

## Supplementary Materials

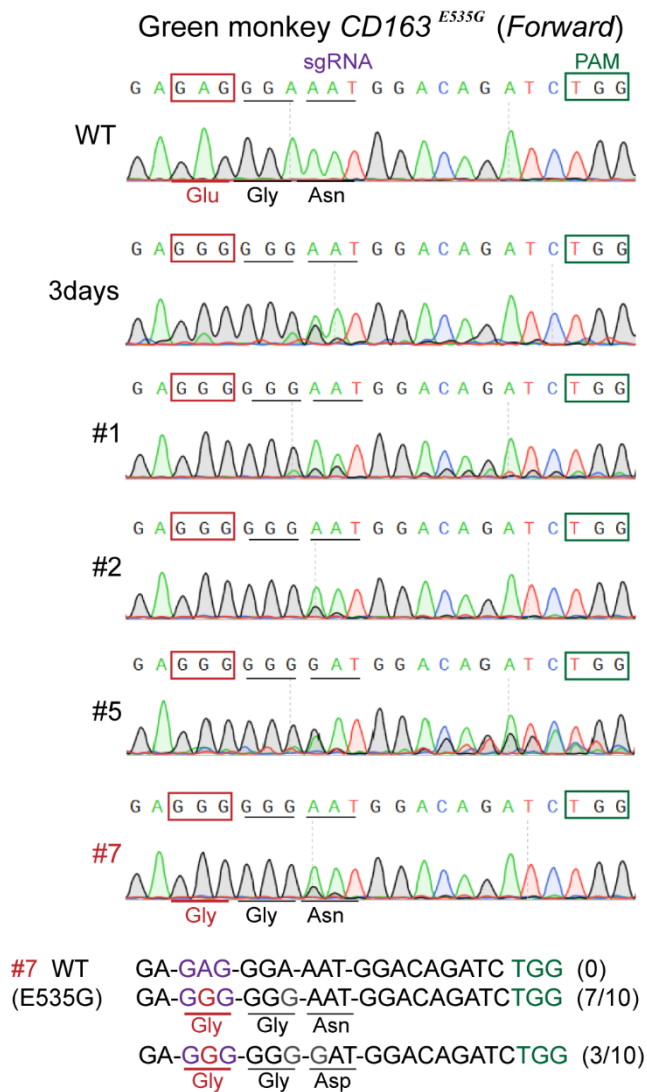
### Genetically modified pigs with CD163 point mutation are resistant to HP-PRRSV infection

