ZR Zoological Research

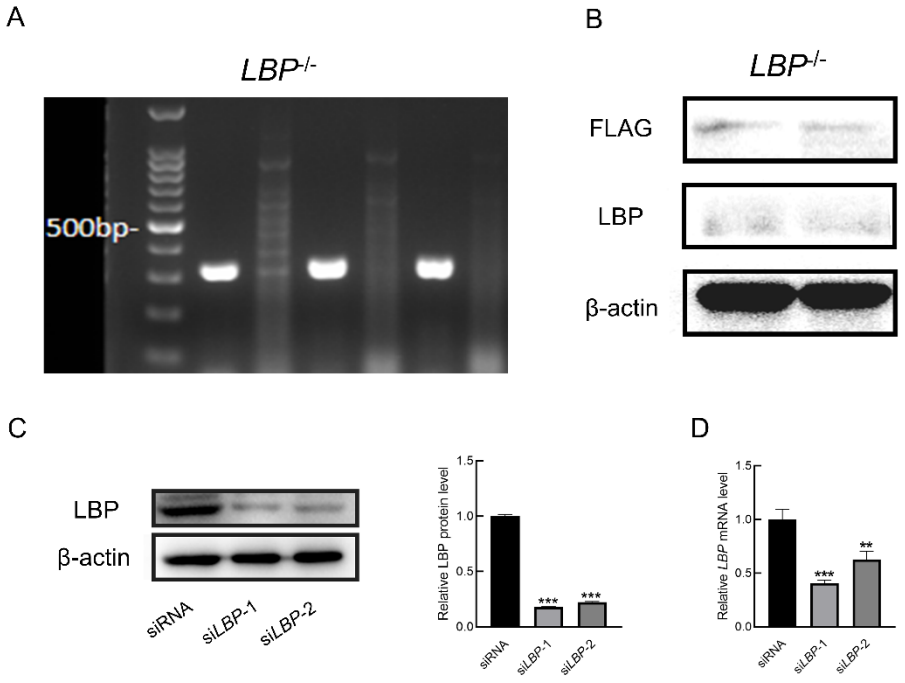
Supplementary Materials

Loss of *LBP* triggers lipid metabolic disorder through H3K27 acetylation-mediated C/EBPβ-*SCD* activation in non-alcoholic fatty liver disease

Ya-Ling Zhu [1,2,#], Lei-Lei Meng [1,#], Jin-Hu Ma [1,#], Xin Yuan [1], Shu-Wen Chen [1], Xin-Rui Yi [1], Xin-Yu Li [1], Yi Wang [1], Yun-Shu Tang [1,2], Min Xue [1], Mei-Zi Zhu [1], Jin Peng [1], Xue-Jin Lu [1], Jian-Zhen Huang [4], Zi-Chen Song [1], Chong Wu [1], Ke-Zhong Zheng [3], Qing-Qing Dai [3], Fan Huang [3,*], Hao-Shu Fang [1,2,*]

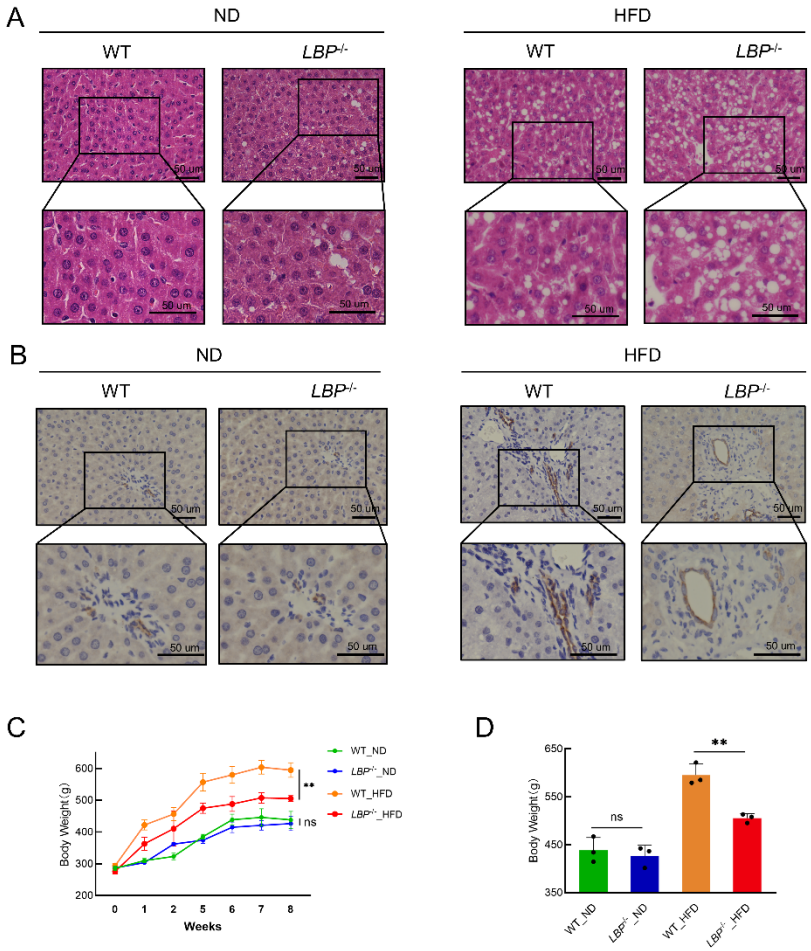
[1] *Department of Pathophysiology, Anhui Medical University, Hefei, Anhui* 230032, *China*
[2] *Laboratory Animal Research Center, School of Basic Medical Sciences, Anhui Medical University, Hefei, Anhui* 230032, *China*
[3] *Department of Hepatobiliary Surgery, First Affiliated Hospital of Anhui Medical University, Hefei, Anhui* 230022, *China*
[4] *College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang, Jiangxi* 330045, *China*

