Monasterium Laboratory
Skin & Hair Research Solutions

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Analyzing the effect of a test compound on wound healing

Question addressed: **Does compound X promote skin wound healing *ex vivo*?**
Does compound X restore wound healing *ex vivo* under pathological conditions (e.g. diabetic ulcer)?

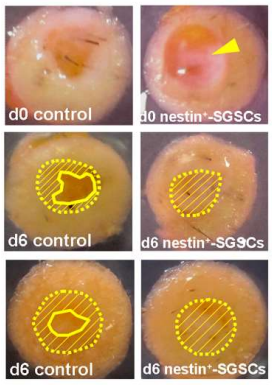
ML approach: Human full-thickness skin harvested from at least 2 healthy or diseased donors and treated *ex vivo* with test compounds of choice. Selected readout parameters are evaluated in the newly formed epithelial tongue, wound bed, or compartments (incl. using laser capture microdissection) and quantified using various techniques, e.g. analysis of the culture medium, immunohistology and quantitative (immuno-)histomorphometry, qRT-PCR, *in situ* zymography, and *in situ* hybridization.

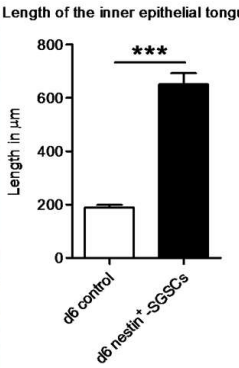
Possible claims: **Compound X stimulates wound healing *ex vivo*,**
Compound X restores wound healing *ex vivo* under pathological conditions

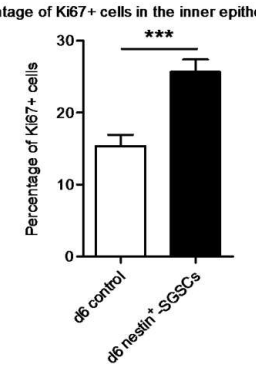
Case study: Nestin+ progenitor cells promote wound healing *ex vivo*

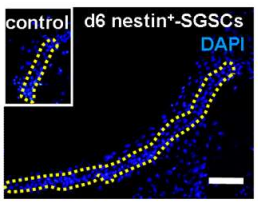
→ allogenic nestin+ progenitor cells were isolated from adult human sweat gland stroma (SGSC) and seeded into the wound bed

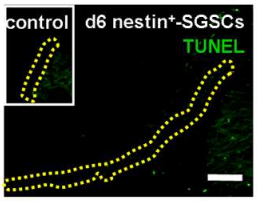
1. Nestin+ progenitor cells promote re-epithelialisation and proliferation of newly generated inner epithelial tongue *ex vivo*

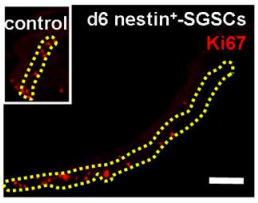


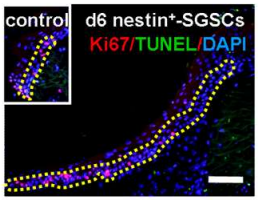






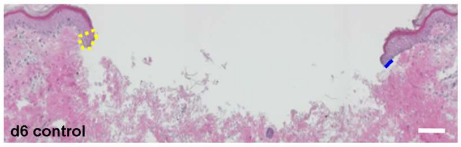


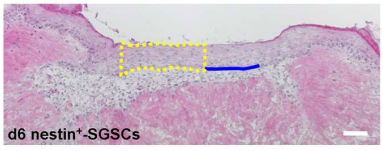




Ki-67: marker for proliferation.
TUNEL: marker for apoptosis.

Pooled data from two independent experiments. Mean±SEM n= 4 punches analysed/group from two different donors.

