

**Supporting information for: Effects of transgenic Bt rice expressing
the Cry1Ab protein on faecal microbiome and pathology of highly
inbred Wuzhishan pigs after two generations of feeding**

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Supplementary Materials

Figure Legends for Figure S1-S6

Figure S1. OTUs Rank abundance curve of the female and male pigs from f0 and f1 generation

Figure S2. Venn diagrams of OTUs distribution of female and male pigs in the f0 and f1 generation

Figure S3. Heatmap hierarchical cluster analysis of female and male pigs in the f0 generation. A,

Heatmap of f0FG and f0ZG groups. B, Heat map of f0FM and f0ZM. The relative levels of abundance are depicted visually from red to blue; blue represents the lowest abundance (min = -5), whereas red (max = 15) represents the highest level of abundance.

Figure S4. Heatmap hierarchical cluster analysis of female and male pigs in the f1 generation. A, Heatmap of f1FG and f1ZG groups. B, Heatmap of f0FM and f0ZM. The relative levels of abundance are depicted visually from red to blue; blue represents the lowest abundance (min = -5), whereas red (max = 15) represents the highest level of abundance.

Figure S5. Microbial community distribution of female and male pigs from different groups in f0 generation. A, C: the relative abundance of the fecal microbiota from f0FG vs f0ZG and f0FM vs f0ZM at the phylum level. B, D: the relative abundance of the fecal microbiota from f0FG vs f0ZG and f0FM vs f0ZM at the family level.

Figure S6. Microbial community distribution of female and male pigs from different groups in f1 generation. A, C: the relative abundance of the fecal microbiota from f1FG vs f1ZG and f1FM vs f1ZM at the phylum level. B, D: the relative abundance of the fecal microbiota from f1FG vs f1ZG and f1FM vs f1ZM at the family level.

Table S1. Comparison of the Nutrient Content of Bt and MH86 Rice (as-is basis)

Nutritional ingredient	Bt	MH86
moisture (%)	11.54	11.78
crude protein (%)	6.44	7.44
crude fat (%)	0.60	0.80
NDF (%)	9.23	10.77
starch (%)	74.12	73.13
crude ash (%)	0.40	0.40
calcium (%)	0.01	0.01
phosphorus (%)	0.09	0.11
zinc (mg/kg)	16.00	17.00
manganese (mg/kg)	9.00	9.30
copper (mg/kg)	2.20	4.80
iron (mg/kg)	10.00	4.60
lysine (mg/kg)	0.24	0.26
methionine (mg/kg)	0.23	0.25
*other amino acids (%)	6.02	6.94

Note: * Including: tryptophan, aspartate, threonine, serine, glutamate, proline, glycine, lactamine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, and arginine.

Table S2. Diet Formulas and Nutrient Composition for the GM and Non-GM Groups at Different Sstage Per the NRC 2012 in Two Generations (as-is basis, kg 100 kg⁻¹)

Different stage	Piglet	Fattening period	Early gestation period (80 days)	Latter period of gestation(35 days)	Lactation						
Group	f1Z	f1F	f0Z/f1Z	f0F/f1F	f0Z/f1Z	f0F/f1F	f0Z/f1Z	f0F/f1F	f0Z/f1Z	f0F/f1F	
Ingredients						%					
Rice	66	66	62	62	56	56	57	57	60	60	

