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Supplemental Data

Topical Enzyme-Replacement Therapy Restores Transglutaminase 1 Activity and Corrects Architecture of Transglutaminase-1-Deficient Skin Grafts

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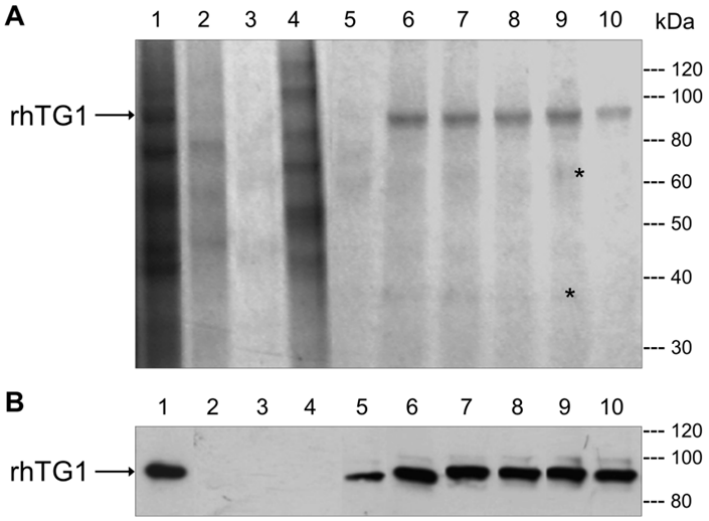

Figure S1. Recombinant Expression and Purification of Human Full-Length Transglutaminase 1

A: Coomassie stained SDS-Gel (10%) and **B**: Western Blot of different expression and purification steps. Lane 1, cell lysate before purification; lanes 2,3, washing steps with 20 mM imidazole; lane 4, washing step with 40 mM imidazole; lanes 5-10 elution steps. Lanes 6-10 in **A** show highly purified rhTG1.

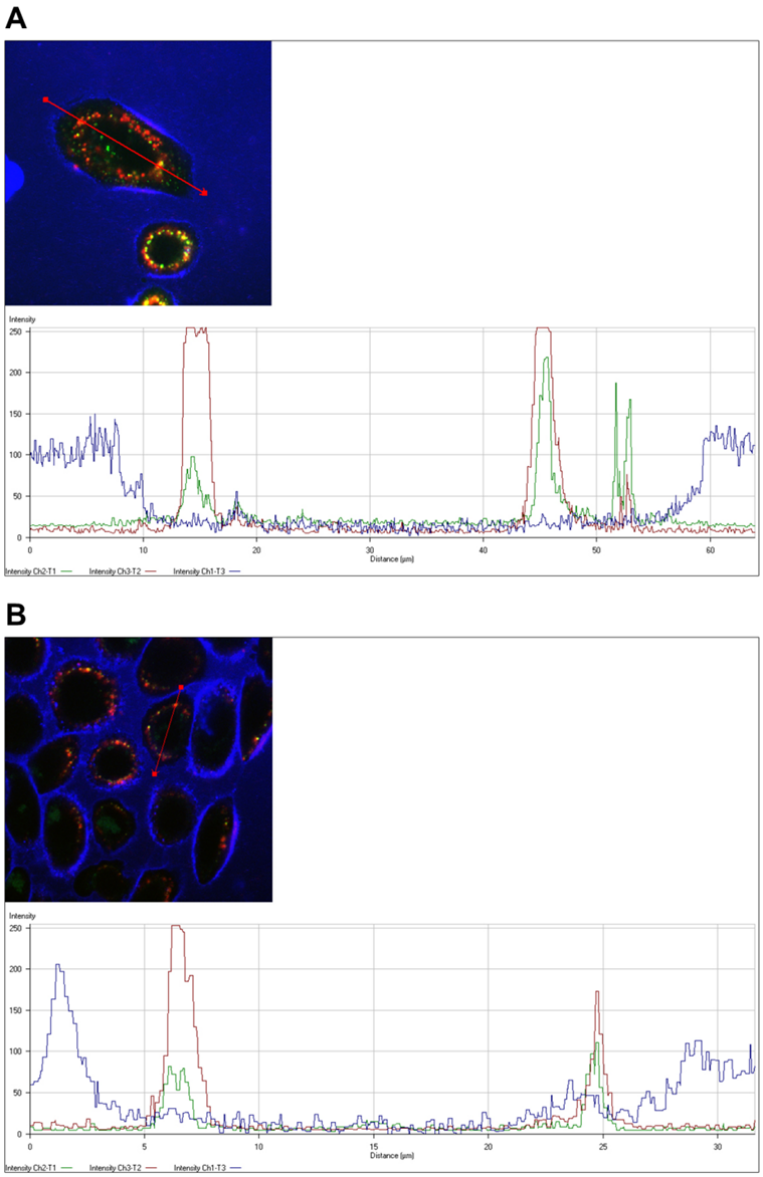

Figure S2. Intensity Scans to Record Intracellular Localization of Liposomes in *TGM1-/-* Keratinocytes

A: Intensity scan along the red line in the equator plane of a *TGM-/-* undifferentiated cell and **B**: of a *TGM-/-* differentiated cell. Traces of the fluorescence intensity of the lipopeptide (green), Rhod-labeled lipid (red) and cell-excluded, membrane-bound trypan blue (blue).

