iScience, Volume *27*

Supplemental information

Hepatokine ITIH3 protects against hepatic steatosis by downregulating mitochondrial bioenergetics and *de novo* lipogenesis

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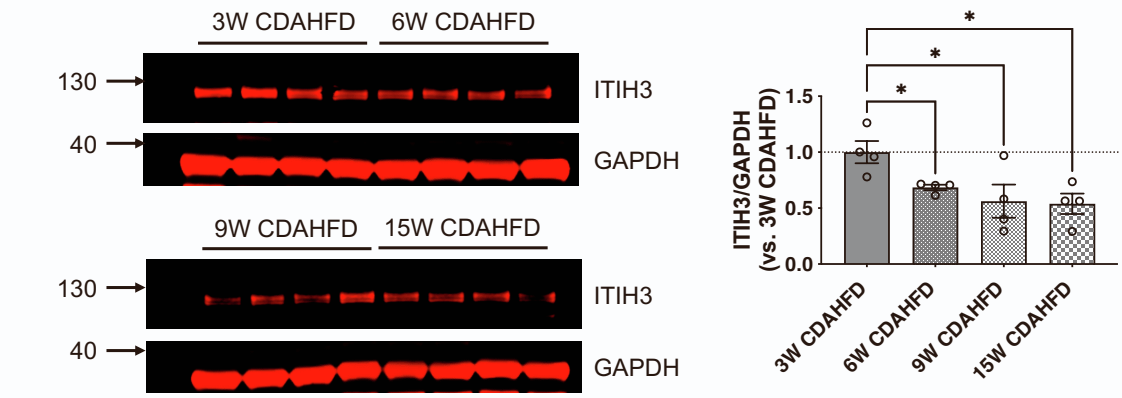

Figure S1. ITIH3 is negatively associated with NAFLD/NASH in mice. *Related to Figure 1.* **(A)** Immunoblot analyses of hepatic ITIH3 and their respective quantification from C57BL/6J mice maintained on a choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD) at different timepoints to develop different stages of NAFLD/NASH. GAPDH was used as a loading control. Data are presented as mean ± SEM (n = 4 mice per group). P values were calculated by one-factor ANOVA corrected by post-hoc 'Holm-Sidak's' multiple comparisons test. *$P < 0.05$.

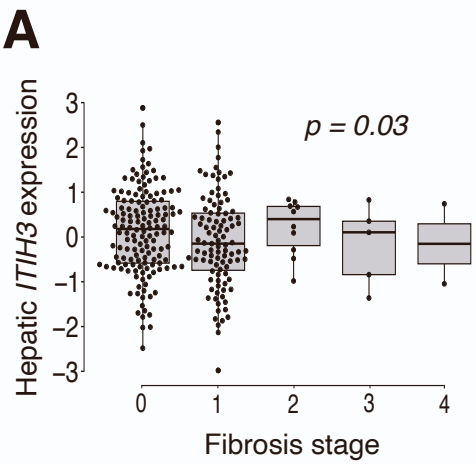

Figure S2. ITIH3 is negatively associated with Fibrosis in humans. *Related to Figure 1.* **(A)** Hepatic *ITIH3* expression from KOBS cohort (n = 262) grouped by Fibrosis stage. Data are presented as median and interquartile range (boxplots). P values were calculated by ANCOVA corrected for age, BMI, and sex. BMI, body mass index.

