

Supplementary materials

***S100a16* Deficiency Prevents Alcohol-induced Fatty Liver Injury via Inducing MANF Expression in Mice**

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Keywords:

Alcoholic liver disease; S100A16; hepatic steatosis; MANF; ER stress.

Material and Methods

Immunohistochemistry (IHC)

Mouse liver samples were fixed, embedded in paraffin, and sectioned at 5 μm thickness for IHC. IHC staining was performed with anti-S100A16 antibody. The primary antibody was incubated at 4 °C overnight, and washed three times with PBS for 10 min. Finally, a horseradish peroxidase-conjugated secondary antibody is applied.

Mouse tail genotyping

Commercially available Mouse Direct PCR Kit (Bimake, B40013) were used to detect mouse tail genotyping according to the instructions provided by the manufacturer, and then agarose gel electrophoresis.

Luciferase reporter gene analysis

Control (pcDNA 3.1) or S100A16 overexpression plasmid was co-transfected with MANF promoter luciferase reporter gene in AML 12 cells. After 48 h, the cells were lysed, and luciferase activities were measured with the Dual-Luciferase Reporter Assay System (Promega, E1910) using an automatic microplate reader (BioTek, Synergy H1). Renilla luciferase plasmid was used as an internal control.

Statistical analysis

All of the experimental results were statistically analyzed using GraphPad Prism 8.0 software. All data are expressed as the mean \pm SEM. Two-tailed student's t-test was used to calculate the statistical significance between two groups and one-way analysis of variance (one way ANOVA) for three or more groups. In all cases, $p < 0.05$ was considered statistically significant.

Supplementary Figures

A

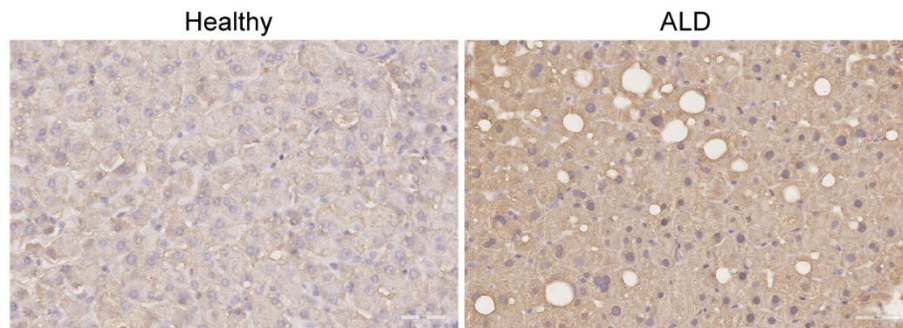


Fig.S1 (A) Representative immunohistochemical staining images of S100A16 in liver paraffin slices from healthy controls and patients with alcoholic-associated liver disease (ALD). Scale bar :50 μ m.

