Supplementary Information

Fig S1a. Schematic showing TREM family genes on human chromosome 6p21 (top) and mouse chromosome 17B3 (bottom). TREM genes are shown in black boxes and TREM-like genes in open boxes. Domains of each protein are shown below the corresponding gene. b and c. Schematic of gene-editing design for mouse TREM2 knockout and R47H knock-in and targeted sequence (top strain) in exon 2. Primer binding sites are highlighted in green in both wild type (WT) and 143-mer single-stranded oligodeoxynucleotides (ssODN) sequence for R47H (nucleotides substitutions highlighted in yellow). Knockouts were generated from 5-bp and 11-bp frame-shift deletions whereas knock-in resulted from the presence of ssODN. Silent mutations (in red letters) were also introduced to prevent cutting as well as to create MluI restriction site for genotyping R47H. Virtual gel of PCR products from wild type (128 bp) and knockout (only 5-bp deletion are shown) mice genomic DNA (left), and PCR products digested with MluI (139 and 100 bp) from R47H knock-in mice (right);

Fig S2a and b. $TREM2^{R47H}$ (a) and $TREM2^{-/-}$ (b) mouse bone marrow derived macrophages (BMDMs) exhibit a survival defect when cultured under limiting conditions of CSF-1. This effect is less pronounced in heterozygous $TREM2^{+/-}$ and $TREM2^{R47H/+}$ BMDMs supportive of a gene-dosage effect on survival.

Fig S3a and b. A significant increase in *Ccl5* was noted on multiple days in both R47H (day 6 and 7, dark blue hash, n=3) and knockout macrophages (days 5, 6 and 7, red hash). A significant increase on days 6 and 7 in the heterozygous knockouts (pink dots) was also noted; c and d. Significant increase of *Ccl22* in R47H macrophages on day 7 and on days 5, 6 and 7 compared to wild-type. n=4 animals for R47H macrophages, n = 3 for WT and heterozygous littermates; n= 2

for knockout and WT litter-mates, n=3 for heterozygous littermates; e and f; No significant changes in *Flt1* in the R47H macrophages, however, significant reductions were observed in the knockout macrophages on days 6 and 7; g. Migration defects were noted for knockout BMDMs in a plug area assay. * $p<0.05$, ** $p < 0.01$, *** $p < 0.001$ and ****$p<.0001$, 2-way ANOVA with Dunnett's correction for multiple comparisons

Fig S4a-b. Significant reduction in CCL3 (a) and CCL4 (b) levels in TREM2^{R47H} microglia following treatment with Abeta 1-42 (blue hash), compared to wildtype littermates (black outline); c. Reduction in CCL2 from peritoneal lavage of TREM2$^{-/-}$, 3.5 hours post treatment with Zymosan; d. Reduction in CCL2 in the TREM2$^{-/-}$ brains treated with LPS 24 hours post treatment does not reach statistical significance.

Fig S5a. Surface expression of TREM2 in bone-marrow derived macrophages (BMDMs) cultured from TREM2$^{+/+}$ (green) and TREM2^{R47H} (blue) mice measured by FACS analysis using antibody 1 conjugated to APC reveals similar levels of surface TREM2 expression in the variant as WT TREM2; b. Antibody 1 does not detect any surface TREM2 in BMDMs from TREM2$^{-/-}$ mice (red) while demonstrating a FACS shift with WT BMDMs (green); c. Surface expression of TREM2 in bone-marrow derived macrophages (BMDMs) cultured from TREM2$^{+/+}$ (green) and TREM2^{R47H} (blue) mice measured by FACS analysis using antibody 2 reveals similar levels of surface TREM2 expression in the variant as WT TREM2; d. Antibody 2 does not detect any surface TREM2 in BMDMs from TREM2$^{-/-}$ mice (red) while detecting a FACS shift with WT BMDMs (purple). Background staining with the corresponding isotype control is shown in dotted black; e. An immunoblot of lysate from BMDMs confirms the specificity of antibody 2.

There are no bands detected in TREM2$^{-/-}$ BMDMs (lanes 1 and 2) but bands corresponding to different glycosylated forms of TREM2 in TREM2^{R47H} and TREM2$^{+/+}$ BMDMs are detected by antibody 2.

