Supplementary Materials: Spinal Cord T-Cell Infiltration in the Rat Spared Nerve Injury Model: A Time Course Study

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Table S1. Genes from qPCR array shown in Figures 4, 5A and S2. Exact *p*-values for genes from qPCR array shown in Figures 4, 5A and S2, *p*-value (Student's *t*-test, compared to control group), in red p < 0.05.

Figure 4										
Symbol	SNI D2	SNI D4	SNI D10	SNI D 21	Sham D2	Sham D4				
Il6	0.2433	0.0039	0.0036	0.0010	0.5957	0.3724				
Il18	0.0016	0.0198	0.0025	0.0071	0.4632	0.4784				
Tnf	0.0170	0.4089	0.8747	0.7619	0.3022	0.1002				
Ccl1	0.3796	0.1863	0.2031	0.0306	0.2854	0.2573				
Ccl22	0.6404	0.2077	0.3666	0.0027	0.6753	0.3053				
Il10	0.3397	0.2279	0.2417	0.0085	0.5318	0.0525				
Tlr1	0.0019	0.0009	0.0000	0.0000	0.0311	0.0580				
Tlr2	0.4178	0.0044	0.0003	0.0004	0.9363	0.2020				
Tlr6	0.2838	0.0454	0.0180	0.0154	0.9007	0.4232				
Tlr7	0.0017	0.0007	0.0000	0.0000	0.4632	0.3094				
Ly96	0.1382	0.0430	0.0032	0.0012	0.9342	0.1205				
Myd88	0.1895	0.0174	0.0018	0.0099	0.4420	0.3298				
Nfkb1	0.9153	0.3075	0.0735	0.0345	0.5872	0.3078				
C3	0.8378	0.0017	0.0004	0.0001	0.5649	0.2429				
C3ar1	0.0448	0.0612	0.0187	0.0352	0.5166	0.3245				
C4b	0.1637	0.0040	0.0052	0.0001	0.9588	0.3336				
Cd68	0.2881	0.0029	0.0000	0.0031	0.1039	0.0952				
Aif1 (Iba1)	0.0230	0.0041	0.0003	0.0013	0.0400	0.2237				
CX3CR1	0.0492	0.0008	0.0003	0.0000	0.8152	0.3263				
Figure 5										
Ccl12	0.0129	0.3837	0.1046	0.1302	0.1134	0.0021				
Ccl22	0.6404	0.2077	0.3666	0.0027	0.6753	0.3053				
Cxcl11	0.0109	0.3697	0.9909	0.8491	0.7270	0.3950				
Figure S2										
Ccl12	0.0129	0.3837	0.1046	0.1302	0.1134	0.0021				
Ccl4	0.0347	0.9222	0.0782	0.1635	0.6786	0.0619				
Cd40	0.0000	0.0517	0.0003	0.0008	0.9316	0.2945				
Cebpb	0.0017	0.1056	0.1136	0.4229	0.3091	0.3591				
Csf1	0.5155	0.2408	0.0925	0.0109	0.5738	0.2444				
Fos	0.2804	0.0502	0.0956	0.0008	0.9424	0.2281				
Il10	0.3397	0.2279	0.2417	0.0085	0.5318	0.0525				
Il18	0.0016	0.0198	0.0025	0.0071	0.4632	0.4784				
Il18rap	0.0037	0.0650	0.0042	0.0000	0.5257	0.9746				
Il1b	0.6978	0.6485	0.1329	0.1768	0.8802	0.9859				
Il1r1	0.1614	0.1969	0.1208	0.0024	0.8188	0.2289				

Table S1. Cont.											
Figure S2											
Il23r	0.7922	0.0153	0.0123	0.0162	0.6401	0.0745					
Il6	0.2433	0.0039	0.0036	0.0010	0.5957	0.3724					
Il6r	0.2254	0.0304	0.0070	0.0363	0.1606	0.8766					
Itgb2	0.0003	0.0160	0.0019	0.0028	0.4380	0.5729					
Ripk2	0.1257	0.2770	0.0265	0.0060	0.1976	0.6640					
Tlr1	0.0019	0.0009	0.0000	0.0000	0.0311	0.0580					
Tlr2	0.4178	0.0044	0.0003	0.0004	0.9363	0.2020					
Tlr3	0.2597	0.1794	0.5503	0.5637	0.5871	0.7104					
Tlr4	0.9409	0.1764	0.0151	0.0023	0.1956	0.2297					
Tlr5	0.7136	0.0198	0.7916	0.2317	0.1417	0.1894					
Tlr6	0.2838	0.0454	0.0180	0.0154	0.9007	0.4232					
Tlr7	0.0017	0.0007	0.0000	0.0000	0.4632	0.3094					
Tnfsf14	0.0285	0.1414	0.0316	0.0366	0.2888	0.4997					



Figure S1. Definition of regions for cell counting.



