Supplementary material

Summary

The supporting information includes 1 supplementary table and 14 supplementary figures.

Table S1. Primers for PCR or quantitative real-time PCR

Primers [1]	Primer sequences (5'-3')	Reference	Annealing temp.(°C)
Beta actin F	AGAGCGCAAGTACTCCGTGT	[1]	60
Beta actin R	ACATCTGCTGGAAGGTGGAC		
GAPDH F	TTTGCGTCAGTGTCATCG	[2]	60
GAPDH R	TGCTCTGCCTTGGGTAAT		
18S rRNA F	CCCACGGAATCGAGAAAGAG	[3]	60
18S rRNA R	TTGACGGAAGGGCACCA		
IL-1β F	TGAAGTGCCGCACCCAAAACCT	[1]	60
IL-1β R	CGGCTCCTCCTTTGCCACAATCA		
IL-6 F	CCCACCACAAATGCCGGCCT	[1]	60
IL-6 R	GAGGGAATGCCCGTGGACGG		
IL-8 F	ACTGGCTGT TGCCTTCTT	[4]	60
IL-8 R	CAGTT CTCTTCAAAAATATCTG		
IL-10 F	GTCCGACTCAACGAAGAAGG	[1]	60
IL-10 R	GCCAGGAAGATCAGGCAATA		
IFN-γ F	TCCAGCGCAAAGCCATCAGTG	[5]	60
IFN-γ R	ATGCTCTCTGGCCTTGGAACATAGT		
TNF-α F	CCACGCTCTTCTGCCTACTGC	[4]	60
TNF-α R	GCTGTCCCTCGGCTTTGAC		
Mucin-1 F	GGTACCCGGCTGGGGCATTG	[1]	60
Mucin-1 R	GGTAGGCATCCCGGGTCGGA		
Mucin-2 F	CTGCTCCGGGTCCTGTGGGA	[1]	60
Mucin-2 R	CCCGCTGGCTGGTGCGATAC		
Occludin F	ATGCTTTCTCAGCCAGCG TA	[4]	60
Occludin R	AAG GTTCCATAGCCTCGGTC		
ZO-1 F	GAGGATGGTCACACCGTGGT	[4]	60
ZO-1 R	GGAGGATGCTGTTGTCTCGG		
gE part F	GGCTTCCACTCGCAGCTCTT	The study	
gE part R	CGCACGTCATCACGAAGGAG		
gE-Sybr-F	GGTGTTTGCATAATTTTGTGGGTGG	[6]	60
gE-Sybr-R	GAAAGGGCCGCATGGTCTCA		

[1]*IL*, interleukin; *IFN-γ*, interferon-γ; *TNF-α*, tumor necrosis factor-α; *ZO-1*, zonula occludens-1.

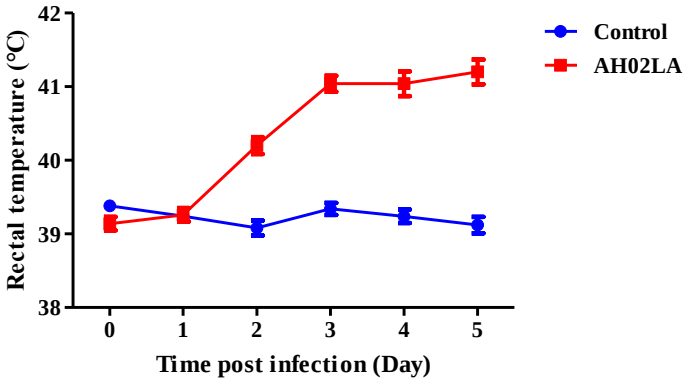

Figure S1. Rectal temperatures post intranasal infection with pseudorabies virus (PRV) AH02LA stain. Average temperatures of five piglets of each group were taken for comparison. Error bars represent the standard deviations.