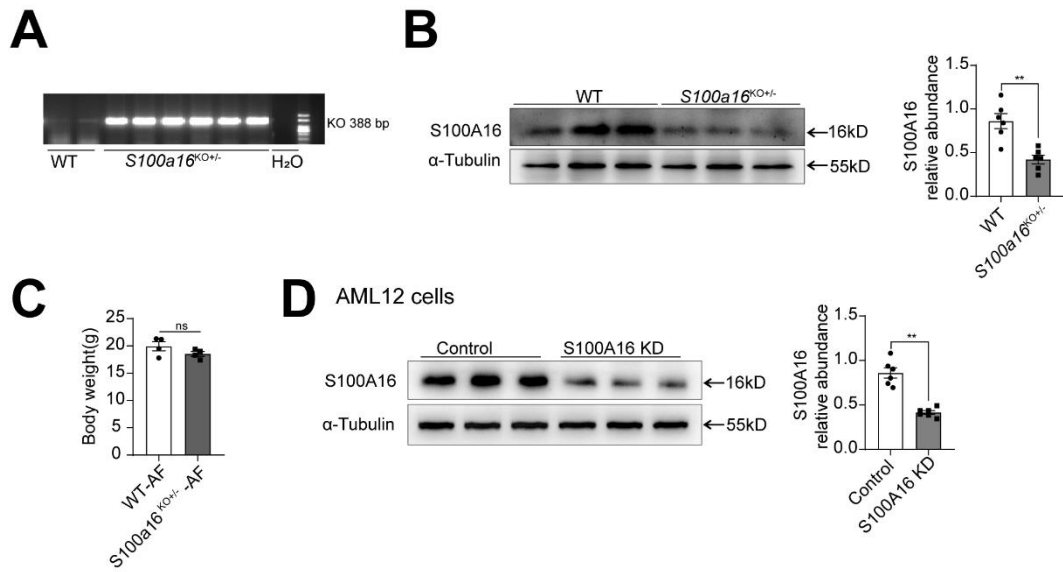


Fig.S2 (A) A representative gel electrophoresis image for genotyping. (B) Western blot analyses of S100A16 in the liver from WT and *S100a16*^{KO+/-} mice. (C) Body weight from WT and *S100a16*^{KO+/-} mice after alcohol feeding. (D) Western blot analyses of S100A16 in AML 12 cells after