[#]Authors contributed equally to this work
[*]Corresponding authors, E-mail: huang_f@vip.126.com; fanghaoshu@ahmu.edu.cn



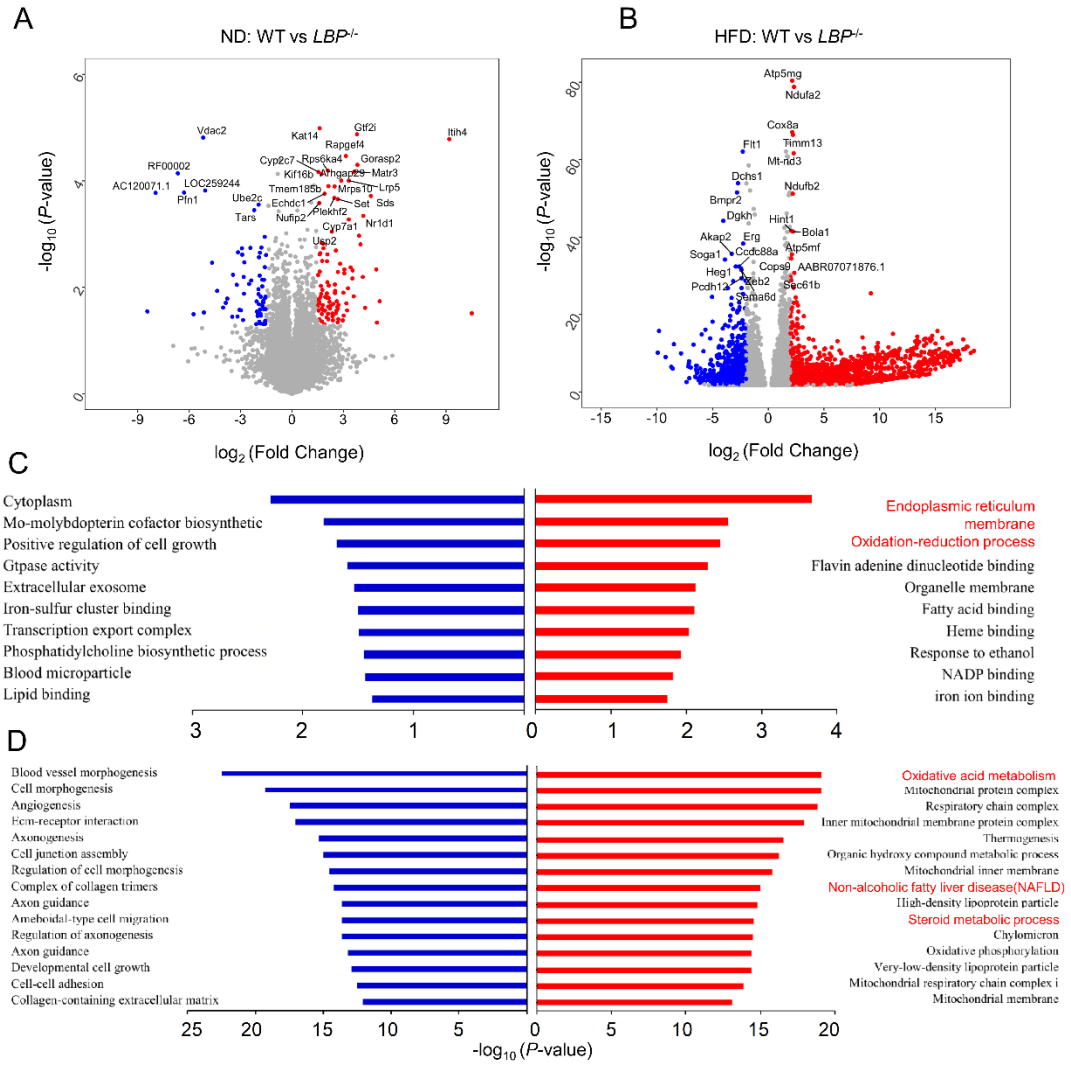
Supplementary Figure S1 Validation of efficiency of *LBP* knockout *in vivo* and *LBP* knockdown *in vitro*

A–B: Agarose electrophoresis (A) and western blot (B) analysis showing significantly decreased RNA and protein concentrations after *LBP* deficiency treatment, respectively. C, D: Western blot (C) and RT-qPCR (D) analysis of *LBP* expression in BRL-3A hepatocytes transfected with siRNA or si*LBP* (n=3/group). Student's t-test was performed for data analysis. **: P<0.01, ***: P<0.001. Data are shown as mean±SD.



