1
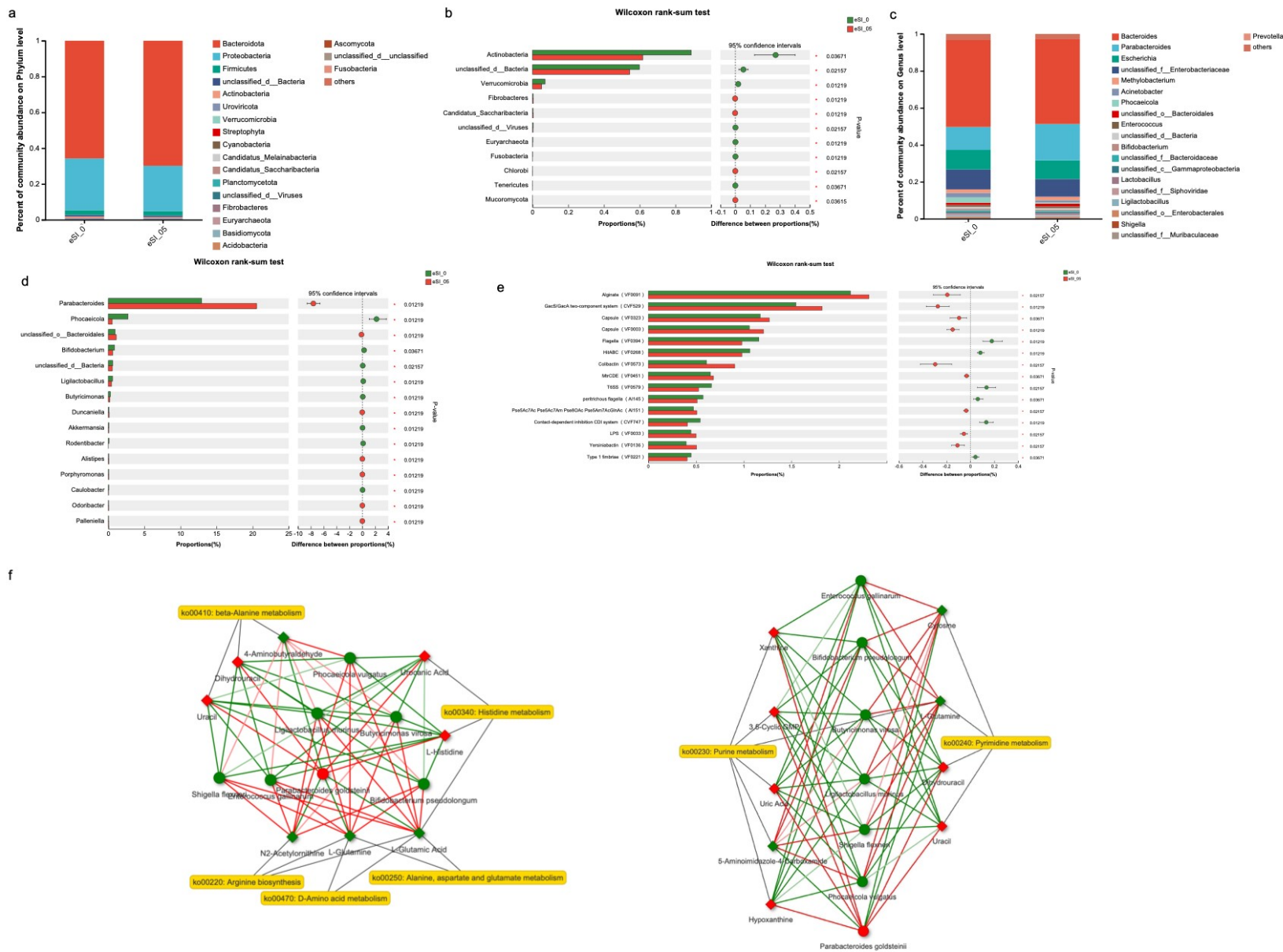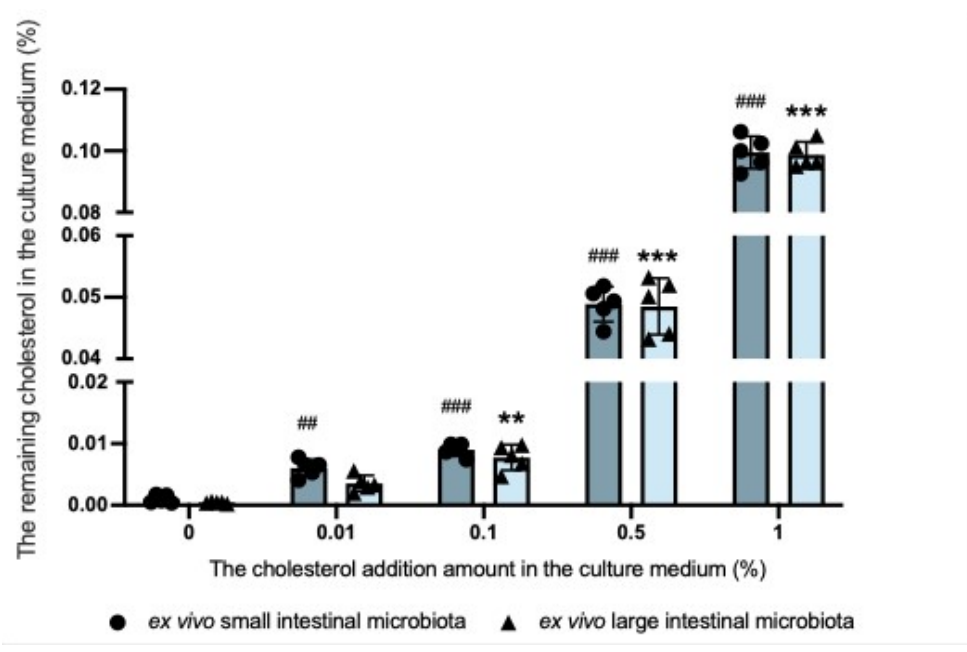
2   Supplementary Fig. S1. Effects of cholesterol on large intestinal microbiota ex vivo. (a) Microbiota composition at the Phylum level for eLI_0 and eLI_05 groups. (b)

