PERSPECTIVE | FOCUS
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nature cell biology

Stem cell dynamics, migration and plasticity during wound healing

Date: 20200320

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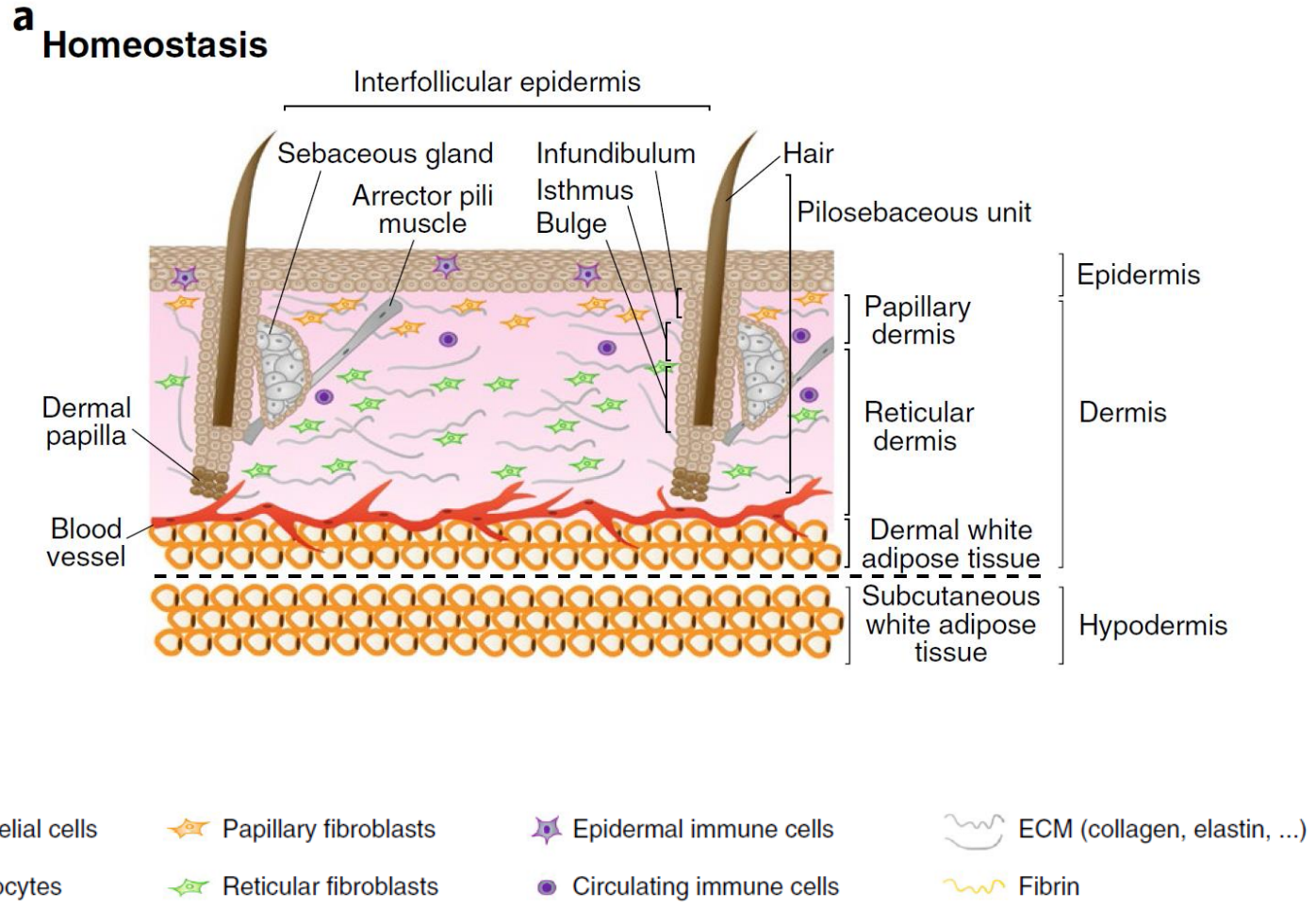
Outline

- Introduction of skin epithelial stem cells during **homeostasis**
  - The structure of skin and hair follicle
  - Resident stem cell
- Introduction of stem cell dynamics during **wound healing**
  - Migration and plasticity
  - Epigenetic modulation
- Link to my thesis

  Epigenetic modulation of wound induced hair follicle neogenesis.

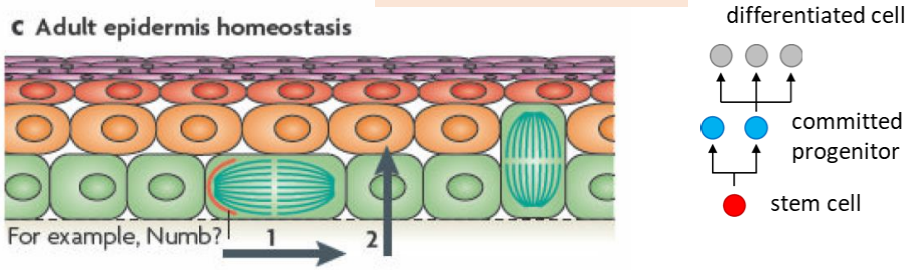
Introduce the structure of the skin



The skin epithelium renews throughout life in a continuous turnover ensured by stem and progenitor cells

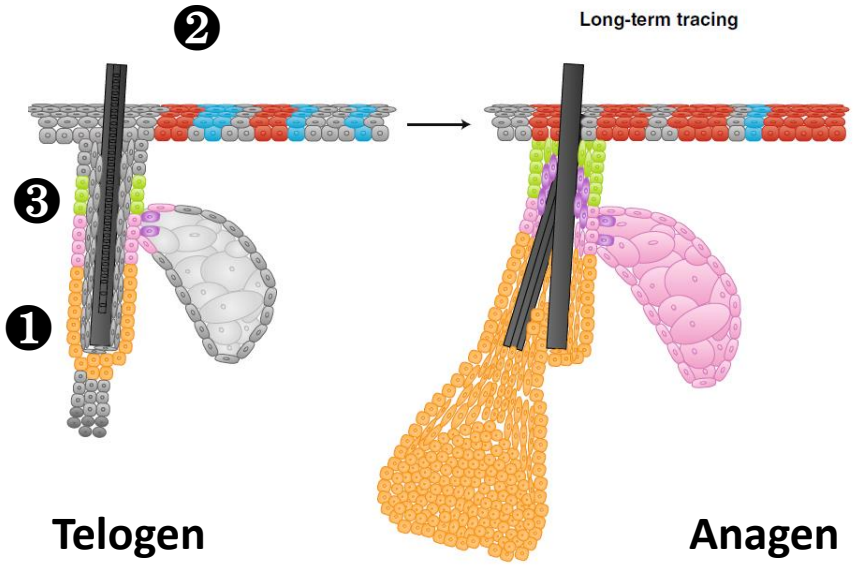
Stem cell populations	Markers	*CreER* tracing
IFE stem cell	Itgα2^{high} , Itgβ1^{high}	*Krt14-CreER*
IFE committed progenitor	Inv, Lgr6	*Inv-CreER* *Lgr6-CreER* *Ah-CreER*
Infundibulum	Lrig1	*Lrig1-CreER*
Sebaceous gland ducts	Gata6	*Gate6-CreER*
Isthmus Sebaceous gland	Lrig1, Lgr6, Blimp1, Plet1	*Lrig1-CreER* *Lgr6-CreER* *Blimp1-Cre*
Bulge (HFSC)	K15, K19, Lgr5, CD34, Sox9, Tcf3	*Krt1-15-CrePR* *K19-CreER* *Lgr5-CreER* *Sox9-Cre* *Sox9-CreER* *Tcf3-CreER*

Asymmetrical

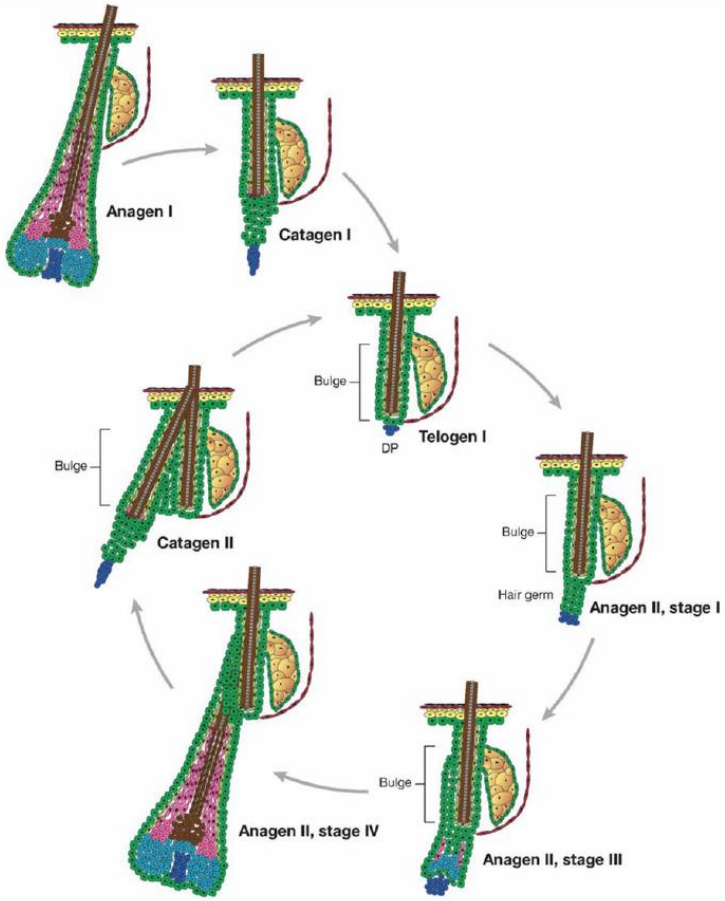


Symmetrical

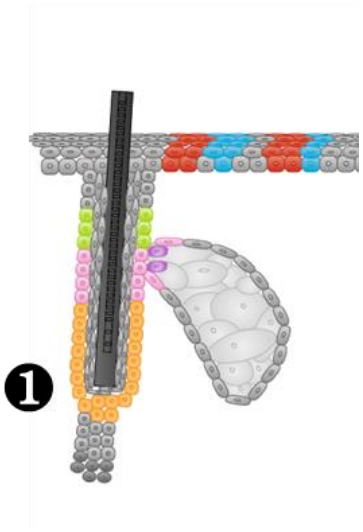
Cedric Blanpain and Elaine Fuchs, 2009



Hair follicle stem cells (HFSC) responsible for cyclic regeneration are located in the bulge



Blanpain, C. & Fuchs, E. 2006

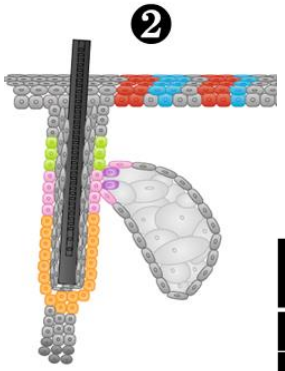


Stem cell populations	*CreER* tracing
Bulge	*Krt1-15-CrePR* *K19-CreER* *Lgr5-CreER* *Sox9-Cre* *Sox9-CreER* *Tcf3-CreER*

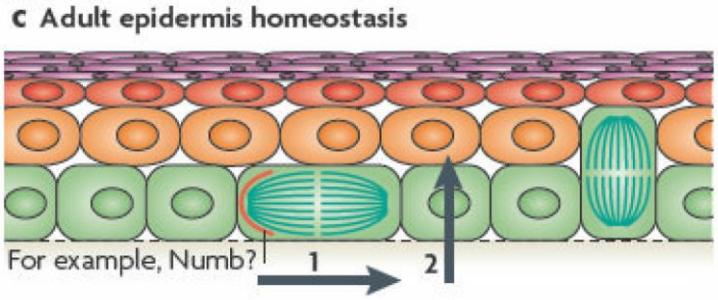
Hair follicle stem cells (HFSCs)

- ✓ hair follicle regeneration during physiological conditions
- X maintain the sebaceous gland, infundibulum or interfollicular epidermis (IFE).

