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Supplemental Information

Clinically Relevant Correction of Recessive Dystrophic Epidermolysis Bullosa by Dual sgRNA CRISPR/Cas9-Mediated Gene Editing

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Supplementary Figure 1

Indel	P1 BR1	P1 BR2	P2 BR1	P2 BR2	
Δ55+insT	62.23	68.95	75.05	75.24	GTGCCCTCTCT----------AACCTTCACCTGTCTTGCC
Δ55	9.76	13.55	12.04	11.8	GTGCCCTCTC----------AACCTTCACCTGTCTTGCC
Δ55+insTA	2.48	1.08	2.68	2.06	GTGCCCTCTCTA----------AACCTTCACCTGTCTTGCC
Δ1	0.47	1.25	0.94	0.35	GTGCCCTCTCT-TGTAGGGTCTGCAGGGTCCAAGAGGCCCCCCGGCCCAGTGGTGAGTACCCAAGAACCTTCACCTGTCTTGCC
Δ56+insT	0.43	0.5	0.47	0.9	GTGCCCTCTCT----------ACCTTCACCTGTCTTGCC
Δ55+insTAT	0.34	0.16	0.47	0.15	GTGCCCTCTCTAT----------AACCTTCACCTGTCTTGCC
Unedited	2.48	0.41	0.16	0.1	GTGCCCTCTCTATGTAGGGTCTGCAGGGTCCAAGAGGCCCCCCGGCCCAGTGGTGAGTACCCAAGAACCTTCACCTGTCTTGCC
∑ΔE80	87.07	93.31	95.88	95.43	

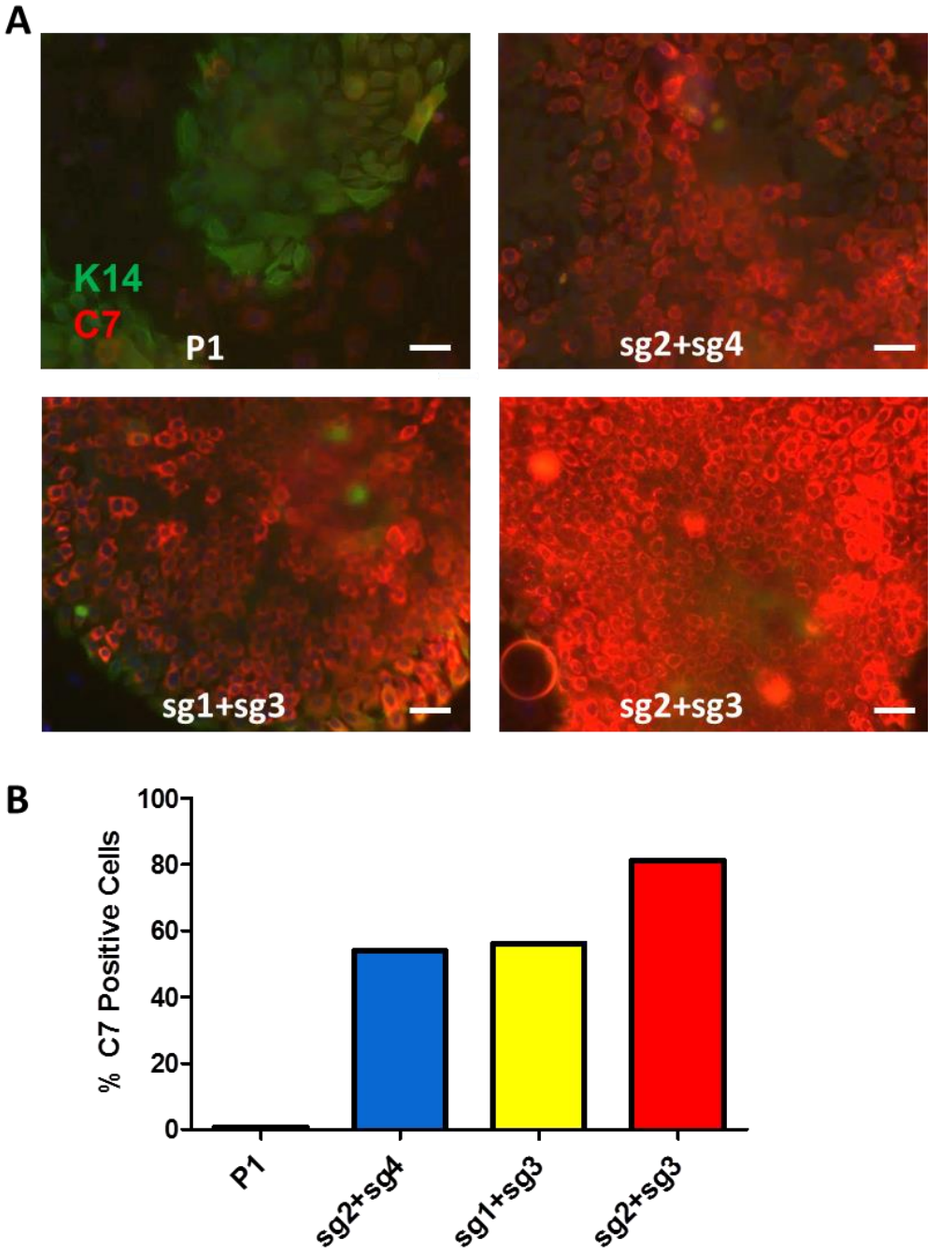
Supplementary Figure 1. Next Generation Sequencing of PCR amplicons spanning the double cleavage site for in depth characterization of repair events at the on-target region. Percentage of reads corresponding to the most frequent indel variants common to all samples are shown. Total percentage of variants lacking E80 (ΣΔE80) are indicated below.

