Supporting Information

Evaluation of the Toxicity and Efficacy of a Multi-Target Polymer-Drug Nano-Polyplex in Undifferentiated SH-SY5Y Cells and *Drosophila* Model of Tauopathy

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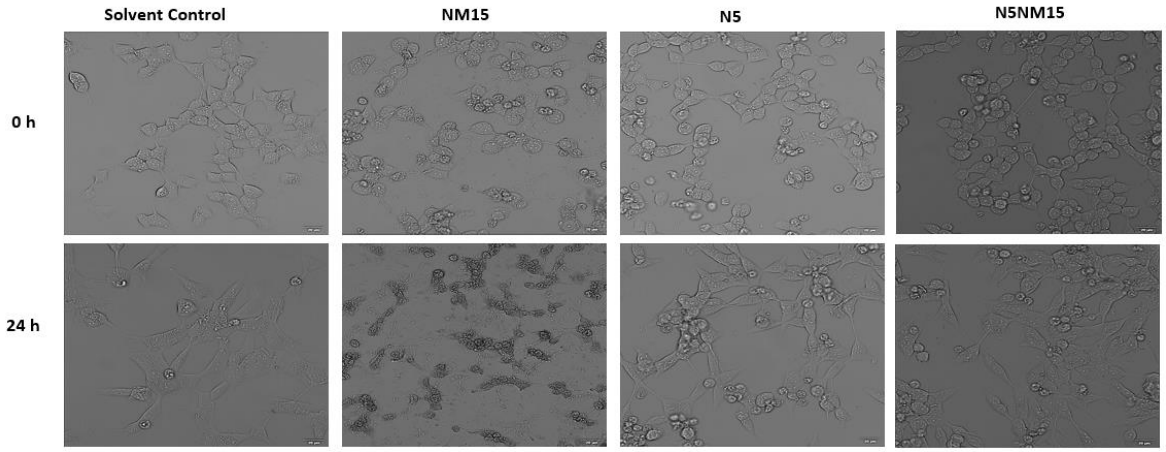


Figure S1. Grey-scale microscopy image depicting cellular uptake of NM15, N5 and N5NM15 nano-polyplex by undifferentiated SH-SY5Y cells incubated in serum-free DMEM media at 37°C, with 5% CO_2, images taken after 0 h and 24 h (Scale bar = 20 µm).

