

**Supplementary Figure 1.** Transfection of *pX330-Hr51* vector.

The cleavage activity of *pX330-Hr51* was examined by traffic reporter system for checking CRISPR activity (10).

(A) The CRISPR target sequence including PAM was inserted between *tdsRed* and first EGFP fragments in *p2color*. This plasmid was designated as *p2color-Hr51*. As the reading frames of the first and second EGFP fragments were different, only *tdsRed* was translated from *p2color* vector. Double-strand break (DSB) by CRISPR/Cas9 from *pX330* in *p2color*, and frameshift occurred often through the non-homologous end joining (NHEJ) pathway. Accordingly, first or second EGFP reading frame mutation occurred. (B) Although only *tdsRed* fluorescence signal was observed in *p2color-Hr51*-transfected HEK293T cells, both *tdsRed* and EGFP signals were observed in cells co-transfected with *p2color-Hr51* and *pX330-Hr51*. Scale bar: 200  $\mu\text{m}$ .

**Supplementary Figure 2.** Temporal changes in male *Hr<sup>em1Utr/em1Utr</sup>* and *Hr<sup>hr/hr</sup>* mice.

(A) Male *Hr<sup>em1Utr/em1Utr</sup>* mice lost ventral hair at 4 weeks of age. After 6 weeks of age, they gradually lost dorsal hair. (B) Male and (C) female *Hr<sup>hr/hr</sup>* mice lost hair around the eyes at 2 weeks of age, and they gradually lost hair from head to tail. They were completely hairless at 4 weeks of age.

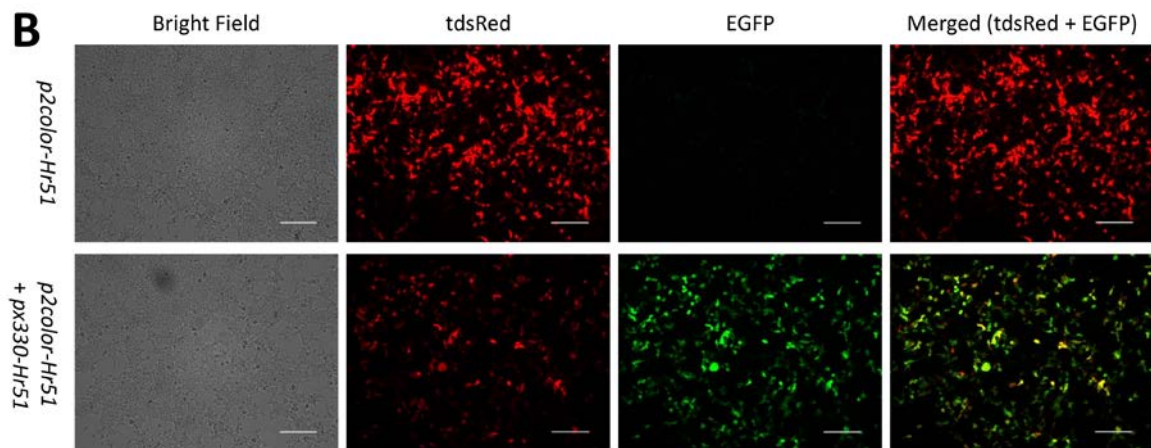
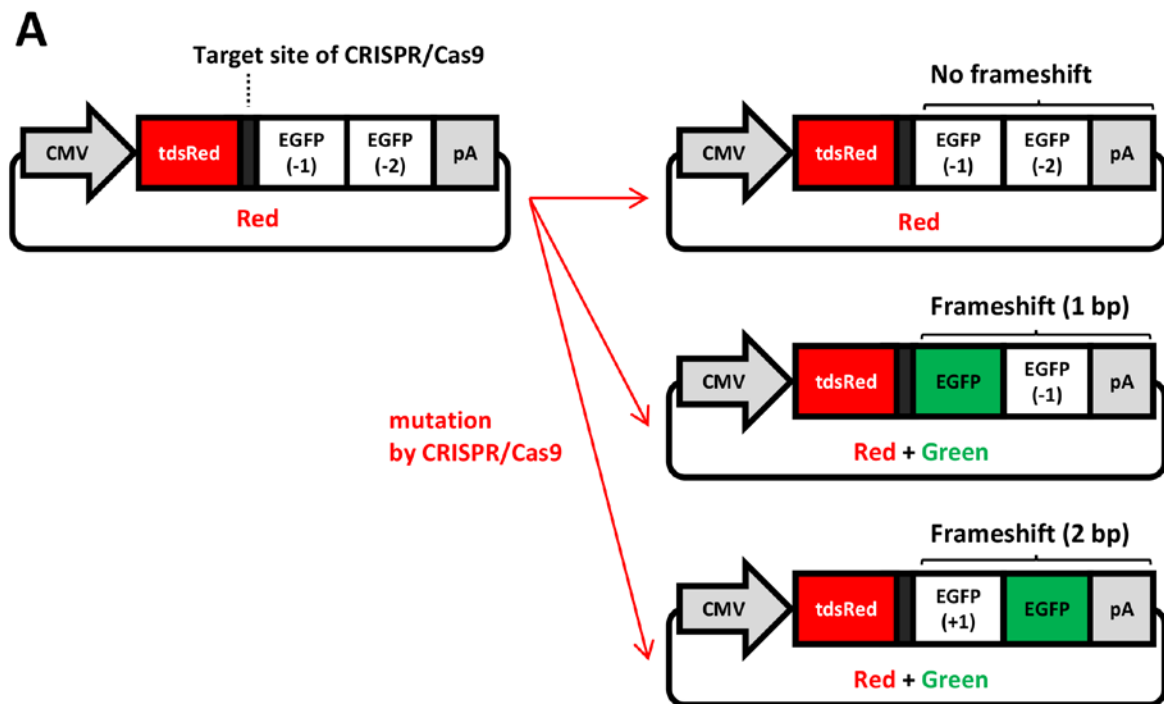
**Supplementary Figure 3.** Body weights of wild-type and  $Hr^{em1Utr/em1Utr}$  mice.

