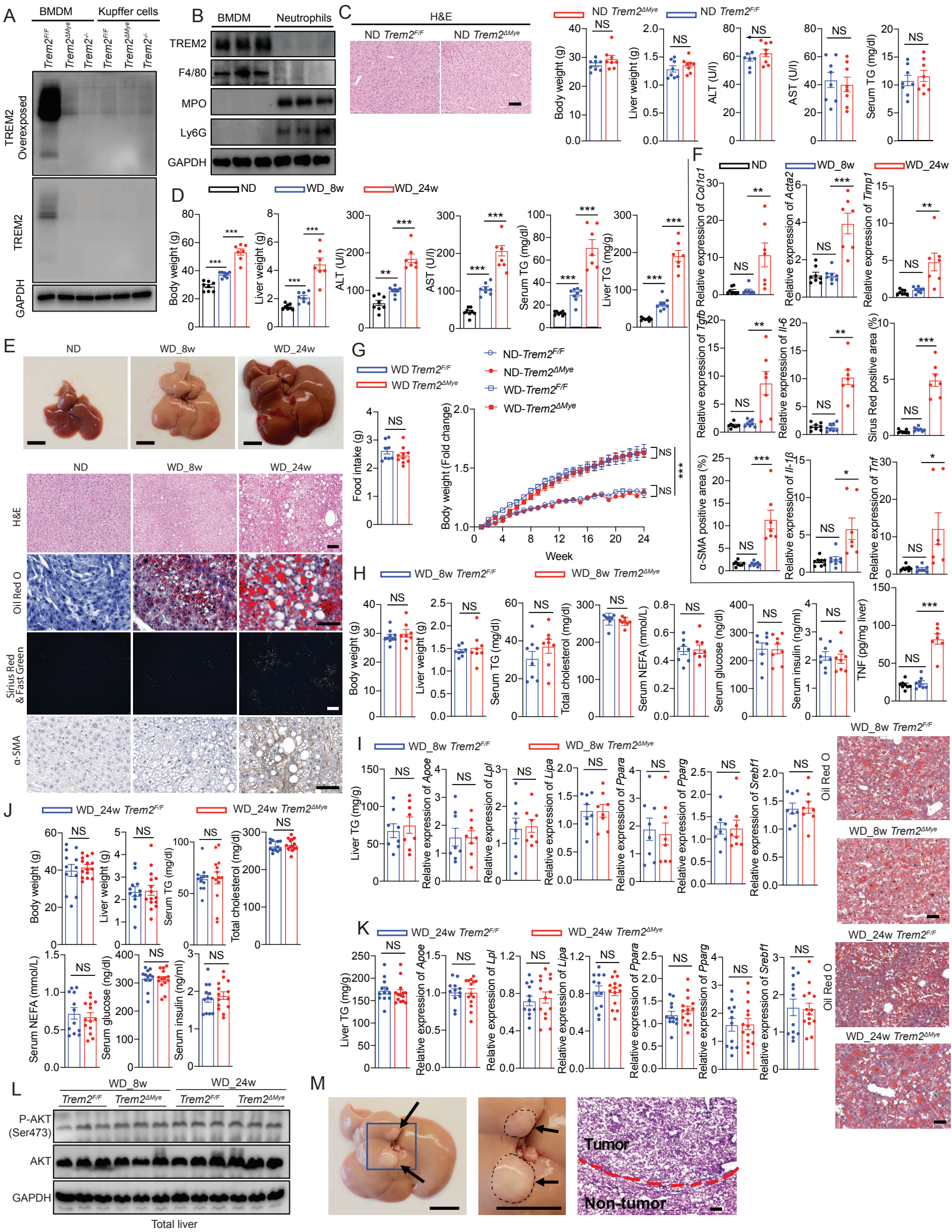
A
BMDM
Kupffer cells
Trem2F/F
Trem2ΔMye
Trem2-/-
TREM2 Overexposed
TREM2
GAPDH
B
BMDM
Neutrophils
TREM2
F4/80
MPO
Ly6G
GAPDH
C
H&E
ND Trem2F/F
ND Trem2ΔMye
Body weight (g)
Liver weight (g)
ALT (U/l)
AST (U/l)
Serum TG (mg/dl)
NS
D
ND
WD_8w
WD_24w
Body weight (g)
Liver weight (g)
ALT (U/l)
AST (U/l)
Serum TG (mg/dl)
Liver TG (mg/g)
E
ND
WD_8w
WD_24w
H&E
Oil Red O
Sirius Red & Fast Green
α-SMA
F
ND
WD_8w
WD_24w
Relative expression of Col1a1
Relative expression of Acta2
Relative expression of Timp1
Relative expression of Tgfb
Relative expression of Il-6
Sirus Red positive area (%)
α-SMA positive area (%)
Relative expression of Il-1β
Relative expression of Tnf
G
WD Trem2F/F
WD Trem2ΔMye
ND-Trem2F/F
ND-Trem2ΔMye
WD-Trem2F/F
WD-Trem2ΔMye
Food intake (g)
Body weight (Fold change)
Week
H
WD_8w Trem2F/F
WD_8w Trem2ΔMye
Body weight (g)
Liver weight (g)
Serum TG (mg/dl)
Total cholesterol (mg/dl)
Serum NEFA (mmol/L)
Serum glucose (ng/dl)
Serum insulin (ng/ml)
TNF (pg/mg liver)
I
WD_8w Trem2F/F
WD_8w Trem2ΔMye
Liver TG (mg/g)
Relative expression of Apoe
Relative expression of Lpl
Relative expression of Lipa
Relative expression of Ppara
Relative expression of Pparg
Relative expression of Srebf1
Oil Red O
J
WD_24w Trem2F/F
WD_24w Trem2ΔMye
Body weight (g)
Liver weight (g)
Serum TG (mg/dl)
Total cholesterol (mg/dl)
Serum NEFA (mmol/L)
Serum glucose (ng/dl)
Serum insulin (ng/ml)
K
WD_24w Trem2F/F
WD_24w Trem2ΔMye
Liver TG (mg/g)
Relative expression of Apoe
Relative expression of Lpl
Relative expression of Lipa
Relative expression of Ppara
Relative expression of Pparg
Relative expression of Srebf1
Oil Red O
L
WD_8w
WD_24w
Trem2F/F
Trem2ΔMye
P-AKT (Ser473)
AKT
GAPDH
Total liver
M
Tumor
Non-tumor

Figure S1. Genetic ablation of *Trem2* in macrophages exacerbates NASH pathology, Related to Figure 1

(A) Immunoblot analysis of TREM2 in BMDMs and liver resident Kupffer cells from *Trem2*$^{F/F}$, *Trem2*$^{\Delta Mye}$, and *Trem2*$^{-/-}$ mice.
(B) Immunoblot analysis of TREM2, F4/80, MPO, and Ly6G in BMDMs and neutrophils from C57BL/6 mice.
(C) Representative H&E staining of liver sections, body weights, liver weights, serum ALT, AST, and serum triglyceride (TG) were measured in *Trem2*$^{F/F}$ and *Trem2*$^{\Delta Mye}$ mice fed with normal diet (ND) for 8 weeks. n = 8 mice per group. Data are shown as mean ± s.e.m.. NS, not significant.
(D) Body weights, liver weights, serum ALT, AST, serum TG, and liver TG were measured in C57BL/6 mice fed with ND or WD for 8 and 24 weeks. ND, n = 8 mice; WD_8w, n = 8 mice; WD_24w, n = 7 mice. Data are shown as mean ± s.e.m.. **p<0.01; ***p<0.001; NS, not significant.
(E) Representative liver picture (scale bar,1 cm), H&E, Oil Red O, Sirius Red/Fast Green, and α-SMA staining of liver sections from C57BL/6 mice fed with ND or WD for 8 and 24 weeks. Sirius Red/Fast Green staining was detected under polarized light. ND, n = 8 mice; WD_8w, n = 8 mice; WD_24w, n = 7 mice. Scale bar, 100 μm.
(F) Relative mRNA expression of fibrosis-related genes and inflammatory genes was analyzed by RT-qPCR, and TNF amounts were measured by ELISA in liver tissue from C57BL/6 mice that were fed with ND or WD for 8 and 24 weeks. Sirius Red/Fast Gree, and α-SMA positive areas shown in (E) were quantified by ImageJ software. ND, n = 8 mice; WD_8w, n = 8 mice; WD_24w, n = 7 mice. Data are shown as mean ± s.e.m.. *p<0.05; **p<0.01; ***p<0.001.
(G) Measurements of food intake and fold changes of body weight in *Trem2*$^{F/F}$ and *Trem2*$^{\Delta Mye}$ mice fed with ND or WD. Data are shown as mean ± s.e.m.. ***p<0.001; NS, not significant.
(H-K) Body weights, liver weights, serum TG, total cholesterol, serum NEFA, serum glucose, and serum insulin were measured in *Trem2*$^{F/F}$ and *Trem2*$^{\Delta Mye}$ mice fed with WD for 8 (H) and 24 weeks (J). Representative Oil Red O staining, liver TG, and relative mRNA abundance of lipid associated genes was analyzed in liver tissue from *Trem2*$^{F/F}$ and *Trem2*$^{\Delta Mye}$ mice fed with WD for 8 (I) or 24 weeks (K). WD_8w, n = 8 mice per group; WD_24w *Trem2*$^{F/F}$, n = 12 mice; WD_24w *Trem2*$^{\Delta Mye}$, n = 14 mice. Scale bar, 100 μm. Data are shown as mean ± s.e.m.. ***p<0.001; NS, not significant.
(L) Immunoblot analysis of AKT and p-AKT (Ser473) in liver tissue from *Trem2*$^{F/F}$ and *Trem2*$^{\Delta Mye}$ mice fed with WD for 8 and 24 weeks.
(M) Gross liver picture (scale bar, 1 cm) and H&E staining (scale bar, 100 μm) of liver sections from a *Trem2*$^{\Delta Mye}$ mouse fed with WD for 24 weeks. Arrows indicate tumors.

