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Supplemental Information

Therapeutic base editing and prime editing of *COL7A1* mutations in recessive dystrophic epidermolysis bullosa

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Figure S1. Genotyping of RDEB patients using Nanopore long-read sequencing. Schematic of *COL7A1* cDNA of Pat1 (A) and Pat2 (B) and Integrative Genomics Viewer (IGV) snapshots showing the alignment data at pathogenic mutations in *COL7A1* gene of Pat1 (A) and Pat2 (B). Each horizontal bar indicates one read and mutations are shown on the bars.

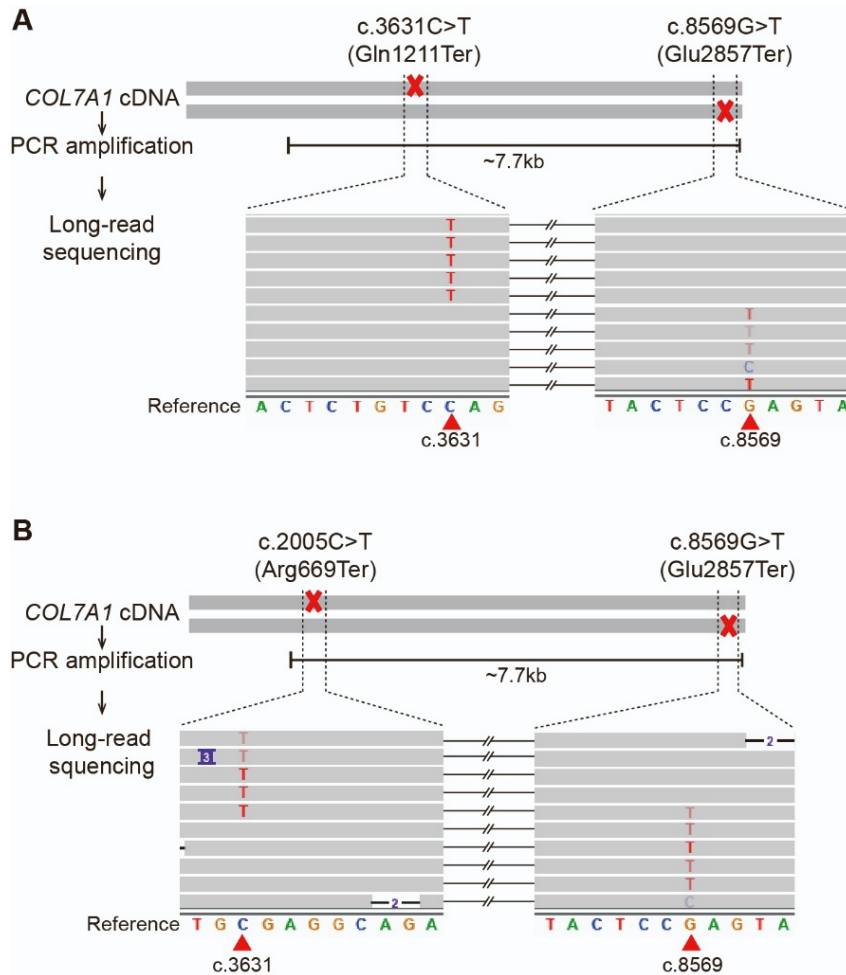


Figure S2. Correction of the c.3631C>T mutation in COL7A1. **A** Results from Sanger sequencing of targeted regions of the *COL7A1* cDNA from ABE-treated patient-derived fibroblasts. **B** Target sequences of pegRNAs and ngRNAs used for prime editing. **C** Results from Sanger sequencing of targeted regions of the *COL7A1* cDNA from PE-treated patient-derived fibroblasts.

