

## SUPPLEMENTARY INFORMATION

### ***Lactobacillus plantarum* ameliorates NASH-related inflammation by upregulating L-arginine production**

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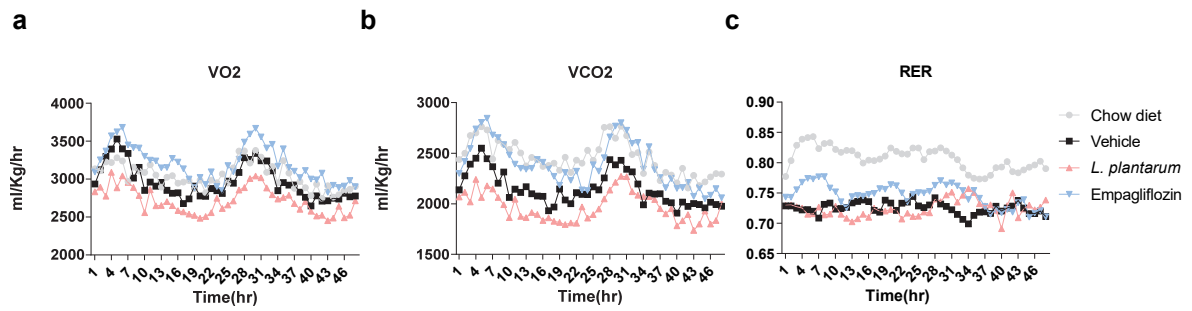
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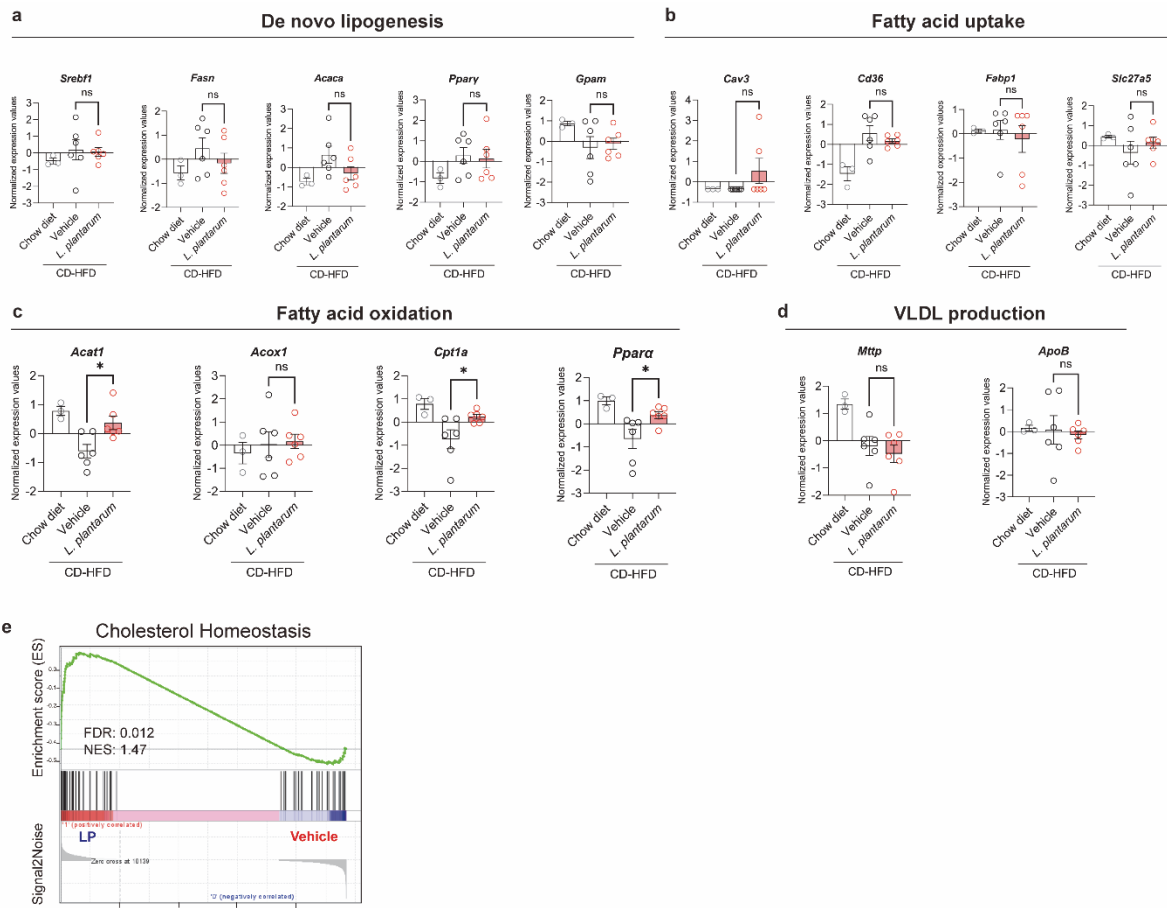
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## SUPPLEMENTARY FIGURES



**Supplementary Fig. 1. Energy homeostasis during treatment.**

(a) Oxygen consumption rate (VO<sub>2</sub>), (b) carbon dioxide release rate (VCO<sub>2</sub>), (c) respiratory exchange ratio measured using a metabolic cage after treatment (n = 3–4 per group).

