Supplementary Material

sgRNA1

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TAR	${\tt taatgctttgcatatgtatatgaatggaaa} {\tt gatgtcccaggtacagggatctgccaatgatcccattt}$	
OLI	TAATGCTTTGCATATGTATATGAATGGAACGATGTCACAAGTACAGGGATCTGCCAATGATCCCATTT	
#B01	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT	
	TAATGCTTTGCATATGTATATGAAATCTGCCAATGATCCCATTT	(-24bp)
#B02	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT	
	TAATGCTTTGCATATGTATATGAATGGAAAGATACAGGGATCTGCCAATGATCCCATTT	(-9bp)
#B03	TAATGCTTTGCATATGTATATGACCAGGTACAGGGATCTGCCAATGATCCCATTT	(-13bp)
	TAATGCTTTGCATATGTATATGAATGGAAAGATCCCAGGTACAGGGATCTGCCAATGATCCCATTT	(-2bp)
#B04	TAATGCTTTGCATATGTATATGAATGGAAAGATGTGTACAGGGATCTGCCAATGATCCCATTT	(-5bp)
	TAATGCTTTGCATATGTATATGAATGGAAAGATGTAGGGATCTGCCAATGATCCCATTT	(-9bp)
#B06	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT	
	TAATGCTTTGCATATGTATATGAATGGTACAGGGATCTGCCAATGATCCCATTT	(-14bp)
	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCAGGTACAGGGATCTGCCAATGATCCCATTT	(-2bp)
#B07	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT	
	TAATGCTTTGCATATGTATATGAATGG//TCTATG	(-170bp)
#B09	TAATGCTTTGCATATGTATATGAATGAGGGATCTGCCAATGATCCCATTT	(-18bp)
	TAATGCTTTGCATATGTATATGAATGGGGATCTGCCAATGATCCCATTT	(-19bp)
#B10	TAATGCTTTGCATATGTATATGAATGGAAAGATCAGGGATCTGCCAATGATCCCATTT	(-10bp)
#B12	${\tt TAATGCTTTGCATATGTATATGAATGGAAA} {\tt GATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT$	
	${\tt taatgctttgcatatgtatatgaatggaaagatcccaggtacagggatctgccaatgatcccattt$	(-2bp)
#B13	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT	
	TAATGCTTTGCATATGTATATGAATGAGGGATCTGCCAATGATCCCATTT	(-18bp)
#B14	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT	
	TAATGCTTTGCATATGTATATGAATGGAACCCAGGTACAGGGATCTGCCAATGATCCCATTT	(-6bp)

