**Supplementary materials**

**Methods**

*Immunohistochemistry and Immunofluorescence*

The antibodies used for immunohistochemistry/immunofluorescence analyses are listed in the Table S5.

Human skin sections were obtained from biopsies frozen in OCT and were fixed in ice-cold methanol. They were incubated with primary antibody before staining with a biotinylated secondary antibody (Vector Laboratories; Burlingame, CA, USA), followed by treatment with fluorescein (FITC)-conjugated streptavidin (UCS Diagnostics; Morlupo, Italy). Cell nuclei were counterstained with DAPI (Vector Laboratories), and the intensity of the green channel was analysed by three-dimensional surface plotting with ImageJ1.34 (<http://rsb.info.nih.gov/ij>).

For immunofluorescence analysis of cultured fibroblasts, cells were seeded on untreated glass coverslips and grown to subconfluence. Fibroblasts were then fixed with 3% paraformaldehyde/phosphate buffer saline (PBS), permeabilized with 0.1% Triton X-100 and non-specific staining blocked with 1% BSA/PBS for 30 minutes, followed by incubation with the primary antibodies. After washing with PBS, cells were incubated with the Alexa FluorTM 488 or 555 secondary antibodies (Invitrogen; Waltham, MA, USA) for 1 hour at room temperature. Cell nuclei were counterstained with DAPI. At least seven randomly selected fields were analysed for fluorescence intensity using ImageJ software.

For immunohistochemistry/immunofluorescence on mouse back skin, tongue and eyes, sections from formalin-fixed paraffin-embedded biopsies were used according to standard procedures and incubated with primary antibodies. After washing they were treated with Alexa FluorTM 555 secondary antibodies (Invitrogen). Cell nuclei were counterstained with DAPI. Staining was quantified using ImageJ software after applying a constant threshold. Images were analyzed with Axioplan2 microscope (Carl Zeiss; Oberkochen, Germany) equipped with an Axiocam camera.

All analyses were performed by blinded observers.

*Collagen lattice contraction assay*

Cells were starved overnight in 0.1% BSA, then cultured under starvation conditions in the presence of Givinostat or VPA for 72 hours. Medium was removed, and cells were detached, counted, and plated in rat tail collagen I (Corning #35429; Glendale, AZ, USA). Collagen lattice was performed as described1. Collagen solution was mixed with 3 x 105 cells/ml in serum-free medium and 1 ml was plated into 12-well cell culture dish. Gel surface area (A) was measured at gel detachment from well after polymerisation (time 0), and after 24 and 48 hours. Gel area was calculated from the acquired images using the Image J program. Gel contraction was expressed as percentage of initial lattice area [(t 24 or 48) \*100/A (t0)\*]. At least two culture plates were used for each treatment and the analysis was repeated at least twice.

After photography, lattices were collected, washed with ice-cold PBS and treated for protein extraction as described1.

*Proliferation analysis*

Proliferation assay was performed using an EdU proliferation kit (Abcam; Cambridge, UK) according to the manufacturer’s protocol. Briefly, RDEB fibroblasts were cultured for 24, 48 and 72 hours in the presence or in absence of Givinostat 250 nM or VPA 2 mM in 24-wells, under starvation conditions (0.1% BSA). At the end of the treatment, the cells were cultured in medium containing 20 µM EdU for 4 hours. After fixation and permeabilization, cells were further stained with iFluor 488 azide. Cell nuclei were counterstained with DAPI (Vector Laboratories) and EdU-positive cells (green) were counted using a UV-microscope. Experiments were performed in duplicate on fibroblasts from four RDEB patients.

*Cell toxicity assay*

The Promega ApoTox-Glo® triplex assay (Promega; Madison, WI, USA) was performed as described by the manufacturer. Briefly, RDEB fibroblasts were treated with Givinostat (250 nM, 500 nM) or VPA (1 mM, 2 mM) for 24, 48 and 72 hours. Next, 20 µL of the cytotoxicity reagent was added to each well and briefly mixed on an orbital shaker. Plates were maintained in culture for further 30 minutes, and the fluorescence was measured at the wavelength of 485Ex/520Em using the Envision plate reader (Perkin Elmer; Waltham, MA, USA). The experiment was performed in triplicate, using fibroblasts of three patients.