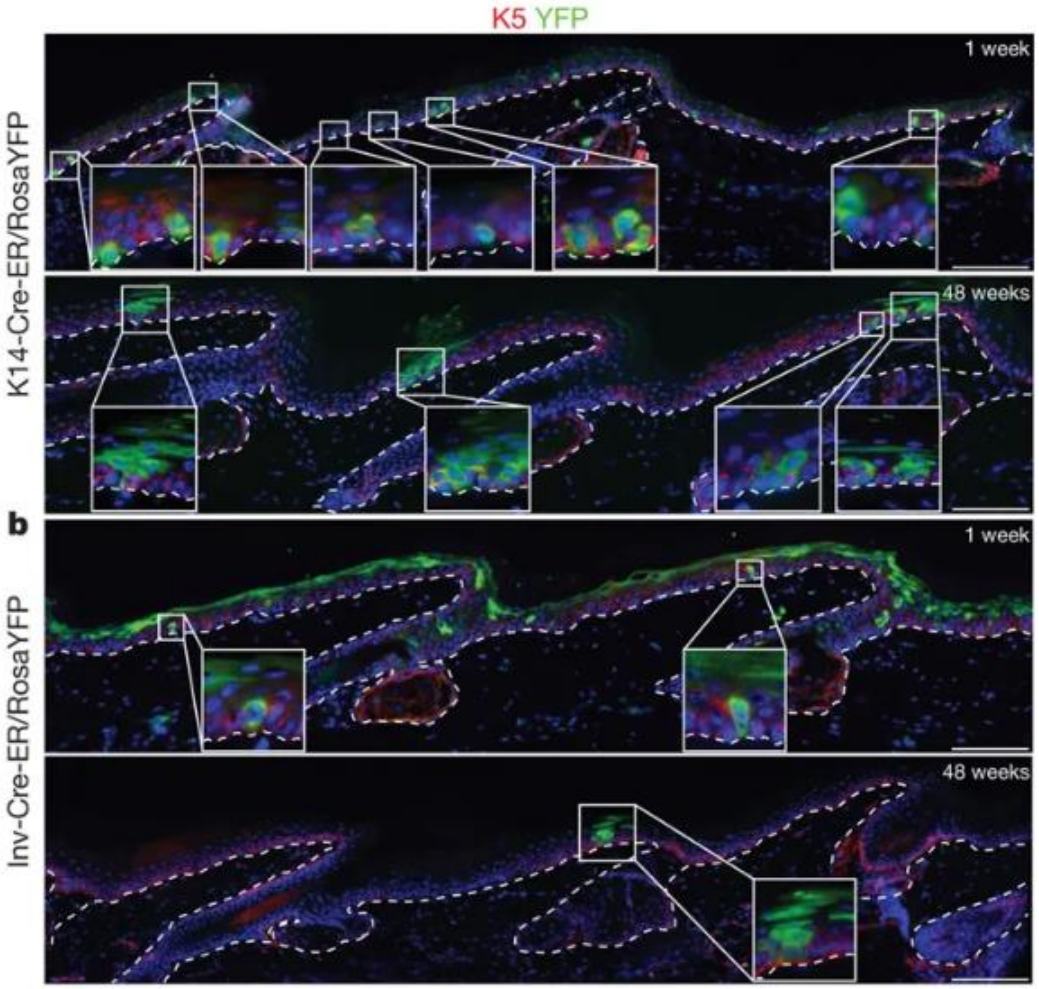
Interfollicular epidermis is maintained by a heterogeneous pool of progenitors with different survival potential



Stem cell populations	*CreER* tracing
IFE stem cell	*K14-CreER*
IFE committed progenitor	*Inv-CreER*

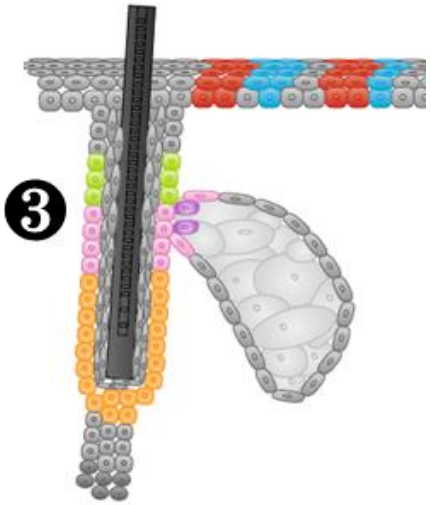


Cedroc Blanpain and Elaine Fuchs, 2009

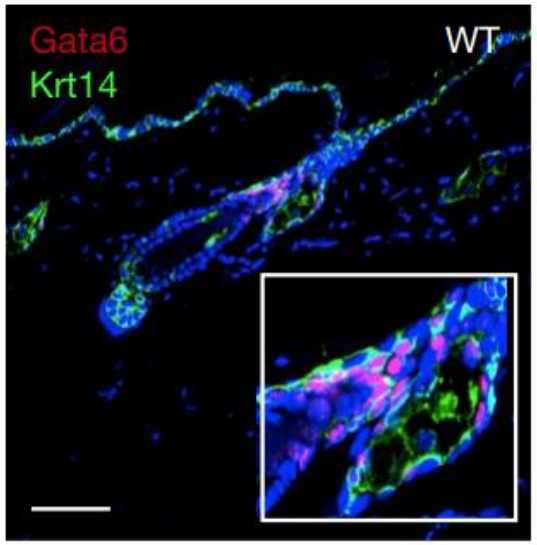


Guilhem Mascré et al. 2012

Isthmus, a region located between the bulge and sebaceous gland, contains its own pool of resident stem cells



Stem cell populations	*CreER* tracing
Sebaceous gland ducts	*Gata6-CreER*
Isthmus and sebaceous gland	*Lrig1-CreER* *Lgr6-CreER* *Blimp1-Cre*



Giacomo Donati et al, 2017

During physiological condition, skin stem cells are confined to restricted compartments.

Outline

- Introduction of skin epithelial stem cells during homeostasis
  - The structure of skin and hair follicle
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- Introduction of stem cell dynamics during **wound healing**
  - Migration and plasticity
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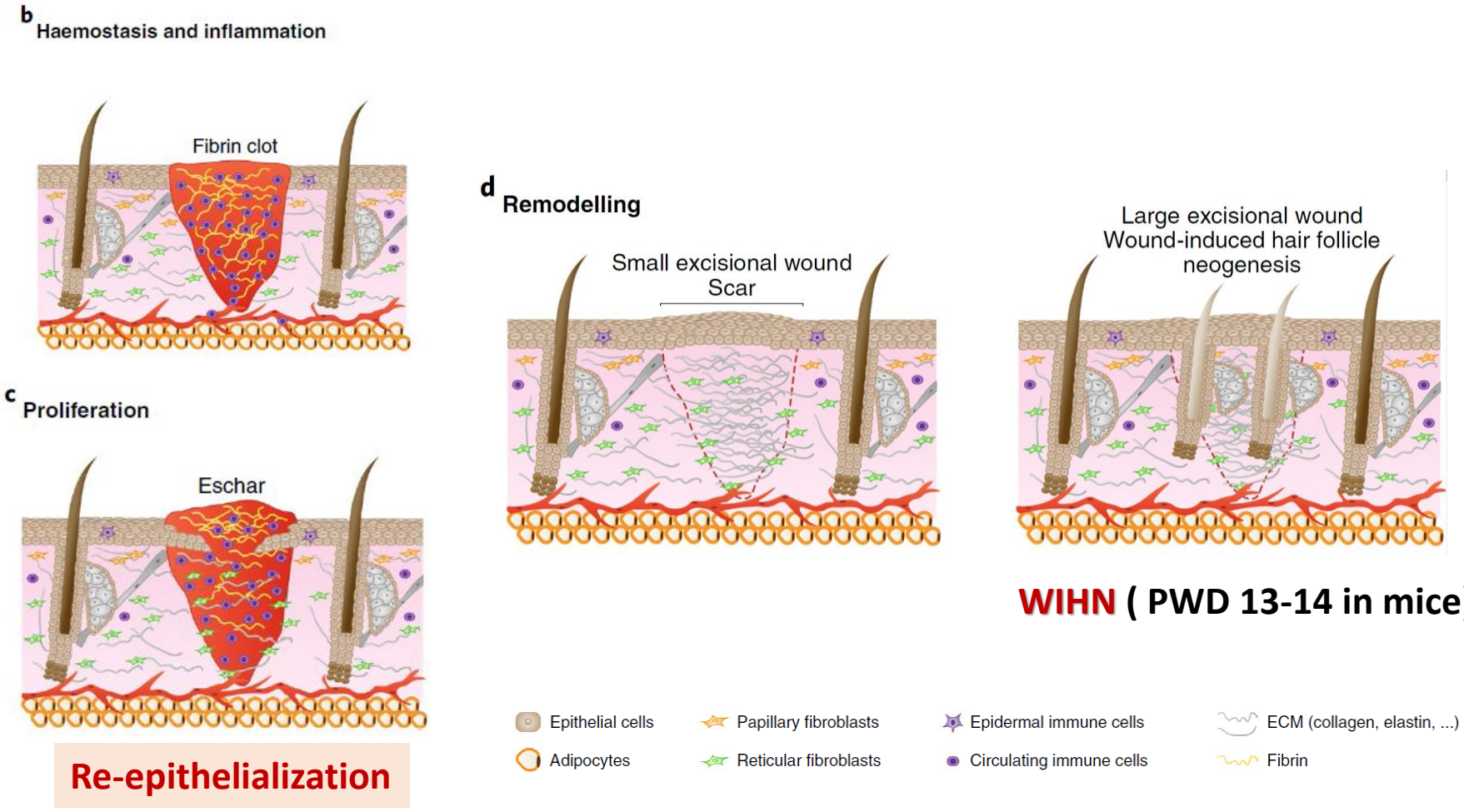
  Epigenetic modulation of wound induced hair follicle neogenesis.