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	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT	
OLI	TAATGCTTTGCATATGTATATGAATGGAACGATGTCACAAGTACAGGGATCTGCCAATGATCCCATTT	
#101	${\tt taatgctttgcatatgtatatgaatggaaagatgtcccaggtacagggatctgccaatgatcccattt$	(WT)
#102	${\tt taatgctttgcatatgtatatgaatggaaagatgtcccaggtacagggatctgccaatgatcccattt$	(WT)
	TAATGCTTTGCATATGTATATGAATGGAAAGATGCCAATGATCCCATTT	(-19bp)
#103	${\tt taatgctttgcatatgtatatgaatggaaagatgtcccaggtacagggatctgccaatgatcccattt$	(WT)
	TAATGCTTTGCATATGTATATGAATGGAAAGATGCCAATGATCCCATTT	(-19bp)
#105	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT	(WT)
	TAATGCTTTGCATATGTATATGAATGGAAAGATCCCAGGTACAGGGATCTGCCAATGATCCCATTT	(-2bp)
#107	TAATGCTTTGCATATGTATATGAATGGAAAGATCCCAGGTACAGGGATCTGCCAATGATCCCATTT	(-2bp)
#201	TAATGCTTTGCATATGTATATGAATGGAAAGATGATCCCATTT	(-25bp)
#202	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT	(WT)
#203	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT	(WT)
	TAATGCTTTGCATATGTATATGAATGGAAGGATCTGCCAATGATCCCATTT	(-18bp)
#205	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT	(WT)
	TAATGCTTTGCATATGTATATGAATGGAAAGATGATCCCATTT	(-25bp)
#301	${\tt taatgctttgcatatgtatatgaatggaaagatgtcccaggtacagggatctgccaatgatcccattt$	(WT)
	TAATGCTTTGCATATGTATATGAATGGAAAGATACAGGGATCTGCCAATGATCCCATTT	(-9bp)
#302	${\tt taatgctttgcatatgtatatgaatggaaagatgtcccaggtacagggatctgccaatgatcccattt$	(WT)
	TAATGCTTTGCATATGTATATGAATGGAAAGGTACAGGGATCTGCCAATGATCCCATTT	(-9bp)
	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCC-AGGTACAGGGATCTGCCAATGATCCCATTT	(-1bp)
#303	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCC-AGGTACAGGGATCTGCCAATGATCCCATTT	(-1bp)
#304	${\tt taatgctttgcatatgtatatgaatggaaagatgtcccaggtacagggatctgccaatgatcccattt$	(WT)
	${\tt taatgctttgcatatgtatatgtatatggaaagatcccaggtacagggatctgccaatgatcccattt$	(-2bp)
	TAATGCTTTGCATATGTATATGAATGG//TCTATG	(-170bp)
#305	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCGTACAGGGATCTGCCAATGATCCCATTT	(-4bp)
#306	TAATGCTTTGCATATGTATATGAATGGTACAGGGATCTGCCAATGATCCCATTT	(-14bp)
#401	${\tt taatgctttgcatatgtatatgaatggaaagatgtcccaggtacagggatctgccaatgatcccattt$	(WT)
	TAATGCTTTGCATATGTATATGAATGGTACAGGGATCTGCCAATGATCCCATTT	(-14bp)
#402	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCCCAGGTACAGGGATCTGCCAATGATCCCATTT	(WT)
	TAATGCTTTGCATATGTATATGAATGGAAAGATGTCAGGTACAGGGATCTGCCAATGATCCCATTT	(-2bp)
	CACAAAGATTAGTTCAATACTCTC//TCTCCTTCACCATGCG	(-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, "+".

