



Identification of Tumor-Reducing Agents to Reveal Potential Downstream Nodes of the Hippo Signaling Pathway

Imaani Easthausen^{1,2}, Julian A. Martinez-Agosto³

¹Bard College, Annandale-on-Hudson, New York

²Amgen Scholars Program, UCLA, Los Angeles, California

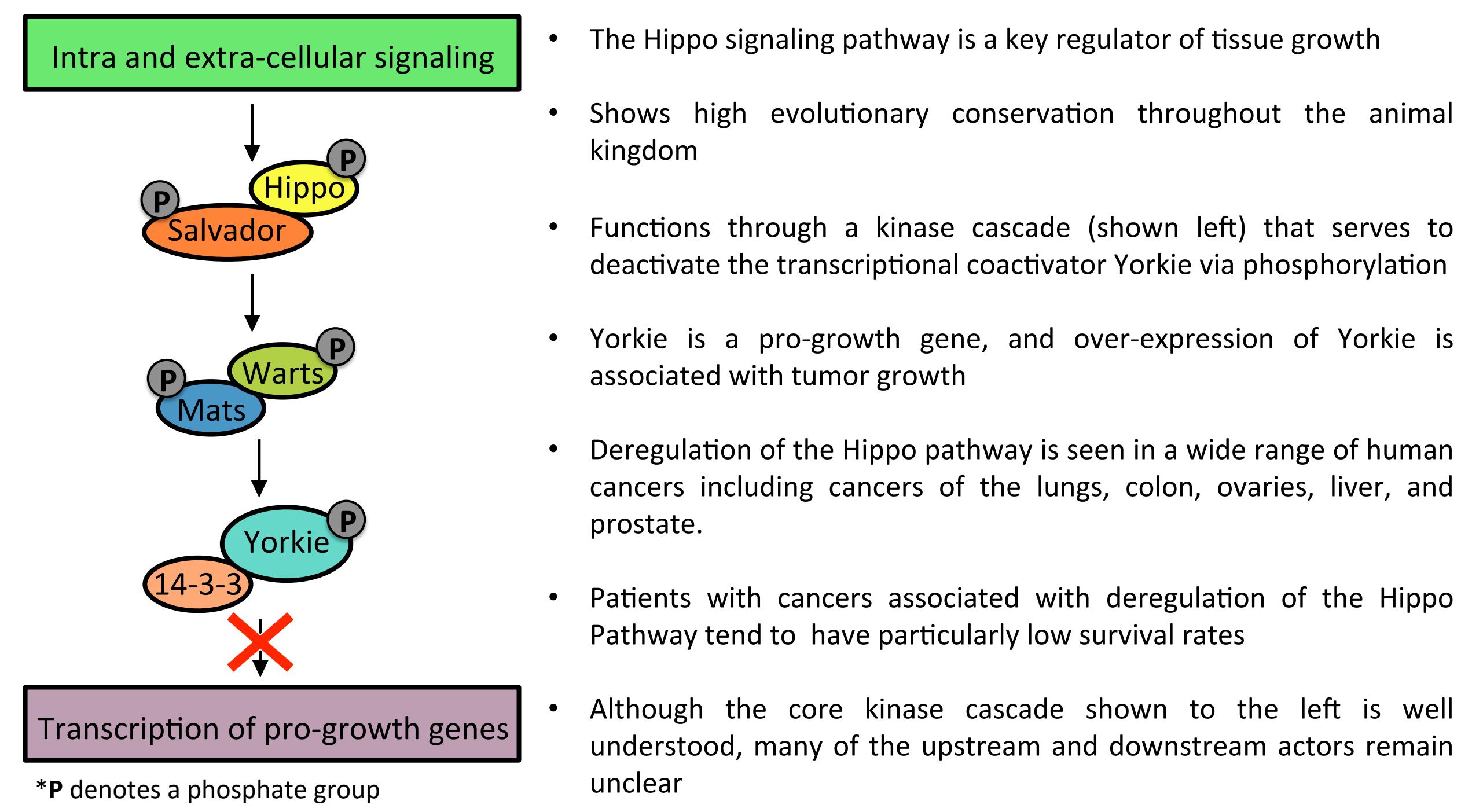
³Department of Human Genetics, UCLA, Los Angeles, California

