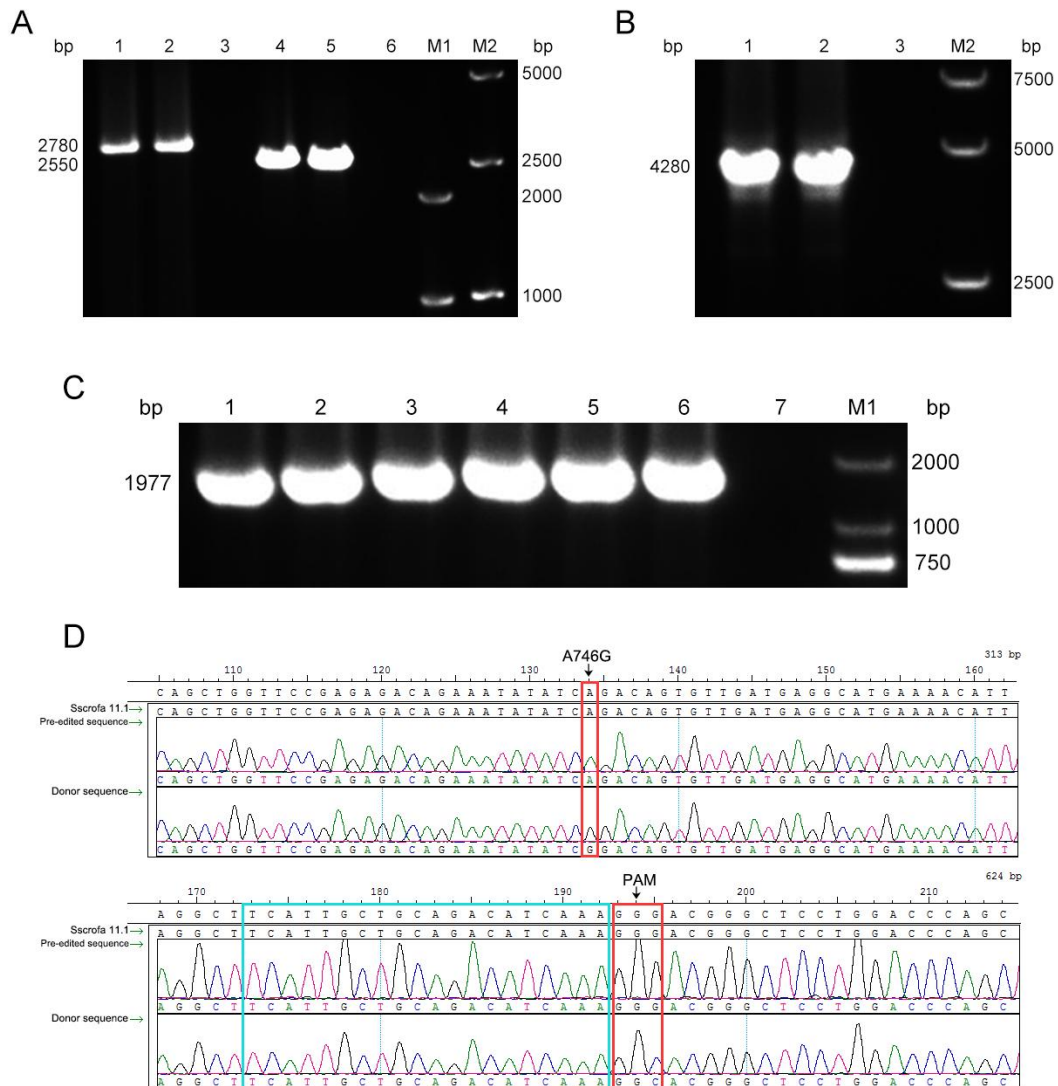
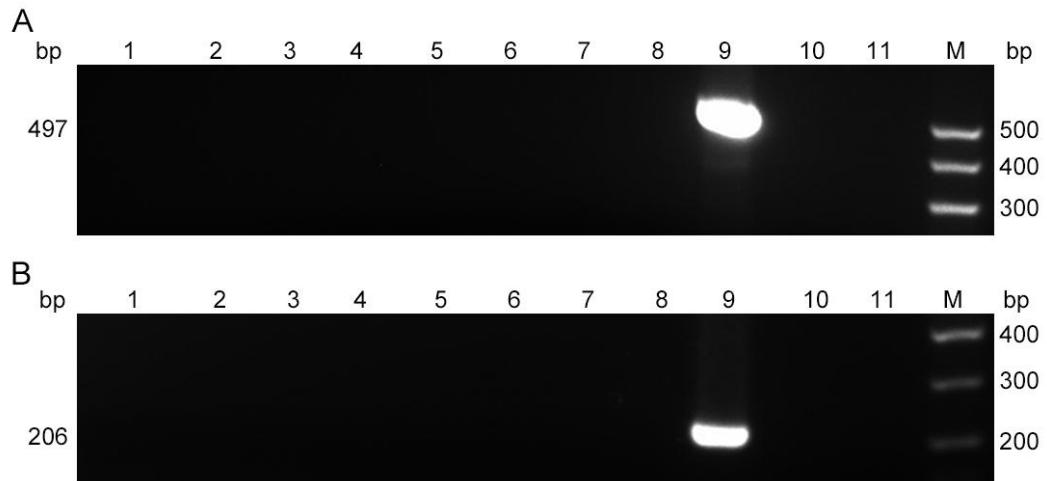