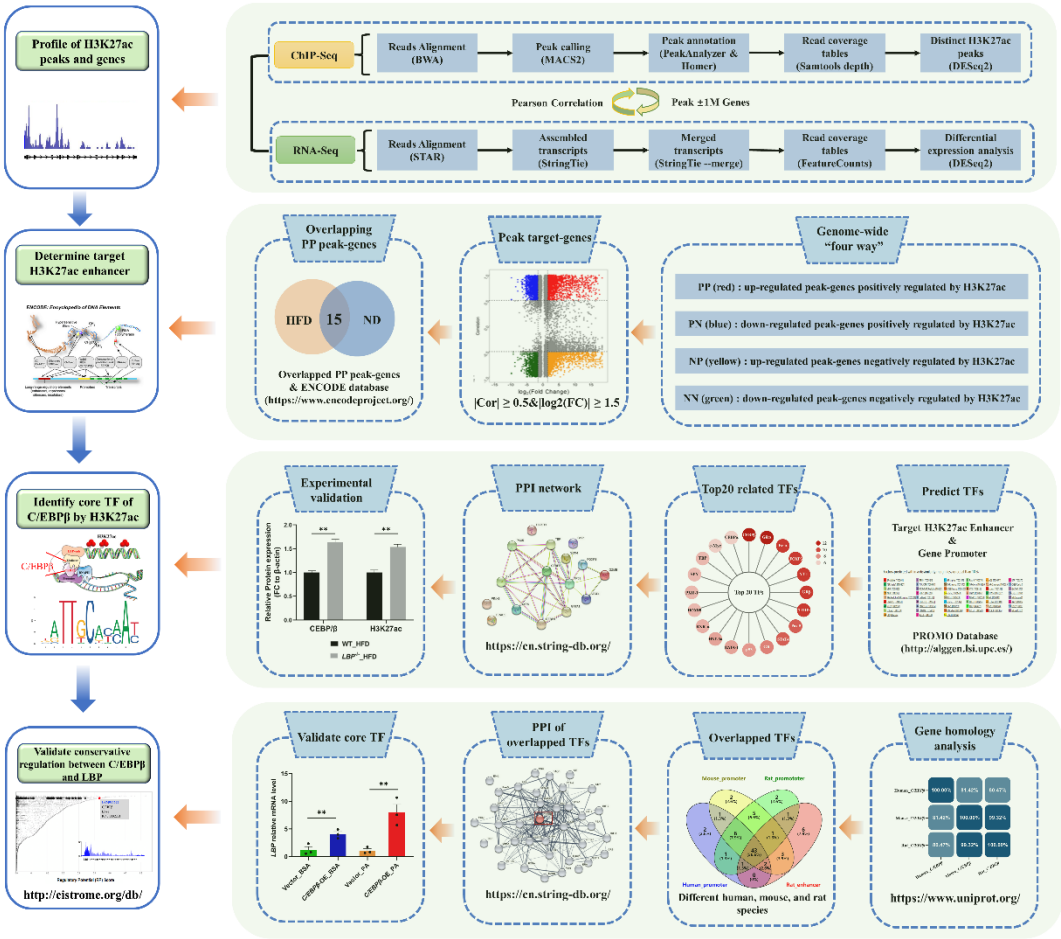
Supplementary Figure S2 Liver lipid accumulation and inflammation and body weight changes induced by *LBP* deficiency

A: Enlargement of H&E-stained liver sections in Figure 1C showing aggravated lipid accumulation induced by *LBP* deficiency and HFD feeding (scale bar, 50 μm), *n*=6/group. B: Representative immunohistochemical staining for Inducible nitric oxide synthase (iNOS) of liver tissues in indicated groups (scale bar, 50 μm), *n*=6/group. C: Body weight change during 8 weeks in WT and *LBP*$^{-/-}$ rats fed with ND and HFD. D: Body weight at time of harvest. *n*=3/group. One-way ANOVA was performed for data analysis. ns: Not significant; **: $P<0.01$. All data are shown as mean±SD.

