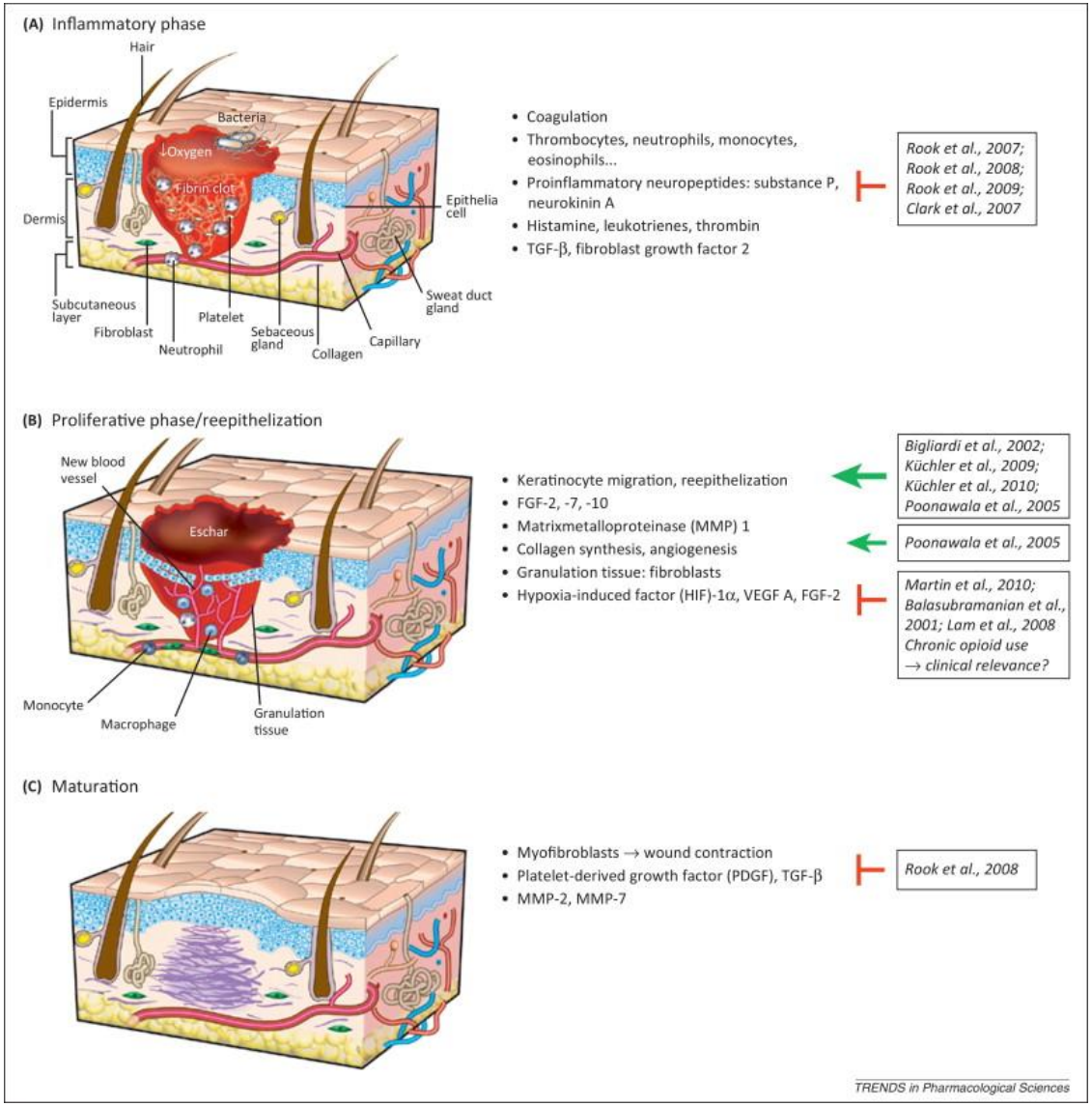
Single-cell analysis reveals fibroblast heterogeneity and myeloid-derived adipocyte progenitors in murine skin wounds

Christian F. Guerrero-Juarez,1,2,3 Priya H. Dedhia,4 Suoqin Jin,3,5 Rolando Ruiz-Vega,1,3 Dennis Ma,6 Yuchen Liu,1,2,3 Kosuke Yamaga,1,2,3 Olga Shestova,4 Denise L. Gay,7 Zaixin Yang,8 Kai Kessenbrock,6 Qing Nie,1,3,5 Warren S. Pear,4 George Cotsarelis, 8 and Maksim V. Plikus

Nat Commun. 2019; 10: 650.

Published online 2019 Feb 8. doi: 10.1038/s41467-018-08247-x

Introduction: Wound healing



Stein C, Küchler S. Targeting inflammation and wound healing by opioids. Trends in Pharmacological Sciences. 2013;34(6):303-12.

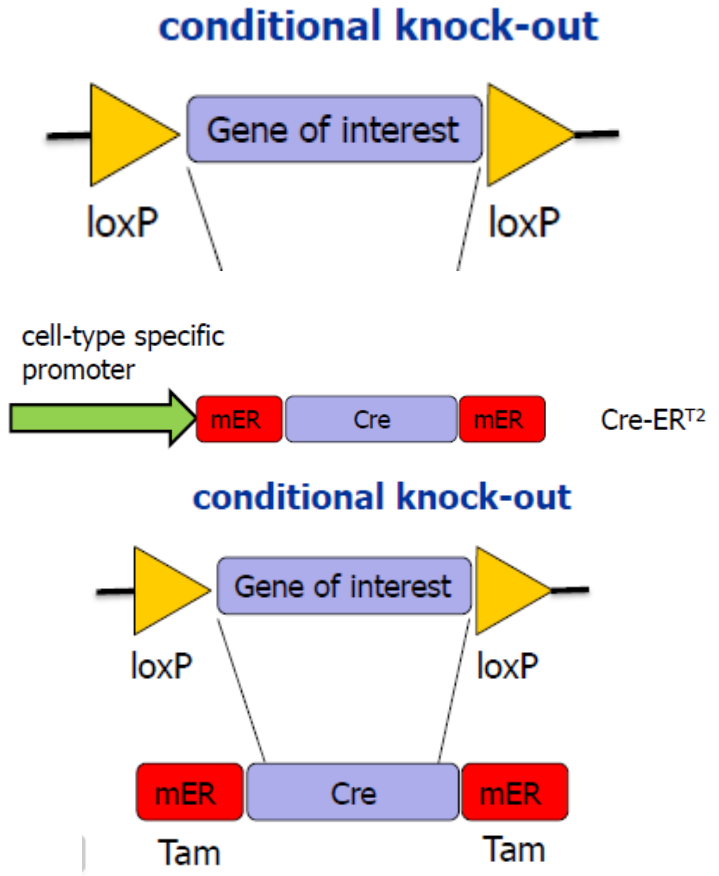
Methods

- C57/BL6 mice; Retn-lacZ, Sm22-Cre, Cd45-Cre, LysM-Cre, Fabp4-Cre, Pparγflox R26R, tdTomato, GFP, RFP, and Rag1−/−
- Full thickness mouse wound model: 1,5x1,5cm square wounds, healing without further intervention
- Bone marrow transplantation:
  - bone marrow flushed from long bones of healthy donor mice
  - HSCs FACS-sorted for Lineage^{neg}, SCA1^{+}, c-KIT^{+}, CD150^{+}, CD48^{neg}
- Single cell sequencing:
  - Pooled mouse wound tissues n=12, digestion for 60min, removal of dead cells by GentleMACS Dead cell removal kit (magnetic bead sorting)
  - Downstream analysis: „Seurat"-package, PC-analsis, tSNE-clustering; „Monocle" for trajectory calculations,
- Single cell western blotting

Method-teaching: transgenic mice

- **Elimination of gene**:
  - Classic „Knock-out mouse“: eliminates a gene in all cells (often embryonically lethal)
- **Conditional Knock-out**: Gene of interest is placed between LoxP-Sites
  - Cre-Recombinase: Cre recombinase cuts out sequences between loxPsites (or inverts sequences between inverted loxPsites)
  - Cre expression cell-type or organ-specific using cell-type specific promoters driving Cre expression (spatial control)
  - Cre expression: inducible by Tamoxifen by using chimeras of Cre with mutated estrogen receptor domains(temporal control: e.g. Cre-ERT2) → presence of Tamoxifen = Cre is expressed

