Supplemental Material

A Unique Subtype of Microglia in Degenerative Thalamus after Cortical Stroke

Supplementary Material and Methods

Distal middle cerebral artery occlusion model

Mice were anesthetized with 5% isoflurane for induction, subsequently maintained with 2% isoflurane in a mixture of 0.8 L/min of air and 0.2 L/min of oxygen. The distal left middle cerebral artery was accessed via a craniotomy, cauterized, and cut just proximal to the anterior and posterior branches [20]. Core body temperature was measured by a rectal probe and maintained at 36.5±0.5°C throughout the surgery. Buprenorphine (0.01mg/kg-body weight) and 0.9% saline were administered subcutaneously before the surgery. Body weights were recorded for three days after the surgery to monitor the recovery.

Histology

Brain section preparation and immunostaining procedure were performed as described previously [19]. Mice were sacrificed and applied with transcardial perfusion of cold phosphate buffered saline (PBS) and 3% paraformaldehyde (PFA). 30 μm coronal cryosections were used in following studies. Histology staining and image quantification were done blinded to the experimental groups.

Fluoro-Jade C (FJC) staining

Degenerative injury was detected by FJC staining, using FJC Ready-to-Dilute Staining Kit (TR-100-FJ; Biosensis Inc., Thebarton, SA, Australia) following the manufacturer's instructions. Briefly, the mounted brain sections were immersed sequentially in solutions of 1% sodium hydroxide diluted in 80% ethanol, 0.06% potassium permanganate and 0.0001% FJC. Slides were then washed with distilled water, cleared in xylene and cover slipped.

Immunofluorescence staining

Brain sections were stained with antibodies including NeuN, MAP2, CD68, GFAP, Tmem119, Iba-1 and CD11c, as listed in **Supplementary Table II**. For NeuN/MAP2 and Tmem119/Iba-

1immunostaining, antigen retrieval was applied in a pre-heated buffer (0.5M sodium citrate, 0.05% Tween-20 in water) for 20 minutes at 60°C. The sections were then processed in the following order: blocking buffer (5% normal goat/donkey serum and 1% bovine serum albumin in 0.3% PBS-triton) for one hour at room temperature, primary antibody diluted in the blocking buffer overnight at 4°C and secondary antibody diluted in the blocking buffer for two hours at room temperature. Hoechst 33342 (1:5000; Invitrogen, Carlsbad, CA, USA) was used for nuclei staining. Sections were then washed in PBS, mounted in Fluoromount (F4680; Sigma-Aldrich, St. Louis, MO) and cover-slipped.

Imaging and image quantification

Brain sections were imaged by Zeiss LSM 800 confocal microscope with the Zen software (Carl Zeiss AB, Stockholm, Sweden). Using coordinates from the Paxinos and Franklin mouse brain atlas [21] full section images at positions of +1mm and -2mm from bregma were acquired by tile imaging. 10x Z-stack images were taken at peri-infarct area of the somatosensory cortex on sections of +1mm from bregma and at ipsilesional somatosensory thalamus on sections of -2mm from bregma. Positively stained areas and contralesional thalamus (cTH) areas were outlined from full section images and measured by Adobe Photoshop CS6 (Adobe Systems Incorporated, San Jose, CA, USA) [8]. Percentage of positively stained areas were calculated by positively stained areas divided by cTH areas. In 10x Z-stack images (areas 0.64x0.64mm^2), neuron numbers were counted using size thresholding 35μm^2 and fluorescent intensity were calculated by integrated density (IntDensity) in ImageJ (NIH). The numbers of CD11c$^+$ and Iba-1$^+$ co-stained cells were counted by Cell Counter in ImageJ (NIH).

RNA extraction

For dissected tissue samples, total RNA was extracted by using Trizol reagent (Invitrogen) followed by QIAGEN RNeasy Plus Mini kit (74134, QIAGEN, Valencia, CA, USA) as described previously [19]. For sorted cell samples, total RNA was extracted using QIAGEN RNeasy Plus Micro Kit (74034, QIAGEN).

Quantitative real-time PCR (qPCR)

For RNA extracted from tissue, cDNA was prepared using High-Capacity cDNA Reverse Transcription Kit (4368814; Applied Biosystems, Foster City, CA). For RNA extracted from sorted microglia, cDNA was prepared using SuperScript™ III Reverse Transcriptase (18080093, Invitrogen). qPCR was performed with TaqMan gene expression assays (listed in **Supplementary Table I**). qPCR data were analyzed by using 2^ (-ΔΔCt) method. GAPDH and Ywhaz were used as housekeeping genes.

NanoString transcriptome analysis

Quality control, normalization and data analysis were performed by nSolver™ Analysis Software (nSolver 4.0 and Advanced Analysis 2.0). Counts for target genes were normalized to the best fitting house-keeping genes as determined by nSolver software. The list of house-keeping genes is listed in **SupTable I**. Differentially expressed genes (DEGs) were defined as normalized counts data that were significantly up or down-regulated relative to naïve samples based on $P< 0.05$ and Benjamini-Hochberg adjusted $P< 0.2$.

Hierarchical cluster, cell type enrichment analysis and pathway analysis

Hierarchical cluster and cell type enrichment analysis were conducted with R package (Version 3.6). Principle component analysis (PCA) and heatmaps with hierarchical clustering were conducted with Manhattan distance and Ward2 method using DEGs as input. To identify enriched cell types, genes sets were tested for over-representation using a hypergeometric method with a

dataset downloaded from MSigDB (SCSig collection: Signatures of Single Cell Identities; https://www.gsea-msigdb.org/gsea/msigdb/supplementary_genesets.jsp). The gene set that most closely resemble our tissue type was used: Manno_Midbrain_Neurotypes_hMgl [22]. The results were ranked by adjusted P-values ($P<0.05$) and top ten cell types were displayed. Further analysis of DEGs was conducted using Ingenuity Pathway Analysis (IPA) software (QIAGEN) to determine disease functions and significant canonical pathways.

Isolation of immune cells from brain

Immune cells were isolated from dissected iTH and cTH tissues as described previously [23]. In brief, a cohort of PD28 mice were performed with transcardial perfusion of 30 mL of cold HBSS under deep anesthesia with isoflurane. Five mice samples were pooled and homogenized in 7 mL glass homogenizer on ice with Douncing buffer (15mM HEPES, 0.5% glucose in Hank's balanced salt solution, HBSS). The suspensions were filtered through cell strainer (70µm, Falcon) and centrifuged (1250rpm for 5 minutes with brake, Centrifuge 5810 R, Eppendorf). Liquid supernatant was removed and 30% Percoll was used to remove myelin with centrifugation (1560rpm for 20 minutes without brake). The collected cells were then washed with 2% fetal bovine serum (FBS) in HBSS buffer.

Flow cytometry analysis

The isolated immune cells were incubated with 100µl Live/Death-Aqua Stain (1:50, L34957, Invitrogen) for 20 minutes on ice in the dark and then washed with 100µl 2% FBS in HBSS buffer. Cells were then incubated with the following antibodies in 100µl FC blocking buffer (Anti-Mouse CD6/CD32, eBioscience, San Diego, CA) for 30 minutes at 4°C: anti-mouse CD45, CD11b, Ly6G and Ly6C (details in **Supplementary Table II**). To ensure proper identification and gating of fluorochromes, fluorescence minus one (FMO) controls on brain immune cells were enlisted when

building multicolor flow cytometry panels. Flow cytometric analysis was performed on the BD LSR-II (Stanford Shared FACS Facility), and FlowJo version 10.5.3 (TreeStar Inc., Ashland, OR) was used for data analysis. The gates were set based on the unstained cells, and the compensation was achieved by single-color stained BD-Comp Beads (BD Biosciences, San Jose, CA).

Supplementary Figure Legends

Supplementary Figure I Degeneration in somatosensory cortex and thalamus after primary cortical ischemic injury. (A) Representative full coronal sections show Fluoro-Jade C (FJC) staining in somatosensory cortex. Enlarged images represent peri-infarct area in stroke and corresponding areas in naïve (square labeled region in full section images). Scale bar = 250μm. (B) Representative full coronal sections show FJC staining in thalamus. Enlarged images represent the square labeled region in full section images. Scale bar = 250μm. The percentage of FJC positive area (C) and the integrated density of FJC (D) in ipsilesional thalamus (iTH) were quantified and compared to naive. N=4-5/time-point. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. Data are expressed as mean ± SEM.

Supplementary Figure II Neuron loss in somatosensory cortex after primary cortical ischemic injury. Representative full coronal sections show NeuN (green) and MAP2 (orange) immunostaining in somatosensory cortex. Enlarged images represent peri-infarct area in stroke and corresponding areas in naïve (square labeled region in full section images). Scale bar = 250μm.

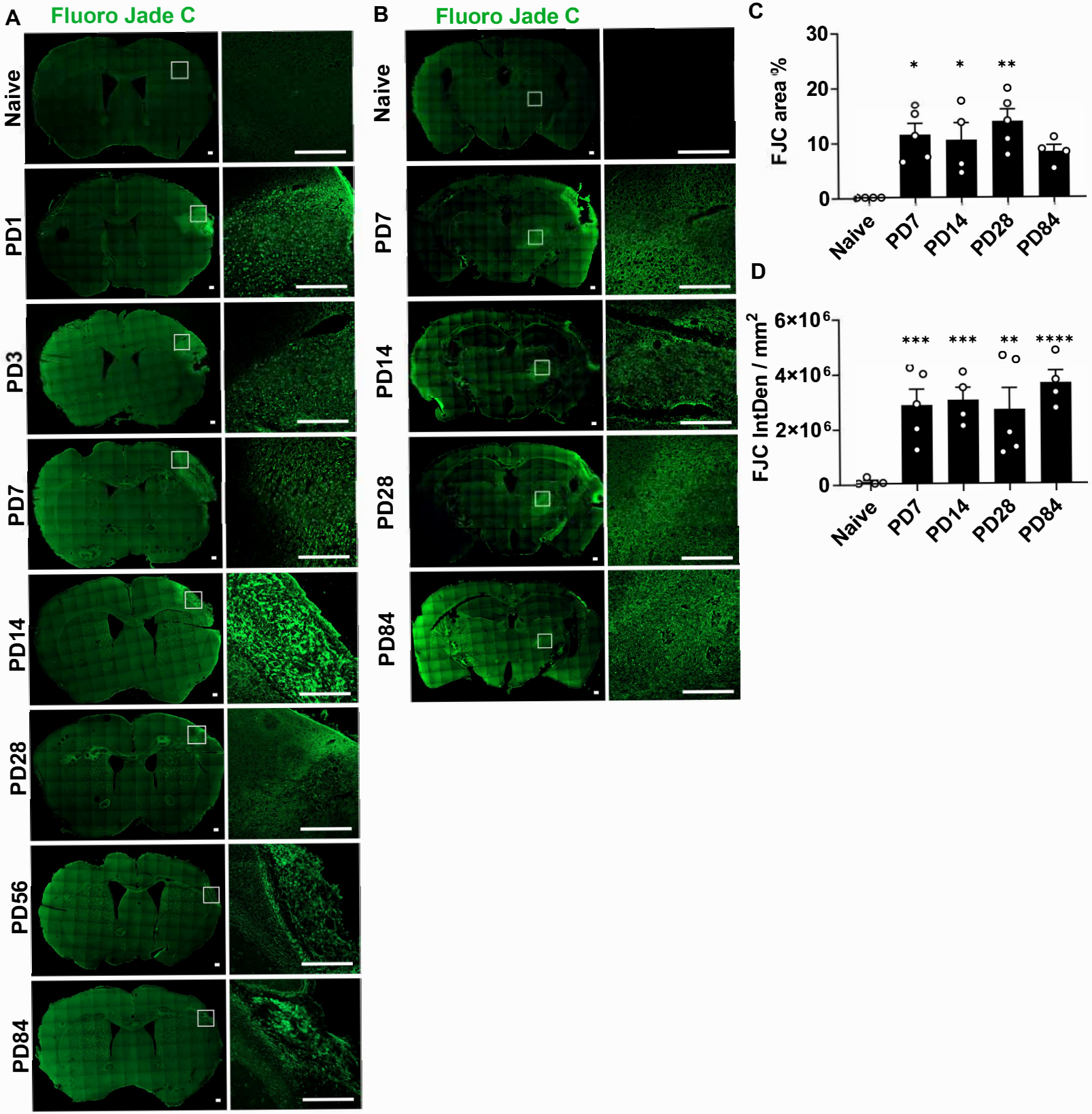
Supplementary Figure III Targeted transcriptome analysis of somatosensory cortex and thalamus after cortical ischemic stroke. (A) Principal component analysis (PCA) plots of DEGs expression show the characteristics of samples in iS1 at PD7, iS1 at PD28, iTH at PD7 and iTH at PD28 compared to naïve samples from corresponding regions respectively. (B) Bar graphs indicate enriched cell types of DEGs in iS1 at PD28 and in iTH at PD7, plotted with -log10(p-value) and number of genes in the cell type. OPC: oligodendrocyte progenitor cell. (C) qPCR validated DEGs in neuroinflammation signaling (*CSF1*, *TLR2* and *TREM2*) in iTH. N=3 in naïve, 4 in PD7 and 4 in PD28. *$P < 0.05$, **$P < 0.01$. Data are expressed as mean ± SEM.

