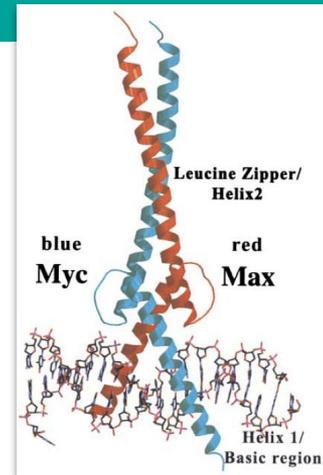
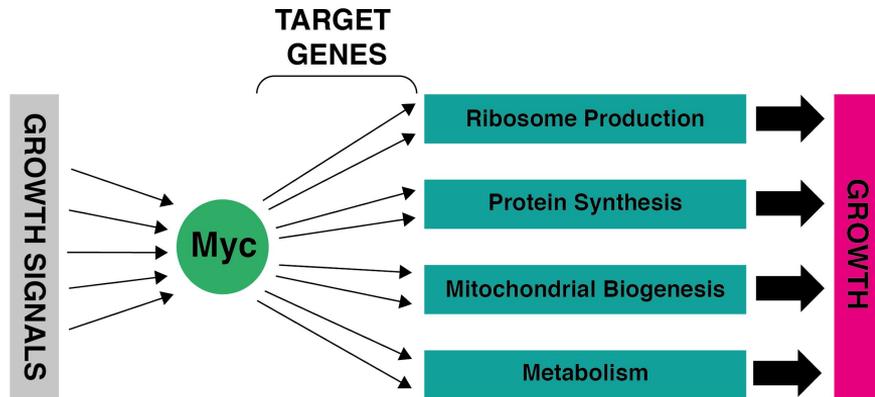


**Upregulation of ribosome  
biogenesis via canonical E-boxes  
is required for Myc-driven  
proliferation**

**Norman Zielke  
Taipale Group**

# Defining the essential downstream targets of the Myc proto-oncogene

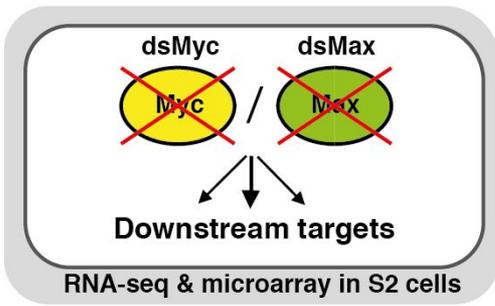
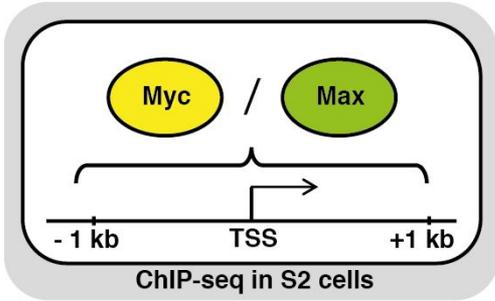
- Myc expression is deregulated in a wide range of human cancers and is often associated with aggressive, poorly differentiated tumors.
- Myc inhibition led to tumor regression in mouse models for various cancer types (Sur et al. 2011; Soucek et al. 2008), qualifying Myc inhibition as a potent strategy 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.



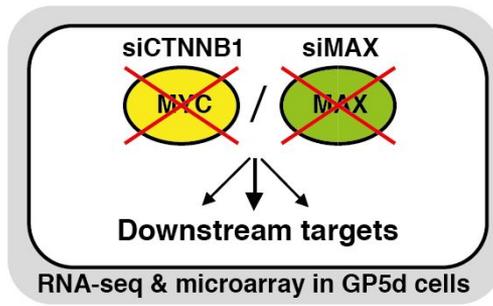
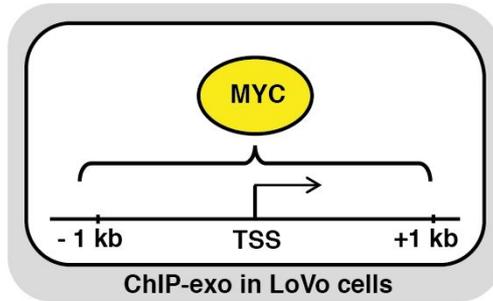