Schmid J; Lecture: „Methods in life sciences“, SS2018



Method-teaching: transgenic mice

Schmid J; Lecture: „Methods in life sciences“, SS2018

- **Conditional Knock-in**:
  - inserting an expression construct headed by a loxP-flanked „Stop cassette“, cut out by Cre recombinase
- **Specific cell ablation**:
  - cross-breeding with a cell-type specific Cre strain
  - DTR is expressed only in specific cell types
  - injection of diphteria toxin leads to specifi killing of these cells
- **Specific cell labelling**:
  - Cell-type specific promotor →LoxP/Stop → fluorescent protein (GFP, EYFP, Tomato red protein)
  - LacZ: reporter system for beta-Gelactosidase expression: blue Color in tissues expressing LacZ

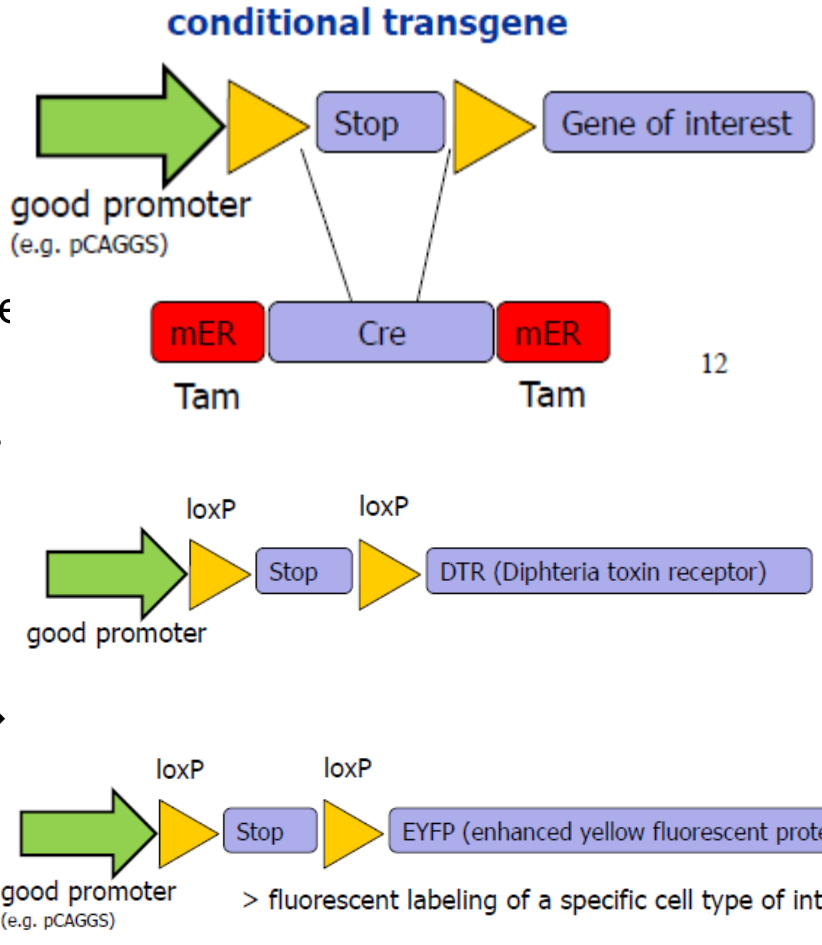


Figure 1: scRNA-seq analysis reveals cellular heterogeneity in day 12 wounds

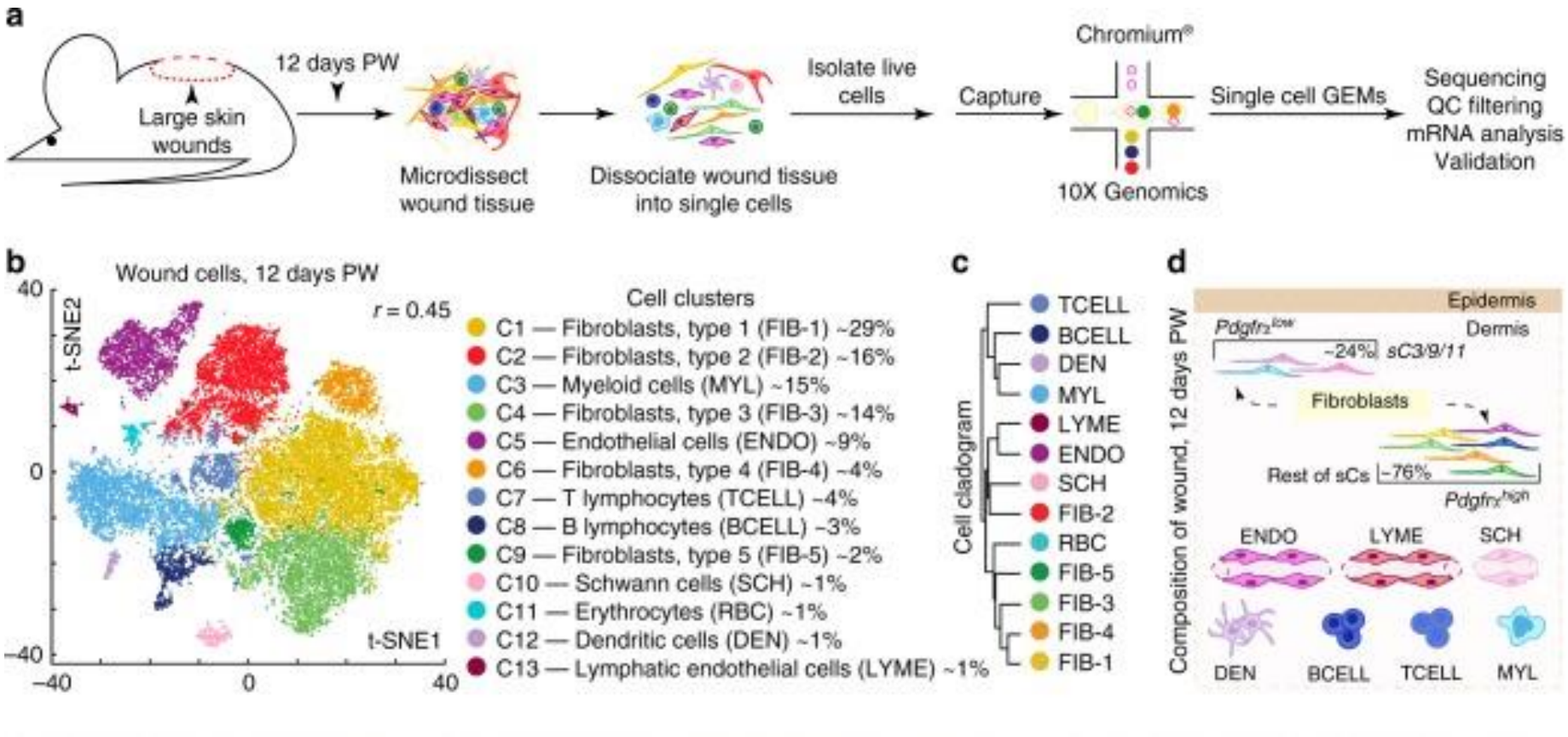


Figure 1:scRNA-seq analysis reveals cellular heterogeneity in day 12 wounds

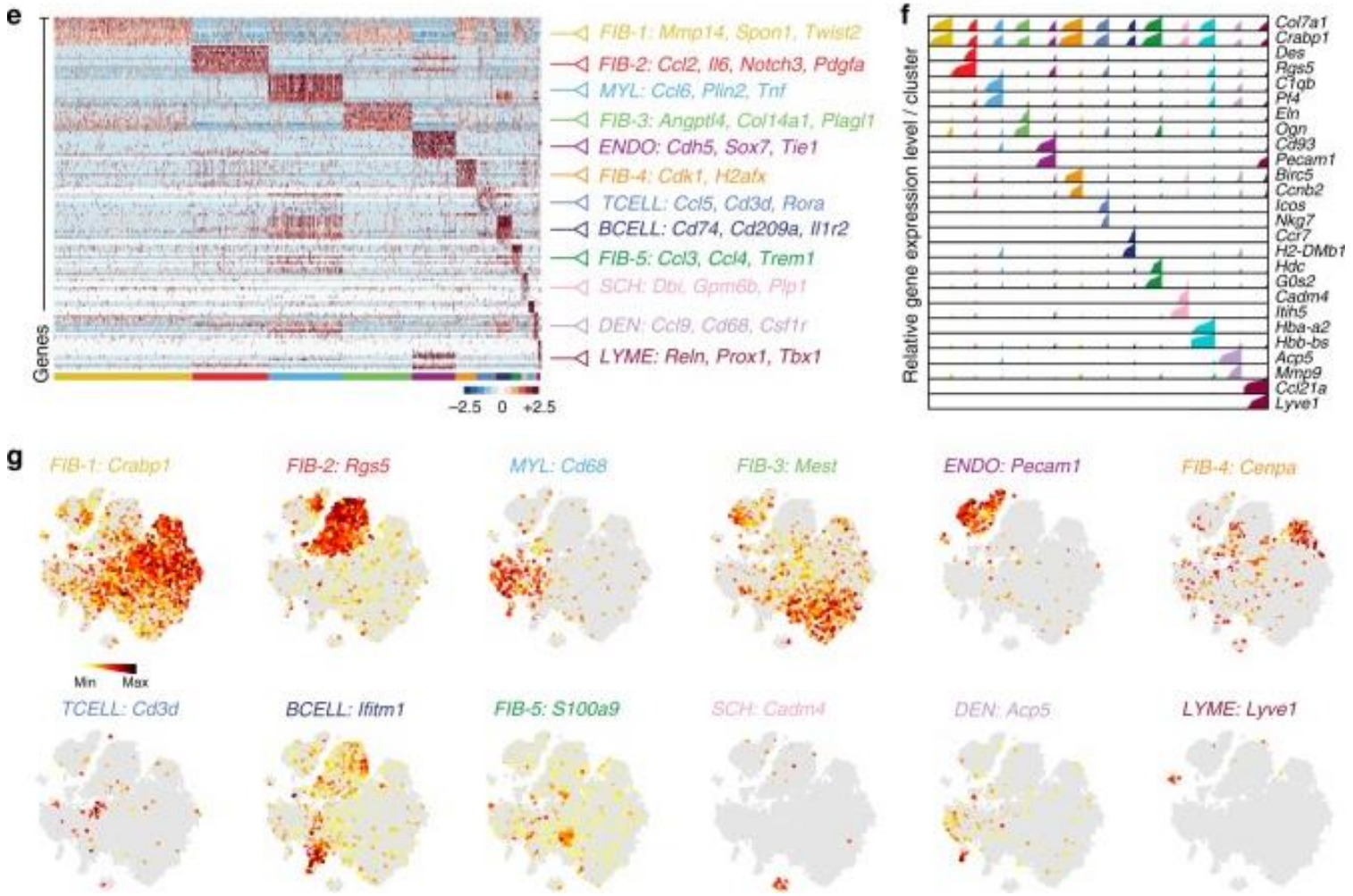


Figure 2:Subclustering of wound fibroblasts reveals cellular heterogeneity.

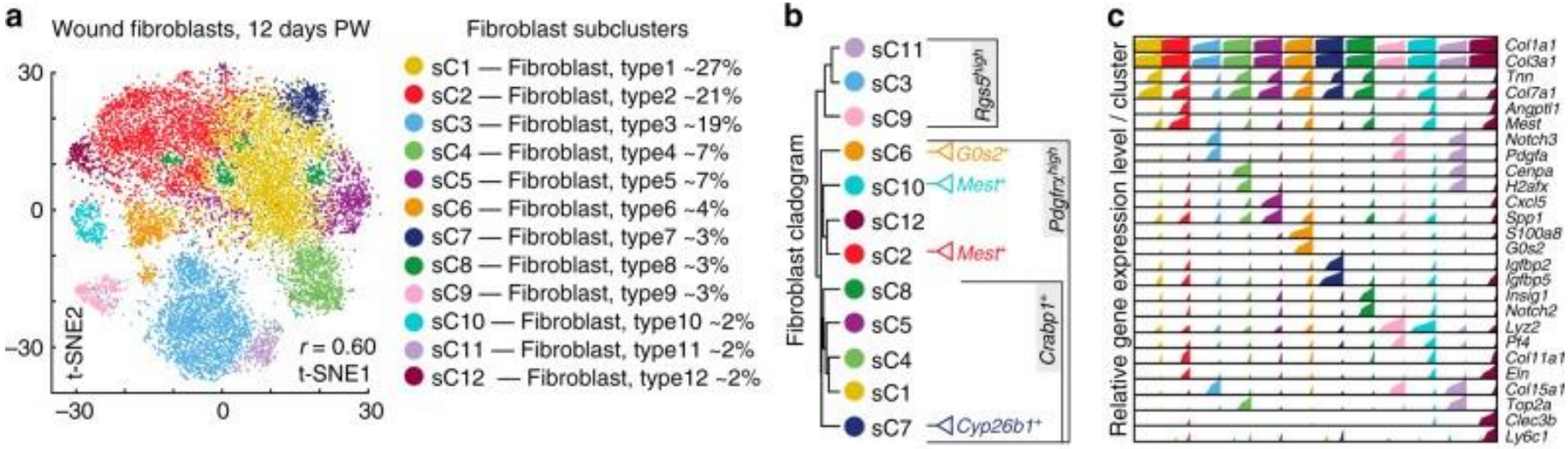


Figure 2:Subclustering of wound fibroblasts reveals cellular heterogeneity.

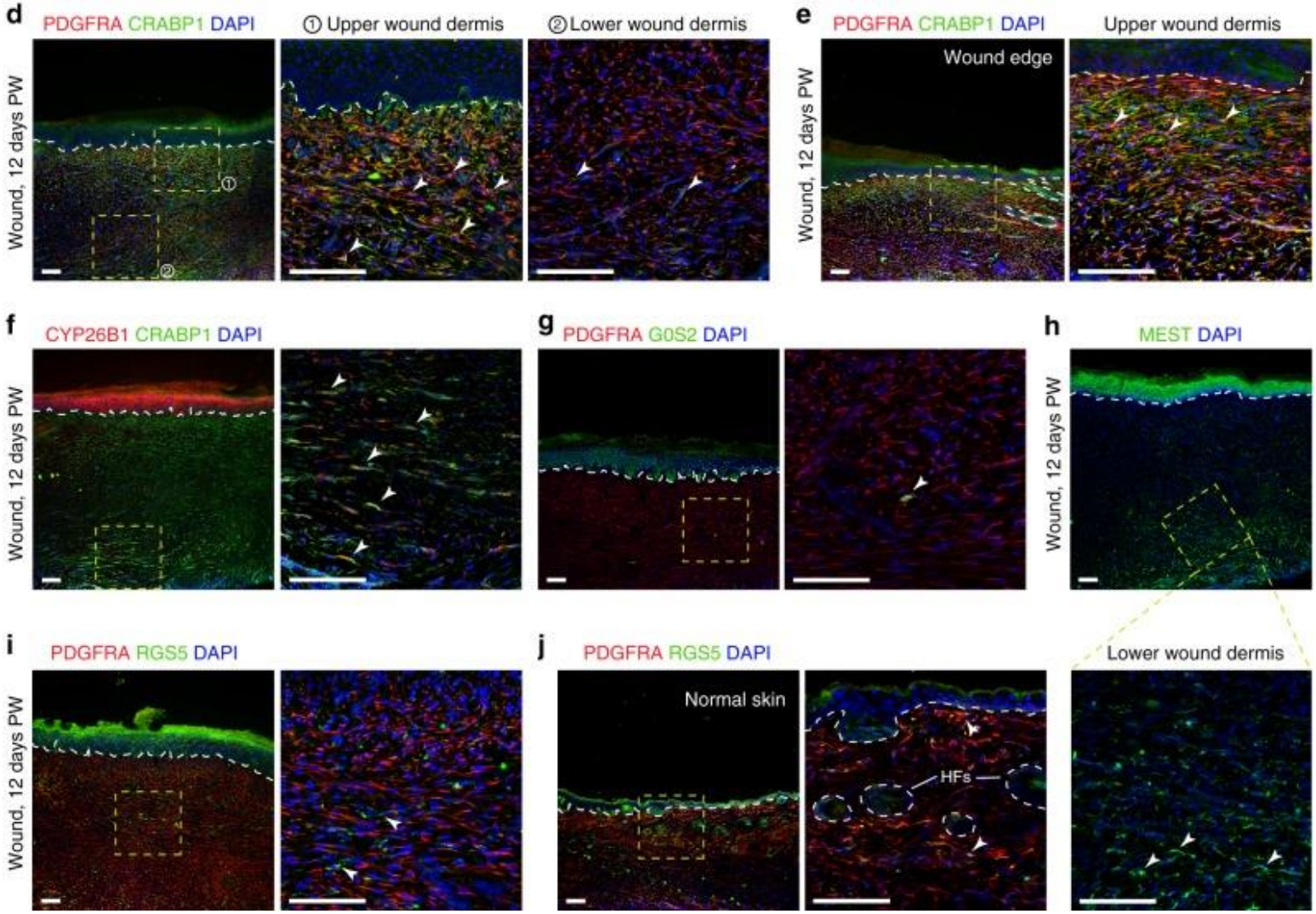


Figure 3: Pseudotime analyses reveal putative fibroblast differentiation trajectories.

