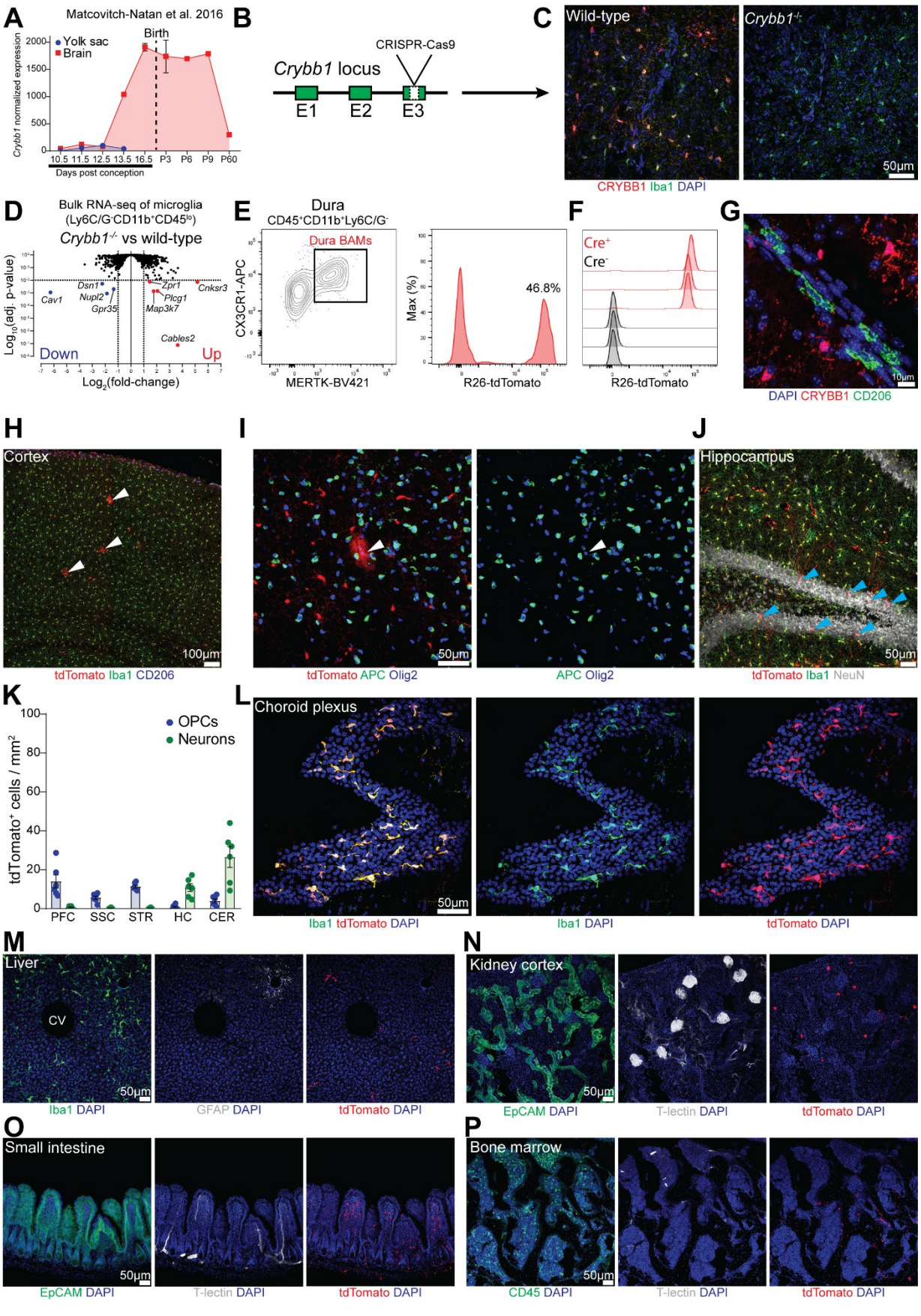
A
Matcovitch-Natan et al. 2016
Yolk sac
Brain
Birth
Crybb1 normalized expression
Days post conception
B
CRISPR-Cas9
Crybb1 locus
E1
E2
E3
C
Wild-type
Crybb1-/-
50μm
CRYBB1 Iba1 DAPI
D
Bulk RNA-seq of microglia
(Ly6C/G-CD11b+CD45lo)
Crybb1-/- vs wild-type
Cav1
Dsn1
Nupl2
Gpr35
Zpr1
Plcg1
Map3k7
Cnksr3
Cables2
Down
Up
Log2(fold-change)
E
Dura
CD45+CD11b+Ly6C/G-
Dura BAMs
CX3CR1-APC
MERTK-BV421
Max (%)
46.8%
R26-tdTomato
F
Cre+
Cre-
R26-tdTomato
G
10μm
DAPI CRYBB1 CD206
H
Cortex
100μm
tdTomato Iba1 CD206
I
50μm
tdTomato APC Olig2
APC Olig2
J
Hippocampus
50μm
tdTomato Iba1 NeuN
K
OPCs
Neurons
tdTomato+ cells / mm2
PFC
SSC
STR
HC
CER
L
Choroid plexus
50μm
Iba1 tdTomato DAPI
Iba1 DAPI
tdTomato DAPI
M
Liver
CV
50μm
Iba1 DAPI
GFAP DAPI
tdTomato DAPI
N
Kidney cortex
50μm
EpCAM DAPI
T-lectin DAPI
tdTomato DAPI
O
Small intestine
50μm
EpCAM DAPI
T-lectin DAPI
tdTomato DAPI
P
Bone marrow
50μm
CD45 DAPI
T-lectin DAPI
tdTomato DAPI

Figure S1. *Crybb1-Cre* produces sparse off-target recombination in OPCs and neurons, Related to Figure 1

(A) Normalized expression of *Crybb1* in yolk sac and brain macrophages at multiple timepoints during development [27].

