

Supplementary Materials

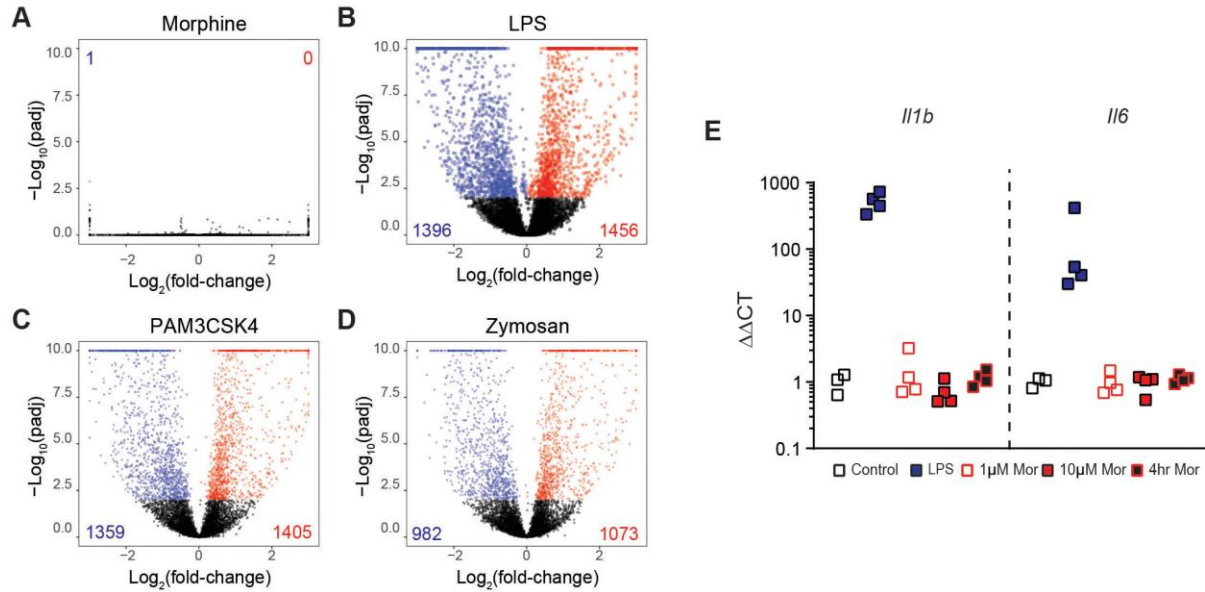


Figure S1: Morphine treatment differs from TLR agonism in cultured microglia.

(A-D) Volcano plots summarizing gene expression changes in cultured rat cortical microglia after 24 hours of treatment with 1 μ M morphine (A), LPS (1 ng/mL) (B), PAM3CSK4 (1 ng/mL) (C), or Zymosan (100 μ g/mL) (D). Blue and red symbols indicate significantly downregulated or upregulated genes, respectively, from DESeq2 ($\text{padj} < 0.01$). The numbers in the lower corners indicate the total numbers of downregulated (blue) and upregulated (red) genes at $\text{padj} < 0.01$.

(E) qPCR for Il1b and Il6 24 hours after LPS (1 ng/mL) or morphine (1 or 10 μ M) exposure and 4 hours after 10 μ M morphine.

