Regulation of adult flight muscle characteristics in *Drosophila* by signals emanating from the wing disc epithelium

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Abstract

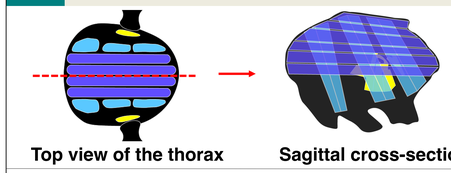
- Adult flight muscles consists of morphologically and physiologically unique direct flight muscles (DFMs) and indirect flight muscles (IFMs)
- Flight muscles derive from adult muscle precursor cells (AMPs) localized basally in the notum portion of the wing disc epithelium
- AMPs exhibit distinct fates in third instar larvae characterized by differential expression of *vestigial* and *cut* along the dorsoventral axis, specifying the IFMs and DFMs
- Notch and Wingless signaling from the disc have also been implicated in AMP development
- However, little else is known about the specification of AMPs

Using single-cell analysis we find:

- Hedgehog (Hh) signaling from the posterior compartment of the epithelium has an important role in the specification of AMPs
- A novel pathway downstream of *smoothened*, distinct from that which functions in the disc epithelium is involved in specifying specific AMP fates

Background

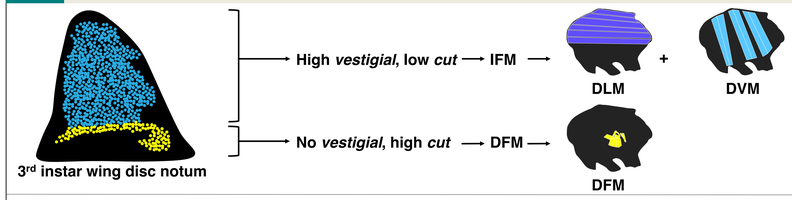
1 *Drosophila* flight muscles consists of IFMs and DFMs



Indirect Flight Muscles (IFMs)
Dorsal-Longitudinal Flight Muscles (DLMs)
Dorsoventral Flight Muscles (DVMs)
Direct Flight Muscles (DFMs)

The DFMs and IFMs are morphologically and physiologically distinct. DFMs exhibit tubular morphology and maneuver wing position to steer flight. IFMs exhibit fibrillar morphology and contract anti-synchronously to generate the mechanical force necessary to propel flight.

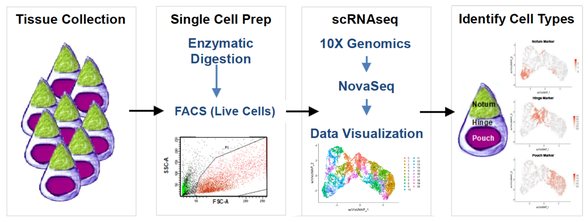
2 Flight muscles are derived from wing disc associated AMPs



Flight muscles are derived from Adult Muscle Precursors (AMPs) localized basally in the notum portion of the wing disc epithelium. By the late 3rd instar, AMPs exhibit distinct fates, characterized by differential expression of *vestigial* and *cut*, specifying the IFM and DFM groups. However, little else is known about the specification of AMPs by the wing disc epithelium.

Method

3 Generating a cell atlas using single-cell RNA sequencing

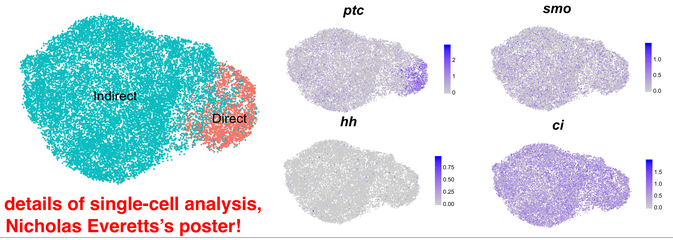


For details of the library preparation and single-cell analysis check out Nicholas Everetts's poster and Melanie Worley's talk!

We collected high quality transcriptomes of wing discs at 96 & 120 hours after egg lay for a total of 7,104 epithelial cells and 20,730 AMPs. The single-cell transcriptomic library was analyzed using scVI to harmonize replicates, impute data, and reduce dimensionality. Cells were clustered based on transcriptional profiles and the data was visualized using Uniform Manifold Approximation and Projection (UMAP). Single-cell analysis was used to cluster and identify cell types.

Results

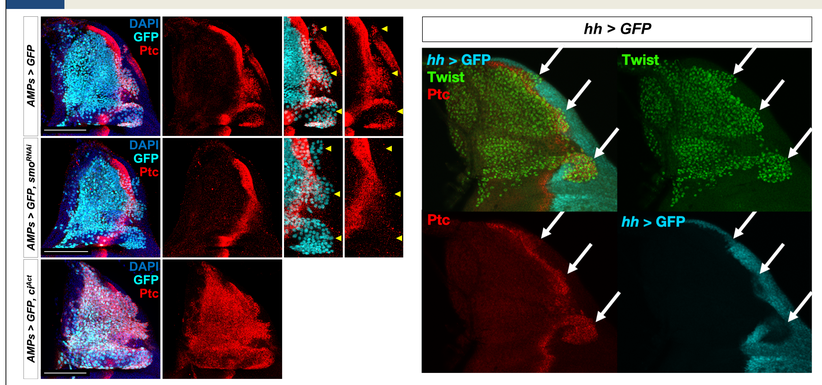
4 Single-cell analysis reveals AMPs with high expression of *patched*



For details of single-cell analysis, see Nicholas Everetts's poster!

