Inventory of Supplemental Information

There are 4 supplemental figures and 1 supplemental table.

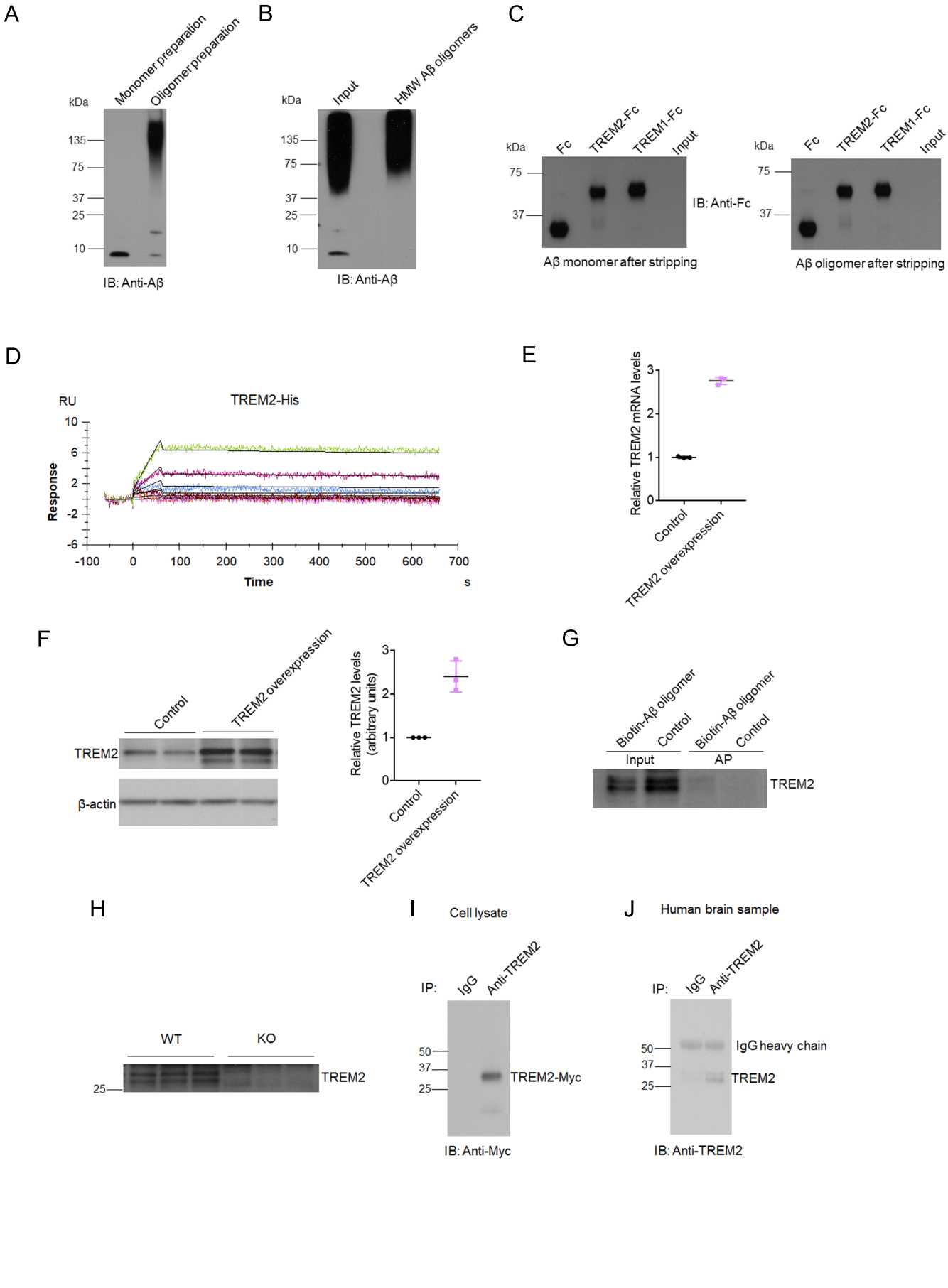
A
Monomer preparation
Oligomer preparation
kDa
135
75
37
25
10
IB: Anti-Aβ
B
Input
HMW Aβ oligomers
kDa
135
75
37
25
10
IB: Anti-Aβ
C
Fc
TREM2-Fc
TREM1-Fc
Input
kDa
75
37
IB: Anti-Fc
Aβ monomer after stripping
Aβ oligomer after stripping
D
RU
TREM2-His
Response
Time
s
E
Relative TREM2 mRNA levels
Control
TREM2 overexpression
F
Control
TREM2 overexpression
TREM2
β-actin
Relative TREM2 levels (arbitrary units)
Control
TREM2 overexpression
G
Biotin-Aβ oligomer
Control
Input
AP
TREM2
H
WT
KO
25
TREM2
I
Cell lysate
IP:
IgG
Anti-TREM2
50
37
25
TREM2-Myc
IB: Anti-Myc
J
Human brain sample
IP:
IgG
Anti-TREM2
50
37
25
IgG heavy chain
TREM2
IB: Anti-TREM2