Supplementary Figure IV Neuroinflammation in somatosensory cortex and thalamus after primary cortical ischemic injury. (A) Representative full coronal sections show CD68 (red) and GFAP (green) immunostaining in somatosensory cortex. Enlarged images represent peri-infarct area in stroke and corresponding areas in naïve (square labeled region in full section images). Scale bar = 250μm. (B) Representative full coronal sections show CD68 (red) and GFAP (green) immunostaining in thalamus. Enlarged images represent the square labeled region in full section images. Scale bar = 250μm. The integrated density of CD68 (C) and the integrated density of GFAP (D) in iTH were quantified and compared to corresponding regions in naïve. N=4-5/time-point. $^{**}P < 0.01$, $^{****}P < 0.0001$. Data are expressed as mean ± SEM.

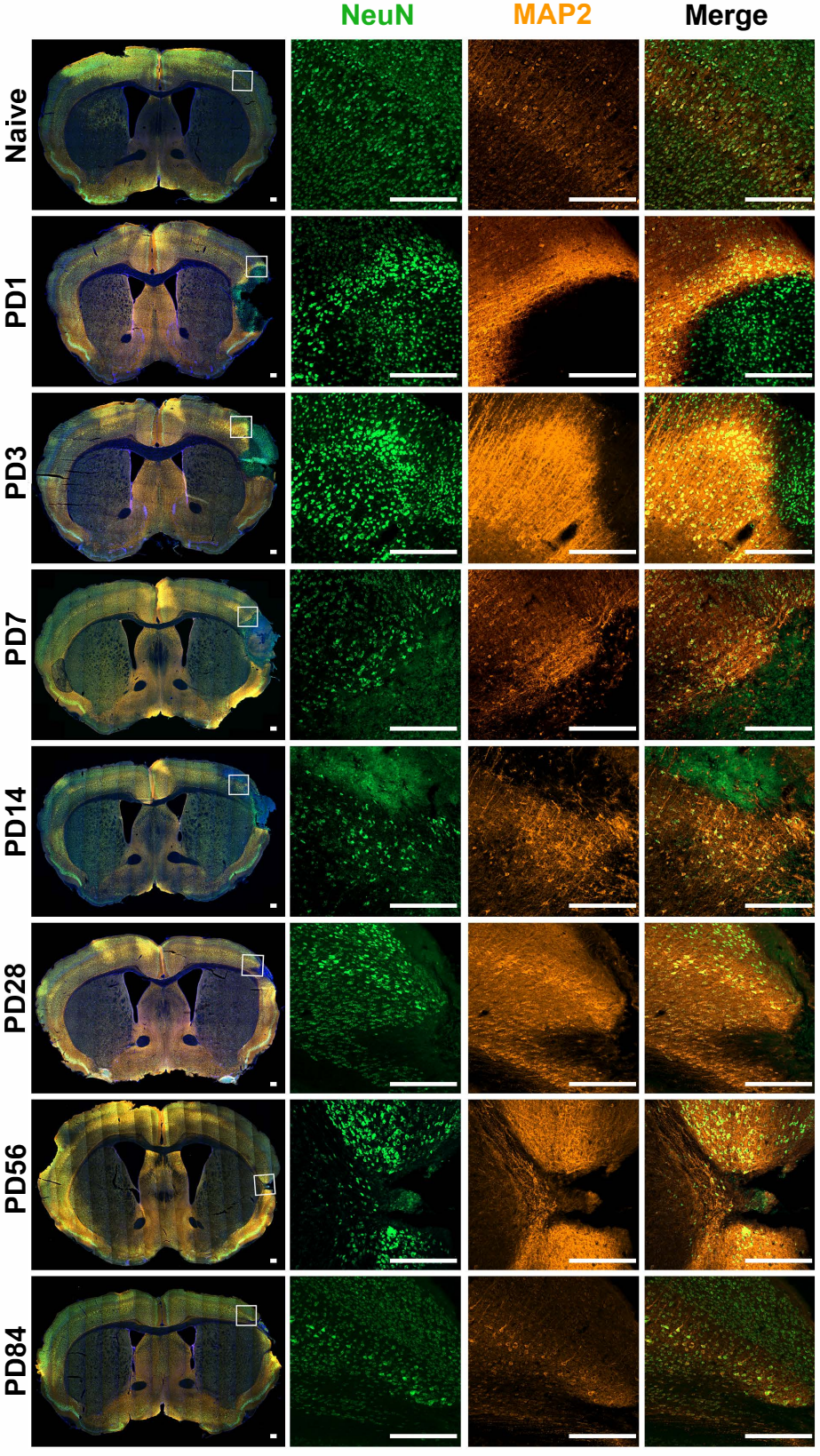
Supplementary Figure V Microglial activation in somatosensory cortex after primary cortical ischemic injury. Representative full coronal sections show Tmem119 (green) and Iba-1 (red) immunostaining in somatosensory cortex. Enlarged images represent peri-infarct area in stroke and corresponding areas in naïve (square labeled region in full section images). Scale bar = 250μm.

Supplementary Figure VI Flow cytometry analysis of leukocytes in primary and secondary injury regions. (A) Gating strategies for analysis leukocytes from iS1 and iTH. Lineage antibodies indicate $CD45^{int}CD11bLy6G^{-}Ly6C^{-}$ as microglia, $CD45^{high}CD11b^{+}$ as pMyeloid cells, $CD45^{high}CD11b^{-}$ as lymphocytes. SSC-A, side scatter area; FSC-A, forward scatter area; FSC-H, forward scatter height; pMyeloid cells, peripheral myeloid cells. Percentage of myeloid cells and lymphocytes at PD28 in (B) iS1 and (C) iTH. N=3 with each sample pooled from five mice. $^{***}P < 0.001$, $^{****}P < 0.0001$. Data are expressed as mean ± SEM.

