

# Aging-dependent regulation of gene expression in a *Drosophila* model of Neurofibromatosis Type 1

Bethany Kear, Elizabeth Brown

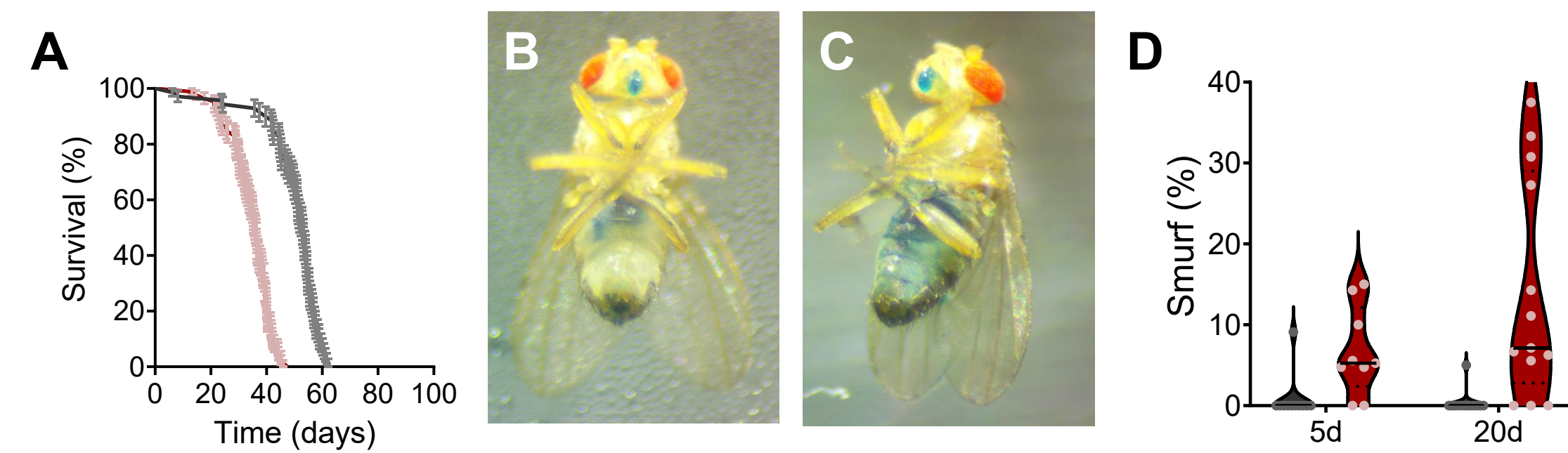
Department of Biological Science, Florida State University, Tallahassee, FL  
Program in Neuroscience, Florida State University, Tallahassee, FL



