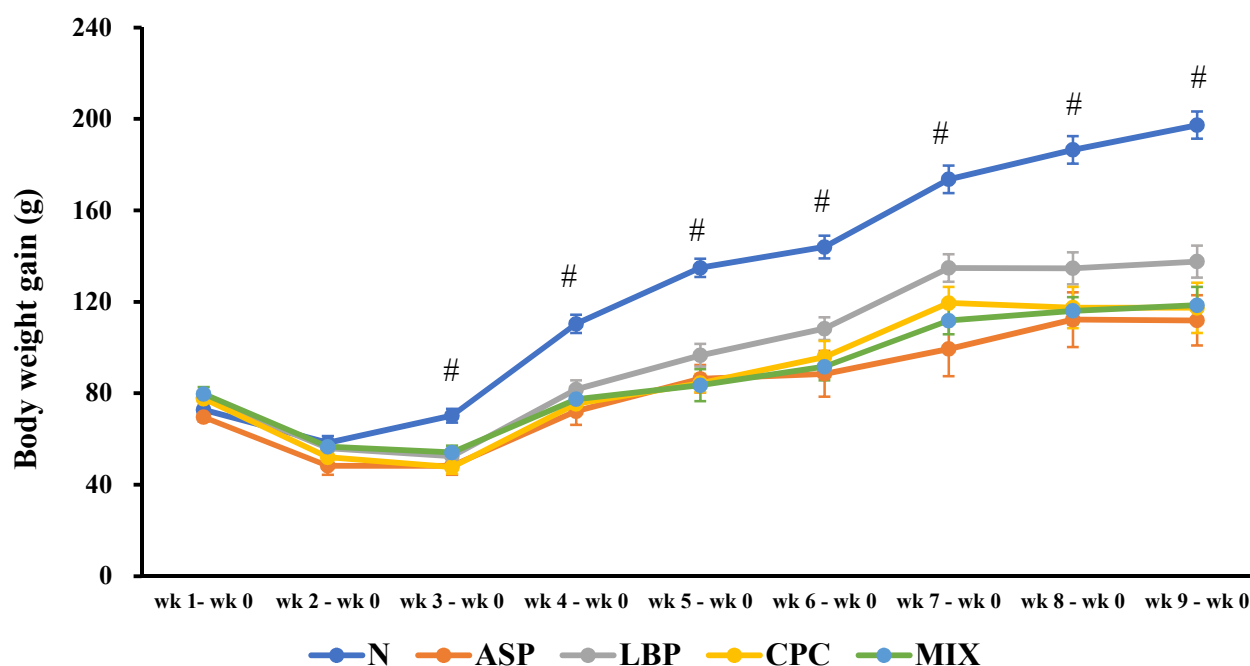
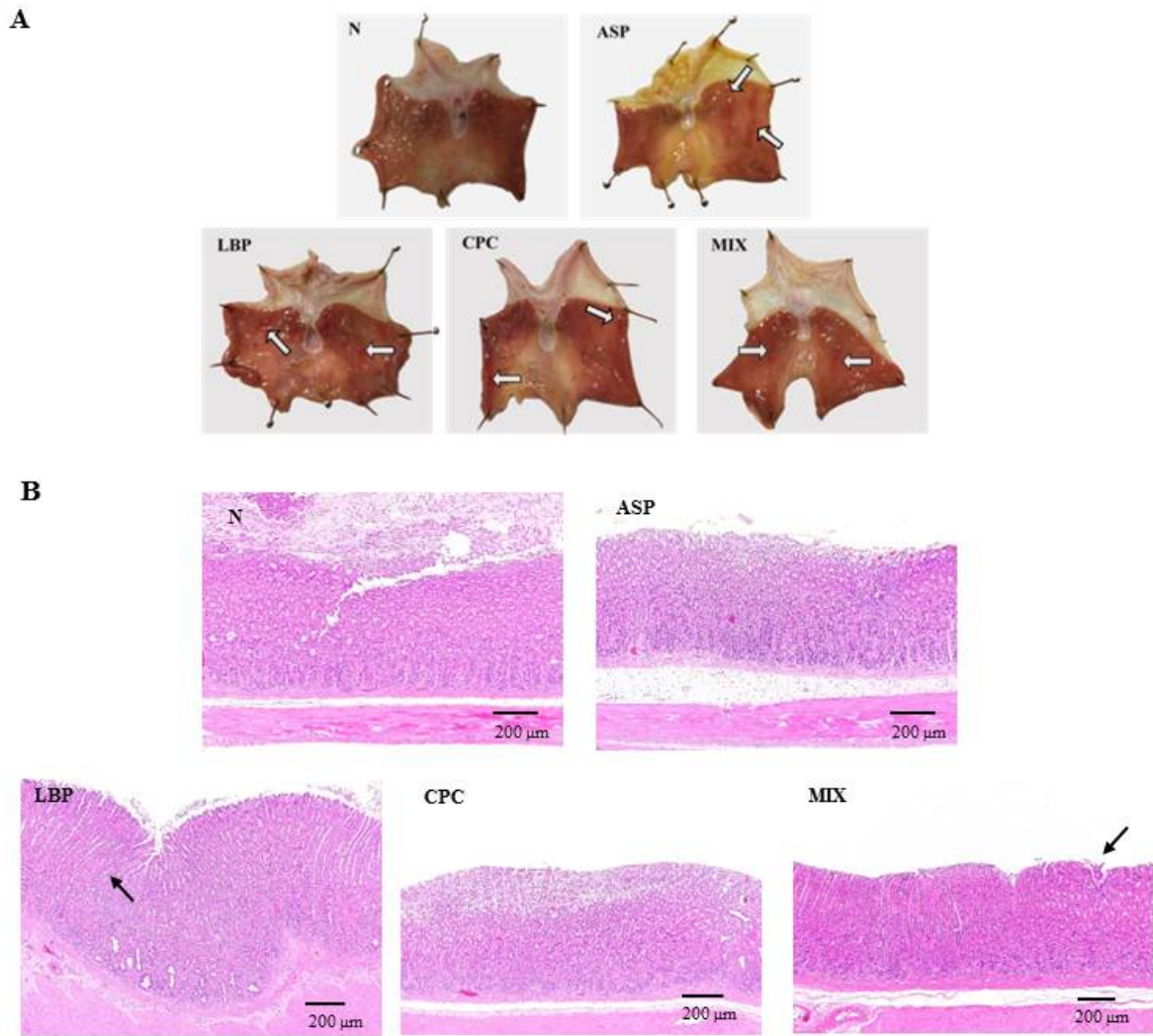


**Combined *Lycium barbarum* polysaccharides and C-phycoerythrin increase gastric *Bifidobacterium* relative abundance and protect against gastric ulcer caused by aspirin in rats**

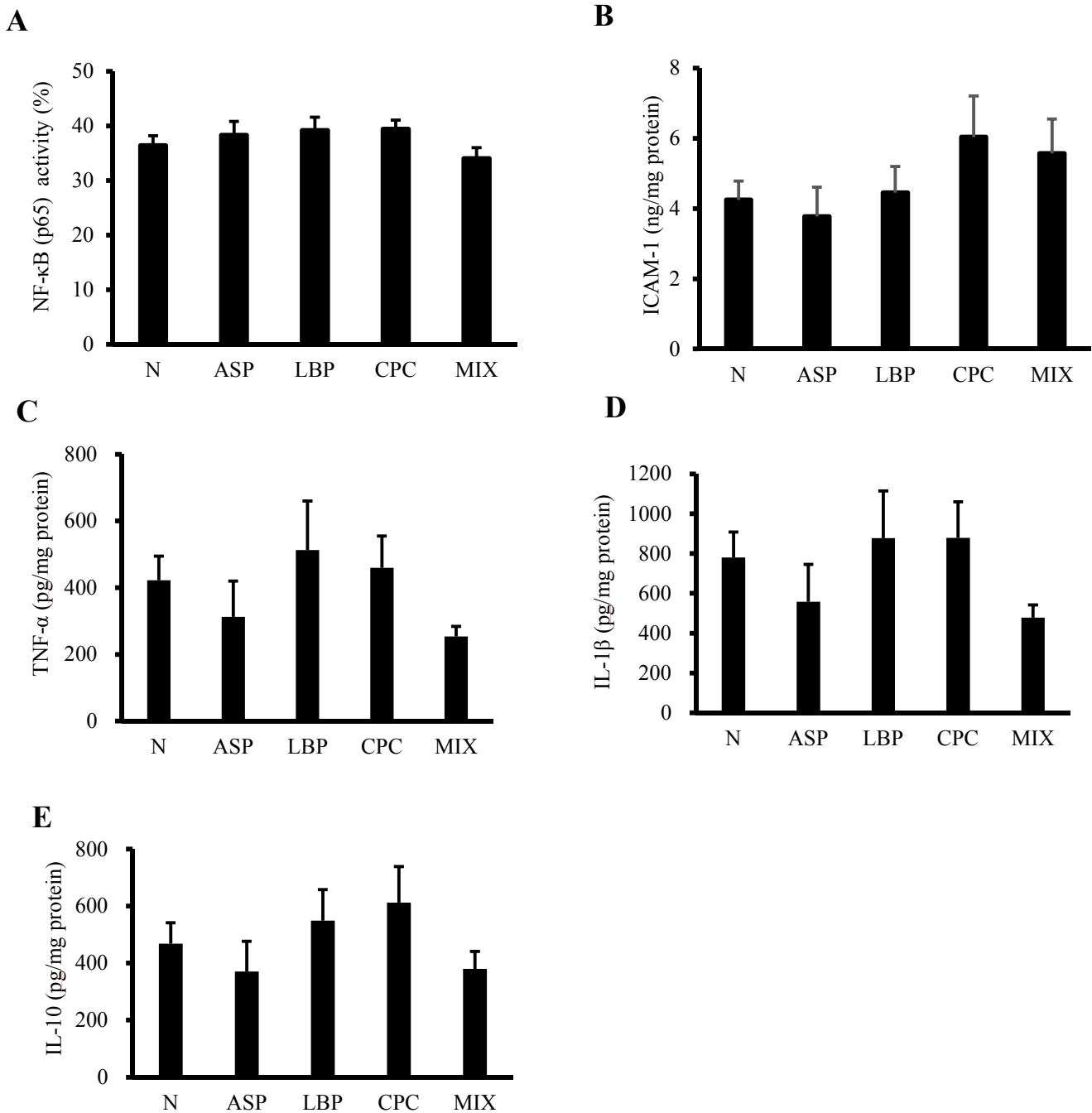
Shu-Yu Hsieh<sup>1</sup>, Yu Zhi Lian<sup>1</sup>, I-Hsuan Lin<sup>2</sup>, Yu-Chen Yang<sup>3</sup>, Alexey A. Tinkov<sup>4,5,6</sup>, Anatoly V. Skalny<sup>5,6,7</sup> and Jane C.-J. Chao<sup>1,8,9\*</sup>



**Supplementary Fig. 1** Accumulated body weight gains of rats from week 1 to week 9. N: standard powder diet; ASP: aspirin; LBP: aspirin + *Lycium barbarum* polysaccharides (LBP); CPC: aspirin + C-phycoerythrin (CPC); MIX: aspirin + LBP + CPC. Data are mean  $\pm$  SEM ( $n = 10-12$  per group). Differences between the groups were determined by one-way ANOVA followed by Fisher's least significant difference test. #  $p < 0.05$  compared with the ASP group.

