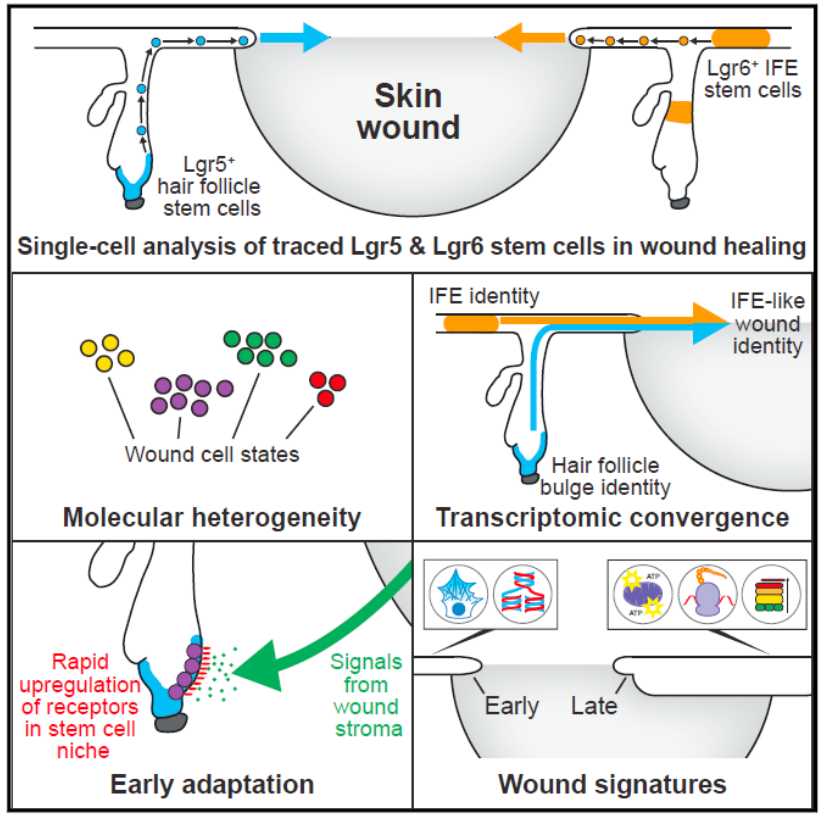
Article

Cell Reports

Single-Cell Transcriptomics of Traced Epidermal and Hair Follicle Stem Cells Reveals Rapid Adaptations during Wound Healing

Graphical Abstract



Authors

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In Brief

Joost et al. elucidate how skin stem cells from different niches respond upon injury. Single-cell transcriptomics revealed that Lgr5 and Lgr6 progeny molecularly converge during wound healing. Instant cell adaptations of Lgr5 cells within their original niche permit interactions with the wound environment, an ability Lgr6 cells already possess before wounding.

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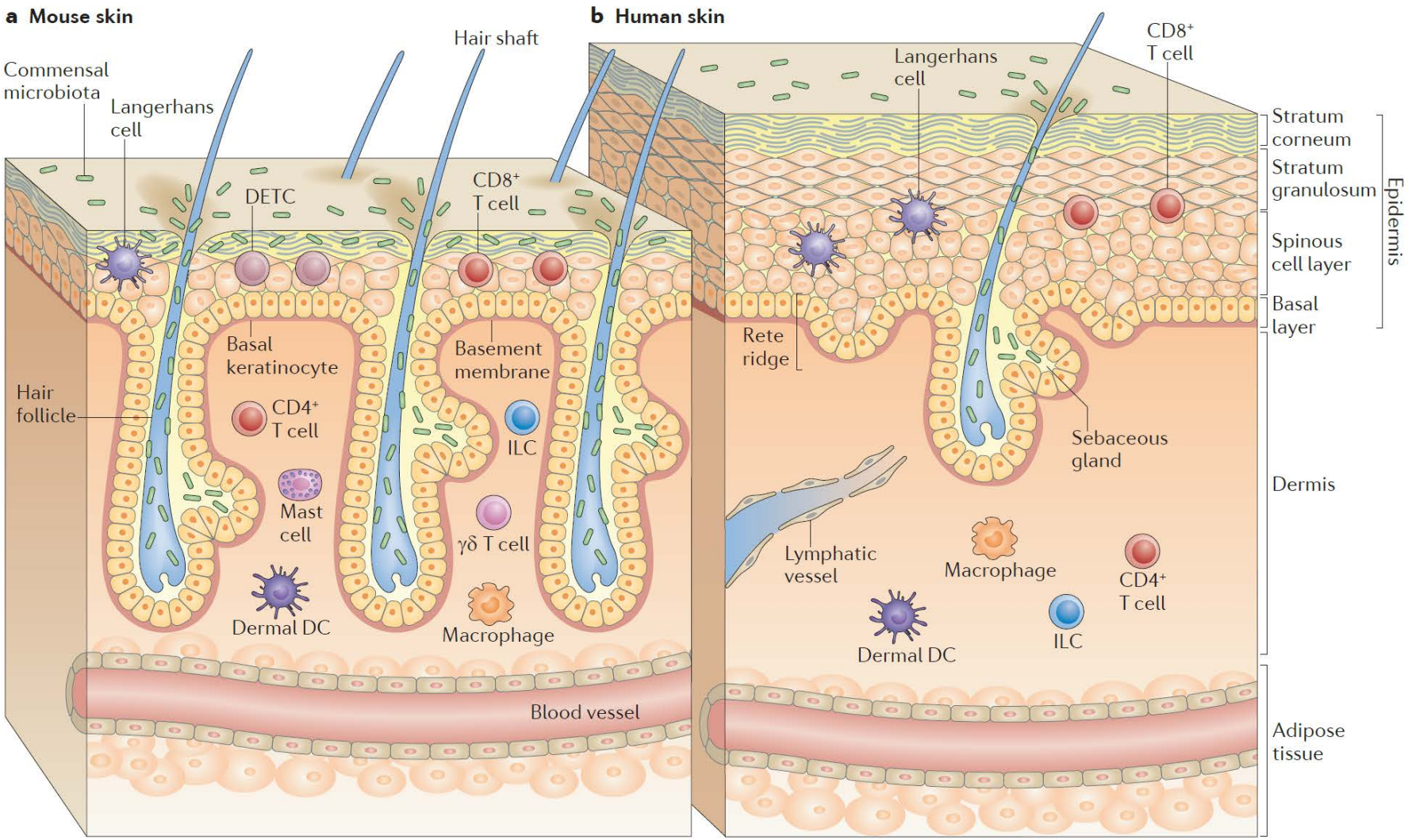
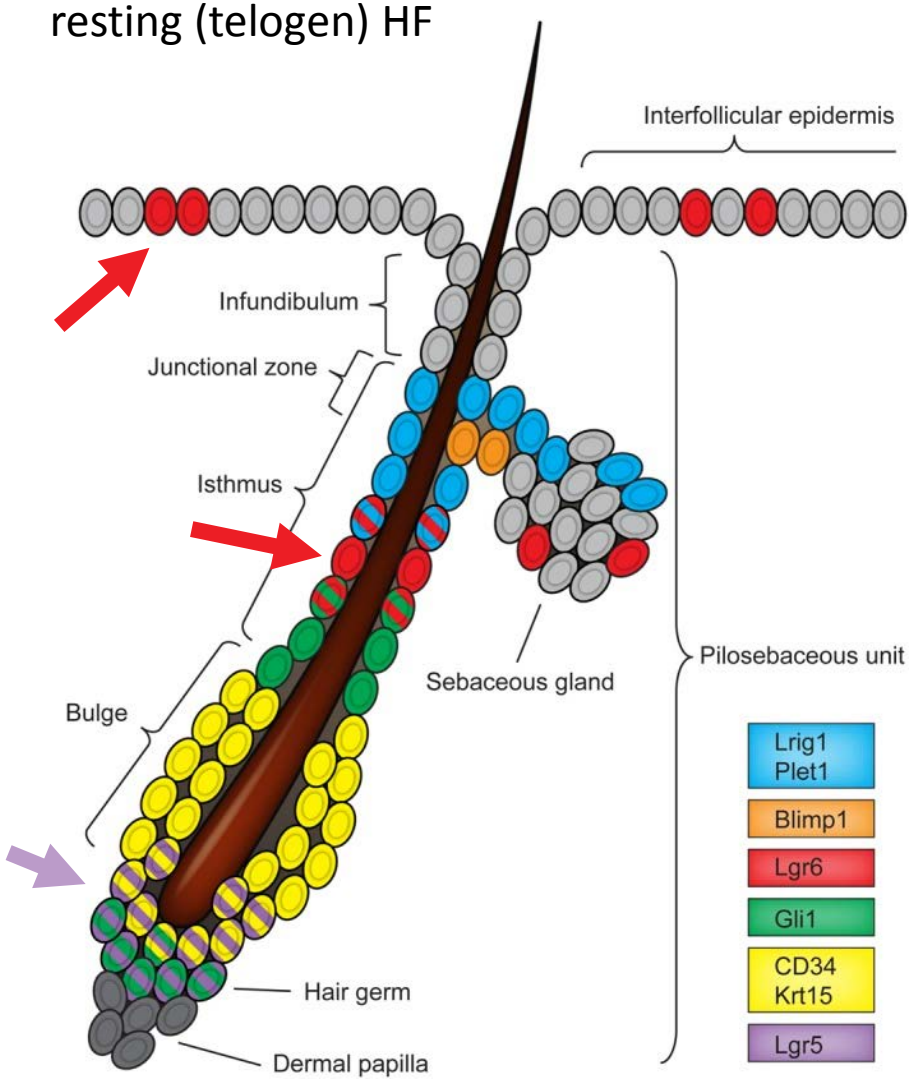


