CRISPR-Cas9 mediated one-step disabling of pancreatogenesis in pigs

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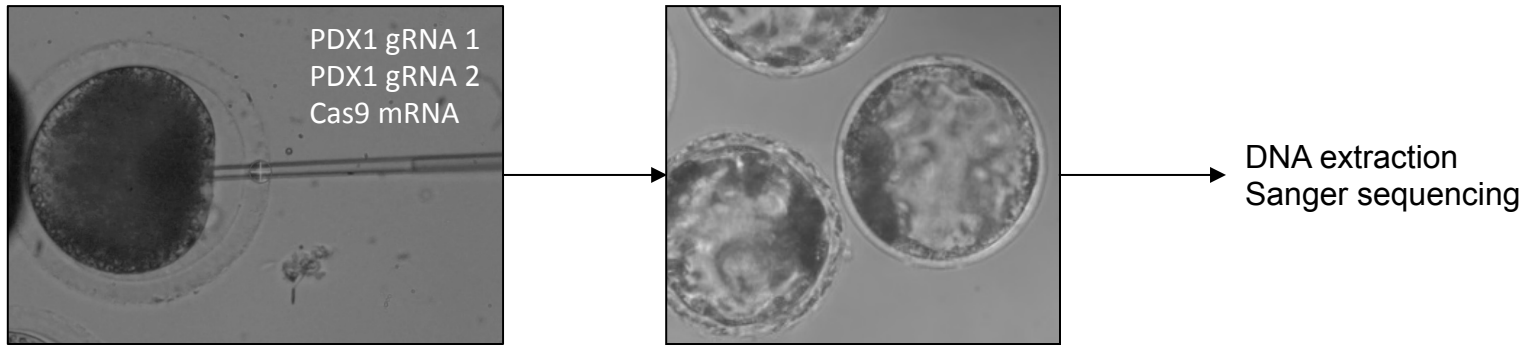* These authors contributed equally to this work

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A

PDX1 gene

Exon 1   Exon 2

5'   3'

gRNA1 target sequence: CCGGACATCTCCCCGTACGA

Zygote → Blastocyst

B   **Bovine *PDX1* locus**

gRNA target site

5'GCTGGAGCAGGGCAGCCCCCGGACATCTCCCCGTACGAGGTGCCTCCGCTAGCCG-3'

Mono-allelic mutations:

```
5'-GCTGGAGCAGGGCAGCCCCCC------TCCCCGTACGAGGTGCCTCCGCTAGCCG-3'  (-6)
5'-GCTGGAGCAGGGCAGCCCCCC------TCCCCGTACGAGGTGCCTCCGCTAGCCG-3'  (-6)
5'-CCCTGGCCTG---------------------------------------GGCTGG-3'  (+4, -124)
5'-GCTGGAGCAGGGCAGCCCCCC------TCCCCGTACGAGGTGCCTCCGCTAGCCG-3'  (-6)
5'-GCTGGAGCAGGGCAGCCCCC-----------TGGTACGAGGTGCCTCCGCTAGCCG-3' (+2, -13)
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Bi-allelic mutations:

```
5'-GCTGGAGCAGGGCAGCCCCCCCGTACATCTCCCCGTACGAGGTGCCTCCGCTA-3'    (1 sub.)
5'-GCTGGAGCAGGGCAGCCCCCCG------------TACGAGGTGCCTCCGCTAGC-3'   (-12)
5'-GCTGGAGCAGGGCAGCCCCCC------------TACGAGGTGCCTCCGCTA-3'      (-12)
5'-TCCCCG-------------------------TACGAGGTGCCTCCGCTAGC-3'      (-47)
5'-GCTGGAGCAGGGCAGCCCCTCCCGTGCACCTCGTACGAGGTGCCTCCGCTAGC-3'   (12 sub.)
5'-GCTGGAGCAGGGCAGCCCCCCGCACATCTCCCCGTACGAGGTGCCTCCGCTAGC-3'  (+1)
5'-GCTGGAGCAGGGCAGCCCCCC-------------------------TGGAGG-3'     (-137)
5'-GCTGGAGCAGGGCAGCCCCCC-------------------------TGGAGG-3'     (-12)
5'-GCTGGAGCAGG------------ACATCTCCCCGTACGAGGTGCCTCCGCTAGCCG-3' (-72)
5'-GCTGGAGCAGGGCAGCCCCCC------------TACGAGGTGCCTCCGCTAGCCG-3'  (-12)
5'-GCTGGAGCAGGGCAGCCCCCC------------TACGAGGTGCCTCCGCTAGCCG-3'  (-12)
```

WT:

```
5'GCTGGAGCAGGGCAGCCCCCCGGACATCTCCCCGTACGAGGTGCCTCCGCTAGCCG-3'
5'GCTGGAGCAGGGCAGCCCCCCGGACATCTCCCCGTACGAGGTGCCTCCGCTAGCCG-3'
```

C   **Pig *PDX1* locus**

gRNA target site

5'-GCGCGCTGGAGCAGGGCGGCCCCCCGGACATCTCCCCGTACGAGGTGCCCCCGCTCGCCGACG-3'

Mono-allelic mutations:

```
5'-GCGCGCTGGAGCAGGGCGACCCCCCGGACATCTCCCCGTACGAGGTGCCCCCGCTCCCCGACG-3' (2 sub.)
5'-TGGGCCCCCGCGACCAGTG-------------GGCCCCCCCGGACATCTCCCCGTACGAGGTG-3'  (+19, -48)
```

WT:

```
5'-GCGCGCTGGAGCAGGGCGGCCCCCCGGACATCTCCCCGTACGAGGTGCCCCCGCTCGCCGACG-3'
5'-GCGCGCTGGAGCAGGGCGGCCCCCCGGACATCTCCCTGTACGAGGTGCCCCCGCTCGCCGATG-3'
5'-GCGCGCTGGAGCAGGGCGGCCCCCCGGACATCTCCCCGTACGAGGTGCCCCCGCTCGCCGACG-3'
5'-GCGCGCTGGAGCAGGGCGGCCCCCCGGACATCTCCCCGTACGAGGTGCCCCCGCTCGCCGACG-3'
5'-GCGCGCTGGAGCAGGGCGGCCCCCCGGACATCTCCCCGTACGAGGTGCCCCCGCTCGCCGACG-3'
5'-GCGCGCTGGAGCAGGGCGGCCCCCCGGACATCTCCCCGTACGAGGTGCCCCCGCTCGCCGACG-3'
5'-GCGCGCTGGAGCAGGGCGGCCCCCCGGACATCTCCCCGTACGAGGTGCCCCCGCTCGCCGACG-3'
5'-GCGCGCTGGAGCAGGGCGGCCCCCCGGACATCTCCCCGTACGAGGTGCCCCCGCTCGCCGACG-3'
5'-GCGCGCTGGAGCAGGGCGGCCCCCCGGACATCTCCCCGTACGAGGTGCCCCCGCTCGCCGACG-3'
5'-GCGCGCTGGAGCAGGGCGGCCCCCCGGACATCTCCCCGTACGAGGTGCCCCCGCTCGCCGACG-3'
5'-GCGCGCTGGAGCAGGGCGGCCCCCCGGACATCTCCCCGTACGAGGTGCCCCCGCTCGCCGACG-3'
```

A

PDX1 gRNA 1
PDX1 gRNA 2
Cas9 mRNA

DNA extraction
Sanger sequencing

B

Pig *PDX1* locus

gRNA1 target site (reverse)   gRNA2 target site (forward)

5'-GGCGGC**CCC**CCGGACATCTCCCCGTACGAGGTGCCC-------158bp-------CAAGGC**TCACGCGTGGAAAGGCCAGTGGG**CAG-3'

Mono-allelic mutations

5'---//--CCCGTACGAGGTGCCCCCCGTACGAGGTGCCCCCGCTCGCCG**TG**--------//---------GGAAAGGCCAGT**GGG**CAG-3' (-37, +2, -165)
5'-GGCGGC**CC**TTA----------//---CCT**GTGGG**CAG-3' (+3, -212)
5'-GGCGGC**CCC**CCGGACA**A**CTCCC**A**GCACGA**A**GA**GCCA**CCG□------------------//--------------------**GGG**CAG-3' (+187, -184)
5'-**GTA**---//------------------------**AGTGGG**CAG-3' (+3, -223)
