

研究例 3

毛の性状制御

An important role and the strict expression control of Hacl-1, a hair follicle specific KAP

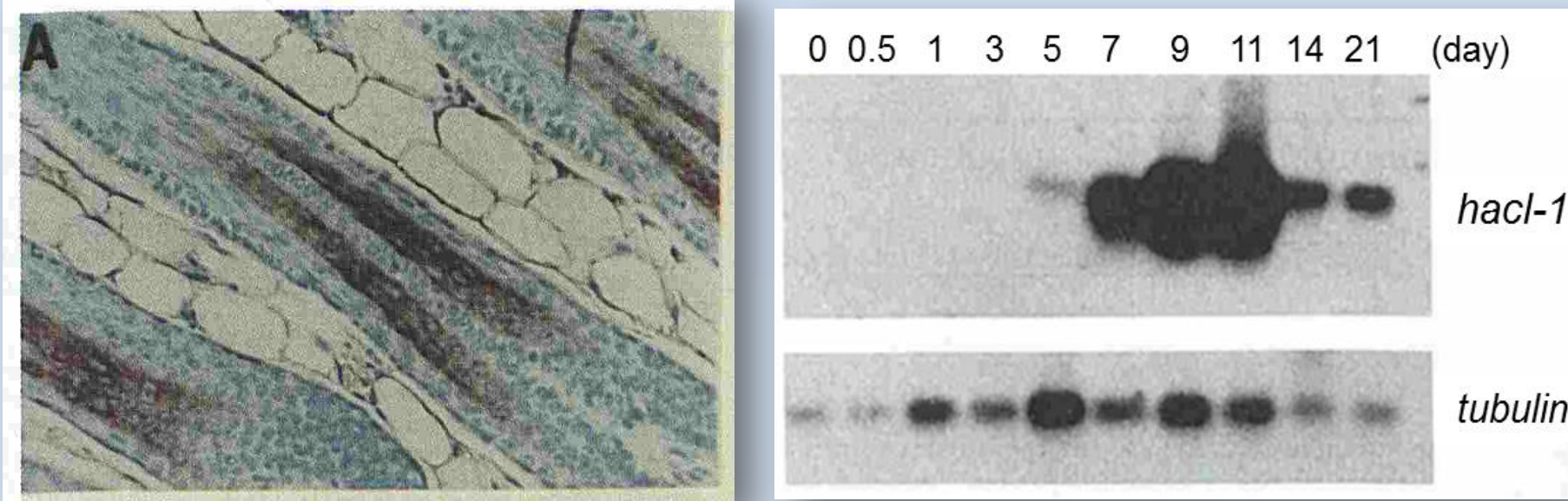
○Shunsuke Fujimoto, Yohei Hirai

Dep. Bioscience., Grad. Sch. Sci. Technol., Kwansai Gakuin Univ.



1. Introduction

The major components of the hair are keratins and keratin associate-proteins (KAPs). KAPs have been supposed to play a roles in the keratin filamentous assembly so as to maintain the physical strength of hair shaft. Previously, Hacl-1, a member of KAPs family, was detected exclusively in the anagenic hair follicle, however, little is known about this protein. In this study, we tested the effect of Hacl-1 overexpression in cultured hair follicle. We also investigated the molecular characteristics and subcellular localization of Hacl-1 and its binding partner.

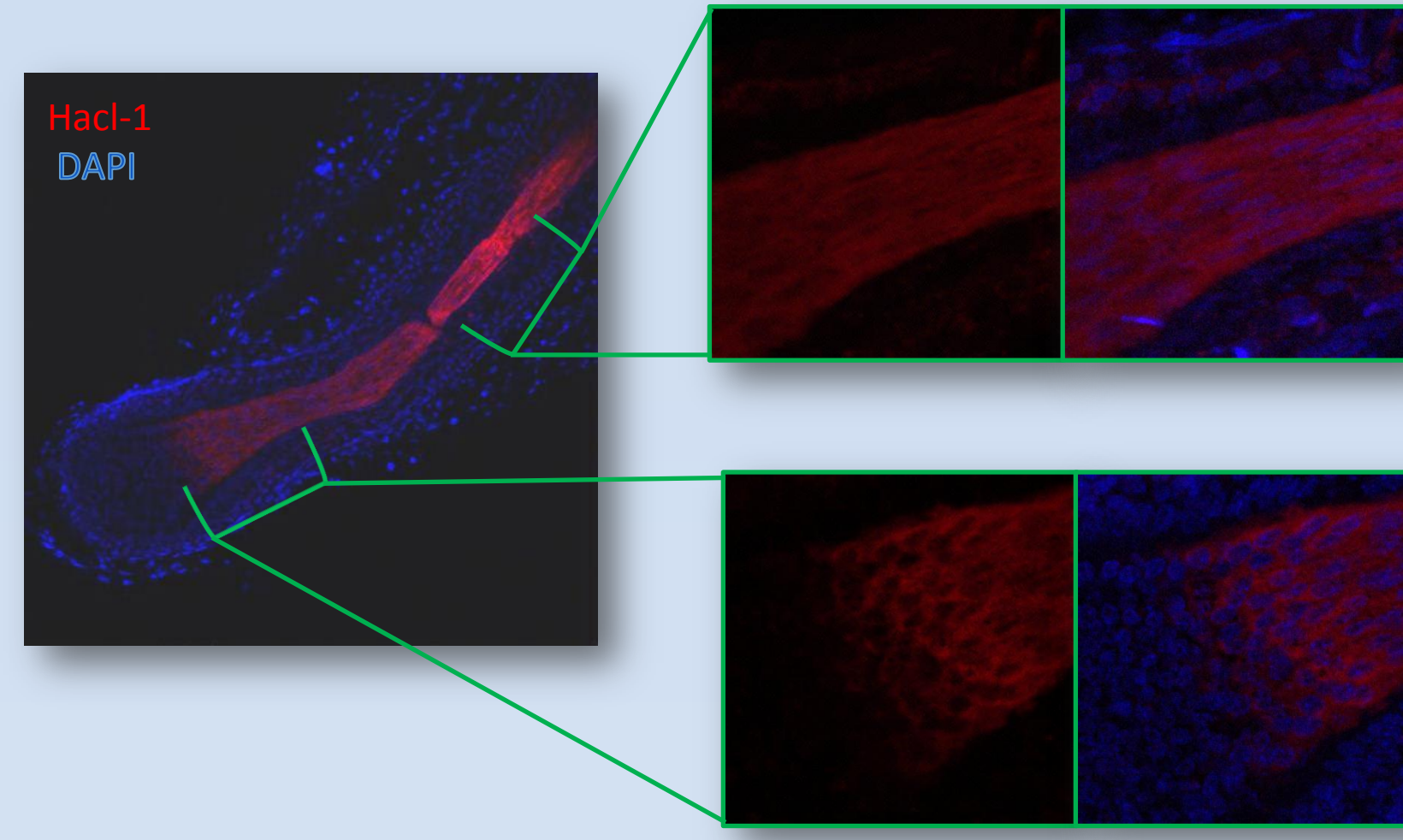


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The previous study demonstrated that Hacl-1 mRNA is expressed in the anagenic hair.