(B) Cartoon describing the generation of *Crybb1*$^{-/-}$ line.

(C) Representative confocal images of CRYBB1 and Iba1 staining in the brain of wild-type and *Crybb1*$^{-/-}$ mice (n=2 mice/genotype, 2 months old, single experiment).

(D) Bulk RNA-seq of microglia sorted from wild-type and *Crybb1*$^{-/-}$ mice (n=3 mice/genotype, 2-4 months old).

(E) Representative gating of dura BAMs in *Crybb1-Cre : R26-tdTomato* mice (n=3 mice, 8 weeks old, single experiment).

(F) Representative tdTomato expression in microglia from *Crybb1-Cre : R26-tdTomato* and *R26-tdTomato* littermate mice (n=3 mice/genotype, 4 weeks old, single experiment).

(G) Representative confocal image of CRYBB1 and CD206 staining in the brain of wild-type mice (n=2 mice, 2 months old, single experiment).

(H) Representative confocal image of Iba1 and CD206 staining in the brain *Crybb1-Cre : R26-tdTomato* mice (white arrowheads = tdTomato^{+}Iba1^{-}CD206^{-} cells, n=6 mice, 2-3 months old, two independent experiments).

(I) Representative confocal images of APC and OLIG2 staining in the brain *Crybb1-Cre : R26-tdTomato* mice (white arrowheads = tdTomato^{+}OLIG2^{+} cells, n=6 mice, 2-3 months old, two independent experiments).

(J) Representative confocal image of Iba1 and NeuN staining in the dentate gyrus (DG) of *Crybb1-Cre : R26-tdTomato* mice (blue arrowheads = tdTomato^{+}NeuN^{+} cells, n=6 mice, 2-3 months old, two independent experiments).

(K) Number of recombinant OPCs and neurons per mm^2 in prefrontal cortex (PFC), somatosensory cortex (SSC), striatum (STR), hippocampus (HC), and cerebellum (CER) from *Crybb1-Cre : R26-tdTomato* mice (n=6 mice, 2-3 months old, pool of two independent experiments).

(L) Representative confocal image of choroid plexus from *Crybb1-Cre : R26-tdTomato* mice (n=4 mice, 2 months old, single experiment).

(M) Representative confocal image of liver from *Crybb1-Cre : R26-tdTomato* mice (CV= central vein, n=4 mice, 2 months old, single experiment).

(N) Representative confocal image of kidney from *Crybb1-Cre : R26-tdTomato* mice (n=4 mice, 2 months old, single experiment).

(O) Representative confocal image of small intestine from *Crybb1-Cre : R26-tdTomato* mice (n=4 mice, 2 months old, single experiment).

(P) Representative confocal image of femoral bone marrow from *Crybb1-Cre : R26-tdTomato* mice (n=4 mice, 2 months old, single experiment).