Figure S1. related to Figure 1: TREM2 binds to Aβ

(A) Monomeric and oligomerized $A\beta_{1-42}$ peptides were immunoblotted with an antibody to detect Aβ (B436, anti-$A\beta_{1-12}$).

(B) Oligomerized $A\beta_{1-42}$ (input) was subjected to size separation using Amicon filters to obtain high molecular weight (HMW) oligomers.

(C) Following co-precipitation of $A\beta_{1-42}$ monomer or oligomers with Fc proteins, blots were stripped and re-probed for precipitated Fc proteins. A representative image is shown. *n* = 3 independent experiments.

(D) Surface Plasmon resonance binding kinetics were measured with the Biacore instrument. TREM2-Fc protein was immobilized in the GAH (goat anti-human) IgG immobilized CM5 sensor surface. The binding of a series dilution of biotin-Aβ oligomers (1 μM to 16 nM, 7 dilutions) to TREM2-Fc was analyzed and fitted to the 1:1 Binding model using BIAevaluation software.

(E and F) TREM2 levels in BV2 cells stably expressing human TREM2 were determined by qRT-PCR (E) or immunoblotting with an antibody against TREM2 (AF1828) (F). *n* = 3 independent experiments.

(G) Lysates from BV2 cells incubated with or without (control) biotin-$A\beta_{1-42}$ oligomers were affinity-precipitated (AP) with streptavidin beads and analyzed for co-precipitation with TREM2.

(H) The molecular weight of endogenous TREM2 is above 25 kDa, as indicated by TREM2 immunoblots probed with sheep anti-mouse TREM2 antibody in samples from WT or TREM2 KO microglia.

(I and J) Anti-TREM2 antibody (D814C) can immunoprecipitate Myc-tagged TREM2 from HEK293T lysates overexpressing TREM2-Myc (I) or endogenous TREM2 from human brain lysates (J). Representative image is shown. *n* = 3 independent experiments.

All values are presented as mean ± s.d.

