

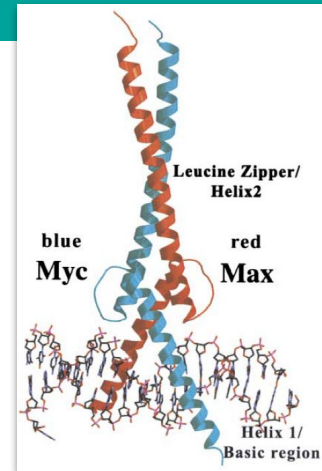
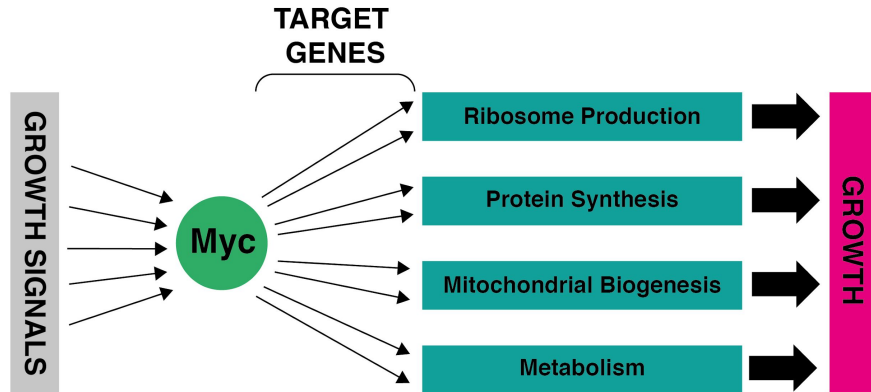


Upregulation of ribosome biogenesis via canonical E-boxes is required for Myc-driven proliferation

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Taipale Group**

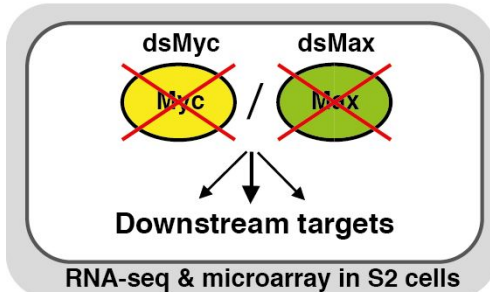
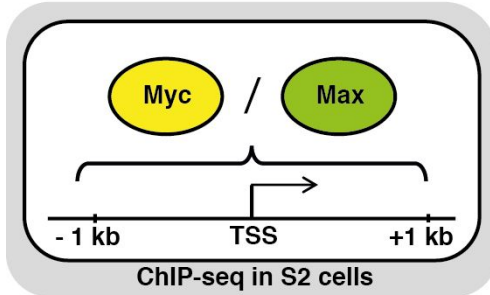
Defining the essential downstream targets of the Myc proto-oncogene

- Myc expression is deregulated in a wide range of human cancers and is often associated with aggressive, poorly differentiated tumors.
- Myc inhibition led to tumor regression in mouse models for various cancer types (Sur et al. 2011; Soucek et al. 2008), qualifying Myc inhibition as a potent strategy for treating diverse types of cancer.
- The Myc protein exhibits no features allowing rational drug design. However, inhibition of crucial downstream targets should be equally effective.

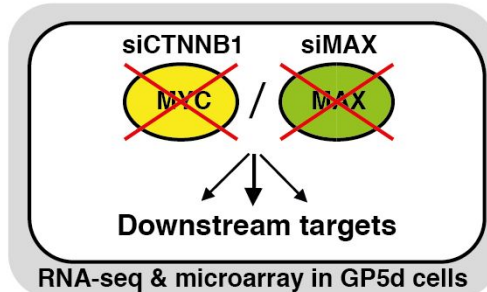
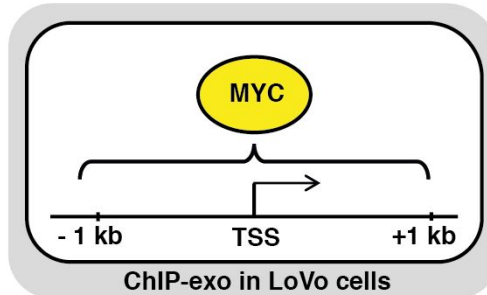