AMGEN

Abstract

The Hippo signaling pathway is a key regulator of tissue size throughout the animal kingdom. In *Drosophila*, it is composed of a core kinase cascade that ultimately serves to deactivate the transcriptional coactivator Yorkie, a pro-growth gene that is associated with stem cell properties. Deregulation of the Hippo pathway and over-expression of Yorkie cause tumorigenesis. Although the core kinase cascade composing the Hippo pathway is well understood, most of the downstream components have not been elucidated and the precise mechanisms by which overexpression of Yorkie leads to tumorigenesis have not yet been delineated. This project utilizes a UAS/GAL4 system to induce overexpression of Yorkie in the wing imaginal discs of fruit flies resulting in the formation of tumors. Thirty-three phosphatase inhibitors are screened against these models for efficacy in reducing tumor size. Here, several phosphatases are presented as both potential therapeutic targets and major downstream regulators of the Hippo signaling pathway.

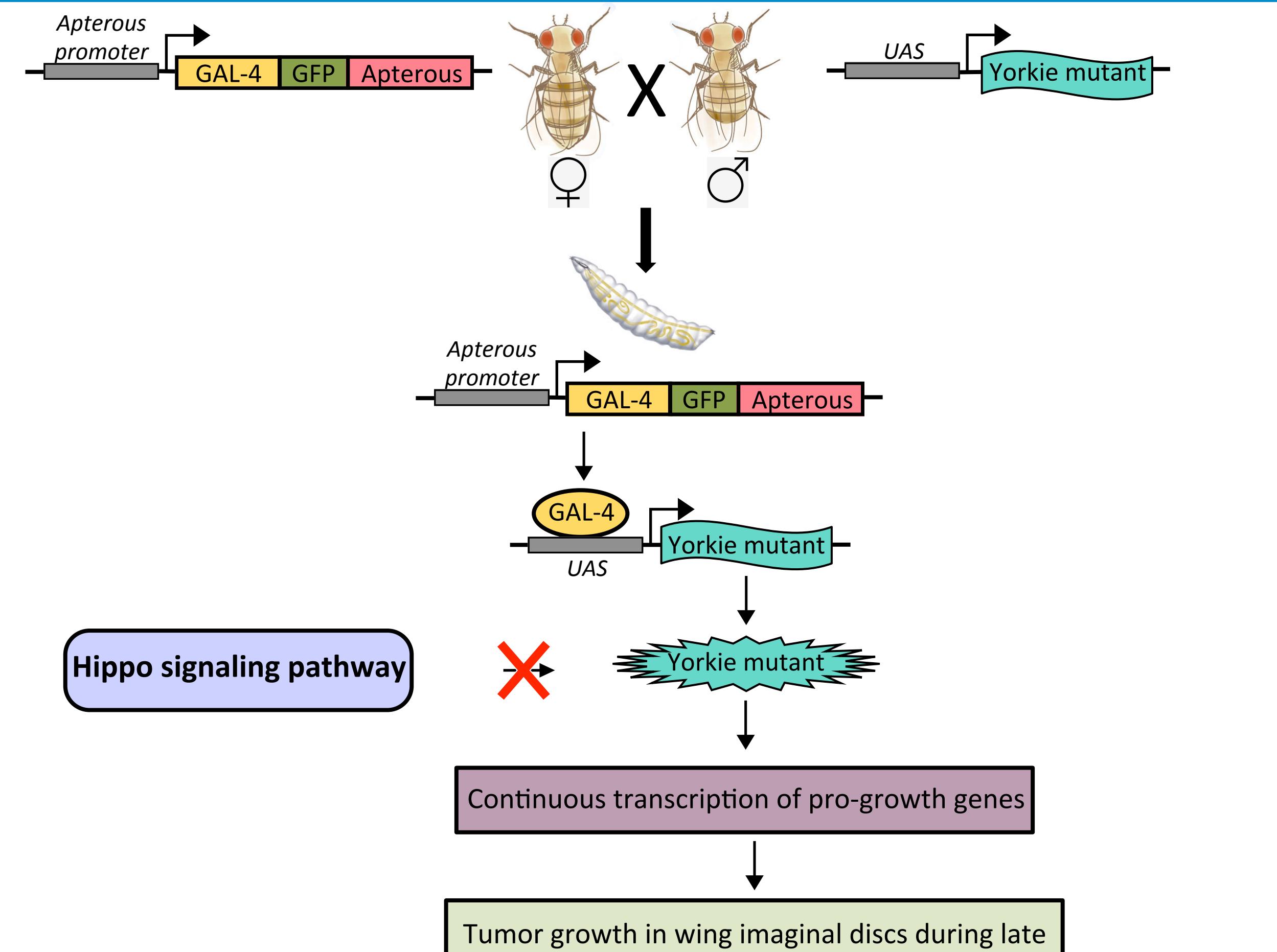
The Hippo Signaling Pathway



Objective

Identify compounds that are effective in reducing tumor growth associated with over-expression of the Yorkie transcriptional coactivator in order to elucidate downstream effectors of the Hippo signaling pathway

