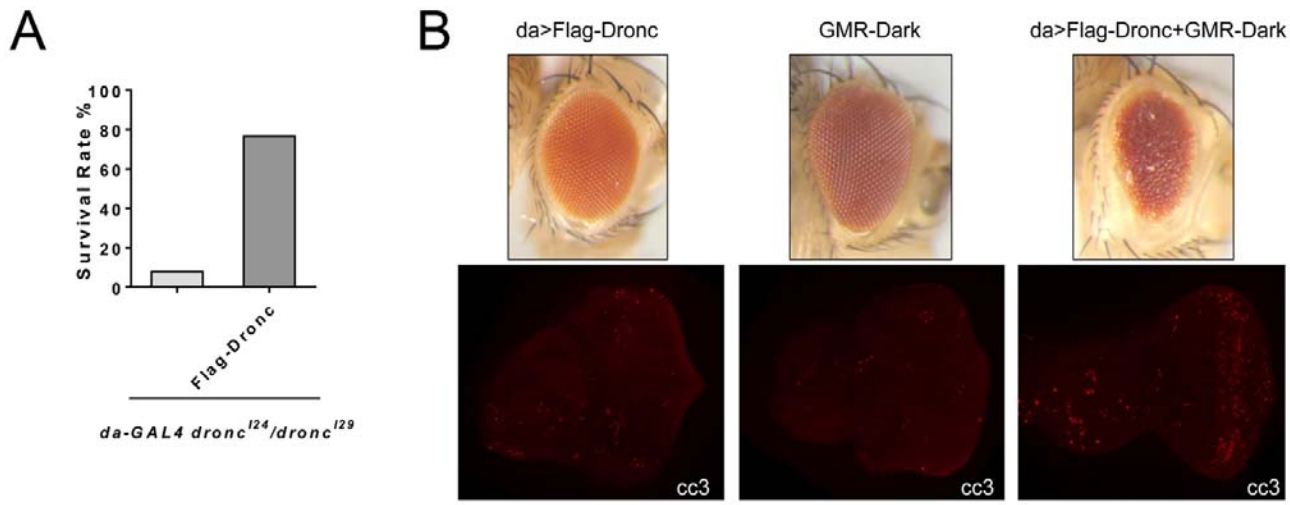
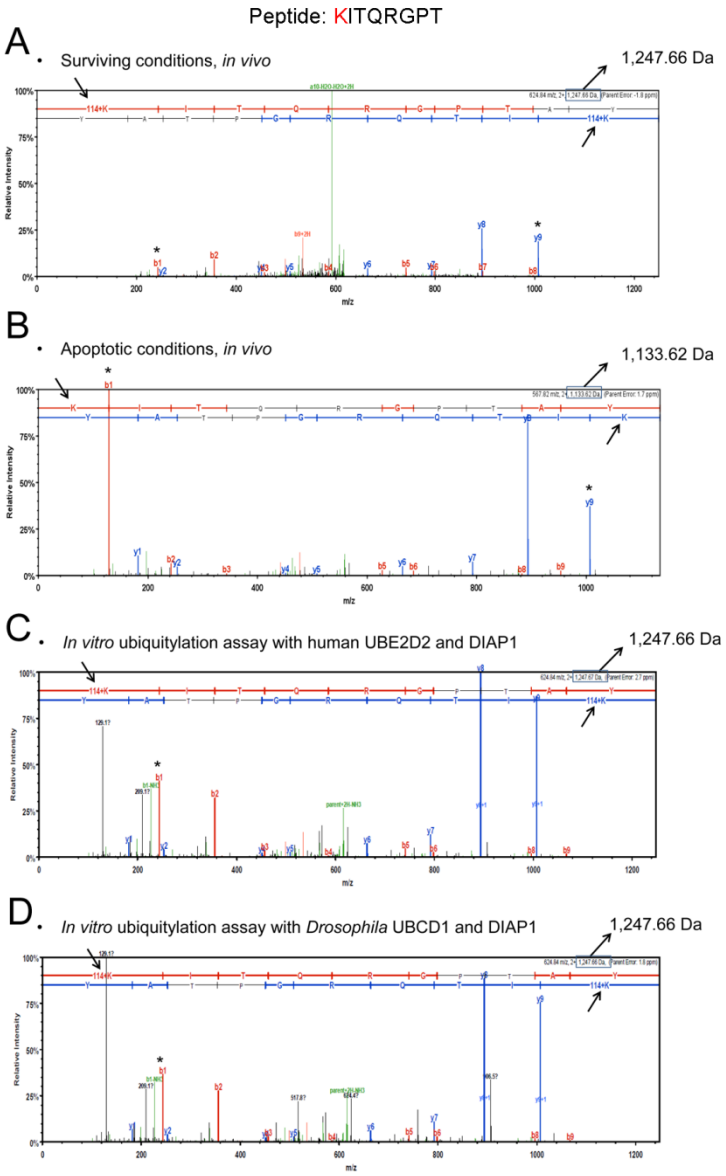
SUPPLEMENTAL FIGURES