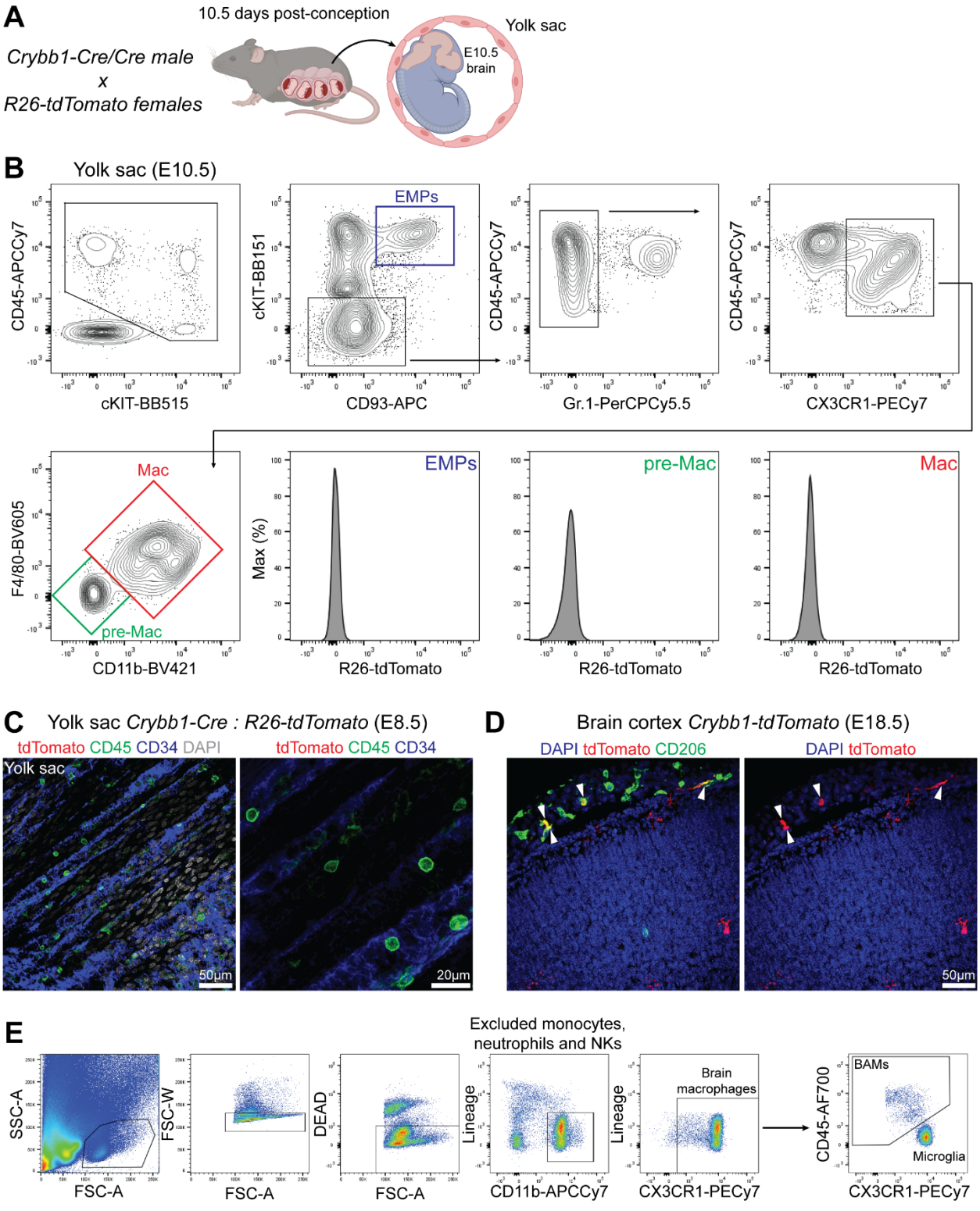
A
10.5 days post-conception
Yolk sac
E10.5 brain
Crybb1-Cre/Cre male
x
R26-tdTomato females
B
Yolk sac (E10.5)
CD45-APCCy7
cKIT-BB515
cKIT-BB151
CD93-APC
EMPs
Gr.1-PerCPCy5.5
CX3CR1-PECy7
F4/80-BV605
CD11b-BV421
Mac
pre-Mac
Max (%)
R26-tdTomato
C
Yolk sac Crybb1-Cre : R26-tdTomato (E8.5)
tdTomato CD45 CD34 DAPI
tdTomato CD45 CD34
Yolk sac
50µm
20µm
D
Brain cortex Crybb1-tdTomato (E18.5)
DAPI tdTomato CD206
DAPI tdTomato
50µm
E
Excluded monocytes, neutrophils and NKs
SSC-A
FSC-A
FSC-W
DEAD
Lineage
CD11b-APCCy7
Brain macrophages
BAMs
Microglia
CD45-AF700

Figure S2. *Crybb1* is expressed in brain macrophages during embryonic development, Related to Figure 2

(A) Cartoon describing the experimental design.

(B) Representative FACS plot of yolk sac EMPs, pre-macrophages (pre-Mac) and macrophages (Mac) in *Crybb1-Cre : R26-tdTomato* E10.5 embryos (n=6 YS, pooled into 3 replicate samples, single experiment).

(C) Representative confocal image of CD45 and CD34 staining in the yolk sac of *Crybb1-Cre : R26-tdTomato* E8.5 embryos (n=5 YS, single experiment).

(D) Representative confocal image of $CD206^{+}$ BAMs in the forebrain cortex of *Crybb1-tdTomato* E18.5 embryos (n=5 embryos, single experiment).

(E) FACS gating strategy for BAMs.