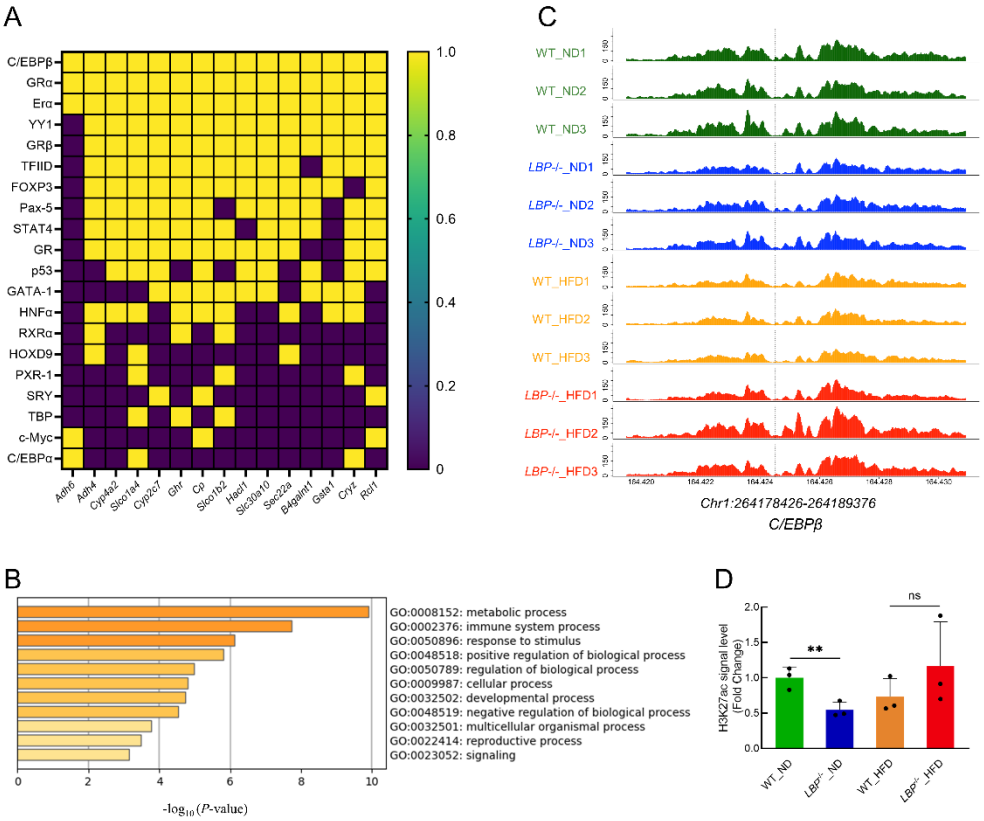


Supplementary Figure S3 *LBP* deficiency aggravates lipid metabolism disorders induced by HFD

A, B: Volcano plots showing significant differentially expressed genes (DEGs) between *LBP*$^{-/-}$ and WT rats fed with ND (A) or HFD (B). Red, blue, and gray indicate up-regulated, down-regulated, and unchanged, respectively. C, D: Regulatory terms of markedly up-regulated (red) and down-regulated DEGs (blue) in liver from *LBP*$^{-/-}$ and WT rats fed with ND (C) and HFD (D) by DAVID (https://david-d.ncifcrf.gov/) (ranked by corrected *P*-values).



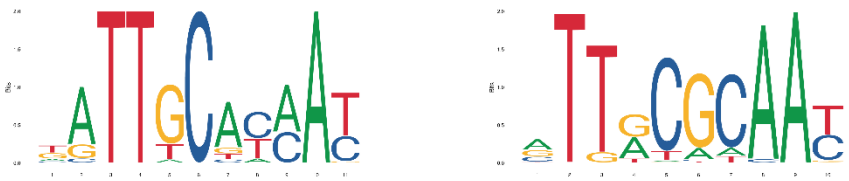
Supplementary Figure S4 Study workflow of core transcription identification



Supplementary Figure S5 C/EBPβ may act as a critical mediator during development of NAFLD with LBP deficiency

A: Heatmap showing regulatory relationship between 15 overlapping PP peak-genes and top 20 predicted TFs. B: GO enrichment analysis of top 20 TFs by Metascape (https://metascape.org/gp/). C, D: Genome browser representation (C) and quantification (D) showing activity of *Chr1:264178426-264189376*, which tracks at C/EBPβ locus in indicated individuals (*n*=3/group). One-way ANOVA was performed for data analysis. ns: Not significant; **: *P*<0.01. Data are shown as mean±SD.

A

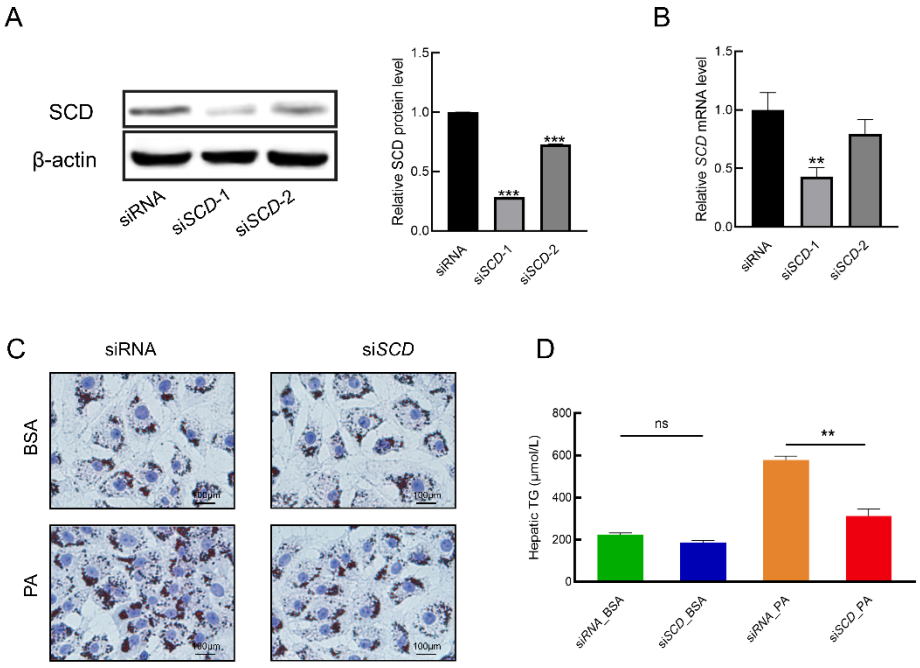


B

Site	Score	Start	End	Strand	Predicted Sequence
Site 1	4.2887635	1380	1390	+	AATTTCCCCAG
Site 2	4.159251	406	415	+	ATTTTAAAAC
Site 3	3.2865922	52	61	+	CTAGCACAAC
Site 4	3.159669	627	636	-	CTTAAAAAAT
Site 5	1.9172345	1381	1390	-	CTGGGGAAAT
Site 6	1.8067557	1859	1868	+	CTGAGGAAAT
Site 7	1.5211531	699	708	+	GTGTCACCAC
Site 8	1.269318	295	304	-	CCTGCGCCAC
Site 9	0.7319348	51	61	+	ACTAGCACAAC
Site 10	0.5881713	359	369	+	AATTTCAAGAC