Soybean meal	22	22	15	15	20	20	20	20	24	24
Wheat bran	5	5	17	17	20	20	18	18	9	9
Fish Flour	2	2	2	2	\	\	\	\	2	2
Soybean oil	\	\	\	\	\	\	1	1	1	1
Granulated sugar	1	1	\	\	\	\	\	\	\	\
Vitamin-mineral premix*	4	4	4	4	4	4	4	4	4	4
Nutrient composition/%										
DE (Kcal/kg) ^a	3544	3542	3513	3511	3387	3385	3459	3457	3589	3587
CP ^b	21.75	21.43	20.89	20.60	20.45	20.23	22.32	22.25	23.25	23.18
Ca ^c	0.94	0.93	0.86	0.83	0.79	0.75	0.85	0.82	0.93	0.92
P ^d	0.95	0.92	0.63	0.63	0.56	0.55	0.78	0.74	0.83	0.80
STTD P ^e	0.27	0.27	0.35	0.34	0.31	0.31	0.29	0.28	0.37	0.35
Lys	1.90	1.84	1.65	1.57	1.63	1.61	1.76	1.75	1.83	1.81
SID Lys ^f	0.98	0.95	0.78	0.73	0.82	0.80	0.89	0.82	0.92	0.91
Met	0.66	0.64	0.58	0.53	0.64	0.62	0.60	0.56	0.70	0.61
dig Met ^g	0.40	0.40	0.45	0.45	0.51	0.51	0.49	0.43	0.53	0.49
Thr	1.15	1.13	1.05	1.02	1.10	1.08	1.18	1.15	1.23	1.19
dig Thr ^h	0.63	0.61	0.64	0.63	0.59	0.52	0.66	0.65	0.70	0.62
Trp	0.35	0.35	0.28	0.25	0.33	0.32	0.43	0.36	0.49	0.40
dig Trp ⁱ	0.26	0.26	0.27	0.24	0.28	0.25	0.26	0.26	0.33	0.32

Note: * In mg/kg diet: Vit.A: 10,000(IU); Vit.D3: 2,000(IU); Vit.E:24(IU); Vit.K: 2; Vit.B2: 6;

Vit.B6: 4; Vit.B12:0.024; Panthothenate: 20; Niacin: 30; Biotin: 0.4; Folic acid: 3.6; Zn: 120; Fe:

96; Mn: 40; Cu: 8; I: 0.56; Se: 0.24; Acetylcholine: 2.0. ^aDE: Digestible energy (kcal kg⁻¹); ^bCP:

Crude protein; ^cCa: Calcium; ^dP: Phosphorus; ^eSTTD P: Standardized total tract digestive

phosphorus; ^fSID lys: Standard ileum digestive lysine; ^gdig Met: Digestive methionine; ^hdig Thr:

Digestive threonine; ⁱdig Trp: Digestive tryptophan.

Table S3. The Statistics of OTU and Reads Carried out with the Threshold Species

Diversity Index of Female and Male in Each Group from f0 and f1 Generation

Groups	SeqsNum ^a	OTUsNum ^b	EvenOTUs Num ^c	Simpson ^d	Shannon ^e	Chao1 ^f	Good's coverage ^g
f0 generation							
f0FG	104851 ± 11992	3357 ± 120	3022 ± 132	0.985 ± 0.005	8.09 ± 0.29	4816 ± 193	0.984 ± 0.001
f0ZG	104498 ± 13612	3500 ± 418	3131 ± 283	0.987 ± 0.003	8.15 ± 0.32	4966 ± 442	0.983 ± 0.001
P	0.961	0.404	0.381	0.423	0.709	0.435	0.457
f0FM	107157 ± 8110	3346 ± 626	2972 ± 538	0.973 ± 0.041	7.85 ± 1.16	4786 ± 889	0.984 ± 0.002
f0ZM	110304 ± 19120	3801 ± 495	3344 ± 243	0.989 ± 0.002	8.47 ± 0.27	5353 ± 468	0.982 ± 0.001
P	0.698	0.179	0.149	0.355	0.229	0.190	0.310
f1 generation							
f1FG	462649 ± 92951	10054 ± 1041	7341 ± 355	0.990 ± 0.004	8.87 ± 0.42	13075 ± 559	0.985 ± 0.001
f1ZG	368025 ± 78603	9613 ± 1312	7728 ± 825	0.993 ± 0.001	9.18 ± 0.24	13842 ± 1853	0.984 ± 0.001
P	0.100	0.559	0.358	0.125	0.160	0.399	0.340
f1FM	462912 ± 127736	10254 ± 904	7519 ± 647	0.988 ± 0.005	8.87 ± 0.33	13611 ± 1003	0.984 ± 0.001
f1ZM	556919 ± 318589	9966 ± 2005	6826 ± 276	0.978 ± 0.012	8.41 ± 0.46	12525 ± 454	0.985 ± 0.001
P	0.540	0.766	0.008	0.149	0.07	0.009	0.027

Note: The results are presented as mean ± SD, f0FG group, n=7; f0ZG group, n=6; f1FG group,

n=5; f1ZG group, n=5; f0FM group, n=7; f0ZM group, n=6; f1FM group, n=6; f1ZM group, n=11

a. The original sequence number of each group.