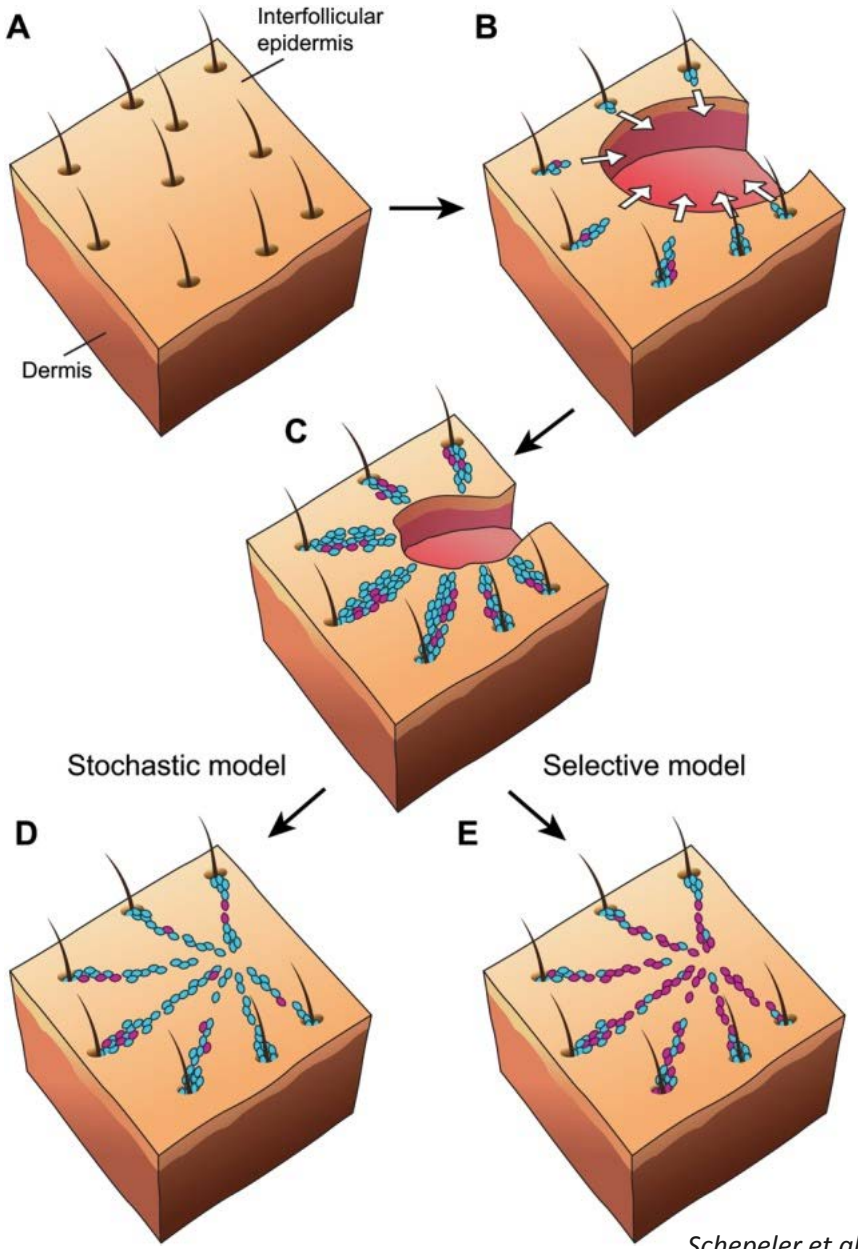
Pasparakis M et al Nat Rev Immunol. 2014 PMID: 24722477

resting (telogen) HF



Schepeler et al Development. PMID: 24961797

- different stem cell reservoirs in adult mouse HF
- timing of SC activation & subsequent long-term contribution to re-epithelialized wound area varies
- majority of Krt15 progeny from bulge SC **rapidly lost** following wound healing
- progeny from Sox9, Gli1, Lrig1 SCs in bulge/isthmus, JZ contribute to **long-term regeneration**



so far two hypothesis of stem cell (SC) contribution to wound healing:

Stochastic model

- fraction of retained SC progeny = proportional to initial contribution

Selective model

- specific SC progeny are retained within regenerated area (at expense of others)

