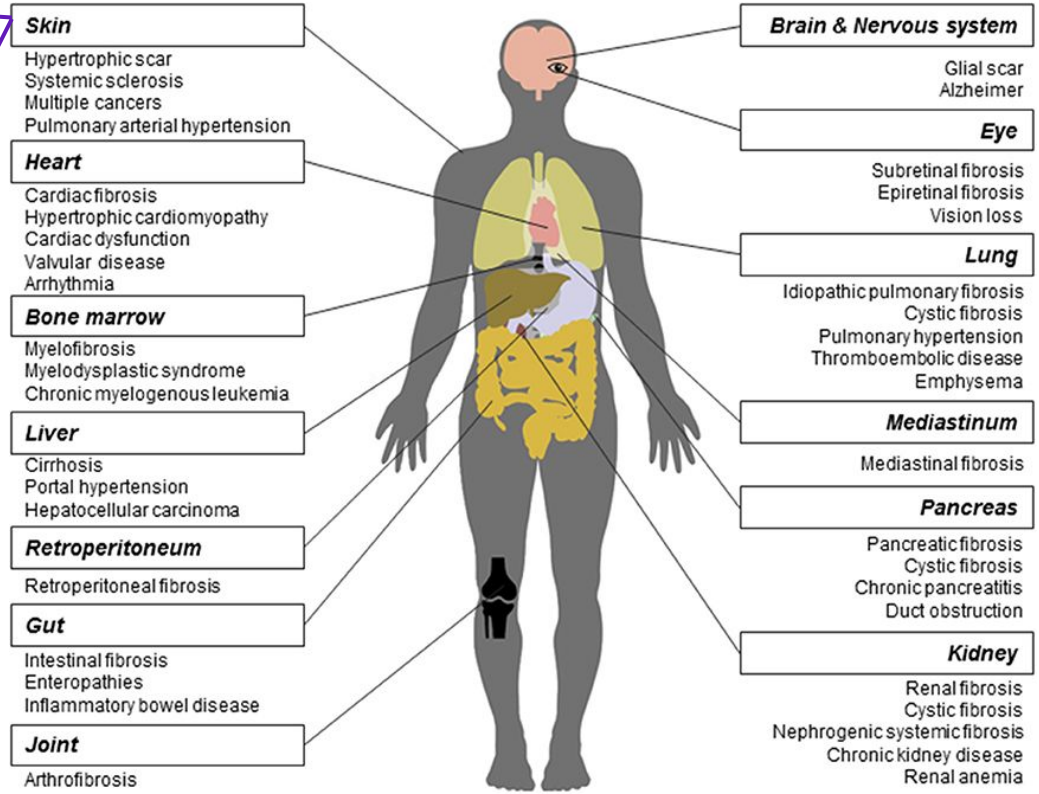
COLLEGE OF ARTS AND SCIENCES
CASE WESTERN RESERVE UNIVERSITY

Arnold and Mabel BECKMAN FOUNDATION

DPP-IV: A mediator for all things fibrosis

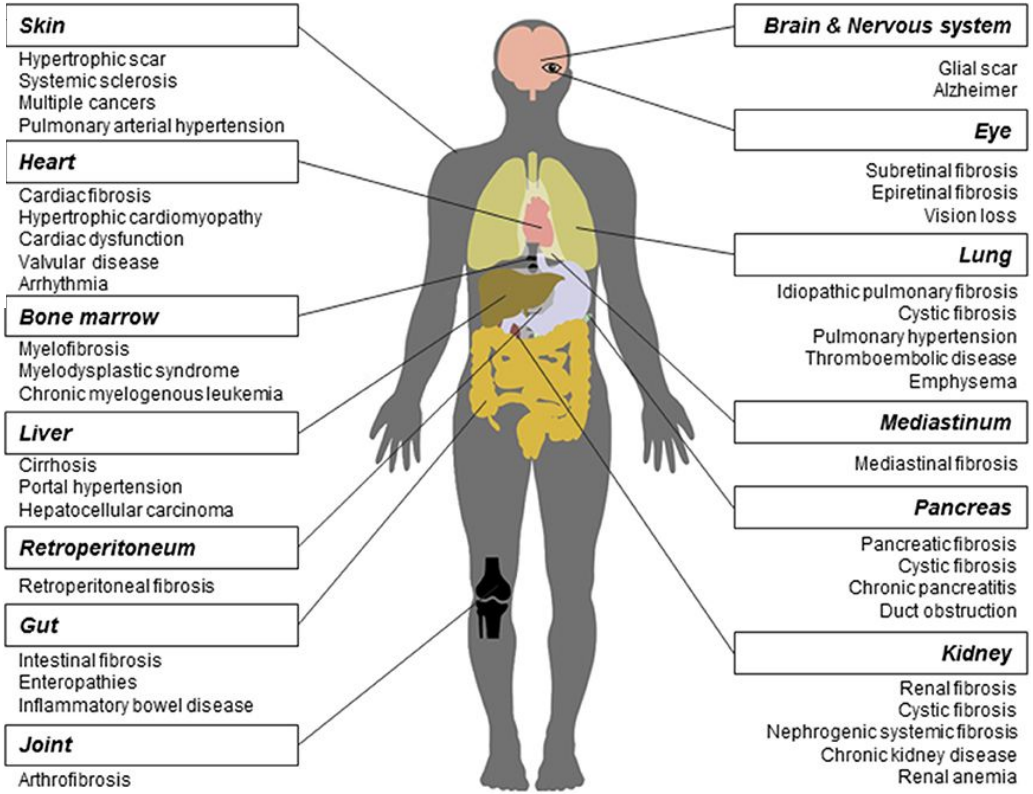
Sakin Kirti
Dr. Radhika Atit Lab

Why study fibrosis?



Li, Drugs and Targets in

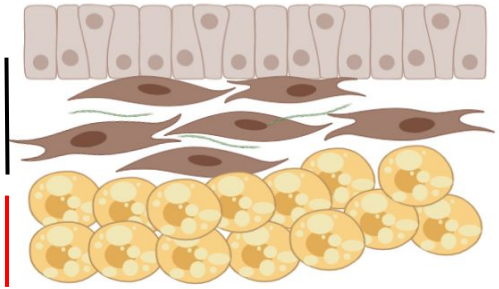
Why study fibrosis?



Li, Drugs and Targets in

The layers of the skin

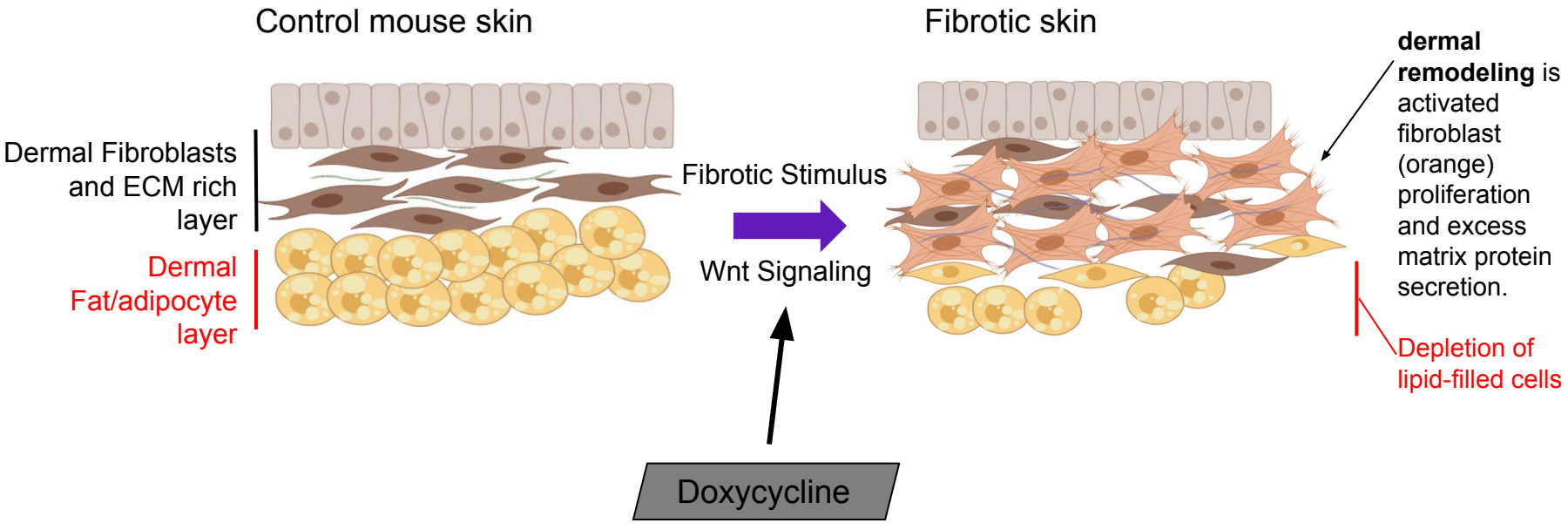
Control mouse skin



Dermal Fibroblasts and ECM rich layer

Dermal Fat/adipocyte layer (DWAT)

What is going on in fibrosis?