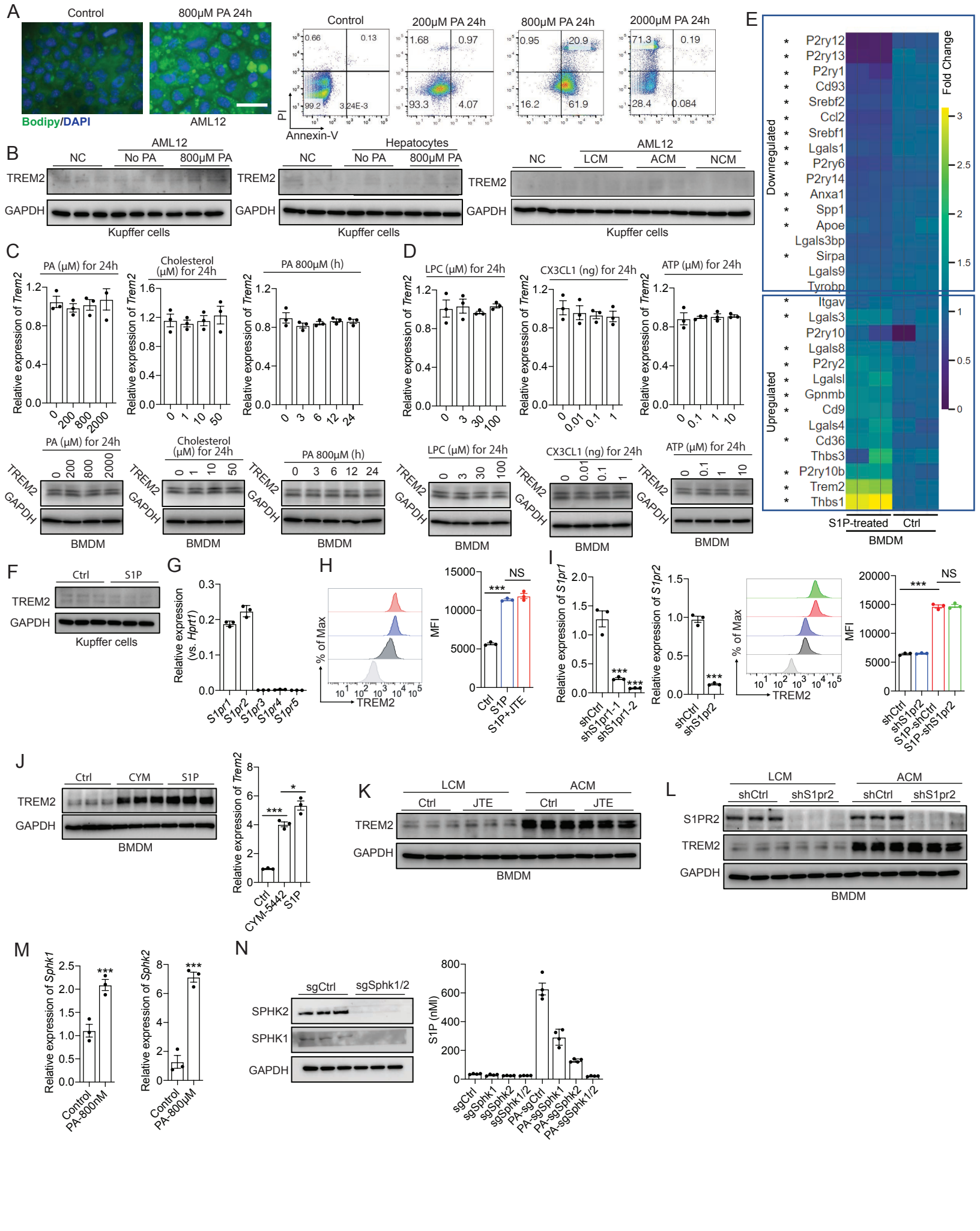
A
Control
800μM PA 24h
Bodipy/DAPI
AML12
Control
200μM PA 24h
800μM PA 24h
2000μM PA 24h
PI
Annexin-V
B
AML12
NC
No PA
800μM PA
Hepatocytes
LCM
ACM
NCM
TREM2
GAPDH
Kupffer cells
C
PA (μM) for 24h
Cholesterol (μM) for 24h
PA 800μM (h)
Relative expression of Trem2
BMDM
D
LPC (μM) for 24h
CX3CL1 (ng) for 24h
ATP (μM) for 24h
E
Fold Change
Downregulated
Upregulated
P2ry12
P2ry13
P2ry1
Cd93
Srebf2
Ccl2
Srebf1
Lgals1
P2ry6
P2ry14
Anxa1
Spp1
Apoe
Lgals3bp
Sirpa
Lgals9
Tyrobp
Itgav
Lgals3
P2ry10
Lgals8
P2ry2
Lgalsl
Gpnmb
Cd9
Lgals4
Cd36
Thbs3
P2ry10b
Trem2
Thbs1
S1P-treated
Ctrl
BMDM
F
Ctrl
S1P
G
Relative expression (vs. Hprt1)
S1pr1
S1pr2
S1pr3
S1pr4
S1pr5
H
% of Max
MFI
NS
S1P+JTE
I
Relative expression of S1pr1
Relative expression of S1pr2
shCtrl
shS1pr1-1
shS1pr1-2
shS1pr2
S1P-shCtrl
S1P-shS1pr2
J
CYM
CYM-5442
K
LCM
ACM
JTE
L
shCtrl
shS1pr2
S1PR2
M
Relative expression of Sphk1
Relative expression of Sphk2
Control
PA-800nM
PA-800μM
N
sgCtrl
sgSphk1/2
SPHK2
SPHK1
S1P (nM)
sgCtrl
sgSphk1
sgSphk2
sgSphk1/2
PA-sgCtrl
PA-sgSphk1
PA-sgSphk2
PA-sgSphk1/2

Figure S2. Obesity upregulates TREM2 in infiltrated liver macrophages via hepatocyte derived S1P, Related to Figure 2

(A) Bodipy staining of AML12 cells treated with vehicle control or 800 μM PA for 24 hours. Scale bar, 100 μm. For flow cytometry analysis, AML12 cells that were treated with vehicle control, 200 μM, 800 μM, or 2000 μM PA for 24 hours were stained with Annexin V and PI (Propidium Iodide) to assess the nature of cell death.
(B) Immunoblot analysis of TREM2 in primary mouse Kupffer cells cocultured with PA-treated AML12 cells (left panel) or primary murine hepatocytes (middle panel) in a transwell system, or directly stimulated with culture medium collected from AML12 cells treated with PA of various concentrations (right panel). LCM, live cell culture medium; ACM, apoptotic cell culture medium; NCM, necrotic cell culture medium.
(C and D) RT-qPCR analysis of *Trem2* mRNA and immunoblot analysis of TREM2 in BMDMs treated with PA or cholesterol for various time durations (C), or with LPC, CX3CL1 or ATP at various concentrations for 24 hours (D).
(E) Heatmap of TREM2 related genes in BMDMs treated with or without S1P (n=4 in each group). *p<0.05.
(F) Immunoblot analysis of TREM2 in primary mouse Kupffer cells that were treated with or without S1P.
(G) Relative mRNA abundance of genes encoding S1P receptors (*S1pr1-5*) in BMDMs.
(H and I) Flow cytometry analysis of cell surface TREM2 in BMDMs treated with S1P. BMDMs were either pretreated with JTE or transduced with lentiviral construct expressing shS1pr1 or shS1pr2 (H). Relative mRNA expression of *S1pr1* and *S1pr2* in BMDMs after lentiviral transduction were also shown in (I). Data are shown as mean ± s.e.m.. ***p<0.001; NS, not significant.
(J) RT-qPCR analysis of *Trem2* mRNA and immunoblot analysis of TREM2 in BMDMs treated with S1P or S1PR1 agonist (CYM-5442) for 24 hours. Data are shown as mean ± s.e.m.. *p<0.05, ***p<0.001.
(K and L) Immunoblot analysis of TREM2 in total cell lysates in BMDMs stimulated with cell culture medium from collected from AML12 cells. BMDMs were either pretreated with JTE (K) or transduced with lentiviral constructs expressing shS1pr2 (L).
(M) Relative mRNA abundance of *Sphk1* and *Sphk2* in AML12 cells treated with vehicle control (LCM) or 800μM of PA (ACM) for 24 hours. Data are shown as mean ± s.e.m.. ***p<0.001.
(N) Immunoblot analysis of SPHK1 and SPHK2 in AML12 cells transduced with lentiviral constructs expressing sgSphk1/2 (left panel). S1P concentrations in culture medium of indicated AML12 cells were measured by ELISA (right panel). AML12 cells were either treated with vehicle or 800μM of PA for 24 hours. Data are shown as mean ± s.e.m.. *p<0.05.
For in vitro experiments in this Figure, data were collected from three samples in each group for analysis and are representative of three independent experiments.

