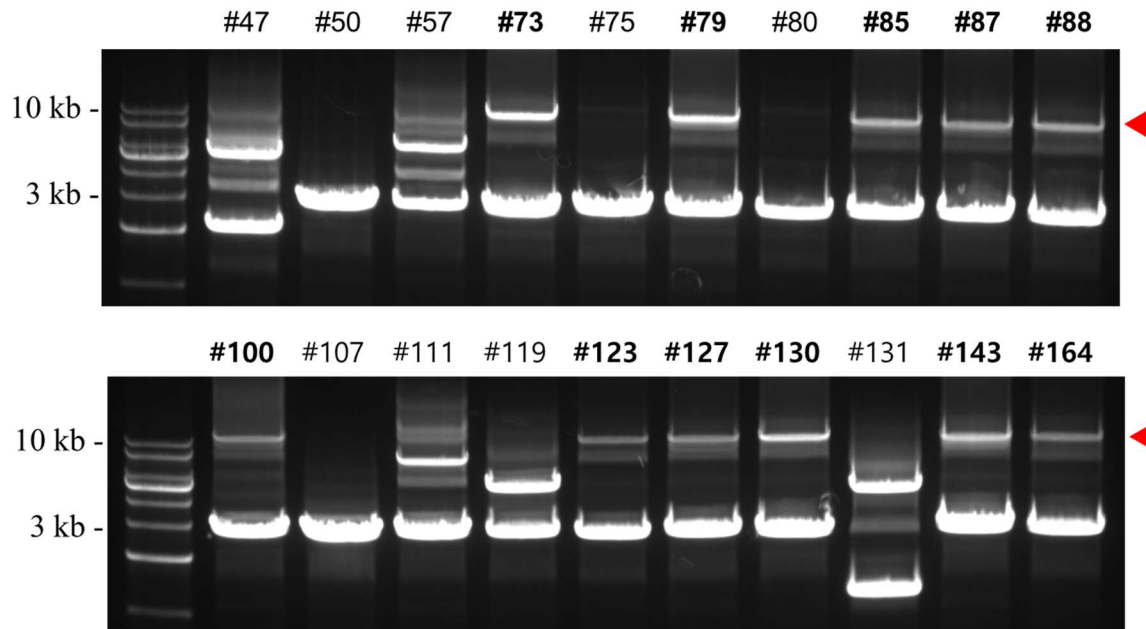
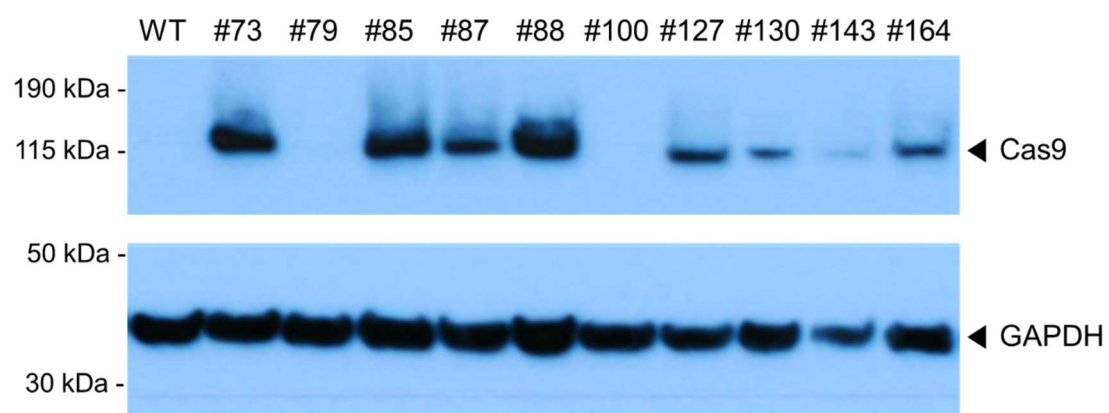


