## Supplementary Material

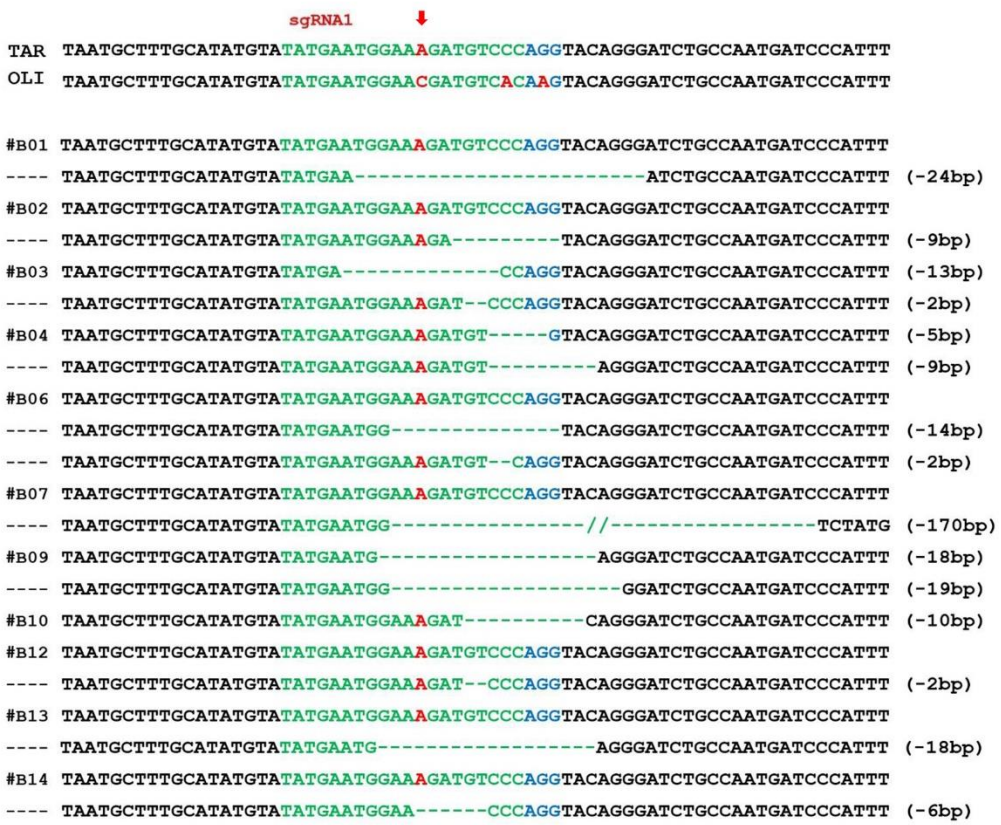


**Figure S1. Editing of the *TYR* gene in blastocysts was verified by T-cloning and Sanger sequencing.** The WT sequence is shown above the target sequence. The sgRNA sequence is marked in green, and the PAM sequences are marked in red and underlined. WT, wild type; deletion, "−"; insertion, "+".


**Figure S2. Editing of the *TYR* gene in rabbits was verified by T-cloning and Sanger sequencing.** The WT sequence is shown above the target sequence. The sgRNA sequence is marked in green, and the PAM sequences are marked in red and underlined. WT, wild type; deletion, "–"; insertion, "+".

