Dissecting Wnt Signaling for Melanocyte Regulation during Wound Healing

Qi Sun, Piul Rabbani, Makoto Takeo, Soung-Hoon Lee, Chae Ho Lim, EN-Nekema Shandi Noel, M. Mark Taketo, Peggy Myung, Sarah Millar and Mayumi Ito

Journal of Investigative Dermatology (2018)

Felix Bergmann

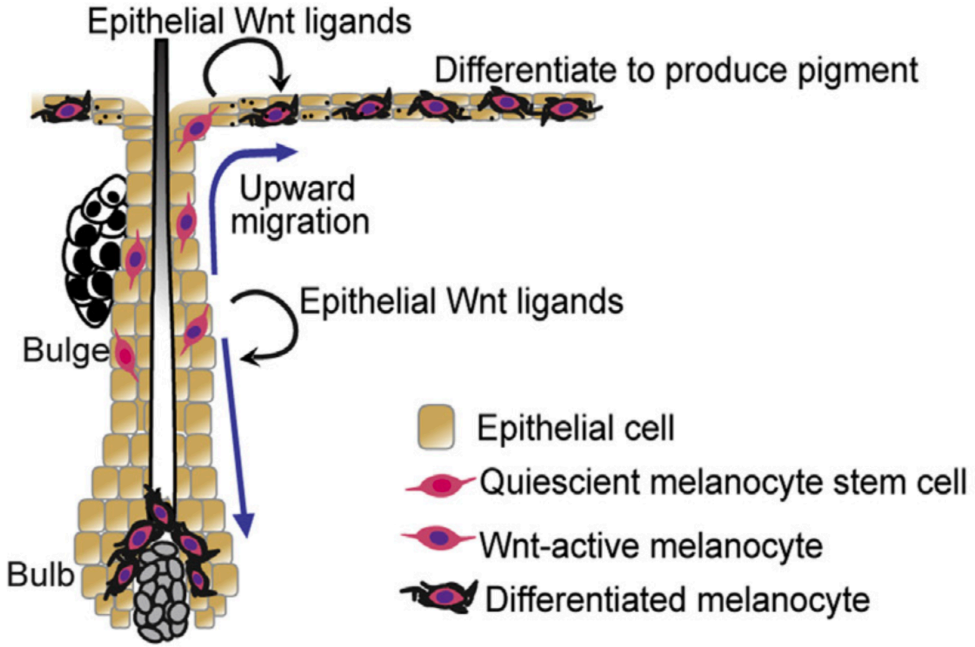
Introduction

- New skin tissue replenished in wound areas typically leave imperfect skin pigmentation after re-epithelialization
- Incomplete understanding of how melanocytes are recruited to the wound site and how they modulate pigment production during wound healing
- Specific inhibition / activation of Wnt signaling in melanocytes

Introduction - Melanocytes

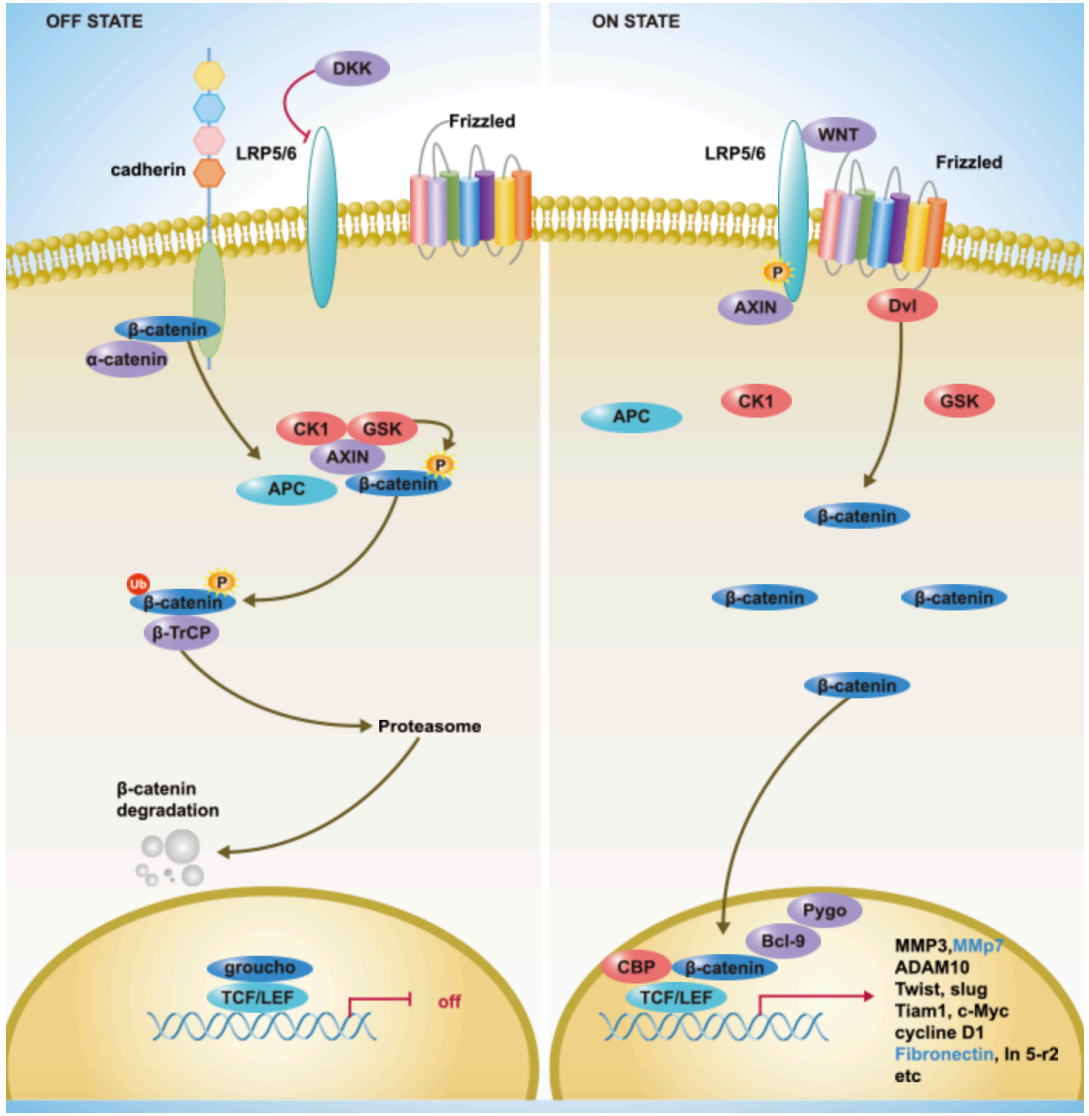
- In Humans: functional melanocytes are continuously repopulated by the differentiation of **melanocyte stem cells (McSCs)** residing in the epidermis and hair follicles- responsible for pigmentation

- In mice: located in the bulge/secondary hair germ and bulb area of the hair follicle.
    - In response to wounding, follicular McSCs exit stem cell niche, migrate towards basal layer of the epidermis - (Mc1r-dependent-MSH)
      —> Differentiate into epidermal melanocytes



Introduction - Melanocytes

- Wnt/beta-Catenin signaling is a central pathway in melanocytes.
- Regulates transcription of:
  - MITF Melanocyte inducing transcription factor
  - DCT Dopachrome Tautomerase / TYRP2 (Tyrosinase related protein)
  - TYR Tyrosinase



Dkk1 expression inhibits the generation of epidermal melanocytes after wounding

Overexpression of Dkk1

- Overexpression of Wnt-inhibitor Dkk1 during wound healing (removes LRP from cell surface)

Dct:Dkk1:GFP Mice
Dct-rtTa; tetO-Dkk1; tetO-H2B-GFP

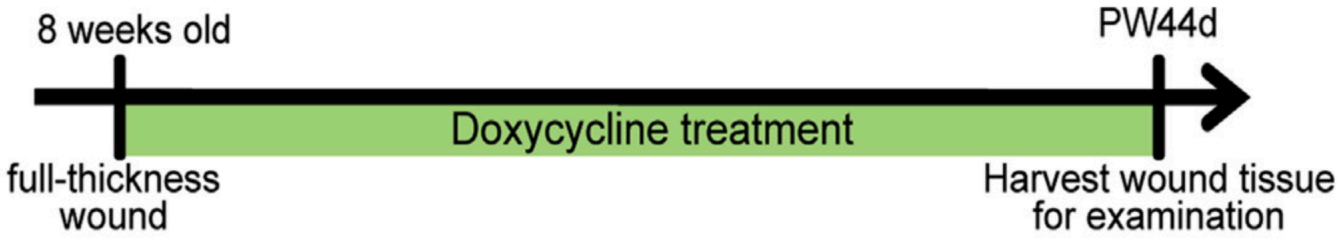
Dkk1 & GFP reporters can inducibly be expressed in Dct+ melanocytes upon doxycycline treatment

