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Figure S1, related to Figure 1

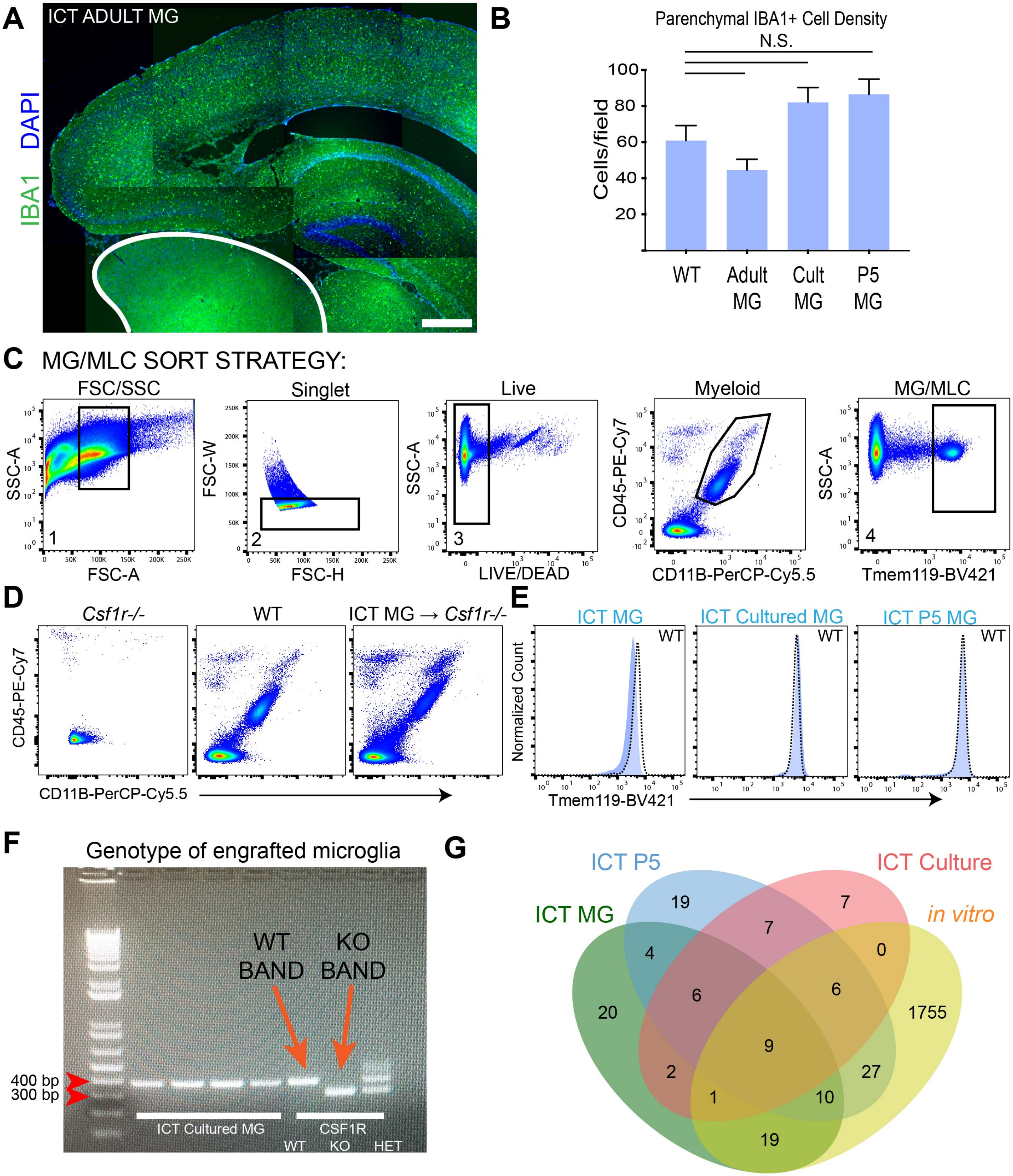
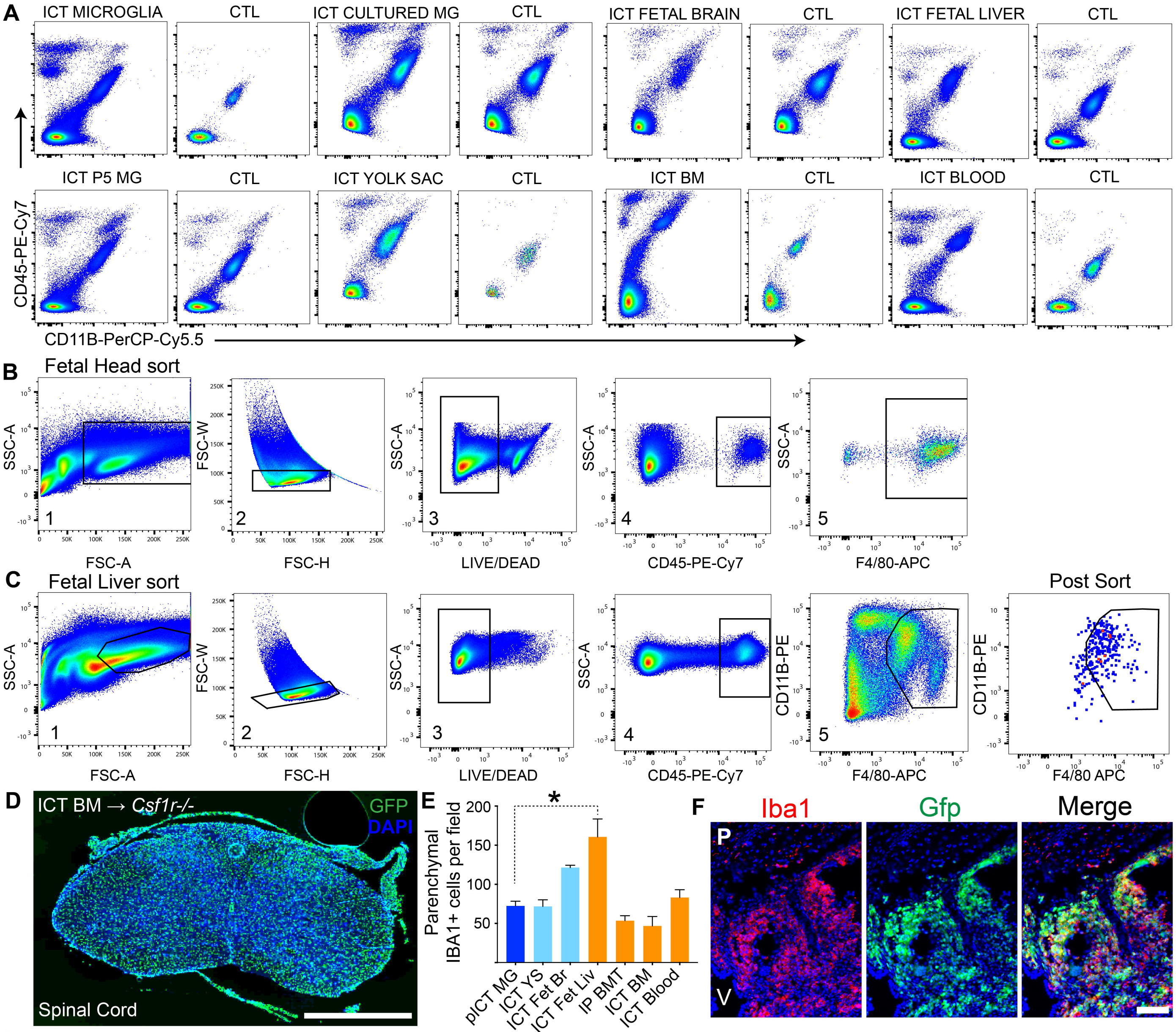


Figure S1, related to Figure 1. Transplantation, sorting and recovery of donor derived WT microglia. A) IBA1 immunostained section of *Csf1r-/-* brain transplanted with adult microglia showing typical widespread engraftment with some unpopulated territories, encircled by white line. Scale bar = 500μm. B) In areas of cell engraftment, transplanted microglia in *Csf1r-/-* hosts reach same density as microglia in a WT brain, $p>0.05$ adjusted for multiple comparisons to WT by Student's t-test, N.S. = not significant. C) Sort strategy for MLC isolation showing FSC/SSC, single cell, live, CD45/11B, and TMEM119 gates. MLCs were sorted based on TMEM119 immunoreactivity. Numbers in lower left of plots shows gate hierarchy (1>2>3>4). Cells were sorted from gate 4. D) Transplantation into *Csf1r-/-* hosts, which lack microglia (left), leads to engraftment of CD45+CD11B+ cells (right) with similar profile to untransplanted microglia in a *Csf1r+/+ (*WT) control (middle) E) Transplanted microglia (blue distribution) express Tmem119 at normal levels (black dotted) by flow cytometric analysis. F) Genotyping gel demonstrating that transplanted, then sorted microglia ("ICT Cultured MG") are WT for the *Csf1r* gene. G) Venn diagram showing differentially expressed genes between transplanted and WT microglia (2-fold cutoff, FPKM >20, FDR<0.05). ICT= intracerebral transplantation, MG=microglia, MLC = microglia-like cell, WT= *Csf1r+/+,* Cult = Cultured, P5 = postnatal day 5.

