INTRODUCTION

Recessive dystrophic epidermolysis bullosa (RDEB) is caused by mutations in the COL7A1 gene resulting in compromised type VII collagen (C7) peptide function. C7 is a key constituent of the dermal–epidermal junction (DEJ), and its impairment leads to a severe blistering phenotype (Mittapalli et al., 2016; Rashidghamat and McGrath, 2017). Allogenic cellular therapies for RDEB include localized fibroblast injections (Wong et al., 2008) or systemic approaches with hematopoietic stem cells (HSCs). Gene therapy and gene editing represent promising strategies for autologous cell engineering. Transposons, retroviral, or lentiviral vectors have been used to deliver the COL7A1 cDNA under the control of exogenous gene regulatory elements (Droz-Georget Lathion et al., 2015; Jacków et al., 2016; Latella et al., 2017; Sebastiano et al., 2014; Siprashvili et al., 2010; Titeux et al., 2010). The integrating properties of these vectors poses an oncogenic risk, which may be magnified in RDEB patients, who are predisposed to aggressive squamous cell carcinoma. (Demeulemeester et al., 2015; Hacein-Bey-Abina et al., 2003; Turchiano et al., 2014). Unregulated overexpression of COL7A1 may also serve as a driver for carcinoma migration and invasion (Pourreyron et al., 2014). The possibility of insertional mutagenesis, and the lack of responsiveness of vector-borne COL7A1 to the endogenous cues that regulate cellular gene expression, render locus-specific targeting for treating RDEB appealing.

Genome editing agents can be used to mediate the precise correction of mutations that cause genetic diseases (Cong et al., 2013; Komor et al., 2017, 2018; Rees and Liu, 2018). The use of nuclease-based reagents leads to a double-stranded DNA break (DSB) that is resolved via non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ typically results in complex mixtures of small insertions and deletions (indels) and has been used for restoring COL7A1 expression (Bonafonte et al., 2019; Bornert et al., 2016; Mencía et al., 2018; Takashima et al., 2019).
However, depending on the indel profile resulting from the stochastic NHEJ process, in-frame deletions can be infrequent, limiting the gene restoration rates. HDR can be used to modify genomic sequences from a donor template; however, the efficiency in therapeutically relevant cells is typically very low, often necessitating antibiotic resistance cassettes to enrich for corrected clones (Hainzl et al., 2017; Osborn et al., 2013; Webber et al., 2016). In addition, DSB repair typically results in an excess of NHEJ/indels accompanying the desired HDR product. For therapeutic applications, the ability to achieve robust allele correction with high efficiency and minimal byproducts (e.g., indels from NHEJ) is often critical.

The adenine base editor (ABE) consists of a Cas9 nickase that does not introduce DSBs, but rather directs a fused laboratory-evolved deaminase to convert target A●T base pairs to GaC within a small window of DNA displaced by the targeting single guide RNA (sgRNA) (Gaudelli et al., 2017). Base editing offers three main advantages over HDR: (1) ABE is generally able to introduce or correct single nucleotide polymorphisms with much higher efficiency, often sufficient to avoid the need for positive selection; (2) ABE activity occurs with minimal indels; and (3) no exogenous donor DNA template is required, as base editing does not rely on HDR or cell division (Rees and Liu, 2018; Yeh et al., 2018).

We investigated the potential of ABE in primary cells from 2 RDEB patients with distinct COL7A1 nonsense mutations. An optimized, current-generation adenine base editor (ABEmax, Koblan et al., 2018) was delivered as mRNA and resulted in efficient gene correction that restored C7 peptide expression in fibroblasts. The 3-dimensional culture of the corrected cells promoted normalized epithelial layer attachment in vitro.

ABE correction was also pursued in induced pluripotent stem cells (iPSCs) that were subsequently differentiated into mesenchymal stromal cells (MSCs), a population being used to treat RDEB patients (Rashidhamat and McGrath, 2017). ABE-corrected iPSC injected in mice formed teratomas with ectodermal derived skin equivalents showed contiguous C7 deposition at the DEJ in vivo. Our data demonstrate the potential of base editing to correct endogenous COL7A1 in autologous cells as a therapeutic strategy for RDEB.