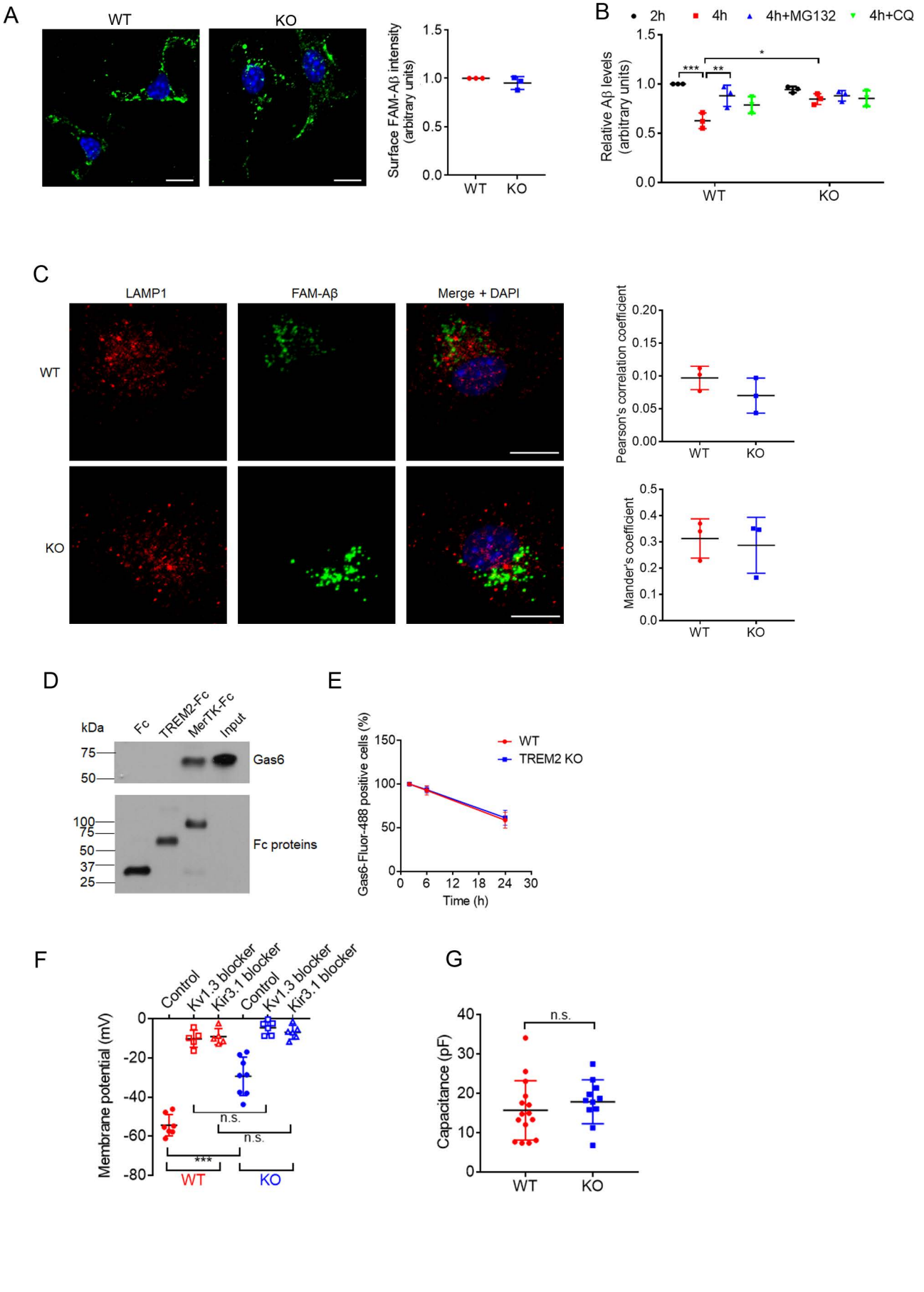
A
WT
KO
Surface FAM-Aβ intensity (arbitrary units)
1.5
1.0
0.5
0.0
WT
KO
B
2h
4h
4h+MG132
4h+CQ
Relative Aβ levels (arbitrary units)
***
**
*
C
LAMP1
FAM-Aβ
Merge + DAPI
WT
KO
Pearson's correlation coefficient
0.20
0.15
0.10
0.05
0.00
Mander's coefficient
0.5
0.4
0.3
0.2
0.1
0.0
D
kDa
Fc
TREM2-Fc
MerTK-Fc
Input
75
50
Gas6
100
75
50
37
25
Fc proteins
E
Gas6-Fluor-488 positive cells (%)
150
100
50
0
0
6
12
18
24
30
Time (h)
WT
TREM2 KO
F
Control
Kv1.3 blocker
Kir3.1 blocker
Control
Kv1.3 blocker
Kir3.1 blocker
Membrane potential (mV)
0
-20
-40
-60
-80
n.s.
n.s.
***
WT
KO
G
Capacitance (pF)
40
30
20
10
0
n.s.
WT
KO