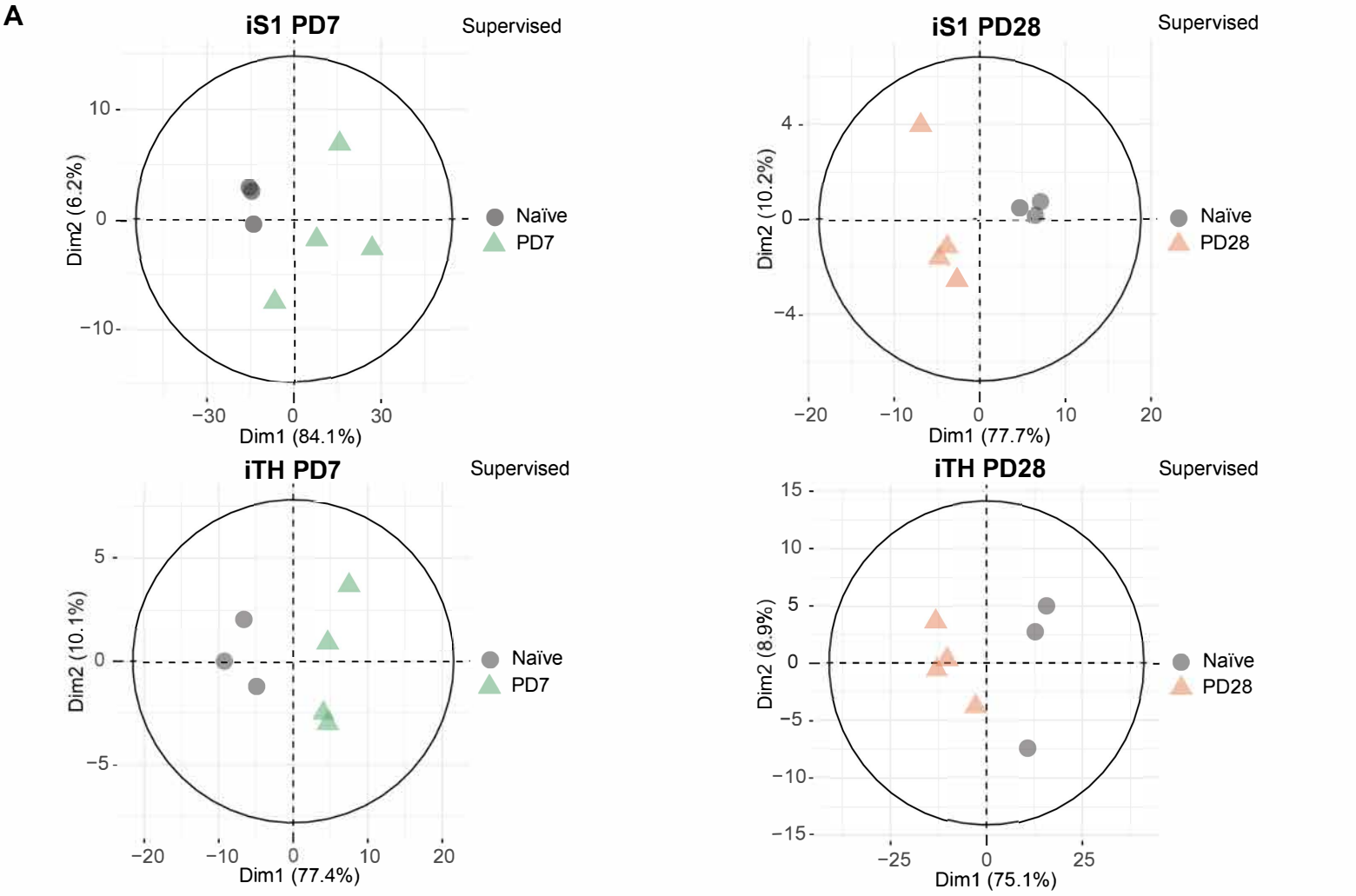
Supplementary Figure I

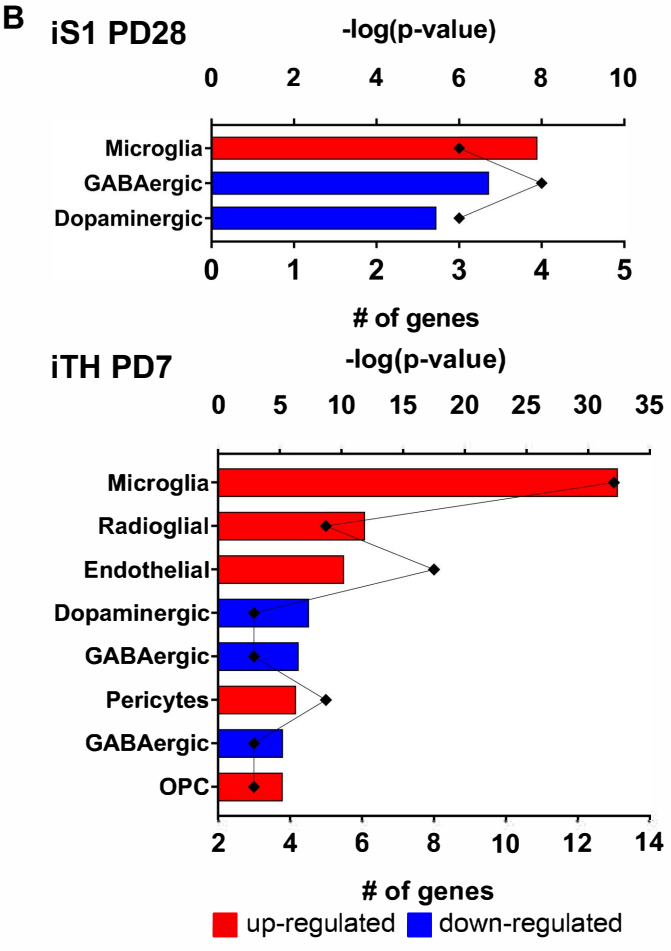


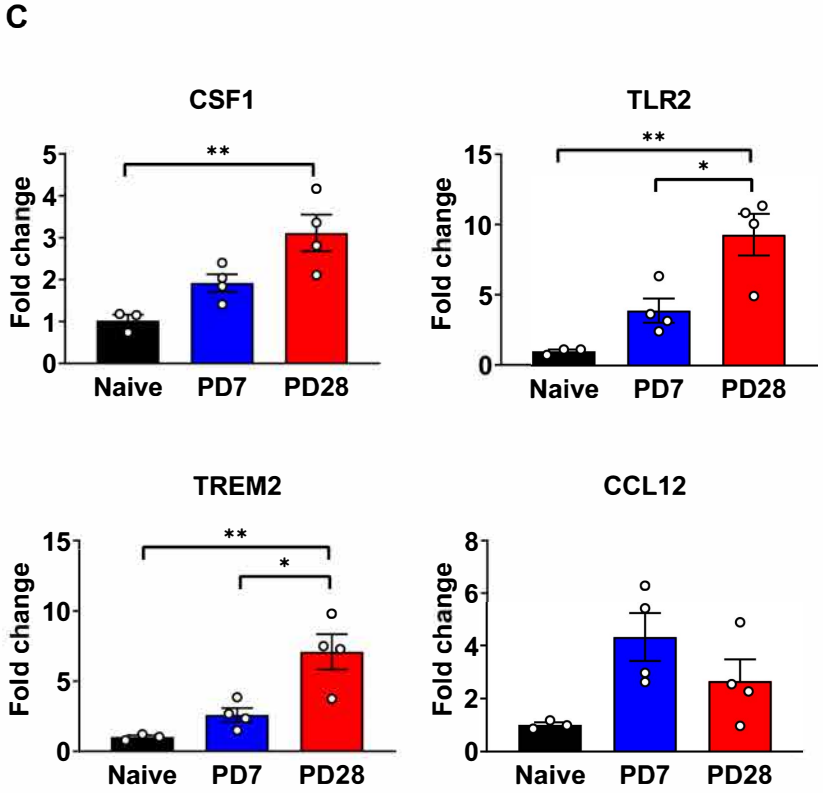
Supplementary Figure II



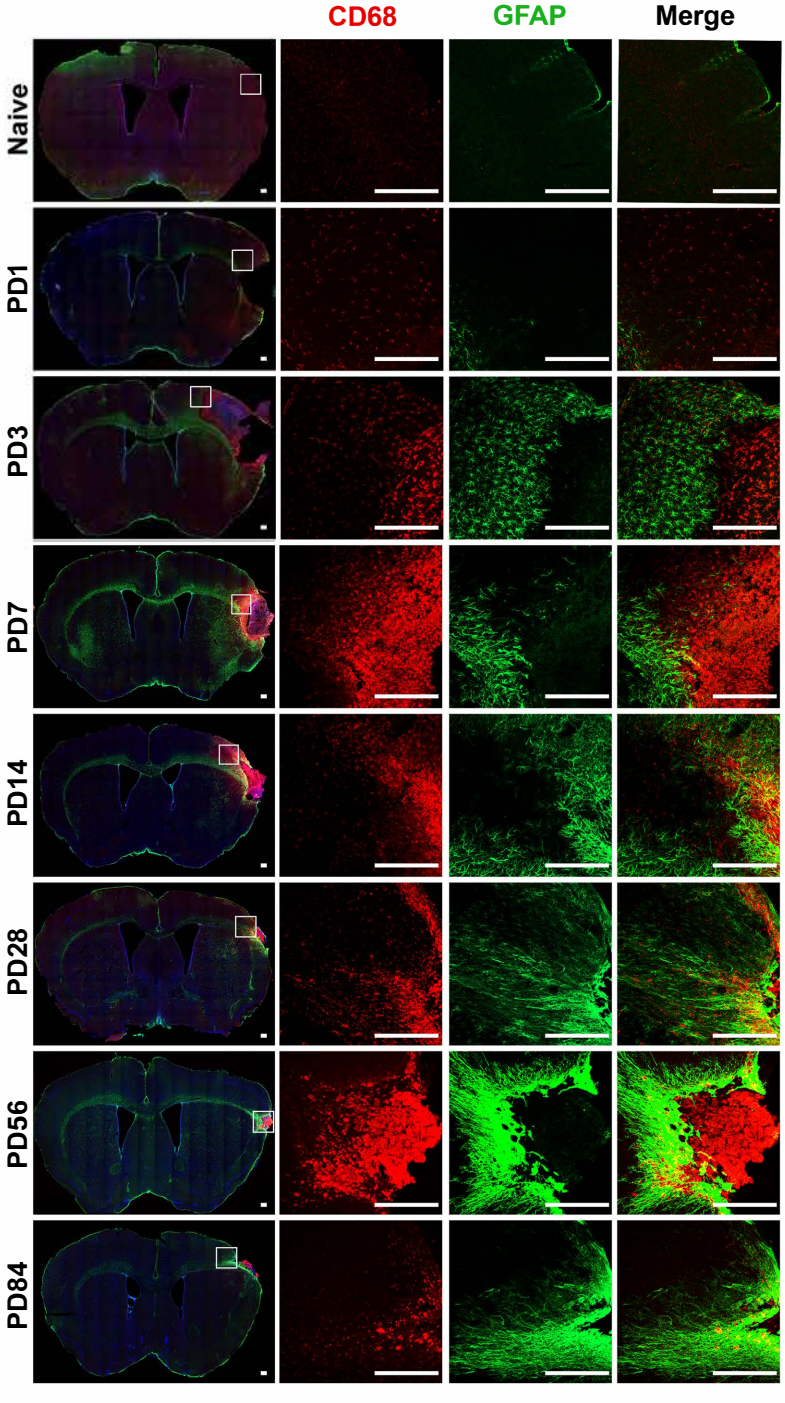
Supplementary Figure III

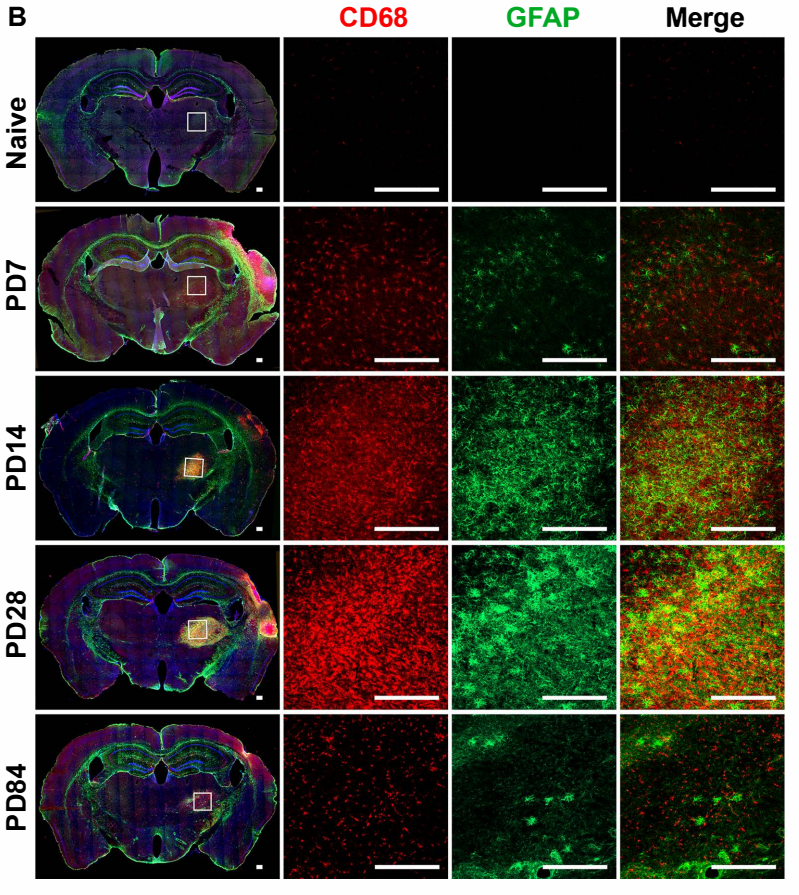


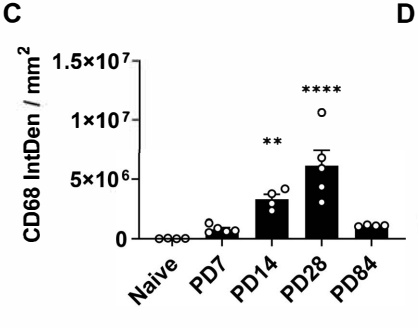


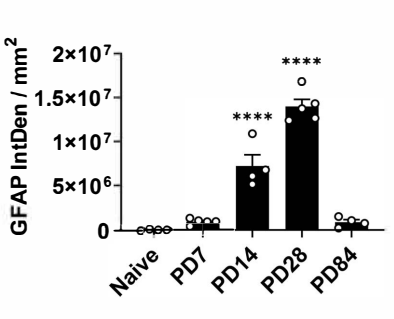


Supplementary Figure IV

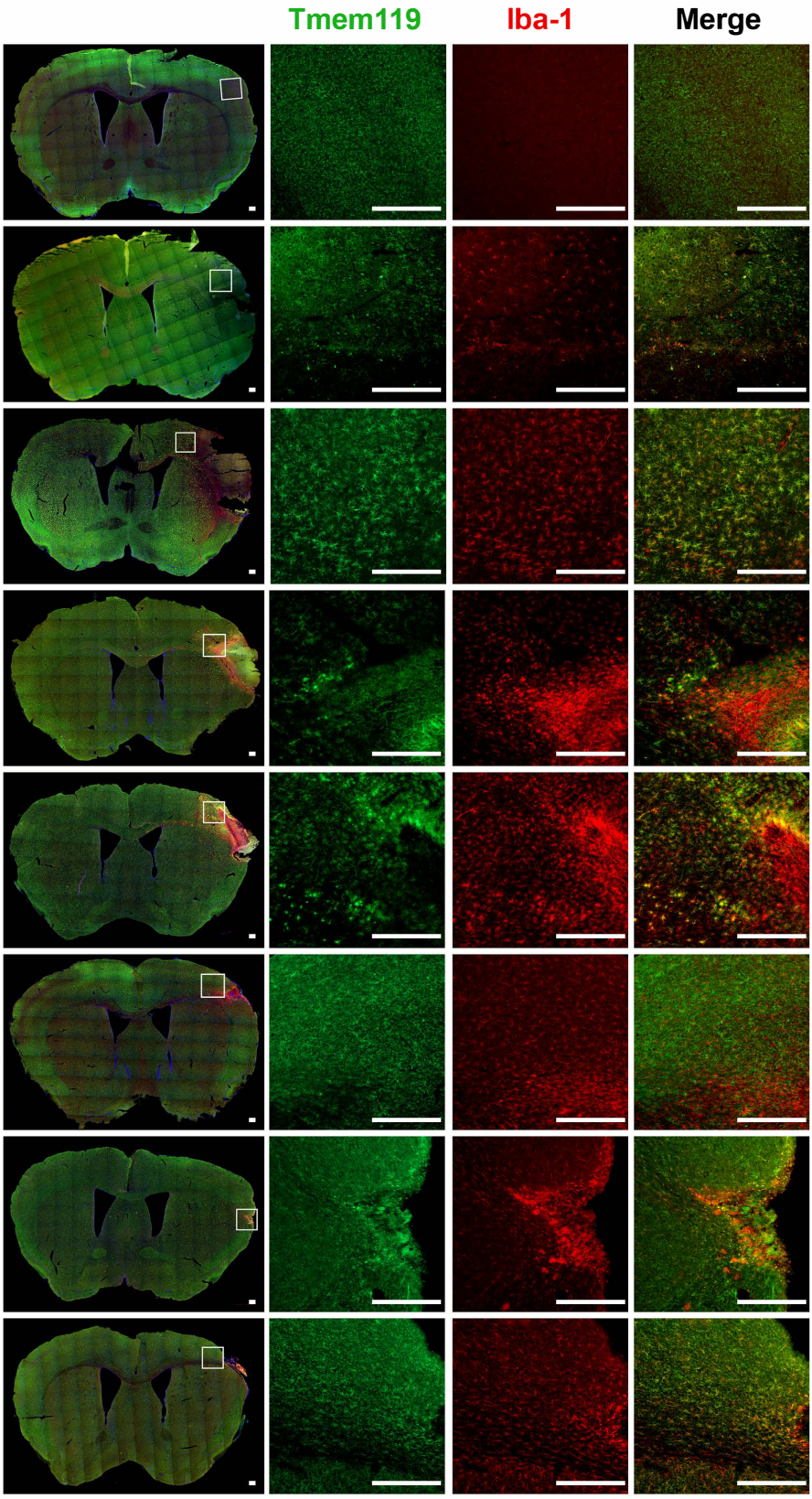




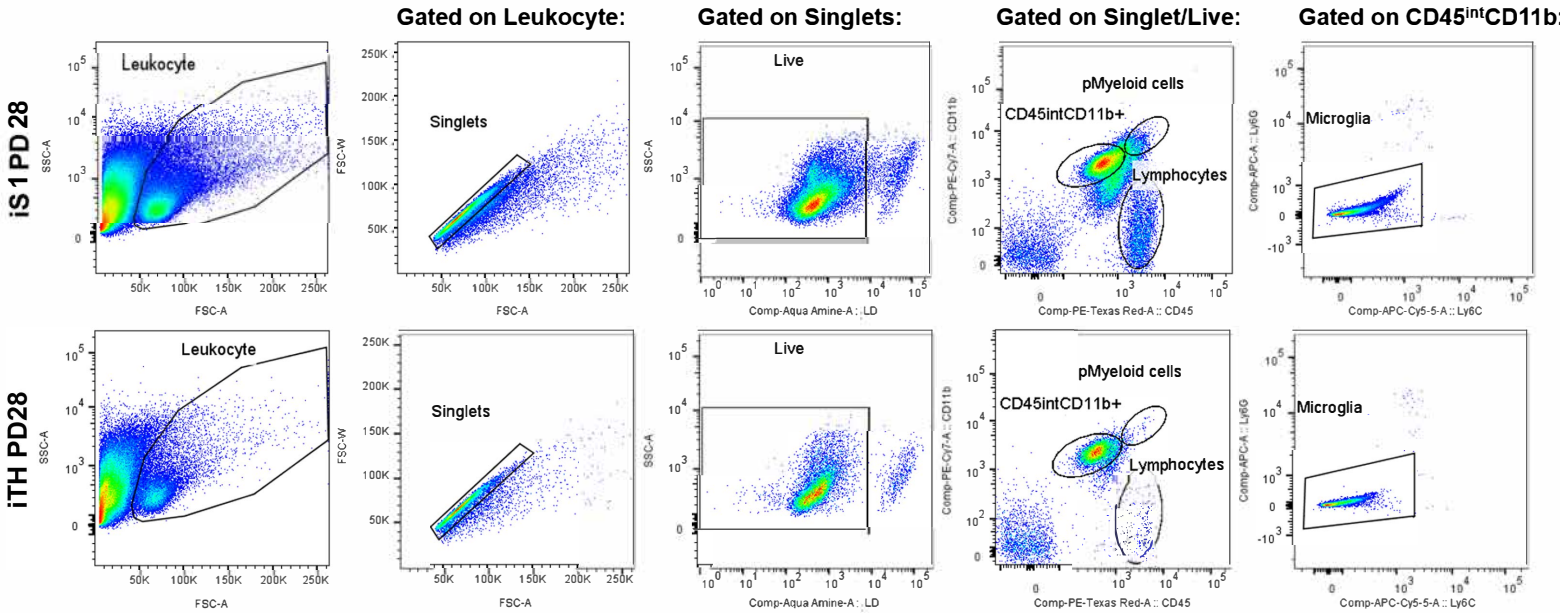


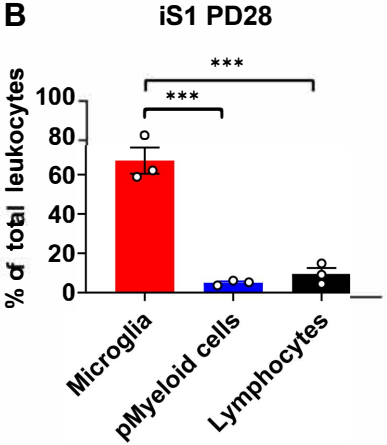


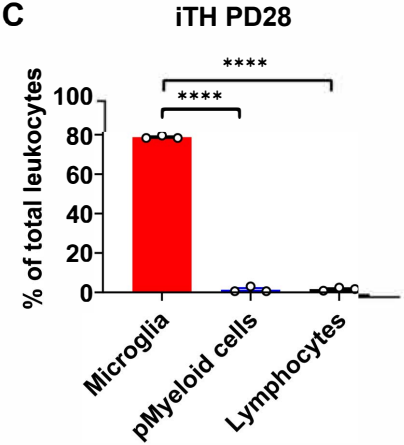
Supplementary Figure V



Supplementary Figure VI







Supplementary Table I Materials related to NanoString study

Spike in Genes added by nCounter CodeSet Design	
Gene Name	**Probe NSID**
Arg1	NM_007482.3:626
CCR3	NM_009914.4:664
CD86	NM_019388.3:251
Ifng	NM_008337.1:95
IL34	NM_001135100.1:848
Ki67	NM_001081117.2:4330
Mx1	NM_010846.1:2485
Psmc4	NM_011874.2:1146
SiglecH	NM_178706.4:446
Ywhaz	NM_011740.2:455
House Keeping Genes for NanoString Analysis	
Gene Name	**Probe NSID**
Chmp2b	NM_026879.2:920
Gtf2h1	NM_008186.4:830
Parp1	NM_007415.2:3020
Pik3cb	NM_029094.3:1970
Prpf3	NM_027541.4:1486
Psen1	NM_008943.2:2770
Stambpl1	NM_029682.4:1040
Tfam	NM_009360.4:790

U2af2	NM_133671.3:131
Primers for qPCR validation	
Gene Name	**Assay ID**
ApoE	Mm01307193_g1
Axl	Mm00437221_m1
CCL12	Mm01617100_m1
CSF1	Mm00432686_m1
CSF1R	Mm01266652_m1
Cst7	Mm00438351_m1
CX3CR1	Mm00438354_m1
GAPDH	Mm99999915_g1
