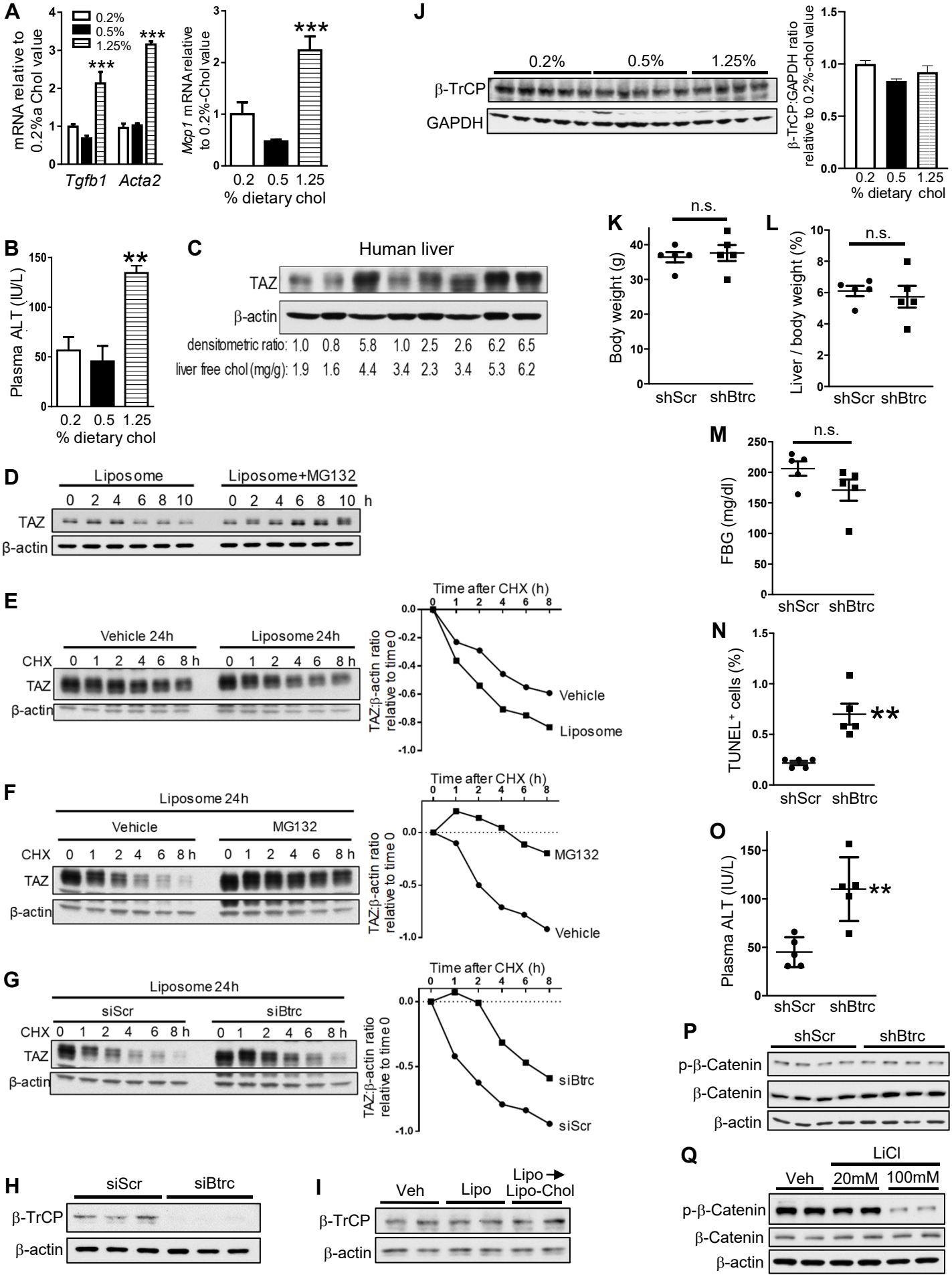
A
0.2%
0.5%
1.25%
mRNA relative to 0.2%a Chol value
***
***
Tgfb1
Acta2
Mcp1 mRNA relative to 0.2%-Chol value
***
0.2 0.5 1.25
% dietary chol
B
Plasma ALT (IU/L)
**
0.2 0.5 1.25
% dietary chol
C
Human liver
TAZ
β-actin
densitometric ratio: 1.0 0.8 5.8 1.0 2.5 2.6 6.2 6.5
liver free chol (mg/g): 1.9 1.6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

