# Supplementary Information

# For

# Porcine gut microbiota in mediating host metabolic adaptation to cold stress

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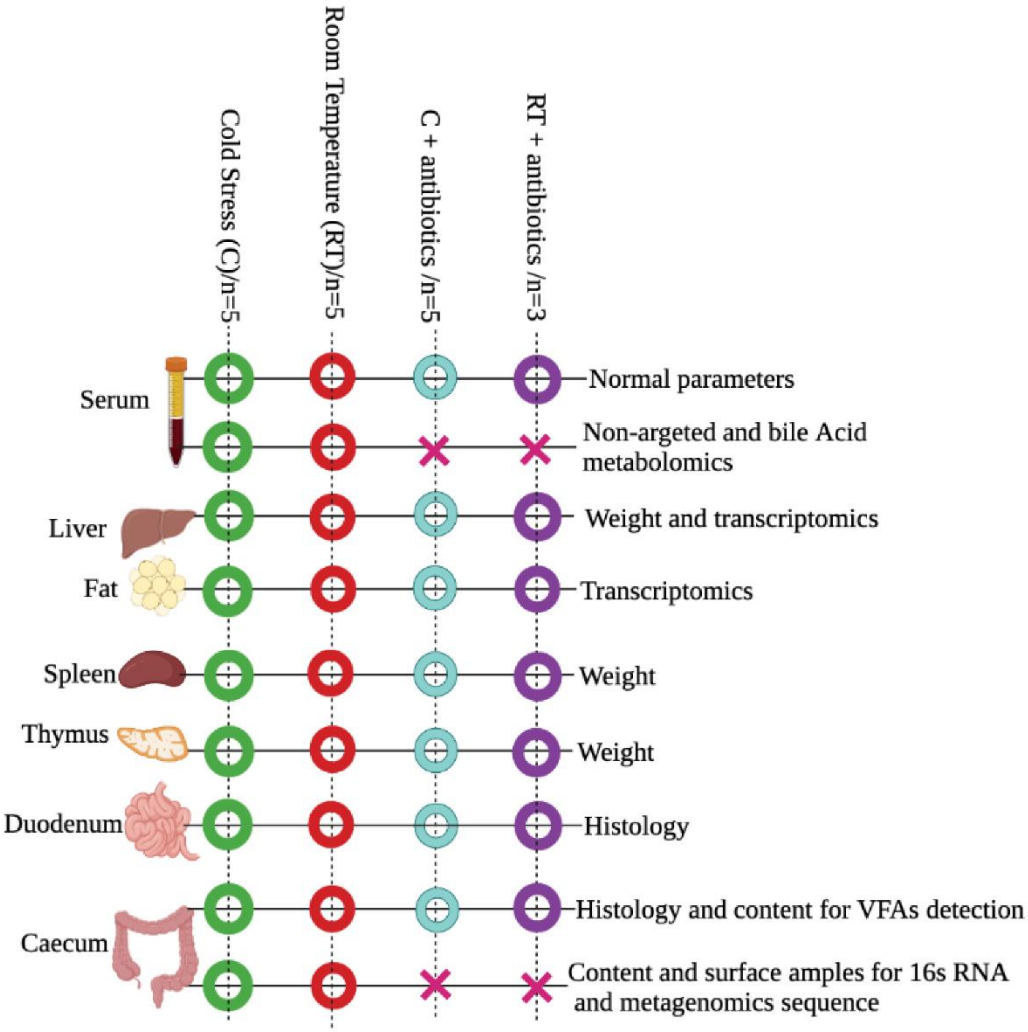
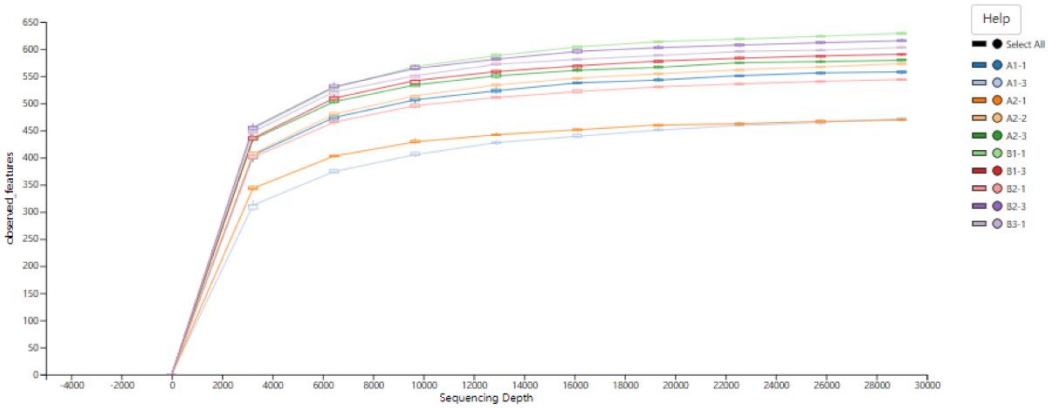
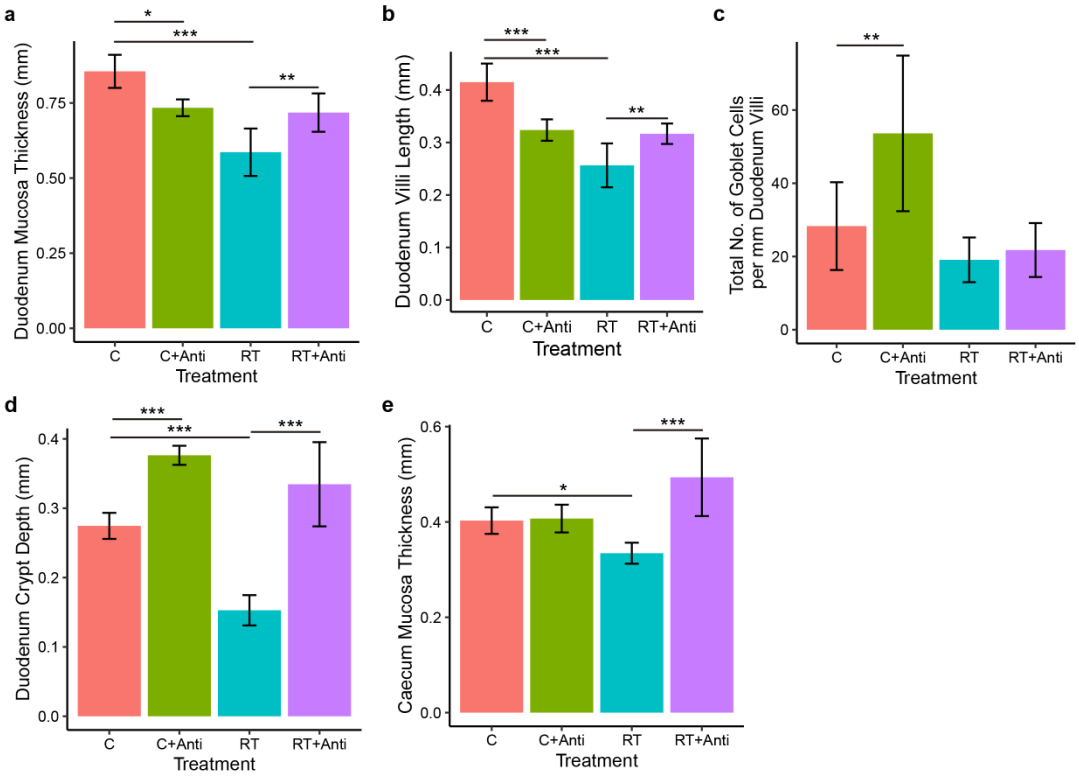
## 12 Supplementary Figures