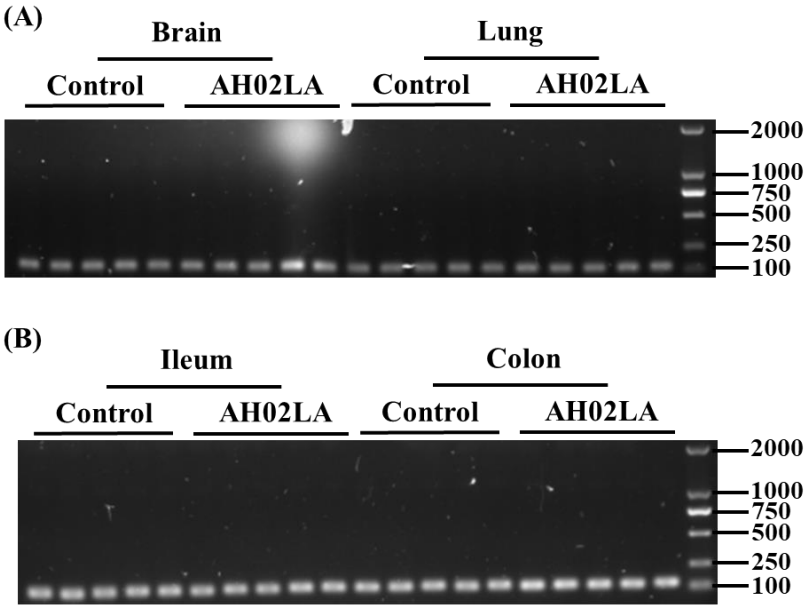

Figure S2. Detection of DNA in brain, lung, ileum and colon of piglets. The total DNAs from brain (A), lung (A), ileum (B) and colon (B) were amplified by PCR with a pair of specific primers for *18S rRNA* gene and analyzed by electrophoresis. M: marker DL2000.

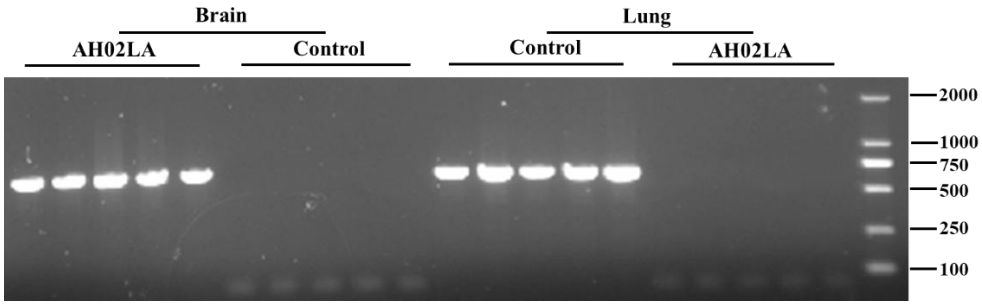

Figure S3. Detection of pseudorabies virus (PRV) DNA in the brain and lung of piglets infected PRV AH02LA. The total DNAs from brain and lung were amplified by PCR with a pair of specific primers for *gE* gene and analyzed by electrophoresis. M: marker DL2000.

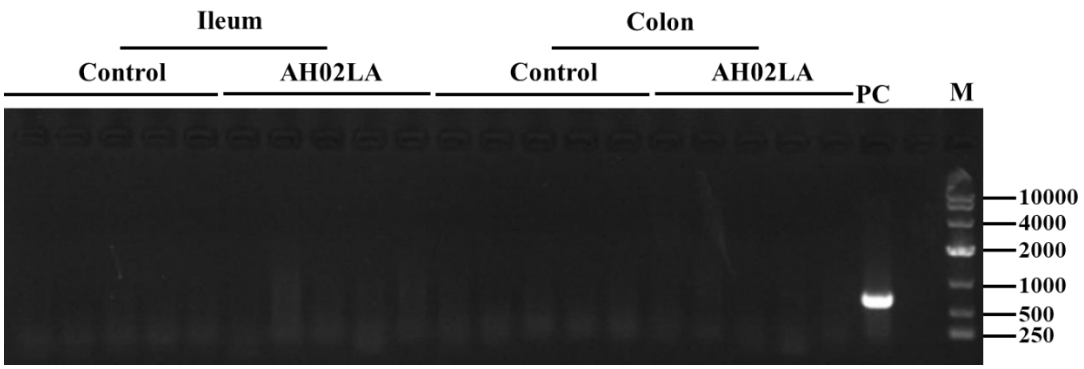

Figure S4. Detection of pseudorabies virus (PRV) DNA in the ileal and colonic mucosa of piglets infected PRV AH02LA. The total DNAs from ileal and colonic mucosa were amplified by PCR with a pair of specific primers for gE gene and analyzed by electrophoresis. PC: positive control, DNA from brain of piglets infected AH02LA. M: marker DL10000.

