Supporting Information

Lactobacillus rhamnosus LRa05 ameliorate hyperglycemia through regulating glucagon-mediated signaling pathway and gut microbiota in type 2 diabetic mice

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Materials and Methods

Evaluation of oxidative stress parameters

Reactive oxygen species (ROS) detection: Briefly, OCT-embedded tissue was cut into 4-μm-thick sections. Tissue sections were incubated with 5μM of DHE for 30 min at 37 °C away from light, and then the sections were coverslipped. The ethidium fluorescence was viewed immediately at Ex535/Em610 with a confocal microscope (Leica TCS SP5 II, Leica Microsystems). The quantitative data of liver ROS were analyzed using Image J software.

16S rRNA Gene Sequencing

The obtained tags were grouped into operational taxonomic units (OTUs) by UPARSE software with a 97% threshold. These OTUs were analyzed by using Mothur method and SILVA138 (http://www.arb-silva.de/) of SSUrRNA database for species annotation analysis (set the threshold of 0.8 ~ 1), which obtained taxonomic information (phylum and genus). The Venn Diagram was drawn by R software (version 3.0.3). The observed-species, Simpson, ACE, and Chao1 index were analyzed by using QIIME software (version 1.7.0). Besides, QIIME software (version 1.9.1) was used to analyze the β-diversity. The principal coordinates analysis (PCoA) was calculated and visualized by R (version 2.15.3), stats, and ggplot2 software package. Linear discriminant analysis (LDA) effect size analysis (LEfSe) was used to reveal the most differentially abundant taxa over-represented in gut microbiota. The heatmap of the top 30 most abundant genus was portrayed using R software

(pheatmap package). A heatmap indicated the relationship between the colonic gut microbiota and the host's genes related to glucagon signaling pathway was were plotted by R packages.

Table S1

Primers sequences for quantitative real time PCR.

Gene	Primer	Sequence (5'–3')	Size(bp)	Annealing temperature (℃)
GCG	forward primer	GCCACTCACAGGGCACATTC	303	60
	reverse primer	CAGAGAAGGAGCCATCAGCG		
GCGR	forward primer	CCTGGGTGGTGGTCAAGTGTC	228	60
	reverse primer	GAGGGATGAGGGTCAGCGTG		
Gnas	forward primer	TGATGAGTCGGAAGAAGGGG	172	60
	reverse primer	GAGGGATCGTGATCGGGTAC		
PKA	forward primer	TTGGAAGGTTCAGTGAGCCC	262	60
	reverse primer	CACCAGTCCACCGCCTTATT		
CRTC2	forward primer	ACATCGGCTCCACACGGTT	214	60
	reverse primer	GGGGACACCATTCTGCGG		
PEPCK	forward primer	GGATGTTCGGGCGGATTG	207	60
	reverse primer	TTTCGTAAGGGAGGTCGGTG		
G6Pase	forward primer	GTGATTGCTGACCTGAGGAACG	95	60
	reverse primer	ACTGCCACCCAGAGGAGATTG		
GLUT 2	forward primer	GGTGGCTCGGGGACAAACT	123	60
	reverse primer	CAGCAATGATGAGGGCGTGT		
GAPDH	forward primer	TTCAGCTCTGGGATGACCTT	129	60
	reverse primer	TGCCACTCAGAAGACTGTGG		

GCG, glucagon; GCGR, glucagon receptor; Gnas, gene encodes the heterotrimeric Gs protein alpha-subunit; cAMP, cellular adenosine-3′-5′-cyclic monophosphate; PKA, cAMP-dependent protein kinase; CREB, cAMP response element-binding protein; CRTC2, CREB-regulated transcriptional coactivator 2; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase; GLUT 2, glucose transporter 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase;

