Fig. S1

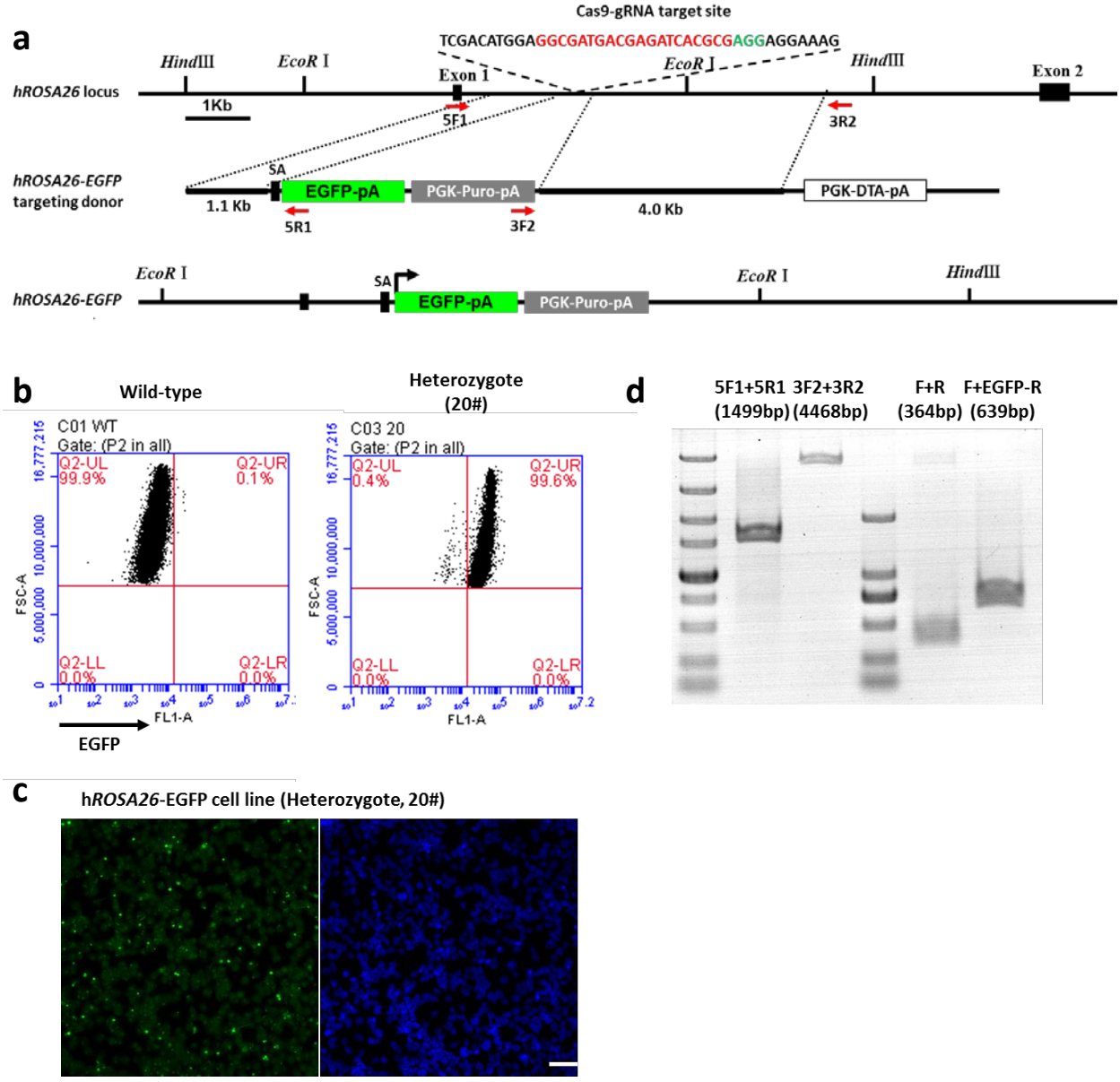


Fig. S1 Identification of HEK293-EGFP cell line. (**a**) A diagram for Cas9-mediated knock-in of *EGFP* into the human *ROSA26* locus via HDR. SA, splice acceptor. (**b**) FACS analysis of HEK293-EGFP cell line. Left: HEK293 WT cell line, right: HEK293-EGFP cell line. (**c**) Knock-in pattern analysis of HEK293-EGFP cell line by PCR. 5F1+5R1 were used for detecting 5-ARM, and 3F2+3R2 detecting 3-ARM. (**d**) Fluorescence microscopy of HEK293-EGFP cell line. Scale bar, 20 μm.

