

CASE STUDY · SKIN BIOLOGY · HEALTHY AGING

From molecules to claims: characterizing the mechanism of an active positioned for skin longevity

How Skin Biology Xplorer combines 2D cells, reconstructed 3D skin and human skin explants with genomic, transcriptomic, proteomic, metabolomic and metagenomic readouts to characterize what an anti-aging ingredient does, through which biological pathways, and which claims the evidence can support, an illustrative case study.

Illustrative case study. This scenario shows how Skin Biology Xplorer can be applied to an anti-aging question. It does not describe a real client project or an existing ingredient: models, results and timelines are illustrative and depend on the active, the target claim and tissue availability. Data from cellular, 3D and explant models can support mechanistic claims; consumer-perceivable efficacy claims require studies in volunteers.

In this case study, “skin longevity” refers to the long-term maintenance of biological functions associated with skin resilience, including redox homeostasis, extracellular-matrix integrity, cellular senescence control, epidermal homeostasis and energy metabolism.

1 Model

2D cells, 3D skin or explants selected to match the question

2 Measure

Genomic, transcriptomic, proteomic, metabolomic and metagenomic readouts

3 Map

A pathway is strengthened when independent layers converge

4 Interpret

Mechanism of action and evidence for claims

At a glance

QUESTION

Does a fermented botanical extract act on the biology of skin aging: and through which mechanism?

CLIENT PROFILE

Cosmetic ingredient supplier preparing claims for an anti-aging active

MODELS

Senescent dermal fibroblasts and keratinocytes (2D) · UV-exposed full-thickness 3D skin · UV-exposed human skin explants, 6 donors aged 52–68

COMPARATORS

Vehicle, non-irradiated control, retinol reference

OMICS LAYERS

Transcriptomics, proteomics, metabolomics/lipidomics (core); epigenomics (exploratory); metagenomics (optional module)

VOLUME

Approximately 200 molecular profiles

MECHANISM

NRF2 antioxidant response + reduced senescence secretome + dermal-matrix preservation, with a non-retinoid molecular profile

CLAIMS

4 supported · 2 supported with restricted wording · 1 not supported

DURATION

Approximately 16 weeks

The challenge

A cosmetic ingredient supplier had developed a fermented botanical extract positioned around “skin longevity.” Early data, a procollagen ELISA and a chemical antioxidant assay, were encouraging, but they did not answer the questions raised by its customers’ regulatory and R&D teams:

- **Does it act on the biology of skin aging** in human-relevant models, rather than only in a test tube?
- **Through which pathways?** A credible mechanism of action can differentiate an active ingredient in a highly competitive market.
- **What can actually be claimed?** In the EU, cosmetic claims must be supported by adequate and verifiable evidence¹, and evidence of the claimed effect forms part of the Product Information File (PIF)².

The request: characterize what the extract does in skin, understand how it does it, and determine which claims the evidence can support, and which it cannot.

01 Choose the model to match the question

No single model answers every question. We built a model ladder in which each level addresses a different question, using the same comparators: vehicle, extract and retinol³, a well-documented anti-aging comparator. Aging biology was modeled in two ways: cellular senescence, one of the hallmarks of aging⁴, and UV-induced photoaging, a major contributor to visible skin aging⁵.

1 2D cells

Is the extract safe and active at cellular level? At which concentrations? Which pathways respond first?

5 non-cytotoxic concentrations + vehicle, n = 4; 4-week extended culture for epigenetic readouts



2 Reconstructed 3D skin

Does the formulated active work after topical application across a living epidermis?

Non-irradiated control, UV + vehicle, UV + 3% extract, UV + 0.1% retinol; n = 4 tissues per arm; optional colonized version for microbiome studies



3 Human skin explants

Does the effect persist in real, aged human skin despite donor-to-donor variability?

7-day culture, daily topical application; same four arms; epidermis and dermis analyzed separately

Figure 1. A model ladder. Each level answers a different question, with the same comparators (vehicle, extract, retinol): 2D cells for activity and dose; reconstructed 3D skin for topical application across a living epidermis; human skin explants from 6 donors aged 52 to 68 for real, aged skin. Illustrative design.

Designed for interpretation. Criteria and pathway modules were defined before data generation, and explants were analyzed using donor-paired comparisons so that each donor served as their own control.

