, including EC, vSMC, and PC (**Fig. 2, Suppl. 2C**).
302 In the 7dpc UMAP plot (**Fig. 2R**), GC and Mo have further declined (**Fig. 2T, 2U**), and *Adgre1*$^+$ Mac
303 remain highly prevalent (**Fig. 2W**). Mac form a connected continuum of clusters, harboring subpopulations
304 Mac-a to Mac-d (**Fig. 2S**). *Ccr2* remains high in most Mac subpopulations, except for Mac-c and is also
305 observed in Mo and DC (**Fig. 2V**). Similar to earlier timepoints, there is no clear one-to-one match of Mac
306 clusters at 7dpc to Mac at 3dpc and are thus labeled Mac-a to Mac-e (**Fig. 2R**). At 7dpc, the number of
307 *Cd209a*$^+$ DC has increased compared to earlier time points (**Fig. 2X**). MHCII (*H2-Aa*) expressing Mac and DC
308 are readily detected (**Fig. 2Y**). Lymphoid cells, identified by *Il2rb*/IL2 receptor subunit β expression, include NK
309 (cluster 15) and TC (cluster 16) (**Fig. 2R**). At 7dpc, the proliferation of structural and vascular cells is reduced
310 compared to 3dpc (**Fig. 2, Suppl. 2C, 2D**), suggesting that cells required for the repair process and wound
311 healing are in place.
312 The sequelae of successful tissue repair requires extensive communication between nerve resident
313 cells and hematogenous immune cells. For a detailed description of the cellular and molecular changes that
314 occur during the first week following PNS injury, we generated and analyzed more than 180,000 high-quality
315 single-cell transcriptomes (**Table S1**). These datasets provide a new resource for understanding cell function
316 and molecules in the degenerating and regenerating mammalian PNS. For widespread distribution of
317 scRNAseq data, we created the injured sciatic nerve atlas (iSNAT), a user-friendly and freely available web-
318 based resource. The "*Expression Analysis*" function in iSNAT provides a readily accessible platform for

comparative analysis of gene expression in naïve and injured nerve at single-cell resolution, https://cdb-



rshiny.med.umich.edu/Giger_iSNAT/.

Screenshot of iSNAT webpage showing the menu with the different tabs/functions*. As an example, the longitudinal expression changes of arginase-1 (Arg1) are shown. Time points include naïve sciatic nerve, 1-day post injury, 3-days post injury, and 7-days post injury. Top row shows UMAP plots with major cell types labeled. The bottom row shows expression of Arg1 during the first week post nerve injury. Note, Arg1 shows highest expression in macrophage (Mac) subpopulations in the 1-day and 3-day injured nerves.*

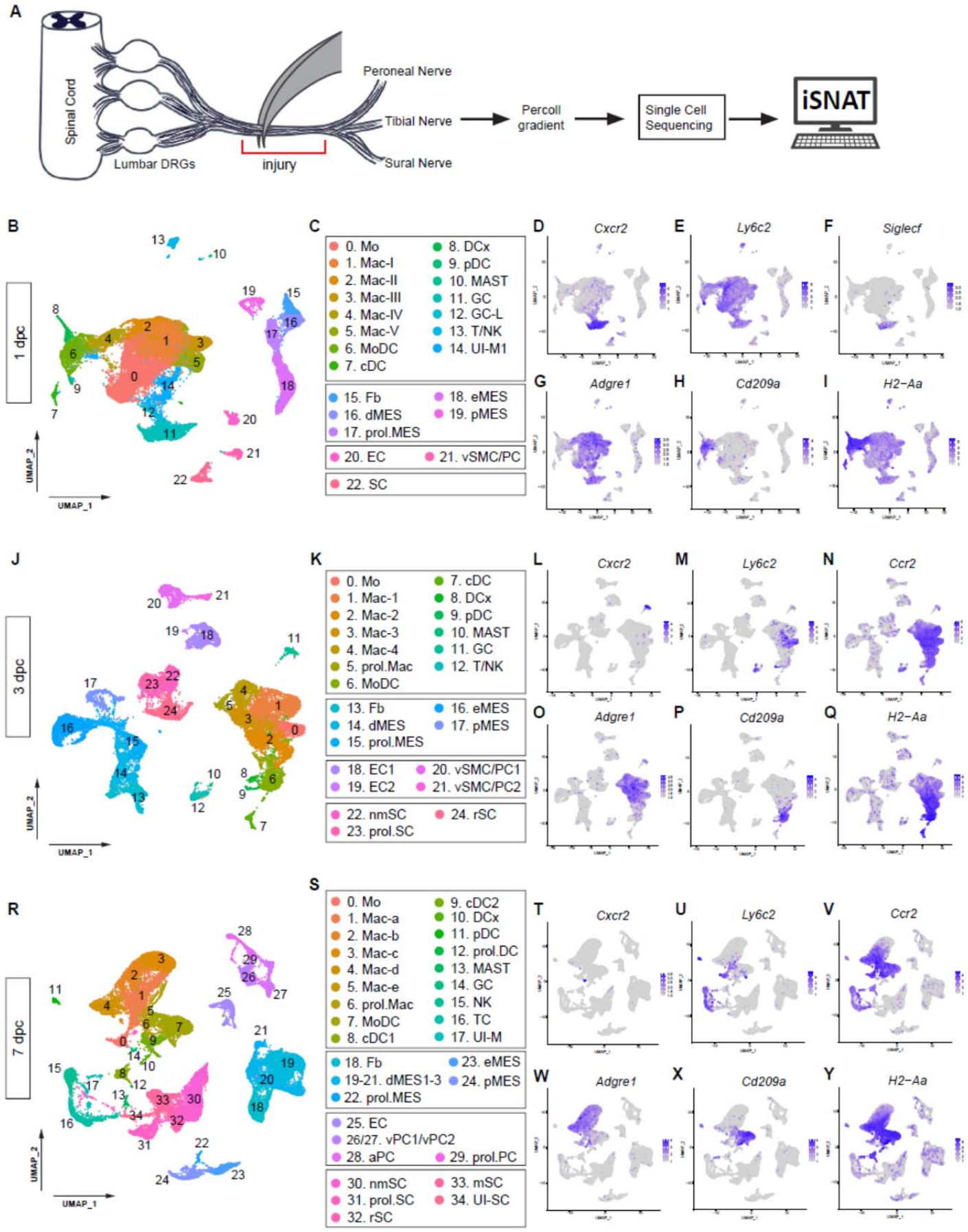
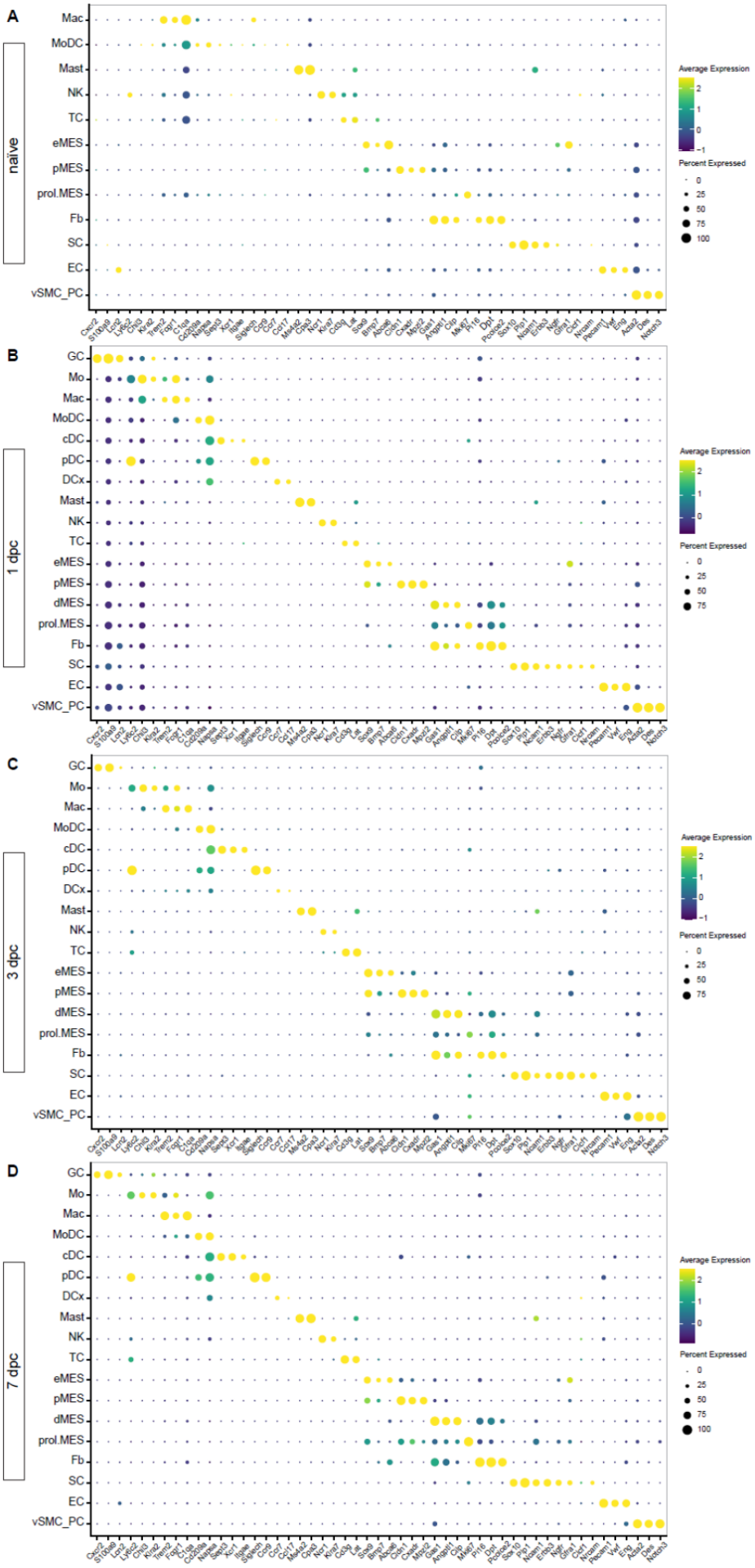


Figure 2

Longitudinal analysis of single-cell transcriptomes of injured PNS

(A) Workflow for single cell analysis of injured mouse sciatic nerve trunk. Cartoon of lumbar spinal cord with DRGs and sciatic nerve. The injury site is shown and the segment marked with the red bracket was harvested at different post-injury time points and analyzed by scRNAseq. **(B)** UMAP plot embedding of sciatic nerve cells at 1dpc. A total of 29,070 high-quality cells (n= 2 biological replicates, 5 technical replates) were subjected to unsupervised Seurat-based clustering resulting in 22 cell clusters. **(C)** List of cell types identified, grouped into immune cells (clusters 0-14), structural cells (clusters 15-19), cells associated with the nerve vasculature (clusters 20 and 21), and Schwann cells (cluster 22). **(D-I)** Feature plots of canonical immune cell markers to identify clusters with GC (*Cxcr2*), including a subset of eosinophils (*Siglecf/*sialic acid binding Ig-like lectin F), Mo (*Ly6c2/*Ly6C), Mac (*Adgre1/*F4/80), MoDC (*Cd209a/*DC-SIGN), and antigen presenting cells (*H2-Aa*/histocompatibility 2, class II antigen A, alpha). **(J)** UMAP plot embedding of sciatic nerve cells at 3dpc. A total of 24,672 high-quality cells (n= 4 biological replicates, 6 technical replates) were subjected to unsupervised Seurat-based clustering resulting in 24 cell clusters. **(K)** List of cell types in the 3-day injured nerve, grouped into immune cells (clusters 0-12), structural cells (clusters 13-17), cells associated with the nerve vasculature (clusters 18-21), and Schwann cells (clusters 22-24). **(L-Q)** Feature plots of canonical markers for immune cells to identify clusters with GC (*Cxcr2*), Mo (*Ly6c2*), Mo/Mac (*Ccr2*), Mac (*Adgre1*), MoDC (*Cd209a*), and antigen presenting cells (*H2-Aa*). **(R)** UMAP plot embedding of sciatic nerve cells at 7dpc. A total of 32,976 high-quality cells (n= 3 biological replicates, 8 technical replates) were subjected to unsupervised Seurat-based clustering resulting in 34 cell clusters. **(S)** List of cell types in the nerve at 7dpc, grouped into immune cells (clusters 0-17), structural cells (clusters 18-24), cells associated with the nerve vasculature (clusters 25-29), and Schwann cells (clusters 30-34). **(T-Y)** Feature plots of canonical markers for immune cells to identify clusters with GC (*Cxcr2*), Mo (*Ly6c2*), Mo/Mac (*Ccr2*), Mac (*Adgre1*), MoDC (*Cd209a*), and antigen presenting cells (*H2-Aa*). Expression levels are projected onto the UMAP with a minimum expression cutoff of 1. Abbreviations for immune cells: Mo, monocytes; Mac, macrophages; prol.Mac, proliferating macrophages; MoDC, monocyte-derived dendritic cells; cDC, conventional dendritic cells; DCx mature/migrating dendritic cells; pDC, plasmacytoid dendritic cells; MAST, mast cells; GC, granulocytes (including neutrophils and eosinophils), GC-L, granule cell-like; TC, T cells; NK, natural killer cells. Abbreviations for structural cells: Fb, fibroblast; dMES, differentiating mesenchymal cells; prol.MES, proliferating mesenchymal cells; eMES, endoneurial mesenchymal cells; pMES, perineurial mesenchymal cells. Abbreviations for vascular cells: EC, endothelial cells, vSMC, vascular smooth muscle cells; PC, pericytes; vPC, venous pericytes; aPC, arterial pericytes; prol.PC, proliferating pericytes. Abbreviations Schwann cells, nmSC, non-myelinating Schwann cells; mSC myelinating Schwann cells; rSC, repair Schwann cells; prol.SC, proliferating Schwann cells. UI, unidentified cells.


A
naïve
Mac
MoDC
Mast
NK
TC
eMES
pMES
prol.MES
Fb
SC
EC
vSMC_PC
Average Expression
Percent Expressed
B
1 dpc
GC
Mo
Mac
MoDC
cDC
pDC
DCx
Mast
NK
TC
eMES
pMES
dMES
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SC
EC
vSMC_PC
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MoDC
cDC
pDC
DCx
Mast
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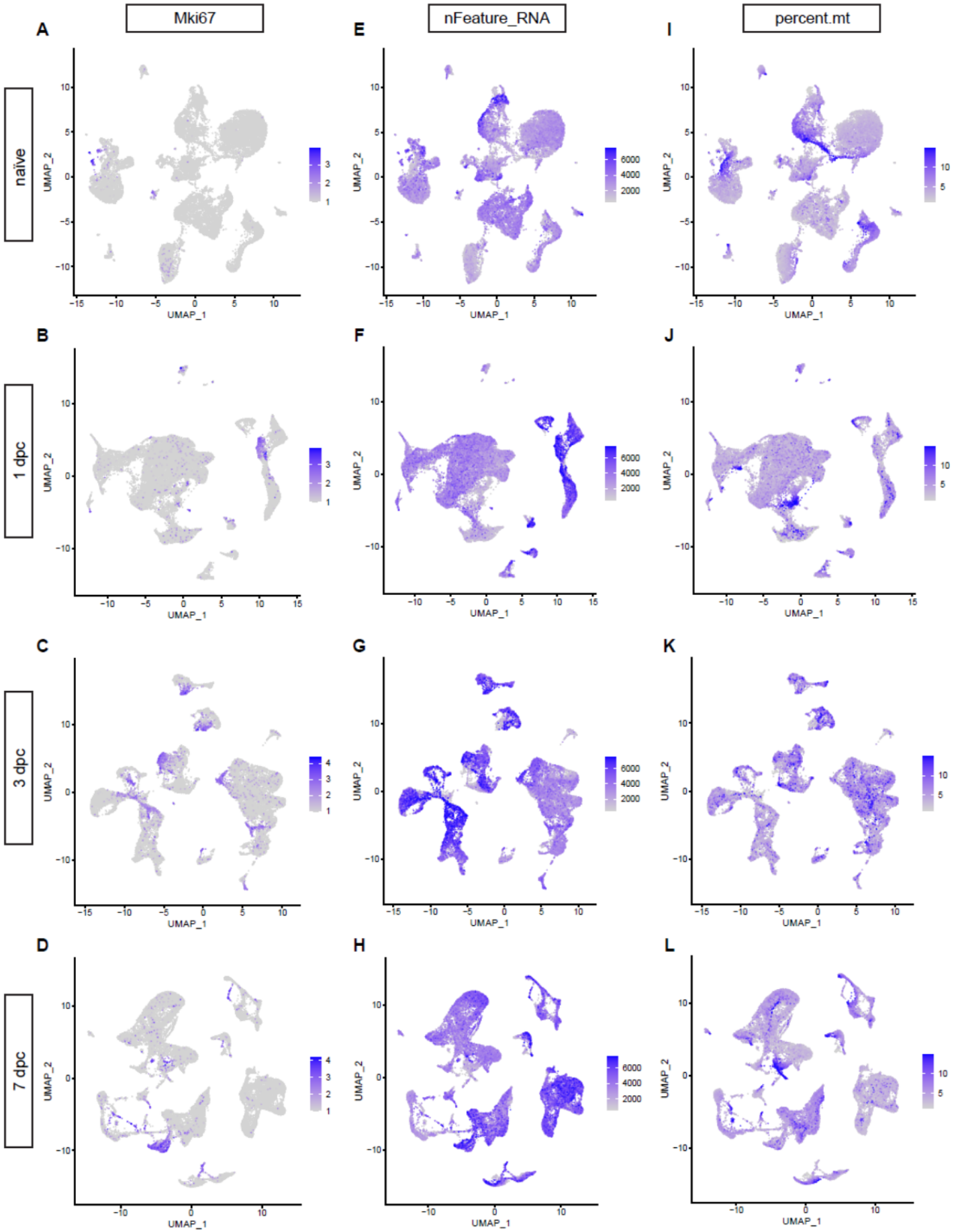
Fb
SC
EC
vSMC_PC
Average Expression
Percent Expressed

362

363 **Figure 2, Suppl Figure 1**

364 **Identification of marker genes for cell type identification in the naïve and injured PNS**

365 Dotplot analysis of scRNAseq datasets of **(A)** naïve nerve, **(B)** 1dpc nerve, **(C)** 3dpc nerve, and **(D)** 7dpc
366 nerve. Expression levels are normalized to average gene expression (color coded). For each cell cluster, the
367 percentile of cells expressing a specific gene product is indicated by the dot size.


Mki67
nFeature_RNA
percent.mt
A
B
C
D
E
F
G
H
I
J
K
L
naïve
1 dpc
3 dpc
7 dpc
UMAP_1
UMAP_2

368

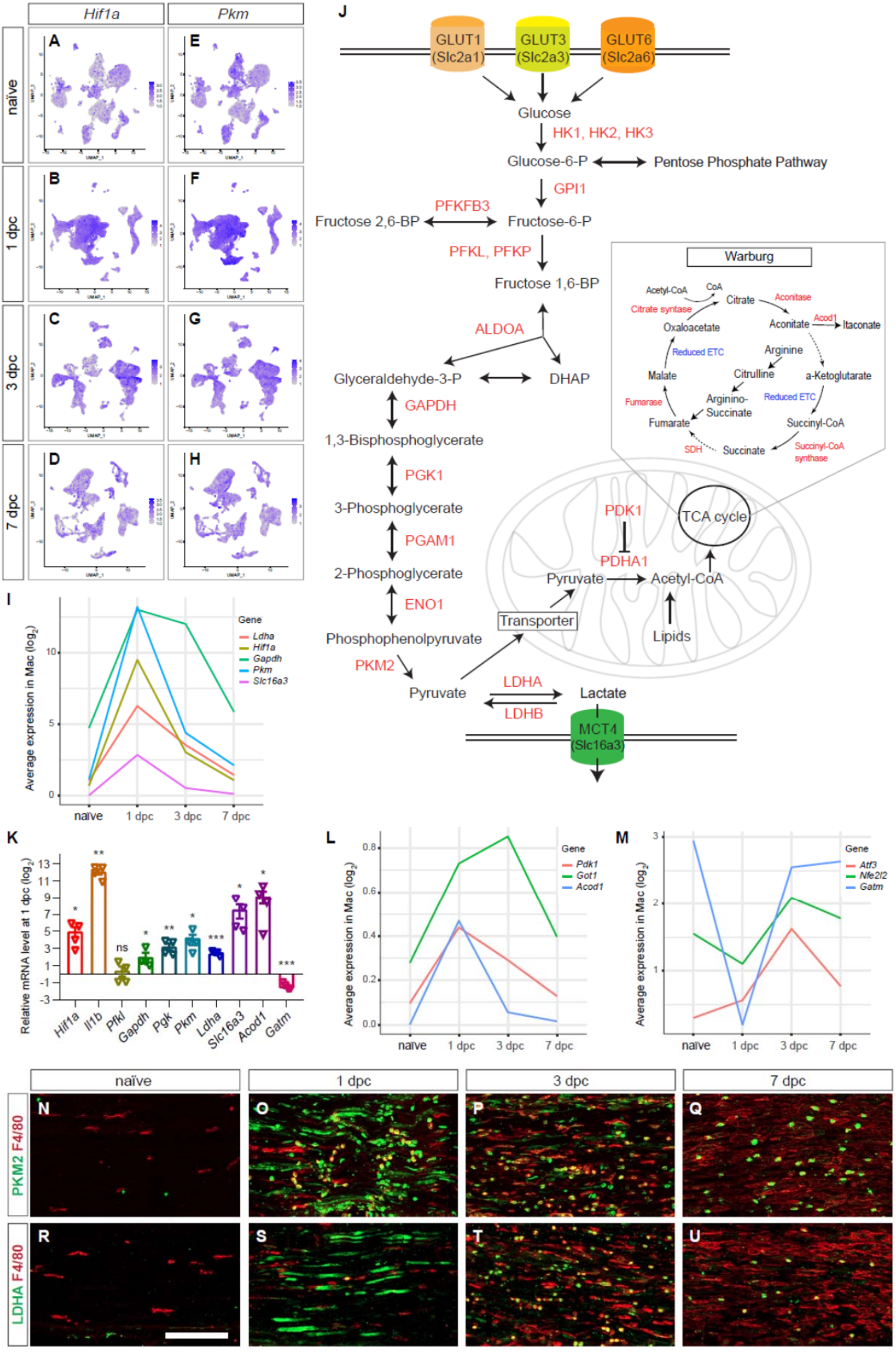
369

370 **Figure 2, Suppl Figure 2**
371 Feature plots for *Mki67* reveals proliferating cells in **(A)** naïve nerve, **(B)** 1dpc, **(C)** 3dpc, and **(D)** 7dpc. The
372 expression levels are projected onto the UMAP with a minimum expression cutoff of 1. **(E-H)** Feature plot
373 showing the number of unique transcripts detected in naïve and injured sciatic nerve cells. Color coded
374 calibration is shown. Note, cells with less than 500 unique features or more than 7500 were excluded from the
375 study. **(I-L)** Feature plots showing the mitochondrial content of cells in the naïve and injured nerves. Color
376 coded calibration is shown. Note, cells with more than 15% mitochondrial content were excluded from the
377 analysis.
378
379 **Myeloid cells in the injured nerve undergo rapid metabolic reprogramming**
380 To demonstrate the application of iSNAT toward understanding how cellular function may change during the
381 repair process, we focused on energy metabolism. Tissue repair is an energetically demanding process,
382 suggesting cells must efficiently compete for limited resources, at the same time, the repair process requires
383 highly coordinated action among diverse cell types. Nerve injury results in strong upregulation of the
384 transcription factor *Hif1a*/hypoxia-induced factor 1α (Hif1α) in immune cells, including Mo, maturing Mac, and
385 GC (**Fig. 3A-3D, 3I**). After a sharp increase at 1dpc, *Hif1a* levels remain elevated in Mo/Mac at 3dpc, before
386 declining to pre-injury levels at 7dpc (**Fig. 3I**). Hif1α is a master regulator of cellular metabolism and the
387 molecular machinery for glycolytic energy production (Nagao et al., 2019; Pearce and Pearce, 2013; Schuster
388 et al., 2021). A metabolic shift, away from oxidative phosphorylation (OXPHOS) and toward aerobic glycolysis
389 for the conversion of glucose into lactate, is known as Warburg effect (**Fig. 3J**). In immune cells the Warburg
390 effect is of interest because it not only regulates metabolic pathways for energy production, but also gene
391 expression to drive Mac toward a proinflammatory, glycolytic state (McGettrick and O'Neill, 2020; Palsson-
392 McDermott et al., 2015).
393 Analysis of gene products implicated in glucose metabolism, revealed that during the early injury
394 response, GC and Mo/Mac express transporters for glucose (*Slc2a1/*GLUT1, *Slc2a3/*GLUT3) and hexose
395 (*Slc2a6/*GLUT6) to import carbohydrates as a means of energy production. Moreover, there is rapid, injury-
396 induced upregulation of most glycolytic enzymes, including hexokinases (*Hk1, Hk2, Hk3*), phospho-
397 fructokinases (*Pfkp, Pfkl*), glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), and pyruvate kinase (*Pkm*)
398 (**Fig. 3E-3H, 3I**). Injury regulated expression of *Hif1a* and key glycolytic enzymes was validated by qRT-PCR
399 (**Fig. 3K**). The *Pkm* gene products, PKM1 and PKM2, convert phosphoenol pyruvate into pyruvate, the rate-
400 limiting enzymes of glycolysis (**Fig. 3J**). PKM2 is of interest because of its nuclear role and interaction with
401 Hif1α to promote expression of glycolytic enzymes and proinflammatory cytokines, including *Il1b* (Palsson-
402 McDermott et al., 2015). Analysis of 1dpc nerve by qRT-PCR revealed a strong upregulation of *Il1b* when
403 compared to naïve nerve (**Fig. 3K**). Mac in the injured nerve express elevated levels of *Ldha*, the enzyme that
404 converts pyruvate to lactate (**Fig. 3I**). Evidence for intracellular lactate build-up, is the sharp increase in
405 *Slc16a3*, encoding the monocarboxylate transporter 4 (MCT4) shuttling lactate out of cells (**Fig. 3I, 3K**). To
406 validate the increase in glycolytic enzymes in injured nerve tissue, longitudinal sections of naïve, 1, 3, and
407 7dpc mice were stained with anti-PKM2 and anti-LDHA (**Fig. 3N-3U**). In naïve nerve, very few cells stain for

408 PKM2 and LDHA. At 1dpc, PKM2 and LDHA are elevated in Mo/Mac at the nerve injury site. At 3dpc, PKM2
409 and LDHA are most abundant and preferentially detected in F4/80$^+$ Mac. At 7dpc, staining is reduced and
410 largely confined to a subset of F4/80$^+$ Mac.