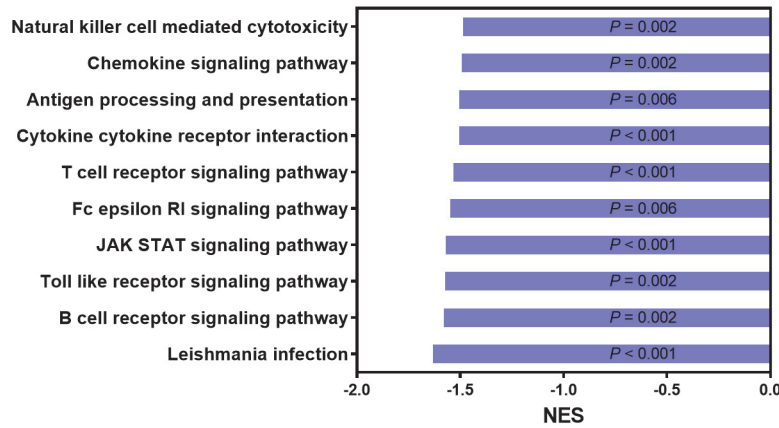


## Supplementary Fig. 2. *L. plantarum* treatment regulates hepatic lipid metabolism.

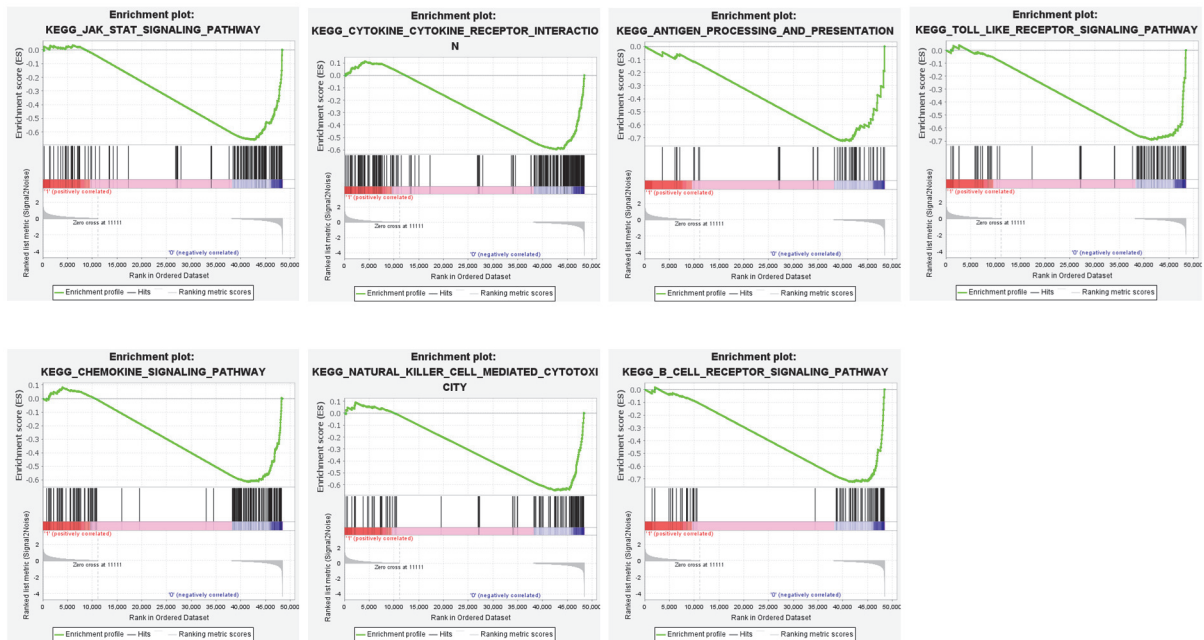
(a-d) mRNA levels of genes implicated in lipid metabolism in the liver, as measured by real-time (RT)-PCR; de novo lipogenesis (a), fatty acid uptake (b), fatty acid oxidation (c) and VLDL generation (d). (e) Enrichment plot for lipid metabolism of the transcriptome data in the *L. plantarum* group relative to the vehicle group. Data are expressed as mean  $\pm$  SD. \* $P < 0.05$  compared to the vehicle-treated group, using the post-hoc pairwise Mann-Whitney U tests after Kruskal-Wallis test with Bonferroni correction. VLDL, very low-density lipoprotein.

a

Top 10 pathway enrichment in KEGG pathway  
between *L. plantarum*-treated NASH vs. vehicle-treated NASH



b



Supplementary Fig. 3. KEGG pathway enrichment analysis results of the transcriptome data in the *L. plantarum* treatment group.

