

Supplementary Material

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                                sgRNA1      ↓
TAR  TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT
OLI  TAATGCTTTGCATATGTATATGAATGGAACGATGTCACAAGTACAGGGATCTGCCAATGATCCCATTT

#B01 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT
---- TAATGCTTTGCATATGTATATGAA-----ATCTGCCAATGATCCCATTT (-24bp)
#B02 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT
---- TAATGCTTTGCATATGTATATGAATGGAAAGA-----TACAGGGATCTGCCAATGATCCCATTT (-9bp)
#B03 TAATGCTTTGCATATGTATATGA-----CCAGGTACAGGGATCTGCCAATGATCCCATTT (-13bp)
---- TAATGCTTTGCATATGTATATGAATGGAAAGAT--CCAGGTACAGGGATCTGCCAATGATCCCATTT (-2bp)
#B04 TAATGCTTTGCATATGTATATGAATGGAAAGATGT----GTACAGGGATCTGCCAATGATCCCATTT (-5bp)
---- TAATGCTTTGCATATGTATATGAATGGAAAGATGT-----AGGGATCTGCCAATGATCCCATTT (-9bp)
#B06 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT
---- TAATGCTTTGCATATGTATATGAATGG-----TACAGGGATCTGCCAATGATCCCATTT (-14bp)
---- TAATGCTTTGCATATGTATATGAATGGAAAGATGT--CAGGTACAGGGATCTGCCAATGATCCCATTT (-2bp)
#B07 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT
---- TAATGCTTTGCATATGTATATGAATGG-----//-----TCTATG (-170bp)
#B09 TAATGCTTTGCATATGTATATGAATG-----AGGGATCTGCCAATGATCCCATTT (-18bp)
---- TAATGCTTTGCATATGTATATGAATGG-----GGATCTGCCAATGATCCCATTT (-19bp)
#B10 TAATGCTTTGCATATGTATATGAATGGAAAGAT-----CAGGGATCTGCCAATGATCCCATTT (-10bp)
#B12 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT
---- TAATGCTTTGCATATGTATATGAATGGAAAGAT--CCAGGTACAGGGATCTGCCAATGATCCCATTT (-2bp)
#B13 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT
---- TAATGCTTTGCATATGTATATGAATG-----AGGGATCTGCCAATGATCCCATTT (-18bp)
#B14 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT
---- TAATGCTTTGCATATGTATATGAATGGAA-----CCAGGTACAGGGATCTGCCAATGATCCCATTT (-6bp)

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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, “+”.

sgRNA1 ↓

TAR TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCAGGTACAGGGATCTGCCAATGATCCCATTT
 OLI TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCAGGTACAGGGATCTGCCAATGATCCCATTT

#101 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCAGGTACAGGGATCTGCCAATGATCCCATTT (WT)
 #102 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCAGGTACAGGGATCTGCCAATGATCCCATTT (WT)
 ---- TAATGCTTTGCATATGTATATGAATGGAAAGATG-----CCAATGATCCCATTT (-19bp)
 #103 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCAGGTACAGGGATCTGCCAATGATCCCATTT (WT)
 ---- TAATGCTTTGCATATGTATATGAATGGAAAGATG-----CCAATGATCCCATTT (-19bp)
 #105 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCAGGTACAGGGATCTGCCAATGATCCCATTT (WT)
 ---- TAATGCTTTGCATATGTATATGAATGGAAAGAT--CCAGGTACAGGGATCTGCCAATGATCCCATTT (-2bp)
 #107 TAATGCTTTGCATATGTATATGAATGGAAAGAT--CCAGGTACAGGGATCTGCCAATGATCCCATTT (-2bp)

#201 TAATGCTTTGCATATGTATATGAATGGAAAGATG-----ATCCCATTT (-25bp)
 #202 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCAGGTACAGGGATCTGCCAATGATCCCATTT (WT)
 #203 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCAGGTACAGGGATCTGCCAATGATCCCATTT (WT)
 ---- TAATGCTTTGCATATGTATATGAATGGAA-----GGATCTGCCAATGATCCCATTT (-18bp)
 #205 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCAGGTACAGGGATCTGCCAATGATCCCATTT (WT)
 ---- TAATGCTTTGCATATGTATATGAATGGAAAGATG-----ATCCCATTT (-25bp)

#301 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCAGGTACAGGGATCTGCCAATGATCCCATTT (WT)
 ---- TAATGCTTTGCATATGTATATGAATGGAAAGA-----TACAGGGATCTGCCAATGATCCCATTT (-9bp)
 #302 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCAGGTACAGGGATCTGCCAATGATCCCATTT (WT)
 ---- TAATGCTTTGCATATGTATATGAATGGAA-----AGGTACAGGGATCTGCCAATGATCCCATTT (-9bp)
 ---- TAATGCTTTGCATATGTATATGAATGGAAAGATGTCC-AGGTACAGGGATCTGCCAATGATCCCATTT (-1bp)
 #303 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCC-AGGTACAGGGATCTGCCAATGATCCCATTT (-1bp)
 #304 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCAGGTACAGGGATCTGCCAATGATCCCATTT (WT)
 ---- TAATGCTTTGCATATGTATATGAATGGAAAGAT--CCAGGTACAGGGATCTGCCAATGATCCCATTT (-2bp)
 ---- TAATGCTTTGCATATGTATATGAATGG-----//-----TCTATG (-170bp)
 #305 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCC---GTACAGGGATCTGCCAATGATCCCATTT (-4bp)
 #306 TAATGCTTTGCATATGTATATGAATGG-----TACAGGGATCTGCCAATGATCCCATTT (-14bp)

