

Supplementary Materials

Depletion of microglia with PLX3397 attenuates MK-801-induced hyperactivity associated with regulating inflammation-related genes in the brain

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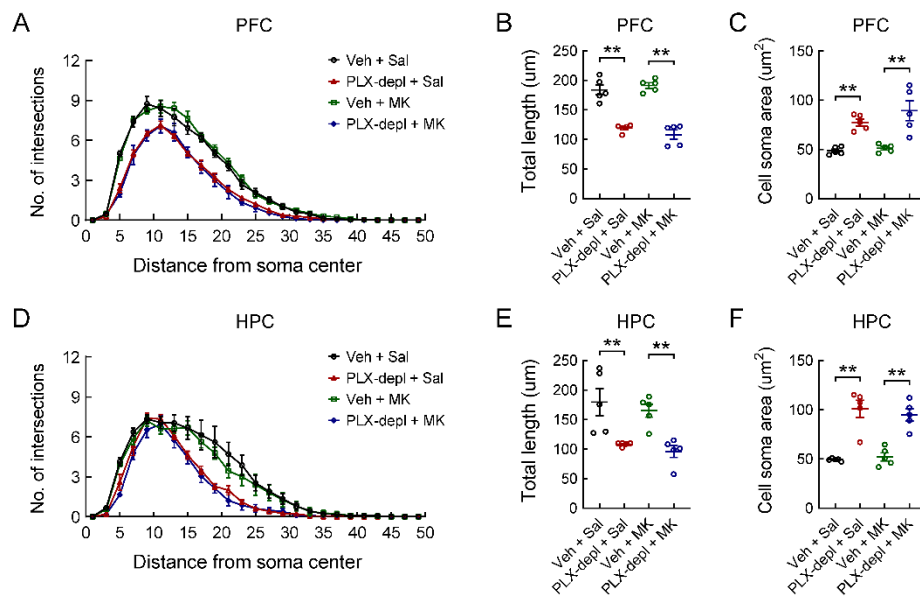
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Supplementary Materials and Methods

