Supplementary Materials for

Human organ rejuvenation by VEGF-A: Lessons from the skin

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**Introduction**

We used immunocompromised young mice for interrogating human tissue rejuvenation by xenotransplanting these animals with aged human skin as an accessible and clinically relevant model for elucidating fundamental molecular principles of human organ rejuvenation *in vivo*.

**Results**

**Maintenance of old human skin xenotransplants in an old SCID/beige mouse environment**

Four weeks after engrafting aged human skin (Table S1, Fig. 1) onto 14±3.2 month-old male and female SCID/beige mice, the aged epidermis phenotype was fully maintained (11) (Figs. 2, 3), namely, thin epidermis including decreased stratum corneum, granulosum and spinosum, a marked flattening of the dermo-epidermal junction, a decreased number of blood vessels, reduced thick collagen bundles, increased epidermal p16<sup>INK4A</sup> and reduced NRF2 expression (Figs. 2, 10 and Fig. S1).

**Rejuvenation of old human skin xenotransplants beyond the epidermis**

The rejuvenation of aged human skin *in vivo* provides the first evidence that the rejuvenation process after transplanting old human skin into a young mouse environment, both on the levels of human skin morphology and key molecular skin aging markers, extends well beyond the epidermis: it impacts dermal collagen production and is notably associated with substantial dermal angiogenesis. The latter originates from human blood vessel-associated cells in the skin xenotransplants themselves.

**VEGF treatment improved skin aging read-outs in organ-cultured aged human skin**

The present study demonstrates that VEGF-mediated signaling is not only required but is also sufficient for human skin rejuvenation *in vivo*. However, our study cannot yet clarify definitively whether VEGF A-driven angiogenesis (a) has senolytic or rejuvenating effects *per se*, or whether (b) it mainly improves tissue perfusion and thus makes aged human skin more accessible to rejuvenating systemic factors provided by a young, but not an old mouse environment. Option (a) would be clinically very attractive, as VEGF or VEGF mimetics could then be used directly as senolytic agents, while option (b) would make it critical to identify the responsible systemic mouse-derived factor[s] and test their direct rejuvenation effects on aged human skin. While this needs to be clarified in follow-up studies, we have attempted here to begin exploring these possibilities by using organ-cultured aged human skin.
Discussion

Our findings that VEGF-A-mediated signaling is both required and sufficient to promote the rejuvenation of aged human skin in vivo are well in line with the “angiogenesis hypothesis of aging,” which postulates that the reduced capillary density and a decline in angiogenic factor production may underlie physiological intrinsic tissue aging and that tissue aging may therefore be reversed by angiogenesis-promoting therapy (66). For example, mice with an accelerated aging syndrome (klotho) present impaired ischemia-induced neovascularization in vivo and marked skin atrophy, (81) further supporting a central role of angiogenesis in skin aging.

However, the angiogenesis hypothesis of aging (66) remains to be conclusively supported by functional and mechanistic data in a major human organ in vivo. The current study not only closes this gap, notably in one of the largest organs of the human body (skin), but also identifies a key role for a single, well-defined pro-angiogenic growth factor, VEGF-A protein, whose coding gene represents a “gerosuppressor” gene (82). This also suggests that much advocated stem cell-based anti-aging strategies (83,84) may be dispensable if well-chosen molecular signaling pathways for human tissue rejuvenation are selectively targeted pharmacologically.

Our findings raise the pertinent question whether VEGF-A or VEGF-A mimetic drugs could be a novel candidate therapy for aging-related human syndromes beyond the reversal of human skin aging. However, important safety and toxicology concerns with this approach must be overcome before clinical pilot studies can be justified. For example, besides their long-recognized role in cancer and multiple other human pathologies (49,75,76), VEGF overexpression may promote UVB-induced skin photodamage (73), psoriasis and its associated cardiovascular defects (85,86), and increased VEGF levels are associated with human senile cataracts and age-related macular degeneration (87). Furthermore, VEGF overexpression in young mice leads to an epidermal hyperplastic phenotype reminiscent of psoriasis and premalignant transformation (88,89), and skin papillomas rely on VEGF to establish a niche and stimulate cancer stemness and renewal (90).

Finally, the rejuvenating properties of VEGF may well be tissue-, dose-, timing- and signaling context-dependent.

For human skin rejuvenation purposes, it would therefore appear to be the most prudent strategy to develop topically applicable VEGF-A mimetics with minimal systemic absorption, and to restrict this therapy to dermatologically closely monitored individuals who resort to adequate sun protection, do not have psoriasis, and do not belong to a skin cancer high-risk group.
Materials and Methods

Human skin xenotransplants into SCID/beige mice
Following ethics approval and informed consent, 1-cm² skin samples of split-thickness (0.4 mm) were obtained from non-UV-exposed areas of the upper thighs. Adult and aged volunteers of both genders (Table S1) were included in the study. Some skin samples were snap-frozen in liquid nitrogen/fixed in 10% saline-buffered formalin overnight and reserved for histological stains. The majority of viable human skin samples were xenotransplanted onto old (OiO) vs. young (OiY) (Table S1) severe combined immunodeficiency SCID/beige mice, as described. Similarly, skin samples of young volunteers were xenotransplanted onto old mice (YiO) (9,10).

Histology, immunohistochemistry (IHC) and immunofluorescence (IF) microscopy of skin before and 30 days post-transplantation
The human skin xenotransplants were harvested and either snap-frozen in liquid nitrogen or fixed in 10% saline-buffered formalin overnight, followed by 70% ethanol. Sections were stained with hematoxylin and eosin, masson fontana, β-Galactosidase (cell signaling), modified picrosirius red procedure (91) using immunohistochemistry (IHC) and immunofluorescence (IF) microscopy (for more details, see Tables S4 and S5).

Quantitative (immuno-) histomorphometry
Expression of the following aging-related marker proteins was investigated by histology, immunohistochemistry or immunofluorescence microscopy in human skin samples obtained before and 30 days post-transplantation, studying these aging-associated read-out parameters as described (Fig. 1, D) (11,16).

Human skin xenotransplants were examined before and 30 days post-transplantation (for details on primary and secondary antibodies, controls and explanations, see Tables S4 and S5), and assessed quantitatively in stringently defined reference areas. Image analysis was performed using Image J software. Staining coverage within the epidermis was measured, calculating density per pixel (%). Picrosirius red staining for thin and thick filaments ratio of collagen fibers was analyzed using polarizing microscopy. As fiber thickness increases, the color changes from green-yellow to orange-red (91).
Intradermal injections of growth factor-neutralizing antibodies, small molecule inhibitors, or VEGF-loaded nanoparticles

One month after transplantation, OiY mice were injected intradermally with either anti-VEGF-A antibody or isotypic controls (Table S2), or anti-HGF/IGF1 antibodies and goat IgG as isotype control. Alternatively, the small molecule SB431542 (TGFβ inhibitor) (78) was injected. Each group included 11 OiY mice transplanted with skin from six human donors (age range 77-83 years, mean 81).

Alternatively, poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) were used for the long-term sustained release of VEGF-A protein since PLGA NPs have been used extensively and have FDA approval as a vehicle for drug delivery (60). NPs containing VEGF-A protein or bovine serum albumin (BSA) (0.1 µg/mg of NPs) were prepared using the double-emulsion solvent evaporation technique (92). The PLGA NP average size was 700 nm, as measured by the dynamic light scattering (DLS) technique (60). The release profile was 40%, 60% and 70% after 6, 13 and 21 days, respectively, measured by the micro bicinchoninic acid (BCA) protein assay kit (data not shown). Thirty-two old mice (mean age 13 months) were transplanted with skin from three aged donors (age range 80-92 years, mean 86). The protein-loaded nanoparticles (Table S3) were administrated intradermally on days 6 and 15 post-engraftment.

In situ hybridization

OCT-embedded skin pieces were sectioned at 7 µm, dried at −20°C for 1 hour, stored at −80°C, and processed for RNA in situ detection by Single-plex RNAscope® Fast Red Assay (Advanced Cell Diagnostics, Newark, CA, USA) following the manufacturer’s instructions. The following probes were used: mouse VEGF-A mRNA custom-designed by Advance Cell Diagnostics to not have cross-reactivity with human VEGF-A mRNA (NM_001110266.1, region: 1029-2435); human VEGF-A mRNA designed to not have cross-reactivity with mouse VEGF-A mRNA (NM_001171623.1, region:1033-2760); human PPIB as a positive control (NM_000942.4, region 139-989); and mouse PPIB as a positive control (NM_011149.2, region: 98-856), DapB as a negative control (EF191515, target 414-862).

Human skin organ culture

Full-thickness human skin organ culture samples (two females, age 76 and 81, 4-mm diameter punch biopsies) were generated and cultured at the air-liquid interface in serum-free Williams’ E medium supplemented with glutamine, insulin and hydrocortisone as described (56,64). Alternatively, the epidermis was separated from the dermis using 2N sodium bromide and
cultured for six days as described above. After a 24-hour preincubation period, the skin was divided randomly into the following treatment groups: Vehicle (William’s E medium); anti-VEGF-A at 0.92 nmol (G6-31, Genentech Inc); VEGF-A protein at 0.1µg/1ml (GenScript); and BSA at 0.1µg/1ml (Sigma-Aldrich).

For the six-day incubation, medium and treatments were changed every second day.

**Serum cortisol measurement**

Serum from six young mice (2 months) and six old mice (14 months) was obtained and analyzed immediately for serum-free cortisol using the Immulite analyzer and DPC kits. Cortisol serum levels were measured by the Laboratory Animal Department, AML, Israel. Cortisol was investigated given its well-recognized use in mammalian tissue aging (93,94).

**Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analyses**

Total RNA was extracted using the miRNeasy Micro Kit (Qiagen, Germany) according to the manufacturer’s protocol. RNA concentration was determined using the Qubit RNA BR assay kit, and RNA quality was measured using the Agilent RNA 6000 Nano Kit compatible with the Agilent 2100 Bioanalyzer. Samples with a RIN value greater than 6.5 were used for qRT-PCR. Reverse transcription was carried out with the High-Capacity RNA-to-cDNA Kit (Applied Biosystems) according to the manufacturer’s protocol using 250 ng of RNA from each sample. One hundred ng of cDNA from each sample was pre-amplified using TaqMan PreAmp Master Mix (Applied Biosystems), two assay pools were made and pre-amplified samples were used for quantitative-PCR in 10 µl of final reaction volume according to the Applied Biosystems protocol (PN4384557).

**RNA-Seq transcriptomics analyses**

Twelve young mice (2.5±0.4 months) were transplanted with skin from two male donors (mean age 82±7). RNA-Sequencing (RNA-Seq) technology was employed after the first, second and fourth weeks following engraftment. Approximately 1 cm² of skin tissue was taken with sterile forceps and quickly cut using sterile scalpel blades. Skin was then stored in RNealater (Thermo Fisher Scientific, USA) for RNA extraction. Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer’s protocol. RNA concentration and quality were determined using a 4200 TapeStation Instrument (Agilent). RNA quantity and quality were measured using a NanoDrop ND-8000 spectrophotometer. Samples with a RIN value greater than 8.0 were used for RNA-Seq. The library preparation was done with the Epicenter ScriptSeqTM
Complete Kit (Human/Mouse/Rat). Sequencing was performed using the Illumina HiSeq 2500 platform at the Technion Genome Center, Infrastructure Unit – Life-Sciences and Engineering (Haifa, Israel). The Illumina TruSeq RNA sample preparation kit (Illumina) was used according to the manufacturer’s protocol.

Raw data quality values were performed using phred+33. Recipe: PE 100bp X 2 and barcode.

**Computational biology analysis**

For details of the RNA-Seq transcriptomics analyses and the subsequent computational biology analysis, RNA-Seq data processing was subjected to Stable clustering of time series trajectories (SCTST) and transcriptomics data post-processing.

The data discussed in this publication were deposited in NCBI’s Gene Expression Omnibus and are accessible through GEO Series accession number GSE138249 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE138249).

**RNA-Seq data processing**

We aligned the raw data to the GRCm38 genome using Tophat and calculated the Fragments Per Kilobase of transcript per Million mapped reads (FPKM) using Cufflinks and in-house software in Python 2.7. Variance stabilization was performed using log2 scaling. To calculate the differentially expressed genes (DEGs), we used a fold change threshold $\Theta_{DEG} = 2$ in log2 scale and a significance level $\alpha_{DEG} = 0.001$. The Gene Ontology (GO) analysis was performed using the molecular signatures taken from the gene set collection C5 of version 3.0 of the Molecular Signatures Database (MSigDB). The significance of the gene set of the DEGs was analyzed using an enrichment approach based on the hypergeometric distribution. The significance ($p$ value) of the gene set enrichment was calculated using the hypergeometric distribution at a significance level $\alpha_{GO} = 0.05$.

**Stable clustering of time series trajectories (SCTST)**

To ensure the clustering stability of the time series trajectories, we designed a new time series clustering algorithm called SCTST. The SCTST algorithm is based on the establishment of a set $\{S\}$ of seed trajectories. Each seed trajectory $s \in \{S\}$ is defined to have a discretized value $v$ at each time point $t$. For the analyzed dataset, we selected four discretized values equally distributed within the range of variation of the normalized transcriptomics signal. We codified such values as $v \in \{0,1,2,3\}$. Since we produced four time point samples $t \in \{\text{Pre},1\text{w},2\text{w},4\text{w}\}$, there are $4^4 = 256$ combinations of possible seed trajectories (pre corresponds to the pre-transplantation and $i$w to
the $i$ week stage). Thus, each seed $s$ trajectory is codified with $v_s = \{v_i, v_j, v_k, v_l\} \subseteq \{0, 1, 2, 3\}$. After filtering out uniform data trajectories, we populated the seed trajectories $s$ by calculating the distance of the remaining data trajectories to the seed trajectories and assigned the closest (in the Euclidean metric sense) seed trajectory to each data trajectory. After filtering out non-populated seed trajectories, 76 trajectory clusters remained. Next, we performed a hierarchical clustering of the remaining trajectories using the Ward method (inner squared distance or minimum variance algorithm). For selecting the clusters, we applied a cutoff $\Theta_{\text{Cut}} = 1.6$, thus clusters were formed when a node and all of its sub-nodes had inconsistent value $< \Theta_{\text{Cut}}$, resulting in a final total of six trajectory clusters.

**Transcriptomics data post-processing**

Hierarchical clustering of genes and samples was performed with one minus correlation metric and the unweighted average distance (UPGMA) (also known as group average) linkage method. Finally, we calculated the prototype representative of each trajectory cluster as the average of the data trajectories members of this cluster. Data post-processing and graphics was performed with in-house developed functions in Matlab.
Supplementary Fig. 1. Expression of the transcription factor NRF2 in in aged skin and xenotransplants. (A) NRF2 expression in the epidermis of OiY, OiO and pre-grafted aged skin (N=6 OiY mice, five OiO mice and four old donors). Expression of the key downstream antioxidant enzymes of NRF2, such as (B), HO-1 (N=6 OiY mice, 5 OiO mice and 4 old donors), (C) PRDX (N=6 OiY mice, 5 OiO mice and 4 old donors). (D) GSR (N=6 OiY mice, 6 OiO mice and 4 old donors). (E) Quantitative analysis. Data were assessed by IHC from three individual donors. Four areas were evaluated per section, and three sections were analyzed per mouse. Following the Shapiro-Wilk test and the Student’s t-test: *p < 0.05, **p < 0.01, ***p < 0.001. EP – epidermis, DER – dermis. Scale bars: 50 µm.
Supplementary Figure S2

Supplementary Fig. 2. Senescence-associated β-gal staining of human and murine skin. (A) Positive blue staining for β-gal staining (indicated by arrows) in OiY compared to (B) OiO and (C) pre-engrafted aged skin (N=7 OiY mice, 6 OiO mice and 4 old donors). (D) β-gal cells present in aged murine epidermis around the human epidermis of OiO xenotransplants, as well as (E) around OiY xenotransplants. (F) β-gal cells in epidermis of OiY xenotransplants, (G) after 12-month aged mice and (H) before transplantation (N=4 OiY mice after 1 month, 4 OiY mice after 12 months and 3 old donors). After staining, the cells were imaged by phase contrast microscopy. Micrographs are shown at x200 magnification. (I) Quantitative analysis of positive β-gal stained cells. Following the Shapiro-Wilk test and Student’s t-test: *p < 0.05, **p < 0.01, ***p < 0.001. Kruskal-Wallis test: #p < 0.05, ##p < 0.01, ###p < 0.001, and Dunn’s test §p < 0.05, §§p < 0.01, §§§p < 0.001. HS – human skin, MS – mouse skin. Scale bars: 50 µm.
**Supplementary Fig. 3.** Biomarkers related to dermal skin aging. (A) Intensity of elastin staining (N=3 old donors, 7 OiO mice and 7 OiY mice) and (B) hyaluronic acid (N=3 old donors, 7 OiO mice and 8 OiY mice) in pre-engrafted aged skin in OiO and in OiY xenotransplants. (C) Quantitation. Data were assessed by IHC from three individual donors. Four areas were evaluated per section, and three sections were analyzed per mouse. Following the Shapiro-Wilk test and Student’s t-test: *p < 0.05, **p < 0.01, ***p < 0.001. **EP** – epidermis, **DER** – dermis, **SB** – stratum basale. Scale bars: 50 μm.
Supplementary Fig. 4. Dermal blood vessels in old and young mice. (A-C) A significant increased number of blood vessels (N=3 old mice and 3 young mice) was observed in old (14 months), versus young mice (7 weeks). Data were assessed by IHC from three mice. Four areas were evaluated per section, and three sections were analyzed per mouse. Following the Shapiro-Wilk test and Student’s t-test: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. EP – epidermis, DER – dermis. Scale bars: 50 µm.
Supplementary Fig. 5. (A-C) Increased number of human mast cells (tryptase/HLA A,B,C) in OiY versus OiO (N=4 OiO mice and 4 OiY mice). Data were assessed by IHC from three individual donors. Three areas were evaluated per section, and three sections were analyzed per mouse. Following the Shapiro-Wilk test and Student’s t-test: *p < 0.05, **p < 0.01, ***p < 0.001. EP – epidermis, DER – dermis. Scale bars: 50 µm.
Supplementary Figure S6