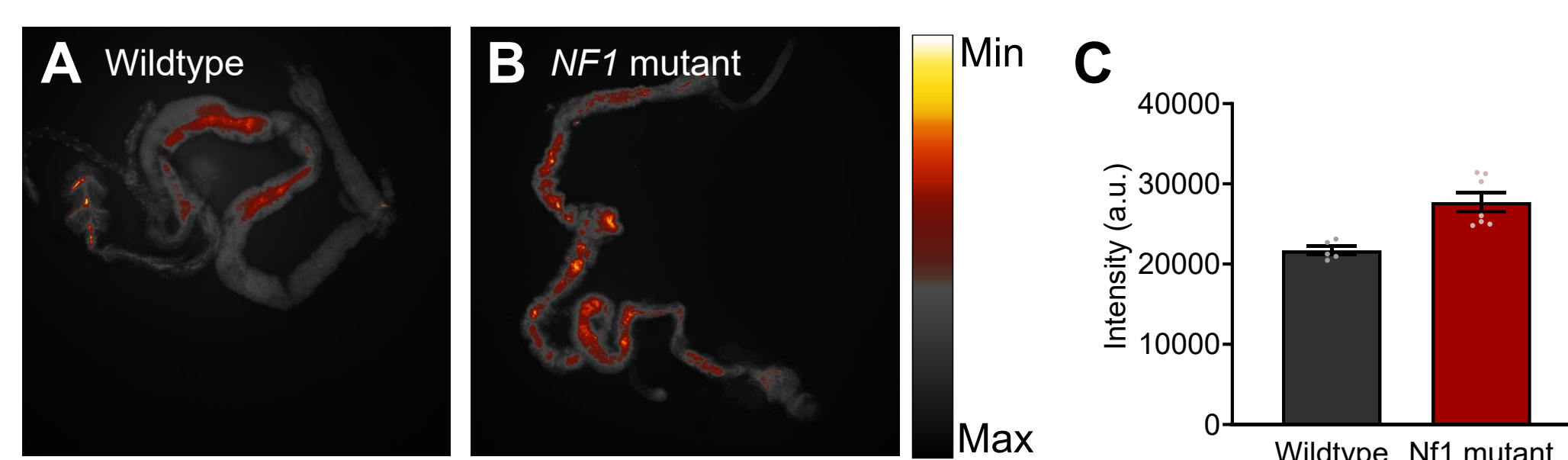
## Abstract

Neurofibromatosis type 1 (NF1) is a genetic disorder resulting from mutations in the *NF1* gene. *NF1* affects 1 in 3000 people and presents with benign tumors of the peripheral nervous system, as well as dysregulation of sleep and metabolic rate. The fruit fly, *Drosophila melanogaster*, is an excellent genetic model to study *NF1*; flies with mutations in the *NF1* gene display decreased sleep and increased metabolic rate. Sleep loss has been associated with changes in gut homeostasis in both flies and mammals, and we previously found that selective knockdown of *NF1* in neurons of the brain increases gut permeability and reactive oxygen species (ROS) in the gut. This raises the possibility that loss of sleep contributes to gut dysregulation in this *NF1* model. To explore the mechanisms that may underlie this link, we conducted an RNA-sequencing analysis of dissected gut tissue from control and *NF1* flies. We examined the gene expression of aged (20 days) *NF1* flies, young (5 days) *NF1* flies, and their aged-matched controls. Relative to controls, 32 genes were upregulated and 20 genes were downregulated in young *NF1* flies, while 15 genes were upregulated and 9 genes were downregulated in the aged condition. Future work will validate these candidate genes regulating sleep and gut homeostasis through genetic screening approaches. Overall, this work contributes to our understanding of the genetic basis of *NF1* and its effect on gut health and physiology.

## Background



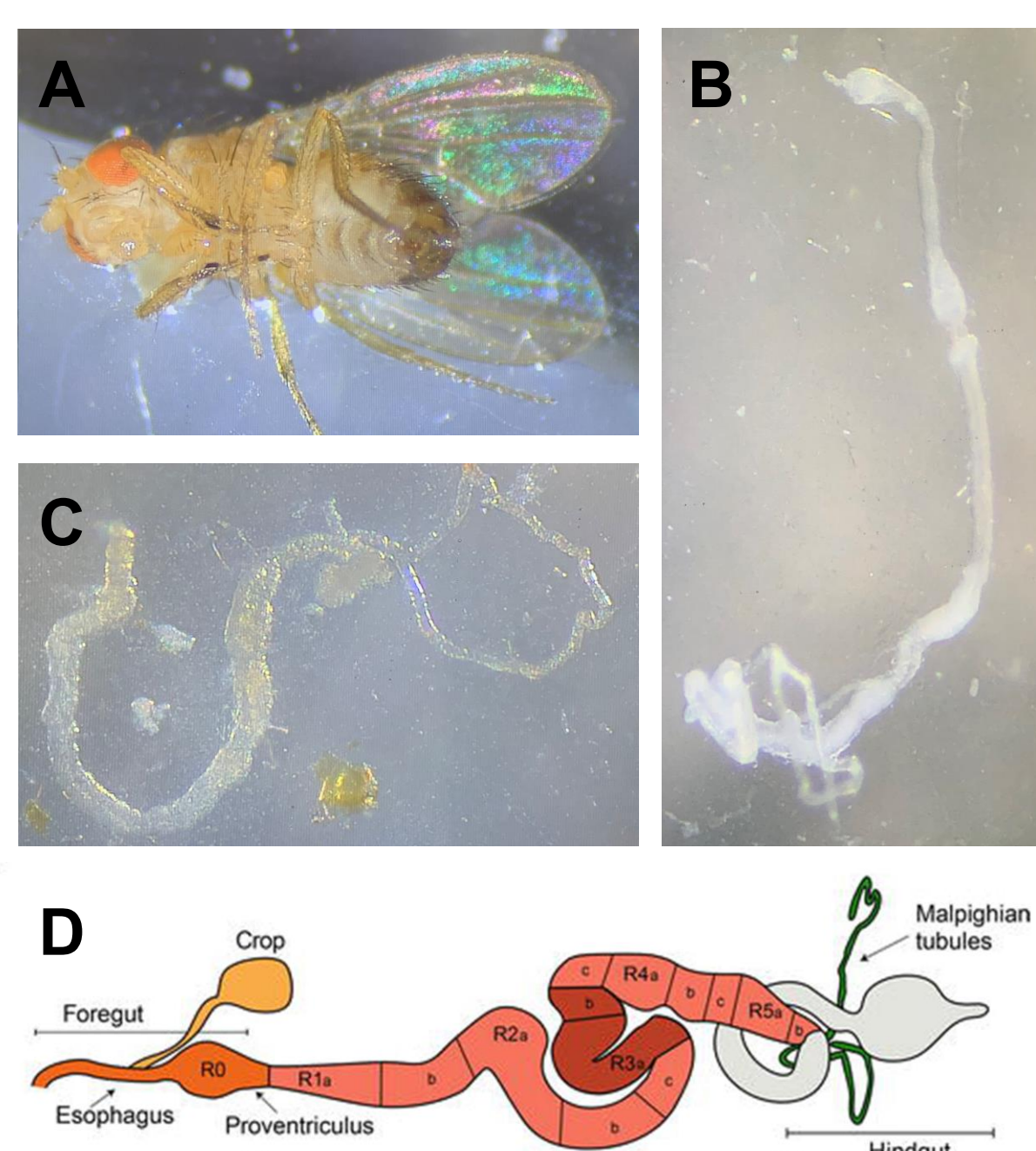
**Figure 1. Mutation of *NF1* decreases survival and promotes aging.** (A) Mutation of *NF1* significantly decreases survival. (B-C) The smurf assay was used to measure intestinal permeability, a marker of aging. (D) Mutation of *NF1* significantly increases intestinal permeability.



