Electronic Supplementary Material (ESI) for Food & Function.
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Supplementary Information for

Dietary *Lactobacillus rhamnosus* GG extracellular vesicles enhance anti-PD-1 immunotherapy efficacy against colorectal cancer

Shun Lu[a], Jing Xu[a], Zihao Zhao[a], Yuheng Guo[a], Hanwen Zhang[a], Peter W. Jurutka[b], Dechun Huang[c], Chongjiang Cao[a], Shujie Cheng[a]*

[a] *Department of Food Nutrition and Safety/National R&D Center for Chinese Herbal Medicine Processing, School of Engineering, China Pharmaceutical University, Nanjing 211198, P. R. China;*

[b] *School of Mathematical and Natural Sciences, Arizona State University, AZ 85306, USA;*

[c] *Department of Pharmaceutical Engineering, School of Engineering, China Pharmaceutical University, Nanjing 211198, P. R. China*

*Corresponding Author: Shujie Cheng

E-mail address: scheng@cpu.edu.cn

Tel: +8618551726017

Department of Food Nutrition and Safety, School of Engineering, China Pharmaceutical University, Nanjing 211198, China

Supplementary figures

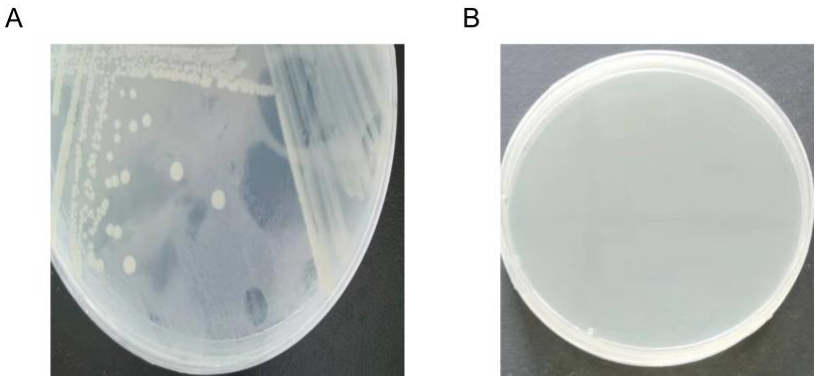


Figure S1. The extracellular vesicles of *Lactobacillus rhamnosus* ATCC 53103 did not contain viable organisms. The delineated colonies on LB solid medium and the extracted LGG-EV were almost free of colonies on solid medium before liquid culture. (A). Colony characteristics of *Lactobacillus rhamnosus* ATCC 53103 cultured in LB solid medium for 24h. (B). Almost no colonies were produced after 100ul of LGG-EV were taken and incubated in solid LB medium coated for 48h.

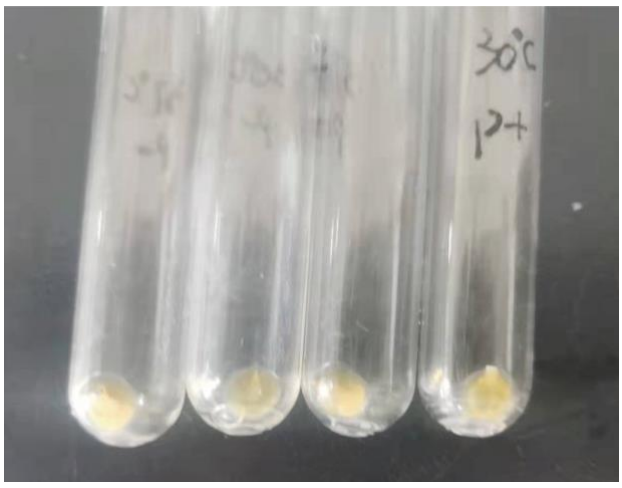


Figure S2. LGG-EV precipitates obtained after ultra-high speed centrifugation of medium supernatants from different culture conditions. Pictures of LGG-EV precipitates after centrifugation at 150,000g for 2h at 37°C/30°C and supernatant of medium without/with PenG addition.

