

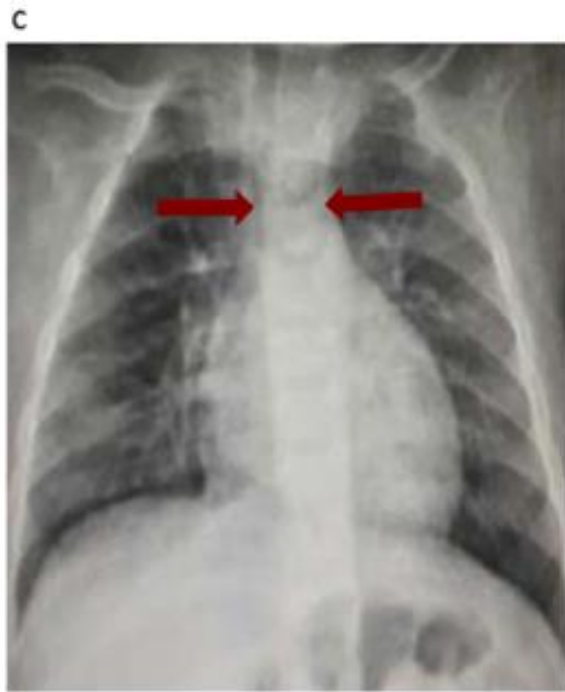
# FOXN1-deficiency and wound healing



**Zoe Zwick**



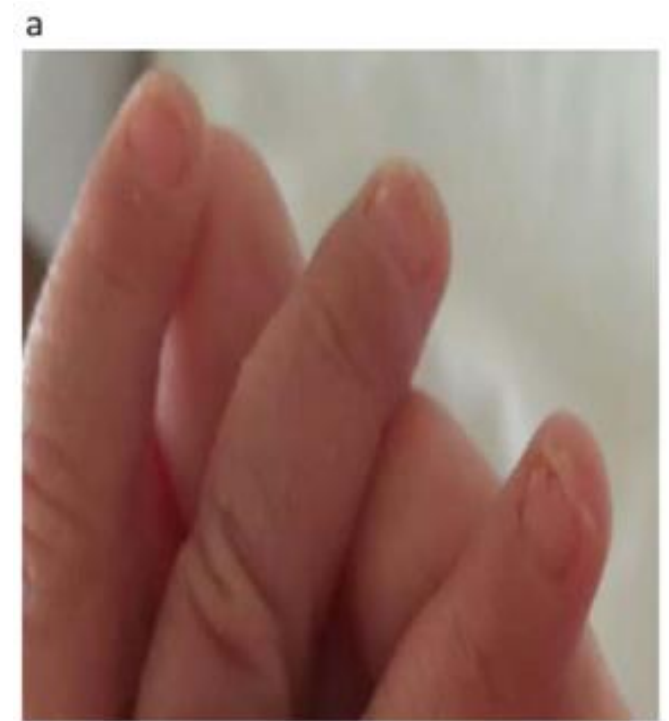
# How are **FOXN1** deficiencies characterized?



Lack of thymus – T cell development organ



Alopecia – no hair growth



nail dystrophy – abnormal nail growth

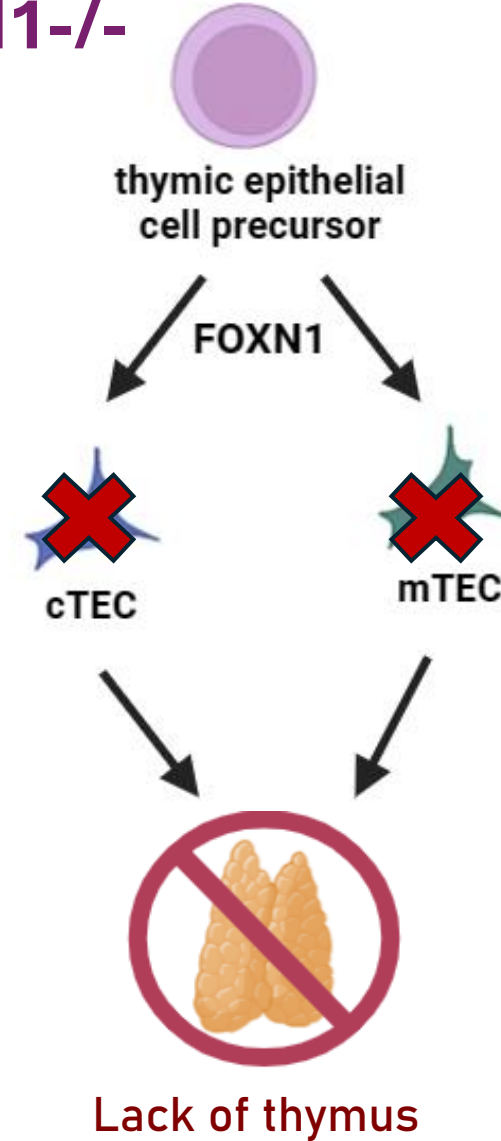
Diagnosed as **T cell immunodeficiency**, **congenital alopecia**, and **nail dystrophy**

# Loss of **FOXN1** leads to epithelial differentiation defects

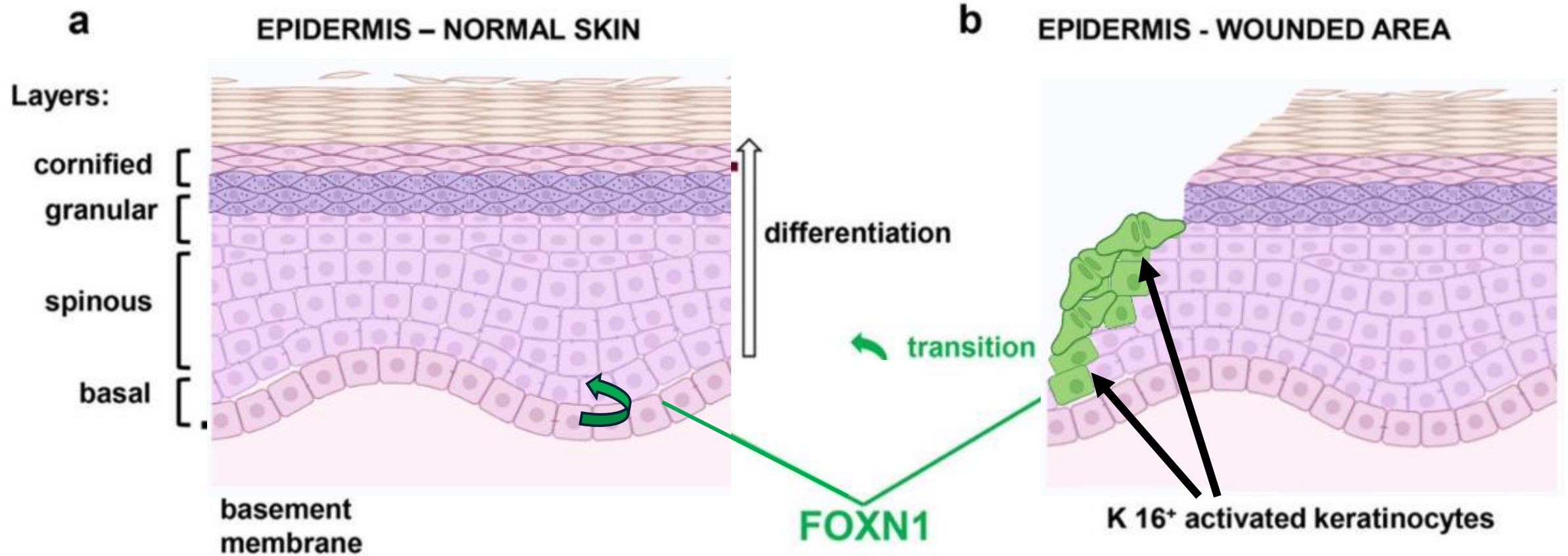
WT



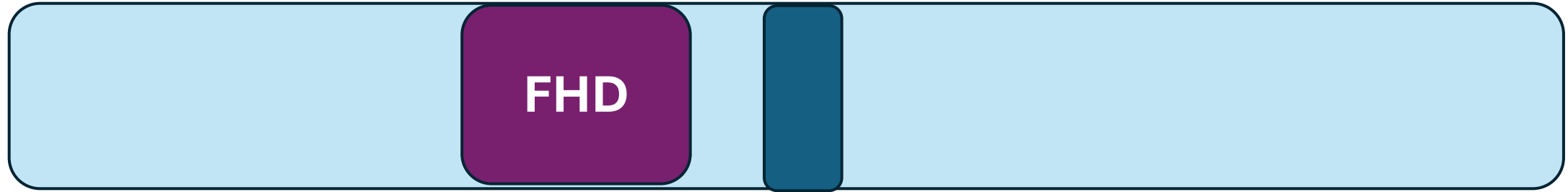
**FOXN1**<sup>-/-</sup>



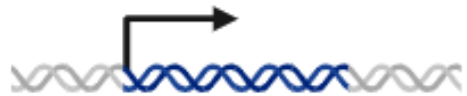
# Epithelial differentiation is important to wound healing



