Supplemental Information

*Ex Vivo COL7A1 Correction for Recessive Dystrophic Epidermolysis Bullosa Using CRISPR/Cas9 and Homology-Directed Repair*

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Figure S1

Detection of CRISPR/Cas9-mediated HDR at the COL7A1 locus by Taqman-ddPCR on the genomic DNA level. (a) Gene editing detection strategy for COL7A1 by ddPCR. Location of a common primer pair and allele-specific probes conjugated with FAM (specific for the corrected COL7A1) or VIC (specific for the mutated COL7A1) fluorophores are indicated. (b) 2-D fluorescence amplitude plot generated by Quantasoft software showing walls containing both of corrected and mutant COL7A1. The black cluster on the plot represents the negative droplets (Ch1-Ch2-), the blue cluster represent the droplets that are positive for the corrected COL7A1 only (Ch1+Ch2-), the green cluster represents the droplets that are positive for the mutant COL7A1 only (Ch1-Ch2+) and the orange cluster represents the droplets that are positive for both (Ch1+Ch2+). (c) The ‘Events’ histogram shows the total number of droplets positive for FAM (in blue) and VIC (in green) signals which correspond to the corrected or mutated COL7A1, respectively. Ch1: channel 1, corresponds to the FAM amplitude; Ch2: channel 2, corresponds to the VIC amplitude.
TaqMan-ddPCR-based detection of corrected COL7A1 mRNA expression after CRISPR/Cas9-mediated HDR. (a) Gene editing detection strategy for COL7A1 by ddPCR at the mRNA level. In the scheme, the same experimental settings as for the Figure 3a, are shown. The VIC-conjugated specific probe recognizes the housekeeping gene RPLP0. (b) Primary RDEB-K and RDEB-F were transduced with indicated doses of IDLVs (pg p24 per cell). 21-days post transduction, mRNA was extracted, subjected for Reverse Transcription and analyzed by Taqman-ddPCR to detect the expression of corrected COL7A1 relative to the expression level of COL7A1 in normal cells. 1-D fluorescence amplitude plots are shown. Yellow lines indicate borders between different samples. Blue dots correspond to the FAM signal and represent droplets containing the corrected COL7A1. Green dots correspond to the VIC signal and represent housekeeping RPLP0. Grey dots correspond to empty droplets. (c-d) Quantification of positive droplets using Quantasoft. The concentration plot, showing gene-edited wells of FAM and VIC amplicons is automatically determined by the software using the total number of events (displayed in Figure S2) by correcting for Poisson distribution. The blue markers indicate corrected COL7A1 copies/µl and the green markers indicate housekeeping RPLP0 copies/µl. The ‘Ratio’ plot shows the percentage of the corrected COL7A1 normalized to the housekeeping RPLP0 background (orange markers). All error bars were generated by QuantaSoft and represent a 95% confidence interval. The percentage of corrected COL7A1 mRNA in RDEB-K and RDEB-F was calculated by considering the Ratio of COL7A1/RPLP0 in normal cells (NHK and NHF, respectively) as 100%. 
Off-target site analysis in genetically corrected RDEB-K 21 days post-transduction.

Genomic DNA from corrected RDEB-K co-treated with IDLVs encoding for the LV-CRISPR-N1 (2 pg p24 per cell) and the LV-Donor (0.5 pg p24 per cell) was extracted and regions corresponding to off-target sequences were amplified by PCR using specific primers (listed in Table S4). The Surveyor cleavage assay was performed at each potential off-target hit. No Surveyor activity indicative of cleavage at predicted off-target sites was detected. nd: non detected.

Absolute quantification the residual Cas9 transcripts in genetically corrected cells and grafted skin equivalents. (a) Standard curve of the lentiCRISPR_v2 plasmid DNA, ranging from 1 to $10^9$ copies/µl. The Ct values were plotted against the logarithm of their initial template copy numbers. The standard curve was generated by linear regression of the plotted points. (b) To evaluate the persistence of Cas9 cDNA in cells after IDLV transduction, total mRNA was extracted and cDNA was synthetized from bulk transduced RDEB-K, RDEB-F and from grafted skin equivalents. Cas9 expression in transduced cells and in grafted skin equivalents was evaluated in triplicates. Three independent experiments were performed.
Type VII collagen rescue, localization and AF formation at the dermal-epidermal junction in serial sections of the genetically corrected skin grafts. Immunofluorescence analysis of grafted SE composed of genetically corrected primary RDEB keratinocytes and fibroblasts at 2 months after deflaping. Skin samples composed of genetically corrected cells showed re-expression and normal localization of C7 at the dermal-epidermal junction in serial sections of SE. Scale bar = 100 µm.
Detection of CRISPR/Cas9-mediated HDR at the COL7A1 locus by Taqman-ddPCR on the genomic DNA level in skin grafts. See Figure S1 for the experimental settings. (a) 1 month or 2 months post-grafting, gDNA was extracted from cryosections and analyzed by ddPCR to assess allelic frequency of corrected COL7A1 on the mutated background in skin grafts. 1-D fluorescence amplitude plots are shown for FAM and VIC signals. Blue dots correspond to the FAM signal amplitude and represent droplets containing the normal or corrected COL7A1 alleles. Green dots correspond to the VIC signal amplitude and represent the mutated COL7A1 alleles. Grey dots correspond to empty droplets. Yellow lines indicate borders between different samples. (b) Quantification of positive droplets using QuantaSoft Software. The concentration plot, showing gene-edited wells of FAM and VIC amplicons is automatically determined by the software using the total number of events by correcting for Poisson distribution. The blue markers indicate corrected COL7A1 copies/μl and the green markers indicate mutated COL7A1 copies/μl. The Fractional abundance plot shows the percentage frequency of the corrected COL7A1 on the mutated COL7A1 background. All error bars were generated by QuantaSoft and represent a 95% confidence interval.
### Table S1. Sequences of guide RNAs