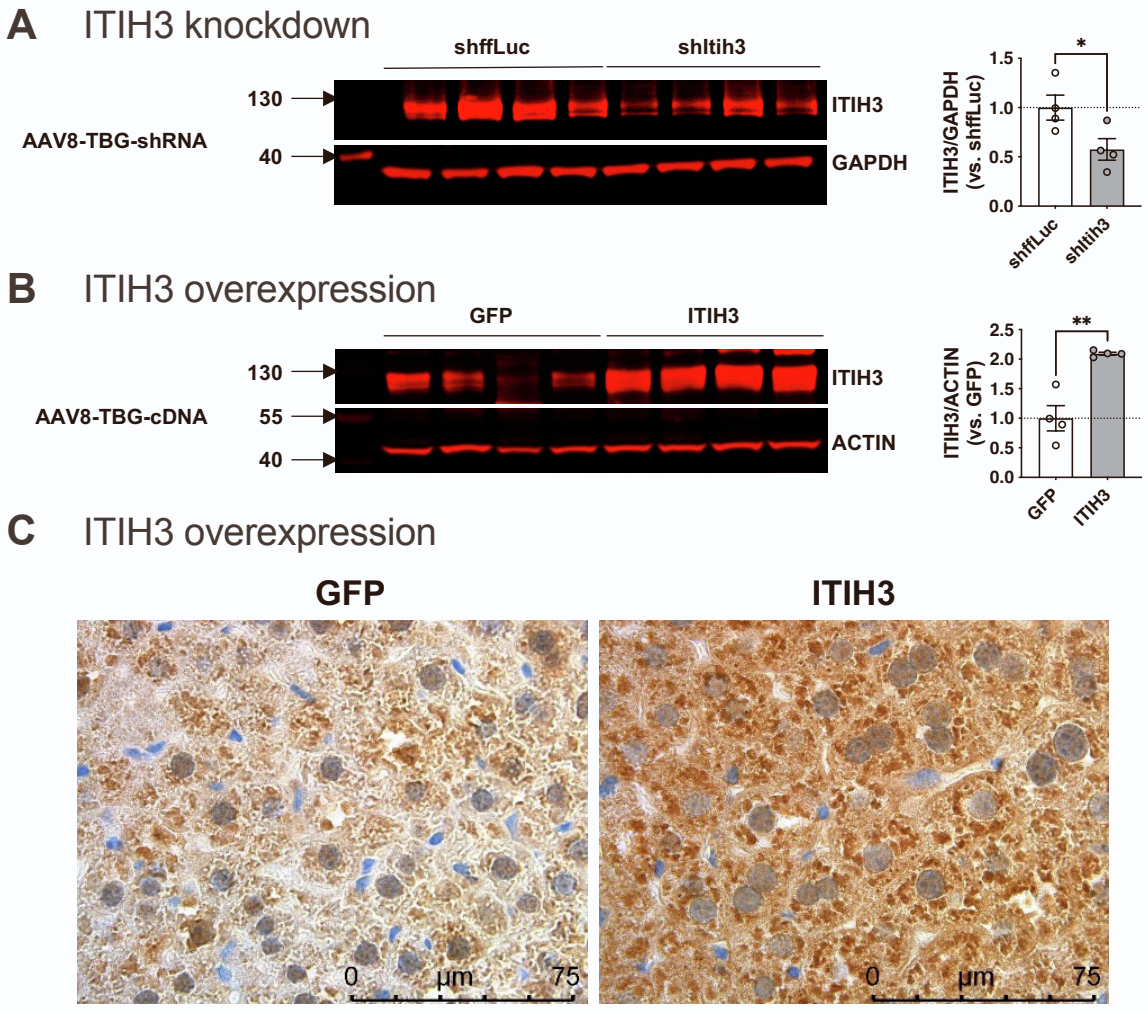

Figure S3. AAV-mediated modulation of hepatic ITIH3 expression. *Related to Figure 2.* Eight-week-old male C57BL/6J mice were injected with either loss-of-function (shffLuc or shItih3) or gain-of-function (GFP or ITIH3) AAV vectors under the control of TBG promoter and fed a HF/HS diet for eight additional weeks. Immunoblot analyses of hepatic ITIH3 in **(A)** ITIH3 knockdown and **(B)** overexpression mice and their respective quantification. GAPDH was used as a loading control. **(C)** Representative immunohistochemical analyses of hepatic ITIH3 in GFP and ITIH3 overexpression mice. Data are presented as mean ± SEM (n = 4 mice per group). P values were calculated by t test. *P < 0.05; **P < 0.01.