Figure S2, related to figure 2



G

ICT Type	Sample	% Recovery (vs WT)	% Tmem119+ of Parenchymal Iba1+	Yield, RIN> 7
Microglia	1	4.8	100	
	2	12.2	100	*
	3	24.8	100	*
	4	3.6	100	
	5	0.0	-	
Cultured MG	1	15.6	100	*
	2	16.3	100	*
	3	28.2	100	*
	4	3.8	-	
	5	10.0	-	
	6	8.3	-	
	7	3.6	-	
	8	2.8	-	
	9	27.6	100	*
	10	10.4	100	*
	11	94.4	-	*
	12	-	100	
	13	-	100	
P5 MG	1	20.7	100	*
	2	39.1	100	*
	3	33.3	100	*
	4	51.9	100	*
	5	23.0	100	*
	6	-	100	
	7	-	100	
Yolk Sac	1	4.8	100	
	2	12.2	100	*
	3	32.5	100	*
	4	44.3	100	*
	5	18.7	100	*
	6	-	100	
Fetal Brain	1	10.4	100	*
	2	9.3	100	*
	3	11.0	100	*
	4	NA	-	*
	5	NA	-	*
Fetal Liver	1	31.4	100	*
	2	35.6	100	*
	3	11.7	100	
	4	35.1	100	*
	5	29.5	100	*
	6	2.9	-	
	7	1.3	-	
	8	74.1	-	*
	9	69.1	-	*
	10	-	100	
	11	-	100	
BM	1	11.3	100	*
	2	9.1	-	*
	3	11.0	-	*
	4	8.2	-	*
	5	10.1	-	*
	6	-	100	
	7	-	100	
	8	-	100	
	9	-	100	
Blood	1	1.9	-	*
	2	13.8	-	*
	3	15.2	-	
	4	12.2	-	
	5	4.4	-	
	6	2.7	100	*
	7	3.6	100	*
	8	8.3	100	*
	9	2.8	100	
	10	-	100	
	11	-	100	
	12	-	100	
	13	-	100	
	14	-	100	

Figure S2, related to Figure 2. MLC transplantation system. A) Example FACS plots showing CD45/11B staining profile of MLCs across all donor types, with WT littermate control (CTL) to the right for comparison. B) Sort strategy for fetal head donor cell isolation showing FSC/SSC, single cell, live, CD45, and F4/80 gates. C) Sort strategy for fetal liver donor cell isolation showing FSC/SSC, single cell, live, CD45, and CD11B/F4/80 gates, and post-sort purity (large dots shown due to small number of cells collected for purity check). For B-C, numbers in lower left corner of plots shows gate hierarchy (1>2>3>4>5). Cells were sorted from gate 5. D) Lumbar spinal cord section from *Csf1r-/-* mouse transplanted by ICT at P3 with GFP-expressing WT bone marrow and harvested 14 days later. Scale bar = 500µm. E) MG and MLC density by Iba1 immunostaining across cortical and subcortical regions of engraftment normalized to area, $p < 0.0001$ for effect of donor type on density by ANOVA, *$p = 0.0001$ for pairwise difference between fetal liver and microglia, adjusted for multiple comparisons, $p > 0.05$ for all other comparisons between MLCs and ICT MG control. F) Pertaining to Figure 2B: IBA1 staining (red) of ventricular donor cells from GFP+ BM, showing that nearly all cells attach to the choroid plexus and are IBA1+. Scale bar = 100µm. G) Table showing relative engraftment levels for ICT experiments (expressed as % recovery of TMEM119+ cells in ICT compared to WT littermate), and percent of parenchymal IBA1+ cells that were also TMEM119+ for histological sections of same samples. Asterisks indicate samples with adequate RNA yield and RNA Integrity Number (RIN) (> 7) for RNAseq. YS = yolk sac, Fet Br = fetal brain, Fet Liv = fetal liver, IP = intraperitoneal, BMT = bone marrow transplant, BM = bone marrow, P = brain parenchyma, V = ventricular space.

Figure S3, related to Figure 3

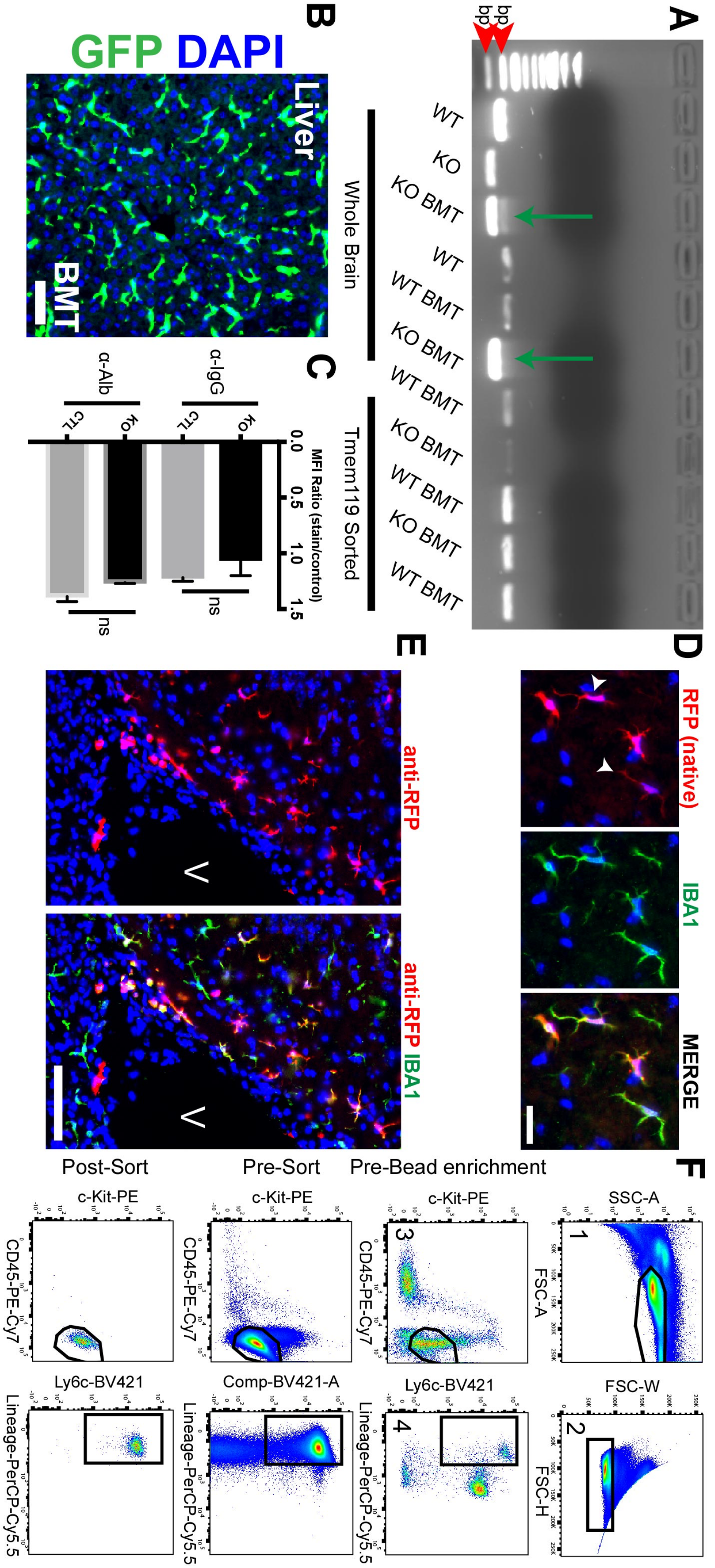
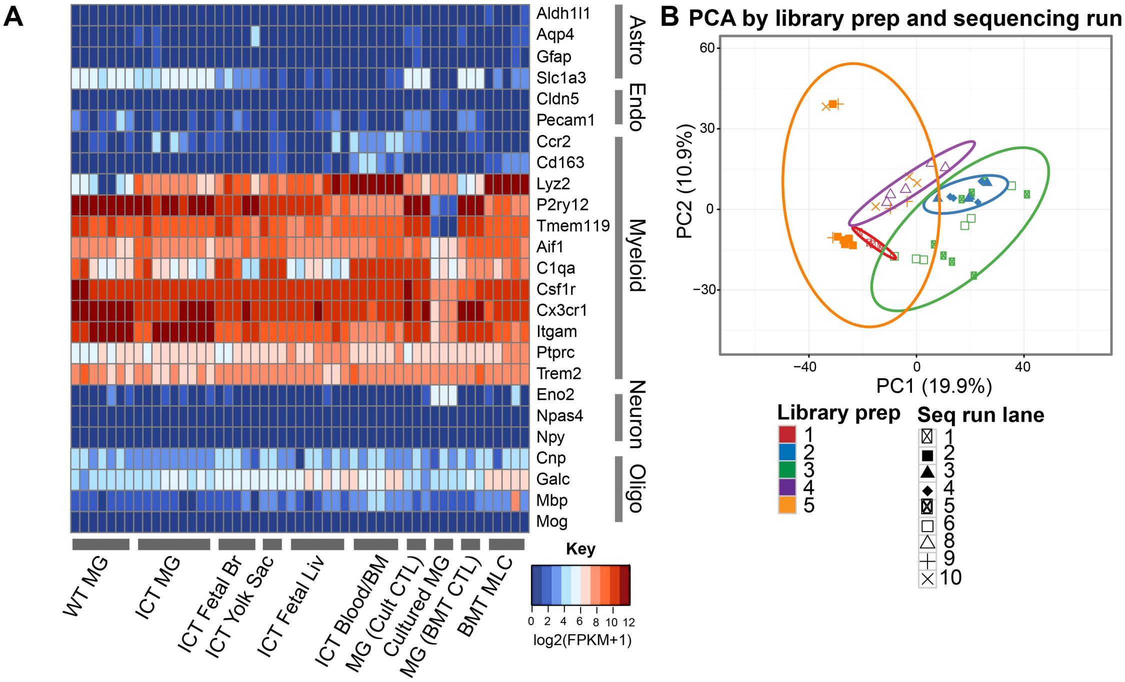
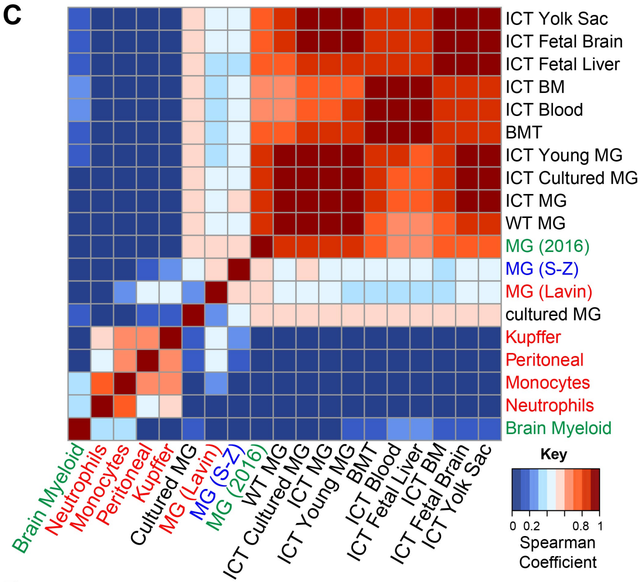