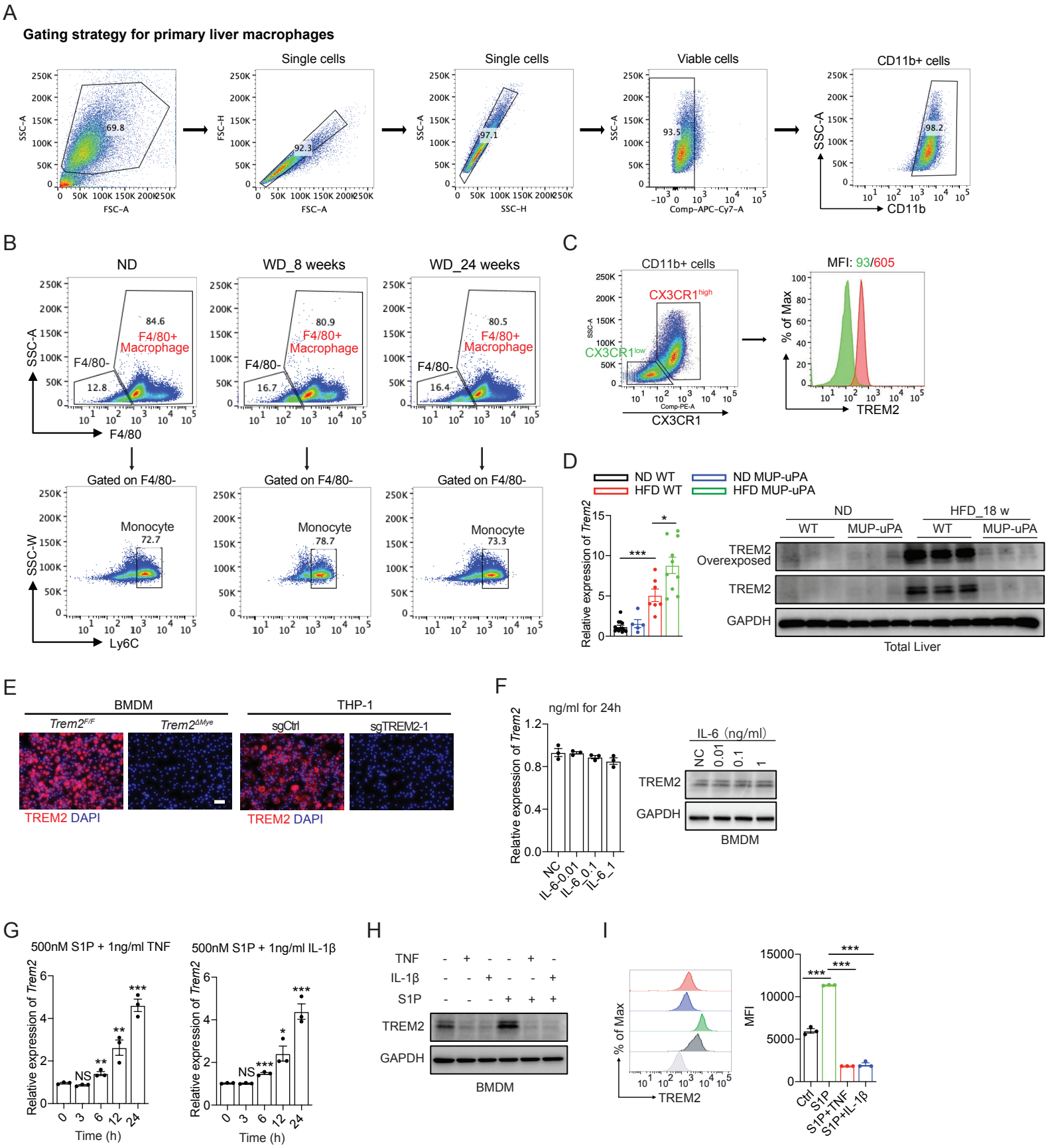
A
Gating strategy for primary liver macrophages
Single cells
Single cells
Viable cells
CD11b+ cells
B
ND
WD_8 weeks
WD_24 weeks
F4/80+ Macrophage
F4/80-
Gated on F4/80-
Monocyte
C
CD11b+ cells
CX3CR1high
CX3CR1low
MFI: 93/605
TREM2
D
ND WT
HFD WT
ND MUP-uPA
HFD MUP-uPA
Relative expression of Trem2
TREM2 Overexposed
GAPDH
Total Liver
E
BMDM
THP-1
Trem2F/F
Trem2ΔMye
sgCtrl
sgTREM2-1
TREM2 DAPI
F
ng/ml for 24h
IL-6 (ng/ml)
BMDM
G
500nM S1P + 1ng/ml TNF
500nM S1P + 1ng/ml IL-1β
Time (h)
H
TNF
IL-1β
S1P
TREM2
GAPDH
BMDM
I
% of Max
MFI
Ctrl
S1P
S1P+TNF
S1P+IL-1β

Figure S3. Uncoupled regulation of *Trem2* mRNA and its protein during NASH development, Related to Figure 3

(A) Flow cytometry gating strategy and analysis of $CD11b^+$ cells freshly isolated from mouse liver by MACS.
(B) Flow cytometry analysis of Ly6C in $F4/80^-$ cell compartment of $CD11b^+$ cells freshly isolated from C57BL/6 mice that were fed with ND or WD for 8 and 24 weeks.
(C) Flow cytometry analysis of TREM2 in $CX3CR1^{low}$ and $CX3CR1^{high}$ liver macrophages freshly isolated from C57BL/6 mice fed with WD for 8 weeks. MFI was used for data analysis.
(D) RT-qPCR analysis of *Trem2* mRNA and immunoblot analysis of TREM2 in liver tissue from WT or *MUP-uPA* mice fed with ND or HFD for 18 weeks. ND WT, n = 13 mice; ND *MUP-uPA*, n = 5 mice; HFD WT, n = 7 mice; HFD *MUP-uPA*, n = 10 mice. Data are shown as mean ± s.e.m.. *p<0.05; ***p<0.001.
(E) Immunofluorescent staining of TREM2 in $Trem2^{F/F}$ and $Trem2^{\Delta Mye}$ BMDMs or TREM2-sufficient (sgCtrl) and -deficient (sgTREM2) THP-1 cells. Scar bars, 100μm.
(F) RT-qPCR analysis of *Trem2* mRNA and immunoblot analysis of TREM2 in BMDMs treated with increasing doses of IL-6 for 24 hours.
(G) RT-qPCR analysis of *Trem2* mRNA in BMDMs pre-treated with 500nM S1P for 2 hours followed by stimulation with 1ng/ml of TNF or IL-1β for various time durations. Data are shown as mean ± s.e.m.. *p<0.05; **p<0.01; ***p<0.001; NS, not significant.
(H and I) Immunoblot analysis of TREM2 (H) and flow cytometry analysis of cell surface TREM2 (I) in BMDMs pre-treated with 500nM S1P for 2 hours followed by stimulation with 1ng/ml of TNF or IL-1β for 24 hours. MFI was used for flow cytometry analysis. Data are shown as mean ± s.e.m.. ***p<0.001.
For in vitro experiments in this Figure, data were collected from three samples in each group for analysis and are representative of three independent experiments.