	т	Α	т	G	Α	А	Т	G	G	А	Α	Α	G	Α	т	G	т	С	С	С	Α	G	G
т	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
с	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
Α	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																							
	Т	Α	т	G	Α	Α	т	G	G	Α	Α	С	G	Α	т	G	т	С	С	С	Α	G	G
т	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
с	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
Α	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
K373	K373T-Het																						
	Т	Α	т	G	Α	Α	т	G	G	Α	Α	Α	G	Α	Т	G	Т	С	С	С	Α	Α	G
т	00.0													0.8265					10		1000		Λ
	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	1.0	0	2.3	2.4	-
G	1	6.2 2.2	90.1 5	1.6 87.8	1.7 1.7	6.3 3.4	85.5 10.4	0.8 97.7	0.5 91.7	2 2.1	2.3 21	1.6 5.4	1.2 88.3	5.3 2.1	6.6	3.4 93.4	2.4	1.4 0.8	1.5	0 1.9	2.3 4.5	2.4 46	93.3
G	1 1.9	6.2 2.2 0.1	90.1 5 2.3	1.6 87.8 0	1.7 1.7 0	6.3 3.4 0	85.5 10.4 2.2	0.8 97.7 0	0.5 91.7 0.5	2 2.1 0.2	2.3 21 1.9	1.6 5.4 41.8	1.2 88.3 0.9	5.3 2.1 0.3	6.6 2.3	3.4 93.4 1	89.1 2.4 7	1.4 0.8 94.8	1.5 60.9	0 1.9 92.5	2.3 4.5 1.7	2.4 46 0.7	93.3 0.2
G C A	1 1.9 6.2	6.2 2.2 0.1 91.5	90.1 5 2.3 2.6	1.6 87.8 0 10.6	1.7 1.7 0 96.7	6.3 3.4 0 90.2	85.5 10.4 2.2 1.9	0.8 97.7 0 1.5	0.5 91.7 0.5 7.3	2 2.1 0.2 95.7	2.3 21 1.9 93.8	1.6 5.4 41.8 51.2	1.2 88.3 0.9 9.6	5.3 2.1 0.3 92.3	6.6 2.3 3.3	3.4 93.4 1 2.2	2.4 7 1.6	1.4 0.8 94.8 3	1.5 60.9 36	0 1.9 92.5 5.6	2.3 4.5 1.7 91.5	2.4 46 0.7 50.9	93.3 0.2 2.4
G C A K373	1 1.9 6.2 T-Chi	6.2 2.2 0.1 91.5	90.1 5 2.3 2.6	1.6 87.8 0 10.6	1.7 1.7 0 96.7	6.3 3.4 0 90.2	85.5 10.4 2.2 1.9	0.8 97.7 0 1.5	0.5 91.7 0.5 7.3	2 2.1 0.2 95.7	2.3 21 1.9 93.8	1.6 5.4 41.8 51.2	1.2 88.3 0.9 9.6	5.3 2.1 0.3 92.3	87.6 6.6 2.3 3.3	3.4 93.4 1 2.2	89.1 2.4 7 1.6	1.4 0.8 94.8 3	1.6 1.5 60.9 36	0 1.9 92.5 5.6	2.3 4.5 1.7 91.5	2.4 46 0.7 50.9	93.3 0.2 2.4
G C A K373	1 19 6.2 T-Chi T	6.2 2.2 0.1 91.5	90.1 5 2.3 2.6 T	1.6 87.8 0 10.6	1.7 1.7 96.7	6.3 3.4 0 90.2	85.5 10.4 2.2 1.9	0.8 97.7 0 1.5 G	0.5 91.7 0.5 7.3	2 2.1 0.2 95.7	2.3 21 1.9 93.8	1.6 5.4 41.8 51.2	1.2 88.3 0.9 9.6	5.3 2.1 0.3 92.3	6.6 2.3 3.3 T	3.4 93.4 1 2.2 G	89.1 2.4 7 1.6 T	1.4 0.8 94.8 3	1.6 1.5 60.9 36	0 1.9 92.5 5.6 C	2.3 4.5 1.7 91.5	2.4 46 0.7 50.9	93.3 0.2 2.4 G
G С А К373 Т	1 19 6.2 T-Chi T 94.2	6.2 2.2 0.1 91.5 A 8	90.1 5 2.3 2.6 T 90.2	1.6 87.8 0 10.6 G 2 2	1.7 1.7 0 96.7 A 2.1	6.3 3.4 0 90.2 A 6.8	85.5 10.4 2.2 1.9 T 87.3	0.8 97.7 0 1.5 G	0.5 91.7 0.5 7.3 G	2 2.1 0.2 95.7 A 1.8	2.3 21 1.9 93.8 A 1.9	1.6 5.4 41.8 51.2 C	1.2 88.3 0.9 9.6 G	5.3 2.1 0.3 92.3 A	87.6 6.6 2.3 3.3 T 71.2	3.4 93.4 1 2.2 G 19.2	89.1 2.4 7 1.6 T 92.4 25	1.4 0.8 94.8 3 C 20	1.6 1.5 60.9 36 A 23	0 1.9 92.5 5.6 C 1.8	2.3 4.5 1.7 91.5 A 1.9	2.4 46 0.7 50.9 A 2.7	93.3 0.2 2.4 G 22.4
G C A K373 T G	1 1.9 6.2 T-Chi T 94.2 0.5 0	6.2 2.2 0.1 91.5 A 8 1.6	90.1 5 2.3 2.6 T 90.2 8.2	1.6 87.8 0 10.6 G 2 88.7	1.7 1.7 0 96.7 A 2.1 2.3	6.3 3.4 0 90.2 A 6.8 0 0.3	85.5 10.4 2.2 1.9 T 87.3 10.4	0.8 97.7 0 1.5 G 97.5	0.5 91.7 0.5 7.3 G 91.5	2 2.1 0.2 95.7 A 1.8 1.8	2.3 21 1.9 93.8 A 1.9 1.6	1.6 5.4 41.8 51.2 C 0.5 7.5 7.2	1.2 88.3 0.9 9.6 G 1.3 89.4	5.3 2.1 0.3 92.3 A 4.3 19.9	87.6 6.6 2.3 3.3 T 71.2 5.2	3.4 93.4 1 2.2 G 19.2 78.3	89.1 2.4 7 1.6 T 92.4 2.5	1.4 0.8 94.8 3 C 20 0	1.6 1.5 60.9 36 A 23 2.2 2.5	0 1.9 92.5 5.6 C 1.8 1.8 1.8	2.3 4.5 1.7 91.5 A 1.9 19.3	2.4 46 0.7 50.9 A 2.7 13	93.3 0.2 2.4 G 22.4 75.8
G C A K373 T G C	1 19 6.2 T-Chi T 94.2 0.5 0 5.4	6.2 2.2 0.1 91.5 A 8 1.6 1.6 88 2	90.1 5 2.3 2.6 T 90.2 8.2 0 1.6	1.6 87.8 0 10.6 G 2 88.7 1.5 7.8	1.7 1.7 96.7 A 2.1 2.3 0	6.3 3.4 0 90.2 A 6.8 0 0.2 93	85.5 10.4 2.2 1.9 T 87.3 10.4 0.5 1.7	0.8 97.7 0 1.5 G 97.5 0.9 0.6	0.5 91.7 0.5 7.3 G 91.5 0.9 0.6	2 2.1 0.2 95.7 A 1.8 1.8 1.8 0	2.3 21 1.9 93.8 A 1.9 1.6 4	1.6 5.4 41.8 51.2 C 0.5 7.5 72.1	1.2 88.3 0.9 9.6 G 1.3 89.4 1.5 7.7	5.3 2.1 0.3 92.3 A 4.3 19.9 0	87.6 6.6 2.3 3.3 T 71.2 5.2 1 22.5	3.4 93.4 1 2.2 G 19.2 78.3 2.2 0.3	89.1 2.4 7 1.6 T 92.4 2.5 4.9 0.2	1.4 0.8 94.8 3 C 20 0 78.1	1.6 1.5 60.9 36 A 23 2.2 9.5 65 2	0 1.9 92.5 5.6 C 1.8 1.8 1.8 1.8	2.3 4.5 1.7 91.5 A 1.9 19.3 1.9 77	2.4 46 0.7 50.9 A 2.7 13 0 84.2	93.3 0.2 2.4 G 22.4 75.8 0.3