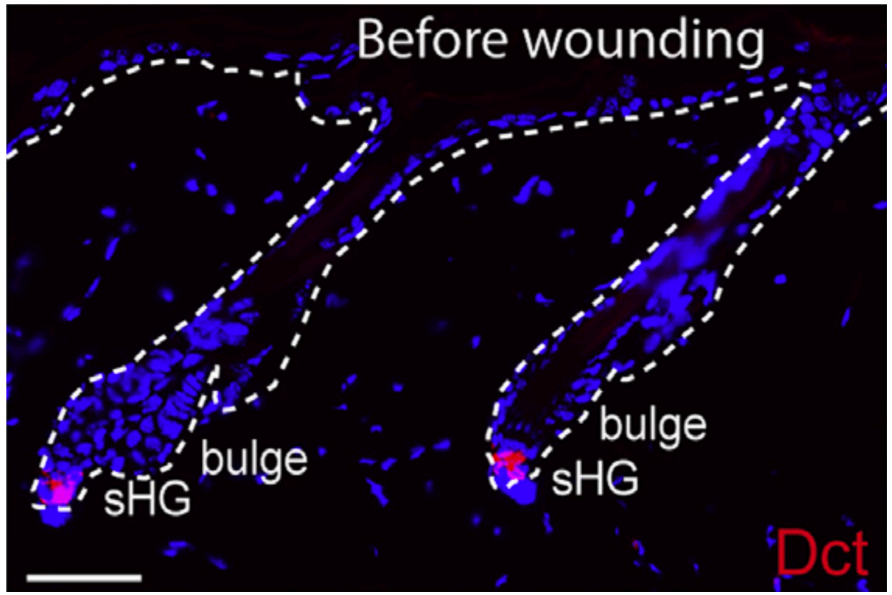
Dct:GFP Mice
Dct-rtTa;tetO-H2B-GFP

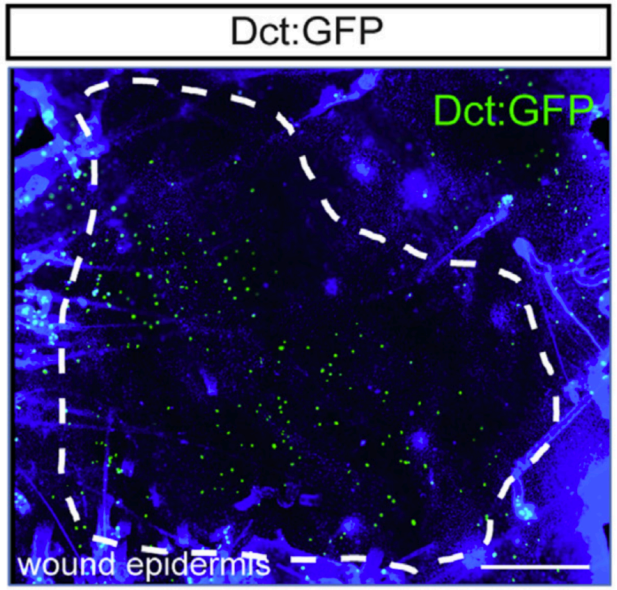
Control Mice only express GFP reporter in DCT+ melanocytes upon Doxycyline treatment

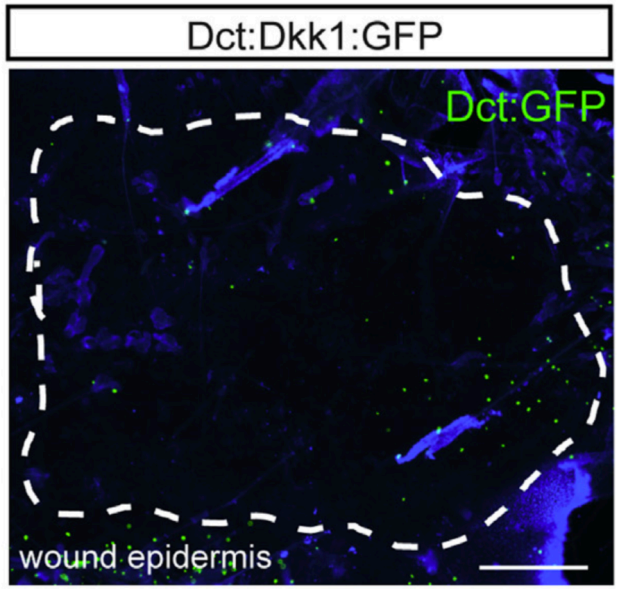
- Dct is expressed by undifferentiated McSCs and differentiated melanocytes

Overexpression of Dkk1

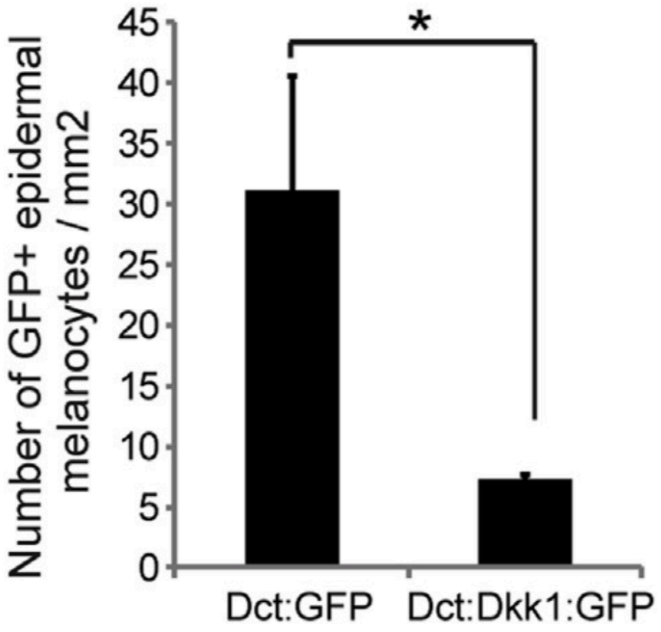








Overexpression of Dkk1

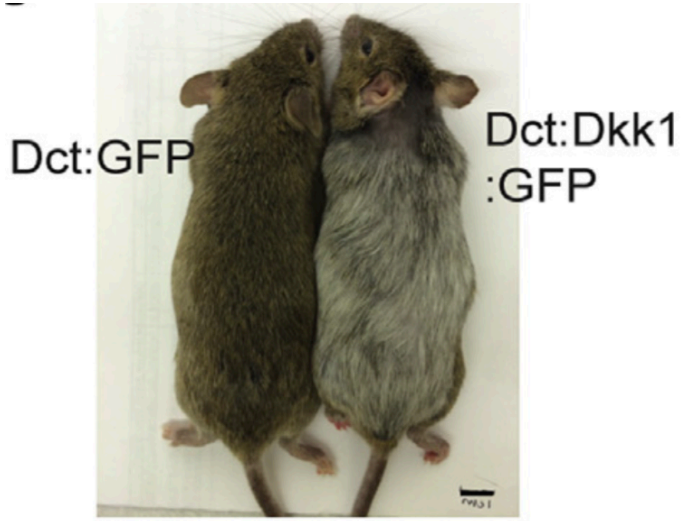


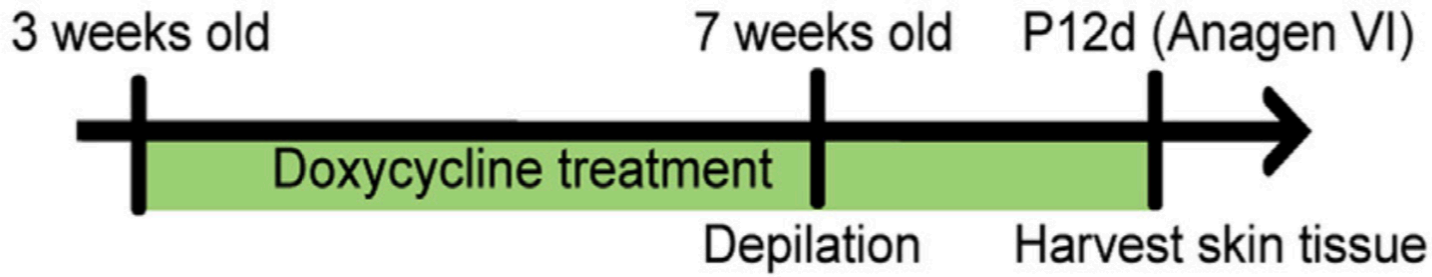
*P < 0.05

"Wnt signaling is required for follicular McSCs to generate epidermal melanocytes during skin wound healing"

