Regeneration of fat cells from myofibroblasts during wound healing

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Science

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Background
Scars

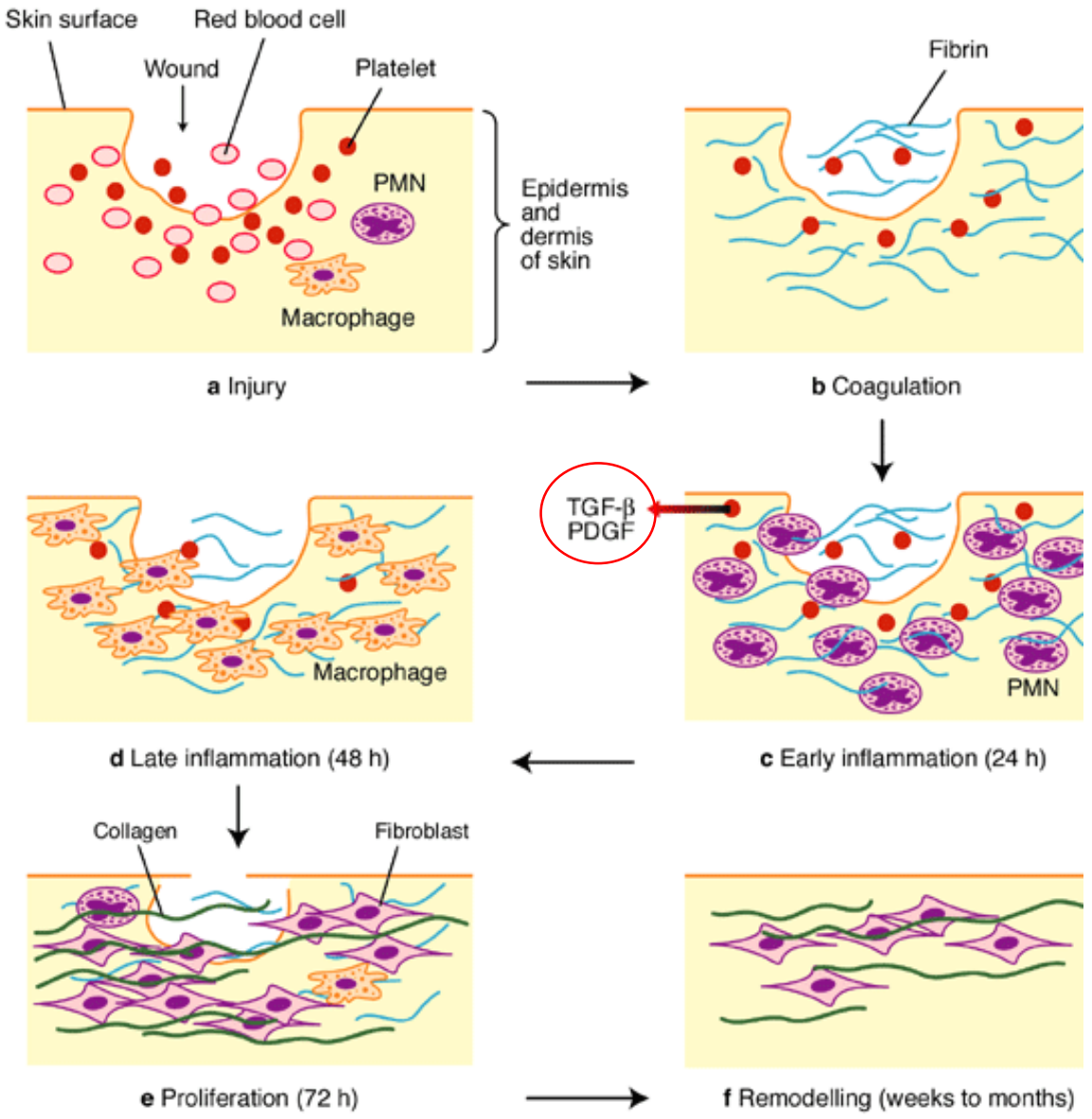
- 100 million people acquire scars every year, approximately 11 million keloid scars and 4 million burn scars
- In the USA, there is an estimated market of 12 Billion Dollars annually on the treatment of skin scarring, and 25 billion dollars were spent related to the treatment of wounds in general in 2015

www.phoenix-society.org

Bayat, A., et al. (2003). "Skin scarring." BMJ : British Medical Journal **326**(7380): 88-92.

Background
Wound healing and scar formation

- Coagulation
- Early inflammation
- Late inflammation
- Proliferation
- Remodelling



The phases of cutaneous wound healing

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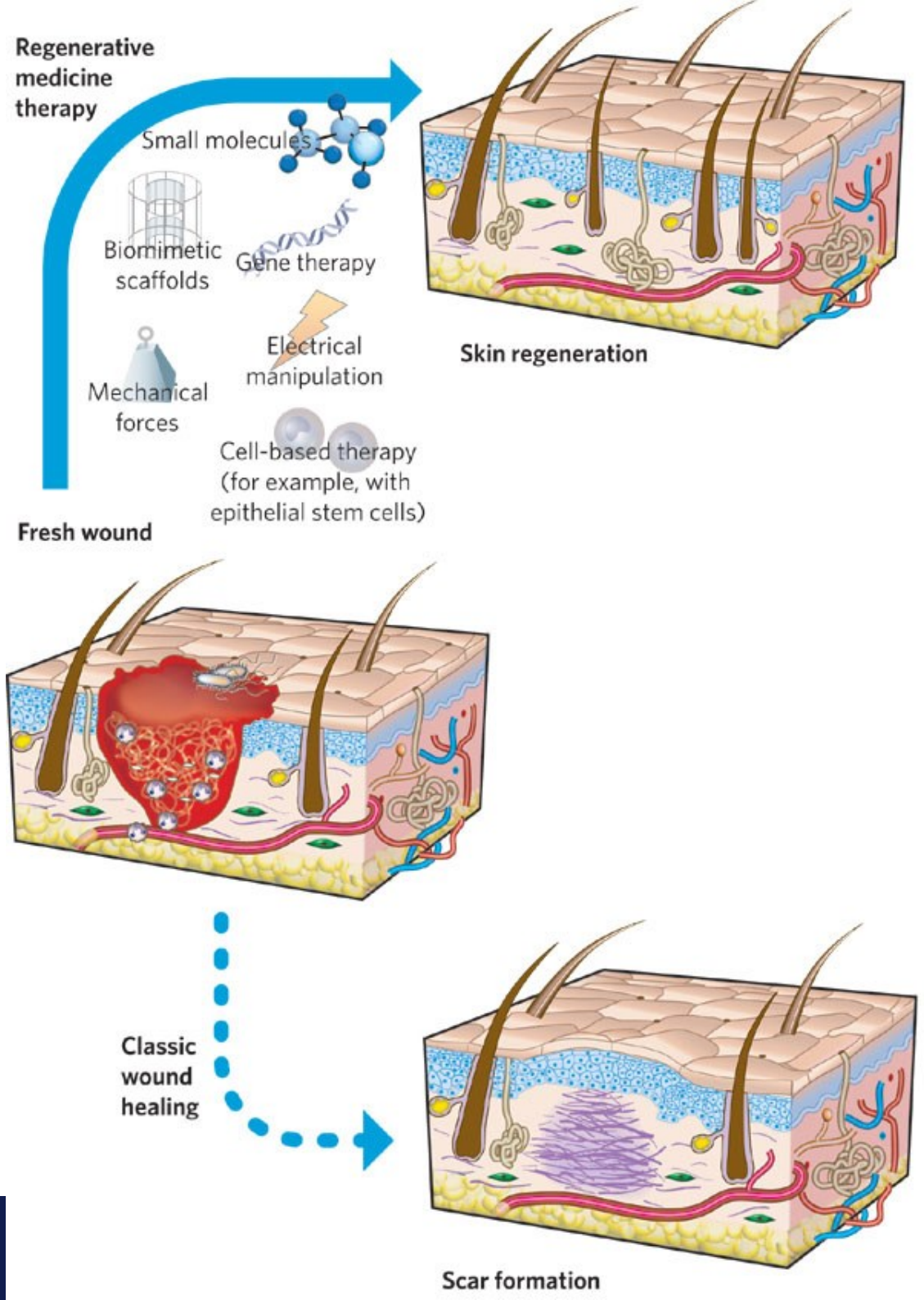
The Bratlie Research Group, Department of Materials Science and Engineering, Department of Chemical and Biological Engineering, Iowa State University, Ames, IA 50011© 2011 Kaitlin Bratlie