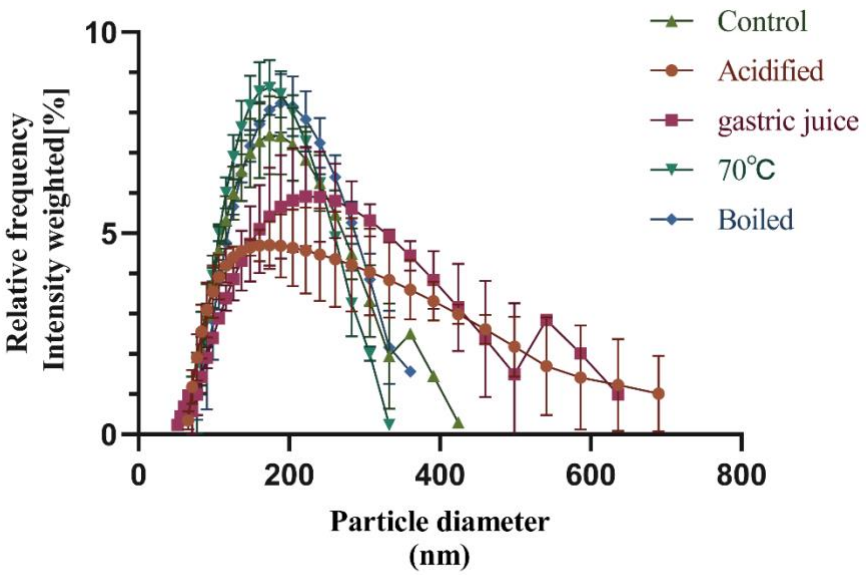


Figure S3. Vesicle particle size distribution after treatment with different extreme conditions. Re-centrifuged LGG-EVs were collected and measured after *in vitro* treatment under acidic conditions (pH=2) and high temperature.

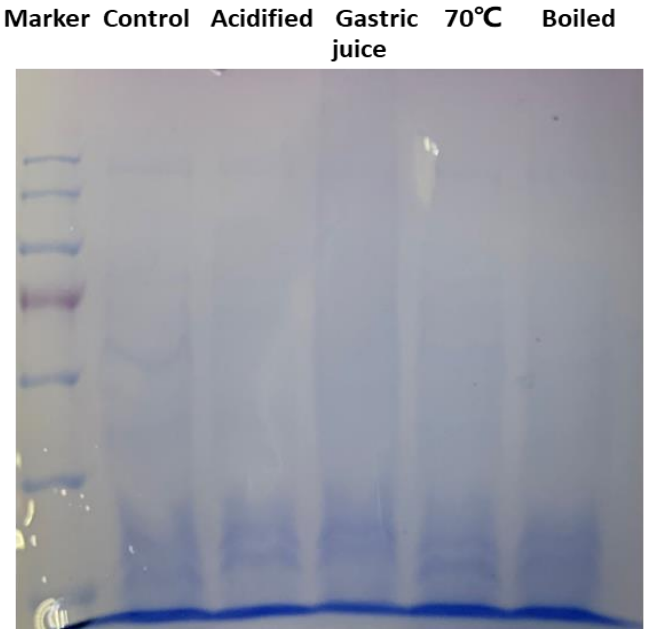


Figure S4. Photographs of the bands of SDS-PAGE movies of LGG-EV before lysis indirectly verified that the proteins within LGG-EV were not released prior to lysis. After *in vitro* treatment with acidic conditions (pH=2) and high temperature, re-centrifuged LGG-EV were collected, sampled at 50ug per well after SDS-PAGE electrophoresis and photographed.

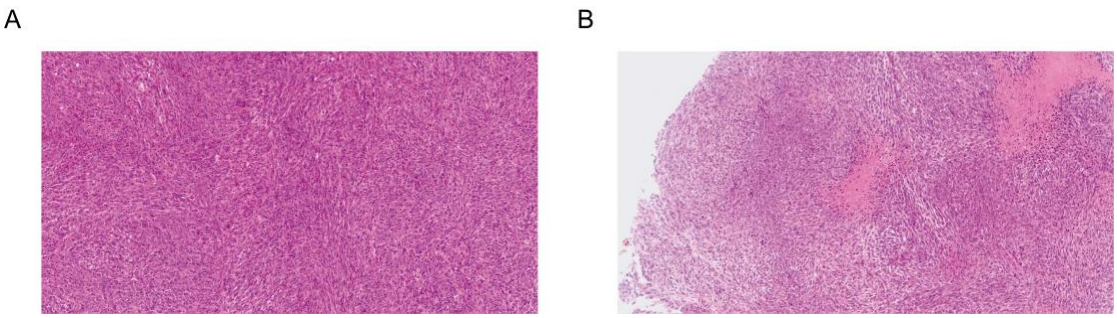


Figure S5. H&E staining images also clarified the expansion of necrotic regions in tumor tissue after treatment with LGG-EV, illustrating LGG-EV against tumor growth. Typical H&E staining images of tumor of control group(A) and LGG-EV treated(B).

