Skin Pharmacology and Physiology
Supplementary Information

Dehydroabietic acid induces regeneration of collagen fibers in ultraviolet B-irradiated human dermal fibroblasts and skin equivalents

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This supplementary information contains supplementary methods and three figures.

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Supplementary Methods

Quantitative real-time polymerase chain reaction (RT-qPCR)

Total RNA was extracted from NHDFn using the RNeasy Mini Kit (#74101, Qiagen GmbH, Hilden, Germany). cDNA was synthesized using SuperScript® III First-Strand Synthesis System (#18080-051, Thermo Fisher Scientific) according to the manufacturer's instructions. For quantitative real-time PCR, we used the ABI 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA) with Taqman Universal Master Mix II (#4364340, Applied Biosystems) and predesigned Taqman primers for *COL1A1* (Hs00164004_m1), *COL3A1* (Hs00943809_m1), *COL4A1* (Hs00266237_m1), *MMP1* (Hs00899658_m1) and *RPLP0* (#4333761F, Applied Biosystems) genes. The level of relative mRNA gene expression was quantified using the comparative $\Delta\Delta C_t$ analysis method and normalized against one of the housekeeping genes (*RPLP0* gene). The *p*-values (*, $p < 0.05$; **, $p < 0.01$) were determined by analyzing triplicate samples.

Human skin equivalent models

Skin equivalents (SEs) were prepared according to our previously described methods. Briefly, the dermal layer was made by mixing type I collagen (Advanced Biomatrix, San Diego, CA, USA) with human dermal fibroblasts (6.0×10^4 cells/well) in a medium cocktail [DMEM, F12, $NaHCO_3$ and NaOH]. The mixture was added to each of the 12 mm Snapwell cell culture inserts (Corning Costar, Corning, NY, USA) and incubated for 2 h at 37°C to allow polymerization. The dermal layer was then cultured in 106 media (Cascade Biologics, Portland, OR, USA) supplemented with 100 μg/ml ascorbic acid (Sigma-Aldrich) and allowed to contract for 7 days at 37°C and 5% CO_2. Human epidermal neonatal keratinocytes (2.0×10^5 cells/well) were seeded on the dermal layer. The SEs were cultured for 1 day in EpiLife media and for 1 day in 3D culture media, CnT-3D-PR (CellNTEC, Bern,

Switzerland); next, the SEs were fed strictly from the bottom in a 3D culture media and the surface was exposed to air to promote the epidermal differentiation for 14 days.