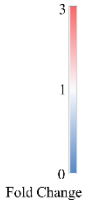
Supplementary Figure S6 C/EBPβ may induce transcription activation of *SCD* by binding to *SCD* promoter

A: Potential motif logos for TF C/EBPβ predicted using JASPAR (https://jaspar.genereg.net/). B: Top 10 potential binding sites of C/EBPβ in *SCD* promoter region predicted using ASPAR (https://jaspar.genereg.net/).



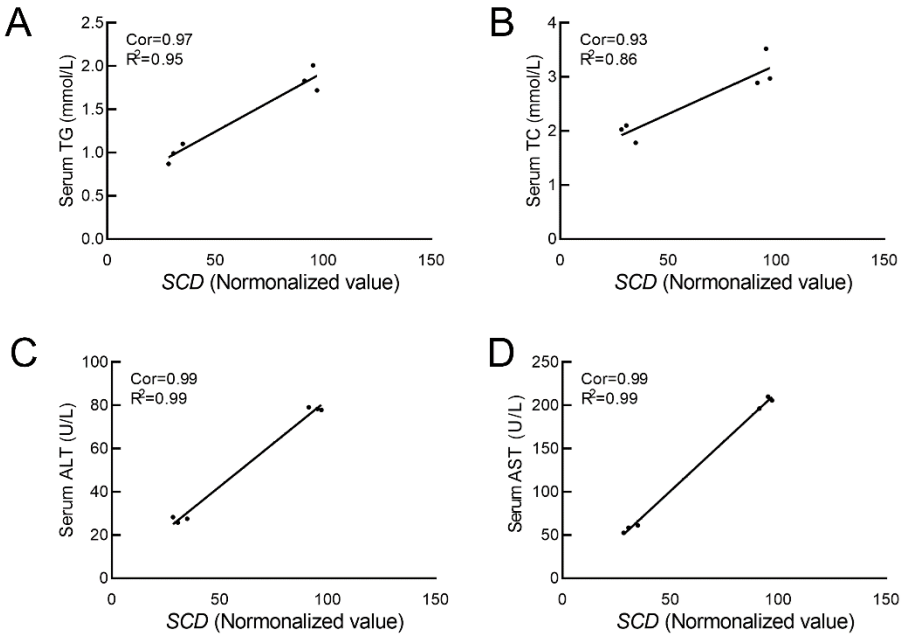
Supplementary Figure S7 si*SCD* significantly reduces PA-induced lipid accumulation in hepatocytes

A, B: Validation of *SCD* knockdown in BRL-3A hepatocytes by western blot analysis (A) and RT-qPCR (B), respectively. *n*=3/group. C: Images of Oil-red O staining of *SCD*-knockdown BRL-3A hepatocytes and controls stimulated with PA or BSA (*n*=3/group). Scale bar, 100 μm. D: Hepatic TG levels after different processing in BRL-3A hepatocytes (*n*=3/group). Student's *t*-test or one-way ANOVA was performed for data analysis as appropriate. **: $P<0.01$; ***: $P<0.001$. Data are shown as mean±SD.



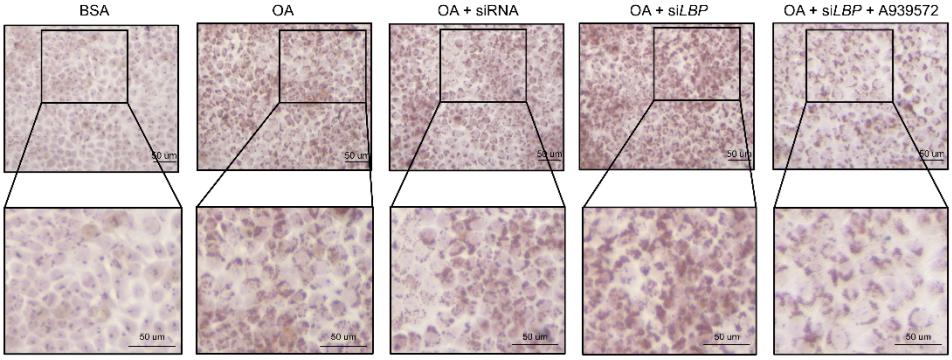
*LBP*_KO	WT	*LBP*_KI	Fatty Acids
3.1421	1.0000	0.5444	Methyl octanoate
1.6834	1.0000	1.0177	Methyl myristate
1.4655	1.0000	0.3411	Methyl dodecanoate
1.4181	1.0000	0.7118	Methyl linolenate
1.3533	1.0000	0.7959	Methyl elaidate
1.3439	1.0000	0.9038	Methyl palmitoleate
1.3415	1.0000	0.6668	Methyl oleate
1.3150	1.0000	0.4657	Methyl undecanoate
1.2168	1.0000	1.1539	Methyl palmitate
1.1422	1.0000	0.9964	Methyl docosatetraenoate
1.1328	1.0000	0.7022	Methyl tridecanoate
1.1159	1.0000	0.7900	cis-11,14-Eicosadienoic acid methyl ester
1.1102	1.0000	0.8093	Methyl linoleate
1.0614	1.0000	0.9184	Methyl stearate
1.0258	1.0000	0.8686	Methyl arachidonate
1.0025	1.0000	0.9279	cis-11,14,17-Eicosatrienoic acid methyl ester