A. Before transplant, Y1O, Y1Y

B. Proliferation

C. Melanocytes

D. Blood vessels

E. p16INK4a

F. Filaggrin

G. Thickness (μm)

| Condition | Before | Y1O | Y1Y |
|-----------|--------|-----|-----|
| Thickness | 95     | 45  | 45  |

| Condition | Before | Y1O | Y1Y |
|-----------|--------|-----|-----|
| Proliferation (%) | 15 | N.S. | N.S. |

| Condition | Before | Y1O | Y1Y |
|-----------|--------|-----|-----|
| Melanocytes (0.66 mm²) | 8 | N.S. | N.S. |

| Condition | Before | Y1O | Y1Y |
|-----------|--------|-----|-----|
| Mean Vessel Density | 7 | ** | N.S. |

| Condition | Before | Y1O | Y1Y |
|-----------|--------|-----|-----|
| p16INK4a (%) | 10 | N.S. | N.S. |

| Condition | Before | Y1O | Y1Y |
|-----------|--------|-----|-----|
| Filaggrin (%) | 100 | N.S. | N.S. |
Supplementary Fig. 6. Epidermal and dermal parameters of human young skin before and one month after transplantation onto old and young mice. (A) Similar epidermal thickness (N=3 young donors, 6 YiO mice and 7 YiY mice) and (B) proliferation (N=3 young donors, 7 YiO mice and 7 YiY mice), (C) melanocytes (N=3 young donors, 8 YiO mice and 7 YiY mice) and (D) dermal blood vessels levels (N=3 young donors, 6 YiO mice and 7 YiY mice), as well as (E) epidermal p16^ink4a (N=3 young donors, 6 YiO mice and 6 YiY mice) and (F) filaggrin expression (N=3 young donors, 8 YiO mice and 7 YiY mice) before transplantation and in YiO and YiY mice. (G) Quantitation. Data were assessed by IHC from three individual donors. Four areas were evaluated per section, and three sections were analyzed per mouse. Following the Shapiro-Wilk test and Student’s t-test: *p < 0.05, **p < 0.01, ***p < 0.001. **EP** – epidermis, **DER** – dermis, **SG**- startum granulosum, **SB** – stratum basale. Scale bars: 50 μm.
Supplementary Fig. 7. Epidermal and dermal parameters of human young skin before and three months after transplantation onto old and young mice. (A) Similar epidermal thickness (N=2 young donors, 5 YiO mice and 5 YiY mice) and (B) proliferation (N=2 young donors, 5 YiO mice and 5 YiY mice), (C) melanocytes (N=2 young donors, 5 YiO mice and 5 YiY mice) and (D) dermal blood vessels levels (N=2 young donors, 5 YiO mice and 5 YiY mice), as well as (E) filaggrin expression (N=2 young donors, 5 YiO mice and 5 YiY mice) before transplantation and in YiO and YiY mice. (F) Quantitation. Data were assessed by IHC from three individual donors. Four areas were evaluated per section, and three sections were analyzed per mouse. Following the Shapiro-Wilk test and Student’s t-test: *p < 0.05, **p < 0.01, ***p < 0.001. EP – epidermis, DER – dermis, SG – stratum granulosum, SB – stratum basale. Scale bars: 50 µm.
Supplementary Figure S8
Supplementary Fig. 8. Epidermal and dermal parameters of human aged skin in pre-transplanted skin, in OiY and after 12 months on the mice. H&E staining: (A) Before transplantation with decreased epidermal thickness, and after transplantation with increased epidermal thickness in OiY 12 months aged mice (N=4 old donors, 8 OiY mice after one month and 6 OiY mice after 12 months). Immunohistochemically staining: (B) Proliferation in pre-transplanted aged skin compared to OiY mice and 12 months aged mice (N=4 old donors, 9 OiY mice after one month and 6 OiY mice after 12 months). (C) Melanocytes in pre-transplants aged skin compared to OiY mice and 12 months aged mice (N=4 old donors, 7 OiY mice after one month and 6 OiY mice after 12 months). (D) epidermal filaggrin (N=3 old donors, 9 OiY mice after one month and 6 OiY mice after 12 months). (E) CD31+ blood (N=4 old donors, 9 OiY mice after one month and 6 OiY mice after 12 months). (F) Epidermal and dermal c-Kit cells (N=4 old donors, 9 OiY mice after one month and 6 OiY mice after 12 months) and (G) quantitation. Data were assessed by IHC from three individual donors. Four areas were evaluated per section, and three sections were analyzed per mouse. Following the Shapiro-Wilk test, Student’s t-test: *p<0.05, **p<0.01, ***p<0.001. Scale bars: 50 µm.
Supplementary Fig. 9. Biomarkers related to epidermis of aged skin in pre-transplanted skin in OiY and after 12 months on the mice. Expression of (A) Collagen 17A (N=3 old donors, 6 OiY mice after one month and 6 OiY mice after 12 months), (B) elastin (N=3 old donors, 6 OiY mice after one month and 6 OiY mice after 12 months) and (C) hyaluronic acid (N=3 old donors, 6 OiY mice after one month and 6 OiY mice after 12 months) in pre-engrafted aged skin, OiY and in 12-month-aged transplants. (D) Quantitation. Data were assessed by IHC from three individual donors. Four areas were evaluated per section, and three sections were analyzed per mouse. Following the Shapiro-Wilk test and Student’s t-test: *p < 0.05, **p < 0.01, ***p < 0.001 or Mann-Whitney U test: #p < 0.05, ##p < 0.01, ###p < 0.001. EP – epidermis, DER – dermis. Scale bars: 50 μm.
Supplementary Figure S10

A

**p-AKT1** (S473)

OiY + control Ab  
OiY + αVEGF Ab

p-AKT1 (per 0.66 mm²)

OiY + control Ab  
OiY + αVEGF Ab

**B**

**p-IRS1** (Tyr966)

OiY + control Ab  
OiY + αGF-1 Ab

p-IRS1 (per 0.66 mm²)

OiY + control Ab  
OiY + αGF-1 Ab

**C**

**p-Smad2** (S255)

OiY + Ethanol  
OiY + αTGF-β

p-Smad2 (per 0.66 mm²)

OiY + Ethanol  
OiY + αTGF-β

**D**

**p-c-Met** (T1349)

OiY + control Ab  
OiY + αHGF Ab

p-c-Met (per 0.66 mm²)

OiY + control Ab  
OiY + αHGF Ab

**E**

**p-Stat3** (T705)

OiY + control Ab  
OiY + αHGF Ab

p-Stat3 (per 0.66 mm²)