02 **Measure the response: select the relevant omics layers**

Skin Biology Xplorer draws on five omics layers. Not every question requires all five: the table shows what each layer can contribute to an anti-aging claim and how it was used in this study.

TABLE 2. OMICS LAYERS FOR AN ANTI-AGING QUESTION

Layer	What it can show	Use in this study
Genomics and epigenomics	DNA-methylation clocks (epigenetic age) ⁶ ; donor genotyping; strain genomes for microbiome studies	Exploratory: methylation arrays in long-term fibroblast cultures
Transcriptomics	Bulk RNA-seq and 3' mRNA-seq for dose-response relationships: matrix, senescence/SASP ⁷ , oxidative-stress, epidermal-differentiation, barrier-homeostasis and dermal-epidermal-junction programs	Core: all three models; epidermis and dermis analyzed separately in explants
Proteomics	timsTOF dia-PASEF ⁸ ; dermal matrisome ⁹ , secretome and senescence-associated secreted proteins	Core: explant dermis and fibroblast secretome
Metabolomics and lipidomics	NAD ⁺ and energy metabolism ¹⁰ , glutathione redox status, oxidized lipids, epidermal ceramides and barrier-related lipid profiles	Core: fibroblasts and 3D skin
Metagenomics	Shotgun sequencing (or 16S/ITS) of a skin microbial community: composition and functional genes ¹¹	Optional module: 3D skin colonized with skin commensals

Pre-analytics determine data quality. Explants are valuable and dense tissues: each sample was immediately separated into epidermis and dermis and stabilized within minutes. RNA quality was assessed using DV200 rather than RIN alone, with rRNA-depletion chemistry used for partially degraded dermal RNA. Dermal proteins were extracted using an extracellular-matrix-adapted protocol to avoid losing insoluble collagens.

In total, approximately 200 molecular profiles: 112 transcriptomes, 32 proteomes, 28 metabolomes/lipidomes, 12 methylomes and 12 metagenomes.

What was measured

≈200 molecular profiles across three model levels

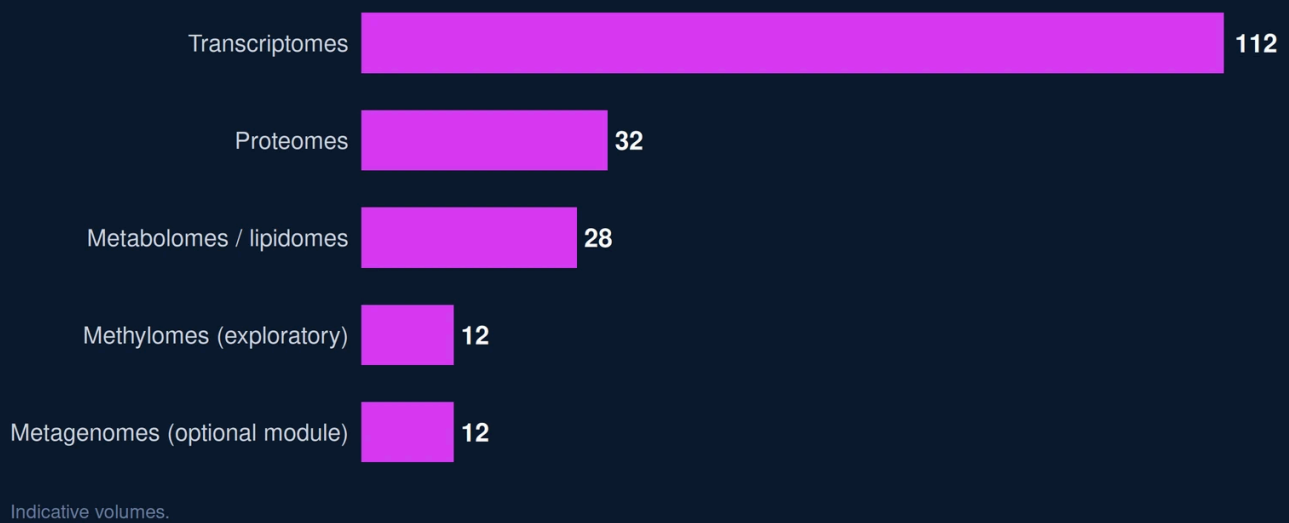


Figure 2. What was measured. Number of molecular profiles per omics layer, about 200 in total across the three model levels. Transcriptomics is the core layer in every model; methylation was exploratory and metagenomics an optional module. Indicative volumes.