3   Top 15 gut microbiota with significant differences in eLI_0 and eLI_05 groups at the Phylum level. (c) Microbiota composition at the Genus level for eLI_0 and eLI_05

4   groups. (d) Top 15 gut microbiota with significant differences in eLI_0 and eLI_05 groups at the Genus level. (e) Top 15 significantly different virulence factor genes

5   in eLI_0 and eLI_05 groups. (f) Network analysis of significantly changed intestinal microbiota and metabolites. The red/green color of nodes indicates

6   up/downregulation. The red/green lines indicate the positive/negative correlations between microbes and metabolites. Light nodes/lines indicate no statistical

7   significance. Dark nodes/lines indicate statistical significance. * $P < 0.05$

8

10 Supplementary Fig. S2. Effects of cholesterol on small intestinal microbiota ex vivo. (a) Microbiota composition at the Phylum level for eSI_0 and eSI_05 groups. (b)

11 Top 15 gut microbiota with significant differences in eSI_0 and eSI_05 groups at the Phylum level. (c) Microbiota composition at the Genus level for eSI_0 and eSI_05

12 groups. (b) Top 15 gut microbiota with significant differences in eSI_0 and eSI_05 groups at the Genus level. (e) Top 15 significantly different virulence factor genes

13 in eSI_0 and eSI_05 groups. (f) Network analysis of significantly changed intestinal microbiota and metabolites. The red/green color of nodes indicates

14 up/downregulation. The red/green lines indicate the positive/negative correlations between microbes and metabolites. Light nodes/lines indicate no statistical

15 significance. Dark nodes/lines indicate statistical significance. * $P < 0.05$

16

The cholesterol addition amount in the culture medium (%)

- ● *ex vivo* small intestinal microbiota
- ▲ *ex vivo* large intestinal microbiota