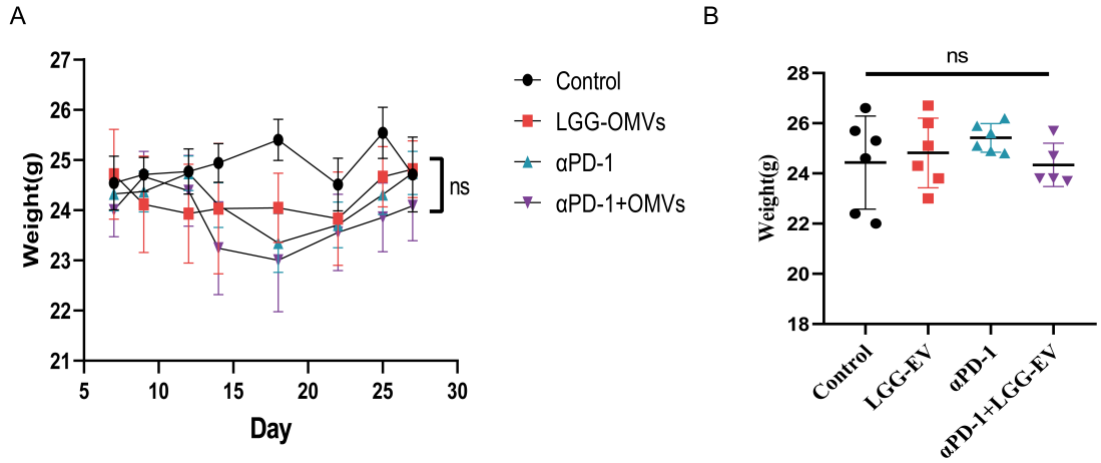
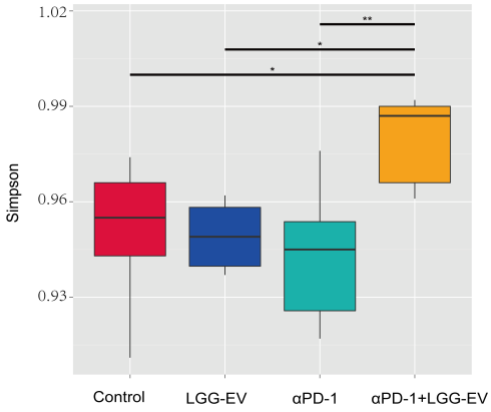


Figure S6. No significant changes in body weight of mice after LGG-EV and PD-1 administration. (A). Body weight curves of the four groups of mice. (B). Body weight of mice in each group after day 28. All data are presented as the means ± SEM.P-value< 0.05, not significant

A



B

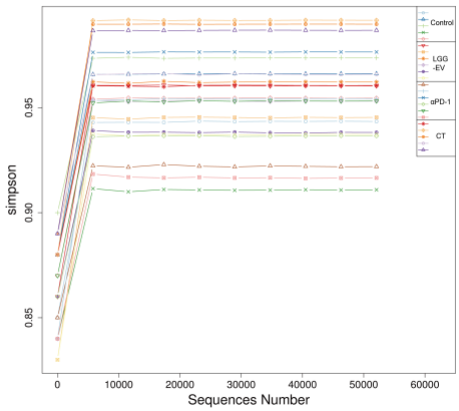
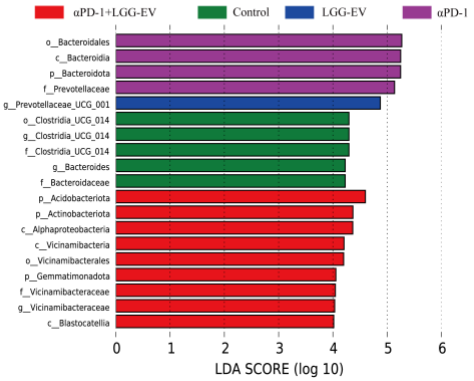


Figure S7. Increase in Simpson's index of flora diversity after co-administration. (A). Simpson's index of flora among the four groups. (B). Simpson curve of all samples.

A



B

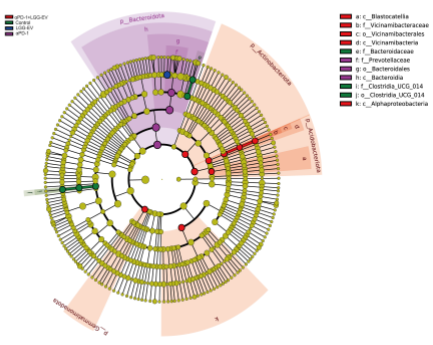


Figure S8. LEfSe analysis for differential abundant taxa of four groups. (A). Histogram of the distribution of LDA values. Threshold parameters were set as p=0.05 for the Mann-Whitney U test and multiclass analysis=all against all. LDA score >4.0. (B). Evolutionary branching diagram of differential taxa.

Table S1. Specific information and dilution ratios of antibodies used in flow cytometry. All antibodies were validated by the manufacturers and by extensive applications in published studies.

Antibody	Clone	Fluorescence channel	Company	Proportion
CD3	17A2	Alexa Fluor 647	Biolegend	1:200
CD8	53-6.7	PE	Biolegend	1:200
CD4	GK1.5	PerCP	Biolegend	1:200
I-A/I-E	M5/114.15.2	Alexa Fluor 488	Biolegend	1:200
CD11c	N418	PE-Cy7	Biolegend	1:200
CD11b	M1/70	APC-Cy7	Biolegend	1:200
CD16/32	93	Non-conjugated	Biolegend	1:300
Zombie Violet	-	BV421	Biolegend	1:300