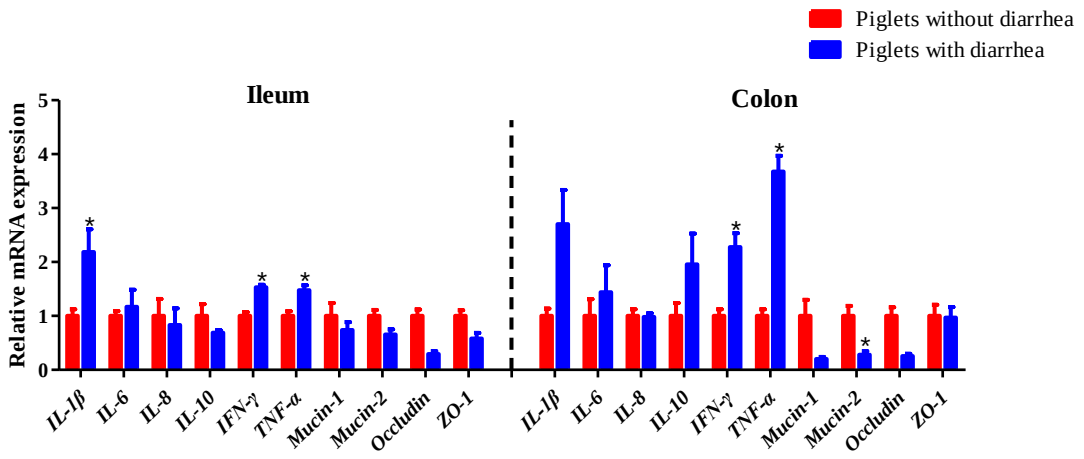

Figure S5. The mRNA expression of genes in the ileal and colonic mucosa of piglets with diarrhea (n=3) and without diarrhea (n=7). The values are expressed as the means ± SEM. Asterisks indicate statistical differences between different group. *$P < 0.05$. The β-actin mRNA level was used to normalize the relative amount of each studied mRNA, and the $2^{-\Delta\Delta Ct}$ method was used to analyze the data. *IL*, interleukin; *IFN-γ*, interferon-γ; *TNF-α*, tumor necrosis factor-α; *ZO-1*, zonula occludens-1.

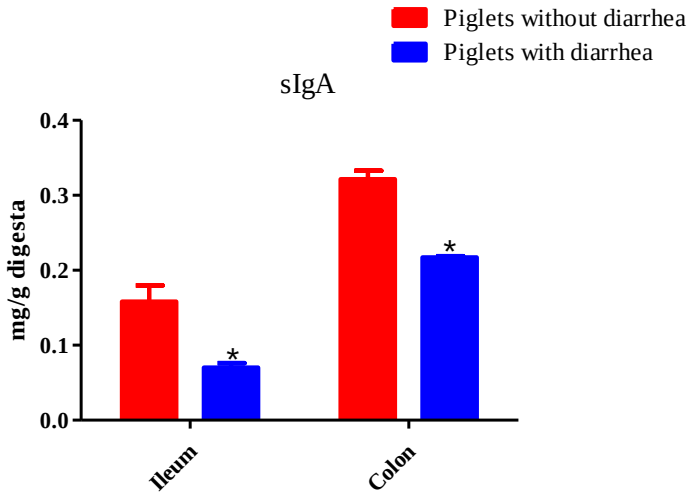

Figure S6. The concentration of secretory immunoglobulin A (sIgA) in the ileal and colonic digesta of piglets with diarrhea (n=3) and without diarrhea (n=7). The values are expressed as the means ± SEM. Asterisks indicate statistical differences between different group. *$P < 0.05$.