# Identification of core Myc targets that are conserved between humans and flies

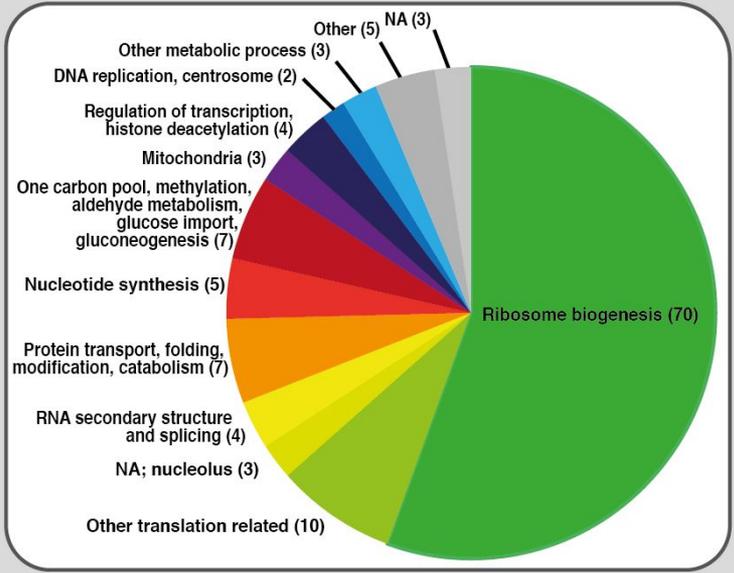
## *Drosophila melanogaster*



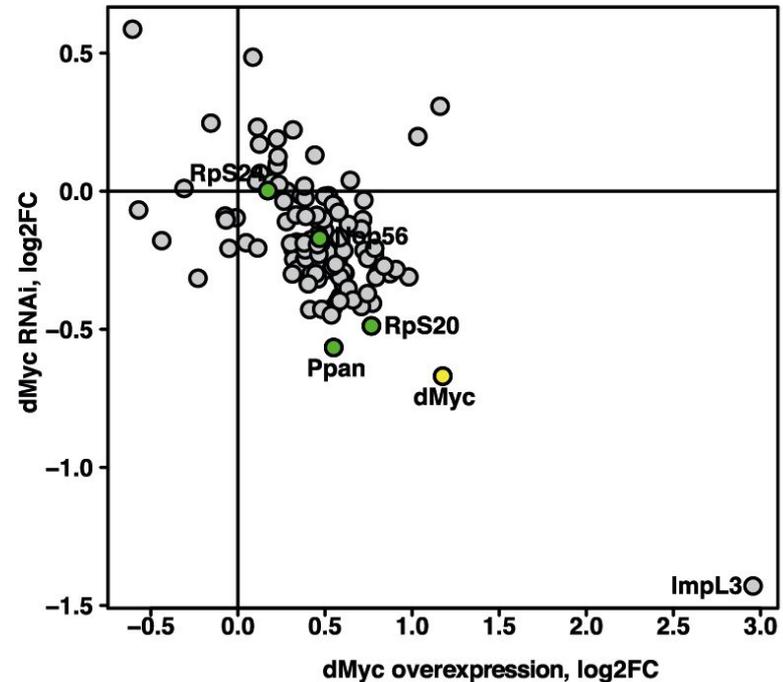
