

Table 1. Antibodies used for cytometric analysis and sorting

Antigen	Label	Clone	Isotype	Supplier	RRID
CD11b	APC/Cy7	M1/70	rat IgG2b, κ	Biolegend	AB_830641
CD11c	PE/Cy7	N418	Armenian hamster	Biolegend	AB_493569
CD16/CD32		93	rat IgG2b, λ	Biolegend	AB_312800
CD172a	AF700	P89	Rat IgG1, κ	Biolegend	AB_2650812
CD4	F488	RM4-5	rat IgG2a, κ	Biolegend	AB_493373
CD45	BV510	30F-11	rat IgG2b, κ	Biolegend	AB_2561392
CD45	APC	30F-11	rat IgG2b, κ	Biolegend	AB_312976
CX3CR1	PE	SA011F11	rat IgG2a, κ	Biolegend	AB_2564314
F4/80	PE-Cy5	BM8	rat IgG2a, κ	Biolegend	AB_893494
I-A/I-E	BV605	M5/114.15.2	rat IgG2b, κ	Biolegend	AB_2565894
Ly6C	FITC	HK1.4	rat IgG2c, κ	Biolegend	AB_1186134
Ly6G	PerCP/Cy5.5	1A8	rat IgG2a, κ	Biolegend	AB_1877272
Ly6G	APC	1A8	rat IgG2a, κ	Biolegend	AB_1877163
TCR β	PerCP/Cy5.5	H57-597	Armenian hamster	Biolegend	AB_1575176
TER-119	Biotin	TER-119	rat IgG2b, κ	Biolegend	AB_313704
XCR1	PerCP/Cy5.5	ZET	mouse IgG2b, κ	Biolegend	AB_2564363
CD197 (CCR7)	PE	4B12	rat IgG2a, κ	Biolegend	AB_2564363
CD209a	PE	MMD3	mouse IgG2c, κ	Biolegend	AB_2721636
Lin:					
CD19	FITC	6D5	rat IgG2a, κ	Biolegend	AB_313640
CD3ε	FITC	145-2C11	Armenian hamster	Biolegend	AB_312670

Ly6G	FITC	1A8	rat IgG2a, κ	Biolegend	AB_1236488
NK1.1	FITC	PK136	mouse IgG2a, κ	Biolegend	AB_313392
TCR β	FITC	H57-597	Armenian hamster	Biolegend	AB_313428

