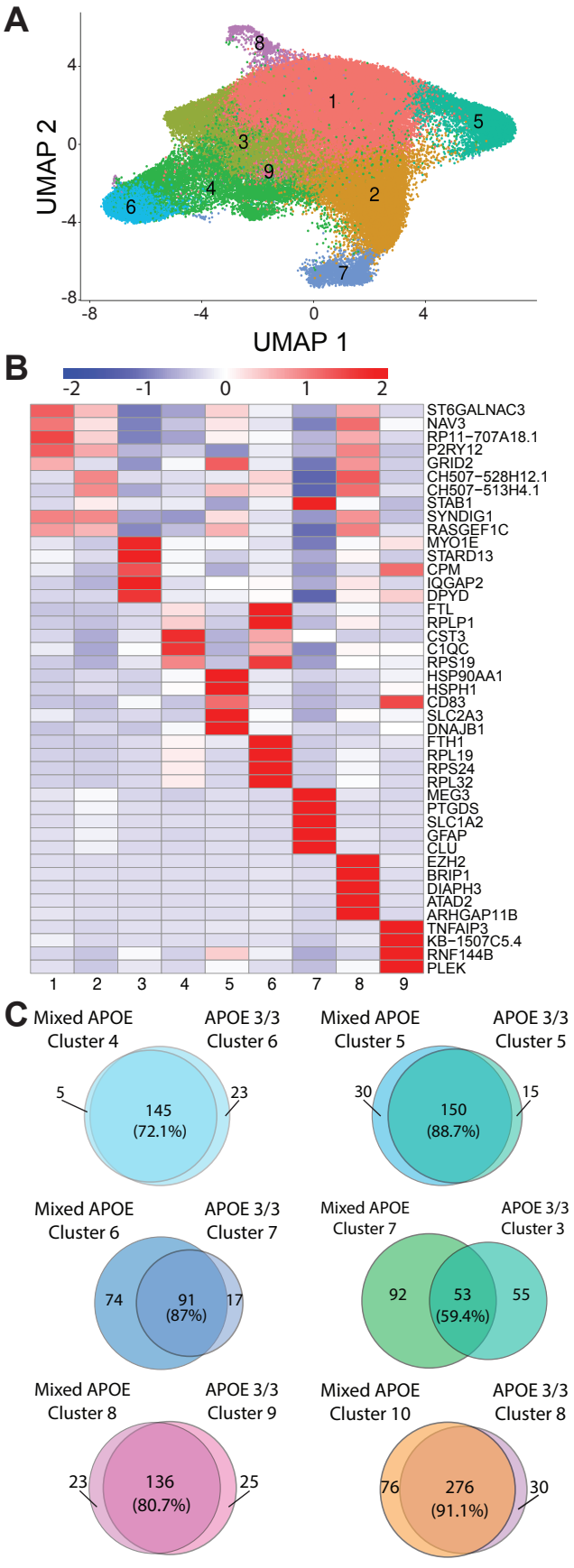
Prater, Green, et al. Supplemental Tables/Figures

Supplemental Table 1: Complete demographic information on the cohort

Sample	Study Designation	Coded AGE	SEX	Race	APOE Genotype	PMI	ADNC Score
1	AD	90+	F	White	3,3	5.08	3
2	AD	74	F	White	3,4	4.42	3
3	AD	90+	F	Mixed	3,3	5.08	2
4	AD	90+	F	White	3,3	3.75	3
5	AD	60	F	Unknown/Unreported	3,3	5.75	3
6	AD	86	F	White	3,3	8.07	2
7	AD	87	F	White	3,4	4.87	3
8	AD	90+	F	White	3,4	4.25	3
9	AD	77	F	White	4,4	3.33	3
10	AD		M	White	3,3	3.33	3
11	AD	83	M	White	3,4	3	3
12	AD	90+	M	White	3,4	4.58	3
13	Ctrl	90+	F	Hispanic / Latino	2,3	4.33	0
14	Ctrl	90+	F	White	3,3	3.75	1
15	Ctrl	90+	F	White	3,3	6.97	0
16	Ctrl	80	F	White	3,3	8.13	0
17	Ctrl	90+	F	White	3,3	7.72	1
18	Ctrl	70	F	White	3,4	7	1
19	Ctrl	74	M	White	2,3	4.83	0
20	Ctrl	84	M	White	3,3	3.92	1
21	Ctrl	90+	M	White	3,3	8.17	1
22	Ctrl	82	M	White	3,3	7.75	1

Ctrl = Control, AD = Alzheimer's Disease pathology, Coded Age = age at death in years. Ages greater than 90 are coded 90+ to maintain anonymity, F = Female, M = Male, Race = self-reported race, *APOE* Genotypes: *APOE* alleles ε2/ε3 (2/3), *APOE* alleles ε3/ε3 (3/3), *APOE* alleles ε3/ε4 (3/4), or *APOE* alleles ε4/ε4 (4/4), PMI = post-mortem interval in hours, ADNC = Alzheimer's Disease Neuropathic Change



Supplemental Figure 1. APOE 3/3 genotype does not substantially alter microglial clustering in human autopsy brain. A) UMAP of unbiased clustering on 13 samples of only APOE 3/3 individuals shows 9 clusters. **B)** Similar to the clusters identified in the mixed *APOE* genotype dataset, the clusters identified in our *APOE* ε3/ε3 genotype dataset are highly distinct by gene expression. The top 5 genes are displayed for each cluster. **C)** Venn diagrams demonstrating overlap between clusters from the Mixed *APOE* and *APOE* ε3/ε3 cohorts demonstrating significant similarity in gene expression profiles.