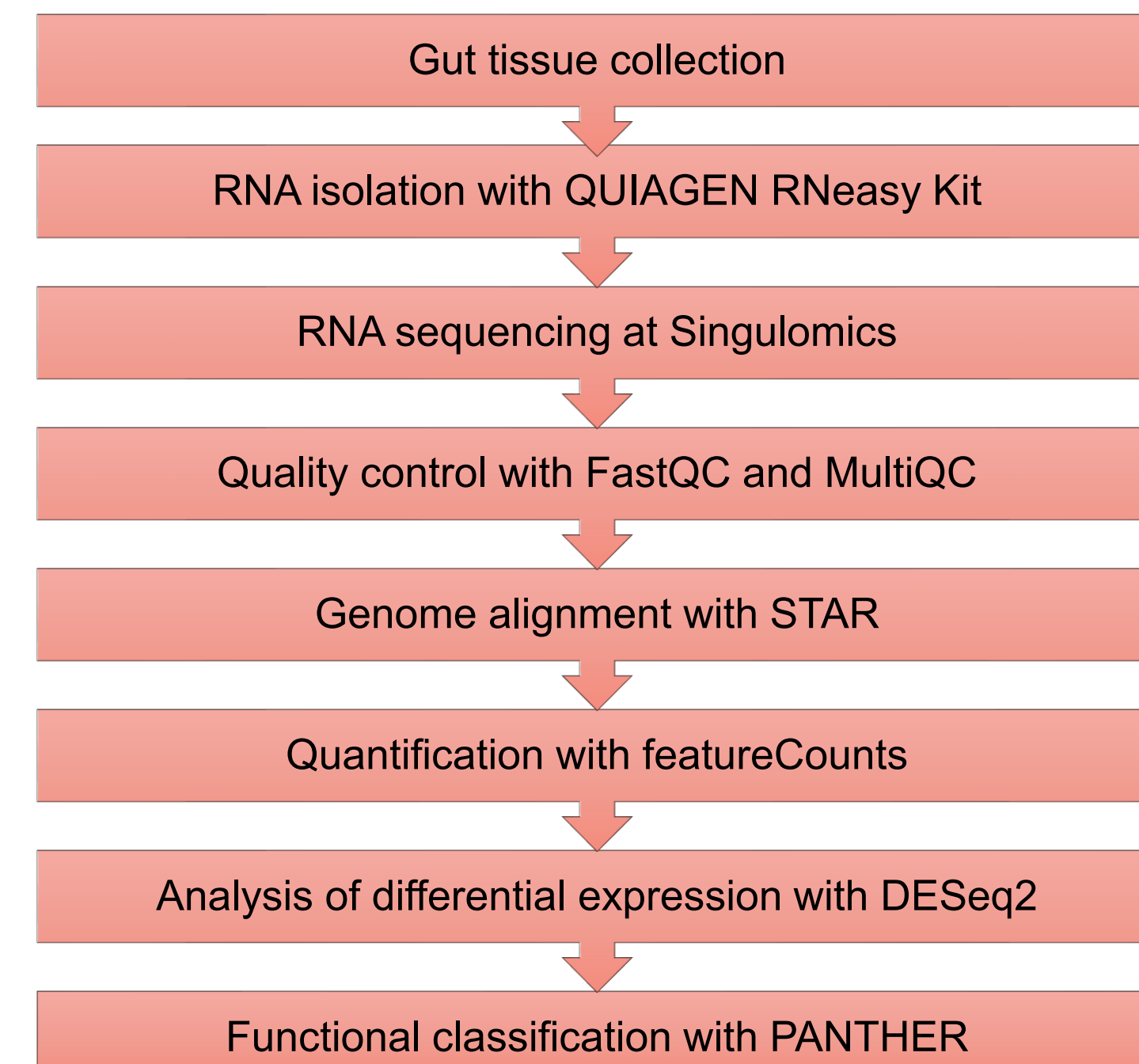
**Figure 2. ROS accumulate in the gut in *NF1* mutant flies.** (A, B) Gut reactive oxygen species (ROS) labeled by oxidized DHE in 20 day-old flies. (C) Quantification of fluorescence intensity.