b. The original OTUs number of each group, OTUs were determined based on 97% of 16S rRNA gene similarity.

c. The Standardized OTUs number of each group.

d. The probability that a random sample of two individuals in a community belongs to a different species. The index is no more than 1, and the larger the diversity, the greater the diversity of species in the community.

e. The index mainly reflects the number of community and the uniformity of individual distribution. The more uniform the individual distribution, the greater the index; If each individual belongs to a different species, the diversity index is the largest; If each individual belongs to the

same species, the diversity index is the smallest;

f. The abundance index of community microorganism mainly reflects the number of species in the ecosystem.

g. The coverage degree of the community microorganism, which is equal to 1 indicating that the sequencing data covers all the microorganisms in the community, and the maximum, is not greater than 1.

Table S4. The Relative Abundances of Genus in Four Groups from f0 and f1 Generation

Genus	Relative abundance		<i>P</i> value
	<i>f0 generation</i>		
	f0F	f0Z	
Oscillospira	0.098017	0.108442	0.571544
Prevotella	0.055038	0.047874	0.958979
Ruminococcus	0.016066	0.012971	0.328443
Comamonas	0.018846	1.04E-06	0.166569
Moryella	0.009402	0.007056	0.25782
Treponema	0.009191	0.005505	0.918053
CF231	0.003561	0.008075	0.017963*
Parabacteroides	0.005241	0.004689	0.99823
Lactobacillus	0.003518	0.00628	0.279923
Phascolarctobacterium	0.004363	0.003399	0.32836
Bacteroides	0.004652	0.002938	0.60695
Escherichia	0.003801	0.003216	0.606827
Blautia	0.003444	0.002178	0.738049
SMB53	0.001646	0.003619	0.001423**
Epulopiscium	0.000618	0.003361	0.625046
Akkermansia	0.000926	0.002506	0.122631
Streptococcus	0.001388	0.001515	0.938345
Others	0.0222	0.021409	0.918067
<i>f1 generation</i>			
	f1F	f1Z	
Oscillospira	0.070967	0.06971375	0.881018
Streptococcus	0.050229	0.04775317	0.907558
Prevotella	0.031966	0.04428712	0.360532
Lactobacillus	0.033431	0.01605944	0.219015
Treponema	0.015466	0.01303999	0.493731
Bacteroides	0.005957	0.01048523	0.277596
Phascolarctobacterium	0.006653	0.00664521	0.997047
Ruminococcus	0.005715	0.00502604	0.616706
p-75-a5	0.00544	0.00467868	0.625601

Sphaerochaeta	0.006058	0.00409692	0.265502
Parabacteroides	0.006169	0.00339756	0.165285
CF231	0.00255	0.00500433	0.215666
Escherichia	0.001217	0.00466938	0.015815*
Butyricicoccus	0.002614	0.00136512	0.389738
Others	0.026568	0.02271551	0.205305

Note: * indicated significant difference; ** indicated extreme difference.