Supplementary Figure S8 Metabolomic analysis reveals increased liver fatty acid content induced by *LBP* knockout in HFD-fed rats



Supplementary Figure S9 Correlation between *SCD* expression and NAFLD parameters in *LBP*$^{-/-}$ rats

A–D: Correlation between *SCD* expression in RNA-Seq data and serum TG (A), TC (B), ALT (C), and AST (D) contents in *LBP*$^{-/-}$ individuals (*n*=3/group).



Supplementary Figure S10 Images of Oil-red O staining of HepG2 hepatocytes in indicated groups (*n*=3/group)

Scale bar, 50 μm.

Supplementary Table S1 Primers for RT-qPCR

Primer	Sequence 5'→3'
Rat *IL6*	F: GTTGCCTTCTTGGGACTG R: ACTGGTCTGTTGTGGGTG
Rat *TNF-α*	F: GCTCCCTCTCATCAGTTCCA R: GCTTGGTGGTTTGCTACGAC
Rat *FASN*	F: GGAGGTGGTGATAGCCGGTAT R: TGGGTAATCCATAGAGCCCAG
Rat *SREBP1c*	F: GGAGCCATGGATTGCACATT R: AGGAAGGCTTCCAGAGAGGA
Rat *LBP*	F: TCAGGCCTTCAACATAGCCA R: TTGGAGTCAGGCGGTAACAT
Rat *SCD*	F: CCAAGAACCTCCTGGGCTAA R: AACTGCCCTTGAGGTAGGTC
Rat *C/EBPβ*	F: CGACTTCCTTTCCGACCTCT R: GAGGCTCACGTAACCGTAGT
Rat *β-actin*	F: CACCATGTACCCAGGCATTG R: CCTGCTTGCTGATCCACATC
Mouse *PPARα*	F: TATTCGGCTGAAGCTGGTGTAC R: CTGGCATTTGTTCCGGTTCT
Mouse *SLC25A20*	F: GACGAGCCGAAACCCATCAG R: AGTCGGACCTTGACCGTGT
Mouse *FATP1*	F: CTGGGACTTCCGTGGACCT R: TCTTGCAGACGATACGCAGAA
Mouse *MLYCD*	F: GCACGTCCGGGAAATGAAC R: GCCTCACACTCGCTGATCTT
Mouse *HADHA*	F: TGCATTTGCCGCAGCTTTAC R: GTTGGCCCAGATTTCGTTCA
Mouse *HADHB*	F: TCGGGTTTGTTGCATCGGA R: GGCCAGAAGCTATCAGACCAA
Mouse *ACADM*	F: AACACAACACTCGAAAGCGG R: TTCTGCTGTTCCGTCAACTCA
Mouse *DECR1*	F: GATCCGGGTCCTCAGAGGTTT R: ATCAGGTGGTAGCATAGGCTT
Mouse *β-actin*	F: GTGACGTTGACATCCGTAAAGA R: GCCGGACTCATCGTACTCC

Supplementary Table S2 Primers for luciferase reporter assay

Primer	Sequence 5'→3'
Site 1	F: AAGAGAAATAGAATGAAAATAT
	R: GCATGCGCCACCACACTTAGCTA
Site 2	F: CACAGAGAGGCTTACAGAAAAC
	R: GACTCCGGCCGCACACACAGG
Site 3	F: GGGTCAGAGCATCTCAGGGACC
	R: TTCACCCAGCAGCAGGCGAAAG

Supplementary Table S3 Top 10 up- and down-regulated genes based on RNA-Seq of *LBP*-/-_ND and WT_ND livers (ranked by *P*-value)

Gene	log_2 (fold-change)	*P*-value	*P*adj	Related function
Up-regulated				
KAT14	1.62	1.03E-05	2.83E-02	Component of histone acetyltransferase activity on histones H3 and H4; Weakening of histone acetyltransferase activity toward histone H4
GTF2I	3.80	1.34E-05	2.83E-02	RNA polymerase II transcription initiation and promoter of Akt signaling; DNA-binding transcription factor activity
ITIH4	9.19	1.65E-05	2.83E-02	Elevated platelet cytosolic Ca^{2+}; Serine-type endopeptidase inhibitor activity and endopeptidase inhibitor activity
RAPGEF4	3.15	3.42E-05	4.01E-02	Cytoskeletal signaling and elevated platelet cytosolic Ca^{2+}; Guanyl-nucleotide exchange factor activity and small GTPase binding
GORASP2	3.82	5.01E-05	4.60E-02	Gene silencing by RNA and cell cycle, mitotic
RPS6KA4	2.09	6.38E-05	4.88E-02	Interferon pathway; Transfer protein tyrosine kinase activity; Gene activation by histone phosphorylation and regulation of inflammatory genes
MATR3	3.63	6.77E-05	4.88E-02	Related to nucleic acid binding and nucleotide binding
CYP2C7	1.55	6.82E-05	4.88E-02	Response to ethanol, lipopolysaccharide
KIF16B	1.72	7.46E-05	4.88E-02	Elevated platelet cytosolic Ca^{2+} and Golgi-to-ER retrograde transport
ARHGAP29	2.88	9.91E-05	5.10E-02	GTPase activator activity and PDZ domain binding; Dampening of ROCK and MYH9 activities in endothelial cells
Down-regulated				
VDAC2	-5.18	1.54E-05	2.83E-02	Metabolite diffusion across mitochondrial outer membrane; Cytoskeletal signaling and deubiquitylation;
RF00002	-6.66	7.22E-05	4.88E-02	/
LOC259244	-5.07	1.52E-04	5.48E-02	Predicted to enable odorant binding activity and active in extracellular space
PFN1	-6.30	1.66E-04	5.76E-02	RNA binding and actin binding
AC120071.1	-7.96	1.68E-	5.76E-	/