Table S2. Drop-Seq run description

Sample Name	Microfluidic Device	Bead Lot Number	Quantine HJ USED?	Bead No. collected (theoretical)	Bead No. (after Wash)	Bead No. (before Amp.)	Amp. Cycles	cDNA (ng/ μ l)	Total cDNA Yield ng	Library (ng/ μ l)	Mouse Strain	Sex	Age	No. of mice pooled	EC10 ³	MGx10 ³	CD45x10 ³	Td-cellsx10 ³	Cell populations within the sample	Treatment	Order Number	Index Primer	Illumina Run ID	Type (Next Seq/10x etc)	Sequencing sample ID	Seurat Designation	
GR200817	12/18/19 0.9mm	101619	YES	120,000	83,163	87,888	15	0.184	77.6	2.26	CB7B6_WT	female	18m	3	18	46	72	N/A	EC (18K); MG154K; CD45H(72);	DO2							
GR210225	12/18/19 0.9mm	101619	YES	93,333	78,107	85,690	15	0.0811	25.952		CB7B6_WT	female	18m	3	20	20	120	N/A	EC (20K); MG120K; CD45H(120K);	DO2		N701					
GR200812	12/18/19 0.9mm	101619	YES	96,666	76,502	69,660	15	0.191	68.76	2.4	CB7B6_WT	female	18m	2	25	124	54	N/A	EC (25K); MG124K; CD45H(54);	D14		N705					
GR200728	12/18/19 0.9mm	101619	YES	113,333	102,773	74,894	15	0.172	67.08	1.7	CB7B6_WT	female	18m	4	32	141	44	N/A	EC (32K); MG141K; CD45H(44);	S02		N704					
GR190610	10/04/18 0.9mm	012819C	YES	104,000	98,662	69,841	15	0.313	100.16	4.06	OXCR1_CreRhet_Tdhet	male	8-12w	4	N/A	N/A	N/A	125	Td-cells (MG125K)	S02002D14	10417514	N704	190624_N5500503_0929_AH NextSeq 500	GR190610_CTACGAGA			
GR190723	12/18/19 0.9mm	101619	YES	53,333	51,386	39,161	15	0.202	40.4	2.8	CB7B6_WT	male	17m	2	12	25	18	N/A	EC (12K); MG125K; CD45H(18);	DO2							
GR180426		83117	no	125,000	78,800	50,780	14	0.124	125.76		CB7B6_WT	male	8w	6	24	24	200	N/A	EC (24K); MG124K; CD45H(200K)	DO2							
GR180413tag		83117	no	152,000	102,773	64,607	15	0.305	122		CB7B6_WT	male	8w	5	25	25	150	N/A	EC (25K); MG125K; CD45H(150K);	D14	10400214	N701	180705_N5500503_0712_AH NextSeq 500	GR180613TAG_TAAGGGGA	D02R1		
GR180414tag		83117	no	164,000	131,550	82,835	15	0.175	70		CB7B6_WT	male	8w	5	25	25	150	N/A	EC (25K); MG125K; CD45H(150K);	DO2	10400214	N702	180705_N5500503_0712_AH NextSeq 500	GR180614TAG_CTACTAG	D14R2		
GR180917AG		83117	YES	112,000	53,442	48,546	15	0.418	102.72	2.1	CB7B6_WT	male	8w	4	25	25	150	N/A	EC (25K); MG125K; CD45H(150K);	D14	10404808	N702	181012_N5500503_0767_AH NextSeq 500	GR180917AG_CTACTAGC	D14R3		
GR180919AG		83117	YES	136,000	92,496	81,210	15	0.443	132.9	2.72	CB7B6_WT	male	8w	5	25	25	150	N/A	EC (25K); MG125K; CD45H(150K);	DO2	10404808	N704	181012_N5500503_0767_AH NextSeq 500	GR180919AG_CTACTAGC	D02R3		
GR190110	10/4/18	72818	YES	160,000	106,884	82,775	15	0.159	47.7	2.6	CB7B6_WT	male	8w	4	33	134	33	N/A	EC (33K); MG1134K; CD45H(33K);	S02	10410132	N704	190205_N5500503_0819_AH NextSeq 500	GR190110_CTACTAGC	S02R4		
GR180716AG	10/4/18	83117	no	157,320	133,605	79,406	15	0.188	75.2	2.18	CB7B6_WT	male	8w	4	35.7	191	28.1	N/A	EC (35.7K); MG1191K; CD45H(28.1K);	S02	10404808	N701	181012_N5500503_0767_AH NextSeq 500	GR180716AG_TGCGCTTA	S02R1		
GR181128	10/4/18	72818	YES	160,000	156,215	82,912	15	0.221	66.3	3.04	CB7B6_WT	male	8w	4	42	133	60	N/A	EC (42K); MG1133K; CD45H(60K);	S02	10408151	N702	181213_N5500503_0808_AH NextSeq 500	GR181128_CTACTAGC	S02R2		
GR181114	10/4/18	72818	YES	160,000	168,548	131,923	15	0.145	69.6	2.4	CB7B6_WT	male	9w	4	45	125	209	N/A	EC (45K); MG1123K; CD45H(209K);	D14	10408151	N702	181213_N5500503_0808_AH NextSeq 500	GR181114_CTACTAGC	D14R4		