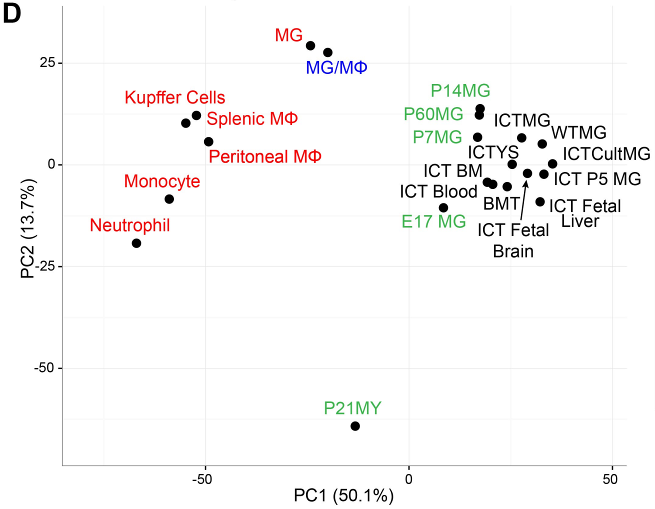
Figure S3, related to Figure 3. Peripheral bone marrow transplantation leads to CCR2-independent engraftment of donor-derived cells in multiple tissues, and results in partial rescue of the *Csf1r-/-* phenotype. A) *Csf1r* genotyping gel for whole brain and TMEM119 sorted microglia and MLCs. Green arrows indicate appearance of WT band in *Csf1r-/-* (KO) whole brain samples following WT donor cell engraftment. WT band has expected migration at 385 BP, KO band at 300 BP. B) Liver section from *Csf1r-/-* mouse following IP transplantation of GFP-labeled BM, scale bar = 50µm. C) Bar graph showing intensity of IgG and albumin (Alb) staining for *Csf1r -/-* (KO, n=2) compared to littermate control (CTL, n=3) animals at age P21, expressed as mean fluorescence intensity ratio between stain and control (no antibody for IgG, secondary only for albumin). D) Representative image of ramified IBA1+ (green) CCR2 -/- (red) cells showing relatively low (arrowheads) and high RFP expressing cells engrafted in the *Csf1r-/-* parenchyma. Scale bar = 25µm. E) Representative image of ramified IBA1+ (green) cells stained for RFP (red), demonstrating typical abundance of RFP+ cells in a periventricular distribution. V = ventricle. Scale bar = 100µm. F) Pertaining to Figure 3F: purification strategy for BM monocyte transplantation, showing FSC/SSC, single cell gates, followed by c-kit/CD45 and Ly6c/lineage plots before bead enrichment, before FACS, and post-sort. The lineage cocktail included antibodies against CD3, B220, NK1.1, Il7Ra, SiglecF, and GR1. Numbers in lower left corner of plots show gate hierarchy (1>2>3>4). Cells were sorted from gate 4.

Figure S4, related to Figure 4





Data	Citation
Neutrophils	Lavin et al
Monocytes	Lavin et al
Kupffer Cells	Lavin et al
Peritoneal Macrophages	Lavin et al
MG	Lavin et al
MG/MΦ	Zhang et al
MG	Bennett et al
Brain Myeloid	Bennett et al



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Splenic MΦ	Lavin et al
Peritoneal MΦ	Lavin et al
MG	Lavin et al
MG/MΦ	Zhang et al
P14MG	Bennett et al
P60MG	Bennett et al
P7MG	Bennett et al
E17MG	Bennett et al
P21MY	Bennett et al
ICT BM	current
ICT Blood	current
ICTMG	current
ICTYS	current
BMT	current
ICT Fetal Brain	current
WTMG	current
ICTCULT MG	current
ICT P5 MG	current
ICT Fetal Liver	current

Figure S4, related to Figure 4. Transcriptomic profiling of highly pure myeloid cells reveals similarities and differences between microglia and MLCs in the CNS environment. A) Sorted cell purity heatmap showing expression of brain cell-type specific markers. B) Principal component analysis (PCA) coded by library prep batch (colors) and sequencing run lane (symbols). Ellipses demarcate 95% confidence intervals for samples grouped by batch. C) Correlation heatmap depicting Spearman coefficients between microglia, MLCs from all sources, and myeloid cell types from published datasets. Table identifies source of datasets. D) Scatterplot showing 2 largest principle components for diverse myeloid cells from current and other studies (PCA performed on 2500 most variant genes). Table identifies source of datasets. ICT = Intracerebral transplant, MG = microglia, fetal br = fetal brain donor tissue, fetal liv = fetal liver donor tissue, BM = bone marrow, MG (Cult CTL) = WT microglia purified in parallel to MGs used for culture ICTs, BMT = MLCs derived from peripheral bone marrow transplantation.