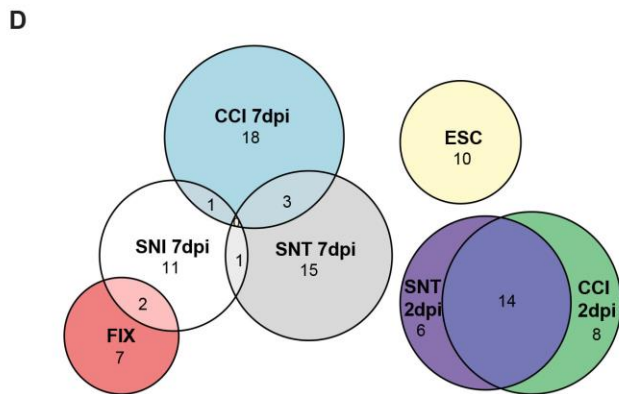
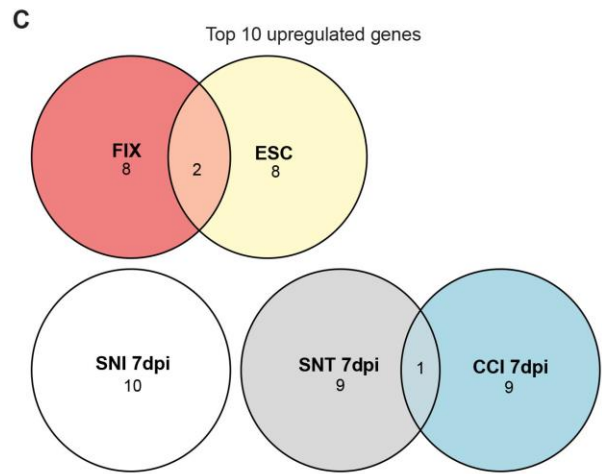
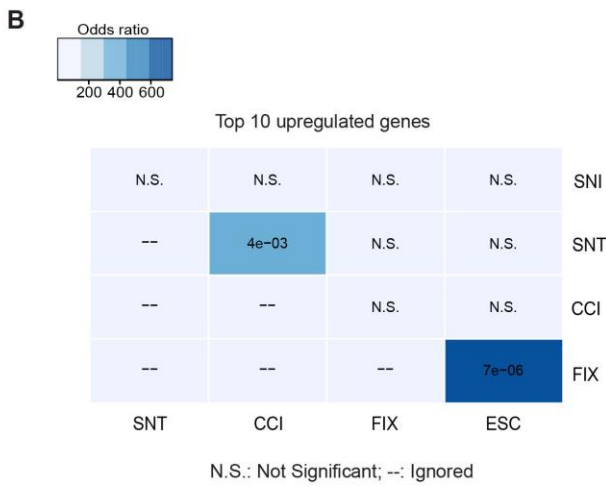
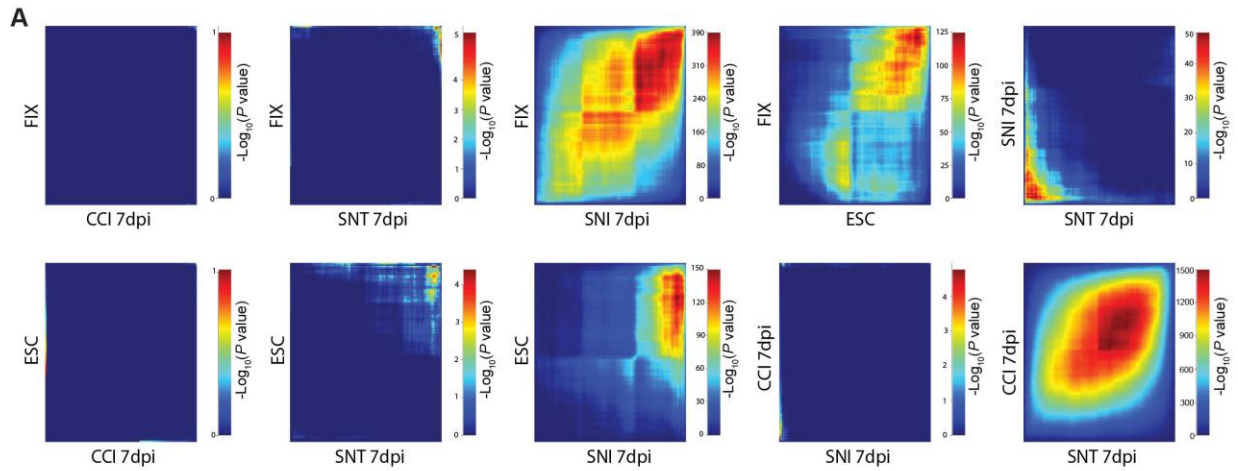


Figure S2: Hypergeometric analysis shows little overlap between injury and morphine conditions.

(A) Heatmaps of the RRHO analysis showing threshold-free comparisons of differentially expressed genes (DEGs) between each condition. Conditions include morphine fixed (FIX), morphine escalating (ESC), CCI 7dpi, SNI 7dpi, and SNT 7dpi. Each pixel represents the overlap between the transcriptome of each comparison as noted, color-coded according to the significance of overlap, $-\log_{10}(P \text{ value})$, of the hypergeometric overlap analysis.

(B) Heatmap of the gene overlap analysis, color-scaled according to odds ratios and including P values when significant overlap is found. Overlap analysis is performed on all comparisons for the top 10 upregulated genes at 7 dpi.

(C) Corresponding Euler plot showing overlap between conditions. Conditions are color-coded. FIX: red, ESC: yellow, SNI: white, SNT: gray, CCI: blue.

