Supporting Information for:

Monocytes maintain central nervous system homeostasis following helminth-induced inflammation.

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Running title: Helminth-induced monocytes prevent neuroinflammation.

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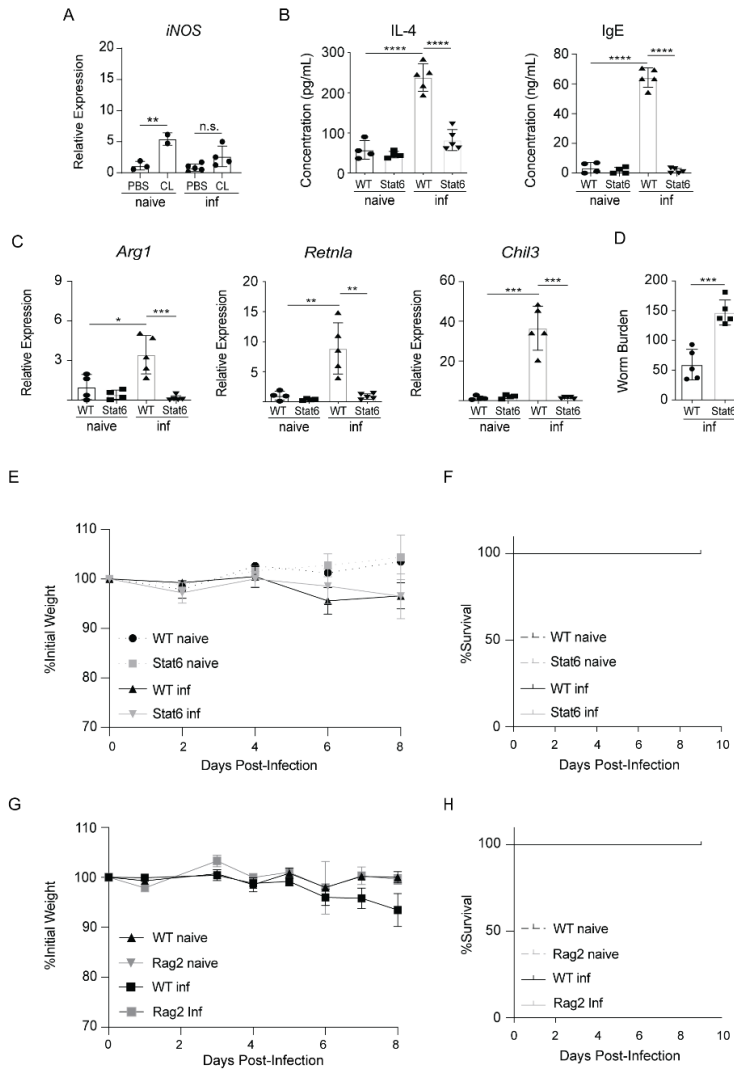QuantiTech primer assay information

Figure S1: Type 2 cytokine responses and adaptive immunity are not required for host survival post-*Trichinella spiralis* infection. **(A)** *iNOS* expression levels in the intestine were determined in naive and *T. spiralis* infected animals that were treated with PBS- or clodronate-loaded (CL) liposomes*.* Results are representative of at least 2 independent experiments with at least 3 biological replicates for test groups and 2-3 biological replicates for naive controls. Wild type (WT) or STAT6KO (Stat6) mice were infected with *T. spiralis* and sacrificed on 8 days post-infection (dpi). Mesenteric lymph node (mLN) cells were stimulated with anti-CD3 and anti-CD28 for 72 hours. **(B)** IL-4 levels from the supernatants of stimulated mLN cells and IgE levels from the plasma (1:50 dilution) were evaluated via ELISA. **(C)** Intestinal expression of M2-associated markers was evaluated via RT-qPCR. **(D)** Intestinal worm burden was evaluated. **(E)** Weights and **(F)** mortality were tracked throughout the course of the infection. WT or RAG2KO (Rag2) mice were infected with *T. spiralis* and sacrificed on 8 dpi. **(G)** Weights and **(H)** mortality were tracked throughout the course of the infection. All panels involving STAT6KO mice are representative of at least 3 independent experiments with at least 3 biological replicates per group per experiment. Statistical analysis was performed using Student's t-test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; n.s., not significant. For panel **(G)**, statistics are comparing WT inf to Rag2KO inf. Error bars represent ±SD. inf, *T. spiralis*-infected.
