Drosophila eyes absent is a Novel mRNA Target of the Tristetraprolin (TTP) Protein DTIS11

Po-An Yeh, et al.

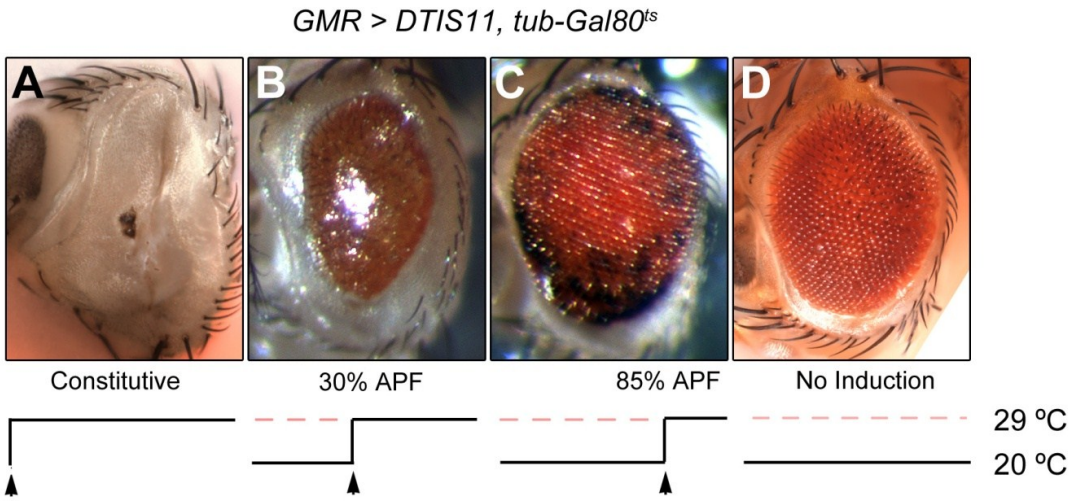

Figure S1. Distinct eye phenotypes caused by temporal control of DTIS11 overexpression. Temperature-sensitive *GMR-Gal4/UAS-DTIS11; tub-Gal80^{ts}/+* flies were cultured at 20ºC before being transferred to the induction temperature (29ºC) as follows: (A) prior to egg-laying (constitutive expression), (B) early pupal stage (30% APF), (C) late pupal stage (~85% APF), or (D) no temperature shift (no induction). Arrowheads indicate the point when DTIS11 was induced by temperature shift.