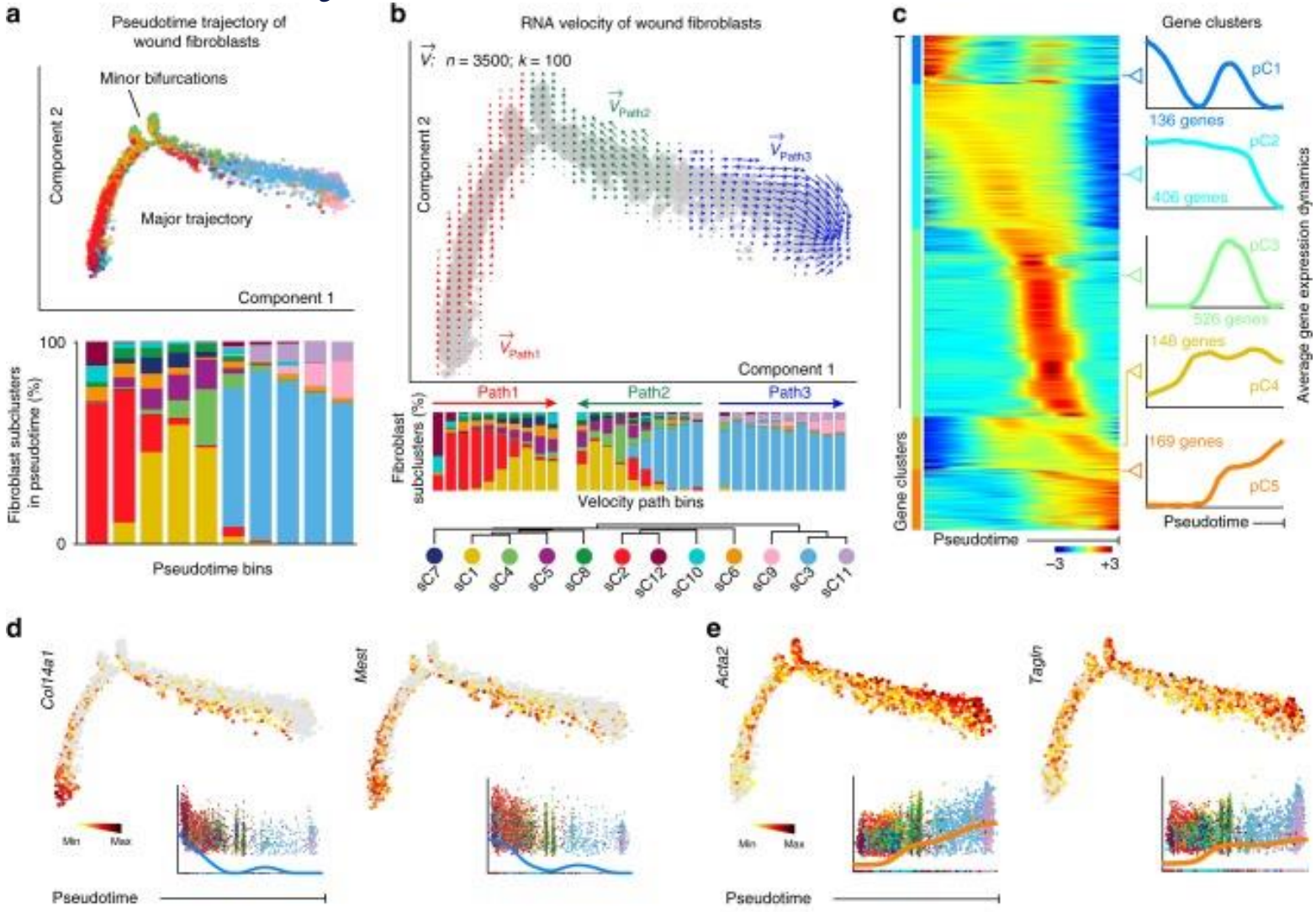


Figure 3: Pseudotime analyses reveal putative fibroblast differentiation trajectories.

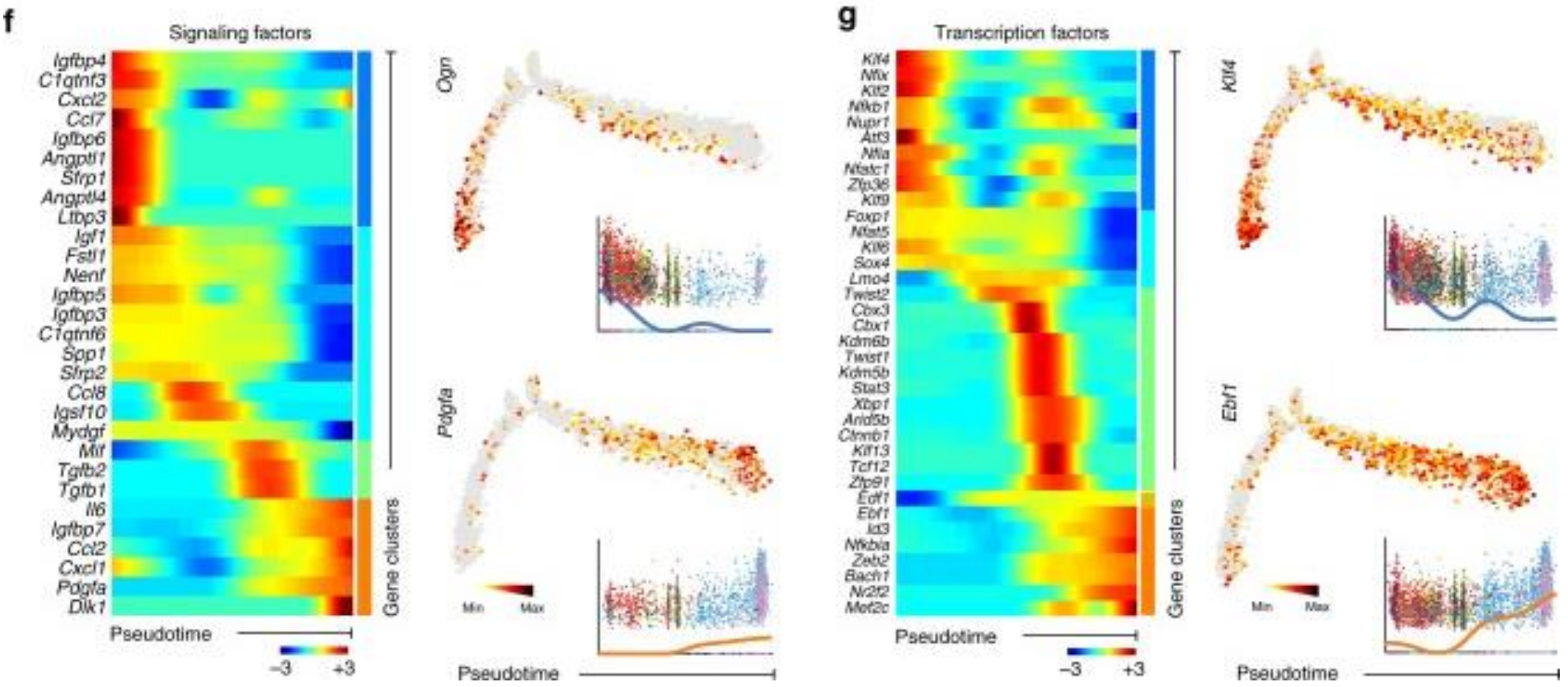


Figure 4: Identification of rare myeloid-derived myofibroblasts in day 12 wounds.