## Methods

### Gut tissue collection followed by RNA sequencing



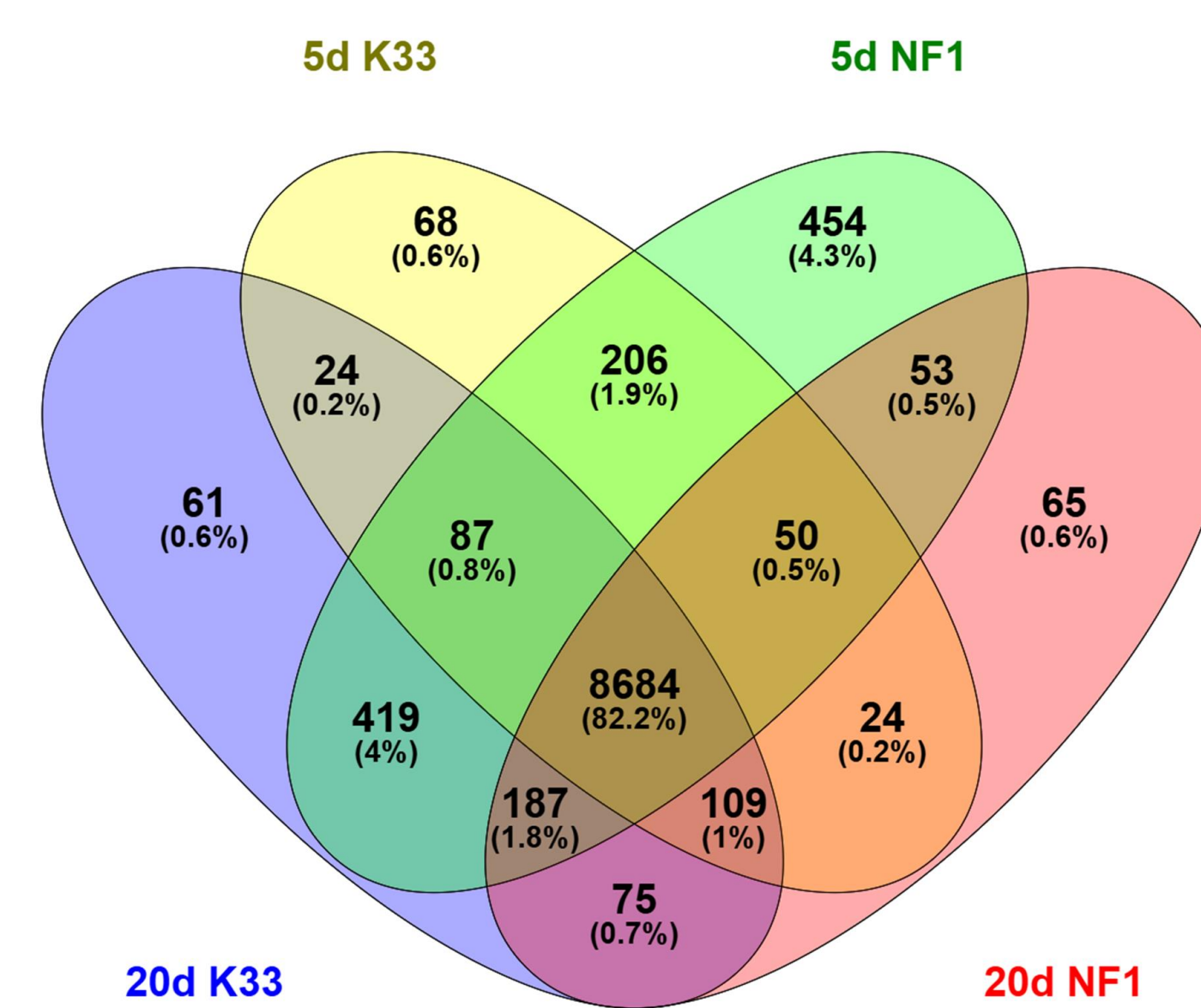
**Figure 3. Gut tissue collection.** (A) Sedated male fly. (B, C) Example dissected guts. (D) Structure of the *Drosophila* gut.



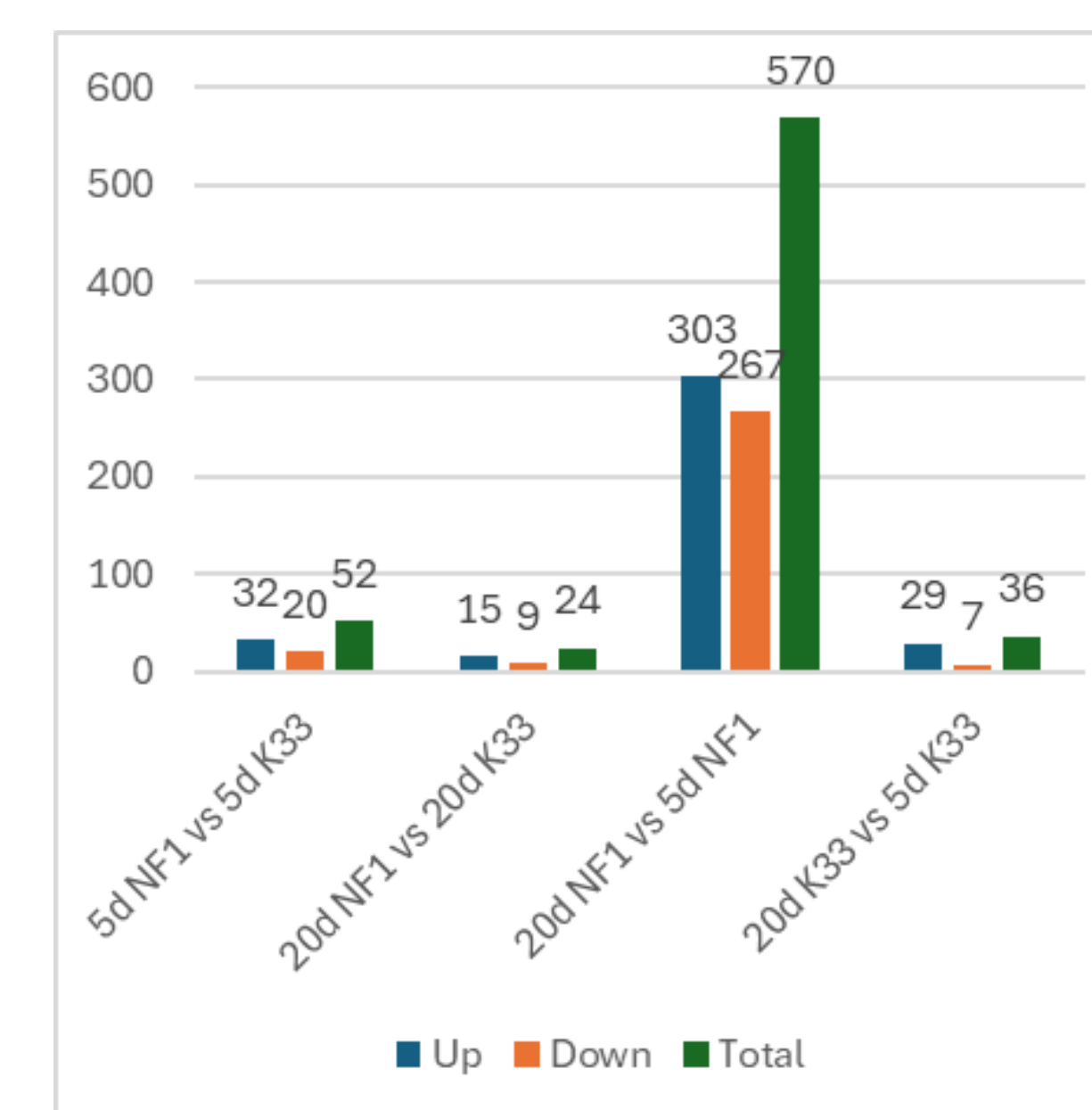
**Figure 4. RNA-seq analysis pipeline and bioinformatics tools utilized at each step.**