Overexpression of Dkk1

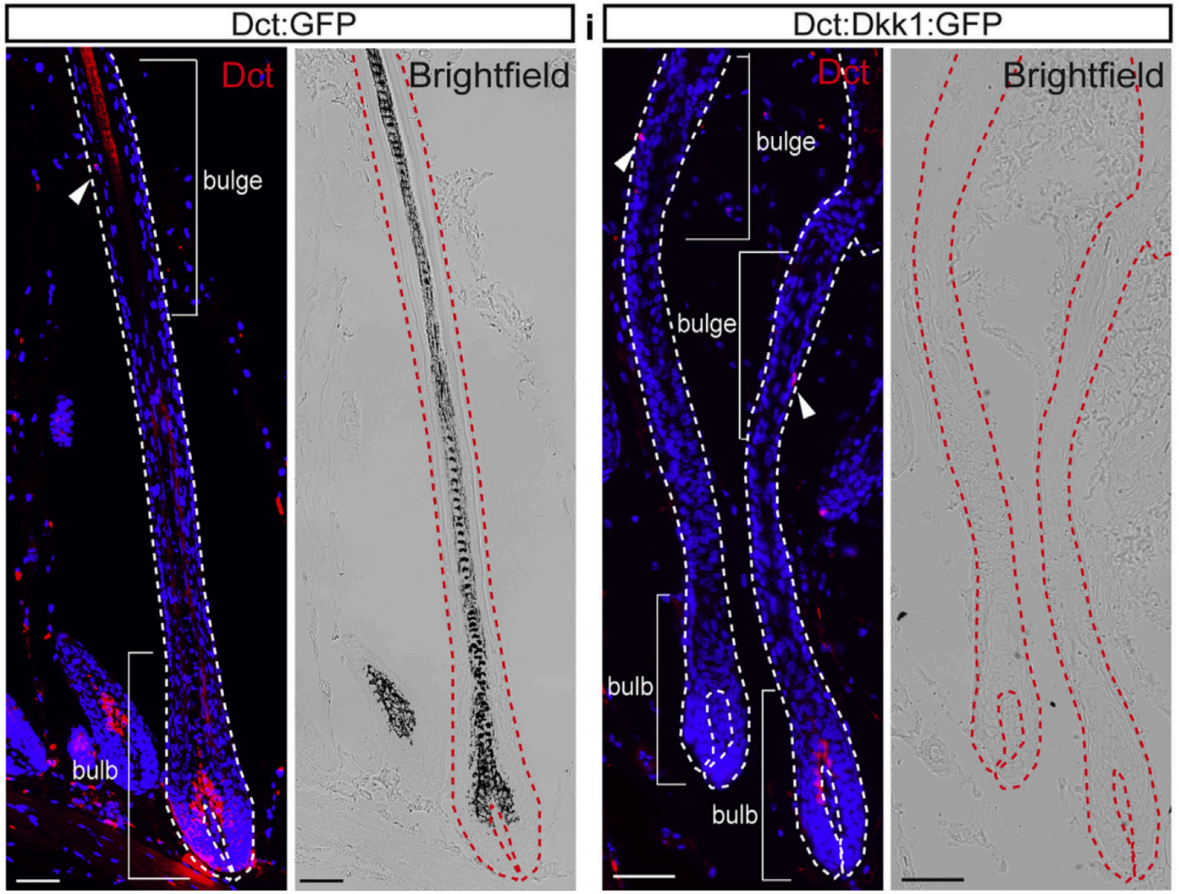
- Intact area of Dkk1-expressing mice show apparent defect in hair pigmentation
- In normal skin without wound healing: McSCs regenerate differentiated melanocytes in hair bulb responsible for hair pigmentation
    - —> Examination of hair bulb





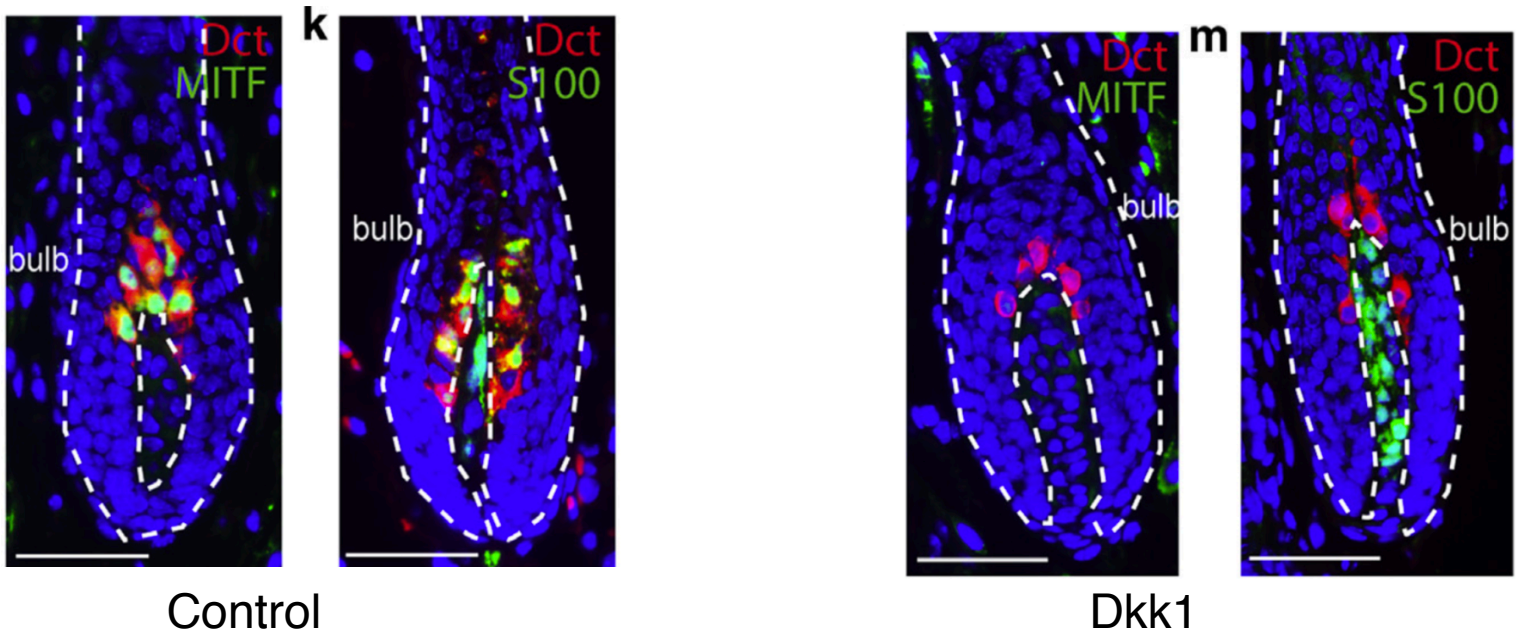
Overexpression of Dkk1

- Significantly reduced number of hair bulb melanocytes in Dkk1 mice

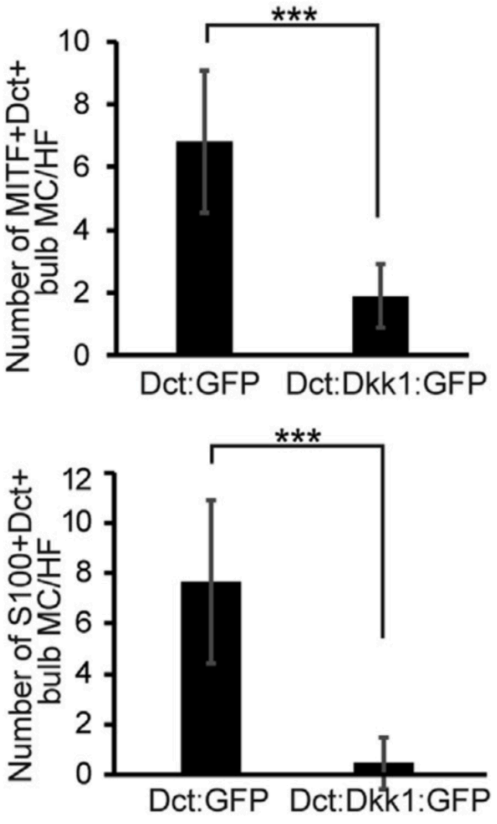


Overexpression of Dkk1

- Hypopigmentation in bulb melanocytes in Dkk1 mice - failed to show immunoreactivity for melanocyte differentiation markers MITF / s100



Control Dkk1



- ***Verifies that the role of β-Catenin in melanocyte differentitation is mainly mediated by Wnt-signaling***

Constitutive activation of Wnt signaling promotes the generation of epidermal melanocytes after wound healing