OiY + control Ab  
OiY + αHGF Ab
Supplementary Fig. 10. Effect of antibodies against VEGF-A, HGF, IGF1 and TGF-β blockers on the downstream molecules in OiY xenotransplants. Phosphorylation of downstream molecules in OiY xenotransplants treated with non-specific Abs. (A) Absence of phosphorylation of Akt in anti-VEGF-A-injected xenotransplants (N=4 OiY mice injected with control Abs and 4 OiY mice injected with VEGF-A blocking Abs), (B) of phospho-IRS1 in anti IGF1-Abs (N=3 OiY mice injected with control Abs and 4 OiY mice injected with IGF-1 blocking Abs), (C) of phospho-SMAD2 in TGF-β inhibition (N=3 OiY mice injected with ethanol and 4 OiY mice injected with SB431542, a specific TGF-β inhibitor), (D) of phospho-c-Met (N=4 OiY mice injected with control Abs and 4 OiY mice injected with HGF blocking Abs) and (E) of phospho-STAT3 (N=4 OiY mice injected with control Abs and 4 OiY mice injected with HGF blocking Abs) in anti- HGF Abs injected xenotransplants. Quantitative analysis. Data were assessed by IHC from two individual donors. Four areas were evaluated per section, and three sections were analyzed per mouse. Following the Shapiro-Wilk test and the Student’s t-test: *p < 0.05, **p < 0.01, ***p < 0.001. Abs – antibodies. Scale bars: 50 µm.
Supplementary Fig. 11. Epidermal expression of key factors in controlling mitochondrial metabolism. (A) Filaggrin (N=4 old donors, 8 OiY mice injected with control Abs and 8 OiY mice injected with VEGF-A blocking Abs), and (B) NRF2 (N=3 old donors, 5 OiY mice injected with control Abs and 5 OiY mice injected with VEGF-A blocking Abs) in pre-transplanted aged skin. In OiY treated with non-specific antibodies compared to decreased levels of (C) elastin (N=3 old donors, 6 OiY mice injected with control Abs and 7 OiY mice injected with VEGF-A blocking Abs) and (D) hyaluronic acid (N=4 old donors, 6 OiY mice injected with control Abs and 7 OiY mice injected with VEGF-A blocking Abs) in OiY treated with VEGF-A blocking antibodies. (E) Quantitation. Data were assessed by IHC from three individual donors. Four areas were evaluated per section, and three sections were analyzed per mouse. Following the Shapiro-Wilk test and Student’s t-test: *p < 0.05, **p < 0.01, ***p < 0.001. EP – epidermis, DER – dermis, SG – Stratum granulosum, Abs- antibodies. Scale bars: 50 µm.
Supplementary Fig. 12. RNA-Seq analyses of PLGA-VEGF-A and VEGF-A neutralization and epidermal thickness and proliferation of aged human skin on young mice treated with HGF, IGF1 blocking antibodies or TGF-β inhibitor. (A) The RNA-Seq analyses revealed upregulation of angiogenesis regulatory factors including STAT3, LCN2, HIF1A, CLC2A1, VEGF-A, FLT3, PRKCB, CXCL1, ID2, CXCL2, FUT2 and NRF2 in aged human skin xenotransplants treated with VEGF-A-loaded nanoparticles. Downregulation of all these genes were detected in the group treated with anti-VEGF-A (N=2 old donors, 3 OiO mice injected with PLGA-BSA, 3 OiO mice injected with PLGA-VEGF, 3 OiY mice injected with control Ab and 4 OiY mice injected with VEGF blocking Ab). (B, C) Significant increased epidermal thickness and proliferation in groups of OiY mice injected with anti-HGF (N=5 mice), anti IGF1 (N=5 mice) and anti TGF-β (N=5 mice) antibody and isotype control (N=5 mice) compared to values of aged skin before transplantation. Following the Shapiro-Wilk test and Student’s t-test: *p < 0.05, **p < 0.01, ***p < 0.001. Kruskal-Wallis test: #p < 0.05, ##p < 0.01, ###p < 0.001, and Dunn’s test §p < 0.05, §§p < 0.01, §§§p < 0.001.
Supplementary Fig. 13. Epidermal and dermal parameters of aged human skin before and six days after culture in an ex vivo model. (A) Decreased epidermal proliferative (N=2 old donors, 4 aged skin cultured with VEGF blocking Ab, 3 aged skin cultured with BSA and 4 aged skin cultured with VEGF recombinant protein), (B) absence of epidermal filaggrin expression (N=2 old donors, 4 aged skin cultured with VEGF blocking Ab, 4 aged skin cultured with BSA and 4 aged skin cultured with VEGF recombinant protein), (C) decreased number of dermal blood vessels (N=2 old donors, 3 aged skin cultured with VEGF blocking Ab, 4 aged skin cultured with BSA and 4 aged skin cultured with VEGF recombinant protein) and of (D) collagen 17A (N=2 old donors, 4 aged skin cultured with VEGF blocking Ab, 4 aged skin cultured with BSA and 4 aged skin cultured with VEGF recombinant protein) were observed in aged skin before culture, or six days after co-culture with anti VEGF-A antibodies or with BSA. However, increased values of all these parameters were observed following co-culture with VEGF-A. (E) Quantitative analysis between the various groups. Data were assessed by IHC from two individual donors. Four areas were evaluated per section. Following the Shapiro-Wilk test and Student’s t-test: *p < 0.05, **p < 0.01, ***p < 0.001. Kruskal-Wallis test: #p < 0.05 ##p < 0.01, ###p < 0.001, and Dunn’s test §p < 0.05, §§p < 0.01, §§§p < 0.001. EP – epidermis, DER – dermis, SG – startum granulosum, SB – stratum basale. Scale bars: 50 µm.
Supplementary Fig. 14. Human epidermal melanocytes in aged skin before and six days after culture in an ex vivo model. (A) Melanocyte (N=2 old donors, 4 aged skin cultured with VEGF blocking Ab, 4 aged skin cultured with BSA and 4 aged skin cultured with VEGF recombinant protein) counts were significantly reduced with (B) c-Kit (N=2 old donors, 4 aged skin cultured with VEGF blocking Ab, 3 aged skin cultured with BSA and 4 aged skin cultured with VEGF recombinant protein), (C) GP-100 (N=2 old donors, 4 aged skin cultured with VEGF blocking Ab, 3 aged skin cultured with BSA and 4 aged skin cultured with VEGF recombinant protein), and (D) Masson Fontana (N=2 old donors, 4 aged skin cultured with VEGF blocking Ab, 4 aged skin cultured with BSA and 4 aged skin cultured with VEGF recombinant protein) staining in aged skin before or six days after co-culture with anti-VEGF-A or co-cultured with BSA compared to increased numbers following co-culture with VEGF-A. Data were assessed by IHC from two individual donors. Four areas were evaluated per section. Following the Shapiro-Wilk test and Student’s t-test: *p < 0.05, **p < 0.01, ***p < 0.001. Kruskal-Wallis
test: \#p < 0.05 \#\#p < 0.01, \#\#\#p < 0.001, and Dunn’s test \$p < 0.05, \$\$p < 0.01, \$\$\$p < 0.001. EP – epidermis, DER – dermis, SB – stratum basale. Scale bars: 50 µm.
Supplementary Fig. 15. **Biomarkers related to epidermal skin aging before and six days after culture in an ex vivo model.** (A) Absence of SIRT1 (N=2 epidermal sheets from old donors, 4 epidermal sheets cultured with VEGF blocking Ab, 4 epidermal sheets cultured with BSA and 4 epidermal sheets cultured with VEGF recombinant protein), (B) PGC1α (N=2 epidermal sheets from old donors, 5 epidermal sheets cultured with VEGF blocking Ab, 4 epidermal sheets cultured with BSA and 4 epidermal sheets cultured with VEGF recombinant protein), (C) MTCO-1 (N=2 epidermal sheets from old donors, 5 epidermal sheets cultured with VEGF blocking Ab, 4 epidermal sheets cultured with BSA and 4 epidermal sheets cultured with VEGF recombinant protein) and (D) MMP1 (N=2 epidermal sheets from old donors, 4 epidermal sheets cultured with VEGF blocking Ab, 4 epidermal sheets cultured with BSA and 4 epidermal sheets cultured with VEGF recombinant protein) expression in epidermal aged skin before and six days after co-culture with anti-VEGF-A antibodies or cultured with BSA. However, downregulation of MMP1 and increased expression of SITR1, PGC1α, MTCO-1 were observed in aged skin cultured with VEGF-A. Data were assessed by IHC from two individual donors. Four areas were evaluated per section. Following the Shapiro-Wilk test and Student’s t-test: *p < 0.05, **p < 0.01, ***p < 0.001. Kruskal-Wallis test: #p < 0.05, ##p < 0.01, ###p < 0.001, and Dunn’s test §p < 0.05, §§p < 0.01, §§§p < 0.001. **EP** – epidermis, **DER** – dermis, **SG** – stratum granulosum. Scale bars: 50 µm.
Table S1. Baseline characteristics of human skin donors and distribution of xenotransplants.

| Human donor | Gender¹ | Age (years; mean±SD) | No. of Xenotransplants Performed¹ |
|-------------|---------|----------------------|----------------------------------|
| Adult (N=12) | F (N=10) | 41±6 | 8 | YiY (N=14) |
|             | M (N=2) | 46±5 | 2 | |
| Aged (N=14) | F (N=5) | 82±6 | 22 | OiY (N=84) |
|             | M (N=9) | 84±3 | 34 | |

¹F-female, M-male

Table S2. Neutralizing antibodies and small molecule inhibitors used in the study.

| Reagent       | Origin & Specificity                          | Brand | Cat. No. | Conc. used (µg/mouse) |
|---------------|-----------------------------------------------|-------|----------|------------------------|
| αHGF Ab       | Goat anti-human                               | R&D   | AF-294-NA| 100                    |
| αIGF1 Ab      | Goat anti-human                               | R&D   | AF-291-NA| 100                    |
| SB431542 (TGFβ inh.) | ---                                          | Tocris | 1614     | 57                     |
| αVEGF Ab (G6-31) | human Fab phage libraries anti-human/mouse | Genentech | 11037    | 0.92 nmol/mouse        |
| Isotype control | Human IgG                                     | Sigma | I4506    | 0.92 nmol/mouse        |
| Isotype control | Goat IgG                                      | R&D   | AB-108-C | 100                    |
Table S3. Proteins used to load PLGA nanoparticles.

| Protein | Brand | Cat. No.   | Conc. used (µg/mouse) |
|---------|-------|------------|-----------------------|
| BSA     | Sigma | A9647      | 1                     |
| VEGF    | A2S   | Z02689-100 | 1                     |
Table S4. Markers/protocols used to assess the aging-related status of xenotransplanted human skin.

| Skin area | Assay      | Process                                      | Marker/Protocol                                      | Motivation                                                                 | No. of Stained Specimens | Ref.    |
|-----------|------------|----------------------------------------------|------------------------------------------------------|---------------------------------------------------------------------------|--------------------------|---------|
| Epidermis | Histology  | Epidermal thickness                         | Average of measurements in H&E sections              | Thinning of the skin is a typical feature of the aged skin               | 46                       | (95)    |
|           | IHC¹       | Epidermal proliferation & senescence         | Ki67+ basal keratinocytes                            | Aged basal keratinocytes proliferate less                                 | 36                       |         |
|           |            | p16⁰⁶⁴⁴A (% color intensity per 0.3 mm²)²     | Cell cycle inhibitor associated to senescence; it is used as an aging biomarker |                                                                           | 16                       | (15,96-99) |
|           |            | NRF2 (mean positive cells per 0.6 mm²)       | Impairment of NRF2 pathway is a key contributor to enhance skin aging |                                                                           | 24                       | (100, 101) |
|           |            | Epidermal differentiation                   | Filaggrin (% color density per pixel)²              | Downregulation of filaggrin is observed in aged skin                      | 28                       | (102)   |
| Basal cells differentiation | COL17A1   | Aged basal keratinocytes are less differentiated |                                                        |                                                                           | 30                       | (19)    |
| Dermis    | Pigmentation (number of melanocytes)       | Melan A                                       | It decreases by 8-20% per decade after age 30        |                                                                           | 32                       | (103)   |
| Number of mast cells | c-KIT     | Known to be reduced in aged skin             |                                                      |                                                                           | 38                       | (104-106) |
|           | Tryptase   |                                              |                                                      |                                                                           | 28                       |         |
| Angiogenesis | CD31(PECAM-1) positive blood vessels with a visible lumen | Decreased number of blood vessels in aged skin |                                                                           |                                                                           | 30                       | (107)   |
| Elastin   | Elastin    | Known to be reduced in aged skin             |                                                      |                                                                           | 28                       | (24)    |
| Hyaluronic acid | Versican G1 domain | Known to be reduced in aged skin |                                                      |                                                                           | 28                       | (25)    |
| Histology | Extracellular matrix (ECM) remodeling | Thin and thick fibrils of collagen (PicroSirius Red stain) | Changes in the thick vs thin collagen ratio are one of the most prominent features of skin aging leading to wrinkles, sagging and laxity | 34 | (108,109) |

