SUPPLEMENTARY FIGURES

Figure S1. Hepatic Tm6sf2 overexpression suppresses plasma lipid levels. (*A*) Changes in plasma lipid levels 2 weeks after AAV-Null or AAV-Tm6sf2 virus injection (n = 8 mice per group). Percent changes relative to pre-injection values are shown. (*B*) Time-course of plasma lipid levels (n = 8 mice per group). (*C*) Assessment of hepatic transduction efficiency 10 days after injection of mice with different doses of AAV-GFP vector. Percent GFP-positive cells were quantified in DAPI-stained liver sections using confocal fluorescence microscopy (n = 4 mice per group). (*D*) Hepatic Tm6sf2 mRNA expression and (*E*) plasma lipid levels 4 weeks after injection with different doses of AAV-Tm6sf2 virus (n = 4-5 mice per group). Viral doses are shown as genome copies per mouse. Control vector (Null) was injected at $1x10^{12}$ gc/mouse. (*F*) Plasma lipid levels after overnight fasting (O/N fast) and overnight fasting followed by re-feeding for 4 hours at 7 weeks after AAV injection (n = 6-8 mice per group). (*G*) Expression of UPR genes in livers of AAV-treated mice 12 weeks after virus injection (uXbp1, unspliced Xbp1; sXbp1, spliced Xbp1; n = 5-8 mice per group). (*H*) Time-course of plasma lipid levels in AAV-injected mice on a high-fat diet for 16 weeks (n = 7-8 mice per group). (*I*) Plasma lipid levels in eight-week-old female $Ldlr^{-/-}$;$ApoB^{100/100}$ mice injected with empty (Null) or Tm6sf2-expressing (Tm6) AAV, maintained on chow diet and analyzed 4 weeks after virus injection (n = 4-5 mice per group). (*J*) FPLC lipoprotein profiles of samples shown in *H* (pooled plasma from 4-5 mice per group). (*K*) Changes in plasma lipid levels in male and female

$Ldlr^{-/-}$;$ApoB^{100/100}$ mice (n = 3-5 mice per group). Percent changes relative to pre-injection values are shown. Data are shown as the mean ± SEM. *, $p < 0.05$ by Student's *t*-test.

