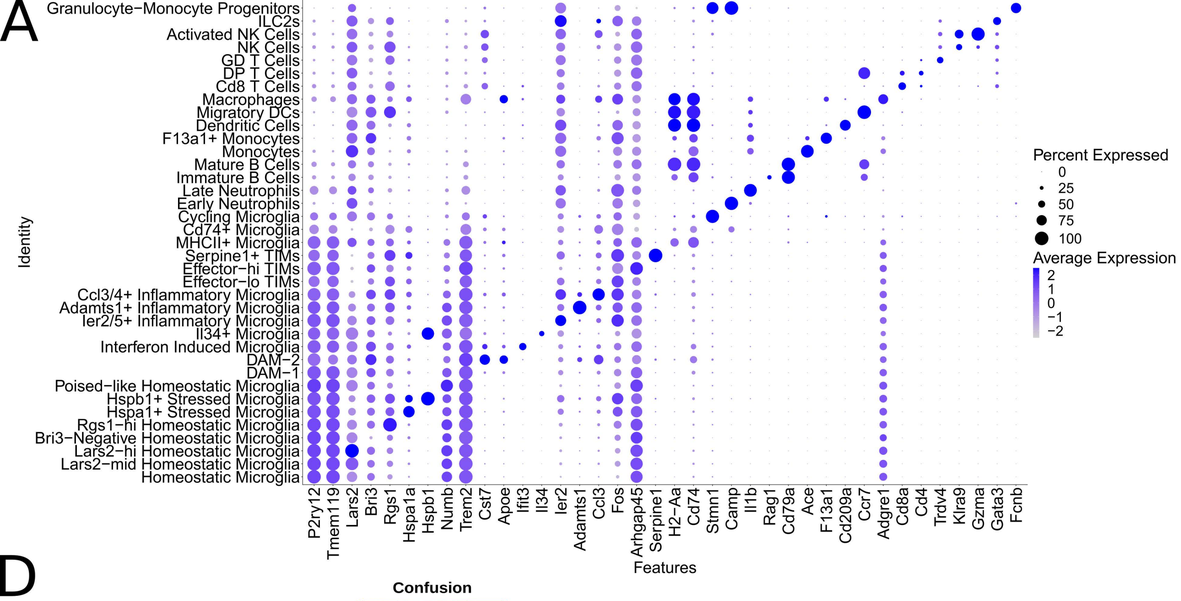
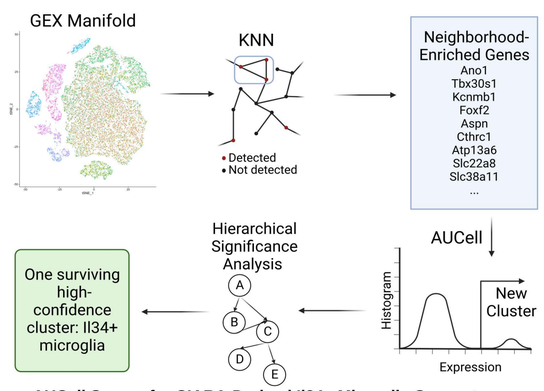
Library Name	Sample	Barcode Sequence
Atlas_10wk	E2	GGTCGAGAGCATTCA
Atlas_10wk	E3	CTTGCCGCATGTCAT
Atlas_10wk	E4	AAAGCATTCTTCACG
Atlas_20wk	E2	GGTCGAGAGCATTCA
Atlas_20wk	E3	CTTGCCGCATGTCAT
Atlas_20wk	E4	AAAGCATTCTTCACG
Aducanumab	E2_isocon	GGTCGAGAGCATTCA
Aducanumab	E3_isocon	CTTGCCGCATGTCAT
Aducanumab	E4_isocon	AAAGCATTCTTCACG
Aducanumab	E2_aducanumab	CTTTGTCTTTGTGAG
Aducanumab	E3_aducanumab	TATGCTGCCACGGTA
Aducanumab	E4_aducanumab	TATAGAACGCCAGGC
Aβ uptake	E2_lo	GGTCGAGAGCATTCA
Aβ uptake	E3_lo	CTTGCCGCATGTCAT
Aβ uptake	E4_lo	AAAGCATTCTTCACG
Aβ uptake	E2_hi	CTTTGTCTTTGTGAG
Aβ uptake	E3_hi	TATGCTGCCACGGTA
Aβ uptake	E4_hi	TATAGAACGCCAGGC

Supplemental Table 1, Related to STAR Methods: Barcode sequences for demultiplexing of single cell libraries. Samples from the indicated library were stained with TotalSeq-C hashing antibodies with the corresponding barcode sequence in the table. These sequences can be used to demultiplex raw reads with Cell Ranger multi, using read R2 and pattern 5PNNNNNNNNNN(BC).

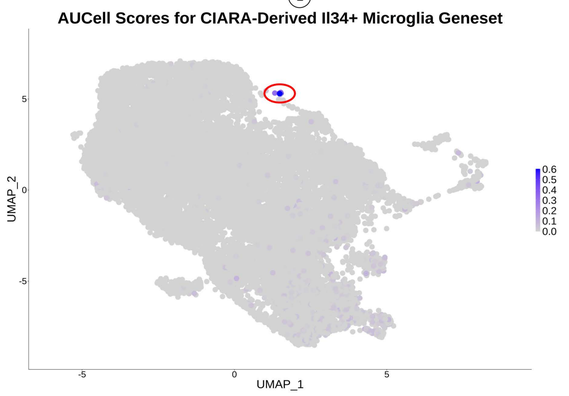
A


Granulocyte−Monocyte Progenitors
ILC2s
Activated NK Cells
NK Cells
GD T Cells
DP T Cells
Cd8 T Cells
Macrophages
Migratory DCs
Dendritic Cells
F13a1+ Monocytes
Monocytes
Mature B Cells
Immature B Cells
Late Neutrophils
Early Neutrophils
Cycling Microglia
Cd74+ Microglia
MHCII+ Microglia
Serpine1+ TIMs
Effector−hi TIMs
Effector−lo TIMs
Ccl3/4+ Inflammatory Microglia
Adamts1+ Inflammatory Microglia
Ier2/5+ Inflammatory Microglia
Il34+ Microglia
Interferon Induced Microglia
DAM−2
DAM−1
Poised−like Homeostatic Microglia
Hspb1+ Stressed Microglia
Hspa1+ Stressed Microglia
Rgs1−hi Homeostatic Microglia
Bri3−Negative Homeostatic Microglia
Lars2−hi Homeostatic Microglia
Lars2−mid Homeostatic Microglia
Homeostatic Microglia
Identity
P2ry12
Tmem119
Lars2
Bri3
Rgs1
Hspa1a
Hspb1
Numb
Trem2
Cst7
Apoe
Ifit3
Il34
Ier2
Adamts1
Ccl3
Fos
Arhgap45
Serpine1
H2−Aa
Cd74
Stmn1
Camp
Il1b
Rag1
Cd79a
Ace
F13a1
Cd209a
Ccr7
Adgre1
Cd8a
Cd4
Trdv4
Klra9
Gzma
Gata3
Fcnb
Features
Percent Expressed
0
25
50
75
100
Average Expression
2
1
0
−1
−2

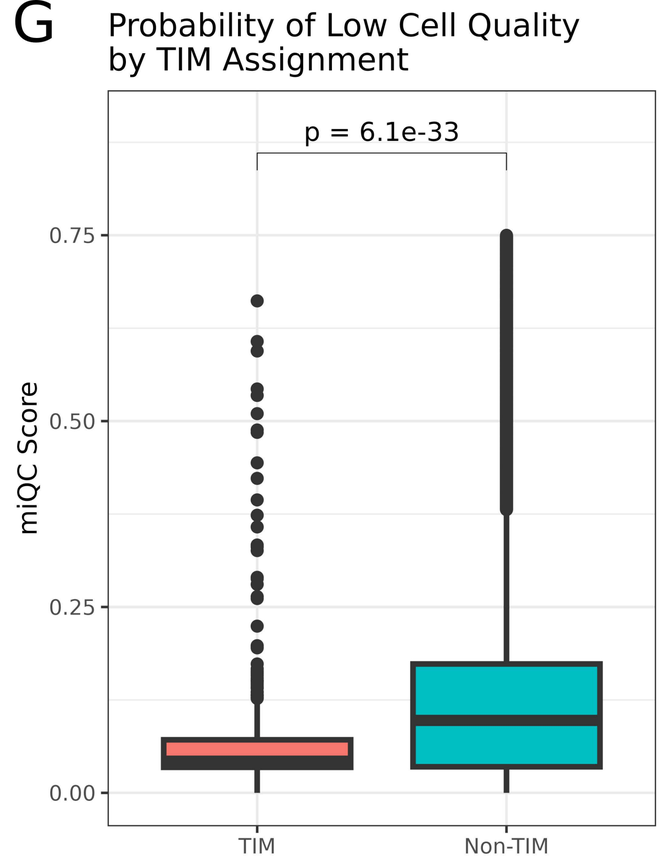
B


GEX Manifold
KNN
Detected
Not detected
Neighborhood-Enriched Genes
Ano1
Tbx30s1
Kcnmb1
Foxf2
Aspn
Cthrc1
Atp13a6
Slc22a8
Slc38a11
...
AUCell
Histogram
New Cluster
Expression
Hierarchical Significance Analysis
A
B
C
D
E
One surviving high-confidence cluster: Il34+ microglia