Introduce the wound healing process

Wound healing process: Haemostasis→ Inflammation→ Proliferation→ Remodeling

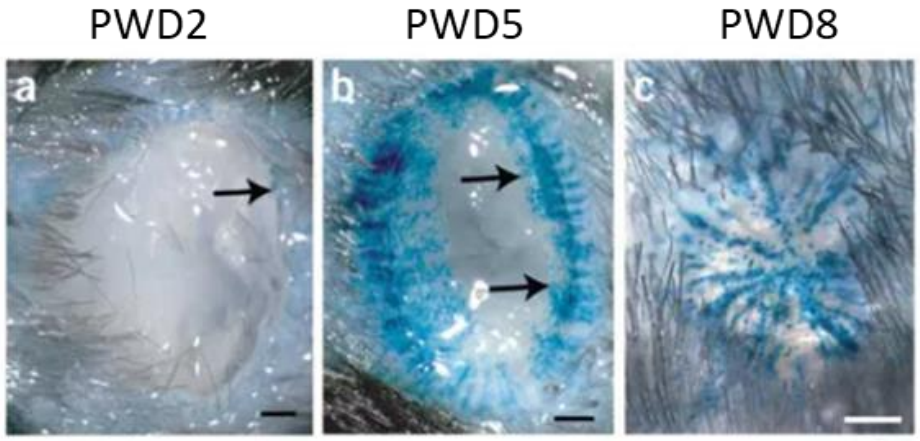


WIHN (PWD 13-14 in mice)

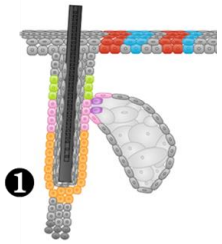
Re-epithelialization

HFSCs and isthmus stem cell rapidly migrate to the wound and contribute to re-epithelialization

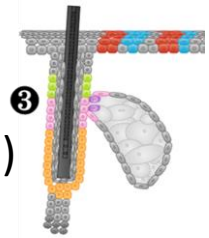
*Tg(**Krt1-15**-cre/PGR)22Cot R26R* mice
(4mm full-thickness wound, dorsal)

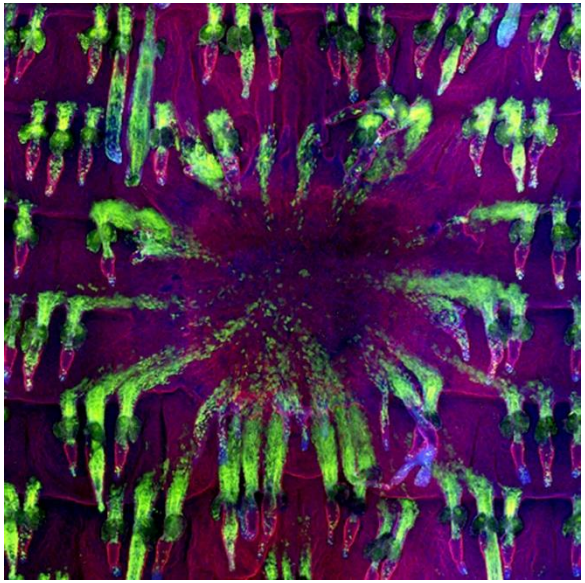


Mayumi Ito et al, 2005



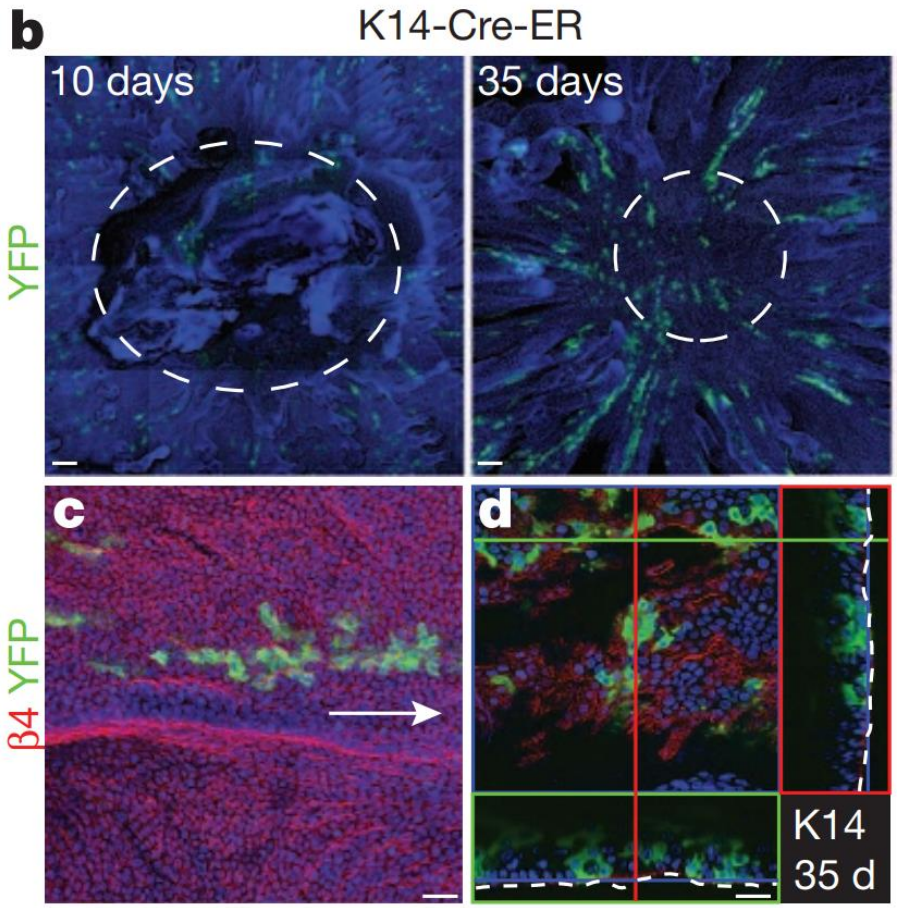
***Lrig1**-EGFP-ires-CreERT2* mice
(2mm full-thickness wound, tail)



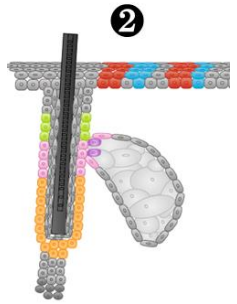


Mahalia E.Page et al, 2013

IFESCs are recruited to the wound, contribute to epidermal repair and persist up to 35 days



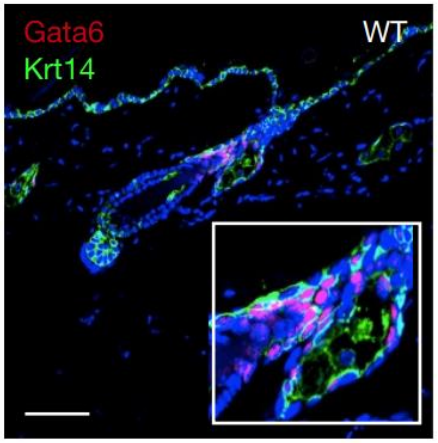
Stem cell populations	*CreER* tracing
IFE stem cell	*K14-CreER*



(3mm full-thickness wound, tail)

Guilhem Mascré et al, 2012

Gata6-expressing cells migrate to the injured IFE and revert from a differentiated to a basal stem cell fate



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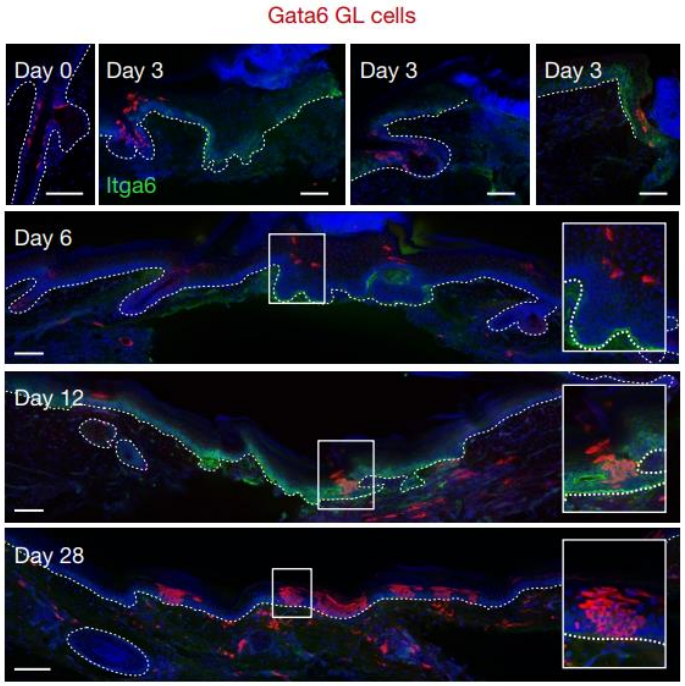
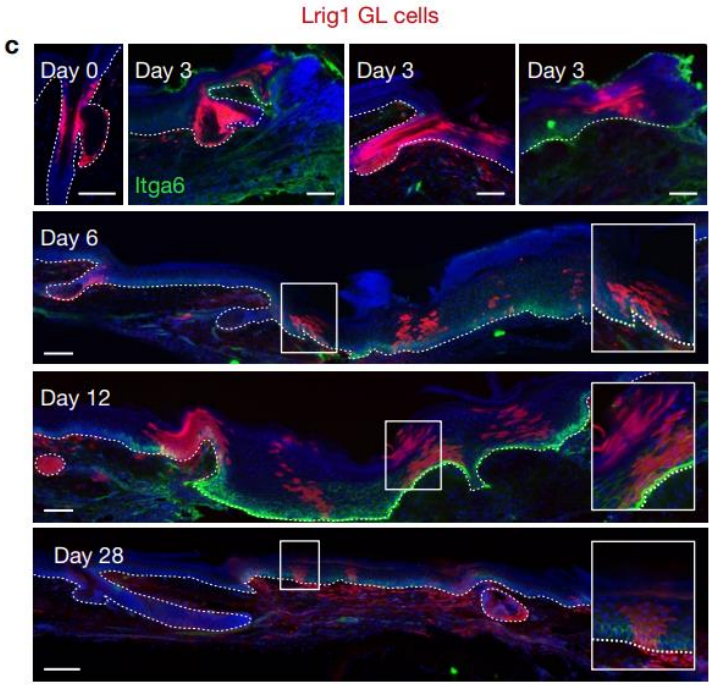
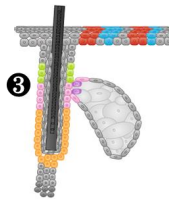
Giacomo Donati et al, 2017





