Exploring the potential of *farudodstat*, a DHODH inhibitor, as an alopecia areata therapeutic in a novel *ex vivo* model of human hair follicle immune privilege collapse

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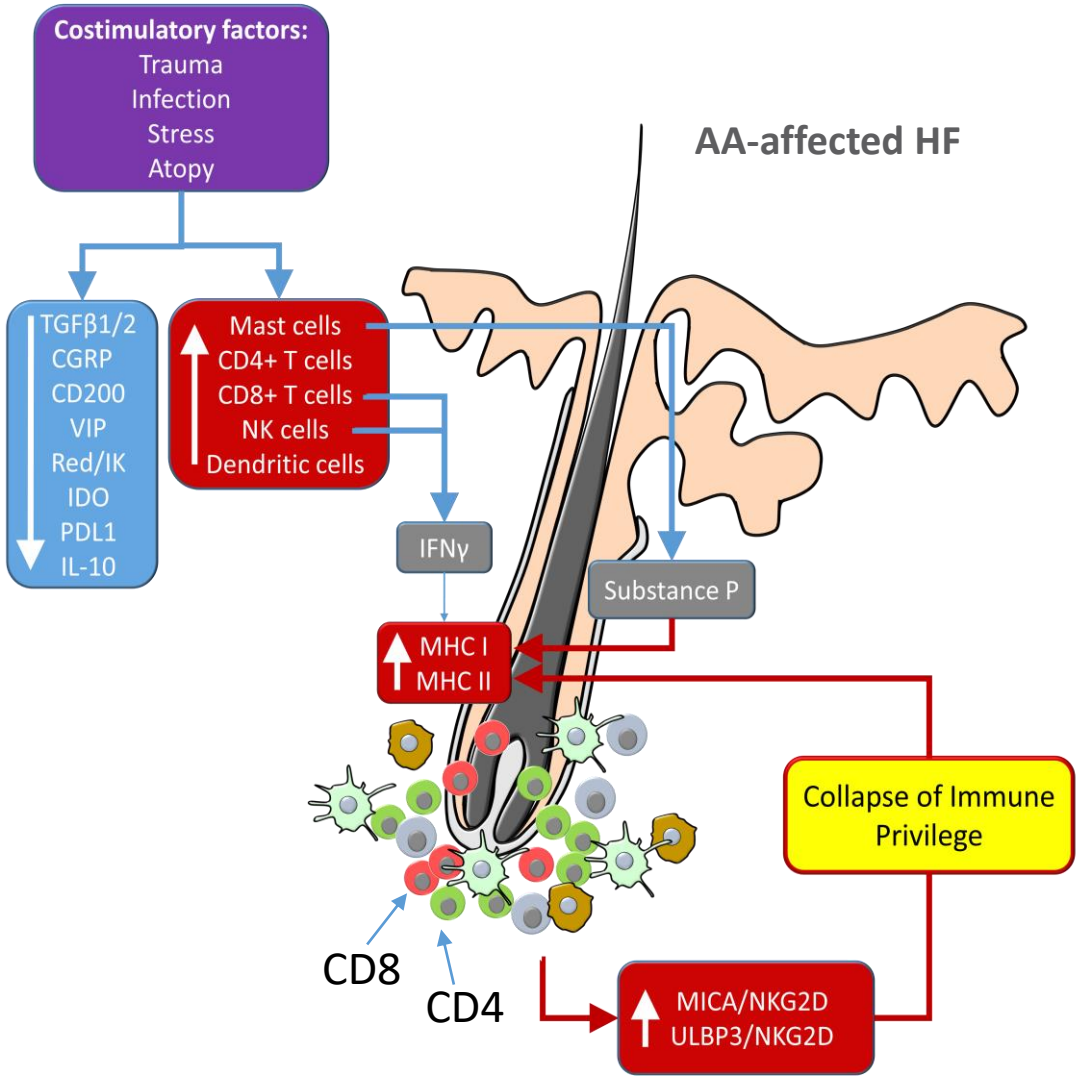
MONASTERIUM LABORATORY
A QIMA Life Sciences Company

ASLAN PHARMACEUTICALS

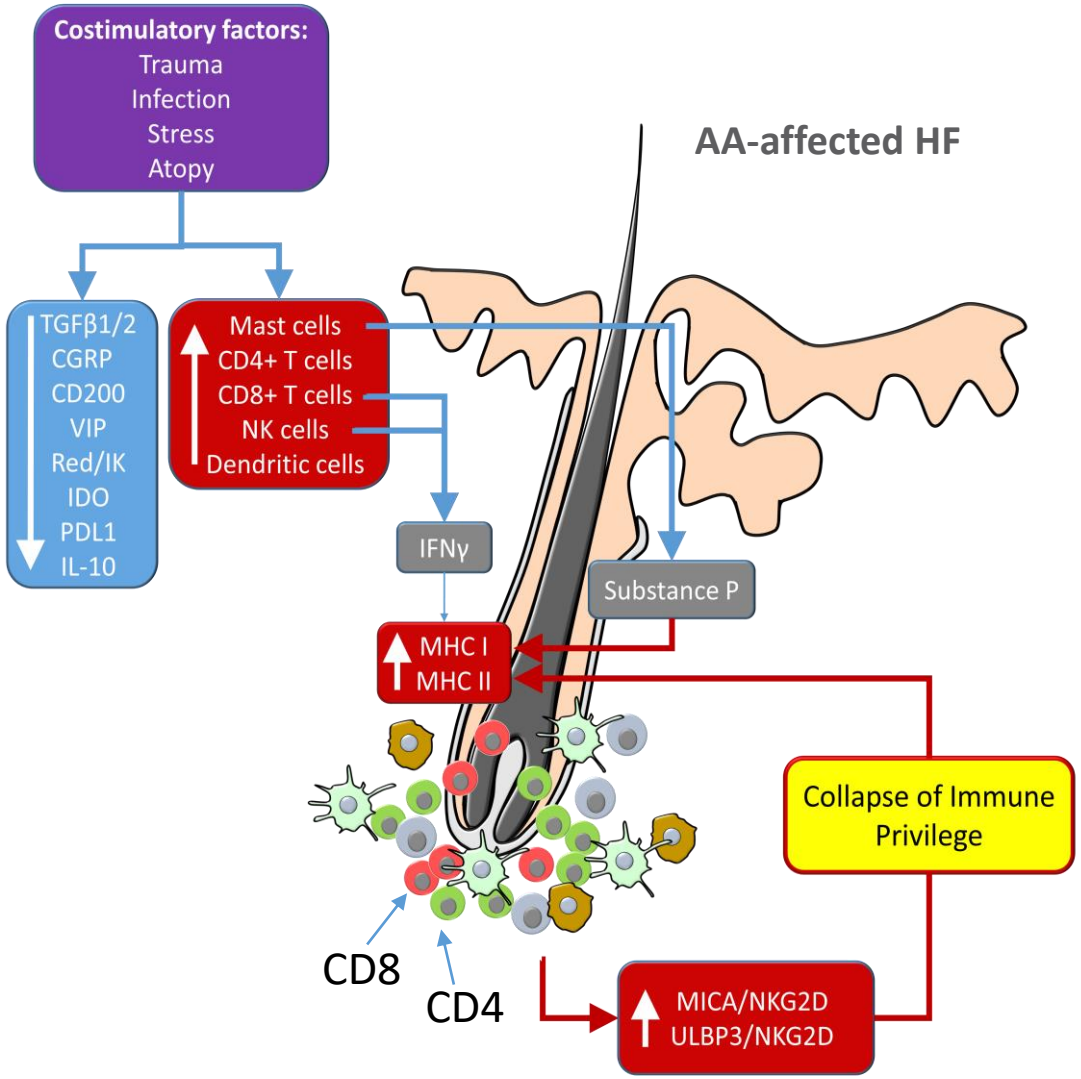
Autoimmune AA (AAA) versus autoantigen-independent non-autoimmune AA (NAIAA)
- One hair loss pattern, two main AA pathobiology pathways -