## Results

### Summary of coexpression and differential gene expression

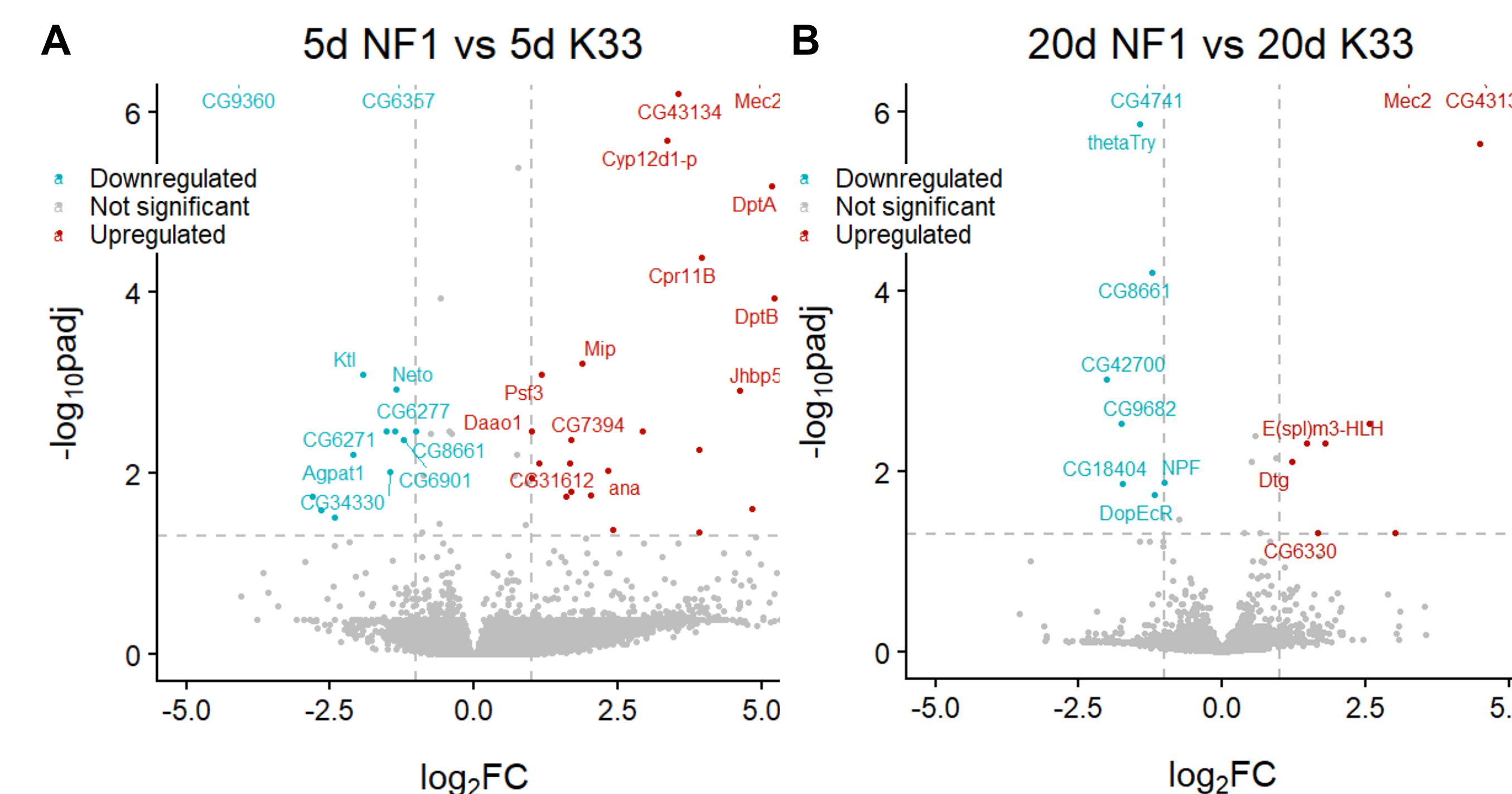


**Figure 5. Number of genes uniquely expressed within a group or co-expressed in two or more groups.**



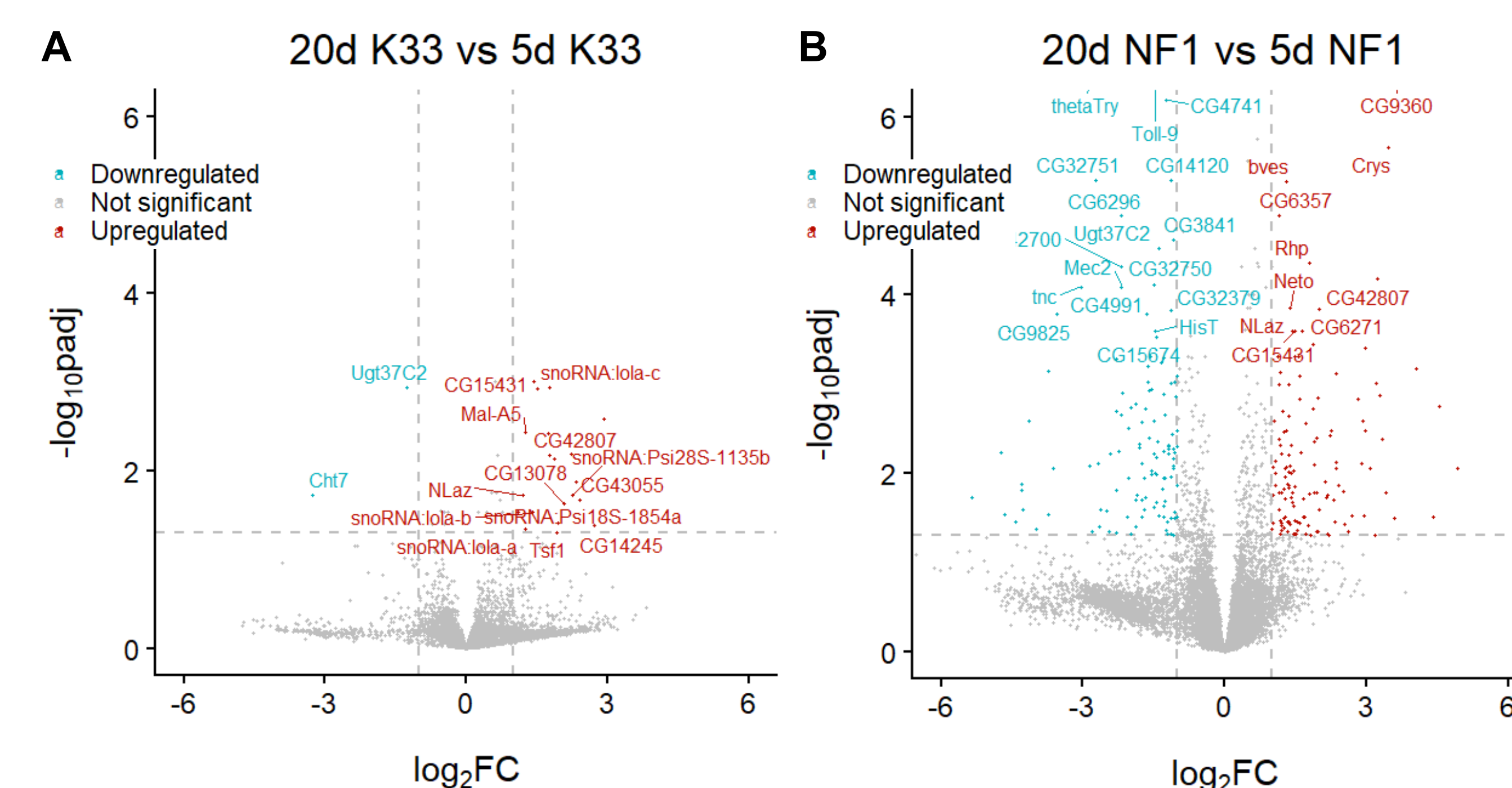
**Figure 6. Number of genes significantly upregulated or downregulated for each condition relative to another.**

### Differential gene expression of *NF1* flies relative to controls



**Figure 7. Volcano plots with the most significant differentially expressed genes (DEGs) labeled.** (A) Genes are significantly upregulated (red) and downregulated (blue) in young *NF1* flies relative to young controls. (B) Genes are significantly upregulated and downregulated in aged *NF1* flies relative to aged controls.