Background
Wound healing and scar formation

Scars:

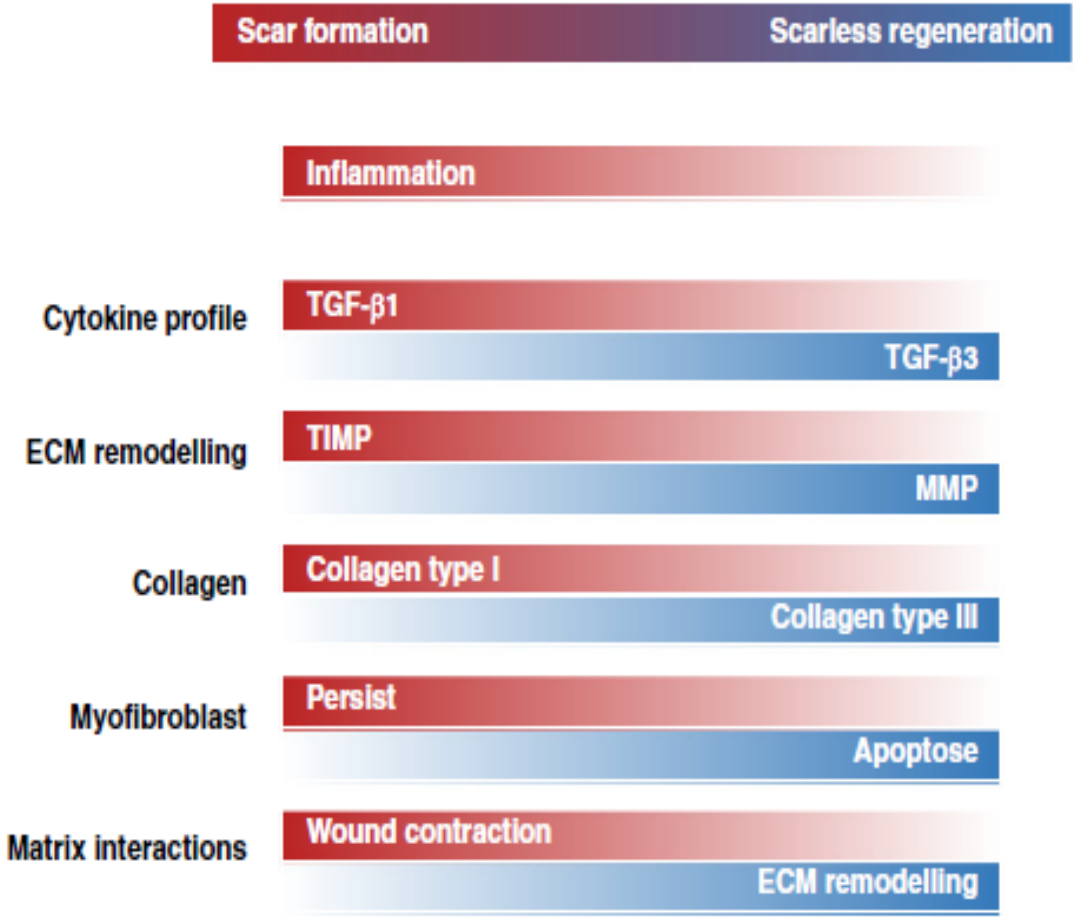
- Excess collagen
- Lack of hair follicles
- Less subcutaneous fat



Geoffrey et al. *Nature* **453**, 314-321 (15 May 2008) | doi:10.1038/nature07039; Published online 14 May 2008

Background
Scar formation

- Scarless wound healing until the end of second trimester
- Differences scar formation-scarless healing:
  - No inflammation
  - Decreased fibrogenic and pro-angiogenic factors
  - Collagen I vs. Collagen III
  - TGF-beta 1 vs. TGF-beta 3

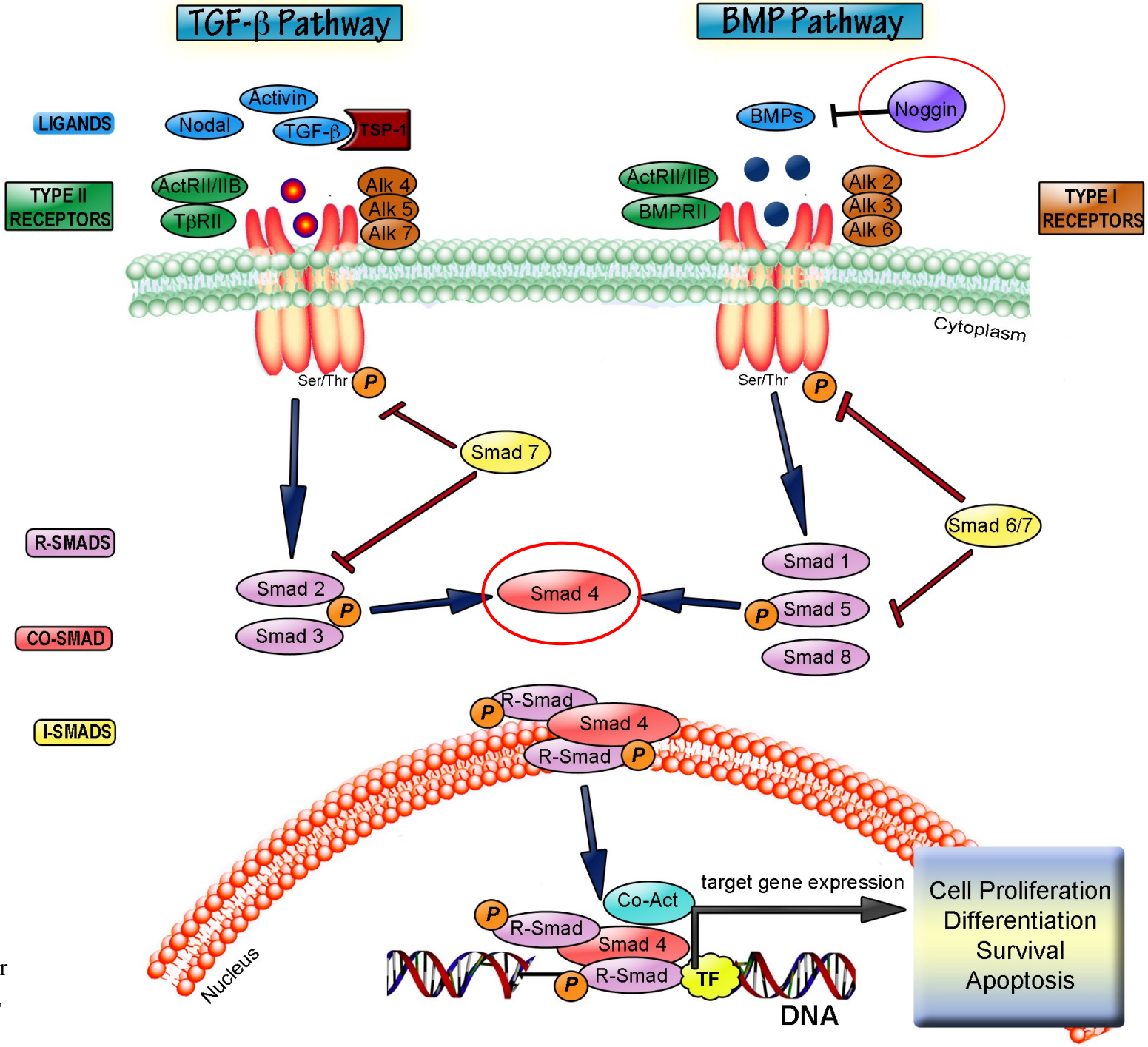


Leavitt, T., et al. (2016). "Scarless wound healing: finding the right cells and signals." Cell Tissue Res **365**(3): 483-493.

