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Supplemental Information

Correction of the Marfan Syndrome Pathogenic *FBN1* Mutation by Base Editing in Human Cells and Heterozygous Embryos

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SUPPLEMENTARY INFORMATION

A

CCGCCAATGGTGTTAACACATAGGAACTGGC (Sequence from NCBI)
CCGCCAATGGTGTTAACACATAGGAACTGGC (WT, 11/20)
CCGCCAATGGTGTTAACACgTAGGAACTGGC (MT, 9/20)

B

CCGCCAATGGTGTTAACACATAGGAACTGGC (Sequence from NCBI)
CCGCCAATGGTGTTAACACATAGGAACTGGC (WT, 13/26)
CCGCCAATGGTGTTAACACgTAGGAACTGGC (MT, 13/26)

Figure S1 The genotype analysis of the patient.

The TA clones of the PCR products of genomic DNA from blood (A) and sperm (B) of one patient with Marfan Syndrome were analyzed by DNA sequencing. The PAM sequences are underlined; the targeted bases (wild type in upper, while mutant in lower case) are highlighted in red; the N/N represents wild type (WT) or mutant (MT) colonies out of total sequenced.

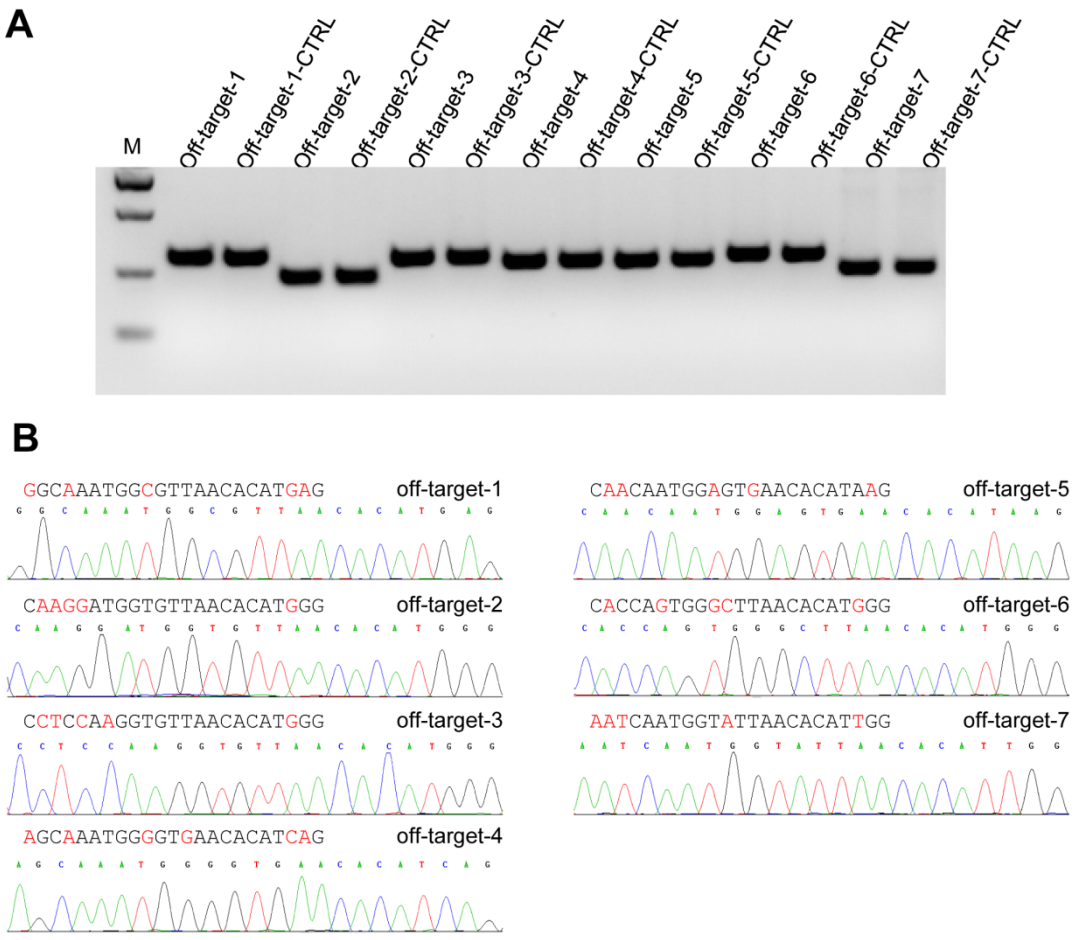


Figure S2 Off-target detection in HEK293T cells.

A. Off-target analysis by T7EN1 cleavage assay. Genomic DNA from base-edited cells was amplified by PCR using primers for 7 potential off-target sites of the sgRNA for the mutagenesis listed in Table S1. The PCR products were subjected to T7EN1 cleavage assay.

B. The representative chromatogram of sequencing of the amplified PCR from A. The red characters were the mismatch sites compared with the mutational sgRNA.

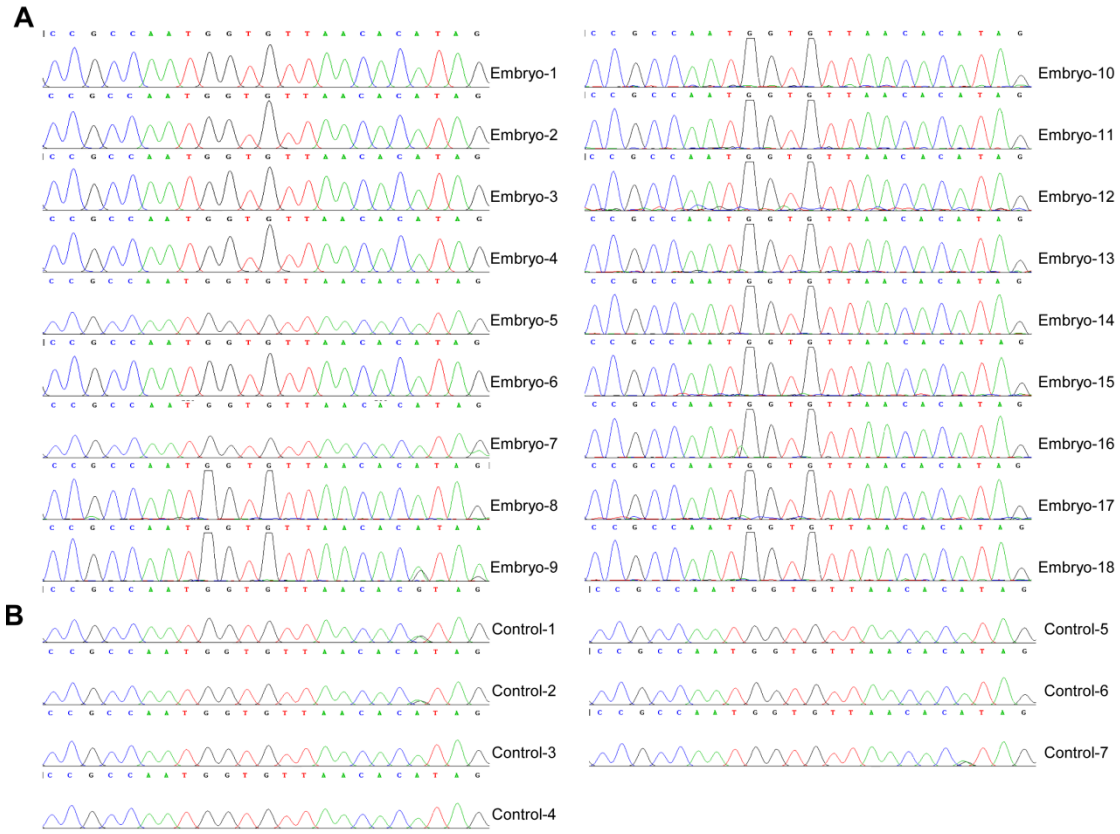
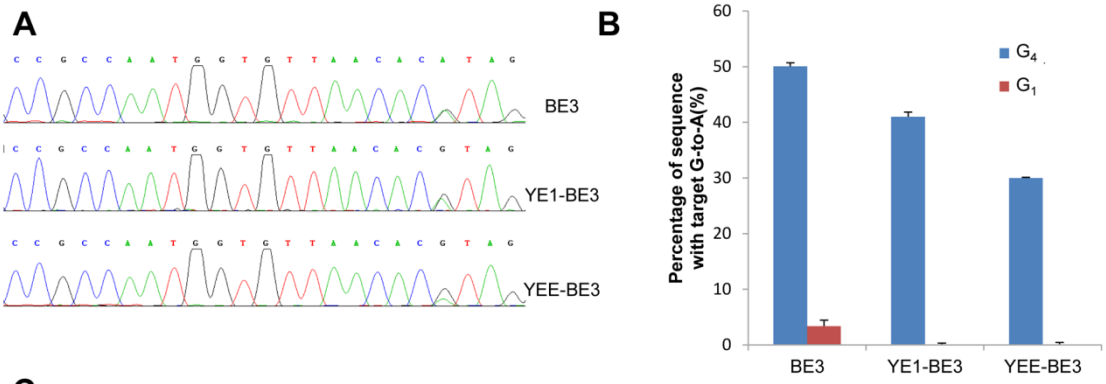


