Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

Identification and isolation of a dermal lineage with intrinsic fibrogenic potential

Rinkevich Y[1], Walmsley GG[2], Hu MS[2], Maan ZN[3], Newman AM[4], Drukker M[4], Januszyk M[3], Krampitz GW[4], Gurtner GC[3], Lorenz HP[3], Weissman IL[5], Longaker MT[6].

Science. 2015 Apr 17;348(6232):aaa2151. doi: 10.1126/science.aaa2151.

Wound healing

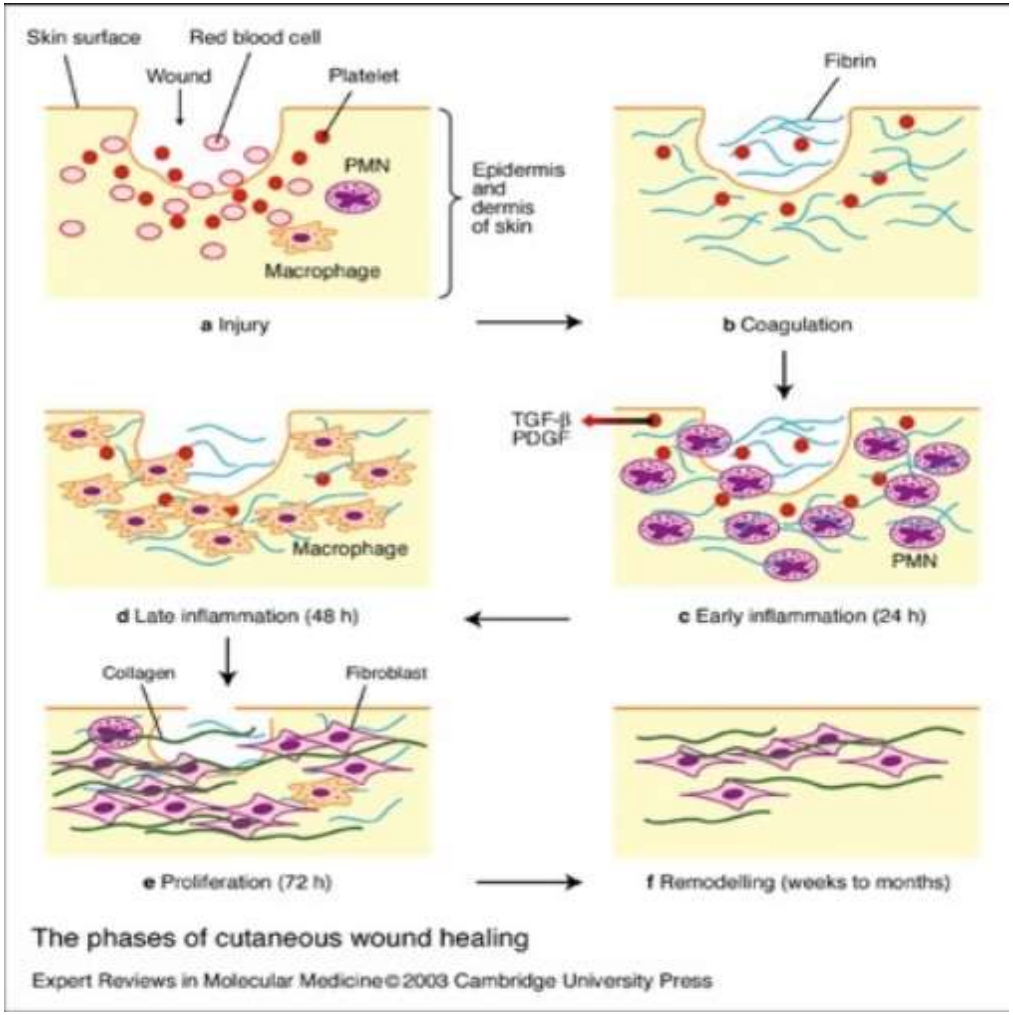

Fig I: Schematic illustration of wound healing mechanisms

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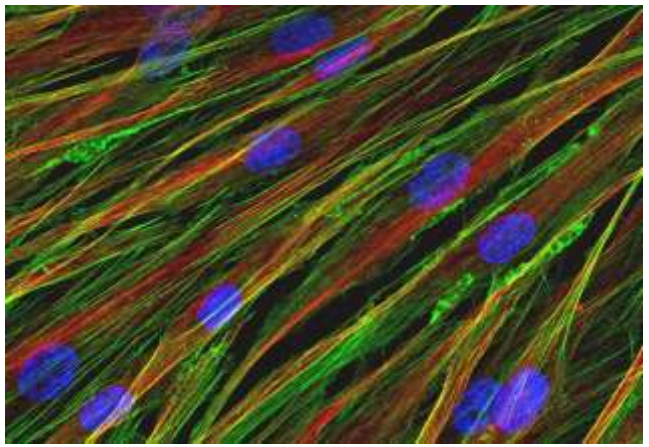
Fibroblasts

- Synthesize and remodel extracellular matrix (ECM)
- Heterogeneous population of cells
- Responsible for tissue and organ fibrosis, atherosclerosis, systemic sclerosis, athermomatous plaques
- Role of fibroblasts in carcinoma progression & scar formation?
- Fibroblast lineages with fibrogenic potential
  → manipulating injury response
- Fibroblast markers:
  Pdgfra, Vim, P4hb, Col1a1, Col3a3, Fbn1



thermofisher.com
Neonatal human dermal fibroblasts

Fibroblasts

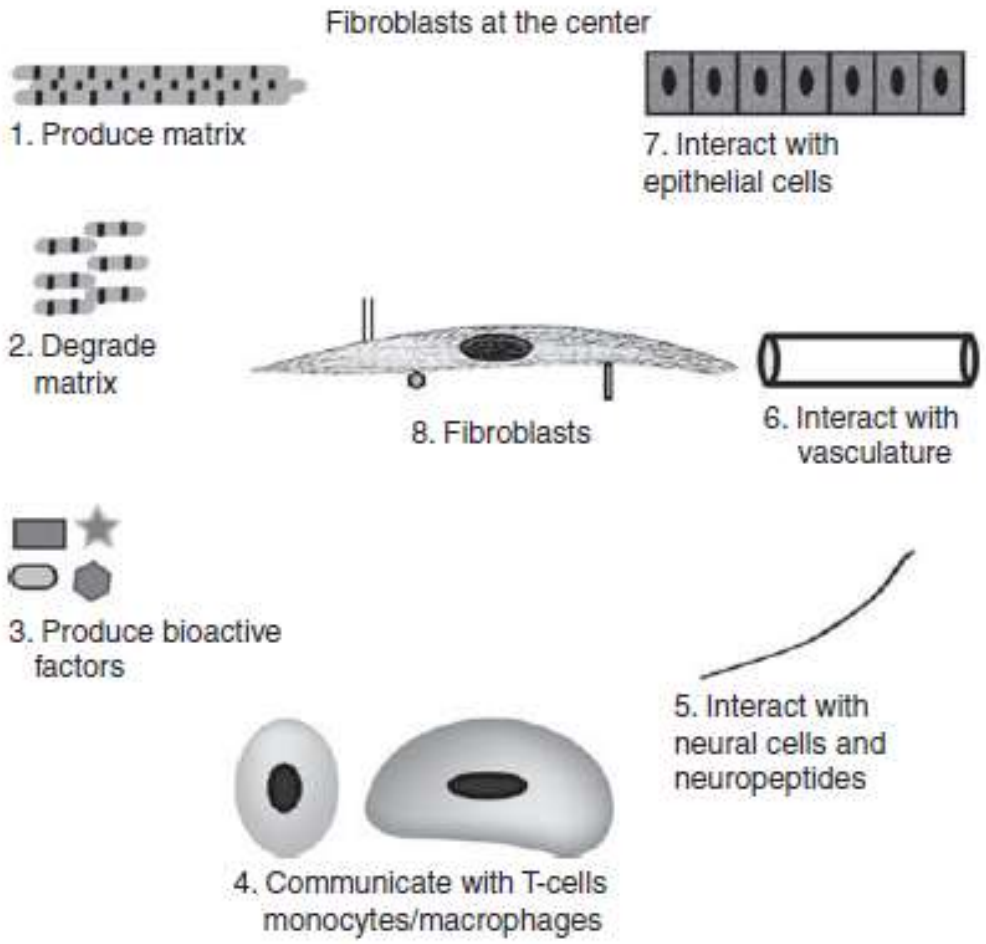


Fig II: the multiple functions of fibroblasts

Sorrell, J. M. and A. I. Caplan (2009). "Fibroblasts-a diverse population at the center of it all." Int Rev Cell Mol Biol **276**: 161-214.