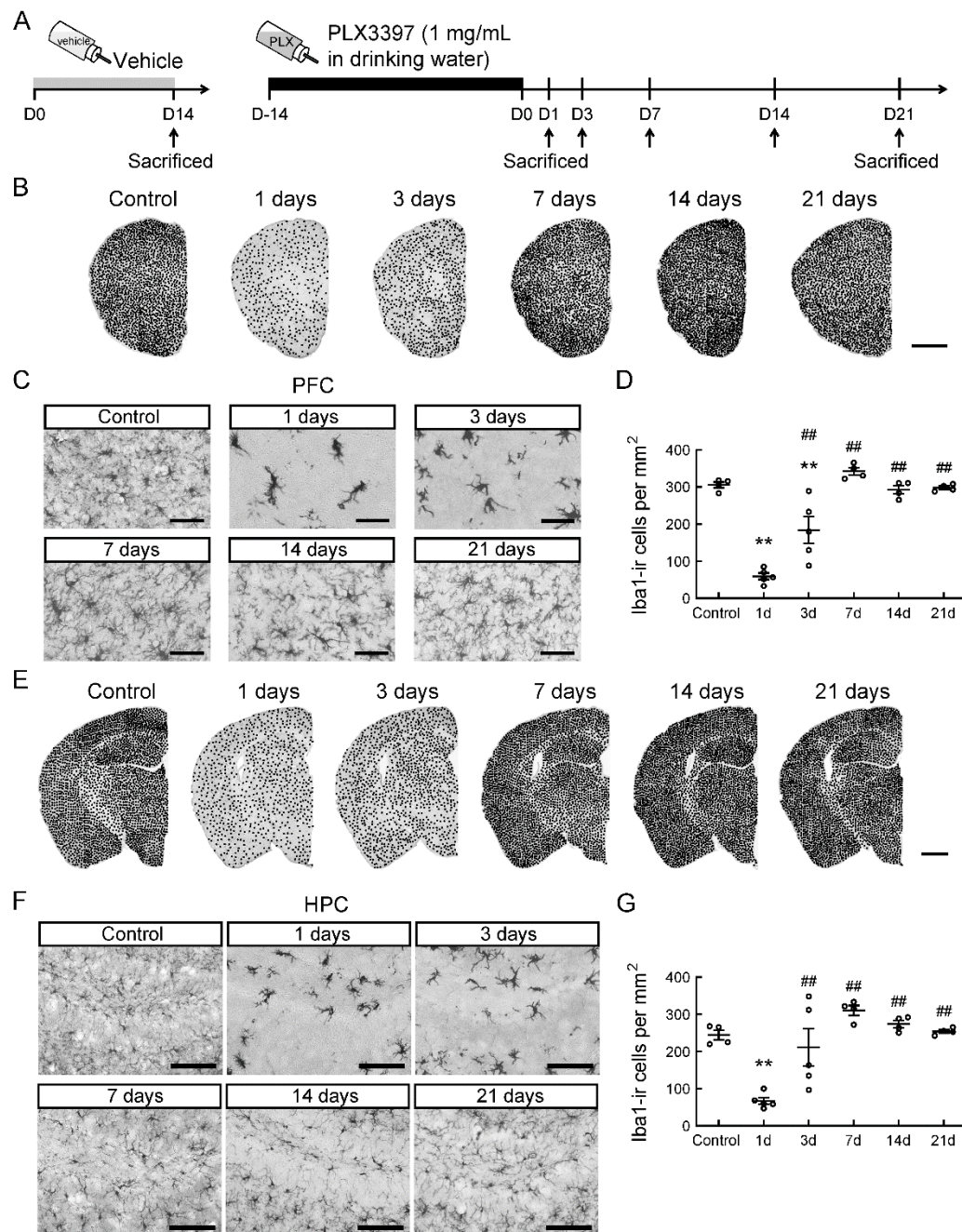
Image processing

All photographs were stored at a size of 1 920×1 080 pixels (219 pixels equals 25 μm). To exclude adjacent microglia or nonspecific immunoreactivity, we sketched the traces and cell bodies manually to obtain skeletonized microglial cell images. This step was conducted using Adobe Illustrator software and a Wacom Intuos CTL-4100 tablet. The skeletonized microglial cell images were then used to quantitatively investigate the branch density and distance from the soma center of the cells using Sholl analysis (Binley et al., 2014). The skeletonized images were also used to calculate microglial density, microglial soma area, total length of microglial processes, and maximum length of microglial processes. To investigate these parameters, we split the skeletonized images into two layers for each cell, one for the traced branches and one for the longest branch. Additionally, a small dot was placed into the trace layer to mark the cell soma center for Sholl analysis. The cell soma sketch was filled with pure black and collected in a single new layer. We then exported these layers from Adobe Illustrator to separate files in JPG format.



Supplementary Figure S1 Microglial morphology after single injection of MK-801 or saline with PLX3397 or vehicle pretreatment, respectively

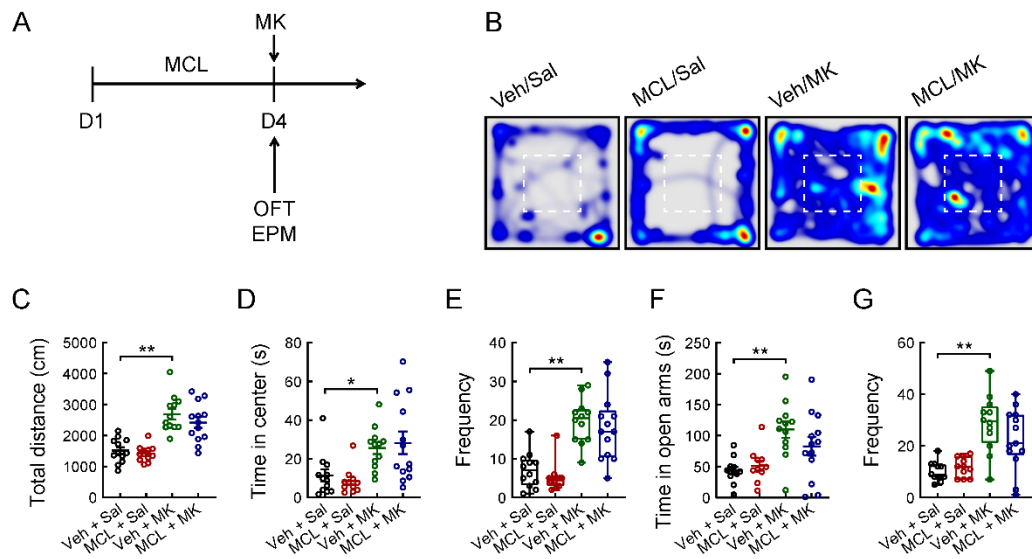
A, D: Quantification of mean number of intersections of microglial processes at distances (in 2 μm increments) from the soma center in the prefrontal cortex (PFC) and hippocampus (HPC). B, E: Quantification of total length of microglial processes. C, F: Quantification of microglial soma area in PFC and HPC of the four groups. Statistical analyses were performed via two-way ANOVA with Bonferroni's *post hoc* test. ** $P < 0.01$. MK, MK-801; PLX-depl, PLX3397-depletion; Sal, saline; Veh, vehicle.



Supplementary Figure S2 Rapid repopulation and replenishment of newborn microglia in mouse brain after removal of CSF1R inhibitor PLX3397