Fig. S2

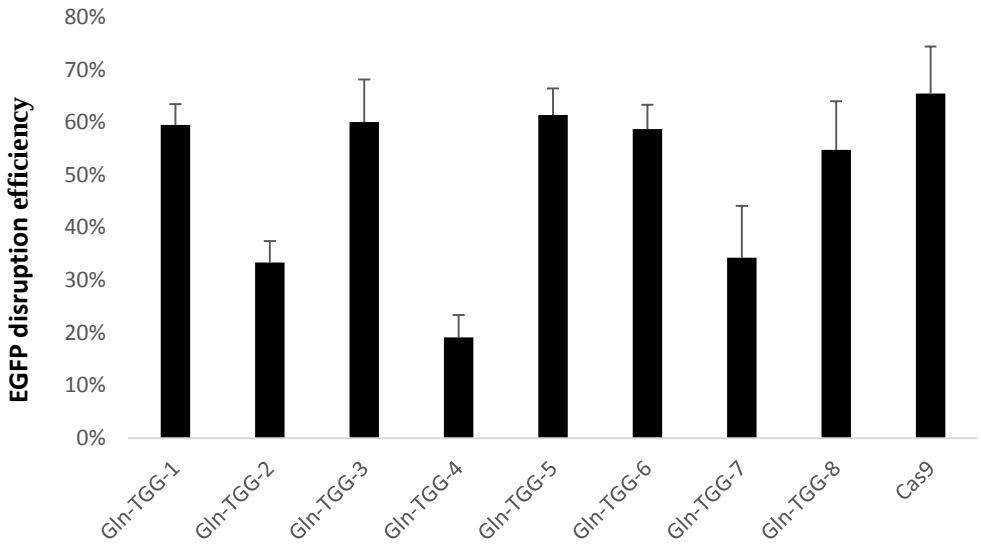


Fig. S2 The EGFP disruption efficiency of Cpf1-gRNA^{tRNA} with the same tRNA sequence but different 5' leader sequence were measured by FACS analysis. Error bars indicate s.e.m. (n = 3 independent experiments).

Fig. S3

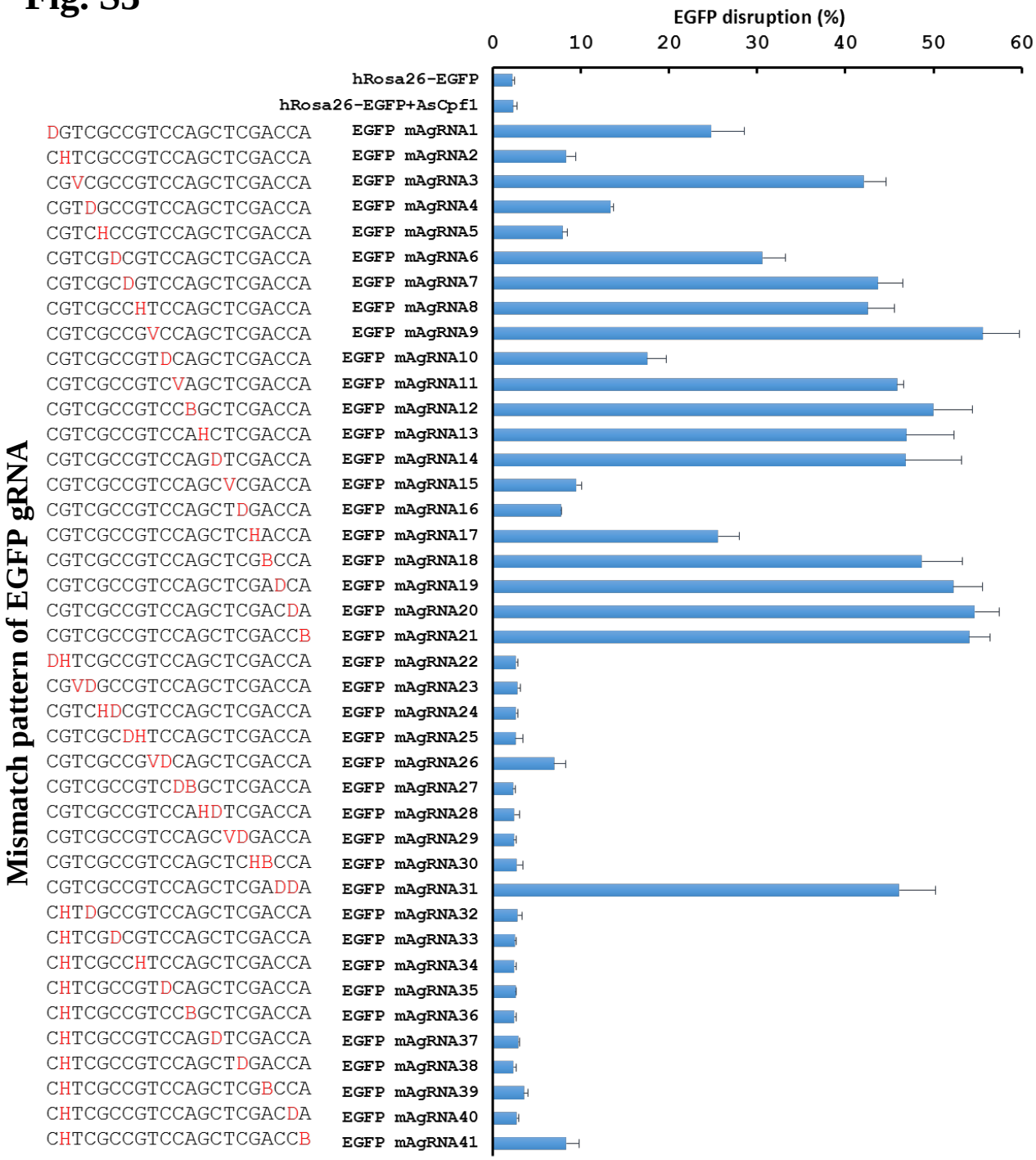


Fig. S3 The targeting efficiency of Cpf1-gRNA^{tRNA} for EGFP harboring single or two mismatches in positions 1 through 21 were measured by FACS analysis. Error bars indicate s.e.m. (n = 3 independent experiments).