Drosophila Models of Tumor Growth

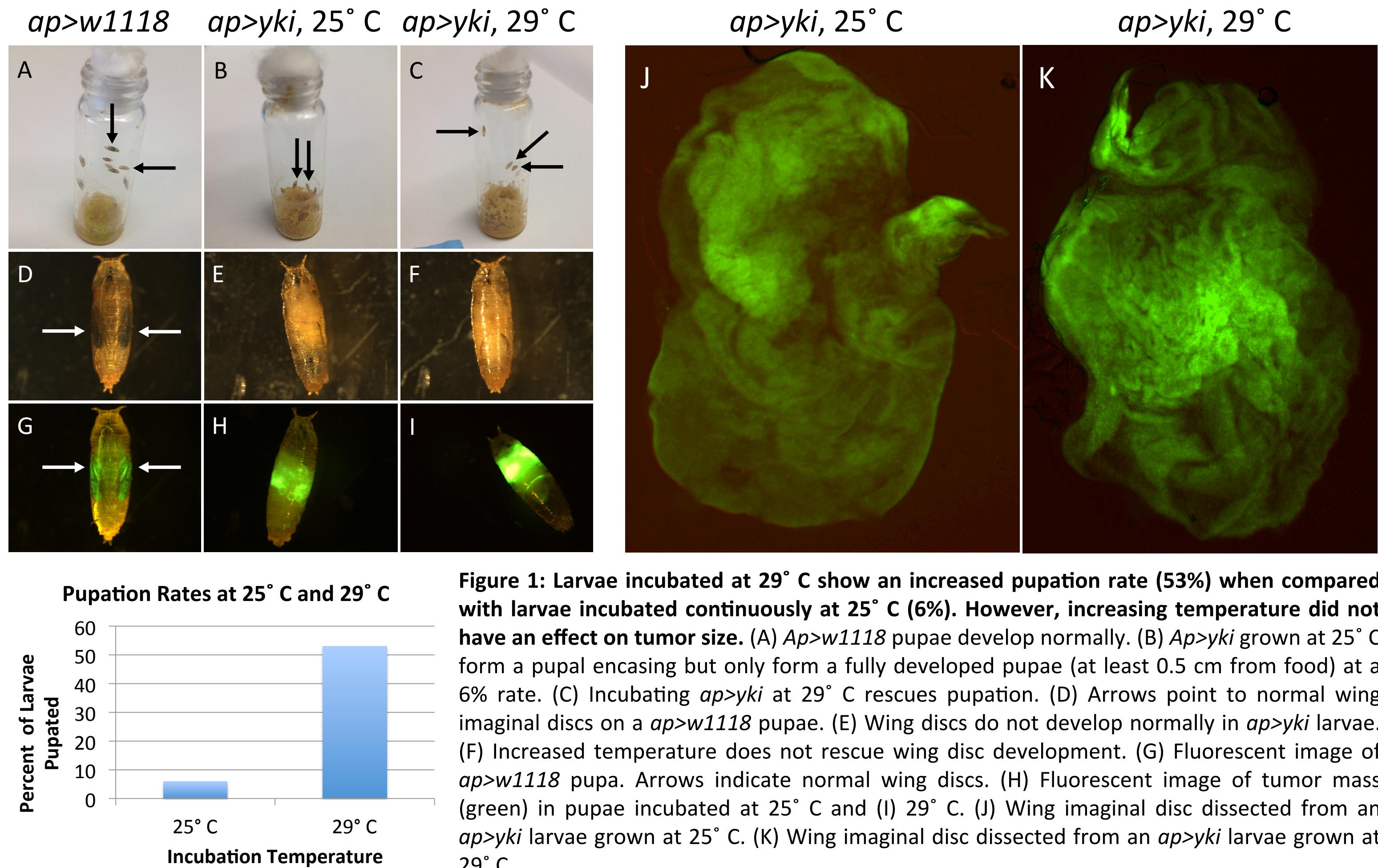


The Drosophila models utilize a UAS/GAL4 system to express a version of Yorkie that has been manipulated at the primary site of phosphorylation in the wing imaginal discs during late larval development. Since the Yorkie clone can't be phosphorylated, it is constantly active and transcribing pro-growth genes, ultimately resulting in tumor growth that inhibits pupation. These models are subjected to various temperature and drug treatments in order to identify conditions and/or compounds that are effective in reducing tumor growth caused by deregulation of the Hippo Pathway.

Drosophila Crosses and Phenotypes

Cross	Phenotype
<i>ap>w1118</i>	Normal
<i>ap>yki</i>	Overexpression of Yorkie and tumor growth in wing imaginal discs

