MEDICAL UNIVERSITY OF VIENNA

Division of Biology and Pathobiology of the Skin

Use of gene silencing in skin models

Michael Mildner

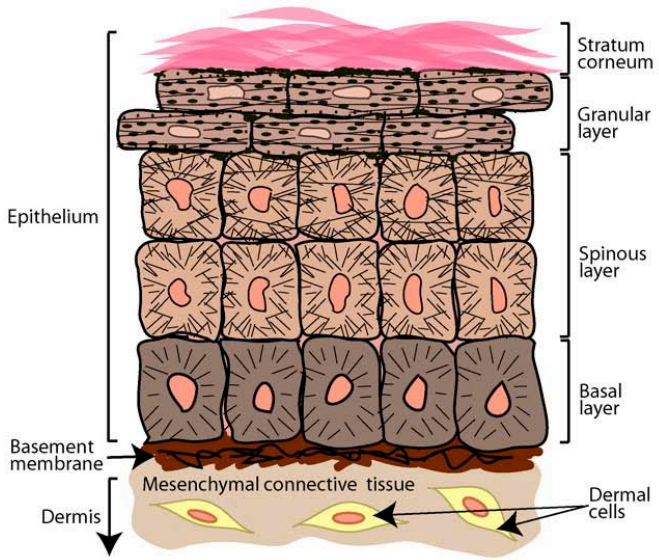
Why gene silencing in an organotypic skin model ?

- It would enable the study of gene deletions in a complex *in vitro* system of human cells.
- We would be able to investigate a direct involvement of target genes in skin development/differentiation, without the influence of other cell types (cells of the immune-system).
- It would strongly reduce the necessity of animal experiments in dermatological research.
- It would reduce time and costs compared to a knock out animal approach.

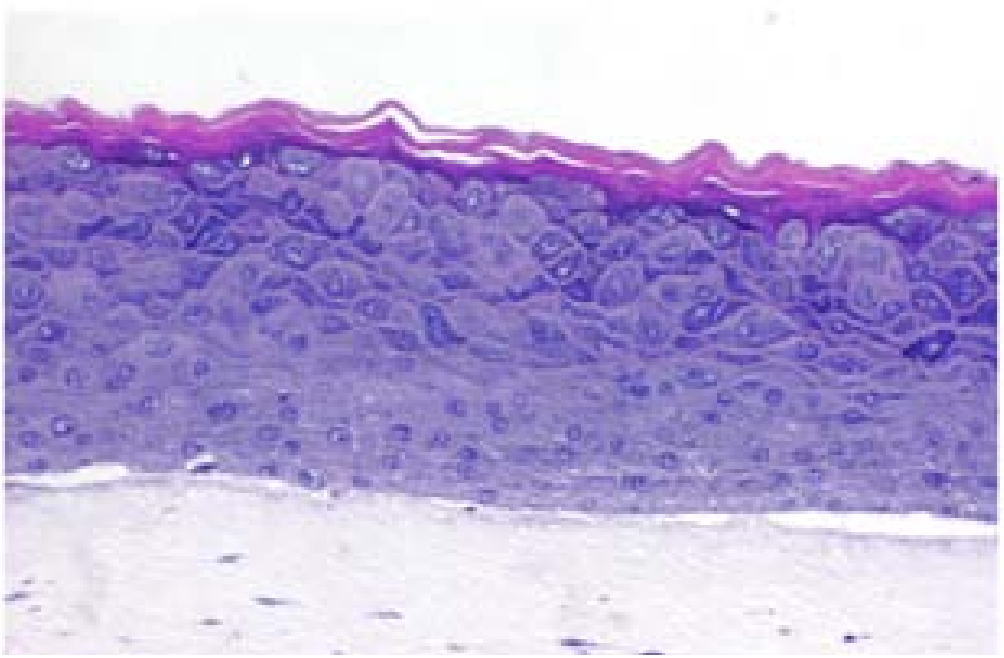
Organotypic skin model

- Differentiating human epidermal keratinocytes
- Growth on a „dermis-like“ support
- Simultaneous analysis of the different steps of terminal KC differentiation
- Studies of the alterations induced by chemicals, pharmaceuticals on this differentiation program

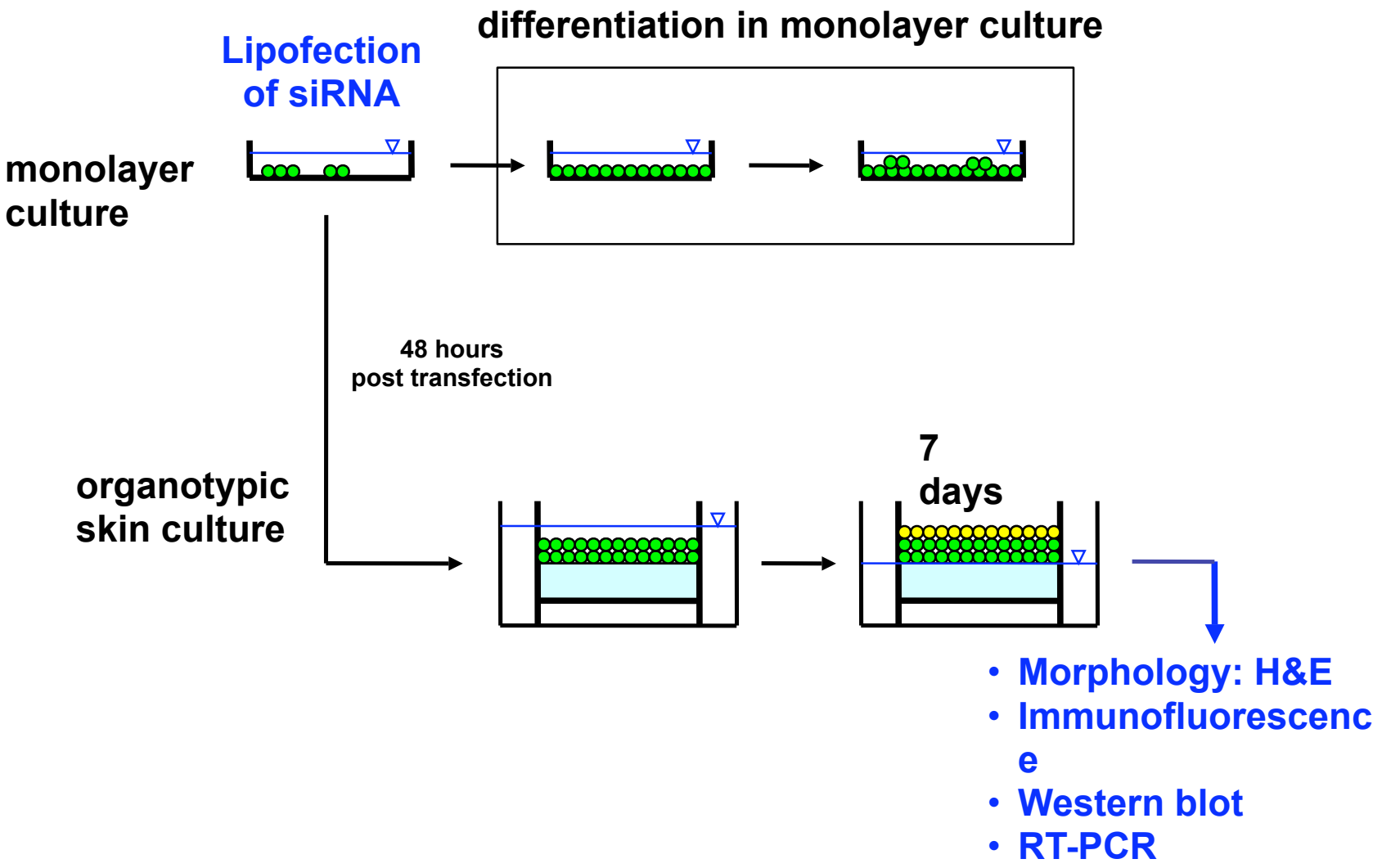
Morphology of the organotypic skin



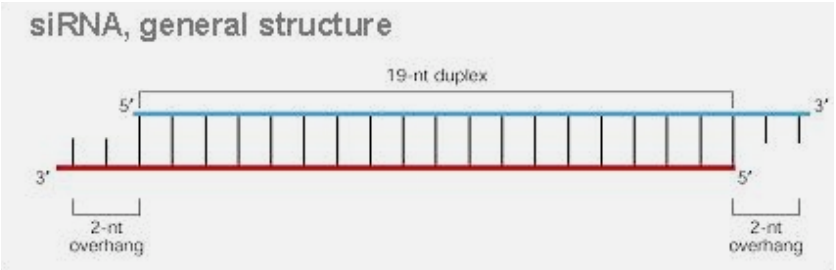
Organotypic skin



Methodology for gene silencing



Gene knockdown by short interfering RNA (siRNA)



siRNA oligonucleotides

transfection with lipofectamin

sequence-specific binding

mRNA

mRNA cleavage by DICER

non-functional mRNA fragments

VEGF
conditional KO-mouse

- No influence on the development of the epidermis

Ref.: Rossiter H et al, Cancer Res. 2004 May

Matriptase-1
KO-mouse

- Mice die 48 hours after birth, due to severe skin problems
- Defect in lipid matrix formation, cornified envelope morphogenesis and stratum corneum desquamation
- Loss of processed filaggrin monomer and filaggrin S-100 protein
- Accumulation of pro-filaggrin
- Transplanted skin shows an ichthyosis like phenotype

