

Supplementary Materials

Thrombospondin 1 is associated with MASH and hepatic macrophage inflammatory responses via CD47

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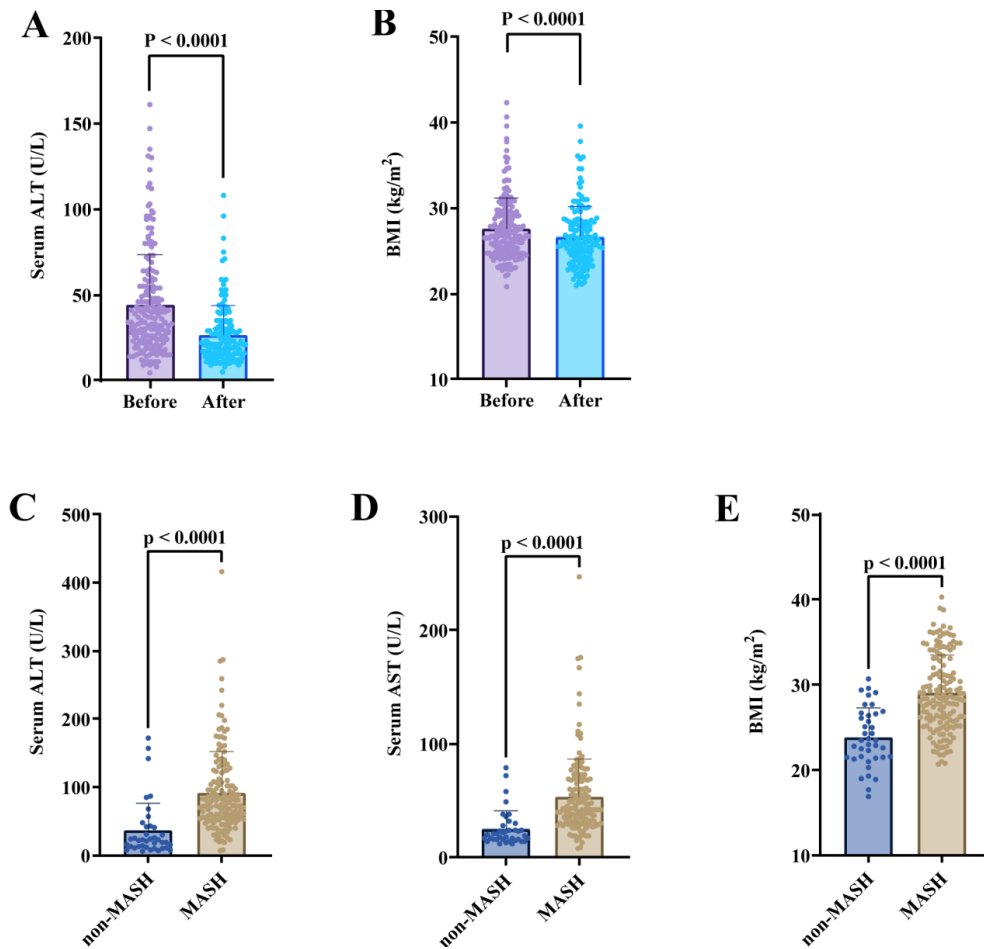