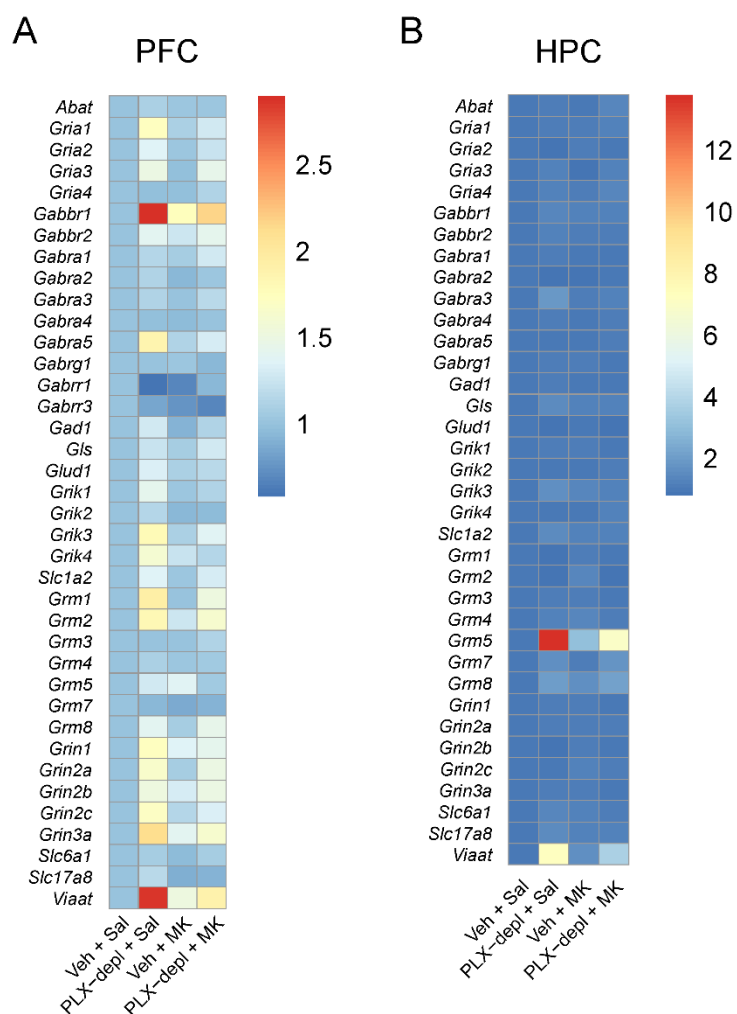
A: Schematic of experimental design. Representative low-magnification and high-magnification photomicrographs illustrating microglial changes (Iba1 immunoreactivity) in PFC (B, C) and HPC (E, F) after PLX3397 (1 mg/mL in drinking water) administration for 14 days to deplete microglia, followed by PLX3397 withdrawal for 1, 3, 7, 14, or 21 days. Control mice were treated with vehicle. Quantification of microglial density in PFC (D) and HPC (G) after PLX3397 withdrawal for 1, 3, 7, 14, or 21 days ($n=4-5$ for each time point). Statistical analyses were performed via one-way ANOVA with Bonferroni's *post hoc* test. ** $P<0.01$

compared to Control; ## $P<0.01$ compared to 1 d. Density of black dots represents relative density of Iba1-ir cells in the brain. Each dot represents approximately two Iba1-ir cells. Means and SEM values in D and G are for combined data from two or more independent experiments. Scale bars=1 000 μm in B, E; 50 μm in C; 100 μm in F.



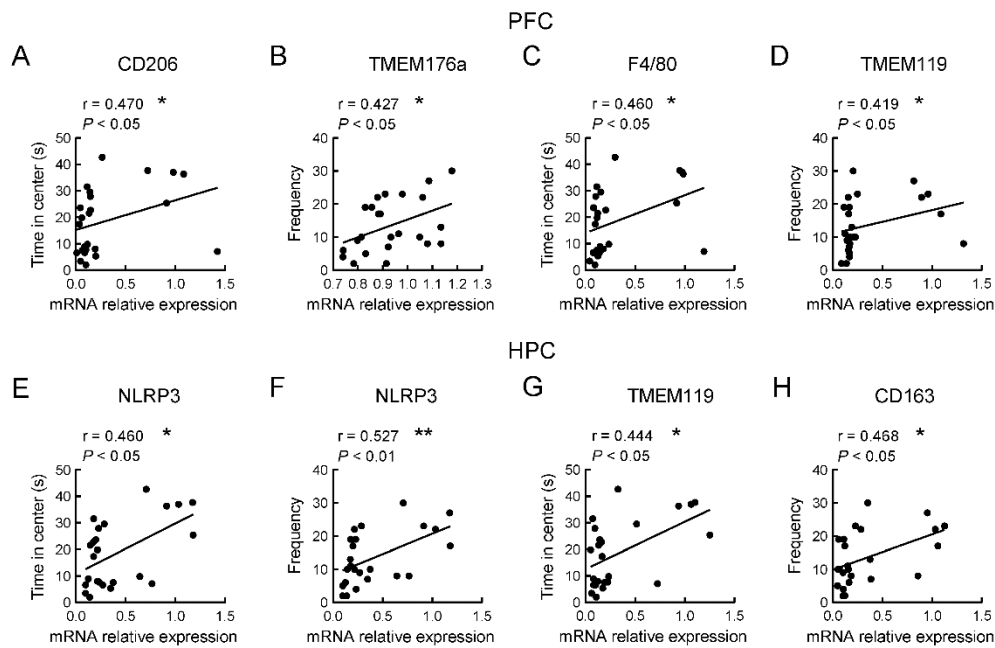
Supplementary Figure S3 Effects of minocycline on MK-801-induced hyperlocomotion in mice

A: Schematic of experimental design. **B:** Heatmaps showing cumulative duration spent by each group in compartment during the open-field test (OFT). Dashed lines represent central areas. **C:** Total distance moved by each group ($n=11-13$ /group) in open-field box. **D:** Time spent by each group in central area during 5 min OFT. **E:** Comparison of frequency of entries into central area of open-field among four groups after MCL and/or MK administration. **F:** Time spent in open arms during 5 min elevated plus-maze (EPM) test in four groups. **G:** Frequency of entries into open arms by each group after MCL and/or MK administration. Statistical analyses were performed via two-way ANOVA with Bonferroni's *post hoc* test ($n=48$). * $P<0.05$; ** $P<0.01$. MCL, minocycline. MK, MK-801; Sal, saline; Veh, vehicle.