GR181024	10/4/18	83117	YES	144,000	174,714	91,317	15	0.394	141.84	2.22	CB7B6_WT	male	9w	5	50	50	100	N/A	EC (50K); MG1100K; CD45H(100K);	DO2	10408151	N701	181213_N5500503_0808_AH NextSeq 500	GR181024_TGCGCTTA	D02R4		
GR181212	10/4/18	72818	YES	160,000	122,906	88,790	15	0.142	42.6	2.56	CB7B6_WT	male	10w	4	60	120	60	N/A	EC (60K); MG1120K; CD45H(60K);	S02	10410132	N701	190205_N5500503_0819_AH NextSeq 500	GR181212_TGCGCTTA	S02R3		
GR180125		83117	no	176,000	147,000	53,780	16				CB7B6_WT	male	8w	5	43.2	47.3	109.5	N/A	EC(43.2K); PMN(47.3K); Macro(109.5K)	D14	10391620		180213_N851191_0108_AH NextSeq 500	GR180125	D14R1		
GR180517		83117	no	150,000	96,000	83,196	16	0.213	85.2	2.372	CB7B6_WT	male	8w	1	N/A	N/A	N/A	N/A	Peripheral blood leukocytes (240K)	N							
GR18110701	10/4/18	83117	YES	160,000	147,993	94,385	15	0.319	114.84	2.86	CB7B6_WT	male	9w	2	N/A	N/A	N/A	N/A	Peripheral blood leukocytes (240K)	S02	10407791	N701	181211_N5500503_0806_AH NextSeq 500	GR18110701_TGCGCTTA	P8502R1		
GR18110702	10/4/18	83117	YES	160,000	137,716	100,702	15	0.283	101.88	2.64	CB7B6_WT	male	9w	2	N/A	N/A	N/A	N/A	Peripheral blood leukocytes (240K)	DO2	10407791	N702	181211_N5500503_0806_AH NextSeq 500	GR18110702_CTACTAGC	P8502R4		
GR181120	10/4/18	72818	YES	160,000	121,272	136,795	15	0.143	68.64	3.46	CB7B6_WT	male	9w	2	N/A	N/A	N/A	N/A	Peripheral blood leukocytes (240K)	D14	10407791	N704	181211_N5500503_0806_AH NextSeq 500	GR181120_CTACTAGC	P8514R1		
GR18121901	10/4/18	72818	YES	160,000	139,771	95,287	15	0.262	94.32	2.54	CB7B6_WT	male	10w	2	N/A	N/A	N/A	N/A	Peripheral blood leukocytes (240K)	S02	10410132	N702	190205_N5500503_0819_AH NextSeq 500	GR18121901_CTACTAGC	P8502R2		
GR18121902	10/4/18	72818	YES	160,000	129,494	85,421	15	0.147	44.1	2.18	CB7B6_WT	male	10w	2	N/A	N/A	N/A	N/A	Peripheral blood leukocytes (240K)	DO2	10414864	N701	190426_N5500503_0819_AH NextSeq 500	GR18121902_TGCGCTTA	P8502R3		
GR190116	10/4/18	72818	YES	160,000	168,548	91,678	15	0.285	98.325	2.44	CB7B6_WT	male	10w	2	N/A	N/A	N/A	N/A	Peripheral blood leukocytes (240K)	DO2	10414864	N702	190426_N5500503_0819_AH NextSeq 500	GR190116_CTACTAGC	P8502R3		
GR190410	10/04/18 0.9mm	012819C	YES	154,666	131,550	98,896	15	0.19	91.2	2.76	CB7B6_WT	male	9w	2	N/A	N/A	N/A	N/A	Peripheral blood leukocytes (240K)	DO2	10416797	N701	190611_N5500503_0919_AH NextSeq 500	GR190410_TGCGCTTA			
GR190415	10/04/18 0.9mm	012819C	YES	160,000	152,104	118,868	15	0.148	82.88	2.22	CB7B6_WT	male	9w	2	N/A	N/A	N/A	N/A	Peripheral blood leukocytes (240K)	D14	10416797	N702	190611_N5500503_0919_AH NextSeq 500	GR190415_CTACTAGC			
GR190417	10/04/18 0.9mm	012819C	YES	160,000	1282 (103.3)	74,714	15	0.309	30.9	2.68	CB7B6_WT	male	9w	2	N/A	N/A	N/A	N/A	Peripheral blood leukocytes (240K)	S02	10416797	N704	190611_N5500503_0919_AH NextSeq 500	GR190417_CTACTAGC			
GR190522	10/04/18 0.9mm	012819C	YES	152,000	127,439	91,437	15	0.351	35.1	2.14	CB7B6_WT	male	8w	2	N/A	N/A	N/A	N/A	Peripheral blood leukocytes (240K)	S02	10417514	N701	190624_N5500503_0929_AH NextSeq 500	GR190522_TGCGCTTA			
GR190529	10/04/18 0.9mm	012819C	YES	160,000	131,550	90,234	15	0.174	83.52	2.56	CB7B6_WT	male	8w	2	N/A	N/A	N/A	N/A	Peripheral blood leukocytes (240K)	D14	10417514	N702	190624_N5500503_0929_AH NextSeq 500	GR190529_CTACTAGC			

