

Supplemental Material

Figure S1. Retinal overexpression of *gl*-Tau^{S2A} produces a smooth eye phenotype compared to the rough eye phenotypes generated by overexpression of *gl*-Tau^{wt} and *gl*-Tau^{S11A}. **A-F**, SEM images of strong and weak lines of three genotypes. The rough eye phenotype is produced by two different lines expressing a single copy of *gl*-Tau^{wt} (**A** and **D**) and a single copy of *gl*-Tau^{S11A} (**C** and **F**). As compared to the above genotypes, *gl*-Tau^{S2A} displays a smooth eye phenotype on chromosomes II and III (**B** and **E**) Scale bar, 100 μm. Genotypes: (**A**) *w*¹¹¹⁸; *gl*-Tau-1.1/CyO, (**D**) *w*¹¹¹⁸; *gl*-Tau-2.1/CyO, (**B**) *w*¹¹¹⁸; *gl*-Tau^{S2A}-2/CyO, (**E**) *w*¹¹¹⁸; *gl*-Tau^{S2A}-3/TM6B, (**C**) *w*¹¹¹⁸; *gl*-Tau^{S11A}-1/+ (**F**) *w*¹¹¹⁸; *gl*-Tau^{S11A}-2/TM6B.

Figure S2. **A** and **C**, Tau^{S2A} when coexpressed with Shaggy displays a relatively high signal as compared to wild type Tau at the disease-related epitope AT100 (**A**) and anti-SggY²¹⁴; the activated form of Shaggy (**C**). **B**, Histogram shows relative levels of AT100 phosphorylation derived from densitometric analysis of three separate blots. The enhancement in the level of tau phosphorylation at the AT100 phosphoepitope is much higher when Shaggy is coexpressed with Tau^{S2A} as compared to Tau^{wt} data set (**B**). Each bar represents mean ± SEM (n = 3). * p < 0.05; **p < 0.01 (one way ANOVA with supplementary Newman-Keuls test).

Figure S3. A-G, Retinal overexpression of Tau^{wt}, Tau^{S2A}, and Tau^{S11A} display no enhancement or suppression when coexpressed with Cdk5/p35. **A-G,** SEM images. The different eye phenotypes produced by one copy of Tau^{wt} (**A**), Tau^{S2A} (**B**), and Tau^{S11A} (**C**) remains unchanged when coexpressed with Cdk5/p35 (**E-G**). Retinal overexpression of Cdk5/p35 by itself produced no obvious phenotype (**D**). Scale bar, 100 μ m. (**H**) Immunoblot using AT270 antibody shows enhancement for both Tau^{S2A} and Tau^{S11A} coexpressed with Cdk5 as compared to wild type; however there is no enhancement observed at the AT8 and PHF1 phosphoepitopes.

Figure S4. Immunoblot analysis of Tau^{wt}, Tau^{S2A} and Tau^{S11A} transgenic head extracts using a series of antibodies against major kinases and their active sites. These data reveal that there are no major differences in the levels of endogenous kinases JNK, Cdk5, or GSK-3 β . However the level of active GSK-3 β , i.e., Sgg^{Y214} is much higher in Tau^{S11A} compared to Tau^{wt} and Tau^{S2A}.

Figure S5. Microtubule binding of Tau^{wt} and Tau^{S2A} when coexpressed with the kinases PAR-1 and Shaggy. **A,** Upper panel, Immunoblot using T46 identifies tau fractions in Tau^{wt} coexpressed with PAR1 and Shaggy that are in the supernatant (S) or bound to microtubules in the pellet (P). Lower panel, Histograms represent the relative levels of 'free' and 'bound' tau in the supernatant (S) and pellet (P) fractions respectively for Tau^{wt}, Tau^{wt} with PAR-1 and Tau^{wt} with Shaggy. Each bar represents mean \pm SEM (n=3). * $p < 0.05$ for