Fig S6a and b. Antibody 1 boosts survival of TREM2$^{+/+}$ (green) macrophages and microglia as demonstrated in a real-time cell confluence assay. Isotype control antibody (blue) is not able to achieve the same boost in survival; c. Antibody 1 was not able to rescue the survival defect observed in TREM2$^{-/-}$ macrophages; d and e. Antibody 2 boosts survival of TREM2$^{+/+}$ macrophages (d, purple) and a trend towards boosting microglia survival (e, purple) as demonstrated in a real-time cell confluence assay. Isotype control antibody (blue) is not able to achieve the same boost in survival; f. Antibody 2 was not able to improve the survival defect observed in TREM2$^{-/-}$ macrophages. Data are plotted as mean +/- s.d. and are from a single representative experiment. The experiment was conducted twice independently.

Fig S7a. Antibody 1 treatment increases the levels of *Ccl2* in BMDMs derived from R47H macrophages at day 5 only; b. Antibody 1 treatment increases CCL2 at the protein level as well; c-e. Antibody 1 causes a significant reduction in *Melk* (c), *Nek2* (d) and *Mmp14* (e) levels in TREM2^{R47H} macrophages compared to isotype control. No significant changes were observed in WT macrophages. * $p<0.05$, ** $p < 0.01$, *** $p < 0.001$ and ****$p<.0001$, 2-way ANOVA with Sidak's correction for multiple comparisons