2. The localization of Hacl-1 protein in the vibrissals hair follicles

Immunostaining

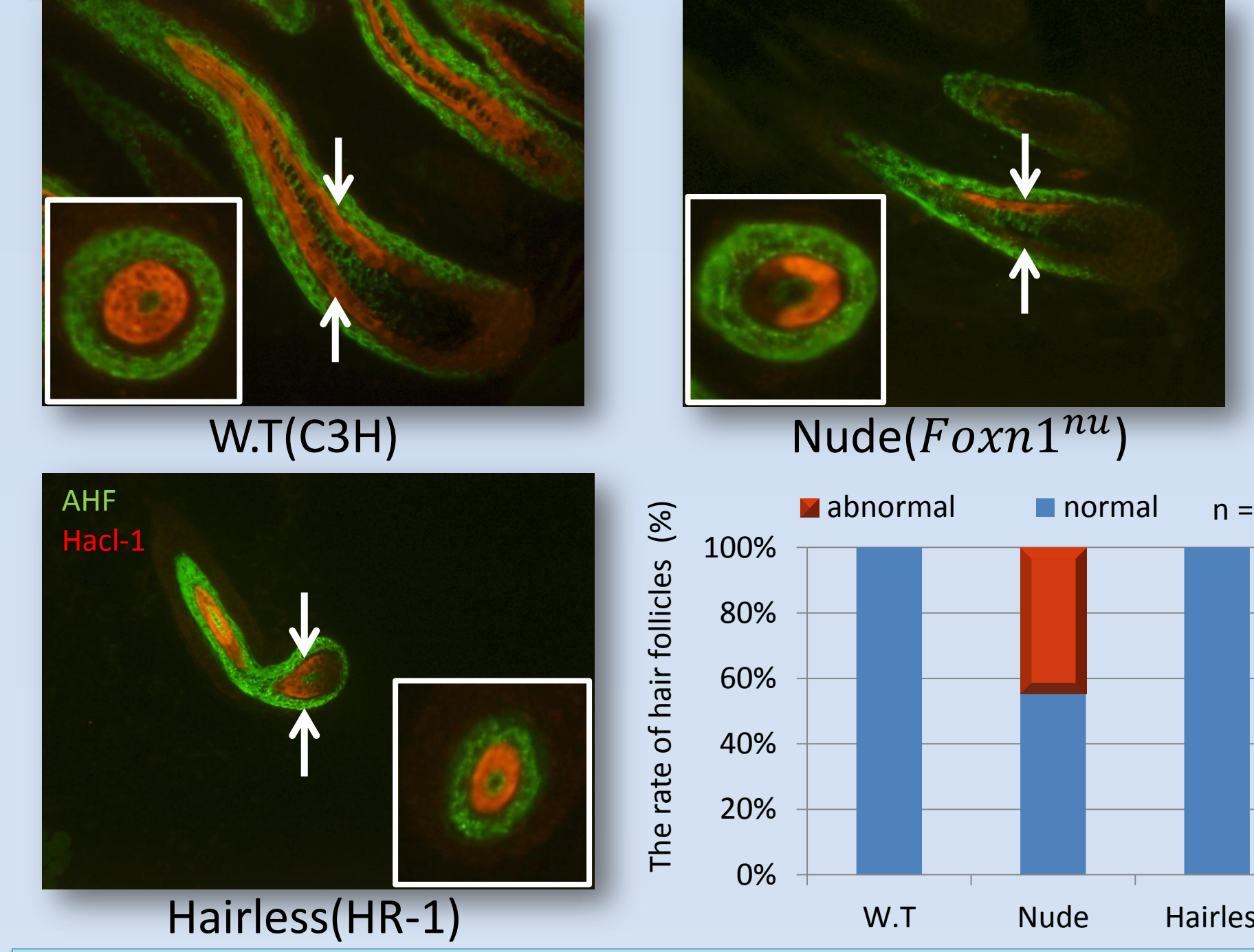


Hacl-1 protein was expressed in the lower and middle portion of the cortex.

Hacl-1 localized specifically in the hair cortex.

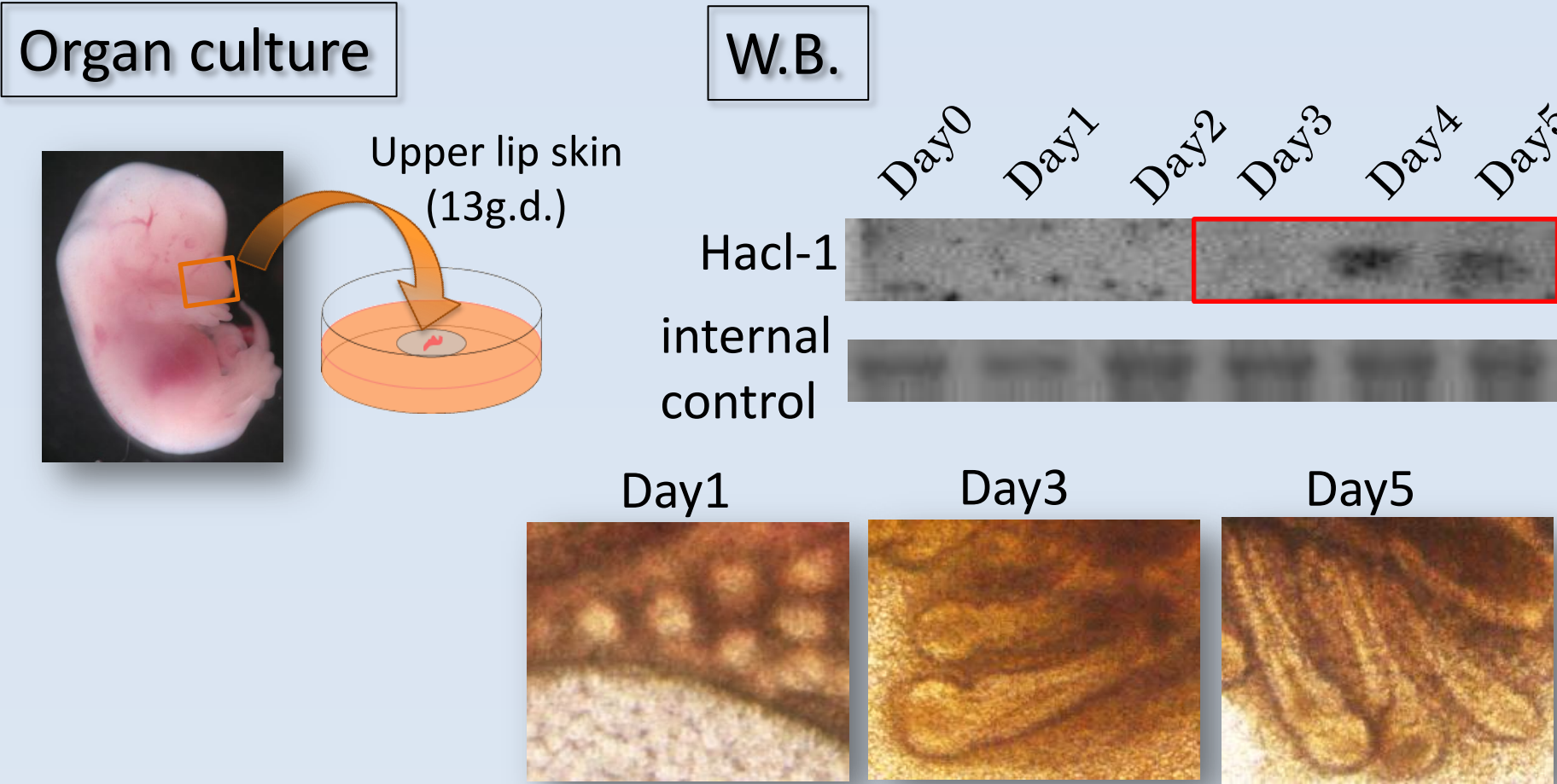
3. The expression pattern of Hacl-1 in mutant mice