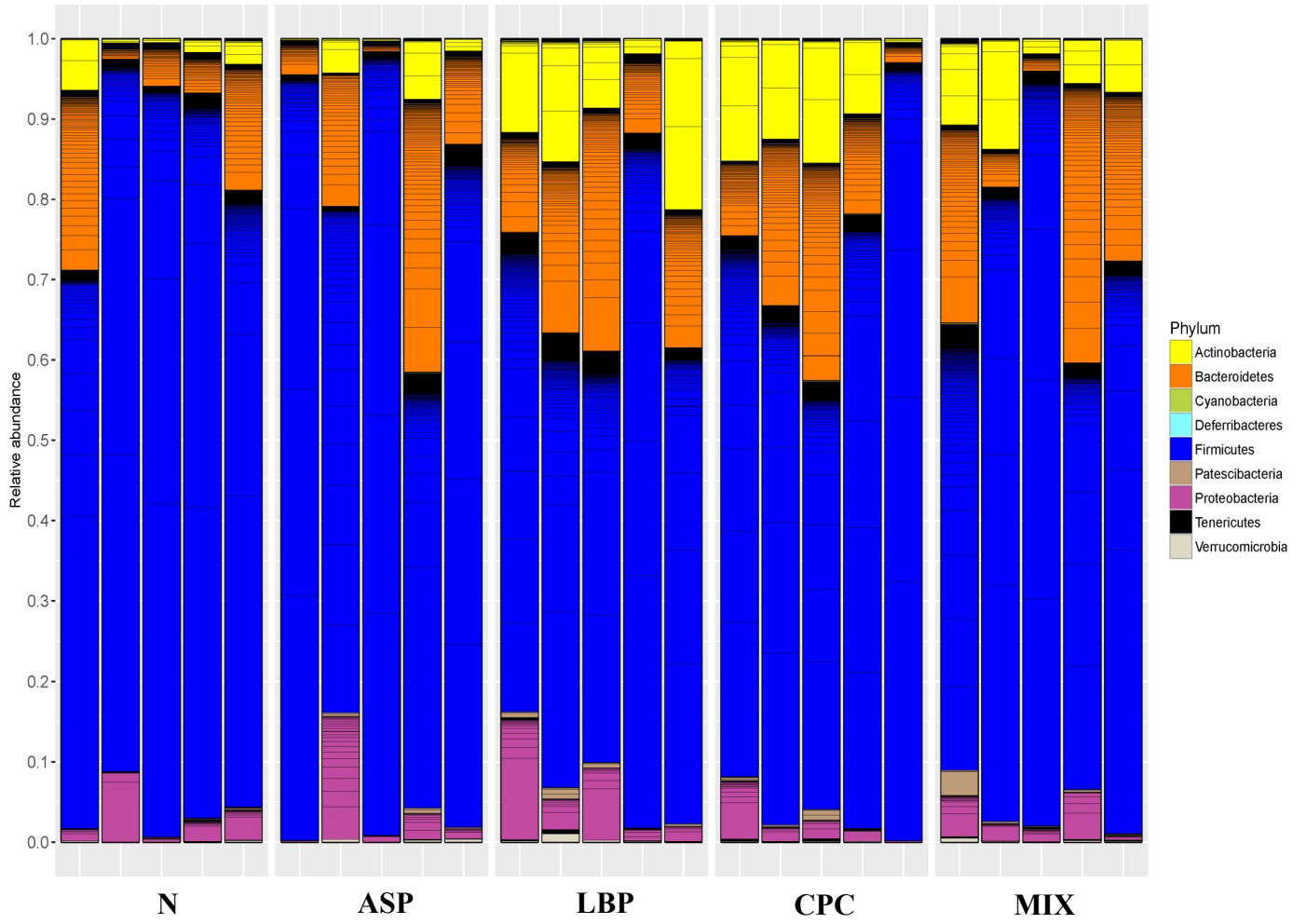


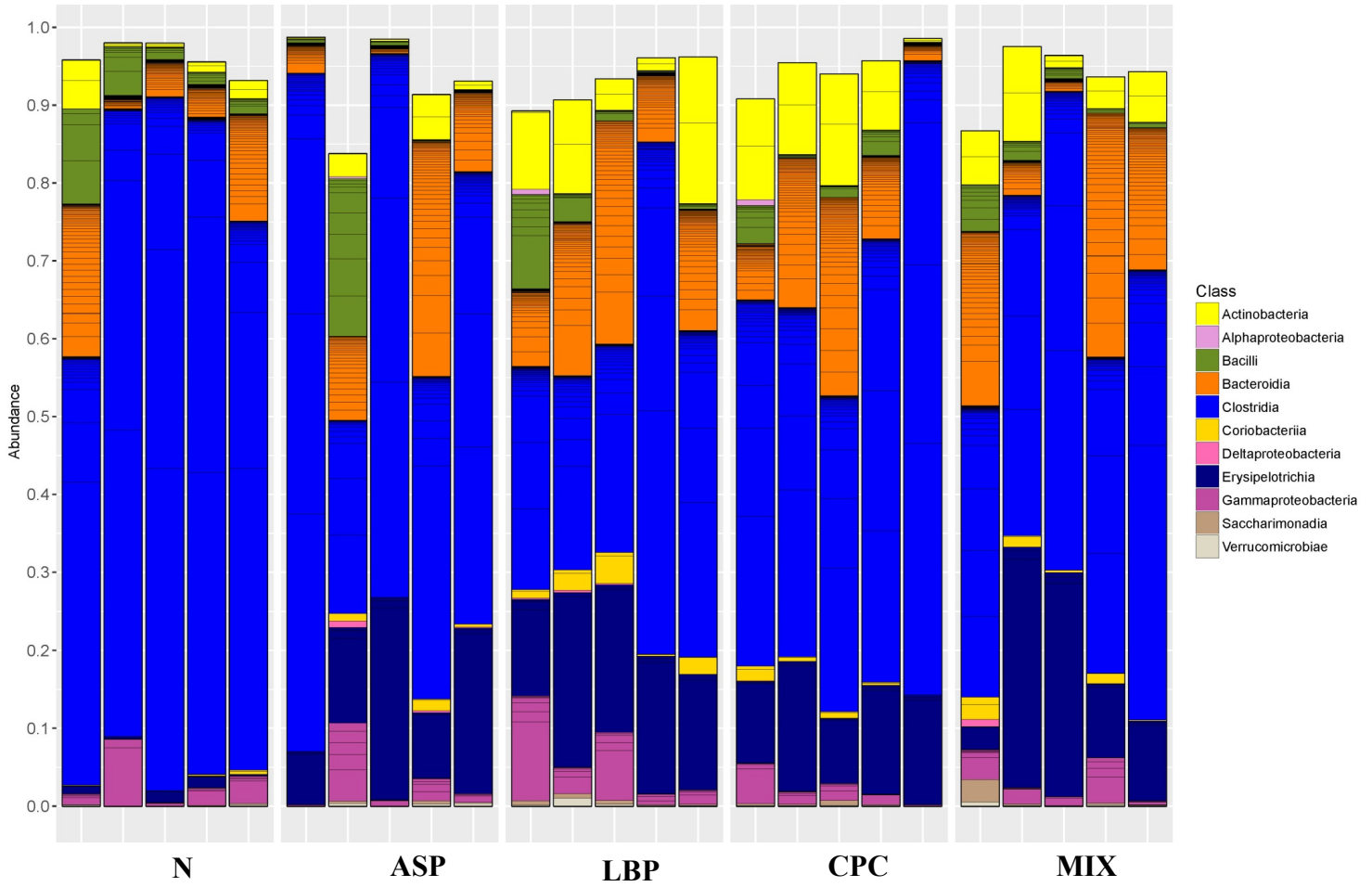
**Supplementary Fig. 2** Macroscopic and microscopic observations of rat stomach tissue. Macroscopic appearance of **a** rat gastric mucosa surface and **b** histological staining by H&E (100×). N: standard powder diet; ASP: aspirin; LBP: aspirin + *Lycium barbarum* polysaccharides (LBP); CPC: aspirin + C-phycoerythrin (CPC); MIX: aspirin + LBP + CPC. Hollow arrows in **a** indicate the red coloration of glandular stomach. Solid arrows in **b** indicate focal epithelial regeneration of gastric mucosa.



