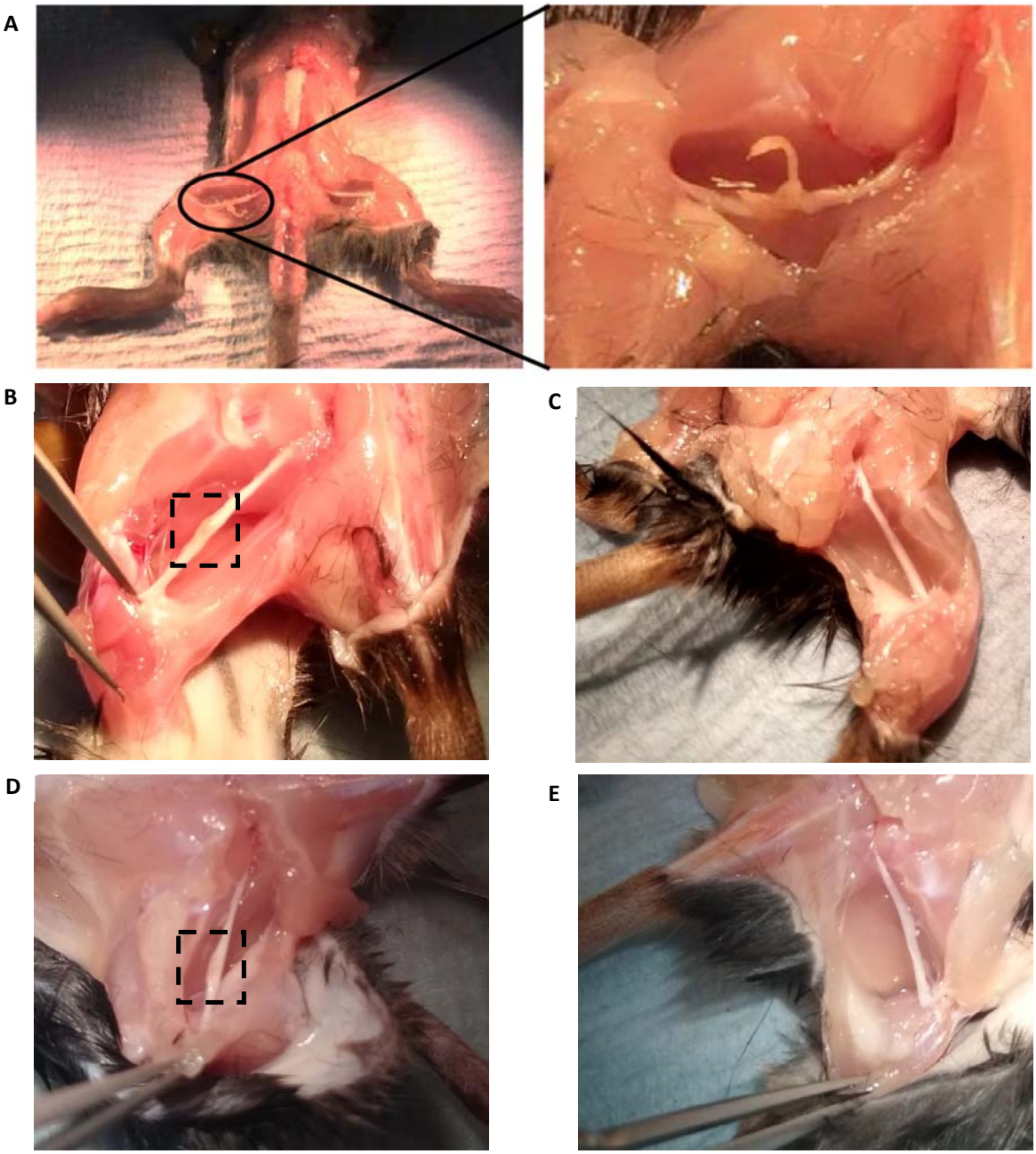
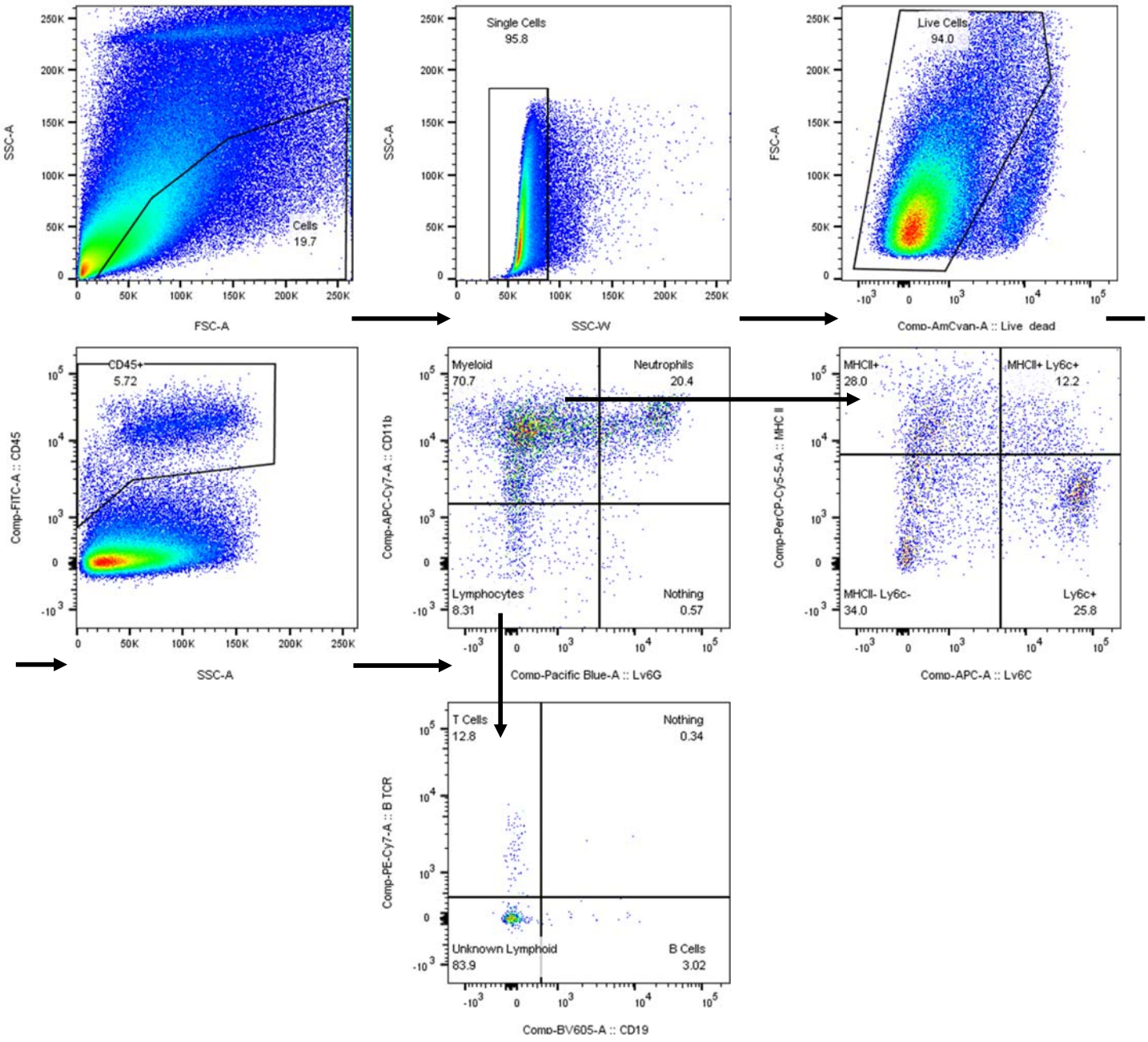
