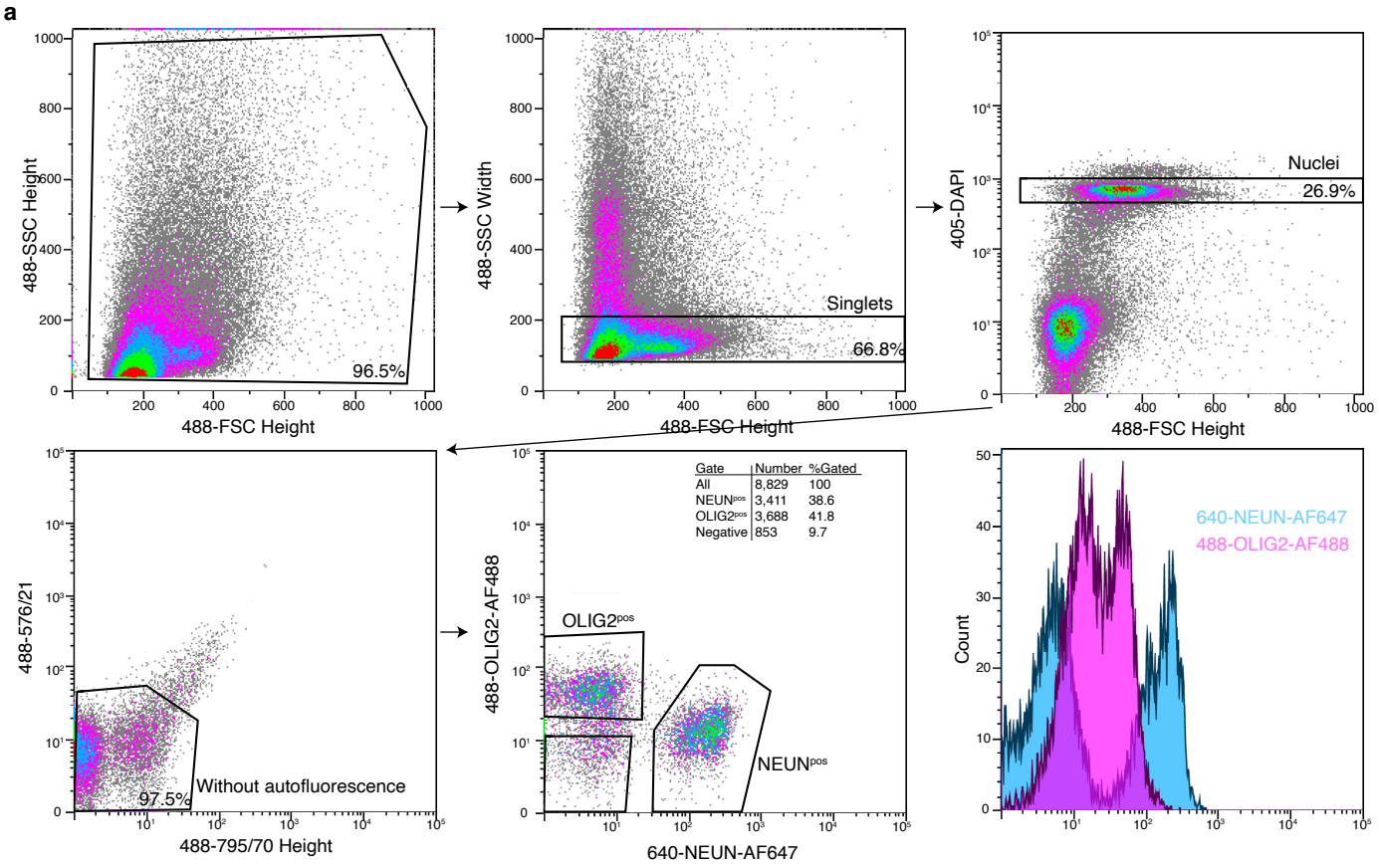
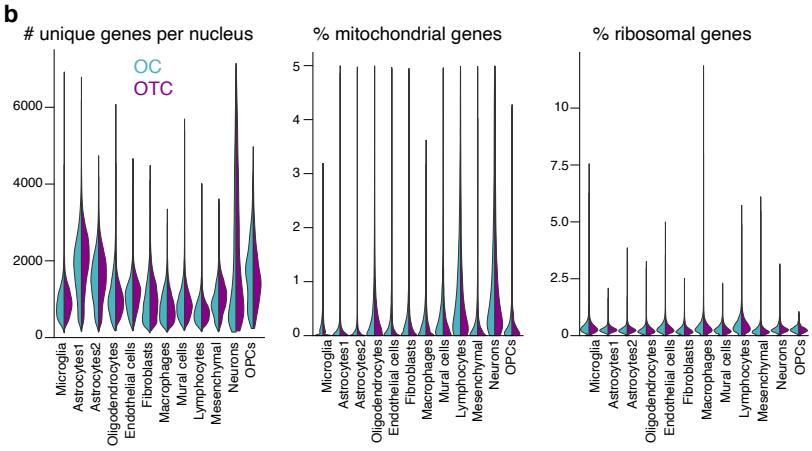
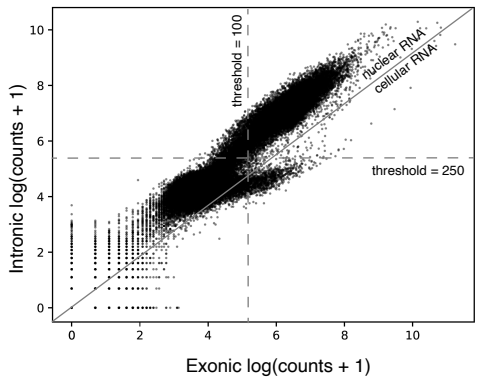
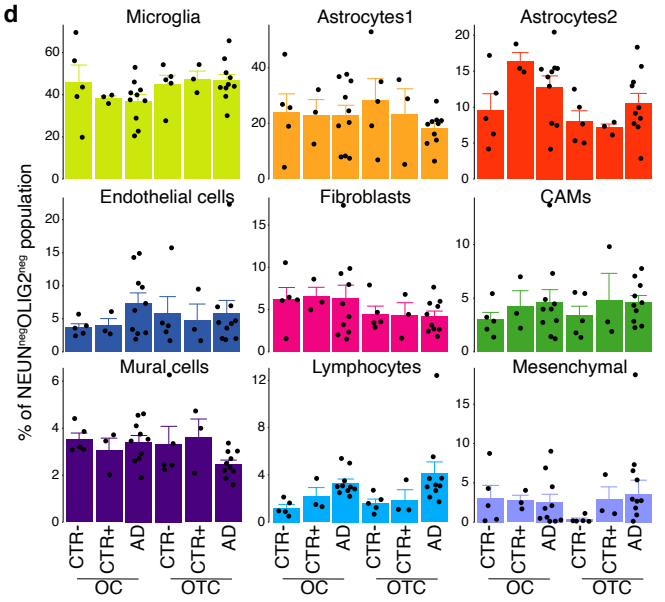
Figure S1


