

Supplemental Information

Correction of the pathogenic mutation in *TGM1* gene by adenine base editing in mutant embryos

Lu Dang, Xueliang Zhou, Xiufang Zhong, Wenxia Yu, Shisheng Huang, Hanyan Liu, Yuanyuan Chen, Wuwen Zhang, Lihua Yuan, Lei Li, Xingxu Huang, Guanglei Li, Jianqiao Liu, and Guoqing Tong

Supplementary information

Correction of the pathogenic mutation in *TGMI* gene by adenine base editing in mutant embryos

Lu Dang^{1,*}, Xueliang Zhou^{1,*}, Xiufang Zhong^{2,*}, Wenxia Yu³, Shisheng Huang³,
Hanyan Liu¹, Yuanyuan Chen², Wuwen Zhang², Lihua Yuan², Lei Li¹, Xingxu
Huang³, Guanglei Li^{3,#}, Jianqiao Liu^{1,#}, Guoqing Tong^{2,#}

¹*Department of Reproductive Medicine, Third Affiliated Hospital of Guangzhou Medical University, Guangzhou 510150, China*

²*Department of Reproductive Center, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*

³*School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China*

**Co-first author*

Correspondence: drivyftongguoqing@shutcm.edu.cn (G.T.), liujqssz@gzhmu.edu.cn (J.L.), ligl@shanghaitech.edu.cn (G.L.)

Short title: Correction of *TGMI* mutation using ABE system

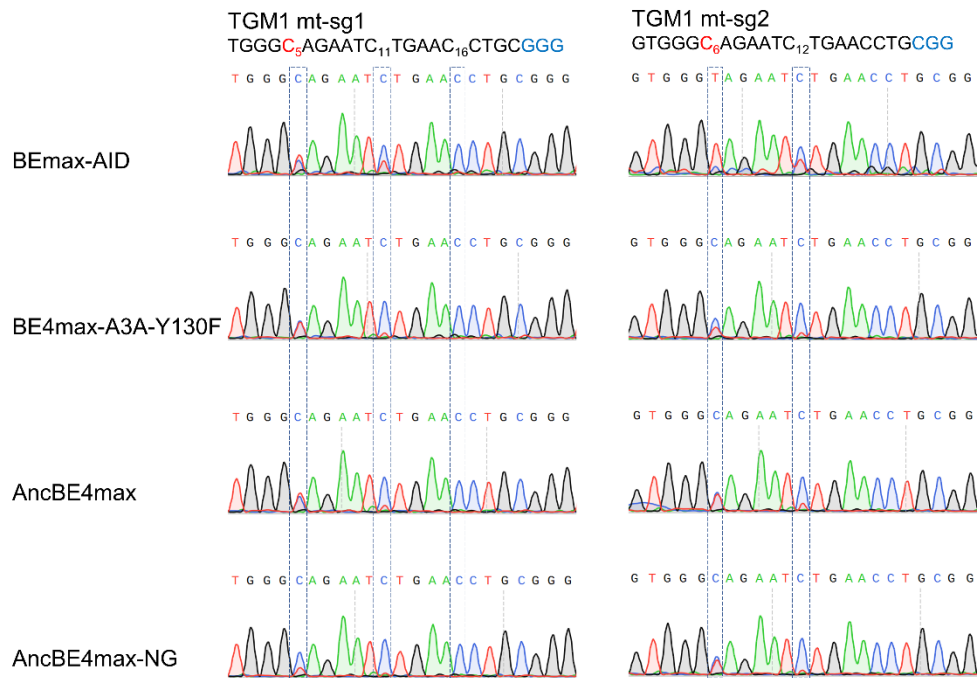


Figure S1. Sanger sequencing chromatogram for the edited HEK293T cell. The target site highlighted in red and with a numeric subscript. The non-target sites are highlighted with a numeric subscript.