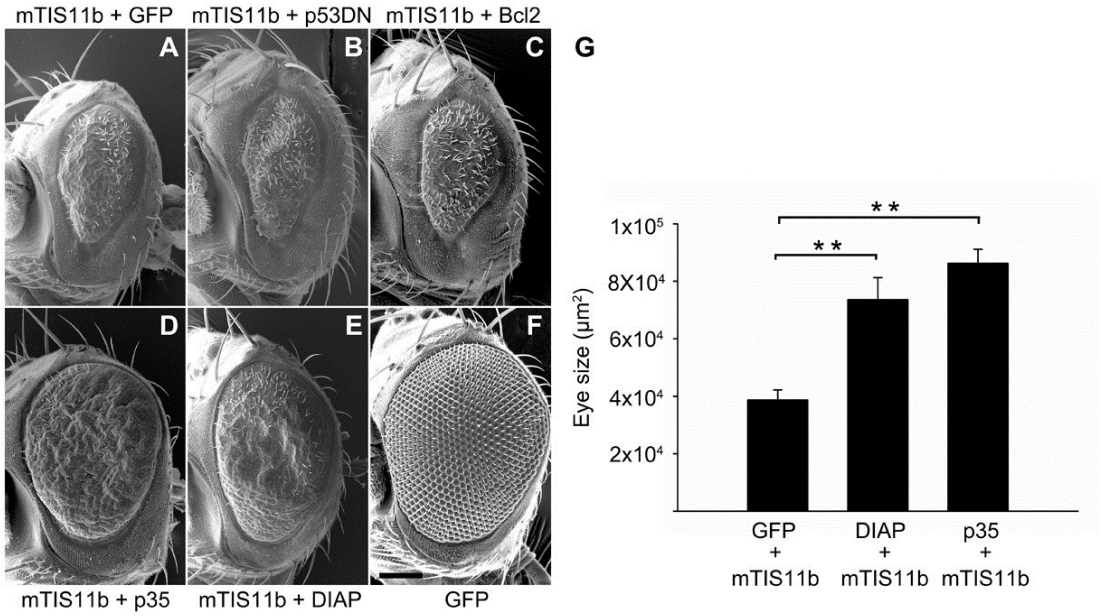

Figure S2. Suppression of apoptotic pathway rescues small eye phenotype elicited by mTIS11b overexpression. Surface scanning electron micrographs of fly eyes following coexpression of mTIS11b with control GFP (A) or several anti-apoptotic genes, including dominant-negative p53DN (B), Bcl2 (C), p35 (D) and *Drosophila* inhibitor of apoptosis (DIAP; E) using the *GMR*-Gal4 driver. (F) Wild-type eye expressing GFP alone. (G) Statistical analysis of eye size associated with various gene interactions ($n = 5$, $^{**}P < 0.001$ performed by Student's *t*- test).

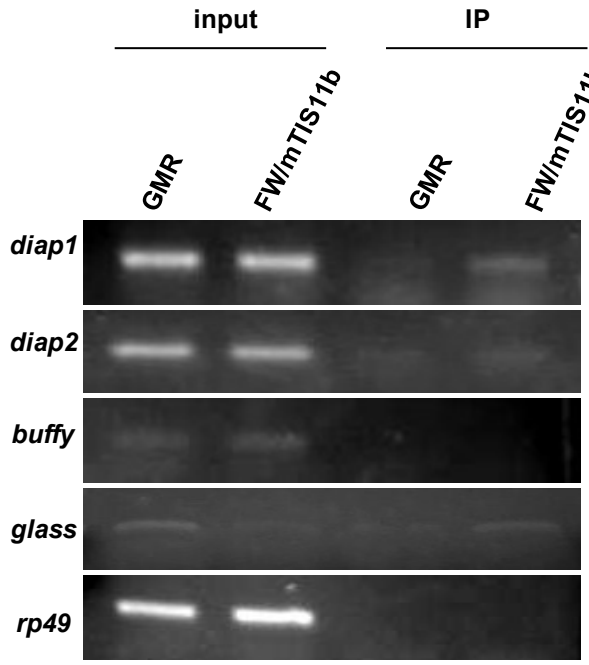

Figure S3. mTIS11b associates with mRNAs of apoptosis-related genes. Cell extracts were isolated from fly heads overexpressing FLAG-mTIS11b or control (GMR). RNA-protein complexes were immunoprecipitated using anti-FLAG (IP), and precipitated RNA was isolated for PCR analysis using the following primers: *diap1*, 5′-CGCATCTTCAACAAGATCGTC-3′ and 5′-ATATACGCGCATCACATCGG-3′; *diap2*, 5′-TATCGCTGGAGGAGGAAAAC-3′ and 5′-ACGAATCCCTTGATGTCTGC-3′; *buffy*, 5′-CCTCGTATCCCACGAATAAC-3′ and 5′-TTCCAGCCAATCGTGTAGG-3′; *glass*, 5′-GCAAGAAGTCCTTCTCGGAC-3′ and 5′-TCATGTGAGCAGGCTGTTGC-3′. Input indicates RNA purified from 1/10 of cell extract.