Tau^{wt} and Tau^{wt} with PAR-1, and ** $p < 0.001$ for Tau^{wt} with Shaggy (one way ANOVA with supplementary Newman-Keuls test). **B**, Upper panel, Similar immunoblot analysis displays the tau fractions in Tau^{S2A} coexpressed with PAR-1 and Shaggy that are present in the supernatant (S) or pellet (P). Lower panel, Histograms represent the relative levels of 'free' and 'bound' tau respectively for Tau^{S2A}, Tau^{S2A} with PAR-1 and Tau^{S2A} with Shaggy respectively. Each bar represents mean \pm SEM (n=3). * $p < 0.05$ for Tau^{S2A} and ** $p < 0.001$ for Tau^{S2A} with Shaggy (one way ANOVA with supplementary Newman-Keuls test). The level of free tau present in the supernatant is increased when Shaggy is coexpressed with Tau^{wt} (**A**) or Tau^{S2A} (**B**) as compared to PAR-1 coexpression.

Figure S6. Microtubule binding of Tau^{S11A} when coexpressed with PAR-1 and Shaggy. **A**, immunoblot using T46 identifies tau fractions in Tau^{S11A} coexpressed with PAR1 and Shaggy that are in the supernatant (S) or bound to microtubules in the pellet (P). **B**, histograms representing the relative levels of 'free' and 'bound' tau in the supernatant (S) and pellet (P) fractions respectively for Tau^{S11A} and for Tau^{S11A} coexpressed with PAR-1 and Shaggy. Each bar represents mean \pm SEM (n = 3). * $p < 0.05$ for Tau^{S11A} with PAR-1, and ** $p < 0.001$ for Tau^{S11A} and Tau^{S11A} with Shaggy. The level of tau bound to microtubules is much higher than the level of free tau in the supernatant even when PAR-1 and Shaggy are coexpressed with Tau^{S11A} (one way ANOVA with supplementary Newman-Keuls test).