Figure S5, related to Figures 4,5

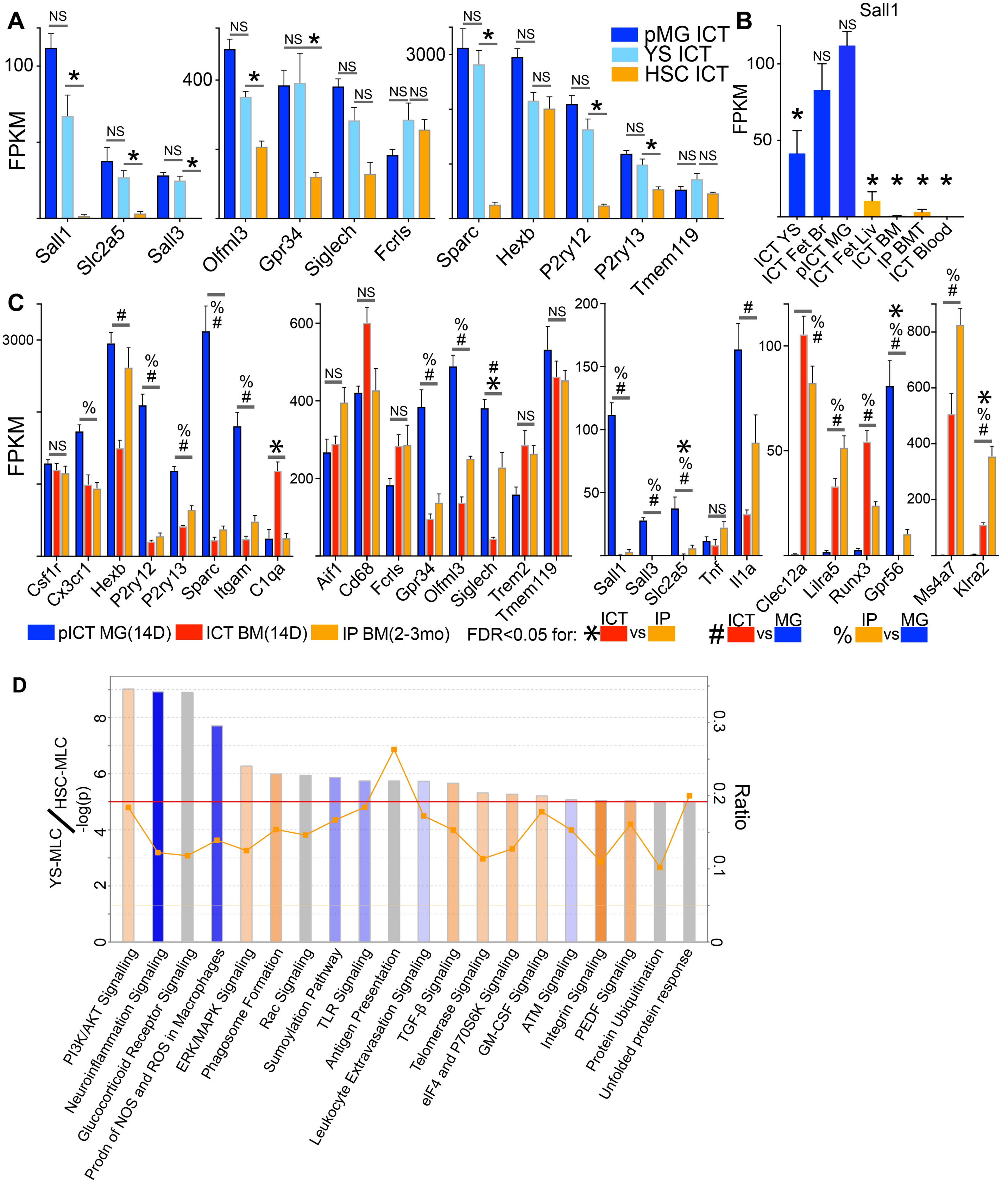

Figure S5, related to Figures 4,5. Ontogeny but not prolonged CNS residence results in faithful expression of microglial genes. A) Signature gene expression in microglia, HSC- and YS-MLCs. * = FDR < 0.05. Error bars depict SEM. B) Sall1 expression (in FPKM) by microglia and MLCs of YS (blue) and HSC (orange) origin. * = FDR<0.05, NS = not significant. Error bars represent SEM. C) Relative expression of myeloid genes in ICT microglia (blue), ICT BM (red, 14 day brain residence), and IP BMT (orange, 2-3 month brain residence). NS indicates no statistical difference between any pairwise comparison. *, FDR < 0.05 comparing ICT BM to IP BMT. #, FDR<0.05 comparing ICT BM to pICT MG. %, FDR<0.05 comparing IP BMT to pICT MG. Error bars depict SEM. D) Ingenuity Pathway Analysis® comparing YS- to HSC-MLCs for pathways with –log(p) >5 cutoff (red line). Orange line shows fraction of genes in pathway that are differentially expressed. Orange bars show positive association of pathway with YS-MLCs, blue bars with HSC-MLCs, gray bars represent indeterminate direction of association.

Figure S6, related to Figure 6

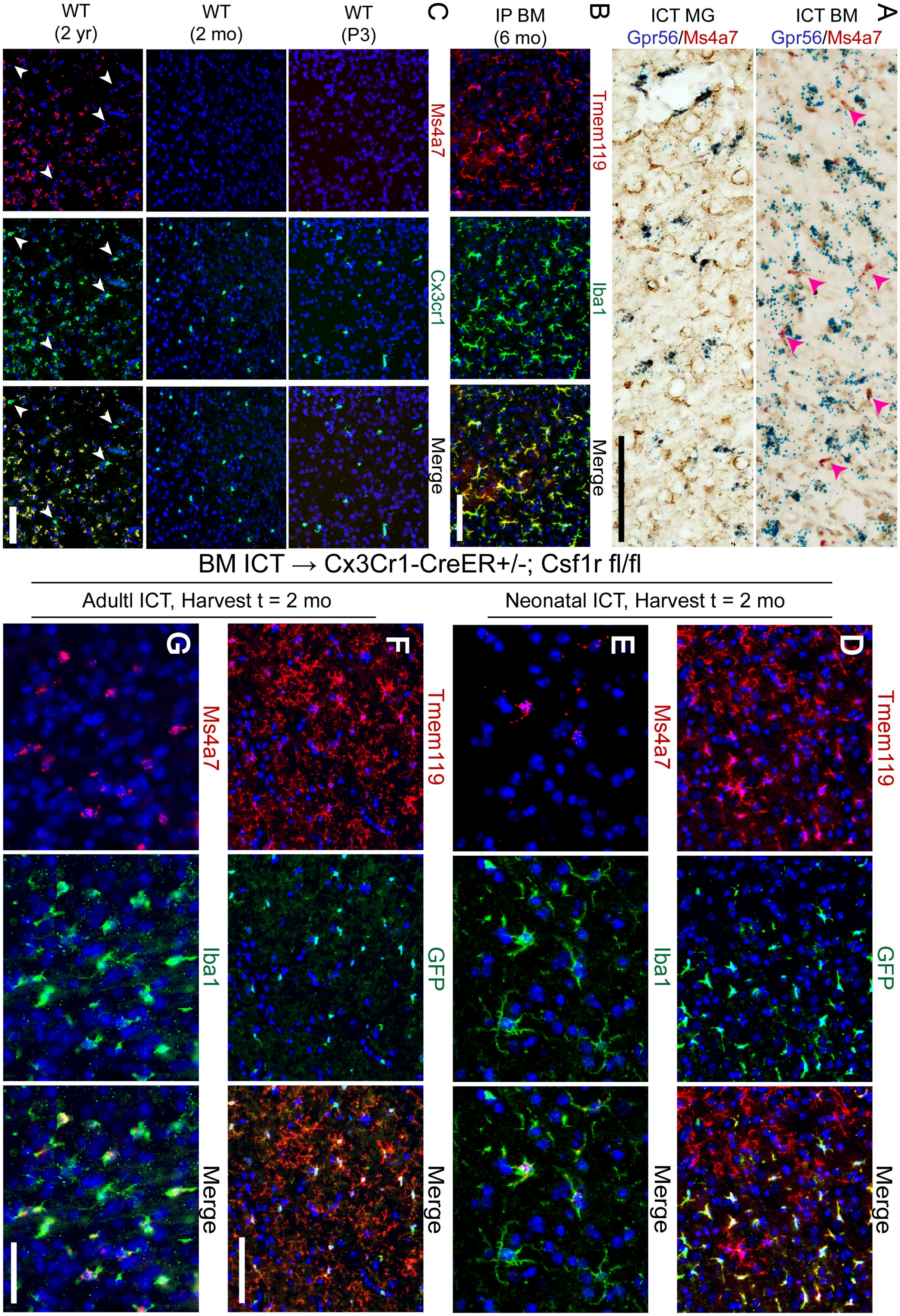

Figure S6, related to Figure 6. HSC-MLCs persist in the brain for long periods, and show distinct expression of origin markers in multiple transplantation systems. (A) RNA *In situ* hybridization for *Gpr56* (blue) and *Ms4a7* (red) in BM-MLCs, ICT MGs. Scale bar = 100μm, arrowheads highlight *Ms4a7*+ cells. (B) Immunostaining shows persistent residence and expression of TMEM119 (red) and IBA1 (green) by MLCs 6 months after transplantation. (C) RNA *In situ* hybridization for *Ms4a7* (red) and *Cx3cr1* (green) shows absence of *Ms4a7*+ cells in the brain parenchyma of WT animals at 3 days, 2 months, and 2 years. Arrowheads depict *Cx3cr1+/Ms4a7-* macrophages in 2 year-old animals, to distinguish from vast autofluorescence. Scale bar = 100μm. (D,F) TMEM119 (red) and donor cell marker (green) expression 2 months after BM ICT into tamoxifen-injected Cx3cr1-CreER;Csf1r fl/fl neonates (D) or adults (F), showing that donor cells intermix with host, ramify and express TMEM119. (E,G) RNA *In situ* hybridization and protein staining of the same tissue for *Ms4a7* (red) and IBA1 protein (green) shows *Ms4a7*+ macrophages in the same pattern as donor cells from nearby immunostained sections, intermixed with *Ms4a7*- cells 2 months after neonatal (E) or adult (G) transplantation. Scale bars = 100μm (D,F), 50μm (E,G)