*ELISA*

Histone extraction and detection of global H3 acetylation were performed using the EpiQuik global histone H3 acetylation assay kit, according to the manufacturer’s instructions (Epigentek; Farmingdale, NY, USA). Samples were assayed in duplicate. The percentage of acetylation was calculated as OD (treated sample - blank)/OD (untreated control - blank) × 100%.

An ELISA kit for human TGF-β1 (ThermoFisher Scientific; Waltham, MA, USA) was used on collagen lattice supernatants. An ELISA assay for mouse TGF-β1 (MB100B, R&D System; Minneapolis, MN, USA) was performed on mouse skin protein extracts. Samples were assayed in triplicate according to manufacturer’s instructions.

For the analysis of cytokines/chemokines, supernatants of vehicle- and HDACi-treated cells were analysed using the ELLA platform, a cartridge-based automated immunoassay system (Bio-techne; Minneapolis, MN, USA). Two multi-analyte cartridges with 4 and 8 analyte panels were used (Bio-techne). Samples were processed in triplicate and run according to the supplier's instructions. Concentration data were automatically provided by the instrument based on calculated factory standard curves.

*Western blot analysis*

Proteins from RDEB and healthy control fibroblasts were extracted using RIPA buffer (50 mM Tris-HCl at pH7.4, 150 mM NaCl, Triton 1%, 0.5% Na deoxycolate, SDS 1%) with the addition of 10 mM NaF, 2 mM sodium orthovanadate and protease cocktail inhibitors (Roche Diagnostic; Mannheim, Germany).

Frozen skin from wild-type mice, vehicle-treated RDEB mice and VPA-treated RDEB mice was crushed in liquid nitrogen with a mortar and pestle, lysed in RIPA buffer containing protease and phosphatase inhibitor cocktails, and incubated on ice for 20 minutes with vortexing every 5 minutes. The samples were then sonicated on ice at 20 kHz (3 x 10 seconds) and centrifuged at 12,000 g for 20 minutes. Protein concentration was quantified using a Qubit photometer (Invitrogen).

Protein extracts were electrophoresed on SDS-PAGE gel and transferred to PVDF or nitrocellulose membranes (Amersham Pharmacia Biotech; Little Chalfont, UK). Blocking was performed with 5% nonfat dry milk or 5% BSA in TBS-T (20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 0.05% Tween 20). The primary antibodies used for Western blot analysis are listed in the Table S5. Detection was performed using horseradish-peroxidase-conjugated secondary antibodies (GE Healthcare; Chicago, IL, USA). Antibody binding was detected using the ClarityTM Western ECL Substrate detection system (Bio-Rad Laboratories; Hercules, CA, USA). The relative intensity of the signals was quantified using Chemidoc (Bio-Rad Laboratories).

*RNA sequencing, data processing and bioinformatics analysis*

Subconfluent RDEB fibroblasts were starved overnight in 0.1% BSA and then treated with 250 mM Givinostat or 2 mM VPA, or DMSO as vehicle for 30 hours. Total RNA was then extracted from fibroblasts using the RNeasy Plus Micro Kit (Qiagen; Germantown, MD, USA), quantified using the Qubit® RNA HS Assay Kit (Thermo Fischer Scientific) and assessed for integrity using the Bioanalyzer 2100 RNA 6000 Nano Kit (Agilent Technologies; Santa Clara, CA, USA). Purified samples were processed using TruSeq RNA-Seq v2 Library Preparation Kit using 300 ng of total RNA. An equimolar library pool, measured by Bioanalyzer High Sensitivity DNA 1000 Assay and Qubit® RNA HS Assay Kit, was loaded onto the Illumina NextSeq 500 platform (Illumina Inc.; San Diego, CA, USA) in order to generate 30M of 75bp paired-end reads. The quality of RNA-Seq data was evaluated using the FASTQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>) and RSeQC (https://doi.org/10.1093/bioinformatics/bts356) tools. Transcript abundance was estimated using Kallisto (2) and differentially expressed (DE) genes were identified using the DeSeq23 R package and a FDR corrected p-value < 0.05. GSEA and MsigDB4 were used to identify gene sets and pathways that were significantly perturbed across conditions. Gene sets with false discovery rate (FDR) q-value (p.adjust) <0.05 were considered significantly enriched.