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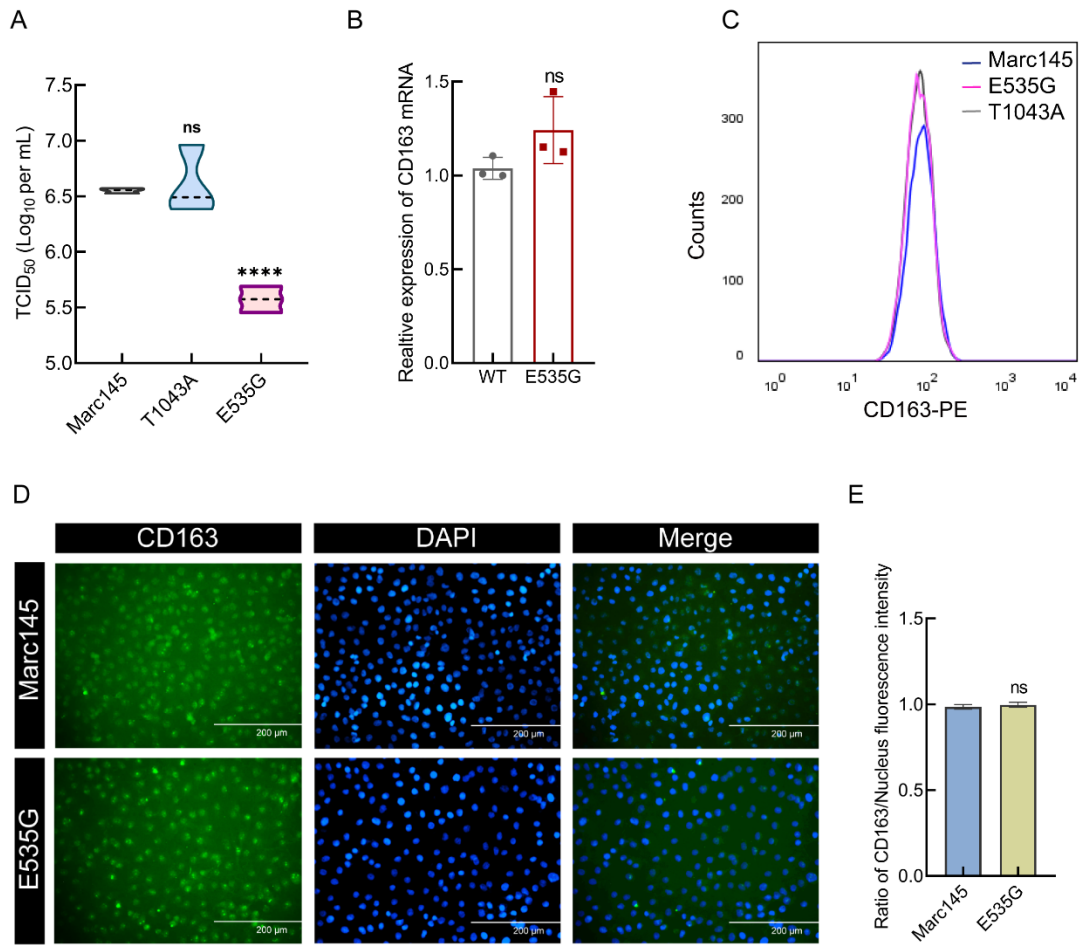
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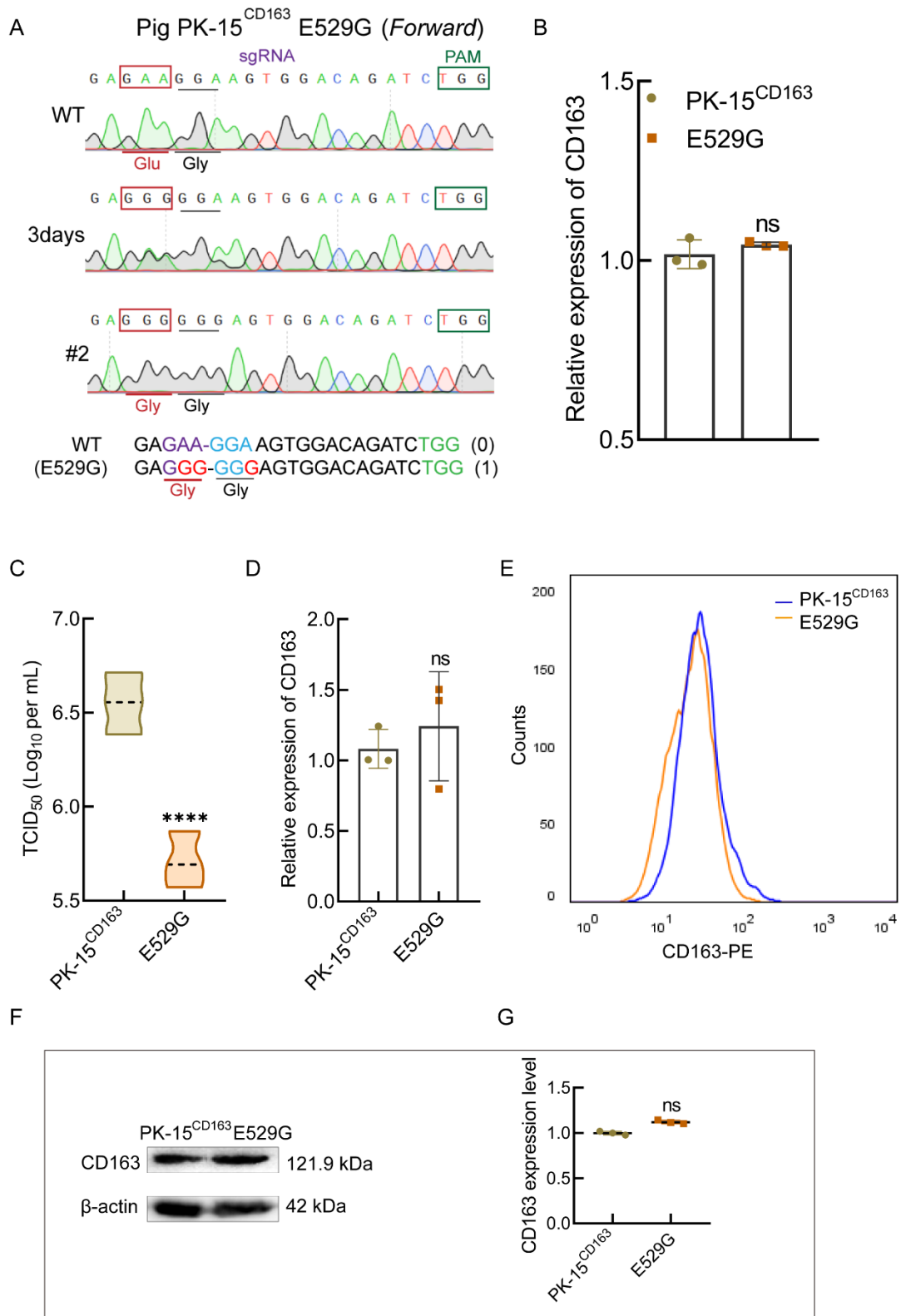
**Supplementary Figure S1. Sanger sequencing results for efficiency analysis of sgRNA target to E535G and single cell clone selection of E535G cells**

