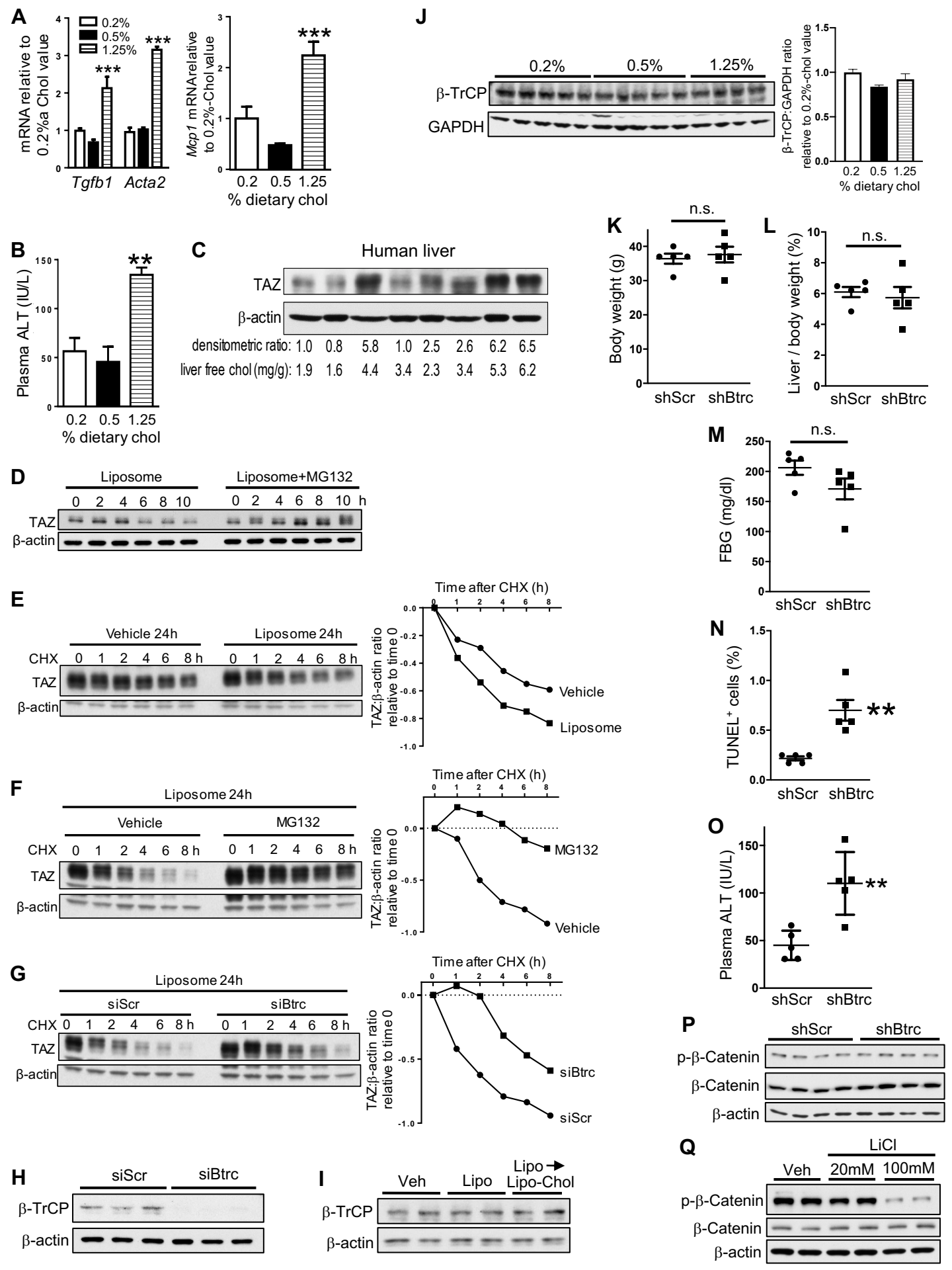
A
0.2%
0.5%
1.25%
mRNA relative to 0.2%a Chol value
***
***
Tgfb1
Acta2
Mcp1 mRNArelative to 0.2%-Chol value
***
0.2 0.5 1.25
% dietary chol
J
0.2%
0.5%
1.25%
β-TrCP
GAPDH
β-TrCP:GAPDH ratio relative to 0.2%-chol value
0.2 0.5 1.25
% dietary chol
B
Plasma ALT (IU/L)
**
0.2 0.5 1.25
% dietary chol
C
Human liver
TAZ
β-actin
densitometric ratio: 1.0 0.8 5.8 1.0 2.5 2.6 6.2 6.5
liver free chol (mg/g): 1.9 1.6 4.4 3.4 2.3 3.4 5.3 6.2
K
n.s.
Body weight (g)
shScr shBtrc
L
n.s.
Liver / body weight (%)
shScr shBtrc
M
n.s.
FBG (mg/dl)
shScr shBtrc
D
Liposome
Liposome+MG132
0 2 4 6 8 10 0 2 4 6 8 10 h
TAZ
β-actin
E
Vehicle 24h
Liposome 24h
CHX 0 1 2 4 6 8 h 0 1 2 4 6 8 h
TAZ
β-actin
Time after CHX (h)
TAZ:β-actin ratio relative to time 0
Vehicle
Liposome
N
TUNEL+ cells (%)
**
shScr shBtrc
F
Liposome 24h
Vehicle
MG132
CHX 0 1 2 4 6 8 h 0 1 2 4 6 8 h
TAZ
β-actin
Time after CHX (h)
TAZ:β-actin ratio relative to time 0
MG132
Vehicle
O
Plasma ALT (IU/L)
**
shScr shBtrc
G
Liposome 24h
siScr
siBtrc
CHX 0 1 2 4 6 8 h 0 1 2 4 6 8 h
TAZ
β-actin
Time after CHX (h)
TAZ:β-actin ratio relative to time 0
siBtrc
siScr
P
shScr
shBtrc
p-β-Catenin
β-Catenin
β-actin
H
siScr
siBtrc
β-TrCP
β-actin
I
Veh
Lipo
Lipo → Lipo-Chol
β-TrCP
β-actin
Q
LiCl
Veh
20mM
100mM
p-β-Catenin
β-Catenin
β-actin