*Real-time PCR*

Total RNA was extracted from primary fibroblasts using Direct-zolTM RNA MicroPrep (Zymo Research; Irvine, CA, USA) according to the manufacturer's instructions, and 1 µg was reverse transcribed using M-MLV reverse transcriptase (RT) (Promega). cDNA was used as template for qRT-PCR using iTaq Universal SYBR™ Green Supermix (Biorad) in a QuantStudio 5 real-time PCR system (Applied Biosystems; Waltham, MA, USA). Relative amounts of mRNA expression levels were calculated using the 2−ΔΔCt method5. Beta-2‑microglobulin, β-actin and hypoxanthine–guanine-phosphoribosyltransferase (HPRT) mRNA levels were analysed and used as endogenous controls. The sequences of the primers used to validate the RNA-seq results are listed in Table S6.

*Mice*

C7-hypomorphic mice (*Col7a1flNeo*) were generated at the University of Freiburg6. Heterozygous *Col7a1flNeo/WT* mice were crossed, and the newborn mice were genotyped by PCR to identify C7-hypomorphic mice (homozygous *Col7a1flNeo/flNeo* mice), as described6. C7 hypomorphic mice are very fragile and have a high mortality rate before weaning. To reduce mortality rate, animals were fed daily from day 10 on a special soft food diet prepared by dissolving powdered infant milk in water supplemented with Nutriplus Gel (Virbac; Carros Cedex, France),.

*HDACi administration and mice monitoring*

At weaning (19-22 days of age), male and female C7-hypomorphic mice were divided into the two experimental groups: 1) vehicle-treated (pastry food only); 2) VPA-treated. Sodium valproate (1.5 g/kg) was provided by adding it daily to the pastry food and monitoring that the amount of food provided was sufficient to sustain mouse growth and completely eaten throughout the day. To estimate the number of mice/experimental group (n=10), the minimum sample size was obtained with the two-sample test for equality of means7, with α = 0.05, β = 0.20. Treated mice were monitored daily for general health status and those showing significant weight loss (> 15% in one week) and/or signs of severe distress were euthanised. Mice were treated for 13 weeks up to 16 weeks of age. Mice were weighted and photographed once a week. Progression of digit length and forepaw mutilation was monitored and quantified using Image J. At the endpoint, mice were treated with sublethal doses of isoflurane inhalation and blood collected from the retroorbital sinus, then they were euthanised with higher doses of isoflurane. Dorsal skin biopsies were collected and snap frozen or fixed in 10% formalin and paraffin embedded. Forepaws, eyes, and tongue were also collected, formalin-fixed and paraffin-embedded.

*Analysis of overt phenotype in mice*

At the end of the treatment period, mice were photographed. Eyes, forepaws and haircoat condition were specifically analysed by two blinded observers, and an arbitrary severity score from 1 to 3 was assigned for each of the three parameters according to Table S4, with score 3 representing the most severe condition.

*Microscopic characterisation of mouse skin, eyes, and tongue*

Tissue samples were analysed by two blinded observers. The thickness of hypodermal and dermal skin tissue was measured on haematoxylin/eosin stained sections using an Axioplan2 microscope (Carl Zeiss) equipped with an Axiocam camera. At least 10 measurements were made along the tissue samples. As for tongue and eye analysis, epithelium was considered hyperplastic if at least 6 cell layers were counted. Sections stained with picrosirius red (Direct Red80; Sigma-Aldrich) were analysed with a polarisation filter, as described8.

