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Supplemental information

A cellular disease model toward gene therapy of *TGM1*-dependent lamellar ichthyosis

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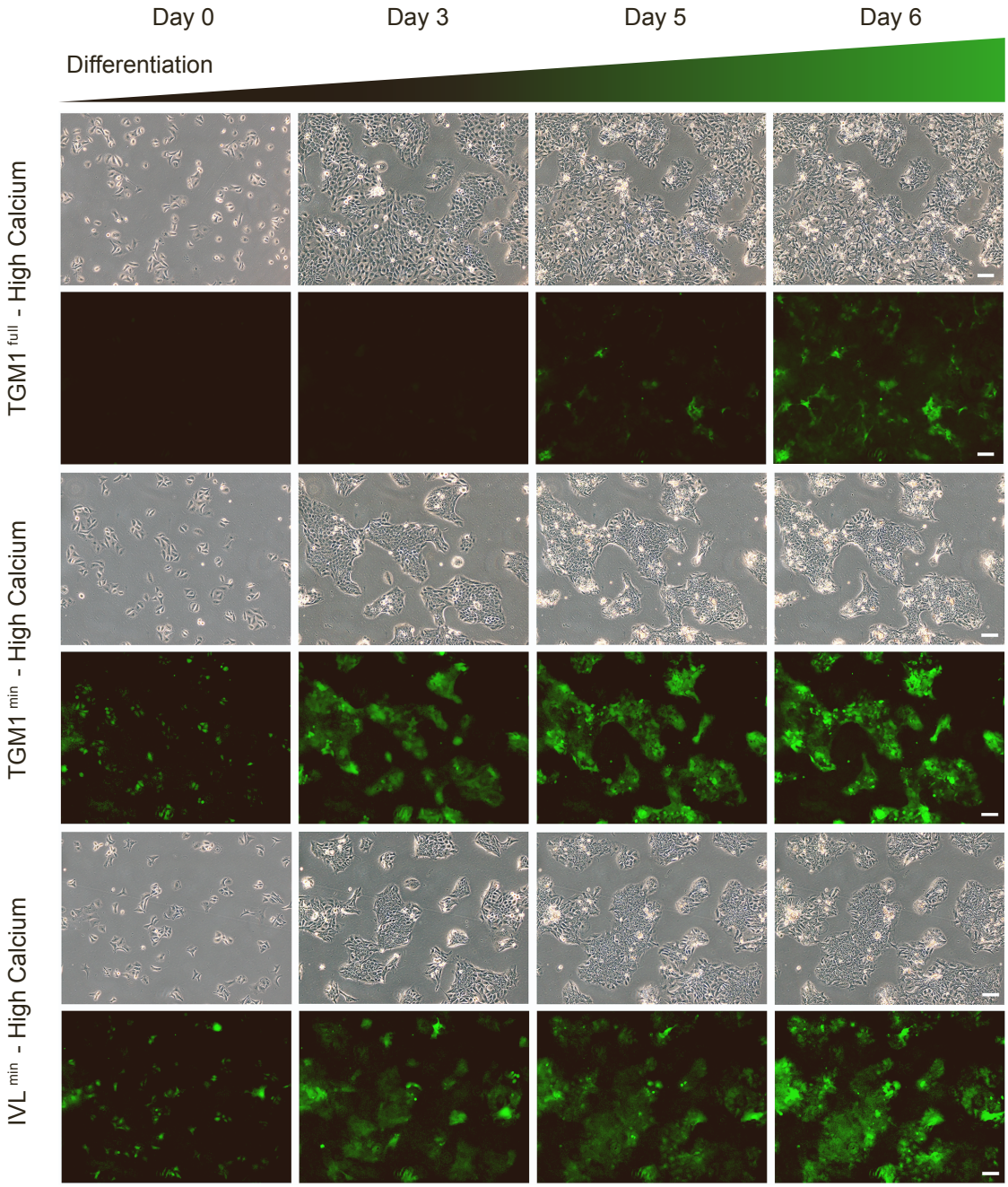


Figure S1. Time-resolved promoter activity by live-cell imaging of HaCaT cells transduced with SINγ-RVs. Representative bright-field and fluorescent micrographs of HaCaT cells transduced with three different promoters, from top to bottom: the 2.2-Kb human TGM1 full length promoter ($TGM1^{full}$), the 367-bp human TGM1 minimal promoter ($TGM1^{min}$), the 637-bp human Involucrin minimal promoter (IVL^{min}), grown in high calcium medium for 6 days. n=3, pictures are representative of what was observed in at least three independent samples or replicates. Scale bars, 50 µm.