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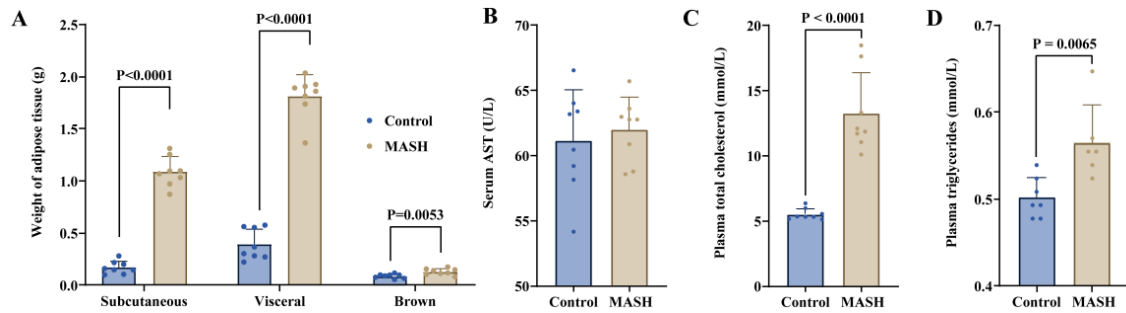
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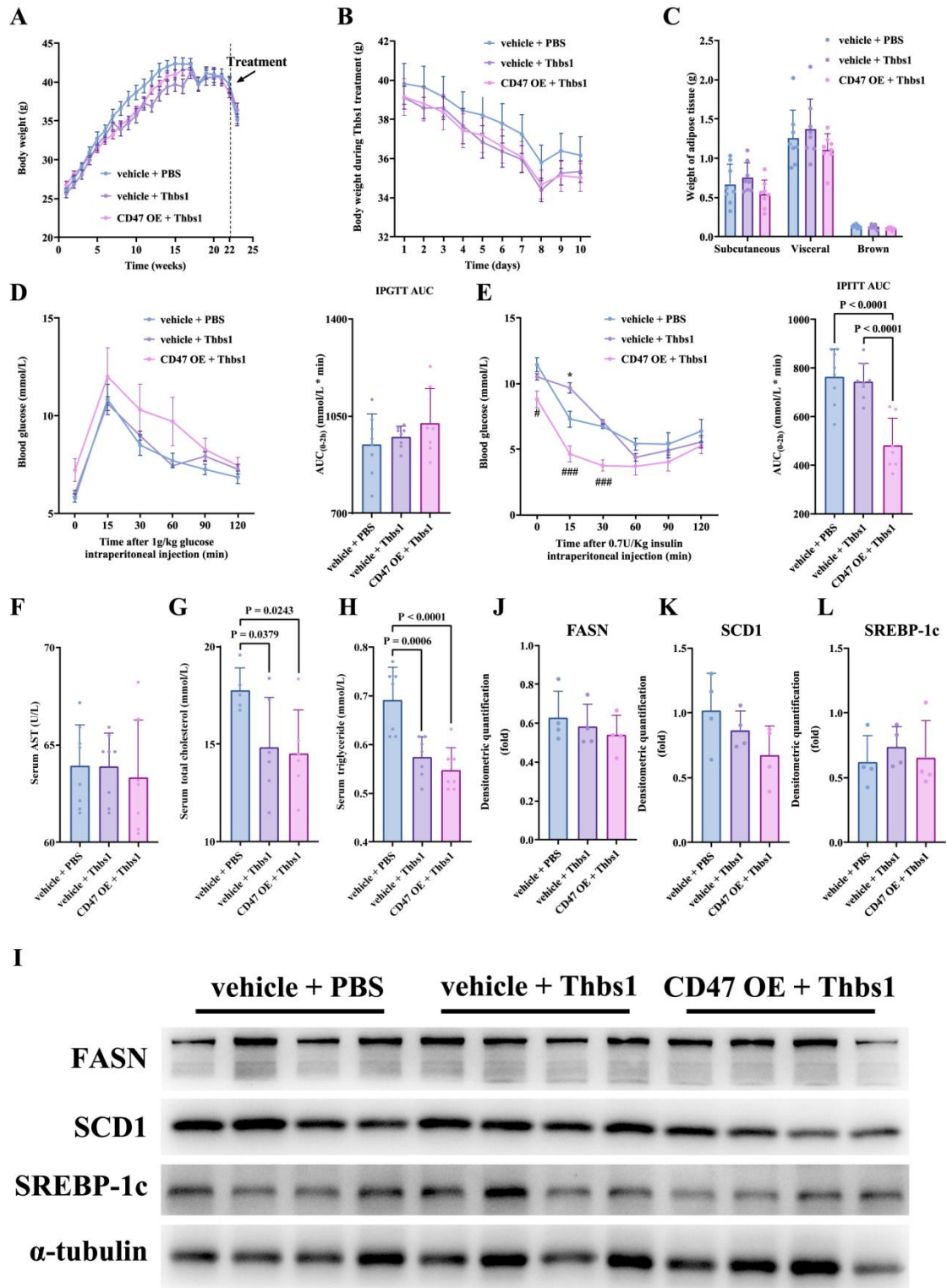