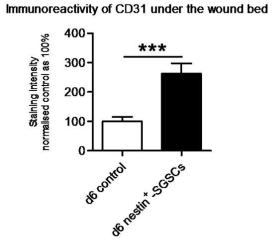


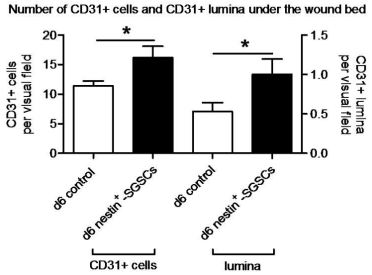


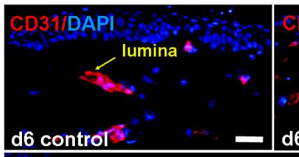
Haematoxylin & Eosin staining: overview and morphology

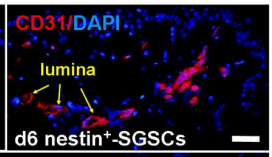
2. Transplanted human sweat gland stroma derived nestin+ cells may stimulate angiogenesis *in situ*

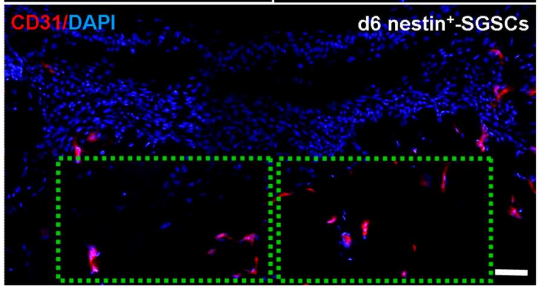
Pooled data from two independent experiments. Mean±SEM n= 4 punches analysed/group from two different donors.











CD31: marker for endothelial cells

➢ **Additional markers available with possibility of establishing 'specific' customised markers**

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Investigating the effect of a test compound on hair follicle growth and anagen-catagen conversion

Question addressed: **Does compound 'X' promote hair follicle elongation (hair shaft production) *ex vivo*?**
Does compound 'X' maintain hair follicles longer in anagen *ex vivo*?

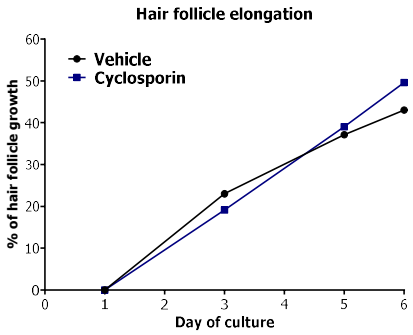
ML approach: Microdissected human scalp hair follicles harvested from at least 2 healthy donors, which spontaneously develop into a catagen-like state when organ cultured. Selected readout parameters can be evaluated in the entire hair follicle or selected compartments (incl. using laser capture microdissection) and quantified using various techniques, e.g. analysis of the culture medium, immunohistology and quantitative (immuno-) histomorphometry, qRT-PCR, *in situ* zymography, *in situ* hybridization, and microarrray.

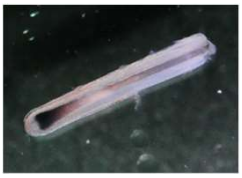
Possible claims: **Compound 'X' stimulates hair shaft production *ex vivo*,**
Compound 'X' inhibits catagen development *ex vivo*

Case study: Cyclosporin maintains anagen *ex vivo*

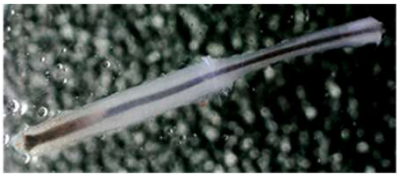
→ **Cyclosporin is well-known for inducing hair growth *in vivo***

1. Cyclosporin maintains the normal rate of hair elongation *ex vivo*





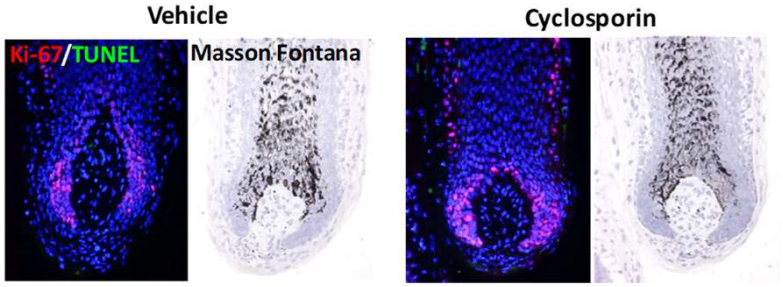
Amputated microdissected hair follicle at day 0, after isolation.