Identification of core Myc targets that are conserved between humans and flies

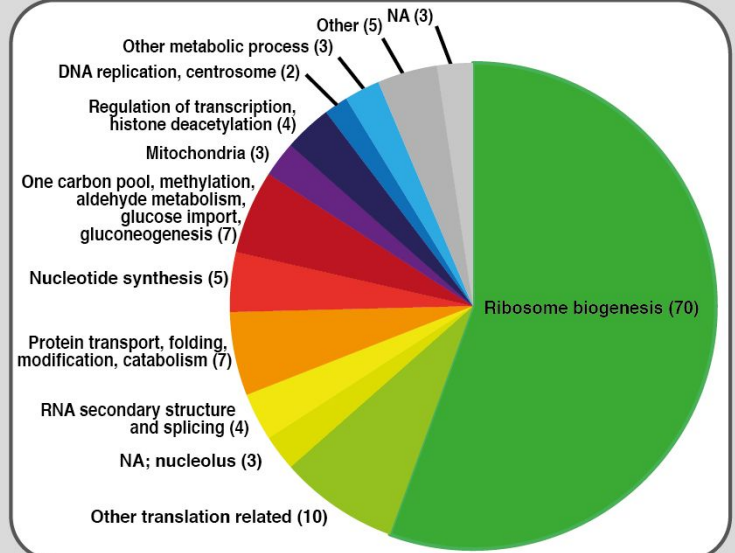
Drosophila melanogaster



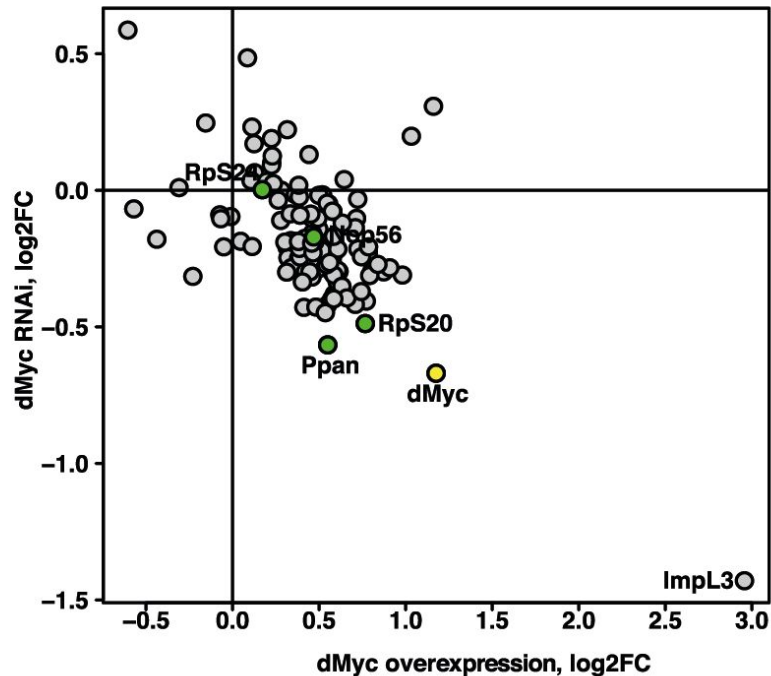
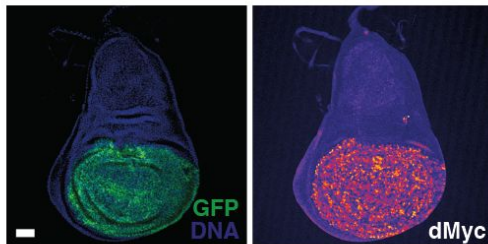
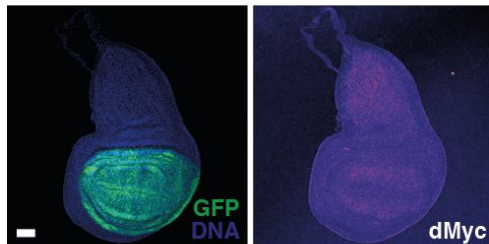
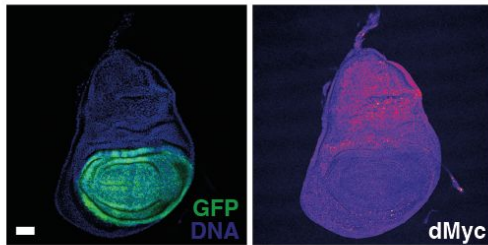
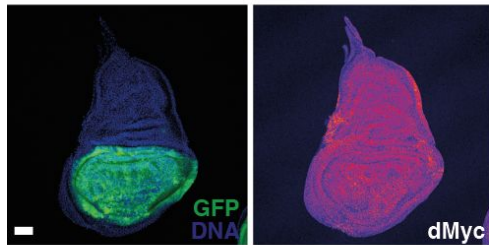
Human