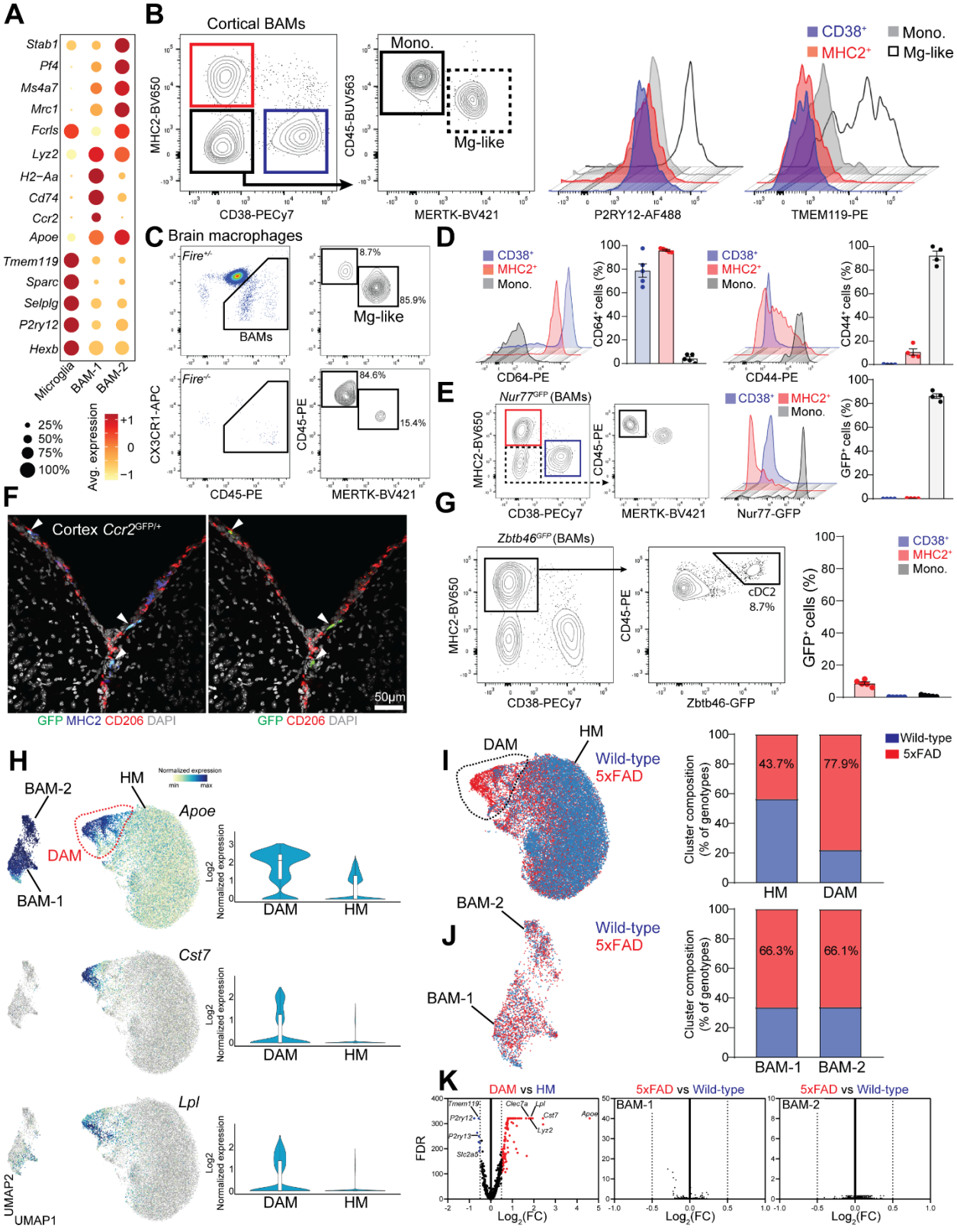
A
Stab1
Pf4
Ms4a7
Mrc1
Fcrls
Lyz2
H2−Aa
Cd74
Ccr2
Apoe
Tmem119
Sparc
Selplg
P2ry12
Hexb
Microglia
BAM-1
BAM-2
25%
50%
75%
100%
Avg. expression
+1
0
−1
B
Cortical BAMs
MHC2-BV650
CD38-PECy7
CD45-BUV563
MERTK-BV421
Mono.
Mg-like
CD38⁺
MHC2⁺
Mono.
Mg-like
P2RY12-AF488
TMEM119-PE
C
Brain macrophages
Fire+/-
BAMs
8.7%
85.9%
Mg-like
Fire-/-
CX3CR1-APC
CD45-PE
84.6%
15.4%
MERTK-BV421
D
CD38⁺
MHC2⁺
Mono.
CD64-PE
CD64⁺ cells (%)
CD44-PE
CD44⁺ cells (%)
E
Nur77GFP (BAMs)
MHC2-BV650
CD38-PECy7
CD45-PE
MERTK-BV421
Nur77-GFP
GFP⁺ cells (%)
F
Cortex Ccr2GFP/+
50μm
GFP MHC2 CD206 DAPI
GFP CD206 DAPI
G
Zbtb46GFP (BAMs)
MHC2-BV650
CD38-PECy7
CD45-PE
cDC2
8.7%
Zbtb46-GFP
GFP⁺ cells (%)
H
BAM-2
HM
DAM
BAM-1
Normalized expression
min
max
Apoe
Cst7
Lpl
Log2
Normalized expression
DAM
HM
UMAP2
UMAP1
I
DAM
HM
Wild-type
5xFAD
Wild-type
5xFAD
Cluster composition
(% of genotypes)
43.7%
77.9%
HM
DAM
J
BAM-2
BAM-1
Wild-type
5xFAD
66.3%
66.1%
BAM-1
BAM-2
K
DAM vs HM
5xFAD vs Wild-type
5xFAD vs Wild-type
FDR
Tmem119
P2ry12
P2ry13
Slc2a5
ClecTa
Lpl
Cst7
Lyz2
Apoe
BAM-1
BAM-2
Log2(FC)

Figure S3, BAM subsets are marginally affected in the 5xFAD model of amyloid pathology, Related to Figure 3

(A) Dot-plot of selected signature genes for microglia, BAM-1 and BAM-2 populations.

(B) Representative FACS plot of different BAM subsets stained for P2RY12 and TMEM119 (n=4, 10-week-old, single experiment).

(C) Representative FACS plot of brain monocytes and Mg-like populations in *Fire*$^{+/-}$ and *Fire*$^{-/-}$ mice (n=2, 3-month-old, single experiment).