a
488-SSC Height
488-FSC Height
96.5%
488-SSC Width
Singlets
66.8%
405-DAPI
Nuclei
26.9%
488-576/21
488-795/70 Height
97.5%
Without autofluorescence
488-OLIG2-AF488
640-NEUN-AF647
OLIG2pos
NEUNpos
Gate | Number | %Gated
All | 8,829 | 100
NEUNpos | 3,411 | 38.6
OLIG2pos | 3,688 | 41.8
Negative | 853 | 9.7
Count
640-NEUN-AF647
488-OLIG2-AF488


b
unique genes per nucleus
% mitochondrial genes
% ribosomal genes
OC
OTC
Microglia
Astrocytes1
Astrocytes2
Oligodendrocytes
Endothelial cells
Fibroblasts
Macrophages
Mural cells
Lymphocytes
Mesenchymal
Neurons
OPCs


c
Intronic log(counts + 1)
Exonic log(counts + 1)
threshold = 100
threshold = 250
nuclear RNA
cellular RNA


d
% of NEUNnegOLIG2neg population
Microglia
Astrocytes1
Astrocytes2
Endothelial cells
Fibroblasts
CAMs
Mural cells
Lymphocytes
Mesenchymal
CTR-
CTR+
AD
OC
OTC

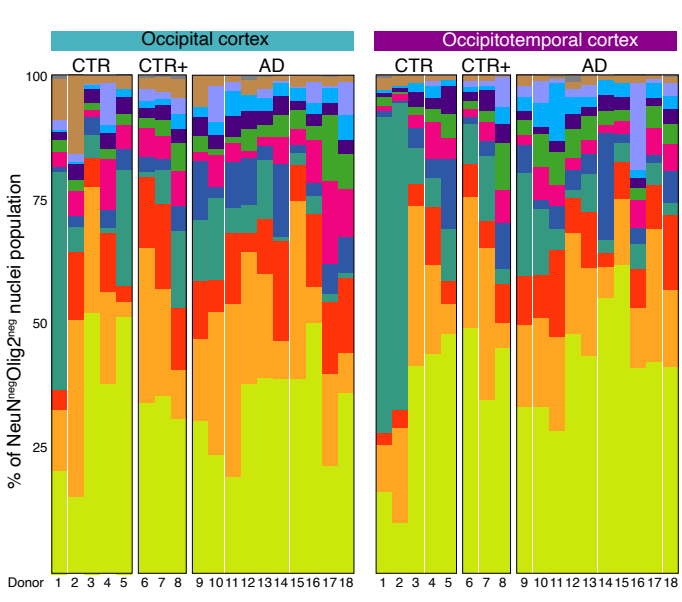

e
Occipital cortex
Occipitotemporal cortex
CTR
CTR+
AD
% of NeuNnegOlig2neg nuclei population
Donor 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Figure S2

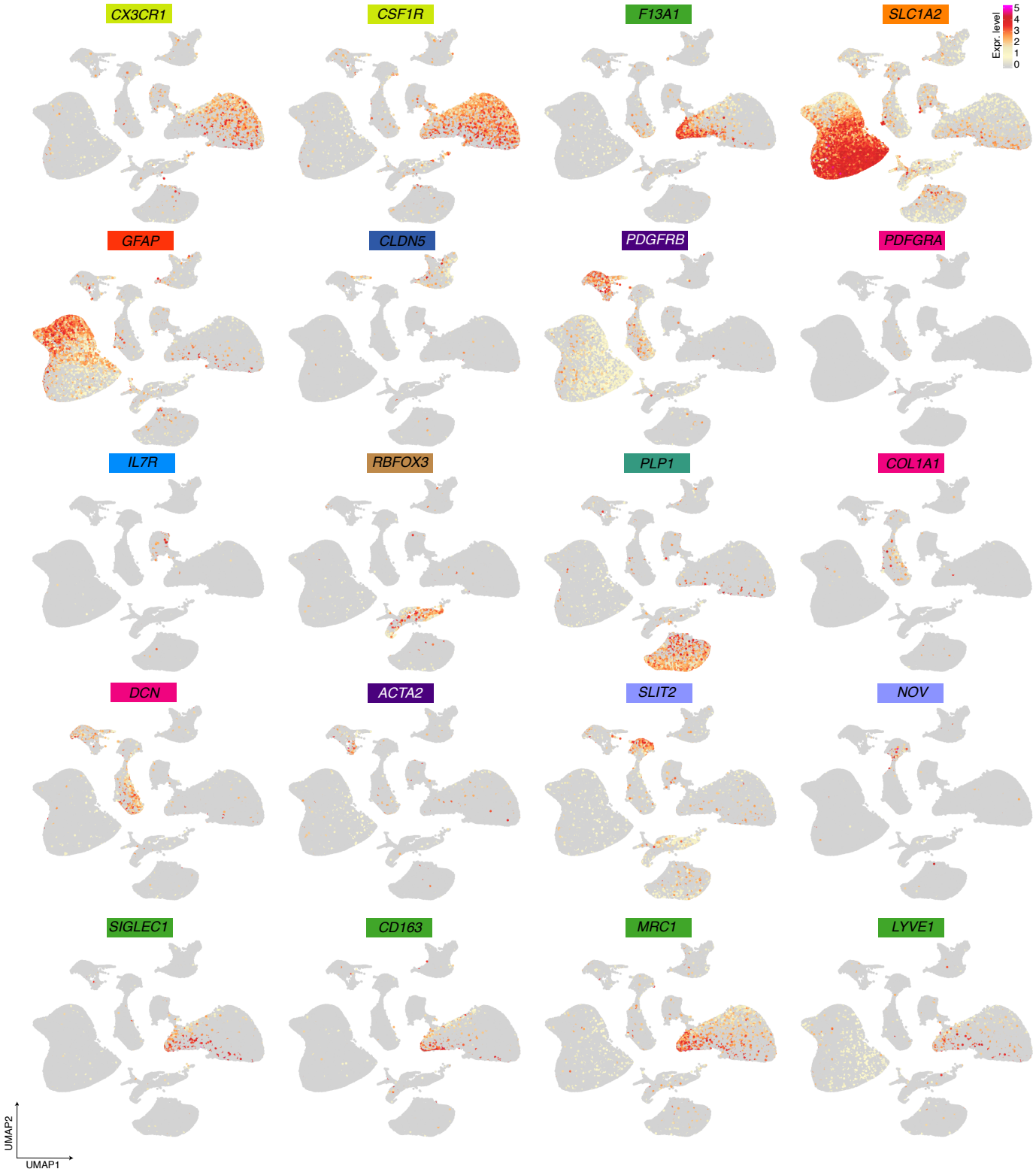

CX3CR1
CSF1R
F13A1
SLC1A2
Expr. level
5
4
3
2
1
0
GFAP
CLDN5
PDGFRB
PDFGRA
IL7R
RBFOX3
PLP1
COL1A1
DCN
ACTA2
SLIT2
NOV
SIGLEC1
CD163
MRC1
LYVE1
UMAP2
UMAP1

