Regulation of skin aging by targeting glycans in epidermal stem cells

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Stem cell dynamics in skin regeneration and aging

Tissue Regeneration

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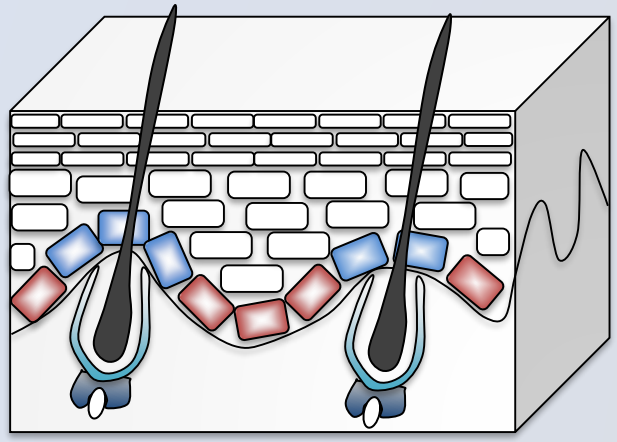
Aging

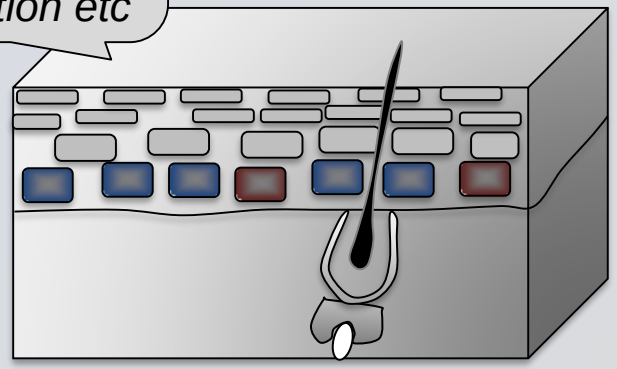
Injury repair ↓
Barrier function ↓
Cancer risk ↑
Dehydration etc

Young skin

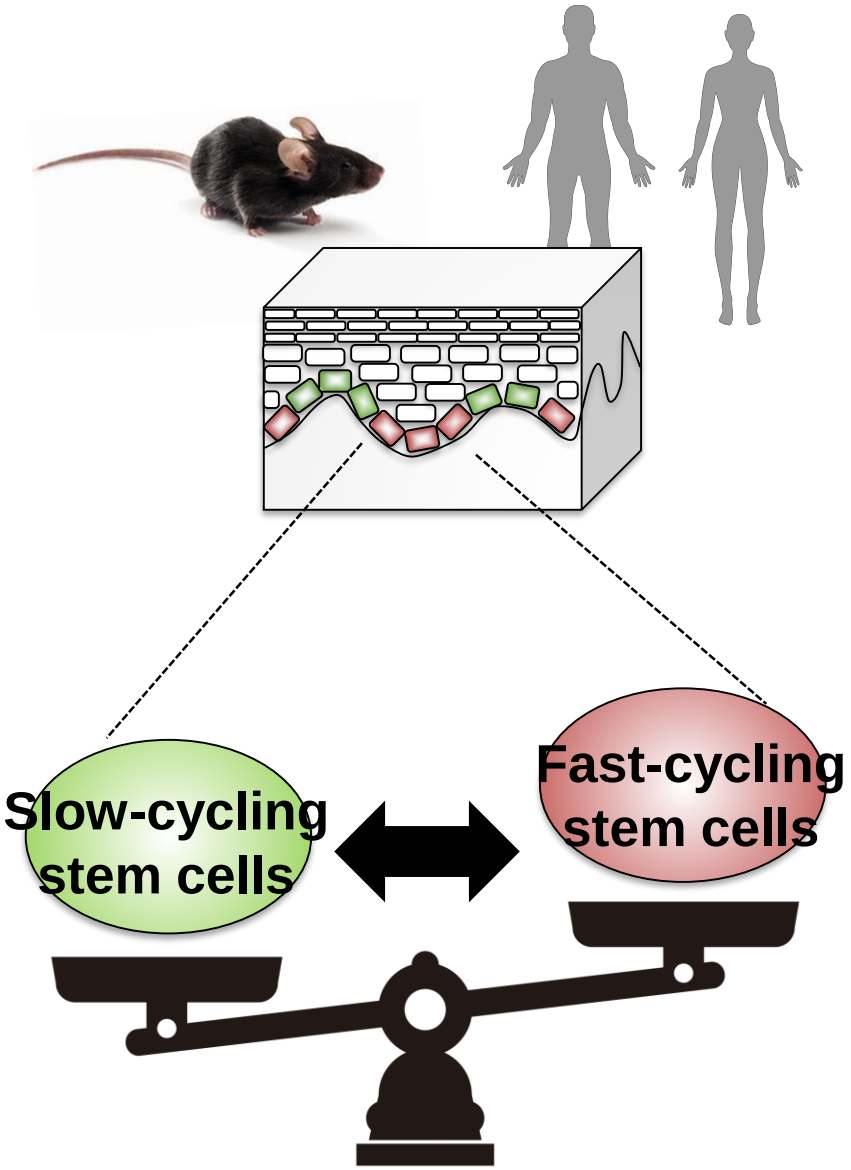


Stem cell aging

Old skin



ECM and glycans provide microenvironment governing skin resilience



Maintenance of skin resilience

Environment

Niche signals
Stressors (inflammation, injury etc)

Extracellular matrix
Glycans

Stem cell intrinsic factors

Epigenome

Metabolism

Transcriptional network

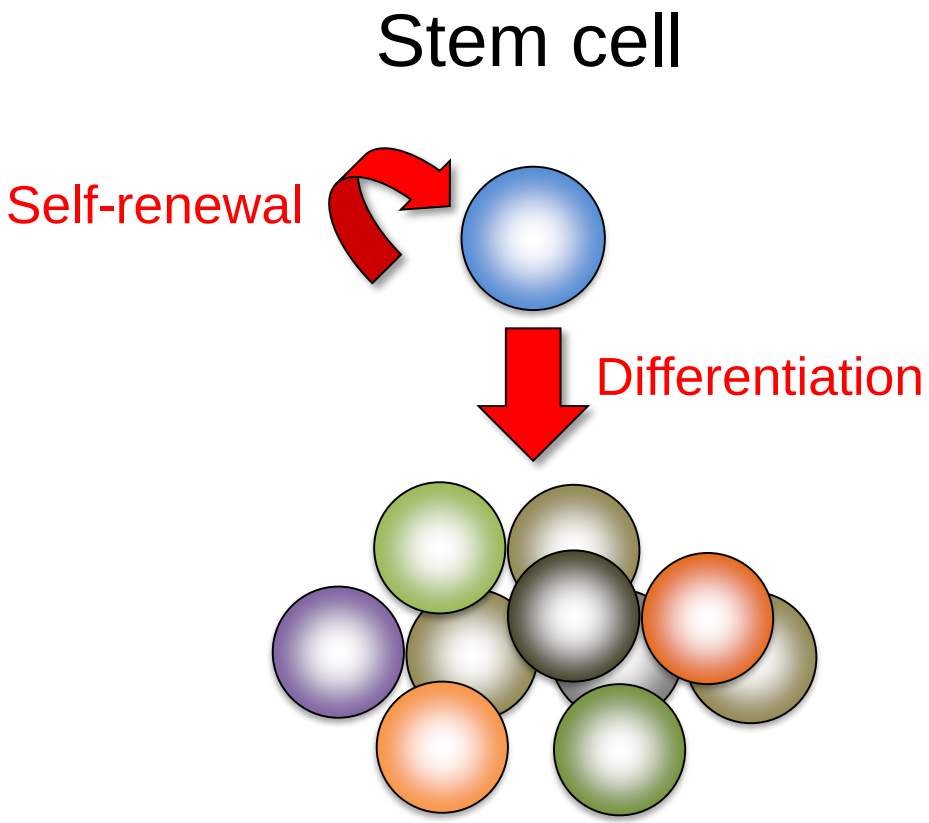
Stem cell fate and identity
Balance of heterogeneous stem cell populations

Topics

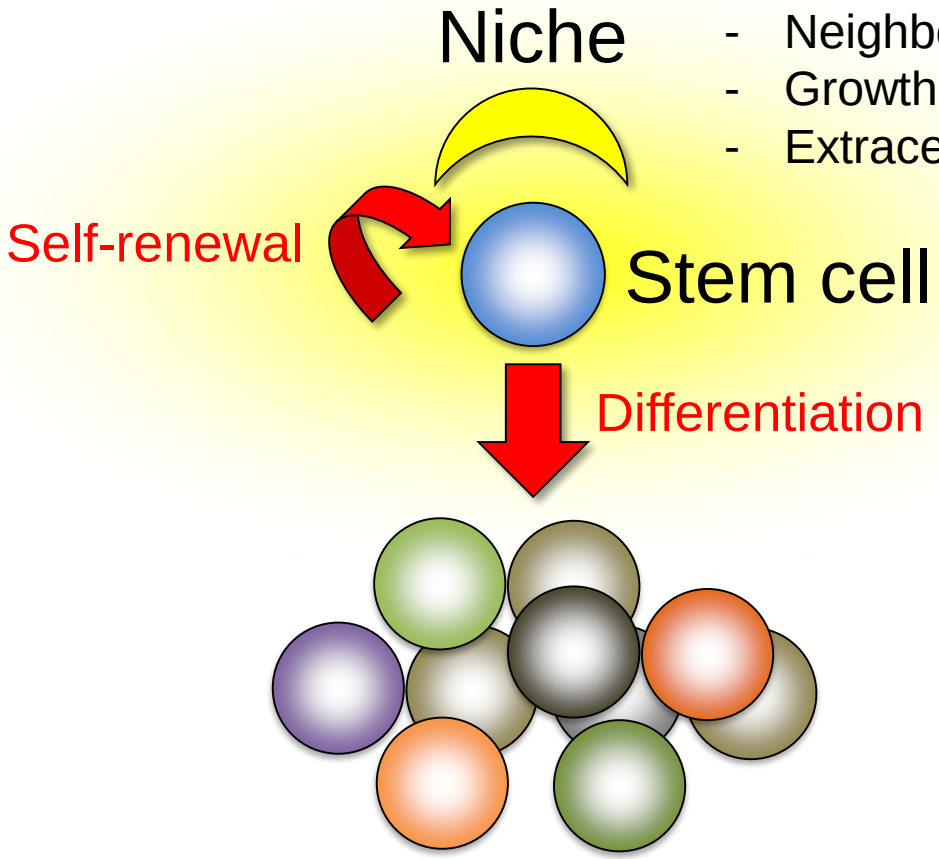
√ • Overview of stem cell biology and recent research trends

• Identification of biomarkers of epidermal stem cell aging using lectin technology

Definition of stem cell



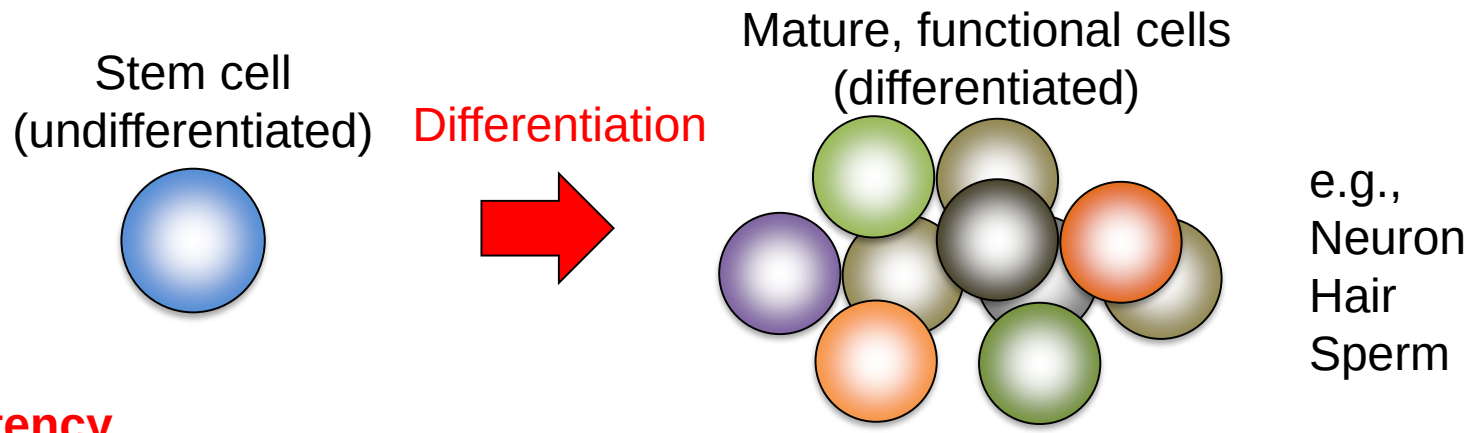
Niche: a specialized microenvironment for stem cells



For examples,
- Neighboring cells
- Growth factors (Wnt, FGF, EGF)
- Extracellular matrix (ECM)