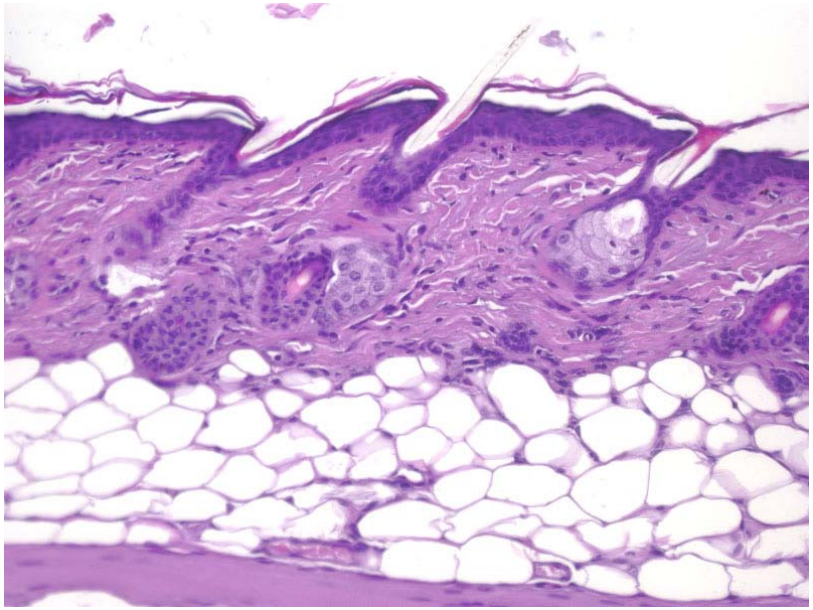
Ref.: List K et al, Oncogene. 2002 May
Ref.: List K et al, J Cell Biol. 2003 Nov

VEGF deficient skin equivalents

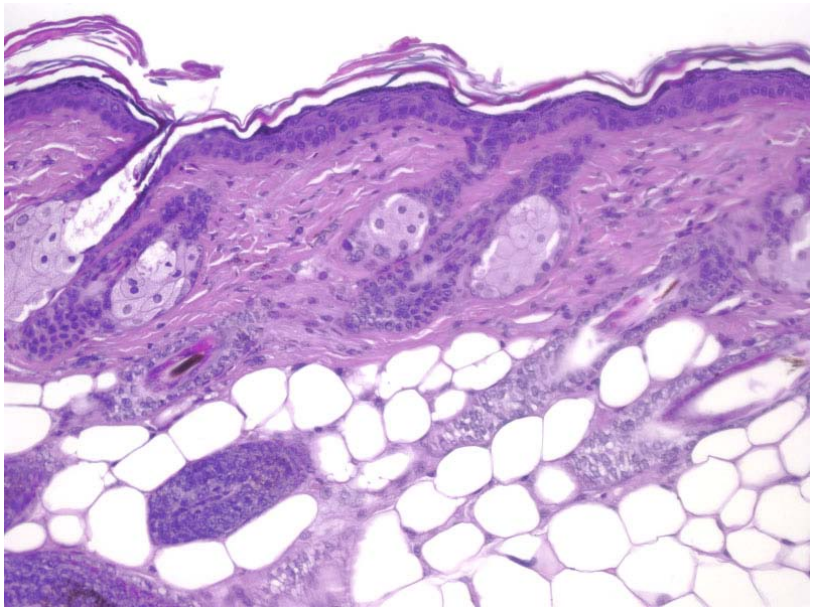
VEGF conditional KO-mouse

normal development of the epidermis

Control

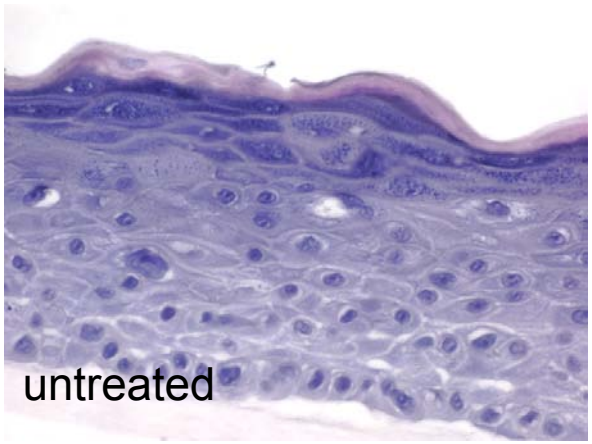


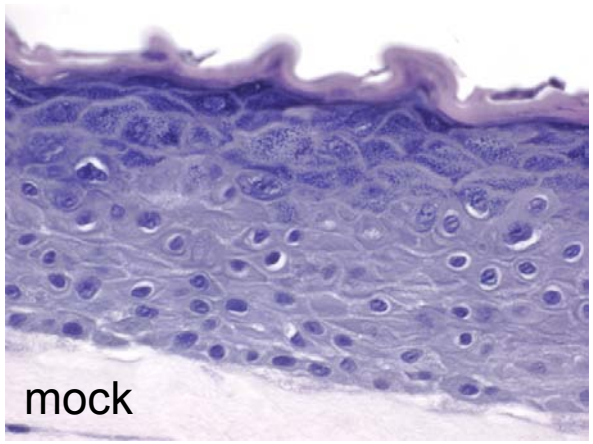
VEGF-deficient

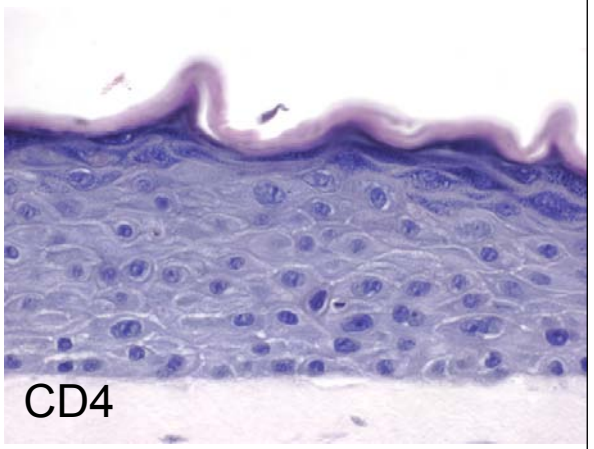


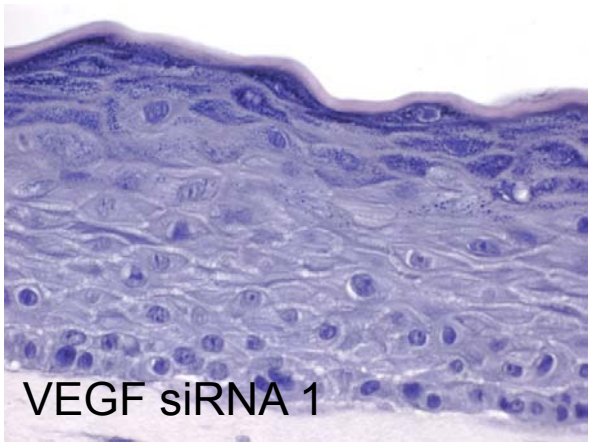
Ref.: Rossiter H et al, Cancer Res. 2004 May

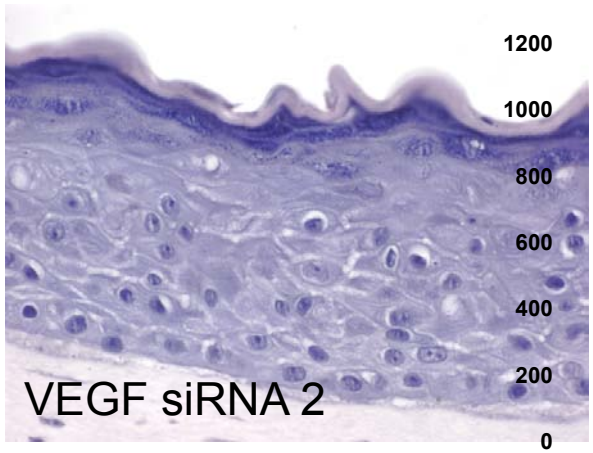
VEGF siRNA in organotypic skin cultures

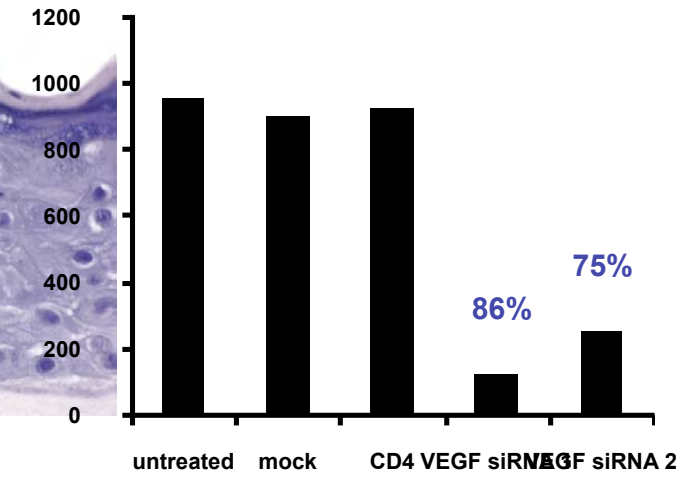












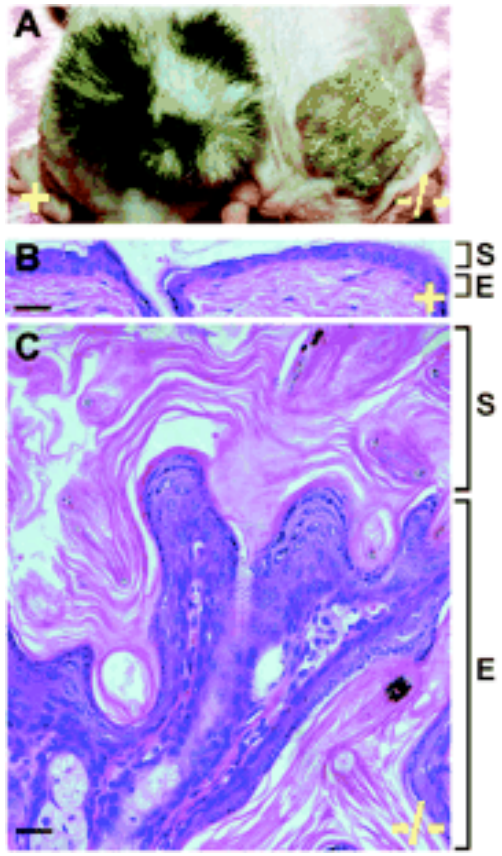
Matriptase-1 deficient organotypic skin cultures