Immunostaining



The concentrically expression pattern was perturbed in nude mice.

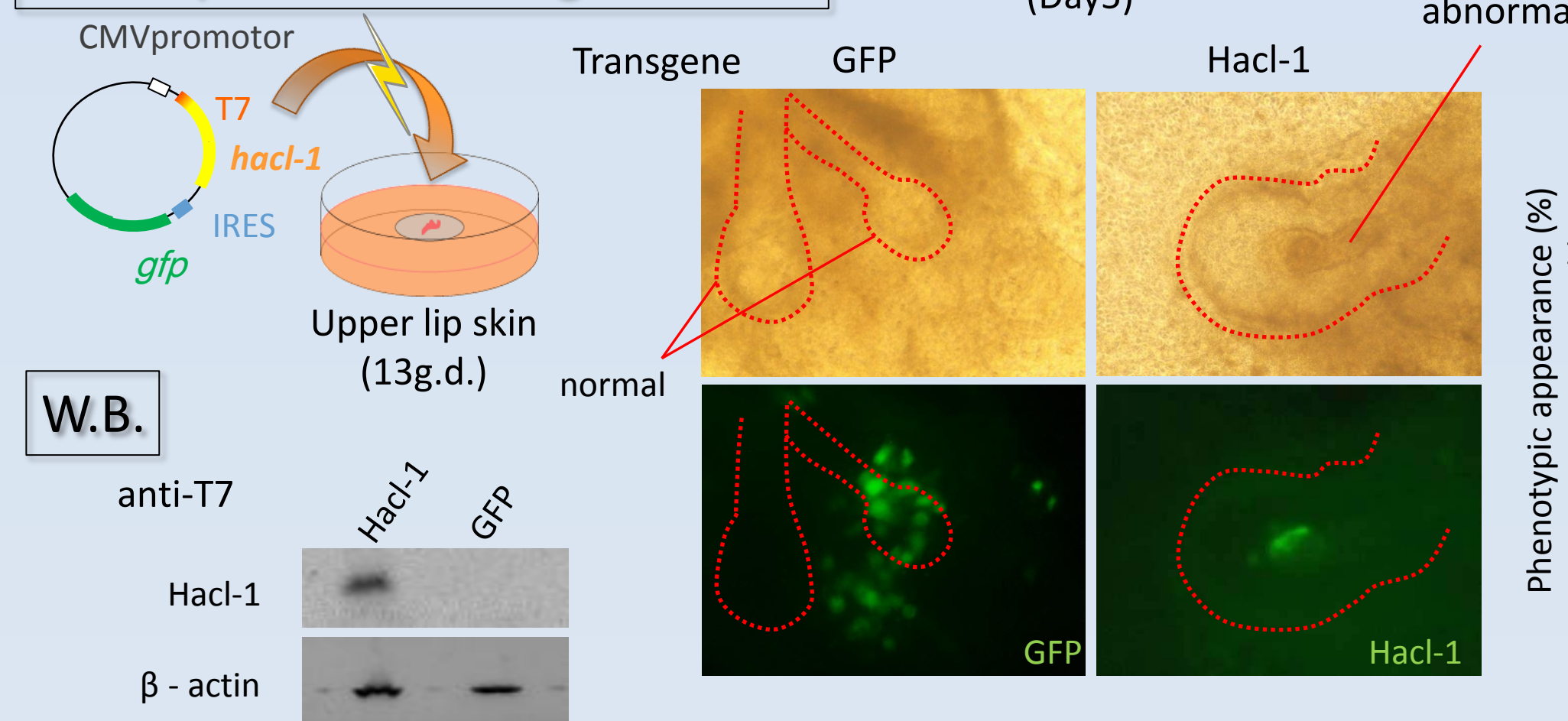
4. The expression of Hacl-1 upon the hair follicle formation in culture



Expression of Hacl-1 was accompanied by the hair follicle formation.