The E535G sgRNA and NG-ABE8e single-base editing plasmid were co-introduced into Marc145 cell line by electroporation and sgRNA efficiency was verified using specific primers mF and mR as shown in Supplementary Table S1 for PCR amplification and sanger sequencing, clone-#7 was selected for subsequent validation.



**Supplementary Figure S2. CD163 expression levels in WT or E535G Marc145 cells after PRRSV infection**

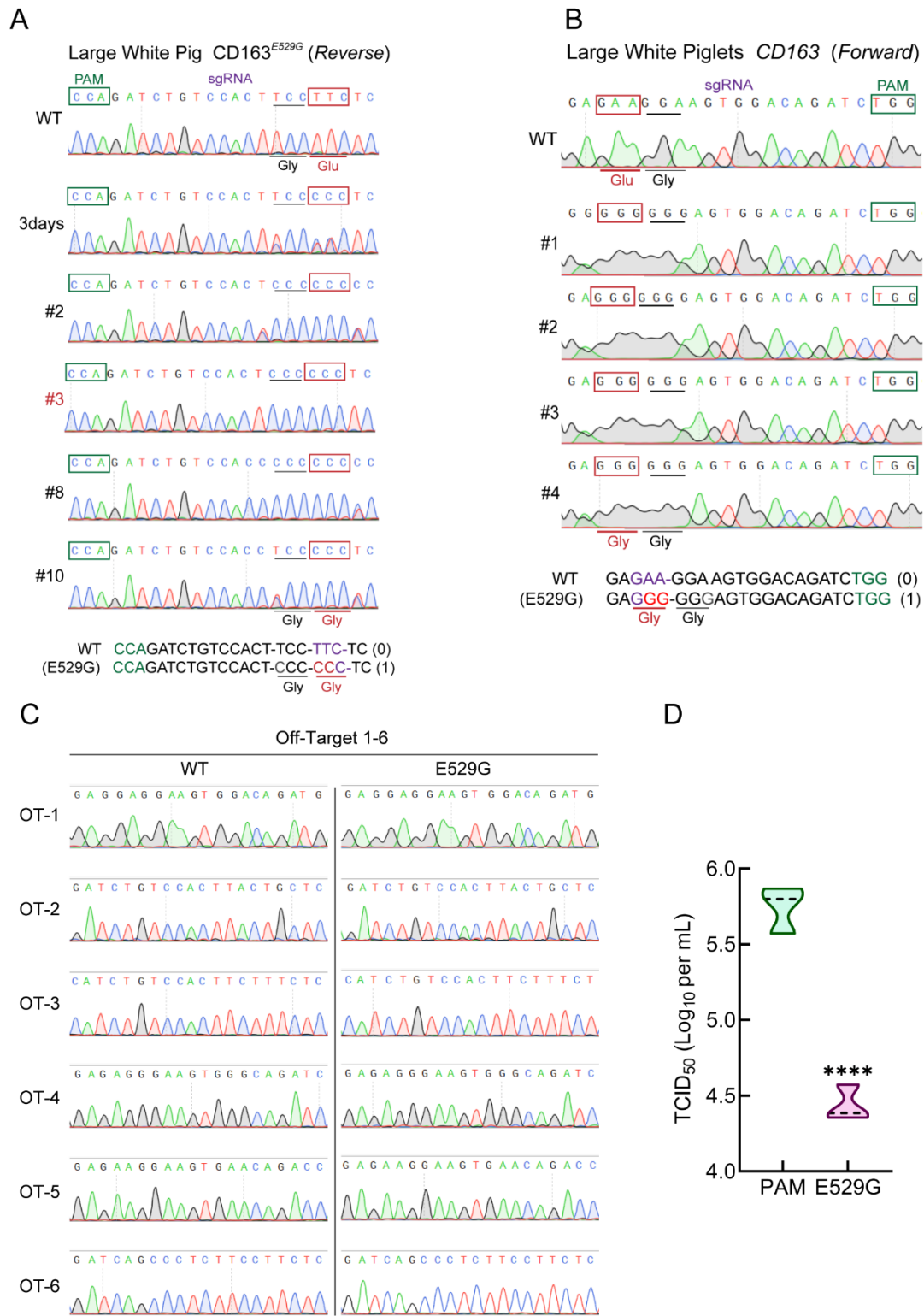
**A:** WT, T1043A, and E535G cells were inoculated with JXA1 and 48 hours later virus titers were determined by TCID<sub>50</sub> assay, n=3. **B–D:** Expression of CD163 in wild-type or E535G Marc145 cells were measured using qPCR (B) and protein were indicated by FACS (C) and IFA (D). **E:** Fluorescence intensity in (D) was quantified by ImageJ analysis.



**Supplementary Figure S3. Identification of PK-15<sup>CD163</sup>-E529G cells as well as CD163 expression states between PK-15<sup>CD163</sup> and PK-15<sup>CD163</sup>-E529G cells with PRRSV inoculated or not**

**A:** Sanger sequencing results for efficiency analysis of sgRNA target to pE529G and selection of PK-15<sup>CD163</sup>-E529G cell clone using specific primers pF and pR shown in

