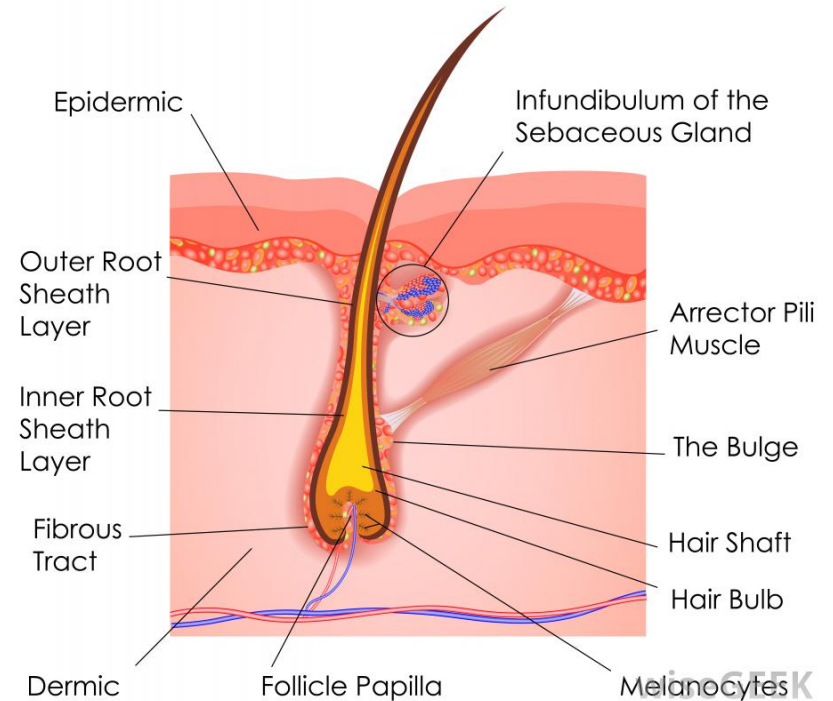


# MicroRNA-214 controls skin and hair follicle development by modulating the activity of the Wnt pathway

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HF undergo cyclic regeneration:  
active growth (anagen)  
regression (catagen)  
relative resting (telogen)

Initiation of a new growth phase  
signaling exchange between  
epithelial stem cells & dermal  
Papilla fibroblasts (e.g. Wnt ligands)



# Introduction

## microRNAs

Fine tuning and buffering the activity of signaling pathways

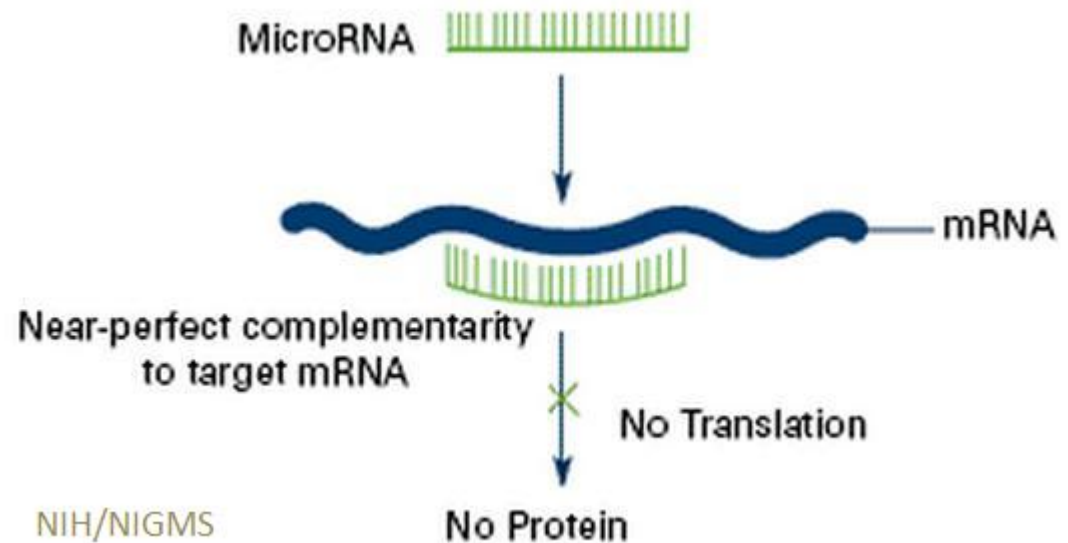
Binding of mRNA → mRNA destabilisation, inhibition of translation initiation

## miR-214

One of the most abundant

miRNAs in HFs at E17.5

Important in controlling  
development of nervous  
system, theeth, pancreas &  
bone formation

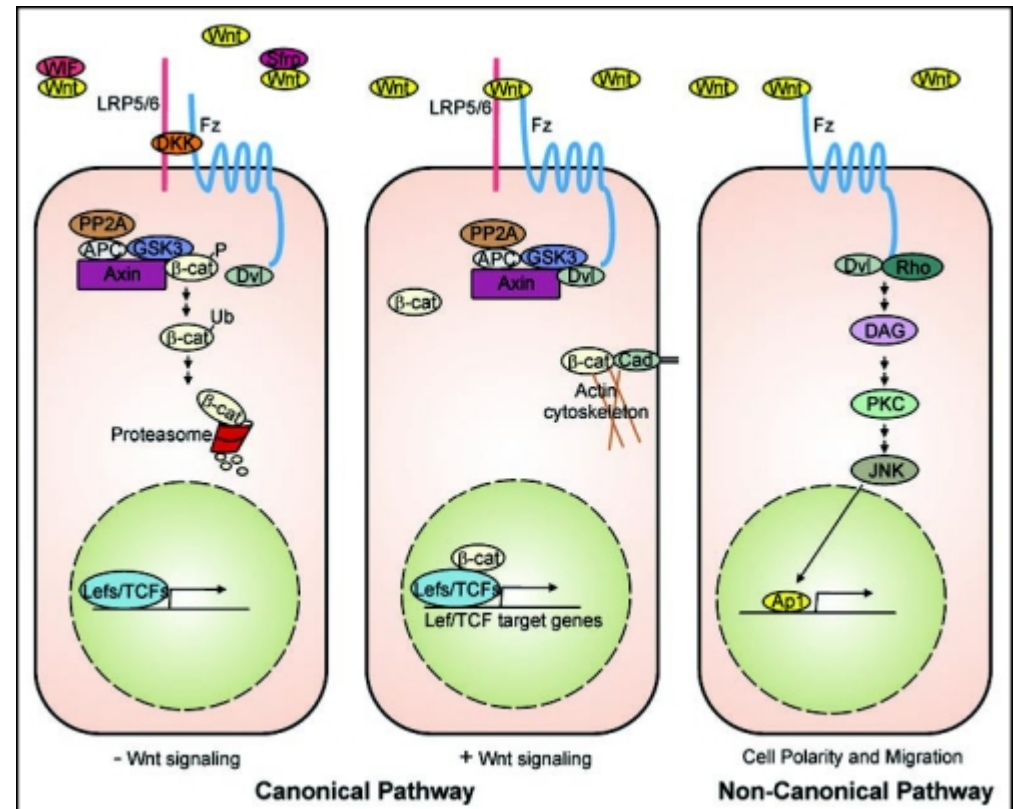


# Introduction

## Wnt-pathway

proliferation, differentiation,  
cell migration, changes in cell  
polarity and cell adhesion

Absence of Wnt-signaling results in  
degradation of-catenin

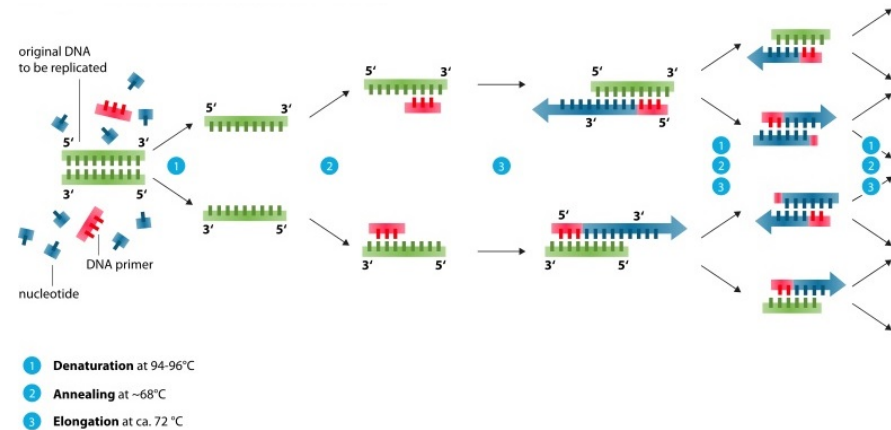
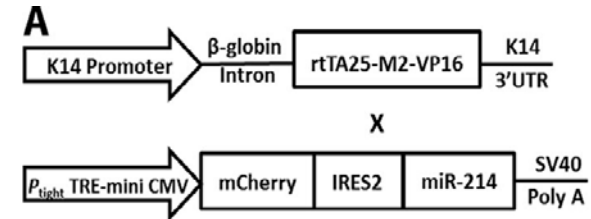