Figure S3

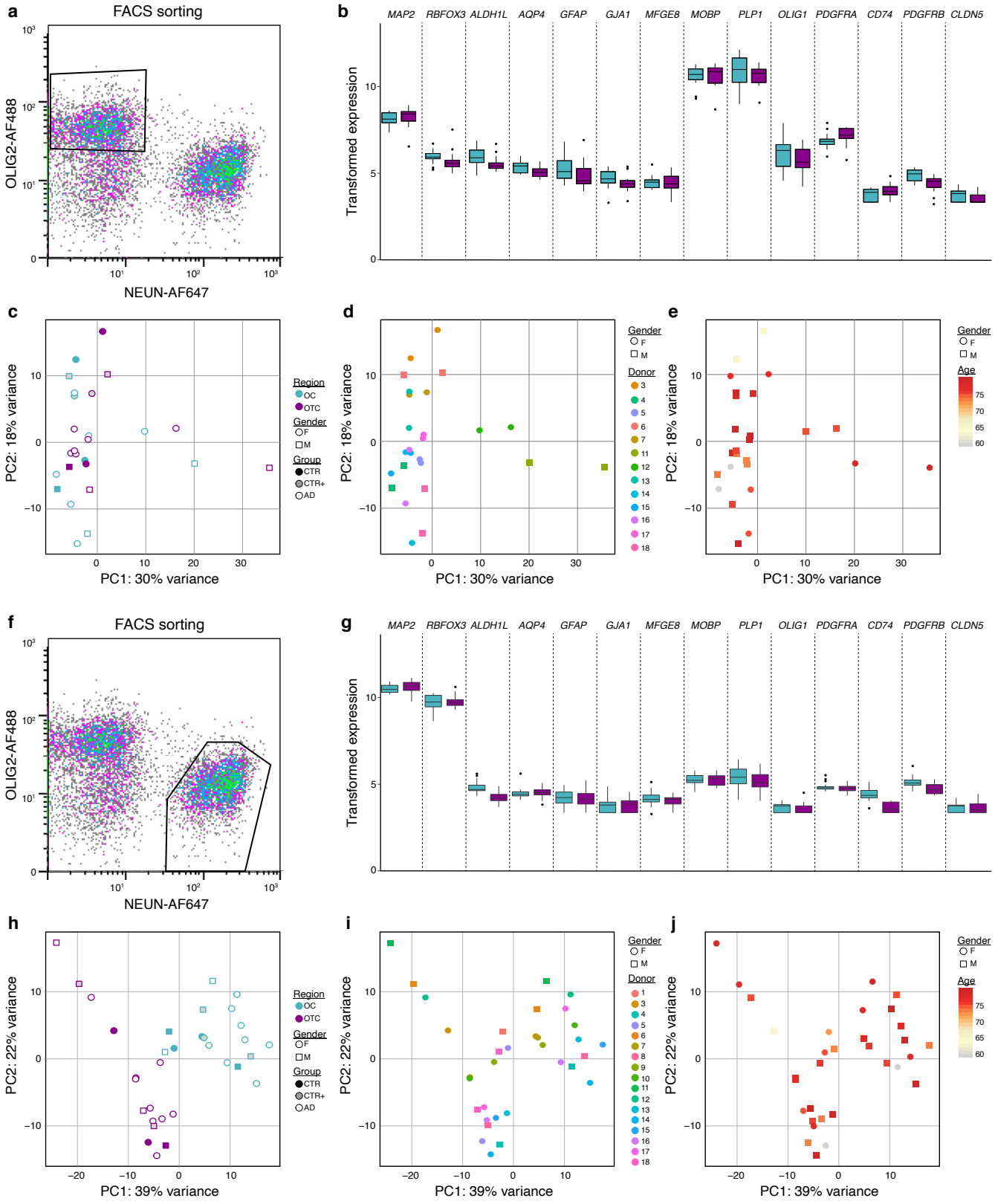

a
FACS sorting
OLIG2-AF488
NEUN-AF647
b
MAP2 RBFOX3 ALDH1L AQP4 GFAP GJA1 MFGE8 MOBP PLP1 OLIG1 PDGFRA CD74 PDGFRB CLDN5
Transformed expression
c
PC2: 18% variance
PC1: 30% variance
Region
OC
OTC
Gender
F
M
Group
CTR
CTR+
AD
d
PC2: 18% variance
PC1: 30% variance
Gender
Donor
e
PC2: 18% variance
PC1: 30% variance
Gender
Age
f
FACS sorting
OLIG2-AF488
NEUN-AF647
g
MAP2 RBFOX3 ALDH1L AQP4 GFAP GJA1 MFGE8 MOBP PLP1 OLIG1 PDGFRA CD74 PDGFRB CLDN5
Transformed expression
h
PC2: 22% variance
PC1: 39% variance
Region
OC
OTC
Gender
F
M
Group
CTR
CTR+
AD
i
PC2: 22% variance
PC1: 39% variance
Gender
Donor
j
PC2: 22% variance
PC1: 39% variance
Gender
Age