→ **Hair follicle dystrophy**
→ **Premature catagen induction**

Bertolini et al. *Exp Dermatol* 2020

Autoimmune AA (AAA) versus autoantigen-independent non-autoimmune AA (NAIAA)
- One hair loss pattern, two main AA pathobiology pathways -



Non-autoimmune Alopecia Areata

- NK cell-, mast cell-, ILC-1- and/or γδ T cell- activity
- HF microbiome dysbiosis
- Neurogenic HF inflammation

Autoimmune Alopecia Areata

- Ectopic expression of HF antigens
- CD8+ T-cell responsive to anagen HF-associated autoantigens
- Insufficient Treg activity

Substance P

IFNγ

Hair follicle IP collapse

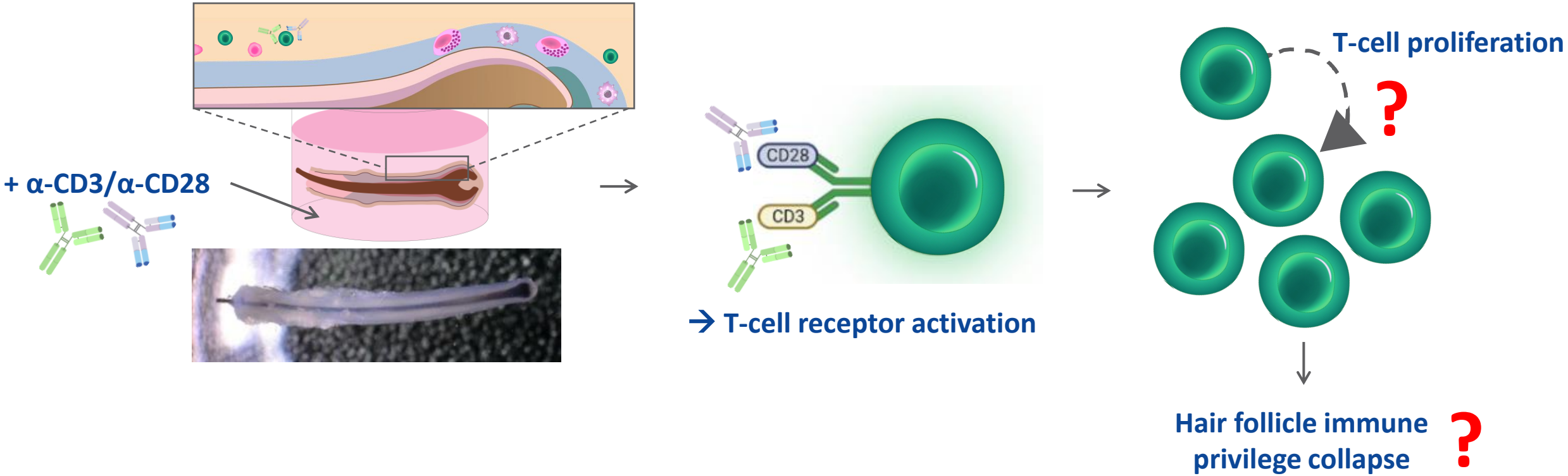
AA therapy: → IP restoration

→ Hair follicle dystrophy
→ Premature catagen induction

Bertolini et al. *Exp Dermatol* 2020

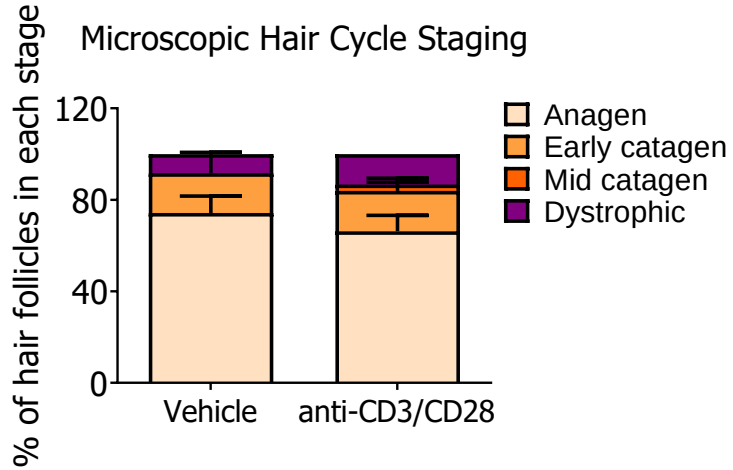
Is HF IP collapse induced by mimicking antigen-presentation in hair follicle organ culture?

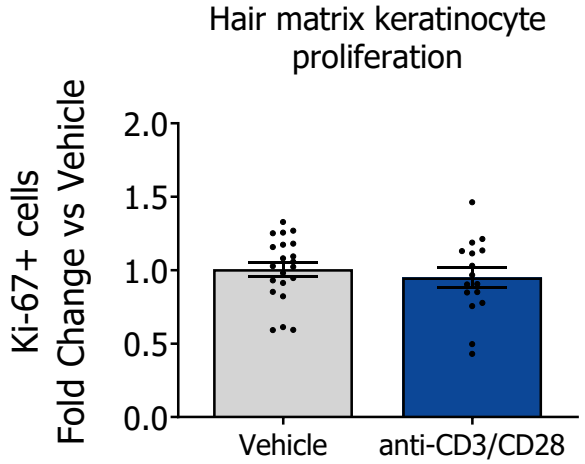
Stimulating healthy human hair follicles *ex vivo* with α-CD3/α-CD28 antibodies to induce T-cell proliferation and IP collapse associated markers



Treatment with α-CD3/α-CD28 antibodies neither affects hair cycle nor hair matrix proliferation in healthy human HFs *ex vivo*

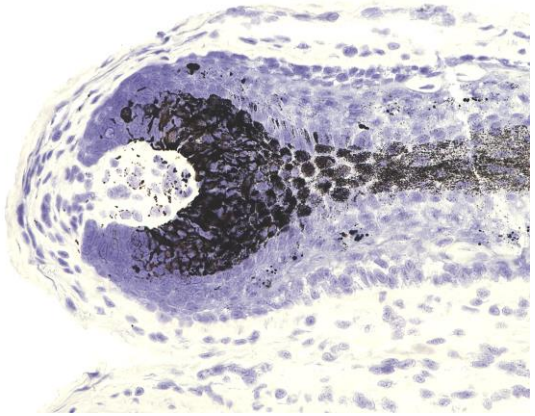
Hair cycle staging & Hair matrix keratinocyte proliferation