Itgax	Mm00498701_m1
LpL	Mm00434764_m1
TGFb1	Mm00441729_g1
TLR2	Mm00442346_m1
Tmem119	Mm00525305_m1
TREM2	Mm04209424_g1
Ywhaz	Mm03950126_s1

Supplementary Table II Antibody details

Antibody	Source	Catalog#	Dilution
Primary antibodies for immunofluorescence staining			
rabbit-MAP2	Cell Signaling	8707	1:200
guinea pig-NeuN	Synaptic Systems	266004	1:200
rat-CD68	Abcam	ab53444	1:500
chicken-GFAP	Abcam	ab4647	1:500
rabbit-Tmem119	Abcam	ab209064	1:300
goat-Iba1	Abcam	ab5076	1:500
hamster-CD11c	Invitrogen	MA1-80129	1:100
rabbit-Iba1	Wako	019-19741	1:200
Secondary antibodies for immunofluorescence staining			
goat anti-rat (AF488)	Invitrogen	A11006	1:500
goat anti-chicken (AF594)	Invitrogen	A11039	1:500
goat anti-guinea pig (AF488)	Invitrogen	A11073	1:500
goat anti-rabbit (AF546)	Invitrogen	A11035	1:500
goat anti-rabbit (AF594)	Invitrogen	A11037	1:500
goat anti-hamster (AF488)	Invitrogen	A21110	1:500
donkey anti-goat (AF594)	Invitrogen	A11058	1:500
donkey anti-rabbit (AF488)	Invitrogen	A21206	1:500
Antibodies for flow cytometry analysis and fluorescence activated cell sorting			
CD11b PE Cy7	BD Pharmingen	552850	1:50
CD11c PE	Biolegend	117307	1:50

CD45 PE Texas Red	ThermoFisher	MCD4517	1:50
CD45 APC	Biolegend	103112	1:50
Ly6C AF700	BD Pharmingen	561237	1:50
Ly6G APC	Biolegend	127613	1:50

Supplementary Table III A list of differentially expressed genes (DEGs) in ipsilesional somatosensory cortex (iS1) at post-stroke day (PD) 7 compared with naïve mice (294 genes).