Figure S7, related to figure 7

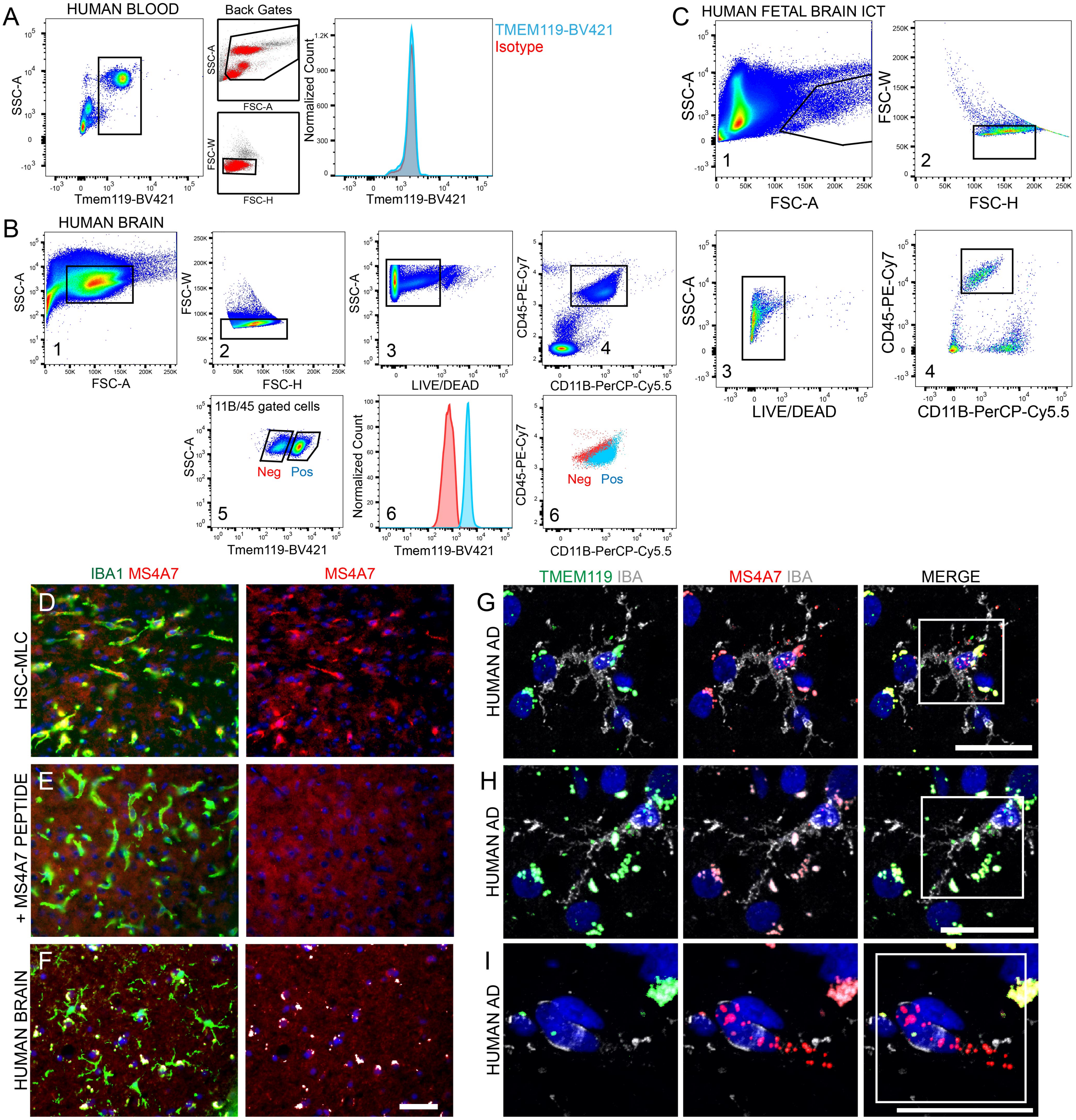

Figure S7, related to Figure 7. Validation of human TMEM119 monoclonal antibody and MS4A7 antibody specificity. (A) Mouse anti-hTMEM119 monoclonal clone A16075D does not stain human blood (blue) above isotype control (red) but (B) does stain most CD45/11B double positive cells from the brain, resolving a mixed double positive population into two otherwise inseparable groups. Numbers in lower left corner of plots shows gate hierarchy (1>2>3>4>5>6). (C) Gating strategy related to Figure 7E, numbers in lower left corner of plots shows gate hierarchy (1>2>3>4). Histogram in main Figure depicts cells in gate 4 from TMEM119 stained vs isotype control stain. D) Engrafted human blood cells immunostained with antibodies against IBA1 (green) and MS4A7 (red). E) Engrafted human blood cells stained with antibodies against IBA1 (green) and MS4A7 pre-incubated with molar excess of immunizing peptide (red) F) Human surgical sample stained with antibodies against IBA1 and MS4A7, and overlaid with signal from unstained channel to identify tissue autofluorescence (light yellow in left panel, white in right panel). Scale bar = 50μm. (G-I) Full images of cells shown in Figures 7I-7K, with area of cropping designated by a white square. Scale bars = 25μm.

Table 1. Differential gene expression in ICT vs WT microglia, related to Figure 1.

Gene	WT MG	pICTMG	log2 (WT/ICT)	FDR
Hlf	30.0	2.0	3.93	0.000
Gpr165	389.1	32.3	3.69	0.000
Serpine2	91.1	10.9	3.16	0.000
Khdrbs3	33.6	5.0	2.49	0.000
Casc4	43.1	9.1	2.39	0.000
Rab6b	32.3	7.3	2.21	0.000
Snx9	25.7	16.8	2.10	0.001
Nav2	28.5	8.0	1.88	0.000
F9	47.9	14.1	1.87	0.002
Lst1	45.4	19.5	1.82	0.001
Ccl9	93.4	29.5	1.78	0.000
Ccl6	44.9	13.4	1.77	0.007
Tspan13	69.6	21.1	1.77	0.000
Csmd3	41.9	13.0	1.73	0.001
Ngrn	25.9	8.0	1.68	0.001
Hnmt	31.7	10.1	1.67	0.004
Tspan7	76.8	27.4	1.57	0.000
Fam102b	139.1	50.6	1.50	0.000
D0H4S114	53.5	21.9	1.46	0.003
Wrb	25.3	9.9	1.43	0.025
Znrf1	37.2	13.4	1.40	0.007
Ets1	47.2	19.7	1.32	0.000
Ccr5	674.4	287.3	1.30	0.000
Gprasp1	26.7	11.8	1.28	0.001
Cst3	20034.9	8776.6	1.28	0.020
Jam2	67.9	28.8	1.27	0.001
Zbtb20	25.3	12.1	1.26	0.045
Tuba1a	73.8	44.3	1.23	0.003
Zfp691	58.7	26.9	1.21	0.003
Basp1	162.0	76.4	1.19	0.002
Prkaca	40.3	19.5	1.17	0.005
0610040J01Rik	40.3	19.2	1.15	0.006
Idua	24.6	12.6	1.13	0.045
Gpr155	153.7	74.8	1.13	0.018
Cdk5r1	20.6	10.7	1.10	0.030
Sema4b	29.2	15.3	1.06	0.010
Fscn1	109.4	56.2	1.04	0.023
Crybb1	113.0	51.1	1.04	0.002
Tppp	24.4	12.7	1.02	0.007
1110004F10Rik	40.2	20.8	1.01	0.035
Bcl9l	36.6	19.1	1.00	0.012
Stab1	16.2	34.7	-1.00	0.044
P2rx4	12.3	22.5	-1.03	0.032
Nfkbiz	47.3	117.4	-1.12	0.007
Vps13c	11.6	27.2	-1.13	0.000
Fth1	313.3	781.4	-1.13	0.001
Tpd52	10.2	22.3	-1.14	0.006
Rpl32	48.5	104.1	-1.15	0.048
C5ar1	17.8	41.8	-1.17	0.020
AU040972	8.8	20.8	-1.20	0.041
Afap1l1	9.4	20.6	-1.21	0.018
Clec5a	13.4	34.7	-1.21	0.003
Il1a	55.6	163.9	-1.22	0.020
Ctsh	48.0	108.6	-1.23	0.000
Alox5	11.0	26.6	-1.24	0.048
Man2b1	136.4	340.0	-1.25	0.000
Gm14023	18.9	55.2	-1.25	0.029
Icosl	18.1	46.1	-1.26	0.020
Apoe	236.5	590.0	-1.26	0.041
D10Wsu52e	14.8	39.6	-1.29	0.008
Rnf150	9.4	23.9	-1.29	0.001
Cd84	62.0	162.1	-1.32	0.001
Rgs1	172.5	474.6	-1.33	0.002
Slc17a5	11.8	30.1	-1.34	0.041
Man2a1	10.2	25.8	-1.37	0.000
Hmox1	8.5	20.8	-1.38	0.004
Myc	12.6	35.1	-1.39	0.010
Csf2ra	41.2	117.6	-1.45	0.001
Tank	9.5	20.7	-1.46	0.006
Ifi30	29.6	89.3	-1.47	0.001
Syt6	13.2	36.6	-1.49	0.001
Osm	6.6	21.6	-1.53	0.017
Neat1	11.8	35.9	-1.54	0.031
3200002M19Rik	14.9	54.0	-1.55	0.004
Lilrb4	4.7	27.1	-1.55	0.001
Cd52	17.9	54.9	-1.56	0.030
Lrrc3	56.4	178.5	-1.57	0.000
C3ar1	42.8	134.0	-1.57	0.000
Tlr2	8.1	24.7	-1.61	0.001
Smc4	10.5	34.7	-1.70	0.011
Ms4a6b	20.1	62.7	-1.77	0.000
C030034L19Rik	9.5	38.1	-1.79	0.000
H60b	2.9	20.4	-1.80	0.000
Ccrl2	5.6	23.2	-1.88	0.001
Ccl2	31.5	182.9	-1.90	0.001
Gpr160	6.7	27.4	-1.94	0.001
Cdkn1a	18.0	74.4	-1.96	0.007
Fmn1	6.1	25.6	-2.02	0.000
Dab2	7.3	35.0	-2.09	0.000
Ccr1	13.0	63.1	-2.17	0.000
Gm16907	3.8	22.7	-2.24	0.000
Ccl12	38.8	254.6	-2.47	0.000
Ms4a6c	5.9	30.7	-2.47	0.000
H2-DMb1	3.8	23.5	-2.48	0.000
Pvt1	3.3	21.3	-2.58	0.000
Myo5a	4.7	29.3	-2.60	0.000
Ctla2b	5.3	33.2	-2.62	0.000
Ctse	2.9	28.4	-3.14	0.000
Lyz2	22.9	230.4	-3.26	0.000
Cd34	6.0	63.4	-3.32	0.000
1810011O10Rik	2.8	41.4	-3.44	0.000
H2-DMa	2.3	25.9	-3.56	0.000
Mki67	2.4	36.5	-3.84	0.002
Mrc1	4.8	60.8	-3.85	0.000
Srgn	7.0	106.6	-3.92	0.000
Niacr1	0.6	22.1	-4.42	0.000
1300014I06Rik	0.4	20.1	-4.71	0.000
Gpr65	1.8	65.3	-5.16	0.000
Fxyd5	0.4	29.6	-6.18	0.000
Msr1	0.2	37.2	-6.69	0.000
Fn1	0.1	27.4	-7.85	0.000

Table S1, related to Figure 1. Differentially expressed genes between ICT-MGs and WT-MGs. Columns (left to right) show gene name, FPKM in WT MG and pooled ICT-MGs, log2(fold change), and false discovery rate (FDR) from edgeR comparison. Differential gene expression (DGE) was filtered for at least 2-fold change, FPKM>20 in at least 1 sample, and FDR <0.05.

Table S2: Expression of microglia enriched genes in MLCs, related to Figure 4.