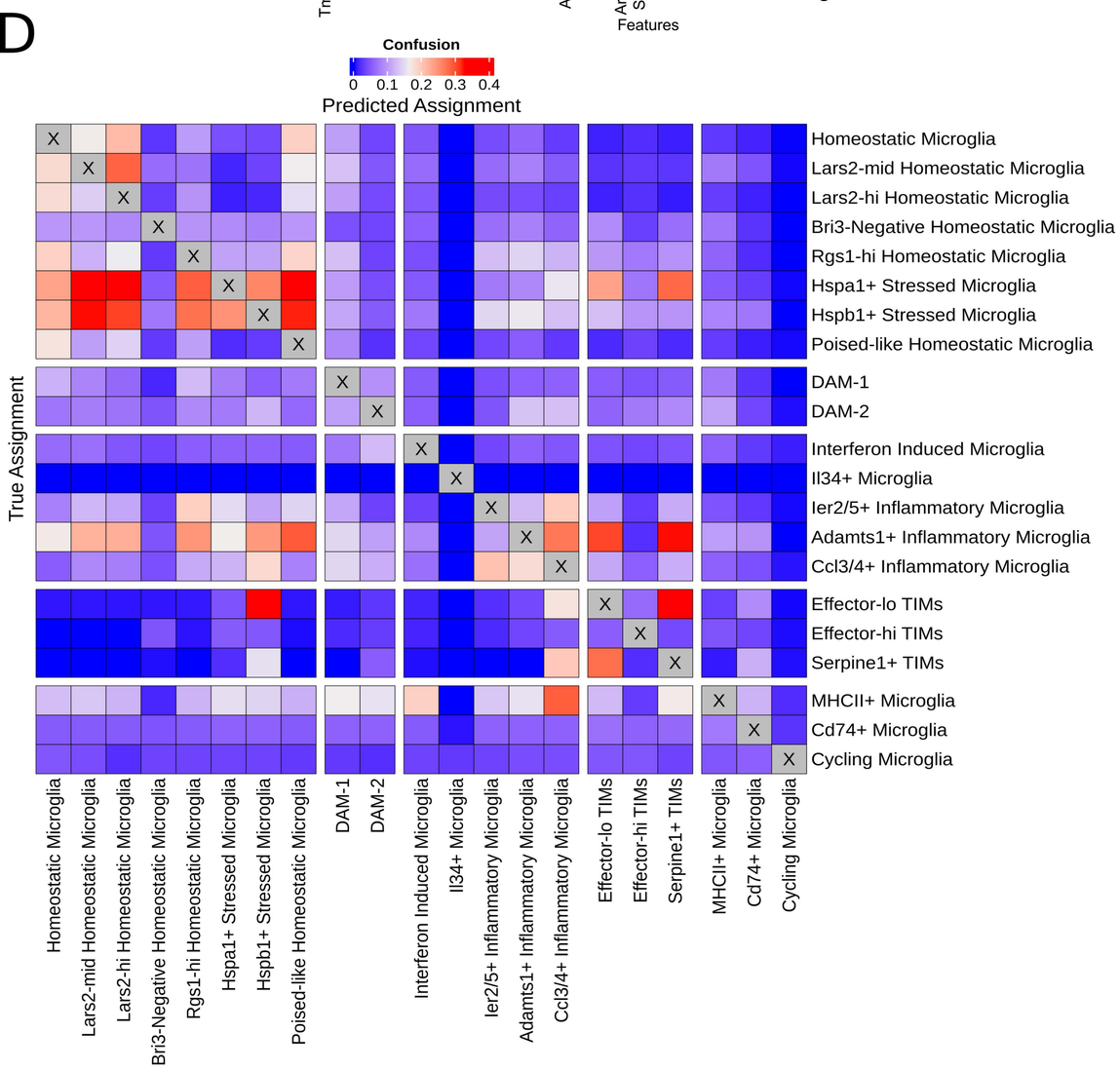
C


AUCell Scores for CIARA-Derived Il34+ Microglia Geneset
UMAP_2
UMAP_1
0.6
0.5
0.4
0.3
0.2
0.1
0.0

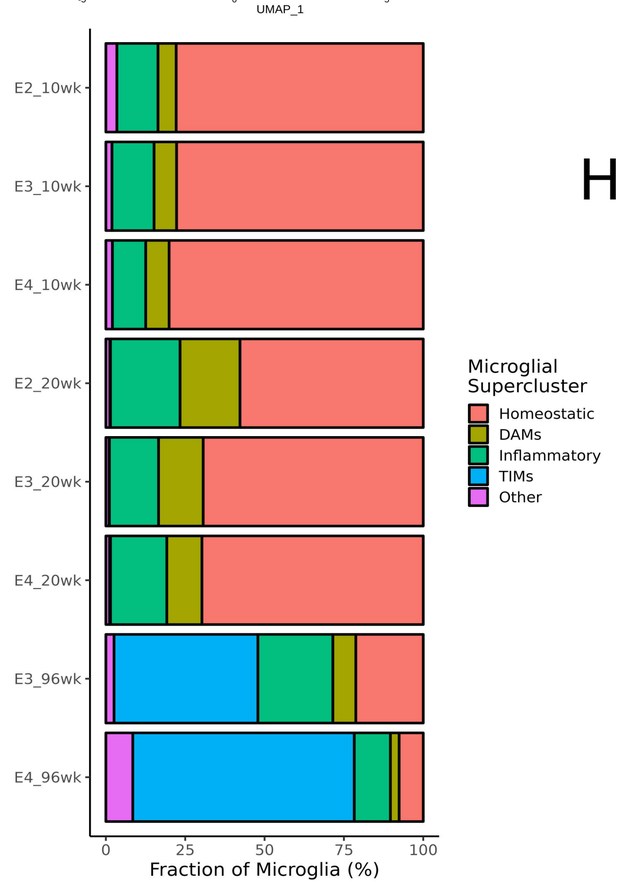
G


Probability of Low Cell Quality by TIM Assignment
p = 6.1e-33
miQC Score
0.75
0.50
0.25
0.00
TIM
Non-TIM

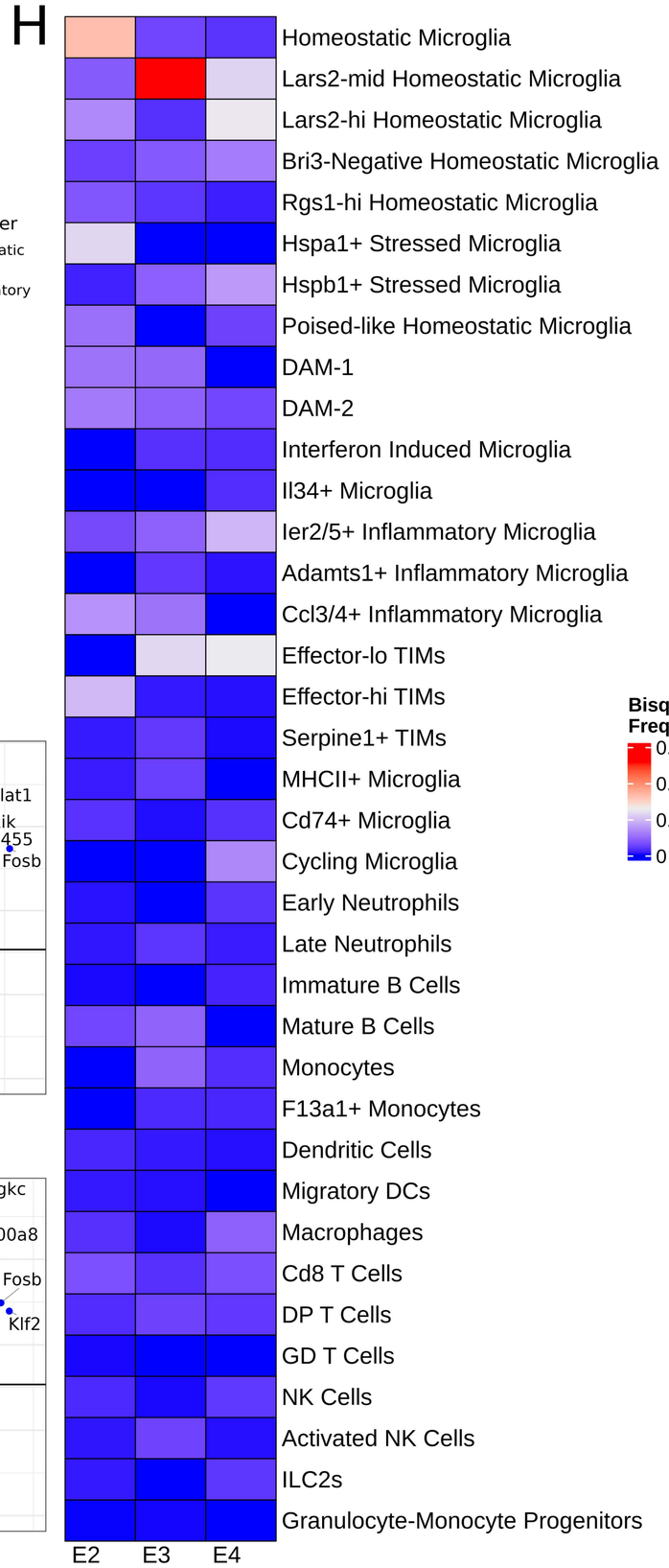
D


Confusion
0 0.1 0.2 0.3 0.4
Predicted Assignment
True Assignment
Homeostatic Microglia
Lars2-mid Homeostatic Microglia
Lars2-hi Homeostatic Microglia
Bri3-Negative Homeostatic Microglia
Rgs1-hi Homeostatic Microglia
Hspa1+ Stressed Microglia
Hspb1+ Stressed Microglia
Poised-like Homeostatic Microglia
DAM-1
DAM-2
Interferon Induced Microglia
Il34+ Microglia
Ier2/5+ Inflammatory Microglia
Adamts1+ Inflammatory Microglia
Ccl3/4+ Inflammatory Microglia
Effector-lo TIMs
Effector-hi TIMs
Serpine1+ TIMs
MHCII+ Microglia
Cd74+ Microglia
Cycling Microglia

F


E2_10wk
E3_10wk
E4_10wk
E2_20wk
E3_20wk
E4_20wk
E3_96wk
E4_96wk
Microglial Supercluster
Homeostatic
DAMs
Inflammatory
TIMs
Other
0
25
50
75
100
Fraction of Microglia (%)