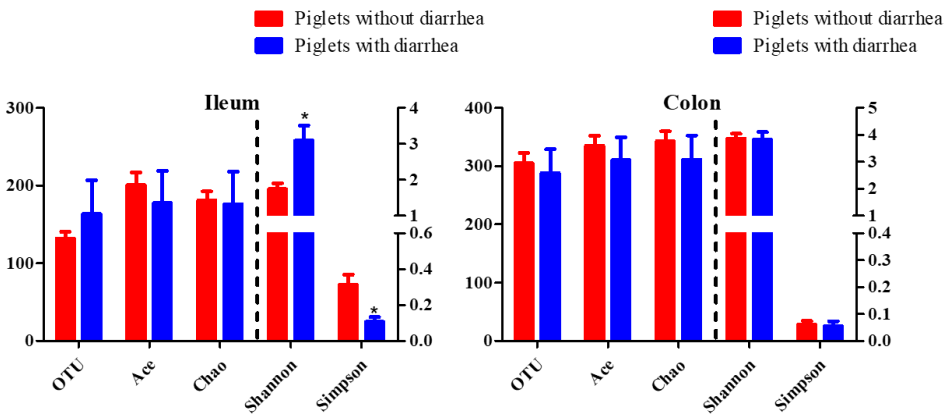

Figure S7. Diversity of ileal and colonic bacterial community in piglets with diarrhea (n=3) and without diarrhea (n=7). The values are expressed as the means ± SEM. Asterisks indicate statistical differences between different group. *$P < 0.05$.

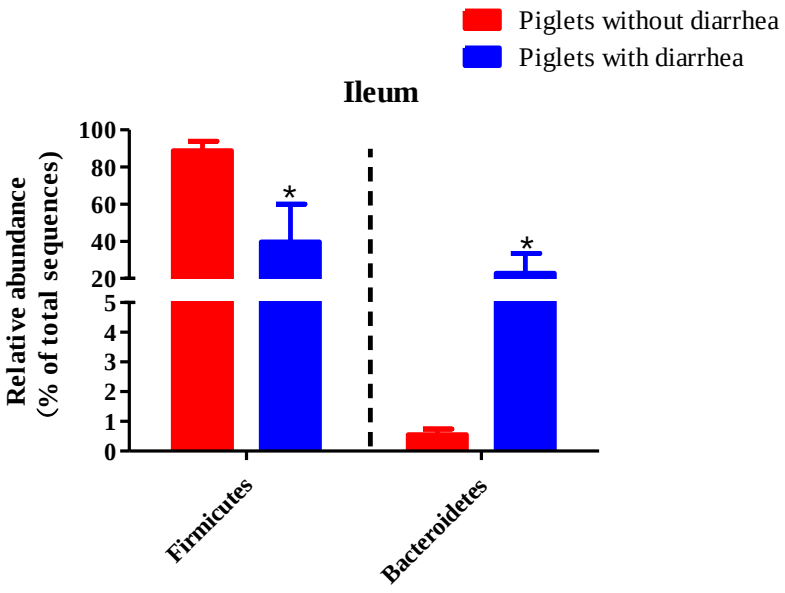

Figure S8. Significantly changed phylum in ileal and colonic digesta of piglets with diarrhea (n=3) and without diarrhea (n=7). The values are expressed as the means ± SEM. Asterisks indicate statistical differences between different group. *$P < 0.05$.

