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Supplemental Information

Correction of recessive dystrophic epidermolysis bullosa by homology-directed repair-mediated genome editing

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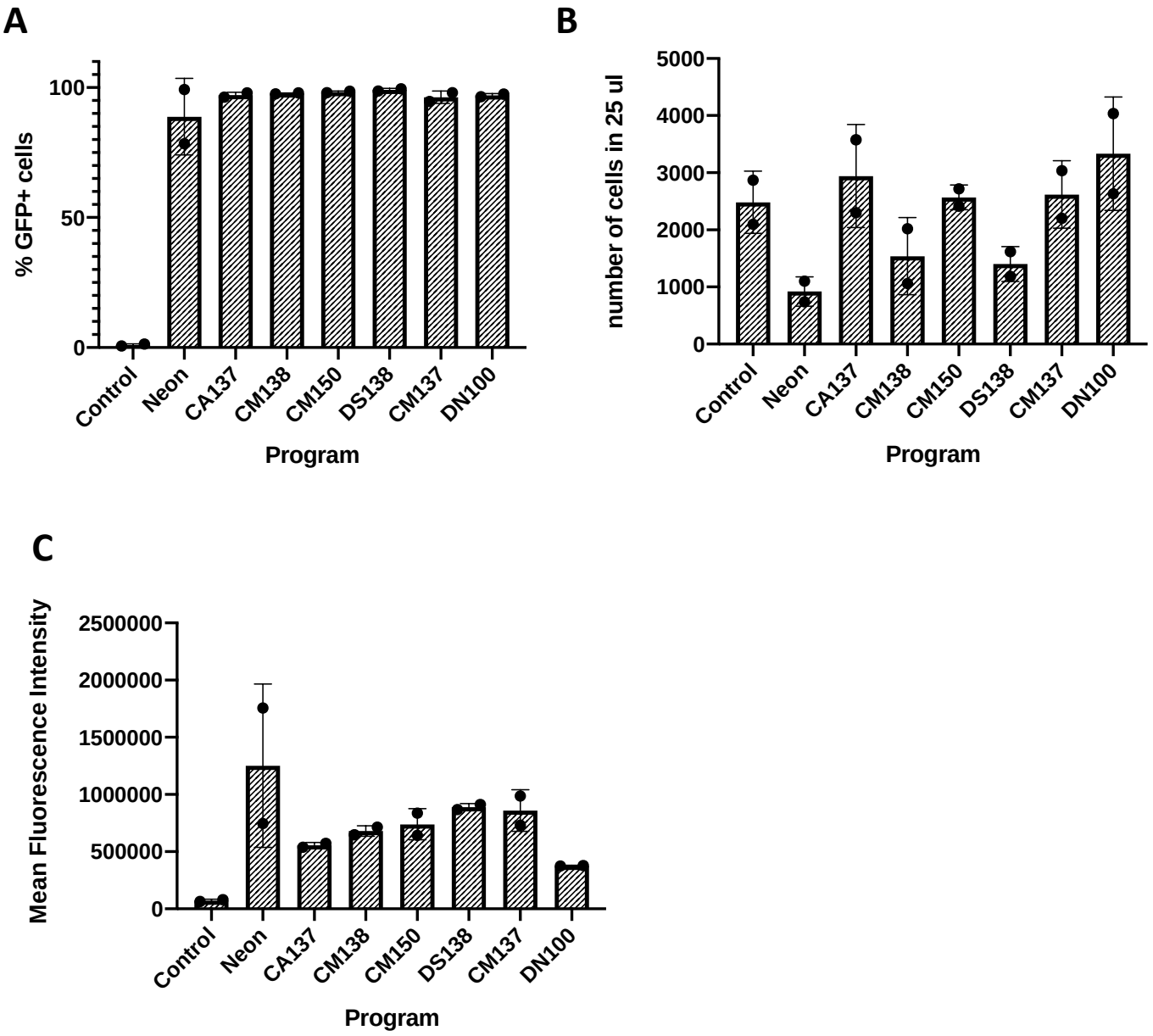
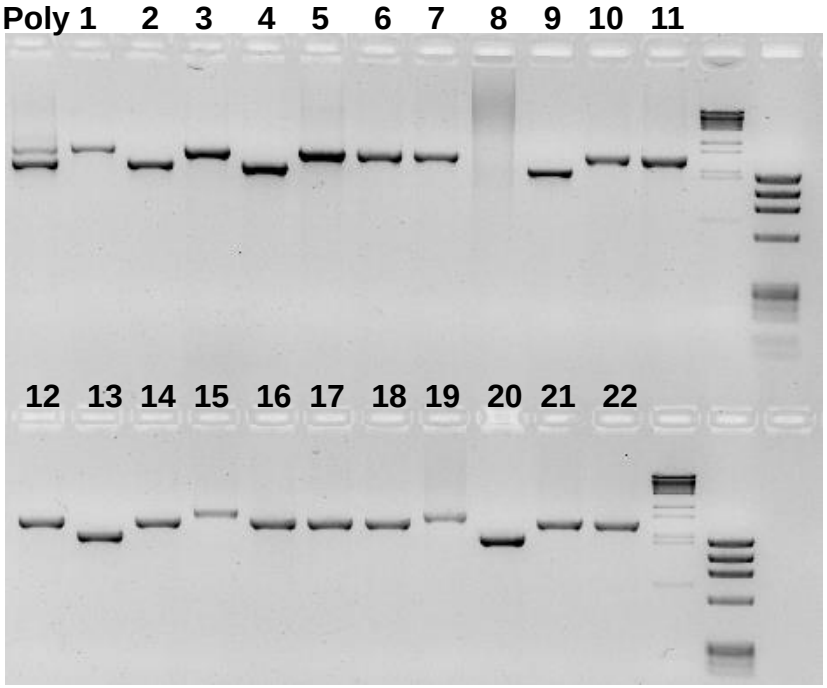
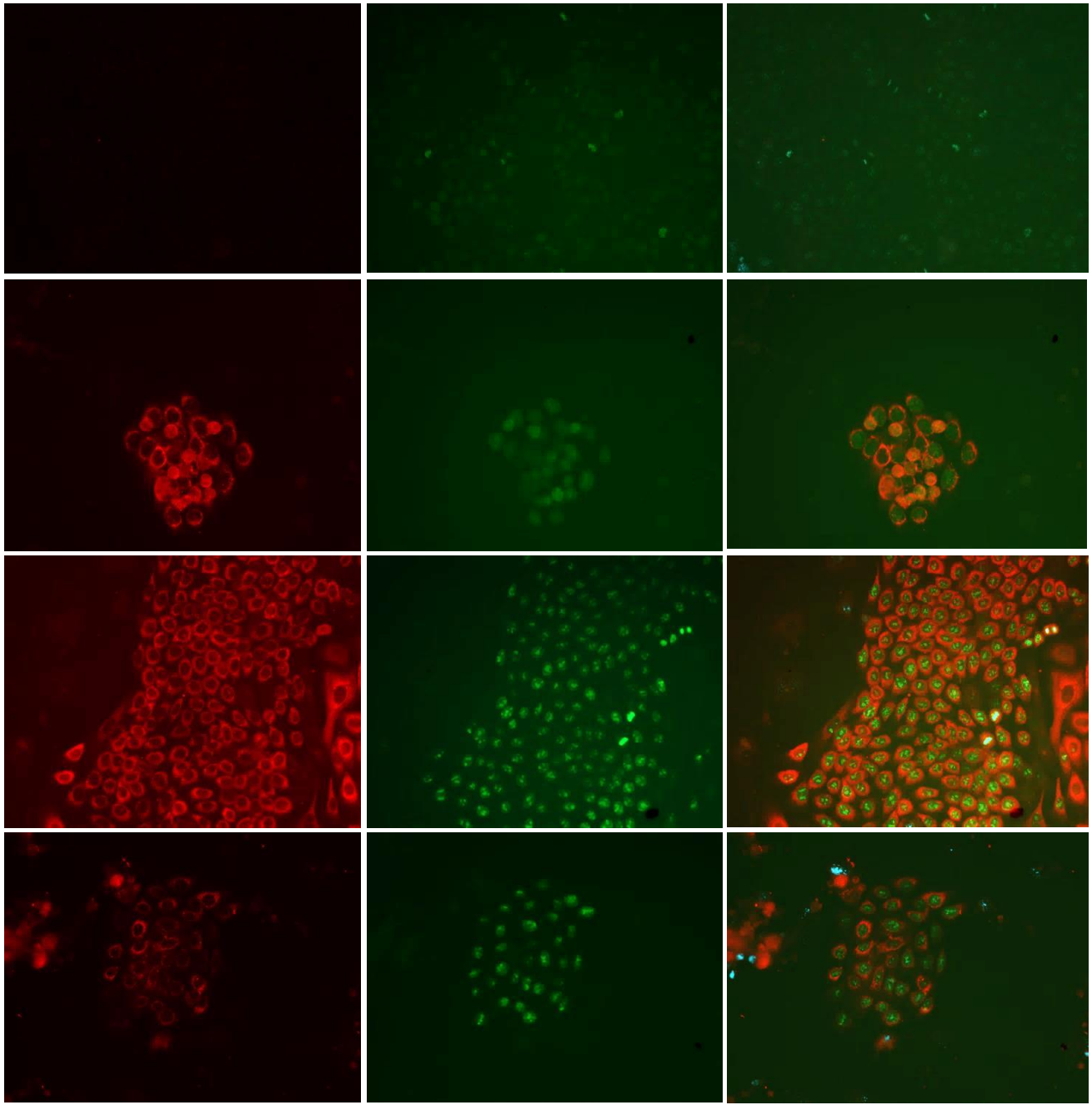