Supplementary Figure S1. *Flag-Dronc^{wt}* is functional.

(**A**) *Flag-Dronc* can rescue the lethality associated with *dronc* null mutations.

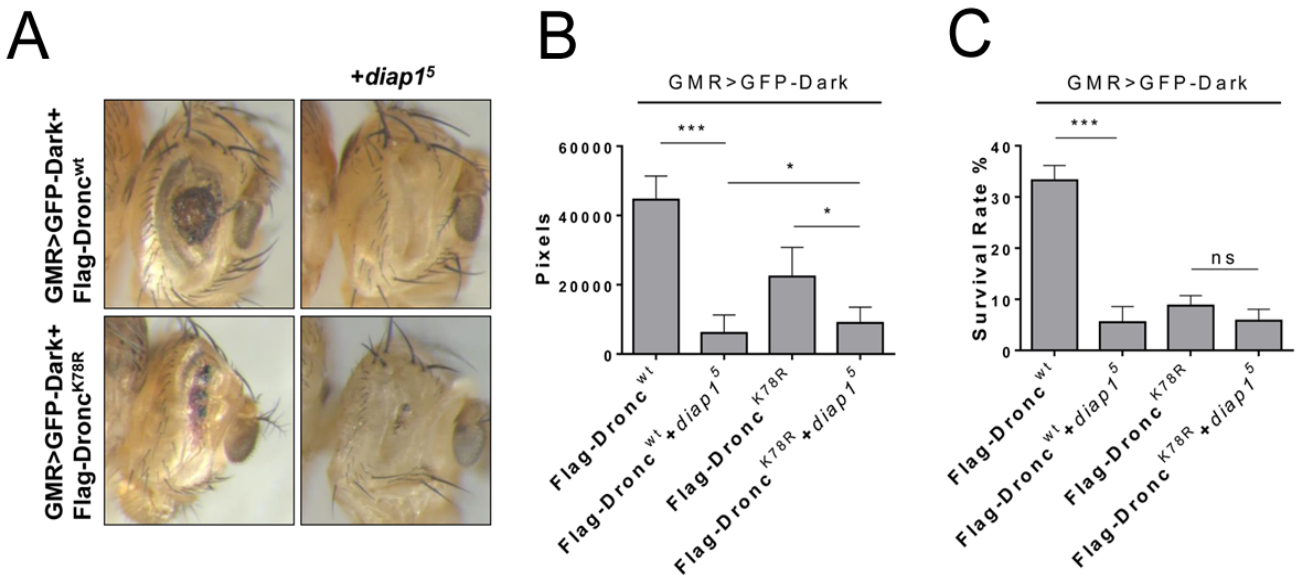
(**B**) *Flag-Dronc* can be activated in the apoptosome. Expression of either *da>Flag-Dronc* or *GMR-Dark* does not lead to any caspase (cleaved caspase-3, cc3) activity. However, when these transgenes are co-expressed (*da>Flag-Dronc+GMR-Dark*), caspase activity is increased in the posterior domain.