		04	02	
UBE2C	-1.95	2.80E-04	8.16E-02	APC-Cdc20 mediated degradation of Nek2A and cell cycle; Ligase activity and ubiquitin protein ligase binding
TARS	-2.20	3.55E-04	9.73E-02	Catalyze the aminoacylation of tRNA; Metabolism of proteins
CHTOP	-1.59	1.13E-03	1.80E-01	Transport of mature transcript to cytoplasm and gene expression; RNA binding
LOC100910979	-1.78	1.79E-03	1.99E-01	/
PACSIN3	-2.92	1.85E-03	1.99E-01	Clathrin-mediated endocytosis and vesicle-mediated transport; Lipid binding and cytoskeletal protein binding

Supplementary Table S4 Top 10 up- and down-regulated genes based on RNA-Seq of *LBP*-/-_HFD and WT_HFD livers (ranked by *P*-value)

Gene	log_2 (fold-change)	*P*-value	*P*adj	Related function
Up-regulated				
ATP5MG	2.15	3.95E-81	6.93E-77	Organelle biogenesis and maintenance and transcriptional activation of mitochondrial biogenesis
NDUFA2	2.31	1.71E-79	1.50E-75	ATP synthesis by chemiosmotic coupling; Complex I biogenesis; NADH dehydrogenase (ubiquinone) activity
COX8A	2.16	7.83E-68	4.58E-64	Cytochrome-c oxidase activity; ATP synthesis by chemiosmotic coupling
TIMM13	2.22	3.76E-67	1.65E-63	Related to peroxisomal lipid metabolism
MT-ND3	2.28	2.38E-62	5.23E-59	ATP synthesis by chemiosmotic coupling; Metabolism; NADH dehydrogenase (ubiquinone) activity
NDUFB2	2.20	6.58E-52	6.41E-49	ATP synthesis by chemiosmotic coupling; Complex I biogenesis; NADH dehydrogenase (ubiquinone) activity
HINT1	2.04	2.25E-42	1.41E-39	Nucleotide binding and protein kinase C binding; Modulation of proteasomal degradation of target proteins
BOLA1	2.22	3.85E-42	2.33E-39	Protect cells against oxidative stress
ATP5MF	2.12	3.13E-36	1.37E-33	Organelle biogenesis and maintenance and transcriptional activation of mitochondrial biogenesis
COPS9	2.06	3.58E-35	1.50E-32	Involved in cellular and developmental processes; Regulator of ubiquitin (UBI)

				conjugation
Down-regulated				
FLT1	-2.29	8.54E-63	2.14E-59	PI3K-Akt signaling pathway and VEGF pathway; Activation of MAP kinase and AKT1 signaling pathway
DCHS1	-2.72	1.26E-54	1.69E-51	ERK signaling; Calcium-dependent cell-adhesion protein
BMPR2	-2.80	2.96E-52	3.17E-49	Akt signaling; Transferase activity and protein tyrosine kinase activity
DGKH	-4.04	6.70E-45	4.89E-42	GPCR downstream signaling; Response to elevated platelet cytosolic Ca^{2+}; NAD+ kinase activity
ERG	-2.26	4.92E-39	2.54E-36	Chromatin regulation/Acetylation; Transcriptional regulation through recruitment of SETDB1 histone methyltransferase and modification
AKAP2	-3.29	2.16E-36	9.70E-34	GPER1 signaling and VEGFA-VEGFR2 signaling pathway
SOGA1	-3.89	6.51E-35	2.65E-32	Insulin receptor signaling pathway; Negative regulation of gluconeogenesis; Regulation of autophagy
HEG1	-2.92	4.05E-33	1.56E-30	Calcium ion binding activity; Negative regulation of Rho protein signal transduction
CCDC88A	-2.60	4.10E-33	1.56E-30	Bifunctional modulator of guanine nucleotide-binding proteins; Regulation of DNA replication and cell proliferation; AKT-mTOR signaling pathway
ZEB2	-2.44	1.41E-32	5.16E-30	Nucleic acid binding and phosphatase regulator activity; Repression of E-cadherin transcription

Supplementary Table S5 Top 10 hypo- and hyper-acetylated peaks identified by ChIP-Seq in *LBP*-/-_ND rats (ranked by *P*-value)