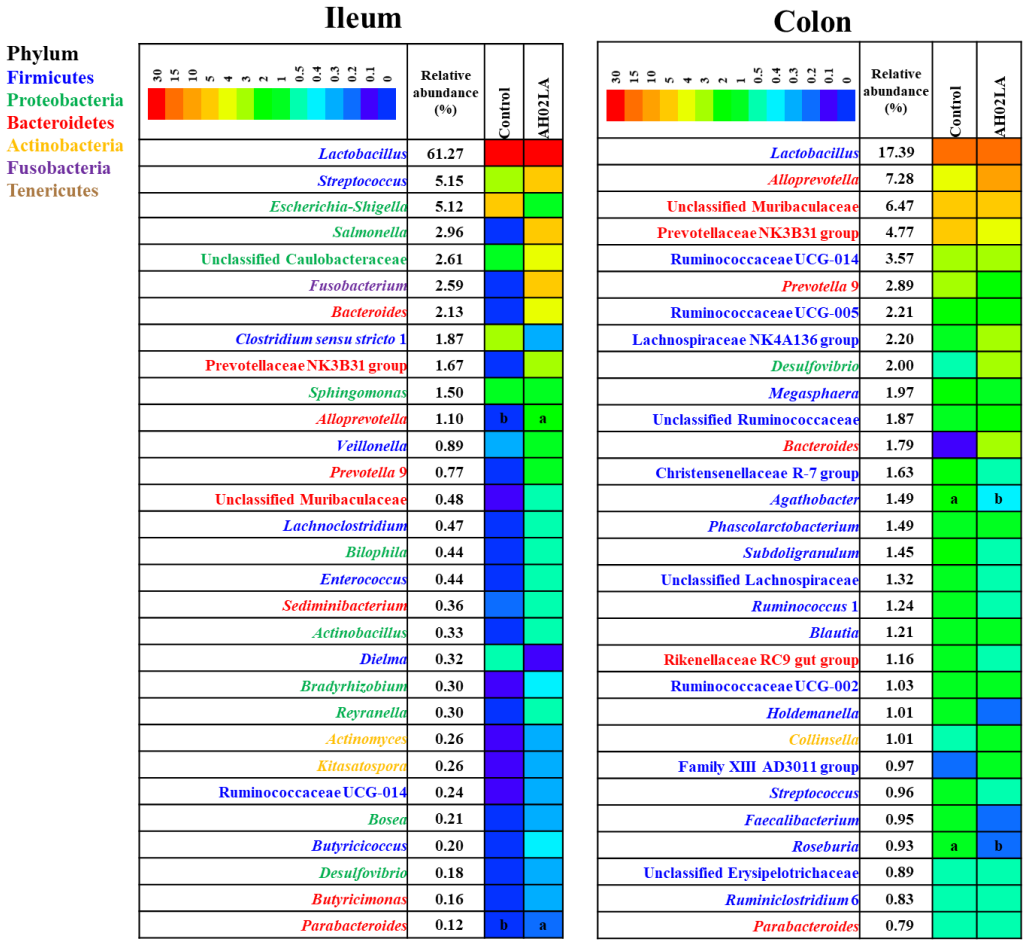

Figure S9. Heatmap of dominant genus in the ileum and colon of piglets with control and AH02LA groups. a, b means in a row with different superscripts differ. (Mann–Whitney U test and a false discovery rate < 0.05).

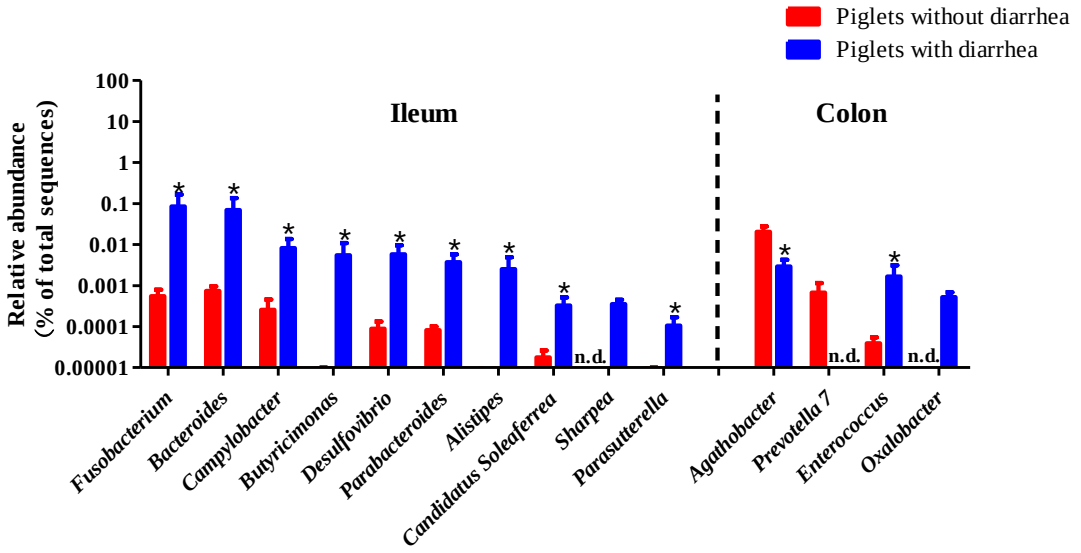

Figure S10. Significantly changed genera in the ileal and colonic digesta of piglets with diarrhea (n=3) and without diarrhea (n=7). The values are expressed as the means ± SEM. Asterisks indicate statistical differences between different group. *$P < 0.05$. n.d., no sequence detected.

