Ankersmit Laboratory

for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

Cyclic Alopecia and Abnormal Epidermal Cornification in Zdhhc13-Deficient Mice Reveal the Importance of Palmitoylation in Hair and Skin Differentiation

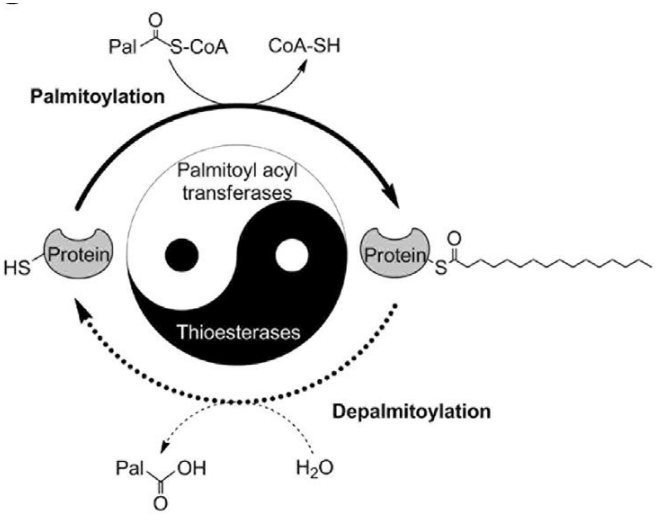
Liu *et al.*, Journal of Investigative Dermatology (2015) 135, 2603–2610

Tanja Wagner

Introduction

S-Palmitoylation

- Post-translational modification
- Palmitoyl acyl transferases (PATs) add palmitate to specific cyteine residues via thioester linkage
- Reversible lipid modification
- modulates protein targeting, trafficking, folding, stability, and protein-protein interactions
- DHHC13, a PAT, encoded by Zdhhc13
- Loss of Palmitoylation leads to diseases



(Han *et al.*, 2004)

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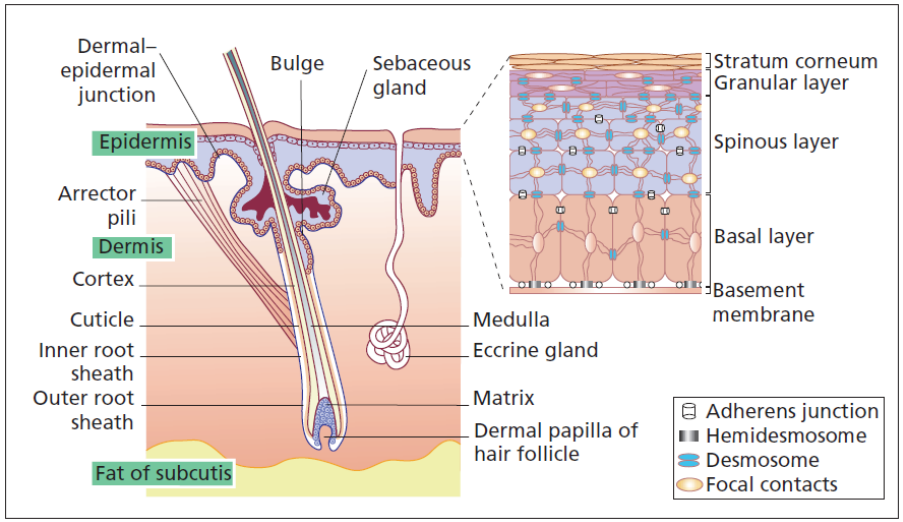
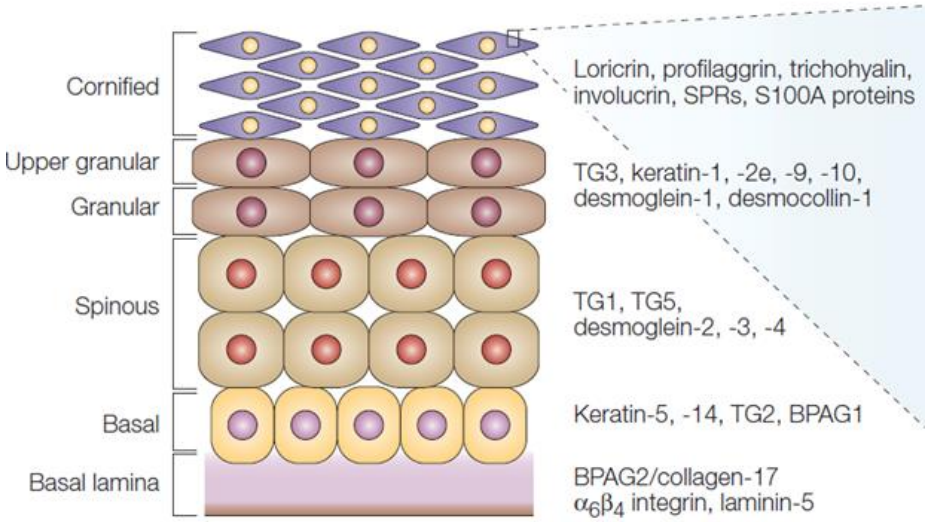
Structure of the skin