Background
BMP & TGF-beta

- BMP = bone morphogenic protein
- TGF-beta = transforming growth factor beta
  - TGF –beta 1-3, Activine, Inhibine
  - BMP= Subgroup of TGF-beta superfamily
  - critical roles in mesoderm formation, heart development, cartilage development and postnatal bone formation.
  - Recombinant BMP-2 and 7 used clinically for interventions such as non-union fractures and spinal fusion

Background
TGFbeta
SMAD pathway
BMP



Sonia Villapol, Trevor T. Logan and Aviva J. Symes (2013). Role of TGF-β Signaling in Neurogenic Regions After Brain Injury, Trends in Cell Signaling Pathways in Neuronal Fate Decision, Dr Sabine Wislet-Gendebien (Ed.), InTech, DOI: 10.5772/53941.

Methods

- **Smart-seq2**: improved single-cell RNA-sequencing, provides expression profile of individual cells
- **Staining of adipose tissue:** Oil Red O dye, skin is viewed from the undersurface in all pictures
- **Meta-analyses.** Transcriptome-wide meta-analyses was performed on microarray and RNA-seq datasets from skin-derived precursors
- FACS, immunostaining, qRT-PCR,
- Mouse adipogenic cell culture, human adipogenic cell culture
- Human scar cell isolation and culture

Results

New adipocytes only regenerate around new hair follicles during wound healing

- Wound healing in humans and in mice: scar with excess collagen and absence of hair follicles
- Large skin wounds in mice regenerate hair follicles via Wnt/FGF pathways

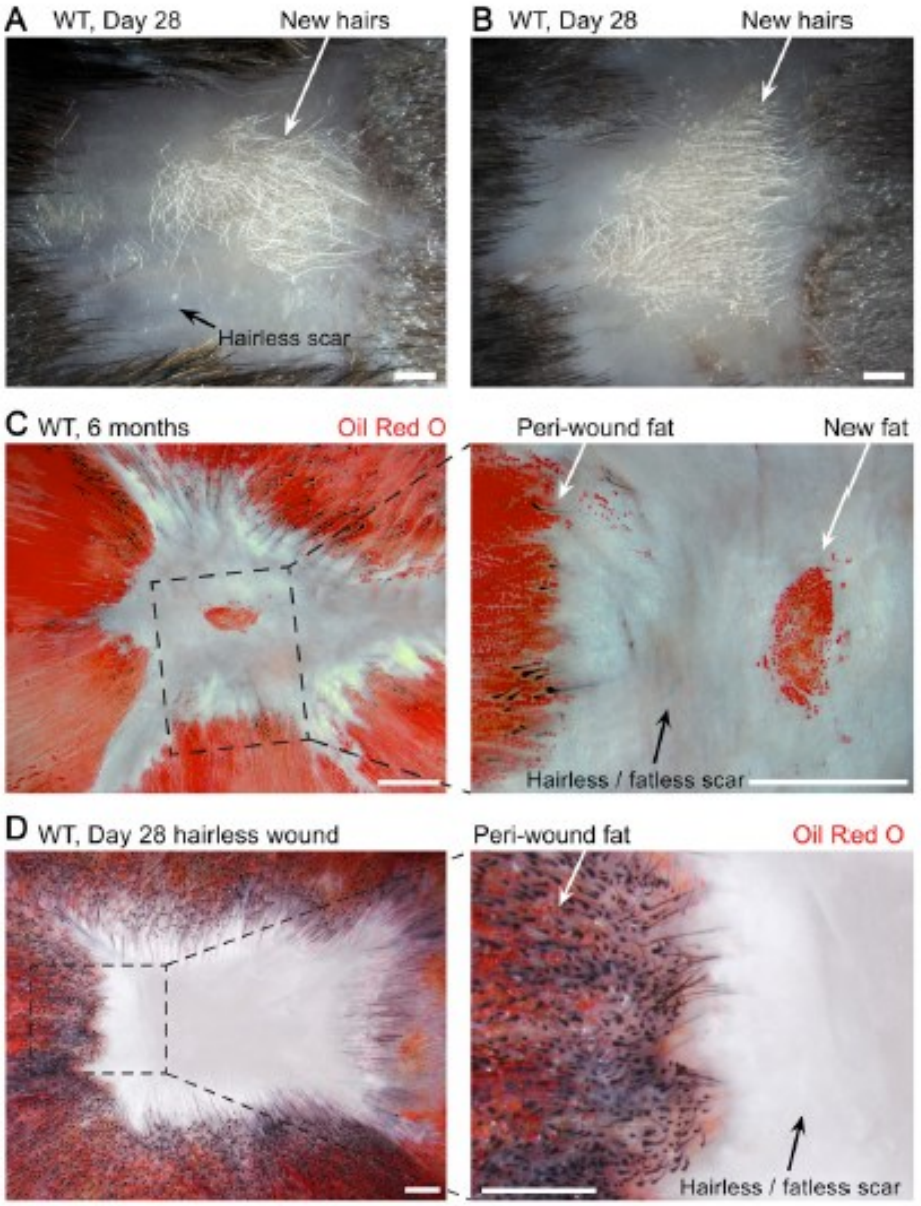


Fig. S1: Wound induced new hair follicles and new fat. (A-B) View from skin surface.

