

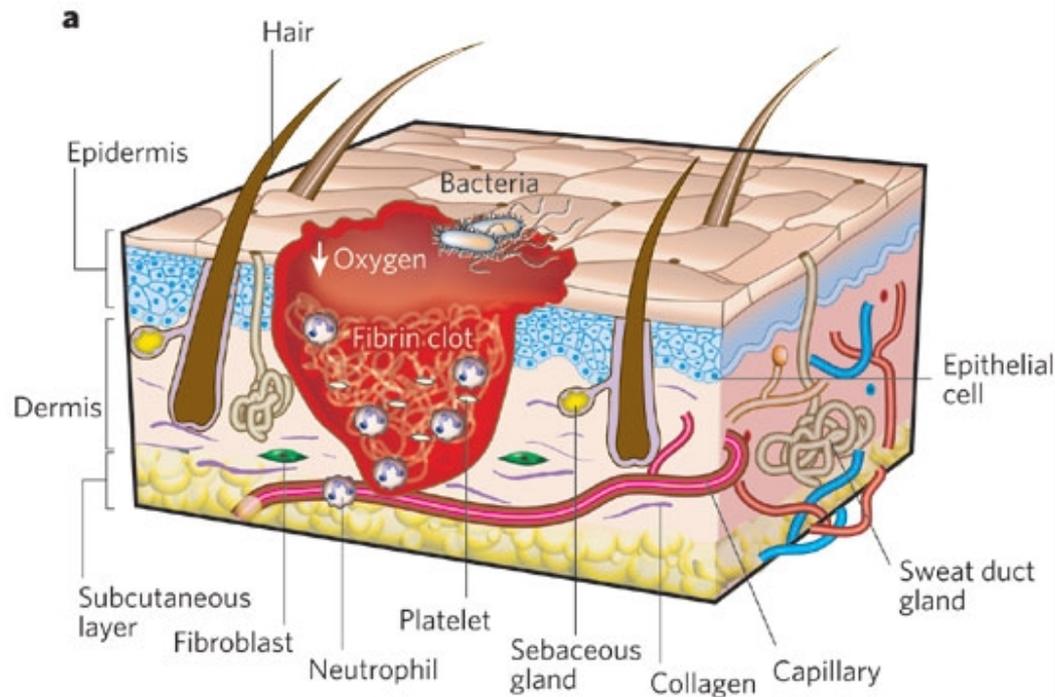
Identification and isolation of a dermal lineage with intrinsic fibrogenic potential

Yuval Rinkevich et al. *Science*
2015;348:aaa2151

Philipp Hacker, 19.10.2015

Introduction

- Wound Healing: Inflammation (48 hours after injury)



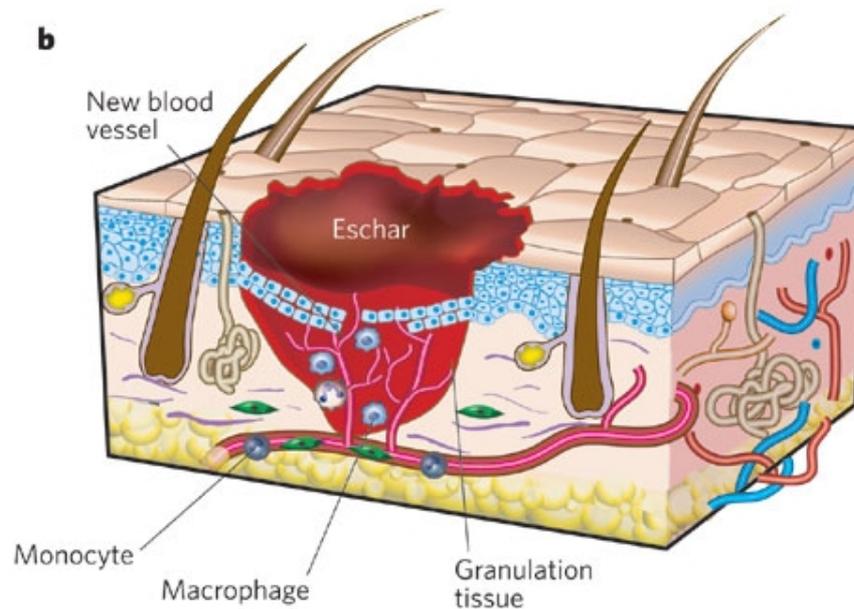
Source: **Wound repair and regeneration**

Geoffrey C. Gurtner, Sabine Werner, Yann Barrandon & Michael T. Longaker

Nature **453**, 314-321(15 May 2008)

Introduction

- Wound Healing: New tissue formation (2-10 days after injury)



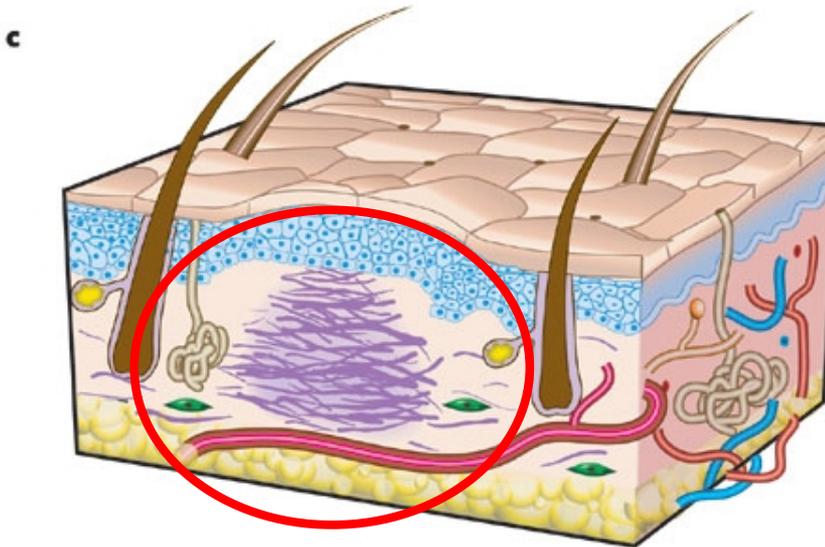
Source: **Wound repair and regeneration**

Geoffrey C. Gurtner, Sabine Werner, Yann Barrandon & Michael T. Longaker

Nature **453**, 314-321(15 May 2008)

Introduction

- Wound Healing: Remodelling (1-12 months after repair)