(D) Representative FACS plot and quantification of different BAM subsets stained for CD64 or CD44 (n=5-4 mice, 2-month-old, single experiment).

(E) Representative FACS plot and quantification of *Nur77 (Nr4a1)* expression in different BAM subsets from *Nur77*^{GFP} mice (n=4 mice, 2-month-old, single experiment)

(F) Representative confocal image of CD206 and MHC2 staining in the brain of *Ccr2*^{GFP} mice (n=5, 3-month-old, single experiment).

(G) Representative FACS plot and quantification of *Zbtb46* expression in different BAM subsets from *Zbtb46*^{GFP} mice (n=5 mice, 10-week-old, single experiment)

(H) UMAP plot of microglia and BAMs from wild-type and 5xFAD littermate mice. Distinct signature of the DAM cluster displayed by violin plots (n=4-5 mice/genotype, 8-month-old).

(I) Genotype composition of HM and DAM clusters.

(J) Genotype composition BAM-1 and BAM-2 clusters.

(K) Differential gene expression analysis of DAM vs HM, and 5xFAD vs wild-type for BAM-1 and BAM-2 clusters.

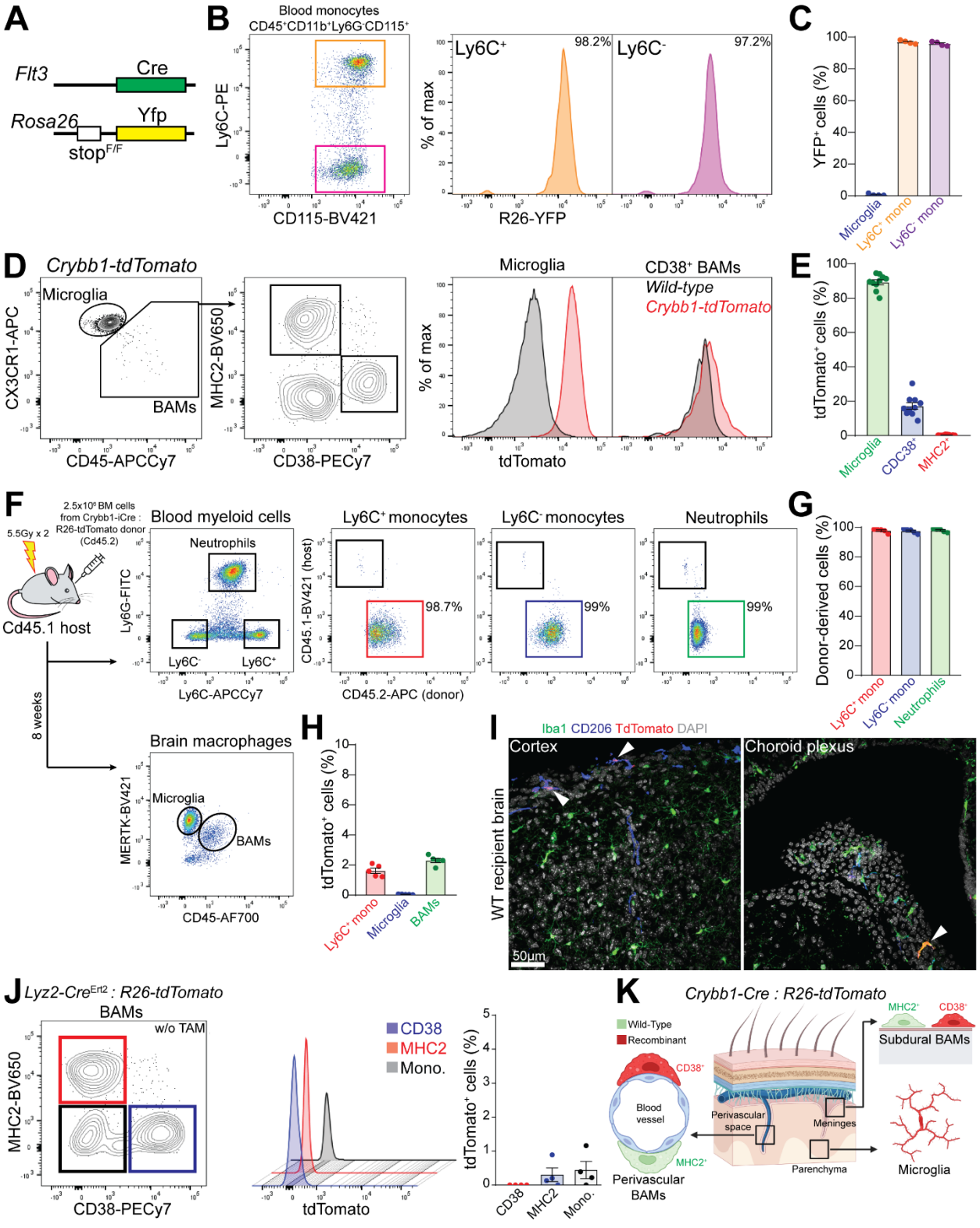
A
Flt3
Cre
Rosa26
Yfp
stop F/F
B
Blood monocytes
CD45+CD11b+Ly6G-CD115+
Ly6C-PE
CD115-BV421
Ly6C+
98.2%
Ly6C-
97.2%
% of max
R26-YFP
C
YFP+ cells (%)
Microglia
Ly6C+ mono
Ly6C- mono
D
Crybb1-tdTomato
Microglia
BAMs
CX3CR1-APC
CD45-APCCy7
MHC2-BV650
CD38-PECy7
Microglia
CD38+ BAMs
Wild-type
Crybb1-tdTomato
% of max
tdTomato
E
tdTomato+ cells (%)
Microglia
CDC38+
MHC2+
F
2.5x10⁶ BM cells
from Crybb1-iCre :
R26-tdTomato donor
(Cd45.2)
5.5Gy x 2
Cd45.1 host
8 weeks
Blood myeloid cells
Neutrophils
Ly6G-FITC
Ly6C-
Ly6C+
Ly6C-APCCy7
Ly6C+ monocytes
CD45.1-BV421 (host)
98.7%
CD45.2-APC (donor)
Ly6C- monocytes
99%
Neutrophils
99%
Brain macrophages
Microglia
BAMs
MERTK-BV421
CD45-AF700
G
Donor-derived cells (%)
Ly6C+ mono
Ly6C- mono
Neutrophils
H
tdTomato+ cells (%)
Ly6C+ mono
Microglia
BAMs
I
Iba1 CD206 TdTomato DAPI
Cortex
Choroid plexus
WT recipient brain
50µm
J
Lyz2-Cre Ert2 : R26-tdTomato
BAMs
w/o TAM
MHC2-BV650
CD38-PECy7
CD38
MHC2
Mono.
tdTomato
tdTomato+ cells (%)
CD38
MHC2
Mono.
K
Crybb1-Cre : R26-tdTomato
Wild-Type
Recombinant
CD38+
Blood vessel
MHC2+
Perivascular BAMs
Perivascular space
Meninges
Parenchyma
MHC2+
CD38+
Subdural BAMs
Microglia