Figure S3 The genotype of the corrected embryos by BE3 and the control human heterozygous embryos.

The representative chromatogram of the sequencing of PCR products was from all the test (A) and control (B) human embryos. All the heterozygous human embryos were collected, and the genomic DNA was extracted and amplified by PCR. The PCR products were analyzed by DNA sequencing. The target bases were highlighted by red (test) or black (control) boxes.



C

	Used embryos	Perfect embryos	Embryos with uncompleted G_4-to-A	Embryos with G_1-to-A	Percentage of perfect embryos	Percentage of edited embryos
BE3	18	16	2	2	89%	100%
YE1-BE3	10	7	3	0	70%	100%
YEE-BE3	11	5	6	0	45%	100%
Control	7	4	-	-	57%	-

Figure S4 BE3 shows the highest correction efficiency for the pathogenic site than YE1-BE3 and YEE-BE3.

A. The FBN1T7498C cells were corrected using three kinds of BE3. The edited cells were used as the template for PCR and Sanger sequence.

B. The deep sequencing was used to detect the editing efficiency, and the percentage of G-to-A at G4 and G1 for correctional sgRNA was analyzed. Data are shown as means ± s.d. from three independent experiments.

C. The summary of the used embryos for correcting the pathogenic sites using three base editors.

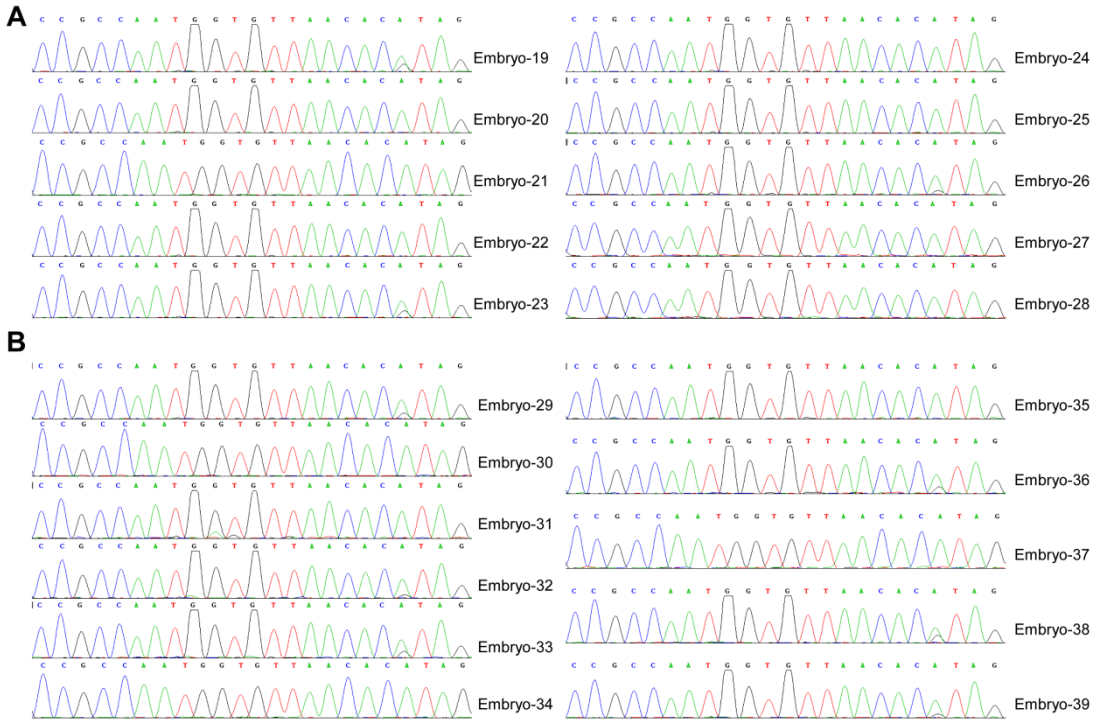


Figure S5 The genotype of the corrected embryos by YE1-BE3 and YEE-BE3

The representative chromatogram of the sequencing of PCR products was from all the YE1-BE3 corrected (A) and YEE-BE3 corrected (B) embryos. All the edited human embryos were collected, and the genomic DNA was extracted and amplified by PCR. The PCR products were analyzed by DNA sequencing.

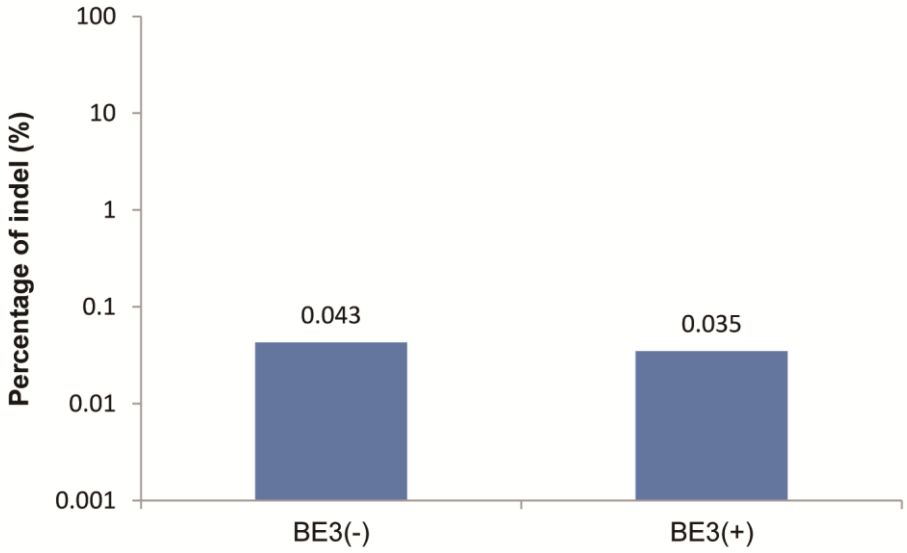


Figure S6 Indel analysis of the corrected and control human heterozygous embryos. Genomic DNA from 7 corrected and 3 control human embryos were collected, the genomic DNA was extracted and amplified by PCR. The PCR products were analyzed by deep sequencing.