Differentiation ability of stem cells

Differentiation：A process by which one cell type become a more specialized cell type



Totipotency

Ability of a cell to produce all differentiated cells in an organism, including extra embryonic tissues. e.g., fertilized egg

Pluripotency

Ability of a cell to produce all differentiated cells in an organism, except extra embryonic tissues. e.g., ES cells, iPS cells

Multipotency

Ability of a cell to produce all differentiated cells in a tissue (multiple types).
e.g., hematopoietic stem cells, hair follicle stem cells, intestinal stem cells

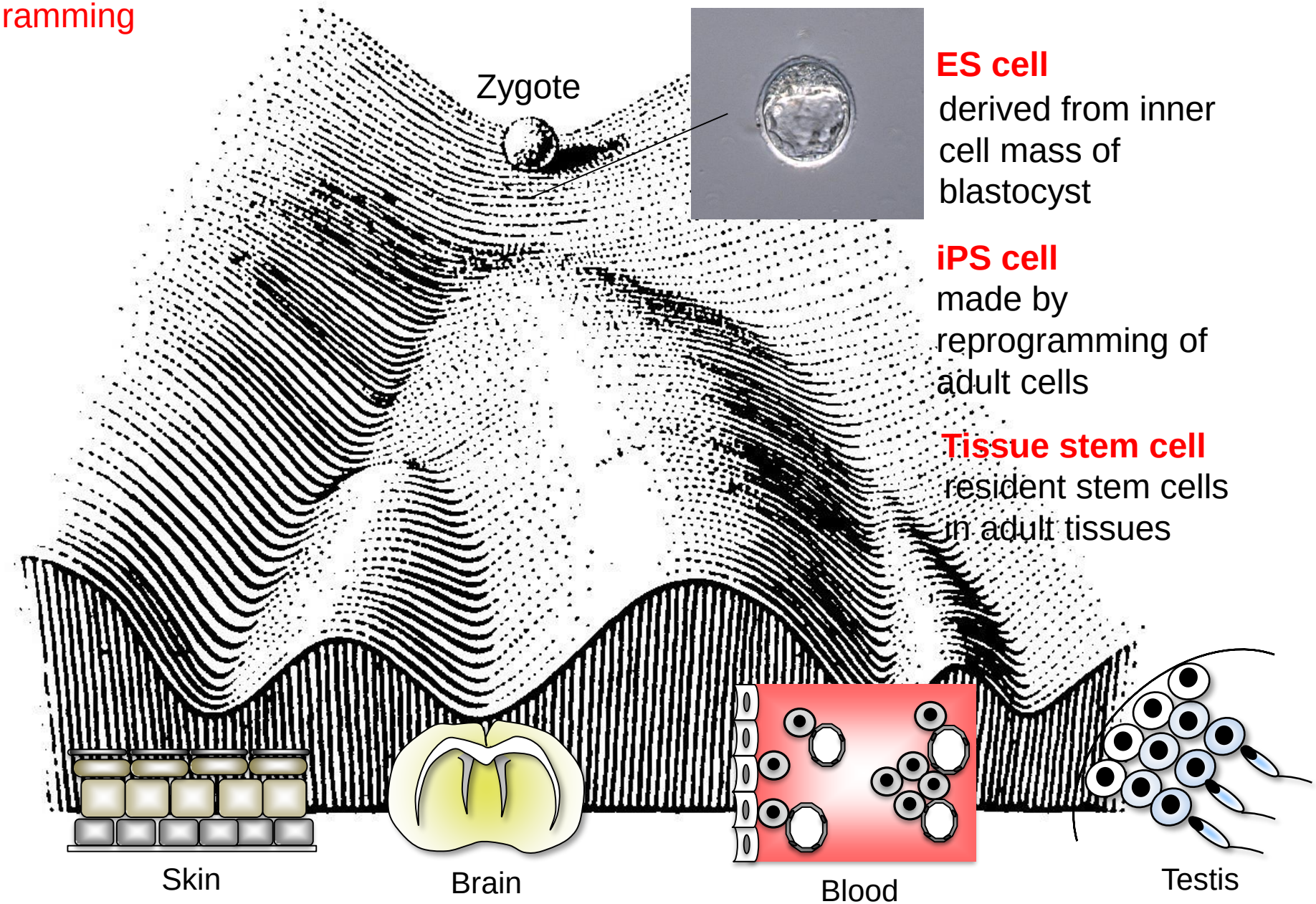
Unipotency

Ability of a cell to produce all differentiated cells in a tissue (single type).
e.g., epidermal stem cells, spermatogonical stem cells

Different types of stem cells

Development

Reprogramming



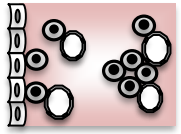
ES cell
derived from inner cell mass of blastocyst

iPS cell
made by reprogramming of adult cells

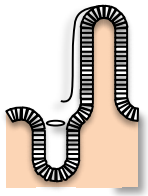
Tissue stem cell
resident stem cells in adult tissues

Stem cells in adult tissues

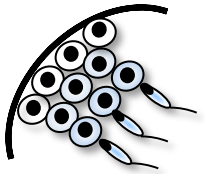
Stem cell-dependent tissue
tissue homeostasis is maintained
by stem cells



Bone marrow

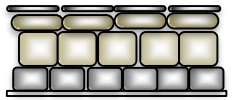


Intestine

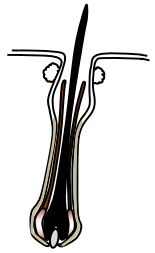


Testis

Neuron

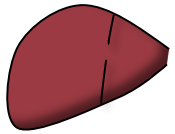


Epidermis

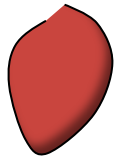


Hair follicle

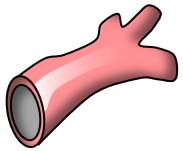
No homeostatic tissue turnover
Regeneration only after injury



Liver
- Proliferation of resident hepatocytes
- Existence of stem/progenitor cells has also been suggested

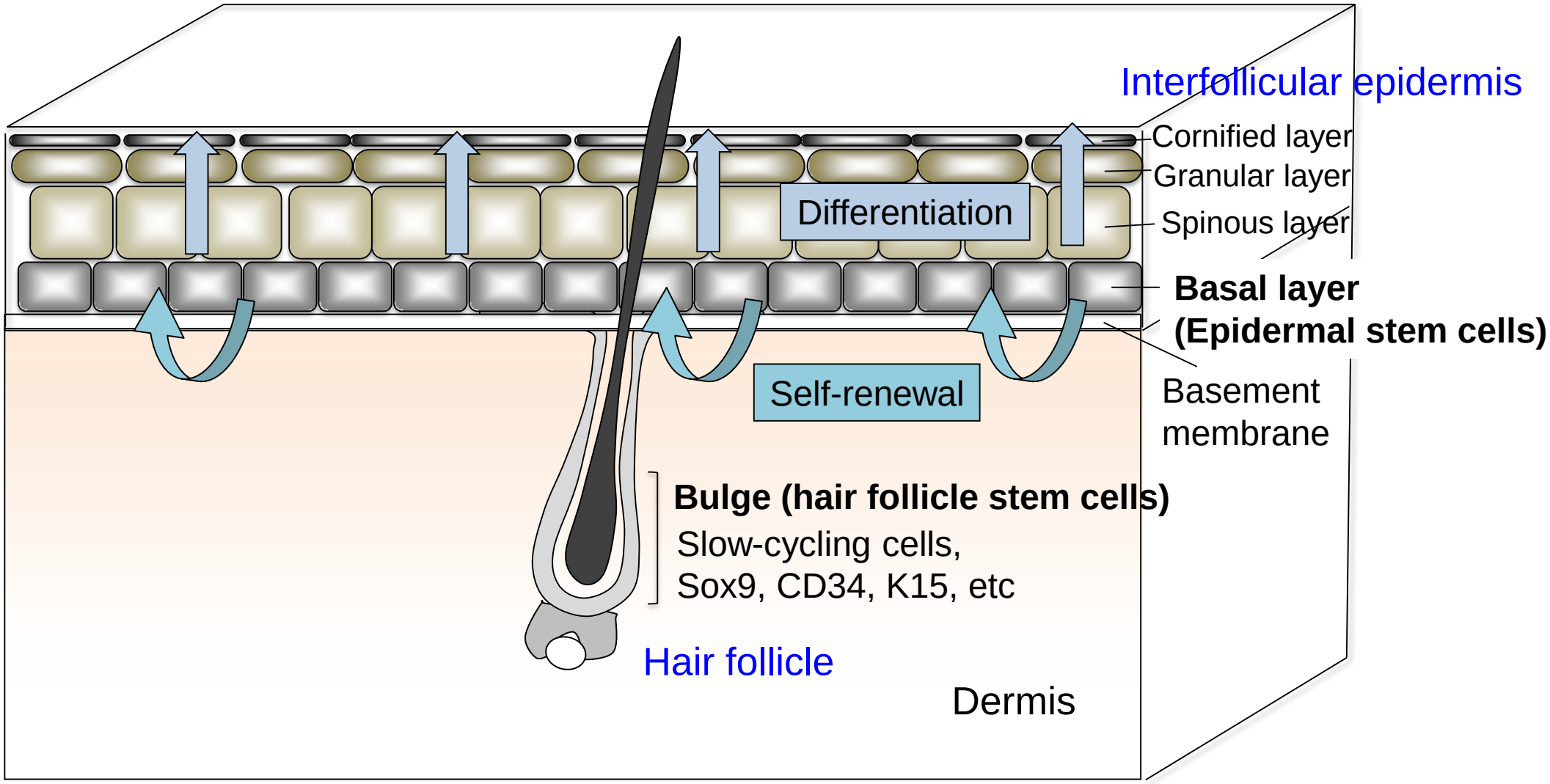


Heart
- Rare, cycling cells exist



Blood vessel
- Proliferative SMCs arise from de-differentiation of mature (post-mitotic) SMCs
- Existence of stem/progenitor cells has also been suggested

Skin structure and its stem cells



ARTICLE

doi:10.1038/nature24487

Regeneration of the entire human epidermis using transgenic stem cells

Tobias Hirsch[1]*, Tobias Rothoeft[2]*, Norbert Teig[2]*, Johann W. Bauer[3]*, Graziella Pellegrini[4,5]*, Laura De Rosa[5]*, Davide Scaglione[6], Julia Reichelt[3], Alfred Klausegger[3], Daniela Kneisz[3], Oriana Romano[7], Alessia Secone Seconetti[5], Roberta Contin[5], Elena Enzo[5], Irena Jurman[8], Sonia Carulli[9], Frank Jacobsen[1], Thomas Luecke[10], Marcus Lehnhardt[1], Meike Fischer[2], Maximilian Kueckelhaus[1], Daniela Quaglino[7], Michele Morgante[8], Silvio Bicciato[7], Sergio Bondanza[9] & Michele De Luca[5]

Hirsch et al., *Nature* 2017

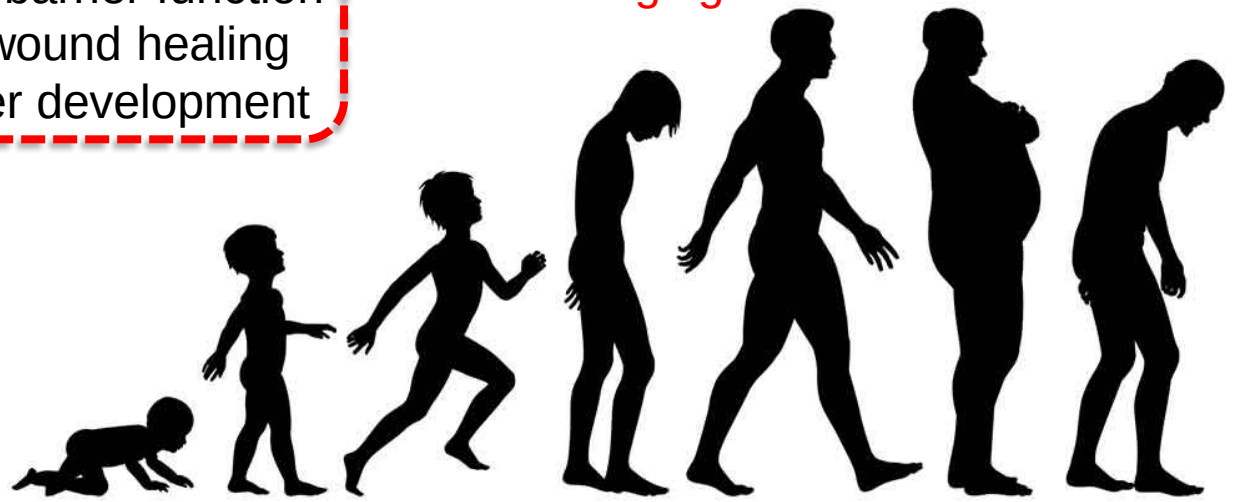
Age-related skin changes and loss of tissue stem cell function (stem cell aging)

Wrinkles, spots, and sagging skin

Hair graying and thinning

Skin dryness
Decreased barrier function
Delayed wound healing
Skin cancer development

Due to epidermal stem cell aging?