Matriptase-1 KO mouse

Severe skin problems

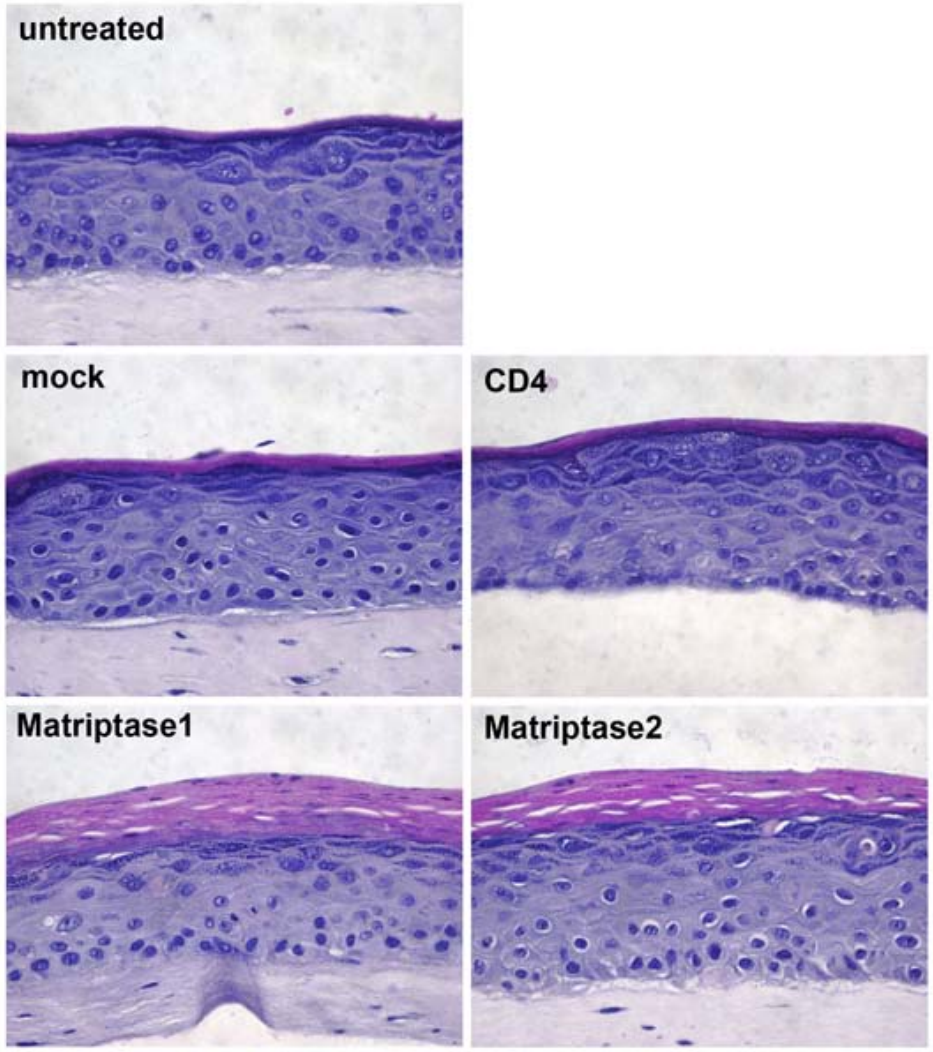
Transplanted skin

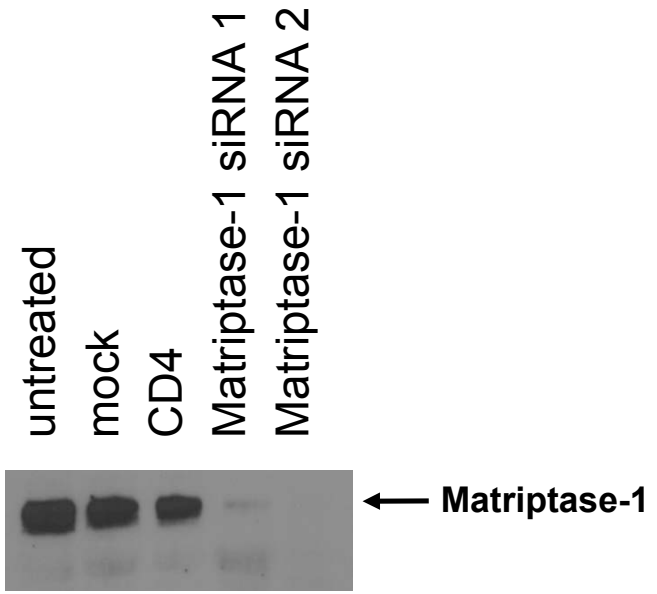
H&E staining



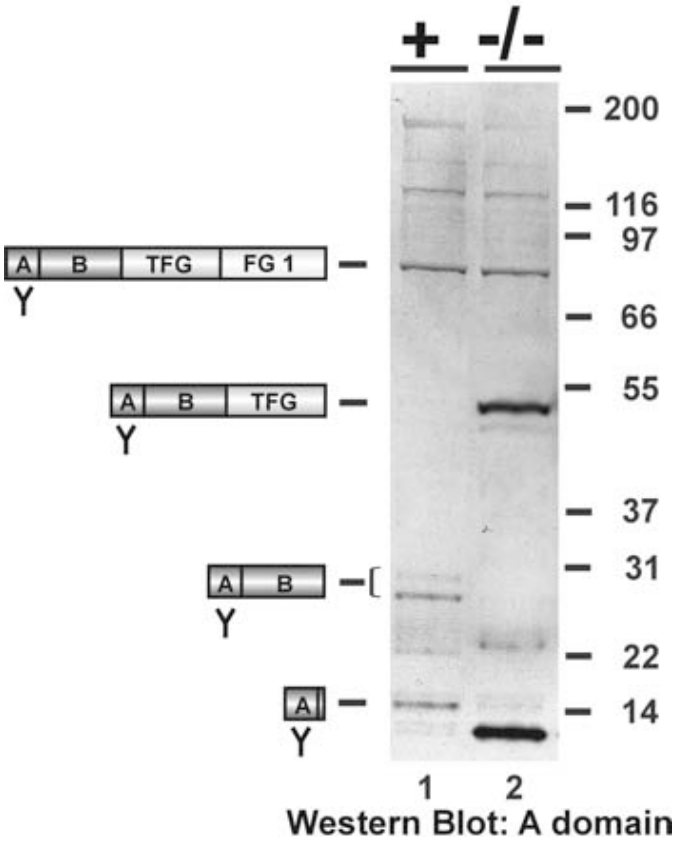
Ref.: List K et al, J Cell Biol. 2003 Nov 24;163(4):901-10.