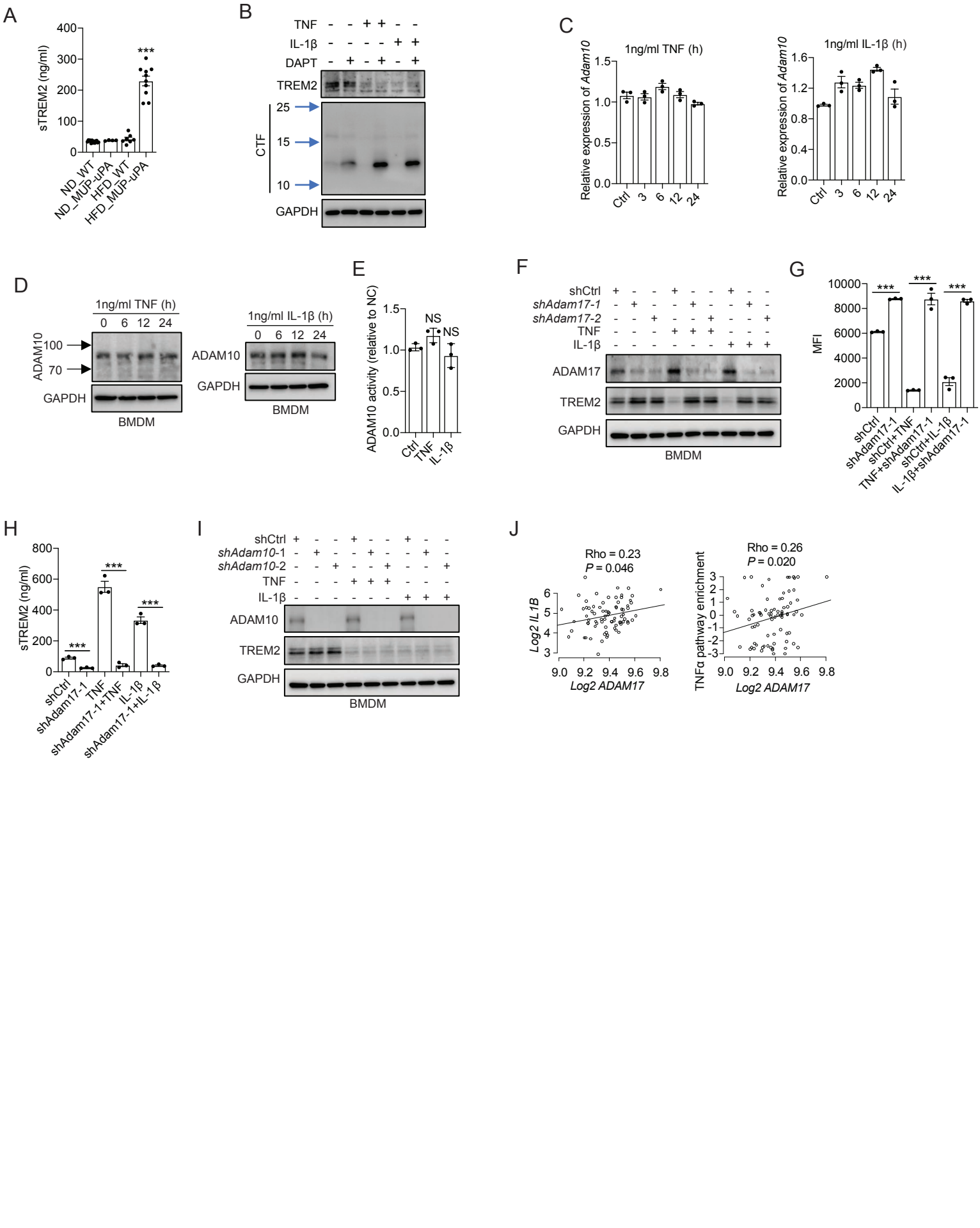
A
sTREM2 (ng/ml)
400
300
200
100
0
***
ND_WT
ND_MUP-uPA
HFD_WT
HFD_MUP-uPA
B
TNF - - + + - -
IL-1β - - - - + +
DAPT - + - + - +
TREM2
CTF
25
15
10
GAPDH
C
1ng/ml TNF (h)
Relative expression of Adam10
1.5
1.0
0.5
0.0
Ctrl 3 6 12 24
1ng/ml IL-1β (h)
Relative expression of Adam10
1.6
1.2
0.8
0.4
0.0
Ctrl 3 6 12 24
D
1ng/ml TNF (h)
0 6 12 24
ADAM10
100
70
GAPDH
BMDM
1ng/ml IL-1β (h)
0 6 12 24
ADAM10
GAPDH
BMDM
E
ADAM10 activity (relative to NC)
1.5
1.0
0.5
0.0
NS
NS
Ctrl
TNF
IL-1β
F
shCtrl + - - + - - + - -
shAdam17-1 - + - - + - - + -
shAdam17-2 - - + - - + - - +
TNF - - - + + + - - -
IL-1β - - - - - - + + +
ADAM17
TREM2
GAPDH
BMDM
G
MFI
10000
8000
6000
4000
2000
0
***
***
***
shCtrl
shAdam17-1
shCtrl+TNF
TNF+shAdam17-1
shCtrl+IL-1β
IL-1β+shAdam17-1
H
sTREM2 (ng/ml)
800
600
400
200
0
***
***
***
shCtrl
shAdam17-1
TNF
shAdam17-1+TNF
IL-1β
shAdam17-1+IL-1β
I
shCtrl + - - + - - + - -
shAdam10-1 - + - - + - - + -
shAdam10-2 - - + - - + - - +
TNF - - - + + + - - -
IL-1β - - - - - - + + +
ADAM10
TREM2
GAPDH
BMDM
J
Rho = 0.23
P = 0.046
Log2 IL1B
6
5
4
3
9.0 9.2 9.4 9.6 9.8
Log2 ADAM17
Rho = 0.26
P = 0.020
TNFα pathway enrichment
3
2
1
0
-1
-2
-3
9.0 9.2 9.4 9.6 9.8
Log2 ADAM17

Figure S4. TNF and IL-1β induce TREM2 proteolytic cleavage by activating ADAM17, Related to Figure 4

(A) sTREM2 concentrations in mouse sera collected from WT or *MUP-uPA* mice fed with ND or HFD for 18 weeks. ND_WT, n = 13 mice; ND_*MUP-uPA*, n = 5 mice; HFD_WT, n = 7 mice; HFD_*MUP-uPA*, n = 10 mice. Data are shown as mean ± s.e.m.. ***p<0.001.
(B) Immunoblot analysis of TREM2 (full-length and C-terminal fragment) in BMDMs stimulated with 1 ng/ml of TNF or IL-1β in the presence or absence of DAPT for 24 hours.
(C and D) RT-qPCR analysis of *Adam10* mRNA (C) and immunoblot analysis of ADAM10 (D) in BMDMs stimulated with 1 ng/ml of TNF or IL-1β for various time durations.
(E) Relative ADAM10 activity was quantified in BMDMs after 24 hours of 1 ng/ml of TNF or IL-1β stimulation. Data are shown as mean ± s.e.m.. NS, not significant.
(F-H) Immunoblot analysis of TREM2 (F), flow cytometry analysis of cell surface TREM2 (G), and ELISA analysis of sTREM2 in culture medium (H) of BMDMs transduced with lentiviral constructs expressing shRNAs against *Adam17* (*shAdam17*-1 and *shAdam17*-2) followed by stimulation with 1 ng/ml of TNF or IL-1β. MFI was used for flow cytometry analysis. Data are shown as mean ± s.e.m.. ***p<0.001.
(I) Immunoblot analysis of ADAM10 and TREM2 in BMDMs transduced with lentiviral constructs expressing shRNAs against *Adam10* (*shAdam10*-1 and *shAdam10*-2) followed by stimulation with 1 ng/ml of TNF or IL-1β.
(J) Association of human *ADAM17* expression with IL1B and TNFα pathway enrichment was analyzed in liver tissue of a clinical cohort (GSE130970, n = 78). Healthy, n = 6; simple steatosis (SS), n = 14; NASH, n = 58.
For in vitro experiments in this Figure, data were collected from three samples in each group for analysis and are representative of at least three independent experiments.