Figure S1. Related to Figure 1. TAZ in Human NASH Liver, Evidence that Cholesterol Stabilizes TAZ Against Proteasomal Degradation, and Further Characterization of NAFLD Mice Treated with AAV8-H1-shBtrc.

(A-B) The following parameters were measured in mice fed the NAFLD diet containing 0.2%, 0.5%, and 1.25% cholesterol for 16 weeks (n = 5 mice/group; means ± SEM; **p < 0.01, ***p < 0.001):

(A) Liver *Tgfb1*, *Acta2* (α-SMA), and *Mcp1* mRNAs.

(B) Plasma ALT.

(C) TAZ immunoblot of human liver sections from the 8 subjects in Figure 1D, with densitometric ratio of TAZ:β-actin and liver free cholesterol content shown below each lane.

(D) TAZ immunoblot of AML12 cells treated for 0-10 h with liposomes ± 10 nM MG132.

(E) TAZ immunoblot, with quantification, of AML12 cells that were incubated for 24 h with vehicle or liposomes, with 10 ng/ml cycloheximide (CHX) included for 0-8 h during the end of this period.

(F) TAZ immunoblot, with quantification, of AML12 cells that were incubated for 24 h with vehicle or liposomes, with 10 ng/ml cycloheximide (CHX) ± 10 nM MG132 included for 0-8 h during the end of this period.

(G) TAZ immunoblot of siSrc- or siBtrc-transfected AML12 cells that were incubated for 24 h with liposomes, with 10 ng/ml cycloheximide (CHX) included for 0-8 h during the end of this period.

(H) β-TrCP immunoblot of AML12 cells transfected with siScr or siBtrc.

(I) β-TrCP immunoblot of AML12 cells that were incubated for 24 h with vehicle or liposomes, or 16 h with liposomes followed by 8 h with Lipo-Chol.

(J) β-TrCP immunoblot of liver extracts, with quantification, from mice fed 16 weeks with the NAFLD diet containing 0.2%, 0.5%, and 1.25% cholesterol.

(K-P) The following parameters were measured in mice fed the NAFLD diet containing 0.2% cholesterol for 16 weeks, with AAV8-H1-shBtrc or AAV8-H1-shScr injected at the 8-week time point (n = 5 mice/group; means ± SEM; **p < 0.01; n.s., non-significant):

(K) Body weight.

(L) Liver weight as a percentage of body weight.

(M) Fasting plasma glucose.

(N) $TUNEL^{+}$ cells in the liver.

(O) Plasma ALT.

(P) Immunoblots of phospho-$Ser^{31,37}Thr^{41}$ and total β-catenin of liver extracts.

(Q) Immunoblots of phospho-$Ser^{31,37}Thr^{41}$ and total β-catenin of AML12 cells that were incubated for 4 h with vehicle or 20-100 mM LiCl.

