Figure S1. Conditional deletion of *Syk* in microglia impairs Aβ plaque-induced microgliosis in *5xFAD* mice, Related to Figure 1

(A and B) FACS analysis detecting SYK expression in distinct cell types from 9-month-old $Syk^{fl/fl}$ and $Syk^{i\Delta MG}$ mice (representative of two independent experiments; n = 4-6 mice/genotype).

(C) Analysis of Iba1 intensity profiles in the cortex of 9-month-old $Syk^{i\Delta MG}$-*5xFAD* and $Syk^{fl/fl}$-*5xFAD* mice.

(D-F) Quantification of the number of microglia per mm^2, as well as the percentage of methoxy-X04$^+$ and Iba1$^+$ areas within the cortex. Each data point represents the average of three technical repeats per mouse (one experiment; n = 6-8 mice/genotype).

(G) Representative confocal images of Iba1, methoxy-X04 and PU.1 in $Syk^{i\Delta MG}$-*5xFAD* mice and $Syk^{fl/fl}$-*5xFAD* mice.

(H-I) Percentage of microglia closer or farther than 20-μm from the plaques border in the analyzed genotypes. Each data point represents the average of three technical repeats per mouse (one experiment; n = 6-8 mice/genotype).

(J) Representative confocal images of Iba1 and PU.1 staining in 9-month-old $Syk^{fl/fl}$ and $Syk^{i\Delta MG}$ mice.

(K-L) Percentage of Iba1$^+$ area, and number of microglia per mm^2 in the two genotypes. Each data point represents the average of three technical repeats per mouse (one experiment; n = 4 mice/genotype).

*, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$ by two-tailed unpaired Student's t test. Data are presented as mean ± SEM.

Figure S2. SYK deficiency in microglia exacerbates AD pathology, Related to Figure 2

(A) Representative confocal images of Iba1, methoxy-X04 and APP in the cortex of 9-month-old *Syk*$^{fl/fl}$*-5xFAD* and *Syk*$^{i\Delta MG}$*-5xFAD* mice. White asterisks indicate the positions of plaques in the zoom-in view.

(B) Number of dystrophic neurites per plaque in 6- and 9-month-old mice from each genotype. Each data point represents the average of two technical repeats per mouse (one experiment; n = 5-9 mice/genotype).

(C) Representative confocal images of 6E10 and methoxy-X04 staining in the cortex from 9-month-old *Syk*$^{fl/fl}$*-5xFAD* mice.

(D) Percentages of filamentous, inert, and dynamic plaques for each genotype in 6- and 9-month-old mice. A total of 1,890 and 1706 plaques were analyzed in 6- and 9-month-old mice, respectively. Each data point represents the average of two technical repeats per mouse (one experiment; n = 5-9 mice/genotype).

(E) Morris water maze test; latency time to visible platform in 6- and 9-month-old *Syk*$^{fl/fl}$ and *Syk*$^{i\Delta MG}$ mice with or without *5xFAD* transgene.

(F) Elevated plus maze test; total traveled distance in 6- and 9-month-old *Syk*$^{fl/fl}$ and *Syk*$^{i\Delta MG}$ mice with or without *5xFAD* transgene.

Sample size of behavioral tests: 6-month-old *Syk*$^{fl/fl}$ (n=6), *Syk*$^{i\Delta MG}$ (n=7), *Syk*$^{fl/fl}$*-5xFAD* (n=12) and *Syk*$^{i\Delta MG}$*-5xFAD* (n=11), as well as 9-month-old *Syk*$^{fl/fl}$ (n=7), *Syk*$^{i\Delta MG}$ (n=6), *Syk*$^{fl/fl}$*-5xFAD* (n=6) and *Syk*$^{i\Delta MG}$*-5xFAD* (n=9) mice. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ by two-way ANOVA with Sidak's multiple comparisons test. Data are presented as mean ± SEM.

Figure S3. Defect in SYK augments autophagy and lipid accumulation in microglia of *5xFAD* mice, Related to Figure 3

(A) Gating strategy used to analyze and/or sort microglia for immunoblotting, Mitotracker Green staining, and TEM analysis. A representative flow cytometry gating strategy for a 6-month-old *Syk*$^{fl/fl}$*-5xFAD* mouse is shown.

(B) Immunoblots for SYK, AKT, pAKT (serine 473), S6K and pS6K from primary *Syk*$^{fl/fl}$ and *Syk*$^{c\Delta MG}$ microglia cultured with or without 10% LCCM. Representative of two independent experiments.

(C) Zoom-in view of LC3 staining in microglia, related to **Figure 3F**. White arrows indicate the LC3^{+} puncta in individual microglia around plaques.

(D) Representative TEM images of lipid accumulation in microglia sorted from 9-month-old of *Syk*$^{i\Delta MG}$ mice with or without *5xFAD* transgene. N indicates cell nucleus.

(E) Average number of large lipid droplets per microglia of each genotype. 30 cells from each genotype were analyzed. Each data point represents one microglia (n = 30 cells/genotype).

*, $P < 0.05$ by two-way ANOVA with Sidak's multiple comparisons test. Data are presented as mean ± SEM.

Figure S4. SYK is required for maintenance of microglial clustering around Aβ plaques in *5xFAD* mice

(A) Schematic of the experimental design.

(B) Representative confocal images of Iba1, TO-PRO3 and methoxy-X04 staining in the cortex and hippocampus of 5- and 9-month-old *Syk$^{fl/fl}$-5XFAD* and *Syk$^{i\Delta MG}$-5xFAD* mice. White circles indicate the contours of the plaques.

(C and D) Quantification of microglia density within a 15-μm radius from the plaque surfaces and Iba1/methoxy-X04 colocalization volume, in either cortex or hippocampus of 5- and 9- month-old mice of each genotype. Each data point represents the average of two technical repeats (one experiment; n = 4-8 mice/genotype).

