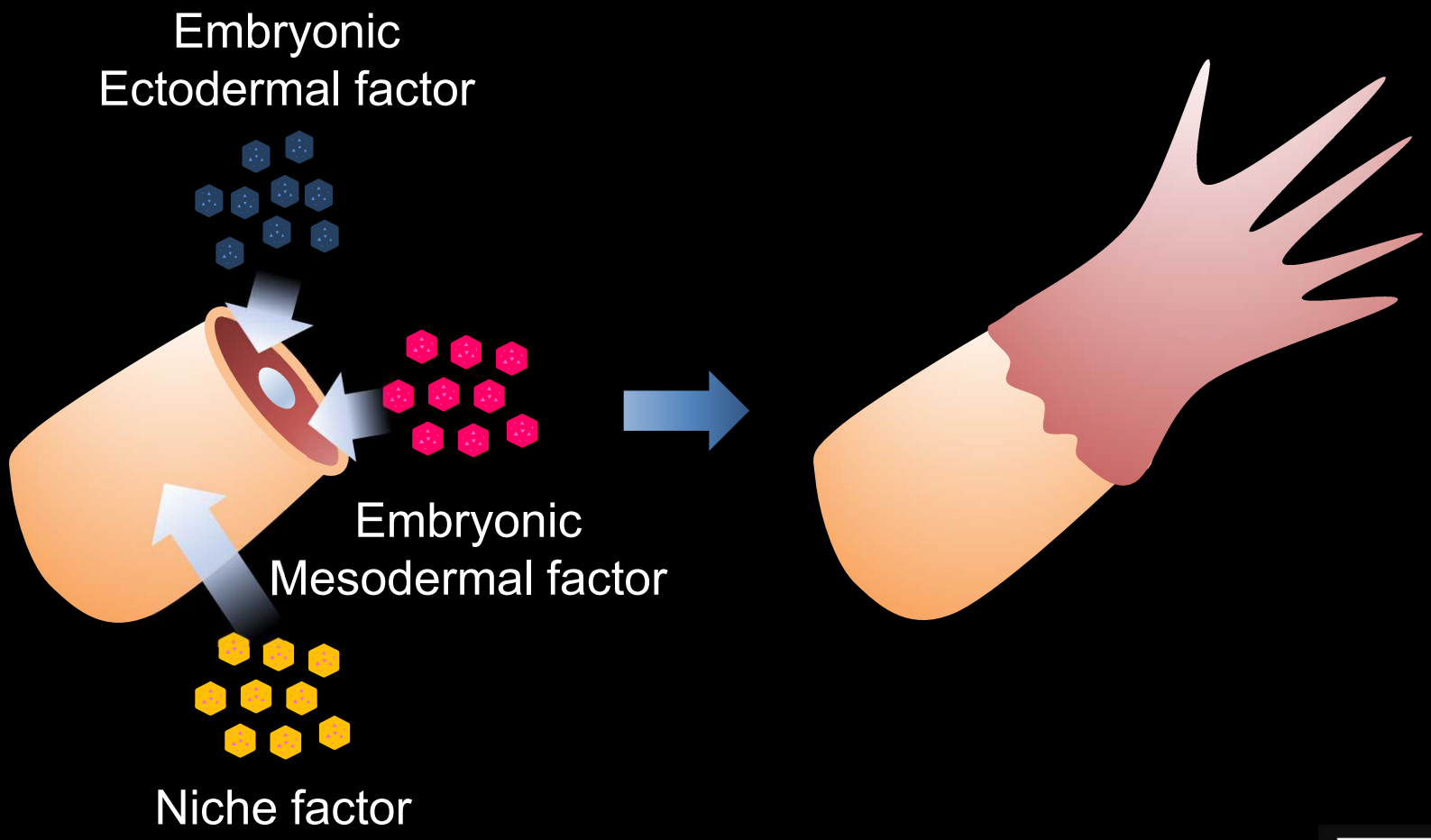
1st international symposium of Moonshot Goal 7

Complex issue regeneration via tissue embryonization

Masakazu KURITA
Department of Plastic Surgery
The University of Tokyo Hospital

Tissue embryonization for Complex tissue regeneration



Limb regeneration

MOONSHOT
RESEARCH & DEVELOPMENT PROGRAM

- Core technologies -

Reprogramming

In vivo gene transduction

Reprogramming
Fibroblasts ⇒ Pluripotent stem cells

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

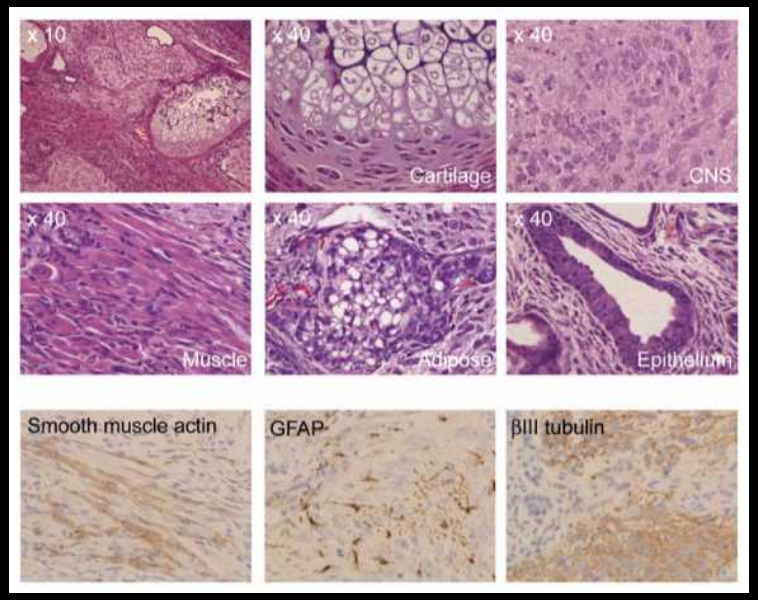
Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024

Vol 448 | 19 July 2007 | doi:10.1038/nature05934 nature

ARTICLES

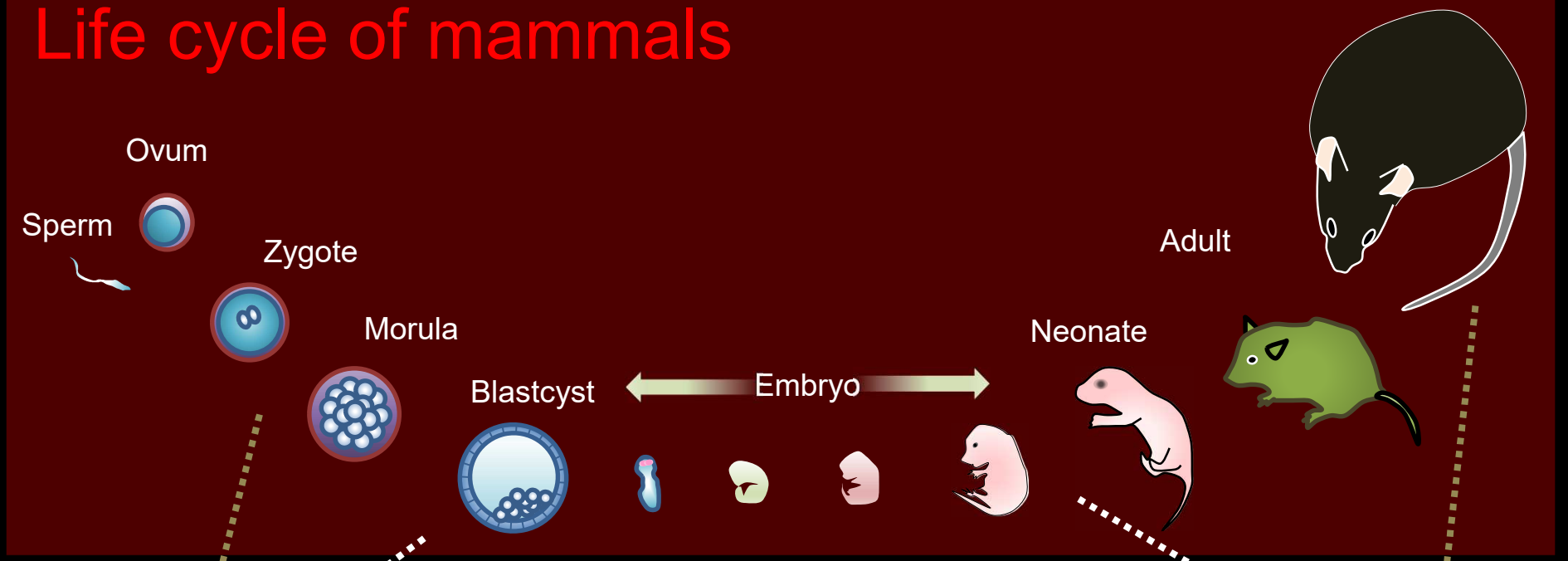
Generation of germline-competent induced pluripotent stem cells

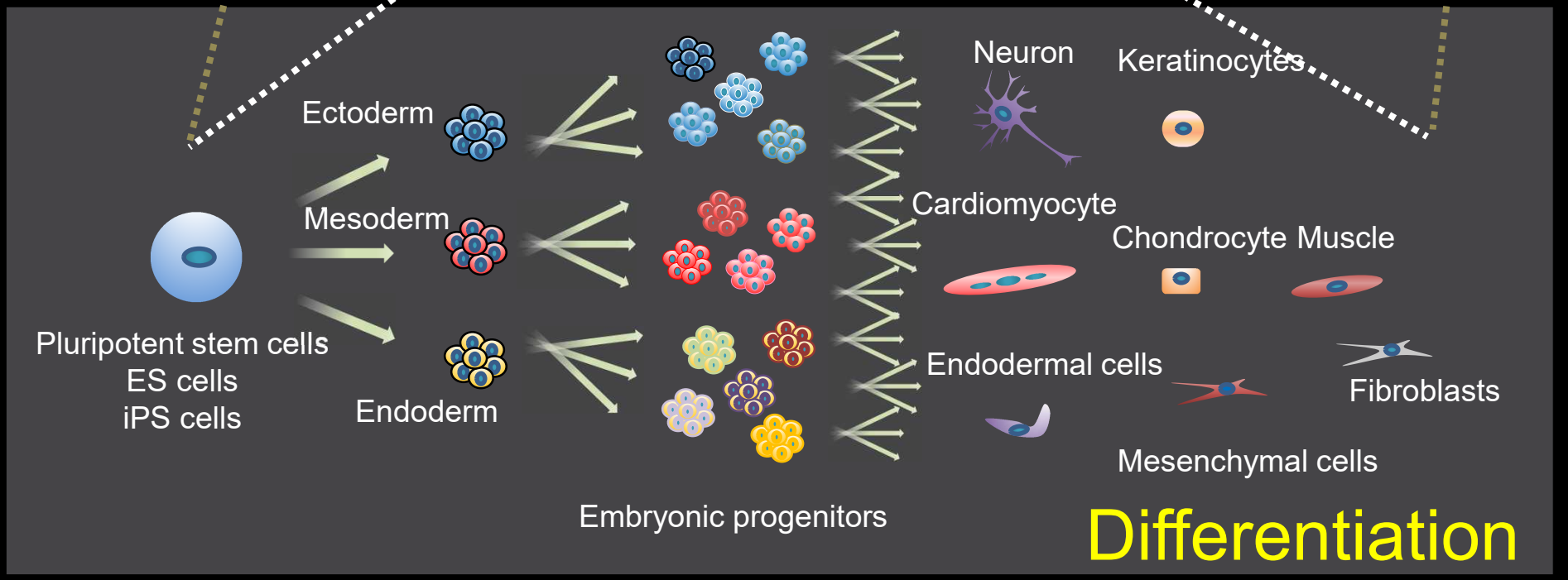
Keisuke Okita[1], Tomoko Ichisaka[1,2] & Shinya Yamanaka[1,2]



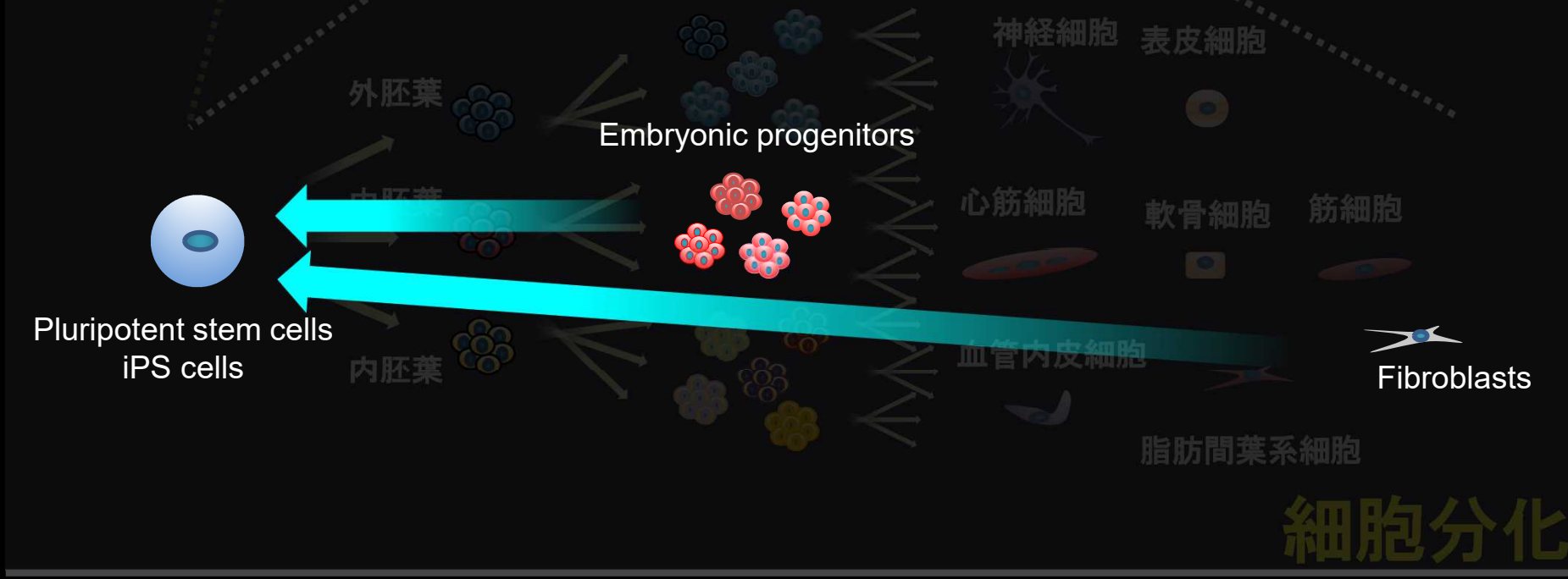
Takahashi & Yamanaka *Cell* 2006
Okita, Ichisaka & Yamanaka *Nature* 2007

Life cycle of mammals





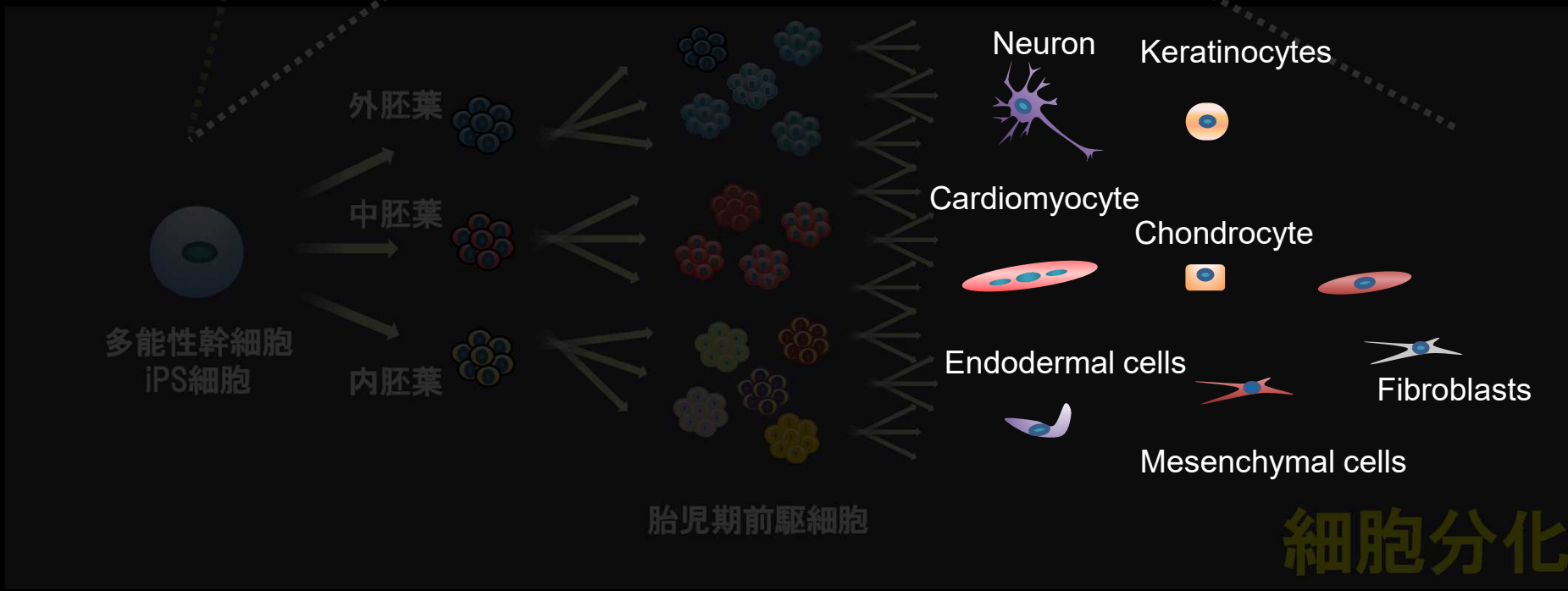
哺乳類の生活環



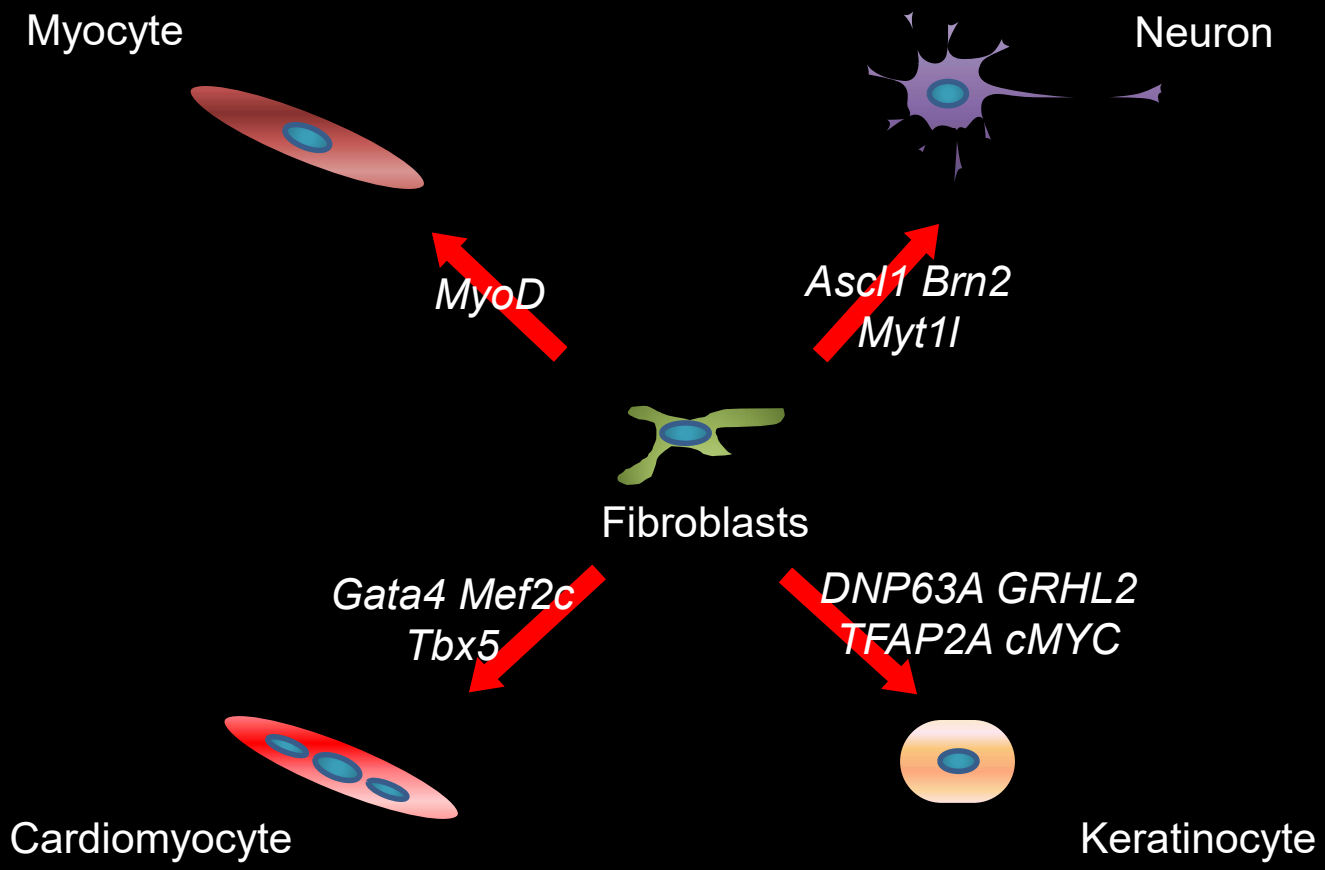
細胞分化

哺乳類の生活環


卵子
精子
受精卵
桑実胚
胚盤胞
胎児
新生児
成体

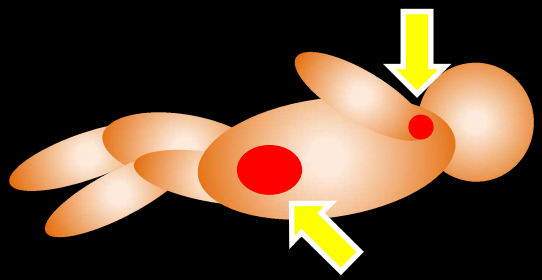

外胚葉
中胚葉
内胚葉
多能性幹細胞
iPS細胞
胎児期前駆細胞
Neuron
Keratinocytes
Cardiomyocyte
Chondrocyte
Endodermal cells
Fibroblasts
Mesenchymal cells
細胞分化

Direct reprogramming
（Direct conversion）

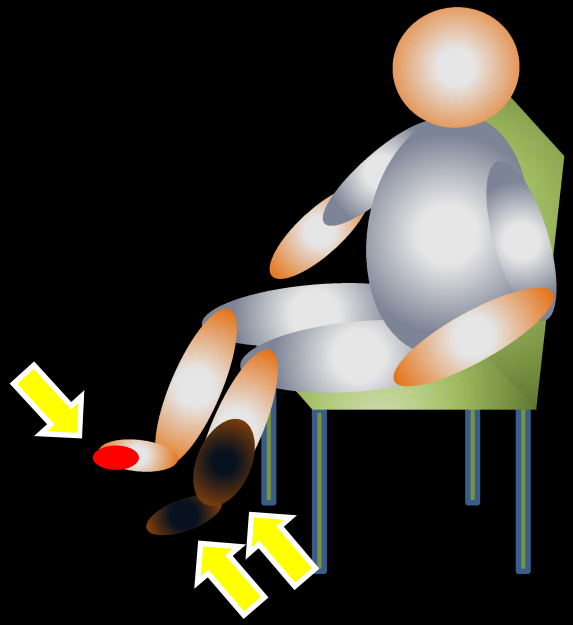


Skin Ulcer

Pressure ulcer



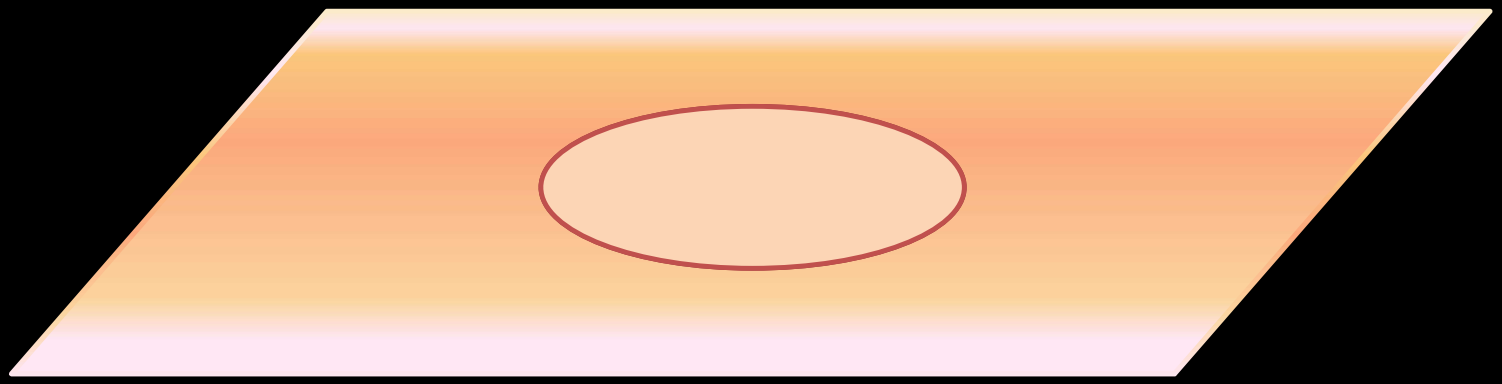
Limb necrosis
（ex. Vascular insufficiency）



Official character of Japan Society of Plastic and Reconstructive Surgery
and Japan Society for Surgical Wound Care
Fairly of Bandage named NAORUN

Possible therapy for ulcers with direct reprogramming

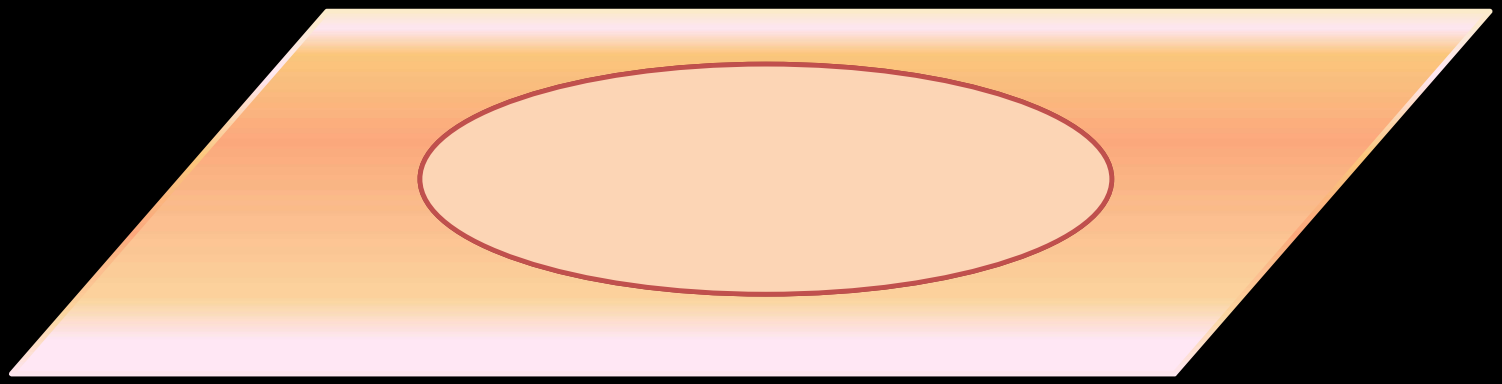
Re-epithelialization can only be attained by migration of surrounding epidermis



Epidermis is regenerated only from epidermis

Possible therapy for ulcers with direct reprogramming

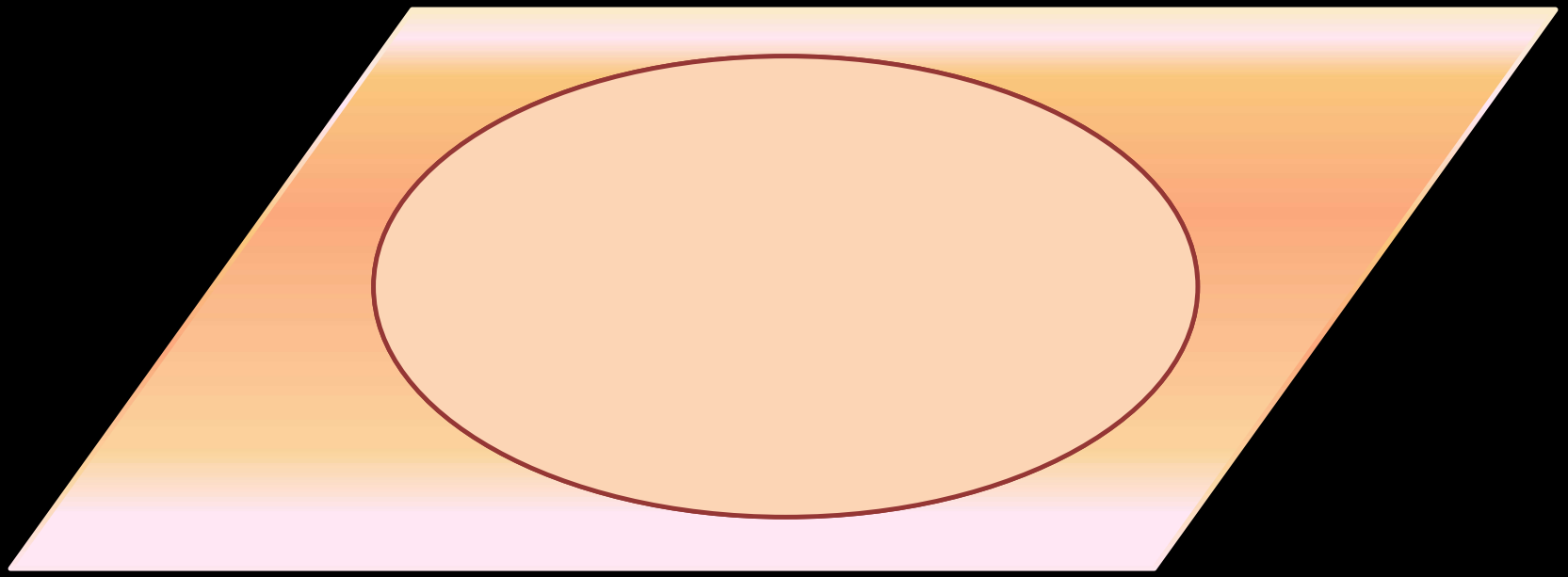
Re-epithelialization can only be attained by migration of surrounding epidermis



Epidermis is regenerated only from epidermis

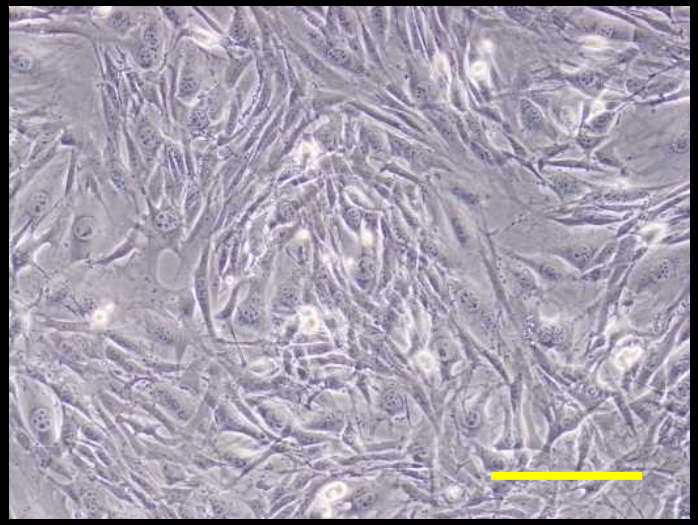
Possible therapy for ulcers with direct reprogramming

Mesenchymal cells to epidermal cells ?