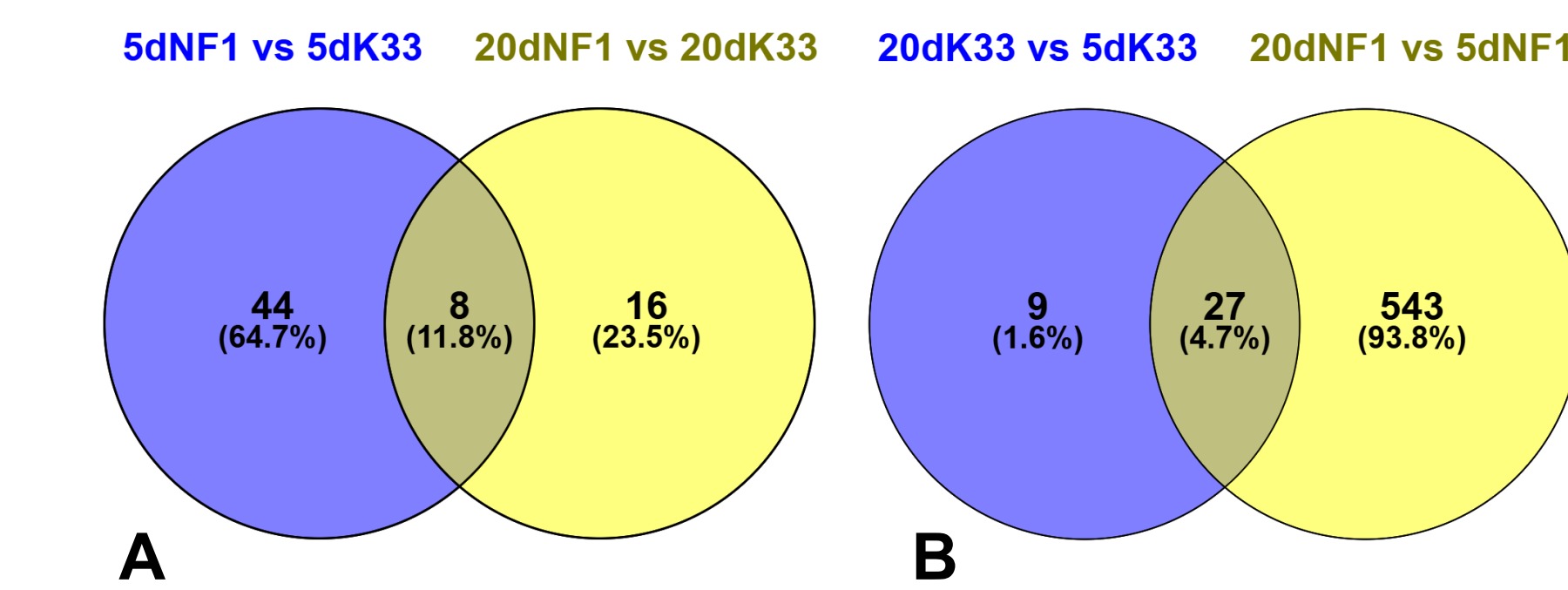
### Differential gene expression of aged flies relative to young controls



**Figure 8. Volcano plots with the most significant DEGs labeled.** (A) Genes are significantly upregulated (red) and downregulated (blue) in aged control flies relative to young control flies. (B) Genes are significantly upregulated and downregulated in aged *NF1* flies relative to young *NF1* flies.

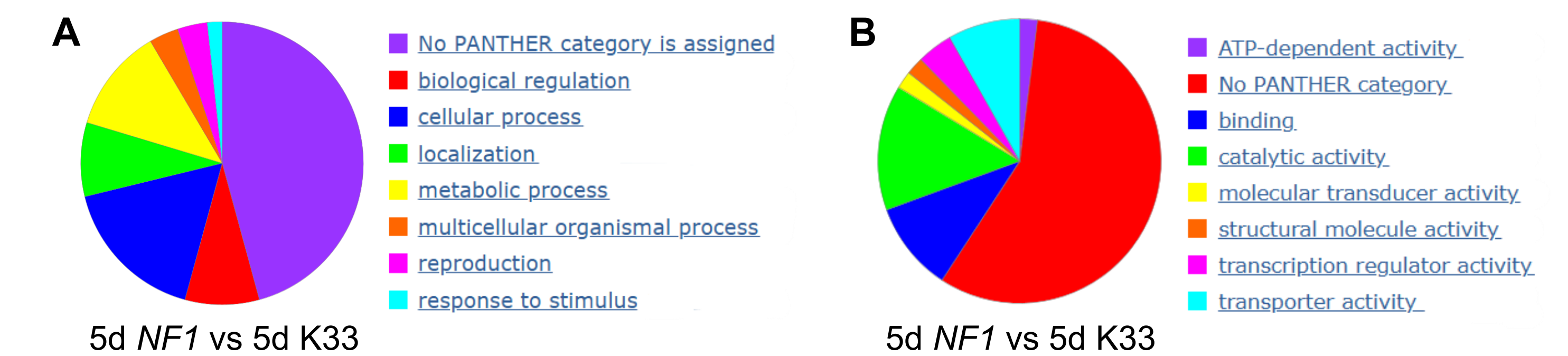
## Results (continued)