## Generation of transgenic mice

K14-rtTA/TRE miR-214 mice on a FVB background

Cloning verified by sequencing

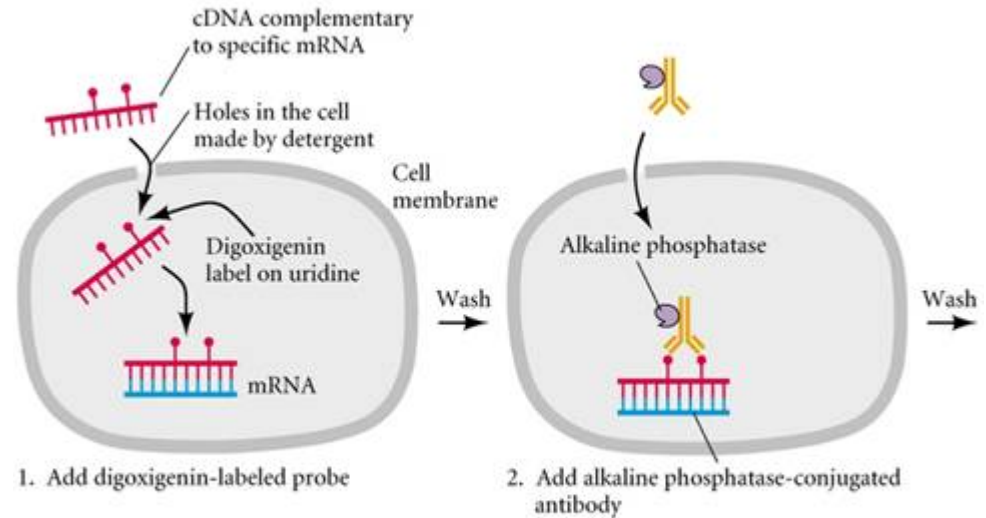
Genotyping by PCR



# Methods

## In situ hybridisation

Skin samples for miR-214

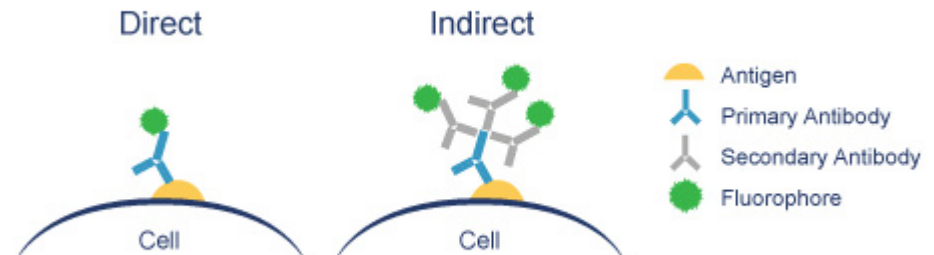


## Immunofluorescence

Keratins + BrdU

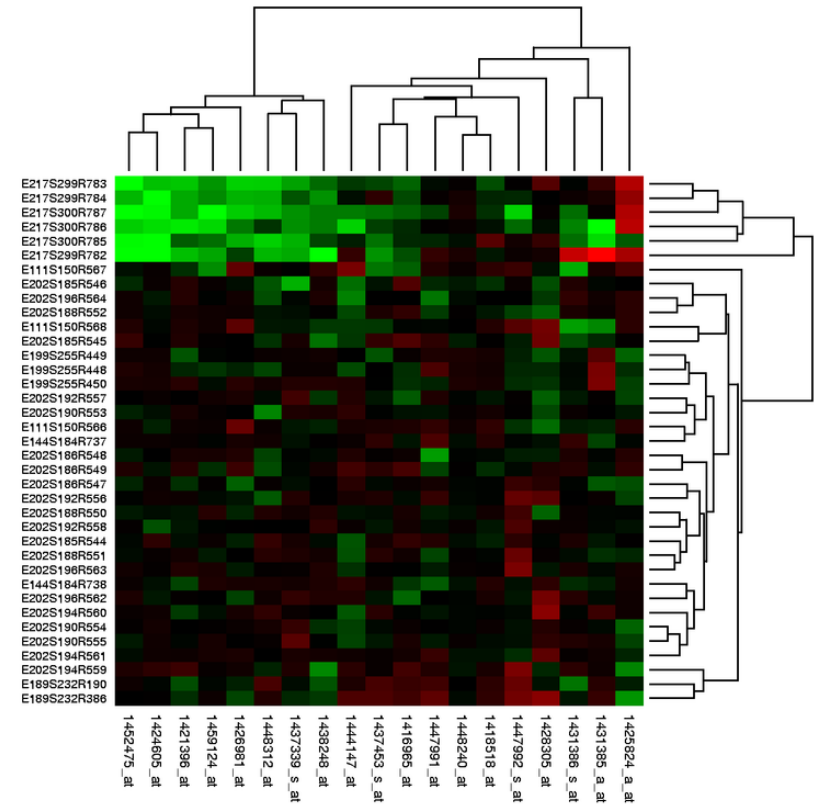
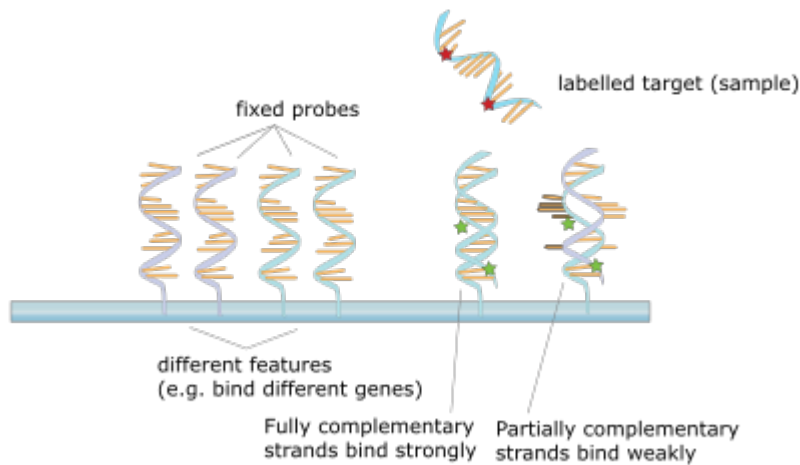
## Combination of IF & ISH