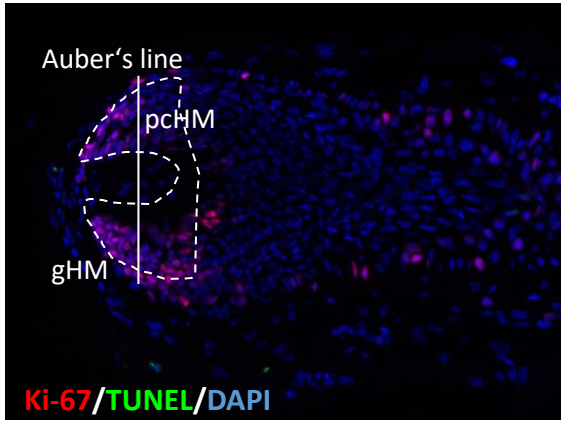


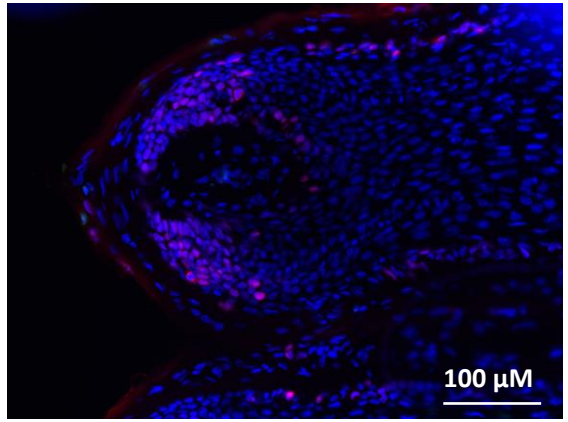


Vehicle

anti-CD3/CD28



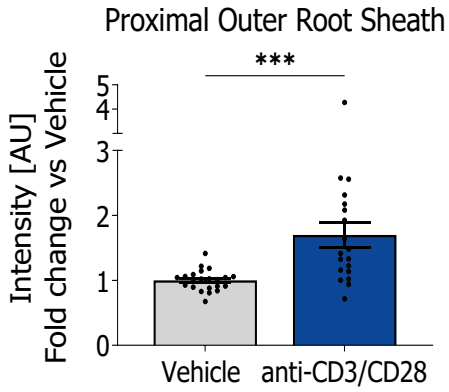


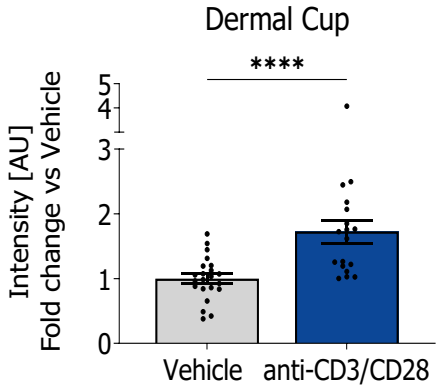


All data are presented as mean±SEM. n = 16-31 HFs from n = 4 donors. Treatment groups were compared using Mann-Whitney test and no statistical significance was observed, scale bar = 100µm. gHM: germinative hair matrix, pcHM: pre cortical hair matrix

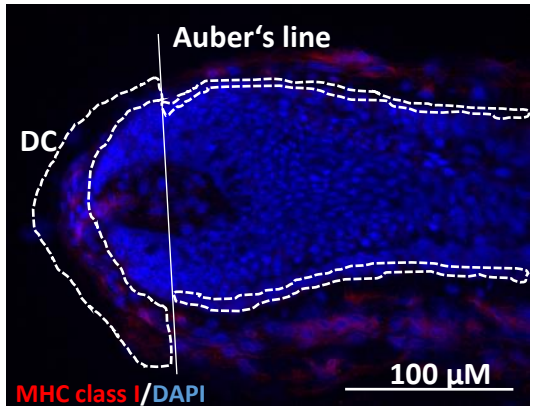
Treatment with α-CD3/α-CD28 antibodies upregulates immune privilege collapse-associated markers in healthy human HFs *ex vivo*

MHC class I expression

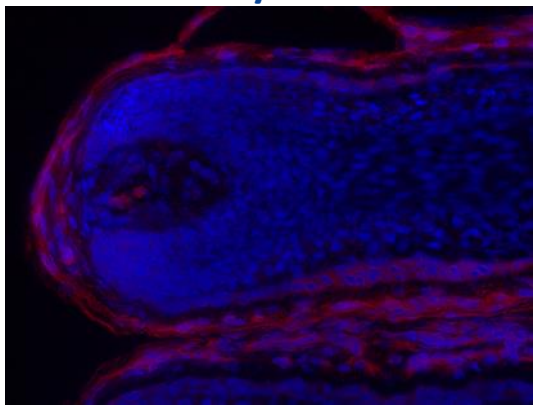




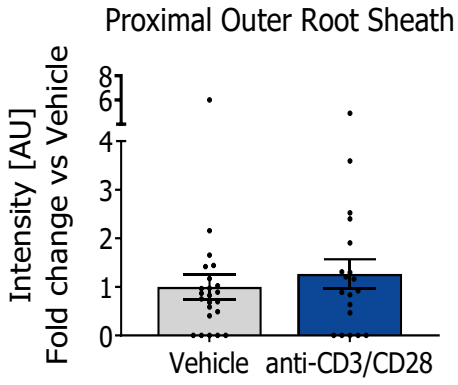
Vehicle

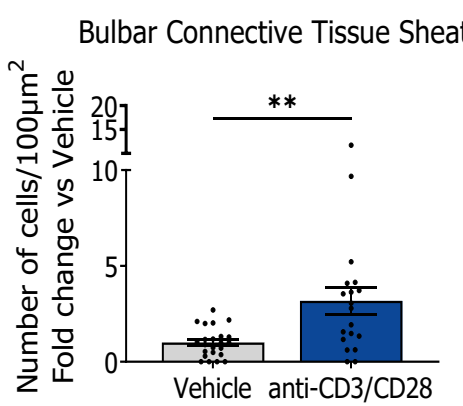


anti-CD3/ CD28

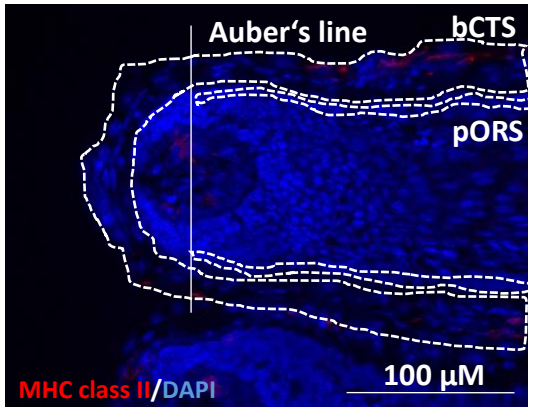


MHC class II expression

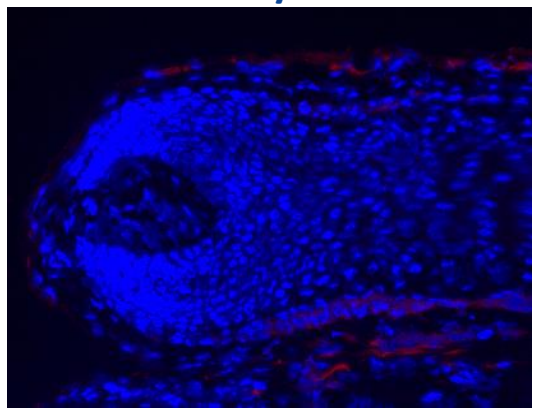




Vehicle

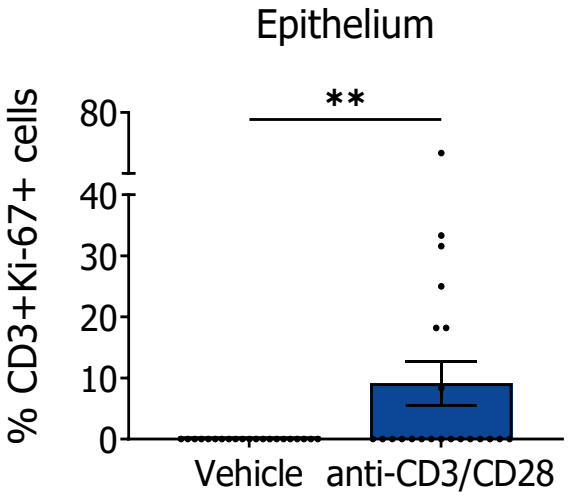


anti-CD3/ CD28

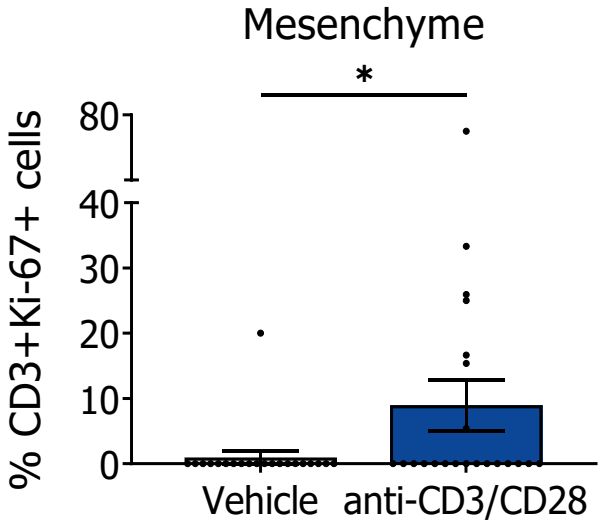


All data are presented as mean±SEM. n = 19-22 HFs from 4 donors. Treatment groups were compared using Mann-Whitney test and ****p<0.0001, ***p<0.001, **p<0.01 scale bars = 100µmbCTS: bulbar connective tissue sheath, DC: dermal cup, pORS: proximal outer root sheath