*Proteomic analysis*

Skin specimens were homogenised in liquid N2 using a MM400 homogeniser (Retsch) and homogenates were dissolved in 4% SDS. 150 µg proteins were loaded onto Novex precast gels (ThermoFisher), gel lanes were cut into small pieces and proteins were digested in-gel using trypsin as described9. The resulting peptide mixtures were purified using STAGE tips and analysed by LC-MS/MS on an Exploris 480 mass spectrometer coupled to an EasyLC 1200 nanoflow-HPLC (all Thermo Scientific). Peptides were separated on a fused silica HPLC-column tip (I.D. 75 μm, New Objective, self-packed with ReproSil-Pur 120 C18-AQ, 1.9 μm (Dr. Maisch) to a length of 20 cm) using a gradient of A (0.1% formic acid in water) and B (0.1% formic acid in 80% acetonitrile in water): samples were loaded with 0% B at a flow rate of 600 nL/min; peptides were separated by 5%-30% B within 85 min with a flow rate of 250 nL/min. The spray voltage was set to 2.3 kV and the ion-transfer tube temperature to 250°C; no sheath and no auxiliary gas were used. The mass spectrometers were operated in the data-independent mode; after each survey scan (mass range m/z = 350 - 1,200; resolution: 120,000) 28 DIA scans with an isolation width of 30.4 m/z were performed covering a total range of precursors from 350-1,200 m/z. The AGC target value was set to 300%, resolution to 30,000 and normalised stepped collision energy to 25.5%, 27% and 30%. The MS raw files were analysed using the Spectronaut software version 16.1 and the directDIA+ workflow with standard settings using a Uniprot full-length mouse database and common contaminants such as keratins and enzymes used for digestion as reference.

Data analysis was performed by Perseus software, version 1.6.6.010. Briefly, normalised protein intensities were log2 transformed, filtered to 9 valid values, and pair-wise comparisons were performed using two sample Student`s T-tests (p<0.05, two-sided). Proteins that were significantly regulated in at least one pairwise comparison were z-score normalized and used for hierarchical clustering using Euclidean distance.

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**Supplementary figure legends**

*Supplementary figure 1. Dose- and time-response to Givinostat and VPA*

1. Toxicity assay showed no difference at all doses for the three time-points analysed (n =3).
2. Left: Representative images of collagen gel contraction after 72 hours of HDACi pre-treatment (patient #13). T0 corresponds to the time of gel detachment from the well. Red dots mark the collagen gel edge. Right: Histograms with values of collagen areas at 24 and 48 hours, expressed as percentage of the initial gel area (T 0), seeded with RDEB fibroblasts pretreated with Givinostat or VPA compared to vehicle-treated cells (CTRL). No differences were found between Givinostat 250 and 500 nM, while VPA 2 mM was more efficient in counteracting spontaneous gel contraction of cultured RDEB fibroblasts compared to VPA 1 mM at both 24 and 48 hours. (\*p<0.05).

*Supplementary figure 2. Contractile activity and α-SMA expression levels of fibroblasts from RDEB patient #13 treated with Givinostat and VPA*

1. Western blot for α-SMA in protein extracts of fibroblasts from the 5 RDEB patients. Patient #13 shows greatly reduced levels as compared to the other patients.
2. Top: Images of collagen lattice assay with fibroblasts of patient #13 untreated (CTRL) or pretreated for 72 hours with 250 nM Givinostat or 2 mM VPA, taken at 48 hours. Red dots mark the edge of the gel. Fibroblasts not treated with HDACi show strong contractile activity despite low levels of α-SMA expression. Bottom: Western blot for α-SMA in proteins extracted from collagen gels. Expression levels are low in the untreated cells and unchanged after treatment with HDACis.

*Supplementary figure 3. Global acetylation of histone 3 in RDEB fibroblasts after pan-HDACi treatment*

Left: Histogram with individual data obtained from an ELISA for acetylated histone 3 (Ac-H3) for 24 or 48 hours. Values are expressed as % of vehicle-treated fibroblasts, considered as 100%. (\*p<0.05; \*\*p<0.01; \*\*\*p<0.005). Right: Immunofluorescence for ac-H3 in RDEB fibroblasts treated with vehicle (CTRL) or Givinostat or VPA for 30 hours.

*Supplementary figure 4. KEGG analysis of differentially expressed genes in VPA- and Givinostat-treated cells*

The highest-ranked categories are shown according to the adjusted p-value (-10 log) (red: positively enriched; blue: negatively enriched). Categories with names highlighted in red or blue are those shared with Hallmark analysis.