H


Homeostatic Microglia
Lars2-mid Homeostatic Microglia
Lars2-hi Homeostatic Microglia
Bri3-Negative Homeostatic Microglia
Rgs1-hi Homeostatic Microglia
Hspa1+ Stressed Microglia
Hspb1+ Stressed Microglia
Poised-like Homeostatic Microglia
DAM-1
DAM-2
Interferon Induced Microglia
Il34+ Microglia
Ier2/5+ Inflammatory Microglia
Adamts1+ Inflammatory Microglia
Ccl3/4+ Inflammatory Microglia
Effector-lo TIMs
Effector-hi TIMs
Serpine1+ TIMs
MHCII+ Microglia
Cd74+ Microglia
Cycling Microglia
Early Neutrophils
Late Neutrophils
Immature B Cells
Mature B Cells
Monocytes
F13a1+ Monocytes
Dendritic Cells
Migratory DCs
Macrophages
Cd8 T Cells
DP T Cells
GD T Cells
NK Cells
Activated NK Cells
ILC2s
Granulocyte-Monocyte Progenitors
E2
E3
E4
Bisque Frequencies
0.3
0.2
0.1
0

E

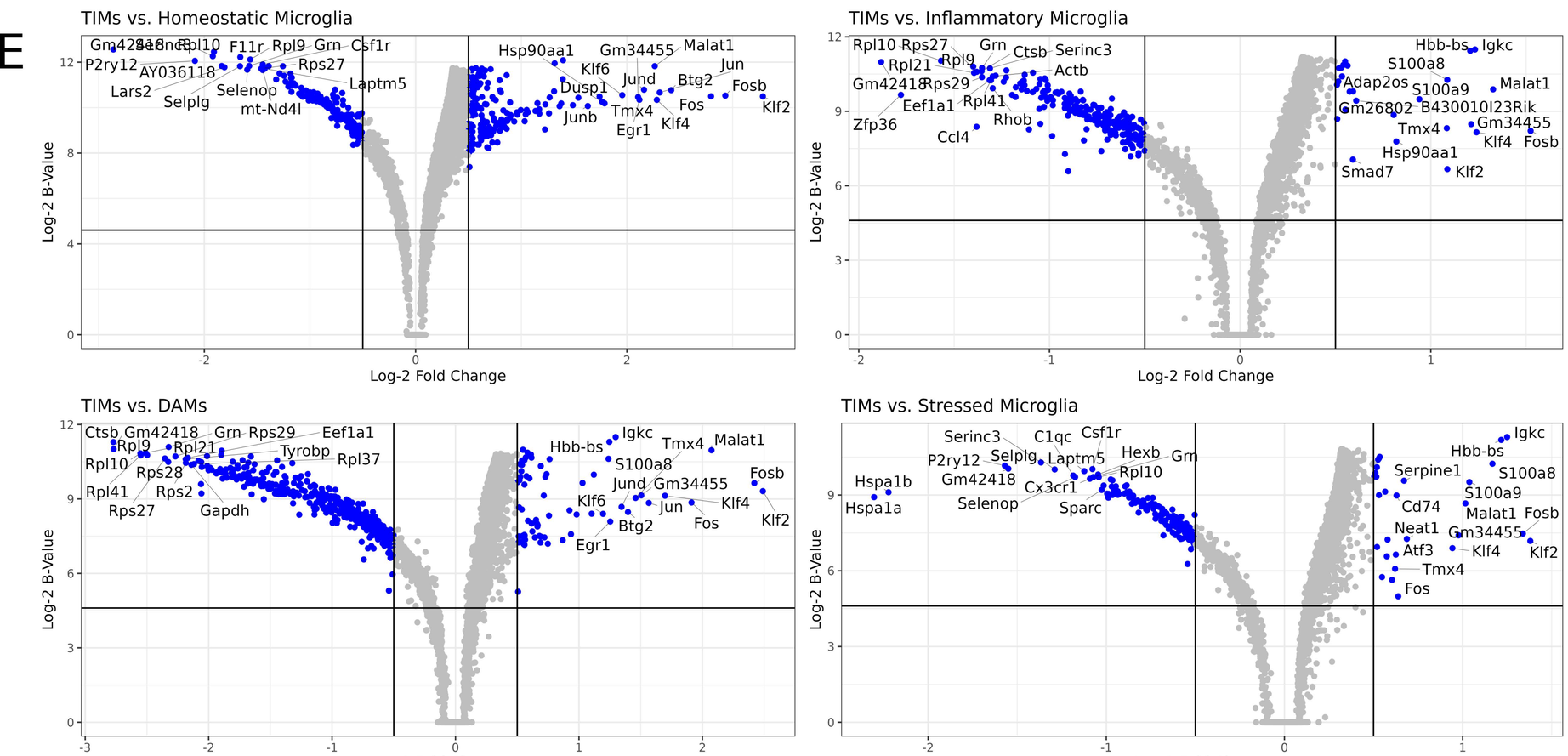
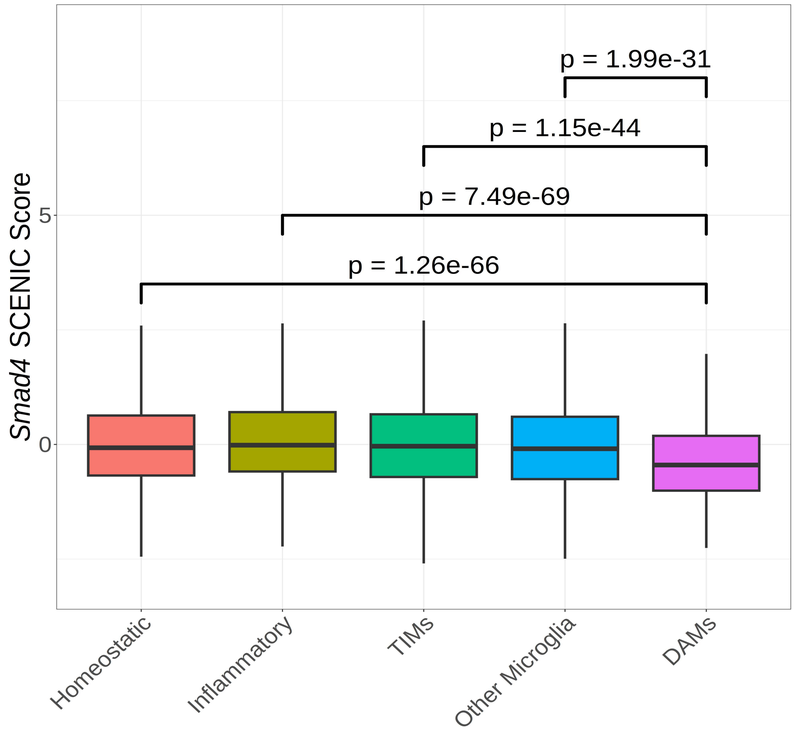
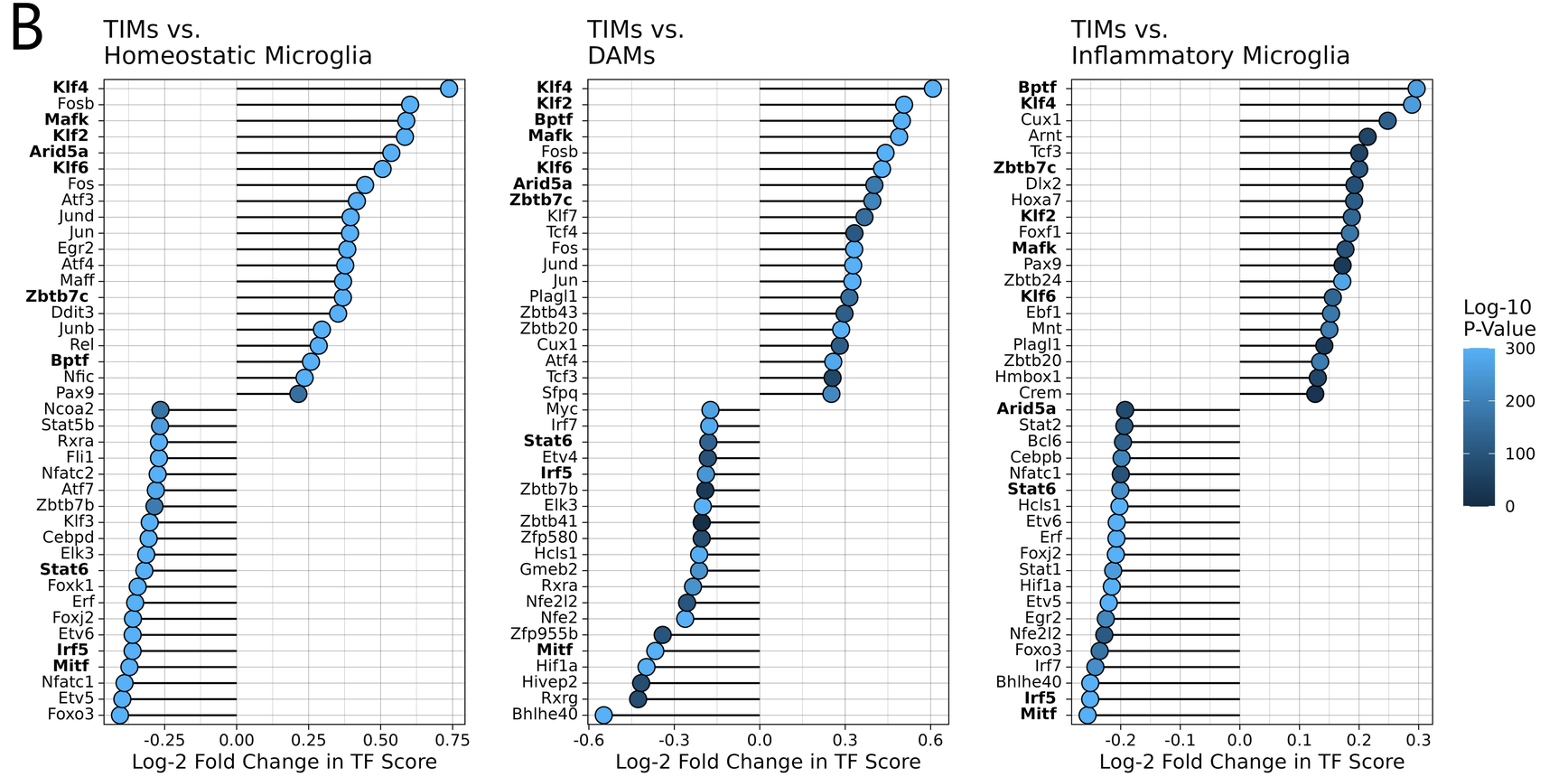

