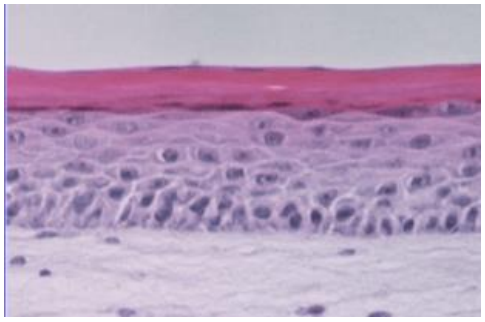
IN VITRO AND *EX VIVO* SKIN MODELS

---- reliable preclinical tests for cosmetic and dermatological products

Axela Biosciences Inc. is a specialized research provider based in New Jersey, committed to supporting life science researchers dedicated to transforming lives through science. We offer advanced models to drive drug discovery and translational research in chronic immuno-inflammatory diseases, including atopic dermatitis, psoriasis, discoid lupus, scleroderma, and other conditions.

In the cosmetics sector, we deliver customized *in vitro* and *ex vivo* testing to substantiate specific product claims. Our core strength lies in close collaboration with our clients, powered by state-of-the-art research facilities and services. Our multidisciplinary team, composed of cell and molecular biologists, immunologists, and dermatologists, collaborates with clients to develop innovative biological models that demonstrate potential cosmetic activities for ingredients or formulas.

Animal models have limitations in dermo-cosmetic testing as they do not accurately replicate the distinctive structure of human skin. *In vitro* and *ex vivo* models using human cells offer valuable alternatives, allowing researchers to study a range of conditions more closely aligned with human skin biology, which supports the development of both cosmetics and therapeutic drugs.



Compared to *in vitro* models, *ex vivo* skin models often provide a more realistic representation of human skin, especially regarding the extracellular matrix (ECM) structure, cell signaling, metabolic processes, and the presence of skin appendages like hair follicles and sweat glands. *Ex vivo* human skin is particularly well-suited for research requiring whole skin biopsies, enabling the assessment of individual ingredients and formulations, as well as transdermal delivery, topical penetration, and percutaneous absorption, all within an environment that closely mirrors normal human skin.

Axela scientists offer highly predictive and cost-effective *in vitro* and *ex vivo* human skin models, along with a range of ingredient and formulation screening services conducted under controlled laboratory conditions. These services provide valuable insights prior to clinical evaluations for cosmetic and dermatological products. Below are some of the research materials and models available to support our clients' diverse needs in the cosmetics sector.

CELLS AND SKIN MATERIALS AVAILABLE FOR TESTING

- **Skin related cell lines**: keratinocytes (HaCAT), melanocytes (B12, HMel-1, HMel-3), immune cells (THP-1), epidermal squamous cells, tracheobronchial epithelial cells, hTERT-immortalized keratinocytes.
- **Primary skin cells** obtained from different types of donors and kept in a 2D culture or co-culture (fibroblasts + keratinocytes with or without stimulation with IL-1β +TNF-β +IFN-β +TGF-β).
- **Primary skin associated immune cells:** such as mast cells, dendritic cell, T cells, etc.
- **Epidermis and dermis spheroids** (3D-cultured primary cells that reflect the micro-environment of human skin more adequately)
- **Tissue engineered human skin equivalent** / human reconstructed tissues: EpiDerm™, MelanoDerm™, EpiDermFT™
- **D-Squame skin tape**: for proteo-transcriptomic analysis of inflammatory cytokines, markers of skin health, etc.
- **Skin models**: NativeSkin
- **Skin biopsies:** frozen tissues, FFPE

IN VITRO AND EX VIVO MODELS AND READOUTS

Skin integrity test

- Evaluation of tissue integrity with hematoxylin and eosin (H&E) staining to examine spongiosis, parakeratosis, necrosis and epidermal/dermal separation
- Examination of tissue metabolism through gene expression markers (r18s, CDKN2A, KRT14, KRT16, TNFα, IFN-γ, MMP9, MMP12, IVL, LOR, Col1a2, and IL-1α, etc.)
- Measurement of trans-epithelial electrical resistance (TEER)
- Evaluation of the formation of functional barrier as a result of differentiation using protein or RNA expression of specific differentiation markers in 2D or 3D skin models