Gene	Log2 fold change	P-value	BH.p.value	probe.ID
Ccl12	5.91	<0.001	0.038	NM_011331.2:173
Cybb	5.18	0.005	0.038	NM_007807.2:1535
Cd68	5.14	<0.001	0.038	NM_009853.1:636
Prl	4.95	0.003	0.038	NM_011164.1:115
Mmp2	4.78	0.002	0.038	NM_008610.2:2376
Tlr2	4.67	0.001	0.038	NM_011905.2:255
C1qa	4.46	<0.001	0.038	NM_007572.2:566
Cd44	4.42	0.004	0.038	NM_009851.2:3075
Cxcl16	4.36	0.003	0.038	NM_023158.6:679
Trem2	4.34	0.001	0.038	NM_031254.2:646
Gusb	4.33	<0.001	0.038	NM_010368.1:1735
C1qc	4.27	0.001	0.038	NM_007574.2:708
Slc11a1	4.25	0.005	0.038	NM_013612.2:945
Fcrls	4.17	<0.001	0.038	NM_030707.3:925
C3	4.17	0.025	0.075	XM_011246258.1:2702
C1qb	4.15	0.001	0.038	NM_009777.2:865
Gfap	4.04	0.001	0.038	NM_001131020.1:610
Cd14	3.95	0.003	0.038	NM_009841.3:235
Icam1	3.95	0.005	0.038	NM_010493.2:2195
Ang	3.92	0.003	0.038	NM_007447.2:425

Tlr4	3.77	0.004	0.038	NM_021297.2:2510
Grn	3.7	0.001	0.038	NM_008175.3:2010
Irf8	3.6	0.002	0.038	NM_008320.3:2274
Hexb	3.52	0.001	0.038	NM_010422.2:805
Spi1	3.48	0.003	0.038	NM_011355.1:200
Psmb8	3.4	0.003	0.038	NM_010724.2:362
Klk6	3.38	0.006	0.040	NM_001164696.1:1053
Itgam	3.37	0.002	0.038	NM_008401.2:155
Ncf1	3.26	0.003	0.038	NM_001286037.1:970
Lrrc25	3.17	0.014	0.056	NM_153074.3:246
CD86	3.16	0.004	0.038	NM_019388.3:251
Cd9	3.09	0.002	0.038	NM_007657.3:620
Tnfrsf1b	3.08	0.006	0.041	NM_011610.3:3270
Msn	3.07	0.002	0.038	NM_010833.2:515
Hpgds	3.06	0.004	0.038	NM_019455.4:194
Tspo	2.96	0.004	0.038	NM_009775.4:241
Stab1	2.92	0.005	0.038	NM_138672.2:5890
Itgax	2.91	0.016	0.061	NM_021334.2:327
Ccr2	2.91	0.033	0.087	NM_009915.2:2965
Hmox1	2.85	0.011	0.052	NM_010442.2:610
Osmr	2.8	0.003	0.038	NM_011019.3:395
Ccr5	2.79	0.020	0.066	NM_009917.5:1340
Casp8	2.78	0.004	0.038	NM_009812.2:1463

Cdk2	2.74	0.002	0.038	NM_016756.4:831
Tcirg1	2.73	0.008	0.046	NM_001136091.1:1345
Igf1	2.69	0.009	0.046	NM_010512.4:6448
Naglu	2.67	0.005	0.038	NM_013792.2:2334
Tnfrsf1a	2.57	0.003	0.038	NM_011609.2:615
Cd33	2.54	0.004	0.038	NM_001111058.1:405
Cx3cr1	2.52	0.003	0.038	NM_009987.3:2696
Csf1r	2.46	0.003	0.038	NM_001037859.1:3655
Sp100	2.38	0.012	0.052	NM_013673.3:410
Mmp14	2.36	0.018	0.062	NM_008608.3:554
Pla2g4a	2.34	0.010	0.050	NM_008869.2:1525
Apoe	2.33	0.009	0.049	NM_001305844.1:903
Psmb9	2.31	0.030	0.082	NM_013585.2:540
Il10ra	2.3	0.008	0.044	NM_008348.2:2522
Tgfbr2	2.25	0.006	0.040	NM_009371.2:475
Tgfb1	2.22	0.018	0.062	NM_011577.1:1470
Npc2	2.13	0.003	0.038	NM_023409.4:3095
Tmem119	2.12	0.003	0.038	NM_146162.2:1550
Il13ra1	2.12	0.008	0.044	NM_133990.4:845
Nfe2l2	2.1	0.006	0.041	NR_132727.1:193
Tnc	2.08	0.012	0.052	NM_011607.1:5665
Trf	2.07	0.005	0.038	NM_133977.2:1940
Stat1	2.02	0.004	0.038	NM_009283.3:1590

Casp7	2.01	0.005	0.038	NM_007611.2:1468
Fn1	2	0.028	0.080	NM_010233.1:2627
Col4a1	1.96	0.040	0.097	NM_009931.2:4116
Man2b1	1.93	0.005	0.038	NM_010764.2:1658
Sgpl1	1.91	0.006	0.041	NM_009163.3:1200
Pmp22	1.88	0.004	0.038	NM_008885.2:395
Casp1	1.88	0.007	0.043	NM_009807.2:259
Ccnd1	1.81	0.004	0.038	NM_007631.1:2000
Ltbr	1.77	0.021	0.068	NM_010736.3:1962
Il4ra	1.73	0.011	0.051	NM_001008700.3:2908
P2ry12	1.7	0.004	0.038	NM_027571.3:439
Aqp4	1.69	0.010	0.049	NM_009700.2:130
Scamp2	1.66	0.009	0.047	NM_022813.3:526
P2rx7	1.55	0.012	0.052	NM_001038839.2:378
Myd88	1.55	0.014	0.057	NM_010851.2:1595
C4a	1.54	0.006	0.041	NM_011413.2:4186
Gsn	1.47	0.011	0.051	NM_146120.3:624
SiglecH	1.46	0.016	0.061	NM_178706.4:446
Casp6	1.41	0.013	0.054	NM_009811.3:360
Gjb1	1.39	0.003	0.038	NM_008124.2:113
Il1r1	1.37	0.013	0.054	NM_001123382.1:820
Csf1	1.37	0.016	0.059	NM_001113530.1:833
Lama2	1.36	0.013	0.055	NM_008481.2:208

P2rx4	1.31	0.020	0.066	NM_011026.2:1655
Atp6v0e	1.3	0.008	0.046	NM_025272.2:585
Gdpd2	1.3	0.020	0.067	NM_023608.3:1438
Myc	1.3	0.021	0.067	NM_010849.4:630
Cers2	1.25	0.004	0.038	NM_029789.1:1422
Atp7a	1.25	0.004	0.038	NM_001109757.2:1200
Il6ra	1.24	0.037	0.093	NM_010559.2:2825
Cln3	1.23	0.007	0.042	NM_001146311.1:378
Lamp1	1.18	0.005	0.039	NM_010684.2:2080
Gba	1.18	0.010	0.050	NM_001077411.1:820
Tnfrsf12a	1.15	0.012	0.052	NM_001161746.1:517
Nqo1	1.15	0.014	0.056	NM_008706.5:430
Mx1	1.11	0.003	0.038	NM_010846.1:2485
Stx2	1.08	0.001	0.038	NM_007941.2:225
Itpr2	1.06	0.014	0.056	NM_010586.1:4365
Serpinb6a	1.06	0.021	0.068	NM_001164117.1:1418
Ugt8a	1.05	0.017	0.061	NM_011674.4:138
Pla2g2f	1.05	0.038	0.094	NM_012045.4:1802
Adrb2	1.03	0.021	0.068	NM_007420.2:680
Stat3	1	0.012	0.052	NM_213659.2:1360
Hspb1	0.991	0.025	0.075	NM_013560.2:630
Pfn1	0.987	0.013	0.054	NM_011072.4:266
Galc	0.927	0.017	0.061	NM_008079.3:1872

Cdkn1a	0.911	0.017	0.061	NM_007669.4:1670
Pllp	0.883	0.009	0.047	NM_026385.3:345
Sncaip	0.879	0.039	0.096	NM_001199151.1:1385
Sirt2	0.861	0.005	0.038	NM_022432.4:435
Trp53	0.853	0.011	0.051	NM_011640.1:1835
Idh1	0.853	0.016	0.059	NM_010497.2:495
Pla2g16	0.848	0.010	0.049	NM_139269.2:568
Pcna	0.837	0.006	0.041	NM_011045.2:590
Hif1a	0.833	0.008	0.045	NM_010431.2:1294
Rras	0.829	0.014	0.055	NM_009101.2:282
Mapk3	0.812	0.007	0.044	NM_011952.2:825
Mbp	0.807	0.023	0.071	NM_010777.3:761
Mag	0.804	0.037	0.092	NM_010758.2:1670
Tnfrsf11b	0.799	0.007	0.044	NM_008764.3:684
Mapkapk2	0.797	0.031	0.083	NM_008551.1:1991
Npc1	0.795	0.012	0.052	NM_008720.2:2645
Plekho2	0.791	0.018	0.062	NM_153119.2:406
Egfr	0.757	0.037	0.092	NM_207655.2:1335
Cln8	0.751	0.010	0.049	NM_012000.3:762
Aldh1l1	0.729	0.005	0.039	NM_027406.1:1340
Hdac1	0.717	0.031	0.083	NM_008228.2:470
Psen2	0.707	0.023	0.071	NM_001128605.1:560
Arrb2	0.68	0.011	0.052	NM_145429.4:725

