



**Figure S5. Misexpression of codon optimized TDP-43 exhibits large vacuoles positive for autophagy markers. (A-D)** Coexpression of *gI-CO-TDP-43* and the autophagic protein Atg5-GFP shows that the large vacuoles present in the 1-day post-eclosion adult retina are positive for Atg5 (scale bar 5  $\mu\text{m}$ ). **(E-G)** Another autophagic marker was coexpressed with *gI-CO-TDP-43*, Atg8-mCherry-GFP, which is pH sensitive and only expresses GFP at a higher pH content. The CO-TDP-43 expressed flies show that few of the relatively smaller puncta were positive for both Atg8-mCherry and GFP (arrowheads), while a majority of the larger puncta were only fluorescent for Atg8-mCherry (white arrows), indicating more acidic puncta (scale bar 10  $\mu\text{m}$ ). Genotypes: **(A-D)**  $w^{1118}$ , *GMR-GAL4/ w^{1118};gI-TDP-43<sup>CO/+</sup>;UAS-Atg5-GFP/+*, **(E-G)**  $w^{1118}$ , *GMR-GAL4/ w^{1118};gI-TDP-43<sup>CO</sup>/UAS-Atg8-mCherry-GFP/+*.