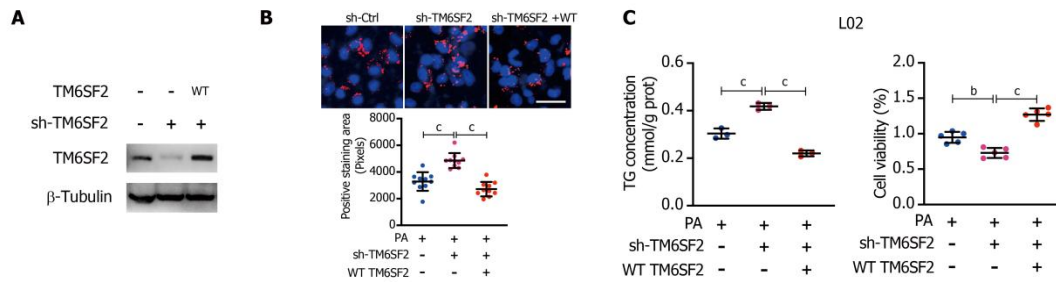
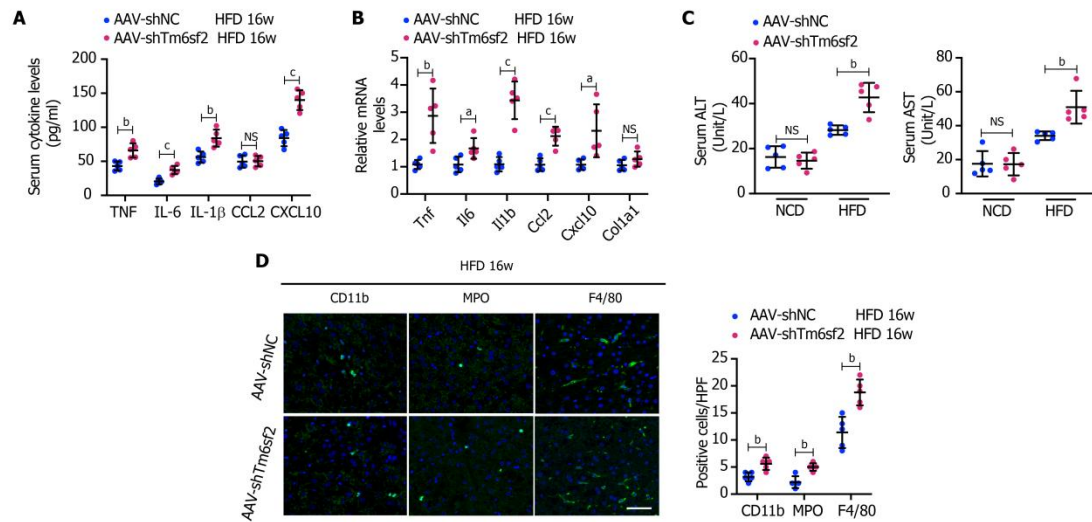


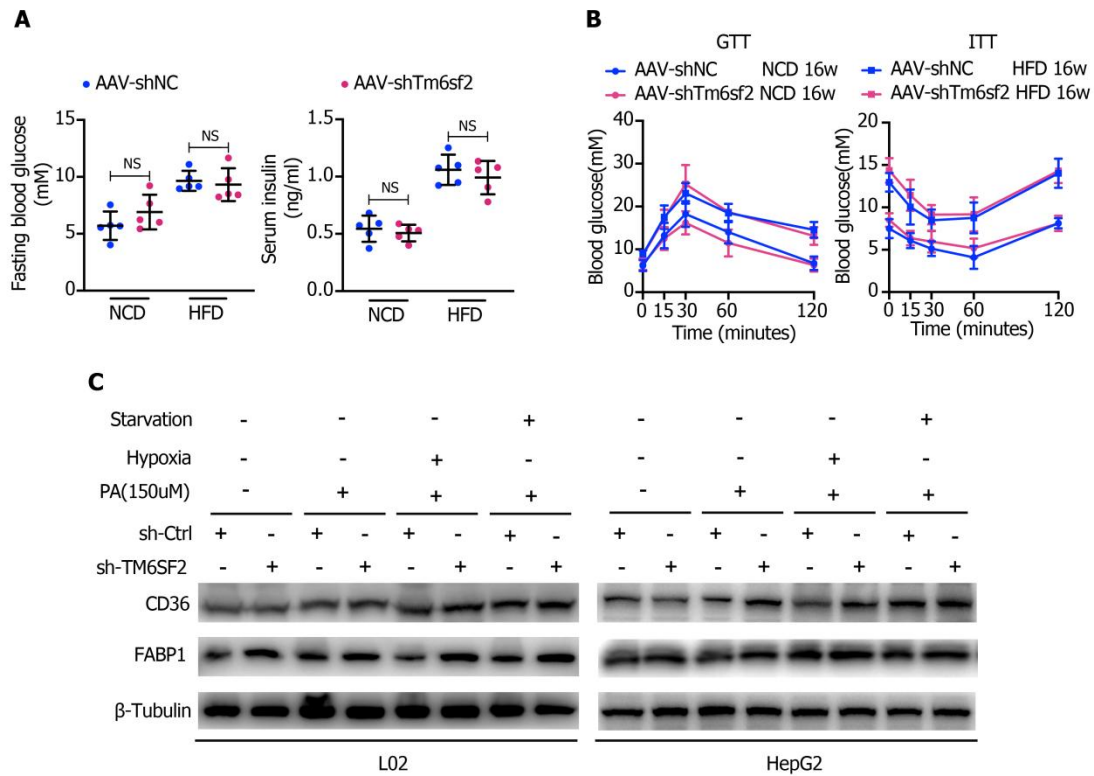
**Supplementary Figure 1 TM6SF2 is upregulated in *in vitro* and *in vivo* models of nonalcoholic fatty liver disease.** A: Protein levels of TM6SF2 in response to palmitic acid (150 μM) treatment in two cell lines; B: Protein (left) and mRNA levels (right) of TM6SF2 in the liver of mice fed with normal chow diet or high-fat diet at the indicated time points. <sup>c</sup>*P* < 0.001.