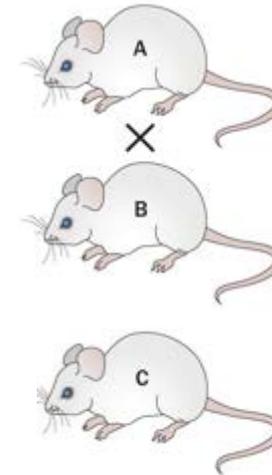
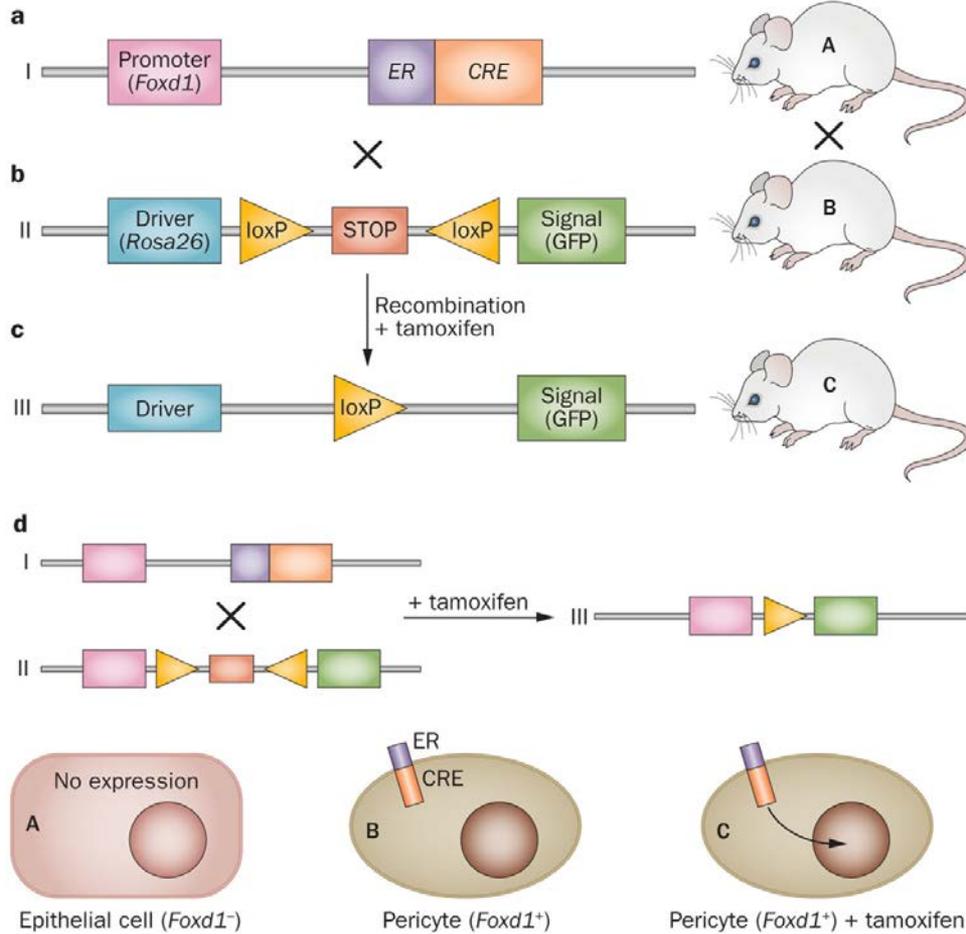
Source: **Wound repair and regeneration**

Geoffrey C. Gurtner, Sabine Werner, Yann Barrandon & Michael T. Longaker

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- Aim of the study: To identify and isolate the fibroblast lineage(s) with fibrogenic potential in vivo

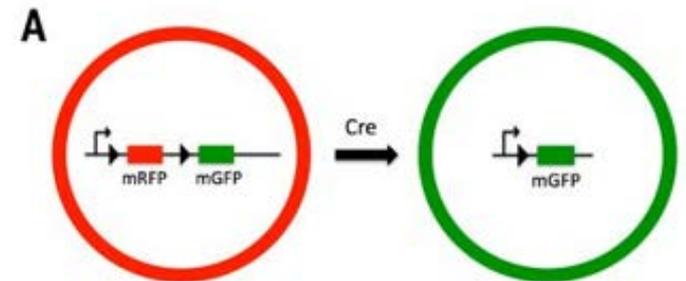
Cre/LoxP Mouse Model



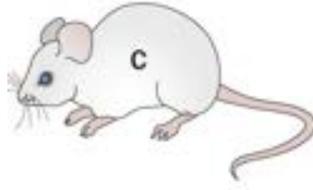
Engrailed-1
(Transcription factor) +
Cre transgenic mouse

