SUPPLEMENTAL FILES

Supplemental figures

Figure S1.

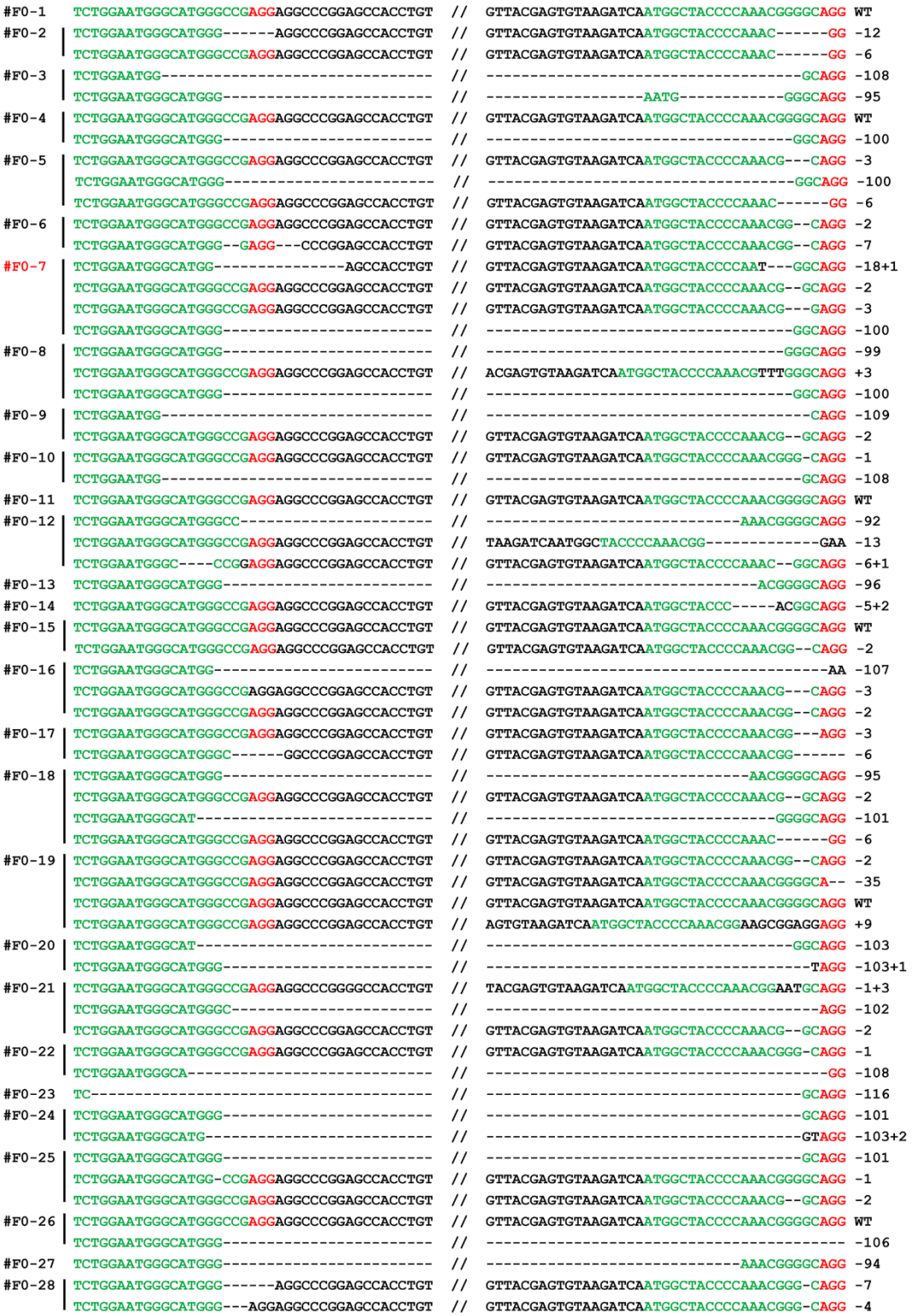

Figure S1. Generation of *FBN1* knockout F0 rabbits.

(A) Sanger sequencing of the targeted region of *FBN1* in F0 rabbits. The highlights of sgRNA sequences and PAM sites are green and red, respectively; Bases of indel are in black within sgRNA sequence; WT represents wild-type *FBN1* sequence was detected.

Figure S2.