Supplementary Figure S2. LC-MS/MS analysis shows that Dronc is ubiquitylated at K78 under surviving, but not apoptotic conditions.

(A,B) Of the peptides obtained by Chymotrypsin digests of immunoprecipated Dronc from larval and pupal extracts under surviving conditions (A), only the peptide K^{78}ITQRGPT was found to carry the di-Glycine signature indicative of ubiquitin modification. di-Glycine is derived from conjugated ubiquitin and adds 114 Da to this peptide. Correspondingly, all b peaks of this peptide obtained under surviving conditions (A) are shifted compared to the b peaks under apoptotic conditions (B); see asterisk at peak b1 as example.

(C,D) LC-MS/MS analyses of *in vitro* ubiquitylated Dronc with Diap1 as E3 ligase and either human UBE2D2 (C) or *Drosophila* UBCD1 (D) as E2 conjugating enzymes show that K78 can be ubiquitylated by DIAP1.
Arrows indicate 114 Da mass shift due to ubiquitylation on K78.

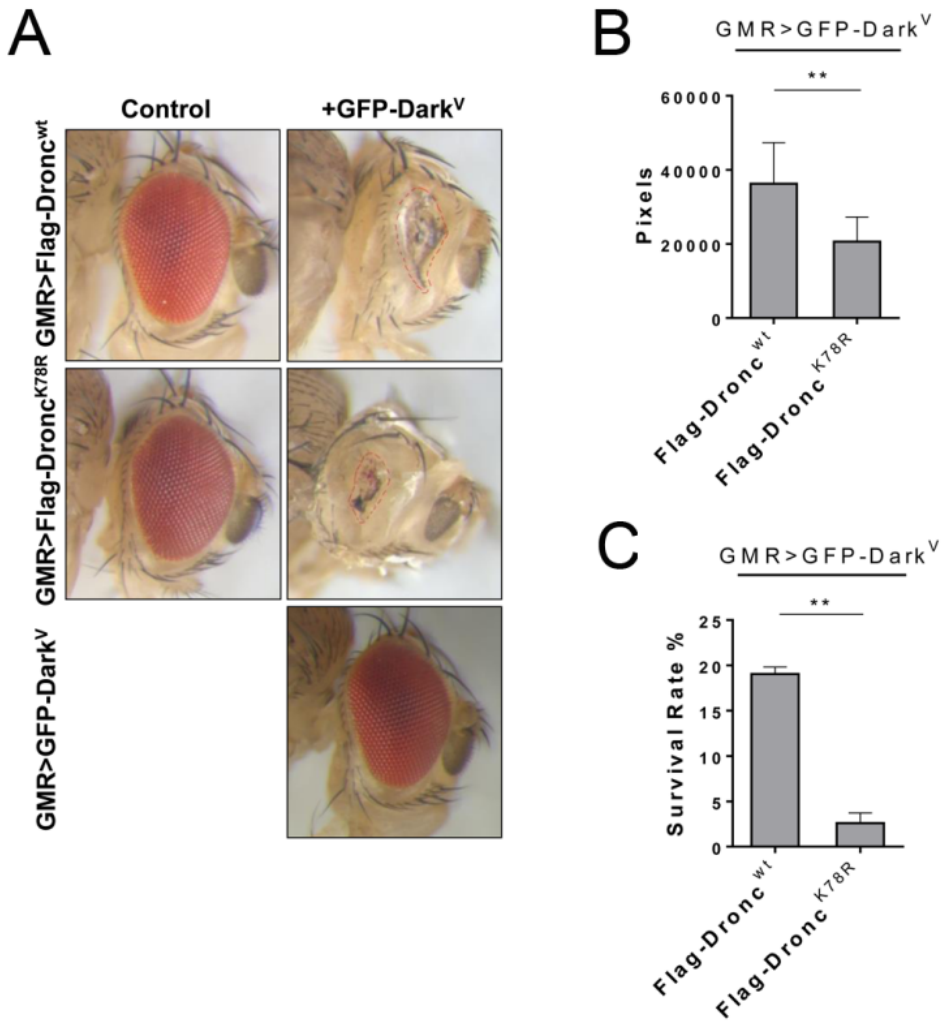


Supplementary Figure S3. Heterozygous *diap1*^{5} mutant strongly enhances *GMR>FlagDronc*^{wt} +*GFP-Dark* eye phenotype, but only weakly enhances *GMR>Flag-Dronc*^{K78R}+*GFP-Dark*.