Figure S2. related to Figure 2: Microglial surface binding of Aβ, degradation of Aβ and Gas6 in microglial cells, and analyses of FAM-Aβ distribution in lysosomes and microglial electrophysiology

(A) WT and TREM2 KO microglia were labeled with FAM-$Aβ_{1-42}$ aggregates at 4°C for 2h and visualized by confocal microscopy. No difference was observed in FAM-Aβ intensity as quantified in the adjacent graph (mean ± s.d., *n* =3 independent experiments; scale bars = 20 μm).

(B) Primary microglia cultures from WT or TREM2 KO mice were incubated with or without 200 nM unlabeled $Aβ_{1-42}$ oligomers at 37°C for 2h. Cells were then washed and re-incubated in Aβ-free media with or without MG132 or chloroquine (CQ) for an additional 2h. Cell-associated Aβ levels were determined by ELISA. All values were normalized to WT-Aβ levels 2h after uptake. *n* = 3 independent measurements. ***P* < 0.01, ****P* < 0.001, two-way ANOVA followed by Tukey *post hoc* test.

(C) Primary microglia cultures from WT or TREM2 KO mice were incubated with 200 nM FAM-$Aβ_{1-42}$ aggregates at 37°C for 2h. After the removal of Aβ, cells were cultured for an additional 2h and then fixed and stained with an antibody against the lysosomal marker LAMP1. Colocalization between FAM-Aβ and LAMP1 were analyzed and quantified using IMARIS software. *n* = 3 independent experiments.

(D) Recombinant Gas6 protein was incubated with Fc, TREM2-Fc or MerTK-Fc and precipitated with protein G. Precipitates were analyzed by immunoblotting with an antibody against Gas6.

(E) Primary microglia cultures from WT or TREM2 KO mice were incubated with 100 nM Alexa-488 labeled Gas6 protein for 2h. Cells were then washed and the degradation of Gas6 was determined by flow cytometry. *n* = 3 independent measurements.

(F) Resting membrane potential in WT and TREM2 KO microglial cells with or without K^+ channel blockers (400 pM Margatoxin for Kv1.3 or 100 nM Tertiapin for Kir3.1) (1h incubation). *n* = 5 -8 cells for each group. ***P < 0.001; n.s., no significance; two-way ANOVA followed by Tukey's post hoc test.

(G) Whole-cell capacitances of WT and TREM2 KO microglial cells at -60 mV. *n* = 11 -15 cells for each group. n.s., no significance, based on unpaired t-test.

All values are presented as mean ± s.d.

