**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  The EGFP disruption efficiency of Cpf1-gRNA \textsuperscript{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).
The targeting efficiency of Cpf1-gRNA 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) EGFP spacer length from 16 bp to 30 bp. (b) EGFP disruption rate analysis by FACS.

**Fig. S4 Effect of EGFP spacer length on AsCpf1 cleavage activity.** (a) EGFP spacer length from 16 bp to 30 bp. (b) EGFP disruption rate analysis by FACS.
Fig. S5 Cpf1-gRNA<sub>trRNA</sub> system mediated efficiently genome editing in human cells and mammal embryos. (a) Schematic depiction of the AsCpf1 and gRNA<sub>trRNA</sub> transcription plasmid. (b) Target sequences of gRNAs used to target human and porcine endogenous gene in this study.
Fig. S6  Genome editing of \textit{WRN} gene in rabbits embryos via the Cpf1-gRNA system. (a) Two target sites in the rabbit \textit{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 \textit{WRN} Exon 4 gRNA.
**Fig. S7** Mutation patterns analysis of rabbit blastocysts when co-injection E4-gRNA and E6-gRNA with AsCpf1 mRNA. (a) Agarose gel electrophoresis assay were performed to identify WRN large fragment deletion in collected blastocysts. 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 Genotype of PA porcine embryos injected with Cpf1-gRNA<sub>rRNA</sub> system targeting porcine DMD and PLN loci.
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  Sanger sequencing of the target sites in all DMD KO (a) and $PLN^{R14del}$ (b) cloned pigs.