Schepeler et al Development. PMID: 24961797

key method: single cell RNA sequencing

Bulk seq

scRNA seq

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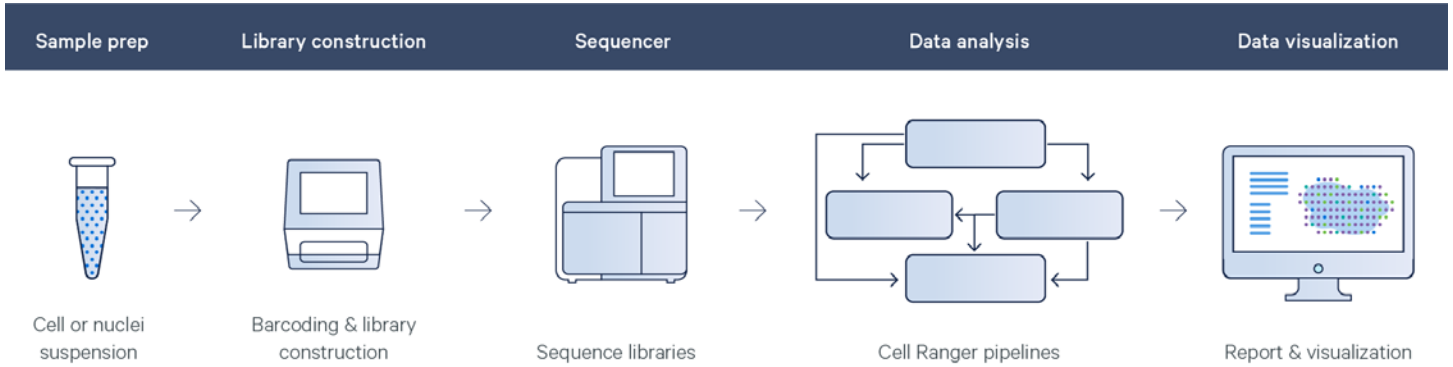
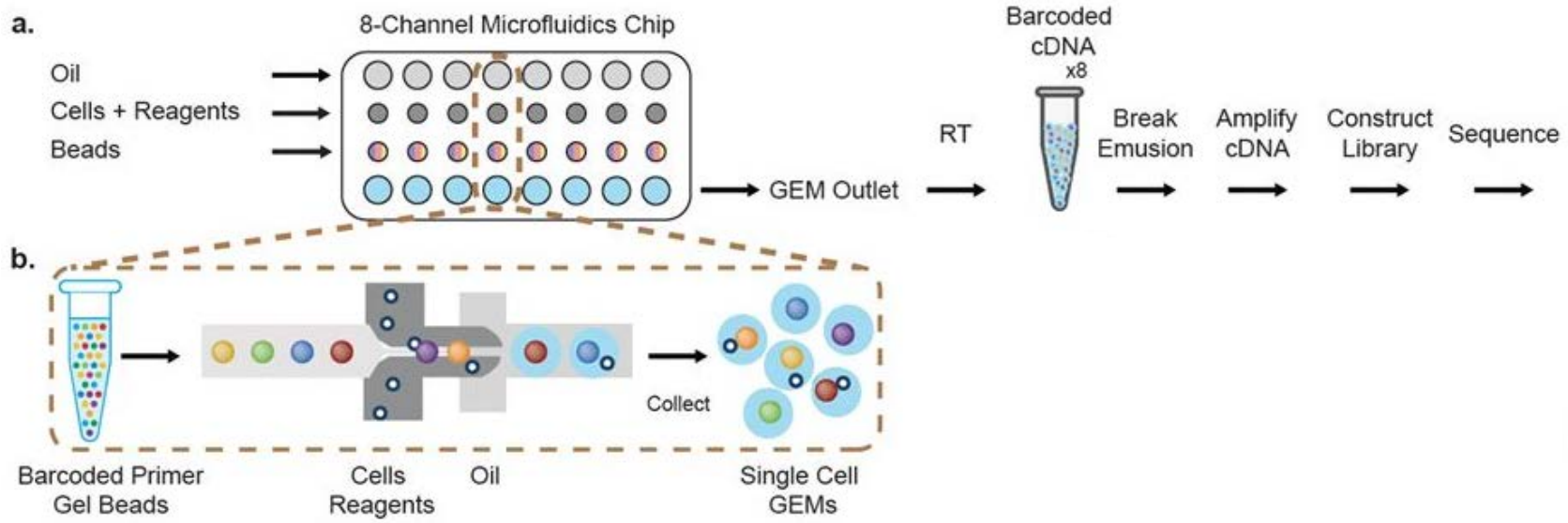
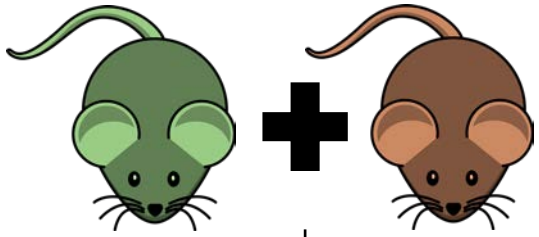