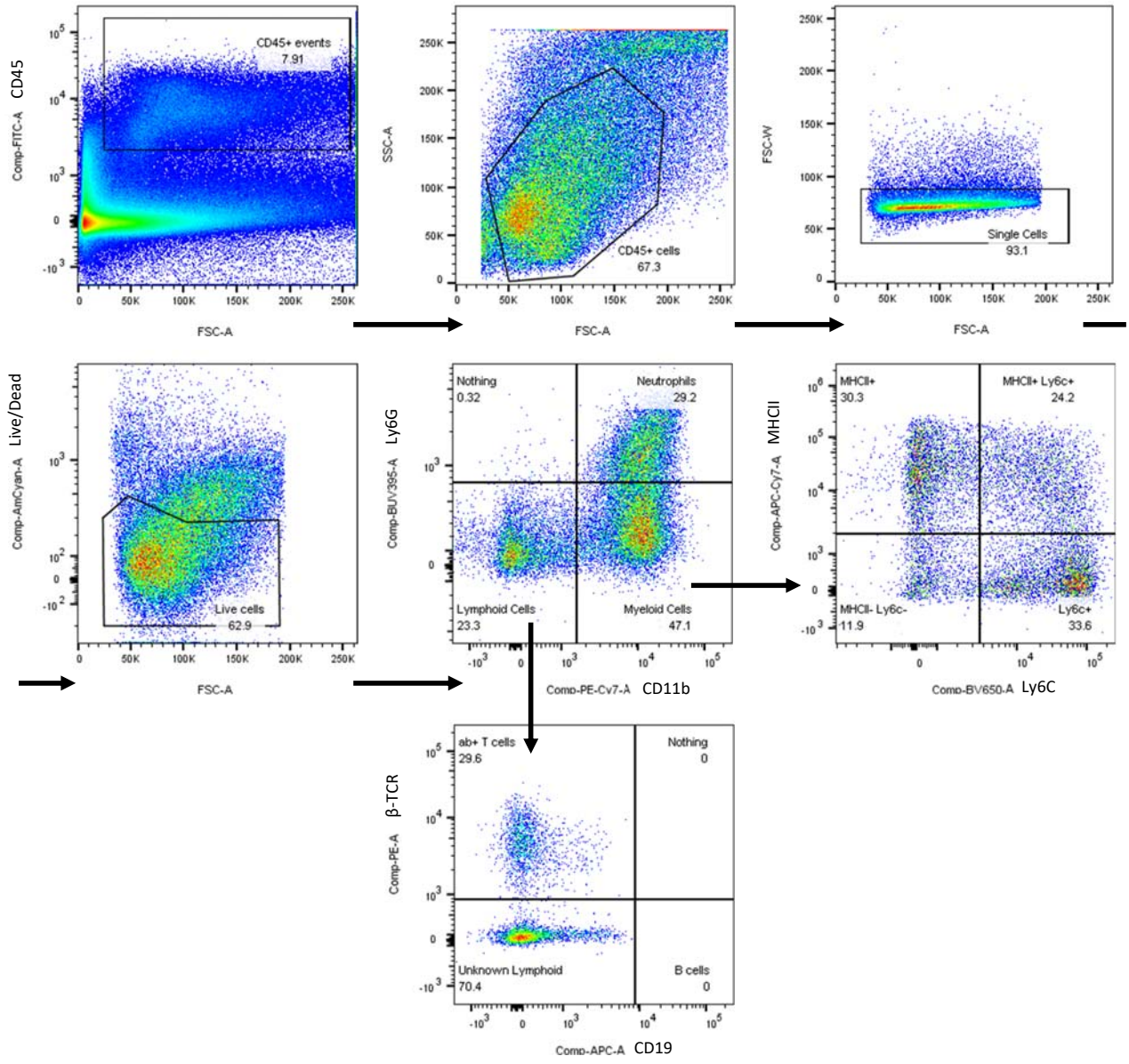


