Supplemental Information

A novel fluorescence-based screen of gene editing molecules for junctional epidermolysis bullosa

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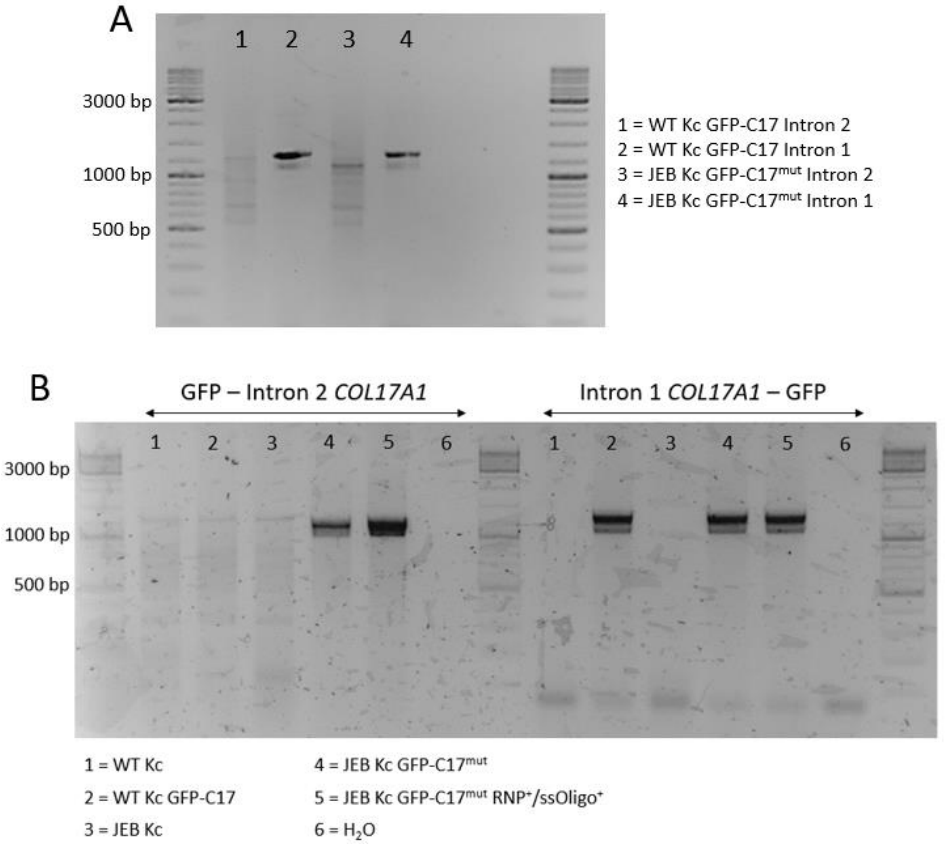

Figure S1. Integration PCRs of WT and JEB GFP-C17 and RNP/ssOligo-treated of GFP-C17^{mut}-expressing JEB cells. A) Primers specific for 1&3) GFP - Intron 2 *COL17A1* and 2&4) Intron 1 of *COL17A1* - GFP were used to amplify the target regions. Gel electrophoresis confirmed the genomic integration of the donor template in both WT and JEB Kc expressing GFP-C17. **B)** Gel electrophoresis of RNP/ssOligo-treated GFP-C17^{mut}-expressing JEB cells confirmed the genomic integration of the donor template. WT and JEB patient keratinocytes were used as control.