# FOXN1's forkhead domain drives epithelial cell differentiation

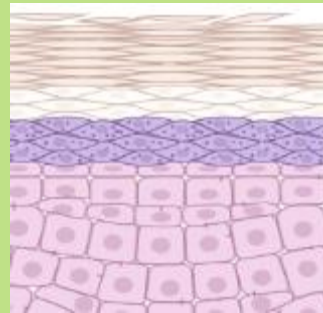


## Molecular Function



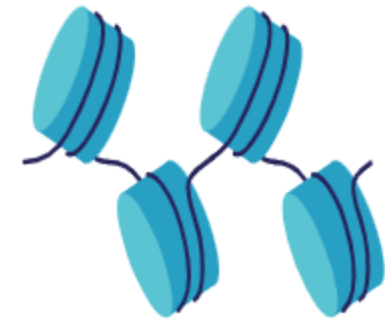
**DNA-Binding  
Transcription Factor  
Activity**

## Biological Process



**Activation of  
Epithelial  
Cell Differentiation**

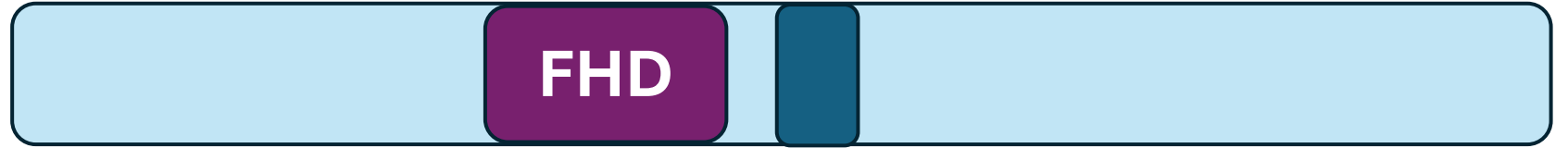
## Cellular Component



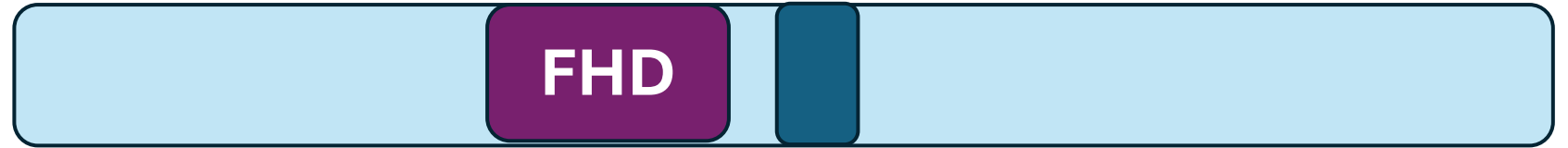
**Chromatin**

# The Forkhead Domain is conserved

**Homo sapien**



**Rattus norvegicus**



**Mus musculus**



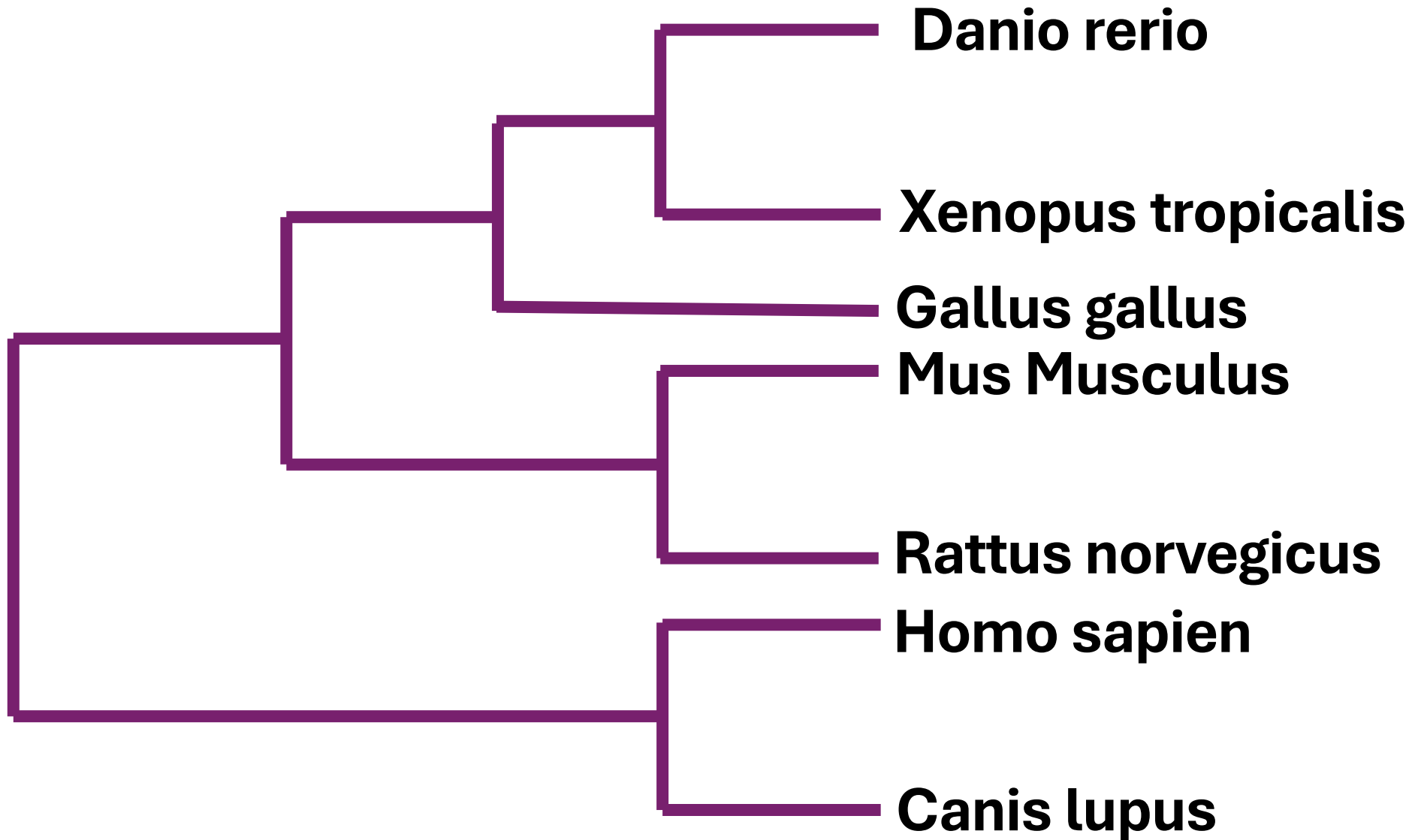