Cluster 4	% replicates in top 10
JUNB	96.30
STAT1	88.89
ZNF768	85.19
E2F6	81.48
PPARA	77.78
JUND	59.26
NFE2L1	44.44
GABPA	33.33
KLF9	33.33
TRIM69	33.33

Cluster 7	% replicates in top 10
NFATC1	96.30
DDIT3	96.30
HIVEP1	92.59
MAFB	85.19
ATF3	77.78
NFIL3	74.07
ATF5	59.26
NR3C1	55.56
E2F3	51.85
ZNF235	37.04

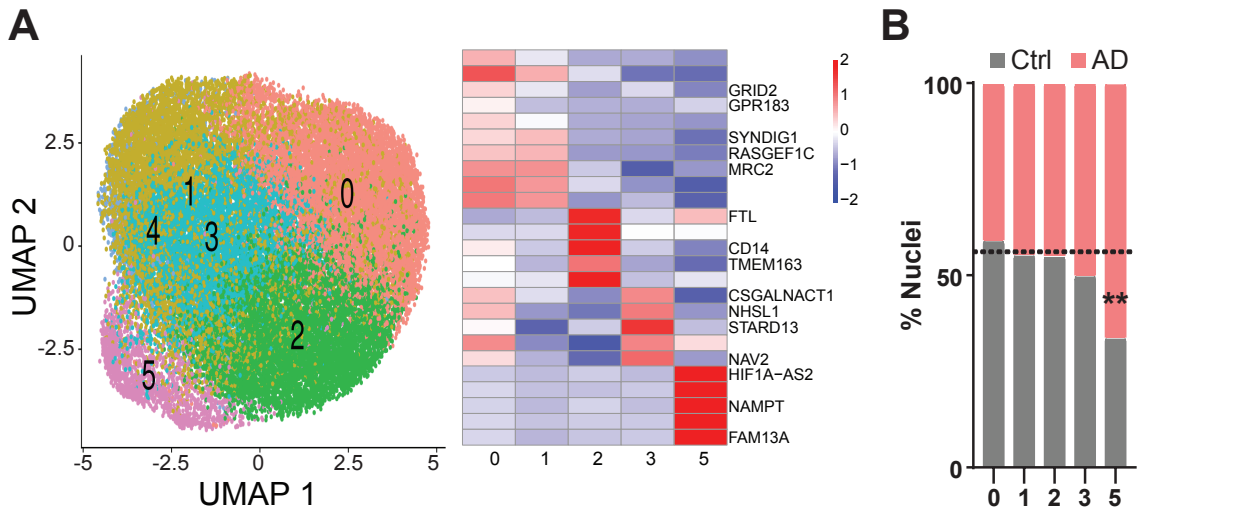
Cluster 9	% replicates in top 10
PRDM1	100.00
CEBPD	96.30
FOXO1	81.48
FOXN2	81.48
FOS	59.26
XBP1	48.15
PPARD	48.15
FOXP2	37.04
XRCC4	37.04
RUNX3	29.63

Cluster 10	% replicates in top 10
EZH2	100.00
BRCA1	96.30
MAZ	85.19
MYBL1	81.48
FOXN2	40.74
THAP1	40.74
ZFP64	40.74
ZNF16	37.04
SREBF2	37.04
ZBTB2	33.33

Supplemental Figure 2. Top transcription factors for each cluster are unique and represent biological function switches. These are the transcription factors that most often drove gene expression in Clusters 4, 7, 9, and 10. Values denote the percentage of replicates of permutations of the dataset where that particular transcription factor was unique to the given cluster. Note that while a few similar transcription factors are seen in multiple clusters, particularly the most prevalent transcription factors are unique, and are representative of gene expression driving biological functions in these clusters.

Supplemental Figure 3. Transcription factor regulatory networks are specific and unique to subpopulations of microglia within the APOE ε3/ε3 genotype clusters. Similar to what

was seen in the larger dataset, transcription factors driving gene expression within clusters are distinct when the data is comprised purely of a single APOE genotype, in this case ε3/ε3 allele carriers. Specific transcription factors drive clusters with similar gene expression phenotypes, such as NFκB1 and NFκB2 in Cluster 9, the inflammatory cluster in this dataset, that also drove expression in Cluster 8 in the larger dataset. This replicates the finding from the full dataset that gene expression and transcription factor regulation of that gene expression are quite distinct between clusters of microglia.