Plxnb3	0.68	0.028	0.080	NM_019587.2:2862
Olig2	0.647	0.022	0.068	NM_016967.2:1740
Car2	0.646	0.026	0.077	NM_009801.4:437
Clu	0.639	0.029	0.080	NM_013492.2:354
Casp3	0.616	0.042	0.100	NM_009810.2:630
Hgf	0.611	0.018	0.063	NM_010427.5:483
Pls1	0.601	0.029	0.080	NM_001033210.3:1815
Pecam1	0.6	0.043	0.101	NM_008816.2:1100
Gnai2	0.597	0.027	0.078	NM_008138.4:971
Adam10	0.596	0.011	0.051	NM_007399.3:2390
Jam3	0.591	0.012	0.052	NM_023277.4:145
Bax	0.591	0.035	0.090	NM_007527.3:735
Ctns	0.587	0.009	0.047	NM_031251.4:1854
Acaa1a	0.581	0.003	0.038	NM_130864.3:626
Sp1	0.578	0.042	0.100	NM_013672.2:6580
S100b	0.56	0.019	0.063	NM_009115.3:1090
Cast	0.549	0.045	0.103	NM_009817.1:1820
Sri	0.548	0.007	0.042	NM_001080974.2:470
Atg5	0.541	0.005	0.038	NM_053069.5:774
Ccs	0.529	0.022	0.068	XM_006531645.1:185
Mmrn2	0.525	0.043	0.100	NM_153127.3:2622
Kcnj10	0.518	0.026	0.076	NM_001039484.1:400
Rela	0.513	0.047	0.105	NM_009045.4:645

Katna1	0.504	0.022	0.068	NM_011835.2:55
Ctnnb1	0.5	0.016	0.059	NM_007614.2:2975
Cdk5rap3	0.497	0.017	0.062	NM_030248.1:248
Rhoa	0.481	0.035	0.089	NM_016802.4:1885
Ppt1	0.475	0.016	0.060	NM_008917.3:1714
Dnm2	0.474	0.013	0.055	NM_001039520.1:1148
Gss	0.465	0.021	0.068	NM_008180.1:728
Gstp1	0.462	0.029	0.080	NM_013541.1:421
Entpd2	0.451	0.041	0.099	NM_009849.2:1016
Fam126a	0.448	0.041	0.099	XM_006535824.2:826
Cldn5	0.441	0.018	0.062	NM_013805.4:975
Arsa	0.434	0.017	0.061	NM_009713.4:2802
Efna1	0.424	0.038	0.093	NM_010107.4:437
Lypla1	0.422	0.004	0.038	XM_006495472.2:648
Atm	0.415	0.004	0.038	NM_007499.2:5543
Arhgef10	0.413	0.042	0.100	NM_001037736.1:1105
Taf10	0.412	0.008	0.044	NM_020024.3:357
Hnrnpm	0.402	<0.001	0.038	NM_001109913.1:606
Ptdss1	0.374	0.002	0.038	NM_008959.3:1830
Rdx	0.374	0.024	0.073	NM_001104617.1:1384
Eif2s1	0.365	0.009	0.047	NM_026114.3:665
Gtf2a1	0.364	0.013	0.054	NM_175335.3:3618
Esam	0.353	0.024	0.074	NM_027102.3:495

Gnptg	0.351	0.008	0.046	NM_172529.3:95
Egfl7	0.334	0.031	0.083	NM_001164564.1:168
Gnptab	0.333	0.003	0.038	NM_001004164.2:1214
Sirt7	0.332	0.006	0.040	NM_153056.2:575
3110043O21Rik	0.331	0.027	0.078	NM_001081343.1:1020
Zfp24	0.32	0.030	0.082	NM_021559.2:2326
Ide	0.314	0.018	0.062	NM_031156.2:500
Twistnb	0.312	0.009	0.047	NM_172253.2:576
Grik2	0.311	0.045	0.103	NM_010349.2:256
Vcp	0.286	0.020	0.066	NM_009503.3:510
Rac1	0.275	0.035	0.090	NM_009007.2:1045
Hmgb1	0.271	0.035	0.089	NM_010439.3:1574
Gfpt1	0.27	0.006	0.041	NM_013528.3:718
Mnat1	0.259	0.037	0.092	NM_008612.2:914
Sod1	0.256	0.003	0.038	NM_011434.1:406
Bcl2	0.256	0.042	0.100	NM_009741.3:1844
Prpf31	0.245	0.045	0.103	NM_001159714.1:1765
Bad	0.234	0.036	0.090	NM_007522.3:1146
Prkcsh	0.229	0.028	0.080	NM_008925.1:1295
Xiap	0.222	0.012	0.053	NM_009688.2:1654
Park7	0.193	0.009	0.049	NM_020569.3:334
Xab2	0.19	0.036	0.090	NM_026156.2:1616
Gtf2b	0.189	0.022	0.070	NM_145546.1:346

Psmc4	0.185	0.008	0.045	NM_011874.2:1146
Polr2b	0.123	0.042	0.100	NM_153798.2:1090
Cd34	0.117	0.030	0.082	NM_001111059.1:560
Bcas2	0.112	0.008	0.044	NM_026602.3:757
Ap3s1	0.103	0.042	0.100	NM_009681.5:610
Chd4	-0.156	0.004	0.038	NM_145979.2:1090
Atf6	-0.221	0.044	0.101	NM_001081304.1:4765
Gucy1b3	-0.251	0.044	0.101	NM_017469.4:372
Ddx23	-0.253	0.029	0.080	NM_001080981.1:2175
Rapgef2	-0.278	0.040	0.098	NM_001099624.2:5580
Cck	-0.294	0.012	0.052	NM_031161.2:351
Ep300	-0.299	0.005	0.038	NM_177821.6:4305
Mecp2	-0.306	0.015	0.057	NM_010788.2:755
Mtor	-0.31	0.012	0.052	NM_020009.2:2432
L1cam	-0.317	0.034	0.089	NM_008478.3:3560
Actn1	-0.321	0.036	0.092	NM_134156.2:2688
Frmpd4	-0.33	0.032	0.085	NM_001033330.2:4690
Camk2b	-0.337	0.046	0.105	NM_001174053.1:2825
Nrxn1	-0.341	0.043	0.101	NM_001346960.1:2013
Cul3	-0.343	0.011	0.051	NM_016716.4:1662
Tcerg1	-0.348	0.017	0.061	NM_001039474.1:468
Nme5	-0.353	0.004	0.038	NM_080637.3:330
Pcsk2	-0.353	0.033	0.086	NM_008792.3:2915

Plcb1	-0.379	0.049	0.109	NM_019677.1:495
Atxn2	-0.381	0.012	0.052	NM_009125.2:3036
Atp6v1g2	-0.381	0.029	0.080	NM_023179.3:994
Ptgs2	-0.387	0.029	0.080	NM_011198.3:675
Ppp3cb	-0.39	0.027	0.078	NM_008914.1:290
Amigo1	-0.39	0.029	0.080	NM_001004293.2:925
Prkce	-0.4	0.033	0.087	NM_011104.2:1510
Phf2	-0.406	0.023	0.070	NM_011078.2:3590
Itpr1	-0.409	0.025	0.075	NM_010585.2:660
Stx1b	-0.413	0.037	0.092	NM_024414.2:570
Vegfa	-0.421	0.026	0.077	NM_001025250.3:3015
Magee1	-0.43	0.019	0.063	NM_053201.3:2362
Gnao1	-0.433	0.008	0.044	NM_010308.3:754
Ppp3ca	-0.442	0.020	0.066	NM_008913.4:1675
Mapt	-0.446	0.010	0.049	NM_001038609.2:1202
Prkca	-0.45	0.042	0.100	NM_011101.3:6965
Cntnap1	-0.457	0.021	0.068	NM_016782.2:1105
Sorcs3	-0.462	0.033	0.086	NM_025696.3:1868
Cadm3	-0.464	0.018	0.062	NM_053199.3:3295
Syt13	-0.465	0.029	0.080	NM_030725.4:2290
Pde4d	-0.467	0.018	0.062	NM_011056.3:4332
Nova1	-0.468	0.006	0.041	NM_021361.1:370
Homer1	-0.469	0.043	0.101	NM_147176.2:1165

Ptprn2	-0.472	0.023	0.071	NM_011215.2:4158
Prkcb	-0.474	0.014	0.056	NM_008855.2:8332
Sncb	-0.477	0.027	0.078	NM_033610.2:676
Cnksr2	-0.479	0.016	0.060	NM_177751.2:628
Cdk5r1	-0.481	0.004	0.038	NM_009871.2:3280
Sf3a2	-0.487	0.005	0.038	NM_013651.4:494
Plcb4	-0.494	0.015	0.059	NM_013829.2:242
Efna5	-0.496	0.015	0.059	NM_207654.2:1130
Calb1	-0.498	0.017	0.061	NM_009788.4:343
Src	-0.518	0.005	0.039	NM_001025395.2:968
Ina	-0.522	0.018	0.063	NM_146100.4:1492
Tenm2	-0.524	0.005	0.038	NM_011856.3:1452
Hap1	-0.531	0.014	0.056	NM_010404.3:2552
Arhgap44	-0.541	0.024	0.071	NM_001099288.1:904
Dlgap1	-0.552	0.022	0.069	NM_027712.3:1654
Synj1	-0.553	0.005	0.038	NM_001045515.1:4090
Acin1	-0.561	0.007	0.043	NM_001085472.2:674
Cacna1b	-0.594	0.007	0.042	NM_001042528.1:4345
Dot1l	-0.595	0.030	0.082	NM_199322.1:5490
Lrrc4	-0.61	0.006	0.041	NM_138682.2:2790
Myh10	-0.613	0.002	0.038	NM_175260.2:2540
Bdnf	-0.664	0.007	0.043	NM_007540.4:3260
Grin1	-0.664	0.010	0.049	NM_008169.2:492

Crebbp	-0.674	<0.001	0.038	NM_001025432.1:3770
Mta1	-0.688	0.001	0.038	NM_054081.2:955
Dagla	-0.707	0.004	0.038	NM_198114.2:685
Slc12a5	-0.727	0.005	0.038	NM_020333.2:5618
Rims1	-0.737	0.013	0.054	NM_053270.1:90
Adcyap1	-0.804	0.029	0.080	NM_001315503.1:2625
Taf4	-0.811	0.003	0.038	NM_001081092.1:3016
Cacna1a	-0.813	<0.001	0.038	NM_007578.3:1655
Kcnb1	-0.836	0.002	0.038	NM_008420.3:3555
Dlx2	-0.843	0.040	0.097	NM_010054.2:1891
Dlg4	-0.869	0.001	0.038	NM_001109752.1:1866
Nefh	-0.909	0.003	0.038	NM_010904.3:3384
Npas4	-0.922	0.002	0.038	NM_153553.4:580
Unc13a	-0.944	0.003	0.038	NM_001029873.2:7755
Palm	-0.968	0.005	0.038	NM_023128.4:548
Syt7	-1.04	<0.001	0.038	NM_018801.3:990
Sptbn2	-1.16	<0.001	0.038	NM_021287.1:6145
Shank2	-1.31	<0.001	0.038	NM_001081370.2:4930
Fos	-1.38	<0.001	0.038	NM_010234.2:1330
Egr1	-1.4	0.002	0.038	NM_007913.5:515
Arc	-2.03	0.003	0.038	NM_018790.2:2715

Supplementary Table IV A list of DEGs in iS1 at PD28 compared with naïve mice (38 genes).