Overexpression of β-catenin

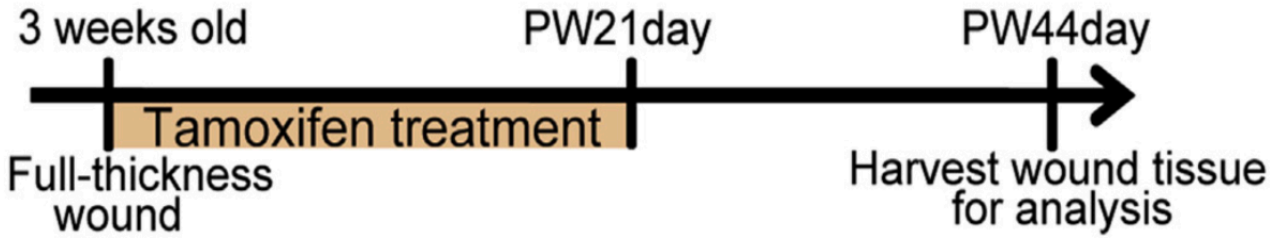
- Overexpression of β-catenin during wound healing

β-catenin-STA Mice
Tyr-CreER;b-Catenin fl(ex3)/+; Dct-LacZ

Specifically express a stabilized mutant form of β-catenin in melanocytes upon tamoxifen (TAM) treatment

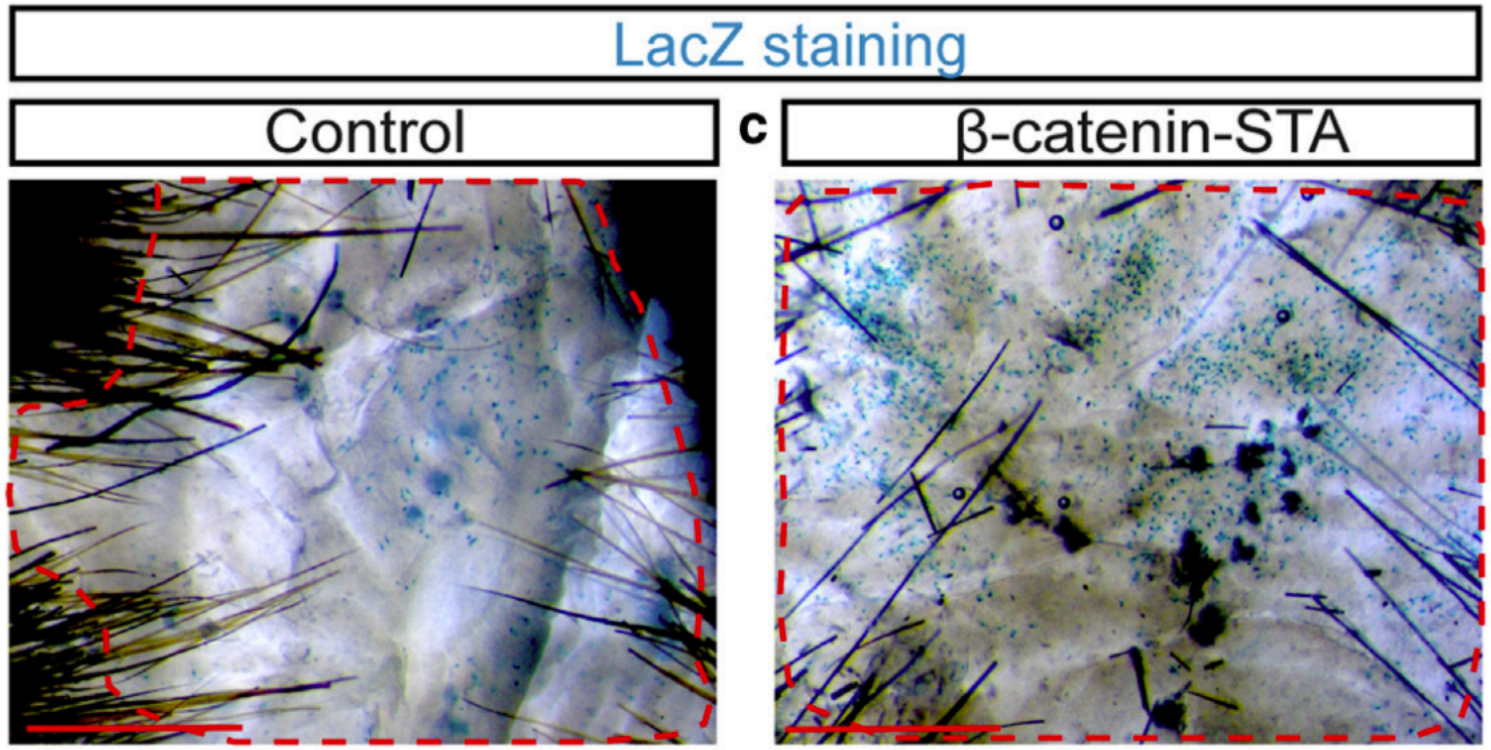
Dct:LacZ Mice

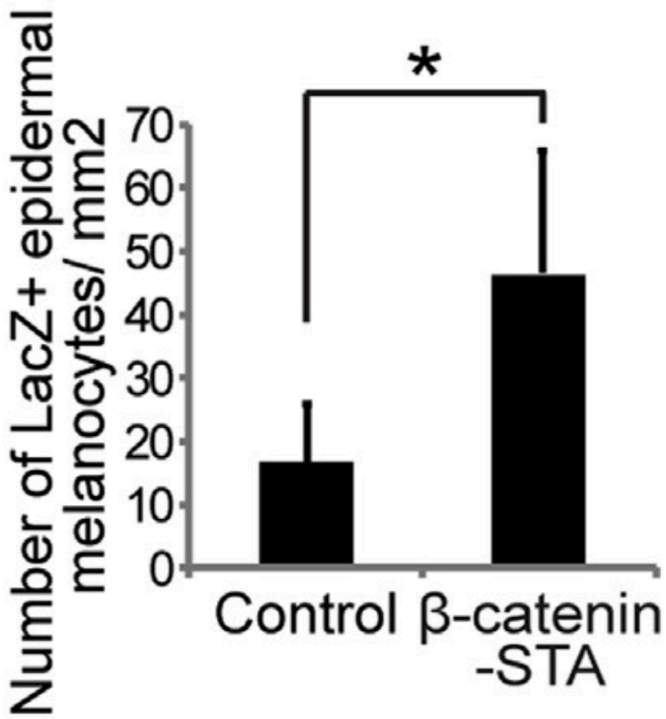
Control



Overexpression of β-catenin

- Forced Wnt activation in melanocytes results in epidermal melanocytes distributed over entire wound scar area