TIMs vs. Homeostatic Microglia
TIMs vs. Inflammatory Microglia
TIMs vs. DAMs
TIMs vs. Stressed Microglia
Log-2 B-Value
Log-2 Fold Change
Gm42418
Rpl10
F11r
Rpl9
Grn
Csf1r
P2ry12
AY036118
Rps27
Laptm5
Lars2
Selplg
Selenop
mt-Nd4l
Hsp90aa1
Gm34455
Malat1
Klf6
Jun
Dusp1
Jund
Btg2
Fosb
Tmx4
Fos
Junb
Klf4
Egr1
Klf2
Rpl10
Rps27
Rpl21
Rpl9
Grn
Ctsb
Serinc3
Actb
Gm42418
Rps29
Eef1a1
Rpl41
Rhob
Zfp36
Ccl4
Hbb-bs
Igkc
S100a8
Adap2os
S100a9
Malat1
Gm26802
B430010I23Rik
Tmx4
Gm34455
Klf4
Fosb
Hsp90aa1
Smad7
Klf2
Ctsb
Gm42418
Grn
Rps29
Eef1a1
Rpl9
Rpl21
Tyrobp
Rpl37
Rpl10
Rps28
Rpl41
Rps2
Rps27
Gapdh
Igkc
Hbb-bs
Tmx4
Malat1
S100a8
Fosb
Jund
Gm34455
Klf6
Klf4
Jun
Btg2
Fos
Klf2
Egr1
Serinc3
C1qc
Csf1r
P2ry12
Selplg
Laptm5
Hexb
Grn
Rpl10
Hspa1b
Hspa1a
Gm42418
Cx3cr1
Selenop
Sparc
Igkc
Hbb-bs
Serpine1
S100a8
S100a9
Cd74
Malat1
Fosb
Neat1
Gm34455
Atf3
Klf4
Klf2
Tmx4
Fos

Figure S1, Related to Figure 1: Clustering of the integrated atlas. (A) Dotplot of markers used in supervised annotation of clusters. (B) Schematic of the CIARA pipeline for identification of rare cell types. (C) Feature plot of scores generated by CIARA, a KNN-based scheme for identification of rare cell types, for the top 14 genes associated with the signature of $Il34^{+}$ microglia, in UMAP space. Note the consistent signal in a region to the top right of the UMAP; after AUCell, 11 cells passed thresholding and were annotated as a microcluster of $Il34^{+}$ microglia. (D) The resulting confusion matrix from training a 100-tree pairwise random forest classifier on raw count data from cells of each cluster for 25 iterations. (E) Volcano plots of differentially expressed genes between TIMs and the indicated microglial superclusters. Superclusters are defined as in Figure 1C. The B-statistic is the log-odds that a gene is differentially expressed. Statistics were calculated using voom normalization and empirical Bayesian estimation through the limma package. (F) Stacked barplot of the fraction of microglia in each sample in the atlas coming from each microglial supercluster. (G) miQC scores for all TIM and non-TIM cells in the atlas. Higher score indicates a higher probability that the cell is low quality or compromised. p-values calculated by Wilcoxon test. (H) Heatmap of decomposition estimates for all clusters made by Bisque.

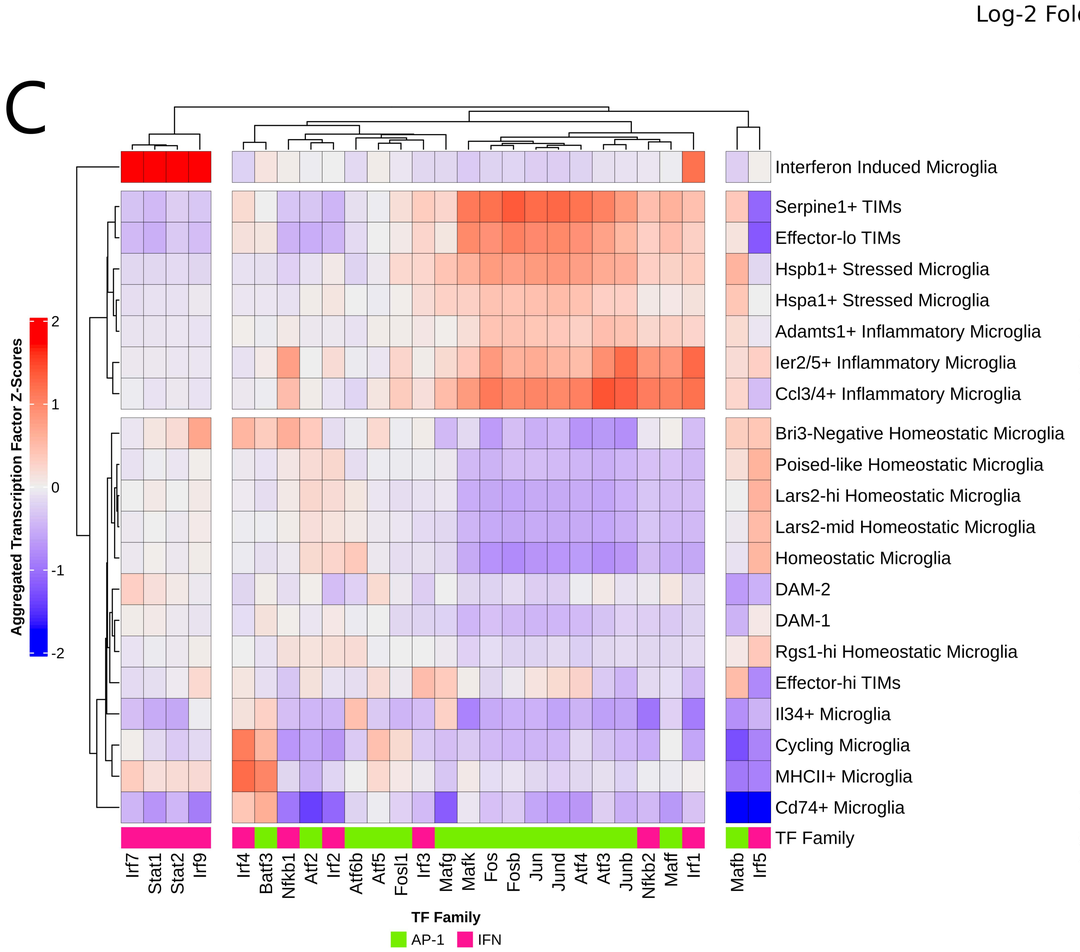
A


p = 1.99e-31
p = 1.15e-44
p = 7.49e-69
p = 1.26e-66
Smad4 SCENIC Score
5
0
Homeostatic
Inflammatory
TIMs
Other Microglia
DAMs

B


TIMs vs.
Homeostatic Microglia
Klf4
Fosb
Mafk
Klf2
Arid5a
Klf6
Fos
Atf3
Jund
Jun
Egr2
Atf4
Maff
Zbtb7c
Ddit3
Junb
Rel
Bptf
Nfic
Pax9
Ncoa2
Stat5b
Rxra
Fli1
Nfatc2
Atf7
Zbtb7b
Klf3
Cebpd
Elk3
Stat6
Foxk1
Erf
Foxj2
Etv6
Irf5
Mitf
Nfatc1
Etv5
Foxo3
-0.25
0.00
0.25
0.50
0.75
Log-2 Fold Change in TF Score
TIMs vs.
DAMs
Klf4
Klf2
Bptf
Mafk
Fosb
Klf6
Arid5a
Zbtb7c
Klf7
Tcf4
Fos
Jund
Jun
Plagl1
Zbtb43
Zbtb20
Cux1
Atf4
Tcf3
Sfpq
Myc
Irf7
Stat6
Etv4
Irf5
Zbtb7b
Elk3
Zbtb41
Zfp580
Hcls1
Gmeb2
Rxra
Nfe2l2
Nfe2
Zfp955b
Mitf
Hif1a
Hivep2
Rxrg
Bhlhe40
-0.6
-0.3
0.0
0.3
0.6
Log-2 Fold Change in TF Score
TIMs vs.
Inflammatory Microglia
Bptf
Klf4
Cux1
Arnt
Tcf3
Zbtb7c
Dlx2
Hoxa7
Klf2
Foxf1
Mafk
Pax9
Zbtb24
Klf6
Ebf1
Mnt
Plagl1
Zbtb20
Hmbox1
Crem
Arid5a
Stat2
Bcl6
Cebpb
Nfatc1
Stat6
Hcls1
Etv6
Erf
Foxj2
Stat1
Hif1a
Etv5
Egr2
Nfe2l2
Foxo3
Irf7
Bhlhe40
Irf5
Mitf
-0.2
-0.1
0.0
0.1
0.2
0.3
Log-2 Fold Change in TF Score
Log-10
P-Value
300
200
100
0