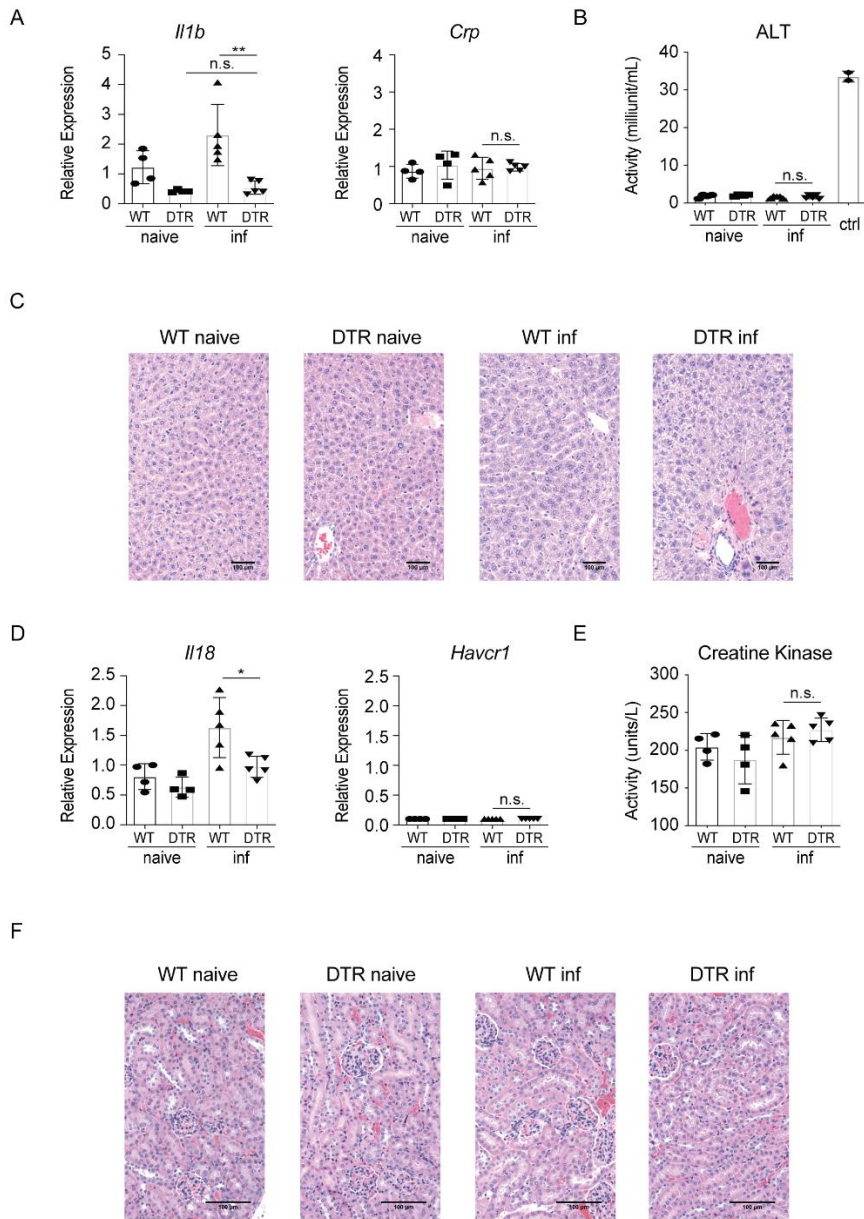
Figure S2: Increased morbidity and mortality exhibited by monocyte-depleted mice following *T. spiralis* infection are not due to liver or kidney injury or dysfunction. Wild type (WT) and CCR2-DTR (DTR) mice were infected with *T. spiralis* and treated with diphtheria toxin (DT) intraperitoneally every other day. Mice were sacrificed 8 dpi. **(A)** RNA was extracted from liver tissue, and expression of markers associated with liver injury were evaluated via RT-qPCR. **(B)** Plasma levels of alanine aminotransferase (ALT) were quantified. **(C)** H&E staining of liver sections. **(D)** RNA was extracted from kidney tissue, and expression of markers associated with kidney injury were evaluated via RT-qPCR. **(E)** Plasma levels of creatine kinase were quantified. **(F)** H&E staining of kidney sections. Results are representative of at least 2 independent experiments with at least 3 biological replicates per group per experiment. Statistical analysis was performed using Student's t-test. *, $p < 0.05$; **, $p < 0.01$; n.s., not significant. Error bars represent ±SD. inf, *T. spiralis*-infected; ctr, positive control.