*Supplementary figure 5. Histone acetylation is decreased in the skin of RDEB mice and VPA systemic administration increases histone acetylation levels*

1. Representative immunofluorescence for acetylated-histone 3 (Ac-H3) in the skin of a wild-type and an RDEB mouse. Staining is weaker in the nuclei of the RDEB skin specimen, in epidermis (E) and within the dermal compartment (D). Bars: 100 µm. Right: Histogram with values for staining intensity in skin specimens of RDEB mice and wild type (WT) littermates (A.U. = arbitrary unit).
2. Western blot for Ac-H3 and total H3 on proteins extracted from the skin of WT or RDEB mice. Right: Histogram with values of Ac-H3 band intensity as compared to total H3.
3. Schematic showing the timing of VPA treatment and analyses. Mice were fed with 1.5 g/kg of VPA daily in the diet for 13 weeks. As control treatments, water was used for VPA and 0.0005% DMSO for Givinostat. Mice were weighed and photographed weekly until 16 weeks of age, when they were sacrificed.
4. Western blot for Ac-H3 and total H3 on proteins extracted from the skin of wild-type, vehicle-treated (CTRL) or Givinostat-treated RDEB mice. Right: Histogram with values of acetylated H3 band intensity as compared to total H3 in wild-type, vehicle-treated (CTRL) and Givinostat-treated RDEB mice.
5. VPA levels in the blood of VPA-treated RDEB and vehicle-treated (CTRL) mice, 4 hours or 24 hours after drug administration.
6. Western blot for Ac-H3 and total H3 on proteins extracted from the skin of wild-type mice, and vehicle-treated (CTRL) or VPA-treated RDEB mice. Right: Histogram with values of acetylated H3 band intensity as compared to total H3 in wild-type (WT), vehicle-treated (CTRL) and VPA-treated RDEB mice.
7. Representative immunohistochemistry for Ac-H3 in the skin of a wild-type mouse, and RDEB mice, treated with vehicle or VPA for 13 weeks. Nuclear staining is weaker in the skin of vehicle-treated RDEB mice compared to both wild-type mice and VPA-treated RDEB ones. Bars: 50 µm. Right: histogram with values of brown staining intensity (diaminobenzidine, DBA). (A.U. = arbitrary unit).

(\* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.005)

*Supplementary figure 6. Systemic antifibrotic effect of VPA administration*

1. Representative histological images of mouse tongue showing loss of filiform papillae (arrowheads) with epidermal hyperplasia in vehicle-treated RDEB mice. Residual filiform papillae are found in VPA-treated RDEB mice, while they are almost absent in the tongue of vehicle-treated mice. Bars: 100 µm.
2. Histogram with the percentage of vehicle-treated (CTRL) or VPA-treated RDEB mice showing presence of filiform papillae, blister formations, and epithelial hyperplasia.
3. Immunofluorescence for TGF-β in tongue sections showing a stronger and more diffuse signal in the tongue of vehicle-treated RDEB mice (CTRL) compared to wild-type and VPA-treated RDEB mice. Bars: 100 µm. Right: Histogram with individual and mean values for TGF-β immunofluorescence intensity in tongue specimens. Values are expressed as arbitrary units (A.U.)
4. Representative histological images of the corneal tissue showing ulceration (arrow) and blistering which are more diffuse in vehicle-treated RDEB mice as compared to VPA-treated mice. Bars: 100 µm.
5. Histogram showing the percentage of mice with corneal tissue abnormalities.
6. Immunofluorescence for TGF-β in corneal sections showing a stronger and more diffuse signal in vehicle-treated RDEB mice. Bars: 50 µm. Right: Histogram with individual and mean values for TGF-β immunofluorescence intensity expressed as arbitrary units (A.U.).

(\*p < 0.05; \*\* p < 0.01; \*\*\* p < 0.005)

*Supplementary figure 7. Proteomic analysis of VPA-treated mouse skin*

1. Heatmap of proteins differentially expressed in the three experimental groups: wild-type (WT), vehicle-treated (CTRL) and VPA-treated RDEB mice. Differences in protein levels identify 5 clusters indicated by bars of different colours on the left. The major pathways within each cluster are shown on the right.
2. Pathways belonging to cluster 1 and cluster 3, those for which expression levels are similar in VPA-treated RDEB mice and wild-type mice.
3. Main cluster 1 network related to ribosomal proteins, downregulated in VPA-treated mice compared to vehicle-treated mice.
4. Cluster 3 network comprising genes involved in the immune response, upregulated in VPA-treated mice compared to vehicle-treated mice.