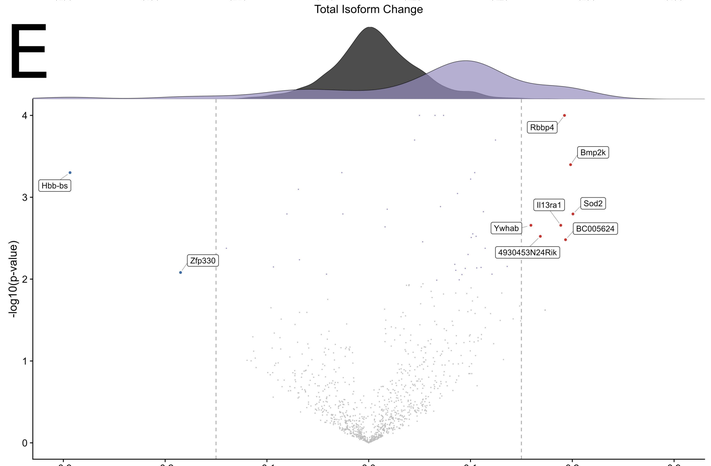
C


Aggregated Transcription Factor Z-Scores
2
1
0
-1
-2
Interferon Induced Microglia
Serpine1+ TIMs
Effector-lo TIMs
Hspb1+ Stressed Microglia
Hspa1+ Stressed Microglia
Adamts1+ Inflammatory Microglia
Ier2/5+ Inflammatory Microglia
Ccl3/4+ Inflammatory Microglia
Bri3-Negative Homeostatic Microglia
Poised-like Homeostatic Microglia
Lars2-hi Homeostatic Microglia
Lars2-mid Homeostatic Microglia
Homeostatic Microglia
DAM-2
DAM-1
Rgs1-hi Homeostatic Microglia
Effector-hi TIMs
Il34+ Microglia
Cycling Microglia
MHCII+ Microglia
Cd74+ Microglia
TF Family
Irf7
Stat1
Stat2
Irf9
Irf4
Batf3
Nfkb1
Atf2
Irf2
Atf6b
Atf5
Fosl1
Irf3
Mafg
Mafk
Fos
Fosb
Jun
Jund
Atf4
Atf3
Junb
Nfkb2
Maff
Irf1
Mafb
Irf5
TF Family
AP-1
IFN

D

Rbbp4
Glmp
Bmp2k
Tubb5
Rab7
4930453N24Rik
Hbb-bs
Ppt1
Ywhab
Il13ra1
Abcg1
Sypl
Mfap1a
BC005624
Apobec3
Atp2b4
Rpl7l1
Srprb
Sod2
Srp9
Mocs2
-log10(p-value)
0
1
2
3
4
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
Total Isoform Change

E


Rbbp4
Bmp2k
Hbb-bs
Il13ra1
Sod2
Ywhab
BC005624
4930453N24Rik
Zfp330
-log10(p-value)
0
1
2
3
4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
dLUI

F

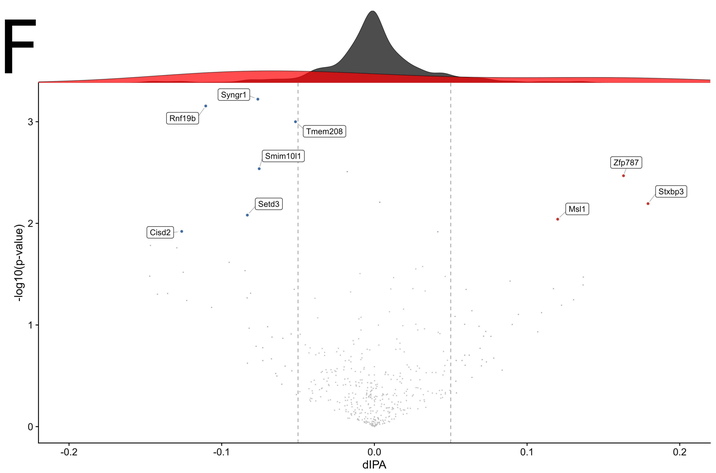
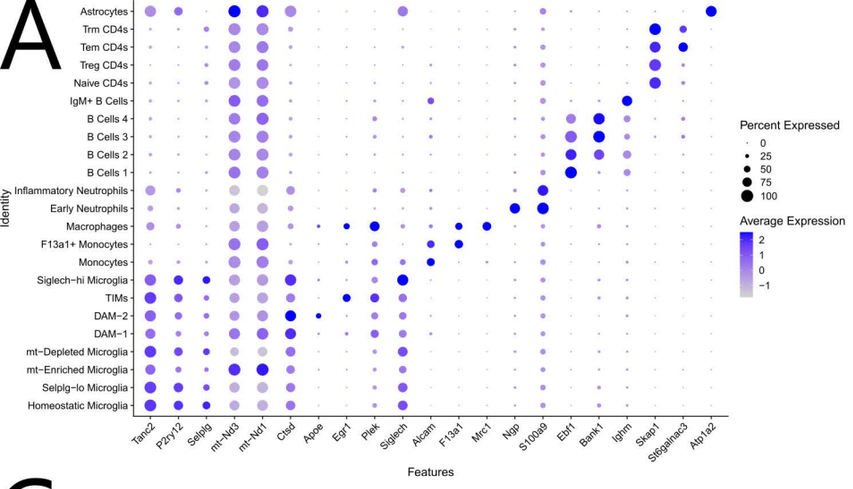
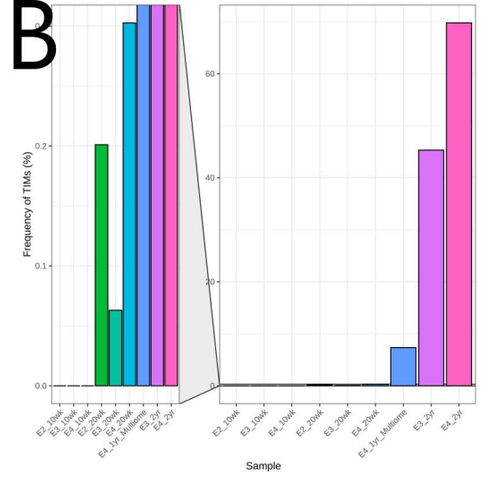
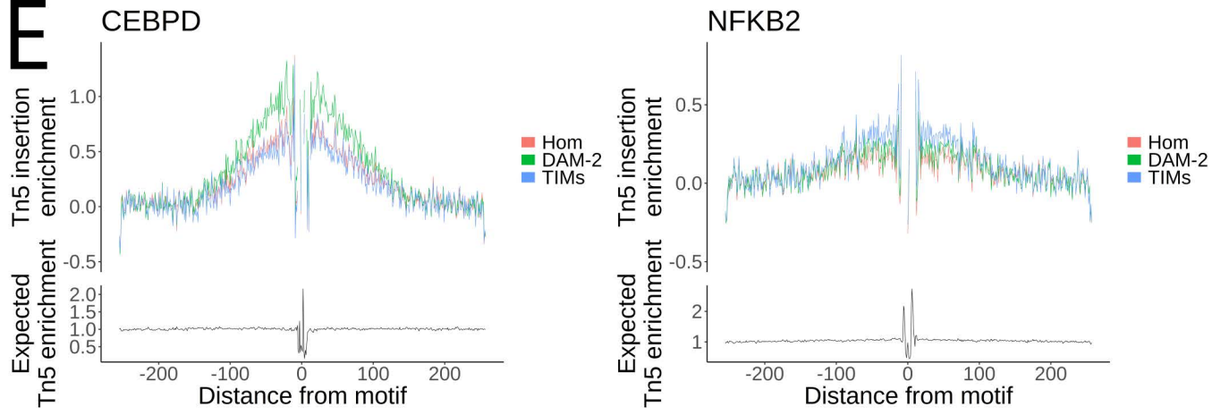

Syngr1
Rnf19b
Tmem208
Smim10l1
Zfp787
Stxbp3
Setd3
Msl1
Cisd2
-log10(p-value)
0
1
2
3
-0.2
-0.1
0.0
0.1
0.2
dIPA

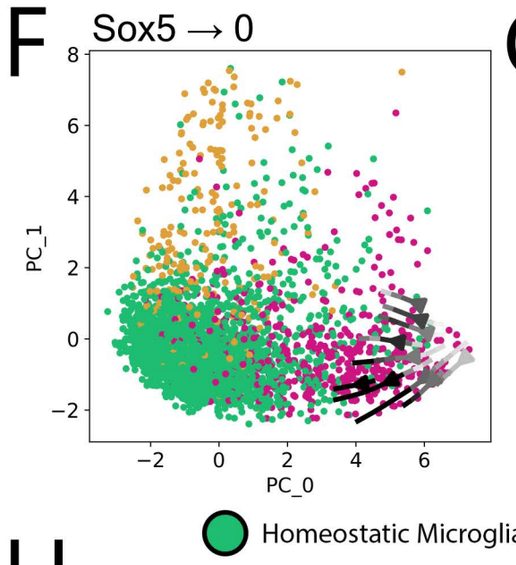
Figure S2, Related to Figure 2: Further analysis of microglia from 96-week-old *APOE3* and *APOE4* mice. (A) Boxplots of *Smad4* regulon scores generated by SCENIC. p-values calculated by Wilcoxon test with Holm-Bonferroni adjustment. Superclusters are mirrored from Figure 2E. (B) Lollipop plots of differentially enriched SCENIC-derived regulons between TIMs and each indicated microglial supercluster. Superclusters are defined as indicated in Figure 1C. Positive values indicate increased strength in TIMs. Bolded transcription factors are shared across all three comparisons. (C) Heatmap of SCENIC scores for all AP-1 factors and interferon-related factors. (D) Plot of the Wasserstein distance in isoform usage of the indicated genes between *APOE3* and *APOE4* mice. Significance calculated via bootstrap resampling. (E) Same as (D) but plotting differential long isoform usage (long 3'UTR index, or LUI). Positive values indicate increased long isoform usage in *APOE4*. (F) Same as (D) but plotting differential intronic polyadenylation. Positive values indicate increased long isoform usage in *APOE4*.