transfection by siRNA (S100A16 KD). α -Tubulin served as the loading control. All data are represented as the mean \pm SEM values. *: $p < 0.05$ vs. the control group, **: $p < 0.01$ vs. the control group.

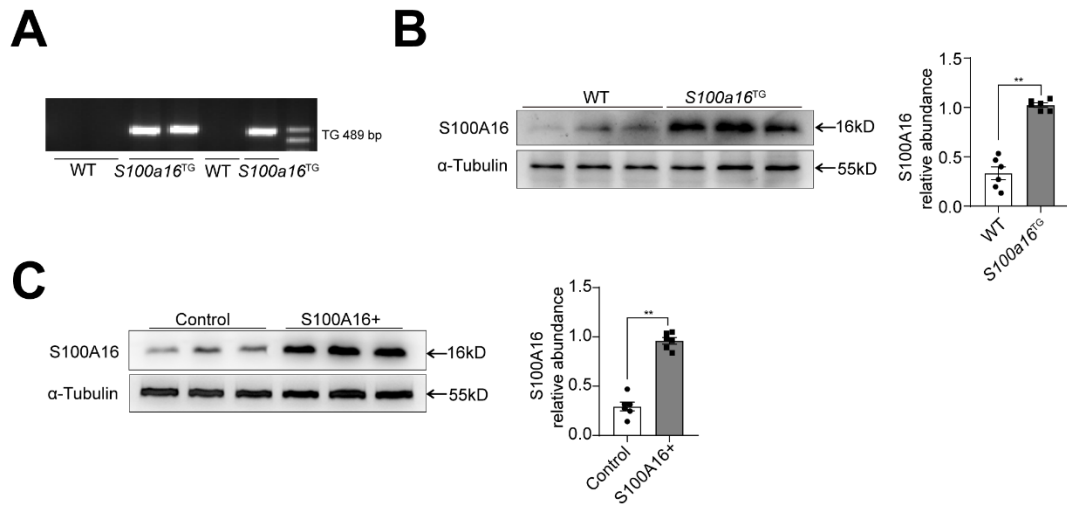
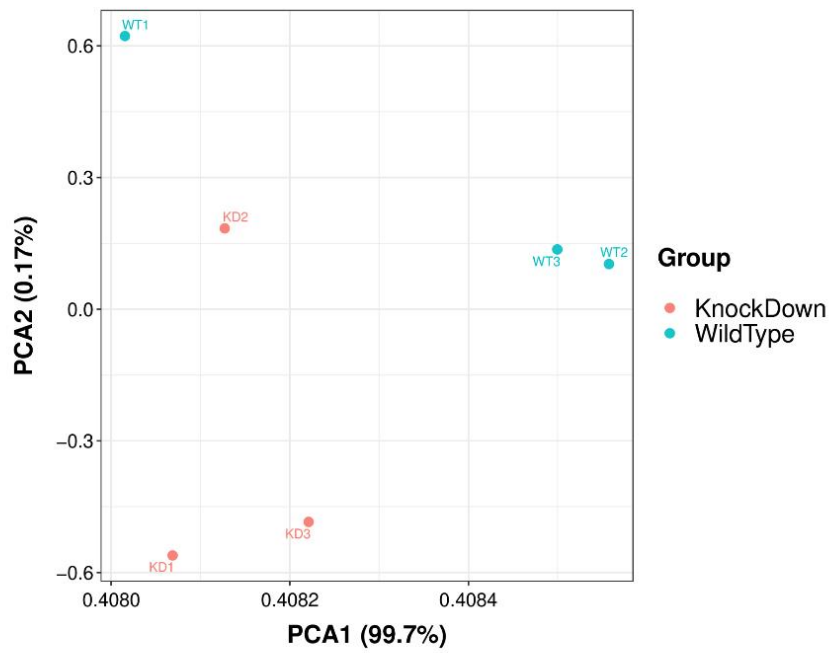
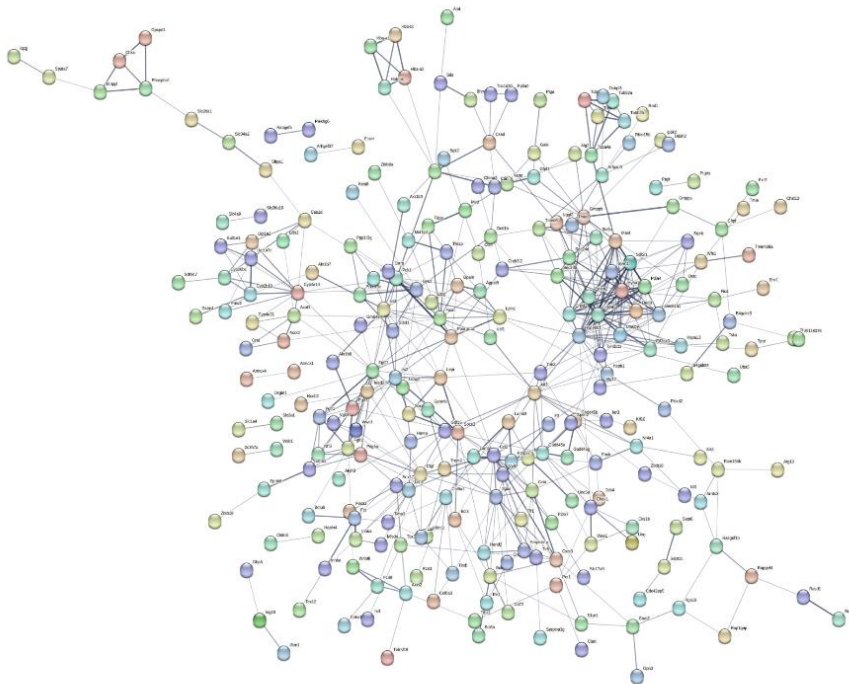