(E) Representative confocal images of Iba1, methoxy-X04 and PU.1 staining in 5- and 9-month-old *Syk$^{fl/fl}$-5XFAD* and *Syk$^{i\Delta MG}$-5xFAD* mice.

(F) Number of microglia per mm^2 in cortex and hippocampus in *Syk$^{fl/fl}$-5XFAD* and *Syk$^{i\Delta MG}$-5xFAD* mice at 5 and 9- months. Data points represent the average of two technical repeats. Sample size: 5-month-old *Syk$^{fl/fl}$-5xFAD* (n=6), 5-month-old *Syk$^{i\Delta MG}$-5xFAD* (n=4), 9-month-old *Syk$^{fl/fl}$-5xFAD* (n=6) and 9-month-old *Syk$^{i\Delta MG}$-5xFAD* (n=8) mice.

*, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.001 by two-way ANOVA with Sidak's multiple comparisons test. Data are presented as mean ± SEM.

Figure S5. Identification of microglia clusters from scRNA-seq of CD45^{+} cells, Related to Figure 4

(A) UMAP plot showing all CD45^{+} cells sorted from *Syk$^{fl/fl}$*, *Syk$^{i\Delta MG}$*, *Syk$^{fl/fl}$-5xFAD* and *Syk$^{i\Delta MG}$-5xFAD* brains.

(B) Frequency of all identified immune populations in each genotype. Each data point represents one mouse.

(C) UMAPs of re-clustered microglia, split by individual samples are also shown.

(D) Gene expression heatmap showing the top enriched genes for each microglia cluster. The number of cells per cluster is denoted above the cluster label.

(E) UMAP plots showing the enrichment of selected markers genes.

(F-H) Violin plots showing the expression levels of *Tmem119*, *Trem2* and *Clec7a* in each cluster.

Figure S6. SYK deficiency in microglia prevents microglial activation and impairs cellular energetic metabolism in *5xFAD* mice, Related to Figure 4

(A) Violin plots of normalized *Trem2* expression in combined TM1, TM2 and DAM clusters from *Syk*$^{fl/fl}$*-5xFAD* and *Syk*$^{i\Delta MG}$*-5xFAD* mice.

(B) Representative confocal images of Iba-1, methoxy-X04, and Trem2 staining in the cortex of 6-month-old *Syk*$^{fl/fl}$*-5xFAD* and *Syk*$^{i\Delta MG}$*-5xFAD* mice.

(C) Mean intensity of TREM2 staining in Iba-1^{+} voxels. Each data point represents the average of two technical repeats (one experiment; n = 6-8 mice/genotype).

(D) Representative confocal images of Iba1, Tmem119 and Clec7a in *Syk*$^{fl/fl}$*-5xFAD* and *Syk*$^{i\Delta MG}$*-5xFAD* mice.

(E and F) Percentage of Clec7a^{+} and Tmem119^{+} areas in 6-month-old *Syk*$^{fl/fl}$*-5xFAD* and *Syk*$^{i\Delta MG}$*-5xFAD* mice. Each data point represents the average of two technical repeats (one experiment; n = 6-8 mice/genotype).

(G) Reconstruction and Sholl analysis of non-plaque associated microglia from 6-month-old *Syk*$^{fl/fl}$*-5xFAD* and *Syk*$^{i\Delta MG}$*-5xFAD* mice (5 cells/brain section; 2 brain sections/mice; n = 6-8 mice/genotype).

(H) Top 15 enriched pathways in *Syk*$^{fl/fl}$*-5xFAD* compared to *Syk*$^{i\Delta MG}$*-5xFAD* mice. GSEA analysis performed on combined TM1, TM2 and DAM clusters. Energetic metabolism related pathways are highlighted in red.

(I) Heatmap of DEGs involved in oxidative phosphorylation, cholesterol homeostasis, glycolysis and mTORC1 signaling pathways.

Figure S7. Assessment of Aβ plaques after short-term treatment with anti-CLEC7A, Related to Figure 7

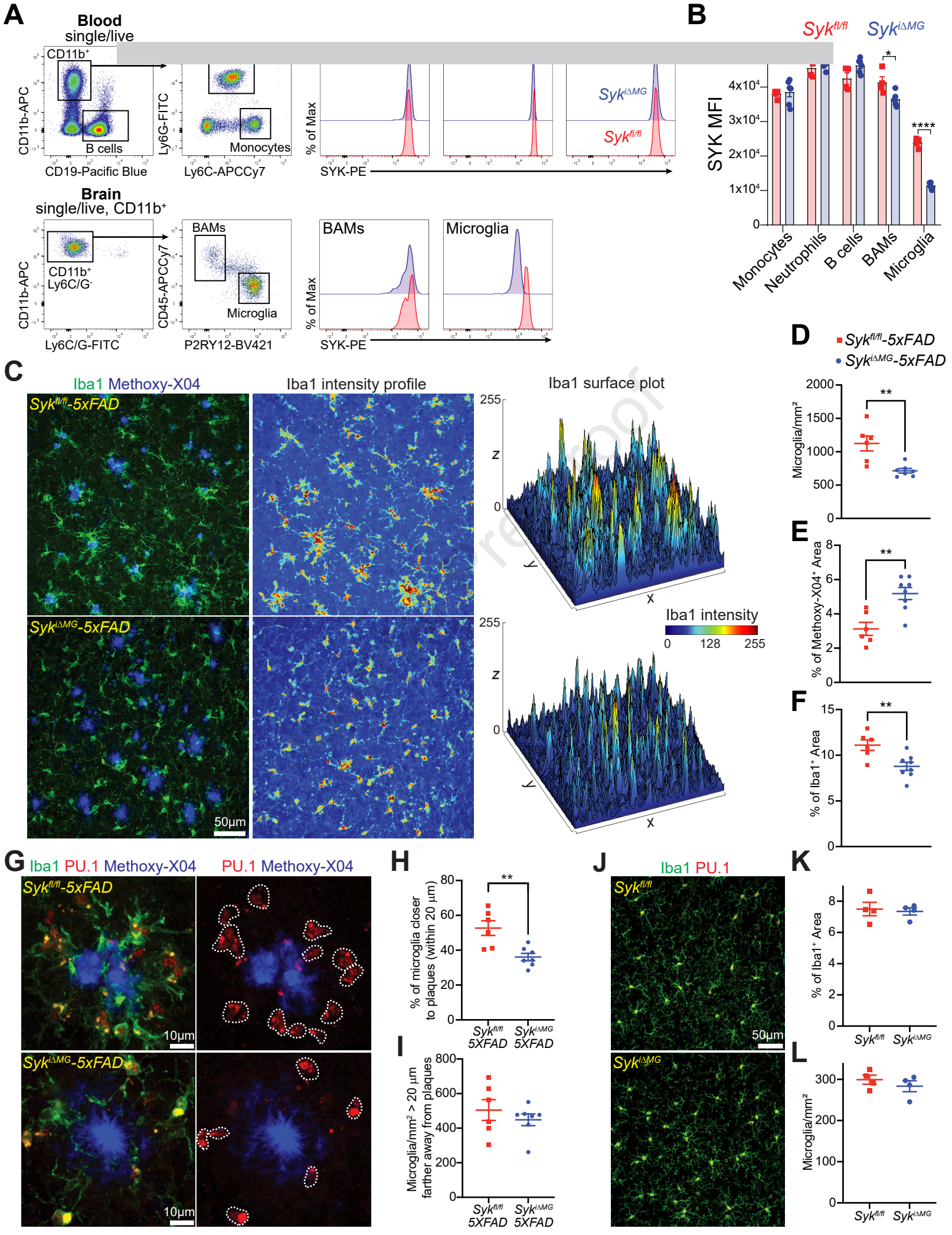
(A) Representative images of $A\beta_{1-42}$ staining in 8-month-old *TREM2^{R47H}-5xFAD* mice treated with 2A11 or isotype control.