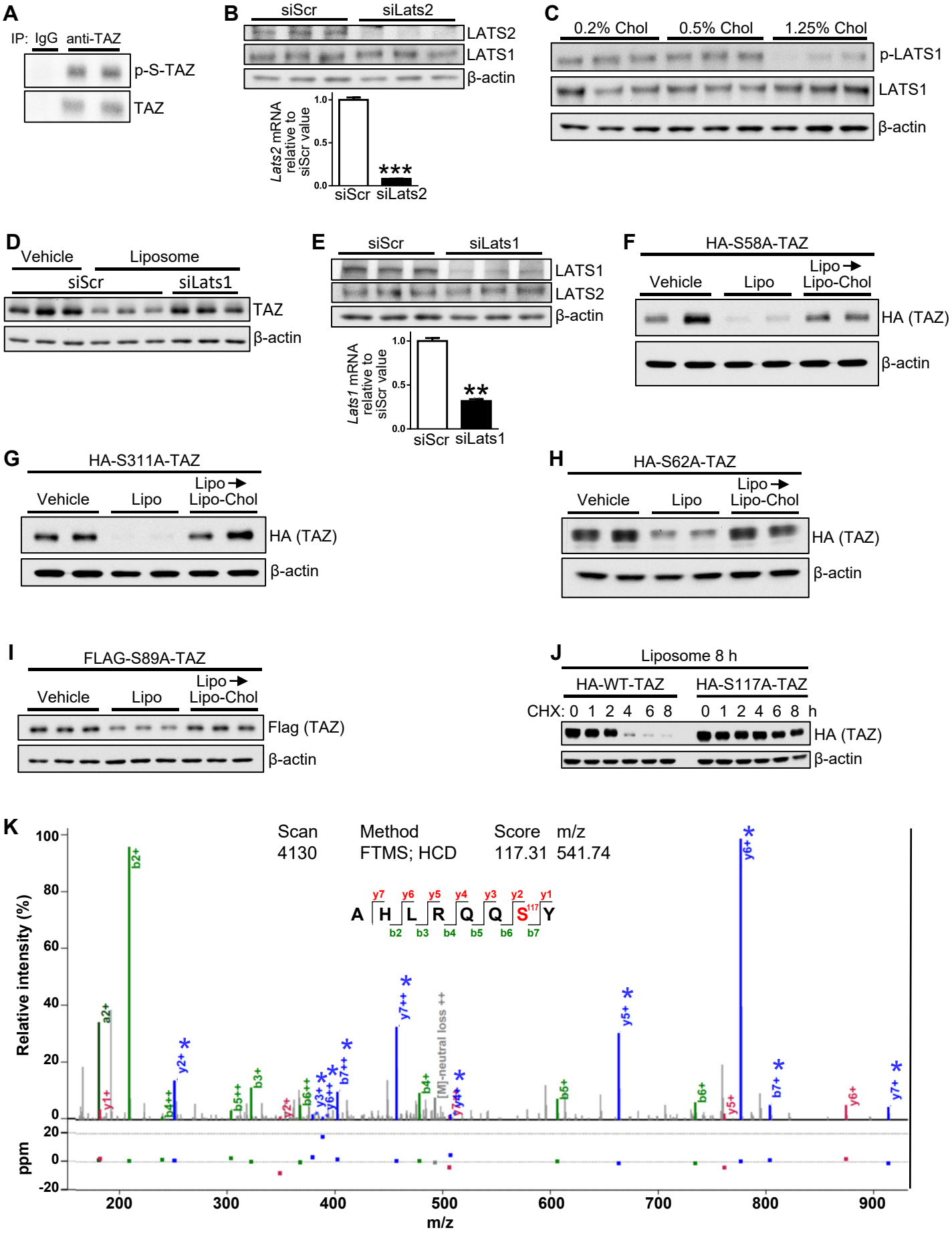
A
IP: IgG anti-TAZ
p-S-TAZ
TAZ
B
siScr siLats2
LATS2
LATS1
β-actin
Lats2 mRNA relative to siScr value
1.0
0.5
0.0
***
siScr siLats2
C
0.2% Chol 0.5% Chol 1.25% Chol
p-LATS1
LATS1
β-actin
D
Vehicle Liposome
siScr siLats1
TAZ
β-actin
E
siScr siLats1
LATS1
LATS2
β-actin
Lats1 mRNA relative to siScr value
1.0
0.5
0.0
**
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
[M]-neutral loss ++

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.