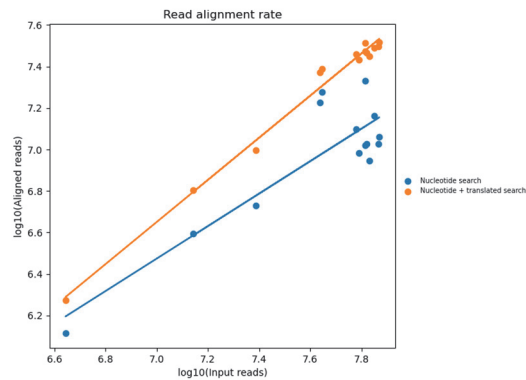
(a) Top 10 enriched pathways of the transcriptome data in the *L. plantarum* treatment group compared with the vehicle group. (b) Enrichment plot for inflammation-related seven terms.

**a**

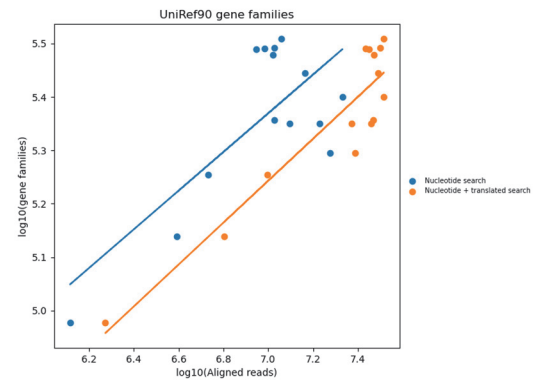
**DNA Paired end reads**

	Raw	Trim	mouse_C57BL_6NJ
Chow diet1	42,701,488	33,089,770	32,277,464
Chow diet2	38,434,691	29,547,908	28,557,021
Chow diet3	42,889,238	32,832,987	32,396,373
Chow diet4	40,790,788	31,442,747	29,735,225
Chow diet5	36,420,531	27,993,824	27,025,363
Vehicle1	38,892,088	29,331,966	19,174,193
Vehicle2	39,867,383	30,505,097	10,712,920
Vehicle3	41,975,933	32,997,000	29,012,758
Vehicle4	41,994,103	37,131,260	33,303,885
LP1	41,234,664	36,518,788	28,312,811
LP2	38,858,437	34,286,349	31,014,413
LP3	39,001,090	34,020,003	0,307,339
LP4	39,753,680	34,619,213	20,355,131
LP5	42,455,157	37,712,656	2,070,638