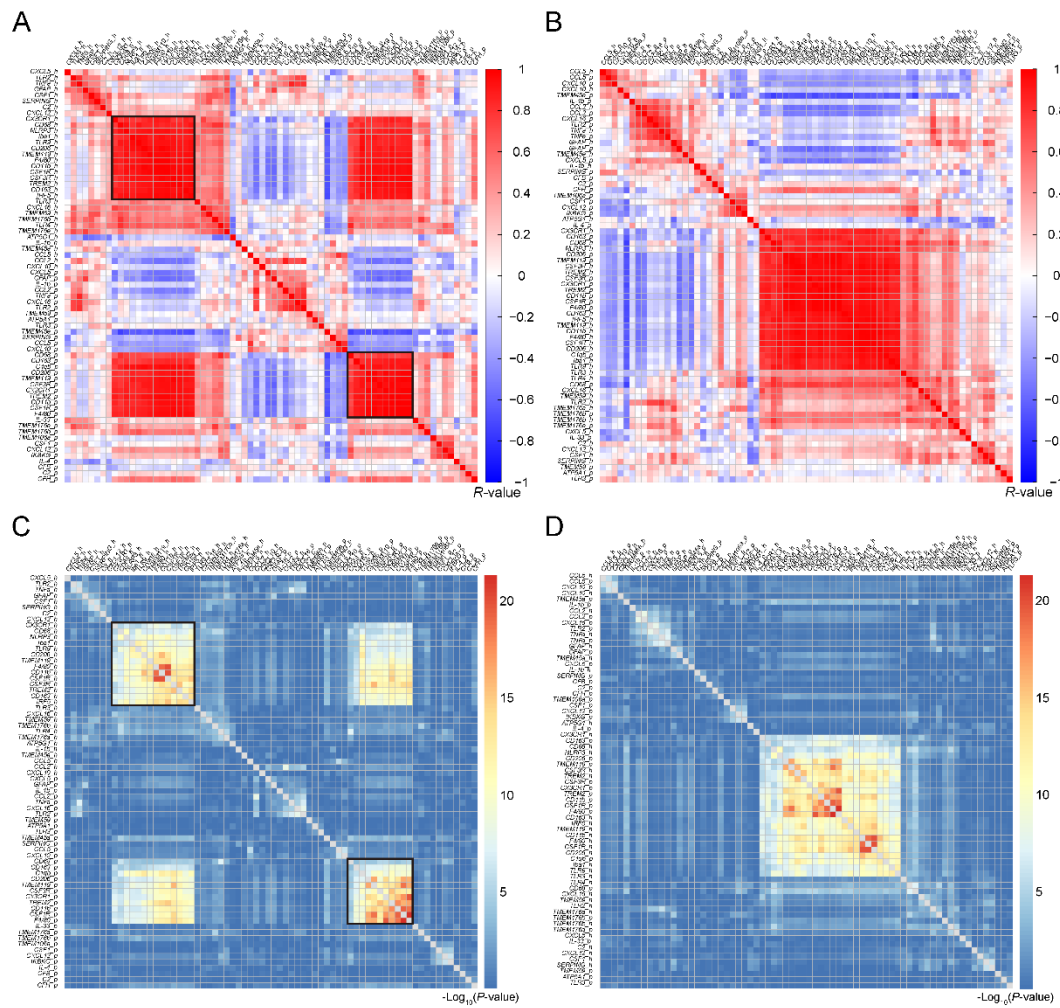
Supplementary Figure S4 Heatmaps showing expression of all candidate glutamate- and GABA-related genes in PFC (A) and HPC (B) after PLX3397 and MK-801 treatment

* Compared to Veh+Sal; * $P < 0.05$; ** $P < 0.01$; # compared to Veh+MK; # $P < 0.05$; ### $P < 0.01$. MK, MK-801; PLX-depl, PLX3397-depletion; Sal, saline; Veh, vehicle.



Supplementary Figure S6 Correlations between inflammation-related gene expression changes in the brain and behaviors during OFT in mice after PLX3397 and MK-801 administration

A-D: Correlation between inflammation-related gene (*CD206*, *TMEM176a*, *F4/80*, and *TMEM119*) expression levels in PFC and anxiolytic-like behaviors in mice. E-H: Correlation between inflammation-related gene (*NLRP3*, *TMEM119*, and *CD163*) expression levels in HPC and behaviors in OFT. Correlation analysis was performed using Spearman correlation ($n=24$). * $P<0.05$; ** $P<0.01$.



Supplementary Figure S7 Correlation matrices of differentially expressed inflammation-related genes in PFC and HPC after PLX3397 and MK-801 administration

A-B: Heatmaps showing pairwise correlations among differentially expressed inflammation-related genes in PFC ("p" suffix) and HPC ("h" suffix). Color corresponds to Spearman correlation coefficients, with red (blue) indicating positive (negative) correlation ($n=24$). C-D: Correlation matrices of differentially expressed inflammation-related genes in PFC and HPC. Color scale indicates $-\log_{10}(P\text{-value})$. P -value was calculated using Spearman correlation test with a significance threshold of $P \leq 0.05$ (indicated by dotted line of $-\log_{10}(P\text{-value})=1.3$). Hierarchical clustering was applied to gene expression data from PFC and HPC, respectively (A, C). Hierarchical clustering was applied to whole matrix (B, D). Black boxes indicate those genes that cluster together. Values in A-D are for combined data from two or more independent experiments.