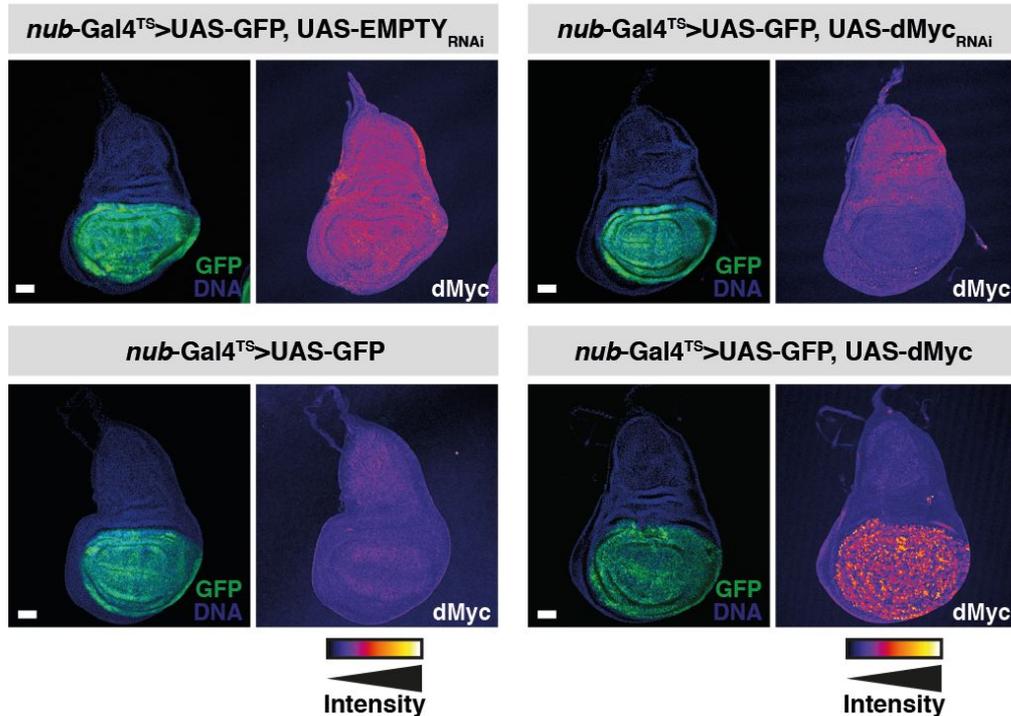
## Human



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

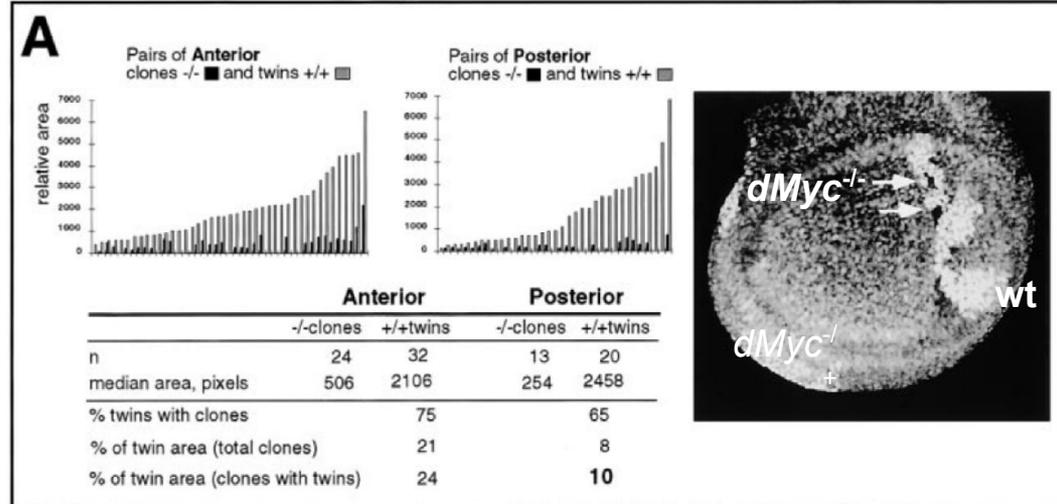
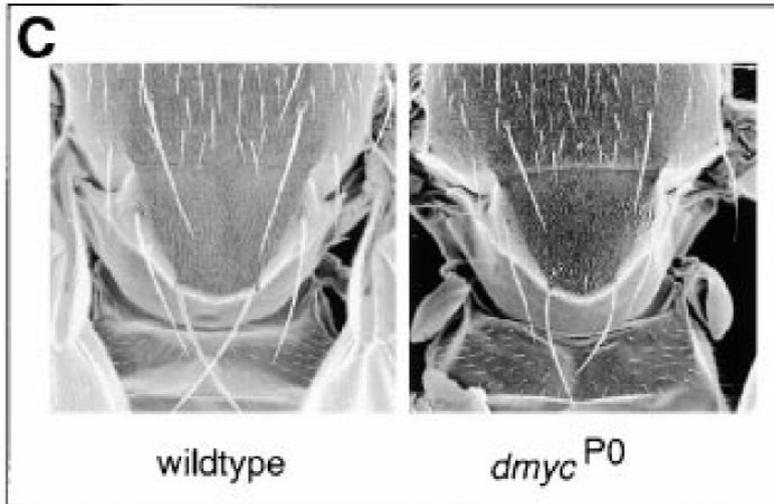