1 IHC, immunohistochemistry

2 Quantified using Image-Pro software analysis
Table S5. Antibodies used for IHC and immunofluorescence staining.

| Antigen (Ag)          | Origin & Specificity | Brand       | Cat. No. | Titer (µg/ml) | Protocol used for Ag Retrieval/Fixation | Detection System |
|-----------------------|----------------------|-------------|----------|---------------|-----------------------------------------|------------------|
| CD31                  | Rat anti-mouse       | BD Pharmingen | 550274  | 0.3          | Sodium citrate                          | Donkey anti-rat, AEC<sup>1</sup> |
|                       | Mouse anti-human     | Dako        | M0823    | 10           | EDTA<sup>2</sup>                         |                  |
| Filaggrin             | Mouse anti-human     | Abcam       | Ab-31356 | 3            | Sodium citrate                          |                  |
| Ki67                  | Mouse anti-human     | Invitrogen  | 18-0192Z | 2            | EDTA                                    |                  |
| Mast cell tryptase    | Mouse anti-human     | Abcam       | Ab-2378  | 0.1          | Sodium citrate                          |                  |
| Melan A               | Mouse anti-human     | Santa Cruz  | sc-20032 | 4            | EDTA                                    |                  |
| Heme oxygenase        |                      | Abcam       | Ab13248  | 2            | EDTA                                    |                  |
| Elastin               |                      | Novus biologicals | BA-4  | 1            | EDTA                                    |                  |
| NRF2<sup>1</sup>      |                      | Santa Cruz  | sc-722   | 0.1          | Sodium citrate                          |                  |
| Peroxiredoxin         |                      | Abcam       | Ab15571  | 1            | EDTA                                    |                  |
| MTCO-1                |                      | Abcam       | Ab45918  | 1            | Sodium citrate                          |                  |
| SIRT1                 |                      | Abcam       | Ab32441  | 0.5          | EDTA                                    |                  |
| Collagen17A           | Rabbit anti-human    | Abcam       | EPR-14758 | 1          | EDTA                                    | Goat anti-rabbit, AEC |
| Smad2 (phospho S255)  |                      | Abcam       | Ab-188334 | 1          | EDTA                                    |                  |
| IRS1 (phospho Y896)   |                      | Abcam       | Ab-4873  | 1            | Sodium citrate                          |                  |
| Met (phospho Y1349)   |                      | Abcam       | Ab-68141 | 2            | EDTA                                    |                  |
| AKT (phospho S473)    |                      | Abcam       | Ab-81283 | 1            | Sodium citrate                          |                  |
| Stat3 (phospho Y705)  |                      | Cell Signaling | 9145  | 1.5          | EDTA                                    |                  |
| p16<sup>INK4a</sup>   | Mouse anti-human     | Ventana     | 725-4713 | 1            | Sodium citrate                          | Horse anti-mouse, AEC |
| VEGF-A                | Mouse anti-human     | Abcam       | Ab1316   | 2            | EDTA                                    | Goat anti-mouse 594, Jackson |
| MMP1                  | Mouse anti-human     | Biolegend   | 634702   | 1            | Sodium citrate                          |                  |
| PGC1α                 | Rabbit anti-human    | Abcam       | Ab54481  | 2            | Sodium citrate                          | Goat anti-rabbit 488, Jackson |
| CD68                  |                      | Abcam       | Ab215212 | 1            | EDTA                                    |                  |
| CD42b                 |                      | Abcam       | Ab183345 | 0.5          | EDTA                                    |                  |
| Cytokeratin10 | Abcam | Ab111447 | 2 | EDTA |
|--------------|-------|----------|---|------|
| HLA A,B,C    | Mouse anti-human | Abcam | Ab-70328 | 0.5 | Sodium citrate |

1AEC, 3-amino-9-ethylcarbazole
2EDTA, Ethylenediaminetetraacetic acid
3NRF2, Nuclear factor erythroid 2-related factor 2
REFERENCES AND NOTES

1. D. A. Sinclair, M. D. LaPlante, *Lifespan: Why We Age—And Why We Don’t Have To* (Atria Books, 2019).

2. G. V. Mkrtchyan, K. P. Abdelmohsen, P. Andreux, I. Bagdonaitė, N. Barzilai, S. Brunak, F. Cabreiro, R. de Cabo, J. Campisi, A. M. Cuervo, M. Demaria, C. Y. Ewald, E. F. Fang, R. Faragher, L. Ferrucci, A. Freund, C. G. Silva-García, A. Georgievskaya, V. N. Gladyshev, D. J. Glass, V. Gorbunova, A. de Grey, W.-W. He, J. Hoeijmakers, E. Hoffmann, S. Horvath, R. H. Houtkooper, M. K. Jensen, M. B. Jensen, A. Kane, M. Kassem, P. de Keizer, B. Kennedy, G. Karsenty, D. W. Lamming, K.-F. Lee, N. M. Aulay, P. Mamoshina, J. Mellon, M. Molenaars, A. Moskalev, A. Mund, L. Niedernhofer, B. Osborne, H. H. Pak, A. Parkhitko, N. Raimundo, T. A. Rando, L. J. Rasmussen, C. Reis, C. G. Riedel, A. Franco-Romero, B. Schumacher, D. A. Sinclair, Y. Suh, P. R. Taub, D. Toiber, J. T. Treebak, D. R. Valenzano, E. Verdin, J. Vijg, S. Young, L. Zhang, D. Bakula, A. Zhavoronkov, M. Scheibye-Knudsen, ARDD 2020: From aging mechanisms to interventions. *Aging (Albany NY)* **12**, 24484–24503 (2020).

3. J. L. Kirkland, T. Tchkonia, Y. Zhu, L. J. Niedernhofer, P. D. Robbins, The clinical potential of senolytic drugs. *J. Am. Geriatr. Soc.* **65**, 2297–2301 (2017).

4. B. Schumacher, T. M. Krieg, The aging skin: From basic mechanisms to clinical applications. *J. Invest. Dermatol.* **141**, 949–950 (2021).

5. R. C. Stone, A. Aviv, R. Paus, Telomere dynamics and telomerase in the biology of hair follicles and their stem cells as a model for aging research. *J. Invest. Dermatol.* **141**, 1031–1040 (2021).

6. T. Wyss-Coray, Ageing, neurodegeneration and brain rejuvenation. *Nature* **539**, 180–186 (2016).

7. S. A. Villeda, K. E. Plambeck, J. Middeldorp, J. M. Castellano, K. I. Mosher, J. Luo, L. K. Smith, G. Bieri, K. Lin, D. Berdnik, R. Wabl, J. Udeochu, E. G. Wheatley, B. Zou, D. A. Simmons, X. Xie, F. M. Longo, T. Wyss-Coray, Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. *Nat. Med.* **20**, 659–663 (2014).
8. P. D. Robbins, D. Jurk, S. Khosla, J. L. Kirkland, N. K. LeBrasseur, J. D. Miller, J. F. Passos, R. J. Pignolo, T. Tchkonia, L. J. Niedernhofer, Senolytic drugs: Reducing senescent cell viability to extend health span. *Annu. Rev. Pharmacol. Toxicol.* **61**, 779–803 (2021).

9. A. Gilhar, T. Pillar, M. David, S. Eidelman, Melanocytes and Langerhans cells in aged versus young skin before and after transplantation onto nude mice. *J. Invest. Dermatol.* **96**, 210–214 (1991).

10. A. Gilhar, Y. Ullmann, R. Karry, R. Shalaginov, B. Assy, S. Serafimovich, R.S. Kalish, Aging of human epidermis: Reversal of aging changes correlates with reversal of keratinocyte fas expression and apoptosis. *J. Gerontol. A Biol. Sci. Med. Sci.* **59**, 411–415 (2004).

11. H. Lee, Y. Hong, M. Kim, Structural and functional changes and possible molecular mechanisms in aged skin. *Int. J. Mol. Sci.* **22**, 12489 (2021).

12. M. Thomsen, S. Galvani, C. Canivet, N. Kamar, T. Böhler, Reconstitution of immunodeficient SCID/beige mice with human cells: Applications in preclinical studies. *Toxicology* **246**, 18–23 (2008).

13. S. Victorelli, A. Lagnado, J. Halim, W. Moore, D. Talbot, K. Barrett, J. Chapman, J. Birch, M. Ogrodnik, A. Meves, J. S. Pawlikowski, D. Jurk, P. D. Adams, D. van Heemst, M. Beekman, P. E. Slagboom, D.A. Gunn, J. F. Passos, Senescent human melanocytes drive skin ageing via paracrine telomere dysfunction. *EMBO J.* **38**, e101982 (2019).

14. K. Itahana, J. Campisi, G. P. Dimri, Methods to detect biomarkers of cellular senescence: The senescence-associated beta-galactosidase assay. *Methods Mol. Biol.* **371**, 21–31 (2007).

15. D. J. Baker, T. Wijshake, T. Tchkonia, N. K. LeBrasseur, B. G. Childs, B. van de Sluis, J. L. Kirkland, J. M. van Deursen, Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* **479**, 232–236 (2011).

16. S. Vidali, J. Chéret, M. Giesen, S. Haeger, M. Alam, R. E. B. Watson, A. K. Langton, M. Klinger, J. Knuever, W. Funk, B. Kofler, R. Paus, Thyroid hormones enhance mitochondrial function in human epidermis. *J. Invest. Dermatol.* **136**, 2003–2012 (2016).
17. S. Rius-Pérez, I. Torres-Cuevas, I. Millán, Á. L. Ortega, S. Pérez, PGC-1α, inflammation, and oxidative stress: An integrative view in metabolism. *Oxid. Med. Cell. Longev.* **2020**, 1452696 (2020).

18. G. Lee, M. J. Uddin, Y. Kim, M. Ko, I. Yu, H. Ha, PGC-1α, a potential therapeutic target against kidney aging. *Aging Cell* **18**, e12994 (2019).

19. K. Shirai, K. Obara, N. Tohgi, A. Yamazaki, R. Aki, Y. Hamada, N. Arakawa, S. R. Singh, R. M. Hoffman, Y. Amoh, Expression of anti-aging type-XVII collagen (COL17A1/BP180) in hair follicle-associated pluripotent (HAP) stem cells during differentiation. *Tissue Cell* **59**, 33–38 (2019).

20. T. Quan, Y. Xiang, Y. Liu, Z. Qin, Y. Yang, G. Bou-Gharios, J. J. Voorhees, A. A. Dlugosz, G. J. Fisher, Dermal fibroblast CCN1 expression in mice recapitulates human skin dermal aging. *J. Invest. Dermatol.* **141**, 1007–1016 (2021).