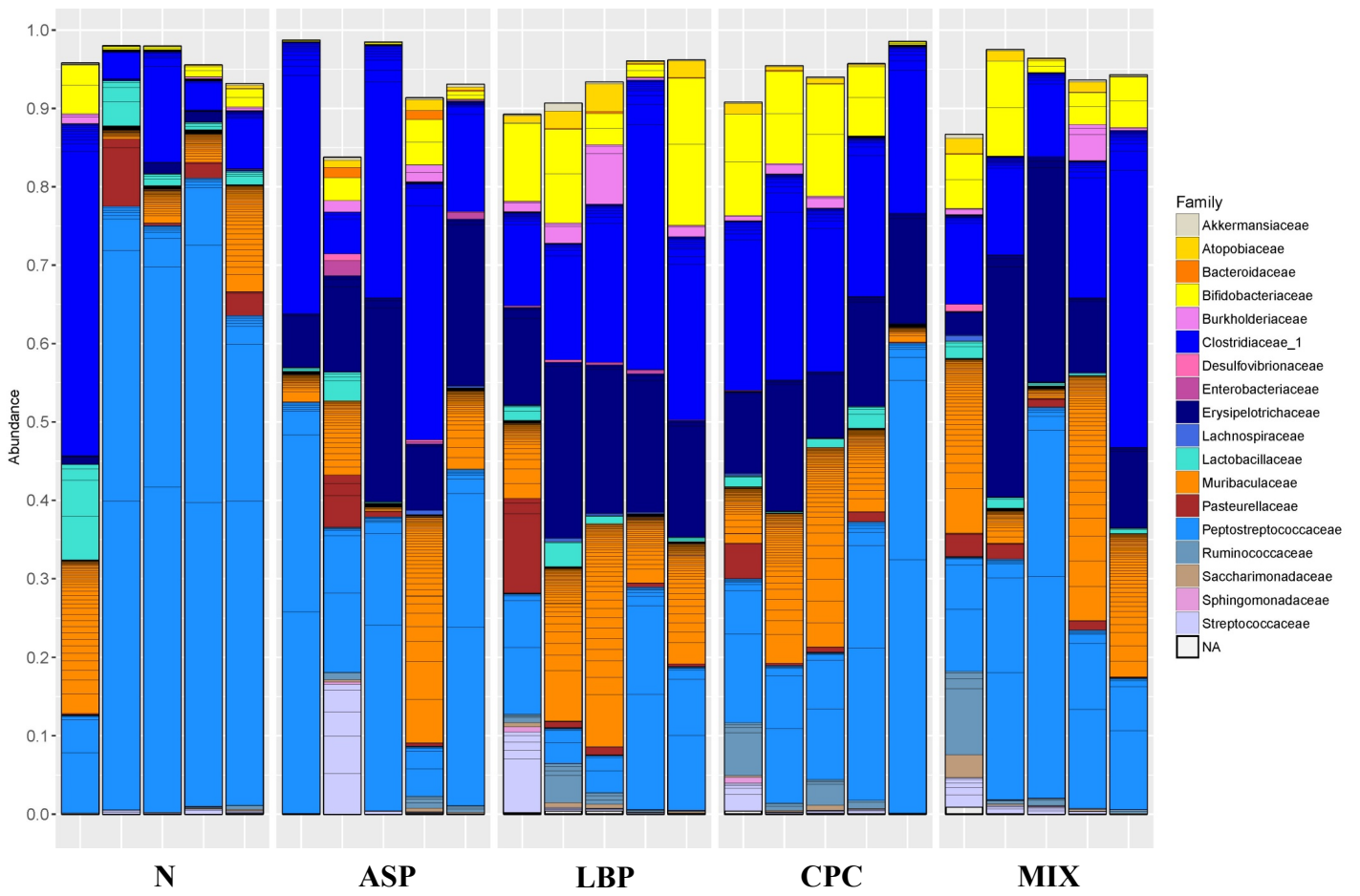
**Supplementary Fig. 3** Effects of *Lycium barbarum* polysaccharides (LBP) and/or C-phycocyanin (CPC) on inflammatory markers in stomach tissues. **a** Nuclear factor- $\kappa$ B (NF- $\kappa$ B) (p65) activity, **b** intercellular adhesion molecule-1 (ICAM-1), **c** tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), **d** interleukin-1 $\beta$  (IL-1 $\beta$ ), and **e** interleukin-10 (IL-10). N: standard powder diet; ASP: aspirin; LBP: aspirin + *Lycium barbarum* polysaccharides (LBP); CPC: aspirin + C-phycocyanin (CPC); MIX: aspirin + LBP + CPC. Data are mean  $\pm$  SEM ( $n = 10-12$  per group). Differences between the groups were determined by one-way ANOVA followed by Fisher's least significant difference test. There were no statistical differences in all inflammatory markers between any two groups.