Fig. S4

a

As-16	GTAATTTCTACTCTTGTAGATCGTCGCCGTCCAGCTC-(htRNA-Gln-TTG-1-1)-polyT
As-17	GTAATTTCTACTCTTGTAGATCGTCGCCGTCCAGCTCG-(htRNA-Gln-TTG-1-1)-polyT
As-18	GTAATTTCTACTCTTGTAGATCGTCGCCGTCCAGCTCGA-(htRNA-Gln-TTG-1-1)-polyT
As-19	GTAATTTCTACTCTTGTAGATCGTCGCCGTCCAGCTCGAC-(htRNA-Gln-TTG-1-1)-polyT
As-20	GTAATTTCTACTCTTGTAGATCGTCGCCGTCCAGCTCGACC-(htRNA-Gln-TTG-1-1)-polyT
As-21	GTAATTTCTACTCTTGTAGATCGTCGCCGTCCAGCTCGACCA-(htRNA-Gln-TTG-1-1)-polyT
As-22	GTAATTTCTACTCTTGTAGATCGTCGCCGTCCAGCTCGACCAG-(htRNA-Gln-TTG-1-1)-polyT
As-23	GTAATTTCTACTCTTGTAGATCGTCGCCGTCCAGCTCGACCAGG-(htRNA-Gln-TTG-1-1)-polyT
As-24	GTAATTTCTACTCTTGTAGATCGTCGCCGTCCAGCTCGACCAGGA-(htRNA-Gln-TTG-1-1)-polyT
As-25	GTAATTTCTACTCTTGTAGATCGTCGCCGTCCAGCTCGACCAGGAT-(htRNA-Gln-TTG-1-1)-polyT
As-26	GTAATTTCTACTCTTGTAGATCGTCGCCGTCCAGCTCGACCAGGATG-(htRNA-Gln-TTG-1-1)-polyT
As-27	GTAATTTCTACTCTTGTAGATCGTCGCCGTCCAGCTCGACCAGGATGG-(htRNA-Gln-TTG-1-1)-polyT
As-28	GTAATTTCTACTCTTGTAGATCGTCGCCGTCCAGCTCGACCAGGATGGG-(htRNA-Gln-TTG-1-1)-polyT
As-29	GTAATTTCTACTCTTGTAGATCGTCGCCGTCCAGCTCGACCAGGATGGGC-(htRNA-Gln-TTG-1-1)-polyT
As-30	GTAATTTCTACTCTTGTAGATCGTCGCCGTCCAGCTCGACCAGGATGGGCA-(htRNA-Gln-TTG-1-1)-polyT

b

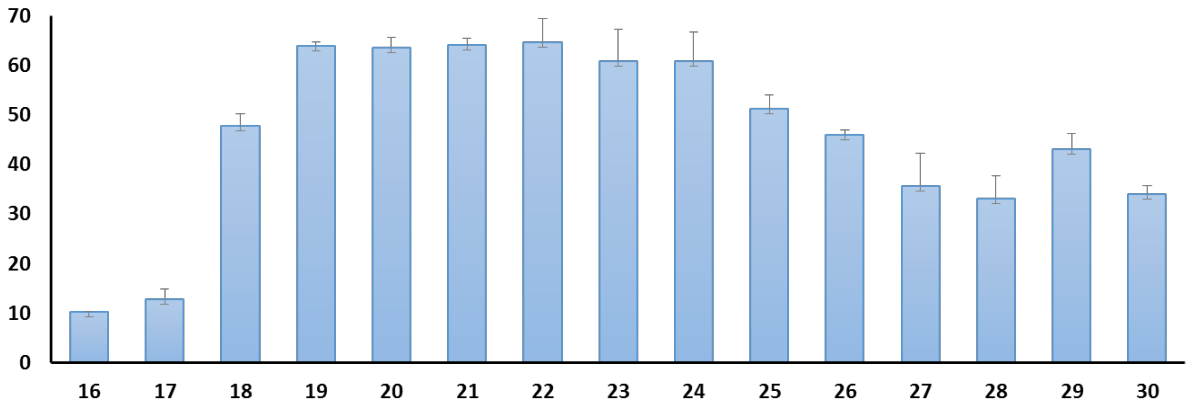
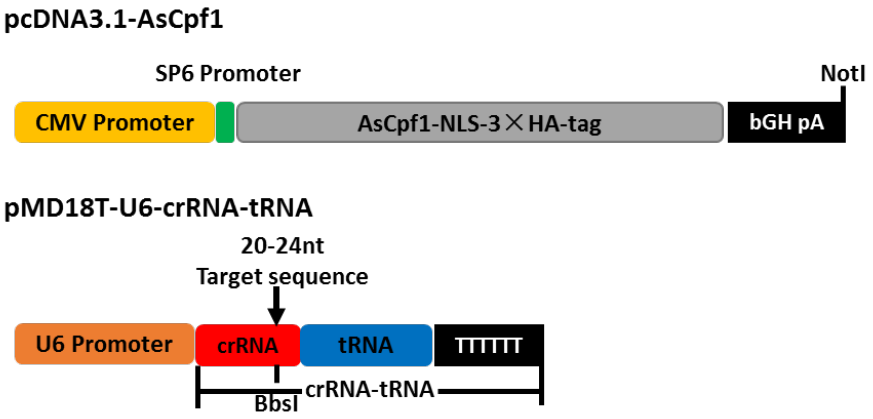


Fig. S4 Effect of EGFP spacer length on AsCpf1 cleavage activity. (**a**) EGFP space length from 16 bp to 30 bp. (**b**) EGFP disruption rate analysis by FACS.