17

18 Supplementary Fig. S3. Cholesterol concentration in the culture medium after co-culturing with the intestinal microbiota. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$,

19 compared with the eSI_0 group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with the eLI_0 group
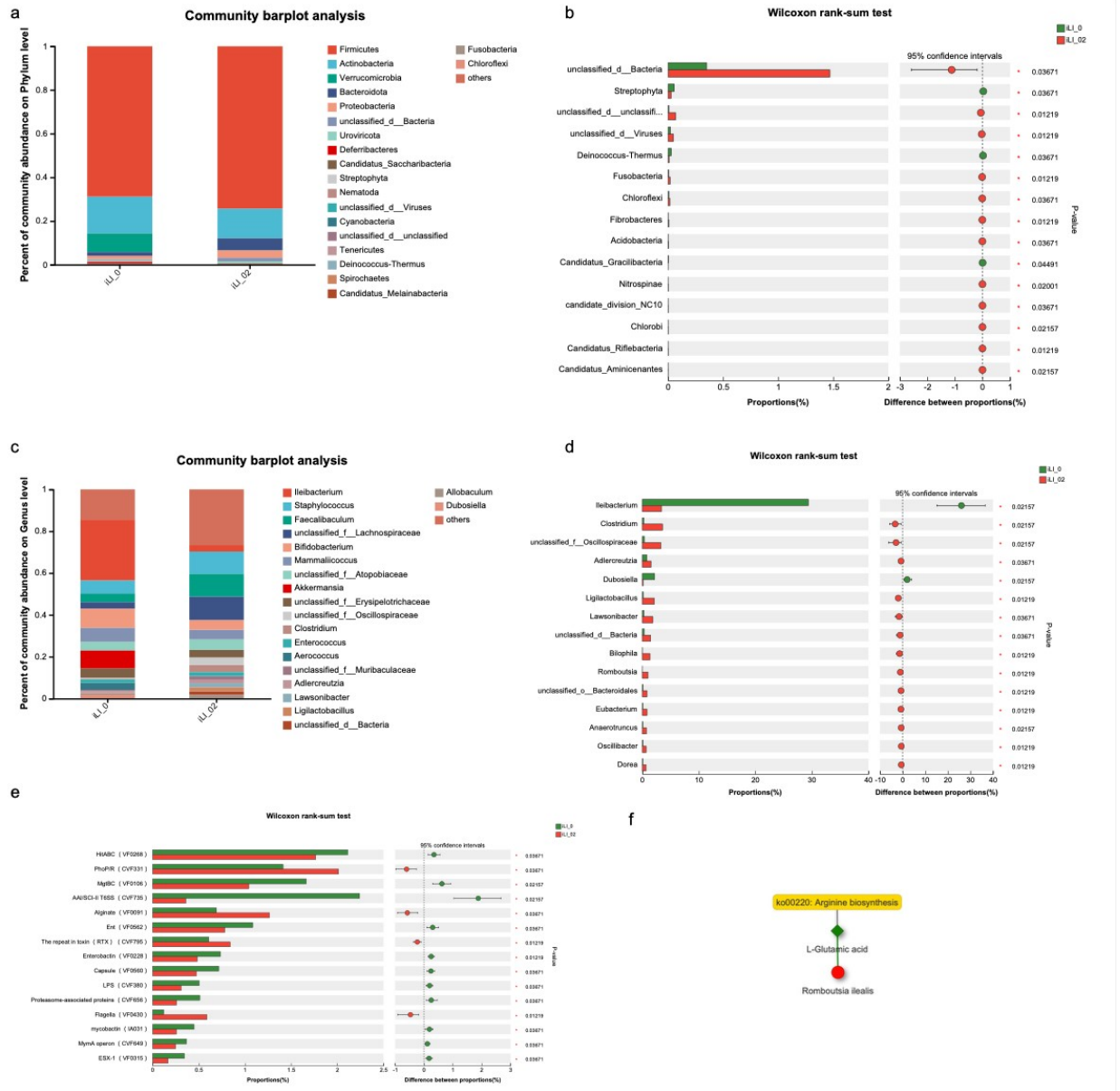
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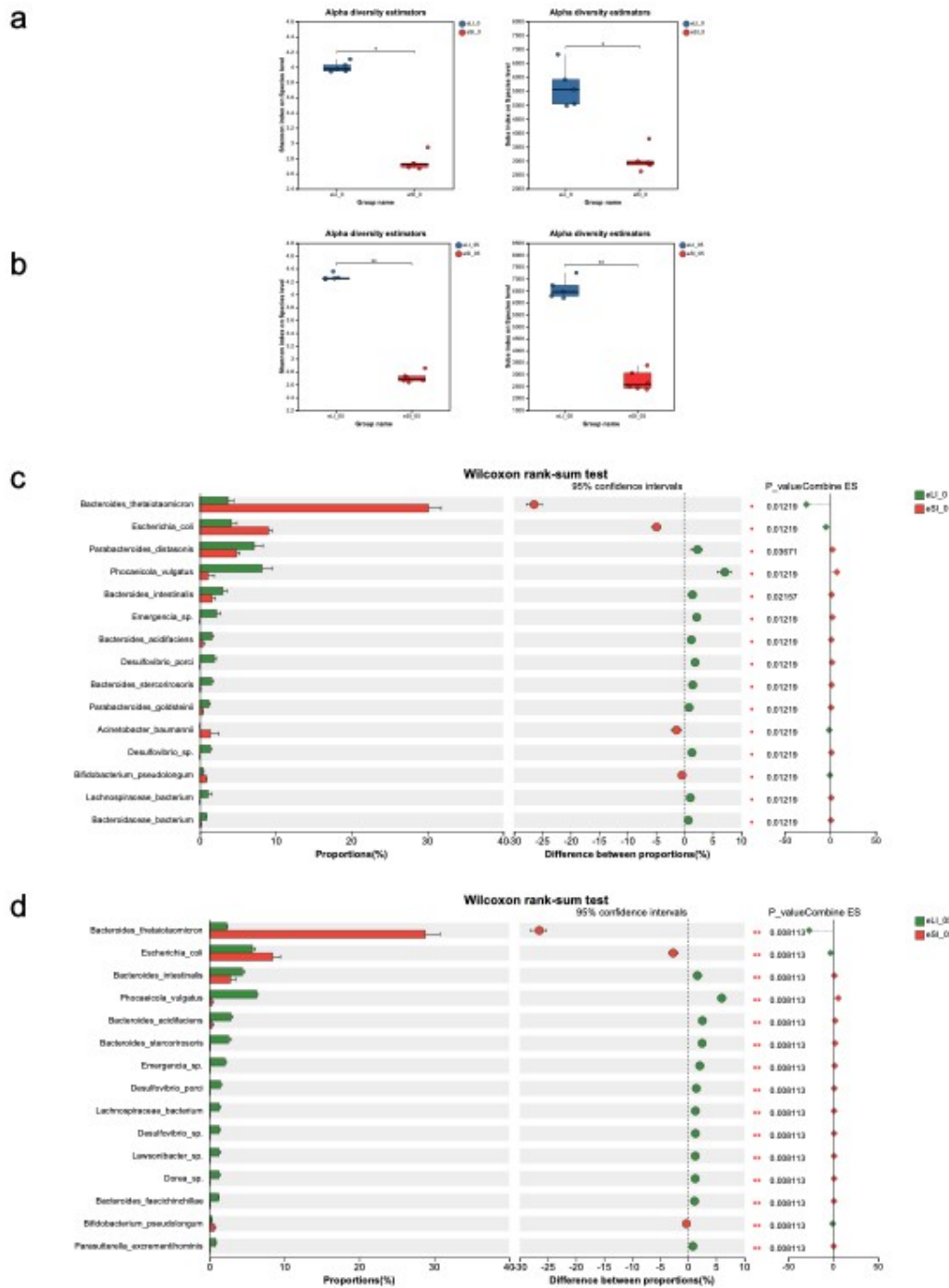
Supplementary Fig. S4. The differences of cholesterol's effects on the small and large intestinal