411 Lactate is far more than a metabolic waste product, since extracellular lactate has been shown to exert
412 immunosuppressive functions, promote angiogenesis, axonal growth, and neuronal health (Chen et al., 2018;
413 Funfschilling et al., 2012; Hayes et al., 2021; Kes et al., 2020). Lactate released by SC has axon protective
414 effects and elevated lactate may be particularly important during the early injury response (Babetto et al.,
415 2020). Evidence for inhibition of OXPHOS in the nerve during the early injury response is the upregulation of
416 *Acod1* (**Fig. 3K, 3L**), an enzyme that converts aconitate into itaconate, thereby disrupting the TCA (**Fig. 3J**).
417 Moreover, itaconate functions as an inhibitor of succinate dehydrogenase (SDH) leading to further inhibition of
418 the TCA (Lampropoulou et al., 2016). Similarly, *Pdk1* (pyruvate dehydrogenase kinase 1) and *Got1* (glutamic-
419 oxaloacetic transaminase), inhibitors of OXPHOS, are high at 1 and 3dpc, but low at 7dpc (**Fig. 3L**).
420 Fragmentation of the TCA is a key feature of pro-inflammatory Mac and a hallmark of the Warburg effect (**Fig.
421 3J**) (Eming et al., 2021). During the inflammation resolution phase, Mac undergo metabolic reprogramming
422 away from glycolysis toward OXPHOS, a switch that coincides with upregulation of the anti-inflammatory
423 transcription factors *Atf3/*activating transcription factor 3 and *Nfe2l2*/Nrf2 (Mills et al., 2018). In the injured
424 nerve, *Atf3* and *Nfe2l2* are low at 1dpc and upregulated at 3dpc (**Fig. 3M**). The mitochondrial enzyme *Gatm*
425 (glycine amidinotransferase) is important for the biosynthesis of creatine, a molecule that facilitates ATP
426 production from ADP. *Gatm* is transiently downregulated in Mo/Mac at 1dpc and increases in Mac at 3dpc and
427 7dpc (**Fig. 3M**).

428 Taken together, application of iSNAT provides multiple lines of evidence that during the early injury
429 response Mo/Mac undergo rapid metabolic reprogramming to increase glycolytic flux and acquire a
430 proinflammatory state. The proinflammatory state is short-lived as Mac rewire their metabolism toward
431 OXPHOS and this is paralleled by a transition toward a pro-resolving phenotype.
432


Hif1a
Pkm
naïve
1 dpc
3 dpc
7 dpc
A
B
C
D
E
F
G
H
J
GLUT1 (Slc2a1)
GLUT3 (Slc2a3)
GLUT6 (Slc2a6)
Glucose
HK1, HK2, HK3
Glucose-6-P
Pentose Phosphate Pathway
GPI1
PFKFB3
Fructose 2,6-BP
Fructose-6-P
PFKL, PFKP
Fructose 1,6-BP
ALDOA
Glyceraldehyde-3-P
DHAP
GAPDH
1,3-Bisphosphoglycerate
PGK1
3-Phosphoglycerate
PGAM1
2-Phosphoglycerate
ENO1
Phosphophenolpyruvate
PKM2
Pyruvate
LDHA
LDHB
Lactate
MCT4 (Slc16a3)
Transporter
Pyruvate
Acetyl-CoA
PDK1
PDHA1
TCA cycle
Lipids
Warburg
Acetyl-CoA
CoA
Citrate
Aconitase
Citrate syntase
Oxaloacetate
Aconitate
Acod1
Itaconate
Reduced ETC
Arginine
Malate
Citrulline
a-Ketoglutarate
Fumarase
Arginino-Succinate
Reduced ETC
Fumarate
Succinyl-CoA
SDH
Succinate
Succinyl-CoA synthase
I
Average expression in Mac (log2)
Gene
Ldha
Hif1a
Gapdh
Pkm
Slc16a3
naïve
1 dpc
3 dpc
7 dpc
K
Relative mRNA level at 1 dpc (log2)
Hif1a
Il1b
Pfkl
Gapdh
Pgk
Pkm
Ldha
Slc16a3
Acod1
Gatm
ns
L
Average expression in Mac (log2)
Gene
Pdk1
Got1
Acod1
M
Average expression in Mac (log2)
Gene
Atf3
Nfe2l2
Gatm
naïve
1 dpc
3 dpc
7 dpc
PKM2 F4/80
LDHA F4/80
N
O
P
Q
R
S
T
U

433

434 **Figure 3**

435 **Application of iSNAT reveals metabolic reprogramming of immune cells**

436 **(A-D)** Feature plots of *Hif1a* expression in naïve and injured sciatic nerve during the first week. **(E-H)** Feature
437 plots of *Pkm* expression in naïve and injured sciatic nerve during the first week. Expression levels are
438 projected onto the UMAP with a minimum expression cutoff of 1. **(I)** Injury regulated gene products associated
439 with glycolysis, as inferred by scRNAseq data. Log2 average expression of genes for cells classified as Mac.
440 **(J)** Metabolic pathways: glycolysis, the catabolism of glucose into pyruvate, and synthesis of nucleotides
441 through the pentose phosphate pathway occur in the cytosol. The tricarboxylic acid cycle (TCA) takes place in
442 mitochondria. The Warburg Effect allows for rapid ATP production through aerobic glycolysis and lactate
443 production. Mitochondrial function is limited because of TCA fragmentation at steps marked with dotted arrows
444 **(K)** Quantification of gene expression by qRT-PCR in the 1dpc nerve relative to naïve nerve. Log2-fold
445 changes relative to naïve nerve are shown. Per gene product, n= 4 replicates. P-values, * <0.05, ** <0.001, ***
446 < 0.0001, Student's *t* test. ns, not significant. **(L, M)** Injury regulated gene products associated with inhibition of
447 mitochondrial energy synthesis (**L**) and inflammation resolution (**M**), as inferred by scRNAseq data. Log2
448 average expression of genes for cells classified as Mac. (**N-U**) Longitudinal sections of naïve and injured
449 sciatic nerves stained for macrophages (anti-F4/80, in red). Nerve sections were co-stained with (**N-Q**) anti-
450 PKM2 (green) and (**R-U**) anti-LDHA (green). Proximal is to the left. Scale bar (**N-U**), 100 µm.

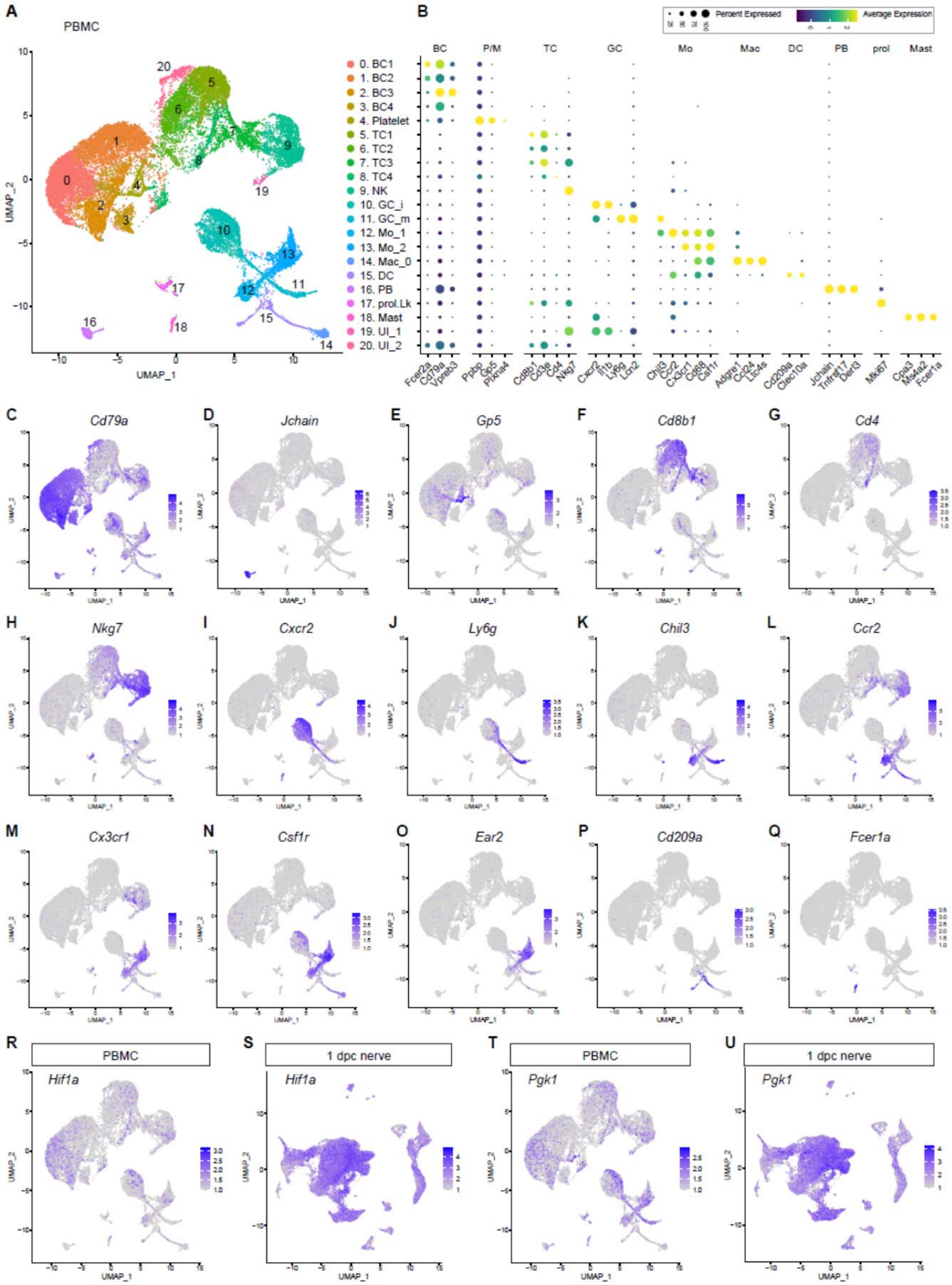
451

452 **Myeloid cells in peripheral blood are not programed for glycolytic energy production**

453 Because large numbers of blood-borne immune cells enter the injured PNS (Kalinski et al., 2020; Ydens et al.,
454 2020), this prompted a deeper analysis of peripheral blood mononuclear cells (PBMC) before they enter the
455 injured nerve. One important goal was to determine which PBMC enter the nerve upon injury and to assess
456 how this impacts gene expression and metabolic profiles. The UMAP plot of 24,842 high-quality PBMC reveled
457 20 clusters (**Fig. 4A,4B**). Four clusters with B cells (BC1-BC4) expressing *Cd79a* (38% of all PBMC);
458 plasmablasts (PB, 1.1%) expressing *Jchain/*joining chain of multimeric IgA and IgM*, Tnfrsf17/*TNF receptor
459 superfamily member 17*,* and *Derl3/*derlin-3; platelets/megakaryocytes (P/M, 2%) expressing, *Pf4/*platelet factor
460 4, *Gp5/*glycoprotein V platelet, and *Plxna4/*PlexinA4; four clusters with T cells (TC1-TC4, 24.2%), expressing
461 *Cd3e* with subsets of *Cd8b1* and *Cd4* cells, and NK cells (8.6%), expressing *Nkg7/*natural killer cell granule
462 protein 7 (**Fig. 4C-4H**). Two clusters with granulocytes, including mature (GCm) (*Ly6g*^{hi}*, Lcn2*^{hi}, *Il1b*$^{-}$) and
463 immature (GCi) (*Cxcr2*^{hi}*, Ly6g*^{low}*, Il1b*^{hi}) make up 10.6% of PBMC (**Fig. 4I,4J**). In addition, there are two Mo
464 subpopulations, Mo1 (2.8%) expressing *Chil3*^{hi}*, Ccr2*^{hi}*, Cx3cr1*^{int} *Ly6c2*^{hi}*, Csf1r*^{low}*,* and *Ear2*$^{-}$; Mo2 (5.3%)
465 expressing *Cx3cr1*^{hi}*, Csf1r*^{hi}*, Ear2*^{hi}*, Ccr2*$^{-}$*, Ly6c2*$^{-}$*,* and *Chil3*$^{-}$, and a cluster with Mac (1.2%) expressing
466 *Adgre1, Ccl24, Ltc4s,* and *Fn1* (**Fig. 4B, 4K-4O**). Mast cells (*Fcer1a, Cpa3*) make up 0.7% and DC (*Cd209a,*
467 *Clec10a*) make up 1.8% of PBMC (**Fig. 4B, 4P,4Q**).

468 Next, we used computational methods to extract blood myeloid cells (GCi, GCm, Mo1, Mo2, Mac, and
469 DC) from the PBMC dataset for comparison with myeloid cells in the injured sciatic nerve at 1dpc, 3dpc, and
470 7dpc. Interestingly, Mo1 (*Ccr2*$^{+}$*, Chil3*$^{+}$) are transcriptionally more similar to 1dpc Mo in the nerve than Mo2
471 (*Ccr2*$^{-}$*, Chil3*$^{-}$*)*, suggesting Mo1 are the primary source of Mo entering the injured nerve. The small population

472 of Mac in blood, is most similar to Mac-III at 1dpc (**Fig. 2B**), suggesting they may enter the injured nerve. GC
473 in the 1dpc nerve are more similar to GCi than GCm in blood, suggesting preferential entry of GCi. Because
474 Mo/Mac in the 1dpc nerve are metabolic programmed for glycolytic energy production (**Fig. 3**), we ask whether
475 their precursors in blood exhibit a similar metabolic profile. Strikingly, when compared to Mo/Mac in the injured
476 nerve, blood Mo1, Mo2, and Mac show either low levels or lack expression of *Hif1a* (**Fig. 4R, 4S**), the glycolytic
477 enzymes *Pgk1*/phosphoglycerate kinase-1 (**Fig. 4T.4U**), *Pfkl*/phosphofructokinase liver type (**Fig. 4, Suppl.**
478 **1A-1E**), and the lactate exporter *Slc16a3* (**Fig. 4, Suppl. 1F-1J**). This suggests that upon nerve entry,
479 circulating Mo/Mac become activated and undergo metabolic reprogramming toward glycolytic energy
480 production. *Arg1,* an important regulator of innate and adaptive immune responses, is not detected in
481 peripheral blood Mo/Mac, however upon nerve entry, Mo/Mac rapidly upregulate *Arg1*. Regulation of *Arg1*
482 expression is very dynamic, levels are highest at 1dpc and 3dpc, then rapidly decline, reaching baseline at
483 7dpc (**Fig. 4, Suppl. 1K-1O**). Thus, our studies show that upon nerve entry, circulating Mo/Mac undergo
484 extensive transcriptional remodeling of glucose metabolism.


A
PBMC
UMAP_1
UMAP_2
0. BC1
1. BC2
2. BC3
3. BC4
4. Platelet
5. TC1
6. TC2
7. TC3
8. TC4
9. NK
10. GC_i
11. GC_m
12. Mo_1
13. Mo_2
14. Mac_0
15. DC
16. PB
17. prol.Lk
18. Mast
19. UI_1
20. UI_2
B
Percent Expressed
Average Expression
BC
P/M
TC
GC
Mo
Mac
DC
PB
prol
Mast
Fcer2a
Cd79a
Vpreb3
Ppbp
Gp5
Pf4
Cd8b1
Cd3e
Cd4
Nkg7
Cxcr2
Il1b
Ly6g
Lcn2
Chil3
Ccr2
Cx3cr1
Cd68
Csf1r
Adgre1
Ccl24
Ltc4s
Cd209a
Clec10a
Jchain
Tnfrsf17
Derl3
Mki67
Cpa3
Ms4a2
Fcer1a
C
Cd79a
D
Jchain
E
Gp5
F
Cd8b1
G
Cd4
H
Nkg7
I
Cxcr2
J
Ly6g
K
Chil3
L
Ccr2
M
Cx3cr1
N
Csf1r
O
Ear2
P
Cd209a
Q
Fcer1a
R
PBMC
Hif1a
S
1 dpc nerve
Hif1a
T
PBMC
Pgk1
U
1 dpc nerve
Pgk1

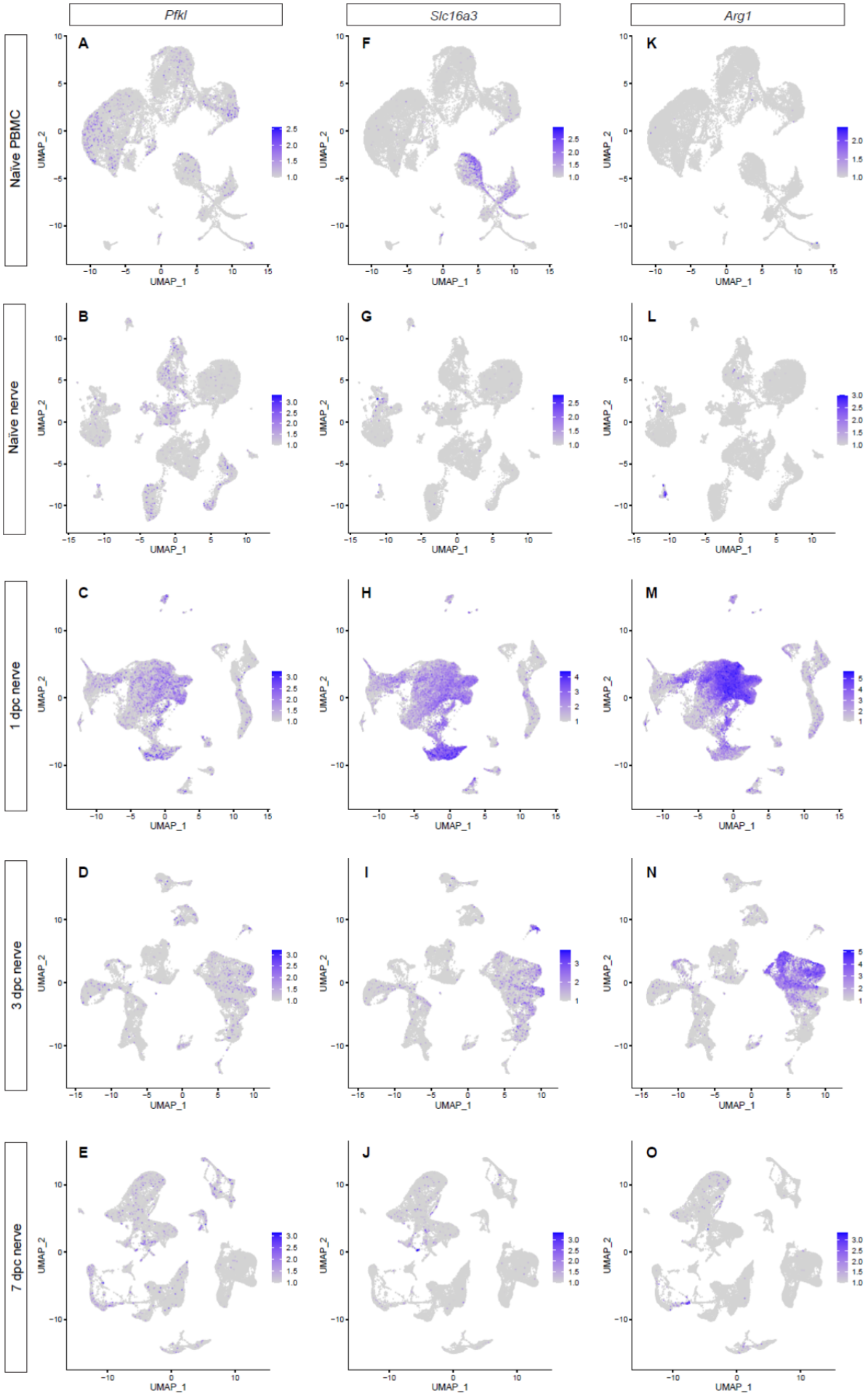
485

486 **Figure 4**

487 **Cellular composition and peripheral blood mononuclear cells**

488 (**A**) UMAP plot embedding of naïve mouse PBMC collected by cardiac puncture. Cell clusters identified harbor
489 B cells (BC1-BC4), Platelets/megakaryocytes (P/M), T cells (TC1-TC4), Natural killer cells (NK), immature and
490 mature granulocytes (mGC and iGC), two subpopulations of monocytes (Mo1 and Mo2), peripheral blood
491 macrophages (MacPB), dendritic cells (DC), plasmablasts (PB), proliferating leukocytes (prol.Lk), mast cells
492 (MAST), and two clusters with unidentified cells (UI1 and UI2). (**B**) A dotplot with marker genes for
493 identification of PBMC. Color coded expression levels are shown. The dot size reflects the percentage of cells
494 that express the gene. (**C-Q**) Feature plots of marker gene expression in PBMC. (**R-U**) Feature plots of *Hif1a*
495 and *Pgk1* (phosphoglycerate kinase 1) expression in PBMC and the injured nerve at 1dpc. Expression levels
496 are projected onto the UMAP with a minimum expression cutoff of 1 Abbreviations: *Cd79a*, CD79A antigen
497 (immunoglobulin-associated alpha); *Gp5*, glycoprotein 5 (platelet); *Cd8b1*, CD8 antigen beta chain 1; *Cd4*,
498 CD4 antigen; *Nkg7*, natural killer cell group 7 sequence; *Cxcr2*, chemokine (C-X-C motif) receptor 2; *Ly6g*,
499 lymphocyte antigen 6 complex locus G; *Chil3*, chitinase-like 3 (Ym1); *Ccr2*, chemokine (C-C motif) receptor 2;
500 *Cx3cr1*, chemokine (C-X3-C motif) receptor 1; *Csf1r*, colony stimulating factor 1 receptor; *Ear2*, eosinophil-
501 associated, ribonuclease A family member 2; *Cd209a*, DC-SIGN (C-type lectin); *Jchain*, joining chain of
502 multimeric IgA and IgM; *Fcer1a*, Fc receptor IgE high-affinity 1 alpha polypeptide.

503


Pfkl
Slc16a3
Arg1
Naïve PBMC
Naïve nerve
1 dpc nerve
3 dpc nerve
7 dpc nerve
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
UMAP_1
UMAP_2

504

505 **Figure 4, Suppl. 1**

506 **Activation of circulating immune cells upon nerve entry**

507 Feature plots for the glycolytic enzyme *Pfkl* (**A-E**), the monocarboxylate transporter *Slc16a3* (**F-J**), and the
508 arginine degrading enzyme *Arg1* (**K-O**), in peripheral blood monocytes (PBMC), naïve nerve, 1dpc, 3dpc, and
509 7dpc nerve. Expression levels are projected onto the UMAP with a minimum expression cutoff of 1.