Fig. S5

a



b

Target gene	Target site	PAM	Target gene	Target site	PAM
h*AAVS1*-sg1	GCAGCCTGTGCTGACCCATCGA	TTTG	h*TET2*-sg1	CCAGACAGAACCTCTGGCTACAAA	TTTG
h*AAVS1*-sg2	CGCTGCCCTCCTCTCGCCCCCGA	TTTG	h*TET2*-sg2	CACAAGAAAGTAGAGGGTATTCCA	TTTA
h*AAVS1*-sg3	TTAGGATGGCCTTCTCCGACGGA	TTTC	h*TET2*-sg3	GTAGCAGTGGAGAGCTACAGGACA	TTTG
h*AAVS1*-sg4	GGCAGCTCCCCTACCCCCCTTA	TTTG	h*TET2*-sg4	ACTAGACAAACCACTGCTGCAGAA	TTTG
h*AAVS1*-sg5	CTGGAGCCATCTCTCTCCTTGCCA	TTTC	p*ROSA26*-sg	TGACACAAACTCAAGACTGTGGGA	TTTG
h*TET1*-sg1	TTTACATCTTCCTTCCTGACTAA	TTTG	p*APP*-sg	GTGATGAACCTCATAGCCTGAA	TTTG
h*TET1*-sg2	GATAGGACTGAGGTTCTTTTTCA	TTTG	p*GGTA1*-sg	GATGGCTTTCATCATGCCACTCG	TTTA
h*TET1*-sg3	GTGCTATCCCACATCAATGGGAA	TTTG	p*NLRP3*-sg	CATTCACTGTCGGGAGGTGAGCC	TTTA
h*TET1*-sg4	GGTATGGGTTGCATCCTGACATG	TTTG			

Fig. S5 Cpf1-gRNA^{tRNA} **system mediated efficiently genome editing in human cells and mammal embryos.** (**a**) Schematic depiction of the AsCpf1 and gRNA^{tRNA} transcription plasmid. (**b**) Target sequences of gRNAs used to target human and porcine endogenous gene in this study.

Fig. S6

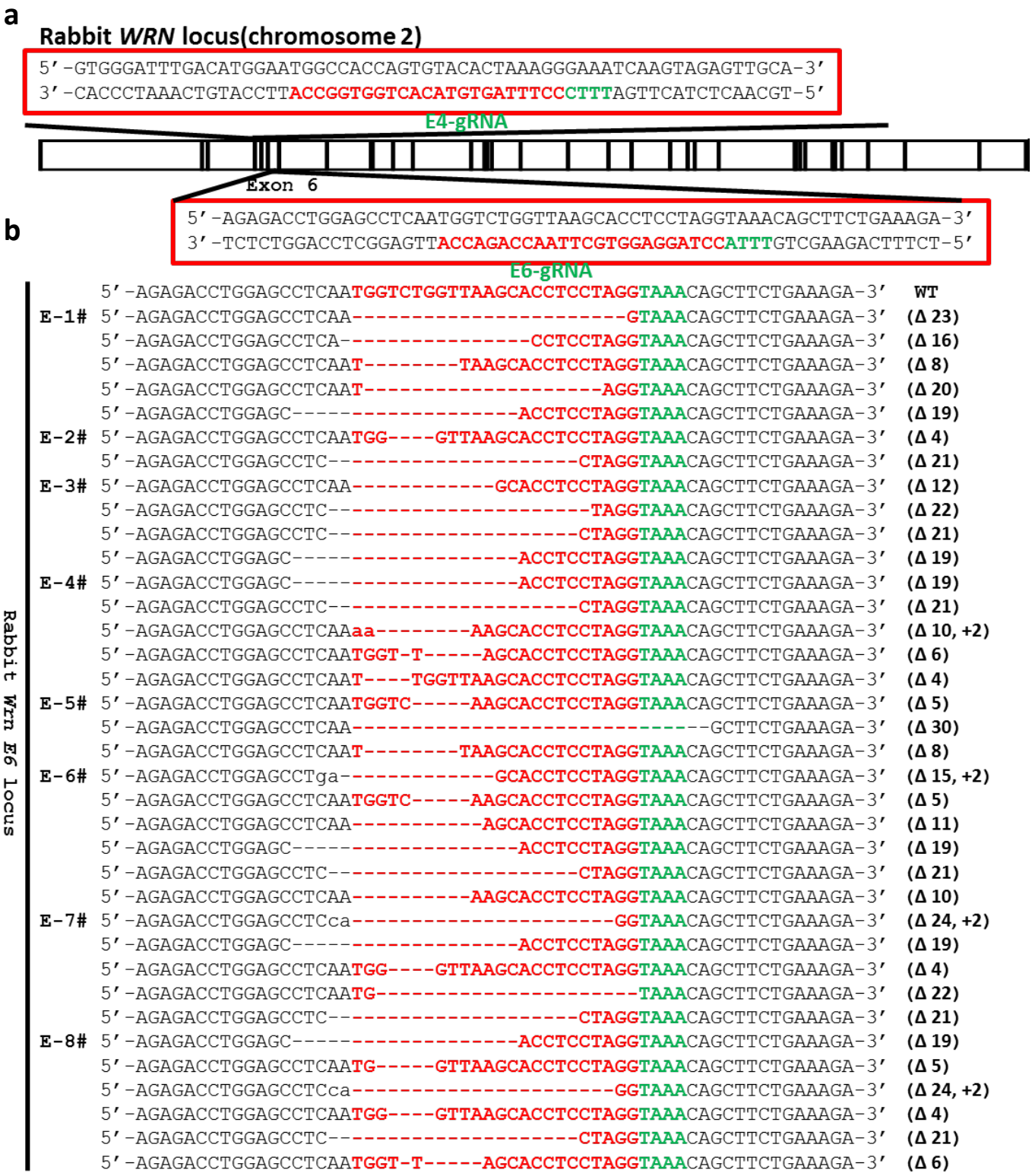
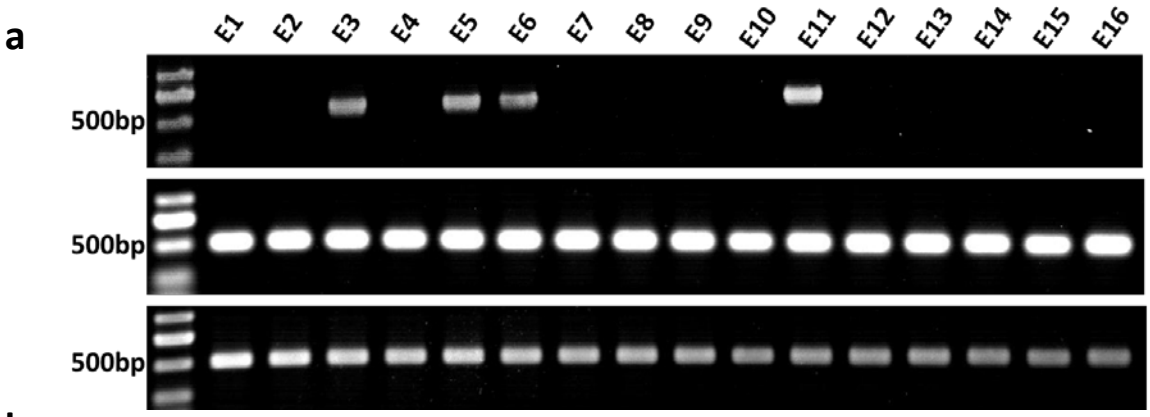