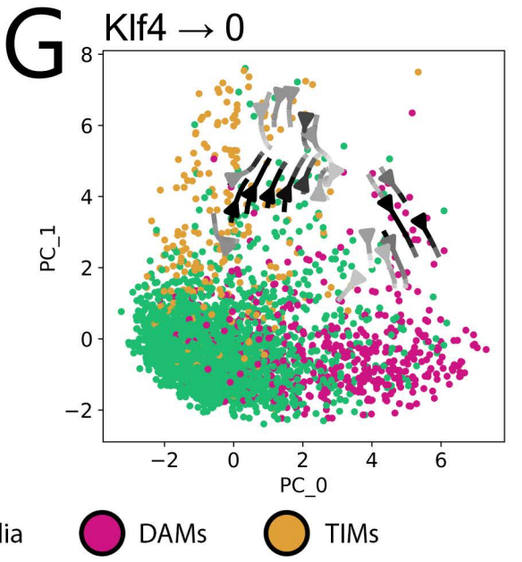

A
Astrocytes
Trm CD4s
Tem CD4s
Treg CD4s
Naive CD4s
IgM+ B Cells
B Cells 4
B Cells 3
B Cells 2
B Cells 1
Inflammatory Neutrophils
Early Neutrophils
Macrophages
F13a1+ Monocytes
Monocytes
Siglech−hi Microglia
TIMs
DAM−2
DAM−1
mt−Depleted Microglia
mt−Enriched Microglia
Selplg−lo Microglia
Homeostatic Microglia
Identity
Tanc2
P2ry12
Selplg
mt-Nd3
mt-Nd1
Ctsd
Apoe
Egr1
Plek
Siglech
Alcam
F13a1
Mrc1
Ngp
S100a9
Ebf1
Bank1
Ighm
Skap1
Slfgalnac3
Atp1a2
Features
Percent Expressed
0
25
50
75
100
Average Expression
2
1
0
−1


B
Frequency of TIMs (%)
E2_10wk
E3_10wk
E4_10wk
E2_20wk
E3_20wk
E4_20wk
E4_1yr_Multiome
E3_2yr
E4_2yr
Sample


E
CEBPD
NFKB2
Tn5 insertion enrichment
Expected Tn5 enrichment
Hom
DAM-2
TIMs
Distance from motif

C
Homeostatic Microglia
Selplg-lo Microglia
mt-Enriched Microglia
mt-Depleted Microglia
DAM-1
DAM-2
TIMs
Siglech-hi Microglia
Normalized signal (range 0 - 68)
Normalized signal (range 0 - 66)
gene
Egr1
Btg2
Genes
Peaks
Links
score
chr18 position (bp)
chr1 position (bp)
D
Normalized signal (range 0 - 18)
Normalized signal (range 0 - 23)
Normalized signal (range 0 - 48)
Normalized signal (range 0 - 80)
Monocytes
F13a1+ Monocytes
Macrophages
Early Neutrophils
Inflammatory Neutrophils
B Cells 1
B Cells 2
B Cells 3
B Cells 4
IgM+ B Cells
Naive CD4s
Treg CD4s
Tem CD4s
Trm CD4s
Astrocytes
Apoe
Skap1
Ngp
Ebf1
Apoc1
Tomm40
2010300F17Rik
Gm11529
Snx11
Klhl18
chr7 position (bp)
chr11 position (bp)
chr9 position (bp)


F
Sox5 → 0
PC_0
PC_1
Homeostatic Microglia
DAMs
TIMs


G
Klf4 → 0
PC_0
PC_1

H
Dehydroepiandrosterone_bySTS
TGFbeta_receptor1
ThromboxaneA2_byTBXAS1
TGFR_BMPR2
PlexinA4_complex1
integrin_aVb5_complex
TREM2_receptor
SIRB1_complex
2arachidonoylglycerol_byDAGLB
IL6_receptor
integrin_a6b1_complex
CCL4_HUMAN
integrin_aVb1_complex
Type_II_IFNR
integrin_a9b1_complex
ACVR1_BMPR2
TGFbeta_receptor2
IL10_receptor
LIFR
BMPR1A_BMPR2
TGFR_AVR2A
ACVR_1A2A_receptor
BMR1A_ACR2A
Testosterone_byHSD17B12
integrin_a5b1_complex
Histamine_byHDC
Mean Fold-Change over Cluster Mean

Figure S3, Related to Figure 3: Further analysis of the multiome dataset. (A) Dotplot of markers used in supervised annotation of clusters. (B) Barplot of the frequency of TIMs from each sample across the integrated atlas and the multiome dataset. (C) Coverage plot showing concordance between RNA and ATAC modalities for *Egr1* and *Btg2*, two TIM markers. (D) Same as (C) but for *APOE*, a DAM marker, *Skap1*, a T cell marker, *Ngp*, a neutrophil marker, and *Ebf1*, a B cell marker. (E) Footprinting of Tn5 insertion frequency around CEBPD and NFKB2 motifs. (F) Perturbation simulation plots ablating *Sox5*. Expression of the transcription factor was set to 0 and the gene regulatory networks were re-initialized to generate new expression profiles for each cell. Cells are projected in a PCA space defined by the gene regulatory net. Arrow shade indicates the magnitude of the transition flow. (G) Same as (F) but for *Klf4*. (H) Boxplot of highest-scoring ligand:receptor complexes across all microglial clusters.

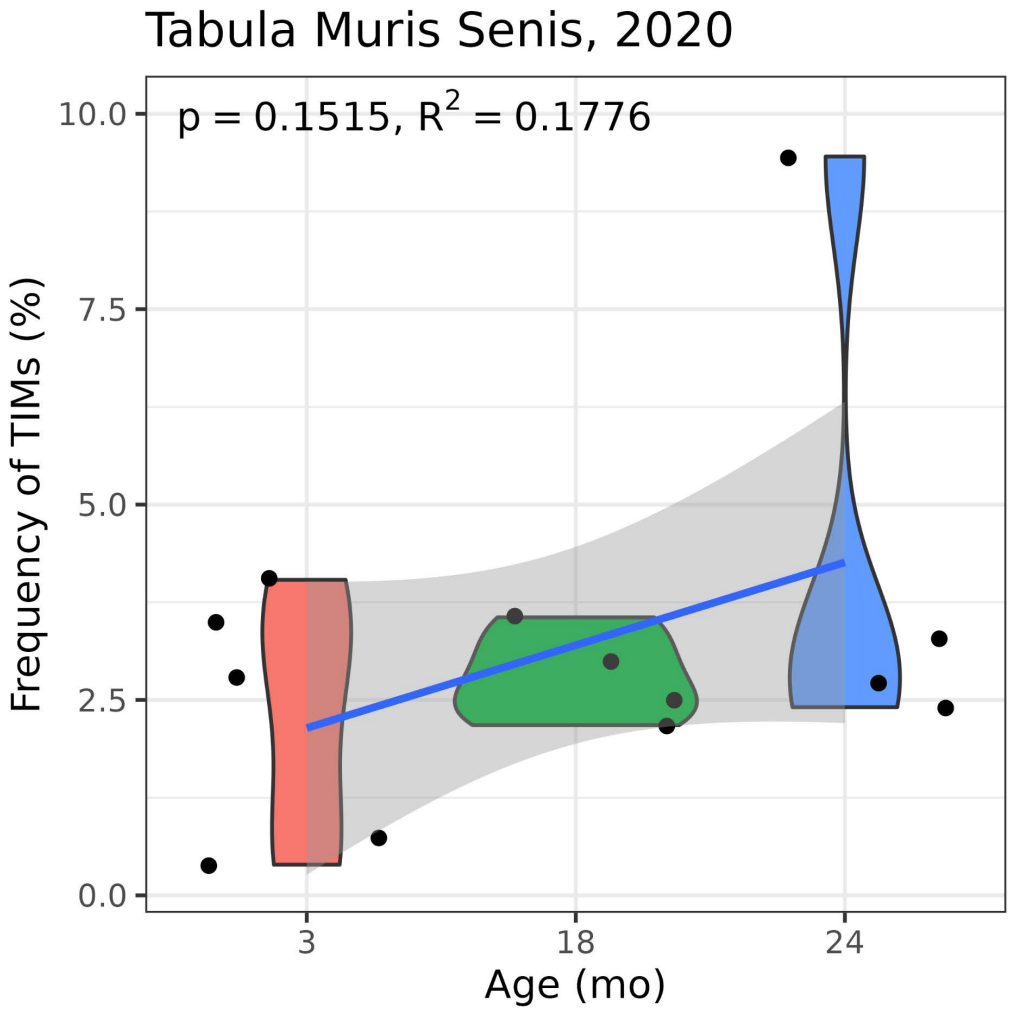
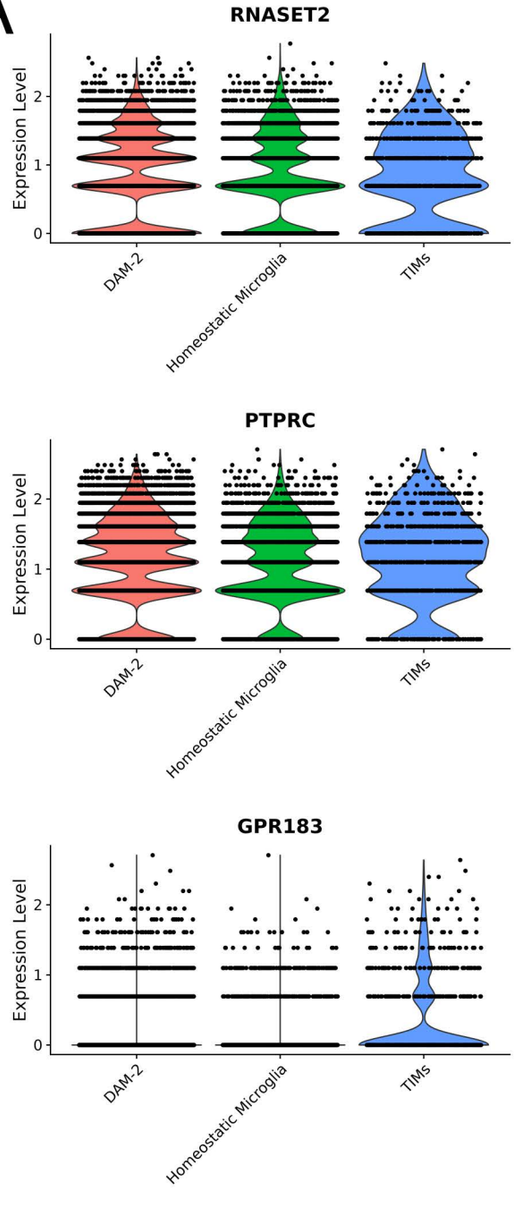
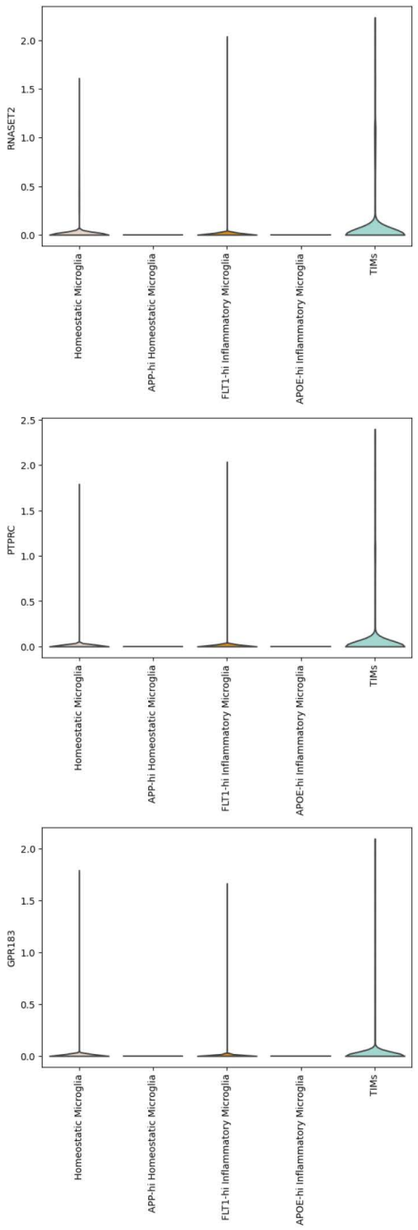