Engrailed-1 (en-1) (gene)

- Homeobox protein; important role in development of brain, limb, sternum
- Primary contributor to
  - connective tissue secretion
  - Organization during embryonic development
  - Fibrosis
  - Cancer stroma formation

→ distinct fibroblasts represent unique cell types

EPFs = engrailed-1 positive fibroblasts
ENFs = engrailed-1 negative fibroblasts

Wnt-1

- Proto-oncogene protein
- Regulation of cell fate and patterning during embryogenesis
- Wnt1^{cre}-cells used for labelling early migratory neural crest populations

WPFs = wnt-1 positive fibroblasts
WNFs = wnt-1 negative fibroblasts

CD26=dipeptidyl peptidase-4

- Cell surface enzyme, expressed on surface of most cell types
- Immune regulation, signal transduction and apoptosis
- cleaves X-proline dipeptides from the N-terminus of polypeptides
- Wide range of substrates:
  - Growth factors, chemokines, neuropept peptides
  - Major role in glucose metabolism

Wikipedia.org

mTmG-Mice

- double-fluorescent Cre reporter mouse
  - Membrane targeted tomato (mT) and
  - Membrane-trageted green fluorescent (mG)
  - Highlight membrane structures and cell morphology

Tool for lineage tracing, transplantation studies and cell morphology

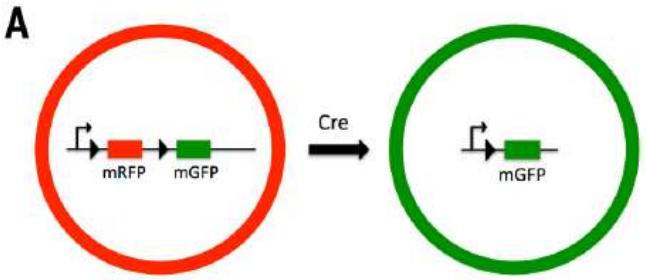


Fig. 1A: schematic illustration showing the mTmG-system for tracing EPFs and WPFs via expression of GFP

Multiple lineages of fibroblasts in the dorsal skin

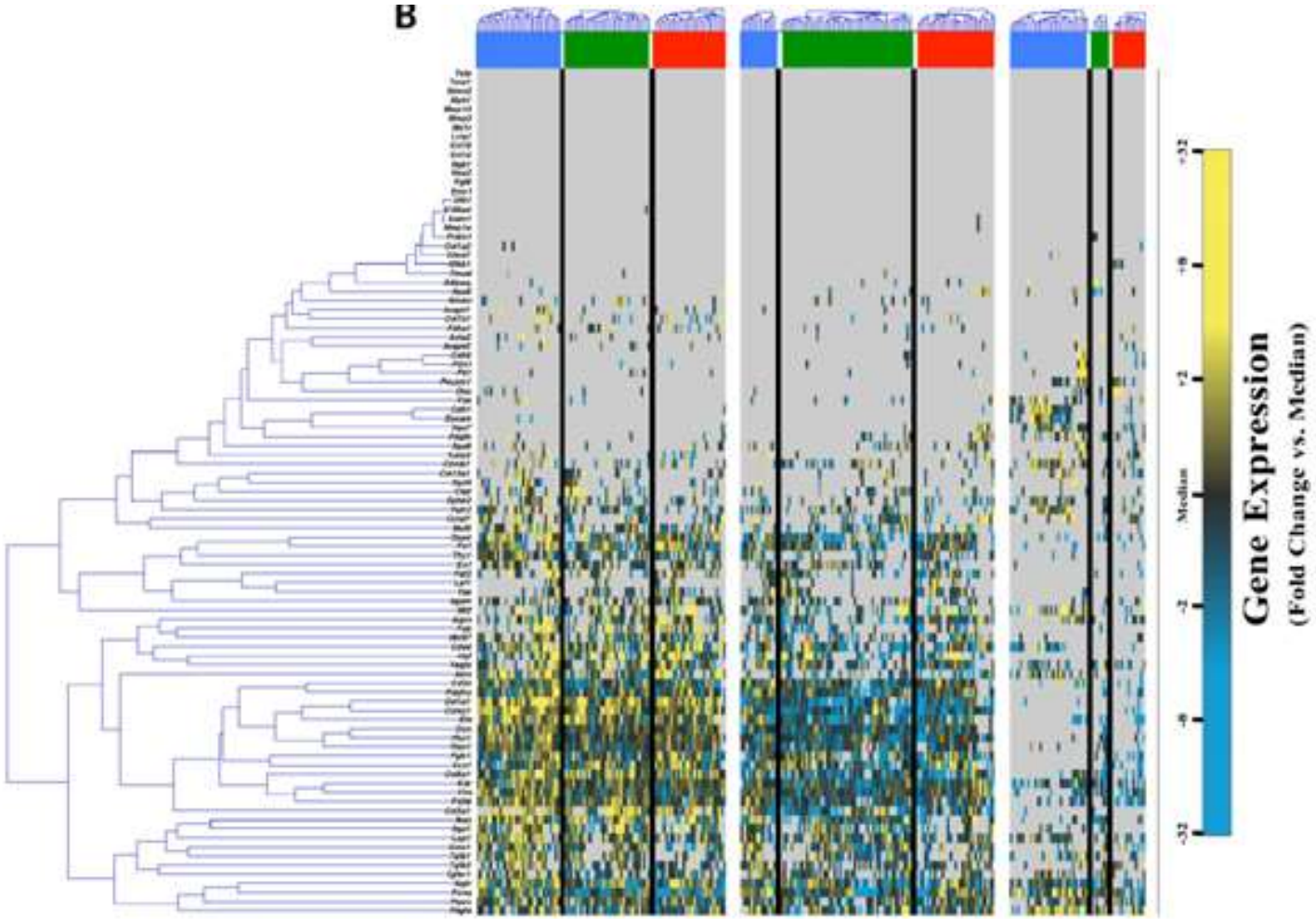


Fig. 1B: clusters of gene expression of EPFs (green), ENFs (red) and lysate (blue)

Multiple lineages of fibroblasts in the dorsal skin

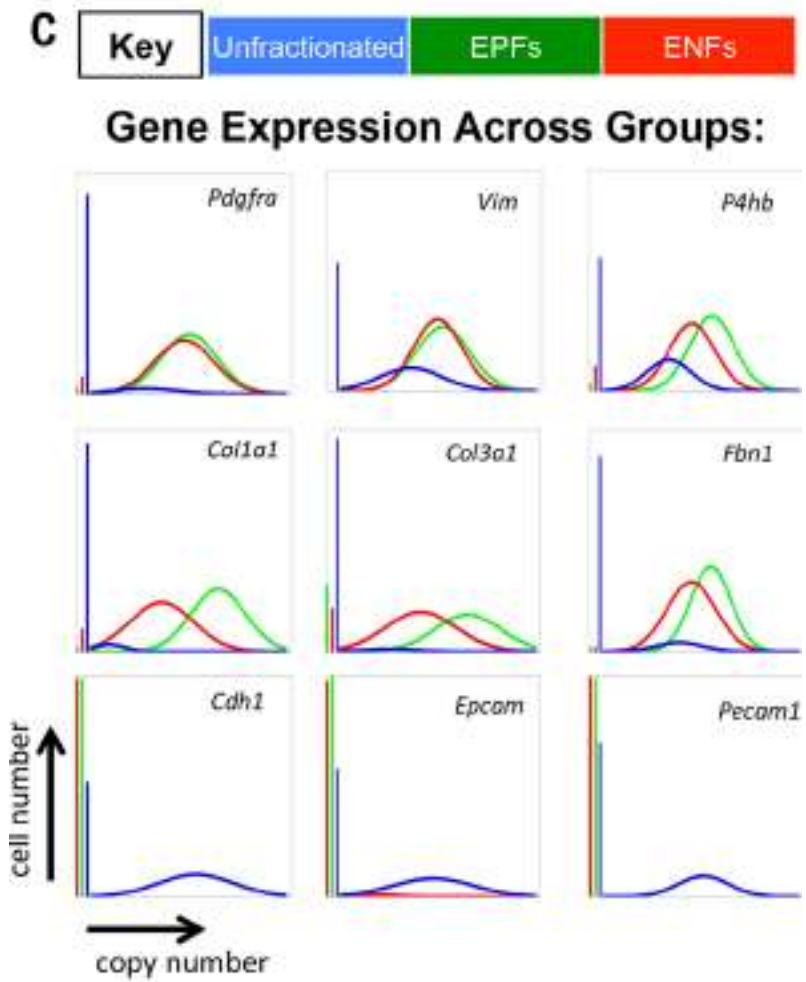


Fig 1B: EPFs and ENFs show typical „fibroblast gene expression“:

- Increased expression of Pdgfra, Vim, P4hb, Col1a1, Col3a3, Fbn1
- Decreased expression of CDH1, Epcam, Pecam

Multiple lineages of fibroblasts in the dorsal skin

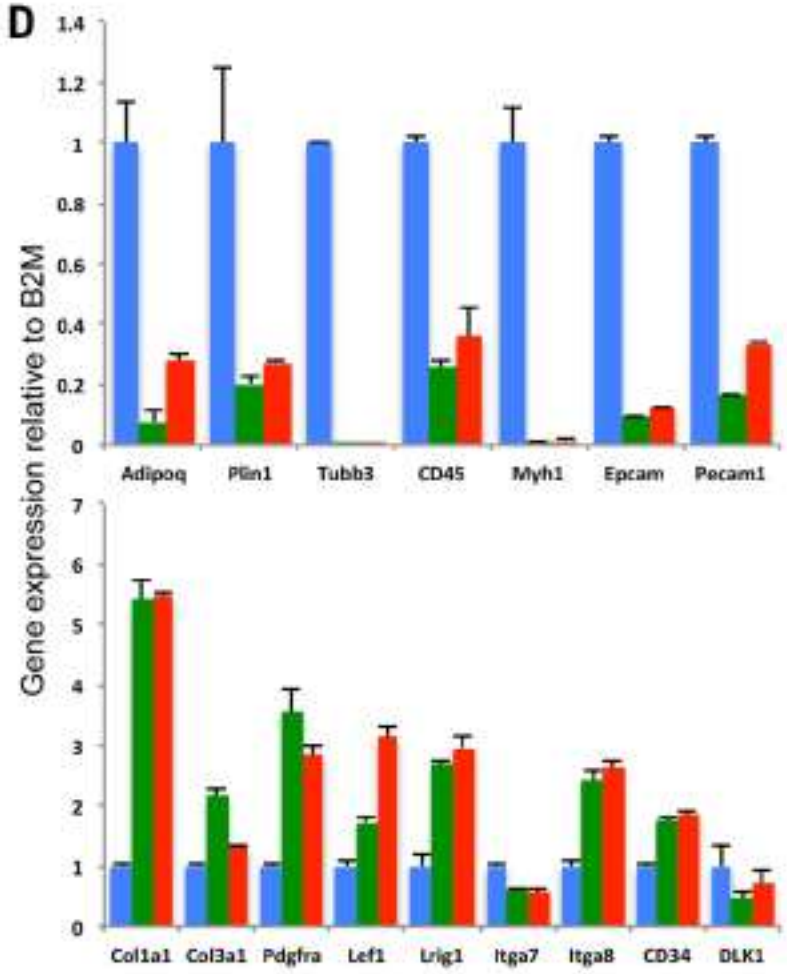


Fig. 1D: qt-PCR analysis of fibroblast and non-fibroblast-associated gene expression in dermal lysate (blue), EPFs (green) and ENFs (red):

„Non-fibroblast genes“ are minimally or not expressed by EPFs and ENFs.