$\beta$ -catenin IF + miR-214 ISH



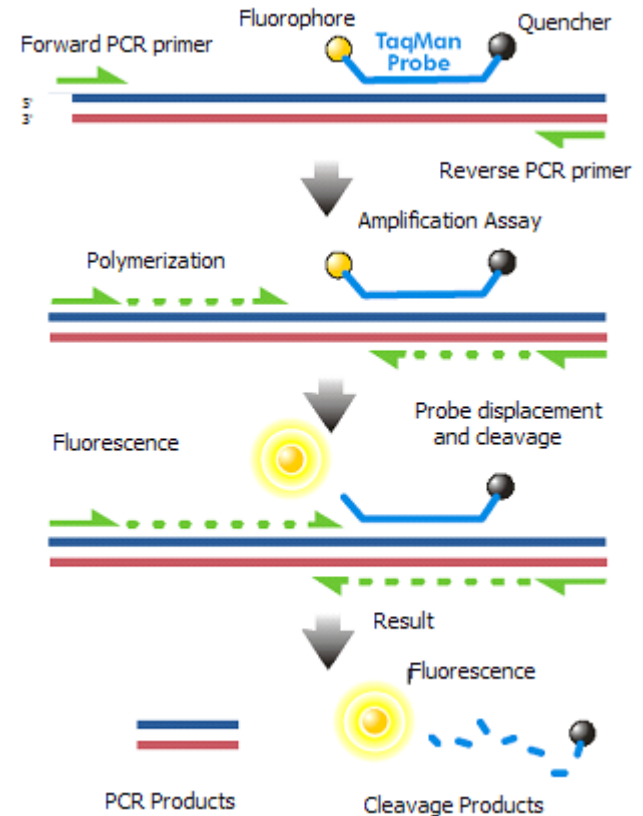
## Microarray

Skin of mice treated with Dox



## Expression of miR-214 with Taq-Man real-time PCR

## Quantitative RT-PCR for mRNA





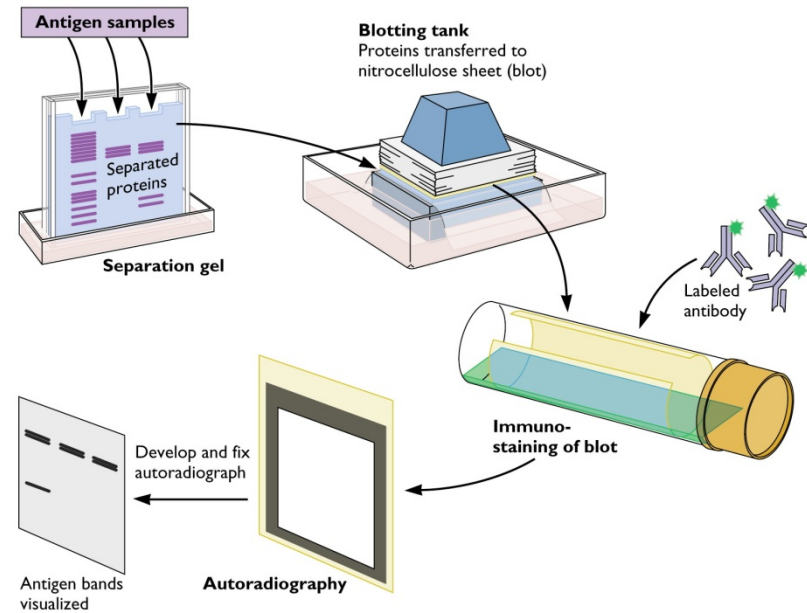
# Methods

## Western Blot

Skin samples or cells

Protein concentration by Bradford assay

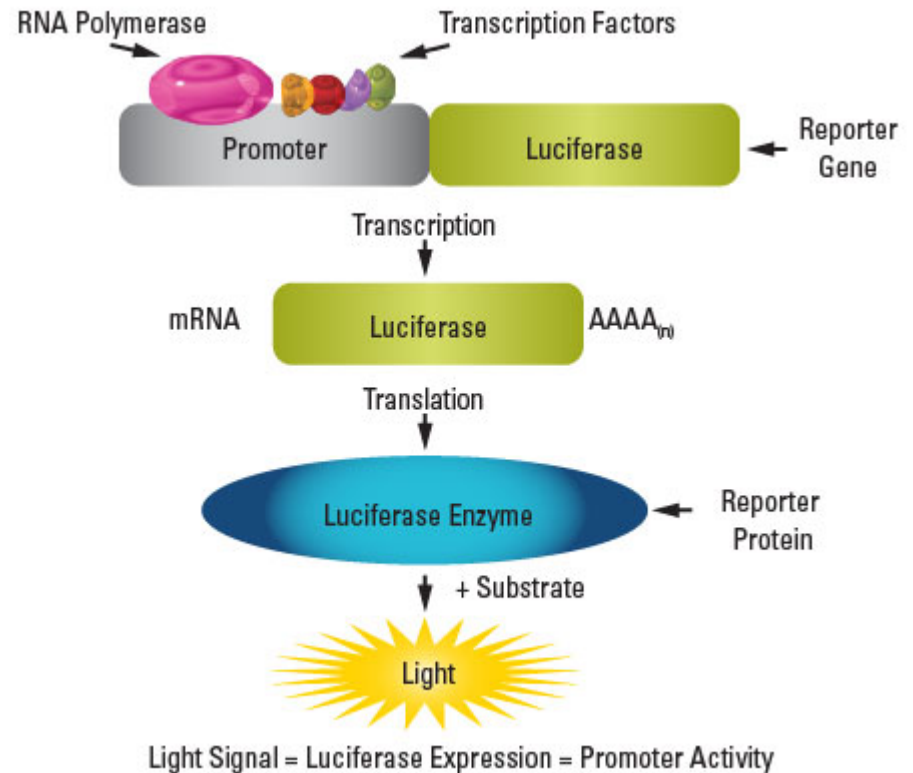
$\beta$ -catenin &  $\beta$ -actin



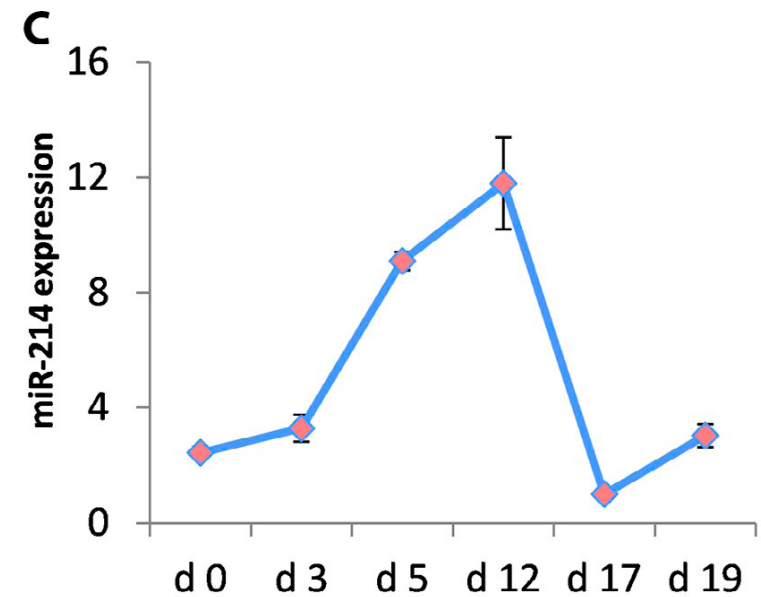
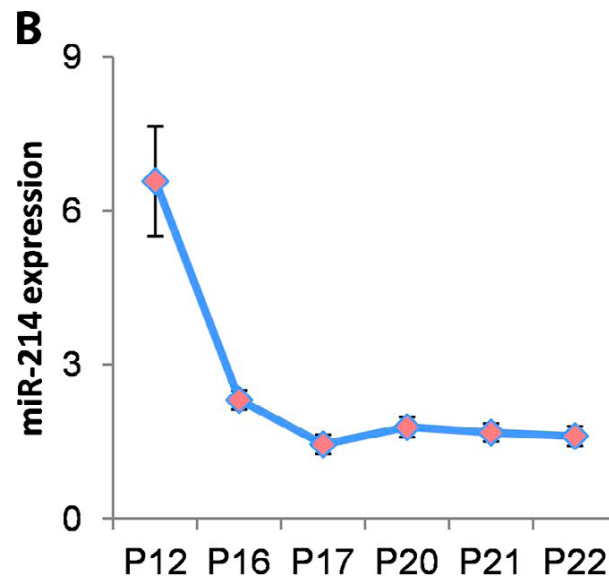
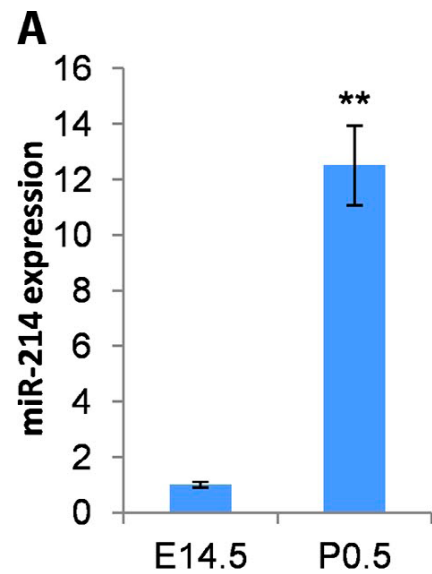
## Luciferase reporter assay

HaCa T cells

Dual luciferase assay:  
cotransfection with miR-214  
mimic or negative control mimic

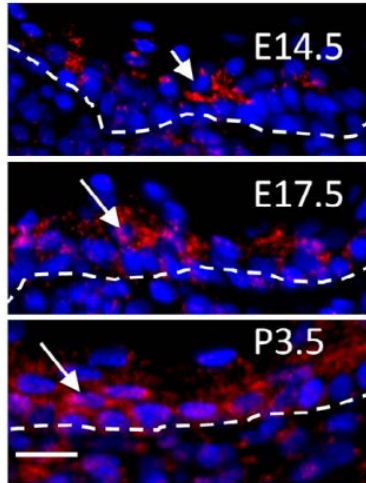


The role of miR-214 in embryonic and adult skin morphogenesis and HF cycling?



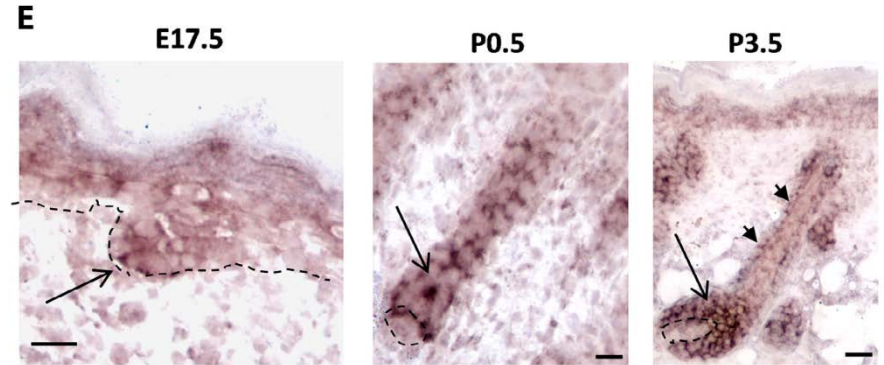
# Results

Where and when is miR-214 expressed?