Supplementary Table S1 Primer sequences used for qRT-PCR experiments

Gene name	Primer name	Primer sequence
4-aminobutyrate transaminase	Abat-F	CTGAACACAATCCAGAATG CAGA
	Abat-R	GGTTGTAACCTATGGGCACA G
allograft inflammatory factor 1	AIF1-F	ATCAACAAGCAATTCCTCGA TGA
	AIF1-R	CAGCATTCGCTTCAAGGACA TA
glutamate receptor, ionotropic, AMPA1 (Gria1)	AMPA1-F	TCCCCAACAATATCCAGATA GGG
	AMPA1-R	AAGCCGCATGTTCTGTGAT T
glutamate receptor, ionotropic, AMPA2 (Gria2)	AMPA2-F	AATGGACGTGTTATGACTCC AGA
	AMPA2-R	CTGACATTCATTCCCATGCC A
glutamate receptor, ionotropic, AMPA3 (Gria3)	AMPA3-F	ACCATCAGCATAGGTGGAC TT
	AMPA3-R	ACGTGGTAGTTCAAATGGA AGG
glutamate receptor, ionotropic, AMPA4 (Gria4)	AMPA4-F	GGGAGGTGACTCCAAGGAC A
	AMPA4-R	CCAGTGATGGATAACCTGG CT
arginase 1	ARG1-F	CCCGACTTCTGGGACTTCTG AGTAGGTTCCGAAGACTGG
	ARG1-R	GT
ATP synthase, H ⁺ transporting, mitochondrial F1 complex, alpha subunit 1	ATP5A1-F	TCTCCATGCCTCTAACACTC G
	ATP5A1-R	CCAGGTCAACAGACGTGTC AG
ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit c1	ATP5G1-F	CCAGAGGCCCCATCTAAGC
	ATP5G1-R	CCCCAGAATGGCATAGGAG AAG
CCAAT/enhancer binding protein, beta	C/EBP- β -F	ACCGGGTTTCGGGACTTGA
	C/EBP- β -R	GTTGCGTAGTCCCGTGTCCA
complement component 1, q subcomponent, beta polypeptide	C1qb-F	ATAAAGGGGGAGAAAGGGC T

complement component 2	C1qb-R	CGTTGCGTGGCTCATAGTT
	C2-F	CGGTGGTAATTTACCCCTCA G
complement component 7	C2-R	GGTGTGATGTGAGCTAGAC CT
	C7-F	CAACTGCAAGTGGGACTCCT A
chemokine (C-C motif) ligand 2	C7-R	CAGCAACTGAACGCCTTCG
	CCL2-F	TTAAAAACCTGGATCGGAA CCAA
chemokine (C-C motif) ligand 5	CCL2-R	GCATTAGCTTCAGATTACG GGT
	CCL5-F	ACTCCCTGCTGCTTTGCCTA C
integrin alpha M	CCL5-R	GAGG TTCCTTCGAGTGACA
	CD11b-F	ATGGACGCTGATGGCAATA CC
cluster of differentiation 163	CD11b-R	TCCCCATTACGTCTCCCA
	CD163-F	CCTTGGAACAGAGACAGG C
mannose receptor (cluster of differentiation 206)	CD163-R	TCCACACGTCCAGAACAGTC
	CD206-F	CTCTGTTCAGCTATTGGACG C
cluster of differentiation 68	CD206-R	CGGAATTTCTGGGATTCAGC TTC
	CD68-F	TGTCTGATCTTGCTAGGACC G
complement factor B	CD68-R	GAGAGTAACGGCCTTTTTGT GA
	CFB-F	GAGCGCAACTCCAGTGCTT
complement component factor h	CFB-R	GAGGGACATAGGTACTCCA GG
	CFH-F	AGGCTCGTGGTCAGAACAA C
complement factor properdin	CFH-R	GTTAGACGCCACCCATTTTC C
	CFP-F	TTCACCCAGTATGAGGAGTC C
carboxypeptidase B1	CFP-R	GCTGACCATTGTGGAGACCT
	CPB1-F	AGGCATGGATTCAACAAGT TGC
	CPB1-R	AGCCTCTCTCACAAACCACT G

colony stimulating factor 1	CSF1-F	GGCTTGGCTTGGGATGATTCT
	CSF1-R	GAGGGTCTGGCAGGTACTC
colony stimulating factor 1 receptor	CSF1R-F	TGTCATCGAGCCTAGTGGC
	CSF1R-R	CGGGAGATTCAGGGTCCAA G
colony stimulating factor 2 receptor, alpha	CSF2RA-F	CTGCTCTTCTCCACGCTACT G
	CSF2RA-R	GAGACTCGCCGGTGTATCC
colony stimulating factor 3 receptor	CSF3R-F	CTGATCTTCTTGCTACTCCC CA
	CSF3R-R	GGTGTAGTTCAAGTGAGGC AG
chemokine (C-X3-C motif) ligand 1	CX3CL1-F	ACGAAATGCGAAATCATGT GC
	CX3CL1-R	CTGTGTCGTCTCCAGGACAA
chemokine (C-X3-C) receptor 1	CX3CR1 - F	GAGTATGACGATTCTGCTGA GG
	CX3CR1 - R	CAGACCGAACGTGAAGACG AG
chemokine (C-X-C motif) ligand 10	CXCL10-F	CCAAGTGCTGCCGTCATTTT C
	CXCL10-R	GGCTCGCAGGGATGATTTC A
chemokine (C-X-C motif) ligand 12	CXCL12-F	TGCATCAGTGACGGTAAAC CA
	CXCL12-R	TTCTTCAGCCGTGCAACAAT C
chemokine (C-X-C motif) ligand 14	CXCL14-F	GAAGATGGTTATCGTCACCA CC
	CXCL14-R	CGTTCCAGGCATTGTACCAC T
chemokine (C-X-C motif) ligand 16	CXCL16-F	CCTTGTCTCTTGCGTTCTTCC
	CXCL16-R	TCCAAAGTACCCTGCGGTAT C
chemokine (C-X-C motif) ligand 5	CXCL5-F	GTTCCATCTCGCCATTCATG C
	CXCL5-R	GCGGCTATGACTGAGGAAG G
EGF-like module containing, mucin-like, hormone receptor-like sequence 1 (Emr1)	F4/80-F	TGACTCACCTTGTGGTCCTA A