Gene	pICT MG	ICT YSs	ICT HSC	Log2 (YSs/ MG)	p-value	Log2 (HSC/ MG)	p-value	Log2 (YSs/ HSC)	p-value
Slc2a5	37.7	27.1	3.2	-0.55	0.19	-3.63	0.00	3.08	0.00
P2ry12	2097.3	1636.9	244.3	-0.39	0.14	-3.05	0.00	2.68	0.00
Asph	196.4	124.1	27.3	-0.60	0.04	-2.91	0.00	2.33	0.00
Gpr34	385.2	392.1	121.4	-0.01	0.99	-1.67	0.00	1.69	0.00
Ccr5	287.3	155.7	50.6	-0.93	0.00	-2.58	0.00	1.67	0.00
Fam110a	15.9	14.7	5.3	-0.17	0.70	-1.67	0.00	1.51	0.00
Cd164	638.0	619.8	231.5	-0.08	0.74	-1.49	0.00	1.43	0.00
Pnp	98.9	87.9	37.8	-0.35	0.13	-1.49	0.00	1.15	0.00
Epb4.1l2	576.2	551.2	265.8	-0.18	0.42	-1.28	0.00	1.11	0.00
Golm1	228.4	241.4	115.3	0.01	0.96	-1.06	0.00	1.09	0.00
9330133O14Rik	10.5	9.9	4.7	-0.13	0.75	-1.15	0.00	1.04	0.02
P2ry13	1188.6	991.0	541.5	-0.30	0.22	-1.20	0.00	0.92	0.00
Commd8	76.8	71.7	41.2	-0.10	0.72	-1.00	0.00	0.92	0.00
Cfh	280.0	236.8	125.8	-0.10	0.32	-1.15	0.00	0.91	0.00
Fam102b	50.6	32.4	17.8	-0.10	0.03	-1.60	0.00	0.89	0.00
Casp8	169.5	141.9	77.1	-0.10	0.34	-1.07	0.00	0.82	0.00
Gal3st4	100.9	87.9	56.5	-0.10	0.35	-1.04	0.00	0.81	0.00
Olfml3	489.7	352.2	208.0	-0.10	0.04	-1.27	0.00	0.80	0.00
Rtn4rl1	56.1	42.4	25.6	-0.10	0.06	-1.25	0.00	0.75	0.01
6230427J02Rik	17.9	36.6	21.2	-0.10	0.01	0.35	0.30	0.73	0.03
Tmem119	533.0	724.2	464.4	-0.10	0.13	-0.26	0.23	0.72	0.00
0610040J01Rik	19.2	18.3	11.4	-0.10	0.82	-0.76	0.01	0.70	0.04
Entpd1	268.5	219.0	141.9	-0.10	0.15	-1.01	0.00	0.69	0.00
Zfp715	38.5	42.7	29.4	-0.10	0.73	-0.47	0.06	0.59	0.03
D15Ertd621e	106.5	87.6	58.9	-0.10	0.11	-0.92	0.00	0.58	0.01
Cmtm6	110.8	99.7	72.7	-0.10	0.51	-0.67	0.00	0.50	0.03
Abca9	226.8	236.5	180.3	-0.10	0.89	-0.48	0.01	0.47	0.01
Ifngr1	319.8	341.2	261.4	-0.10	0.82	-0.36	0.10	0.43	0.05
Srgap2	125.4	97.9	78.0	-0.10	0.08	-0.80	0.00	0.42	0.02
Plekho1	217.3	210.4	162.9	-0.10	0.39	-0.61	0.00	0.41	0.08
Tmem173	93.0	84.4	66.6	-0.10	0.52	-0.57	0.01	0.40	0.08
Rnase4	650.4	1106.7	870.1	-0.10	0.00	0.40	0.07	0.39	0.05
Serinc3	1876.7	1984.5	1596.0	-0.10	0.81	-0.30	0.10	0.37	0.05
Elmo1	149.4	117.1	95.1	-0.10	0.06	-0.80	0.00	0.36	0.06
Smap2	139.7	113.9	93.7	-0.10	0.12	-0.71	0.00	0.34	0.09
Tgfbr1	569.2	513.8	416.1	-0.10	0.45	-0.51	0.02	0.34	0.10
Tnfaip8l2	33.0	55.9	48.2	-0.10	0.04	0.45	0.15	0.33	0.32
Gna15	22.0	26.3	24.5	-0.10	1.00	-0.30	0.20	0.32	0.21
B4galt4	15.9	12.6	8.9	-0.10	0.19	-0.80	0.02	0.30	0.45
Mef2c	171.4	172.0	148.1	-0.02	0.95	-0.29	0.09	0.29	0.11
Mylip	59.2	60.7	48.4	-0.14	0.62	-0.40	0.06	0.28	0.27
4632428N05Rik	244.0	237.5	205.0	-0.08	0.73	-0.34	0.05	0.28	0.11
Slco2b1	280.3	323.1	277.3	0.16	0.55	-0.09	0.67	0.26	0.26
Fcrls	183.5	286.2	257.4	0.66	0.03	0.50	0.05	0.17	0.56
Hexb	2956.8	2161.0	2016.1	-0.54	0.02	-0.68	0.00	0.16	0.51
Rgs10	292.9	203.7	207.0	-0.50	0.03	-0.63	0.00	0.14	0.50
Srsf9	74.4	82.1	79.9	0.14	0.64	0.03	0.89	0.12	0.63
0610007C21Rik	50.3	72.3	66.8	0.55	0.20	0.45	0.15	0.11	0.78
Orai1	25.2	24.4	23.7	-0.13	0.66	-0.20	0.47	0.09	0.76
Tmem55b	47.1	48.0	43.1	-0.29	0.33	-0.36	0.13	0.08	0.77
Fam105a	166.9	183.6	184.2	0.13	0.61	0.10	0.61	0.04	0.85
Itpripl1	35.4	39.3	40.5	0.16	0.53	0.14	0.59	0.04	0.88
Ophn1	74.2	76.1	74.7	-0.13	0.62	-0.12	0.56	0.01	0.96
Sft2d1	38.0	36.4	39.8	-0.41	0.27	-0.40	0.18	0.00	1.00
Rab3il1	35.7	47.8	52.8	0.48	0.15	0.51	0.09	-0.02	0.95
Lair1	329.9	198.1	213.3	-0.77	0.01	-0.72	0.00	-0.03	0.91
Inpp5d	118.6	121.9	131.7	-0.10	0.72	-0.04	0.84	-0.05	0.85
Itm2b	608.1	658.4	718.9	0.05	0.83	0.15	0.50	-0.08	0.74
Pycard	29.5	16.6	18.4	-0.86	0.01	-0.77	0.01	-0.08	0.81
Lpar6	126.8	139.9	156.3	0.11	0.67	0.24	0.30	-0.11	0.69
Asb2	5.0	4.9	5.4	-0.21	0.59	-0.10	0.76	-0.11	0.78
Itgb5	120.3	141.7	155.8	0.15	0.55	0.28	0.21	-0.12	0.64
Ctsd	4098.4	3168.3	3629.6	-0.39	0.10	-0.19	0.42	-0.18	0.48
Sft2d2	53.2	53.9	64.3	-0.09	0.75	0.18	0.42	-0.25	0.18
AF251705	95.3	105.3	116.4	-0.14	0.57	0.17	0.41	-0.29	0.23
Blnk	74.7	66.9	77.5	-0.47	0.19	-0.15	0.49	-0.31	0.33
Il10ra	77.7	94.9	123.3	0.19	0.53	0.52	0.05	-0.32	0.16
BC004004	14.0	12.0	18.0	-0.19	0.62	0.18	0.58	-0.35	0.35
Gpr160	27.4	39.1	49.9	0.39	0.24	0.77	0.01	-0.36	0.19
Cysltr1	30.2	29.7	37.2	-0.11	0.75	0.28	0.28	-0.37	0.20
Trem2	159.3	199.4	271.6	0.27	0.32	0.71	0.00	-0.42	0.04
Ptafr	17.6	17.9	27.7	0.00	1.00	0.54	0.04	-0.52	0.10
Zfp658	20.7	19.7	32.0	-0.17	0.60	0.51	0.05	-0.67	0.02
Tmem37	11.1	23.5	42.0	1.08	0.01	1.78	0.00	-0.69	0.08
Pmepa1	165.6	170.4	353.3	0.02	0.95	1.04	0.00	-1.01	0.00
Ldhb	2.4	0.8	16.0	NA	NA	2.45	0.00	-3.78	0.00
Liph	0.8	0.9	0.0	NA	NA	NA	NA	NA	NA

Table S2, related to Figure 4. Expression of microglia-enriched gene cassette from Bennett et al 2016 in ICT-MGs, HSC- and YS-MLCs. Columns (left to right) show gene name, FPKM values, Log2(fold change) for 3 relevant comparisons, and associated p-values. Colored genes indicate DGE between YS- and HSC-MLCs (2 fold cutoff, FDR<0.05, FPKM >20). Genes colored blue were closer to MG levels in YS-MLCs, while genes colored orange were closer in HSC-MLCs.

Table S3: Expression of TMEM119- myeloid cell enriched gene cassette in MLCs, related to Figure 4.