Figure S1

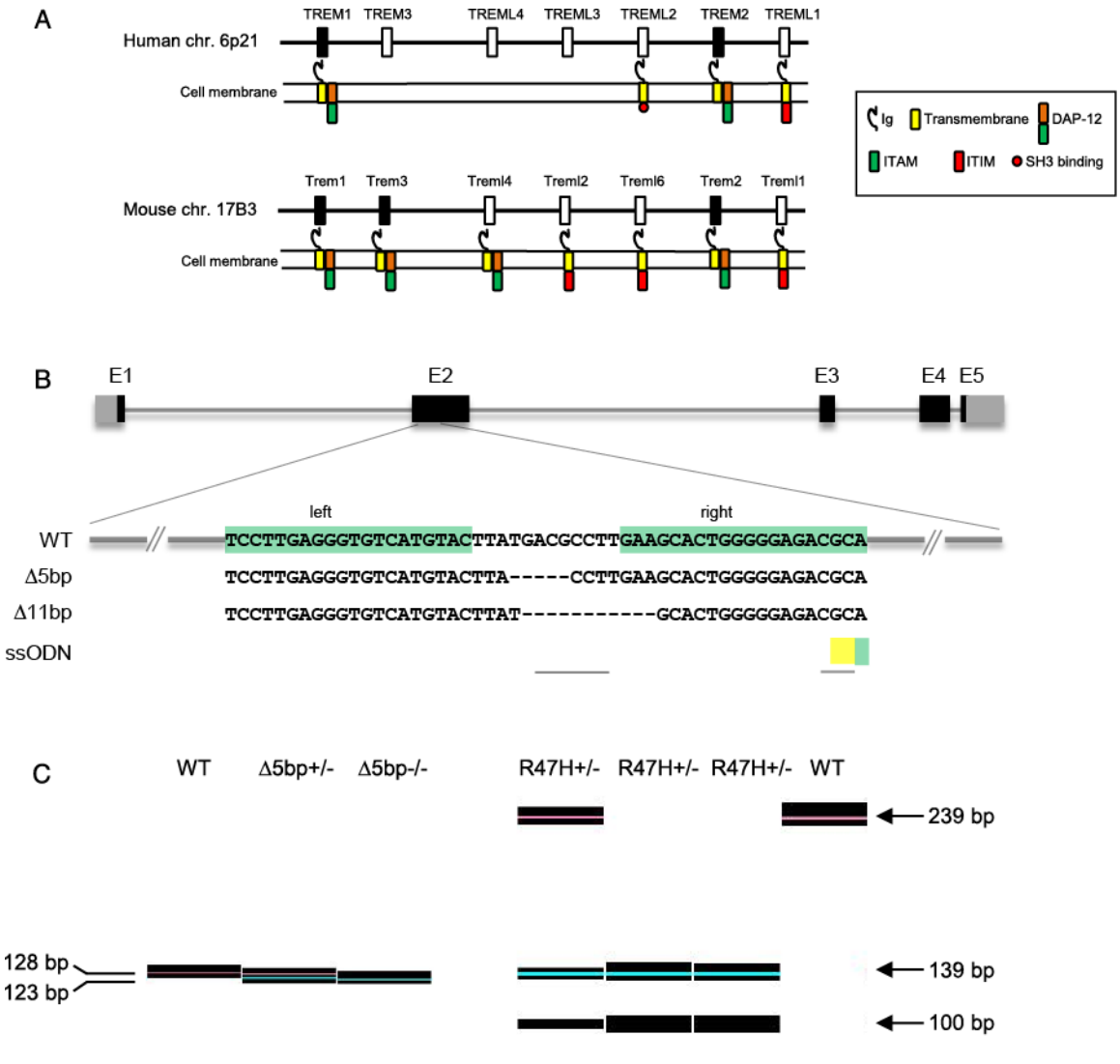

A
TREM1 TREM3 TREML4 TREML3 TREML2 TREM2 TREML1
Human chr. 6p21
Cell membrane
Trem1 Trem3 Treml4 Treml2 Treml6 Trem2 Treml1
Mouse chr. 17B3
Cell membrane
Ig
Transmembrane
DAP-12
ITAM
ITIM
SH3 binding
B
E1 E2 E3 E4 E5
left
right
WT TCCTTGAGGGTGTCATGTACTTATGACGCCTTGAAGCACTGGGGGAGACGCA
Δ5bp TCCTTGAGGGTGTCATGTACTTA-----CCTTGAAGCACTGGGGGAGACGCA
Δ11bp TCCTTGAGGGTGTCATGTACTTAT----------GCACTGGGGGAGACGCA
ssODN
C
WT Δ5bp+/- Δ5bp-/- R47H+/- R47H+/- R47H+/- WT
239 bp
128 bp
123 bp
139 bp
100 bp

Figure S2

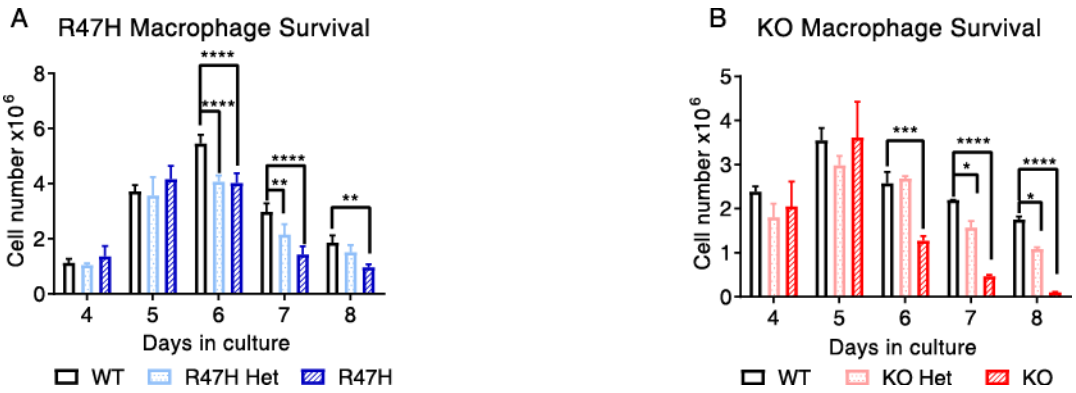

A
R47H Macrophage Survival
Cell number x10^6
Days in culture
WT
R47H Het
R47H
B
KO Macrophage Survival
Cell number x10^6
Days in culture
WT
KO Het
KO