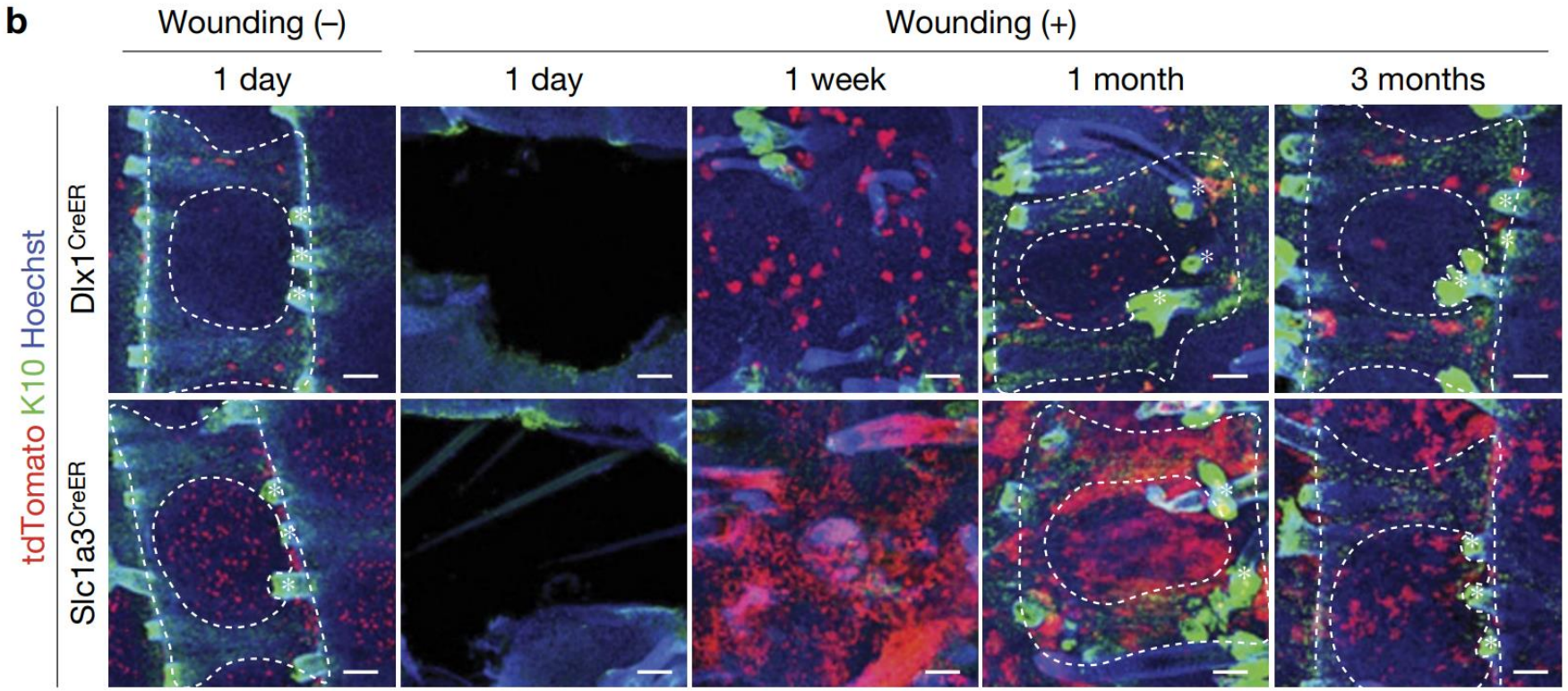


Gata6: sebaceous duct (2mm full-thickness wound, tail)
GL: genetically labelled cells

Slow-cycling and rapidly-cycling stem cell only persist in their region of origin

Dlx1: infrequently dividing basal cells **Slc1a3**: short-lived progenitors

Aiko Sada et al 2016



During repair, all basal cells present some degree of plasticity, a change in behavior and functional contribution, but the wound does not reset the clock completely and cells keep a memory of their original location and hierarchy.

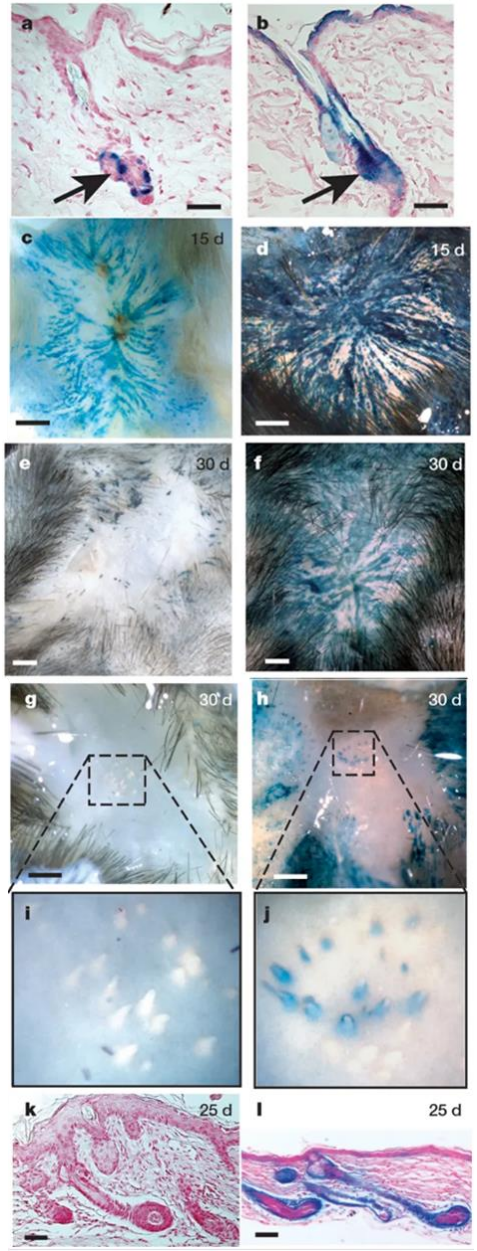
The degree of damage can also influence cellular plasticity

De novo hair follicle formation is apparent in large wounds

Experiment	Mice	Mean No. Regenerated Follicles	SD	n	range	p-value
Age	3 week old wild type (1.0 x 1.0 cm wound)	40	23	40	10-102	0.07
	7-8 week old wild type (1.5 x 1.5 cm wound)	30	28	32	0-91	0.6
	10 month old wild type (1.5 x 1.5 cm wound)	36	26	10	0-70	0.5

WIHN assay
Create 1*1 cm^2 wound on dorsal skin

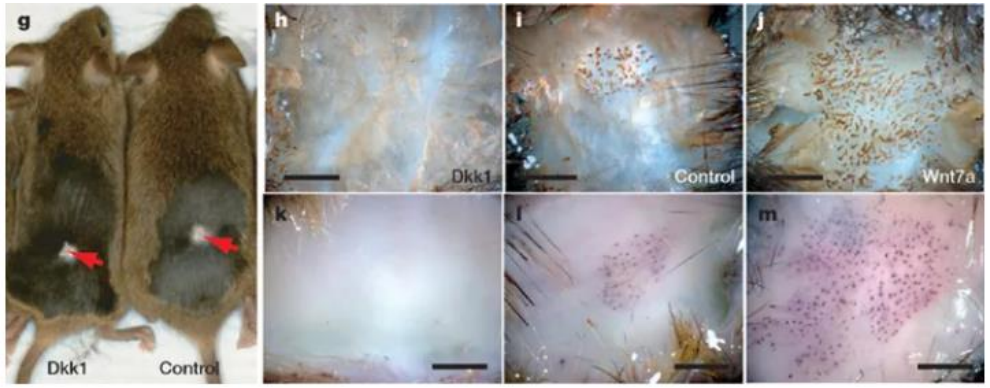
C57BL/6J
(3-4 weeks old)