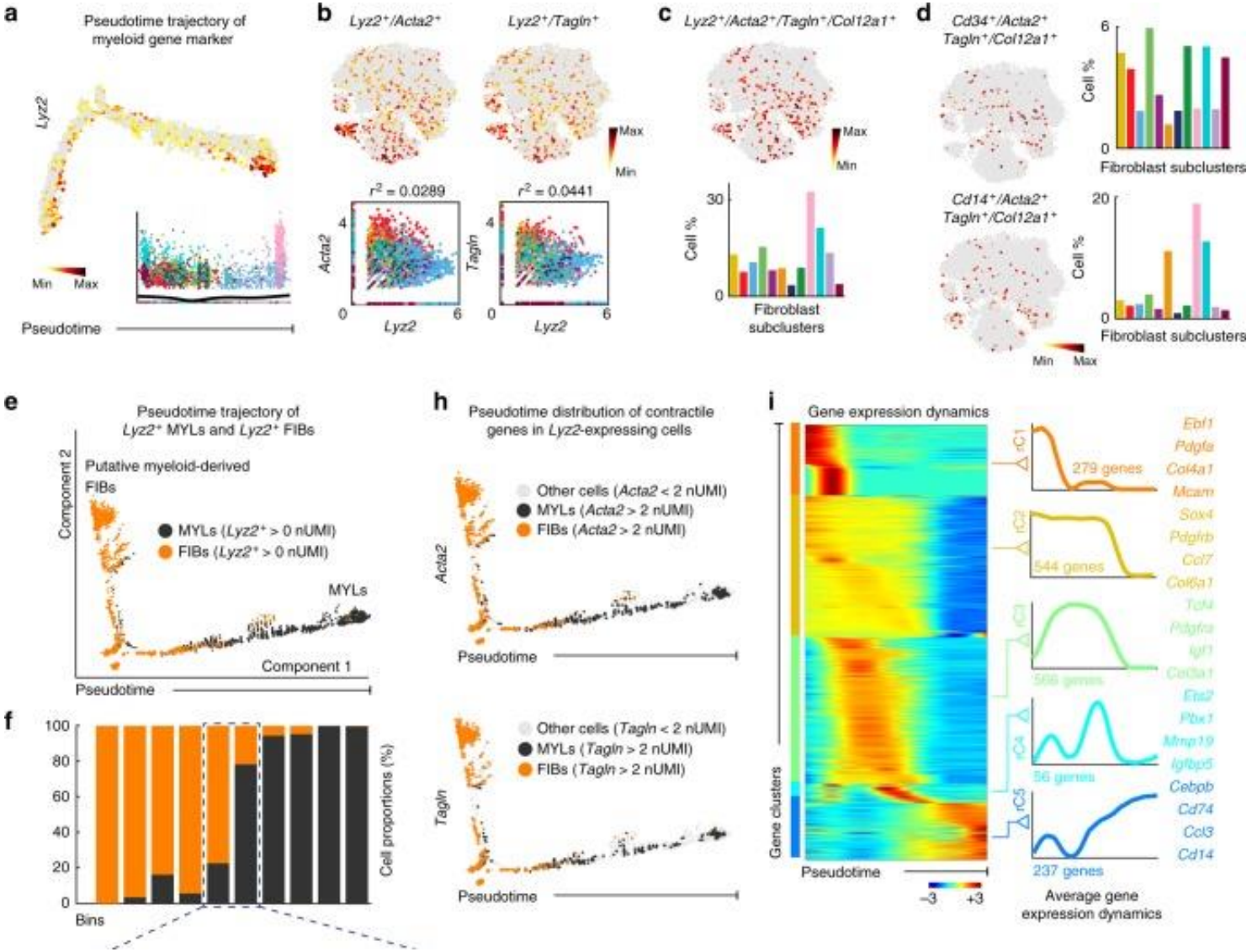


Figure 4: Identification of rare myeloid-derived myofibroblasts in day 12 wounds.

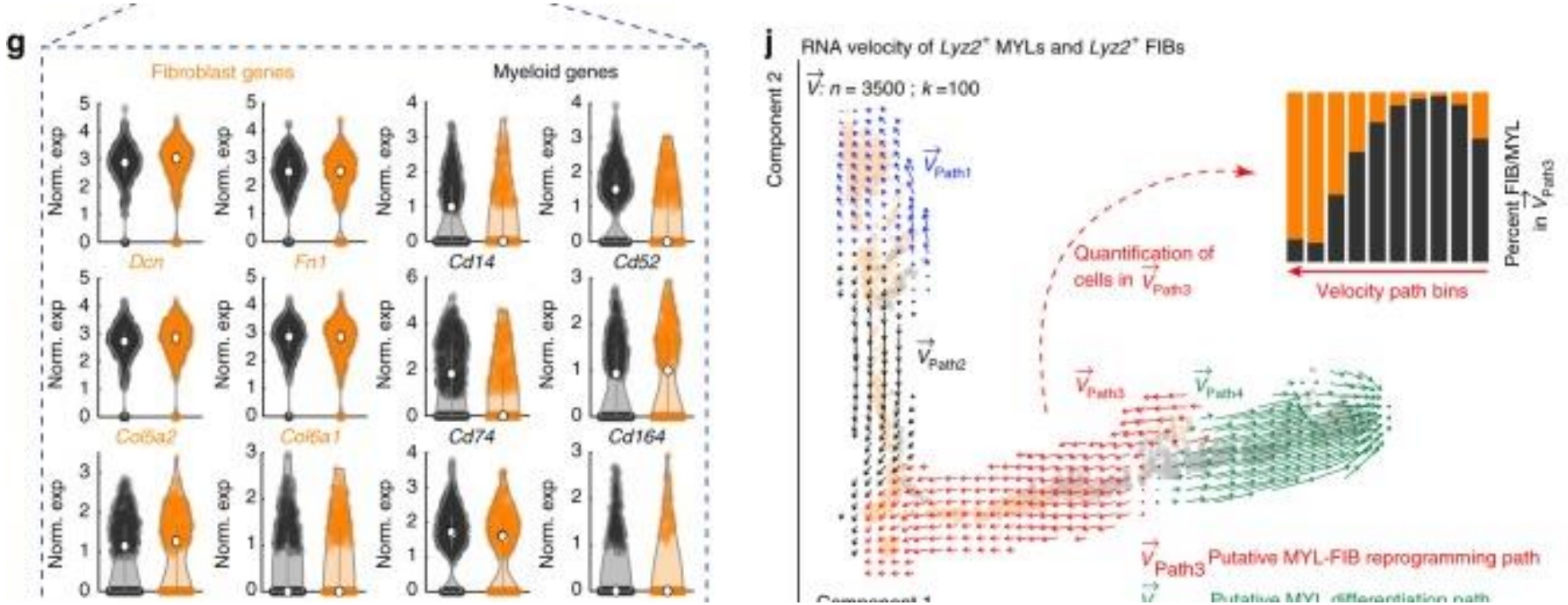


Figure 5: Validation of myeloid-derived myofibroblasts in day 12 wounds.