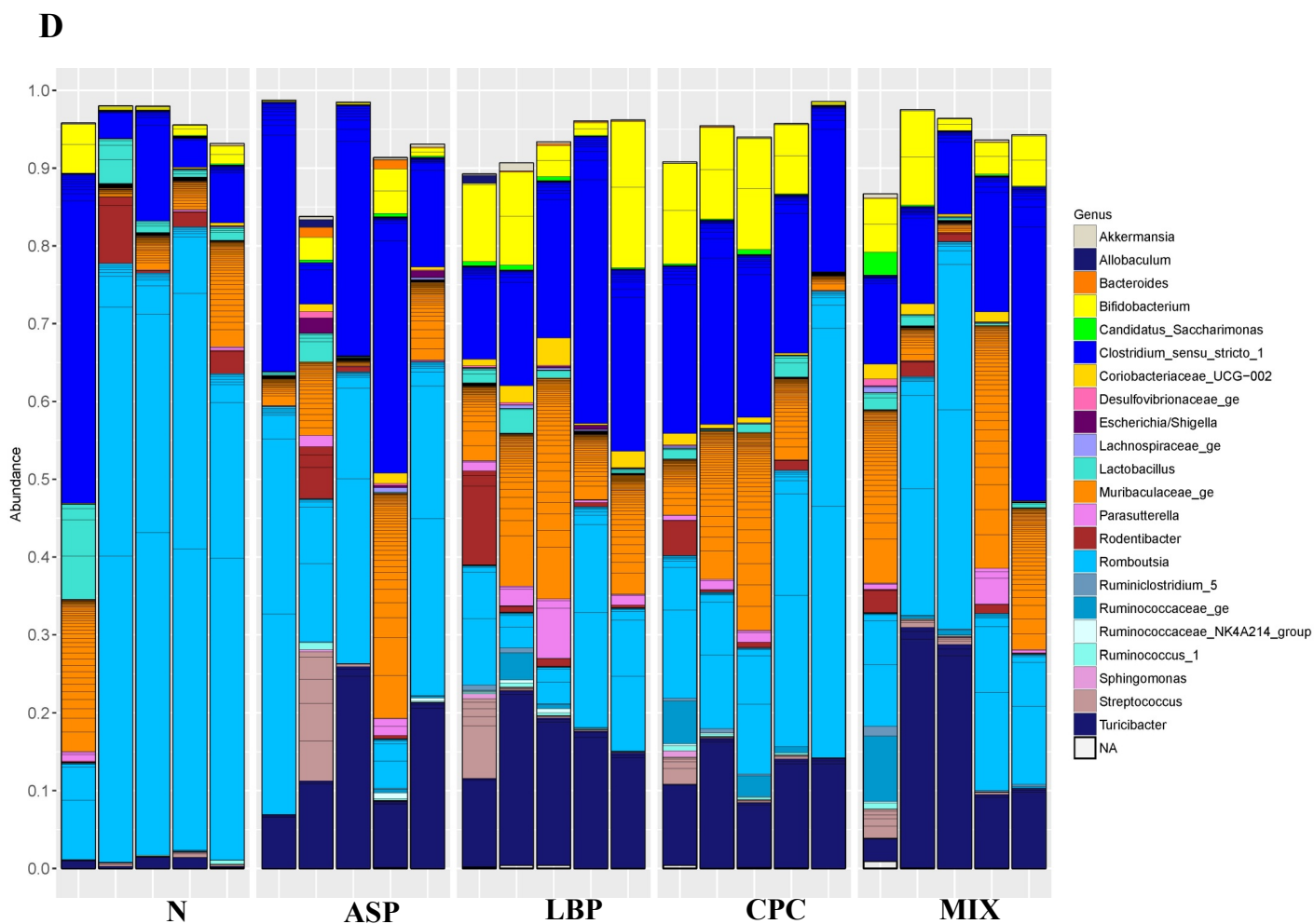
**A**



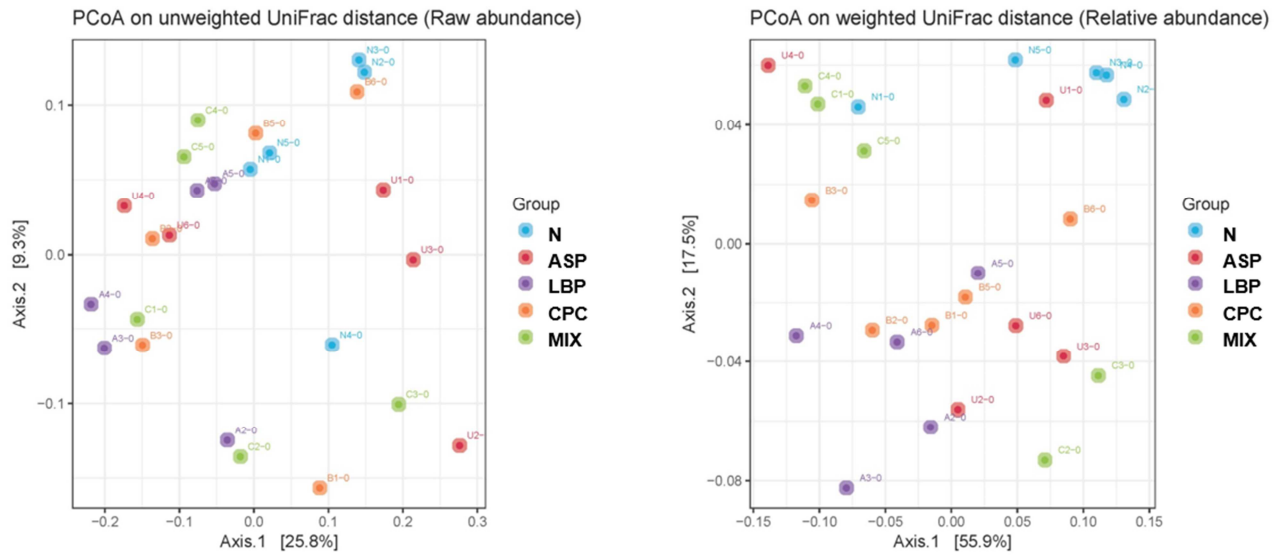
**B**

C





**Supplementary Fig. 4** The taxa distribution of rat gastric microbiota. **a** Taxa by phylum, **b** top 100 taxa by class, **c** top 100 taxa by family, and **d** top 100 taxa by genus. N: standard powder diet; ASP: aspirin; LBP: aspirin + *Lycium barbarum* polysaccharides (LBP); CPC: aspirin + C-phycoyanin (CPC); MIX: aspirin + LBP + CPC. Each bar represents an individual sample, and 5 samples are randomly selected from each group ( $n = 5$  per group).



**Supplementary Fig. 5** Beta diversity of gastric microbiota. N: standard powder diet (N); ASP: aspirin (U); LBP: aspirin + *Lycium barbarum* polysaccharides (LBP) (A); CPC: aspirin + C-phycocyanin (CPC) (B); MIX: aspirin + LBP + CPC (C). The left and right panels indicate raw abundance by unweighted UniFrac distance and relative abundance by weighted UniFrac distance, respectively, using principal coordinate analysis (PCoA). The 5 dots