(A-C) Loss of one copy of *diap1* strongly enhances eye phenotype of *GMR>Flag-Dronc*^{wt}+*GFP-Dark* (quantified in B) and causes a significant increase in lethality (quantified in C). In contrast, *diap1* heterozygosity only weakly enhances *GMR>Flag-DroncK78R+GFP-Dark* eye phenotype (quantified in B) and lethality (quantified in C). (**B**) Quantification of eye size phenotypes in (A). n=9 for *GMR>Flag-Dronc*^{wt}+*GFP-Dark* , n=11 for *GMR>Flag-Dronc*^{wt}+*GFP-Dark+diap1*^{5}, n=8 for *GMR>Flag-Dronc*^{K78R}+*GFP-Dark, n=11 for GMR>Flag-Dronc*^{K78R}+*GFP-Dark+diap1*^{5}

(C) Quantification of eclosion rates of *GMR>Flag-Dronc*^{wt}+*GFP-Dark and GMR>Flag-Dronc*^{K78R}+*GFP-Dark* with or without loss of one copy of *diap1*.

For quantifications, the student's t-test was used. Error bars are SD. * P<0.05; *** P<0,001; ns – not significant.



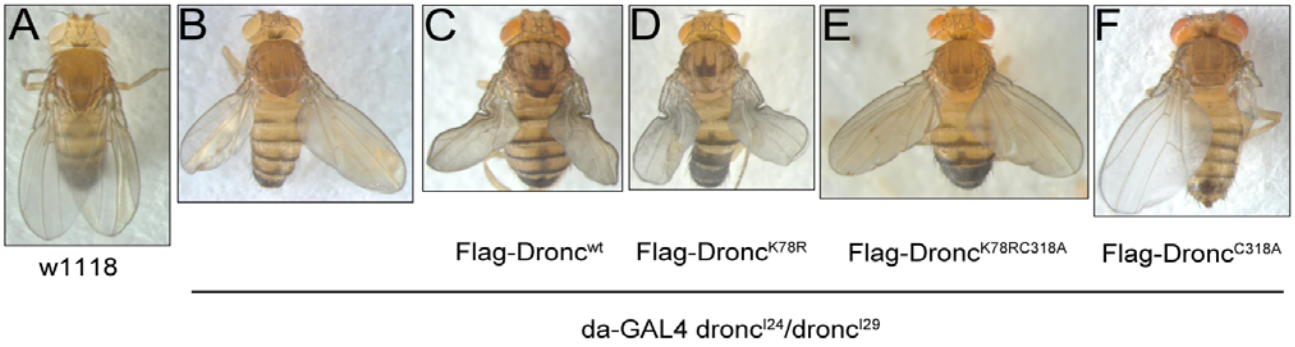
Supplementary Figure S4. Cleavage resistant Dark^V, but not cleaved-mimic Dark^{CC}, can form a more functional apoptosome with Flag-Dronc^{K78R} than with Flag-Dronc^{wt}.

(A) Expression of *GMR>Flag-Dronc^{K78R}+GFP-Dark* resulted in significantly smaller eyes than *GMR>Flag-Dronc^{wt} +GFP-Dark*. Expression of *GMR>GFP-Dark^V* alone does not have any eye phenotype.

