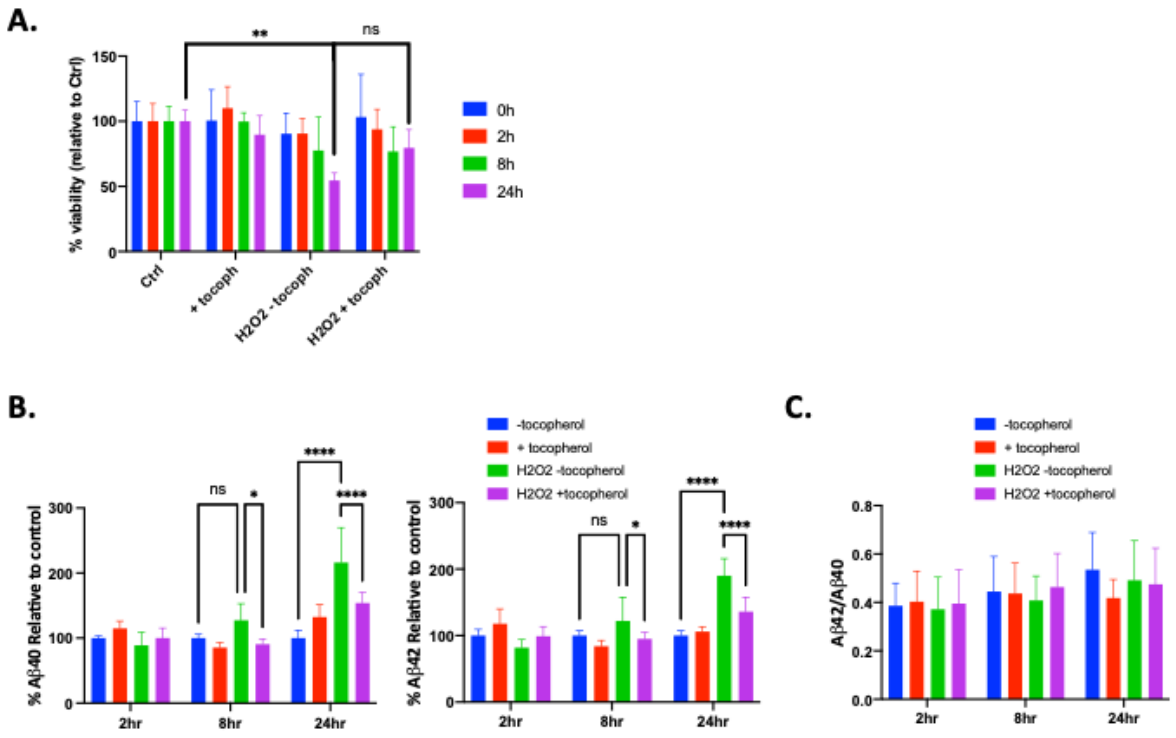
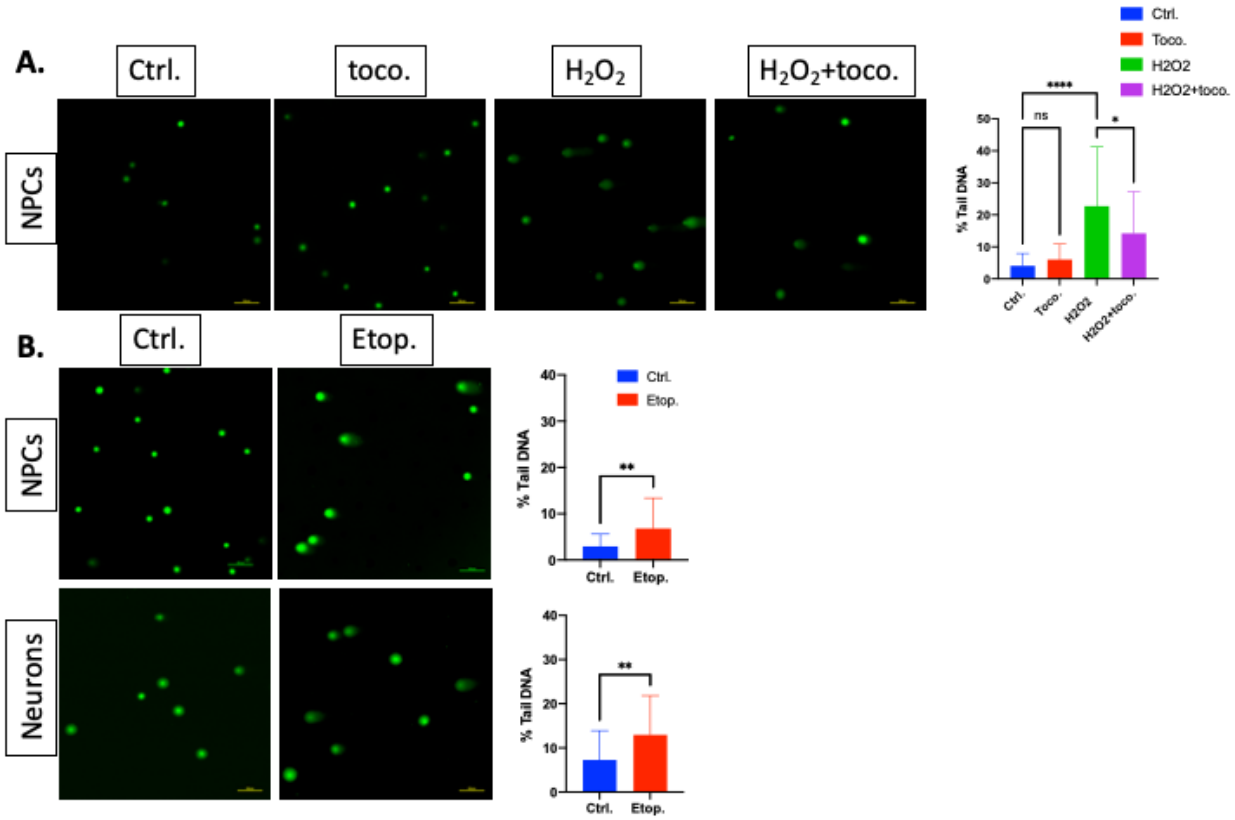
Supplementary Material

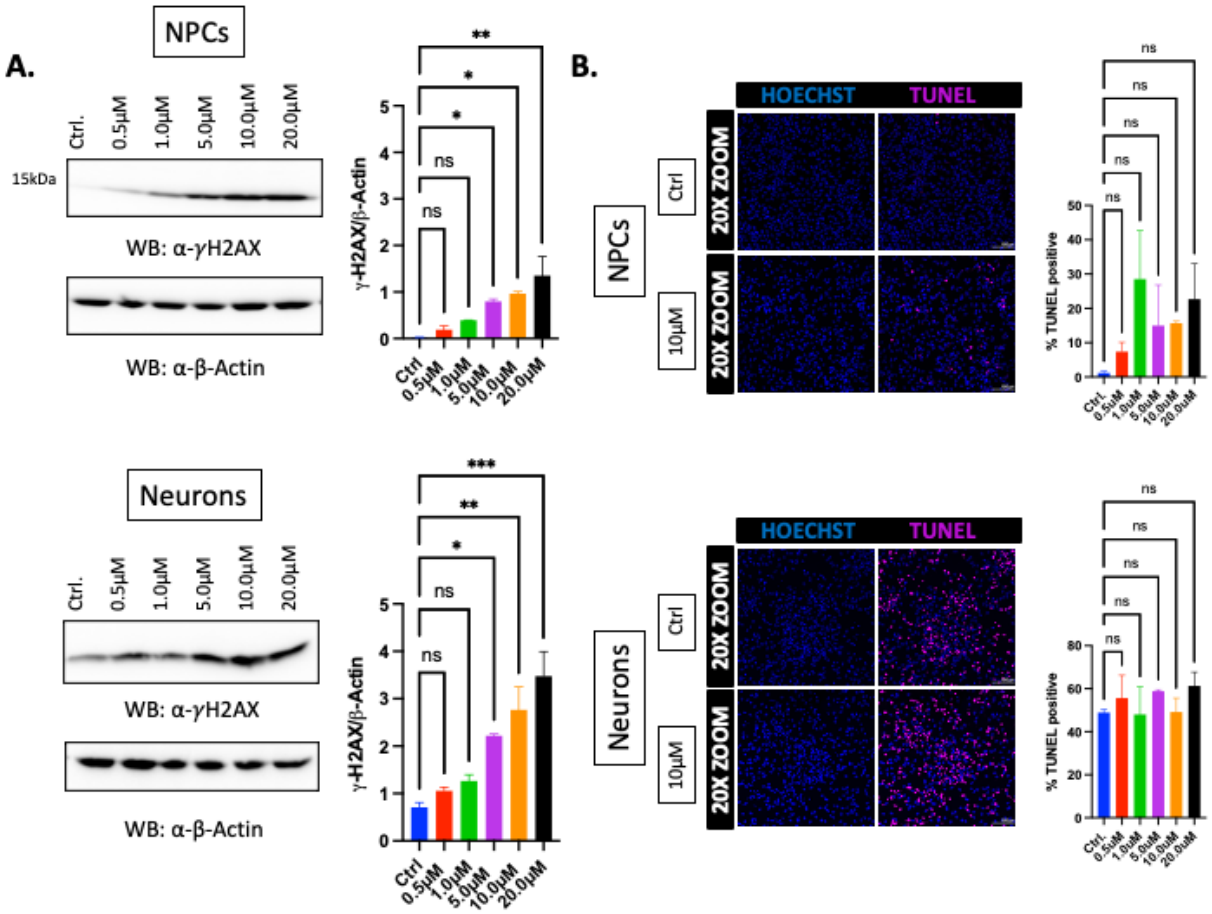
DNA Damage Increases Secreted Aβ$_{40}$ and Aβ$_{42}$ in Neuronal Progenitor Cells: Relevance to Alzheimer's Disease



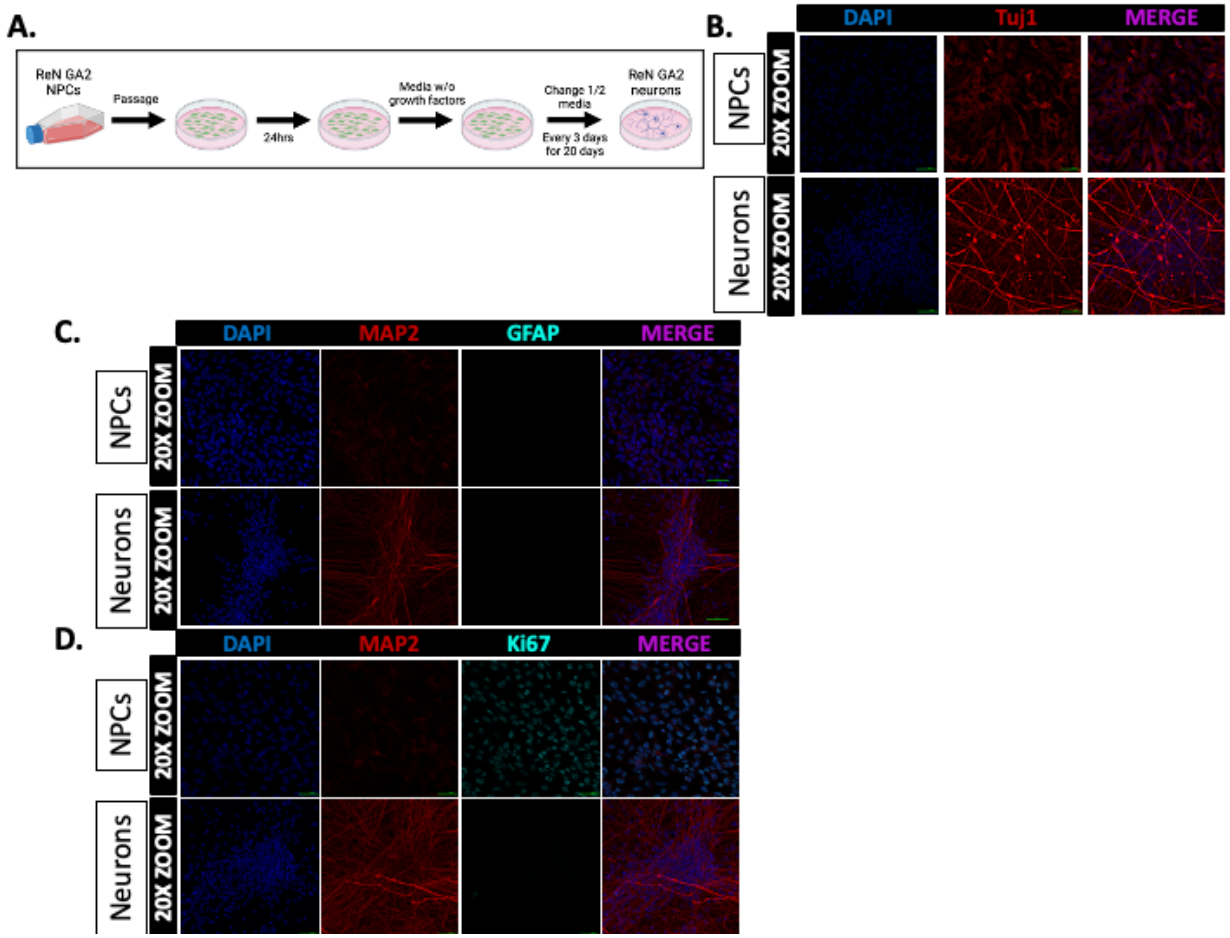
Supplementary Figure 1. Hydrogen peroxide damage increases Aβ$_{40}$ and Aβ$_{42}$ secretion in ReN GA2 NPCs, an effect that is mitigated by α-tocopherol treatment. A) Percent cell viability after designated treatment and recovery times. B) Percent secreted Aβ$_{40}$ and Aβ$_{42}$ relative to controls after designated treatments and recovery times. C) Aβ$_{42}$/Aβ$_{40}$ ratio calculated from raw values. Error bars represent means ± SD of three separate experiments, and