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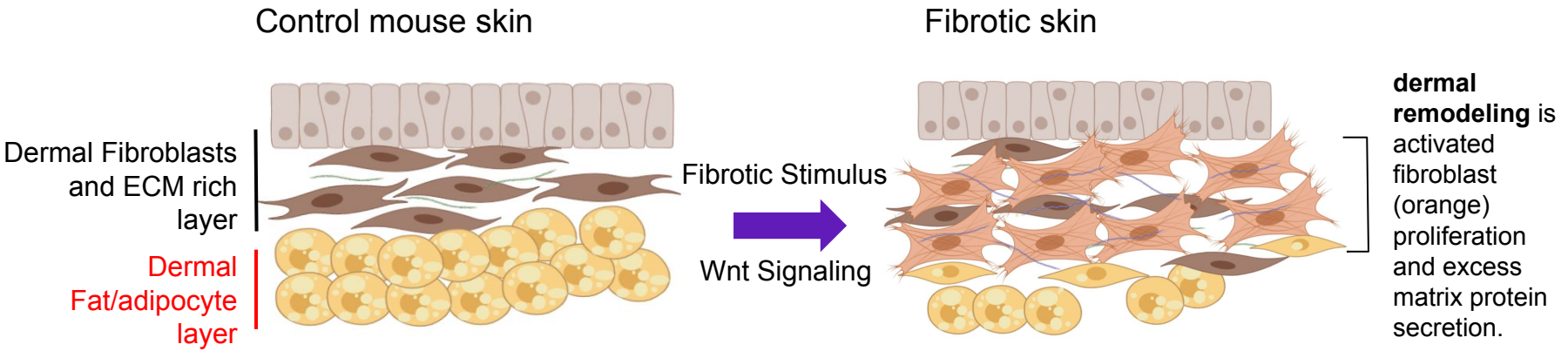
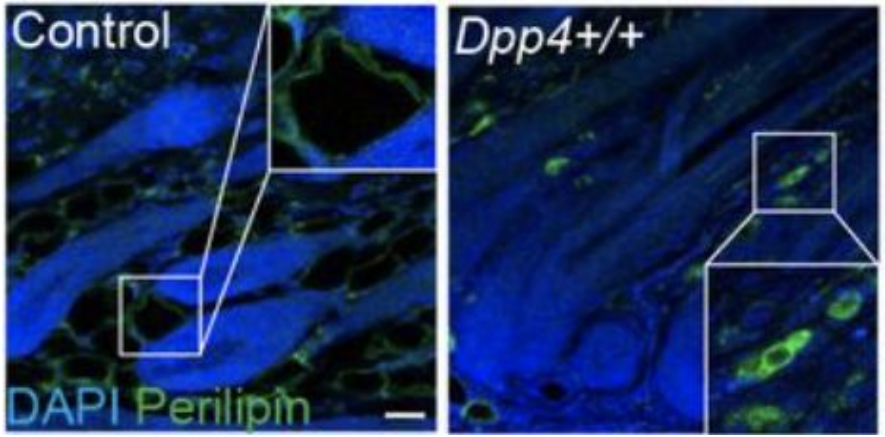
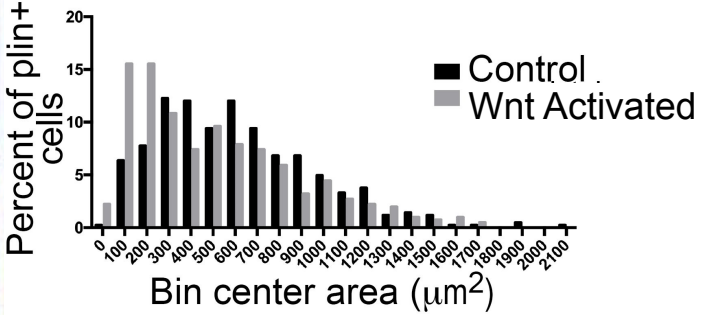


Before jumping into mediators...

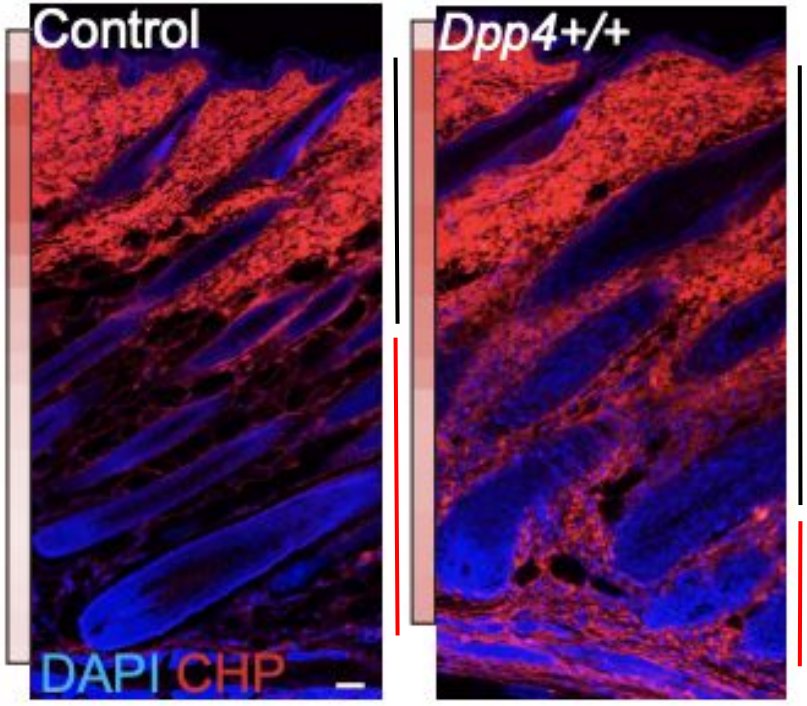
1. Does collagen production increase during fibrosis?
2. Does the quality of collagen change during fibrosis?

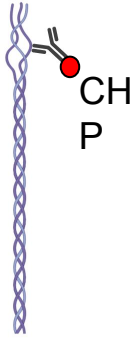
How is fat lost in fibrosis?



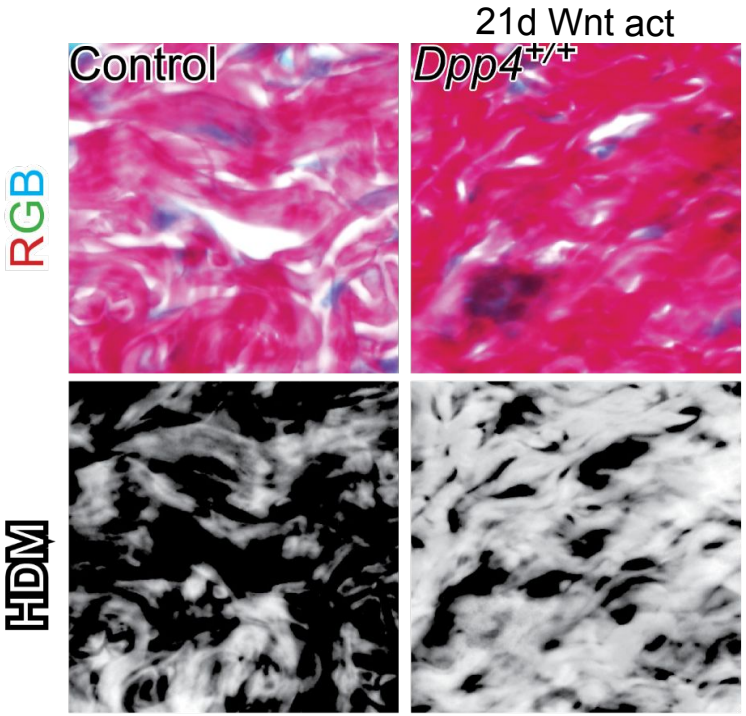


Wnt activation elevates collagen remodeling in the DWAT layer





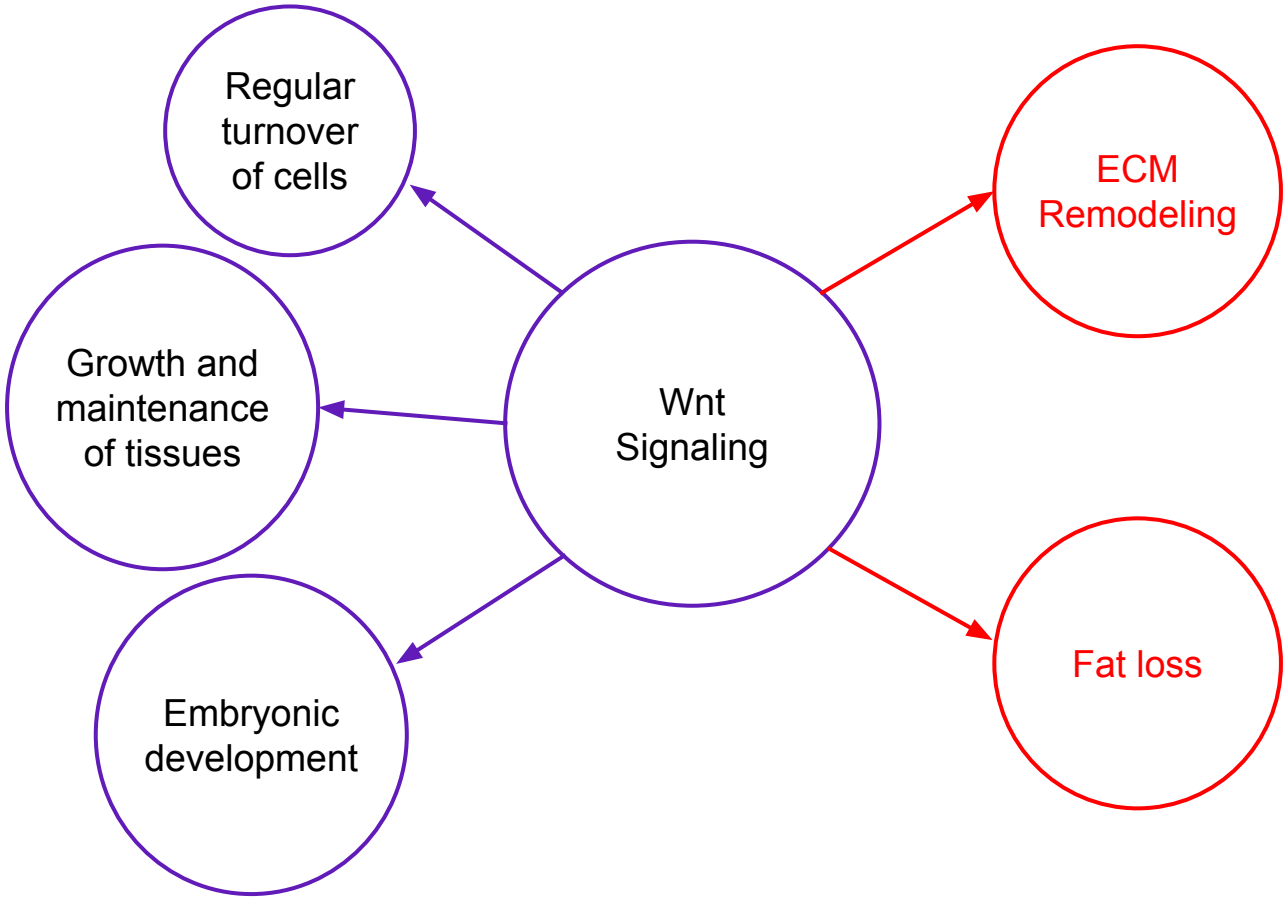
Wnt activation changes collagen quality



Back to scarring...