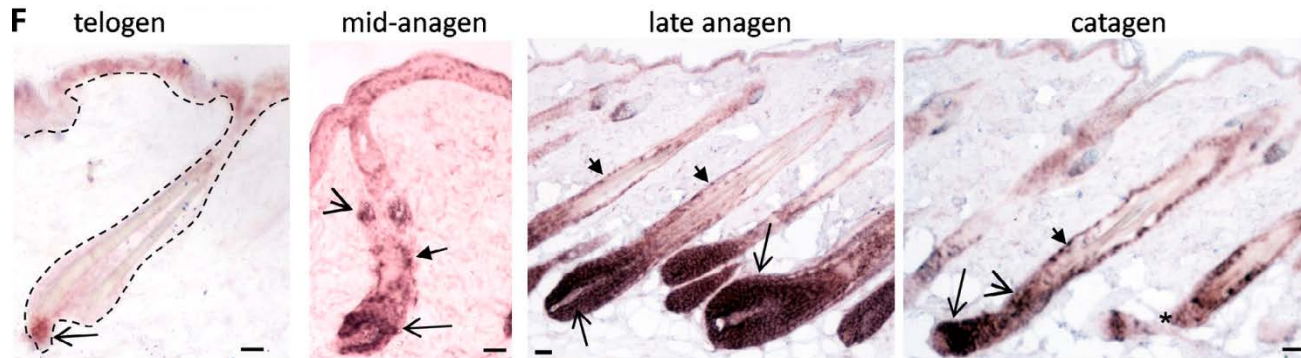
embryonic skin:  
suprabasal

postnatal:  
suprabasal +  
basal



embryonic skin:  
hair placodes

postnatal: hair matrix +  
outer root sheath



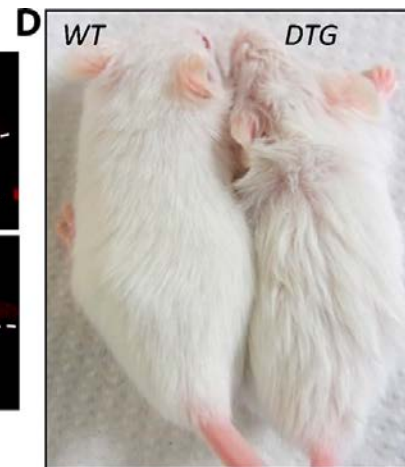
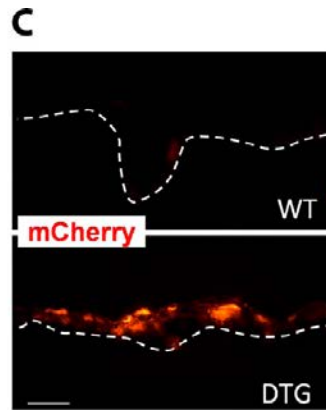
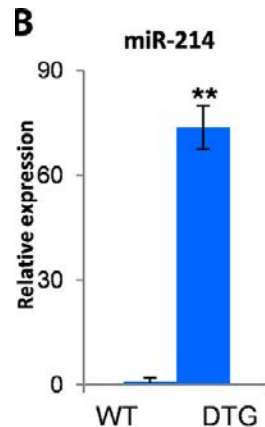
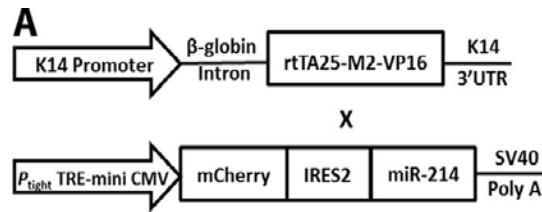
secondary  
germ

growing hair  
matrix, outer  
root sheath,  
bulge area

hair matrix, outer root  
sheath

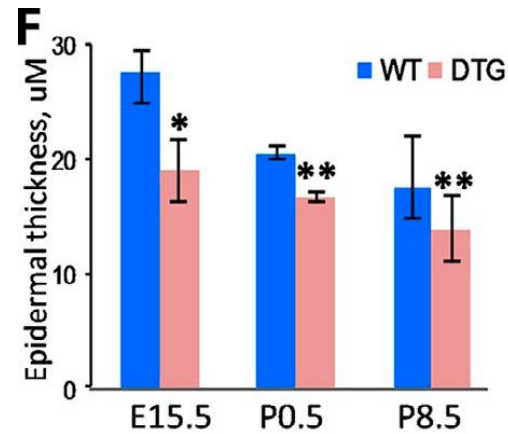
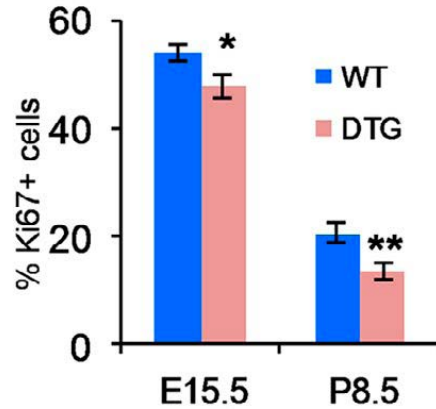
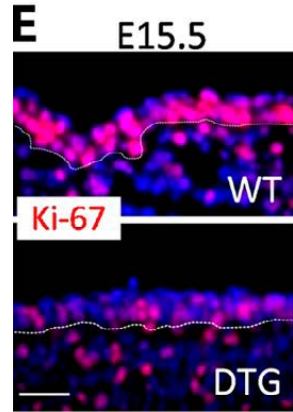
hair matrix, outer root sheath  
epithelial strand

Which effect has overexpression of miR-214 in epithelial progenitor cell population in basal epidermal layer + HF outer root sheath?

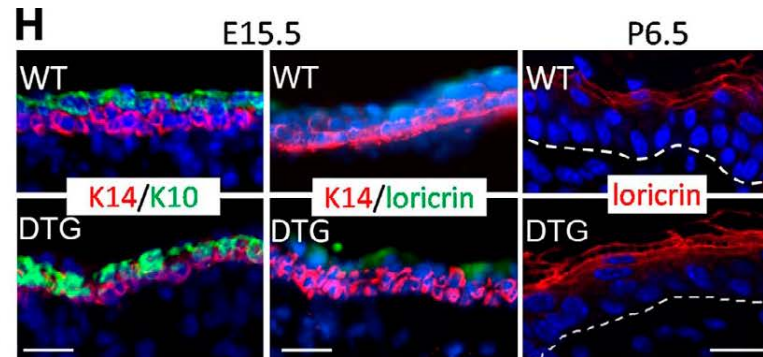
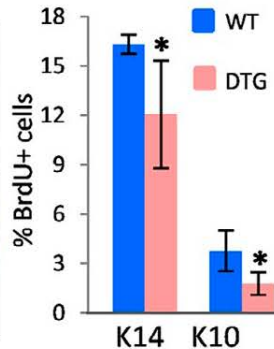
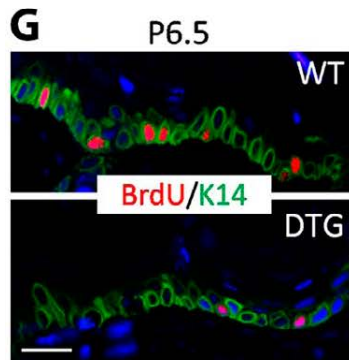




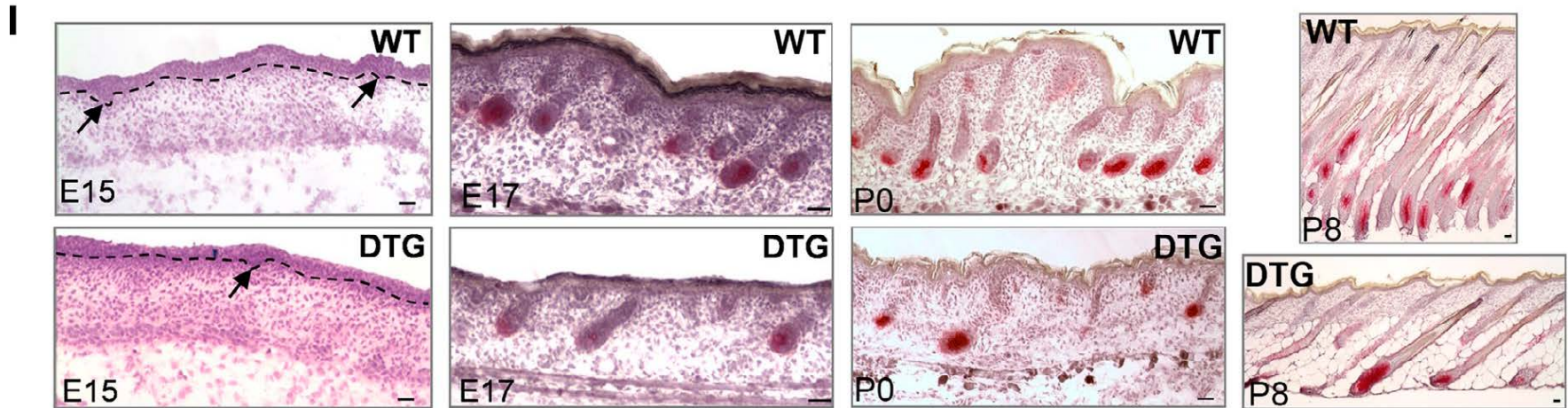
# Results



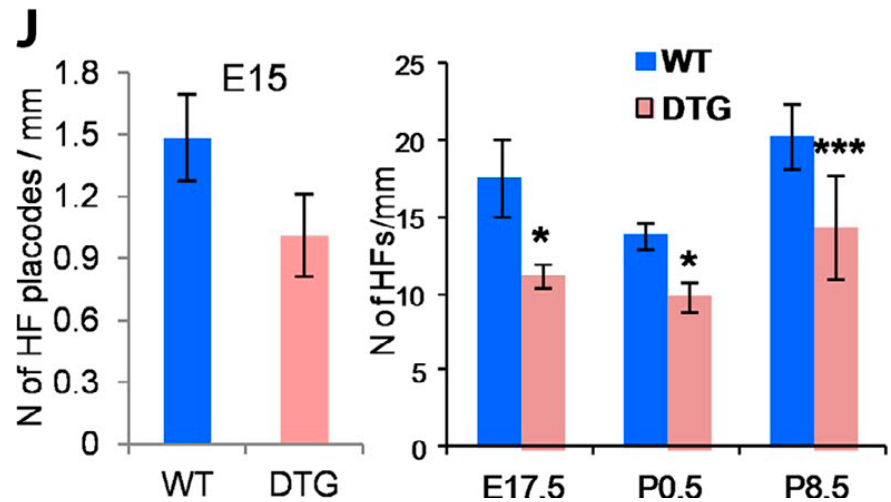
Decreased epidermal cell proliferation resulting in a thinner epidermis