**b**



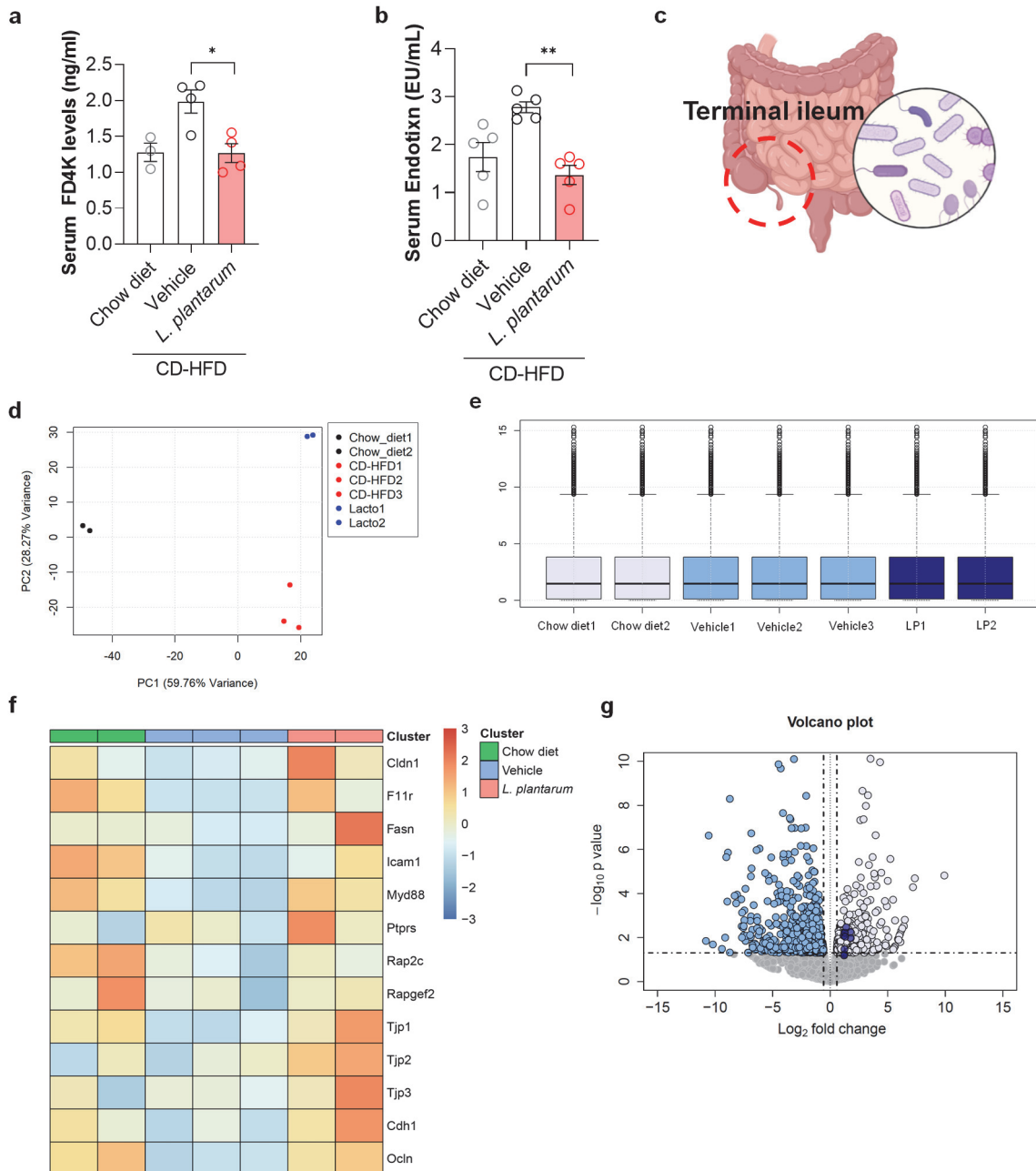
**c**



**Supplementary Fig. 4. Quality control for metagenomic shotgun analysis.**

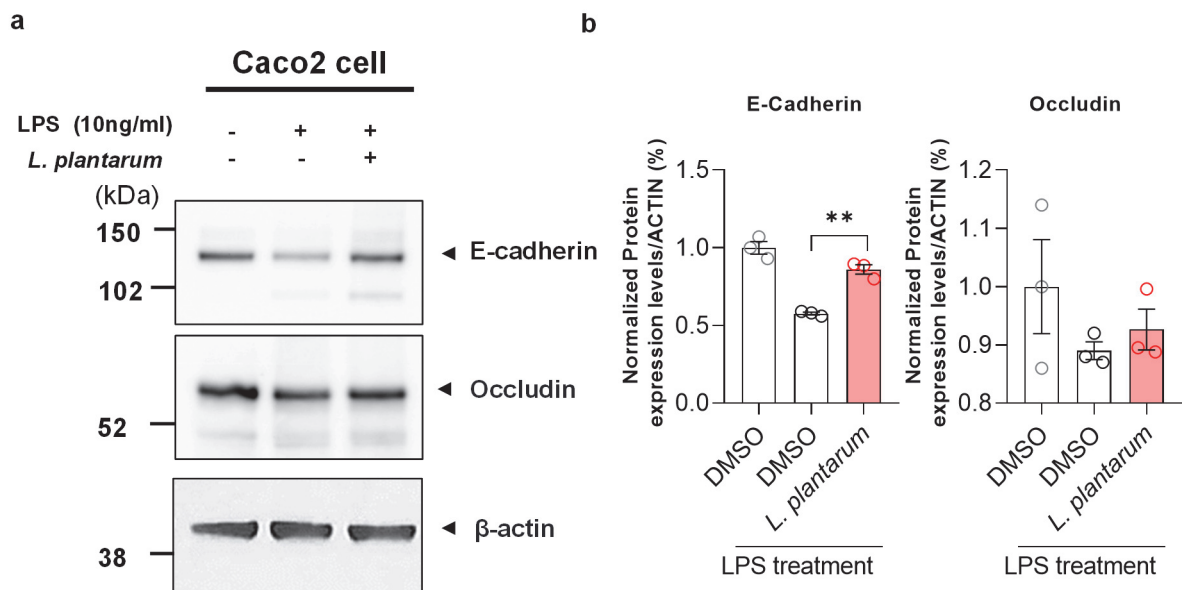
(a) The number of reads filtered at each QC step. Summary read values of metagenomic data after filtering. (b) Map reads to alignment rate. (c) Align reads to a UniRef90 database.

Abbreviation: LP, *Lactobacillus plantarum*.