Fig. S6 Genome editing of *WRN* gene in rabbits embryos via the Cpf1- gRNA^{tRNA} system. (**a**) Two target sites in the rabbit *WRN* locus at exons 4 and 6, respectively. The target sequence and PAM are indicated by red and green. (**b**) Genotype of rabbit blastocysts injection AsCpf1 mRNA and *WRN* Exon 4 gRNA^{tRNA}.

Fig. S7



b

E4-gRNA E6-gRNA

```
5′-GATTTGACATGGAATGGCCACCAGTGTACACTAAAGGGAAAT(1750bp)ATGGTCTGGTTAAGCACCTCCTAGGTAAAC-3′  WT
E3 5′-GATTTGA----------ACCTCCTAGGTAAAC-3′  (Δ 1803)
E5 5′-GATTTGACATGGA----------TCCTAGGTAAAC-3′  (Δ 1080)
E6 5′-GATTTGACATG----------GCACCTCCTAGGTAAAC-3′  (Δ 1794)
E11 5′-GATTTGACATGGAA----------ACCTCCTAGGTAAAC-3′  (Δ 1793)
```

c

E4-gRNA

```
5′-GTGGGATTTGACATGGAATGGCCACCAGTGTACACTAAAGGGAAATCAAGTAGAGTTGCA-3′  WT
E1 5′-GTGGGATTTGACATGGAA-------CAGTGTACACTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 7)
E2 5′-GTGGGATTTGACATGGAA--------AGTGTACACTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 8)
5′-GTGGGATTTG----------TACACTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 20)
E3 5′-GTGGGATTTGACATG----------TACACTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 15)
5′-GTGGGATTTGACA----------CTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 21)
E5 5′-GTGGGATTTGACATGGAA-------CAGTGTACACTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 7)
5′-GTGGGATTTGACATGGA--------CAGTGTACACTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 8)
E6 5′-GTGGGATTTGACATG----------TACACTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 15)
5′-GTGGGATTTGACA----------CTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 21)
E7 5′-GTGGGATTTGACATGGAA--------AGTGTACACTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 8)
E9 5′-GTGGGATTTGACATG----------CTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 19)
E11 5′-GTGGGATTTGACATG----------ACACTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 16)
E12 5′-GTGGGATTTGACATGGAA----------GTACACTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 11)
5′-GTGGGATTTGACATGG----------CTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 18)
E14 5′-GTGGGATTTGACA----------CTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 21)
E15 5′-GTGGGATTTGACATGGA--------CAGTGTACACTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 8)
5′-GTGGGATTTG----------TACACTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 20)
E16 5′-GTGGGATTTGACA----------CACTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 19)
5′-GTGGGATTTGACA----------CTAAAGGGAAATCAAGTAGAGTTGCA-3′  (Δ 21)
```