We compared the body weights of wild-type and  $Hr^{em1Utr/em1Utr}$  mice from 4 to 10 weeks of age. Body weights of wild-type and  $Hr^{em1Utr/em1Utr}$  mice at 5 and 6 weeks of age were significantly different.

**Supplementary Figure 4.** Amino acids of normal and mutated *Hr* gene.

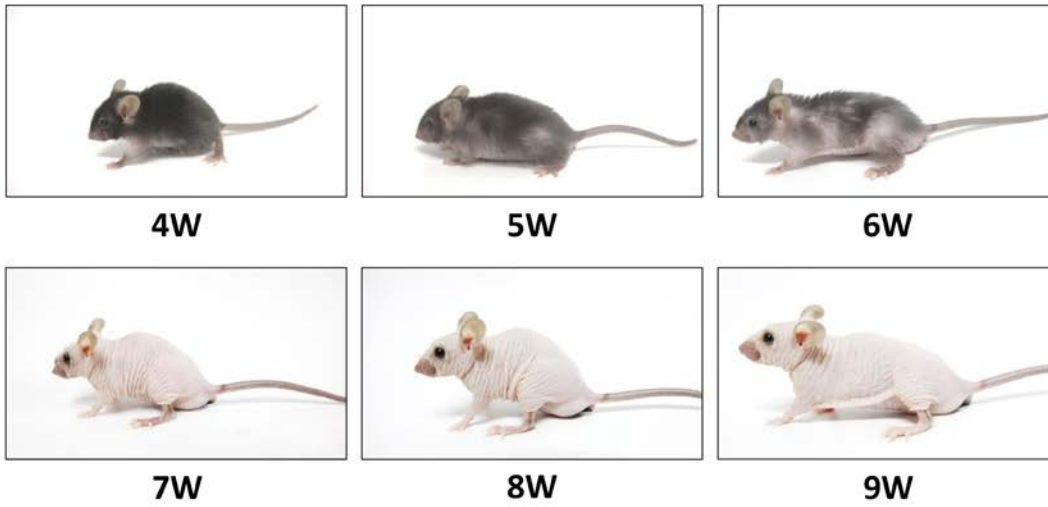
(A) Schematic for length of amino acid. Red letter (M) and sign (@) indicate start codon and stop codon, respectively. Blue sign (&) indicates first stop codon after mutation. Orange letter (M) indicates the predicted first start codon after the mutation. The white arrow indicates the region of mutation. The Length of normal, mutated, and N-terminal truncated amino acid is indicated, respectively. (B) Amino acid sequence of *Hr* gene. Red letter (M) and sign (@) indicate start codon and stop codon, respectively. Orange letter (M) indicates the predicted first start codon after the mutation.

