

1 *Lactobacillus casei* reduces susceptibility to type 2 diabetes via
2 microbiota-mediated body chloride ion influx
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7 **Supplemental Tables**

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9 Table S1 Primers for amplifying target intestinal bacteria
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Bacterial Target	Primer	Sequence (5'-3')	Reference
<i>C. scindens</i>	scindF	GCAACCTGCCTGCACT	1
	scindR	ACCGAATGGCCTTGCCA	
<i>C. sordellii</i>	sordF	TCGAGCGACCTTCGG	
	sordR	CACCACCTGTCAC	
<i>C. coccoides-E. rectale</i> group	CEF	CGGTACCTGACTAAGAAGC	2
	CER	AGTTTCATTCTTGCGAACG	
<i>E. rectale</i>	RectaleF	CGGTACCTGACTAAGAAGC	3
	RectaleR	CCTAGTATTCATCGTTTACGGCGTG	
<i>Lactobacillus</i>	Lac-F	AGCAGTAGGGAATCTTCCA	
	Lac-R	CACCGCTACACATGGAG	
<i>Bifidobacterium</i>	Bifid-F	CTCCTGGAAACGGGTGG	4
	Bifid-R	GGTGTCTTCCCGATATCTACA	
<i>Clostridium</i> cluster IV	C4F	TACCHRAGGAGGAAGCCAC	
	C4R	GTTCTTCCTAATCTCTACGCAT	

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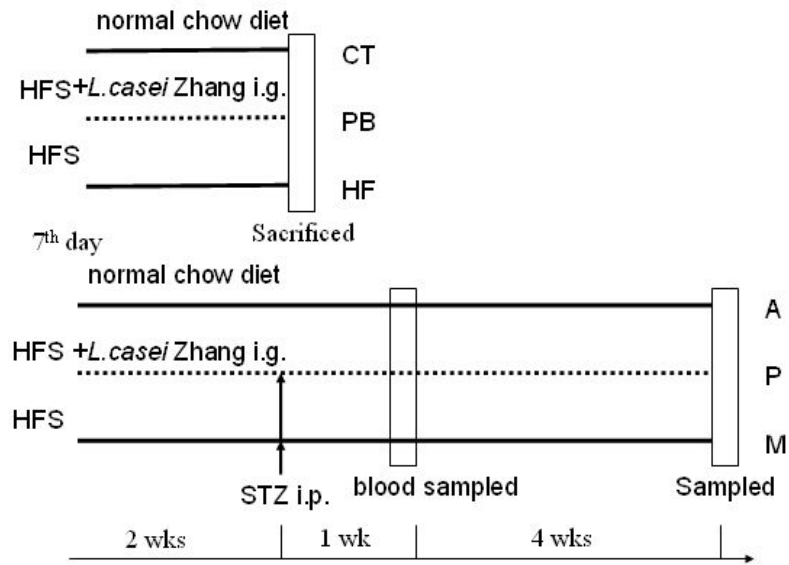
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Table S2 Primer sequences for real-time PCR