(**B**) Quantification of eye size phenotypes in (A). n=10 for each genotype

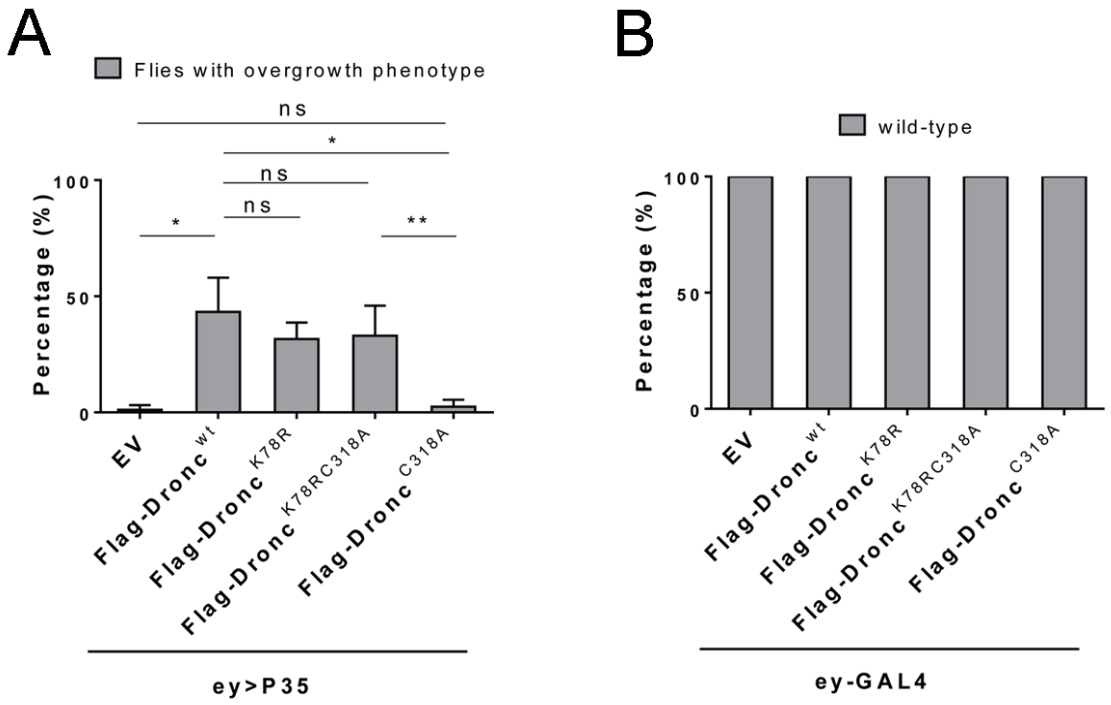
(C) Eclosion rates of flies expressing *GMR>Flag-Dronc^{K78R} +GFP-Dark^V* are significantly smaller than *GMR>Flag-Dronc^{wt} +GFP-Dark^V*.

For quantifications, the student's t-test was used. Error bars are SD. ** P<0.01.



Supplementary Figure S5. Both *Flag-Dronc^{K78RC318A}* and *Flag-Dronc^{C318A}* cannot rescue the wing phenotype of *dronc* null mutants.

Compared to control flies (A, *w^{1118}*), wings from *dronc* null mutants are held-out, often irregularly shaped and less transparent (B). Often one wing is missing (see (F)). *da>Flag-Dronc^{K78RC318A}* (E) and *da>Flag-Dronc^{C318A}* (F) do not rescue this phenotype. In contrast, Flag-Dronc^{wt} and Flag-Dronc^{K78R} rescue the wing phenotype of *dronc* null mutants (C,D). However, these wings do not appear normal because of ectopic apoptosis (for details see reference [1]).