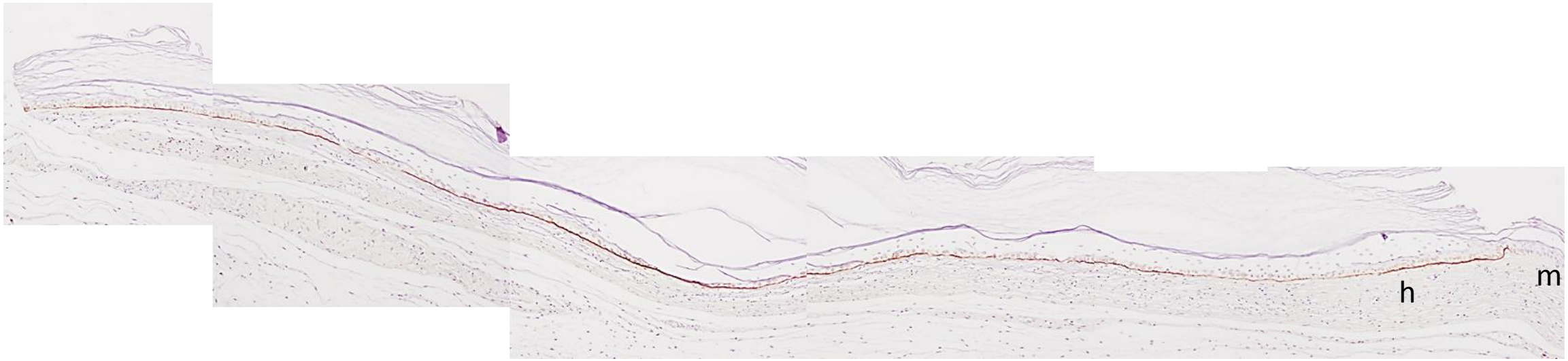
Supplementary Figure 1. Optimization of electroporation conditions in primary RDEB keratinocytes. A) Percentage of GFP-positive cells after GFP-mRNA electroporation with different programs and platforms. X axis, Neon and different 4D-Nucleofector™ System codes. B) Cell survival after electroporation. Cell number determined by flow cytometry 72h after treatment. C) GFP Mean Fluorescence Intensity for each electroporation condition.