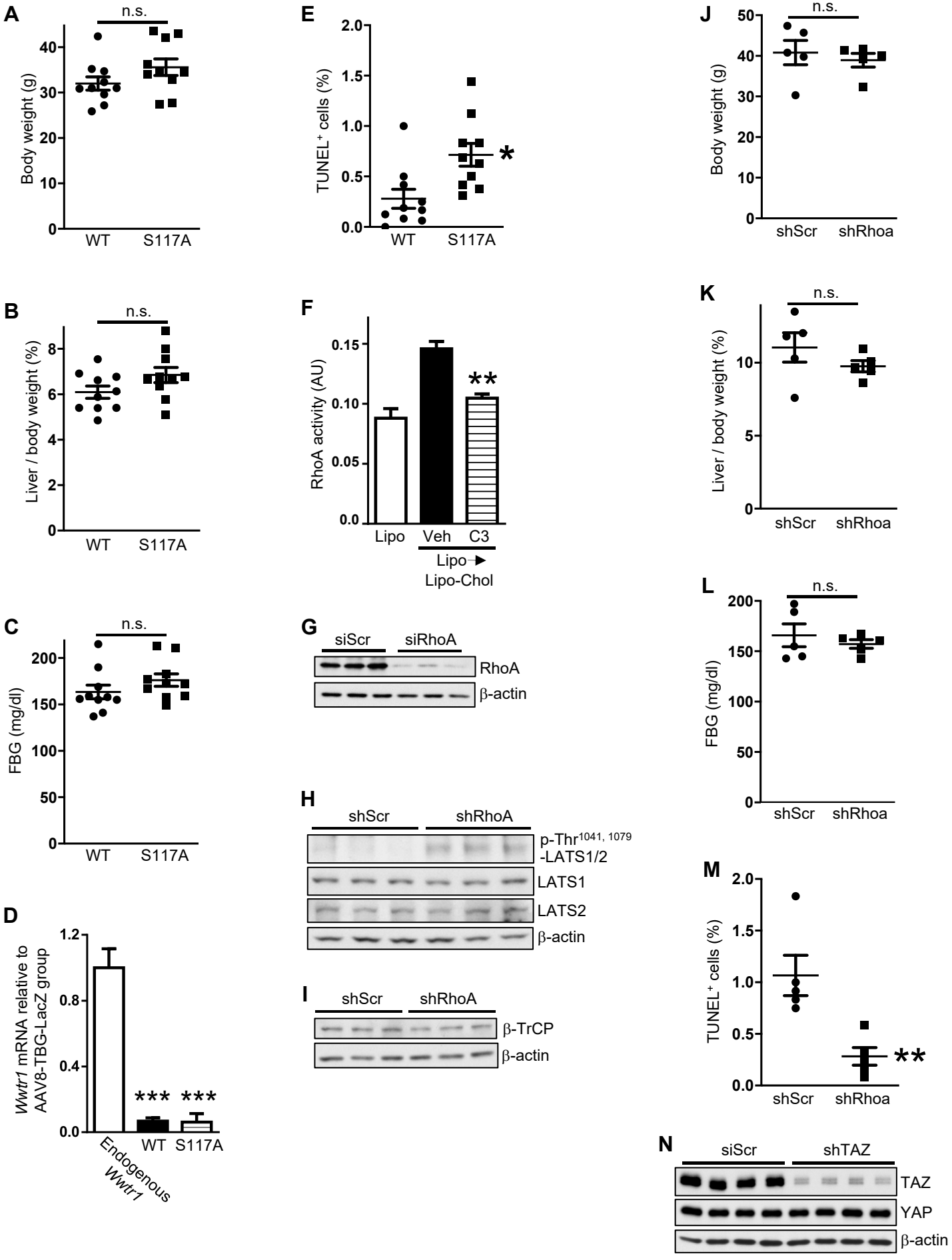
A
n.s.
Body weight (g)
WT
S117A
B
n.s.
Liver / body weight (%)
WT
S117A
C
n.s.
FBG (mg/dl)
WT
S117A
D
Wwtr1 mRNA relative to AAV8-TBG-LacZ group
***
***
WT
S117A
Endogenous Wwtr1
E
TUNEL+ cells (%)
*
WT
S117A
F
RhoA activity (AU)
**
Lipo
Veh
C3
Lipo
Lipo-Chol
G
siScr
siRhoA
RhoA
β-actin
H
shScr
shRhoA
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
siScr
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-L) 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.