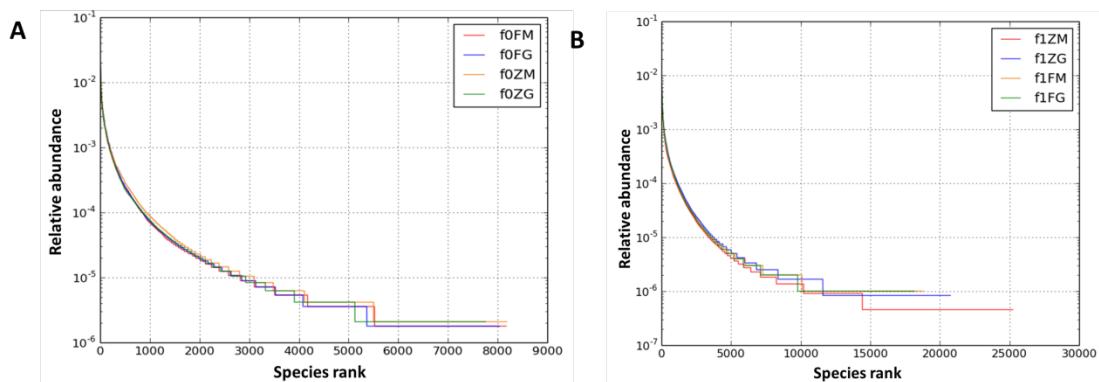


Figure S1

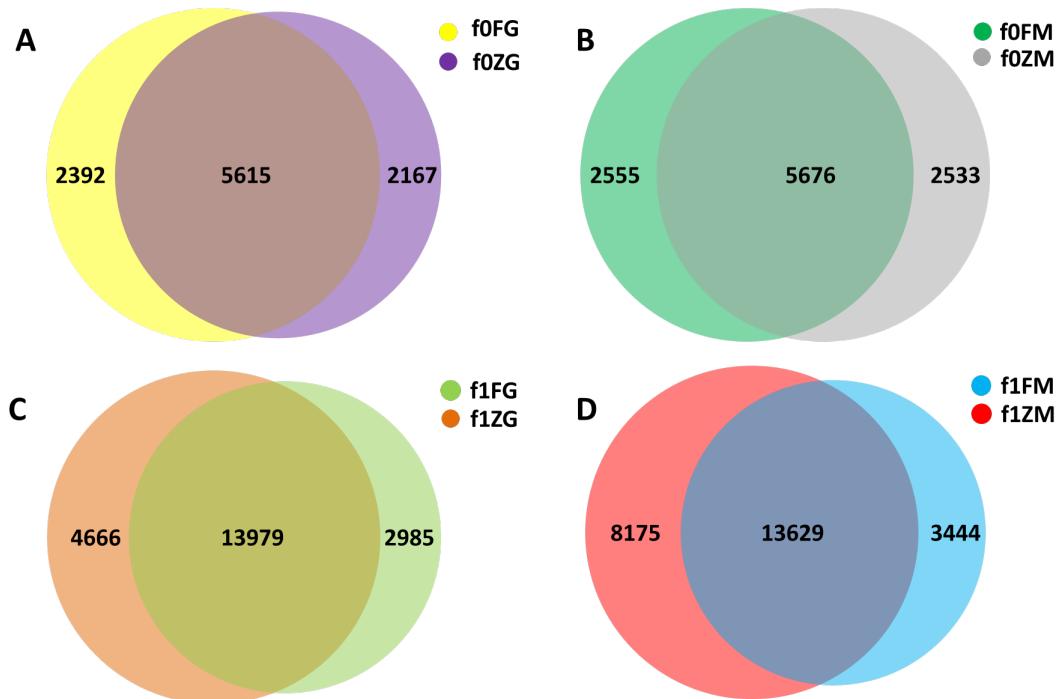


Figure S2