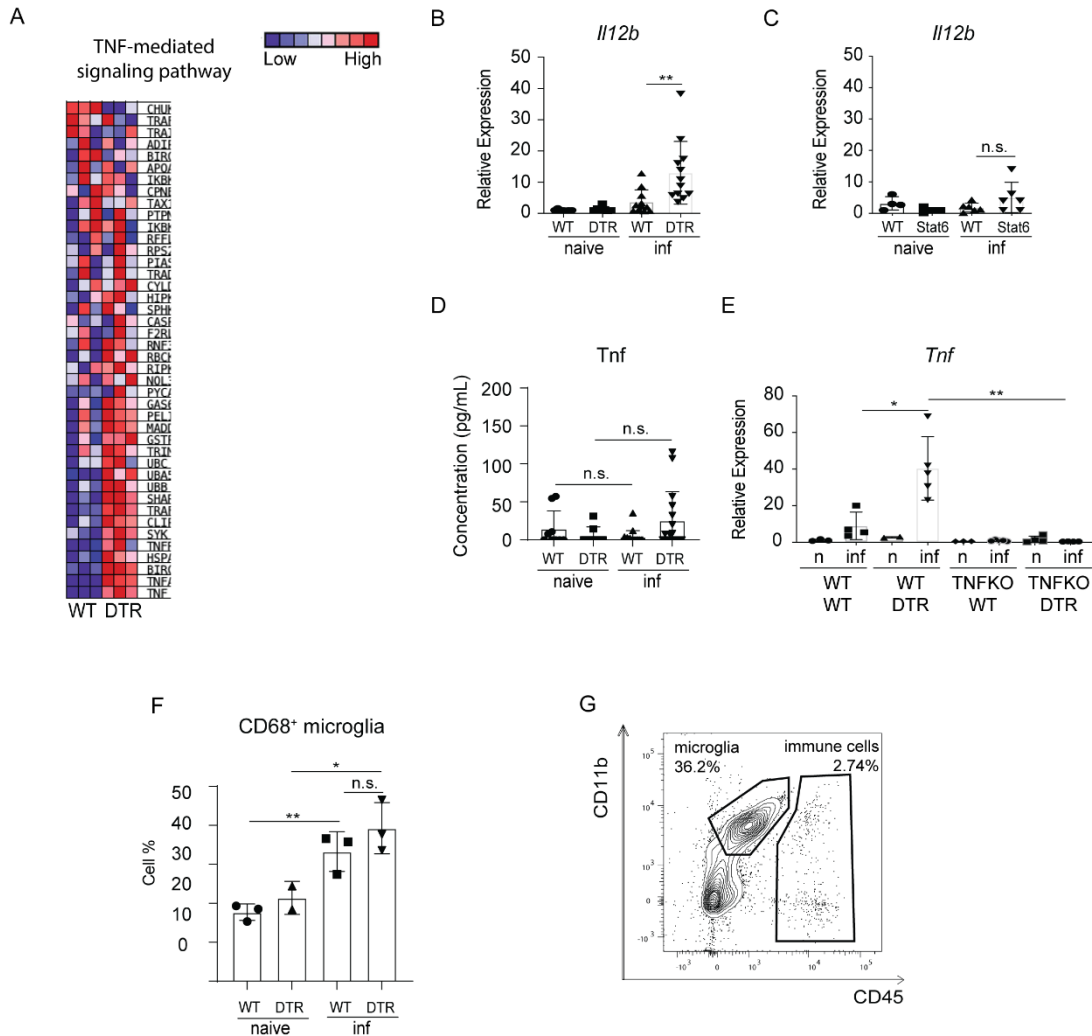
Figure S3: Loss of CCR2+ monocytes results in a proinflammatory signature in the brain. Wild type (WT), monocyte-depleted (DTR), STAT6KO (Stat6), TNFKO, and monocyte-depleted TNFKO mice (TNFKO DTR) mice were infected with *T. spiralis*. Mice were sacrificed 8 dpi. **(A)** Gene sequencing enrichment analysis (GSEA) heat map for TNF-mediated signaling pathway comparing infected WT and infected DTR mice. RNA was extracted from whole brain, and *Il12b* expression was evaluated in **(B)** monocyte-depleted and **(C)** STAT6KO mice compared to control mice following infection. **(D)** Serum TNF from naive and infected WT and DTR mice was measured by ELISA (pooled from 4 independent experiments). **(E)** RNA was extracted from the whole brains of WT, DTR, TNFKO, and TNFKO DTR mice following infection, and *Tnf* expression was evaluated via RT-qPCR. **(F)** CD68 expression levels were evaluated by flow cytometric analysis on microglia from naive and infected WT and monocyte-deplete mice. **(G)** Gating strategy of microglia (CD45^{mid}CD11b$^+$) compared to immune cells (CD45^{hi}). Results are representative of at least 2 independent experiments with at least 3 biological replicates for test groups and 2-3 biological replicates for naive controls. Statistical analysis was performed using Student's t-test. *, p < 0.05; **, p < 0.01; n.s., not significant. Error bars represent SD. inf, *T. spiralis*-infected. n, naive.