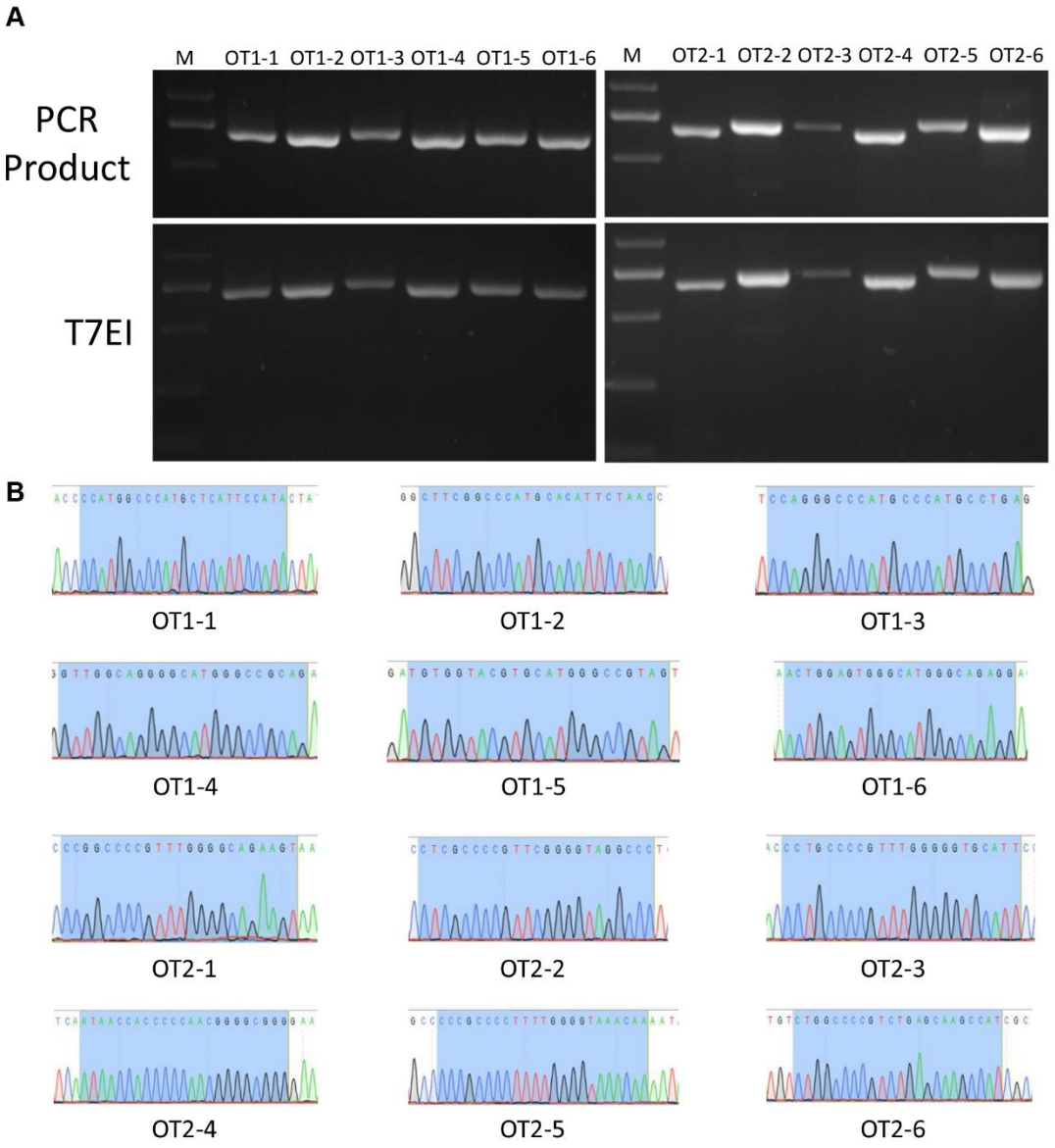

Figure S2. Off-target detection of *FBN1* KO rabbits.

(A) T7E1 analysis of POTS for sgRNA1 and sgRNA2. M, DL2000; OT1represent POTS of sgRNA1; OT2 represent POTS of sgRNA2. (B) Chromatogram sequence analysis of 12 POTS for sgRNA1 and sgRNA2 using PCR products. The sequences of the POTS and the PAM are

represented in shadow.

Figure S3.