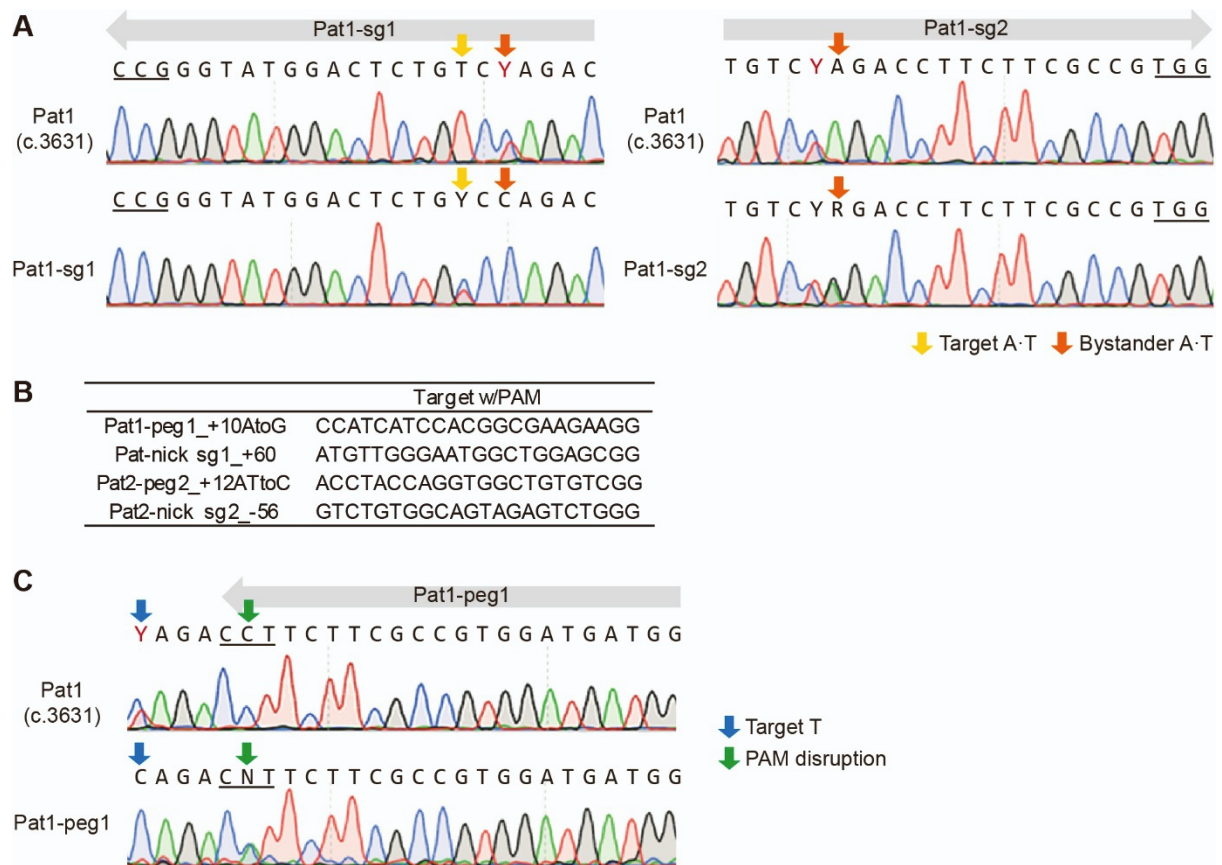


Figure S3. Uncropped western blot images presented in Figure 2. Western blot analysis of proteins from cell lysates (left) and conditioned media (right) of NHDF (lane 1), non-edited fibroblasts from Pat1 (lane 2), ABE/Pat1-sg1-treated RDEB fibroblasts (lane 3), and ABE/Pat1-sg2-treated RDEB fibroblasts (lane 4). The position of the band corresponding to full-length 290-kD type VII collagen (C7) is indicated. GAPDH was included as an internal control. Beta-actin is not detected in the culture supernatants of fibroblasts.