# RNA-seq profiling of Myc-responsive genes in *Drosophila* wing discs



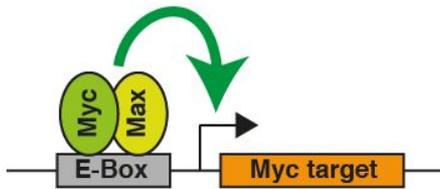
# Hypomorphic dMyc shows attributes of the Minute phenotype

- 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

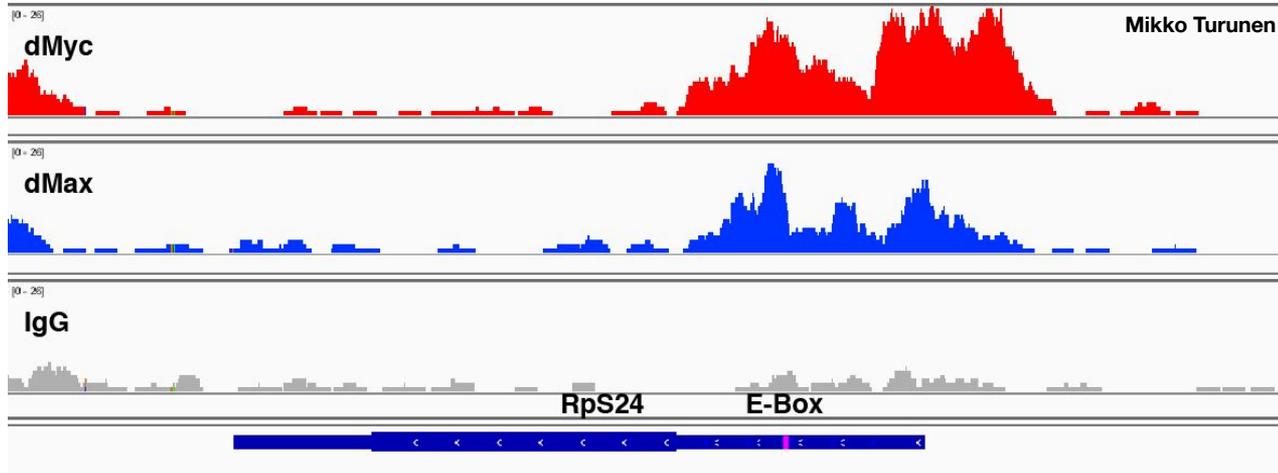
WILD TYPE



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.