**Supplementary Figure 2 Overexpression of TM6SF2 improved hepatic lipid accumulation.** A: TM6SF2-knockdown L02 cells were restored with the expression of wild type TM6SF2. Protein levels of TM6SF2 were assessed by immunoblotting; B: Representative Nile red staining (top) and quantification of lipid accumulation (bottom) of L02 cells were shown. Scale bars: 50  $\mu$ m; C: TM6SF2-knockdown L02 cells were restored with the expression of wild type TM6SF2 or empty vector. Left, intracellular TG levels in cells were examined after palmitic acid (PA) (150  $\mu$ M) treatment for 24 h ( $n = 3$ ). Right, the reconstituted cells were incubated with PA (150  $\mu$ M) for 24 h and then the cellular viability analysis was performed ( $n = 5$ ). <sup>b</sup> $P < 0.01$ ; <sup>c</sup> $P < 0.001$ .



**Supplementary Figure 3 Knockdown of TM6SF2 promotes high-fat diet-induced inflammation in liver.** A-C: Mice were injected with AAV-shNC and AAV-shTm6sf2 viruses *via* the tail vein and fed a high-fat diet or normal chow diet for 16 wk ( $n = 5$  mice per group). The serum concentrations of inflammatory cytokines (A), hepatic mRNA levels of inflammation-related genes (B) and serum levels of ALT and AST (C) were shown; D: Representative images of immunofluorescent staining (left) and their quantification (right) were shown (8 fields of each mouse were examined). Scale bar: 50  $\mu\text{m}$ . <sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ ; <sup>c</sup> $P < 0.001$ . NS: Not significant.



**Supplementary Figure 4 Hepatic TM6SF2 deficiency increases de novo lipogenesis.** A and B: Levels of fasting blood glucose and plasma insulin (A) were examined and the glucose tolerance test and insulin tolerance test (B) were also performed in the indicated mice during normal chow diet or high-fat diet feeding; C: The effect of starvation or hypoxia condition on CD36 and FABP1 protein expression was determined in TM6SF2-knockdown cells with or without palmitic acid treatment. NS: Not significant.

**Supplementary Table 1 The information of materials used in this study**

<b>Antibodies</b>	<b>Source</b>	<b>Identifier</b>
Mouse anti-TM6SF2 (1:1500)	Abcam	Cat#ab169629
Mouse anti-Beta-tubulin (1:10000)	Proteintech	Cat#10094-1-AP
Rabbit anti-SREBP-1c (1:500)	Proteintech	Cat#66875-1-Ig
Rabbit anti-p-ACC (1:1000)	ABclonal	Cat#AP0298
Rabbit anti-ACC (1:1000)	ABclonal	Cat#A15606
Rabbit anti-SCD-1 (1:1000)	ABclonal	Cat#A16429
Rabbit anti-FASN (1:500)	ABclonal	Cat#A0461
Rabbit anti-LaminA/C (1:1500)	ABclonal	Cat#A19524
Rabbit anti-PPAR-alpha (1:1500)	ABclonal	Cat#A6697
Rabbit anti-CPT1a (1:500)	ABclonal	Cat#A5307
Rabbit anti-ACOX1 (1:500)	ABclonal	Cat#A8091
Rabbit anti-CD36 (1:1500)	ABclonal	Cat#A5792
Rabbit anti-FABP1 (1:1000)	ABclonal	Cat#A5311
<b>Chemicals, Reagent or Resource</b>	<b>Source</b>	<b>Identifier</b>
Palmitic acid	Sigma-Aldrich	Cat#P0500
Glucose	Sigma-Aldrich	Cat#G8270
Novolin R	Novo Nordisk	N/A
Mouse and Rabbit Specific HRP/DAB IHC Detection Kit - Micro-polymer	Abcam	Cat#ab236466.
Oil Red O	Solarbio	Cat#O8010
Hematoxylin and Eosin Staining Kit	Beyotime	Cat# C0105M
High-Fat (60 kcal%) Purified Rodent Diet	Dyets	Cat#HF60