Gene	Primer	Sequence (5'-3')	Reference
18S	18SF	TAAGTCCCTGCCCTTTGTACACA	5
	18SR	ATCCGAGGGCCTCACTAAAC	
CIC1	CIC1F	TGTGGAACGCTCAGAACTGCAGTC	6
	CIC1R	TCTAGTGCCAAGACACCTCTGAGC	
CIC-2	CIC-2F	GCAGTGATTGTCTTCGAGCTCACCGG	7
	CIC-2R	CCAGCTCAGGCAGGTAGGGCAGCT	
CIC-3	CIC-3F	TTGCCTACTATCACCACGAC	8
	CIC-3R	GCATCTCCAACCCATTTACT	
CIC4	CIC4F	GAGACTCGGAGCGTCTCATCGG	9
	CIC4R	CTGTTGGCAGGCAGCTCAGGAG	
CIC5	CIC5F	TGCTGACTGTCCTTACTCAG	
	CIC5R	CAGGATGTTCCGAAGCTTCA	
CIC7	CIC7F	ATGAGCACGCCTGTGACCTGCCTG	10
	CIC7R	CGAGGAAGAGATGCCTCCTGTGGC	
GlyR α 1	GlyR1F	GCACCAAGCACTACAACAC	11
	GlyR1R	AGGACAGGATGACGATAAGC	
GABAA α 1	GABAA1F	CTCCTACAGCAACCAGCTATACCC	12
	GABAA1R	GCGGTTTTGTCTCAGGCTTGAC	
GABAA α 2	GABAA2F	AAGAGAAAGGCTCCGTCATG	
	GABAA2R	GCTTCTTGTTGGTTCTGGAGTAG	
F4/80	F4/80F	ATGACCACACTTGCCATCCT	13
	F4/80R	GGCGAGTCGCTTCTAAGACA	
CD68	CD68F	TGGA CT CAGCAGCTCTACCA	
	CD68R	CCTGTGGGTGGTCGTAGG	
CFTR	CFTRF	AATATCCTTAGCCCCTCGGA	14
	CFTRR	TGGTGGAACAATGGCACTA	
FoxA2	FoxA2F	GTATGCTGGGAGCCGTGAAG	15
	FoxA2R	AGCCTGCGCTCATGTTGC	
SLC26A3	SLC26A3F	TTCTACCCTGTCTTCGTC	16
	SLC26A3R	ACACCGTAGGAAATAGCG	
SLC26A6	SLC26A6F	CGGGAGGCAACACGCAGAT	
	SLC26A6R	GGTGGCTGAGGAACGGAAGAC	
CYP7A1	CYP7A1F	GAGCTCATGCATGCCAATGAGAAGAG	17
	CYP7A1R	AATCCACATACCTCAGAGC	
Bestrophin-3	BEST3F	CTCATCTCCAGCAGTGTCCA	18
	BEST3R	CAGATGAGGCGACTTGAGG	
TGR5	TGR5F	TTGCTCCTGTCAGTCTTGGCCTAT	19
	TGR5R	TTGGGTCTTCCTCGAAGCACTTGT	
T-BET	T-BETF	TCCTGTCTCCAGCCGTTTCT	
	T-BETR	CGCTCACTGCTCGGA ACTCT	
GATA-3	GATA-3 F	GGCGGCAGATGGTACTG	20
	GATA-3 R	TCTGCCCATTCATTTTATGGTAGA	