Mikko Turunen

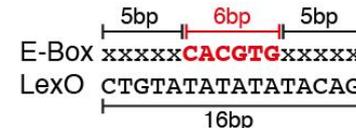
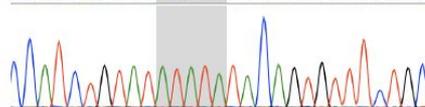
RpS24<sup>1xLexO\_B2</sup>

```

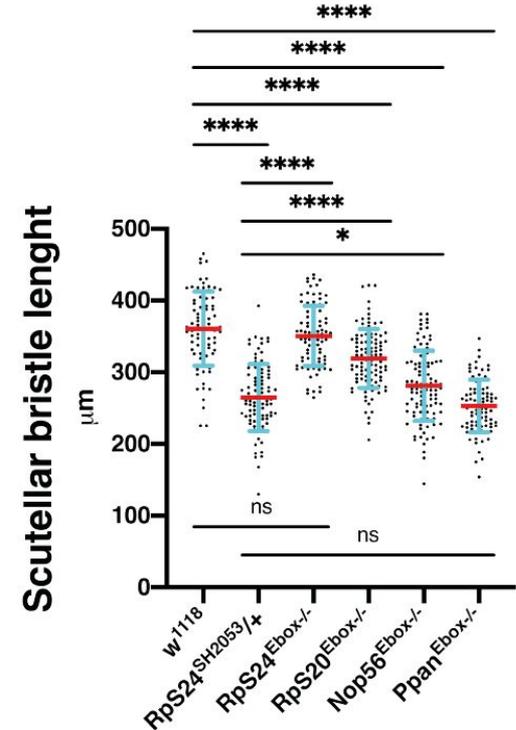
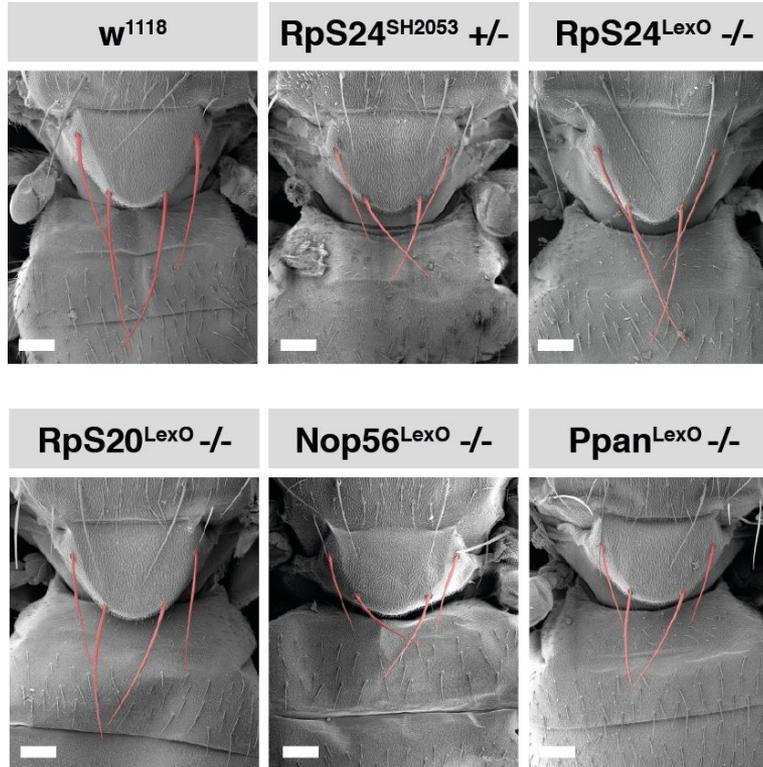
2700      2710      2720      2730
GGTGCTCCA--TGTCTCCAGGTGACA-TGTTCTGCGCTCATTGG
GGTGCTCCATCTGTAT--ATATAT-ACAGTGTTTCTGCGCTCATTCCG
GGTGCTCCATCTGTAT--ATATAT-ACAGTGTTTCTGCGCTCATTCCG
GGTGCTCCATCTGTAT--ATATAT-ACAGTGTTTCTGCGCTCATTCCG
GGTGCTCCA--TGTCTCCAGGTGACA-TGTTCTGCGCTCATTCCG
  