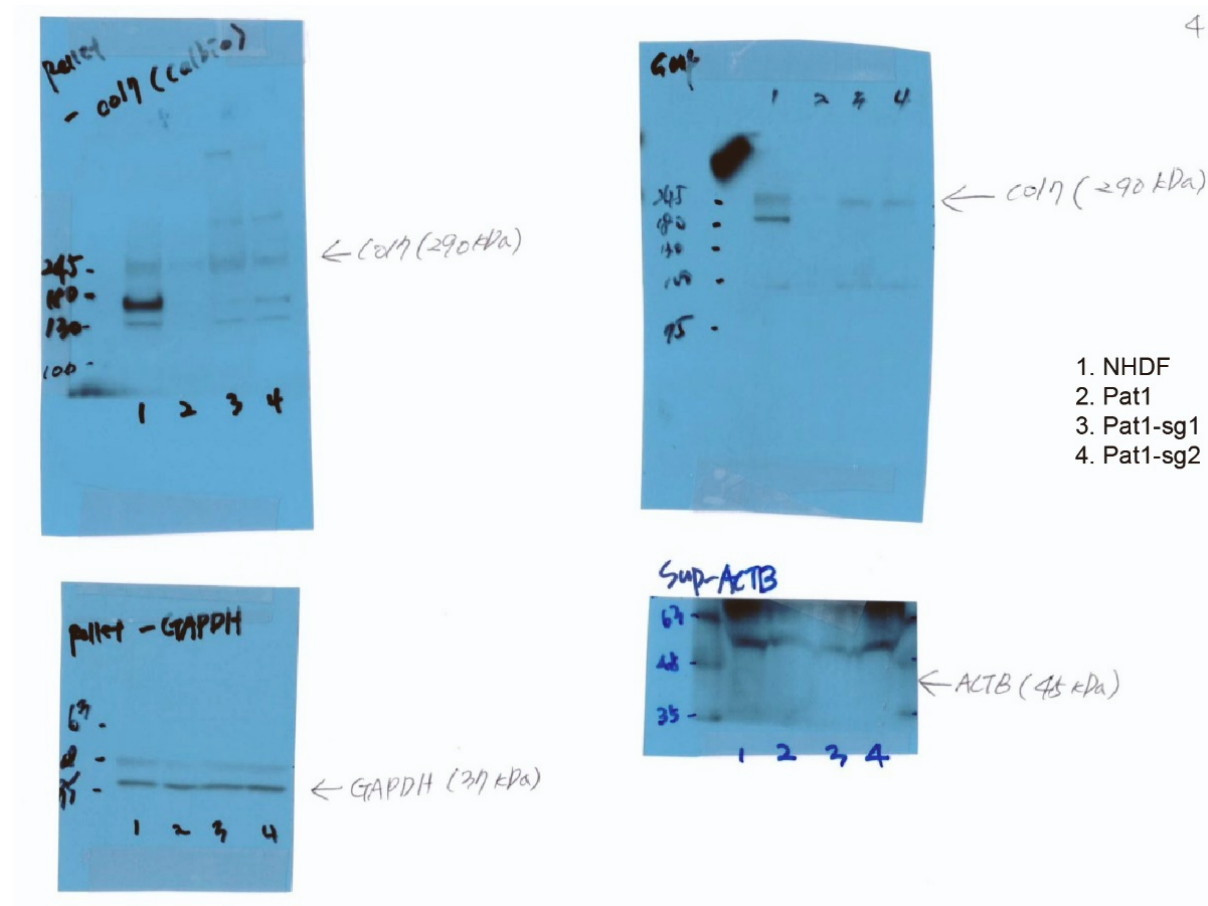


Figure S4. Uncropped western blot images presented in Figure 3. Western blot analysis of proteins from cell lysates (left) and conditioned media (right) of NHDF (lane 1), non-edited fibroblasts from Pat1 (lane 2), and PE3-treated RDEB fibroblasts (lane 3). The position of the band corresponding to full-length 290-kD type VII collagen (C7) is indicated. GAPDH was included as an internal control. Beta-actin is not detected in the culture supernatants of fibroblasts.