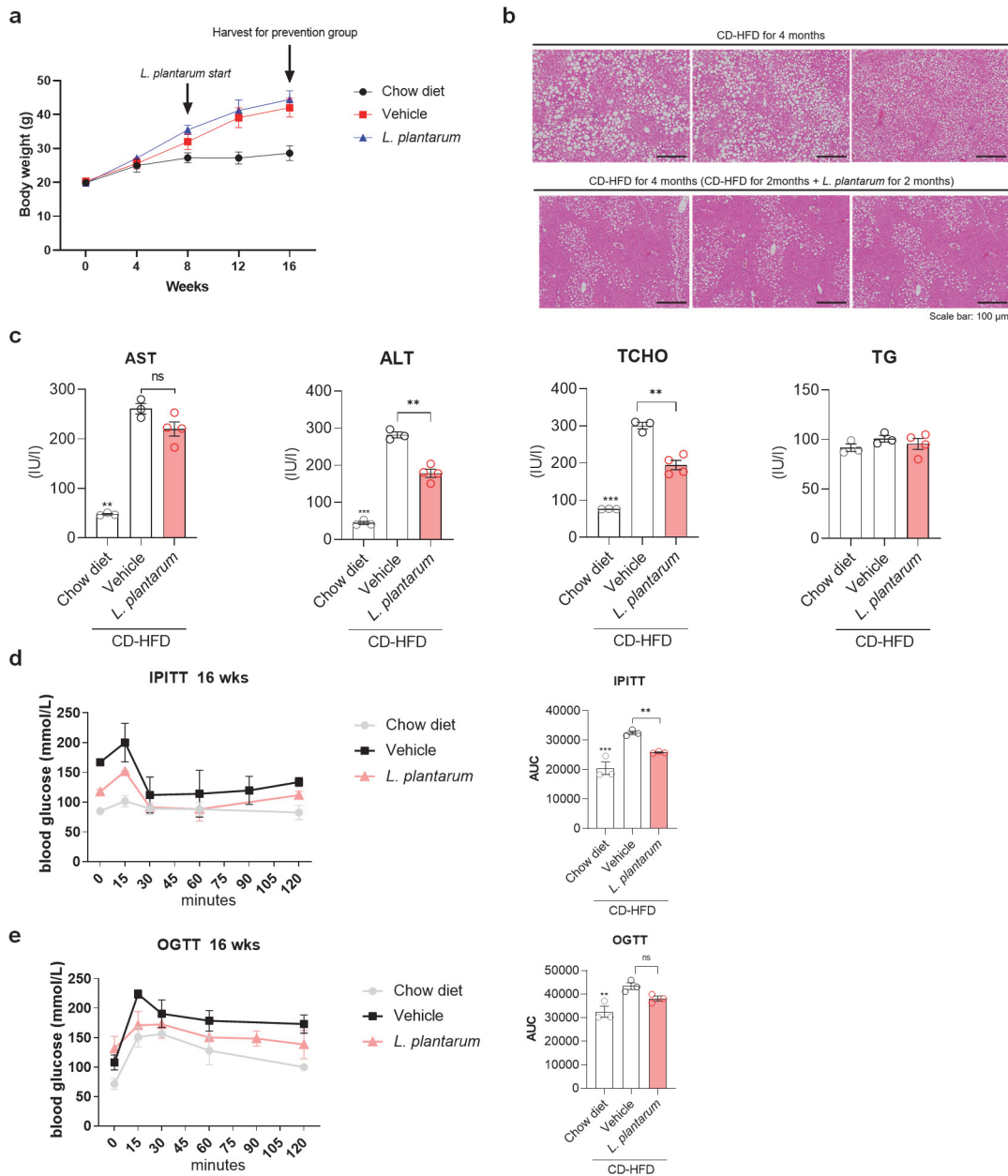
**Supplementary Fig. 5. Improved intestinal permeability and transcriptomic changes of *L. plantarum* treatment in intestinal tissues.** (a) Serum concentrations of FD4K 4 h after feeding with FD4K by gavage. (b) Serum endotoxin levels. (c) The terminal ileum was utilized to see the transcriptomic changes induced by *L. plantarum* treatment. (d) Principal component analysis plot. (e) Box plot for normalized counts. (f) Heatmap representation of 13 genes belonging to the “establishment of endothelial intestinal barrier” (GO:0090557). (g) Volcano plot depicting overall gene expression change of *L. plantarum* group compared with vehicle

mice. Intestinal barrier function-related genes in (f) were marked in deep blue dots. \* $P < 0.05$ , \*\*  $P < 0.01$  compared with the vehicle-treated mice.