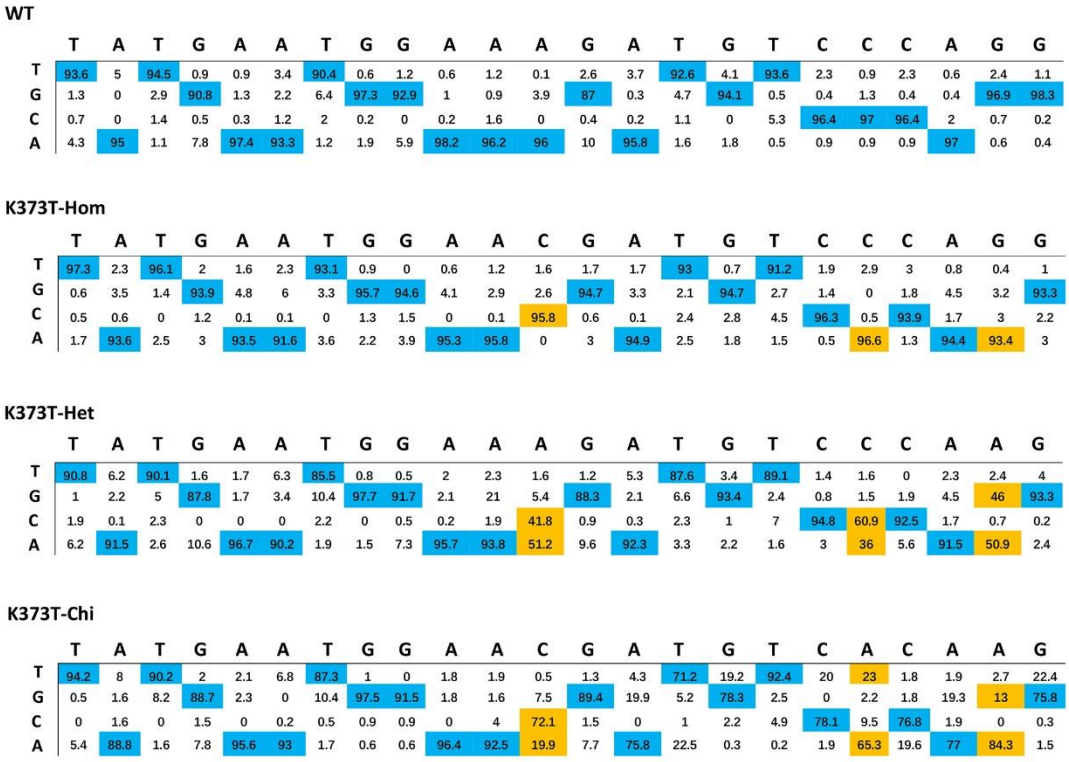


**Figure S3. GUIDE-seq results at sgRNA-targeted genomic sites in TYR-K373T mutant rabbits.** The number represents the percentage of bases in this position. The blue is represented by unaltered base, and yellow represents directed mutation base. K373T-Hom, homozygous mutation of K373T; K373T-Chi, chimaeric mutation of K373T; K373T-Het, heterozygous mutation of K373T; WT, New Zealand white rabbit with a natural T373K mutation.