Suppl Fig. 1

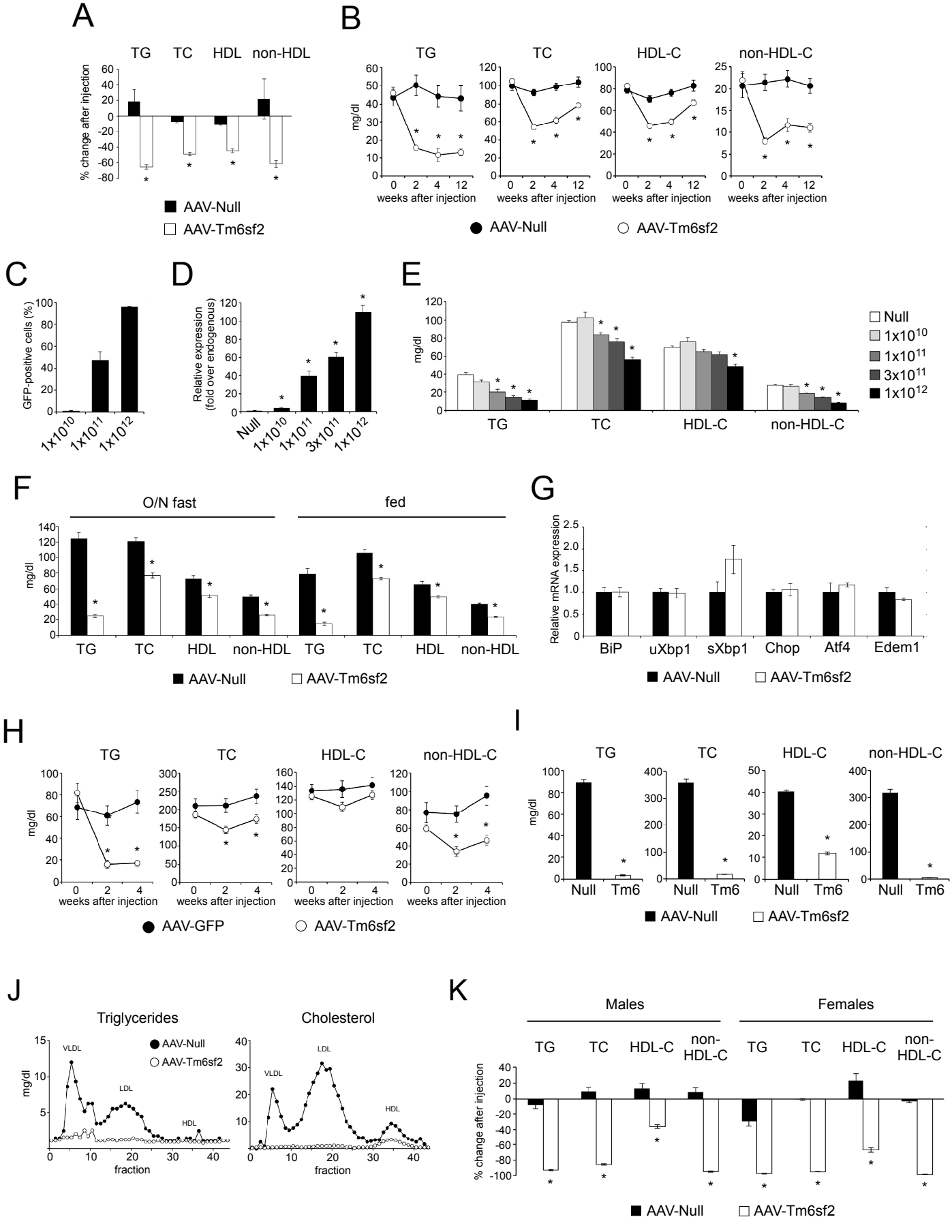

A
TG
TC
HDL
non-HDL
% change after injection
AAV-Null
AAV-Tm6sf2
B
TG
TC
HDL-C
non-HDL-C
mg/dl
weeks after injection
AAV-Null
AAV-Tm6sf2
C
GFP-positive cells (%)
1x10^10
1x10^11
1x10^12
D
Relative expression (fold over endogenous)
Null
1x10^10
1x10^11
3x10^11
1x10^12
E
mg/dl
Null
1x10^10
1x10^11
3x10^11
1x10^12
TG
TC
HDL-C
non-HDL-C
F
O/N fast
fed
mg/dl
TG
TC
HDL
non-HDL
AAV-Null
AAV-Tm6sf2
G
Relative mRNA expression
BiP
uXbp1
sXbp1
Chop
Atf4
Edem1
AAV-Null
AAV-Tm6sf2
H
TG
TC
HDL-C
non-HDL-C
mg/dl
weeks after injection
AAV-GFP
AAV-Tm6sf2
I
TG
TC
HDL-C
non-HDL-C
mg/dl
Null
Tm6
AAV-Null
AAV-Tm6sf2
J
Triglycerides
Cholesterol
AAV-Null
AAV-Tm6sf2
VLDL
LDL
HDL
mg/dl
fraction
K
Males
Females
TG
TC
HDL-C
non-HDL-C
% change after injection
AAV-Null
AAV-Tm6sf2

Figure S2. Hepatic overexpression of human TM6SF2, but not mouse Tm6sf2, is associated with inflammation and reduced expression of UPR genes. Male mice were injected with AAV-GFP, AAV-Tm6sf2 and AAV-TM6SF2 vectors ($1x10^{12}$ gc/mouse) and livers were analyzed 2 weeks later. (*A*) Representative histological sections showing pericentral inflammatory infiltrate in liver overexpressing TM6SF2, but not GFP or Tm6sf2. Three sections per liver representing different tissue regions were analyzed with similar results (n = 2-4 mice per group). (*B*) Relative mRNA levels of UPR genes (n = 2-4 mice per group). Data are shown as the mean ± SEM. *, p(*vs*. GFP) < 0.05 by Kruskal-Wallis test followed by Dunn's post hoc test.