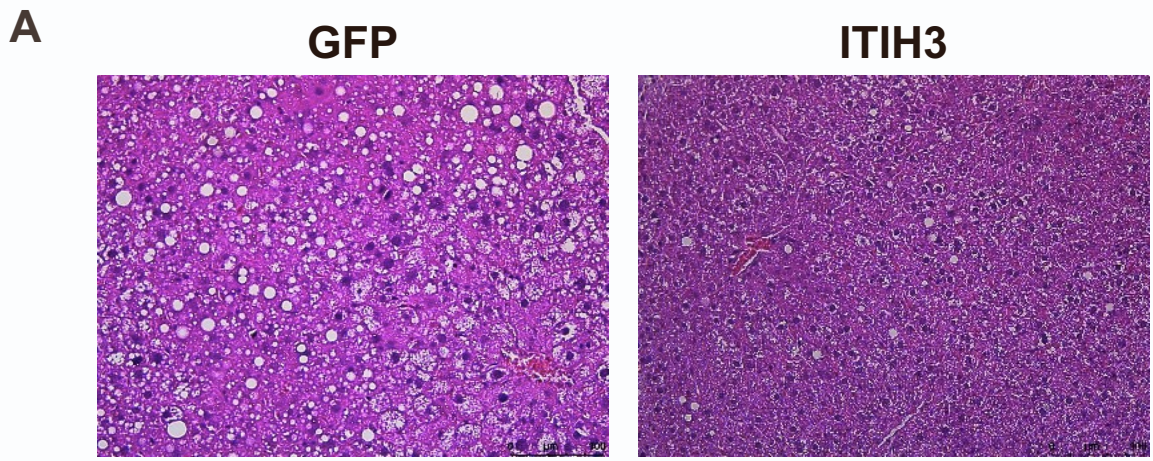

Figure S4. ITIH3 lowers liver lipid droplet accumulation *in vivo*. *Related to Figure 2.* Eight-week-old male C57BL/6J mice were injected with gain-of-function (GFP or ITIH3) AAV vectors under the control of TBG promoter and fed a HF/HS diet for eight additional weeks. **(A)** Representative images of H&E staining of livers overexpressing GFP or ITIH3 are shown.

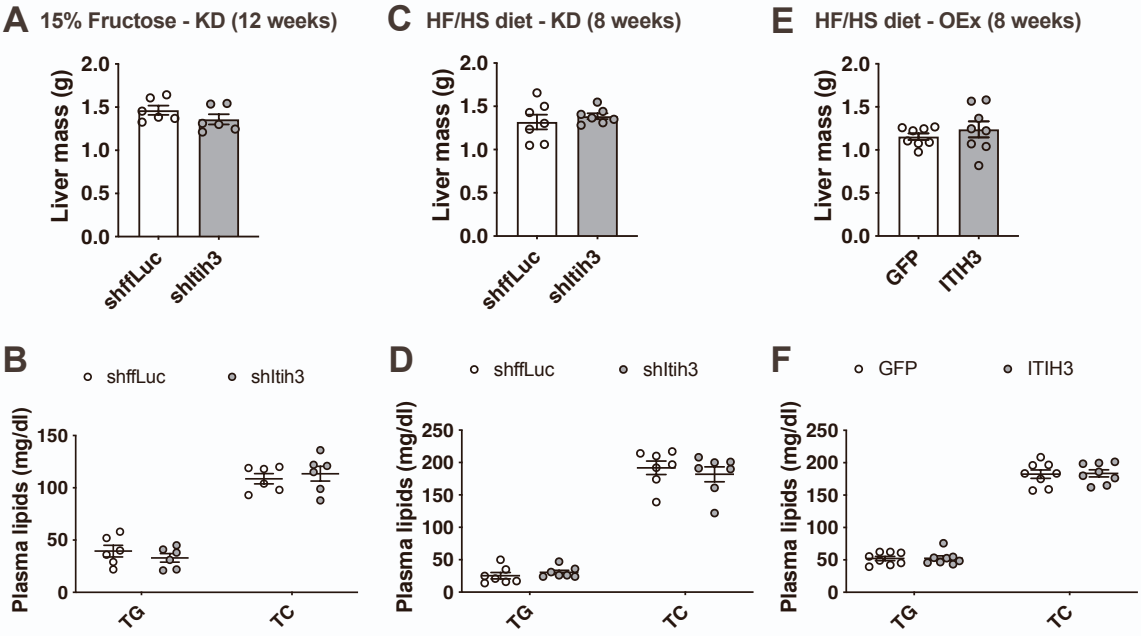

Figure S5. ITIH3 did not alter liver mass and plasma lipids. *Related to Figure 2.* Comparisons of **(A, C and E)** liver mass and **(B, D and F)** plasma TG and TC levels from **(A and B)** 15% fructose in drinking water or **(C and D)** HF/HS fed ITIH3 silencing (KD) mice or **(E and F)** HF/HS fed ITIH3 overexpressing (OEx) mice, respectively. Data are presented as mean ± SEM (n = 6-8 mice per group). TG, triglyceride; TC, total cholesterol.