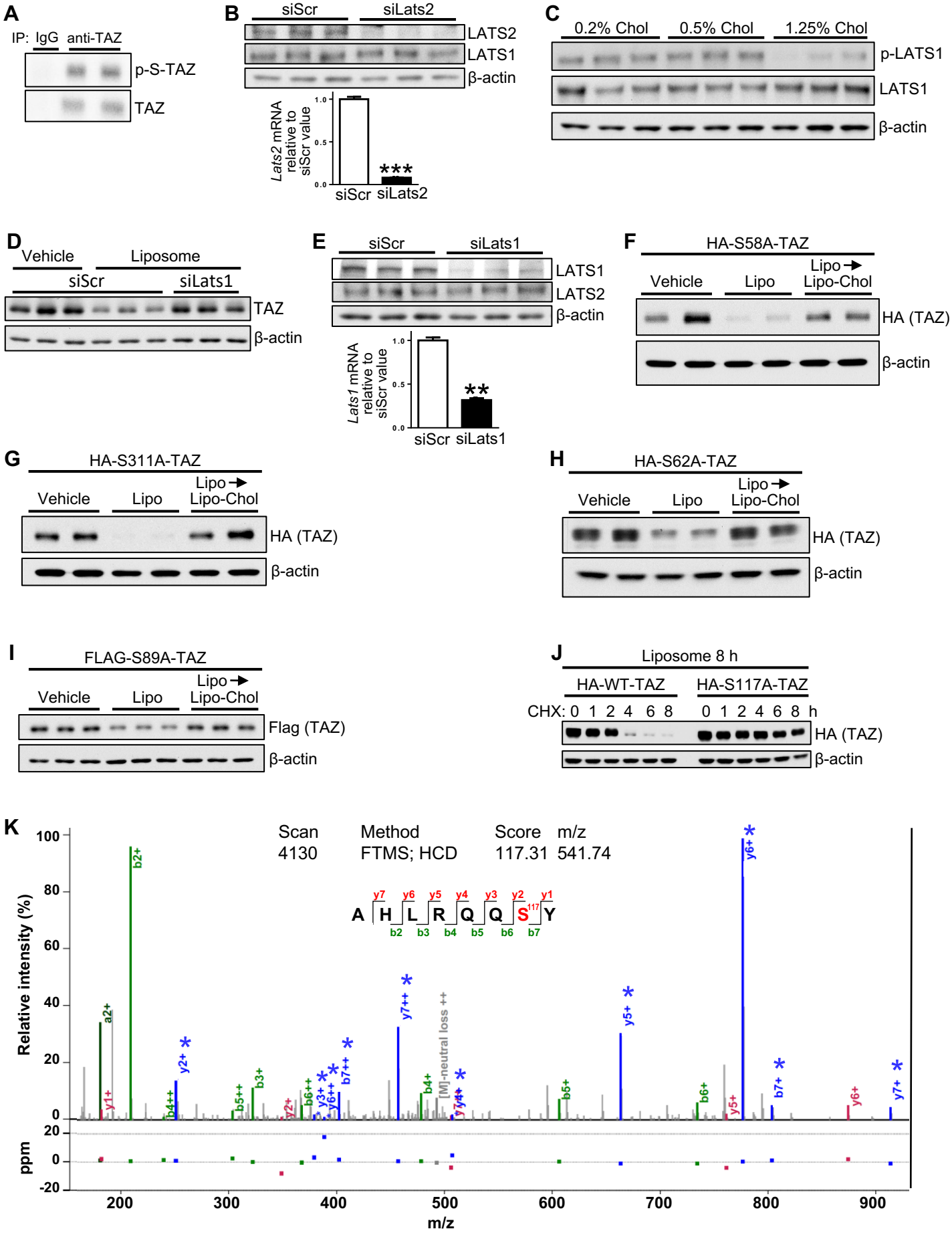
A
IP: IgG anti-TAZ
p-S-TAZ
TAZ
B
siScr siLats2
LATS2
LATS1
β-actin
Lats2 mRNA relative to siScr value
***
siScr siLats2
C
0.2% Chol 0.5% Chol 1.25% Chol
p-LATS1
LATS1
β-actin
D
Vehicle Liposome
siScr siLats1
TAZ
β-actin
E
siScr siLats1
LATS1
LATS2
β-actin
Lats1 mRNA relative to siScr value
**
siScr siLats1
F
HA-S58A-TAZ
Vehicle Lipo Lipo → Lipo-Chol
HA (TAZ)
β-actin
G
HA-S311A-TAZ
Vehicle Lipo Lipo → Lipo-Chol
HA (TAZ)
β-actin
H
HA-S62A-TAZ
Vehicle Lipo Lipo → Lipo-Chol
HA (TAZ)
β-actin
I
FLAG-S89A-TAZ
Vehicle Lipo Lipo → Lipo-Chol
Flag (TAZ)
β-actin
J
Liposome 8 h
HA-WT-TAZ HA-S117A-TAZ
CHX: 0 1 2 4 6 8 0 1 2 4 6 8 h
HA (TAZ)
β-actin
K
Scan 4130
Method FTMS; HCD
Score 117.31
m/z 541.74
A H L R Q Q S117 Y
Relative intensity (%)
ppm
m/z

Figure S2. **Related to Figure 2. Effect of Individual Serine-Alanine Mutations on Cholesterol-Mediated Increase in TAZ, and Mass Spectroscopy Evidence that TAZ is Phosphorylated on S117.**

(A) Phospho-serine and total TAZ immunoblots in TAZ immunoprecipitates of AML12 cell extracts.

(B) LATS1 and LATS2 immunoblots and *Lats2* mRNA of siScr- or siLats2-transfected AML12 cells (n = 4 biological replicates; values shown are means ± SEM; ***p < 0.001).

(C) Phospho- and total LATS1 immunoblots in liver extracts from mice fed 16 weeks with the NAFLD diet containing 0.2%, 0.5%, or 1.25% cholesterol.

(D) TAZ immunoblot of siScr- or siLats1-transfected AML12 cells that were incubated for 24 h with vehicle or liposomes.

(E) LATS1 and LATS2 immunoblots and *Lats1* mRNA (n = 4 biological replicates; means ± SEM; **p < 0.01).

(F-I) HA- or FLAG-TAZ immunoblots of AML12 cells transfected with the indicated mutant TAZ constructs and then incubated for 24 h with vehicle, or liposomes for 16 h and then Lipo-Chol for 8 h.

(J) HA-TAZ immunoblot of AML12 cells transfected with HA-WT-human TAZ or HA-S117A-human TAZ and then incubated for 8 h, with 10 ng/ml cycloheximide (CHX) included for the indicated times during this period.

(K) LC-MS/MS spectrum analysis of TAZ peptides from HepG2 cells that had been incubated for 4 h with 10 nM MG132 and liposomes. The analysis identified phosphopeptide AHLRQQS^{117}(P)Y, whose sequence is shown with y and b ions indicated and with the phosphorylated serine (S117) indicated in red. The numbers paired with each ion identification, e.g., b3 and y5, indicate the number of amino acids present on N-terminal fragments for b ions and C-terminal fragments for y ions. Peaks in the spectrum that are green correspond to matched b ions and peaks that are red correspond to matched y ions, with good sequence coverage of spectral lines for the y2, y3, y4, y5, y6, y7, and b7 fragments, noted by the blue asterisks.