```

```

350      340      330
CCATCTGTATATATATACA GTGTTTCTGC
  
```

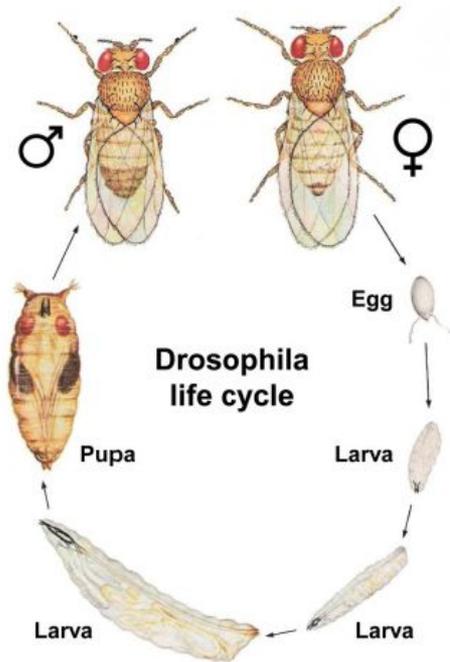


# Homozygotes of E-Box-deleted Ppan display a *Minute*-like bristle phenotype, but E-Box deletions in genes encoding ribosomal proteins are essentially normal

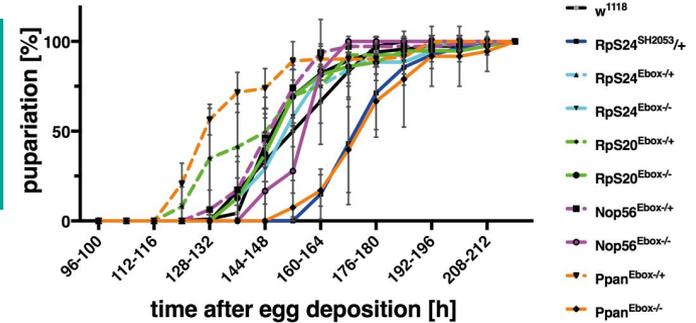


# Deletion of the E-Box of Ppan causes a developmental delay

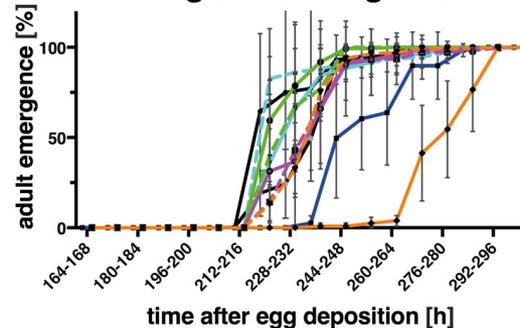
*Minute* mutants are characterized by a delay of 2–3 days in the emergence of adult flies due to an extension of larval and pupal development by 20%–30%.