Supplementary Figure 1. Other clinical or serological indicators in clinical studies.

(A and B) Patients with MASLD showed significantly reduced BMI and serum ALT after intervention; (C-E) Patients with MASH showed significantly higher BMI and serum ALT and AST than patients in the non-MASH group. Data are presented as mean \pm SD. *P* values are indicated in figures. Statistical analyses were performed using paired or unpaired two-tailed Student's *t*-tests, as appropriate. $P < 0.05$ was considered statistically significant.



Supplementary Figure 2. Long-term administration of a Western diet and fructose solution to mice induces a MASH model. (A) The mice in the MASH group exhibited a notable increase in fat mass; (B) No significant differences were observed in serum AST levels between the control and MASH groups; (C and D) Mice in the MASH group exhibited notably elevated serum levels of triglycerides and total cholesterol in comparison to those in the control group. Data are presented as mean \pm SD. For animal experiments, $n = 8$ mice per group were initially included; after outlier exclusion using ROUT ($Q = 5\%$), 6-8 samples per group were used for statistical analysis. P values are indicated in figures. $P < 0.05$ was considered statistically significant.



Supplementary Figure 3. Liver-specific CD47 overexpression attenuated the effects of Thbs1 in diet-induced MASH mice. (A) The trend of weight changes in mice of each group during dietary intervention; (B) The trend of weight change in mice of each group during Thbs1 treatment; (C) Among the three groups, no statistically significant differences in body fat mass were observed; (D) No significant differences

were observed in the IPGTT index across the three groups; (E) The difference in IPITT index across the three groups; (F-H) The variations observed in serum AST, total cholesterol, and triglycerides among the three groups; (I-L) Western blot analysis and quantification of proteins involved in *de novo* lipid synthesis among the three groups. Data are presented as mean \pm SD; $n = 8$ mice per group were initially included for physiological and biochemical analyses ($n = 4$ per group for Western blot); after outlier exclusion using ROUT ($Q = 5\%$), 6-8 samples per group were used for statistical analysis. P values are indicated in the figures. Statistical differences among multiple groups were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. $*P < 0.05$ between the vehicle + PBS and vehicle + Thbs1 groups; $^{\#}P < 0.05$ between the vehicle + Thbs1 and CD47 OE + Thbs1 groups; $^{\#\#\#}P < 0.001$ between the vehicle + Thbs1 and CD47 OE + Thbs1 groups. $P < 0.05$ was considered statistically significant.

Supplementary Table 1. Sequences of primers for qRT-PCR

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
<i>Thbs1</i>	GGTAGCTGGAAATGTGGTGCG T	GCACCGATGTTCTCCGTTGTG A
<i>Cd47</i>	GGTGGGAAACTACACTTGCGA AG	CTCCTCGTAAGAACAGGCTGA TC
<i>Fasn</i>	GCTGCGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGAC TT
<i>Scd1</i>	TTCTTCTCTCACGTGGGTTG	CGGGCTTGTAGTACCTCCTC
<i>Cpt1a</i>	GGCATAAACGCAGAGCATTCC TG	CAGTGTCCATCCTCTGAGTAG C
<i>Cd36</i>	GGACATTGAGATTCTTTTCCTC TG	GCAAAGGCATTGGCTGGAAG AAC
<i>Ccl2</i>	GCTACAAGAGGATCACCAGCA G	GTCTGGACCCATTCTTCTTGG
<i>Tgfb1</i>	TGATACGCCTGAGTGGCTGTCT	CACAAGAGCAGTGAGCGCTG AA
<i>Colla1</i>	CCTCAGGGTATTGCTGGACAA C	CAGAAGGACCTTGTTTGCCAG G
<i>Colla2</i>	TTCTGTGGGTCCTGCTGGGAAA	TTGTCACCTCGGATGCCTTGA G
<i>Acaca</i>	GTTCTGTTGGACAACGCCTTCA C	GGAGTCACAGAAGCAGCCCAT T
<i>Ppara</i>	ACCACTACGGAGTTCACGCAT G	GAATCTTGCAGCTCCGATCAC AC
<i>Ccn2</i>	TGCGAAGCTGACCTGGAGGAA A	CCGCAGAACTTAGCCCTGTAT G
<i>Acta2</i>	TGCTGACAGAGGCACCACTGA A	CAGTTGTACGTCCAGAGGCAT AG
<i>Il-6</i>	TACCACTTCACAAGTCGGAGG C	CTGCAAGTGCATCATCGTTGT TC
<i>Il-1b</i>	TGGACCTTCCAGGATGAGGAC A	GTTTATCTCGGAGCCTGTAGT G