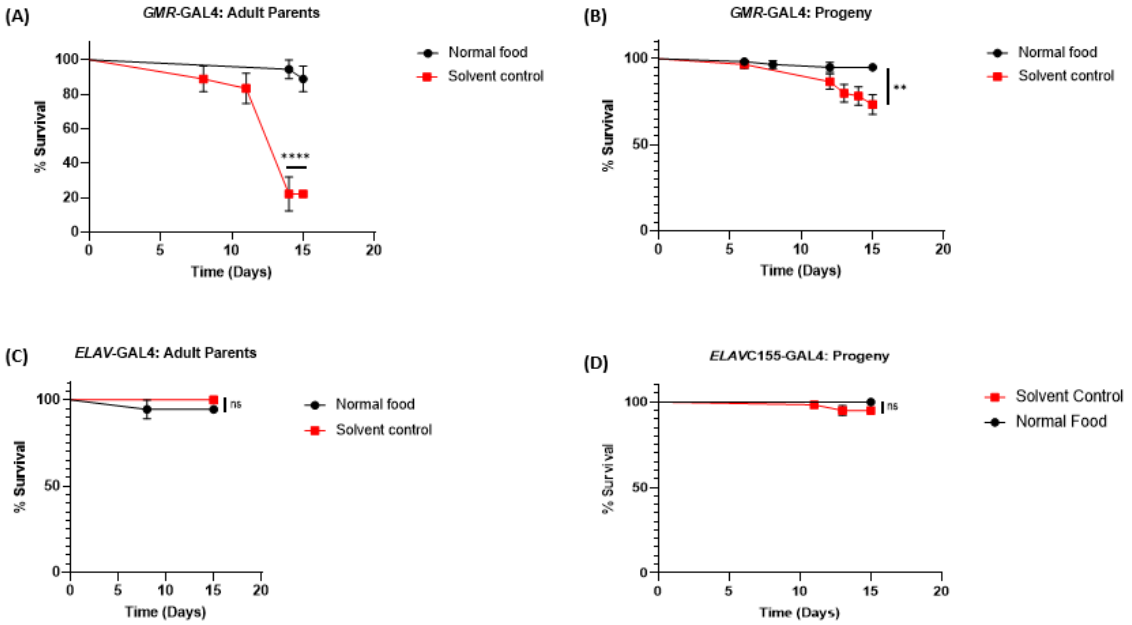


Figure S2. Toxicity study was assessed in *GMR*-GAL4 and *ELAV*-GAL4 adult flies and their progeny after feeding with the solvent control (1.2 mM Tris-HCl, pH 7.4) (**A-D**). Statistical analysis was performed using the Mantel-Cox test, with n=18 adult flies (6 flies per group in 3 separate tubes, maintaining a 1:1 male-to-female ratio) and n=60 progeny (20 flies per group in 3 separate tubes, maintaining a 1:1 male-to-female ratio).

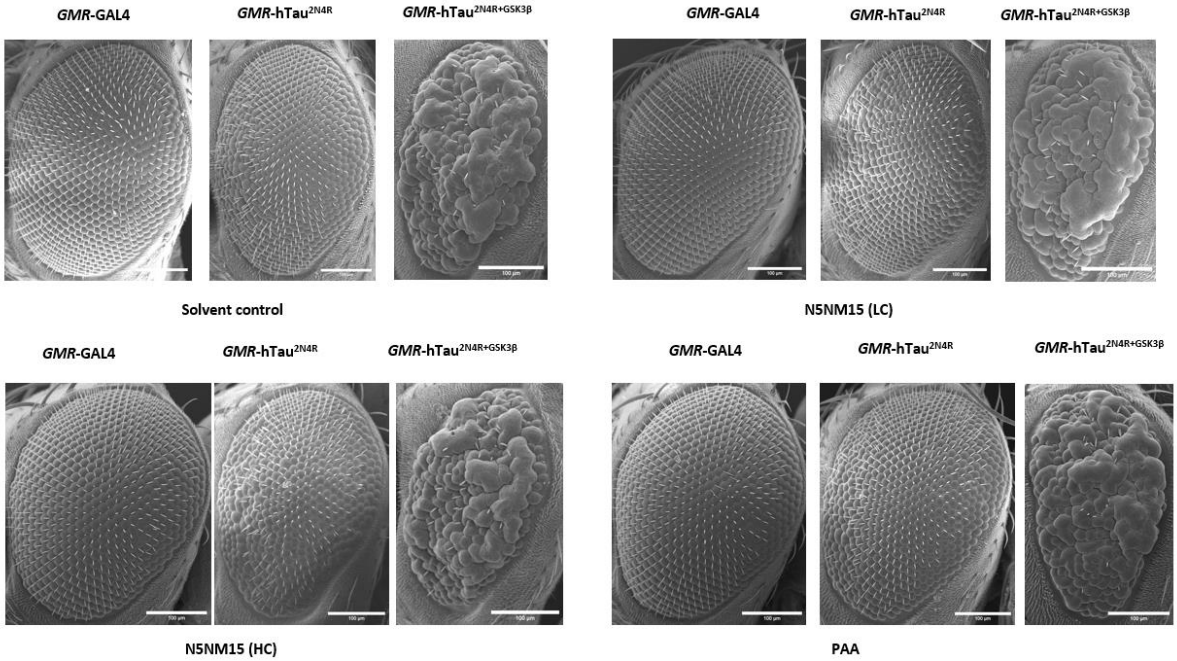


Figure S3. SEM images of *GMR-GAL4, GMR*-hTau^{2N4R} and *GMR*-hTau$^{2N4R+GSK3\beta}$ flies treated with solvent control, LC and HC of N5NM15, and PAA illustrating their effects on eye morphology (Scale bar = 100 µm).