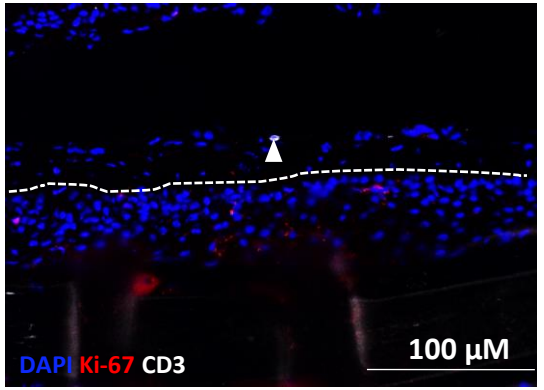
Treatment with α-CD3/α-CD28 antibodies stimulates T-cell proliferation in healthy human HFs *ex vivo*



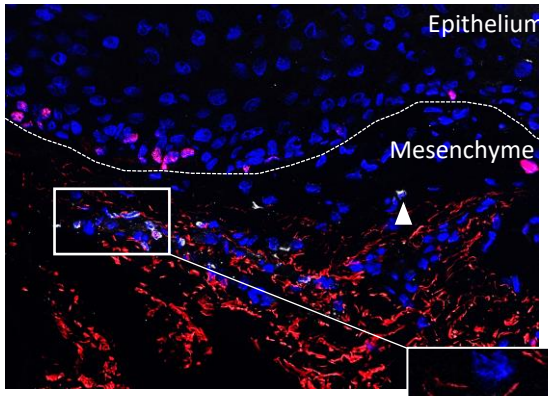
T-cell proliferation

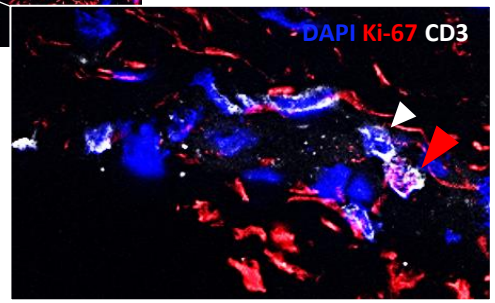


vehicle



anti-CD3/ CD28



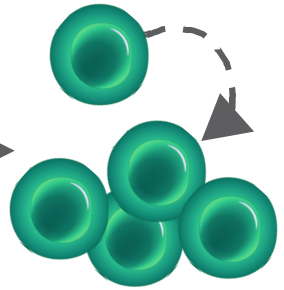


All data are presented as mean±SEM. n = 21-24 HFs from 3 donors. Treatment groups were compared using Mann-Whitney test and *p<0.05, ** p<0.01, scale bars = 100µm:

Treatment with α-CD3/α-CD28 induces T-cell proliferation and IP collapse in human hair follicles ex vivo

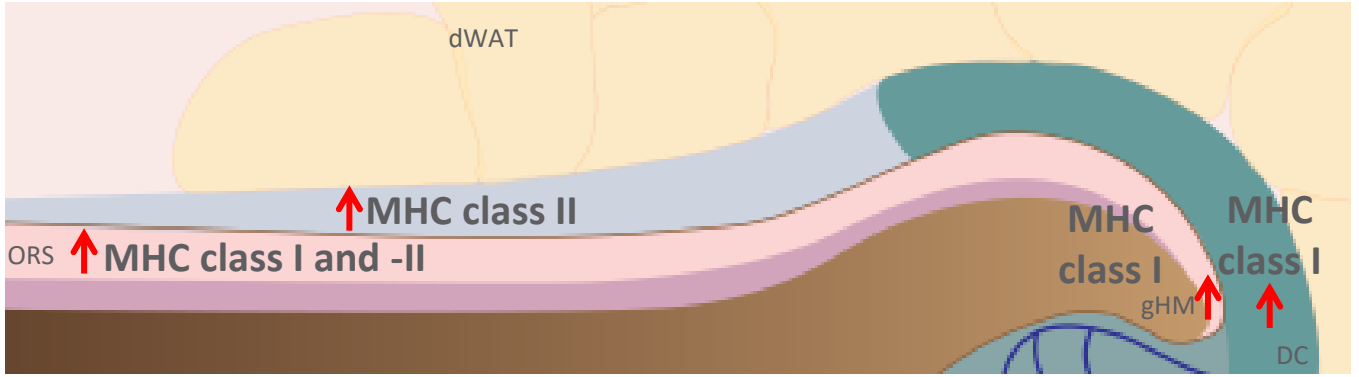
T-cell proliferation ↑

+ α-CD3/α-CD28 →



→ Cytokine release?

Hair follicle immune privilege collapse ↑



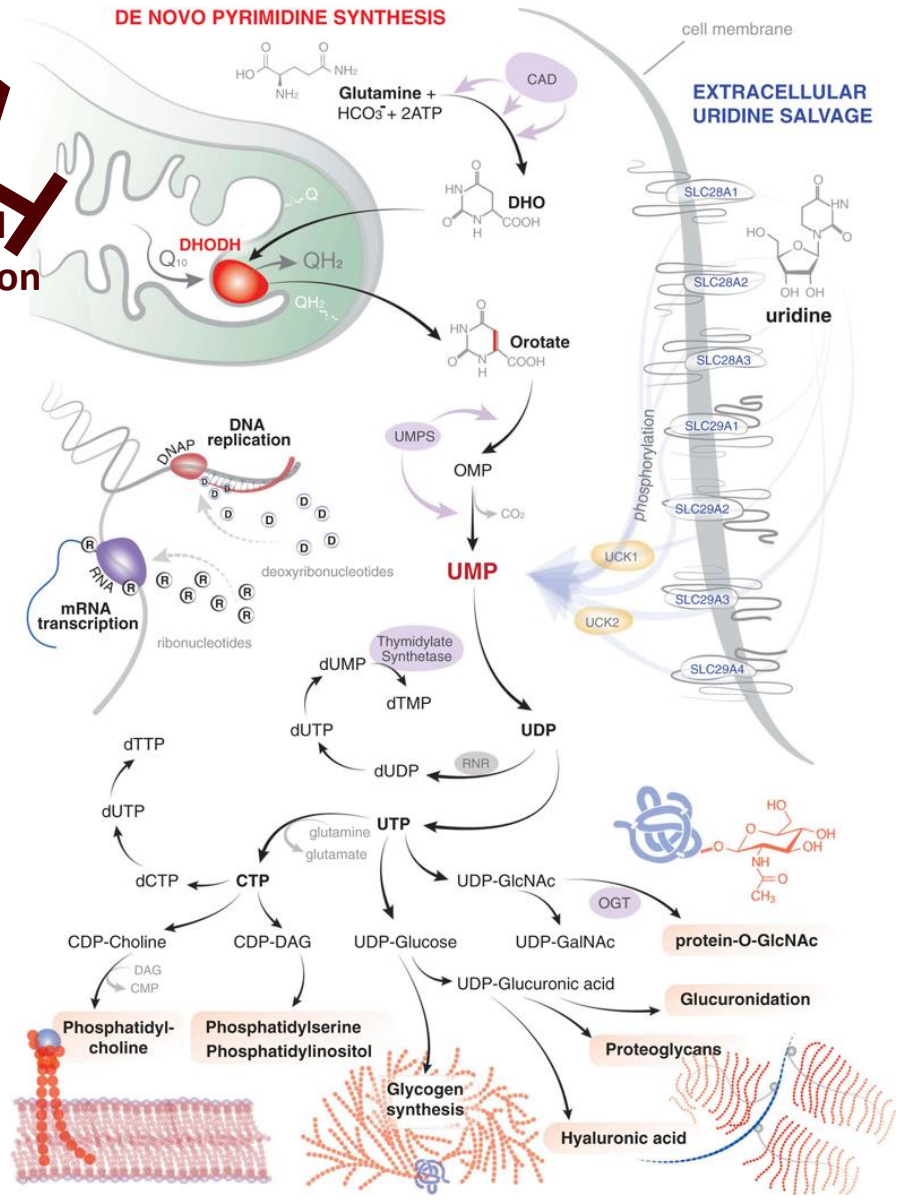
dWAT: dermal white adipose tissue; DC: dermal cup; gHM: germinative hair matrix, ORS: outer root sheath