Supplementary Figure 2

	P1 Control	P1 BR1	P1 BR2	P2 Control	P2 BR1	P2 BR2
ON-TARGET	1,83	97,17	99,58	0,72	99,61	99,65
OFFT 1	0,46	0,60	0,56	0,19	0,45	0,26
OFFT 2	0,10	0,17	0,53	0,05	0,20	0,07
OFFT 3	0,21	0,49	0,52	0,13	0,28	0,58
OFFT 4	0,10	0,17	0,45	0,03	0,00	0,06
OFFT 5	0,12	0,15	0,39	0,06	0,20	0,25
OFFT 6	0,18	0,20	0,36	0,05	0,02	0,00
OFFT 7	0,15	0,11	0,34	0,16	0,07	0,09
OFFT 8	0,19	0,16	0,31	0,12	0,04	0,14
OFFT 9	0,20	0,14	0,31	0,51	0,18	0,07
OFFT 10	0,00	0,00	0,29	0,00	0,00	0,00
OFFT 11	0,03	0,09	0,29	0,18	0,21	0,05
OFFT 12	0,09	0,15	0,28	0,04	0,14	0,07
OFFT 13	0,10	0,30	0,28	0,19	0,23	0,09
OFFT 14	0,29	0,19	0,28	0,52	0,23	0,12
OFFT 15	0,14	0,30	0,28	0,02	0,00	0,00
OFFT 16	0,17	0,12	0,27	0,27	0,31	0,19
OFFT 17	0,14	0,20	0,26	0,16	0,21	0,11
OFFT 18	0,00	0,28	0,25	0,35	0,15	0,08
OFFT 19	0,08	0,22	0,25	0,08	0,29	0,33
OFFT 20	0,18	0,13	0,25	0,28	0,11	0,05
OFFT 21	0,24	0,17	0,24	0,09	0,09	0,00
OFFT 22	0,04	0,05	0,23	0,08	0,06	0,13
OFFT 23	0,29	0,30	0,23	0,24	0,62	0,36
OFFT 24	0,03	0,23	0,23	0,15	0,03	0,14
OFFT 25	0,39	0,00	0,21	0,39	0,14	0,17
OFFT 26	0,21	0,09	0,21	0,05	0,00	0,14
OFFT 27	0,11	0,13	0,21	0,10	0,17	0,17
OFFT 28	0,22	0,17	0,21	0,04	0,14	0,02
OFFT 29	0,18	0,18	0,20	0,00	0,26	0,03
OFFT 30	0,16	0,23	0,20	0,06	0,10	0,05
OFFT 31	0,00	0,00	0,19	0,00	0,04	0,00
OFFT 32	0,14	0,08	0,19	0,09	0,15	0,05
OFFT 33	0,12	0,17	0,19	0,12	0,45	0,30
OFFT 34	0,08	0,17	0,19	0,00	0,05	0,04
OFFT 35	0,15	0,10	0,19	0,12	0,18	0,16
OFFT 36	0,00	0,04	0,19	0,00	0,17	0,08
OFFT 37	0,07	0,15	0,18	0,09	0,23	0,20
OFFT 38	0,10	0,11	0,18	0,23	0,09	0,11
OFFT 39	0,20	0,29	0,18	0,05	0,07	0,10
OFFT 40	0,11	0,30	0,17	0,14	0,07	0,16
OFFT 41	0,10	0,12	0,17	0,12	0,30	0,10
OFFT 42	0,11	0,09	0,17	0,03	0,14	0,00
OFFT 43	0,00	0,05	0,17	0,03	0,03	0,10
OFFT 44	0,03	0,14	0,16	0,14	0,15	0,07
OFFT 45	0,03	0,09	0,16	0,00	0,07	0,00
OFFT 46	0,06	0,07	0,16	0,02	0,04	0,04
OFFT 47	0,06	0,12	0,15	0,04	0,07	0,01
OFFT 48	0,03	0,03	0,15	0,04	0,08	0,12
OFFT 49	0,03	0,11	0,15	0,02	0,05	0,01
OFFT 50	0,03	0,09	0,15	0,04	0,12	0,06