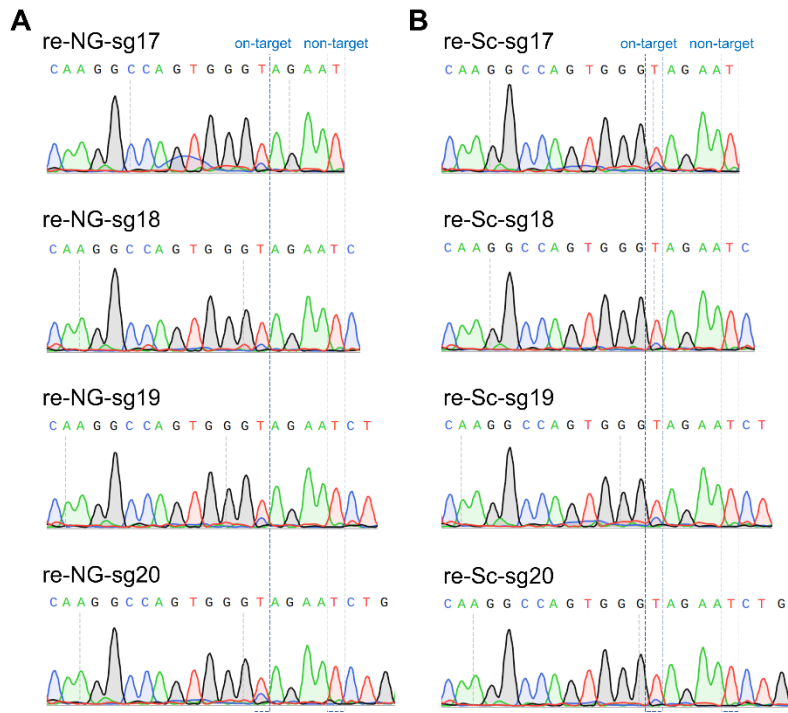


Figure S2. Sanger sequencing chromatogram for the correction of the pathogenic mutation by ABEmax-NG (A) and Sc-ABEmax (B) combined with truncated sgRNA.

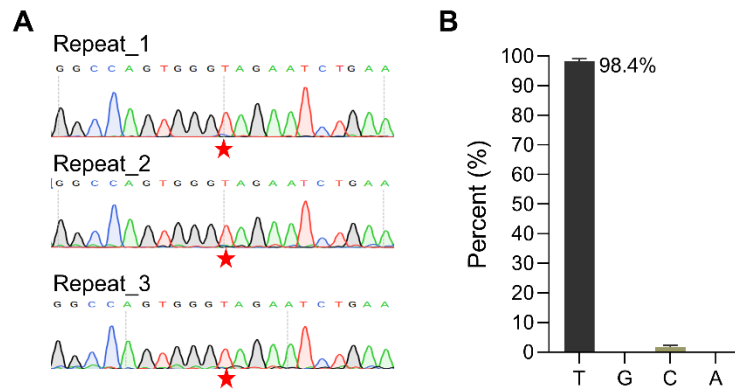


Figure S3. Analysis of the patient sperm. (A) The sequence chromatogram of the sperm from the patient. The red star indicated the pathogenic point mutation. (B) The genotype analysis of sperm sample by deep sequencing.

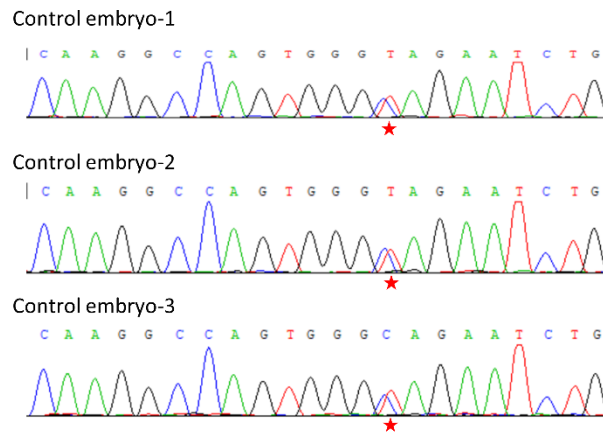


Figure S4. The sequence chromatogram for control embryos. The red star shows the pathogenic mutations.