	F4/80-R	CTTCCCAGAATCCAGTCTTT CC
parasite-induced macrophage novel gene 1 protein	FIZZ1-F	CCAATCCAGCTAACTATCCC TCC
	FIZZ1-R	ACCCAGTAGCAGTCATCCCA
gamma-aminobutyric acid (GABA) B receptor 1	GABBR1-F	ACGTCACCTCGGAAGGTTG
	GABBR1-R	CACAGGCAGGAAATTGATG GC
gamma-aminobutyric acid (GABA) B receptor 2	GABBR2-F	AAGACCCCATAGAGGACAT CAA
	GABBR2-R	GGGTGGTACGTGTCTGTGG
gamma-aminobutyric acid (GABA) A receptor, subunit alpha 1	GABRA1-F	AAAAGTCGGGGTCTCTCTGA C
	GABRA1-R	CAGTCGGTCCAAAATTCTTG TGA
gamma-aminobutyric acid (GABA) A receptor, subunit alpha 2	GABRA2-F	GGACCCAGTCAGGTTGGTG
	GABRA2-R	TCCTGGTCTAAGCCGATTAT CAT
gamma-aminobutyric acid (GABA) A receptor, subunit alpha 3	GABRA3-F	ATGTGGCACTTTTATGTGAC CA
	GABRA3-R	CCCCAGGTTCTTGTCGTCTT G
gamma-aminobutyric acid (GABA) A receptor, subunit alpha 4	GABRA4-F	ACAATGAGACTCACCATAA GTGC
	GABRA4-R	GGCCTTTGGTCCAGGTGTAG
gamma-aminobutyric acid (GABA) A receptor, subunit alpha 5	GABRA5-F	TGACCCAAACCCTCCTTGTC T
	GABRA5-R	GTGATGTTGTCATTGGTCTC GT
gamma-aminobutyric acid (GABA) A receptor, subunit gamma 1	GABRg1-F	TGTGGAGTCAAAGTAGAGG AGTG
	GABRg1-R	TTCCCAGATGCAGGGTTAGT A
gamma-aminobutyric acid (GABA) C receptor, subunit rho 1	GABRR1-F	CGAGGAGCACACGACGATG
	GABRR1-R	GTGAAGTCCATGTCAACCTC TG
gamma-aminobutyric acid (GABA) receptor, rho 3	GABRR3-F	TTACCTCAGGCACTATTGGA AGG

	GABRR3-R	CGGGATGTACTCGAAGCAT GATA
glutamic acid decarboxylase 1	Gad1-F	CACAGGTCACCCTCGATTTT T
	Gad1-R	ACCATCCAACGATCTCTCTC ATC
glial fibrillary acidic protein	GFAP-F	TGGAGGAGGAGATCCAGTT C
	GFAP-R	AGCTGCTCCCGGAGTTCT
glutaminase	Gls-F	CTACAGGATTGCGAACATCT GAT
	Gls-R	ACACCATCTGACGTTGTCTG A
glutamate dehydrogenase 1	Glud1-F	CCCAACTTCTTCAAGATGGT GG
	Glud1-R	AGAGGCTCAACACATGGTT GC
high mobility group box 1	HMGB1-F	GGCGAGCATCCTGGCTTATC
	HMGB1-R	GGCTGCTTGTCATCTGCTG
hypoxanthine guanine phosphoribosyl transferase	HPRT-F	GACCGGTCCCGTCATGC
	HPRT-R	TCATAACCTGGTTCATCATC GC
ionized calcium binding adapter molecule 1	Iba1-F	GGATTTCAGGGAGGAAAA
	Iba1-R	TGGGATCATCGAGGAATTG
inhibitor of kappaB kinase beta	IKBKB-F	ACAGCCAGGAGATGGTACG
	IKBKB-R	CAGGGTGACTGAGTCGAGA C
inhibitor of kappaB kinase gamma	IKBKG-F	AAGCACCCCTGGAAGAACC
	IKBKG-R	CCTGCTCTGAAGGCAGATGT A
interleukin 12a	IL-12p35-F	ATGACCCTGTGCCTTGGTAG
	IL-12p35-R	GATTCTGAAGTGCTGCGTTG
interleukin 18	IL-18-F	AGAAGACTCTTGCCTCAACT TC
	IL-18-R	AACGAAGAGAACTTGGTCA TTTAT
interleukin 1 beta	IL-1 β -F	GATCCACACTCTCCAGCTGC A
	IL-1 β -R	CAACCAACAAGTGATATTCT CCATG