with the same color represent 5 samples from each group ( $n = 5$  per group). There were no significant differences in beta diversity by unweighted and weighted UniFrac distances among five groups using Kruskal-Wallis one-way ANOVA.



**Supplementary Table 1** Correlation coefficients between the relative abundance of the genus *Bifidobacterium* and the levels of gastroprotective factors in the stomach

	Relative abundance of <i>Bifidobacterium</i> (n = 25)	COX-1 (n = 25)	COX-2 (n = 23)	PGE <sub>2</sub> (n = 25)	NOx (n = 25)
Relative abundance of <i>Bifidobacterium</i>	-	0.235	0.324	-0.239	-0.083
COX-1	0.235	-	0.892 <sup>***</sup>	0.186	-0.083
COX-2	0.324	0.892 <sup>***</sup>	-	-0.082	-0.195
PGE <sub>2</sub>	-0.239	0.186	-0.082	-	-0.092
NOx	0.085	-0.083	-0.195	-0.092	-

COX-1: cyclooxygenase-1; COX-2: cyclooxygenase-2; PGE<sub>2</sub>: prostaglandin E<sub>2</sub>; NOx: total nitrite and nitrate (NOx). Correlation was determined by Pearson's correlation coefficient. <sup>\*\*\*</sup> $p < 0.001$ .