- Excess collagen production
- Denser collagen

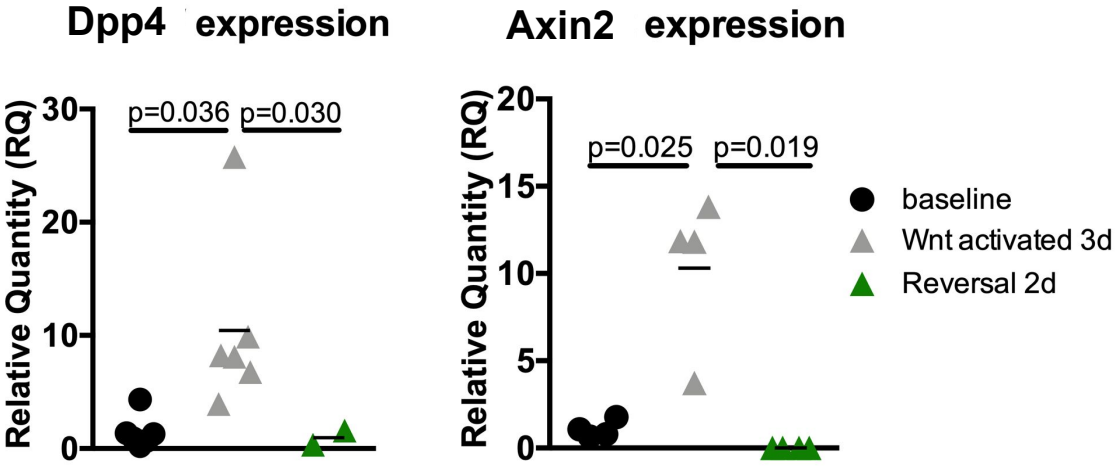
Wnt signaling leads to fibrosis… and other things



Dpp4 - a possible mediator

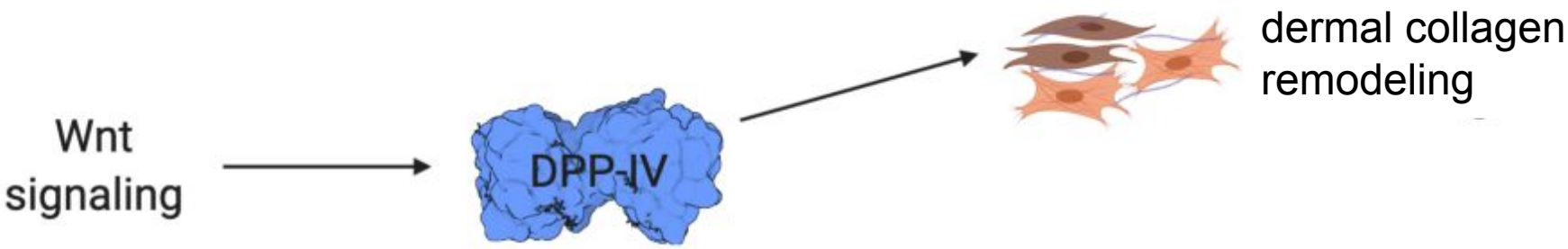
- Upregulated in dermal mouse fibrosis
- Activation of fibroblasts
- Fat biology of other organs

Dpp4 mRNA expression is a Wnt-signaling responsive



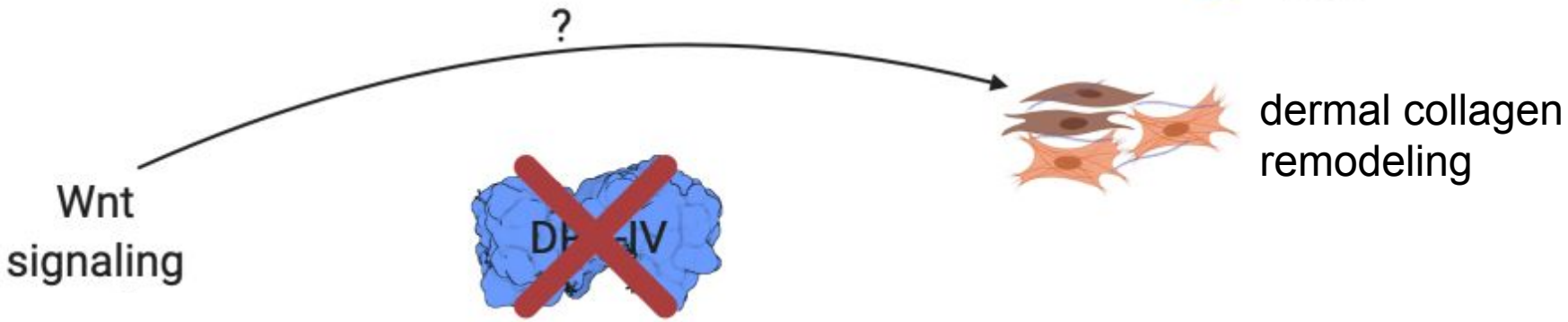
Results from Anna in the Atit

Hypothesis:



I hypothesize that Wnt signaling/DPP-IV promotes dermal remodeling and collagen quality changes in skin fibrosis.

How will I test my hypothesis?

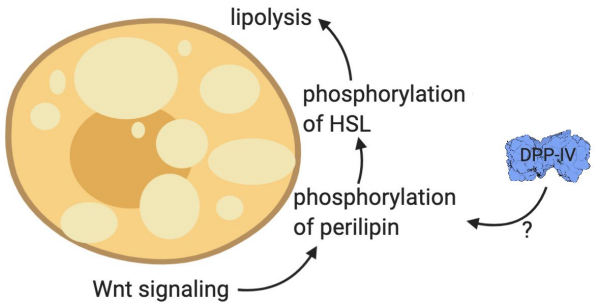


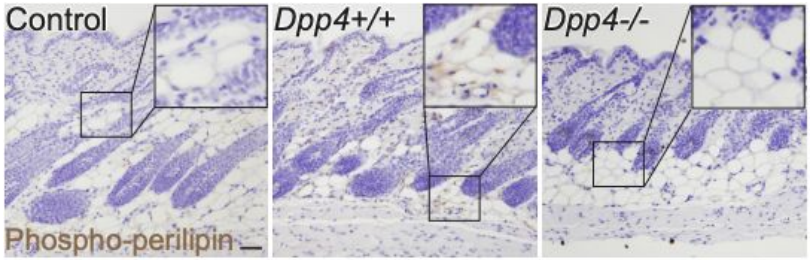
Aim 1: How does DPP-IV affect lipolysis in fibrosis?

Does DPP-IV mediate lipolysis directly?