Table S1 The primers used in the research

Primer name	Sequence (5'- 3')	Product length (bp)	Usage
FBN1-ON-200-F	ACTCACCAATGCAGGACGTA	200	Amplification for the on-target sites
FBN1-ON-200-R	AGCTGCTTCATAGGGTCAGC		
FBN1-ON-676-F	GCTGAAGTCTCCACCCACC	676	
FBN1-ON-676-R	TGTCTCTCCTTGCCTTTTG		
FBN1-mutation-off1-301-F	TCAAGGGACAGGAGTAGGCA	301	Amplification for the off-target sites of the mutation sgRNA
FBN1-mutation-off1-301-R	TTGGGGCAGGAGGTTTTGTT		
FBN1-mutation-off2-223-F	ATCTTAATCAGGGCCTTGA	223	
FBN1-mutation-off2-223-R	GCCTTCATTCCATCAACTG		
FBN1-mutation-off3-296-F	CAGGTTCGTGTCGCAGTAGC	296	
FBN1-mutation-off3-296-R	CTGTGTTGCCAGCACGAAA		
FBN1-mutation-off4-282-F	TGGTAGTGGTTGGTGACACT	282	
FBN1-mutation-off4-282-R	CGTTACATTGGGAAGCGGAA		
FBN1-mutation-off5-272-F	GGATTCAACATAGATTGGAA	272	
FBN1-mutation-off5-272-R	CCCGTTTACACATTGCTA		
FBN1-mutation-off6-302-F	TTCTAGTAGGTGAAAAAGGG	302	
FBN1-mutation-off6-302-R	TTGGACACCACATAGACAG		
FBN1-mutation-off7-252-F	TATTATTGCTAAACCGAAACCA	252	
FBN1-mutation-off7-252-R	AGCCCCTCACCCACTCAT		
FBN1-BE-OFF1-370-F	AGAGGCTTGCGAAGGACATC	370	Amplification for the off-target sites of the base editor sgRNA
FBN1-BE-OFF1-370-R	ATTTGGTCTAGGGCAGAGGC		
FBN1-BE-OFF2-247-F	ATTATTCACAAGTTATGGTA	247	
FBN1-BE-OFF2-247-R	TAACCCTCTTCTTTGTAA		
FBN1-BE-OFF3-235-F	AAGGGACTGTTTTTGTCCTGTCA	235	
FBN1-BE-OFF3-235-R	GTGAAACCACCATGACATGAAGT		
FBN1-BE-OFF4-262-F	GTCATACTTGGCCAGGGTCC	262	
FBN1-BE-OFF4-262-R	CCCACGTGAGCTGGCTAAAA		
FBN1-BE-OFF5-448-F	TGATCAGCATGTGGAGCCTG	448	
FBN1-BE-OFF5-448-R	GAAGTCAGCCAGGAGCCATT		
FBN1-BE-OFF6-296-F	GAGTTAGGAGTGGGAAGG	296	
FBN1-BE-OFF6-296-R	ACAAAGGACAGTAATGAAGAG		
FBN1-BE-OFF7-208-F	TTTGCCTCCTTGATTCCCCC	208	
FBN1-BE-OFF7-208-R	GTGGATGGTGTGGAGGTGAG		
FBN1-BE-OFF8-208-F	CGCAGAACCAGACATCTTTAG	208	
FBN1-BE-OFF8-208-R	TTTGTTAGTAGCACAGGGGC		
FBN1-BE-OFF9-223-F	AAATTTGGAGAATATAGCTAGG	223	
FBN1-BE-OFF9-223-R	GAAAGTGCTTGAAACATAGTAA		
FBN1-BE-OFF10-229-F	ACAGGCATAAGTCACCGCA	229	
FBN1-BE-OFF10-229-R	CACTGGGTTACCTGGCATTT		