ROSA26mTmG
transgenic mouse

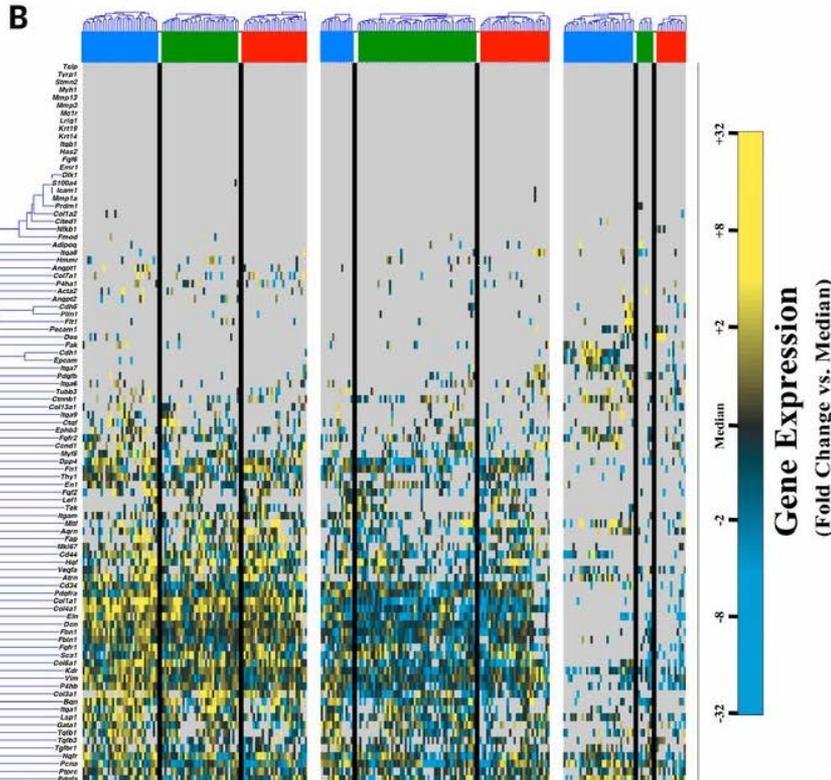
En1Cre;R26mTmG
mouse



Cre/LoxP Mouse Model

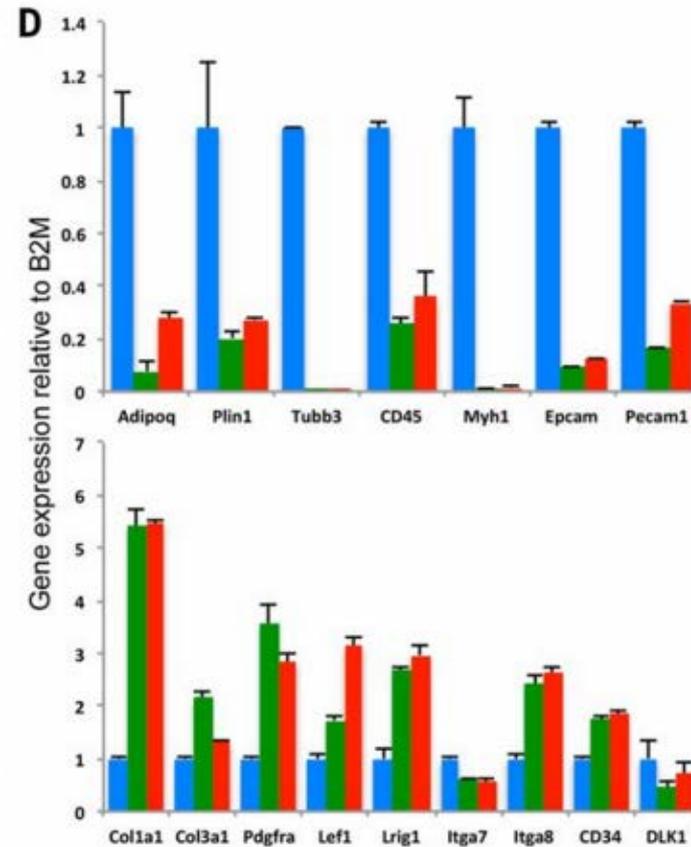
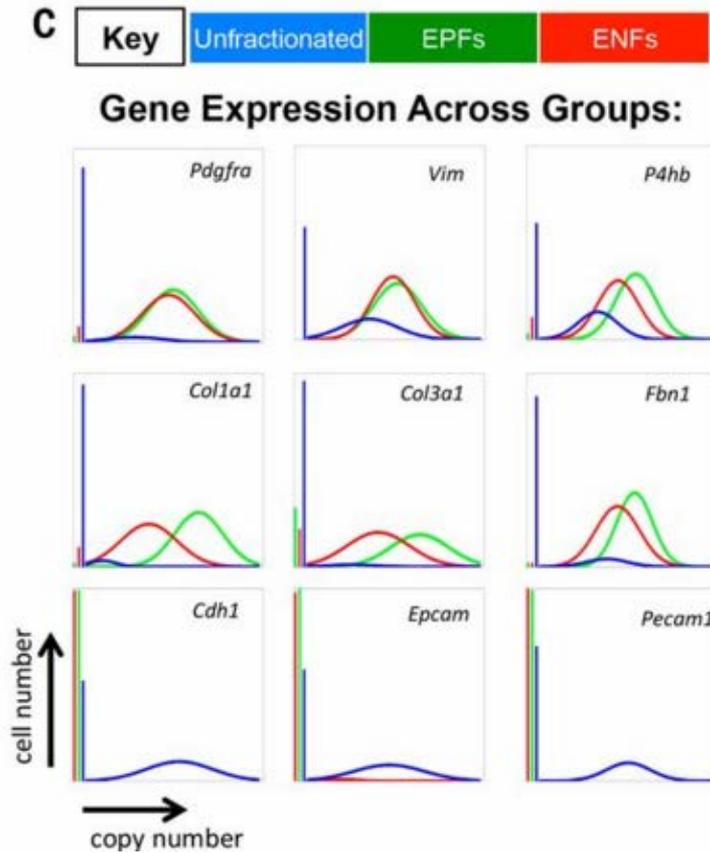


FACS – based isolation of Engrailed-1
positive Fibroblasts (EPFs) and Engrailed-1
negative Fibroblasts (ENFs) from dorsal
skin samples

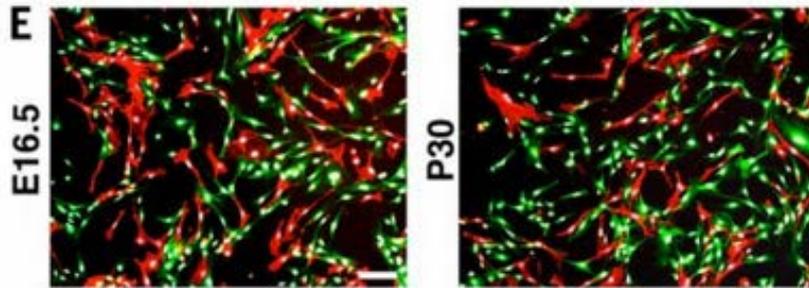


Gene expression
analysis

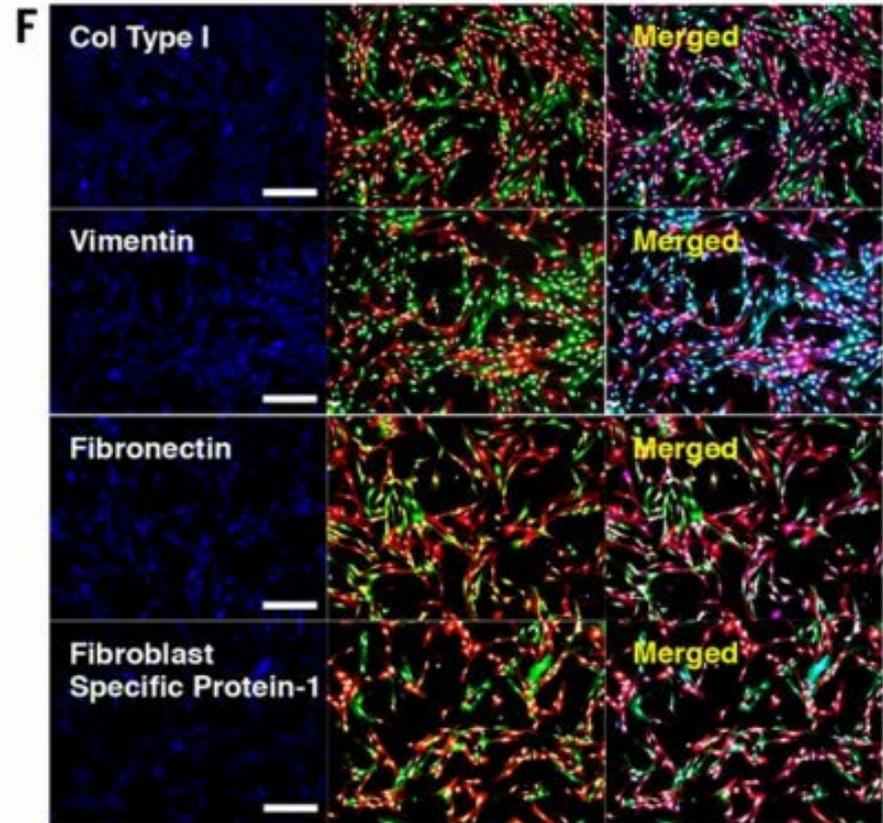
I. Engrailed-1 lineage positive (EPFs) vs Engrailed-1 lineage negative (ENFs) fibroblasts – Gene expression analysis