21. C. J. Schmidlin, M. B. Dodson, L. Madhavan, D. D. Zhang, Redox regulation by NRF2 in aging and disease. *Free Radic. Biol. Med.* **134**, 702–707 (2019).

22. K. N. Luu Hoang, J. E. Anstee, J. N. Arnold, The diverse roles of heme oxygenase-1 in tumor progression. *Front. Immunol.*, **12**, 658315 (2021).

23. M. J. Smallwood, A. Nissim, A. R. Knight, M. Whiteman, R. Haigh, P. G. Winyard, Oxidative stress in autoimmune rheumatic diseases. *Free Radic. Biol. Med.* **125**, 3–14 (2018).

24. J.W. Shin, S.H. Kwon, J.Y. Choi, J.I. Na, C.H. Huh, H.R. Choi, K.C. Park, Molecular mechanisms of dermal aging and antiaging approaches. *Int. J. Mol. Sci.* **20**, 2126 (2019).

25. H. Yoshida, A. Nagaoka, A. Komiya, M. Aoki, S. Nakamura, T. Morikawa, R. Ohtsuki, T. Sayo, Y. Okada, Y. Takahashi, Reduction of hyaluronan and increased expression of HYBID (alias CEMIP and KIAA1199) correlate with clinical symptoms in photoaged skin. *Br. J. Dermatol.* **179**, 136–144 (2018).
26. H. Watanabe, E. Ichihara, H. Kayatani, G. Makimoto, K. Ninomiya, K. Nishii, H. Higo, C. Ando, S. Okawa, T. Nakasuka, H. Kano, N. Haru, A. Hirabae, Y. Kato, T. Ninomiya, T. Kubo, K. Rai, K. Ohashi, K. Hotta, M. Tabata, Y. Maeda, K. Kiura, VEGFR2 blockade augments the effects of tyrosine kinase inhibitors by inhibiting angiogenesis and oncogenic signaling in oncogene-driven non-small-cell lung cancers. *Cancer Sci.*, **112**, 1853–1864 (2021).

27. M. Wroblewski, R. Bauer, M. Cubas Córdova, F. Udonta, I. Ben-Batalla, K. Legler, C. Hauser, J. Egberts, M. Janning, J. Velthaus, C. Schulze, K. Pantel, C. Bokemeyer, S. Loges, Mast cells decrease efficacy of anti-angiogenic therapy by secreting matrix-degrading granzyme B. *Nat. Commun.* **8**, 269 (2017).

28. N. Sayed, Y. Huang, K. Nguyen, Z. Krejciowa-Rajaniemi, A.P. Grawe, T. Gao, R. Tibshirani, T. Hastie, A. Alpert, L. Cui, T. Kuznetsova, Y. Rosenberg-Hasson, R. Ostan, D. Monti, B. Lehallier, S.S. Shen-Orr, H.T. Maecker, C.L. Dekker, T. Wyss-Coray, C. Franceschi, V. Jovic, F. Haddad, J.G. Montoya, J.C. Wu, M.M. Davis, D. Furman, An inflammatory aging clock (iAge) based on deep learning tracks multimorbidity, immunosenescence, frailty and cardiovascular aging. *Nat. Aging* **1**, 598–615 (2021).

29. Y. I. Lee, S. Choi, W. S. Roh, J. H. Lee, T. G. Kim, Cellular senescence and inflammaging in the skin microenvironment. *Int. J. Mol. Sci.* **22**, 3849 (2021).

30. A. Sodhi, T. Ma, D. Menon, M. Deshpande, K. Jee, A. Dinabandhu, J. Vancel, D. Lu, S. Montaner, Angiopoietin-like 4 binds neuropilins and cooperates with VEGF to induce diabetic macular edema. *J. Clin. Invest.* **129**, 4593–4608 (2019).

31. A. Ridiandries, J. T. M. Tan, C. A. Bursill, The role of chemokines in wound healing. *Int. J. Mol. Sci.* **19**, 3217 (2018).

32. F. Cuomo, A. Coppola, C. Botti, C. Maione, A. Forte, L. Scisciola, G. Liguori, I. Caiafa, M. V. Ursini, U. Galderisi, M. Cipollaro, L. Altucci, G. Cobellis, Pro-inflammatory cytokines activate hypoxia-inducible factor 3α via epigenetic changes in mesenchymal stromal/stem cells. *Sci. Rep.* **8**, 5842 (2018).
33. J. Rebollo, J. Geliebter, N. Reyes, ESM-1 siRNA knockdown decreased migration and expression of CXCL3 in prostate cancer cells. *Int. J. Biomed Sci.* **13**, 35–42 (2017).

34. I. Uribesalgo, D. Hoffmann, Y. Zhang, A. Kavirayani, J. Lazovic, J. Berta, M. Novatchkova, T.-P. Pai, R. A. Wimmer, V. László, D. Schramek, R. Karim, L. Tortola, S. Deswal, L. Haas, J. Zuber, M. Szűcs, K. Kuba, B. Dome, Y. Cao, B. J. Haubner, J. M. Penninger, Apelin inhibition prevents resistance and metastasis associated with anti-angiogenic therapy. *EMBO Mol. Med.* **11**, e9266 (2019).

35. M. Vilà-González, S. Kelaini, C. Magee, R. Caines, D. Campbell, M. Eleftheriadou, A. Cochrane, D. Drehmer, M. Tsifaki, K. O’Neill, E. Pedrini, C. Yang, R. Medina, D. McDonald, D. Simpson, A. Zampetaki, L. Zeng, D. Grieve, N. Lois, A. W. Stitt, A. Margariti, Enhanced function of induced pluripotent stem cell-derived endothelial cells through ESM1 signaling. *Stem Cells* **37**, 226–239 (2019).

36. A. Doğan. Apelin receptor (Aplnr) signaling promotes fibroblast migration. *Tissue Cell* **56**, 98–106 (2019).

37. V. R. Kay, L. S. Cahill, A. Hanif, J. G. Sled, P. Carmeliet, C. Tayade, B. A. Croy, Adult Pgf−/− mice behaviour and neuroanatomy are altered by neonatal treatment with recombinant placental growth factor. *Sci. Rep.* **9**, 9285 (2019).

38. L. Tudisco, A. Orlandi, V. Tarallo, S. De Falco, Hypoxia activates placental growth factor expression in lymphatic endothelial cells. *Oncotarget* **8**, 32873–32883 (2017).

39. R. P. Santiago, M. O. S. Carvalho, C. V. B. Figueiredo, L. M. Fiuza, R. M. Oliveira, S. C. M. A. Yahouédéhou, V. M. L. Nascimento, I. M. Lyra, T. Araujo-Santos, N. F. Luz, M. M. Aleluia, C. C. Guarda, V. M. Borges, M. S. Goncalves, Associations between TGF-β1 levels and markers of hemolysis, inflammation, and tissue remodeling in pediatric sickle cell patients. *Mediators Inflamm.* **2021**, 4651891 (2021).
40. N. Guttmann-Raviv, N. Shraga-Heled, A. Varshavsky, C. Guimaraes-Sternberg, O. Kessler, G. Neufeld, Semaphorin-3A and semaphorin-3F work together to repel endothelial cells and to inhibit their survival by induction of apoptosis. *J. Biol. Chem.* **282**, 26294–26305 (2007).

41. L. M. Acevedo, S. Barillas, S. M. Weis, J. R. Göthert, D. A. Cheresh, Semaphorin 3A suppresses VEGF-mediated angiogenesis yet acts as a vascular permeability factor. *Blood* **111**, 2674–2680 (2008).

42. H. J. Choi, H. Park, H. W. Lee, Y. G. Kwon, The Wnt pathway and the roles for its antagonists, DKKS, in angiogenesis. *IUBMB Life* **64**, 724–731 (2012).

43. S. F. Rocha, M. Schiller, D. Jing, H. Li, S. Butz, D. Vestweber, D. Biljes, H. C. A. Drexler, M. Nieminen-Kelhä, P. Vajkoczy, S. Adams, R. Benedito, R. H. Adams, Esm1 modulates endothelial tip cell behavior and vascular permeability by enhancing VEGF bioavailability. *Circ. Res.* **115**, 581–590 (2014).

44. V. T. Nguyen, N. Farman, R. Palacios-Ramirez, M. Sbeih, F. Behar-Cohen, S. Aractingi, F. Jaisser, Cutaneous wound healing in diabetic mice is improved by topical mineralocorticoid receptor blockade. *J. Invest. Dermatol.* **140**, 223–234.e7 (2020).

45. Y. Liu, H. Zhang, L. Yan, W. Du, M. Zhang, H. Chen, L. Zhang, G. Li, J. Li, Y. Dong, D. Zhu, MMP-2 and MMP-9 contribute to the angiogenic effect produced by hypoxia/15-HETE in pulmonary endothelial cells. *J. Mol. Cell. Cardiol.* **121**, 36–50 (2018).

46. G. Zhao, X. W. Cheng, L. Piao, L. Hu, Y. Lei, G. Yang, A. Inoue, S. Ogasawara, H. Wu, C. N. Hao, K. Okumura, M. Kuzuya, The soluble VEGF receptor sFlt-1 contributes to impaired neovascularization in aged mice. *Aging Dis.* **8**, 287–300 (2017).

47. A. Rattner, J. Williams, J. Nathans, Roles of HIFs and VEGF in angiogenesis in the retina and brain. *J. Clin. Invest.* **129**, 3807–3820 (2019).

48. Z. Arany, S. Y. Foo, Y. Ma, J. L. Ruas, A. Bommi-Reddy, G. Girnun, M. Cooper, D. Laznik, J. Chinsomboon, S. M. Rangwala, K. H. Baek, A. Rosenzweig, B. M. Spiegelman, HIF-independent
regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha. *Nature* **451**, 1008–1012 (2008).