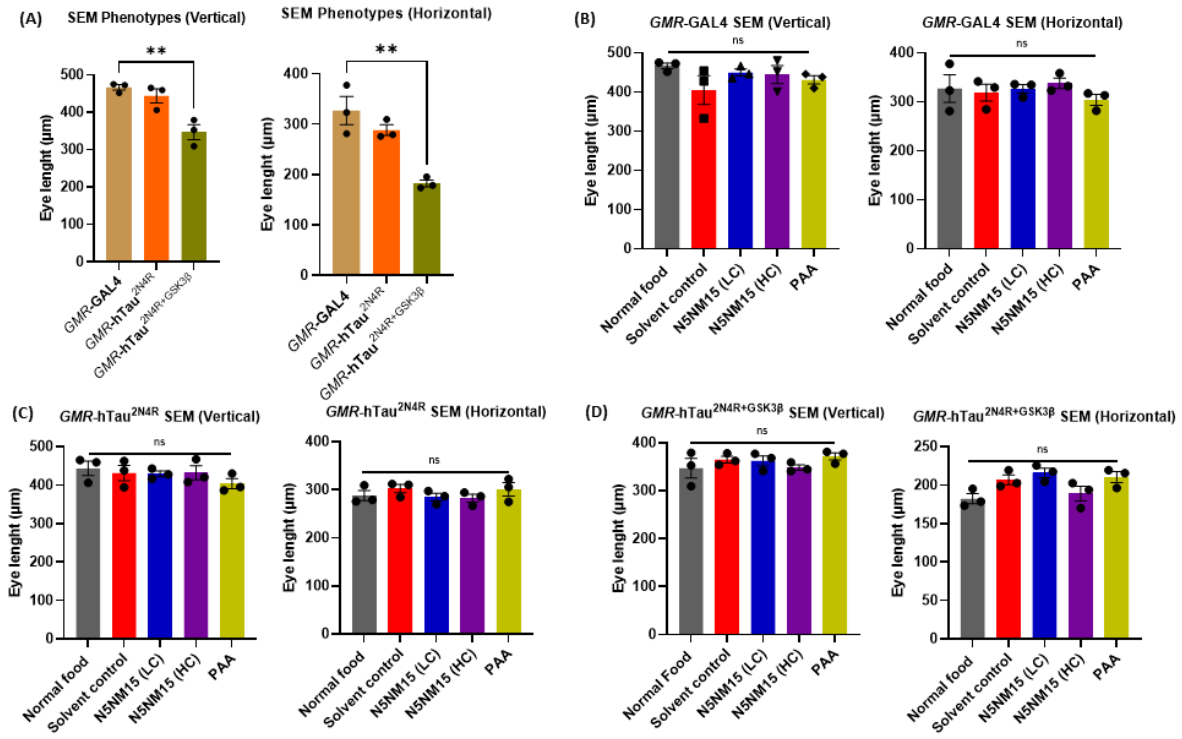


Figure S4. Quantitative analysis of eye length based on SEM images in *GMR*-GAL4, *GMR*-hTau^{2N4R}, and *GMR*-hTau$^{2N4R+GSK3\beta}$ Flies (**A**). Eye length measurements of (**B**) *GMR*-GAL4, (**C**) *GMR*-hTau^{2N4R} and (**D**) hTau$^{2N4R+GSK3\beta}$ flies treated with LC and HC of N5NM15 and PAA. Data are presented as mean ± SEM (n=3). Statistical analysis was performed using one-way ANOVA (**p ≤ 0.01 for Figure A), with Dunnett's multiple comparisons test.