**Danio rerio**



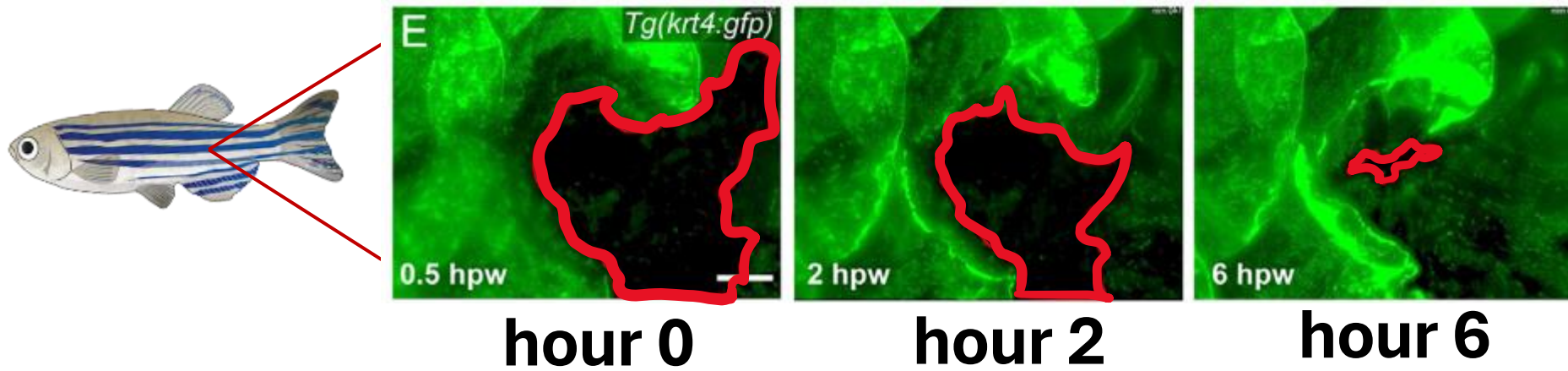
**Xenopus Tropicalis**



# FOXN1 is only found in vertebrates



# Zebrafish is a good model organism for wound healing and development



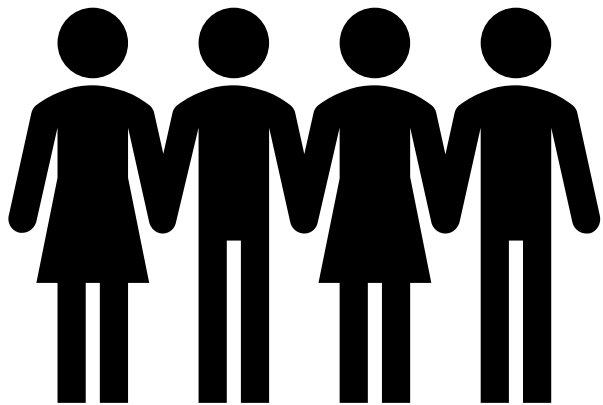
Individual cells are identifiable via **fluorescence**



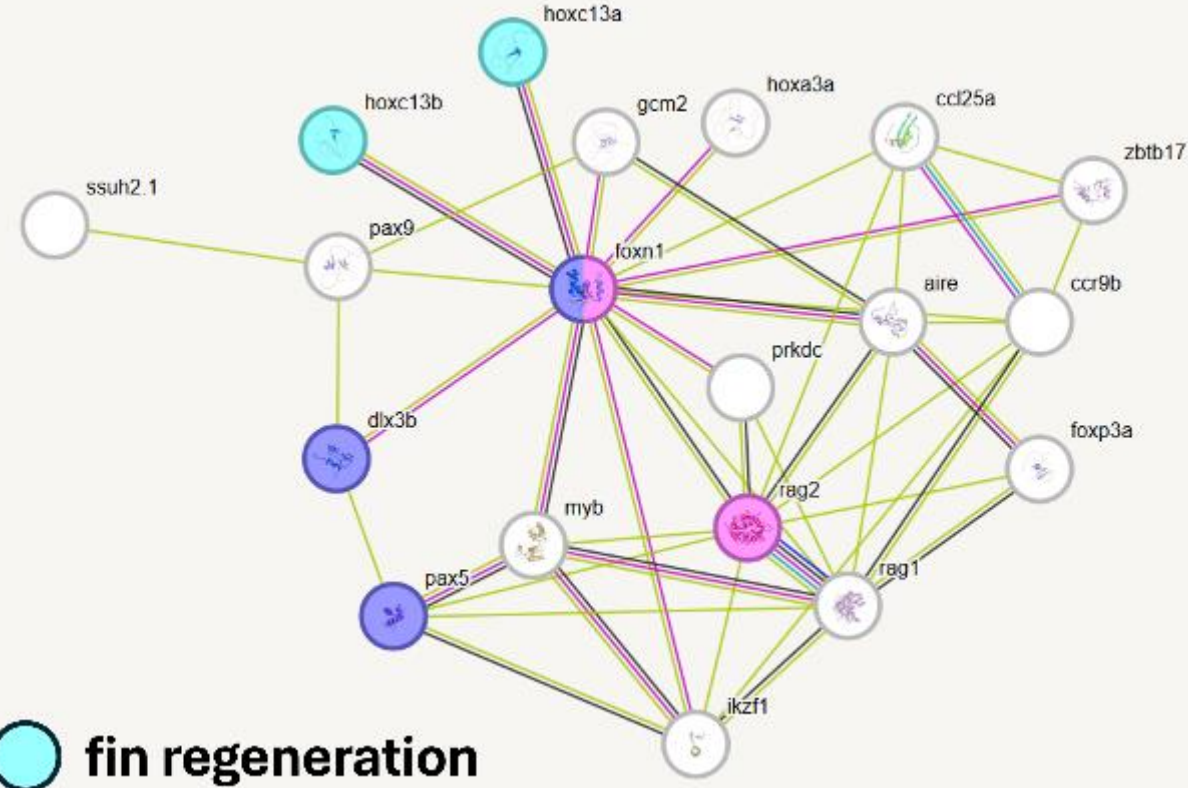
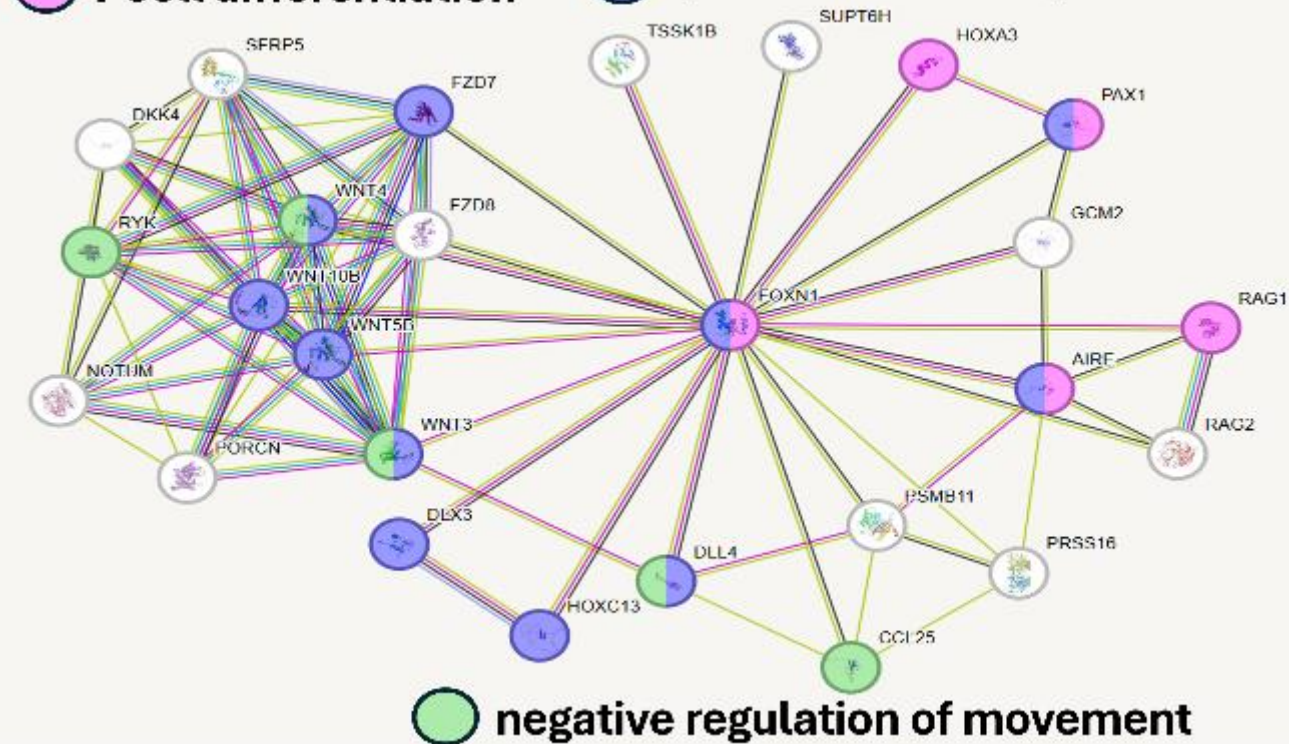
Zebrafish are transparent, and develop outside of a uterus