Skin inflammation test

- Loricrin and filaggrin expression by immunohistochemistry (IHC) staining
- Immune cell activation/infiltration (T cells, macrophages mast cells, all immune cells) by IHC staining
- Multiplex immunoassays to quantify cytokine release (IL-1α, IL-1β, TNFα, IFN-γ, ICAM1, MIP1β, MCP-1, IP-10, MMP-9, uPA) and angiogenic growth factor release (Angiopoietin-1, EGF, FGF2, CXCL1)

Skin pigmentation test

- Microscopic and macroscopic darkening analysis
- Histology analysis

- Melanin synthesis using a colorimetric assay,
- Tyrosinase expression and activity using a colorimetric assay to examine treatment with active ingredients for whitening or tanning application

Phototoxicity test

- Morphological changes after UVA or UVB exposure by H&E staining of skin samples and DNA damage by IHC staining.

Genotoxicity test

- Examination of DNA damage pathway activation (e.g. p53 activation).

Cosmetogenomic screening

- Barrier function (differentiation, adhesion, lipids synthesis)
- Elasticity
- Firmness
- Extracellular matrix protection
- Pigmentation
- Anti-microbial characteristics
- Moisturizing effect
- Soothing, calming or anti-inflammatory properties
- Antioxidant defense
- Energetic metabolism
- Detoxifying properties
- Longevity

Oxidative stress test

- Glutathione metabolism
- Telomere length measurement (RT-PCR)
- AGE formation (Advanced Glycation End) assay
- DPPH free radical and hydrogen peroxide scavenging assays
- ROS assays
- Nitrite and nitric oxide metabolism assays
- Oxidase/peroxidase assays
- Poly (ADP-ribose) Polymerase (PARP) assays

Wound healing test

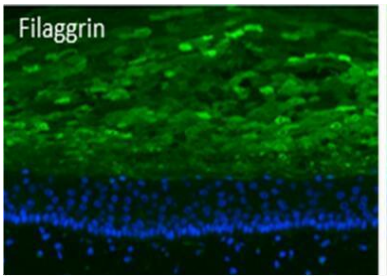
- Scratch assay with monolayer of keratinocytes or dermal fibroblasts using live cell imaging
- Evaluation of gene expression of pro-inflammatory cytokines, growth factors, transcription factors, heat sock protein desmogleins and collagen markers (IL1A, IL6, CXCL8, TGFB1, PDGFC, NFKB1, TP53, HSP90AA1, HSPA1A, HSPD1, DSG1, DSG3, COL1A1, COL3A1)

Anti-aging

- Aging-related enzyme assays (elastase, collagenase, hyaluronidase, and tyrosinase)
- Proliferation (Ki67) and differentiation (Keratin 10 and Filaggrin) assays
- Immunoassays to quantify senescence-associated secretory phenotype (SASP) factors (IGFBP6, IGFBP2, CCL4, IL-1β, GM-CSF, PLGF, Angiogenin, MIF-1, MIP-1A, Gro-α, IL-6, MCP-4, Gp130, ICAM-1, MCP-1, IL-8, MIP-3A, Osteoprotegerin, TIMP-1, uPAr, TNFRI, and TNFRII)
- Beta-Galactosidase activity (correlates with senescence of the cells)
- mRNA expression of anti-aging biomarkers (Collagen 1A1, Collagen 3A1, Elastin, MMPs, TIMPs, Pro-collagen, Hyaluronic Acid, Inflammatory Mediators)

Epidermal and dermal markers

- Expression of specific differentiation markers in 2D or 3D models
- Collagen production in human dermal fibroblasts, MMPs activities (enzymatic assays), and expression (mRNA or protein levels).



NativeSkin model

This model provides the closest alternative to direct testing on human skin. Human skin biopsies are maintained within a patented matrix, preserving their viability and functionality for up to 7 days.