Matriptase-1 siRNA knock down in organotypic skin cultures





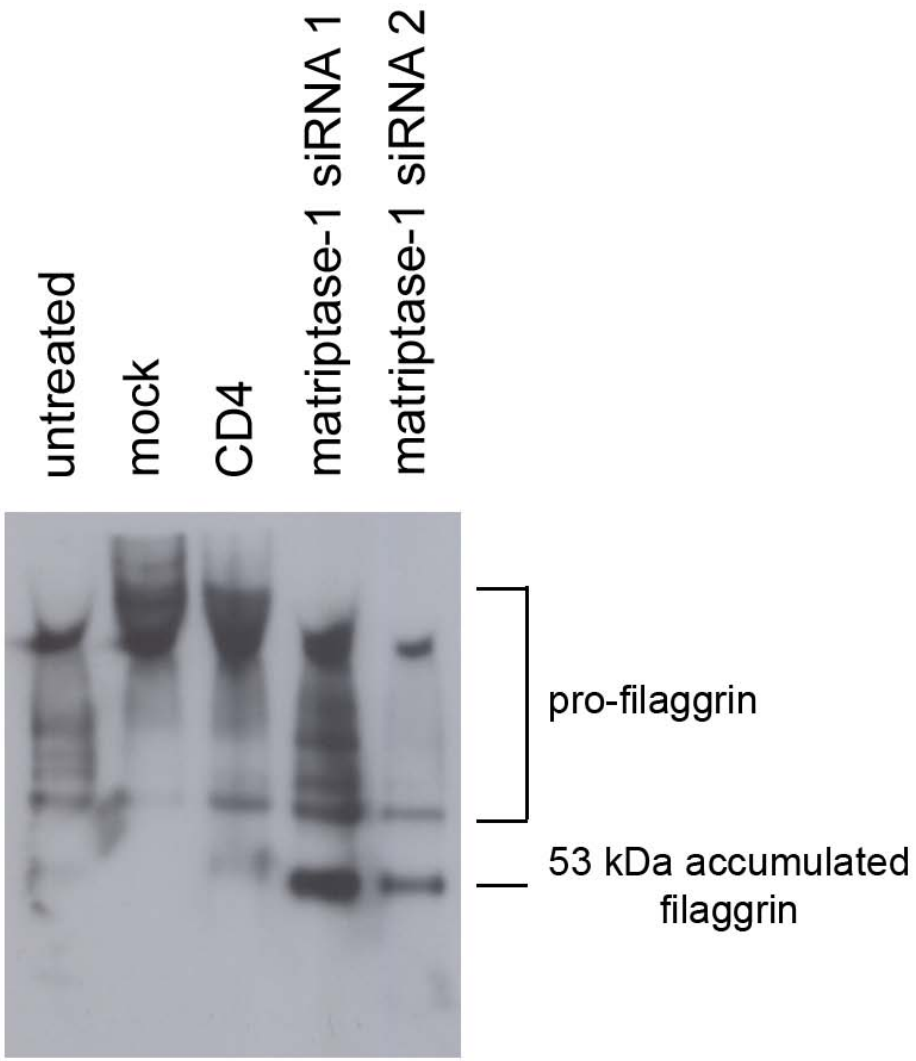
Filaggrin processing



Western Blot: A domain

List K et al, J Cell Biol. 2003 Nov 24;163(4):901-1

Impaired filaggrin processing



Summary

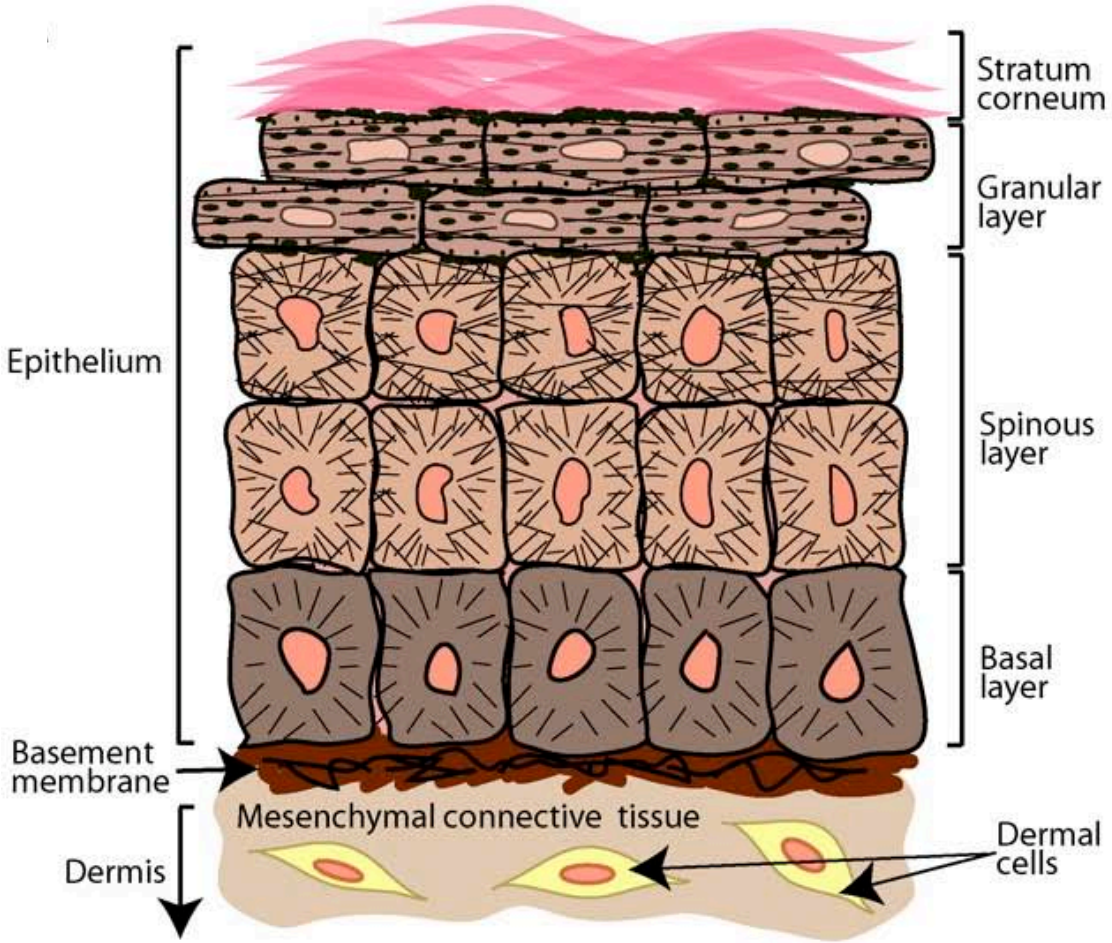
- Organotypic skin cultures of Matriptase-1 and VEGF siRNA treated keratinocytes show comparable results to the corresponding KO-mice.
- Organotypic skin cultures of keratinocytes transfected with VEGF siRNA show:
  - no phenotype
- Organotypic skin cultures of keratinocytes transfected with Matriptase-1 siRNA show:
  - Hyperkeratosis
  - Parakeratosis
  - Impaired filaggrin processing

DNase1L2 degrades nuclear DNA during corneocyte formation

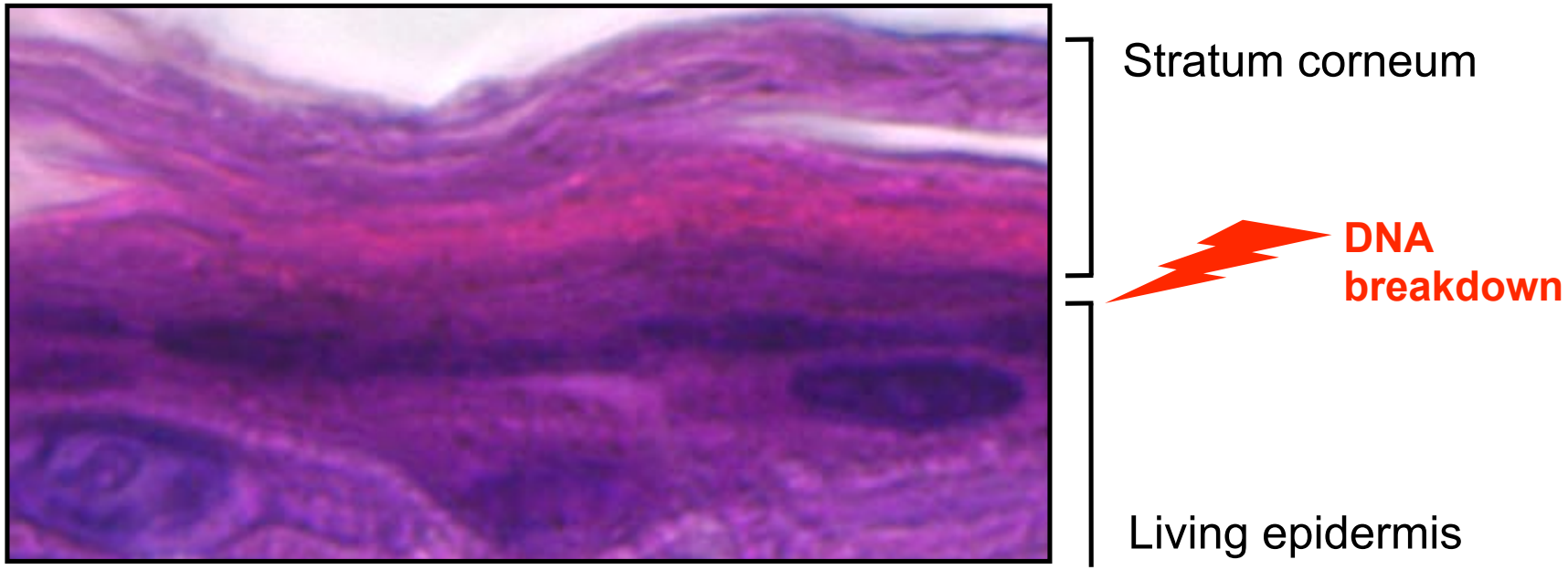
Heinz Fischer, Leopold Eckhart, Michael Mildner, Karin Jaeger, Maria Buchberger, Minoo Ghannadan, Erwin Tschachler

J Invest Dermatol. 2007 Jan;127(1):24-30.