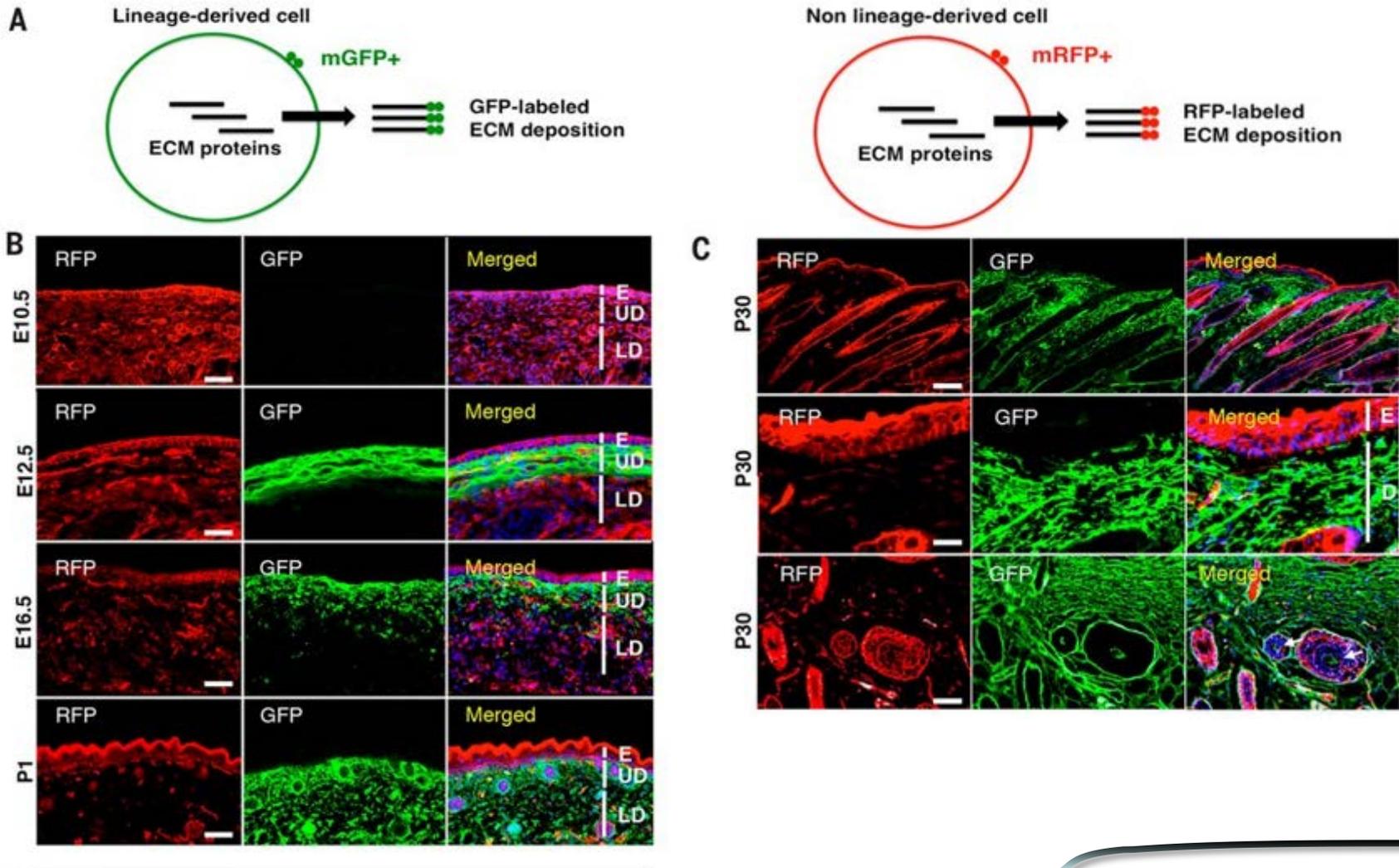
I. Morphologie of EPFs and ENFs



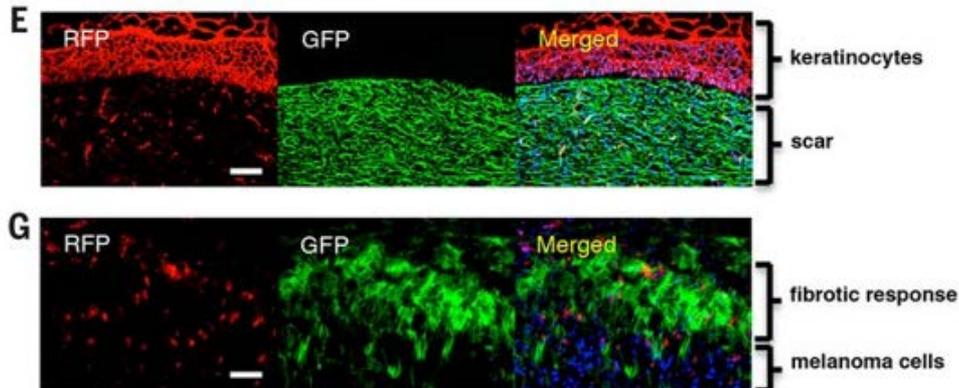
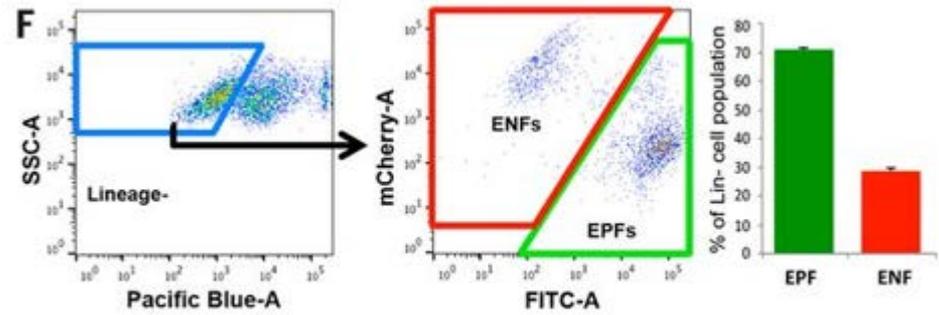
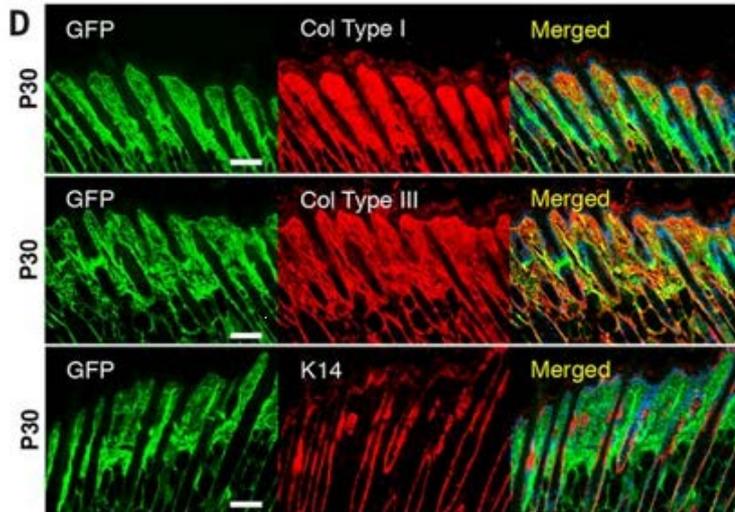
EPFs green (GFP)
ENFs red (RFP)



II. Fibrogenic potential is lineage-restricted



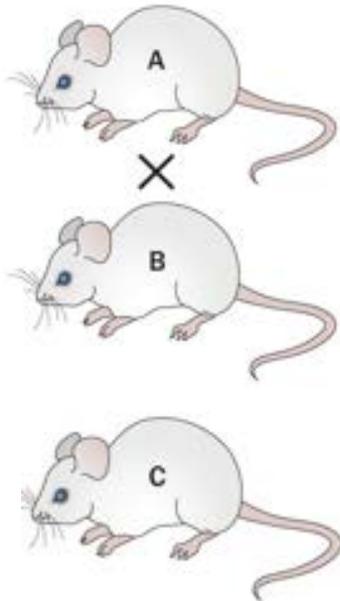
II. Fibrogenic potential is lineage-restricted



At least two embryonic lineages exist (EPFs and ENFs), but **EPFs** are the only effectors of connective tissue secretion and formation in vivo during embryonic development, postnatal wound healing, cancer stroma formation and radiation fibrosis of the skin.

III. Fibrogenic potential of dermal fibroblasts is cell- intrinsic

- Oral mucosa wounds show more rapid healing and less scarring formation than cutaneous wounds – is this property cell-intrinsic or owed to the environmental conditions of the host tissue?