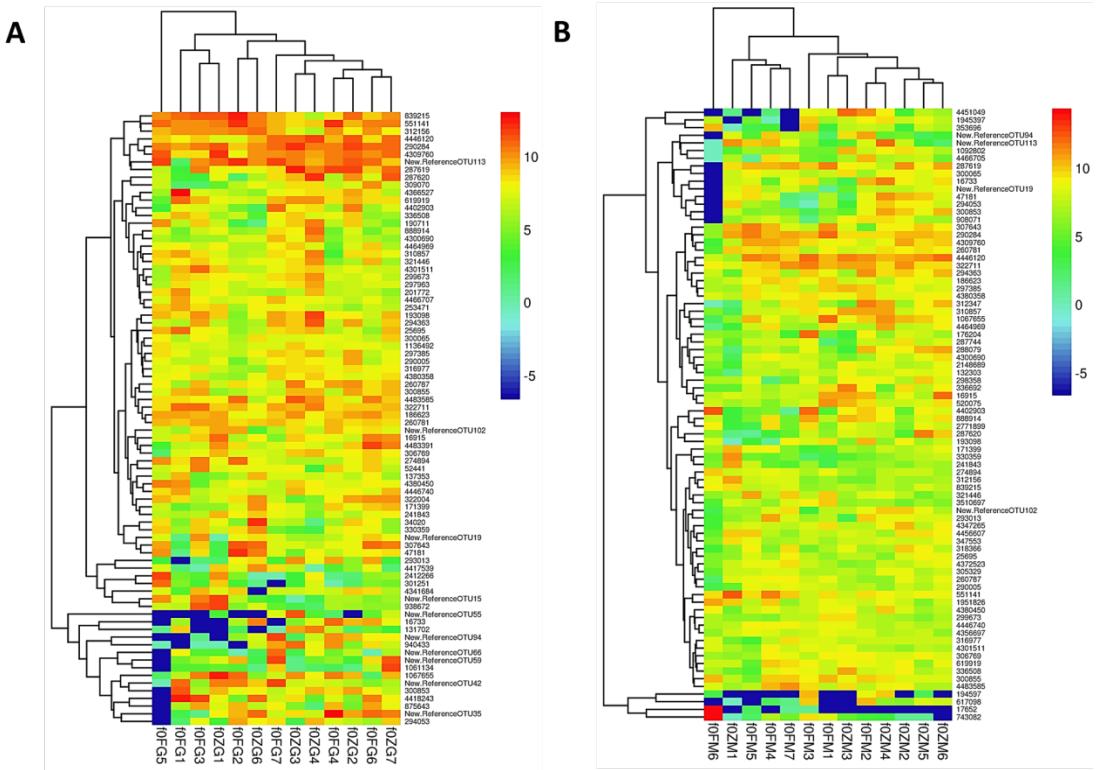


Figure S3

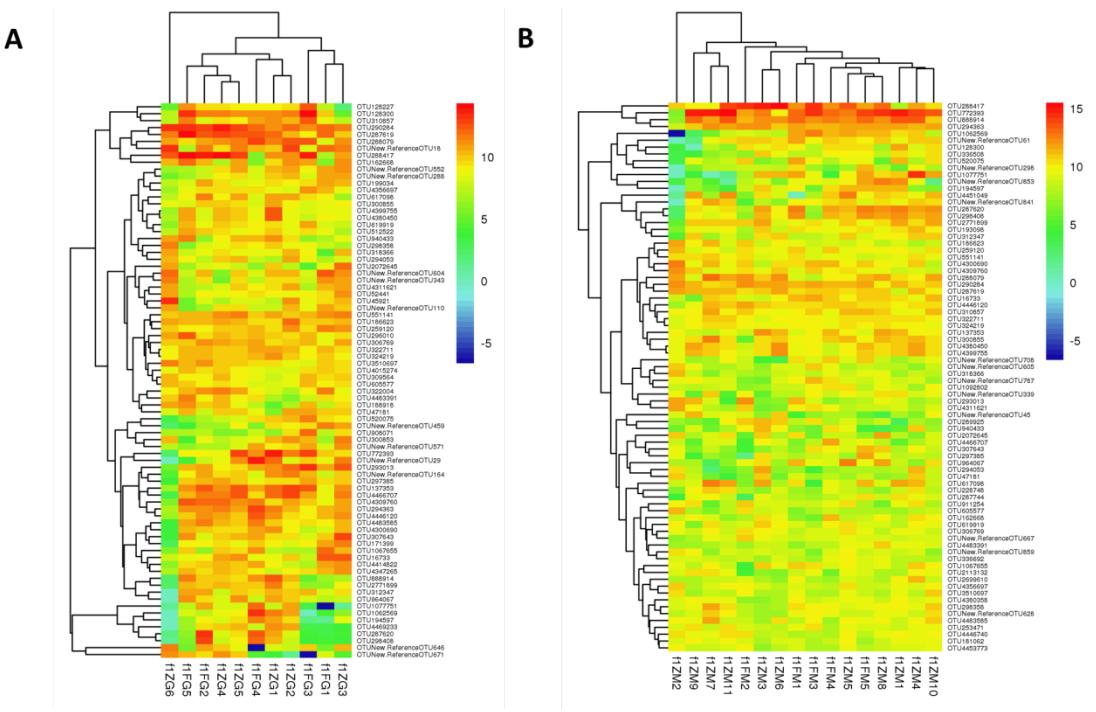


Figure S4