Burns *et al.*, 2004



Candi *et al.*, 2005

Keratin10 → spinous and granular layer

Keratin14 → basal layer

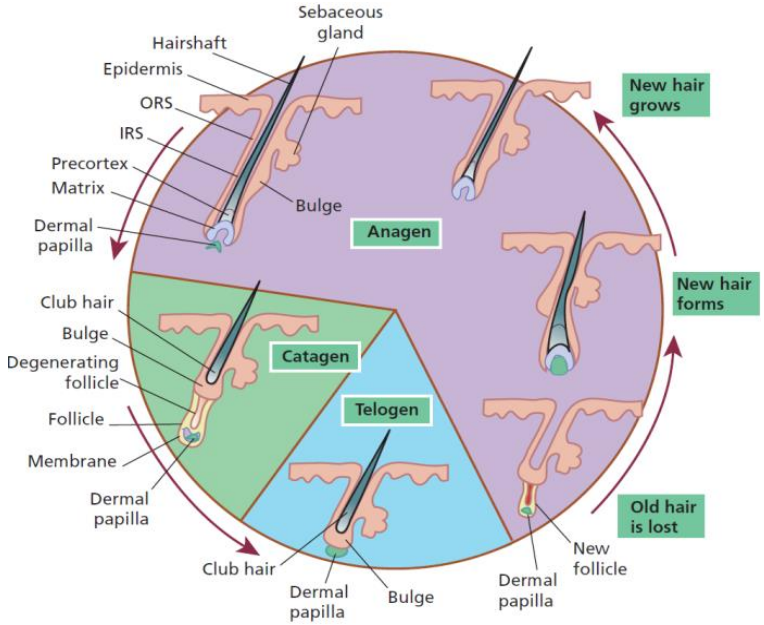
Keratin72 → inner root sheath of hair follicles

Keratin82 → hair cuticle-specific keratin

https://www.ncbi.nlm.nih.gov/gene/140807
https://www.ncbi.nlm.nih.gov/gene/3888

- Burns T, Breathnach S, Cox N, Griffiths C: Rook`s Textbook of Dermatology, 7 ed (Blackwell Publishing, 2004)
- Candi E, Schmidt R, Melino G: The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 6:328–340 (2005).

Hair cycle



Burns *et al.*, 2004

→ **Anagen**: hair growth

→ **Catagen**: hair regression

→ **Telogen**: resting phase

- Burns T, Breathnach S, Cox N, Griffiths C: Rook`s Textbook of Dermatology, 7 ed (Blackwell Publishing, 2004)

Aims of the study

- Exploration of the role of palmitoylation in the skin and hair differentiation
- Investigation of the pathogenic mechanisms of alopecia and skin hyperkeratosis in Zdhhc^{13skc4} mice

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Methods

Mouse model: $Zdhhc13^{skc4}$ mice

Nonsense Zdhh13 mutation:

→ point mutation resulting in a premature stop codon

→ leading to a truncated form of DHHC13

→ no palmitoylation of cysteines

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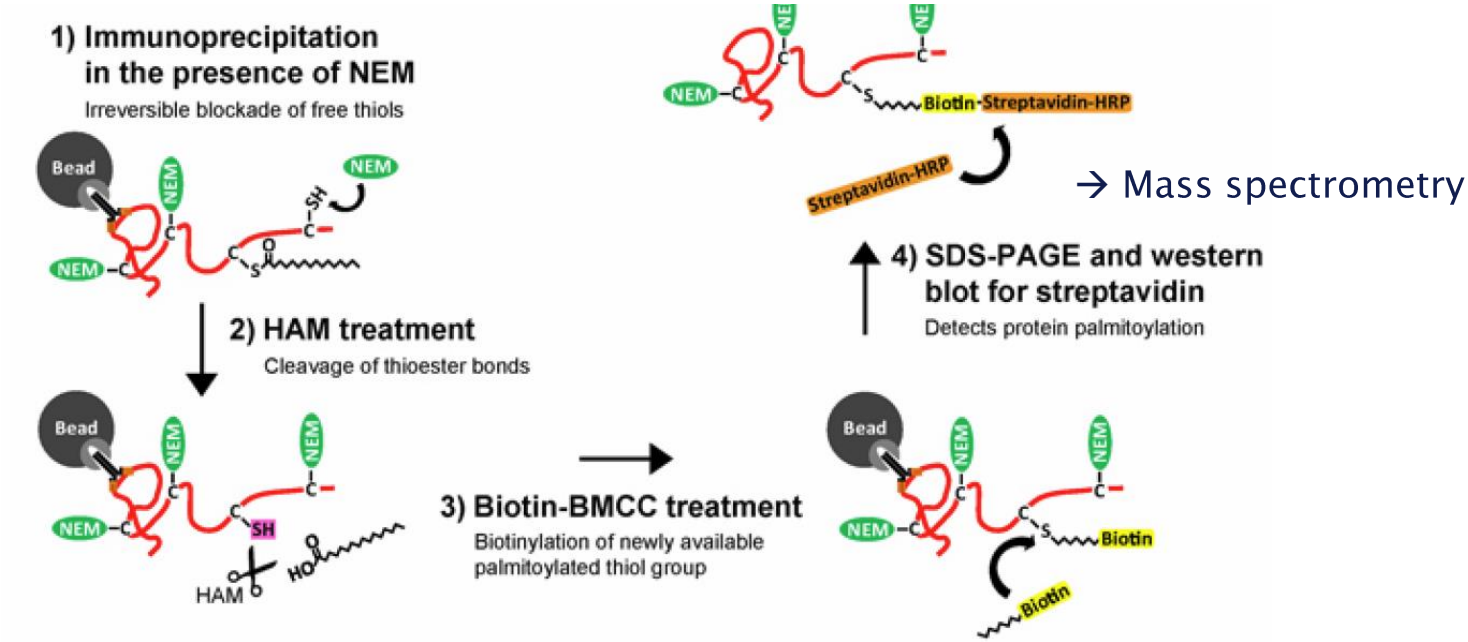
Acyl-biotinyl exchange (ABE)-proteomic approach