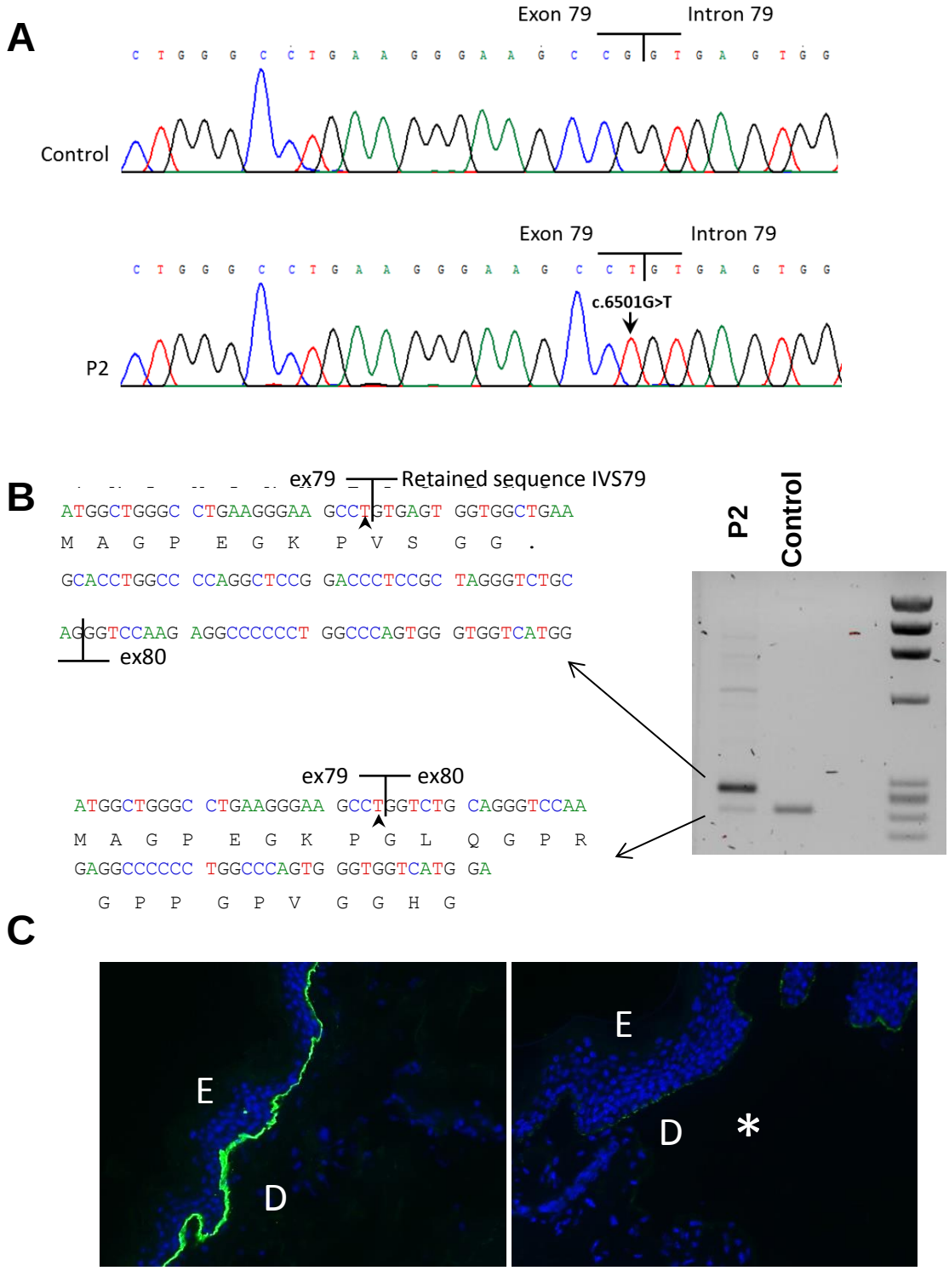
Supplementary Figure 2. HDR PCR genotyping of isolated clones from treated primary P1 keratinocytes. 22 clones are shown. Poly, bulk edited culture. All recombinant (5 out of 21, lower band) clones were bi-allelically corrected.