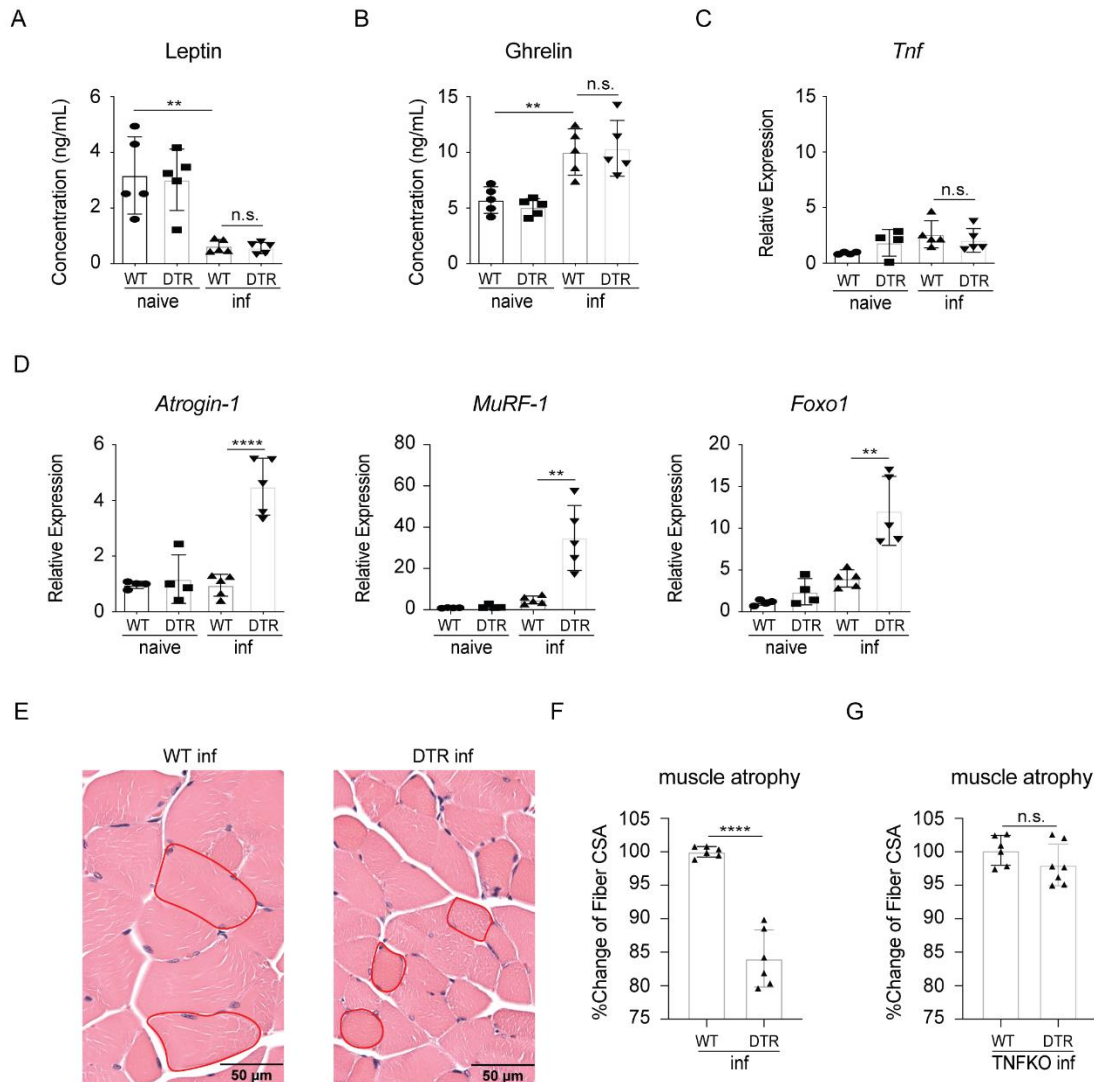
Figure S4: Expression of proinflammatory cytokines in the brain is associated with muscle atrophy. Wild type (WT) or CCR2-DTR (DTR) mice were treated with diphtheria toxin intraperitoneally every other day and sacrificed 8 dpi. Plasma **(A)** leptin and **(B)** ghrelin levels were evaluated. RNA from gastrocnemius tissue was extracted, and expression of **(C)** proinflammatory- and **(D)** atrophy-associated markers were evaluated via RT-qPCR. H&E staining of gastrocnemius tissue was evaluated via **(E)** microscopy and **(F)** cross-sectional areas (CSA) was quantified. Representative muscle fibers are encircled in red. **(G)** The CSA of gastrocnemius muscle fibers was quantified in infected TNFKO mice. Panels are representative of at least 3 independent experiments with at least 3 biological replicates per group per experiment. Statistical analysis was performed using Student's t-test. **, $p < 0.01$; ****, $p < 0.0001$; n.s., not significant. Error bars represent SD. inf, *T. spiralis*-infected.