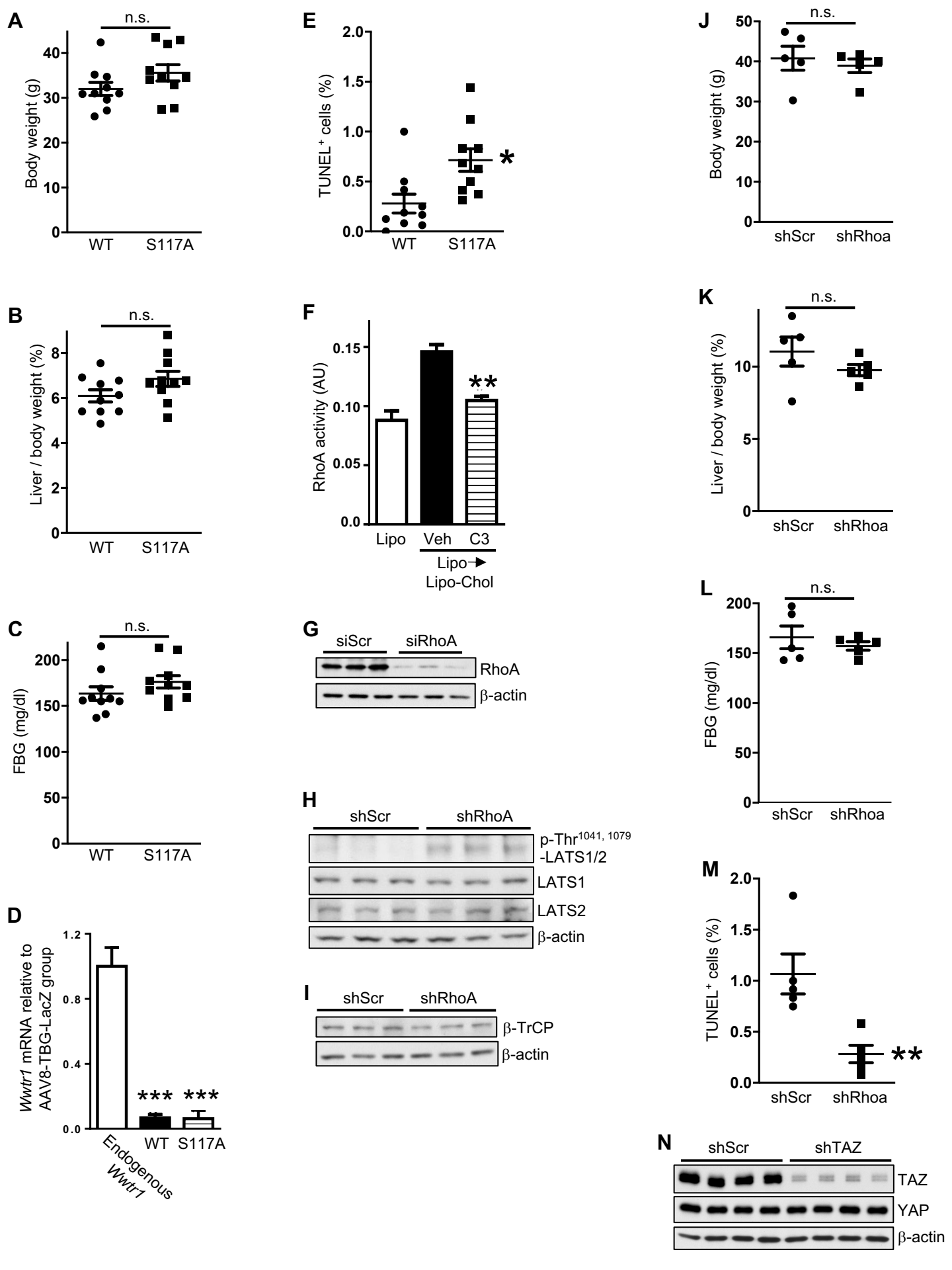
A
n.s.
Body weight (g)
WT
S117A
B
n.s.
Liver / body weight (%)
WT
S117A
C
n.s.
FBG (mg/dl)
WT
S117A
D
Wwtr1 mRNA relative to AAV8-TBG-LacZ group
***
***
WT
S117A
Endogenous Wwtr1
E
TUNEL+ cells (%)
*
WT
S117A
F
RhoA activity (AU)
**
Lipo
Veh
C3
Lipo→
Lipo-Chol
G
siScr
siRhoA
RhoA
β-actin
H
shScr
shRhoA
p-Thr1041, 1079 -LATS1/2
LATS1
LATS2
β-actin
I
shScr
shRhoA
β-TrCP
β-actin
J
n.s.
Body weight (g)
shScr
shRhoa
K
n.s.
Liver / body weight (%)
shScr
shRhoa
L
n.s.
FBG (mg/dl)
shScr
shRhoa
M
TUNEL+ cells (%)
**
shScr
shRhoa
N
shScr
shTAZ
TAZ
YAP
β-actin

Figure S3. Related to Figures 2 and 3. Further Characterization of NASH Mice Transduced with AAV8-TBG-S117A-TAZ or with AAV8-H1-shRhoa.

(A-E) The following parameters were measured in *Wwtr1*$^{fl/fl}$ mice fed the NAFLD diet containing 0.2% cholesterol for 16 weeks, with AAV8-TBG-Cre plus either AAV8-TBG-HA-WT-hTAZ (WT) or AAV8-TBG-HA-S117A-hTAZ (S117A) injected at the 8-week time point (n = 10 mice/group; means ± SEM; *p < 0.05; ***p < 0.001; n.s., non-significant):

(A) Body weight.