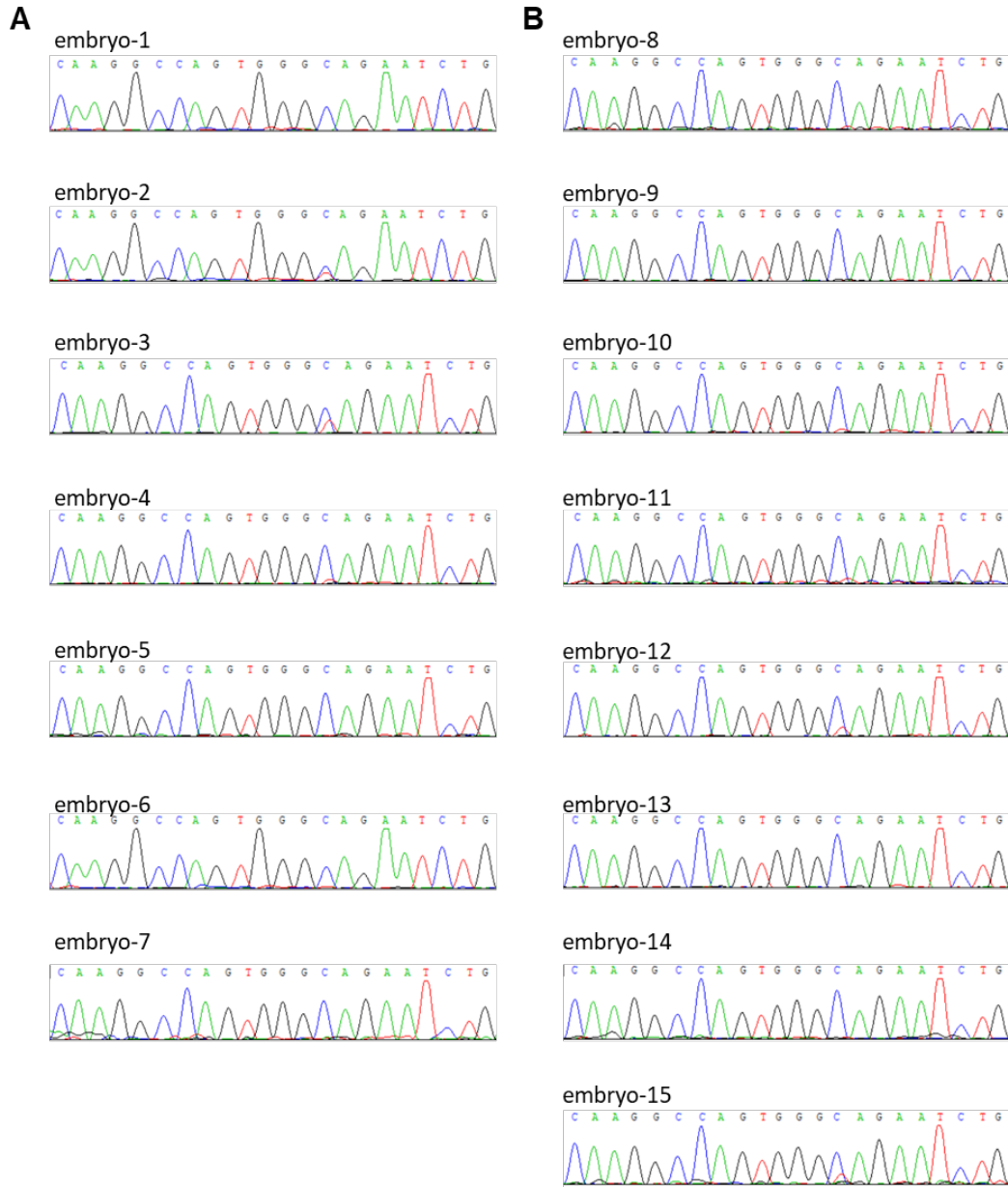


Figure S5. (A, B) The sequence chromatogram for all edited embryos in ABEmax-NG group (A) and Sc-ABEmax group (B).