RESULTS
Targeting COL7A1 mutations with base editors
We pursued base editing in 2 RDEB patients with premature termination codon mutations in the COL7A1 gene. One patient possessed a homozygous c.553C>T (R185X) mutation, and the second was a compound heterozygote with c.1573C>T (R669X) and c.2005C>T (R669X) mutations (Figure 1a-c). A skin biopsy was obtained from each patient, and tissue staining with a polyclonal C7 antibody showed that the untreated cells did not show any C7 at the DEJ, demonstrating that these patients are null for C7 (Figure 1d).

ABE consists of a single protein containing a wild-type TadA adenosine deaminase, an evolved TadA* deoxyadenosine deaminase, and a Cas9(D10A) nickase (Figure 1e) (Gaudelli et al., 2017). Depending on which DNA strand is targeted, ABE mediates A>G or T>C transitions. sgRNAs were designed that positioned the target nucleotides at position 7 (c.553C>T) or 8 (c.1573C>T) of the protospacer (Figure 1f). The c.2005 site was not readily accessed by ABE because of the lack of an appropriately positioned protospacer adjacent motif (PAM). ABEmax mRNA and sgRNAs were delivered to primary patient fibroblasts and iPSC; the latter of which served as an engineering template for directed differentiation into MSCs and an in vivo model for skin equivalent generation from teratomas (Figure 1g).

COL7A1 restoration in null patient cells
ABE mRNA and chemically modified sgRNAs (Hendel et al., 2015) were electroporated into primary fibroblasts, and base editing was assessed in genomic DNA and mRNA by Sanger and Illumina deep sequencing. c.553 C>T nonsense mutation correction rates were 24±1.0% and 45%±5.8% among the genomic DNA and mRNA, respectively (Supplementary Figure S1a and Figure 2a).

Sequencing of the c.1573C>T site showed 50% wild-type (WT) sequence in unedited cells, as expected for a compound heterozygote (Supplementary Figure S1b). This locus also has putative “bystander” adenine bases capable of being modified by ABE at positions 4 and 10 of the sgRNA. ABE activity resulted in detectable modification of all 3 possible bases in PCR-amplified genomic DNA and mRNA (Supplementary Figure S1b and Figure 2b). The frequency of the desired nucleotide at position 8 in place of the stop codon, subtracting out the 50% contribution from the other allele that has a WT sequence at this site, was 8.2±1.6% (Figure 2b). Position 10 was edited to approximately the same extent, whereas position 4 was the most efficiently edited site with a frequency of 30±4.6% (Figure 2b). Deep sequencing of the COL7A1 mRNA showed that the frequency of edits at positions 4 and 10 matched the editing efficiency in genomic DNA. Transcripts with the nonsense mutation corrected at position 8 were enriched in mRNA to 17.8±1.1% (Figure 2b).

We next assessed whether the observed base editing resulted in restored C7 peptide expression. Wild type, RDEB c.553 and c.1573 C>T untreated, and ABE-treated fibroblasts were analyzed by immunofluorescence for vimentin and C7. As expected, all of the cells showed expression of the vimentin cytoskeletal protein (Figure 2c). In agreement with the biopsy sample analysis that was null for C7, staining with a polyclonal anti-C7 antibody showed that the untreated cells were completely devoid of C7 expression (Figure 1d and 2c). ABE treatment resulted in C7 restoration with the expression frequency correlating with the observed molecular rates of base editing (Figures 2a-c). To show that the immunoreactive C7 observed in cells represented the full-length C7 peptide, we performed a western blot analysis. Cell lysates from the uncorrected cells did not show any C7, and base editing restored the ~290 kD full-length C7 (Figure 2d). Edited c.553 cells also showed secretion of ~290 kD C7, as well as larger fragments consistent with previous reports (Christiano et al., 1996; Shinkuma et al., 2016; Titeux et al., 2010), representing the putative non-reduced C7 polypeptide/homotrimer (Figure 2e).