Figure S4

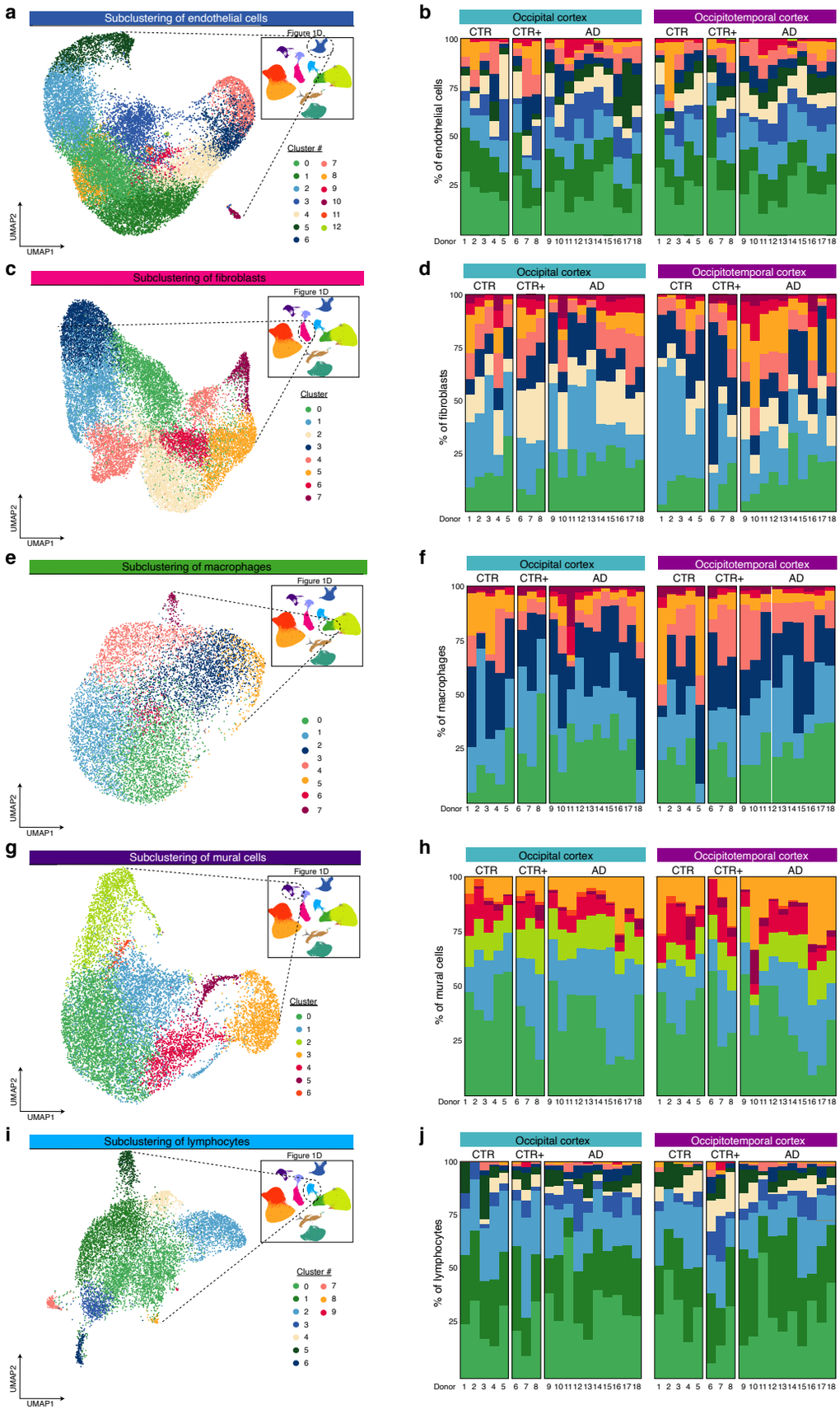

a
Subclustering of endothelial cells
Figure 1D
Cluster #
0 1 2 3 4 5 6 7 8 9 10 11 12
UMAP2
UMAP1
b
Occipital cortex
Occipitotemporal cortex
CTR
CTR+
AD
% of endothelial cells
100
75
50
25
Donor 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
c
Subclustering of fibroblasts
Figure 1D
Cluster
0 1 2 3 4 5 6 7
UMAP2
UMAP1
d
Occipital cortex
Occipitotemporal cortex
CTR
CTR+
AD
% of fibroblasts
100
75
50
25
Donor 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
e
Subclustering of macrophages
Figure 1D
0 1 2 3 4 5 6 7
UMAP2
UMAP1
f
Occipital cortex
Occipitotemporal cortex
CTR
CTR+
AD
% of macrophages
100
75
50
25
Donor 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
g
Subclustering of mural cells
Figure 1D
Cluster
0 1 2 3 4 5 6
UMAP2
UMAP1
h
Occipital cortex
Occipitotemporal cortex
CTR
CTR+
AD
% of mural cells
100
75
50
25
Donor 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
i
Subclustering of lymphocytes
Figure 1D
Cluster #
0 1 2 3 4 5 6 7 8 9
UMAP2
UMAP1
j
Occipital cortex
Occipitotemporal cortex
CTR
CTR+
AD
% of lymphocytes
100
75
50
25
Donor 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Figure S5