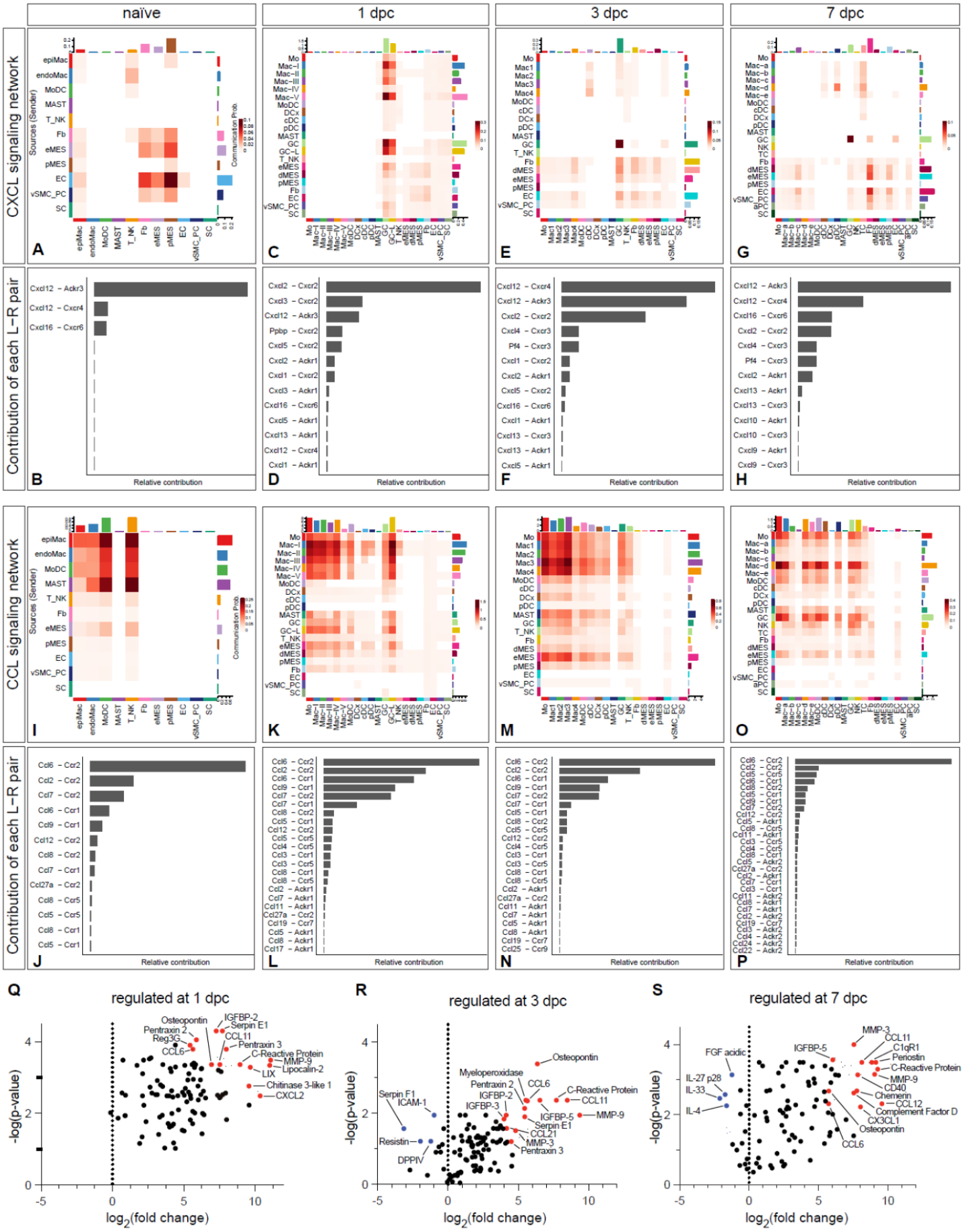
510

511 **CellChat identifies large cell-cell communication networks activated during nerve repair**

512 To understand cell-cell communication networks during the repair process, we interrogated cell surface protein
513 interactions using CellChat (Jin et al., 2021). To facilitate identification and mining of predicted protein-protein
514 interactions, we added CellChat as a feature to iSNAT. The output of CellChat is the probability for cell-cell
515 communication to occur via specific ligand-receptor systems. In naïve and injured nerve, CellChat identified
516 hundreds of ligand-receptor pairs among different cell groups, which were further categorized into 64 major
517 signaling networks. Prominent examples of injury regulated networks include CXCL- (**Fig. 5A-5H**), CCL- (**Fig.**
518 **5I-5P**), CX3C-type chemokine, serum amyloid A (SAA), progranulin (GRN), and osteopontin/secreted
519 phosphoprotein 1 (SPP1). Many of the ligand-receptor pairs in these networks function in leukocyte
520 chemotaxis (SenGupta et al., 2019), and thus, can be mined to determine cellular sources and receptor
521 mechanisms that promote GC and Mo infiltration into the injured nerve. For example, at 1dpc, Mac-II and Mac-
522 III express *Cxcl2, Cxcl3, Cxcl5*, and *Ppbp*/pro-platelet basic protein/CXCL7, encoding activators and
523 chemoattractants for GC that signal through the receptor *Cxcr2*. Mac-I show highest levels of *Ccl2*, Mac-II
524 express *Pf4/*CXCL4*, Grn/*progranulin, and Mac-V express *Ccl24, Spp1*, and *Il1rn*. Analysis of chemotactic
525 receptors further revealed that GC express *Cxcr2*, the formyl peptide receptors *Fpr1* and *Fpr2*, *Tnfrsf1a/*TNF
526 receptor 1*, Ltb4r1*/leukotriene B4 receptor 1, and *Ccr1*. Mo strongly express the chemotactic receptors *Ccr2*,
527 *Ccr5,* and *Ltb4r1,* indicating that GC and Mo use overlapping, yet distinct mechanisms for entering the injured
528 nerve. Many of the chemotactic molecules including *Ccl2, Ccl7*, *Ccl9*, and *Ccl12* show highest expression at
529 1dpc. At 3dpc, chemokines are reduced compared to peak levels, and by 7dpc have declined further,
530 approaching steady state levels comparable to naïve nerves (**Fig. 5A-5P**).

531 In addition to immune cells, CellChat identifies structural cells as a major hub for chemotactic factors,
532 providing evidence for prominent immune-stroma crosstalk. In particular, eMES rapidly increase the
533 production of *Ccl2, Ccl7, Il6, Il11, Cxcl1, Cxcl5, Cxcl12, Cx3cl1, Spp1,* and *Lif*. In addition, injured eMES show
534 elevated expression of serum amyloid A apolipoproteins (*Saa1, Saa2, Saa3*), chemotactic molecules for GC
535 and Mo/Mac. *Ptx3*/pentraxin 3, a factor implicated in wound healing (Erreni et al., 2017), is regulated by injury
536 and rapidly increases in eMES and dMES at 1dpc and 3dpc. Distinct immune molecules are expressed by
537 pMES, including *Serping1* (complement C1 inhibiting factor), *Cfh* (complement factor H), *Lbp*
538 (lipopolysaccharide binding protein). Upon injury, dMES upregulate *Ccl2, Ccl7, Cxcl1, Cxcl5, Cxcl12,*
539 underscoring the importance of diverse stromal cells in orchestrating the immune response (**Fig. 5, Suppl 1**).
540 Because SNC inflicts vascular damage, we searched CellChat for injury induced angiogenic signaling
541 pathways, top hits include VEGF, ANGPT (angiopoietin), ANGPTL (angiopoietin-like proteins), SEMA3

542 (semaphorin 3), EPHA, and EPHB (ephrin receptors). While the importance of angiogenesis and angiogenic
543 factors in PNS repair has been appreciated, CellChat informs on specific ligand-receptor systems and shows
544 which cell types produce these factors.
545


naïve
1 dpc
3 dpc
7 dpc
CXCL signaling network
Contribution of each L−R pair
CCL signaling network
Contribution of each L−R pair
Sources (Sender)
Communication Prob.
Relative contribution
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
regulated at 1 dpc
regulated at 3 dpc
regulated at 7 dpc
-log(p-value)
log2(fold change)

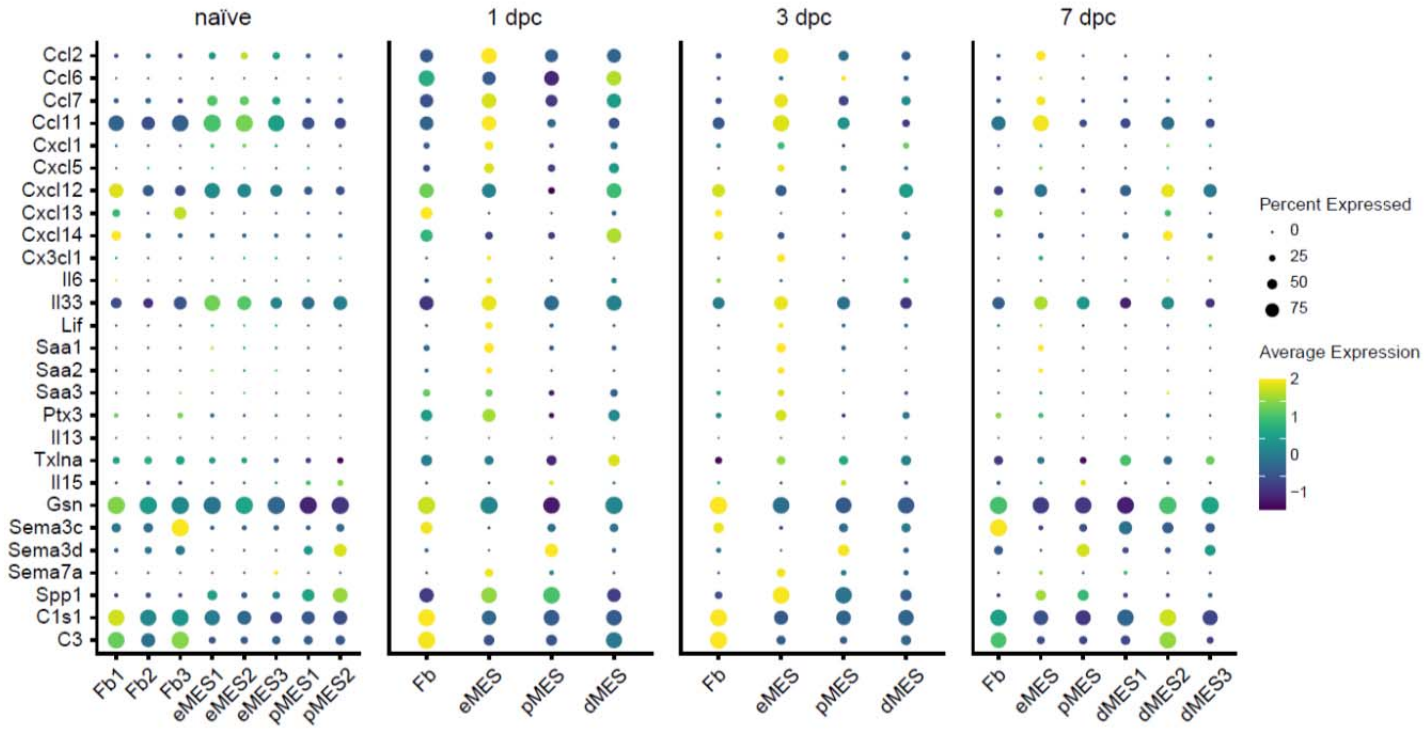
546

547

548 **Figure 5**

549 **CellChat reveals chemotactic cell-cell communication networks in the injured PNS**

550 Hierarchical plots of CellChat analysis showing the inferred intercellular communication network for (**A-H**)
551 CXCL-chemokines and (**I-P**) CCL-cytokines in naïve nerve and during the first week following injury. The
552 sender cells (ligand sources) are shown on the y-axis and receiving cells on the x-axis. The communication
553 probabilities for cells that communicate with each other are indicated. (**B,D,F,H**) The bar graphs show the
554 contributions of CXCL ligand receptor pairs for each time point. (**J,L,N,P**) The bar graphs show the
555 contributions of CCL ligand receptor pairs for each time point. (**Q-S**) Volcano plot of extracellular proteins
556 detected by ELISA compared to naïve nerve. The most abundant and strongly upregulated proteins in the 1dpc
557 nerve (**Q**), the 3dpc nerve (**R**), and the 7dpc nerve (**S**) are shown. The normalized signal on the x-axis shows
558 the log2 fold-change and the y-axis shows the -log(p-value), normalized to naïve nerve.



559

560

561 **Figure 5, Suppl. 1**

562 **Structural cells in the injured nerve directly shape the immune microenvironment**

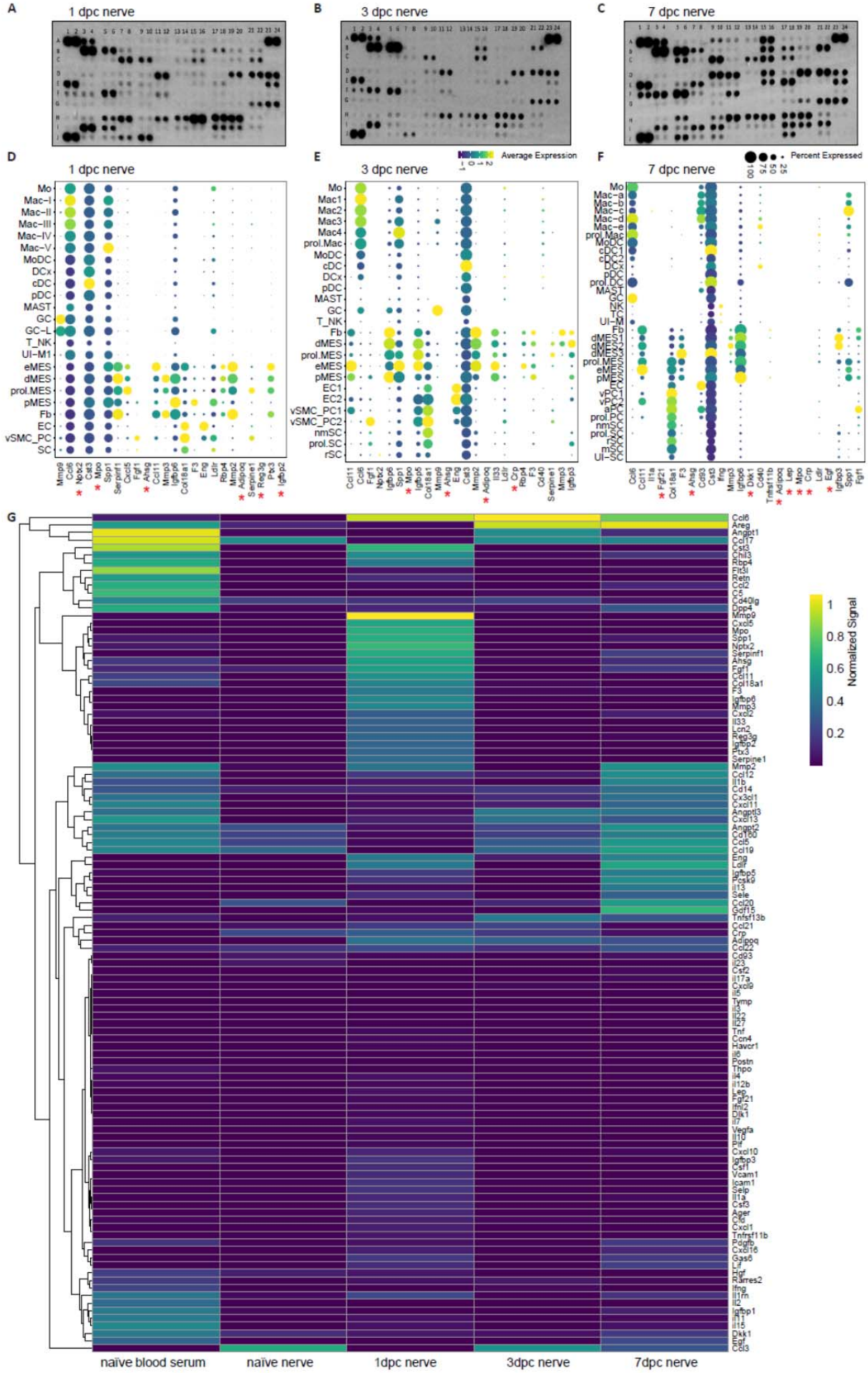
563 Dotplots of structural cells, including Fb (fibroblasts), eMES (endoneurial mesenchymal cells), pMES
564 (perineurial mesenchymal cells), and dMES (differentiating mesenchymal cells). Expression of extracellular
565 immune molecules, as detected by scRNAseq, during the first week following nerve injury are shown. Color
566 coded gene expression levels, normalized to average gene expression is shown. The dot size reflects the
567 percentage of cells that express the gene.

568

569 **Identification of sciatic nerve injury regulated extracellular proteins**

570 While CellChat predicts a large number of ligand-receptor interactions to occur in the injured nerve, we used
571 ELISA to probe nerve lysates to ask whether some of the corresponding extracellular proteins are present at 1,

572 3, and 7dpc (**Fig. 5, Suppl. 2**). ELISA data obtained from naïve nerve and serum were used for comparison
573 (**Fig. 1, Suppl. 3**). Proteins strongly upregulated by SNC include chemokines (CCL6, CCL11, CCL12, CXCL2,
574 LIX, and osteopontin), serpin family members (Serpin E1/PAI-1, Serpin F1/PEDF), pentraxins (NPTX2, PTX3,
575 CRP), ECM degrading matrix metallopeptidases (MMP9, MMP3, MMP2), and IGF binding proteins (IGFBP2,
576 IGFBP3, IGFBP5, IGFBP6) (**Fig. 5 Suppl. 2**). In the 1dpc nerve, prominently detected proteins include MMP9,
577 CCL6, NPTX2, PTX3, CST3, MPO, Osteopontin, SerpinF1, CXCL5, FGF1, AHSG, CCL11, MMP3, IGFBP2,
578 (**Fig. 5Q, Fig 5, Suppl. 2D**). Top proteins detected in the nerve at 3dpc include CCL11, CCL6, FGF1, NPTX2,
579 PTX3, IGFBP6, Osteopontin, MPO, IGFBP5, MMP9, CST3, MMP3, Endostatin (**Fig. 5R, Fig 5, Suppl. 2E**).
580 Top proteins detected in the nerve at 7dpc include CCL6, CCL11, IL1α, FGF21, Endostatin, F3, AHSG, ENG,
581 CST3, MMP2, Adipoq, INFγ, IL33, and LDLR (**Fig. 5S, Fig 5, Suppl. 2F).**
582 To examine whether the corresponding transcripts are expressed in the injured nerve, and to identify
583 the cellular sources, we generated dotplots from scRNAseq datasets (**Fig. 5, Suppl. 2D-2F**). For the top 25
584 proteins detected by ELISA, there is a close correlation at the transcriptional level with many gene products
585 strongly expressed by structural cells. Some of the top gene products detected by ELISA but not by
586 scRNAseq, are serum proteins (**Fig. 1, Suppl. 3D)**. Specifically, they include fetuin A (AHSG), CRP (C reactive
587 protein), IGFBP-2, Reg3g (regenerating family member 3γ), and CFD (complement factor D). Because mice
588 were transcardially perfused before nerves were harvested, this shows that numerous systemic factors
589 accumulate in the injured nerve. CRP is of interest, because it binds to the surface of dead cells and activates
590 complement C1q (Alnaas et al., 2017). Collaboration between CRP and C1q is supported by the strong
591 expression of C1q components (*C1qa, C1qb, and C1qc*) in most Mac subpopulations. CFD is a serine
592 protease required for the formation of C3 convertase. Soluble C1qR1/CD93 functions as a bridging molecule
593 that aids apoptotic cell binding to professional phagocytes for removal by efferocytosis (Blackburn et al., 2019).
594 Taken together, we provide validation for many injury-regulated gene products identified by scRNA-seq and
595 show that serum proteins that function in opsonization feature prominently in the PNS following nerve crush
596 injury. Interestingly, several abundantly detected serum proteins (Angpt1, Angpt2, Angptl3, IGFBP-1) seem
597 not to enter the injured nerve parenchyma (**Fig. 5, Suppl. 1**). This suggests that these proteins are either
598 rapidly degraded or that SNC causes a partial breakdown of the BNB, allowing only some serum proteins to
599 enter the injured nerve.


A
1 dpc nerve
B
3 dpc nerve
C
7 dpc nerve
D
1 dpc nerve
E
3 dpc nerve
F
7 dpc nerve
Average Expression
Percent Expressed
G
Normalized Signal
naïve blood serum
naïve nerve
1dpc nerve
3dpc nerve
7dpc nerve

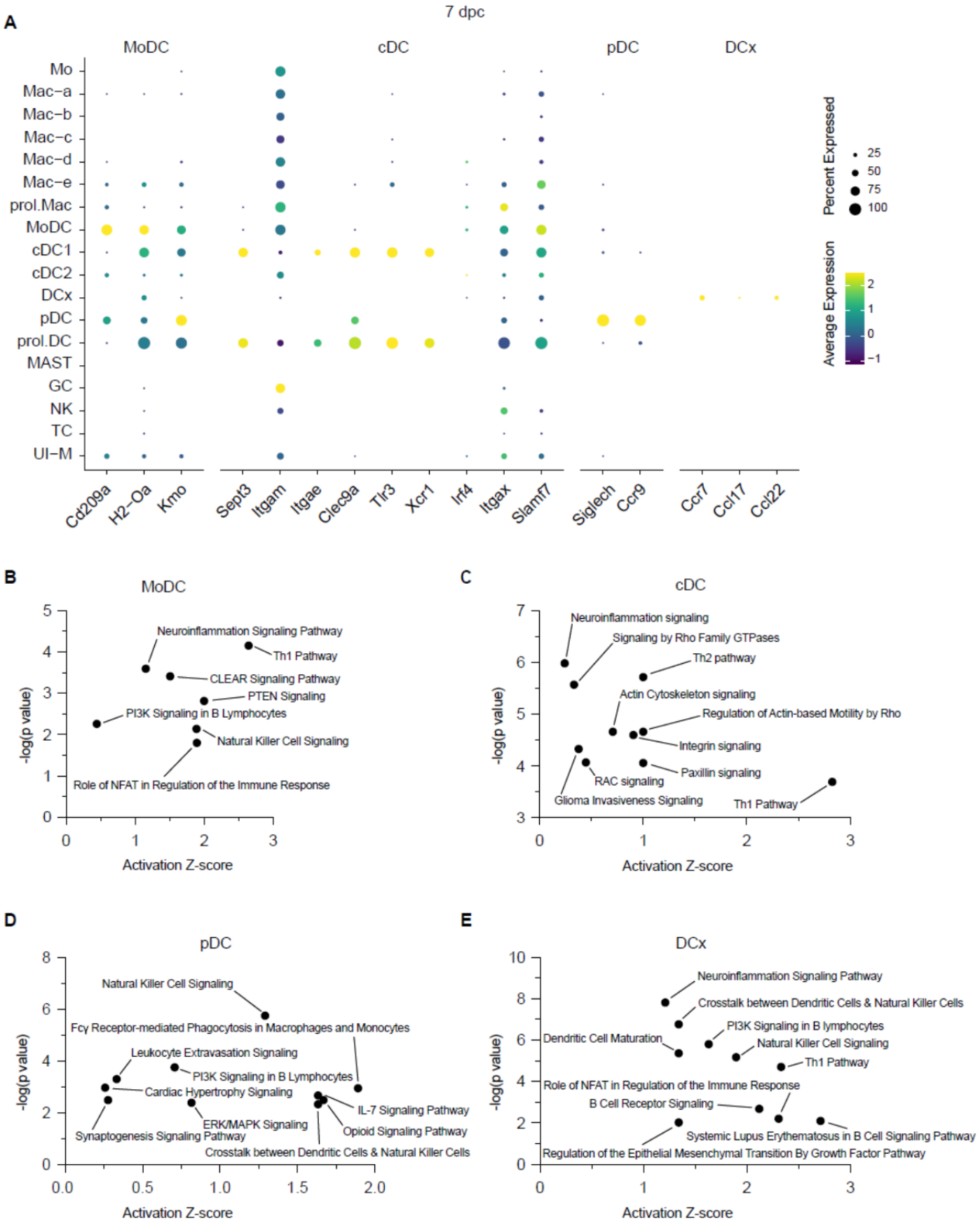
600

601 **Figure 5, Suppl. 2**

602 **Identification of extracellular proteins in the injured sciatic nerve by ELISA**

603 (**A-C**) ELISA membranes probed with injured nerve lysates prepared at (A) 1dpc, (B) 3dpc, and (C) 7dpc. (**D-F**)
604 Dotplots of scRNAseq data from 1dpc, 3dpc, and 7dpc, for the top 25 gene products detected by ELISA.
605 Relative RNA expression levels, normalized to average gene expression (color coded) are shown. For each
606 cell cluster, the percentile of cells expressing a specific gene product is indicated by the dot size. Gene
607 products marked with a red star are detected by ELISA but not by scRNAseq. (**G**) Heatmap shows proteins
608 detected by ELISA in serum of naïve mice, naïve sciatic nerve trunk, and injured sciatic nerve trunk at 1dpc,
609 3dpc, and 7dpc. Number of biological replicates for ELISA, n = 1. Relative protein levels were normalized to
610 internal controls on ELISA membranes shown at coordinates (A1,A2), (A23,A24), (J1,J2). Coordinates of
611 proteins that can be detected by the ELISA: (A3, A4) Adiponectin [*Adipoq*], (A5, A6) Amphiregulin [*Areg*], (A7,
612 A8) Angiopoientin-1 [*Angpt1*], (A9, A10) Angiopoientin-2 [*Angpt2*], (A11, A12) Angiopoientin-like 3 [*Angptl3*],
613 (A13, A14) BAFF [*Tnfrsf13b*], (A15, A16) C1qR1 [*Cd93*], (A17, A18) CCL2 [*Ccl2*], (A19, A20) CCL3 [*Ccl3*],
614 (A21, A22) CCL5 [*Ccl5*], (A23, A24) Reference spots, (B3, B4) CCL6 [*Ccl6*], (B5, B6) CCL11 [*Ccl11*], (B7, B8)
615 CCL12 [*Ccl12*], (B9, B10) CCL17 [*Ccl17*], (B11, B12) CCL19 [*Ccl19*], (B13, B14) CCL20 [*Ccl20*], (B15, B16)
616 CCL21 [*Ccl21*], (B17, B18) CCL22 [*Ccl22*], (B19, B20) CD14 [*Cd14*], (B21, B22) CD40 [*Cd40*], (C3, C4)
617 CD160 [*Cd160*], (C5, C6) Chemerin [*Rarres2*], (C7, C8) Chitinase 3-like 1 [*Chil3*], (C9, C10) Coagulation
618 Factor III [*F3*], (C11, C12) Complement Component C5 [*C5*], (C13, C14) Complement Factor D [*Cfd*], (C15,
619 C16) C-Reactive Protein [*Crp*], (C17, C18) CX3CL1 [*Cx3cl1*], (C19, C20) CXCL1 [*Cxcl1*], (C21, C22) CXCL2
620 [*Cxcl2*], (D1, D2) CXCL9 [*Cxcl9*], (D3, D4) CXCL10 [*Cxcl10*], (D5, D6) CXCL11 [*Cxcl11*], (D7, D8) CXCL13
621 [*Cxcl13*], (D9, D10) CXCL16 [*Cxcl16*], (D11, D12) Cystatin C [*Cst3*], (D13, D14) DKK-1 [*Dkk1*], (D15, D16)
622 DPPIV [*Dpp4*], (D17, D18) EGF [*Egf*], (D19, D20) Endoglin [*Eng*], (D21, D22) Endostatin [*Col18a1*], (D23, D24)
623 Fetuin A [*Ahsg*], (E1, E2) FGF acidic [*Fgf1*], (E3, E4) FGF-21 [*Fgf21*], (E5, E6) Flt-3 Ligand [*Flt3l*], (E7, E8)
624 Gas 6 [*Gas6*], (E9, E10) G-CSF [*Csf3*], (E11, E12) GDF-15 [*Gdf15*], (E13, E14) GM-CSF [*Csf2*], (E15, E16)
625 HGF [*Hgf*], (E17, E18) ICAM-1 [*Icam1*], (E19, E20) IFN-gamma [*Ifng*], (E21, E22) IGFBP-1 [*Igfbp1*], (E23, E24)
626 IGFBP-2 [*Igfbp2*], (F1, F2) IGFBP-3 [*Igfbp3*], (F3, F4) IGFBP-5 [*Igfbp5*], (F5, F6) IGFBP-6 [*Igfbp6*], (F7, F8) IL-
627 1alpha [*Il1a*], (F9, F10) IL-1Beta [*Il1b*], (F11, F12) IL-1ra [*Il1rn*], (F13, F14) IL-2 [*Il2*], (F15, F16) IL-3 [*Il3*], (F17,
628 F18) IL-4 [*Il4*], (F19, F20) IL-5 [*Il5*], (F21, F22) IL-6 [*Il6*], (F23, F24) IL-7 [*Il7*], (G1, G2) IL-10 [*Il10*], (G3, G4) IL-
629 11 [*Il11*], (G5, G6) IL-12 p40 [*Il12*], (G7, G8) IL-13 [*Il13*], (G9, G10) IL-15 [*Il15*], (G11, G12) IL-17A [*Il17a*],
630 (G13, G14) IL-22 [*Il22*], (G15, G16) IL-23 [*Il23*], (G17, G18) IL-27 p28 [*Il27*], (G19, G20) IL-28 [*Ifnl3*], (G21,
631 G22) IL-33 [*Il33*], (G23, G24) LDL R [*Ldlr*], (H1, H2) Leptin [*Lep*], (H3, H4) LIF [*Lif*], (H5, H6) Lipocalin-2 [*Lcn2*],
632 (H7, H8) LIX [*Cxcl5*], (H9, H10) M-CSF [*Csf1*], (H11, H12) MMP-2 [*Mmp2*], (H13, H14) MMP-3 [*Mmp3*], (H15,
633 H16) MMP-9 [*Mmp9*], (H17, H18) Myeloperoxidase [*Mpo*], (H19, H20) Osteopontin [*Spp1*], (H21, H22)
634 Osteoprotegerin [*Tnfrsf11b*], (H23, H24) PD-ECGF [*Tymp*], (I1, I2) PDGF-BB [*Pdgfb*], (I3, I4) Pentraxin 2
635 [*Nptx2*], (I5, I6) Pentraxin 3 [*Ptx3*], (I7, I8) Periostin [*Postn*], (I9, I10) Pref-1 [*Dlk1*], (I11, I12) Proliferin [*Prl2c2*],
636 (I13, I14) Proprotein Convertase 9 [*Pcsk9*], (I15, I16) RAGE [*Ager*], (I17, I18) RBP4 [*Rbp4*], (I19, I20) Reg3G
637 [*Reg3g*], (I21, I22) Resistin [*Retn*], (J1, J2) Reference spots, (J3, J4) E-Selectin [*Sele*], (J5, J6) P-Selectin
638 [*Selp*], (J7, J8) Serpin E1 [*Serpine1*], (J9, J10) Serpin F1 [*Serpinf1*], (J11, J12) Thrombopoietin [*Thpo*], (J13,