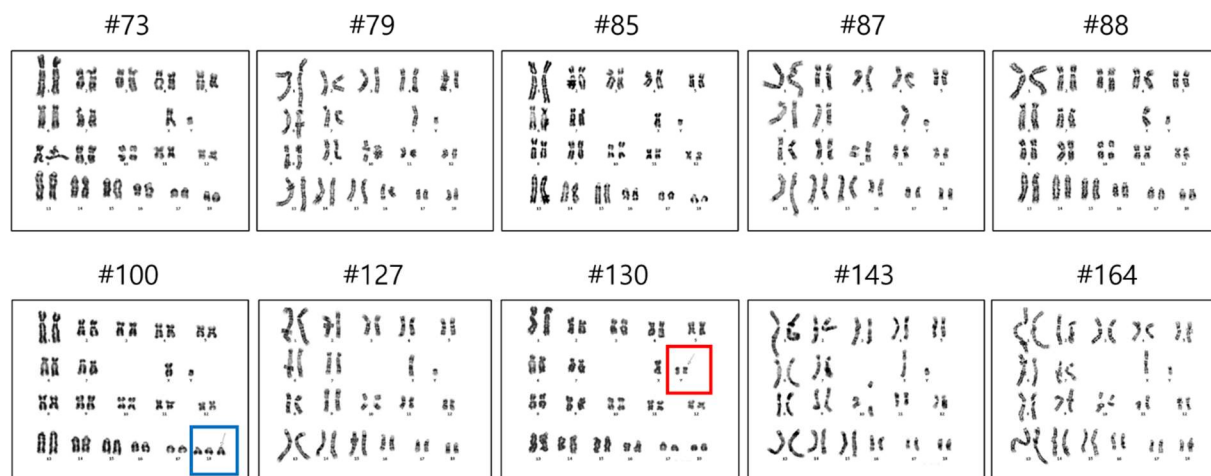
Supplemental Materials



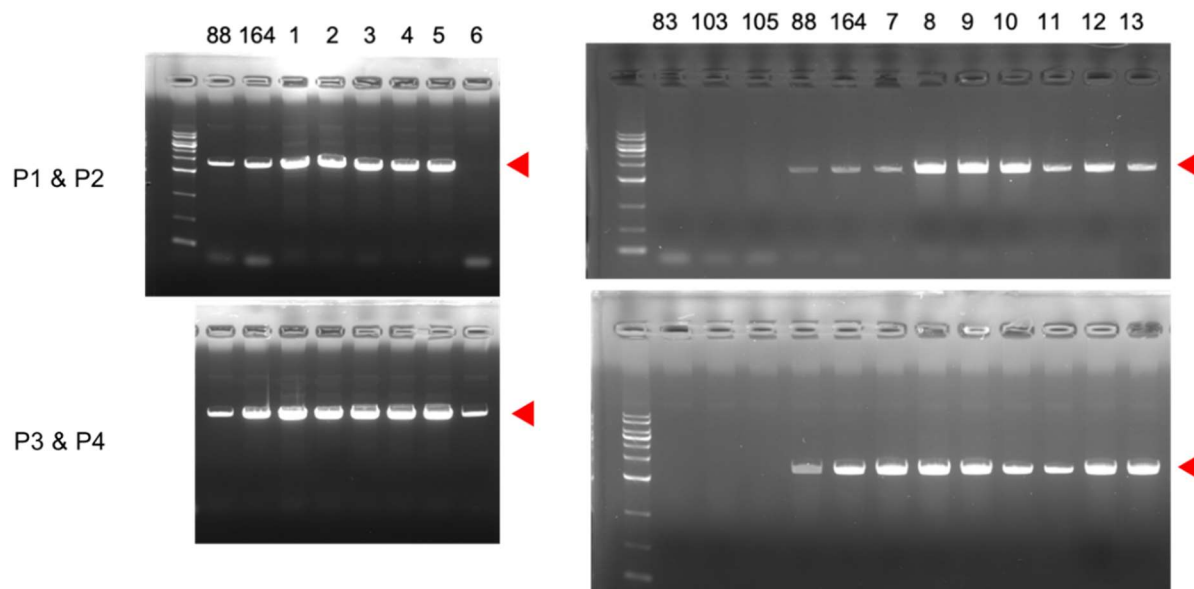
Supplemental Fig. 1. Genotyping of Cas9 gene knock-in colonies. Long-range (P1+P4) PCRs were performed to identify stable knock-in colonies at the porcine ROSA26 locus. The red triangle indicates the correct size (10.8 kb) of the targeted Cas9 gene cassette, while the blue triangle indicates no insertion or the wild type (2.9 kb) in kilobases (kb). Primer pairs are shown in Supplemental Table 2. Red triangles indicate colonies containing Cas9 gene correctly.