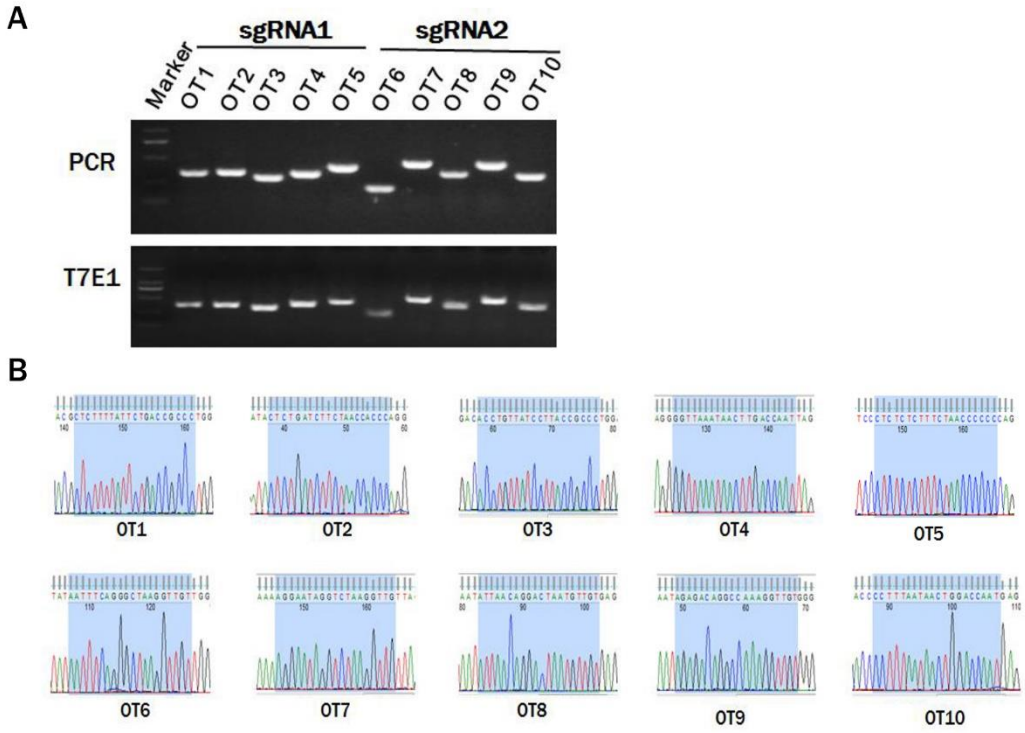


**Figure S4. Identification of off-target effects in TYR K373T mutant rabbits.**

(A) PCR and T7E1 cleavage analysis of POTS for sgRNA. M, DL2000; OT1–10, ten POTS. No fragment was detected in T7EI assays. (B) Chromatogram sequence analysis of 10 POTS for sgRNA in TYR-K373T mutant rabbits; double peaks were not observed in any of the sequencing chromatograms. The blue area represents the POTS sequence.

**Table S1: Oligonucleotide used for sgRNA, PCR and qRT-PCR .**

| Name | Primers | Sequence(5’-3’) | Produce size (bp) |
|---|---|---|---|
| **SgRNA** | SgRNA -1-F | TAGG TATGAATGGAAAGATGTCCC | 24 |
| | SgRNA -1-R | AAAC GGGACATCTTTCCATTCATA | |
| **TYR-cell** | TYR-cell-F | GGCTAGCCATGCGTCTGACTGTTTTATA | |
| | TYR-cell-R | GGAATTCCTTATAGATGGCTCTGATACA | |
| **TYR** | TYR-F | CAGGAGAGAGAGCAAATTGGC | 695 |
| | TYR-R | AGATCTGGCTGAGTCTGAGAC | |
| **TYR-Q** | TYR-Q-F | GGATAGCAGATGCCACTCAAA | 100 |
| | TYR-Q-R | AACGCATGGTGAAGGAGAAA | |
| **GAPDH** | GAPDH-F | TTCCACGGCAGGTCAAGGC | 99 |
| | GAPDH-R | GGGCACCAGCATCACCCCAC | |

* TYR-cell were used for the cell experiment SgRNA were used for the construction of sgRNA expression plasmids; TYR was used for the PCR mutation detection; TYR-Q was used to test the mRNA expression of founder rabbit.

**Table S2.10 Potential off-target sites examined by PCR and the primers used.**

| | Potential off-target site | Number of mismatches | Position | PCR primer |
|---|---|---|---|---|
| 1 | GATTAATGTTACGATGTCCCCGG | 2 | chr7: -148540069 | F: CTAGTCTGGGATCAGACAGGTA<br>R: TCTCATGTCACCTTCACTTTCC |
| 2 | TGTTCATGGACCGATGTCCCTGG | 2 | chrUN0: -1215984 | F: ACTGGGTGTGATTGTGAAGTAA<br>R: CGGAGTGTCTTTGTAGCATCA |
| 3 | TGTTAATTGAACGATGGCCCTGG | 3 | chr18: -17497659 | F: AATATTCCAGGTGGCTTCCC<br>R: CATGGCAATTTGTGTTGCATTT |
| 4 | TATGAAAGGGACGATGTCTCAGG | 3 | chr2: +134076656 | F: GCTACTCTTAGGATCATTGGTTACT<br>R: ATTTGGGATGCTGTGCATAAAG |
| 5 | TATCCATGGAACGCTGTCCCAGG | 3 | chr3: -143791182 | F: CCTCTTTCTGCCTTCCGATTT<br>R: AGATAGACCCTCTCCCTTGTTC |
| 6 | TATCAATCCAACCATGTCCCAGG | 2 | chr3: +57083673 | F: GCCGTCATAAAGGCTCATCT<br>R: ATGCCAGATCCTCTGCTTTC |
| 7 | TATAAGAGGAAGGATGTCCCTGG | 2 | chr3: -31667645 | F: GCAGAGGATCAGAGCAAAGAA<br>R: GGTGAGACGCACTTCCATAAA |
| 8 | GATGAATGGAGGGATGTCGCTGG | 3 | chr17: +28450946 | F: GGTGCCCACACATTCATTTAG<br>R: TGGCAGAAACTGTACAACAAAG |
| 9 | TATGAATGTAAAGATGTCTCTGG | 4 | chr12: -63613896 | F: GGGCAGTGAAGGGCATTTA<br>R: CAGTGGATGACGCAGAAAGA |
| 10 | TATTAATGCAGCGATGTCCAAGG | 4 | chr12: +140753137 | F: GCATGGATCTGAAGCAAACATC<br>R: CTTCTCACGTACACCGTCTTTAT |