- **WT DHHC13**
  → Palmitoylation
  → Biotin signal

- **Mutant DHHC13**
  → no Palmitoylation
  → No signal



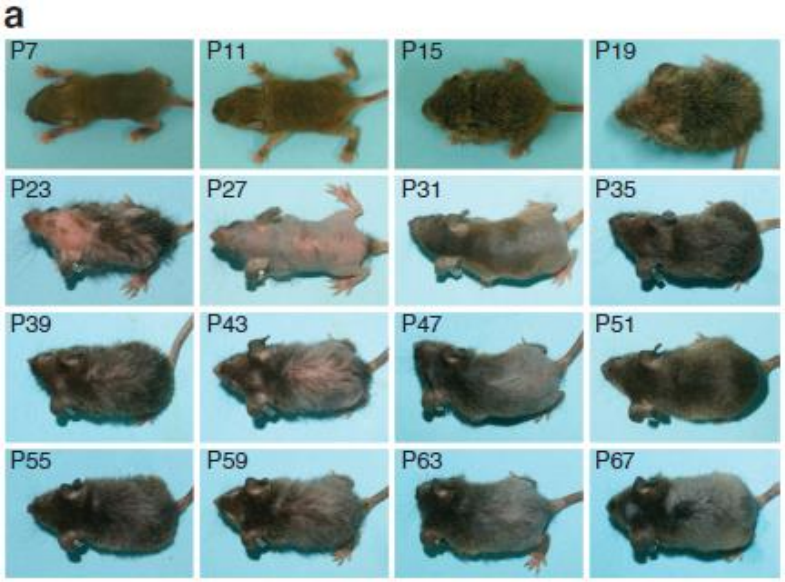
Brigidi and Bamji., 2013

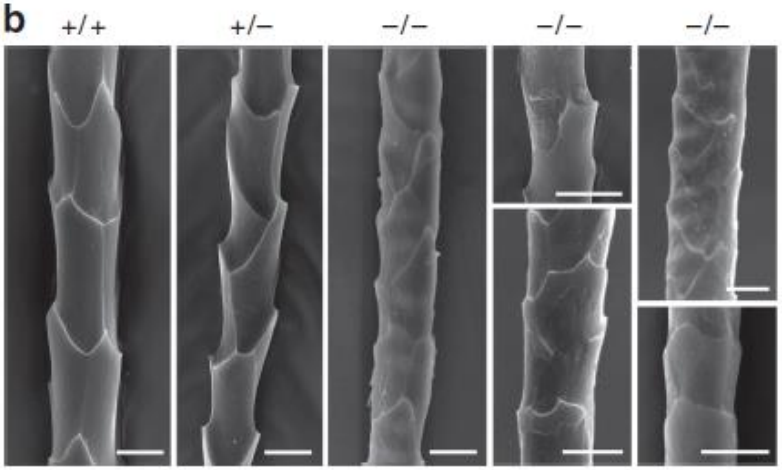
1) Purification of target protein using a target-specific antibody, then treatment with **N-ethylmaliemide** (NEM; green) → irreversibly bind and *block* free thiol (-SH) groups along *unmodified cysteines* (C)
2) Treatment with **hydroxylamine (HAM)** → specific cleavage of thioester bonds at palmitoylated cysteines
3) Treatment with **thiol-reactive biotin** molecule → specific *biotinylation of the palmitoylated cysteine*
4) Detection of *palmitoylated cysteine(s) tagged with biotin*

Brigidi, G.S., Bamji, S.X. Detection of Protein Palmitoylation in Cultured Hippocampal Neurons by Immunoprecipitation and Acyl-Biotin Exchange (ABE). J. Vis. Exp. (72), e50031, doi:10.3791/50031 (2013).

Results

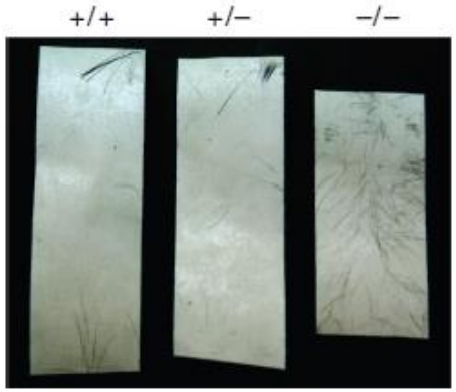
Hair abnormalities in Zdhhc13^{skc4} mice