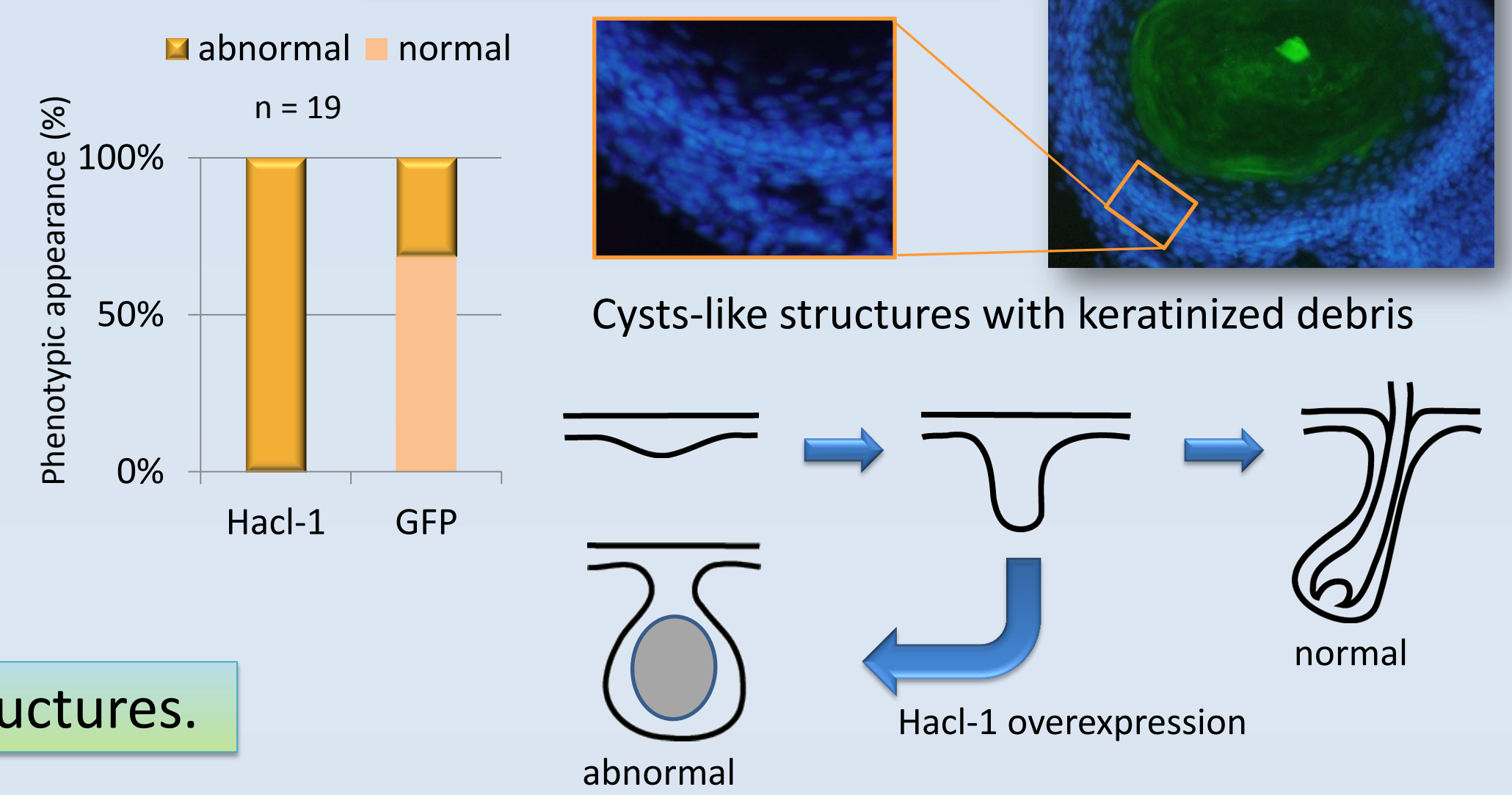
5. The Effect of Hacl-1 on the hair follicle formation

Electroporation and organ culture



Hacl-1 overexpression caused abnormal follicular structures.

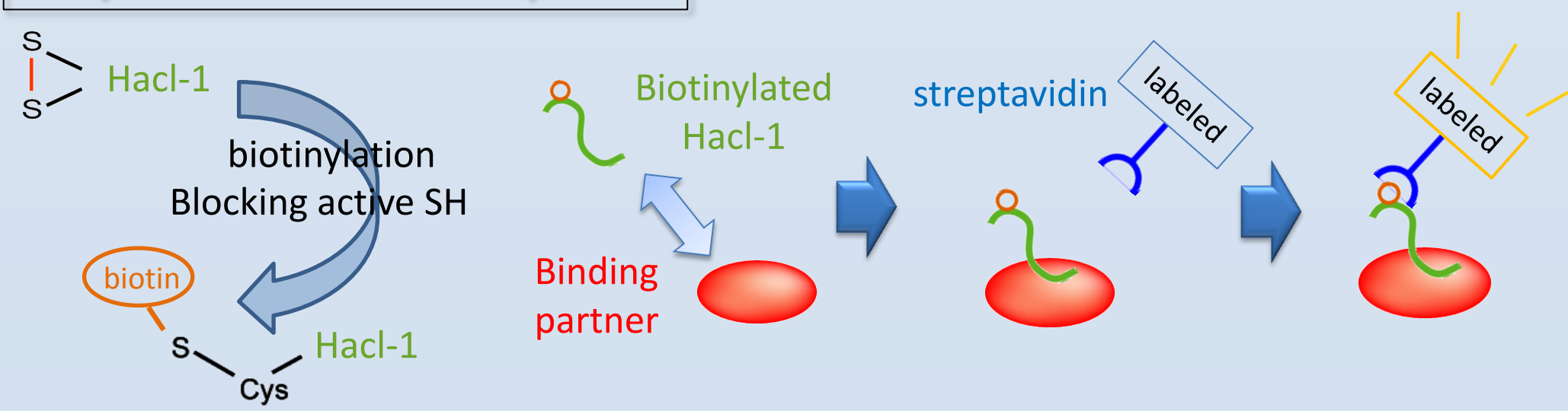
Abnormal hair follicles with Hacl-1 overexpression



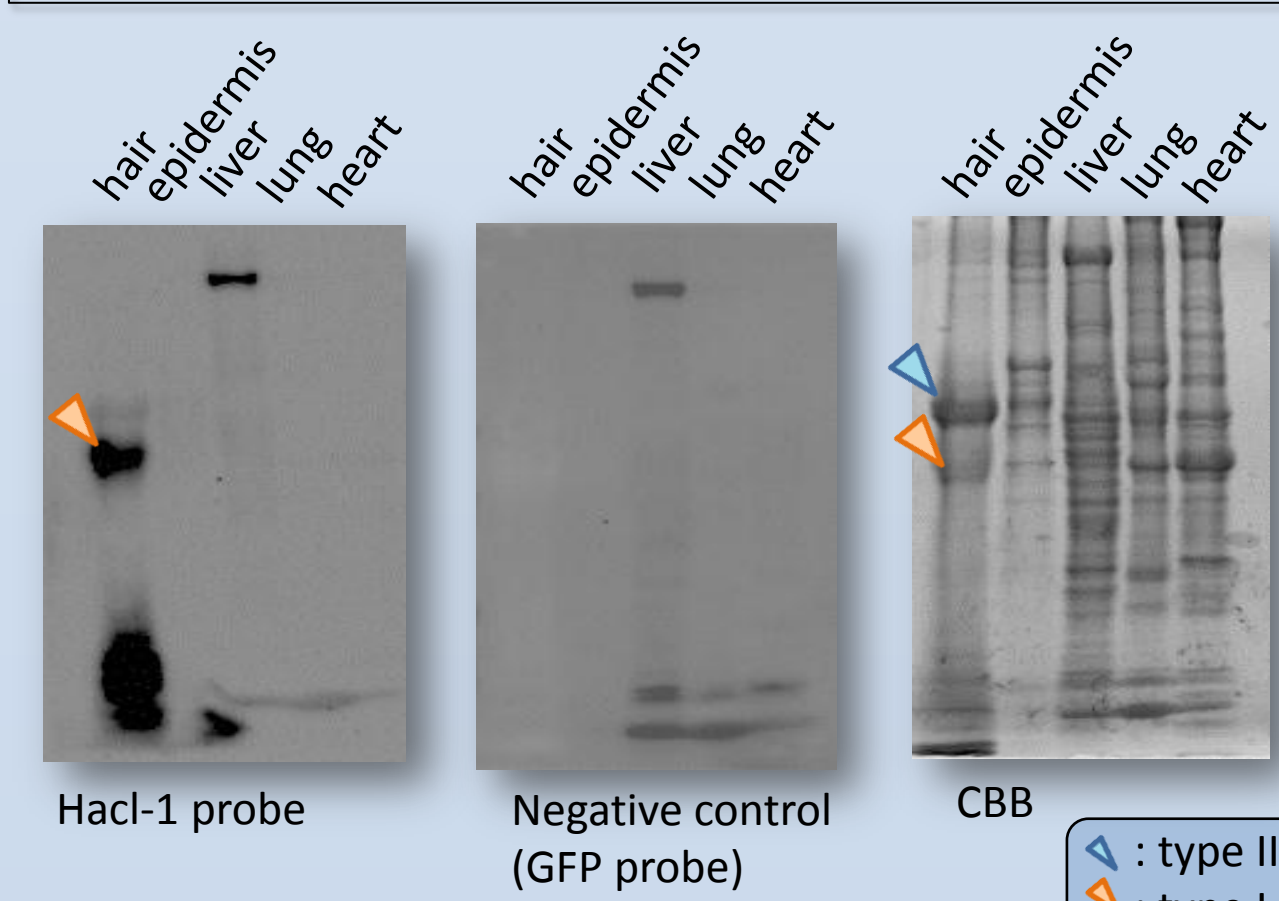
Cysts-like structures with keratinized debris