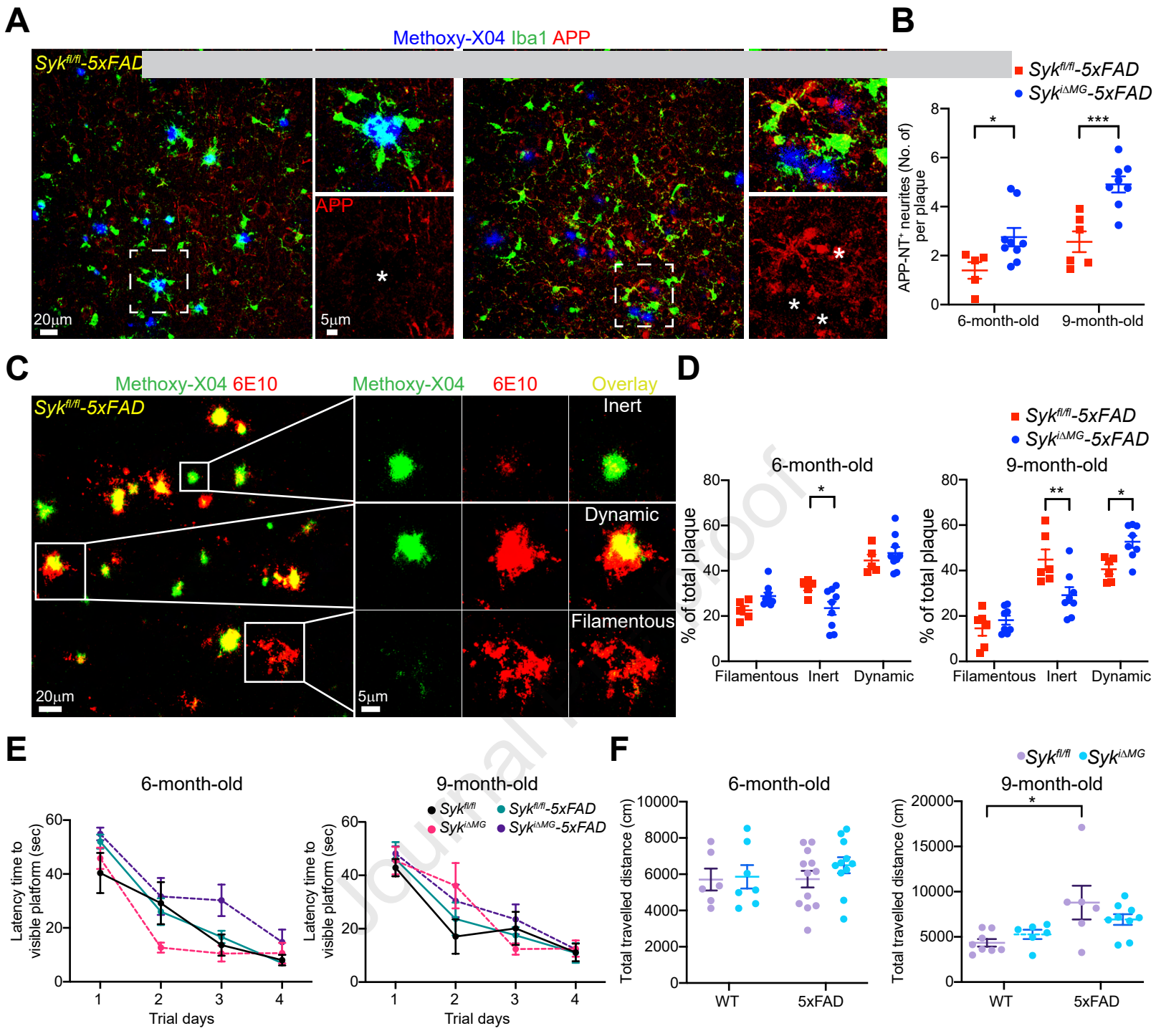
(B) Percentage of $A\beta_{1-42}$ stained areas in cortex and hippocampus of 8-month-old *TREM2^{R47H}-5xFAD* mice receiving either 2A11 or isotype control. Each data point represents the average of two technical repeats (one experiment, n = 6 mice/group).