Results

New adipocytes only regenerate around new hair follicles during wound healing

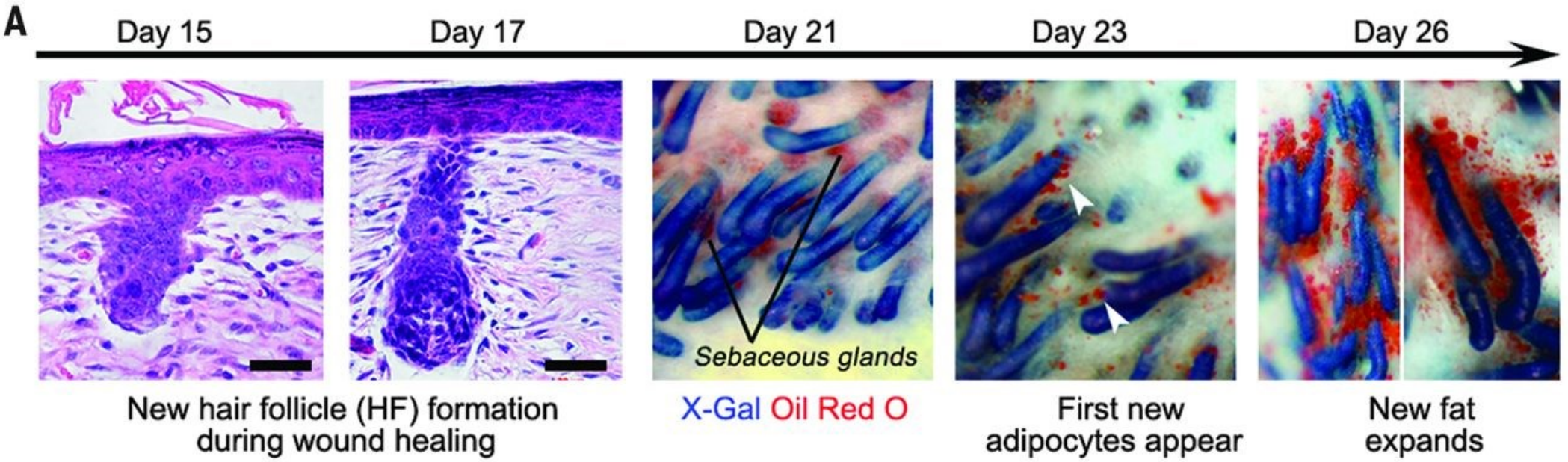


Fig. 1A New adipocytes (orange) only regenerate around new hair follicles (blue) during wound healing.

"New adipocytes are undistinguishable in size, density, and depth from skin surface."

Results

New adipocytes only regenerate around new hair follicles during wound healing

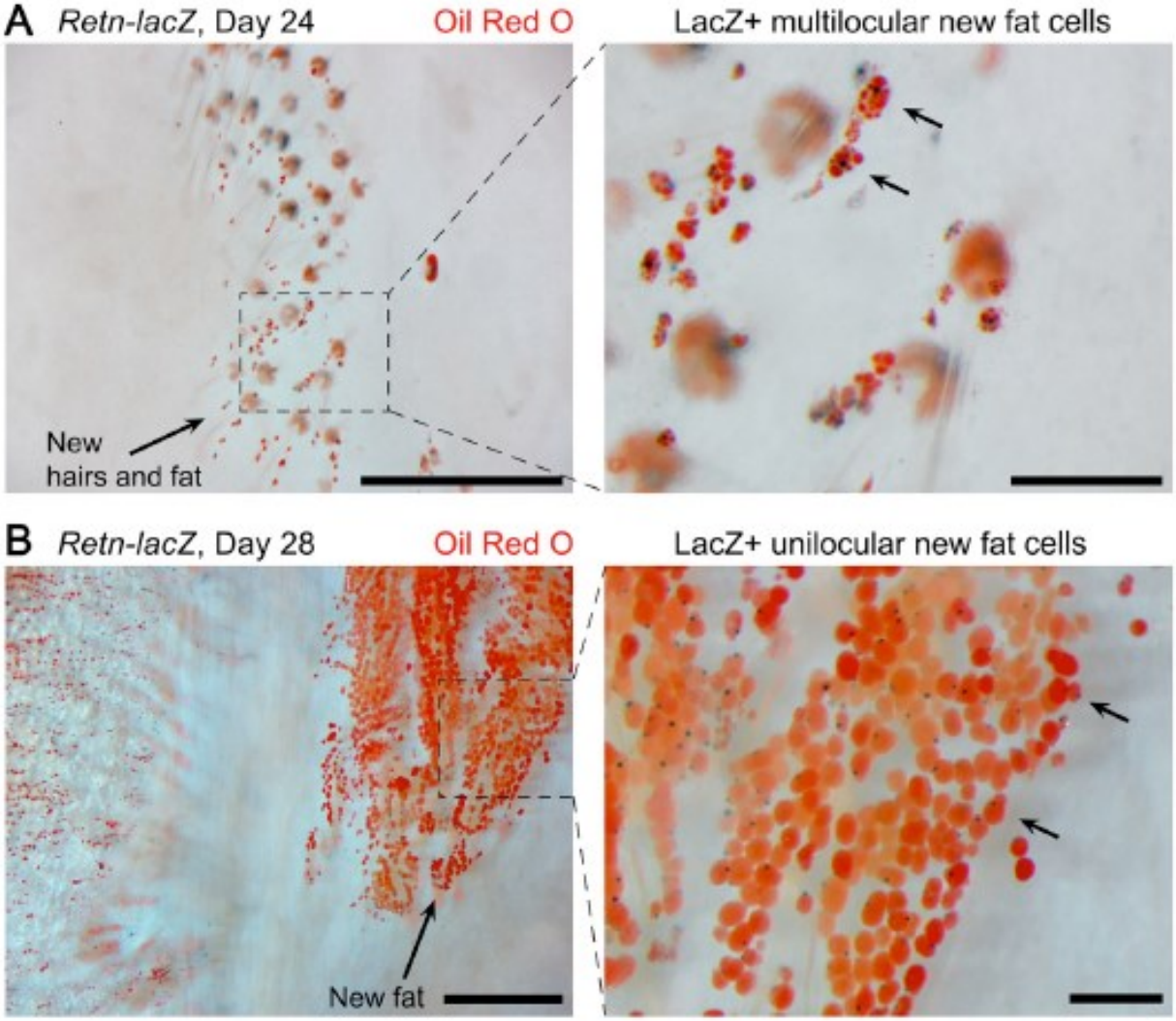


Fig. S2:
Maturation of new adipocytes.

Mature adipose tissue cells:

- Resistin
- Adiponectin
- lacZ-positive cells

„Are hair follicles necessary to establish adipocyte precursors?“

Results

New adipocytes only regenerate around new hair follicles during wound healing

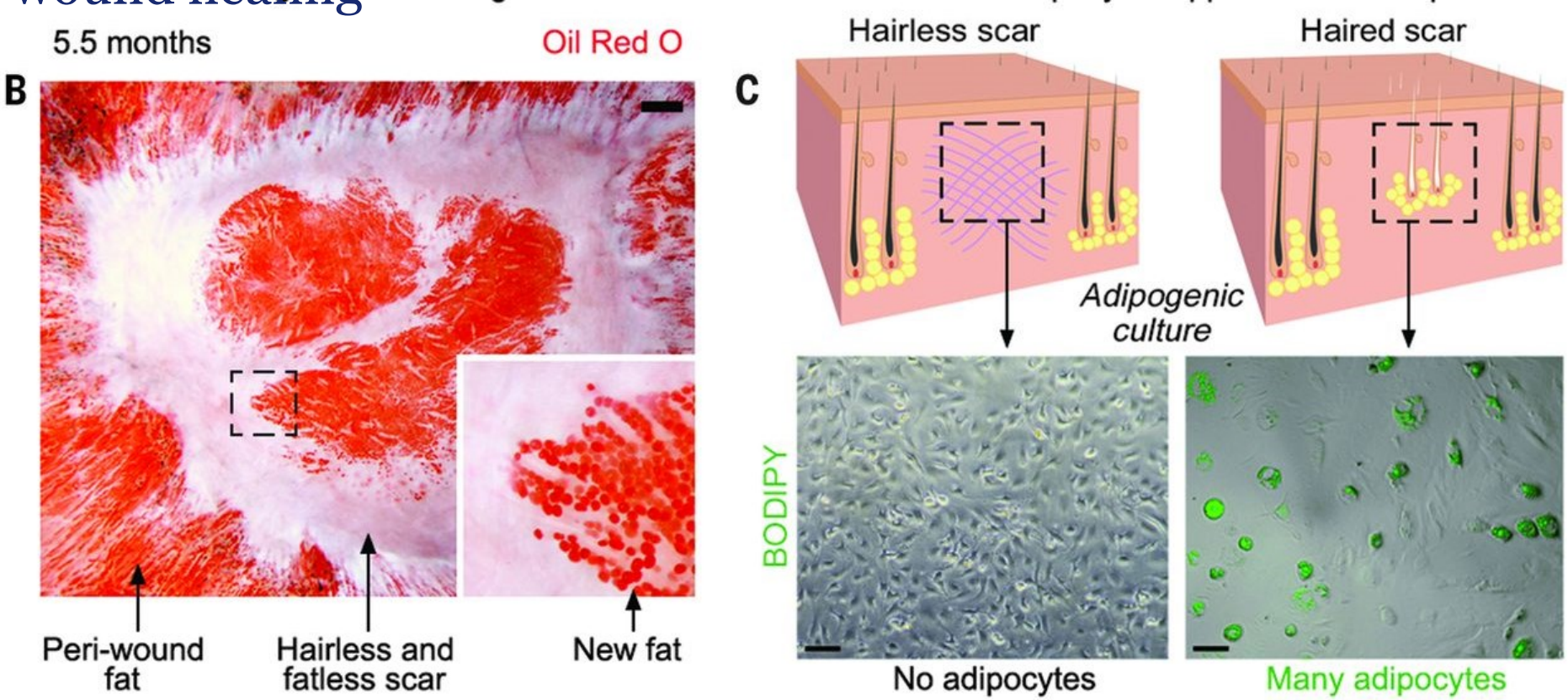


Fig. 1B, C:

- dermal cells from wounds with regenerated hair follicles differentiated into adipocytes
- Cells from wounds without hair follicles did not differentiate into adipocytes

Results

New adipocytes originate from wound myofibroblasts.

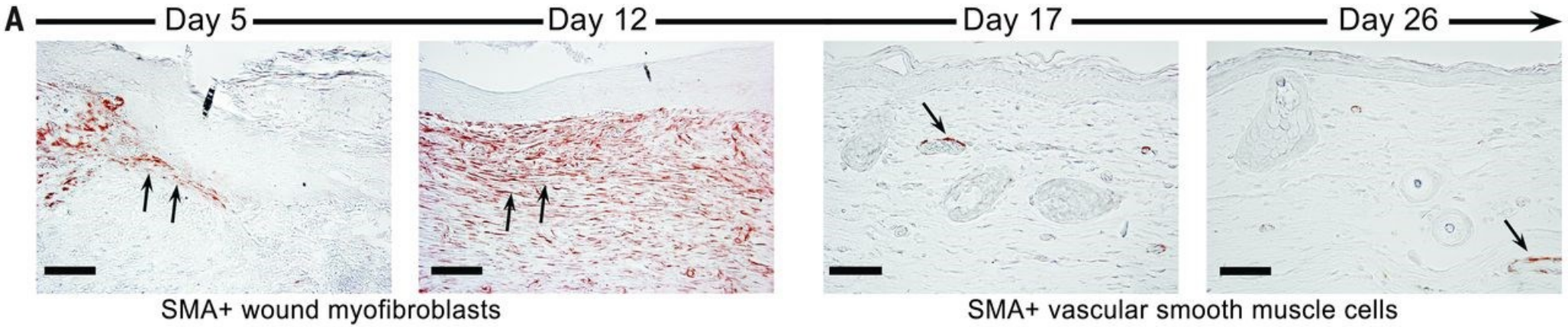


Fig 2A:

- Myofibroblasts appear in wounds on day 5, abundant in scar by day 12
- No expression of smooth muscle actin by day 12

Results

New adipocytes originate from wound myofibroblasts.

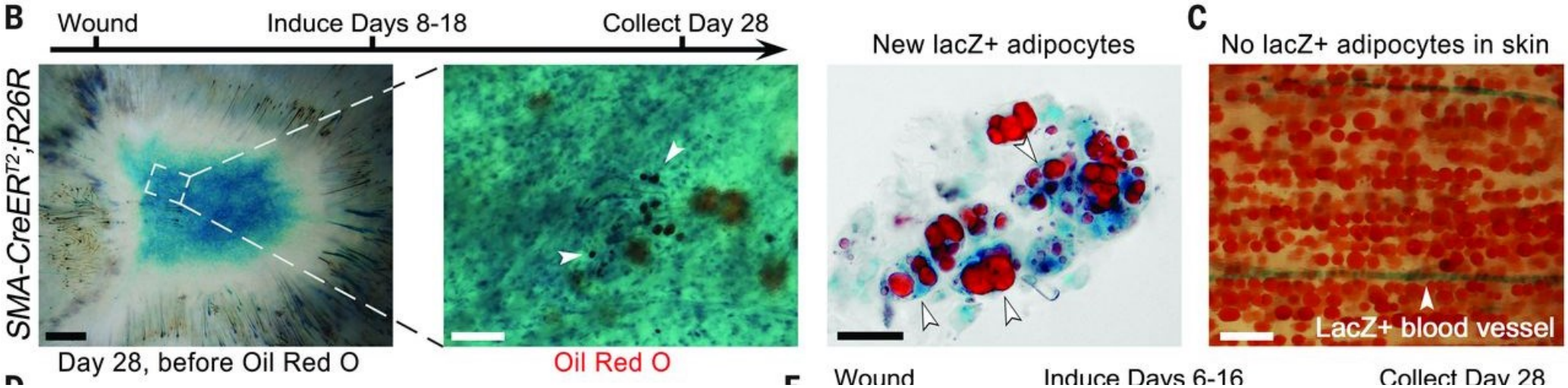


Fig. 2B, C: Cellular origin of adipocytes?

- SM22Cre and SMACre are not activated in normal white fat
- in SM22Cre and SMACreER Mice: new adipocytes in wounds express LacZ
- LacZ in adipocytes indicates myofibroblast origin

Results

New adipocytes originate from wound myofibroblasts.

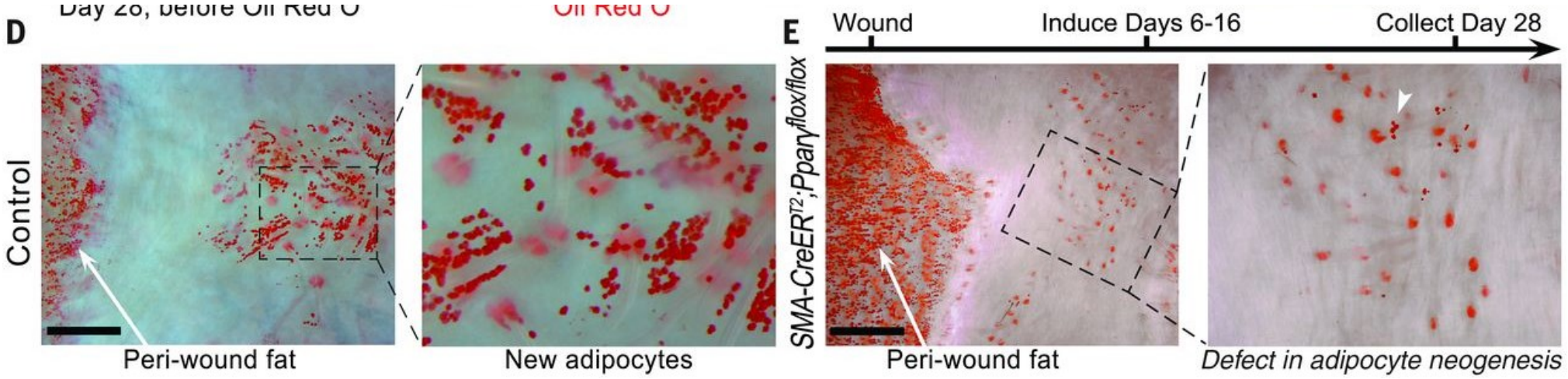


Fig 2D, E:

- Deletion of Pparγ in myofibroblasts resulted in near-complete loss of new adipocytes
- normal cutaneous adipocytes at the wound edge remained intact.