03 Map the pathways

Each data layer was first analyzed independently and then integrated. A biological pathway was considered strengthened when independent layers or models converged on the same direction of effect.

- **Donor-paired statistics.** Explant comparisons were paired by donor (DESeq2¹² for RNA; linear models for proteins and metabolites), with false-discovery-rate control.
- **Predefined aging modules.** Matrix synthesis and degradation, senescence/SASP, oxidative stress, epidermal-barrier homeostasis and energy metabolism were tested as gene modules (GSEA¹³) defined before inspection of the results.
- **Upstream regulators.** Transcription-factor activity inferred from curated regulons¹⁴ identified candidate regulators of the response; multi-omics factor analysis¹⁵ identified axes of variation shared across data layers.
- **Converging-evidence rule.** A pathway was considered supported when at least two independent data layers or models agreed on the direction of effect.

	Epigenomics	Transcriptomics	Proteomics	Metabolomics	Metagenomics	Phenotypic test	Conclusion
Dermal matrix synthesis	–	↑	↑	–	–	↑	Supported
Matrix degradation (MMP-1)	–	↓	↓	–	–	–	Supported
Oxidative-stress response (NRF2)	–	↑	–	↑	–	↓	Supported
Senescence and SASP	–	↓	↓	–	–	↓	Supported
Energy metabolism (NAD ⁺)	–	=	–	↑	–	–	In vitro only
Epigenetic age	=	–	–	–	–	–	Not supported
Microbial-community balance	–	–	–	–	=	–	Preserved (model)
Retinoid signaling	–	=	–	–	–	–	Not activated

Figure 3. Evidence map. Rows are ageing-related pathways, columns are data layers. A blue arrow marks a significant change and its direction, = no significant change, – not measured. A pathway counts as supported only when at least two independent layers or models agree: four pathways meet that rule, energy metabolism rests on a single layer in vitro, and epigenetic age did not change. Illustrative results.

Main results (extract vs vehicle, UV-exposed explants and 2D/3D models; illustrative):

- **Dermal matrix.** COL1A1 (×1.6) and COL3A1 (×1.4) increased, while MMP1 decreased (×0.55). Among approximately 3,800 dermal proteins, including 248 matrisome proteins, collagen I α1 (+22%) and decorin (+15%) increased and MMP-1 decreased (–31%); collagen staining was concordant.
- **Oxidative stress.** NRF2 target genes (HMOX1, NQO1, GCLM) were induced¹⁶; the GSH/GSSG ratio increased (×1.4), while oxidized phospholipids and intracellular ROS decreased after UV exposure.
- **Senescence.** SA-β-gal-positive fibroblasts decreased by 28%¹⁷; SASP transcripts (IL6, CXCL8, MMP3) and the corresponding secreted proteins were reduced.
- **Energy metabolism.** The NAD⁺/NADH ratio increased (×1.3) in fibroblasts, one data layer, one model.
- **Epigenetic age.** After 4 weeks, methylation age was 0.8 years lower than vehicle, but the 95% CI (–2.1 to +0.5) included no effect.
- **Microbiome compatibility.** In colonized 3D skin, no statistically significant change in community composition or functional profiles was detected (PERMANOVA p = 0.41).

- **Retinol comparator.** Retinol produced a similar matrix response (normalized enrichment score 2.4 vs 2.1 for the extract), together with a retinoid-response signature (e.g. CRABP2) and epidermal genes associated with irritation; these signatures were not detected with the extract.