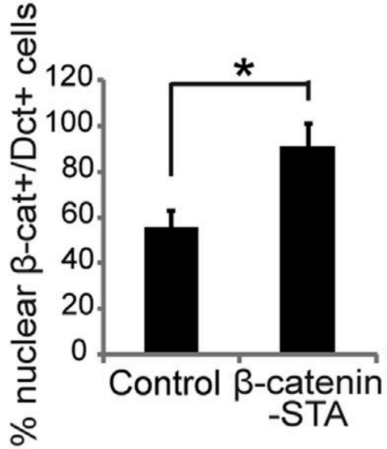


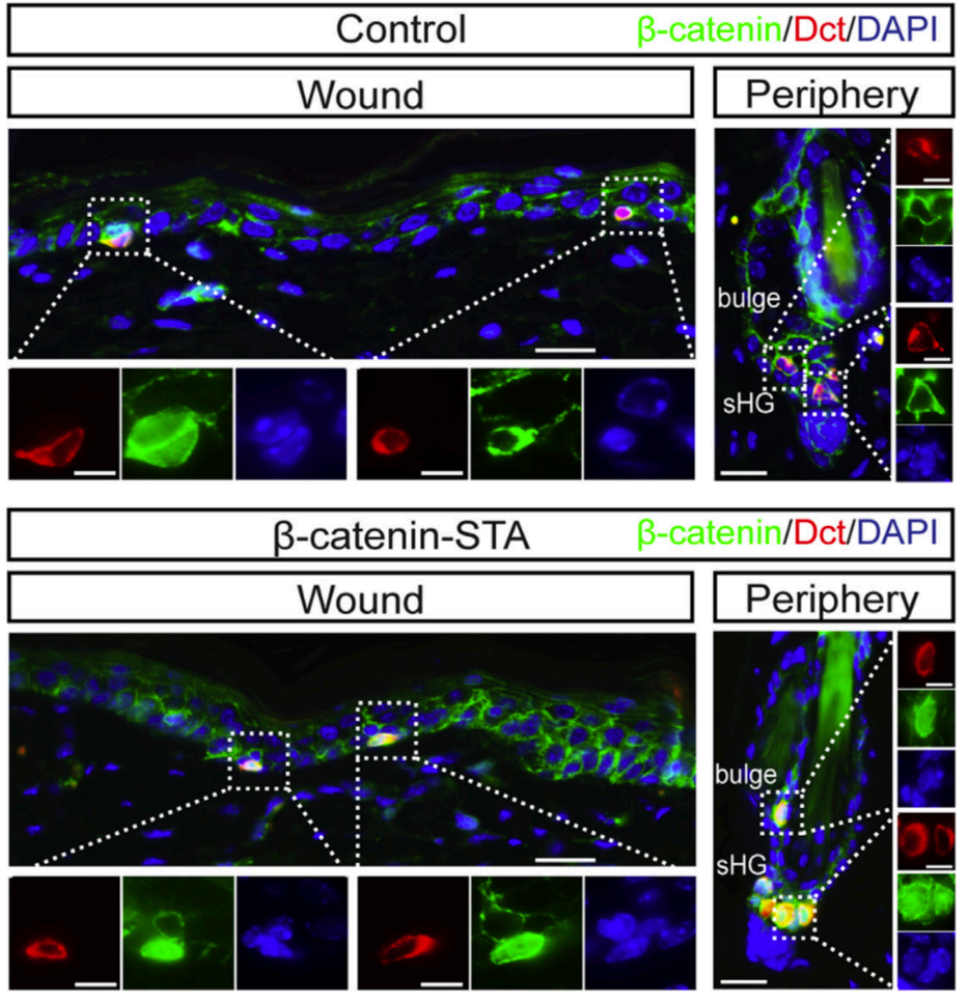


Overexpression of β-catenin

- Significant increase of the % of nuclear β-catenin+ *epidermal melanocytes* in β-catenin-STA Mice
- Increased expression of nuclear β-catenin in McSCs in the *hair follicles* of β-catenin-STA mice

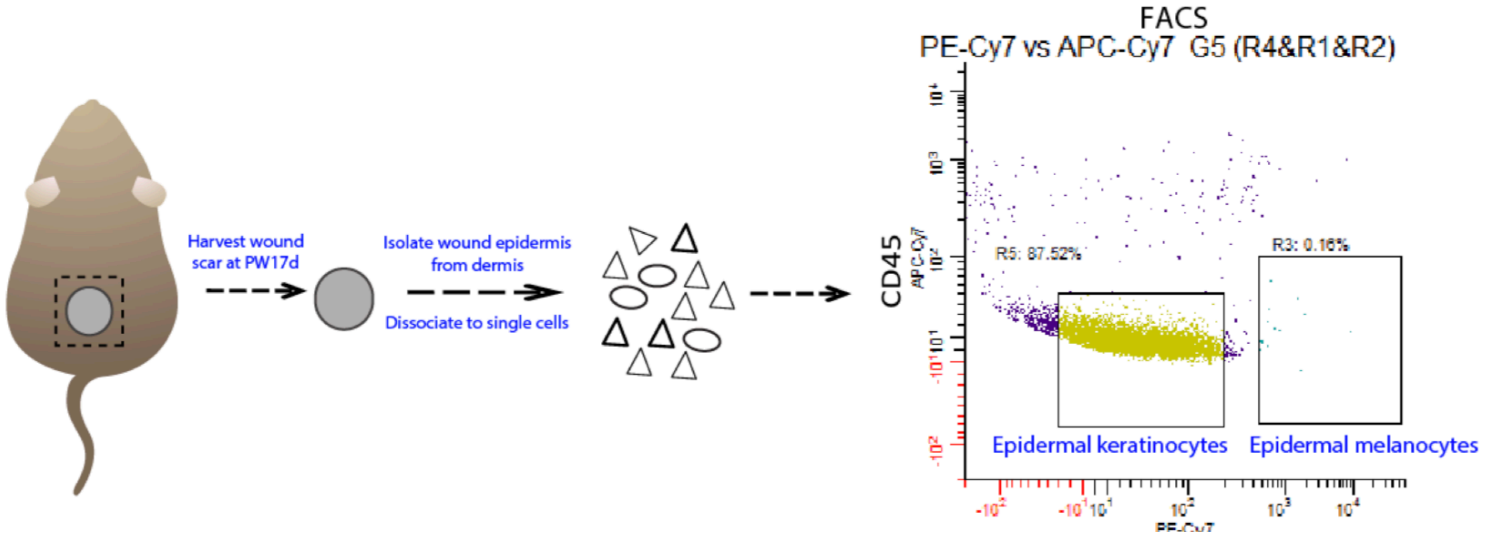
- ***Forced activation of Wnt signaling via β-catenin stabilization enhances the number and distribution of melanocytes within the wound epidermis.***

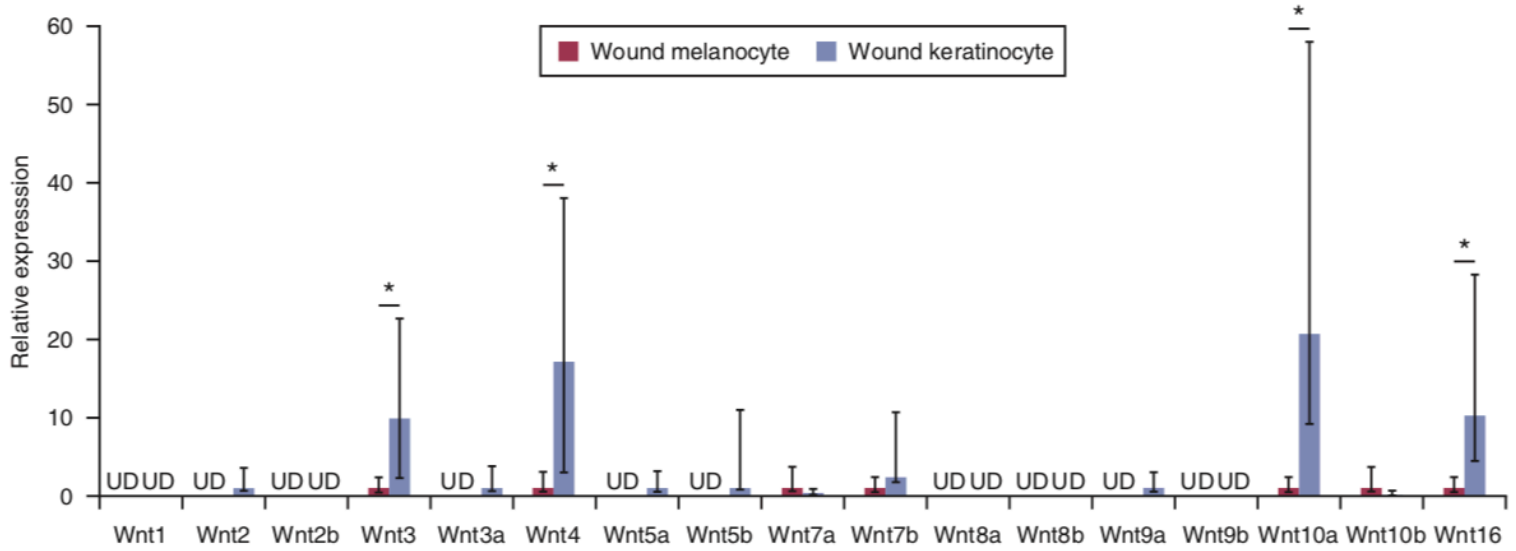




Wnt ligands secreted by epithelial cells are essential for the activation of Wnt/β-catenin signaling in epidermal melanocytes

Origin of Wnt ligands





Epithelial cells may be the major source of Wnt ligands in the wound area

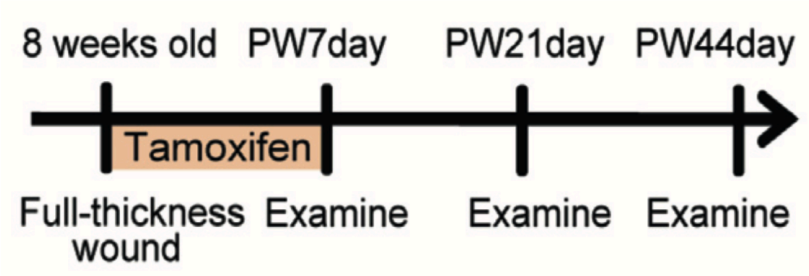
Inhibition of Wnt ligand secretion in epithelial cells

K14-Wls cKO mice
K14-CreER; Wls fl/fl; Dct-LacZ

Wls is essential for Wnt ligand secretion and is ablated in K14 basal epithelial cells after TAM treatment