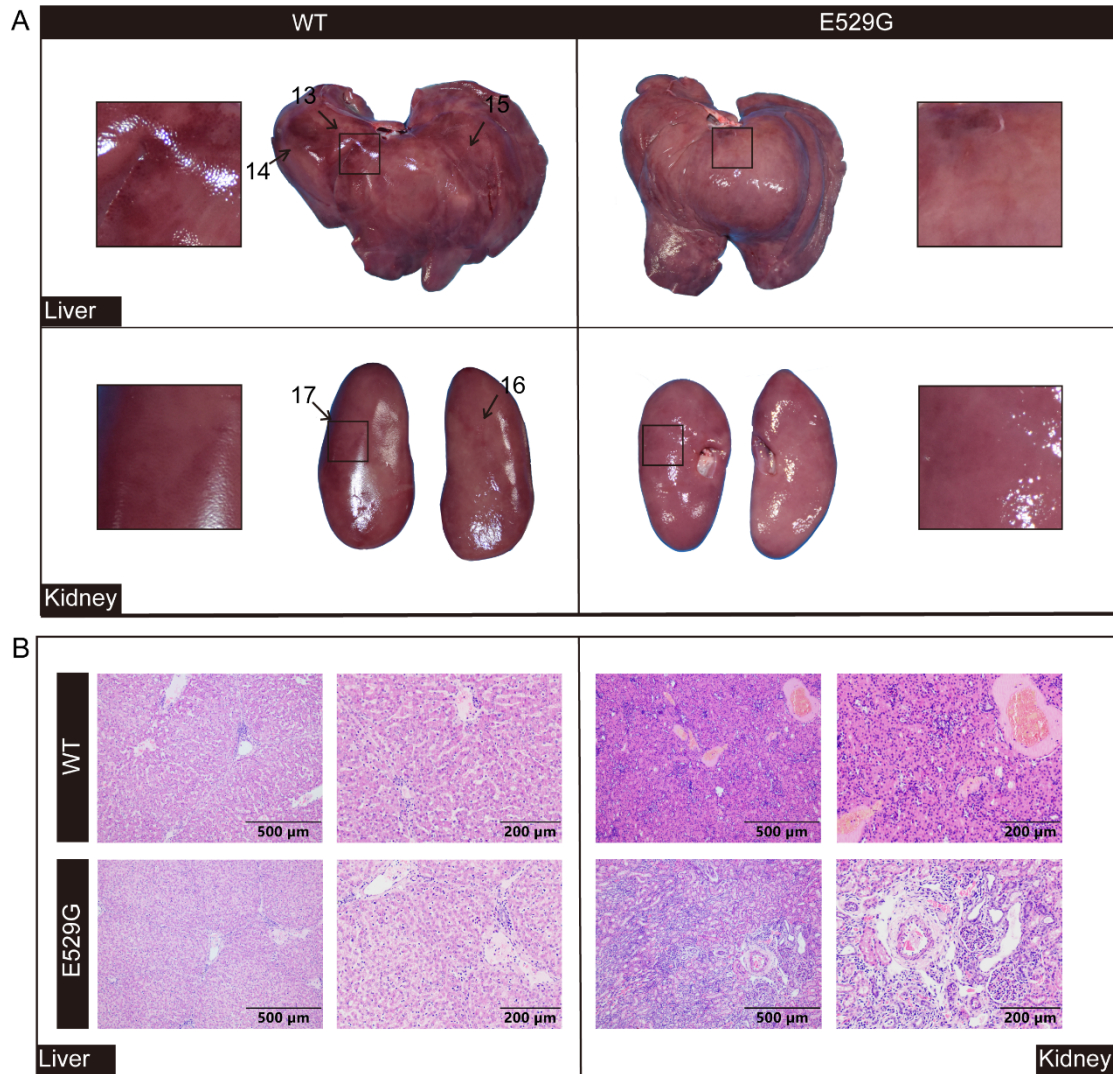
Supplementary Table S1. **B:** Relative expression of CD163 mRNA in PK-15<sup>CD163</sup> and PK-15<sup>CD163</sup>-E529G cells were measured by qPCR using primers pCD163-F/R. **C:** PK-15<sup>CD163</sup> and PK-15<sup>CD163</sup>-E529G cells were inoculated with JXA1 and 48 hours later virus titers were determined by TCID<sub>50</sub> assay, n=3. **D:** Relative expression of CD163 mRNA in PK-15<sup>CD163</sup> and PK-15<sup>CD163</sup>-E529G cells were tested by qPCR 48h post infection of GD strain at MOI=0.1. **E-G:** Expression of CD163 in PK-15<sup>CD163</sup> and PK-15<sup>CD163</sup>-E529G cells were further indicated by FACS (E) and WB (F). ImageJ analysis of WB results were shown in (G).