**Supplementary tables**

*Table S1. RDEB and control skin specimens used for in vivo histone acetylation analysis*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Patient #** | **Age at biopsy (Sex)** | **Biopsy Site** | **RDEB subtype** | **Mutations (consequences)** | **COLVII expression \*** |
| R1 | 30 (M) | Right arm | Intermediate | c.425A>G (altered splicing) / c.7344G>A (altered splicing) | Reduced |
| R2 | 12 (F) | Right arm | Intermediate | c.4448G>A / c.4448G>A (p.Gly1483Asp) | Strongly reduced |
| R3 \*\* | 41 (F) | Left arm | Inversa | c.8323G>A (p.Gly2775Ser) / c.7651G>C (p.Gly2551Arg) | Reduced |
| R4 \*\* | 41 (M) | Left arm | Localized | c.8323G>A (p.Gly2775Ser) / c.7651G>C (p.Gly2551Arg) | Reduced |
| R5 | 34 (F) | Right arm | Localized | c.425A>G (altered splicing) / c.7344G>A (altered splicing) | Reduced |
| R6 | 19 (F) | Right leg | Localized | c.425A>G (altered splicing)/ c.4402-16A>G (altered splicing) | Slightly reduced |
| R7 | 39 (M) | Left iliac crest area | Intermediate | c.806delT p.Leu269Arg*fs*\*15) / c.6074G>C (p.Gly2025Ala) | Slightly reduced |
| R8 | <1 (F) | Right subscapularis area | Severe | c.3840delC (p.Gly1281Val*fs*\*44) / c.6751-1G>T (altered splicing) | Absent |
| R9 | 50 (M) | Right arm | Pruriginosa | c.3355C>T (p.Gln1119\*)/ c.5820G>A (altered splicing) | ND |
| R10 | 49 (F) | Right buttock | Inversa | c.6206G>T (p.Arg2069Leu) / c.7069-25A>G (altered splicing) | Reduced |
| R11 | <1 (F) | Left leg | Severe | c.4351delG (p.Glu1451Arg*fs*\*259) / c.2314+5G>C (altered splicing) | Absent |
| C1 | 18 (F) | Thorax | N.A. | N.A. | Normal |
| C2 | 35 (?) | Abdomen | N.A. | N.A. | Normal |
| C3 | 40 (F) | Abdomen | N.A. | N.A. | Normal |
| C4 | 69 (F) | Right arm | N.A. | N.A. | Normal |
| C5 | 44 (?) | Right arm | N.A. | N.A. | Normal |

\* Type VII collagen expression in biopsy of perilesional skin (immunofluorescence antigen mapping); \*\* dizygotic twins. N.A. = not applicable; N.D. = not determined