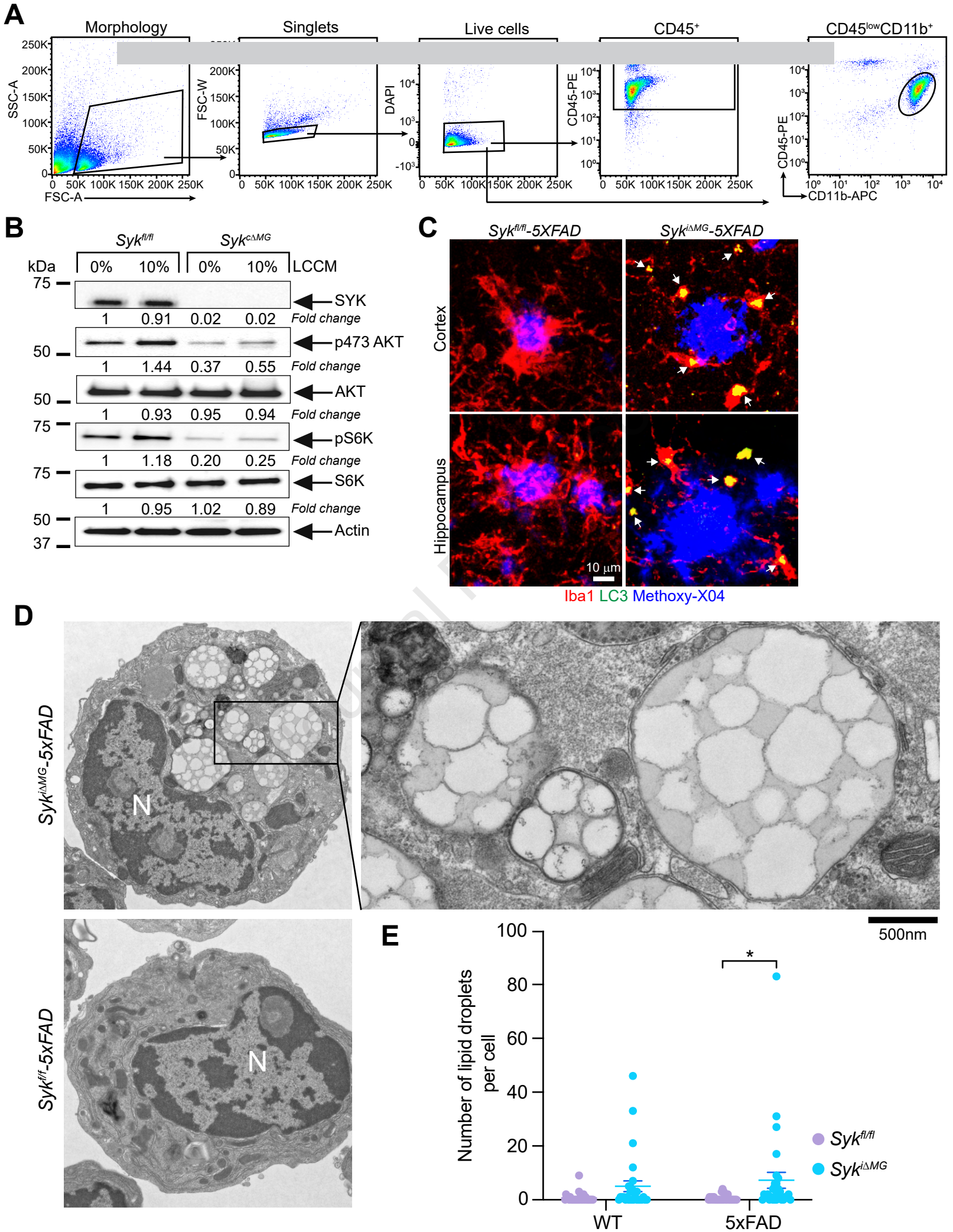
(C) Representative confocal images of filamentous, dynamic, and inert plaques in the cortex of 8-month-old *TREM2^{R47H}-5xFAD* mice treated with 2A11 or isotype control.