Structure of the epidermis



Nuclear DNA is degraded during formation of the cornified layer

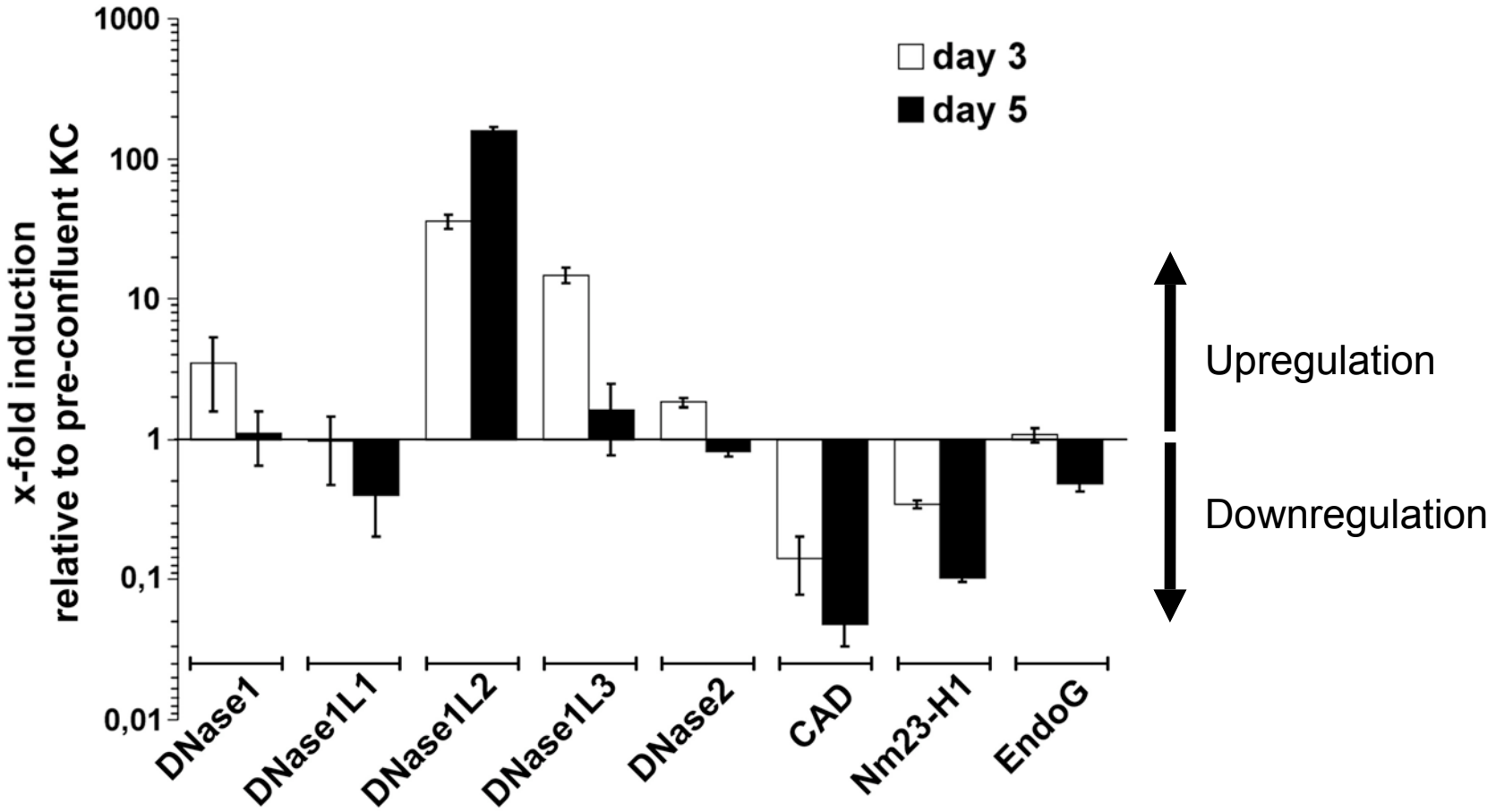


Hypotheses

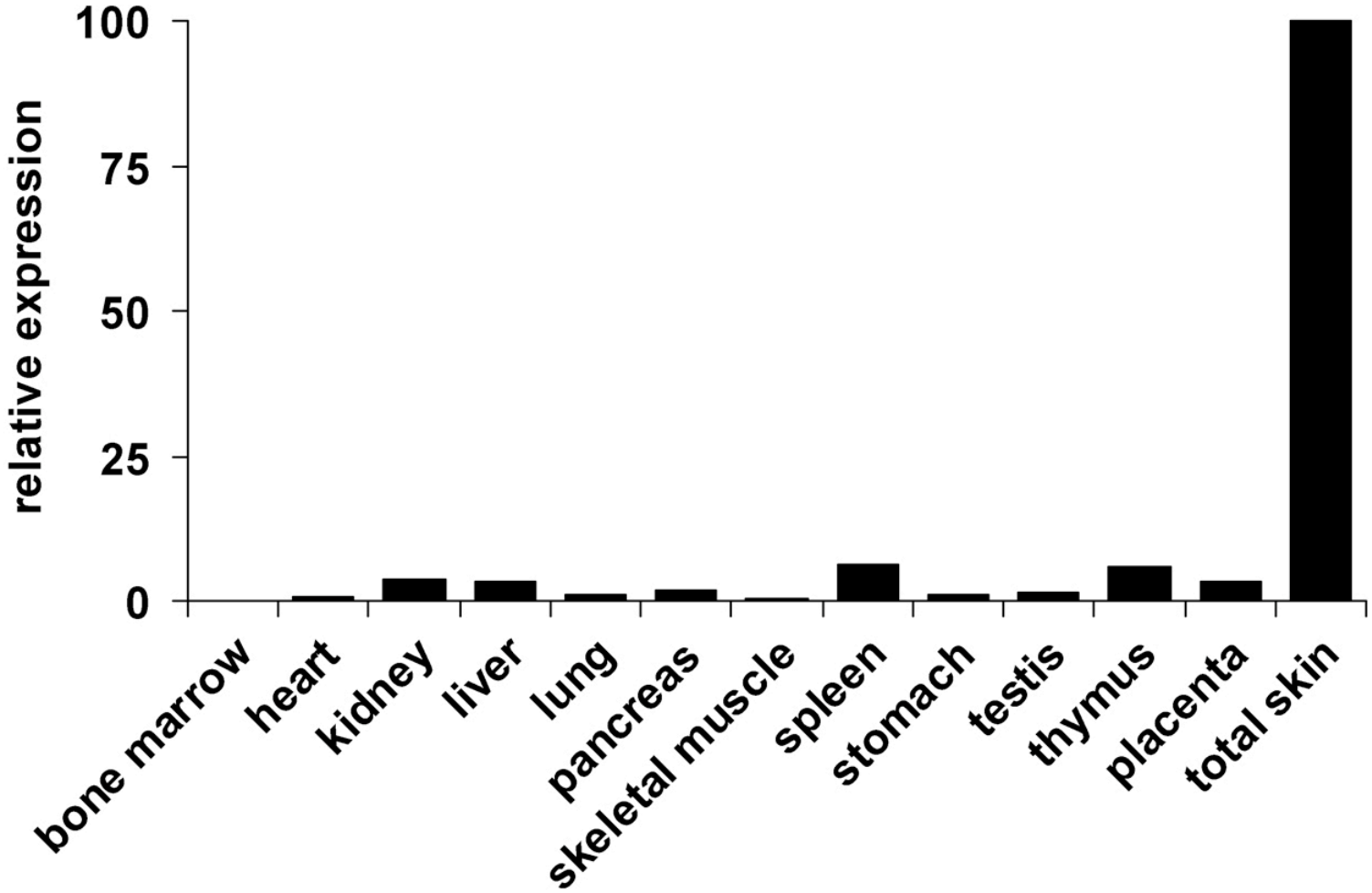
1. During keratinocyte differentiation nuclear DNA is degraded by a specific DNase.

2. The expression of this DNase is upregulated during keratinocyte differentiation.

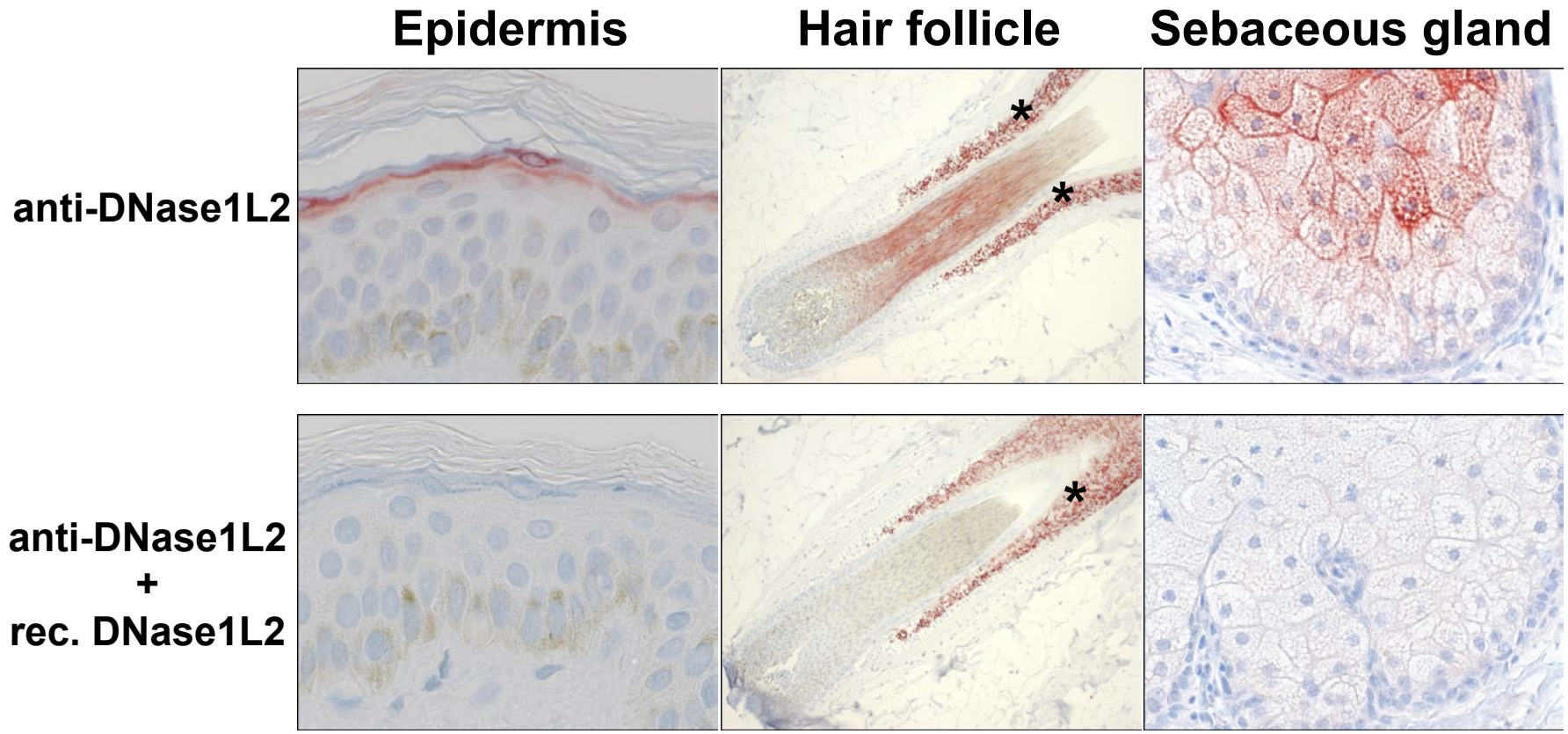
Keratinocyte differentiation is associated with upregulation of DNase1L2 mRNA