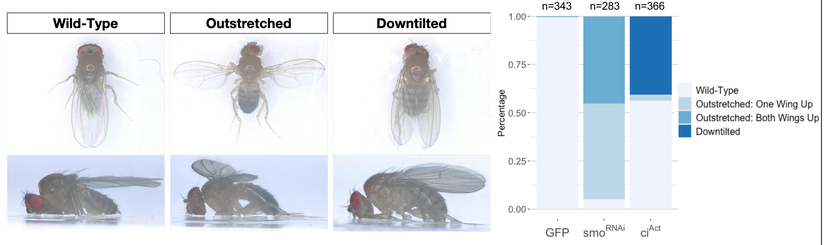
(Left) AMPs cluster into direct and indirect AMPs based on differential expression of *vestigial* and *cut*. (Right) A subset of AMPs express high levels of *patched*, the receptor and transcriptional target of Hedgehog (Hh) signaling. However, very few cells express the ligand Hedgehog and the expression of *smoothened*, a component of the pathway, and *cubitus interruptus*, the transcriptional effector, is not restricted to any distinct clusters.

5 Posterior AMPs transduce Hh signaling, but do not express the ligand



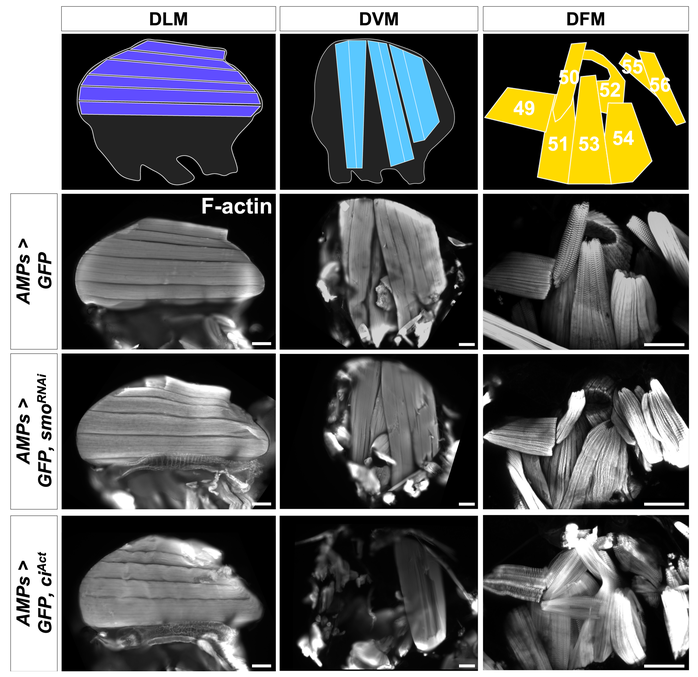
(Left) A *15B03-Gal4* FlyLight driver is used to identify and manipulate AMPs. AMPs underlying the posterior compartment of the epithelium stain for Ptc, indicating active Hedgehog signaling. Expression of smo^{RNAi} and ci^{Act} in AMPs eliminates and activates Ptc staining, respectively. The bright dorsoventral stripe of Ptc is the disc epithelium. (Right) AMPs are visualized with Twist staining and arrows point to posterior AMPs that stain for Ptc. Expression of *hh-Gal4* is not detected in AMPs, suggesting that AMPs are receiving Hh from elsewhere, perhaps the disc epithelium.

6 Hh signaling perturbation in AMPs causes adult wing posture defects



(Left) Three main phenotypes were observed in our study: wild-type, outstretched, and downtilted. (Right) Expression of GFP in AMPs generates flies with Wild-Type posture. Expression of smo^{RNAi} in AMPs generates mostly adults with an outstretched phenotype with either one or both wings held up perpendicular to the anterior-posterior axis of their body. Expression of ci^{Act} in AMPs generates a majority of wild-type flies, but also a considerable proportion of adults exhibiting the downtilted phenotype.

7 Hh signaling perturbation in AMPs disrupts flight muscle morphology



(Top) Schematics of muscle types. (Left) Neither loss nor activation of Hh signaling alters DLM morphology. (Middle) Loss of Hh signaling in AMPs does not noticeably alter DVM morphology. Activation of Hh signaling in all AMPs leads to loss of the two anterior-most major DVM fibers, or complete loss of DVMs. (Right) Loss of Hh signaling in AMPs leads to abnormal DFM morphology. Muscles 53 and 54 lose distinction and muscles 55 and 56 appear enlarged. The posterior edge of muscle 52 appears to be truncated. Activation of Hh signaling in AMPs also generates disrupted DFM morphology. Thus, Hh signaling appears necessary for the DFMs to be specified properly. Conversely, ubiquitous Hh signaling in the AMPs is detrimental to a subset of the IFMs

More from the Hariharan Lab at TAGC2020

Hedgehog signaling between the wing-disc epithelium and muscle precursors revealed by single-cell analysis in *Drosophila*	→	**#1547B Poster by Nicholas Everetts**
Localized and tissue-wide gene expression changes during regeneration of *Drosophila* imaginal discs revealed by single-cell analysis	→	**#196 Talk at 4/24 12pm by Melanie Worley**
Learn more about *Drosophila* development and mentoring	→	**#1482C Maya Emmons-Bell #1494C Sophia Friesen #147 & #462C Jamie Lahvic**

Acknowledgments

Thank you members of the Hariharan Lab, NIGMS, MCB at UC Berkeley, and GSA. Thank you Seulki Kim for the beautiful poster format. This project was made possible by the NIH grant R35 GM122490.