Lineage tracing experiments: myofibroblasts are the source for new regenerating adipocytes.

Results

Molecular profiling and functional studies of adipocyte regeneration reveal that ZFP423 and BMP signaling are necessary for adipocyte regeneration

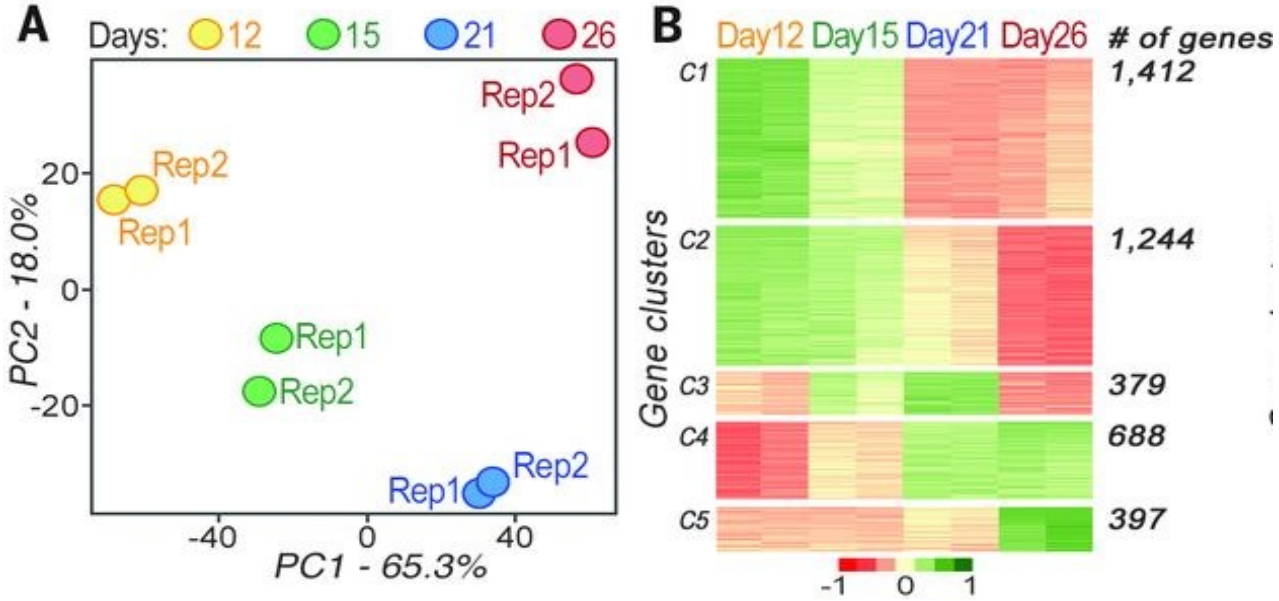


Fig 3A: myofibroblast transcriptome reveals distinct changes across four postwounding time points

Fig. 3B: 4120 differentially expressed genes are expressed in 5 clusters

- Upregulated: Regulators of adipocyte lineage (Zfp 423, Cerbl2, Stat5b
- Downregulated: chondro/osteogenic: Sox9, Runx1/2,…

Results

Molecular profiling and functional studies of adipocyte regeneration reveal that ZFP423 and BMP signaling are necessary for adipocyte regeneration

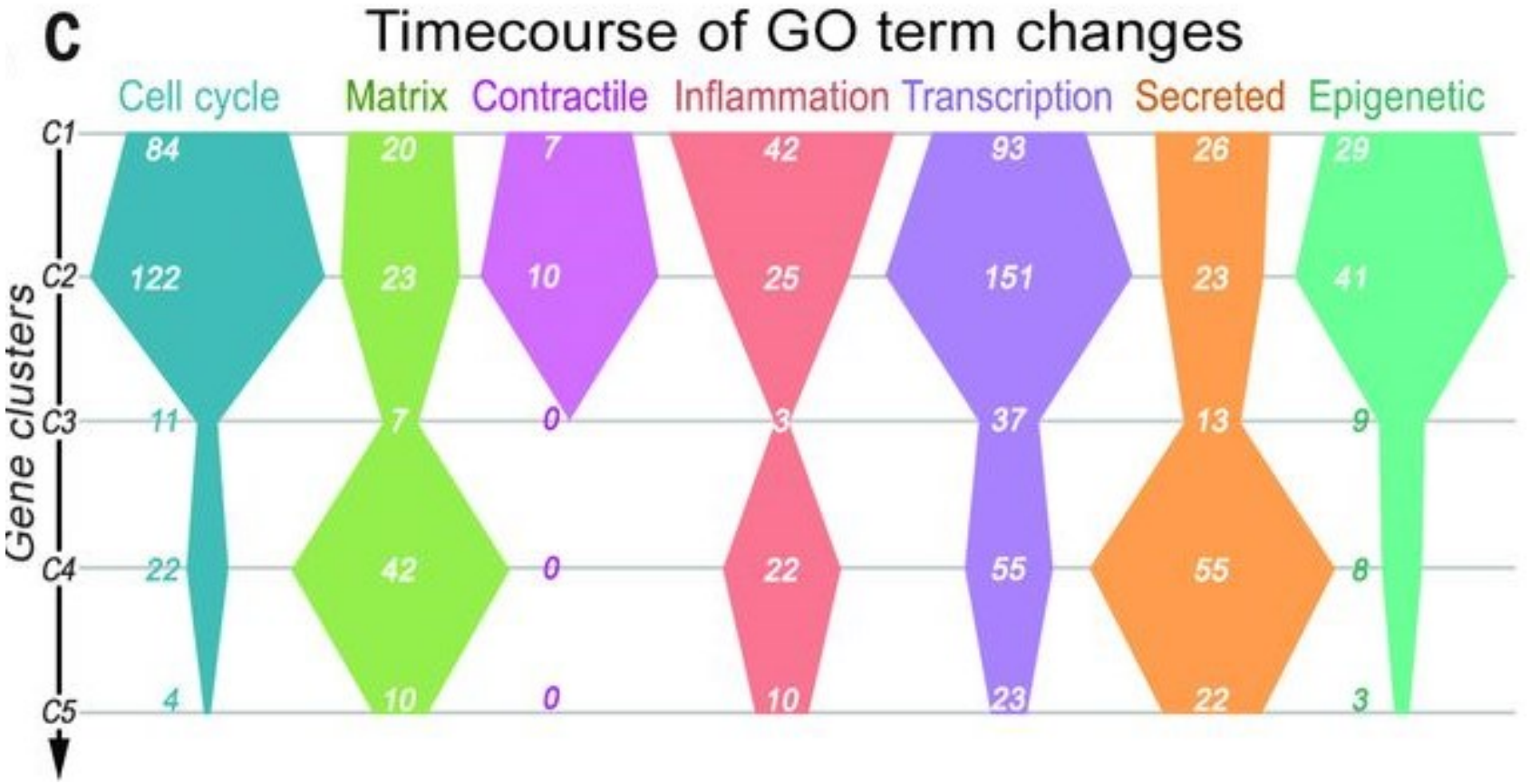


Fig 3C: temporal changes of gene expression in myofibroblasts

Results

Molecular profiling and functional studies of adipocyte regeneration reveal that ZFP423 and BMP signaling are necessary for adipocyte regeneration