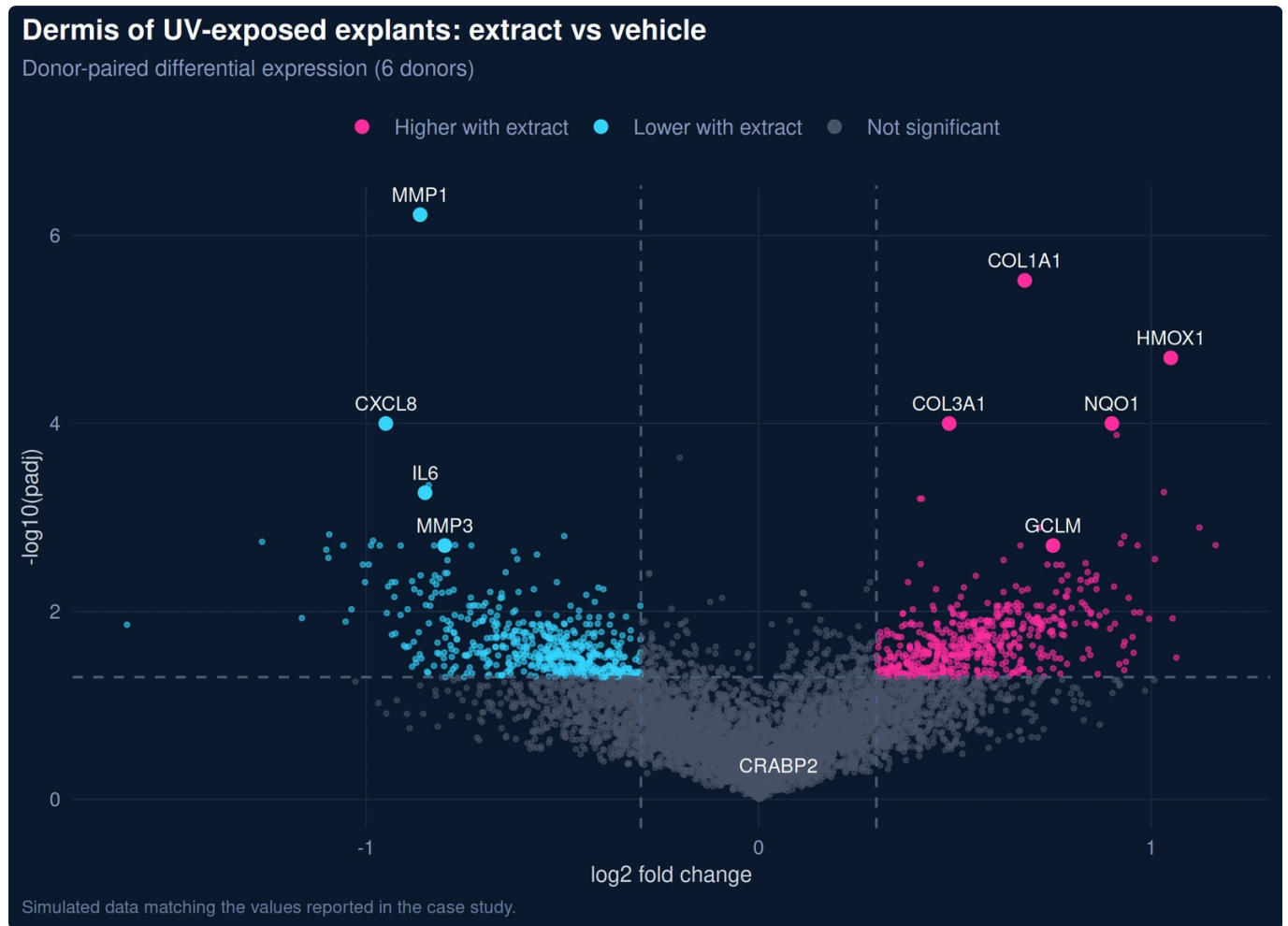