| gRNA | Sequence                  | gRNA length (bp) | Strand    | Cut-to-mutation distance* (bp) | Off target activity | Surveyor digestion product (bp) |
|------|---------------------------|------------------|-----------|-------------------------------|---------------------|---------------------------------|
| N1   | GTCCGCAGCTTTCGCTGA        | 17               | First Strand | 5 (downstream)               | 0 MMs = 1 1 MMs = 0 2 MMs = 3 | 684 (non cleaved) 370 (cleaved) 305 (cleaved) |
| N2   | GAAAGCTGCGGACCTCG         | 17               | Reverse Strand | 21 (downstream)            | 0 MMs = 1 1 MMs = 0 2 MMs = 6 | 684 (non cleaved) 391 (cleaved) 293 (cleaved) |
| N3   | GATGGCTCCCTCATCCAT        | 17               | First Strand  | 43 (downstream)             | 0 MMs = 1 1 MMs = 0 2 MMs = 18 | 684 (non cleaved) 340 (cleaved) 344 (cleaved) |
| N4   | GGCACACCGCTGTGAC          | 17               | Reverse Strand | 31 (upstream)               | 0 MMs = 1 1 MMs = 0 2 MMs = 14 | 684 (non cleaved) 444 (cleaved) 240 (cleaved) |
| N5   | GCTCGCGCAATGGATG          | 17               | Reverse Strand | 44 (downstream)             | 0 MMs = 1 1 MMs = 1 2 MMs = 14 | 684 (non cleaved) 368 (cleaved) 318 (cleaved) |
Table S2. Absolute quantification of residual Cas9 cDNA expression in genetically corrected RDEB keratinocytes, fibroblasts and in grafted skin equivalents (SE)

| Sample Name                        | Target gene | Experience N1 | Experience N2 | Experience N3 | Mean Experiences N1-N2-N3 | Cas9 copies/µl |
|------------------------------------|-------------|---------------|---------------|---------------|---------------------------|----------------|
| RDEB-K (IDLVs : 1.5 / 0.5)         | Cas9        | 27.4          | 27.76         | 27.42         | 27.52                     | 1.39           |
| RDEB-K (IDLVs : 2 / 0.5)           | Cas9        | 26.07         | 26.97         | 25.54         | 26.19                     | 1.8            |
| RDEB-K                             | Cas9        | 32.52         | 32.98         | 32.01         | 32.5                      | -              |
| NHK                                | Cas9        | 35.9          | 34.05         | 34.04         | 34.66                     | -              |
| RDEB-F (IDLVs : 1.5 / 0.5)         | Cas9        | 25.63         | 26.22         | 25.91         | 25.92                     | 1.89           |
| RDEB-F (IDLVs : 2 / 0.5)           | Cas9        | 26.07         | 26.09         | 25.73         | 25.96                     | 1.87           |
| RDEB-F                             | Undetermined| Undetermined  | Undetermined  | Undetermined  | Undetermined              | -              |
| Corrected-SE-1 month - N1          | Cas9        | 32.33         | Undetermined  | Undetermined  | 32.33                     | -              |
| Corrected-SE-1 month - N2          | Undetermined| Undetermined  | Undetermined  | Undetermined  | Undetermined              | -              |
| Corrected-SE-1 month - N3          | Undetermined| Undetermined  | Undetermined  | Undetermined  | Undetermined              | -              |
| Corrected-SE-2 months - N1         | Cas9        | 39.34         | Undetermined  | Undetermined  | 39.34                     | -              |
| Corrected-SE-2 months - N2         | Undetermined| Undetermined  | Undetermined  | Undetermined  | 35.06                     | -              |
| Corrected-SE-2 months - N3         | Undetermined| Undetermined  | Undetermined  | Undetermined  | 31.4                      | -              |
| RDEB-SE-1 month - N1               | Undetermined| 37.27         | Undetermined  | Undetermined  | 37.27                     | -              |
| RDEB-SE-1 month - N2               | Undetermined| 30.88         | 34.83         | 32.85         |                            |                |
| WT-SE-1 month - N1                 | Undetermined| Undetermined  | Undetermined  | Undetermined  | 39.44                     | -              |
| WT-SE-1 month - N2                 | Undetermined| Undetermined  | Undetermined  | Undetermined  | 20-26%                    |                |