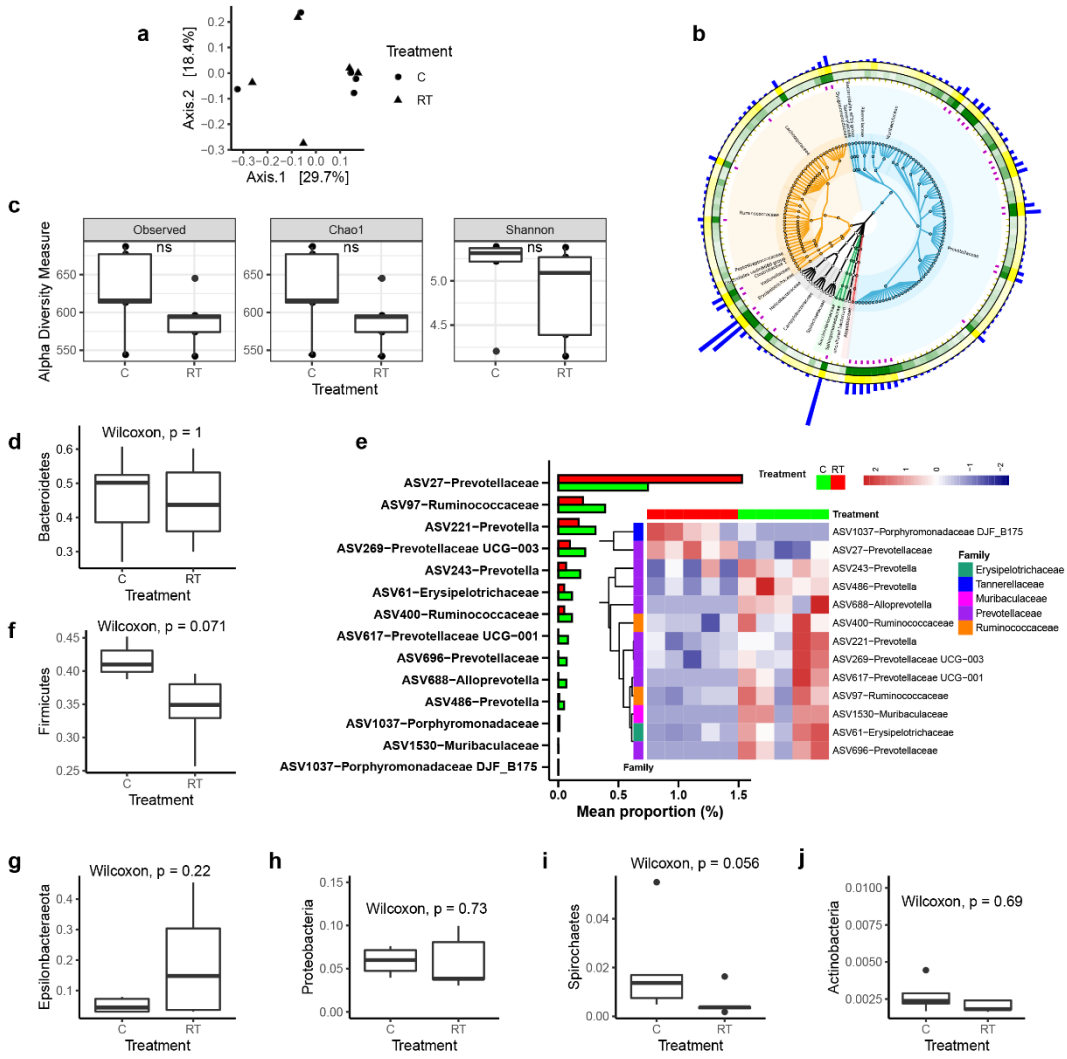

**Supplementary Figure 1** Details of sampling and analysis. Circles represent sampling types and analysis items; forks indicate that the corresponding items were not analyzed.