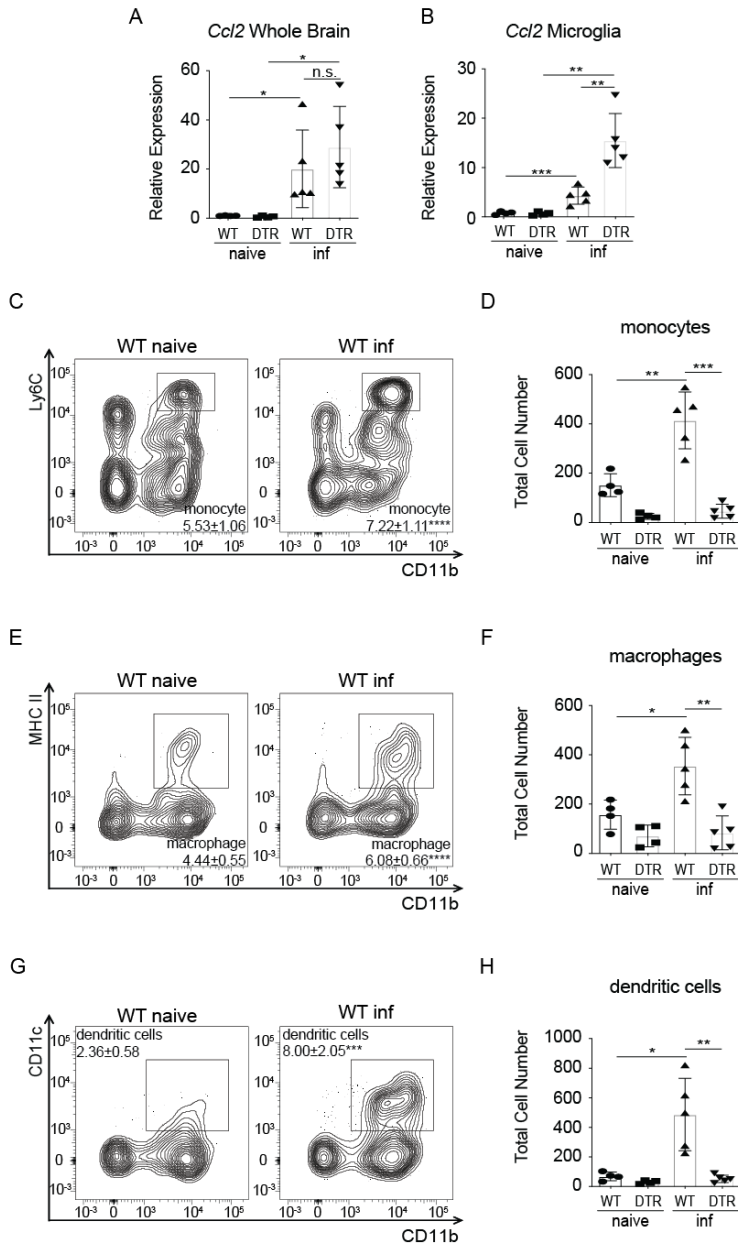
Figure S5: CCR2⁺ monocyte populations increase in the brain following _Trichinella_ infection. wild type (WT) or DTR mice were treated with DT (i.p.) every other day and sacrificed 8 dpi. RNA from **(A)** whole brain or **(B)** sort-purified microglia was extracted, and expression of _Ccl2_ was evaluated via RT-qPCR reaction. Single cell suspensions were generated from brain tissue and evaluated via flow cytometry. Gating strategy and total cell number of **(C,D)** monocytes (CD3⁻CD19⁻Ly6G⁻MHCII⁻CD11c⁻ cells) **(E,F)** macrophages (gated on CD3⁻CD19⁻Ly6G⁻CD11c⁻ cells), and **(G,H)** dendritic cells (gated on CD3⁻CD19⁻Ly6G⁻ cells). Numbers in cytometry plots represent mean percentages (±SD) of cells out of CD45ʰⁱ cells, and statistics are comparing WT naive and WT _T. spiralis_-infected mice. All panels are representative of at least 3 independent experiments with at least 3 biological replicates per group per experiment. Statistical analysis was performed using Student's t-test. *, p < 0.05; **, p < 0.01; ***, p < 0.001; ****, p < 0.0001; n.s., not significant. Error bars represent ±SD. inf, _T. spiralis_-infected.