Gene	Log2 fold change	P-value	BH.p.value	probe.ID
C3	6.3	0.003	0.152	XM_011246258.1:2702
Itgax	3.29	0.009	0.193	NM_021334.2:327
Cd14	3.14	0.010	0.193	NM_009841.3:235
Gfap	2.74	0.010	0.196	NM_001131020.1:610
S100b	0.647	0.009	0.193	NM_009115.3:1090
Ptgs2	0.588	0.004	0.152	NM_011198.3:675
Prkcg	0.468	0.011	0.196	NM_011102.3:1580
Bcl2	0.393	0.006	0.167	NM_009741.3:1844
Atm	0.371	0.007	0.177	NM_007499.2:5543
Hnrnpm	0.3	0.003	0.152	NM_001109913.1:606
Gfpt1	0.254	0.008	0.193	NM_013528.3:718
Gtf2b	0.222	0.011	0.196	NM_145546.1:346
Erlec1	-0.264	0.005	0.161	NM_025745.3:940
Ep300	-0.264	0.010	0.193	NM_177821.6:4305
Smn1	-0.271	0.010	0.193	NM_011420.2:390
Ring1	-0.286	0.001	0.104	NM_009066.3:243
Fus	-0.316	0.004	0.152	NM_139149.2:15
Chd4	-0.345	<0.001	0.012	NM_145979.2:1090
Cul3	-0.358	0.008	0.193	NM_016716.4:1662
Keap1	-0.378	<0.001	0.072	NM_016679.4:4140
Crebbp	-0.429	0.007	0.172	NM_001025432.1:3770

Gtf2ird1	-0.464	0.002	0.114	NM_001081464.1:1430
Cacna1a	-0.595	0.002	0.114	NM_007578.3:1655
Flt1	-0.604	<0.001	0.072	NM_010228.3:1550
Jun	-0.607	0.005	0.161	NM_010591.2:2212
Slc12a5	-0.614	0.011	0.196	NM_020333.2:5618
Hdac7	-0.634	0.005	0.161	NM_019572.2:3706
Phf2	-0.653	0.002	0.114	NM_011078.2:3590
Syt7	-0.669	0.006	0.167	NM_018801.3:990
Sf3a2	-0.69	<0.001	0.072	NM_013651.4:494
Dlg4	-0.693	0.005	0.161	NM_001109752.1:1866
Sncb	-0.709	0.004	0.152	NM_033610.2:676
Mta1	-0.856	<0.001	0.072	NM_054081.2:955
Shank2	-0.992	<0.001	0.082	NM_001081370.2:4930
Palm	-1.01	0.004	0.152	NM_023128.4:548
Pla2g4e	-1.05	<0.001	0.082	NM_177845.4:1016
Taf4	-1.15	<0.001	0.072	NM_001081092.1:3016
Sox10	-1.38	0.006	0.167	XM_128139.6:2646

Supplementary Table V A list of DEGs in ipsilesional thalamus (iTH) at PD7 compared with naïve mice (50 genes).

Gene	Log2 fold change	P-value	BH.p.value	probe.ID
Cd14	2.8	0.002	0.162	NM_009841.3:235
Ccl12	2.58	0.002	0.162	NM_011331.2:173
Slc11a1	2.52	0.008	0.162	NM_013612.2:945
Gfap	1.84	<0.001	0.162	NM_001131020.1:610
Tlr2	1.83	0.009	0.163	NM_011905.2:255
Fcrls	1.37	0.002	0.162	NM_030707.3:925
CD86	1.36	0.014	0.191	NM_019388.3:251
Cxcl16	1.3	0.006	0.162	NM_023158.6:679
Irf8	1.23	0.009	0.163	NM_008320.3:2274
Cd68	1.18	0.008	0.162	NM_009853.1:636
C1qa	1.14	0.004	0.162	NM_007572.2:566
Trem2	1.14	0.012	0.177	NM_031254.2:646
Tgfb1	1.13	0.010	0.172	NM_011577.1:1470
C1qc	1.03	0.007	0.162	NM_007574.2:708
C1qb	1.01	0.007	0.162	NM_009777.2:865
Spi1	0.887	0.007	0.162	NM_011355.1:200
Hpgds	0.788	0.004	0.162	NM_019455.4:194
Hexb	0.726	0.011	0.177	NM_010422.2:805
Csf1r	0.712	0.011	0.177	NM_001037859.1:3655
Grn	0.679	0.006	0.162	NM_008175.3:2010

Cx3cr1	0.662	0.004	0.162	NM_009987.3:2696
SiglecH	0.637	0.013	0.182	NM_178706.4:446
Eng	0.599	0.007	0.162	NM_001146350.1:574
Aqp4	0.519	0.012	0.182	NM_009700.2:130
Sox10	0.516	0.012	0.177	XM_128139.6:2646
Tnfrsf1a	0.49	0.009	0.163	NM_011609.2:615
Lrp1	0.397	0.010	0.172	NM_008512.2:1310
Lsm2	0.36	0.014	0.191	NM_001110101.2:641
Mapkapk2	0.344	0.011	0.177	NM_008551.1:1991
Olig2	0.341	0.004	0.162	NM_016967.2:1740
Sox9	0.341	0.008	0.162	NM_011448.4:3540
Arhgef10	0.341	0.011	0.177	NM_001037736.1:1105
Efna1	0.337	0.006	0.162	NM_010107.4:437
AI464131	0.292	0.005	0.162	NM_001085515.2:1232
Trpm2	0.273	0.005	0.162	NM_138301.2:2106
Lama2	0.264	0.001	0.162	NM_008481.2:208
Flt1	0.212	0.006	0.162	NM_010228.3:1550
Jam3	0.208	0.006	0.162	NM_023277.4:145
Akt1s1	0.197	0.002	0.162	NM_026270.4:946
Ep300	0.191	0.005	0.162	NM_177821.6:4305
Nwd1	0.169	<0.001	0.124	NM_176940.5:3564
Gnao1	0.154	0.005	0.162	NM_010308.3:754
Comt	0.131	0.003	0.162	NM_007744.3:625

Rhoa	0.108	<0.001	0.127	NM_016802.4:1885
Trim37	-0.153	0.003	0.162	NM_197987.2:972
Taf9	-0.173	0.004	0.162	NM_027139.5:324
Slc17a6	-0.189	0.007	0.162	NM_080853.3:2825
Slc2a1	-0.231	0.002	0.162	NM_011400.3:2190
Bdnf	-0.604	0.007	0.162	NM_007540.4:3260
Adcyap1	-0.692	0.014	0.191	NM_001315503.1:2625

Supplementary Table VI A list of DEGs in iTH at PD28 compared with naïve mice (187 genes).

Gene	Log2 fold change	P-value	BH.p.value	probe.ID
Itgax	4.99	<0.001	0.007	NM_021334.2:327
Slc11a1	3.04	0.003	0.034	NM_013612.2:945
Gfap	2.95	<0.001	0.007	NM_001131020.1:610
Tlr2	2.84	<0.001	0.014	NM_011905.2:255
Cd14	2.8	0.002	0.025	NM_009841.3:235
C3	2.56	<0.001	0.011	XM_011246258.1:2702
Fcrls	2.41	<0.001	0.007	NM_030707.3:925
Cybb	2.4	<0.001	0.015	NM_007807.2:1535
C1qa	2.35	<0.001	0.007	NM_007572.2:566
CD86	2.27	<0.001	0.015	NM_019388.3:251
Trem2	2.24	<0.001	0.011	NM_031254.2:646
C1qc	2.17	<0.001	0.007	NM_007574.2:708
Cxcl16	2.11	<0.001	0.011	NM_023158.6:679
Cd68	2.01	<0.001	0.012	NM_009853.1:636
C1qb	2	<0.001	0.008	NM_009777.2:865
Irf8	1.87	<0.001	0.015	NM_008320.3:2274
Tgfb1	1.82	<0.001	0.014	NM_011577.1:1470
Ccl12	1.68	0.022	0.113	NM_011331.2:173
Tlr4	1.45	0.004	0.047	NM_021297.2:2510
Grn	1.43	<0.001	0.007	NM_008175.3:2010

Hpgds	1.38	<0.001	0.008	NM_019455.4:194
Ncf1	1.35	0.001	0.022	NM_001286037.1:970
Spi1	1.34	<0.001	0.014	NM_011355.1:200
Hexb	1.28	<0.001	0.012	NM_010422.2:805
Ang	1.16	0.014	0.088	NM_007447.2:425
Cx3cr1	1.13	<0.001	0.008	NM_009987.3:2696
Hmox1	1.13	<0.001	0.015	NM_010442.2:610
Csf1r	1.12	<0.001	0.015	NM_001037859.1:3655
Psmb8	1.11	0.008	0.062	NM_010724.2:362
Il10ra	1.08	0.009	0.066	NM_008348.2:2522
Cd34	1.07	<0.001	0.011	NM_001111059.1:560
Itgam	1.05	0.001	0.020	NM_008401.2:155
Cd33	1.03	0.008	0.063	NM_001111058.1:405
Gusb	1.02	0.001	0.021	NM_010368.1:1735
Aqp4	1.01	<0.001	0.011	NM_009700.2:130
Osmr	1.01	0.011	0.073	NM_011019.3:395
Cd9	1	0.002	0.023	NM_007657.3:620
Csf1	0.97	0.001	0.021	NM_001113530.1:833
Tspo	0.965	0.023	0.117	NM_009775.4:241
Myd88	0.927	0.024	0.119	NM_010851.2:1595
Apoe	0.923	<0.001	0.011	NM_001305844.1:903
Psmb9	0.819	0.027	0.128	NM_013585.2:540
Casp8	0.798	0.012	0.075	NM_009812.2:1463

Msn	0.792	0.022	0.113	NM_010833.2:515
Cd44	0.787	0.043	0.167	NM_009851.2:3075
Tnfrsf1a	0.778	<0.001	0.014	NM_011609.2:615
Tcirg1	0.778	0.004	0.044	NM_001136091.1:1345
Mmp2	0.778	0.038	0.156	NM_008610.2:2376
Col4a1	0.731	0.012	0.075	NM_009931.2:4116
Hspb1	0.689	0.003	0.035	NM_013560.2:630
Man2b1	0.656	0.003	0.034	NM_010764.2:1658
Pla2g4a	0.626	0.034	0.148	NM_008869.2:1525
Ltbr	0.588	0.003	0.035	NM_010736.3:1962
Tgfbr2	0.561	0.007	0.057	NM_009371.2:475
Stat1	0.539	0.038	0.156	NM_009283.3:1590
Fgf2	0.532	0.004	0.044	NM_008006.2:509
Naglu	0.522	0.009	0.066	NM_013792.2:2334
Nfe2l2	0.52	0.023	0.117	NR_132727.1:193
Npc2	0.51	0.012	0.075	NM_023409.4:3095
Igf1	0.504	0.006	0.054	NM_010512.4:6448
Il4ra	0.5	0.043	0.167	NM_001008700.3:2908
Erbb3	0.461	0.016	0.096	NM_010153.1:1290
Fn1	0.458	0.018	0.103	NM_010233.1:2627
Sgpl1	0.445	0.018	0.103	NM_009163.3:1200
P2rx4	0.437	0.021	0.113	NM_011026.2:1655
Pkn1	0.407	0.023	0.118	NM_001199593.1:1380