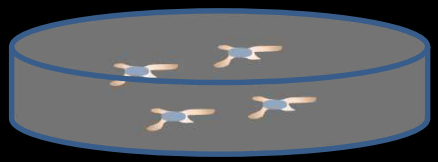
Identification of reprogramming factors

Fibroblasts



Keratinocytes

200um



Fibroblasts

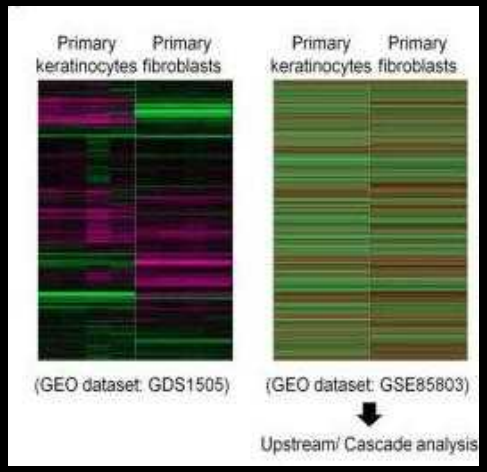
Gene transduction

?

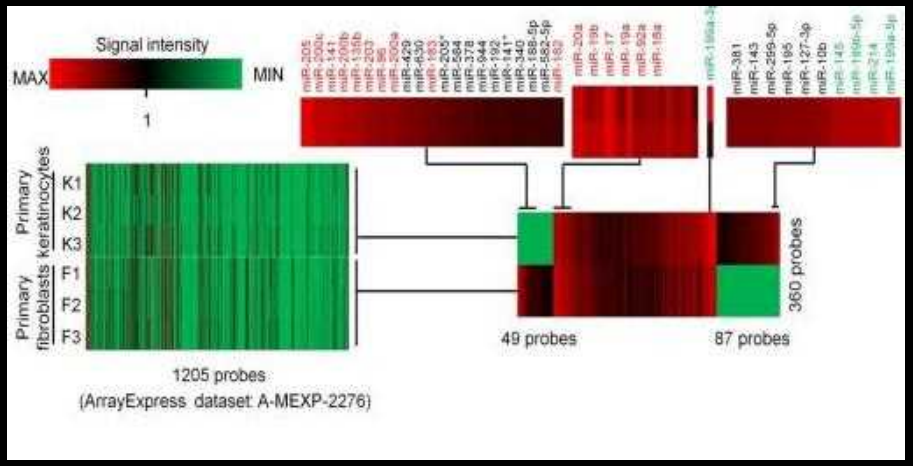
Kurita et al. *Nature*. 2018;561:243-247. modified

Identification of reprogramming factors

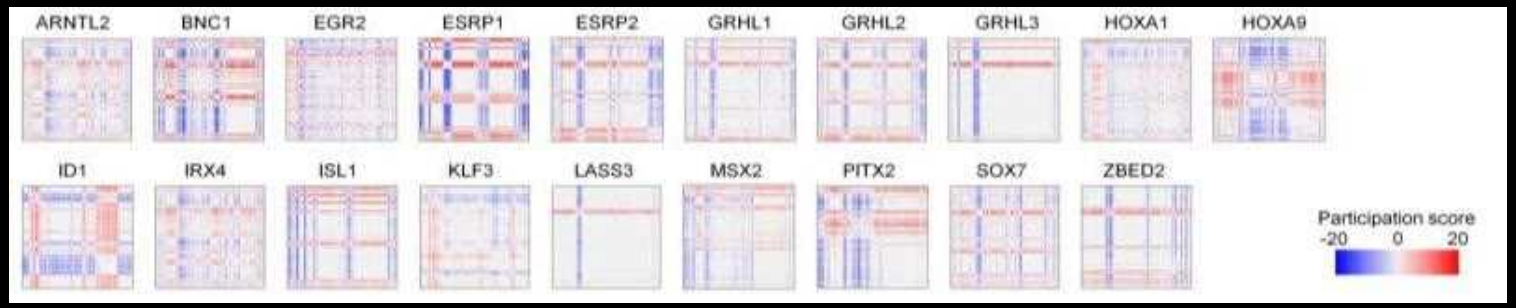
Microarray



Micro RNA microarray



Gene expression reversal analysis



55 transcription factors
31 microRNAs

Kurita et al. *Nature*. 2018;561:243-247. modified

Gene transduction experiments(～200 sets)