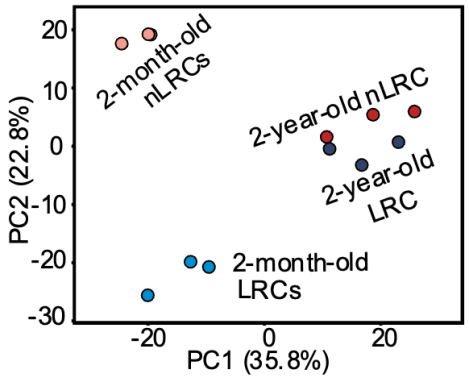
Alterations in extracellular environment of epidermal stem cells during aging

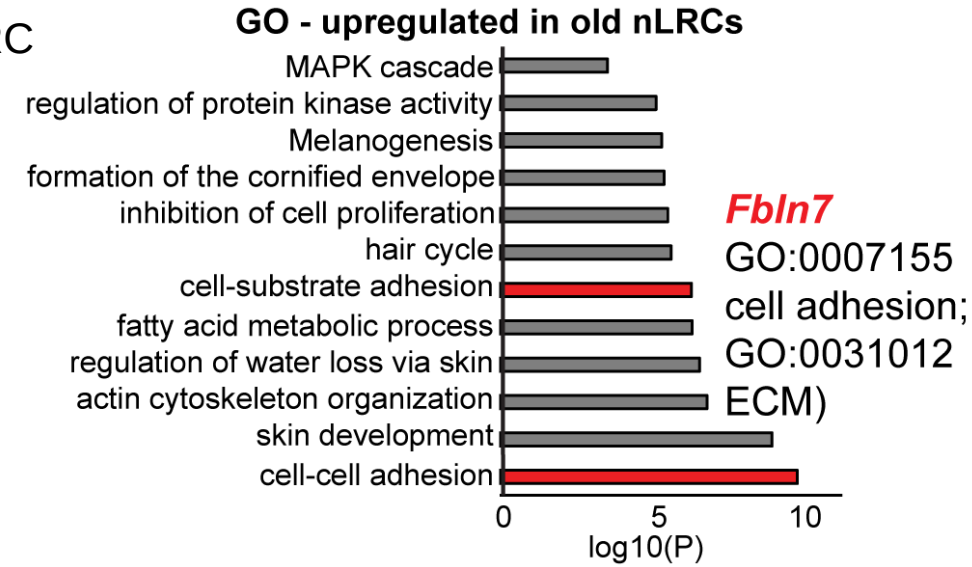
RNA sequencing

Young (2 months old), LRC, non-LRC

Vs

Old (2 years old), LRC, non-LRC

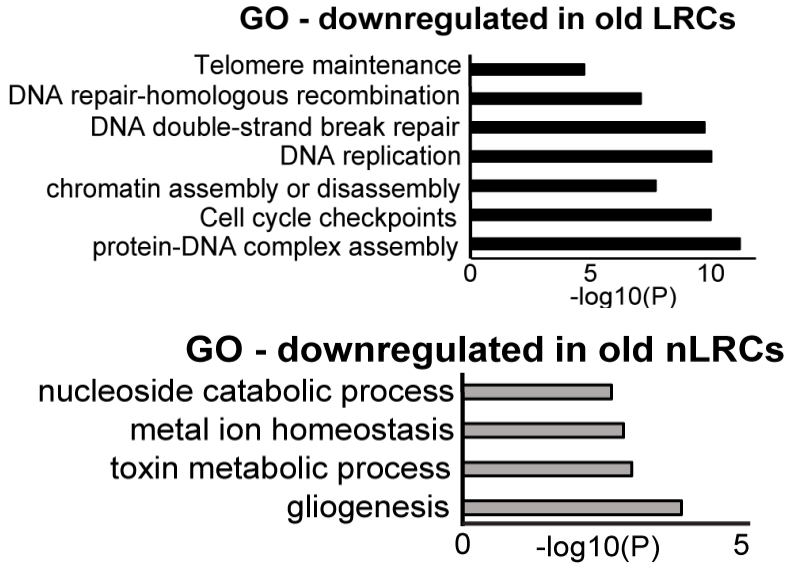




Fbln7

GO:0007155 cell adhesion;

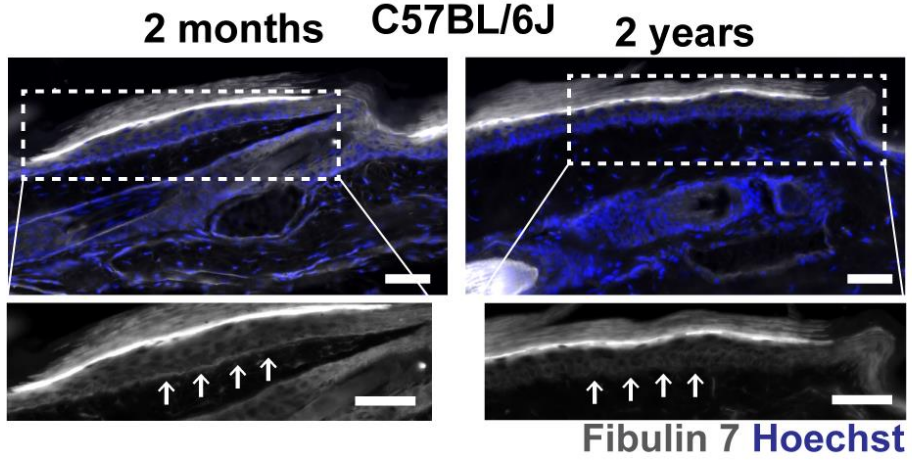
GO:0031012 ECM)

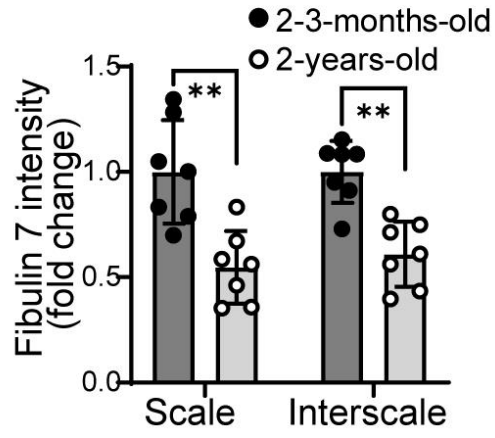


Fibulin-7 protein

- Coiled-coil (Heparin-binding activity)
- EGF-like module
- Sushi domain
- Fibulin-type module

de Vega et al., J. *Biol. Chem.* 2007





Raja et al., *EMBO Reports* 2022

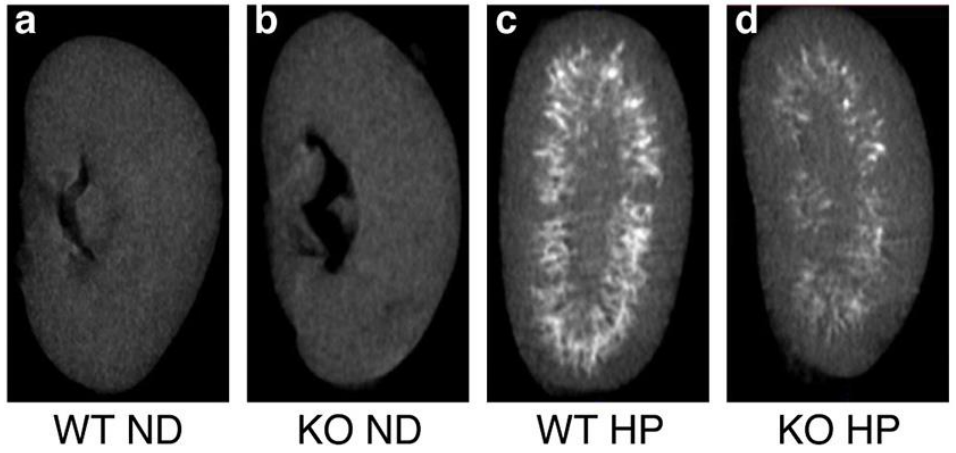
Fibulin-7, a heparin binding matricellular protein, promotes renal tubular calcification in mice

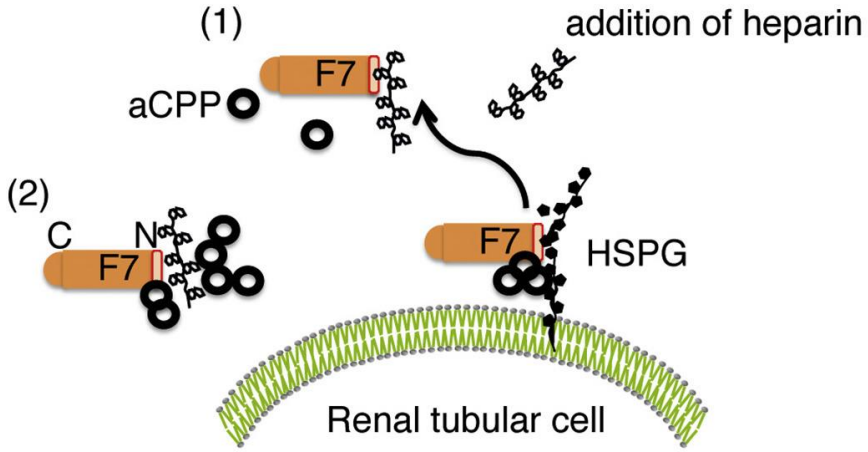
Fibulin-7 protein

- Coiled-coil (Glycan-binding activity)
- EGF-like module
- Sushi domain
- Fibulin-type module

de Vega et al., J. *Biol. Chem.* 2007

Calcification↓ in *Fbln7* KO

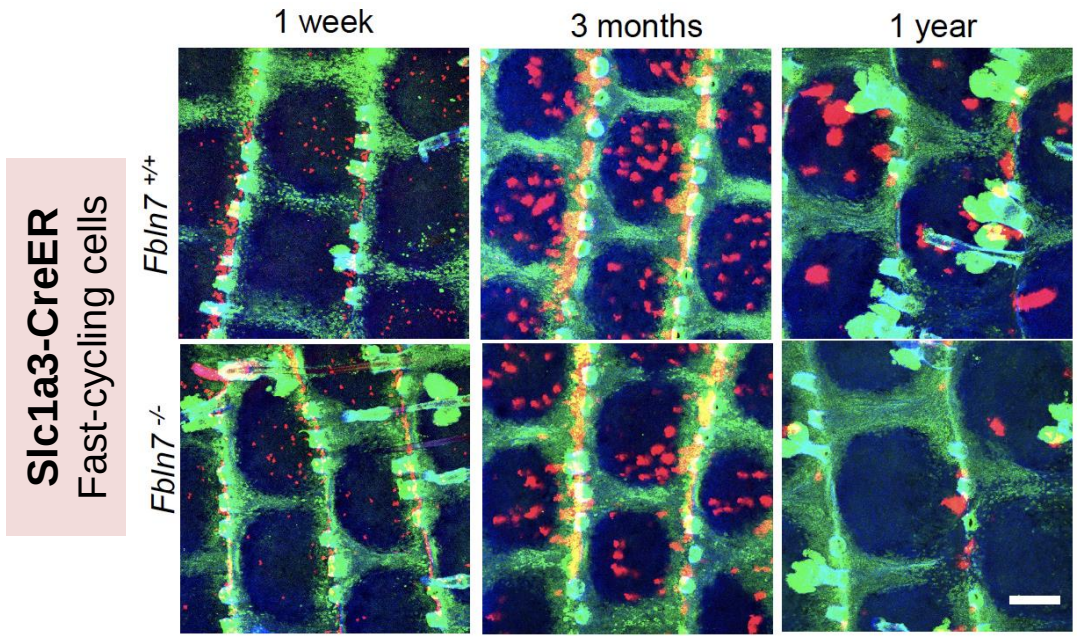




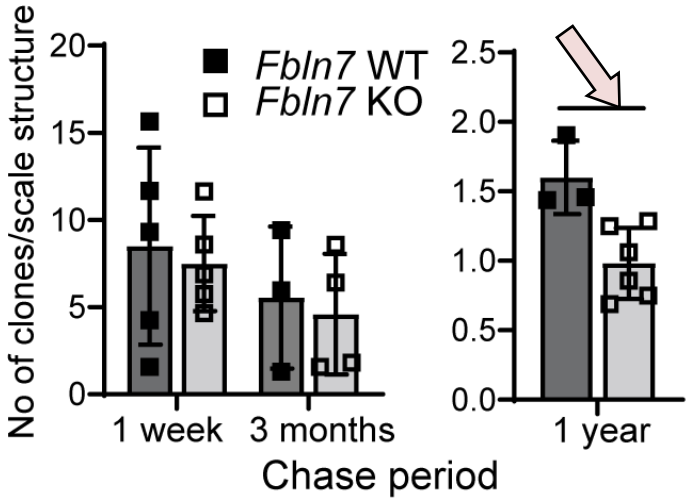
HP: phosphate diet
HSPG: heparan sulfate proteoglycan
aCPP: artificial calcium phosphate particles