639 J14) TIM-1 [*Havcr1*], (J15, J16) TNF-alpha [*Tnf*], (J17, J18) VCAM-1 [*Vcam1*], (J19, J20) VEGF [*Vegf*], (J21,
640 J22) WISP-1 [*Ccn4*], (J23, J24) negative control.
641
642 **The injured sciatic nerve harbors distinct dendritic cell populations**
643 Only few DC are present in the naïve nerve, and they can be distinguished from resident Mac by their
644 prominent expression of *Flt3* (FMS-like tyrosine kinase 3) and *Napsa* (**Fig. 1L**). During the first week following
645 nerve injury, MoDC, identified by *Cd209a* expression, gradually increase (**Fig. 2H, 2P, 2X**). Gene expression
646 analysis in iSNAT identifies additional DC subpopulations, including conventional dendritic cells (cDC,
647 *Sept3/*septin 3 GTPase, *Clec9a*/C-type lectin domain containing 9A), plasmacytoid dendritic cells (pDC*, Ccr9*
648 and *Siglech*), and DCx (*Ccr7/*chemokine receptor 7, *Ccl17, Ccl22*, *Fscn1*/fascin an actin-binding protein) (**Fig.
649 5, Suppl. 3A**). DCx are reminiscent of Langerhans cells and express *Ccr7*, suggesting they are mature cells
650 destined for homing, the migration from injured nerve to draining lymph nodes (Bros et al., 2011; Liu et al.,
651 2021). At 7dpc, two subpopulations of conventional DC (cDC1 and cDC2) are detected. Previous work has
652 established that cDC1 are specialized in cross presentation and activation of cytotoxic TC, and cDC2 are
653 specialized in driving helper TC responses (Steinman, 2012). The two populations can be distinguished based
654 on preferential expression of marker genes, cDC1 (*Itgam*$^-$/CD11b, *Itgae*$^+$/integrin αE, *Clec9a*$^+$*, Tlr3*$^+$/toll-like
655 receptor 3*, Xcr1*$^+$/X-C motif chemokine receptor 1$^+$) and cDC2 (*Itgam*$^+$*, Clec9a*$^-$, *Xcr1*$^-$*, Irf4*$^+$/interferon regulatory
656 factor 4, *Slamf7*/CD2-like receptor activating cytotoxic cells).
657 CellChat identifies an FLT3 signaling network in the injured nerve and predicts communication between
658 TC, NK, and all four DC populations (data not shown). Nerve injury triggers strong expression of interferon-
659 inducible genes in MoDC (*Ifi30, Ifitm1, Ifitm6*) , cDC1 (*Ifi30, Ifi205, Irf8*), and cDC2 (*Ifi30*). MoDC express
660 high levels of macrophage galactose-type lectin, encoded by the C-type lectin receptor *Clec10a*, typically found
661 on tolerogenic antigen-presenting cells (van Kooyk et al., 2015). The *Clec9a* gene product is expressed by
662 cDC and binds to filamentous actin exposed by damaged or ruptured cells (Zhang et al., 2012). The strong
663 expression of *Clec9a* by cDC1 at 7dpc suggests a role in antigen uptake and presentation. While DC are
664 superior to Mac in presenting antigen and express high levels of *MHC class II* components (*H2-Aa, H2-Ab1,
665 H2-DMa, H2-Eb1, Cd74*), most Mac in the naïve nerve, and some Mac in the injured nerve, express *MHC
666 class II* and *Cd74*, suggesting they are endowed with antigen presenting capabilities. Mac in the injured nerve
667 can be distinguished from DC by their preferential expression of complement C1q (*C1qa, C1qb, C1qc*),
668 *C3ar1/*complement C3a receptor 1, *Fcgr1/*CD64*, Trem2, Slc11a1*, *Adgre1*, and *Apoe*/apolipoprotein E.
669 To infer potential functions for DC in the injured nerve, we carried out ingenuity pathway analysis (IPA)
670 (**Fig. 5, Suppl. 3B-3E**). At 7dpc, the top positively regulated pathways include, *Th1 pathway* for MoDC and
671 *Th2 pathway* for cDC1, suggestive of proinflammatory and inflammation resolving functions, respectively. The
672 top pathways for pDC are *NK cell signaling* and *Fcγ receptor-mediated phagocytosis* and for DCx
673 *neuroinflammation signaling* and *crosstalk between DC and NK cells*. Taken together, we identify different DC
674 populations in the injured PNS and pathway analysis predicts extensive crosstalk between DC-NK and DC-TC.



675

676

677

678

679 **Figure 5, Suppl. 3**

680 **Identification of distinct dendritic cell populations in the injured PNS**

681 (**A**) Dotplot for DC marker genes at 7dpc identifies MoDC (monocyte-derived dendritic cells), cDC
682 (conventional dendritic cells)**,** pDC (plasmacytoid dendritic cells), and DCx (mature dendritic cells destined for
683 homing to draining lymph nodes)**.** Relative RNA expression levels, normalized to average gene expression
684 (color coded) are shown. For each cell cluster, the percentile of cells expressing a specific gene product is
685 indicated by the dot size. Ingenuity pathway analysis at 7dpc for (**B**) MoDC, (**C**) cDC, (**D**) pDC, and (**E**) DCx.
686 Top-scoring enriched canonical pathways in each cell type are represented by activation z-scores and p-
687 values.

688

689 **PNS injury triggers a gradual T cell response**

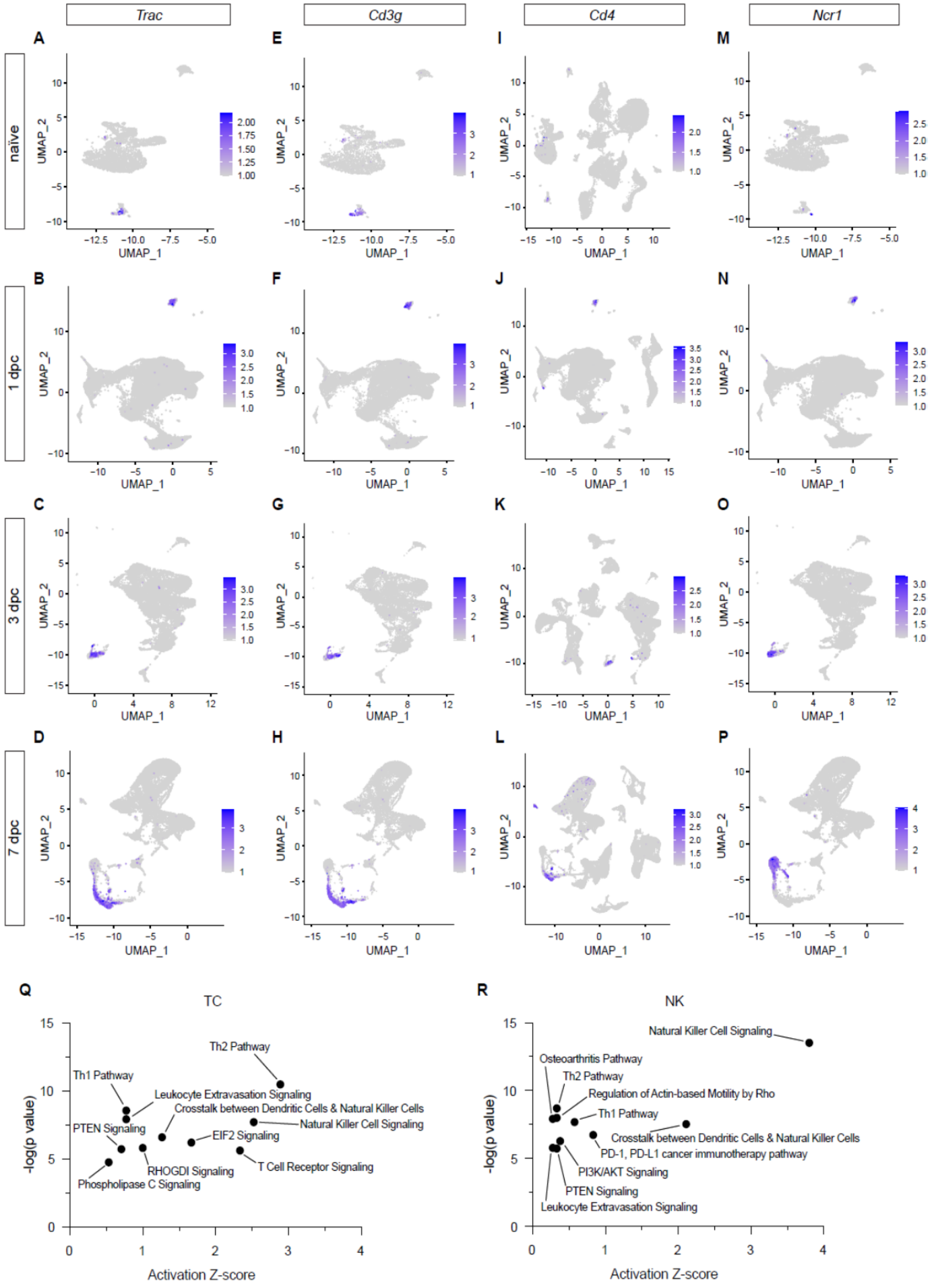
690 For TC classification, we used the "*Expression Analysis*" function in iSNAT. Most TC express the T cell
691 receptor (TCR) α-chain constant (*Trac*) and β-chains (*Trbc1* and *Trbc2*), suggesting they are αβ TC (**Fig. 5,**
692 **Suppl. 4A-4D**). Only few TC are present in the naïve sciatic; however, TC gradually increase upon injury, and
693 by 7dpc make up ~10% of immune cells. Few *Mki67*/Ki67$^+$ TC are observed in the injured nerve and the
694 majority expresses *Ms4a4b*, a negative regulator of cell proliferation (**Fig. 2, Suppl. 2F-2H**). This suggests
695 that TC expansion is primarily due to nerve infiltration, rather than local proliferation. In support of this, TC
696 strongly express *Cxcr6*, a chemotactic receptor for *Cxcl16* expressed by Mac and DCx in the injured nerve.
697 Nearly all TC express CD3 (*Cd3g, Cd3d, Cd3e,* and *Cd3z/Cd247*), a key component of the TCR-CD3 complex
698 (**Fig. 5, Suppl. 4E-4H**). At 7dpc, the TC population is comprised of CD4$^+$ T helper cells (Th), expressing *Cd4*,
699 (**Fig. 5, Suppl. 4I-4L**) and CD8 cells expressing *Cd8a* and *Cd8b1,* suggesting they are CD8αβ$^+$ cells (data not
700 shown). Differentiation of CD4$^+$ Th into pro-inflammatory Th1 or anti-inflammatory Th2 effector cells is
701 controlled by the transcription regulators T-bet (*Tbx21*) and GATA3 (*Gata3*), respectively (Jenner et al., 2009).
702 *Gata3*$^+$ Th2 cells are observed in naïve and injured nerves, while *Tbx21*$^+$ Th1 are largely absent from naïve
703 nerve but increase following injury and express the killer cell lectin like receptors *Klrd1* and *Klrc1*, markers of
704 an activated pro-inflammatory state. Pathway analysis of TC at 7dpc, identified *Th2 pathway* and *Th1 pathway*
705 as top hits (**Fig. 5, Suppl. 4Q**). It is well established that the Th1 response plays a key role in neuropathic pain
706 development and persistence (Davies et al., 2020; Moalem and Tracey, 2006). At 7dpc, there is a small
707 population of γδ TC (*Trdc*), and some T regulatory cells (Tregs), a specialized subpopulation of TC that acts to
708 suppress the immune response. In the PNS, Tregs (*Cd4*, *Il2ra*/CD25, *Foxp3)* are of interest, because they
709 may function in self-tolerance and pain mitigation (Davoli-Ferreira et al., 2020).

710

711 **Natural Killer cells increase following nerve injury**

712 In contrast to TC, NK do not express *Cd3g, Cd3d, Cd3e*, but strongly express natural cytotoxicity receptor
713 (*Ncr1*), killer cell lectin-like receptor subfamily B member 1C (*Klrb1c*/NK1.1), the pore-forming glycoprotein
714 perforin (*Prf1*), and the granzyme family serine proteases (*Gzma, Gzmb*) (**Fig. 5, Suppl. 4M-4P**). Granzymes
715 are delivered into target cells through the immunological synapse to cause cell death. While the full spectrum
716 of cells targeted by NK has yet to be determined, NK cytotoxic factors have been shown to accelerate

717 degeneration of damaged axons (Davies et al., 2019). Compared to NK, $CD8^+$ TC express low levels of
718 granulysin (*Gnly*), perforin (*Prf1*), and granzymes (*Gzma, Gzma*) suggesting limited cytotoxic activity. However,
719 similar to NK, many TC express *Nkg7*, a natural killer cell granule protein, and the killer cell lectin like receptor
720 K1 (*Klrk1*/NKG2D), indicative of some cytotoxic abilities. NK (and some TC) strongly express *Klrc1*/NKG2A
721 (CD94), an immune inhibitory checkpoint gene. The ligand for NKG2A is the non-classical MHC1 molecule
722 Qa-2 (encoded by *H2-q7*), expressed by NK and TC. CellChat predicts a high probability for an MHC-I
723 signaling network between $CD8^+$ TC, NK, and pDC, and an MHC-II signaling network between $CD4^+$ TC and
724 with DC and Mac in the injured nerve. IPA for NK at 7dpc, identifies *NK signaling*, *Th2 pathway*, and *crosstalk*
725 *between DC and NK* as top hits (**Fig. 5, Suppl. 4R**). The majority of NK and TC in the injured nerve produce
726 interferon γ (*Ifng*), some TC produce TNFα (*Tnf*), and few produce GM-CSF (*Csf2*). In addition, NK and TC
727 produce chemoattractants and survival signals for DC, including *Flt3l*, *Ccl5, Xcl1/*lymphotactin, suggesting they
728 directly regulate DC migration and function.


Trac
Cd3g
Cd4
Ncr1
naïve
1 dpc
3 dpc
7 dpc
UMAP_1
UMAP_2
TC
Th2 Pathway
Th1 Pathway
Leukocyte Extravasation Signaling
Crosstalk between Dendritic Cells & Natural Killer Cells
Natural Killer Cell Signaling
PTEN Signaling
EIF2 Signaling
T Cell Receptor Signaling
RHOGDI Signaling
Phospholipase C Signaling
-log(p value)
Activation Z-score
NK
Natural Killer Cell Signaling
Osteoarthritis Pathway
Th2 Pathway
Regulation of Actin-based Motility by Rho
Th1 Pathway
Crosstalk between Dendritic Cells & Natural Killer Cells
PD-1, PD-L1 cancer immunotherapy pathway
PI3K/AKT Signaling
PTEN Signaling
Leukocyte Extravasation Signaling
-log(p value)
Activation Z-score

730 **Figure 5, Suppl. 4**

731 **Description of TC and NK populations in the injured PNS**

732 Feature plots for (**A-D**) *Trac*/T cell receptor alpha constant, (**E-H**) *Cd3g*/CD3 gamma subunit of T cell receptor
733 complex, (**I-L**) *Cd4*/T cell surface glycoprotein CD4, and (**M-P)** *Ncr1*/Natural cytotoxicity triggering receptor-1 of
734 naïve, 1, 3, and 7dpc nerve identify TC subpopulations and NK cells. Expression levels are projected onto the
735 UMAP with a minimum expression cutoff of 1. (**Q, R**) Ingenuity pathway analysis for TC and NK at 7dpc. Top-
736 scoring enriched canonical pathways are represented by activation z-scores and p-values.

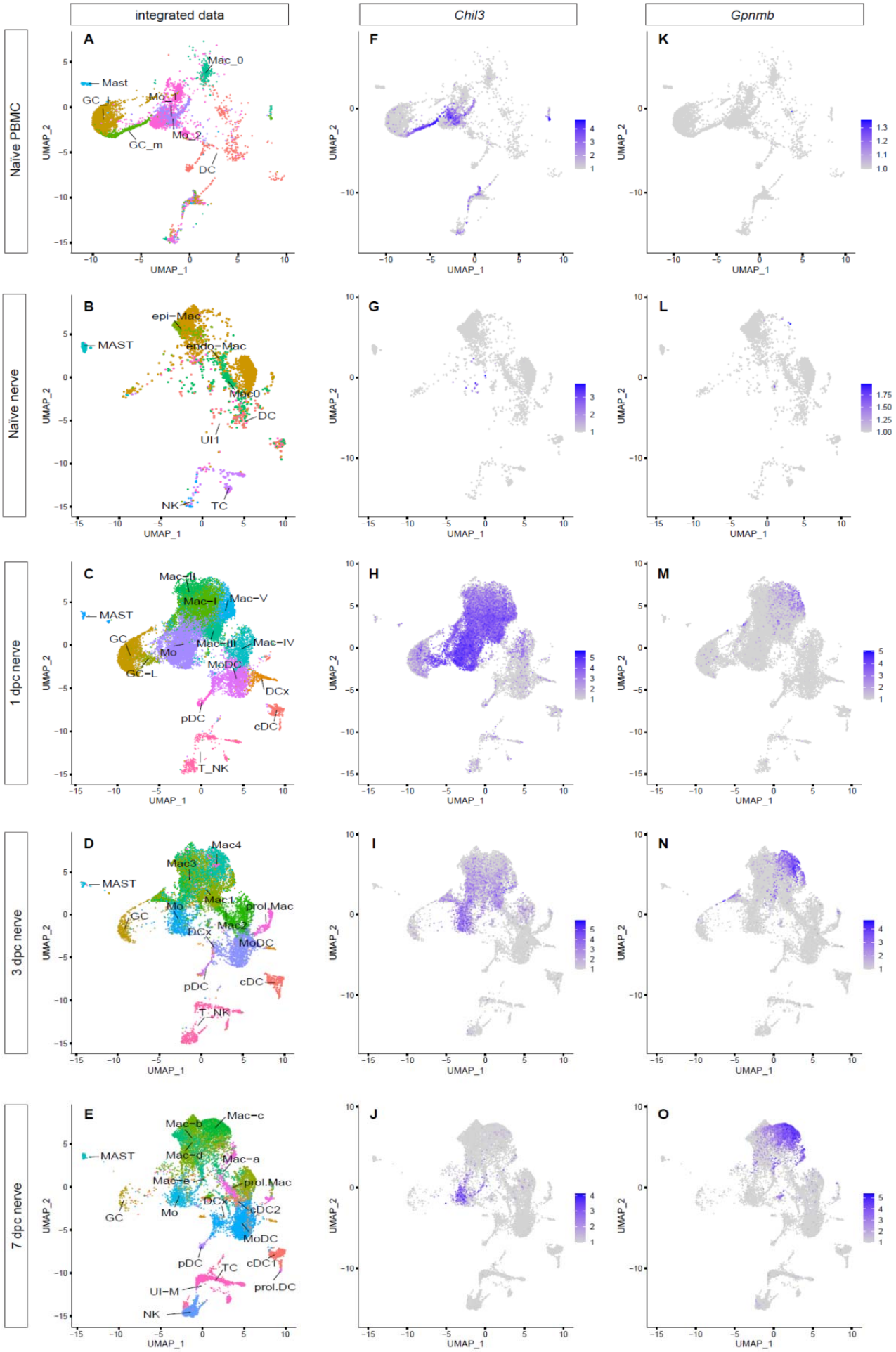
737

738 **Tracking myeloid cell transcriptional states and maturation in the injured nerve**

739 Mo/Mac are often described as highly "plastic" cells, educated by the local tissue environment. While Mac
740 subpopulations in the injured PNS have been cataloged (Kalinski et al., 2020; Ydens et al., 2020), tracking
741 them over time, as they mature, has not yet been attempted. To better understand Mo/Mac maturation in the
742 injured nerve, we took advantage of our longitudinal scRNAseq datasets. Computational methods were used to
743 extract myeloid cells from PBMC, naïve nerve, and the three post-injury time points to carry out an integrated
744 data analysis (**Fig. 6A-E**). When comparing the UMAP plots of naïve sciatic nerve and PBMC, there is little
745 overlap, indicative of largely distinct immune compartments (**Fig. 6A,6B**). When comparing myeloid cells in
746 blood with 1dpc nerve, the rapid entry of GC into the nerve is readily detected and GC are largely absent from
747 the 7dpc nerve (**Fig. 6, Suppl. 1F-1J**). Interestingly, 1dpc GC are more similar to *Il1b*$^{+}$ GCi than *Il1b*$^{-}$ GCm in
748 blood (**Fig. 6, Suppl. 1K-1O**). In a similar vein, Mo in the 1dpc nerve are transcriptionally more similar to *Chil3*$^{+}$
749 Mo1 than to *Ear2*$^{+}$ Mo2 in blood. This suggests that following SNC, select GC and Mo subpopulations
750 preferentially extravasate and enter the injured nerve. Moreover, the integrated analysis clearly shows the
751 rapid increase of *Chil3*$^{+}$ Mo in the 1dpc nerve, followed by a decline over the next six days (**Fig. 6F-6J**).
752 Conversely, few *Gpnmb*$^{+}$ Mac are detected in blood or naïve nerve, however *Gpnmb*$^{+}$ gradually increase
753 following SNC. Only a small number of *Gpnmb*$^{+}$ Mac is detected at 1dpc, they have increased at 3dpc, and
754 further increased at 7dpc, suggesting these are more mature Mac (**Fig. 6K-6O**).