**Supplementary Figure S4. Genotype identification of E529G PFFs as well as F0 piglets**

**A:** Sanger sequencing results for efficiency analysis of sgRNA target to pCD163-E529G and selection of PFFs-E529G cell clone by specific primers pF and pR shown in Supplementary Table S1. **B:** Genotype identification of F0 piglets by Sanger

sequencing. **C:** The potential off-target sites (Supplementary Table S5 ) were predicted and analyzed by sanger sequencing using primers in Supplementary Table S6. **D:** Virus titers of WT and E529G PAMs in Figure 4H were determined by TCID<sub>50</sub> assay, n=3.



**Supplementary Figure S5. Supplement materials of lesions in tissues and organs from viral challenged pigs**

**A:** Macroscopic observation of tissue damage of liver (above) and kidney (below) from WT (left) and E529G (right) pigs. **B:** H&E staining of liver (above) and kidney (below), 10×magnification is on the left and corresponding 20×magnification is on the right.

**Supplementary Table S1.** Sequence of primers in this study. (DOCX)

<b>Primer name</b>	<b>sequence</b>
mGAPDH-F	CACCAGGGCTGCTTTTAACTC
mGAPDH-R	TGGTGAAGACGCCAGTGGAC
pGAPDH-F	CCTTCCGTGTCCCTACTGCCAAC
pGAPDH-R	GACGCCTGCTTCACCACCTTCT
mCD163-F	ATGGGGTGGACTTACCTGTG
mCD163-R	GTCCATTTCCCTCTCCAAAG
pCD163-F	ATGGACACGAGTCTGCTCTC
pCD163-R	TGAATTTCCACCTCCACTGT
PRRSV-ORF7-F	AAACCAGTCCAGAGGCAAGG
PRRSV-ORF7-R	GCAAATAAACTCCACAGTGTA
mF	TGAGACATGTCCATTGAGCAG
mR	CTCAGTCCCAGTGCAGTGAA
pF	GGCATTGAGAAAGTCCAATTTTC
pR	AGGACCCAAGAATGTTGAGCTCC
CD163-CDS-F	TCTAGAATGGACAAACTCAGAATGGTGC
CD163-CDS-R	ACCGGTTTGTACTTCAGAGTGGTCTCC

**Supplementary Table S2.** Blastocyst development rate of reconstructed embryos *in vitro*. (DOCX)

<b>SCNT (n = 3)</b>	<b>Donor Cell</b>	<b>Numbers of oocytes</b>	<b>Numbers of blastocyst</b>	<b>Blastocyst Rate</b>
<b>1</b>	<b>Wild type PFFs</b>	200	31	18.2%
	<b>E529G PFFs</b>	200	29	17.7%
<b>2</b>	<b>Wild type PFFs</b>	200	30	17.8%
	<b>E529G PFFs</b>	200	31	19.0%
<b>3</b>	<b>Wild type PFFs</b>	200	27	16.3%
	<b>E529G PFFs</b>	200	33	19.3%

**Supplementary Table S3.** Whole-blood-count results for E529G (animals #1 to #3) and wild-type (animals WT-1 to 3) piglets at 2 weeks of age. (DOCX)