We performed isotype antibody staining and short tandem repeat-based cell line validation for quality control (Supplementary Figures S2 and S3). Short tandem repeat
validation showed that the pre- and post-ABE-modified cells were derived from the same donor, demonstrating the ability of ABE to correct the COL7A1 mutations in primary cells.

Base editing allelic variance

We dissected the editing outcomes using CRISPResso2 (Clement et al., 2019) bioinformatical analysis of the Illumina Mi-Seq sequencing data to quantify each distinct allele in the genomic DNA and mRNA PCR amplicons. As expected, the homozygous null patient harboring the c.553C>T mutation showed the uniform presence of the mutant thymine nucleotide in unedited cells. Allele-specific analysis showed that 3 unique amplicons were present following ABE treatment. The unedited, disease-causing thymine, and the edited cytosine were by far the most common alleles, whereas a T>C transition at position 3 leading to a V186A alteration was present at a frequency < 0.5% (Figure 3a).

Two major alleles were observed at the c.1573C>T target site in the unedited cells from the c.1573C>T and c.2005C>T compound heterozygous patient (Supplementary Figure S1b). Following editing, 8 different alleles were all detected at frequencies above 0.2% (Figure 3b). Bystander edits resulted in conservative Val→Ala amino acid changes (Figure 3b), but their effect on C7 function or immunogenicity is not known. In addition, the frequency of the purely WT sequence in this amplicon pool containing no stop codon or bystander mutation decreased from 50% in untreated genomic DNA to only 34±6.5% following editing, indicating that the non-targeted allele was also edited at bystander positions, despite a mismatch with the sgRNA.

ABE activity can lead to the introduction of low levels of indels at the target site, thought to be caused by the nick used to direct DNA repair to the non-edited strand (Koblan et al., 2018). We observed 1.5% indels for the c.553C>T editor and 1.9% for the c.1573C>T editor (Figure 3c); rates consistent with previous work (Koblan et al., 2018). Collectively, these data demonstrate the greater purity of
ABE editing that can be expected when only a single target nucleotide is present in the editing window, as is the case for the c.533C>T mutation, compared with multiple target nucleotides in the editing window, as is the case for the c.1573C>T mutation. We also observed a general enrichment of nonsense-corrected transcripts in the mRNA pool, suggesting that the corrected mRNA population is stabilized relative to the mutant one that is potentially subjected to nonsense-mediated decay.

**Base editing is precise with a low incidence of editing at off-target sites**
The specificity of genome editing via Cas9 depends on how well a given sgRNA is able to precisely recognize its unique target sequence in the genome. To assess the OT profile of the 2 ABE sgRNAs employed, we used CIRCLE-seq (Tsai et al., 2017), an unbiased method using cell free DNA, and the CRISPOR in silico predictive algorithm (Haeussler et al., 2016) (Supplementary Figures S4 and S5 and Figure 4a). We amplified 20 identified loci from the ABE-edited cells, then used Illumina deep sequencing to assess the frequency of OT editing. Off-target editing was not observed using the c.553C>T reagent at any of the sites assessed (Supplementary Figure S4b and Figure 4b). The c.1573C>T reagent yielded A>G editing at 1 of the 20 evaluated sites, which fell in an exon encoding the ubiquitin modifying enzyme UBA7 (Figure 4c and Supplementary Figure S4c).