Multiple lineages of fibroblasts in the dorsal skin

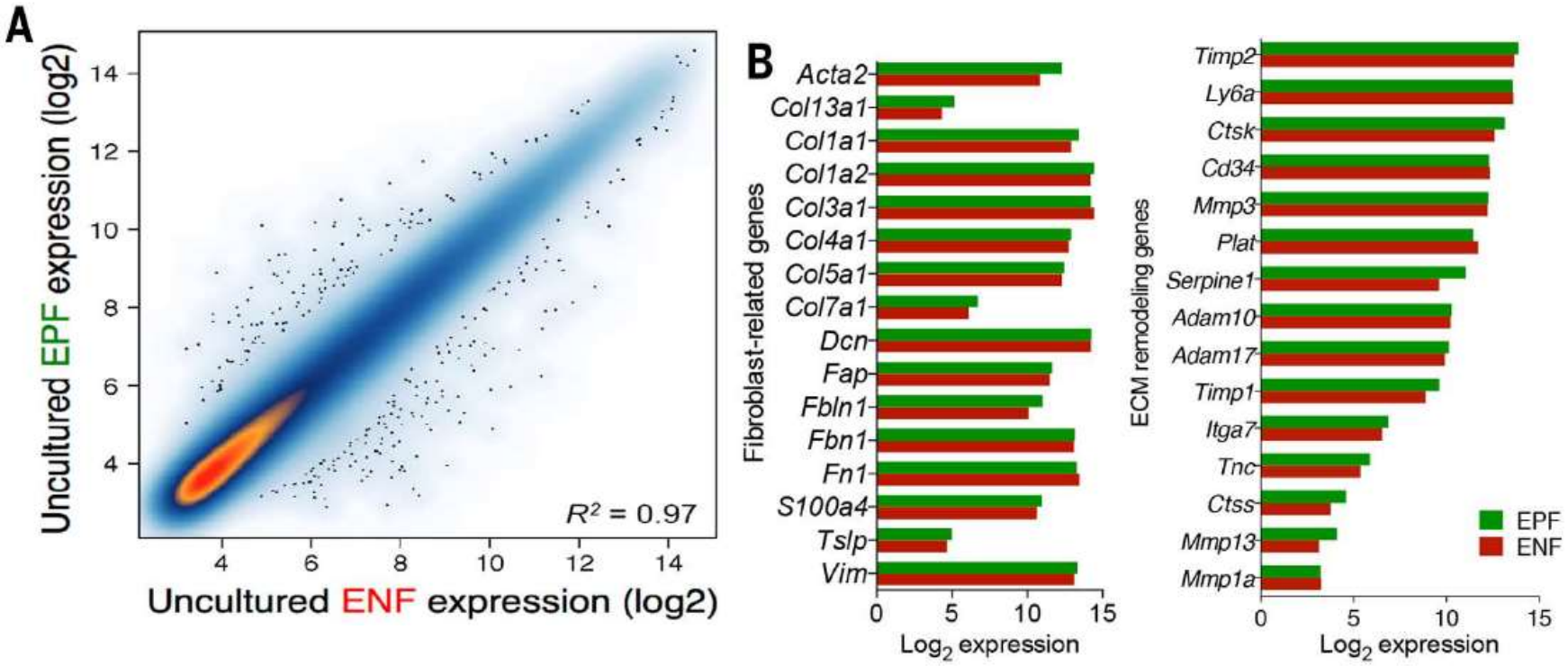


Fig 2A,B: EPFs and ENFs share a transcriptome-wide similarity and fibroblast-gene expression

Multiple lineages of fibroblasts in the dorsal skin

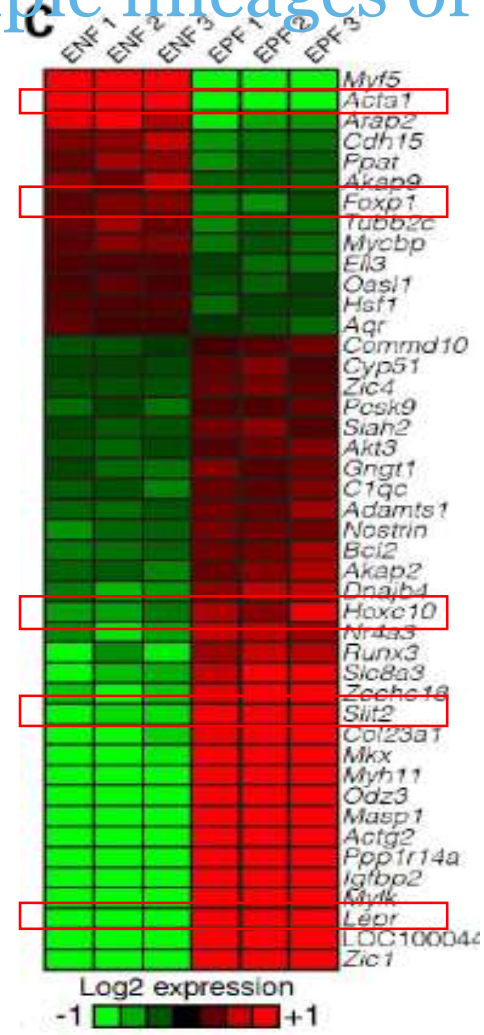


Fig 2C: key differences in transcript expression (EPFs vs. ENFs:

HOXC10, Slit2, Foxp1, leptin receptor, Mylk, actin alpha1

Multiple lineages of fibroblasts in the dorsal skin

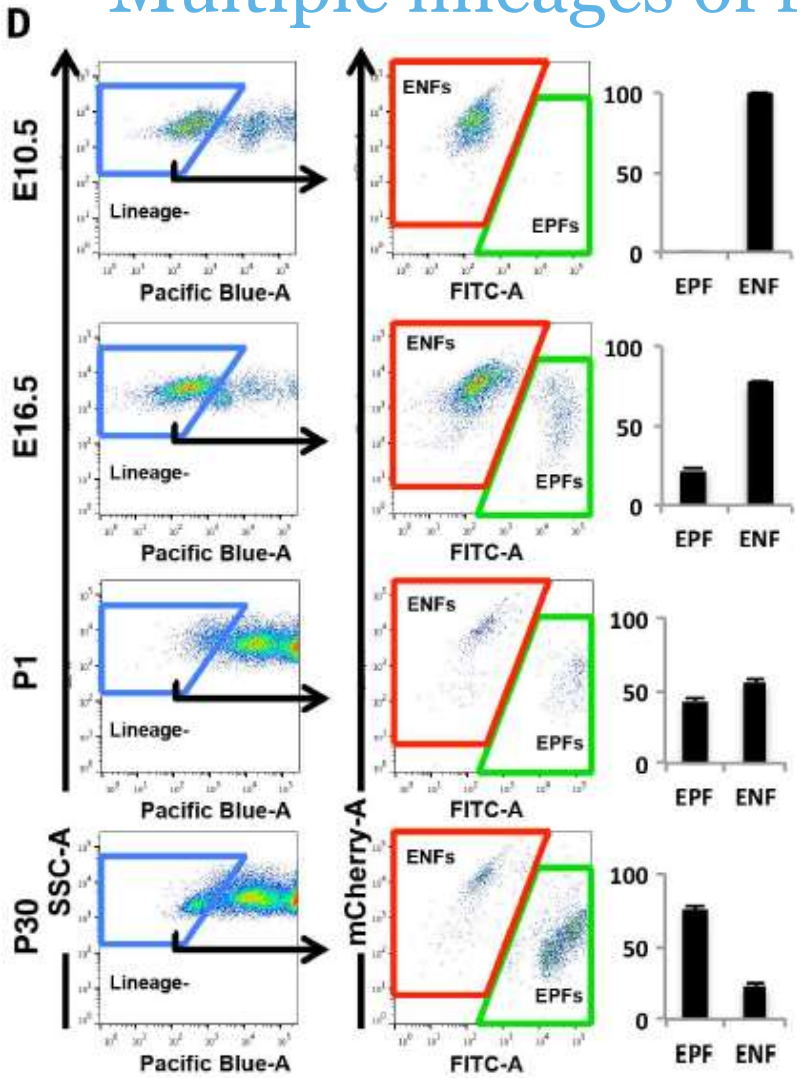


Fig 2D: dynamics of ENF- and EPF-presence during development

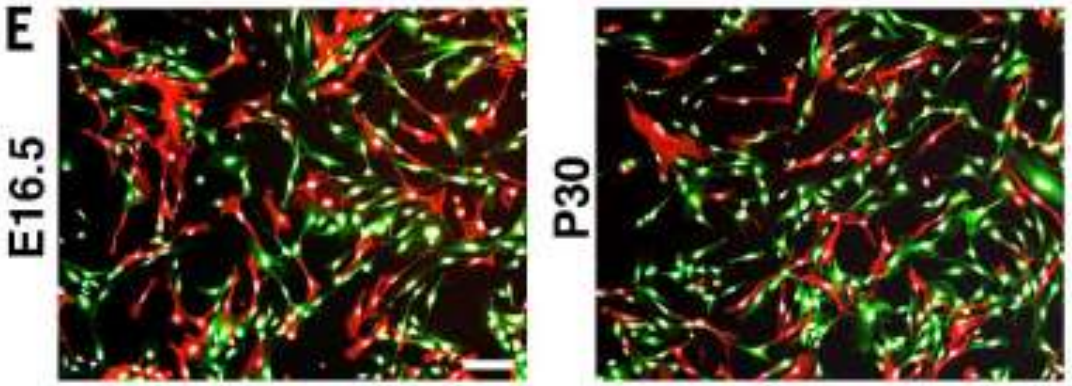


Fig 2E: EPFs and ENFs display similar morphology

Multiple lineages of fibroblasts in the dorsal skin

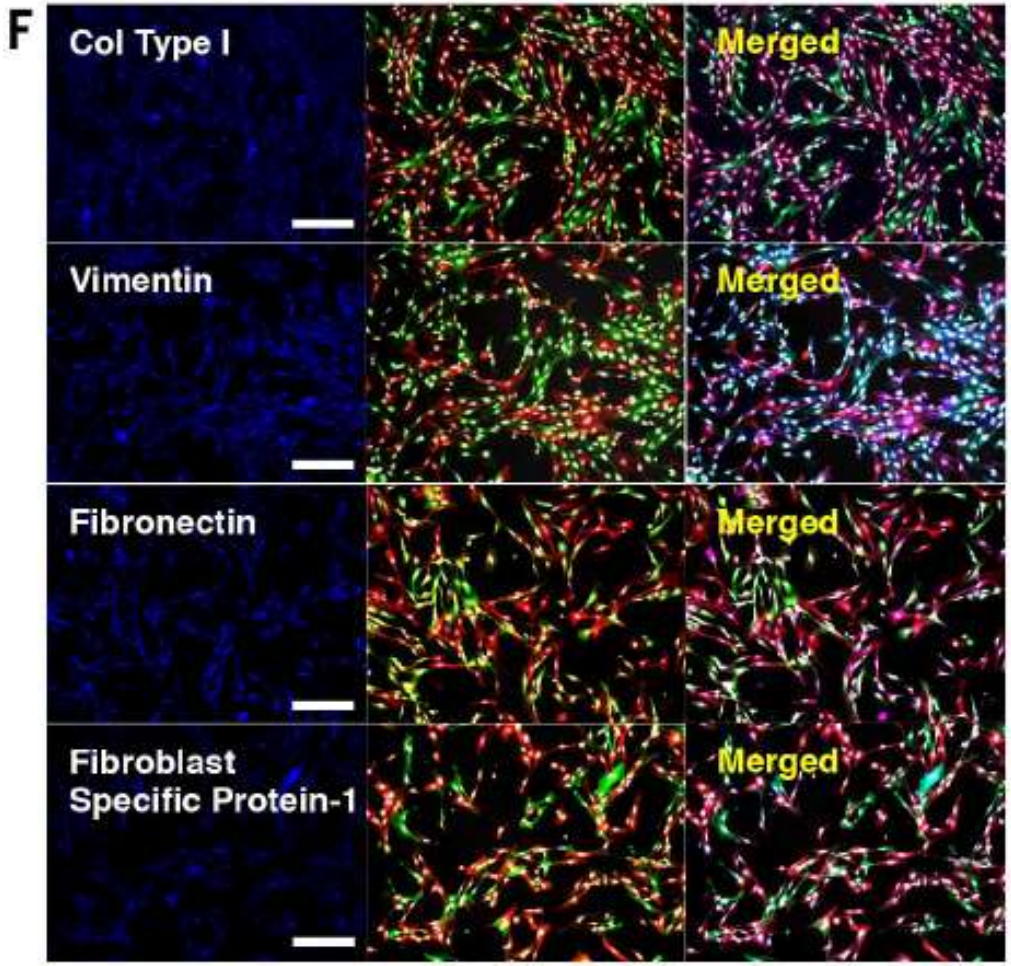
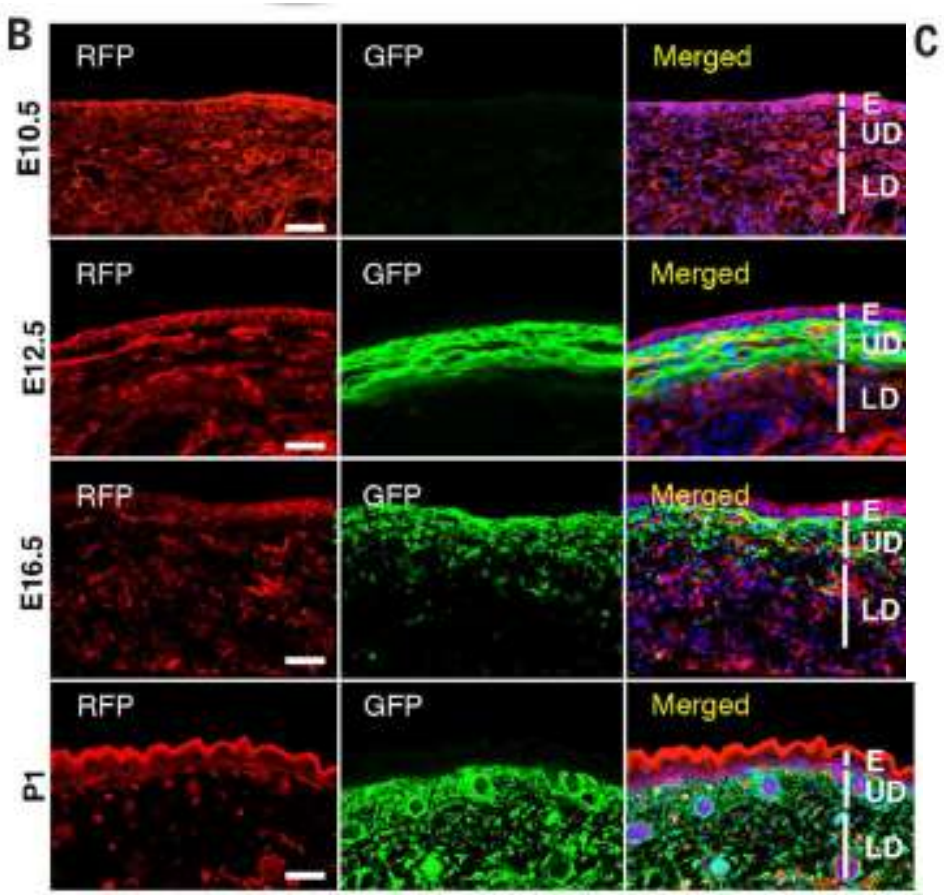


Fig 2F: EPFs and ENFs stained positive for Col type I, Vimentin, Fibronectin and FSP-1

Results

Fibrogenic potential of dermal fibroblasts is lineage-restricted



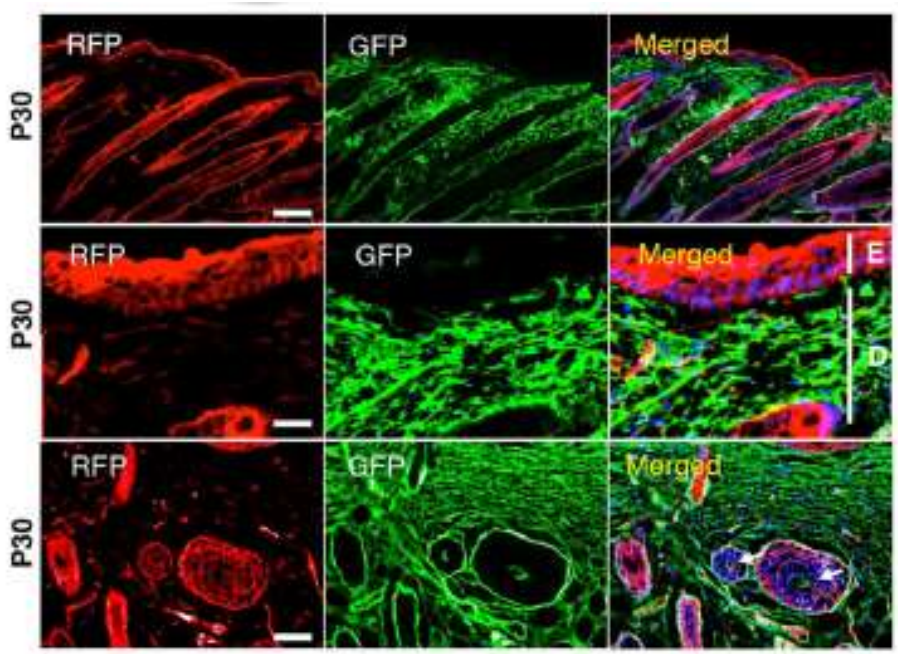


Fig 3B: GFP-labeled (EPF-derived) and RFP-labeled (ENF-derived) dermal ECM-deposition during development

Results

Fibrogenic potential of dermal fibroblasts is lineage-restricted

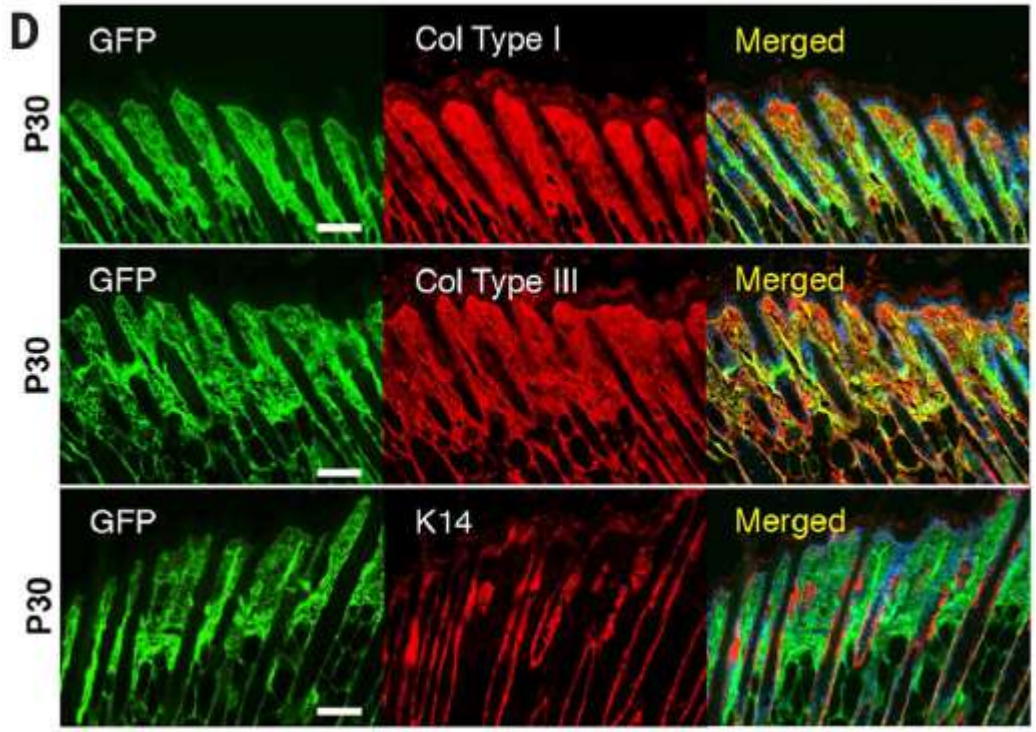


Fig 3D:

- overlapping staining of Collagen I and GFP (EFPs) confirms EFP-derivation of observed ECM
- K14 (keratinocytes) shows no overlapping pattern

Only EPFs function as in vivo effectors of connetive tissue secretion and formation.