20 Supplemental Figures



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Fig. S1 Experimental design

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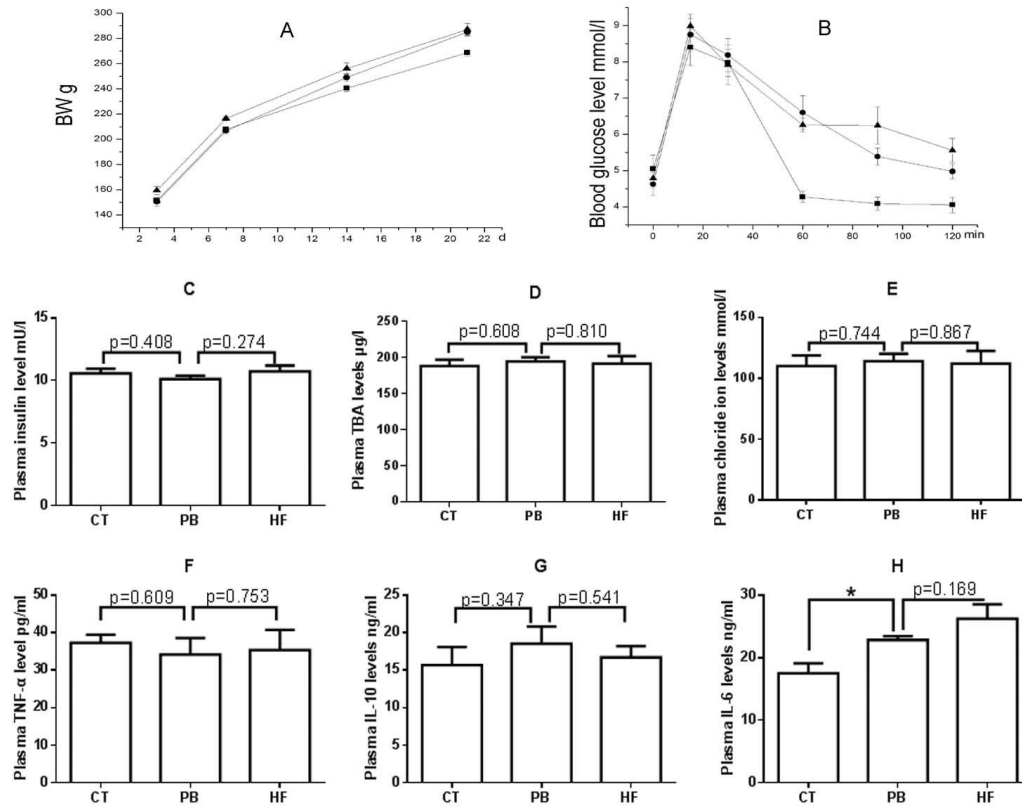
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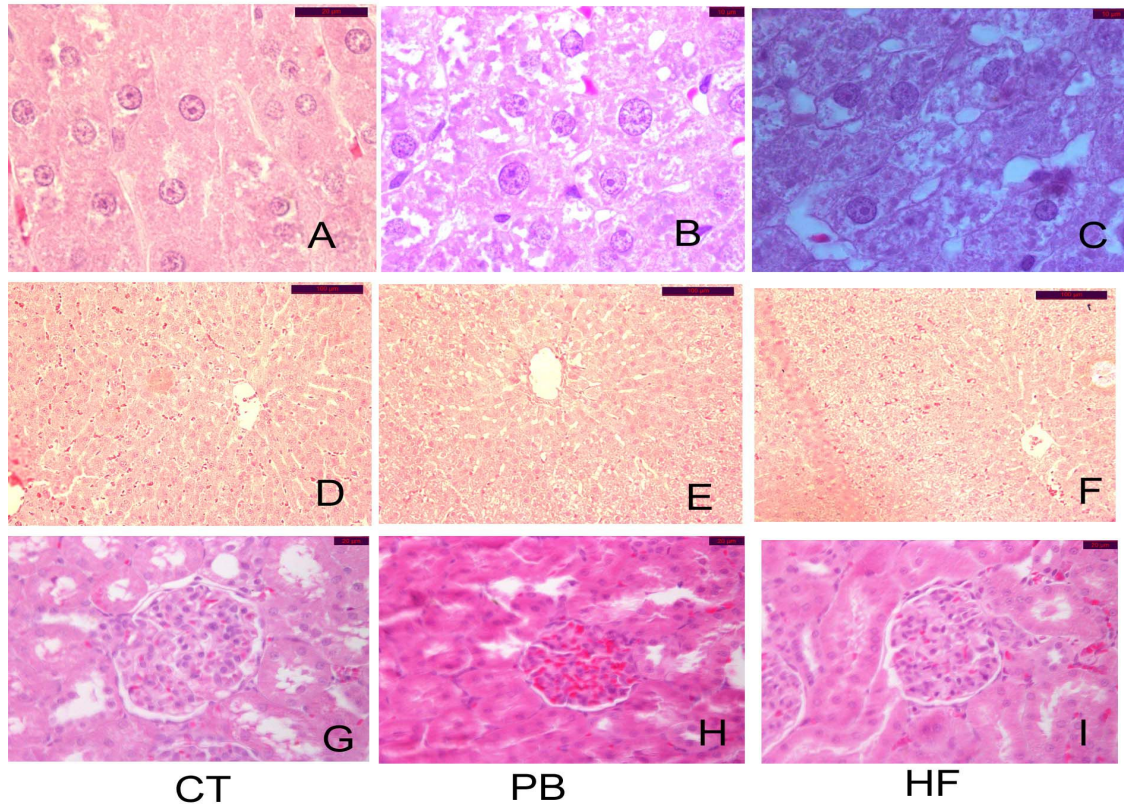
35 Fig. S2 Comparison of body weight (A), OGTT (B), plasma insulin levels (C), plasma TBA

36 levels (D), plasma chloride ion levels (E), plasma TNF- α levels (F), plasma IL-10 levels (G), and

37 plasma IL-6 levels (H) between different groups. Black triangles = HF group; black circles = PB

38 group; black squares = CT group. * represents $p < 0.05$.