DNase1L2 is predominantly expressed in skin

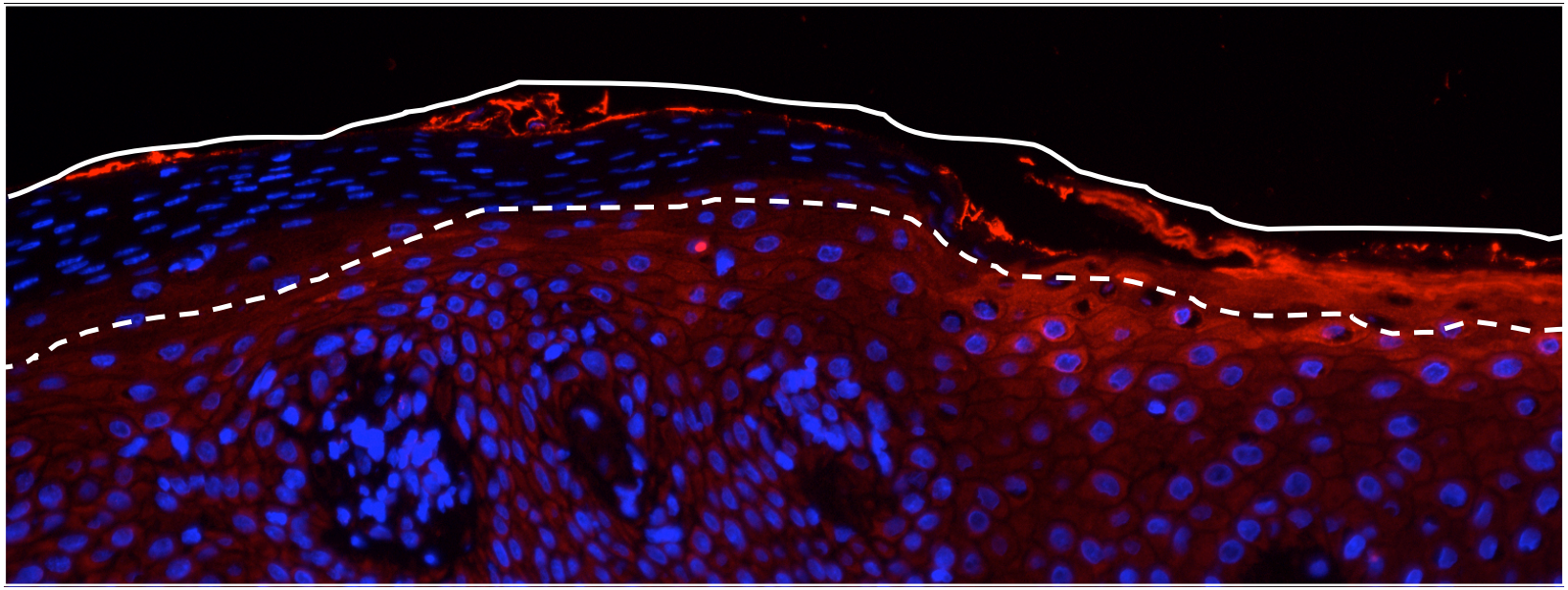


DNase1L2 is expressed in differentiated epidermal keratinocytes

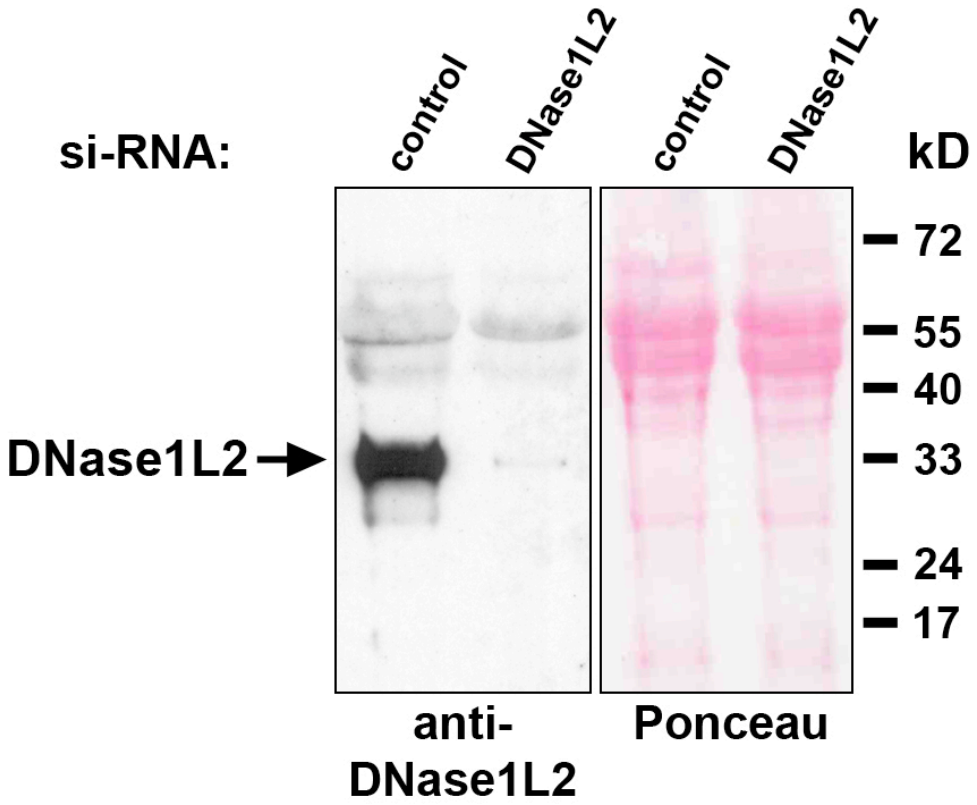


In skin with parakeratotic stratum corneum DNase1L2 expression is reduced

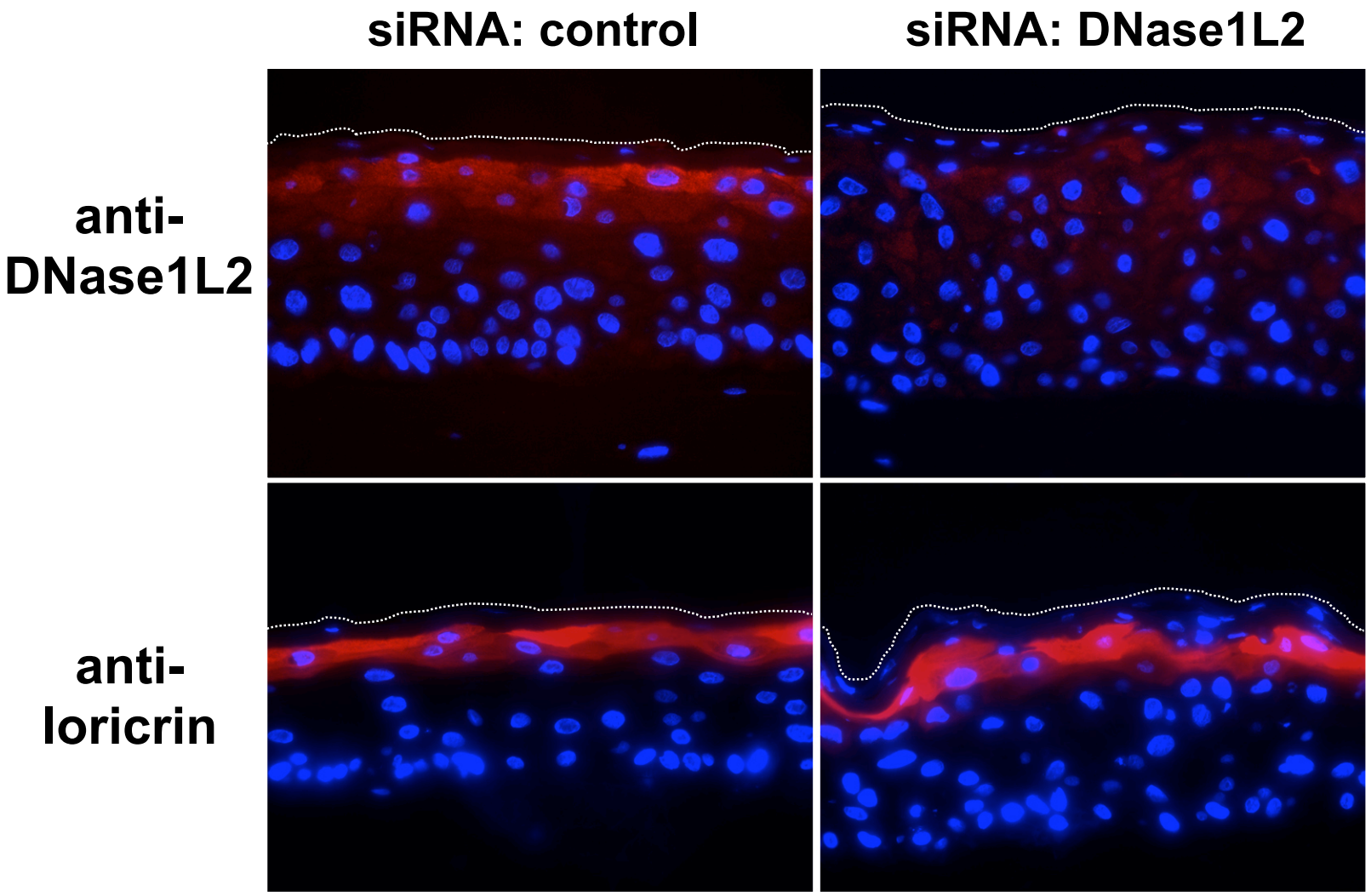
Immunofluorescence: anti-DNase1L2



Knockdown of DNase1L2 in skin equivalents is highly efficient



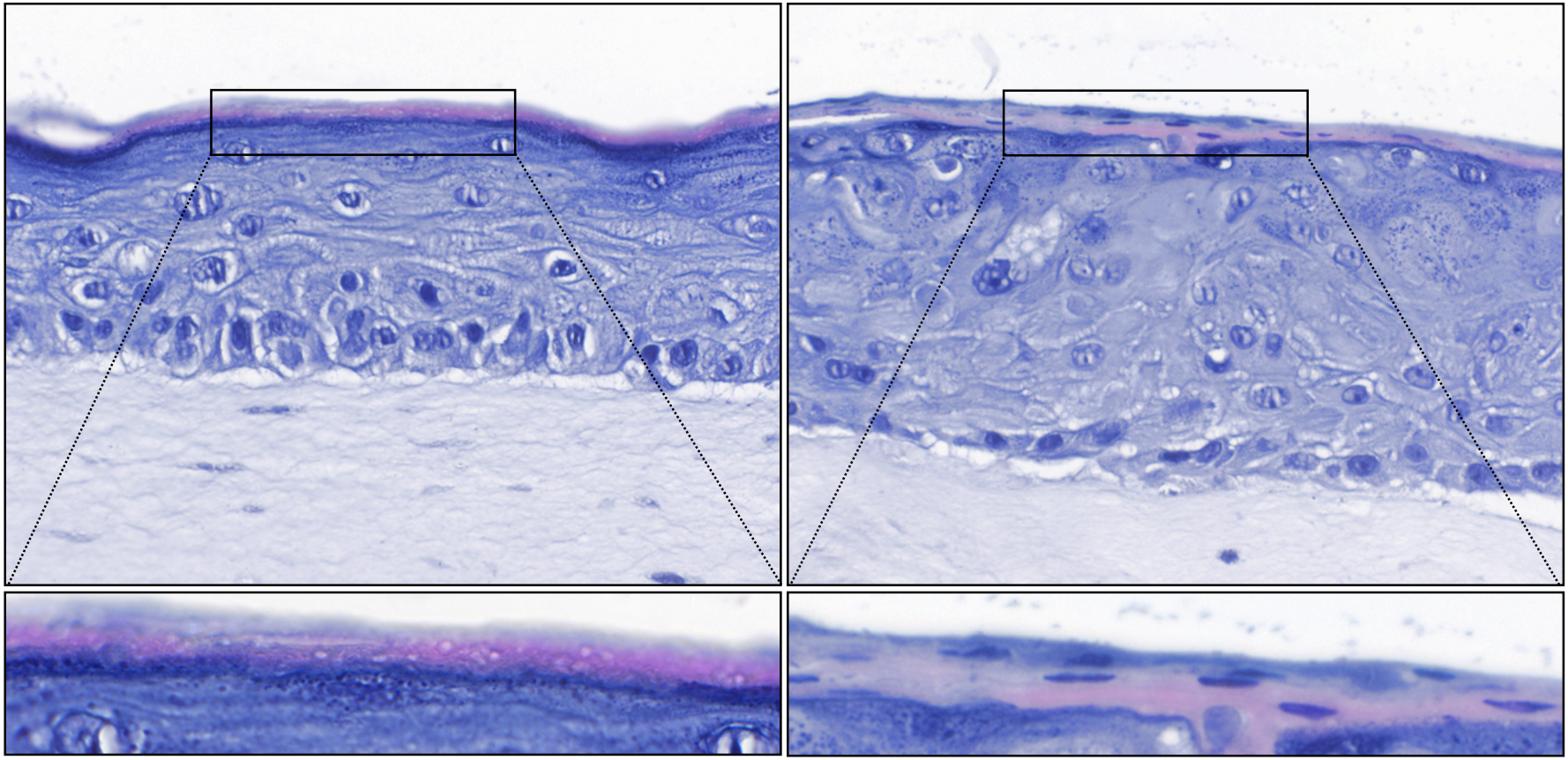
Knockdown of DNase1L2 in skin equivalents results in parakeratosis



Knockdown of DNase1L2 in skin equivalents results in parakeratosis

siRNA: control

siRNA:DNase1L2



Summary: DNase1L2

- specifically expressed in the epidermis
- expression correlates with keratinocyte differentiation