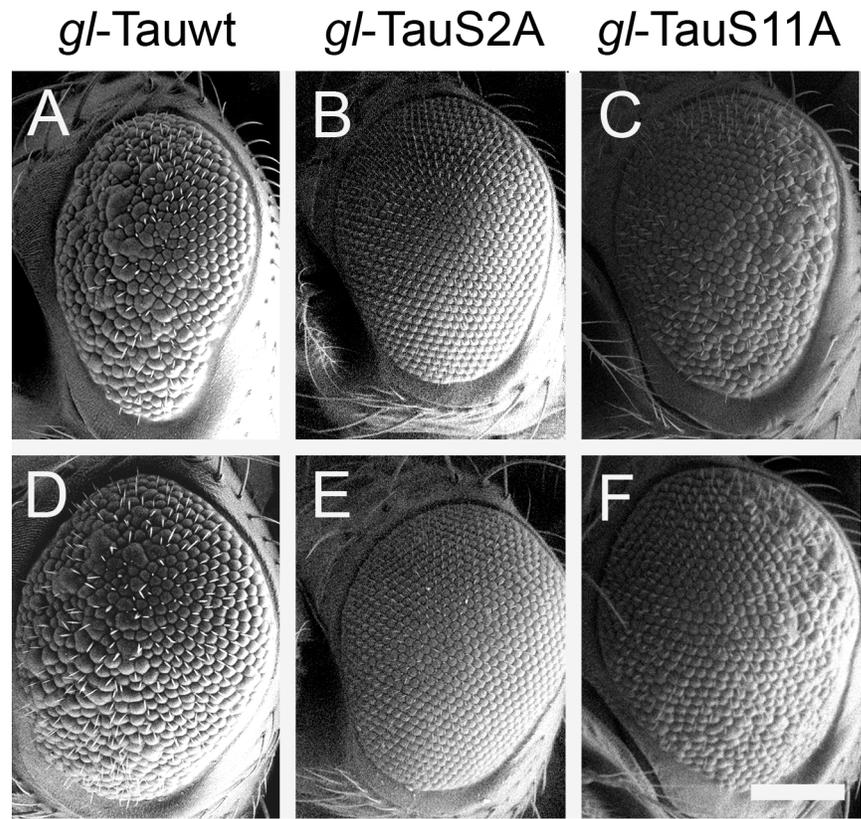


Fig. S1

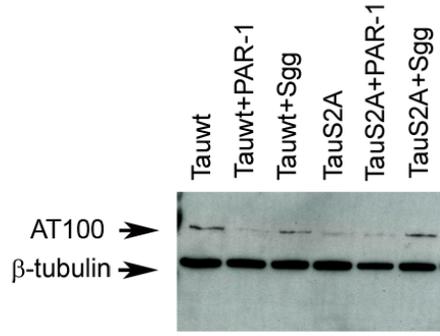
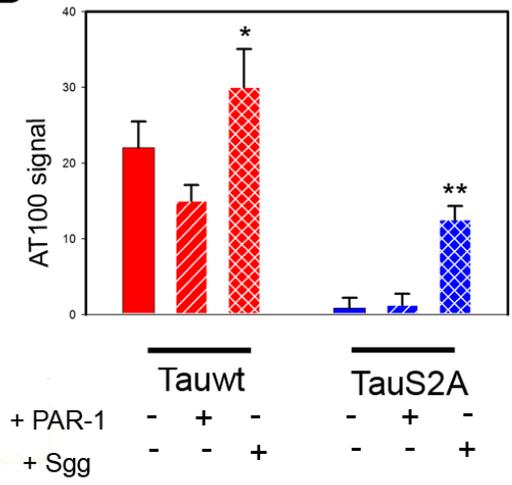