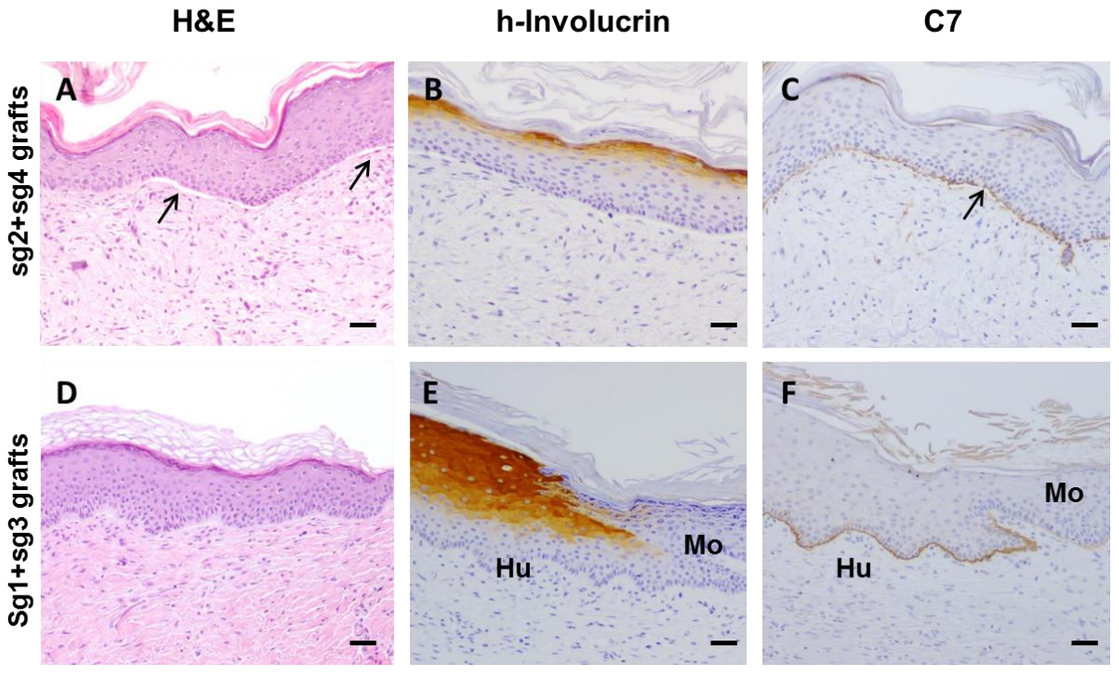
Supplementary Figure 2. Frequencies of indel detection for the 50 highest ranking off-target sites. Percentages of indel-containing NGS reads are shown for each site and sample. Frequencies above the 0.52% threshold are highlighted in red. On-target NGS frequencies are shown in grey (top row).