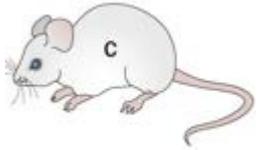


Wnt1Cre transgenic mouse (labels early migratory neural crest populations)

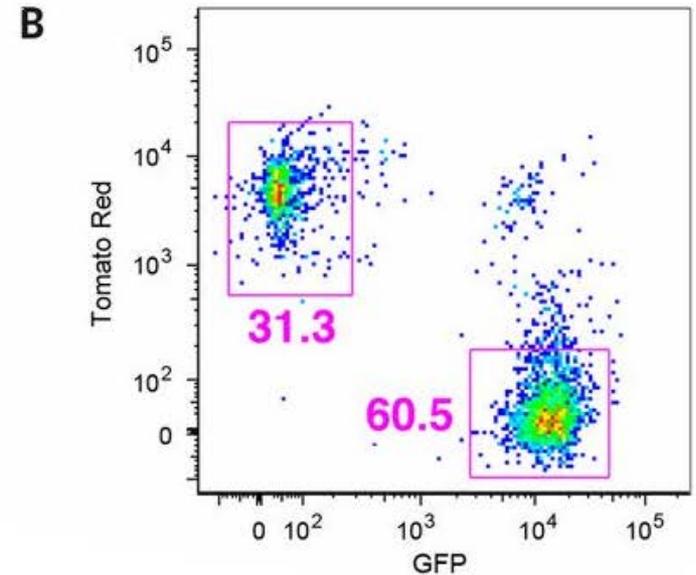
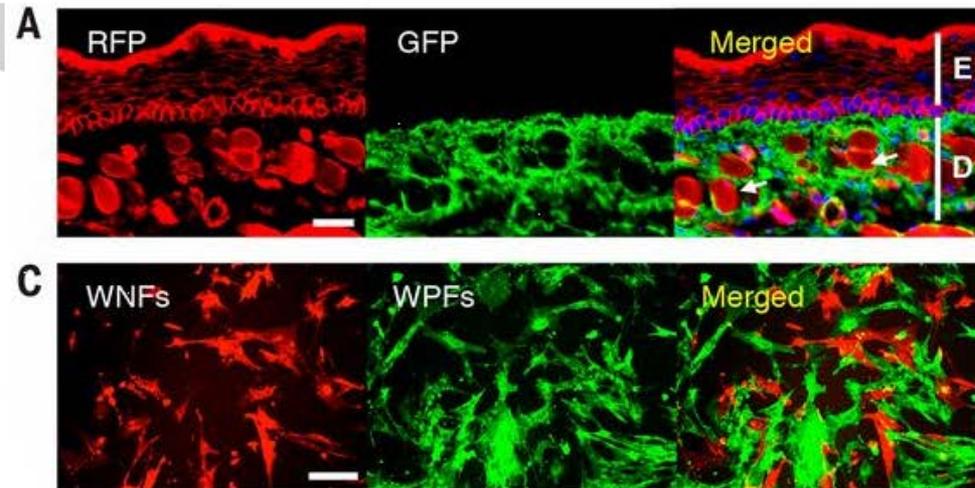
R26mTmG transgenic mouse

Wnt1Cre;R26mTmG transgenic mouse

*III. Fibrogenic potential of
dermal fibroblasts is cell-
intrinsic*

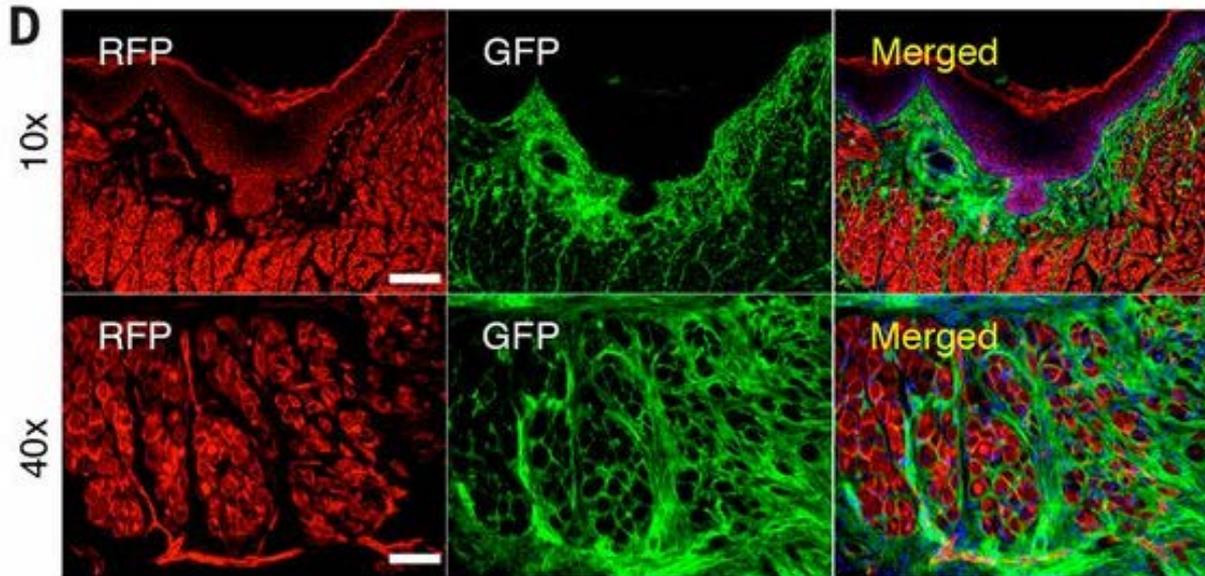


FACS – based isolation of Wnt-1 positive
Fibroblasts (WPFs) and Wnt-1 negative
Fibroblasts (WNFs) from oral dermis
samples

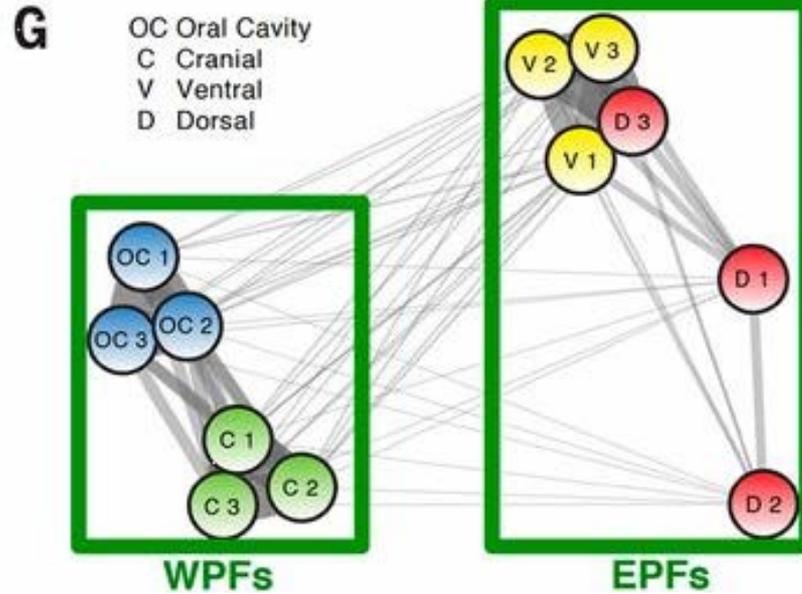
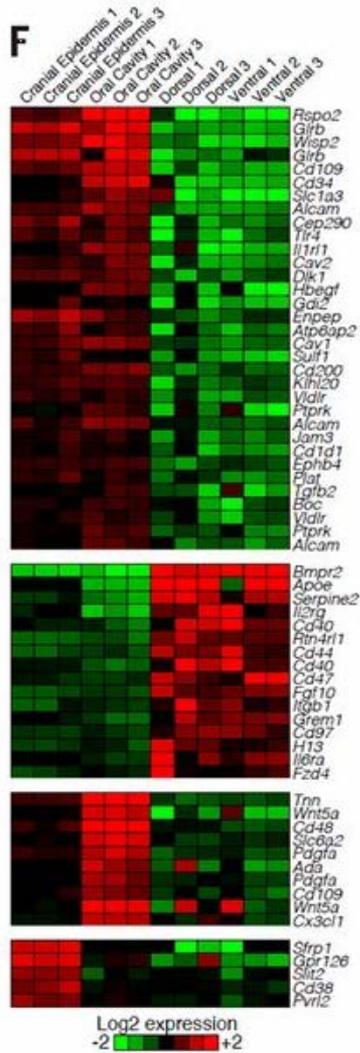