↑ Upregulation/induction

Farudodstat, the highly selective DHODH inhibitor, that inhibits T-cell proliferation

farudodstat

DHODH inhibition



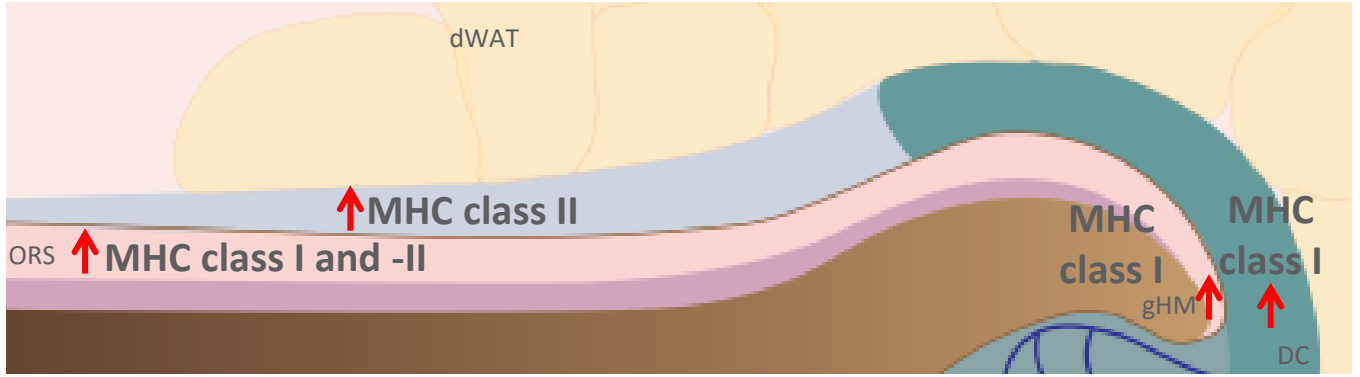
- Dihydroorotate dehydrogenase (DHODH) is an ubiquitous enzyme located within the inner membrane of the mitochondria essential for the *de novo* production of pyrimidines, which is induced during cell proliferation
- DHODH inhibitors are currently used for the treatment of autoimmune disease, e.g. Teriflunomide in MS
- DHODH inhibitors preferentially block T-cell proliferation and, as consequence, cytokine production
- **Farudodstat** is a highly selective DHODH inhibitor with a well-tolerated safety profile *in vitro* and *in vivo*
- **Farudodstat** is approximately 30 times more potent (IC50:35nM) than first-generation DHODH inhibitors in limiting T cell proliferation and IFNg release
- **Farudodstat** is currently in a Phase 2a proof-of-concept trial for AA. FAST-AA is a 2:1 randomized trial, with 60 alopecia areata patients receiving oral doses of either farudodstat or placebo twice daily for 12 weeks, followed by a crossover treatment period

Can dihydroorotate dehydrogenase (DHODH) inhibition by farudodstat protect HFs *ex vivo* from the α-CD3/α-CD28 induced IP collapse?

T-cell proliferation ↑

+ α-CD3/α-CD28

Hair follicle immune privilege collapse ↑



dWAT: dermal white adipose tissue; DC: dermal cup; gHM: germinative hair matrix, ORS: outer root sheath

DHODH inhibition

farudodstat

???

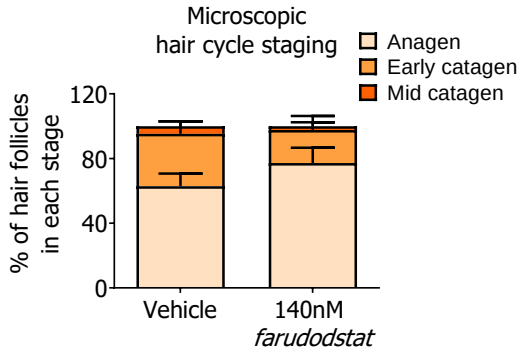
T-cell proliferation

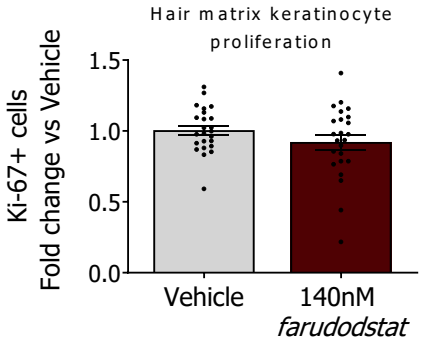
???

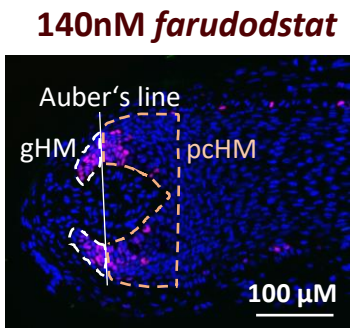
mitochondria DHODH ↑ Upregulation/induction

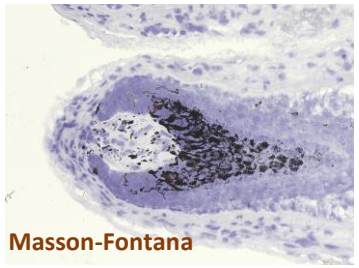
Farudodstat maintains anagen phase without impacting hair matrix keratinocyte proliferation or expression of IP collapse-associated markers in healthy HFs *ex vivo*

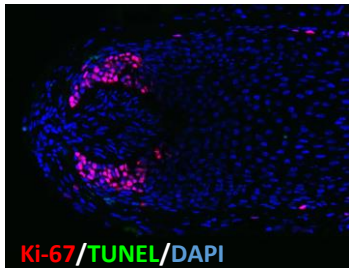
Hair cycle staging & Hair matrix keratinocyte proliferation