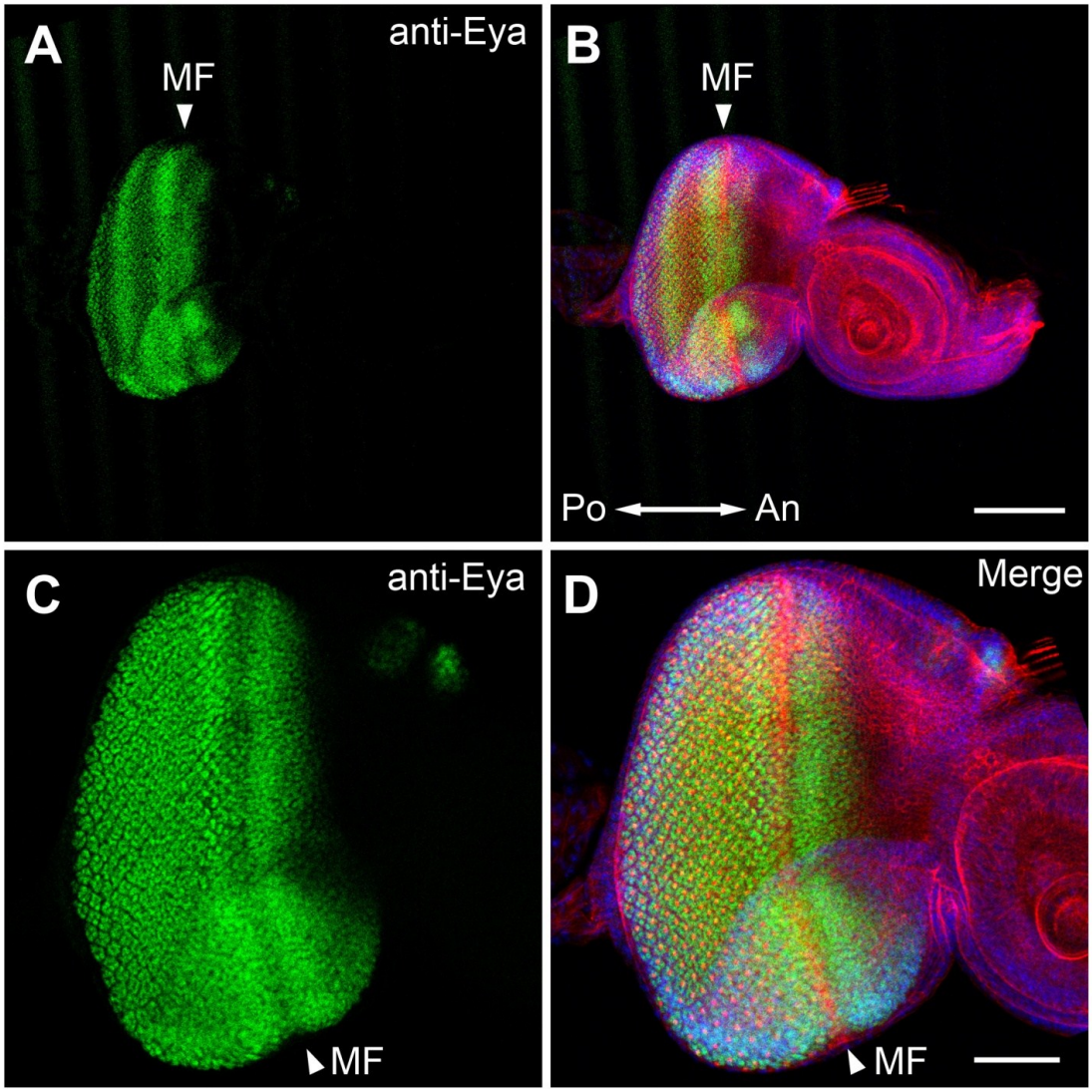

Figure S4. Eya is expressed behind and in front of the morphogenetic furrow in fly eye-antennal discs. (A,C) Eya expression (green) in eye-antennal disc of third-instar larva. (B,D) Merged images of same discs showing counterstaining with phalloidin (red) and DAPI (blue). Phalloidin highlights the morphogenetic furrow (MF, arrowhead). Anterior (An) and posterior (Po) directions are indicated. Scale bars, 100 μm (B), 50 μm (D).