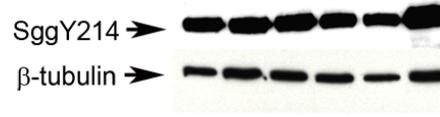
A**B****C**

Fig. S2

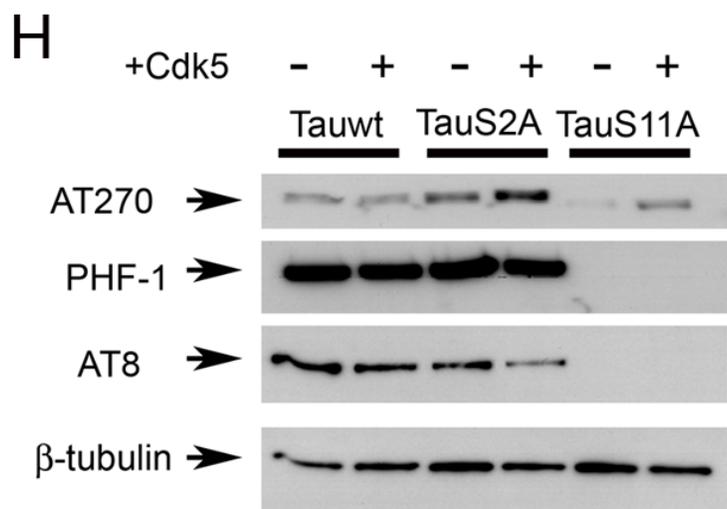
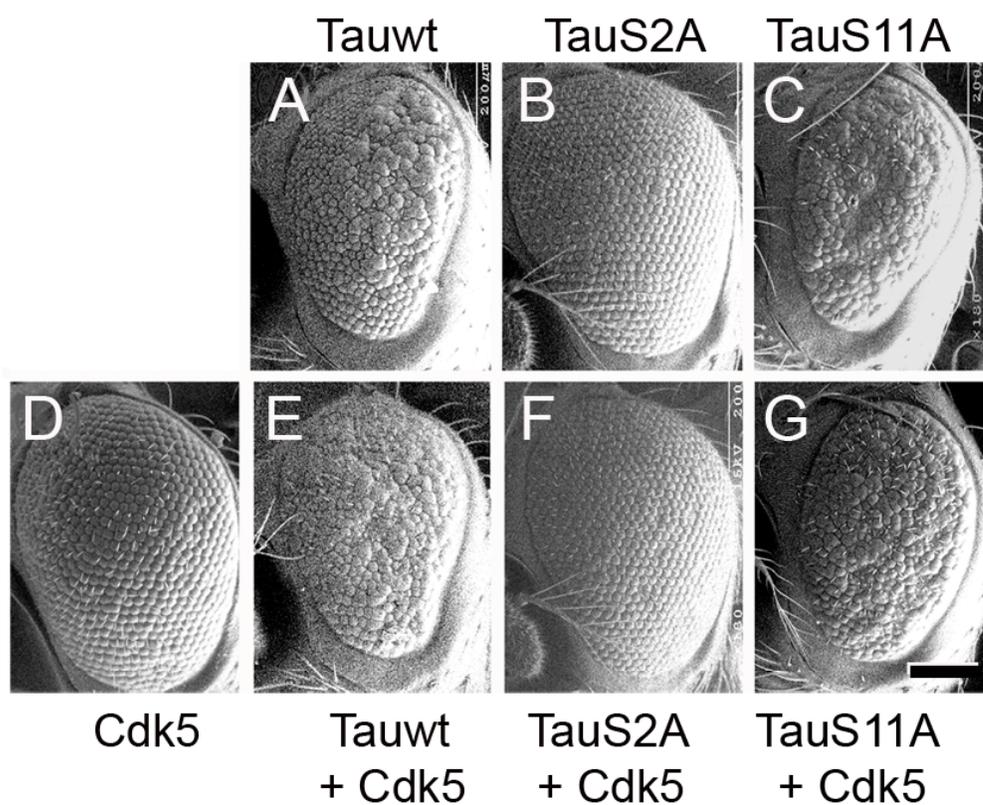