Supplementary Figure 1: 2-3 months after partial sciatic nerve ligation, the suture has been completely absorbed.
Representative images of a mouse sciatic nerve after PSNL.
A) Seven days after surgery, the suture placed through the nerve is clearly visible.
B) 10 weeks after surgery only a small area of swelling of yellowish hue remains visible on the ipsilateral side. The uninjured contralateral side is shown for comparison (**C**).
D) Slight swelling and discoloration is still visible 14 weeks after surgery compared to the contralateral side (**E**).



Supplementary Figure 2: Gating strategy used for first batch of flow cytometry experiments (time points day 1 – day 15).

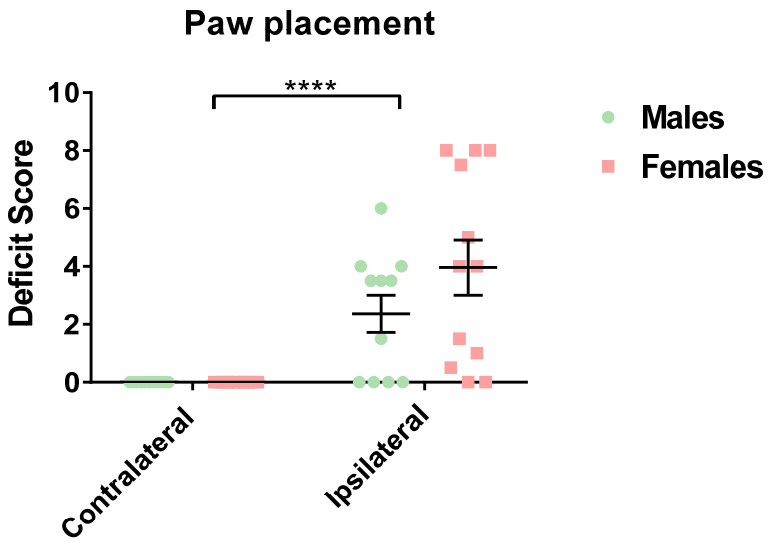
Single live CD45 positive cells were further split into CD11b+/Ly6G-, CD11b+/Ly6G+ and CD11b-/Ly6G- populations. CD11b+/Ly6G- were further split into four populations: MHCII+ single positive (putative antigen presenting macrophages), MHCII-/LyC6- double negative (putative resident macrophages), Ly6C+ single positive cells (likely infiltrating monocytes), and MHCII+/Ly6C+ double positive cells (only present during injury). CD11b-/Ly6G- populations were further split into βTCR expressing T cells and CD19-expressing B cells. Representative gating from an injured ipsilateral nerve sample harvested 8 days following PSNL.
This gating strategy was the first we developed at the start of our series of studies spanning a year and a half of work. It has since evolved, and we recommend that future work employ our second gating strategy (Supplementary Figure 7).



Supplementary Figure 3: Gating strategy used for all other flow and FACS experiments

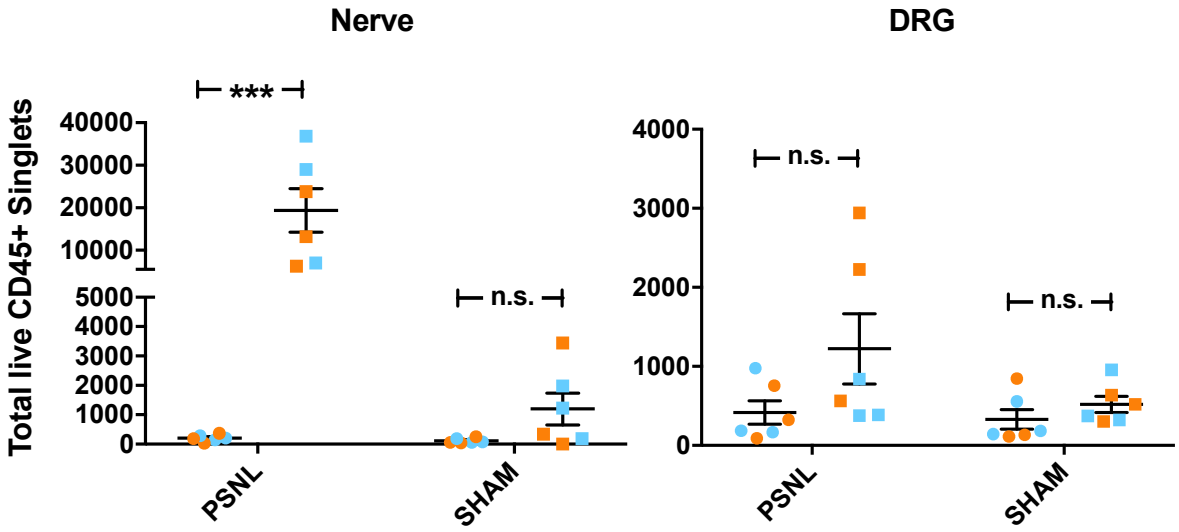
Live single cells were selected from CD45 positive cells, which were then further split into CD11b+/Ly6G-, CD11b+/Ly6G+ and CD11b-/Ly6G- populations. CD11b+/Ly6G- were further split into four populations: MHCII+ single positive (putative antigen presenting macrophages), MHCII-/LyC6- double negative (putative resident macrophages), Ly6C+ single positive cells (likely infiltrating monocytes), and MHCII+/Ly6C+ double positive cells (only present during injury). CD11b-/Ly6G- populations were further split into αβTCR expressing T cells and CD19-expressing B cells. *NB: βTCR and CD19 were omitted from the panel in the 2 vs. 10 week flow and 1 week FACS experiments.* Representative gating from an injured ipsilateral nerve sample harvested 24 hours following PSNL.

We recommend this gating strategy (antibody concentrations provided in Supplementary Table 1C) for future work in this area.



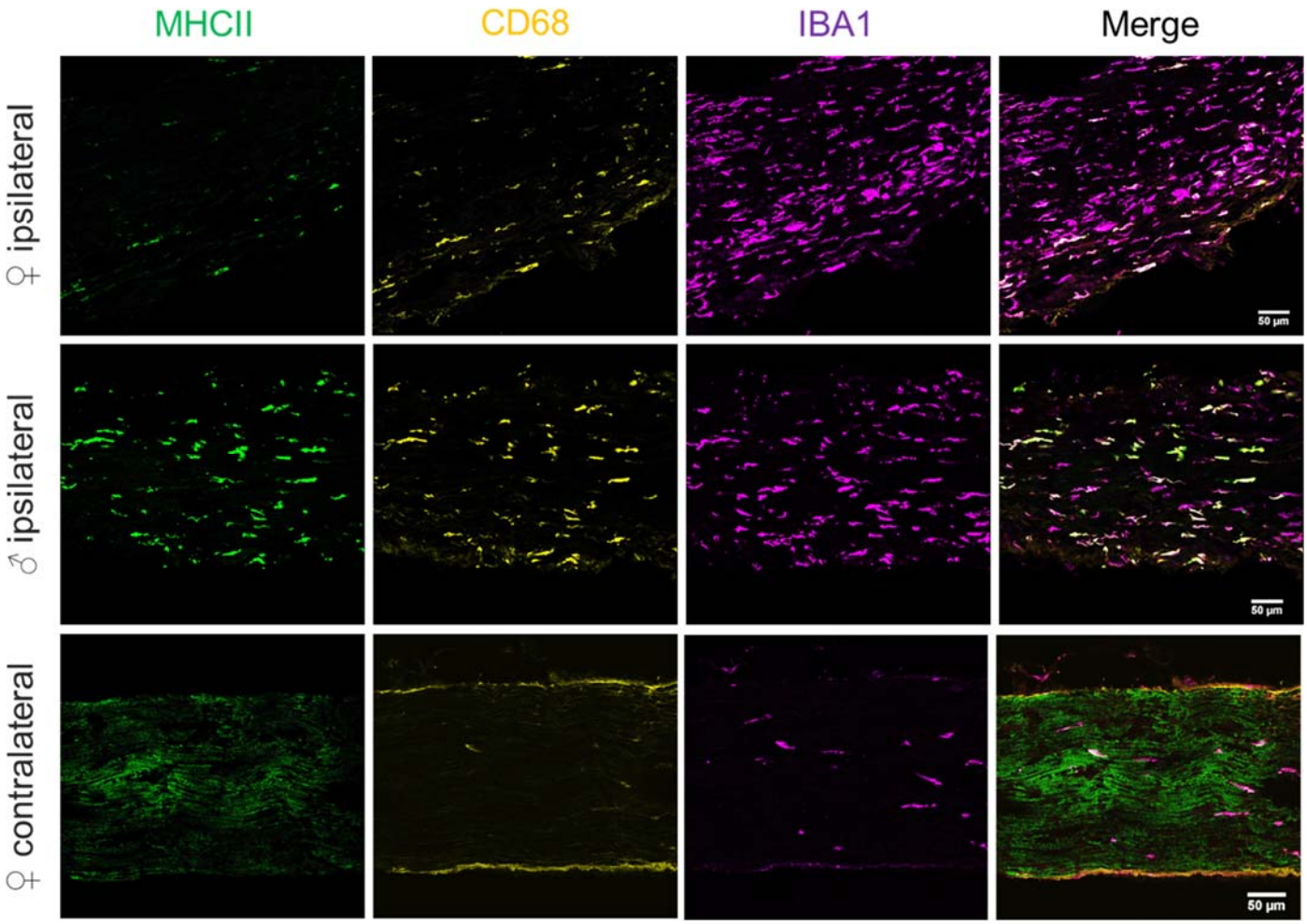
Supplementary Figure 4: 14 weeks post PSNL mice still display behaviours indicating pain in their ipsilateral paw.