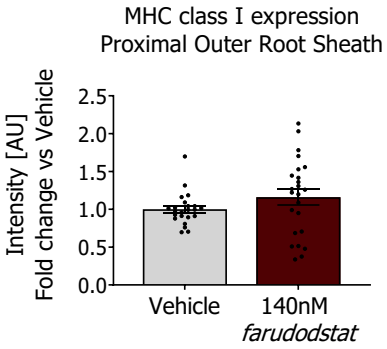


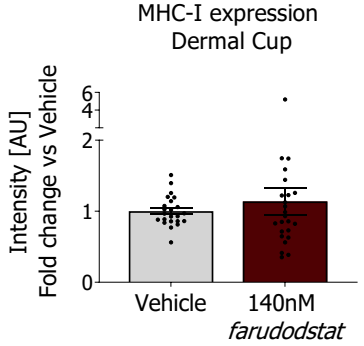


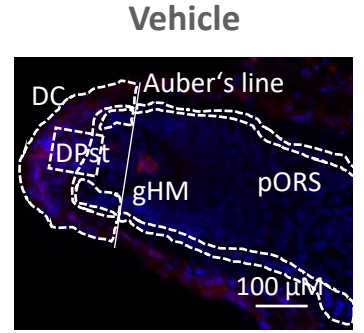


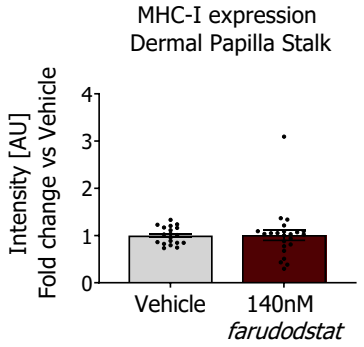


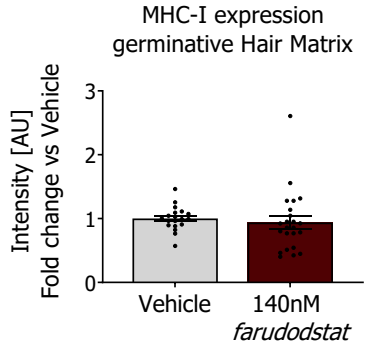
MHC class I expression

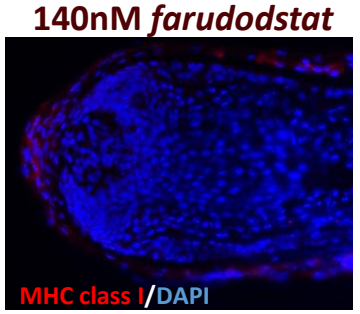






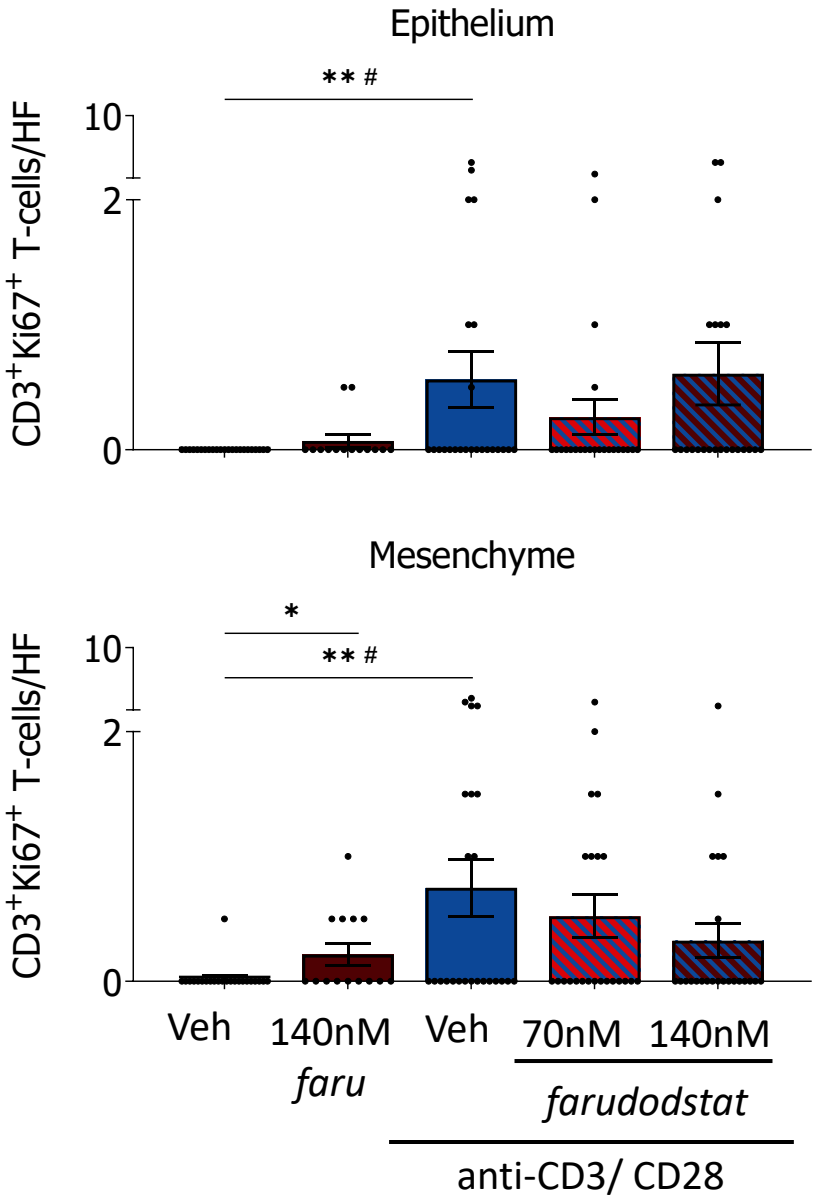




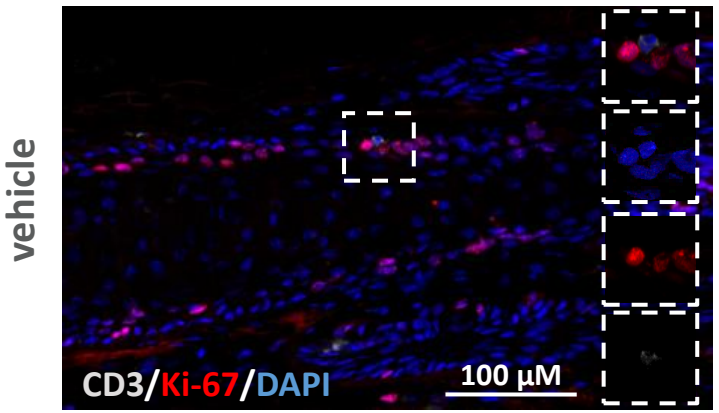


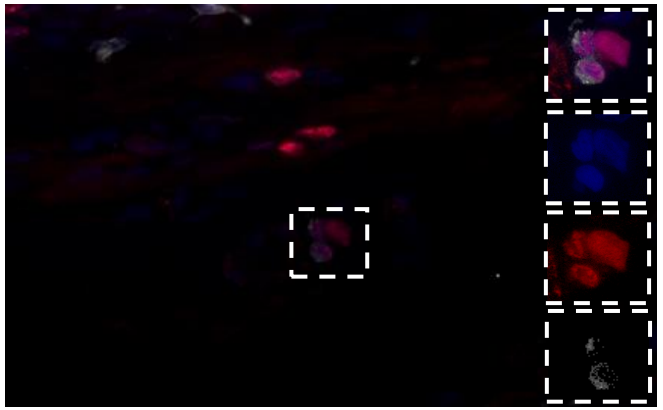
All data are presented as mean±SEM. n = 19-38 HFs from n = 6 donors Treatment groups were compared using Mann-Whitney test and no statistical significance was observed, scale bar = 100µm. bCTS: bulbar connective tissue sheath, DC: dermal cup, DPst: dermal papilla stalk, faru: farudodstat, gHM: germinative hair matrix, pcHM: pre cortical hair matrix, pORS: proximal outer root sheath

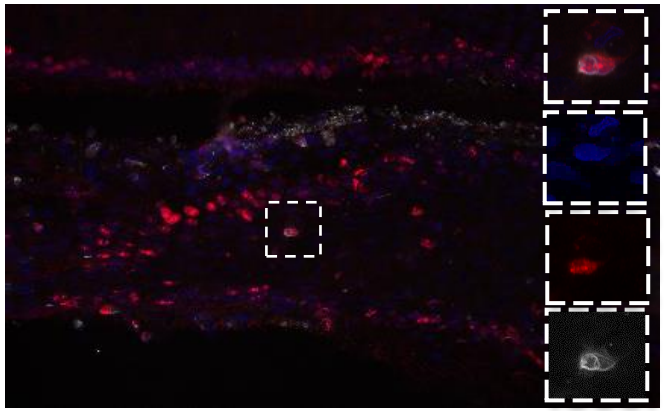
Farudodstat tendentially reduces α-CD3/α-CD28 induced T-cell proliferation in HFs *ex vivo*