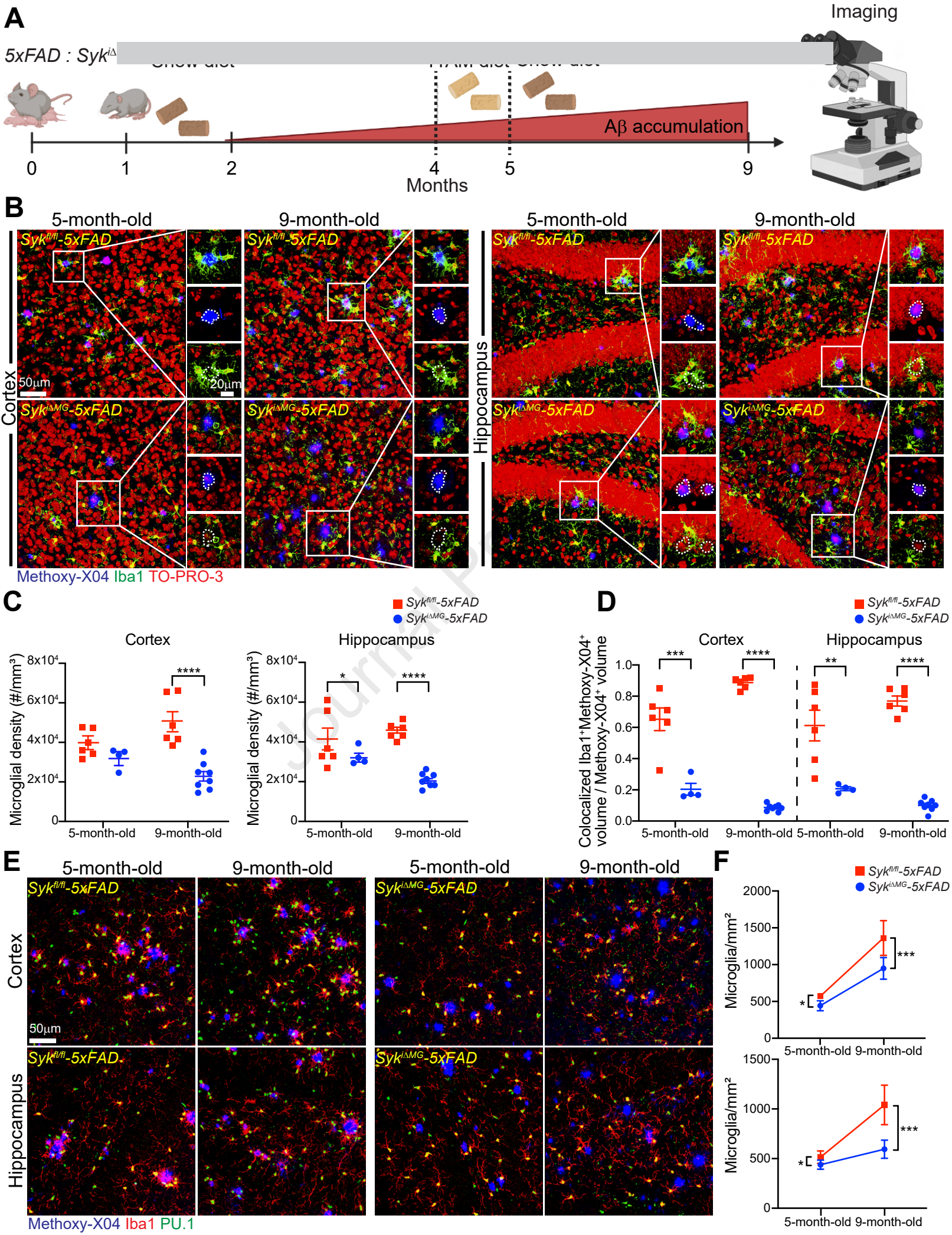
(D) Percentages of filamentous, inert, and dynamic plaques in 2A11 or isotype control treatments. A total of 649 and 788 plaques were analyzed in 2A11 and isotype control treated mice, respectively. Each data point represents the average of two technical repeats (one experiment; n = 6 mice/group).

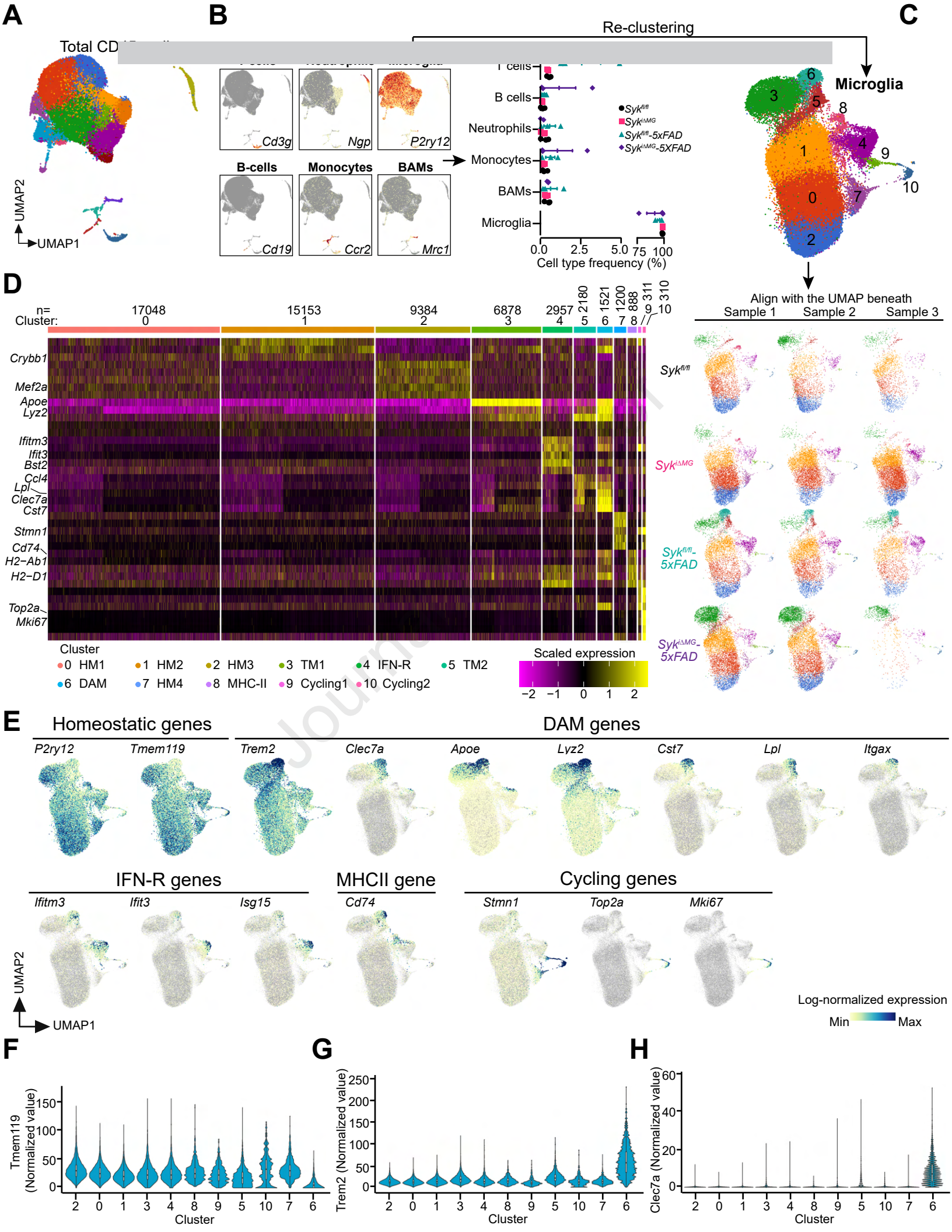
*, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$ by two-way ANOVA with Sidak's multiple comparisons test. Data are presented as mean ± SEM.