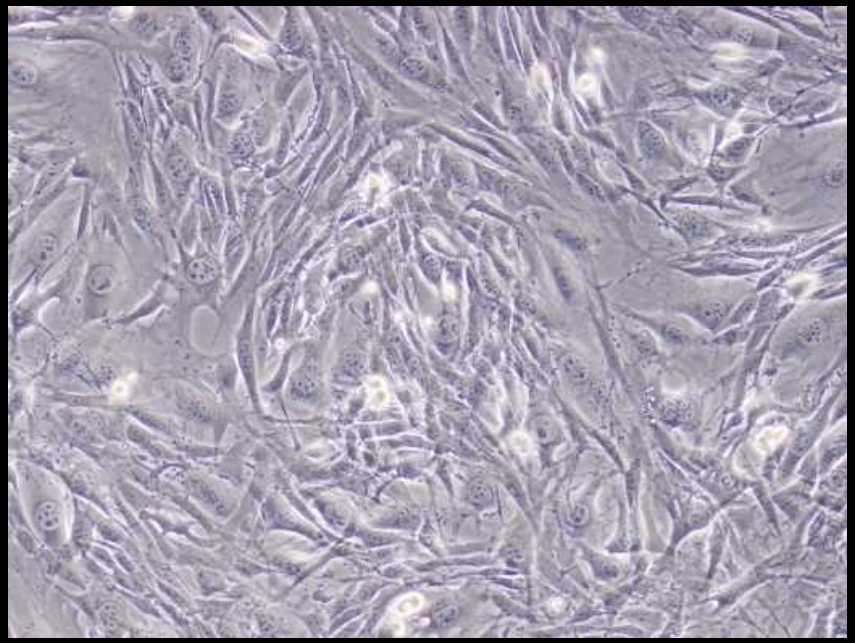
58 TFs

31 miRs

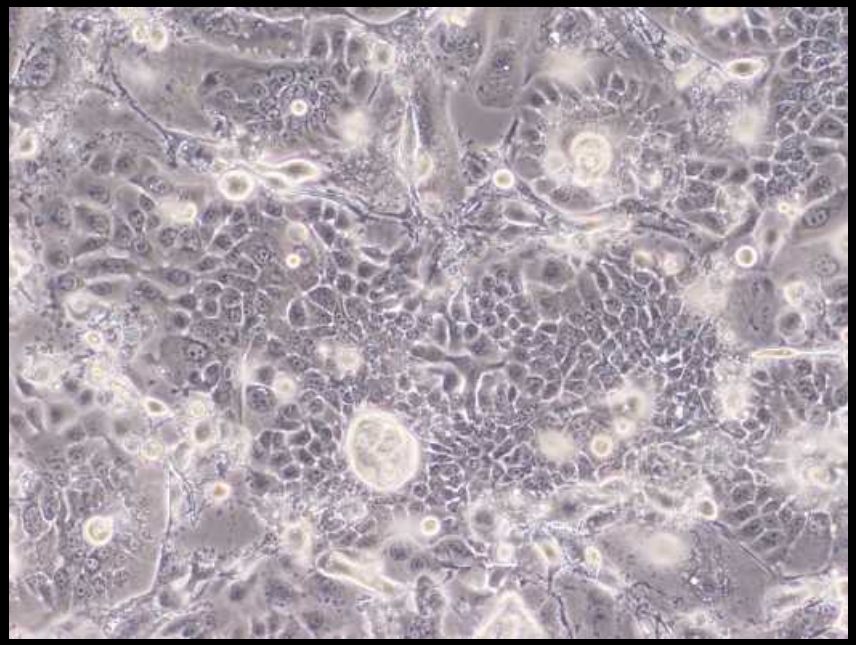
Reporter

Y2012 Y2013 Y2014

Dermal fibroblasts



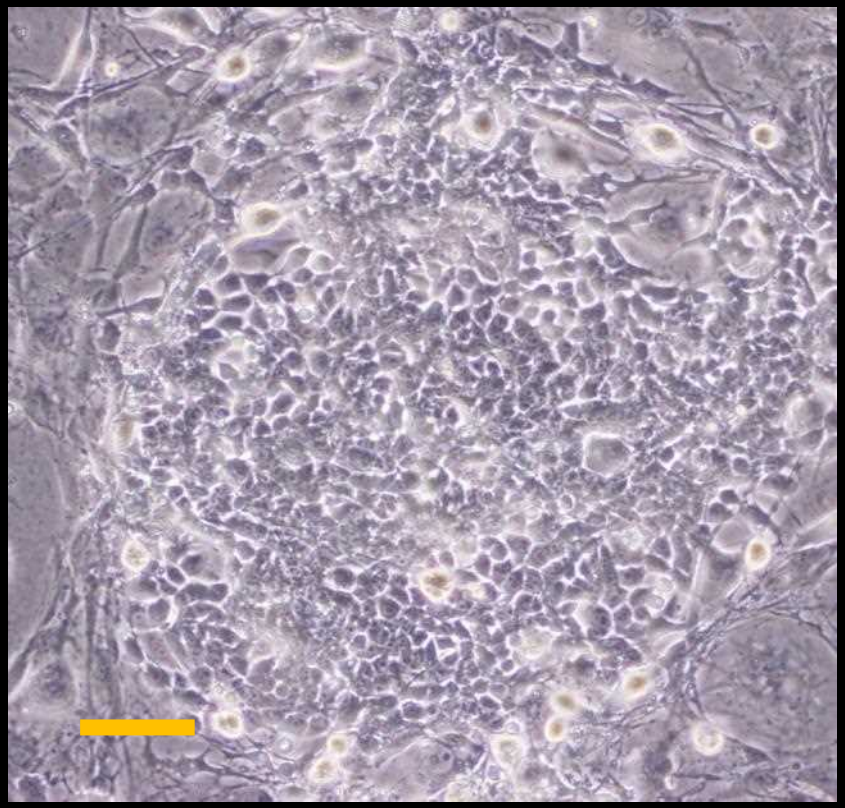
Keratinocytes

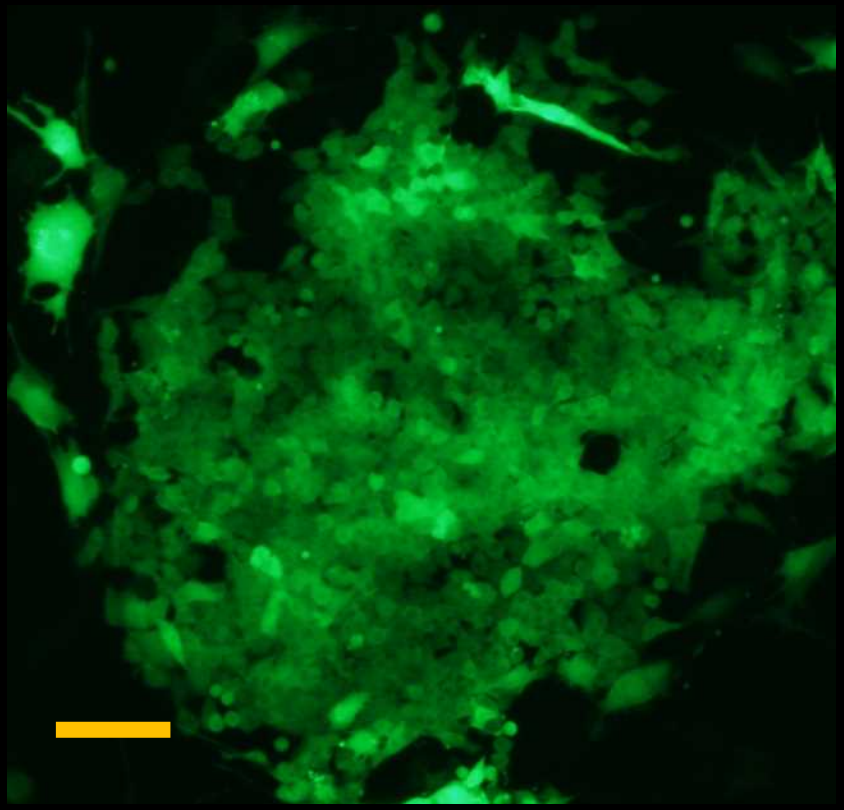


Kurita et al. *Nature*. 2018;561:243-247. modified

18 genes

200 μm

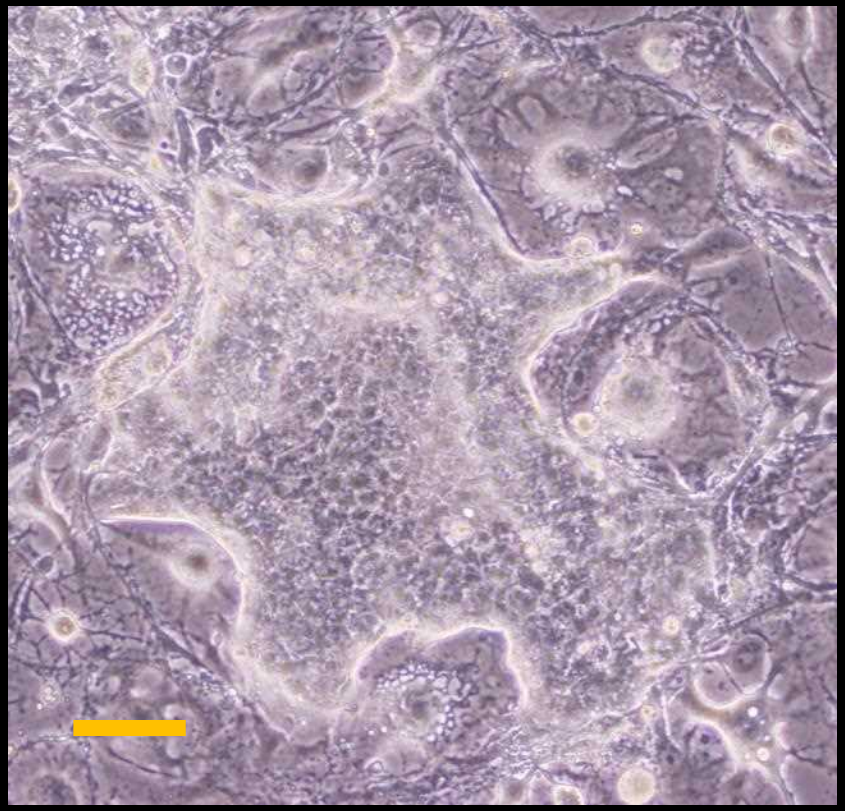


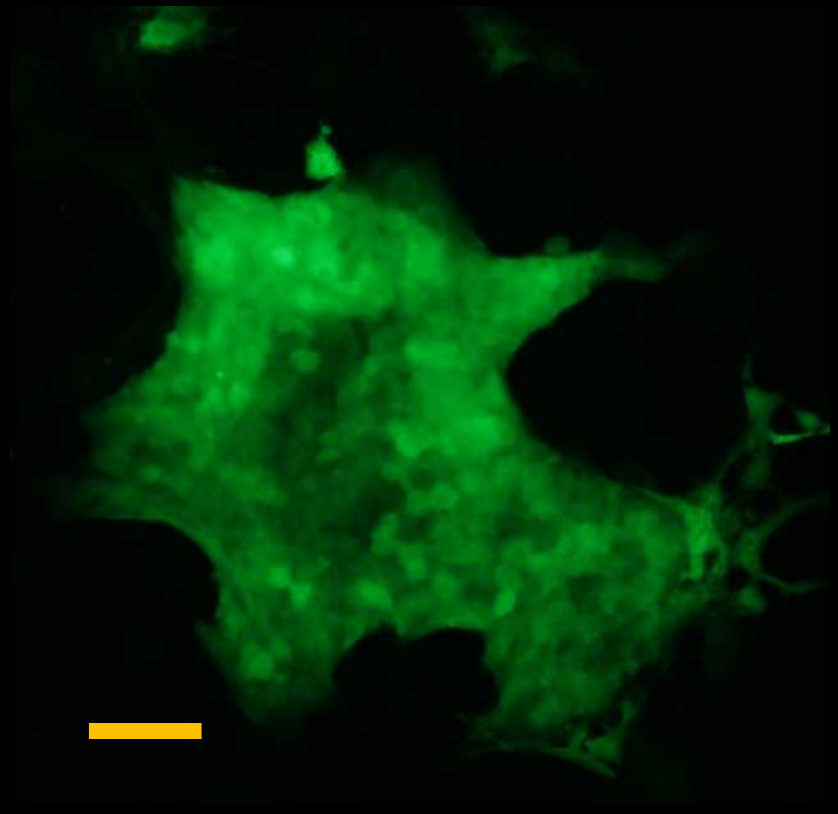


Jan 14 2014

Kurita et al. *Nature*. 2018;561:243-247. modified

17 genes — 200 μm



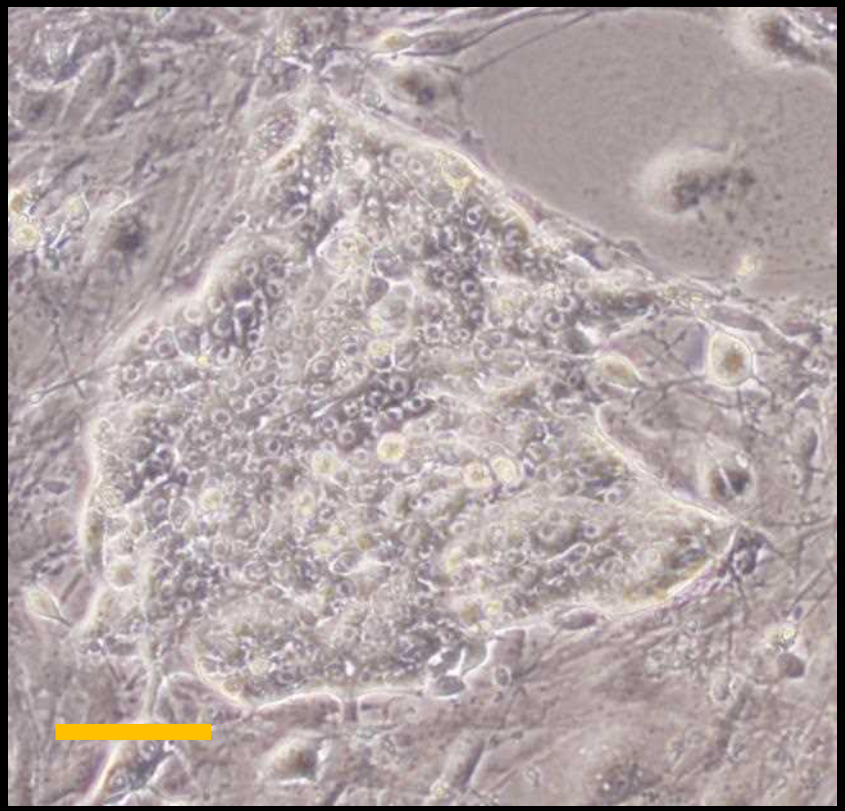


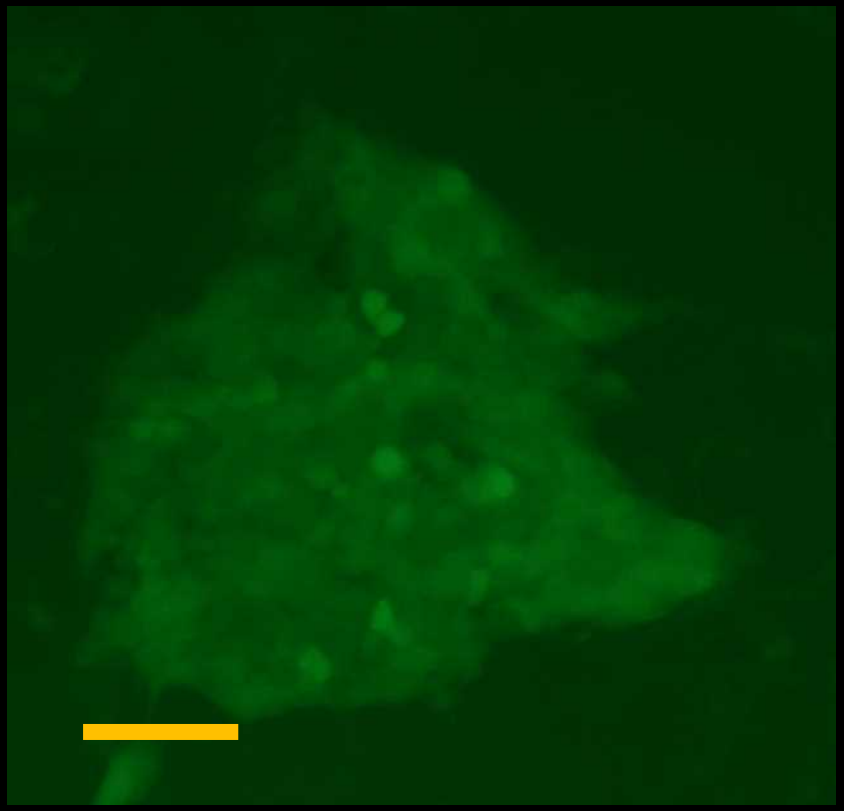
Feb 27 2014

Kurita et al. *Nature*. 2018;561:243-247. modified

42 genes

200 μm



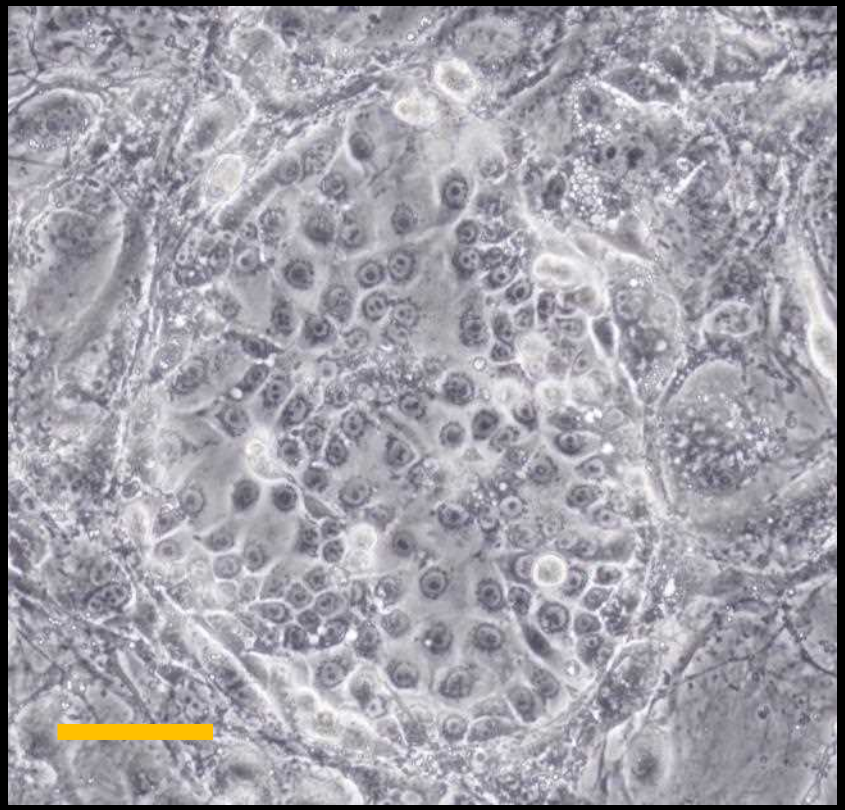


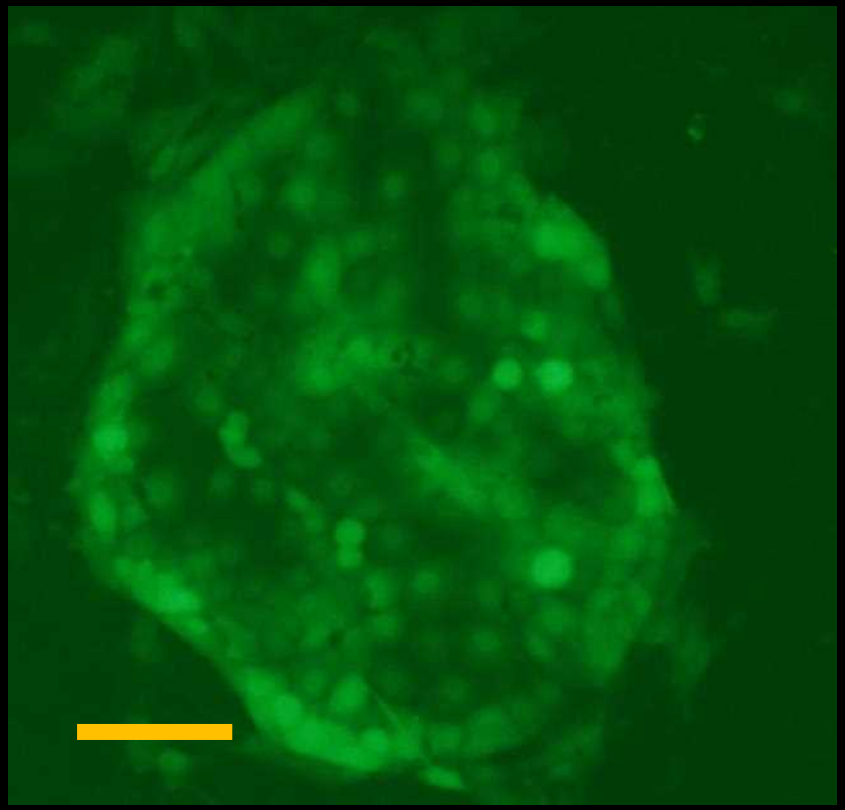
May 31 2014

Kurita et al. *Nature*. 2018;561:243-247. modified

42 genes

200 μm



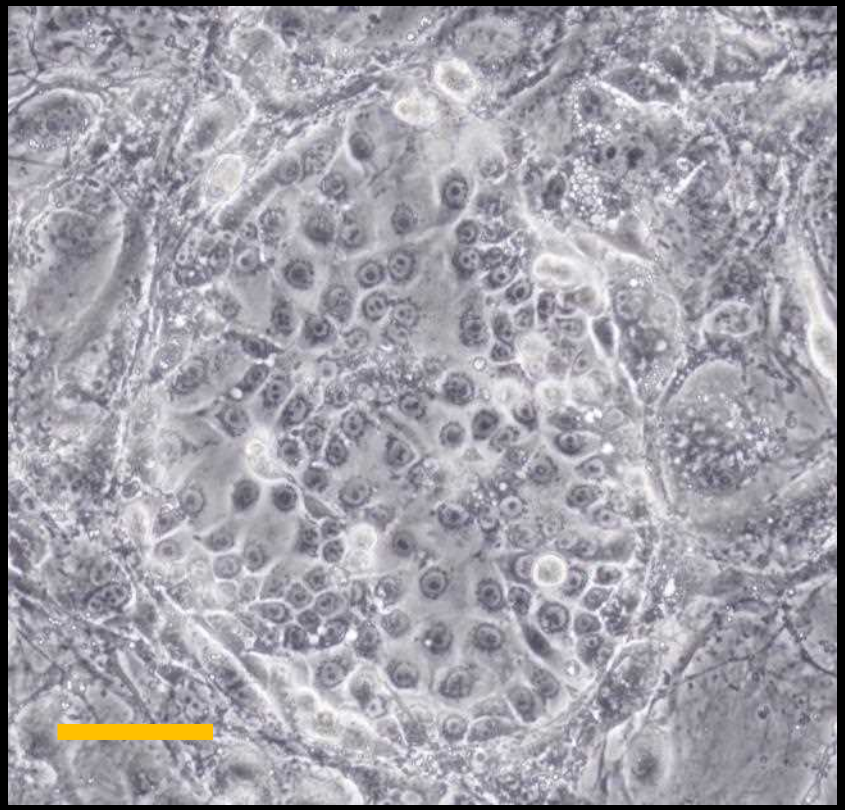


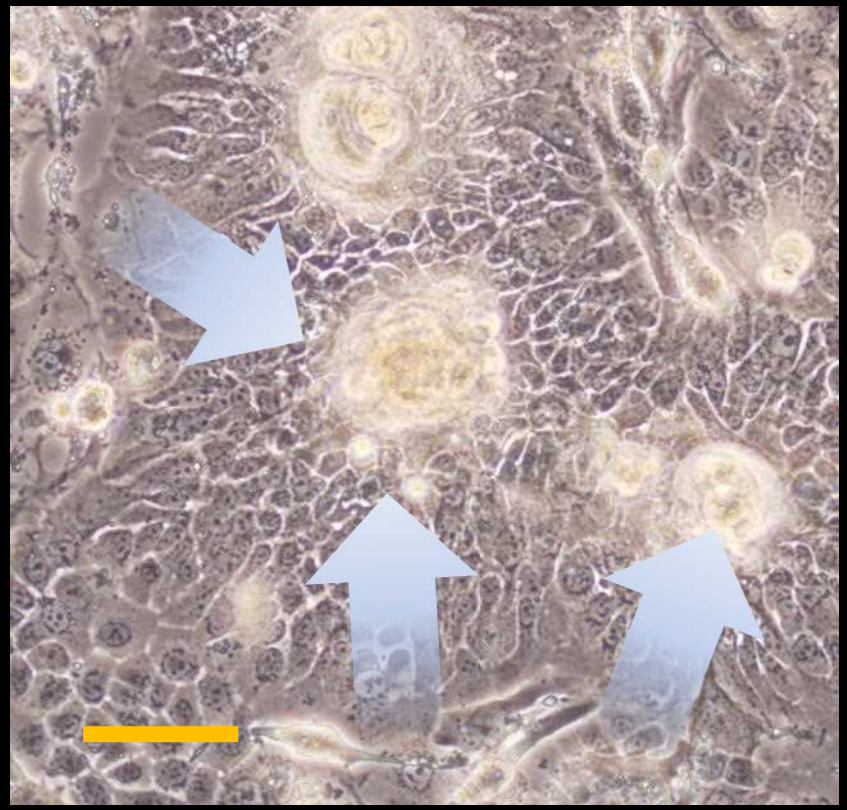
July 9 2014

Kurita et al. *Nature*. 2018;561:243-247. modified

42 genes

200 μm

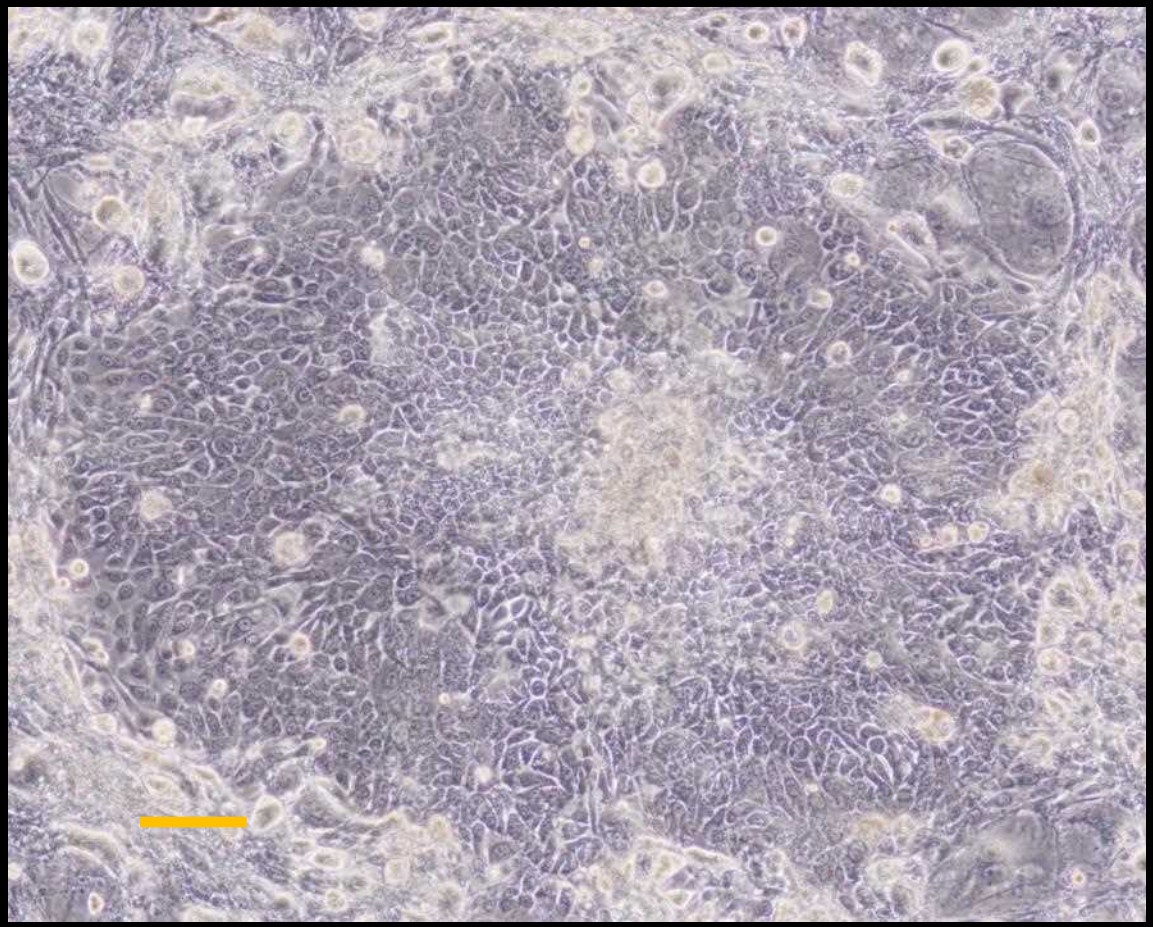




keratinocytes

Kurita et al. *Nature*. 2018;561:243-247. modified

28 genes



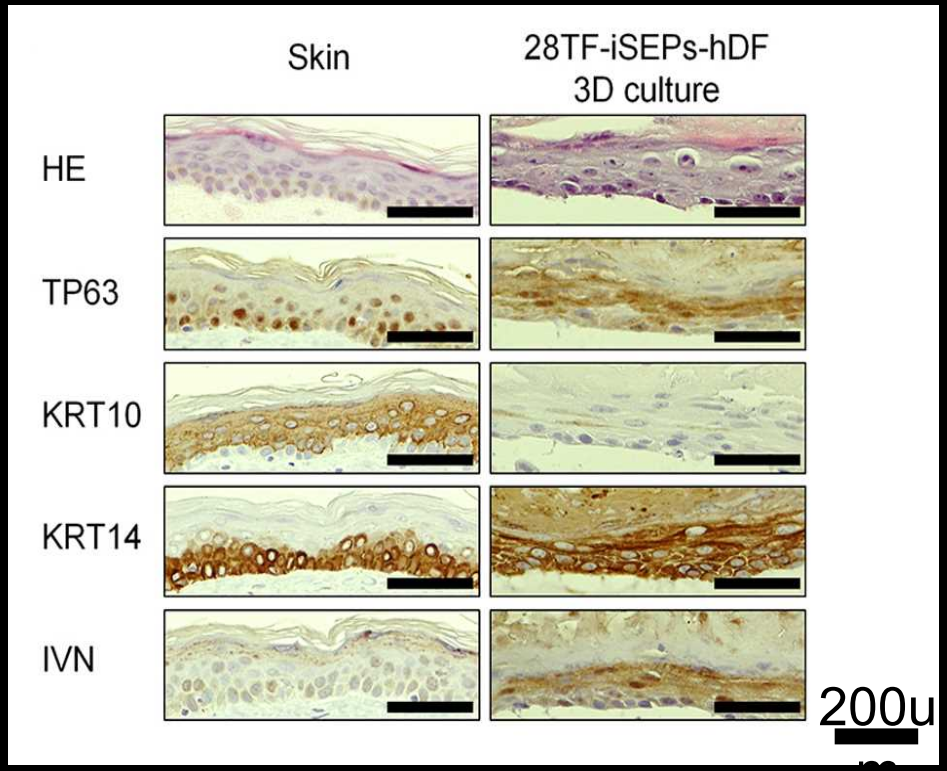
200 μm

Sep 12 2014

Kurita et al. *Nature*. 2018;561:243-247. modified

28 genes

3D culture



iSEPs
induced Stratified Epithelium Progenitors

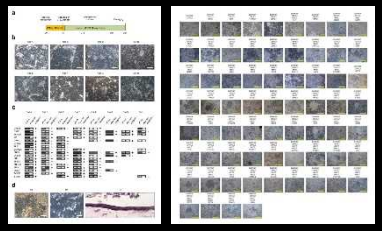
Kurita et al. *Nature*. 2018;561:243-247. modified

Identification of optimized factors

Cell/ colony shape
Efficiency
Proliferation
3D structure

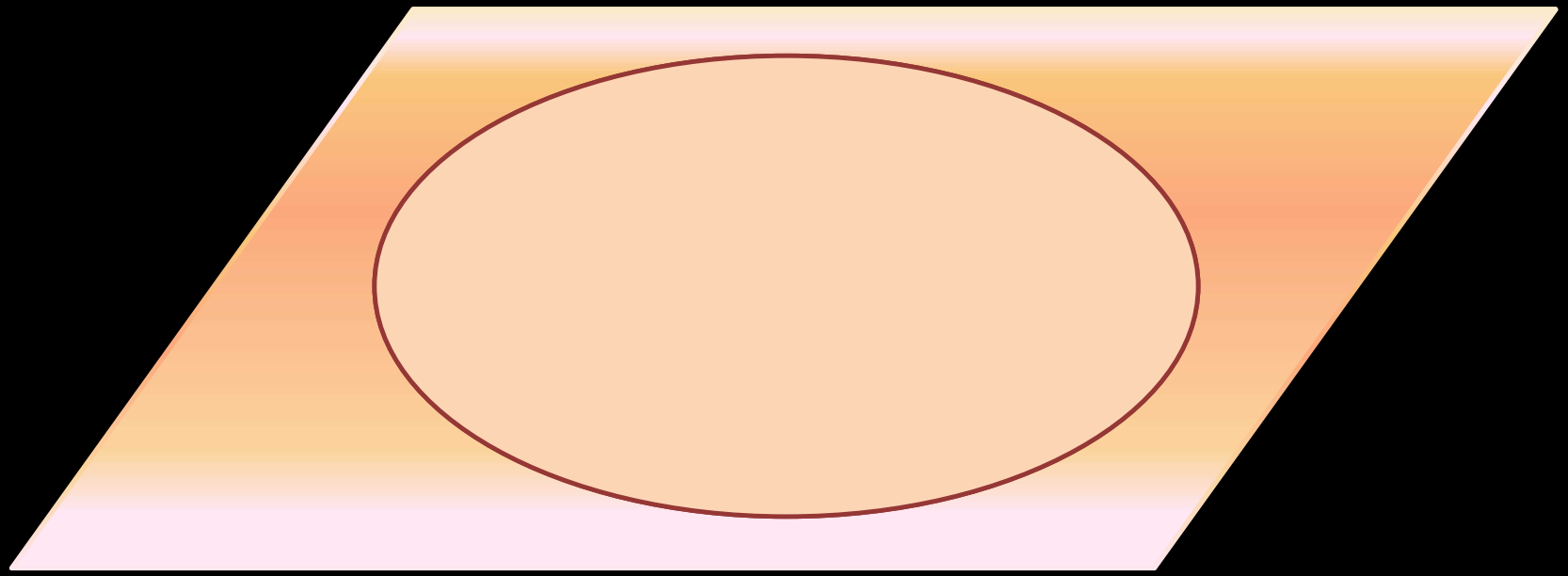
28 genes

DNP63A
GRHL2
TFAP2A
c-MYC

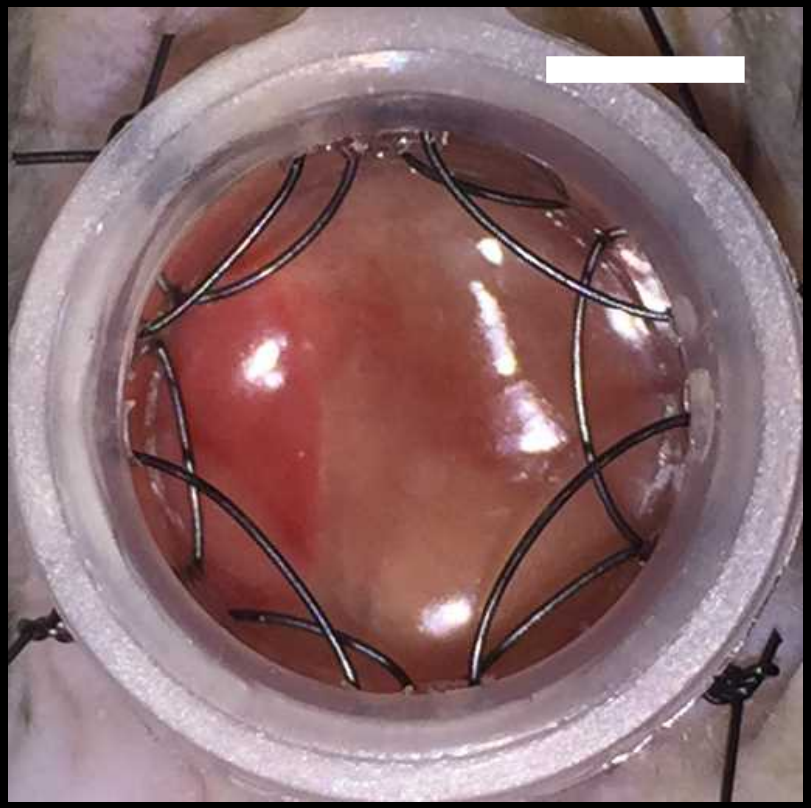


DGTM factors

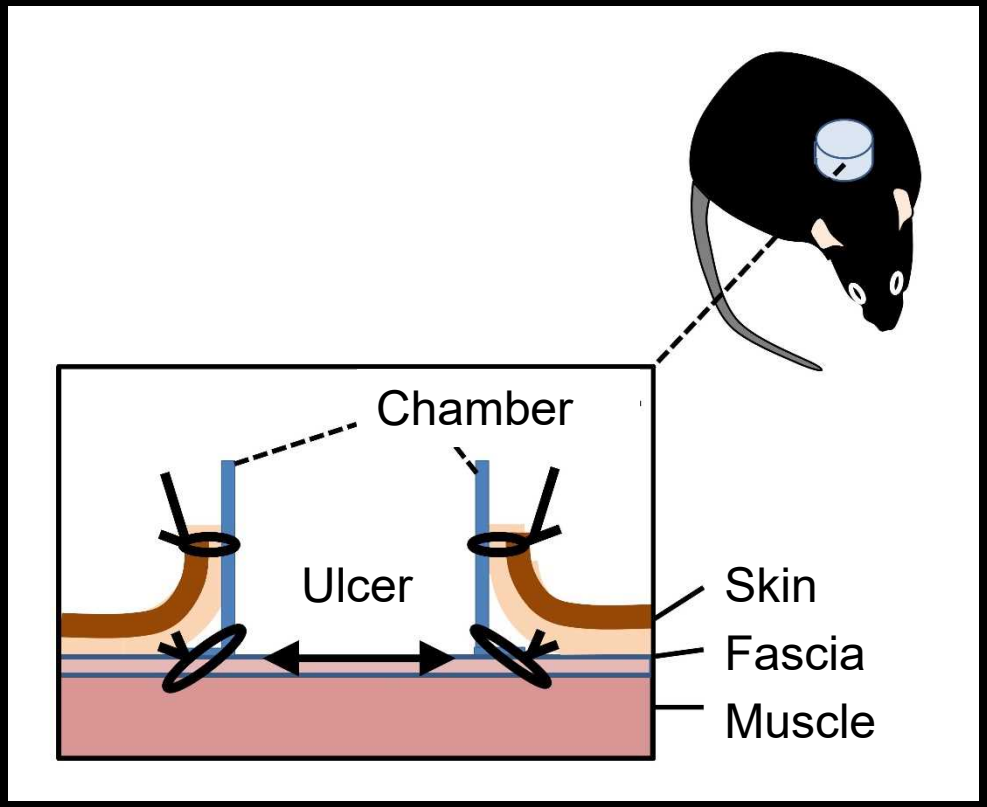
Kurita et al. *Nature*. 2018;561:243-247. modified



Isolated skin ulcer model



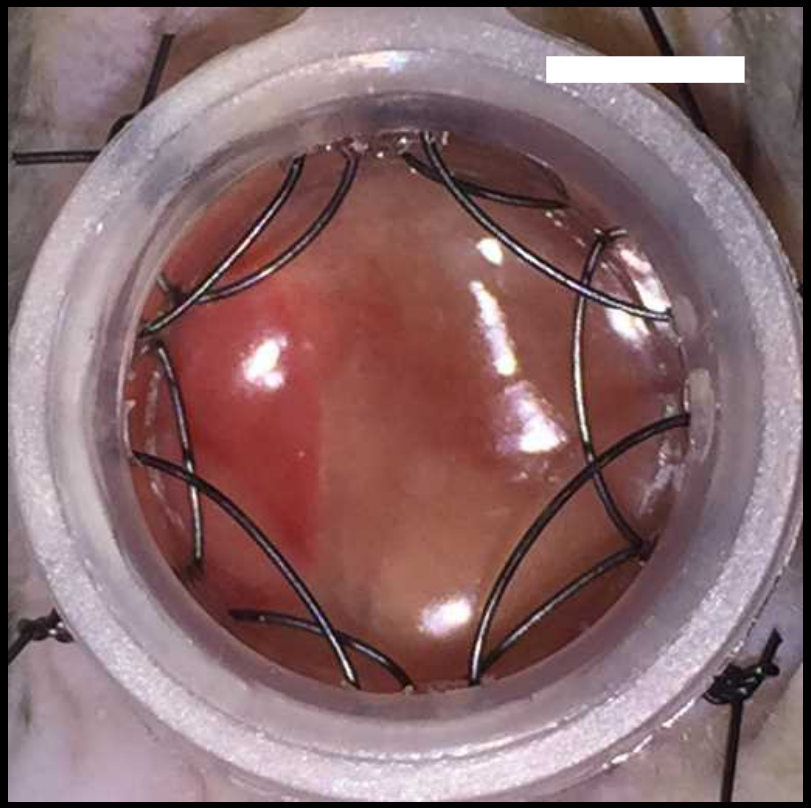
3mm

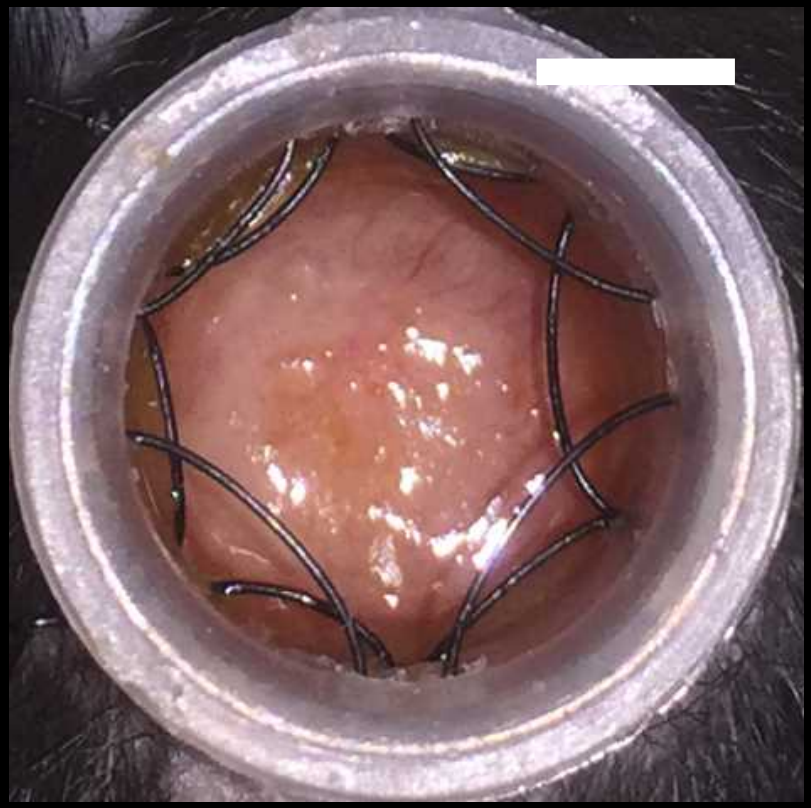


Kurita et al. *Nature*. 2018;561:243-247. modified

Isolated skin ulcer model

DAY28





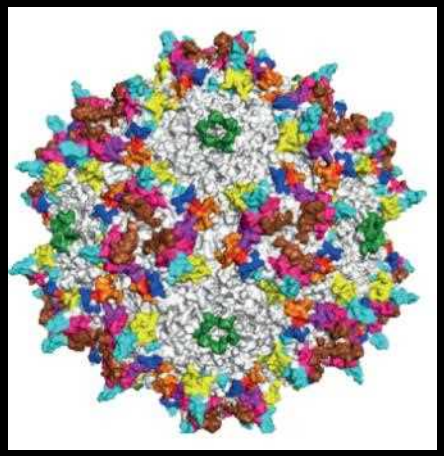
3mm

(n=20, no exception)

Kurita et al. *Nature*. 2018;561:243-247. modified

DGTM transduction

AAV: Adeno-associated virus vector



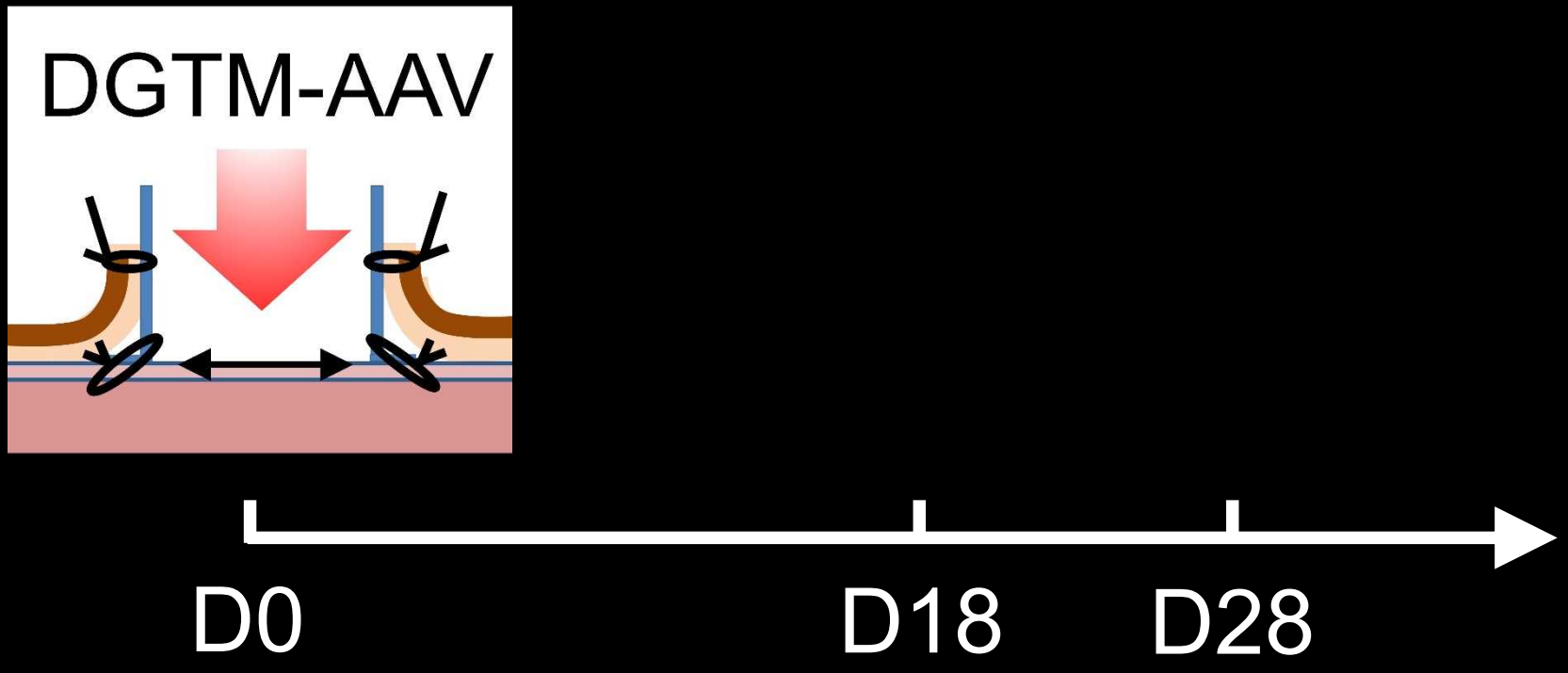
Kotterman et al.
Nat Rev Genet.
2014 ;15(7):445-51.

1) The strongest vector for *in vivo* gene transduction

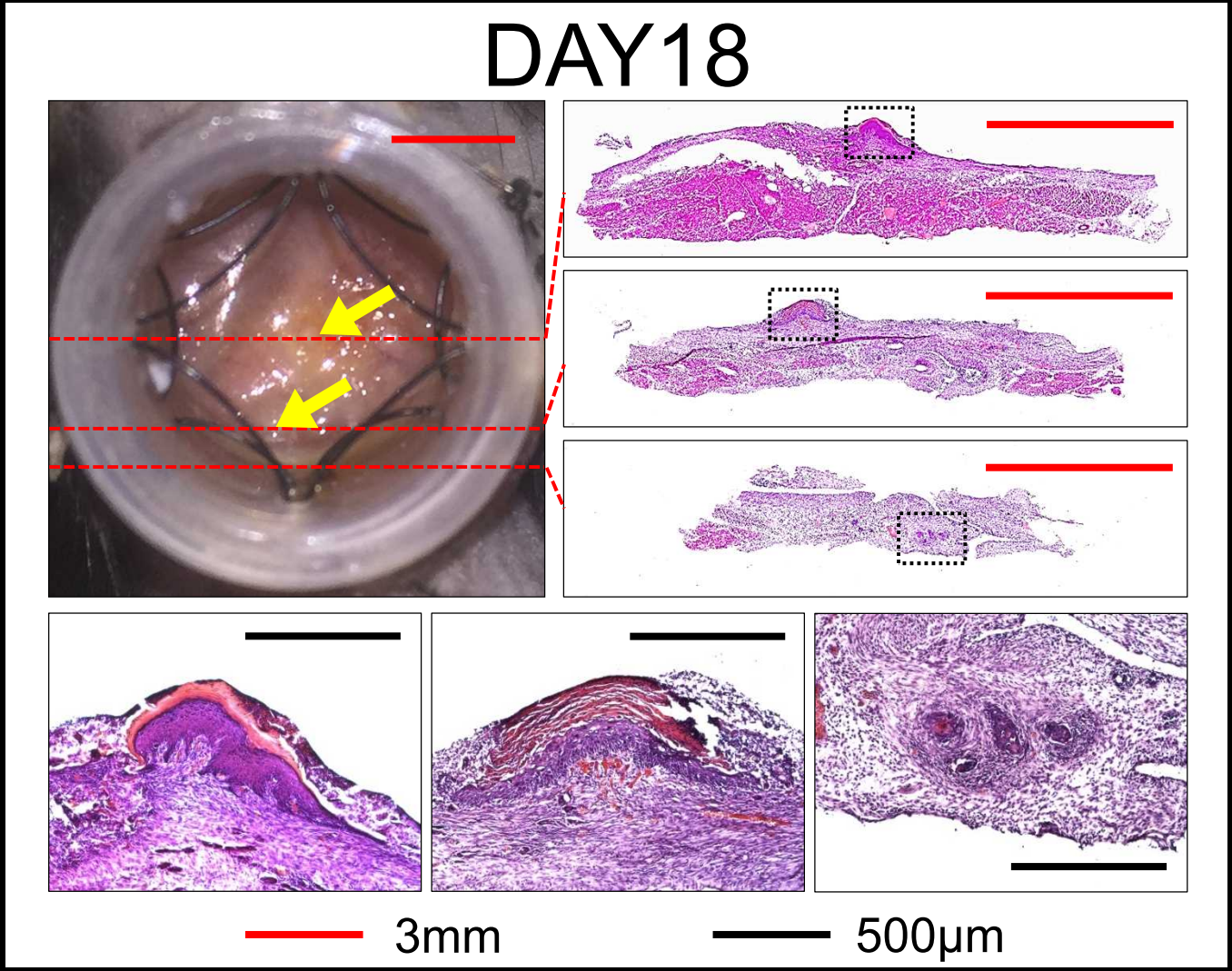
2) Relatively safe and clinically utilized