Fig.S3 (A) A representative gel electrophoresis image for genotyping. (B) Western blot analyses of S100A16 in the liver from WT and *S100a16^{TG}* mice. (C) Western blot analyses of S100A16 in AML 12 cells after transfection by overexpression plasmid (*S100A16+*). α -Tubulin served as the loading control. All data are represented as the mean \pm SEM values. *: $p < 0.05$ vs. the control group, **: $p < 0.01$ vs. the control group.

A**Principal Components Analysis****B****Fig.S4** (A) A PCA plot. (B) Protein-protein interaction network picture.

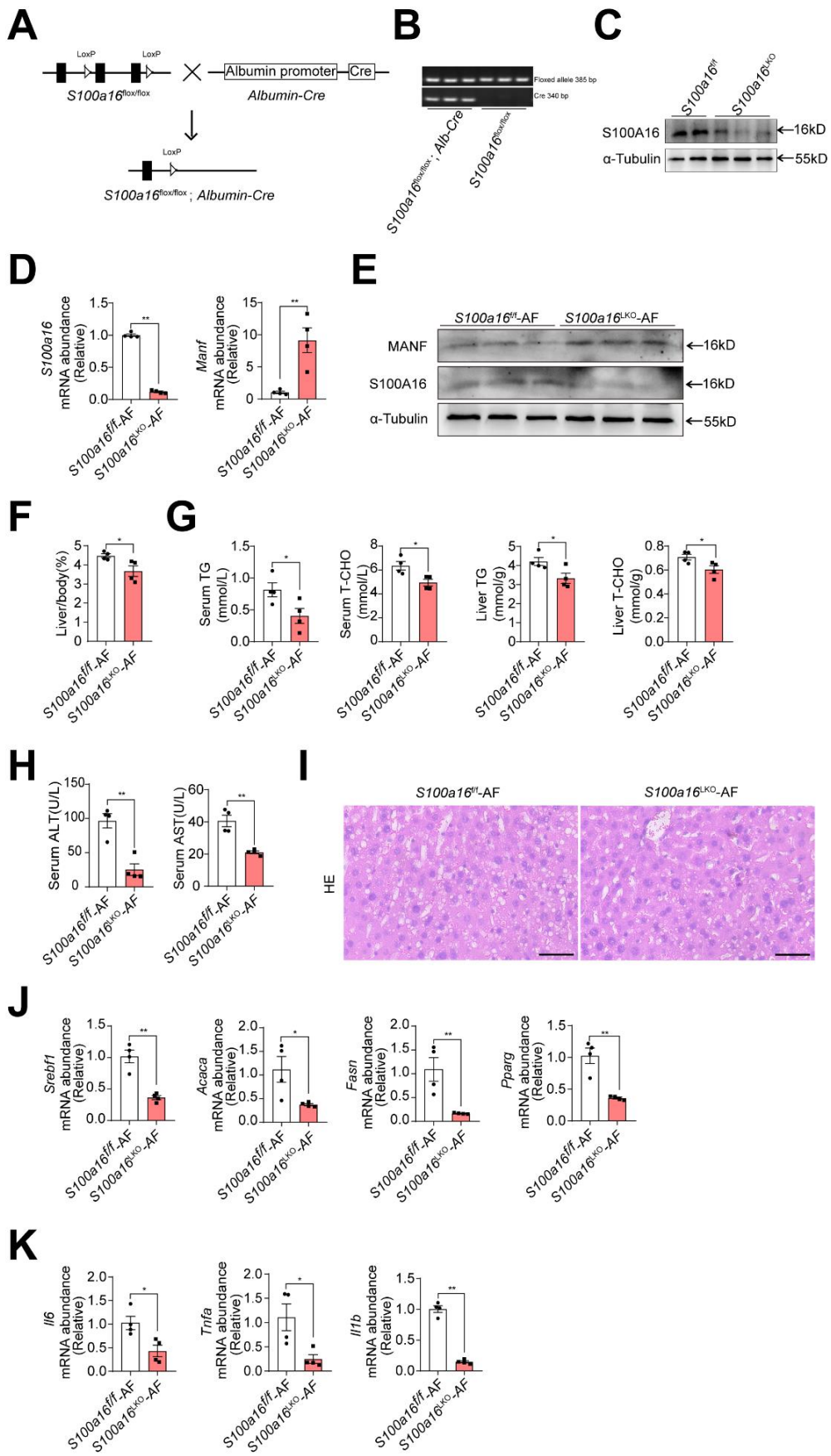


Fig.S5 (A) The strategy for creating *S100a16* hepatocyte-specific knockout mice. (B) A representative gel electrophoresis image for genotyping. (C) Western blot analyses of S100A16 in the liver from *S100a16^{fl/fl}* and *S100a16^{LKO}* mice. (D-K) *S100a16^{fl/fl}* or *S100a16^{LKO}* mice were fed separately with the corresponding liquid diet 10 days and administered a single binge of ethanol. (D) mRNA and (E) protein levels of S100A16, MANF in the liver from *S100a16^{fl/fl}* and *S100a16^{LKO}* mice. (F) Liver weight/body weight (%). (G) Serum TG, TC levels and liver TG, TC levels. (H) Serum ALT, AST levels. (I) Representative H&E staining of mice livers. Scale bars: 50 μ m. (J) Relative mRNA levels of *Srebf1*, *Acaca*, *Fasn*, and *Pparg*. (K) Relative mRNA levels of *Il6*, *Tnfa*, and *Il1b*. α -Tubulin served as the loading control. All data are represented as the mean \pm SEM values. *: $p < 0.05$ vs. the control group, **: $p < 0.01$ vs. the control group.

