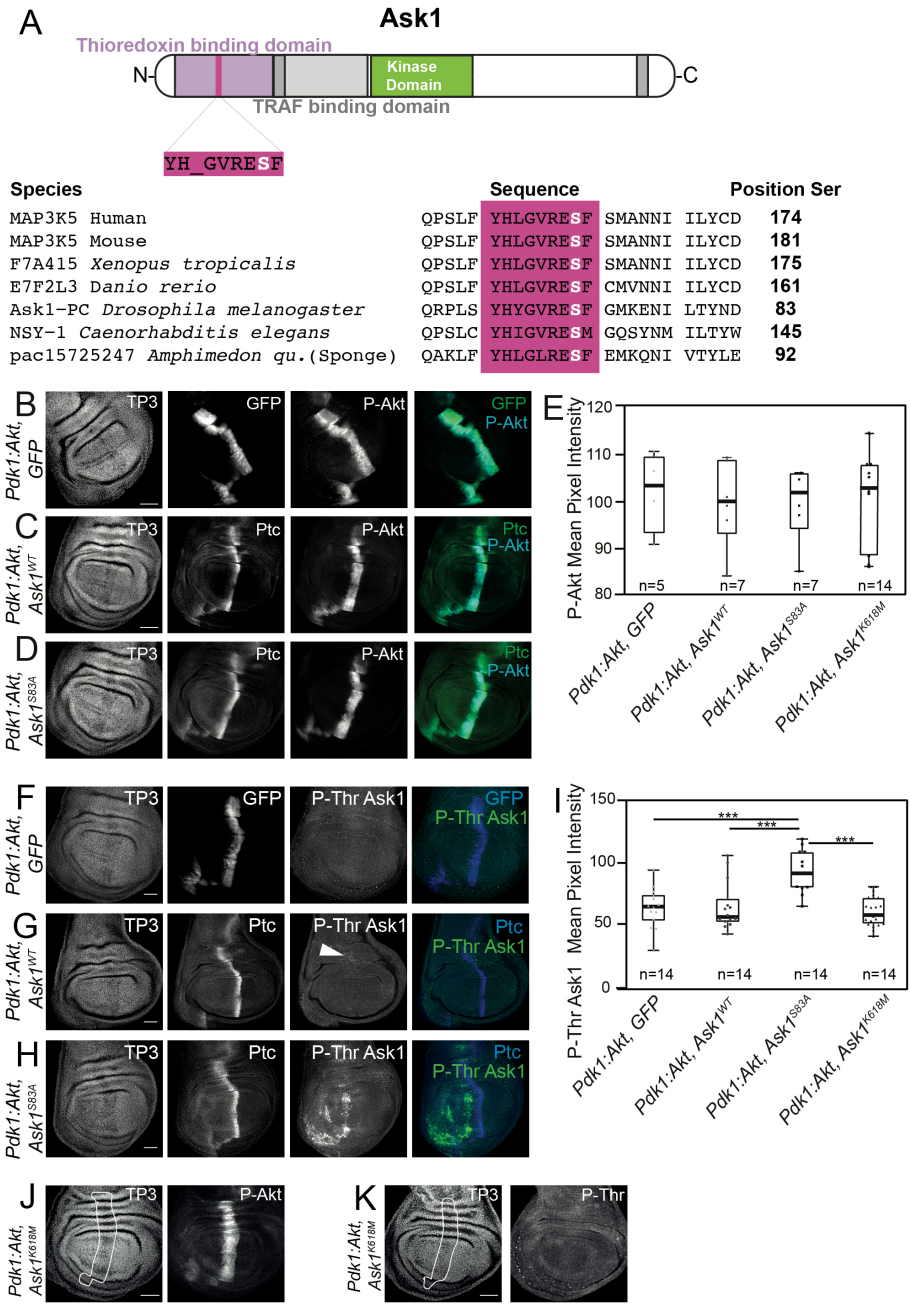

Figure S1. (A) The main domains of a canonical Ask1 protein. The position of the YH_GVRESF sequence is indicated, as well as the Ser83 residue of the *Drosophila* Ask1 protein. Conservation between some organisms is shown. Note the consensus YH_GVRESF sequence. Full comparative sequences of the Ask1 proteins are published in (Santabárbara-Ruiz et al., 2019). Thioredoxin is responsive to oxidative stress and dissociates from the thioredoxin-domain to promote Ask1 activation. Dark gray, the C- and N-terminal coiled-coil domains involved in homodimerization. (B-E) Anti-P-Akt antibody staining to show the expression of *Pdk1:Akt* of the transgene