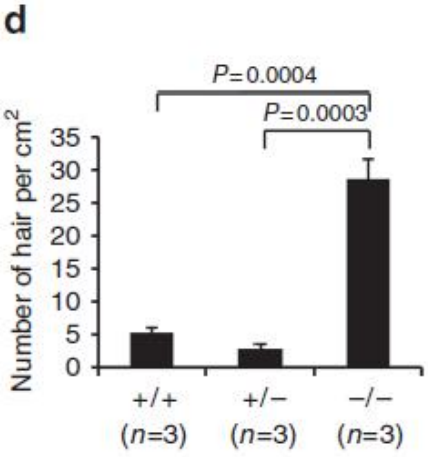




smooth ragged and dilapidated

c Hair pull test





→ hair loss at the telogen phase

→ poor hair anchoring with a defective cuticle hair shaft

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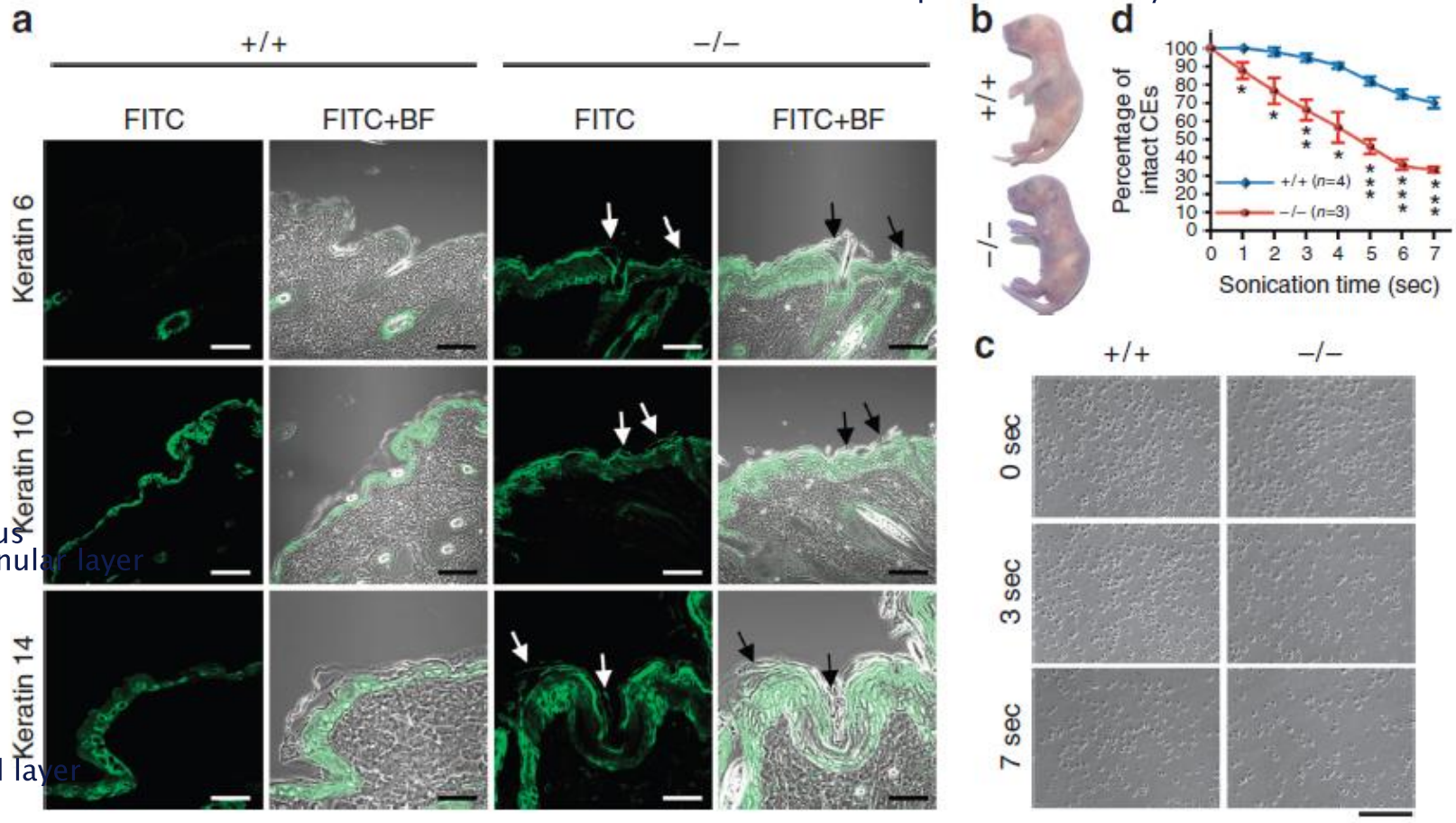
Skin abnormalities in Zdhhc13^{skc4} mice

Toluidine blue dye penetration assay



Prolif ha

&

Spinous granular layer

basal layer

Zdhhc13^{skc4} mice

→ keratinocyte hyperpoliferation and disorganized cornification

→ impaired skin barrier

→ cornified envelope had poor resistance against physical stress

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Cornifelin as a candidate substrate of DHHC13

Protein name	Peptide sequence	Start position	End position	WT1 – HA	WT1 +HA	WT2 +HA	WT3 +HA	M1 – HA	M1 +HA	M2 +HA	M3 +HA
Cornifelin	ISDDFGE**C**CCAPYLPGGLHSLR	51	72	0	537	455	1,355	0	0	0	0
Cornifelin	ISDDFGEC**C**CAPYLPGGLHSLR	51	72	0	536	454	1,355	0	0	0	0
Cornifelin	ISDDFGECC**C**APYLPGGLHSLR	51	72	0	537	455	1,356	0	0	0	0
Cornifelin	ISDDFGEC**CC**APYLPGGLHSLR	51	72	0	57	41	78	0	0	0	0
Cornifelin	ISDDFGE**CCC**APYLPGGLHSLR	51	72	0	98	22	69	0	0	0	0
Cornifelin	YHIQGSVGHDWAALTF**C**LPCALCQMAR	79	105	0	37	49	156	0	0	0	0
Cornifelin	YHIQGSVGHDWAALTFCLPCAL**C**QMAR	79	105	0	37	49	156	0	0	0	0

Negative controls: Hydroxyalamine (HA)-untreated groups
(WT-HA; M-HA)