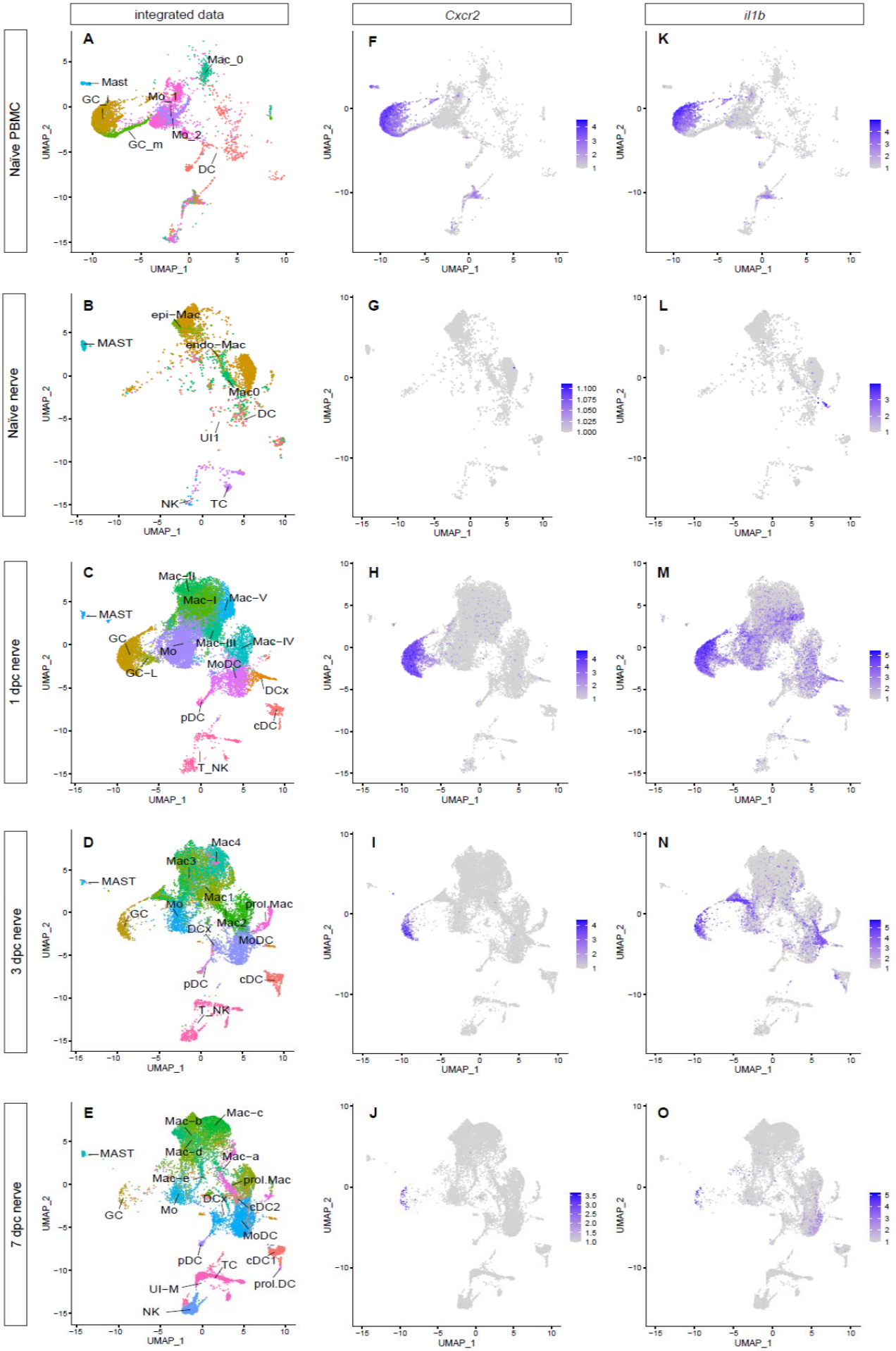
755 To predict trajectories of Mo maturation into their descendants, Mac and MoDC, we carried out
756 slingshot, pseudotime analysis of integrated myeloid cells. Using Mo as starting cells, revealed a bifurcated
757 differentiation trajectory and indicates that Mo give rise to Mac and MoDC in the injured nerve **(Fig. 7; Fig. 7,**
758 **Suppl. 1-3).** The high degree of Mo/Mac plasticity is evident when cell cluster identified at 1dpc (Mac-I to Mac-
759 V) (**Fig. 2A)**, 3dpc (Mac1-Mac4) (**Fig. 2J**), and 7dpc (Mac-a to Mac-d) (**Fig. 2R**) are visualized in the integrated
760 dataset (**Fig. 7, Suppl. 1**). To facilitate access to integrated immune cell datasets, we added these data to
761 iSNAT.

762


integrated data
Chil3
Gpnmb
Naïve PBMC
Naïve nerve
1 dpc nerve
3 dpc nerve
7 dpc nerve
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
Mast
GC_l
Mo_1
Mac_0
Mo_2
GC_m
DC
MAST
epi-Mac
endo-Mac
Mac0
UI1
NK
TC
Mac-II
Mac-I
Mac-V
GC
Mo
Mac-III
Mac-IV
MoDC
GC-L
DCx
pDC
cDC
T_NK
Mac4
Mac3
Mac1
prol.Mac
Mac2
Mac-b
Mac-c
Mac-d
Mac-a
Mac-e
cDC2
cDC1
prol.DC
UI-M
NK
UMAP_1
UMAP_2

764 **Figure 6**

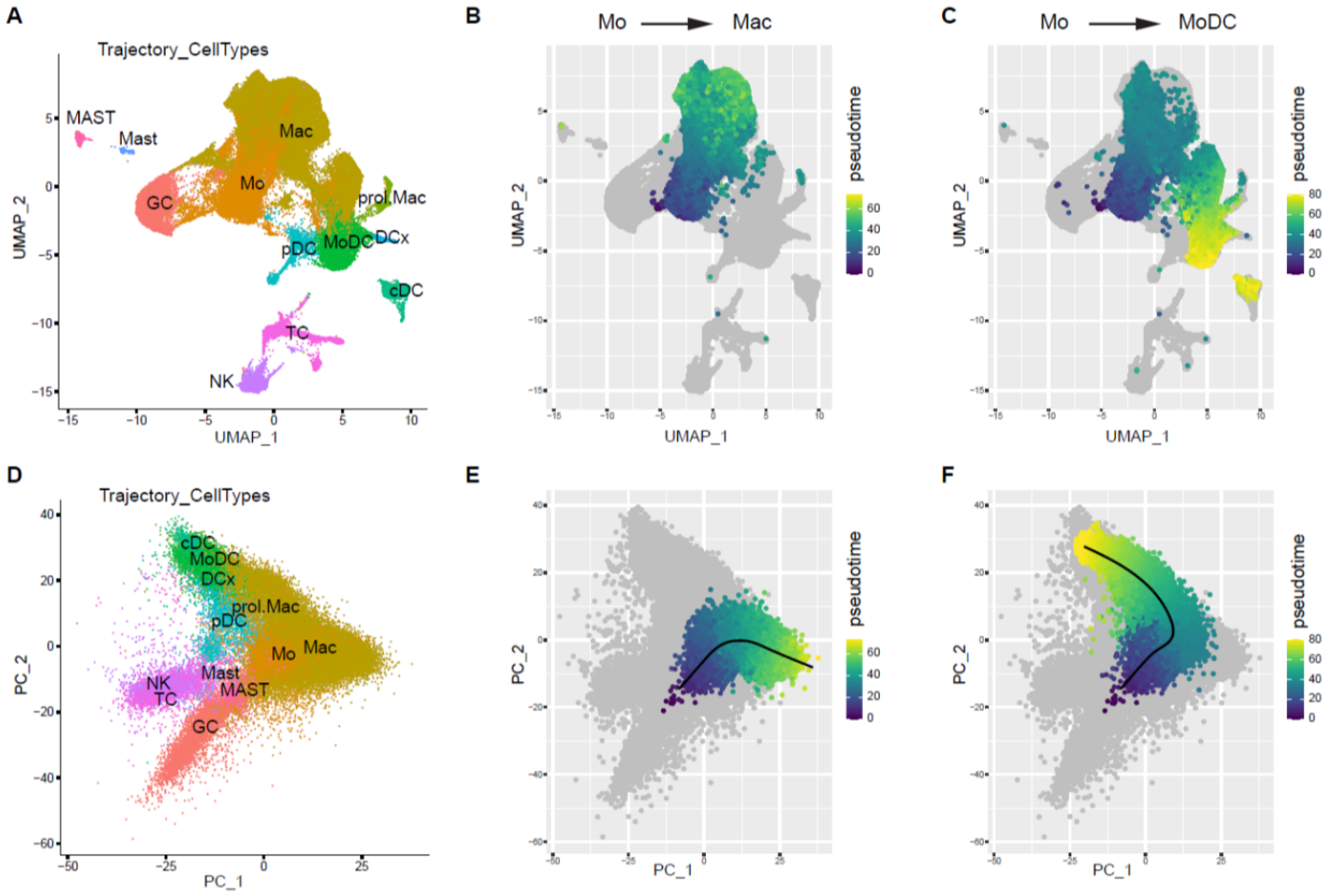
765 **Integrated analysis of single cell transcriptomes generated from PBMC, naïve nerve, and injured nerve.**
766 (**A-E**) UMAP plots of integrated myeloid cells split into (**A**) PBMC (of naïve mice), (**B**) naïve sciatic nerve trunk,
767 (**C**), 1dpc nerve, (**D**) 3dpc nerve, and (**E**) 7dpc nerve. (**F-J**) Integrated analysis of *Chil3*$^{+}$ Mo in (**F**) PBMC, (**G**)
768 naïve nerve, (**H**) 1dpc nerve, (**I**) 3dpc nerve, (**J**) 7dpc nerve. (**K-D**) Integrated analysis of *Gpnmb*$^{+}$ Mac in (**K**)
769 PBMC, (**L**) naïve nerve, (**M**) 1dpc nerve, (**N**) 3dpc nerve, (**O**) 7dpc nerve. For feature plots (**F-O**), expression
770 values are projected onto the integrated UMAP with a minimum expression cutoff of 1. Abbreviations: GC,
771 granulocytes; Mo, monocytes; Mac, macrophages: DC, dendritic cells; MAST, mast cells; T_NK, T cells and
772 natural killer cells; UI, unidentified cells.


integrated data
Cxcr2
il1b
Naïve PBMC
Naïve nerve
1 dpc nerve
3 dpc nerve
7 dpc nerve
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
Mast
GC_j
Mo_1
Mo_2
GC_m
Mac_0
DC
MAST
epi-Mac
endo-Mac
Mac0
UI1
NK
TC
Mac-II
Mac-I
Mac-V
GC
Mo
Mac-III
Mac-IV
MoDC
GC-L
DCx
pDC
cDC
T_NK
Mac4
Mac3
Mac1
prol.Mac
Mac2
Mac-b
Mac-c
Mac-d
Mac-a
Mac-e
cDC2
cDC1
prol.DC
UI-M
UMAP_1
UMAP_2

774 **Figure 6, Suppl. 1**

775 (**A-E**) UMAP plots of integrated myeloid cells split into (**A**) PBMC (of naïve mice), (**B**) naïve sciatic nerve trunk,
776 (**C**) 1dpc nerve, (**D**) 3dpc nerve, and (**E**) 7dpc nerve. Integrated analysis to track *Cxcr2*$^{+}$ GC (**F-J**) and
777 expression of *Il1b* (**K-O**) in myeloid cells of PBMC, naïve nerve, 1dpc, 3dpc, and 7dpc nerve. For feature plots
778 (**F-O**), expression values are projected onto the integrated UMAP with a minimum expression cutoff of 1
779 Abbreviations: GC, granulocytes; Mo, monocytes; Mac, macrophages: DC, dendritic cells; MAST, mast cells;
780 T_NK, T cells and natural killer cells; UI, unidentified cells.

781

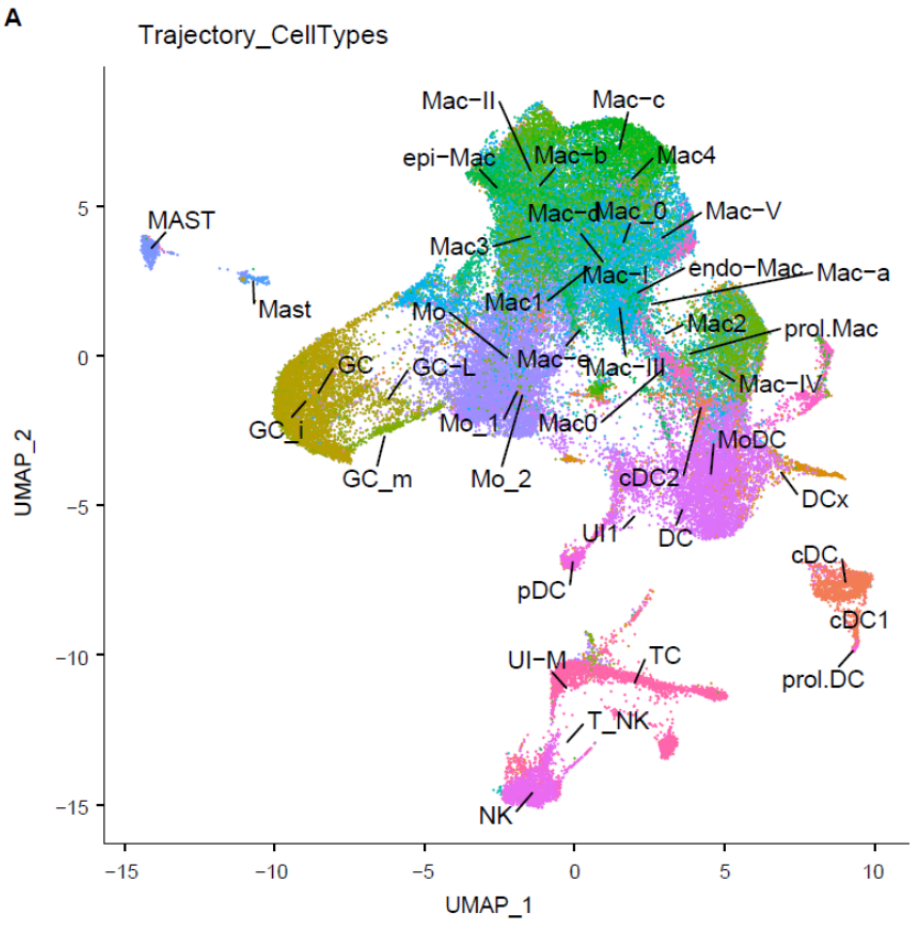


782

783 **Figure 7**

784 **Tracking of myeloid cells before and after entering the injured nerve**

785 **(A)** UMAP of integrated immune cells with simplified cluster labels **(D)** PCA of integrated immune cells using
786 the same cluster labels as A. The first four principal components were used as input to slingshot pseudotime
787 analysis (**B**) Slingshot pseudotime, for Mo to Mac trajectory, projected onto the UMAP. **(E)** Pseudotime
788 projected onto the PCA showing the predicted trajectory, starting from Mo and differentiating toward Mac (**C**) A
789 separate trajectory, starting from Mo shows differentiation toward MoDC; psuedotime projected on UMAP. (**F**)
790 Mo to MoDC pseudotime projected on PCA.


A
Trajectory_CellTypes
Mac−II
Mac−c
epi−Mac
Mac−b
Mac4
MAST
Mac−d
Mac_0
Mac−V
Mac3
Mac−I
endo−Mac
Mac−a
Mast
Mo
Mac1
Mac2
prol.Mac
GC
GC−L
Mac−e
Mac−III
Mac−IV
GC_i
Mo_1
Mac0
MoDC
GC_m
Mo_2
cDC2
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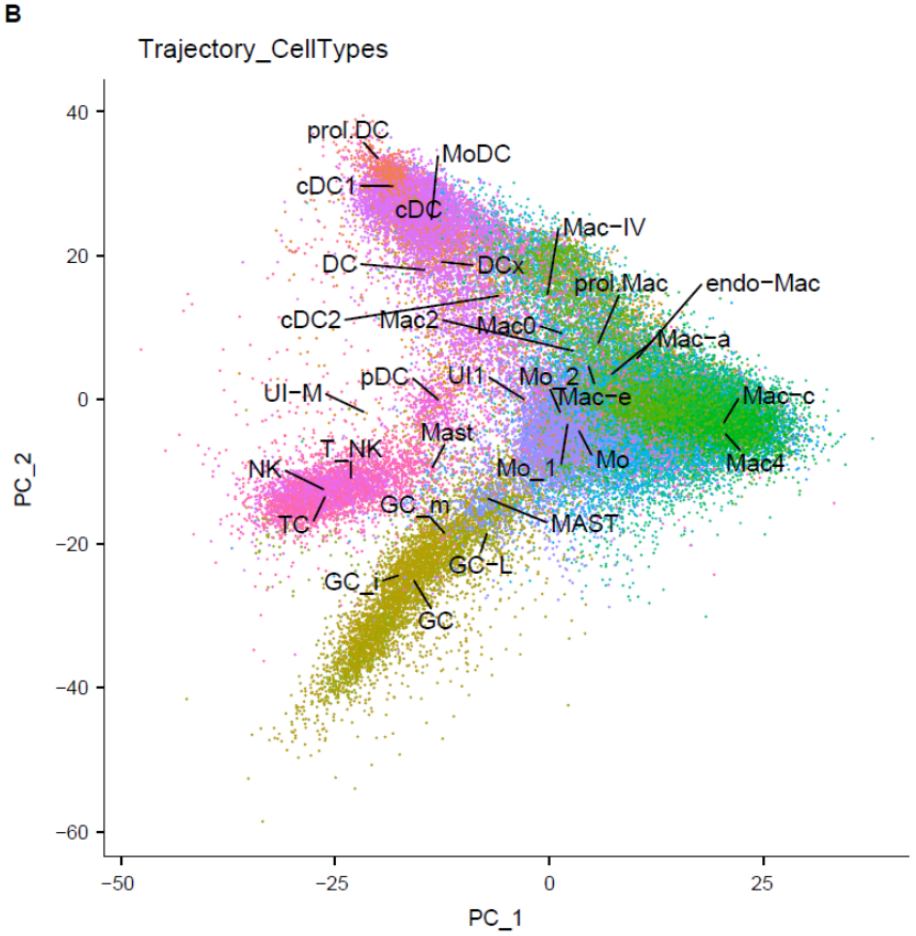
NK
UMAP_2
UMAP_1


B
Trajectory_CellTypes
prol.DC
MoDC
cDC1
cDC
Mac−IV
DC
DCx
prol.Mac
endo−Mac
cDC2
Mac2
Mac0
Mac−a
pDC
UI1
Mo_2
UI−M
Mac−e
Mac−c
Mast
T_NK
Mo
Mac4
NK
Mo_1
GC_m
MAST
TC
GC−L
GC_i
GC
PC_2
PC_1

791

792 **Figure 7, Suppl 1.**

793 (**A**) Integrated immune cells UMAP with all cluster labels from each time points individual analysis.

794 (**B**) Integrated immune cells PCA with all cluster labels from each time points individual analysis.

795 Abbreviations: GC, granulocytes [GC-i and GC-m (blood), GC-L (1dpc nerve), GC (1dpc, 3dpc and 7dpc

796 nerve)], Mo, monocytes (Mo_1 and Mo_2 (blood), Mo (1dpc, 3dpc, and 7dpc nerve)], Mac, macrophages

797 [endo-Mac and epi-Mac (naïve nerve), (Mac-I, Mac-II, Mac-III, Mac-IV, Mac-V (1dpc nerve), Mac0, Mac1,

798 Mac2, Mac3, Mac4 (3dpc nerve), Mac-a, Mac-b, Mac-c, Mac-d, Mac-e (7dpc nerve)], MoDC (monocyte-derived

799 dendritic cells), cDC (conventional dendritic cells), pDC, plasmacytoid dendritic cells, DCx (mature dendritic

800 cells), Mast (mast cells), TC (T cells), NK (natural killer cells), UI (unidentified).

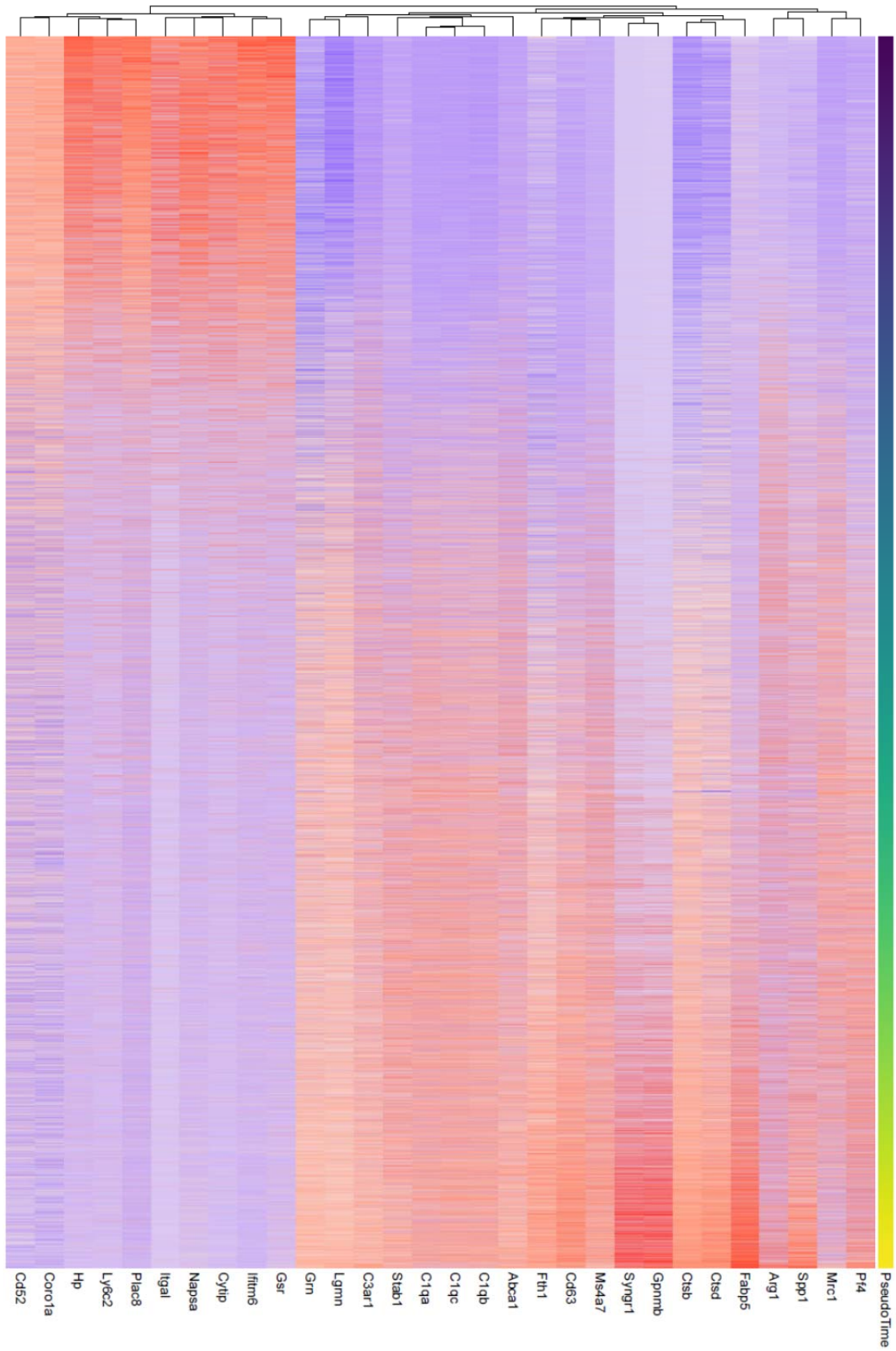
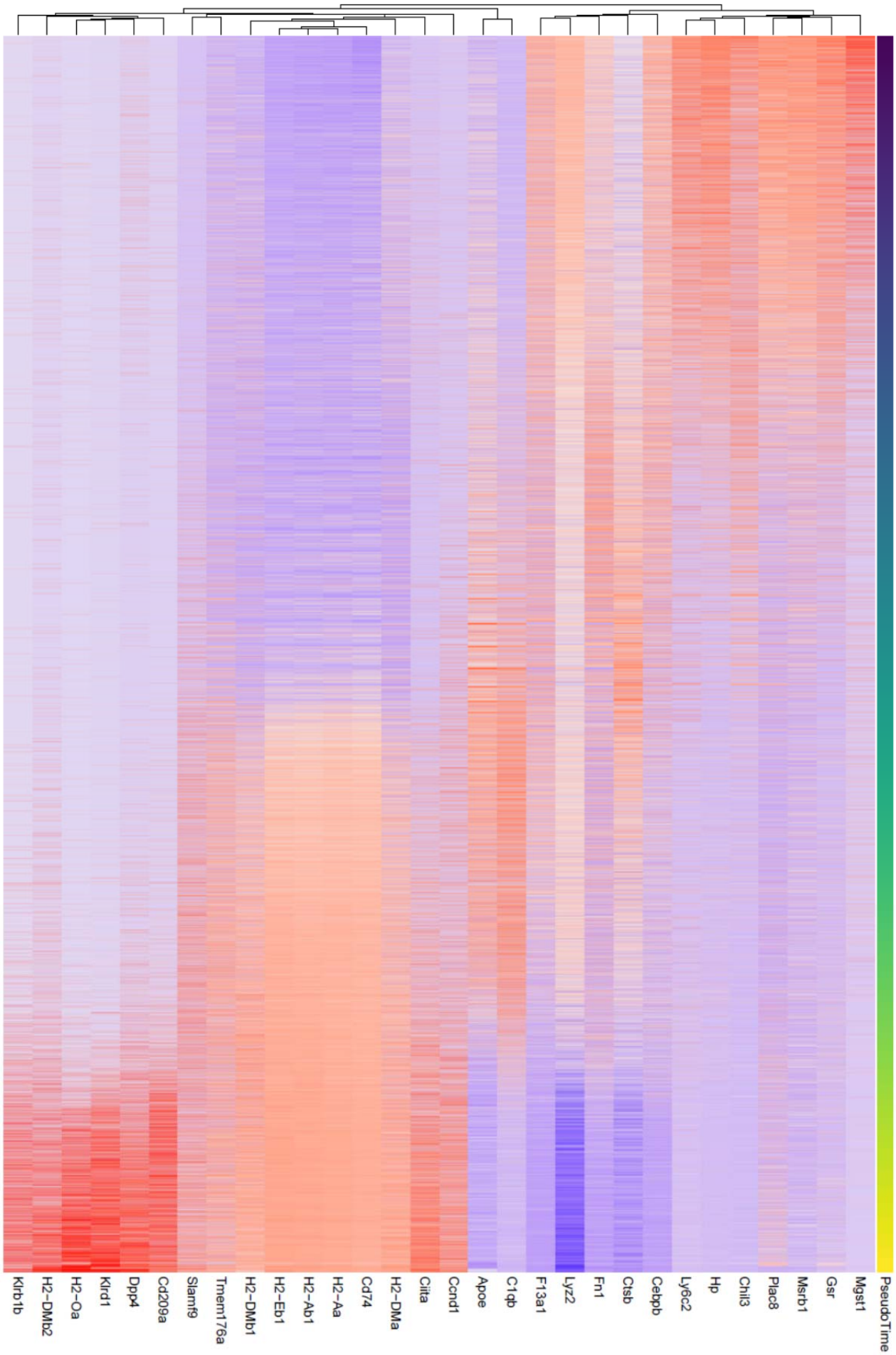


Figure 7, Suppl 2.