(B) Liver weight as a percentage of body weight.

(C) Fasting plasma glucose.

(D) Liver *Wwtr1* mRNA in *Wwtr1*$^{fl/fl}$ mice treated with AAV8-TBG-LacZ instead of AAV8-TBG-Cre and without AAV8-TBG-hTAZ ("Endogenous *Wwtr1*").

(E) TUNEL$^+$ cells in the liver.

(F) RhoA activity of AML12 cells that were incubated for 24 h with liposomes (Lipo) or for 16 h with liposomes and then 8 h with Lipo-Chol ± 1 μg/ml C3 transferase Rho inhibitor (C3) included during the last 4 h (n = 4 biological replicates; means ± SEM; **p < 0.01 versus Veh).

(G) RhoA immunoblot of AML12 cells that were transfected with siScr or siRhoa.

(H-M) The following parameters were assayed in mice fed the NASH diet for 16 weeks, with AAV8-H1-shRhoa or AAV8-H1-shScr administered at the 8-week time point (n = 5 mice/group; values shown are means ± SEM; **p < 0.01; *n.s.*, non-significant):

(H) Liver phospho-Thr$^{1041,\ 1079}$-LATS1/2, LATS1, and LATS2 immunoblots.

(I) Liver β-TrCP immunoblot.

(J) Body weight.

(K) Liver weight as a percentage of body weight.

(L) Fasting plasma glucose.

(M) TUNEL$^+$ cells in the liver.

(N) TAZ and YAP immunoblots in liver extracts from mice fed 16 weeks with the NASH diet, with AAV8-H1-shTAZ or AAV8-H1-shScr administered at the 8-week time point.

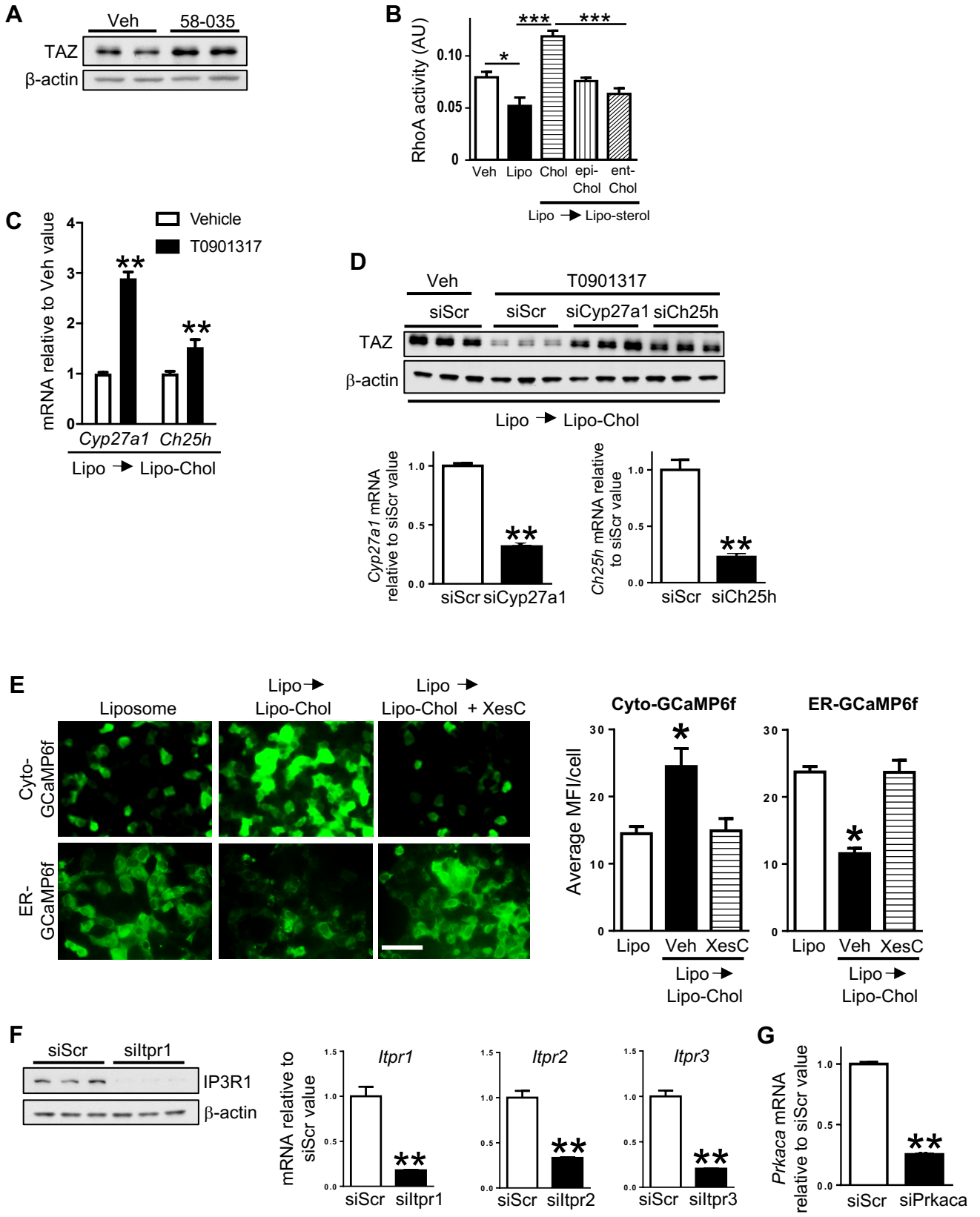
A
Veh
58-035
TAZ
β-actin
B
RhoA activity (AU)
0.10
0.05
0
*
***
***
Veh
Lipo
Chol
epi-Chol
ent-Chol
Lipo → Lipo-sterol
C
Vehicle
T0901317
mRNA relative to Veh value
4
3
2
1
0
**
**
Cyp27a1
Ch25h
Lipo → Lipo-Chol
D
Veh
T0901317
siScr
siScr
siCyp27a1
siCh25h
TAZ
β-actin
Lipo → Lipo-Chol
Cyp27a1 mRNA relative to siScr value
1.0
0.5
0.0
**
siScr
siCyp27a1
Ch25h mRNA relative to siScr value
1.0
0.5
0.0
**
siScr
siCh25h
E
Liposome
Lipo → Lipo-Chol
Lipo → Lipo-Chol + XesC
Cyto-GCaMP6f
ER-GCaMP6f
Cyto-GCaMP6f
ER-GCaMP6f
Average MFI/cell
30
20
10
0
*
*
Lipo
Veh
XesC
Lipo → Lipo-Chol
F
siScr
siItpr1
IP3R1
β-actin
mRNA relative to siScr value
Itpr1
Itpr2
Itpr3
1.5
1.0
0.5
0.0
**
**
**
siScr
siItpr1
siScr
siItpr2
siScr
siItpr3
G
Prkaca mRNA relative to siScr value
1.0
0.5
0.0
**
siScr
siPrkaca