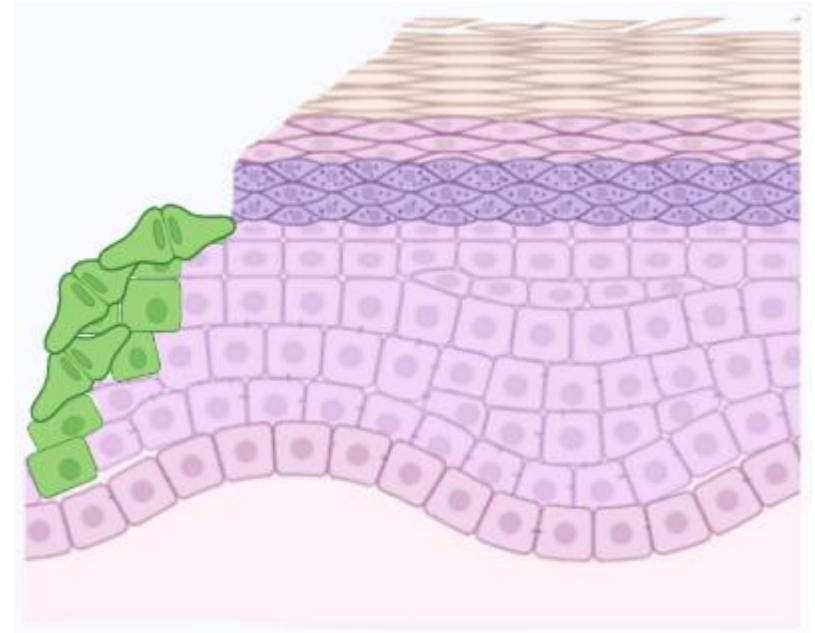
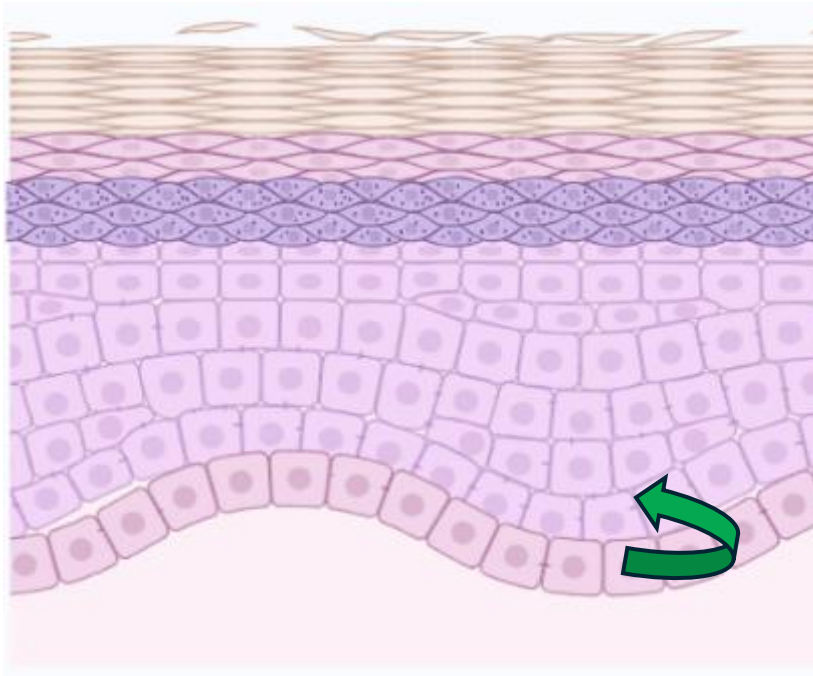
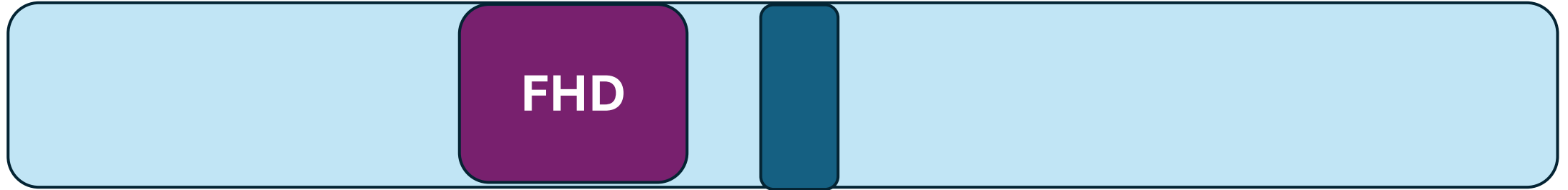
# FOXP1 in humans and zebrafish have similar interaction networks



**● T cell differentiation**    **● epithelium development**



# GAP: How does FOXP1 regulate epithelial cell differentiation in skin?



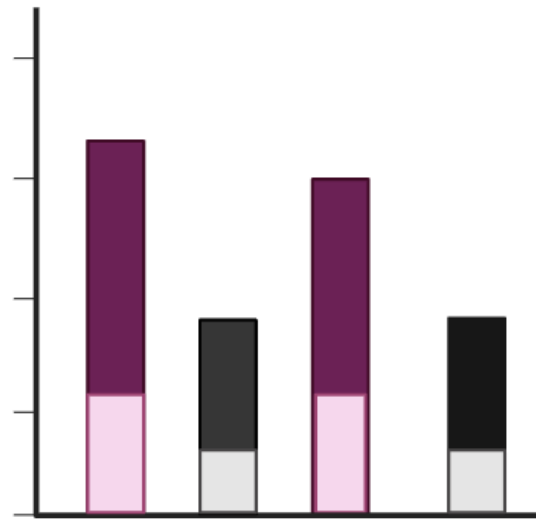
# Goal: elucidate mechanisms related to epithelial differentiation involved in FOXN1 deficiency rapid wound healing

AIM 1



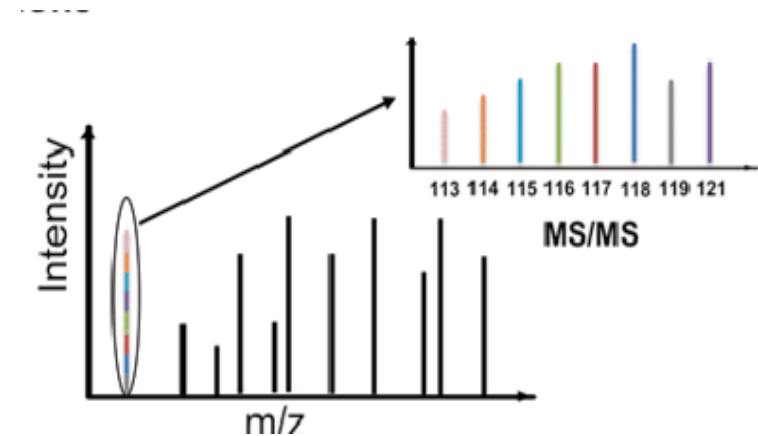
Find conserved **amino acids** important for wound healing

AIM 2



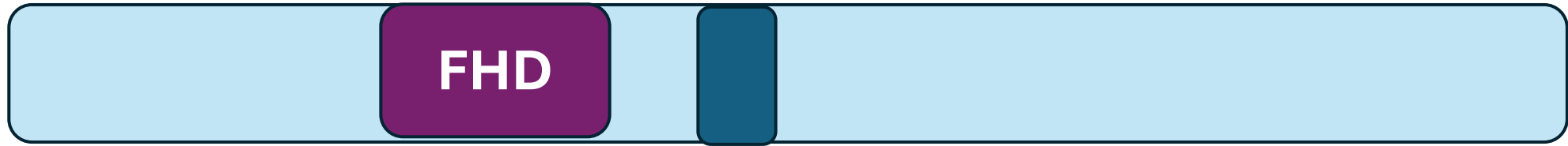
Characterize differentially expressed genes during wound healing