Results

Fibrogenic potential of dermal fibroblasts is lineage-restricted

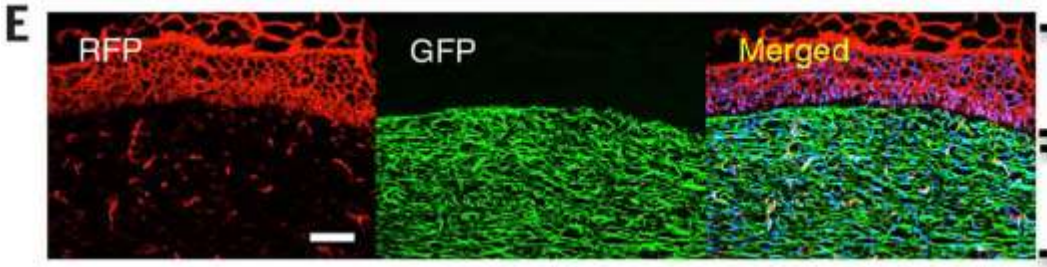


Fig 3E: contribution of EPFs and EPFs to scar formation.

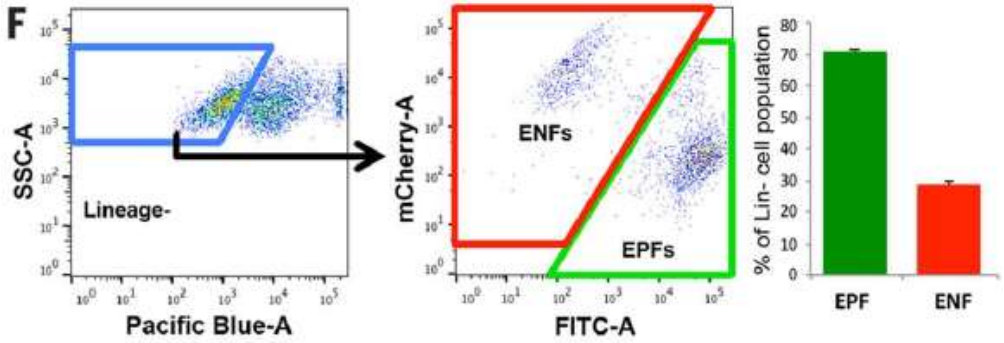


Fig 3F: majority of EPFs in healed skin 14 days after wounding
(FACS analysis)

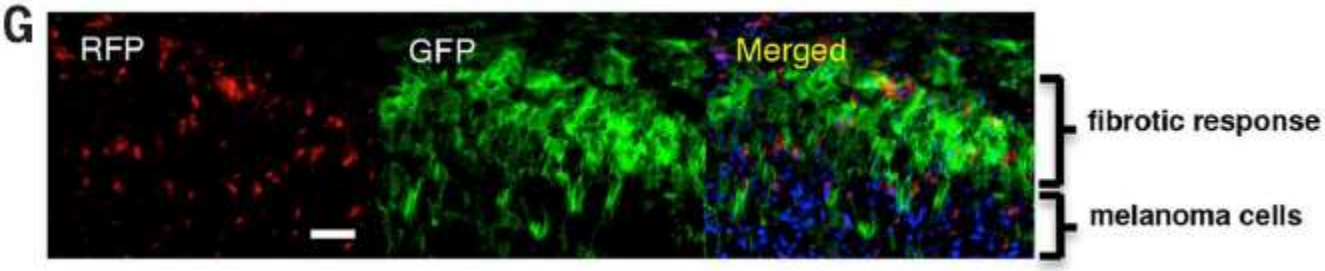


Fig 3G: the stroma of a melanoma is predominantly EPF-derived

Results

Fibrogenic potential of dermal fibroblasts is lineage-restricted

S1K

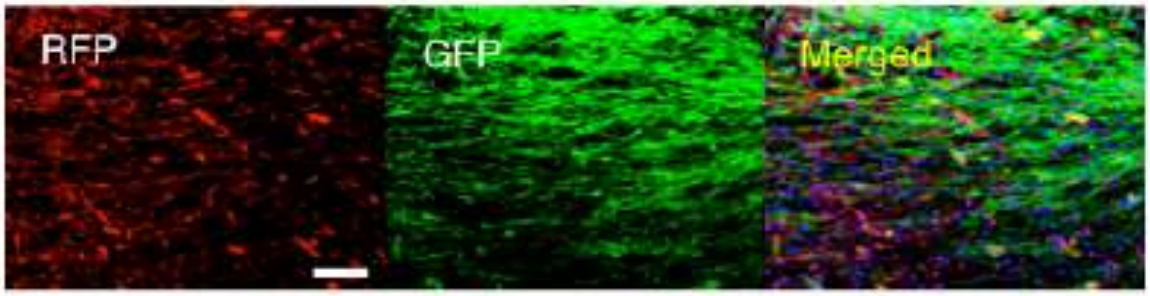


Fig S1K: EPFs contribute majorily to skin fibrosis after irradiation

Data indicate:

- EPFs are the primary lineage contributing to connective tissue deposition during embryonic development, postnatal wound healing, cancer stroma formation and radiation fibrosis of the skin

Fibrogenic potential of dermal fibroblasts is cell-intrinsic

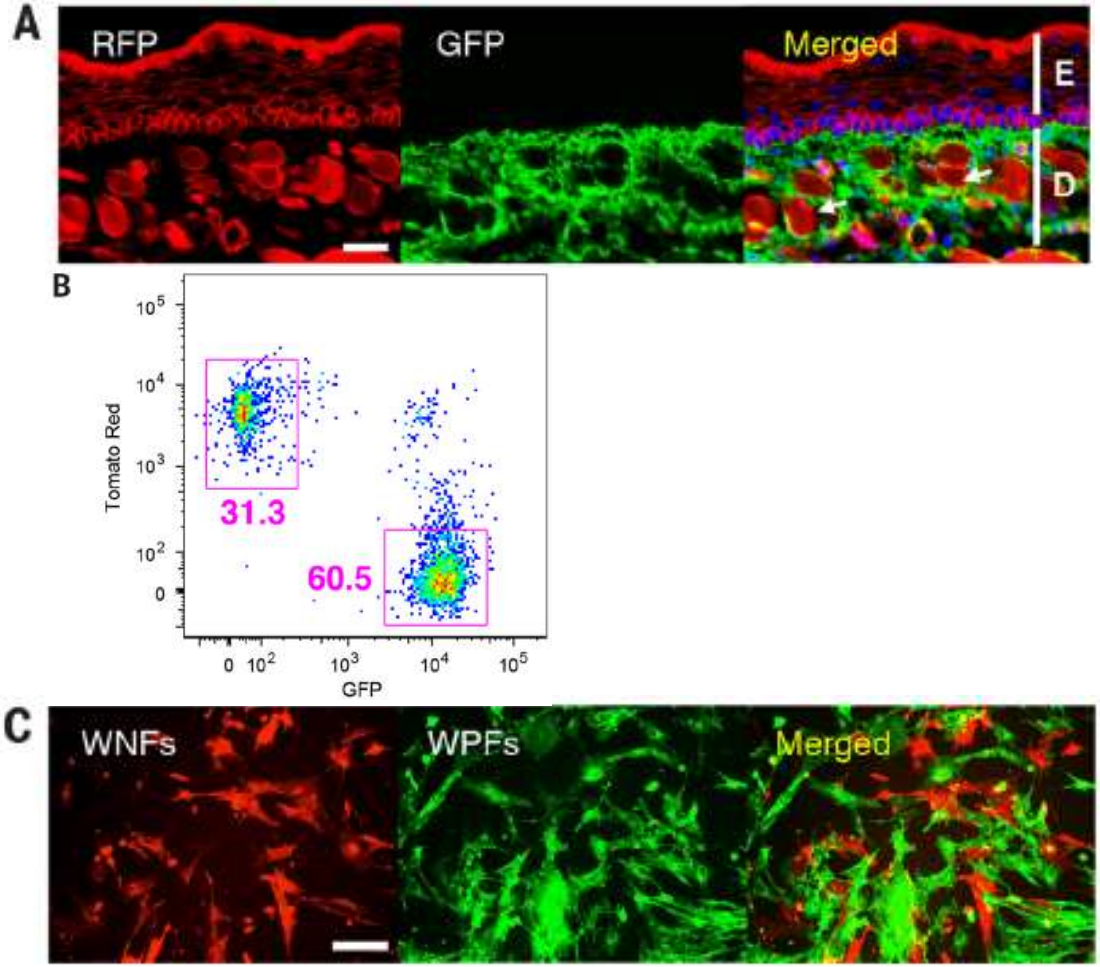


Fig 4A,B:

- Wnt-1-positive fibroblasts (WPFs) show significant connective tissue secretion in oral dermis (GFP-marked)
- Wnt-1 negative fibroblasts were also present in the oral dermis (RFP-marked)