Two independent observers unfamiliar with pain models, the specific experimental question and its expected outcomes scored videotaped paw placement of mice in an open field over a period of 2 minutes. A two-way ANOVA revealed a main effect of paw (ipsi- vs. contralateral): $F(1,21) = 28.97$, $p < 0.0001$, which was significant for both male (Sidak post-hoc test, $p = 0.02$) and female ($p = 0.0002$) mice. n = 11 male and n = 12 female mice were tested, with scores for each shown here as separate data points with mean and SEM.



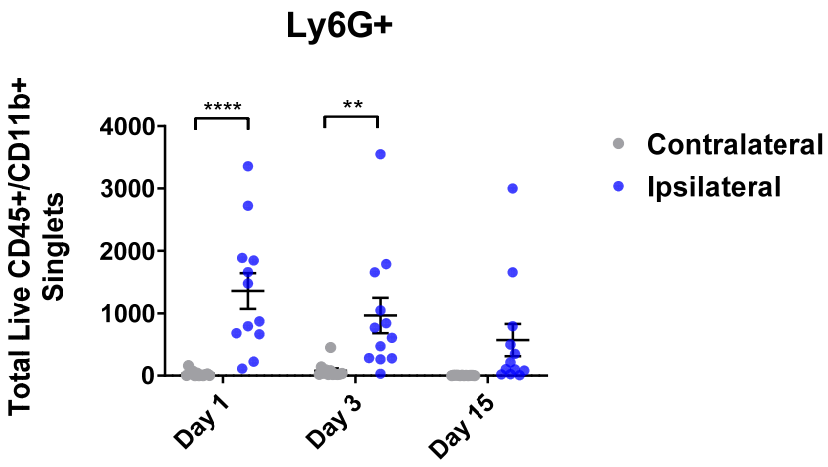
Supplementary Figure 5: Total live CD45+ singlets in nerve and DRG one day after partial sciatic nerve ligation (PSNL) or sham surgery.

Plotted are the total number of live CD45+ immune cells isolated from ipsilateral (squares) and contralateral (circles) sciatic nerve and DRG. n = 6 mice each were processed for PSNL and sham surgery, shown here as separate data points with mean and SEM (3 males in blue, 3 females in orange). In nerve, only PSNL surgery resulted in significantly upregulated immune cell numbers (two-way ANOVA, significant interaction between surgery & paw, $F(1, 20) = 12.48$, $p < 0.0021$, Sidak post-hoc test between ipsilateral and contralateral PSNL numbers, $p = 0.0002$). In DRG, none of the changes were significant.



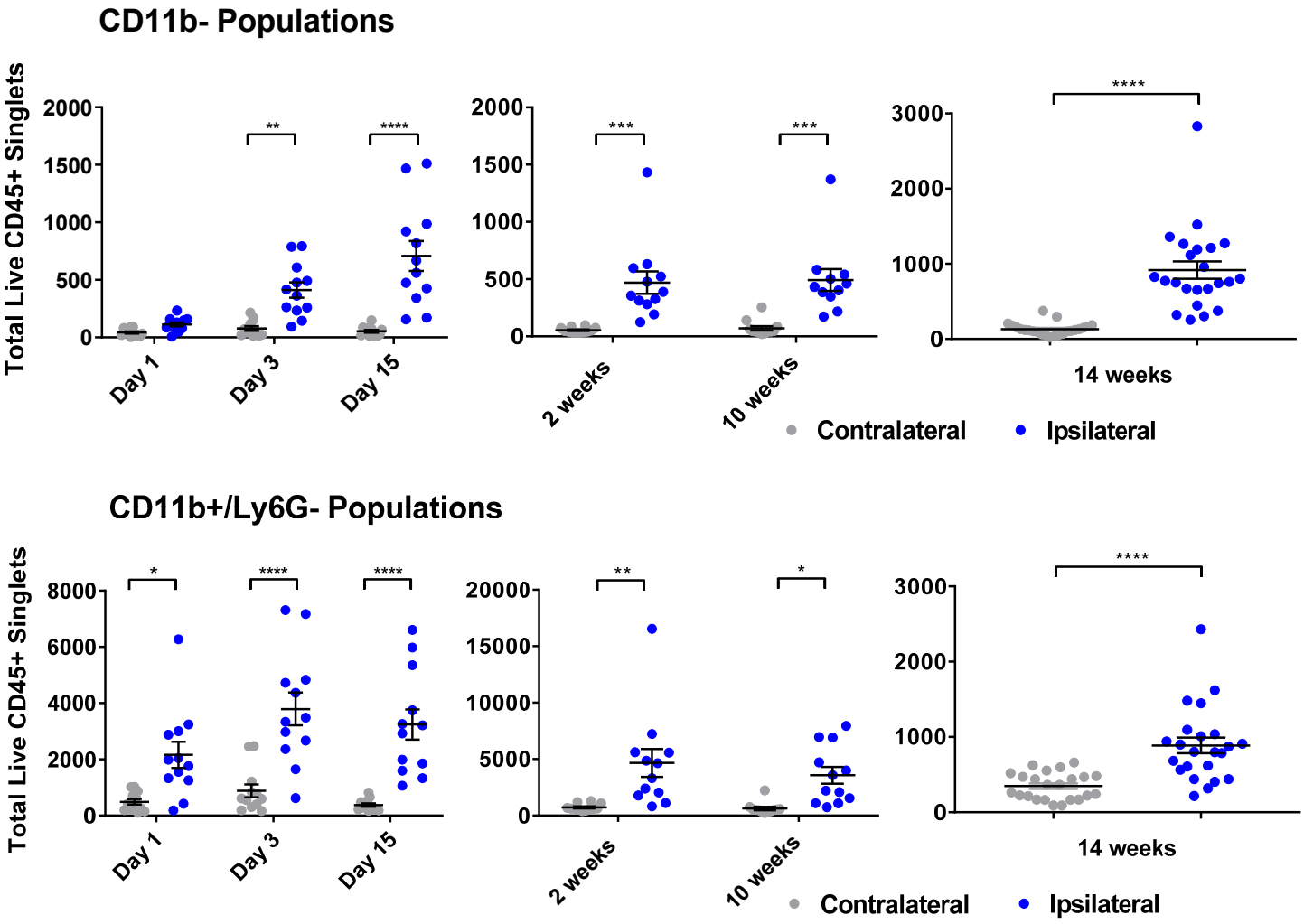
Supplementary Figure 6: Mirroring our flow cytometry data, immunostaining likewise shows increased expression of macrophages and MHCII+ antigen presenting cells at 14 weeks following injury.