Functionally conserved MYC targets between human (126) and fly (124)

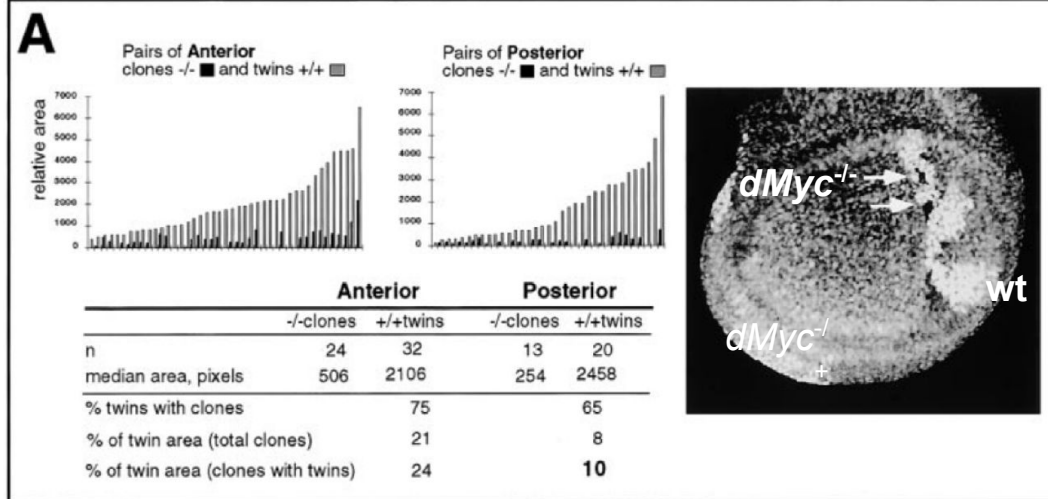
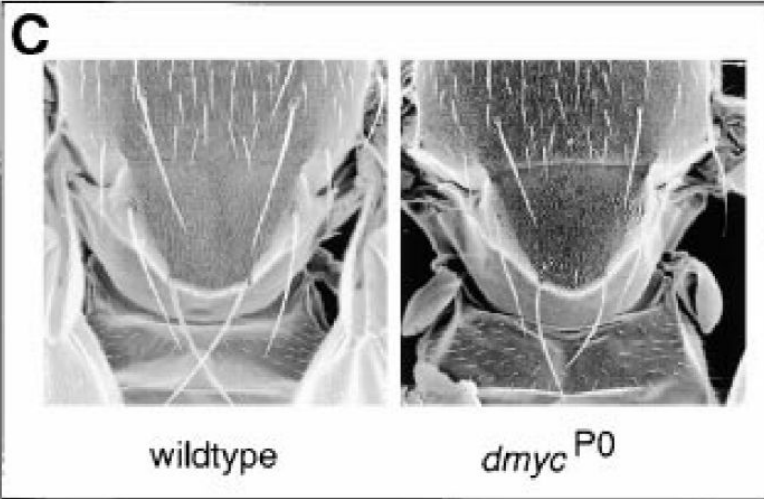


RNA-seq profiling of Myc-responsive genes in *Drosophila* wing discs



Hypomorphic dMyc shows attributes of the Minute phenotype

- Minute phenotypes are caused by more than 60 genes, of which many encode ribosomal genes (Marygold et al., 2007).
- Minutes are haploinsufficient and heterozygous adult flies have small, thin cuticular bristles (Bridges and Morgan, 1923).
- Minute mutants are characterized by a delay of 2–3 days in the emergence of adult flies due to an extension of larval and pupal development by 20%–30%.