Increased Incubation Temperature Causes Increased Pupation Rates



Compound 2B Extended the Amount of Time Spent as a Larva

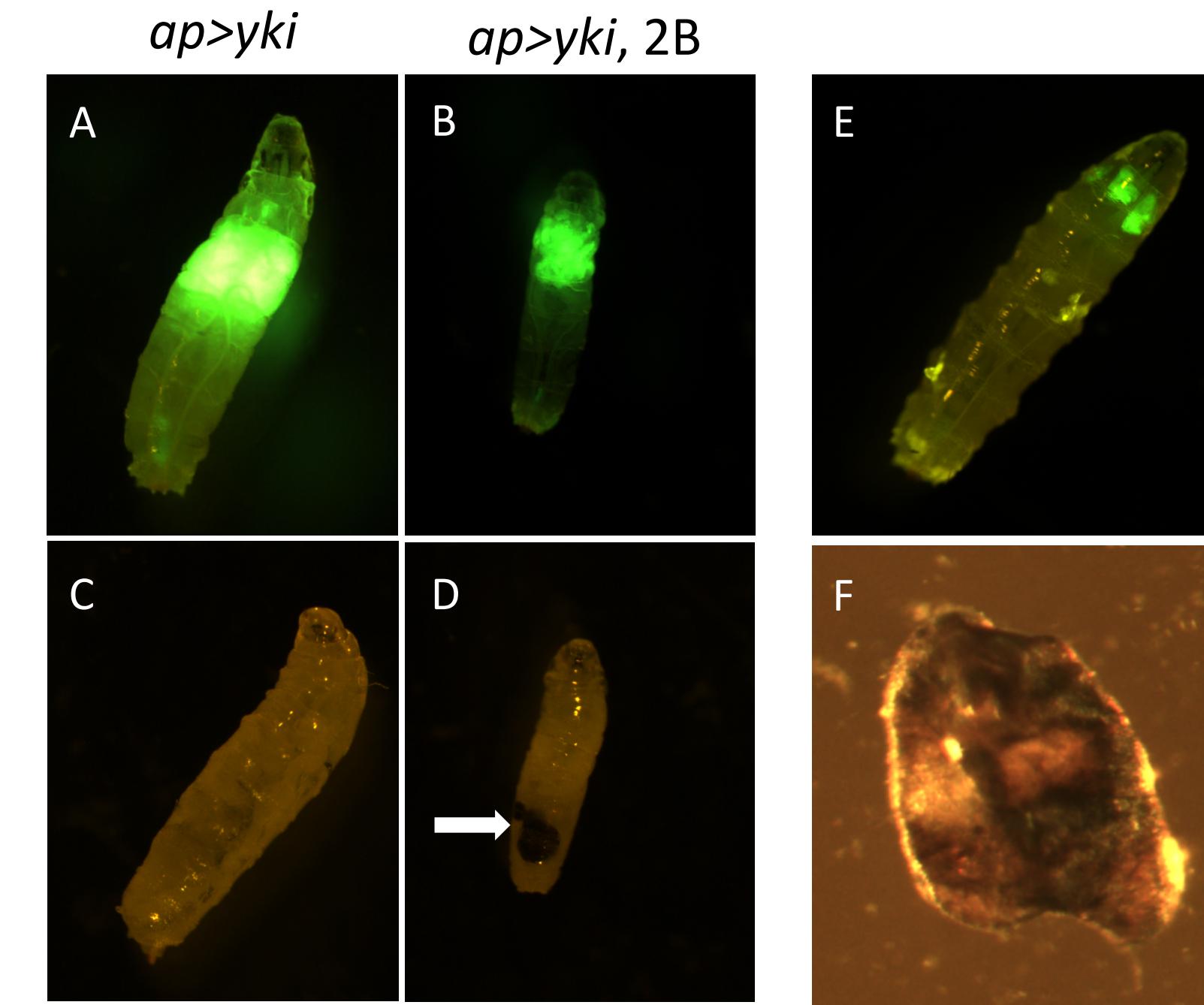


Figure 5: Larvae treated with compound 2B rarely formed any kind of pupal encasing, and remained in the larval stage for as long as 22 days or 18 days after normal larvae pupate. (A) Tumor mass (green) in an *ap>yki* larva that is 6 days old (third instar). (B) Tumor mass (green) in an *ap>yki* larva that has been treated with compound 2B at 13 days old. (C) White light image of *ap>yki* larva at six days old (third instar). (D) White light image of *ap>yki* larva treated with compound 2B at 13 days. (E) *Ap>w1118* larva at 3 days (third instar). (F) Melanotic mass dissected from *ap>yki* larva treated with compound 2B for 13 days.