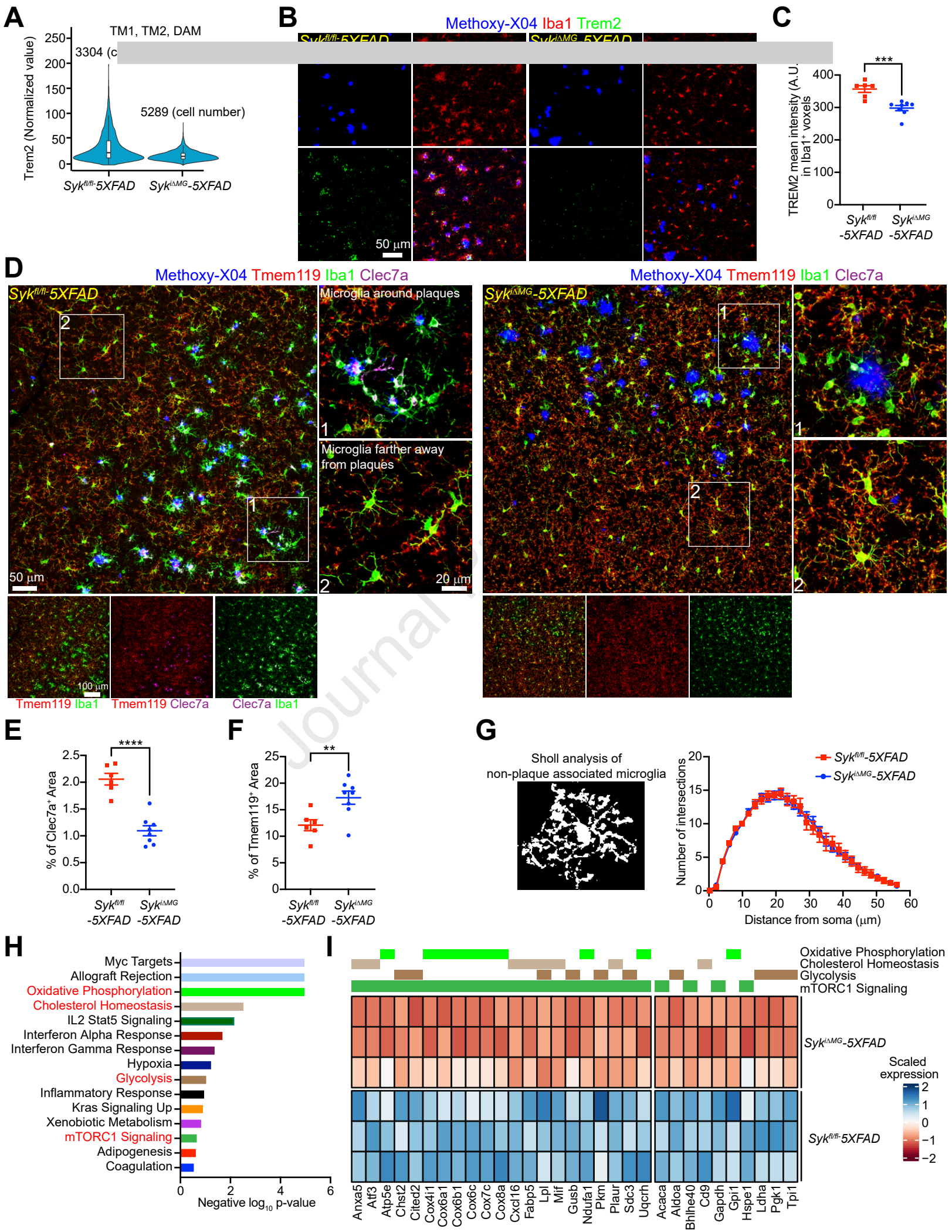

A
Blood
single/live
CD11b+
CD11b-APC
B cells
CD19-Pacific Blue
Ly6G-FITC
Monocytes
Ly6C-APCCy7
% of Max
SYK-PE
Sykfl/fl
SykiΔMG
Brain
single/live, CD11b+
CD11b+
Ly6C/G-
Ly6C/G-FITC
CD45-APCCy7
BAMs
Microglia
P2RY12-BV421
B
Sykfl/fl
SykiΔMG
SYK MFI
4x10^4
3x10^4
2x10^4
1x10^4
0
*
****
Monocytes
Neutrophils
B cells
BAMs
Microglia
C
Iba1 Methoxy-X04
Iba1 intensity profile
Iba1 surface plot
Sykfl/fl-5xFAD
SykiΔMG-5xFAD
50μm
255
z
0
y
x
Iba1 intensity
0
128
255
D
Sykfl/fl-5xFAD
SykiΔMG-5xFAD
Microglia/mm²
2000
1500
1000
500
0
**
E
% of Methoxy-X04+ Area
6
4
2
0
**
F
% of Iba1+ Area
15
10
5
0
**
G
Iba1 PU.1 Methoxy-X04
PU.1 Methoxy-X04
Sykfl/fl-5xFAD
SykiΔMG-5xFAD
10μm
H
% of microglia closer to plaques (within 20 μm)
80
60
40
20
0
**
Sykfl/fl 5XFAD
SykiΔMG 5XFAD
I
Microglia/mm² > 20 μm farther away from plaques
800
600
400
200
0
Sykfl/fl 5XFAD
SykiΔMG 5XFAD
J
Iba1 PU.1
Sykfl/fl
SykiΔMG
50μm
K
% of Iba1+ Area
8
6
4
2
0
Sykfl/fl
SykiΔMG
L
Microglia/mm²
300
200
100
0
Sykfl/fl
SykiΔMG