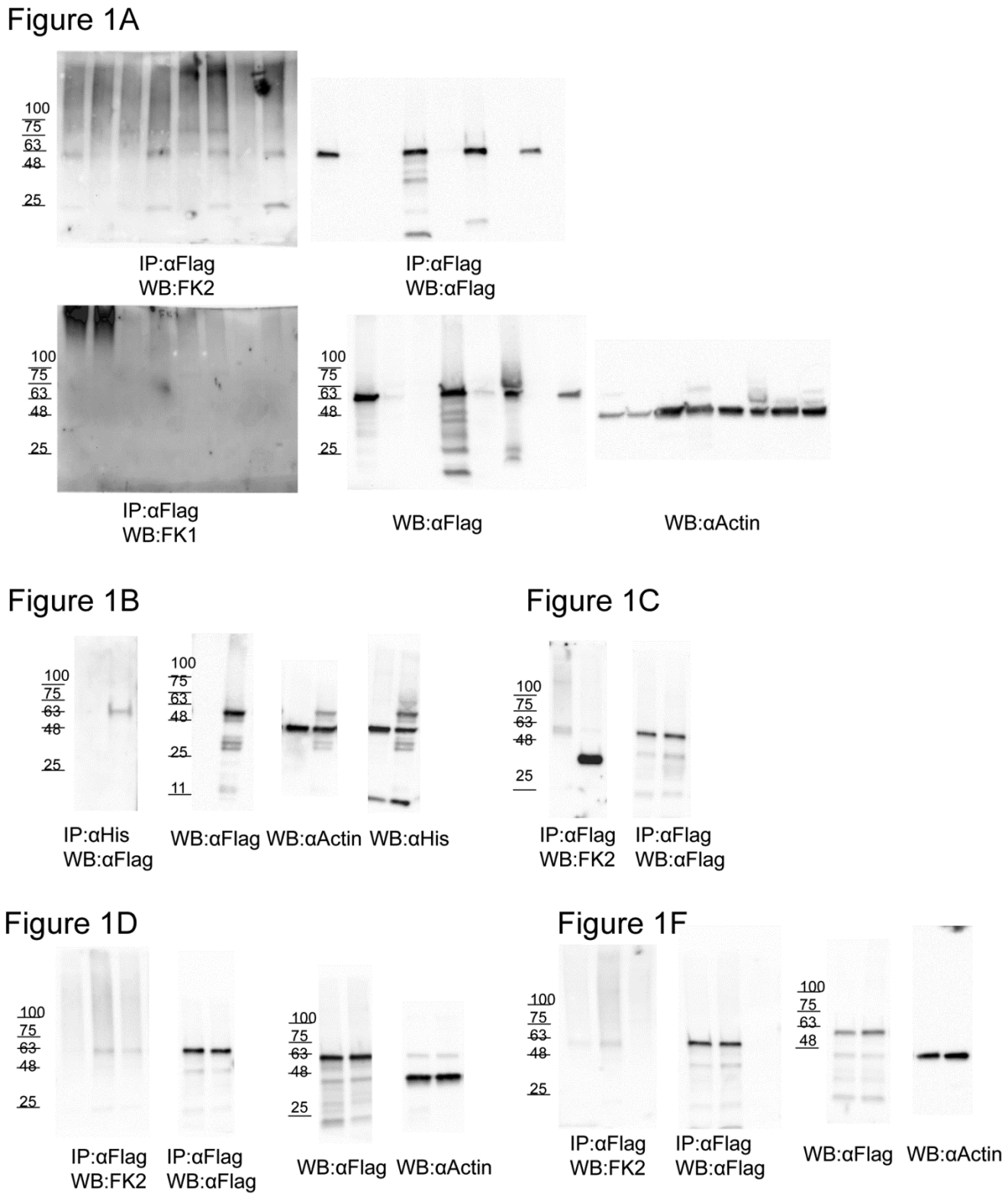


Supplementary Figure S6. *Flag-Dronc*^{K78R} and *Flag-Dronc*^{K78RC318A} can induce a head capsule overgrowth phenotype.