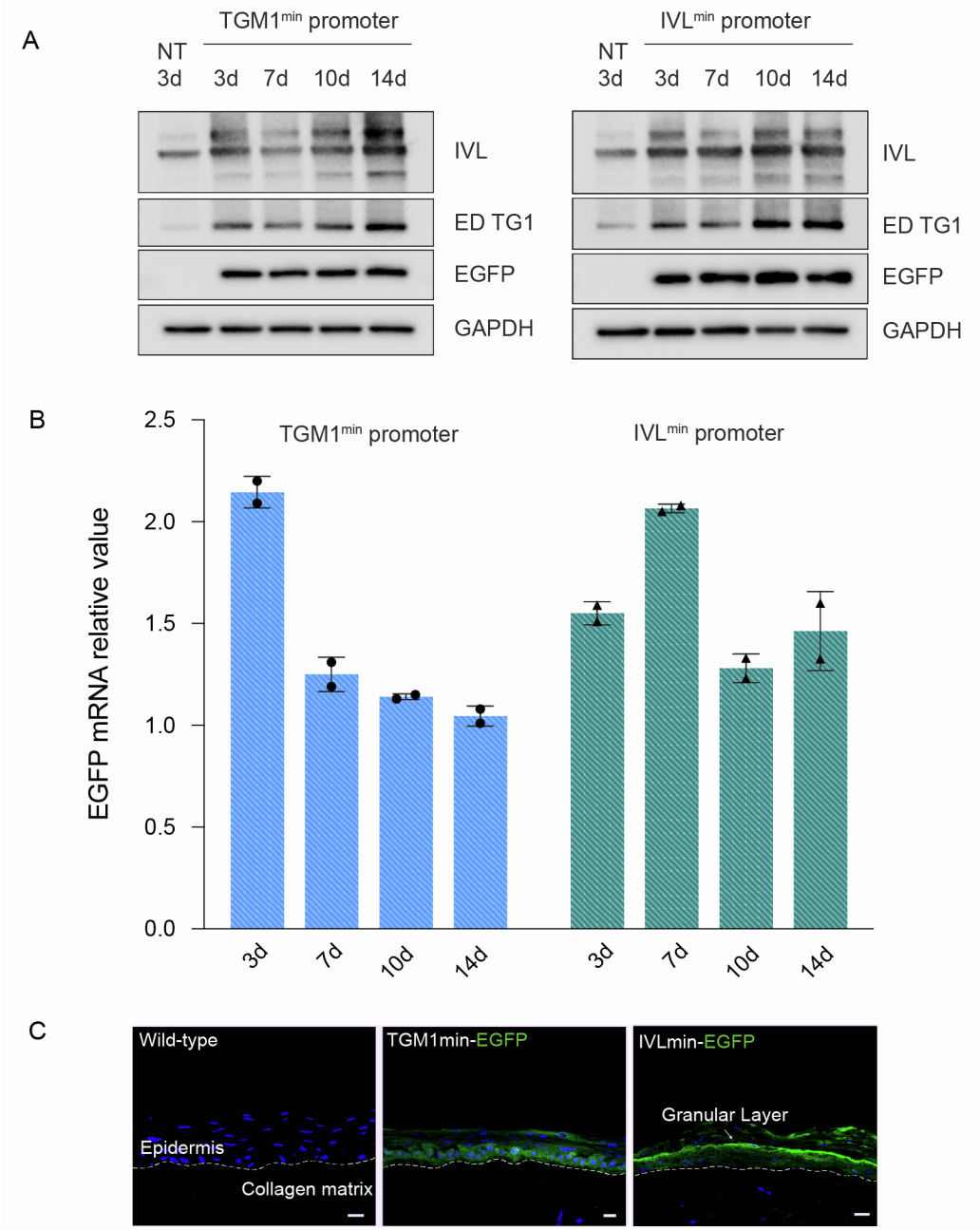


Figure S2. TGM1^{min} and IVL^{min} promoters activity in keratinocytes. A) Western blot analysis of keratinocyte differentiation markers (IVL and endogenous TG1) and EGFP in growing (3d), confluent (7-10d) and over-confluent (14d) K82 keratinocytes transduced with (left) a SINγ-RV carrying EGFP under the control of TGM1^{min} promoter or, (right) a SINγ-RV carrying EGFP under the control of IVL^{min} promoter. B) EGFP mRNA expression during transgenic keratinocyte differentiation (3d-14d). GAPDH mRNA was used to normalize the qRT-PCR (* $p < 0.0001$). C) Representative immunofluorescent images of 7 µm-thick cryosections of 3D skin equivalents obtained with TGM1^{min} or IVL^{min} promoter-transduced keratinocytes showing the EGFP fluorescent signal. White dotted line marks the epidermal-dermal junction. Scale bars 20 µm.