d

E6-gRNA

```
5′-AGAGACCTGGAGCCTCAATGGTCTGGTTAAGCACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  WT
E1 5′-AGAGACCTGGAG----------CACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 19)
5′-AGAGACCTGGAGCCTCAATGGT-----TAAGCACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 5)
E2 5′-AGAGACCTGGAG----------CCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 21)
5′-AGAGACCTGGAGCCTCAAT----------AGCACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 10)
E3 5′-AGAGACCTGGAGCCTCAAT----------AGCACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 10)
E5 5′-AGAGACCTGGAGCCTCAATGGT-----TAAGCACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 5)
E6 5′-AGAGACCTGGAGCCTCAA--------TTAAGCACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 8)
E7 5′-AGAGACCTGGAGCCTCAAT----------AGCACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 10)
5′-AGAGACCTGG----------AGCACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 19)
E9 5′-AGAGACCTGGAGCCTCA----------CACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 14)
5′-AGAGACCTGGAGCCTCAATGGT-----TAAGCACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 5)
E11 5′-AGAGACCTGGAGCCTCAA--------TTAAGCACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 8)
E12 5′-AGAGACCTGGAGCCTCAATGGT-----TAAGCACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 5)
5′-AGAGACCTGGAGCCTCAA--------TTAAGCACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 8)
E14 5′-AGAGACCTGGAGCCTCAAT----------AGCACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 10)
E15 5′-AGAGACCTGGAGCCTCA----------CACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 14)
5′-AGAGACCTGG----------AGCACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 19)
E16 5′-AGAGACCTGGAGCCTCAAT----------AGCACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 10)
5′-AGAGACCTGGAGCCTCA----------CACCTCCTAGGTAAACAGCTTCTGAAAGA-3′  (Δ 14)
```

Fig. S7 Mutation patterns analysis of rabbit blastocysts when co-injection E4-gRNA^{tRNA} and E6-gRNA^{tRNA} with AsCpf1 mRNA. (**a**) Agarose gel electrophoresis assay were performed to identify *WRN* large fragment deletion in collected blastcysts. Embryos 3#, 5#, 6#, and 11# harbored large fragment deletion. (**b**) T-cloning and Sanger sequencing of deletion of *WRN* in 3#, 5#, 6#, and 11# embryos. PAM sites are highlighted in green; target sequences are red. (**c**) and (**d**) T-cloning and Sanger sequencing of the target site for each gRNA in injected embryos. PAM sites are labeled in green; target sequences are red.