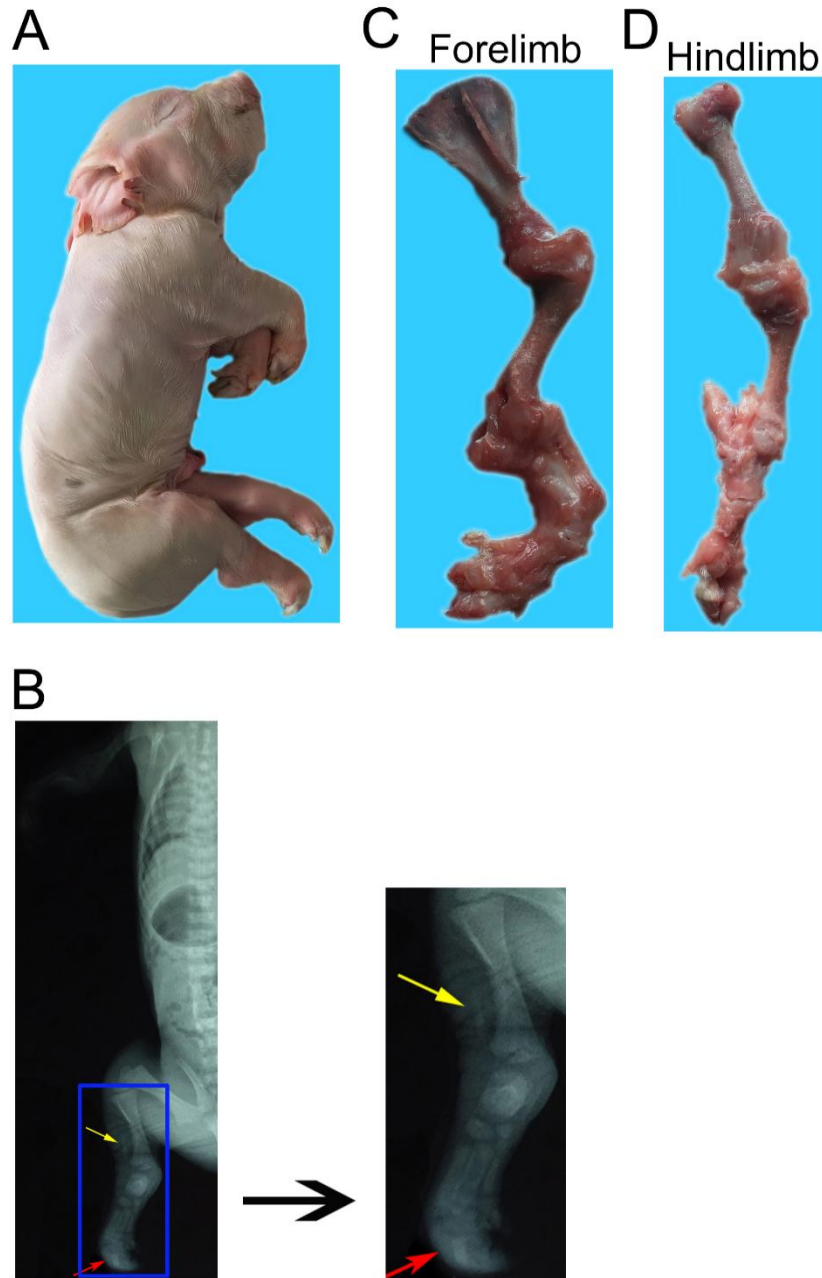
Supplementary Materials



Supplementary Figure S1 Generation of DNA donor template. A-C: Amplification of DNA donor template that encompassed the target point mutation by bridging PCR. D: Sequencing chromatograms of *BMPR-IB* A746G mutation (red box in upper panel) and sgRNA sequence (blue box) in DNA donor template.