Suppl Fig. 2

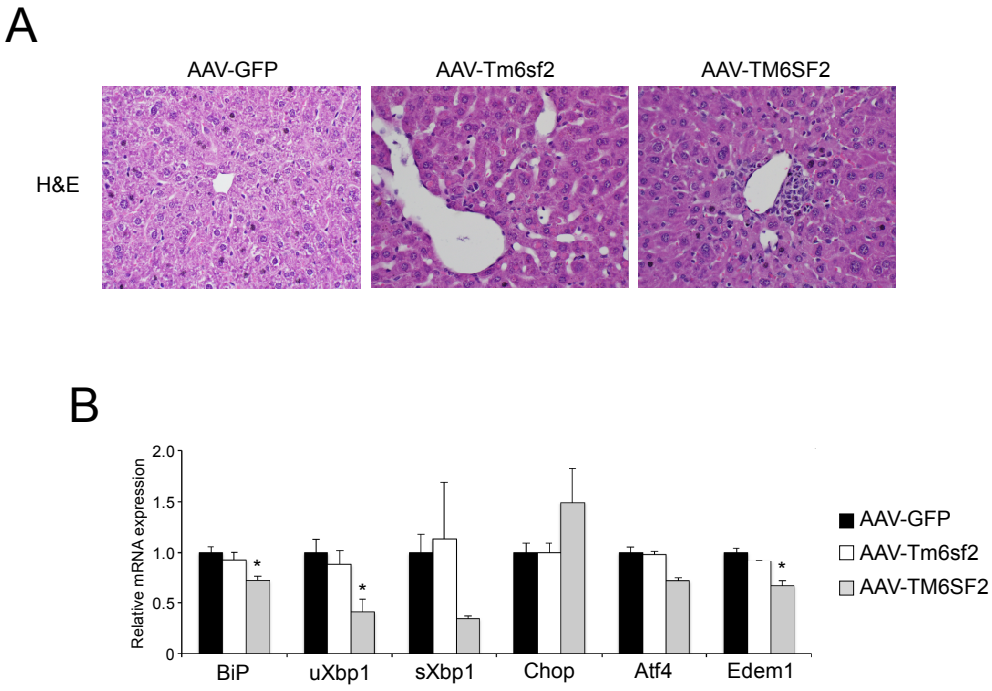

A
AAV-GFP
AAV-Tm6sf2
AAV-TM6SF2
H&E
B
Relative mRNA expression
2.0
1.5
1.0
0.5
0
BiP
uXbp1
sXbp1
Chop
Atf4
Edem1
AAV-GFP
AAV-Tm6sf2
AAV-TM6SF2

Figure S3. Hepatic phenotype of mice overexpressing Tm6sf2. (*A*) Hepatic cholesterol secretion was assessed after Pluronic F-127 injection in plasma samples shown in Fig. 3A. Changes in cholesterol concentration from preinjection (0 h) to 2 hours after Pluronic injection are shown (n = 4-5 mice per group). (*B*) Representative Trichrome-stained liver sections from mice shown in Fig. 3B, 12 weeks after AAV injection on chow diet. AAV-Tm6sf2 liver shows no sign of increased fibrosis. (*C*) Relative hepatic mRNA levels of selected genes involved in fatty acid (FA), triglyceride (TG), cholesterol and VLDL metabolism 12 weeks after injection with AAV-Null or AAV-Tm6sf2 virus (n = 5-8 mice per group). (*B*) Mttp activity in the livers of mice 12 weeks after injection with AAV-Null (Null) or AAV-Tm6sf2 (Tm6) virus (n = 5-8 mice per group). Data are shown as the mean ± SEM. *, $p < 0.05$ by Student's *t*-test.

