Supplementary Figures

Figure S1	Surgical approaches for excision of non-affected brain tissue.
Figure S2	Validations of sorted cells indicate pure microglial population.
Figure S3	Correlation of our microglial signature vs bulk surgical tissue.
Figure S4	Co-expression networks of WGCNA modules significantly associated with age, sex or *APOE*.
Figure S5	Expression of established microglial marker genes in single cell data.
Figure S6	Heatmap of top cluster marker genes across single cell clusters.
Figure S7	Hypergeometric overlap of module genes across all single cell clusters.

Supplementary Figure S1

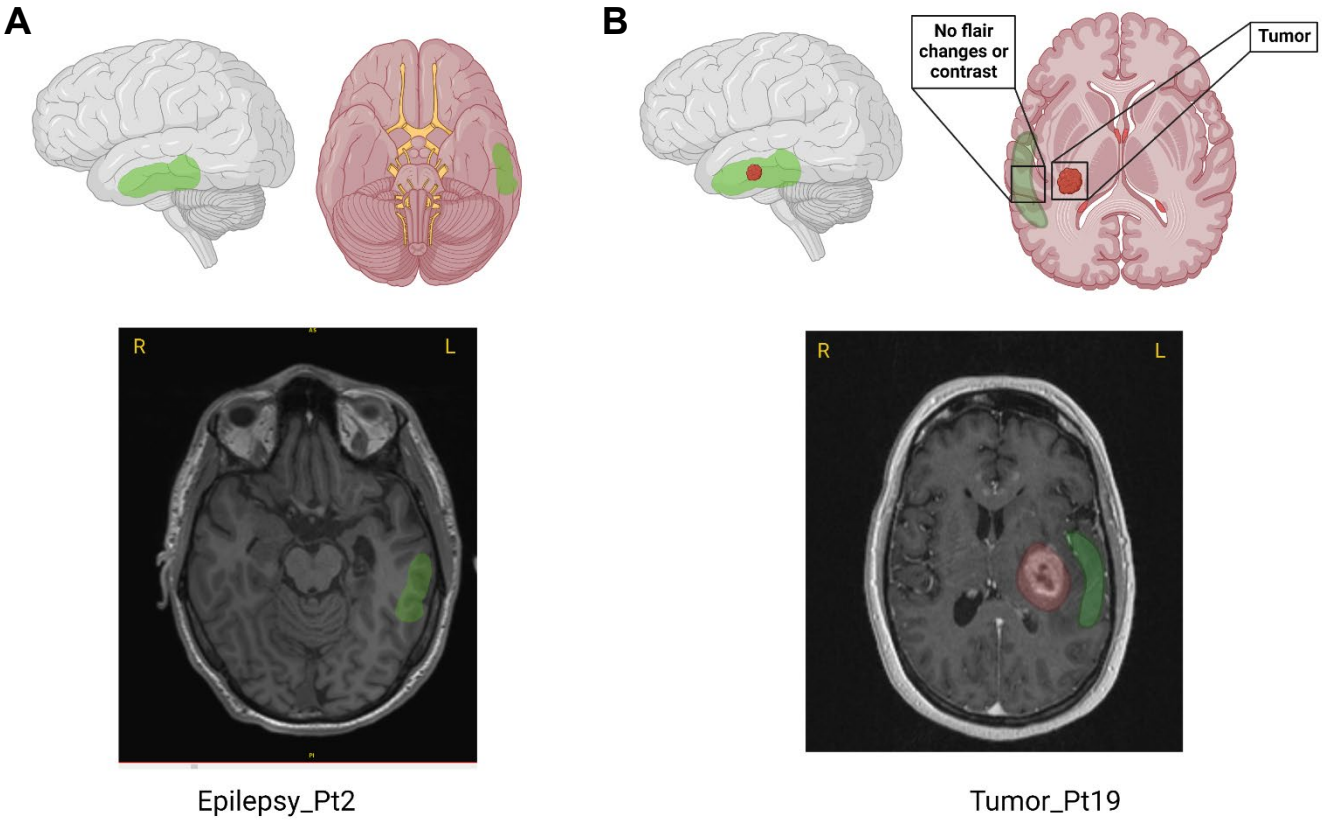


Figure S1. Surgical approaches for excision of non-affected brain tissue. (A) 3-D model and axial T1 MRI image highlighting general areas where normal tissue was obtained in epilepsy cases (green). (B) 3-D model and axial T1 MRI image highlighting the area for normal tissue removal in patients with oncologic resections (green) [Created with BioRender.com].

Supplementary Figure S2

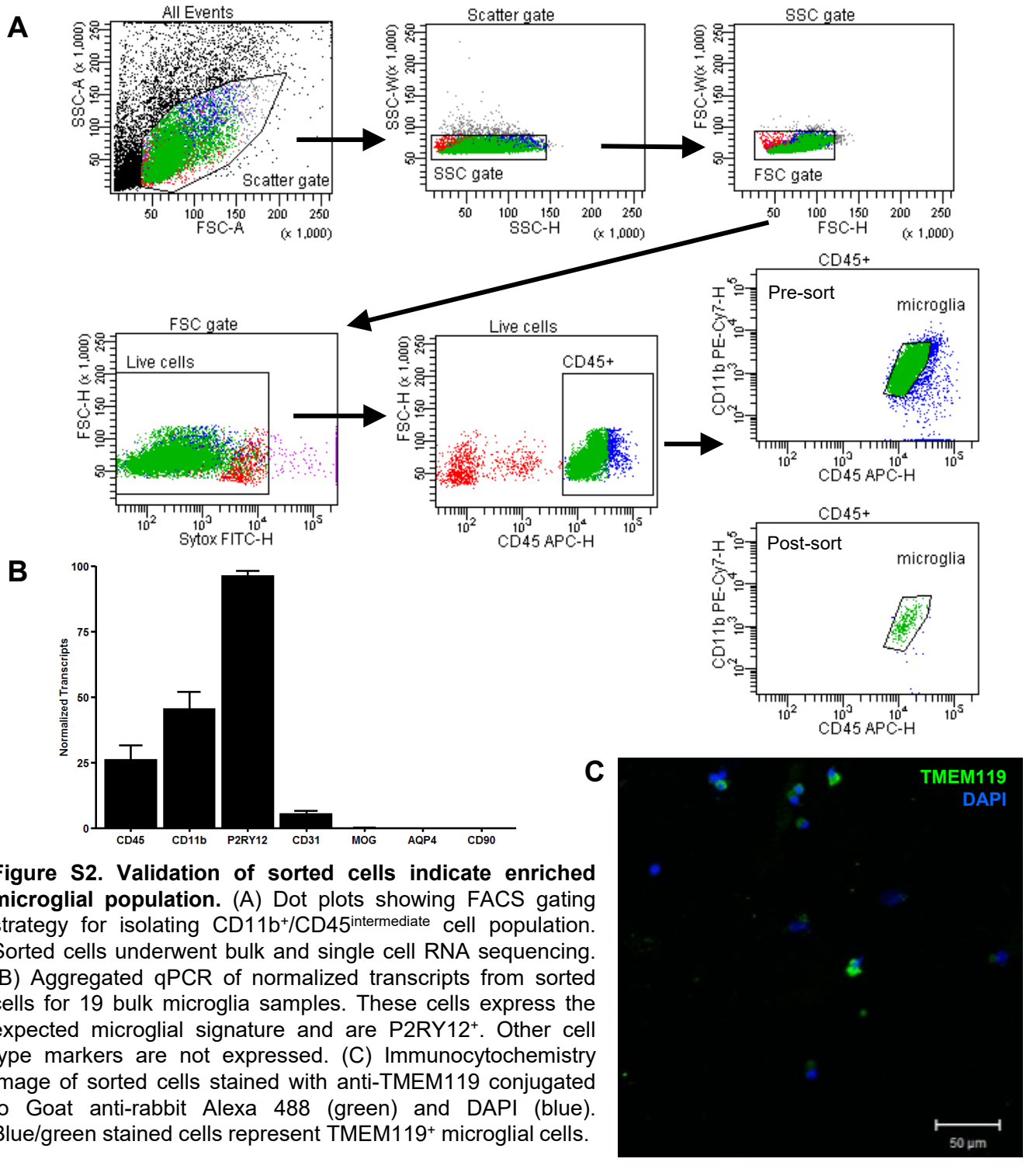
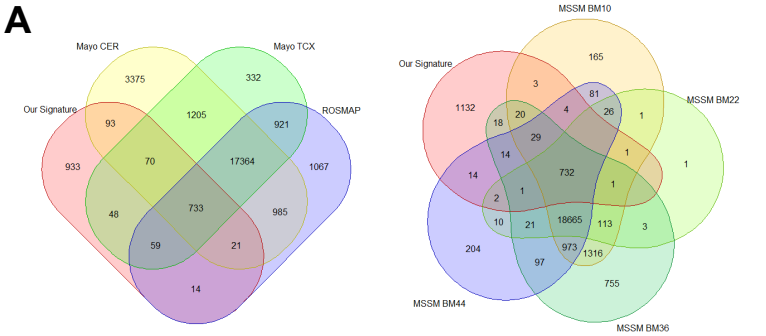