49. J. Yang, B. McNeish, C. Butterfield, M. A. Moses, Lipocalin 2 is a novel regulator of angiogenesis in human breast cancer. *FASEB J.* **27**, 45–50 (2013).

50. P. S. Tsou, J. H. Ruth, P. L. Campbell, T. Isozaki, S. Lee, H. Marotte, S. E. Domino, A. E. Koch, M. A. Amin, A novel role for inducible Fut2 in angiogenesis. *Angiogenesis* **16**, 195–205 (2013).

51. C. Vogel, R. de Sousa Abreu, D. Ko, S.-Y. Le, B. A. Shapiro, S. C. Burns, D. Sandhu, D. R. Boutz, E. M. Marcotte, L. O. Penalva, Sequence signatures and mRNA concentration can explain two-thirds of protein abundance variation in a human cell line. *Mol. Syst. Biol.* **6**, 400 (2016).

52. W. Choi, L. Yin, C. Smuda, J. Batzer, V. J. Hearing, L. Kolbe, Molecular and histological characterization of age spots. *Exp. Dermatol.* **26**, 242–248 (2017).

53. C.-Y. Lu, K. B. Santosa, A. Jablonka-Shariff, B. Vannucci, A. Fuchs, I. Turnbull, D. Pan, M. D. Wood, A. K. Snyder-Warwick, Macrophage-derived vascular endothelial growth factor-A is integral to neuromuscular junction reinnervation after nerve injury. *J. Neurosci.* **40**, 9602–9616 (2020).

54. V. Gorenjak, A. M. Petrelis, M. G. Stathopoulou, S. Toupance, S. Kumar, C. Labat, C. Masson, H. Murray, J. Lamont, P. Fitzgerald, A. Benetos, S. Visvikis-Siest; TELARTA Consortium. A genetic determinant of VEGF-A levels is associated with telomere attrition. *Aging* **13**, 23517–23526 (2021).

55. M. H. Nisancioglu, C. Betsholtz, G. Genové, The absence of pericytes does not increase the sensitivity of tumor vasculature to vascular endothelial growth factor-A blockade. *Cancer Res.* **70**, 5109–5115 (2010).

56. A. Luengas-Martinez, J. Hardman-Smart, D. Rutkowski, T. S. Purba, R. Paus, H. S. Young, Vascular endothelial growth factor blockade induces dermal endothelial cell apoptosis in a clinically relevant skin organ culture model. *Skin Pharmacol. Physiol.* **33**, 110–118 (2020).
57. T. Hongu, Y. Funakoshi, S. Fukuhara, T. Suzuki, S. Sakimoto, N. Takakura, M. Ema, S. Takahashi, S. Itoh, M. Kato, H. Hasegawa, N. Mochizuki, Y. Kanaho, Arf6 regulates tumour angiogenesis and growth through HGF-induced endothelial β1 integrin recycling. Nat. Commun. 6, 7925 (2015).

58. S. Lin, Q. Zhang, X. Shao, T. Zhang, C. Xue, S. Shi, D. Zhao, Y. Lin, IGF-1 promotes angiogenesis in endothelial cells/adipose-derived stem cells co-culture system with activation of PI3K/Akt signal pathway. Cell Prolif. 50, e12390 (2017).

59. S. Muppala, R. Xiao, I. Krukovets, D. Verbovetsky, R. Yendamuri, N. Habib, P. Raman, E. Plow, O. Stenina-Adognravi, Thrombospondin-4 mediates TGF-β-induced angiogenesis. Oncogene 36, 5189–5198 (2017).

60. X. Jiang, H. Lin, D. Jiang, G. Xu, X. Fang, L. He, M. Xu, B. Tang, Z. Wang, D. Cui, F. Chen, H. Geng, Co-delivery of VEGF and bFGF via a PLGA nanoparticle-modified BAM for effective contracture inhibition of regenerated bladder tissue in rabbits. Sci. Rep. 6, 20784 (2016).

61. L. Y. Chen, X. Wang, X. L. Qu, L. N. Pan, Z. Y. Wang, Y. H. Lu, H. Y. Hu, Activation of the STAT3/microRNA-21 pathway participates in angiotensin II-induced angiogenesis. J. Cell. Physiol. 234, 19640–19654 (2019).

62. F. Liu, S. Chen, Y. Yu, C. Huang, H. Chen, L. Wang, W. Zhang, J. Wu, Y. Ye, Inhibitor of DNA binding 2 knockdown inhibits the growth and liver metastasis of colorectal cancer. Gene 819, 146240 (2022).

63. R. Paus, The skin and endocrine disorders, in Rook’s Textbook of Dermatology, C. E. M. Griffiths, J. Barker, T. O. Bleiker, R. Chalmers, D. Creamer Eds. (Wiley/Blackwell, ed. 9, 2016), chap. 149, vol. 4.

64. L. Zhou, X. Zhang, R. Paus, Z. Lu, The renaissance of human skin organ culture: A critical reappraisal. Differentiation 104, 22–35 (2018).

65. M. Grunewald, S. Kumar, H. Sharife, E. Volinsky, A. Gileles-Hillel, T. Licht, A. Permyakova, L. Hinden, S. Azar, Y. Friedmann, P. Kupetz, R. Tzuberi, A. Anisimov, K. Alitalo, M. Horwitz, S. Leebhoff, O. Z. Khoma, R. Hlushchuk, V. Djonov, R. Abramovitch, J. Tam, E. Keshet,
Counteracting age-related VEGF signaling insufficiency promotes healthy aging and extends lifespan. *Science* **373**, eabc8479 (2021).

66. C.T. Ambrose, Pro-angiogenesis therapy and aging: A mini-review. *Gerontology* **63**: 393–400 (2017).

67. A. J. Covarrubias, R. Perrone, A. Grozio, E. Verdin, NAD\(^+\) metabolism and its roles in cellular processes during ageing. *Nat. Rev. Mol. Cell Biol.* **22**, 119–141 (2021).

68. I. Bieche, S. Vacher, D. Vallerand, S. Richon, R. Hatem, L. De Plater, A. Dahmani, F. Némati, E. Angevin, E. Marangoni, S. Roman-Roman, D. Decaudin, V. Dangles-Marie, Vasculature analysis of patient derived tumor xenografts using species-specific PCR assays: Evidence of tumor endothelial cells and atypical VEGFA-VEGFR1/2 signalings. *BMC Cancer*, **14**, 178 (2014).

69. S. Chatterjee, L. C. Heukamp, M. Siobal, J. Schöttle, C. Wieczorek, M. Peifer, D. Frasca, M. Koker, K. König, L. Meder, D. Rauh, R. Buettner, J. Wolf, R. A. Brekken, B. Neumaier, G. Christofori, R.K. Thomas, R.T. Ullrich, Tumor VEGF:VEGFR2 autocrine feed-forward loop triggers angiogenesis in lung cancer. *J. Clin. Invest.* **123**, 1732–1740 (2013).

70. M. E. Swift, H. K. Kleinman, L.A. DiPietro, Impaired wound repair and delayed angiogenesis in aged mice. *Lab. Invest.* **79**, 1479–1487 (1999).

71. L. Li, H. Pan, H. Wang, X. Li, X. Bu, Q. Wang, Y. Gao, G. Wen, Y. Zhou, Z. Cong, Y. Yang, C. Tang, Z. Liu, Interplay between VEGF and Nrf2 regulates angiogenesis due to intracranial venous hypertension. *Sci. Rep.* **6**, 37338 (2016).

72. T. A. Wilgus, A. M. Matthies, K. A. Radek, J. V. Dovi, A. L. Burns, R. Shankar, L. A. DiPietro, Novel function for vascular endothelial growth factor receptor-1 on epidermal keratinocytes. *Am. J. Pathol.* **167**, 1257–1266 (2005).

73. J. W. Zhu, Y. J. Ni, X. Y. Tong, X. Guo, X. P. Wu, Activation of VEGF receptors in response to UVB promotes cell proliferation and melanogenesis of normal human melanocytes. *Exp. Cell Res.* **387**, 111798 (2020).
74. M. A. Rahat, J. Khyshkowska, V. Iragavarapu-Charyulu, Editorial: Targeting angiogenesis to treat autoimmune diseases and cancer. *Front. Immunol.* **11**, 1005 (2020).

75. A. G. Marneros, Effects of chronically increased VEGF-A on the aging heart. *FASEB J.* **32**, 1550–1565 (2018).

76. S. Dai, Y. Mo, Y. Wang, B. Xiang, Q. Liao, M. Zhou, X. Li, Y. Li, W. Xiong, G. Li, C. Guo, Z. Zeng, Chronic stress promotes cancer development. *Front. Oncol.* **10**, 1492 (2020).

77. J. Campisi, P. Kapahi, G. J. Lithgow, S. Melov, J. C. Newman, E. Verdin, From discoveries in ageing research to therapeutics for healthy ageing. *Nature* **571**, 183–192 (2019).

78. J. Liu, Y. Zhang, C. Yu, J. Li, W. Liu, B. Luo, Epstein-Barr virus-encoded latent membrane protein 2A downregulates GCNT3 via the TGF-β1/Smad-mTORC1 signaling axis. *J. Virol.* **95**, e02481-20 (2021).

79. F. Hakuno, S. I. Takahashi, IGF1 receptor signaling pathways. *J. Mol. Endocrinol.* **61**, T69-T86 (2018).

80. S. Zakaria, M. W. Helmy, A. Salahuddin, G. Omran, Chemopreventive and antitumor effects of benzyl isothiocynate on HCC models: A possible role of HGF/pAkt/STAT3 axis and VEGF. *Biomed. Pharmacother.* **108**, 65–75 (2018).