Suppl Fig. 3

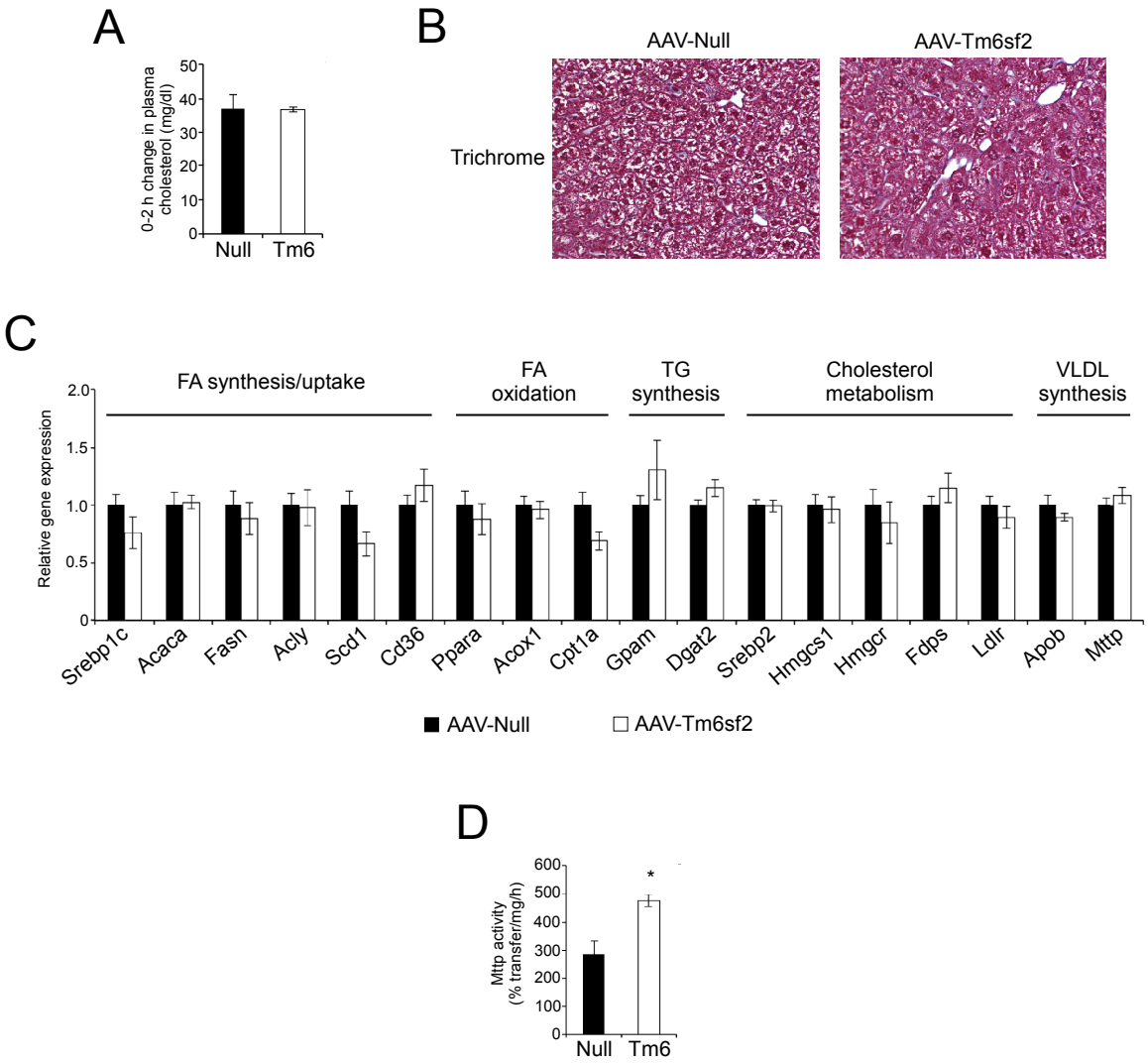
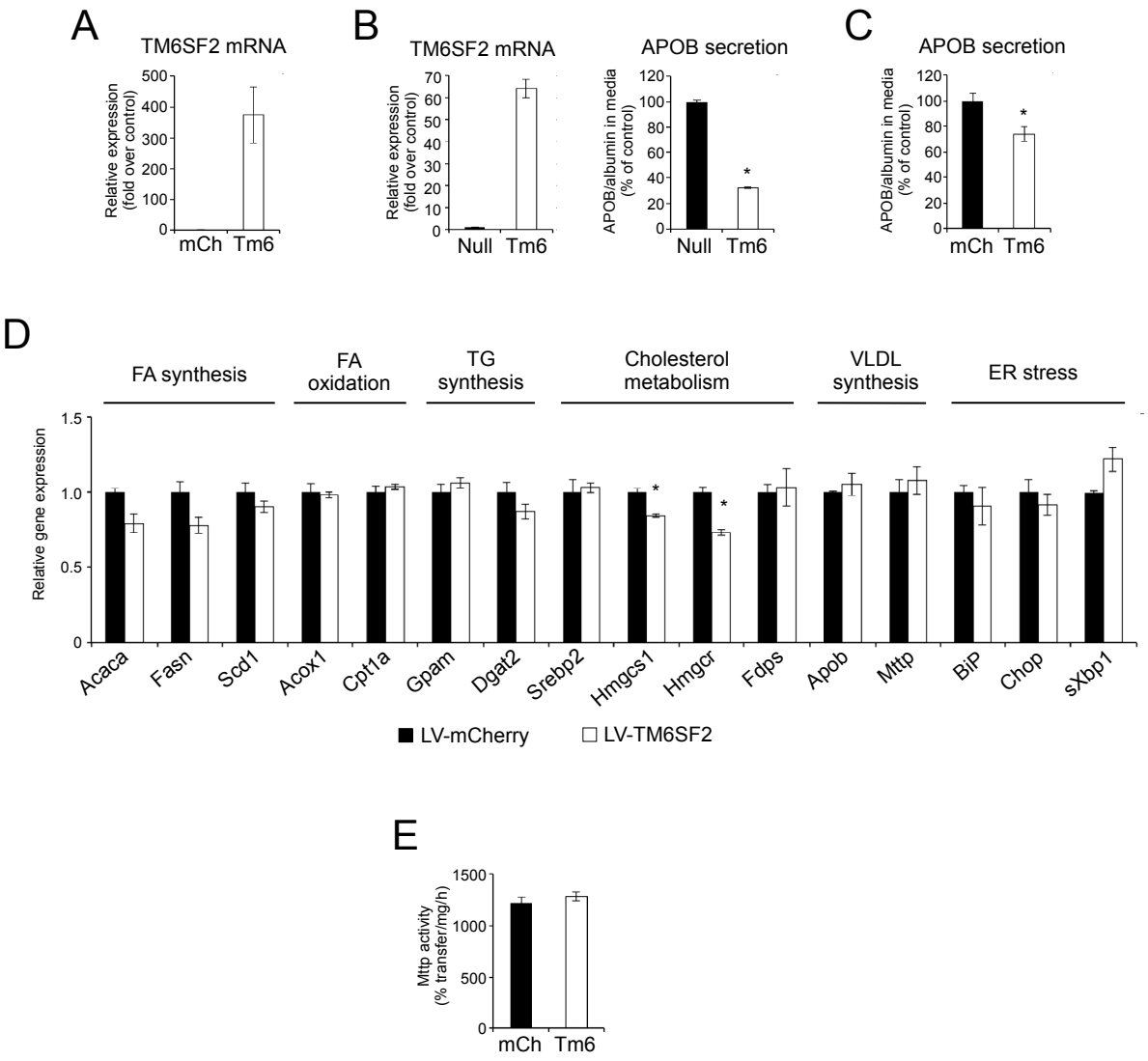