microbiota in an *ex vivo* model. (a-b) Shannon and Sobs index. (c-d) Significantly different

microbiota between eSI and eSI model (the top 15 species by relative abundance). * *P* < 0.05, ** *P*
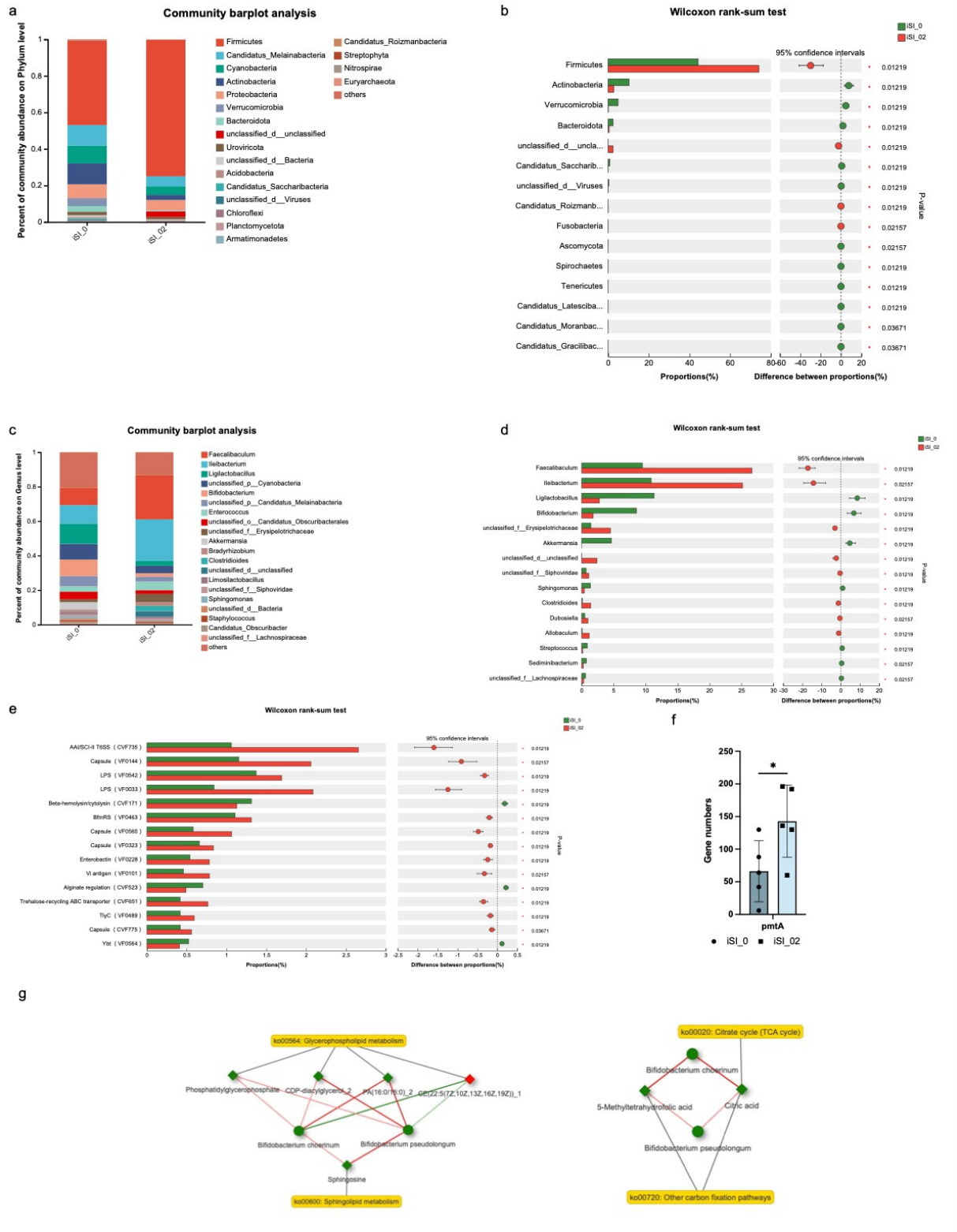
<   0.01