Fig 4C: WPFs and WNFs showed similar morphology and motility

Fibrogenic potential of dermal fibroblasts is cell-intrinsic

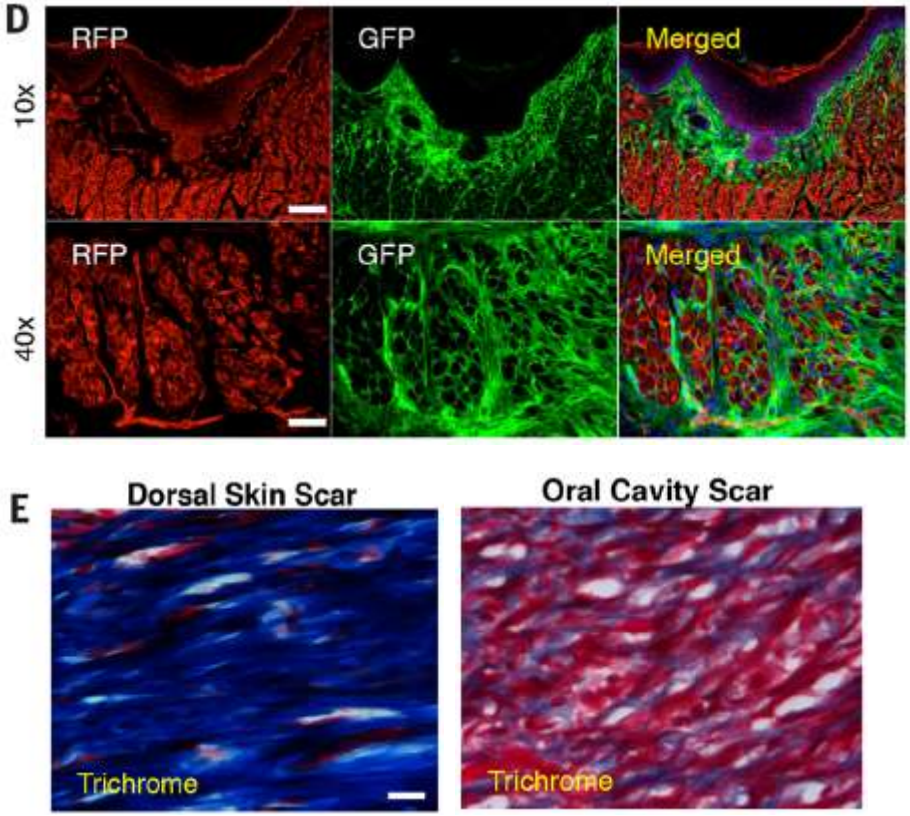


Fig 4D: oral scar tissue after wounding is GFP-positive and hence WFP-derived

Fig 4E: significant differences in oral vs. Dorsal skin scar: diminished collagen content in oral wounds

Transcriptome analysis: differences betweens EPFs and WPFs mainly in global expression signatures (according to somitic vs. neural crest origins)

Fibrogenic potential of dermal fibroblasts is cell-intrinsic

Are the site-specific differences (between EPFs and WPFs) cell-intrinsic properties or an outcome of anatomic microenvironment?

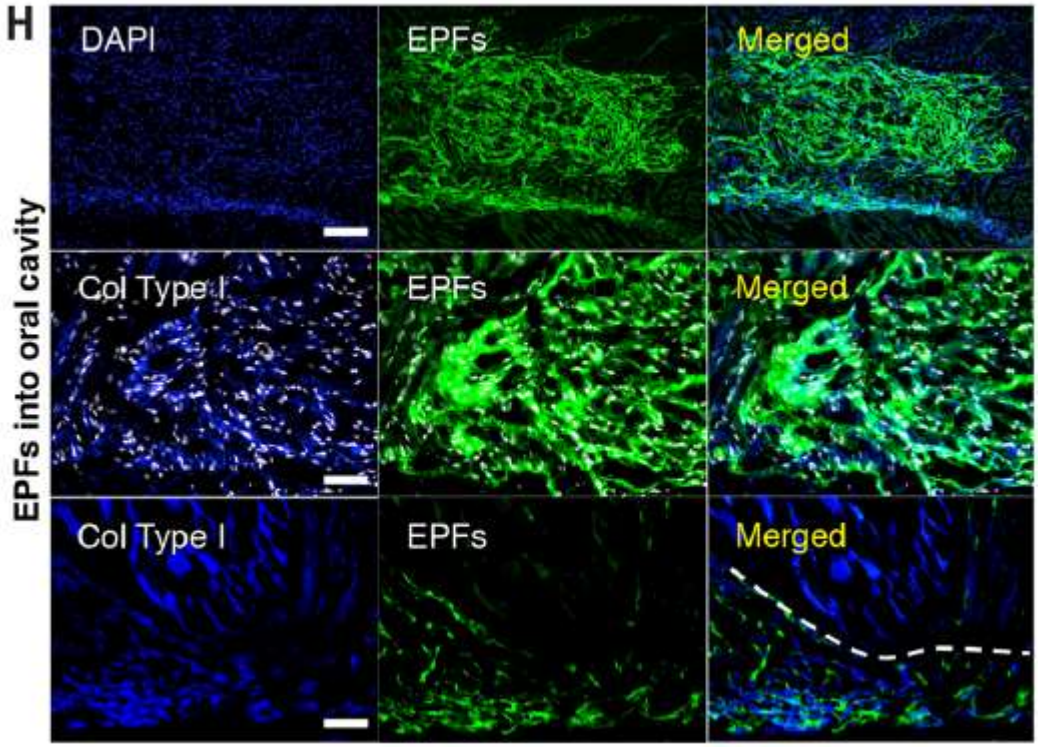


Fig 4H: reciprocal transplantation of WPFs (from the oral cavity) to the dorsum:

- significantly reduced scarring
- WPFs dispersed around hair follicles, „beehive“ pattern of collagen secretion
- mimicking oral scar

DTR-based ablation of EPFs reduces cutaneous scarring during wound healing

DTR/EPF-GFP-expressing mice

- treated with DT
- difference in healing time scar size and –architecture?

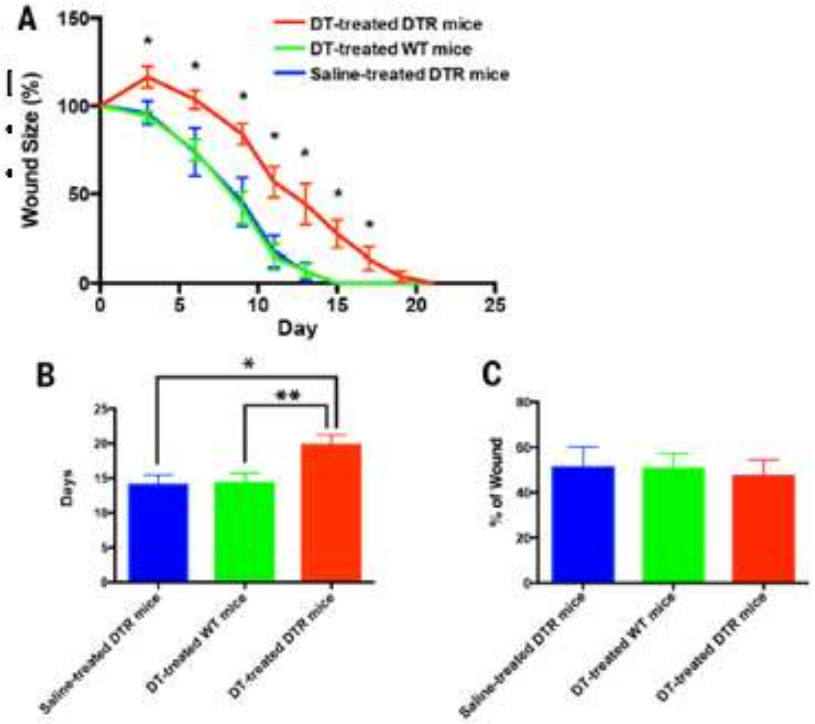


Fig 5A-C:

- Wound healing time in DTR-treated in DTR-treated mice is significantly elongated
- There is no significant difference in scar size

(DTR = diphteria toxin receptor,
DT = diphteria toxin)

DTR-based ablation of EPFs reduces cutaneous scarring

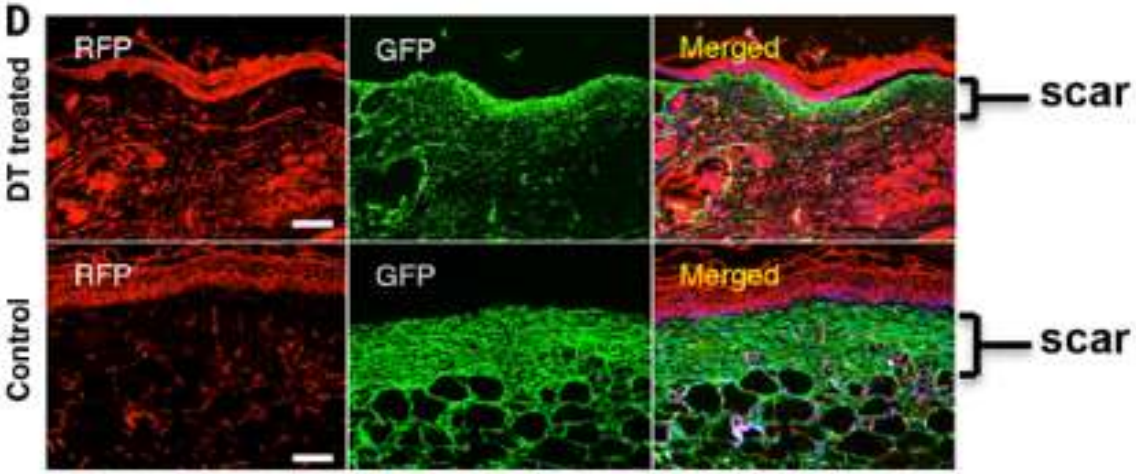


Fig 5D: greatly reduced connective tissue deposition in DTR-treated mice (GFP-labeled)