Figure S4. **Related to Figure 4. Cholesterol Specificity of the TAZ Pathway and IP3R-Mediated Calcium Changes.**

(A) TAZ immunoblot of AML12 cells that were incubated for 8 h with vehicle or 30 μM of the ACAT inhibitor 58-035.

(B) RhoA activity of AML12 cells that were incubated for 18 h with vehicle or liposomes, or 16 h with liposomes and then 2 h with sterol-rich liposomes (Lipo → Lipo-sterol) containing cholesterol (Chol), *epi*-cholesterol (epi-Chol), or *ent*-cholesterol (ent-Chol) (*n* = 4 biological replicates; means ± SEM; *p < 0.05, ***p < 0.001).

(C) *Cyp27a1* and *Ch25h* mRNAs in AML12 cells that were incubated for 16 h with liposomes and then 24 h with Lipo-Chol ± 20 μM T0901317 (n = 4 biological replicates; means ± SEM; **p < 0.01).

(D) TAZ immunoblot and *Cyp27a1*, and *Ch25h* mRNA levels of AML12 cells were transfected with siScr, siCyp27a1, or siCh25h and then incubated for 16 h with liposomes followed by 24 h with Lipo-Chol ± 20 μM T0901317 (n = 4 biological replicates; means ± SEM; **p < 0.01).

(E) Images and mean fluorescence intensity (MFI) per cell of AML12 cells transduced with cyto-GCaMP6f or ER-GCaMP6f and then incubated for 16.5 h with liposomes or 16 h with liposomes and then 30 minutes with Lipo-Chol ± 2 μM xestospongin C (XesC) (n = 3 biological replicates; means ± SEM; *p < 0.05 versus other groups). Scale bar, 100 μm.

(F) IP3R1 immunoblot and *Itpr1*, *Itpr2*, and *Itpr3* mRNA levels of AML12 cells that were transfected with siScr, siItpr1, siItpr2, or siItpr3 (n = 4 biological replicates; means ± SEM; **p < 0.01).

(G) *Prkaca* mRNA in AML12 cells that were transfected with siScr or siPrkaca (*n* = 4 biological replicates; means ± SEM; **p < 0.01).