Supplementary Figure 3



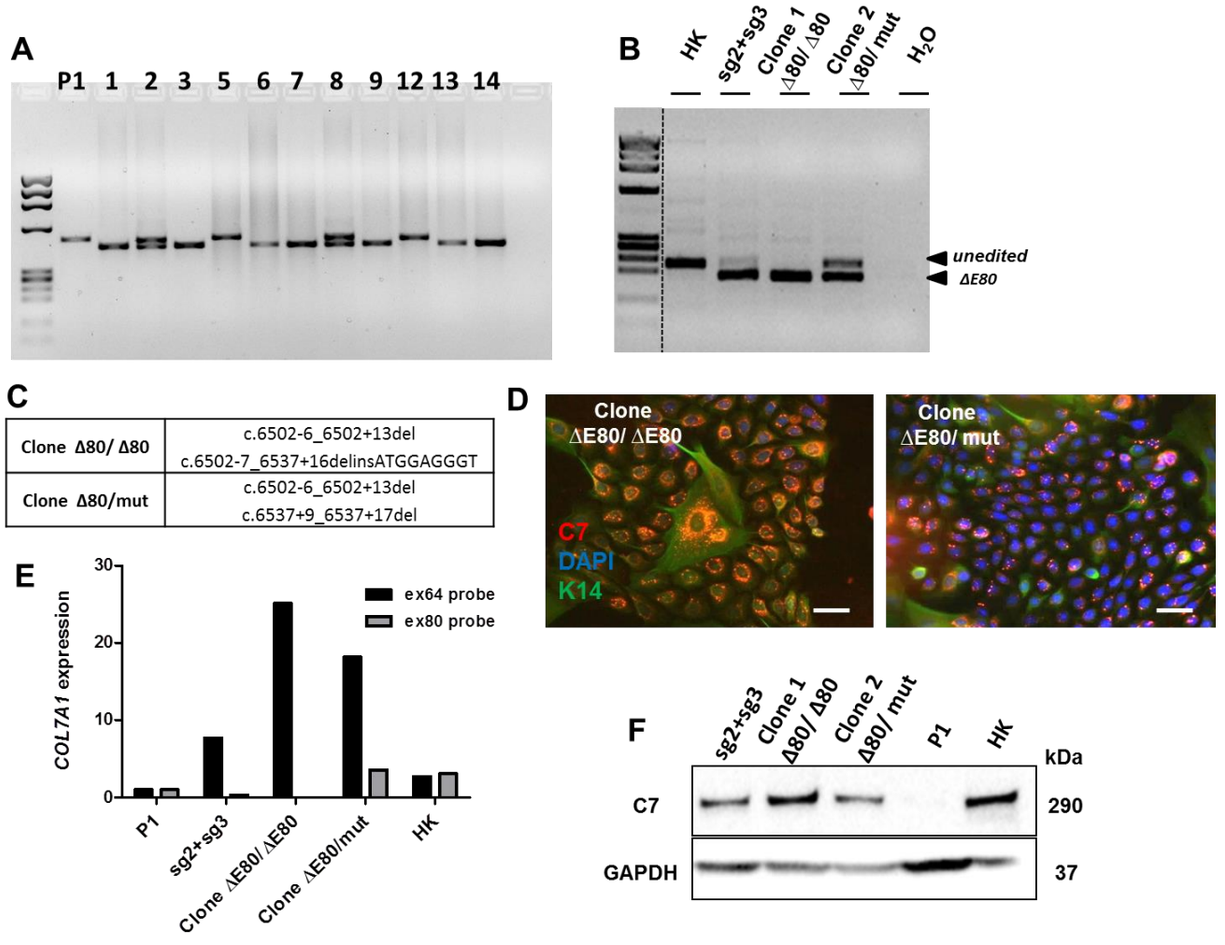
Supplementary Figure 3. Immunodetection of C7 in primary RDEB patient P1 keratinocytes treated with different RNP combinations. A) Double immunofluorescence C7/K14 staining showing the extent of C7 expressing cells after treatment with the different RNPs. Keratin-14 (green) was used to distinguish keratinocytes. Bars: 50µm. B) Percentage of C7-expressing RNP-treated keratinocytes detected by flow cytometry after immunolabeling with anti-C7 antibody.