Compound 2B Caused Significant Reduction in Tumor Size

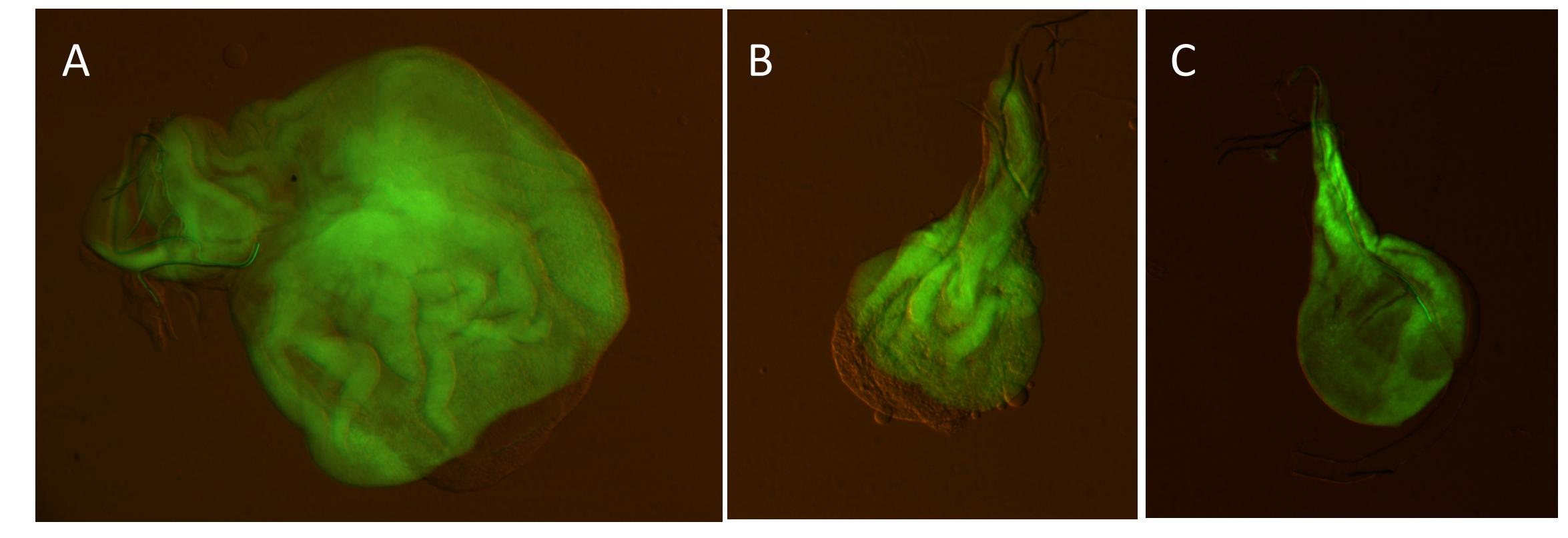


Figure 6: Compound 2B significantly reduced tumor growth in wing imaginal discs. (A) Wing imaginal discs dissected from *ap>yki* larvae treated with DMSO. (B) Wing imaginal discs dissected from *ap>w1118* larvae treated with DMSO. (C) Tumors in wing imaginal discs dissected from *ap>yki* larvae treated with 2B are significantly smaller when compared with *ap>yki* larvae treated with DMSO.

Other Drugs Targeting the Same Phosphatase Resulted in Less Dramatic Tumor Reduction