III. Fibrogenic potential of
dermal fibroblasts is cell-
intrinsic

Wounded oral dermis

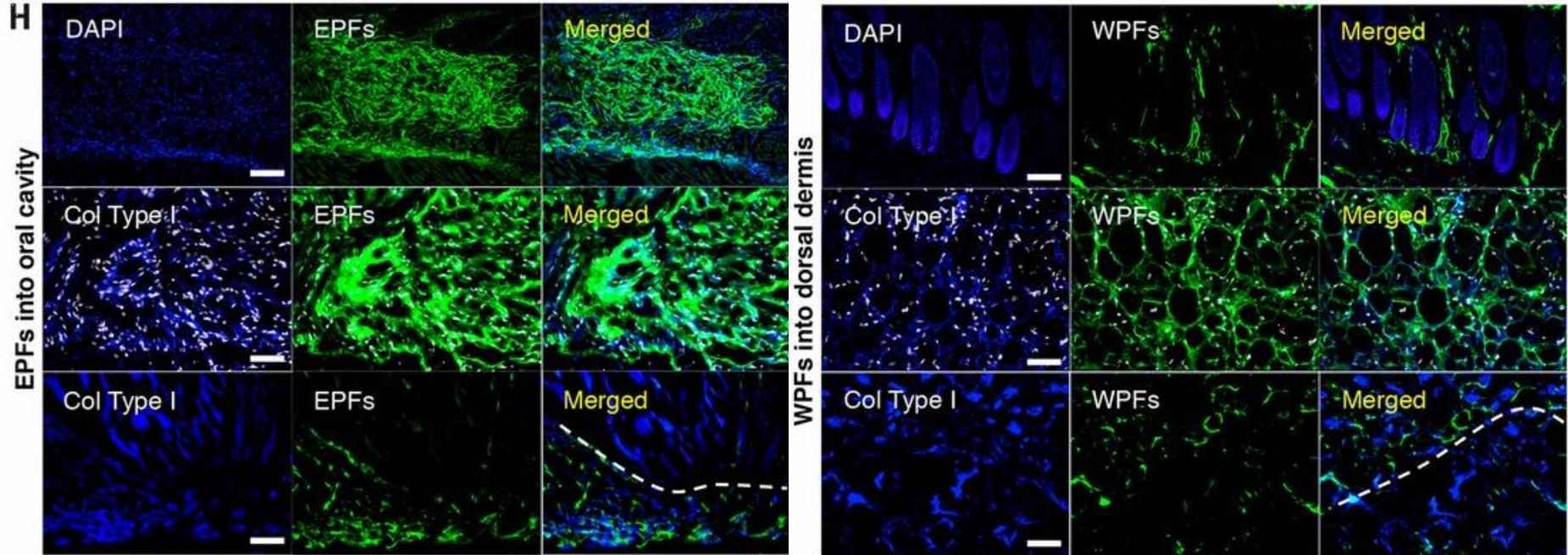


III. Fibrogenic potential of dermal fibroblasts is cell- intrinsic



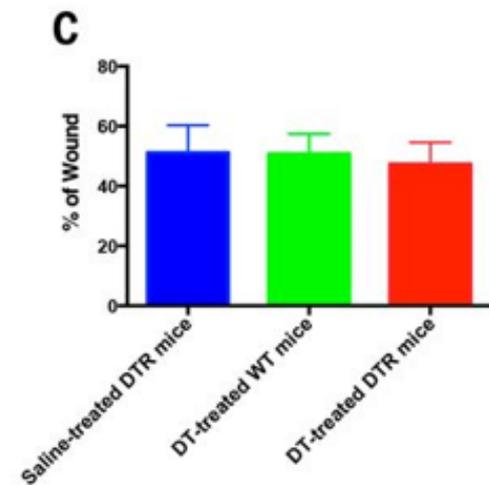
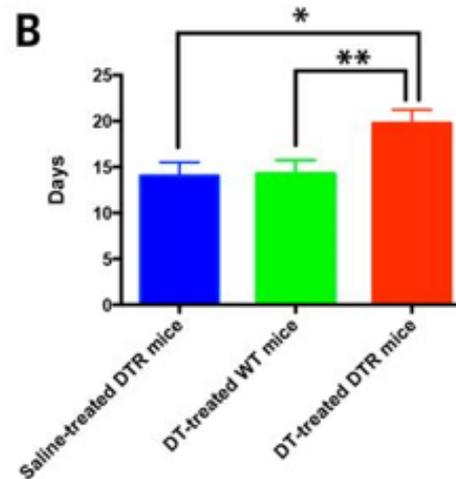
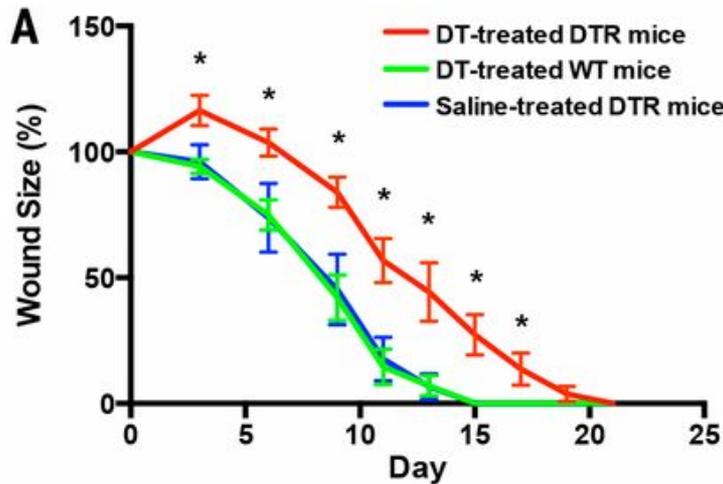
III. Fibrogenic potential of
dermal fibroblasts is cell-
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New approach: Reciprocal transplantation experiments