Supplementary Figure

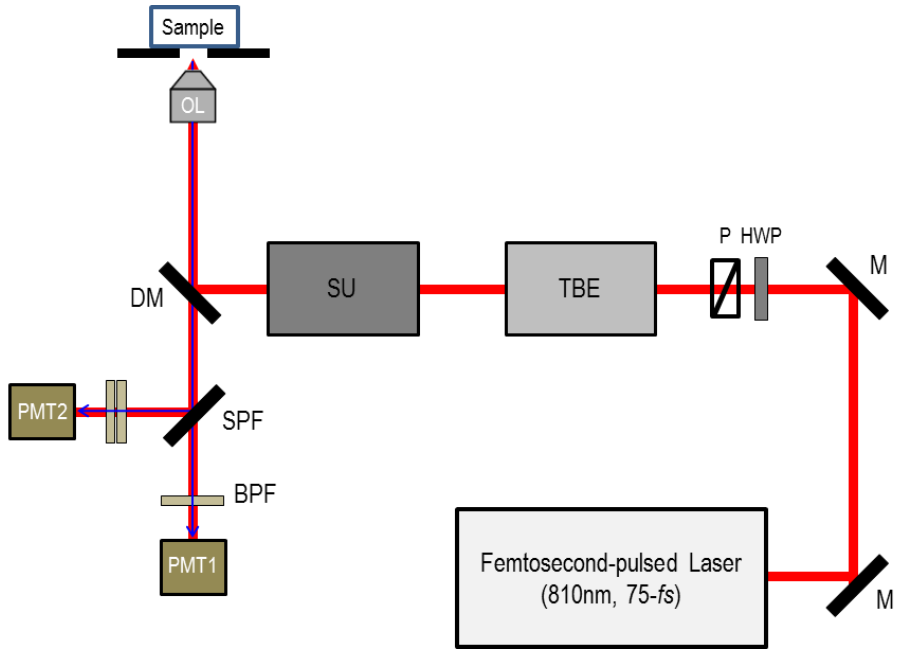

Fig. S1. Schematic diagram of optical setup of a nonlinear optical microscopy. Blue arrows indicate backward signals emitted from the samples. HWP: half-wave plate, P: polarizer, TBE: telescope beam expander, SU: scanning unit, OL: objective lens, DM: dichroic mirror, SPF: short-pass filter, BPF: bandpass filter, and PMT: photomultiplier tube.

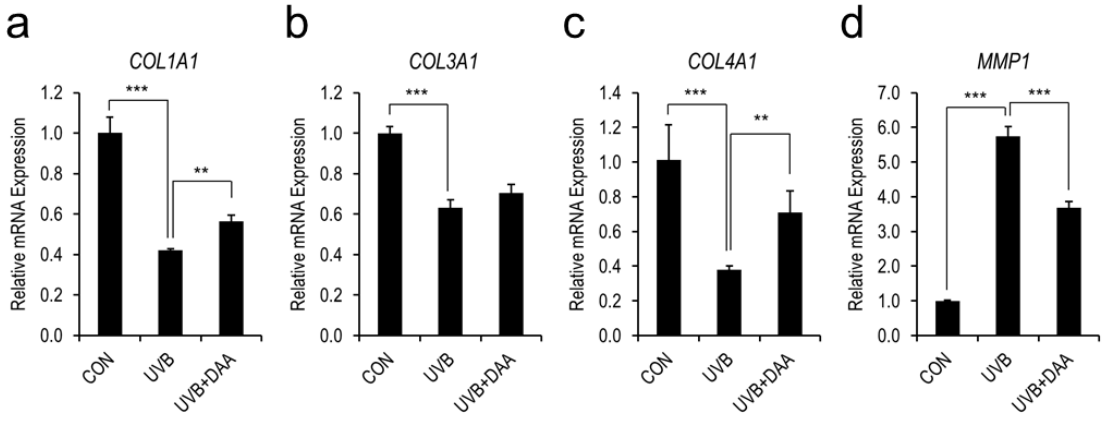

Fig. S2. NHDFn were irradiated with UVB (25mJ/cm^2) and treated with DAA (10 μM) for 18 h. Relative mRNA levels for *COL1A1* (a), *COL3A1* (b), *COL4A1* (c), and *MMP-1* (d) genes were quantified by real-time PCR analysis (n=3 per group). **, $p < 0.01$; ***, $p < 0.001$