Tsunezumi et al., *Matrix Biol* 2018

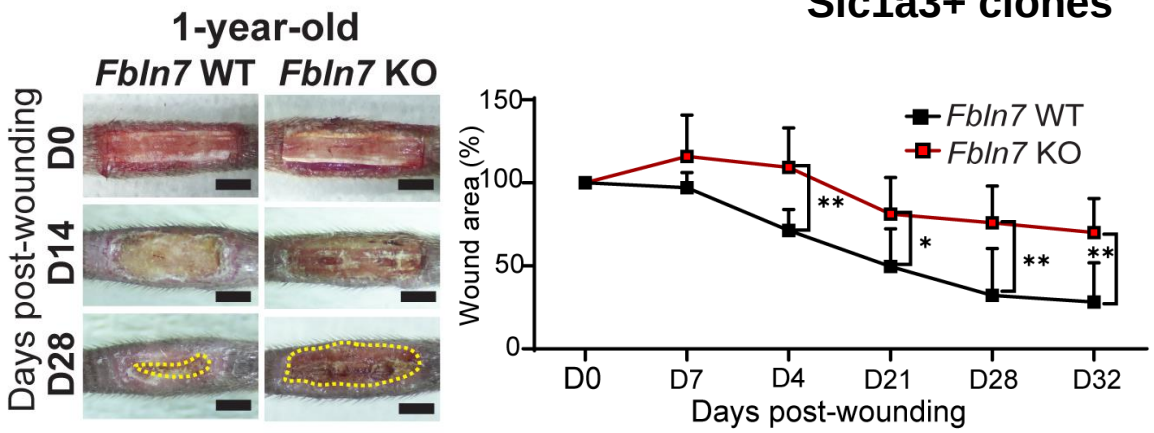
Loss of *Fbln7* accelerates age-dependent depletion of fast-cycling stem cell clones and delays wound healing



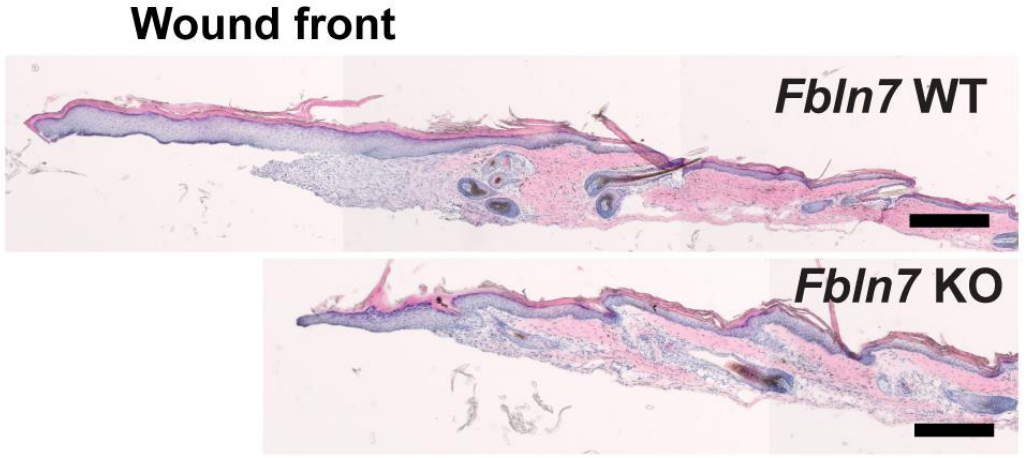
tdTomato K10 (interscale)Hoechst



-> early loss of Slc1a3+ clones



-> delayed wound healing



Raja et al., *EMBO Reports* 2022

Fbln7 KO shows elevated inflammation-related genes and impaired stem cell lineage