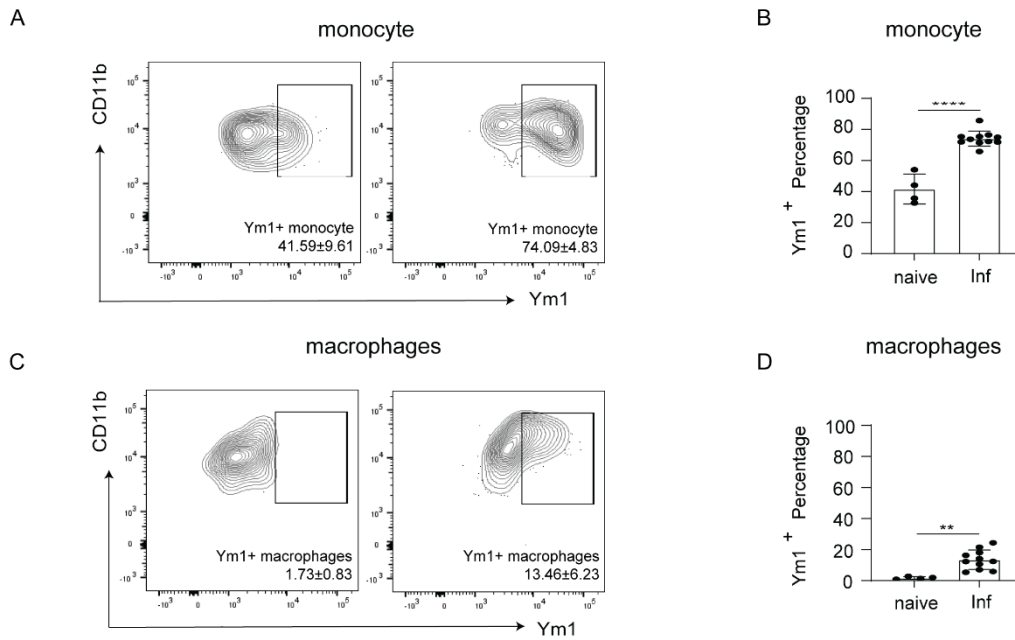
Figure S6: Intracellular staining of Ym1 in infected mouse brain. (A) Representative gating of intracellular staining of Ym1 in brain monocytes from infected WT mice 8 dpi. **(B)** Ym1+ monocyte percentage in brains of infected WT mice 8 dpi. **(C)** Representative gating of intracellular staining of Ym1 in WT infected brain macrophages 8 dpi. **(D)** Ym1+ macrophage percentage in brains of infected WT mice 8 dpi. Results are representative of at least 2 independent experiments with at least 3 biological replicates per group per experiment. Statistical analysis was performed using Student's t-test. *, $p < 0.05$; **, $p < 0.01$; n.s., not significant. Error bars represent SD. inf, *T. spiralis*-infected.

Table 1: Upregulated (green, fold change > 1.5) and downregulated (red, fold change < -1.5) genes in infected wild type versus infected monocyte-depleted mice.

Name	ENSEMBL	Log$_2$ fold change	Fold change	FDR p-value
Ear2	ENSMUSG00000072596	5.304526	39.52042	7.37E-10
Fam205a3	ENSMUSG00000093996	5.005028	32.11173	3.94E-10
Gm26718	ENSMUSG00000097367	3.269005	9.63981	1.29E-05
Gm6436	ENSMUSG00000061330	3.231516	9.392544	0.029057
H2-Aa	ENSMUSG00000036594	2.935299	7.649147	0
Ccr2	ENSMUSG00000049103	2.770431	6.823118	0
H2-Eb1	ENSMUSG00000060586	2.762616	6.786255	0
Sirpb1c	ENSMUSG00000074677	2.670894	6.368236	0.001775
H2-Ab1	ENSMUSG00000073421	2.577319	5.968296	0
Chil3	ENSMUSG00000040809	2.304529	4.940062	1.63E-09
Gm48283	ENSMUSG00000114436	2.297991	4.917724	0.000312
Gm11427	ENSMUSG00000083161	2.249898	4.756491	0.008457
Gm26964	ENSMUSG00000098196	2.101937	4.292852	0.023004
Plbd1	ENSMUSG00000030214	2.025178	4.070421	4.65E-09
Gm15929	ENSMUSG00000086032	1.954938	3.876994	0.001204
F10	ENSMUSG00000031444	1.890935	3.708755	0.009371
Ccr7	ENSMUSG00000037944	1.656662	3.152862	0.001896
Retnla	ENSMUSG00000061100	1.449134	2.730442	1.31E-05
Cd74	ENSMUSG00000024610	1.280404	2.42907	0
Gm10645	ENSMUSG00000074228	1.153722	2.224871	4.47E-05
Plac8	ENSMUSG00000029322	1.05925	2.083848	0.039914
Spn	ENSMUSG00000051457	1.017955	2.025047	0.035097
1700016P03Rik	ENSMUSG00000085609	0.876306	1.835669	1.9E-06
Mgl2	ENSMUSG00000040950	0.799879	1.740955	0.01748
Gm42047	ENSMUSG00000110631	0.774176	1.710212	2.6E-05
Xlr3b	ENSMUSG00000073125	0.756189	1.689023	2.41E-07
Btg2	ENSMUSG00000020423	0.737627	1.667431	1.14E-08
Tsc22d2	ENSMUSG00000027806	0.729865	1.658484	0
Npas4	ENSMUSG00000045903	0.679316	1.60138	0.000333
Hba-a1	ENSMUSG00000069919	0.653162	1.572611	1.6E-06
Arc	ENSMUSG00000022602	0.632251	1.549981	4.17E-07
Ctla2a	ENSMUSG00000044258	-0.585563	-1.500624	0.021471
Tgm2	ENSMUSG00000037820	-0.586866	-1.50198	1.46E-05
Relb	ENSMUSG00000002983	-0.590557	-1.505828	0.000128