---

Normal chow diet	Research Diets	Cat# D12450B
Nile red	Solarbio	Cat#N8440
Antifade Polyvinylpyrrolidone	Beyotime	Cat#P0123
Mounting Medium		
RIPA Lysis Buffer	Beyotime	Cat#P0013B
SDS-PAGE Sample Loading Buffer, 5X	Beyotime	Cat#P0015L
BCA Protein Assay Kit	Beyotime	Cat#P0011
Triglyceride assay kit	Nanjing Jiancheng	Cat#A110-1-1
Total cholesterol assay kit	Nanjing Jiancheng	Cat#A111-1-1
Aspartate aminotransferase Assay Kit	Nanjing Jiancheng	Cat#C010-2-1
Alanine aminotransferase Assay Kit	Nanjing Jiancheng	Cat#C009-2-1
Acetoacetate Assay	Abcam	Cat#ab180875
B-Hydroxybutyrate (beta-HB) Assay	Abcam	Cat#ab180876
Nonesterified Free fatty acids assay kit	Nanjing Jiancheng	Cat#A042-2-1
Mouse malonyl-CoA ELISA test kit	Jianglai, Shanghai	Cat#JL47416
Mouse TNF ELISA test kit	Abcam	Cat#ab208348
Mouse IL-1 $\beta$ ELISA test kit	Abcam	Cat#ab197742
Mouse IL-6 ELISA test kit	Abcam	Cat#ab222503
Mouse CCL2 ELISA test kit	Gelatins	Cat#JLC049
Mouse CXCL10 ELISA test kit	Gelatins	Cat#JLC5800
Seahorse XF Palmitate Oxidation Stress Test Kit	Agilent	Cat#103693-100
Seahorse XF Palmitate-BSA	Agilent	Cat#102720-100

---

---

FAO Substrate

Blood Glucose Meter	Yuwell	Cat#590
MK-4074	MedChemExpress	Cat#HY-107709
GW7647	MedChemExpress	Cat#HY-13861
Etomoxir (Eto)	MedChemExpress	Cat#HY-50202
PrimeScript™ RT Master Mix (Perfect Real Time)	Takara Biotechnology	Cat#RR036A
Hieff® qPCR SYBR Green Master Mix (No Rox)	Yeasen	Cat#11201ES08
Enhanced Chemiluminescent C57BL/6	NCM Biotech GemPharmatech Co.,Ltd	Cat#P10100 N/A

Oligonucleotides

Human <i>TM6SF2</i>	F: 5'-GCATTGATGAGCGCCCTAAT C-3' R: 5'-AGTGGGTCATAGGAGACCTC G-3'	N/A
Human <i>ACTB</i>	F: 5'-CATGTACGTTGCTATCCAGGC -3' R 5'-CTCCTTAATGTCACGCACGAT -3'	N/A
Mouse <i>Tm6sf2</i>	F: 5'-GATCACCTACGACCCTCTCTA TG-3' R:	N/A

---

---

	5'-TGGAGTGCAATGACAAGGTC	
	C-3'	
Mouse <i>Actb</i>	F:	N/A
	5'-GGCTGTATTCCCCTCCATCG-3'	
	,	
	R	
	5'-CCAGTTGGTAACAATGCCAT	
	GT-3'	
Mouse <i>Tnf</i>	F:	N/A
	5'-CAGGCGGTGCCTATGTCTC-3'	
	R:	
	5'-CGATCACCCCGAAGTTCAGT	
	AG-3'	
Mouse <i>Il6</i>	F:	N/A
	5'-CTGCAAGAGACTTCCATCCA	
	G-3'	
	R:	
	5'-AGTGGTATAGACAGGTCTGT	
	TGG-3'	
Mouse <i>Il1b</i>	F:	N/A
	5'-GAAATGCCACCTTTTGACAG	
	TG-3'	
	R:	
	5'-TGGATGCTCTCATCAGGACA	
	G-3'	
Mouse <i>Ccl2</i>	F:	N/A
	5'-TAAAAACCTGGATCGGAACC	
	AAA-3'	
	R:	