AIM 3

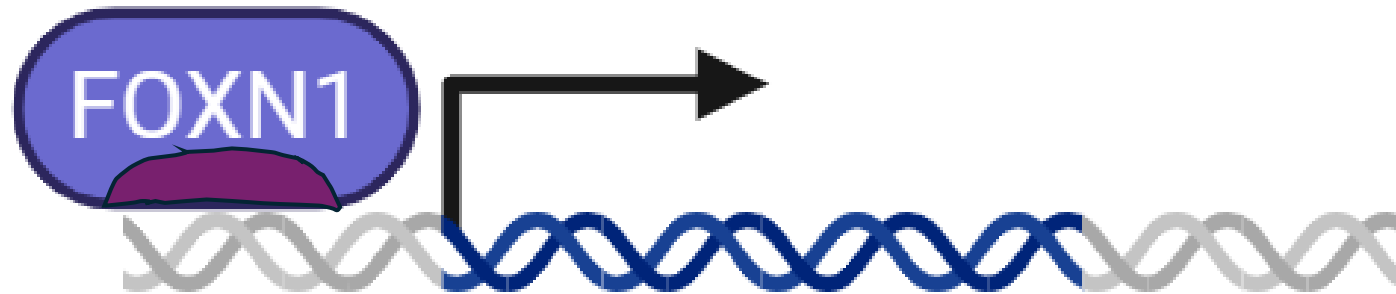


Identify proteins important for epithelial development that cause faster re-epithelialization

## Aim 1: Find conserved amino acids important for wound healing



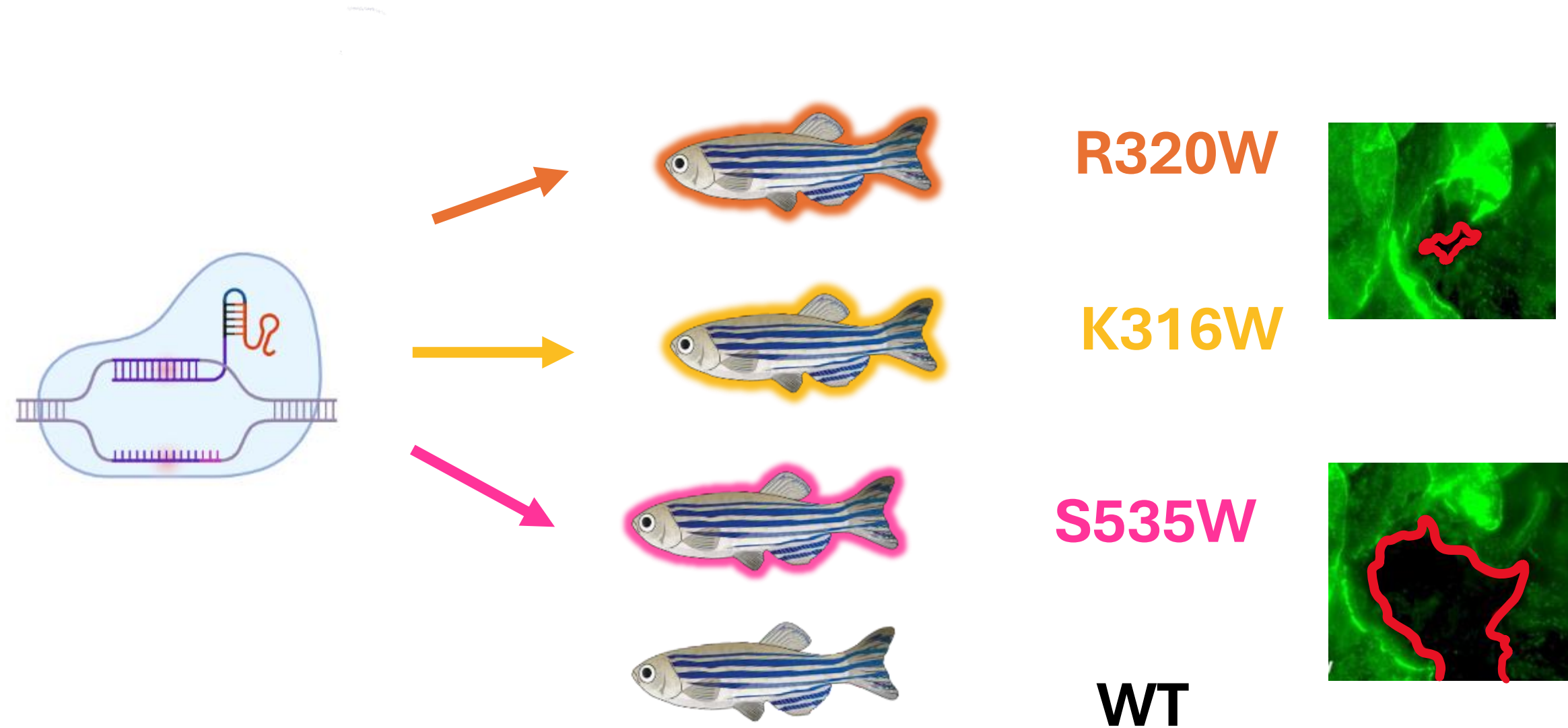
**Hypothesis:** Mutations in the **FHD** will cause faster re-epithelialization



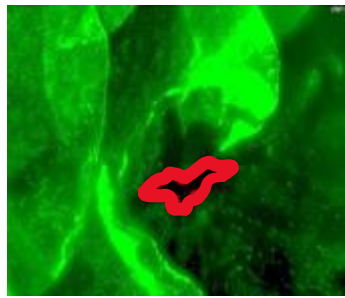
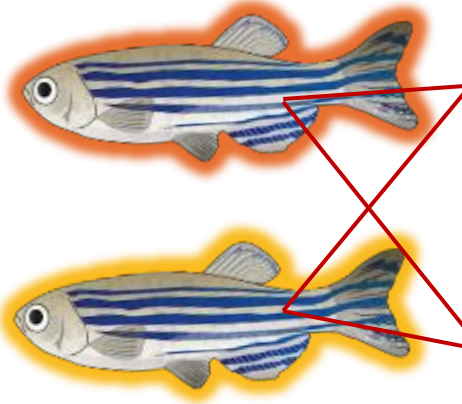
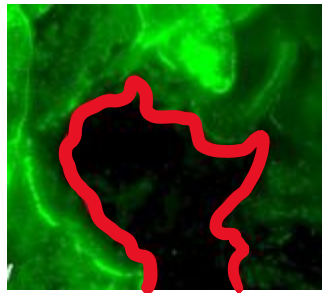
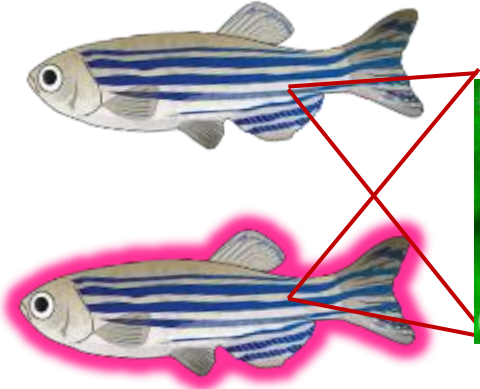
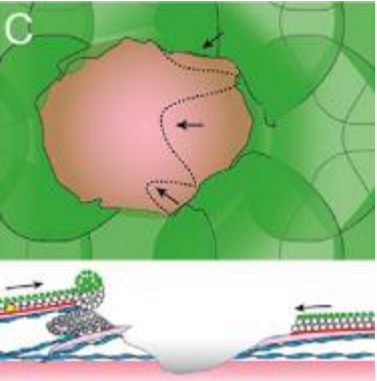
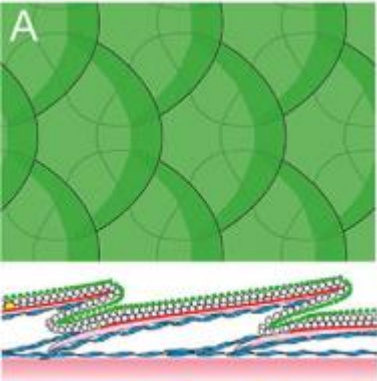
**Rationale:** The **FHD** is important for DNA binding, so without it, it will not be able to activate transcription