Figure S4. *Crybb1-Cre* does not recombine in bone-marrow derived BAMs, Related to Figure 4

(A) Cartoon describing the generation of the *Flt3-Cre : R26-Yfp* line.

(B) Representative FACS plot of blood monocytes from *Flt3-Cre : R26-Yfp* mice.

(C) Percentage of YFP^+ monocytes and microglia from *Flt3-Cre : R26-Yfp* mice (n=4 mice, 3-months-old, single experiment).

(D) Representative FACS plot of microglia and BAMs from *Crybb1-tdTomato* mice.

(E) Percentage of $tdTomato^+$ microglia and BAMs from *Crybb1-tdTomato* mice (n=10 mice, 2-months-old, pool of three independent experiments).

(F) Representative FACS plot of blood myeloid cells and brain macrophages in *Cd45.1* Bl6 recipient mice, reconstituted with $2.5x10^6$ *Crybb1-Cre : R26-tdTomato* BM cells. Mice analyzed 8 weeks after transplant.

(G) Percentage of donor-derived ($CD45.2^+$) blood myeloid cells 8 weeks after transplantation of *Crybb1-Cre : R26-tdTomato* BM (n=5 mice, 4-month-old, single experiment).

(H) Percentage of $tdTomato^+$ blood monocytes, microglia, and BAMs 8 weeks after transplantation of *Crybb1-Cre : R26-tdTomato* BM (n=5 mice, 4-month-old, single experiment).

(I) Representative confocal image of Iba1 and CD206 staining in the brain of *Cd45.1* Bl6 recipient mice 8 weeks after transplantation of *Crybb1-Cre : R26-tdTomato* BM (n=3 mice, 4-month-old, single experiment).

(J) Representative FACS plot and percentage of recombination in different BAM subsets from *Lyz2*^{CreErt2} *: R26-tdTomato* mice under TAM-free condition (n=4 mice, 2-3-month-old, single experiment).

(K) Cartoon summarizing the recombination pattern in *Crybb1-Cre : R26-tdTomato* mice.