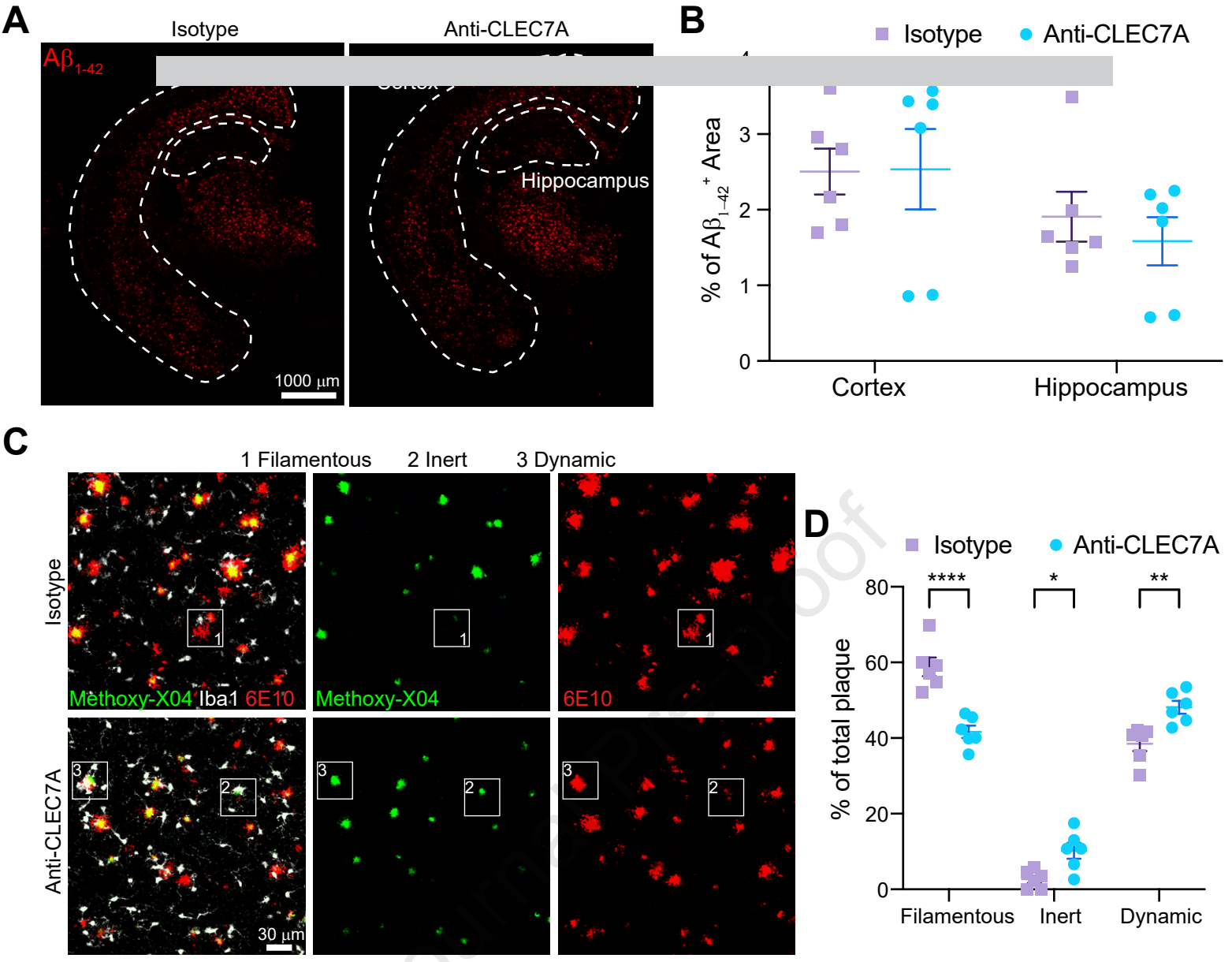

A
Methoxy-X04 Iba1 APP
Sykfl/fl-5xFAD
APP
20μm
5μm
B
Sykfl/fl-5xFAD
SykiΔMG-5xFAD
APP-NT+ neurites (No. of) per plaque
6-month-old
9-month-old
C
Methoxy-X04 6E10
Methoxy-X04
6E10
Overlay
Sykfl/fl-5xFAD
Inert
Dynamic
Filamentous
20μm
5μm
D
Sykfl/fl-5xFAD
SykiΔMG-5xFAD
6-month-old
9-month-old
% of total plaque
Filamentous
Inert
Dynamic
E
6-month-old
9-month-old
Sykfl/fl
SykiΔMG
Sykfl/fl-5xFAD
SykiΔMG-5xFAD
Latency time to visible platform (sec)
Trial days
F
6-month-old
9-month-old
Sykfl/fl
SykiΔMG
Total travelled distance (cm)
WT
5xFAD


A
Morphology
Singlets
Live cells
CD45+
CD45lowCD11b+
SSC-A
FSC-A
FSC-W
DAPI
CD45-PE
CD11b-APC
B
Sykfl/fl
SykcΔMG
kDa
0%
10%
0%
10%
LCCM
75
SYK
1
0.91
0.02
0.02
Fold change
50
p473 AKT
1
1.44
0.37
0.55
Fold change
50
AKT
1
0.93
0.95
0.94
Fold change
75
pS6K
1
1.18
0.20
0.25
Fold change
75
S6K
1
0.95
1.02
0.89
Fold change
50
37
Actin
C
Sykfl/fl-5XFAD
SykiΔMG-5XFAD
Cortex
Hippocampus
10 μm
Iba1 LC3 Methoxy-X04
D
SykiΔMG-5xFAD
Sykf/f-5xFAD
N
N
500nm
E
Number of lipid droplets per cell
100
80
60
40
20
0
*
WT
5xFAD
Sykfl/fl
SykiΔMG