Gene	pICT MG	ICT YSs	ICT HSCs	Log2 (YSs/ MG)	p-value	Log2 (HSC/ MG)	p-value	Log2 (YSs/ HSC)	p-value
H2-Aa	9.4	13.3	469.7	0.43	0.53	5.50	0.00	-5.04	0.00
H2-Ab1	5.1	7.4	194.4	0.53	0.46	5.20	0.00	-4.65	0.00
AI607873	1.5	3.5	68.8	1.29	0.03	5.64	0.00	-4.31	0.00
Klra2	3.5	12.7	233.9	1.70	0.06	5.90	0.00	-4.17	0.00
C3	2.6	0.9	10.9	-1.94	0.01	1.97	0.00	-3.90	0.00
Cd300ld	0.8	1.1	16.7	NA	NA	5.03	0.00	-3.84	0.00
Lyz2	230.4	446.8	5880.4	0.90	0.01	4.67	0.00	-3.76	0.00
Cd74	76.2	78.5	966.8	-0.07	0.89	3.60	0.00	-3.65	0.00
Clec12a	0.6	8.8	81.3	3.69	0.00	6.99	0.00	-3.36	0.00
Ccr2	3.7	1.1	6.6	-1.98	0.02	1.21	0.24	-3.18	0.00
Cpne2	1.5	2.7	20.6	0.67	0.24	3.78	0.00	-3.13	0.00
Lilrb4	27.1	35.0	246.1	0.01	0.99	3.12	0.00	-3.10	0.00
Gp49a	7.0	6.1	38.6	-1.54	0.00	1.40	0.00	-2.93	0.00
Cd97	0.8	0.9	6.9	NA	NA	3.28	0.00	-2.91	0.00
Arhgap15	3.5	11.4	88.5	1.89	0.00	4.68	0.00	-2.76	0.00
Cxcl2	15.5	23.7	131.0	0.59	0.44	3.17	0.00	-2.56	0.00
Rara	0.5	1.5	8.5	NA	NA	3.64	0.00	-2.52	0.00
Mndal	0.9	3.5	19.1	NA	NA	3.51	0.00	-2.45	0.00
Plbd1	1.8	2.4	22.7	0.52	0.46	2.92	0.00	-2.38	0.00
Syne1	0.0	0.2	3.9	2.40	0.00	4.51	0.00	-2.07	0.00
Cybb	11.3	92.5	359.2	2.94	0.00	4.96	0.00	-2.00	0.00
Neat1	35.9	34.4	125.9	-0.10	0.74	1.69	0.00	-1.79	0.00
Ifitm3	10.8	29.3	106.6	1.36	0.06	3.15	0.00	-1.77	0.00
Fxyd5	29.6	35.8	98.8	0.28	0.59	1.84	0.00	-1.55	0.00
Il1b	2.8	3.3	8.6	-0.30	0.65	NA	NA	-1.50	0.00
Rasgrp4	2.4	1.5	4.4	-0.94	0.10	0.51	0.18	-1.41	0.00
Ifitm2	12.7	29.1	79.4	1.09	0.02	2.47	0.00	-1.35	0.00
Emb	1.5	5.8	13.7	1.84	0.01	3.14	0.00	-1.29	0.11
Sp100	3.9	13.2	30.8	1.97	0.00	3.11	0.00	-1.13	0.00
Fam65b	3.5	3.9	11.1	0.40	0.48	1.49	0.00	-1.06	0.01
Cd44	2.3	8.6	17.6	2.01	0.00	3.07	0.00	-1.04	0.00
Igsf6	44.3	70.5	146.1	0.64	0.04	1.66	0.00	-1.00	0.00
Sepx1	8.8	13.7	28.6	0.76	0.30	1.78	0.00	-0.98	0.04
Lilrb3	7.4	7.7	20.0	0.13	0.81	1.11	0.00	-0.96	0.01
Ipcef1	6.4	4.9	8.6	-0.43	0.41	0.43	0.21	-0.85	0.10
Itga4	1.9	14.5	25.0	2.74	0.00	3.58	0.00	-0.82	0.00
Adpgk	14.2	15.2	26.4	-0.20	0.69	0.61	0.15	-0.79	0.02
F630028O10Rik	8.4	22.3	34.3	1.33	0.00	1.93	0.00	-0.59	0.13
Iqgap1	4.5	15.7	23.2	1.78	0.00	2.34	0.00	-0.55	0.13
Pilra	35.6	48.9	52.1	0.36	0.32	0.44	0.12	-0.06	0.87
Vim	11.6	38.1	29.3	1.42	0.00	1.36	0.02	0.07	0.89
Cnn2	2.2	7.9	7.3	1.62	0.01	NA	NA	0.07	0.88
Mxd1	13.7	8.3	6.3	-0.83	0.06	-1.13	0.00	0.32	0.42
Evi2b	69.1	63.3	53.3	-0.17	0.63	-0.57	0.01	0.42	0.18
Pram1	4.2	7.6	5.5	0.65	0.09	0.19	0.60	0.47	0.20
Lsp1	19.7	82.4	64.1	2.07	0.00	1.57	0.00	0.50	0.29
Cd24a	1.2	4.2	2.3	1.85	0.01	NA	NA	0.73	0.34
Ceacam1	45.0	23.0	10.5	-1.02	0.00	-2.08	0.00	1.09	0.00
Lyz1	0.9	0.9	22.1	NA	NA	NA	NA	NA	NA
Crip1	3.1	7.6	20.6	NA	NA	NA	NA	NA	NA

Gene	pICT MG	ICT YSs	ICT HSCs	Log2 (YSs/ MG)	p-value	Log2 (HSC / MG)	p-value	Log2 (YSs /HSC)	p-value
Gm11428	3.9	6.1	17.4	NA	NA	NA	NA	NA	NA
S100a11	5.7	8.1	7.4	NA	NA	NA	NA	NA	NA
Lilra6	2.7	2.4	6.7	NA	NA	NA	NA	NA	NA
S100a9	4.1	4.2	5.5	NA	NA	NA	NA	NA	NA
Pygl	2.3	3.3	4.9	0.44	0.44	NA	NA	NA	NA
S100a8	1.8	1.0	4.1	NA	NA	NA	NA	NA	NA
2310001H17Rik	0.6	0.6	4.1	NA	NA	NA	NA	NA	NA
Fpr1	0.2	0.4	3.5	NA	NA	NA	NA	NA	NA
Anxa1	0.6	7.6	3.4	3.18	0.00	NA	NA	NA	NA
Pilrb2	0.2	0.9	3.1	NA	NA	NA	NA	NA	NA
H2-Eb1	0.0	0.2	2.7	NA	NA	NA	NA	NA	NA
Fpr2	0.2	0.0	2.3	NA	NA	NA	NA	NA	NA
Avpi1	0.9	1.2	2.0	NA	NA	NA	NA	NA	NA
Ngp	0.2	0.9	1.7	NA	NA	NA	NA	NA	NA
1810033B17Rik	0.4	1.8	1.7	NA	NA	NA	NA	NA	NA
H2-Q10	0.1	0.4	1.6	NA	NA	NA	NA	NA	NA
Gsr	1.4	0.9	1.5	NA	NA	NA	NA	NA	NA
S100a6	1.1	3.4	1.3	NA	NA	NA	NA	NA	NA
Gda	0.2	0.4	1.2	NA	NA	NA	NA	NA	NA
Stfa2l1	1.4	0.2	1.1	NA	NA	NA	NA	NA	NA
Ifitm6	2.7	1.5	1.1	NA	NA	NA	NA	NA	NA
Plac8	0.0	0.9	1.1	NA	NA	NA	NA	NA	NA
Retnlg	0.0	1.9	1.0	NA	NA	NA	NA	NA	NA
Lcn2	0.0	0.2	0.9	NA	NA	NA	NA	NA	NA
Sell	1.0	0.1	0.8	NA	NA	NA	NA	NA	NA
Gm5483	1.8	0.0	0.7	NA	NA	NA	NA	NA	NA
Samd9l	1.2	0.9	0.6	-0.47	0.62	NA	NA	NA	NA
Gm16894	0.5	0.8	0.6	NA	NA	NA	NA	NA	NA
Slfn4	0.0	0.0	0.5	NA	NA	NA	NA	NA	NA
Atp8b4	0.4	0.6	0.5	NA	NA	NA	NA	NA	NA
Camp	0.0	0.0	0.5	NA	NA	NA	NA	NA	NA
Dok2	0.9	1.3	0.5	NA	NA	NA	NA	NA	NA
Ear2	0.0	0.4	0.4	NA	NA	NA	NA	NA	NA
Ifitm1	0.0	0.0	0.4	NA	NA	NA	NA	NA	NA
Pglyrp1	0.4	0.9	0.4	NA	NA	NA	NA	NA	NA
Chi3l3	0.0	0.5	0.2	NA	NA	NA	NA	NA	NA
Slfn1	0.0	0.3	0.2	NA	NA	NA	NA	NA	NA
Mmp8	0.0	0.2	0.1	NA	NA	NA	NA	NA	NA
Cxcr2	0.2	1.2	0.1	NA	NA	NA	NA	NA	NA
Ltf	0.1	0.0	0.1	NA	NA	NA	NA	NA	NA
Trim30b	0.6	0.3	0.1	NA	NA	NA	NA	NA	NA
Ly6c2	0.0	0.0	0.1	NA	NA	NA	NA	NA	NA
Hdc	0.0	0.2	0.1	NA	NA	NA	NA	NA	NA
Mmp9	0.0	0.0	0.0	NA	NA	NA	NA	NA	NA
1100001G20Rik	0.0	0.0	0.0	NA	NA	NA	NA	NA	NA
Elane	0.0	0.0	0.0	NA	NA	NA	NA	NA	NA
Hsd11b1	0.0	0.0	0.0	NA	NA	NA	NA	NA	NA
Mpo	0.0	0.0	0.0	NA	NA	NA	NA	NA	NA
Padi4	0.0	0.0	0.0	NA	NA	NA	NA	NA	NA
Slpi	1.7	1.7	0.0	NA	NA	NA	NA	NA	NA

Table S3, related to Figure 4. Expression of non-microglial myeloid cell-enriched gene cassette from Bennett et al 2016 in ICT-MGs, HSC- and YS-MLCs. Columns (left to right) show gene name, FPKM values, Log2(fold change values) for 3 relevant comparisons, and associated p-values. Colored genes indicate DGE between YS- and HSC-MLCs (2 fold cutoff, FDR<0.05, FPKM >20). Genes colored blue were closer to MG levels in YS-MLCs, while genes colored orange were closer in HSC-MLCs. NA = unable to achieve meaningful statistical comparison based on read counts by edgeR.