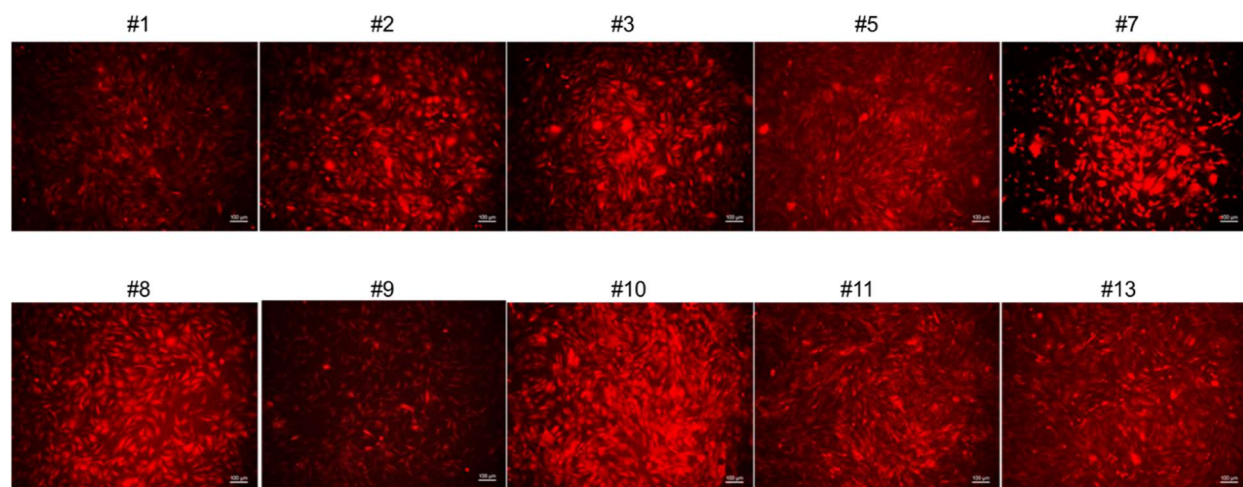
Supplemental Fig. 2. Expression profile of Cas9 gene. Cas9 gene expression was assessed through western blot analysis using cell extracts from stable knock-in colonies. Beta-actin served as the loading control.



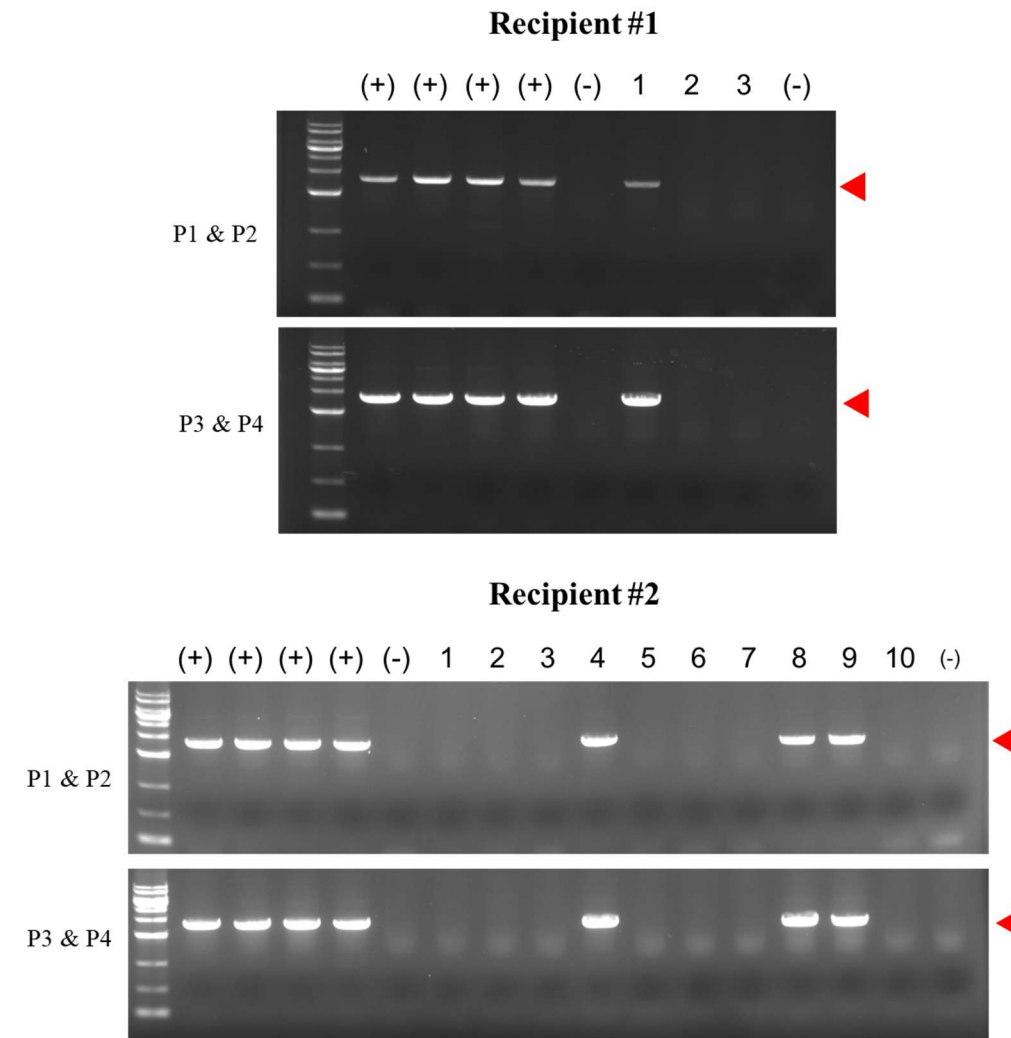
Supplemental Fig. 3. Karyotyping of Cas9 gene knock-in colonies. Two colonies (#100, 130) show abnormal karyotypes, trisomy in chromosome 18 (blue box), and duplication in X chromosomes (red box), respectively. All other colonies have normal karyotypes.