A
5xFAD : Syk$^{i\Delta}$
Imaging
Aβ accumulation
0
1
2
4
5
9
Months
B
5-month-old
9-month-old
5-month-old
9-month-old
Syk$^{fl/fl}$-5xFAD
Syk$^{i\Delta MG}$-5xFAD
Cortex
Hippocampus
50μm
20μm
Methoxy-X04 Iba1 TO-PRO-3
C
Syk$^{fl/fl}$-5xFAD
Syk$^{i\Delta MG}$-5xFAD
Cortex
Hippocampus
Microglial density (#/mm^3)
8x10^4
6x10^4
4x10^4
2x10^4
0
****
*
5-month-old
9-month-old
D
Syk$^{fl/fl}$-5xFAD
Syk$^{i\Delta MG}$-5xFAD
Cortex
Hippocampus
Colocalized Iba1$^+$Methoxy-X04$^+$ volume / Methoxy-X04$^+$ volume
1.0
0.8
0.6
0.4
0.2
0.0
***
****
**
****
5-month-old
9-month-old
5-month-old
9-month-old
E
5-month-old
9-month-old
5-month-old
9-month-old
Syk$^{fl/fl}$-5xFAD
Syk$^{i\Delta MG}$-5xFAD
Cortex
Hippocampus
50μm
Methoxy-X04 Iba1 PU.1
F
Syk$^{fl/fl}$-5xFAD
Syk$^{i\Delta MG}$-5xFAD
Microglia/mm^2
2000
1500
1000
500
0
***
*
5-month-old 9-month-old
1500
1000
500
0
***
*
5-month-old 9-month-old


A
B
C
D
E
F
G
H
Re-clustering
Microglia
Cd3g
Ngp
P2ry12
B-cells
Monocytes
BAMs
Cd19
Ccr2
Mrc1
T cells
B cells
Neutrophils
Monocytes
BAMs
Microglia
Cell type frequency (%)
UMAP1
UMAP2
n=
Cluster:
17048
15153
9384
6878
2957
2180
1521
1200
888
311
310
Crybb1
Mef2a
Apoe
Lyz2
Ifitm3
Ifit3
Bst2
Ccl4
Lpl
Clec7a
Cst7
Stmn1
Cd74
H2-Ab1
H2-D1
Top2a
Mki67
Cluster
0 HM1
1 HM2
2 HM3
3 TM1
4 IFN-R
5 TM2
6 DAM
7 HM4
8 MHC-II
9 Cycling1
10 Cycling2
Scaled expression
Align with the UMAP beneath
Sample 1
Sample 2
Sample 3
Homeostatic genes
DAM genes
P2ry12
Tmem119
Trem2
Clec7a
Apoe
Lyz2
Cst7
Lpl
Itgax
IFN-R genes
MHCII gene
Cycling genes
Ifitm3
Ifit3
Isg15
Cd74
Stmn1
Top2a
Mki67
Log-normalized expression
Min
Max
Tmem119 (Normalized value)
Trem2 (Normalized value)
Clec7a (Normalized value)
Cluster


A
TM1, TM2, DAM
3304 (c
5289 (cell number)
Trem2 (Normalized value)
250
200
150
100
50
0
Sykfl/fl-5XFAD
SykiΔMG-5XFAD
B
Methoxy-X04 Iba1 Trem2
Sykfl/fl-5XFAD
SykiΔMG-5XFAD
50 μm
C
***
TREM2 mean intensity (A.U.) in Iba1+ voxels
400
300
200
100
0
Sykfl/fl -5XFAD
SykiΔMG -5XFAD
D
Methoxy-X04 Tmem119 Iba1 Clec7a
Sykfl/fl-5XFAD
Microglia around plaques
Microglia farther away from plaques
50 μm
20 μm
SykiΔMG-5XFAD
100 μm
Tmem119 Iba1
Tmem119 Clec7a
Clec7a Iba1
E
****
% of Clec7a+ Area
2.5
2.0
1.5
1.0
0.5
0.0
Sykfl/fl -5XFAD
SykiΔMG -5XFAD
F
**
% of Tmem119+ Area
25
20
15
10
5
0
Sykfl/fl -5XFAD
SykiΔMG -5XFAD
G
Sholl analysis of non-plaque associated microglia
Number of intersections
20
15
10
5
0
0
10
20
30
40
50
60
Distance from soma (μm)
Sykfl/fl-5XFAD
SykiΔMG-5XFAD
H
Myc Targets
Allograft Rejection
Oxidative Phosphorylation
Cholesterol Homeostasis
IL2 Stat5 Signaling
Interferon Alpha Response
Interferon Gamma Response
Hypoxia
Glycolysis
Inflammatory Response
Kras Signaling Up
Xenobiotic Metabolism
mTORC1 Signaling
Adipogenesis
Coagulation
0
2
4
6
Negative log10 p-value
I
Oxidative Phosphorylation
Cholesterol Homeostasis
Glycolysis
mTORC1 Signaling
SykiΔMG-5XFAD
Sykfl/fl-5XFAD
Scaled expression
2
1
0
−1
−2
Anxa5
Atf3
Atp5e
Chst2
Cited2
Cox4i1
Cox6a1
Cox6b1
Cox6c
Cox7c
Cox8a
Cxcl16
Fabp5
Lpl
Mif
Gusb
Ndufa1
Pkm
Plaur
Sdc3
Uqcrh
Acaca
Aldoa
Bhlhe40
Cd9
Gapdh
Gpi1
Hspe1
Ldha
Pgk1
Tpi1


A
Isotype
Anti-CLEC7A
Aβ1-42
Hippocampus
1000 μm
B
Isotype
Anti-CLEC7A
% of Aβ1-42+ Area
0
1
2
3
Cortex
Hippocampus
C
1 Filamentous
2 Inert
3 Dynamic
Isotype
Anti-CLEC7A
Methoxy-X04 Iba1 6E10
Methoxy-X04
6E10
30 μm
D
Isotype
Anti-CLEC7A
% of total plaque
0
20
40
60
80
****
*
**
Filamentous
Inert
Dynamic