5'-GGCGGC**CCC**CCGGACATCTCCCCGTACGAGGTGCCC□//□------//-------------------------CCAGT**GGG**CAG-3' (+10, -11, +38, -39)
5'---//-----------------------------------3' (-298)
5'---//-------------------------------------3' (-254)
5'-GGCGGC**CCC**CCG--------//---------//--------------------------------------AGT**GGG**CAG-3' (-208)
5'-□-----------------------------//--AGT**GGG**CAG-3' (+72, -265)
5'--------------------------------//--**GT GGG**CAG-3' (-243)

Bi-allelic mutations

5'- ---//-----------------------------GGG**C**CAGT**GGG**CAG-3' (**-255**)
5'-GGCGGC**CCC**CCG-------------------//---AGT**GGG**CAG-3' (**-208**)
5'- ---//-----------------------------AGT**GGG**CAG-3' (**-242**)

Wild-type

5'-GGCGGC**CCC**CCGGACATCTCCCCGTACGAGGTGCCC-------158bp-------CAAGGC**TCACGCGTGGAAAGGCCAGTGGG**CAG-3'
5'-GGCGGC**CCC**CCGGACATCTCCCCGTACGAGGTGCCC-------158bp-------CAAGGC**TCACGCGTGGAAAGGCCAGTGGG**CAG-3'
5'-GGCGGC**CCC**CCGGACATCTCCCCGTACGAGGTGCCC-------158bp-------CAAGGC**TCACGCGTGGAAAGGCCAGTGGG**CAG-3'
5'-GGCGGC**CCC**CCGGACATCTCCCCGTACGAGGTGCCC-------158bp-------CAAGGC**TCACGCGTGGAAAGGCCAGTGGG**CAG-3'
5'-GGCGGC**CCC**CCGGACATCTCCCCGTACGAGGTGCCC-------158bp-------CAAGGC**TCACGCGTGGAAAGGCCAGTGGG**CAG-3'
5'-GGCGGC**CCC**CCGGACATCTCCCCGTACGAGGTGCCC-------158bp-------CAAGGC**TCACGCGTGGAAAGGCCAGTGGG**CAG-3'
5'-GGCGGC**CCC**CCGGACATCTCCCCGTACGAGGTGCCC-------158bp-------CAAGGC**TCACGCGTGGAAAGGCCAGTGGG**CAG-3'
5'-GGCGGC**CCC**CCGGACATCTCCCCGTACGAGGTGCCC-------158bp-------CAAGGC**TCACGCGTGGAAAGGCCAGTGGG**CAG-3'
5'-GGCGGC**CCC**CCGGACATCTCCCCGTACGAGGTGCCC-------158bp-------CAAGGC**TCACGCGTGGAAAGGCCAGTGGG**CAG-3'

C

E28 pig embryos
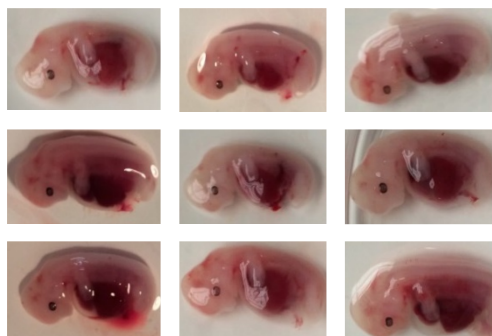
Supplementary figure legends