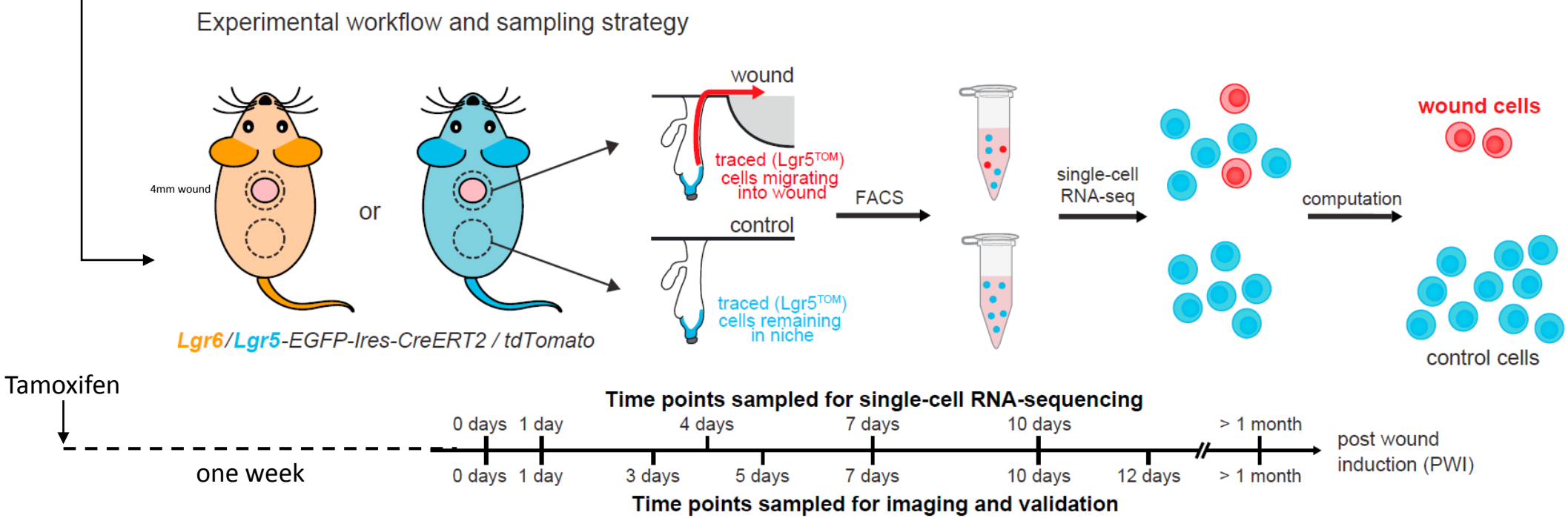


Lgr5-EGFP-Ires-Cre-ERT2 /
Lgr6-EGFP-Ires-CreERT2

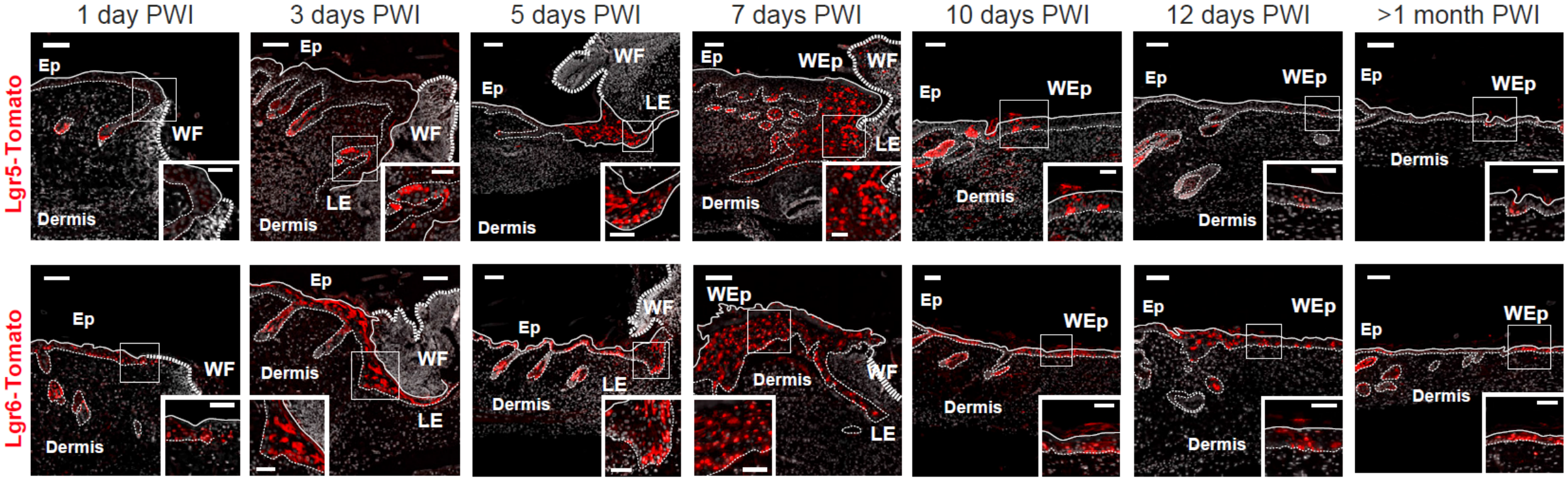
R26-tdTomato



- breeding of tomato-mice to cre-recombinase harbouring mice → STOP cassette deleted in offspring in the *cre*-expressing tissue/cells → tdTomato (red) fluorescence
- Tamoxifen treatment (7wks) induces cre-expression
- Post wound; tomato traced cells were sorted and sequenced

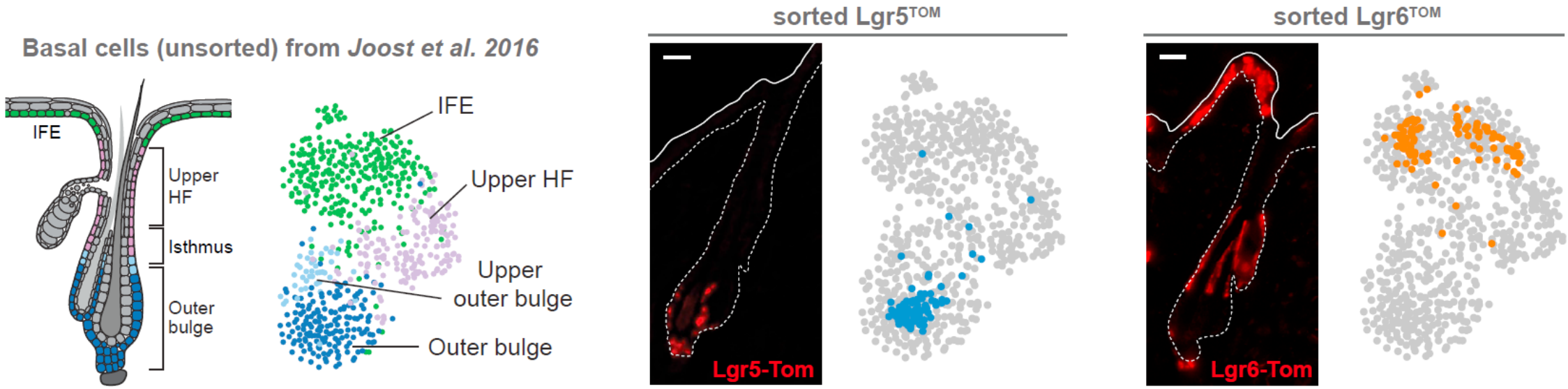


Spatial distribution of traced cells during wound healing



- sign. difference in temporospatial distribution
  - Lgr6 at leading edge (wound front) from beginning on
  - Lgr5 progeny reached WF after 3-5 days
  - Lgr5 progeny less abundant in closed wound/remodeled wound

Comparing transcriptome profiles of sorted Lgr5/6 from unwounded skin

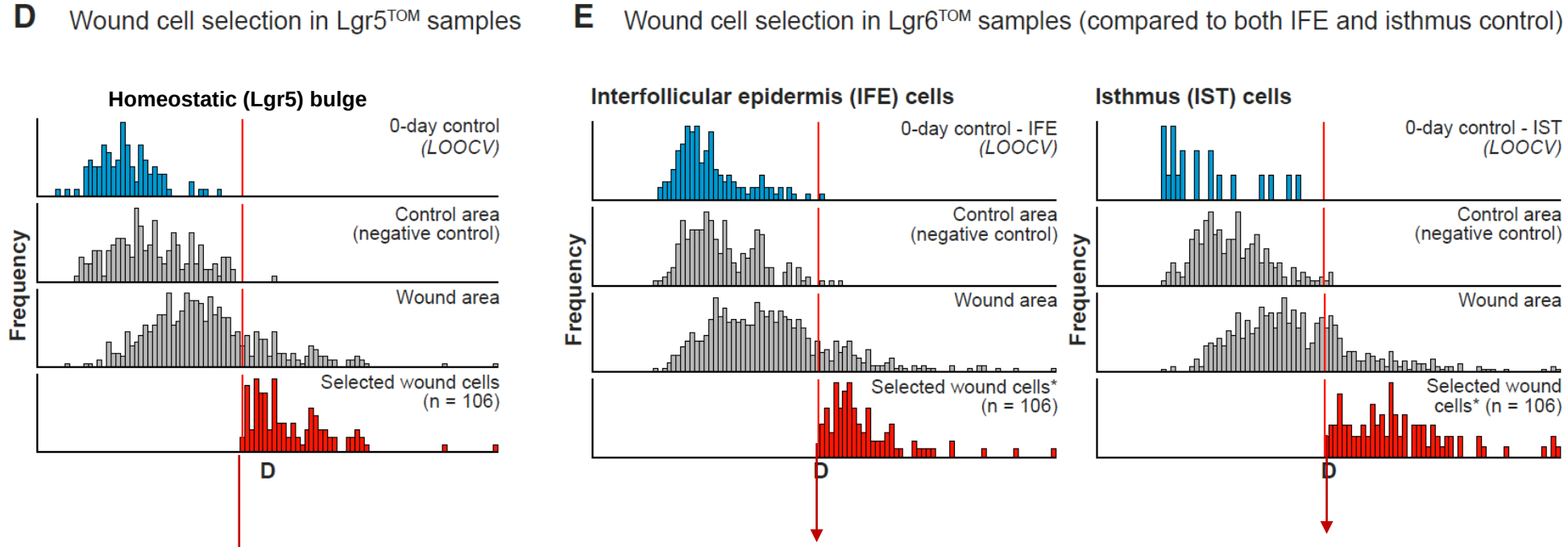


- Lgr6 showed almost exclusively interfollicular epidermis (IFE)-like signature
- Only few Lgr6 showed isthmus-like signature