interleukin 33	IL-33-F	TCCAACCTCCAAGATTTCCTCCG
	IL-33-R	CATGCAGTAGACATGGCAGAA
interleukin 4	IL-4-F	GTCTGTAGGGCTTCCAAGGT
	IL-4-R	CGAAAGAGTCTCTGCAGCTC
interleukin 6	IL-6-F	TAGTCCTTCCTACCCCAATT
	IL-6-R	TTGGTCCTTAGCCACTCCTTC
inducible nitric oxide synthase	iNOS-F	TGACGGCAAACATGACTTCAG
	iNOS-R	GCCATCGGGCATCTGGTA
interferon regulatory factor 5	IRF5-F	AGAGACAGGGAAGTACACTGAAG
	IRF5-R	TGGAAGTCACGGCTTTTGTTAAG
glutamate receptor, ionotropic, kainate 1 (Grik1)	KA1-F	TCACACCCTACGAGTGGTATAAC
	KA1-R	AGCTCCAACGCCAAACCAG
glutamate receptor, ionotropic, kainate 2 (Grik2)	KA2-F	CAGCGTCGGCTCAAACATAAG
	KA2-R	GGTTTCTTTACCTGGCAACCTT
glutamate receptor, ionotropic, kainate 3 (Grik3)	KA3-F	AGGTCCTAATGTCACTGACTCTC
	KA3-R	TGCCATAAAGGGTCCTATCAGAC
glutamate receptor, ionotropic, kainate 4 (Grik4)	KA4-F	CCCTGAGGATTGCTGCTATCT
	KA4-R	CACCCTTGGGGAGGATCTGA
solute carrier family 1 (glial high affinity glutamate transporter) (Slc1a2)	mGLT1-F	GCACGAGAGCTATGGTGTA
	mGLT1-R	GTCTGGGATTACCTGGGTGGA
glutamate receptor, metabotropic 1 (Grm1)	mGluR1-F	TGGAACAGAGCATTGAGTTCATC
	mGluR1-R	CAATAGGCTTCTTAGTCCTGCC
glutamate receptor, metabotropic 2 (Grm2)	mGluR2-F	GCTCCACAGCTATCACCG
	mGluR2-R	TCATAACGGGACTTGTCGCTC
glutamate receptor, metabotropic 3 (Grm3)	mGluR3-F	CTGGAGGCCATGTTGTTTGC

glutamate receptor, metabotropic 4 (Grm4)	mGluR3-R	CATCCACTTTAGTCAACGAT GCT
	mGluR4-F	CCCATACCCATTGTCAAGTT GG
	mGluR4-R	TGTAGCGCACAAAAGTGAC CA
glutamate receptor, metabotropic 5 (Grm5)	mGluR5-F	ACGACCATGACGACCTTCG
	mGluR5-R	GGCAGGTGATACCCCTGTC
glutamate receptor, metabotropic 7 (Grm7)	mGluR7-F	ACACGGATCGCAAATGCAC
	mGluR7-R	CTCCCCGGTAGTCAGCACA
glutamate receptor, metabotropic 8 (Grm8)	mGluR8-F	ATGGTTTGTGAGGGAAAGC G
	mGluR8-R	GAATGGGCATACTCCTGGCT
nuclear factor of kappa light polypeptide gene enhancer in B cells 1	NFKB1-F	ATGGCAGACGATGATCCCT AC
	NFKB1-R	TGTTGACAGTGGTATTTCTG GTG
nuclear factor of kappa light polypeptide gene enhancer in B cells (NF-kB) inhibitor, beta	NFKBIB-F	GCGGATGCCGATGAATGGT
	NFKBIB-R	TGACGTAGCCAAAGACTAA GGG
NLR family, pyrin domain containing 3	NLRP3-F	ATTACCCGCCCGAGAAAGG
	NLRP3-R	TCGCAGCAAAGATCCACAC AG
glutamate receptor, ionotropic, NMDA1 (zeta 1) (Grin1)	NMDA1-F	ATGCACCTGCTGACATTCG
	NMDA1-R	TATTGGCCTGGTTTACTGCC T
glutamate receptor, ionotropic, NMDA2A (epsilon 1) (Grin2a)	NMDA2A-F	ACGTGACAGAACGCGAACT T
	NMDA2A-R	TCAGTGCGGTTCAATAA CG
glutamate receptor, ionotropic, NMDA2B (epsilon 2) (Grin2b)	NMDA2B-F	GCCATGAACGAGACTGACC C
	NMDA2B-R	GCTTCCTGGTCCGTGTCATC
glutamate receptor, ionotropic, NMDA2C (epsilon 3) (Grin2c)	NMDA2C-F	GGGATCTGCCATAACGAGA AG
	NMDA2C-R	GCACTGAGTGTCGAAGTTTC CA

glutamate receptor ionotropic, NMDA3A (Grin3a)	NMDA3A	AGAGCCAGGGCGAAATGAT
	-F	G
	NMDA3A	GGAAACTCGTGGCGCACTA
	-R	
tumor protein p53	p53-F	GCGTAAACGCTTCGAGATGT
		T
	p53-R	TTTTTATGGCGGGAAGTAGA
		CTG
nuclear factor NF-kappa-B p65 subunit	p65-F	GATTGAAGAGAAGCGCAAA
		A
	p65-R	CAGAAAGTTGAGTTTCGGGTA
serine peptidase inhibitor, clade G, member 1	SERPING	TAGAGCCTTCTCAGATCCCG
	1-F	A
	SERPING	ACTCGTTGGCTACTTTACCC
	1-R	A
solute carrier family 6 (neurotransmitter transporter), member 1 (Slc6a1)	SLC6-F	GAAAGCTGTCTGATTCTGAG
		GTG
	SLC6-R	AGCAAACGATGATGGAGTC
		CC
suppressor of cytokine signaling 2	SOCS2-F	AGTTCGCATTTCAGACTACCT
		ACT
	SOCS2-R	TGGTACTCAATCCGCAGGTT
		AG
signal transducer and activator of transcription 6	STAT6-F	CTCTGTGGGGCCTAATTTC
		A
	STAT6-R	GCATCTGAACCGACCAGGA
		AC
toll-like receptor 1	TLR1-F	TGAGGGTCCTGATAATGTCC
		TAC
	TLR1-R	AGAGGTCCAAATGCTTGAG
		GC
toll-like receptor 2	TLR2-F	GCAAACGCTGTTCTGCTCAG
		AGGCGTCTCCCTCTATTGTA
	TLR2-R	TT
toll-like receptor 3	TLR3-F	GTGAGATACAACGTAGCTG
		ACTG
	TLR3-R	TCCTGCATCCAAGATAGCAA
		GT
toll-like receptor 4	TLR4-F	ACTGTTCTTCTCCTGCCTGA
		CA
	TLR4-R	GGACTIONGCTGAGTTTCTGA
		TCC
toll-like receptor 9	TLR9-F	ATGGTTCTCCGTCGAAGGAC
		T