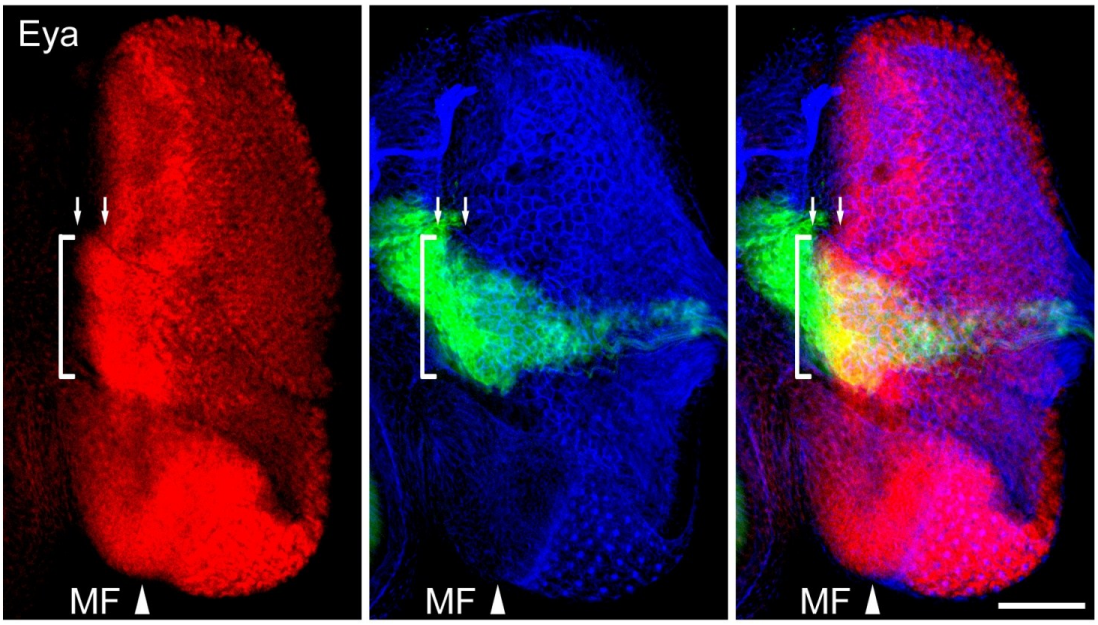

Figure S5. Knockdown of DTIS11 elevates Eya protein levels. An increase and expansion in Eya (red) is seen in the area with DTIS11 knockdown (green, GFP), as indicated by the white bracket. Elevated staining is especially noted in an area marked by small arrows before the morphogenetic furrow (MF, arrowhead). Blue represents phalloidin counterstaining. Genotype: *eq-Gal4, UAS-mCD8GFP/UAS-DTIS11*^{RNAi}. Scale bar, 50 μm.

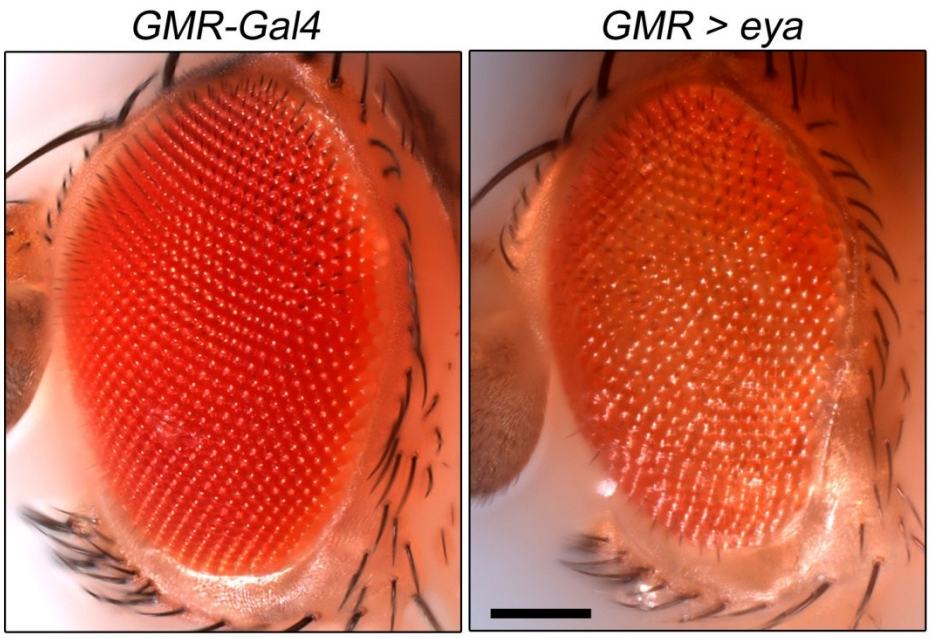

Figure S6. Overexpression of Eya does not elicit big eye morphology. Control (GMR-Gal4) and GMR-Gal4/UAS-Eya eyes are shown. Scale bar, 100 μm.