Figure S2. Validation of sorted cells indicate enriched microglial population. (A) Dot plots showing FACS gating strategy for isolating CD11b^{+}/CD45^{intermediate} cell population. Sorted cells underwent bulk and single cell RNA sequencing. (B) Aggregated qPCR of normalized transcripts from sorted cells for 19 bulk microglia samples. These cells express the expected microglial signature and are P2RY12^{+}. Other cell type markers are not expressed. (C) Immunocytochemistry image of sorted cells stained with anti-TMEM119 conjugated to Goat anti-rabbit Alexa 488 (green) and DAPI (blue). Blue/green stained cells represent TMEM119^{+} microglial cells.

Supplementary Figure S3

A



B

Dataset	# Samples	# Expressed Genes	% Overlap	Region
Our Signature	19	1971		
Mayo CER	144	20732	4.4%	Cerebellum
Mayo TCX	148	23846	3.8%	Temporal cortex
MSSM BM10	160	22130	3.6%	Frontal pole
MSSM BM22	133	19577	3.8%	Superior temporal gyrus
MSSM BM36	119	22758	3.6%	Parahippocampal gyrus
MSSM BM44	133	20873	3.8%	Inferior frontal gyrus
ROSMAP DLPFC	469	21164	3.9%	Dorsolateral prefrontal cortex