- scRNAseq on fluorescence-marked contractile cells

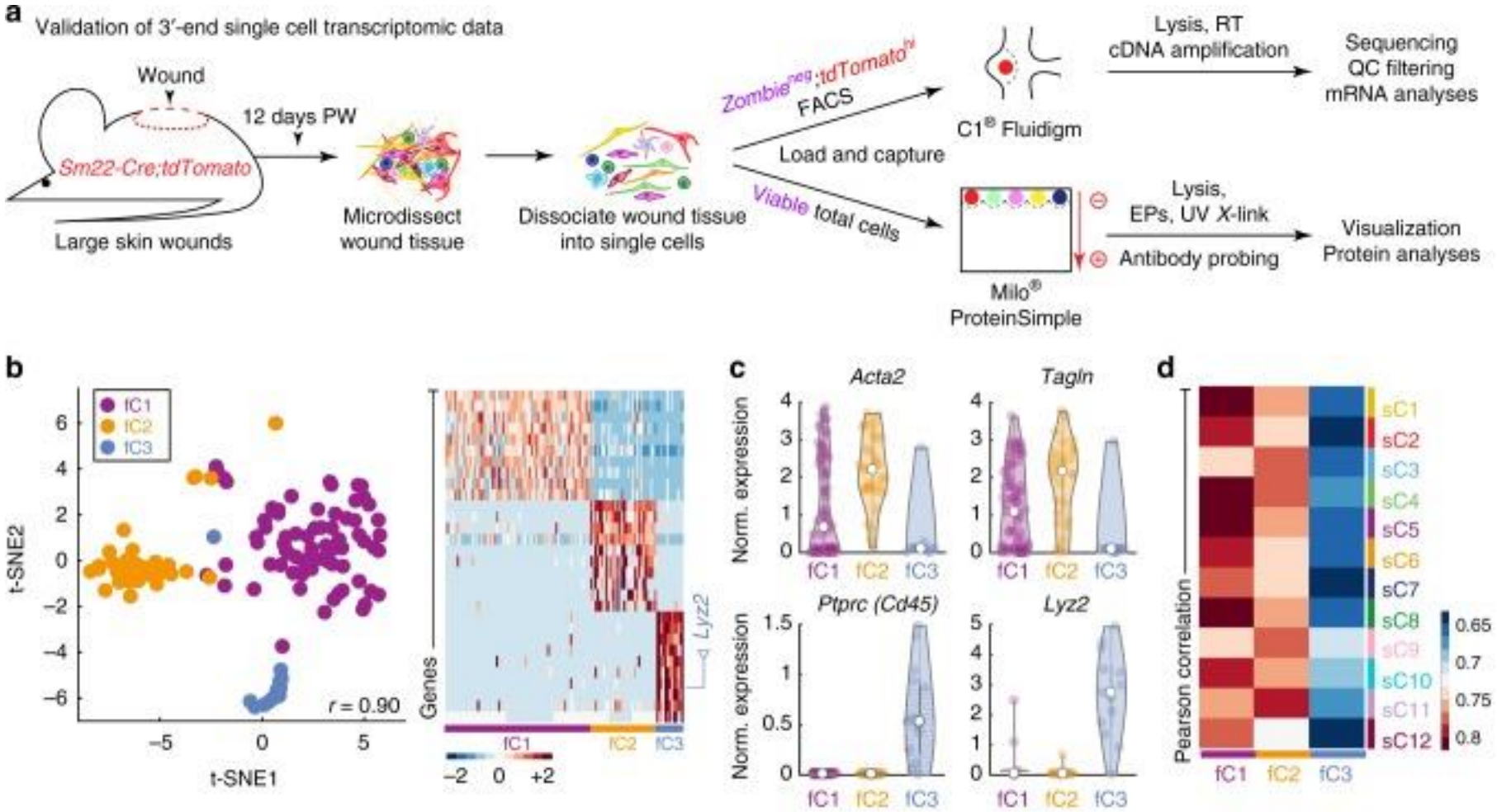


Figure 5: Validation of myeloid-derived myofibroblasts in day 12 wounds.

- Confirmation on protein level

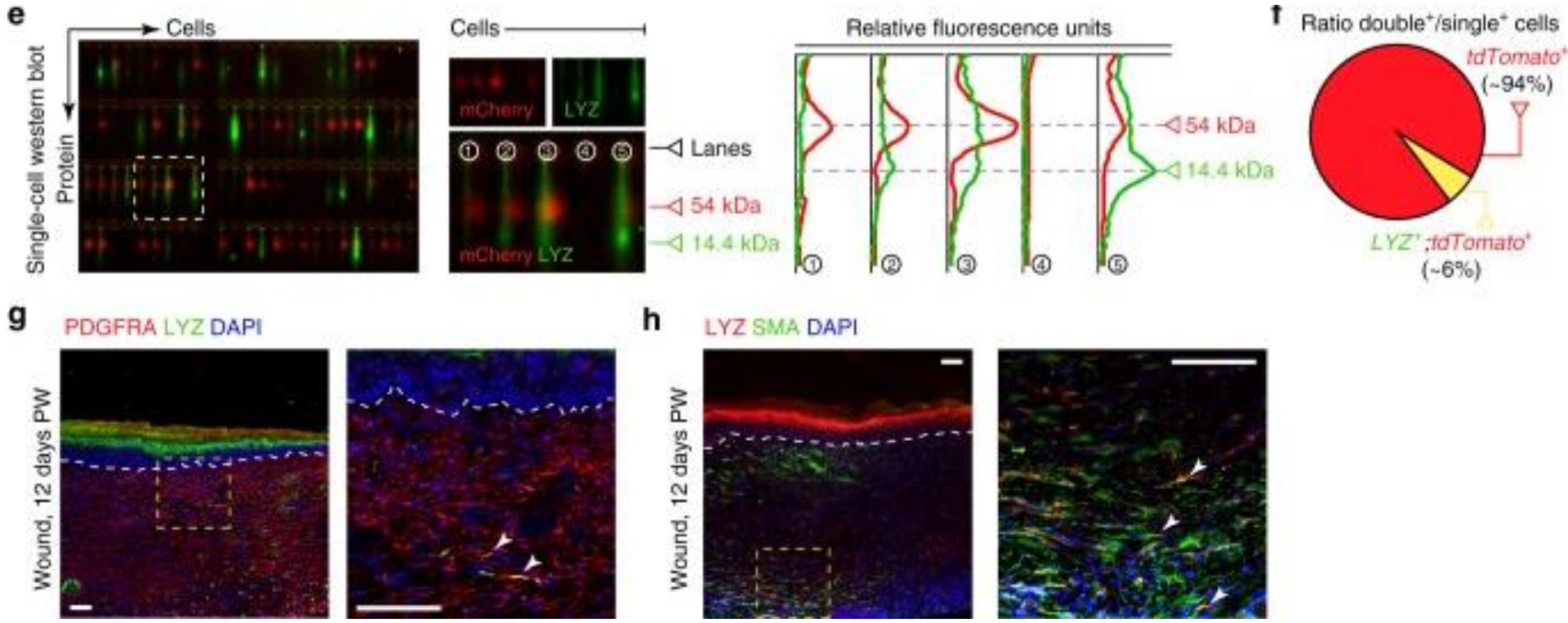
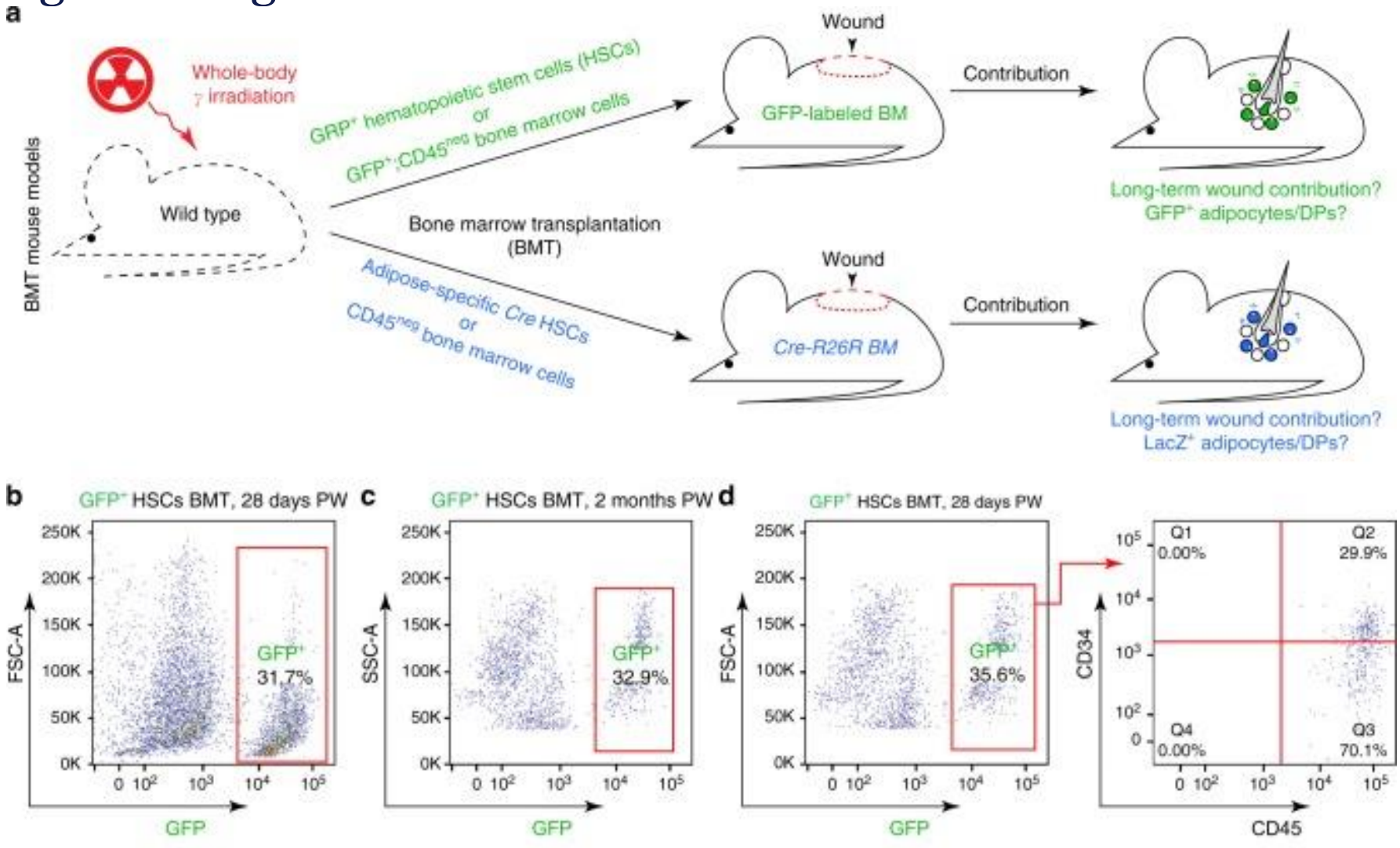