Supplementary Figure 1. Transfection of *pX330-Hr51* vector.

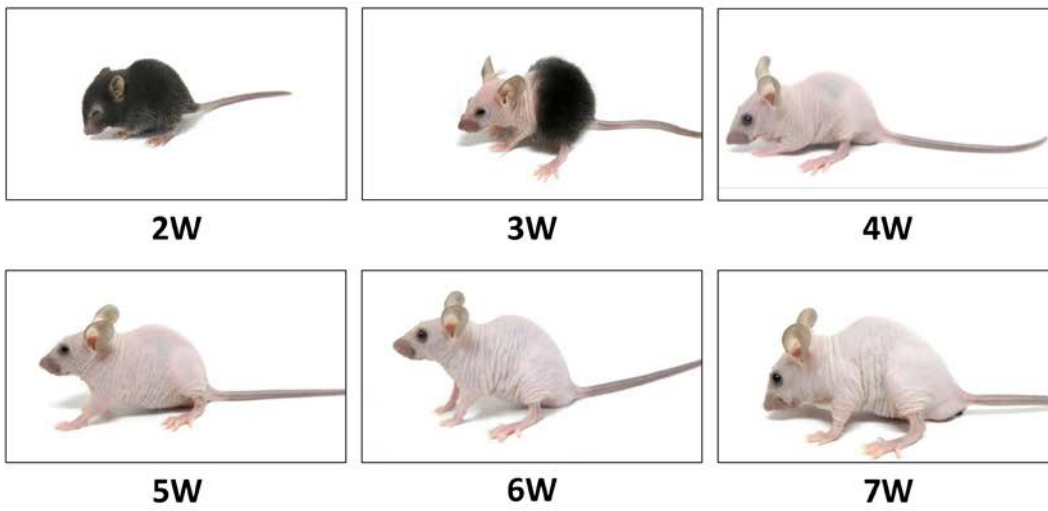


Supplementary Figure 2. Temporal changes in male  $Hr^{em1Utr/em1Utr}$  and  $Hr^{hr/hr}$  mice.

