Functional genomics of gut homeostasis in *Nf1* mutant *Drosophila*

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Introduction

- The *Nf1* gene provides instructions for producing neurofibromin, a tumor suppressant protein. Lack of neurofibromin typically leads to Neurofibromatosis Type 1, a disorder characterized by benign tumors growing under the skin as well as metabolic and developmental disfunction (Friedman, 2022).
- Recent research indicates a potential link between the *Nf1* gene, aging, and gut homeostasis. Using the fruit fly, D*rosophila melanogaster,* as a model, Brown et al. (2023) found increased gut permeability and concentration of reactive oxidative species in the guts of *Nf1* mutant flies.
- Our goal is to investigate the mechanisms that regulate this link. To do this, we will perform RNA-sequencing on the guts of wildtype and Nf1 mutant flies across the lifespan.
- These experiments will identify differentially expressed genes. We expect to see differences in mRNA expression across age groups and genotype, and we aim to use this data to identify the genes associated with aging in the gut.
- This data will increase current understanding of the relationship between gut homeostasis, longevity, and the Nf1 function.

Background

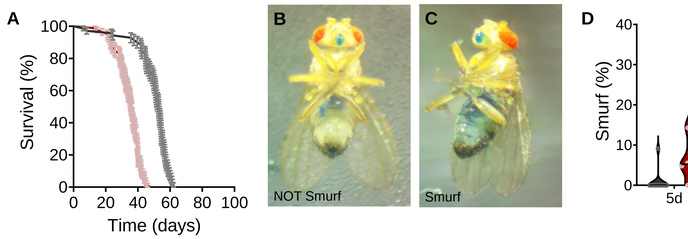

Figure 1. Mutation of Nf1 decreases survival and promotes aging. (A) Mutation of *NF1* significantly decreases survival. **(B-C)** The smurf assay was used to measured intestinal permeability, a marker of aging. **(D)** Mutation of *NF1* significantly increases intestinal permeability.

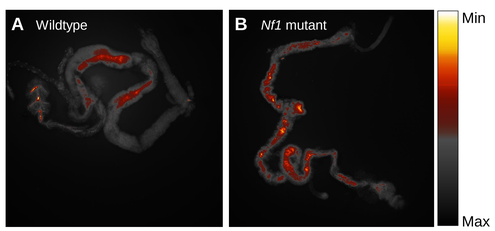

Figure 2. ROS accumulate in the gut in Nf1 mutant flies. (A,B) Gut reactive oxygen species (ROS) labeled by oxidized DHE in 20 day-old flies. **(C)** Quantification of fluorescence intensity.

Methods

Fly maintenance:
Drosophila melanogaster fly lines of wildtype and *Nf1* mutant flies were maintained on standard fly food. Isolated males were aged to either 5 days or 20 days, resulting in four experimental groups:

- 5 day-old *Nf1* mutants
- 20 day-old *Nf1* mutants
- 5 day-old wildtype flies
- 20 day-old wildtype flies

Gut Dissections:

- Flies were sedated on ice and then suspended in Schneider's *Drosophila* medium.
- Using forceps, the abdomen was separated from the thorax to expose the gut.
- The crop and Malpighian tubules were discarded; the foregut, midgut, and hindgut were extracted and then transferred to Eppendorf tubes.
- For each of the four experimental groups, guts from twenty flies were collected and then immediately flash frozen. This process was repeated five times, generating five independent replicates of gut tissue from each group for RNA extraction and analysis.

RNA Sequencing:

- For each sample, we will extract RNA and prepare sequencing libraries. These libraires will be sequenced using Illumina NovaSeq 6000.
- Alignment of mRNA to the *Drosophila* genome and quantification of gene expression will be performed using the program STAR (Dobin et al., 2013).

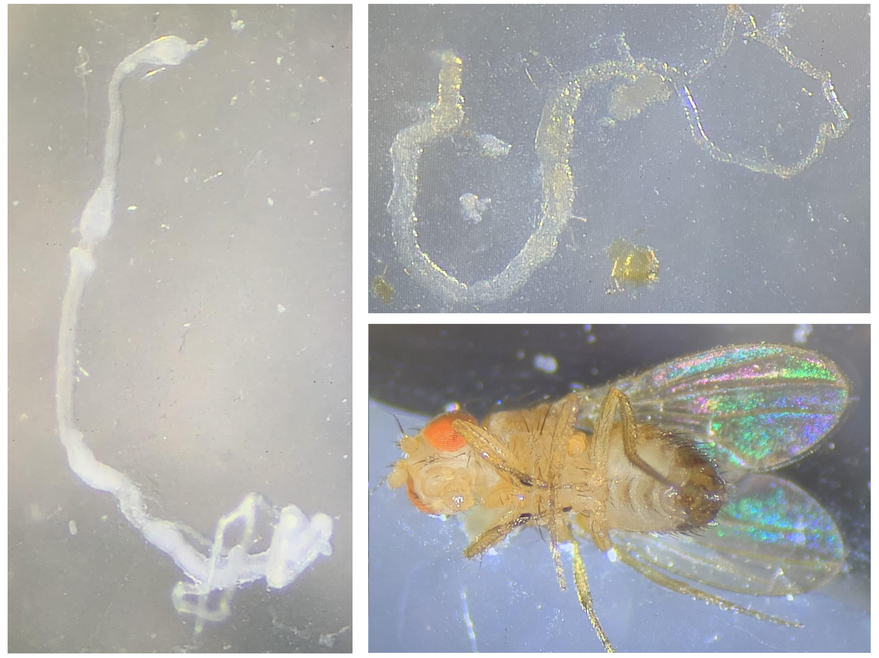

Figure 3. Features of the experimental design. (A,B) Example dissected guts. **(C)** Sedated male fly.

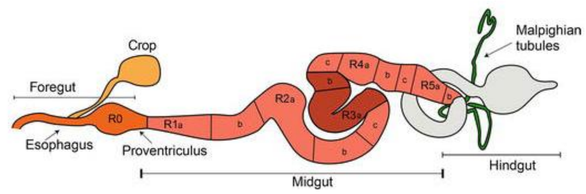

Figure 4. Structure of the Drosophila gut.

Discussion

- Once results are available, we will identify changes in gene expression associated with Nf1 and aging in gut tissue.
- Future studies will functionally characterize the candidate genes identified in regulating gut homeostasis and aging.

References

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