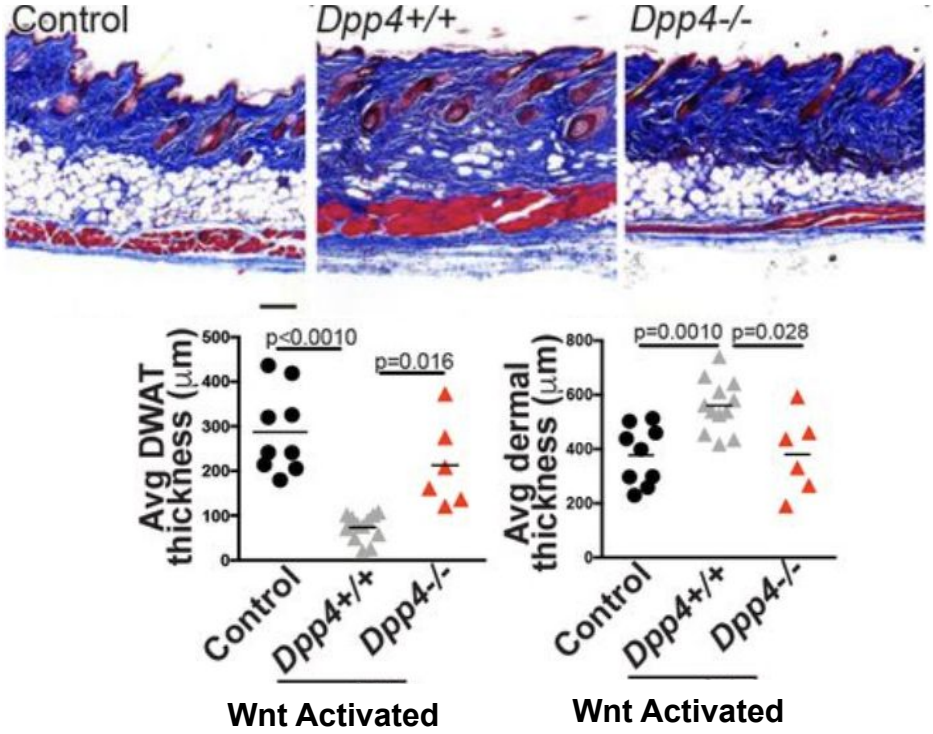
-p-perilipin, p-HSL IF

Ind. var	Dep. var	Time pt.
Presence of *Dpp4*	Presence of… -p-plin	5 days

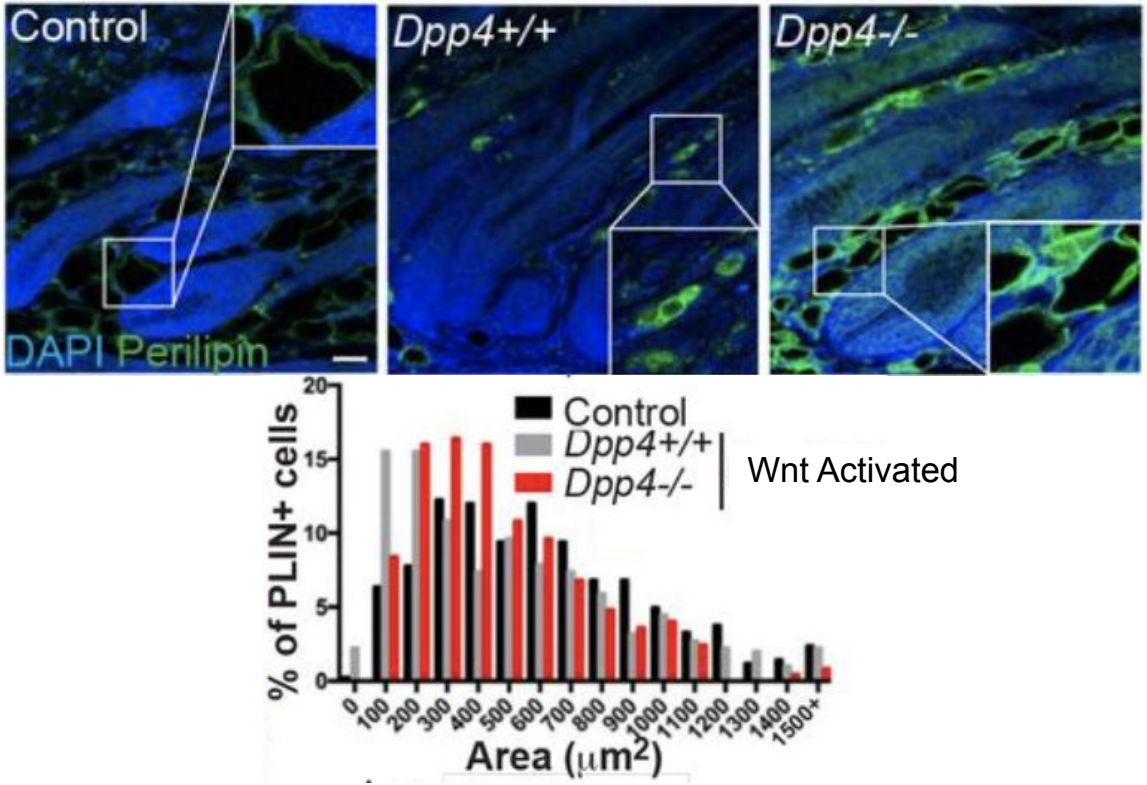




DPP-IV presence required for fibrosis

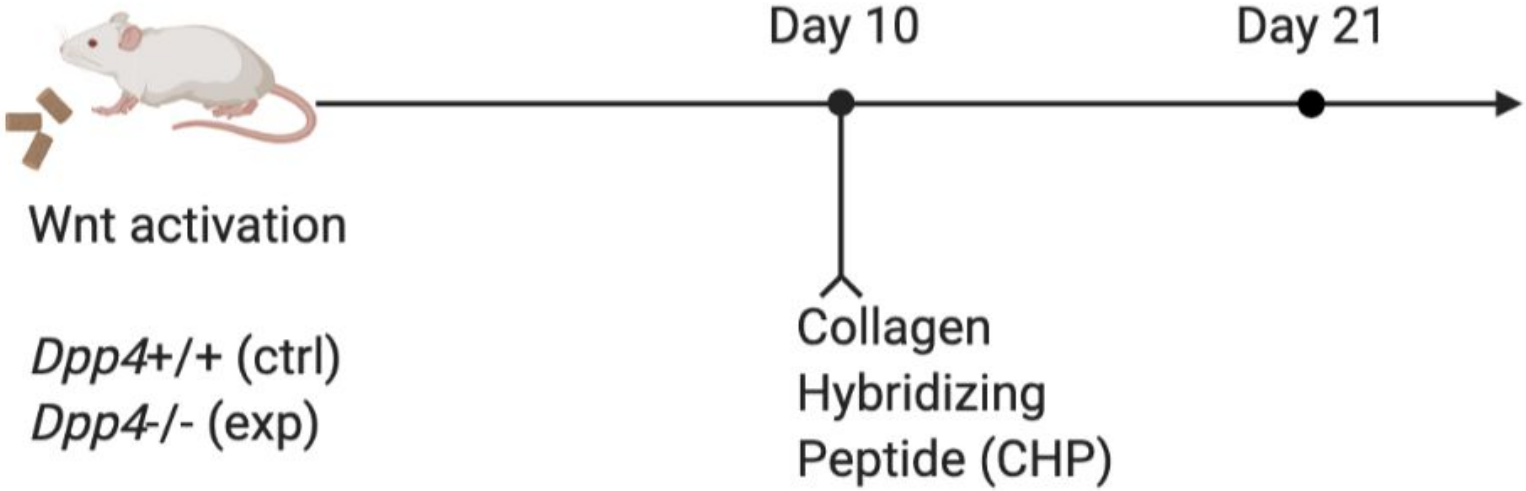


Aim 1: DPP-IV presence leads to fibrotic fat loss

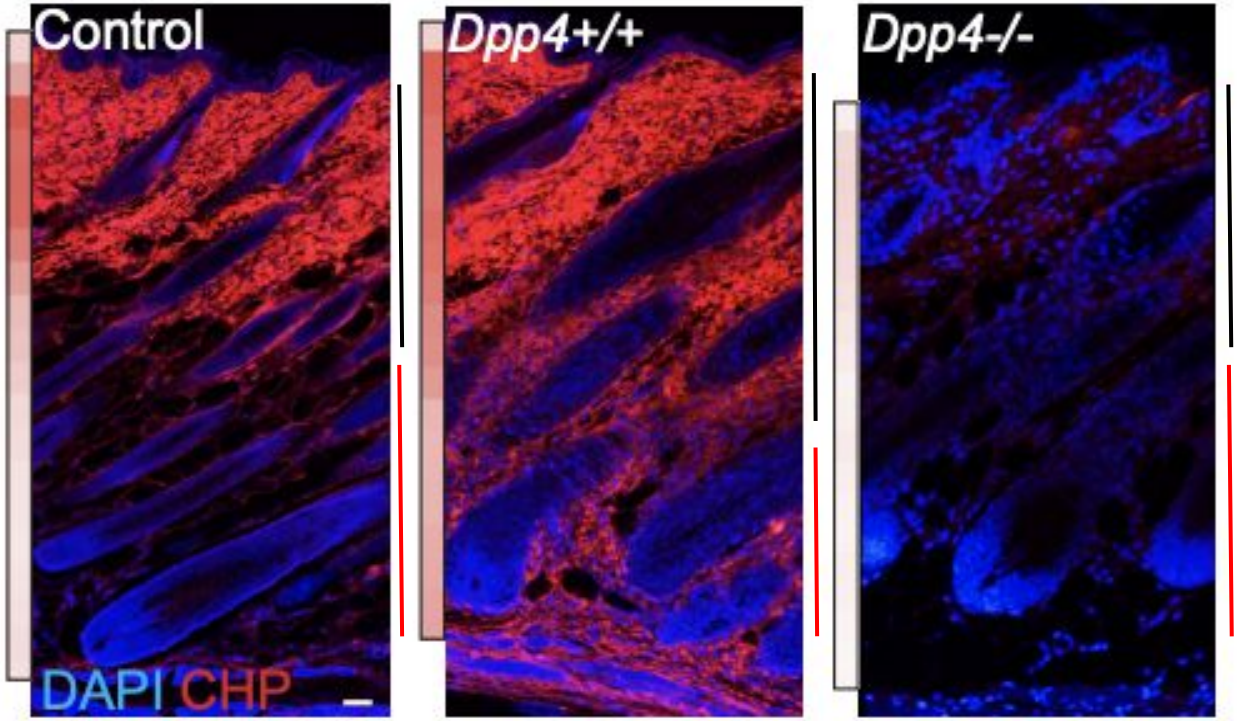


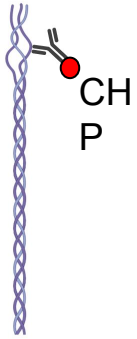
Experimental Design: Overview of Aim 2

Dermal Collagen Remodeling Measurements:

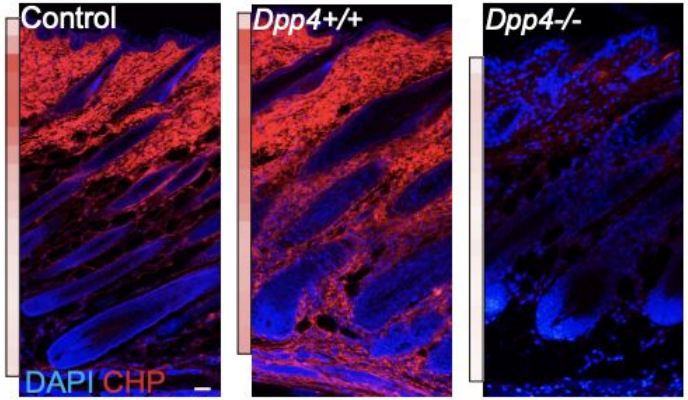


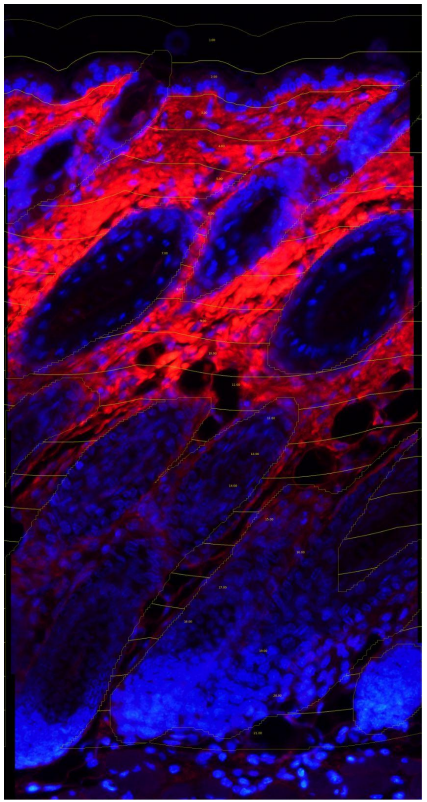
How does DPP-IV impact ECM protein production?





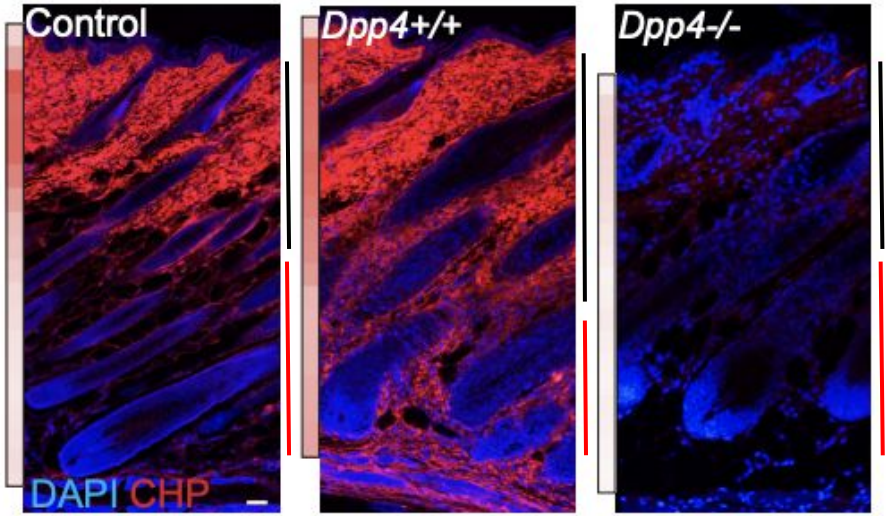
Quantification of Collagen Remodelling Assay