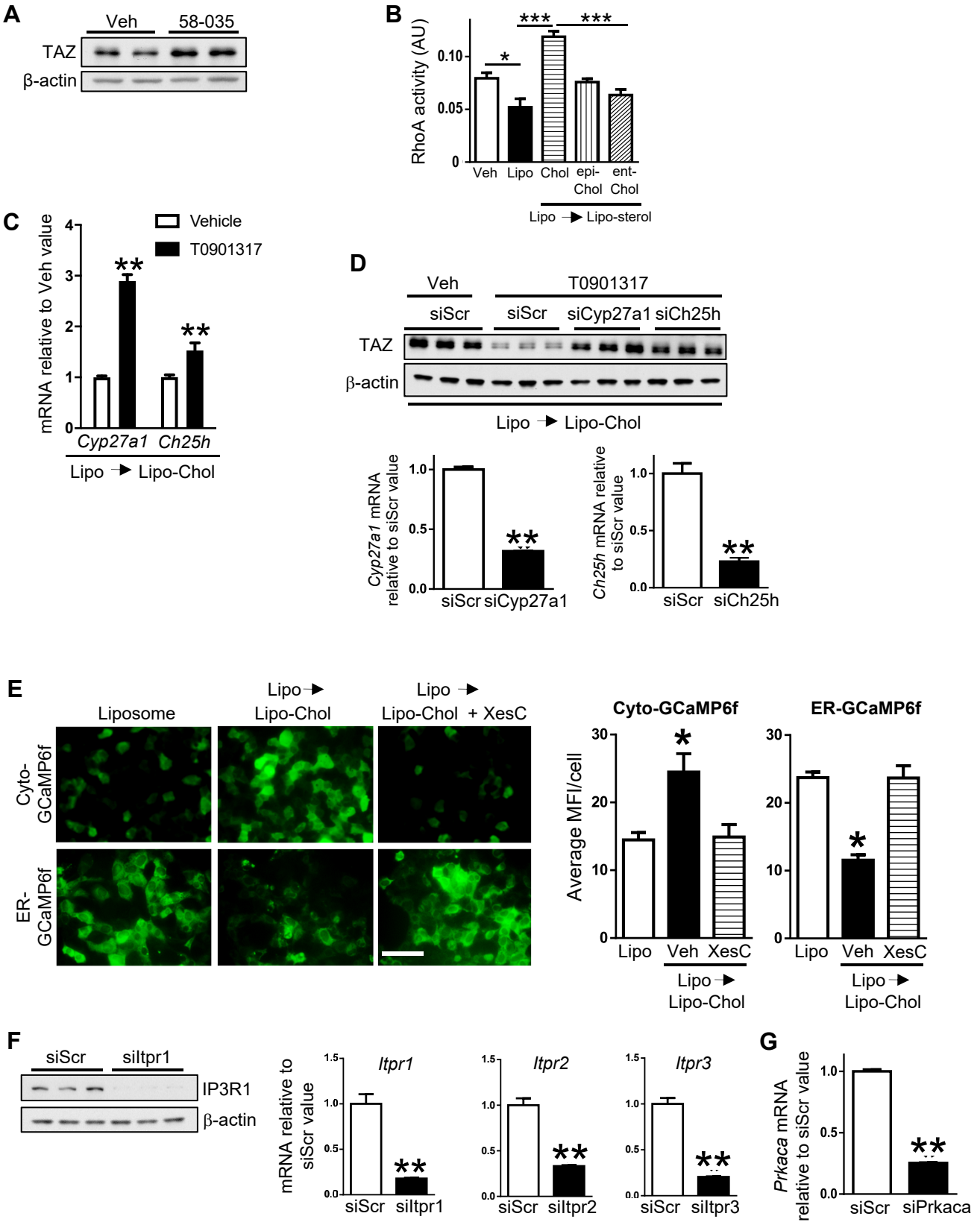
A
Veh
58-035
TAZ
β-actin
B
RhoA activity (AU)
0.10
0.05
0
*
***
***
Veh
Lipo
Chol
epi-Chol
ent-Chol
Lipo → Lipo-sterol
C
Vehicle
T0901317
mRNA relative to Veh value
4
3
2
1
0
**
**
Cyp27a1
Ch25h
Lipo → Lipo-Chol
D
Veh
T0901317
siScr
siScr
siCyp27a1
siCh25h
TAZ
β-actin
Lipo → Lipo-Chol
Cyp27a1 mRNA relative 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
siItpr2
siItpr3
G
Prkaca mRNA relative to siScr value
1.0
0.5
0.0
**
siScr
siPrkaca

Figure S4. **Related to Figures 4 and 5. 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).