DGTM transduction

AAV: Adeno-associated virus vector

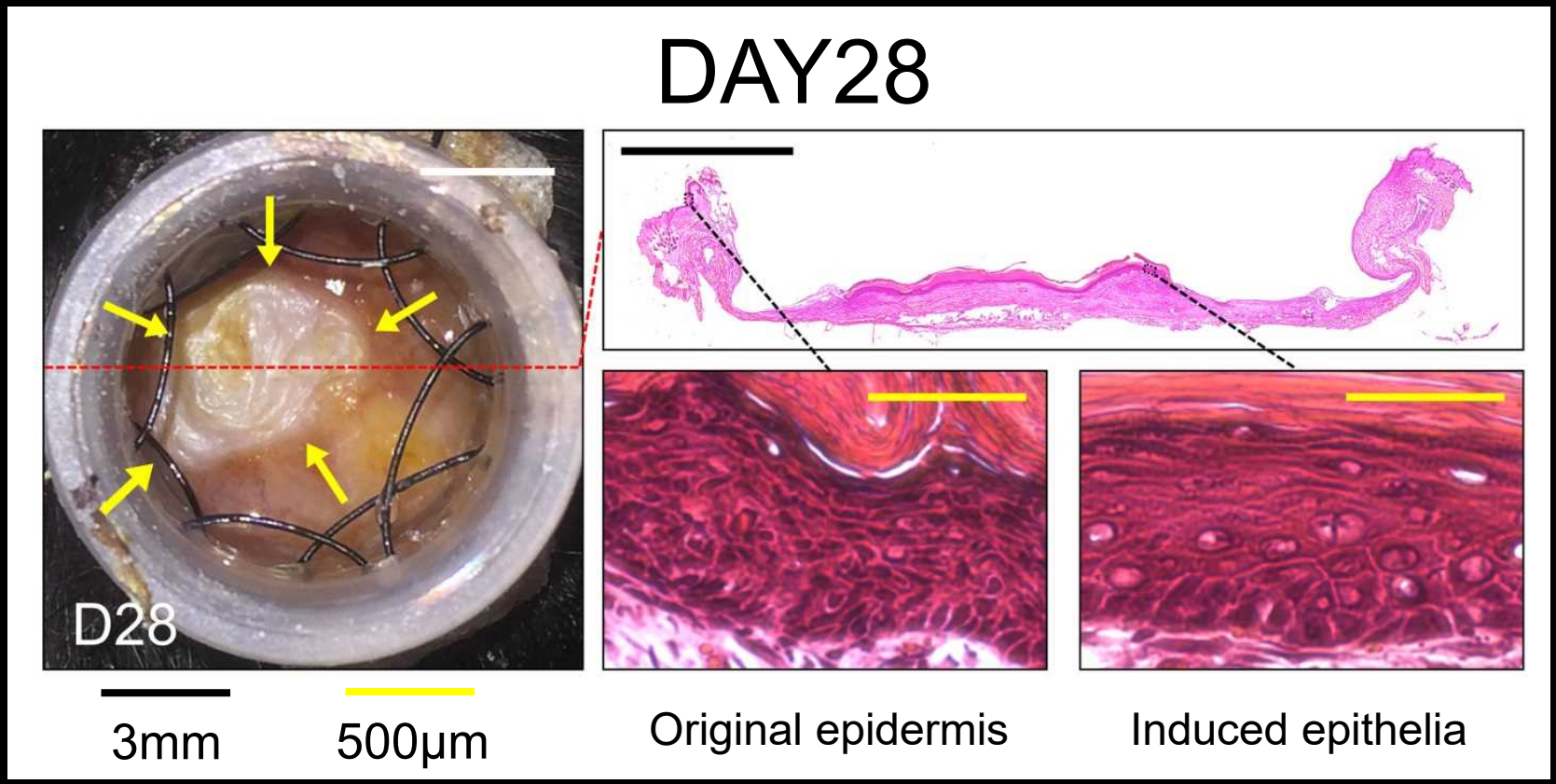


Induction of stratified epithelia with DGTM transduction



Kurita et al. *Nature*. 2018;561:243-247. modified

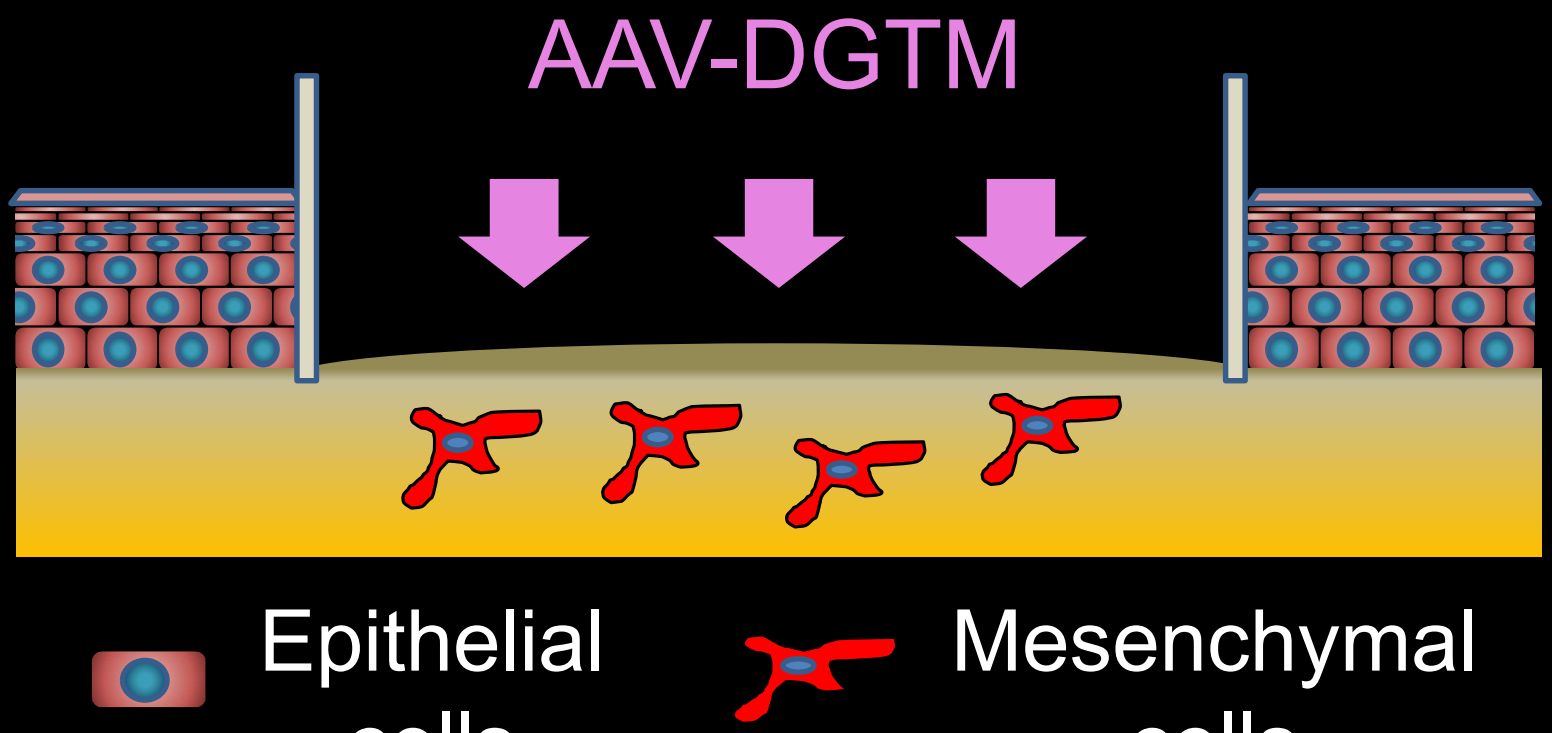
Induction of stratified epithelia with DGTM transduction



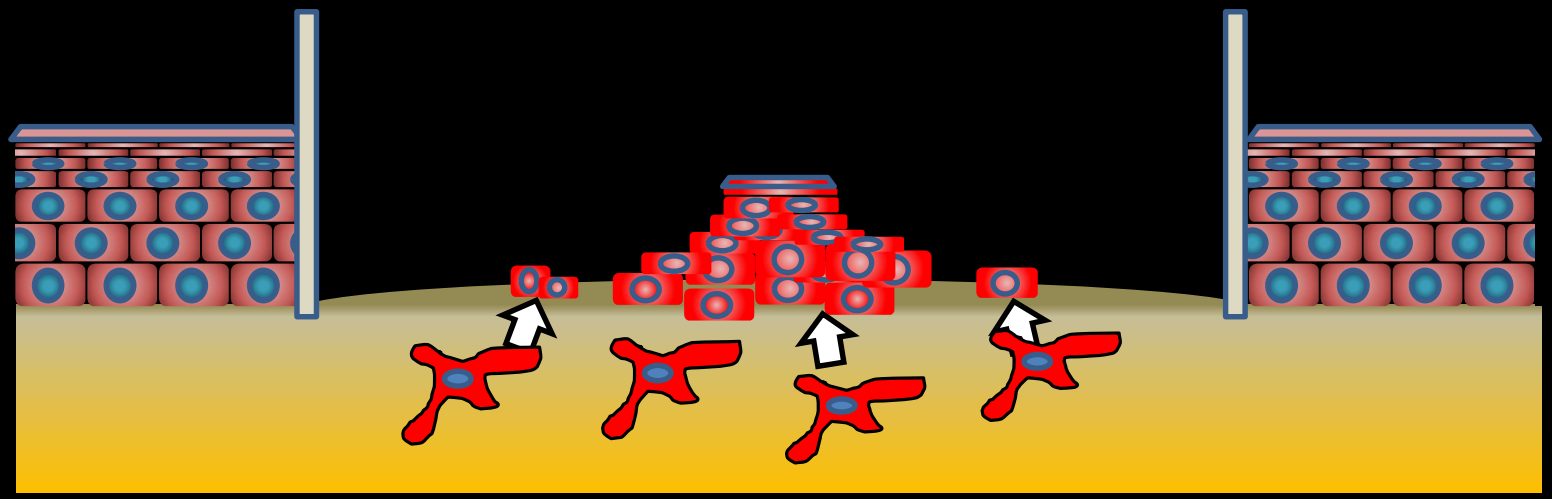
(n=21, no exception)

Kurita et al. *Nature*. 2018;561:243-247. modified

Induction of epithelial tissue via direct reprogramming



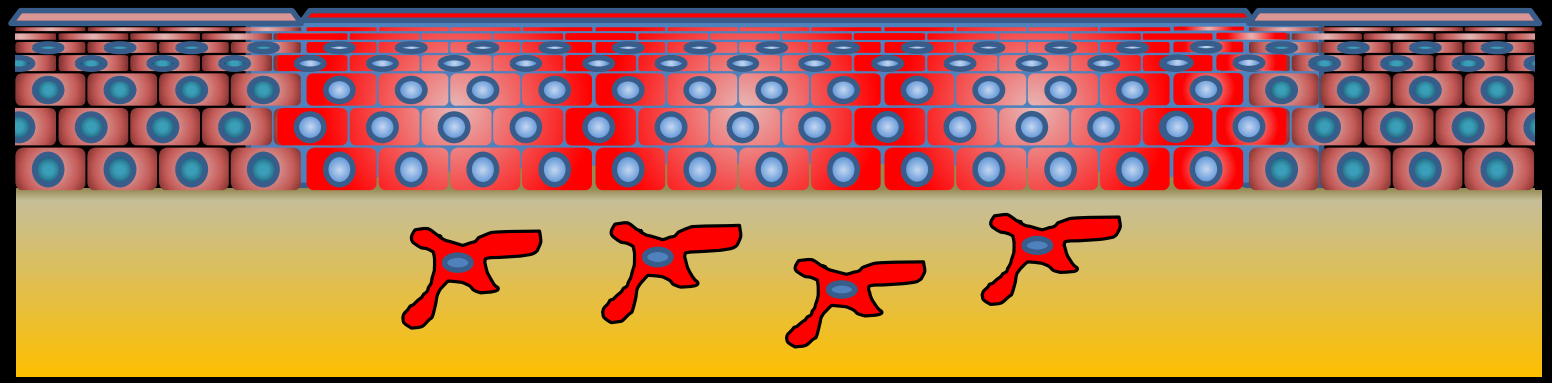
Induction of epithelial tissue via direct reprogramming



Epithelial cells

Mesenchymal cells

Induction of epithelial tissue via direct reprogramming



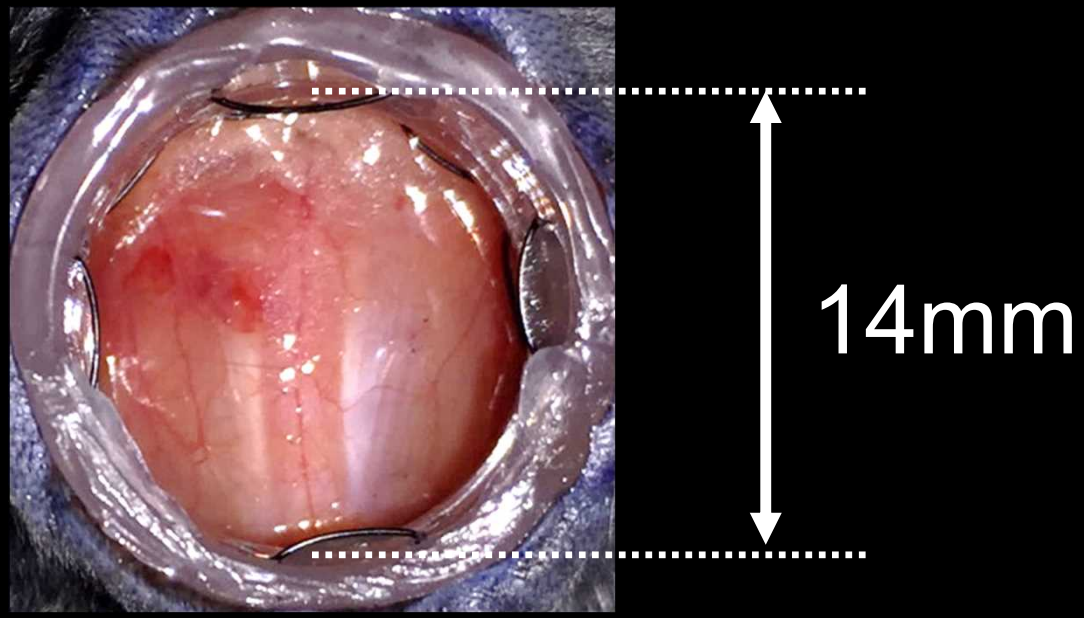
Epithelial cells

Mesenchymal cells

With promotion of epithelialization

D0 : AAV-DGTM + collagen
D4- : FGF2 + Rock inhibitor

D0

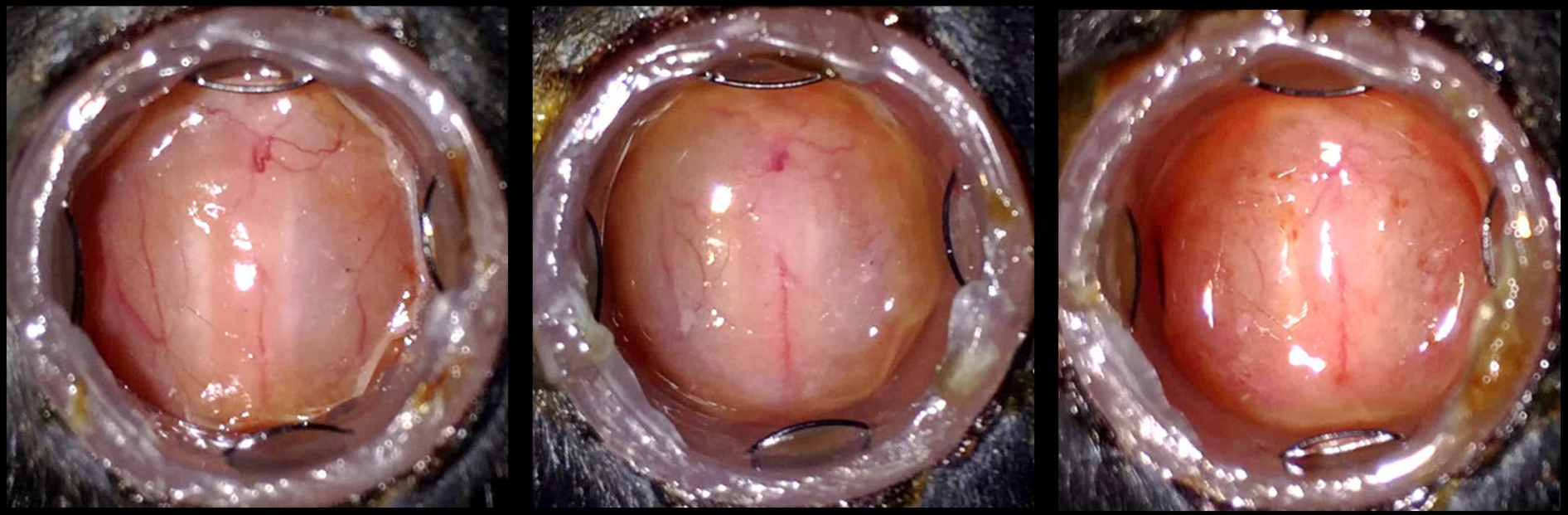


Kurita et al. *Nature*. 2018;561:243-247. modified

With promotion of epithelialization

D0 : AAV-DGTM + collagen
D4- : FGF2 + Rock inhibitor

D4 D5 D6

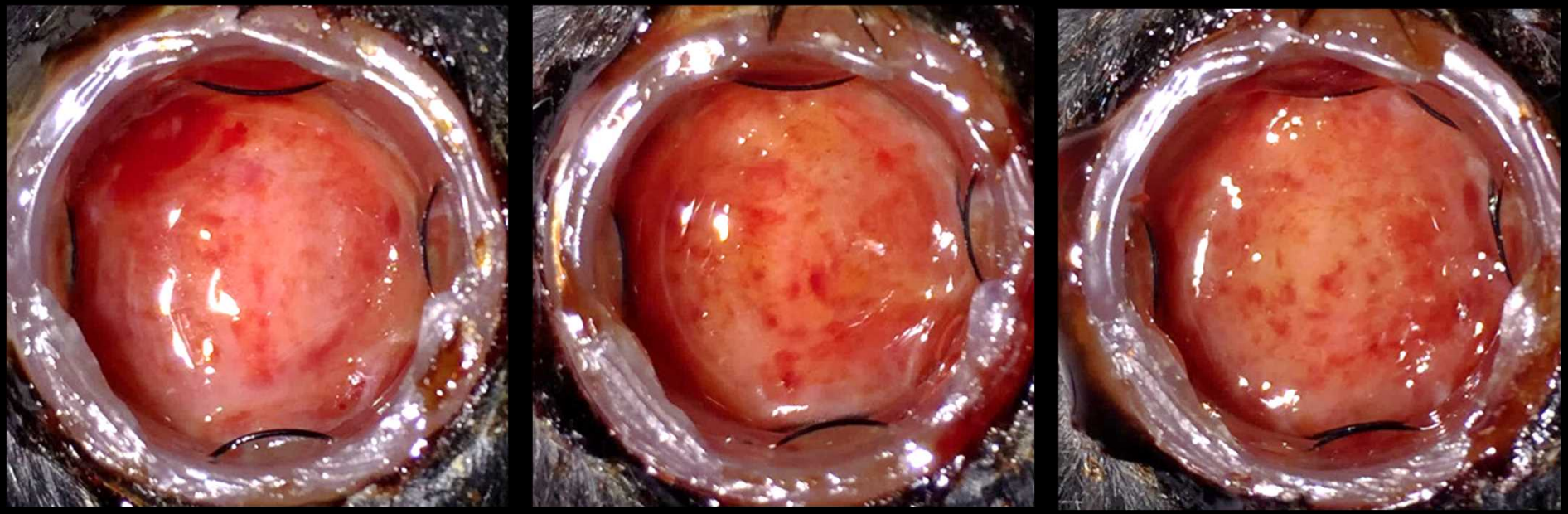


Kurita et al. *Nature*. 2018;561:243-247. modified

With promotion of epithelialization

D0 : AAV-DGTM + collagen
D4- : FGF2 + Rock inhibitor

D7 D8 D9

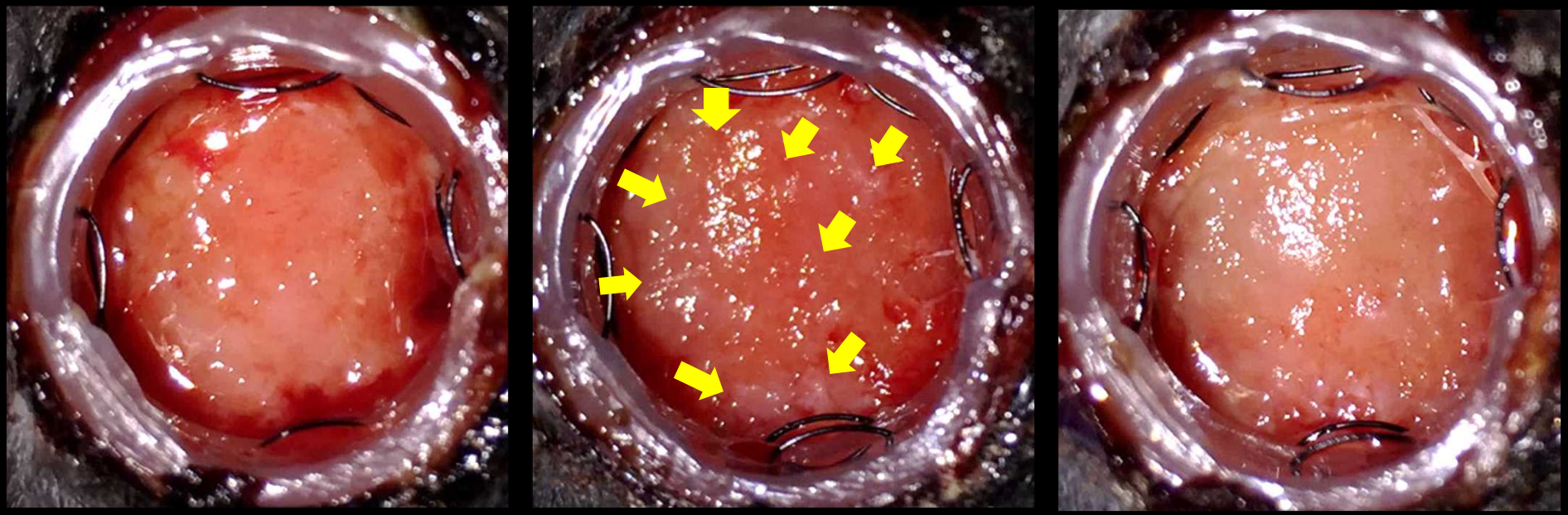


Kurita et al. *Nature*. 2018;561:243-247. modified

With promotion of epithelialization

D0 : AAV-DGTM + collagen
D4- : FGF2 + Rock inhibitor

D10 D11 D12

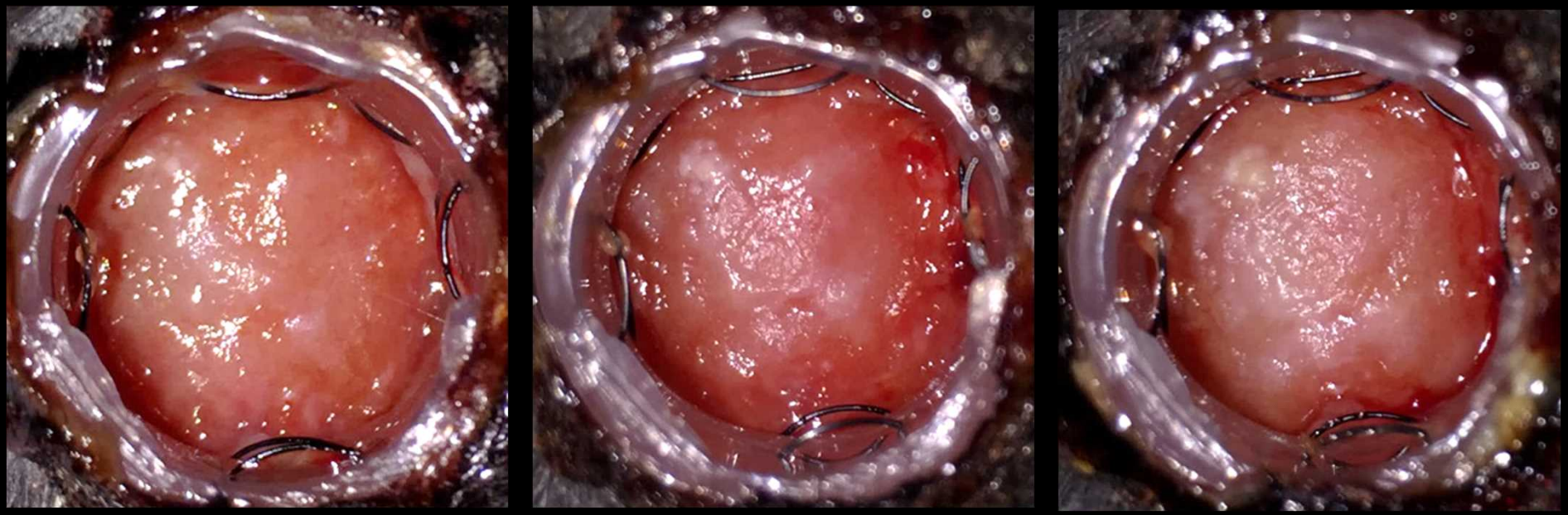


Kurita et al. *Nature*. 2018;561:243-247. modified

With promotion of epithelialization

D0 : AAV-DGTM + collagen
D4- : FGF2 + Rock inhibitor

D13 D14 D15

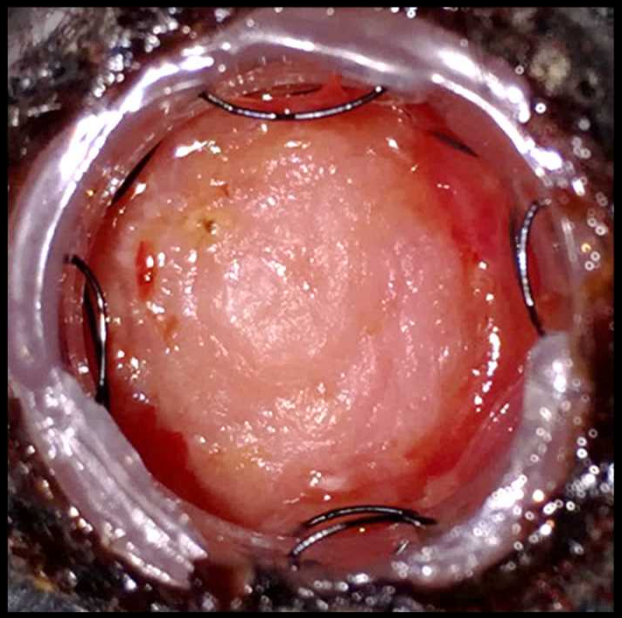


Kurita et al. *Nature*. 2018;561:243-247. modified

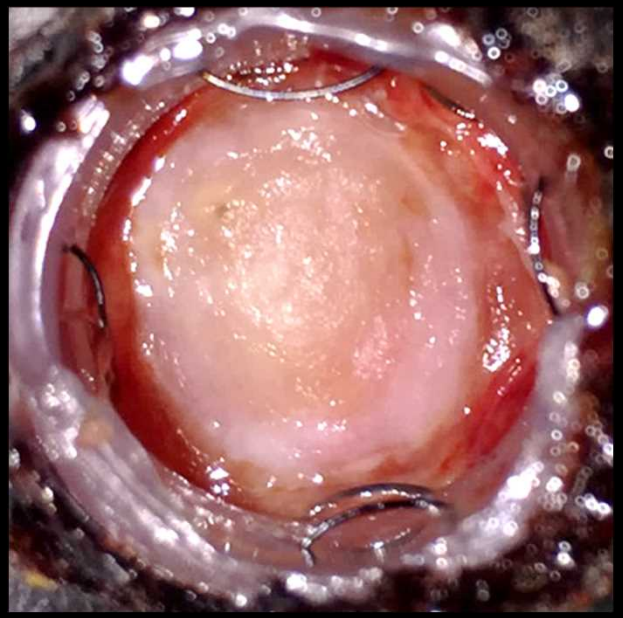
With promotion of epithelialization

D0 : AAV-DGTM + collagen
D4- : FGF2 + Rock inhibitor

D16



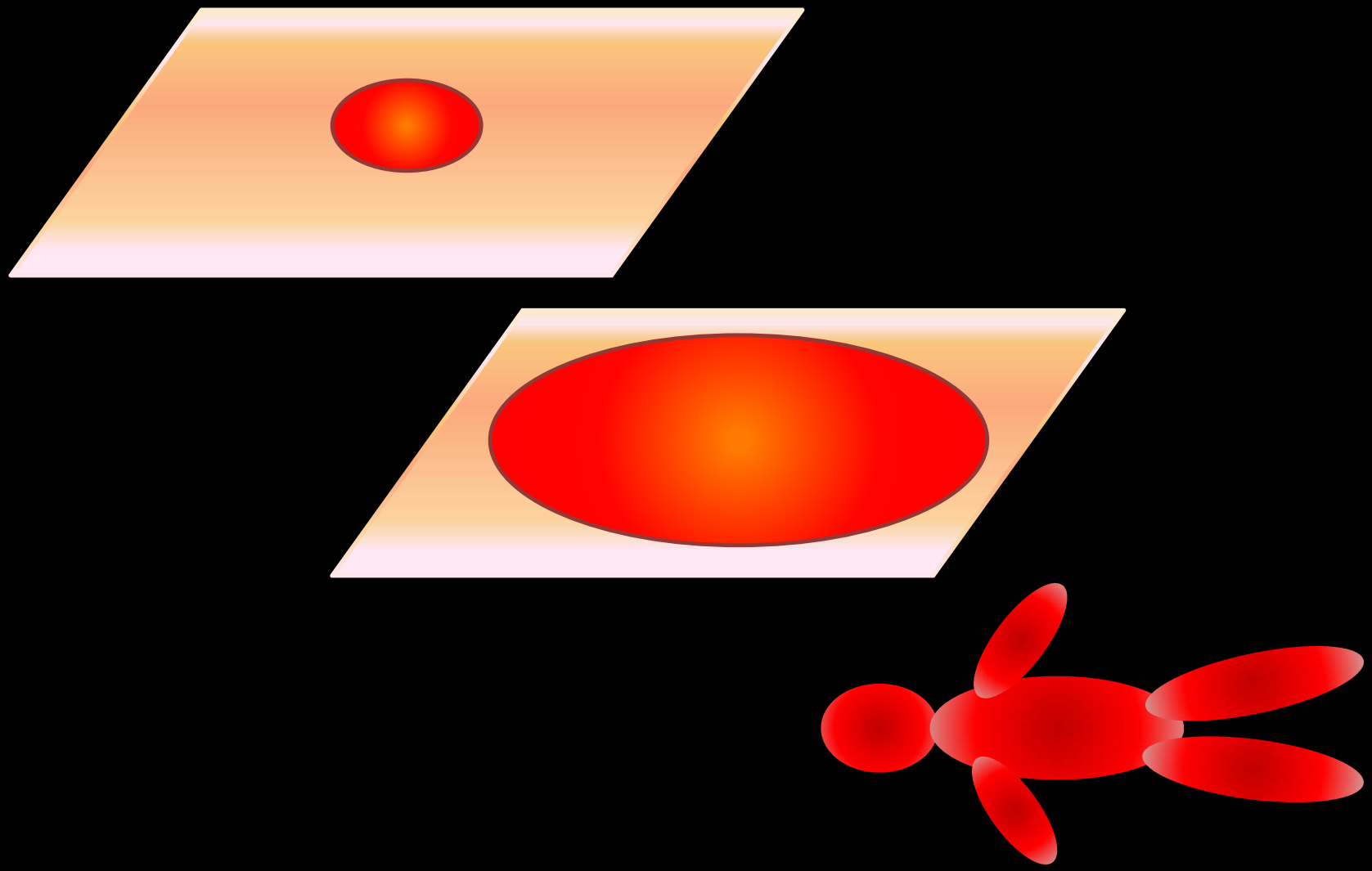
D17



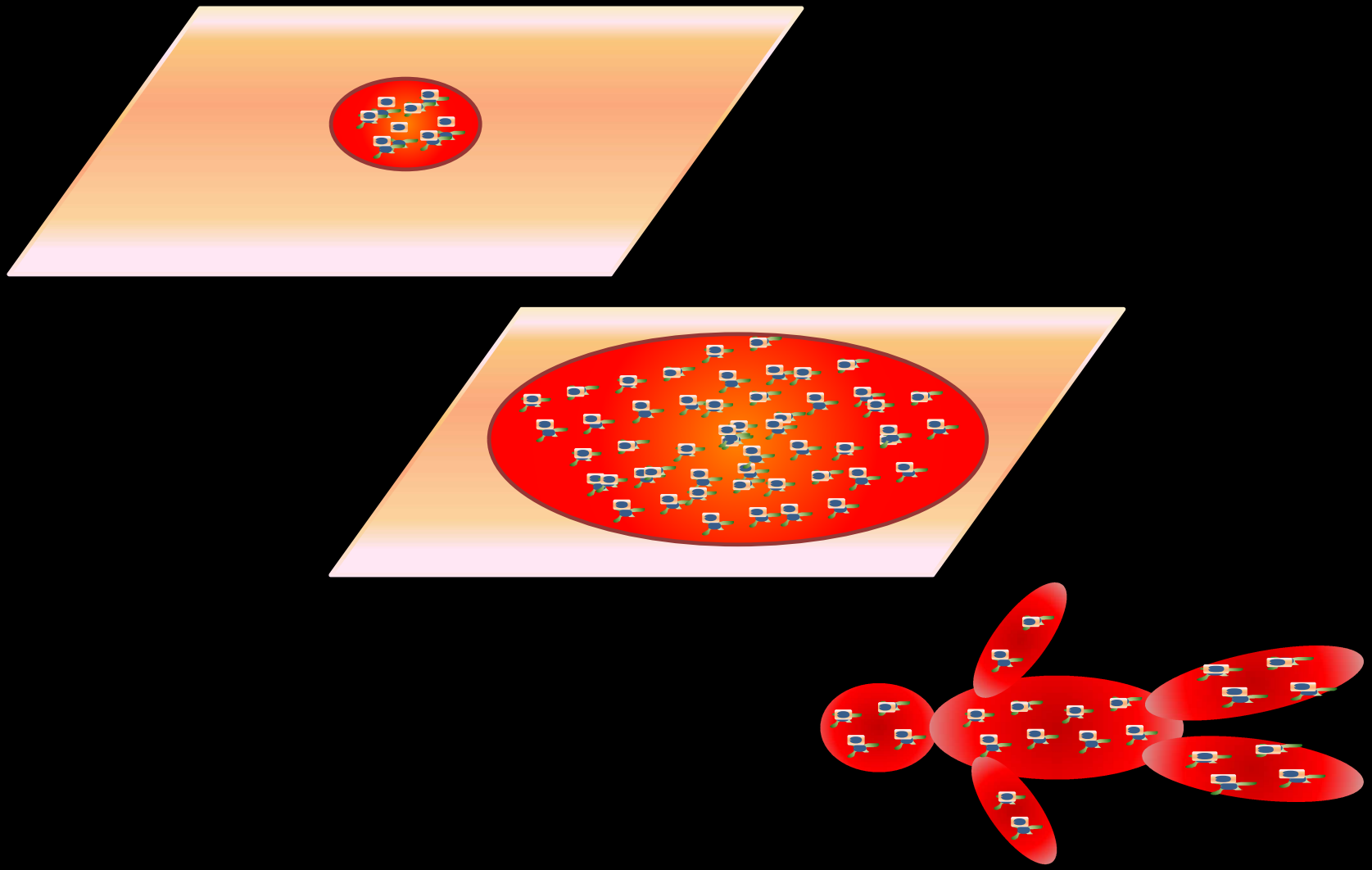
(n=5, no exception)