combinations indicated. (B) *Pdk1:Akt* and *GFP* co-expression; (C) *Pdk1:Akt* and $Ask1^{WT}$ co-expression; and (D) *Pdk1:Akt* and $Ask1^{S83A}$ co-expression. (E) The mean pixel intensity and quantification of the P-Akt immunofluorescence for the genotypes indicated. (F-H) Anti-P-Thr Ask1 antibody staining to show the expression of Ask1 of the transgene combinations indicated. (F) *Pdk1:Akt* and *GFP* co-expression; (G) *Pdk1:Akt* and $Ask1^{WT}$ co-expression; and (H) *Pdk1:Akt* and $Ask1^{S83A}$ co-expression. (I) The mean pixel intensity and quantification of the P-Thr Ask1 immunofluorescence for the genotypes indicated. The GFP staining corresponds to the controls. The others were stained with anti-Ptc antibody to identify the expression zone. Arrowhead points to a zone with some weak P-Thr Ask1 staining. (J) P-Akt immunofluorescence following the co-expression of *Pdk1:Akt* and $Ask1^{K618M}$. (K) P-Thr Ask1 immunofluorescence following the co-expression of *Pdk1:Akt* and $Ask1^{K618M}$. The stripe outlined in white indicates the zone of transgene expression. TP-3 (TO-PRO-3): nuclear staining. Scale bars: 50 μm. ***$p < 0.001$.