Mayumi Ito et al, 2007

Wnt signal and Sonic hedgehog participate in hair follicle neogenesis

KRT17 protein

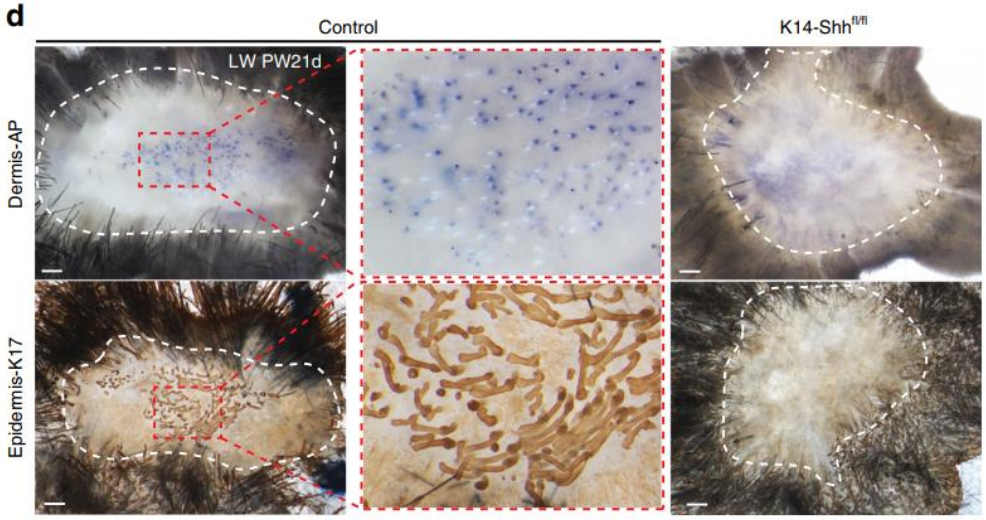


DKK --| Wnt signal ➔WIHN decrease

Mayumi Ito et al, 2007

Alkaline phosphatase activity

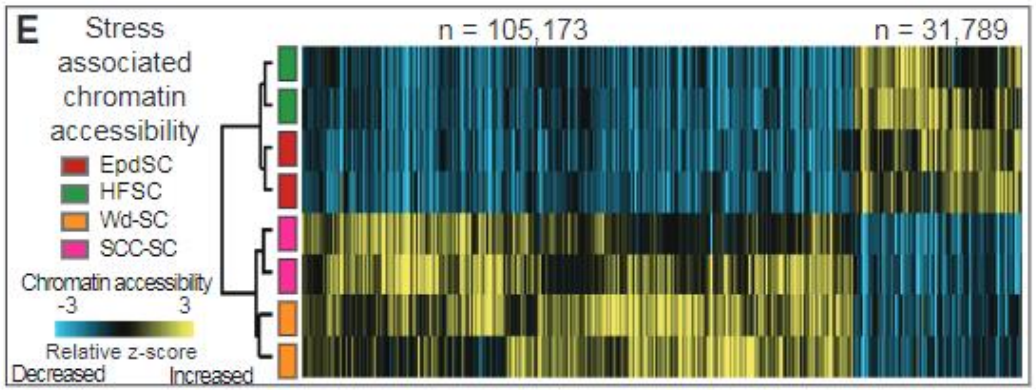
Shh signal ➔WIHN increase

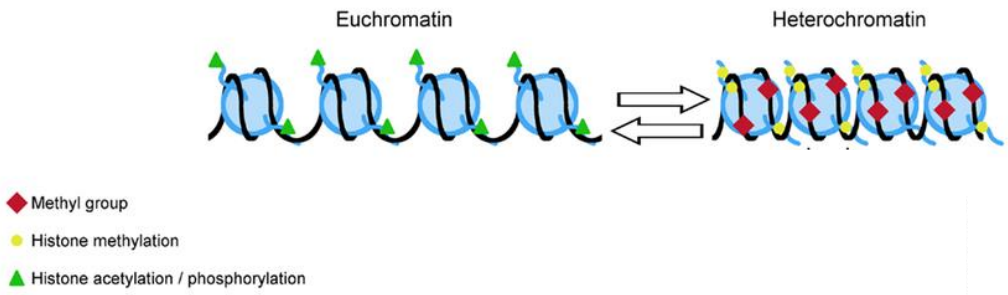


Chae Ho Lim et al, 2018

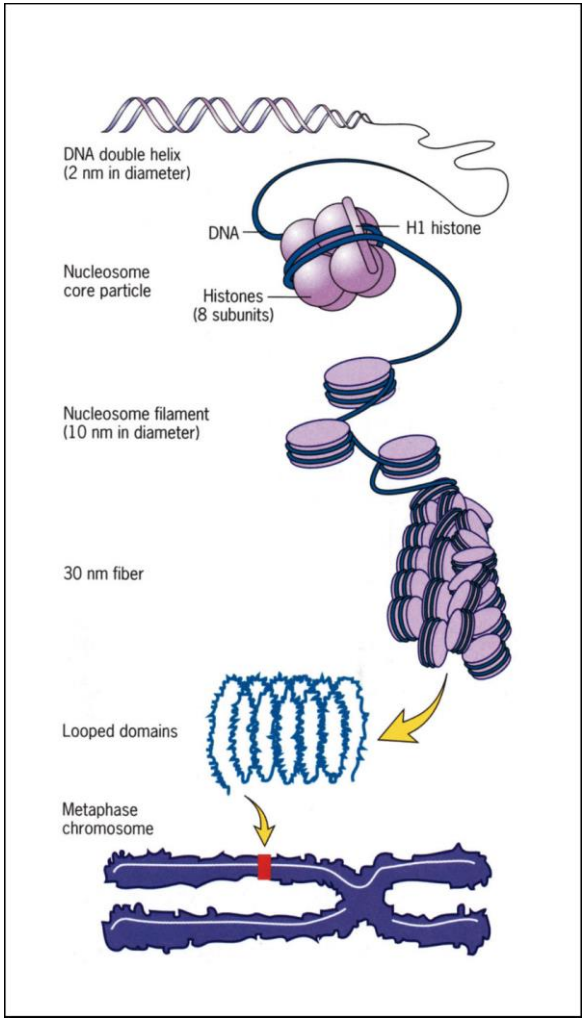
Histone modification regulate the gene expression and surpression

Yejing Ge et al, 2017





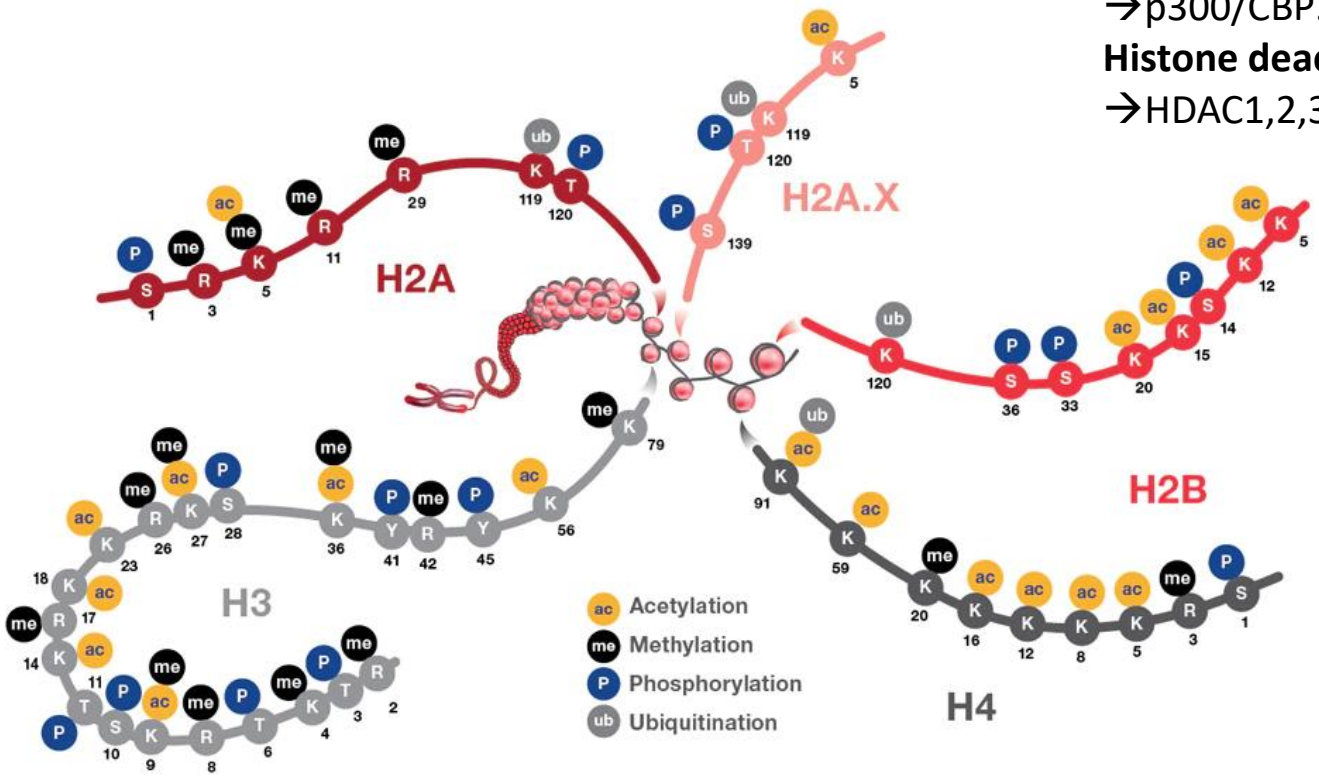
Florian Raabe and Dietmar Spengler, 2013



Nithyha Parameswaran, 2016

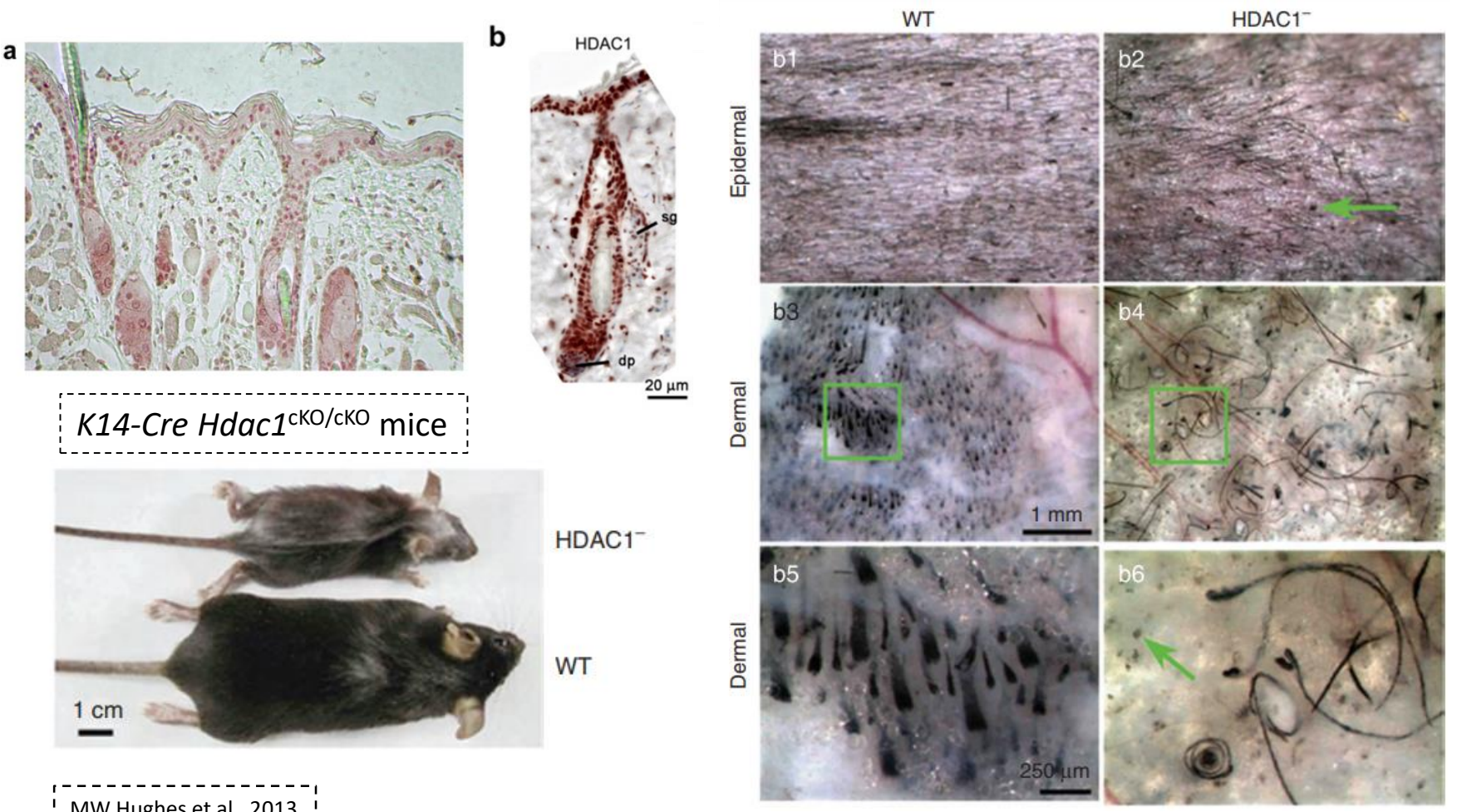
Post-translation modification of histone tail

Histone methyltranferase (HMT)
→Ezh2,1,SUV39H1…
Histone acetyltransferase (HAT)
→p300/CBP…
Histone deacetylatransferase (HDAC)
→HDAC1,2,3…



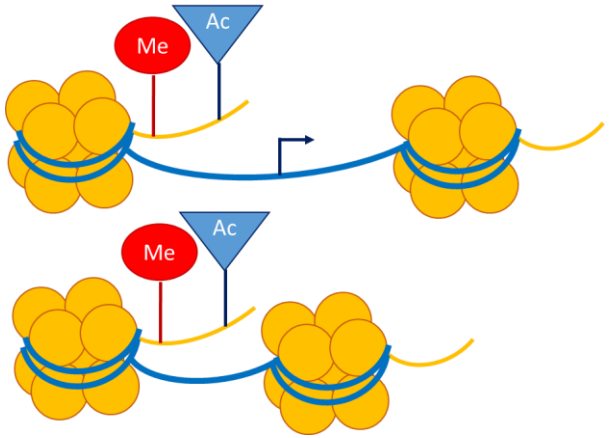
Thermo fisher-epigenetic

HDAC1 knockout mice developed abnormal hair patterning, ingrown hairs, and follicular dystrophy



MW Hughes et al., 2013

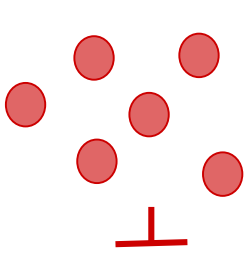
Does histone modification regulate hair follicle neogenesis?