➔ absence of biotinylated peptides in the skin of -/- mice

Cornifelin

→ contains 5 palmitoylated cysteines

→ specific PAT substrate of DHHC13

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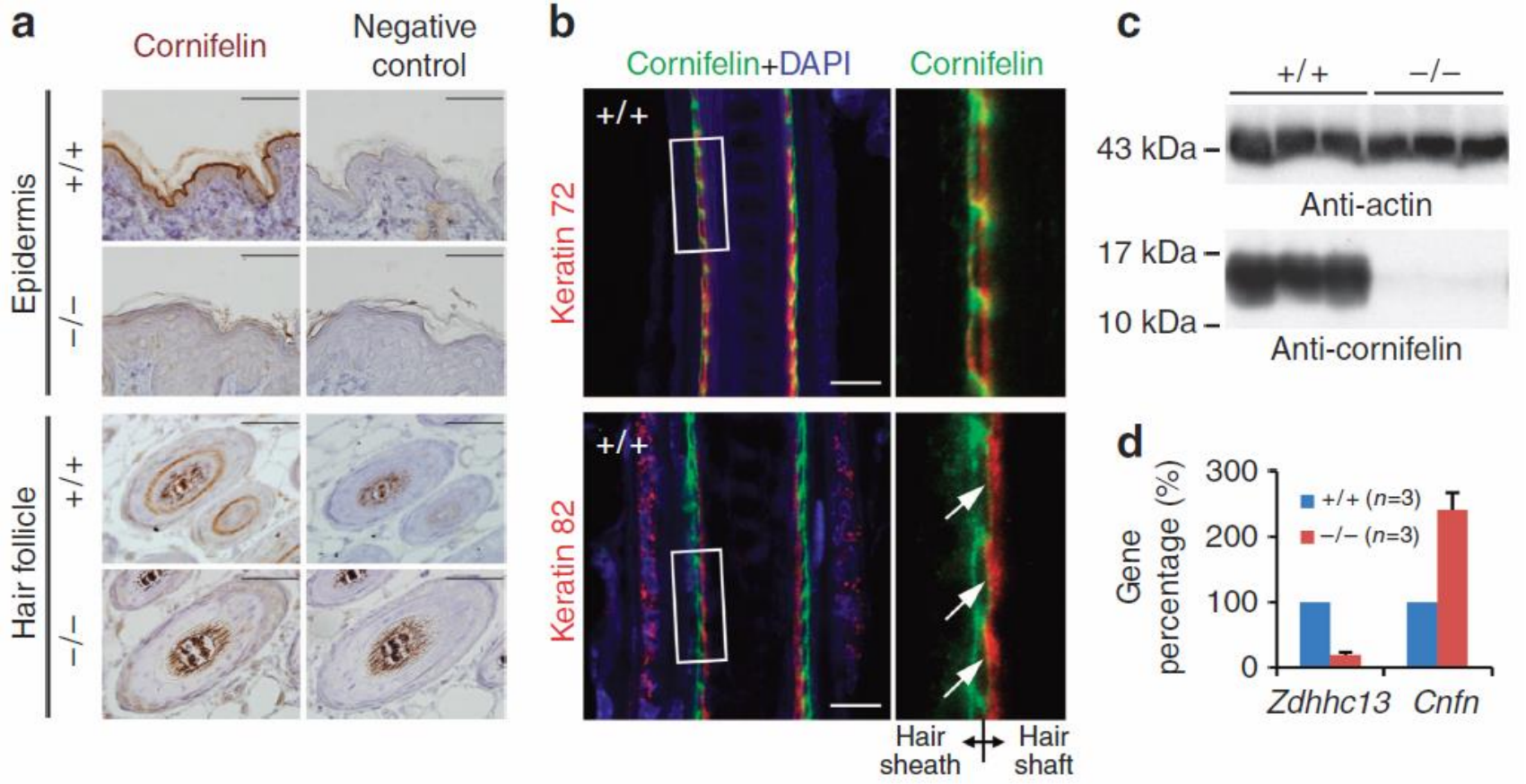
Expression of Cornifelin



→ Cnfn expressed in the **cuticle of the inner root sheat** and the **surface layer of the cuticle of the hair shaft**