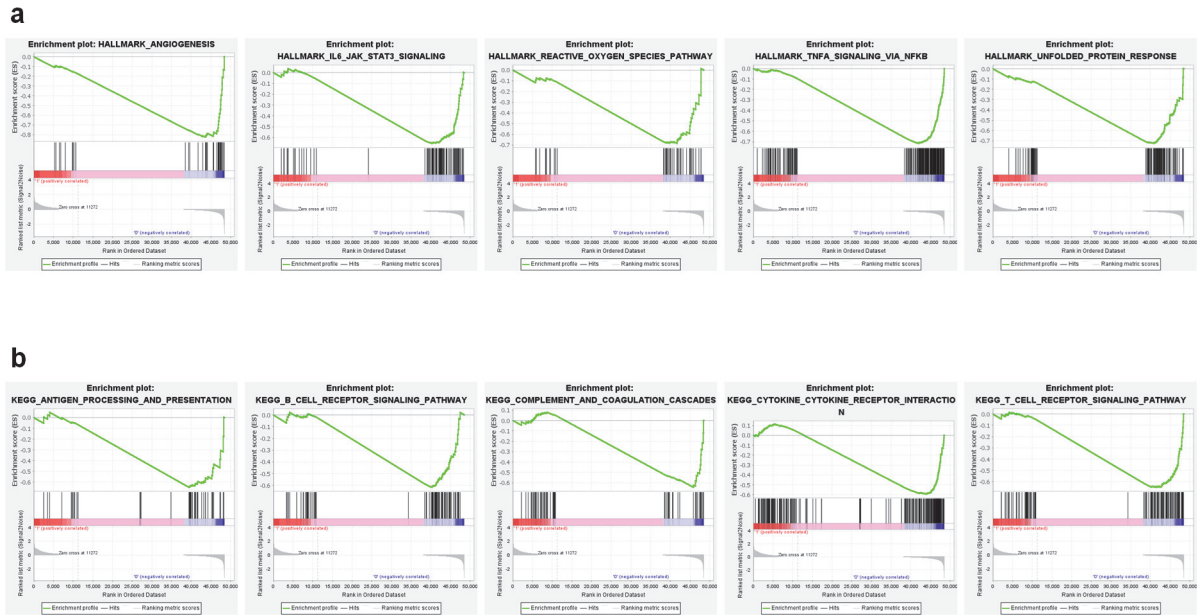


**Supplementary Fig. 6. Tight junction protein levels in Caco-2 cells.** (a) Representative immunoblot analyses of E-cadherin and occludin. (b) Bar graphs represent band intensities of the blot. Data is the mean  $\pm$  SD of more than three independent experiments. Band intensities were normalized to  $\beta$ -actin. \*\*  $P < 0.01$ . Data points were logarithmically transformed to obtain normality before testing. LPS, lipopolysaccharide.

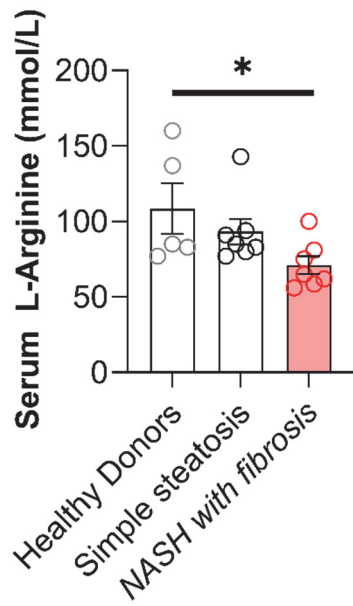




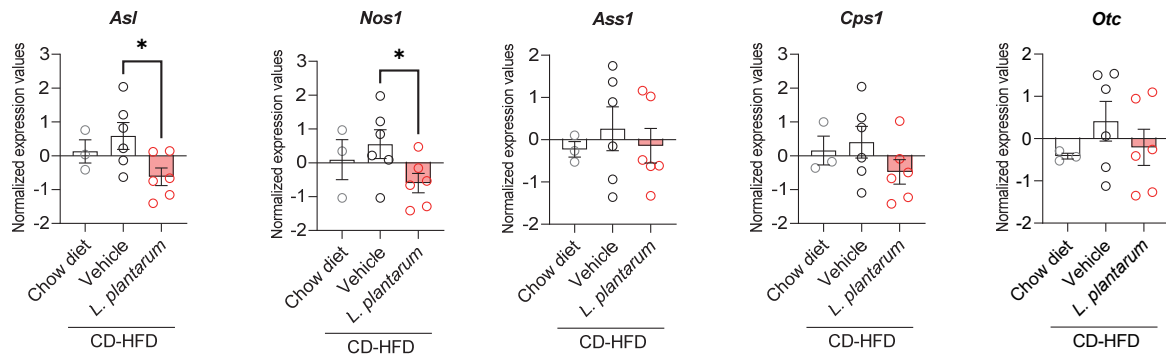
**Supplementary Fig. 7. Effect of *L. plantarum* on NAFL murine models.** (a) Body weight over time. (b) Representative microphotographs of H&E-stained liver sections. Scale bar = 100  $\mu$ m. (c) Serum biochemistry levels. (d, e) Comparing OGTT and IPITT at 16 weeks, mice were subsequently fed with CD-HFD for eight weeks and administered *L. plantarum* for eight weeks. \* $P < 0.05$ , \*\*  $P < 0.01$  compared with the CD-HFD vehicle-treated group, using the posthoc Tukey test after ANOVA. CD-HFD, choline-deficient high fat diet; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TCHO, total cholesterol; TG, triglyceride; IPITT, intraperitoneal insulin tolerance test; OGTT, oral glucose tolerance test.



**Supplementary Fig. 8. GSEA enrichment plots for the top enriched category in L-arginine treatment related to Figure 6. (a, b) Inflammation-related terms in L-arginine treated group compared with the vehicle group. Based on the Hallmark (a) and the KEGG pathway (b).**



**Supplementary Fig. 9. Levels of L-arginine across various stages of NAFLD in human NAFLD. L-arginine levels decreased as NAFLD progressed.**



**Supplementary Fig. 10. Relative mRNA levels of genes related to L-arginine biosynthesis in liver transcriptomic data.**

## SUPPLEMENTARY TABLES

### Supplementary Table 1. List of antibodies used for Immunohistochemistry analysis and

#### Western blot assay

<i>Name</i>	<i>Supplier</i>	<i>Cat no.</i>
F4/80	Invitrogen	Cat# MA5-16363
Myeloperoxidase (MPO)	Invitrogen	Cat# PA5-16672
E-cadherin	Cell Signaling Technology	Cat# 3195
Occludin	Novus	Cat# 87402
HNF-4alpha	Santa Cruz Biotechnology	Cat# sc-374229
Albumin	R & D systems	Cat# MAP-1455
$\beta$ -Actin	Abcam	Cat# ab20272