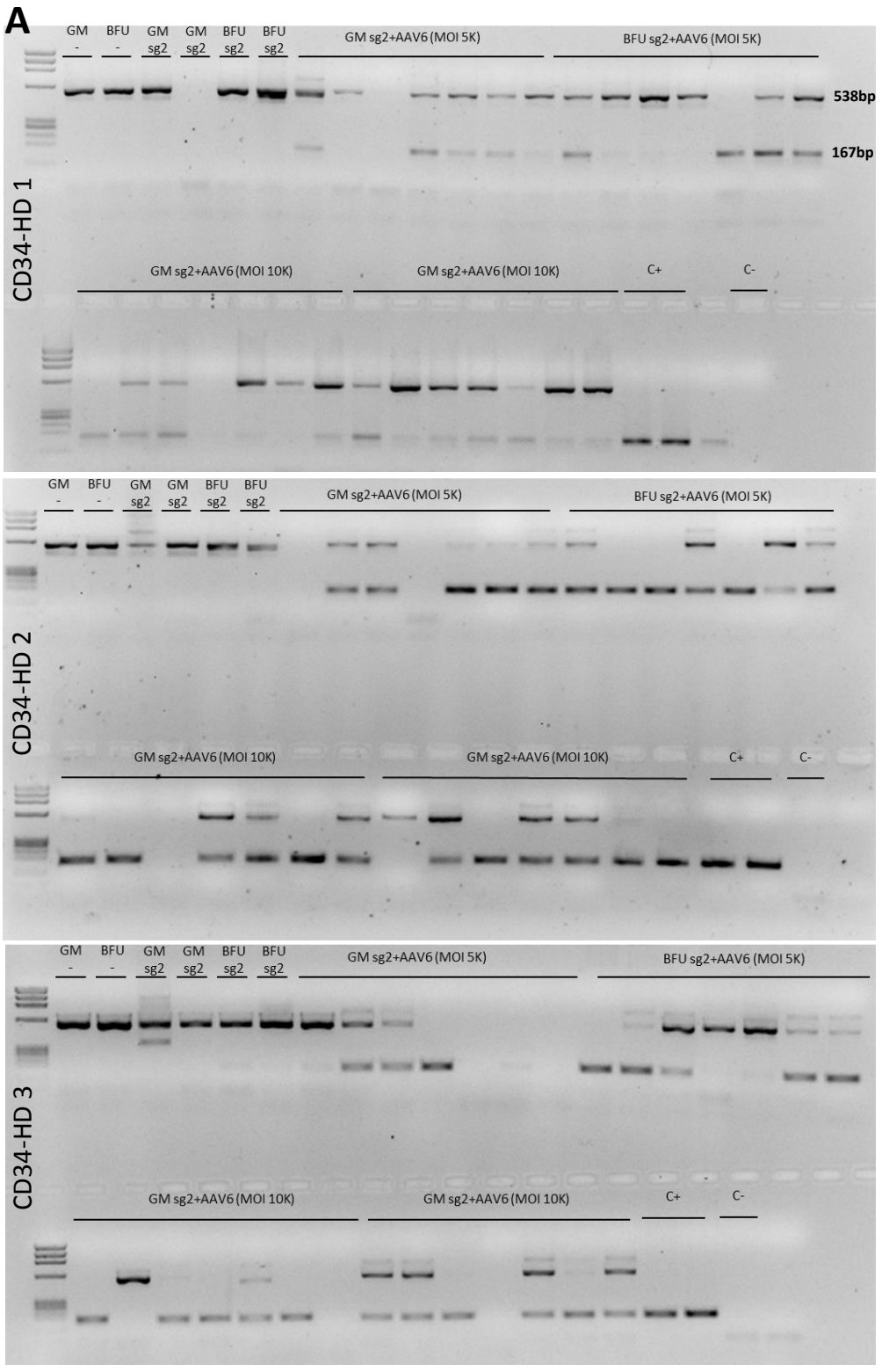
Supplementary Figure 3. C7 restoration after HDR treatment in proliferative P1 keratinocytes. C7 immunofluorescence (red) detection after genome editing treatment (Sym and Asym). KI67 immunostaining (green) shows proliferative status. Co, Normal Human Keratinocytes; P1, untreated patient keratinocytes.

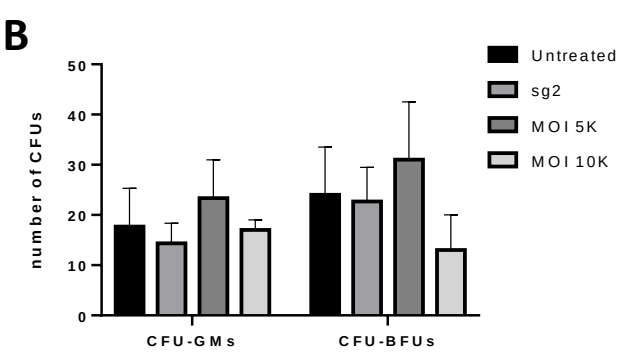


Supplementary Figure 4. C7 Immunohistochemistry in grafts from gene-edited P1 keratinocytes. Consecutive fields were assembled to show continuous C7 deposition along the BMZ throughout the human skin graft. h, human tissue; m, mouse tissue



Supplementary Figure 5. Patient P2 genotyping and C7 expression characterization.
A) c. 6501G>T homozygous variation detected by Sanger sequencing of a PCR fragment spanning exon 79 and adjacent intronic regions. Control sequence shown for comparison. B) *COL7A1* transcription analysis. RT-PCR bands corresponding to exons 79-80 were cloned and sequenced. Splice donor alteration causes retention of a fragment of intron 79. The presence of the smaller band corresponding to the normal splice variant containing the c.6501G>T change indicates that this is a hypomorphic allele. C) Immunofluorescence detection of C7 in a frozen section of P2 skin. Very faint, dotted staining indicates greatly decreased C7 expression. Asterik denotes blíster. E, Epidermis; D, Dermis.





Supplementary Figure 6. Analysis of individual colonies from methylcellulose clonal cultures from genome edited CD34+ cells. A) PCR genotyping of edited human burst-forming unit erythroid (BFU) and colony-forming unit granulocyte-macrophage (GM) from three healthy different donors (CD34-HD 1-3). B) Colony Forming Units (CFU-GMs and CFU-BFUs) quantification in methylcellulose culture of edited CD34+ cells.