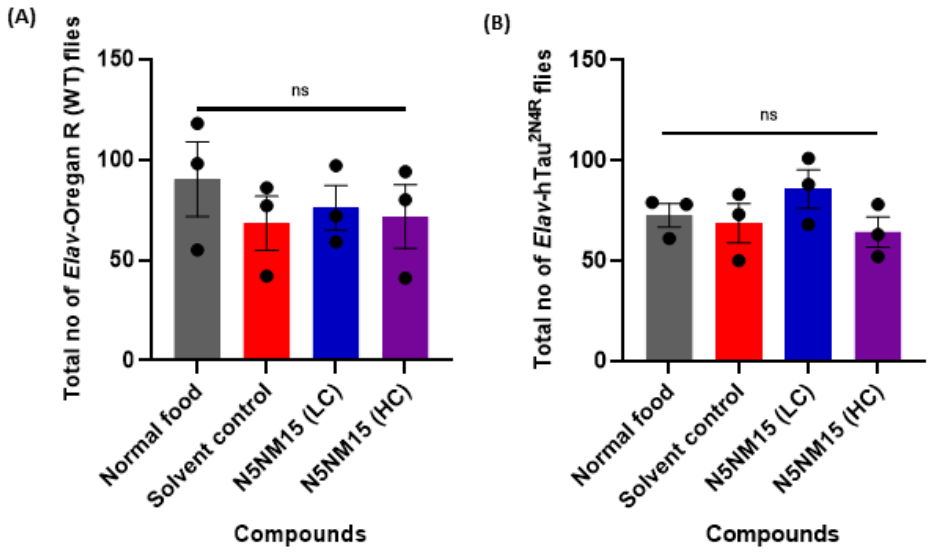


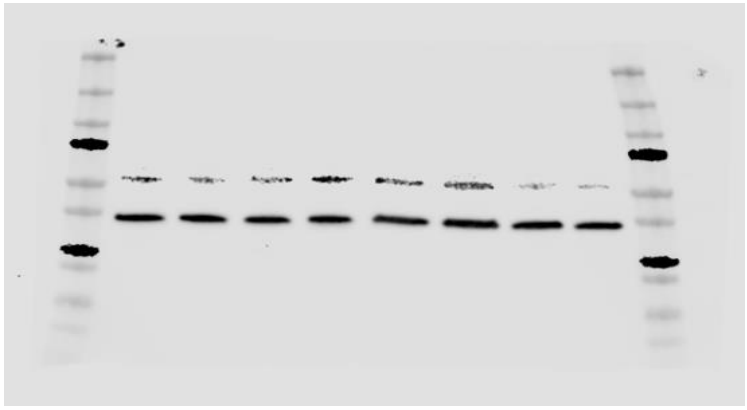
Figure S5. Total number of F1 progeny flies collected after 11 days to evaluate the effect of N5NM15 at varying concentrations (LC (11:3 µg/mL), and HC (44:12.5 µg/mL)) on progeny production. (**A**) *Elav*-Oregon-R (WT) flies were reared on normal food, solvent control, or food containing LC and HC of N5NM15. (**B**) *Elav*-hTau^{2N4R} progeny flies, expressing human tau protein, were reared under the same conditions. Data are presented as mean ± SEM (n=3). No significant differences (p > 0.05, one-way ANOVA) were observed.

Media1.mp4

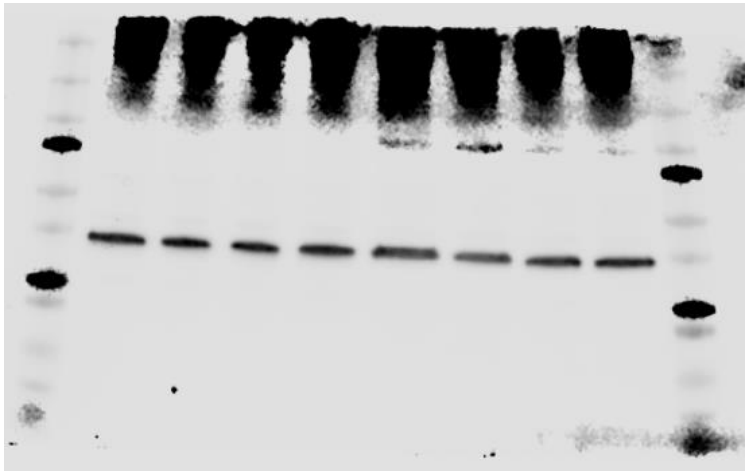
Video S6. Negative geotaxis assay: *ELAV*-hTau^{2N4R} vs *ELAV*-Oregon-R (WT) progeny flies in normal food.

Full Blot images

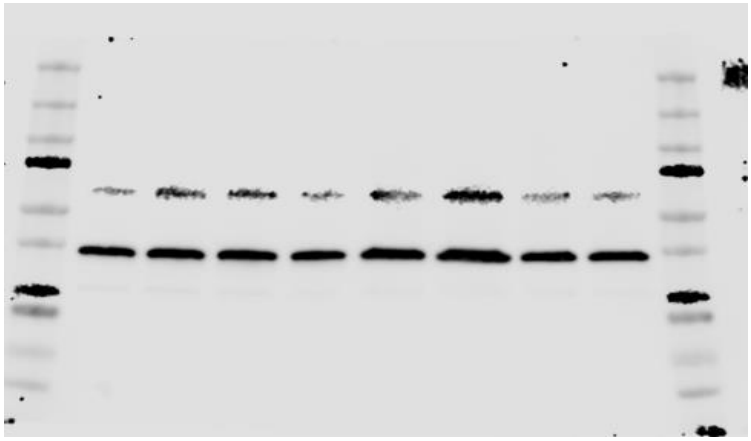
1. Tau5- Figure 3A in the manuscript



2. AT8- Figure 3B in the manuscript



3. AT180- Figure 3C in the manuscript



4. PHF-13- Figure 3D in the manuscript