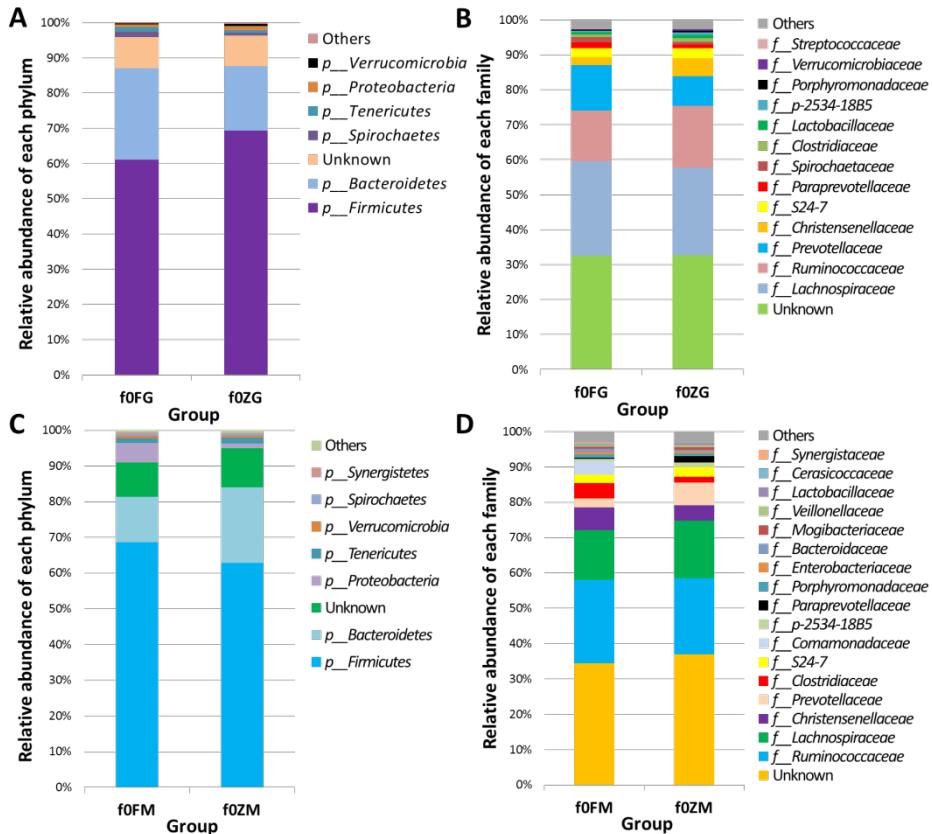


Figure S5

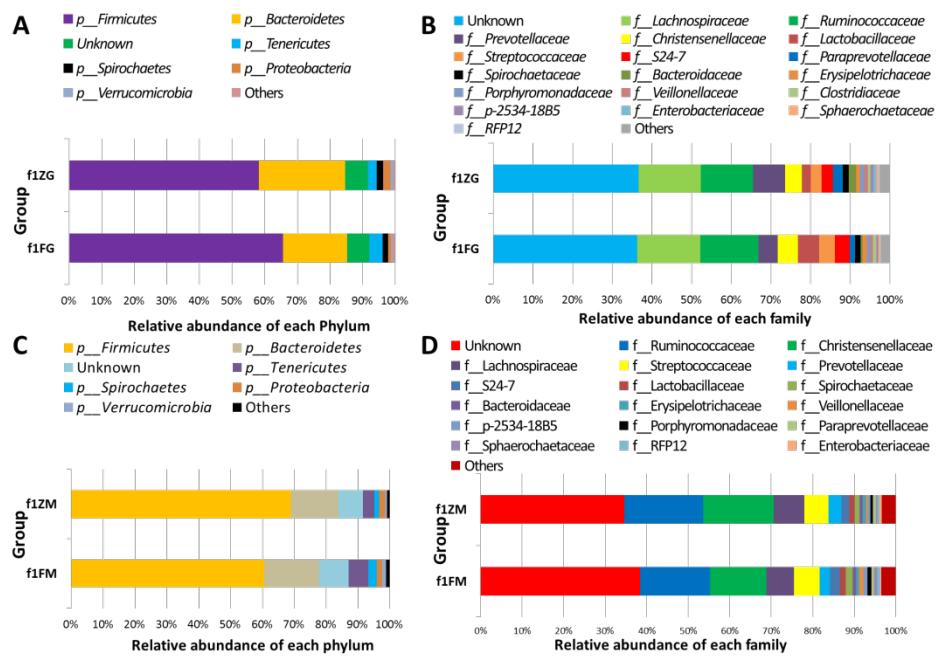


Figure S6