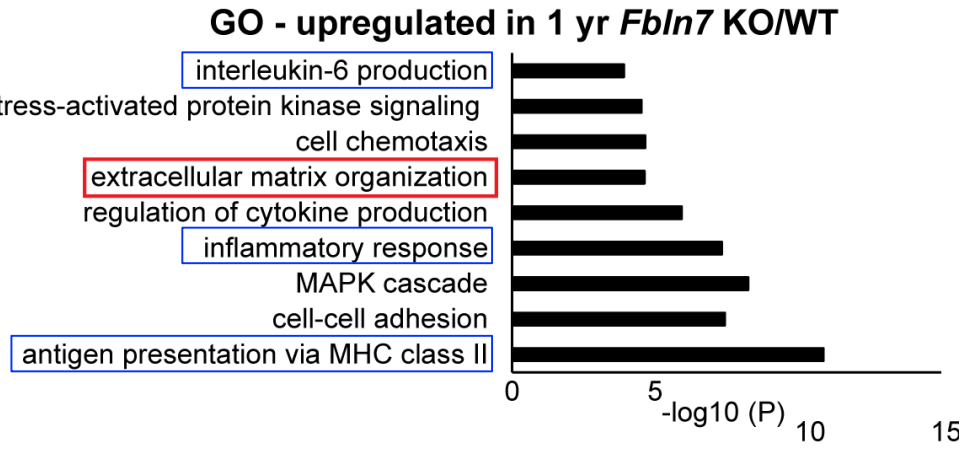
***Fbln7* KO**

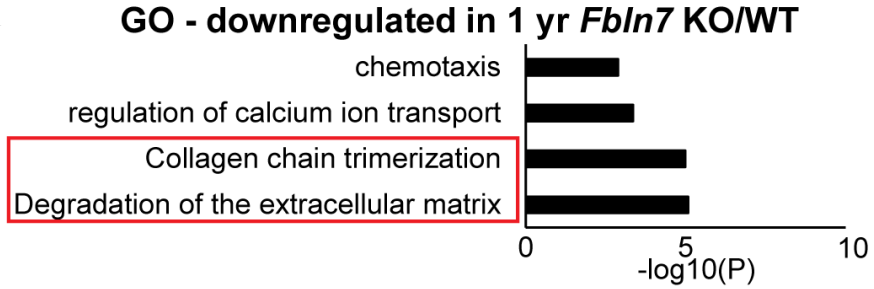
ECM alteration

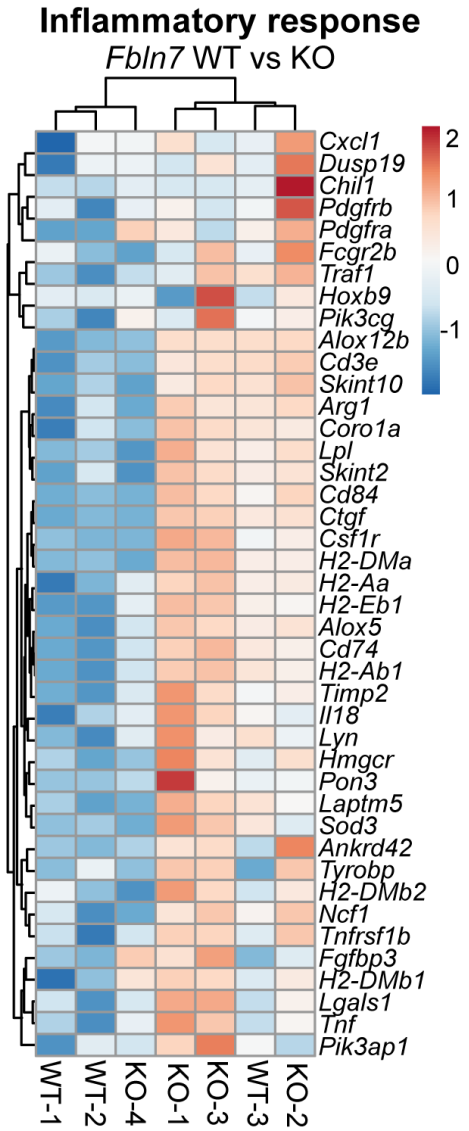
Inflammatory response↑

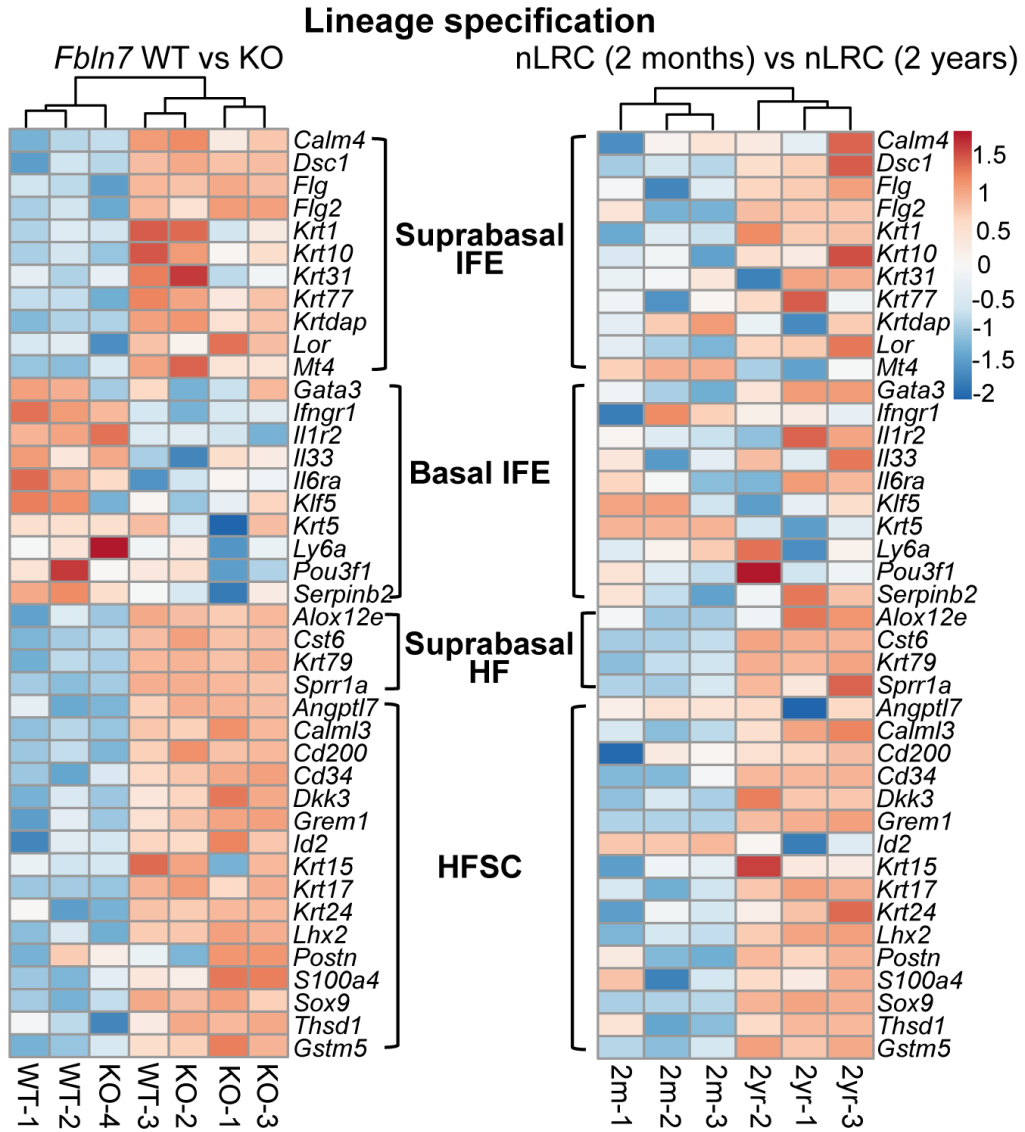
Differentiation↑

Lineage mis-specification



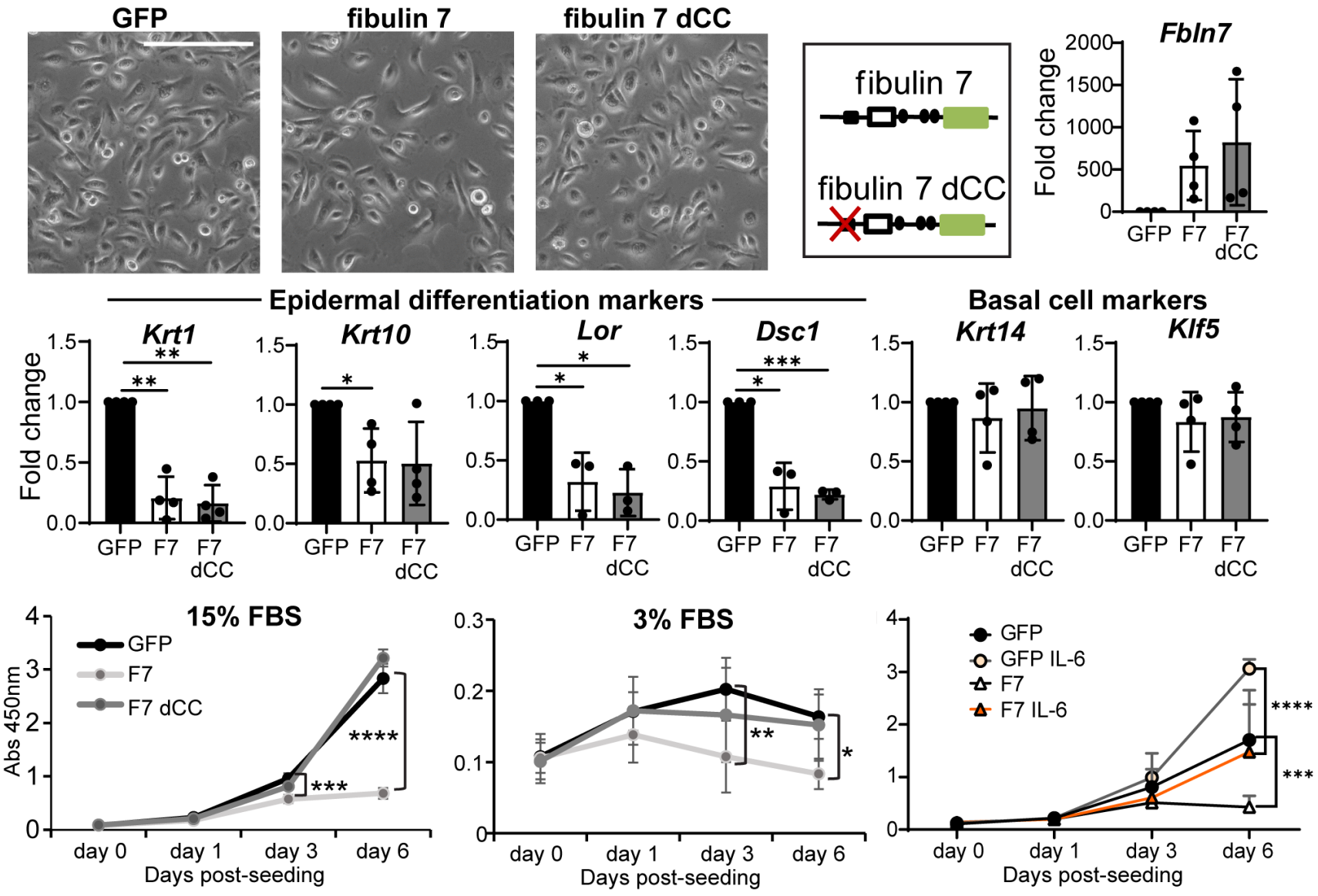






Raja et al., *EMBO Reports* 2022

Fibulin-7 maintains epidermal stem cells in an undifferentiated, slower proliferation state



Fbln7 OE

Differentiation↓ (CC-independent)

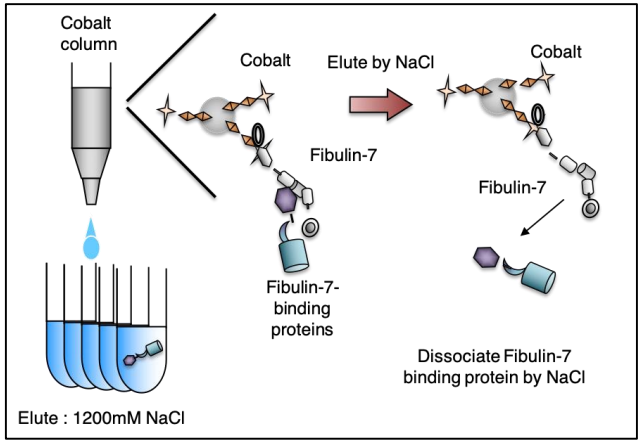
Proliferation↓ (CC-dependent)

Inflammatory response ↓

Raja et al., *EMBO Reports* 2022

Fibulin-7 binds with collagen IV and other extracellular molecules

Metal ion affinity chromatography

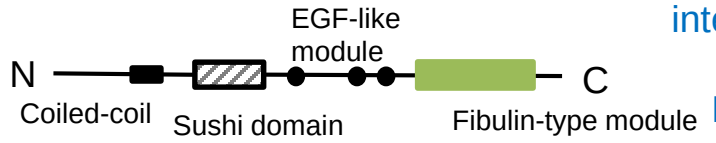


Mass spectrometry

Protein-protein interactions by Solid phase binding assay (ELISA)

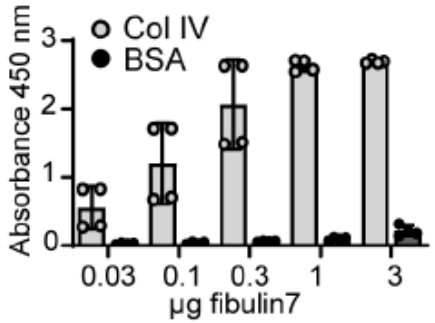
Fibulin-7 protein

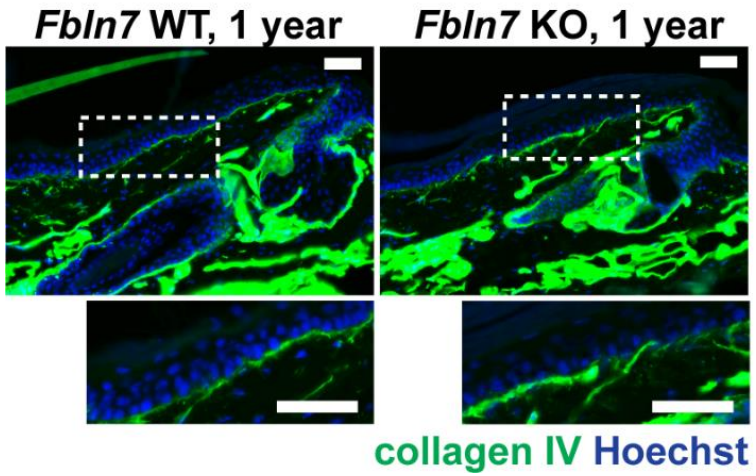
Tethering growth factors?



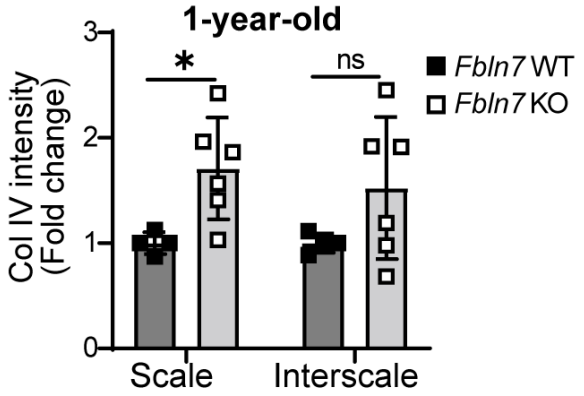
ECM structural integrity to suppress differentiation
Integrin binding

fibulin 7 - binding proteins					
	Collagen-related	growth factors signaling	basement membrane	ECM/matricellular proteins	proteases
Full Length fibulin 7 Proliferation & differentiation	COL3A1 LOXL3 PLOD1 PLOD3	granulins neuropilin-1 semaphorin-3E semaphorin-3C semaphorin-3A semaphorin-3B HGF	BM-HSPC LAMA5 LAMC1 nidogen-1 LAMB-2	fibronectin periostin netrin-4 CCDC80 THBS3 emilin-1	cathepsin Z legumain BMP1
delta CC fibulin 7 Differentiation	COL2A1 COL4A2 COL5A2 COL14A1	neuropilin-2 IGF2BP1 IGFBP-2 SLIT2 FSTL1	fibulin 1	clusterin SMOC1 APP CILP2 fibrillin-2 cochlin tenascin C	PLAT aminopeptidase B BMP1 dermcidin





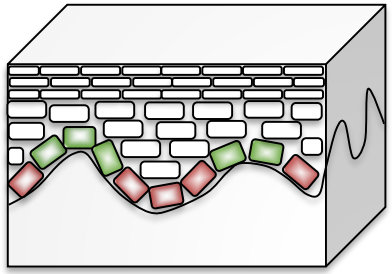
collagen Ⅳ staining

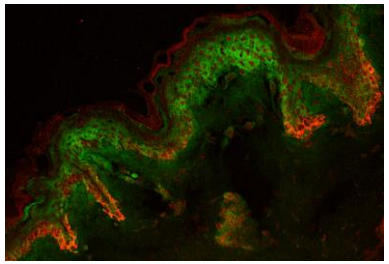


Raja et al., *EMBO Reports* 2022

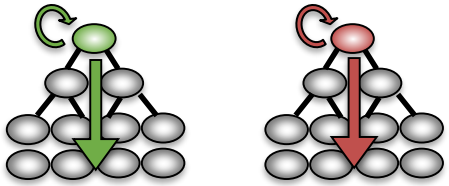
Summary: Fibulin-7 maintains epidermal stem cell heterogeneity by maintaining ECM integrity in aging skin

Young skin



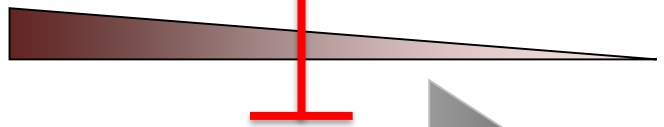


Slow-cycling stem cells **Fast-cycling stem cells**



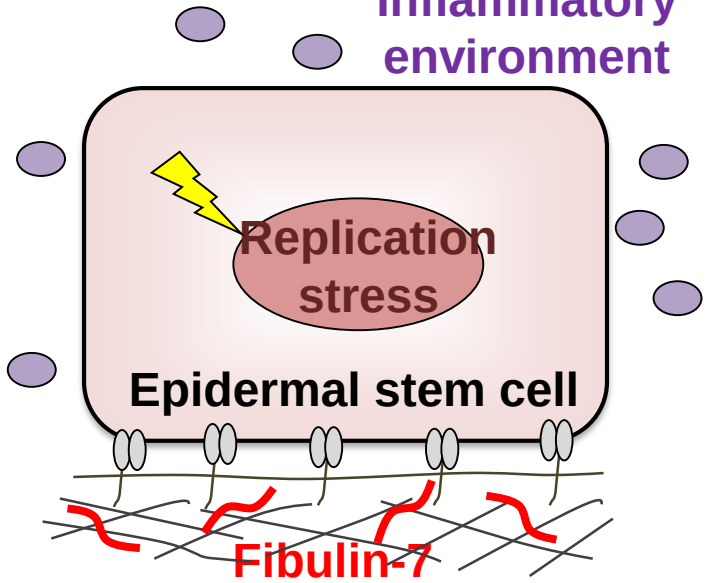
Tissue homeostasis/regeneration by two stem cell populations