→ higher mRNA expression level of Cnfn in -/- mice

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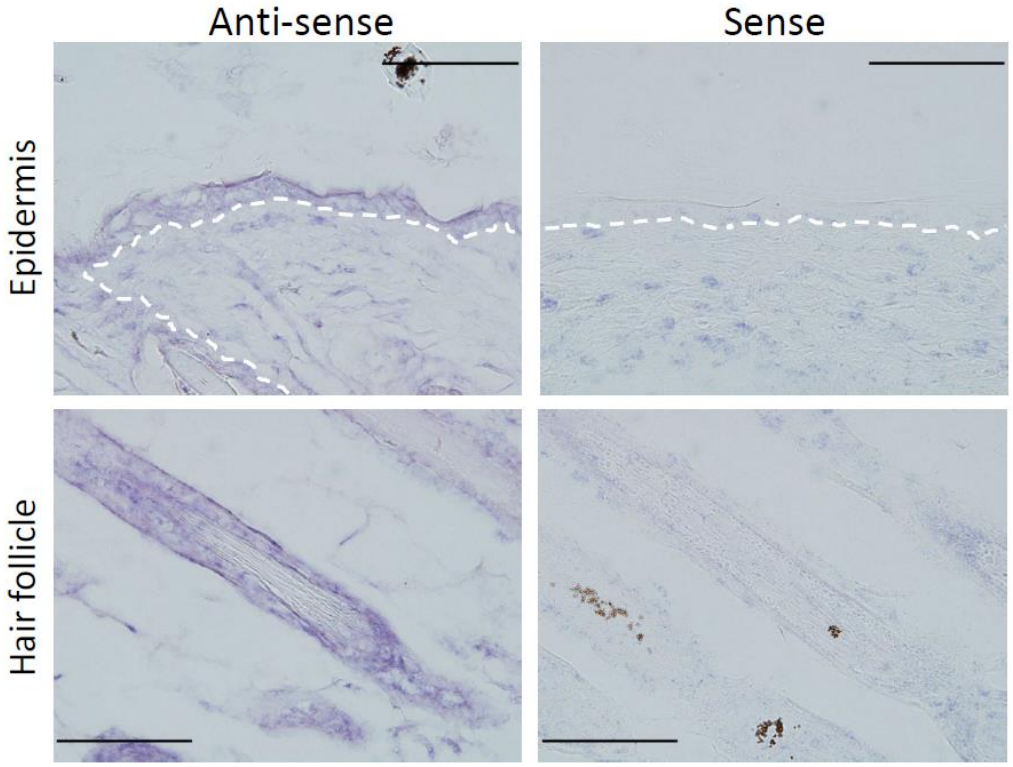
Expression of Zdhhc13



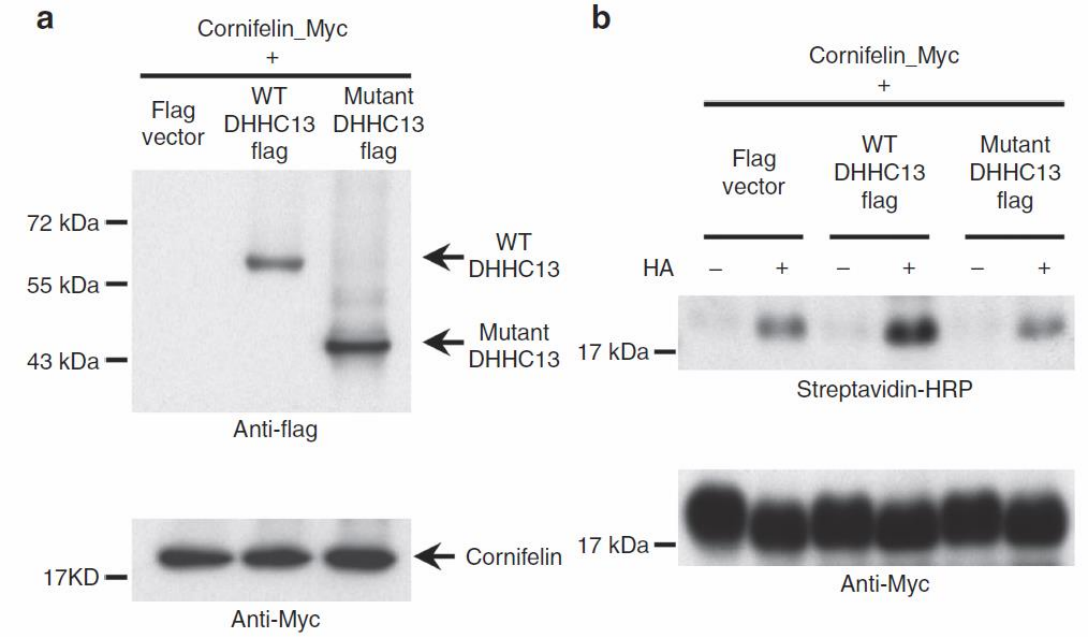
→ Zdhhc13 mRNA expression in the epiderms and hair follicles of WT mice

Liu *et al.*, Journal of Investigative Dermatology (2015) 135, 2603–2610 (Supplementary)

DHHC13 palmitoylates cornifelin

Co-transfection with cornifelin-myc: +Flag vector
+WT DHHC13-Flag
+mutated DHHC13-Flag