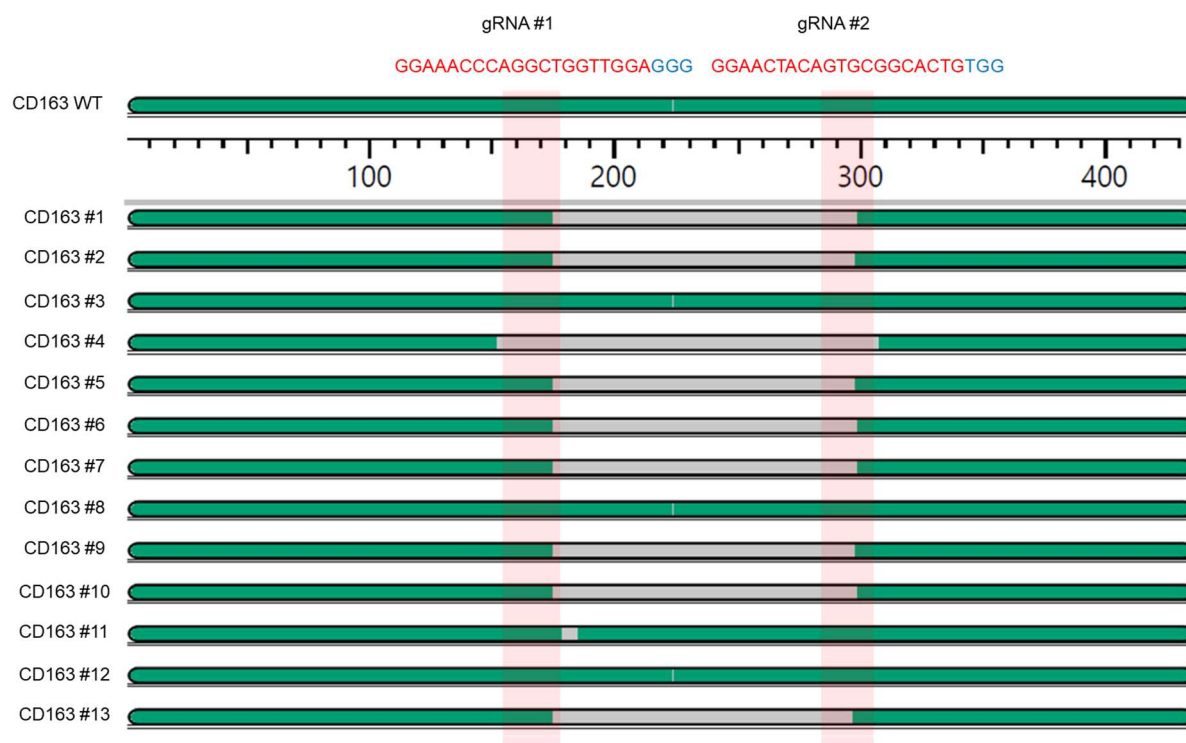
Supplemental Fig. 4. Genotyping of Cas9 gene-expressing piglets. 5'-junction (P1+P2) and 3'-junction (P3+P4) PCR analyses confirmed that 12 of 13 cloned piglets exhibited the correct homologous recombination at the ROSA26 locus. Red triangles indicate expected PCR product size. Positive control: 88 and 164, Negative control: 83, 103 and 105, Cas9-expressing piglets: 1–13. Primer pairs are listed in Supplemental Table 2.