Fibulin-7



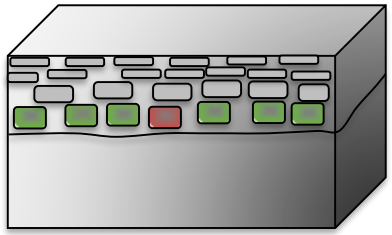
Stem cell aging

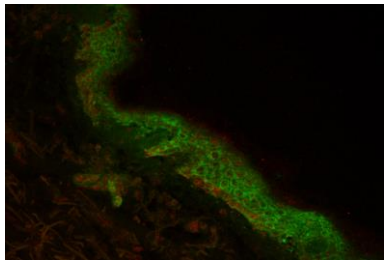
Inflammatory environment



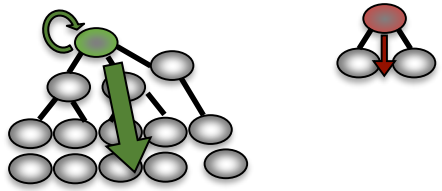
ECM integrity

Aged skin





Slow-cycling stem cells **Fast-cycling stem cells**



Loss of fast-cycling stem cells
Impaired tissue resilience

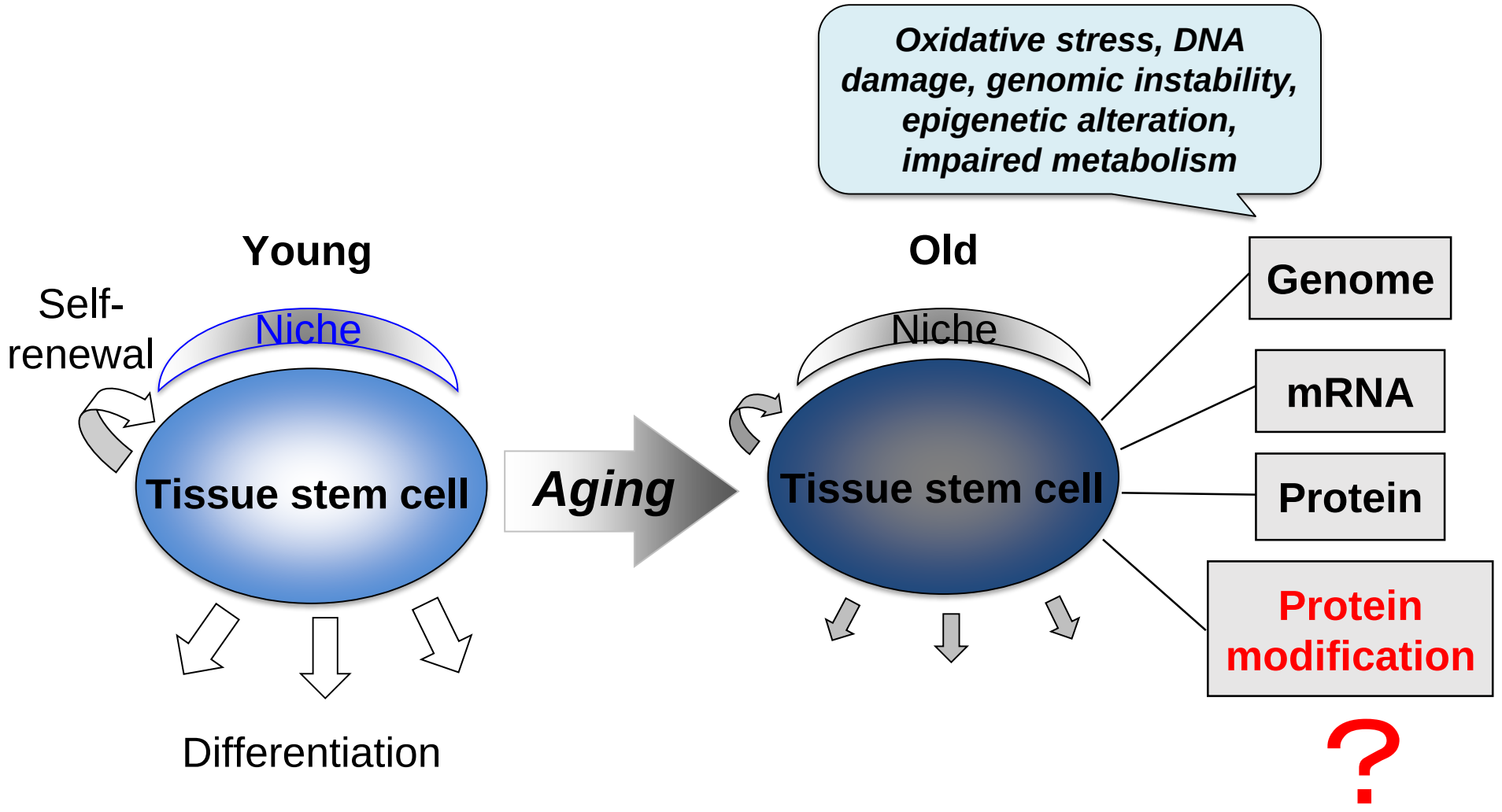
Raja et al., *EMBO Reports* 2022

Topics

- Overview of stem cell biology and recent research trends

√ • Identification of biomarkers of epidermal stem cell aging using lectin technology

Stem cell aging, a potential driver of age-associated tissue disfunction



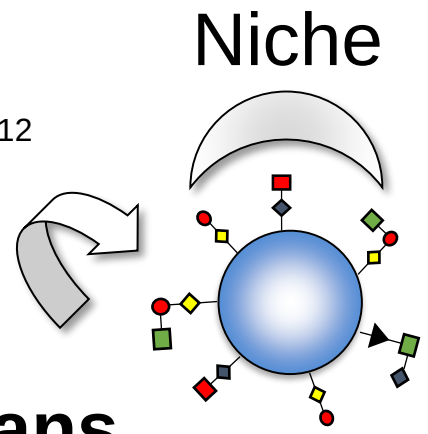
Biological function of glycans

Protein function

Moremen et al., *Nat Rev Mol Cell Biol.* 2012

Cell-cell or cell-ECM interaction

Zhao et al., *Cancer Sci.* 2008

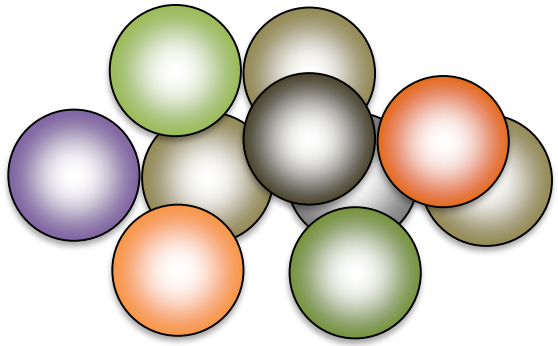


Development Stem cells

Haltiwanger et al., *Annu Rev Biochem.* 2004
Nishihara, *FEBS Letters.* 2018

Cancer and diseases

Ohtsubo & Marth, *Cell.* 2006



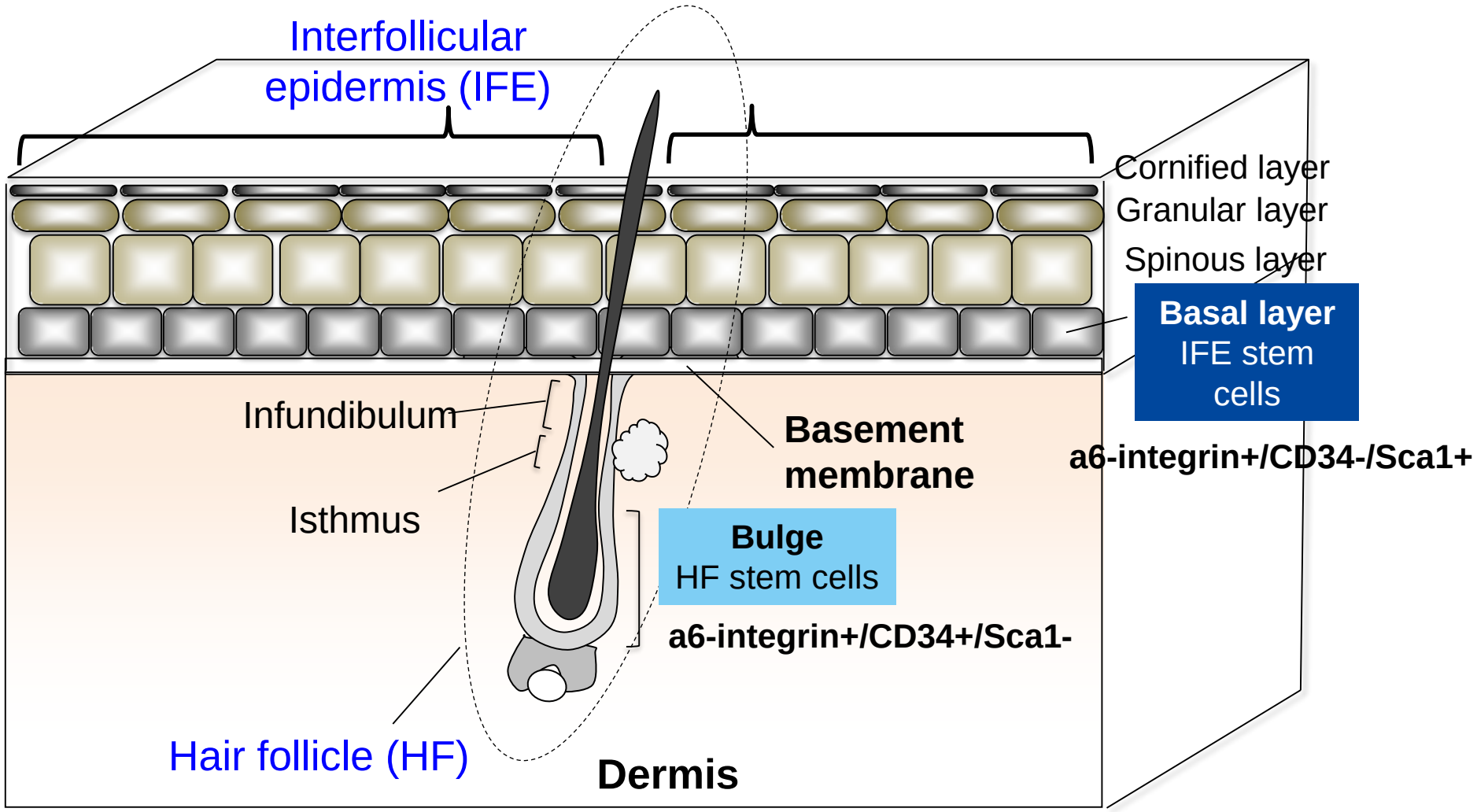
Biomarker

Adewumi et al., *Nat Biotechnol.* 2007
Hasehira et al., *Mol Cell Proteomics*, 2012
Tateno et al., *J Biol Chem*, 2011

Aging

Cindrić et al., *Adv Exp Med Biol*. 2021
Paton et al., *Int Mol Sci.* 2021

Stem cell compartments in adult mouse skin



Advances in Stem Cells and their Niches, 2019

Lectin microarray, a high throughput glycome analysis

Lectin microarray

In collaboration with Dr. Hiroaki Tateno (AIST)

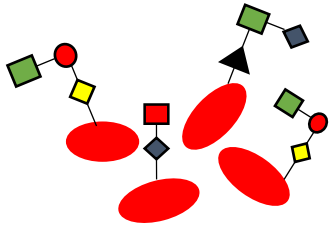
FACS-isolation of stem cells

IFE young IFE old

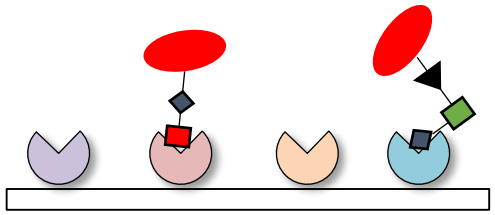
HF young HF old

2 months 22-24 months

Isolation and labeling of membrane proteins

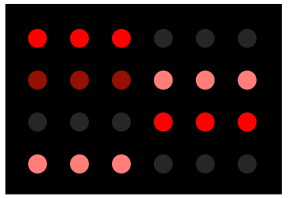


Incubation in lectin array



Immobilized 96 lectins (glycan-binding proteins)

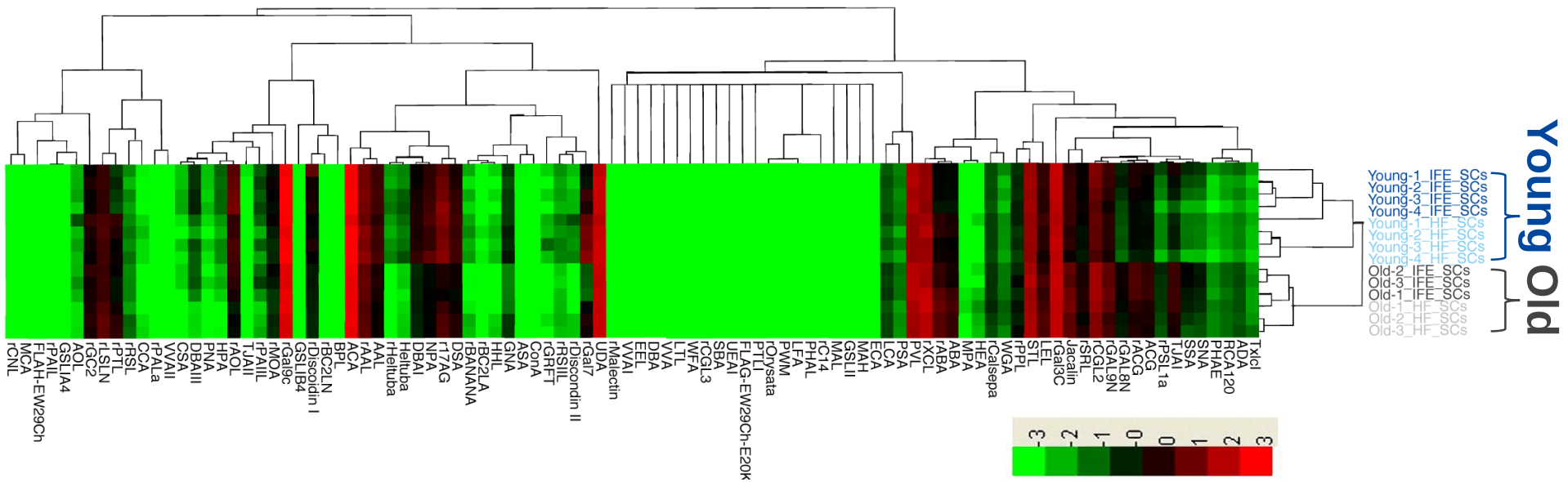
Signal detection and analysis



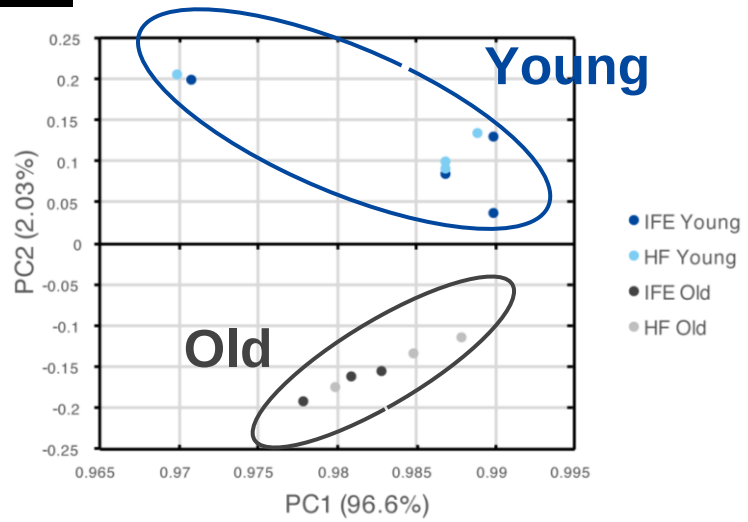
Hirabayshi et al., *Chem Soc Rev*, 2013
Tateno et al., *JBC* 2011
Tateno et al., *Glycobiology* 2007