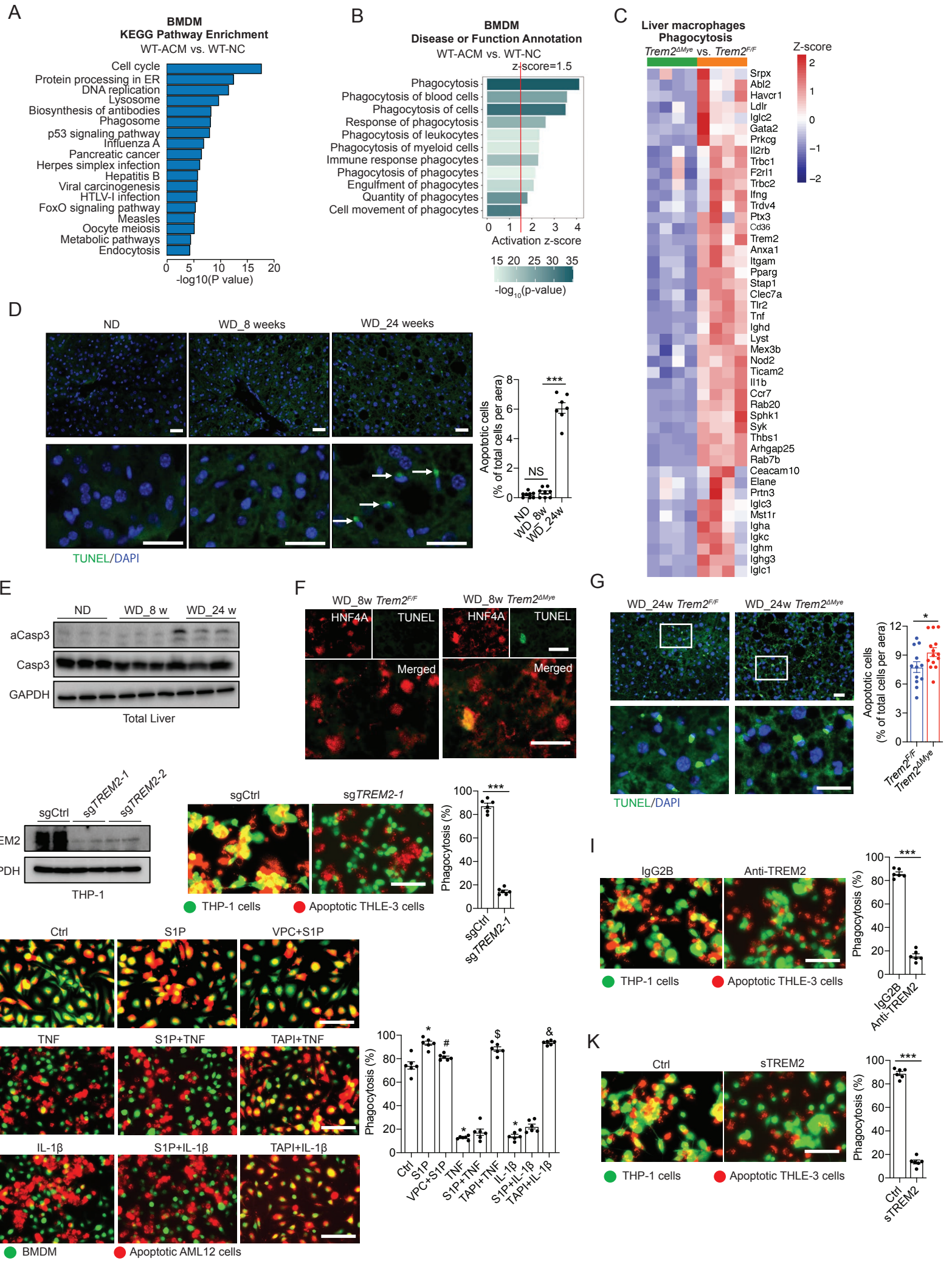
A
BMDM
KEGG Pathway Enrichment
WT-ACM vs. WT-NC
Cell cycle
Protein processing in ER
DNA replication
Lysosome
Biosynthesis of antibodies
Phagosome
p53 signaling pathway
Influenza A
Pancreatic cancer
Herpes simplex infection
Hepatitis B
Viral carcinogenesis
HTLV-I infection
FoxO signaling pathway
Measles
Oocyte meiosis
Metabolic pathways
Endocytosis
0 5 10 15 20
-log10(P value)
B
BMDM
Disease or Function Annotation
WT-ACM vs. WT-NC
z-score=1.5
Phagocytosis
Phagocytosis of blood cells
Phagocytosis of cells
Response of phagocytosis
Phagocytosis of leukocytes
Phagocytosis of myeloid cells
Immune response phagocytes
Phagocytosis of phagocytes
Engulfment of phagocytes
Quantity of phagocytes
Cell movement of phagocytes
0 1 2 3 4
Activation z-score
15 20 25 30 35
-log10(p-value)
C
Liver macrophages
Phagocytosis
Trem2ΔMye vs. Trem2F/F
Z-score
2
1
0
−1
−2
Srpx
Abl2
Havcr1
Ldlr
Iglc2
Gata2
Prkcg
Il2rb
Trbc1
F2rl1
Trbc2
Ifng
Trdv4
Ptx3
Cd36
Trem2
Anxa1
Itgam
Pparg
Stap1
Clec7a
Tlr2
Tnf
Ighd
Lyst
Mex3b
Nod2
Ticam2
Il1b
Ccr7
Rab20
Sphk1
Syk
Thbs1
Arhgap25
Rab7b
Ceacam10
Elane
Prtn3
Iglc3
Mst1r
Igha
Igkc
Ighm
Ighg3
Iglc1
D
ND
WD_8 weeks
WD_24 weeks
TUNEL/DAPI
Aopototic cells
(% of total cells per aera)
***
NS
ND
WD_8w
WD_24w
E
ND
WD_8 w
WD_24 w
aCasp3
Casp3
GAPDH
Total Liver
F
WD_8w Trem2F/F
WD_8w Trem2ΔMye
HNF4A
TUNEL
Merged
G
WD_24w Trem2F/F
WD_24w Trem2ΔMye
TUNEL/DAPI
Aopototic cells
(% of total cells per aera)
*
Trem2F/F
Trem2ΔMye
H
sgCtrl
sgTREM2-1
sgTREM2-2
TREM2
GAPDH
THP-1
sgCtrl
sgTREM2-1
Phagocytosis (%)
***
THP-1 cells
Apoptotic THLE-3 cells
I
IgG2B
Anti-TREM2
Phagocytosis (%)
***
THP-1 cells
Apoptotic THLE-3 cells
J
Ctrl
S1P
VPC+S1P
TNF
S1P+TNF
TAPI+TNF
IL-1β
S1P+IL-1β
TAPI+IL-1β
Phagocytosis (%)
Ctrl
S1P
VPC+S1P
TNF
S1P+TNF
TAPI+TNF
IL-1β
S1P+IL-1β
TAPI+IL-1β
BMDM
Apoptotic AML12 cells
K
Ctrl
sTREM2
Phagocytosis (%)
***
THP-1 cells
Apoptotic THLE-3 cells

Figure S5. TREM2 is essential for macrophage efferocytosis of lipid-laden apoptotic hepatocytes, Related to Figure 5

(A and B) KEGG pathway enrichment analysis of differentially expressed genes (A) and IPA analysis on phagocytosis related functions (B) in WT BMDMs treated with control medium (NC) or cell culture medium collected from apoptotic AML12 cells (ACM). n = 4 per group.
(C) Heatmap of phagocytosis-related genes (GO:0006909) that were downregulated in primary liver macrophages isolated from WD fed *Trem2*$^{\Delta Mye}$ mice compared to cells from WD fed *Trem2*$^{F/F}$ mice. n = 4 mice per group.
(D) Representative TUNEL staining of liver sections from C57BL/6 mice that were fed with ND (n = 8 mice), WD for 8 (n = 8 mice), and 24 (n = 7 mice) weeks. Scale bar, 100 μm. Apoptotic cells were quantified as percentage of total cells per area. Data are shown as mean ± s.e.m.. ***p<0.001; NS, not significant.
(E) Immunoblot analysis of aCasp3 (cleaved caspase-3), Casp3 (caspase-3), and GAPDH in liver tissue from C57BL/6 mice fed with ND or WD for 8 and 24 weeks (n = 3 mice per group).
(F) Representative HNF4A and TUNEL co-staining of liver sections from *Trem2*$^{F/F}$ and *Trem2*$^{\Delta Mye}$ mice fed with WD for 8 weeks. Scale bar, 100μm.
(G) Representative TUNEL staining of liver sections from *Trem2*$^{F/F}$ and *Trem2*$^{\Delta Mye}$ mice fed with WD for 24 weeks. Apoptotic cells were quantified as percentage of total cells per area. Scale bar, 100μm. Data are shown as mean ± s.e.m.. *p<0.05.
(H) PA-treated apoptotic THLE-3 cells (labeled in red) were cocultured with THP-1 cells (labeled in green) transduced with lentiviral constructs expressing sgCtrl or *sgTREM2*. Immunoblot analysis (left panel) of TREM2 and GAPDH indicates successful deletion using two different sgRNAs against *TREM2*. Efferocytosis was quantified as the percentage of THP-1 cells engulfing apoptotic THLE-3 cells. n = 6 per group; scale bar, 100 μm. Data are shown as mean ± s.e.m.. ***p<0.001; NS, not significant.
(I) PA-treated apoptotic THLE-3 cells (labeled in red) were cocultured with THP-1 cells (labeled in green) pretreated with control (IgG2B) or TREM2 neutralizing antibody (Anti-TREM2, 200 ng/ml). Efferocytosis was quantified as the percentage of THP-1 cells engulfing apoptotic THLE-3 cells. n = 6 per group; scale bar, 100 μm. Data are shown as mean ± s.e.m.. ***p<0.001.
(J) PA-treated apoptotic AML12 cells (labeled in red) were cocultured with WT BMDMs (labeled in green) pretreated with VPC (S1PR1/3 inhibitor, 10 μM), S1P (500 nM) or TAPI (ADAM17 inhibitor, 10 μM) for 1 hour followed by stimulation with S1P (500 nM), TNF (1 ng/ml), or IL-1β (1 ng/ml). Efferocytosis was quantified as the percentage of BMDMs engulfing apoptotic AML12 cells. n = 6 per group; scale bar, 100 μm. Data are shown as mean ± s.e.m.. *p < 0.05 vs. Ctrl group; #p < 0.05 vs. S1P treated group; $p < 0.05 vs. TNF treated group; &p < 0.05 vs. IL-1β treated group.
(K) THP-1 cells (labeled in green) were cocultured with THLE-3 cells (labeled in red) treated with PA to induce apoptosis followed by incubation with recombinant sTREM2 (200 ng/ml). Efferocytosis was quantified as the percentage of THP-1 cells engulfing apoptotic THLE-3 cells. n = 6 per group; scale bar, 100 μm. Data are shown as mean ± s.e.m.. ***p<0.001.
The in vitro experiments in this Figure are representative of at least three independent experiments.