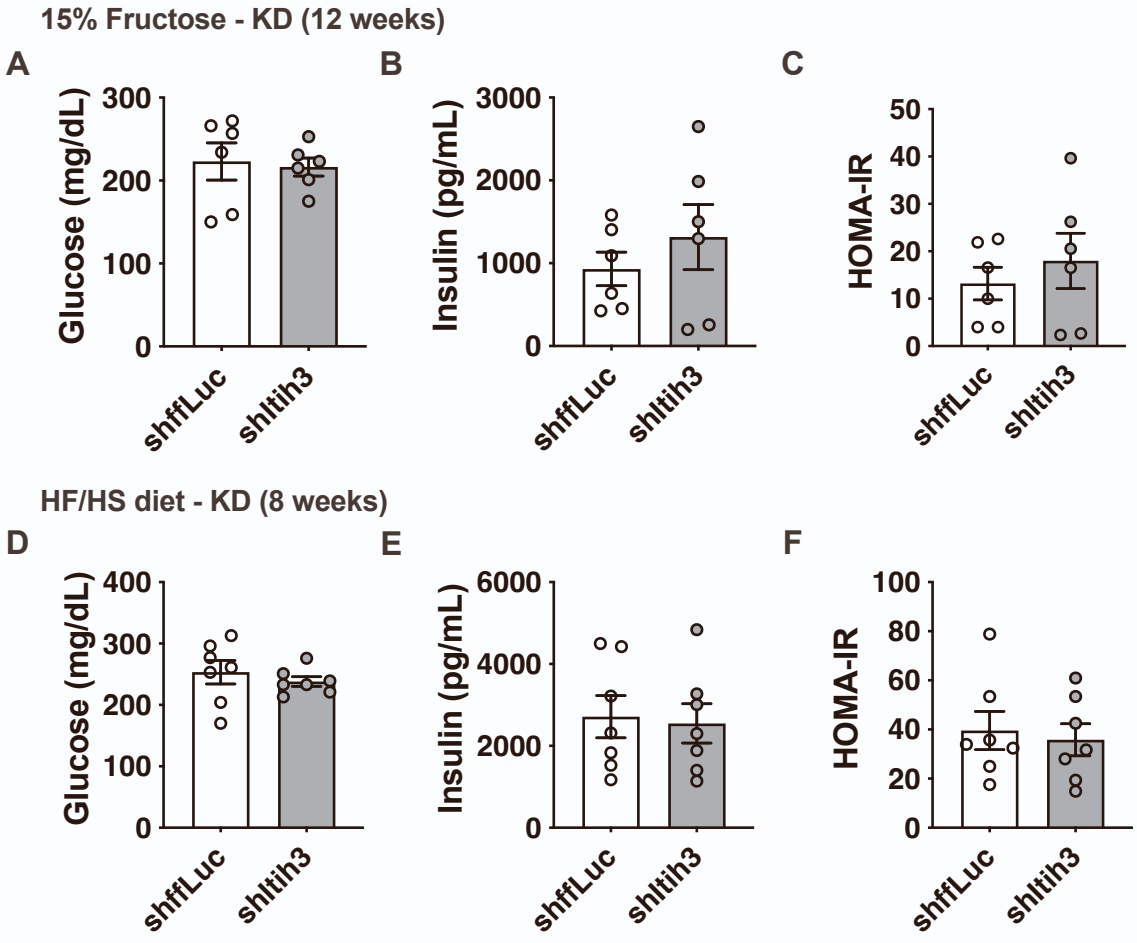

Figure S6. ITIH3 did not alter fasting plasma glucose and insulin levels as well as HOMA-IR. *Related to Figure 2.* Comparisons of fasting plasma **(A and D)** glucose and **(B and E)** insulin levels and **(C and F)** HOMA-IR from **(A, B and C)** 15% fructose in drinking water or **(D, E and F)** HF/HS fed ITIH3 silencing (KD) mice, respectively. Data are presented as mean ± SEM (n = 6-7 mice per group). HOMA-IR, Homeostatic Model Assessment for Insulin Resistance.