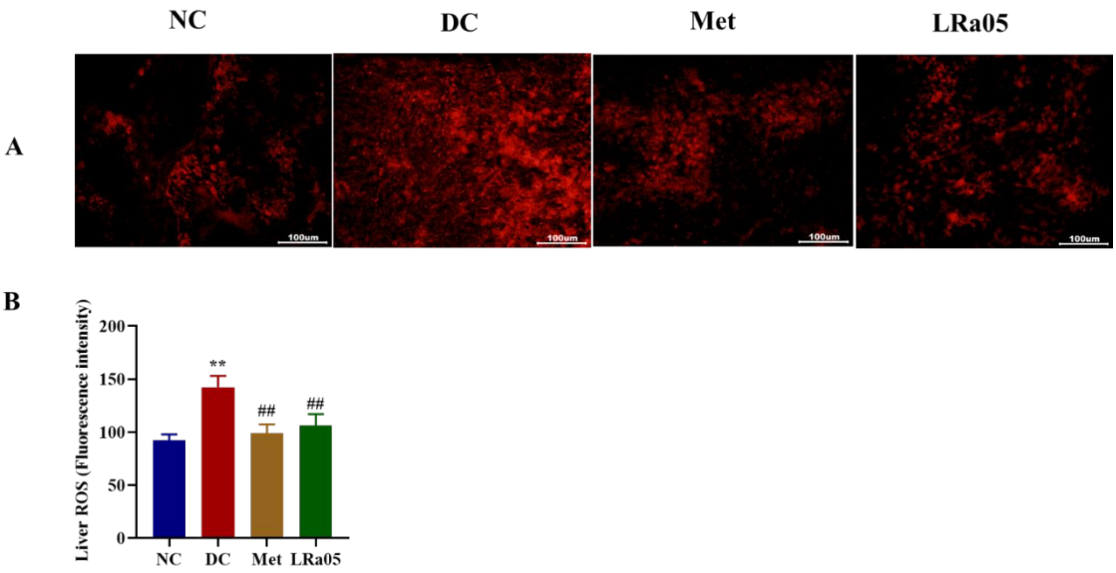

Fig. S1. Effect of LRa05 on relieving liver ROS. ROS (A), detected by DHE staining (×100); Liver ROS (Fluorescence intensity) (B), analyzed using Image J software. Data expressed as mean ± SD. $^{**}p<0.01$ *vs.* NC group. $^{\#\#}p<0.01$ *vs.* DC group.

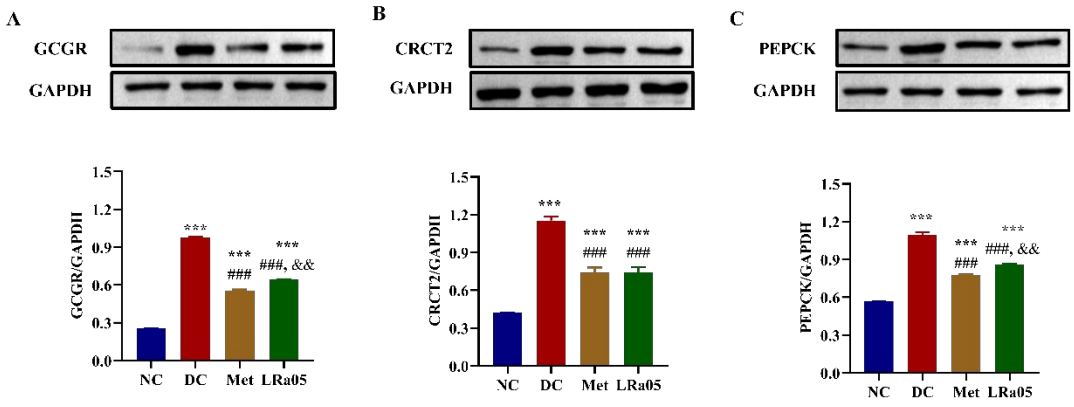

Fig. S2. Effects of LRa05 on the expression of key proteins in the glucagon-mediated signaling pathway in the liver of T2DM mice. Protein expression of GCGR (A), protein expression of CRCT2 (B), protein expression of PEPCK (C). Data expressed as mean ± SD. ***p<0.001 *vs.* NC group. ###p <0.001 *vs.* DC group. &&p<0.05 *vs* Met group.