6. Detection of the binding partner of Hacl-1

Preparation of Hacl-1 probe

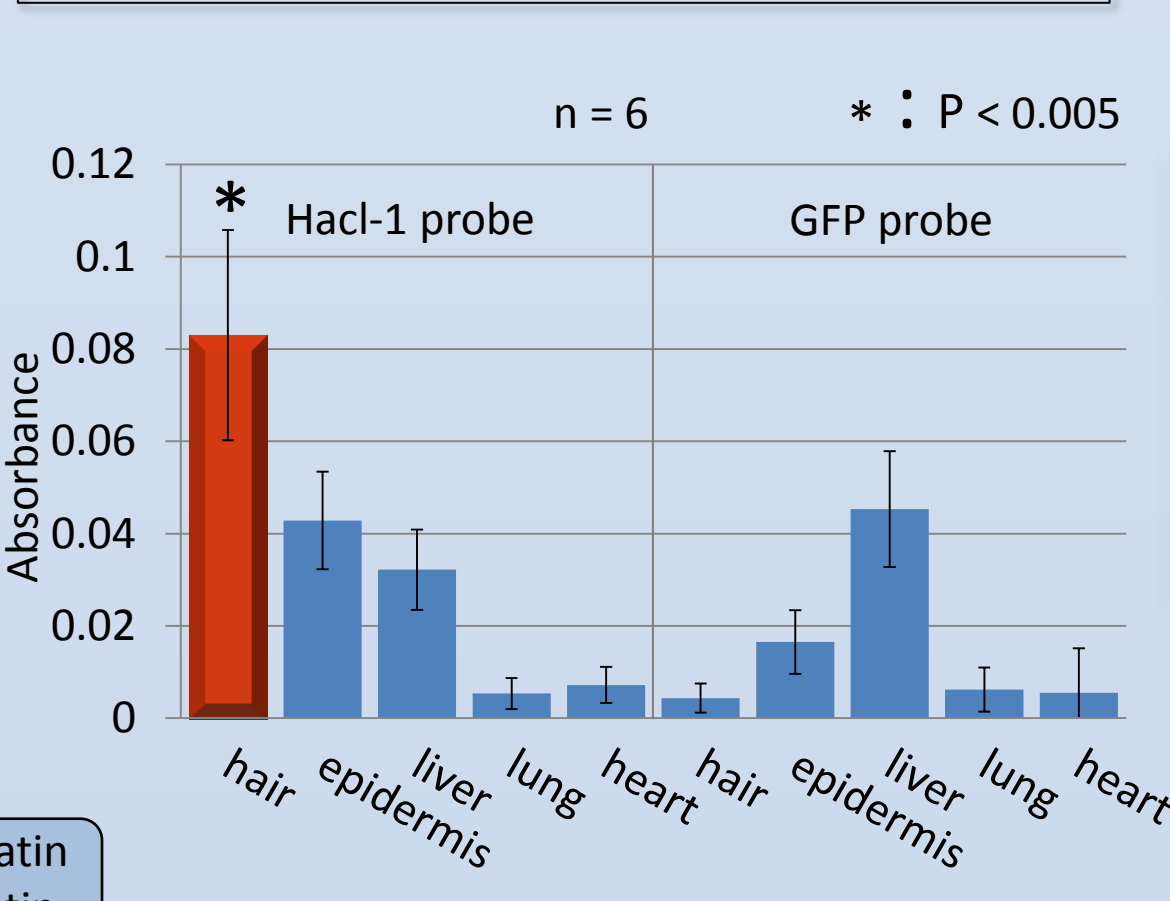


West-Western analysis with Hacl-1 probe



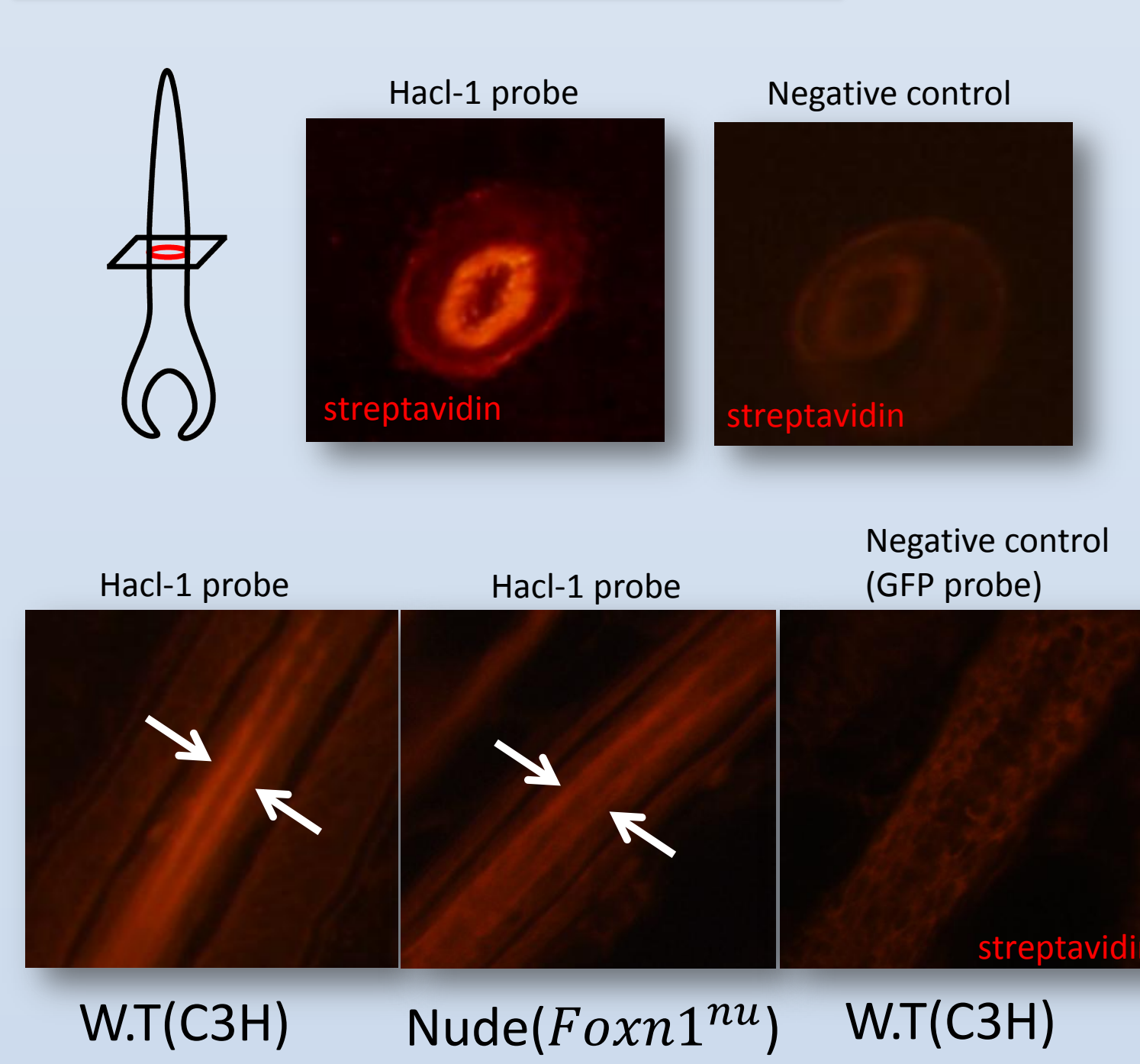
The binding partner specifically localized in the hair and was probably keratin type I. Hacl-1 may interact with other KAPs.