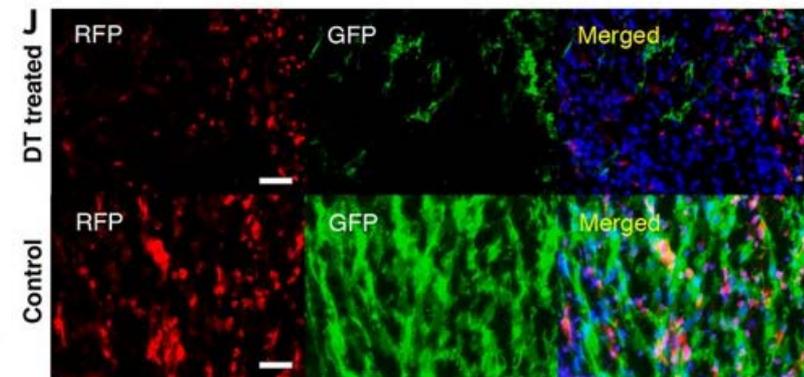
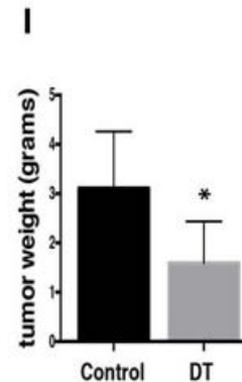
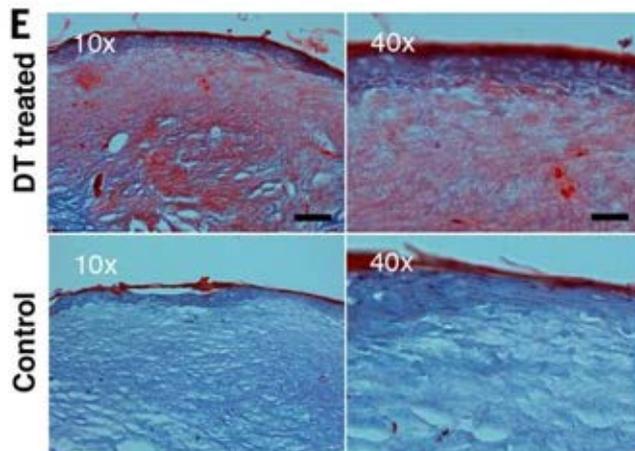
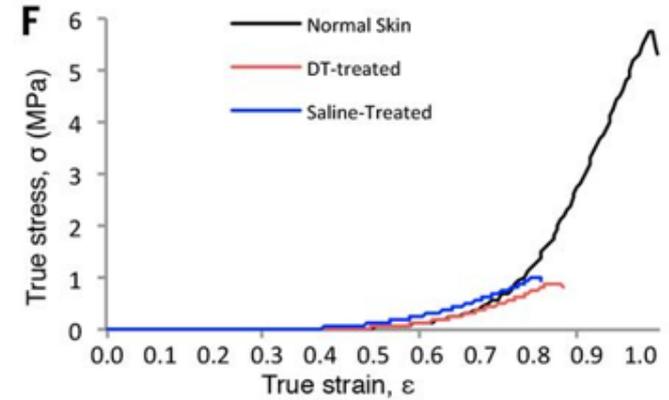
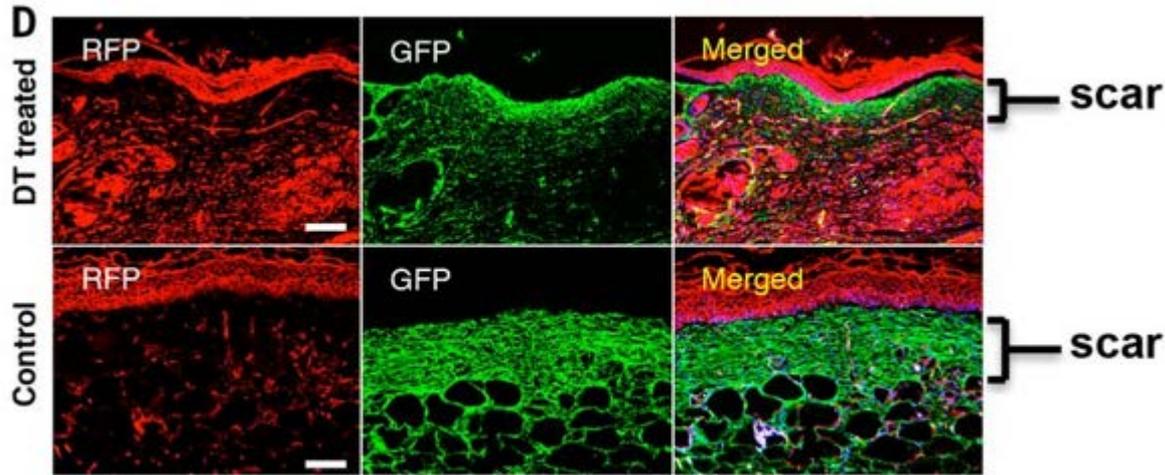


IV. Ablation of EPF-lineage reduces scarring?

- Different mouse model: **En1Cre;R26mTmG** mice were crossed with **R26tm1(HBEGF)Awai** mice which express simian diphtheria toxin receptor (DTR) → EPFs are GFP and DTR positive



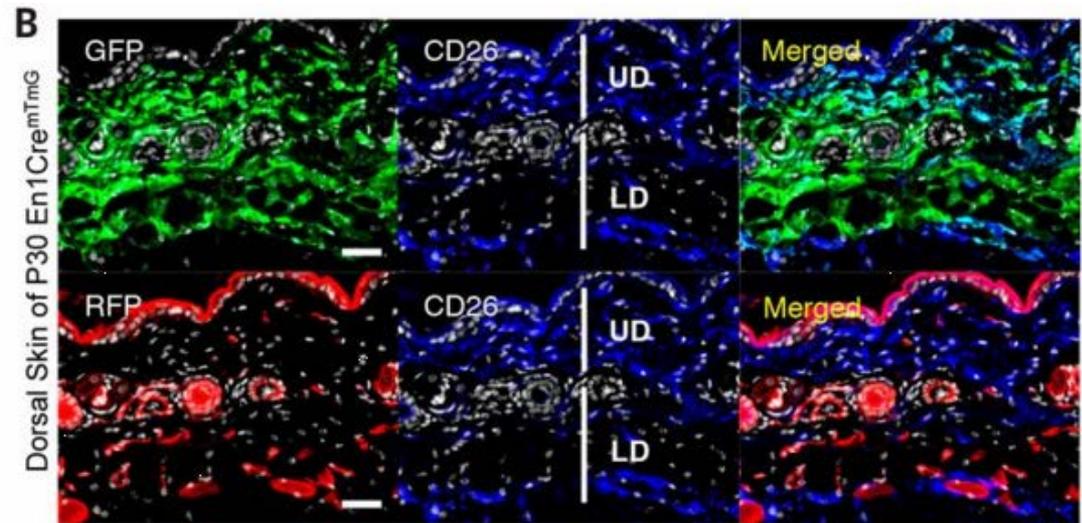
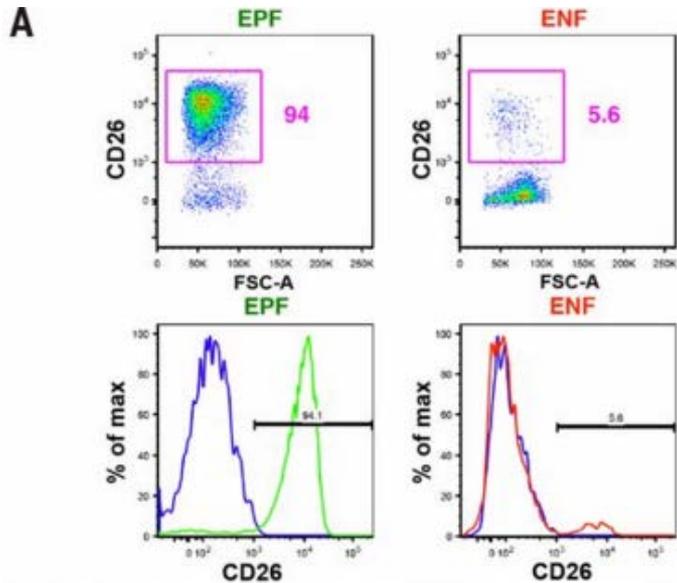
IV. Ablation of EPF-lineage reduces scarring?