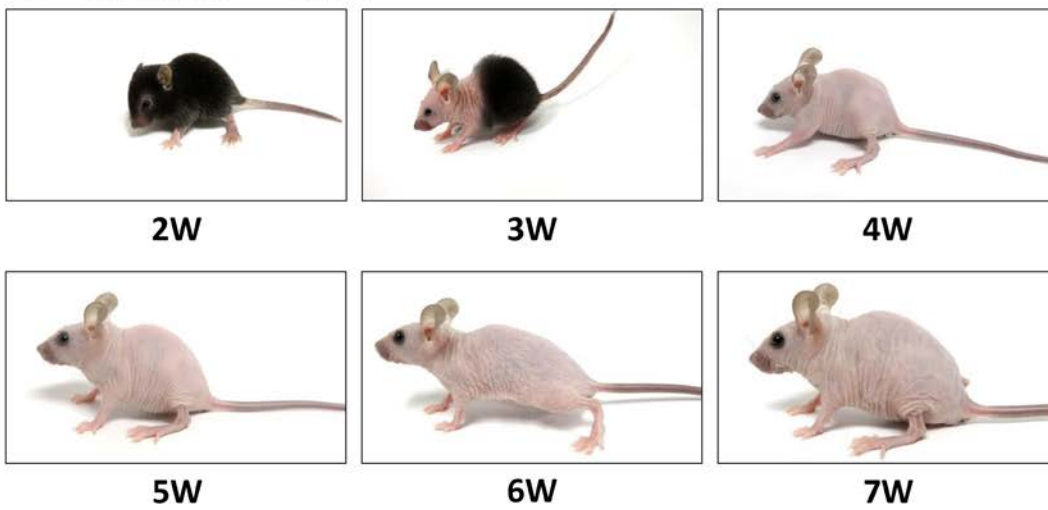
**A** Male  $Hr^{em1Utr/em1Utr}$  mice



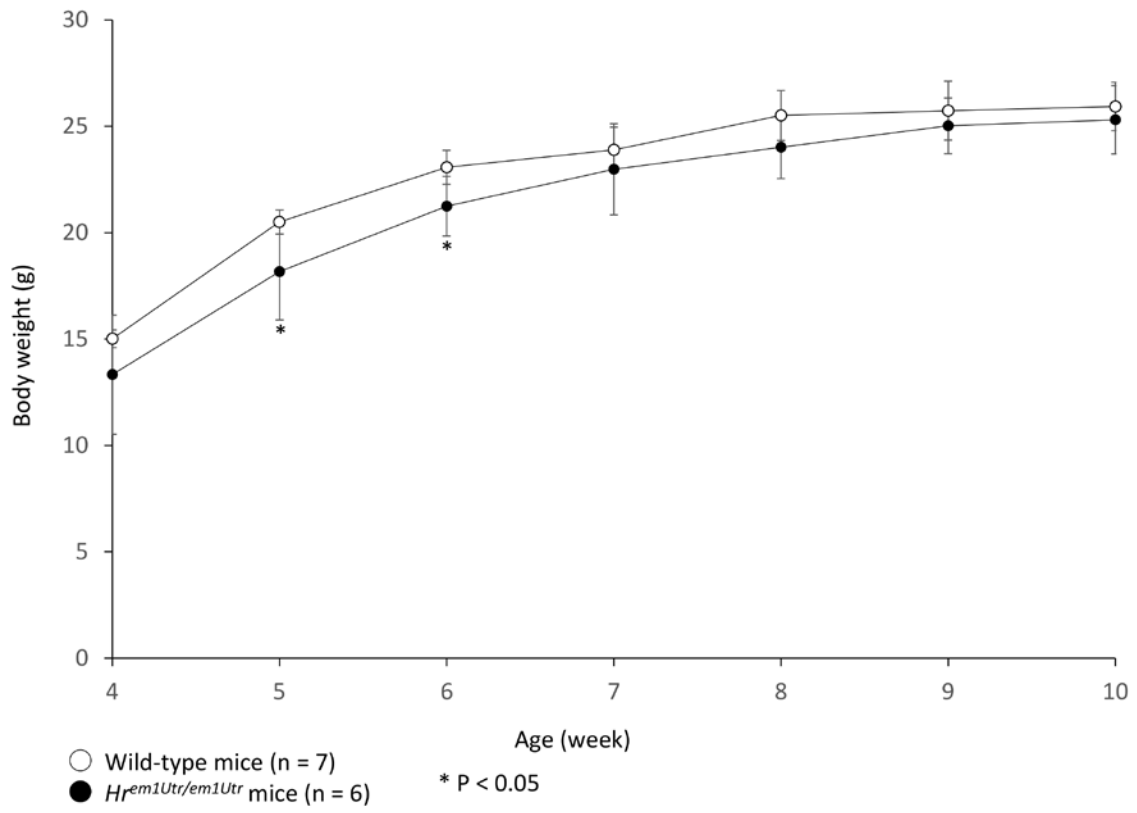
**B** Male  $Hr^{hr/hr}$  mice



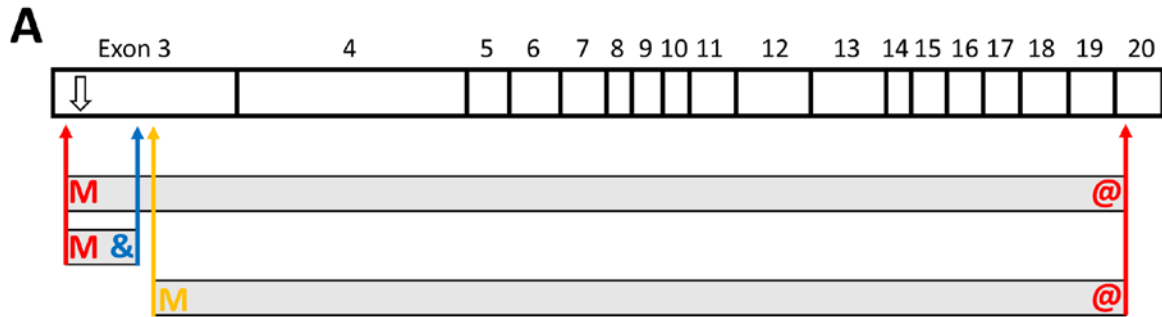
**C** Female  $Hr^{hr/hr}$  mice



**Supplementary Figure 3.** Body weights of wild-type and *Hr<sup>em1Utr/em1Utr</sup>* mice.



**Supplementary Figure 4.** Amino acids of normal and mutated *Hr* gene.



**B**

Amino acid sequence of *Hr* gene

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M E S M P S F L K D T P A W E K T A P V N G I V G Q E P G T S P Q D G L R H G A L C L G E P A P F W R G V L S T P D S W L P P G F L Q G P K D T L S L V E G E G P R N G E R K G S W L G
G K E G L R W K E A M L A H P L A F C G P A C P P R Y G L I P E H S G G H P K S D P V A F R P L H C P F L L E T K I L E R A P F W V P T C L P P Y L M S S L P P E R P Y D W P L A P N P W V
Y S G S Q P K V P S A F L G S K G F Y H K D P N I L R P A K E P L A E S G M L G L A P G G H L Q Q A C E S E G P S L H Q R D G E T G A G R Q Q N L C P V F L G Y P D T V P R A P W P S C P
P G L V H S L G N I W A G P G S N S L G Y Q L G P P A T P R C P S P G P P T P P G G C C S S H L P A R E G D L G P C R K C Q D S P E G G S S G P G E S S E E R N K A D S R A C P P S H H T K L
K K T W L T R H S E Q F E C P G G C S G K E E S P A T G L R A L K R A G S P E V Q G A S R G P A P K R P S H P F P G T G R Q G A R A W Q E T P E T I I G S K A E A E Q Q E E Q R G P R D G R