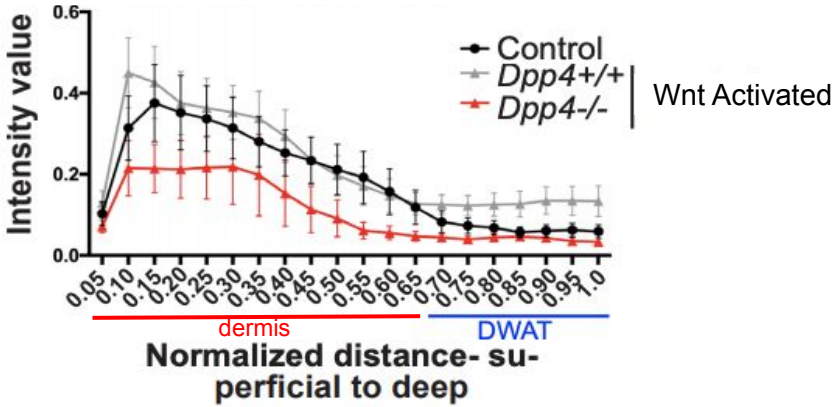




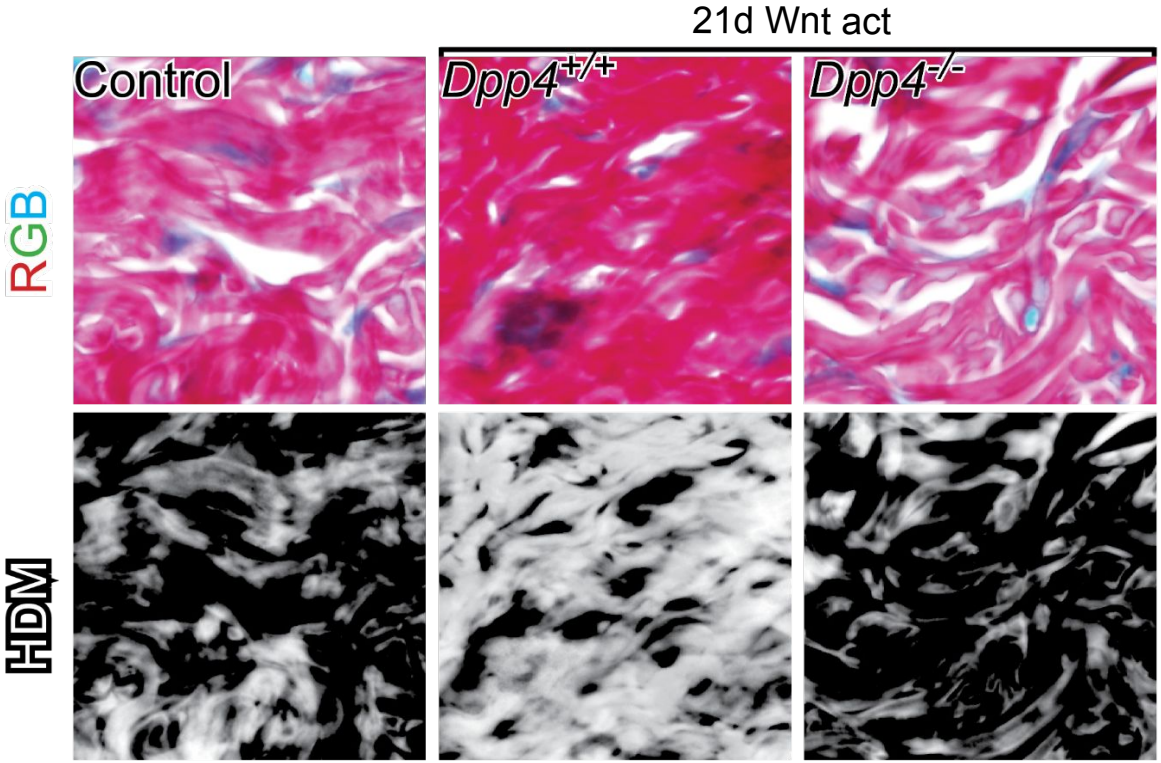
$$\frac{\text{Intensity Measurements}}{\text{Area in each layer}}$$

How does DPP-IV impact ECM protein production?

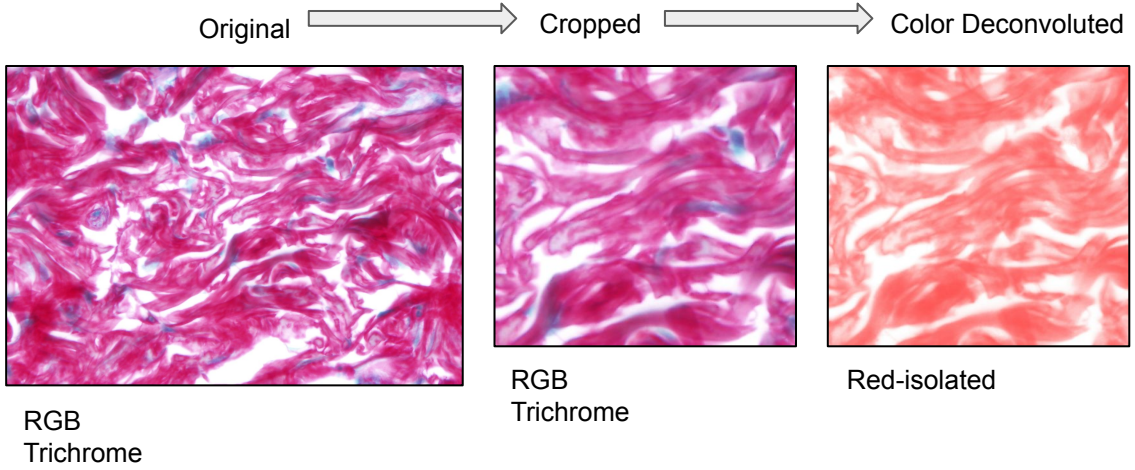




How does DPP-IV impact collagen matrix changes?



Quantification using TWOMBLI



Area (micror	Lacunarity	Total Length	Endpoints	HGU (micror	Branchpoints	Box-Counting	Curvature_40	% High Dens	Alignment	TotalImageArea
14405	13.335	6810	152	44.803	166	1.377	41.353	0.762	0.08248	220900
12255	16.025	6023	148	40.696	83	1.352	49.246	0.885	0.05131	220900
17508	10.617	8054	149	54.054	223	1.432	36.72	0.736	0.1638	220900
19495	9.331	8760	140	62.571	209	1.455	41.838	0.654	0.2461	220900

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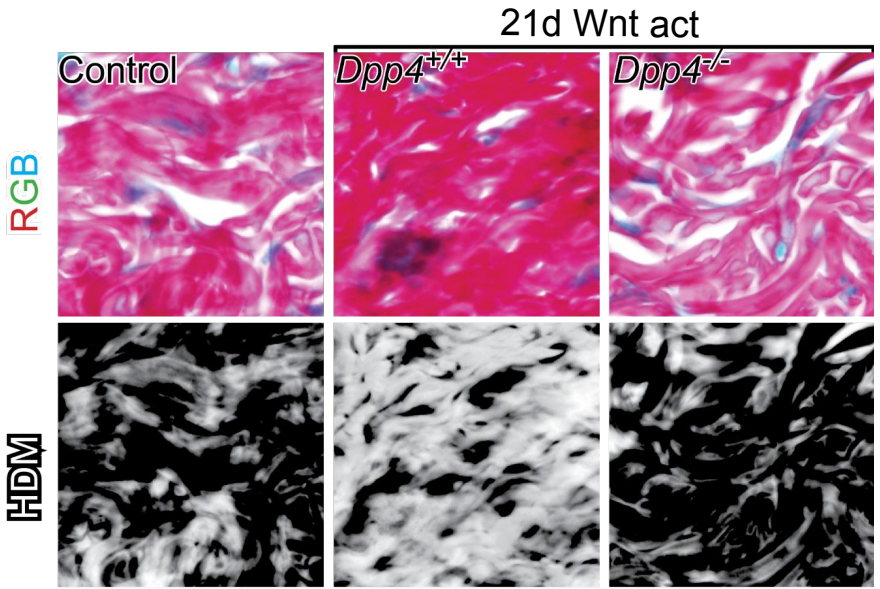
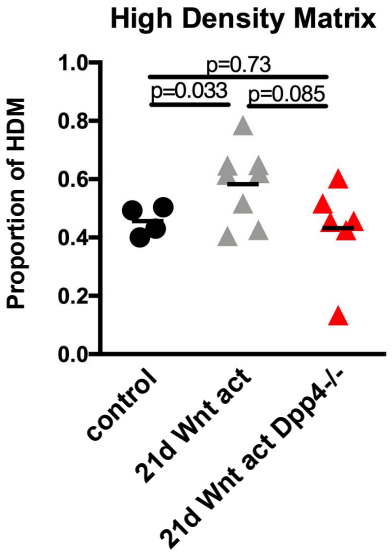
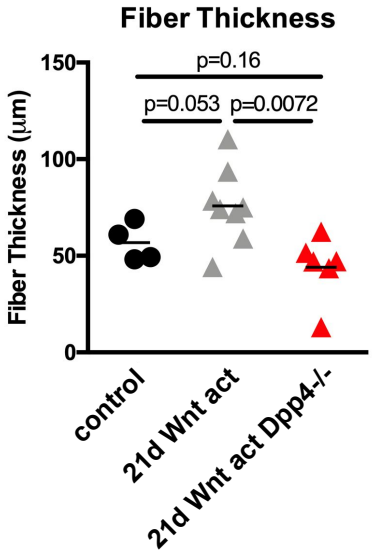




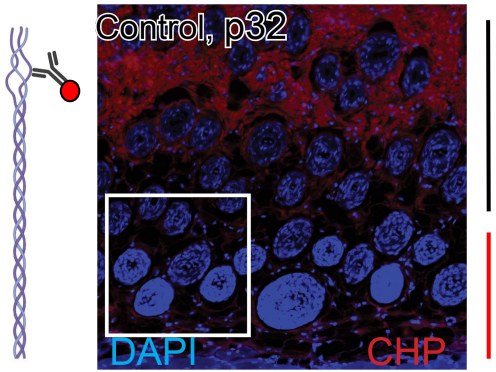


Aim 2: How does DPP-IV affect fibroblast activation?

Does DPP-IV affect the remodeling of collagen?

-collagen hybridizing peptide (CHP)

Ind. var	Dep. var	Time pt.
Presence of *Dpp4*	Presence of CHP	10 days



Does DPP-IV make fibroblasts multiply?