**iPSC base editing and MSC derivation**

iPSCs represent a potentially inexhaustible source of cells for regenerative medicine. Following the co-electroporation of iPSCs with ABE mRNA and sgRNA, we isolated colonies and sequenced the COL7A1 c.553 region to assess the editing. Corrected and unedited clones were characterized for iPSC markers, and pluripotency was unaltered by base editing (Figure 5a and b and Supplementary Figure S5). We differentiated c.553 uncorrected and edited iPSC clones into MSCs, and they expressed the MSC antigens CD90, CD73, and CD105 similar to primary MSCs derived from normal adult bone marrow (Figure 5c). Corrected, but not uncorrected, MSCs expressed C7 as detected by immunofluorescence, and full-length C7 was observed following western blotting (Figure 5d-g). These data show
that COL7A1 base-edited iPSCs can differentiate into MSCs in vitro, representing a renewable cell population for RDEB treatment.

Deposition of type VII collagen in vitro and in vivo
To assess the architectural properties of base-edited C7, we performed an in vitro 3-dimensional epithelial tissue attachment assay (Dabelsteen et al., 2009; Dickson et al., 2000). The use of C7 null, uncorrected cells resulted in structural failure at the DEJ and detached epithelia, whereas base-edited cells restored normalized tissue architecture (Figures 6a-c). To further validate the ability of the base-edited cells to contribute to the DEJ, we used the ectodermal differentiation capabilities of the iPSCs to form skin equivalents following injection into immune-deficient mice (Osborn et al., 2013). Teratomas from both base-edited and uncorrected iPSCs showed the presence of human cytokeratin 5, whereas only base-edited cells deposited C7 at the DEJ in vivo (Figure 6d and e and Supplementary Figure S7). These data show that base editing results in C7 that is functionally competent for fulfilling its role at the DEJ.

DISCUSSION
In this study, we sought to determine the potential of base editors to correct COL7A1 gene mutations. Cells from 2 patients with nonsense mutations were used, both of which were completely unreactive to a polyclonal C7 antibody. This suggests that mutant COL7A1 mRNA, or any produced peptide fragments, are degraded, and the total absence of C7 is a crucial aspect to this study. Many RDEB patients show residual/partial C7 expression levels that can confound analyses (Wagner et al., 2010). Therefore, these two C7 null patient samples were ideal to assess and optimize COL7A1 DNA base editing in RDEB.

Base editors can convert C>T, G>A, A>G, or T>C (Gaudelli et al., 2017; Komor et al., 2016) without the need...
for a donor DNA template, typically with much higher efficiencies than HDR, and with dramatically reduced indels (Koblan et al., 2018; Rees and Liu, 2018). The current optimized version of the adenine base editor, ABEmax (Koblan et al., 2018), was co-delivered with sgRNAs designed for opposite-strand targeting converting T>C at protospacer position 7 (c.553) or 8 (c.1573). The average T>C mutation correction rates in primary fibroblasts were 23.8% at the c.553 target and 8.2% for c.1573 in genomic DNA. Deep sequencing following RT-PCR showed an increased presence of corrected transcripts averaging 45% and 17.8% in c.553 or c.1573 patient cells, respectively. Enrichment of T>C modified transcripts suggests that the edited mRNA molecules were stabilized, whereas the mutant transcripts were subjected to nonsense-mediated decay.

The homozygous c.553 target could be efficiently edited with little or no bystander mutations. In contrast, the compound heterozygous c.1573 target had two bystander nucleotides within the editing window, one edited at a much greater efficiency than the disease-causing mutation. ABE also modified the non-targeted, c.2005C>T allele containing a mismatch to the protospacer and introduced C4 and/or C10 bystander edits; however, these mutations on the already null allele should not have any negative impact. Nevertheless, for other applications or patients with hypomorphic mutations, the possibility of introducing additional mutations in the non-targeted allele despite a mismatch with the protospacer should be carefully considered. Each of the bystander edits results in Val→Ala amino acid changes in the C7 protein, and their impact on C7 function requires further assessment prior to the translational application of the c.1573C>T reagent.