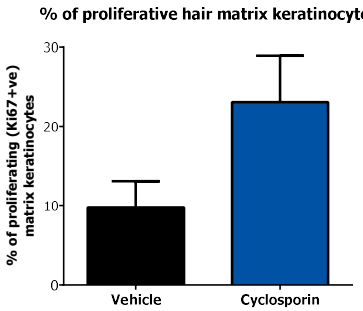


Amputated microdissected hair follicle at day 6 of organ culture: Note the newly formed hair shaft and outer root sheath

Data from 2 experiments, Mean, n=16-17 HFs from 2 donors.

2. Cyclosporin maintains hair matrix keratinocyte proliferation *ex vivo*

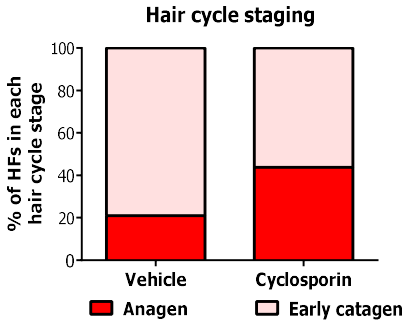


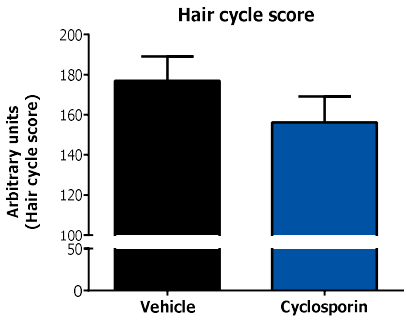


Pooled data from two independent experiments. Mean±SEM n=13-16HFs analysed/group from two donors.

Ki-67: marker for proliferation. TUNEL: marker for apoptosis. Masson Fontana: histochemical staining revealing melanin content

2. Cyclosporin maintains hair follicles longer in anagen *ex vivo*





Hair cycle staging and score analysed as previously published (Langan et al., Exp. Dermatol 2015)

Pooled data from two independent experiments. Mean or Mean±SEM n=13-16HFs analysed/group from two different donors.

➢ **Additional markers available with possibility of establishing 'specific' customized markers**

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Investigating the effect of a test compound on gray/white hair follicle pigmentation

Question addressed: **Does compound X stimulate re-pigmentation of gray/white hair follicles *ex vivo*?**

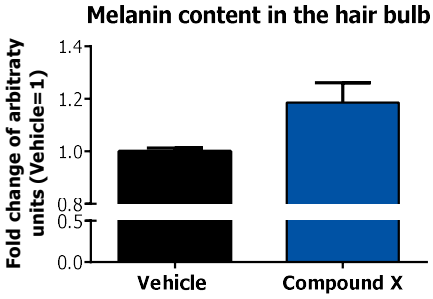
ML approach: Microdissected gray/white human scalp hair follicles harvested from at least 2 healthy donors treated *ex vivo*. Selected readout parameters are evaluated in the entire anagen VI hair follicle or selected compartments (incl. using laser capture microdissection) and quantified using various techniques, e.g. analysis of the culture medium, immunohistology and quantitative (immuno-) histomorphometry, qRT-PCR, *in situ* zymography, *in situ* hybridization, and microarrray.