T-cell proliferation

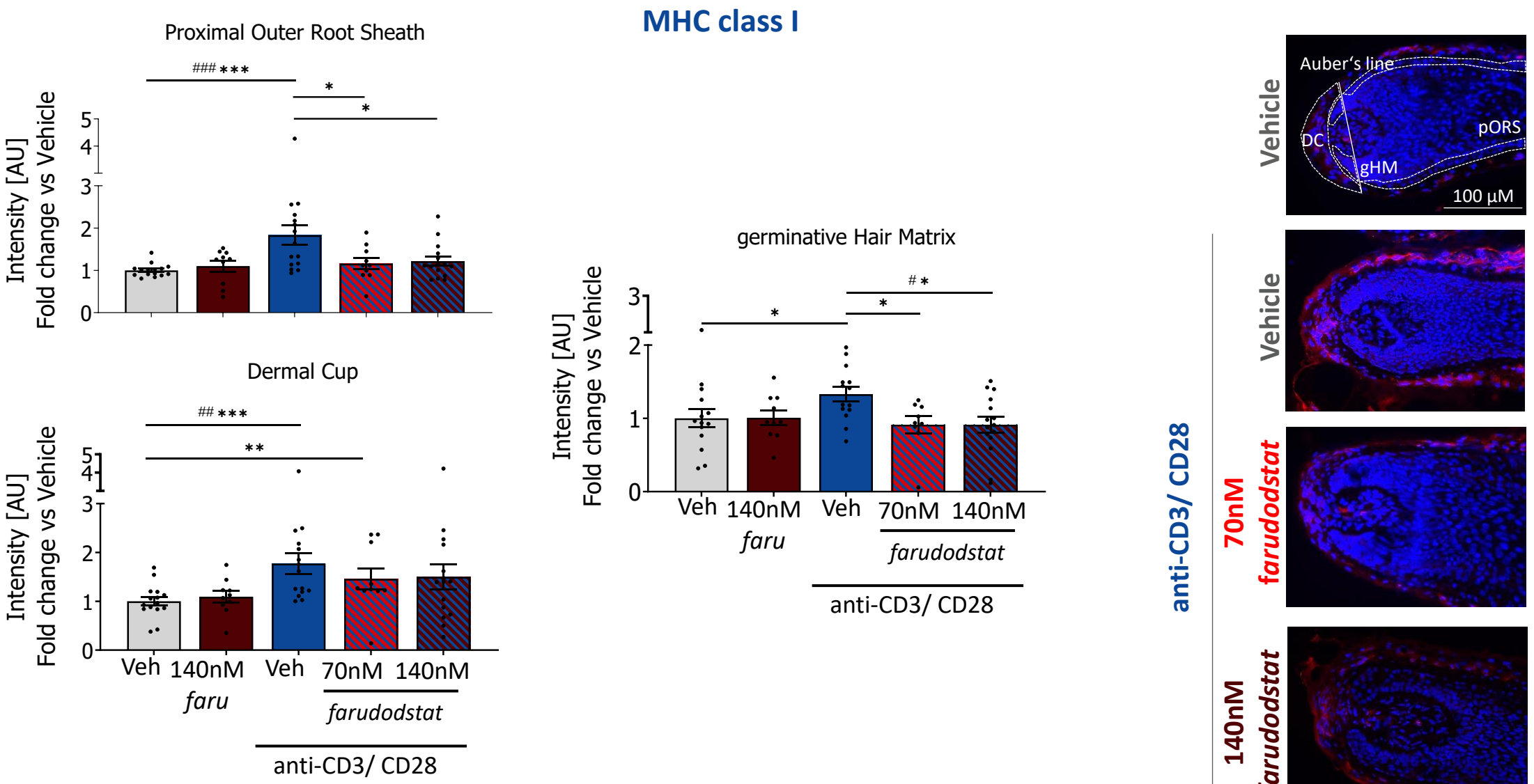






n = 14-24 HFs from n = 2-3 donors. Data are presented as mean±SEM, treatment groups were compared using D'Agostino & Pearson omnibus normality test, no Gaussian distribution; Kruskal-Wallis test with Dunn´s multiple comparison test #p<0.05; Mann-Whitney, *p<0.05, **p<0.01.DC: dermal cup, faru: farudodstat, gHM: germinative hair matrix, pORS: proximal outer root sheath, veh: vehicle

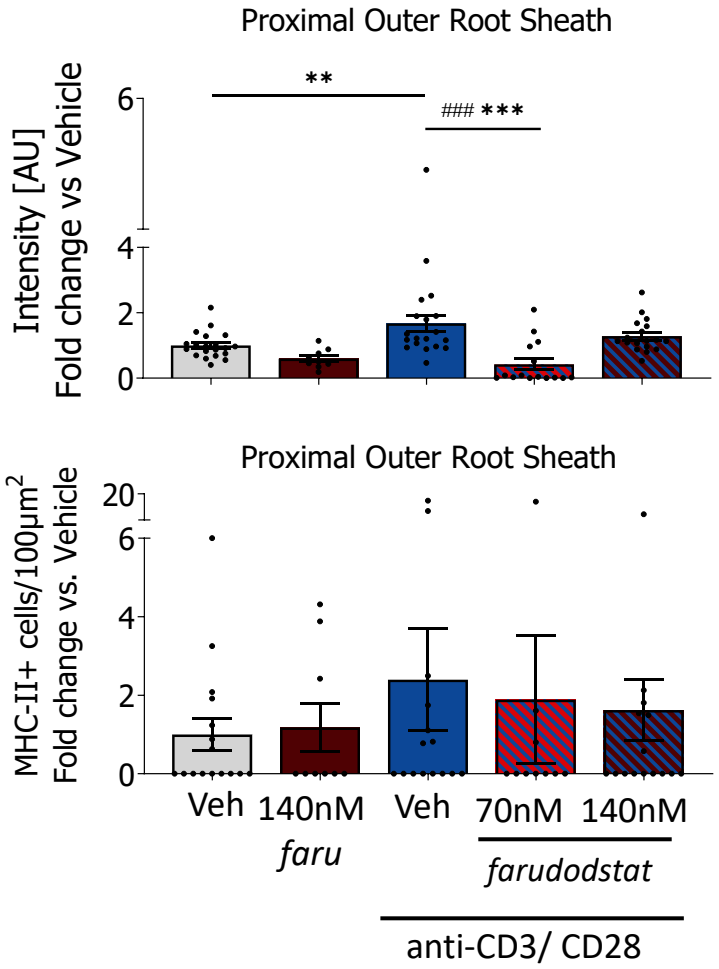
Farudodstat protects HFs *ex vivo* from α-CD3/α-CD28 induced upregulation of MHC class I expression

