

## Summary

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

Primers <sup>1</sup> Primer sequences (5'-3')		Reference	Annealing temp.(°C)		
Beta actin F AGAGCGCAAGTACTCCGTGT		[4]	(0)		
Beta actin R	ACATCTGCTGGAAGGTGGAC	[1]	60		
GAPDH F	TTTGCGTCAGTGTCATCG	[0]	(0)		
GAPDH R	TGCTCTGCCTTGGGTAAT	[2]	60		
18S rRNA F	CCCACGGAATCGAGAAAGAG	[2]	(0)		
18S rRNA R	TTGACGGAAGGGCACCA	[3]	60		
<i>IL-1β</i> F	TGAAGTGCCGCACCCAAAACCT	[1]	60		
IL-1 $\beta$ R	CGGCTCCTCCTTTGCCACAATCA	[1]			
<i>IL-6</i> F	CCCACCACAAATGCCGGCCT	[1]	(0)		
<i>IL-6</i> R	GAGGGAATGCCCGTGGACGG	[1]	60		
<i>IL-8</i> F	ACTGGCTGT TGCCTTCTT	[4]	(0)		
<i>IL-8</i> R	CAGTT CTCTTCAAAAATATCTG	[4]	60		
<i>IL-10</i> F	GTCCGACTCAACGAAGAAGG	[1]	(0		
<i>IL-10</i> R	GCCAGGAAGATCAGGCAATA	[1]	60		
IFN-γ F	TCCAGCGCAAAGCCATCAGTG	[5]	(0		
<i>IFN-γ</i> R	ATGCTCTCTGGCCTTGGAACATAGT	[5]	00		
<i>TNF-α</i> F	CCACGCTCTTCTGCCTACTGC	[4]	60		
<i>TNF-α</i> R	GCTGTCCCTCGGCTTTGAC	[4]	60		
Mucin-1 F	GGTACCCGGCTGGGGCATTG	[1]	(0)		
Mucin-1 R	GGTAGGCATCCCGGGTCGGA	[1]	60		
Mucin-2 F	CTGCTCCGGGTCCTGTGGGA	[1]	(0		
Mucin-2 R	CCCGCTGGCTGGTGCGATAC	[1]	60		
Occludin F	ATGCTTTCTCAGCCAGCG TA	[4]	(0		
Occludin R	AAG GTTCCATAGCCTCGGTC	[4]	60		
<i>ZO-1</i> F	GAGGATGGTCACACCGTGGT	[4]	(0		
<i>ZO-1</i> R	GGAGGATGCTGTTGTCTCGG	[4]	60		
gE part F	GGCTTCCACTCGCAGCTCTT				
gE part R	CGCACGTCATCACGAAGGAG	The study			
gE-Sybr-F	GGTGTTTGCATAATTTTGTGGGTGG	[7]	(0)		
gE-Sybr-R	GAAAGGGCCGCATGGTCTCA	[6]	60		

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

<sup>1</sup>*IL*, interleukin; *IFN*- $\gamma$ , interferon- $\gamma$ ; *TNF*- $\alpha$ , tumor necrosis factor- $\alpha$ ; *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  $\pm$  SEM. Asterisks indicate statistical differences between different group. \**P* < 0.05. The  $\beta$ -actin mRNA level was used to normalize the relative amount of each studied mRNA, and the2- $\Delta\Delta$ Ct method was used to analyze the data. *IL*, interleukin; *IFN-* $\gamma$ , interferon- $\gamma$ ; *TNF-* $\alpha$ , tumor necrosis factor- $\alpha$ ; *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  $\pm$  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  $\pm$  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  $\pm$  SEM. Asterisks indicate statistical differences between different group. \**P* < 0.05.

	Ileum					Colon					
Phylum Firmicutes Proteobacteria Bacteroidetes	30 15 10 10 10 1 1 1 1 0 3 0 0 3 0 10	Relative abundance (%)	Control	AH02LA		30 15 10 10 2 2 1 1 1 0.5 0.5 0.5 0.5 0.5 0.2	Relative abundance (%)	Control	AH02LA		
Actinobacteria	Lactobacillus	61.27				Lactobacillus	17.39				
Fusobacteria	Streptococcus	5.15				Alloprevotella	7.28				
Tenericutes	Escherichia-Shigella	5.12				Unclassified Muribaculaceae	6.47				
	Salmonella	2.96				Prevotellaceae NK3B31 group	4.77				
	Unclassified Caulobacteraceae	2.61				Ruminococcaceae UCG-014	3.57				
	Fusobacterium	2.59				Prevotella 9	2.89				
	Bacteroides	2.13				Ruminococcaceae UCG-005	2.21				
	Clostridium sensu stricto 1	1.87				Lachnospiraceae NK4A136 group	2.20				
	PrevotellaceaeNK3B31 group	1.67				Desulfovibrio	2.00				
	Sphingomonas	1.50				Megasphaera	1.97				
	Alloprevotella	1.10	b	а		Unclassified Ruminococcaceae	1.87				
	Veillonella	0.89				Bacteroides	1.79				
	Prevotella 9	0.77				Christensenellaceae R-7 group	1.63				
	Unclassified Muribaculaceae	0.48				Agathobacter	1.49	a	b		
	Lachnoclostridium	0.47				Phascolarctobacterium	1.49				
	Bilophila	0.44				Subdoligranulum	1.45				
	Enterococcus	0.44				Unclassified Lachnospiraceae	1.32				
	Sediminibacterium	0.36				Ruminococcus 1	1.24				
	Actinobacillus	0.33				Blautia	1.21				
	Dielma	0.32				Rikenellaceae RC9 gut group	1.16				
	Bradvrhizobium	0.30				Ruminococcaceae UCG-002	1.03				
	Revranella	0.30				Holdemanella	1.01				
	Actinomyces	0.26				Collinsella	1.01				
	Kitasatospora	0.26				Family XIII AD3011 groun	0.97				
	Ruminococcaceae UCG-014	0.24				Streptococcus	0.96				
	Bosen	0.21				Faecalibacterium	0.95				
	Butyricicoccus	0.20				Roseburia	0.93	а	b		
	Desulfavibria	0.18				Unclassified Erysipelotrichaceae	0.89				
	Butyricimonas	0.16				Ruminiclostridium 6	0.83				
	Parabacteroides	0.12	b	a		Parabacteroides	0.79				