Figure S3

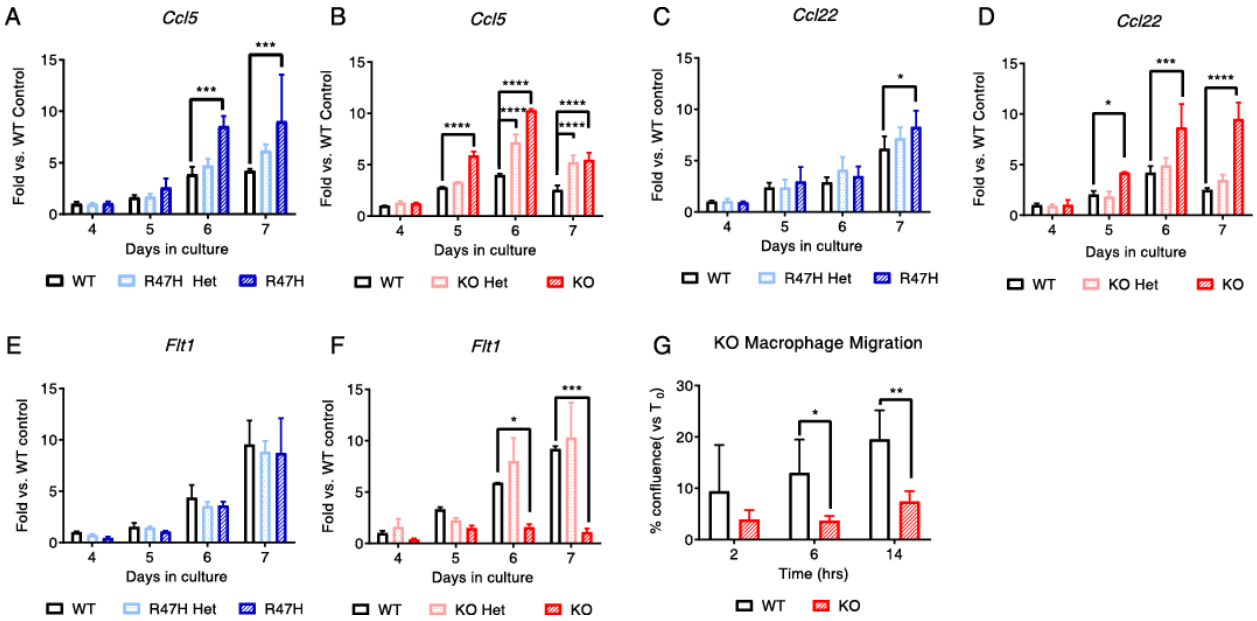

A
Ccl5
Fold vs. WT Control
Days in culture
WT
R47H Het
R47H
B
Ccl5
Fold vs. WT Control
Days in culture
WT
KO Het
KO
C
Ccl22
Fold vs. WT control
Days in culture
WT
R47H Het
R47H
D
Ccl22
Fold vs. WT Control
Days in culture
WT
KO Het
KO
E
Flt1
Fold vs. WT control
Days in culture
WT
R47H Het
R47H
F
Flt1
Fold vs. WT control
Days in culture
WT
KO Het
KO
G
KO Macrophage Migration
% confluence(vs T 0)
Time (hrs)
WT
KO