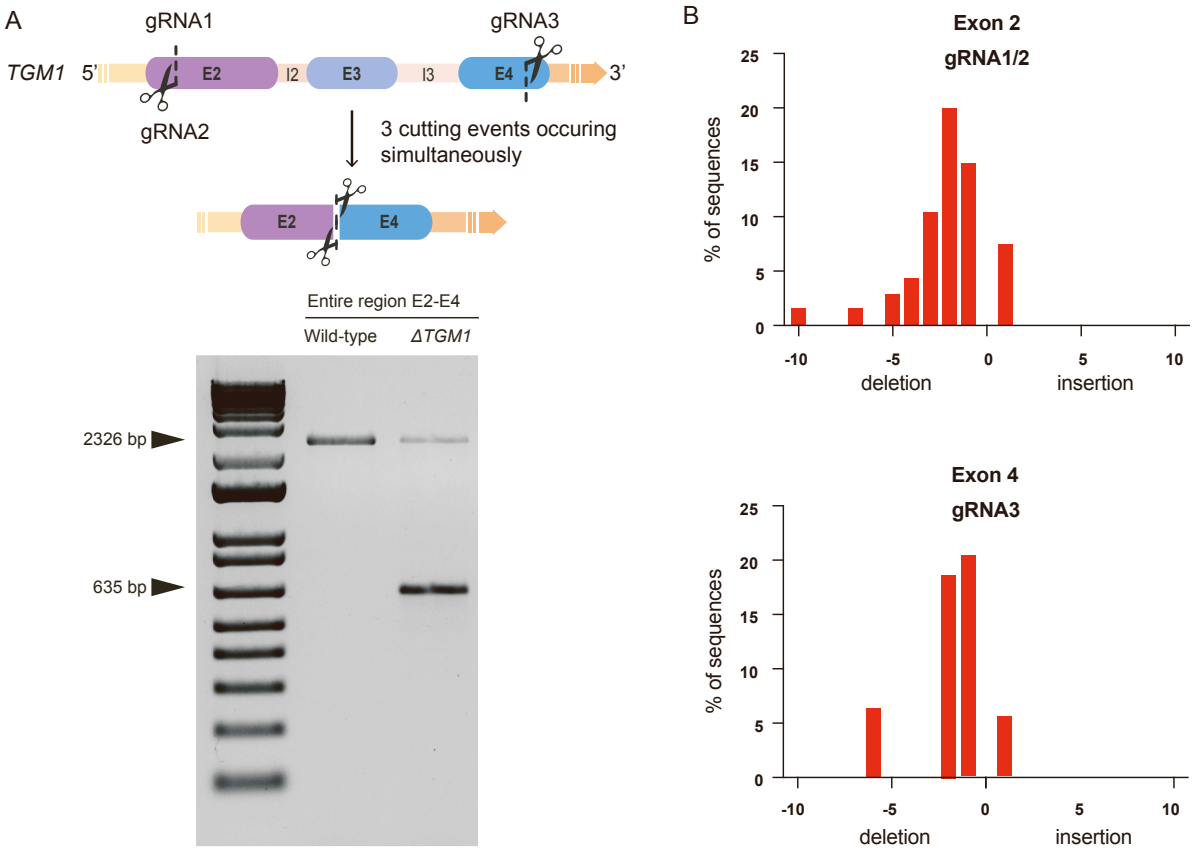


Figure S3. Genotype of *ΔTGM1* cellular model. A) Top: *TGM1*-knockout strategy design. gRNA1 and gRNA2 target Exon 2, exploiting the presence of an SNP in a wild-type strain (K81), while gRNA3 targets Exon 4 of the *TGM1* gene. If the three cutting events occur simultaneously the resulting *TGM1* gene should be shorter as depicted in figure. Bottom: PCR analysis of genomic DNA from wild-type and *ΔTGM1* keratinocytes (K81), spanning both the Cas9 target sites to evaluate the excision of the 1.7 Kb genomic region between Exon 2 and Exon 4. Note that the excision of the entire genomic region is a high frequency event. B) TIDE decomposition analysis through Sanger sequencing of edited genomic sequences in *ΔTGM1* keratinocytes (K81) with gRNA1/2 (editing *TGM1*-E2) and gRNA3 (editing *TGM1*-E4) showing the overall editing efficiencies and the percentage of indel formation (insertions and deletions) in both edited exons, as compared to a wild-type sequence. The overall editing efficiencies, reaching 100%, is indicated by the absence of non-edited sequences (point 0 of the graphs) and the presence of a significant amount of InDels abolishing the open reading frame and expression of *TGM1*.