A
0-2 h change in plasma cholesterol (mg/dl)
50
40
30
20
10
0
Null
Tm6
B
AAV-Null
AAV-Tm6sf2
Trichrome
C
FA synthesis/uptake
FA oxidation
TG synthesis
Cholesterol metabolism
VLDL synthesis
Relative gene expression
2.0
1.5
1.0
0.5
0
Srebp1c
Acaca
Fasn
Acly
Scd1
Cd36
Ppara
Acox1
Cpt1a
Gpam
Dgat2
Srebp2
Hmgcs1
Hmgcr
Fdps
Ldlr
Apob
Mttp
AAV-Null
AAV-Tm6sf2
D
Mttp activity (% transfer/mg/h)
600
500
400
300
200
100
0
*
Null
Tm6

Figure S4. Tm6sf2 overexpression suppresses APOB secretion without significantly impacting transcription of metabolic genes or MTTP activity. (*A*) Relative TM6SF2 mRNA levels in Huh7 cells expressing lentiviral mCherry (mCh) or TM6SF2 (Tm6) (n = 3 wells per group). (*B*) Relative TM6SF2 expression (left panel) and APOB secretion (right panel) in a Huh7 cell line derived independently from the one shown in *A*. Secreted APOB mass was normalized by concomitantly secreted albumin mass (n = 3 wells per group). (*C*) Albumin-normalized APOB secretion in primary iPSC-derived hepatocyte-like cells transduced with adenovirus expressing mCherry (mCh) or TM6SF2 (Tm6) (n = 3 wells per group). (*D*) Relative mRNA expression of selected genes involved in fatty acid (FA), triglyceride (TG), cholesterol and VLDL metabolism and ER stress in Huh7 cells overexpressing mCherry (LV-mCherry) or TM6SF2 (LV-TM6SF2) (n = 3 wells per group). (*E*) MTTP activity in Huh7 cells stably expressing mCherry (mCh) or TM6SF2 (Tm6) (n = 3 wells per group). Data are presented as the mean ± SEM. *, $p < 0.05$ by Student's *t*-test.

Suppl Fig. 4


A
TM6SF2 mRNA
Relative expression (fold over control)
mCh
Tm6
B
TM6SF2 mRNA
Relative expression (fold over control)
Null
Tm6
APOB secretion
APOB/albumin in media (% of control)
Null
Tm6
C
APOB secretion
APOB/albumin in media (% of control)
mCh
Tm6
D
FA synthesis
FA oxidation
TG synthesis
Cholesterol metabolism
VLDL synthesis
ER stress
Relative gene expression
Acaca
Fasn
Scd1
Acox1
Cpt1a
Gpam
Dgat2
Srebp2
Hmgcs1
Hmgcr
Fdps
Apob
Mttp
BiP
Chop
sXbp1
LV-mCherry
LV-TM6SF2
E
Mttp activity (% transfer/mg/h)
mCh
Tm6