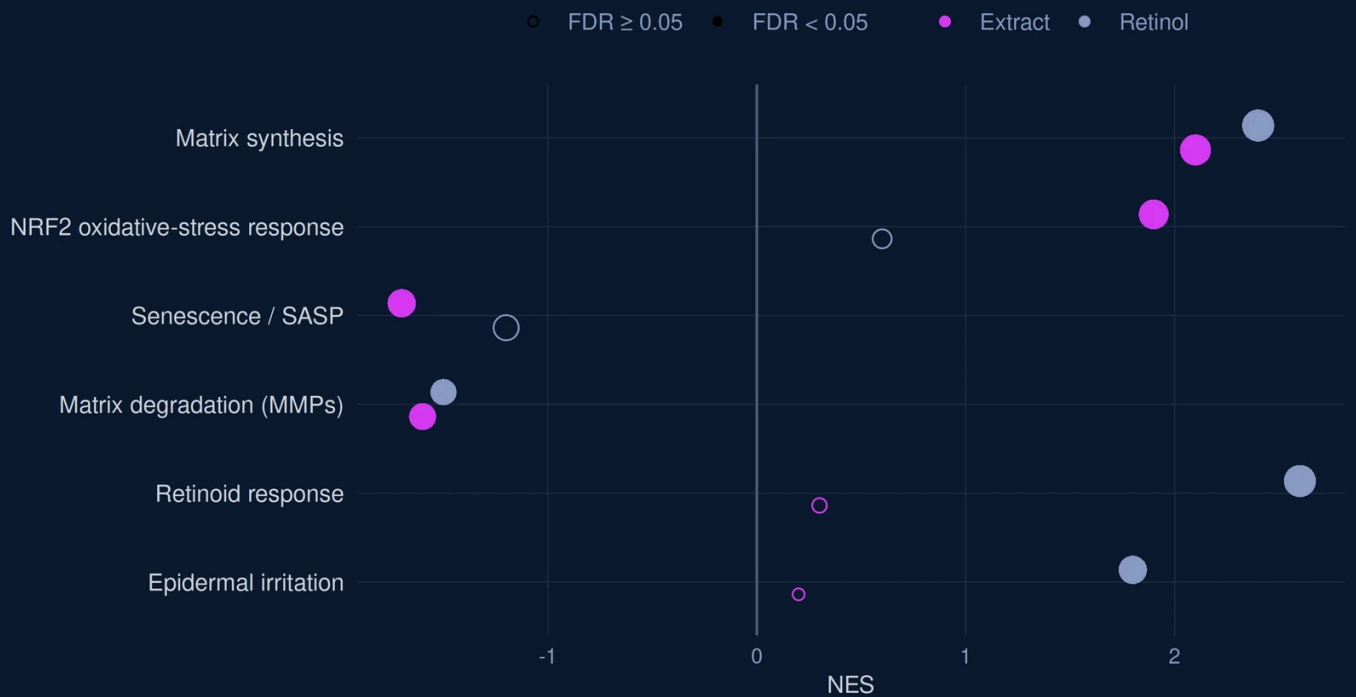