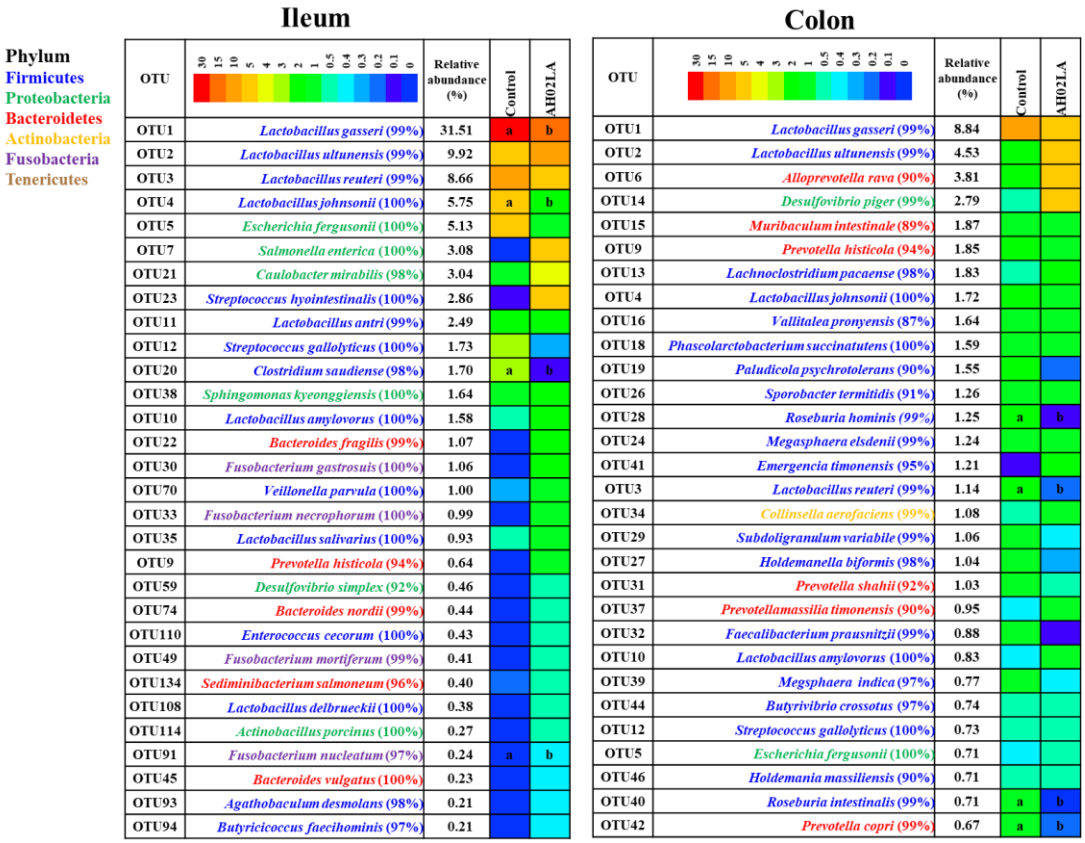

Figure S11. Heatmap of dominant OTUs in the ileum and colon of piglets with control and AH02LA groups. a,b means in a row with different superscripts differ. (Mann–Whitney U test and a false discovery rate < 0.05)

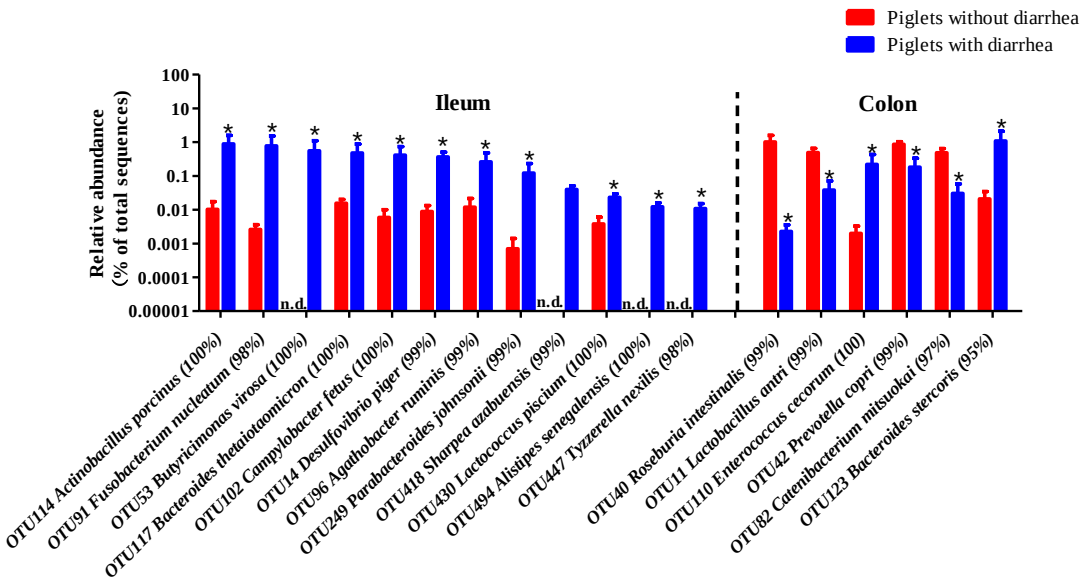

Figure S12. Significantly changed bacteria OTUs in the ileal and colonic digesta of piglets with diarrhea (n=3) and without diarrhea (n=7). The values are expressed as the means ± SEM. Asterisks indicate statistical differences between different group. *$P < 0.05$. n.d., no sequence detected.