-EdU proliferation assay

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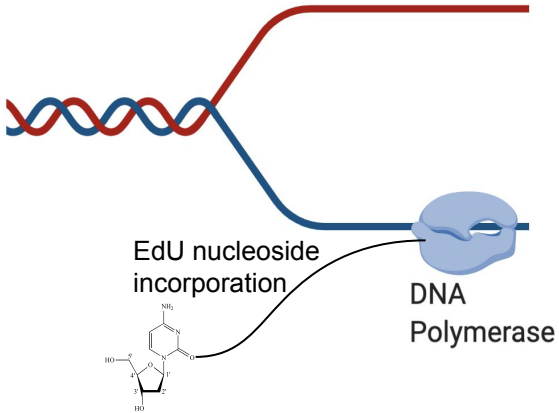

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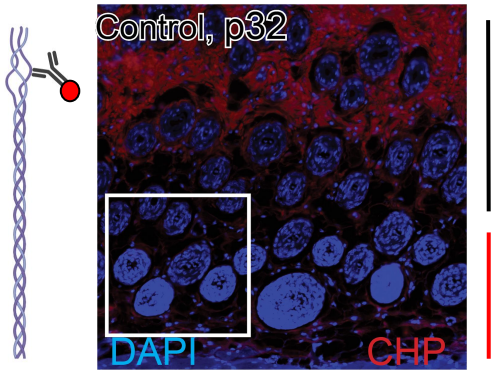

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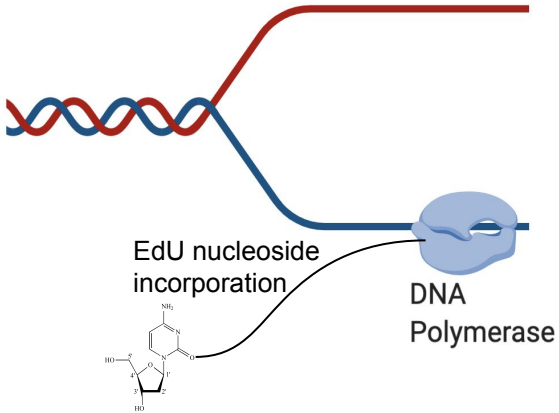
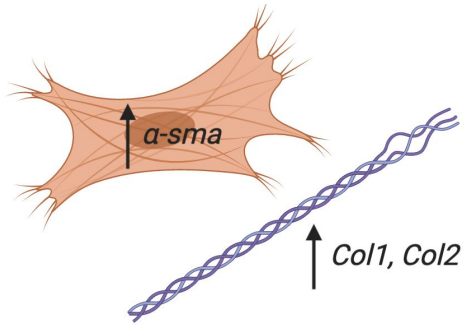


Does DPP-IV affect production of fibrotic genes?

-fibrotic gene qPCR: *Col1, Col2, α-sma*

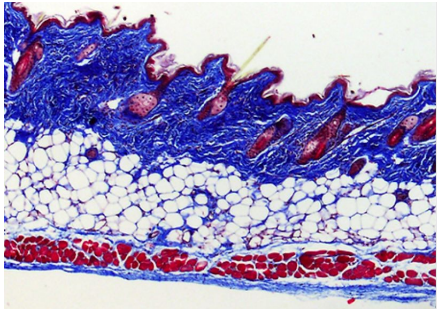
Ind. var	Dep. var	Time pt.
Presence of *Dpp4*	Relative DNA quantity	10 days



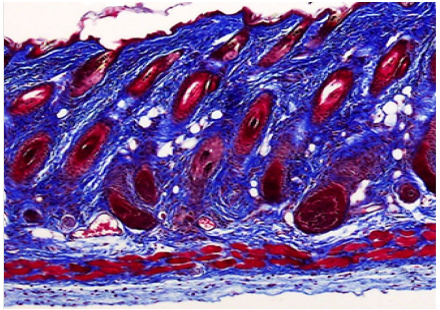
To summarize...

Dermal Fibroblasts and ECM rich layer

Dermal Fat/adipocyte layer



DPP-IV



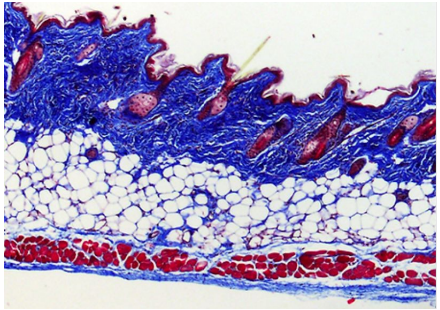
Expansion of Matrix (blue) layer

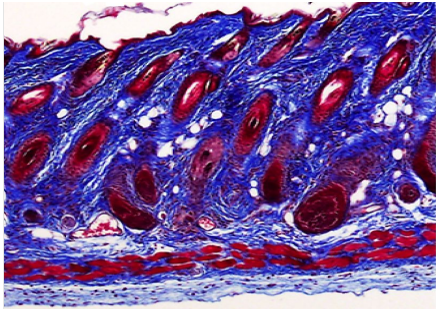
Loss of dermal fat

To summarize...

Dermal Fibroblasts and ECM rich layer

Dermal Fat/adipocyte layer





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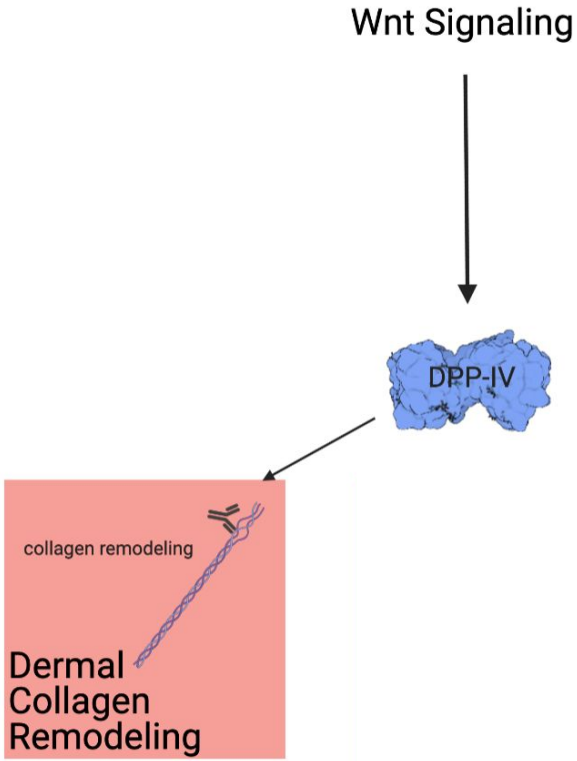
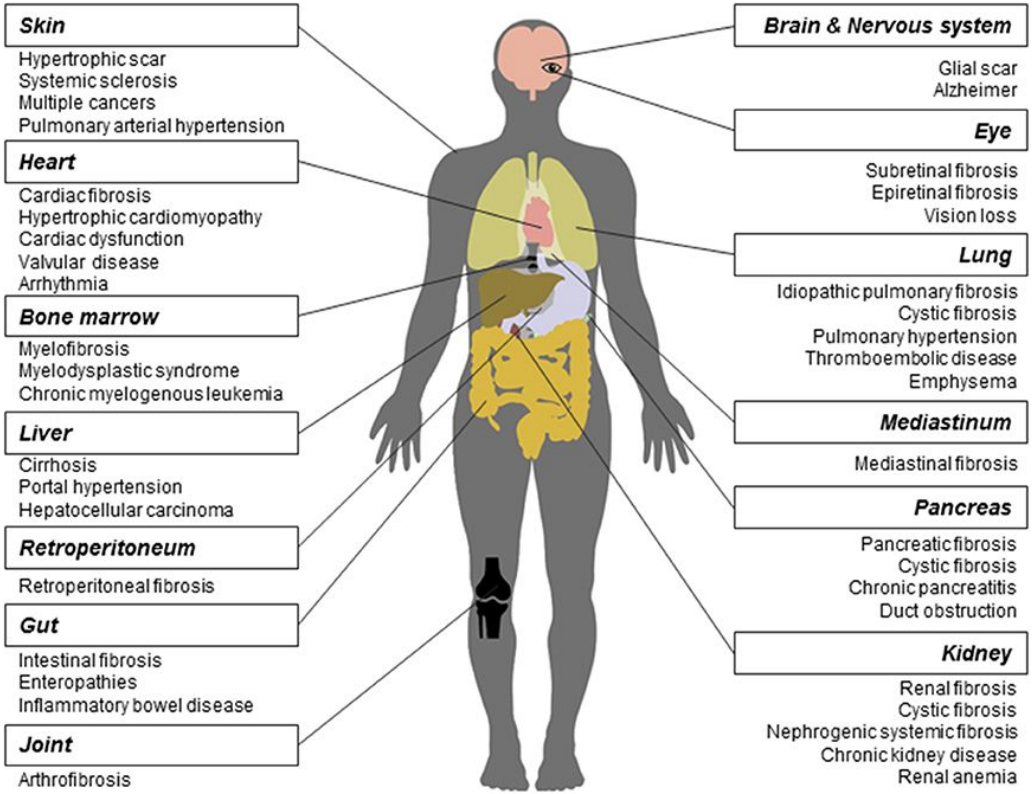
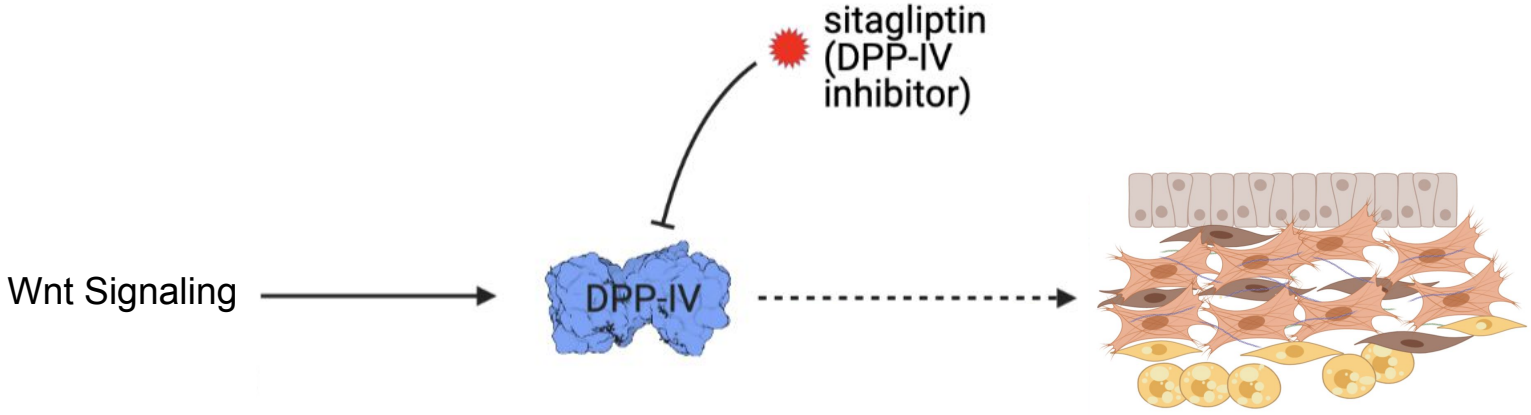