Identification of activated vs. non-activated Lgr5/6 cells

Transcriptome of unwounded day 0 cells

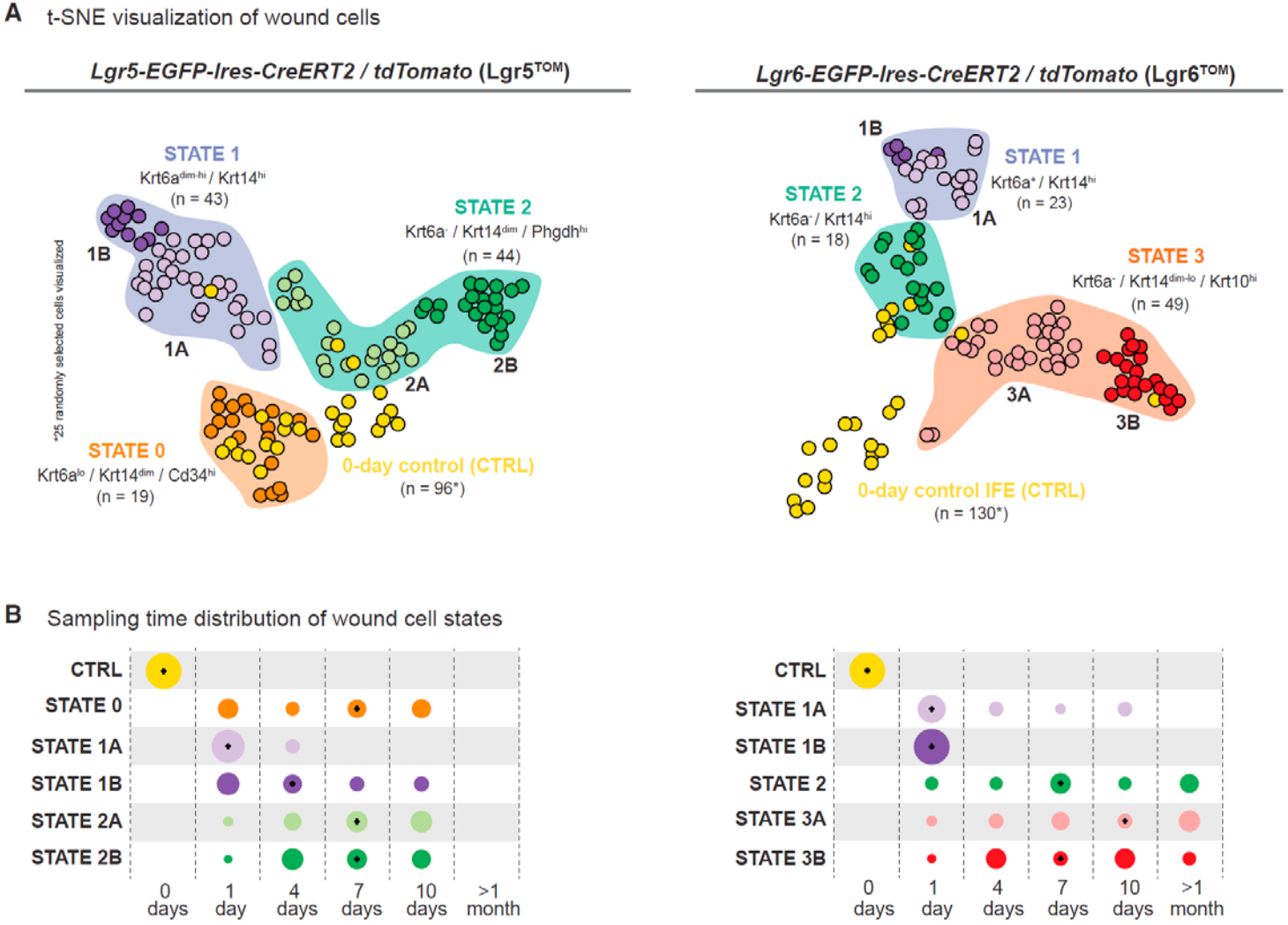
Definition of expected gene expression variability



Wound area cells with transcriptome profile ≠ homeostatic Lgr5/Lgr6 niche → classified as (activated) wound cells

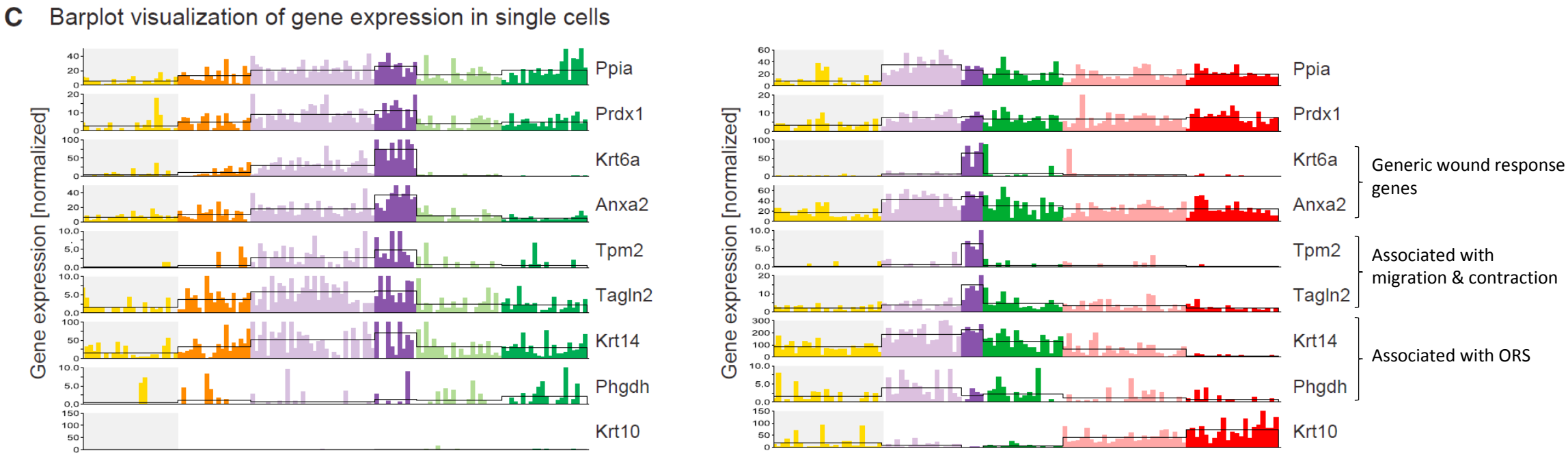
Profiling transcriptional heterogeneity of Lgr5/Lgr6 SC progeny

- Lgr5 & Lgr6 cells did not only cluster by time point (PWI)
  - each cluster contained different wound healing time points



A and B not necessarily imply temporal order but expression of early wound signature