(D) Euler plot of the proliferation-associated genes identified for each condition in the top 100 upregulated genes. Conditions are color-coded. FIX: red, ESC: yellow, SNI 7dpi: white, SNT 7dpi: gray, CCI 7dpi: blue, SNT 2dpi: purple, CCI 2dpi: green.

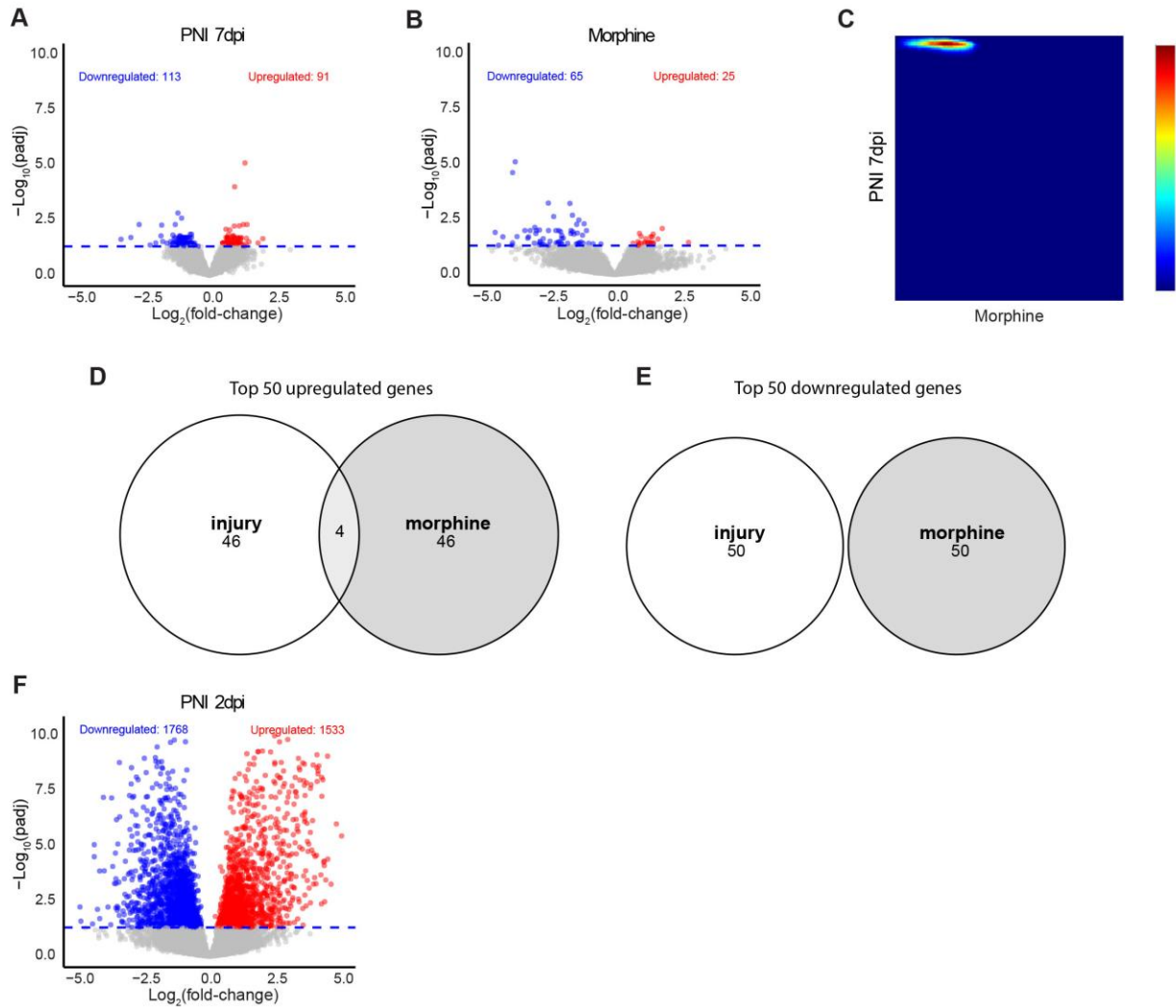


Figure S3: Diverse transcriptional responses of spinal microglia in chronic neuropathic pain versus opioid exposure.

(A) Volcano plots summarizing gene expression changes in microglia 7 days after PNI, with all PNI conditions pooled together. Blue and red dots indicate significantly downregulated or upregulated genes, respectively, from DESeq2 ($\text{padj} < 0.05$). The numbers in the lower corners indicate the total numbers of downregulated (blue) and upregulated (red) genes at $\text{padj} < 0.05$.