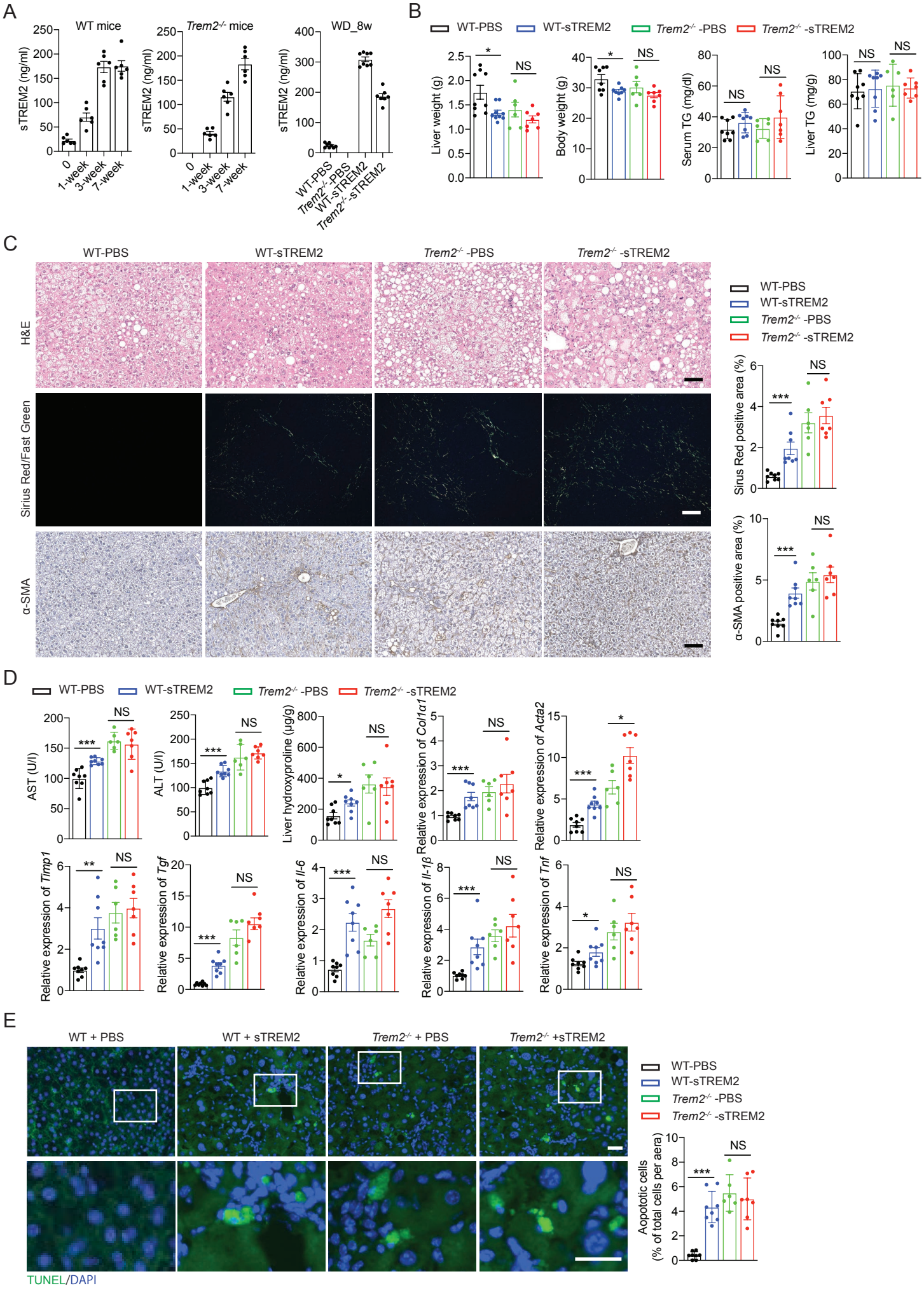
A
WT mice
Trem2-/- mice
WD_8w
sTREM2 (ng/ml)
0
1-week
3-week
7-week
WT-PBS
Trem2-/- -PBS
WT-sTREM2
Trem2-/- -sTREM2
B
WT-PBS
WT-sTREM2
Trem2-/- -PBS
Trem2-/- -sTREM2
Liver weight (g)
Body weight (g)
Serum TG (mg/dl)
Liver TG (mg/g)
NS
C
WT-PBS
WT-sTREM2
Trem2-/- -PBS
Trem2-/- -sTREM2
H&E
Sirius Red/Fast Green
α-SMA
Sirus Red positive area (%)
α-SMA positive area (%)
D
AST (U/l)
ALT (U/l)
Liver hydroxyproline (μg/g)
Relative expression of Col1α1
Relative expression of Acta2
Relative expression of Timp1
Relative expression of Tgf
Relative expression of Il-6
Relative expression of Il-1β
Relative expression of Tnf
E
WT + PBS
WT + sTREM2
Trem2-/- + PBS
Trem2-/- +sTREM2
TUNEL/DAPI
Aopototic cells (% of total cells per aera)

Figure S6. Absence of sTREM2 did not contribute to the exacerbated NASH pathology in WD-fed *Trem2*$^{-/-}$ mice, related to Figure 6

Trem2$^{-/-}$ mice and their WT littermates were fed with WD for 8 weeks and treated with either PBS or sTREM2 (weekly i.v. injection, 1 μg/mouse). WT-PBS, n = 7 mice; *Trem2*$^{-/-}$-PBS, n = 6 mice; WT-sTREM2, n = 7 mice; *Trem2*$^{-/-}$-sTREM2, n = 7 mice.

(A) sTREM2 amounts in mouse sera were quantified by ELISA.

(B) Liver weights, body weights, serum TG, and liver TG were analyzed. Data are shown as mean ± s.e.m.. *p<0.05; NS, not significant.

(C) Representative H&E, Sirius Red/Fast Green, and α-SMA staining of liver sections. Sirius Red/Fast Green staining was recorded under polarized light. Sirius Red and α-SMA positive areas were quantified by ImageJ Software. Scale bar, 100 μm. Data are shown as mean ± s.e.m.. ***p<0.001; NS, not significant.

(D) Serum ALT, AST and hepatic hydroxyproline in liver tissue were measured. Relative mRNA expression of fibrosis-related genes, inflammatory genes, and *Trem2* was measured by RT-qPCR in liver tissue. Data are shown as mean ± s.e.m.. *p<0.05; **p<0.01; ***p<0.001; NS, not significant.

(E) Apoptotic cells in liver tissue were stained with TUNEL and quantified as the percentage of total cells per area (TUNEL, green; DAPI, blue). Scale bar, 100 μm. Data are shown as mean ± s.e.m.. ***p<0.001; NS, not significant.