Table S1 The editing frequency analysis of the corrected embryos.

| | Sample name | Percentage of wild genotype on target site (%) | Editing frequency on non-target site (%) |
|-----------------|-------------|--|--|
| ABEmax-NG group | embryo-1 | 94.18359 | 0.206321 |
| | embryo-2 | 70.01231 | 0.191813 |
| | embryo-3 | 71.18183 | 0.207506 |
| | embryo-4 | 86.02006 | 0.219498 |
| | embryo-5 | 97.86279 | 0.285806 |
| | embryo-6 | 91.58933 | 0.2553 |
| | embryo-7 | 97.50425 | 0.206666 |
| Sc-ABEmax group | embryo-8 | 97.66458 | 0.285655 |
| | embryo-9 | 97.16523 | 0.211075 |
| | embryo-10 | 81.65478 | 0.310669 |
| | embryo-11 | 97.4 | 0.240646 |
| | embryo-12 | 67.82205 | 0.314222 |
| | embryo-13 | 97.06223 | 0.346686 |
| | embryo-14 | 96.45348 | 0.265436 |
| | embryo-15 | 79.42178 | 0.257861 |

Table S2 The sgRNA sequence used in this study.

| sgRNA | Sequence (5'-3') |
|-------------|----------------------|
| TGM1 mt-sg1 | TGGGCAGAATCTGAACCTGC |
| TGM1 mt-sg2 | GTGGGCAGAATCTGAACCTG |
| re-NG-sg20 | AGGCCAGTGGGTAGAATCTG |
| re-NG-sg19 | AGGCCAGTGGGTAGAATCT |
| re-NG-sg18 | AGGCCAGTGGGTAGAATC |
| re-NG-sg17 | AGGCCAGTGGGTAGAAT |
| re-Sc-sg20 | GGCCAGTGGGTAGAATCTGA |
| re-Sc-sg19 | GGCCAGTGGGTAGAATCTG |
| re-Sc-sg18 | GGCCAGTGGGTAGAATCT |
| re-Sc-sg17 | GGCCAGTGGGTAGAATC |

Table S3 The primer used for PCR or deep sequencing.

| Primer | Sequence Sequence (5'-3') |
|-------------------|------------------------------|
| TGM1-PCR-F | CCTACTCTAGGAAACAACCC |
| TGM1-PCR-R | GAAGAGGATGTAGATCTCATTG |
| TGM1-deep seq-F1 | atcacgCCTACTCTAGGAAACAACCC |
| TGM1-deep seq-F2 | cgatgttCCTACTCTAGGAAACAACCC |
| TGM1-deep seq-F3 | ttaggcagCCTACTCTAGGAAACAACCC |
| TGM1-deep seq-F4 | tgaccagCCTACTCTAGGAAACAACCC |
| TGM1-deep seq-F5 | acagtgtCCTACTCTAGGAAACAACCC |
| TGM1-deep seq-F6 | gccaatCCTACTCTAGGAAACAACCC |
| TGM1-deep seq-F7 | cagatctCCTACTCTAGGAAACAACCC |
| TGM1-deep seq-F8 | acttgaacCCTACTCTAGGAAACAACCC |
| TGM1-deep seq-F9 | gatcaggCCTACTCTAGGAAACAACCC |
| TGM1-deep seq-F10 | tagcttcCCTACTCTAGGAAACAACCC |
| TGM1-deep seq-F11 | ggctacCCTACTCTAGGAAACAACCC |
| TGM1-deep seq-F12 | cttgtaCCTACTCTAGGAAACAACCC |
| OT-F1 | ACCTTTCTGTCTTTGGATGT |
| OT-R1 | TCTCCTTGTGTCTGGTGTA |
| OT-F2 | TGAGGACCCATCCAACCTC |
| OT-R2 | GCAAGCGTATATGTGAATCC |
| OT-F3 | TGACTAGAAAGTGTGTGCTT |
| OT-R3 | CATGTGTACCTGGTCCATT |
| OT-F4 | TTGGATAACGCCATTGATG |
| OT-R4 | CCTCCTATAAATAACACCCTGA |
| OT-F5 | TATGATGATCCTTCCTTTGC |
| OT-R5 | TATAGTTGTTGGACTCAGGA |
| OT-F6 | TGCTGCCTGTGATTTATGA |
| OT-R6 | GGTCAACTGTGGTGTCTC |
| OT-F7 | TCTTCACTGATCCTATTGACC |
| OT-R7 | CTTAGAGATGATGCTGGAGT |
| OT-F8 | AGTCCCATCTTGTCCACATCA |
| OT-R8 | TAGAGGTGCAGCATGGCCTC |
| OT-F9 | CTGACCTTCGCTGAGATAG |
| OT-R9 | TCACACCAATTCTGATTCCA |
| OT-F10 | GAATCATTCCACAACCTCTAGG |
| OT-R10 | TGATGAACAGCAGGAAGG |
| OT-F11 | CATCGGTCATACTGTATTAGG |
| OT-R11 | GTTGGAATGTGGTGAATGTA |
| OT-F12 | CCCTCAACTTTAGAAAGAAGT |
| OT-R12 | TGTAGAAGAGAGATCAGATGTG |
| OT-F13 | CTAATCAGTGTCTTACTGGAGAG |
| OT-R13 | GCTCCTCTAACTCTAATGTATG |
| OT-F14 | ACTGAGGTGATTAACAAAGC |
| OT-R14 | TTGGTTAGAGGTTAGGTGTG |