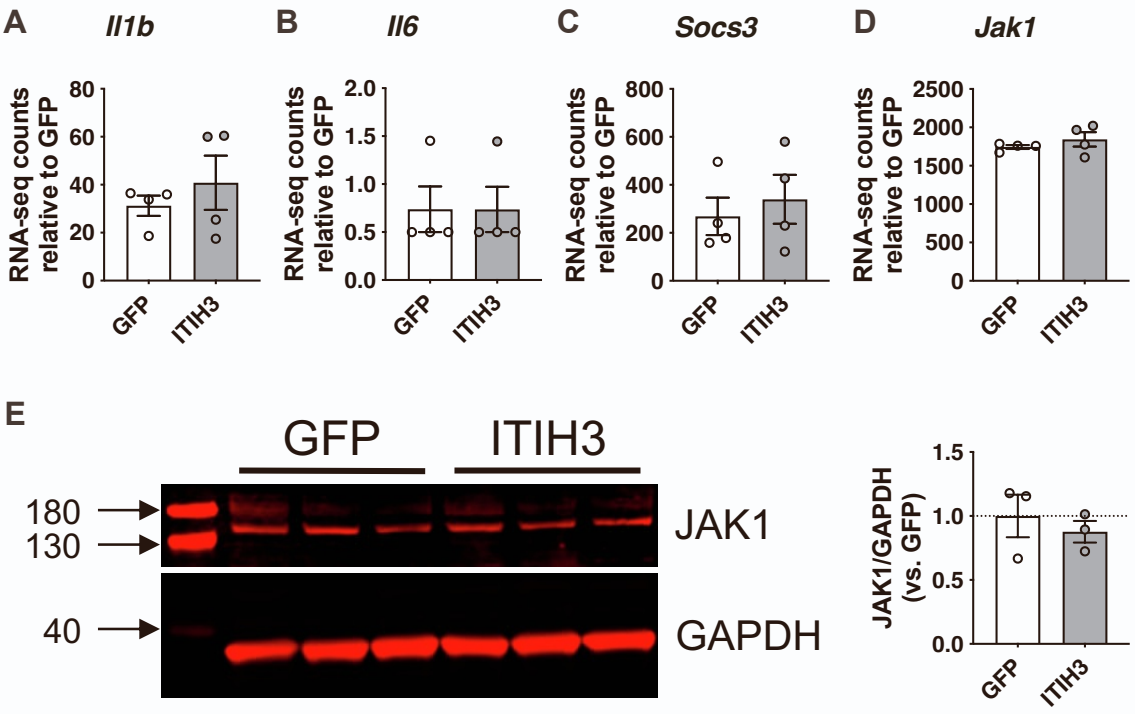

Figure S7. ITIH3 did not alter IL-6/JAK pathway-related transcripts. *Related to Figure 4.* RNA counts of **(A)** *Il1b*, **(B)** *Il6*, **(C)** *Socs3*, and **(D)** *Jak1* in ITIH3 overexpressing mice. Follow-up **(E)** immunoblot analyses and their respective quantification of liver JAK1 in GFP or ITIH3 overexpressing mice. GAPDH was used as a loading control. Data are presented as mean ± SEM (n = 4 mice for transcriptomics and n = 3 mice for immunoblot analyses per group). P values were calculated by (A – D) Wald test; (E) t test. *P < 0.05; **P < 0.01; ***P < 0.001.

Table S3. Clinical characteristics and liver histology of 262 KOBS study participants with gene expression data. *Related to STAR Methods.*

	N
Ethnicity	Finns - white all from Kuopio city area
Sex (F/M)	182/80
T2D, N (%)	94 (35.9)
Age (years)	48.71 [42.32, 55.38]
BMI (kg/m^2)	42.47 [39.15, 46.16]
Cholesterol (mmol/L)	4.10 [3.50, 4.70]
HDL (mmol/L)	1.07 [0.90, 1.27]
LDL (mmol/L)	2.25 [1.73, 2.80]
TG (mmol/L)	1.38 [1.06, 1.88]
Glucose (mmol/L)	6.00 [5.40, 6.70]
Insulin (pmol/L)	111.11 [73.61, 162.50]
Steatosis grade, N (%)	
0	105 (40.1)
1	95 (36.3)
2	33 (12.6)
3	9 (11.1)
Lobular inflammation, N (%)	
0	179 (68.3)
1	67 (25.6)
2	16 (6.1)
Hepatocellular ballooning grade, N (%)	
0	195 (74.7)
1	65 (24.9)
2	1 (0.4)
Fibrosis stage, N (%)	
0	146 (55.7)
1	99 (37.8)
2	10 (3.8)
3	5 (1.9)
4	2 (0.8)

Data are shown as median (interquartile range) or n (%) according to distribution of variables. BMI: body mass index; HDL: high density lipoprotein; LDL: low density lipoprotein.