(B) Volcano plots summarizing gene expression changes in microglia after morphine exposure, with all morphine conditions pooled together. Blue and red dots indicate significantly downregulated or upregulated genes, respectively, from DESeq2 ($\text{padj} < 0.05$). The numbers in the lower corners indicate the total numbers of downregulated (blue) and upregulated (red) genes at $\text{padj} < 0.05$.

(C) Heatmap of the RRHO analysis showing threshold-free comparisons of DEGs between injury and morphine conditions. Each pixel represents the overlap between the transcriptome of each comparison as noted, color-coded according to the significance of overlap, $-\log_{10}(\text{P value})$, of the hypergeometric analysis.

(D) Euler plot of the top 50 upregulated genes for injury and morphine conditions. Conditions are color-

coded. injury: white, morphine: gray.

(E) Euler plot of the top 50 downregulated genes for injury and morphine conditions. Conditions are color-coded. injury: white, morphine: gray.

(F) Volcano plots summarizing gene expression changes in microglia 2 days after PNI, with all PNI conditions pooled together. Blue and red dots indicate significantly downregulated or upregulated genes, respectively, from DESeq2 ($\text{padj} < 0.05$). The numbers in the lower corners indicate the total numbers of downregulated (blue) and upregulated (red) genes at $\text{padj} < 0.05$.