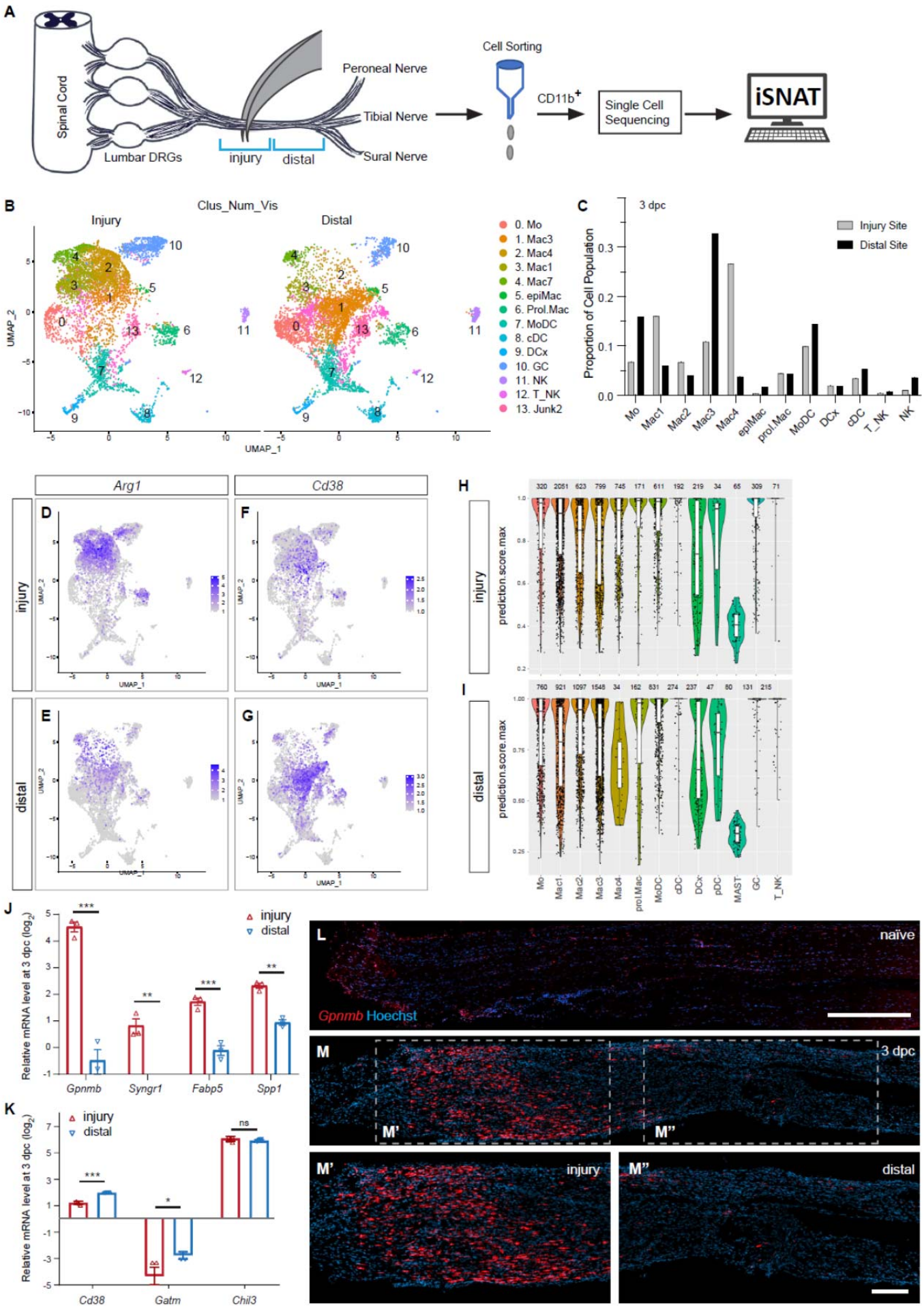
Heatmap showing the top 30 genes by importance in predicting pseudotime (Figure 7E) as determined by random forest analysis. Colors represent the Z-score calculated across genes.


PseudoTime
Mgst1
Gsr
Msrb1
Plac8
Chil3
Hp
Ly6c2
Cebpb
Ctsb
Fn1
Lyz2
F13a1
C1qb
Apoe
Ccnd1
Ciita
H2-DMa
Cd74
H2-Aa
H2-Ab1
H2-Eb1
H2-DMb1
Tmem176a
Slamf9
Cd209a
Dpp4
Klrd1
H2-Oa
H2-DMb2
Klrb1b

805

806 **Figure 7, Suppl 3.**
807 Heatmap showing the top 30 genes by importance in predicting pseudotime (Figure 7F) as determined by
808 random forest analysis. Colors represent the Z-score calculated across genes.
809
810 **Nerve trauma generates spatial differences in immune cell composition**
811 A crush injury divides a nerve into three distinct compartments: the proximal nerve, the injury site, and the
812 distal nerve (**Fig. 8A**). While specialized immune cells have been identified at the site of nerve injury (Cattin et
813 al., 2015; Kalinski et al., 2020; Shin et al., 2018), a comparative analysis of cells at the injury site and the distal
814 nerve has not yet been carried out. Here we harvested sciatic nerves at 3dpc and microdissected ~ 3mm
815 segments that either harbor the injury site or distal nerve. Innate immune cells were then captured with anti-
816 CD11b and further analyzed by scRNAseq (**Fig. 8A**). The resulting UMAP plots revealed location specific
817 enrichment of select immune cell populations (**Fig. 8B**). For example, GC (cluster 10) are more abundant at
818 the nerve injury site, than in the distal nerve and Mo (cluster 0) are more abundant in the distal nerve (**Fig. 8C**).
819 Most notably, is the location specific enrichment of select Mac subpopulations (**Fig. 8C**). For example, *Arg1*
820 expressing Mac4 (cluster 2) and Mac1 (cluster 3) are enriched at the nerve injury site (**Fig. 8D, 8E**), while
821 *Cd38*$^{+}$ Mac3 (cluster 1) are more abundant in the distal nerve (**Fig. 8F, 8G**). The number of proliferating Mac is
822 comparable between the injury site and distal nerve (**Fig. 8C**). Similarly, the distribution of MoDC, DCx, cDC,
823 and T/NK is comparable between the injury site and the distal nerve (**Fig. 8C**). However, NK are more
824 abundant in the distal nerve (**Fig. 8C**).
825 For an unbiased cell cluster identification at the injury site and in the distal nerve, we compared the
826 scRNAseq datasets generated from the 3dpc injury site and distal nerve to the "whole nerve" 3dpc reference
827 scRNAseq data (**Fig. 2J**). We projected the whole nerve principal component analysis (PCA) structure onto
828 "query" scRNA-seq data generated from the injury site of distal nerve, implement through Seurat v3 (Stuart et
829 al., 2019). Our 'TransferData' pipeline finds anchor cells between the 3dpc whole nerve reference data and the
830 query dataset, then uses a weighted vote classifier based on the known reference cell labels to yield a
831 quantitative score for each cell's predicted label in the query dataset. A prediction score of 1 means maximal
832 confidence, all votes, for the predicted label, and a score of 0 means no votes for that label (**Fig. 8H, 8I**). Most
833 notable is the strong enrichment of Mac4 at the 3dpc injury site (**Fig. 8H**), when compared to distal nerve (**Fig.
834 8I**). Similarly, distribution of the Mac1 population is skewed toward the injury site, however to a lesser extent
835 than Mac4. Conversely, Mac3 cells are enriched in the distal nerve (**Fig. 8H,8I**). To confirm the location
836 specific distribution of different Mac subpopulations at 3dpc, we analyzed the injury site and distal nerve for
837 Mac subpopulation enriched transcripts using qRT-PCR. Because *Gpnmb/*glycoprotein non-metastatic
838 melanoma protein B, and *Syngr1*/synaptogyrin 1*, Fabp5/*fatty binding protein 5*,* and *Spp1* are highly enriched
839 in Mac4, we assessed their expression by qRT-PCR and found significantly higher expression at the injury site
840 (**Fig. 8J**). Conversely, scRNAseq reveled preferential expression of *Cd38*/ADP-ribosyl cyclase 1 in Mac3 in
841 the distal nerve and this was independently confirmed by qRT-PCR (**Fig. 8K**). Expression of the Mo marker
842 *Chil3* is not significantly different between the injury site and distal nerve. Compared to naïve nerve, *Gatm*
843 (creatine biosynthesis) is reduced in the nerve at 3dpc, both at the injury site and the distal nerve (**Fig. 8K**).

844 To assess the spatial distribution of Mac4 in the 3dpc nerve, we used *in situ* hybridization with an
845 RNAscope probe specific for *Gpnmb.* Very few *Gpnmb*$^{+}$ cells are detected in naïve sciatic nerve (**Fig. 8L**),
846 however at 3dpc, *Gpnmb*$^{+}$ cells are enriched in the endoneurium at the site of nerve injury site and far fewer
847 labeled cells are detected in the distal nerve (**Fig. 8M-M''**). Together, these studies reveal spatial differences in
848 Mac subpopulation distribution within the injured nerve.
849


A
Spinal Cord
Lumbar DRGs
injury
distal
Peroneal Nerve
Tibial Nerve
Sural Nerve
Cell Sorting
CD11b+
Single Cell Sequencing
iSNAT
B
Clus_Num_Vis
Injury
Distal
UMAP_1
UMAP_2
0. Mo
1. Mac3
2. Mac4
3. Mac1
4. Mac7
5. epiMac
6. Prol.Mac
7. MoDC
8. cDC
9. DCx
10. GC
11. NK
12. T_NK
13. Junk2
C
3 dpc
Injury Site
Distal Site
Proportion of Cell Population
Mo
Mac1
Mac2
Mac3
Mac4
epiMac
prol.Mac
MoDC
DCx
cDC
T_NK
NK
Arg1
Cd38
injury
distal
D
E
F
G
H
I
prediction.score.max
MAST
GC
pDC
J
Relative mRNA level at 3 dpc (log2)
Gpnmb
Syngr1
Fabp5
Spp1
K
Cd38
Gatm
Chil3
ns
L
naïve
Gpnmb Hoechst
M
3 dpc
M'
M''
injury
distal

850

851 **Figure 8**

852 **Spatial differences in the immune landscape of the injured sciatic nerve**

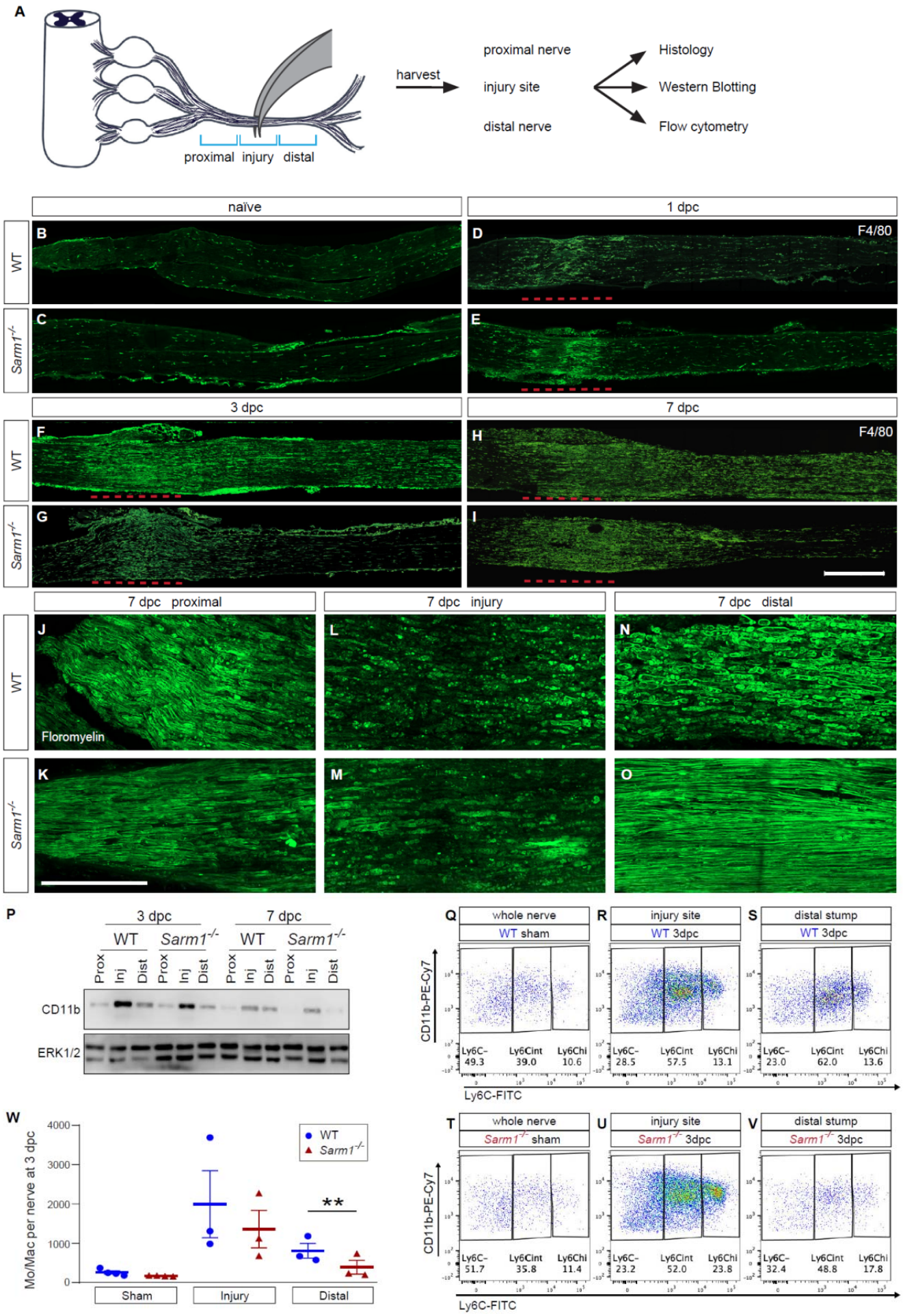
853 **(A)** Cartoon of a mouse lumbar spinal cord, dorsal root ganglia (DRGs) and major branches of the sciatic
854 nerve. Nerve segments ~ 3 mm in length, with the injury site and distal nerve are marked with blue brackets.
855 Immune cells from the injury site and distal nerve were captured with anti-CD11b magnetic beads and
856 analyzed in separate scRNAseq runs. **(B)** UMAP plot of sciatic nerve myeloid cells captured at the injury site
857 (left) and the distal nerve (right) at 3dpc. A total of 17,404 high quality cells were subjected to unsupervised
858 Seurat-based clustering resulting in 13 cell clusters. (**C**) Bar graph of population size at the injury site versus
859 distal nerve for 3d injured nerve immune cells. (**D, E**) Feature plots for *Arg1* expressing immune cells at the 3d
860 injury site and distal nerve (**F, G**). Feature plots for *Cd38* expressing immune cells at the injury site and distal
861 nerve. Expression levels are color coded and calibrated to average gene expression. (**H, I**) Projection of the
862 3d "whole nerve" reference data onto cells at the 3d injury site (**H**) and 3d distal nerve (**I**) onto 3d "whole nerve"
863 reference data. The y-axis shows the prediction score for each cell's top predicted cell population. The number
864 of cells identified to each population is shown on top. (**J, K**) Quantification of gene expression by qRT-PCR in
865 the 3d injured nerve injury site versus distal nerve (n= 3). P-values, * <0.05, ** <0.001, *** < 0.0001, Student's *t*
866 test. ns, not significant. (**L**) Longitudinal sections of naïve sciatic nerve stained for *Gpnmb* expression by
867 RNAscope. (**M**) Longitudinal sections of 3d injured nerve stained for *Gpnmb* expression by RNAscope,
868 proximal is to the left. Sections were counterstained with Hoechst. High power images of injury site (**M'**) and
869 distal nerve (**M''**) are shown. Scale bar: 200 μm (**L**, **M''**).

870

871 **Nerve trauma causes a strong inflammatory response independently of Wallerian degeneration**

872 To separate the immune response to mechanical nerve wounding from the immune response to WD, we
873 employed *Sarm1-/-* mice, in which WD is greatly delayed (Osterloh et al., 2012). WT and *Sarm1-/-* mice were
874 subjected to SNC and nerves harvested at 1, 3, and 7dpc. Longitudinal nerve sections were stained with anti-
875 F4/80 to assess abundance and distribution of Mac proximal to the injury site, at the injury site, and distal to
876 the injury site (**Fig. 9A**). In naive WT and *Sarm1-/-* mice, few F4/80$^+$ Mac are detected (**Fig. 9B,9C**). Following
877 nerve crush injury, WT and *Sarm1-/-* mice show a rapid increase in F4/80$^+$ Mac at the nerve injury site at 1dpc
878 (**Fig. 9D,9E**), 3dpc (**Fig. 9F,9G**) and, 7dpc (**Fig. 9H,9I**). In the distal nerve, however *Sarm1-/-* mice show far
879 fewer F4/80$^+$ cells at 3dpc and 7dpc (**Fig. 9F-9I**). At 7dpc, fluoromyelin staining of the proximal WT and *Sarm1-*
880 */-* nerves shows intact myelin (**Fig. 9J, 9K**). Myelin ovoids emanating from disintegrated myelinated axons are
881 observed in a ~3mm segment around the site of nerve injury, both in WT and *Sarm1-/-* mice (**Fig. 9L,9M**). In
882 the distal nerve of the same mice, myelin ovoids are observed only in WT (**Fig. 9N**), but not *Sarm1-/-* mutants
883 showing that at 7dpc fiber fragmentation has not yet occurred (**Fig. 9O).** For an independent assessment of
884 WD elicited nerve inflammation, nerve trunks were isolated from naïve and injured WT and *Sarm1-/-* mice,
885 divided into proximal nerve, the injury site, or distal nerve, and analyzed by Western blotting (**Fig. 9P**).
886 Independently of *Sarm1* genotype, the injury sites show elevated levels of CD11b compared to proximal nerve.
887 In the distal nerve, CD11b was more abundant in WT than in *Sarm1-/-* mice.

888 To quantify immune cell profiles in WT and *Sarm1-/-* mice, we used flow cytometry (**Fig. 9Q-9W)**, the
889 gating strategy is illustrated in **Fig. 9, Suppl. 1**. In sham operated mice, no significant differences in Ly6C-high
890 ($Ly6C^{hi}$), Ly6C-intermediate ($Ly6C^{int}$), or Ly6C-negative ($Ly6C^{-}$) Mo/Mac were observed (**Fig. 9Q,9T**). For
891 quantification of immune cell profiles that respond to traumatic nerve wounding versus WD, we separately
892 harvested the site of nerve injury and distal nerve for analysis by flow cytometry. At the 3dpc injury site, the
893 total number of Mo/Mac is comparable between WT and *Sarm1-/-* mice (**Fig. 9R,9U,9W**). However, within the
894 distal nerve, significantly more Mo/Mac cells are present in WT mice than in *Sarm1-/-* mice (**Fig. 9S,9V,9W**).
895 Taken together, these studies show that nerve trauma causes a highly inflamed wound microenvironment,
896 independently of WD, and a distinct inflammatory response in the distal nerve, that is WD dependent.


A
proximal
injury
distal
harvest
proximal nerve
injury site
distal nerve
Histology
Western Blotting
Flow cytometry
naïve
1 dpc
3 dpc
7 dpc
WT
Sarm1-/-
F4/80
7 dpc proximal
7 dpc injury
7 dpc distal
Floromyelin
P
3 dpc
7 dpc
WT
Sarm1-/-
Prox
Inj
Dist
CD11b
ERK1/2
Q
whole nerve
WT sham
R
injury site
WT 3dpc
S
distal stump
WT 3dpc
CD11b-PE-Cy7
Ly6C-FITC
Ly6C- 49.3
Ly6Cint 39.0
Ly6Chi 10.6
Ly6C- 28.5
Ly6Cint 57.5
Ly6Chi 13.1
Ly6C- 23.0
Ly6Cint 62.0
Ly6Chi 13.6
W
Mo/Mac per nerve at 3 dpc
4000
3000
2000
1000
0
WT
Sarm1-/-
**
Sham
Injury
Distal
T
whole nerve
Sarm1-/- sham
U
injury site
Sarm1-/- 3dpc
V
distal stump
Sarm1-/- 3dpc
Ly6C- 51.7
Ly6Cint 35.8
Ly6Chi 11.4
Ly6C- 23.2
Ly6Cint 52.0
Ly6Chi 23.8
Ly6C- 32.4
Ly6Cint 48.8
Ly6Chi 17.8

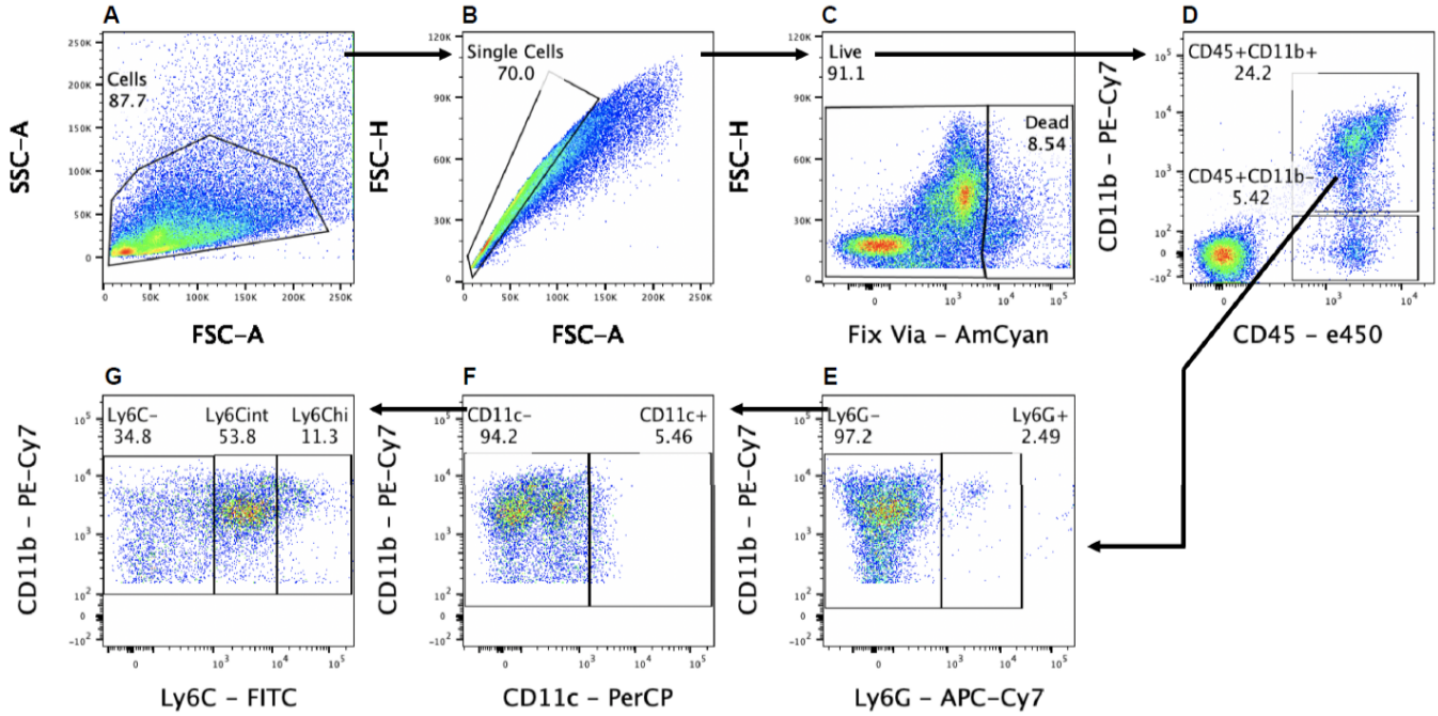
897

898 **Figure 9**

899 **Nerve trauma causes WD independent nerve inflammation**

900 **(A)** Cartoon of a mouse lumbar spinal cord, dorsal root ganglia (DRGs) and major branches of the sciatic
901 nerve. A nerve injury divides the nerve trunk into a proximal segment, the injury site, and distal segment, each
902 marked with blue brackets. Nerve segments were harvested and subjected to analysis. (**B-I**) Longitudinal
903 sciatic nerve sections from WT and *Sarm1*$^{-/-}$ mice, stained with anti-F4/80 for identification of Mac.
904 Representative examples of (**B, C**) naïve nerve, (**D, E**), 1dpc (**F, G**), 3dpc, and at (**H, I**) 7dpc. Injury site is
905 marked with a dashed red line, proximal is to the left. Scale bar, 500 µm. (**J-O**) Longitudinal sciatic nerve
906 sections from WT and *Sarm1*$^{-/-}$ mice at 7dpc, stained with fluoromyelin. Representative images of proximal
907 nerve, the injury site and distal nerve are shown. Scale bar, 200 µm. **(P)** Western blots of sciatic nerve
908 segments collected at 3dpc and 7dpc from WT and *Sarm1*$^{-/-}$ mice. Nerves were divided into proximal, injury
909 site, and distal segments and blots probed with anti-CD11b, and anti-ERK1/2. **(Q-V)** Flow cytometry dotplots
910 for Mo/Mac of sham operated WT and *Sarm1*$^{-/-}$ sciatic nerve trunks, the 3dpc nerve injury site and distal nerve.
911 (**W**) Quantification of Mo/Mac (Ly6C^{hi} + Ly6C^{int} + Ly6C^{-}) in sham operated mice, the 3dpc injury site and 3dpc
912 distal nerve of WT and *Sarm1*$^{-/-}$ mice. N= 3, with 3-5 mice per genotype per replica. Flow data are represented
913 as mean ± SEM. Statistical analysis was performed in GraphPad Prism (v9) using two-way, paired *t*-test. **
914 p<0.01.