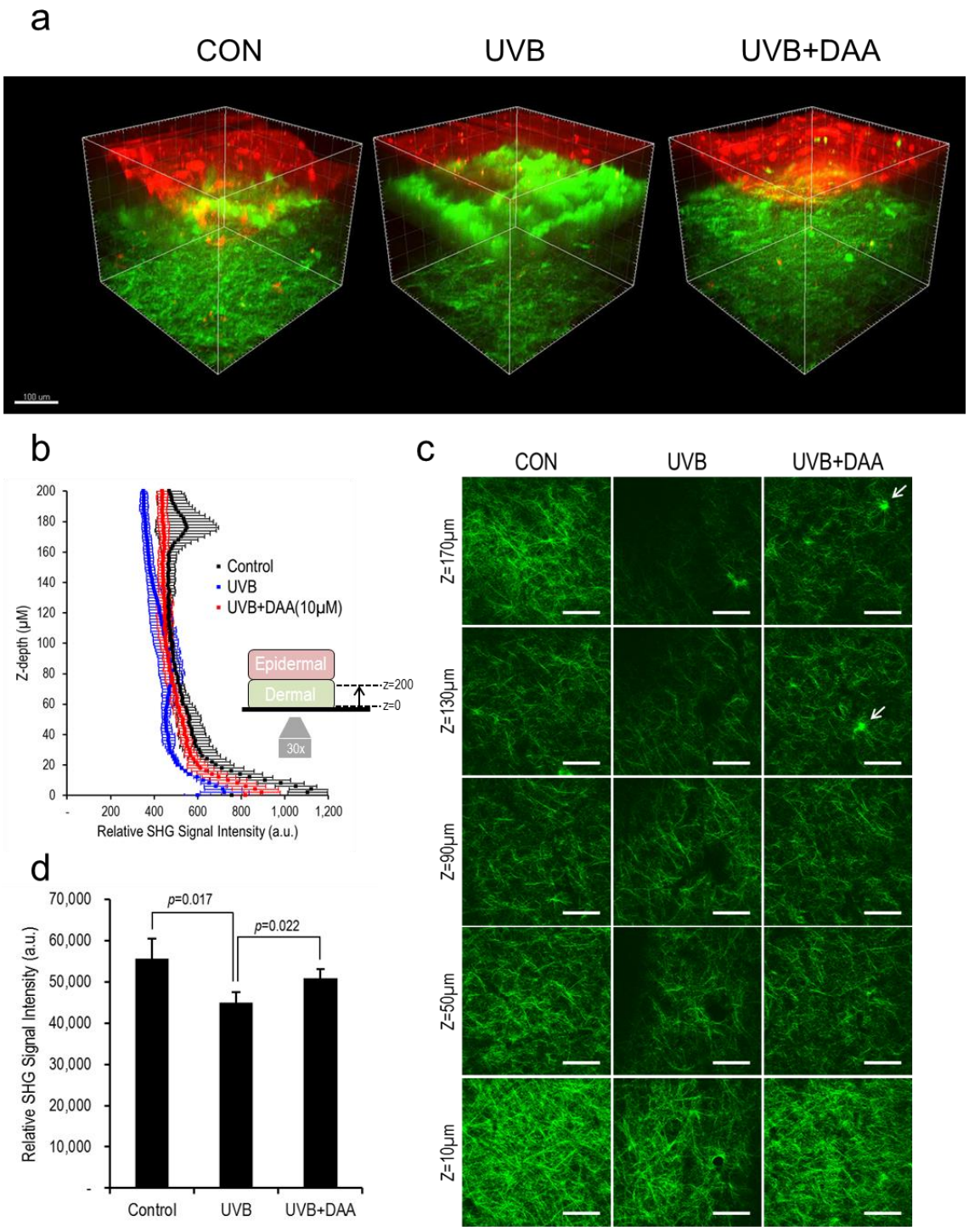

Fig. S3. DAA promotes the synthesis of collagen fibrils in the UVB-irradiated human skin equivalent. (a) Nonlinear optical imaging of 3D full-thickness human skin equivalents including the epidermis and the dermis. Epidermal and dermal sides of skin equivalents were separately measured and then were three dimensionally merged. The total measurement

volume is 400 (x) × 400 (y) × 400 (z) μm^3. The red and green colors indicate TPEF signals for autofluorescence in the SC layer and SHG signals for collagen in the dermal layers, respectively. (B) Z-depth profiling of SHG signal intensities ranged from z=0 μm on the bottom end to z=200 μm (n=3 per group). (C) XY-axis SHG image gallery at different z-depths. White arrows indicate collagen-rich regions. Scalebars; 100 μm. (D) Relative SHG signal intensities for measurement volume, 400 (x) × 400 (y) × 200 (z) μm^3.