**Supplementary Figure 2** Rarity curves for 86 samples.

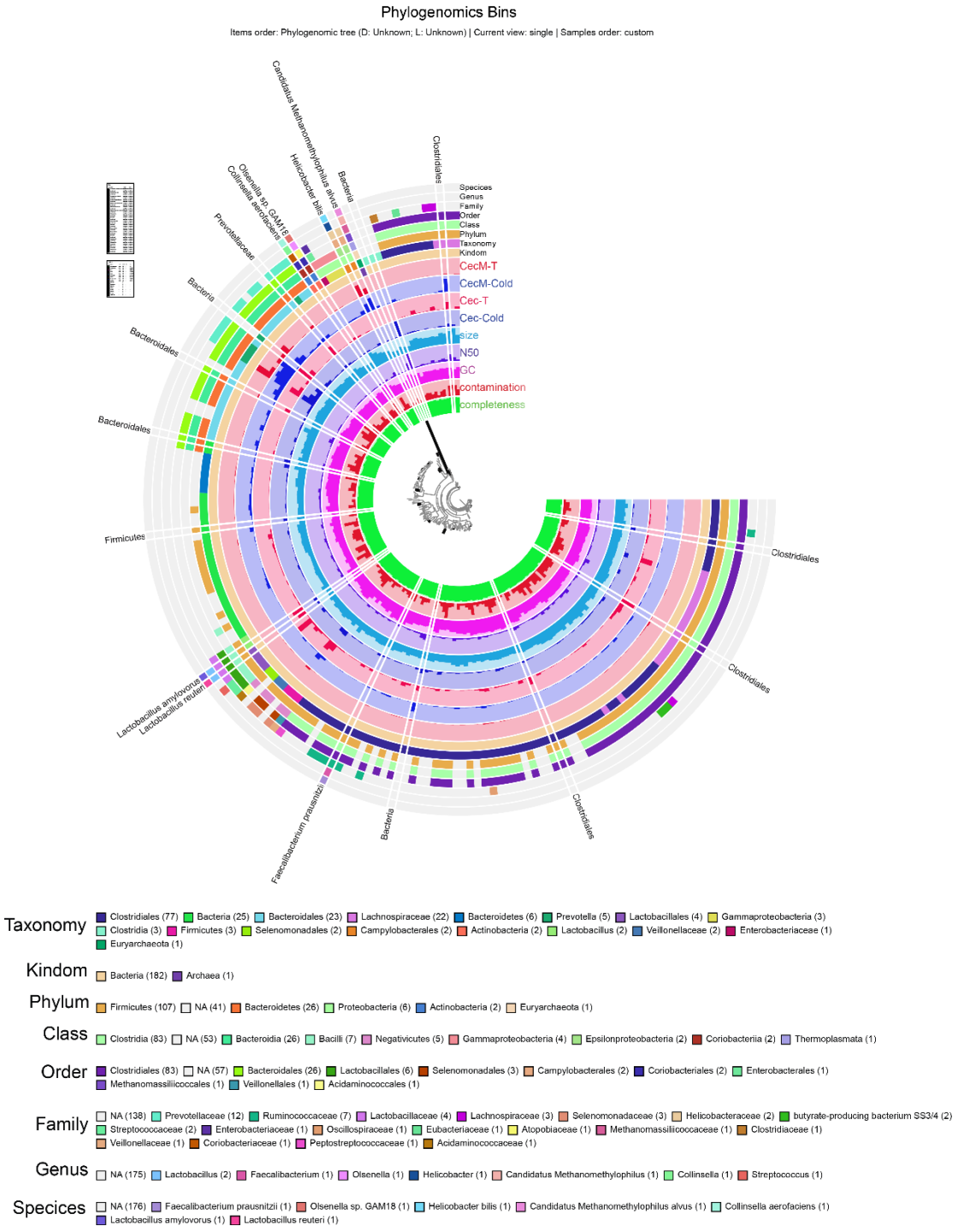



**Supplementary Figure 3** Cold-exposed piglets show an increased index for the duodenum. **a** Duodenal mucosa thicknesses. **b** Duodenal villus length. **c** Total number of goblet cells per mm duodenal villi. **d** Duodenal crypt depth. **e** Cecal mucosa thickness. The data are the means ± SEMs. Statistical significance was determined using Wilcoxon test (*$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$).

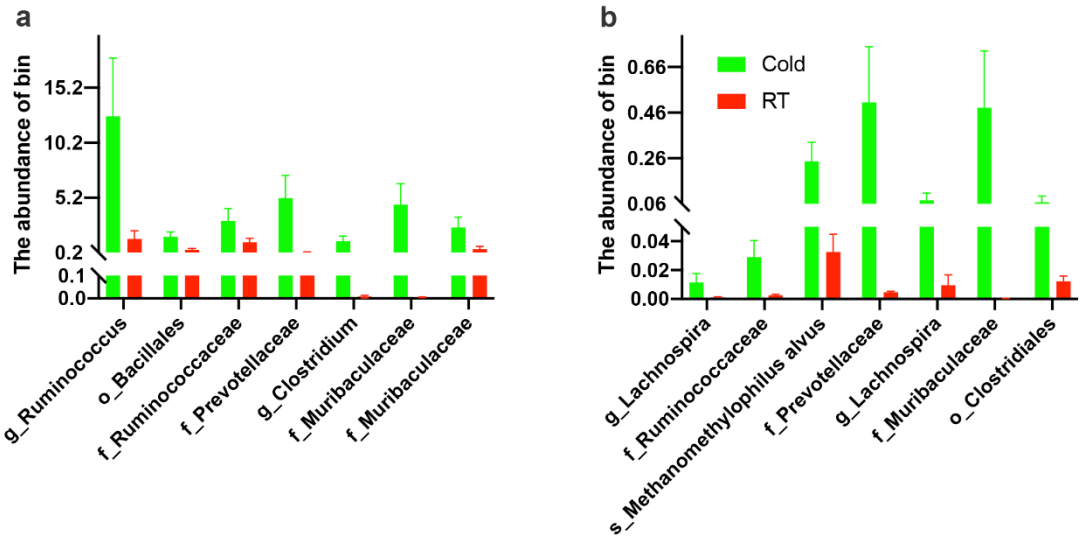



**Supplementary Figure 4** Cold exposure changes the composition of the epithelial surface of the cecum. **a** Principal coordinates analysis (PCoA) based on weighted UniFrac analysis. Each symbol represents a single sample of cecal content after 48 h of cold stress (n = 5) or RT (n = 5 per group). **b** Heatmap tree comparing the most abundant ASVs from the cecal content of 48 h cold-stressed animals (n = 5, inner green rings) and RT controls (n = 5, outer yellow rings) and their phylogenetic relationships. The bar represents abundant ASVs. **c** Alpha diversity of ASVs. **d** Boxplot of the microbiota of the cecal content at the phylum level. **e** The different ASVs with bars and heatmaps with $P < 0.05$. **f-j** Boxplot of the microbiota of the cecal content at the phylum level.