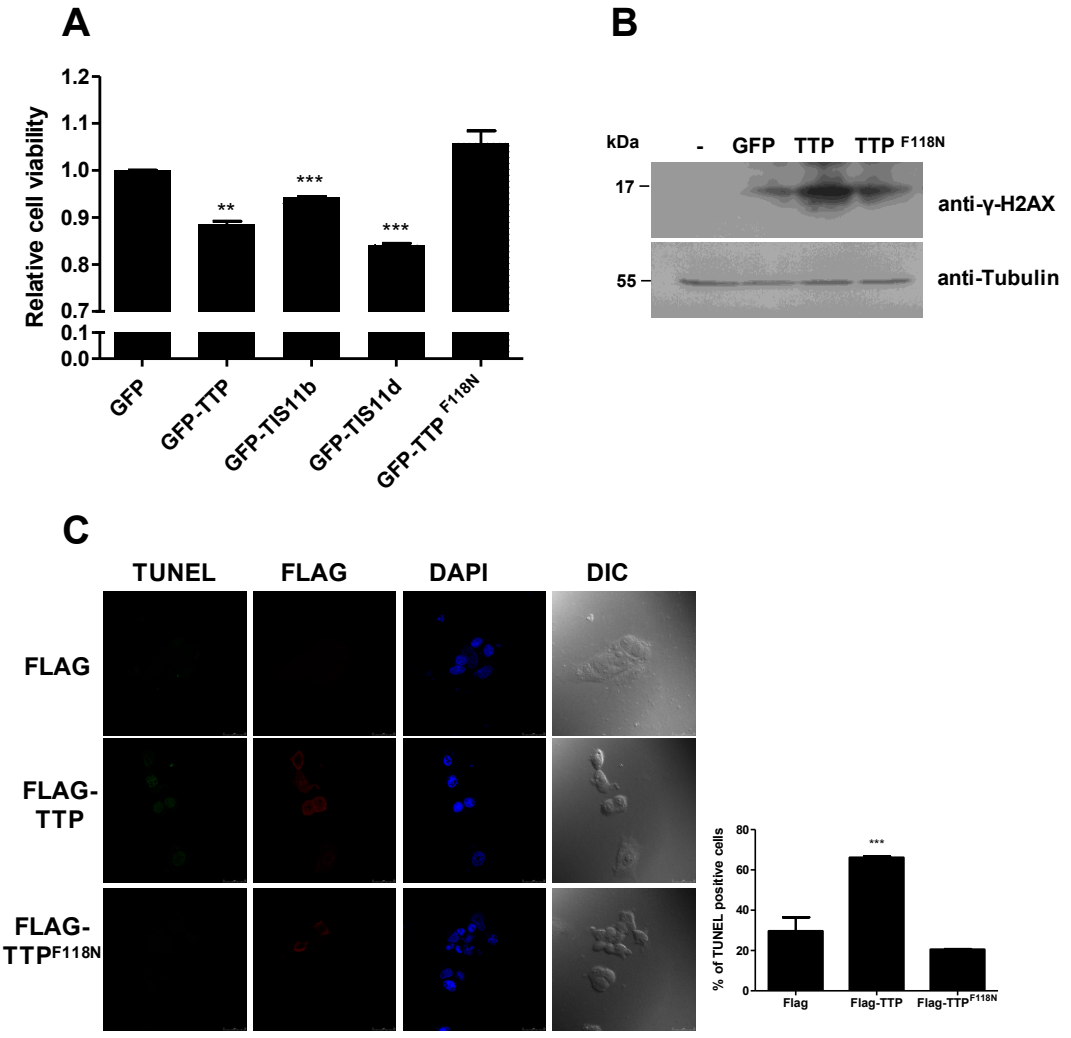

Figure S7. Overexpression of TTP increases apoptosis. (A) MTS cell viability analysis in MCF7 cells. MCF7 cells were seeded into 96-well plates and transiently transfected with GFP vector or GFP-tagged TTP, TIS11b, TIS11d or TTP^{F118N} expression plasmids. After 2 days, cell viability was determined by MTS assay and normalized to vector control (**$P < 0.01$, ***$P < 0.001$). Each treatment was performed in triplicate, and the experiment was repeated two more times. (B) Phosphorylation of histone H2AX. MCF7 cells were transfected with GFP vector or with GFP-tagged TTP or TTP^{F118N} expression plasmids. After 24 h, whole-cell extracts were isolated and γ-H2AX protein was detected by immunoblotting. (C) TUNEL assay (TdT-mediated dUTP nick end-labeling). MCF7 cells were cultured on slides and transfected with vector control or with wild-type TTP or TTP^{F118N} expression plasmid. After fixation, TUNEL staining was performed according to the manufacturer's procedure (Roche). Red, FLAG-tagged TTP; green, TUNEL staining; blue, DAPI; DIC, Differential interference contrast microscopy. The percentage of cells with TUNEL stained were evaluated and presented in the right panel (***$P < 0.001$).