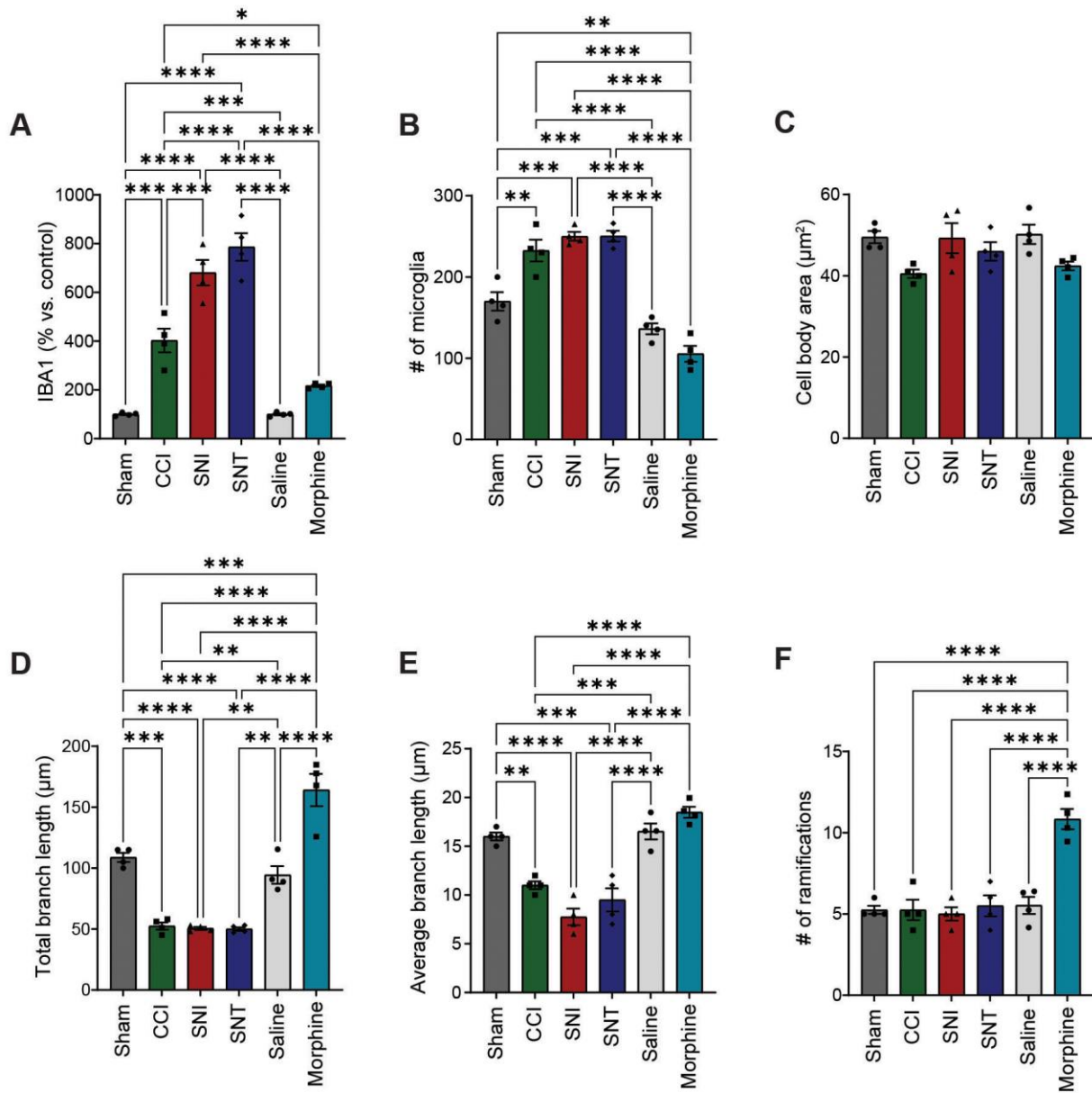


Figure S4: Microglia display different histological and morphological signatures following PNI versus chronic morphine exposure.

(A) Quantification of IBA1 density (% area) in the dorsal horn, normalized as % vs. control. Ipsilateral for PNI conditions, bilateral for the morphine condition. Control = sham for PNI conditions, Control = saline for the morphine condition.

(B) Quantification of the number of microglia in the dorsal horn for each condition.

(C-F) Analysis of microglia morphology, including number of ramifications, microglia body area, total branch length, and average branch length. One-way ANOVA and Holm-Sidak's test (I, N); * indicates $p < 0.05$; ***, $p < 0.001$; ****, $p < 0.0001$ vs. CCI, SNT, SNI, sham, saline, or morphine. Dots: $n = 4$ mice/condition.

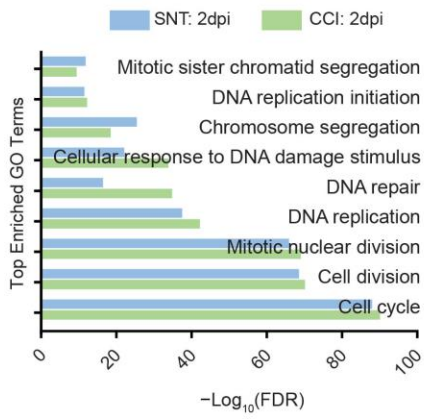
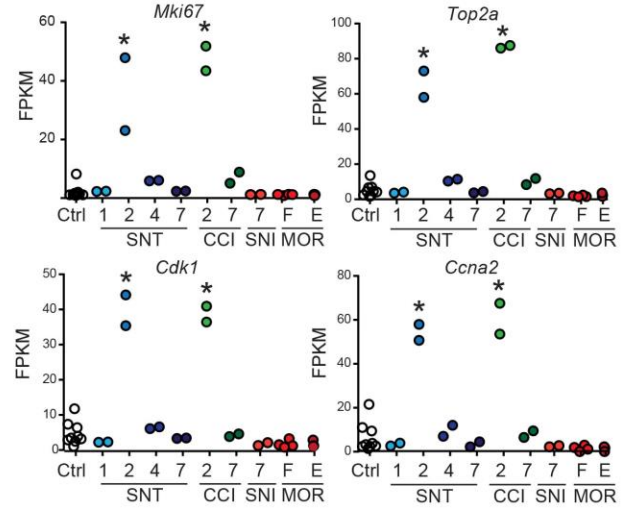
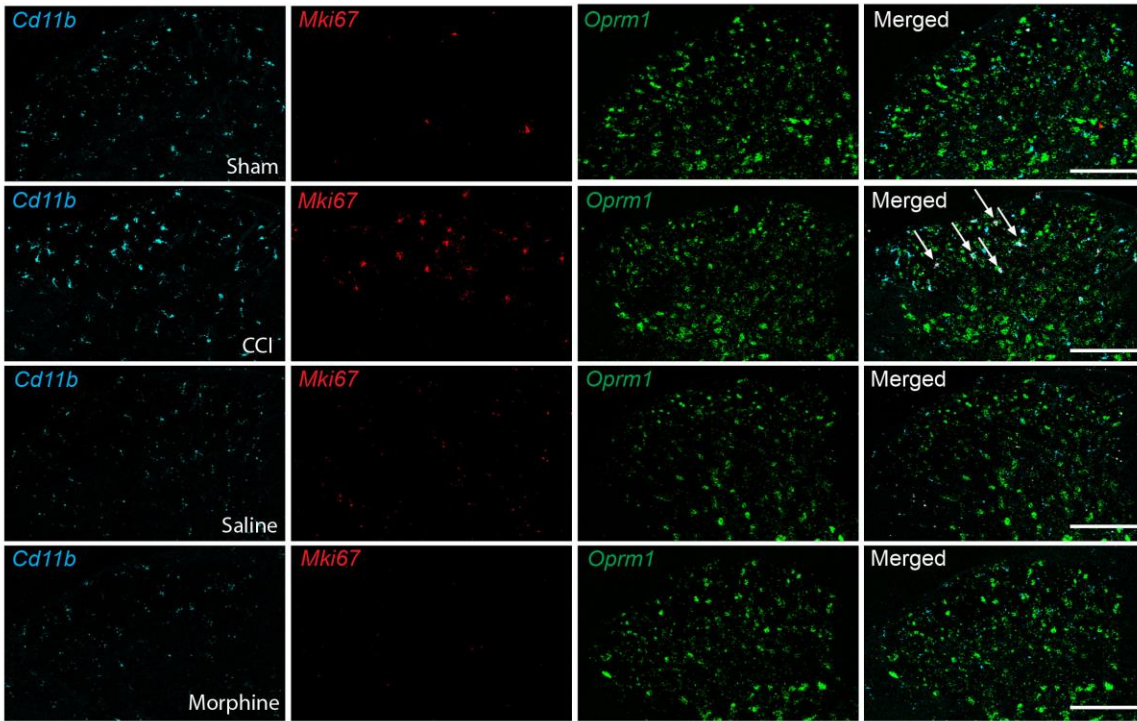
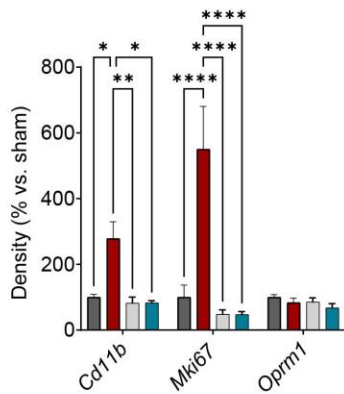
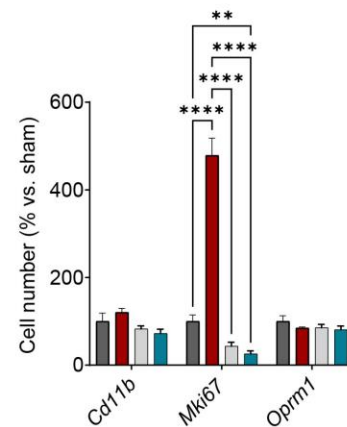
A**B****C****D****E**