Supplementary Fig. S5. Effects of cholesterol on large intestinal microbiota *in vivo*. (a) Microbiota composition at the Phylum level for iLI_0 and iLI_02 groups. (b) Top 15 gut microbiota with significant differences in iLI_0 and iLI_02 groups at the Phylum level. (c) Microbiota composition at the Genus level for iLI_0 and iLI_02 groups. (b) Top 15 gut microbiota with significant differences in iLI_0 and iLI_02 groups at the Genus level. (e) Top 15 significantly different virulence factor genes in iLI_0 and iLI_02 groups. (f) Network analysis of significantly changed intestinal microbiota and metabolites. The red/green color of nodes indicates up/downregulation.

The red/green lines indicate the positive/negative correlations between microbes and metabolites.

Light nodes/lines indicate no statistical significance. Dark nodes/lines indicate statistical significance. * $P < 0.05$
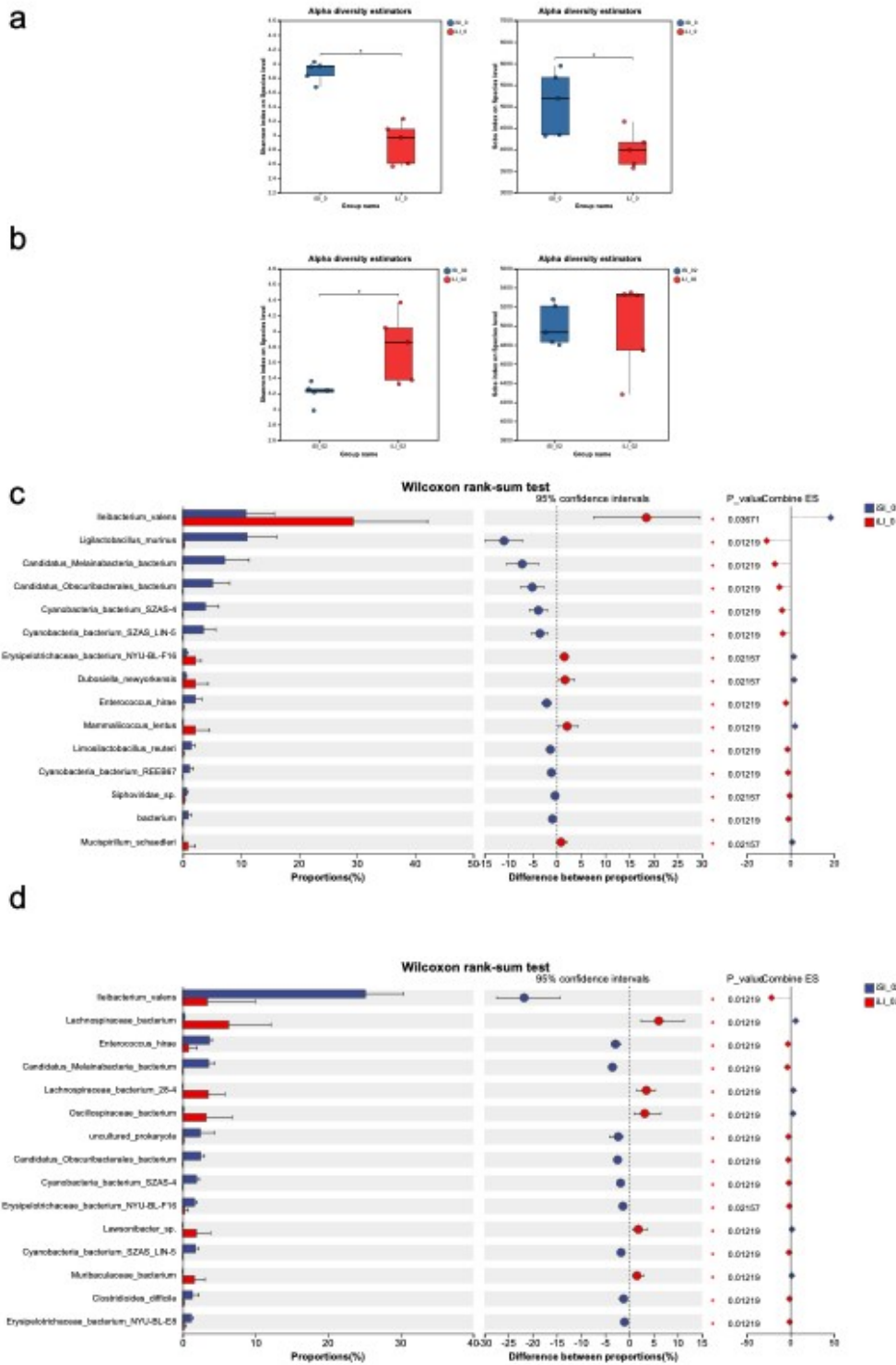
Supplementary Fig. S6. Effects of cholesterol on small intestinal microbiota *in vivo*. (a) Microbiota composition at the Phylum level for iSI_0 and iSI_02 groups. (b) Top 15 gut microbiota with significant differences in iSI_0 and iSI_02 groups at the Phylum level. (c) Microbiota composition