Supplementary Figure 4



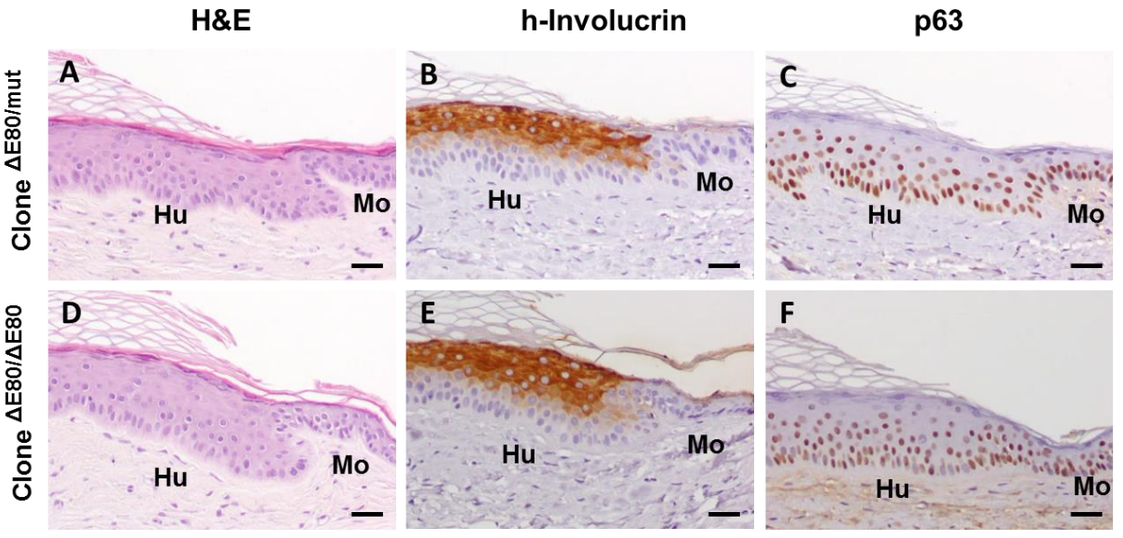
Supplementary Figure 4. Histopathological and immunohistochemical analysis of grafts produced with keratinocytes modified with additional RNP combinations. (A, D) H&E staining of 12-week-old grafts from RDEB keratinocytes modified with sg2+sg4 (A) and sg1+sg3 (D) RNP pairs. (B, E) h-Involucrin immunohistochemical detection in grafts from polyclonal keratinocytes treated with sg2+sg4 (B) and sg1+sg3 (E). (C, F) C7 immunoperoxidase detection in grafts from polyclonal keratinocytes treated with sg2+sg4 (C) and sg1+sg3 (F) treated RDEB keratinocytes. Grafts generated with RDEB keratinocytes treated with the combination sg2+sg4 show scattered microblisters (arrows) (A, C) concomitant with the lower and patchy C7 expression (C). Grafts generated with the combination sg1+sg3 (D, F) do not show blistering signs (D) and display continuous C7 expression (F). Hu and Mo correspond to human and mouse tissue, respectively. Bars: 100 µm.