Profiling transcriptional heterogeneity of Lgr5/Lgr6 SC progeny



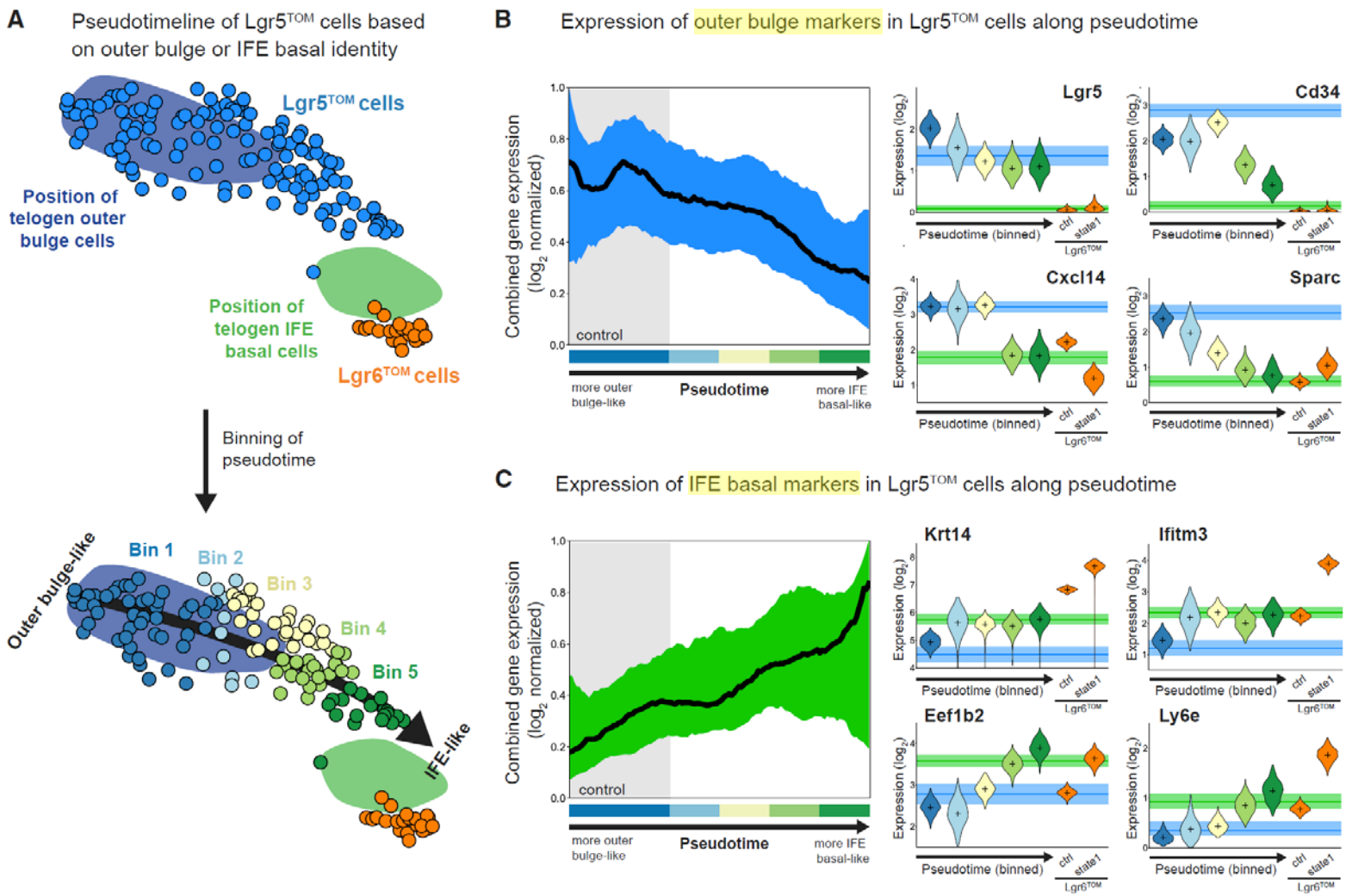
- Several marker genes converged in Lgr5 and Lgr6 cells
- Lgr5 cells showed higher expression of outer bulge markers

Identification of gene signature switch upon wound response

- Lgr5 but not Lgr6 cell span entire expression axis from bulge to IFE
  → Indicating gradual change in cellular identity

- Genes enriched in outer bulge = downregulated over wound healing course

- Most basal IFE markers = induced in Lgr5 during wound healing + remained high
  → Certain genes already upregulated to acute injury; others due to location change from niche

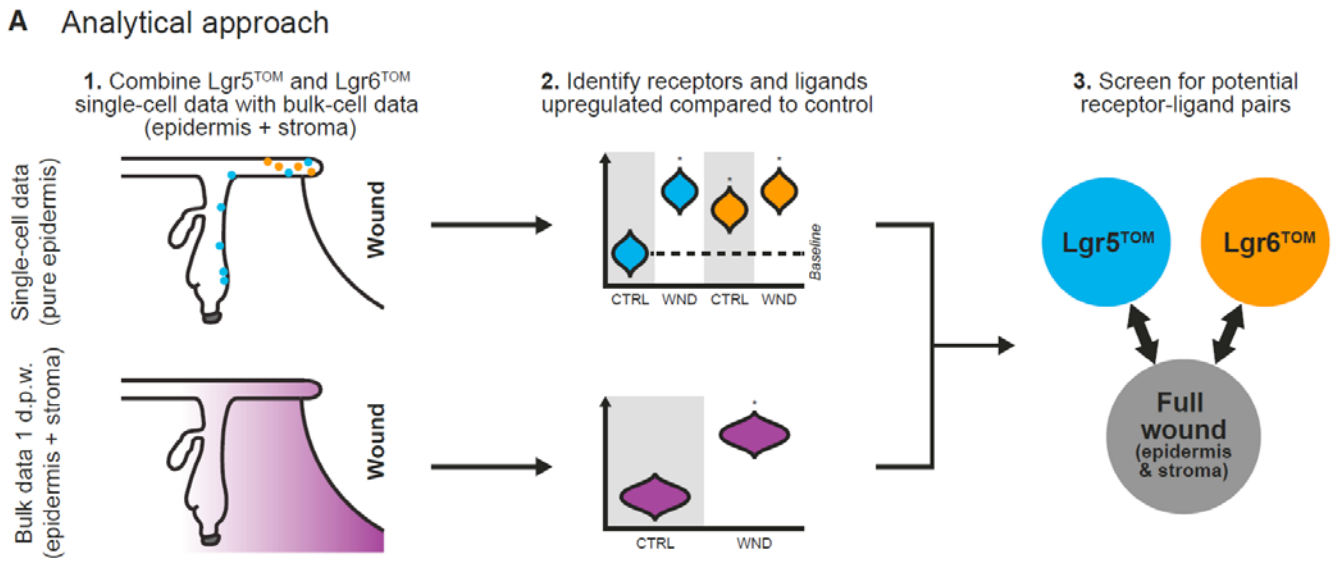


Molecular interactions of Lgr5/Lgr6 SC progeny in homeostasis & early wound response

Crosstalk between epidermis & wound environment = central in orchestrating cell recruitment into wound → re-epithelialization

Combination of **sequencing data** of early wound cell populations **+ bulk seq** containing stromal and epithelial wound-parts

→ Identification of potential **receptor-ligand interactions** between activated wound cells & environment



Molecular interactions of Lgr5/Lgr6 SC progeny in homeostasis & early wound response

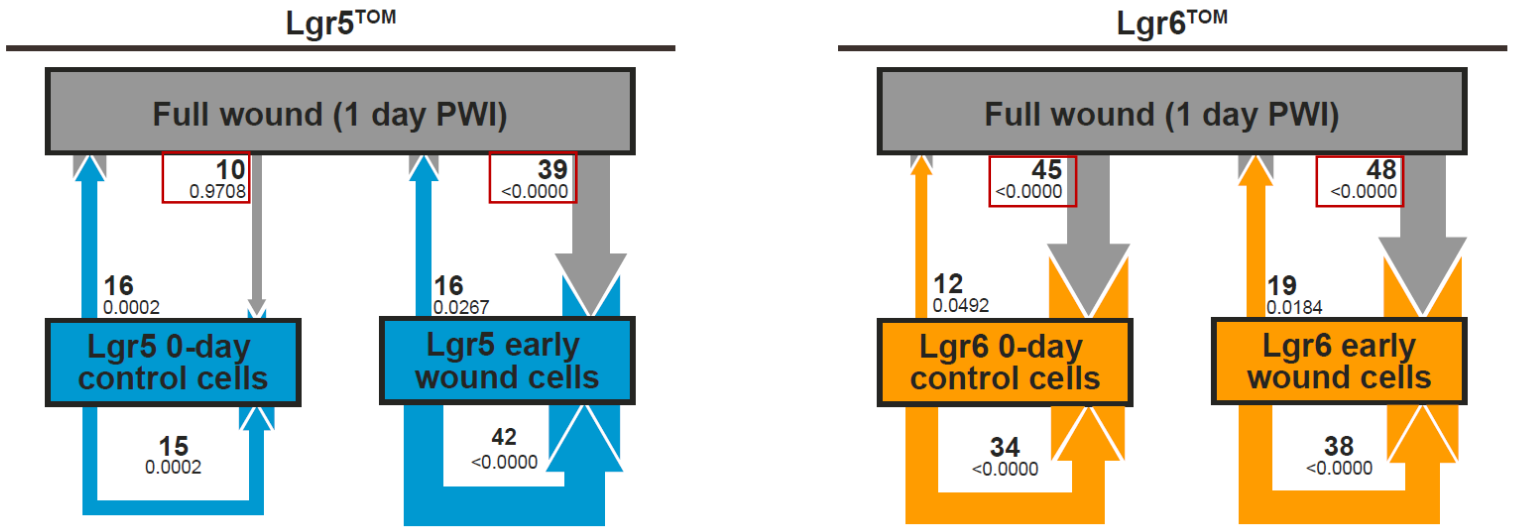
- Primarily receptors expressed in epithelial cells
  ligands originating from wound environment