#401 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCAGGTACAGGGATCTGCCAATGATCCCATTT (WT)
 ---- TAATGCTTTGCATATGTATATGAATGG-----TACAGGGATCTGCCAATGATCCCATTT (-14bp)
 #402 TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCAGGTACAGGGATCTGCCAATGATCCCATTT (WT)
 ---- TAATGCTTTGCATATGTATATGAATGGAAAGATGT--CAGGTACAGGGATCTGCCAATGATCCCATTT (-2bp)
 ---- CACAAAGATTAGTTCAATACTCTC-----//-----TCTCCTTACCATGCG (-278bp)

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, “+”.

WT

	T	A	T	G	A	A	T	G	G	A	A	A	G	A	T	G	T	C	C	C	A	G	G
T	93.6	5	94.5	0.9	0.9	3.4	90.4	0.6	1.2	0.6	1.2	0.1	2.6	3.7	92.6	4.1	93.6	2.3	0.9	2.3	0.6	2.4	1.1
G	1.3	0	2.9	90.8	1.3	2.2	6.4	97.3	92.9	1	0.9	3.9	87	0.3	4.7	94.1	0.5	0.4	1.3	0.4	0.4	96.9	98.3
C	0.7	0	1.4	0.5	0.3	1.2	2	0.2	0	0.2	1.6	0	0.4	0.2	1.1	0	5.3	96.4	97	96.4	2	0.7	0.2
A	4.3	95	1.1	7.8	97.4	93.3	1.2	1.9	5.9	98.2	96.2	96	10	95.8	1.6	1.8	0.5	0.9	0.9	0.9	97	0.6	0.4

K373T-Hom

	T	A	T	G	A	A	T	G	G	A	A	C	G	A	T	G	T	C	C	C	A	G	G
T	97.3	2.3	96.1	2	1.6	2.3	93.1	0.9	0	0.6	1.2	1.6	1.7	1.7	93	0.7	91.2	1.9	2.9	3	0.8	0.4	1
G	0.6	3.5	1.4	93.9	4.8	6	3.3	95.7	94.6	4.1	2.9	2.6	94.7	3.3	2.1	94.7	2.7	1.4	0	1.8	4.5	3.2	93.3
C	0.5	0.6	0	1.2	0.1	0.1	0	1.3	1.5	0	0.1	95.8	0.6	0.1	2.4	2.8	4.5	96.3	0.5	93.9	1.7	3	2.2
A	1.7	93.6	2.5	3	93.5	91.6	3.6	2.2	3.9	95.3	95.8	0	3	94.9	2.5	1.8	1.5	0.5	96.6	1.3	94.4	93.4	3

K373T-Het

	T	A	T	G	A	A	T	G	G	A	A	A	G	A	T	G	T	C	C	C	A	A	G
T	90.8	6.2	90.1	1.6	1.7	6.3	85.5	0.8	0.5	2	2.3	1.6	1.2	5.3	87.6	3.4	89.1	1.4	1.6	0	2.3	2.4	4
G	1	2.2	5	87.8	1.7	3.4	10.4	97.7	91.7	2.1	2.1	5.4	88.3	2.1	6.6	93.4	2.4	0.8	1.5	1.9	4.5	46	93.3
C	1.9	0.1	2.3	0	0	0	2.2	0	0.5	0.2	1.9	41.8	0.9	0.3	2.3	1	7	94.8	60.9	92.5	1.7	0.7	0.2
A	6.2	91.5	2.6	10.6	96.7	90.2	1.9	1.5	7.3	95.7	93.8	51.2	9.6	92.3	3.3	2.2	1.6	3	36	5.6	91.5	50.9	2.4

K373T-Chi

	T	A	T	G	A	A	T	G	G	A	A	C	G	A	T	G	T	C	A	C	A	A	G
T	94.2	8	90.2	2	2.1	6.8	87.3	1	0	1.8	1.9	0.5	1.3	4.3	71.2	19.2	92.4	20	23	1.8	1.9	2.7	22.4
G	0.5	1.6	8.2	88.7	2.3	0	10.4	97.5	91.5	1.8	1.6	7.5	89.4	19.9	5.2	78.3	2.5	0	2.2	1.8	19.3	13	75.8
C	0	1.6	0	1.5	0	0.2	0.5	0.9	0.9	0	4	72.1	1.5	0	1	2.2	4.9	78.1	9.5	76.8	1.9	0	0.3
A	5.4	88.8	1.6	7.8	95.6	93	1.7	0.6	0.6	96.4	92.5	19.9	7.7	75.8	22.5	0.3	0.2	1.9	65.3	19.6	77	84.3	1.5

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.