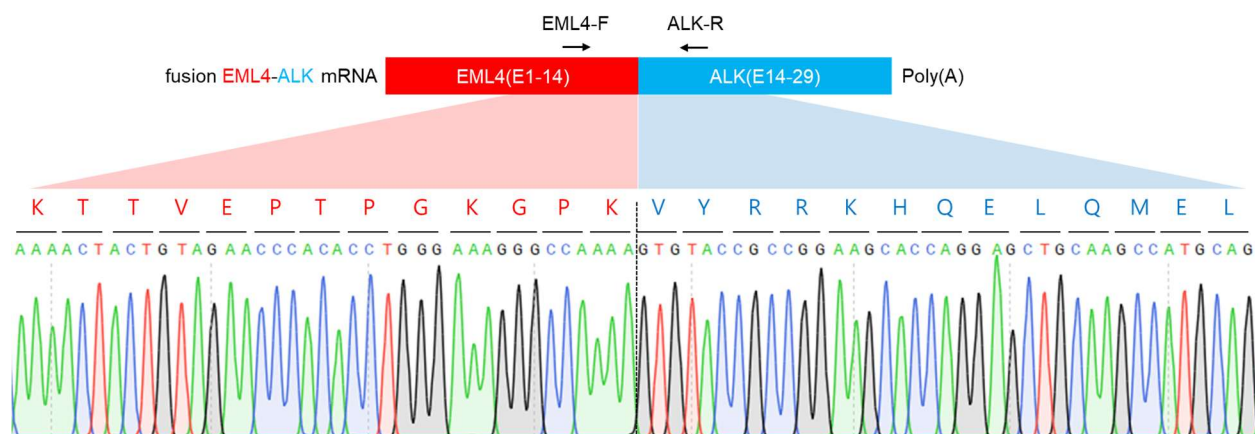
Supplemental Fig. 5 Expression analysis of the tdTomato gene. Fluorescence images of ear fibroblasts from Cas9-expressing piglets were captured using a red filter under a fluorescence microscope. The numbers indicate the label numbers of the piglets



Supplemental Fig. 6. Genotyping of piglets generated from recipient female pigs by artificial insemination with sperm from a Cas9-expressing male pig. 5'-junction (P1+P2) and 3'-junction (P3+P4) PCR analyses confirmed that 3 of 10 piglets carried the Cas9 gene cassette at the ROSA26 locus. Recipient indicates female pig. Red triangles indicate the expected PCR product size. (+) Positive control, (-) Negative control, Piglets: 1–3(recipient #1), 1–10(recipient #2). Primer pairs are listed in Supplemental Table 2.



Supplemental Fig. 7. Schematic for Sanger sequencing analysis of the CD163 gene knock-out. Green color indicates sequences identical to the reference CD163 gene locus, while gray color indicates mutations, including deletions. The red box marks the gRNA target sites.



Supplemental Fig. 8. Sanger sequencing analysis of EML4–ALK mRNA fusion transcripts. Sanger sequencing results of RT-PCR products confirmed that the sequences of EML4–ALK mRNA fusion transcripts matched exactly with the predicted sequences

Target Gene	gRNA Sequence (5' to 3')
Porcine ROSA26	ACCACCCAGAAGCCTCGGCC CGG
CD163 #1	GGAAACCCAGGCTGGTTGGA GGG
CD163 #2	GGAACCTACAGTGCGGCACTGT TGG
EML4	TGAAGTGCCAGAGCATACA AGG
ALK	GATTAGAACACAAGTCCTC GGG

Supplemental Table 1. Target genes and sgRNA sequences. PAM sequences are highlighted in red.

Name	Primers Sequence (5' to 3')
P1	TGG GAA AGG AAG CAA TCA TC
P2	ATG GTG GGG TAC TTC TCG TG
P3	AGT CTG GAG CAT GCG CTT TA
P4	GGT CCA ATC GCA GTG GTA GT
P5	GGA GGT CTA GAA TCG GCT AAG CC
P6	GGC TAC ATG TCC CGT CAG GG
P7	GCC CCC TCC CCA TGA ATC AT
P8	TAG GGG CCA AAG TCA GCC ATC
P9	GCC TTA CTC CTG CTC AAG CA
P10	CCA ACA CCA CAC AGT TAG CTA G
EML4-F	TGG GGA ATG GAG ATG TGC TT
ALK-R	TGA GGG TGA TGT TTT TCC GAG
GAPDH-F	ATG TTC CAG TAT GAT TCC ACC C
GAPDH-R	ACC AGC ATC ACC CCA TTT G

Supplemental Table 2. List of primers used in the study