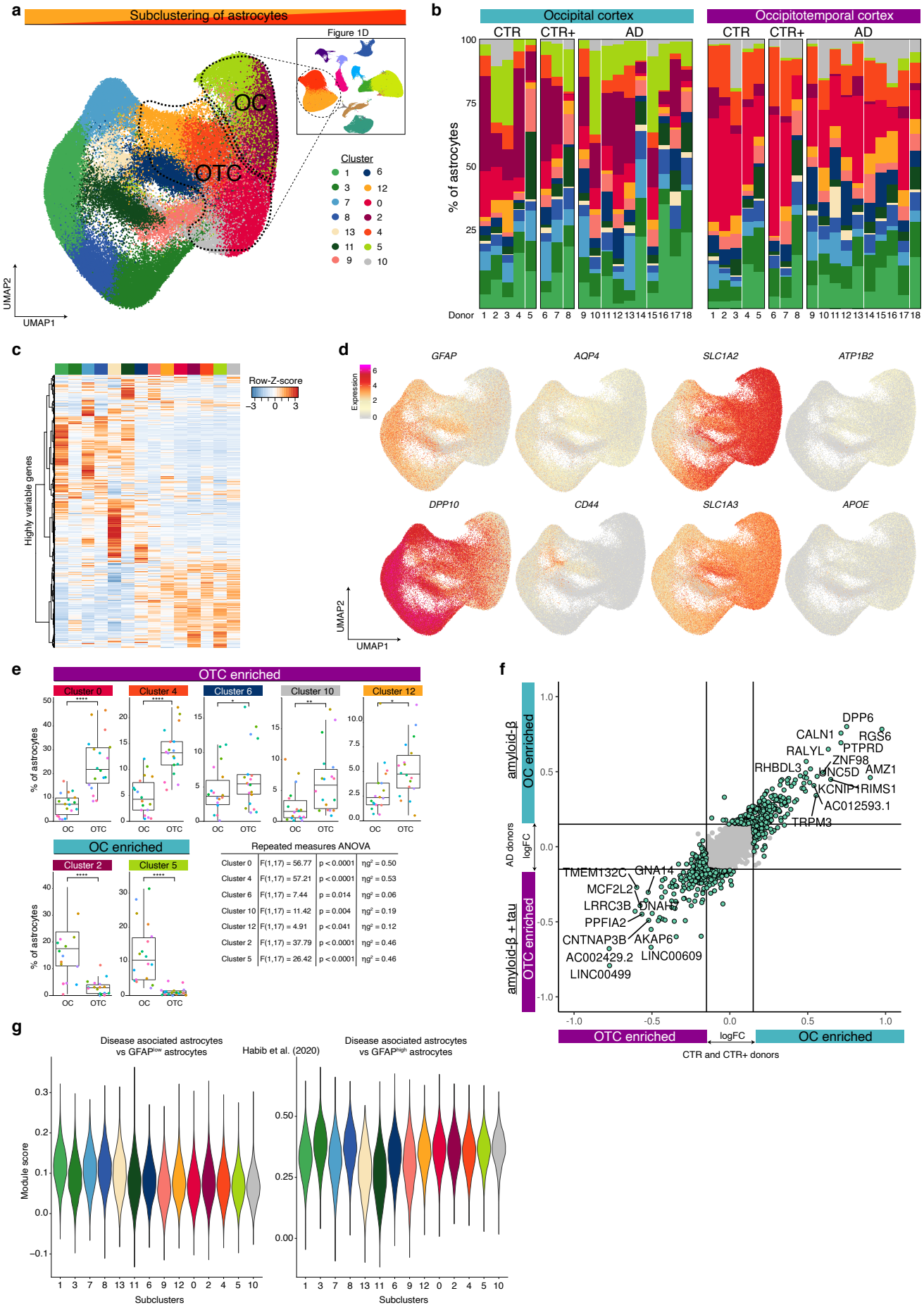

a
Subclustering of astrocytes
Figure 1D
OC
OTC
Cluster
1 6
3 12
7 0
8 2
13 4
11 5
9 10
UMAP2
UMAP1
b
Occipital cortex
Occipitotemporal cortex
CTR CTR+ AD
CTR CTR+ AD
% of astrocytes
100
75
50
25
Donor 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
c
Row-Z-score
−3 0 3
Highly variable genes
d
GFAP
AQP4
SLC1A2
ATP1B2
Expression
6
4
2
0
DPP10
CD44
SLC1A3
APOE
UMAP2
UMAP1
e
OTC enriched
Cluster 0
Cluster 4
Cluster 6
Cluster 10
Cluster 12
% of astrocytes
OC OTC
OC enriched
Cluster 2
Cluster 5
Repeated measures ANOVA
Cluster 0 F(1,17) = 56.77 p < 0.0001 ηg² = 0.50
Cluster 4 F(1,17) = 57.21 p < 0.0001 ηg² = 0.53
Cluster 6 F(1,17) = 7.44 p = 0.014 ηg² = 0.06
Cluster 10 F(1,17) = 11.42 p = 0.004 ηg² = 0.19
Cluster 12 F(1,17) = 4.91 p < 0.041 ηg² = 0.12
Cluster 2 F(1,17) = 37.79 p < 0.0001 ηg² = 0.46
Cluster 5 F(1,17) = 26.42 p < 0.0001 ηg² = 0.46
f
amyloid-β
OC enriched
AD donors logFC
amyloid-β + tau
OTC enriched
DPP6
CALN1
RGS6
RALYL
PTPRD
RHBDL3
ZNF98
UNC5D
AMZ1
KCNIP1
RIMS1
AC012593.1
TRPM3
TMEM132C
GNA14
MCF2L2
LRRC3B
GNAI3
PPFIA2
CNTNAP3B
AKAP6
AC002429.2
LINC00609
LINC00499
OTC enriched
logFC
OC enriched
CTR and CTR+ donors
g
Disease asociated astrocytes vs GFAP^low astrocytes
Habib et al. (2020)
Disease asociated astrocytes vs GFAP^high astrocytes
Module score
Subclusters
1 3 7 8 13 11 6 9 12 0 2 4 5 10