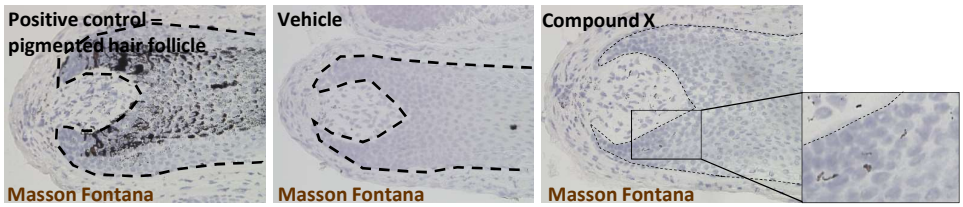
Possible claims: **Compound X stimulates pigmentation of gray/white hair follicles *ex vivo***

Study example: Compound X stimulates pigmentation of white hair follicles *ex vivo*

Human microdissected amputated white hair follicles

1. Compound X stimulates melanin production *ex vivo*

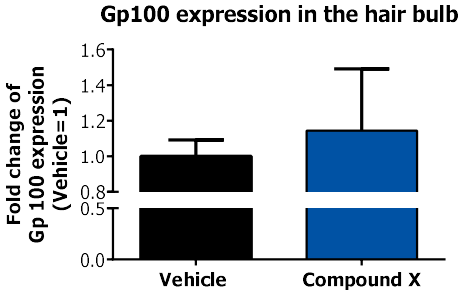


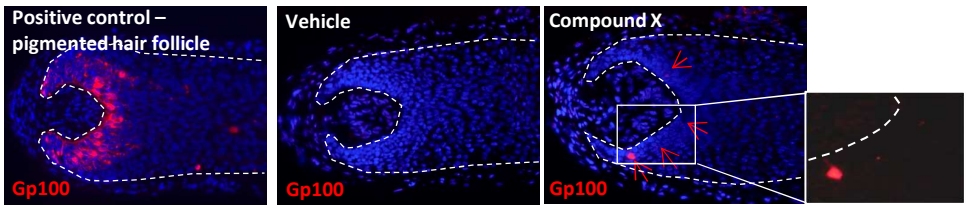


Masson Fontana: Histochemical staining revealing melanin content

Pooled data from two independent experiments. Mean±SEM, n=4-6 anagen IV hair follicles from two donors.

2. Compound X tendentially promotes the formation of new melanosomes *ex vivo*

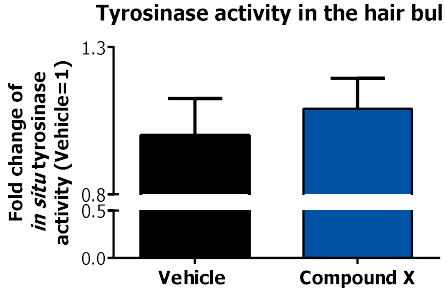


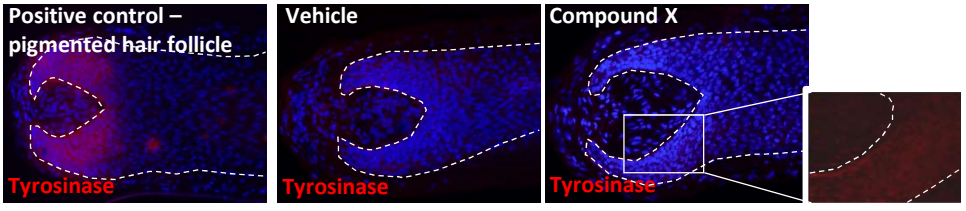


Gp100 immunofluorescence revealing melanosomes, the melanin-producing organelle

Pooled data from two independent experiments. Mean±SEM, n=5-8 anagen IV hair follicles from two donors.

3. Compound X tendentially increases the activity of tyrosinase *ex vivo*





In situ assay revealing the enzymatic activity of tyrosinase, the rate-limiting enzyme regulating melanin production

Pooled data from two independent experiments. Mean±SEM, n=4-5 anagen IV hair follicles from two donors.