The data are the means ± SEMs. Statistical significance was determined using Wilcoxon test (*$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$).

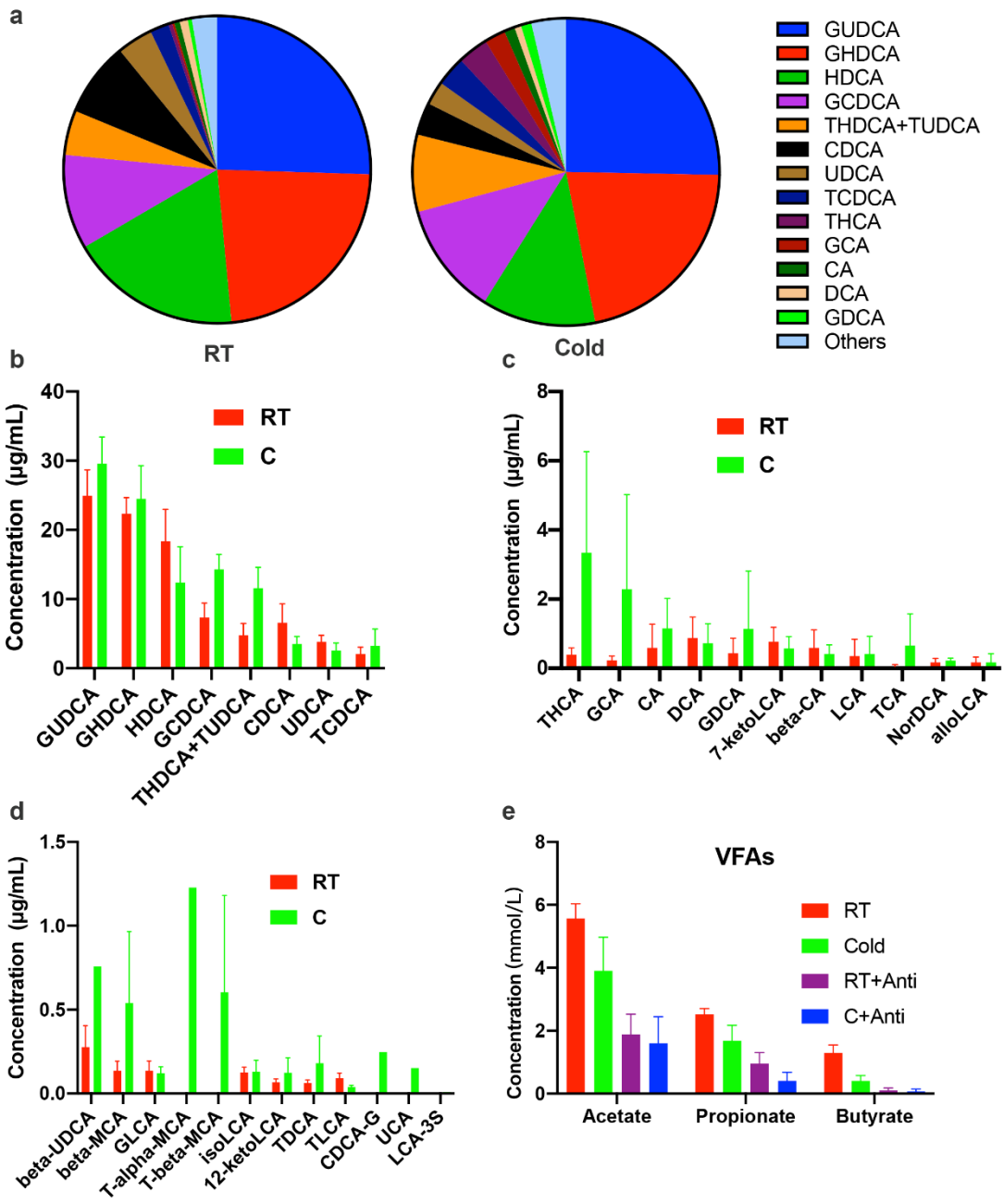



**Supplementary Figure 5** Phylogenomic tree with 191 bins with a completeness ≥ 90% and contamination ≤5%.

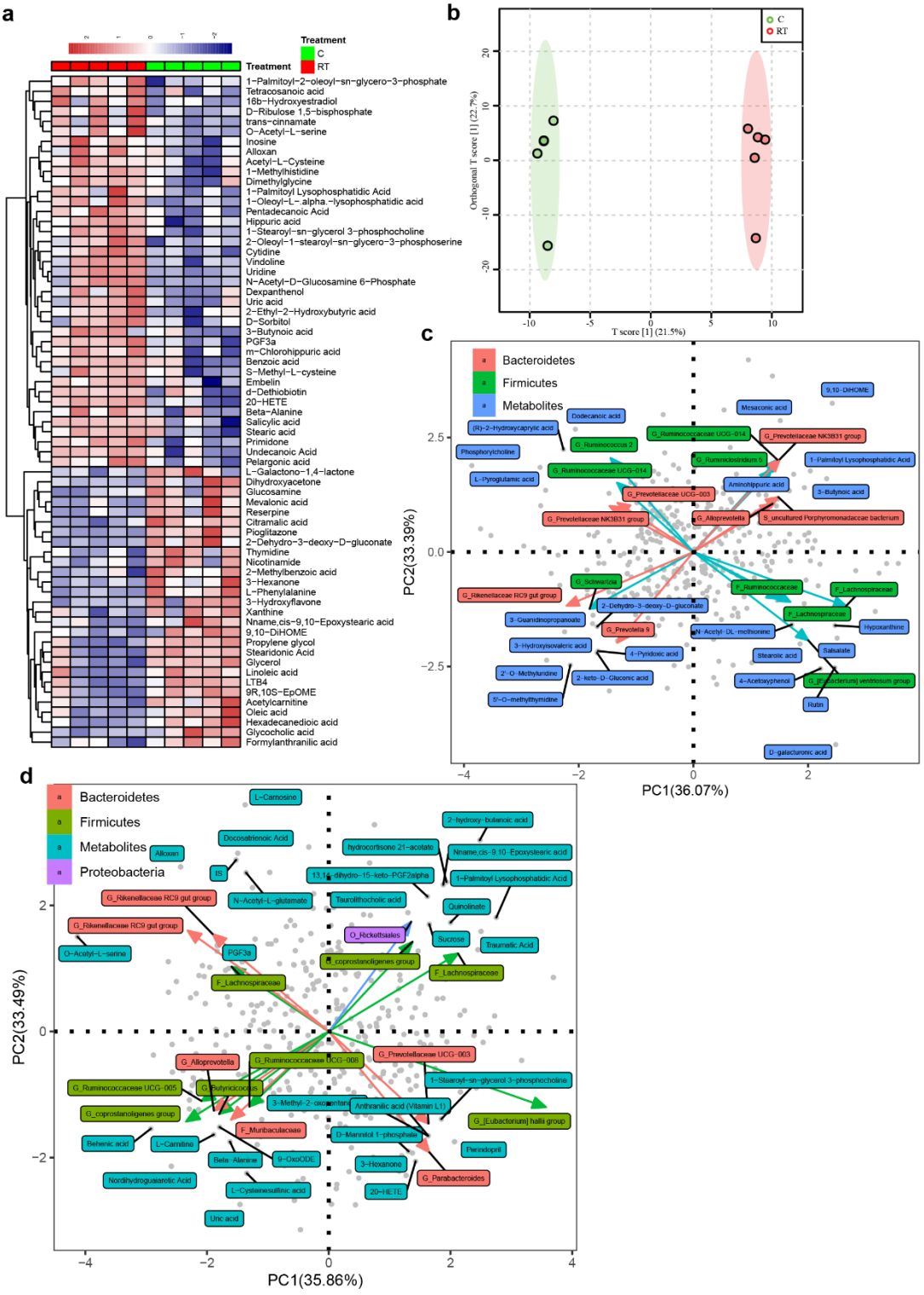



**Supplementary Figure 6** Significant bins after STAMP analysis between the RT and cold-stressed groups. The different bins **(a)** in the content and **(b)** on the epithelial surface of the cecum.


**Supplementary Figure 7** Cold exposure changes other functions of the microbiota in the cecum. Heatmap of the differences in the microbiota in the cecum at other functional levels **(a)** in the content and (**b)** on the epithelium surface of the cecum.