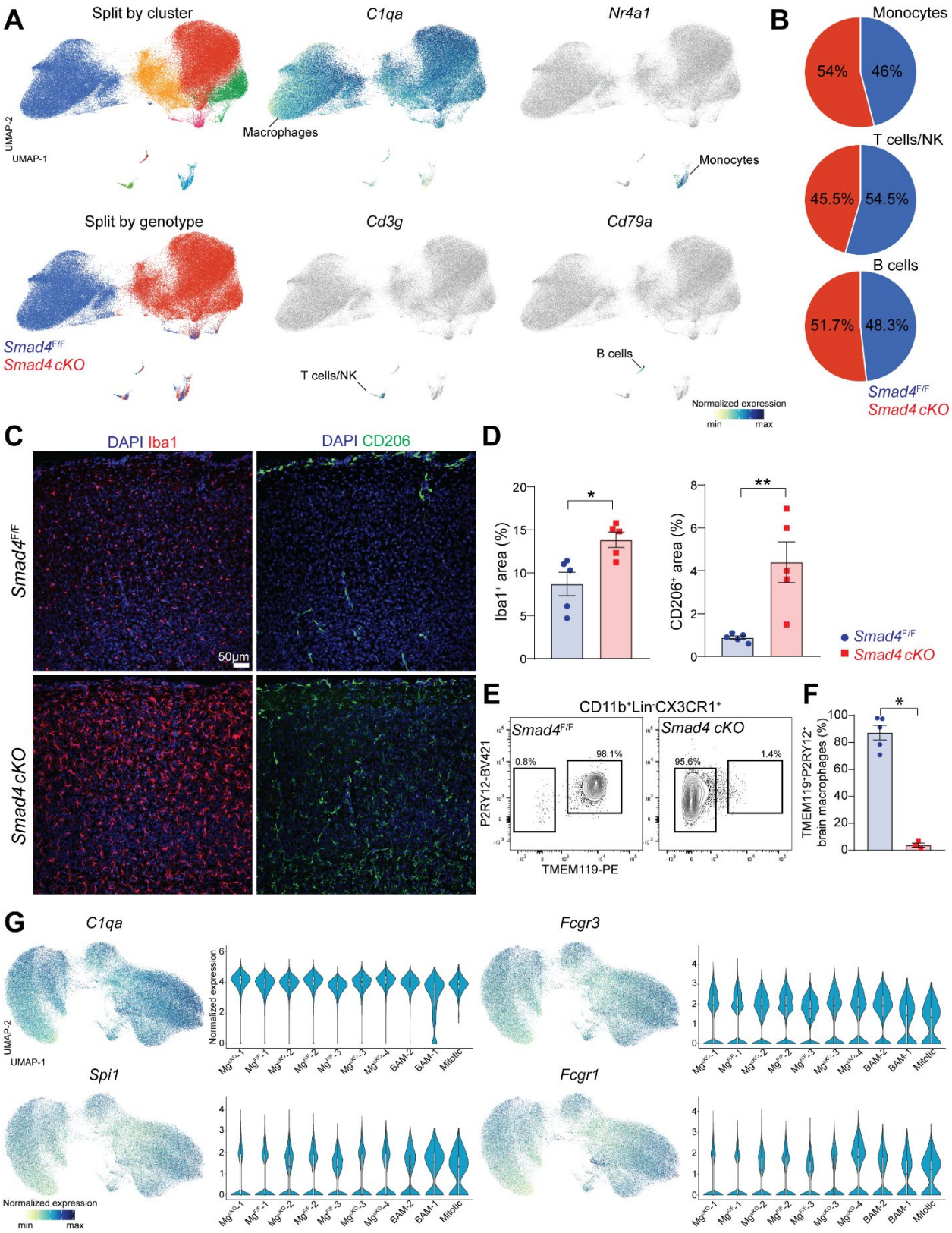
A
Split by cluster
C1qa
Nr4a1
UMAP-2
UMAP-1
Macrophages
Monocytes
Split by genotype
Cd3g
Cd79a
Smad4F/F
Smad4 cKO
T cells/NK
B cells
Normalized expression
min
max
B
Monocytes
54%
46%
T cells/NK
45.5%
54.5%
B cells
51.7%
48.3%
Smad4F/F
Smad4 cKO
C
DAPI Iba1
DAPI CD206
Smad4F/F
Smad4 cKO
50μm
D
Iba1+ area (%)
CD206+ area (%)
*
**
Smad4F/F
Smad4 cKO
E
CD11b+Lin-CX3CR1+
Smad4F/F
Smad4 cKO
0.8%
98.1%
95.6%
1.4%
P2RY12-BV421
TMEM119-PE
F
TMEM119+P2RY12+ brain macrophages (%)
*
G
C1qa
Fcgr3
Spi1
Fcgr1
Normalized expression
min
max
Mgfl/fl-1
Mgfl/fl-2
Mgfl/fl-3
Mgko-1
Mgko-2
Mgko-3
Mgko-4
BAM-2
BAM-1
Mitotic

Figure S5. SMAD4-deficient microglia exhibit a loss of homeostatic signature; however, the macrophage core signature is not affected, Related to Figure 5

(A) UMAP plot of $CD45^{+}Ly6G^{-}$ brain immune cells from *Smad4*$^{F/F}$ and *Smad4 cKO* littermate mice, split either by cluster or genotype. Enrichment of chosen lineage marker genes is displayed (n=4 mice/genotype, 6-8 weeks old).

(B) Genotype composition of monocytes, T/NK cells and B cells clusters.

(C) Representative confocal images of Iba1 and CD206 staining in the brain of *Smad4*$^{F/F}$ and *Smad4 cKO* mice.

(D) Percentage of Iba1 and CD206 covered areas in the brain of *Smad4*$^{F/F}$ and *Smad4 cKO* mice (n=5 mice/genotype, 4-week-old, displayed mean values of three sections per mouse, Mann-Whitney U test, *P<0.05, **P<0.01, pool of two independent experiments)

(E) Representative FACS plot of P2RY12 and TMEM119 staining in brain macrophages from *Smad4*$^{F/F}$ and *Smad4 cKO* mice.

(F) Percentage of $P2RY12^{+}TMEM119^{+}$ brain macrophages from *Smad4*$^{F/F}$ and *Smad4 cKO* littermate mice (n=3-5 mice/genotype, 4-week-old, Mann-Whitney U test, *P<0.05, single experiment).

(G) UMAP and violin plots displaying the expression of macrophage lineage genes in microglia and BAMs from *Smad4*$^{F/F}$ and *Smad4 cKO* mice.