Fig. S8

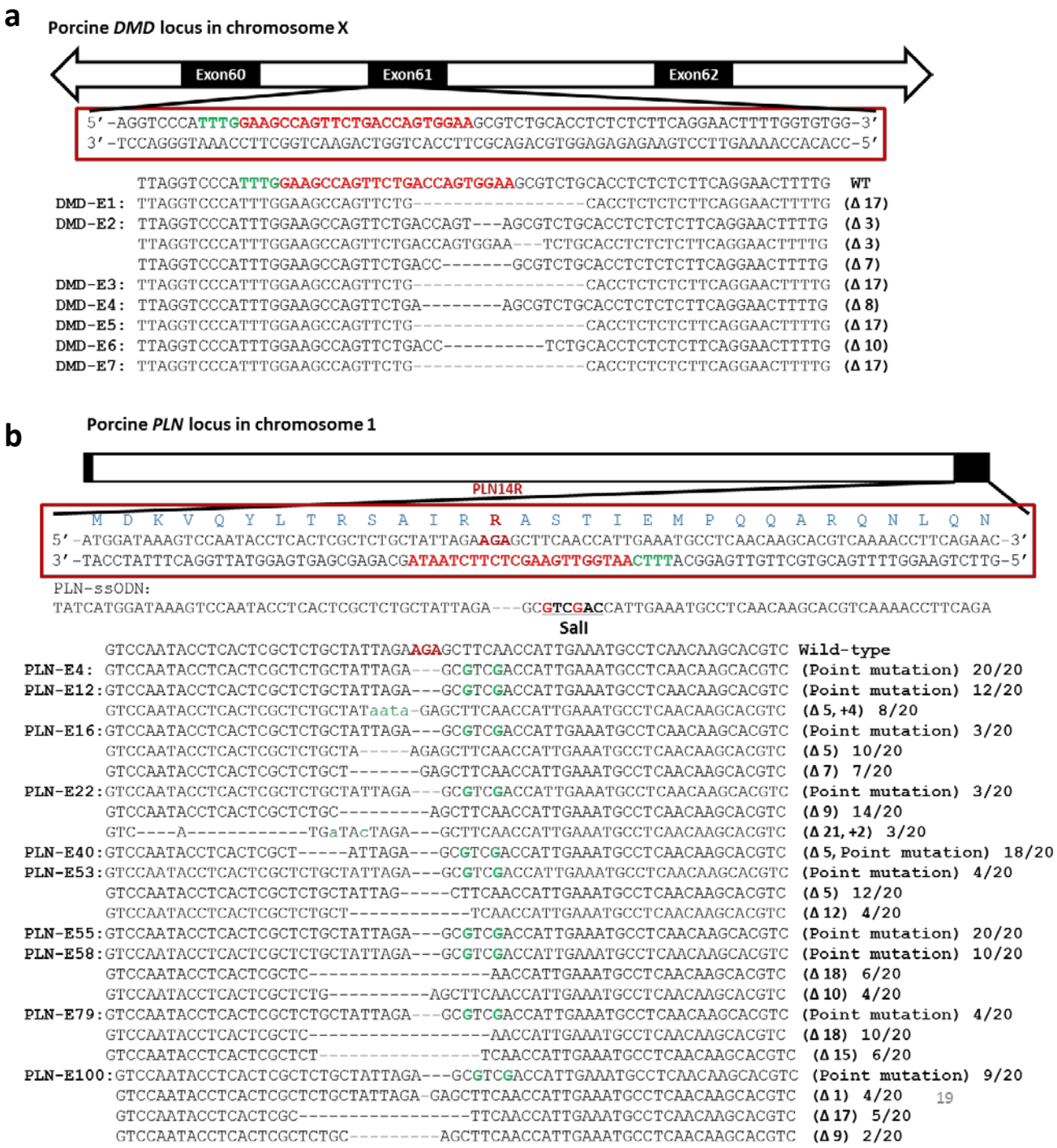


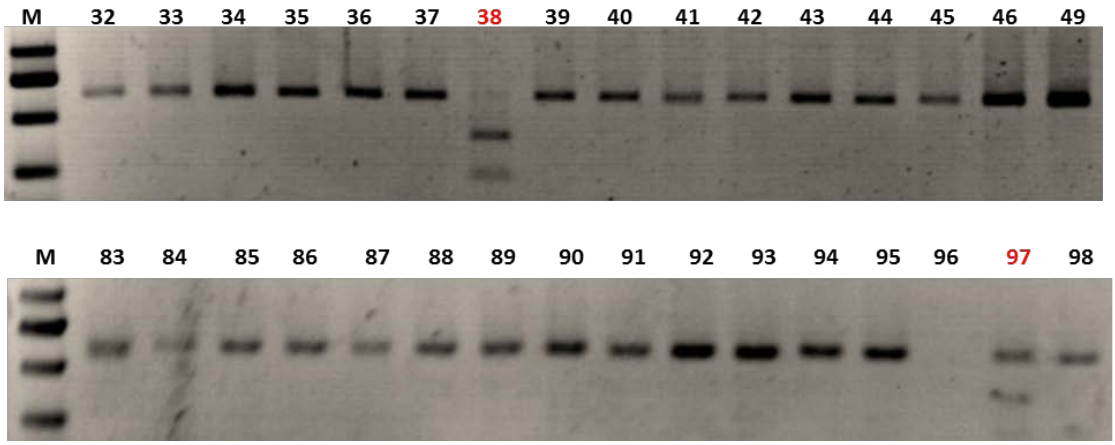
Fig. S8 Genotype of PA porcine embryos injected with Cpf1-gRNA^{tRNA} system targeting porcine *DMD* and *PLN* loci.

Fig. S9

a *DMD* mutant colonies

```
                TTAGGTCCCATTTGGAAGCCAGTTCTGACCAGTGGAAGCGTCTGCACCTCTCTCTTCAGGAACTTTTG Wild-type
DMD-C2:         TTAGGTCCCATTTGGAAGCCAGTTCTG----------------CACCTCTCTCTTCAGGAACTTTTG (Δ 17)
DMD-C10:        TTAGGTCCCATTTGGAAGCCAGTTCTG----------------CACCTCTCTCTTCAGGAACTTTTG (Δ 17)
DMD-C13:        --------------------------------TGGAAGCGTCTGCACCTCTCTCTTCAGGAACTTTTG (Δ 94)
DMD-C16:        TTAGGTCCCATTTGGAAGCCAGTTCTGA--------AGCGTCTGCACCTCTCTCTTCAGGAACTTTTG (Δ 8)
DMD-C20:        TTAGGTCCCATTTGGAAGCCAGTTCTG----------------CACCTCTCTCTTCAGGAACTTTTG (Δ 17)
DMD-C38:        TTAGGTCCCATTTGGAAGCCAGTTCTG----------------CACCTCTCTCTTCAGGAACTTTTG (Δ 17)
DMD-C44:        TTAGGTCCCATTTGGAAGCCAGTTC--------------------------------AGGAACTTTTG (Δ 32)
DMD-C52:        TTAGGTCCCATTTGGAAGCCAGTTCTGACC----------TCTGCACCTCTCTCTTCAGGAACTTTTG (Δ 10)
DMD-C53:        TTAGGTCCCATTTGGAAGCCAGTTCTG----------------CACCTCTCTCTTCAGGAACTTTTG (Δ 17)
```

b



c *PLN* mutant colonies carrying point mutations

```
                ACCTCACTCGCTCTGCTATTAGAAGAGCTTCAACCATTGAAATGCCTCAACAAGCACGTCAAAACCTT Wild-type
PLN-C38:        ACCTCACTCGCTCTGCTATTAGA---GCGTCGACCATTGAAATGCCTCAACAAGCACGTCAAAACCTT PLN14Rdel
                ACCTCACTCGCTCTGCTATTAGA---GCGTCGACCATTGAAATGCCTCAACAAGCACGTCAAAACCTT PLN14Rdel
PLN-C97:        ACCTCACTCGCTCTGCTATTAGA---GCGTCGACCATTGAAATGCCTCAACAAGCACGTCAAAACCTT PLN14Rdel
                ACCTCACTCGCTCTGC--------GAGCTTCAACCATTGAAATGCCTCAACAAGCACGTCAAAACCTT (Δ 8)
```