*Table S2. RDEB and non-RDEB primary fibroblasts used in the study*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Patient #** | **Age (sex)** | **Biopsy Site** | **RDEB subtype** | **Mutations (consequences)** | **COLVII expression \*** |
| R2 | 12 (F) | Right arm | Intermediate | c.4448G>A / c.4448G>A (p.Gly1483Asp) | Strongly reduced |
| **R3** \*\* | 41 (F) | Left arm | Inversa | c.8323G>A (p.Gly2775Ser) / c.7651G>C (p.Gly2551Arg) | Reduced |
| R4 \*\* | 41 (M) | Left arm | Localized | c.8323G>A (p.Gly2775Ser) / c.7651G>C (p.Gly2551Arg) | Reduced |
| **R7** | 39 (M) | Left iliac crest area | Intermediate | c.806delT (p.Leu269Arg*fs*\*15) / c.6074G>C (p.Gly2025Ala) | Slightly reduced |
| R12 | 17 (F) | Not reported | Severe | c.425A>G (altered splicing) / c.7369delAAAG (p.Lys2457Glu*fs*\*8) | Absent |
| **R13** | 29 (M) | Not reported | Localized | c.267-1G>C (altered splicing) / c.5096C>T (p.Pro1699Leu) | Altered and reduced |
| R14 | 35 (F) | Left buttock | Intermediate | c.3986G>T (p.Gly1329Val) / c.3986G>T (p.Gly1329Val) | Normal |
| **R15** \*\*\* | 30 (M) | Left forearm | Intermediate | c.4965C>T (altered splicing) / c.8117delC (p.Pro2706Gln*fs*\*80) | Reduced |
| R16 \*\*\* | 30 (M) | Left forearm | Localized | c.4965C>T (altered splicing) / c.8117delC (p.Pro2706Gln*fs*\*80) | Reduced |
| R17 | 24 (M) | Left wrist (Abdomen) | Intermediated | c.8505dupC (p.Val2836Arg*fs*\*13) / c.6831+3A>G (altered splicing) | Slightly reduced |
| **R18** | <1 (F) | Not reported | Intermediated | c.497dupA (p.Val168Gly*fs*\*12) / c.4965C>T (altered splicing) | Reduced |
| R19 | 50 (M) | Right arm | Pruriginosa | c.3355C>T (p.Gln1119\*) / c.5820G>A (altered splicing) | N.D. |
| R20 | 2 (M) | Left buttock | Severe | c.553C>T (p.Arg185\*) / c.553C>T (p.Arg185\*) | Absent |
| R21 | 13 (M) | Left buttock | Severe | 8441-15del20 (altered splicing) / 8505dupC (p.Val2836Arg*fs*\*13) | Extremely reduced |
| C6 | 27 (M) | Not reported | N.A. | N.A. | Normal |
| C7 | 46 (M) | Left shoulder | N.A. | N.A. | Normal |
| C8 | 34 (F) | Right pectoral | N.A. | N.A. | Normal |
| C9 | 26 (F) | Breast | N.A. | N.A. | Normal |
| C10 | 31 (F) | Left lumbar | N.A. | N.A. | Normal |
| C11 | ? | ? | N.A. | N.A. | Normal |
| C12 | 42 (F) | Abdomen | N.A. | N.A. | Normal |
| C13 | 10 (M) | Foreskin | N.A. | N.A. | Normal |

*Table S3. Characteristics of RDEB mice treated with vehicle (CTRL) or VPA*

For severity score, the following parameters were considered: weight (0, > 8 g; 1, between 4 and 8 g; 2, <4 g) and forepaw appearance (0, normal digit without oedema; 1, initial digit contraction; 2, digit loss, digit contraction and/or oedema). The first day of treatment is reported (days).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Ctrl #** | **Sex** | **Days** | **Grams** | **Gravity** | **VPA #** | **Sex** | **Days** | **Grams** | **Gravity** |
| **165** | F | 20 | 5.4 | 2 | **166** | M | 20 | 3.8 | 4 |
| **168** | F | 19 | 7.0 | 1 | **171** | M | 21 | 7.4 | 1 |
| **170** | F | 21 | 4.0 | 3 | **172** | F | 21 | 7.3 | 1 |
| **177** | F | 20 | 8.4 | 0 | **174** | F | 21 | 5.3 | 1 |
| **185** | F | 22 | 6.0 | 1 | **179** | F | 19 | 5.5 | 3 |
| **190** | M | 22 | 9.1 | 0 | **183** | F | 22 | 6.5 | 1 |
| **195** | M | 21 | 7.1 | 2 | **187** | F | 22 | 8.5 | 0 |
| **196** | F | 21 | 7.1 | 2 | **193** | M | 21 | 6.6 | 2 |
| **197** | M | 21 | 7.2 | 2 | **198** | F | 21 | 7.5 | 1 |
| **200** | F | 21 | 7.2 | 1 | **199** | F | 21 | 6.3 | 1 |
| **229** | M | 21 | 3.3 | 3 | **227** | M | 21 | 9 | 2 |
|  |  |  |  |  | **230** | F | 21 | 5.4 | 2 |
| **Sex** | 9F/7M |  |  |  | **Sex** | 12F/4M |  |  |  |
| **Mean** |  | **20.9** | **6.2** | **1.69** | **Mean** |  | **20.8** | **6.3** | **1.63** |
| **SD** |  | 0.9 | 1.7 | 1.20 | **SD** |  | 0.9 | 1.5 | 1.02 |