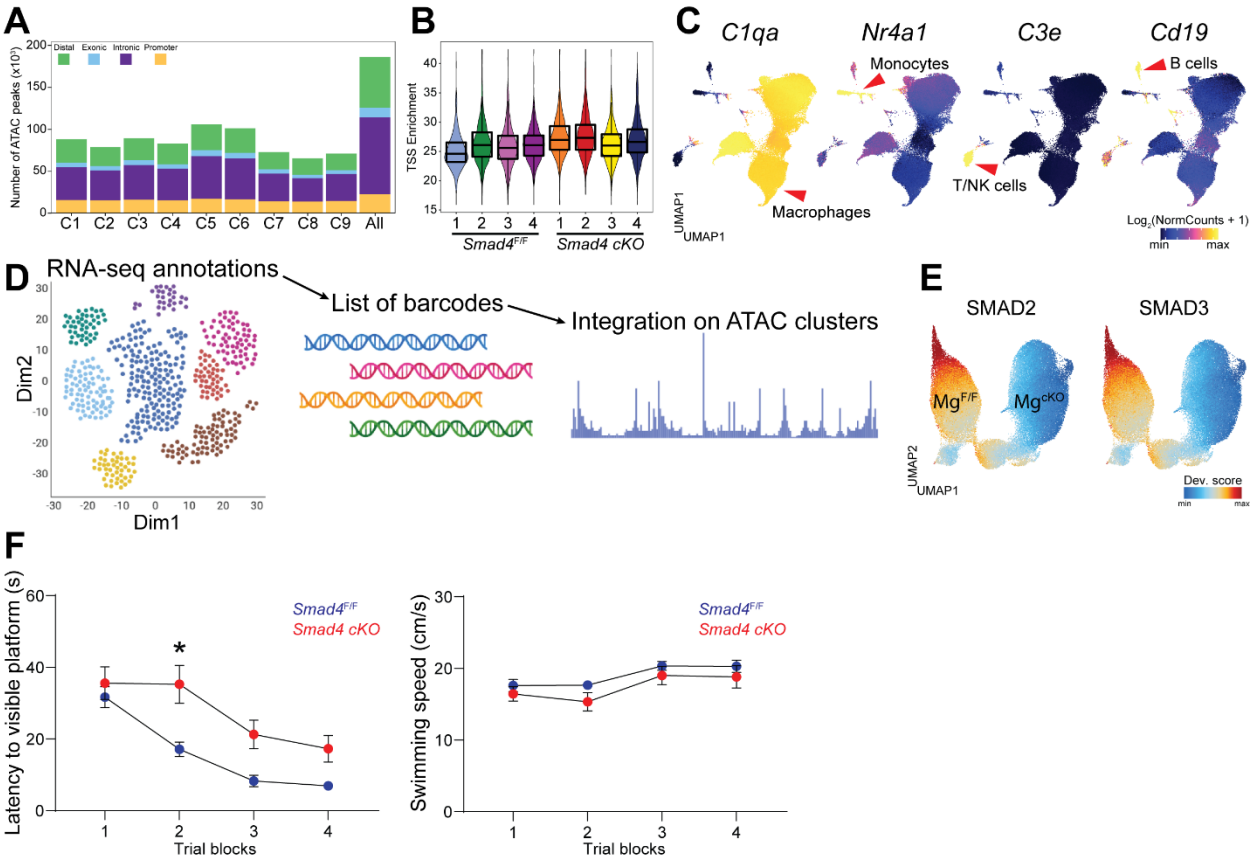
A
Distal
Exonic
Intronic
Promoter
Number of ATAC peaks (x10³)
C1 C2 C3 C4 C5 C6 C7 C8 C9 All
B
TSS Enrichment
Smad4F/F
Smad4 cKO
C
C1qa
Nr4a1
C3e
Cd19
Monocytes
B cells
T/NK cells
Macrophages
UMAP1
Log2(NormCounts + 1)
min
max
D
RNA-seq annotations
List of barcodes
Integration on ATAC clusters
Dim2
Dim1
E
SMAD2
SMAD3
MgF/F
MgcKO
UMAP2
UMAP1
Dev. score
F
Latency to visible platform (s)
Trial blocks
Swimming speed (cm/s)
Smad4F/F
Smad4 cKO

Figure S6. scATAC-seq recapitulates the same populations identified by scRNA-seq and reveals altered enrichment of TFs binding motifs in SMAD4-deficient microglia, Related to Figure 6

(A) Number of peaks called for each cluster.

(B) Number of fragments per cell and enrichment of Tn5 insertions in transcription start sites (TSS) for each sample.

(C) scATAC-seq profiles colored by accessibility of the indicated gene (red arrowhead = cell population).

(D) Cartoon describing the scATAC-seq annotation strategy

(E) UMAP of the scATAC-seq profiles colored by enrichment of the SMAD2/3 motifs.

(F) Morris Water Maze test on *Smad4*$^{F/F}$ and *Smad4 cKO* littermate mice assessing latency time to visible platform and swimming speed (n=14 *Smad4*$^{F/F}$ and n=8 *Smad4 cKO*, 3-4-month-old, two-way ANOVA with Bonferroni post-hoc test, *P<0.05, pool of three independent experiments).