the p values were determined using 2-way ANOVA and Tukey's multiple comparisons test. *p<0.05, **p<0.01, ****p<0.0001. ns, not significant.



Supplementary Figure 2. Hydrogen peroxide and etoposide damage induce DNA DSBs differentially in ReN GA2 NPCs and neurons. A) Cultures of ReN GA2 NPCs were assessed for DSB levels by neutral comet assay with and without 5 µM α-tocopherol pre-treatment for 0.5 hr, treated with and without 2.5 µM peroxide for 0.5 h. % tail DNA was quantified using 30 nuclei per experiment (100x tail DNA intensity/cell DNA intensity). B) Cultures of ReN GA2 NPCs and 20 day differentiated neurons were assessed for DSB levels by neutral comet assay with and without 6 h 10 µM etoposide treatment. % tail DNA was quantified using 30 nuclei per experiment (100x tail DNA intensity/cell DNA intensity). Error bars represent means ± SD of three separate experiments, and the p values were determined using one-way ANOVA and Tukey's multiple comparisons test, or Students T test. *p<0.05, **p<0.01, ****p<0.0001.



Supplementary Figure 3. Etoposide induces increased γ-H2AX expression in ReN GA2 NPCs and neurons, but not apoptosis. A) Western blot of endogenous γ-H2AX expression in ReN GA2 NPCs and 20 day differentiated neurons treated with and without etoposide at designated concentrations for 6 h and allowed to recover for 2 h. B) Click-IT terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay performed on ReN GA2 NPCs and 20 day differentiated neurons exposed to indicated etoposide concentrations for 6 h and allowed to recover for 2 h. Representative images show control and 10 μM etoposide treatments with Hoechst nuclei stain (blue) and overlaid TUNEL labeling (pink). Error bars represent means ± SD of two separate experiments, and the p values were determined using one-way ANOVA and Tukey's multiple comparisons test. *p<0.05, **p<0.01, ***p<0.001.



Supplementary Figure 4. Profiles of ReN GA2 NPCs and 20-day differentiated neurons. A) Treatment schematic of 20-day differentiation protocol. (Schematic created in BioRender.com.) Immunostaining of ReN GA2 NPCs and 20-day differentiated neurons with antibodies for (B) anti-Tuj1 (early neuron marker), (C) anti-MAP2 (neuron marker) and anti-GFAP (astrocyte marker), and (D) anti-MAP2 (neuron marker) and anti-Ki67 (proliferation marker).