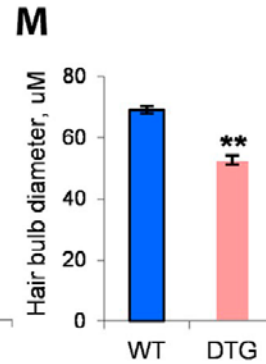
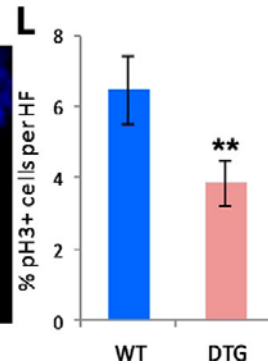
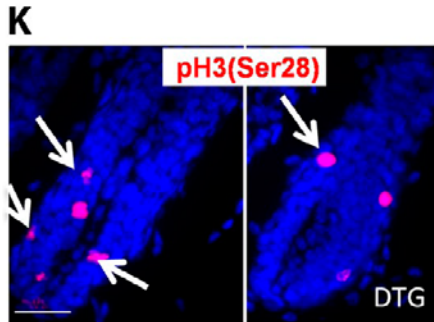
Reduced keratinocyte proliferation + accelerated terminal differentiation caused by increased expression of loricrin may be causative for reduced epidermal thickness



Decrease of HF by miR-214  
overexpression & reduced length  
+ total skin thickness

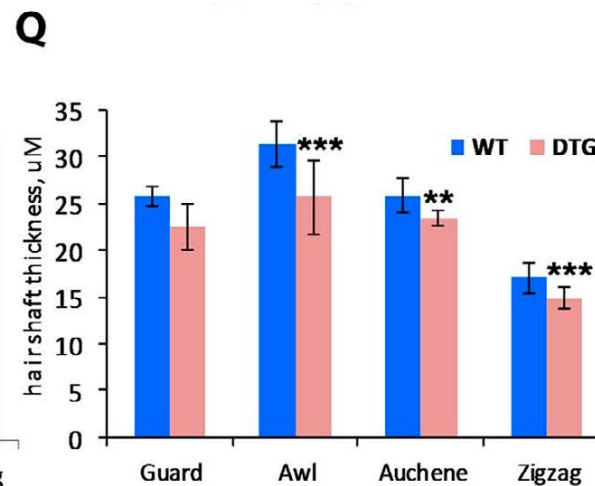
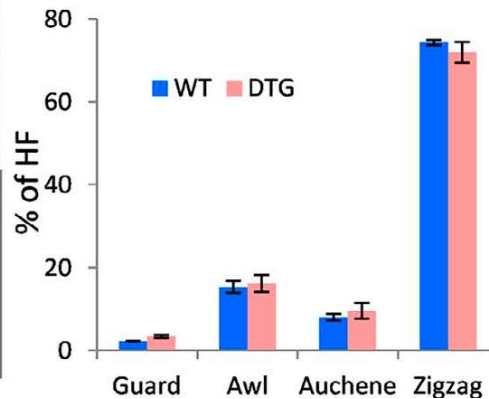
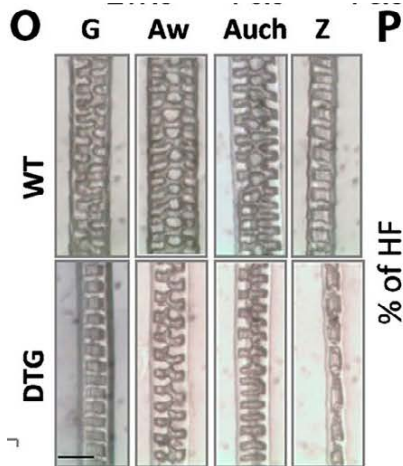
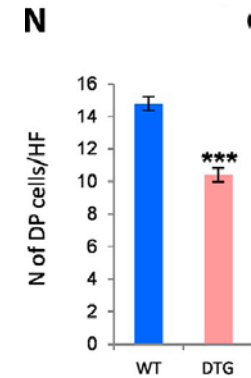


# Results



Reduced cell proliferation, but no apoptotic cells

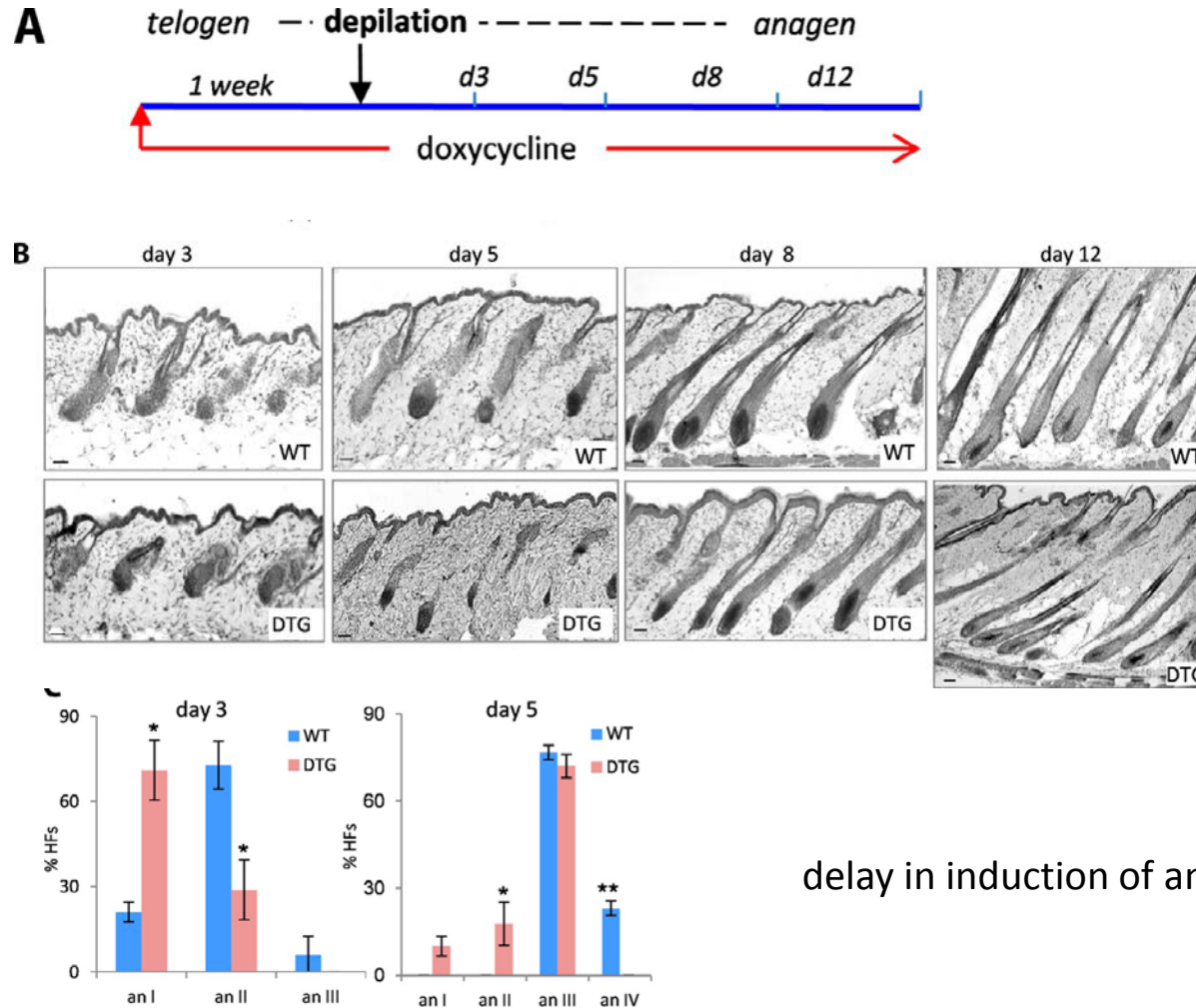
Reduced number of dermal papilla cells



Different proportions of hair types not the reason for phenotype change, but maybe reduction in hair thickness