Table S3. Cell numbers among cell type classes of down-sampled young and aged brain datasets

	celltype	Aged	Young
1	Mg	5970	5241
2	BAM	295	316
3	MdC	2112	2875
4	DC	470	921
5	Gran	301	324
6	Tc	836	380
7	NK	44	125
8	Bc	96	115
9	MaC	0	10
10	EC	1747	1611
11	MC	92	36
12	Epi	20	27
13	OD	16	18

Table S4. Gene lists of module scores

DAM	DIM	ISG	SAM	Foamy macrophages	monocytes
Dkk2	Atf3	Rsad2	Spp1	Fabp4	S100a4
Fabp5	Btg2	Socs1	Fabp5	Ctsl	Ms4a6c
Gm1673	Ccl4	Ddx60	Gpnmb	Atp6v0d2	Crip1
Gpnmb	Ctss	Ifit2	Ctsb	Gpnmb	Ctss
Igf1	Dusp1	Ifit3	Ctsl	Fabp5	Ccl9
Itgax	Egr1	Ifit3b	Lgals3	Htra1	F13a1
Mamdc2	Fos	Herc6	Lpl	Epb41l3	Plac8
Spp1	Icam1	Rtp4	Fth1	Pld3	Fn1
	Ier2	Spats2l	Cd63		Ccr2
	Ier5	Ifi44l	Ctsd		Psap
	Il1a	Oas3			Ms4a4c
	Il1b	Cxcl10			Npc2
	Itga6	Mx1			Ifi30
	Jun	Mx2			Lyz2
	Junb	Isg15			Pld4
	Klf6	Oasl1			Lamp1
	Tnf	Ifi44			Ifitm3
	Zfp36	Oas2			Smpdl3a
	Cd83	Usp18			Ly86
	Fosb	Epsti1			Ctsc
	Cd14	Oas1e			Vim
	Fth1	Oas1c			Lgals1
	Nfkbia	Oas1b			Clec4a3
	Nfkbiz	Oas1f			S100a10
		Oas1h			Prdx1
		Oas1g			Anxa5
		Oas1a			Ctsb
		Oas1d			Dbi
		Siglec1			Napsa

Module	Reference
DAM	Silvin et al. Immunity 2022 Aug 9;55(8):1448-1465.e6. doi: 10.1016/j.immuni.2022.07.004.
DIM	Silvin et al. Immunity 2022 Aug 9;55(8):1448-1465.e6. doi: 10.1016/j.immuni.2022.07.004.
ISG	Kim et al. Interferon Cytokine Res. 2018 Apr;38(4):171-185. doi: 10.1089/jir.2017.0127
SAM	Beuker et al. Nat. Commun. 2022 Feb 17;13(1):945. doi: 10.1038/s41467-022-28593-1.
Foamy macrophages	Williams et al. Nat Immunol. 2020 Oct;21(10):1194-1204. doi: 10.1038/s41590-020-0768-4.
monocytes	Ballesteros et al. Cell 2020 Nov 25;183(5):1282-1297.e18. doi: 10.1016/j.cell.2020.10.003

Table S5. Antibodies used for immunohistochemistry

Antigen	Biological source	Clone	Isotype	Supplier	Cat. Num.	RRID	Dilution
IGF1	mouse	Sm1.2	IgG1k	Millipore	05-172	AB_309 643	1:100
CD206	rat	MR5D3	IgG2a	BioRad	MCA2235	AB_324 622	1:200
Iba1	rabbit	polyclonal		Wako	019- 19741	AB_839 504	1:200
DsRed (TdTomato)	rabbit	polyclonal		Takara	632496	AB_100 13483	1:200
Anti-MHC II (I-a/I-E)	rat	M5/114	IgG2bk	Millipore	MABF33	AB_108 07702	1:200

Table S6. RNA scope probe list

	Reference	Name
1	437511	RNAscope Probe - Mm-Mrc1
2	480311-C2	RNAscope Probe - Mm-Cd209a-C2
3	423381	RNAscope Probe - Mm-Lrg1
4	426371-C2	RNAscope Probe - Mm-Ms4a4c-C2
5	498711	RNAscope Probe - Mm-Cst7
6	432871	RNAscope Probe - Mm-Ccr7
7	403431-C3	RNAscope Probe - Mm-Arg1-C3
8	408921-C2	RNAscope Probe - Mm-Cxcl10-C2
9	546211-C2	RNAscope Probe - Mm-Ccl8-C2
10	468421-C2	RNAscope® Probe- Mm-Car4-C2