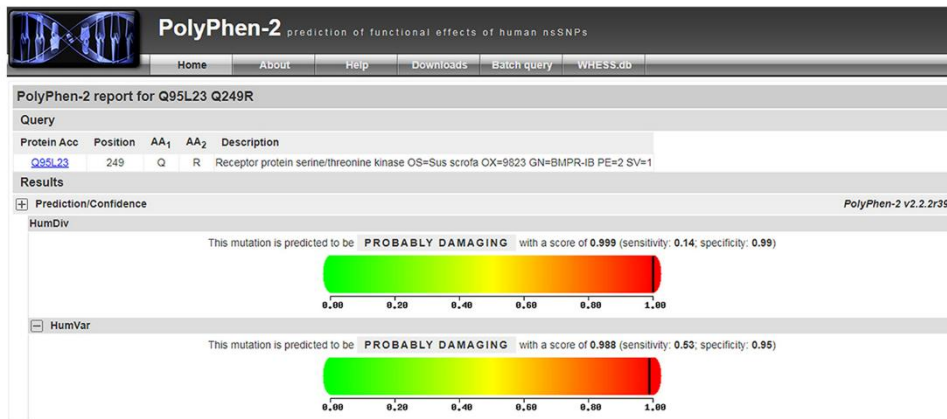


Supplementary Figure S2 Routine PCR detection of integration of CRISPR plasmids in cloned piglets. A: Cas9. B: sgRNA. Lanes 1 to 8 indicate amplification of DNA from cloned piglets (1, *BMPR-IB*^{-/-}; 2–5, *BMPR-IB*^{-/746G}; 6–8, WT). 9, PX458 DNA; 10, DNA from WT pig; 11, water. M indicates DL 500 DNA marker.