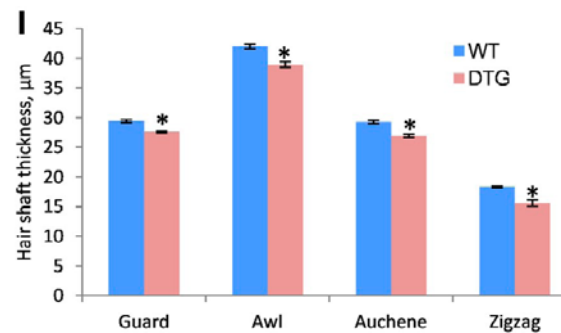
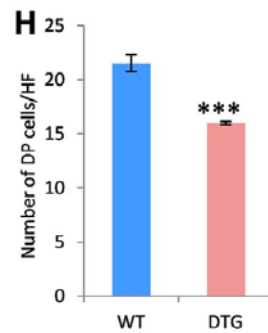
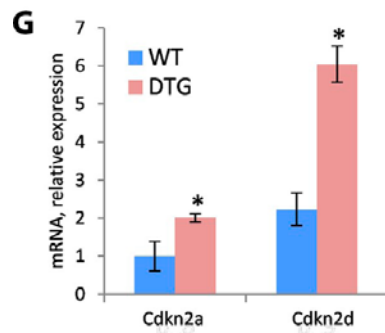
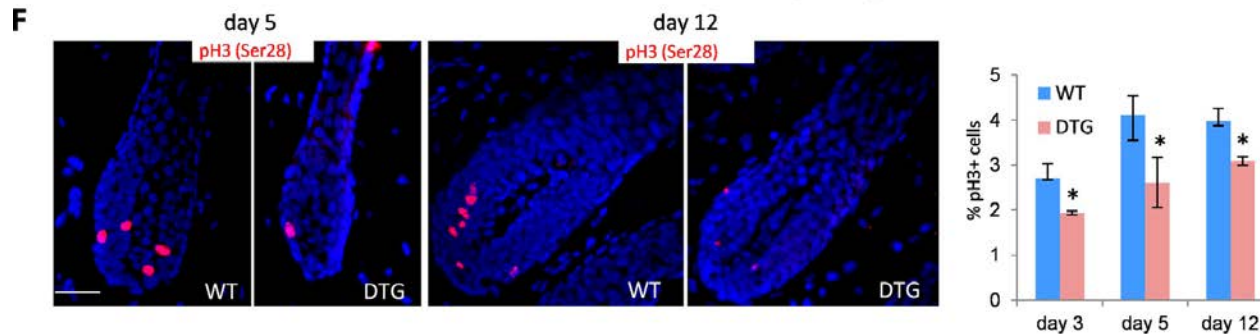
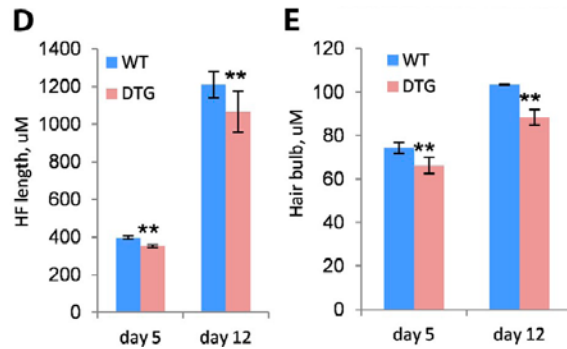
Which effect does miR-214 have on HF cycling?



delay in induction of anagen phase

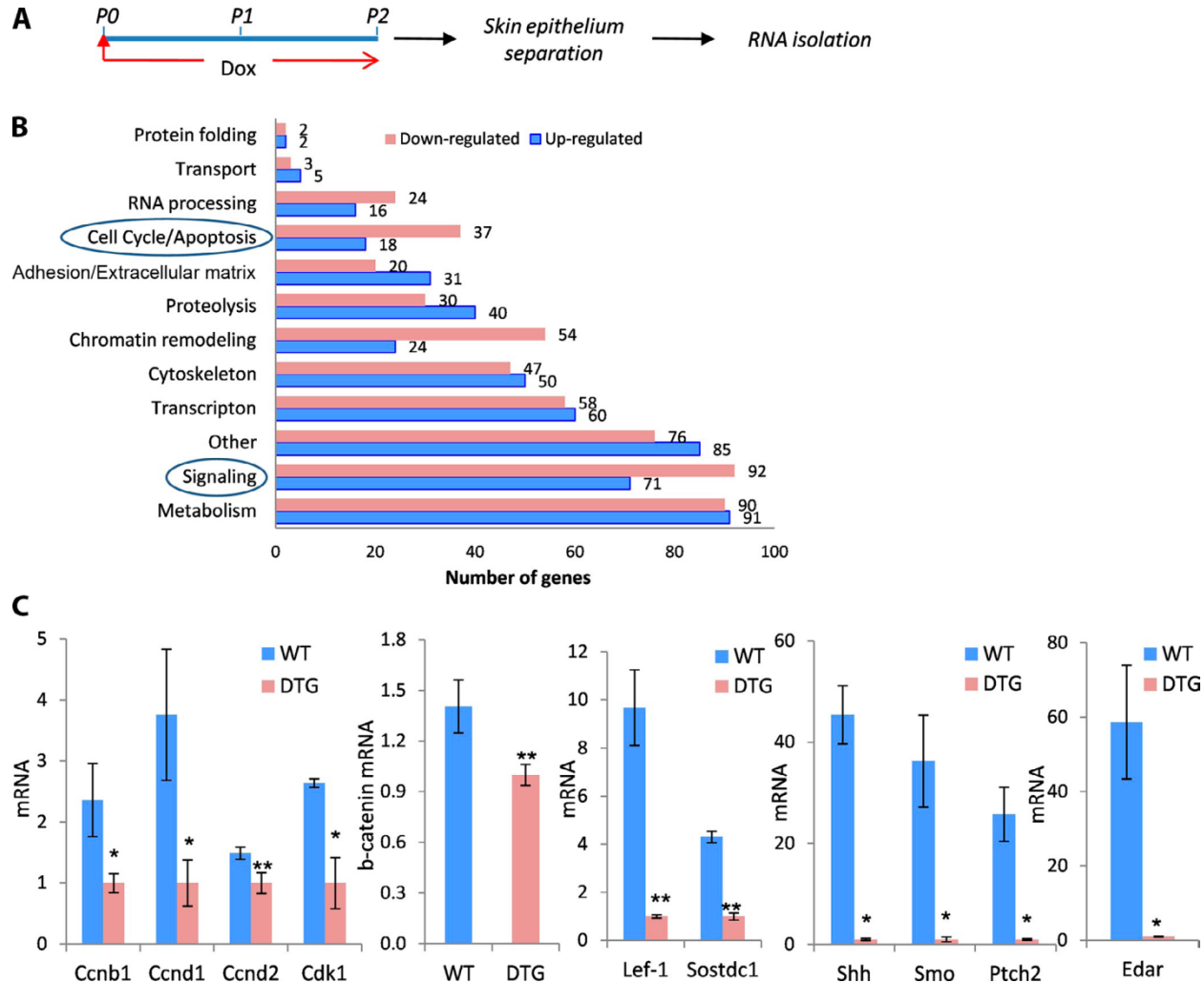
# Results

effect of miR-214 may be mediated by a modulation of keratinocyte proliferation/differentiation + regulating epithelial –mesenchymal interactions (control of dermal papilla cell number)