I R L Q E S R L V D T S C Q H H L A G V T Q C Q S C V Q A A G E V G V L T G H S Q K S R R S P L E E K Q L E E E D S S A T S E E G G G G P G P E A S L N K G L A K H L L S G L D R L C R L L R
K E R E A L A W A Q R E G Q G P A M T E D S P G I P H C C S R C H H G L F N T H W R C S H C S H R L C V A C G R I A G A G K N R E K T G S Q E Q H T D D C A Q E A G H A A C S L I L T Q
F V S S Q A L A E L S T V M H Q V W A K F D I R G H C F C Q V D A R V W A P G D G G Q Q K E P T E K T P P T P Q P S C N G D S N R T K D I K E E T P D S T E S P A E D G A G R S P L P C P S
L C E L L A S T A V K L C L G H D R I H M A F A P V T P A L P S D D R I T N I L D S I I A Q V V E R K I Q E K A L G P G L R A G S G L R K G L S L P L S P V R T R L S P P G A L L W L Q E P R P K H G
F H L F Q E H W R Q G Q P V L V S G I Q K T L R L S L W G M E A L G T L G G Q V Q T L T A L G P P Q P T N L D S T A F W E G F S H P E T R P K L D E G S V L L H R T L G D K D A S R V Q N
L A S S L P L P E Y C A H Q G K L N L A S Y L P L G L T L H P L E P Q L W A A Y G V N S H R G H L G T K N L C V E V S D L I S I L V H A E A Q L P P W Y R A Q K D F L S G L D G E G L W S P G S
Q T S T V W H V F R A Q D A Q R I R R F L Q M V C P A G A G T L E P G A P G S C Y L D A G L R R R L R E E W G V S C W T L L Q A P G E A V L V P A G A P H Q V Q G L V S T I S V T Q H F L
S P E T S A S A Q L Y H Q G A S L P P D H R M L Y A Q M D R A V F Q A V K A A V G A L Q E A K @
    
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**Supplementary Table 1.** List of primers used in this study