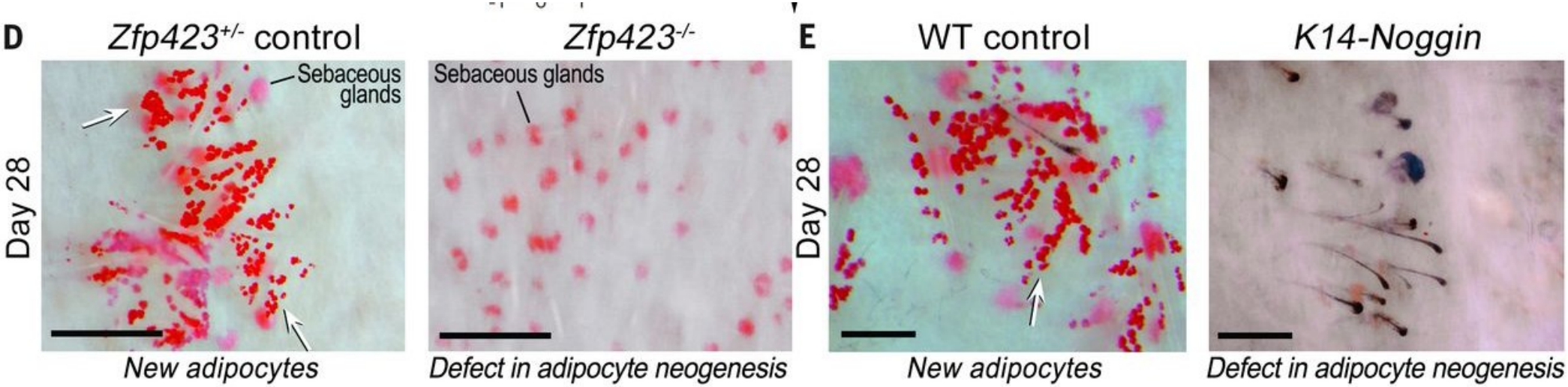


Fig 3D: Zfp 423 mutant mice fail to regenerate fat completely but show no difference of adipocytes during development.

Fig 3E: Noggin = soluble BMP-antagonist
K14-mice overexpress Noggin; they fail to regenerate adipocytes but form hair follicles

Results

Molecular profiling and functional studies of adipocyte regeneration reveal that ZFP423 and BMP signaling are necessary for adipocyte regeneration

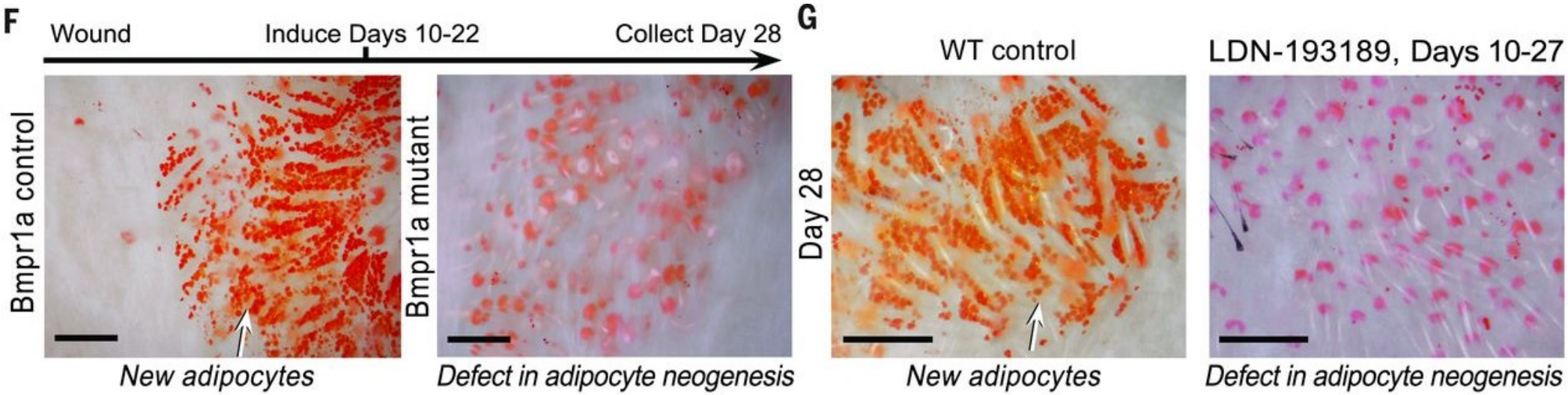


Fig 3F: deletion of BMP-receptor leads to lack of new adipocytes, but does not impede hair growth

Fig 3G: inhibition of SMAD-1/5/8 prevents adipocyte regeneration in hair-bearing wounds

Results

4 BMP drives reprogramming of mouse myofibroblasts and human keloid fibroblasts into adipocytes.

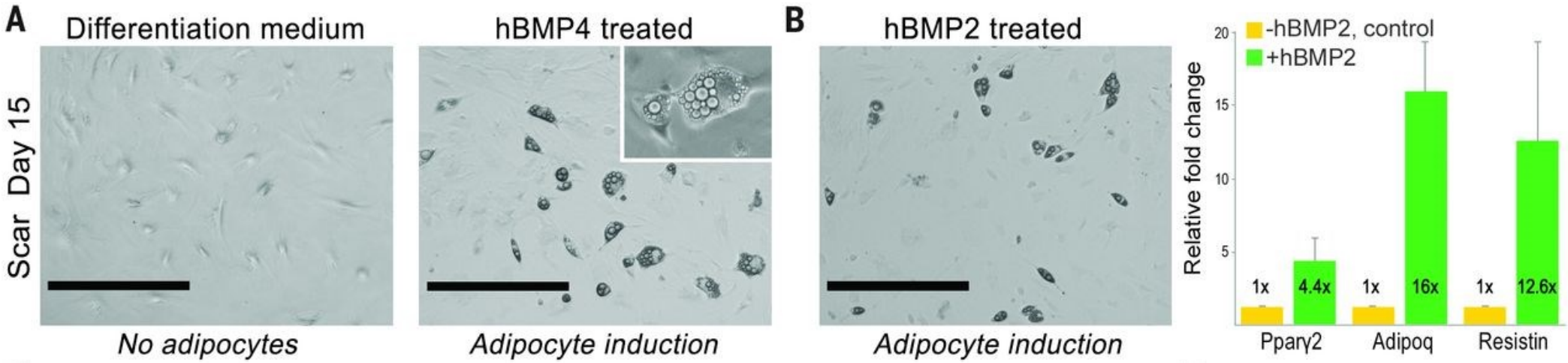


Fig 4A: human keloid scar cells treated with BMP4 induce conversion to adipocytes

Fig 4B: mouse dermal wound cells treated with BMP2 activate adipocyte-specific genes