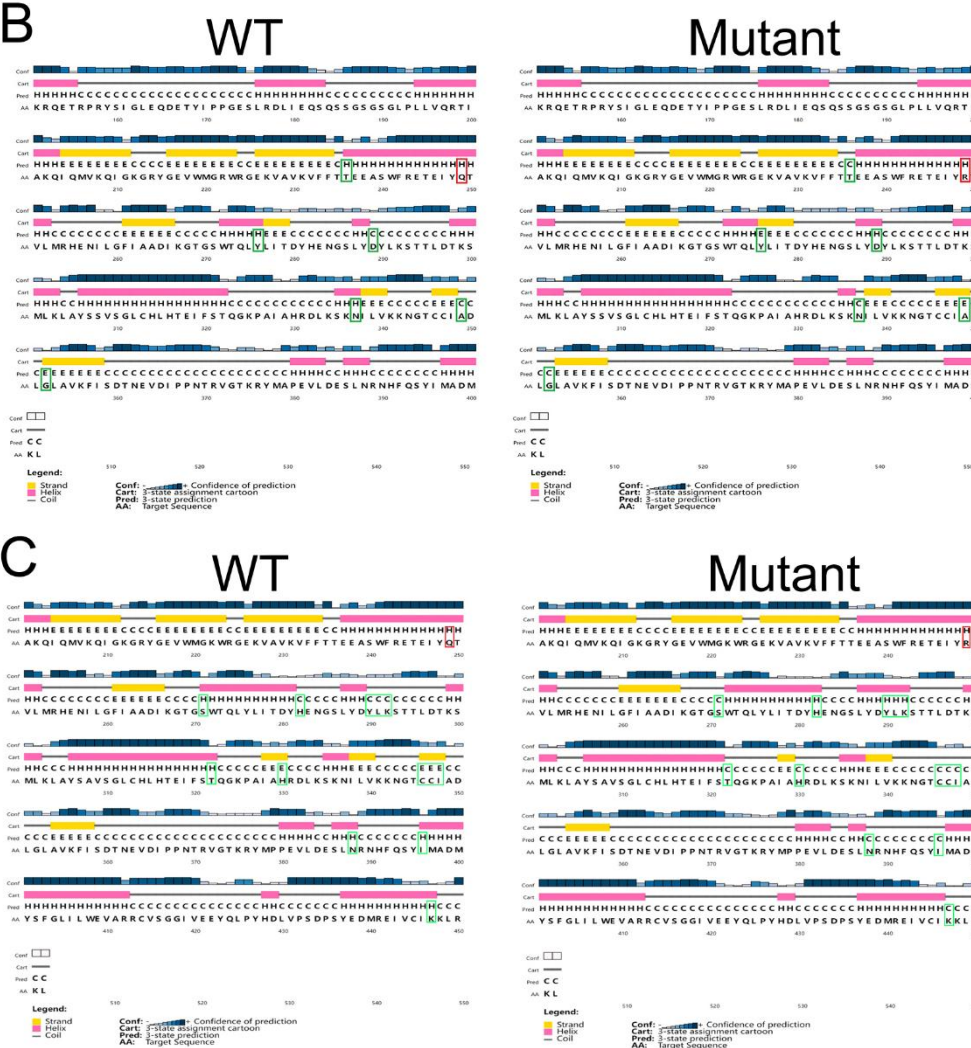


Supplementary Figure S3 Phenotypic characterization of *BMPR-IB*-disrupted *BMPR-IB*^{-/746G} piglet (different from that in Figure 2). A: Whole-body photograph. B: Radiograph of hindlimb. C: Anatomical view of forelimb. D: Anatomical view of hindlimb. Missing fibula and third proximal phalanx (os compedale) are marked with yellow and red arrows, respectively.

A



B



Supplementary Figure S4 Prediction of functional effect of *BMPR-IB*-A746G mutation. A: Prediction based on PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>). This score represents the probability that a substitution is damaging, ranging from 0.0 (tolerated) to 1.0 (deleterious). Analysis suggested that A746G mutation is a deleterious substitution: HumDiv, probably damaging, score 0.999; HumVar, probably damaging, score 0.988. B: Structural comparison of *BMPR-IB*-746AA and

BMPR-IB-746GG protein in porcine using PSIPRED (<http://bioinf.cs.ucl.ac.uk/psipred/>). C: Structural comparison of BMPR-IB-746AA and BMPR-IB-746GG protein in sheep using PSIPRED. H, C, and E represent alpha helix, random coil, and extended strand, respectively.

Supplementary Table S1 PCR primers, excluding those for detecting off-target sites

Primer No.	Primer name	Forward primer (5'-3')	Reverse primer (5'-3')	Amplicon (bp)
1	BMPRIB-sgRNA	CACCGTCATTGCTGCAGACATCAAA	AAACTTTGATGTCTGCAGCAATGAC	25
2	sgRNA-F/R	CAGTGTTTCTCTACCCCTCCTG	GTTCTTCTTTTTCCCTGCAA	653
3	BMPRIB-long-1	AACTAAATGGTTTCTGGTGTCTGTCA	TCATGCCTCATCAAACTGTCCGATAT	2864
4	BMPRIB-long-2	AGACAGAAATATATCGGACAGTGTT	GATACTGCCAGATGTTTTCTCTTGAAG	2536
5	BMPRIB-long-3	TTTCGGAAACGGTGATAACTCCTCGT	CTTTAGAAACAAATCCGTCAGAGGGTC	4337
6	BMPRIB-donor	AAAGTATTCTAACCTGCCATTACCA	ATGATCCTTCTGTACCTGTGATTGC	1977
7	BMPRIB-A746G	CAGATGGTGAAACAGATTGGA	TAGGAAACCCTGAACATTGC	205
8	BMPRIB-del-lon α-1	CGTGGTAAGATAGCGGATTGCTGGAT	TGATGGAAGACGACACAAACAAATGG	9245
9	Cas9	CGAGGAAGTGGTGGACAAG	CGAACAGGTGGGCATAGG	497
10	gRNA	CAAGGCTGTTAGAGAGATAATTGG	GTGTTTCGTCCTTTCCACAA	206
11	BMPRIB-SYBR	CCTGGGTCTGGCTGTCAAAT	GGTGTTGGGTGGTATGTCG	66
12	GAPDH-SYBR	CACTCTTCCACTTTTGATGCTG	CCTGTTGCTGTAGCCAAATTC	99