Figure S6

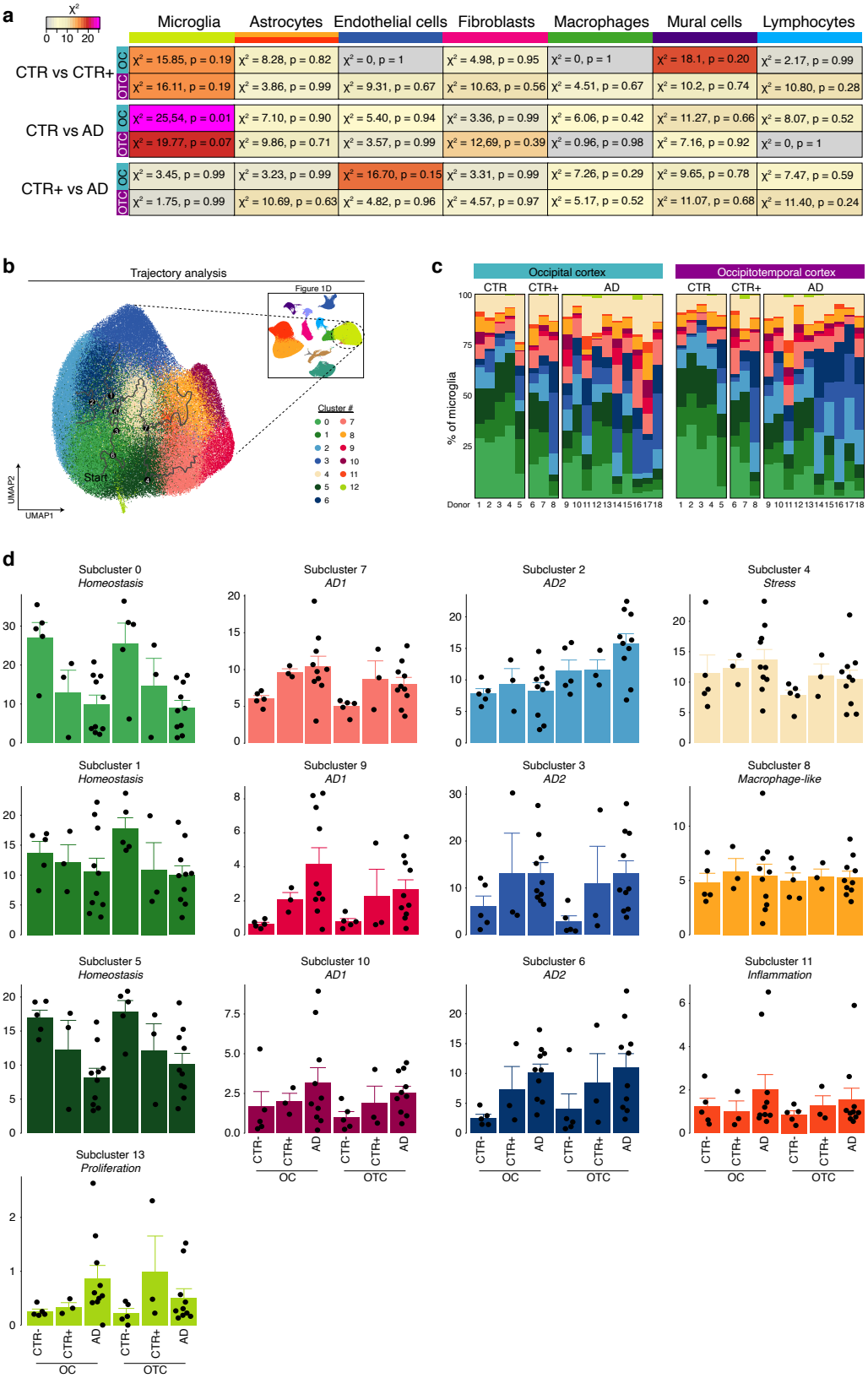
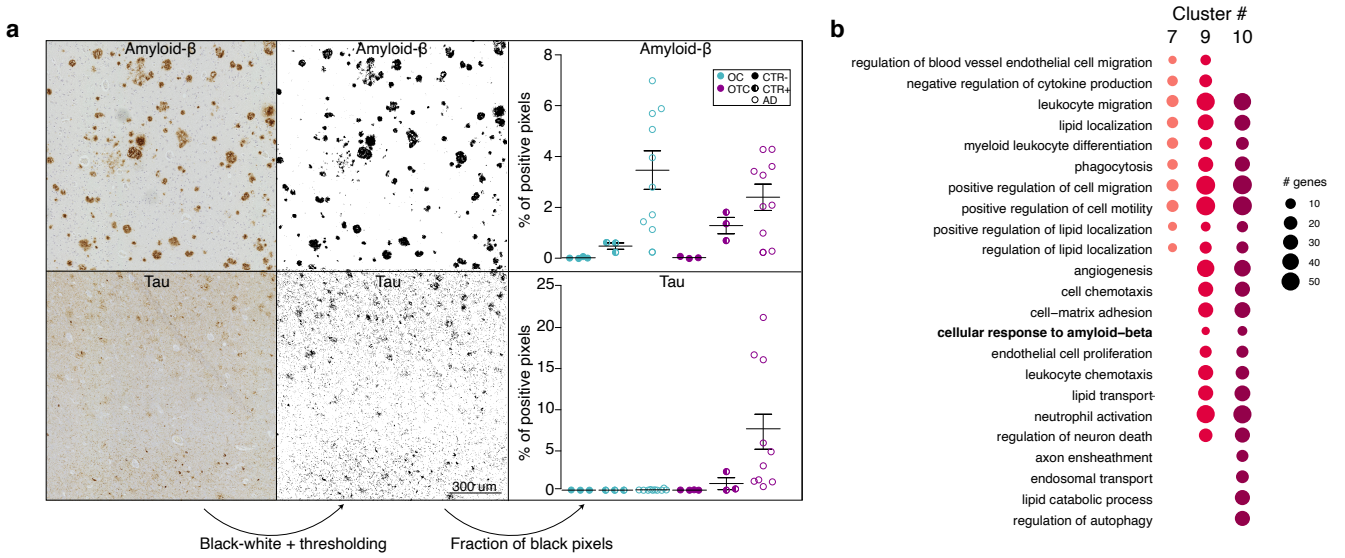
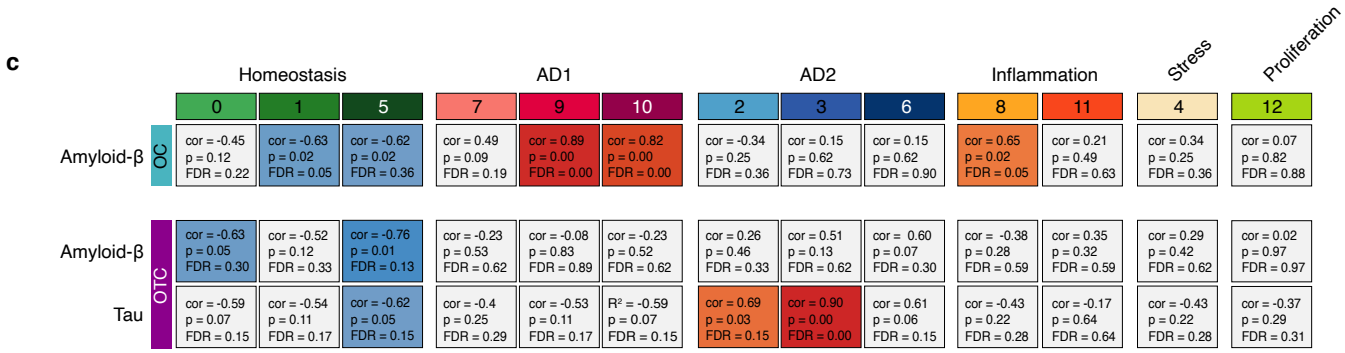
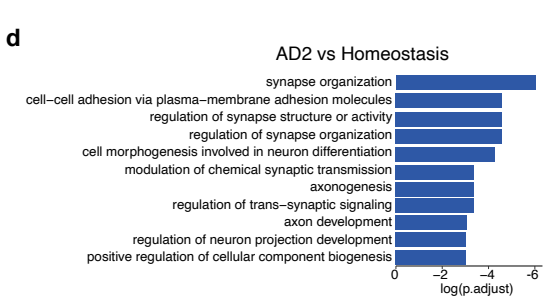
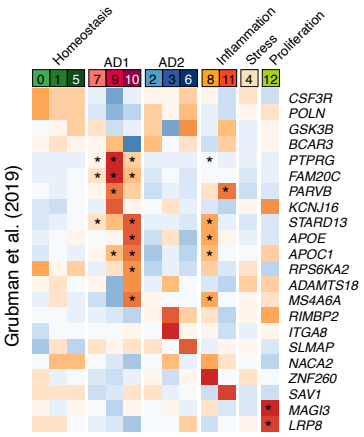

a
Microglia
Astrocytes
Endothelial cells
Fibroblasts
Macrophages
Mural cells
Lymphocytes
CTR vs CTR+
CTR vs AD
CTR+ vs AD
OC
OTC
b
Trajectory analysis
Figure 1D
Cluster #
Start
UMAP1
UMAP2
c
Occipital cortex
Occipitotemporal cortex
CTR
CTR+
AD
% of microglia
Donor
d
Subcluster 0
Homeostasis
Subcluster 7
AD1
Subcluster 2
AD2
Subcluster 4
Stress
Subcluster 1
Homeostasis
Subcluster 9
AD1
Subcluster 3
AD2
Subcluster 8
Macrophage-like
Subcluster 5
Homeostasis
Subcluster 10
AD1
Subcluster 6
AD2
Subcluster 11
Inflammation
Subcluster 13
Proliferation
% of microglia
CTR-
CTR+
AD
OC
OTC