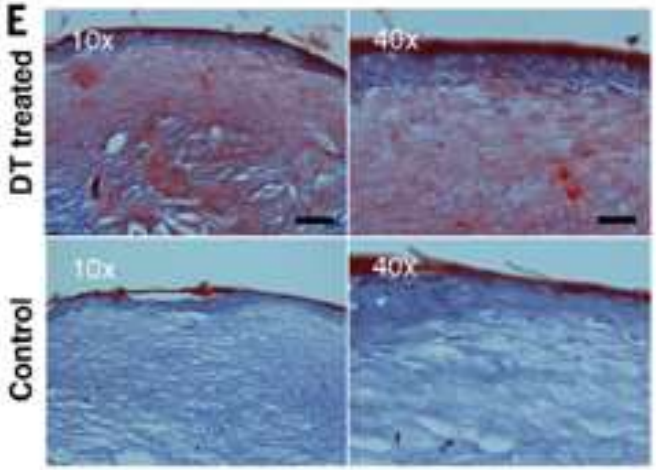


Fig 5E: reduced collagen density in DTR-treated wounds

DTR-based ablation of EPFs reduces cutaneous scarring

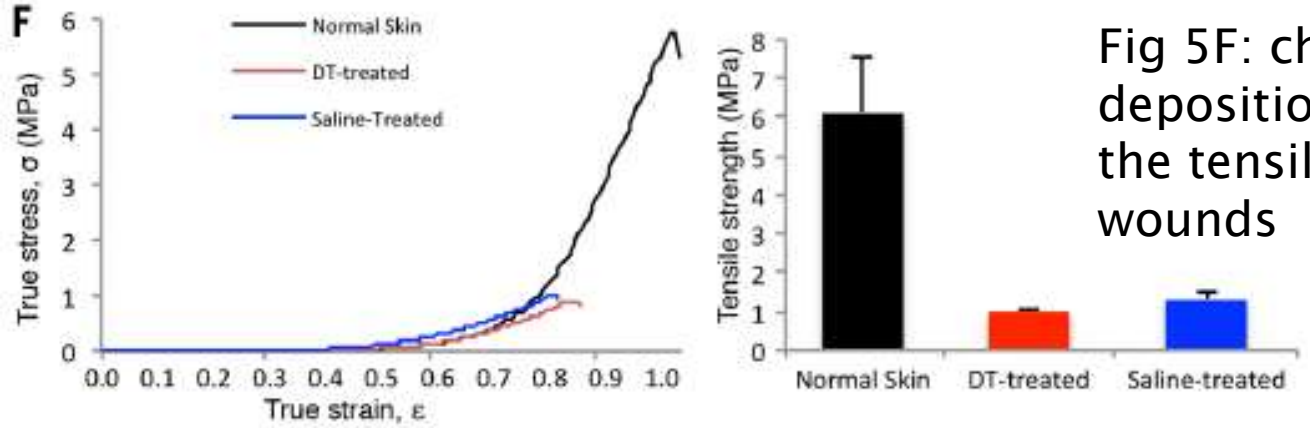
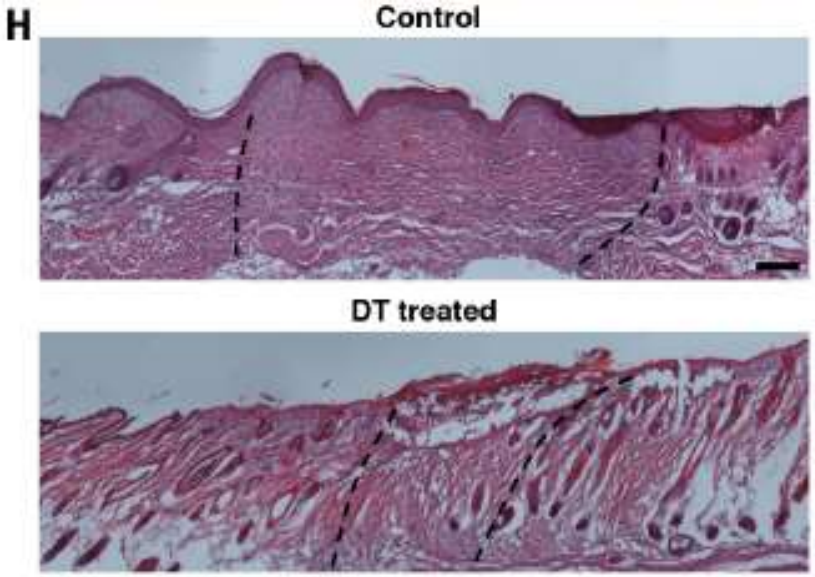


Fig 5F: change in collagen deposition does not influence the tensile strength of healed wounds



5H: change in ECM-deposition does not influence regeneration of adipocytes or hair follicles

DTR-based ablation of EPFs reduces melanoma growth

I

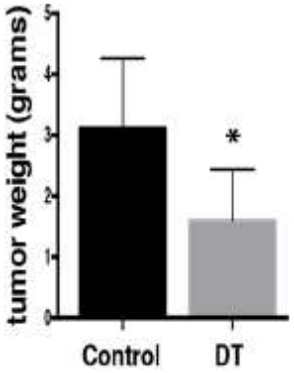


Fig 5I: tumor burden was significantly lower in mice treated with DT

J

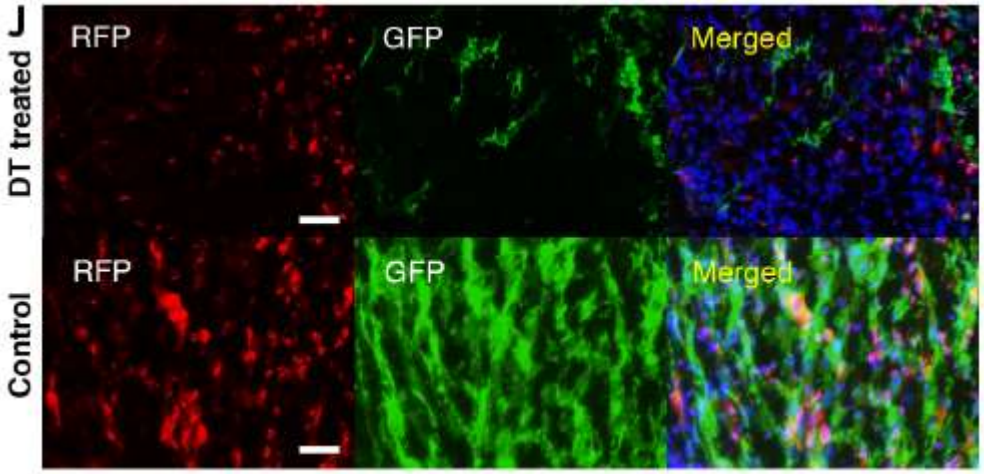
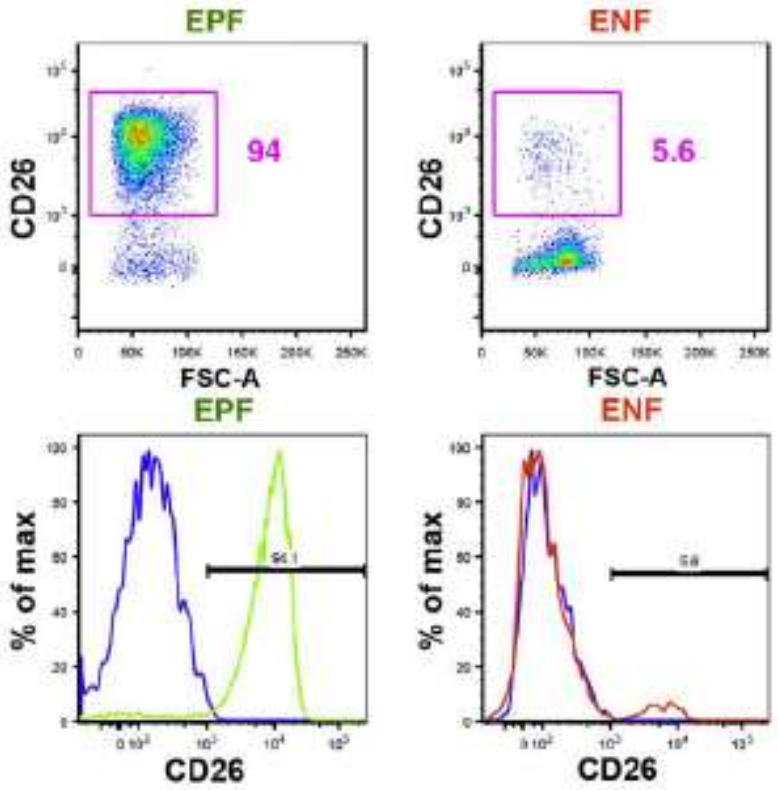


Fig 5J: histological analysis shows significant reduction of EFP-derived tissue deposition in DT-treated melanoma (GFP-labeled)

Results

Surface profiling and prospective isolation of EPFs

- FACS-isolation of fibroblasts
- 176 potential surface molecules
- Most surface molecules similarily present in both EPFs and ENFs
- Most prominent in EPFs vs. ENFs: CD26 (94% in EPFs), WPFs similar
- Surface profiling and prospective isolation of EPFs



Results

Surface profiling and prospective isolation of EPFs

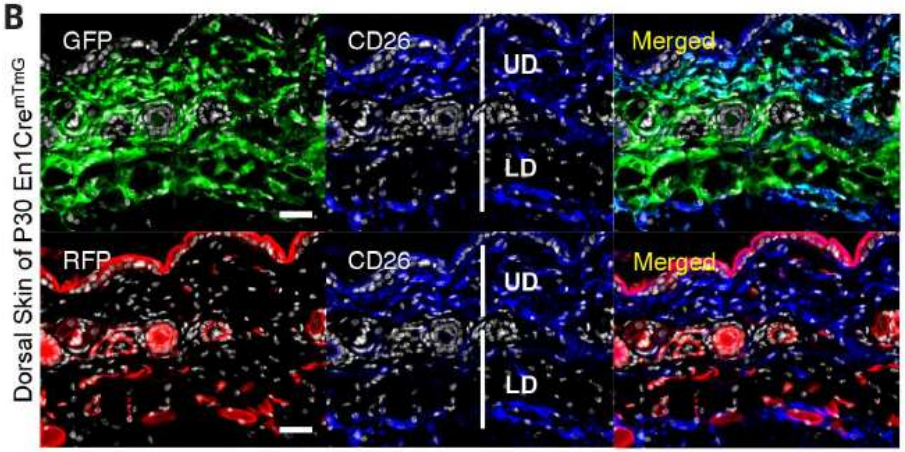


Fig 6B: overlapping immunopositivity of CD26 and GFP (marking EPFs) confirms FACS analysis