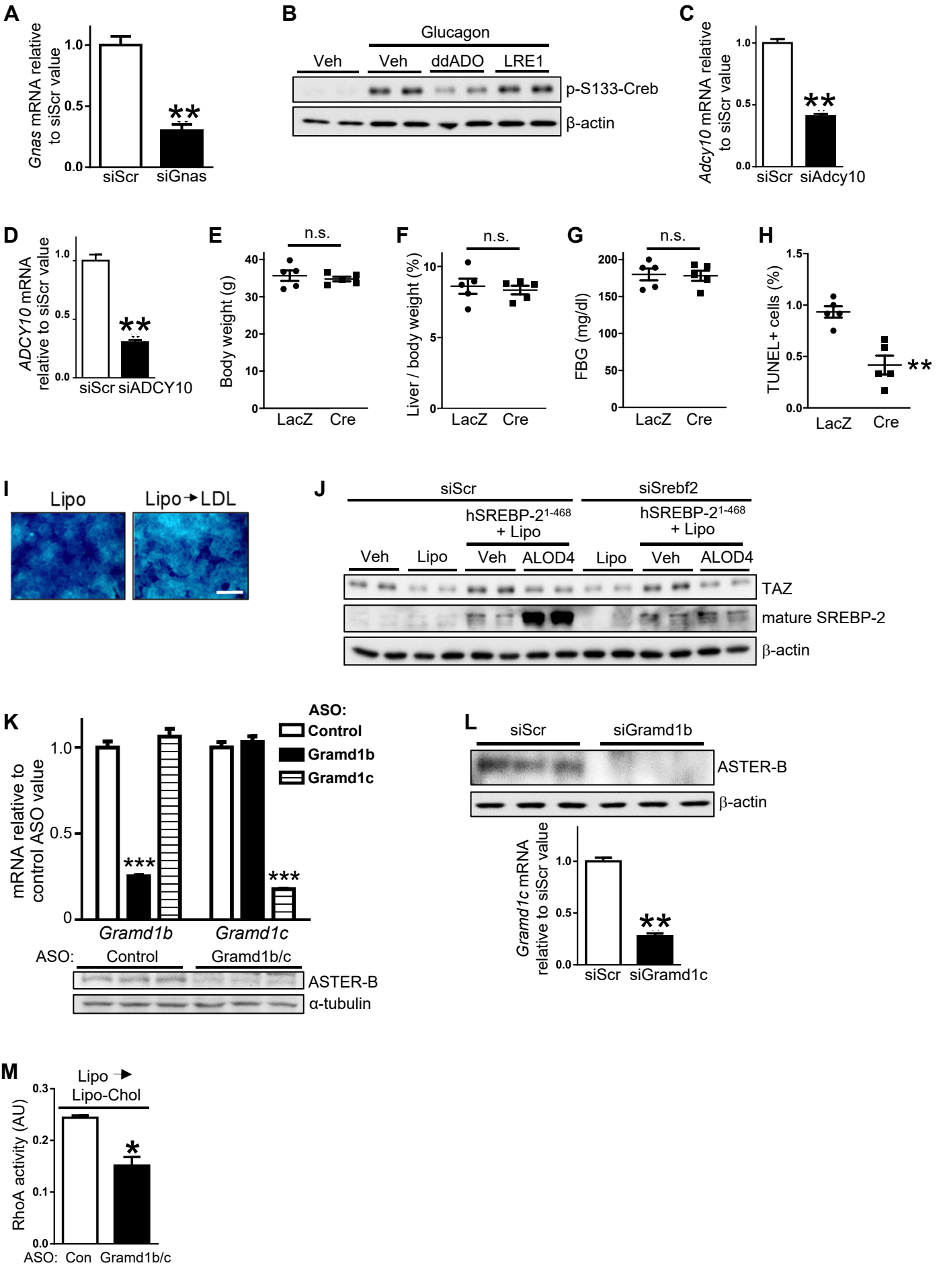
A
Gnas mRNA relative to siScr value
1.0
0.5
0.0
**
siScr
siGnas
B
Glucagon
Veh
Veh
ddADO
LRE1
p-S133-Creb
β-actin
C
Adcy10 mRNA relative to siScr value
1.0
0.5
0.0
**
siScr
siAdcy10
D
ADCY10 mRNA relative to siScr value
1.0
0.5
0.0
**
siScr
siADCY10
E
n.s.
Body weight (g)
40
30
20
10
0
LacZ
Cre
F
n.s.
Liver / body weight (%)
10
5
0
LacZ
Cre
G
n.s.
FBG (mg/dl)
200
150
100
50
0
LacZ
Cre
H
TUNEL+ cells (%)
1.5
1.0
0.5
0.0
**
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
1.0
0.5
0
***
***
Gramd1b
Gramd1c
ASO:
Control
Gramd1b/c
ASTER-B
α-tubulin
L
siScr
siGramd1b
ASTER-B
β-actin
Gramd1c mRNA relative to siScr value
1.0
0.5
0.0
**
siScr
siGramd1c
M
Lipo →
Lipo-Chol
RhoA activity (AU)
0.3
0.2
0.1
0.0
*
ASO:
Con
Gramd1b/c

Figure S5. **Related to Figure 5. 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).