Figure 4. Differential expression in the dermis of UV-exposed explants. Each point is a gene, extract versus vehicle, compared within each of the 6 donors. To the right, genes higher with the extract: matrix genes COL1A1 and COL3A1, and NRF2 targets HMOX1, NQO1 and GCLM. To the left, genes lower with the extract: MMP1 and the senescence-associated genes IL6, CXCL8 and MMP3. CRABP2, a marker of retinoid response, does not change. Dashed lines: significance and fold-change thresholds. Simulated data matching the case study.

Predefined ageing modules: extract vs retinol

GSEA, normalised enrichment score vs vehicle



Simulated data matching the values reported in the case study.

Figure 5. Predefined ageing modules, extract versus retinol. Each point is the normalised enrichment score (NES) of a gene module defined before the analysis: positive means the module goes up versus vehicle, negative that it goes down. Filled points are significant (FDR < 0.05). Both arms raise matrix synthesis (NES 2.1 for the extract, 2.4 for retinol), but only retinol activates the retinoid-response and irritation modules. Simulated data; only the matrix values are reported in the case study.

Selected effects of the extract

Extract vs vehicle, UV-exposed models (illustrative)

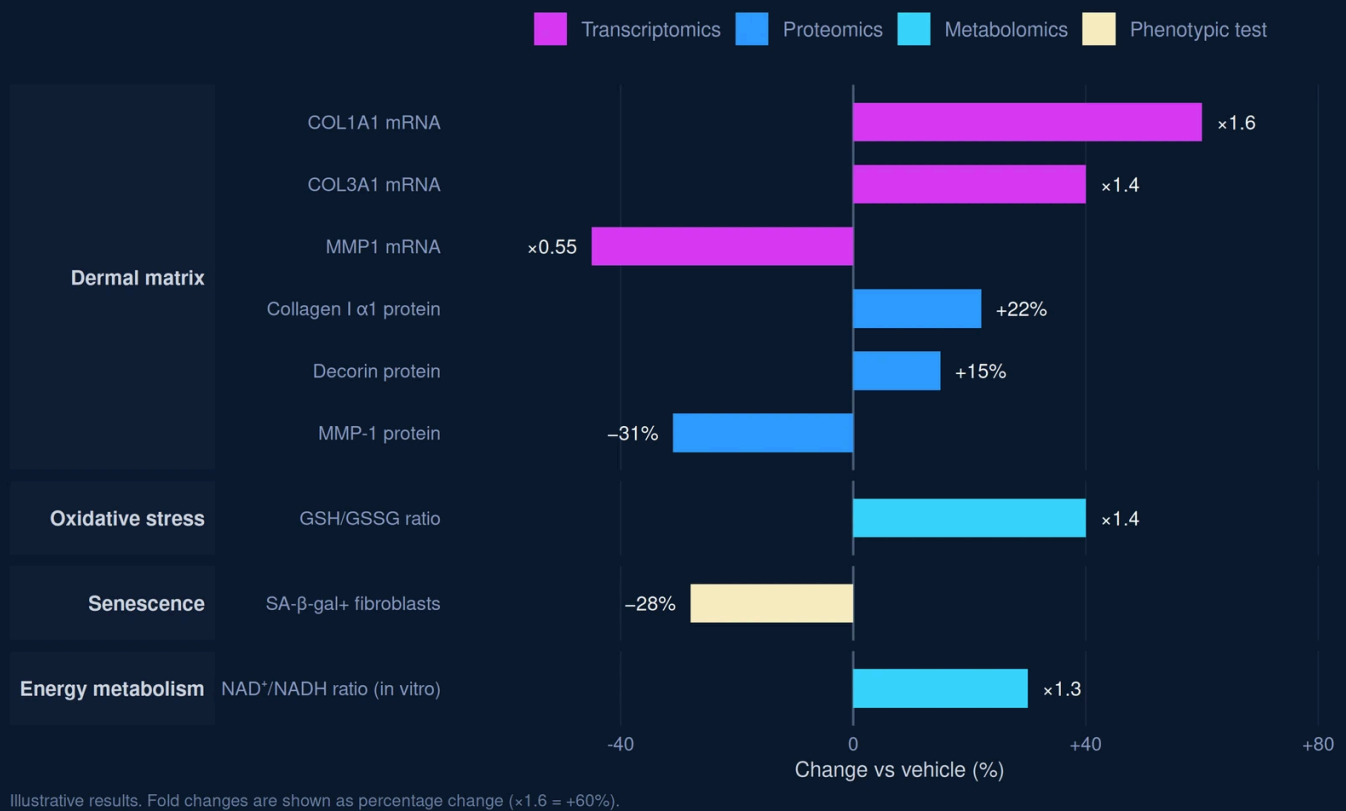
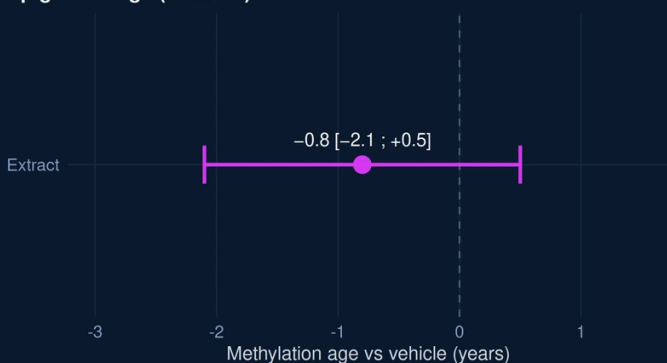


Figure 6. Selected effects of the extract. Change versus vehicle for key readouts, coloured by data layer and grouped by pathway: collagen and decorin up and MMP-1 down at gene and protein level, a higher GSH/GSSG ratio (antioxidant capacity), fewer senescent fibroblasts, and a higher NAD⁺/NADH ratio in vitro. Fold changes are shown as percentage change (x1.6 = +60%). Illustrative results.

What the extract does not do

Epigenetic age (4 weeks)



Illustrative results. Bar = 95% CI.

Matrix response vs retinol



Figure 7. What the extract does not do. Left: after 4 weeks, methylation age is 0.8 years lower than with vehicle, but the 95% CI (-2.1 to +0.5) includes zero, so no effect on epigenetic age is claimed. Right: the extract's matrix response is close to retinol's (NES 2.1 versus 2.4), without the retinoid signature that retinol carries. Illustrative results.

94 Interpret: mechanism of action and evidence for claims

Mechanism of action. Taken together, the data support a three-part mechanism: (1) activation of the NRF2 antioxidant response, limiting UV-induced oxidative damage; (2) attenuation of the senescence-associated secretory phenotype, reducing matrix degradation by MMPs; and (3) support of collagen and

decorin synthesis in the dermis. A retinoid-response signature was not detected: the extract reproduces part of the matrix response observed with retinol through a distinct molecular profile.