**Supplementary Table 2. Primers used for real-time PCR in this study**

<i>Gene</i>	<i>Species</i>	<i>Sequence (5'-3')</i>
IL-1 $\beta$ _F	Mouse	CCAGCTTCAAATCTCACAGCAG
IL-1 $\beta$ _R	Mouse	CTTCTTTGGGTATTGCTTGGGATC
IL-12_F	Mouse	GAGCACTCCCCATTCTACT
IL-12_R	Mouse	GCATTGGACTTCGGTAGATG
GAPDH_F	Mouse	ACTCCACTCACGGCAAATTC
GAPDH_R	Mouse	TCTCCATGGTGGTGAAGACA
Srebf1_F	Mouse	TGA CCC GGC TAT TCC GTG A
Srebf1_R	Mouse	CTG GGC TGA GCA ATA CAG TTC
Fasn_F	Mouse	GCG GGT TCG TGA AAC TGA TAA
Fasn_R	Mouse	GCA AAA TGG GCC TCC TTG ATA
Acaca_F	Mouse	CAG TAA CCT GGT GAA GCT GGA
Acaca_R	Mouse	GCC AGA CAT GCT GGA TCT CAT
Pparg_F	Mouse	GGT GTG ATC TTA ACT GCC GGA
Pparg_R	Mouse	GCC CAA ACC TGA TGG CAT TG
Gpam_F	Mouse	CCA CAG AGC TGG GAA AGG TT
Gpam_R	Mouse	GTG CCT TGT GTG CGT TTC AT
Cav3_F	Mouse	AGC CGC GTC TAC TCC ATC TA
Cav3_R	Mouse	TCT CTT TCT GCG TGC TGA TG
CD36_F	Mouse	GCA AAG AAG GAA AGC CTG TG
CD36_R	Mouse	CCA ATG GTC CCA GTC TCA TT
Fabp1_F	Mouse	CAT CCA GAA AGG GAA GGA CA
Fabp1_R	Mouse	CCC AGT CAT GGT CTC CAG TT
Slc27a5_F	Mouse	TCG GAT CTG GGA ATT CTA CG
Slc27a5_R	Mouse	CAG GAA TGC AAA AAC CCT GT
Acox1_F	Mouse	CAC GGC TAT TCT CAC AGC AG
Acox1_R	Mouse	CAG GCT GTT AAT GTC CAC CA
PPara_F	Mouse	ACG ATG CTG TCC TCC TTG ATG
Ppara_R	Mouse	GTG TGA TAA AGC CAT TGC CGT

<b>Acat1_F</b>	Mouse	CCC CAT TGA TTT TCC ACT TG
<b>Acat1_R</b>	Mouse	AGC ACA ACC ACA CTG AAT GC
<b>Cpt1a_F</b>	Mouse	CCT GCA TTC CTT CCC ATT TG
<b>Cpt1a_R</b>	Mouse	TGC CCA TGT CCT TGT AAT GTG
<b>Mttp_F</b>	Mouse	GAA AGA AGT GCT CCC TCA G
<b>Mttp_R</b>	Mouse	CCT TTG AAC TTA CTA AGG AGG G
<b>ApoB_F</b>	Mouse	TAC TTC CAC CCA CAG TCC CCT
<b>ApoB_R</b>	Mouse	CCT TAG AAG CCT TGG GCA CAT

**Supplementary Table 3. Basic characteristics of the participants in the human liver transcriptomic cohort**

<i>Clinical features</i>	<i>NAFLD (n = 28)</i>	<i>Healthy control (n = 5)</i>
Age (year)	43.07 ± 15.06	26 ± 5.36
Sex, male (%)	17 (60.7)	5 (100)
DM (%)	11 (39.2)	0
Height (cm)	170.46 ± 8.59	176.53 ± 6.49
Weight (cm)	87.46 ± 20.42	79.12 ± 10.82
BMI (kg/m <sup>2</sup> )	29.82 ± 5.21	25.31 ± 2.49
AST (U/L)	76.16 ± 30.61	16.12 ± 3.79
ALT (U/L)	91.25 ± 53.59	15.22 ± 3.65
γ-GTP (U/L)	139.03 ± 79.61	20.23 ± 3.96
Total cholesterol (mg/dL)	188.21 ± 40.01	181.21 ± 38.85
Triglyceride (mg/dL)	195.35 ± 96.24	97.80 ± 25.21
HDL-cholesterol (mg/dL)	43.33 ± 7.52	42.80 ± 9.94
LDL-cholesterol (mg/dL)	127.40 ± 39.57	98.73 ± 43.63
<b><i>Liver Histology</i><sup>†</sup></b>		
Steatosis	1.85 (1-3)	NA
Inflammation	1.57 (1-3)	NA
Ballooning	1.25 (0-2)	NA
NAFLD Activity Score	4.67 (2-7)	NA

**Notes:** Data are expressed as number (%) or mean ± standard deviation. <sup>†</sup>Values for liver histology are expressed as mean (min-max).