C

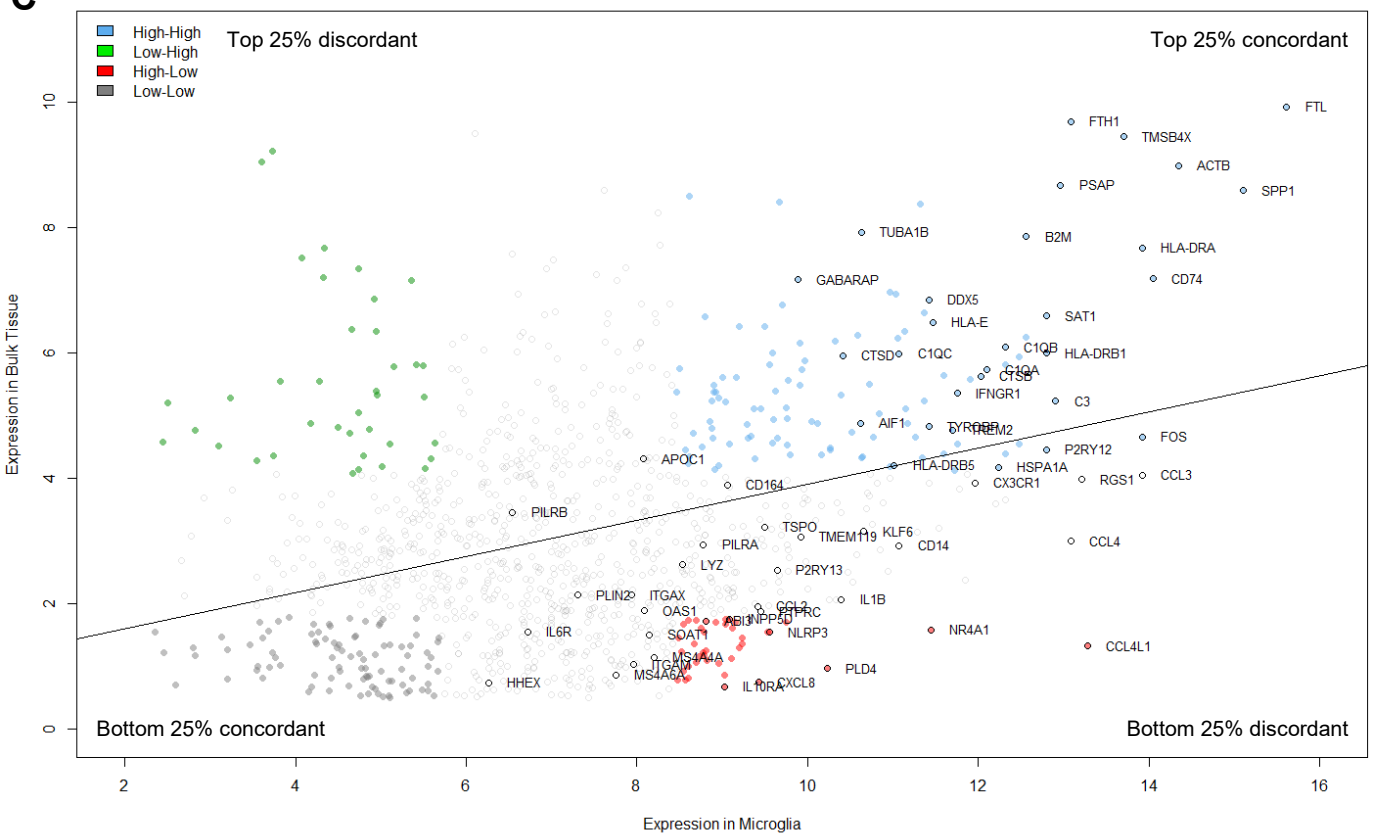


Figure S3. Correlation of our microglial signature vs bulk surgical tissue. (A) Venn diagrams showing the number of overlapping genes between our microglial signature and the 7 AMP-AD brain regions. (B) Total number of genes expressed per region and % bulk genes representing the microglial signature (overlap). (C) Scatter plot showing correlation of expression in CQN values for our microglial signature genes in bulk microglia vs. bulk surgical fresh brain tissue. Concordant and discordant genes in the highest and lowest expression quadrants are highlighted in blue (high-high), green (low-high), red (high-low) and gray (low-low). Select genes are annotated (o).