CrispR/Cas9-mediated deletion of the E-Box of *Drosophila* RpS24

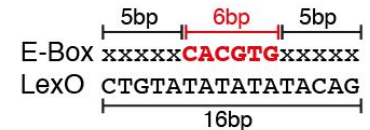
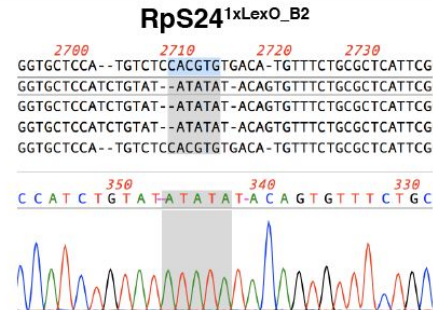
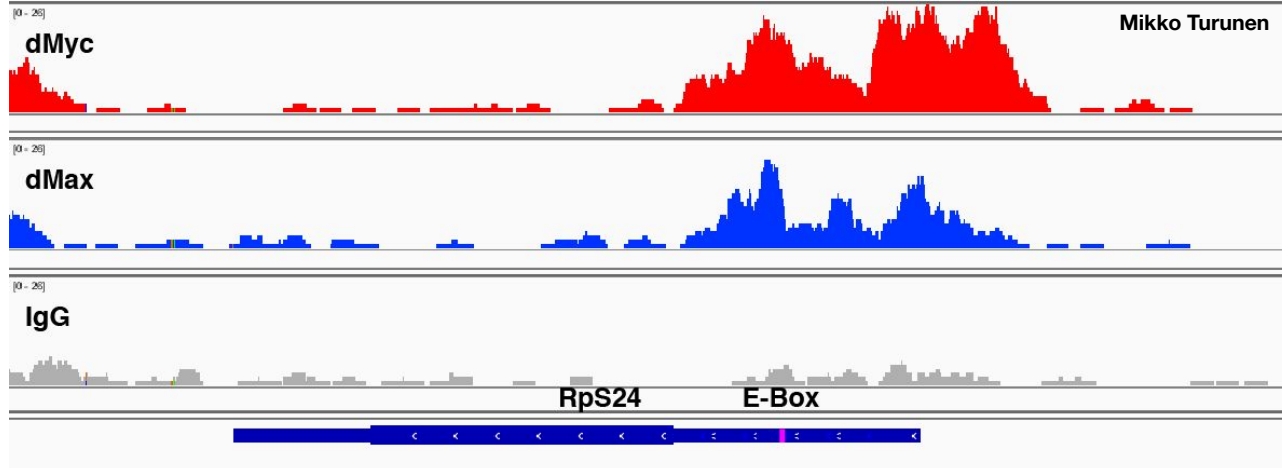
WILD TYPE



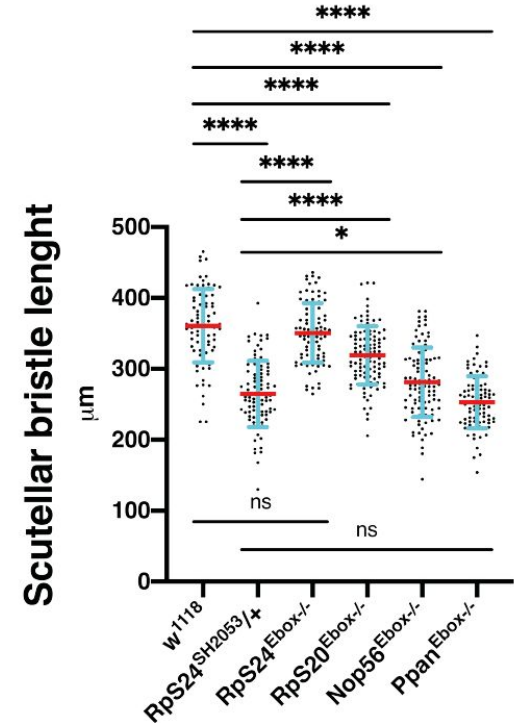
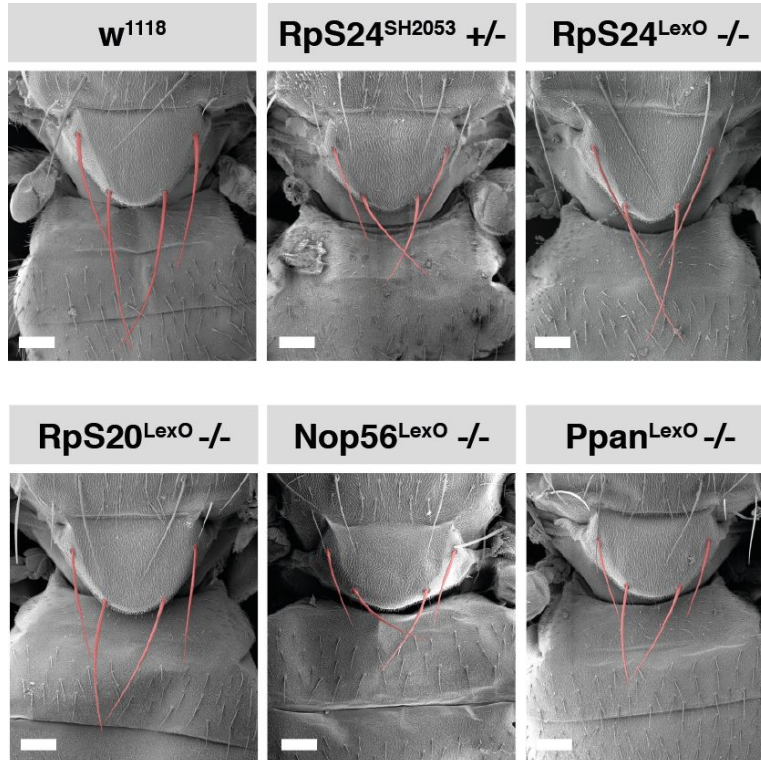
E-BOX MUTANT