Supplemental Figure 4. The APOE ε3/ε3 genotype cohort also demonstrates a subcluster of the homeostatic cluster increased in AD brain. A) The results from the full dataset were replicated in the pure APOE ε3/ε3 allele cohort. Again suggesting multiple subpopulations within the "homeostatic" cluster. These subpopulations are once again distinct by gene expression despite being comprised of one "homeostatic" subpopulation. **B)** Within the APOE ε3/ε3 cohort "homeostatic" Cluster 1, there is again a Cluster 1.5 that is increased in AD brain. Unlike the full cohort, this population is not specific to AD brain, but is increased in AD. These results suggest that within the "homeostatic" subpopulation of microglia there may be additional subpopulations of particular interest for AD research and therapeutic development.

Table S4: Recipes for Nuclei Extraction and FANS Buffers:

Myelin gradient buffer (Store at 4°C)

Reagent	Volume for 1 Liter
$NaH_2PO_4 \cdot H_2O$ (Fisher Scientific, S369-500), adjust to pH 7.4 with 3.56g/L $Na_2HPO_4 \cdot H_2O$ (Fluka, 71643)	0.78 g
NaCl (Fisher Scientific, S271-3)	8.0 g
KCl (Fisher Scientific, P217-500)	0.4 g
Glucose (Sigma, G7021-1KG)	2.0 g
BSA (VWR, EM-2930)	0.2%

Nuclei buffer (NB) (Store at 4°C)

Reagent	Volume for 10mL
Nuclease-free water into a 15 ml conical tube. (Fisher Scientific, M46000)	9.85 mL
1 M Tris-HCl, pH 7.5 (ThermoFisher, 15567027)	100 µL
5 M NaCl (ThermoFisher AM9760G)	20 µL
1 M MgCl2 (ThermoFisher Scientific, AM9530G)	30 µL

Nuclei lysis buffer (NLB) (make same-day)

Reagent	Volume for 1 Sample
Nuclei Buffer	727 µL
10% NP-40 alternative (final concentration 0.1%)	10 µL
Protease inhibitors in DPBS (Sigma-Aldrich, 4693124001)	142.9 µL
1mM ATA in NB (make fresh evening prior)	112.5 µL
PMSF (Tocris Bioscience, 4486)	10 µL
Phosphatase inhibitors (Sigma-Aldrich, P5726-1ML)	5 µL
Protector RNase inhibitor (final concentration 1 U/µl). (Sigma, 3335402001)	28.25 µL
Total Volume	1,035.5 µL

Nuclei suspension solution (NSS) (make same-day) (10X Genomics calls this "Wash Buffer")

Reagent	Volume for 1 Sample
DPBS (Sigma-Aldrich, D8537-500ML)	637 µL
10% BSA (final concentration 1%) (Sigma-Aldrich, A1595-50mL)	100 µL
1mM ATA in DPBS (make fresh evening prior)	100 µL
7x Protease inhibitors in DPBS (Sigma-Aldrich, 4693124001)	142.9 µL
1M PMSF (Tocris Bioscience, 4486)	10 µL
Protector RNase inhibitor (final concentration 1.0 U/µl) (Sigma, 3335402001)	5 µL
Phosphatase inhibitors (Sigma-Aldrich, P5726-1ML)	5 µL
Total Volume	1 mL

Percoll/myelin gradient buffer solution (PMB) (make same day)

Reagent	Volume for 1 Sample
Myelin gradient buffer	412 µL
10x HBSS (Fisher Sci., 14185052)	30 µL
1mM ATA in myelin gradient buffer (make fresh evening prior)	100 uL
1.5M NaCl	25 µL
Percoll (Fisher Sci., 17-089-101) (Do not use if crystals have precipitated out)	270 µL

Protector RNase Inhibitor (final concentration 1.0 U/µl) (Sigma Aldrich, 3335402001)	5 µL
Phosphatase inhibitors (Sigma-Aldrich, P5726-1ML)	5 µL
7x Protease inhibitors in myelin gradient buffer (Sigma-Aldrich, 4693124001)	142.9 µL
PMSF (Tocris Bioscience, 4486)	10 µL
Total Volume	1 mL

FACS Media (FM) (store at 4°C)

Reagent	**Volume for 1 sample**
Nuclease free H_2O (Fisher Scientific, M46000)	7.7 mL
HEPES (Invitrogen, 15630080)	100 uL
10x HBSS without Mg/Ca (Fisher Scientific, 14185052)	1 mL
FBS 10%	1 mL
Protease inhibitors (Sigma-Aldrich, 4693124001) (dissolve tablet in 9.8mL of the above media at room temp and chill on ice before adding the solutions below)	"~200 uL"
PMSF (Tocris Bioscience, 4486)	100 uL
Phosphatase inhibitors (Sigma-Aldrich, P5726-1ML)	50 uL
Protector RNase Inhibitor (Sigma Aldrich, 3335402001)	50 uL
100 uM ATA in dPBS (Sigma-Aldrich, A1895) good at -20C for 1 month	1 mL
Total Volume	11.2 mL