Supplementary Figure S4

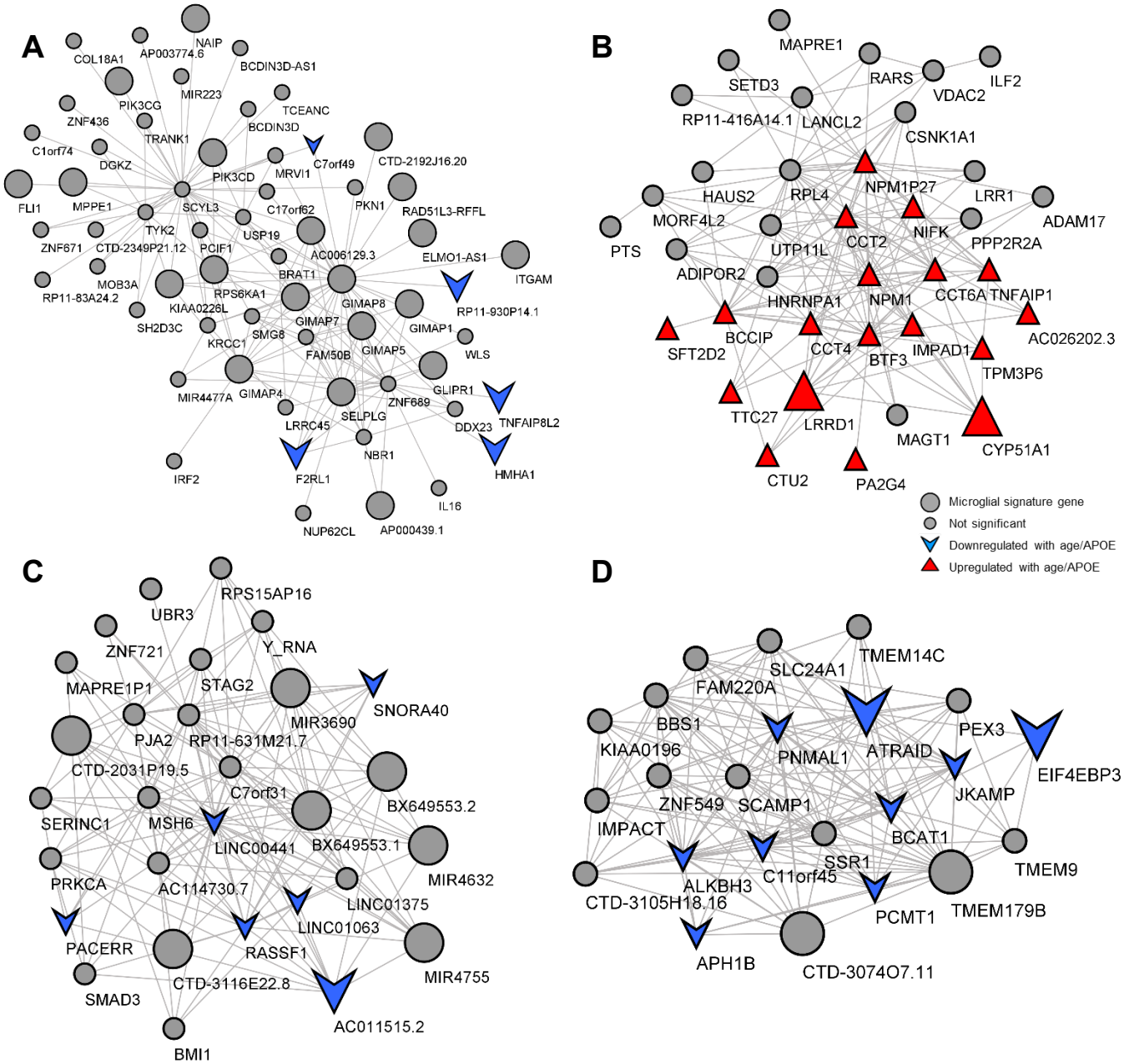


Figure S4. Co-expression networks of WGCNA modules significantly associated with age, sex or *APOE*. Genes with module membership > 0.7 are shown here with microglial signature genes denoted with larger circles, upregulated genes with age or *APOE* shown as red triangles and downregulated genes shown as as blue inverted arrows. (A) Module 4 co-expression network which was significantly downregulated in *APOE* ε4 carriers. (B) Module 28 gene co-expression network was upregulated in *APOE* ε4 carriers. (C) Module 34 gene co-expression network was downregulated in *APOE* ε4 carriers. (D) Module 36 gene co-expression network was downregulated in *APOE* ε4 carriers.