Representative images of ipsilateral and contralateral sciatic nerve cryosections from C57B/6J mice of both sexes at 14 weeks post-PSNL surgery. Sections were stained with antibodies against MHCII (green), CD68 (yellow) and IBA1 (purple). Scale bar = 50 µm.



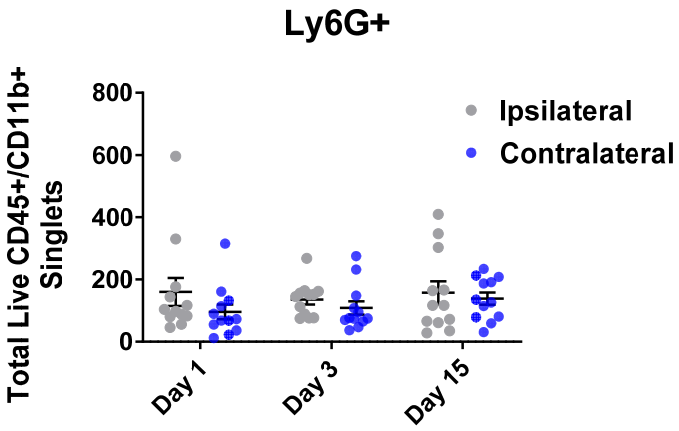
Supplementary Figure 7: Neutrophil numbers in sciatic nerve peak at day 1 after partial sciatic nerve ligation

Plotted are the total number of live neutrophils (CD45+/CD11b+/Ly6G+ singlets) isolated from sciatic nerve after partial sciatic nerve ligation at different time points. Each time, n = 12 mice per time point were processed (6 males & 6 females), shown here as separate data points with mean and SEM. Neutrophils were significantly upregulated one and three days after PSNL (two-way ANOVA, main effect of *injury*, $F(1,66) = 33.47$, $p < 0.0001$, significant at day 1 and day 3, $p < 0.0001$ and $p = 0.0065$, respectively).



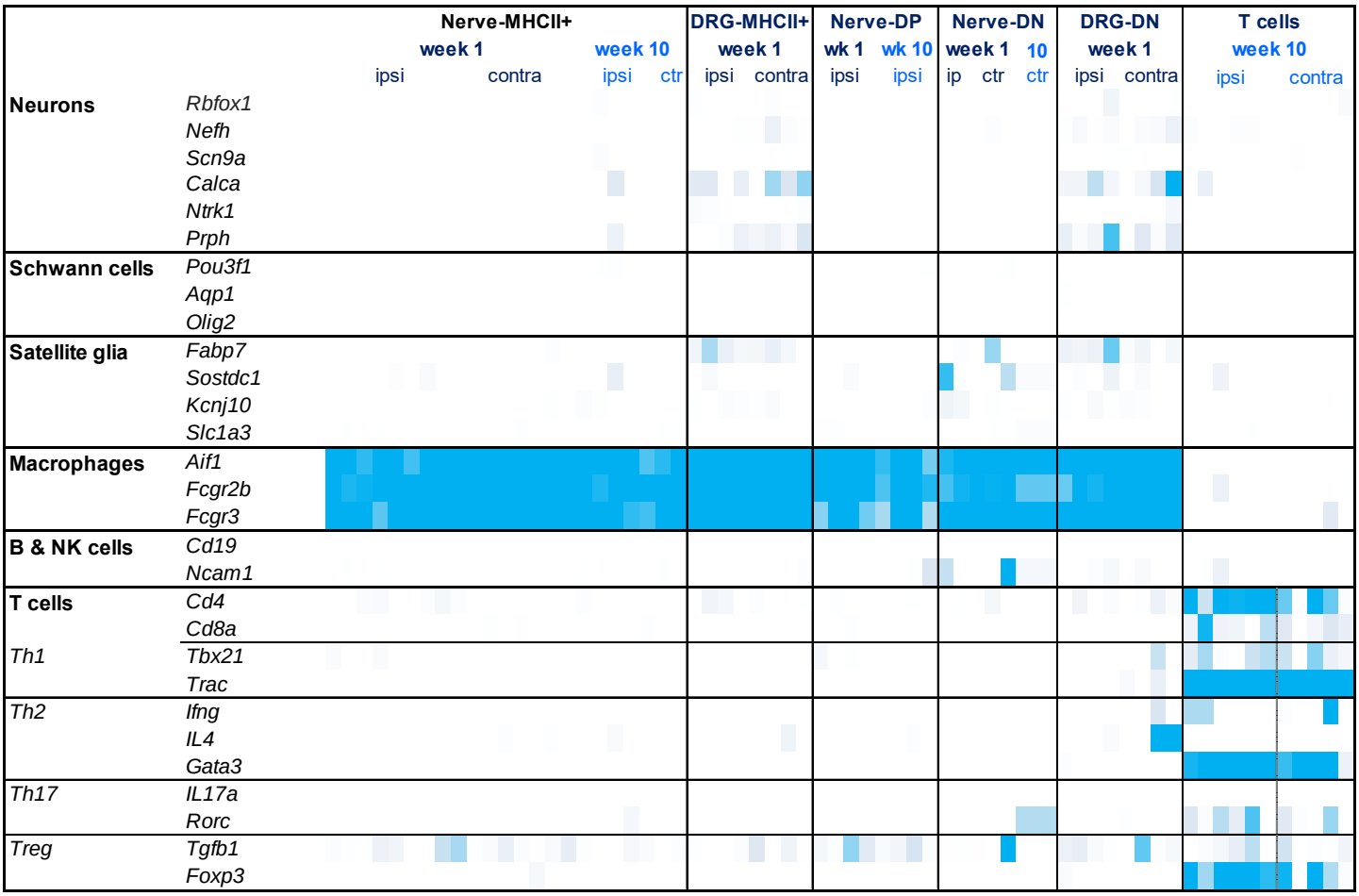
Supplementary Figure 8: Both myeloid and non-myeloid populations are persistently increased in ipsilateral sciatic nerve until at least 14 weeks post PSNL.