FBN1-BE-OFF11-238-F	CCTTTACAGGCTCACATCTT	238	Amplification for the off-target sites of the base editor sgRNA
FBN1-BE-OFF11-238-R	GTAGTTTTGAGATAAGATAACCG		
FBN1-BE-OFF12-232-F	TAGCATTTGTTGGCAGTTAC	232	
FBN1-BE-OFF12-232-R	GCGATTGTTTTCTTGTTCAT		
FBN1-BE-OFF13-206-F	AATCAGCTTTGACAAATATTGTA	206	
FBN1-BE-OFF13-206-R	AGTAACTGGAATCCGTGCTA		
FBN1-BE-OFF14-222-F	CCTGGTCATAATGTGGGTC	222	
FBN1-BE-OFF14-222-R	CTGCCTGGCTGAGGAATA		
FBN1-BE-OFF15-223-F	CATTTATCTGGTTTTTCTTGTT	223	
FBN1-BE-OFF15-223-R	ACAAGTGTAATCACCATAGTCC		
FBN1-BE-OFF16-239-F	GGCAAGAGAAGGAGAGAGG	239	
FBN1-BE-OFF16-239-R	TTTGGGAAGTTTGAGAAAAA		
FBN1-BE-OFF17-248-F	CAGCAGGTGTGGTCGTTTT	248	
FBN1-BE-OFF17-248-R	TTCACCTCATCAACACCCC		
FBN1-BE-OFF18-249-F	TAAAATGTATGTAGGGAAAGC	249	
FBN1-BE-OFF18-249-R	ATAAGTCAAAGGAAAAGTGAAA		
FBN1-BE-OFF19-233-F	AAAAAGAGAAAGAGGGAGTCA	233	
FBN1-BE-OFF19-233-R	CAAGGGATAGGAGACATCG		
FBN1-BE-OFF20-285-F	GAGAAGCAAATGGTTTATGGT	285	
FBN1-BE-OFF20-285-R	TTAGATTAGCAGATACTCAGGGA		
FBN1-BE-OFF21-288-F	GATGTAAATGTGAAAATGGAAAC	288	
FBN1-BE-OFF21-288-R	CTCTGTTGGGTTATCGTGC		
FBN1-BE-OFF22-243-F	GGCTGGTTTATTTTTCTTCAA	243	
FBN1-BE-OFF22-243-R	GAGTCAAAAGTAGTGCCTGGA		
FBN1-BE-OFF23-206-F	GAAAAGAGGCTCTAATTGTAGG	206	
FBN1-BE-OFF23-206-R	GTCTGAGGCAGCACTTTGT		
FBN1-BE-OFF24-229-F	TCAATAAGAAAAAGTCTCAACAG	229	
FBN1-BE-OFF24-229-R	CGCTTTTCCTGATATGCTA		
FBN1-BE-OFF25-217-F	AAATCTTTGACCTTTTCATACTC	217	
FBN1-BE-OFF25-217-R	ATGAACCCAGATGAGCCA		
FBN1-BE-OFF26-249-F	TAATGGGATGGCTGGGTC	249	
FBN1-BE-OFF26-249-R	AAATGCTTATCATCACTGGTCA		
FBN1-BE-OFF27-248-F	GCTTCAAGTAATAACAGTCCGTA	248	
FBN1-BE-OFF27-248-R	CCAAAGTGCTGGGATTACA		
FBN1-BE-OFF28-267-F	ATCTTTTTTATGTATCCACGG	267	
FBN1-BE-OFF28-267-R	ACTGACCTTTGGTGAGTAATAA		
FBN1-BE-OFF29-206-F	CAAAACCTCTAGTTCCCTGA	206	
FBN1-BE-OFF29-206-R	ACAGAACAGATGCCTCAAAA		
FBN1-BE-OFF30-228-F	TACCATCTCACGCCAGTTAG	228	
FBN1-BE-OFF30-228-R	GCATGATTTACAATCCTTTGG		
FBN1-BE-OFF31-242-F	CAAGGAGAGTGCTGTAAAGAG	242	
FBN1-BE-OFF31-242-R	GCATAGGAATGTAGAGGAGTTTA		

FBN1-BE-OFF32-370-F	CGATAAAGGGATCAGTCACTAA	370	
FBN1-BE-OFF32-370-R	GCTCCAGGTCCACAAACAC		