The potential for off-target editing is a key consideration for the use of genome editing agents as potential therapeutics. The Cas9 nickase in ABE mitigates off-target concerns in comparison with previous methods employing nucleases because indels and translocations are much less likely to result from off-target ABE activity. Indeed, the on-target indel frequency was < 2%, consistent with previous reports (Koblan et al., 2018). To identify off-target sites, we used an experimental methodology (CIRCLE-seq) and a predictive software tool (CRISPOR). The top 10 sites for each method were analyzed by deep sequencing, and no off-target editing was observed using the c.553C>T sgRNA. A single off-target site in the UBA7 gene was detected for the c.1573C>T sgRNA. UBA7 has a potential role as a tumor suppressor in lung cancer (Yin et al., 2009); however, the impact on fibroblasts and MSCs is unknown. Interestingly, the computational identification method identified this bona fide off-target site, whereas CIRCLE-seq did not. Similar to the on-target activity, the off-target site had multiple edits within the sgRNA sequence leading to V991A and L990S.
substitutions in UBA7. Collectively, the on- and off-target data highlight the benefits of choosing a base editor and protospacer combination with only a single editable nucleotide in the target window. This approach is favorable not only because it leads to more uniform mutation correction at the target site but also because it decreases the likelihood of introducing non-synonymous changes at off-target loci.

C7 peptide expression was rescued in fibroblasts from both patients following ABE editing. C7 was detected by immunofluorescence, and full-length C7 was observed in cell lysates and supernatant via western blotting. Corrected fibroblasts are of immediate therapeutic benefit and have shown efficacy in RDEB patients (Petrof et al., 2013). MSCs have also been used to treat RDEB patients (Petrof et al., 2015); however, MSC numbers decline with age, and they are prone to senescence, making primary MSC engineering applications challenging (Serakinci et al., 2008; Stolzing et al., 2008). To circumvent these issues, we performed base editing on c.553 RDEB patient iPSCs, which in principle represent an abundant MSC source following directed differentiation. iPSCs exposed to MSC induction media acquired cell surface markers present on bone marrow–derived MSCs and showed restored, full-length C7 expression.

In vitro and in vivo assays were performed to demonstrate the functionality of the base-edited cells. Using a 3-dimensional organotypic in vitro skin culture system, we observed normalized attachment of the epithelial layer when using base-edited cells but not uncorrected controls. A hallmark of iPSC is their ability to form tissues of all 3 germ layers, and we previously observed that the ectodermal component forms skin equivalents in mice in vivo (Osborn et al., 2013). Similarly, we observed cytokeratin 5-positive skin structures with a contiguous band of C7 at the DEJ in base-edited but not C7 null, iPSC-treated animals. Collectively, our study shows the feasibility of autologous cellular engineering using base editing to correct COL7A1 mutations in cell populations currently employed clinically.
for RDEB. ABE mRNA electroporation facilitated gene correction without selection or the need of a repair template and with minimal off-target effects. We successfully base-edited fibroblasts and iPSCs and showed rescued C7 expression and function. These findings suggest that base editing represents a promising potential strategy for autologous RDEB cell therapy.

MATERIALS AND METHODS
Cell culture and gene transfer
RDEB fibroblasts were obtained following written informed patient consent, IRB approval, and in accordance with the Declaration of Helsinki Principles. Cell culture conditions were as previously reported (Tolar et al., 2014). Sendai virus reprogramming was performed to create iPSCs that were characterized as previously described (Tolar et al., 2014; Webber et al., 2016). Polyadenylated ABEmax mRNA with a 5’ cap-1 motif was produced by Aldevron (Fargo, ND), and Neon transfection was used for electroporation (fibroblasts: 1500 V, 20 ms pulse width, 1 pulse iPSC: 1100 V, 20 ms, and 1 pulse) with 2 μg of ABEmax mRNA and 1 μg of phosphorothioate modified (Hendel et al., 2015) sgRNA R185X target: 5’-CAACUCACUCAGCUCCUCA-3’ or R525X target 5’-GACACUCACACCCGCUGCCC-3’ (Synthego, Menlo Park, CA). PCR primers are listed in the Supplementary Materials.

Three-dimensional organotypic cultures were performed as described previously and scored and quantified by an expert reviewer (Dabelsteen et al., 2009; Dickson et al., 2000).