- absence correlates with parakeratosis in psoriasis
- specific knockdown of DNAse1L2 by si RNA technology *in SE culture* results in retention of nuclei in corneocytes

Conclusion

DNase1L2 is essential for the degradation of nuclear DNA during stratum corneum formation

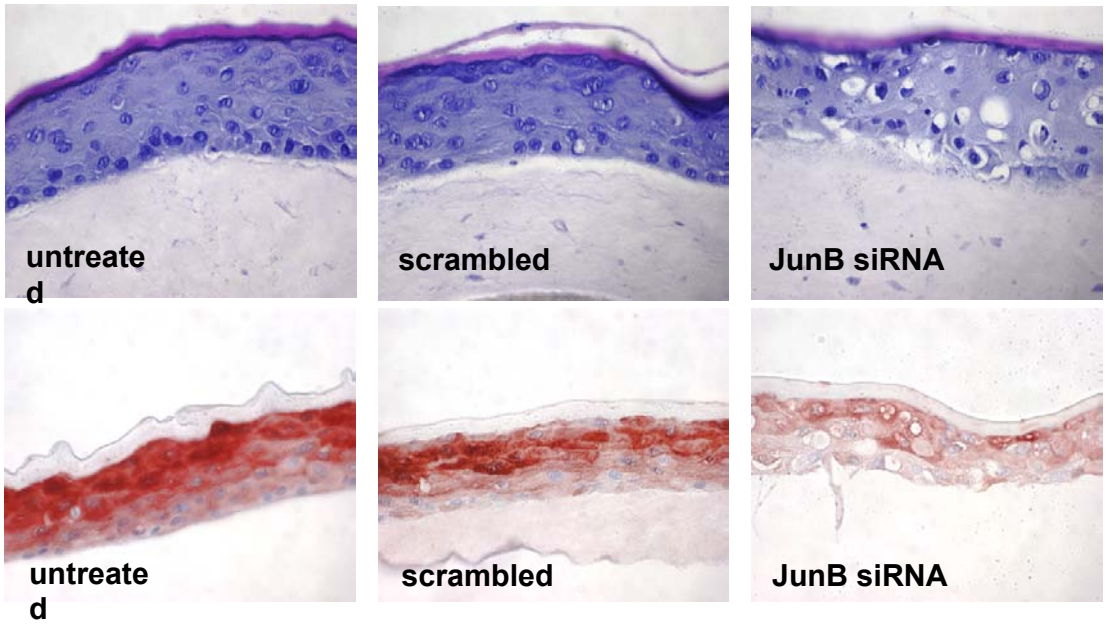
Other Genes examined so far:

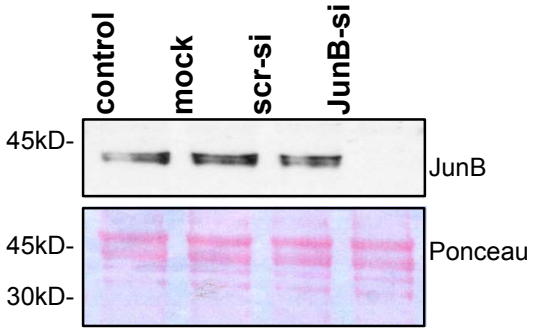
- JunB
- Filaggrin

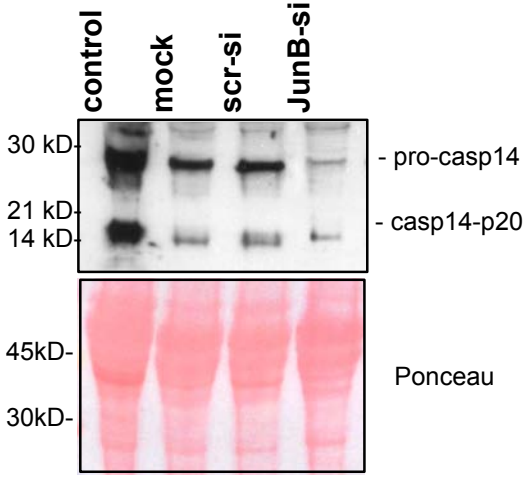
Transcription of the caspase-14 gene in human epidermal keratinocytes requires AP-1 and NFkappaB

Claudia Ballaun, Susanne Karner, Paul Mrass, Michael Mildner, Maria Buchberger, Jürgen Bach, Jozef Ban, Hanna Harant, Erwin Tschachler and Leopold Eckhart

Biochem Biophys Res Commun. 2008 Jun 27;371(2):261-6.