TABLE 3. FROM EVIDENCE TO CLAIMS

Candidate claim (model wording)	Evidence	Conclusion
“Helps preserve the dermal matrix of UV-exposed human skin (ex vivo)”	Collagen I and decorin ↑ at gene and protein levels; MMP-1 ↓; collagen staining ↑	Supported: 3 layers, 2 models
“Helps protect skin cells against oxidative stress (in vitro, ex vivo)”	NRF2 targets ↑; GSH/GSSG ↑; oxidized lipids and ROS ↓	Supported: 2 layers + phenotype
“Reduces markers of cellular senescence (in vitro)”	SA-β-gal ↓; SASP transcripts and secreted proteins ↓	Supported
“Acts through a non-retinoid molecular profile”	No retinoid-response signature detected, unlike the retinol comparator	Supported: mechanistic positioning
“Supports cellular energy metabolism (in vitro)”	NAD ⁺ /NADH ↑ in fibroblasts only	Restricted: one layer, one model; wording strictly limited to in vitro
“Does not significantly alter the composition or functional profile of a skin microbial community in this model”	No statistically significant change in composition or function in colonized 3D skin	Restricted: model-specific; volunteer study required for an in vivo claim
“Reverses epigenetic / biological age”	−0.8 years; 95% CI −2.1 to +0.5	Not supported: not claimed

Under Commission Regulation (EU) No 655/2013, cosmetic claims must meet six common criteria, including evidential support and honesty. Laboratory models can substantiate mechanistic claims framed as in vitro or ex vivo findings; consumer-perceivable efficacy claims, such as visible wrinkle reduction, require studies in volunteers. Final claim wording remains the responsibility of the client’s regulatory team.

What the client received

- A **claims–evidence matrix** linking each candidate claim to its data, model and level of support.
- A **mechanism-of-action summary** and publication-ready figures for the technical dossier, customer presentations and Product Information File (PIF).
- **Integrated, quality-controlled datasets** (FASTQ, raw MS files, processed matrices) and a complete statistical report.
- A **documented list of claims not supported by the data**, including epigenetic-age reversal and NAD⁺ statements extending beyond the in vitro model, helping protect the brand from poorly substantiated claims.
- A **recommendation for the next step**: a volunteer study using tape strips and swabs to connect the molecular findings with in vivo skin.

INDICATIVE TIMELINE (APPROXIMATELY 16 WEEKS)

Weeks 0–2	Study design, model selection, cytotoxicity and dose finding
Weeks 2–7	2D and 3D treatments; explant collection and culture (subject to tissue availability)
Weeks 5–12	Omics data generation: RNA-seq, proteomics, metabolomics, methylation, metagenomics
Weeks 10–14	Layer-specific analysis, integration and mechanism inference
Weeks 14–16	Claims–evidence matrix, final report and figures

Why this approach works

- **Start from the claim, then select the model.** Each model level answers a different question; together, they build the evidence package.
- **Measure more than one layer.** RNA, proteins and metabolites each tell part of the story; convergence across layers makes a mechanism more credible.
- **Define pathways before looking at the data.** Predefined aging modules reduce the risk of building a narrative after seeing the results.
- **Benchmark against a reference.** A retinol arm shows how an active compares with a well-documented reference and where its biological profile differs.
- **State what the data do not support.** Excluding a weak claim costs nothing in the laboratory; an indefensible claim can be costly for a brand.

Beyond this example

The same approach can be applied to other cosmetic and dermatology questions:

- **Other claims:** barrier repair and hydration (ceramide lipidomics, epidermal differentiation and barrier markers such as FLG, LOR and CLDN1), soothing and sensitive skin (neurosensory and inflammatory mediators), tone evenness (melanogenesis), protection from pollution and blue light, and microbiome balance.
- **Other models:** pigmented or immunocompetent 3D skin, hair follicles, sebocytes, co-cultures and microbiome-colonized models.
- **Bridge to in vivo:** tape strips (transcriptomics, proteomics, lipidomics) and swabs (metagenomics) collected from volunteer panels to connect laboratory mechanisms with real skin.
- **Ingredient types:** botanical and fermented extracts, peptides, postbiotics and other biotechnology-derived actives.

References

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Need molecular evidence for a claim?

Tell us about your active ingredient, target claim and available models. We will propose an evidence plan, model ladder, omics layers and comparators, before processing a single sample. genxmap.com/contact