	TLR9-R	GAGGCTTCAGCTCACAGGG
transmembrane protein 106a	Tmem106	AGCTCACCTCTCGGAAGGAT
	a-F	
	Tmem106	AGTCACAAAGCTGGAACTA
transmembrane protein 106b	a-R	GC
	Tmem106	ATGGCGTCTGTGTTTGTCTG
	b-F	
transmembrane protein 119	Tmem106	CGTAGCTGACATAGGCTGAT
	b-R	TTT
	Tmem119	CCTACTCTGTGTCACTCCCG
transmembrane protein 132d	-F	
	Tmem119	CACGTACTGCCGGAAGAAA
	-R	TC
transmembrane protein 163	Tmem132	CCGTGCTCATCAGCCTAGC
	d-F	
	Tmem132	GGTGACCGGGAGGTAGGTA
transmembrane protein 168	d-R	G
	Tmem163	GGGTTGGAAGACCGAGGTT
	-F	TA
transmembrane protein 16a	Tmem163	GCCAGGGTGACAATAATGG
	-R	ACA
	Tmem168	TCACTGCGATACTGTGTTAG
transmembrane protein 16b	-F	TCA
	Tmem168	GCCACAAGTAAGTTGATCCT
	-R	GG
transmembrane protein 175	Tmem16a-	CCCGTGCCAGTCACCTTTTT
	F	
	Tmem16a-	TCATCTGCTTCCGTTTCCAG
transmembrane protein 176a	R	T
	Tmem16b	TTTATGATTGCCCTGACGTT
	-F	CTC
transmembrane protein 176b	Tmem16b	GAGGTTGATGATGACTGCTG
	-R	TT
	Tmem175	TCCCCTTGTCATACCTGCTG
transmembrane protein 176a	-F	A
	Tmem175	AGACTCCCCTTTGGCCCATCA
	-R	
transmembrane protein 176b	Tmem176	GCCGGATGCTCATTGCTAAG
	a-F	
	Tmem176	ATGGCCTATGTAGAGGGTTC
transmembrane protein 176b	a-R	C
	Tmem176	CCAGTCCGCTCACATCAGC
	b-F	

	Tmem176	CTGGGTCACCCCTAGAGAC
	b-R	AG
transmembrane protein 229a	Tmem229	GCCTCTACTTCTACGGGATG
	a-F	C
	Tmem229	GGTAGGGTGAAGAGAAACC
	a-R	CA
transmembrane protein 229b	Tmem229	ATGCTATCCACGGCTACTTC
	b-F	T
	Tmem229	TGGAGGTGCCATAGATGAA
	b-R	GA
transmembrane protein 230	Tmem230	CTCCCCAGCAGTAAAGTCA
	-F	AAT
	Tmem230	ACGGTAGCAAGGGCAATGG
	-R	
transmembrane protein 45a	Tmem45a-	TGGGCTTTTGGTGGACTATG
	F	A
	Tmem45a-	CCAGCTATACCAGTGAGAG
	R	ACA
transmembrane protein 59	Tmem59-	CGCTGCTGCTGTTGACTATG
	F	
	Tmem59-	GTAGGTGTGCAAGGGGTAG
	R	G
transmembrane protein 97	Tmem97-	TCTACTTCGTCTCGCACATC
	F	C
	Tmem97-	GAAGGACTTGAACCACACT
	R	GG
tumor necrosis factor alpha	TNF α -F	AATGGCCTCCCTCTCATCAG
		T
	TNF α -R	CTACAGGCTTGTCACTCGAA
tumor necrosis factor receptor-associated factor 6	TRAF6-F	AAAGCGAGAGATTCTTTCCC
		TG
	TRAF-6-R	ACTGGGGACAATTCACTAG
		AGC
triggering receptor expressed on myeloid cells 2	TREM2-F	CTGGAACCGTCACCATCACT
		C
	TREM2-R	CGAAACTCGATGACTCCTCG
		G
vesicular glutamate transporter-3 (Slc17a8)	VGLUT3-	AGAATGCCGTGGGAGACTC
	F	
	VGLUT3-	CAGTCACAGATGTACCGCTT
	R	G
vasicular inhibitory amino acid transporter	VIAAT-F	ACCTCCGTGTCCAACAAGTC
	VIAAT-R	CAAAGTCGAGATCGTCGCA
		GT

secretory protein precursor	Ym1-F	CAGGTCTGGCAATTCTTCTG AA
	Ym1-R	GTCTTGCTCATGTGTGTAAG TGA

References

Binley KE, Ng WS, Tribble JR, Song B, Morgan JE. 2014. Sholl analysis: a quantitative comparison of semi-automated methods. *Journal of Neuroscience Methods*, **225**: 65–70.