Vwf	ENSMUSG00000001930	-0.597701	-1.513303	0.003516
Slfn2	ENSMUSG00000072620	-0.60057	-1.516315	0.030876
Ikbke	ENSMUSG00000042349	-0.600756	-1.516511	0.038138
Bcl2a1b	ENSMUSG00000089929	-0.60232	-1.518156	0.010206
Ncf4	ENSMUSG00000071715	-0.604757	-1.520723	0.043844
Cxcl16	ENSMUSG00000018920	-0.608435	-1.524604	5.72E-05

Grrp1	ENSMUSG00000050105	-0.612446	-1.52885	0.010562
Pim1	ENSMUSG00000024014	-0.627263	-1.544632	0.00239
Adm	ENSMUSG00000030790	-0.638448	-1.556654	0.029484
Gadd45b	ENSMUSG00000015312	-0.638487	-1.556696	1.49E-11
Lcp2	ENSMUSG00000002699	-0.639582	-1.557878	1.06E-05
Slc4a11	ENSMUSG00000074796	-0.64871	-1.567766	0.015759
Fkbp5	ENSMUSG00000024222	-0.648825	-1.567891	3.32E-07
Acer2	ENSMUSG00000038007	-0.652691	-1.572098	0.003507
Socs3	ENSMUSG00000053113	-0.653012	-1.572448	0.000355
Plin4	ENSMUSG00000002831	-0.669275	-1.590274	8.53E-05
Gm2260	ENSMUSG00000098428	-0.674341	-1.595868	0.046934
Slc15a3	ENSMUSG00000024737	-0.675015	-1.596613	2.03E-06
Nfkbia	ENSMUSG00000021025	-0.67917	-1.601218	9.17E-08
Igsf6	ENSMUSG00000035004	-0.688357	-1.611448	0.018079
Galnt15	ENSMUSG00000021903	-0.688406	-1.611502	2.7E-05
Fas	ENSMUSG00000024778	-0.705039	-1.630189	0.001848
H2-Q6	ENSMUSG00000073409	-0.706413	-1.631742	0.002954
C5ar1	ENSMUSG00000049130	-0.713506	-1.639784	1.53E-06
Sh3tc1	ENSMUSG00000036553	-0.721401	-1.648783	0.000792
Xdh	ENSMUSG00000024066	-0.726106	-1.654168	0.001513
Alox5	ENSMUSG00000025701	-0.731389	-1.660237	0.012351
Cdkn1a	ENSMUSG00000023067	-0.737348	-1.667109	9.2E-09
Birc3	ENSMUSG00000032000	-0.742136	-1.67265	1.85E-06
Ier3	ENSMUSG00000003541	-0.742495	-1.673066	4.55E-08
Runx1	ENSMUSG00000022952	-0.798933	-1.739814	6.02E-08
Srarp	ENSMUSG00000070637	-0.801233	-1.74259	0.045678
Cxcl10	ENSMUSG00000034855	-0.807935	-1.750704	0.009536
Map3k6	ENSMUSG00000028862	-0.839294	-1.789174	0.000171
Ifitm1	ENSMUSG00000025491	-0.860342	-1.815469	0.003022
Tnfaip2	ENSMUSG00000021281	-0.878738	-1.838766	1.13E-07
Nlrp3	ENSMUSG00000032691	-0.908906	-1.877621	9.02E-05
Scn10a	ENSMUSG00000034533	-0.908939	-1.877664	0.022408
Tlr2	ENSMUSG00000027995	-0.910471	-1.879659	1.33E-11
Maff	ENSMUSG00000042622	-0.934081	-1.910673	1.52E-06
Ch25h	ENSMUSG00000050370	-0.935144	-1.912082	0.002509
Bcl3	ENSMUSG00000053175	-0.943843	-1.923646	1.18E-05
Ccl3	ENSMUSG00000000982	-0.967297	-1.955174	0.004901
Nfkbie	ENSMUSG00000023947	-0.997374	-1.996363	1.85E-06
Fgr	ENSMUSG00000028874	-0.998523	-1.997954	0.012361
Rab20	ENSMUSG00000031504	-1.003711	-2.005151	0.003347
Ccl12	ENSMUSG00000035352	-1.005073	-2.007045	2.84E-07
Gm26508	ENSMUSG00000097614	-1.045895	-2.064648	0.002183
Lrg1	ENSMUSG00000037095	-1.058826	-2.083236	2.12E-06
Gpr84	ENSMUSG00000063234	-1.060232	-2.085267	7.37E-10
Ccl2	ENSMUSG00000035385	-1.062011	-2.08784	0.000728