<b>Value for animal</b>
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Indicator	WT-1	WT-2	WT-3	#1	#2	#3	Reference value (range)
<b>WBC count (10<sup>9</sup>/L)</b>	6.06	6.12	6.09	13.91	13.66	14	10.00-22.00
<b>Neutrophil count (10<sup>9</sup>/L)</b>	3.35	3.41	3.32	11.84	11.25	11.63	2.00-15.00
<b>% neutrophils</b>	55.2	55.7	54.4	85.1	82.3	83	20.0-70.0
<b>Lymphocyte count (10<sup>9</sup>/L)</b>	2.09	2.07	2.14	0.87	0.9	0.88	0.8-18.70
<b>% lymphocytes</b>	34.4	33.7	35.1	34.3	36.1	35.8	35.0-75.0
<b>Monocyte count (10<sup>9</sup>/L)</b>	0.54	0.57	0.54	1.1	1.44	1.43	0.20-2.20
<b>% monocytes</b>	9	9.4	8.8	7.9	10.5	10.2	0.0-10.0
<b>Eosinophil count (10<sup>9</sup>/L)</b>	0.06	0.06	0.07	0.02	0.01	0.01	0-2.00
<b>% eosinophils</b>	1	1	1.3	0.2	0.1	0.1	0.0-15.0
<b>Basophil count</b>	0.02	0.01	0.02	0.08	0.06	0.05	0.00-0.30
<b>% basophils</b>	0.4	0.2	0.4	0.6	0.5	0.4	0.0-4.0
<b>RBC count (10<sup>12</sup>/L)</b>	6.93	6.9	6.87	3.98	4	4.02	5.00-9.50
<b>% HCT (hematokrit)</b>	39.5	39.5	39.2	27.8	27.8	28	32.0-50.0
<b>MCV (fl)</b>	57	57.1	57.1	69.8	69.6	69.7	50.0-68.0
<b>Hb level (g/dl)</b>	133	135	134	85	85	88	90-180
<b>MCH (fl)</b>	19.2	19.6	19.5	21.4	21.2	21.8	16.0-22.0
<b>MCHC (g/dl)</b>	338	342	341	307	304	313	290-380
<b>RDW (fl)</b>	38.5	38.3	38.4	55.6	55	54.9	0.1-99.9
<b>No. of platelets (10<sup>9</sup>/L)</b>	290	290	301	672	689	695	120-720
<b>MPV (fl)</b>	10.4	10.4	10.4	10.5	10.6	10.6	3.8-14.1
<b>PDW (fl)</b>	18.7	18.7	18.5	18.5	18.9	18.9	0.1-30.0
<b>%PCT</b>	0.303	0.301	0.312	0.703	0.729	0.733	0.010-9.990

WBC, white blood cell; RBC, red blood cell; PCV, packed cell volume; Hb, hemoglobin; HC, Hematocrit; MCV, mean corpuscular volume; MCH, Mean Corpuscular Hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; MPV, Mean Platelet Volume; PDW, platelet distribution width; PCT, Thrombocytosis.

**Supplementary Table S4.** Standard for scoring of clinical signs. (DOCX)

Clinical signs	Scores	Clinical signs	Scores
Normal	0	Rubefaction	2
Poor appetite	1	Dyspnea	2
Lethargy	1	Skin rash	2
Conjunctivitis	1	Coarse hair	2
Red skin	1	Cough	2
Sneezing	1	Asthma	4
Dry feces	1	Lameness	4
Diarrhea	1	Blue ears	4
Emaciation	2	Stopping feed intake	4

**Supplementary Table S5.** Off-target ID of E529G pigs. (DOCX)

<b>Off-target ID</b>	<b>Off-target Sequence</b>	<b>mismatch Pos</b>	<b>mismatch Count</b>
1	GAGGAGGAAGTGGACAGATGAAG	...*.....*	2
2	GAGCAGTAAGTGGACAGATCTGA	...*.....*	2
3	GAGAAAGAAGTGGACAGATGTGG	....*.....*	2
4	GAGAGGGAAGTGGGCAGATCCAG	....*.....*	2
5	GAGAAGGAAGTGAACAGACCAGG	.....*.....*	2
6	GAGAAGGAAGAGGGCTGATCGGG	.....*.....*	3

**Supplementary Table S6.** Off-target primers. (DOCX)

<b>Off-Target-Primer name</b>	<b>sequence</b>
OT-1-F	ATGGGAAAGGGAGCAGATTT
OT-1-R	ACCCAGCACTCTGCTTTTGT
OT-2-F	CCAGATTGCCAGCCTTTATGC
OT-2-R	GTGAAGGGACTGAGAGTGTTGT
OT-3-F	AGCATGGAGACCAGCATAGC
OT-3-R	TCTTTTCCCACCCCTTTCCC
OT-4-F	CTGAGAGGAGGGGGATCTGG
OT-4-R	ATTCCATGGATGGGTGTGCC
OT-5-F	CGTCCTCTCCCTTCCCTATC
OT-5-R	GAACTCGGGAGCCTTTCTCT
OT-6-F	GTGTAACCCTCCTTCGTCGG
OT-6-R	CCCTGTGGGTTGTGGGAATG