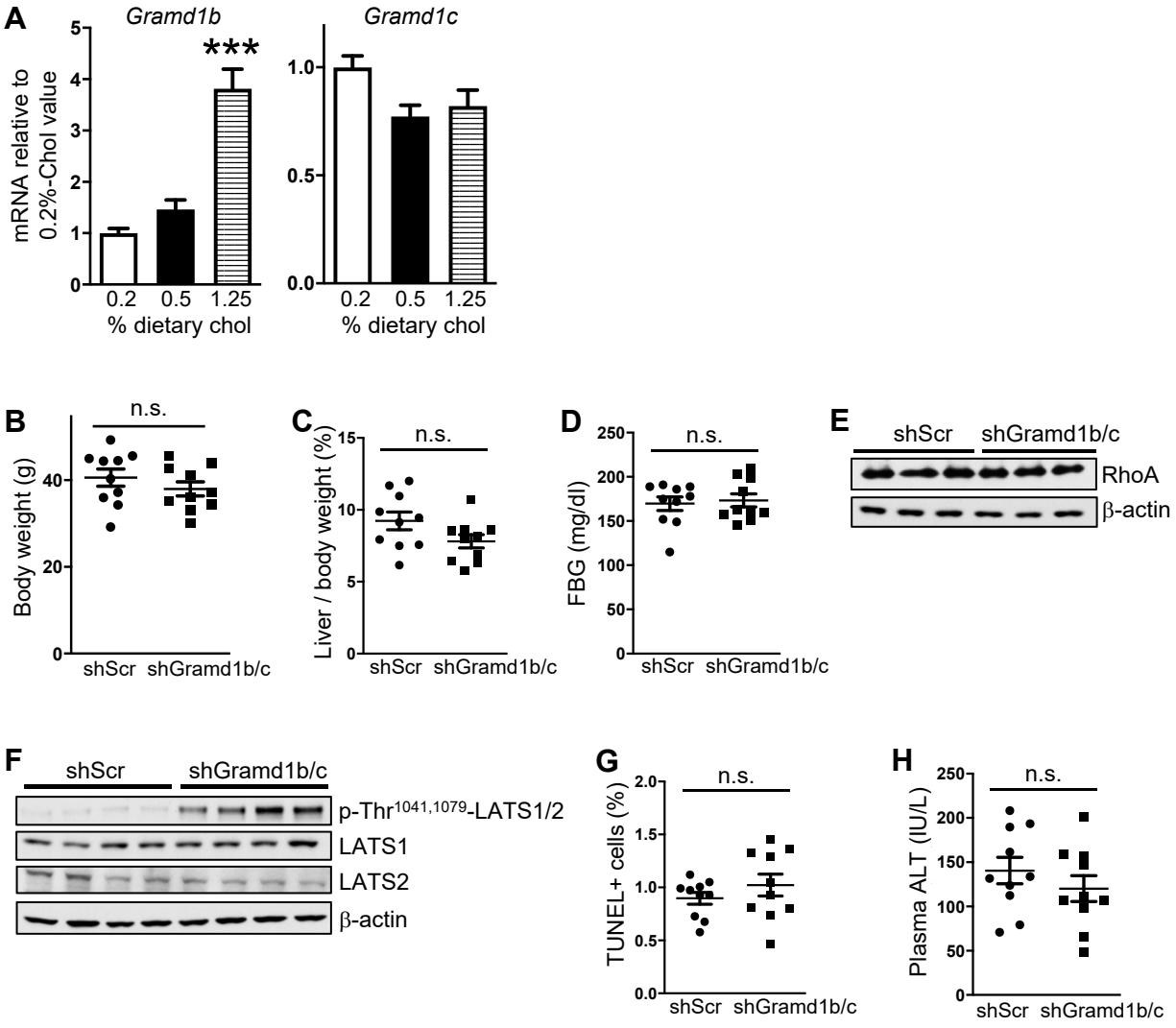

Figure S6. **Related to Figures 6 and 7. Further Characterization of NASH Mice Treated with AAV8-H1-shGramd1b/c.**

(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.

Table S1 (related to Figures 1F and 3F). Pathology of human NASH liver samples.

ID	Age	Gender	Pathological diagnoses
1	68	Male	Non-alcoholic steatohepatitis; cirrhosis, mixed macronodular and micronodular type. Minimal inflammatory activity, with minimal or no ongoing steatosis. No evidence of hepatocyte dysplasia or malignancy.
2	56	Female	Type 2 Diabetes. Obesity. Advanced chronic liver disease (stage 4: "cirrhosis"). Marked fibrosis throughout the entire liver. The residual hepatocellular parenchyma shows abundant well-formed Mallory hyaline, consistent with advanced chronic liver disease from steatohepatitis.
3	53	Male	NASH. Cirrhosis. Mild steatosis and focal active steatohepatitis. Focal Mallory hyaline is identified.
4	67	Female	Cirrhosis, NASH. Active steatohepatitis, grade 2 of 3.
5	64	Female	NASH. Cirrhosis with steatohepatitis and moderate macrovesicular steatosis.
6	62	Female	Fatty liver. Cirrhosis. Cirrhosis with moderate macrovesicular steatosis (35%) and focal steatohepatitis, consistent with fatty liver disease.
7	43	Female	Fatty liver. Cirrhosis with focal steatosis and mild activity.
8	57	Female	NASH cirrhosis

Table S2 (related to Figure 2 and Figure S2). Primers used for *WWTR1* mutagenesis.

Primers	5'- Sequence -3'
S58A F	CCTGATgcgGGCTCGCACTCGC
S58A R	CTCCTTAAAG AAAGACTCCG GCAGGATCTT CT
S62A F	TCGCACgcgCGCCAGTCCAG
S62A R	GCCCGAATCA GGCTCCTTAA AGAAAGACTC C
S89A F	TCGCACgcgTCGCCCGCGTCC
S89A R	GCGGACATGC TGGGCACCC
S117A F	CAGCAGgccTACGACGTGACCGACG
S117A R	GCGGAGGTGC GCGTGCTGC
S311A F	GAGCAGgccACTGACAGTGGCCTG
S311A R	CCTCGAATGA TATGGCCCTC CATTGAGGAA AG

Note: lower case letters indicate the mutated nucleotide based on the sequence of WT human *WWTR1* mRNA.