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Fig. S3 Hepatic (A-F) and renal (G-I) tissue sections of three groups of rat

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CT: A($\times 1000$), D($\times 200$), G($\times 1000$); PB: B($\times 1000$), E($\times 200$), H($\times 1000$); HF: C ($\times 1000$), F ($\times 200$), I ($\times 1000$).

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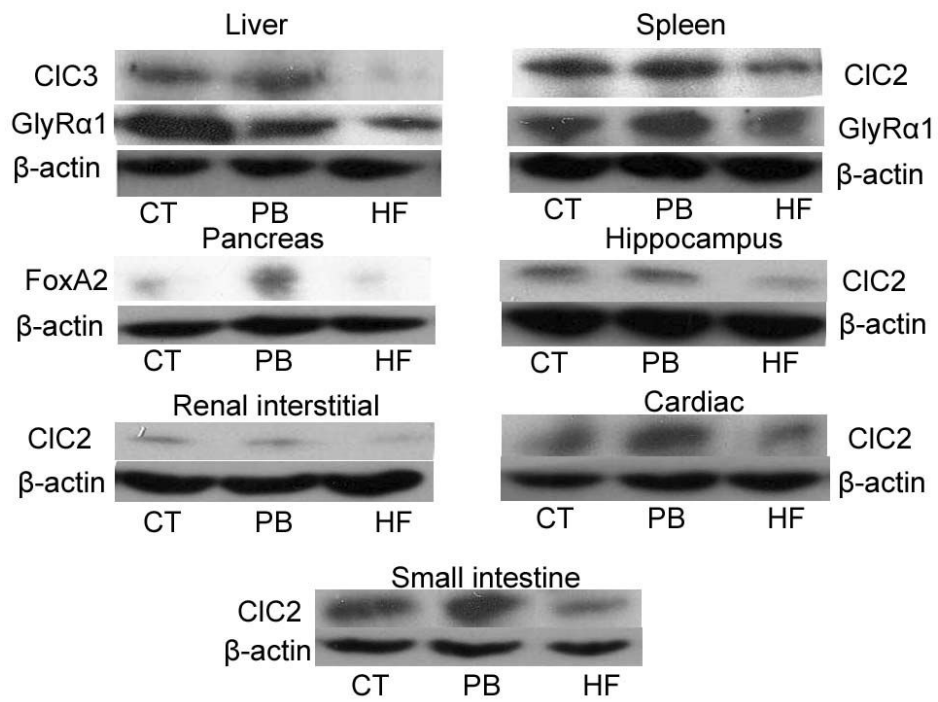
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The CT rats exhibited an intact structure of hepatic lobule (A and D), whereas HF rats showed preliminary fatty liver morphology with compressed liver sinusoids, disarranged hepatic cords and large areas of fatty vacuolization but no inflammatory infiltration (C and F). Compared to HF rats, hepatic structure was clear and areas of fatty vacuolization were reduced in PB (B and E). No significant lesion was observed in renal glomerulus of all three groups (G, H and I). However, both the kidneys of PB and HF rats exhibited fewer renal tubular cavities (white areas) compared with the CT rats (G, H and I).



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54 Fig. S4 The levels of target proteins in the three groups of rats

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66 **Supplemental references**