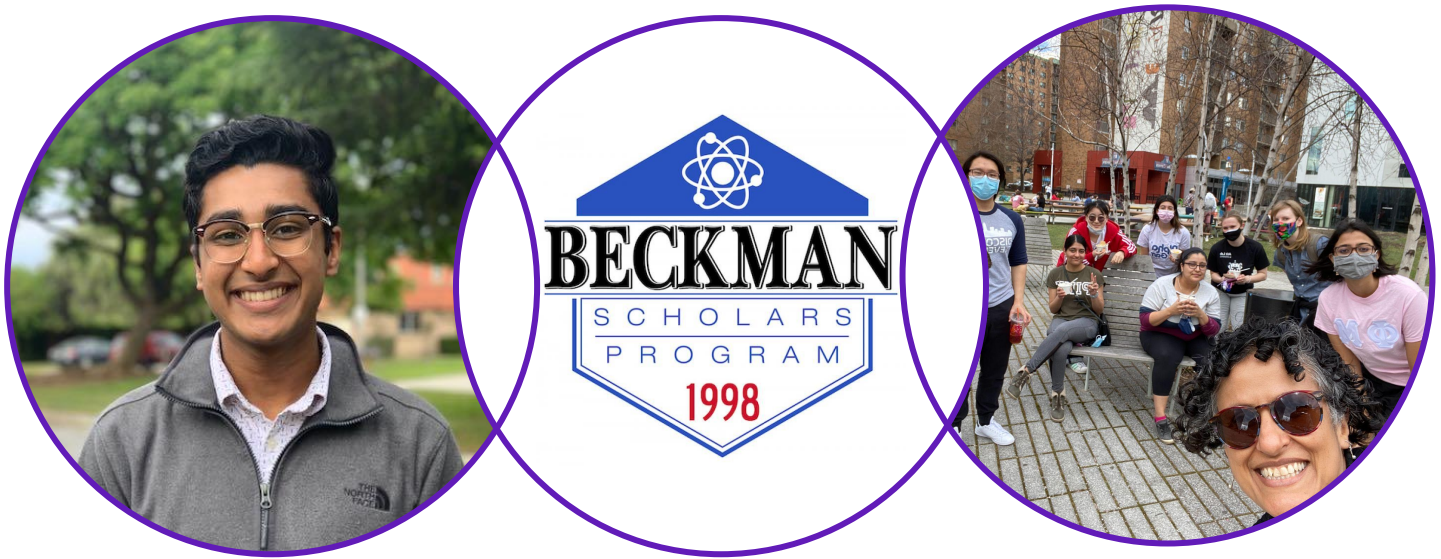
In the grand scheme of things...



Impact and future direction



Acknowledgments to the Atit Lab and the Beckman Program



Beckman Scholars Program (SK), NIH-NIAMS: R01 (RA), NIAMS T32 Orthopedics Training Grant and Dermatology

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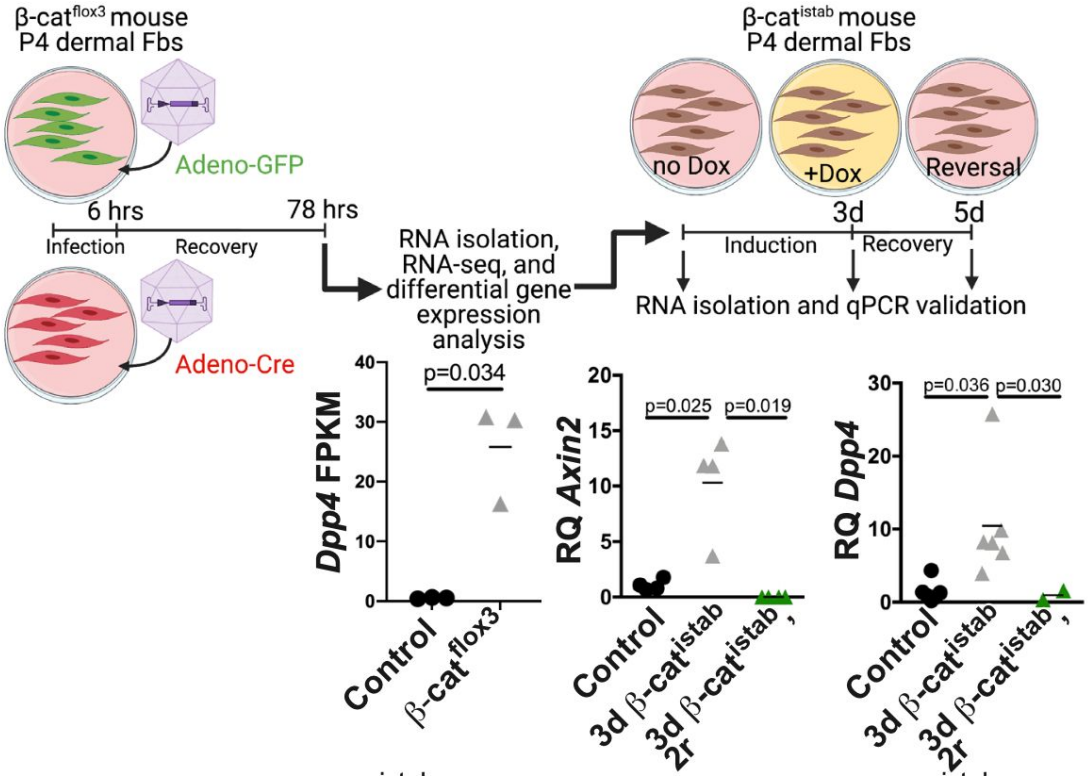
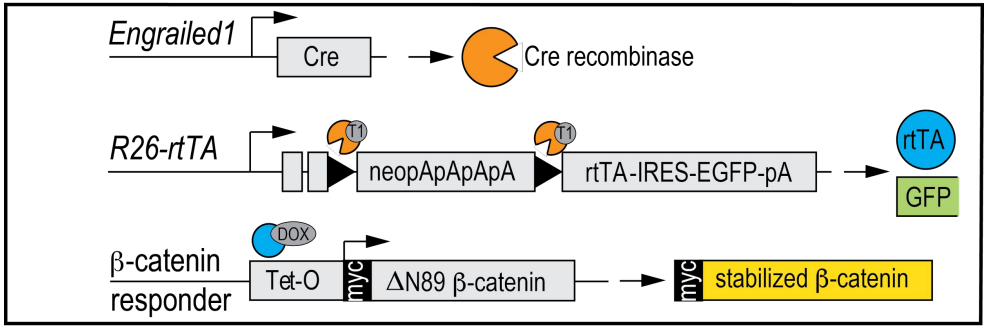
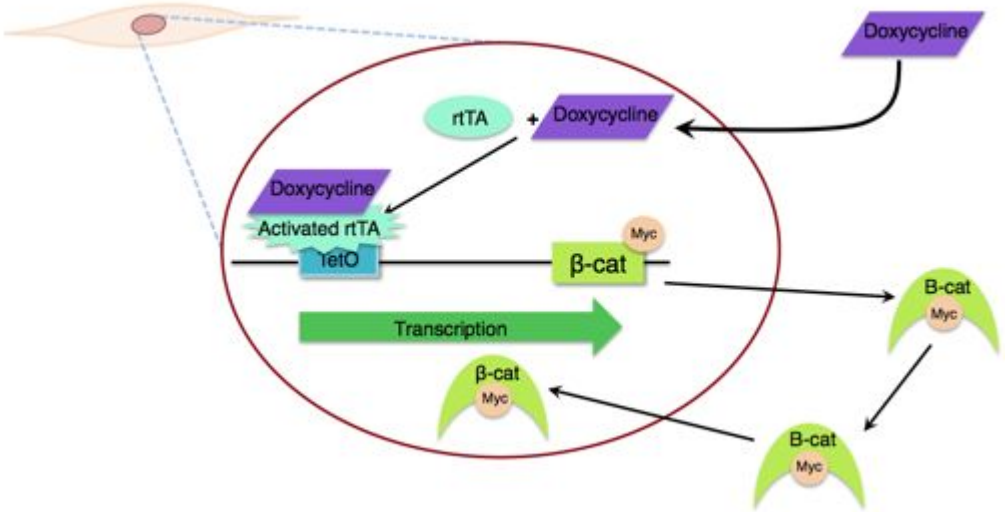
DPP-IV is upregulated in mouse fibroblasts



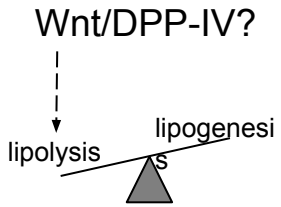
Mouse Model



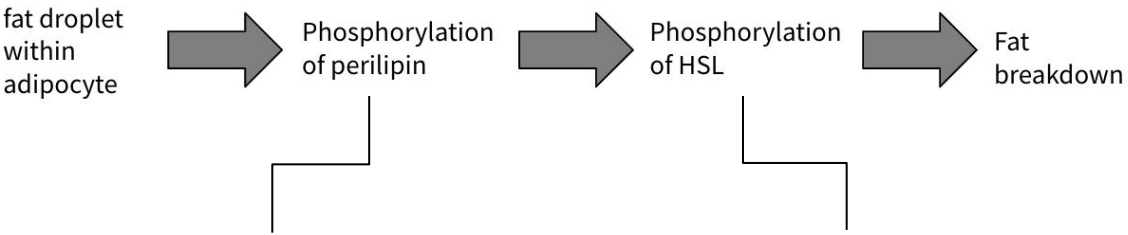
Gives us spatial and temporal resolution to study the onset of fibrosis and also what happens to existing fibrosis in the absence of sustained Wnt activation, key to therapy



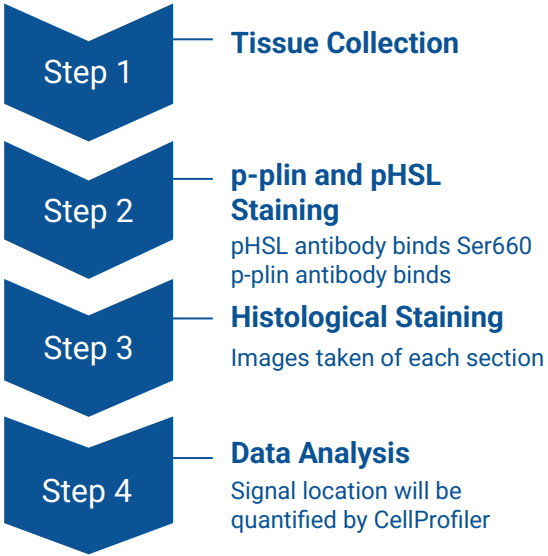
Experimental Design: Does DPP-IV mediate lipolysis directly?