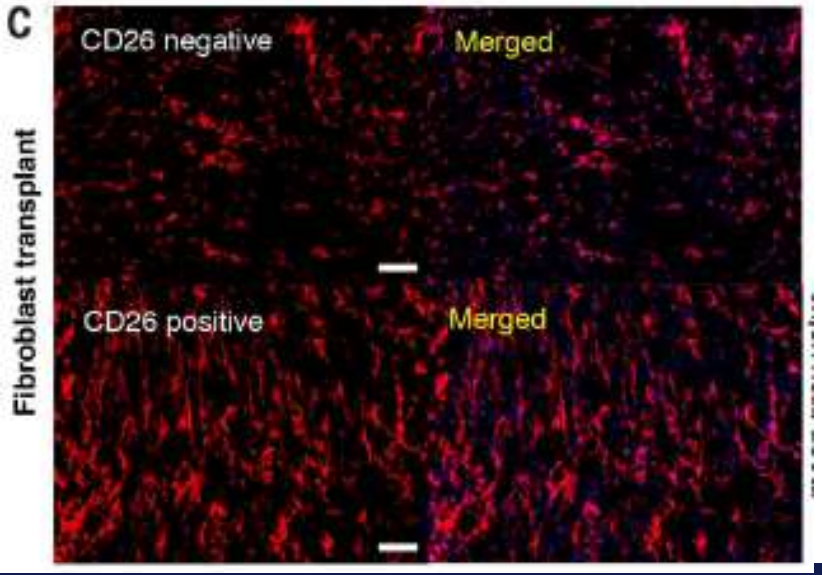
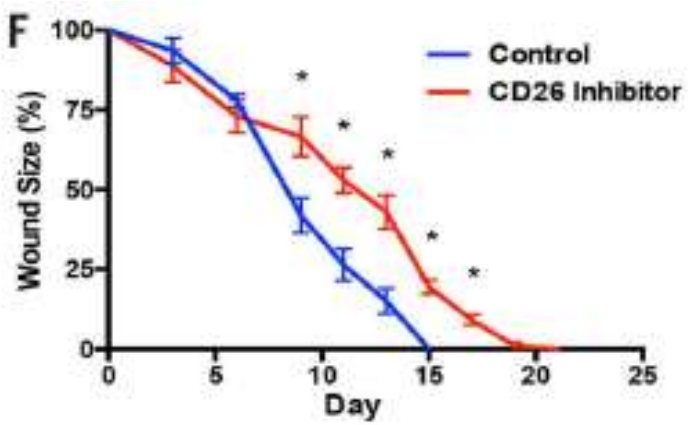
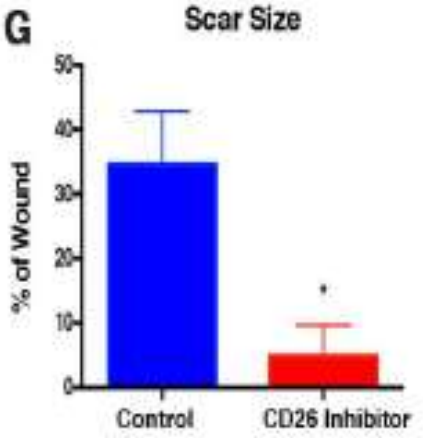


Fig 6C: fibrosis in-vivo assay for ECM-deposition
RFP-labeled = ECM
Blue = CD26+

Results

Inhibition of CD26 reduces cutaneous scarring during wound healing





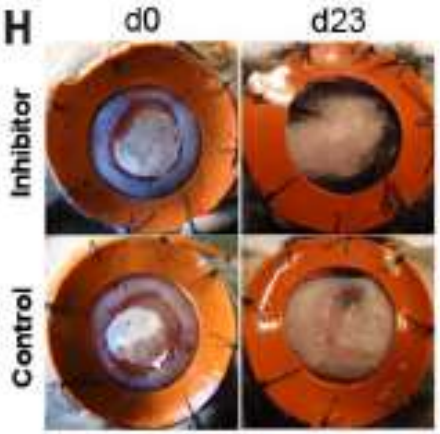
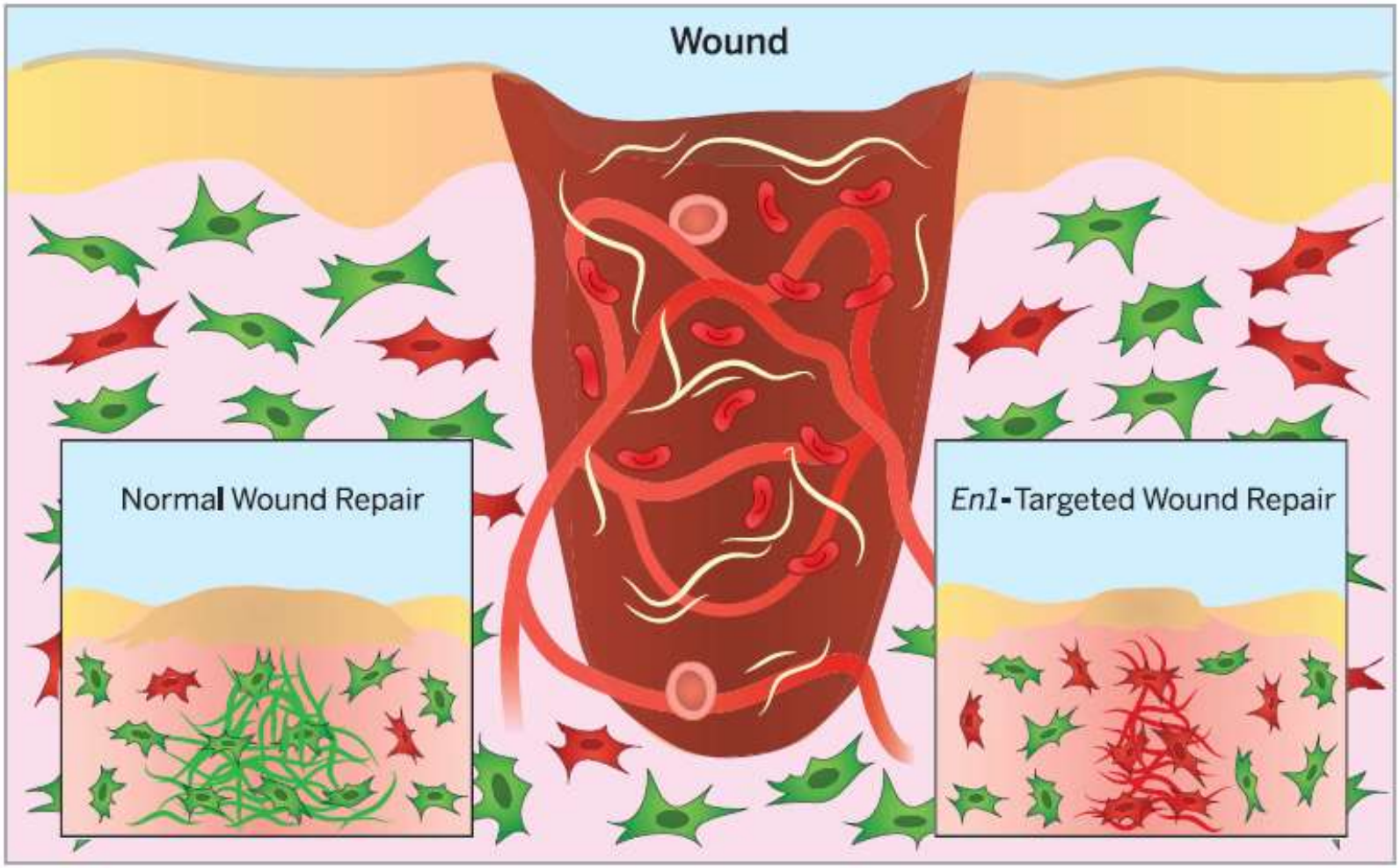


Fig 6F-H: Diprotin-A (CD26-inhibitor)-treated wounds show slower wound healing but significantly reduced scar size

Conclusion



Fibroblast functional properties: Intrinsic or extrinsic?

- Reciprocal transplantation shows that dermal architecture and wound healing are independent of local microenvironment
- Where do scar-forming dermal fibroblasts come from?

  → Distinct local lineages of resident fibroblasts are responsible for fibrosis

  →no ECM-contribution from other mesenchymal/nonmesenchymal cells

Targeting the fibroplast culprit behind cutaneous scarring

- CD26/DPP4-inhibitors (Sitagliptin, Vildagliptin) already approved by FDA for Diabetes II →
  - effects on wound healing?
  - Oral systemic dose sufficient to affect wound healing and fibrosis?
  - systemic or topical effects?

Personal comments

- Why En1/Wnt1in the first place?
  - No further work on the role of Engrailed-1 by the authors
- CD26 is also present in numerous other cell types
- Perhaps more clinical relevance for treating melanoma than for scar prevention?

  → delayed wound healing

Personal comments

- Use of Diptrotin-A as CD26-inhibitor?

  → Why not use Gliptins?

- Slower wound healing in Gliptin-treated patients?

→potential problem for diabetes patients?

No further literature available for clincal results of use of gliptins in wound healing!

Marfella A et al. 2014: **Dipeptidyl peptidase 4 inhibition may facilitate healing of chronic foot ulcers in patients with type 2 diabetes.**

Any questions?

Thank you!

Sources

- Sorrell, J. M. and A. I. Caplan (2009). "Fibroblasts-a diverse population at the center of it all." Int Rev Cell Mol Biol 276: 161-214.
- Matteucci, E. and O. Giampietro (2009). "Dipeptidyl peptidase-4 (CD26): knowing the function before inhibiting the enzyme." Curr Med Chem 16(23): 2943-2951.
- Doupis, J. (2014). "Linagliptin: from bench to bedside." Drug Des Devel Ther 8: 431-446.
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