Immunodetection
C7 and iPSC immunofluorescence and isotype staining was performed as previously described (Takashima et al., 2019; Tolar et al., 2014), and images were taken using confocal microscopy (Olympus BX61, Olympus Optical, Japan). Western blotting was performed with a polyclonal anti-human type VII collagen (red) and anti-human cytokeratin (CK5; green) antibodies, as well as DAPI nuclear stain. Scale bars = 50 μm. At inset, lower right is the base-edited nucleotide that was observed following the amplification of human COL7A1 DNA from the in vivo teratoma tissue. ABE, adenine base editor; iPSC, induced pluripotent stem cells; RDEB, recessive dystrophic epidermolysis bullosa.

High-throughput sequencing
PCR amplicons for on- and off-targets were generated using the primers in the Supplementary Materials and were sequenced on an Illumina Mi-Seq (San Diego, CA). A bioinformatic analysis was performed with CRISPRESSo2 (Clement et al., 2019).

iPSC
MSCs were differentiated from iPSC using the STEmdiff Mesenchymal Progenitor Kit (STEMCELL Technologies, Cambridge, MA) following the manufacturer’s instructions and stained at day 28 with mouse anti-human CD73, CD90 (BD Pharmingen, San Jose, CA) and CD105 antibodies (ThermoFisher, Waltham, MA), and data was
acquired on a BD FACSARia (San Jose, CA). The iPSC were injected into the flank of immune-deficient NSG mice, and teratomas were harvested for C7 immunofluorescence as described above.

Data availability statement

Datasets related to this article can be found in the Supplementary Materials or by request from the corresponding author.

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CONFLICT OF INTEREST

GAN has filed patents relating to base editor use. DRL is a consultant and co-founder of Editas Medicine, Pairwise Plants, and Beam Therapeutics, companies that use genome editing. BRW is a consultant and has financial interests in Beam Therapeutics. BRB is the co-founder of Tmunity. The other authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: MJ, GAN, BB, DRL, JT; Methodology: MJ, GAN, FK, ANM, SCN, MR, LX, WC, CRE, BB, SD; Formal Analysis: MJ, GAN, FK; Funding Acquisition: MJ, GAN, HHH, SD, BB, DRL, JT; Investigation: MJ, GAN, FK, ANM, SCN, MR, LX, WC, SD; Resources: MJ, GAN, HHH, BB, SD, DRL, JT; Writing: MJ, GAN, ANM, FK, SCN, MR, LX, WC, CRE, BB, HHH, SD, BRB, DRL, and JT.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2019.07.701.

REFERENCES

SUPPLEMENTARY METHODS: CIRCLE-SEQ

CIRCLE-seq was performed as previously described (Tsai et al., 2017). PCR amplification before sequencing was conducted using PhusionU polymerase, and the products were gel-purified and quantified with a Qubit High-sensitivity kit before loading onto an Illumina Mi-Seq. Data was processed using the CIRCLE-seq analysis pipeline (Tsai et al., 2017) with parameters: “read_threshold: 4; window_size: 3; mapq_threshold: 50; start_threshold: 1; gap_threshold: 3; mismatch_threshold: 6; merged_analysis: True”. The top 10 most common sites based on the CIRCLE-seq read count were chosen for PCR amplification and high-throughput sequencing.

Abbreviation: CIRCLE-seq, Circularization for In vitro Reporting of CLeavage Effects by sequencing.

Supplementary Figure S1. Sanger sequencing of primary RDEB cell PCR products. Sanger sequencing chromatograms of genomic DNA and mRNA of uncorrected and base-edited (a) COL7A1 c.553 C>T and (b) COL7A1 c.1573C>T primary fibroblast cells. The bases amenable to base editing are underlined and enumerated in relation to the 5’ end of the guide RNA. RDEB, recessive dystrophic epidermolysis bullosa.
Supplementary Figure S2. Fibroblast immunofluorescence controls. Edited and untreated RDEB fibroblasts were stained with isotype control antibodies for vimentin (chicken) and C7 (rabbit). DAPI nuclear stain and merged images are shown at right. Scale bar = 50 μm. RDEB, recessive dystrophic epidermolysis bullosa.