Kurita et al. *Nature*. 2018;561:243-247. modified

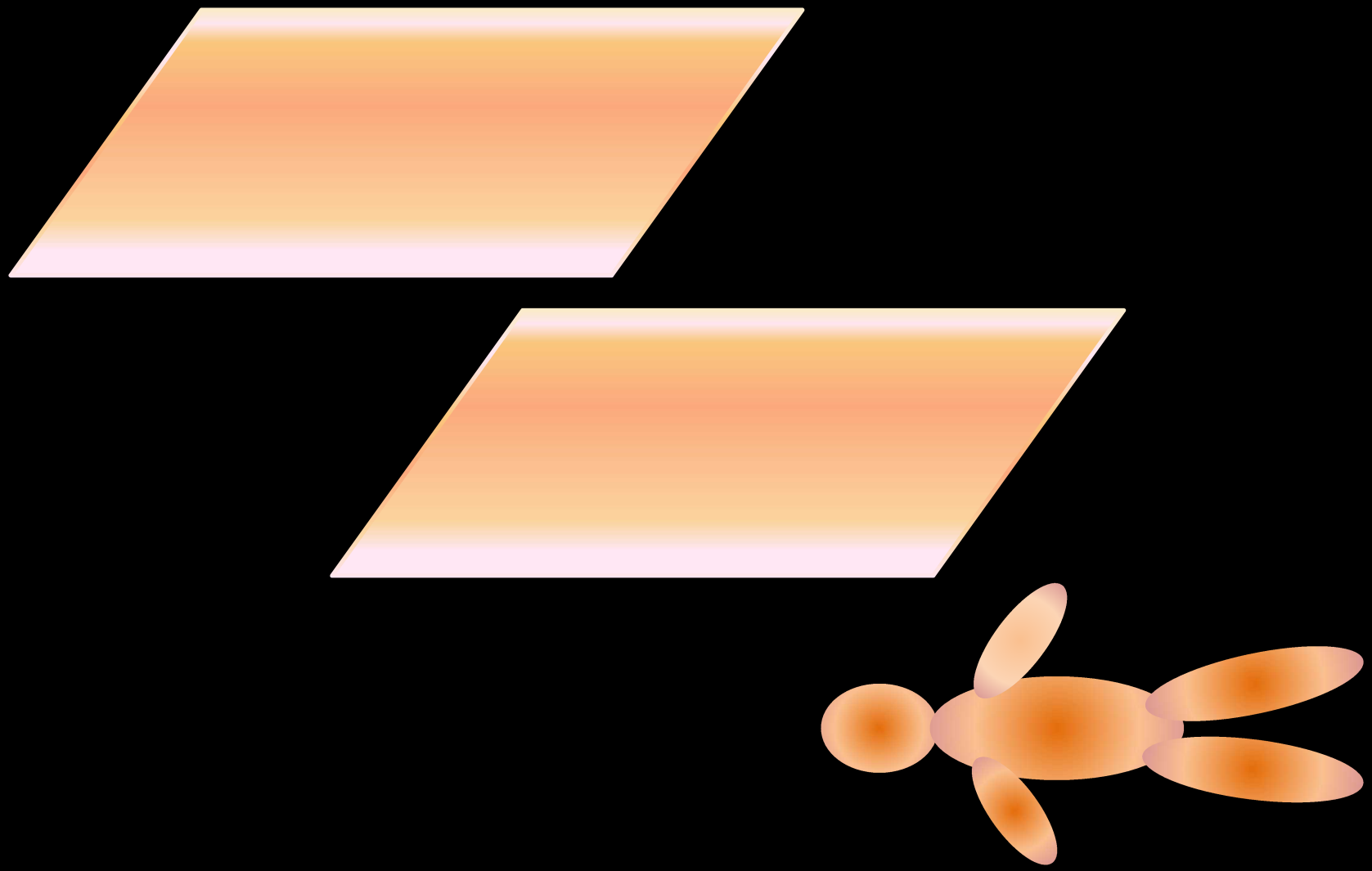
Wound closure via *de novo* epithelialization



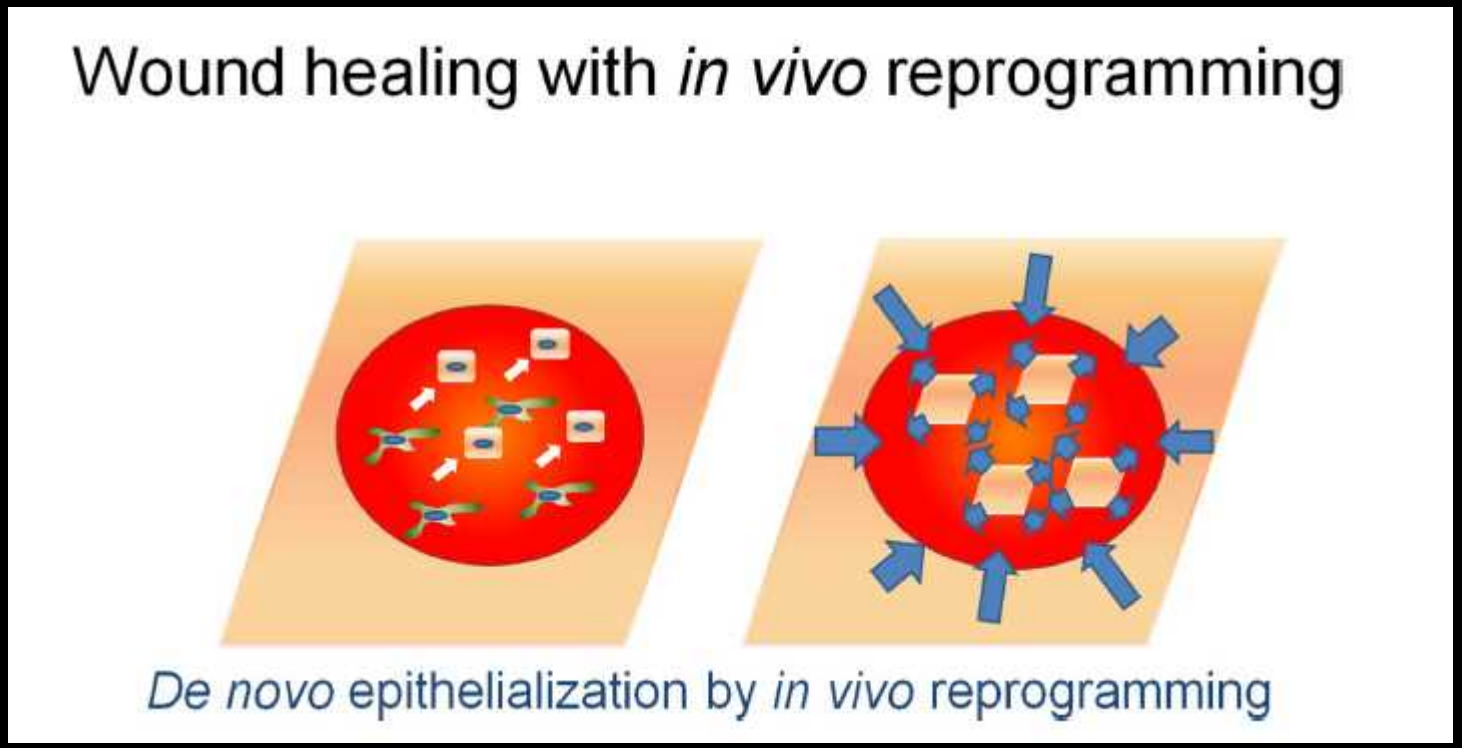
Wound closure via *de novo* epithelialization



Wound closure via *de novo* epithelialization



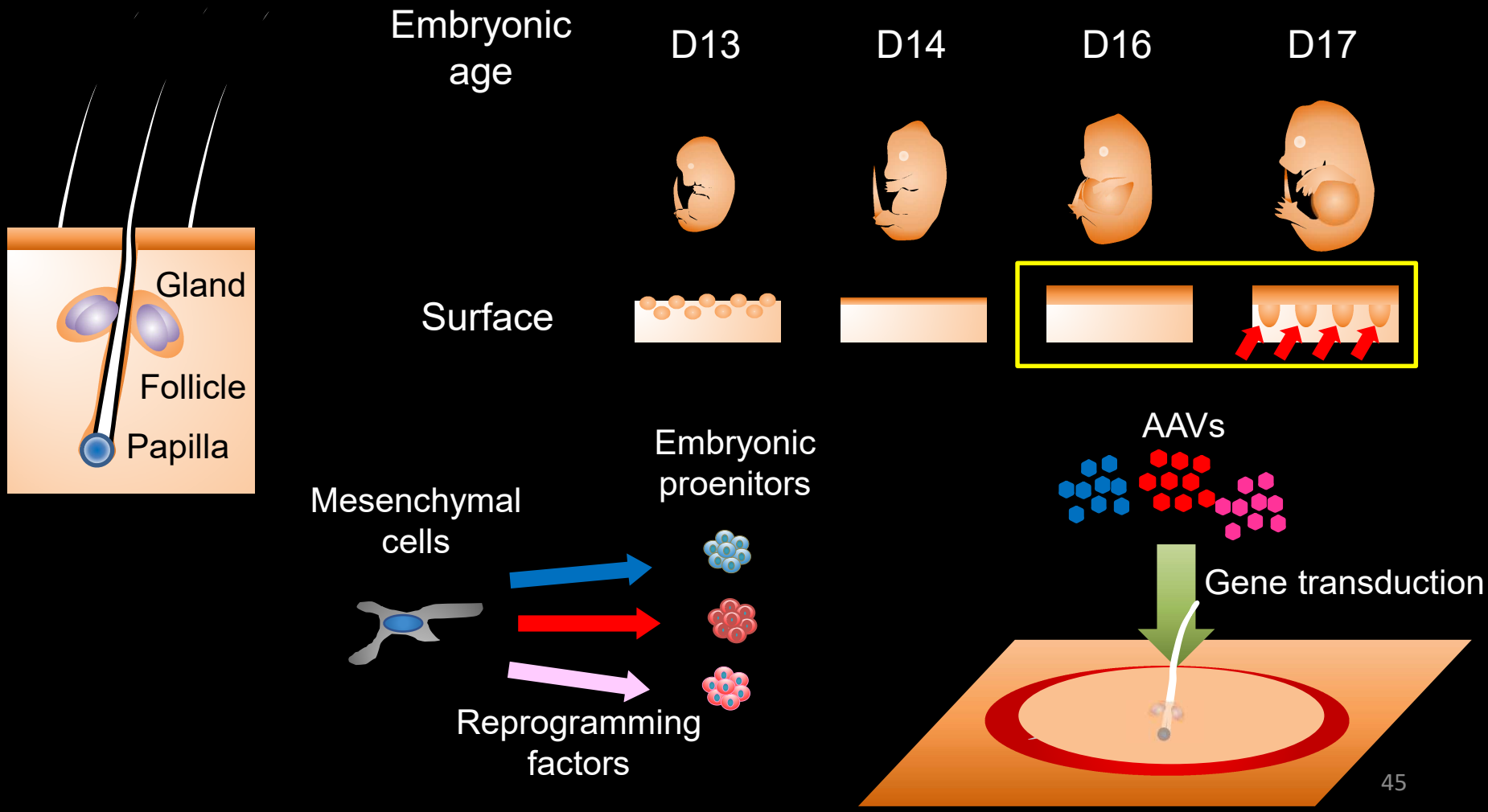
Limitation



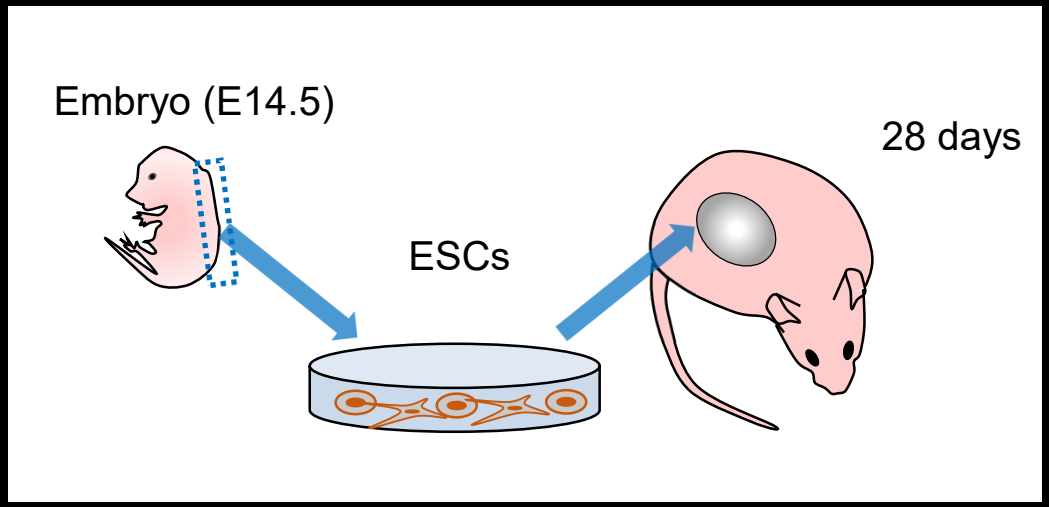
Epithelial tissue only

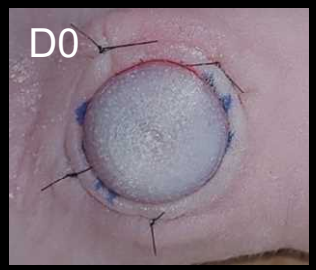
Kurita et al. *Nature*. 2018;561:243-247. modified

Generation of skin with appendage via tissue embryonization (2018-)



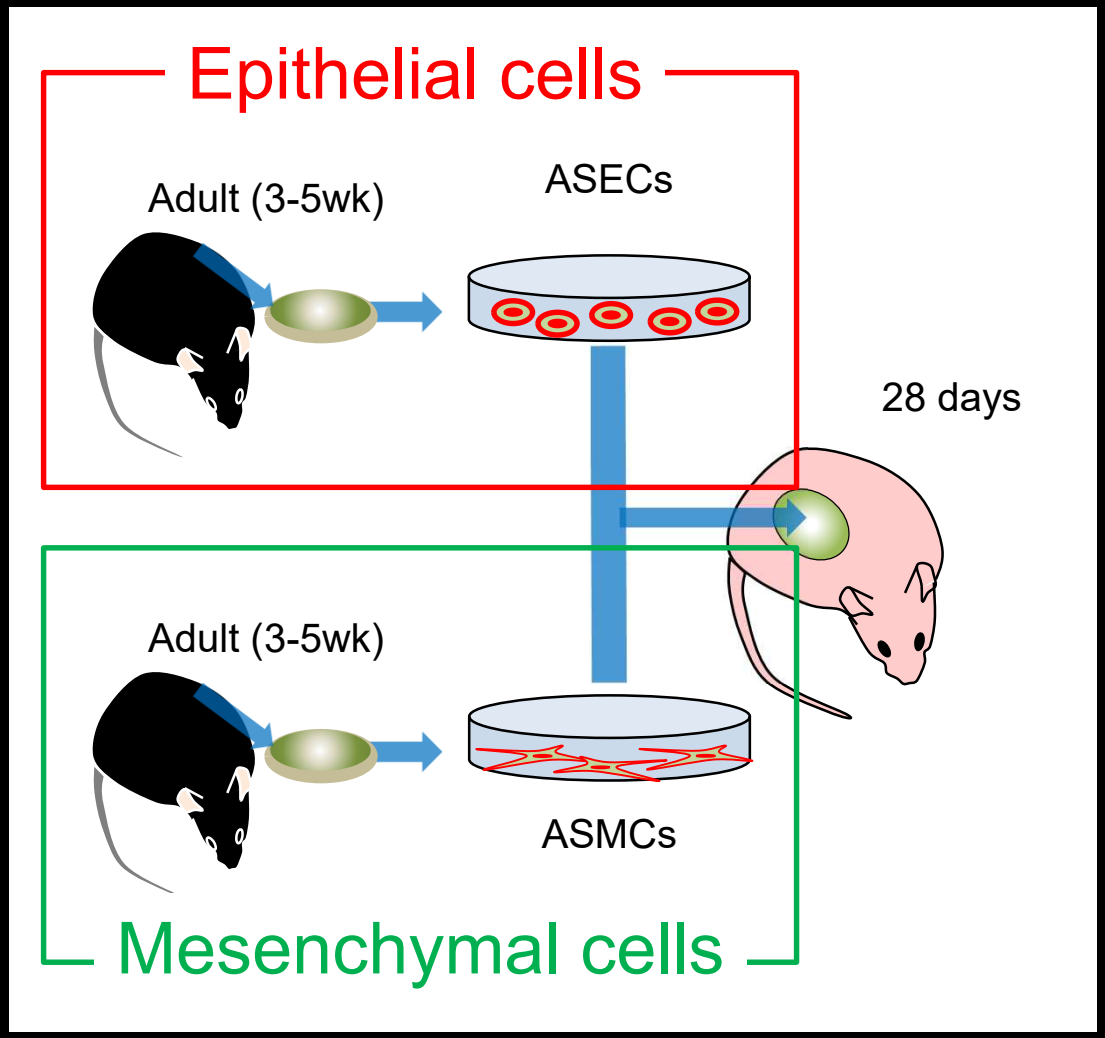
Skin reconstitution with embryonic skin cells





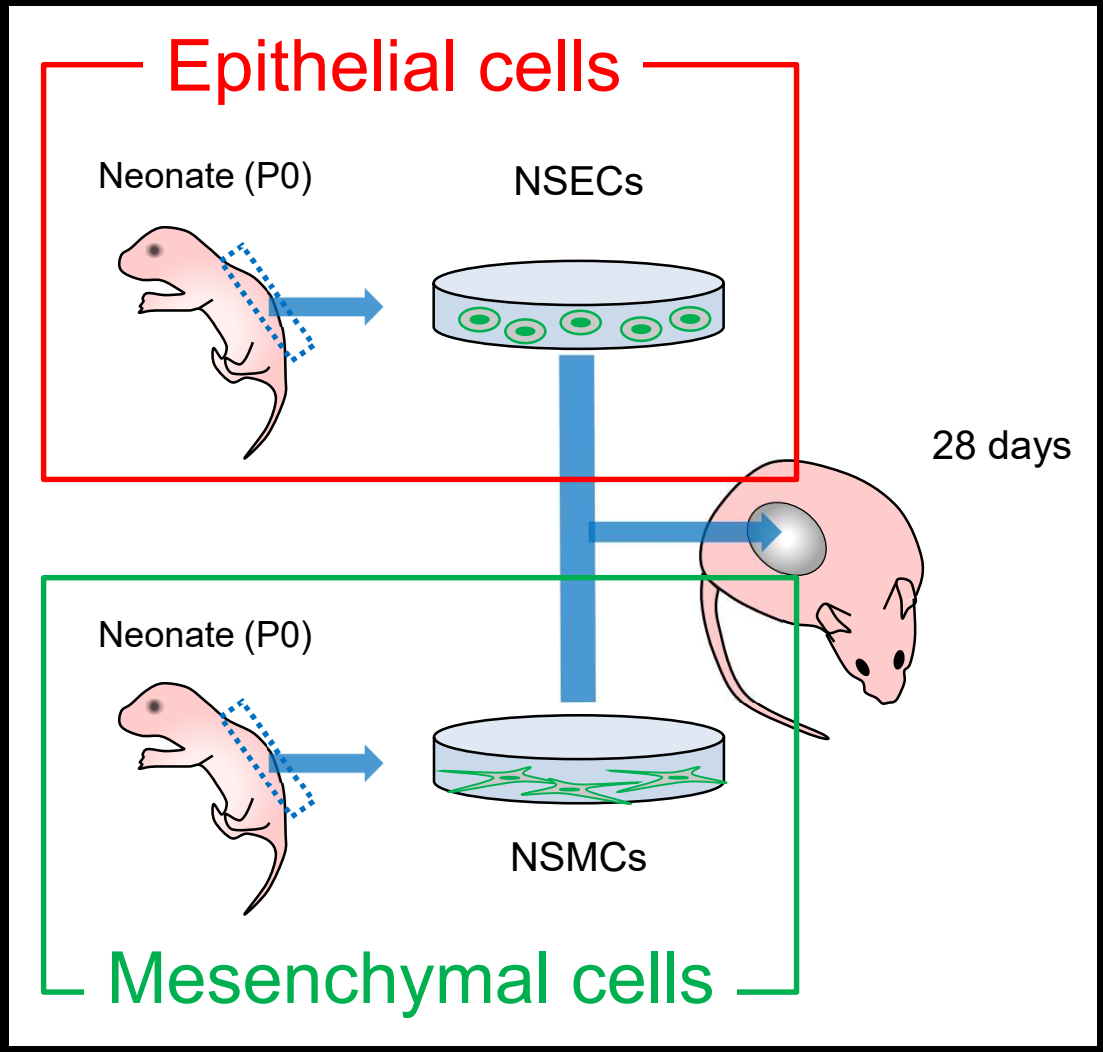
Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

Skin reconstitution with adult mouse skin cells



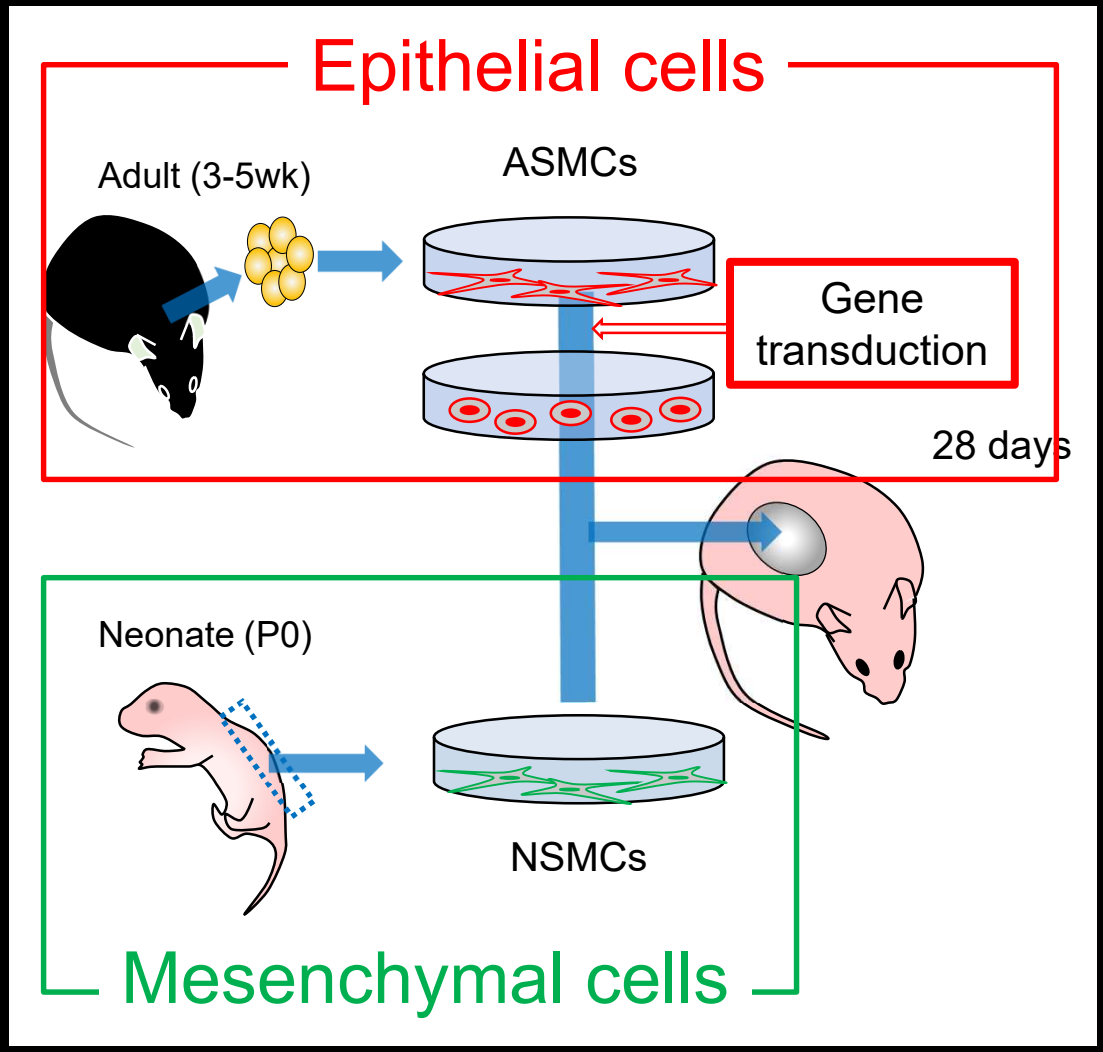
Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

Skin reconstitution with new born mouse skin cells



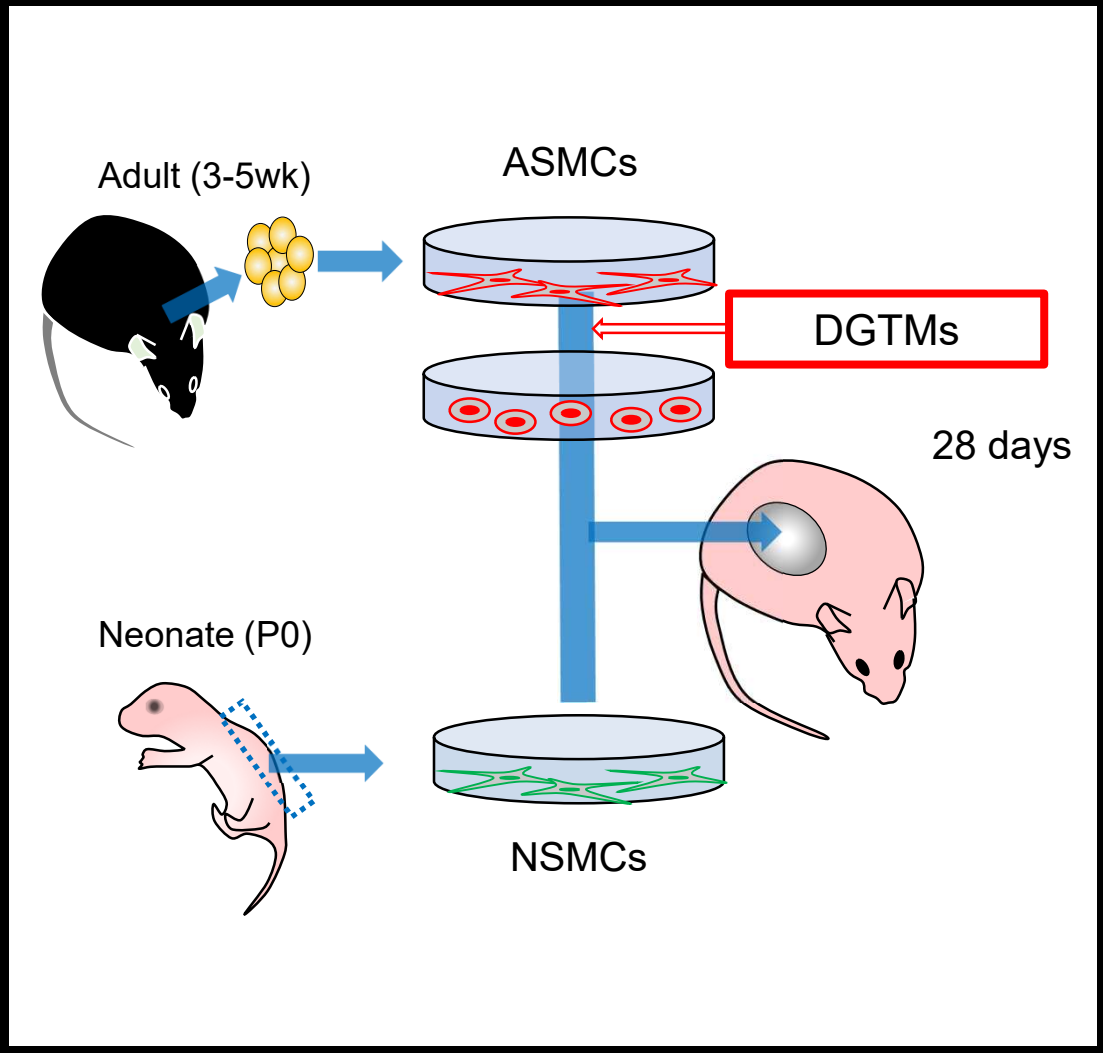
Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