Table S3 (related to all figures). siRNA and shRNA sequences used in this study.

Target Gene	siRNA or shRNA	siRNA Sense Sequences (5' to 3')
Btrc (β-TrCP)	siRNA	rGrArGrCrUrArArArUrUrGrUrGrArUrArCrCrUrUrCrCrUGT
Btrc (β-TrCP)	shRNA	GCGACATAGTTTACAGAGAAT
Lats2	siRNA	rArArGrArUrUrGrUrArUrUrUrArUrGrGrUrArArArArGrGAA
Rhoa	siRNA	rCrUrArCrCrArGrUrArUrUrUrArGrArArGrCrCrArArCrCAC
Rhoa	shRNA	GTCAAGCATTTCTGTCCAAAT
Itpr1 (IP3R1)	siRNA	rGrUrUrUrCrArUrCrUrGrCrArArGrCrUrArArUrArArArACA
Itpr2 (IP3R2)	siRNA	rGrCrUrUrUrGrArArGrUrArUrUrArCrGrCrCrArArCrCrACA
Itpr3 (IP3R3)	siRNA	rGrUrCrCrUrGrCrUrUrArGrUrArCrCrGrUrUrGrArArGrAGA
Prkaca (PKA)	siRNA	rGrGrArUrCrArGrUrUrUrGrArUrArGrArArUrCrArArGrACC
Gramd1b (ASTER-B)	siRNA	rGrGrCrGrUrUrUrCrUrCrUrGrArUrArUrCrArUrCrUrUrCCA
Gramd1b (ASTER-B)	shRNA	GATGAAGGACTCGCTTATCAA
Gramd1c (ASTER-C)	siRNA	rCrArArGrUrCrArCrUrGrGrArCrUrUrGrArArUrArArGrAAT
Gramd1c (ASTER-C)	shRNA	GGGAAAGAGATGAGAAGTTCT
Adcy10 (sAC)	siRNA	rGrArArArUrCrUrCrUrGrArCrGrArArUrGrArArGrArUrUCT
ADCY10 (human sAC)	siRNA	rCrArArUrCrArUrUrUrCrUrArArCrArUrGrUrCrArArArGAA
Cyp27a1	siRNA	rGrUrUrCrCrArGrArArCrUrCrArGrUrCrUrArUrArUrCrACT
Ch25h	siRNA	rArCrCrUrGrArUrUrUrCrUrGrArCrUrCrUrUrUrArArArUAA
Gnas	siRNA	rCrUrUrCrCrCrArCrCrUrGrArArUrUrCrUrArUrGrArGrCAT
Srebp2 (Srebf2)	siRNA	rArGrGrCrArArGrArCrUrGrArUrUrGrUrUrCrUrGrArGrCTG

Table S4 (Related to all figures). Primers used for qPCR.

Primers	Organism	5'- Sequence -3'
Hprt F	mouse	TCAGTCAACGGGGGACATAAA
Hprt R	mouse	GGGGCTGTACTGCTTAACCAG
HPRT F	human	CCTGGCGTCGTGATTAGTGAT
HPRT R	human	AGACGTTCAGTCCTGTCCATAA
Taz (*Wwtr1*) F	mouse	CATGGCGGAAAAAGATCCTCC
Taz (*Wwtr1*) R	mouse	GTCGGTCACGTCATAGGACTG
WWTR1 F	human	TCCCAGCCAAATCTCGTGATG
WWTR1 R	human	AGCGCATTGGGCATACTCAT
Tgfb1 F	mouse	CTCCCGTGGCTTCTAGTGC
Tgfb1 R	mouse	GCCTTAGTTTGGACAGGATCTG
Acta2 F	mouse	ATGCTCCCAGGGCTGTTTTCCCAT
Acta2 R	mouse	GTGGTGCCAGATCTTTTCCATGTCG
Btrc F	mouse	AAGACTGTAATAATGGCGAACCC
Btrc R	mouse	TCTCTTGGTTTATGCAAAGCCTG
Rhoa F	mouse	AGCTTGTGGTAAGACATGCTTG
Rhoa R	mouse	GTGTCCCATAAAGCCAACTCTAC
Col1a1 F	mouse	GCTCCTCTTAGGGGCCACT
Col1a1 R	mouse	CCACGTCTCACCATTGGGG
Col1a2 F	mouse	GTAACTTCGTGCCTAGCAACA
Col1a2 R	mouse	CCTTTGTCAGAATACTGAGCAGC
Col3a1 F	mouse	CTGTAACATGGAAACTGGGGAAA
Col3a1 R	mouse	CCATAGCTGAACTGAAAACCACC
Dpt F	mouse	TGGATGGGTGAATCTTAACCGC
Dpt R	mouse	TCAGAGCCTTCCTTCTTGCTA
Adgre1 (*F4/80,Emr1*) F	mouse	ACCACAATACCTACATGCACC
Adgre1 (*F4/80,Emr1*) R	mouse	AAGCAGGCGAGGAAAAGATAG
Tnfa F	mouse	CTTCTGTCTACTGAACTTCGGG
Tnfa R	mouse	CAGGCTTGTCACTCGAATTTTG
Mcp1 F	mouse	TTAAAAACCTGGATCGGAACCAA
Mcp1 R	mouse	GCATTAGCTTCAGATTTACGGGT
Ihh F	mouse	CTCTTGCCTACAAGCAGTTCA
Ihh R	mouse	CCGTGTTCTCCTCGTCCTT
Spp1 (Opn) F	mouse	CTGACCCATCTCAGAAGCAGAATCT
Spp1 (Opn) R	mouse	TCCATGTGGTCATGGCTTTCATTGG
Timp1 F	mouse	CTCAAAGACCTATAGTGCTGGC
Timp1 R	mouse	CAAAGTGACGGCTCTGGTAG
Last1 F	mouse	TGGTGACTCTGGGGATAAAGAA
Lats1 R	mouse	GGGAGTAACTCTGAATCCGAGAC
Lats2 F	mouse	GGACCCCAGGAATGAGCAG