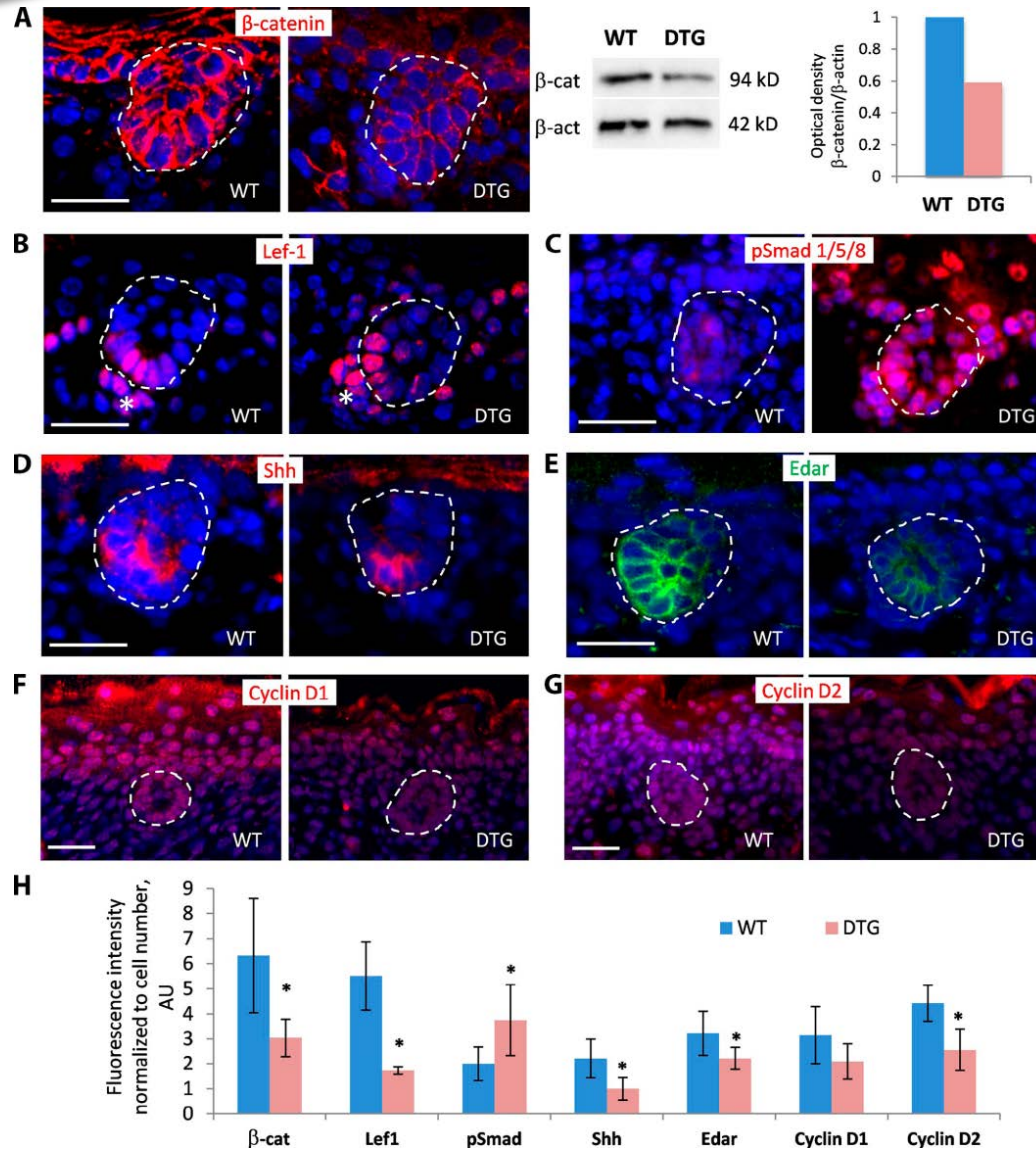


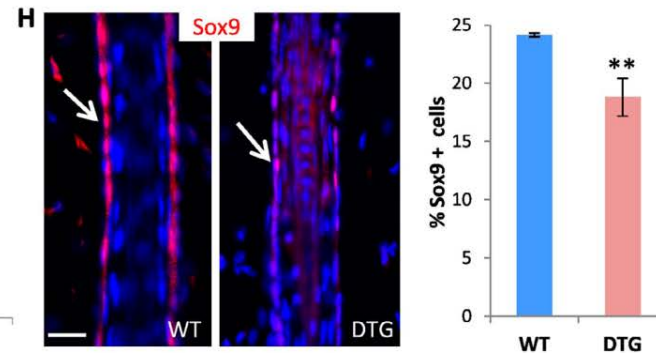
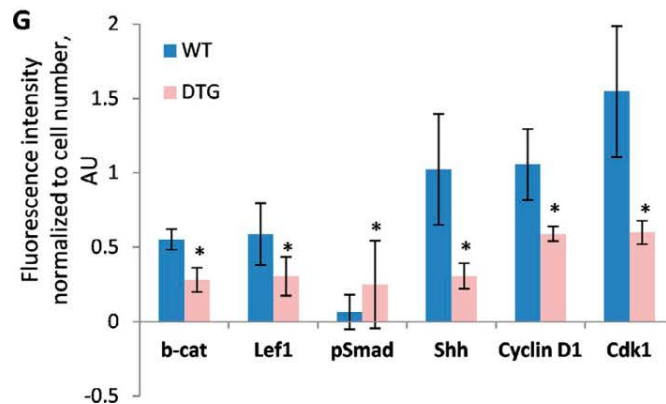
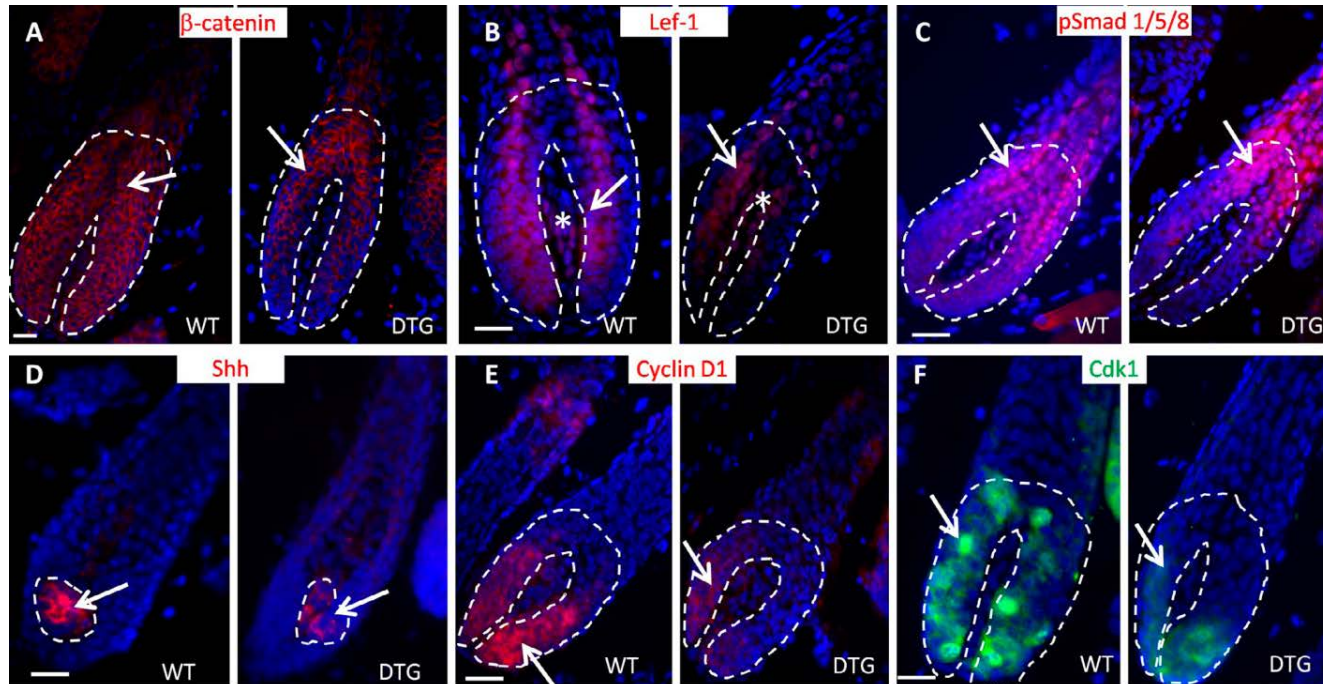
# Results

Which molecular mechanisms are lying behind?



# Results

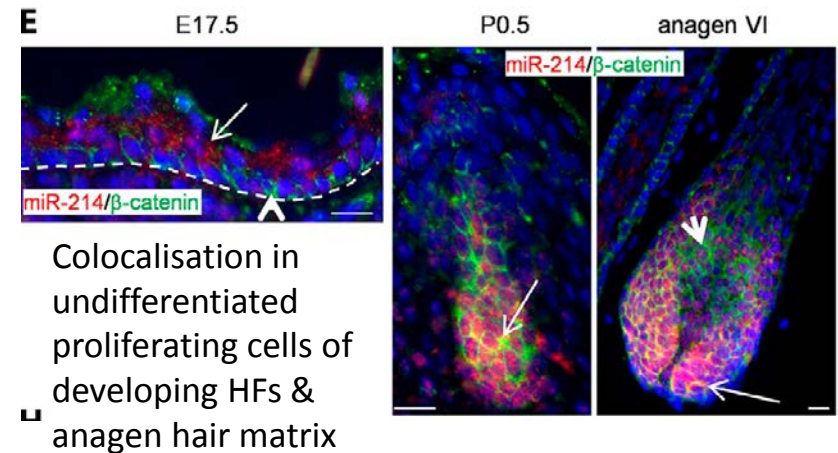
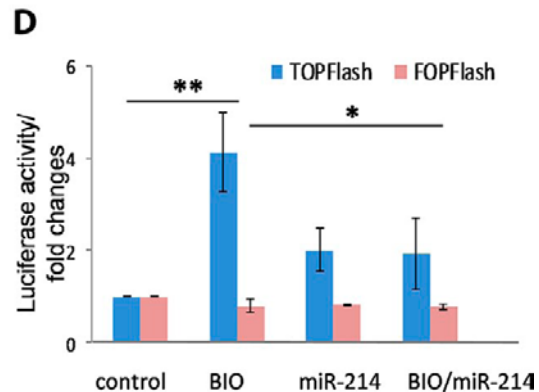
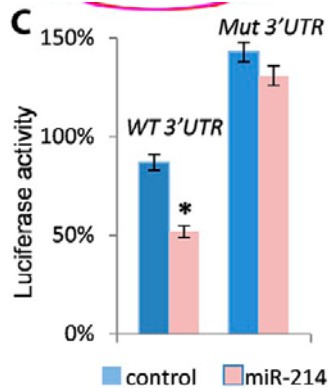
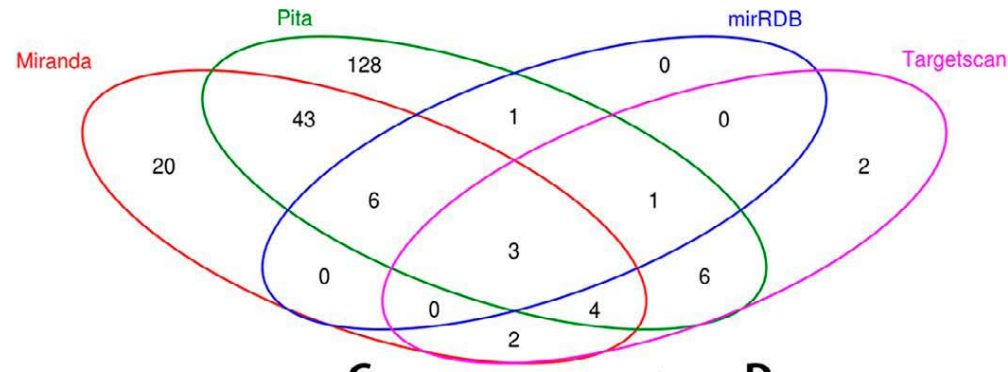