Identification of hair generatable epithelial cell factor



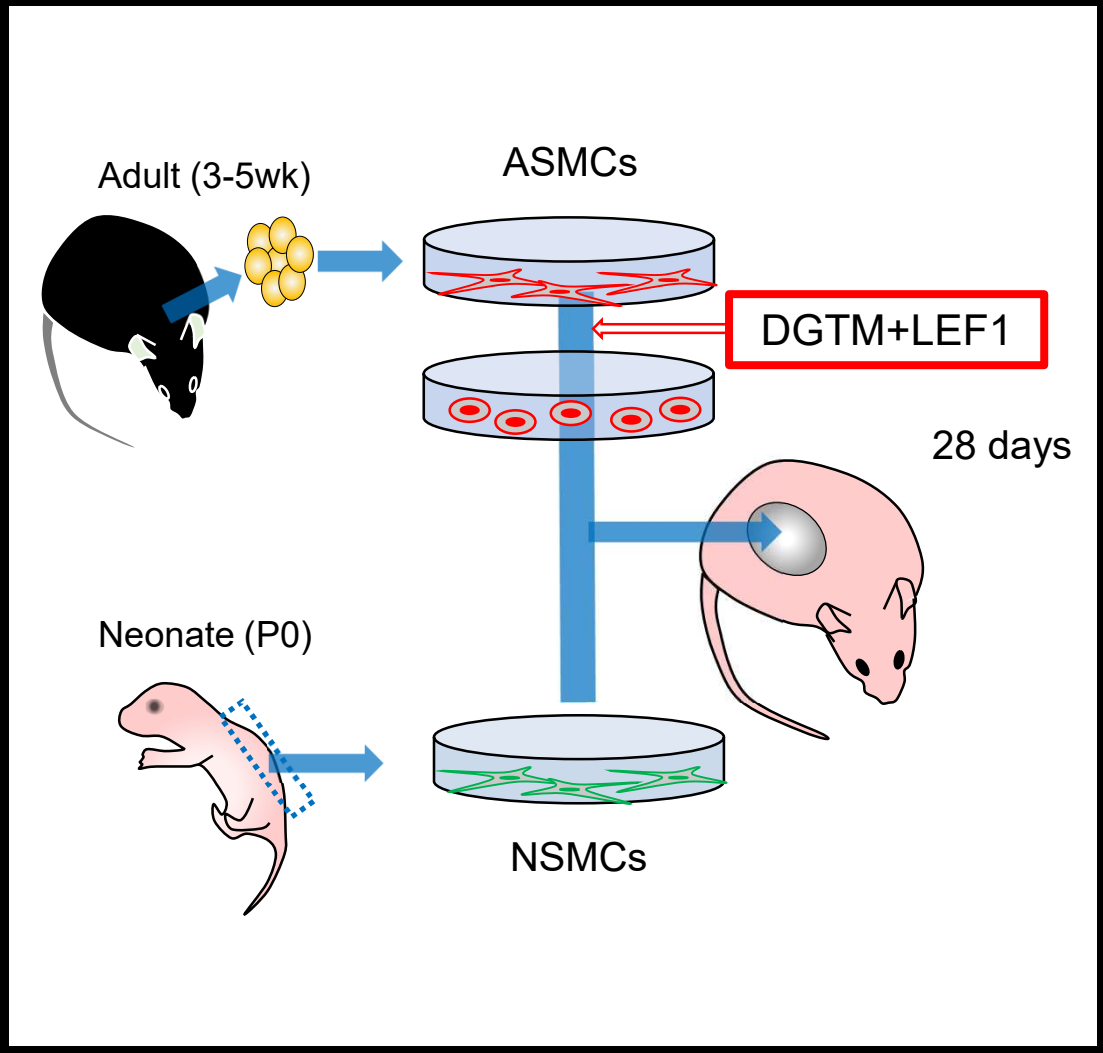
Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

Identification of hair generatable epithelial cell factor



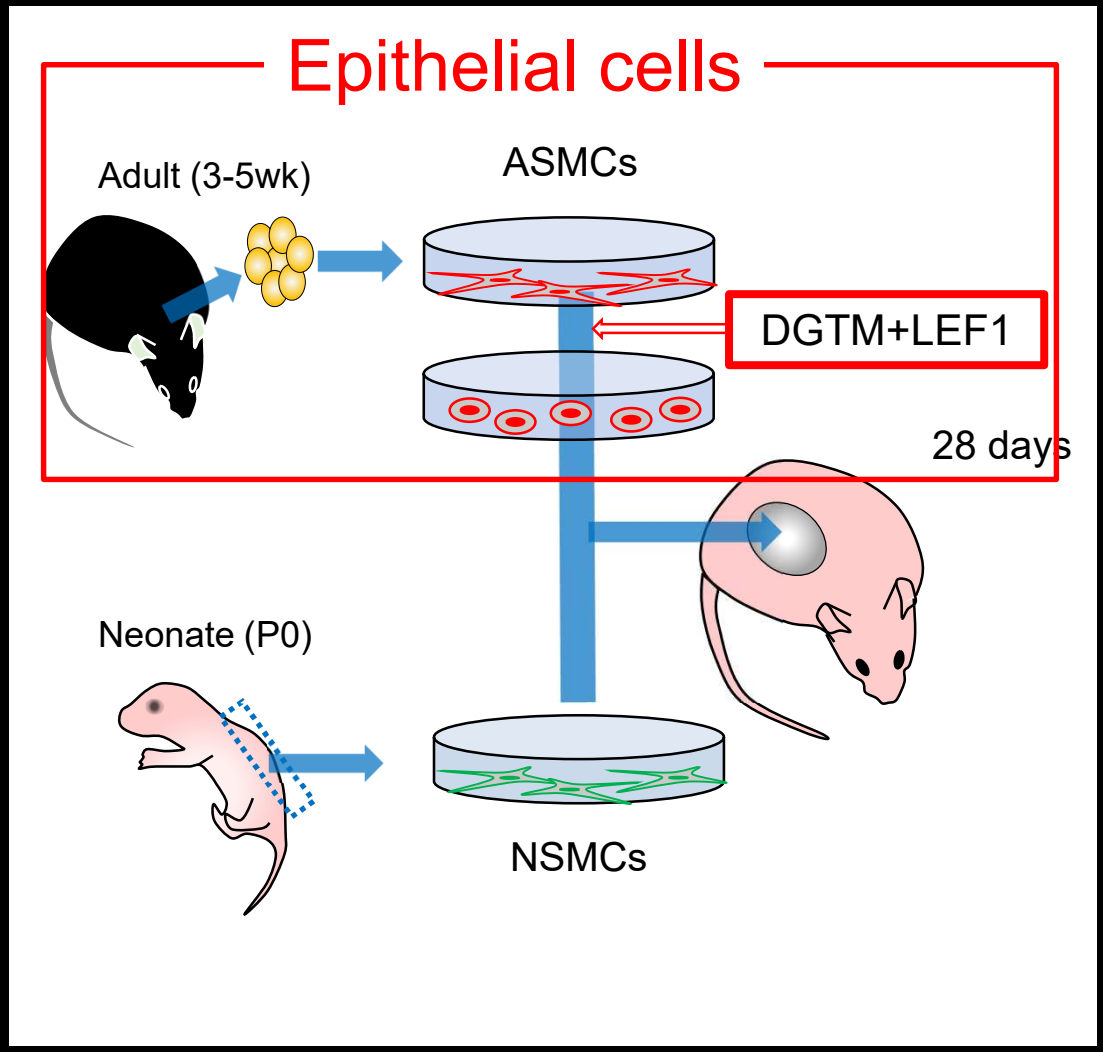
Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

Identification of hair generatable epithelial cell factor



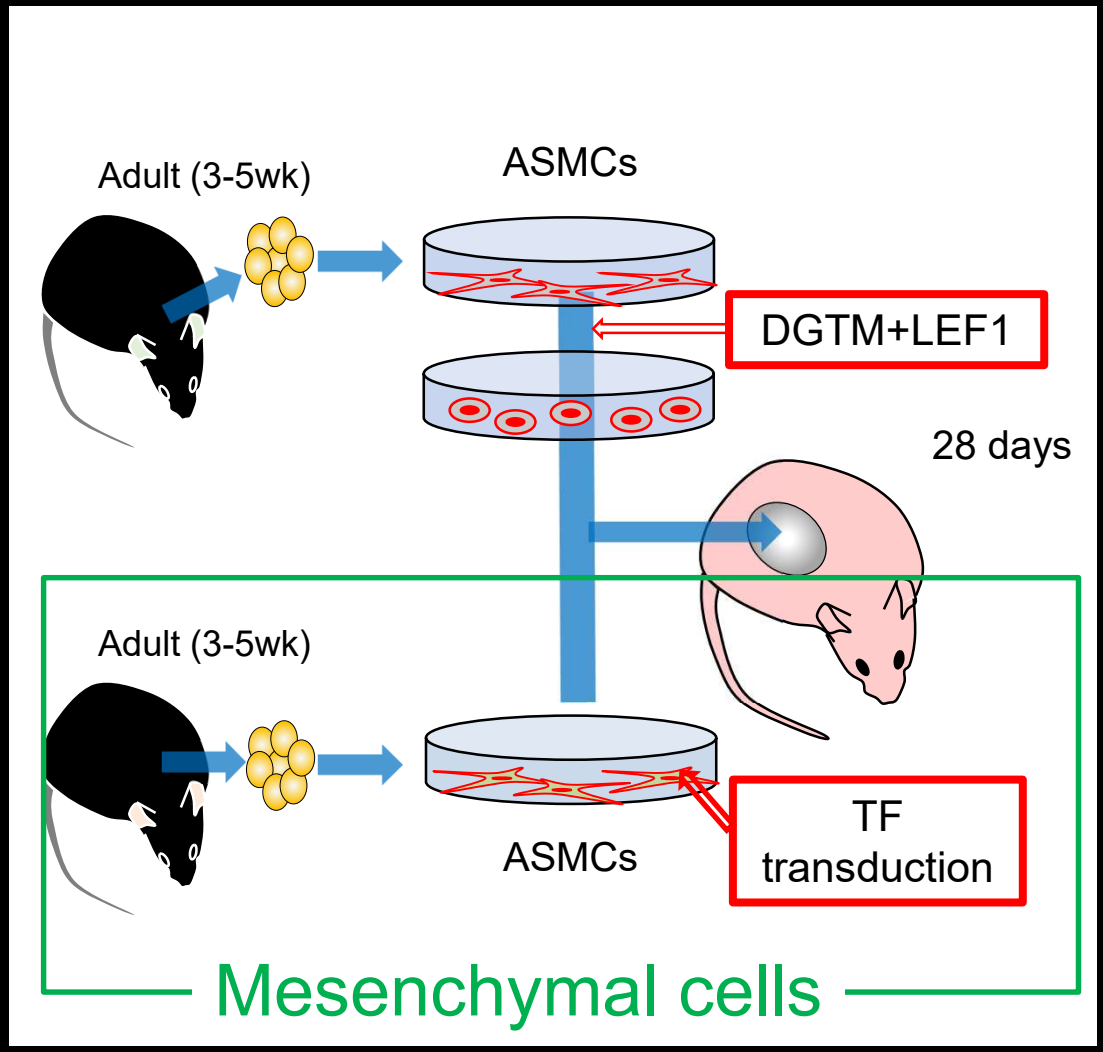
Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

Identification of hair generatable mesenchymal cell factor



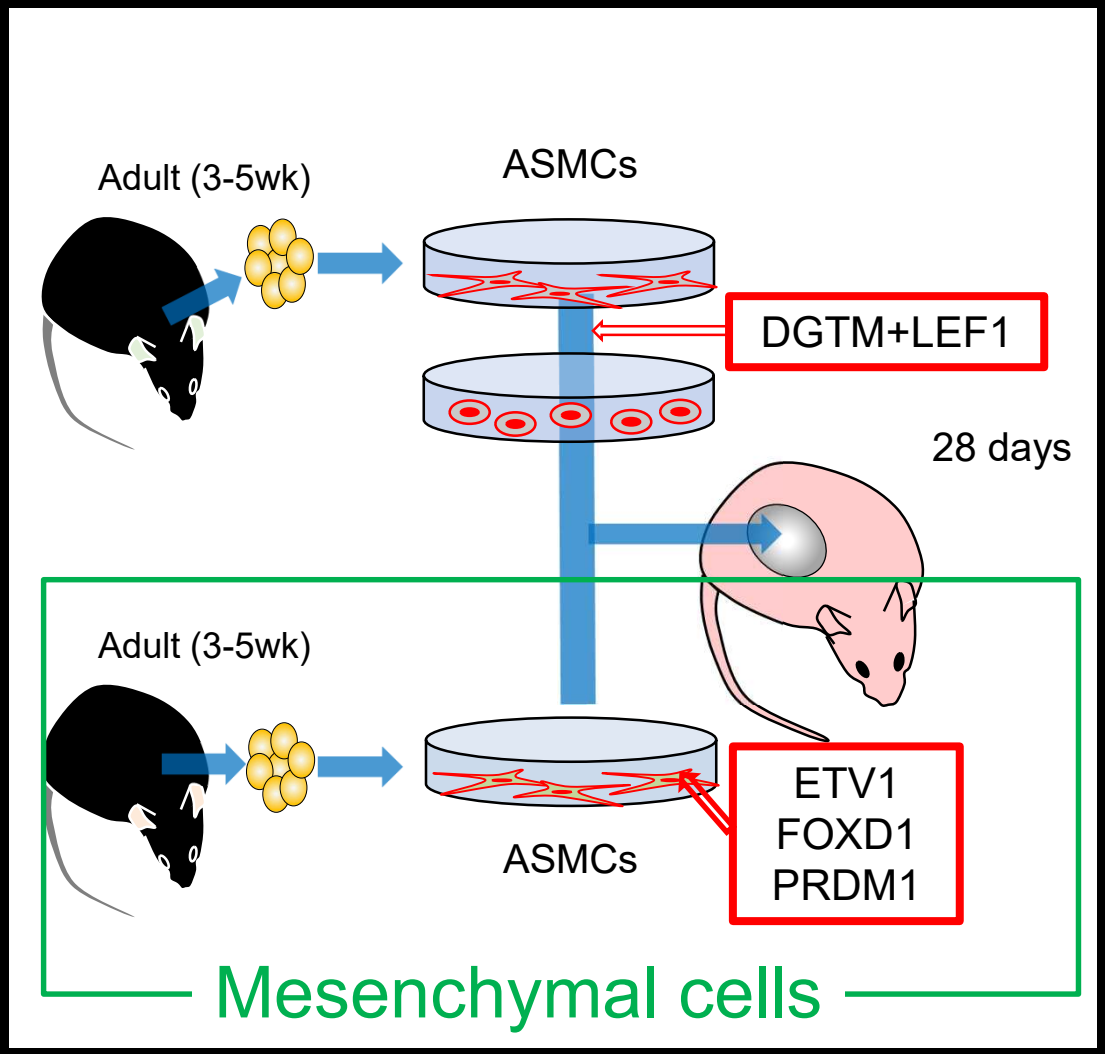
Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

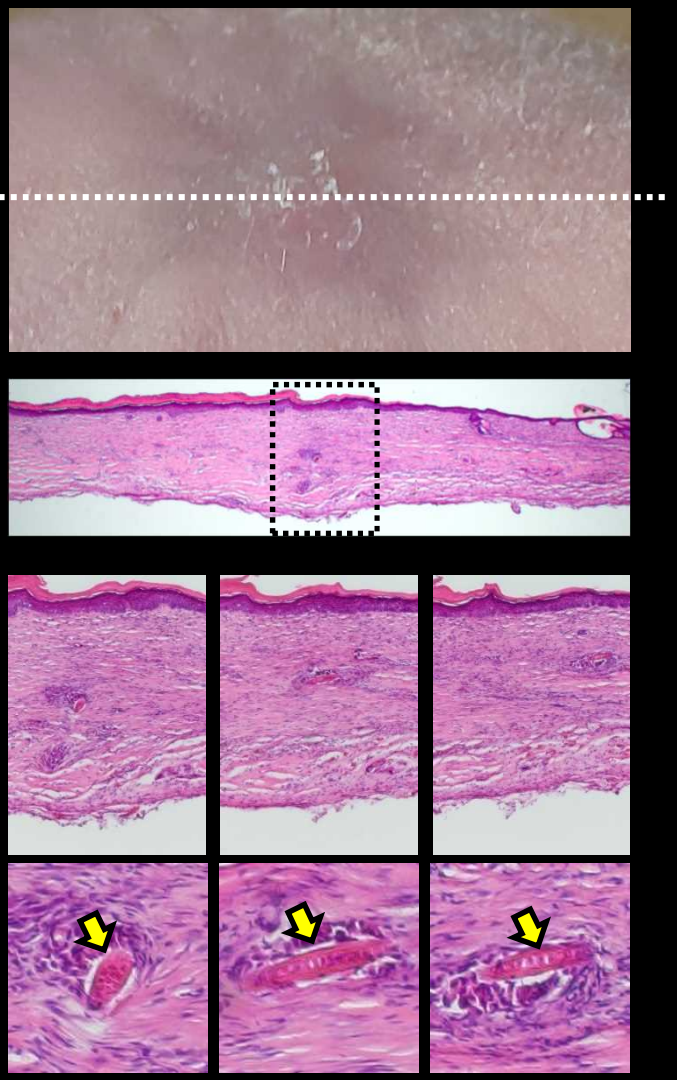
Identification of hair generatable mesenchymal cell factor



Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

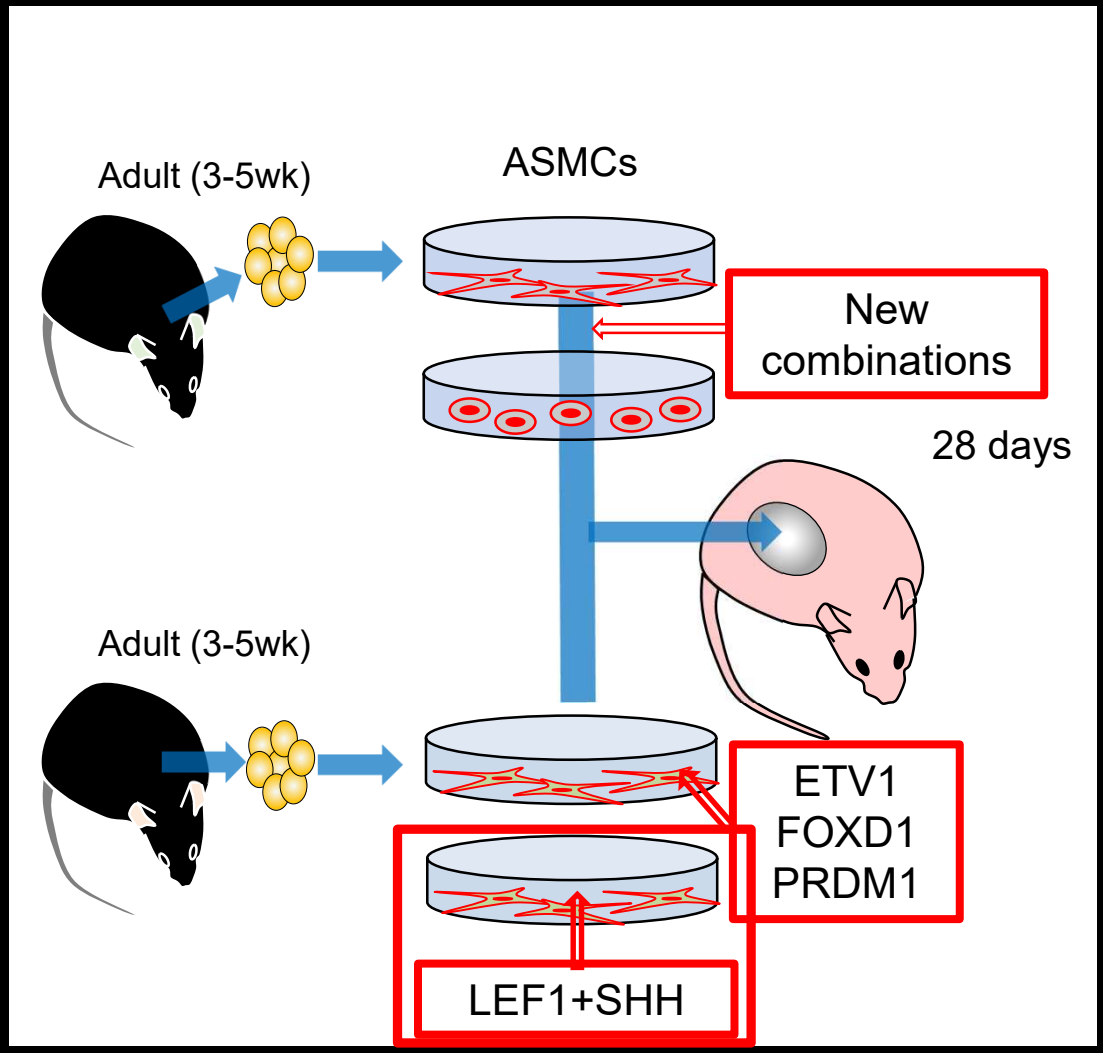
Identification of hair generatable mesenchymal cell factor





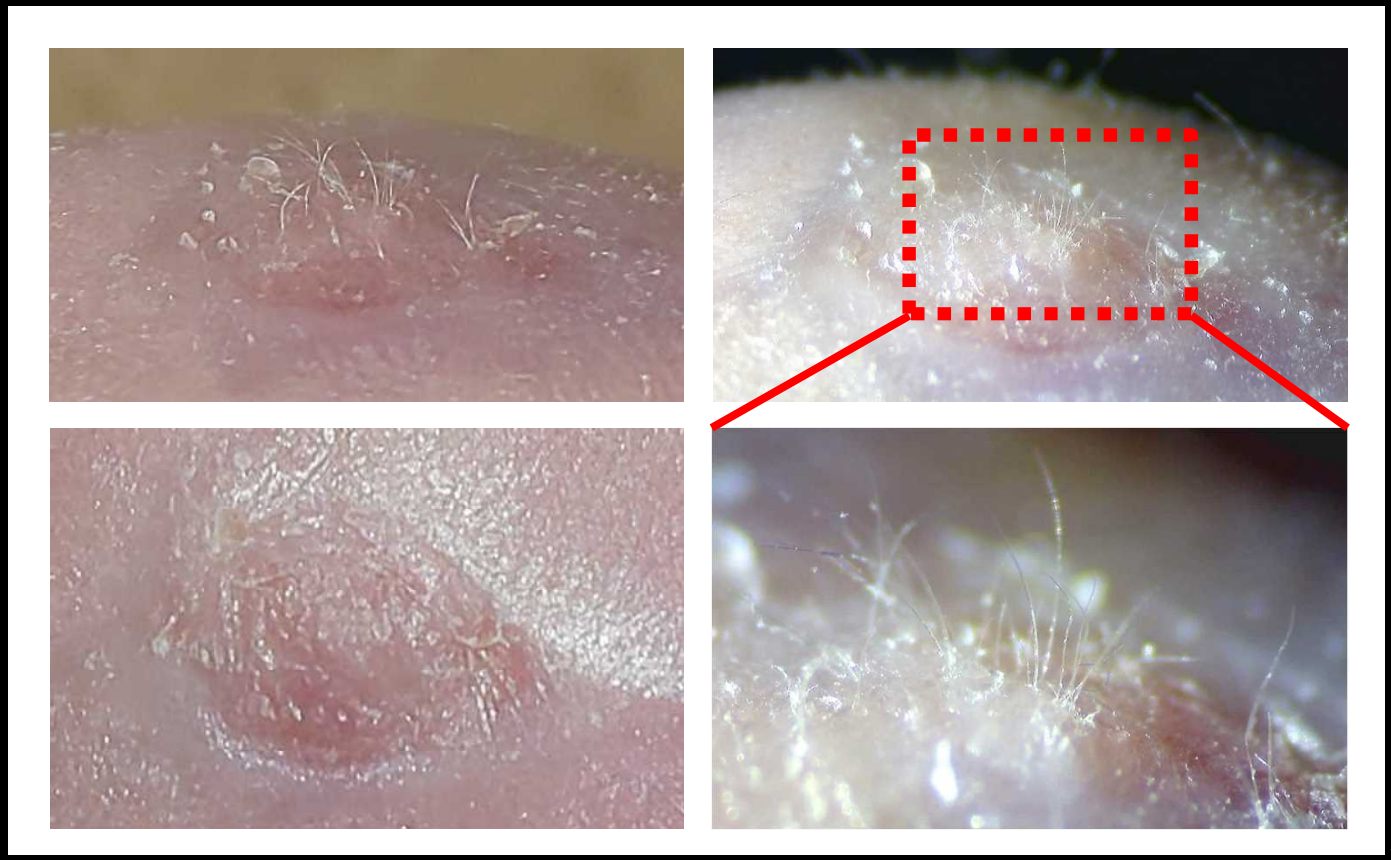
Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

Identification of hair generatable mesenchymal cell factor



Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

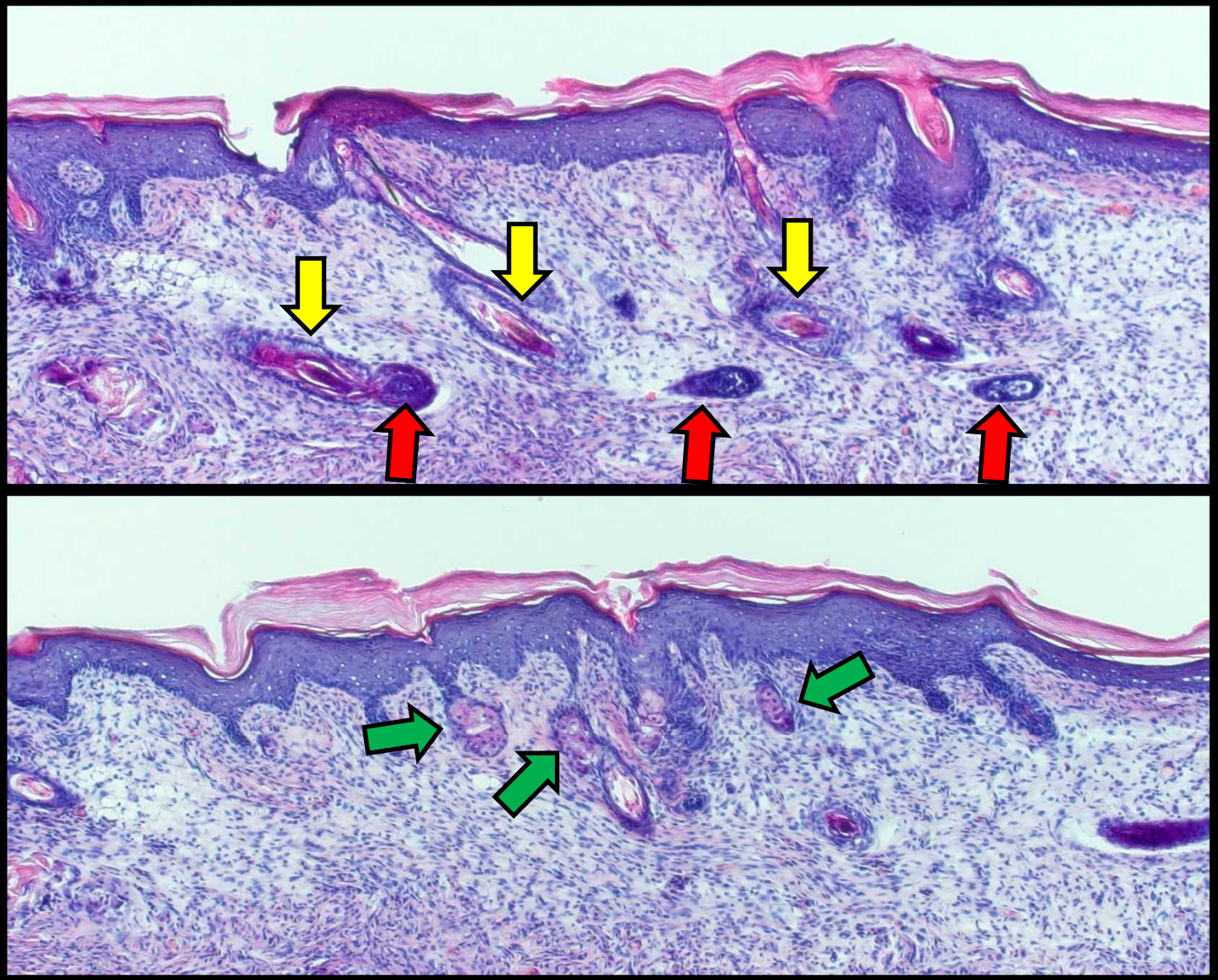
Identification of hair generatable mesenchymal cell factor



Epithelial cell : *DNP63A+GRHL2+TFAP2A+cMYC+LEF1*
Mesenchymal cell1: *ETV1+FOXD1+PRDM1*
Mesenchymal cell2: *LEF1+SHH*