Lats2 R	mouse	CCCTCGTAGTTTGCACCACC
Itpr1 F	mouse	CGTTTTGAGTTTGAAGGCGTTT
Itpr1 R	mouse	CATCTTGCGCCAATTCCCG
Itpr2 F	mouse	CCTCGCCTACCACATCACC
Itpr2 R	mouse	TCACCACTCTCACTATGTCGT
Itpr3 F	mouse	GGGCGCAGAACAACGAGAT
Itpr3 R	mouse	GAAGTTTTGCAGGTCACGGTT
Gnas F	mouse	CAGAGCCTCCATTGGGGTC
Gnas R	mouse	GCTTCTCGCTCAACTGGGG
Cyp27a1 F	mouse	CCAGGCACAGGAGAGTACG
Cyp27a1 R	mouse	GGGCAAGTGCAGCACATAG
Ch25h F	mouse	TGCTACAACGGTTCGGAGC
Ch25h R	mouse	AGAAGCCCACGTAAGTGATGAT
Prkaca F	mouse	AGATCGTCCTGACCTTTGAGT
Prkaca R	mouse	GGCAAAACCGAAGTCTGTCAC
Gramd1b F	mouse	ACACAATGGGCTACTGTGAGG
Gramd1b R	mouse	GGCTTGGTCTCGATGCTACT
Gramd1c F	mouse	AACAAAGATCAGGCCCACCG
Gramd1c R	mouse	AGTGAGCTCTTCAGCTGTTCC
Adcy10 F	mouse	TGCCAGTGGGATTGTCTTC
Adcy10 R	mouse	TGAGGCCCAAACACTGATAC
ADCY10 F	human	ACAAAGTGTACGACCTTCATGC
ADCY10 R	human	CGAAGCTCAGATAAATAGCCCTG

Hprt, hypoxanthine guanine phosphoribosyl transferase; *Taz* (*Wwtr*1), WW domain containing transcription regulator 1; *Tgfb1*, transforming growth factor, beta 1; *Acta2*, α-smooth muscle actin; *Btrc*, beta-transducin repeat containing protein; *Rhoa*, ras homolog family member A; *Col1a1*, collagen type I alpha 1; *Col1a2*, collagen type I alpha 2; *Col3a1*, collagen, type III, alpha 1; *Dpt*, dermatopontin; *Adgre1* (*F4/80*), adhesion G protein-coupled receptor E1; *Tnfa*, tumor necrosis factor-α ; *Mcp1*, monocyte chemoattractant protein-1; *Ihh*, Indian hedgehog; *Spp1*, secreted phosphoprotein 1; *Timp1*, tissue inhibitor of metalloproteinase 1; *Lats1,* large tumor suppressor; *Lats2,* large tumor suppressor 2; *Itpr1,* inositol 1,4,5-trisphosphate receptor 1; *Itpr2,* inositol 1,4,5-trisphosphate receptor 2; *Itpr3,* inositol 1,4,5-trisphosphate receptor 3; *Gnas,* GNAS (guanine nucleotide binding protein, alpha stimulating) complex locus; *Cyp27a1,* cytochrome P450 family 27 subfamily a polypeptide 1; *Ch25h,* cholesterol 25-hydroxylase; *Prkaca,* protein kinase, cAMP dependent, catalytic, alpha; *Gramd1b*, GRAM domain containing 1B; *Gramd1c*, GRAM domain containing 1C; Adcy10/ADCY10, adenylate cyclase 10.