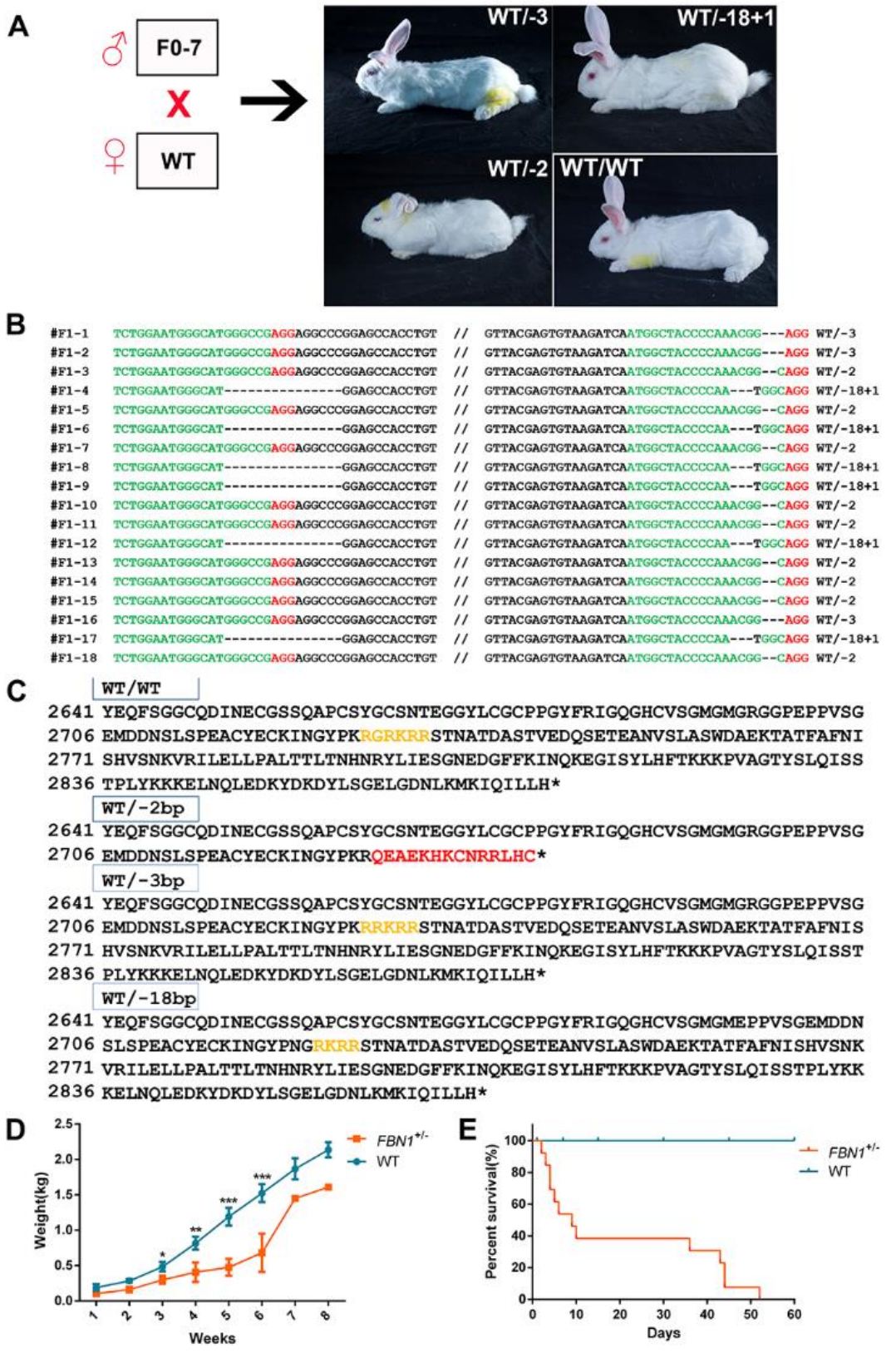

Figure S3. Hereditary of *FBN1* KO rabbits.

(A) Generation of F1 rabbits by #F0-7 mated with WT rabbits. (B) T-cloning sequence analysis of the *FBN1* Het rabbits. Target sites of the two sgRNA sequences, sgRNA1 and sgRNA2, are highlighted in green, protospacer-adjacent motif (PAM) sequence is highlighted in red; WT represents gene sequence of rabbit. (C) The predicted protein sequences of *FBN1* Het rabbits, showing the premature termination codon (PTC) mutation in *FBN1*. (D) The weight comparation shows the growth retarded were determined in the *FBN1* Het rabbits (n=12). (E) Survival curves shows the high mortality of *FBN1* Het rabbits (WT, n=6; *FBN1* Het, n=12). Data are presented as mean ± SEM and analyzed using student's t-tests with Graphpad Prism software 6.0. *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Scale bars: 50 μm.