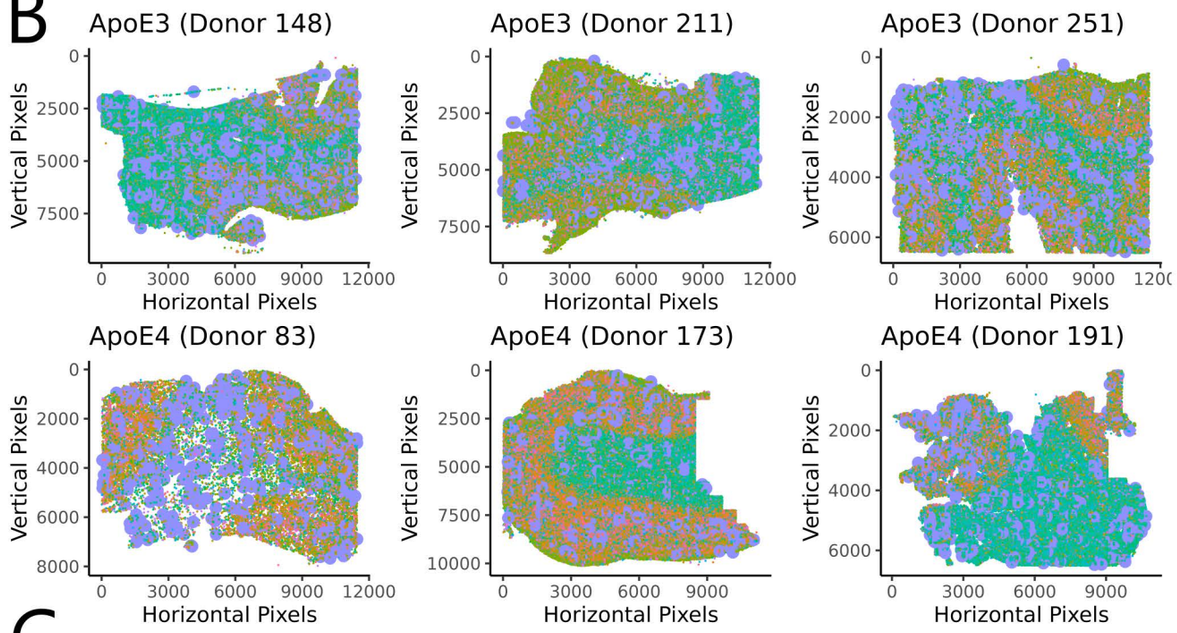
Figure S4, Related to Figure 4: Tabula Muris Senis reanalysis. Violin plot of the frequency of TIMs across each sample in the Tabula Muris Senis, a publicly available single-cell dataset of murine tissues across age. Data is overlaid with a linear regression.