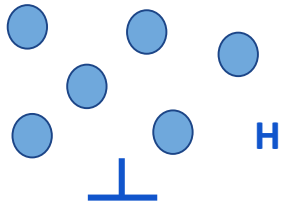
“?”

Hair follicle neogenesis

Epigenetic modification of gene expression



HMT inhibitor

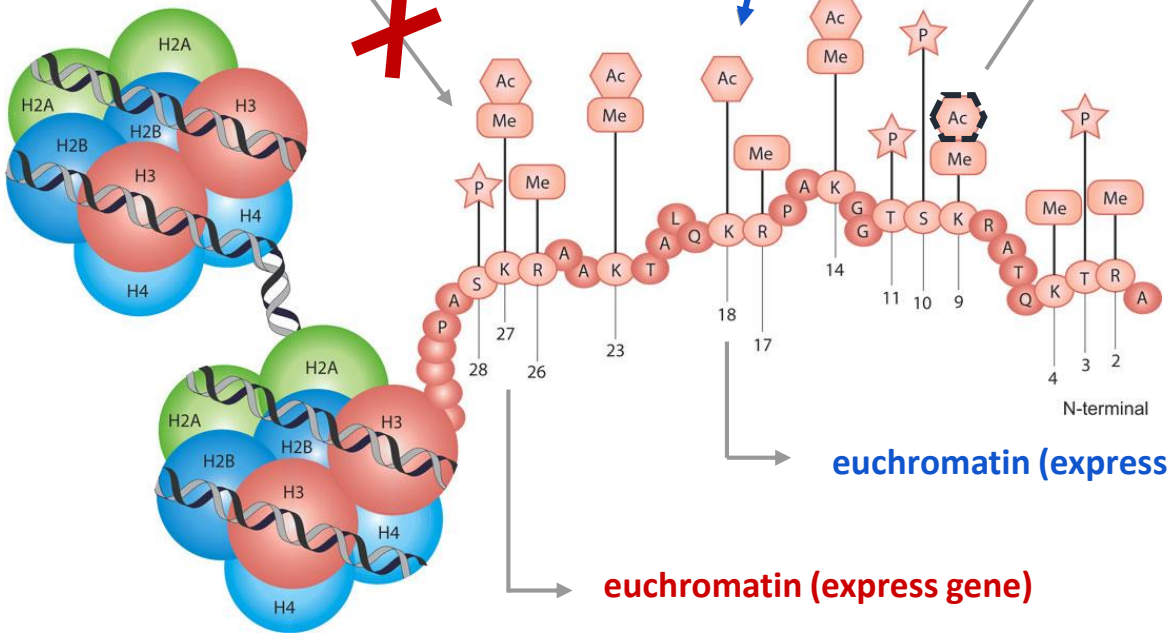


HDAC inhibitor

histone methyltransferase (HMT)

histone acetyltransferase (HAT)

histone deacetyltransferase (HDAC)

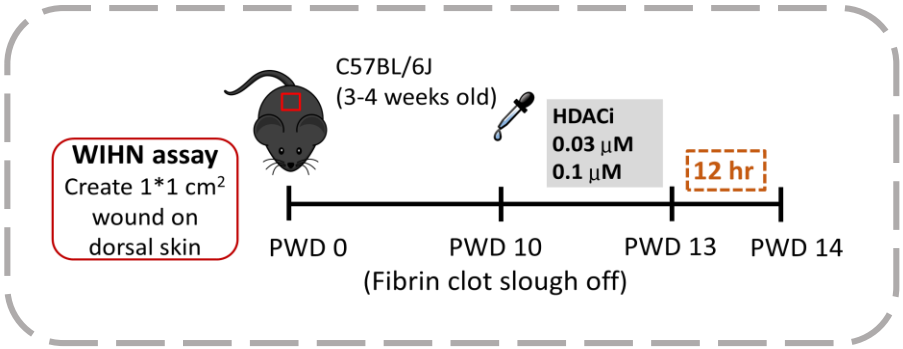


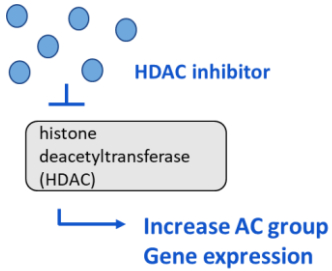
Post translational modification

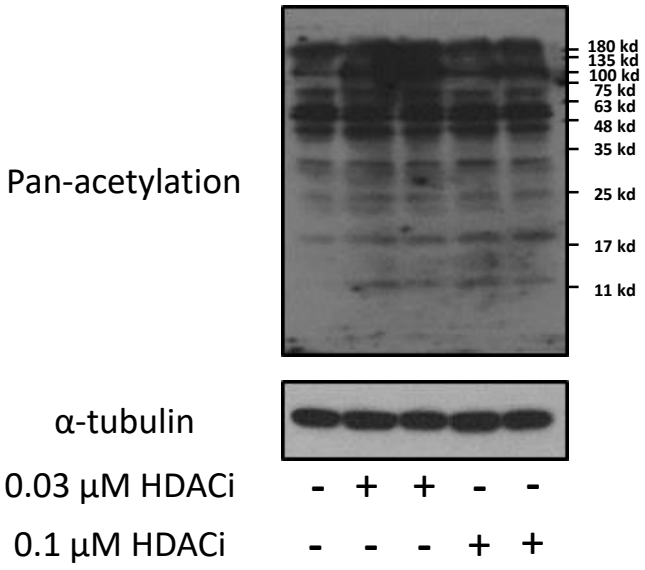
1. Acetylation
2. Methylation
3. Phosphorylation
4. Ubiquitination ….

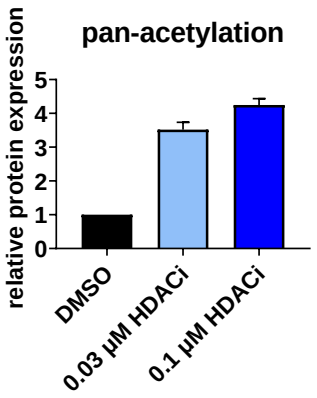
Elizabeth M. Adler et al, 2008, science signaling

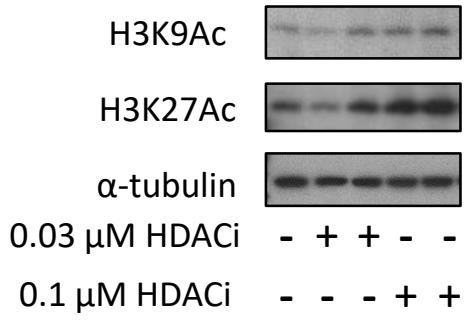
The expression level of acetylation increased after 12 hours treat with HDAC inhibitor