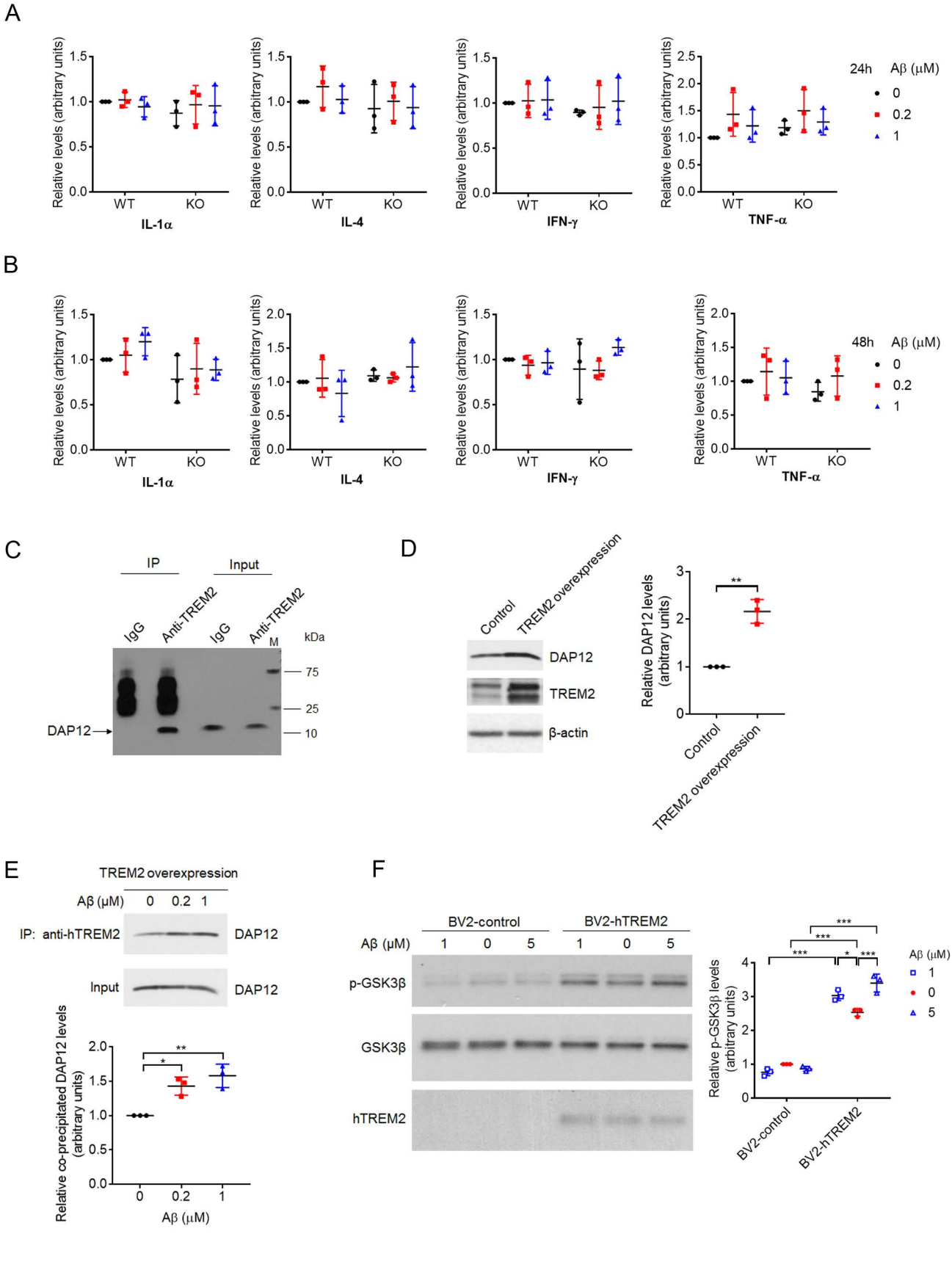
A
Relative levels (arbitrary units)
WT
KO
IL-1α
IL-4
IFN-γ
TNF-α
24h
Aβ (μM)
0
0.2
1
B
48h
C
IP
Input
IgG
Anti-TREM2
M
kDa
75
25
10
DAP12
D
Control
TREM2 overexpression
DAP12
TREM2
β-actin
Relative DAP12 levels (arbitrary units)
E
TREM2 overexpression
IP: anti-hTREM2
Input
Relative co-precipitated DAP12 levels (arbitrary units)
F
BV2-control
BV2-hTREM2
p-GSK3β
GSK3β
hTREM2
Relative p-GSK3β levels (arbitrary units)

Figure S3. related to Figure 3: Cytokines unaffected by Aβ and TREM2 deficiency; TREM2 is involved in Aβ-induced signaling changes

(A and B) Primary microglia cultures from WT or TREM2 KO mice were incubated with various $A\beta_{1-42}$ oligomer concentrations as indicated for 24h (A) or 48h (B). Conditioned medium was collected and cytokine levels were determined using an ELISA-based cytokine multiplex assay. All values were normalized to WT-Aβ (0 μM) levels for each time point. *n* = 3 independent experiments.