- 65 of the 124 conserved Myc targets have canonical E-Boxes (CACGTG).
- 24 genes have been targeted with CRISPR/Cas9 to replace the E-Boxes by LexO sites
- 15 E-Box deleted strains have been validated by WGS and cloning of the locus.

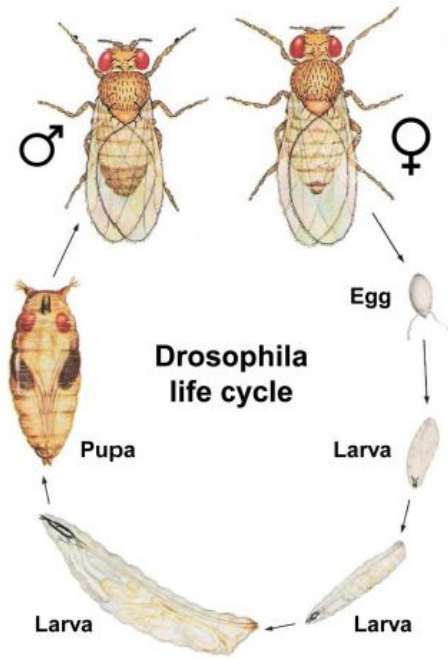


Homozygotes of E-Box-deleted Ppan display a *Minute*-like bristle phenotype, but E-Box deletions in genes encoding ribosomal proteins are essentially normal

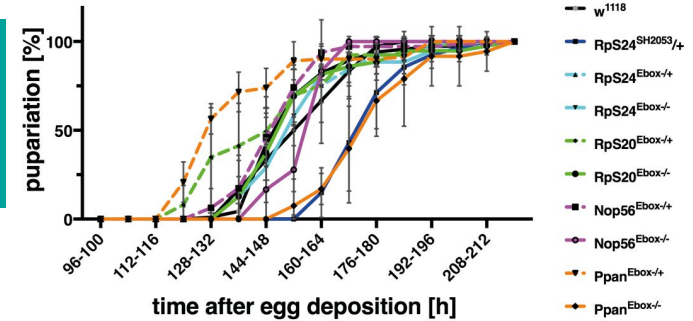


Deletion of the E-Box of Ppan causes a developmental delay

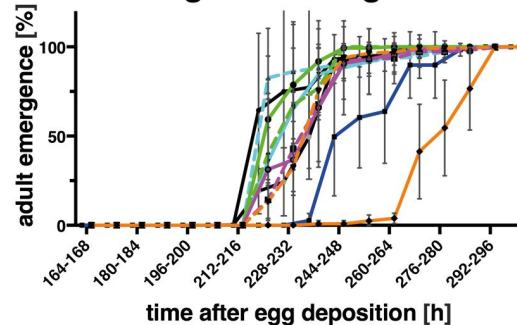
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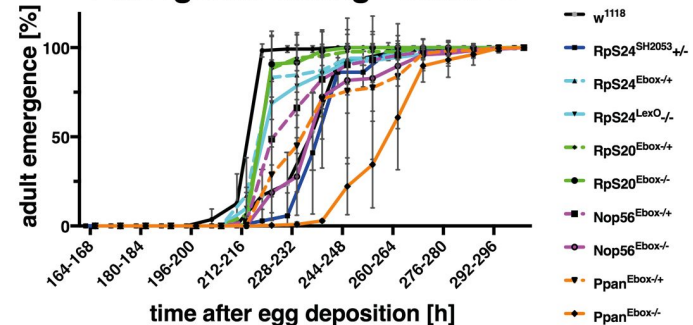
Pupariation timing