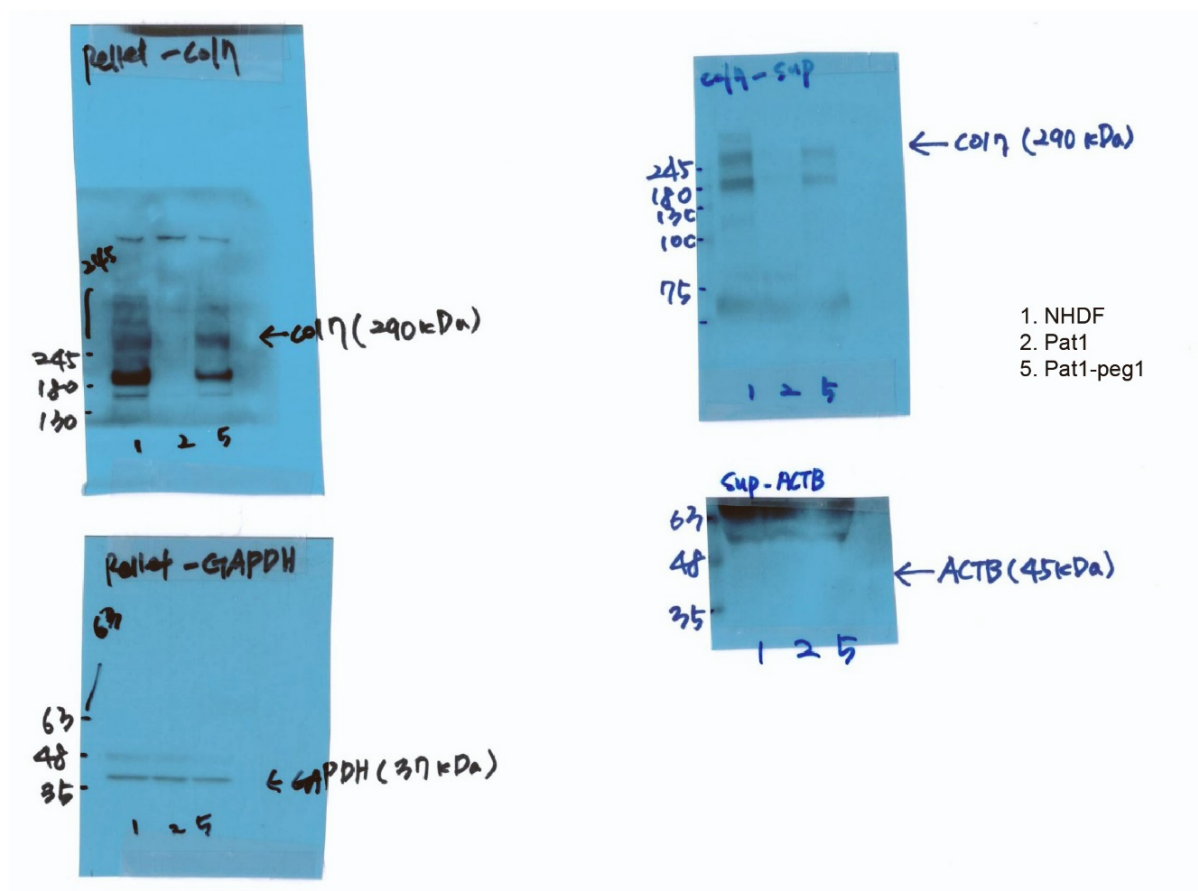


Figure S5. Analysis of off-target effects in ABE-/PE-treated patient-derived fibroblasts. **A** List of potential off-target sites with 1~3 mismatches relative to the target site. The potential off-target sites were found using Cas-OFFinder software. **B-D** A-to-G conversion rates (**B, C**) and indel frequencies (**D**) at *in silico* predicted off-target sites in ABE- or PE-treated patient-derived fibroblasts.