Supplementary Figure S3. Cell line authentication. Genomic DNA from fibroblasts before and after gene editing were submitted for validation via STR analysis. The analyzed loci are shown at left, and the ‘pre’ sample was archived at the time of the initial experimental onset. ‘Post’ cells were analyzed from the same genomic DNA used for Sanger and high-throughput sequencing following ABE mRNA delivery. Analytics were performed at the same time on duplicate pre- and post-samples by IDEXX BioAnalytics (Columbia, MO). Green ‘X’ indicates that the STR pattern was identical for pre- and post-ABE-treated samples. ABE, adenine base editor; STR, short tandem repeat.
Supplementary Figure S4. CIRCLE-seq Off-target analysis. (a) CIRCLE-seq was performed, and the top 10 identified off-target genomic loci are shown with highlighted bases representing differences between the off-target site and the COL7A1 target. The gene name and location in relation to the coding or non-coding sites is indicated. (b, c) Illumina Mi-Seq sequencing to assess OT base editing in c.553 or c.1573 RDEB cells. The CIRCLE-seq identified off-target sites were amplified and deep sequenced to assess off-target editing in primary patient fibroblasts treated with ABE mRNA. Data are from 3 independent biological replicates of edited cells. GFP mRNA-treated patient cells were used as a control. CIRCLE-seq: Circularization for In vitro Reporting of CLeavage Effects by sequencing; GFP, green fluorescent protein; OT, off-target; RDEB, recessive dystrophic epidermolysis bullosa.
Supplementary Figure S5. Primary data of the sequences identified by CIRCLE-seq that were deep sequenced for OT analysis. Guide RNA sequences are shown at the top for the 553 site (left) and 1573 site (right). The alignments of the protospacers to the off-target sites detected by CIRCLE-seq are shown below. Nucleotides that match the protospacer are indicated with a dot, and nucleotides that differ are shown for each site. Dashes indicate a skipped nucleotide in the alignment, and small nucleotide letters indicate an insertion relative to the protospacer. The read count of the observed sequence is written at right along with the ID used in Supplementary Figure S4, if sequencing was possible. Brackets indicate 2 different alignments of the protospacer to the same off-target site. “ND” indicates that editing at that off-target site was not determined either because the site could not be amplified or because the control sample showed sequence heterogeneity, indicating that the same primers amplified homologous sites elsewhere in the genome. CIRCLE-seq, Circularization for In vitro Reporting of CLeavage Effects by sequencing; ND, not determined; OT, off-target.

Supplementary Figure S6. iPSC and MSC immunofluorescence controls. (a-b) Uncorrected or ABE-edited RDEB iPSC cells were stained with isotype (iso) control antibodies in the indicated fluorescent channel. The DAPI nuclear stain for each condition is shown at the bottom for each cell population. (c-e) MSC isotype antibody controls. (c) wild type, (d) uncorrected, and (e) ABE-edited 553 iPSC-derived MSCs were stained with C7 rabbit isotype control, as well as DAPI nuclear stain. Scale bars = 20 μm for iPSC and 50 μm for MSC. ABE, adenine base editor; iPSC, induced pluripotent stem cells; MSC, mesenchymal stromal cells; RDEB, recessive dystrophic epidermolysis bullosa.
Supplementary Figure S7. Teratoma immune fluorescence. (a) Collagen type VII null or (b) ABE-edited RDEB iPSC cells were stained with primary and isotype control antibodies for the indicated antigen. The individual image slices represent the un-merged experimental images that are merged and shown in Figure 6. Scale bar = 50 μm. ABE, adenine base editor; iPSC, induced pluripotent stem cells; MSC, mesenchymal stromal cells; RDEB, recessive dystrophic epidermolysis bullosa.