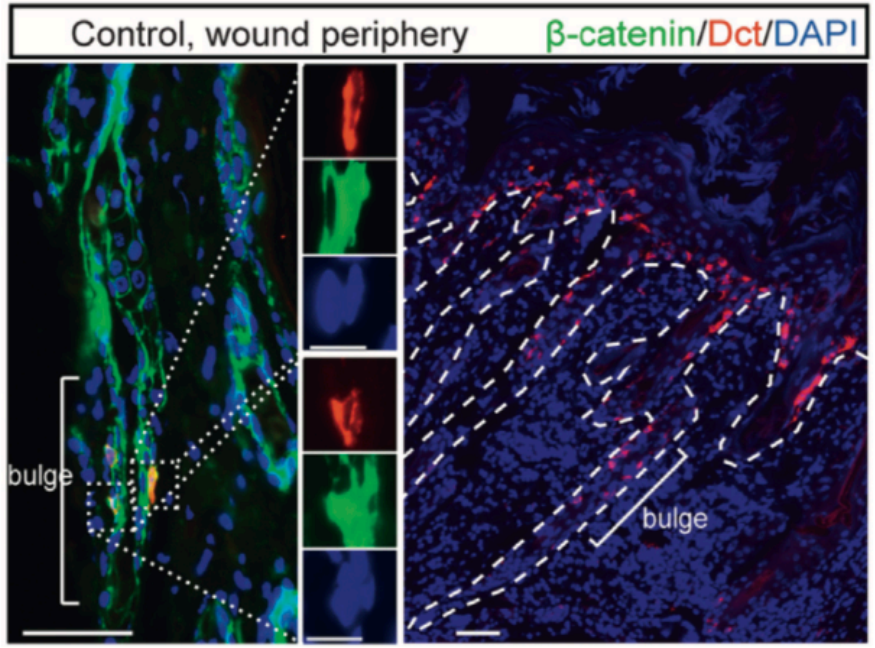
Dct:LacZ Mice

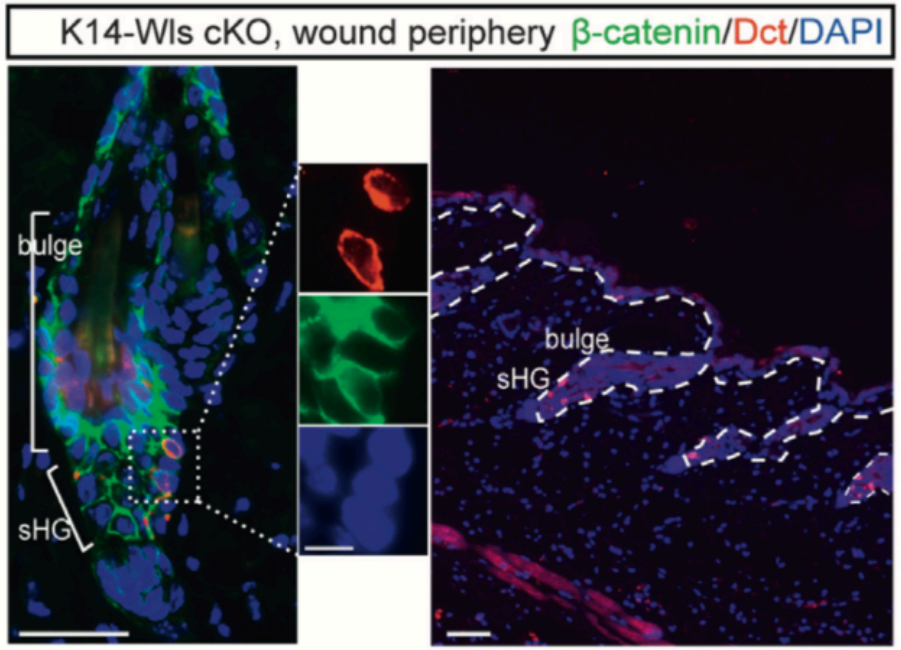
Control



Inhibition of Wnt ligand secretion in epithelial cells

PW7d





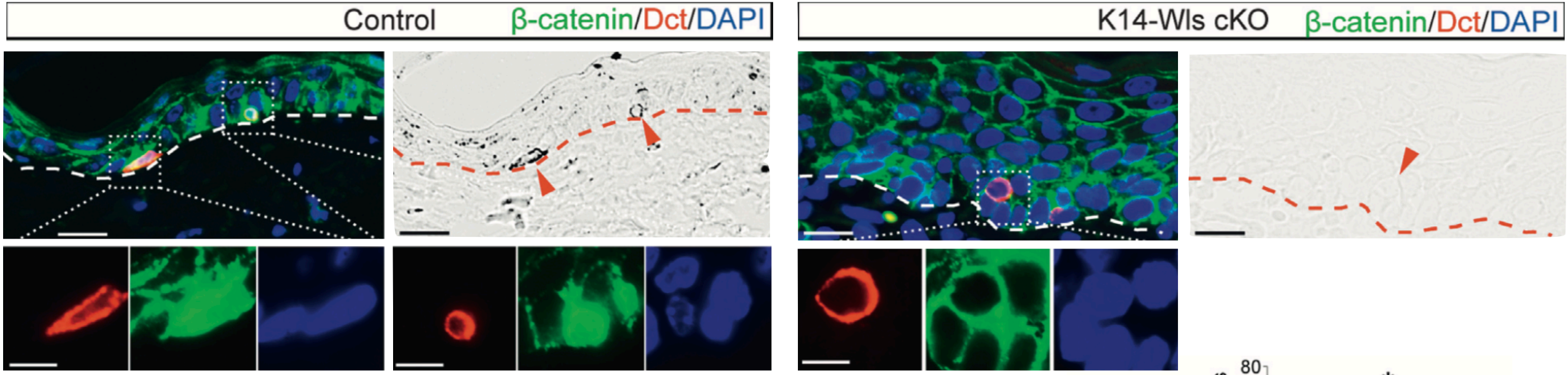
Expansion of Dct+ McSCs in hair follicle of wound periphery area + migration upward into epidermis

Some Dct+ Melanocytes are Wnt active —> nuclear β-catenin signals

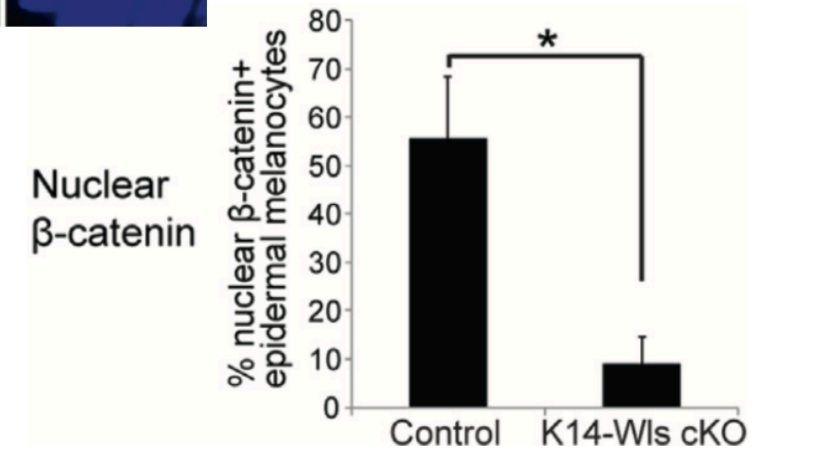
Loss of Wnt ligands inhibit Wnt activation in Dct+ McSCs and their expansion into the epidermis