Figure S1. Gene disruption with single sgRNA-guided Cas9 at pig and cattle *PDX1* loci. (A) Schematic diagram showing the location and sequence of a gRNA (gRNA1) designed to target exon1 of both pig and cattle *PDX1* gene. Bovine and porcine zygotes were co-injected with Cas9 mRNA and sgRNA1 and *in vitro* cultured until the blastocyst stage for blastocyst genotyping. (B) Sanger sequencing results for bovine blastocysts. The results were from two independent experiments (C) Sanger sequencing results for porcine blastocysts. The results were from two independent experiments. Red dashes represent deletions and red letters indicate insertions or substitutions; insertions (+) or deletions (-) are shown to the right of each allele. For cattle, the mutated blastocysts (83.3%, 10/12) are composed of 41.7% (5/12) of mono-allelic mutations and 41.7% (5/12) of bi-allelic mutations. For pigs they were 15% (2/13) of the embryos with mono-allelic mutations.

Figure S2. Gene disruption with dual sgRNA-guided Cas9 at pig *PDX1* loci. (A) The workflow in testing the efficacy and efficiency of *PDX1* disruption with dual sgRNA-guided Cas9. Cas9 mRNA and PDX1 sgRNA1 and sgRNA2 were co-injected into a pig zygote and cultured until the blastocyst stage. Genomic DNA was isolated from the blastocysts and subjected to Sanger sequencing to determine the genotypes. (B) Sanger sequencing results at the *PDX1* locus of porcine blastocysts developed from zygotes injected with two sgRNAs. The results were from two independent experiments. The PAM sequence is underlined, and the targeted sequences complementary to PDX1 gRNA1 and gRNA2 are shown in blue. Red dashes and // represent deletions and red letters and [] insertions; insertions (+) or deletions (-) are shown to the right of each allele. (C) Left, a representative bright field image showing the porcine female reproductive tract at day 28 of pregnancy. Right, nine pig embryos were collected at day 28 of development.

Full gel image for Figure 1B

Pig embryos injected with PDX1 gRNAs (g1/g2)
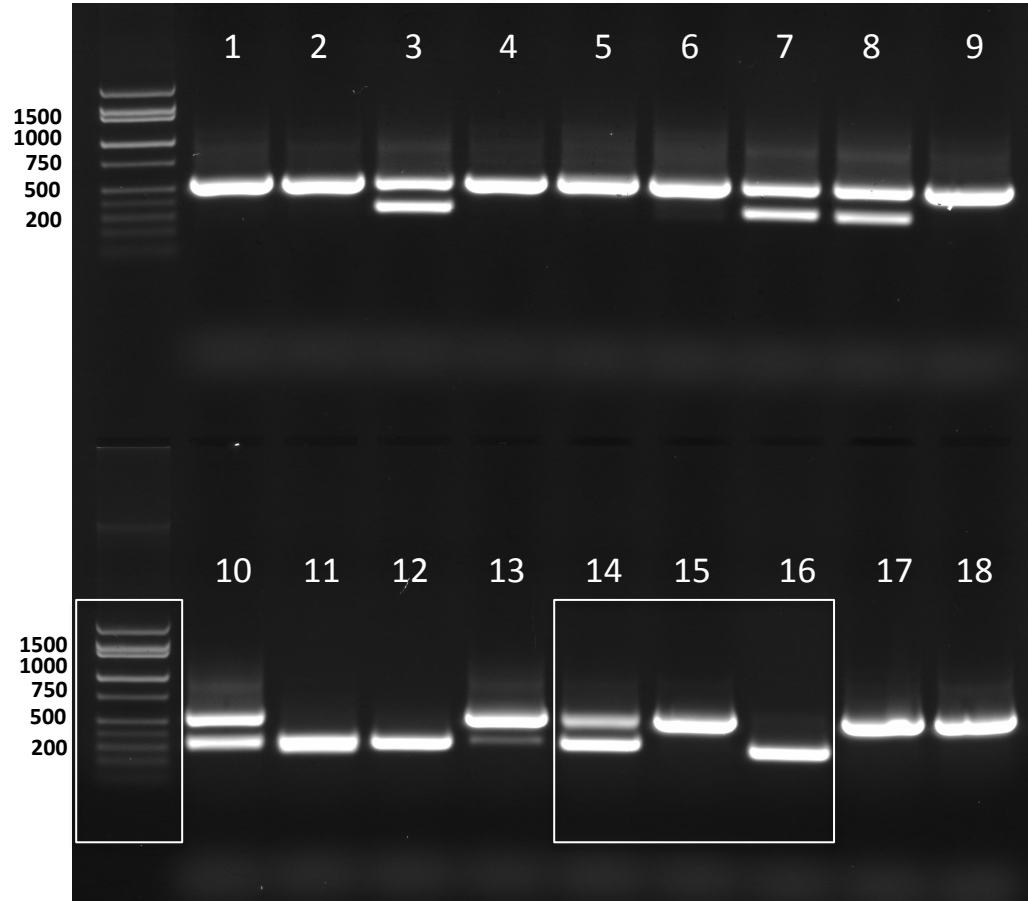
1-2 & 4-5: PDX1-g1
3 & 6-17: PDX1-g1+g2
18: Control pig genomic DNA