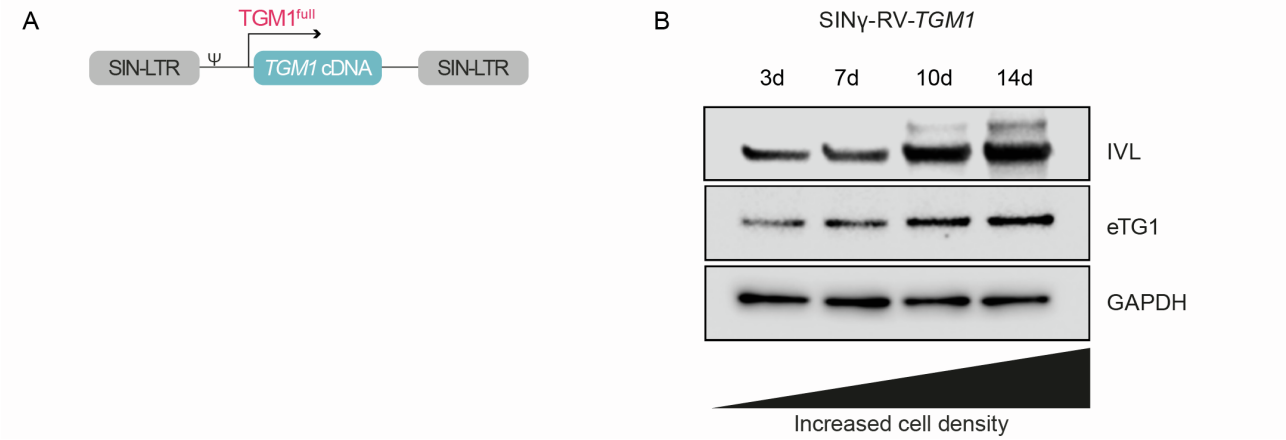


Figure S4. TG1 expression in *ΔTGM1* keratinocytes cultures at increased cell densities. A) SINγRV vector expressing *TGM1* cDNA under the control of its own promoter (SINγ-RV-*TGM1*). B) Western blot analysis of exogenous TG1 (eTG1) and IVL differentiation marker in growing (3d), confluent (7-10d) and over-confluent (14d) keratinocytes transduced with SINγ-RV-*TGM1*. n=3, picture is representative of what was observed in at least three independent replicates.