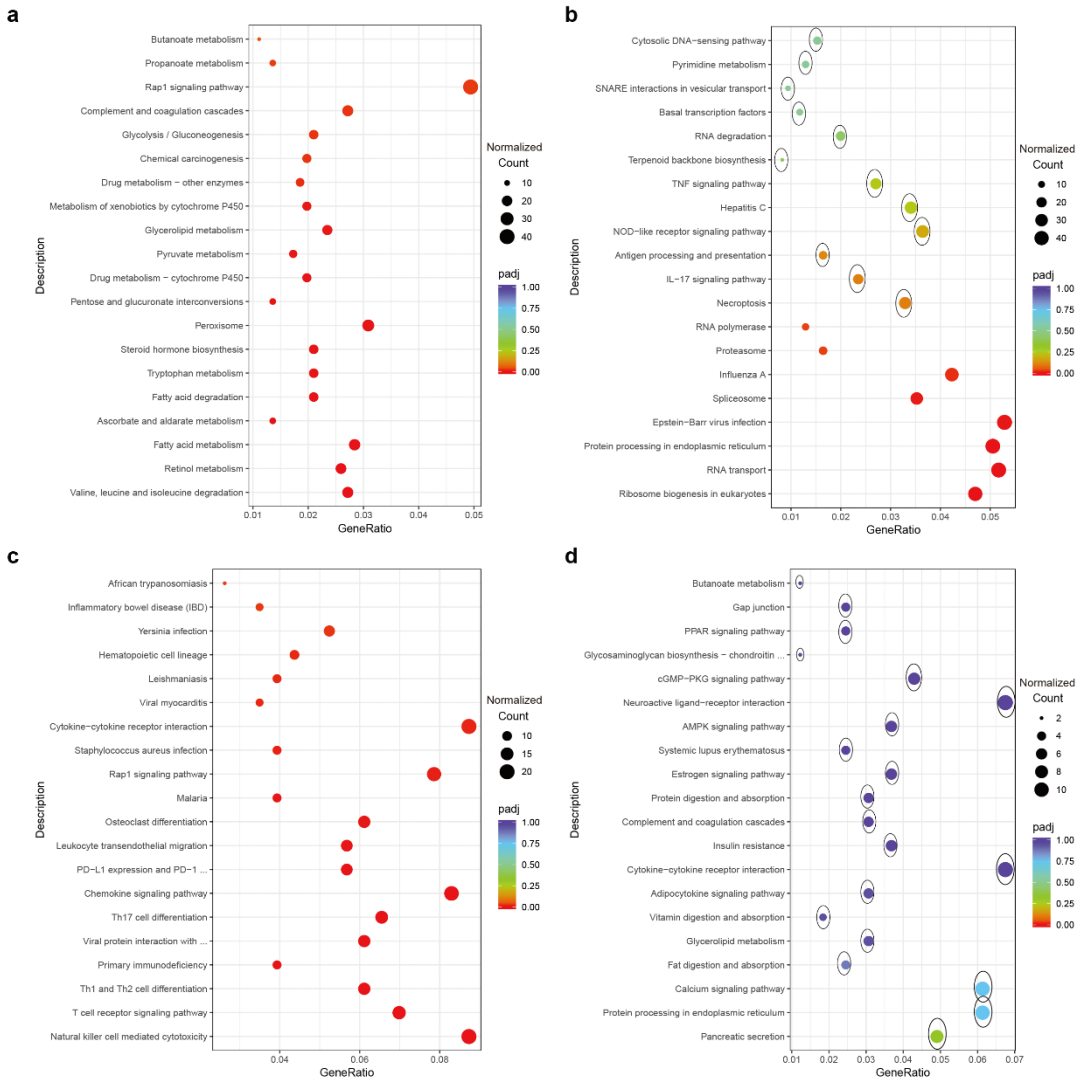



**Supplementary Figure 8** Cold exposure changes bile acid concentrations in serum (**a-d**) and SCFA levels (**e**) in the cecal content.

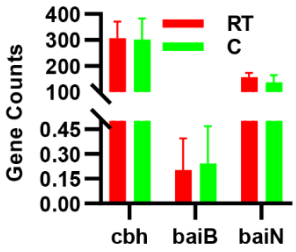



**Supplementary Figure 9** Different metabolomic alterations in the negative model in serum and strong correlation with the microbiome of the contents and epithelial surface of the cecum. **a** Heatmap of the differences in serum metabolomic metabolites under