Inhibition of Wnt ligand secretion in epithelial cells

PW21d

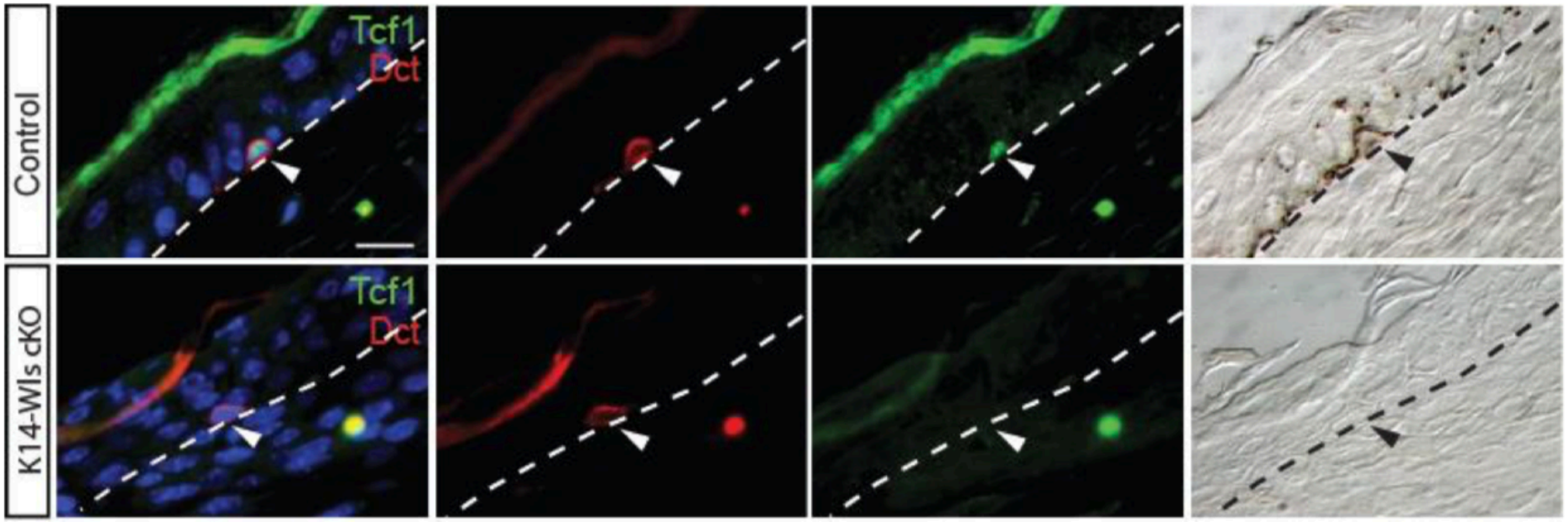


Defect in Wnt signal activation in epidermal melanocytes, shown by lack of nuclear β-catenin signals.



Inhibition of Wnt ligand secretion in epithelial cells

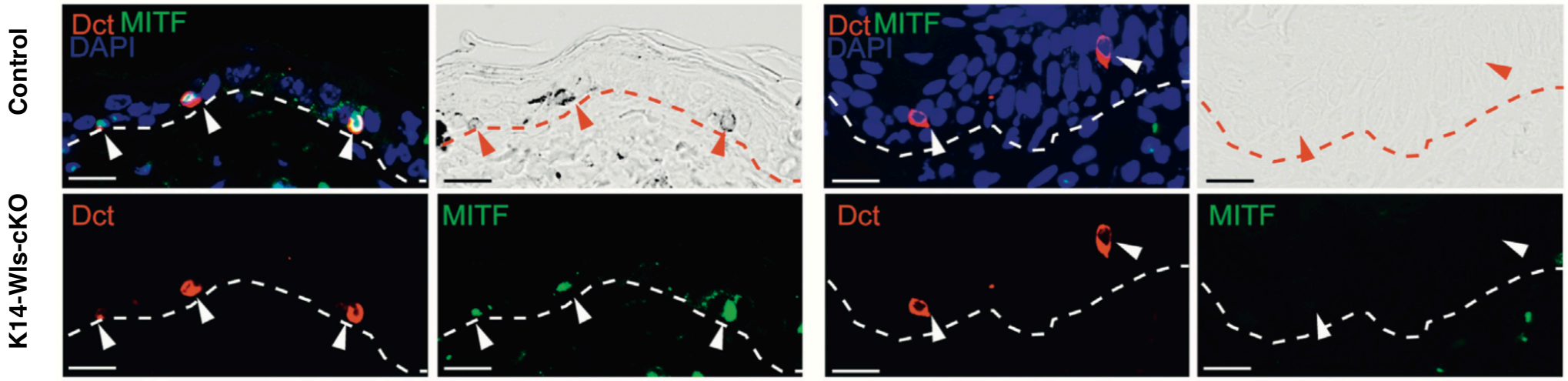
PW21d



Epithelial Wnt ligands are required for **Tcf1** expression in epidermal melanocytes
Tcf1: Wnt activation marker

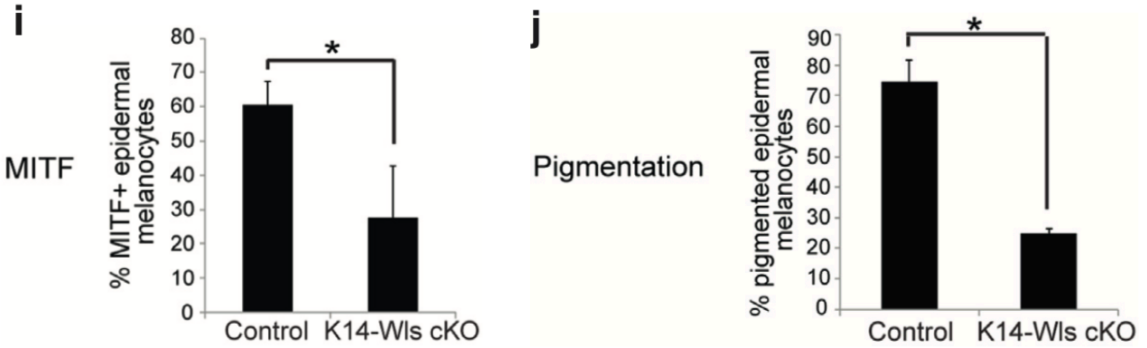
Inhibition of Wnt ligand secretion in epithelial cells

PW21d



Epidermal Melanocytes in K14-Wls-cKO mice show **defect in pigment production**

Fail to express melanocyte differentiation marker **MITF** (normally expressed in epidermal melanocytes)

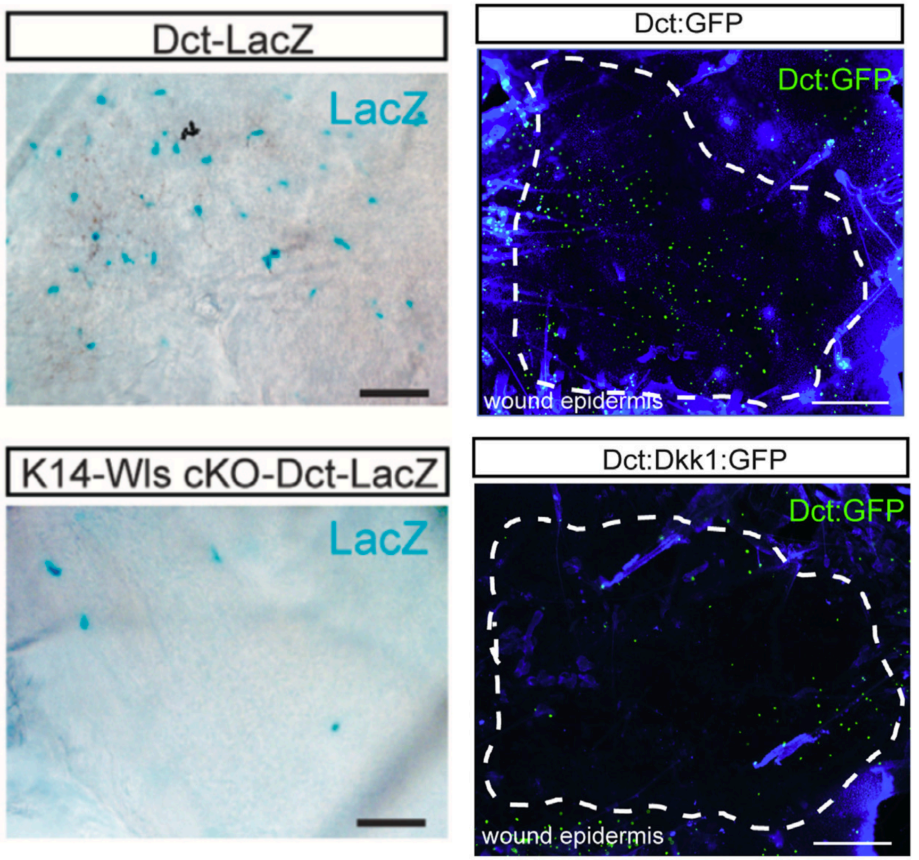


Inhibition of Wnt ligand secretion in epithelial cells

PW44d

Whole wound samples show significant reduction of epidermal melanocytes in wound area of K14-Wls-cKO

Epithelium derived Wnt ligands are essential for Wnt activation of McSCs and epidermal melanocytes, which is required to recruit functional melanocytes to the wound area.