Supplemental tables

Table S1: Generation of *FBN1* knockout rabbits using CRISPR/Cas9

Recipient	gRNA/Cas9 (ng/μl)	No. embryos transferred	No. newborns (%)	No. mutant rabbits (%)
1	30/200	56	3(5)	2(67)
2	30/200	56	10(18)	9(90)
3	30/200	42	10(24)	10(100)
4	30/200	43	5(12)	5(100)
Total (average)		197	28(14)	26(93)

Table S2: Genotype and phenotype in F1 generation rabbits

NO.F1 generation	Genotype	Transcription is terminated prematurely or not	Phenotype
#1-1	WT/-100	√	√
#1-2	WT/-3	×	×
#1-3	WT/-3	×	×
#1-4	WT/-2	√	√
#1-5	WT/-18+1	×	×
#1-6	WT/-2	√	√
#1-7	WT/-18+1	×	×
#1-8	WT/-2	√	√
#2-1	WT/-18+1	×	×
#2-2	WT/-18+1	×	×
#2-3	WT/-2	√	√
#2-4	WT/-2	√	√
#2-5	WT/-18+1	×	×
#2-6	WT/-100	√	√
#2-7	WT/-2	√	√
#3-1	WT/-2	√	√
#3-2	WT/-2	√	√
#3-3	WT/-3	×	×
#3-4	WT/-18+1	×	×
#3-5	WT/-100	√	√
#3-6	WT/-2	√	√

Table S3: Oligonucleotide used for sgRNA, PCR and qPCR.

NO.	Name	Primers	Sequence(5'-3')	Produce size (bp)
1	SgRNA1	gRNA-1-F	TAGGTCTGGAATGGGCATGGGCCG	20
		gRNA-1-R	AAACCGGCCCATGCCCATTCCAGA	
2	SgRNA2	gRNA-2-F	TAGGATGGCTACCCCAAACGGGGC	20
		gRNA-2-R	AAACGCCCCGTTTGGGGTAGCCAT	
3	FBN1	FBN1-F	TGCCTCACATCTAGCTCCCT	745
		FBN1-R	AGAAACTCCAGAAAGCCCCG	
4	qPCR-FBN1	FBN1-qPCR-F	TAGCTCCTTCCTGTGGCTCC	90
		FBN1-qPCR-R	GGCATAGACAGTGGTCGTCA	
5	qPCR-GAPDH	GAPDH-F	ATCCATTCATTGACCTCCACTAC	179
		GAPDH-R	GTACTGGGCACCAGCATCAC	

1 and 2 were used for the construction of sgRNA expression plasmids; 3 was used for the PCR mutation detection; 4 and 5 were used for the qPCR detection.

Table S4. 12 potential off-target sites examined by PCR and primers used for list.

sgRNA	Potential Off Target Site	Number of mismatch	Position	PCR Primer
s1	TATGGAATGAGCATGGGCCA TGG	3	chr1:-130169142	F: ATCTCCGAAGCCAACAGAAG R: TCTGGCATGGTAAGGAGAAAC
	GTTAGAATGTGCATGGGCCG AAG	4	chr12:-93730077	F: GTCAAGTGGCAATGGGTTTC R: CCACAAGATGTCCCTGAGATG
	TCAGGCATGGGCATGGGCCC TGG	3	chr9:-64499502	F: ACACCATTGGCCCAACTAA R: TTAGGAAAGTACAACGGGCTAC
	GTTGGCAGGGGCATGGGCCG CAG	4	chr17:+81565876	F: GGGATACCACCTTGTACCAATAA R: GAAGAGGGCTTGATCCACAA
	TGTGGTACGTGCATGGGCCG TAG	4	chr13:+141566422	F: AACTGTGACAGGGAATCCTAAC R: CCTGTAGCTCACACACAAACTA
	ACTGGAGTGGGCATGGGCAG AGG	3	chr20:+32533869	F: GAGCCAGGATGTGGTTTCTT R: CCTGTATCTGCTGGGTTGTG
s2	ACTTCTGCCCCAAACGGGGC CGG	4	chr19:-39585556	F: CGCAGGAAGCAATGGAAATG R: TGTGTGACAGGGCAAAGAG
	GGGCCTACCCCGAACGGGGC GAG	4	chr7:-43683785	F: CACACACAGTCTCTCTGTTCAC R: TTCCTTCTGCACACCATGTC
	AATGCACCCCCAAACGGGGC AGG	4	chrUN0:-1338853	F: CATGTCTGTCTCTCTGCCTTAT R: TCTCTTCCCTGCTAACTCTTTG
	ATAACCACCCCCAACGGGGC GGG	4	chr8:+14988562	F: CAAGACACAAAGAAGCACCATC R: CCCACCAAGTTATTGGTCTGTA
	TTGTTTACCCCAAAAGGGGC GGG	4	chr1:-18383438	F: AGAGCAGCTTTAGGAACCTTTG R: TCCTGTCTCCCTGCAGTTT
	ATGGCTTGCTCAGACGGGGC CAG	4	chr3:-24783207	F: AGGGAGTGTCCAGGAGTAAA R: TAGCAAGCAGAGAACACTTCAG