d

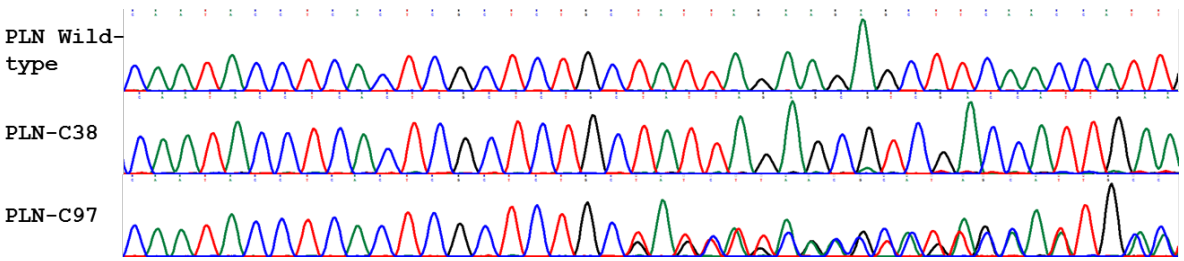


Fig. S9 Genotype analysis of selected PFF individual colonies. (**a**) Genotype of *DMD* mutant colonies. The WT sequence is shown at the top. The target sequence and PAM are indicated by red and green, respectively. (**b**) Identification of selected colonies by PCR-Sal I digestion. No.39 and 97 colony can be digested by Sal I restriction enzyme. (**c**) Genotype of *PLN*^{R14del} mutant colonies carrying point mutations. (**d**) Sanger sequencing of the target sites in the two *PLN*^{R14del} mutant colonies.

Fig. S10

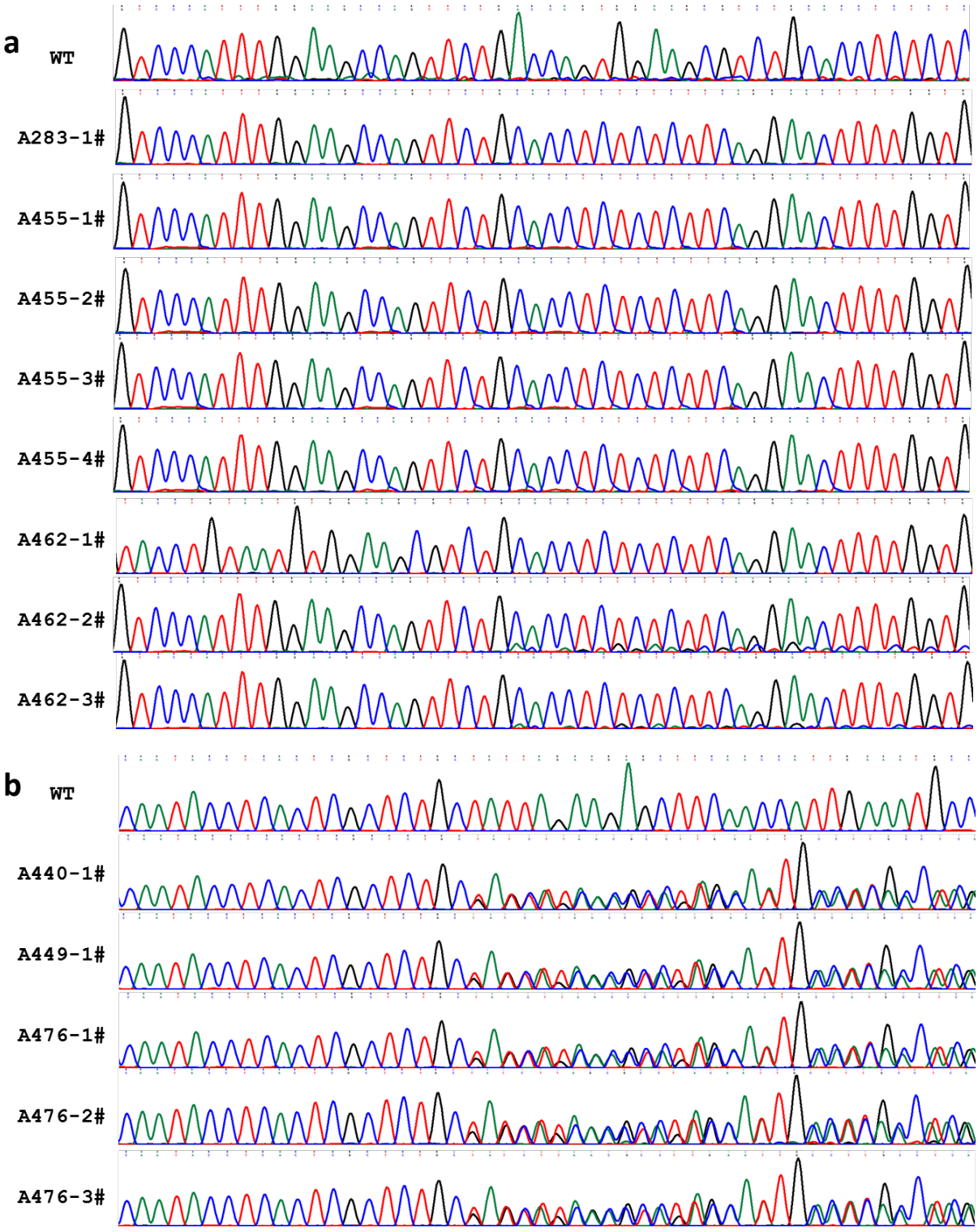


Fig. S10 Sanger sequencing of the target sites in all DMD KO (**a**) and *PLN*^{R14del} (**b**) cloned pigs.