Figure 6: Hematopoietic lineage contributes toward regenerating wounds in BMT mice



- FACS-analysis from wound tissue

Figure 6: Hematopoietic lineage contributes toward regenerating wounds in BMT mice

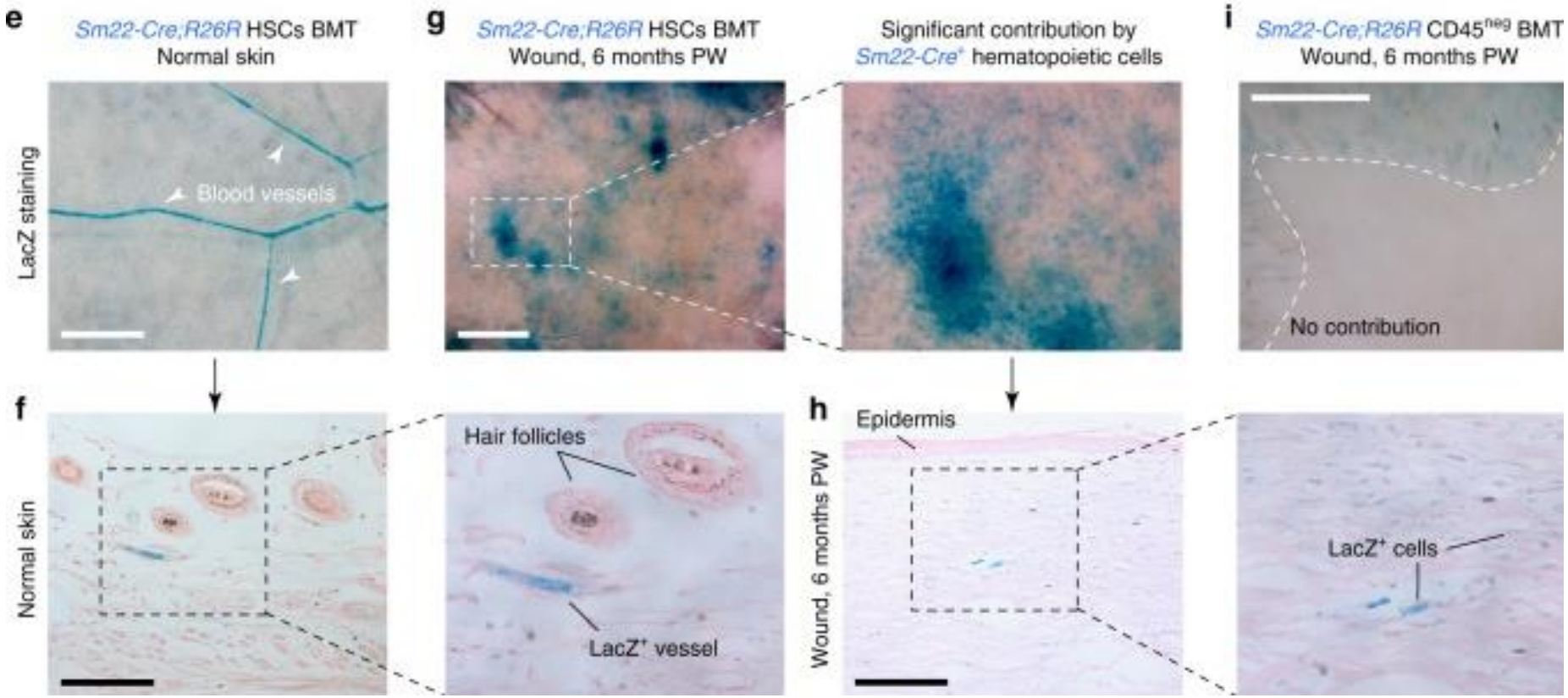


Figure 7: Hematopoietic lineage cells contribute to rare de novo adipocytes

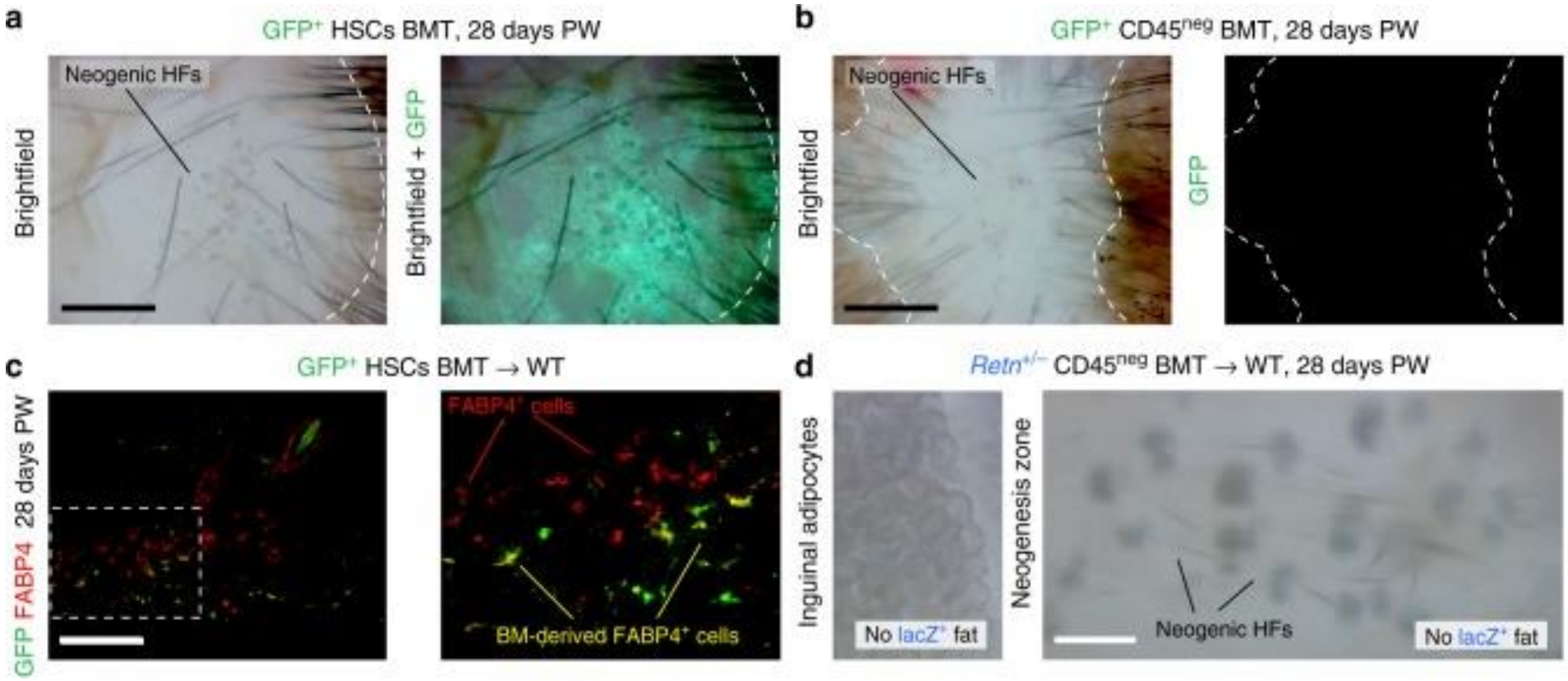


Figure 7: Hematopoietic lineage cells contribute to rare de novo adipocytes

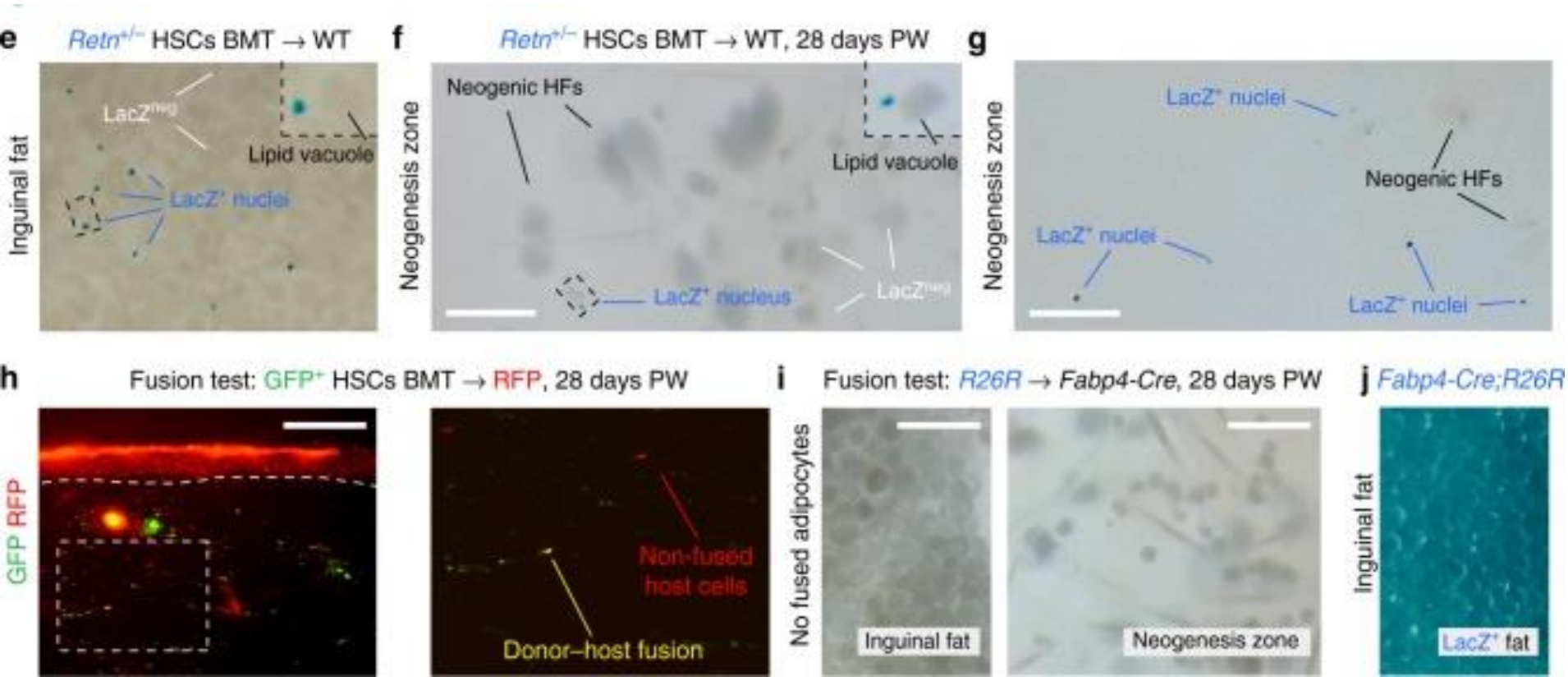
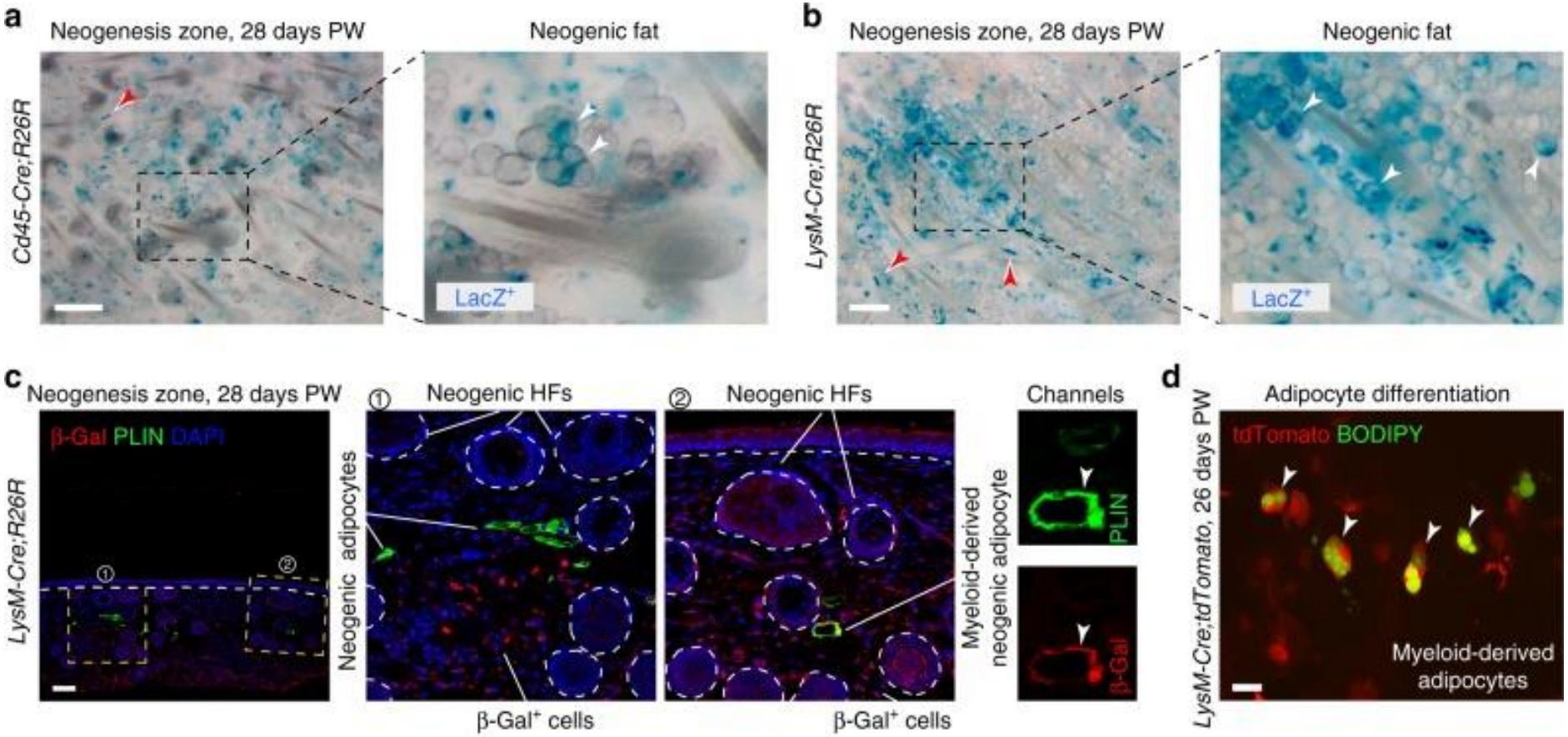
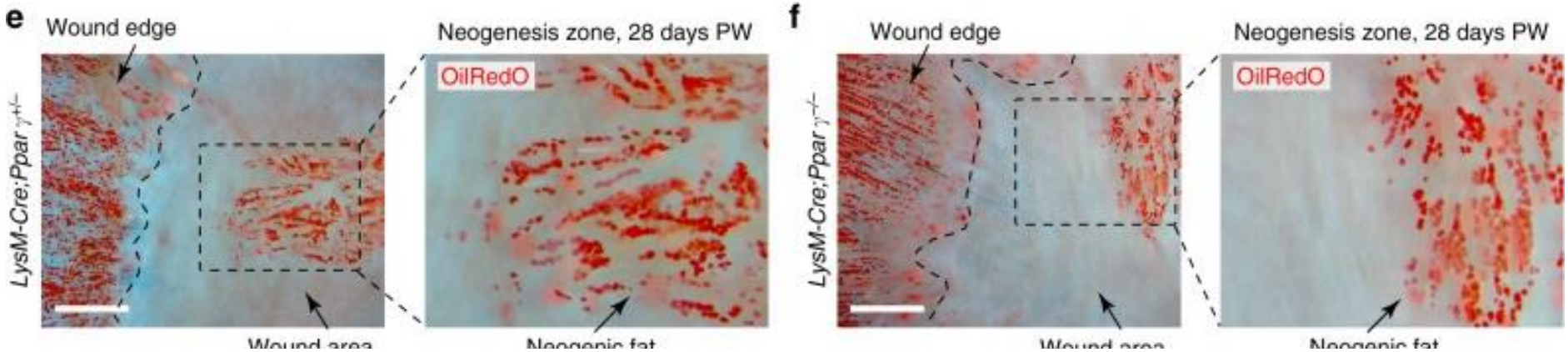


Figure 8: Myeloid lineage cells contribute to rare de novo adipocytes



- myeloid-specific marker Lyz2 (Lysozyme 2, aka LysM)

Figure 8: Myeloid lineage cells contribute to rare de novo adipocytes



Discussion

- Main finding: hematopoietic stem cells can differentiate into myofibroblasts, adipocytes and neogenic heir follicles during wound healing
- Discrepancy to previous studies: due to different timepoints, extent of wound
- Implication: findings as basis to identify treatments for scarfree wound healing/reduced scarring
- Translation to human wound healing?

Danke!