- 67 1. Miyata, M., Yamakawa, H., Hamatsu, M., Kuribayashi, H., Takamatsu, Y., & Yamazoe, Y.
68 Enterobacteria modulate intestinal bile acid transport and homeostasis through apical
69 sodium-dependent bile acid transporter (SLC10A2) expression. *J. Pharmacol. Exp. Ther.* **336**,
70 188-196 (2011).
- 71 2. Rinttilä, T., Kassinen, A., Malinen, E., Krogius, L. & Palva, A. Development of an extensive set
72 of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal
73 samples by real-time PCR. *J. Appl. Microbiol.* **97**,1166-117 (2004).
- 74 3. Ahmed, S., Macfarlane, G.T., Fite, A., McBain, A.J., Gilbert, P. & Macfarlane, S.
75 Mucosa-associated bacterial diversity in relation to human terminal ileum and colonic biopsy
76 samples. *Appl. Environ. Microbiol.* **73**,7435-7442 (2007).
- 77 4. Nakamura, N. et al. Molecular ecological analysis of fecal bacterial populations from term
78 infants fed formula supplemented with selected blends of prebiotics. *Appl. Environ. Microbiol.*
79 **75**,1121-1128 (2009).
- 80 5. Bhushan, S. Tchatalbachev, S. Klug, J. Fijak, M., Pineau, C., Chakraborty, T. & Meinhardt.
81 A.Uropathogenic *Escherichia coli* block MyD88-dependent and activate MyD88-independent
82 signaling pathways in rat testicular cells. *J. Immunol.* **180**,5537-5547 (2008).
- 83 7. Enz, R., Ross, B.J. & Cutting, G.R. Expression of the voltage-gated chloride channel ClC-2 in
84 rod bipolar cells of the rat retina. *J. Neurosci.* **19**, 9841-9847 (1999).
- 85 8. Britton, F.C. et al. Functional characterization of novel alternatively spliced ClC-2 chloride
86 channel variants in the heart. *J. Biol. Chem.* **280**, 25871-25880 (2005).
- 87 9. Mao, J. et al. Suppression of ClC-3 channel expression reduces migration of nasopharyngeal

88 carcinoma cells. *Biochem. Pharmacol.* **75**,1706-1716 (2008).

89 10. Kulka, M., Schwingshackl, A. & Befus, A.D. Mast cells express chloride channels of the ClC
90 family. *Inflamm. Res.* **51**,451-456 (2002).

91 11. Yoshikawa, S., Oguchi, T., Funahashi, Y., de Groat, W.C. & Yoshimura, N. Glycine transporter
92 type 2 (GlyT2) inhibitor ameliorates bladder overactivity and nociceptive behavior in rats. *Eur.*
93 *Urol.* **62**, 704-712 (2012).

94 12. Mendu, S.K., Bhandage, A., Jin, Z. & Birmir, B. Different subtypes of GABA-A receptors are
95 expressed in human, mouse and rat T lymphocytes. *PLoS One* **7**, e42959 (2012).

96 13. Huang, W. et al. Depletion of liver Kupffer cells prevents the development of diet-induced
97 hepatic steatosis and insulin resistance. *Diabetes* **59**, 347-357 (2010).

98 14. Kanno, T. & Nishizaki, T. CFTR mediates noradrenaline-induced ATP efflux from DRG
99 neurons. *Mol. Pain* **7**, 72 (2011).

100 15. Chen, F., Zhu, Y., Tang, X., Sun, Y., Jia, W., Sun, Y. & Han, X. Dynamic regulation of PDX-1
101 and FoxO1 expression by FoxA2 in dexamethasone-induced pancreatic β -cells dysfunction.
102 *Endocrinology* **152**, 1779-1788 (2011).

103 16. Zhang, G.H. et al. Dopamine stimulates Cl⁻ absorption coupled with HCO₃⁻ secretion in
104 rat late distal colon. *Eur. J. Pharmacol.* **570**,188-195 (2007).

105 17. Pfaffl, M.W., Gerstmayer, B., Bosio, A. & Windisch, W. Effect of zinc deficiency on the
106 mRNA expression pattern in liver and jejunum of adult rats: monitoring gene expression using
107 cDNA microarrays combined with real-time RT-PCR. *J. Nutr. Biochem.* **14**, 691-702 (2003).

108 18. Lee, W.K., Chakraborty, P.K., Roussa, E., Wolff, N.A. & Thévenod, F. ERK1/2-dependent
109 bestrophin-3 expression prevents ER-stress-induced cell death in renal epithelial cells by reducing

110 CHOP. *Biochim. Biophys. Acta* **1823**,1864-1876 (2012).

111 19. Xie, G., et al. Alteration of bile acid metabolism in the rat induced by chronic ethanol
112 consumption. *FASEB J.* **27**,3583-3593 (2013).word pdf

113 20. Gremy, O., Benderitter, M., Linard, C. Acute and persisting Th2-like immune response after
114 fractionated colorectal gamma-irradiation. *World J. Gastroenterol.* **14**,7075-7085 (2008).

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