(C) Lysates from primary WT microglial cells were immunoprecipitated with an antibody against TREM2 or control IgG. The expression of DAP12 in the precipitates was determined by Western blot. Only the TREM2 antibody successfully co-precipitated endogenous DAP12.

(D) DAP12 expression in BV2 cells stably expressing human TREM2 and control BV2 cells was determined by Western-blot. n = 3 independent experiments. **P < 0.01, unpaired t-test.

(E) BV2 cells stably expressing human TREM2 were exposed to $A\beta_{1-42}$ oligomers for 2h. DAP12 co-precipitation with TREM2 was determined by IP-Western-blot, and normalized to the samples without Aβ treatment. n = 3 independent experiments. *P < 0.05, **P < 0.01, one-way ANOVA followed by Tukey's post hoc test.

(F) BV2 cells stably expressing human TREM2 (BV2-hTREM2) and control BV2 cells (BV2-control) were pretreated with mouse TREM2 shRNA and exposed to various concentrations of $A\beta_{1-42}$ oligomers as indicated for 24h. Levels of phospho- and total GSK3β were determined by Western blot. *n* = 3 independent experiments. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, two-way ANOVA followed by Tukey's *post hoc* test.

All values are presented as mean ± s.d.

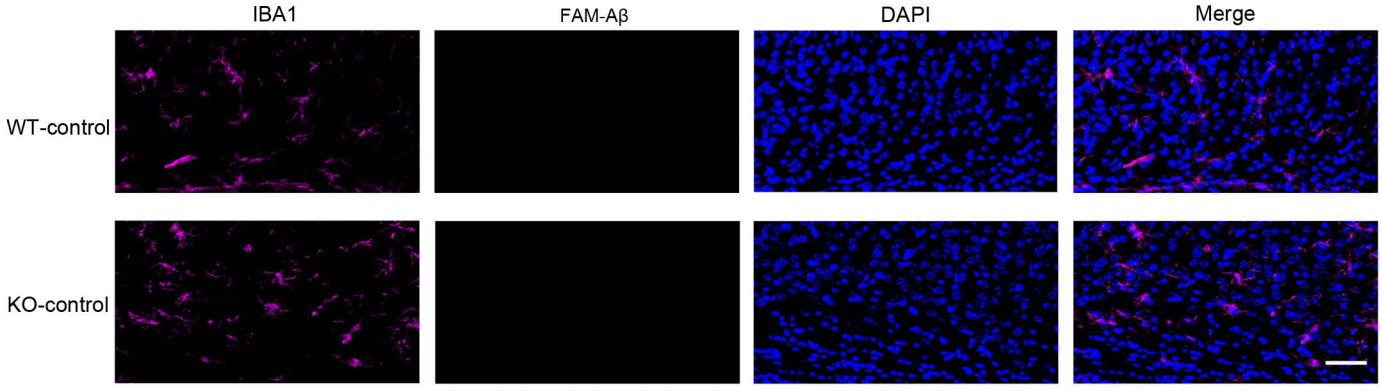

Figure S4. related to Figure 4: Microglia distribution is not affected by control vehicle injection

Representative images of WT or TREM2 KO mouse brain 16 hours after control vehicle injection. Eight WT and 10 KO animals were used. Scale bar = 50 μm.

Table S1 List of human brain tissues used

Number	Source	Identifier	Diagnosis	Gender	Age	Braak
1	UMiami	HBCG_16_003	AD	F	90	III-IV
2	UMiami	HBED_16_004	AD	F	71	III-IV
3	UMiami	HBFF_16_004	AD	M	85	III-IV
4	UMiami	HBFR_16_001	AD	F	69	V-VI
5	UCSD	X5754	AD	M	92	6.2
6	UCSD	X5743	AD	M	87	6.2