Binding analysis with Hacl-1 probe



Hacl-1 interacted with type I hair keratin in hair cortex without disulfide bridges.

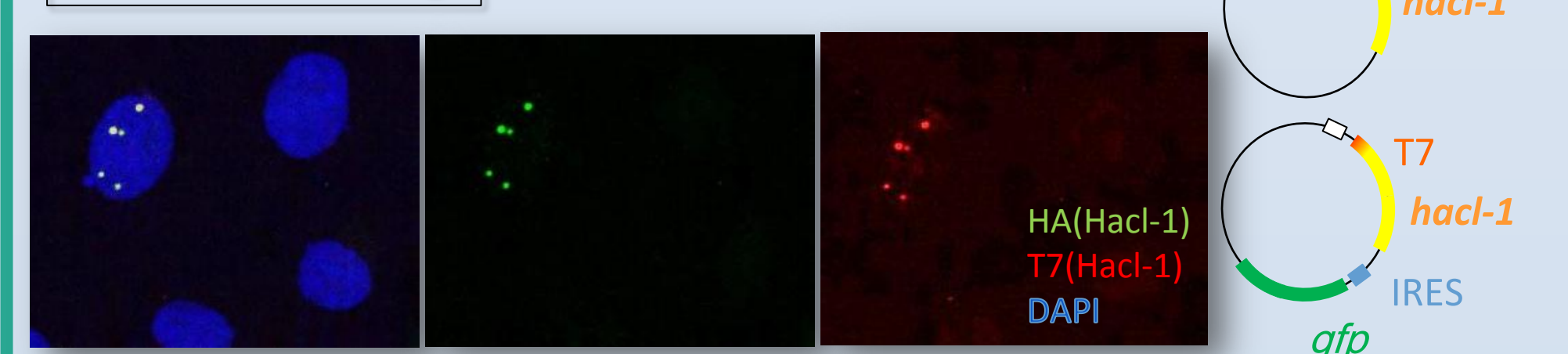
Histochemistry with Hacl-1 probe



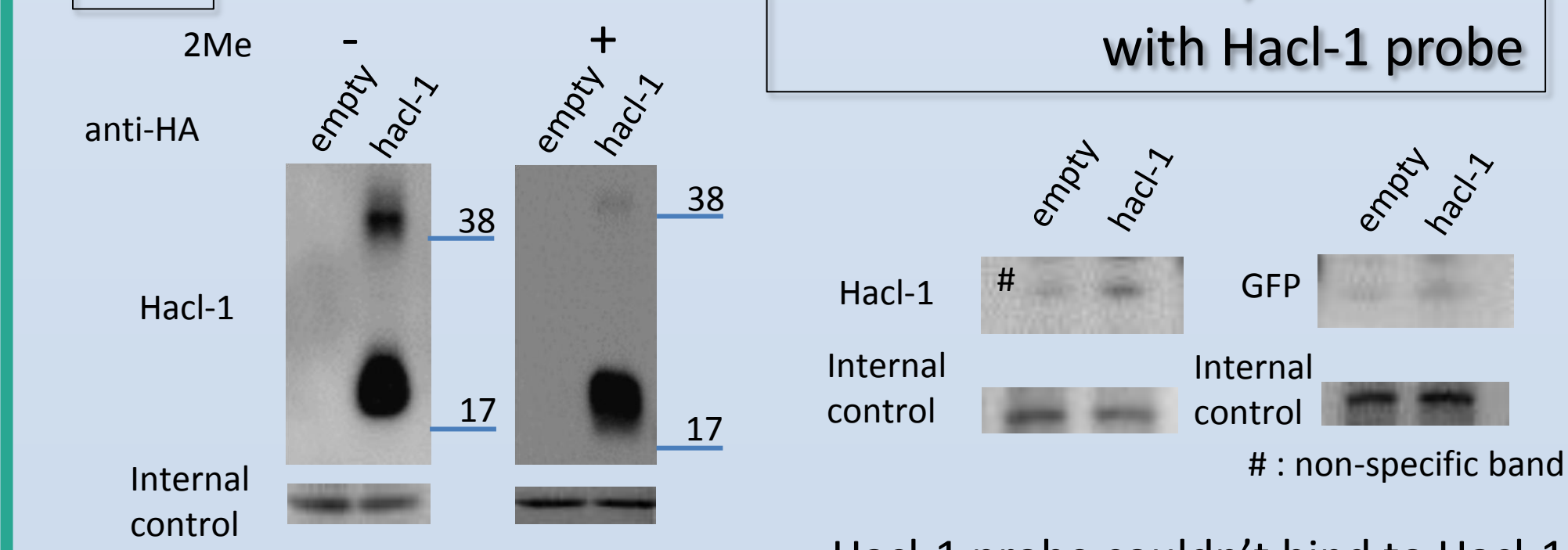
The binding partner of Hacl-1 in Nude mice appeared to distribute similarly to WT mice.

