

WT	
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	
#5	
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	21/30
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTGAGAACTAACTGGGATGAGAACTTCACT	3/30
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTAGAGAACTAACTGGGATGAGAACTTCACT	6/30
#9	
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	24/30
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTAGAGAACTAACTGGGATGAGAACTTCACT	6/30
#12	
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	9/30
TGCGCTTGGCACAGACTTTTCTTGTTATTGTGAATAAGAAATTGGAGAACTAACTGGGATTAGAACTTCACT	6/30
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTAGAGAACTAACTGGGATGAGAACTTCACT	6/30
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTGAAAACTAACTGGGATGAGAACTTCACT	3/30
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTAGAGAACTAACTGGGATGAGAACTTCACT	3/30
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTGAGAACTAACTGGGATGAGAACTTCACT	3/30
#13	
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	5/30
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTCAGAACTAACTGGGATGAGAACTCACT	3/30
CCGCCTTGGCACAGACTTTTCTTGTTATTGTAAAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	15/30
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTAGAGAACTAACTGGGATGAGAACTTCACT	5/30
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTCAGAACTAACTGGGATGAGAACTTCACT	2/30
#14	
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	6/30
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGAACAAGAAATTGAGAACTAACTGGGATGAGAACTTCACT	12/30
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTGGAGAACTAACTGGGATGAGAACTTCACT	9/30
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTGAGAACTAACTGGGATGAGAACTTCACT	3/30
#17	
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	23/30
CCGCCTTGGCACAGACTTTTCTTGTTATTGTGGGAACAAGAAATTGAGAACTAACTGGGATGAGAACTTCACT	7/30

Figure S1. Analysis of the embryo edited using gRNA-2 and HF2-BE2. PCR amplicons of the target region from the embryos were cloned into the pGEM-T vector for Sanger sequencing. The number of clones for each pattern is indicated. gRNA-2 target sequence is underlined. PAM, green. Point mutation, red.

A G A A A T T C G A G A A C T A A C T G G G G A G A A A T T T G A G A A C T A A C T G G G G

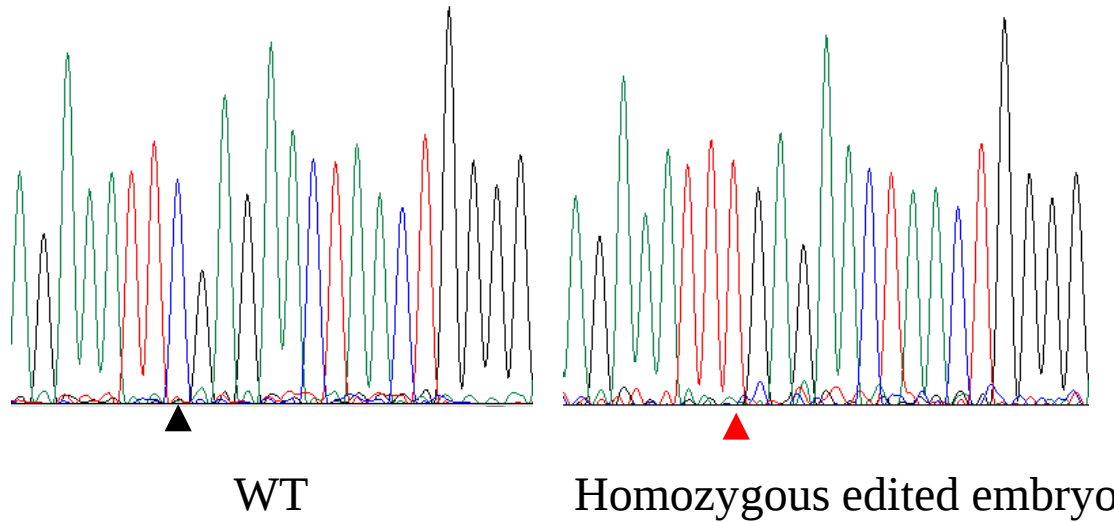


Figure S2. Sequencing results of the homozygous edited embryo (#21) using gRNA-2. Sanger sequencing chromatographs of a wild-type (WT) embryo and the homozygous edited embryo are shown.



gRNA-1

WT	GGTGATGGGAGTCCCTGCGGCCAGCTTTCAGGCAGAGGTTCTGCCAGGATATCC	
P1	GGTGATGGGAGTCCCTGCGGCCAGCTTTCAGGCAGAGGTTCTGCCAGGATATCC	30/30
P2	GGTGATGGGAGTCCCTGCGGCCAGCTTTCAGGCAGAGGTTCTGCCAGGATATCC	30/30
P3	GGTGATGGGAGTCCCTGCGGCCAGCTTTCAGGCAGAGGTTCTGCCAGGATATCC	30/30
P4	GGTGATGGGAGTCCCTGCGGCCAGCTTTCAGGCAGAGGTTCTGCCAGGATATCC	30/30
P5	GGTGATGGGAGTCCCTGCGGCCAGCTTTCAGGCAGAGGTTCTGCCAGGATATCC	30/30
P6	GGTGATGGGAGTCCCTGCGGCCAGCTTTCAGGCAGAGGTTCTGCCAGGATATCC	30/30
P7	GGTGATGGGAGTCCCTGCGGCCAGCTTTCAGGCAGAGGTTCTGCCAGGATATCC	30/30
P8	GGTGATGGGAGTCCCTGCGGCCAGCTTTCAGGCAGAGGTTCTGCCAGGATATCC GGTGATGGGAGTCCCTGCGGTTAGCTTTCAGGCAGAGGTTCTGCCAGGATATCC	15/30 15/30
P9	GGTGATGGGAGTCCCTGCGGCCAGCTTTCAGGCAGAGGTTCTGCCAGGATATCC GGTGATGGGAGTCCCTGCGGTCAGCTTTCAGGCAGAGGTTCTGCCAGGATATCC	13/30 17/30
P10	GGTGATGGGAGTCCCTGCGGCCAGCTTTCAGGCAGAGGTTCTGCCAGGATATCC	30/30
P11	GGTGATGGGAGTCCCTGCGGCCAGCTTTCAGGCAGAGGTTCTGCCAGGATATCC	30/30