Dynamic glycan alterations during epidermal stem cell aging

Heat map and hierarchical clustering



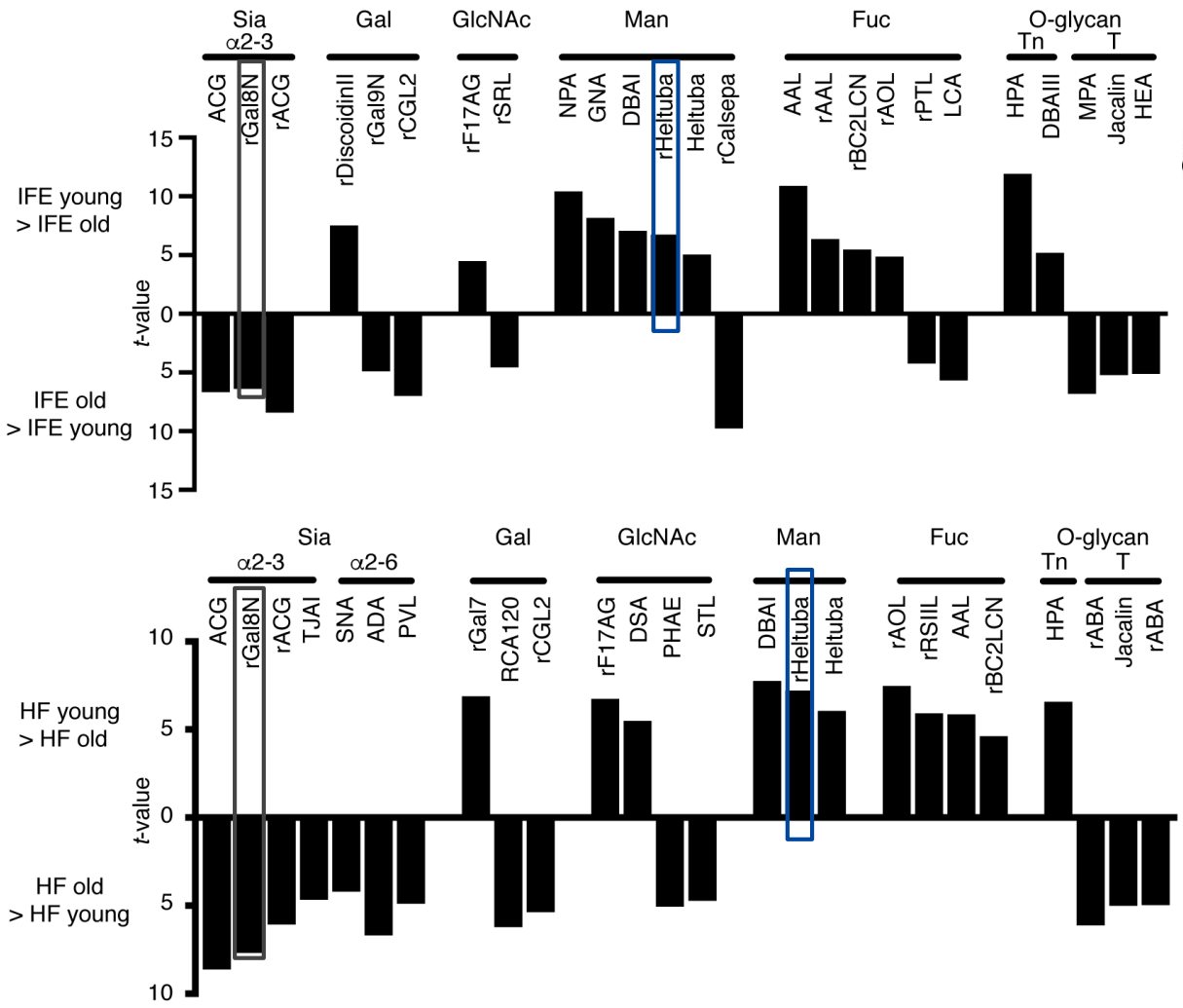
Principle component analysis

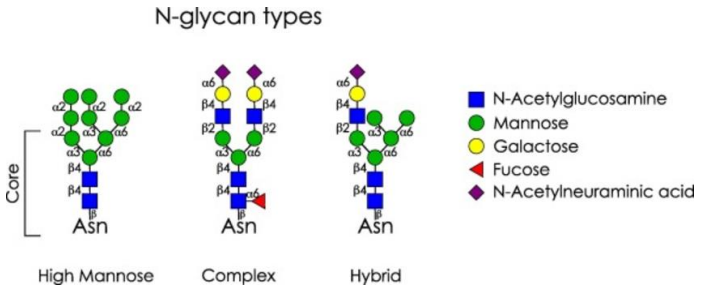


Oinam et al., *Aging Cell 2020*

Classes of lectins that differentially identified glycans in young and old stem cells

Glycome shift: High mannose type -> Complex type





Higel et al., 2016

Higher in young

rHeltuba
Mannose-binding lectin

Higher in old

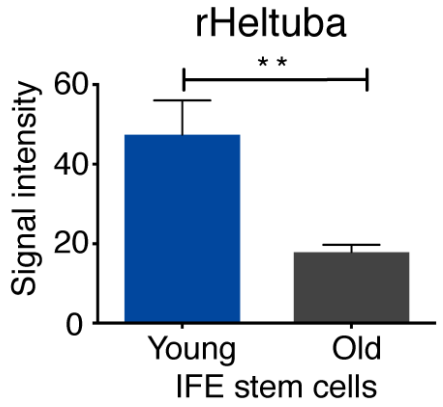
rGal8N
α2,3 Sia-binding lectin

Oinam et al., *Aging Cell 2020*

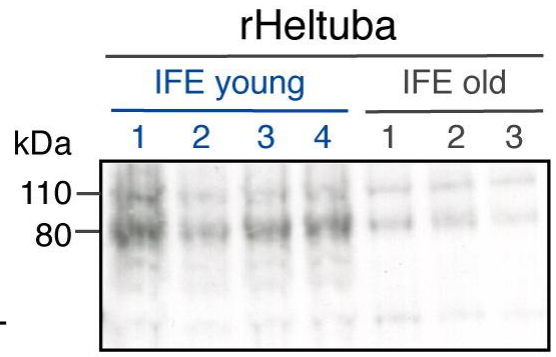
Old epidermal stem cells display decreased mannose and increased Sia modifications