Figure S5: Proliferation drives microglial activation following PNI but not morphine treatment.

- (A) Top enriched GO Terms associated with SNT (blue) or CCI (green) at 2 dpi.
- (B) Time course of normalized (FPKM) expression across time and injury for genes associated with proliferation. * Indicates significantly differentially expressed with DESeq2 $p < 0.01$ compared to relevant control groups.
- (C) Example images of spinal cord dorsal horns in situ hybridization 2 days after procedure (CCI or sham) or treatment (escalating morphine or saline). Markers include Cd11b, mKi67, Oprm1 RNAscope probes and a merge image. Scale bars = 200 μm .
- (D) Quantification of hybridization density (% area) for each marker on the ipsilateral side. Normalization was made as % vs. control sham.
- (E) Quantification of cell number positive for each marker. Normalization was made as % vs. control sham. One-way ANOVA and Holm-Sidak's test (B, C); * indicates $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$ vs. sham or saline or morphine. $n = 4$ mice.

gene_id	baseMean	log2FoldC	lfcSE	stat	pvalue	padj
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1110059E2 4Rik	64.681434 8	- 1.0131173 71	0.258024	-3.92645	8.62E-05	0.019748
1600010M 07Rik	48.013624 56	- 1.0668116 92	0.31401	-3.39738	0.00068	0.04093
2210015D1 9Rik	49.733330 71	- 0.7834578 18	0.233917	-3.3493	0.00081	0.044283
2810013P0 6Rik	53.171944 82	- 1.0953316 55	0.33255	-3.29374	0.000989	0.047227
3110056K0 7Rik	42.457238 66	- 1.7579147 81	0.476972	-3.68557	0.000228	0.03008
4933434E2 0Rik	468.44794 07	- 0.6006977 47	0.174151	-3.44929	0.000562	0.038356
6030468B1 9Rik	73.611997 32	- 1.2657547 25	0.382222	-3.31157	0.000928	0.046356
6330416G1 3Rik	317.42514 46	0.9220803 06	0.252397	3.653294	0.000259	0.031092
Abca1	686.95468 83	0.9529662 23	0.170339	5.594531	2.21E-08	9.92E-05
Abca2	213.76749 81	0.7160621 13	0.190824	3.752476	0.000175	0.024845
Acy1	80.689179 97	- 0.9278986 45	0.251638	-3.68744	0.000227	0.03008
Adar	340.15634 94	0.4927370 4	0.13991	3.521809	0.000429	0.035057
Adnp2	102.45090 74	0.8791434 9	0.247926	3.545993	0.000391	0.035057
Afg3l2	178.35877 9	0.6881821 45	0.196934	3.494482	0.000475	0.035689
AI607873	47.616224 15	1.0825451 7	0.331157	3.268982	0.001079	0.048889