---



---

	5'-GCATTAGCTTCAGATTTACGG GT-3'	
Mouse <i>Cxcl10</i>	F: 5'-CCAAGTGCTGCCGTCATTTTC -3'	N/A
	R: 5'-GGCTCGCAGGGATGATTCA A-3'	
Mouse <i>Col1a1</i>	F: 5'-GCTCCTCTTAGGGGCCACT-3'	N/A
	R: 5'-ATTGGGGACCCTTAGGCCAT- 3'	
Mouse <i>Sreb1</i>	F: 5'-TGACCCGGCTATTCCGTGA-3'	N/A
	R: CTGGGCTGAGCAATACAGTTC-3 ,	
Mouse <i>Fasn</i>	F: 5'-GGAGGTGGTGATAGCCGGTA T-3'	N/A
	R: 5'-TGGGTAATCCATAGAGCCCA G-3'	
Mouse <i>Scd1</i>	F: 5'-TTCTTGCGATACTCTGGTG C-3'	N/A
	R: 5'-CGGGATTGAATGTTCTTGTCG	

---

---

	T-3'	
Mouse <i>Acaca</i>	F:	N/A
	5'-CTCCCGATTCATAATTGGGTC	
	TG-3'	
	R:	
	5'-TCGACCTTGTTTTACTAGGTG	
	C-3'	
Mouse <i>Hmgcr</i>	F:	N/A
	5'-TG TTCACCGGCAACAACAAG	
	A-3'	
	R:	
	5'-CCGCGTTATCGTCAGGATGA-	
	3'	
Mouse <i>Cd36</i>	F:	N/A
	5'-ATGGGCTGTGATCGGAACTG-	
	3'	
	R:	
	5'-TTTGCCACGTCATCTGGGTTT-	
	3'	
Mouse <i>Slc27a1</i>	F:	N/A
	5'-CTGGGACTTCCGTGGACCT-3'	
	R:	
	5'-TCTTGCAGACGATACGCAGA	
	A-3'	
Mouse <i>Fabp1</i>	F:	N/A
	5'-ATGAACTTCTCCGGCAAGTA	
	CC-3'	
	R:	
	5'-GGTCCTCGGGCAGACCTAT-3'	

---

---

Mouse <i>Ppara</i>	F: 5'-AACATCGAGTGTCGAATATG TGG-3' R: 5'-CCGAATAGTTCGCCGAAAGA A-3'	N/A
Mouse <i>Cpt1a</i>	F: 5'-TGGCATCATCACTGGTGTGTT -3' R: GTCTAGGGTCCGATTGATCTTT G-3'	N/A
Mouse <i>Acox1</i>	F: 5'-TAACTTCCTCACTCGAAGCC A-3' R: 5'-AGTTCCATGACCCATCTCTGT C-3'	N/A
Mouse <i>Acadl</i>	F: 5'-TTTCCTCGGAGCATGACATTT T-3' R: 5'-GCCAGCTTTTTCCAGACCT-3 ,	N/A
Mouse <i>Acadm</i>	F: 5'-AACACAACACTCGAAAGCG G-3' R: 5'-TTCTGCTGTTCCGTCAACTCA-	N/A

---

---

	3'	
Mouse <i>Cyp4a10</i>	F:	5'- N/A
		TTCCCTGATGGACGCTCTTTA-3'
	R:	5'-
		GCAAACCTGGAAGGGTCAAAC-
	3'	
The sequences of shRNAs		
sh-TM6SF2	TGACCTGGCCCTTGTCATATA	N/A
AAV-shTm6sf2	GCACAGCACATGGCATCTTCA	N/A

---

**Supplementary Table 2 The information of Gene Expression Omnibus series cited in this study**

<b>GEO series</b>	<b>Contributors</b>	<b>Overall design</b>
GSE130970	Hoang SA, Oseini A, Feaver RE, Cole BK <i>et al</i>	6 cases are histologically normal, and 72 cover the full spectrum of nonalcoholic fatty liver disease (activity score 0-6, fibrosis stage 0-4)
GSE48452	Ahrens M, Ammerpohl O, von Schönfels W, Kolarova J <i>et al</i>	73 samples of human liver grouped into Control = 14, Healthy obese = 27, Steatosis = 14 and NASH = 18
GSE83452	Lefebvre P, Lalloyer F, Baugé E, Pawlak M <i>et al</i>	Microarray data were obtained for 152 patients at baseline (44 no NASH, 104 NASH, 4 undefined) and 79 patients (54 no NASH, 22 NASH, 3 undefined) at 1 yr follow-up [38 diet restriction (Diet), 41 bariatric surgeries]
GSE89632	Arendt BM, Comelli EM, Ma DW, Lou W <i>et al</i>	Cross-sectional study, including patients with non-alcoholic fatty liver disease (25 SS, 23 NASH) and 24 healthy living liver donors as HC