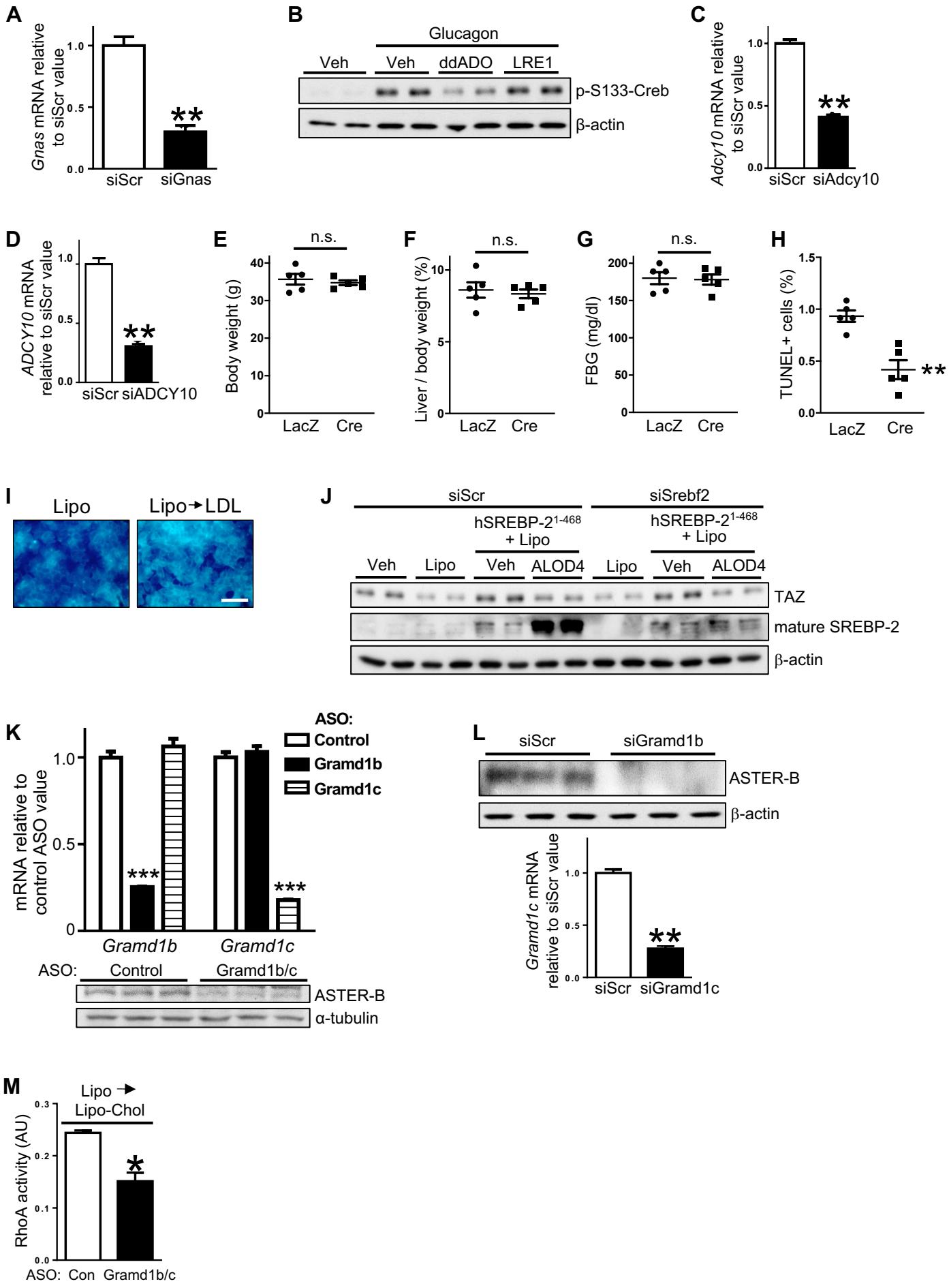
A
Gnas mRNA relative to siScr value
siScr
siGnas
B
Glucagon
Veh
Veh
ddADO
LRE1
p-S133-Creb
β-actin
C
Adcy10 mRNA relative to siScr value
siScr
siAdcy10
D
ADCY10 mRNA relative to siScr value
siScr
siADCY10
E
n.s.
Body weight (g)
LacZ
Cre
F
n.s.
Liver / body weight (%)
LacZ
Cre
G
n.s.
FBG (mg/dl)
LacZ
Cre
H
TUNEL+ cells (%)
LacZ
Cre
I
Lipo
Lipo→LDL
J
siScr
siSrebf2
hSREBP-2 1-468 + Lipo
hSREBP-2 1-468 + Lipo
Veh
Lipo
Veh
ALOD4
Lipo
Veh
ALOD4
TAZ
mature SREBP-2
β-actin
K
ASO:
Control
Gramd1b
Gramd1c
mRNA relative to control ASO value
Gramd1b
Gramd1c
ASO:
Control
Gramd1b/c
ASTER-B
α-tubulin
L
siScr
siGramd1b
ASTER-B
β-actin
Gramd1c mRNA relative to siScr value
siScr
siGramd1c
M
Lipo →
Lipo-Chol
RhoA activity (AU)
ASO: Con
Gramd1b/c

Figure S5. **Related to Figures 5 and 6. Additional Data Related to Adenylyl Cyclase and Gramd1b/c.**

(A) *Gnas* mRNA in AML12 cells were transfected with siScr or siGnas (*n* = 4 biological replicates; means ± SEM; **p < 0.01).

(B) Phospho-S133-Creb immunoblot of primary mouse hepatocytes that were incubated for 30 min with vehicle or 200 nM glucagon ± 10 μM 2′,5′-dideoxyadenosine (ddAdo) or 30 μM LRE1.

(C) *Adcy10* mRNA of AML12 cells that were transfected with siScr or siAdcy10 (n = 4 biological replicates; means ± SEM; **p < 0.01).

(D) *ADCY10* mRNA of primary human hepatocytes that were transfected with siScr or siADCY10 (n = 6 biological replicates; means ± SEM; **p < 0.01).

(E-H) The following parameters were assayed in *Adcy10*$^{fl/fl}$ mice fed the NASH diet for 16 weeks, with AAV8-TBG-Cre or AAV8-TBG-LacZ administered at the 8-week time point (n = 5 mice/group; means ± SEM, ***p* < 0.01, n.s., non-significant):

(E) Body weight.

(F) Liver weight as a percentage of body weight.

(G) Fasting plasma glucose.

(H) TUNEL^{+} cells in the liver.

(I) Filipin staining of AML12 cells that were incubated for 24 h with liposomes or 24 h with liposomes and then 8 h with 100 μg/ml LDL. Scale bar, 100 μm.

(J) TAZ and mature SREBP-2 immunoblots of AML12 cells transfected with siScr or siSrebf2 targeting endogenous SREBP-2 and treated as follows: incubated for 24 h with vehicle or liposomes; or transfected with human SREBP-2(1-468) and then incubated for 24 h with liposomes ± 10 μM ALOD4 included during the last 4 h.