*Table S4. Parameters used to evaluate the gravity score at the endpoint*

|  |  |  |  |
| --- | --- | --- | --- |
| **Score** | **Hair coat** | **Corneal opacity** | **Mitten deformities** |
| **1** | Normal | No | No |
| **2** | Sparse fur coat/small area of alopecia | One eye | One forepaw |
| **3** | Large areas of alopecia | Both eyes | Both forepaws |

*Table S5. List of antibodies*

(WB,Western blot; IF, immunofluorescence; IHC, immunohistochemistry)

|  |  |  |  |
| --- | --- | --- | --- |
| **Antibody** | **Host and Code** | **Company** | **Application, dilution** |
| α-SMA | Mouse, A2547 | Sigma-Aldrich | WB, 1:1000  IF, 1:100 ICF, 1:200 |
| Acetylated α-tubulin | Rabbit, 5335 | Cell Signaling Technology | WB, 1:1000 |
| α-tubulin | Mouse, sc-5286 | Santa-Cruz Biotechnology | WB, 1:1000 |
| β-tubulin | Mouse, T4026 | Sigma-Aldrich (Merck) | WB, 1.1000 |
| β-catenin | Mouse, 610153 | BD Transduction Laboratories | IF, 1:100 |
| Hystone 3 | Rabbit, 9715 | Cell Signaling Technology | WB, 1:1000 |
| Acetylated Hystone H3 | Rabbit, 9677 | Cell Signaling Technology, | WB, 1:1000 IF, 1:400 IHC, 1:400 |
| Ki67 | Rabbit, MA5-14520 | Termo Fisher Scientific | IHC, 1:100 |
| Tenascin-C | Mouse, NB110-68136 | Novus Biological | IF, 1:50 |
| TGF-β | Mouse, sc-130348 | Santa-Cruz Biotechnology | IF, 1:50 |
| Vinculin | Mouse, sc-73614 | Santa-Cruz Biotechnology | WB, 1:1000 |

*Table S6. List of primers used for validation of RNA-seq*

|  |  |  |
| --- | --- | --- |
| **Gene** | **Forward (5’-3’)** | **Reverse (5’-3’)** |
| *AURKA* | TTGGAAGACTTGGGTCCTTG | TGGAGCTGTAGCCTTAACAGG |
| *CDK1* | CTTGGCTTCAAAGCTGGCTC | GGGTATGGTAGATCCCGGCT |
| *CDK3* | GCTTCCCTAAGTGGACCAGG | TCATACTGCAGGAGTTGCATGA |
| *COL5A1* | TGCTGCTTCTCCTGATCTGC | CAGATCCTCTCCCTCCCGAA |
| *DKK1* | TGACAACTACCAGCCGTACC | CAGGCGAGACAGATTTGCAC |
| *DNMT1* | GGCGGCTCAAAGATTTGGAA | CAGGTAGCCCTCCTCGGATA |
| *DSG2* | CCTGACAAGAAGTCCGAAGGG | CGTCCACATAGGAGAGCAGTTT |
| *JUP* | CGGATGGTGTGAGGATGGAG | CGAGTACAGGAGCTGCACAA |
| *ITGA6* | AACGGGCTCATTCAGCGG | CGACAGGTAGAGCAAGCACA |
| *LAMA3* | TTATCCATCCTGGGGTGCTG | AGGGACAGTGACAAAGGCTG |
| *LAMB3* | TTGTACCCAGTTTGCTTTGCTG | CCAGGCAGGGCAAAACAC |
| *NFKB* | AGAAGATGGGAAAGGGCTGC | TCGCAATCTTCCATTCCAATAGT |
| *NKD1* | CTTTCGGCTGGAAGTGGC | GTCGCACTGGAGCTCTTCAA |
| *P53* | TCAACAAGATGTTTTGCCAA | ATGTGCTGTGACTGCTTGTA |
| *PCNA* | CAAGTAATGTCGATAAAGAGGAGG | GTGTCACCGTTGAAGAGAGTGG |
| *SMAD3* | GCCTGCTGGGCTGGA | TCCAGGGACCTGGGGAT |
| *TGFB2* | CTTCCCCTCCGAAACTGTCTG | TCAAGGTACCCACAGAGCAC |