



Figure S1. Off-target detection in piglets. Chromatogram sequence analysis of ten POTS for sgRNA using PCR products in P53. Red box labeled base indicates mutation. The piglets with off-target was shown in red. WT wild-type.

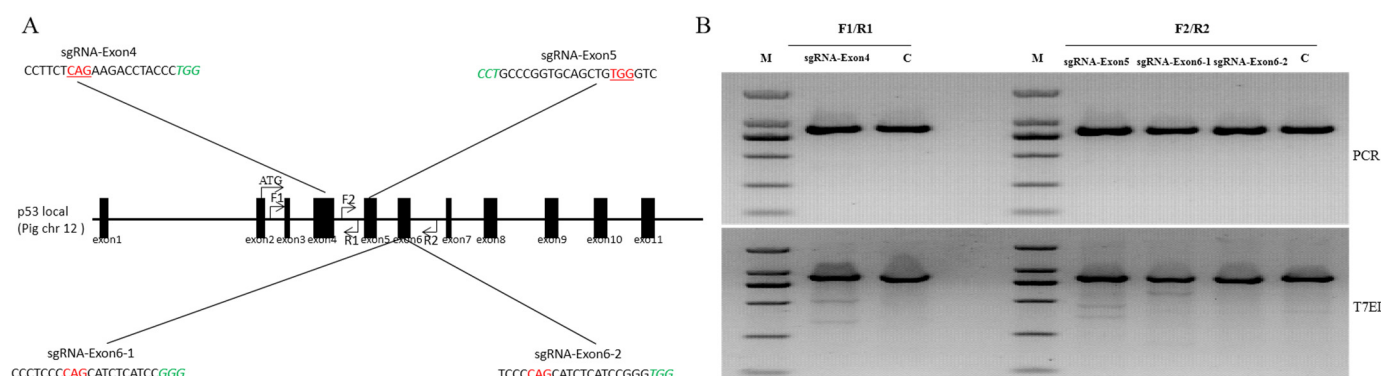


Figure S2. The information of sgRNAs design and the editing activity detected. (A) Schematic diagram of the target site at the P53 locus. sgRNA sequences are presented in black. PAM sequences are highlighted in green. The BE3-mediated nucleotide substitutions are marked in red and underlined. (B) Detection of sgRNA-Exon4, sgRNA-Exon5, sgRNA-Exon6-1 and sgRNA-Exon6-2: BE3-mediated base editing of P53 by PCR and T7EN1 cleavage assay. M, DNA marker; sgRNA-Exon4, P53sgRNA-Exon4; sgRNA-Exon5, P53sgRNA-Exon5; sgRNA-Exon6-1, P53sgRNA-Exon6-1; sgRNA-Exon6-2, P53sgRNA-Exon6-2; C, control.

Table S1. The primers of potential off-target sites (POTS).

NO.	Target	Position	Mismatches	Primer sequence
OT1	crRNA: CCTTCTCAGAAGACCTACCCNGG DNA: CCTTCTCAGATGACCTGCCAAGG	chr1:191778281-191778303	3	F: GCTACAAGGAGCGTTAACTCAG R: AGTTCATAATGTAGAACGCCAGT
OT2	crRNA: CCTTCTCAGAAGACCTACCCNGG DNA: CCTTCTCAGGAGCCCTCCCCTGG	chr1:195746835-195746857	3	F: AAACACCTGCTATCTAAGCTG R: CCCGAATACTCATGGTTGC
OT3	crRNA: CCTTCTCAGAAGACCTACCCNGG DNA: CATTCTCAGCAGACCCACCCCGG	chr14:5758517-5758539	3	F: GCCCTCTCTGAATGAGTCTCGT R: CCACCATCGGGAAAATACCACT
OT4	crRNA: CCTTCTCAGAAGACCTACCCNGG DNA: CCTGCTCAGAAGGCCTCCCCCGG	chr4:2993758-2993780	3	F: GTCCGTGGTCACCACTGGTC R: GCCACCTAAGTCTCACTGTTGC
OT5	crRNA: CCTTCTCAGAAGACCTACCCNGG DNA: CCTTCCCAGAAGGCCTTCCCTGG	chr7:38386638-38386660	3	F: GCCCTATAAAGAGTTTAGCAT R: TCTGTACCTCTACATCCCC
OT6	crRNA: CCTTCTCAGAAGACCTACCCNGG DNA: CCTTCTCTTAAGTCTACCCAGG	chr7:43009897-43009919	3	F: TATATCCAGAGCCAACTAGTCA R: TAGGCCAGTCAATAACAGT
OT7	crRNA: CCTTCTCAGAAGACCTACCCNGG DNA: CCTTCCCAGAAGACCCAGCCAGG	chr10:53780587-53780609	3	F: TGCACATCCTTTGTCAACTCA R: TGCCTTTTCTTCCCTATGCTT
OT8	crRNA: CCTTCTCAGAAGACCTACCCNGG DNA: TCTTCTCAGAAGCCCTTCCCAGG	chr11:74692873-74692895	3	F: GCTGCACATTTTCATCATCCC R: GGCCAGTAAACAAACATCACC
OT9	crRNA: CCTTCTCAGAAGACCTACCCNGG DNA: CCTGCTCAGAAGCCCTGCCCTGG	chr17:39846377-39846399	3	F: CCCAGCACCTTTATACTCAC R: GTGCAGTCAACAAAACACCATC
OT10	crRNA: CCTTCTCAGAAGACCTACCCNGG DNA: GCTTCTCAAGAGAGCTACCCAGG	chr9:58847217-58847239	4	F: TGGCATTTTCTCTGAACCAT R: CCACCTAAGCCAGATGTTCC