- Striking difference in # receptors expressed in Lgr5 & Lgr6 cells

# receptors	Lgr5	Lgr6
Before wounding	10	45
upon injury	39	48

→ Lgr5 cells capable of gaining competence to react to signals from wound environment

B Potential interactions between Lgr5^{TOM} and Lgr6^{TOM} control and early wound cells (state 1) and the wound environment (1 day PWI)



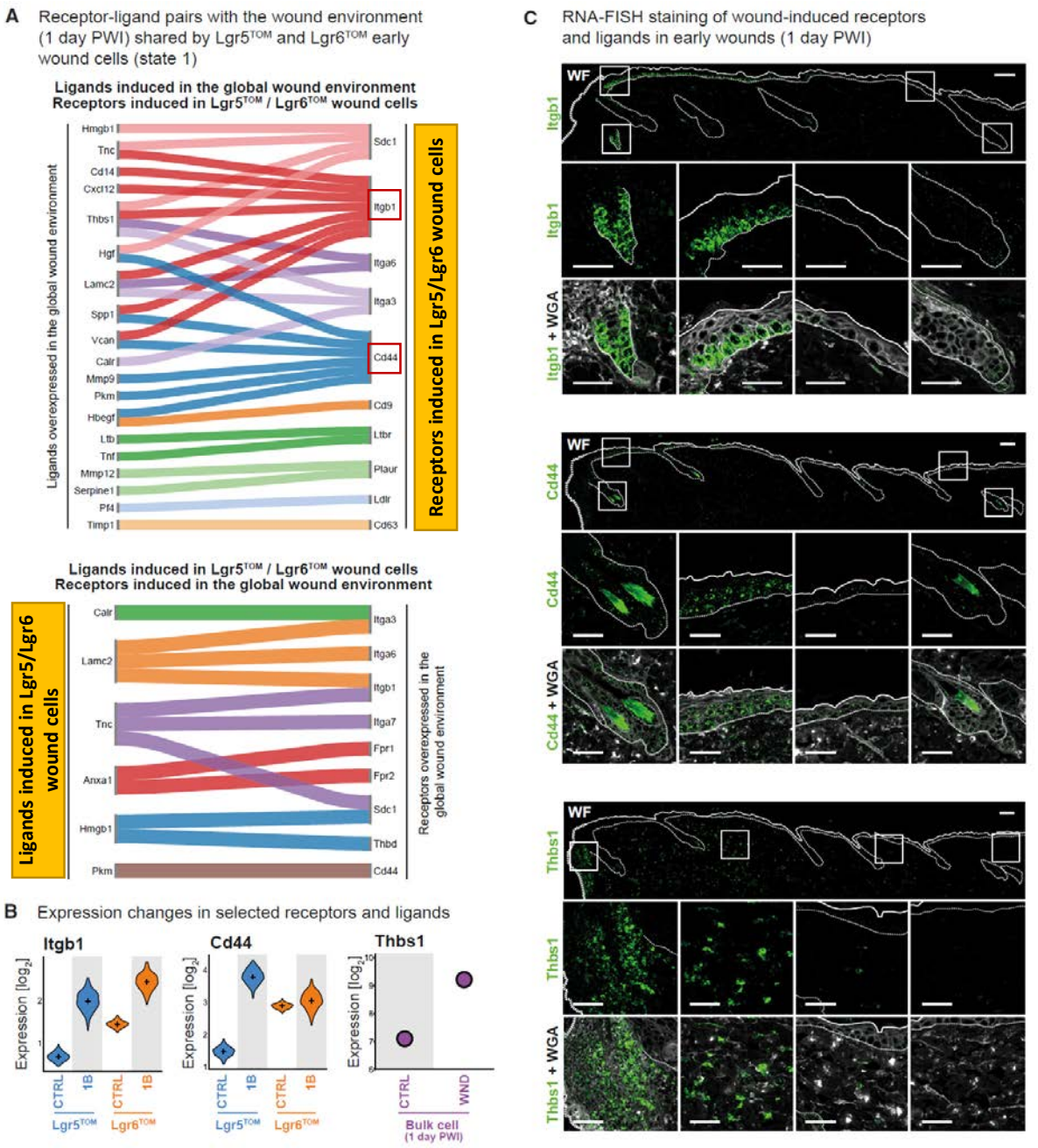
Key ligand-receptor interactions

- Most commonly found epithelial receptors for wound signals:
  - Cd44
  - Alpha & beta integrins

- Thbs1 = Itgb1 interaction partner
  - Highest expression in newly forming wound matrix (1 day PWI)

→ Lgr6 cells in IFE = already primed for interactions with wound environment

→ Lgr5 cells actively shape their responsiveness upon wounding
adaptation starts within 24hrs upon wounding while still residing in natural niche