In the NativeSkin model, a full-thickness human skin biopsy is embedded in a solid, nutrient-rich matrix, with the epidermal surface exposed to air. This design keeps the biopsy stable within the matrix, preventing any lateral diffusion of topically applied formulations. NativeSkin serves as a robust platform for studying the response of live human skin to compounds, drugs, cosmetics, and medical devices, following either topical or systemic administration.

EpiDerm model

EpiDerm is an advanced, laboratory-grown human tissue model that serves as a reliable, ethical alternative to animal testing, ideal for dermatology and skincare applications. By mimicking the structure and function of human skin, EpiDerm provides precise insights into skin responses, drug efficacy, and cosmetic product safety, with multiple regulatory validations supporting its use.

The EpiDerm model replicates in vivo-like morphology and growth characteristics, exhibiting uniformity and high reproducibility. It comprises organized basal, spinous, granular, and cornified layers, closely mirroring those in human skin. EpiDerm is both mitotically and metabolically active and expresses markers of epidermal differentiation, such as profilaggrin, the K1/K10 cytokeratin pair, involucrin, and type I epidermal transglutaminase. Ultrastructural analysis further reveals the presence of keratohyalin granules, tonofilament bundles, desmosomes, and a multi-layered stratum corneum with intercellular lamellar lipid layers arranged similarly to those in vivo. With its close parallels to human skin, EpiDerm offers a robust in vitro platform for evaluating dermal irritancy, toxicology, and other skin-related assessments.

Psoriasis skin model

The co-culture of keratinocytes, fibroblasts, dendritic cells, and T-cells enables the screening of candidate drugs and enhances our understanding of the biological mechanisms underlying psoriatic disease.

The 3D Psoriasis Skin Model offers an intermediate approach between traditional cell cultures and animal models. Unlike standard cell cultures, this model more accurately replicates the cell differentiation and metabolic changes characteristic of psoriatic lesions. Additionally, 3D models avoid nonspecific influences from surrounding organs and tissues, making them a preferred choice over animal models for some studies, as animal skin differs significantly from human skin. This model is well-suited for drug screening and evaluating new pharmacological strategies.

Advantages of our psoriasis skin model:
- Psoriatic human tissue phenotype
- 3-dimensional, highly differentiated
- Highly reproducible and ready to set up
- Ideal for drug screening and basic researches
- Cost effective alternative to animal and clinical testing
- Similar to *in vivo* psoriasis skin conditions

THE FEATURES OF OUR *EX VIVO* SKIN MODELS

Ex vivo skin explants represent an experimental model that closely replicates both health and diseased skin physiology. These models retain the complete native skin cell population, including keratinocytes, melanocytes and Langerhans cells, alongside a dermal matrix containing fibroblasts, collagen, elastin, glycosaminoglycans, and other components. Standardized in thickness and size and cultured at the air–liquid interface (ALI), these explants are invaluable for studying mechanisms underlying cutaneous disorders and assessing the effects of topically applied products.

In collaboration with our partners, Axela scientists offer a range of *ex vivo* human skin models. Our skin explants enable the evaluation of compounds, chemicals, cosmetic ingredients, and final formulations across various applications, including:

- Anti-Aging
- Pigmentation
- Stress / Inflammation
- UV Protection

Our *ex vivo* skin models are suited for diverse testing purposes such as efficacy assessments, topical applications, dermal and epidermal studies, percutaneous absorption, metabolism, long-term studies, repeated-dose assays, as well as investigations into Langerhans cells, skin-resident T cells, immune responses, melanogenesis, and melanocyte permeability studies.

Advantages of our *ex vivo* human skin models include:

- Models are highly relevant to clinical testing conditions
- Tissues are excised from different body areas
- Allows for investigating of both systemic administration and true topical application for skin absorption and barrier function studies.
- Donor customization available such as restrictions regarding age, gender or phototype,
- 2D or 3D dermatoscope available

OUR UNIQUE TECHNOLOGY PLATFORMS

NanoString GeoMx Digital Spatial Proteomics and Genomics

- GeoMx DSP merges multiplex immunofluorescence (IF) and in situ hybridization (ISH) with digital optical barcoding and subsequent next-generation sequencing (NGS) to perform protein and transcriptomic profiling on intact tissues
- Digital Spatial Profiling for in situ expression of hundreds of proteins and RNAs in different skin tissue compartments or cell population in skin biopsies.