Tmem252	ENSMUSG00000048572	-1.108489	-2.156198	3.81E-05
Tnfaip3	ENSMUSG00000019850	-1.118203	-2.170763	0
Hrct1	ENSMUSG00000071001	-1.144907	-2.211318	0.009134
Ccl7	ENSMUSG00000035373	-1.145499	-2.212226	0.035223
Batf	ENSMUSG00000034266	-1.160242	-2.23495	0.007694
Ccl4	ENSMUSG00000018930	-1.177159	-2.261311	0.000853
Icam1	ENSMUSG00000037405	-1.198012	-2.294234	0
Il1a	ENSMUSG00000027399	-1.257676	-2.391103	4.29E-11
Slamf8	ENSMUSG00000053318	-1.333755	-2.520579	0.000151
8430408G22Rik	ENSMUSG00000048489	-1.355146	-2.55823	2.41E-07
Osm	ENSMUSG00000058755	-1.376904	-2.597105	0.00111
Lcn2	ENSMUSG00000026822	-1.396404	-2.632446	0
Gcat_2	ENSMUSG00000116378	-1.564348	-2.957438	8.4E-06
Il1b	ENSMUSG00000027398	-1.598562	-3.028412	1.52E-07
Hcar2	ENSMUSG00000045502	-1.61907	-3.07177	6.41E-07
AL592169.1	ENSMUSG00000116069	-1.747376	-3.357474	0.043938
Acod1	ENSMUSG00000022126	-1.754791	-3.374774	0.011074
Retnlg	ENSMUSG00000022651	-1.79957	-3.481163	0.012351
Serpina3f	ENSMUSG00000066363	-1.803389	-3.490392	0.001113
Lao1	ENSMUSG00000024903	-1.805633	-3.495825	0.007474
Cd40	ENSMUSG00000017652	-1.814809	-3.51813	2.52E-09
Il1rn	ENSMUSG00000026981	-1.823029	-3.538233	3.47E-07
Cd69	ENSMUSG00000030156	-1.982615	-3.952088	0.041086
S100a8	ENSMUSG00000056054	-2.083062	-4.237054	0.000233
AL732309.2	ENSMUSG00000115074	-2.301766	-4.930609	0.02417
S100a9	ENSMUSG00000056071	-2.307743	-4.951079	6.85E-06
Tnf	ENSMUSG00000024401	-2.446487	-5.450872	0
Cxcl2	ENSMUSG00000058427	-2.852437	-7.222194	2.96E-09
Il12b	ENSMUSG00000004296	-2.901498	-7.472016	0.028447
Gm20441	ENSMUSG00000092360	-5.065622	-33.48916	0.045595
Ccr3	ENSMUSG00000035448	-6.214271	-74.2475	0

Table 1: Upregulated (green, fold change > 1.5) and downregulated (red, fold change < -1.5) genes in infected wild type versus infected monocyte-depleted mice. Wild type and CCR2-DTR mice were infected with *T. spiralis* and treated with diphtheria toxin intraperitoneally every other day. Mice were sacrificed 8 days post-infection and brain tissue was harvested. Results are representative of 3 biological replicates per naive and infected group.

QuantiTech primer assay information:

Gene Target	SOURCE	IDENTIFIER
Actb	Qiagen	Cat. # QT01136772
Tnf	Qiagen	Cat. # QT00104006
Il1b	Qiagen	Cat. # QT01048355
Il6	Qiagen	Cat. # QT00098875
Il12b	Qiagen	Cat. # QT00153643
Nlrp3	Qiagen	Cat. # QT00122458
Arg1	Qiagen	Cat. # QT00134288
Retnla	Qiagen	Cat. # QT00254359
Chil3	Qiagen	Cat. # QT00108829
Ear2	Qiagen	Cat. # QT00265965
Mgl2	Qiagen	Cat. # QT00143640
Foxo1	Qiagen	Cat. # QT00116186
Fbxo32	Qiagen	Cat. # QT00158543
Trim63	Qiagen	Cat. # QT00291991
Ccl2	Qiagen	Cat. # QT00167832
F10	Qiagen	Cat. # QT02524935
Crp	Qiagen	Cat. # QT00255444
Havcr1	Qiagen	Cat. # QT00112427
Il18	Qiagen	Cat. # QT00171129