### Differentially expressed genes shared by comparison groups

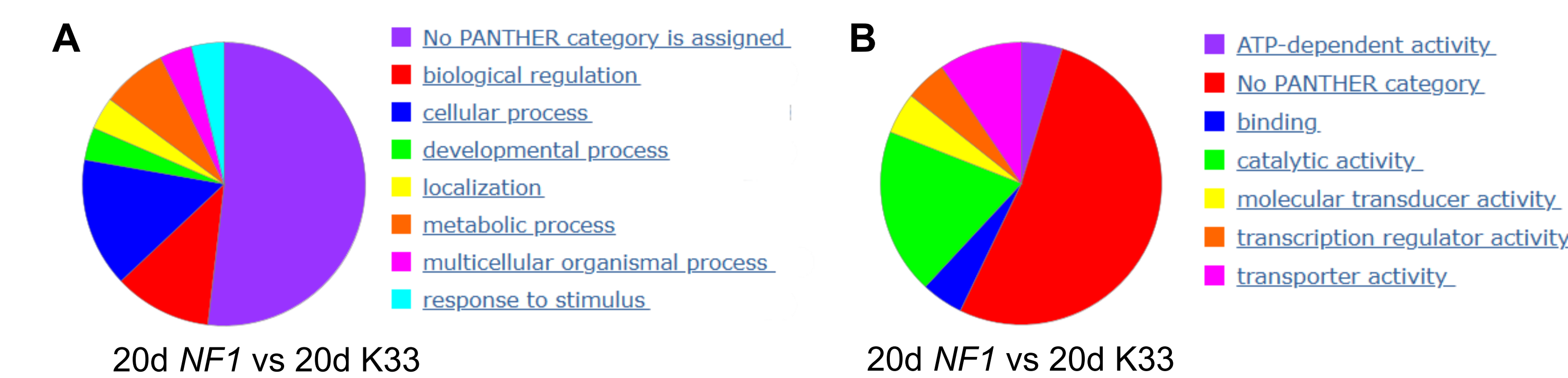


**Figure 9. Overlap between comparison groups.** (A) 8 genes are significantly differentially expressed with *NF1* mutation regardless of age. (B) 27 genes are significantly differentially expressed with age regardless of genotype.

### Functional classification of significant differentially expressed genes



**Figure 10. Functional classifications of significant DEGs of 5d *NF1* flies relative to age-matched controls.** (A) Classified by biological process. (B) Classified by molecular function.



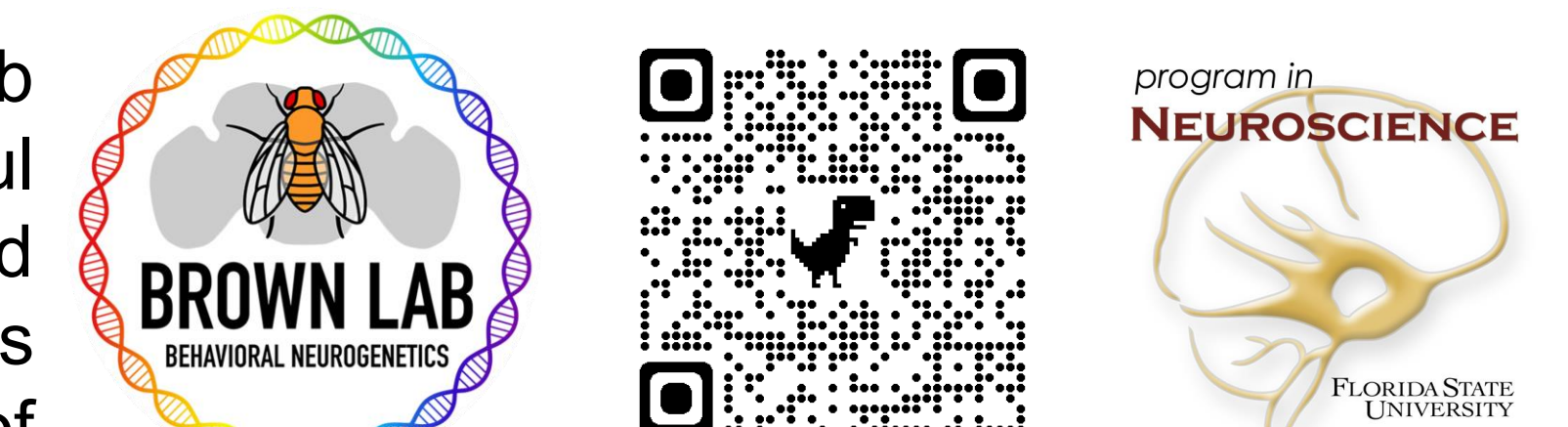
**Figure 11. Functional classifications of significant DEGs of 20d *NF1* flies relative to age-matched controls.** (A) Classified by biological process. (B) Classified by molecular function.

## Conclusions

- Young *NF1* mutant flies have 32 genes significantly upregulated and 20 genes significantly downregulated.
- Aged *NF1* mutant flies have 15 genes significantly upregulated and 9 genes significantly downregulated.
- Among these, 8 genes are differentially expressed regardless of age.
- The proportion of differentially expressed genes associated with various biological processes and molecular functions changes with age.
- Future studies will use genetic screening approaches to better understand the role of these differentially expressed genes in mediating longevity and intestinal homeostasis in the *NF1* disease state.

## Acknowledgments

We thank members of the Brown lab for technical assistance and helpful discussions. This work was supported by the Bess H. Ward Honors Thesis Award and the National Institutes of Health (R00AG071833).



## References

- Brown, E. B., Zhang, J., Lloyd, E., Lanzon, E., Botero, V., Tomchik, S., & Keene, A. C. (2023). Neurofibromin 1 mediates sleep depth in *Drosophila*. *PLoS genetics*, 19(12).