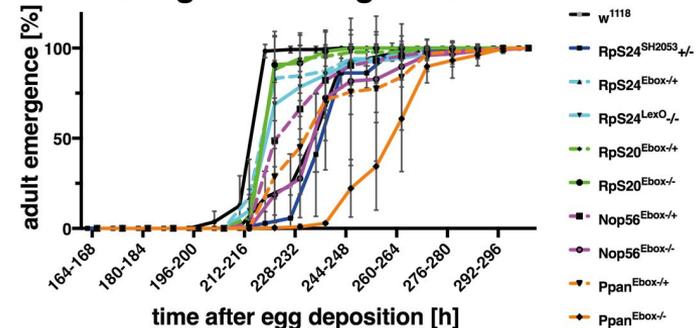
### Pupariation timing



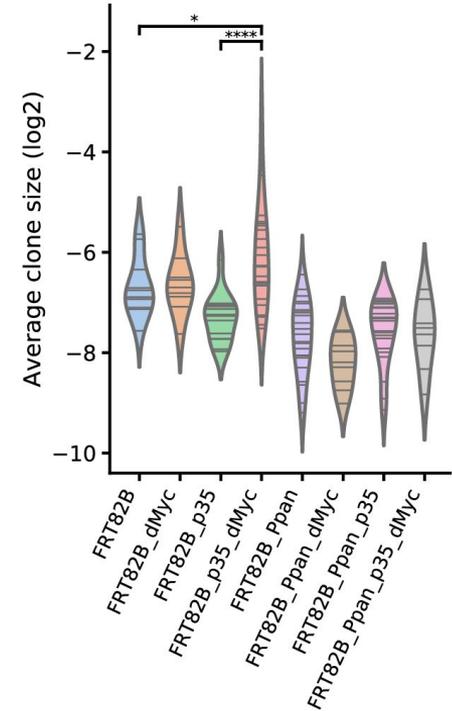
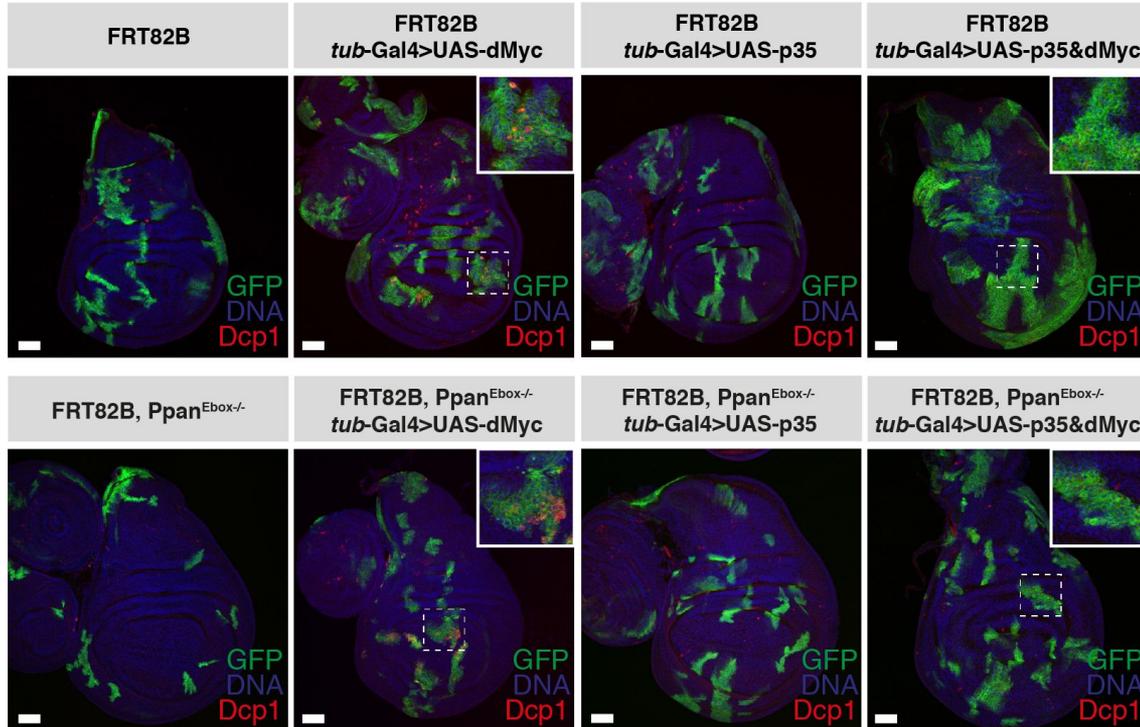
### Emergence timing - males



### 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.