Supplementary Figure 5



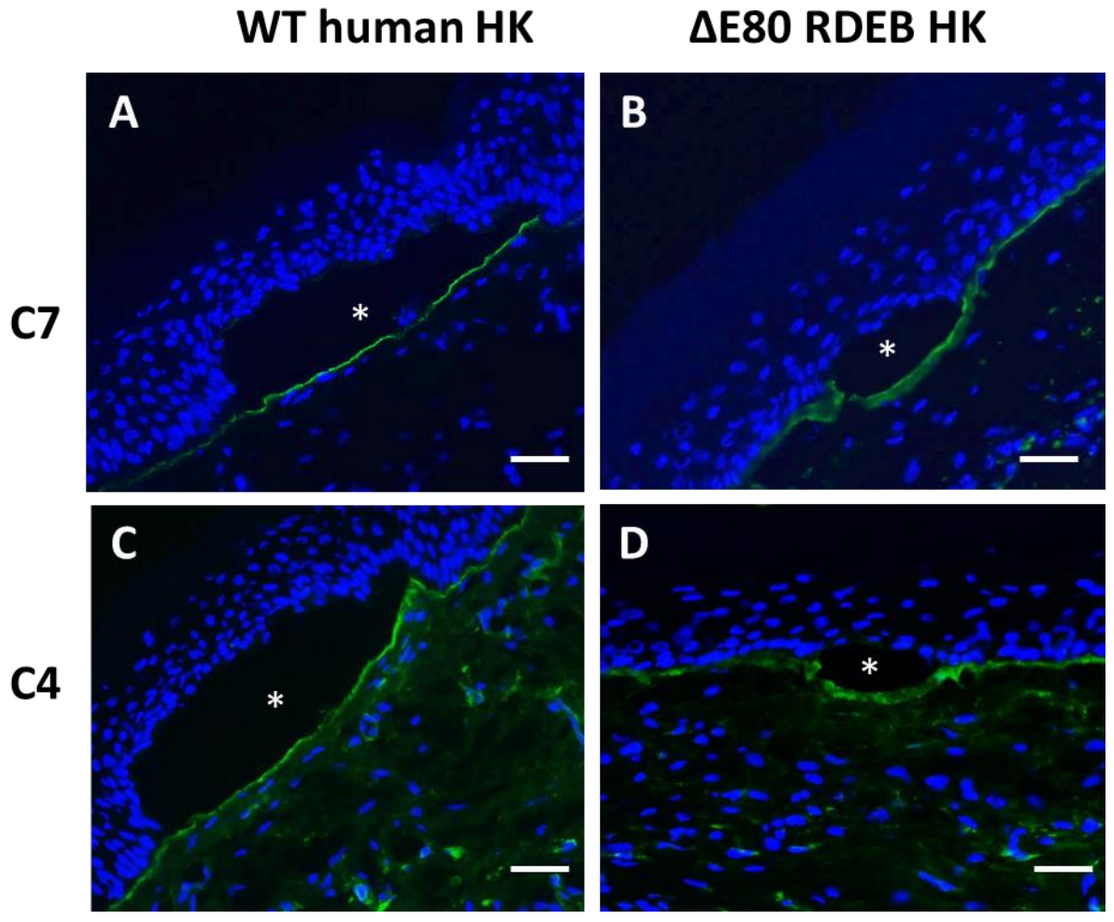
Supplementary Figure 5. DNA, RNA and protein characterization of monoallelically and biallelically E80-deleted clones. (A) PCR genotype of the 11 isolated E80-deleted clones, showing alleles lacking E80 (lower band). (B) RT-PCR of variants of transcripts present in the two clones. Biallelically modified clone showed only shorter ΔE80-transcripts. (C) *COL7A1* alleles revealed by Sanger sequencing in each edited clone. (D) Immunofluorescence detection of C7 expression in both edited clones Bars: 50 µm. (E) qPCR quantification of *COL7A1* expression with two different exon-specific probes. (F) Western Blot protein expression quantification of the sg2+sg3 pool and the two isolated clones.

Supplementary Figure 6



Supplementary Figure 6. Additional phenotypic characterization of clonal mono and bi-allelic ΔE80 grafts. (A, D), Histology (H&E staining). (B, E) human involucrin expression. (C, F) p63 expression at the Human (Hu), mouse (Mo) boundary. These markers demonstrate that clonal grafts maintain normal features of epidermal differentiation and long-term skin regenerative performance. Top panels: Monoallelic correction (ΔE80/mut) clone. Lower panels: Biallelic correction (ΔE80/ ΔE80) clone. Bars: 100 µm.

Supplementary Figure 7



Supplementary Figure 7. Cleavage plane analysis in suction-induced blisters in control and gene edited RDEB grafts. Immunofluorescence of C7 (A, B) and C4 (C, D) was performed on consecutive frozen tissue sections from blisters induced in normal human (A, C) and ΔE80 RDEB (B, D) keratinocyte grafts. C7 and C4 at the floor of the blister demonstrate cleavage between basal keratinocyte and *lamina lucida*. The asterisks indicate dermal-epidermal separation. Nuclei are stained with DAPI. Bars: 100 μm.