| | |
|--------|------------------------|
| OT-F15 | TCAGAACCCAGAGCAATCA |
| OT-R15 | AGAGATGGAGTCCGTGTG |
| OT-F16 | CACATGGATGCTCTTCAATC |
| OT-R16 | CATGGTAAGACATACTCATTGG |
| OT-F17 | CGACTGAACATCTCTGTGT |
| OT-R17 | CCTCTAAGTCCTCCACCTT |

Table S4 The information of predicted off-target sites.

| Name | DNA | Chr | Position | Direction | Mismatches |
|------|--------------------------|-------|-------------|-----------|------------|
| OT1 | GGCCAGTGGGTAGAATagGAGGG | chr15 | 94,745,816 | - | 2 |
| OT2 | GGCCcaTGGGTAGAATCTGAAGG | chr5 | 8,503,050 | - | 2 |
| OT3 | GGCCAGTGGGcAGAAgCTGACAG | chr5 | 135,355,669 | + | 2 |
| OT4 | GGCCAGTGGGTAGAAaCTtAAGG | chr4 | 73,013,365 | - | 2 |
| OT5 | GGCCAGTGGGctGAATCaGAGAG | chr8 | 13,409,077 | - | 3 |
| OT6 | GGCCAGgGGGTgGAATCTGtGAG | chr8 | 28,250,565 | + | 3 |
| OT7 | GGCCAGaGGGTAGAcTCTtACAG | chr5 | 24,402,175 | - | 3 |
| OT8 | GGCCtGTGGGTAGcATgTGACGG | chr5 | 180,112,311 | + | 3 |
| OT9 | GGgCAGTGGGgAGAcTCTGATGG | chr20 | 63,027,610 | - | 3 |
| OT10 | GGCCtGTGGGgAGAtTCTGAGGG | chr1 | 160,135,818 | - | 3 |
| OT11 | GGCCAGaGGGTAGAATgTtAGGG | chr1 | 160,810,629 | - | 3 |
| OT12 | GaCCAGTGGGTAGAAaCTGtAGG | chr1 | 173,804,224 | - | 3 |
| OT13 | atCCAGTGGGTAAaAATCTGAAAG | chr1 | 223,358,725 | - | 3 |
| OT14 | GGCCAGTtGGTgGAATCTGcAAG | chr7 | 110,235,678 | - | 3 |
| OT15 | GaCCAtTGGGTAGAATCTaACAG | chr7 | 158,652,020 | + | 3 |
| OT16 | GGCCAGTtGGaAGAATCTtACAG | chr12 | 10,679,571 | - | 3 |
| OT17 | GGCCAGaaGGTAGAAgCTGAAAG | chr4 | 25,043,394 | + | 3 |