**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  $\pm$  SEM. Asterisks indicate statistical differences between different group. \**P* < 0.05. n.d., no sequence detected.

Ileum							Colon					
Phylum Firmicutes Proteobacteria	οτυ	30 15 10 10 10 10 04 0.4 0.4 0.2 0.3	Relative abundance (%)	Control	AH02LA		οτυ	30 15 6 4 3 3 1 0 5 0 0 5 0 0 1 0 0	Relative abundance (%)	Control	AH02LA	
Actinobactoria	OTU1	Lactobacillus gasseri (99%)	31.51	а	b		OTU1	Lactobacillus gasseri (99%)	8.84			
Fusobacteria	OTU2	Lactobacillus ultunensis (99%)	9.92				OTU2	Lactobacillus ultunensis (99%)	4.53			
Tenericutes	OTU3	Lactobacillus reuteri (99%)	8.66				OTU6	Alloprevotella rava (90%)	3.81			
	OTU4	Lactobacillus johnsonii (100%)	5.75	a	b		OTU14	Desulfovibrio piger (99%)	2.79			
	OTU5	Escherichia fergusonii (100%)	5.13				OTU15	Muribaculum intestinale (89%)	1.87			
	OTU7	Salmonella enterica (100%)	3.08			1 [	OTU9	Prevotella histicola (94%)	1.85			
	OTU21	Caulobacter mirabilis (98%)	3.04			1 [	OTU13	Lachnoclostridium pacaense (98%)	1.83			
	OTU23	Streptococcus hyointestinalis (100%)	2.86			1 [	OTU4	Lactobacillus johnsonii (100%)	1.72			
	OTU11	Lactobacillus antri (99%)	2.49			[	OTU16	Vallitalea pronyensis (87%)	1.64			
	OTU12	Streptococcus gallolyticus (100%)	1.73			1 [	OTU18	Phascolarctobacterium succinatutens (100%)	1.59			
	OTU20	Clostridium saudiense (98%)	1.70	a	b		OTU19	Paludicola psychrotolerans (90%)	1.55			
	OTU38	Sphingomonas kyeonggiensis (100%)	1.64			[	OTU26	Sporobacter termitidis (91%)	1.26			
	OTU10	Lactobacillus amylovorus (100%)	1.58			[	OTU28	Roseburia hominis (99%)	1.25	а	b	
	OTU22	Bacteroides fragilis (99%)	1.07			[	OTU24	Megasphaera elsdenii (99%)	1.24			
	OTU30	Fusobacterium gastrosuis (100%)	1.06			[	OTU41	Emergencia timonensis (95%)	1.21			
	OTU70	Veillonella parvula (100%)	1.00			[	OTU3	Lactobacillus reuteri (99%)	1.14	а	b	
	OTU33	Fusobacterium necrophorum (100%)	0.99				OTU34	Collinsella aerofaciens (99%)	1.08			
	OTU35	Lactobacillus salivarius (100%)	0.93				OTU29	Subdoligranulum variabile (99%)	1.06			
	OTU9	Prevotella histicola (94%)	0.64			[	OTU27	Holdemanella biformis (98%)	1.04			
	OTU59	Desulfovibrio simplex (92%)	0.46			1 [	OTU31	Prevotella shahii (92%)	1.03			
	OTU74	Bacteroides nordii (99%)	0.44			[	OTU37	Prevotellamassilia timonensis (90%)	0.95			
	OTU110	Enterococcus cecorum (100%)	0.43			1 [	OTU32	Faecalibacterium prausnitzii (99%)	0.88			
	OTU49	Fusobacterium mortiferum (99%)	0.41			1 [	OTU10	Lactobacillus amylovorus (100%)	0.83			
	OTU134	Sediminibacterium salmoneum (96%)	0.40			1 [	OTU39	Megsphaera indica (97%)	0.77			
	OTU108	Lactobacillus delbrueckii (100%)	0.38			1 [	OTU44	Butyrivibrio crossotus (97%)	0.74			
	OTU114	Actinobacillus porcinus (100%)	0.27			1 [	OTU12	Streptococcus gallolyticus (100%)	0.73			
	OTU91	Fusobacterium nucleatum (97%)	0.24	а	b	1 [	OTU5	Escherichia fergusonii (100%)	0.71			
	OTU45	Bacteroides vulgatus (100%)	0.23				OTU46	Holdemania massiliensis (90%)	0.71			
	OTU93	Agathobaculum desmolans (98%)	0.21				OTU40	Roseburia intestinalis (99%)	0.71	а	b	
	OTU94	Butyricicoccus faecihominis (97%)	0.21			] [	OTU42	Prevotella copri (99%)	0.67	а	b	

**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  $\pm$  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  $\pm$  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-* $\gamma$ , interferon- $\gamma$ ; *TNF-* $\alpha$ , tumor necrosis factor- $\alpha$ ; sIgA, secretory immunoglobulin A.

## References

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