Figure S4

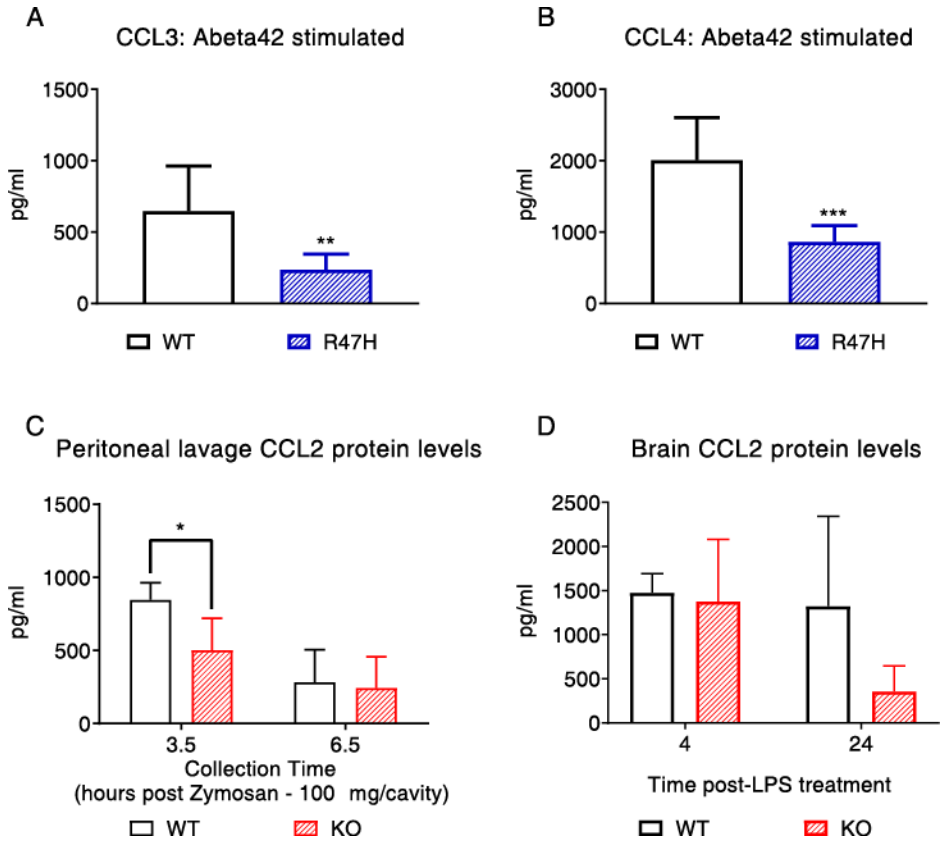

A
CCL3: Abeta42 stimulated
pg/ml
1500
1000
500
0
**
WT
R47H
B
CCL4: Abeta42 stimulated
pg/ml
3000
2000
1000
0
***
WT
R47H
C
Peritoneal lavage CCL2 protein levels
pg/ml
1500
1000
500
0
*
3.5
6.5
Collection Time
(hours post Zymosan - 100 mg/cavity)
WT
KO
D
Brain CCL2 protein levels
pg/ml
2500
2000
1500
1000
500
0
4
24
Time post-LPS treatment
WT
KO