# Aim 1b: Utilize CRISPR/Cas9 to create mutations in candidate sites



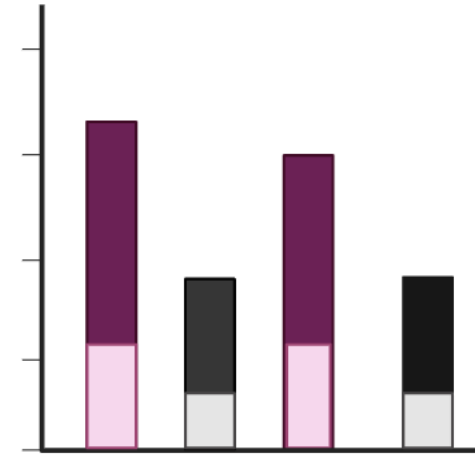
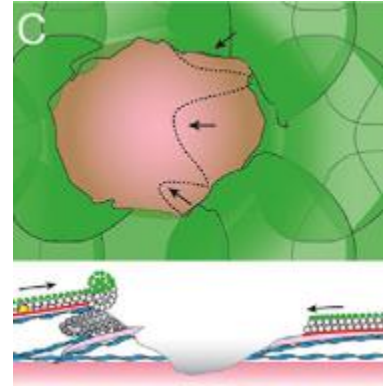
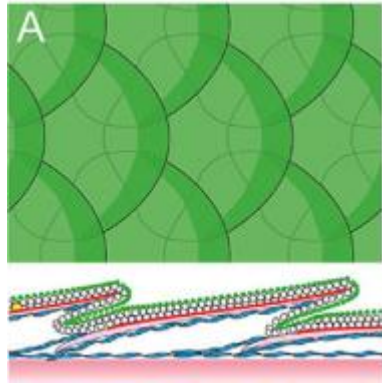
# Aim 1c: Complete **wound healing assay** to determine **re-epithelialization rates**



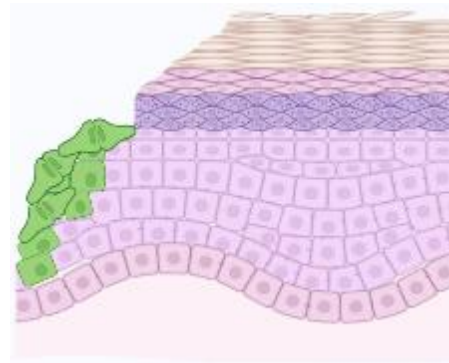
**hour 0**

**hour 2**

## AIM 2: Identify differentially expressed genes in FOXN1 mutants during wound healing using RNA-seq



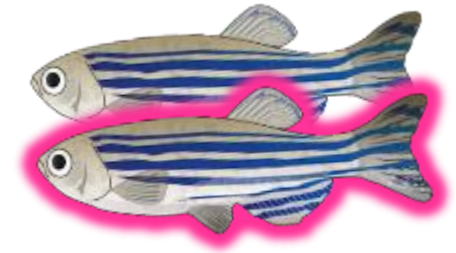
**Hypothesis:** FOXN1 mutants will have increased expression of genes related to **cell movement** and **proliferation**



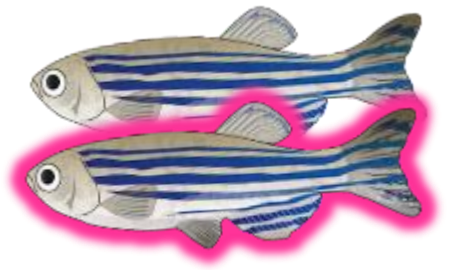
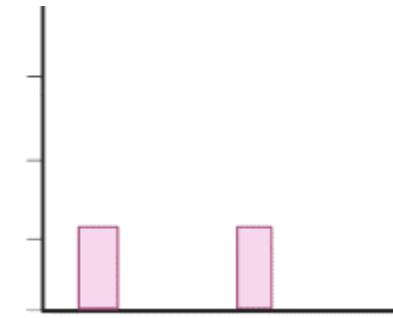
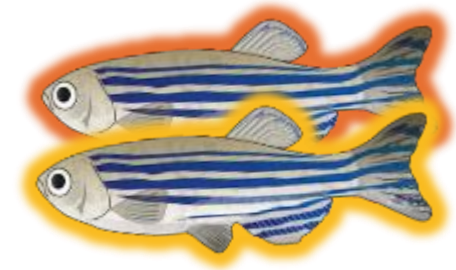
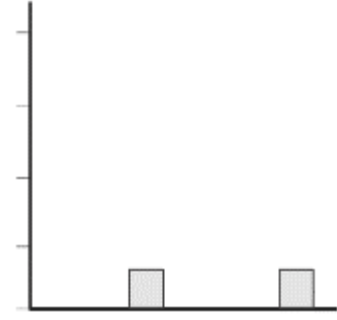
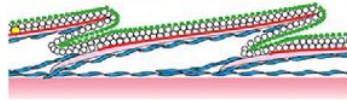
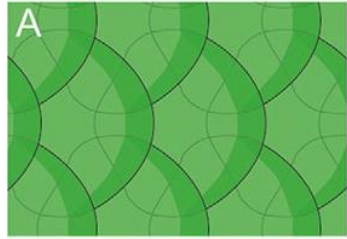
**Rationale:** re-epithelialization requires skin cells to move to cover wounds and differentiate into new cell layers



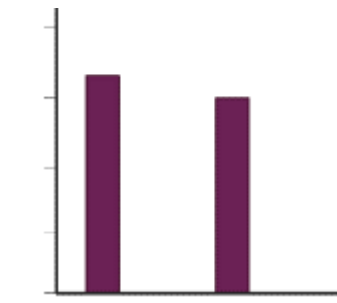
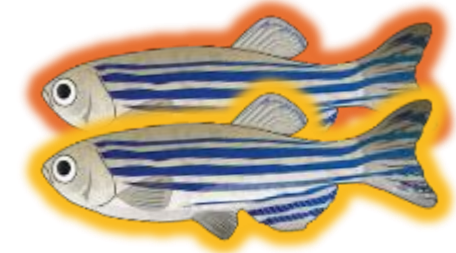
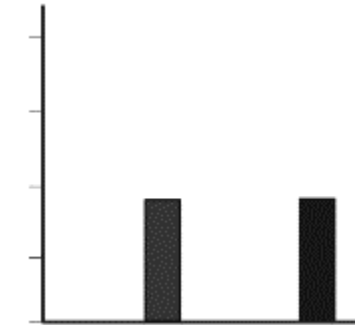
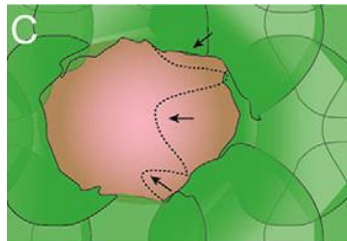
# AIM 2a: collect epithelial cells before and after wounding for RNA-seq