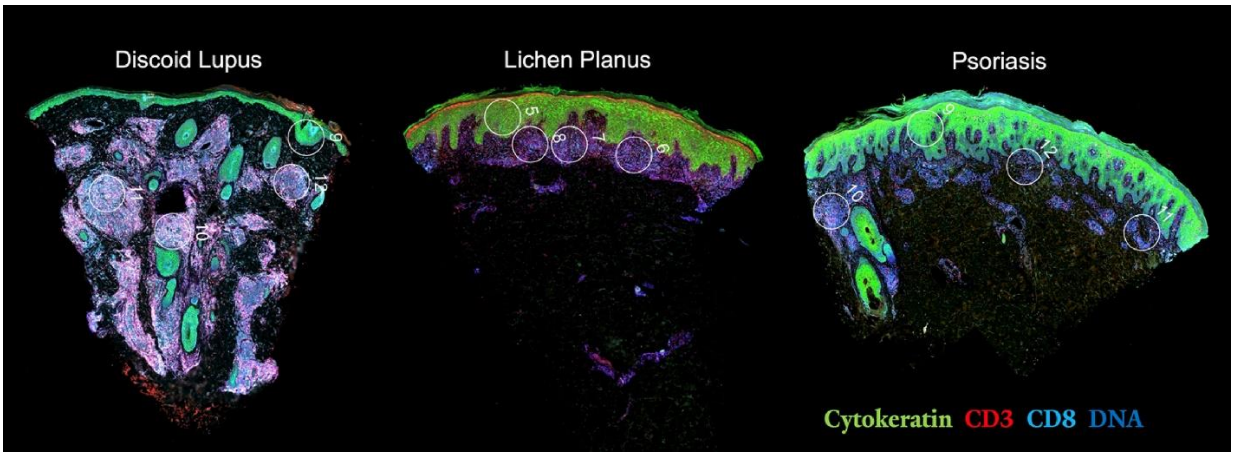
NanoString nCounter Transcriptomics

- Get insights into immune system dysfunction quickly with a comprehensive 770 gene multiplex panel for human or mouse to evaluate the pathways, processes, and cell types involved in chronic inflammatory disease, and dermatology-related conditions.
- Development of predictive signatures of response to various treatments
- Compatible with challenging sample types such as PBMCs and FFPE