CD45+/CD11b- populations (lymphoid and other non-myeloid) are significantly upregulated from day 3 post PSNL-onwards, and remain upregulated at comparable numbers 2 weeks and 10 weeks after injury. 14 weeks after injury, the absolute number of events recorded was even higher (~1000 vs. 500 at 2 & 10 weeks), but given that these experiments were not conducted in the same batch, it is unclear whether this constitutes a biologically significant upregulation over time.
CD45+/CD11b+/Ly6G- myeloid cell populations show a very similar pattern, with significant upregulation from day 1 PSNL which lasts up to 3 and a half months later. The size of the increase is comparable 2 weeks and 10 weeks after injury, but appears lower 14 weeks after injury in absolute terms. Again, we cannot comment on whether this equates to reduction in inflammation, or whether this is due to differences in experimental batches. At each time point, n = 12 mice were processed (6 males & 6 females), shown here as separate data points with mean and SEM.



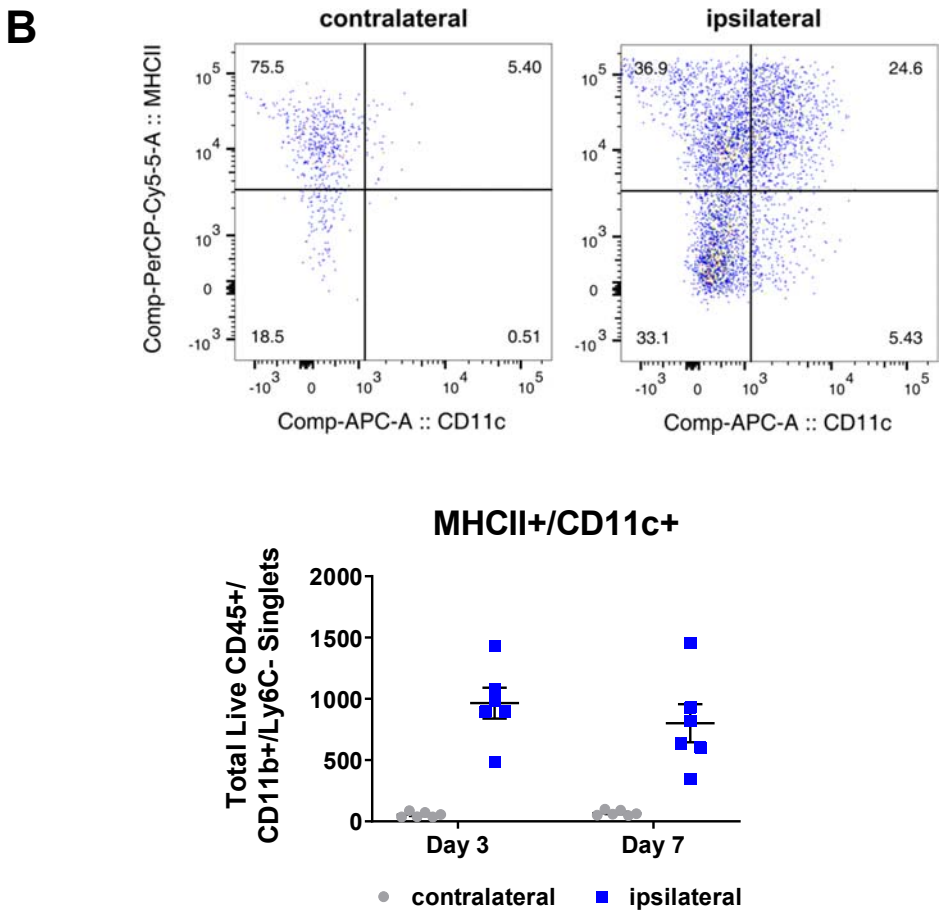
Supplementary Figure 9: DRG contains a permanent CD45+/CD11b+/Ly6G+ population that does not vary with injury.

Plotted are the total number of live neutrophils (CD45+/CD11b+/Ly6G+ singlets) isolated from DRG after partial sciatic nerve ligation at different time points. Each time, n = 12 mice per time point were processed (6 males & 6 females); shown here as separate data points with mean and SEM. No significant differences in live cell numbers positive for these markers were detected in ipsilateral vs. contralateral DRG.



Supplementary Figure 10: TPM expression levels for select marker genes to assess purity of sequenced samples.