Figure S5

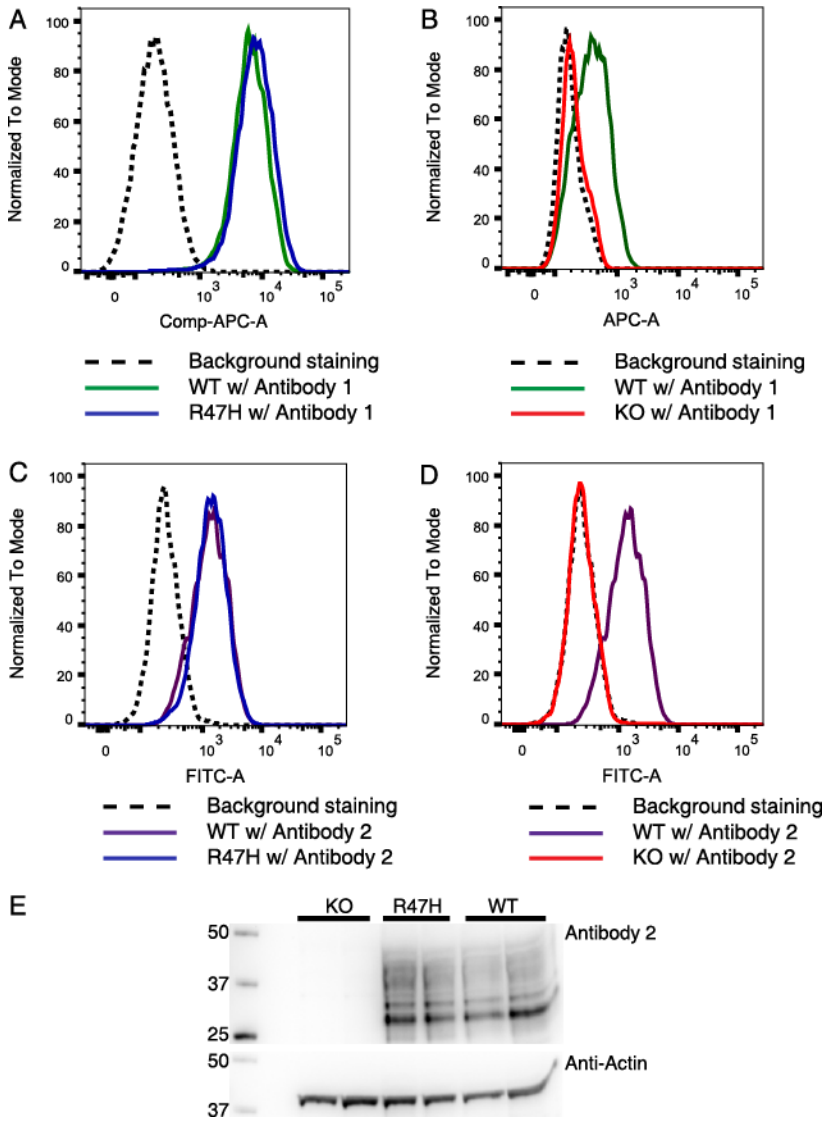

A
Normalized To Mode
Comp-APC-A
Background staining
WT w/ Antibody 1
R47H w/ Antibody 1
B
Normalized To Mode
APC-A
Background staining
WT w/ Antibody 1
KO w/ Antibody 1
C
Normalized To Mode
FITC-A
Background staining
WT w/ Antibody 2
R47H w/ Antibody 2
D
Normalized To Mode
FITC-A
Background staining
WT w/ Antibody 2
KO w/ Antibody 2
E
KO
R47H
WT
Antibody 2
Anti-Actin

Figure S6

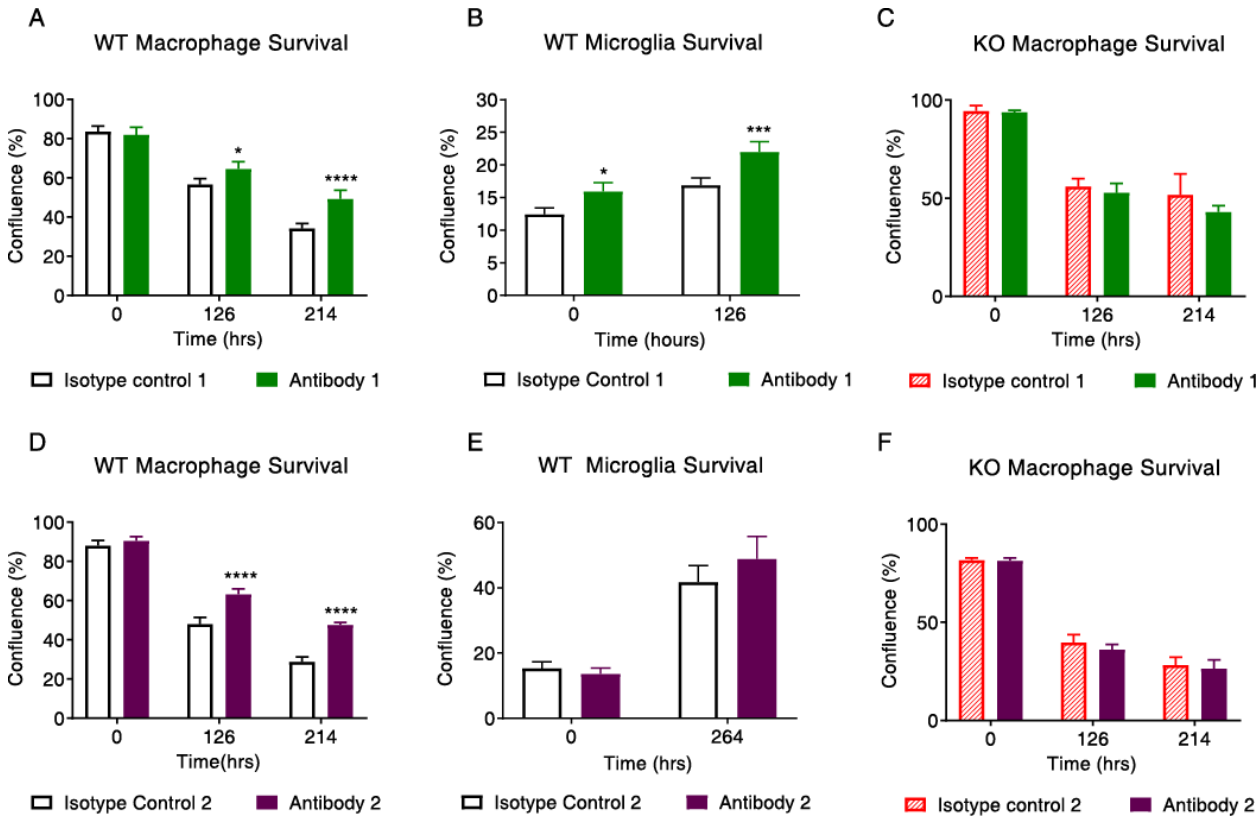
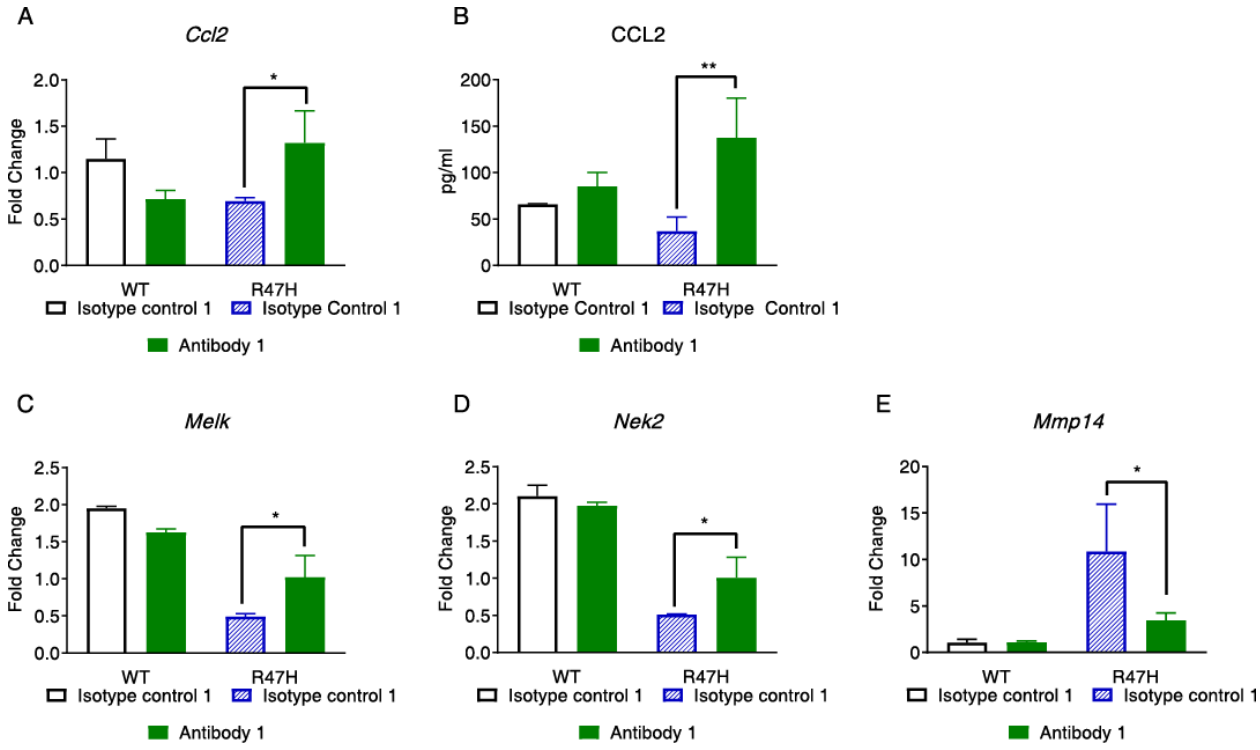