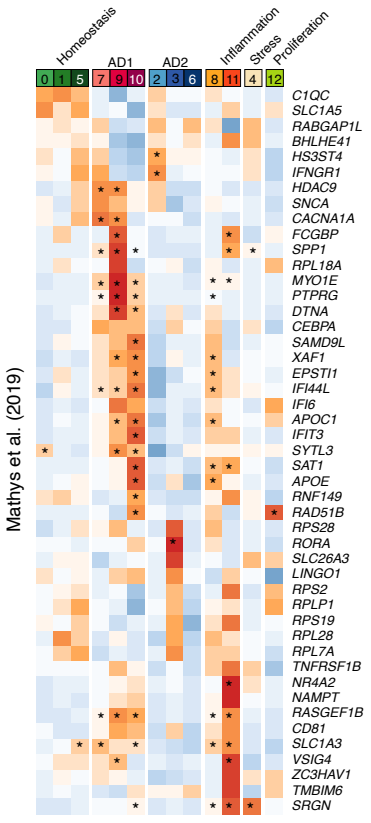
Figure S7


a
Amyloid-β
Tau
% of positive pixels
OC
OTC
CTR-
CTR+
AD
300 um
Black-white + thresholding
Fraction of black pixels
b
Cluster #
7
9
10
regulation of blood vessel endothelial cell migration
negative regulation of cytokine production
leukocyte migration
lipid localization
myeloid leukocyte differentiation
phagocytosis
positive regulation of cell migration
positive regulation of cell motility
positive regulation of lipid localization
regulation of lipid localization
angiogenesis
cell chemotaxis
cell–matrix adhesion
cellular response to amyloid–beta
endothelial cell proliferation
leukocyte chemotaxis
lipid transport
neutrophil activation
regulation of neuron death
axon ensheathment
endosomal transport
lipid catabolic process
regulation of autophagy
genes
10
20
30
40
50


c
Homeostasis
AD1
AD2
Inflammation
Stress
Proliferation
0
1
5
7
9
10
2
3
6
8
11
4
12
Amyloid-β
OC
cor = -0.45 p = 0.12 FDR = 0.22
cor = -0.63 p = 0.02 FDR = 0.05
cor = -0.62 p = 0.02 FDR = 0.36
cor = 0.49 p = 0.09 FDR = 0.19
cor = 0.89 p = 0.00 FDR = 0.00
cor = 0.82 p = 0.00 FDR = 0.00
cor = -0.34 p = 0.25 FDR = 0.36
cor = 0.15 p = 0.62 FDR = 0.73
cor = 0.15 p = 0.62 FDR = 0.90
cor = 0.65 p = 0.02 FDR = 0.05
cor = 0.21 p = 0.49 FDR = 0.63
cor = 0.34 p = 0.25 FDR = 0.36
cor = 0.07 p = 0.82 FDR = 0.88
Amyloid-β
OTC
cor = -0.63 p = 0.05 FDR = 0.30
cor = -0.52 p = 0.12 FDR = 0.33
cor = -0.76 p = 0.01 FDR = 0.13
cor = -0.23 p = 0.53 FDR = 0.62
cor = -0.08 p = 0.83 FDR = 0.89
cor = -0.23 p = 0.52 FDR = 0.62
cor = 0.26 p = 0.46 FDR = 0.33
cor = 0.51 p = 0.13 FDR = 0.62
cor = 0.60 p = 0.07 FDR = 0.30
cor = -0.38 p = 0.28 FDR = 0.59
cor = 0.35 p = 0.32 FDR = 0.59
cor = 0.29 p = 0.42 FDR = 0.62
cor = 0.02 p = 0.97 FDR = 0.97
Tau
cor = -0.59 p = 0.07 FDR = 0.15
cor = -0.54 p = 0.11 FDR = 0.17
cor = -0.62 p = 0.05 FDR = 0.15
cor = -0.4 p = 0.25 FDR = 0.29
cor = -0.53 p = 0.11 FDR = 0.17
R² = -0.59 p = 0.07 FDR = 0.15
cor = 0.69 p = 0.03 FDR = 0.15
cor = 0.90 p = 0.00 FDR = 0.00
cor = 0.61 p = 0.06 FDR = 0.15
cor = -0.43 p = 0.22 FDR = 0.28
cor = -0.17 p = 0.64 FDR = 0.64
cor = -0.43 p = 0.22 FDR = 0.28
cor = -0.37 p = 0.29 FDR = 0.31


d
AD2 vs Homeostasis
synapse organization
cell–cell adhesion via plasma–membrane adhesion molecules
regulation of synapse structure or activity
regulation of synapse organization
cell morphogenesis involved in neuron differentiation
modulation of chemical synaptic transmission
axonogenesis
regulation of trans–synaptic signaling
axon development
regulation of neuron projection development
positive regulation of cellular component biogenesis
log(p.adjust)


e
Homeostasis
AD1
AD2
Inflammation
Stress
Proliferation
Row Z–Score
Grubman et al. (2019)
CSF3R
POLN
GSK3B
BCAR3
PTPRG
FAM20C
PARVB
KCNJ16
STARD13
APOE
APOC1
RPS6KA2
ADAMTS18
MS4A6A
RIMBP2
ITGA8
SLMAP
NACA2
ZNF260
SAV1
MAGI3
LRP8


f
Homeostasis
AD1
AD2
Inflammation
Stress
Proliferation
Mathys et al. (2019)
C1QC
SLC1A5
RABGAP1L
BHLHE41
HS3ST4
IFNGR1
HDAC9
SNCA
CACNA1A
FCGBP
SPP1
RPL18A
MYO1E
PTPRG
DTNA
CEBPA
SAMD9L
XAF1
EPSTI1
IFI44L
IFI6
APOC1
IFIT3
SYTL3
SAT1
APOE
RNF149
RAD51B
RPS28
RORA
SLC26A3
LINGO1
RPS2
RPLP1
RPS19
RPL28
RPL7A
TNFRSF1B
NR4A2
NAMPT
RASGEF1B
CD81
SLC1A3
VSIG4
ZC3HAV1
TMBIM6
SRGN