unwounded

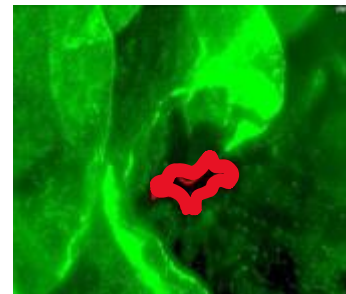
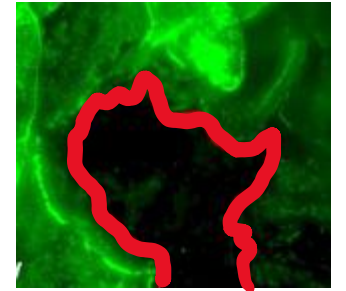
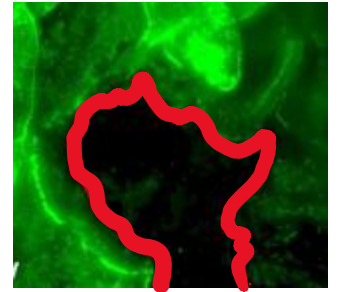
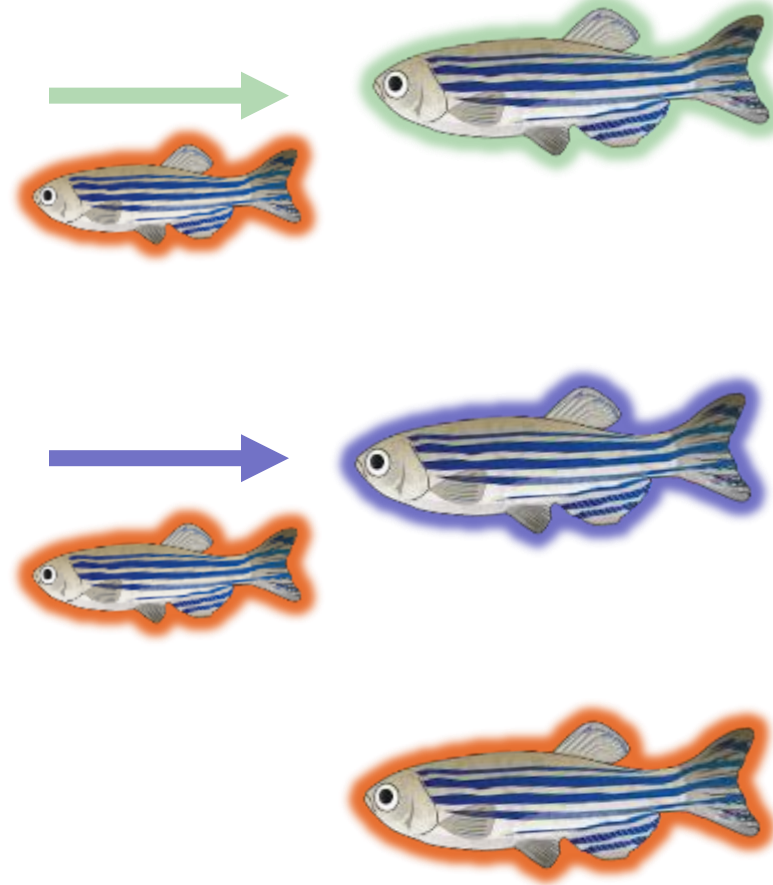
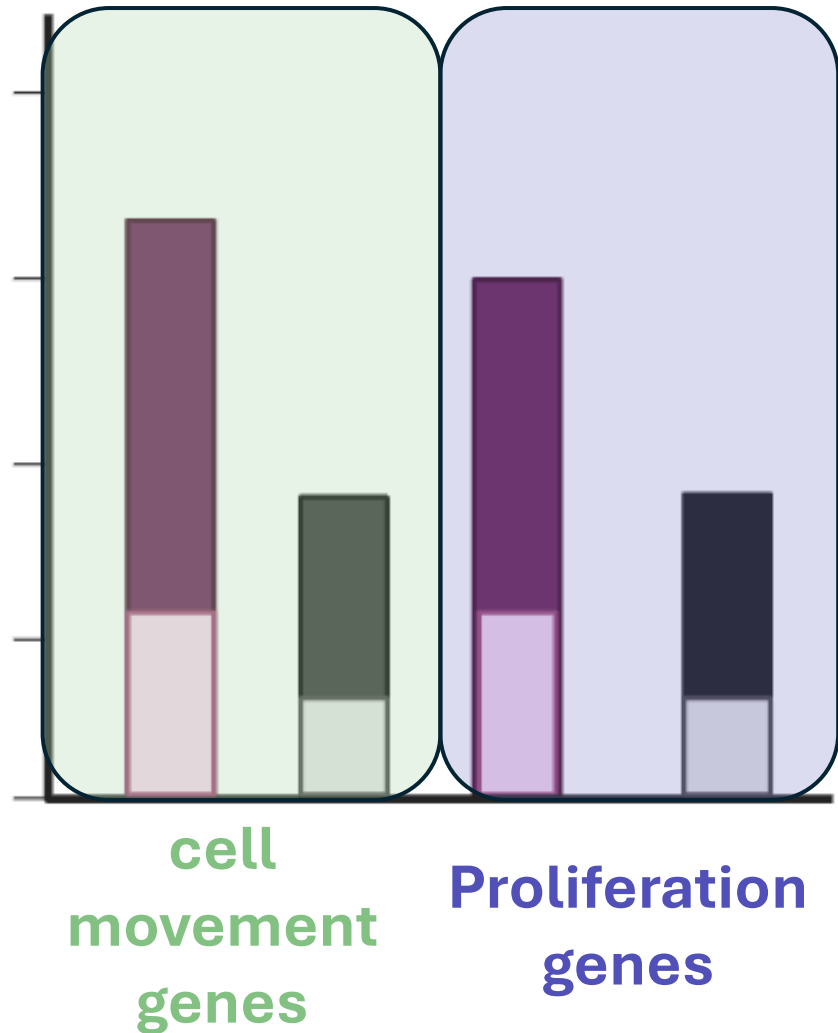


wounded



**AIM 2b:** use GO to sort differentially expressed genes and validate using CRISPR/Cas9 + wounding assay

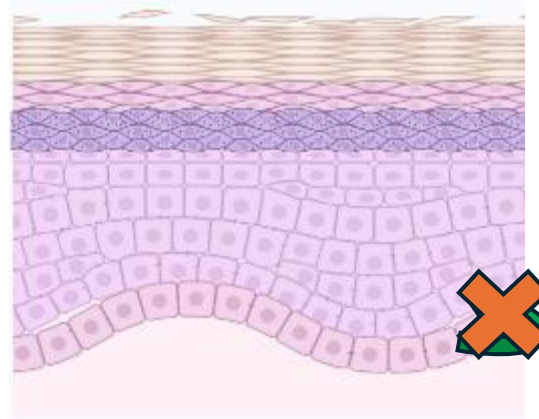
**FOXN1<sup>-/-</sup>**  
**WT**



**AIM 3:** Identify proteins important for epithelial development that cause faster re-epithelialization during wound healing



**Hypothesis:** FOXP1 mutant epithelial cells will show decreased levels of proteins associated with **differentiation** throughout **development**