➢ **Additional markers available with possibility of establishing 'specific' customized markers**

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Analyzing the effect of a test compound on skin rejuvenation

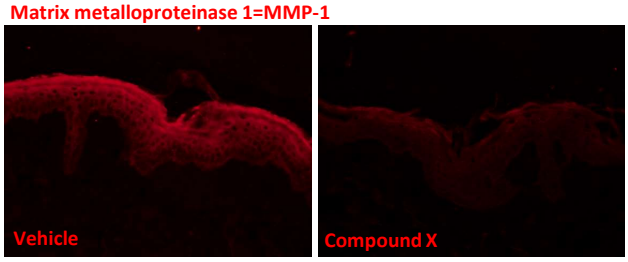
Question addressed: **Does compound X promote skin rejuvenation *ex vivo*?**

ML approach: Human full-thickness skin harvested from at least 2 elderly donors and treated *ex vivo* with test compounds of choice. Selected readout parameters are evaluated in the entire epidermis, dermo-epidermal junction or selected compartments (incl. using laser capture microdissection) and quantified using various techniques, e.g. analysis of the culture medium, immunohistology and quantitative (immuno-)histomorphometry, qRT-PCR, *in situ* zymography, *in situ* hybridization, and microarrray.

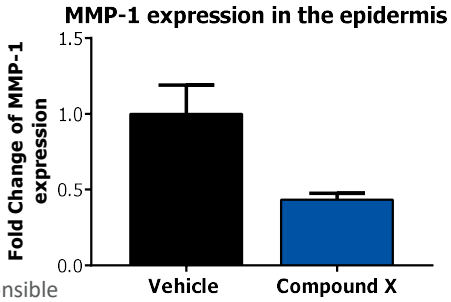
Possible claims: **Compound X stimulates youthful skin phenotype: youthful skin has lower MMP1, higher Sirt1, and an organized fibrillin fiber structure.**

Study example: Compound X promotes skin rejuvenation *ex vivo*

1. Compound X decreases matrix metalloproteinase (MMP-1) expression *ex vivo*



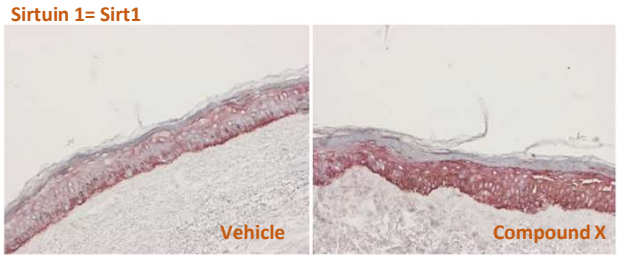
MMP-1 immunofluorescence reveals proteinase expression, responsible for degrading collagen, elastic, and fibrillin-rich microfibers.

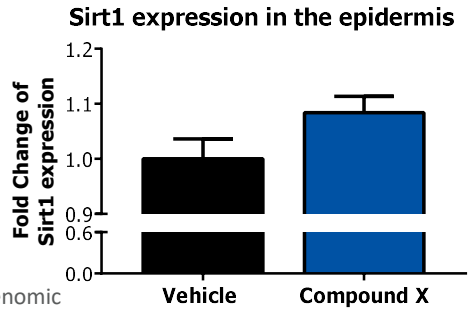


Data from one experiment. Mean±SEM n= 2 punches analysed/group from one donor.

2. Compound X increases Sirt1 expression *ex vivo*

Pooled data from two independent experiments. Mean±SEM n= 2 punches analysed/group from two different donors.

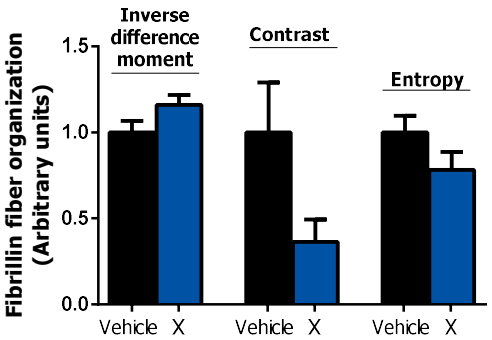


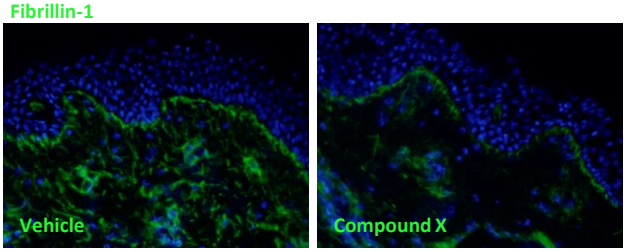


Sirt1 immunoreactivity indicates mitochondrial homeostasis, regulating senescence, aging, and genomic stability.