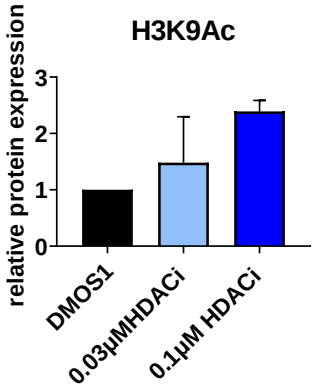


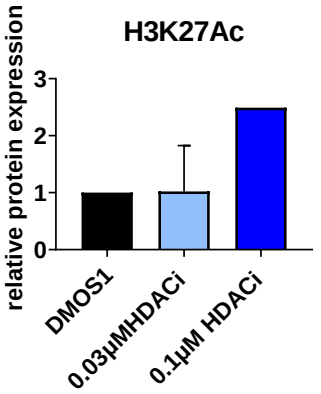




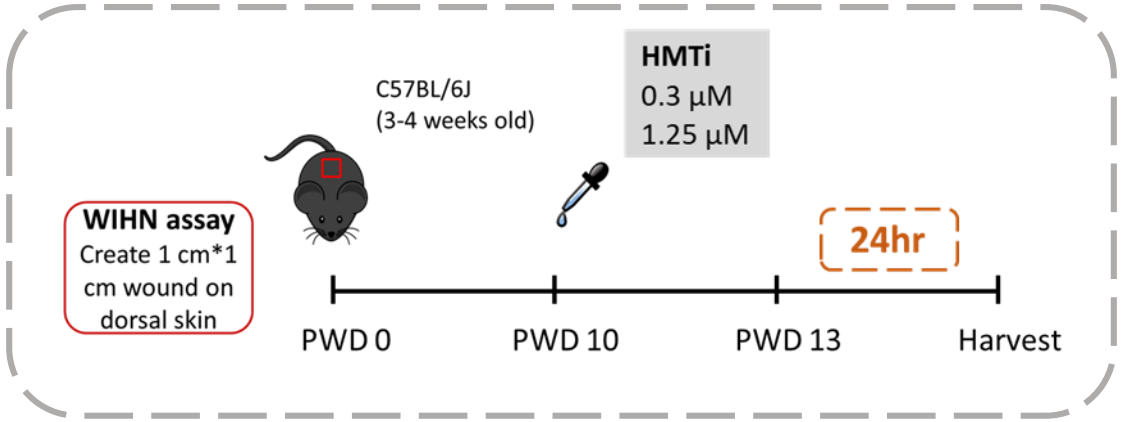


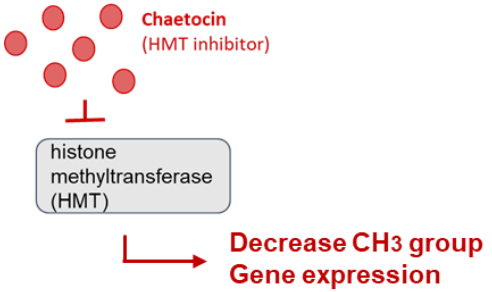


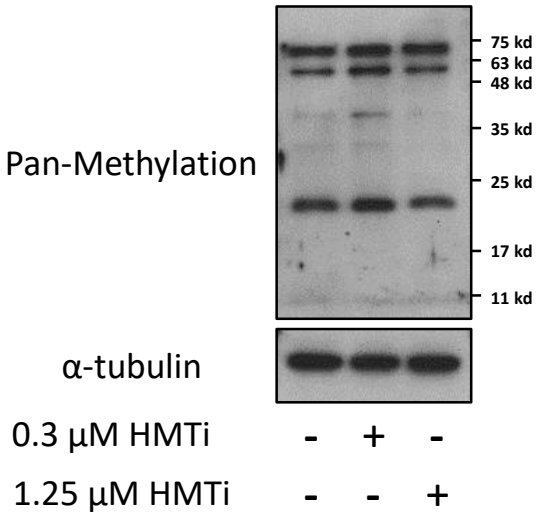


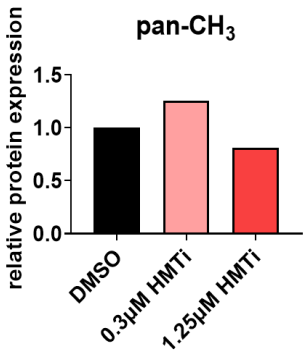


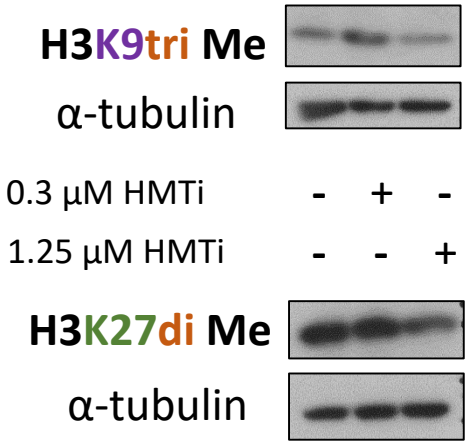
The high dose of HMT inhibitor decrease the expression level of pan-methylation

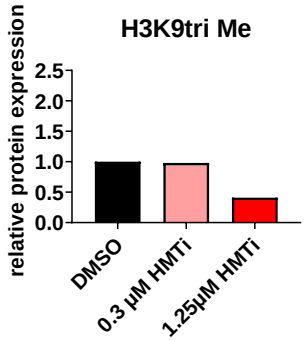


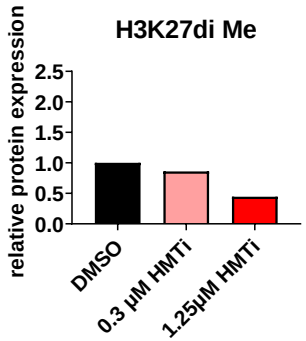




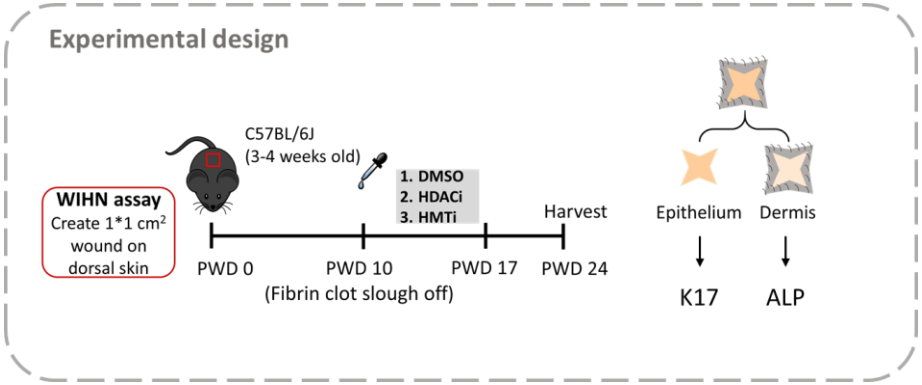




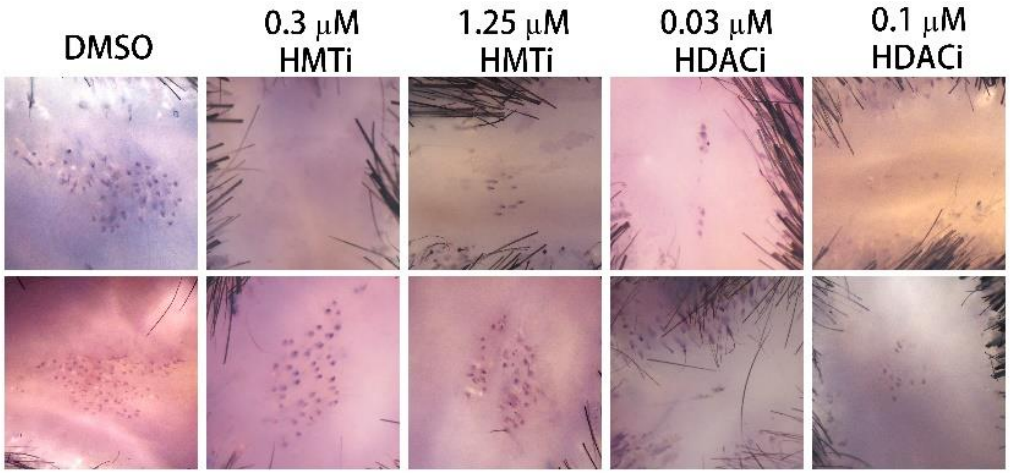


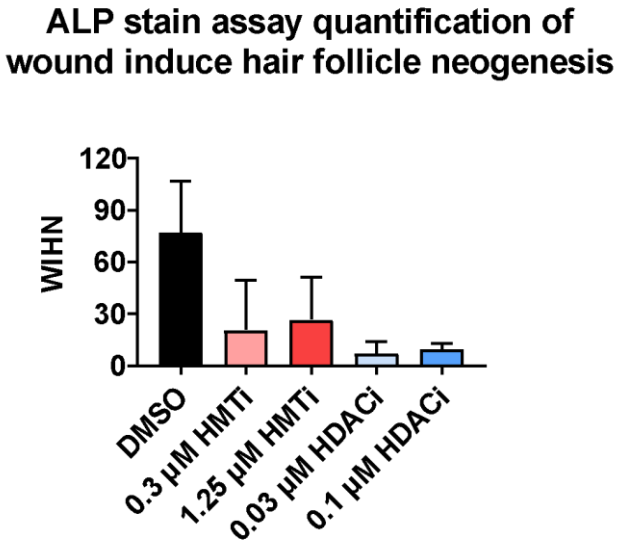


HMT, HDAC inhibitor decrease the numbers of hair follicle during WIHN



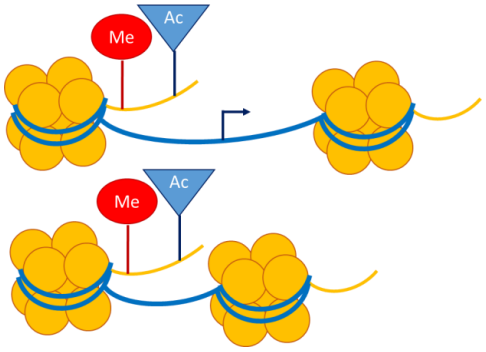
ALP stain





Hypothesis

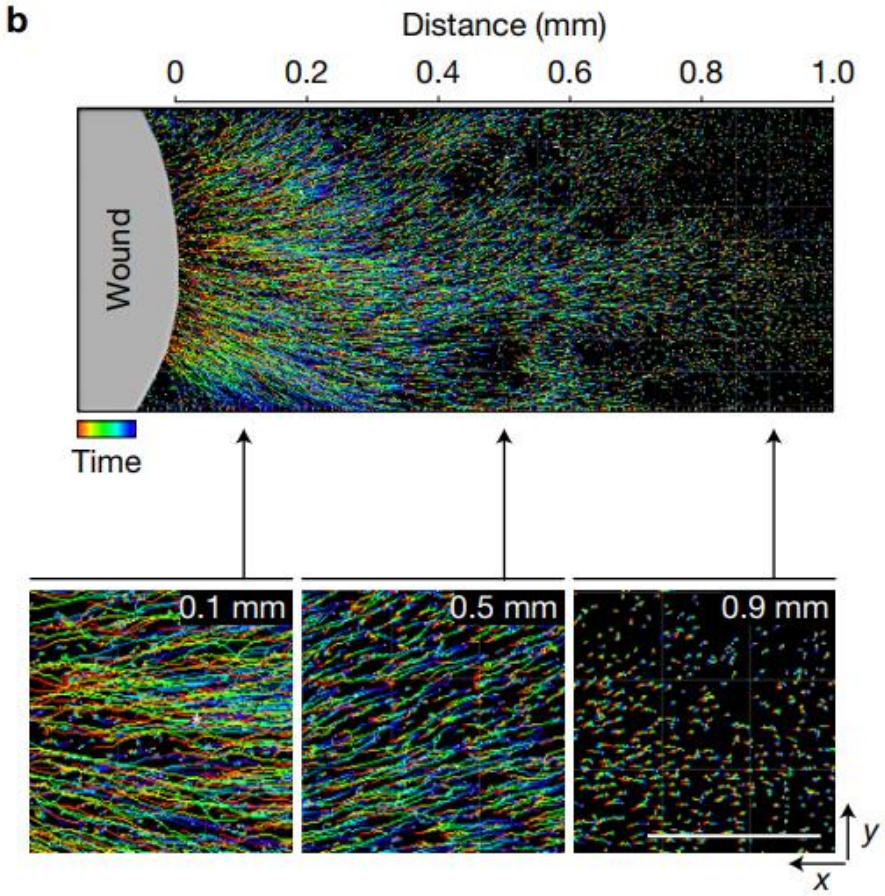
Regeneration ability is controlled by epigenetic mechanisms and these mechanisms modulate WIHN.