Results

4 BMP drives reprogramming of mouse myofibroblasts and human keloid fibroblasts into adipocytes.

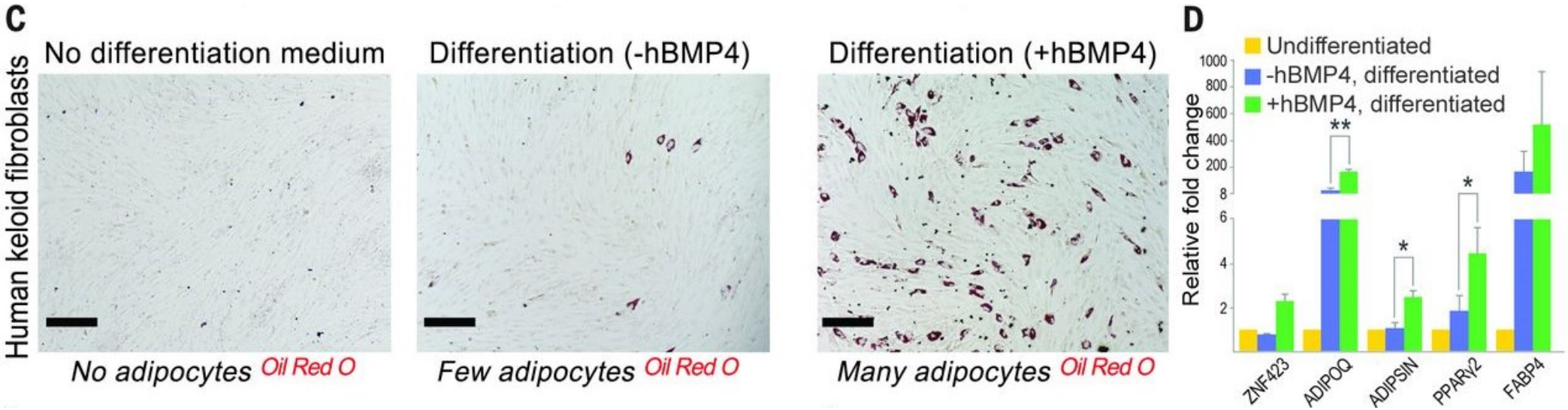


Fig 4C:Treatment of cultured human keloid scar cells with human recombinant BMP4 induces reprogramming into adipocytes

Fig 4D: BMP4-induced activation of adipocyte-specific genes

Results

4 BMP drives reprogramming of mouse myofibroblasts and human keloid fibroblasts into adipocytes.

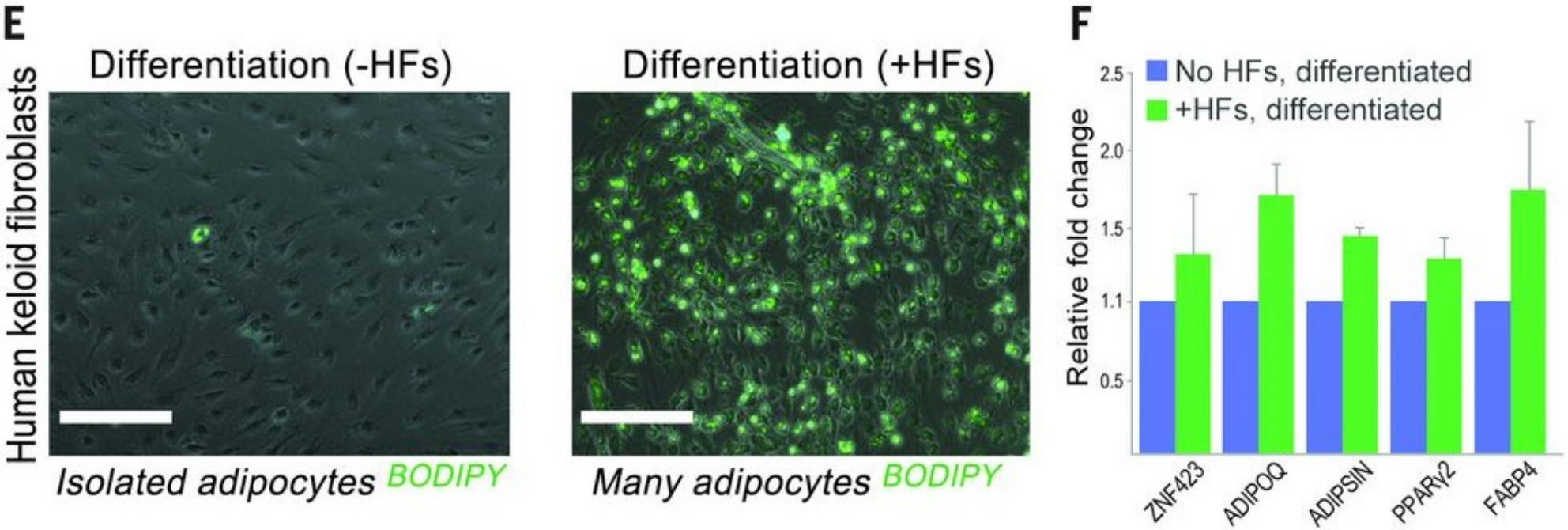


Fig 4E: human scalp hair follicles induced adipogenic conversion of human keloid scar cells

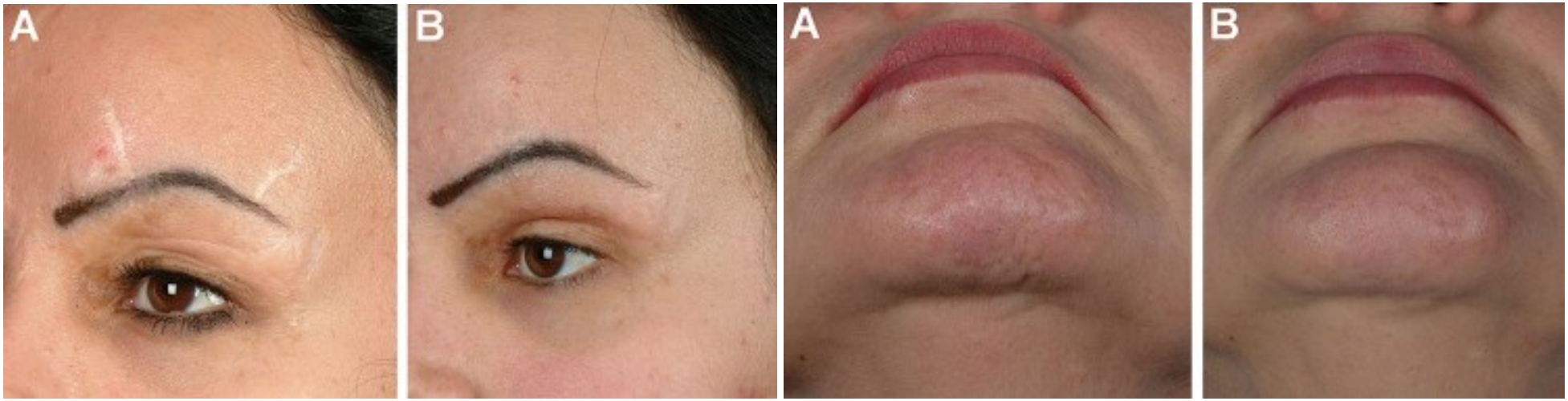
Fig 4F: increase in adipocyte genes in coculture with hair follicles

Discussion

- new hair follicles in a wound reprogram myofibroblasts to an adipocyte fate by activation of the BMP-ZFP423 pathway
- observed conversion of myofibroblasts to adipocytes demonstrates lineage reprogramming in vivo in an adult mammal
- Window of opportunity after wounding to influence regeneration rather than scarring?
- Hair follicles grow independently of fat
- BMP plays a key role for regenerating hair follicles

Discussion

- Regenerating hair follicles could benefit all patients with disorders due to lack of fat
  - Scars Lipodystrophies
  - Keloids Ageing



Pallua, N., et al. (2014). "Improvement of facial scar appearance and microcirculation by autologous lipofilling." J Plast Reconstr Aesthet Surg **67**(8): 1033-1037.

Comments

- Very complex paper- majority of information provided in supplementary material only

- Major part of scar tissue is in dermis, not subcutaneously
- Does improvement of fat regeneration really improve scar quality?
- Significance in human model?
  - Less hair follicles in human skin than in mouse

Questions?
Thank you!