<i>Tnf</i>	GGTGCCTATGTCTCAGCCTCTT	GCCATAGAACTGATGAGAGG GAG
<i>Adgre1</i>	CGTGTTGTTGGTGGCACTGTGA	CCACATCAGTGTTCCAGGAGA C
<i>Cd4</i>	G TTCAGGACAGCGACTTCTGG A	GAAGGAGAACTCCGCTGACTC T
<i>Nos2</i>	GAGACAGGGAAGTCTGAAGCA C	CCAGCAGTAGTTGCTCCTCTT C
<i>Cd8a</i>	ACTACCAAGCCAGTGCTGCGA A	ATCACAGGCGAAGTCCAATCC G
<i>Arg1</i>	CATTGGCTTGCGAGACGTAGA C	GCTGAAGGTCTCTTCCATCAC C
<i>Cd68</i>	GGCGGTGGAATACAATGTGTC C	AGCAGGTCAAGGTGAACAGCT G
<i>Cd86</i>	ACGTATTGGAAGGAGATTACA GCT	TCTGTCAGCGTTACTATCCCG C
<i>Il-10</i>	CGGGAAGACAATAACTGCACC C	CGGTTAGCAGTATGTTGTCCA GC
<i>Il-12a</i>	ACGAGAGTTGCCTGGCTACTA G	CCTCATAGATGCTACCAAGGC AC
<i>Il-23a</i>	CATGCTAGCCTGGAACGCACA T	ACTGGCTGTTGTCCTTGAGTC C
<i>Rplp0</i>	AGATTCGGGATATGCTGTTGGC	TCGGGTCCTAGACCAGTGTTCC
<i>Actb</i>	CCACAGCTGAGAGGGAAATC	AAGGAAGGCTGGAAAAGAGC

Primers used for qRT-PCR were specifically designed for mouse (*Mus musculus*) sequences. Thbs1: Thrombospondin 1; Cd47: cluster of differentiation 47; Fasn: fatty acid synthetase; Scd1: stearyl-coenzyme A desaturase 1; Cpt1a: carnitine palmitoyl transferase 1 α ; Cd36: cluster of differentiation 36; Ccl2: C-C motif chemokine ligand 2; Tgfb1: transforming growth factor β 1; Colla1: collagen type I α 1; Colla2: collagen type I α 2; Acaca: acetyl coenzyme A carboxylase; Ppara: peroxisome proliferator activated receptor α ; Ccn2: cellular communication network factor 2; Acta2: actin α 2; Il-6: interleukin 6; Il-1b: interleukin 1 beta; Tnf: tumor necrosis factor; Adgre1: adhesion G protein-coupled receptor E1; Cd4: cluster of differentiation 4; Nos2: nitric

oxide synthase 2; Cd8a: cluster of differentiation 8a; Arg1: arginase-1; Cd68: cluster of differentiation 68; Cd86: cluster of differentiation 86; Il-10: interleukin 10; Il-12a: interleukin 12A; Il-23a: interleukin 23A; Rplp0: ribosomal protein lateral stalk subunit P0; Actb: actin beta.

Supplementary Table 2. Multivariate linear regression analysis for NAS

Variable	β (95%CI)	<i>P</i> value
Thbs1 (per unit increase)	0.048 (0.010-0.087)	0.013
BMI (kg/m ²)	0.095 (0.049-0.141)	< 0.001
ALT (U/L)	0.005 (-0.001-0.011)	0.112
AST (U/L)	0.002 (-0.009-0.013)	0.782
Age (year)	-0.015 (-0.030-0.000)	0.056
Sex	0.333 (-0.070-0.737)	0.105
Diabetes mellitus	0.483 (0.072-0.893)	0.021
Triglycerides (mmol/L)	0.124 (-0.034-0.283)	0.124
Total cholesterol (mmol/L)	-0.298 (-0.721-0.125)	0.168
HDL cholesterol (mmol/L)	0.147 (-0.387-0.680)	0.590
LDL cholesterol (mmol/L)	0.089 (-0.376-0.554)	0.707
Fasting plasma glucose (mmol/L)	0.069 (-0.030-0.167)	0.174
Fibrosis stage	0.719 (0.508-0.931)	< 0.001

Multivariate linear regression was conducted using multiply imputed datasets. All covariates were mutually adjusted within the same model, and β coefficients represent adjusted changes in NAS. β : Regression coefficient; CI: confidence interval; BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; NAS: NAFLD Activity Score.