Table 4: Genes decreased in IP BM MLCs (2-3 months brain residence) compared to ICT BM MLCs (2 weeks brain residence), related to Figure 4.

Gene	ICT BM	IP BM	Log2 (ICT/ IP)	FDR
4833417C18Rik	20.51	0.82	4.06	0.000
Ddit4	31.18	1.81	4.02	0.000
Amica1	34.95	2.35	3.99	0.000
2810008D09Rik	45.15	5.44	3.19	0.000
Hist1h1e	32.06	3.59	3.01	0.000
Samd1	36.97	6.06	2.81	0.000
Cbr2	20.64	4.84	2.76	0.010
Adam19	69.85	10.3	2.76	0.000
C030034L19Rik	127.09	19	2.71	0.000
U2af1l4	29.14	5.59	2.69	0.003
Dok3	44.82	7.67	2.66	0.000
Clec4a1	22.59	3.4	2.63	0.004
Spp1	50.64	7.66	2.61	0.003
Snhg3	60.39	15.4	2.52	0.001
Ier2	209.03	37.5	2.50	0.000
Arl10	41.57	7.63	2.48	0.004
2410004N09Rik	35.35	5.05	2.46	0.024
Ssna1	43.99	7.81	2.45	0.008
Pnpla2	45.86	9.17	2.37	0.000
Per1	119.81	24.9	2.36	0.000
Fkbp5	197.23	30.1	2.34	0.001
Rps16	40.51	49.7	2.34	0.038
Stab1	156.37	33.1	2.31	0.000
Rps27a	34.36	11.9	2.31	0.015
Serpinf1	125.35	26.4	2.30	0.000
Dctpp1	59.52	9.98	2.30	0.028
Htra2	33.98	26.5	2.29	0.006
Klf2	146.66	30.7	2.29	0.001
Mpdu1	20.08	6.72	2.28	0.022
C1qa	1187.1	253	2.25	0.000
Ppia	134.48	142	2.22	0.012
Lsp1	83.81	18	2.17	0.001
Zfp414	31.63	6.01	2.17	0.002
Slc38a7	22.27	4.27	2.14	0.002
Fau	70.92	56	2.13	0.050
Rps29	285.31	218	2.13	0.017
Sult1a1	184.48	37	2.13	0.003
Sh3gl1	26.03	9.41	2.09	0.006
Fcgrt	25.74	6.22	2.07	0.016
Exosc5	20.24	4.59	2.06	0.031
Arrdc1	62	16.1	2.05	0.000
Dhrs4	22.91	5.82	2.05	0.029
Mitd1	22.78	5.21	2.02	0.049
Prkab1	65.28	19.2	2.00	0.006
Neat1	225.88	56.9	1.98	0.008
Ugt1a7c	21.19	7.43	1.97	0.040
Zfp513	23.22	5.76	1.97	0.003
Sf3b4	22.66	7.8	1.97	0.012
Asna1	32.25	6.18	1.92	0.014
Cryba4	49.54	10.16	1.92	0.011
Dnase2a	20.29	8.91	1.91	0.012
Snrpa	32.89	10.62	1.89	0.015
Slfn2	38.11	11.01	1.88	0.006
Bcl2l1	25.87	7.15	1.87	0.029
Irf3	53.89	15.83	1.83	0.003
Slc2a3	22.72	6.67	1.79	0.047
Lyl1	181.1	53.34	1.77	0.000
Ppp4c	45.09	12.73	1.77	0.029
Miip	21.75	8.19	1.74	0.028
H2-DMa	240.8	82.49	1.74	0.012
H2-DMb2	77.88	22	1.69	0.049
Polg	24.02	7.23	1.69	0.015
Atp13a1	23.52	9.19	1.69	0.006
Ap1b1	44.13	13.47	1.67	0.004
Shmt2	20.51	6.66	1.66	0.027
Glt25d1	65.77	20.3	1.64	0.002
Ltc4s	117.7	37.2	1.64	0.044
Anapc16	74.34	21.78	1.63	0.012
Isyna1	40.01	13.86	1.63	0.017
Araf	44.17	18.17	1.63	0.011
Sdc4	50.53	15.66	1.61	0.034
Rogdi	142.9	52.51	1.60	0.012
1810031K17Rik	52.81	17.67	1.56	0.047
Slc35c2	56.31	20.12	1.56	0.036
Ptms	139.7	50.17	1.56	0.030
Rnf169	71.47	23.1	1.54	0.002
Traf3ip3	30.69	10.03	1.52	0.029
Scamp5	52.12	17.72	1.52	0.026
Naga	37.44	13.26	1.50	0.021
Hlx	27.79	11.47	1.47	0.027
Ggta1	80.25	29.43	1.45	0.027
Gpr108	42.63	12.44	1.44	0.031
B4galnt1	90.39	31.23	1.42	0.049
Hist1h1c	41.66	12.64	1.41	0.035
Tsc22d3	252.6	96.9	1.40	0.039
Ak2	134.5	51.24	1.38	0.028
Tspyl2	39.75	14.85	1.38	0.037
Zfp36l1	627.7	248.9	1.35	0.016
P2ry6	222.2	88.71	1.35	0.033
Tmem104	82.77	29.97	1.34	0.017
Fbrs	69.97	30.96	1.32	0.030
Cd14	550.6	226.4	1.32	0.029
Kcnk6	94.49	37.25	1.30	0.022
Atp6v0b	283.4	126.7	1.29	0.048
Hk3	24.55	10.92	1.28	0.049
Kdm6b	41.14	18.33	1.28	0.033
Ppcdc	121.4	56.03	1.23	0.046
Slc43a2	71.13	29.77	1.21	0.031

Table S4, related to Figure 4. Genes more highly expressed in MLCs from BM ICT at 2 weeks than MLCs from BM IP at 2-3 months. Columns (left to right) show gene name, FPKM values, Log2(fold change), and associated FDRs, for all genes with FPKM>20 in at least 1 sample, and Log2(FC) of 1 or higher.

Table S5: Genes increased in IP BM MLCs (2-3 months brain residence) compared to ICT BM MLCs (2 weeks brain residence), related to Figure 4.

Gene	ICT BM	IP BM	Log2 (ICT/IP)	FDR
Eif2s3y	0.59	35.8	-6.47	0.000
Ddx3y	0.31	21.53	-6.17	0.000
Lyz1	10.17	31.49	-5.93	0.010
St8sia6	0.99	30.67	-5.30	0.000
Atp1b1	1.54	50.09	-5.01	0.015
Rps23	85.35	112.7	-4.55	0.000
Cdr1	2.75	53.75	-4.16	0.041
Ndrg2	1.28	20.2	-3.67	0.037
1810011O10Rik	1.01	29.9	-3.51	0.000
G530011O06Rik	3.19	29.62	-3.46	0.000
Qpct	4.34	34.97	-3.19	0.012
Kcnj13	4.67	32.77	-2.79	0.004
Tgm2	4.26	38.35	-2.76	0.002
Galnt3	5.4	27.46	-2.66	0.000
Siglech	45.59	229.05	-2.34	0.000
Cab39l	5.19	23.24	-2.33	0.023
Ccnd2	8.37	37.82	-2.22	0.003
Inpp4b	6.57	26.8	-2.21	0.035
Hmgb2	10.17	31.12	-2.18	0.008
AI504432	8.96	43.19	-2.18	0.012
H2-M3	11.55	44.83	-2.12	0.009
Cmpk1	14.61	61.48	-2.12	0.000
Tmed5	16.64	60.49	-2.06	0.012
Rab6b	5.58	22.57	-2.05	0.016
Camk2n1	9.41	38.75	-1.96	0.004
Myo1b	7.92	30.06	-1.90	0.009
Peli1	15.53	54.4	-1.85	0.012
Abcc9	8.87	31.52	-1.85	0.006
Niacr1	6.96	26.93	-1.83	0.037
Rtn1	24.9	91.44	-1.79	0.030
Pkib	8.66	29.93	-1.78	0.027
Klra2	110.98	355.86	-1.77	0.003
Arhgap5	22.81	69.58	-1.70	0.027
Ids	23.7	74.67	-1.67	0.031
B3gnt2	22.14	67.73	-1.67	0.024
Cfh	70.15	176.16	-1.67	0.020
Plxna4	10.95	31.68	-1.67	0.012
Tex2	9.25	29.49	-1.61	0.022
Fam98b	11.54	34.38	-1.61	0.026
Basp1	29.04	78.35	-1.60	0.027
Htra3	7.22	21.16	-1.58	0.050
Dnajc28	9.05	25.28	-1.56	0.049
Leprel1	8.84	23.64	-1.56	0.048
Ifi204	14.1	30.23	-1.52	0.048
Tlr3	13.16	34.91	-1.50	0.037
H2-K1	320.31	874.72	-1.49	0.024
Ier5	107.84	295.01	-1.48	0.038
Arhgap15	50.11	122.93	-1.45	0.031
Lrrc3	87.08	222.08	-1.39	0.029
Sgpp1	33.38	82.43	-1.38	0.030

Table S5, related to Figure 4. Genes more highly expressed in MLCs from BM IP at 2-3 months than MLCs from BM ICT at 2 weeks. Columns (left to right) show gene name, FPKM values, Log2(fold change), and associated FDRs, for all genes with FPKM>20 in at least 1 sample, and Log2(FC) of -1 or lower.