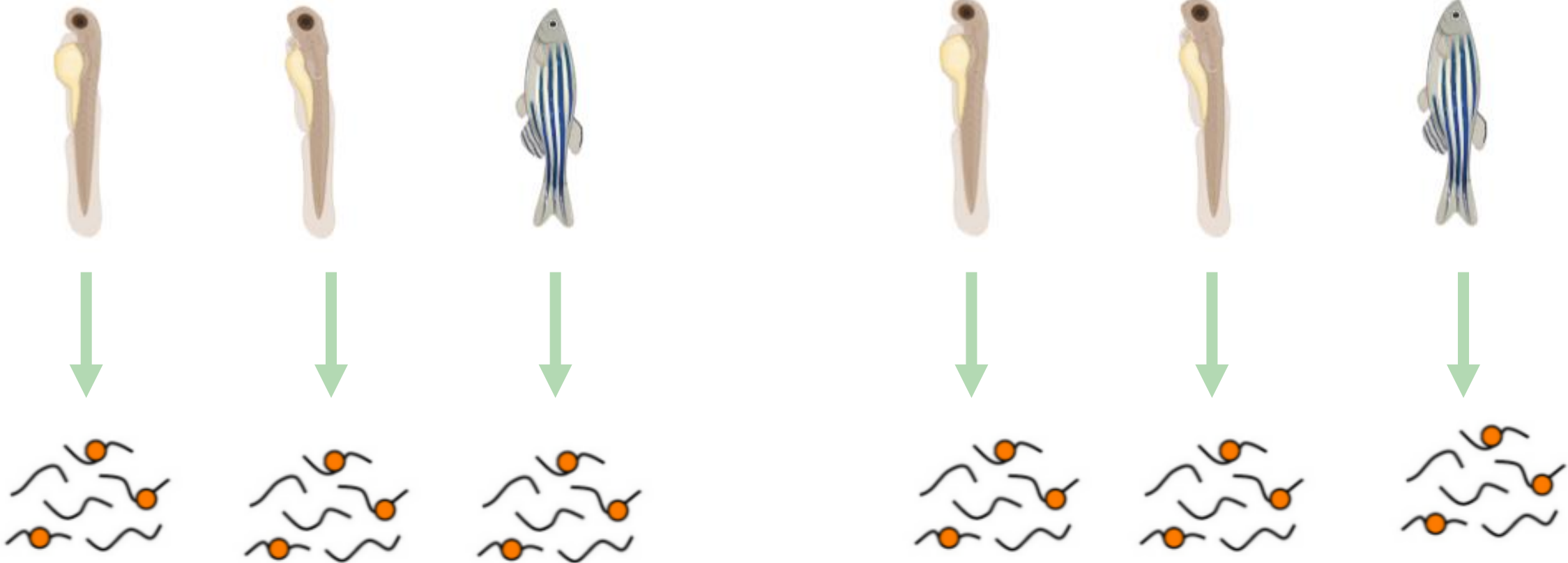


**Rational:** Proteins important for epithelial development may be important for wound healing as well

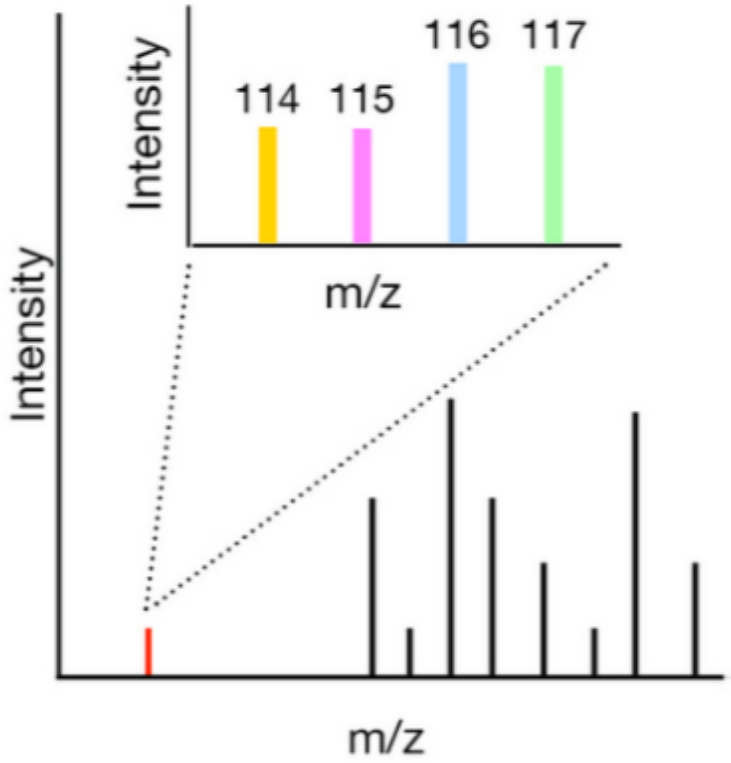
**AIM 3a: Isolate proteins** from epithelial samples from WT and mutant zebrafish at different stages of development for **labeling**

**WT**

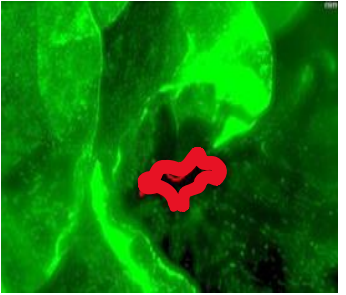
**FOXN1<sup>-/-</sup>**



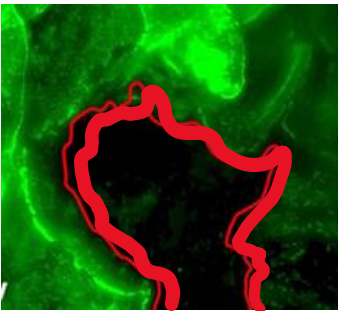
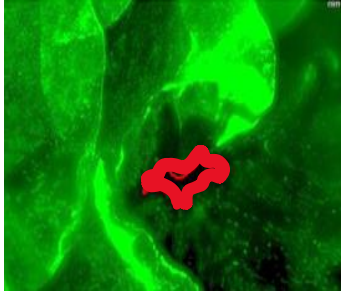
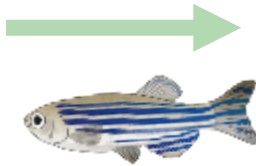
# AIM 3b: Analyze differential protein abundances using mass spec and validate using CRISPR/Cas9



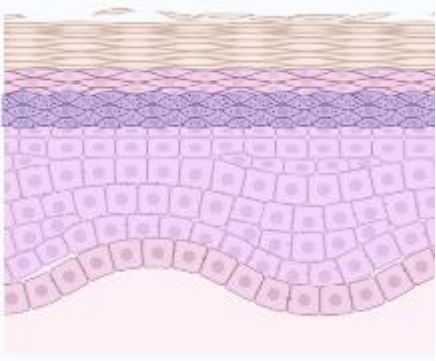
Quantify  
iTRAQ reporter ions



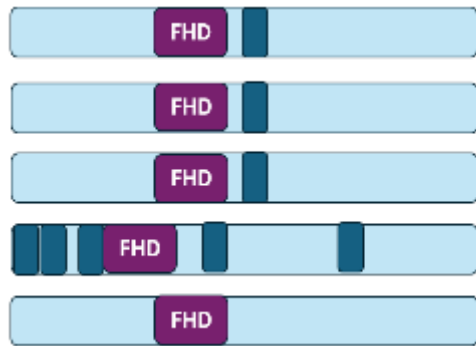
Identify  
MS/MS fragmentation



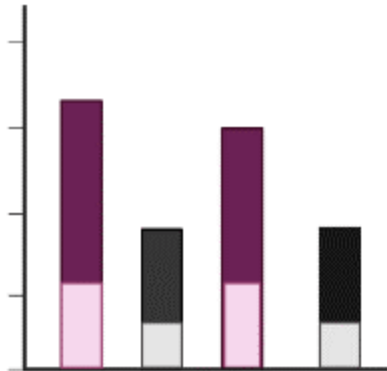
# SUMMARY



**FOXN1 is an important gene for epithelial development**

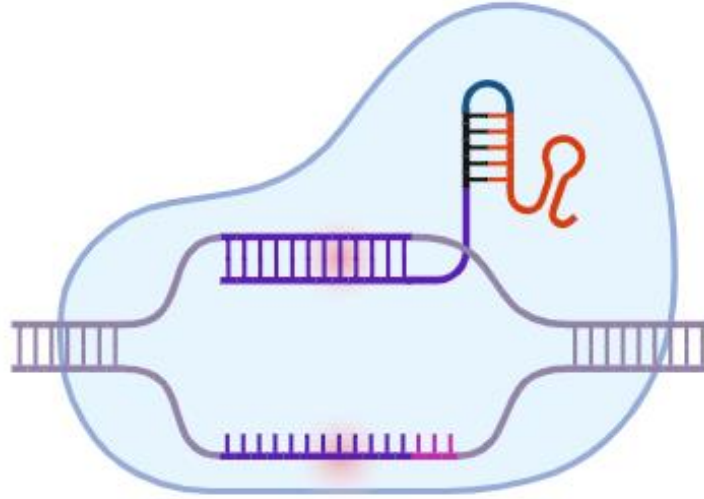


**FOXN1 is well conserved across model organisms with a common Forkhead Box Domain important for DNA binding**



**Genomic and proteomic techniques can be used to elucidate the role of FOXN1 in wound healing**

# Future Directions



Utilize CRISPRa to identify if it is possible to rescue epithelial differentiation in mutant FOZN1 zebrafish, and measure rescued expression levels using RNA-seq

# Sources

[\(PDF\) A Novel FOXP1 Variant Is Identified in Two Siblings with Nude Severe Combined Immunodeficiency \(researchgate.net\)](#)

[Genetic interplays between Msx2 and Foxp1 are required for Notch1 expression and hair shaft differentiation – ScienceDirect](#)

[Regulatory T cells in skin regeneration and wound healing | Military Medical Research | Full Text \(biomedcentral.com\)](#)

[Foxp1 in Skin Development, Homeostasis and Wound Healing - PMC \(nih.gov\)](#)

[Impairment of the Hif-1 \$\alpha\$  regulatory pathway in Foxp1-deficient \(Foxp1 \$^{-/-}\$ \) mice affects the skin wound healing process - PubMed \(nih.gov\)](#)

[Adult Zebrafish as a Model System for Cutaneous Wound-Healing Research – ScienceDirect](#)

[FOXP1 Transcription Factor in Epithelial Growth and Wound Healing: Molecular and Cellular Biology: Vol 37, No 17 \(tandfonline.com\) content \(mpg.de\)](#)

[RNA-Seq Expression Analysis: 5 Dirty Secrets - Genevia Technologies](#)

[Informatics for RNA Sequencing: A Web Resource for Analysis on the Cloud | PLOS Computational Biology](#)

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