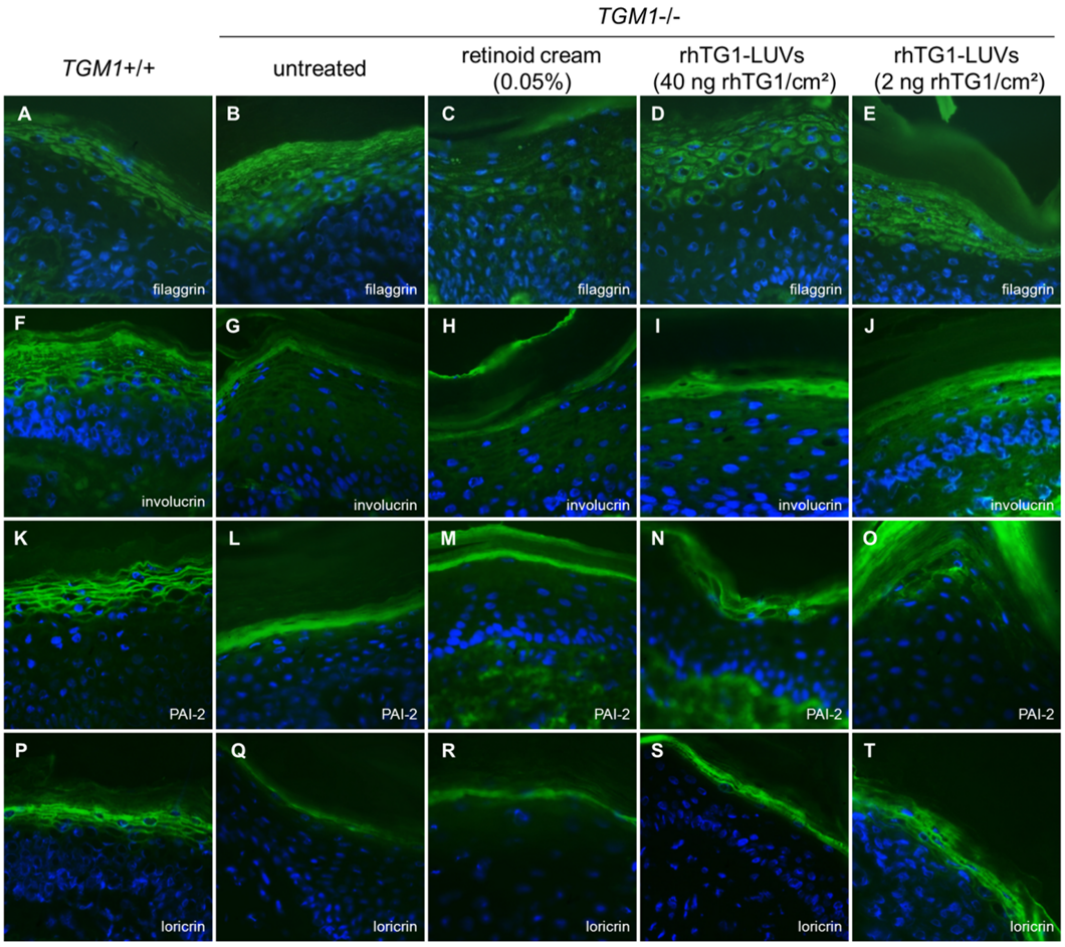

Figure S3. Immunohistochemical Detection of Differentiation Markers and TG1-Substrates after Treatment with rhTG1-LUV Preparations

In regenerated TG1-deficient grafts as well as in human TG1-deficient skin the investigated differentiation markers show a more diffuse and shifted distribution when compared to normal skin. Staining of filaggrin (A-E), involucrin (F-J), plasminogen activator inhibitor-2 (PAI-2) (K-O) and loricrin (P-T) in mice treated with rhTG1-LUV formulations reveal a normalization of the distribution patterns when compared to untreated or retinoid treated mice.

Table S1. Characterisation of Liposomal Preparations of Different Diameters

	MLVs*	LUVs#	LUVs#	SUVs¶
Diameter [nm] (Dynamic light scattering)	> 1500 nm	200 nm	100 nm	< 50 nm
Inner volume (calculated) (V=1 ml, 10 mM lipid)	> 200 µl	~ 180 µl	~ 55 µl	~ 10 µl
Amount of encapsulated protein (Dialysis, HPLC)	+++	++	++	+
Stability (Dynamic light scattering)	+	+++	+++	++
Coupling of lipopeptide vector (Fluorescence-spectroscopy)	+++	+++	+++	+++
Polydispersity-Index/Uniformity (Dynamic light scattering)	> 1.0	0.108	0.075	0.548

*MLVs: multilamellar large vesicles; #LUVs: large unilamellar vesicles; ¶SUVs: small unilamellar vesicles; +++ high; ++ moderate; + low.