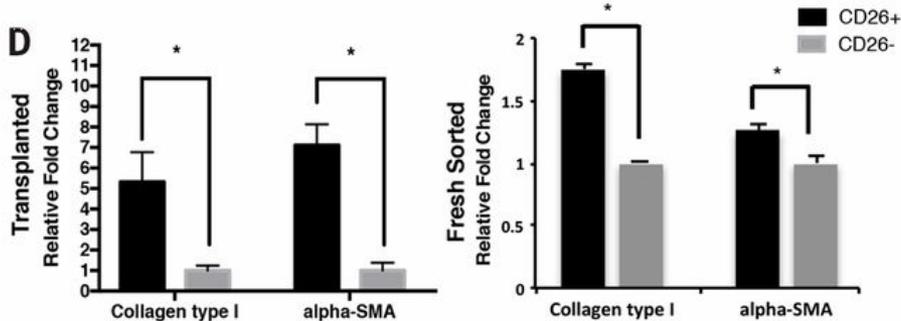
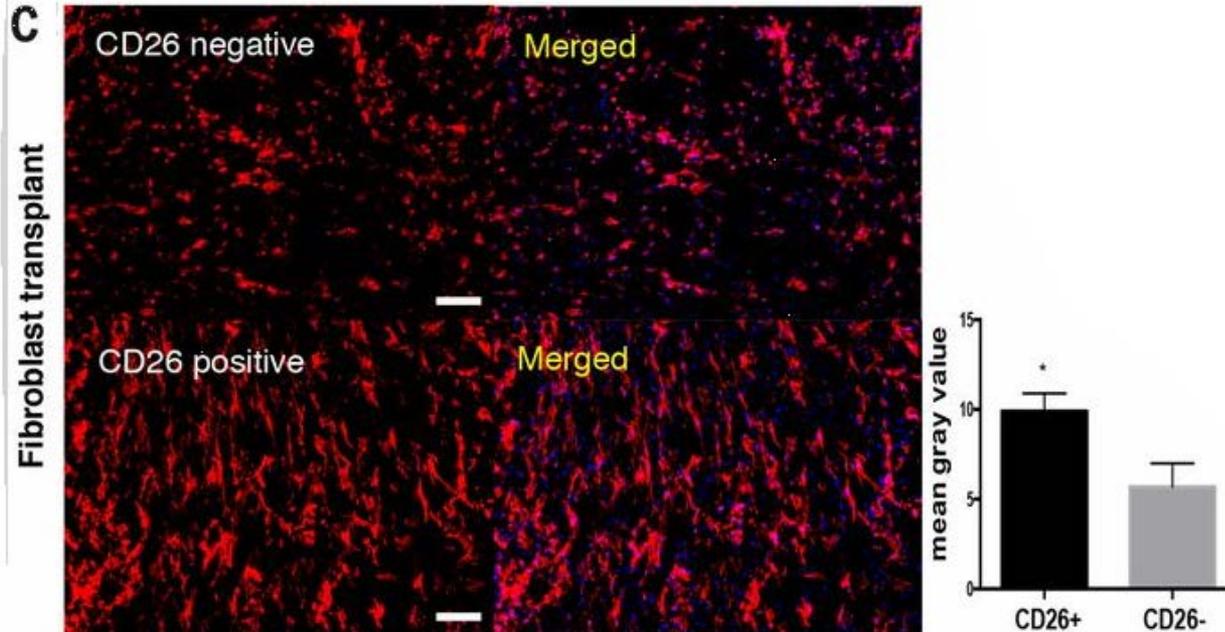
Trichrome staining: showing
reduced collagen deposition in
DT-treated mice.

V. How to isolate EPFs

- FACS-based fibroblast purification from skin lysates of wild-type mice aged 8 weeks
- Cell surface marker screening (including 176 monoclonal antibodies) revealed **CD26** as the marker with the biggest difference between EPFs and ENFs



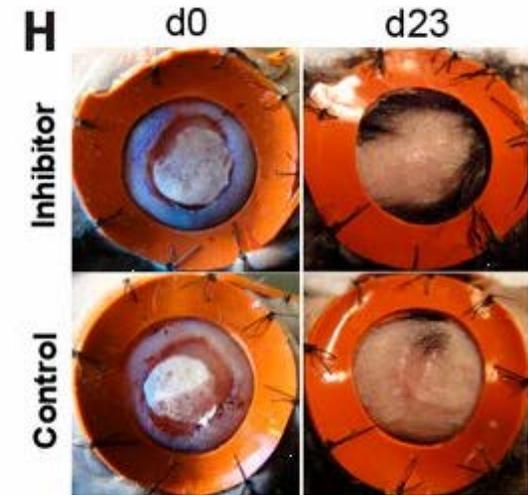
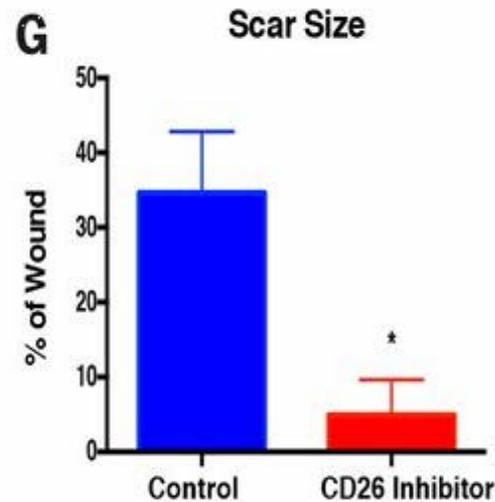
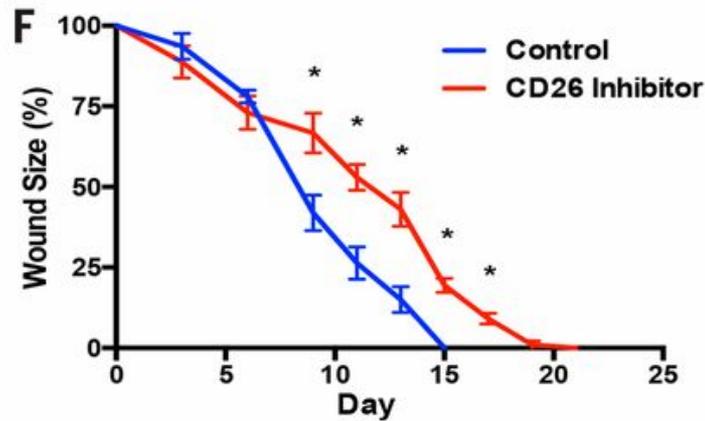
V. How to isolate EPFs



At baseline ECM expression of EPFs and ENFs is similar, whereas the response to a stimuli and the following upregulation of ECM gene expression is much higher in EPFs.

*VI. Inhibition of CD26 reduces
cutaneous scarring during
wound healing*

- CD26 is a surface marker for a fibroblast lineage responsible for ECM deposition
- Diprotin A was used to inhibit CD26



Conclusion/Discussion

- Distinct fibroblast lineages represent unique cell types
- EPFs are the primary lineage contributing to connective tissue deposition during embryonic development, postnatal wound healing, cancer stroma formation
- EPFs fibrogenic potential is cell intrinsic
- CD26 is a marker for the distinct lineage of fibroblasts that contributes the bulk of connective tissue in scarring and other conditions involving fibrosis (EPFs)
- Inhibition of CD26 might be a promising step forward to regulating fibrosis

Personal Comments/ Critics

- Why did they use Engrailed-1 mice?
- Where do the Engrailed-1 positive fibroblasts come from?
- Mouse model is used in this study – to what extent are the findings applicable in humans?
- CD26 maybe marker for other cells?
- In my opinion a very credible and sophisticated paper

Thank you for your
attention!