Abbreviations: DM, diabetes mellitus; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transferase; HDL, high-density lipoprotein; LDL, low density lipoprotein; NA, not applicable.



**Supplementary Table 4. Relative abundance of bacterial composition estimated by genome-based profiling using shotgun sequencing data**

<i>Species</i>	<i>Chow diet</i>	<i>Vehicle group</i>	<i>L. plantarum group</i>
<i>Faecalibaculum_rodentium</i>	35.47243	0.635758	4.894642
<i>Muribaculaceae_bacterium_DSM_103720</i>	14.60647	1.879778	0.596436
<i>Bacteroides_vulgatus</i>	14.26879	56.24696	20.85911
<i>Muribaculum_intestinale</i>	13.58573	0.095878	1.900638
<i>Lactobacillus_intestinalis</i>	3.820192	0.253843	0
<i>Bacteroides_caccae</i>	3.278392	3.728053	1.567714
<i>Lactobacillus_reuteri</i>	3.204472	0.38465	0
<i>Bifidobacterium_pseudolongum</i>	2.450714	5.072518	0.398562
<i>Lactobacillus_murinus</i>	1.977508	1.759658	3.247004
<i>Mucispirillum_schaedleri</i>	1.777474	3.83332	29.54553
<i>Enterorhabdus_caecimuris</i>	1.382062	0.59501	0.609636
<i>Parabacteroides_goldsteinii</i>	1.183696	3.83323	6.924694
<i>Anaerotruncus_sp_G3_2012</i>	0.883488	1.218993	1.156412
<i>Lachnospiraceae_bacterium_28_4</i>	0.432074	1.361583	8.261528
<i>Lactobacillus_johnsonii</i>	0.329346	0.41756	0.625662
<i>Clostridium_sp_ASF502</i>	0.23002	0.158865	0.361566
<i>Akkermansia_muciniphila</i>	0.207396	12.42145	4.322594
<i>Adlercreutzia_equolifaciens</i>	0.159166	0.011948	0.01259
<i>Dorea_sp_5_2</i>	0.154344	0.015433	0.108972
<i>Lachnospiraceae_bacterium_A4</i>	0.141234	0.34071	0
<i>Lachnospiraceae_bacterium_A2</i>	0.111222	0.99498	0.002626
<i>Lachnospiraceae_bacterium_COE1</i>	0.086364	0	0
<i>Asaccharobacter_celatus</i>	0.05564	0.004845	0.004928
<i>Firmicutes_bacterium_ASF500</i>	0.051598	0.109635	0.009174
<i>Oscillibacter_sp_1_3</i>	0.04001	0.113228	0.093656
<i>Acutalibacter_muris</i>	0.03684	0.071655	0.071282
<i>Clostridium_sp_ASF356</i>	0.029736	0	0
<i>Lachnospiraceae_bacterium_3_1</i>	0.021034	0.005305	0.002918
<i>Lachnospiraceae_bacterium_M18_1</i>	0.014164	0.003703	0.027728
<i>Lachnospiraceae_bacterium_10_1</i>	0.00416	1.653088	0.028392
<i>Escherichia_coli</i>	0.003472	0.003335	0.30838
<i>Clostridium_cocleatum</i>	0.000544	0.374628	0
<i>Eubacterium_plexicaudatum</i>	0.000126	0.000393	0.028072
<i>Eubacterium_sp_14_2</i>	0.000102	0.021123	0.013282
<i>Bacteroides_caecimuris</i>	0	0	0.980864
<i>Bacteroides_sartorii</i>	0	0	0.031734
<i>Bacteroides_uniformis</i>	0	0	0.085286
<i>Prevotella_sp_MGM1</i>	0	0.01246	0
<i>Prevotella_sp_MGM2</i>	0	0	1.10701
<i>Parabacteroides_distasonis</i>	0	0.070713	0.392228
<i>Parabacteroides_merdae</i>	0	0	0.001358
<i>Staphylococcus_aureus</i>	0	0	0.008852
<i>Staphylococcus_sciuri</i>	0	0.000253	0.008886
<i>Enterococcus_faecalis</i>	0	0.00489	0.288056
<i>Lactobacillus_plantarum</i>	0	0.018343	9.655816
<i>Lactococcus_lactis</i>	0	1.660193	0.85178
<i>Acetatifactor_muris</i>	0	0.169973	0.074428
<i>Lachnospiraceae_bacterium_3_2</i>	0	0.271855	0.19921
<i>Romboutsia_ilealis</i>	0	0.116015	0.104724

<i>Anaerotruncus_colihominis</i>	0	0.001098	0.005262
<i>Ruthenibacterium_lactatiformans</i>	0	0.00275	0
<i>Ileibacterium_valens</i>	0	0.033863	0.021378
<i>Proteus_hauseri</i>	0	0.016508	0.070356
<i>Proteus_penneri</i>	0	0	0.129058

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