Fig. S3

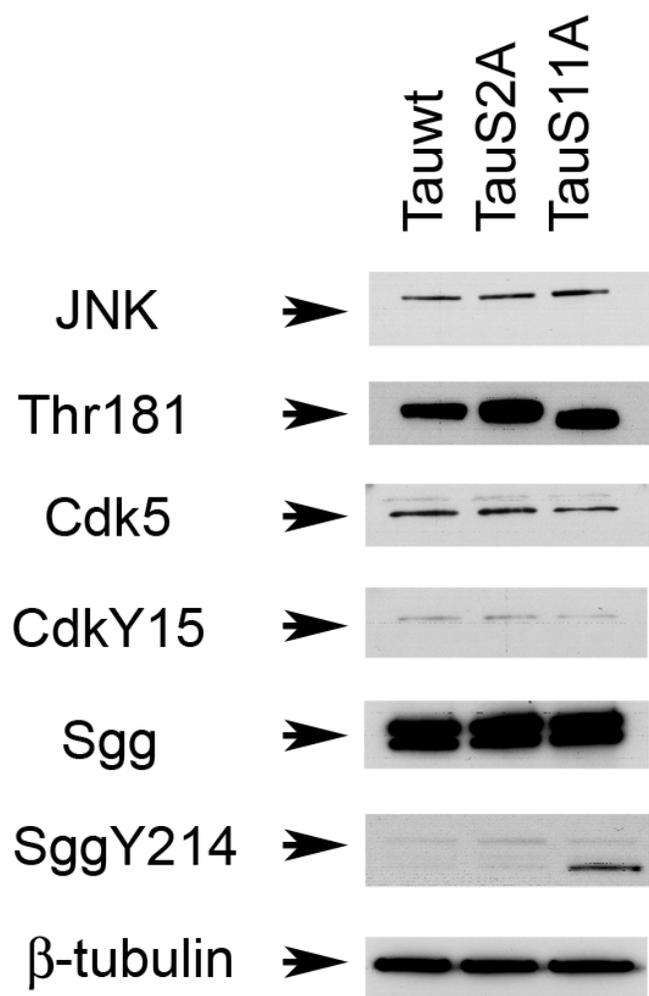


Fig. S4

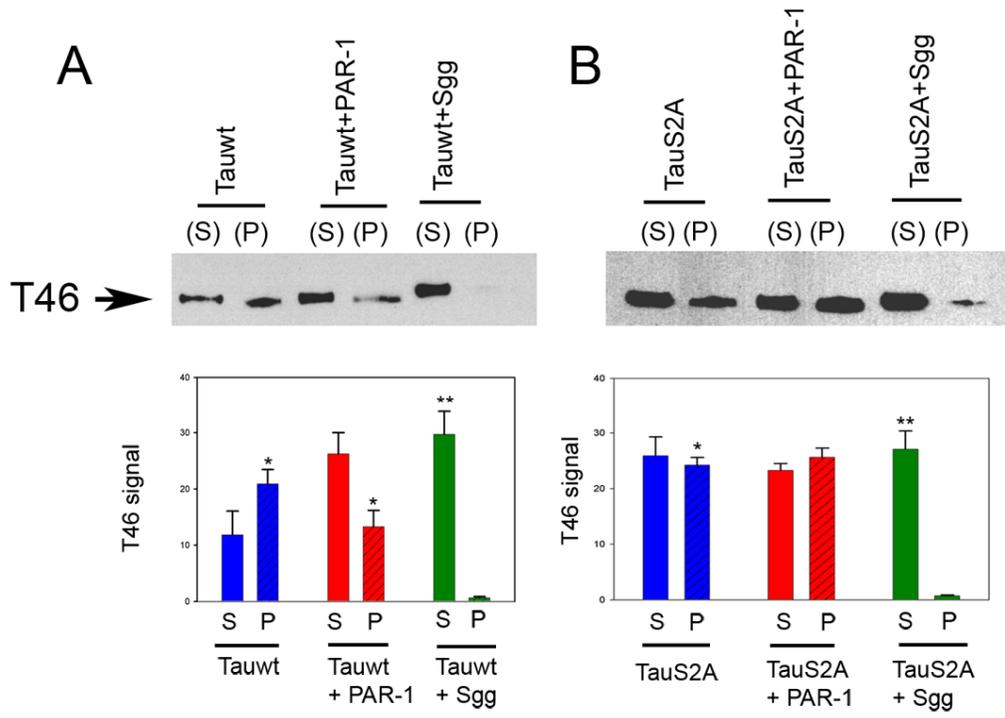


Fig. S5

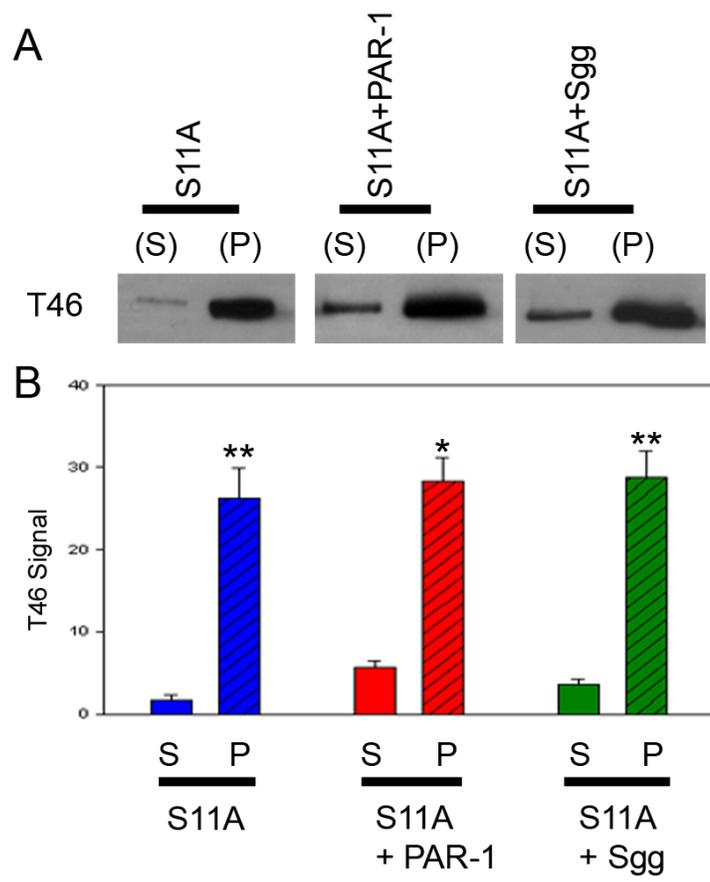


Fig. S6