7. The cohesive self-assembly of Hacl-1

Immunostaining



W.B.



Hacl-1 formed dimer via S - S bonds. Hacl-1 probe couldn't bind to Hacl-1 protein without active cysteines.

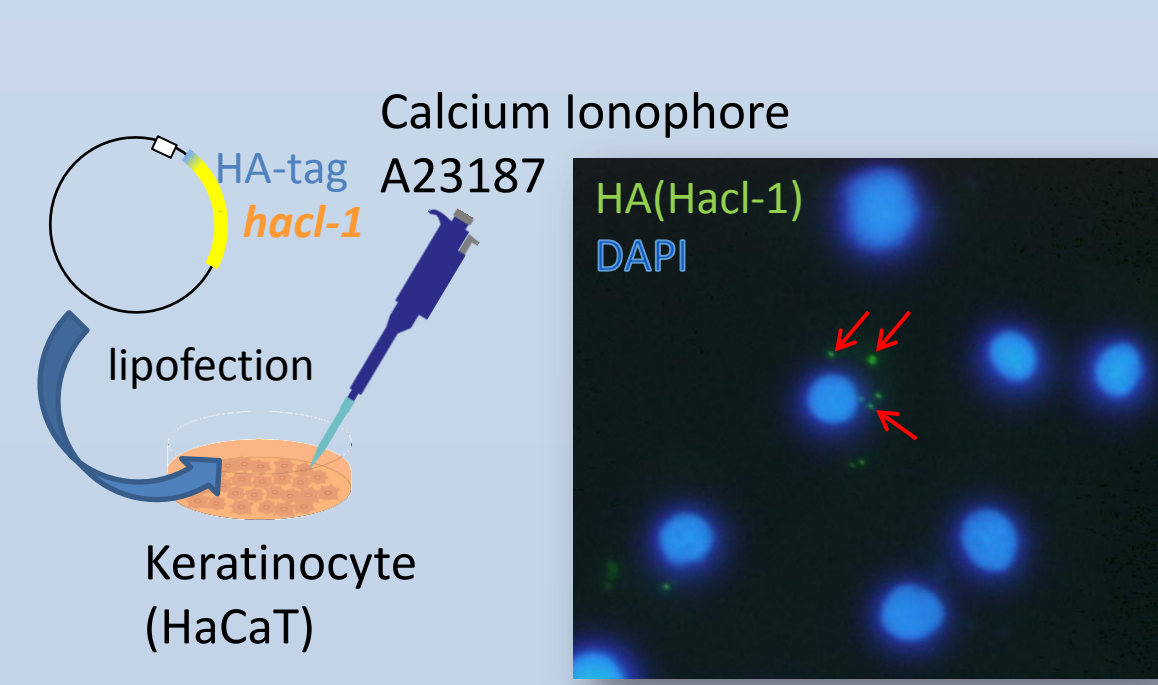
Hacl-1 may have the ability to form dimeric products with disulfide bonds.

8. The subcellular localization of exogenous Hacl-1 in non-trichocyte cells

Immunostaining

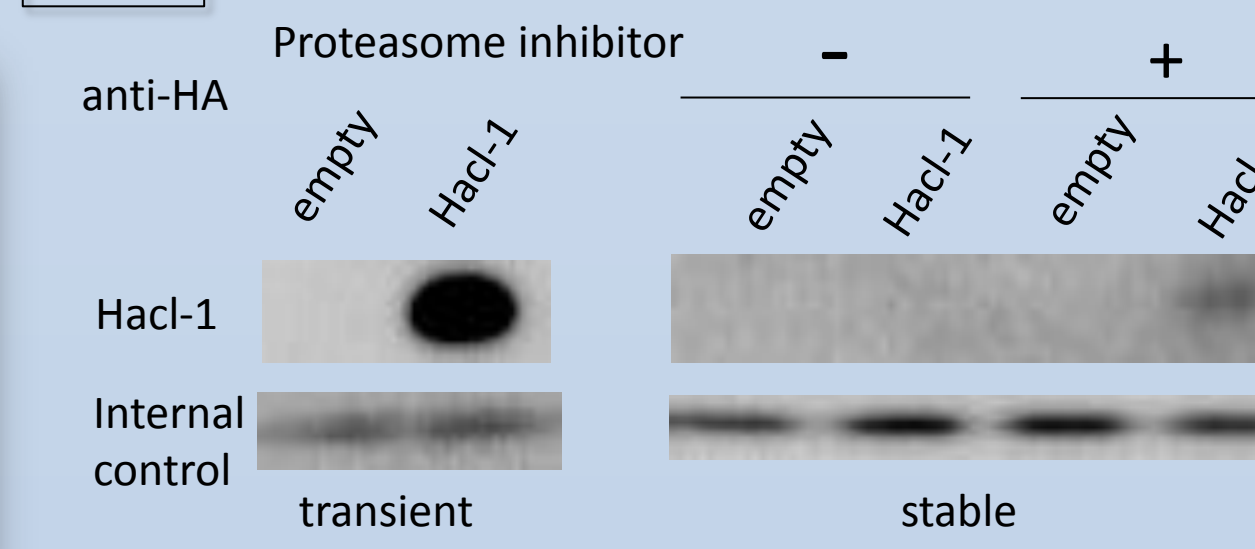


The exogenous Hacl-1 was localized in the cell nuclei as granules.