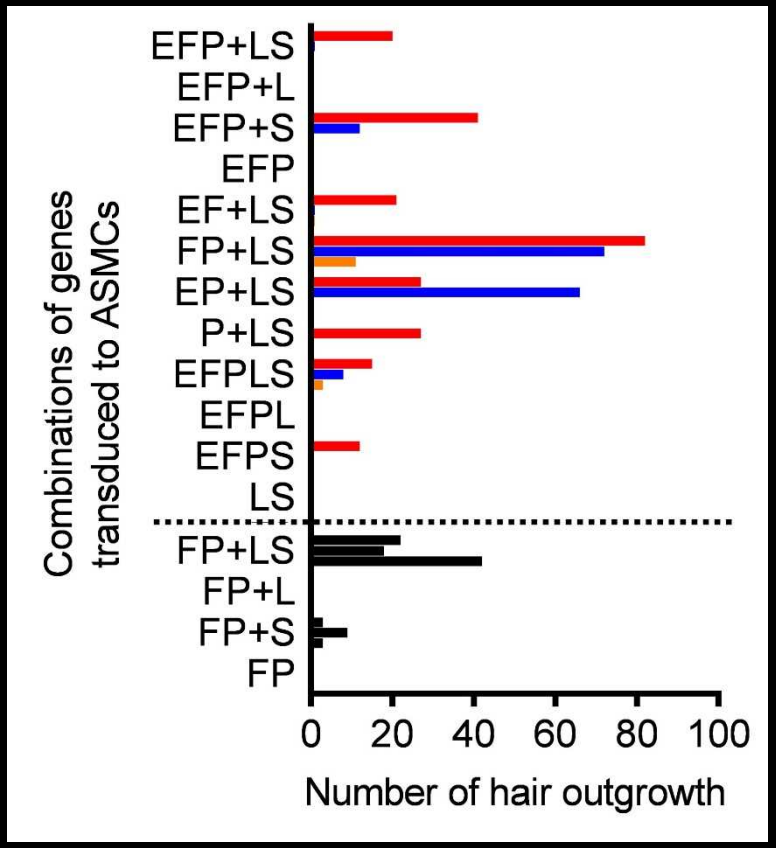
Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

Identification of hair generatable mesenchymal cell factor



Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

Minimization of reprogramming factors

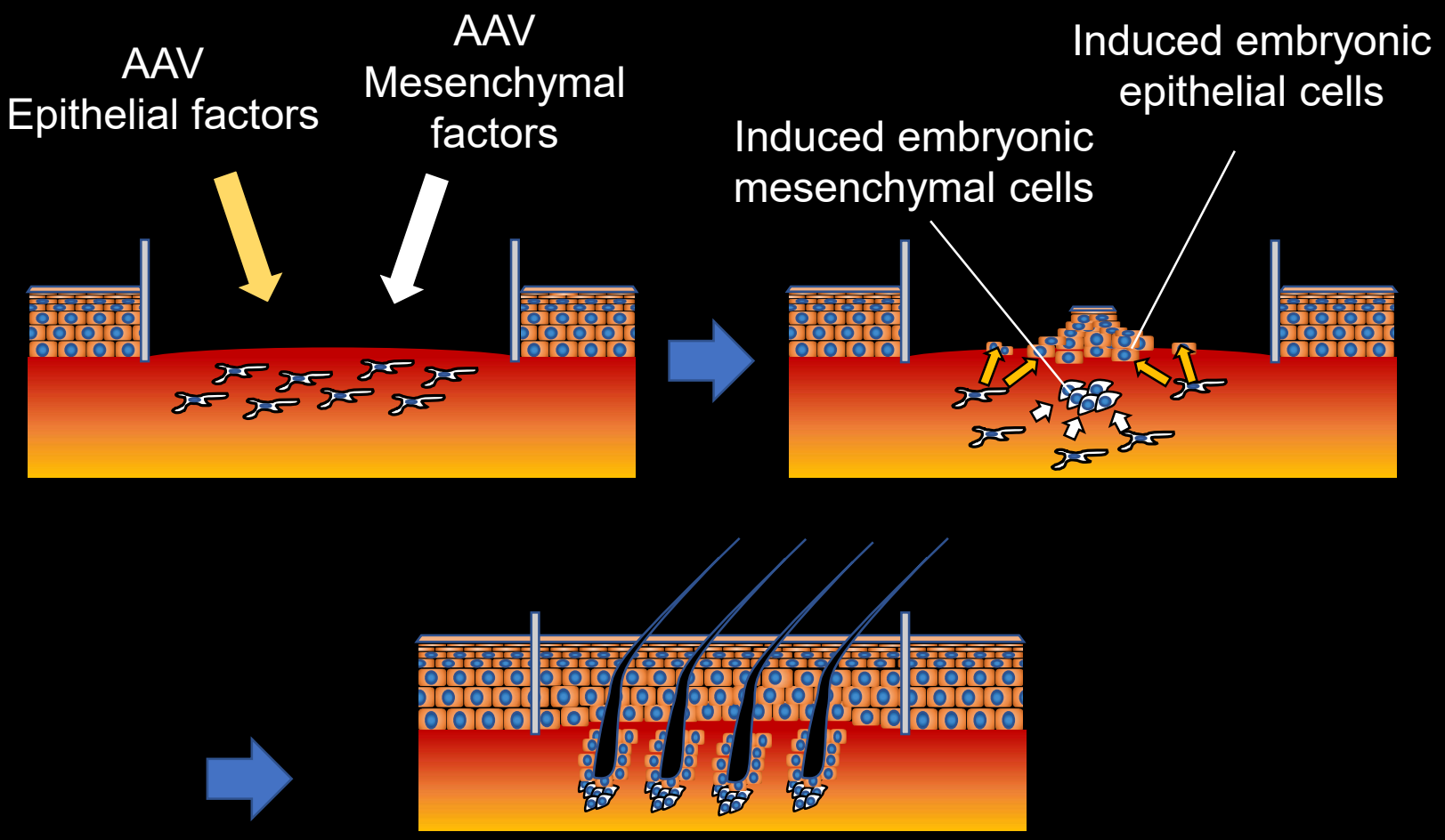


DGTM+*LEF1*
ETV1+FOXD1+PRDM1
LEF1+SHH

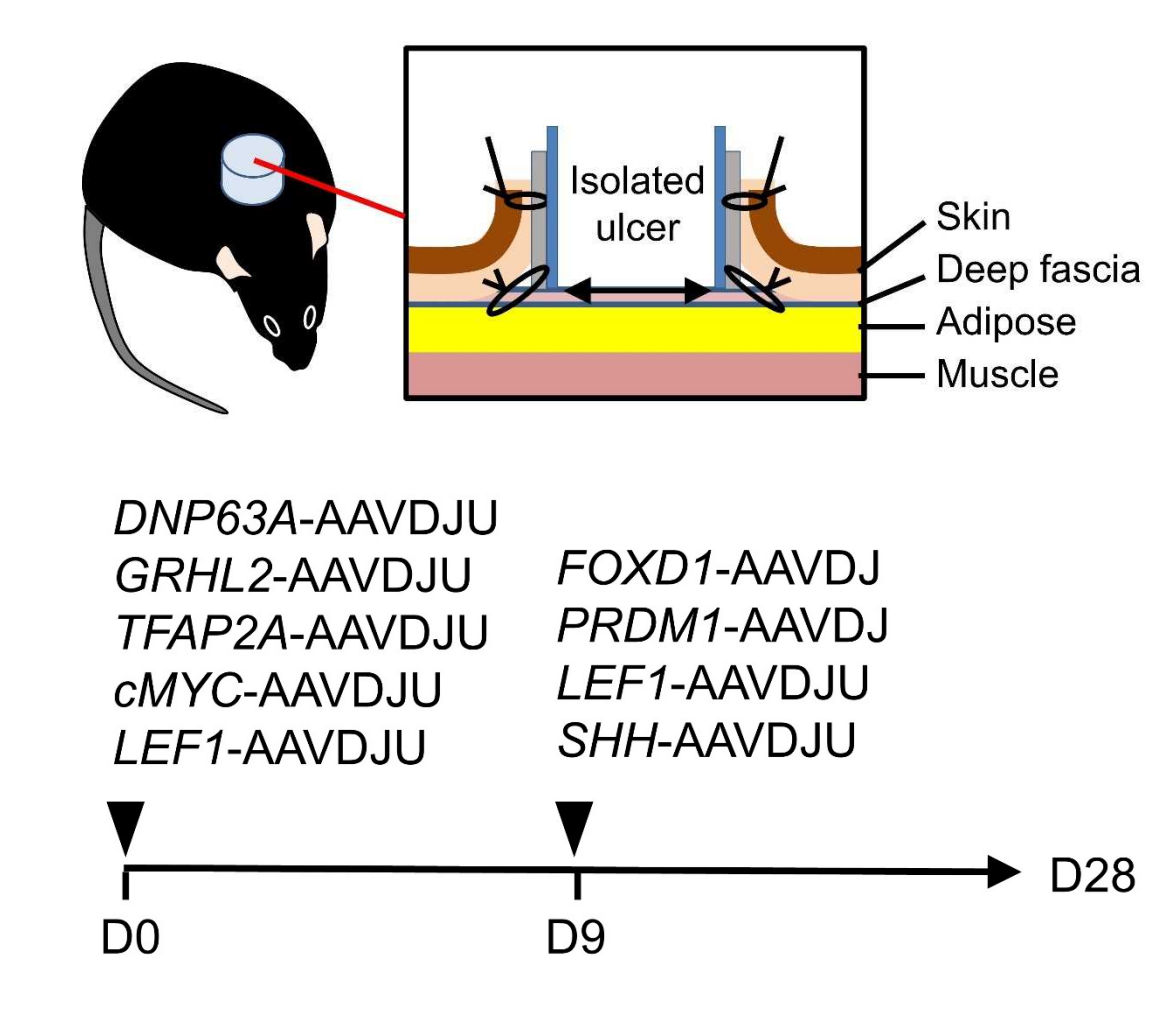
DGTM+*LEF1* **DGTML**
FOXD1+PRDM1 **FP**
LEF1+SHH **LS**

Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

Skin appendage regeneration via tissue embryonization



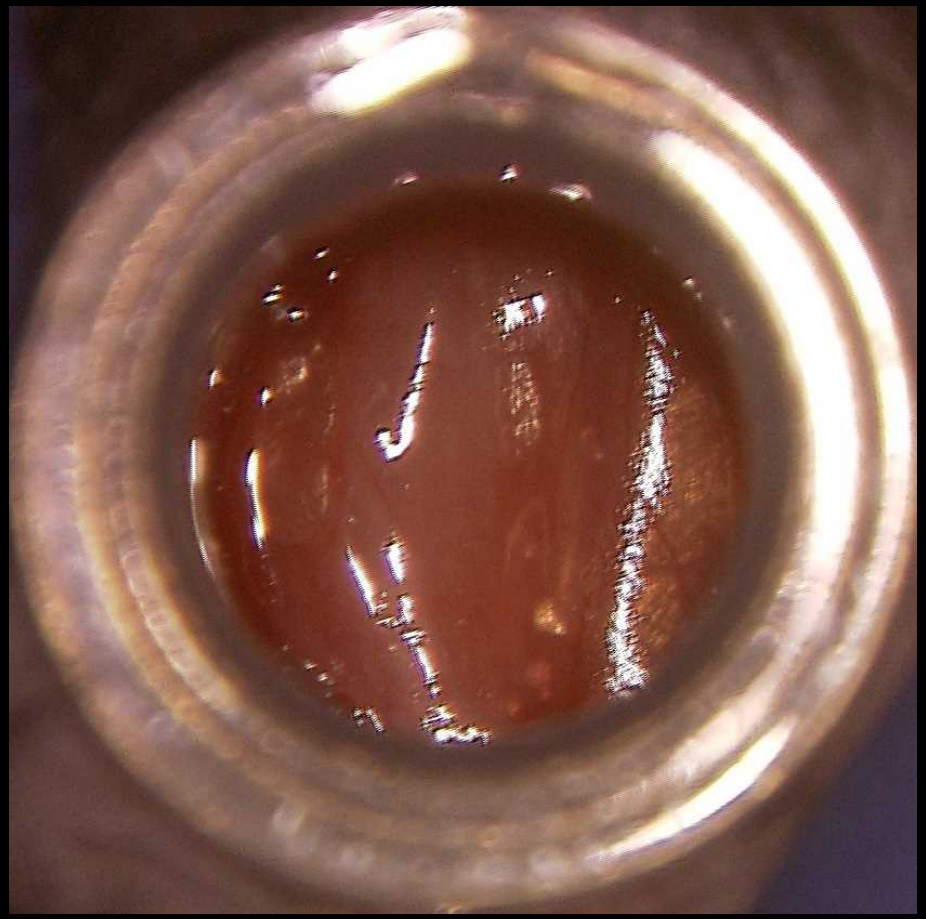
Induction in isolated skin ulcer model



Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

Induction in isolated skin ulcer model

D0

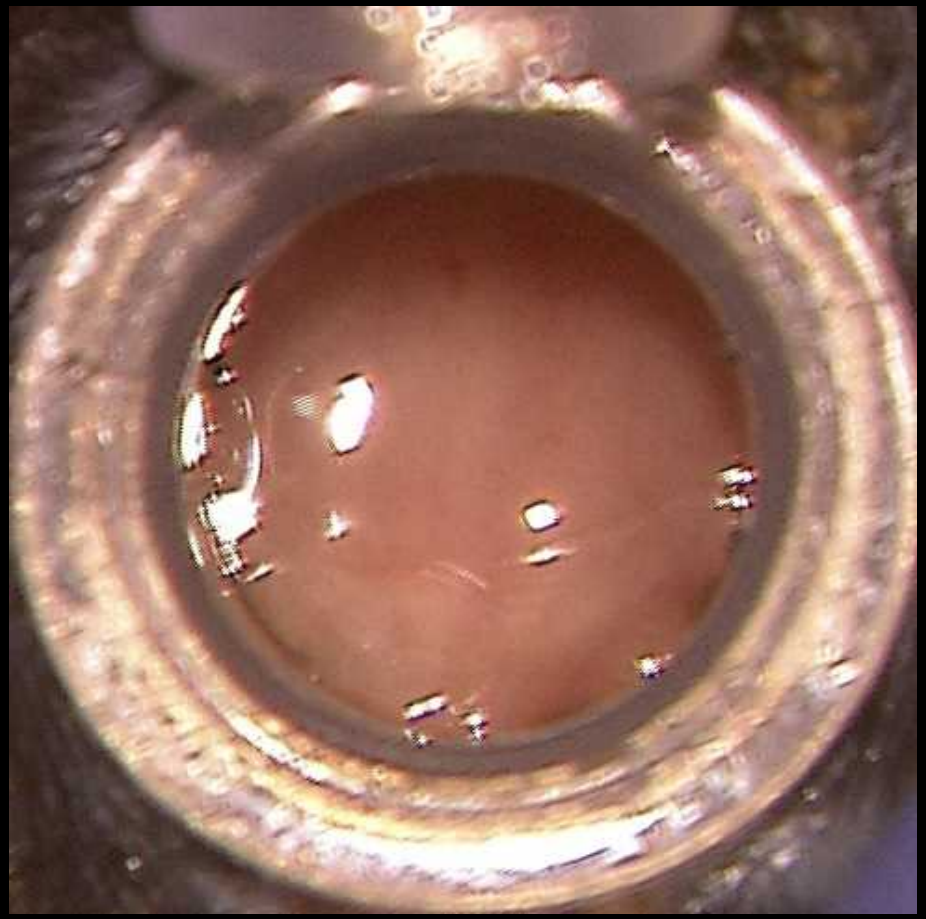


+ Epithelial factor AAVs

Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

Induction in isolated skin ulcer model

D9

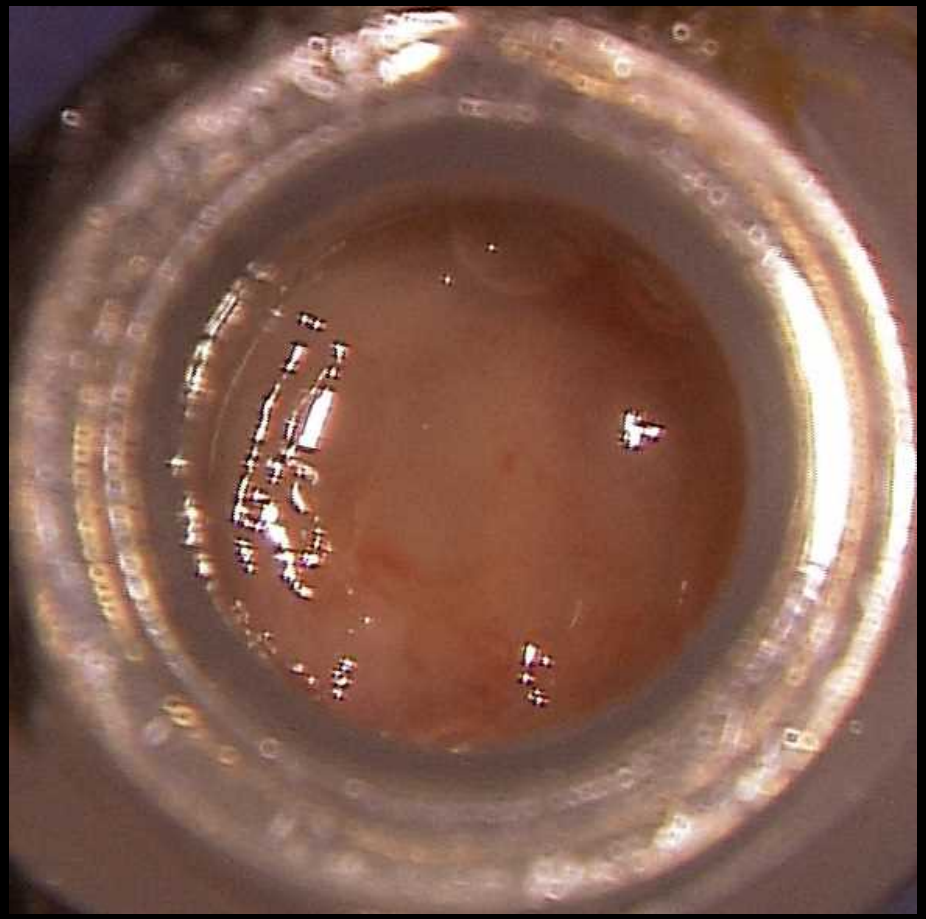


+ Mesenchymal factor AAVs

Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

Induction in isolated skin ulcer model

D14



Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

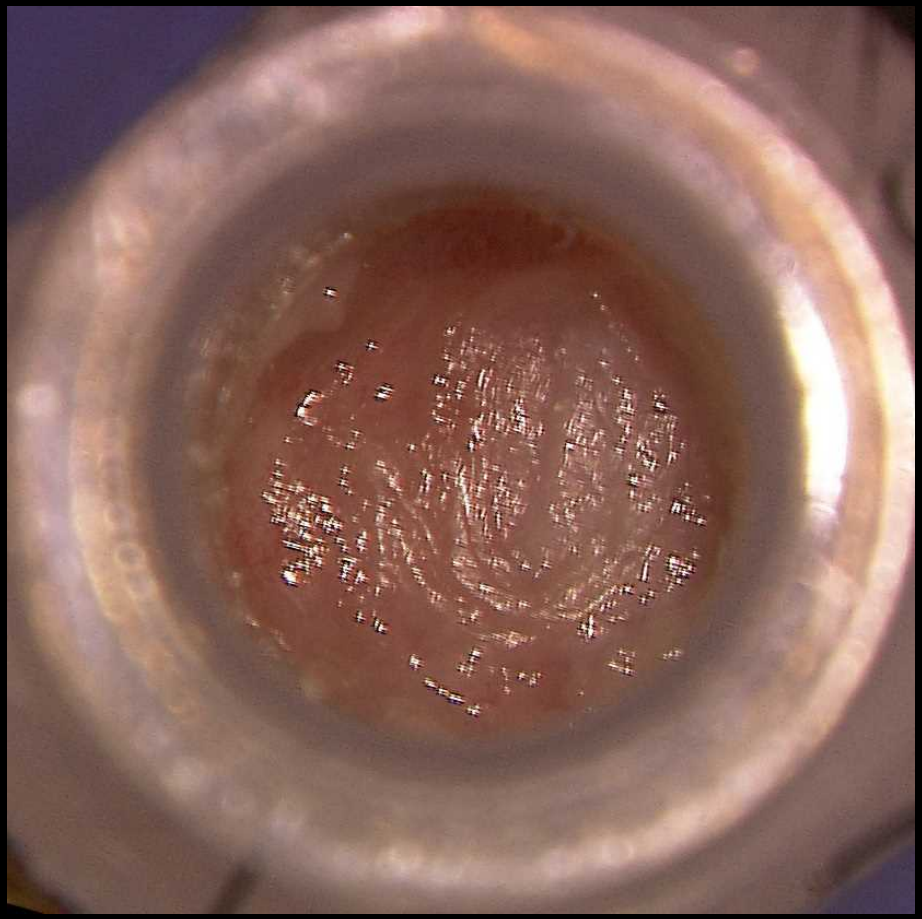
Induction in isolated skin ulcer model

D21

Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

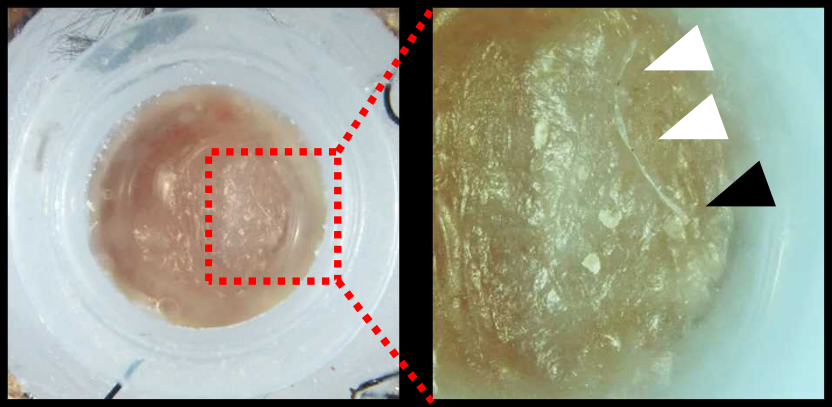
Induction in isolated skin ulcer model

D28

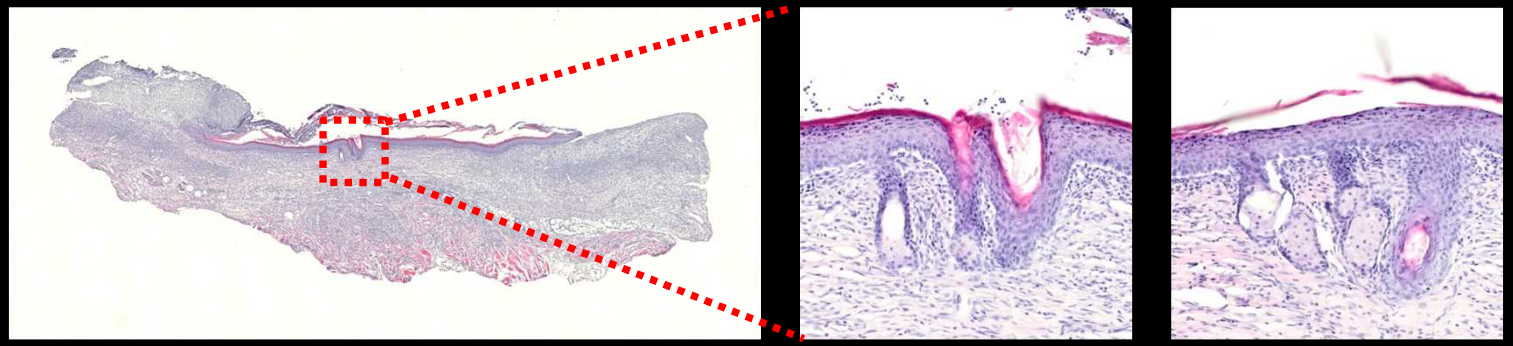


Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

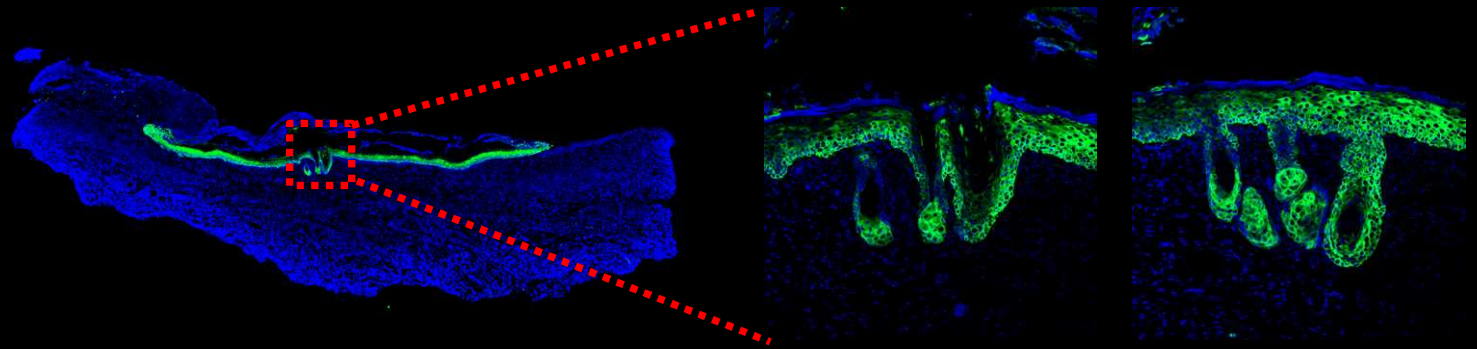
Induction in isolated skin ulcer model



HE

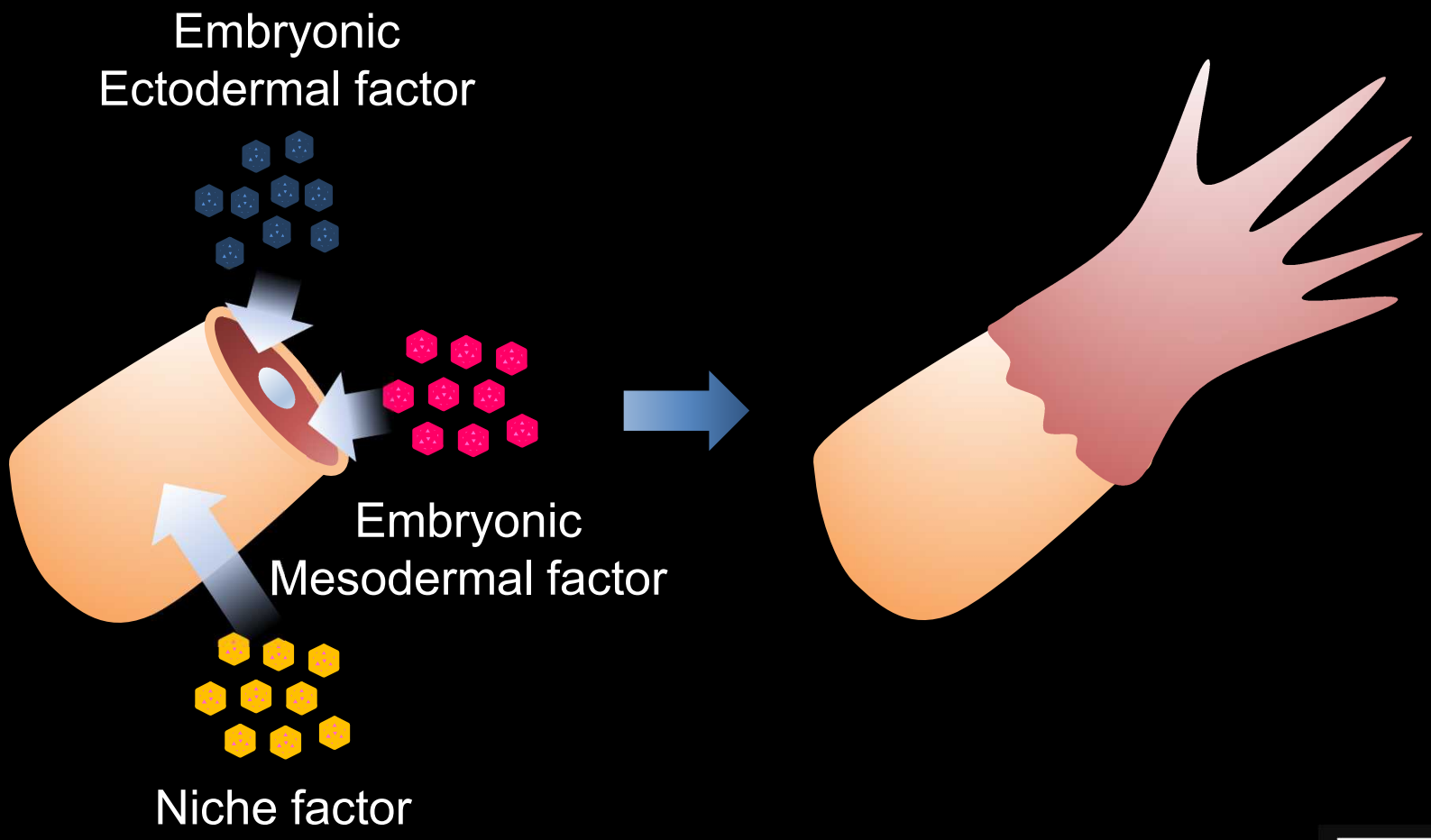


Keratin14
DAPI



Moriwaki et al. *bioRxiv* 2023.03.05.531138 modified

Tissue embryonization for Complex tissue regeneration



Limb regeneration

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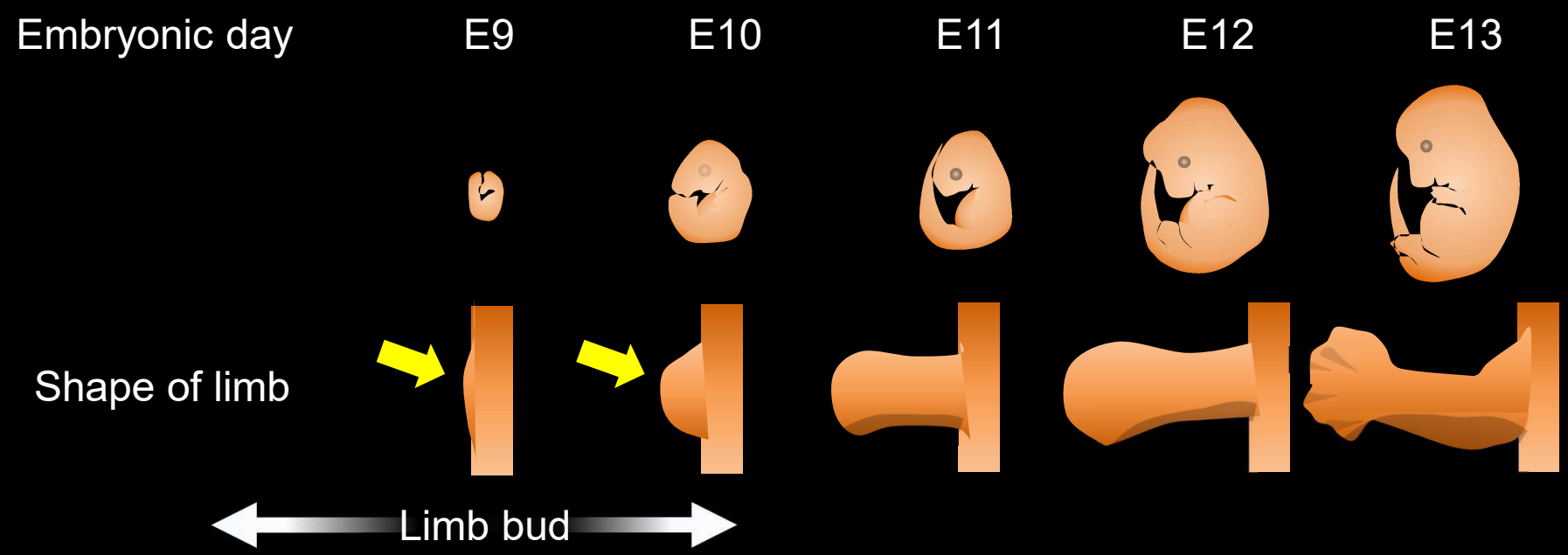
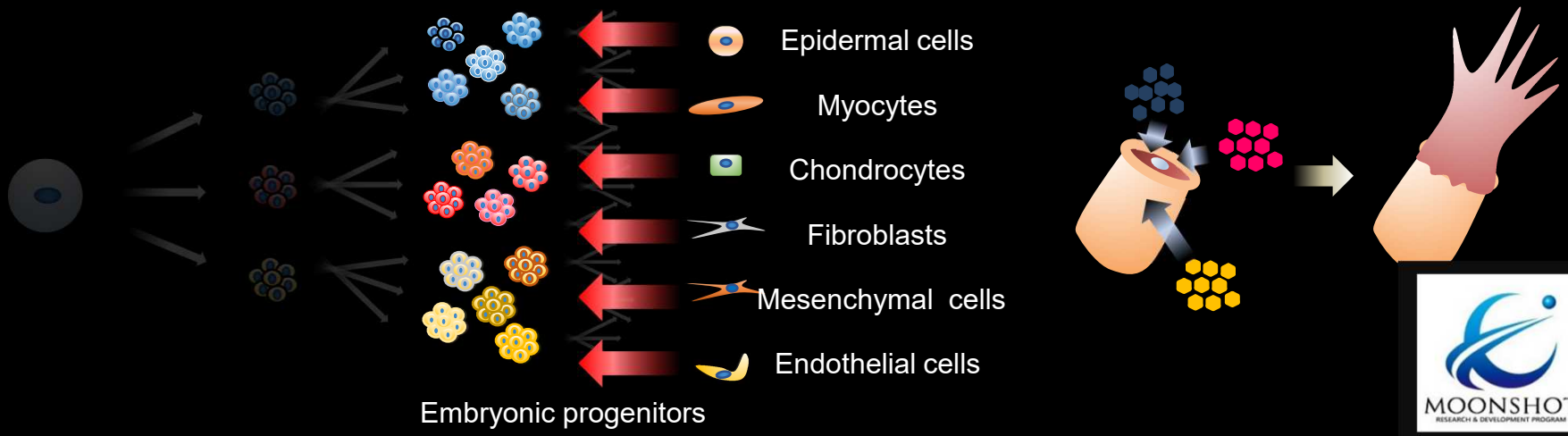
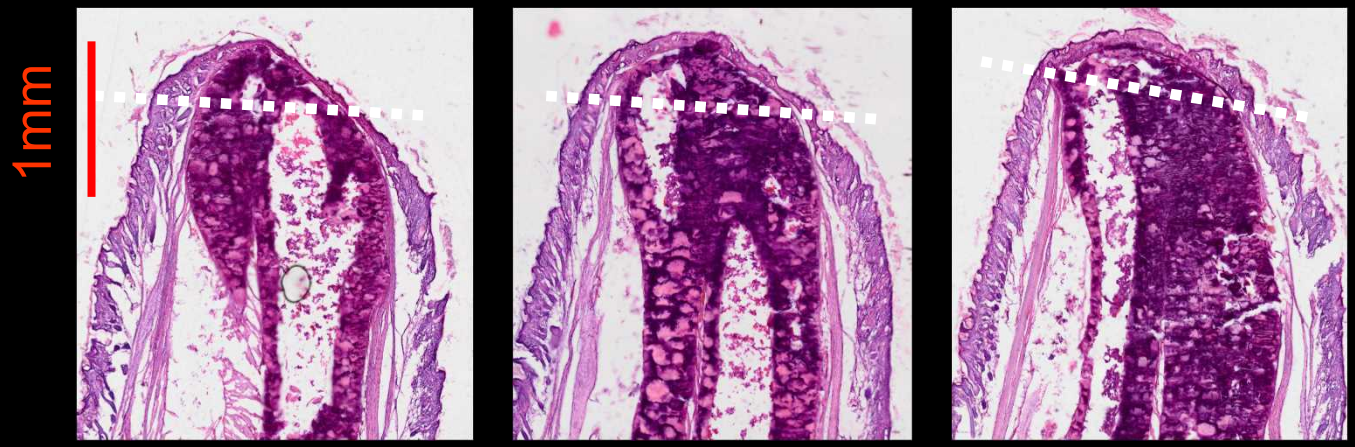
Limb regeneration via tissue embryonization





Induction of regeneration in amputated forelimb



Amputation only

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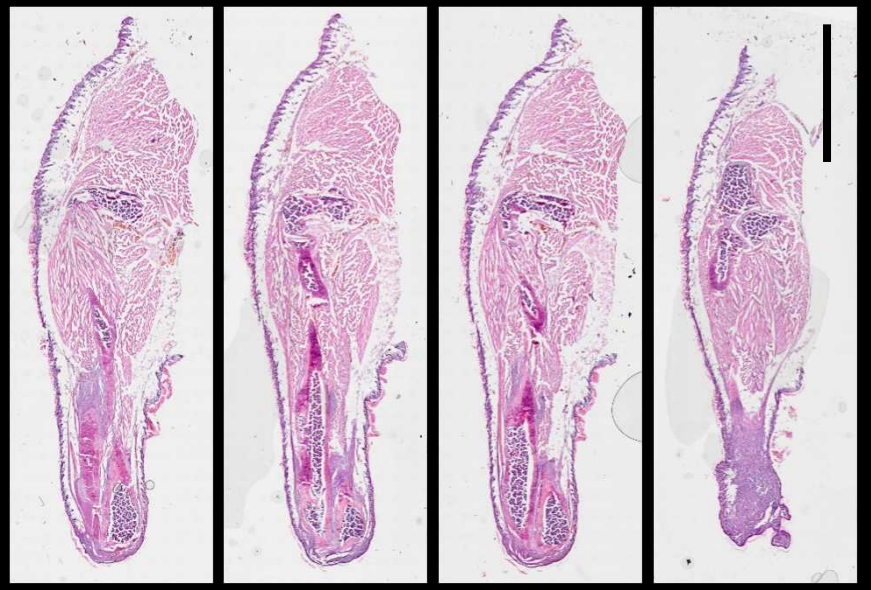
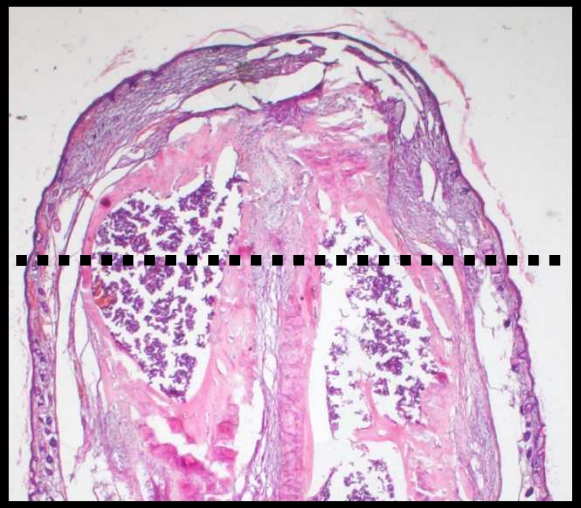
Induction of regeneration in amputated forelimb





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Induction of regeneration in amputated forelimb

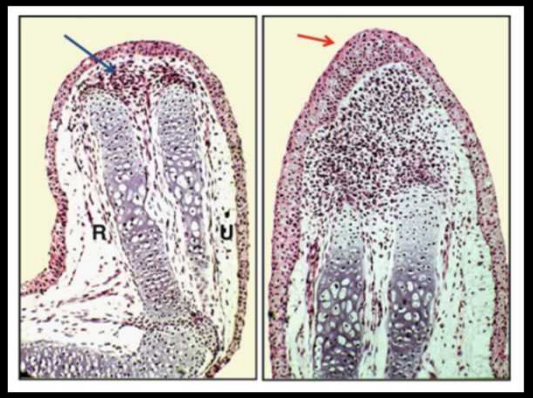
Salamander

McCusker et al. *Regeneration* 2015;2:54-71. modified

Frog

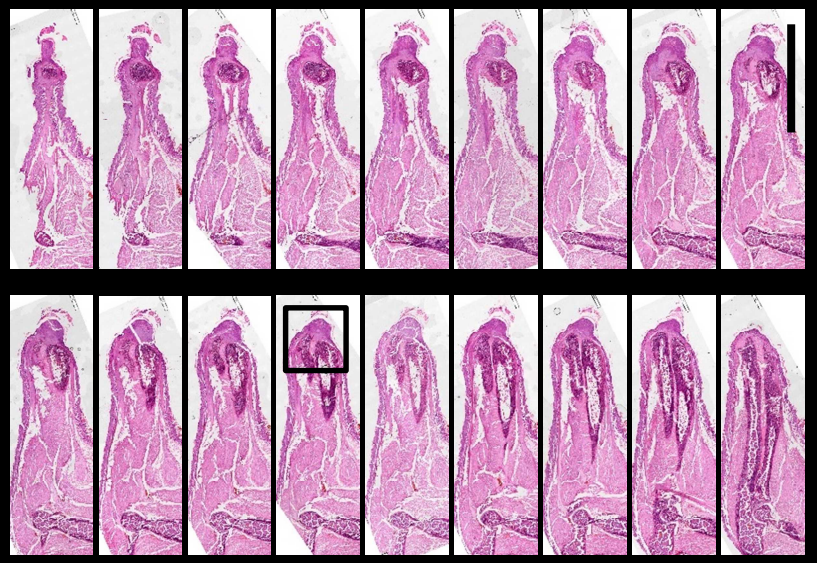
Suzuki et al. *ScientificWorldJournal*. 2006;6 Suppl 1:26-37.

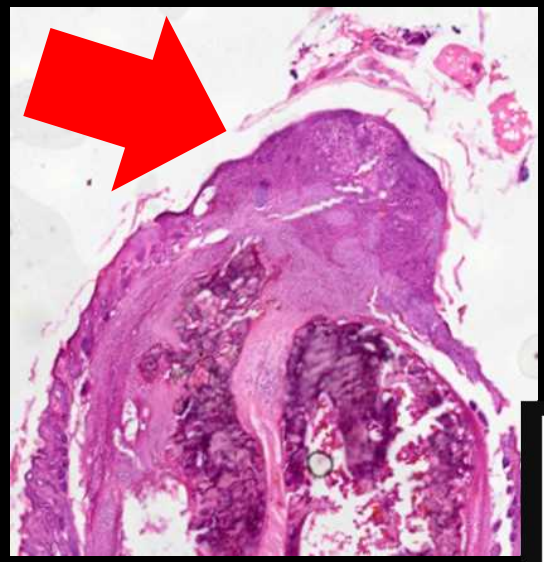
Blastema



Heber-Katz et al. *Curr Top Microbiol Immunol.* 2013;367:253-76.

Induction of regeneration in amputated forelimb





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Induction of regeneration in amputated forelimb

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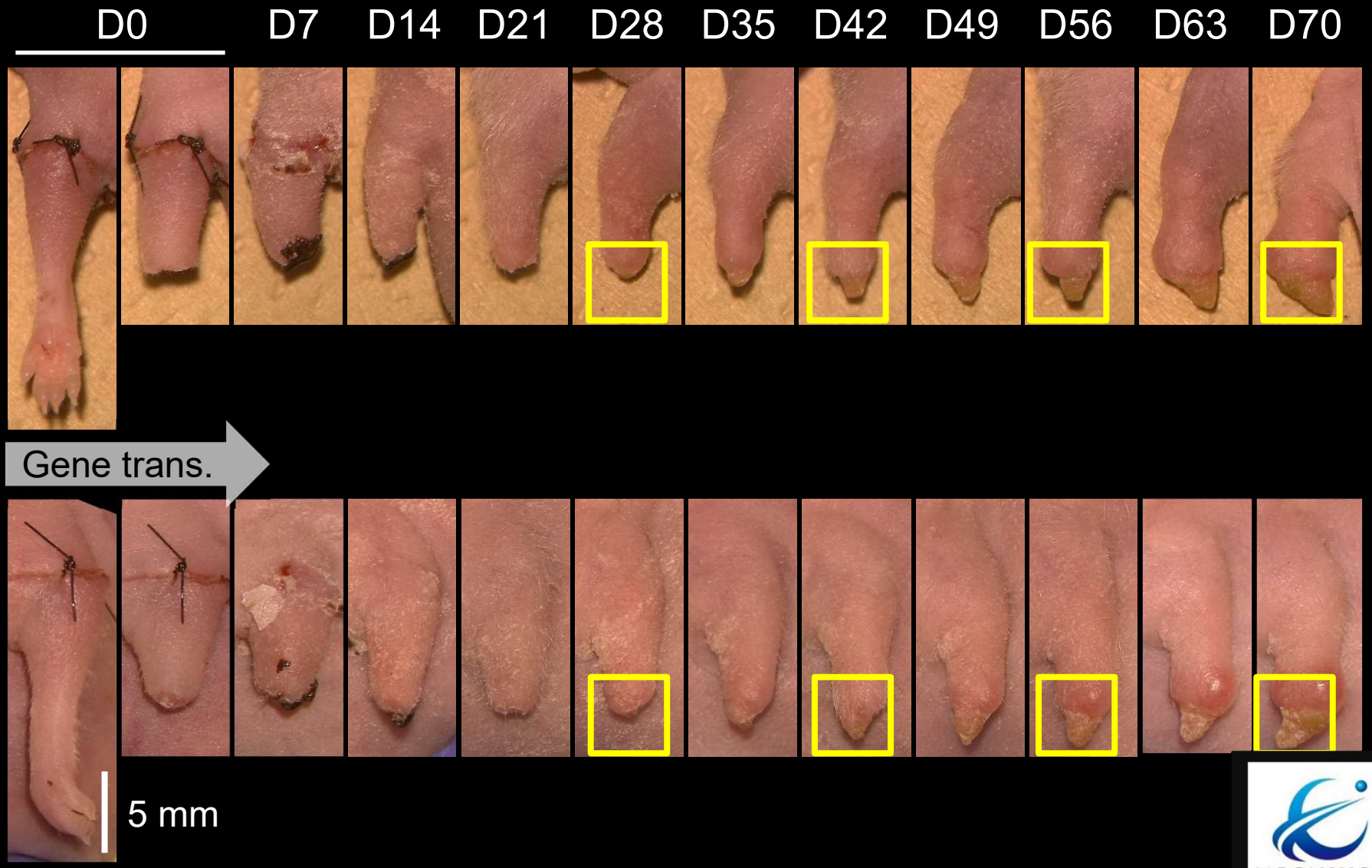
Induction of regeneration in amputated forelimb



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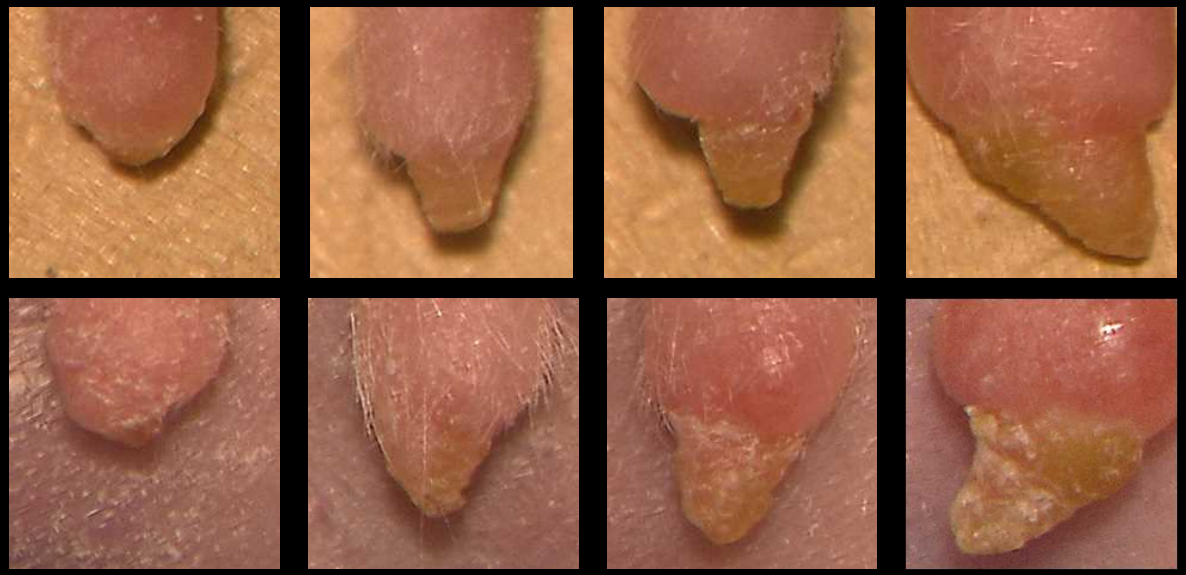
Induction of regeneration in amputated forelimb

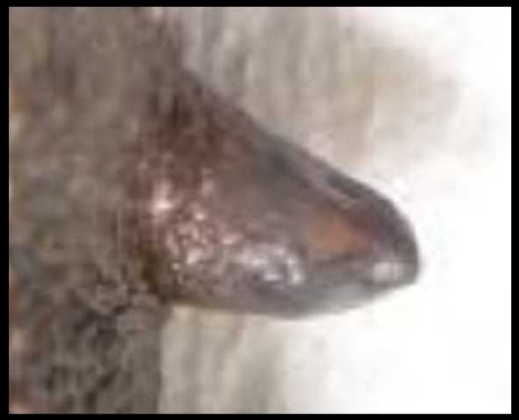
D28 D42 D56 D70



Lizard Tail

Gilbert et al. *Regeneration (Oxf)*. 2015;2(2):45-53.

Newt Limb



Casco-Robles et al. *Biomedicines*. 2021;9(10):1426.

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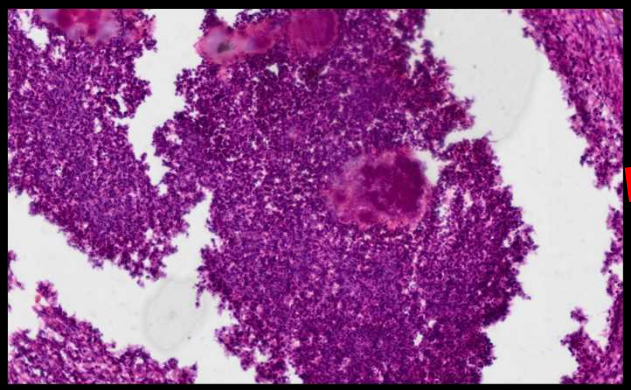
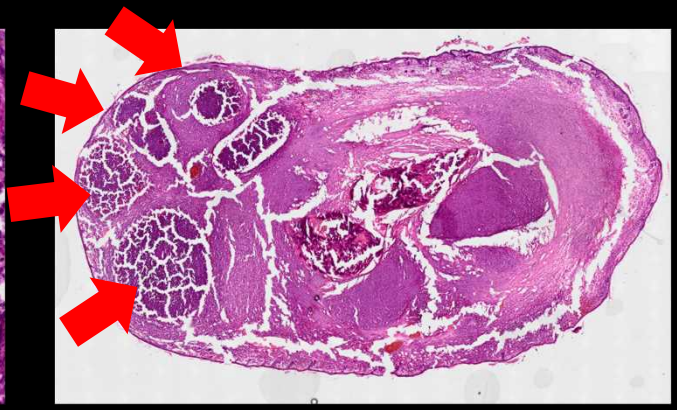
Induction of regeneration in amputated forelimb





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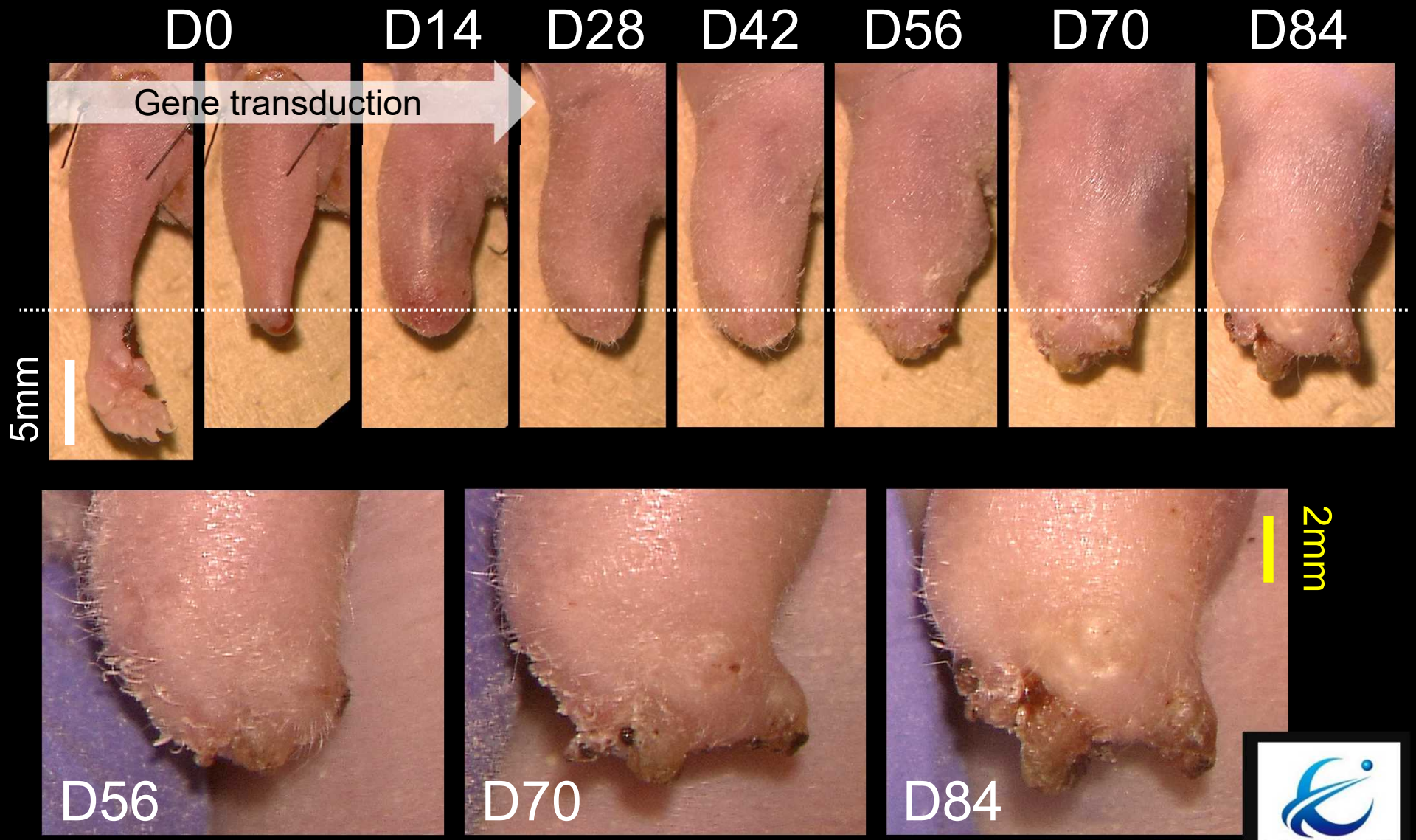
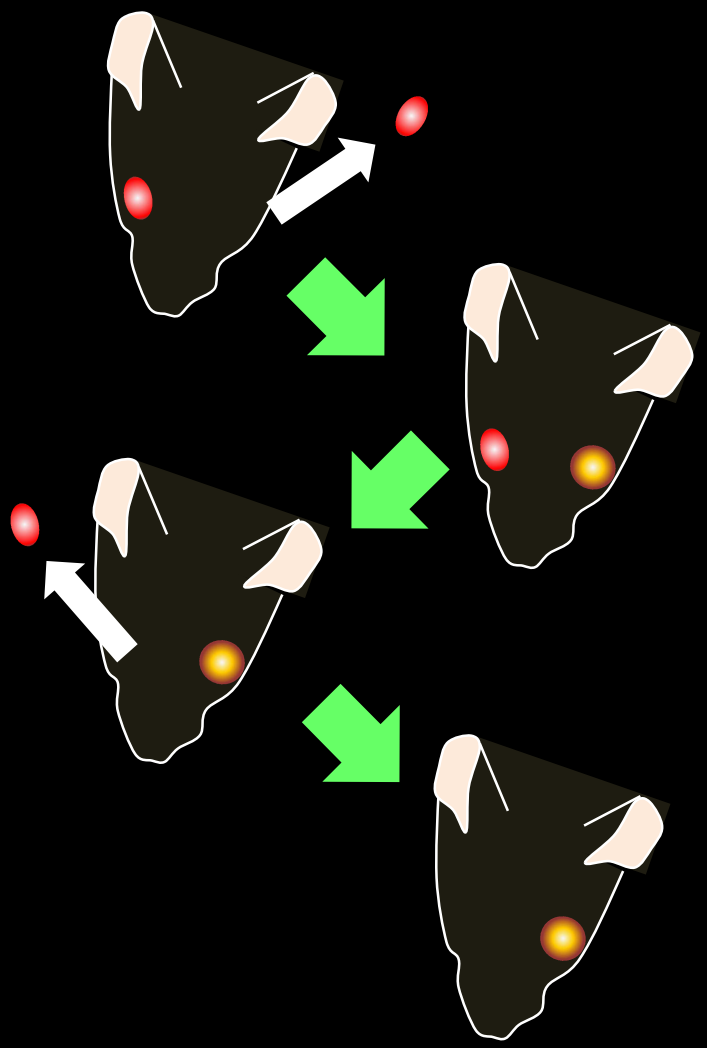
Induction of regeneration in amputated forelimb

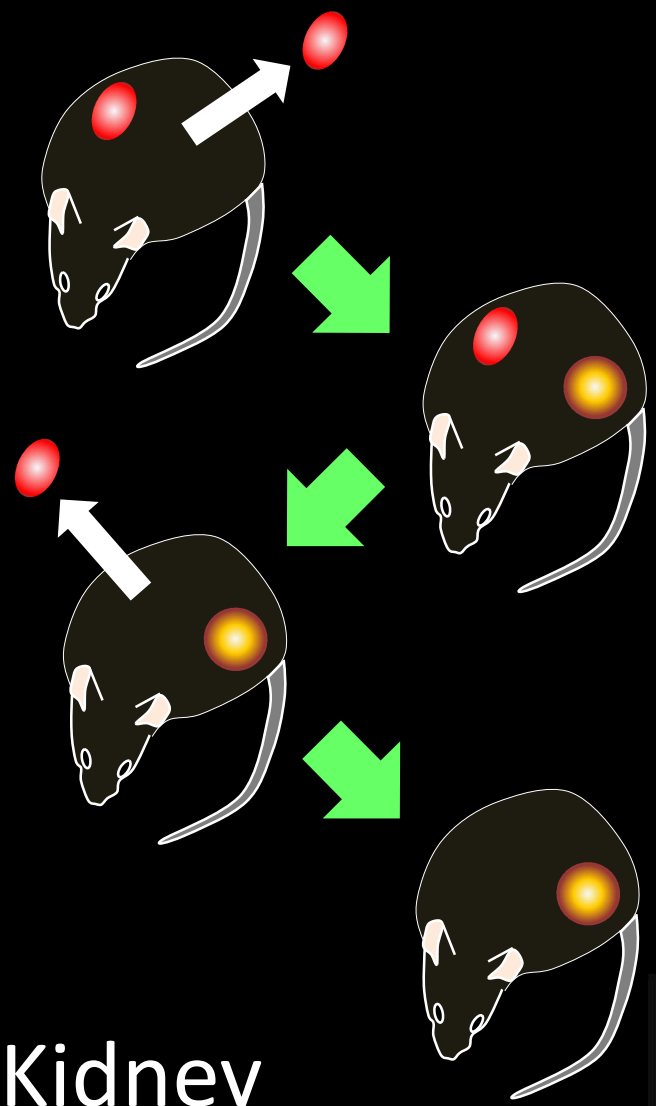


Unpublished data

Complex tissue generation via tissue embryonization



Orbital content



Kidney

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Moonshot program collaborators

The University of Tokyo
Faculty of medicine

Hironori Hojo

Bone regeneration
Bio infomatics

Hiroyuki Okada

The University of Tokyo
Faculty of engineering

Takamasa Sakai

Bio material

Takuya Katashima
Shohei Ishikawa

The University of Tokyo
Faculty of medicine

Gojiro Nakagami

Geriatric nursing
Nursing science
and engineering

Qin Qi
Daijiro Haba
Mao Kunimitsu

Osaka University
Institute for Advanced
Co-Creation Studies

Keiichiro Suzukii

Genome editing
Molecular Biology

MOONSHOT
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Moonshot program collaborators

DDS/ Carrier development

Ishikawa et al. *ACS Macro Lett.* 2023;12(4):510-517.

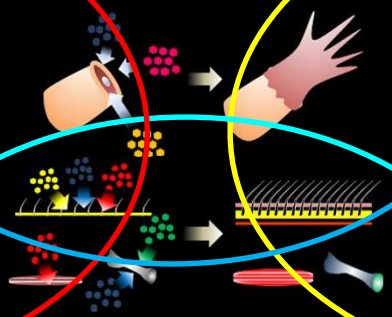
Kato et al. *Commun Biol.* 2023;6(1):508.

Ishikawa et al. *Nature materials.* In press

Searching factors

Hojo et al. *Cell Rep.* 2022;40:111315.

Tani et al. *Cell Rep.* Cell Rep. 2023:112276.



Vector development

Skin & soft tissue rejuvenation

Thank you for listening