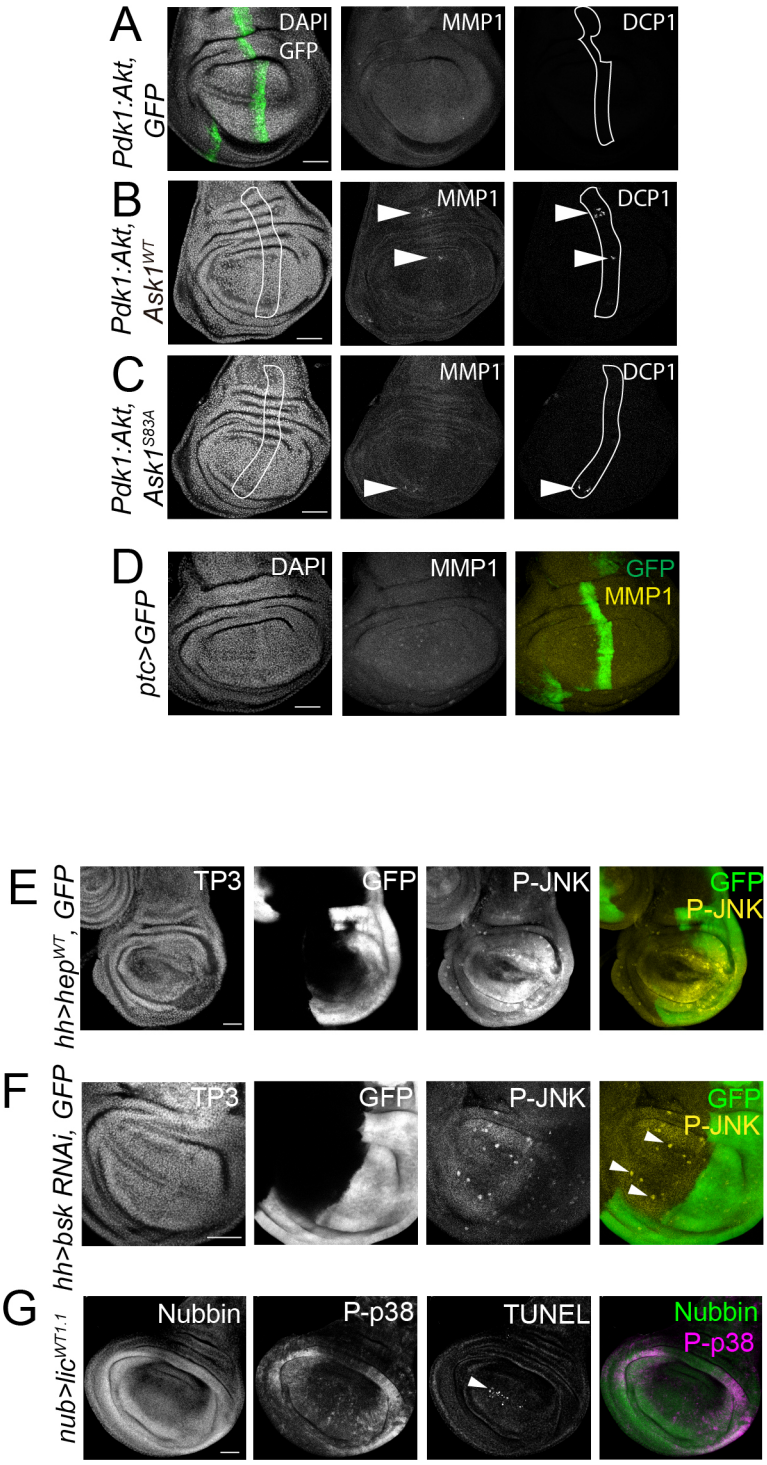

Figure S2. Apoptosis measured by assessing the expression of the caspase DCP1 and MMP1 after the co-expression of (A) *Pdk1:Akt* and *GFP*, (B) *Pdk1:Akt* and $Ask1^{WT}$, or (C) *Pdk1:Akt* and $Ask1^{S83A}$, using the *ptc>* driver (outline). The arrows indicate some apoptotic cells, which were also positive for MMP1. (D) Endogenous expression of MMP1 in *ptc>GFP* wing discs. (E-G) Validation of antibodies against proteins in the JNK and p38 pathways. (E) Anti-P-JNK antibody staining after the ectopic expression of the wild-type JNKK *hemipterous* (hep^{WT}). (F) Anti-P-JNK antibody staining after the administration of RNAi against the JNK *basket (bsk RNAi*). The Gal4 driver used in

these experiments was *hh-Gal4*, which controls expression in the posterior compartment, allowing us to compare the staining with that of the anterior compartment. Note that for hep^{WT}, the posterior compartment was smaller, likely due to apoptosis, with strong P-JNK staining. Following the expression of the *bsk RNAi*, endogenous P-JNK levels decreased. Moreover, P-JNK in mitoses (arrowheads) was reduced in the posterior (GFP) compartment. Note that both transgenes in (E) and (F) were co-expressed with *UAS-GFP*. (G) Expression of the wild-type MAPKK *licorne* (lic^{WT}), stained with the anti-P-p38 antibody. The driver used here was *nub-Gal4*, which operates in the entire wing pouch. The same driver was used for the rescue experiments in Figures 3 and 4. The arrowhead indicates dead cells. An antibody against Nubbin was used to identify the *nub-Gal4* domain. TP-3 (TO-PRO-3): nuclear staining. Scale bars: 50 μm.

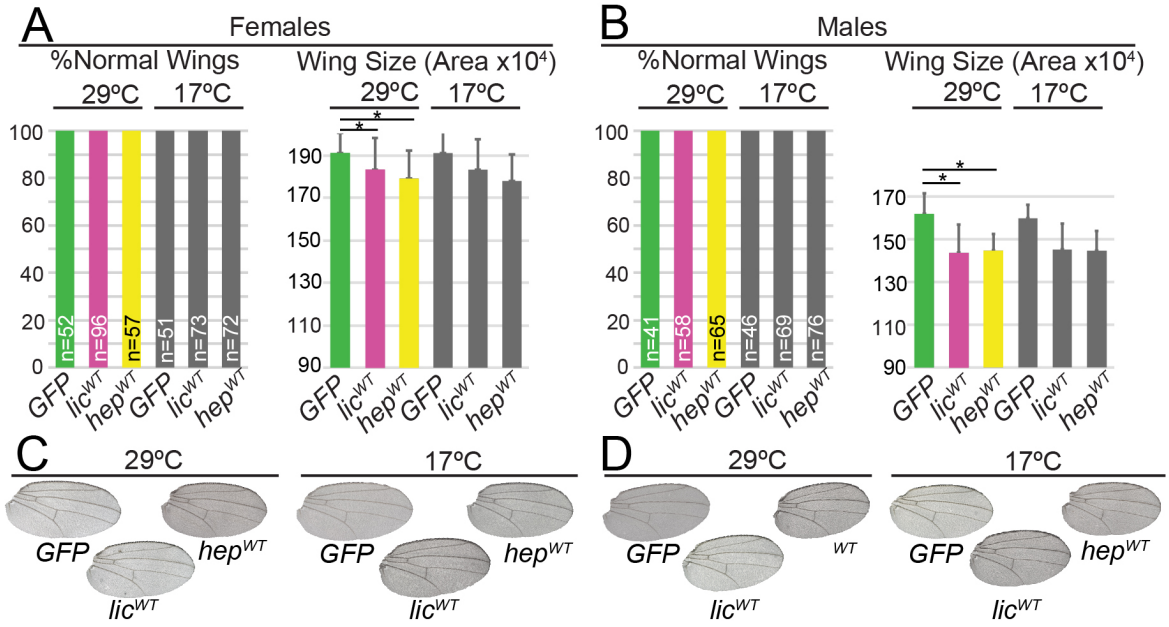

Figure S3. Control wing analysis of transgene expression in the absence of genetic ablation. The *nub-Gal4 UAS-GFP, nub-Gal4 UAS-lic^{WT} and nub-Gal4 UAS-hep^{WT}* genotypes were kept at the same temperature as those in Figures 3 and 4. (A-B) Percentage of regenerated wings and wing areas, as an indication of wing size, in females and males. Colored bars correspond to the experiments with a temperature shift to 29ºC. Gray bars correspond to the experiments performed at 17ºC. *p < 0.05. (C-D) Examples of wings obtained from the indicated genotypes and temperatures.