915



916

917 **Figure 9, Suppl. 1**

918 Gating strategy for flow cytometry. **(A)** Cells were first gated with forward scatter (FSC-A) and side scatter
919 (SSC-A) to exclude debris. **(B)** Cells were then gated with forward scatter height (FSC-H) and FSC-A to find
920 single cells and to exclude doublets. **(C)** Live cells were isolated by negative staining for fixed viability dye (Fix
921 Via). **(D-G)** Leukocytes (D) were analyzed as follows: lymphocytes were isolated as CD45^{+}, CD11b^{-}. Myeloid

922 cells ($CD45^+$, $CD11b^+$) were further separated into $Ly6G^+$ granulocytes (E). The remaining cells ($CD45^+$,
923 $CD11b^+$, $Ly6G^-$) were characterized as DC (F) ($CD45^+$, $CD11b^+$, $CD11c^+$, $Ly6G^-$), and Mo/Mac (G) ($CD45^+$,
924 $CD11b^+$, $CD11c^-$, $Ly6G^-$).
925
926 **In *Sarm1-/-* mice, monocytes enter the distal nerve stump but fail to mature into macrophages**
927 Because we used *Sarm1* global knock-out mice for our studies and *Sarm1* has been shown to function in Mac
928 (Gurtler et al., 2014), a potential confounding effect is *Sarm1* deficiency in Mac. To assess nerve entry of
929 circulating WT immune cells, in a *Sarm1-/-* background, we employed parabiosis (**Fig. 10A**). *Sarm1-/-* host
930 mice were surgically fused to tdTomato (tdT) donor mice and allowed to recover for three weeks. For
931 comparison, WT/tdT parabiosis complexes were generated and processed in parallel. Flow cytometry was
932 used to analyze blood samples of host parabionts for tdT^+ leukocytes and revealed ~30% chimerism (**Fig. 10B,**
933 **10C**). In each complex, both parabionts were subjected to bilateral nerve crush. At 7dpc, analysis of
934 longitudinal nerve sections of the *Sarm1-/-* parabiont revealed that many tdT^+ leukocytes entered the site of
935 nerve injury, comparable to injured WT parabionts (**Fig. 10D, 10E, 10F**). In the distal nerve of the *Sarm1-/-*
936 parabiont, at 1mm, 2mm, and 3mm distal to the injury site, some tdT^+ leukocytes are present, however at
937 significantly reduced numbers when compared to WT parabionts (**Fig. 10F**). Interestingly, only few tdT^+ cells in
938 the *Sarm1-/-* distal nerve stained for F4/80, a marker for Mac (**Fig. 10G,10H**) or Ly6G, a marker for neutrophils
939 (**Fig. 10, Suppl. 1**). To further investigate the blood-borne immune cells that enter the 7dpc distal nerve of
940 *Sarm1-/-* mice, we separately harvested and analyzed the 7dpc injury site and distal nerve segments from WT
941 and *Sarm1-/-* single mice using flow cytometry. The abundance of Mo and Mac, identified as $Ly6C^{hi}$, $Ly6C^{int}$,
942 and $Ly6C^-$ cells, at the nerve injury site is comparable between WT and *Sarm1-/-* mice (**Fig. 10I, 10J**). In the
943 distal nerve however, $Ly6C^{hi}$ cells in *Sarm1-/-* nerves are significantly elevated compared to $Ly6C^{int}$ and $Ly6C^-$
944 populations (**Fig. 10I,10J**). This stands in contrast to WT distal nerves, where $Ly6C^-$ and $Ly6C^{int}$ cells
945 outnumber the $Ly6C^{hi}$ population **(Fig. 10K).** Because WT and *Sarm1-/-* mice show similar baselines of Mac in
946 the naïve nerve (**Fig. 9Q,9T**), this shows that in the absence of WD, SNC is sufficient to trigger a strong
947 immune response to nerve wounding, but fails to elicit WD associated nerve inflammation, except for the
948 appearance of Mo.
949 Taken together, these data show that in *Sarm1-/-* mice $Ly6C^{hi}$ Mo enter the distal nerve prior to WD but
950 fail to differentiate into Mac. This suggests that chemoattractive signals for Mo are released from severed
951 axons prior to WD and that fiber degeneration in the distal nerve is required for Mo maturation. Moreover, WD
952 is required for rapid, full-blown inflammation of the distal nerve.

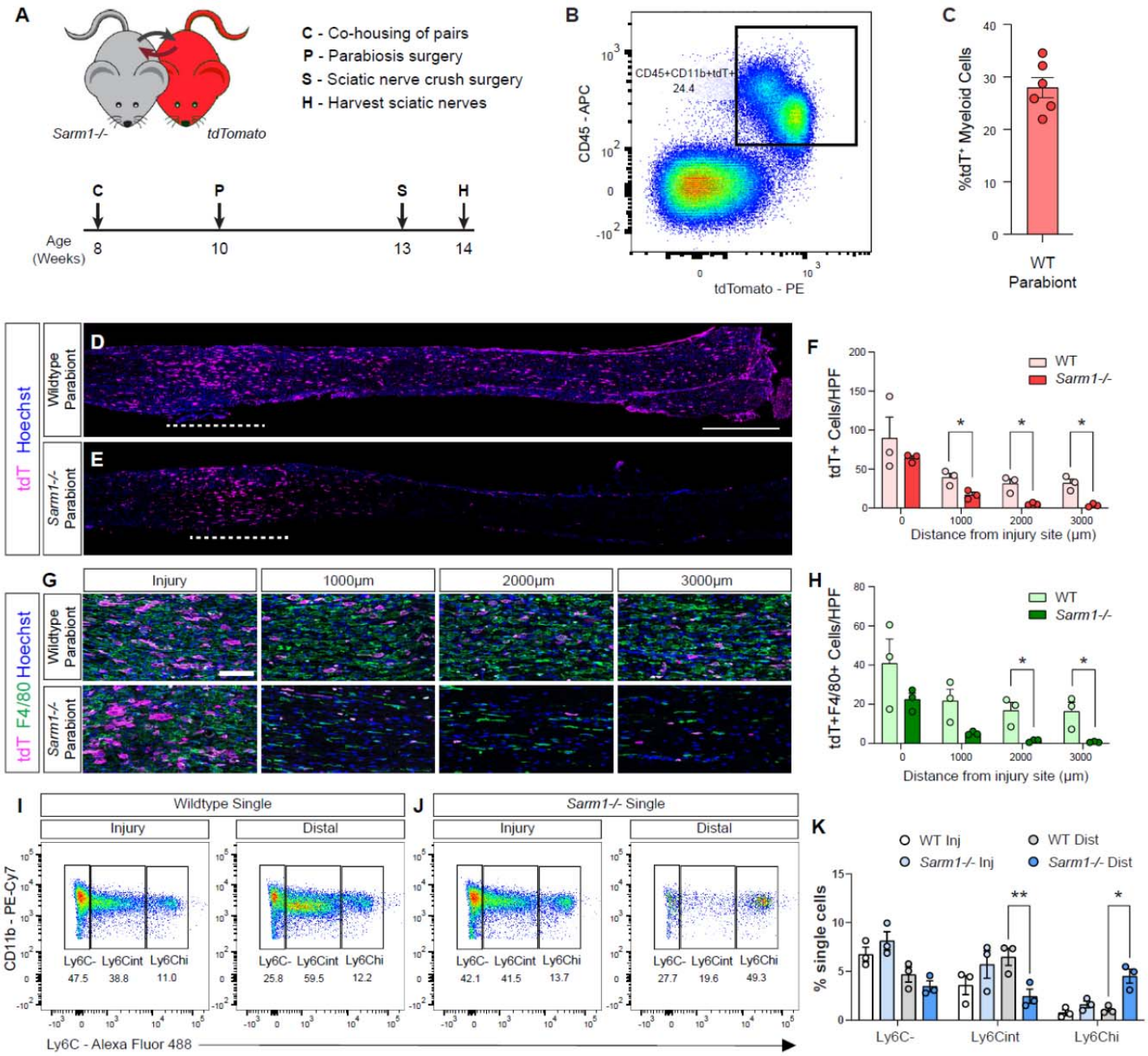


Figure 10

Evidence for WD dependent and WD independent nerve inflammation

(**A**) Timeline for parabiosis experiments. After a two-week co-housing period, 10-week-old WT or *Sarm1*$^{-/-}$ and -*tdTomato* mice were surgically paired. (**B**) To assess chimerism, blood was harvested and analyzed by flow cytometry. Dotplot of tdT^{+} myeloid cells (CD45^{+}CD11b^{+}) is shown. (**C**) Quantification of tdT^{+} myeloid cells in host parabionts (n= 6), revealed chimerism of 28± 2%. (**D, E**) Bilateral SNC was performed 3 weeks after pairing and tissue harvested at 7dpc. Longitudinal sciatic nerve sections from (**D**) WT and (**E**) *Sarm1*$^{-/-}$ parabionts showing infiltrating tdT^{+} leukocytes (magenta). Nuclei (blue) were labeled with Hoechst dye. The nerve crush site is marked by the white dashed line, proximal is to the left. Scale bar, 500 µm. (**F**) Quantification of tdT^{+} cells per high power field (HPF, 500 µm x 250 µm) at the injury site (0 µm) and at 1000, 2000, and 3000 µm distal to the injury site. The average cell number ± SEM is shown, n = 3 mice per

genotype, average of 4 HPF per 2 nerves. Student's *t* test, $p < 0.05$ (*). (**G**) HPF of sciatic nerves from WT and *Sarm1*$^{-/-}$ parabionts 7dpc taken from the injury site, 1000, 2000, and 3000 µm distal to the injury site showing infiltrating tdT^{+} leukocytes (magenta), F4/80^{+} macrophages (green), and nuclei (blue). Scale bar, 100 µm. (**H**) Quantification of tdT^{+}F4/80^{+} cells per HPF ± SEM at indicated distances distal to the injury site, n = 3 mice, average of 4 HPF per 2 nerves. Student's *t* test, $p < 0.05$ (*). (**I, J**) Flow cytometric analysis of sciatic nerves from single (not part of parabiosis complex) WT and *Sarm1*$^{-/-}$ mice 7dpc. Sciatic nerve trunks were microdissected and separated in 3 mm injury site and distal nerve segments. Dotplots showing Mo/Mac maturation assessed by Ly6C surface staining, Mo (Ly6C^{hi}), Mo/Mac (Ly6C^{int}), Mac (Ly6C^{-}), previously gated as CD45^{+}CD11b^{+}Ly6G^{-}CD11c^{-} cells. (**K**) Quantification of Mo/Mac shown in panels **I** and **J,** as a percentage of single cells ± SEM, n = 3, injury and distal sites were pooled from 5 mice per genotype per biological replicate. Two-way ANOVA with Tukey's post-hoc test for multiple comparisons, $p < 0.05$ (*), $p < 0.01$ (**).

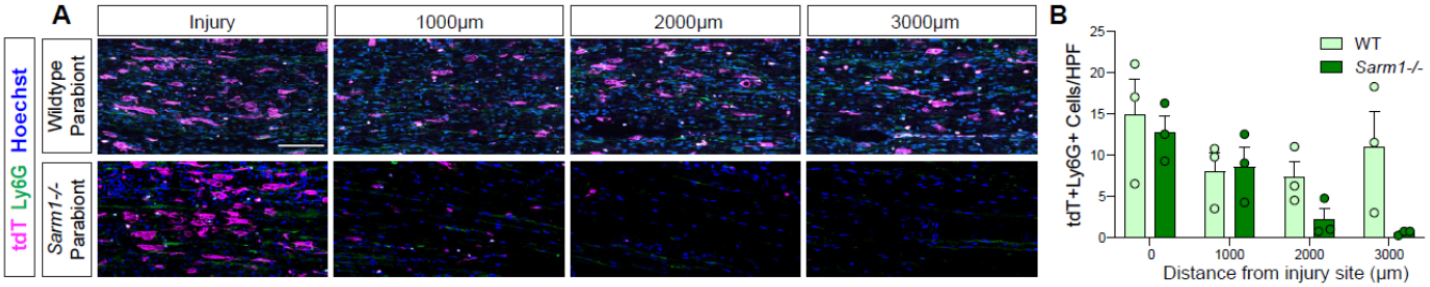


Figure 10, Suppl. 1

Quantification of tdT,Ly6G double-positive neutrophils in the 7dpc nerve of host parabionts (n= 6). (**A**) High power filed (HFP) of longitudinal sections of a WT and *Sarm1-/-* parabionts at 7dpc. Representative images taken from the injury site, 1000, 2000, and 3000 µm distal to the injury site. Infiltrating tdT^{+} leukocytes (magenta), Ly6G^{+} neutrophils (green), and nuclei (Hoechst) are labeled. Scale bar, 100 µm. (**B**). Quantification of tdT^{+}Ly6G^{+} cells per (HPF) ± SEM at indicated distances distal to the injury site, n = 3 mice, an average of 4 HPF per 2 nerves. Student's *t* test.

985 ## Discussion

986 The injured adult murine PNS exhibits a remarkable degree of spontaneous axonal regeneration and functional
987 recovery. To better understand the cellular and molecular events associated with PNS repair, we carried out a
988 longitudinal scRNAseq study. Analysis of the immune response to nerve crush injury, during the first week,
989 revealed a highly dynamic microenvironment. The early immune response is pro-inflammatory and dominated
990 by GC and Mo/Mac, metabolically programmed for glycolytic energy production. The elevated expression of
991 nearly all glycolytic enzymes, lactate dehydrogenase, and the lactate export channel MCT4 indicates that a
992 Warburg-like effect is at play, coupling glycolytic energy production with a proinflammatory Mo/Mac phenotype.
993 This stands in marked contrast to the low glycolytic activity of circulating Mo/Mac in blood of naïve mice and
994 indicates a rapid metabolic shift upon nerve entry. The glycolytic burst is short-lived, however, at 3dpc
995 expression of glycolytic enzymes begins to decline, and at 7dpc has reached levels similar to naïve nerve. As
996 glycolytic activity declines, there is evidence for a metabolic shift toward OXPHOS, and this coincides with the
997 appearance of Mac with a resolving phenotype. Separation of the nerve injury site from distal nerve revealed
998 that mechanical nerve injury creates two separate immune microenvironments. A wound repair response at the
999 crush site, and an inflammatory response to WD in the distal nerve. This was further corroborated in injured
1000 *Sarm1-/-* mice. At the injury site of *Sarm1-/-* and WT mice, nerve crush results in a strong immune response,
1001 dominated by hematogenous leukocytes. In the distal nerve of *Sarm1-/-* mice, full-blown nerve inflammation is
1002 delayed, and thus, WD dependent. Hematogenous immune cells are largely missing from the *Sarm1-/-* distal
1003 nerve, except for Mo, suggesting that chemotactic signals are released from severed fibers prior to physical
1004 disintegration. Taken together, we describe a framework of cell types and single-cell transcriptomes for a
1005 neural tissue with a high degree of spontaneous morphological and functional regeneration. The datasets
1006 reported provide an essential step toward understanding the dynamic nature of complex biological processes
1007 such as neural tissue degeneration and regeneration.
1008

1009 **iSNAT**

1010 To facilitate mining of scRNAseq datasets of naïve nerve and at different post-SNC time points, we generated
1011 the injured sciatic nerve atlas (iSNAT). The "*Expression Analysis*" function is a search tool to assess which
1012 cells in the nerve express a gene of interest. The output is four feature pots (naïve, 1, 3, and 7dpc nerve)
1013 shown side-by-side to assess which cells express a gene of interest and whether the gene is regulated by
1014 SNC. In addition, any cell type or time point during the first week can be selected to identify the top 50 enriched
1015 gene products. The *two Genes* function quantifies co-expression of two genes in the same cell. Higher
1016 resolution UMAP plots of select cell types, such as immune cells and structural cells (stromal cells), can be
1017 accessed and mined separately for analysis of subcluster specific gene expression. We added single cell
1018 transcriptomes of PBMC and used dataset integration to show how immune cell clusters change during the first
1019 week following nerve injury. Embedded in iSNAT is the CellChat function, designed for identification of
1020 intercellular signaling networks. Families of surface or secreted molecules, e.g. CXCL family members, can be
1021 searched for cells in the naïve and injured nerve that express the corresponding receptor(s) and the probability

1022 for this interaction to occur is calculated. This provides a powerful tool for understanding interactions among
1023 different cell types in the nerve. The *Spatial Distribution* function shows gene expression at the injury site
1024 versus distal nerve at 3dpc. We have validated many gene products identified by scRNAseq, using a
1025 combination of qRT-PCR, RNAscope, immunofluorescence staining, reporter gene expression, and ELISA.
1026 While iSNAT is expected to facilitate data analysis and functional studies in the injured PNS, there are some
1027 notable limitations. The reading depth at the single cell level is still limited. We have sequenced and analyzed
1028 ~150,000 high-quality cells. On average we detect ~2000 unique features per cell, well below the estimated
1029 total of 5000-10000. Thus, if a gene of interest is not found in iSNAT, it may either not be expressed, or be
1030 expressed below the detection sensitivity of scRNAseq. We acknowledge that enzymatic tissue digestion
1031 combined with mechanical tissue dissociation may lead to cell loss or variable capturing efficiency for different
1032 cell types. Most notably, mSC are sparse in our naïve nerve dataset. Additional cell types that may be lost
1033 include B cells and adipocytes, both of which have previously been detected in the rodent PNS (Chen et al.,
1034 2021). Our gene expression atlas is work in progress and we anticipate that future studies will overcome these
1035 limitations, allowing us to generate improved, next generations of iSNAT.
1036
1037 **Structural cells in the injured nerve shape the immune microenvironment**
1038 A nerve crush injury triggers proliferation of stromal cells, including epineurial Fb, identified as *Mki67*$^{+}$*Pdgfra*$^{+}$
1039 cells that mature into Fb. dMES are abundantly found in the injured nerve. In addition, pMES and eMES begin
1040 to proliferate following SNC. In the UMAP plot of 3dpc nerve, clusters with proliferating structural cells are
1041 connected and give rise to pMES, eMES, and dMES (**Fig. 2**). This suggests that in addition to damaging the
1042 epineurium, SNC damages protective cell layers within the nerve, including the perineurium, a thin cell layer of
1043 epithelioid myofibroblasts that surrounds nerve fascicles, and the endoneurium, a delicate layer of connective
1044 tissue that covers individual myelinated nerve fibers and contains the endoneurial fluid. Proliferation of eMES
1045 and pMES likely reflects damage to the BNB and is supported by the accumulation of some serum proteins in
1046 the crushed nerve. In addition to IgG (Vargas et al., 2010), we detected other serum proteins that function in
1047 opsonization. These include complement components, soluble C1qR1/CD93, adiponectin (Adipoq), and
1048 pentraxins (Nptx2, Crp), molecules that aid in the clearance of cellular debris and apoptotic cells and push Mac
1049 toward an anti-inflammatory resolving phenotype (Blackburn et al., 2019; Casals et al., 2019; Guo et al., 2012).
1050 Of interest, structural cells in the injured nerve consistently show the highest number of unique
1051 transcripts, indicative of a strong injury response. MES are a major source of immune modulatory factors,
1052 shaping the injured nerve microenvironment in a paracrine manner through release of soluble factors.
1053 CellChat, identified important roles in chemotaxis, angiogenesis, ECM deposition and remodeling, suggestive
1054 of extensive stroma-immune cell communication. In particular, eMES are a major signaling hub and show
1055 strong interactions with Mo, Mac, SC, pMES, and EC. The presence of several chemokines, growth factors,
1056 and immune proteins identified at the transcriptional level was independently validated by ELISA, providing
1057 confidence in the quality of scRNAseq datasets.
1058
1059

1060 **Cellular metabolism and macrophage functional polarization in the injured PNS**
1061 Evidence from injured non-neural tissues shows that immune cell metabolism is directly linked to cell plasticity
1062 and function, thereby affecting tissue repair and scarring (Eming et al., 2021; Eming et al., 2017). Little is
1063 known about the metabolic adaptions associated with successful neural tissue repair. Here we compared
1064 immune cell metabolism of bone-marrow derived circulating myeloid cells before and after nerve entry. Once in
1065 the injured nerve, neutrophils, Mo, and Mac undergo rapid metabolic reprogramming, greatly increasing gene
1066 products that drive glycolysis. This is similar to the metabolic shift observed in non-neural tissues with high
1067 regenerative capacities, such as skeletal muscle (Eming et al., 2021). Interestingly, rapid upregulation of
1068 glycolytic activity in myeloid cells in the injured nerve is reminiscent of the injury-regulated glycolytic shift in SC,
1069 involving the mTORC/Hif1α/c-Myc axis (Babetto et al., 2020). This suggests that enhanced glycolytic flux and
1070 lactate extrusion from both, SC and innate immune cells, is axoprotective.
1071 Many cells use aerobic glycolysis during rapid proliferation, since glycolysis provides key metabolites
1072 for the biosynthesis of nucleotides and lipids (Lunt and Vander Heiden, 2011). In the 1dpc nerve, myeloid cells
1073 show highest levels of glycolytic enzymes, however only few *Mki67* (encoding Ki67^{+}) proliferating cells are
1074 detected. In immune cells, alterations in metabolic pathways couple to immune cell effector function, most
1075 notably the production of different cytokines (O'Neill et al., 2016). Glucose is a main source for cellular energy
1076 (ATP) production through two linked biochemical pathways, glycolysis and the mitochondrial TCA (O'Neill et
1077 al., 2016; Voss et al., 2021). Glycolysis converts glucose into pyruvate and pyruvate is converted into acetyl-
1078 CoA to enter the TCA and fuel OXPHOS in mitochondria as an efficient means of ATP production.
1079 Alternatively, pyruvate can be converted into lactate and NAD^{+}, creating a favorable redox environment for
1080 subsequent rounds of glycolysis. The upregulation of *Ldha* and *Slc16a3*/MCT4 in myeloid cells of the injured
1081 nerve is striking and resembles the Warburg effect described for cancer cells (Schuster et al., 2021; Zhu et al.,
1082 2015). The transient increase in extracellular lactate may not only be axon protective (Babetto et al., 2020;
1083 Funfschilling et al., 2012), but additionally regulate immune cell reprogramming (Morioka et al., 2018) and
1084 trigger pain (Rahman et al., 2016). The injury induced increase of *Hif1α* suggests that hypoxia is a main driver
1085 of metabolic reprogramming, however, the Hif1α pathway can also be activated by pattern recognition
1086 receptors recognizing DAMPs released by injured cells following trauma (Corcoran and O'Neill, 2016).
1087 The molecular basis for Mac reprogramming into an anti-inflammatory state remains incompletely
1088 understood. A resolving Mac phenotype may be initiated by Mac mediated engulfment and digestion of
1089 apoptotic cell corpses (Boada-Romero et al., 2020; Greenlee-Wacker, 2016). Mac in the injured sciatic nerve
1090 are fully equipped with the molecular machinery for efferocytosis, including phagocytic receptors, enzymes,
1091 and transporters to cope with elevated cholesterol load and other metabolic challenges (Kalinski et al., 2020).
1092 Parabiosis experiments, combined with SNC revealed that clearing of apoptotic leukocytes, through
1093 efferocytosis, takes place in the injured PNS (Kalinski et al., 2020). Professional phagocytes that undergo
1094 multiple rounds of efferocytosis experience metabolic stress such as accumulation of intracellular lipids (Schif-
1095 Zuck et al., 2011). Growing evidence suggests that Mac leverage efferocytotic metabolites for anti-
1096 inflammatory reprogramming to promote tissue repair (Zhang et al., 2019). During the resolution phase Mac
1097 are equipped with the machinery for fatty acid oxidation and OXPHOS as a means of energy production. The

1098 rapid reprogramming of Mac is likely key for wound healing, axon regeneration, and restoration of neural
1099 function. Timely resolution of inflammation protects from excessive tissue damage and fibrosis. Interestingly,
1100 perineural cells may function as lactate sink in the injured nerve, since pMES express high levels of
1101 *Slc16a1*/MCT1 for cellular import, as well as *Ldhb* for conversion of lactate into pyruvate. This suggests that
1102 different cell types in the injured nerve employ different strategies to cover their bioenergetic needs. It will be
1103 interesting to examine whether Mo/Mac metabolic reprogramming, efferocytosis, and inflammation resolution
1104 are altered under conditions where nerve health and axon regeneration are compromised (Sango et al., 2017).
1105 Because nerve inflammation has been linked to the development of neuropathic pain (Davies et al., 2020),
1106 prolonged and poorly resolving nerve inflammation, due to impaired metabolic reprogramming, may directly
1107 contribute to pain syndromes.
1108

1109 **Identification of distinct immune compartments in the injured PNS**

1110 Mo/Mac are highly plastic cells that are educated by the microenvironment. Considering the complexity of
1111 injured PNS tissue, it is perhaps not surprising that Mac subpopulations were identified that are not uniformly
1112 distributed within the injured nerve. At the site of injury, nerve trauma is caused by compression or transection,
1113 resulting in cell destruction, release of damage associated molecular patterns (DAMPs), vascular damage,
1114 nerve bleeding, and disruption of tissue homeostasis. In the distal nerve, where physical trauma is not directly
1115 experienced, severed fibers rapidly undergo WD. Thus, mechanical nerve injury results in temporally and
1116 spatially distinct microenvironments. This was demonstrated by single cell RNAseq of immune cells captured
1117 at the nerve injury site or the distal nerve. We identified distinct, yet overlapping immune compartments,
1118 suggesting functions associated with wound healing and WD. Experiments with *Sarm1-/-* mice demonstrate
1119 that SNC triggers a spatially confined inflammatory response and accumulation of blood-borne immune cells
1120 independently of WD. Thus, physical nerve wounding and the resulting disruption of tissue homeostasis are
1121 sufficient to trigger robust local nerve inflammation. Our findings are reminiscent of a study in zebrafish larvae
1122 where laser transection of motor nerves resulted in Mac accumulation at the lesion site prior to axon
1123 fragmentation. Moreover, delayed fragmentation of severed zebrafish motor axons expressing the *Wld(s)*
1124 transgene did not alter Mac recruitment (Rosenberg et al., 2012). In injured *Sarm1-/-* mice, distal nerves are
1125 much less inflamed when compared to parallel processed WT mice. Our observation is consistent with studies
1126 in *Wld(s)* mice, where reduced nerve inflammation has been reported (Chen et al., 2015; Coleman and Hoke,
1127 2020; Lindborg et al., 2017; Perry and Brown, 1992). Parabiosis experiments show that hematogenous WT
1128 immune cells readily enter the *Sarm1-/-* injury site, and to a much lesser extent, the distal nerve. Interestingly,
1129 Mo enter the *Sarm1-/-* distal nerve prior to fiber disintegration, however, fail to mature into Mac. This suggests
1130 that severed, but physically intact PNS fibers in the *Sarm1-/-* distal nerve release chemotactic signals for Mo.
1131 Our studies show that SNC is sufficient to trigger Mo recruitment to the distal nerve, but WD is required for Mo
1132 differentiation and full-blown nerve inflammation.
1133

1134 **What drives WD associated nerve inflammation?**

Studies with injured *Sarm1-/-* mice show that full-blown inflammation of the distal nerve requires WD, the underlying molecular signals, however, remain incompletely understood. Because WD results in axon disintegration and simultaneous breakdown of myelin sheaths into ovoids, it is not clear whether myelin debris, SC activation, or axon fragmentation is the main trigger for WD associated nerve inflammation. Of interest, transgenic expression of Raf-kinase in mSC in adult mice is sufficient to drive SC dedifferentiation into p75-positive progenitor-like state without compromising axon integrity. SC dedifferentiation resulted in cytokine expression and nerve inflammation, however analysis of immune cell composition and comparison to SNC triggered nerve inflammation, has not yet been carried out (Napoli et al., 2012). In the healthy PNS, the endoneurial milieu is protected by the BNB, a selectively permeable barrier formed by the specialized EC along with the perineurial barrier. The BNB creates an immunologically and biochemically privileged space harboring nerve fibers and endoneurial fluid (Lim et al., 2014). ELISA of injured nerve tissue revealed that the BNB is at least partially compromised following SNC, resulting in local disturbances of vascular permeability, allowing access of serum proteins that function in opsonization and phagocytosis to the endoneurium. This suggests that in addition to degenerating axons and myelin debris, disruption of the BNB may be a driver of trauma inflicted nerve inflammation. Additional studies are needed to fully define the mechanisms that underly WD-associated nerve inflammation.