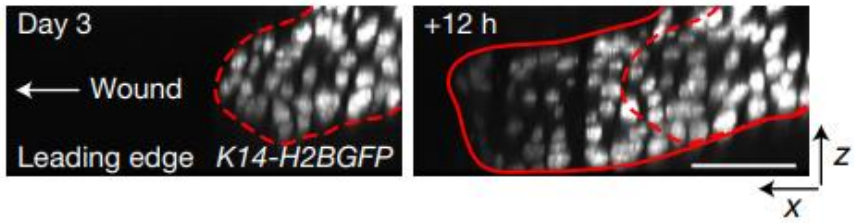


Hair follicle neogenesis

Thank you

Migration of differentiated cells contributes to re-epithelialization

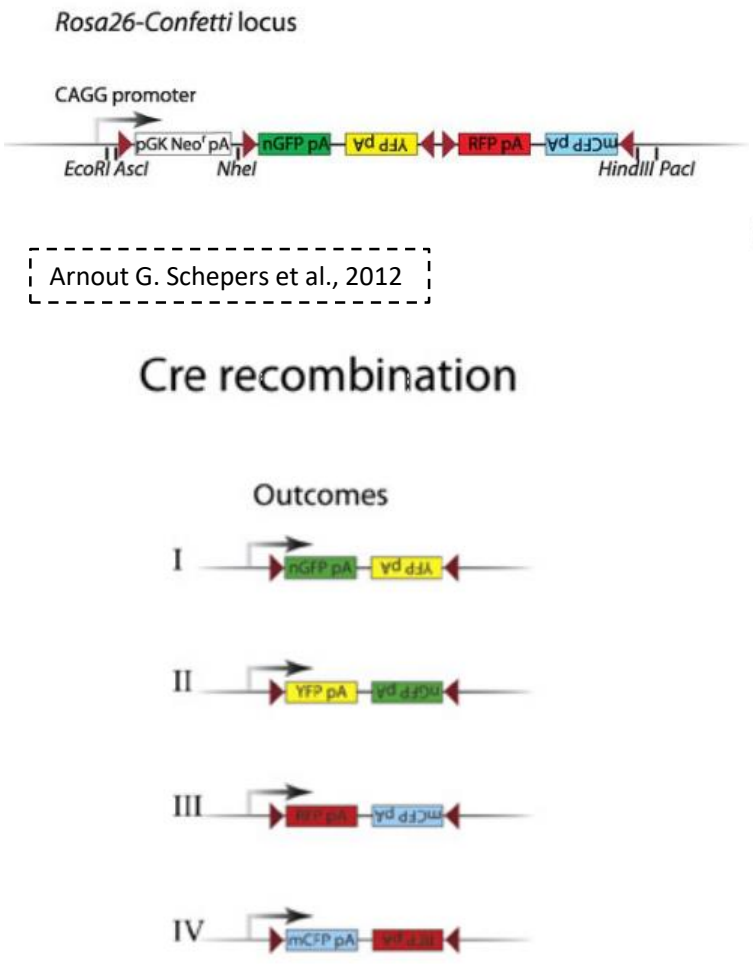




time-lapse microscopy the repair of 1-mm-wide wounds by tracking fluorescently marked epithelial cell nuclei

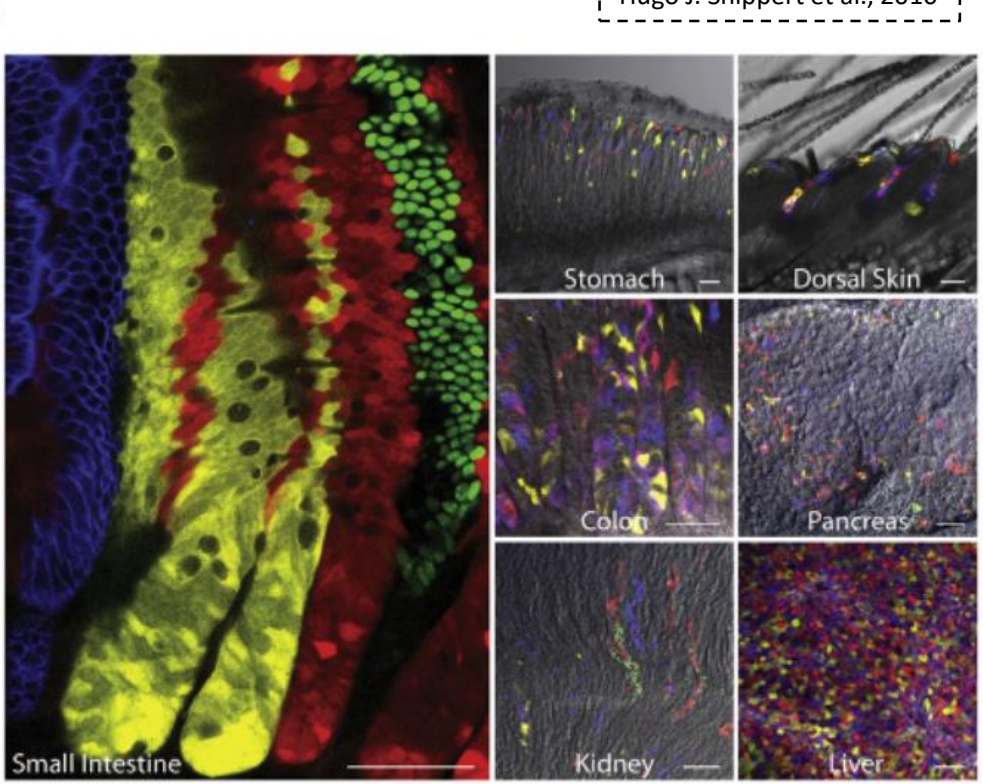
Sangbum Park et al, 2017

Intestinal Crypt Homeostasis



Arnout G. Schepers et al., 2012

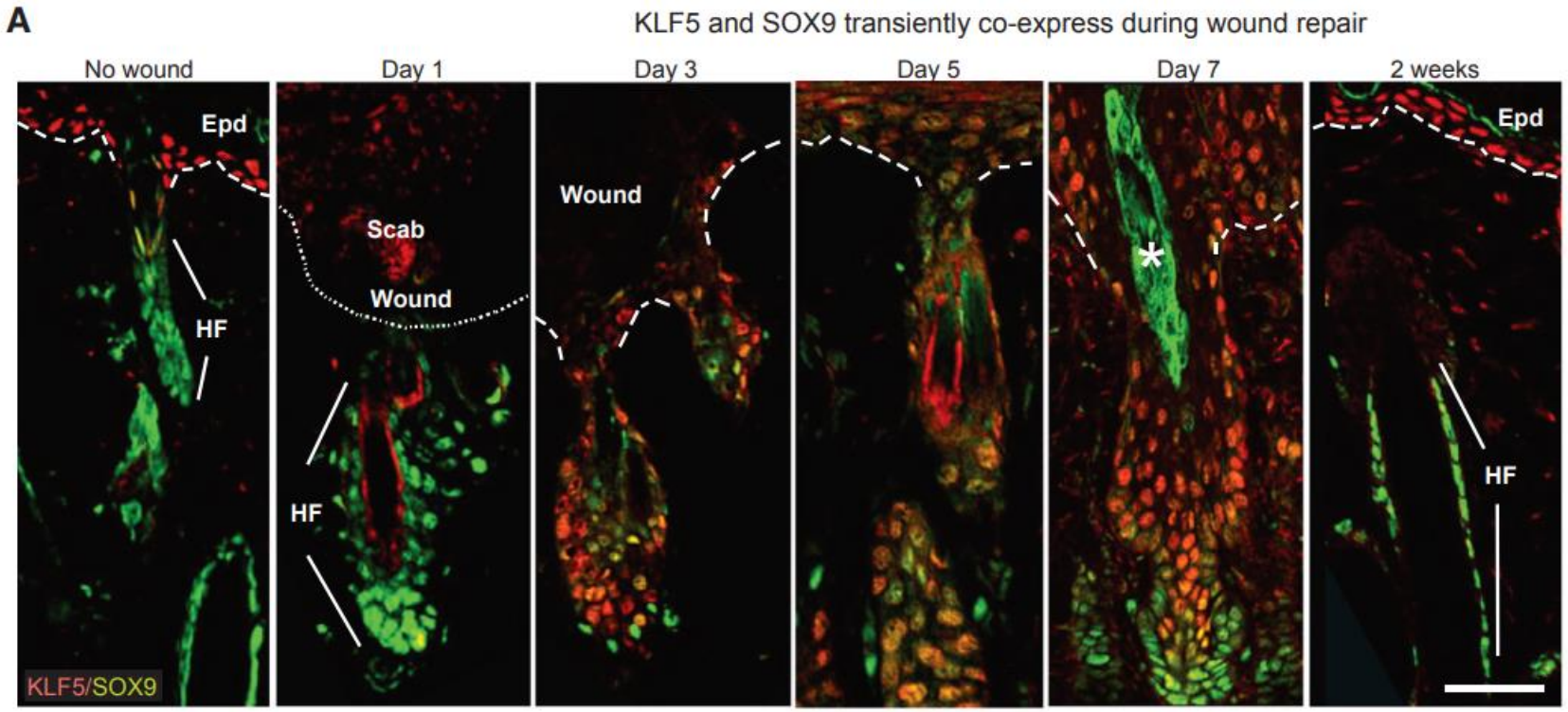
Hugo J. Snippert et al., 2010



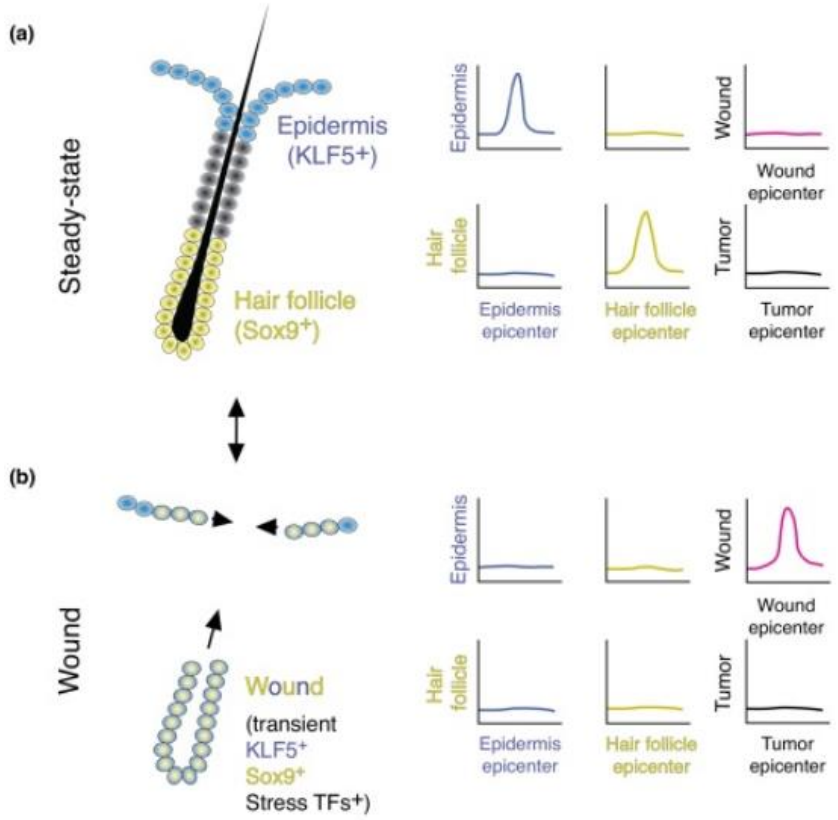
Wounded IFE exhibited a hybrid signature between HFSCs and IFESCs

IFESC HFSC

KLF5 Sox9 → cell proliferation and differentiation



Yejing Ge et al, 2017



(1) A new wound epicenter
(2) The loss of epidermal and hair follicle epicenters
(3) The expression of activating stress-associated transcription factors

Azadeh Paksa and Jayaraj Rajagopal, 2017