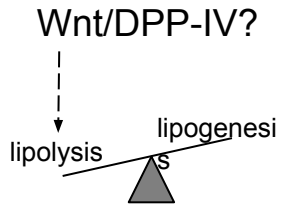
Measurements of Lipolysis



If DPP-IV causes lipolysis, genetic deletion should rescue it	
↓ p-plin, ↓ pHSL (Dpp4-/-) DPP-IV acts on p-plin and/or pHSL in lipolysis	Similar p-plin, pHSL (Dpp4+/+) DPP-IV does not act on p-plin or pHSL



Experimental Design: DPP-IV - fewer adipocytes or smaller adipocytes?



Perilipin Immunofluorescent Staining

1

Control Group	Experimental Group	Independent Variable	Dependent Variable	Time Point
Dpp4+/+ Wnt activated	Dpp4-/- Wnt activated	Presence of Dpp4?	fat response	10 days

if Dpp4 affects how lipid is lost (loss of fat content within adipocytes)

2

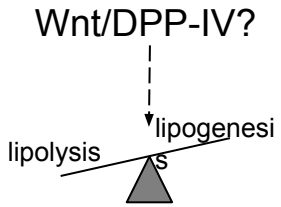
Step 1 — **Tissue Collection**

Step 2 — **Perilipin Antibody Staining**
Primary:
Secondary:

Step 3 — **Data Collection**
Images taken of each section

Step 4 — **Data Analysis**
CellProfiler marks area at which perilipin is present

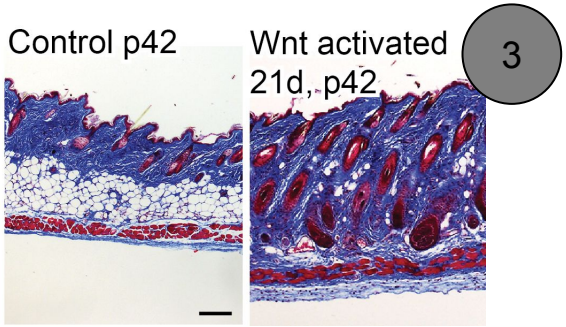
Experimental Design: Does DPP-IV mediate fat depletion in adipocytes?



Adipocyte Number and Area

1

Control Group	Experimental Group	Independent Variable	Dependent Variable	Time Point
Dpp4+/+ Wnt activated	Dpp4-/- Wnt activated	Presence of Dpp4	fat response	21 days



3

2

Step 1 — **Tissue Collection**

Step 2 — **Hematoxylin and Eosin Staining**

Step 3 — **Data Collection**
Adipocyte numbers counted and adipocyte area calculated

Step 4 — **Data Analysis**
Compare number of adipocytes present to area that adipocytes take up

Experimental Design: Does DPP-IV affect the remodeling of collagen?

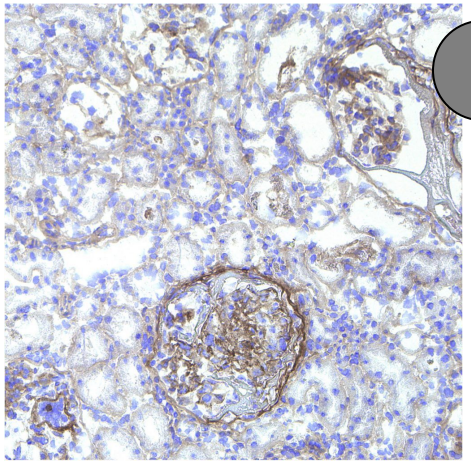
Fb activation:
1. Collagen remodeling
2. Proliferation
3. Fibrotic gene expression

Wnt/DPP-IV
?

Collagen Remodeling

1

Control Group	Experimental Group	Independent Variable	Dependent Variable	Time Point
Dpp4+/+ Wnt activated	Dpp4-/- Wnt activated	Presence of Dpp4?	Dermal response	10 days



3

2

- Step 1 — **Tissue Collection**
- Step 2 — **bCHP Staining**
  Stain with bCHP
- Step 3 — **Data Collection**
  Fluorescent images will be taken
- Step 4 — **Data Analysis**
  CellProfiler marks intensity of bCHP signal

3helix, Collagen Hybridizing Peptide, Biotin Conjugate

Experimental Design: Does DPP-IV make fibroblasts multiply?

Fb activation:
1. Collagen remodeling
2. Proliferation
3. Fibrotic gene expression↑

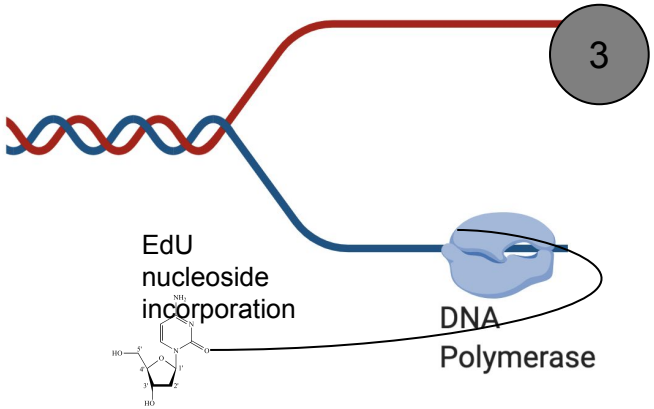
Wnt/DPP-IV ?

EdU Proliferation Assay

1

Control Group	Experimental Group	Independent Variable	Dependent Variable	Time Point
Dpp4+/+ Wnt activated	Dpp4-/- Wnt activated	Presence of Dpp4?	Dermal response	10, 21 days

3



2

Step 1 — **Tissue Preparation**
Inject mice with EdU 6 hours prior to sacrifice

Step 2 — **Tissue Collection**
Dissections, sectioning

Step 3 — **Data Collection**
Fluorescent images will be taken

Step 4 — **Data Analysis**
CellProfiler marks proliferating cells

Experimental Design: Does DPP-IV affect the production of fibrotic genes?

Fb activation:
1. Collagen remodeling
2. Proliferation
3. Fibrotic gene expression↑

Wnt/DPP-IV
?

Fibrosis Gene Production

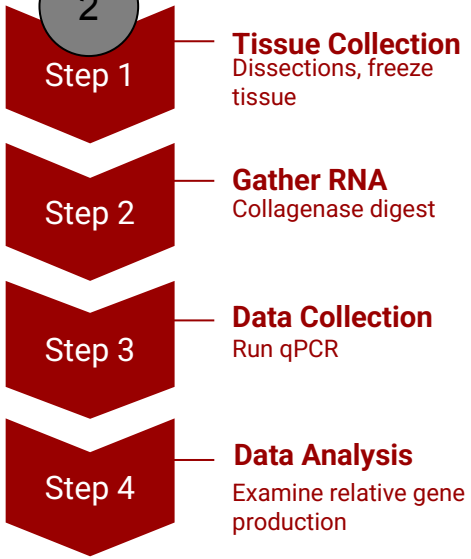
1

Control Group	Experimental Group	Independent Variable	Dependent Variable	Time Point
Dpp4+/+ Wnt activated	Dpp4-/- Wnt activated	Presence of Dpp4?	DNA quantity	10, 21 days

- qPCR quantifies amount of DNA
- marks duplicating DNA

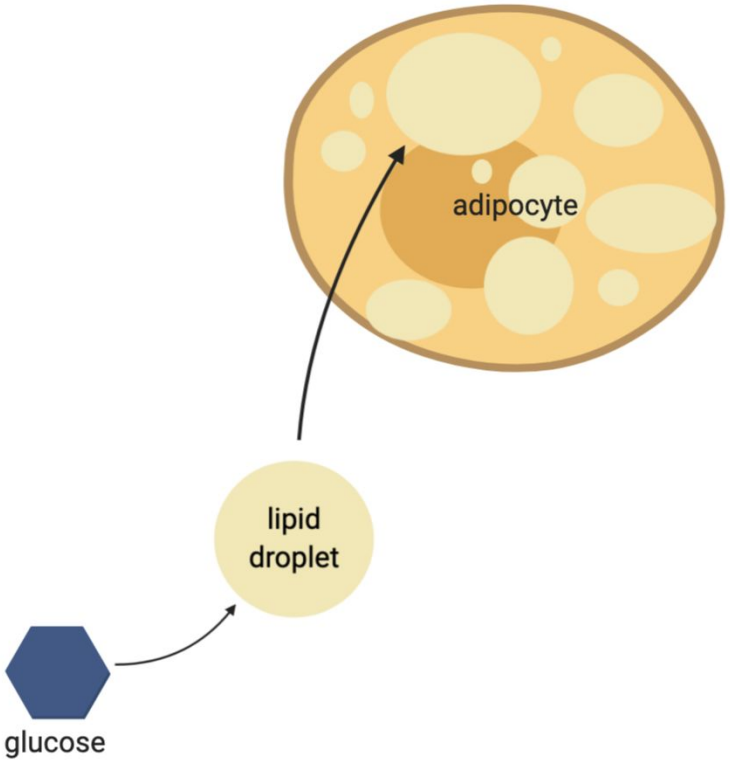
- understand whether DPP-IV plays
  role in fibroblast gene production
- qPCR is a relatively easy way to
  gather genetic data

2



Aim 1 - Alternative

- If results show lack of DPP-IV in lipolysis, may affect lipogenesis
- Measuring uptake of tagged glucose



Aim 2 - Alternative

- Rather than DPP-IV affecting all ECM expansion, may be due to intercellular communications
- In vitro experiments to understand communication between adipocytes and fibroblasts