(K) *Grand1b* and *Gramd1c* mRNA levels and ASTER-B immunoblot of AML12 cells were transfected with control ASO or ASO targeting *Gramd1b* or *Gramd1c* (n = 4 biological replicates; means ± SEM; ***p < 0.001 versus other groups).

(L) ASTER-B immunoblot and *Gramd1c* mRNA level of AML12 cells were transfected with siScr or siGramd1b (n = 4 biological replicates; means ± SEM; **p < 0.01).

(M) RhoA activity of AML12 cells that were treated with control ASO or ASO targeting *Gramd1b* and *Gramd1c* and then incubated for 24 h with liposomes followed by 2 h with Lipo-Chol (n = 4 biological replicates; means ± SEM; *p < 0.05).

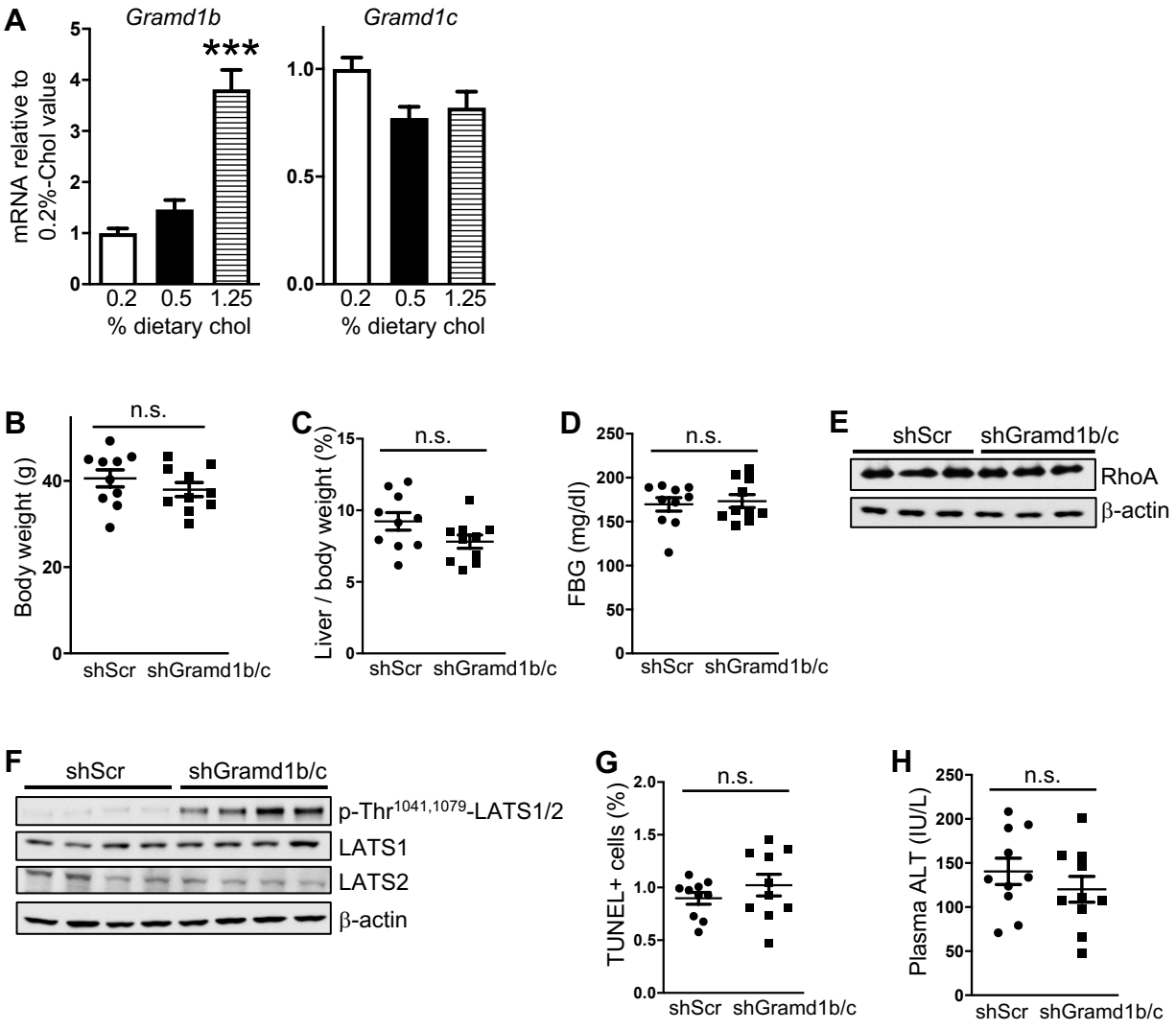

Figure S6. **Related to Figure 7. Further Characterization of NASH Mice Treated with AAV8-H1-shGramd1b/c.**

(A) *Gramd1b* and *Gramd1c* mRNA levels of liver extracts from mice fed 16 weeks with the NAFLD diet containing 0.2%, 0.5%, and 1.25% cholesterol (n = 5; means ± SEM; ***p < 0.001).

(B-H) The following parameters were assayed in mice fed the NASH diet for 16 weeks, with AAV8-H1-shGramd1b/c or AAV8-H1-shSrc administered at the 8-week time point (*n* = 10 mice/group; means ± SEM; *n.s.*, non-significant):

(B) Body weight.

(C) Liver weight as a percentage of body weight.

(D) Fasting plasma glucose.

(E) Liver RhoA immunoblot.

(F) Liver phospho-Thr^{1041,1079}-LATS1/2, LATS1, and LATS2 immunoblots.

(G) TUNEL^{+} cells in the liver.

(H) Plasma ALT.