Taken together, we carried out a longitudinal analysis of injured mouse PNS, naïve nerve, and PBMC transcriptomes at single cell resolution. The study provides unprecedented insights into the dynamic cellular landscape, cell-cell interaction networks, and immune cell metabolic reprograming during the first week following nerve crush injury. To facilitate dataset mining, we developed the injured sciatic nerve atlas (iSNAT), a novel tool to navigate the cellular and molecular landscape of a neural tissue endowed with a high regenerative capacity.

Material and Methods

Mice and genotyping

All procedures involving mice were approved by the Institutional Animal Care and Use Committees (IACUC) of the university of Michigan (PRO 00009851) and performed in accordance with guidelines developed by the National Institutes of Health. Young adult male and female mice (8-16 weeks) on a C57BL/6 background were used throughout the study. Transgenic mice included, *Sarm1/Myd88-5-/-* (Jackson labs Stock No: 018069) and *ROSA26-mTdt/mGFP* (Jackson labs Stock No: 007576). Mice were housed under a 12 h light/dark cycle with regular chow and water ad libitum. For genotyping, ear biopsies were collected, and genomic DNA extracted by boiling in 100 µl alkaline lysis buffer (25 mM NaOH and 0.2 mM EDTA in ddH_2O) for 30 min. The pH was neutralized with 100 µl of 40 mM Tris-HCl (pH 5.5). For PCR, 1–5 µl of gDNA was mixed with 0.5 µl of 10 mM dNTP mix (Promega, C1141, Madison, WI), 10 µl of 25 mM $MgCl_2$, 5 µl of 5X Green GoTaq Buffer (Promega, M791A), 0.2 µl of GoTaq DNA polymerase (Promega, M3005), 1 µl of each PCR primer stock (100 µM each), and ddH_2O was added to a total volume of 25 µl. The following PCR primers, purchased from *Integrated DNA Technologies,* were used: *Sarm1* WT Fwd: 5'GGG AGA GCC TTC CTC ATA CC 3'; *Sarm1* WT Rev: 5'TAA GAA TGA GCA GGG CCA AG 3'; *Sarm1* KO Fwd: 5'CTT GGG TGG AGA GGC TAT TC 3'; *Sarm1* KO Rev: 5'AGG TGA GAT GAC AGG AGA TC 3'; *Rosa26* WT Fwd: 5'-CGT GAT CTG CAA CTC CAG TC-3'; *Rosa26* WT Rev: 5'-GGA GCG GGA GAA ATG GAT ATG-3'. PCR conditions were as follows: Hot start 94°C 3 minutes; DNA denaturing at 94°C 30 seconds; annealing 60°C 1 minute; extension 72°C 1-minute, total cycles 34. Final extension for 6 min at 72°C.

Surgical procedures

Mice were deeply anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) or with isoflurane (5% induction, 2-3% maintenance, SomnoSuite Kent Scientific). Buprenorphine (0.1 mg/kg) was given as an analgesic. For SNC, thighs were shaved and disinfected with 70% isopropyl alcohol and iodine (PDI Healthcare). A small incision was made on the skin, underlying muscles separated, and the sciatic nerve trunk exposed. For sham operated mice, the nerve was exposed but not touched. For SNC, the nerve was crushed for 15 seconds, using a fine forceps (Dumont #55). The wound was closed using two 7mm reflex clips (Cell Point Scientific). Parabiosis surgery was performed as described (Kalinski et al., 2020). Briefly, before parabiosis surgery, similar aged, same sex mice were housed in the same cage for 1-2 weeks. Mice were anesthetized and their left side (host) or right side (donor) shaved and cleaned with iodine pads. A unilateral skin-deep incision was made from below the elbow to below the knee on the host and donor mouse. Mice were joined at the knee and elbow joints with non-absorbable nylon sutures. Absorbable sutures were used to join the skin around the shoulders and hindlimbs. Reflex wound clips (7 mm) were used to join the remainder of the skin between the two mice. Mice were allowed to recover for 3-4 weeks before use for SNC surgery.

Histological procedures

Mice were euthanized with an overdose of Xylazine/Ketamine and transracially perfused for 5 min with ice-cold PBS followed by 5 min with freshly prepared ice-cold 4% paraformaldehyde in PBS. Sciatic nerve trunks were

harvested and postfixed for 2 hours in ice-cold perfusion solution, followed by incubation in 30% sucrose in PBS solution at 4°C overnight. Nerves were covered with tissue Tek (Electron Microscopy Sciences, 62550–01) and stored at −80°C. Nerves were cryo-sectioned at 14 µm thickness and mounted on Superfrost^{+} microscope slides, air dried overnight, and stored in a sealed slide box at -80ºC. For antibody staining, slides were brought to RT and rinsed in 1x PBS three times, 5 min each. Slides were incubated in 0.3% PBST (1X PBS plus 0.3% Tween 20) for 10 min, followed by incubation for 1h in 5% donkey serum solution in 0.1% PBST (blocking buffer). Primary antibodies at appropriate dilutions were prepared in blocking solution, added to microscope slides, and incubated at 4ºC overnight. The next day, sections were rinsed three times in PBS, 5 min each. Appropriate secondary antibodies in blocking buffer were added for 1h at a dilution of 1:1000 at room temperature. Slides were rinsed three times in PBS, 5 min each, once in 0.1% PBST for 5 min, and briefly with milliQ water. Sections were mounted in DAPI containing mounting medium (Southern biotec (Cat. No.0100-20)), and air dried. Images were acquired with a Zeiss Apotome2 microscope equipped with an Axiocam 503 mono camera and ZEN software. Image processing and analysis were conducted using the ZEN software.

For *in situ* mRNA detection, the RNAscope Multiplex Fluorescent Reagent Kit v2 (ACD, 323100) was used. Microscope slides with serially cut nerves were rinsed in 1x PBS for 5 min and air dried by incubation at 60ºC for 45 min in an oven (VWR Scientific, Model 1525 incubator). Next, tissue sections were fixed in 4% Paraformaldehyde (PFA)/PBS for 45 mins at RT and dehydrated by incubation in a graded series of 50%, 70%, and 100% ethanol for 5 min each. Sections were air dried for 5 min at RT and one drop of hydrogen peroxide solution (ACD catalog number: PN 322381) was added to each nerve section on each slide and incubated at RT for 10 min. Sections were then submerged in 99ºC RNase free water for 1 min, followed by incubation in 99ºC 1x antigen retrieval solution (ACD catalog number: 322000) for 20 min. Next, slides were air dried by incubation for 45 min in an oven at 60ºC. Protease III solution (ACD catalog number: PN 322381) was applied to tissue sections followed by incubation at 40ºC in an ACD hybridization oven (ACD catalog number: 321710) for 45 min. RNA probes were mixed at appropriate ratios and volumes (typically 50:1 for C1:C2) for complex hybridization. For single RNA probe hybridization, RNA probes were diluted with probe dilutant at 1:50-1:100 (ACD catalog number: 300041). Appropriate probes or the probe mixtures were applied to tissue sections and incubated for 2h in the hybridization oven at 40ºC. 1X wash buffer was prepared from a 50X stock solution (ACD catalog number: PN 310091) and sections rinsed for 2 min. The slides were then stored in 5X SSC buffer overnight. The next morning, sections were rinsed in 1X wash buffer and amplification probes, corresponding to the primary RNA probes, were applied starting with the C1 probe (ACD catalog number: 323100). Slides were incubated in the hybridization oven for 30 min 40ºC and then rinsed twice with 1X wash buffer. Next, the A2 and A3 probes were applied. For development, the TSA system (AKOYA, Cy3: NEL744001KT; Cy5: NEL745001KT; Fluorescein: MEL741001KT) was used. Once the color for probe C1 was selected, HRPC1 solution (ACD catalog number: 323120), it was applied to the appropriate sections and incubated for 15 mins in the hybridization oven at 40ºC. The sections were then rinsed in 1x washing buffer. Designated TSA color for probe C1, diluted in the TSA dilutant (ACD catalog number: 322809) between

1233 1:1000-1:2000 was applied to the respective sections and incubated for 30 mins in the ACD hybridization oven
1234 at 40ºC. Sections were rinsed in 1X wash buffer and then HRP blocker (ACD catalog number: 323120) was
1235 applied and incubated for 15 min in the ACD hybridization oven at 40ºC. This procedure was repeated for
1236 probes C2 and C3 as needed using HRPC2 and HRPC3 respectively. Sections were mounted in DAPI
1237 southern biotech mounting media (Cat. No.0100-20), air dried, and imaged or stored at 4ºC in the dark. For
1238 quantification of labeled cells in nerve tissue sections, a field of view (FoV) was defined, 200µm X 500µm at
1239 the injury site and the distal nerve. The FoV in the distal nerve was 2000 µm away from the injury site. The
1240 number of labeled cells per FoV was counted. Only labeled cells with a clearly identifiable nucleus were
1241 included in the analysis. The number of cells counted per FoV was from n= 2 mice and n= 2 technical
1242 replicates per mouse.
1243
1244 **Preparation of single-cell suspensions for flow cytometry and scRNAseq**
1245 Mice were euthanized and transcardially perfused with ice-cold PBS to reduce sample contamination with
1246 circulating leukocytes. Sciatic nerve trunks from naïve and injured mice were harvested. For injured mice, a
1247 segment was collected that includes the injury site and distal nerve just before to the trifurcation of the tibial,
1248 sural, and peroneal nerves. Nerves were placed in ice-cold PBS containing actinomycin D [45 µM, Sigma
1249 Aldrich, A1410]. Some nerves were further dissected into 3 mm segments, either encompassing the site of
1250 nerve injury, ~1.5 mm proximal to ~1.5 mm distal of the crush site, or distal nerve, located between +1.5 to
1251 +4.5 mm away from the crush site. Nerves from 3 mice (6 mm segments) or 5-6 mice (3 mm segments) were
1252 pooled for each biological replicate. Nerves were minced with fine scissors and incubated in 1 ml PBS
1253 supplemented with collagenase (4 mg/ml Worthington Biochemical, LS004176) and dispase (2 mg/ml, Sigma-
1254 Aldrich, D4693) and incubated for 30–45 min at 37°C in a 15-mL conical tube. For scRNAseq, the digestion
1255 buffer also contained actinomycin D (45 µM). Nerves were triturated 20x with a 1000 µl pipette every 10 min
1256 and gently agitated every 5 min. Next, nerves were rinsed in DMEM with 10% FBS, spun down at 650 g for 5
1257 min, the resulting pellet resuspended, and fractionated in a 30% Percoll gradient. For flow cytometry, the cell
1258 fraction was collected and filtered through a pre-washed 40 µm Falcon filter (Corning, 352340) and cells were
1259 pelleted at 650 g for 5 min at 4°C. Immune cell populations were identified with established antibody panels as
1260 described (Kalinski et al., 2020). For scRNAseq, the cell suspension was cleared of myelin debris with myelin
1261 removal beads (Miltenyi, 130-096-733), and cells resuspended in Hanks balanced salt solution (Gibo,
1262 14025092) supplemented with 0.04% BSA (Fisher Scientific, BP1600). To enrich for immune cells in nerve
1263 specimens or from peripheral blood, some samples were run over an anti-CD45 or anti-CD11b column
1264 (Miltenyi, 130-052-301). Cells were counted and live/dead ratio determined using propidium iodine staining and
1265 a hemocytometer (Kalinski et al., 2020). Blood was collected from adult naïve mice by cardiac puncture and
1266 collected into K2 EDTA coated tubes (BD 365974) to prevent coagulation. Approximately 500 µl of blood was
1267 passed through a 70 µm cell strainer in 5 ml ACK (Ammonium-Chloride-Potassium) lysis buffer. Blood was
1268 incubated at room temperature for 5 min and erythrocyte lysis stopped by addition of 15 mL FACS buffer,
1269 followed by leukocyte spin down in a clinical centrifuge. This process was repeated 3 times for complete
1270 erythrocyte lysis.

1271

1272 **Barcoding and library preparation**

1273 The Chromium Next GEM Single Cell 3' Reagent kit v3.1 (Dual Index) was used. Barcoding and library
1274 preparation was performed following the manufacturer's protocols. Briefly, to generate single-cell gel-bead-in-
1275 emulsion (GEMs) solution, approximately 15,000 cells, in a final volume of 43 µl, were loaded on a Next GEM
1276 Chip G (10x Genomics) and processed with the 10x Genomics Chromium Controller. Reverse transcription
1277 was performed as follows: 53°C for 45 minutes and 85°C for 5 minutes in a Veriti Therml Cycler (Applied
1278 Biosystems). Next, first strand cDNA was cleaned with DynaBeads MyOne SILANE (10x Genomics,
1279 2000048). The amplified cDNA, intermedium products, and final libraries were prepared and cleaned with
1280 SPRIselect Regent kit (Beckman Coulter, B23318). A small aliquot of each library was used for quality control
1281 to determine fragment size distribution and DNA concentration, using a bioanalyzer. Libraries were pooled for
1282 sequencing with a NovaSeq 6000 (Illumina) at an estimated depth of 50,000 reads per cell, yielding 11.3 billion
1283 reads. Novaseq control software version 1.6 and Real Time Analysis (RTA) software 3.4.4. were used to
1284 generate binary base call (BCL) formatted files.

1285

1286 **Data availability**

1287 All scRNA-seq datasets are available online in the Gene Expression Omnibus (GEO) database (GSE198582).

1288

1289 **scRNAseq data analysis**

1290 Raw scRNAseq data were processed using the 10x Genomics CellRanger software version 3.1.0. The
1291 CellRanger "mkfastq" function was used for de-multiplexing and generating FASTQ files from raw BCL. The
1292 CellRanger "count" function with default settings was used with the mm10 reference supplied by 10x
1293 Genomics, to align reads and generate single cell feature counts. CellRanger filtered cells and counts were
1294 used for downstream analysis in Seurat version 4.0.5 implemented in R version 4.1.2. Cells were excluded if
1295 they had fewer than 500 features, more than 7500, or the mitochondrial content was more than 15%. For each
1296 post-injury time point, reads from multiple samples were integrated and normalized flowing a standard Seurat
1297 SCTransform+CCA integration pipeline (Hafemeister and Satija, 2019). The mitochondrial mapping percentage
1298 was regressed out during the SCTransform normalization step. Principal component analysis was performed
1299 on the top 3000 variable genes and the top 30 principal components were used for downstream analysis. A K-
1300 nearest neighbor graph was produced using Euclidean distances. The Louvain algorithm was used with
1301 resolution set to 0.5 to group cells together. Non-linear dimensional reduction was done using UMAP. The top
1302 100 genes for each cluster, determined by Seurat's FindAllMarkers function and the Wilcoxon Rank Sum test,
1303 were submitted to Qiagen's Ingenuity Pathway Analysis (IPA) software – version 70750971 (Qiagen Inc.,
1304 https://digitalinsights.qiagen.com/IPA) using core analysis of up- and down-regulated expressed genes. Top-
1305 scoring enriched pathways, functions, upstream regulators, and networks for these genes were identified
1306 utilizing the algorithms developed for Qiagen IPA software (Kramer et al., 2014), based on Qiagen's IPA
1307 database of differentially expressed genes.

1308

1309 **Comparative analysis of cell identities at different post-injury time points**
1310 Comparison of cell identities between time points was done using the Seurat technique for classifying Cell
1311 Types from an integrated reference. This technique projects the PCA structure of the reference time point onto
1312 the query time point. This is similar to Seurat's implementation of Canonical Correlation Analysis (CCA) in that
1313 it creates anchors between the two data sets, but it stops short of modifying the expression values of the
1314 query. The output of this technique is a matrix with predicted IDs and a prediction score between 0 and 1. For
1315 each reference cell type we used the geometric mean of prediction scores for cells predicted with that type in
1316 the query set. This single prediction score was used as a surrogate for confidence in the same cell state
1317 existing in the query cells. Alternatively, using the overlapping, top 3,000, highly variable genes from each
1318 dataset, we computed the Pearson correlation between the genes' average expression (log2 of uncorrelated
1319 "RNA" assay counts) from each reference cell type and its predicted query cells. As reported in Results, we
1320 projected 1dpc cell types onto 3dpc as well as 3dpc onto 7dpc.
1321
1322 **Cell Chat**
1323 CellChat version 1.1.3, with its native database, was used to analyze cell-cell communication (Jin et al., 2021)
1324 **(**Suoqin Jin et al., https://doi.org/10.1038/s41467-021-21246-9)
1325 A truncated mean of 25% was used for calculating average mean expression, meaning a gene was considered
1326 to have no expression in a cell type if fewer than 25% of cells in that cell type expressed the gene.
1327
1328 **SlingShot**
1329 Slingshot version 2.2.1 was used to model trajectories in the integrated myeloid dataset. The PCA embeddings
1330 from the first 4 principal components were used as input and the beginning of the trajectory anchored at the
1331 Monocytes. The Mo to Mac and Mo to MoDC trajectories were selected as interesting. To predict the genes
1332 that contribute most to pseudotime we used the tidymodels R package (Hadley Wickham and Max Kuhn
1333 www.tidymodels.org); specifically, regression models in random forests with 200 predictors (genes) randomly
1334 samples at each split and 1,400 trees. The Impurity method was used to calculate a genes importance in
1335 predicting the pseudotime. Heatmaps of the top 30 genes by importance were used to visually examine how
1336 the gene changes in cells ordered by their pseudotime.
1337
1338 **Code Availability**
1339 iSNAT, an interactive web application was generated with the RStudio's shiny package
1340 (http://github.com/rstudio/shiny). The Dashboard format was generously supplied by the RStudio group
1341 https://rstudio.github.io/shinydashboard/ The code for all analysis and Rshiny server is available from
1342 GitHub(https://github.com/GigerLab/iSNAT) --Not yet populated).
1343
1344 **Protein analysis**
1345 For protein analysis, mice (naïve, and at 1, 3 and 7dpc) were euthanized and transcardially perfused with ice-
1346 cold PBS for 5 minutes. Naïve and injured sciatic nerves were dissected, with nerves from 3 mice pooled per

1347 time point in ice-cold PBS with 1% protease inhibitor cocktail (Sigma-Aldrich, P8340). Samples were minced,
1348 homogenized in 1% Triton X-100 (Sigma-Aldrich, T8787). Samples were frozen at -80ºC, thawed, centrifuged
1349 at 10,000 x g for 5 min to pellet cellular debris. The protein concentrations of the nerve supernatants and
1350 serum were determined using a BCA protein concentration assay. Western blot analysis of sciatic nerve tissue
1351 was carried out as described previously (Kalinski et al., 2020). For some WT and *Sarm1-/-* mice, injured nerves
1352 were divided into proximal, injury site, and distal segments. For the proteome Profiler Mouse XL Cytokine
1353 array, 200 µg of protein was applied to each ELISA membrane and developed according to the manufacturer's
1354 instructions (Proteome Profiler Mouse XL Cytokine Kit, ARY028, R&D Systems, Minneapolis, MN, USA).
1355 Cytokine array signals were detected by X-ray film, scanned, and quantified using LI-COR Image Studio
1356 software – version 5.2.5. Cytokine signals (pixel-density value) from duplicate spots were averaged then
1357 normalized to the reference spots in the upper right and left corners and the lower left corner on the
1358 membrane. Representative images of array membranes are shown (n = 1-2 biological replicates per condition).
1359

1360 **qRT-PCR**

1361 Quantitative PCR (qPCR) was carried out in triplicate with SYBR Green Fluorescein Master Mix (Thermo
1362 Scientific Cat. No. 4364344) on a QuantStudio 3 real-time PCR system (Applied Biosystems Cat. No A28567).
1363 The ΔΔCt method was used to determine the relative expression of mRNAs, which was normalized to *Rlp13a*
1364 mRNA levels. Mice were transcardially perfused with ice-cold PBS, sciatic nerve trunks harvested, and
1365 collected in an RNase free 1.5 mL Eppendorf tube in 1 ml TRIzol solution (Thermofisher Cat. No. 15596026).
1366 Nerves were minced into small pieces with RNase free spring scissors, homogenized using a pestle motor
1367 mixer (RPI, 299200) and frozen at -80ºC overnight. The next day, specimens were thawed, placed on ice, and
1368 0.2 ml of chloroform was added to the TRIzol mix and shaken thoroughly for 5 min. Samples were centrifuged
1369 at 12000 x g, 4ºC for 15 min in a tabletop centrifuge. The aqueous phase was removed and placed in a new
1370 Eppendorf tube. Glycogen (2 µl) and isopropanol (0.5 ml) were added to the aqueous solution and mildly
1371 shaken with a shaker at 4ºC for 10 mins. Samples were centrifuged at 12000 x g at 4ºC for 10 min to
1372 precipitate total RNA. The supernatant was discarded, and the pellet resuspended in 1 ml 75% ethanol,
1373 vortexed briefly and then centrifuged 4ºC for 5 mins at 7500 x g. The supernatant was discarded, the pellet
1374 airdried for 1-2 h, and resuspended in 20 µl of RNase free water. RNA yield was quantified with a nanodrop
1375 (Thermo Scientific, Nanodrop One), RNA aliquoted, and stored at -80ºC.

1376 RNA was reverse transcribed into first stand cDNA using the Invitrogen SuperScript™ III First-Strand
1377 Synthesis System kit (Cat. No. 18080051). Briefly, to 250 ng of total RNA, 1 µl of 50 µM oligo(dT)20 primer, 1
1378 µl of 10 mM dNTP mix were added and the final volume adjusted to 10 µl using RNase free water. The RNA
1379 mix was incubated at 65ºC for 5 min and quickly placed on ice for at least 5 mins. In a separate tube, a master
1380 mix was prepared containing 2 µl of 10x RT buffer, 4 µl of 25 mM $MgCl_2$ and 2 µl of 0.1M DTT. 8 µl of the
1381 master mix was added to 10 µl of RNA mix with 1 µl of SuperScript III reverse transcriptase and 1 µl of
1382 RNaseOut enzyme. The reverse transcription reaction (final volume 20 µl) was carried out at 50ºC for 50 min,
1383 followed by a 5 min incubation at 85ºC for 5 mins. RNase H (1 µl) was added to the first stand cDNA and
1384 incubated at 37ºC for 20 mins. First strand cDNA was quantified using a Nanodrop One and stored in aliquots

at -20ºC. The cDNA was diluted to 50 ng/ul and used for qPCR in a 96 well plate format. SYBR Green Fluorescein Master Mix 10 µl (Thermo Scientific Cat. No. 4364344), 0.4 µl of forward primer, 0.4 µl of reverse primer, first strand cDNA (50 ng) was mixed, and the final volume adjured to 20 µl per well with milliQ water. The plate was sealed, centrifuged at 2500 x g for 1 min, and then placed in QuantStudio 3 real-time PCR system (Applied Biosystems Cat. No A28567) to run the qPCR. The protocol for running was as follows. Data was exported in Excel format and analyzed in Excel using the ΔΔCt method where the gene control was *Rlp13a* while the reference control was the sciatic nerve in naïve condition.

Statistical Methods

Data are presented as mean ±SEM. Statistical analysis was performed in GraphPad Prism (v7) using paired or unpaired 2-tailed Student's t test, or 1-way or 2-way ANOVA with correction for multiple comparisons with Tukey's post-hoc test, as indicated in the figure legends. A p-value < 0.05 (*) was considered significant. $p<0.01$ (**), $p<0.001$ (***), and $p<0.0001$ (****). Data acquisition and analysis was carried out by individuals blinded to experimental groups.

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Competing interests

Except for Gabriel Corfas, the authors declare no competing financial or non-financial interests. Gabriel Corfas is a scientific founder of Decibel Therapeutics; he has an equity interest in and has received compensation for consulting. The company was not involved in this study.

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