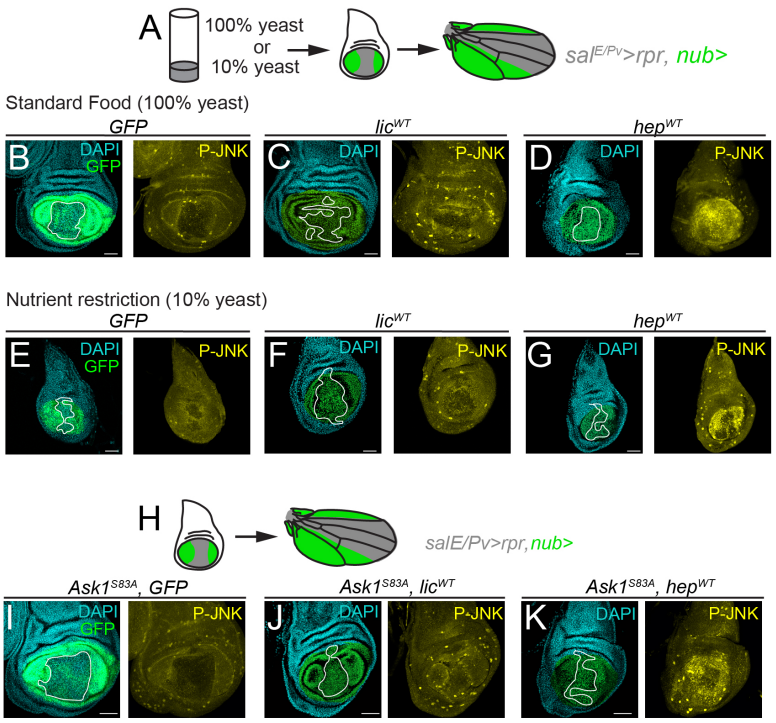

Figure S4. Anti-P-JNK antibody staining corresponding to the experiments in Figures 3 and 4. (A) Scheme (top) of the zones of dual transactivation in the wing disc and its corresponding zone in the adult wing in conditions of standard food or nutrient restriction. Gray, apoptotic zone generated by *sal*$^{E/Pv}$*>rpr*; green, transgene activation under *nub-Gal4* (*nub>*) to drive the expression of the indicated UAS transgene. Three UAS transgenes were used: *UAS-GFP*, as a control; *UAS-lic*^{WT}, to express the MAPKK *licorne*; and *UAS-hep*^{WT}, to express the JNKK *hemipterous*. (B-D) Anti-P-JNK antibody staining of *nub>GFP* (B), *nub>lic*^{WT} (C) and *nub>hep*^{WT} (D) wing discs in standard food conditions. (E-G) Anti-P-JNK antibody staining of *nub>GFP* (E), *nub>lic*^{WT} (F) and *nub>hep*^{WT} (G) wing discs in conditions of nutrient restriction. (H) Scheme (top) of the zones of dual transactivation in the wing disc and their corresponding zones in the adult wing. Gray, apoptotic zone (*sal*$^{E/Pv}$*>rpr*); green, zone of transgene activation under the *nub-Gal4* (*nub>*) driver. Three UAS transgenes were expressed together with the Ser83 mutant construct *UAS-Ask1*^{S83A} in standard food conditions: *UAS-GFP*, as a control; *UAS-lic*^{WT}, to express *licorne*; and *UAS-hep*^{WT}, to express *hemipterous*. (I-K) Anti-P-JNK antibody staining of *nub>Ask1*^{S83A}*,GFP* (I), *nub>Ask1*^{S83A}*, lic*^{WT} (J) and *nub>Ask1*^{S83A}*, hep*^{WT} (K) wing discs. The white line in the confocal images outlines the zone of apoptotic cells indicated by the pyknotic nuclei. Nuclei were stained with TO-PRO-3 (TP-3). *UAS-GFP* (*GFP*) was used to monitor the *nub>* zone. In (C), (D), (F), (G), (J) and (K), the *nub>* zone is colored in green. Scale bars: 50 μm.