A
WT Macrophage Survival
Confluence (%)
Time (hrs)
Isotype control 1
Antibody 1
B
WT Microglia Survival
Confluence (%)
Time (hours)
Isotype Control 1
Antibody 1
C
KO Macrophage Survival
Confluence (%)
Time (hrs)
Isotype control 1
Antibody 1
D
WT Macrophage Survival
Confluence (%)
Time(hrs)
Isotype Control 2
Antibody 2
E
WT Microglia Survival
Confluence (%)
Time (hrs)
Isotype Control 2
Antibody 2
F
KO Macrophage Survival
Confluence (%)
Time (hrs)
Isotype control 2
Antibody 2

Figure S7


A
Ccl2
Fold Change
2.0
1.5
1.0
0.5
0.0
*
WT
R47H
Isotype control 1
Isotype Control 1
Antibody 1
B
CCL2
pg/ml
200
150
100
50
0
**
WT
R47H
Isotype Control 1
Isotype Control 1
Antibody 1
C
Melk
Fold Change
2.5
2.0
1.5
1.0
0.5
0.0
*
WT
R47H
Isotype control 1
Isotype control 1
Antibody 1
D
Nek2
Fold Change
2.5
2.0
1.5
1.0
0.5
0.0
*
WT
R47H
Isotype control 1
Isotype control 1
Antibody 1
E
Mmp14
Fold Change
20
15
10
5
0
*
WT
R47H
Isotype control 1
Isotype control 1
Antibody 1