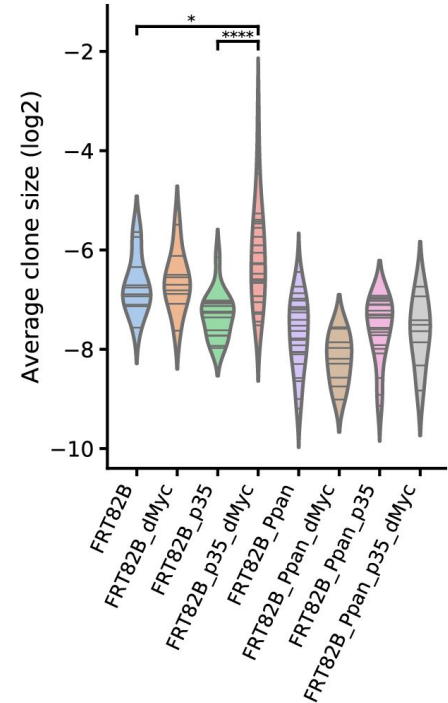
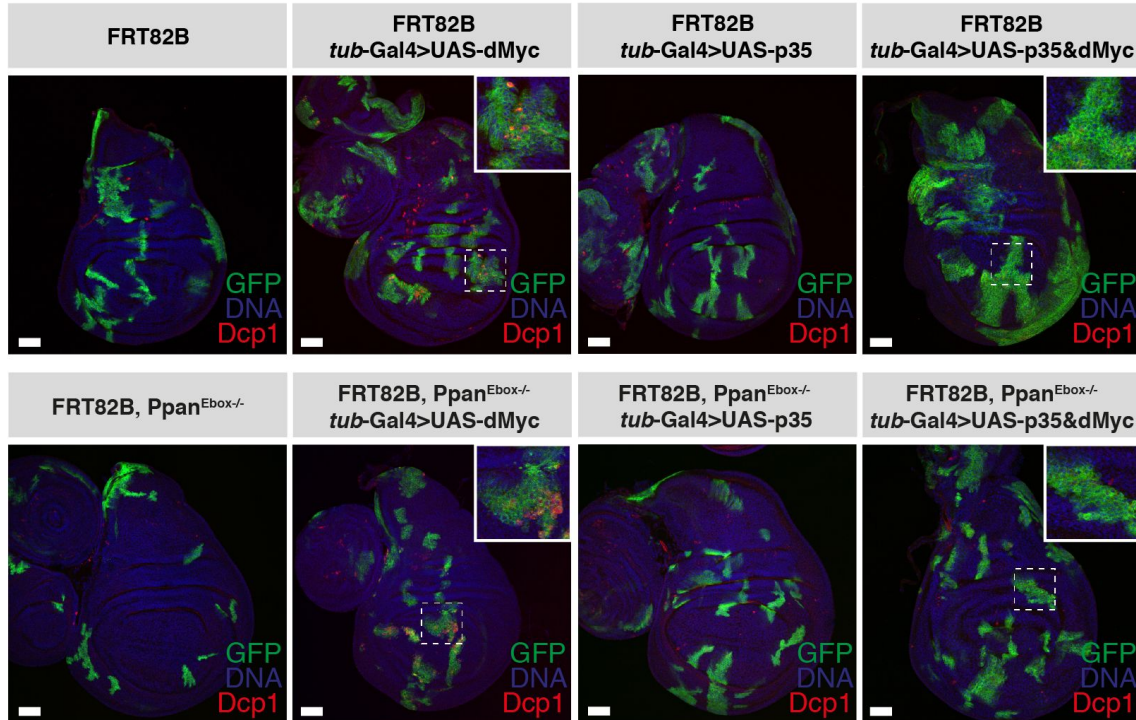
Emergence timing - males



Emergence timing - females



Ppan is critical for Myc-induced growth



Summary

- CRISPR/Cas9-mediated deletion of regulatory elements allows to delineate complex processes such as cellular growth, which was difficult with traditional genetic approaches.
- Despite the complexity of the MYC regulated network, it is possible to specifically prevent regulation of a single MYC target gene in such a way that development proceeds largely normally, yet the ability of MYC to drive growth is impeded.
- Deletion of the E-Box of SSF1/2 ortholog Peter Pan (Ppan) results in a Minute-like phenotype, similar to Myc hypomorphic mutants
- Ppan is the bonafide ortholog of yeast SSF1/SSF2, which is involved in rRNA processing.
- Induction of Ppan expression is crucial for Myc-induced growth in *Drosophila* wing discs.