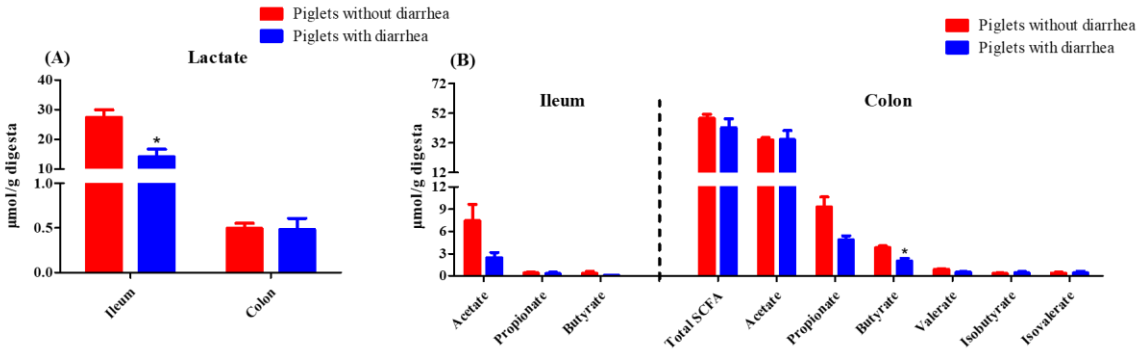

Figure S13. The concentrations of lactate (A) and SCFA (B) in the ileal and colonic digesta of piglets with diarrhea (n=3) and without diarrhea (n=7). The values are expressed as the means ± SEM. Asterisks indicate statistical differences between different group. *$P < 0.05$. SCFA, short-chain fatty acid.

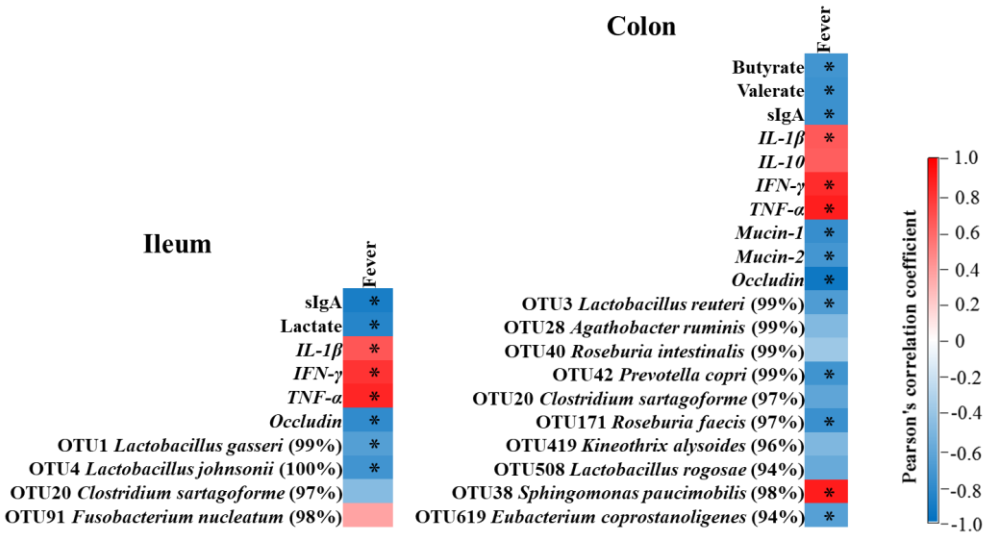

Figure S14. Correlation analysis between rectal temperature with immune markers, bacteria or bacterial fermentation products in the ileum and the colon. The red represents a significant positive correlation, and the blue represents a significant negative correlation. *P < 0.05. *IL*, interleukin; *IFN-γ*, interferon-γ; *TNF-α*, tumor necrosis factor-α; sIgA, secretory immunoglobulin A.

References

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