β-galactosidase staining of whole mount wound tissue

Dct:GFP signals in whole mount wound epidermis

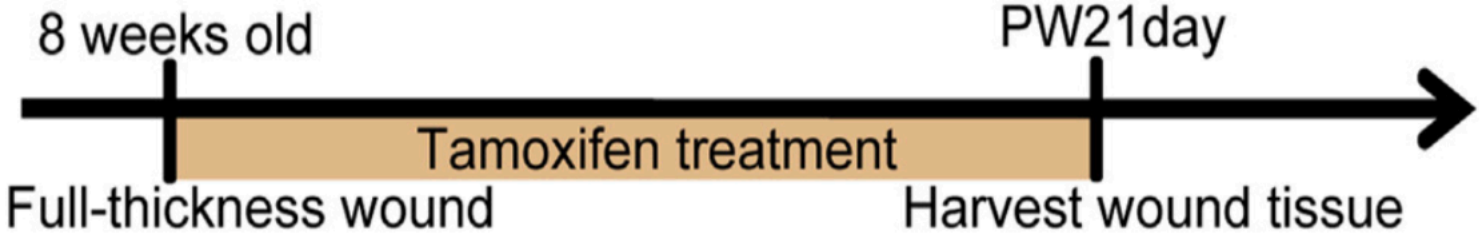
Inhibition of Wnt ligand secretion from melanocytes does not affect their Wnt activation

Inhibition of Wnt ligand secretion in melanocytes

Tyr-Wls-cKO
Tyr-CreEr,Wls fl/fl

Control Mice

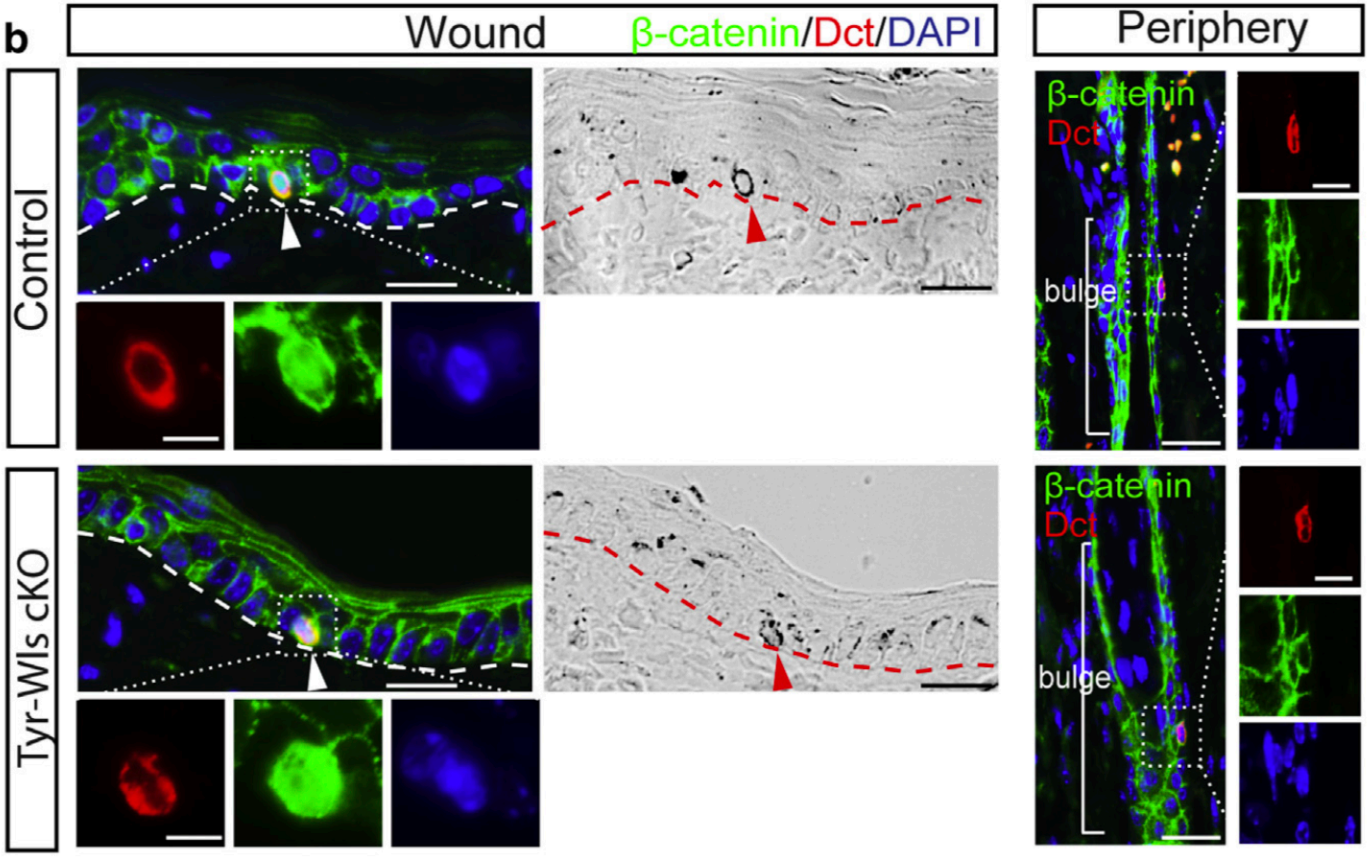
Wls is essential for Wnt ligand secretion and is ablated in Melanocytes after TAM treatment



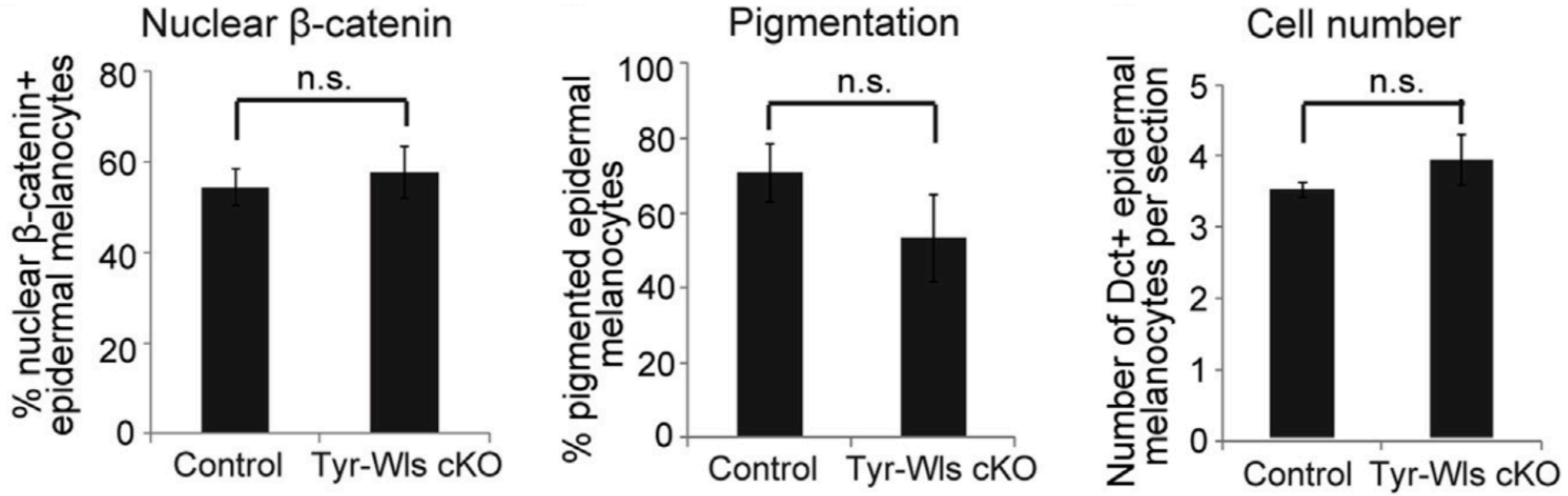
Inhibition of Wnt ligand secretion in melanocytes

After wounding and TAM treatment, there was:

- No difference in the pigmentation and number of epidermal melanocytes
- No difference in Wnt activation of epidermal melanocytes of control, and Tyr-Wls-cKO mice (nuclear β-catenin)



Inhibition of Wnt ligand secretion in melanocytes



Although melanocytes have the potential to express Wnt ligands in some conditions, such intrinsic Wnt ligands are dispensable for Wnt signal activation of epidermal melanocytes during skin wound healing.

Final Thoughts

- Differential fate choice for McSCs to become epidermal vs. Hair melanocytes may NOT be determined by Wnt signaling.
    - Wnt signaling may, however, function to enhance proliferation and differentiation of McSCs, thereby reinforcing recruitment of melanocytes to wound site.
- Results suggest that modulation of Wnt signaling in melanocytes may promote the recovery phase of vitiligo

Conclusion

- **Dkk1 expression inhibits the generation of epidermal melanocytes after wounding**

- **Constitutive activation of Wnt signaling promotes the generation of epidermal melanocytes after wound healing**

- **Wnt ligands secreted by epithelial cells are essential for the activation of Wnt/β-catenin signaling in epidermal melanocytes**

- **Inhibition of Wnt ligand secretion from melanocytes does not affect their Wnt activation**