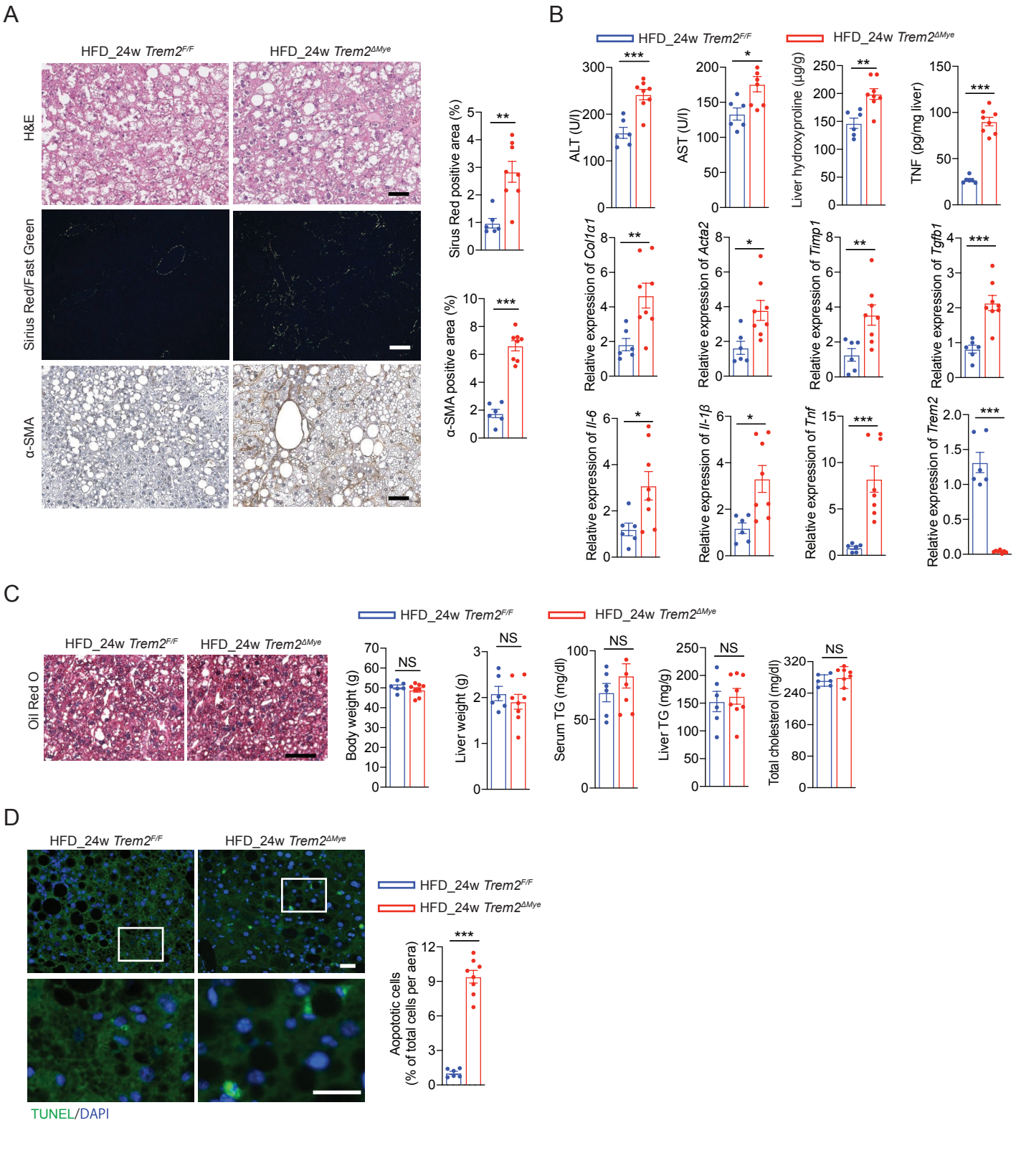
A
HFD_24w Trem2F/F
HFD_24w Trem2ΔMye
H&E
Sirius Red/Fast Green
α-SMA
Sirus Red positive area (%)
α-SMA positive area (%)
B
HFD_24w Trem2F/F
HFD_24w Trem2ΔMye
ALT (U/l)
AST (U/l)
Liver hydroxyproline (μg/g)
TNF (pg/mg liver)
Relative expression of Col1α1
Relative expression of Acta2
Relative expression of Timp1
Relative expression of Tgfb1
Relative expression of Il-6
Relative expression of Il-1β
Relative expression of Tnf
Relative expression of Trem2
C
HFD_24w Trem2F/F
HFD_24w Trem2ΔMye
Oil Red O
Body weight (g)
Liver weight (g)
Serum TG (mg/dl)
Liver TG (mg/g)
Total cholesterol (mg/dl)
NS
D
HFD_24w Trem2F/F
HFD_24w Trem2ΔMye
Aopototic cells (% of total cells per aera)
TUNEL/DAPI

Figure S7. HFD feeding is sufficient to induce NASH development in *Trem2$^{\Delta Mye}$* mice, related to Figure 7

(A) Representative H&E, Sirius Red/Fast Green, and α-SMA staining of liver sections from *Trem2$^{F/F}$* and *Trem2$^{\Delta Mye}$* mice that were fed with HFD for 24 weeks. Scale bar, 100 μm. *Trem2$^{F/F}$*, n = 6 mice; *Trem2$^{\Delta Mye}$*, n = 8 mice. Sirius Red/Fast Green staining was detected under polarized light. Sirius Red and α-SMA positive areas were quantified by ImageJ Software. Data are shown as mean ± s.e.m.. **p<0.01; ***p<0.001.
(B) Serum ALT, AST, hepatic hydroxyproline, and TNF amounts in liver tissue from mice as in (A) were quantified. Relative mRNA expression of fibrosis-related genes, inflammatory genes, and *Trem2* were measured by RT-qPCR in liver tissue. *Trem2$^{F/F}$*, n = 6 mice; *Trem2$^{\Delta Mye}$*, n = 8 mice. Data are shown as mean ± s.e.m.. *p<0.05; **p<0.01; ***p<0.001.
(C) Representative Oil Red O staining of liver sections from mice as in (A), scale bar, 100 μm. Body weights, liver weights, serum TG, liver TG, and total cholesterol in these mice were also analyzed. *Trem2$^{F/F}$*, n = 6 mice; *Trem2$^{\Delta Mye}$*, n = 8 mice. Data are shown as mean ± s.e.m.. NS, not significant.
(D) Apoptotic cells in liver tissue from mice as in (A) were stained with TUNEL and quantified as the percentage of total cells per area (TUNEL, green; DAPI, blue). Scale bar, 100 μm. *Trem2$^{F/F}$*, n = 6 mice; *Trem2$^{\Delta Mye}$*, n = 8 mice. Data are shown as mean ± s.e.m.. ***p<0.001.

Table S1. NAFLD activity score and fibrosis stage of mice treated with western diet, related to Figure 1.

Treatment	ND	WD_8 weeks	WD_24 weeks
Steatosis	0.0 (0.0)	1.9 (0.3)	3.0 (0.0)**
Lobular inflammation	0.0 (0.0)	0.6 (0.3)	1.9 (0.1)**
Ballooning score	0.0 (0.0)	0.0 (0.0)	1.1(0.3)**
NAFLD activity score	0.0 (0.0)	2.1 (0.4)	6.0 (0.3)***
Fibrosis stage	0.0 (0.0)	0.1(0.1)	2.4 (0.2)***

Results were expressed as mean ± SEM and were compared by t test. ND: n = 8 mice, WD_8 weeks: n = 8 mice, WD_24 weeks: n = 7 mice. NAFLD, non-alcoholic fatty liver disease; ND, normal diet; WD, western diet. **p <0.01, ***p <0.001 WD_24 weeks vs. WD_8 weeks.

Table S2. Patient samples used for immunofluorescence staining, related to Figure 3.

Histology	ID	Date of collection	Gender	Age	Steatosis	Ballooning	Fibrosis	Inflammation	NAS score	Source
Normal	4859712	11/14/18	Female	42	0	0	0	0	0	NJDH
	4861745	11/21/18	Female	52	0	0	0	0	0	NJDH
	4862183	11/21/18	Male	46	0	0	0	0	0	NJDH
	4862845	11/28/18	Male	53	0	0	0	0	0	NJDH
	4865098	12/4/18	Female	32	0	0	0	0	0	NJDH
	4865259	12/5/18	Female	46	0	0	0	0	0	NJDH
	4845682	12/12/18	Female	59	0	0	0	0	0	NJDH
	4867190	12/19/18	Female	56	0	0	0	0	0	NJDH
	4869307	12/19/18	Male	56	0	0	0	0	0	NJDH
	4868954	12/27/18	Female	65	0	0	0	0	0	NJDH
	4871906	12/28/18	Male	53	0	0	0	0	0	NJDH
	4870173	12/28/18	Male	42	0	0	0	0	0	NJDH
	4870135	12/28/18	Male	56	0	0	0	0	0	NJDH
	4871156	12/28/18	Female	38	0	0	0	0	0	NJDH
	4872029	1/8/19	Male	60	0	0	0	0	0	NJDH
	4874283	1/14/19	Male	63	0	0	0	0	0	NJDH
	4881381	2/13/19	Female	45	0	0	0	0	0	NJDH
	4881887	2/15/19	Female	61	0	0	0	0	0	NJDH
	4880990	2/15/19	Female	51	0	0	0	0	0	NJDH
	4882146	2/19/19	Male	44	0	0	0	0	0	NJDH
	ev341L	7/31/17	Female	52	0	0	0	0	0	MSSM
	ev355L	9/11/17	Female	61	0	0	0	0	0	MSSM
	ev377L	11/2/17	Female	NA	0	0	0	0	0	MSSM
	ev404L	2/8/18	Male	80	0	0	0	0	0	MSSM
	ev421L	4/12/18	Male	72	0	0	0	0	0	MSSM
	ev497L	11/27/19	Male	50	0	0	0	0	0	UTSW
Steatosis	4940478	11/1/19	Female	40	NA	NA	NA	NA	NA	NJDH
	4947448	12/4/19	Male	44	NA	NA	NA	NA	NA	NJDH
	4950436	12/20/19	Male	47	NA	NA	NA	NA	NA	NJDH
	4951557	12/26/19	Female	60	NA	NA	NA	NA	NA	NJDH
	4969248	5/15/20	Female	59	NA	NA	NA	NA	NA	NJDH
	4905743	5/15/20	Female	45	NA	NA	NA	NA	NA	NJDH
	4484117	6/12/20	Male	54	NA	NA	NA	NA	NA	NJDH
	4977176	6/23/20	Male	33	NA	NA	NA	NA	NA	NJDH
	4980541	7/10/20	Male	37	NA	NA	NA	NA	NA	NJDH
	4981115	7/14/20	Female	32	NA	NA	NA	NA	NA	NJDH
	4986296	8/7/20	Male	60	NA	NA	NA	NA	NA	NJDH
	4986857	8/14/20	Male	67	NA	NA	NA	NA	NA	NJDH
	4993740	9/11/20	Female	60	NA	NA	NA	NA	NA	NJDH
	4878616	10/16/20	Female	64	NA	NA	NA	NA	NA	NJDH
	5019914	1/26/21	Female	36	NA	NA	NA	NA	NA	NJDH
	5027712	3/5/21	Male	53	NA	NA	NA	NA	NA	NJDH
	4899542	4/26/19	Male	57	NA	NA	NA	NA	NA	NJDH
	4709041	7/12/19	Male	31	NA	NA	NA	NA	NA	NJDH
	4922136	7/26/19	Female	38	NA	NA	NA	NA	NA	NJDH
	4922513	7/30/19	Female	45	NA	NA	NA	NA	NA	NJDH
	ev064L	2/12/15	Male	62	1	1	2	2	4	MSSM
	ev279L	4/13/17	Female	55	2	0	0	1	3	MSSM
	ev288L	4/25/17	Female	54	1	0	0	0	1	MSSM
	ev313L	6/8/17	NA	NA	1	0	0	0	1	MSSM
	ev320L	6/19/17	Female	72	1	0	0	0	1	MSSM
	ev328L	7/13/17	Male	61	2	0	1c	1	3	MSSM
	ev494L	NA	NA	NA	1	0	1c	0	1	UTSW
	ev516L	2/6/20	NA	NA	2	0	4	1	3	UTSW
NASH	4475275	11/11/16	Female	43	3	1	3	2	9	NJDH
	4992833	9/8/20	Female	45	3	1	3	1	8	NJDH
	4722154	1/3/17	Female	67	3	2	2	2	9	NJDH
	4743297	5/4/17	Female	35	3	2	2	2	9	NJDH
	4725350	8/11/17	Female	48	3	2	2	2	9	NJDH
	4766251	9/8/17	Female	51	3	2	2	2	9	NJDH
	4980849	7/14/20	Male	37	3	1	2	2	8	NJDH
	4421813	3/23/17	Male	44	3	1	2	1	7	NJDH