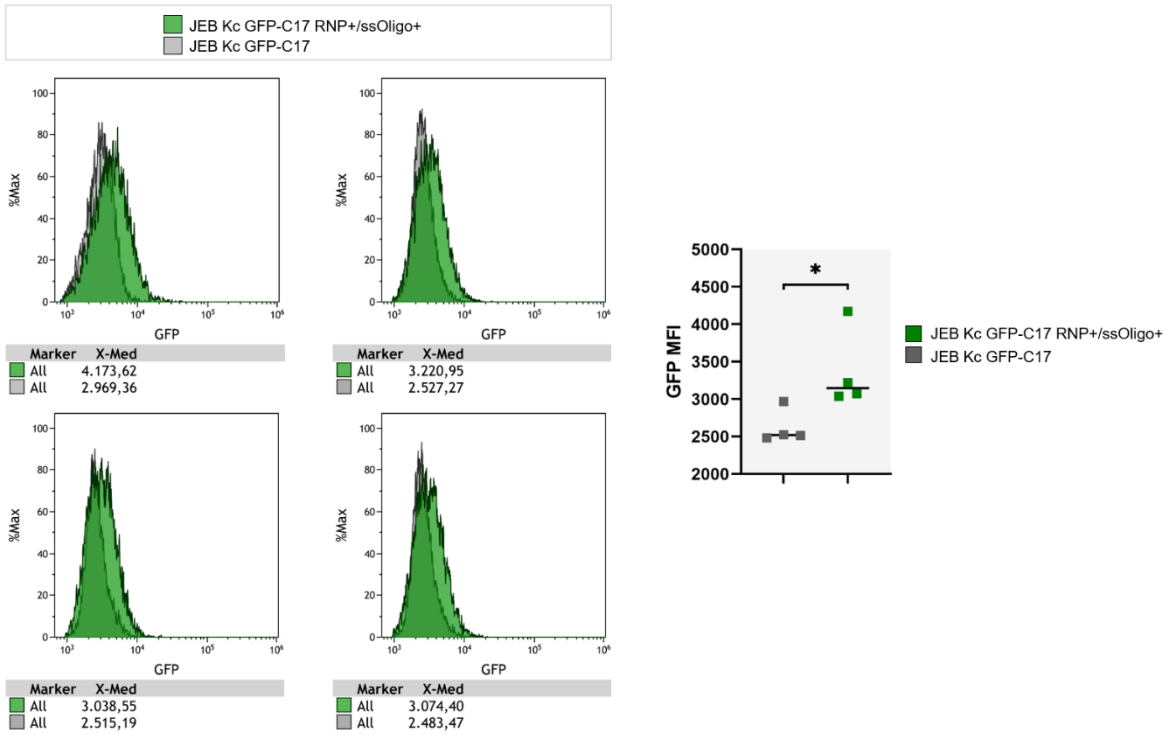

Figure S2. CRISPR/Cas9-mediated GFP-C17 restoration in JEB keratinocytes.

Flow cytrometric analyses of RNP⁺/ssODN⁺-treated (dark green) and untreated (ligray) GFP-C17 JEB cells, showing a significant increase in GFP median fluorescence intensity (MFI, denoted by X-Med in histograms) upon gene repair in 4 individual experiments. Statistical analysis (paired Student's t test) was performed using GraphPad Prism 9.

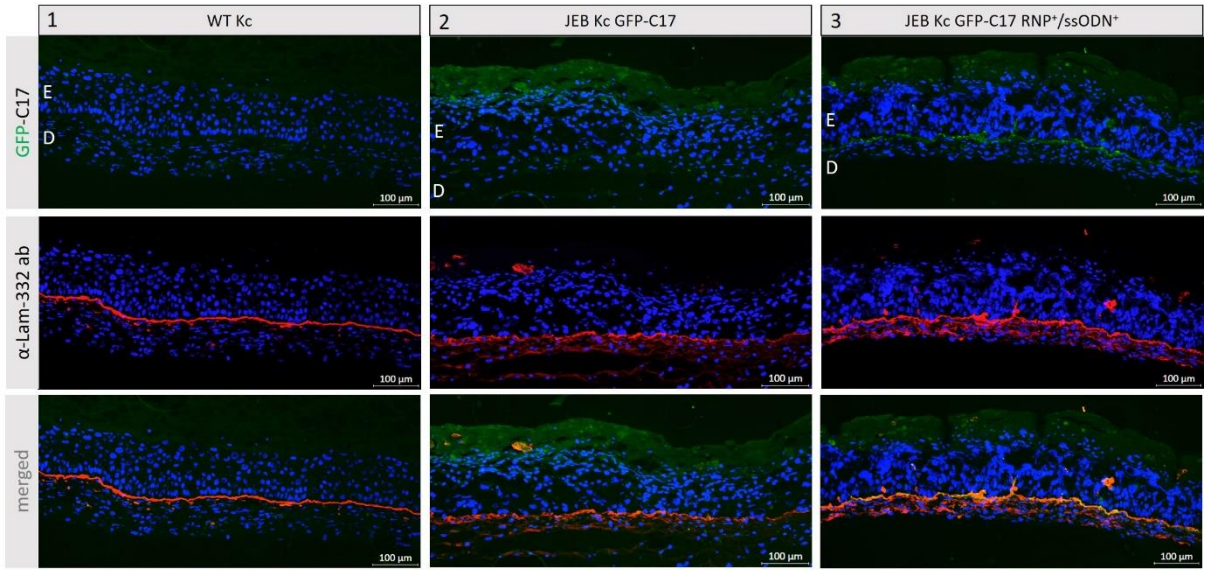

Figure S3. Laminin-332 IF stainings of JEB-derived skin equivalents after RNP/ssODN-treatment. Immunofluorescence staining performed on cryosections showed an accurate laminin-332 expression (red fluorescence) in the BMZ of SEs derived from WT keratinocytes (1). SEs from untreated GFP-C17^{mut}-expressing JEB

Kc showed fewer laminin-332 and no visible GFP-C17 along the BMZ (2), whereas immunofluorescence staining of 3D SEs expanded from RNP/ssODN-treated JEB cells revealed accurate colocalization of GFP-C17 (green fluorescence) with laminin-332 (red fluorescence) within the BMZ (3). Cell nuclei were stained with 4', 6-Diamidino-2-phenylindol (DAPI, blue). E= Epidermis ; D= Dermis.