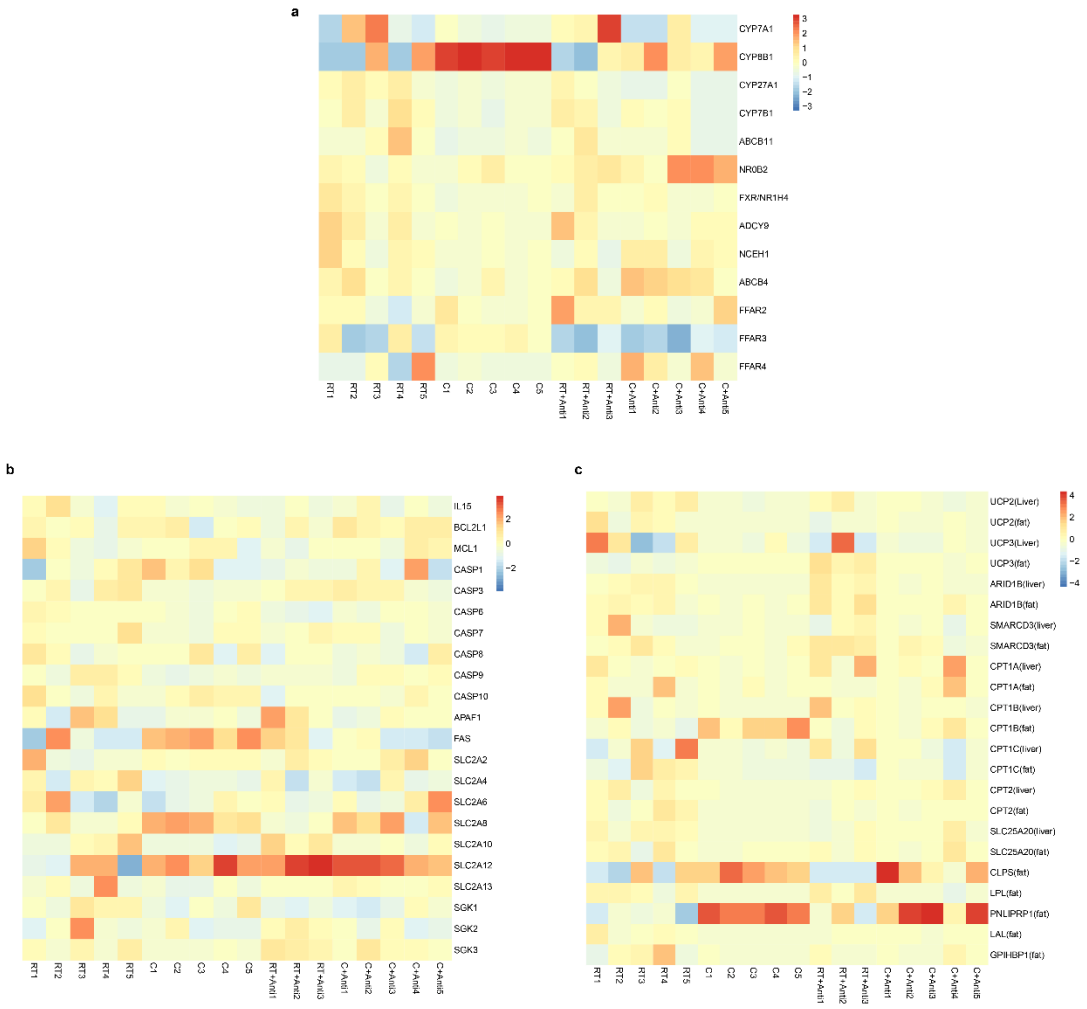
cold stress. **b** Partial least-squares discriminant analysis (PLS-DA) of microbial metabolites in serum. **c** Correlation of the metabolites in serum with the microbiome of the cecal contents. **d** Correlation of the metabolites with the microbiome of the epithelial surface of the cecum.



**Supplementary Figure 10** Summary of pathways identified at the RNA expression level by RNA seq. **a** Downregulated pathways under cold stress compared with RT in the liver of piglets. **b** Upregulated pathways under cold stress compared with RT in the liver of piglets. **c** Downregulated pathways under cold tress compared with RT in the fat of piglets. **d** Upregulated pathways under cold stress compared with RT in the fat of piglets. The circled nodes indicate results that are not significant (padj > 0.05), and unmarked nodes show significant findings (padj <0.05).




**Supplementary Figure 11** The counts of genes involved in secondary bile acid biosynthesis in the gut microbiota of the cecum content by metagenomic sequencing. Cbh, conjugated bile acid hydrolase, baiB, bile acid-coenzyme A ligase, baiN, 3-dehydro-bile acid delta (4,6)-reductase.




**Supplementary Figure 12** Heatmaps showing the variability between samples, (**a, b, c**) related to Figure 6.