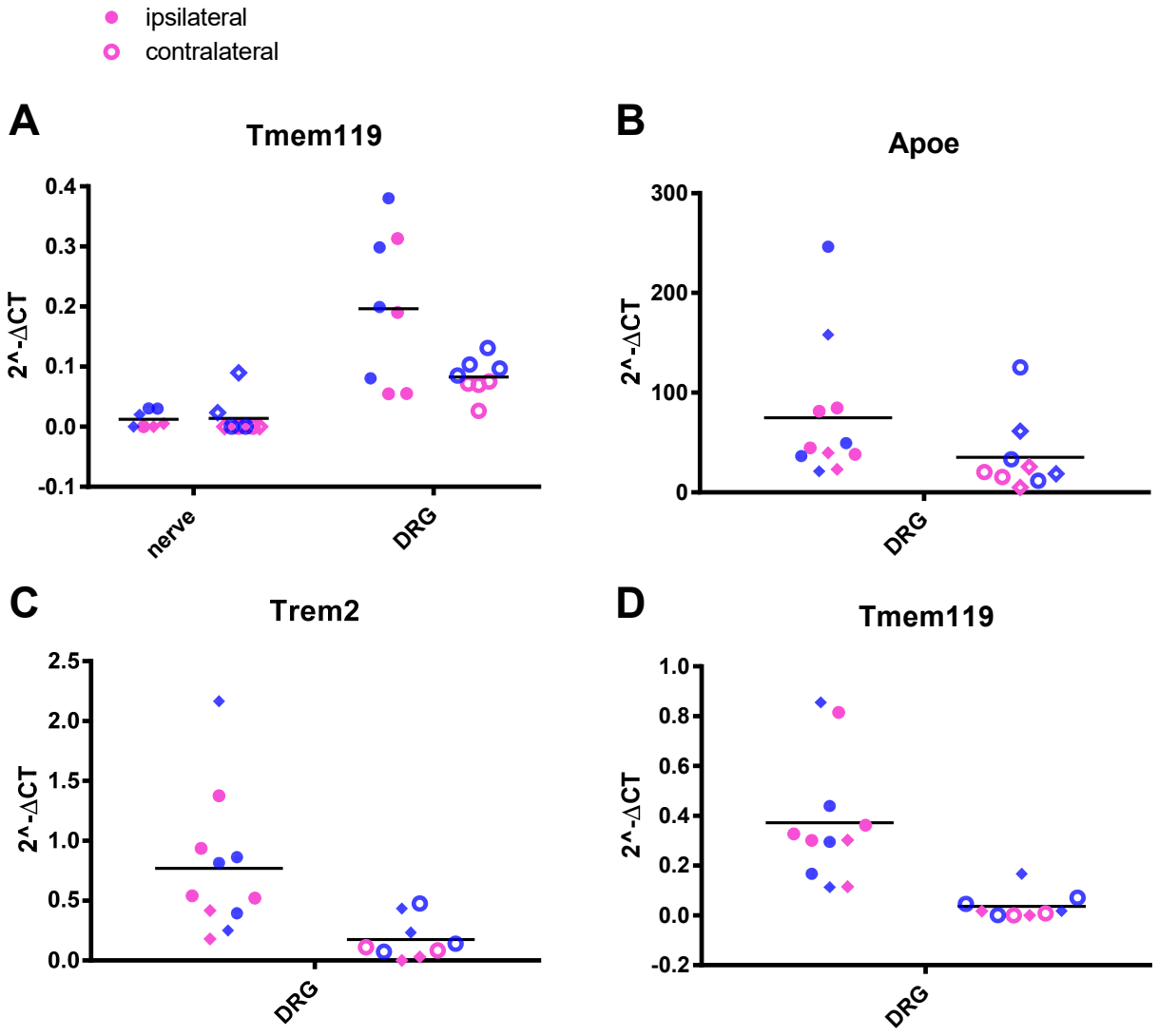
Myeloid and T cell samples from nerve were largely clean, with very little detectable contamination from neurons, Schwann cells and other lymphocytes. Samples from DRG had a small amount of neuronal and satellite glial cell contamination, likely caused by satellite glia “pulling down” the neurons they envelope and being sorted on CD45+, which a small percentage of them is known to express.



Supplementary Figure 11: CD11c is upregulated at protein level in MHCII+/Ly6C- sciatic nerve macrophages seven days after PSNL.

A) Representative dot plots of immune cells extracted from sciatic nerve seven days after PSNL. Live single cells were gated to be CD45+, CD11b+ and Ly6C-. CD11c was significantly upregulated in the MHCII+ population in ipsilateral samples.

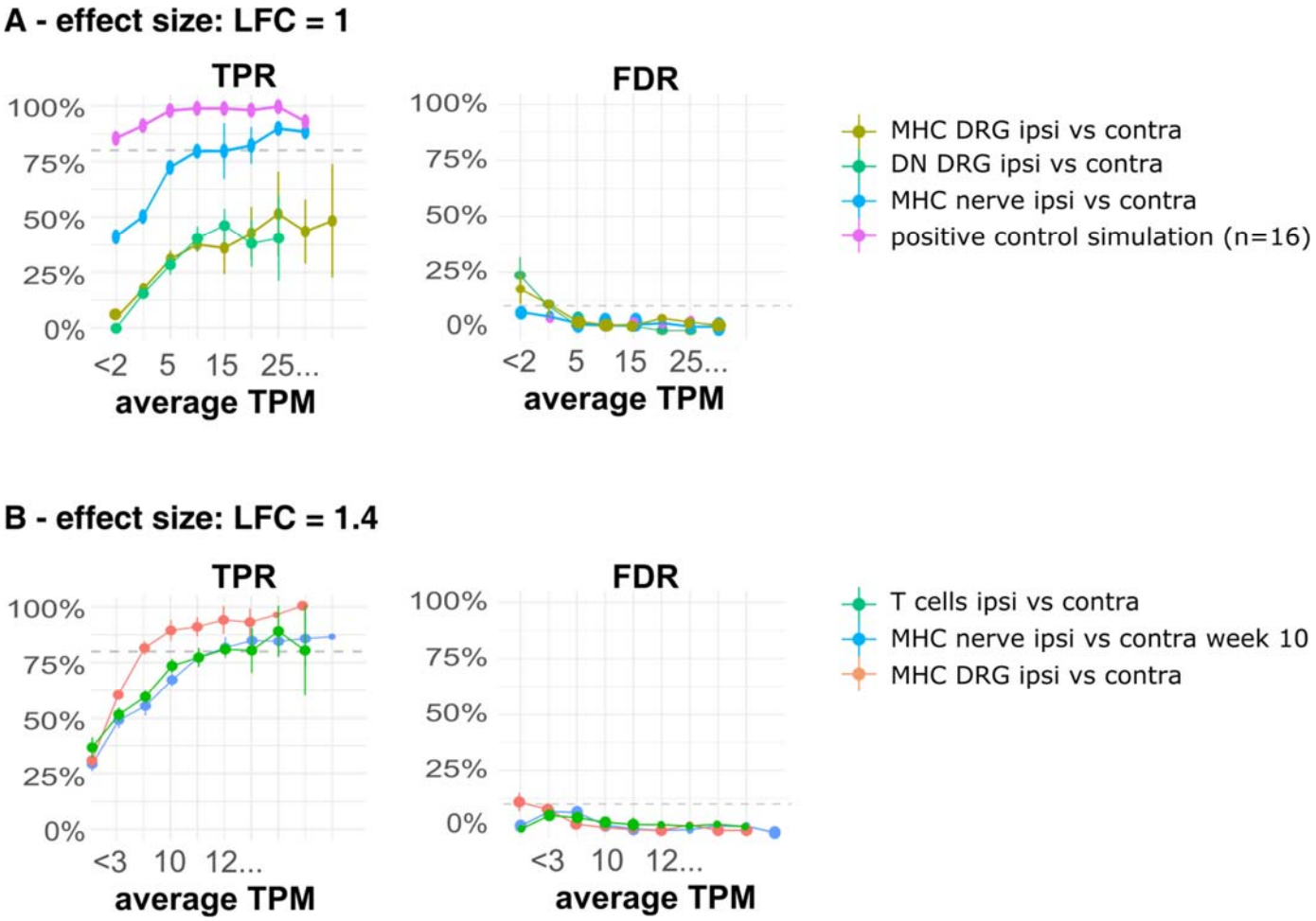
B) Quantification of the number of MHCII+/CD11c+ immune cells (gated on CD45+, CD11b+, Ly6C- live singlets) three and seven days after nerve injury: two-way ANOVA, $F(1,20)=67.78$, $p< 0.0001$. Each dot is an animal (n = 6, 3 male, 3 female) from which immune cells were obtained from ipsi- and contralateral nerves. Lines represent means and SEM.