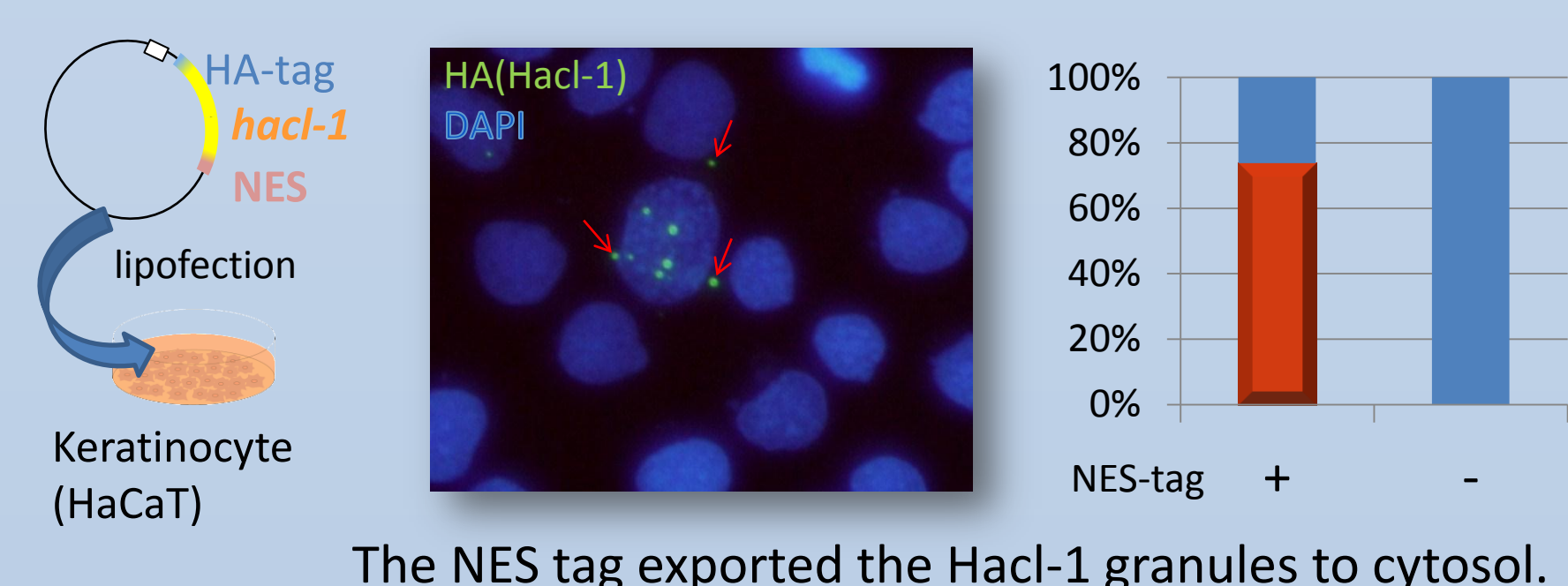


Localization of Hacl-1 in the keratinized HaCaT cells.

W.B.

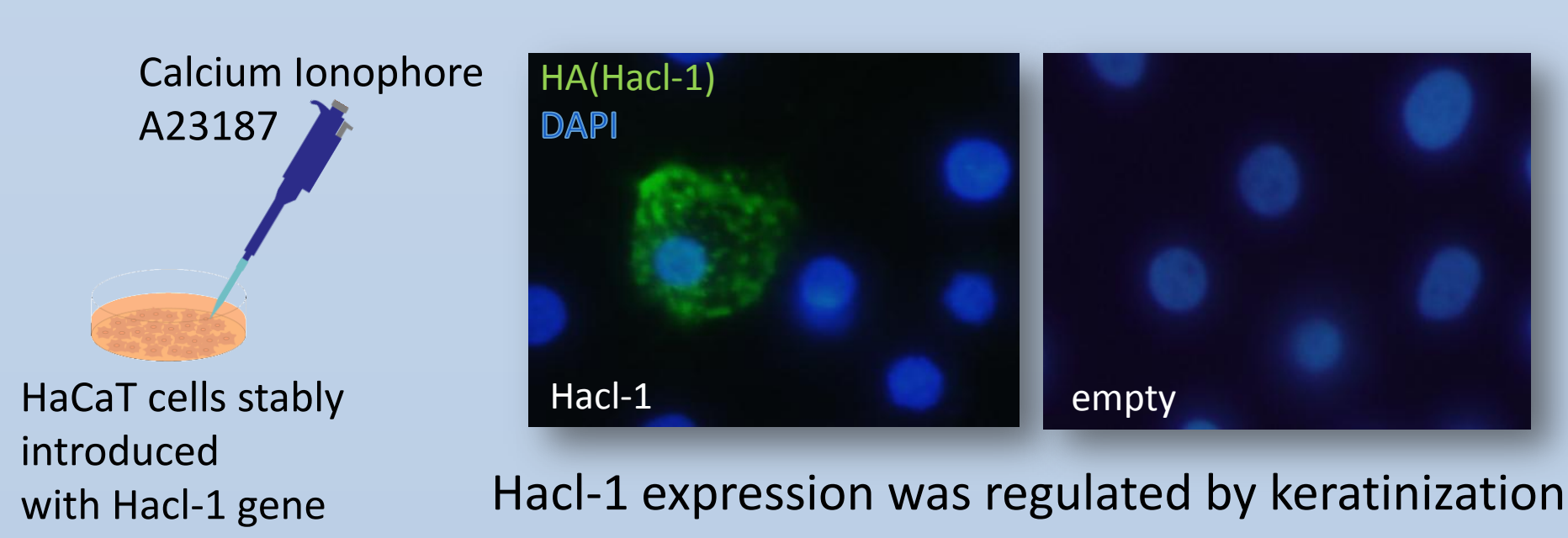


Hacl-1 was actively degraded by the proteasome protein-eliminating system.



The NES tag exported the Hacl-1 granules to cytosol.

NES : nuclear export signal
-(CDLTLRELPLPLQL)



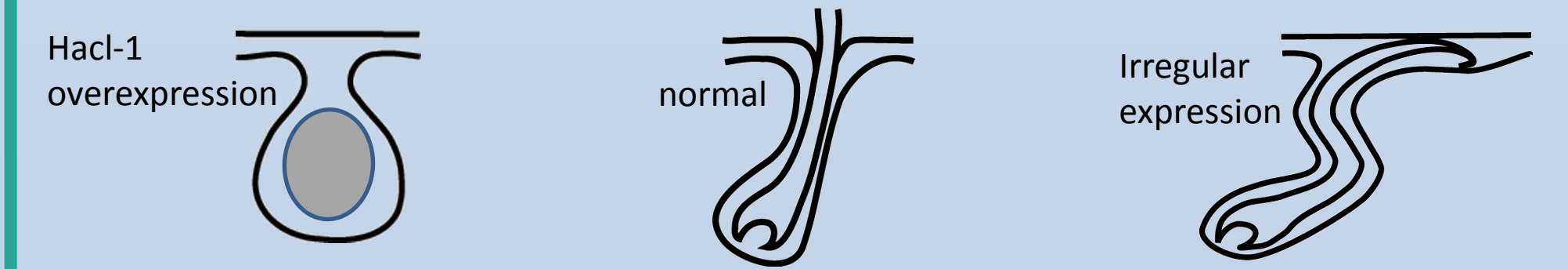
Hacl-1 expression was regulated by keratinization.

Expression and localization of Hacl-1 was regulated by keratinization.

9. Conclusion

- The expression pattern of Hacl-1 was perturbed in nude mice.
- Hacl-1 overexpression caused abnormal morphogenesis/differentiation of the hair follicle.

→ Expression control of Hacl-1 protein may be crucial for normal development of hairs and their follicles.



→ The expression pattern of the Hacl-1 may be controlled by keratinization progression.

- The binding partner of Hacl-1 localized in hair cortex and the intermolecular interaction didn't require active cysteine. (It may be hair keratin type I)
- Hacl-1 may have the ability for the cohesive self-assembly.

→ Hacl-1 may confer the mechanical strength upon hair keratin IFs.