➔Immunoprecipitatin with anti-myc antibody
➔ ABE assay



Staining for biotin detection
→ palmitoylated Cnfn

→ Higher Cnfn-myc Palmitoylation levels in WT DHHC13 flag

→ **Mutant DHHC13** could **not palmitoylate Cnfn**

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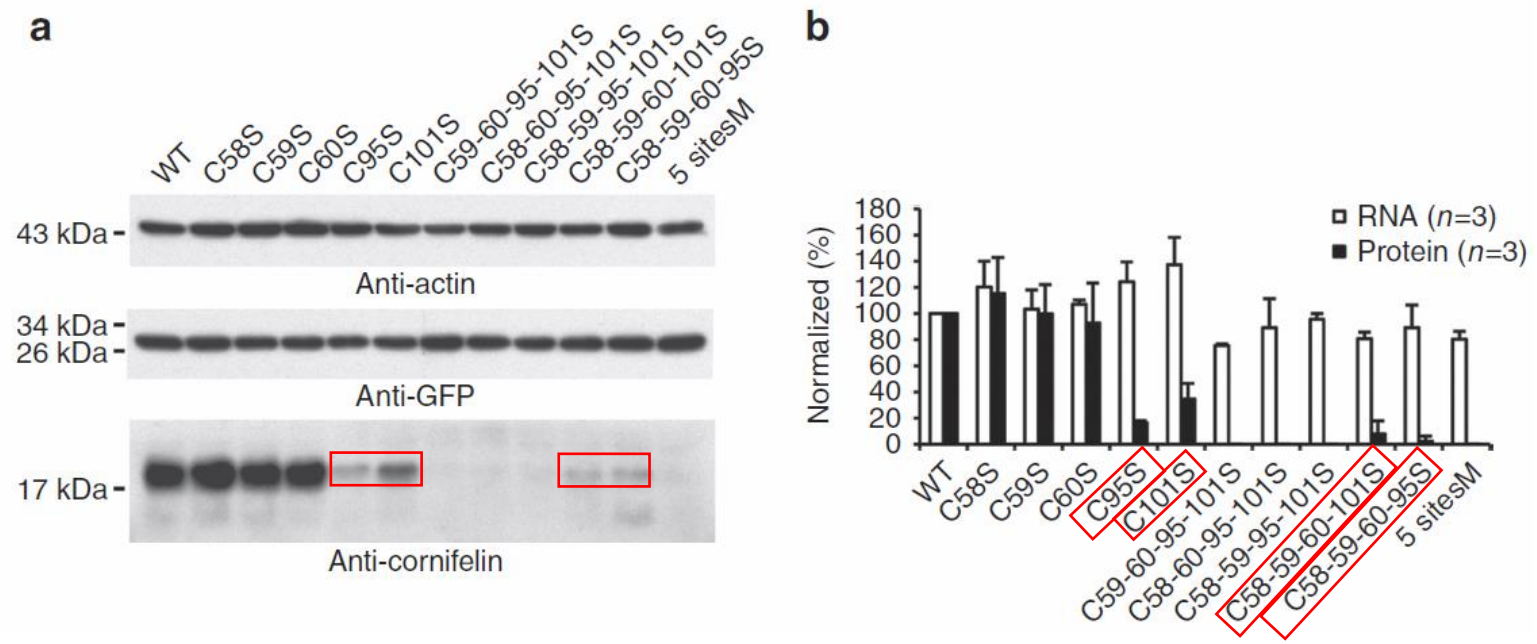
Palmitoylation of Cys95 and Cys101 is required for Cnfn expressoin

Cornifelin-myc constructs:

*single-site mutations

*four-site mutations

*full five-site mutation



GFP
→ Control for transfection efficiency

→ Cnfn was significantly reduced in **C95S** and **C101S**

→ Important for **Cnfn expression**

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Discussion

Loss of Cnfn

- lead to ragged and dilapidated cuticle hair shaft
- poor hair anchoring ability resulting in cyclic alopecia
- affected skin barrier function
- hyperkeratosis
- defective Palmitoylation resulted in the loss of Cnfn

➔ Palmitoylation was essential for Cnfn protein stability

➔ Cnfn has an important role in the skin barrier and hair anchoring

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Discussion

- other specific PAT substrates of DHHC13, not only Cnfn
- function of Cnfn reamins unclear
- lack of functional studies
- evidence of a causal relationship between Cnfn in the hair and skin abnormalities

➔ **Generation of a Cnfn-deficient mouse model!**

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References

- Brigidi, G.S., Bamji, S.X. Detection of Protein Palmitoylation in Cultured Hippocampal Neurons by Immunoprecipitation and Acyl-Biotin Exchange (ABE). J. Vis. Exp. (72), e50031, doi:10.3791/50031 (2013).
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- Candi E, Schmidt R, Melino G: The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 6:328–340 (2005).
- https://www.ncbi.nlm.nih.gov/gene/140807
- https://www.ncbi.nlm.nih.gov/gene/3888

Thank you for your attention!