wт

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.

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	

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

* 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.

	Potential off-target site	Number of mismatches	Position	PCR primer
1	GATTAATGTTACGATGTCCCCCGG	2	chr7:	F: CTAGTCTGGGATCAGACAGGTA
±	011111110111100110100000	2	-148540069	R: TCTCATGTCACCTTCACTTTCC
2	ͲϹͲͲϹϪͲϹϹϪϹϹϹϪͲϹͲϹϹϹͲϹϹ	2	chrUN0:	F: ACTGGGTGTGATTGTGAAGTAA
	IGIICAIGGACCGAIGICCCIGG	2	-1215984	R: CGGAGTGTCTTTGTAGCATCA
2		2	chr18:	F: AATATTCCAGGTGGCTTCCC
5	IGIIAAIIGAACGAIGGCCCIGG	5	-17497659	R: CATGGCAATTTGTGTTGCATTT
	Ψ λΨCλλλCCCλCCλΨCΨC λCC	2	chr2:	F: GCTACTCTTAGGATCATTGGTTACT
-	INIGAAAGGACGAIGICICAGG	5	+134076656	R: ATTTGGGATGCTGTGCATAAAG
E		2	chr3:	F: CCTCTTTCTGCCTTCCGATTT
5	INICONIGGANCGCIGICCCAGG	5	-143791182	R: AGATAGACCCTCTCCCTTGTTC
6	₩ ₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩₩	2	chr3:	F: GCCGTCATAAAGGCTCATCT
	TATCAATCCAACCATGTCCCAGG	2	+57083673	R: ATGCCAGATCCTCTGCTTTC
7	Ψ λΨλλ ΟλΟΟλλΟΟλΨΟΨΟΟΟΨΟΟ	2	chr3:	F: GCAGAGGATCAGAGCAAAGAA
	TATAAGAGGAAGGATGTCCCTGG	Z	-31667645	R: GGTGAGACGCACTTCCATAAA
0	C \ T C \ \ T C \ \ T C C \ C C \ T C T C	2	chr17:	F: GGTGCCCACACATTCATTTAG
0	GAIGAAIGGAGGGAIGICGCIGG	5	+28450946	R: TGGCAGAAACTGTACAACAAAG
9	ТАТСААТСТАААСАТСТСТСТСС	л	chr12:	F: GGGCAGTGAAGGGCATTTA
	111044101444041010101010	4	-63613896	R: CAGTGGATGACGCAGAAAGA
1.0	ͲϪͲͲϪϪͲϹϹϪϹϹϹϪͲϹͲϹϹϪϪϹϹ	Λ	chr12:	F: GCATGGATCTGAAGCAAACATC
T.0	INTIANIOCACCATOTOCAAGO	7	+140753137	R: CTTCTCACGTACACCGTCTTTAT

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