81. M. Yamauchi, Y. Hirohashi, T. Torigoe, Y. Matsumoto, K. Yamashita, M. Kayama, N. Sato, T. Yotsuyanagi, Wound healing delays in α-Klotho-deficient mice that have skin appearance similar to that in aged humans - Study of delayed wound healing mechanism. *Biochem. Biophys. Res. Commun.* **473**, 845–852 (2016).

82. J.M. Freije, C. López-Otín, Reprogramming aging and progeria. *Curr. Opin. Cell Biol.* **24**, 757–764 (2012).

83. D. K. Tran, T. N. T. Phuong, N. L. Bui, V. Singh, Q. H. Looi, B. Koh, B. M. Shahrin, U. M. Zaman, J. B. Foo, C. C. Wu, P. L. Show, D. T. Chu, Exploring the potential of stem cell-based therapy for aesthetic and plastic surgery. *IEEE Rev. Biomed. Eng.* 3134994 (2021).
84. T. H. Ambrosi, O. Marecic, A. McArdle, R. Sinha, G. S. Gulati, X. Tong, Y. Wang, H. M. Steininger, M. Y. Hoover, L. S. Koepke, M. P. Murphy, J. Sokol, E. Y. Seo, R. Tevlin, M. Lopez, R. E. Brewer, S. Mascharak, L. Lu, O. Ajanaku, S. D. Conley, J. Seita, M. Morri, N. F. Neff, D. Sahoo, F. Yang, I. L. Weissman, M. T. Longaker, C. K. F. Chan, Aged skeletal stem cells generate an inflammatory degenerative niche. *Nature*, 597, 256–262 (2021).

85. M. R. Pourani, F. Abdollahimajd, O. Zargari, M. S. Dadras, Soluble biomarkers for diagnosis, monitoring, and therapeutic response assessment in psoriasis. *J. Dermatolog. Treat.* 2021, 1–8 (2021).

86. A. Luengas-Martinez, R. Paus, H. S. Young, A novel personalised treatment approach for psoriasis: Anti-VEGF-A therapy. *Br. J. Dermatol.* 186, 782–791 (2021).

87. R. S. Apte, Age-related macular degeneration. *N. Engl. J. Med.* 385, 539–547 (2021).

88. F. Larcher, R. Murillas, M. Bolontrade, C. J. Conti, J. L. Jorcano, VEGF/VPF overexpression in skin of transgenic mice induces angiogenesis, vascular hyperpermeability and accelerated tumor development. *Oncogene* 17, 303–311 (1998).

89. F. Benhadou, E. Glitzner, A. Brisebarre, B. Swedlund, Y. Song, C. Dubois, M. Rozzi, C. Paulissen, V. Del Marmol, M. Sibilia, C. Blanpain, Epidermal autonomous VEGFA/Flt1/Nrp1 functions mediate psoriasis-like disease. *Sci. Adv.* 6, eaax5849 (2020).

90. S.-W. Lai, M.-Y. Chen, O. A. Bamodu, M.-S. Hsieh, T.-Y. Huang, C.-T. Yeh, W.-H. Lee, Y.-G. Cherng, Exosomal IncRNA PVT1/VEGFA axis promotes colon cancer metastasis and stemness by downregulation of tumor suppressor miR-152-3p. *Oxid. Med. Cell. Longev.* 2021, 9959807 (2021).

91. C. Greiner, S. Grainger, S. Farrow, A. Davis, J. L. Su, M. D. Saybolt, R. Wilensky, S. Madden, S.T. Sum, Robust quantitative assessment of collagen fibers with picrosirius red stain and linearly polarized light as demonstrated on atherosclerotic plaque samples. *PLOS ONE* 16, e0248068 (2021).

92. L. Pusch, R. Brox, K. Scheuer, T. Yokosawa, M. Wu, B. A. Zubiri, E. Spiecker, K. D. Jandt, D. Fischer, H. Hackstein, Distinct endocytosis and immune activation of poly(lactic-co-glycolic) acid
nanoparticles prepared by single- and double-emulsion evaporation. *Nanomedicine (Lond.)* **16**, 2075–2094 (2021).

93. J. V. Valbuena Perez, R. Linnenberger, A. Dembek, S. Bruscoli, C. Riccardi, M. H. Schulz, M. R. Meyer, A. K. Kiemer, J. Hoppstädter, Altered glucocorticoid metabolism represents a feature of macroph-aging. *Aging Cell* **19**, e13156 (2020).

94. M. L. Barca, R. S. Eldholm, K. Persson, G. H. Bjørkløf, T. Borza, E. Telenius, A.-B. Knapskog, A. Brækhus, I. Saltvedt, G. Selbæk, K. Engedal, Cortisol levels among older people with and without depression and dementia. *Int. Psychogeriatr.* **31**, 597–601 (2019).

95. Z. Wang, M.-Q. Man, T. Li, P. M. Elias, T. M. Mauro, Aging-associated alterations in epidermal function and their clinical significance. *Aging (Albany NY)* **12**, 5551–5565 (2020).

96. K. E. Rentscher, J. E. Carroll, S. W. Cole, R. L. Repetti, T. F. Robles, Relationship closeness buffers the effects of perceived stress on transcriptomic indicators of cellular stress and biological aging marker p16\(^{\text{INK4a}}\). *Aging (Albany NY)* **12**, 16476–16490 (2020).

97. J. Voisin, F. Farina, S. Naphade, M. Fontaine, K.-T. Tshilenge, C. G. Aguirre, A. Lopez-Ramirez, J. Dancourt, A. Ginisty, S. Sasidharan Nair, K. L. Madushani, N. Zhang, F.-X. Lejeune, M. Verny, J. Campisi, L. M. Ellerby, C. Neri, FOXO3 targets are reprogrammed as Huntington's disease neural cells and striatal neurons face senescence with p16\(^{\text{INK4a}}\) increase. *Aging Cell* **19**, e13226 (2020).

98. P. Patil, Q. Dong, D. Wang, J. Chang, C. Wiley, M. Demaria, J. Lee, J. Kang, L J. Niedernhofer, P.D. Robbins, G. Sowa, J. Campisi, D. Zhou, N. Vo, Systemic clearance of p16\(^{\text{INK4a}}\)-positive senescent cells mitigates age-associated intervertebral disc degeneration. *Aging Cell* **18**, e12927 (2019).

99. M. E. C. Waaijer, W. E. Parish, B. H. Strongitharm, D. van Heemst, P. E. Slagboom, A. J. M. de Craen, J. M. Sedivy, R. G. J. Westendorp, D. A. Gunn, A. B. Maier, The number of p16\(^{\text{INK4a}}\) positive cells in human skin reflects biological age. *Aging Cell* **11**, 722–725 (2012).
100. M. Schäfer, H. Farwanah, A.H. Willrodt, A.J. Huebner, K. Sandhoff, D. Roop, D. Hohl, W. Bloch, S. Werner, Nrf2 links epidermal barrier function with antioxidant defense. *EMBO Mol. Med.* **4**, 364–379 (2012).

101. M. Telorack, M. Meyer, I. Ingold, M. Conrad, W. Bloch, S. Werner, A glutathione-Nrf2-thioredoxin cross-talk ensures keratinocyte survival and efficient wound repair. *PLOS Genet.* **12**, e1005800 (2016).

102. K. Bojanowski, W. R. Swindell, S. Cantor, R. K. Chaudhuri, Isosorbide di-(linoleate/oleate) stimulates prodifferentiation gene expression to restore the epidermal barrier and improve skin hydration. *J. Invest. Dermatol.* **141**, 1416–1427.e12 (2021).

103. E. Csekes, L. Račková, Skin aging, cellular senescence and natural polyphenols. *Int. J. Mol. Sci.* **22**, 12641 (2021).

104. M. Cavinato, P. Jansen-Dürr, Molecular mechanisms of UVB-induced senescence of dermal fibroblasts and its relevance for photoaging of the human skin. *Exp. Gerontol.* **94**, 78–82 (2017).

105. J. Feru, E. Delobbe, L. Ramont, B. Brassart, C. Terryn, A. Dupont-Deshorgue, C. Garbar, J.C. Monboisse, F. X. Maquart, S. Brassart-Pasco, Aging decreases collagen IV expression in vivo in the dermo-epidermal junction and in vitro in dermal fibroblasts: Possible involvement of TGF-β1. *Eur. J. Dermatol.* **26**, 350–360 (2016).

106. G. J. Fisher, Y. Shao, T. He, Z. Qin, D. Perry, J. J. Voorhees, T. Quan, Reduction of fibroblast size/mechanical force down-regulates TGF-β type II receptor: Implications for human skin aging. *Aging Cell* **15**, 67–76 (2016).

107. Y. Hachmo, A. Hadanny, S. Mendelovic, P. Hillman, E. Shapiro, G. Landau, H. Gattegno, A. Zrachya, M. Daniel-Kotovsky, M. Catalagna, G. Fishlev, E. Lang, N. Polak, K. Doenyas, M. Friedman, Y. Zemel, Y. Bechor, S. Efrati, The effect of hyperbaric oxygen therapy on the pathophysiology of skin aging: A prospective clinical trial. *Aging (Albany NY)* **13**, 24500–24510 (2021).
108. R. Gref, C. Deloménie, A. Maksimenko, E. Gouadon, G. Percoco, E. Lati, D. Desmaële, F. Zouhiri, P. Couvreur, Vitamin C-squalene bioconjugate promotes epidermal thickening and collagen production in human skin. *Sci. Rep.* **10**, 16883 (2020).

109. M. J. Lee, G. Agrahari, H.-Y. Kim, E.-J. An, K.-H. Chun, H. Kang, Y.-S. Kim, C. W. Bang, L.-J. Tak, T.-Y. Kim, Extracellular superoxide dismutase prevents skin aging by promoting collagen production through the activation of AMPK and Nrf2/HO-1 cascades. *J. Invest. Dermatol.* **141**, 2344–2353.e7 (2021).