Figure S2. Gene regulation in the spinal cord following SNI- other upregulated genes. RT-qPCR array with fold change of gene expression in adult male rats at D2, D4, D10 and D21 after SNI compared to naive animals. Values are expressed as mean \pm 95% CI. Dotted line at 1 fold regulation (naive animals). * p < 0.05, ** p < 0.01, *** p < 0.001. Student's *t*-test: Naive (not shown) *vs*. SNI. *N* = 6 for each time point (D2, D4, D10 and D21).

Genes from qPCR array in Sham animals







Chemokines : T-Lymphocytes Attractors



⊂ Sham D2 SNI D2 SM D4 SNI D4

32

16

Microglial Markers

MYD88



Figure S3. Genes from qPCR array in sham animals. RT-qPCR array with fold change of gene expression of adult male rats at D2 and D4 after sham and SNI compared to naive animals (not shown). Values are expressed as mean \pm 95% CI. Dotted line at one fold regulation (naive animals). * p < 0.05, ** p < 0.01, *** p < 0.001. Student's *t*-Test naive versus sham or SNI, N=6 for SNI D2 and D4. N= 4 for sham D2 and D4.

Sham D2

Sham D4

SNI D2

SNI D4

IL-10

NF-kB

Anti-inflammatory Factors

CCL22

Genes interacting with TLRs

Supplementary Materials and Methods: Western Blotting

One week after SNI, rats were terminally anesthetized by an i.p. injection of pentobarbital and then sacrificed by decapitation. Spinal cords were dissected to collect the L4–L5 lumbar enlargement. Following extraction, the protein concentration for each sample was measured by a Bradford protein assay. After migration and transfer onto Immuno-Blot PVDF membranes (Bio-Rad, Germany), blocking was performed in 5% non-fat milk for 5 min at room temperature. Incubation with 1:200 Rabbit anti-CD2 (Santa-Cruz, CL, USA) was done in 4 °C milk overnight.

For detection, membranes were incubated for 30 min at room temperature in horseradish peroxidase-conjugated secondary antibody (1:10,000 goat anti-rabbit/HRP, Dako, Denmark). Washings were made 5 times between each incubation in tween 0.1% 2× PBS. Signals were revealed using enhanced chemiluminescence (ECL) reagent (West Dura, Pierce, Rockford, IL, USA). For imaging, we used a luminescent image analyzer (LAS-4000, Fujifilm, Japan).



Figure S4. Antibody sensitivity tests. (**A**) Immunochemistry on rat thymus with (**left**), or without (**right**) primary anti-CD8 antibody; (**B**,**C**) Immunofluorescence on neuroma two days after SNI with green-labeled CD2 or CD8 T-cell marker (**left**), and control without primary antibody (**right**). Small cells consistent with a lymphocytic morphology are targeted; (**D**) Western blot analysis seven days after SNI of thymus protein extract with anti-CD2 antibody, showing a single band around 45 kDa. Scale bar represents 100 μm.



Figure S5. Time course of CD8-positive T-cell distribution in the lumbar spinal cord. (**A**–**D**): Bar histograms showing the number of CD8+ cells detected in sham (white) or SNI (black) animals at days 2, 7, 21 and 42, ipsilateral (hatched) or contralateral (full) to injury. Four regions of the spinal cords were analyzed: (**A**) the dorsal horn (DH); (**B**) the ventral horn (VH); (**C**) the dorsal white matter (DW) and (**D**) the ventral white matter (VW). In several cases, no cells were detected (where no bar is shown). N = 4/groups. Values are expressed as mean \pm 95% CI of cells counted in two 30µm lumbar sections per animal. 2-way ANOVA with Sidak correction. No significant difference was found between sham and SNI.

Supplementary Materials and Methods: IB4 Immunostaining

To assess the part of the spinal cord with afferent injured nerves, a staining with isolectin (Griffonia simplicifolia) B4 FITC conjugated has been performed on D2, D7, D21 and D42 SNI and sham slices. After blocking the slice for half an hour, the IB4 was incubated for 2 hours, the slides were then washed in PBS and mounted in mowiol. One of each of the 5 slices in mean was stained, therefore, scanning the area of interest.



Figure S6. IB4 staining on D42 Sham and SNI rat dorsal horn. The Griffonia (Bandeiraea) simplicifolia (isolectin B4) staining which shows the typical loss of staining in the superficial laminae of the dorsal horn after peripheral nerve injury in the ipsilateral side (**bottom left**). Scale bar represents 50 µm.