A

| | OT# | DNA | Chr:position | Direction | Mismatches | #Bulge type | Bulge Size |
|-----------|-----|--------------------------|-----------------|-----------|------------|-------------|------------|
| Pat1-sg1 | 1 | GTCTAGACAGAGTCCiTA-CAGG | chr2:222668063 | - | 1 | RNA | 1 |
| | 2 | cTCTAGACAGAGTCCcTcCCTGG | chr16:65885523 | - | 3 | X | 0 |
| | 3 | GTCTAGtCAGAcTCCcTACCTGG | chr3:194986751 | + | 3 | X | 0 |
| | 4 | GTCaAGaAaAGAGTCCAcACCAGG | chr2:237287245 | + | 3 | X | 0 |
| | 5 | GTCTAGiCAGAGTCCaAaICCGG | chr10:44910865 | + | 3 | X | 0 |
| | 6 | aTCTAGCACAGAGaCCATACCAGG | chr15:54428712 | - | 2 | DNA | 1 |
| | 7 | GTCATAGAaAGAGTCCAgACCTGG | chr8:128411500 | + | 2 | DNA | 1 |
| | 8 | GTCTAGaAaAcAG-CCATACCTGG | chr11:64201596 | + | 2 | RNA | 1 |
| | 9 | GTCcAGACAGAcTCC-TACCAGG | chr11:125939426 | + | 2 | RNA | 1 |
| | 10 | GTCTAG-CAGAGcCCATgCCAGG | chr17:78370455 | + | 2 | RNA | 1 |
| | 11 | GTCTA-ACAGAGTgtATACCTGG | chr12:25153928 | + | 2 | RNA | 1 |
| | 12 | GTCT-GgCAGAGTCCATcCCAGG | chr19:37454846 | + | 2 | RNA | 1 |
| | 13 | GTCTAGACAcAGT-aATACCTGG | chr20:46249490 | - | 2 | RNA | 1 |
| | 14 | GTCTgGAACAGAGTCCATgCCAGG | chr4:133163104 | - | 2 | DNA | 1 |
| | 15 | tTaTAGACAGAGTcCTATACCAGG | chr2:168602331 | - | 2 | DNA | 1 |
| | 16 | GTCcAGACAGAGT-CATACiTGG | chr6:13201451 | + | 2 | RNA | 1 |
| | 17 | GTCTAaACAGAGTiCATACCCTGG | chr1:176920267 | - | 2 | DNA | 1 |
| | 18 | GTaTAGAt-GAGTCCATACCTGG | chr1:81450137 | + | 2 | RNA | 1 |
| | 19 | aTC-AGiCAGAGTCCATACCCTGG | chr5:82994985 | - | 2 | RNA | 1 |
| | 20 | GTCT-GACAGAtTCaATACCAGG | chr5:128797239 | + | 2 | RNA | 1 |
| Pat1-sg2 | 1 | TGTCTAaACCTTCTTC-CCiTGG | chr8:137586826 | - | 2 | RNA | 1 |
| | 2 | TGTCaAGACCTTCTTC-CaGAGG | chr14:83115514 | - | 2 | RNA | 1 |
| | 3 | TGTtTAiACCTTCTTC-CCGGGG | chr17:45224139 | + | 2 | RNA | 1 |
| | 4 | TiTCTAGACCTTCTT-GCaGTGG | chr19:18447708 | + | 2 | RNA | 1 |
| | 5 | TGT-TAGACCTaCTTiCCGGTGG | chr22:22319369 | - | 2 | RNA | 1 |
| Pat1-peg1 | 1 | CCATC-TCCACGGCaAAGAAGG | chr17:50569186 | - | 1 | RNA | 1 |
| | 2 | CCiTCATCCACGGCcAAGcAGG | chr16:47884695 | + | 3 | X | 0 |
| | 3 | CCATgtTCCACGGCiAAGATGG | chr15:68833313 | + | 3 | X | 0 |
| | 4 | gCATgATCCACGGGaGAAGAAGG | chr3:33989747 | - | 3 | X | 0 |
| | 5 | tCAgCAgCCACGGCGAAGAGGG | chr13:113920748 | - | 3 | X | 0 |
| | 6 | CCATCATCCACGGCGggaAAGG | chr2:219638783 | - | 3 | X | 0 |
| | 7 | CCATCATCCctCiGCGAgGAAGG | chr1:7492183 | + | 3 | X | 0 |
| | 8 | CCATCATCCAtGGC-AgGAAGG | chrX:18936163 | + | 2 | RNA | 1 |
| | 9 | CCATCAT-CAaGgTGAAGATGG | chrX:140206918 | + | 2 | RNA | 1 |
| | 10 | CC-TCATCCAgGGaGAAGACGG | chr16:73436845 | - | 2 | RNA | 1 |
| | 11 | CCATCAT-CACaGCcAAGAAGG | chr15:40931409 | + | 2 | RNA | 1 |
| | 12 | CC-TCATCCACGGiGAAGiAGG | chr11:119664880 | + | 2 | RNA | 1 |
| | 13 | CCATCATCCACca-GAAGATGG | chr3:162552242 | - | 2 | RNA | 1 |
| | 14 | CCATCATCCA-GGtGgAGAAGG | chr17:32990613 | + | 2 | RNA | 1 |
| | 15 | CCATCAT-CAgGGiGAAGAAGG | chr19:58435338 | + | 2 | RNA | 1 |
| | 16 | CCATCATCCACGG-GAgGIAGG | chr20:50499905 | - | 2 | RNA | 1 |
| | 17 | CCA-CATCCAgGGaGAAGAGGG | chr20:33019730 | - | 2 | RNA | 1 |
| | 18 | CCATCATCCA-GGtGcAGAGGG | chr6:42178418 | + | 2 | RNA | 1 |
| | 19 | CC-TCATCCACiGgGAAGATGG | chr10:78155109 | + | 2 | RNA | 1 |