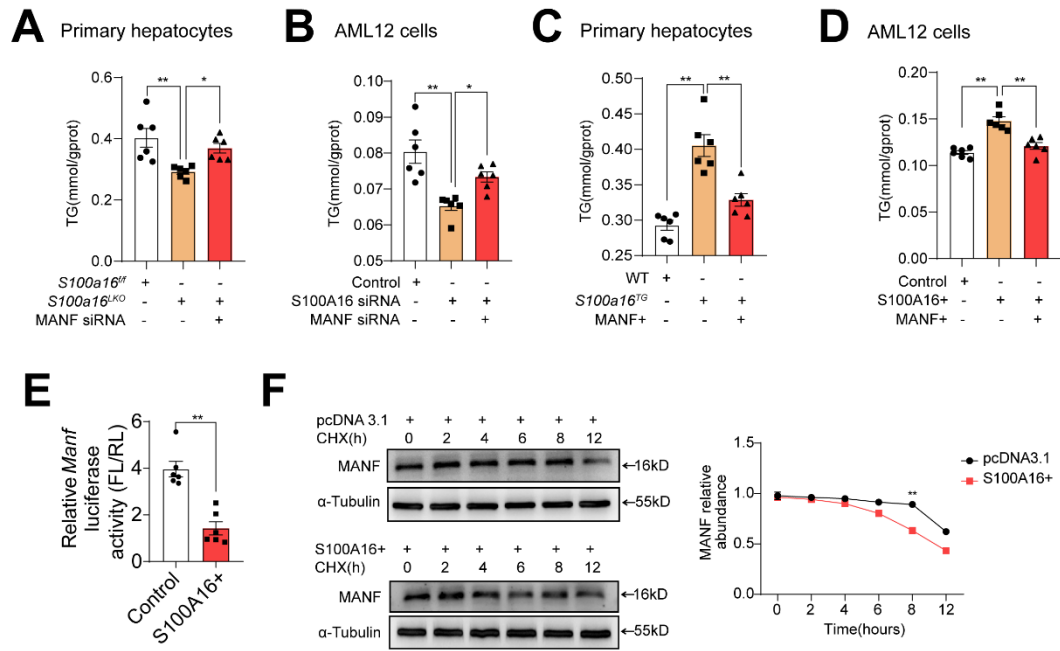


Fig.S6 (A) TG contents in ethanol-induced primary hepatocytes from *S100a16^{fl/fl}* or *S100a16^{LKO}* mice transfected with *Manf* siRNA. (B) TG contents in ethanol-induced AML 12 cells transfected with *S100a16* siRNA and *Manf* siRNA. (C) TG contents in ethanol-induced primary hepatocytes from WT or *S100a16^{TG}* mice transfected with *Manf* overexpression plasmid. (D) TG contents in ethanol-induced AML 12 cells transfected with *S100A16* and *MANF* overexpression plasmid. (E) Luciferase reporter gene assay for effect of *S100A16* overexpression plasmid (*S100A16+*) on *MANF* gene promoter. The *MANF* luciferase reporter plasmid was co-transfected with *S100A16* full-length or pcDNA 3.1 plasmid in AML 12 cells. The luciferase activity was normalized with renilla luciferase as the internal control. (F) *MANF* protein expression in AML 12 cells transfected with *S100A16* overexpression or vector plasmid treated with CHX (25 μ g/mL) at predict time. Quantification of western blots on the right. α -Tubulin served as the loading control. All data are represented as the mean \pm SEM values. *: $p < 0.05$ vs. the control group, **: $p < 0.01$ vs. the control group.

Supplementary Tables

Table S1

The information of the primary antibodies used in this study.

Antibody	Company	Cat#	Species	Dilution
MANF	Abcam	Ab67271	rabbit	1:1000
S100A16	Proteintech	11456	rabbit	1:500
α -Tubulin	Proteintech	11224	rabbit	1:1000
GAPDH	Proteintech	HRP60004		1:5000
BIP	CST	3177	rabbit	1:1000
p-IRE1	Abcam	Ab48187	rabbit	1:1000
IER1	CST	3294	rabbit	1:1000
ATF4	Proteintech	11815	rabbit	1:500
P-eIF2 α	CST	3398	rabbit	1:1000
eIF2 α	CST	5324	rabbit	1:1000
ATF6	CST	65880	rabbit	1:1000

Table S2**The sequence information of the primers used for RT-qPCR.**

Genes	Species	Forward	Reverse
<i>S100a16</i>	mouse	CGGACACAGGGAACCGAAAG	GTCCAGTATTCGTCAAAGCAGA
<i>Manf</i>	mouse	TCTGGGACGATTTTACCAGGA	TCTTGCTTCACGGCAAAACTTTA
<i>I8s</i>	mouse	GTAACCCGTTGAACCCCAT	CCATCCAATCGGTAGTAGCG
<i>Srebfl</i>	mouse	GATGTGCGAACTGGACACAG	CATAGGGGGCGTCAAACAG
<i>Fasn</i>	mouse	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
<i>Acaca</i>	mouse	GATGAACCATCTCCGTTGGC	GACCCAATTATGAATCGGGAGTG
<i>Ppara</i>	mouse	CATTTGTATGACTCATAATAAAGT	CGGATGGCCACCTCTTTGCTCTG
<i>Il6</i>	mouse	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
<i>Il1b</i>	mouse	GCAACTGTTCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
<i>Tnf</i>	mouse	CTGAACTTCGGGGTGATCGG	GGCTTGTCACTCGAATTTGAGA