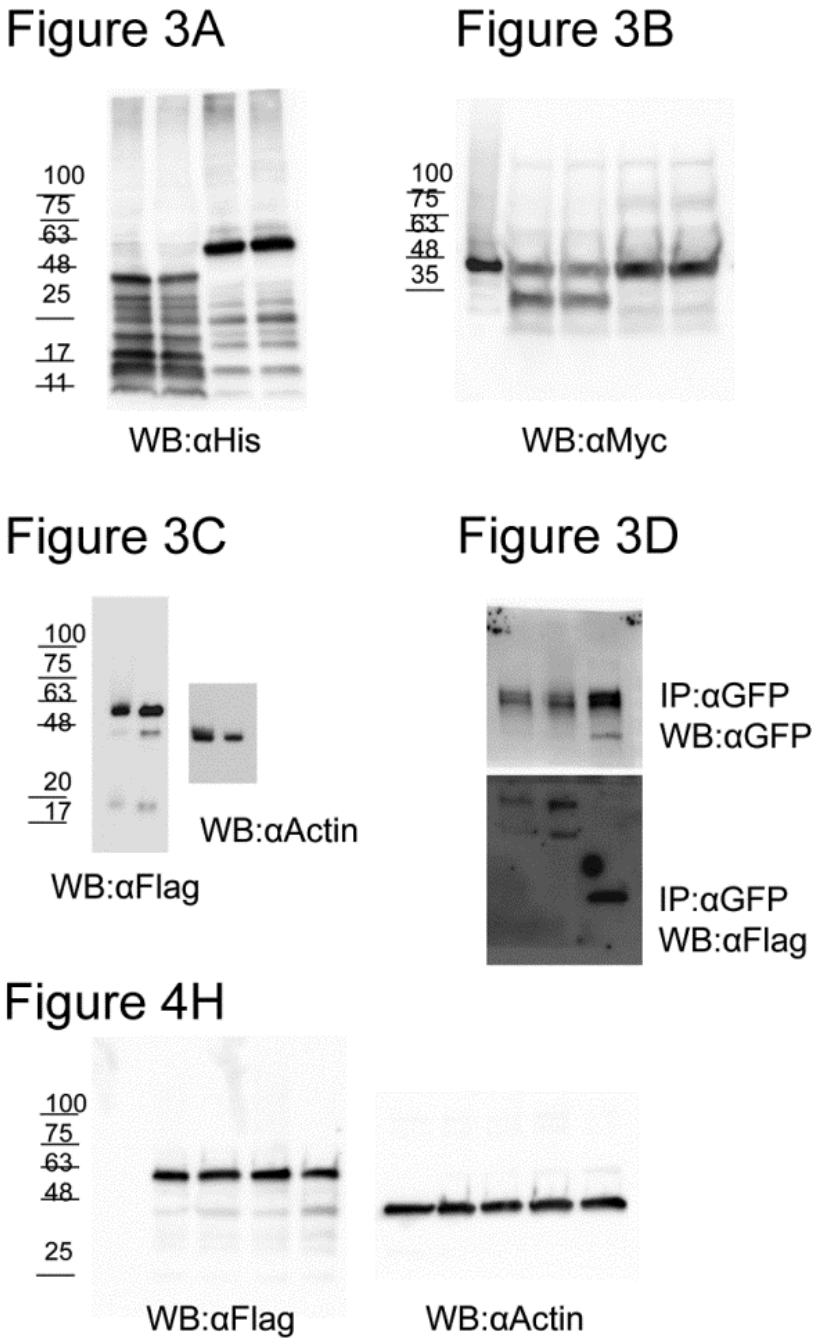
(A) Expression of *Flag-Dronc*^{wt}, *Flag-Dronc*^{K78R} and *Flag-Dronc*^{K78RC318A} in *ey>p35* background can induce overgrowth phenotypes. Overgrowth is characterized by expanded head cuticle with pattern duplications such as bristles and ocelli. In contrast, *Flag-Dronc*^{C318A} cannot induce this phenotype.

(B) Expression of indicated *Flag-Dronc* constructs with *ey-GAL4* does not lead to any eye phenotype.

For quantifications, the student's t-test was used. Error bars are SD. * P<0.05; ** P<0.01; ns – not significant.



Supplementary Figure S7. Uncropped immunoblots of the blots in Figure 1.



Supplementary Figure S8. Uncropped immunoblots of the blots in Figures 3 and 4.

Supplemental References:

1. Yang CS, Thomenius MJ, Gan EC, Tang W, Freel CD, et al. (2010) Metabolic regulation of Drosophila apoptosis through inhibitory phosphorylation of Dronc. EMBO J 29: 3196-3207.