Primer for detecting mutations	Primer sequence (5'- to 3')
<i>Cas9</i> detection primer F	AGTTCATCAAGCCCATCCTG
<i>Cas9</i> detection primer R	GAAGTTTCTGTTGGCGAAGC
Primer for detecting mutations	Primer sequence (5'- to 3')
detection primer of MultiNA F	GGCAGGAGAGTGATGGAGAG
detection primer of MultiNA R	GAGCAGGTTCTCCTAGGCACAG
<i>Hr</i> exon3 genotyping F	CATTCAGATAGGGGCCAGAA
<i>Hr</i> exon3 genotyping R	ACAGGGTCACTCTTGGGATG
Primer for analyzing off-targets	Primer sequence (5'- to 3')
Off-target 1 detection primer F	GCATGCCCCCTGTATTTATG
Off-target 1 detection primer R	TTCCAGGTCTCCATTGCTTC
Off-target 2 detection primer F	GCACACACACACACACCTCA
Off-target 2 detection primer R	TTGCCACTCACCATCAACTC
Primer for reverse transcription (RT) PCR	Primer sequence (5'- to 3')
<i>Hr</i> exon3-4 RT primer F	ATGAGGGCAGGAGAGTGATG
<i>Hr</i> exon3-4 RT primer R	AAGTGAGGGGCCTTCTGATT
<i>Hr</i> exon7-12 RT primer F	ACCACGACTCTTCAACACC
<i>Hr</i> exon7-12 RT primer R	TGCTGTCCAGGATGTTGGTA
<i>Hr</i> exon13-19 RT primer F	AGCATTCTGGGAGGGATTCT
<i>Hr</i> exon13-19 RT primer R	GGCATAAAGCATACGGTGGT

**Supplementary Table 2.** Hematology

	Wild-type mice (n=12)	<i>Hr<sup>em1Utr/em1Utr</sup></i> mice (n=12)
WBC ( $\times 10^2/\mu\text{l}$ )	33.7 $\pm$ 10.5	28.8 $\pm$ 9.7
RBC ( $\times 10^4/\mu\text{l}$ )	705.0 $\pm$ 157.4	925.7 $\pm$ 55.5*
HGB (g/dl)	11.0 $\pm$ 2.3	14.4 $\pm$ 1.0*
HCT (%)	34.0 $\pm$ 6.9	45.8 $\pm$ 3.1*
MCV (fl)	47.9 $\pm$ 1.0	49.4 $\pm$ 0.7*
MCH (pg)	15.5 $\pm$ 0.4	15.6 $\pm$ 0.3
MCHC (g/dl)	32.2 $\pm$ 0.5	31.5 $\pm$ 0.3*
PLT ( $\times 10^4/\mu\text{l}$ )	59.5 $\pm$ 21.3	55.5 $\pm$ 13.9

Values are given as mean  $\pm$  SD

\* P<0.05 compared with wild-type mice



**Supplementary Table 3.** Blood chemistry

	Wild-type mice (n=7)	<i>Hr<sup>em1Utr/em1Utr</sup></i> mice (n=7)
Aspartate aminotransferase (U/l)	48.3 ± 6.0	71.3 ± 15.2*
Alanine aminotransferase (U/l)	24.7 ± 2.6	24.9 ± 3.5
Alkaline phosphatase (U/l)	375.4 ± 99.0	298.3 ± 30.9
Blood glucose (mg/dl)	116.3 ± 22.4	127.7 ± 35.5
Blood urea nitrogen (mg/dl)	37.7 ± 2.7	36.0 ± 4.6
Creatinine (mg/dl)	0.2 ± 0.1	0.2 ± 0.1
Total protein (g/dl)	4.6 ± 0.2	4.4 ± 0.3
Albumin (g/dl)	2.1 ± 0.2	2.0 ± 0.2
Total bilirubin (mg/dl)	0.4 ± 0.1	0.4 ± 0.1
Calcium (mg/dl)	8.9 ± 0.2	8.3 ± 0.3*
Inorganic phosphorus (mg/dl)	13.9 ± 1.5	13.7 ± 2.2
Total cholesterol (mg/dl)	92.7 ± 14.5	54.3 ± 10.6*
Triglycerides (mg/dl)	107.7 ± 10.5	54.3 ± 10.0*
Na <sup>+</sup> (mEq/l)	153.0 ± 2.9	150.9 ± 1.1
K <sup>+</sup> (mEq/l)	4.4 ± 0.8	3.6 ± 0.4
Cl <sup>-</sup> (mEq/l)	119.1 ± 2.5	116.9 ± 2.7

Values are given as mean ± SD

\* P<0.05 compared with wild-type mice

**Supplementary Table 4.** Off-targets of *pX330-Hr51* vector

Off-target candidate loci		12-bp PAM-proximal seed
chr8: 109209355-109209369	Off-target 1	GTGAACGGCATTGGGG
	Sequencing result	GTGAACGGCATTGGGG
chr5: 20146003-20146017	Off-target 2	<u>CCT</u> CAATGCCGTTCA
	Sequencing result	CCTCAATGCCGTTCA