# Results

What are downstream targets of miR-214?

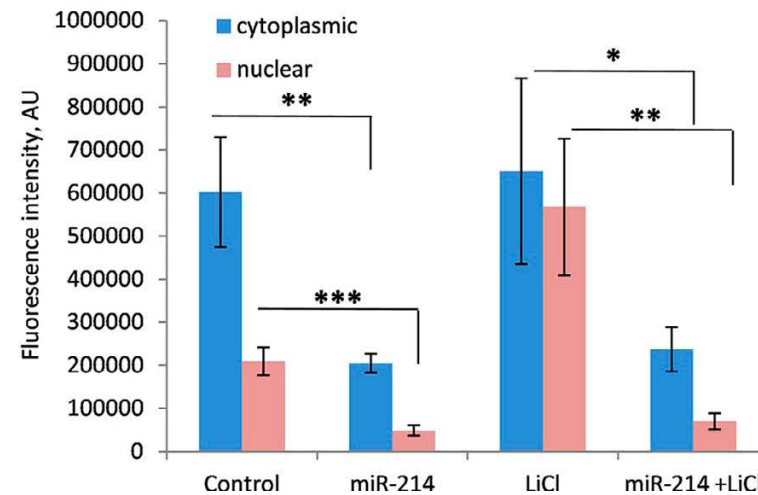
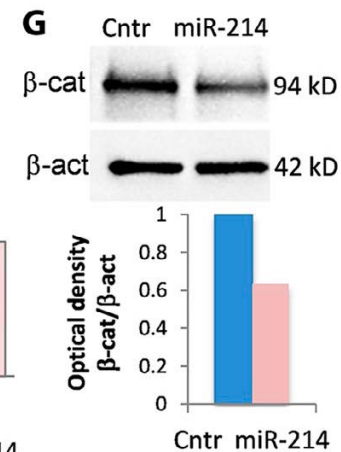
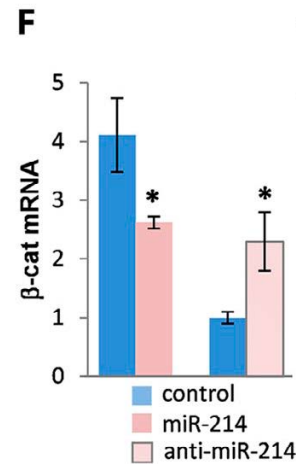
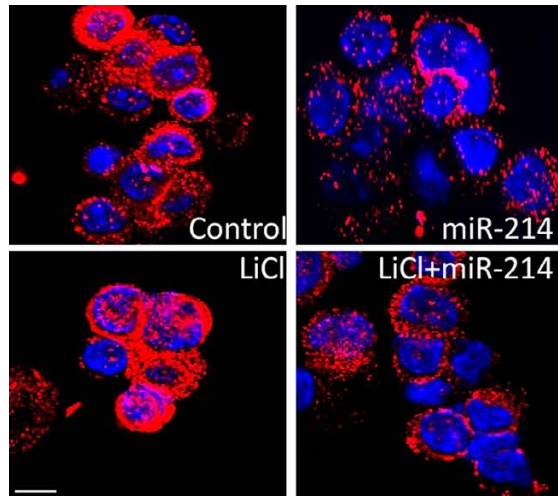
**A**



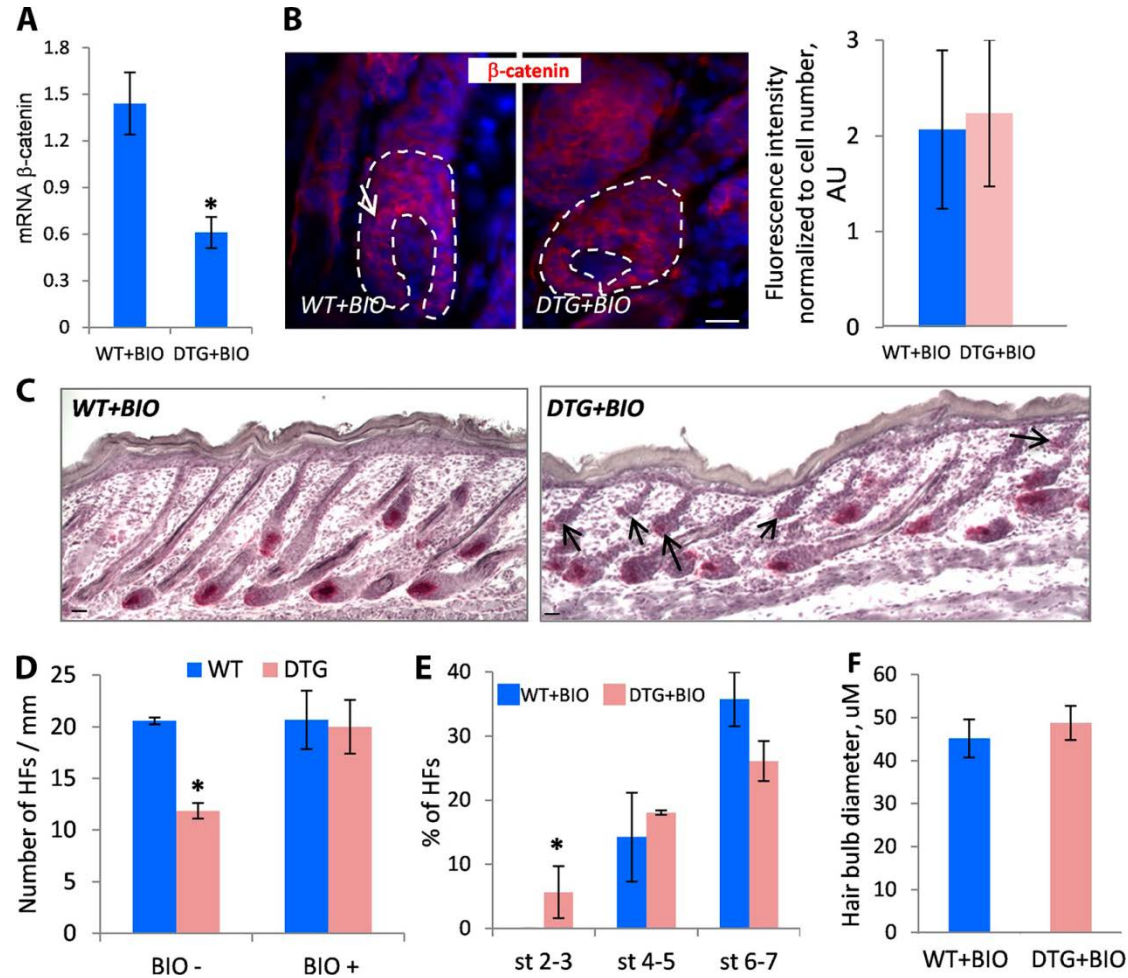


# Results

Transfection of PMEKs with miR-214 mimic or inhibitor resulted in a decrease or increase of  $\beta$ -catenin, lithium chloride cannot rescue  $\beta$ -catenin expression



How is miR-214 functionally linked with  $\beta$ -catenin?





# Conclusion

miR-214 shows spatial temporal changes in expression pattern in the skin during HF morphogenesis and cycling

Overexpression of miR-214 in keratinocytes inhibits cell proliferation resulting in fewer HFs, decreased size of hair bulb and thinner hair

miR-214 regulates the balance of multiple signaling including Wnt in the skin

$\beta$ -catenin is a direct target of miR-214 in keratinocytes