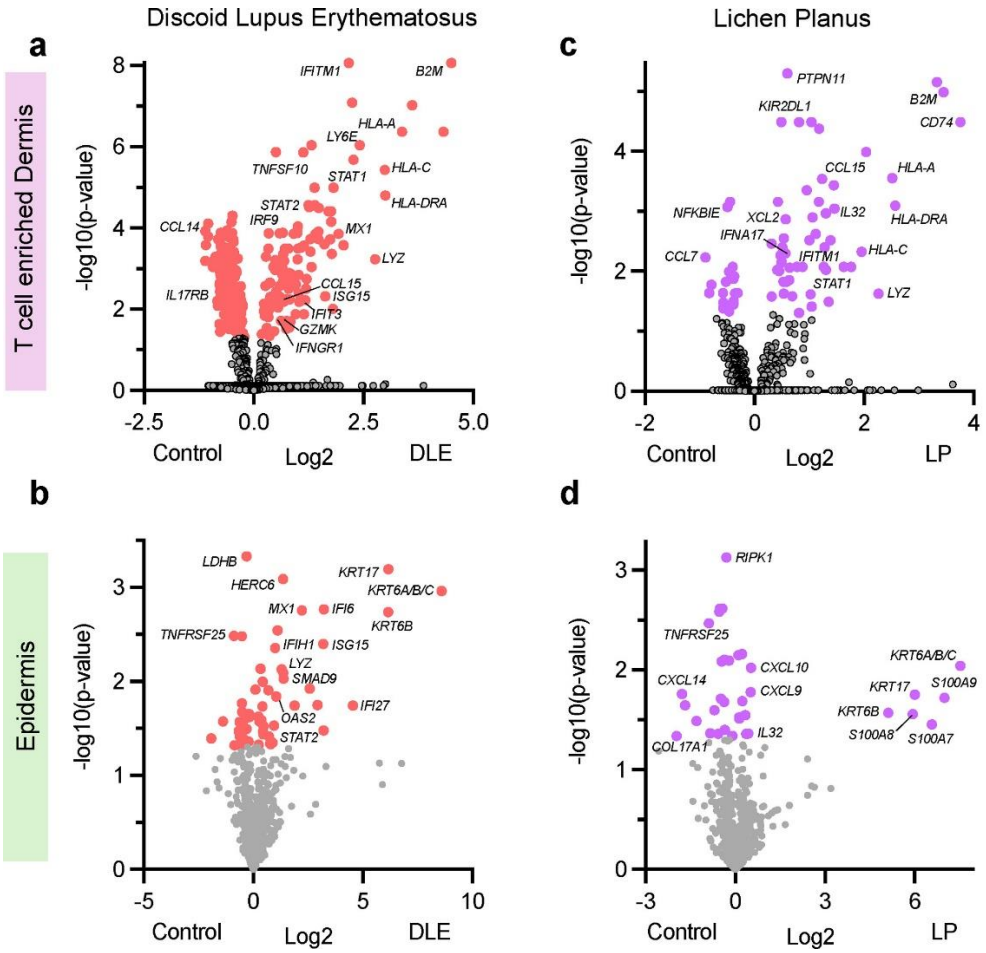
OTHERS TO SUPPORT R&D OF COSMETIC AND DERMATOLOGICAL PRODUCTS

- qPCR array, RT-PCR
- Multiplex IHC
- Multicolor flow cytometry
- Epidermal separation
- Immunofluorescence staining
- Luminex multiplex immunoassay
- RNA and Protein extraction and quantitation
- Live cell imaging system
- 2D or 3D dermatoscope
- Automatic western blot (quantitation of any biomarker)