gRNA-2

WT	CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	
P1	CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT CTTGTTATTGTGGAACAATAAATTCGAGAACTAACTGGGATGAGAACTTCACT CTTGTTATTGTGAACAATAAATTCGAGAACTAACTGGGATGAGAACTTCACT TTTGTTATTGTGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	13/30 5/30 10/30 2/30
P2	CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	30/30
P3	CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT CTTGTTATTGTGAACAATAAATTCGAGAACTAACTGGGATGAGAACTTCACT CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	0/30 10/30 13/30 7/30
P4	CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	13/30 17/30
P5	CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	12/30 18/30
P6	CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT CTTGTTATTGTGGAACAATAAATTCGAGAACTAACTGGGATGAGAACTTCACT CTTGTTATTGTGGAACAATAAATTCGAGAACTAACTGGGATGAGAACTTCACT	0/30 12/30 18/30
P7	CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	8/30 22/30
P8	CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	30/30
P9	CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	30/30
P10	CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT	30/30
P11	CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT CTTGTTATTGTGGGAACAAGAAATTCGAGAACTAACTGGGATGAGAACTTCACT CTTGTTATTGTGAACAATAAATTCGAGAACTAACTGGGATGAGAACTTCACT	0/30 25/30 5/30

Figure S4. Mutant spectra in founder mice. PCR amplicons from gRNA-1 and gRNA-2 founder mice were subcloned into the pGEM-T vector and sequenced. The number of clones for each pattern is indicated. gRNA target sequence is underlined. PAM, green. Point mutation, red.

P3

GGGAACAAGAAATT <u>CGAGAACTAACTG</u> GGGATGAGAACTTCACTGTTCCATACTGGGATTGGAGAGATGCAG	0/30
AATACAAGAAATT <u>CGAGAACTAACTG</u> GGGATGAGAACTTCACTGTTCCATACTGGGATTGGAGAGATGCA	10/30
GGGAACAAGAAATTGAGAACTAACTGGGATGAGAACTTCACTGTTCCATACTGGGATTGGAGAGATGCAG	13/30
GGGAACAAGAAATTGAGAACTAACTGGGATGAGAACTTCACTGTTCCATACTGGGATTGGAGAGATGCAG	7/30

Figure S5. Deamination occurs outside the gRNA target site.

PCR amplicons of the P3 founder mouse from the gRNA-2 group were cloned into the pGEM-T vector and verified by Sanger sequencing. 4 out of the 12 clones analyzed show obvious deamination on the target strand outside the gRNA-2 target site. gRNA-2 target sequence is underlined. PAM, green. Point mutation, red.

TTGTGGGAACAAGAAATTCGAGAACTAACTGGGG
T T G T G G G A A C A A G A A A T T C G A G A A C T A A C T G G G G

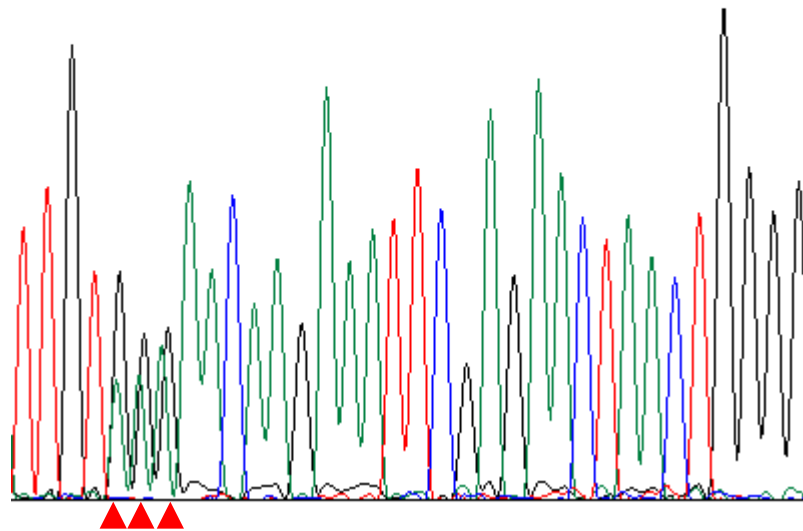


Figure S6. Germline transmission of the mutant allele.

PCR amplicons of the target region in F1 offspring from the P11 founder mouse were sequenced. Sanger sequencing chromatographs of the edited F1 pup is shown. Red arrowhead, the base edited by HF2-BE2.