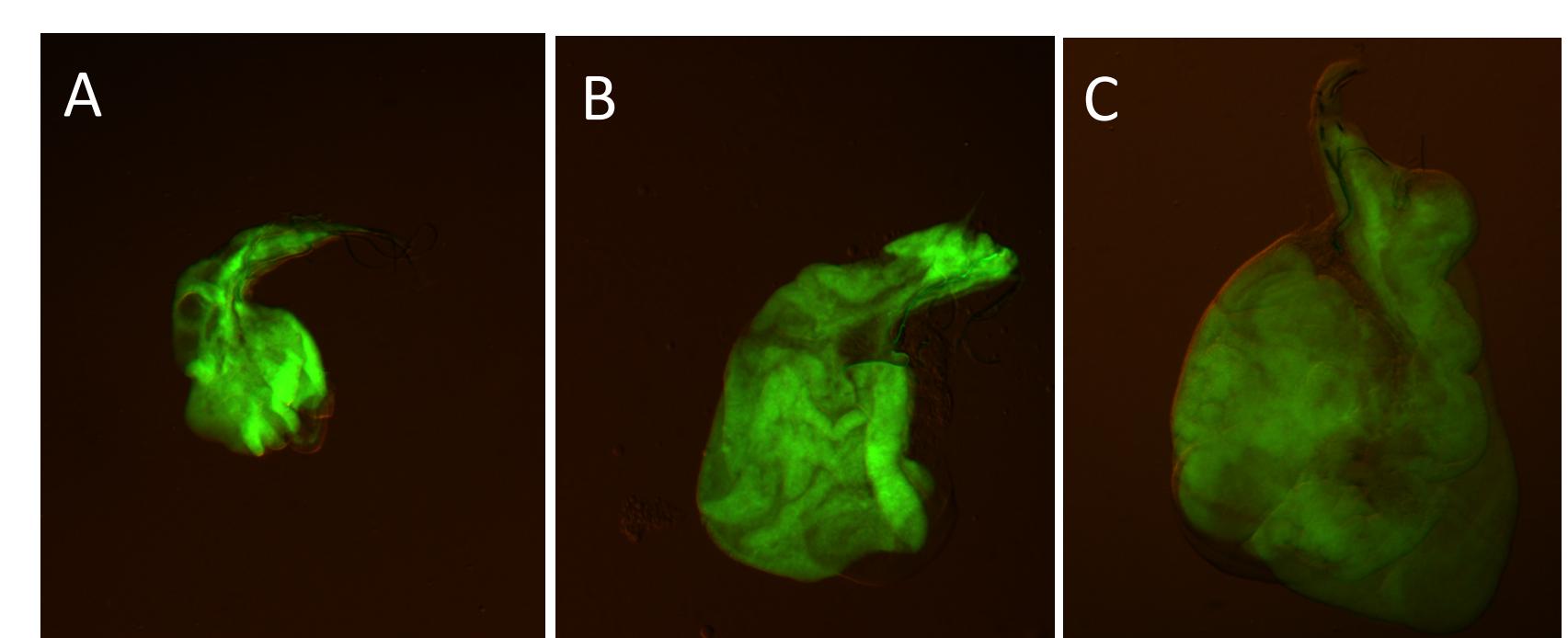


Figure 7: Phosphatase inhibitors that target the same phosphatase as compound 2B reduced tumor growth in the wing imaginal discs, but showed reduced efficacy when compared with compound 2B. (A) Wing disc was dissected from a larva treated with compound 2B. (B) Wing disc was dissected from a larva treated with compound 1B. (C) Wing disc was dissected from a larva treated with 3B.

Conclusions

- Six phosphatase inhibitors were shown to be effective in reducing Yorkie-induced tumor growth
- Pupation of *ap>yki* larvae is temperature-dependent
- Compound 2B may be involved in regulation of ecdysone (hormone required for pupation)
- DMSO may be involved in regulating pupation

Acknowledgements

Special thanks to:

- The Amgen Foundation for making this research possible
