Supplemental Information

Efficient CRISPR-Cas9-Mediated Gene Ablation in Human Keratinocytes to Recapitulate Genodermatoses: Modeling of Netherton Syndrome

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S1. Sanger sequencing of the alleles present in the ΔSPINK5 polyclonal population and in the selected clone. Chromatograms show very high frequencies of precise deletion in all gRNA combinations (a-d), containing also other repairing events in very low frequencies. Clone 1+4.2 sequencing (a) confirms homozygosis for disrupting deletion. Exon 1 is underlined. The deleted sequences appear in red and delimited by forward slashes.
S2. Characterization of immortalized ΔSPINK5 clone 1+4.2. Western blot analysis confirms expression of HPV16E7 protein in HKΔSPINK5+E6E7 at passage 20.

S3. Histopathological features of the NS patient skin. Top panels, H&E staining of a biopsy from a healthy donor and from the patient whose cells were used to generate NS grafts. Note the typical acanthotic, papillomatous epidermis (right) as compared with the skin from a healthy donor (left). Lower panels, LEKTI immunoperoxidase staining. NS patient skin (right) shows highly reduced LEKTI staining as compared with healthy donor skin (left) showing a continuous suprabasal staining.
S4. Recapitulation of an inflammatory phenotype in ΔSPINK5 grafts. Skin grafts produced with healthy donor keratinocytes (HK Ctrl), NS patient keratinocytes (HKNS), SPINK5-knockout clone (HKΔSPINK5) and immortalized ΔSPINK5 clone (HKΔSPINK5+E6E7). Histological analysis (H&E staining) of grafts produced either with HKNS, HKΔSPINK5 and HKΔSPINK5+E6E7 reveals the presence of a vascular reaction in the dermis, with blood vessels (*) oriented perpendicular to epidermis and mainly located in upper dermis in most of the sections analyzed, as compared to control skin grafts (HK Ctrl). The presence of inflammatory cells in HKNS, HKΔSPINK5 and HKΔSPINK5+E6E7 grafts is also observed (eosinophils marked with red arrows). Scale bar: 25 µm.
S5. Restoration of LEKTI expression in SPINK5-transduced immortalized ΔSPINK5 clone (HKΔSPINK5+E6E7+SPINK5). (a) LEKTI immunofluorescence and (b) western blot analysis confirm the efficient ex vivo lentiviral approach. (c) Densitometry of the bioactive LEKTI fragment, relative to loading control (β-actin), shows a recovery of 61.3% of LEKTI expression after gene transfer.
S6. Macroscopic appearance of the regenerated skin after grafting to immunodeficient mice of skin equivalents generated with gene corrected and not corrected immortalized ΔSPINK5 cells. Cells were genetically modified for expression of either eGFP or SPINK5/eGFP by lentiviral vector gene transfer. The panels show a representative 6 weeks-old graft produced with ΔSPINK5+E6E7 cells (top panels) and with ΔSPINK5+E6E7+SPINK5 cells (lower panels), observed under white light illumination (left) and under blue light illumination showing eGFP fluorescence (right). Note the desquamation area in the not-corrected graft (arrow). None of the grafts shows any macroscopic sign of neoplastic development.