Supplementary Table S2 Primers for PCR detection of predicted off-target sites

Off-target site	Primer direction	Primer sequence
1	FP	5'- ACAAGCCTCTGGGTGACAG -3'
	RP	5'- CTGGGGTGCCTCCTTCTAAC -3'
2	FP	5'- AGATGAACGGGAAGGAGGAG -3'
	RP	5'- AGGGACAGAAAAGGGGAATG -3'
3	FP	5'- TATGGGGTCAAGGGATGTGT -3'
	RP	5'- GGGAAACAGAAGAAGCAGGA -3'
4	FP	5'- GGTTTGGGTGTTTCAGGAGA -3'
	RP	5'- TCCCTACATGCCAGCAGATT -3'
5	FP	5'- CTGTTTTACCGACACCAAG -3'
	RP	5'- ATTCTTCCACTCCCAGACCT -3'
6	FP	5'- CTGTTTTACCGACACCAAG -3'
	RP	5'- CGTTCCTCAAGCATAAGACAA -3'
7	FP	5'- GAGATGGTGGTGGGAGAAGA -3'
	RP	5'- TCAGAACAGGGGAACTCAGC -3'
8	FP	5'- GAGGGTGAGGTTTCTGGTTG -3'
	RP	5'- GTGACAGCGAAGCAGGTACA -3'
9	FP	5'- TGCCCTATGCTGTTGTATGC -3'
	RP	5'- ATTGGCTGGAATCACTGCTG -3'
10	FP	5'- CCCTCGGGAAACTAAACCTC -3'
	RP	5'- AGCTCTGTGCCTCTGGATGT -3'
11	FP	5'- AGCCTGAGCAAGAAGGAAGA -3'
	RP	5'- AGAGGAAGATGGAGCAATGG -3'
12	FP	5'- GTGGCTAGAAGGATGGTGGGA -3'
	RP	5'- GGCCCTAAGTATGGATGGTT -3'
13	FP	5'- GAGGGAAGGGAAGCAAAGAG -3'
	RP	5'- TGGGAACCTTGGAAAACTG -3'
14	FP	5'- CGGCGTCCCTCTGTTAGTG -3'
	RP	5'- CCACCCCATTCCTTCCTG -3'
15	FP	5'- CCAGGTGGAGAGGAGGAAGT -3'
	RP	5'- GGTCCTTAACCCACTGAGCA -3'

Supplementary Table S3 Summary of *BMPR-IB* A746G mutation in cell colonies

Type	Total	Homozygotes	Heterozygotes	Indels	Wild type
Number of cell colonies	113	16	23	30	44
Proportion	100%	14.16%	20.35%	26.55%	38.94%

Supplementary Table S4 Detection of predicted off-target sites by Sanger sequencing

Off-target site	Sequence	Gene	Locus	Result
1	TCCATGCTGCAGACATCA AG		Chr2: +74435012	-
2	ACATAGCAGGAGACATCA AA		Chr1: -41849987	-
3	ACATAGCAGGAGACATCA AA		Chr1: -41719398	-
4	TTAGTGCCGGAGACATCA AA	ENSSSCG000000161 48	Chr15: +123162535	-
5	TCTCAGCAGCAGACATCA AA	ENSSSCG000000100 30	Chr14: +51329154	-
6	GGGTGGCTGCAGACATCA AA		Chr16: -18569987	-
7	ATATTGCTGCAGCCATCAA A		Chr3: -139007881	-
8	TCATAGCTGGACACATCA AA		Chr3: -5611747	-
9	TGATTGGTGCATACATCAA A		Chr2: +133467423	-
10	TGAAGGATGCAGACATCA AA		Chr6: -83149645	-
11	TCATTCATGGAGACATCAA A		Chr8: +7594530	-
12	GCTTGGCTACAGACATCA AA		Chr15: -18948461	-
13	TCCTTGTGGGAGACATCA AA		Chr1: +184885654	-
14	ACACTACAGCAGACATCA AA		Chr2: +1605477	-
15	CCTTTTCTGGAGACATCAA A		Chr14: +153156809	-

Note: BENCHILING online search address: <https://www.benchling.com/>. “-” indicated no off-target effect.