Supplementary Table 1. The various overexpression phenotypes of our transgenic lines. Table shows the phenotypes of overexpressing Baldspot, FKBP39, dIPK, cathD and Atg8a, respectively. The Gal4 drivers used are listed in the first column. eyGal4 drives expression of transgenes mainly in the dividing cells of the eye; GMRGal4 is expressed in the post-mitotic cells of the eye; cgGal4 is expressed in the larval fat body and hemocytes; actGal4 and tubGal4 show constitutive expression in most tissues; hsGal4 directs ubiquitious transgene expression following a heat shock.

Supplementary Figure 1. *FKBP39*$^{5\text{-}HA\text{-}2590}$ mutants show higher level of autophagy in larval fat body cells than controls, and this effect is reversed in a clean excision line.
a: RT-PCR shows that FKBP39 mRNA levels are reduced in adult flies of the genotypes *FKBP39*$^{5\text{-}HA\text{-}2440}$*/Df(3R)Exel6194* and *FKBP39*$^{5\text{-}HA\text{-}2590}$*/Df(3R)Exel6194* (top panel, lanes 2 and 4, respectively; compare to the control w^{1118} in lane 1). Clean excisions of the P elements restore *FKBP39* expression levels in *FKBP39*^{exA3}*/Df(3R)Exel6194* and *FKBP39*^{exB1}*/Df(3R)Exel6194* animals (lanes 3 and 5, respectively). Actin is shown as a control (bottom panel). *b, c*: Lysotracker staining of fat bodies. Short, 80-minute starvation induces higher levels of autophagy in fat bodies of *FKBP39*$^{5\text{-}HA\text{-}2590}$*/Df(3R)Exel6194* mutant larvae than in *Df(3R)Exel6194/TM6* controls (*b*, compare to Figure 1m). Starvation response is restored to wild-type levels in the precise excision line *FKBP39*^{exB1}*/Df(3R)Exel6194* (*c*). *d* shows quantitation of the results. Asterisk marks a significant change (p=0.001 for columns 1-2, p=0.002 for columns 2-3, p=0.1 for columns 1-3).

Supplementary Figure 2. FKBP39 inhibits Ras-mediated activation of MAPK by proline-directed kinases, but Ras itself is not required for developmental autophagy.

Although phospho-MAPK can be hardly detected in wild-type or FKBP39-overexpressing animals (*a*, lanes 1-2), fat body-specific expression of the hyperactive Ras^{V12} mutant results in a strong induction of MAPK phosphorylation (lane 3). This activation is almost completely blocked by co-overexpression of FKBP39 (lane 4). Right panel shows Coomassie Brillant Blue staining as a loading control. Loss of function clones for the null mutation Ras^{c40b} in the wandering third instar fat body are marked by loss of GFP expression, whereas twin spots can be identified by higher GFP expression (*b*, left panel). Clonal loss of *Ras* function does not interfere with developmental autophagy in fat body cells (delineated by a white line), as shown by Lysotracker staining (right panel).