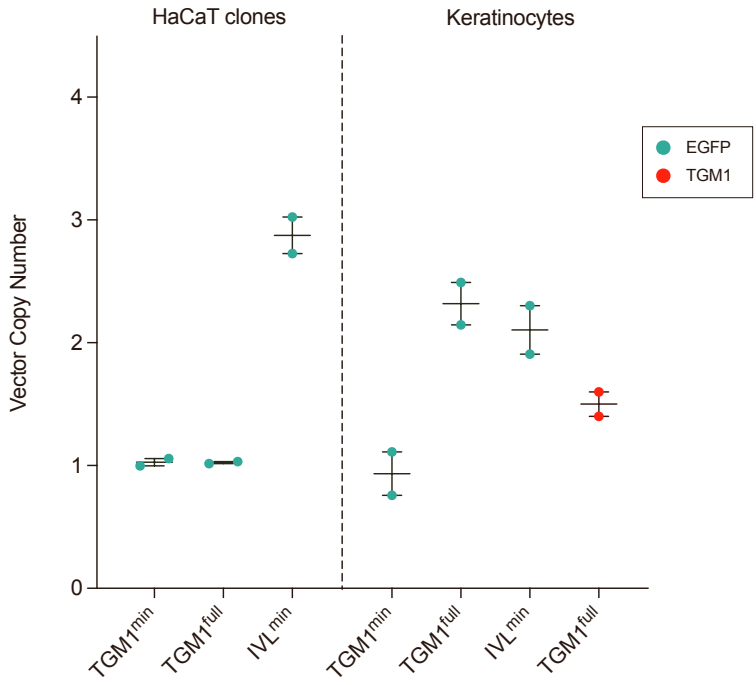
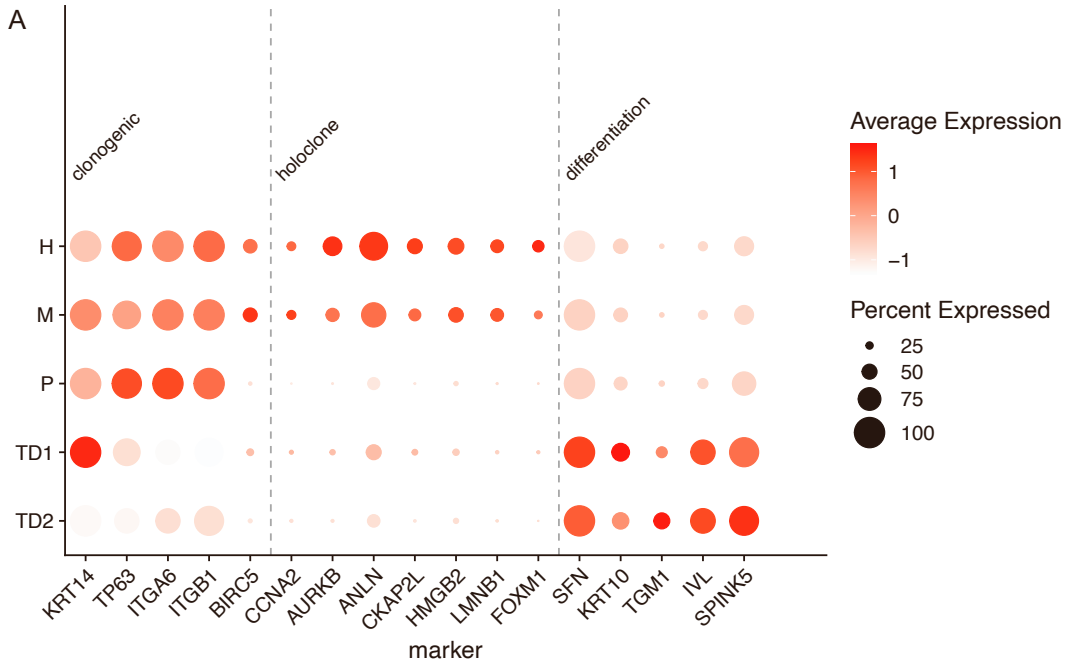


Figure S5. Vector Copy Number (VCN) of transduced HaCaT clones and keratinocytes cultures. ddPCR analysis of EGFP (green) and exogenous *TGM1* (red) Vector Copy Number in HaCaT clones (right) and bulk K82 keratinocytes (left) transduced with SINγ-RV carrying $TGM1^{min}$, $TGM1^{full}$ or IVL^{min} promoters. For the exogenous *TGM1* (red) VCN evaluation was used a custom probe design to recognize the provirus integration, that binds an exon-exon junction. VCN was absolutely quantified considering an internal reference gene (hGAPDH, VCN=2), n=2. Dots represent the single values, while middle line represents the mean value.

A



B

Cell label vs *TGM1* pos cells

	NEG	POS	% POS
H	151	48	**24,1 %**
M	556	127	**18,6 %**
P	531	143	**21,2 %**
TD1	299	118	**28,3 %**
TD2	248	118	**32,2 %**

C

Cell label vs # *TGM1* fragments

	1	2	3	4	5	>5
H	29	13	2	2	2	0
M	81	25	16	4	1	0
P	103	25	9	4	2	0
TD1	77	27	7	5	1	1
TD2	65	26	15	6	2	4

Figure S6. Single-cell multiomic analysis of *TGM1*-transduced keratinocytes. A) DotPlot showing the expression of clonogenic, holoclone and differentiation markers in the five SINγ-RV-*TGM1* keratinocytes populations. Dots size indicates the percentage of cells expressing that gene, the average expression color scale refers to scaled data. B) Percentages of positive cells (bold), containing at least one provirus fragment and negative cells, without provirus integration, for each keratinocytes population. C) Number of SINγ-RV-*TGM1* provirus fragments retrieved in individual cells, for each keratinocytes population.

Table S1. List of primers used with specified name, sequence, and application.