- Drs. Tama Hasson and Patty Phelps for their guidance and mentorship
- All members of the Martinez-Agosto lab for their assistance and support
- Josh Cary for his indispensable feedback

Compounds 7C, 8B, and 11C Caused a Reduction in Tumor Size

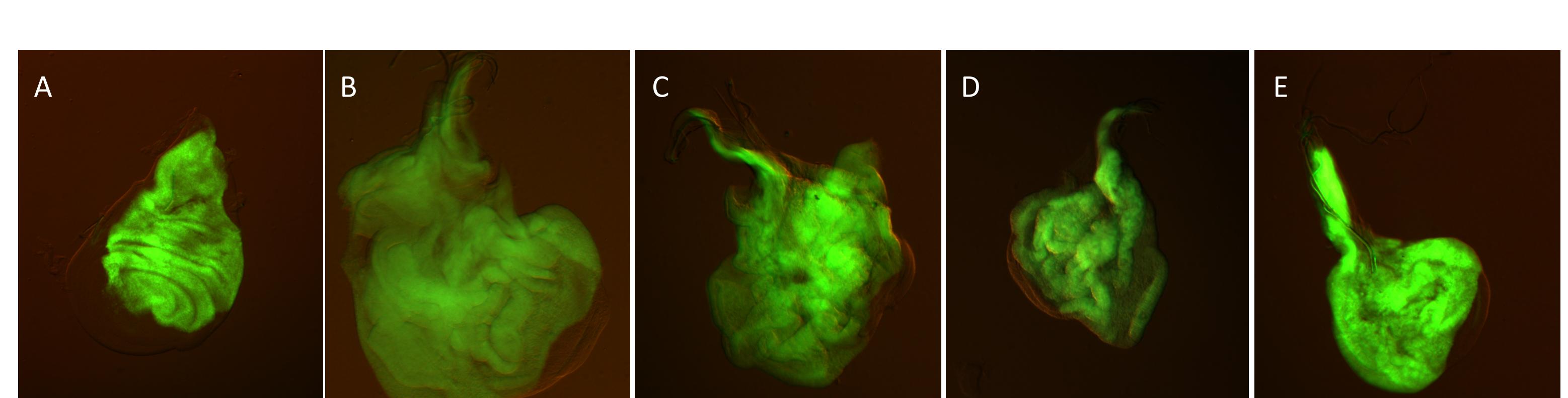


Figure 4: The three compounds that increased pupation rates by 30% or more, reduced tumor size in the wing imaginal discs. (A) Normal wing disc dissected from *ap>w1118* larvae. Larvae were grown at 25°C and treated with DMSO. (B) Tumorous wing disc dissected from *ap>yki* larvae. Larvae were grown at 25°C and treated with DMSO. (C) Wing discs dissected from *ap>yki* larvae treated with compound 7C show a slight reduction in tumor size. (D) Wing discs dissected from *ap>yki* larvae treated with compound 11C and (E) 8B show a moderate reduction in tumor size.