Ak6	45.276440 12	- 1.1909675 88	0.331635	-3.5912	0.000329	0.035057
Alkbh3	106.54310 01	- 0.8736062 59	0.246173	-3.54875	0.000387	0.035057
Ano7	63.716858 66	- 1.1731102 2	0.338858	-3.46195	0.000536	0.037285
Arhgap31	323.78165 61	1.0370545 98	0.295413	3.510528	0.000447	0.035057
Atp13a2	671.96235 5	0.6781524 83	0.207733	3.264543	0.001096	0.0491
Atp6v0a2	1106.7336 09	0.9304114 59	0.239526	3.884385	0.000103	0.021091
Axl	448.82399 61	0.8319259 8	0.244094	3.408215	0.000654	0.040424
BC003965	107.18559 99	- 0.8525824 97	0.241258	-3.5339	0.000409	0.035057
Bcl2a1a	101.12349 08	- 1.1550668 91	0.30318	-3.80983	0.000139	0.022664
Bcl2a1b	350.16371 98	- 1.0086564 33	0.280738	-3.59288	0.000327	0.035057
Bcor	142.42964 94	0.7635054 24	0.22282	3.426556	0.000611	0.039997
C2cd2	67.766721 73	1.2030298 72	0.3482	3.455	0.00055	0.037945
C2cd2l	98.256898 66	1.0722761 56	0.308695	3.473576	0.000514	0.036537
Calm2	2282.3499 31	- 0.6495880 38	0.19348	-3.3574	0.000787	0.043931
Capg	475.60757 95	- 0.8882722 23	0.226807	-3.91643	8.99E-05	0.019748

Cask	241.30759 84	- 0.7122183 55	0.203855	-3.49375	0.000476	0.035689
Ccdc115	214.30219 55	- 0.7552512 13	0.224829	-3.35922	0.000782	0.043931
Ccl6	1091.7351 62	- 1.0485549 98	0.215679	-4.86165	1.16E-06	0.002609
Ccl9	742.16076 22	- 1.0310976 1	0.281171	-3.66715	0.000245	0.030966
Cd22	52.165071 12	1.2070148 49	0.353793	3.411638	0.000646	0.040424
Cd9	2846.6046 31	- 0.6586501 99	0.201177	-3.27398	0.00106	0.048889
Cfl2	102.93847 85	- 0.6616505 62	0.196135	-3.37344	0.000742	0.043296
Chchd1	75.451983 23	- 1.0765068 38	0.31718	-3.394	0.000689	0.041163
Chst2	82.749552 61	2.0161043 5	0.52713	3.824683	0.000131	0.022322
Cklf	141.67443 33	- 1.1244629 22	0.317177	-3.54522	0.000392	0.035057
Cks2	41.727141 94	- 1.2819508 14	0.36431	-3.51884	0.000433	0.035057
Clec4d	139.42195 46	- 2.9620856 8	0.742229	-3.9908	6.59E-05	0.019748
Clec5a	897.20133 11	- 0.5535356 4	0.155891	-3.55079	0.000384	0.035057

Table S1: Differentially expressed genes after nerve injury.

gene_id	baseMean	log2FoldC hange	lfcSE	stat	pvalue	padj
1600010M 07Rik	77.2284118 5	1.23506575 9	0.3335235	3.703085	0.000213	0.038849
AB124611	103.277683 5	- 1.18619714 2	0.3203603	-3.7027	0.000213	0.038849
Abca7	183.398529 9	- 0.97924008 7	0.2673556	-3.66269	0.00025	0.03979
Acap1	65.3554991 9	- 2.13772720 3	0.5824726	-3.67009	0.000242	0.039714
Acpp	19.2707665 6	- 2.96756770 7	0.7158614	-4.14545	3.39E-05	0.01405
Adam8	137.356093 2	- 2.08828243	0.5495513	-3.79998	0.000145	0.034323
Anxa2	388.106847	- 1.44597348	0.3972289	-3.64015	0.000272	0.041769
Aqp9	18.7645557 7	- 2.73271773 3	0.7527931	-3.6301	0.000283	0.042338