Chr.	Position (Start-End)	log_2 (fold-change)	*P*-value	*P*adj	Regulation
Hypoacetylated peaks					
1	99044194-99047503	-12.58	4.30E-23	1.15E-18	DR
17	19194861-19202125	-1.41	2.11E-21	2.81E-17	DR
1	99051650-99052303	-12.14	1.90E-20	1.69E-16	DR
6	95378485-95389760	-1.69	1.04E-15	6.96E-12	DR
6	43207373-43211188	-2.21	3.07E-13	1.63E-09	DR
1	177006246-177010630	-1.89	2.54E-12	9.75E-09	DR
8	75555207-75575491	-1.72	2.63E-12	9.75E-09	DR
1	204616297-204632772	-1.34	2.93E-12	9.75E-09	DR
6	92596092-92601011	-2.76	4.43E-11	1.18E-07	DR
3	127393649-127395538	-3.81	8.55E-11	2.02E-07	DR
Hyperacetylated peaks					
17	69452402-69462956	1.77	6.79E-09	7.51E-06	UR
5	77246433-77247383	1.91	1.86E-08	1.42E-05	UR
5	77569030-77573406	1.74	7.25E-08	4.20E-05	UR
8	38142909-38155473	1.51	8.02E-08	4.55E-05	UR
4	168207524-168212107	1.98	5.57E-07	2.39E-04	UR
2	243715676-243721043	1.31	1.72E-06	5.67E-04	UR
8	41762881-41770021	2.33	1.96E-06	6.08E-04	UR
2	243506520-243532810	1.28	3.83E-06	1.08E-03	UR
2	243689941-243696236	2.00	4.93E-06	1.27E-03	UR
9	49566950-49567828	1.73	5.27E-06	1.33E-03	UR

Supplementary Table S6 Top 10 hypo- and hyper-acetylated peaks identified by ChIP-Seq in *LBP*-/-_HFD rats (ranked by *P*-value)

Chr.	Position (Start-End)	log_2 (Fold Change)	*P*-value	*P*adj	Regulation
Hypoacetylated peaks					
4	152884936-152888430	-2.69	2.27E-46	1.27E-41	DR
8	41650552-41653034	-11.37	1.68E-25	4.70E-21	DR
1	213242398-213243753	-6.53	1.66E-22	3.08E-18	DR
8	41646554-41649230	-10.52	1.27E-21	1.77E-17	DR
1	259165910-259167705	-11.40	7.89E-19	7.33E-15	DR
8	41639540-41641028	-7.53	1.73E-18	1.13E-14	DR
9	73995039-73997415	-11.40	1.82E-18	1.13E-14	DR
2	263802363-263803642	-10.82	5.36E-18	2.99E-14	DR
3	154788368-154791693	-10.41	5.27E-16	2.45E-12	DR
8	112634080-112641325	-1.89	1.09E-15	4.66E-12	DR
Hyperacetylated peaks					
7	124987182-124992573	7.79	1.83E-19	2.04E-15	UR
18	55379481-55382440	2.73	1.63E-18	1.13E-14	UR
9	96760981-96764971	2.63	2.34E-17	1.19E-13	UR
5	23427224-23428654	10.25	1.55E-15	6.18E-12	UR
11	88948329-88949962	10.17	4.22E-15	1.47E-11	UR
5	104362635-104363698	9.99	1.56E-14	4.58E-11	UR
2	232585919-232587723	6.12	7.43E-14	1.97E-10	UR
3	154812697-154814599	6.29	1.33E-12	2.86E-09	UR
19	27855994-27856351	9.69	3.83E-12	7.36E-09	UR
18	55373240-55374482	2.85	2.31E-11	3.68E-08	UR

Supplementary Table S7 Fatty acid oxidation (FAO)-related genes indicated in this study

Gene	Symbol	FAO-related function	Correlation with FAO activity
PPARα	Peroxisome proliferator-activated receptor α	*PPARα*, as a regulator, is widely reported to positively control expression of genes involved in liver peroxisomal FAO.	Positive
SLC25A20	Solute carrier family 25 member 20	*SLC25A20* encoded protein mediates transport of acylcarnitines into mitochondrial matrix for oxidation by mitochondrial FAO pathway.	Positive
FATP1	Fatty acid transport protein 1	*FATP1* encoded protein is evolutionarily conserved and localizes to plasma membrane to enhance transportation of fatty acids (FAs), involving in FAO.	Positive
MLYCD	Malonyl-CoA decarboxylase	Product of *MLYCD* catalyzes breakdown of malonyl-CoA to acetyl-CoA and carbon dioxide and inhibits transport of fatty acyl CoAs into mitochondria, increasing rate of FAO.	Positive
HADHA	Hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha	*HADHA* encodes alpha subunit of mitochondrial trifunctional protein, which catalyzes the last three steps of mitochondrial beta-oxidation of long chain FAs.	Positive
HADHB	Hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit beta	*HADHB* encodes beta subunit of mitochondrial trifunctional protein, which catalyzes the last three steps of mitochondrial beta-oxidation of long chain FAs.	Positive
ACADM	Acyl-CoA dehydrogenase medium chain	*ACADM* encodes medium-chain specific (C4 to C12 straight chain) acyl-Coenzyme A dehydrogenase, which catalyzes initial step of mitochondrial FA beta-oxidation pathway.	Positive
DECR1	Mitochondrial 2,4-dienoyl-CoA reductase 1	*DECR1* encodes accessory enzyme, which participates in beta-oxidation and metabolism of unsaturated fatty enoyl-CoA esters.	Positive