Clu	0.406	0.005	0.051	NM_013492.2:354
Sox10	0.392	0.040	0.159	XM_128139.6:2646
Sox9	0.367	0.006	0.053	NM_011448.4:3540
Il13ra1	0.367	0.044	0.169	NM_133990.4:845
Lsm2	0.347	0.016	0.096	NM_001110101.2:641
Mapkapk2	0.343	0.012	0.075	NM_008551.1:1991
Lrp1	0.339	0.021	0.111	NM_008512.2:1310
Cldn5	0.333	0.011	0.073	NM_013805.4:975
Xbp1	0.331	0.004	0.044	NM_013842.2:825
Jun	0.295	0.044	0.169	NM_010591.2:2212
Olig2	0.287	0.011	0.073	NM_016967.2:1740
Nwd1	0.183	<0.001	0.008	NM_176940.5:3564
Ikbkb	0.181	0.029	0.135	NM_010546.2:498
Hnrnpm	0.17	0.039	0.158	NM_001109913.1:606
Tnr	0.162	0.048	0.175	NM_022312.3:3195
Adcy8	0.154	0.036	0.153	NM_009623.2:2655
Flt1	0.136	0.045	0.171	NM_010228.3:1550
Rhoa	0.108	<0.001	0.012	NM_016802.4:1885
Amigo1	-0.0817	0.046	0.172	NM_001004293.2:925
Ppp2r5c	-0.0863	0.048	0.175	NM_001135001.1:1400
Gls	-0.0951	0.043	0.167	NM_001113383.1:976
Akt3	-0.0994	0.035	0.150	NM_011785.3:2494
Ccnh	-0.103	0.017	0.099	NM_023243.2:960

Calm1	-0.109	0.028	0.132	NM_009790.4:1575
Cab39	-0.114	0.029	0.135	NM_133781.4:2830
Pcsk2	-0.116	0.026	0.124	NM_008792.3:2915
Mapk10	-0.116	0.046	0.172	NM_001081567.1:1496
Ugcg	-0.116	0.049	0.175	NM_011673.3:610
Cntn1	-0.121	0.004	0.043	NM_001159647.1:1070
App	-0.127	0.024	0.121	NM_007471.2:511
Gabrb2	-0.129	0.033	0.144	NM_008070.3:4335
Ddx23	-0.129	0.046	0.173	NM_001080981.1:2175
Dnaja2	-0.13	0.037	0.153	NM_019794.4:1045
Prkaca	-0.132	0.042	0.166	NM_008854.3:699
Mfn2	-0.133	0.005	0.051	XM_006535920.1:692
Ppp3cb	-0.133	0.009	0.066	NM_008914.1:290
Pak1	-0.135	0.002	0.028	NM_011035.2:1615
Sod2	-0.135	0.006	0.054	NM_013671.3:1495
Nell2	-0.135	0.026	0.124	NM_016743.2:500
Rad23b	-0.136	0.023	0.118	NM_009011.4:1585
Erlec1	-0.14	0.045	0.171	NM_025745.3:940
Ppp2ca	-0.143	0.019	0.106	NM_019411.4:975
Dnm1l	-0.148	0.019	0.105	NM_001025947.1:2075
Atp6v0e2	-0.148	0.040	0.160	NM_133764.3:882
Atp6v1e1	-0.149	0.011	0.075	NM_007510.2:1025
Snap91	-0.151	0.007	0.058	NM_001277986.1:3170

Prpf31	-0.157	0.016	0.096	NM_001159714.1:1765
Park7	-0.157	0.049	0.175	NM_020569.3:334
Nptn	-0.158	0.005	0.050	NM_009145.2:1110
Atp6v0d1	-0.159	0.003	0.040	NM_013477.3:640
Slc8a1	-0.159	0.029	0.134	NM_011406.2:6665
Lypla1	-0.16	0.026	0.124	XM_006495472.2:648
Ntng1	-0.161	0.040	0.159	NM_001163351.1:1090
Nmnat2	-0.164	0.021	0.111	NM_175460.3:67
Ap4s1	-0.165	0.011	0.073	NM_021710.3:476
Ppp2r5e	-0.165	0.014	0.088	NM_012024.2:4010
Pgam1	-0.166	0.009	0.067	NM_023418.2:466
Stx1b	-0.172	0.006	0.054	NM_024414.2:570
Syt1	-0.172	0.017	0.100	NM_009306.2:3320
Atp6v1a	-0.173	0.037	0.153	NM_007508.5:434
Gria4	-0.174	0.003	0.034	NM_001113180.1:1274
Gsr	-0.174	0.021	0.111	NM_010344.4:1507
Tbpl1	-0.175	0.050	0.177	NM_011603.5:714
Thy1	-0.177	0.006	0.055	NM_009382.3:425
Rab2a	-0.177	0.020	0.108	NM_021518.3:450
Bnip3	-0.178	0.009	0.065	NM_009760.4:1108
Efr3a	-0.181	0.001	0.020	NM_133766.3:1062
Nsf	-0.181	0.002	0.031	NM_008740.2:395
Mapk9	-0.182	0.003	0.037	NM_207692.1:260

Entpd4	-0.182	0.025	0.122	NM_026174.2:845
Ube2k	-0.183	0.007	0.058	NM_016786.3:1212
Opa1	-0.184	0.008	0.065	NM_001199177.1:2845
Dlat	-0.185	0.005	0.051	NM_145614.4:2046
Sqstm1	-0.185	0.016	0.096	NM_011018.2:1430
Ap1s1	-0.186	0.036	0.153	NM_007457.2:848
Slc17a6	-0.19	0.007	0.058	NM_080853.3:2825
Hras	-0.19	0.012	0.075	NM_001130443.1:240
Atp6v1h	-0.191	0.008	0.062	XM_006495434.2:1296
Gabra1	-0.192	0.012	0.077	NM_010250.4:905
Cul1	-0.205	0.010	0.068	NM_012042.3:919
Cdk5	-0.208	0.001	0.020	NM_007668.3:77
Sucla2	-0.208	0.007	0.058	NM_011506.1:955
Sorcs3	-0.211	0.034	0.149	NM_025696.3:1868
Cadps	-0.212	<0.001	0.014	NM_001042617.1:3524
Ube2n	-0.212	0.001	0.020	NM_080560.3:2376
Dld	-0.212	0.002	0.031	NM_007861.4:252
Rab3a	-0.215	0.005	0.051	NM_009001.6:1272
Insr	-0.215	0.032	0.144	NM_010568.2:7814
Fgf12	-0.218	<0.001	0.014	NM_001276419.1:632
Trim37	-0.226	<0.001	0.011	NM_197987.2:972
Ppp3cc	-0.226	0.005	0.050	NM_008915.2:1020
Pgk1	-0.227	<0.001	0.015	NM_008828.2:36

Chrnb2	-0.227	0.005	0.051	NM_009602.4:4470
Rit2	-0.232	0.006	0.056	NM_009065.2:626
Nrg1	-0.241	0.035	0.150	NM_178591.2:1116
Cds1	-0.243	0.007	0.056	NM_173370.3:2232
Xab2	-0.245	0.006	0.056	NM_026156.2:1616
Prkaa2	-0.245	0.008	0.060	NM_178143.1:1890
Tcerg1	-0.246	0.009	0.066	NM_001039474.1:468
Glrb	-0.247	<0.001	0.014	NM_010298.5:704
Nova1	-0.25	0.026	0.124	NM_021361.1:370
Ran	-0.251	0.020	0.108	NM_009391.3:1755
Polr2j	-0.254	0.006	0.054	NM_011293.2:70
Ache	-0.254	0.048	0.175	NM_009599.3:1073
Lmna	-0.259	0.030	0.138	NM_001002011.2:1611
Scn1a	-0.263	0.012	0.075	NM_018733.2:1030
Chrna7	-0.263	0.049	0.177	NM_007390.3:335
Taf9	-0.265	<0.001	0.011	NM_027139.5:324
Lsm7	-0.272	0.003	0.035	NM_025349.2:37
Twistnb	-0.28	0.025	0.122	NM_172253.2:576
Txnl1	-0.281	0.043	0.167	NM_016792.2:1635
Slc2a1	-0.284	<0.001	0.014	NM_011400.3:2190
Cacnb4	-0.302	0.002	0.025	NM_146123.2:6510
Bid	-0.303	0.032	0.144	NM_007544.3:1307
Mmp16	-0.358	0.038	0.155	NM_019724.3:3075

Ninj2	-0.427	0.030	0.138	NM_016718.2:244
Fus	-0.429	<0.001	0.012	NM_139149.2:15
Tie1	-0.468	<0.001	0.014	NM_011587.2:2715
Adcyap1	-0.615	0.023	0.118	NM_001315503.1:2625
Erg	-0.769	0.031	0.138	NM_133659.2:992
Pla2g4e	-0.819	0.010	0.068	NM_177845.4:1016

*** Preclinical Checklist**

Preclinical Checklist: Prevention of bias is important for experimental cardiovascular research. ***This short checklist must be completed, and the answers should be clearly presented in the manuscript.*** *The checklist will be used by reviewers and editors and it will be published. See "Reporting Standard for Preclinical Studies of Stroke Therapy" and "Good Laboratory Practice: Preventing Introduction of Bias at the Bench" for more information.*

This study invovles animal models:
Yes

Experimental groups and study timeline

The experimental group(s) have been clearly defined in the article, including number of animals in each experimental arm of the study:	Yes
An account of the control group is provided, and number of animals in the control group has been reported. If no controls were used, the rationale has been stated:	Yes
An overall study timeline is provided:	Yes

Inclusion and exclusion criteria

A priori inclusion and exclusion criteria for tested animals were defined and have been reported in the article:	Yes

Randomization

Animals were randomly assigned to the experimental groups. If the work being submitted does not contain multiple experimental groups, or if random assignment was not used, adequate explanations have been provided:	Yes
Type and methods of randomization have been described:	Yes
Methods used for allocation concealment have been reported:	Yes

Blinding

Blinding procedures have been described with regard to masking of group/treatment assignment from the experimenter. The rationale for nonblinding of the experimenter has been provided, if such was not feasible:	Yes
Blinding procedures have been described with regard to masking of group assignment during outcome assessment:	Yes

Sample size and power calculations

Formal sample size and power calculations were conducted based on a priori determined outcome(s) and treatment effect, and the data have been reported. A formal size assessment was not conducted and a rationale has been provided:	Yes

Data reporting and statistical methods

Number of animals in each group: randomized, tested, lost to follow-up, or died have been reported. If the experimentation involves repeated measurements, the number of animals assessed at each time point is provided, for all experimental groups:	Yes
Baseline data on assessed outcome(s) for all experimental groups have been reported:	Yes
Details on important adverse events and death of animals during the course of experimentation have been provided, for all experimental arms:	Yes
Statistical methods used have been reported:	Yes
Numeric data on outcomes have been provided in text, or in a tabular format with the main article or as supplementary tables, in addition to the figures:	Yes

Experimental details, ethics, and funding statements

Details on experimentation including stroke model, formulation and dosage of therapeutic agent, site and route of administration, use of anesthesia and analgesia, temperature control during experimentation, and postprocedural monitoring have been described:	Yes
Different sex animals have been used. If not, the reason/justification is provided:	Yes

Statements on approval by ethics boards and ethical conduct of studies have been provided:	Yes
Statements on funding and conflicts of interests have been provided:	Yes

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