2. Compound X improves fibrillin fiber organization *ex vivo*

Pooled data from two independent experiments. Mean±SEM n= 2 punches analysed/group from two different donors.





Fibrillin fiber organization is a marker for intrinsic and extrinsic aging.

➢ **Additional markers available with possibility of establishing 'specific' customised markers**

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Investigating the effect of a test compound on experimentally induced epithelial-mesenchymal-transition (EMT) in human hair follicles

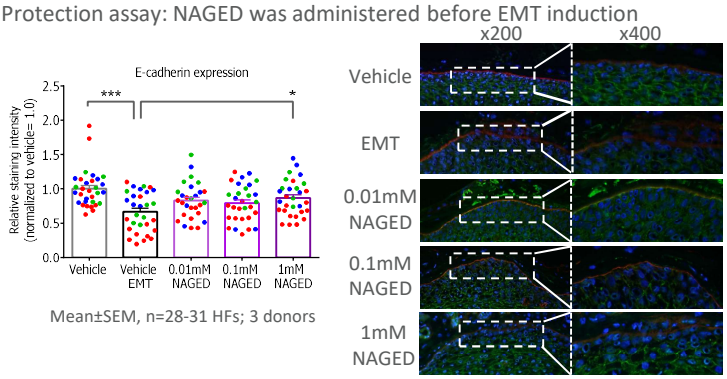
Question addressed: **Does compound 'X' restore human hair follicle *ex vivo* after EMT induction?**
Does compound 'X' protect human hair follicle *ex vivo* from EMT induction?

ML approach: Microdissected full-length human scalp hair follicles harvested from at least 2 healthy donors, subjected to experimentally-induced EMT in *ex vivo* organ culture. Selected readout parameters can be evaluated in the bulge region of the hair follicle or selected compartments (incl. using laser capture microdissection) and quantified using various techniques, e.g. analysis of the culture medium, immunohistology and quantitative (immuno-) histomorphometry, qRT-PCR, *in situ* hybridization, and microarrray.

Possible claims: **Compound 'X' restores human hair follicle *from* EMT *ex vivo,***
Compound 'X' protects human hair follicle *from* EMT *ex vivo*

Case study: . N-acetyl-GED-0507-34-Levo (NAGED) protects and partially rescues from EMT

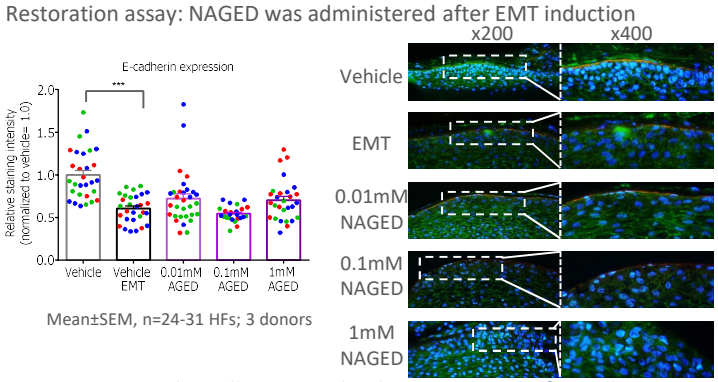
Protection assay: NAGED was administered before EMT induction