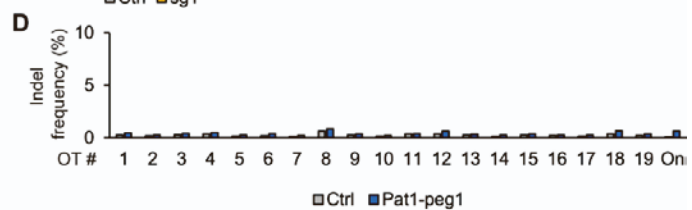
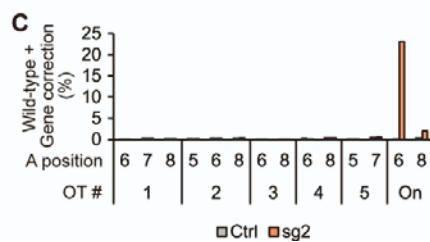
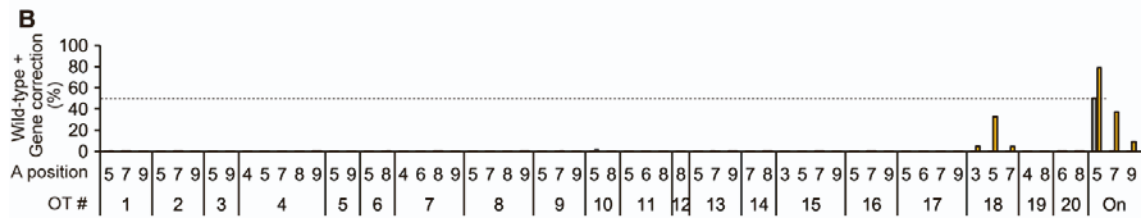


Figure S6. Correction of the c.3631C>T mutation in COL7A1 in patient-derived keratinocytes. A Information about the RDEB patient whose keratinocytes were used for the experiment. **B** Results from Sanger sequencing of targeted regions of the COL7A1 of genomic DNA from patient's blood. Red arrows indicate the mutations. **C, D** Conversion rate and indel frequency calculated by deep sequencing of genomic DNA from RDEB fibroblasts treated with ABE (**B**) and PE (**C**).