	Primer name	Sequence (5'-3')	Application	Notes
#1	TGM1 PromP1_F	ACCTAGTTAACGCTGAGTGTCTGCTCCCATG	PCR amplification	distal region (-1.6 Kb / -1.4 Kb)
#2	TGM1 PromP1_R	TACAGGGCCGGCCTCCCAGAGAACCAGTAGGATG	PCR amplification	
#3	TGM1 PromP2_F	ATTCTGGCCGGCCTGCTCCCTCCCTAGC	PCR amplification	proximal region (90 bp / +67 bp)
#4	TGM1 PromP2_R	GATTTCCATGGTCAGGATGGATGGGAC	PCR amplification	
#5	IVL PromP1_F	TTGGGTTAACAGCTTCTCCATGTGTCATG	PCR amplification	distal region (-2.473 Kb / -2.036 Kb)
#6	IVL PromP1_R	GAAGGCCGGCCGGTCTTATGGGTTAGC	PCR amplification	
#7	IVL PromP2_F	ATTAGGCCGGCCAGGAATAGTTGAGCTACCAG	PCR amplification	proximal region (221 bp / +6 bp)
#8	IVL PromP2_R	GAAATCCATGGTGCTGAGCTGAGCAGGAG	PCR amplification	
#9	$TGM1^{full}$ Prom_F	AACTAGTTAACGCCAAGGCTTCAGTGTTTG	PCR amplification	full length promoter (2.2 Kb)
#10	$TGM1^{full}$ Prom_R	ATTATGGCCGGCCGAGGTCTGGGGGCTTAGG	PCR amplification	
#11	TGM1_Exon2_F	GATGGGCCACGTTCCGATG	KO analysis	
#12	TGM1_Exon3_R	CGGGACAGGAGGAGGAGC	KO analysis	
#13	TGM1_Intron3_F	CAGTGGCTCATACACATTGTG	KO analysis	
#14	TGM1_Exon5_R	CTGCCGCCAATCCTCATGG	KO analysis	

Table S2. List of TaqMan® probes used for qRT-PCR and catalog number.

TaqMan® probes	Company	Catalog number
EGFP	Thermo Fisher	Mr00660654_cn
GAPDH	Thermo Fisher	4352665
Genomic GAPDH	Thermo Fisher	Hs03929097_g1
K10	Thermo Fisher	Hs00166289_m1
TGM1 Exon 14-15	Thermo Fisher	Hs00165929_m1
Exogenous TGM1	Thermo Fisher	Custom made

Table S3. List of antibodies, source, and concentration.

Antibody information				Dilution/amount	
Antibody	**Company**	**Catalog number**	**Description**	**WB**	**IF**
anti-TGM1	Invitrogen	PA5-59088	Rabbit polyclonal	1/1000 - 1/4000	
anti-TGM1	Merk	HPA040171	Rabbit polyclonal		1/2000
anti-Cytokeratin 14	BioLegend	905301	Rabbit polyclonal		1/150,000
anti-Collagen XVIIA1	Genetex	GTX54647	Mouse monoclonal		1/100
anti-SPINK5	Merk	HPA011351	Rabbit polyclonal		1/2000
anti-human GAPDH	Merk	ZRB374 - clone 10B13	Rabbit monoclonal	1/1000	
anti-GAPDH	Abcam	ab8245	Mouse monoclonal	1/10,000	
anti-Involucrin	Leica	NCL-INV	Mouse monoclonal	1/2000	1/1000
Anti-Loricrin	Abcam	Ab240187	Rabbit monoclonal		
anti-Cytokeratin 10	BioLegend	905403	Rabbit polyclonal	1/500	1/1000
anti-GFP	Santa Cruz Biotechnology	sc-9996	Mouse monoclonal	1/250	
anti-GFP	Merk	AB10145	Rabbit polyclonal	1/1000	1/500
anti-Vimentin	BioLegend	699302	Rat		1/1000
Alexa Fluor 568 anti-rat	Thermo Fisher Scientific	A11077	secondary antibody		1/1000
Donkey anti-rabbit IgG HRP	Santa Cruz Biotechnology	sc-2313	secondary antibody	1/2000	
Donkey anti-mouse IgG HRP	Santa Cruz Biotechnology	sc-2314	secondary antibody	1/10,000 - 1/20,000	
Donkey anti-Mouse IgG (H+L) Alexa Fluor 568	Thermo Fisher Scientific	A10037	secondary antibody		1/2000 - 1/1000
Donkey anti-Rabbit IgG (H+L) Alexa Fluor 488	Thermo Fisher Scientific	A21206	secondary antibody		1/2000 - 1/1000