Supplementary Figure S5

Figure S5. Expression of established microglial marker genes in single cell data. Dot plot showing expression of established microglial marker genes from the literature across all clusters.

Supplementary Figure S6

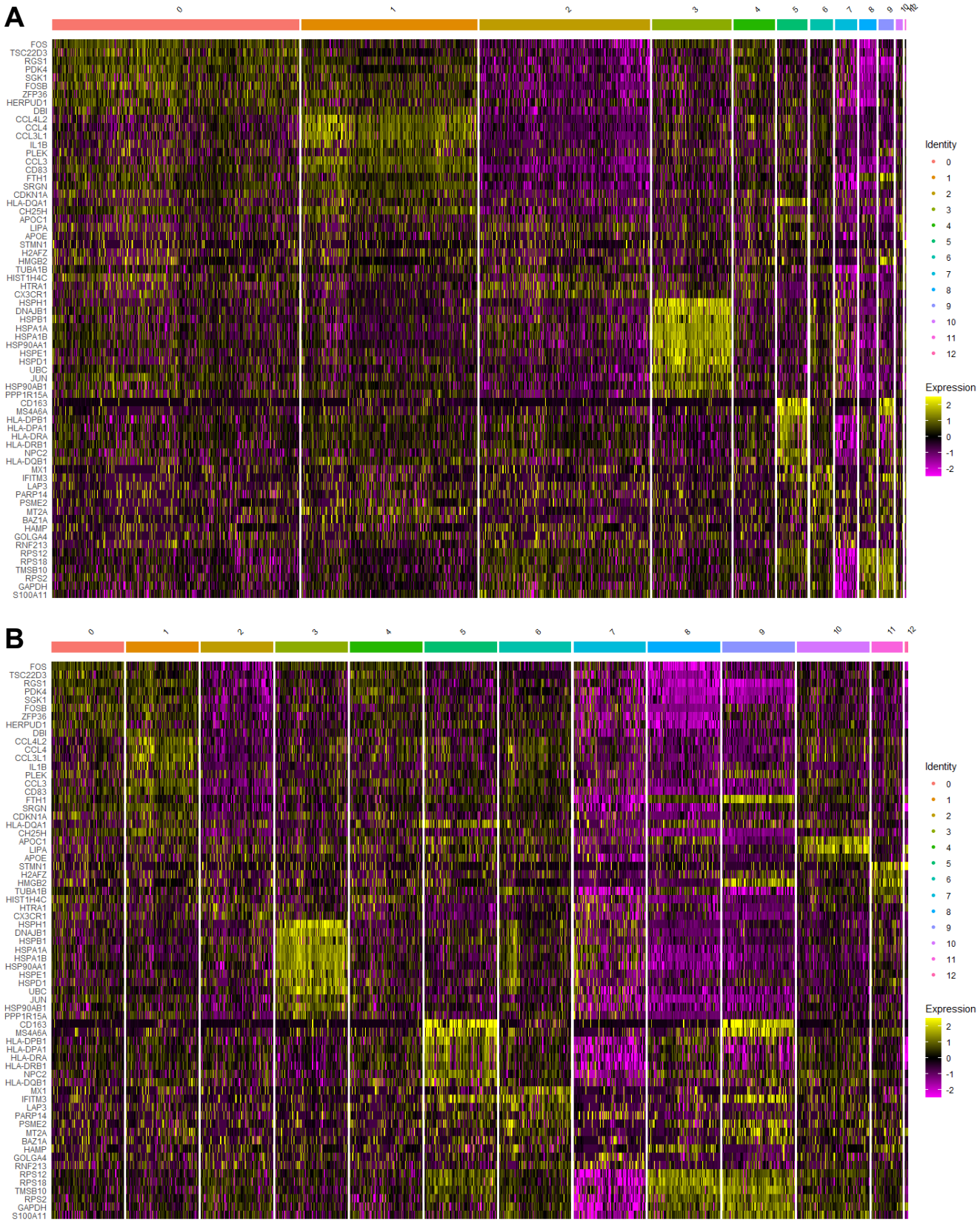


Figure S6. Expression of top cluster marker genes across single cell clusters. (A) Heatmap showing expression of selected marker genes (log fold change < -0.1 or > 0.1; q<0.05) for myeloid clusters. (B) Same heatmap downsampled to 100 cells.

Supplementary Figure S7

	cl0	cl1	cl2	cl3	cl4	cl5	cl6	cl7	cl8	cl9	cl10
M40	5	5	4	5	5	6	5	2	1	11*	5
M39	5	5	5	6*	4	5	5	3	2	3	8*
M38	12*	13*	13*	13*	13*	14*	15*	1	9*	12*	14*
M37	5	7	5	5	5	7	11*	0	1	5	9
M36	7	5	7	6	7	7	6	1	4	7	10
M35	5	10	6	5	5	7	10	1	1	5	10
M34	9	9	7	7	5	7	10	5	1	3	9
M33	8	12	7	8	7	11	16	3	3	4	15
M32	9	10	9	9	8	8	13	3	5	12	12
M31	4	3	5	5	3	6	5	2	1	8	6
M30	4	10	4	4	4	10	15	1	3	33*	5
M29	10	16	12	11	10	14	17	3	4	14	14
M28	32*	41*	31*	33*	30*	39*	40*	6	20*	29*	38*
M27	5	3	3	3	10	4	5	1	0	3	4
M26	15	18	13	14	12	18	22	5	6	22*	19
M25	6	11	9	7	6	13	18	1	5	12	13
M24	14	19	17	14	12	20	23	6	8	11	18
M23	26	22	30	27	25	26	27	12	12	23	36
M22	28	24	31	30	27	30	30	8	18	21	44
