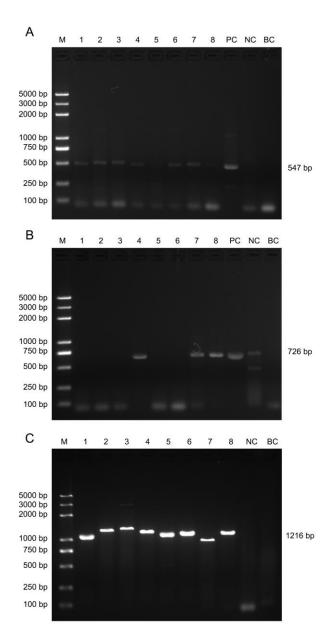


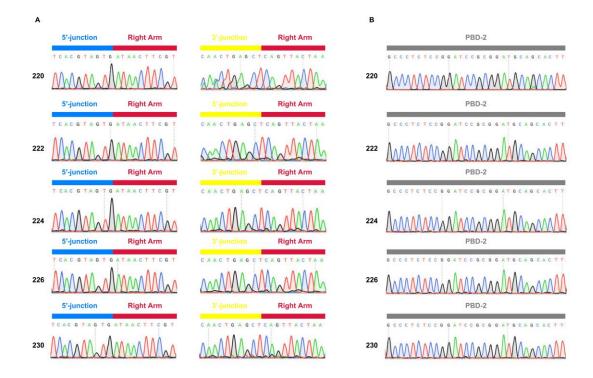
**Figure S1.** Analysis for the T2A-mediated dual *pbd-*2 expression. (**A**) Map of *pcCAG-nPBD2*. (**B**) Map of *pcCAG-PBD2-T2A-PBD2*. (**C**) PK-15 cells were transfected with *pcCAG-nPBD2* and *pcCAG-PBD2-T2A-PBD2*, respectively. The cell culture supernatant was collected 48 h after transfection and then was incubated with *Actinobacillus pleuropneumoniae* for 1 h, the surviving bacteria were then plated on agar for counting. Cell culture supernatant of PK-15 was used as a blank control and porcine β-defensin 2 (PBD-2) of 60 μg/mL served as a positive control. Data are presented as mean  $\pm$  SD and are plotted from three independent experiments. \*\*P < 0.01, \*\*\*\*P < 0.0001, unpaired one tailed Student' s t-test.



**Figure S2.** Map of *pX330-pRosa26*. A plasmid expressing Cas9 nuclease and sgRNA targeting the *pRosa26* locus (5'-GCTCCTTCTCGATTATGGGC-3').



**Figure S3.** PCR analysis for the identification of site-specific *pbd-*2 knock-in porcine fetal fibroblasts (PFFs). (**A**) Amplification for the dual *pbd-*2 gene; (**B**) Amplification for the selectable marker gene *neoR*; (**C**) Amplification for the fragment which represents on-target insertion of the transgene. M: Molecular mass marker; Lane 1-8: Numbers for different cell colonies; PC: Positive control; NC: Negative control; BC: Blank control.



**Figure S4.** Sanger sequencing analyses to confirm on-target integration of *pbd-2* in cloned pigs. Numbers represent different transgenic pigs. (**A**) Sequencing results of the On-target fragment amplified with primers OT-F and OT-R. Red line indicates the sequence in the right homology arm; blue line indicates the sequence upstream of the right homology arm; yellow line indicates the sequence downstream of the right homology arm. (**B**) Sequencing results of the PBD-2 fragment amplified with primers vTG-F and vTG-R. Gray line indicates the sequence in the *pbd-2* gene.

**Table S1.** Information of primer pairs for identification of transgenic PFFs.

Fragment	Primer	Sequence (5'-3')	Length (bp)
PBD-2	vTG-F	GCTGGTTGTTGTGCTGTCTCATCA	547
	vTG-R	CCCTCTAGACTCGAGTCAGGGTCAGC	
Marker	marker-F	GATGGATTGCACGCAGGTTCTCC	726
	marker-R	GATACCGTAAAGCACGAGGAAGCG	
Locus	f-locus	CCTTCTATCGCCTTCTTGACGAGTT	1216
	r-locus	AACTGTCCCAGCAACACCTAAGATT	

**Table S2.** Information of primer pairs for amplification of predicted off-target loci.

Fragment	Primer	Sequence (5'-3')	Length (bp)
OT-1	OT1-F	ATAAAACAATGGGGGAATGAGAC	429
	OT1-R	AGCCAGAGGCAAAGTGATAGATA	
OT-2	OT2-F	CACAGAAGGAATTCCCTGAGAGA	389
	OT2-R	CATGCTTCTCTGCACACACTA	
OT-3	OT3-F	TCGGTGTCCTTGCCAAGTCG	417
	OT3-R	CACCTTCCTGGCATTAATCCCT	
OT-4	OT4-F	AGTTAATGATTCCTAGGACATGCTT	367
	OT4-R	TCAAAGTGGCGGGTAAGGGAA	
OT-5	OT5-F	GGGTCTGTGTTGTTGCATGAGAA	350
	OT5-R	CACAGTGGGTGAGGTAAGAAATT	