rHeltuba (Mannose-binding lectin), **Higher in young**

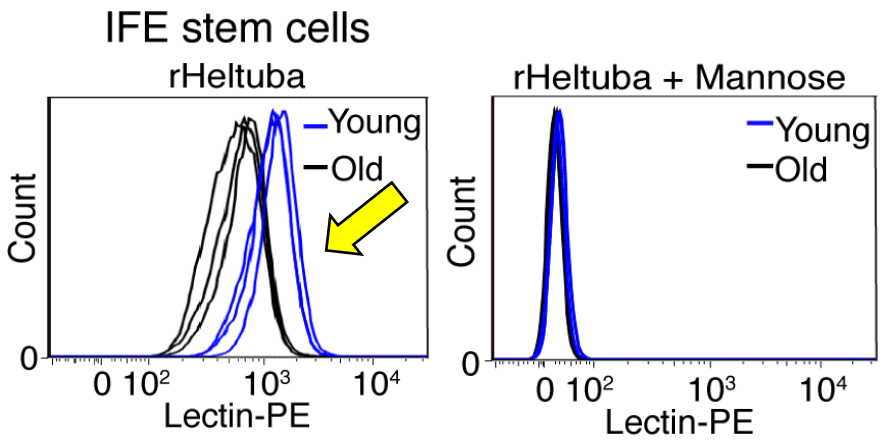
Lectin microarray



Lectin blotting

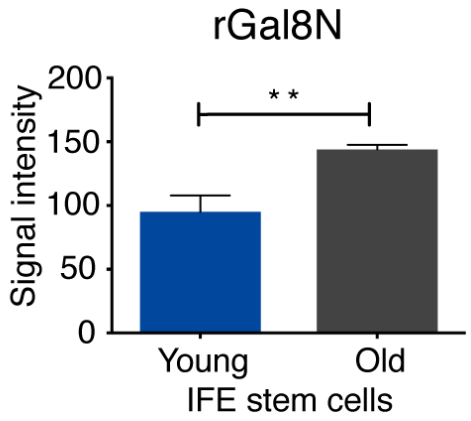


Lectin FACS

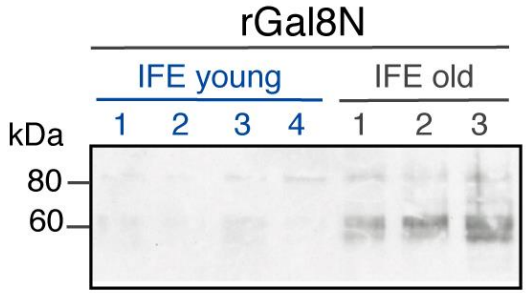


rGal8N (α2,3 Sia-binding lectin), **Higher in old**

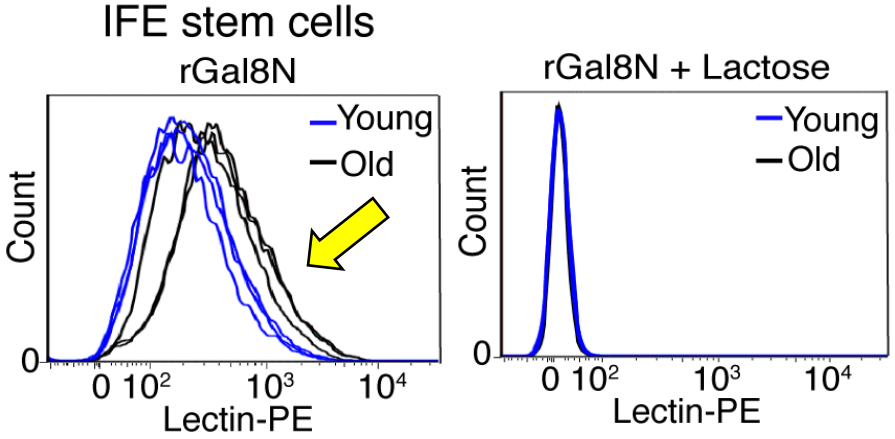
Lectin microarray



Lectin blotting

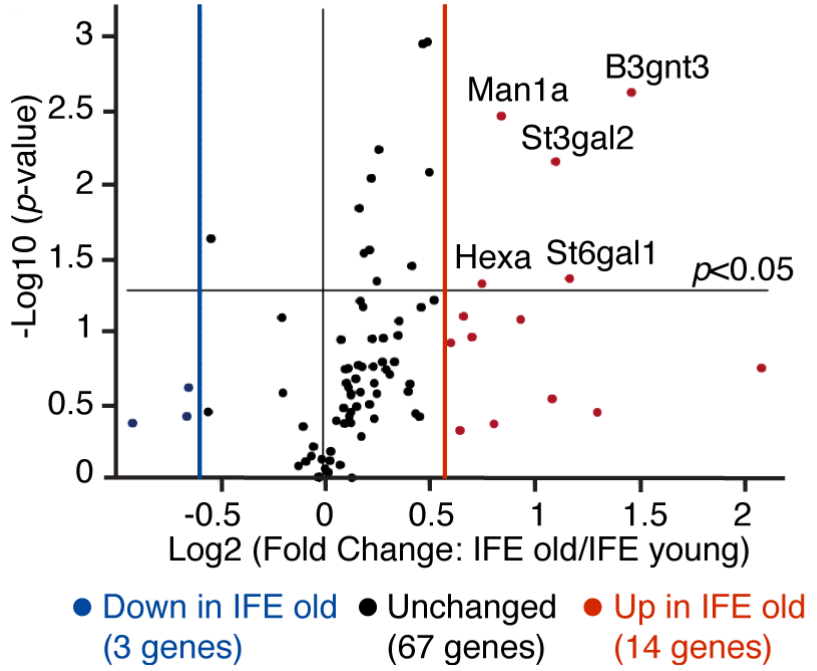


Lectin FACS



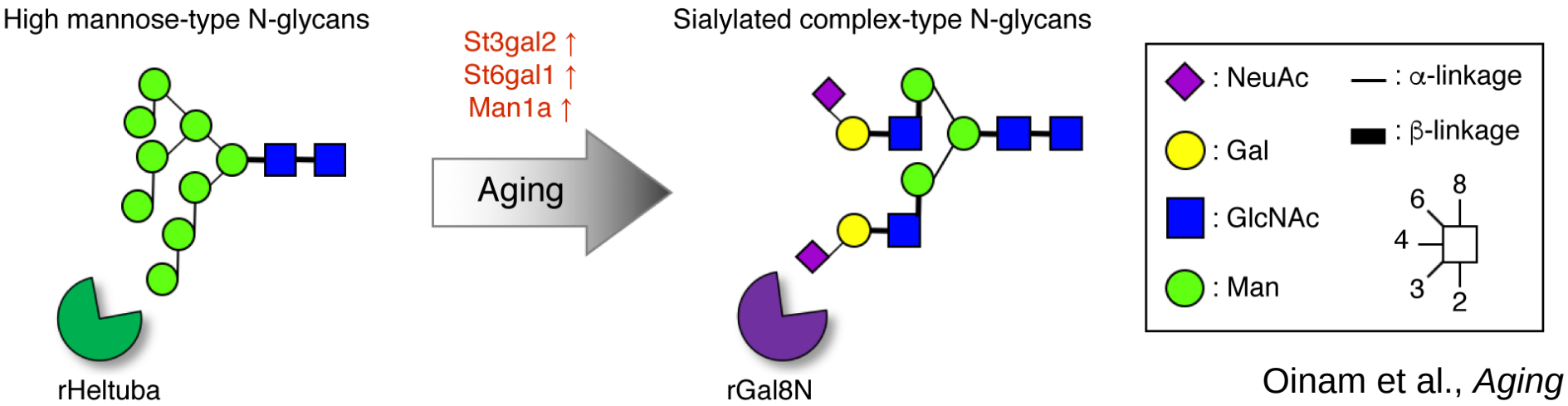
Oinam et al., *Aging Cell 2020*

Up-regulation of sialyltransferase and mannosidase genes in old epidermal stem cells



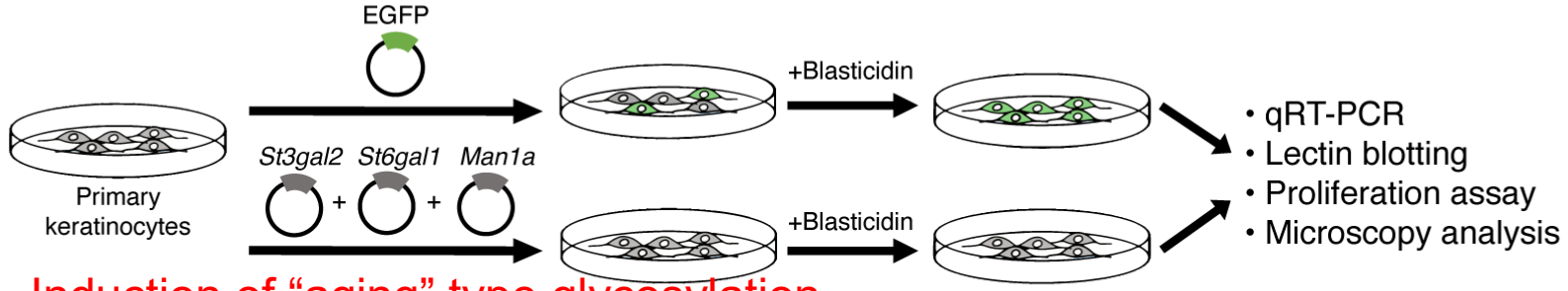
Gene symbol	Fold change	*p*-value	Function
St3gal2	2.16	0.0064	Addition of sialic acid from cyclic monophosphase (CMP)-sialic acid to b-galactosides and forms α-2,3 sialylated glycoconjugates.
St6gal1	2.26	0.0414	Addition of sialic acid from CMP-sialic acid to galactose-containing substrates and forms α-2,6 sialylated glycoconjugates.
Man1a	1.81	0.0031	Removal of mannose from N-glycans

Glycome shift during epidermal stem cell aging

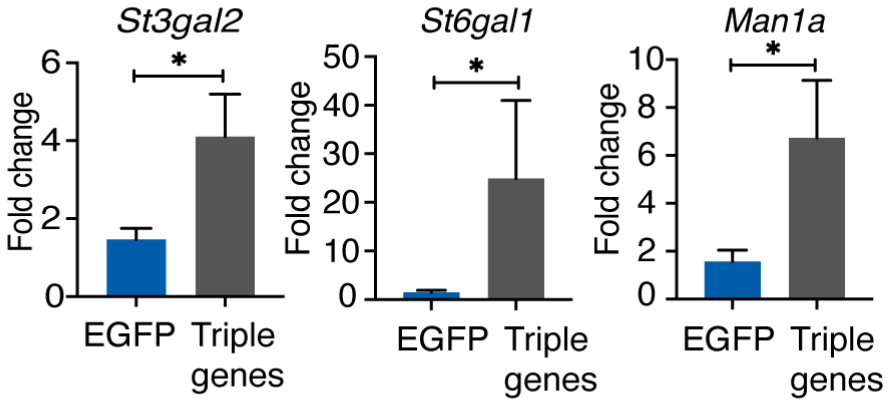


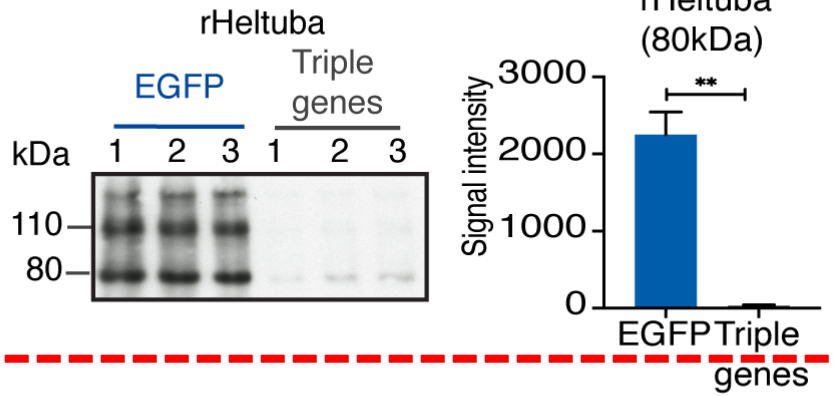
Oinam et al., *Aging Cell 2020*

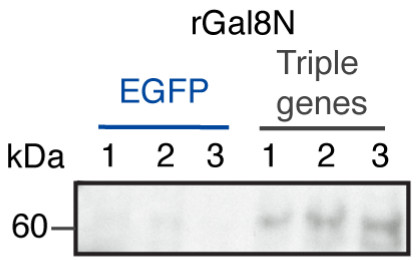
Aging-associated glycogene overexpression leads to an impaired keratinocyte growth

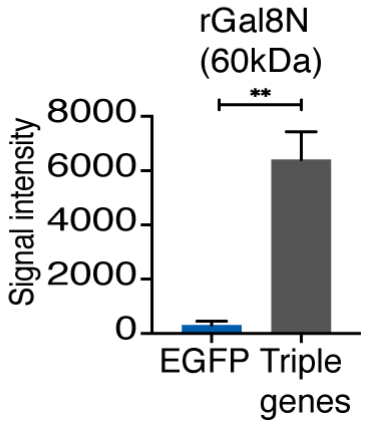


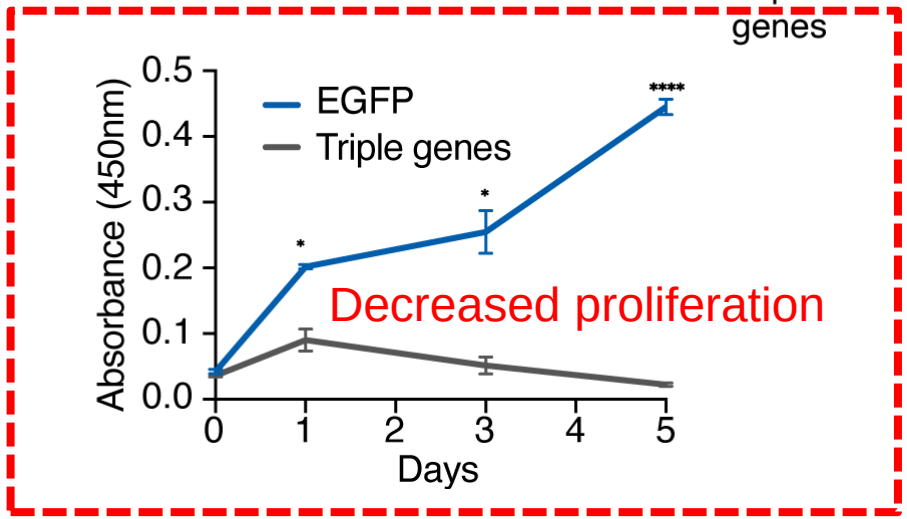
Induction of “aging” type glycosylation





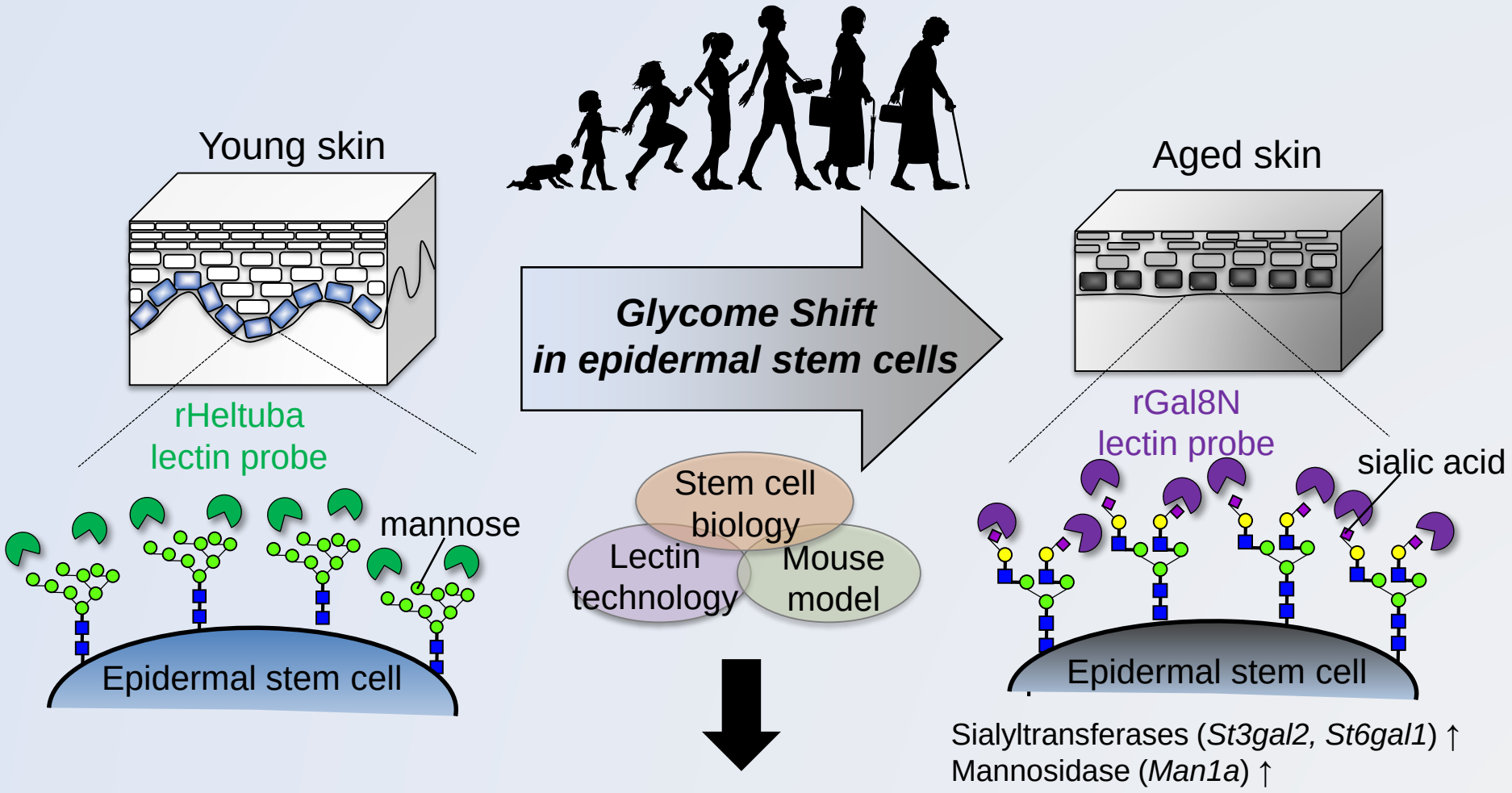






Oinam et al., *Aging Cell 2020*

Summary and future perspective



The identified lectins may serve as a molecular probe for aging
and functional studies will lead to a better understanding of skin and stem cell aging

Oinam et al., *Aging Cell 2020*