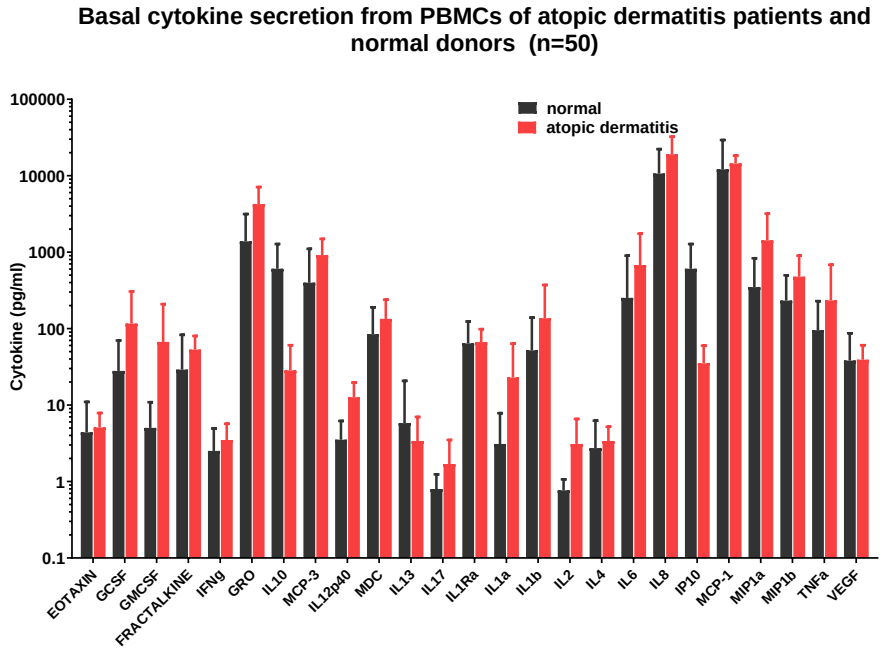
DATA EXAMPLES



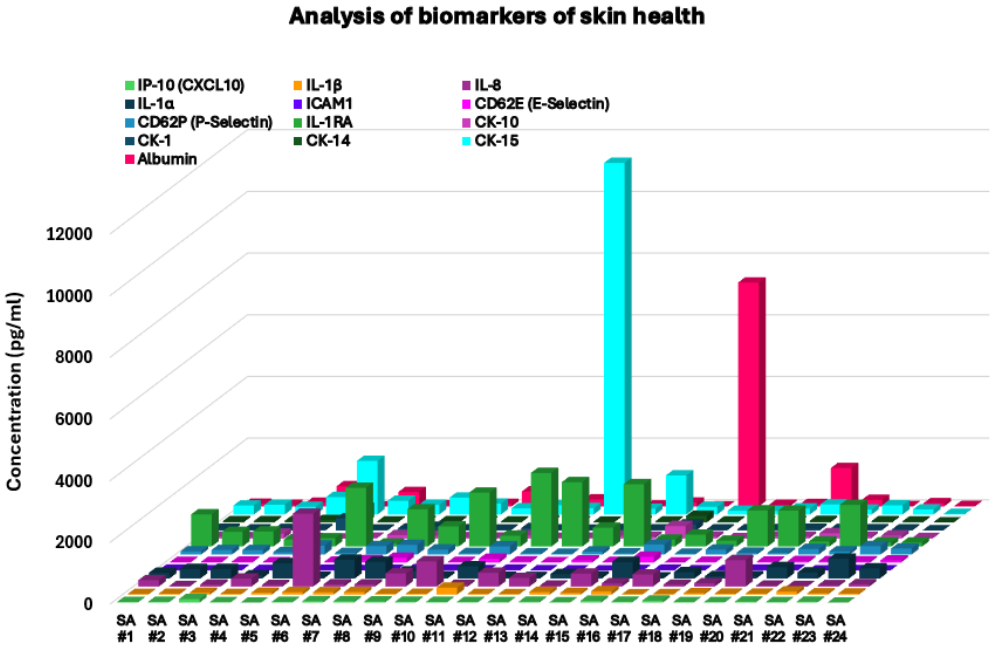
Example 1. Visualization of T lymphocytes using CD3 and CD8 as morphology markers for selection of ROIs (region of interest). Representative images of discoid lupus, lichen planus and psoriasis after scanning image in GeoMx DSP instrument. Tissues were stained with pan-cytokeratin (green), CD3e (red), CD8a (cyan), and Syto83 (DNA, blue) for visualization and ROI selection. Each ROI is a geometric circle measuring 300μM in diameter.