Mean±SEM, n=28-31 HFs; 3 donors

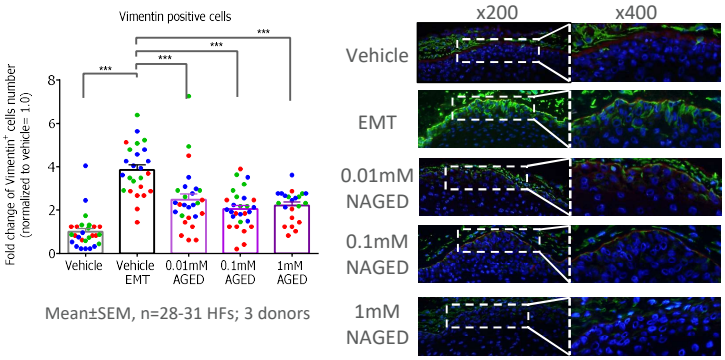
Pretreatment with NAGED prevents downregulation of E-cadherin expression in the bulge region

Restoration assay: NAGED was administered after EMT induction



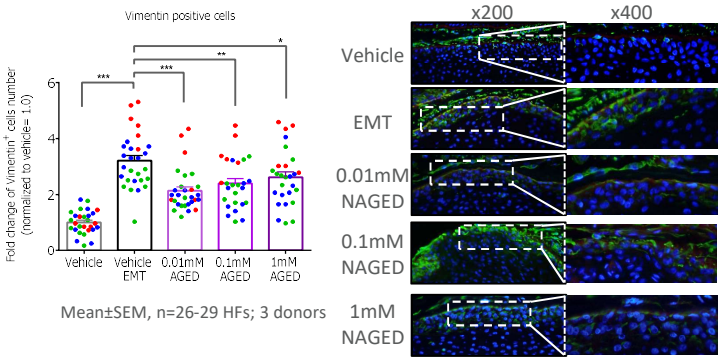
Mean±SEM, n=24-31 HFs; 3 donors

NAGED tendentially reverts the downregulation of E-cadherin expression in the bulge region



Mean±SEM, n=28-31 HFs; 3 donors

Pretreatment with NAGED significantly prevents increase of Vimentin positive cells number in the bulge region



Mean±SEM, n=26-29 HFs; 3 donors

NAGED significantly reverses the increase of Vimentin positive cells number in the bulge region

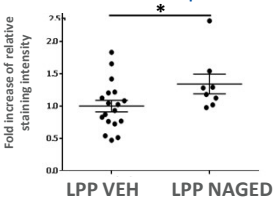
Graph Pad Prism 6, Mann-Whitney-test, * $p < 0.05$, *** $p < 0.001$

Graph Pad Prism 6, Mann-Whitney-test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

NAGED reverses established HFeSCs EMT in scalp skin samples from lichen planopilaris (LPP) patients *ex vivo*

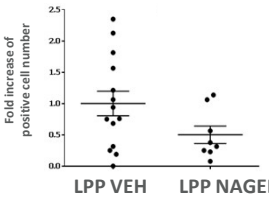
Restoration assay: punch biopsies from the LPP lesional scalp were treated with vehicle or with 0.1mM NAGED

NAGED increases E-Cadherin expression



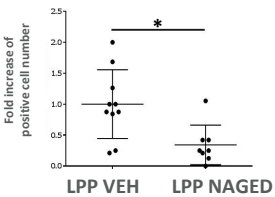
Mean±SEM, n=8-18, 2 donors,
Mann Whitney test, *p<0.05

NAGED reduces Vimentin+ cells number



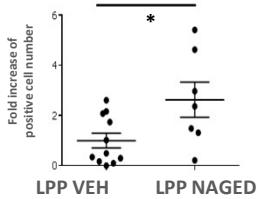
Mean±SEM, n=8-14 HFs,2 donors,
Students' t-test, n.s.

NAGED reduces SLUG+ cells number



Mean±SEM, n=8-10, 2 donors,
Mann Whitney test, *p<0.05

NAGED increases K15+ cells number



Mean±SEM, n=6-11 HFs, 2 donors
Students' t-test, *p<0.05

➢ **Additional markers available with possibility of establishing 'specific' customized markers**