M21	13	11	16	13	12	11	14	6	7	14	18
M20	25	32	23	25	21	29	34	14	5	26	37
M19	45	69*	39	38	39	65*	75*	9	25	48*	66*
M18	47	48	48	49*	38	48	56	27*	14	44	58
M17	30	35	30	29	29	34	38	11	21	31	35
M16	17	15	20	18	15	16	15	6	11	14	28
M15	30	52	36	32	30	48	59	8	23	68*	56
M14	49	71*	47	48	48	71*	83*	15	28	41	88*
M13	56	55	62*	56	55*	62	63	11	40*	64*	70
M12	51	61	57*	50	49	72*	80*	11	45*	84*	73*
M11	60	60	48	58	60	55	65	33*	16	37	73
M10	44	87	41	40	42	107*	116*	16	21	145*	85
M9	84*	83	85*	87*	74	82	86	46*	23	57	112*
M8	85*	124*	77	85	75	114*	138*	30	33	112*	134*
M7	181*	231*	139*	168*	159*	197*	224*	87*	57	140*	229*
M6	135*	118	129*	135*	110	122	129	69*	25	112	168*
M5	120	122	141	128	117	152	147	25	89*	119	165
M4	79	63	98	82	73	74	82	20	26	90	115
M3	259*	309*	264*	259*	254*	313*	333*	106*	236*	266*	315*
M2	178	249	208	198	154	239	289	76	89	196	295
M1	346	303	372*	360	332	289	321	169*	143	164	406

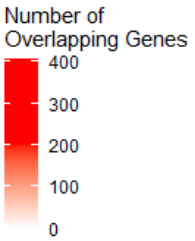


Figure S7. Hypergeometric overlap of module genes across all single cell clusters. Hypergeometric distribution of enrichment between module genes and clusters showing numbers of overlapping genes. * represents module genes that were significantly enriched in the cluster ($p < 0.05$).