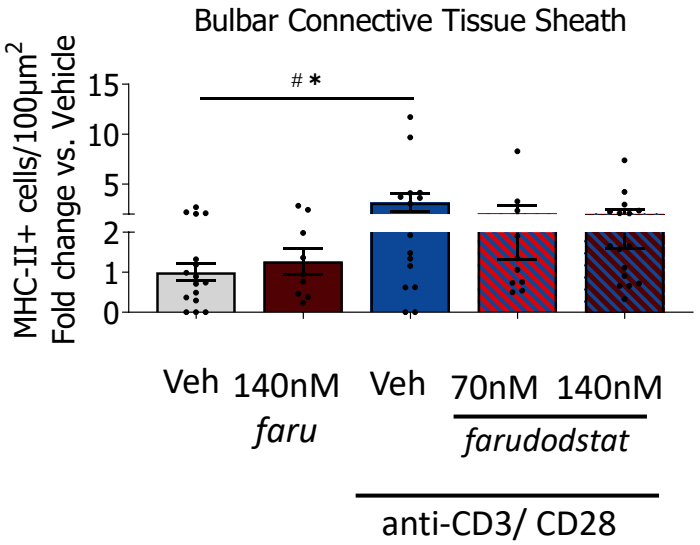


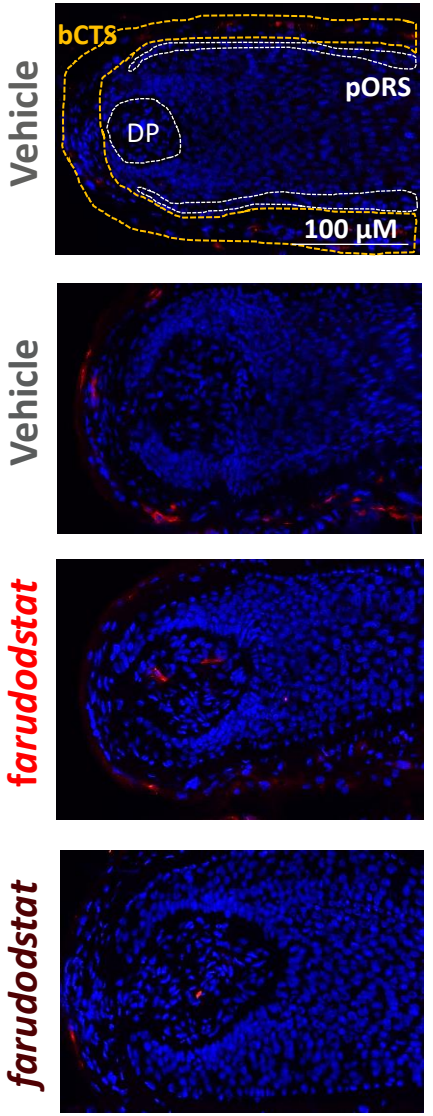
n = 9-15 HFs from n = 2-3 donors. Data are presented as mean±SEM, treatment groups were compared using D'Agostino & Pearson omnibus normality test, no Gaussian distribution; Kruskal-Wallis test with Dunn´s multiple comparison test #p<0.05, ##p<0.05, ### p<0.001; Mann-Whitney, *p<0.05, **p<0.01, ***p<0.001 DC: dermal cup, faru: farudodstat, gHM: germinative hair matrix, pORS: proximal outer root sheath, veh: vehicle

Farudodstat reduces α-CD3/α-CD28 induced upregulation of MHC class II+ cell number and expression

MHC class II







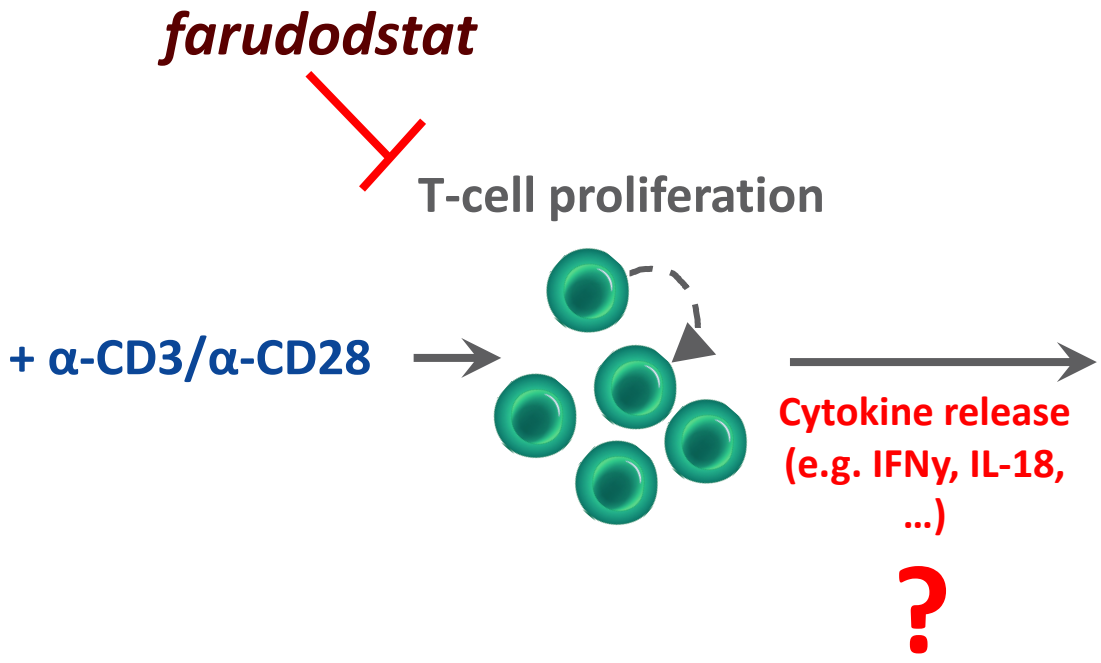
n = 9-16 HFs from n = 2-3 donors. Data are presented as mean±SEM, treatment groups were compared using D'Agostino & Pearson omnibus normality test, no Gaussian distribution; Kruskal-Wallis test with Dunn´s multiple comparison test, ### p<0.001 ## p<0.01, #p<0.05; Mann-Whitney, *p<0.05, **p<0.01, ***p<0.001. DP: dermal papilla, faru: farudodstat, pORS: proximal outer root sheath, veh: vehicle

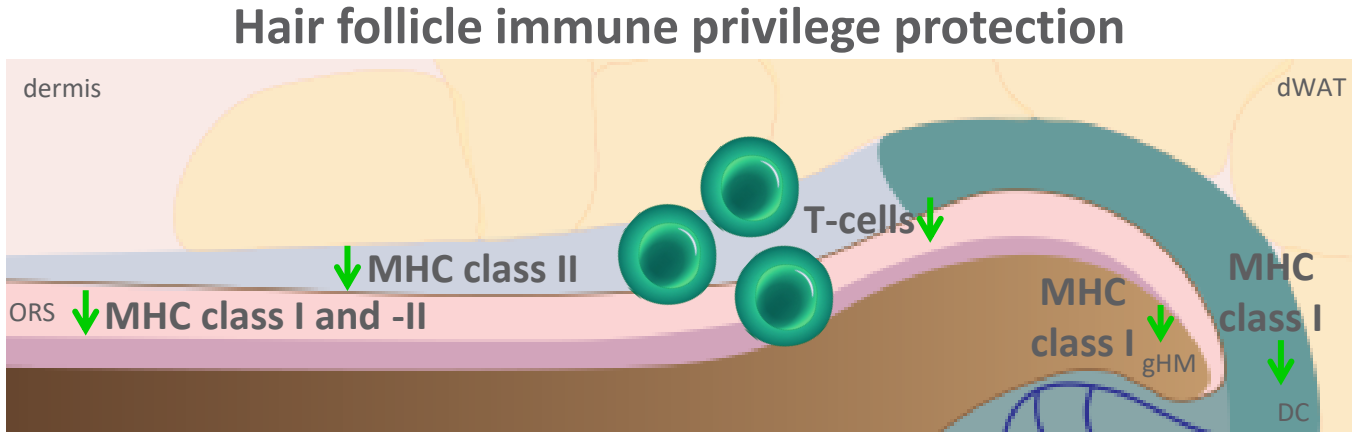
Conclusion & Perspective

→ TCR stimulation *ex vivo* is enough to induce hair follicle immune privilege collapse

→ DHODH inhibition with *farudodstat* might offer a novel therapy for AA management (proof-of-concept study on study in AA patients ongoing)

→ Our data invite for (pre)-clinical investigation of further read-out parameters in HFs under *farudodstat* treatment





dWAT: dermal white adipose tissue; DC: dermal cup; gHM: germinative hair matrix, ORS: outer root sheath

→ Other AA associated markers (e.g. MICA, CD1d, B2M,…)
→ IP guardians? (e.g. α-MSH, TGFβ1/2,…)
→ Cytokine and Chemokine release (e.g. IL-15, CXCL10, CXCL12,…)
→ Cytotoxicity/HF dystrophy,…
→ …

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