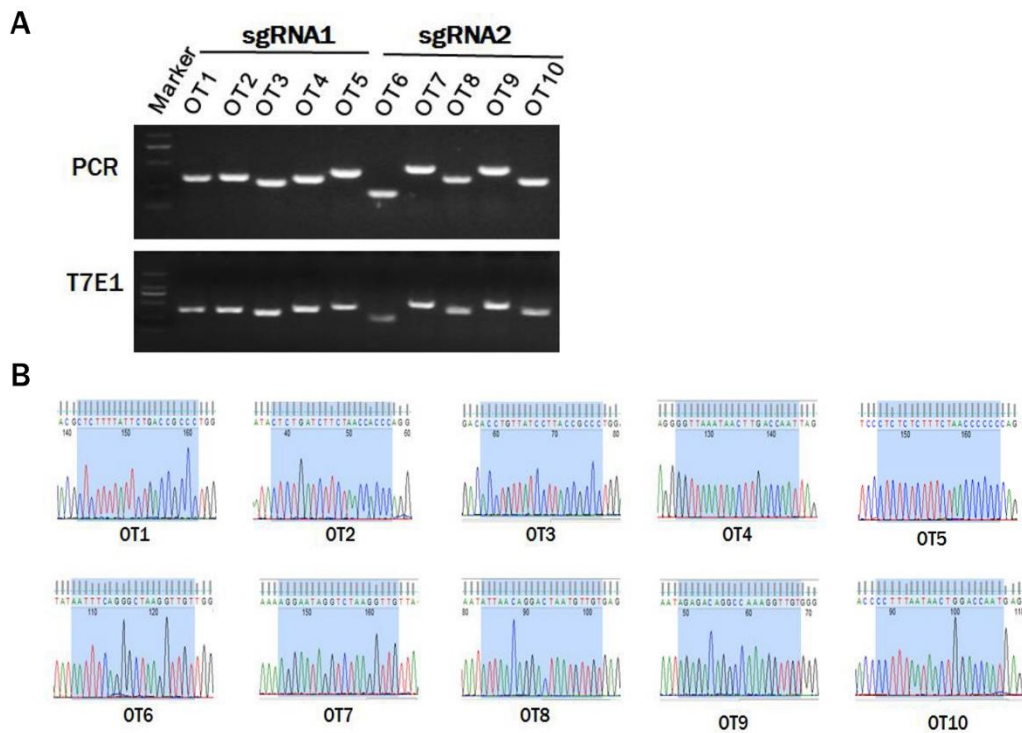


Figure S4. Identification of off-target effects in TYR K373T mutant rabbits.

(A) PCR and T7E1 cleavage analysis of POTS for sgRNA. M, DL2000; OT1–10, ten POTS. No fragment was detected in T7E1 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	GGATAGCAGATGCCACTCAA	100
	TYR-Q-R	AACGCATGGTGAAGGAGAAA	
GAPDH	GAPDH-F	TTCCACGGCAGGTCAAGGC	99
	GAPDH-R	GGGCACCAGCATCACCCAC	

* 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 R: TCTCATGTCACCTTCACTTTCC
2	TGTTTCATGGACCGATGTCCCTGG	2	chrUN0: -1215984	F: ACTGGGTGTGATTGTGAAGTAA R: CGGAGTGTCTTTGTAGCATCA
3	TGTTAATTGAACGATGGCCCTGG	3	chr18: -17497659	F: AATATTCCAGGTGGCTTCCC R: CATGGCAATTTGTGTTGCATT
4	TATGAAAGGGACGATGTCTCAGG	3	chr2: +134076656	F: GCTACTCTTAGGATCATTGGTTACT R: ATTTGGGATGCTGTGCATAAAG
5	TATCCATGGAACGCTGTCCCAGG	3	chr3: -143791182	F: CCTCTTTCTGCCTTCCGATTT R: AGATAGACCCTCTCCCTTGTTT
6	TATCAATCCAACCATGTCCCAGG	2	chr3: +57083673	F: GCCGTCATAAAGGCTCATCT R: ATGCCAGATCCTCTGCTTTC
7	TATAAGAGGAAGGATGTCCCTGG	2	chr3: -31667645	F: GCAGAGGATCAGAGCAAAGAA R: GGTGAGACGCACTTCCATAAA
8	GATGAATGGAGGGATGTCGCTGG	3	chr17: +28450946	F: GGTGCCACACATTCAATTAG R: TGGCAGAACTGTACAACAAAG
9	TATGAATGTAAAGATGTCTCTGG	4	chr12: -63613896	F: GGGCAGTGAAGGGCATTTA R: CAGTGGATGACGCAGAAAGA
10	TATTAATGCAGCGATGTCCAAGG	4	chr12: +140753137	F: GCATGGATCTGAAGCAAACATC R: CTTCTCACGTACACCGTCTTTAT