Supplementary Figure 12: qRT-PCR confirming differences in expression of certain markers between nerve and DRG macrophages.

A) qRT-PCR for *Tmem119* confirmed our sequencing results which indicated increased expression of this gene in MHCII+ macrophages from DRG compared to nerve: a two-way ANOVA revealed a significant main effect of *tissue* $F(1, 27) = 26.4$, $p<0.0001$ and significant interaction between *tissue* x *injury* $F(1, 27) = 5.54$, $p = 0.0261$. Each dot represents the 2^-ΔCT value of a biological replicate (blue = male, pink = female, n = 4). Samples drawn as diamonds were already included in our sequencing, so constitute technical replicates, while samples drawn as circles were independent biological replicates now additionally tested.

B-D) qRT-PCR from MHCII-/Ly6C- macrophages extracted from ipsilateral and contralateral DRG (n = 11,9): two-way ANOVAs with *sex* and *injury* as factors revealed a significant main effect of *injury* for *Trem2* - $F(1, 16) = 8.91$, $p = 0.0088$, and *Tmem119* - $F(1, 16) = 13.95$, $p = 0.0018$, but not *Apoe*, in line with the p value in sleuth only passing false-discovery rate correction at $p < 0.15$ (Suppl. Table 5). Each dot represents the 2^-ΔCT value of a biological replicate (blue = male, pink = female). Samples drawn as diamonds were already included in our sequencing, so constitute technical replicates, while samples drawn as circles were independent biological replicates now additionally tested.



Supplementary Figure 13: Results from powsimR package analyses, which employs our data as a seed to estimate the true positive effects and false discovery rates one would likely encounter with datasets of similar dispersion.

A) The R package powsimR was fed raw count data from week 1 MHCII+ nerve and DRG, as well as MHCII-/Ly6C- (=DN) samples. The package estimates mean-dispersion relationships from these data and uses them as the basis for power analyses. TPR = true positive rate: the lines indicate the % chance to detect an effect size of log fold change (LFC) = 1, if one were to conduct differential expression analysis on data with similar properties, using the DESeq2 algorithm with n = 8 (the number of samples we had for MHCII nerve ipsi vs. contra comparisons), n = 4 (MHC DRG and DN DRG) and n = 16 (picked as a positive control). FDR = false discover rate: the lines indicate the chance of encountering false positives with the same simulation parameters. Data are split according to expression level: average TPM values were estimated from the mean log2 counts that are provided by powsimR.

B) As in A, but this time the simulation was run to enable detection of a much larger effect size: log fold change (LFC) = 1.4. n numbers corresponding to week 10 T cell and MHCII comparisons and week 1 MHC DRG comparisons were used.

A

Laser	Colour	Epitope	Cell Type	Final Dilution	Cat #
Violet	BV605	CD19	B cells	1:300	BioLegend, #128037
	AmCyan	Live/Dead	-	1:1000	Invitrogen, #L34959
	Pacific Blue	Ly6G	Neutrophils	1:300	BioLegend, #127612
Blue	FITC	CD45	Leukocytes	1:300	BioLegend, #103108
	PerCP-Cy5.5	MHCII	Activated macrophages & dendritic cells	1:300	BioLegend, #107625
Yellow	PE-Cy7	αβ -TCR	T cells (αβ chain)	1:300	BioLegend, #101215
Red	APC-Cy7	CD11b	Myeloid lineage	1:300	BioLegend, #107628
	APC	Ly6C	Monocytes	1:300	BioLegend, #152409
-	-	Fc block	CD16/32	1:20	BioLegend, #101302

B

Laser	Colour	Epitope	Cell Type	Final Dilution	Cat #
Violet	Pacific Blue	Ly6G	Neutrophils	1:300	BioLegend, #127612
	AmCyan	Live/Dead	-	1:1000	Invitrogen, #L34959
	BV711	Ly6C	Monocytes	1:2400	BioLegend, #128037
Blue	FITC	CD45	Leukocytes	1:300	BioLegend, #103108
Yellow	PE-Cy7	CD11b	Myeloid lineage	1:1200	BioLegend, #101215
Red	APC-Cy7	MHCII	Activated macrophages & dendritic cells	1:4800	BioLegend, #107628
-	-	Fc block	CD16/32	1:20	BioLegend, #101302

C

Laser	Colour	Epitope	Cell Type	Final Dilution	Cat #
UV	BUV395	Ly6G	Neutrophils	1:300	BD Bioscience, #563978
Violet	AmCyan	Live/Dead	-	1:1000	Invitrogen, #L34959
	BV650	Ly6C	Monocytes	1:1500	BioLegend, #128049
Blue	FITC	CD45	Leukocytes	1:1200	BioLegend, #103108
Yellow	PE-Cy7	CD11b	Myeloid lineage	1:1200	BioLegend, #101215
	PE	αβ -TCR	T cells (αβ chain)	1:300	BioLegend, #109207
Red	APC-Cy7	MHCII	Activated macrophages & dendritic cells	1:1200	BioLegend, #107628
	APC	CD19	B cells	1:300	BioLegend, #115511
-	-	Fc block	CD16/32	1:20	BioLegend, #101302

Supplementary Table 1: Panels used for flow cytometry and FACS experiments

Experiments were conducted over the span of a year and a half, over which our panels evolved. **A)** Original panel used for 1 day to 2 weeks post-PSNL flow cytometry **B)** Updated panel used for 1 week post-PSNL FACS and 2 vs. 10 weeks post-PSNL flow cytometry experiments. **C)** Final panel – our current choice - used for 10 weeks post-PSNL FACS and 14 weeks post-PSNL flow cytometry experiments.