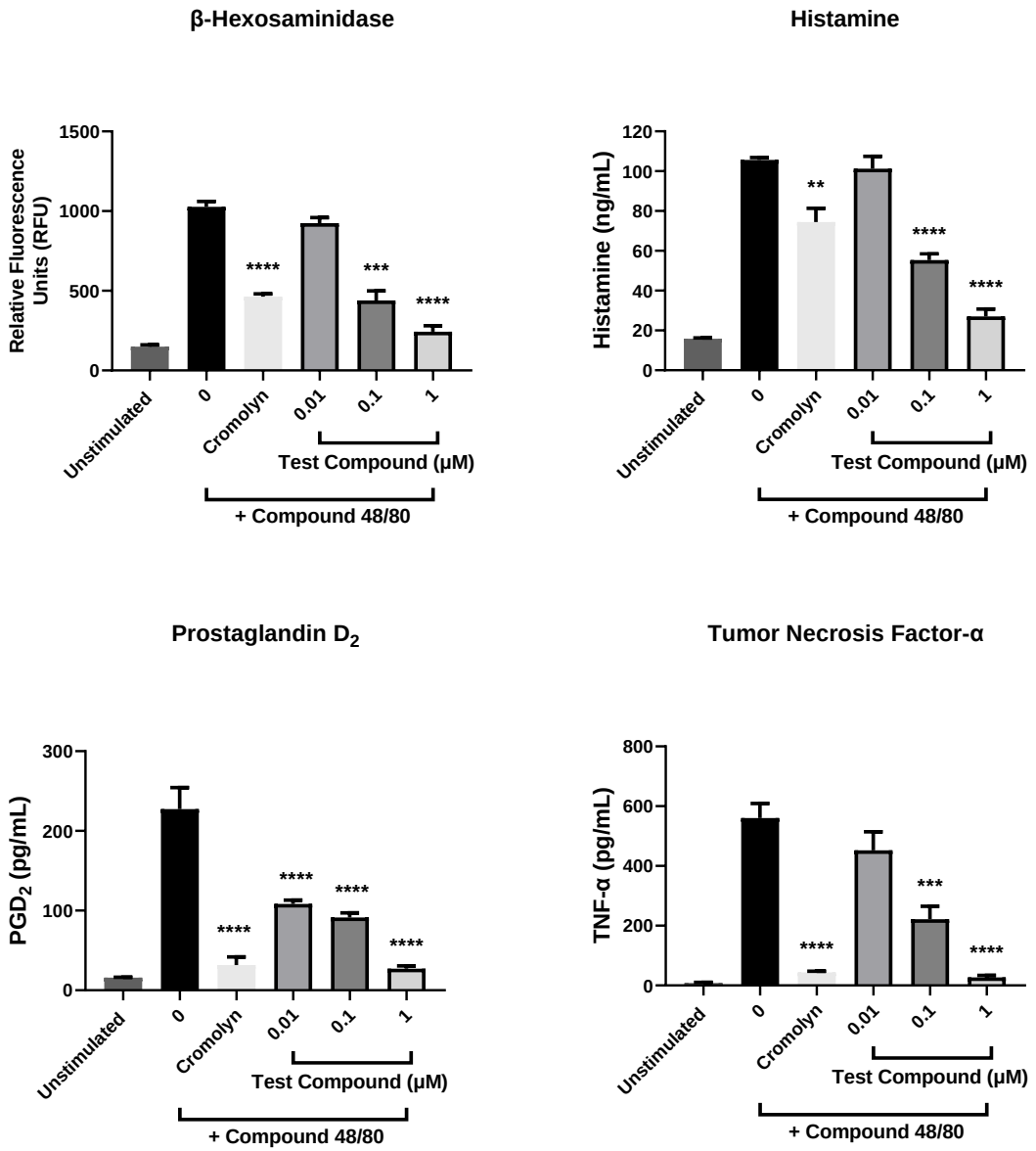
Example 2. Differential gene expression of discoid lupus erythematosus and lichen planus compared to controls. Significant genes colored (FDR<0.05). **Left:** (DLE vs healthy controls**)** volcano plot comparing CD3/CD8-enriched ROIs in dermis (a) and Pan-CK positive ROI in epidermis (b). **Right:** (LP vs healthy controls) volcano plot comparing CD3/CD8-enriched ROIs in dermis (c) and Pan-CK positive ROI in epidermis (d)



Example 3. Differential cytokine/chemokine secretion from PBMC cell cultures from 50 atopic dermatitis patients and 50 normal donors. PBMCs were cultured in RPMI-1640/10%FBS medium for 24 h and cell culture supernatants were collected for Luminex-based multiplex immunoassays.



Example 4. Characterization of skin surface biomarkers from minimally invasive D-Squame® tape skin samples (Stratum Corneum Tape Stripping). Luminex-based immunoassays were used for analysis of various inflammatory cytokines and protein markers of keratinocytes differentiation (CK1, 10, 11) and barrier integrity (Albumin) in extracts obtained from D-Squame ® strips from 24 human subjects (n=24).



Example 5 Effect of a cosmetic ingredient on Compound 48/80 induced mast cell degranulation
Normal skin-derived mast cells were incubated with varying concentrations of test compound or cromolyn (mast cell stabilizer) during stimulation with compound 48/80 (mast cell activator). After treatment, β-hexosaminidase, histamine, prostaglandin D2 and tumor necrosis factor (TNF)-α in the cell-free supernatants were quantified. Data shown are mean ± SEM (3 donors). Statistical analysis was performed using one-way ANOVA with pairwise comparisons made to cells that received no compound treatment. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.