at the Genus level for iSI_0 and iSI_02 groups. (b) Top 15 gut microbiota with significant differences in iSI_0 and iSI_02 groups at the Genus level. (e) Top 15 significantly different virulence factor genes in iSI_0 and iSI_02 groups. (f) Gene numbers of pmtA in iSI_0 and iSI_02 groups. (g) Network analysis of significantly changed intestinal microbiota and metabolites. The red/green color of nodes indicates up/downregulation. The red/green lines indicate the positive/negative correlations between microbes and metabolites. Light nodes/lines indicate no statistical significance. Dark nodes/lines indicate statistical significance. $*$ $P < 0.05$
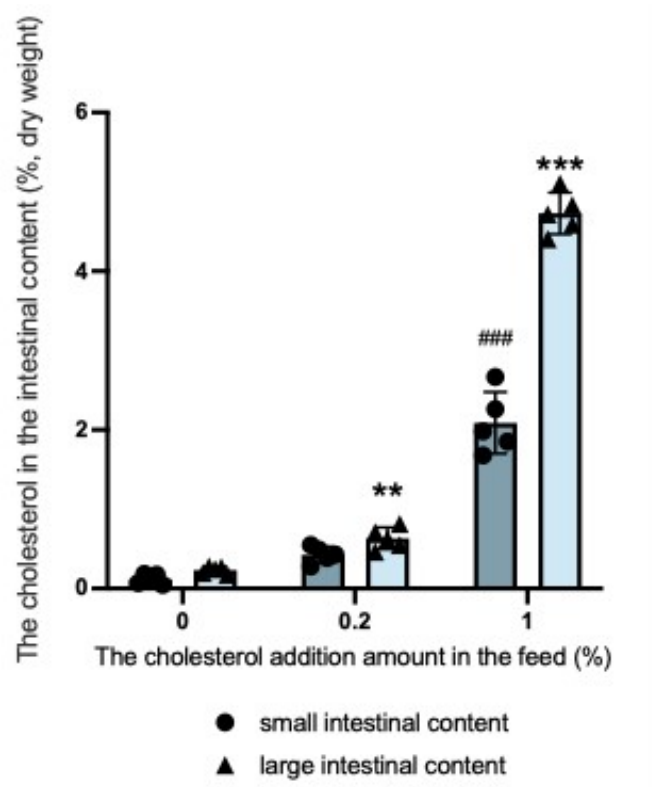
Supplementary Fig. S7. Cholesterol concentration in the intestinal content. # $P < 0.05$, ## $P < 0.01$,

$P < 0.001$, compared with the iSI_0 group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared

with the iLI_0 group

Supplementary Fig. S8. The differences of cholesterol's effects on the small and large intestinal microbiota in an *in vivo* model. (a-b) Shannon and Sobs index. (c-d) Significantly different microbiota between iSI and iSI model (the top 15 species by relative abundance). * *P* < 0.05, ** *P* < 0.01