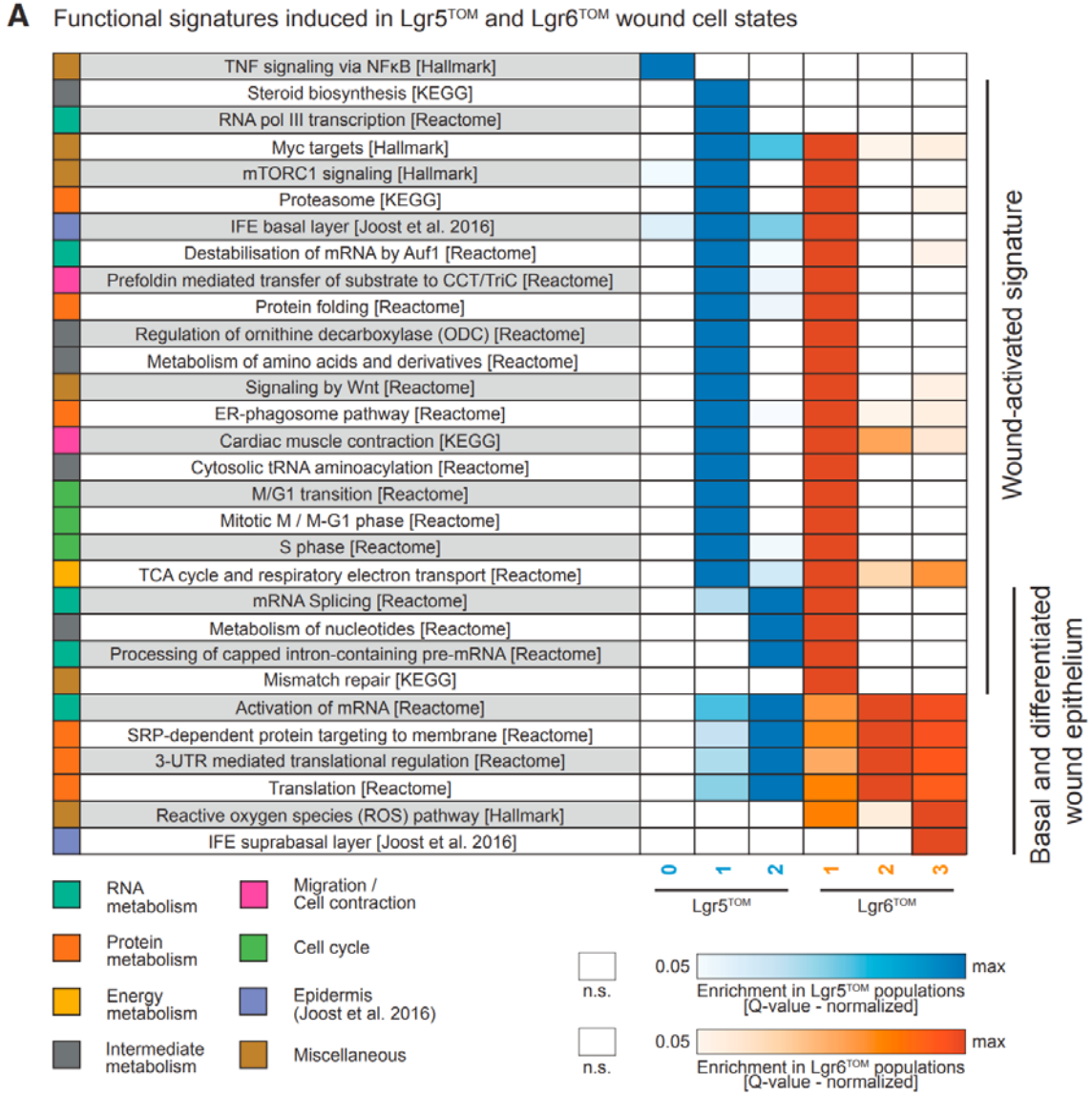
Profiling common wound-healing transcriptome signature

- Gene enrichment analysis revealed enrichment in both Lgr5 & Lgr6 progeny

- Early wound-cell state associated with basic cell-physiological processes
  - Energy production
  - Cell cycle
  - Migration
  - mRNA synthesis

- Later wound-cell states associated with
  - Ribosome formation
  - Translation
  - Protein processing

- **Only** NF*k*B significantly enriched in Lgr5 state 0
  => probably cells that remained in bulge but sensed injury

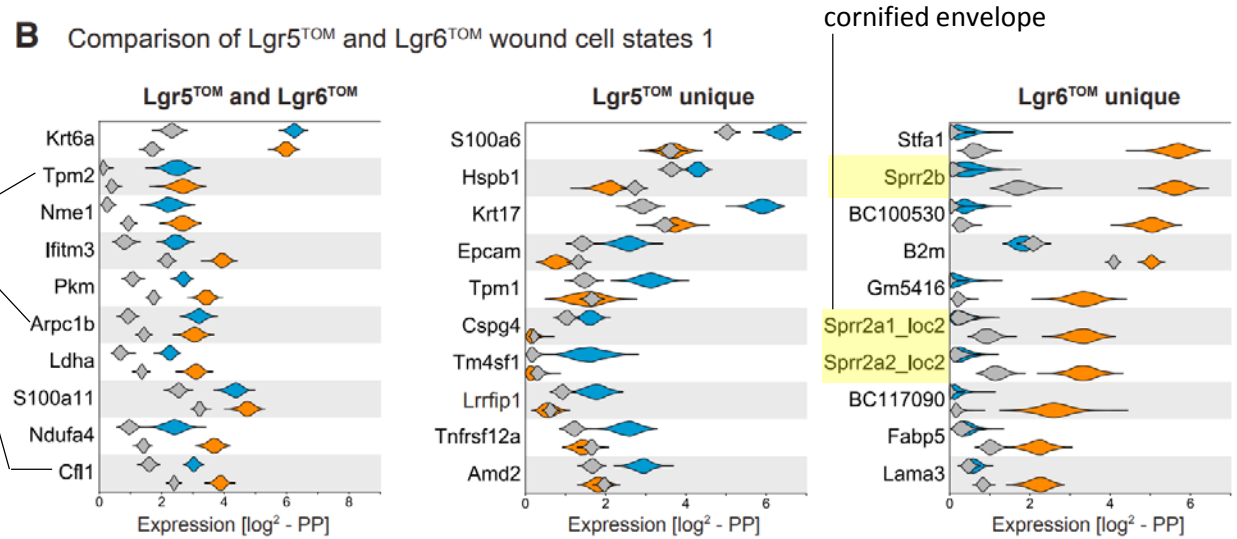


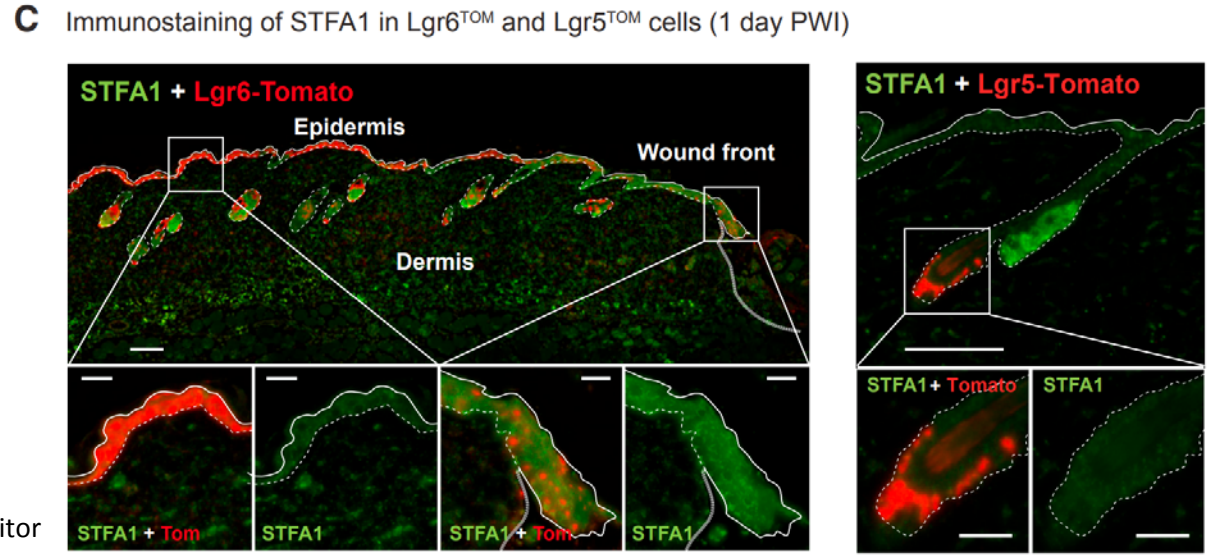
Convergent & niche-specific gene expression

- High number of “shared genes” in Lgr5 & Lgr6

migration

cornified envelope





Stfa1 = cysteine proteinase inhibitor

conclusion

- Lgr5 & Lgr6 exhibit at each time point high degree of cellular heterogeneity
- Rapid receptor remodeling in Lgr5 cells
  → Early upregulation of receptors could serve as “initial priming” rendering Lgr5 cells susceptible for migration
- Lgr6 cells = already primed for potential interaction with wound environment
- Transient lineage adaptation during wound repair

Thank you! ☺