	4236607	4/26/17	Female	65	2	2	2	1	7	NJDH
	4781173	11/15/17	Female	43	2	1	2	2	7	NJDH
	4852038	10/11/18	Male	51	3	2	1	2	8	NJDH
	4995309	12/29/20	Male	28	3	1	1	3	8	NJDH
	4679596	5/13/16	Male	35	3	2	1	1	7	NJDH
	4714550	11/25/16	Female	57	3	1	1	2	7	NJDH
	4807723	7/27/18	Female	29	3	2	1	1	7	NJDH
	4847573	9/17/18	Female	37	3	1	1	2	7	NJDH
	4965941	4/28/20	Male	41	3	1	1	2	7	NJDH
	4989971	8/25/20	Female	49	3	1	1	2	7	NJDH
	5000705	10/20/20	Male	54	3	1	1	2	7	NJDH
	4733307	3/7/17	Male	32	3	1	1	1	6	NJDH
	ev112L	6/29/15	Male	64	2	1	2	2	5	MSSM
	ev310L	6/2/17	Male	71	2	1	2	2	5	MSSM
	ev311L	6/5/17	Female	52	2	1	2	2	5	MSSM
	ev344L	8/8/17	Female	66	3	1	2	2	5	MSSM
	ev399L	1/11/18	Male	39	3	1	2	1	4	MSSM
	ev549L	11/3/20	Male	25	3	1	2	2	5	UTSW

In the study cohort for TREM2 expression analysis by immunofluorescence assay, 26 healthy controls (20 from NJDH, 5 from MSSM, 1 from UTSW), 28 steatosis (20 from NJDH, 6 from MSSM, 2 from UTSW) and 26 steatohepatitis (20 from NJDH, 5 from MSSM, 1 from UTSW) patient samples were collected during surgical operation between 2015 and 2021. The histological grading and staging of NAFLD and fibrosis were determined according to the published criteria[1]. NJDH, Nanjing Drum Tower Hospital, Nanjing, China; MSSM, Mount Sinai Hospital, New York, NY, USA; UTSW, University of Texas Southwestern Medical Center, Dallas, TX, USA.

Table S3. Patient samples used for ELISA analysis, related to Figure 4.

Histology	ID	Date of collection	Gender	Age	Steatosis	Ballooning	Fibrosis	Inflammation	NAS score	Source
Normal	4859712	11/14/18	Female	42	0	0	0	0	0	NJDH
	4861745	11/21/18	Female	52	0	0	0	0	0	NJDH
	4862183	11/21/18	Male	46	0	0	0	0	0	NJDH
	4862845	11/28/18	Male	53	0	0	0	0	0	NJDH
	4865098	12/4/18	Female	32	0	0	0	0	0	NJDH
	4865259	12/5/18	Female	46	0	0	0	0	0	NJDH
	4845682	12/12/18	Female	59	0	0	0	0	0	NJDH
	4867190	12/19/18	Female	56	0	0	0	0	0	NJDH
	4869307	12/19/18	Male	56	0	0	0	0	0	NJDH
	4868954	12/27/18	Female	65	0	0	0	0	0	NJDH
	4871906	12/28/18	Male	53	0	0	0	0	0	NJDH
	4870173	12/28/18	Male	42	0	0	0	0	0	NJDH
	4870135	12/28/18	Male	56	0	0	0	0	0	NJDH
	4871156	12/28/18	Female	38	0	0	0	0	0	NJDH
	4872029	1/8/19	Male	60	0	0	0	0	0	NJDH
	4874283	1/14/19	Male	63	0	0	0	0	0	NJDH
	4881381	2/13/19	Female	45	0	0	0	0	0	NJDH
	4881887	2/15/19	Female	61	0	0	0	0	0	NJDH
	4880990	2/15/19	Female	51	0	0	0	0	0	NJDH
	4882146	2/19/19	Male	44	0	0	0	0	0	NJDH
Steatosis	4940478	11/1/19	Female	40	NA	NA	NA	NA	NA	NJDH
	4947448	12/4/19	Male	44	NA	NA	NA	NA	NA	NJDH
	4950436	12/20/19	Male	47	NA	NA	NA	NA	NA	NJDH
	4951557	12/26/19	Female	60	NA	NA	NA	NA	NA	NJDH
	4969248	5/15/20	Female	59	NA	NA	NA	NA	NA	NJDH
	4905743	5/15/20	Female	45	NA	NA	NA	NA	NA	NJDH
	4484117	6/12/20	Male	54	NA	NA	NA	NA	NA	NJDH
	4977176	6/23/20	Male	33	NA	NA	NA	NA	NA	NJDH
	4980541	7/10/20	Male	37	NA	NA	NA	NA	NA	NJDH
	4981115	7/14/20	Female	32	NA	NA	NA	NA	NA	NJDH
	4986296	8/7/20	Male	60	NA	NA	NA	NA	NA	NJDH
	4986857	8/14/20	Male	67	NA	NA	NA	NA	NA	NJDH
	4993740	9/11/20	Female	60	NA	NA	NA	NA	NA	NJDH
	4878616	10/16/20	Female	64	NA	NA	NA	NA	NA	NJDH
	5019914	1/26/21	Female	36	NA	NA	NA	NA	NA	NJDH
	5027712	3/5/21	Male	53	NA	NA	NA	NA	NA	NJDH
	4899542	4/26/19	Male	57	NA	NA	NA	NA	NA	NJDH
	4709041	7/12/19	Male	31	NA	NA	NA	NA	NA	NJDH
	4922136	7/26/19	Female	38	NA	NA	NA	NA	NA	NJDH
	4922513	7/30/19	Female	45	NA	NA	NA	NA	NA	NJDH
NASH	4475275	11/11/16	Female	43	3	1	3	2	9	NJDH
	4992833	9/8/20	Female	45	3	1	3	1	8	NJDH
	4722154	1/3/17	Female	67	3	2	2	2	9	NJDH
	4743297	5/4/17	Female	35	3	2	2	2	9	NJDH
	4725350	8/11/17	Female	48	3	2	2	2	9	NJDH
	4766251	9/8/17	Female	51	3	2	2	2	9	NJDH
	4980849	7/14/20	Male	37	3	1	2	2	8	NJDH
	4421813	3/23/17	Male	44	3	1	2	1	7	NJDH
	4236607	4/26/17	Female	65	2	2	2	1	7	NJDH
	4781173	11/15/17	Female	43	2	1	2	2	7	NJDH
	4852038	10/11/18	Male	51	3	2	1	2	8	NJDH
	4995309	12/29/20	Male	28	3	1	1	3	8	NJDH
	4679596	5/13/16	Male	35	3	2	1	1	7	NJDH
	4714550	11/25/16	Female	57	3	1	1	2	7	NJDH
	4807723	7/27/18	Female	29	3	2	1	1	7	NJDH
	4847573	9/17/18	Female	37	3	1	1	2	7	NJDH
	4965941	4/28/20	Male	41	3	1	1	2	7	NJDH
	4989971	8/25/20	Female	49	3	1	1	2	7	NJDH
	5000705	10/20/20	Male	54	3	1	1	2	7	NJDH
	4733307	3/7/17	Male	32	3	1	1	1	6	NJDH

In the study cohort for quantification of circulating sTREM2 amounts by ELISA assay, 20 healthy, 20 steatosis and 20 steatohepatitis patient samples were collected from NJDH during surgical operation between 2015 and 2021. The histological grading and staging of NAFLD and fibrosis were determined according to the published criteria[1]. NJDH, Nanjing Drum Tower Hospital, Nanjing, China.

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