Atp6v1f	763.010611 7	1.13094055 3	0.3034881	3.726475	0.000194	0.038062
AW112010	119.928273 5	1.29442101 8	0.3443811	3.758688	0.000171	0.03647
B430306N 03Rik	106.594376 4	- 1.34752162 9	0.2852753	-4.72358	2.32E-06	0.003463
Bola3	49.8225694 6	1.79580106 7	0.4030983	4.454996	8.39E-06	0.008359
Bst1	27.1216774 1	- 2.81128488 8	0.732149	-3.83977	0.000123	0.031975
C3	262.745391	- 2.10567857 8	0.4881515	-4.31358	1.61E-05	0.010339
Cbr1	123.590767 7	0.69452660 9	0.188094	3.692444	0.000222	0.039055
Ccna2	50.1417224 9	- 2.00106316 6	0.5505856	-3.63443	0.000279	0.042167
Cd177	843.296306	- 1.41162623 4	0.3920553	-3.60058	0.000318	0.045196
Cdc25b	22.5811426 3	- 2.95047487 6	0.6811514	-4.3316	1.48E-05	0.010339
Chdh	4.03533526 2	- 4.21253581 7	1.0505243	-4.00994	6.07E-05	0.019936
Chst13	8.04763985 4	- 4.50461896 3	1.0716457	-4.20346	2.63E-05	0.012572
Cldn15	29.7981188 5	- 2.21076109 8	0.5922126	-3.73305	0.000189	0.038062
Cybb	676.408834 9	- 1.34015622 2	0.3648782	-3.67289	0.00024	0.039714

E2f8	14.6534326 2	- 3.84684294 9	0.6540803	-5.8813	4.07E-09	2.43E-05
Emilin2	108.081522 6	- 1.99806767 9	0.5081715	-3.93188	8.43E-05	0.023995
Gpsm2	12.9718793 8	- 3.67922597 4	0.914216	-4.02446	5.71E-05	0.019509
Gsn	265.824446	- 1.20916321 1	0.2964488	-4.07883	4.53E-05	0.016913
Gsto1	152.808238 6	0.99686139	0.2468748	4.037923	5.39E-05	0.018965
Hax1	137.767656 8	0.88059450 7	0.2305788	3.819061	0.000134	0.03337
Hbs1l	106.486631 1	- 0.81271429 7	0.2232557	-3.64029	0.000272	0.041769
Hgf	10.5414876 1	- 2.81034203 8	0.785353	-3.57844	0.000346	0.047978
Ifit1bl2	10.4594992	- 3.27571240 4	0.8283868	-3.95433	7.68E-05	0.022943
Ifitm1	33.9932253 7	- 2.69143395	0.6861646	-3.92243	8.77E-05	0.024375
Il1f9	74.9911085 3	- 2.83500164 5	0.6438079	-4.40349	1.07E-05	0.009798
Iqgap1	260.643829 8	- 1.42970253 3	0.3118645	-4.58437	4.55E-06	0.005445
Irf7	75.7168031 5	- 1.27310624 9	0.3352837	-3.7971	0.000146	0.034323
Kif20a	22.4399337 8	- 2.21559828	0.5127256	-4.32122	1.55E-05	0.010339

Kndc1	13.7800345 6	2.78680320 6	0.7397177	3.767388	0.000165	0.035863
Kntc1	10.7464973 8	- 2.66754622 4	0.7392645	-3.60838	0.000308	0.044863
Larp7	113.140584 6	1.35647281	0.3389124	4.00243	6.27E-05	0.019936
Ltf	4393.50788 9	- 1.64385660 5	0.3824976	-4.29769	1.73E-05	0.010339
Ly75	22.6785928 2	- 3.74736848 3	0.6068458	-6.17516	6.61E-10	7.90E-06
Mefv	19.4772630 6	- 3.38359763 7	0.7884753	-4.29132	1.78E-05	0.010339
Megf9	62.0012820 2	- 2.13306152 3	0.5627186	-3.79064	0.00015	0.034552

Table S2: Differentially expressed genes after morphine treatment.