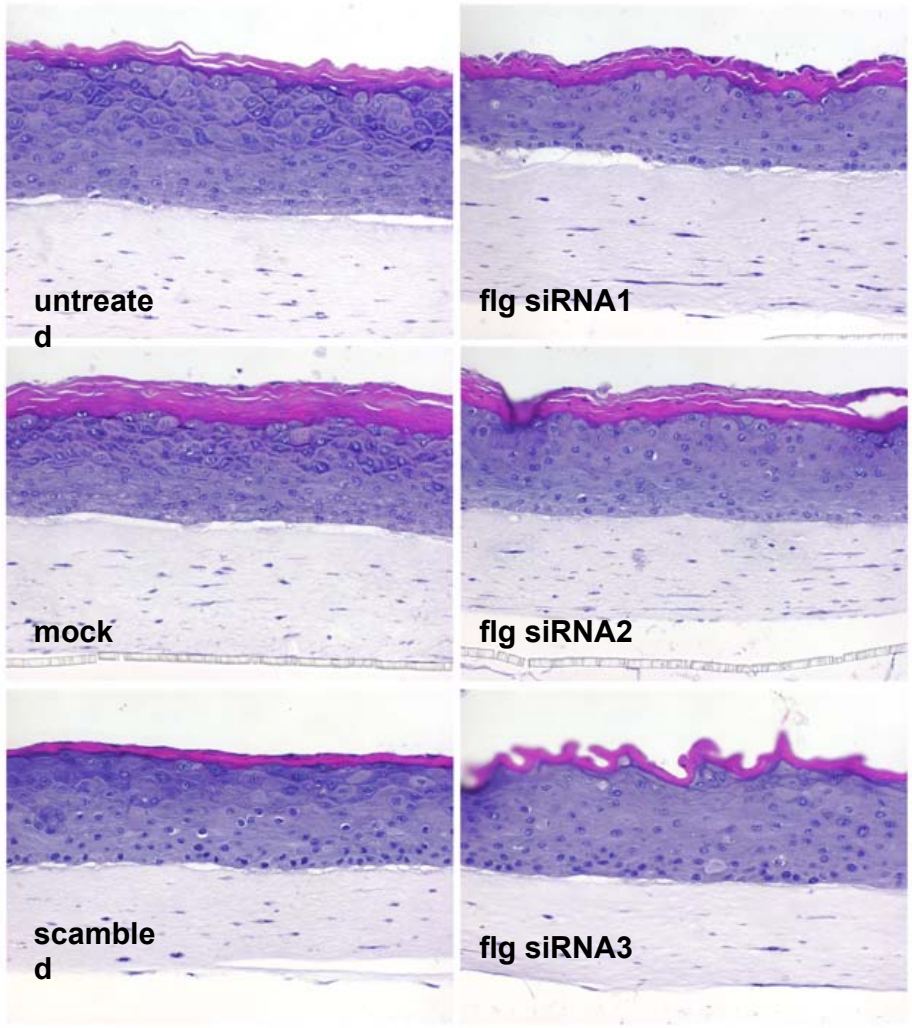


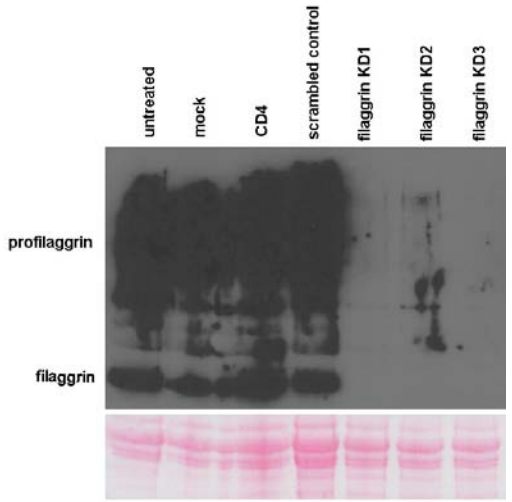




JunB knock down led to an altered epidermal architecture with an irregular stratification, a reduced or absent granular layer and the appearance of foamy cells and vacuoles in the suprabasal layers

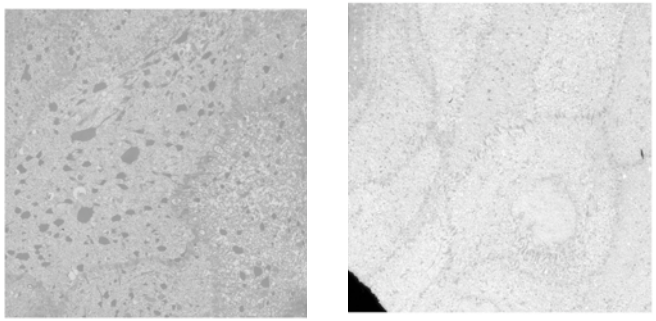
(Pro)filaggrin knock out in an organotypic skin model



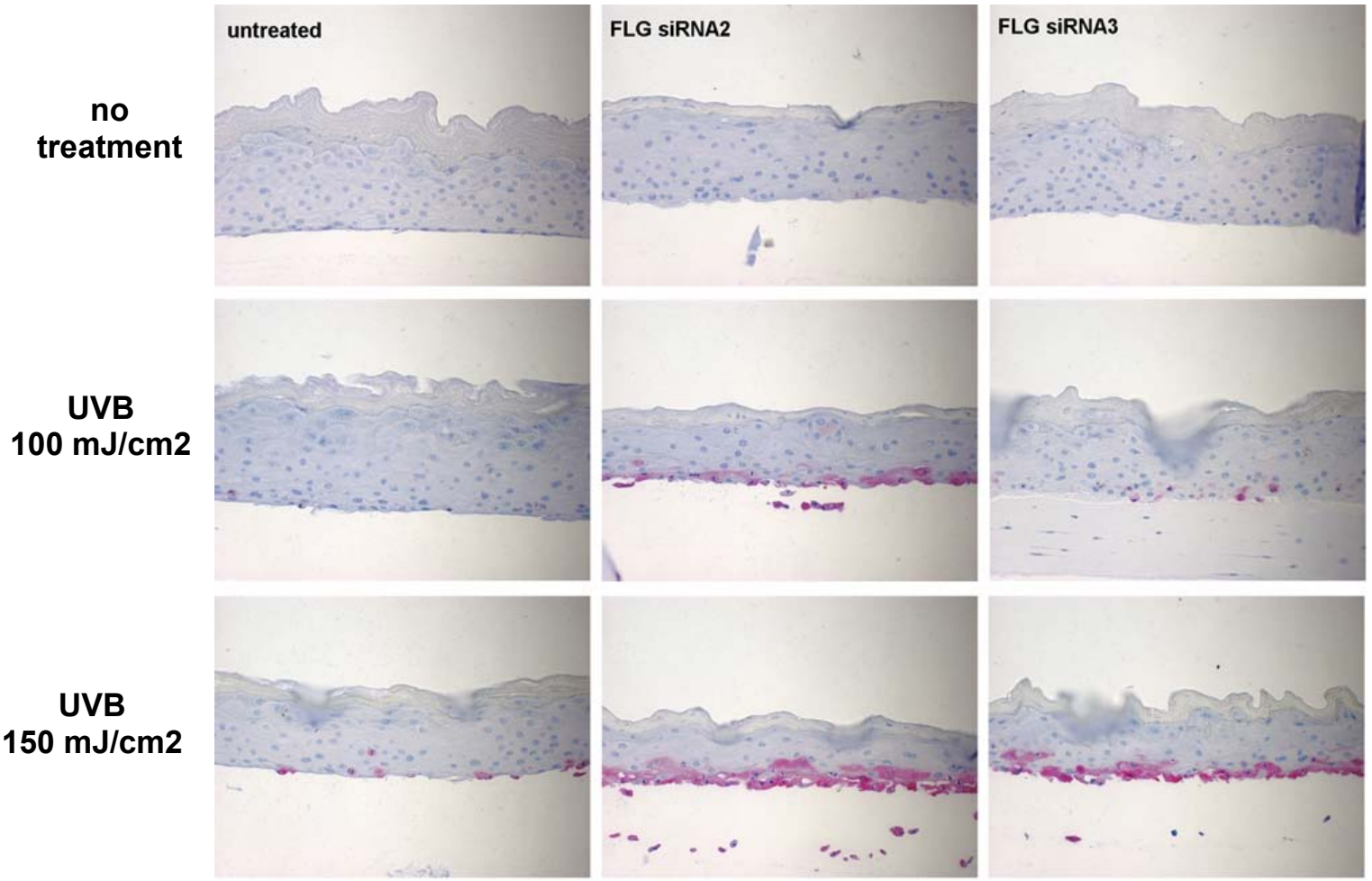


Ultra-structure

scrambled **filg siRNA**



Filaggrin knock-down increases sensitivity to UVB-irradiation



Potential of this technique

- It enables the study of deletion of individual genes in a complex system (cell-cell interaction, cell-matrix interaction,...) of **human cells.**
- This technique could strongly reduce the necessity of animal experiments in dermatological research.
- The *in vitro* knock down is less expensive and much less time consuming than animal models

Improvement of this model

- Generation of organotypic skin cultures containing other cell-types of the skin
  - Melanocytes
  - Langerhans cells
  - Microvascular endothelial cells
- Generation of organotypic skin tumors
  - Squamous cell carcinoma
  - Basal cell carcinoma
  - Melanoma

Acknowledgements

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