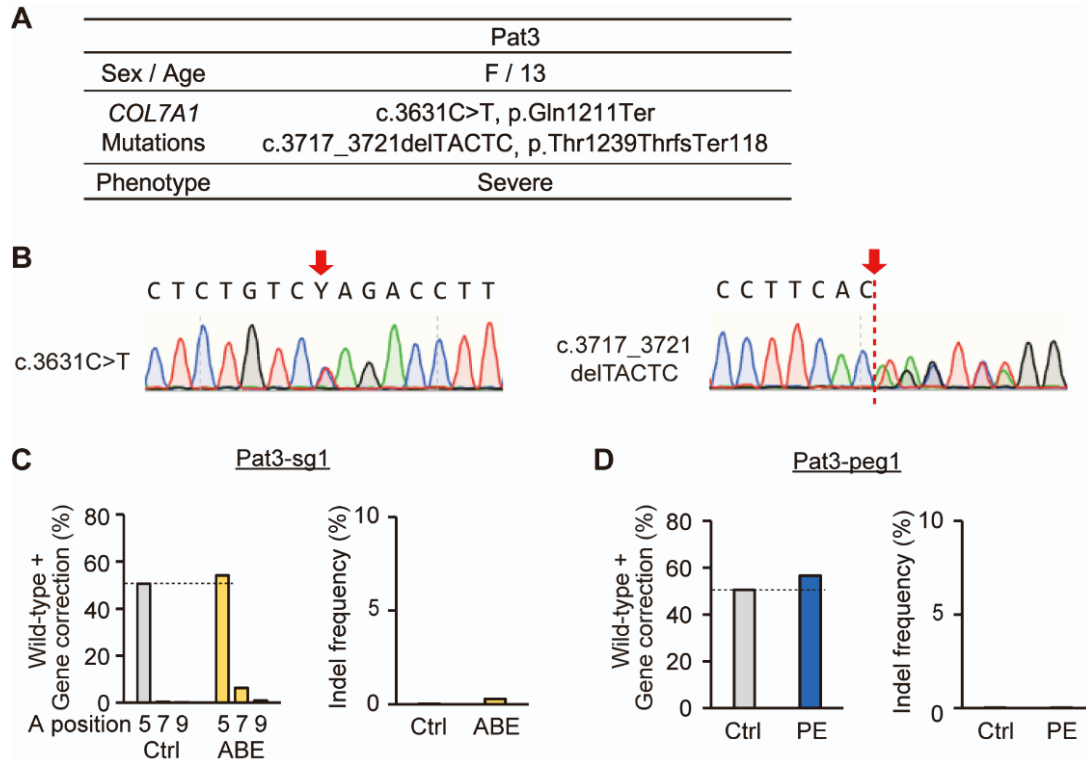


Table S1. Phenotypes and genotypes in Korean patients with RDEB. (Additional datasheet)

Table S2. Primers used for sequencing in this study. (Additional datasheet)

Table S3. Oligos used for sgRNA- and pegRNA-expressing plasmids in this study.

| Oligo name | Sequence (5'to3') |
|---------------------------|---|
| Pat1-sg1_up | CACCGGTCTAGACAGAGTCCATAACC |
| Pat1-sg1_down | AAACGGTATGGACTCTGTCTAGACC |
| Pat1-sg2_up | CACCGTGTCTAGACCTTCTTCGCCG |
| Pat1-sg2_down | AAACCGGCGAAGAAGGTCTAGACAC |
| Pat1-peg1_up | CACCGCCATCATCCACGGCGAAGAGTTTT |
| Pat1-peg1_down | CTCTAAAACCTTTCGCCGTGGATGATGGC |
| Pat1-peg1_RTT14PBS15_up | GTGCTGTCCAGACATTCTTCGCCGTGGATGATG |
| Pat1-peg1_RTT14PBS15_down | AAAACATCATCCACGGCGAAGAATGTCTGGACA |
| Pat1-peg1_RTT14PBS13_up | GTGCTGTCCAGACATTCTTCGCCGTGGATGA |
| Pat1-peg1_RTT14PBS13_down | AAAATCATCCACGGCGAAGAATGTCTGGACA |
| Pat1-peg1_RTT14PBS11_up | GTGCTGTCCAGACATTCTTCGCCGTGGAT |
| Pat1-peg1_RTT14PBS11_down | AAAATCCACGGCGAAGAATGTCTGGACA |
| Pet1-peg1_nick_+60_up | CACCGATGTTGGGAATGGCTGGAG |
| Pet1-peg1_nick_+60_down | AAACCTCCAGCCATTCCCAACATC |
| Pat2-peg2_up | CACCGACCTACCAGGTGGCTGTGTGTTTT |
| Pat2-peg2_down | CTCTAAAACACACAGCCACCTGGTAGGTC |
| Pat2-peg2_RTT14PBS15_up | GTGCTCGCAGTACGGACACAGCCACCTGGTAGG |
| Pat2-peg2_RTT14PBS15_down | AAAACCTACCAGGTGGCTGTGTCCGTACTGCGA |
| Pat2-peg2_RTT14PBS13_up | GTGCTCGCAGTACGGACACAGCCACCTGGTA |
| Pat2-peg2_RTT14PBS13_down | AAAATACCAGGTGGCTGTGTCCGTACTGCGA |
| Pat2-peg2_RTT14PBS11_up | GTGCTCGCAGTACGGACACAGCCACCTGG |
| Pat2-peg2_RTT14PBS11_down | AAAACCAGGTGGCTGTGTCCGTACTGCGA |
| Pat2-peg2_nick_-56_up | CACCGGTCTGTGGCAGTAGAGTCT |
| Pat2-peg2_nick_-56_down | AAACAGACTCTACTGCCACAGACC |
| Scaffold_up | AGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATC AACTTGAAAAAGTGGCACCGAGTCG |
| Scaffold_down | GCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAG CCTTATTTTAACTTGCTATTTCTAG |