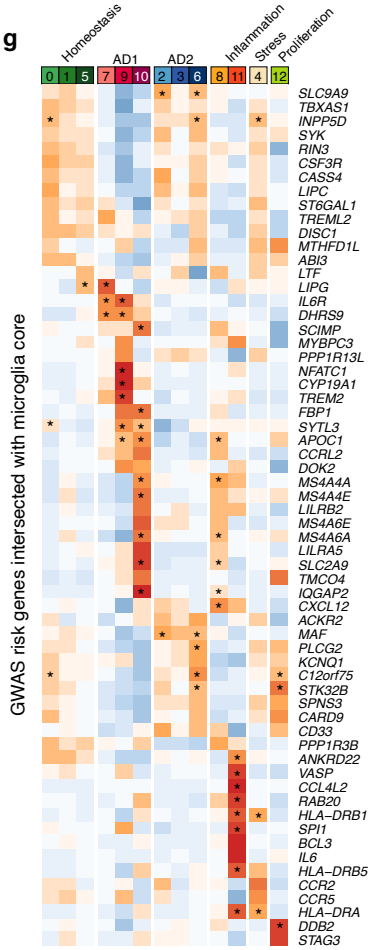
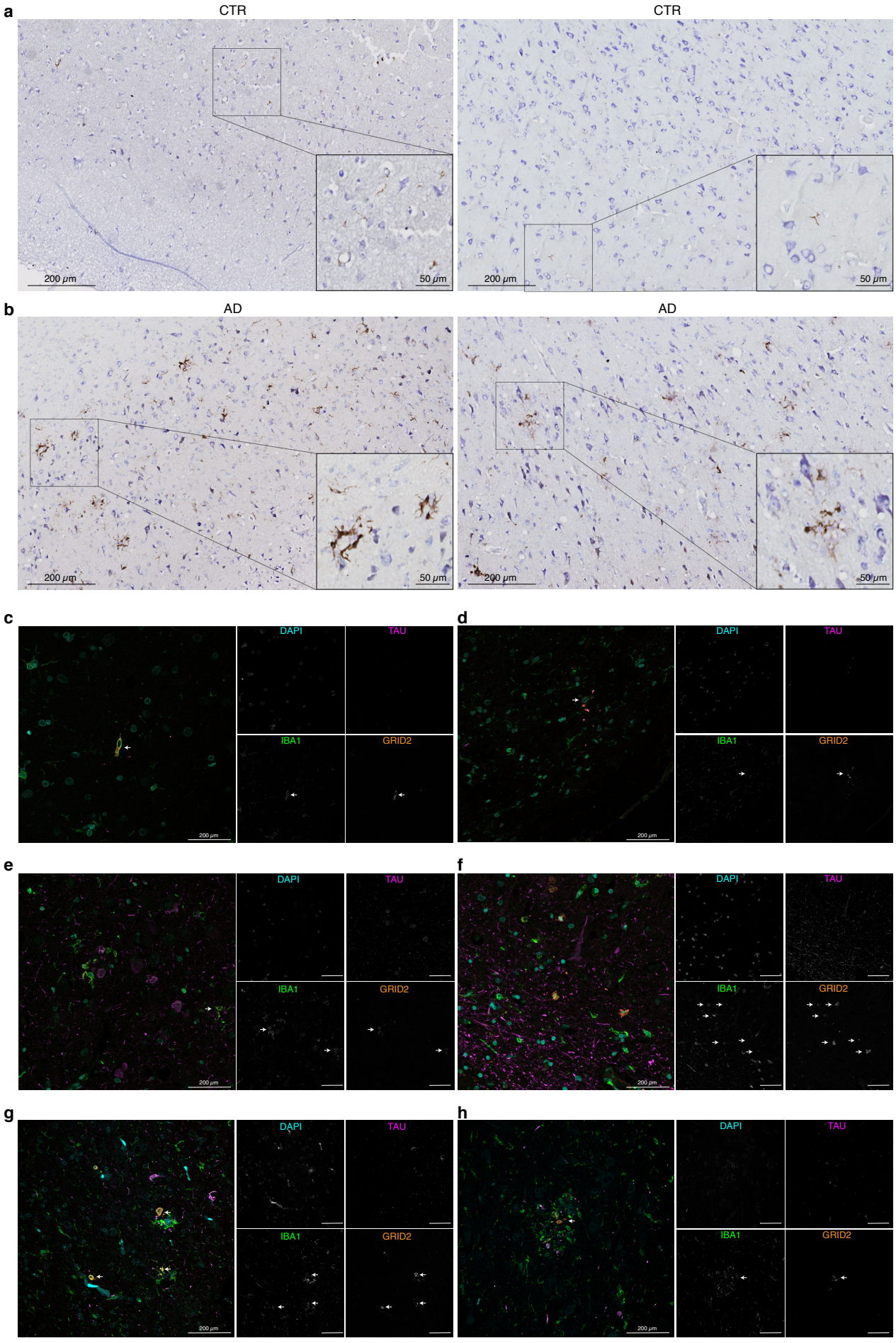

g
Homeostasis
AD1
AD2
Inflammation
Stress
Proliferation
GWAS risk genes intersected with microglia core
SLC9A9
TBXAS1
INPP5D
SYK
RIN3
CSF3R
CASS4
LIPC
ST6GAL1
TREML2
DISC1
MTHFD1L
ABI3
LTF
LIPG
IL6R
DHRS9
SCIMP
MYBPC3
PPP1R13L
NFATC1
CYP19A1
TREM2
FBP1
SYTL3
APOC1
CCRL2
DOK2
MS4A4A
MS4A4E
LILRB2
MS4A6E
MS4A6A
LILRA5
SLC2A9
TMCO4
IQGAP2
CXCL12
ACKR2
MAF
PLCG2
KCNQ1
C12orf75
STK32B
SPNS3
CARD9
CD33
PPP1R3B
ANKRD22
VASP
CCL4L2
RAB20
HLA–DRB1
SPI1
BCL3
IL6
HLA–DRB5
CCR2
CCR5
HLA–DRA
DDB2
STAG3

Figure S8


a
CTR
CTR
200 μm
50 μm
200 μm
50 μm
b
AD
AD
200 μm
50 μm
200 μm
50 μm
c
d
DAPI
TAU
IBA1
GRID2
200 μm
e
f
g
h