A

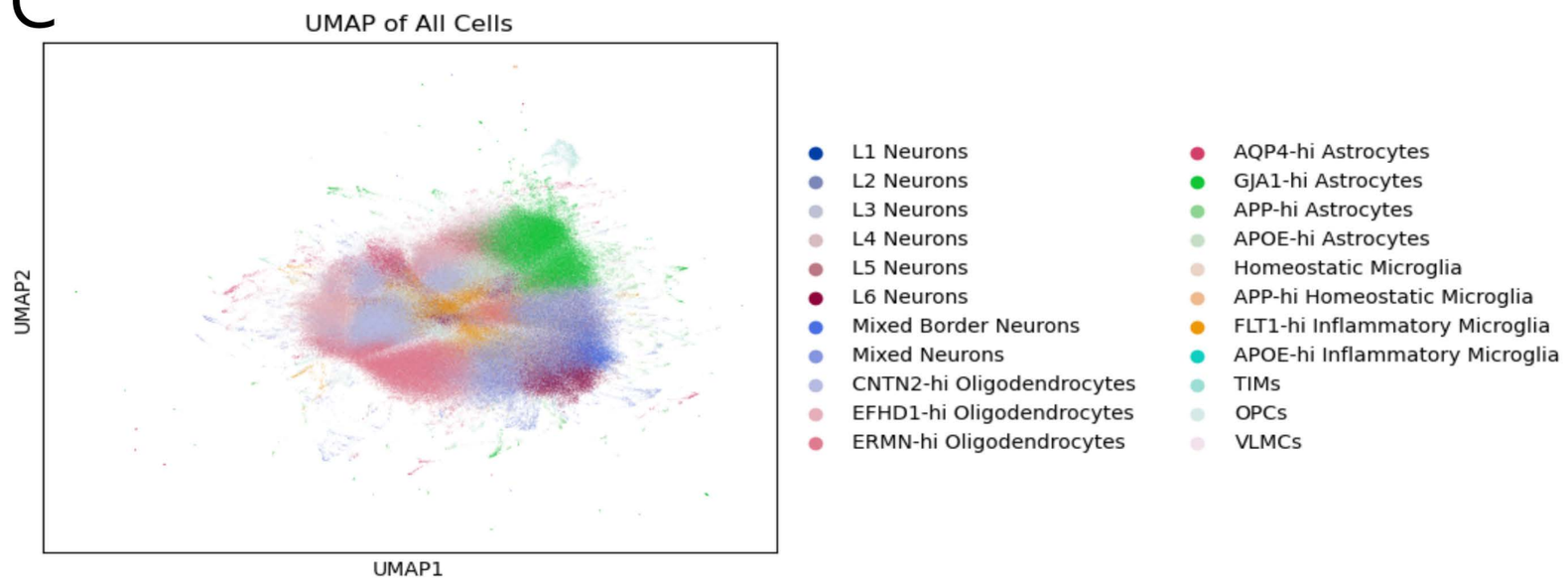

RNASET2
Expression Level
DAM-2
Homeostatic Microglia
TIMs



B



C

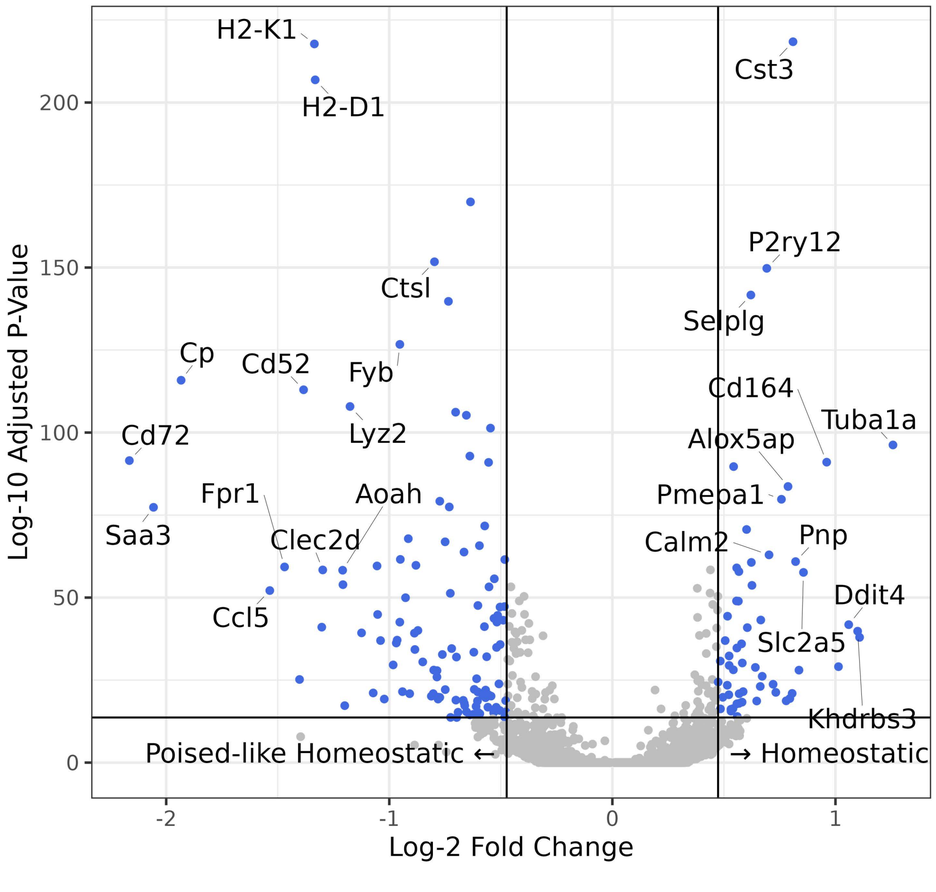


D

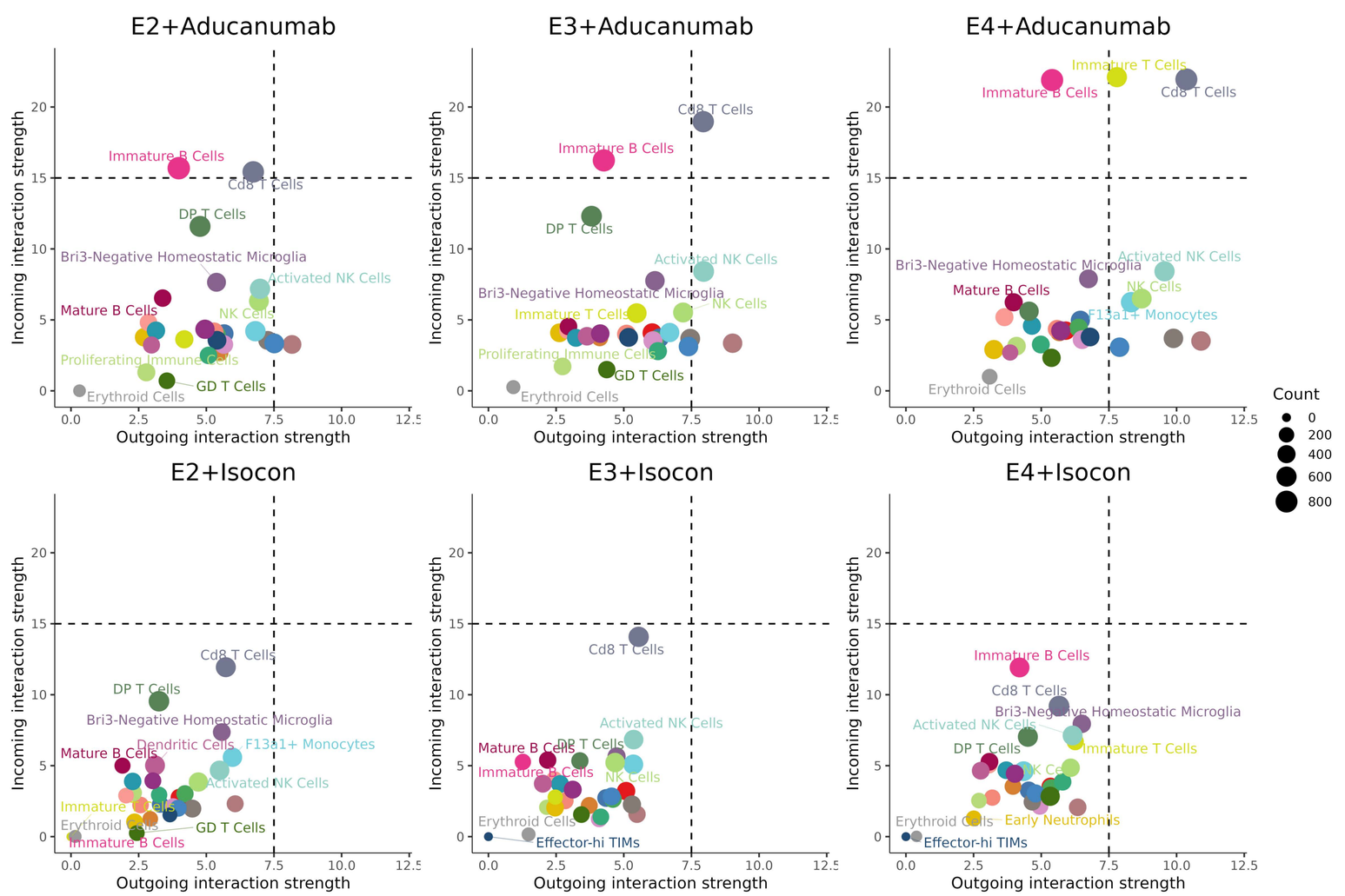
E

Figure S5, Related to Figure 5: Further analysis of the Xenium dataset. (A) Violin plots of three representative genes used to annotate TIMs. At left is their expression in the DLPFC-2 dataset, while at right is their expression in the Xenium data. (B) Spatial scatter plots of all six tissue sections. (C) UMAP of all 494,376 cells across all six sections, colored by final annotation. (D) Cell-cell neighborhood adjacency matrix showing the relative frequency at which cells are found adjacent to other cells *in situ* across all samples. (E) Boxplots of the increased frequency of finding a given cell within a 1000 pixel-radius circle centered around a TIM compared to a null distribution based on cell frequencies.

A


H2-K1
H2-D1
Cst3
P2ry12
Ctsl
Selplg
Cp
Cd52
Fyb
Cd164
Tuba1a
Cd72
Lyz2
Alox5ap
Fpr1
Aoah
Pmepa1
Saa3
Clec2d
Calm2
Pnp
Ddit4
Ccl5
Slc2a5
Khdrbs3
Poised-like Homeostatic ←
→ Homeostatic
Log-10 Adjusted P-Value
Log-2 Fold Change

B


E2+Aducanumab
E3+Aducanumab
E4+Aducanumab
E2+Isocon
E3+Isocon
E4+Isocon
Incoming interaction strength
Outgoing interaction strength
Immature B Cells
Cd8 T Cells
DP T Cells
Bri3-Negative Homeostatic Microglia
Activated NK Cells
Mature B Cells
NK Cells
Immature T Cells
Proliferating Immune Cells
GD T Cells
Erythroid Cells
F13a1+ Monocytes
Dendritic Cells
Effector-hi TIMs
Early Neutrophils
Count
0
200
400
600
800

C

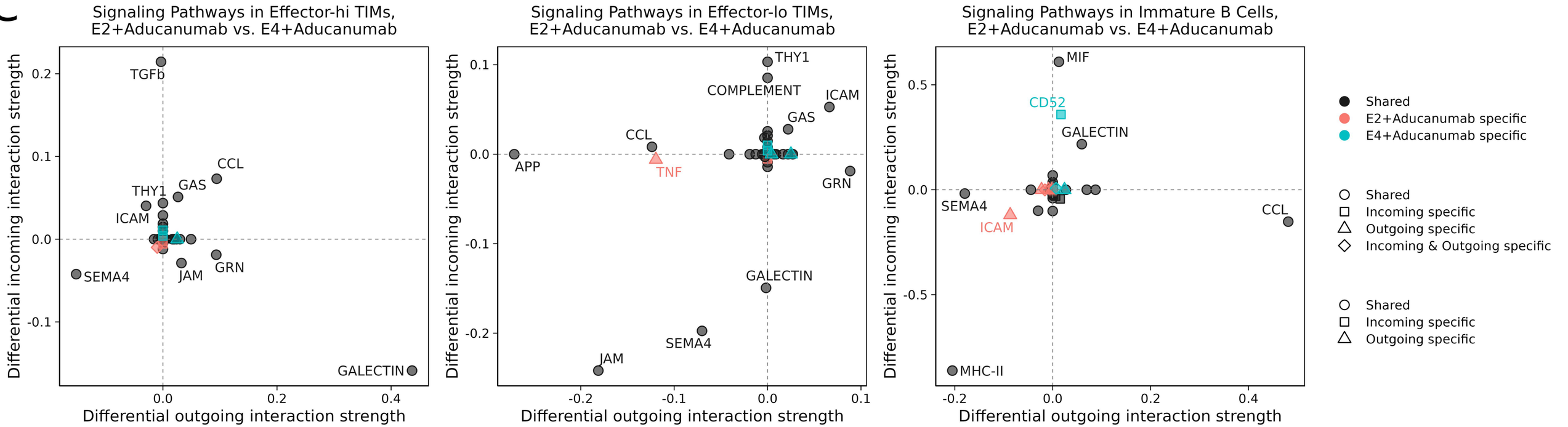

Signaling Pathways in Effector-hi TIMs, E2+Aducanumab vs. E4+Aducanumab
Signaling Pathways in Effector-lo TIMs, E2+Aducanumab vs. E4+Aducanumab
Signaling Pathways in Immature B Cells, E2+Aducanumab vs. E4+Aducanumab
Differential incoming interaction strength
Differential outgoing interaction strength
TGFb
CCL
THY1
GAS
ICAM
SEMA4
JAM
GRN
GALECTIN
COMPLEMENT
APP
TNF
MIF
CD52
MHC-II
Shared
E2+Aducanumab specific
E4+Aducanumab specific
Incoming specific
Outgoing specific
Incoming & Outgoing specific

Figure S6, Related to Figure 7: Further analysis of the aducanumab dataset. (A) Violin plot of differentially expressed genes between homeostatic microglia and poised-like homeostatic microglia. (B) Dotplot of clusters in each library within the aducanumab dataset plotting incoming signaling against outgoing signaling as estimated by CellChat. (C) Dotplot of differentially enriched signaling pathways in effector-hi TIMs, effector-lo TIMs, and immature B cells between AD***APOE2* and AD***APOE4* aducanumab-treated animals by both incoming and outgoing signal strength. Pathways are color- and shape-coded by directionality and sample specificity. Positive numbers indicate a greater strength in AD***APOE4*.