Table S3. HDR efficiency in genetically corrected RDEB cells and grafted skin equivalents (SE)

| Cells/Grafts | Genetically corrected RDEB-K | Genetically corrected RDEB-F | Grafted SE |
|--------------|------------------------------|----------------------------|------------|
| Sample       | gDNA | cDNA | Protein | gDNA | cDNA | Protein | gDNA | Protein |
| Assay        | ddPCR | ddPCR | WB      | ddPCR | ddPCR | WB      | ddPCR | IF      |
| Figure       | N3   | S2   | N5      | N3   | S2   | N5      | N6   | S6      |
| Correction   | 19.6% | 11%  | 11%     | 22.1%| 15.7%| -       | 17-19%| 20-26%  |
Table S4. Oligonucleotides and Probes sequences

| Figure | Assay                      | Primer name  | Sequence                  |
|--------|----------------------------|--------------|---------------------------|
| N1     | NHEJ activity              | Fw_Surveyor | GTCCCCCTGCTTATGCCAA       |
| N1     | NHEJ activity              | Rev_Surveyor| GACCTTCTCTGTCTTGCAGT      |
| N2     | Allele specific PCR        | Commun_P1   | GATTCCTCCTAATTCTGGGACTC   |
| N2     | Allele specific PCR        | Mutant_P2   | GACCTTCTCTGTCTTGCAGTAG    |
| N2     | Allele specific PCR        | Corrected_P3| GACCTTCTCTGTCAAGAAGTA     |
| N2     | Allele specific PCR        | Fw_GAPDH    | TCCATGCCAT CACTGCCACCCAG  |
| N2     | Allele specific PCR        | Rev_GAPDH   | CATAACAGGAATGAGCTTGACAAAGT|
| N3; S1, 2, 5 | TaqMan-ddPCR | Fw_Exon2   | CATGGCCGCGCAATTT         |
| N3; S1, 2, 5 | TaqMan-ddPCR | Rev_Exon2 | CTGGCTGCTCCAGAAAAGAG     |
| N3; S1, 2, 5 | TaqMan-ddPCR | FAM_Probe  | TTTCTCGAAGGGCT-G-MGB      |
| N3; S1, 5 | TaqMan-ddPCR | VIC_Probe  | CTTCTCGAAGGGCTGT-MGB      |
| N6, S3 | Off-target activity        | Fw_OT1      | GCTGCTTCTCTGTACTCACA      |
| N6, S3 | Off-target activity        | Rev_OT1     | TGCCCTTCATAGGAGTGCTG      |
| N6, S3 | Off-target activity        | Fw_OT2      | AATTCGGTTTGCTGAGCAC       |
| N6, S3 | Off-target activity        | Rev_OT2     | ACCACGATGGACTAGAAGGC      |
| N6, S3 | Off-target activity        | Fw_OT3      | AGGTTCAGAGGCTGTAAACG      |
| N6, S3 | Off-target activity        | Rev_OT3     | TCTGCTAGACACCCCTCCTC      |
| N6, S3 | Off-target activity        | Fw_OT4      | GTCGCTTTGCTCTGCTCTG       |
| N6, S3 | Off-target activity        | Rev_OT4     | ACTTCAGAAGTTGAGAGGCC      |
| N6, S3 | Off-target activity        | Fw_OT5      | GATAAGAAATGAGGTAAAGC      |
| N6, S3 | Off-target activity        | Rev_OT5     | CACAGCAAGATACATCATCTA     |
| N6, S3 | Off-target activity        | Fw_OT6      | CCAGGGAAGGCTGTCTTTCTC     |
| N6, S3 | Off-target activity        | Rev_OT6     | TTTGTGGGTCACTTGTGCAG      |
| N6, S3 | Off-target activity        | Fw_OT7      | TCCCAAGTAAGGAGGGCTCA      |
| N6, S3 | Off-target activity        | Rev_OT7     | CCAGAAATGGAGGCTGT         |
| N6, S3 | Off-target activity        | Fw_OT8      | GGGACATGTGCAGACTCA        |
| N6, S3 | Off-target activity        | Rev_OT8     | GAGCCATCTGCGAGGTTTGT      |
| S4     | Absolute qPCR              | Fw_Cas9     | GGACTCGAGAGTAACACTAAG     |
| S4     | Absolute qPCR              | Rev_Cas9    | AAAGTGCGCGAGATCAACAC      |