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Supplemental Material to:

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Inhibition of glycolysis attenuates 4-hydroxynonenal-dependent autophagy and exacerbates apoptosis in differentiated SH-SY5Y neuroblastoma cells

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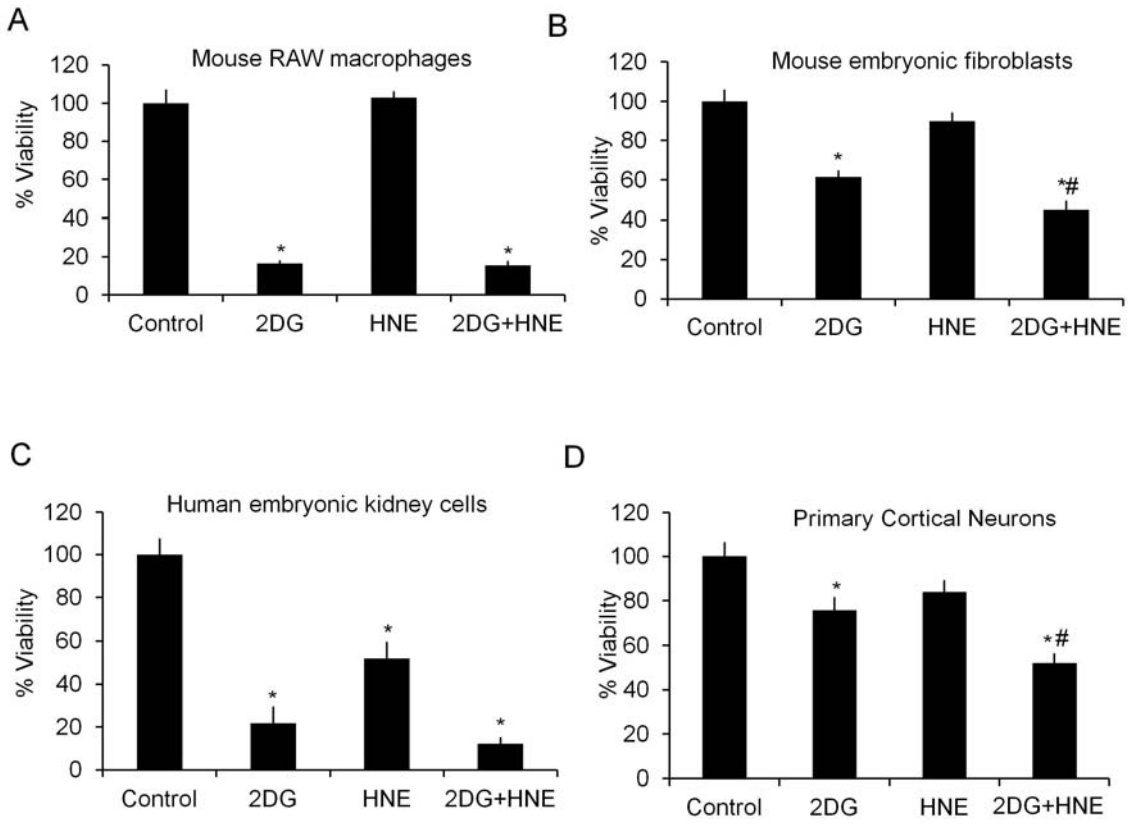

Figure S1. 2DG and HNE induce cell death in several different cell lines and primary neurons. Cell viability was assessed by the trypan blue exclusion method after exposure to 20 mM 2DG for 24 h, followed by HNE at 30 μM for 2 h. (**A**) Cell viability following exposure to 2DG and HNE in Mouse RAW macrophages. (**B**) Cell viability following exposure to 2DG and HNE in mouse embryonic fibroblasts. (**C**) Cell viability following exposure to 2DG and HNE in human embryonic kidney cells. (**D**) Cell viability following exposure to 2DG and HNE in rat primary cortical neurons. Data = mean ± SEM (n=3), normalized to Control. *p<0.05, compared to Control. #p<0.05, compared to 2DG. Student's t-test.

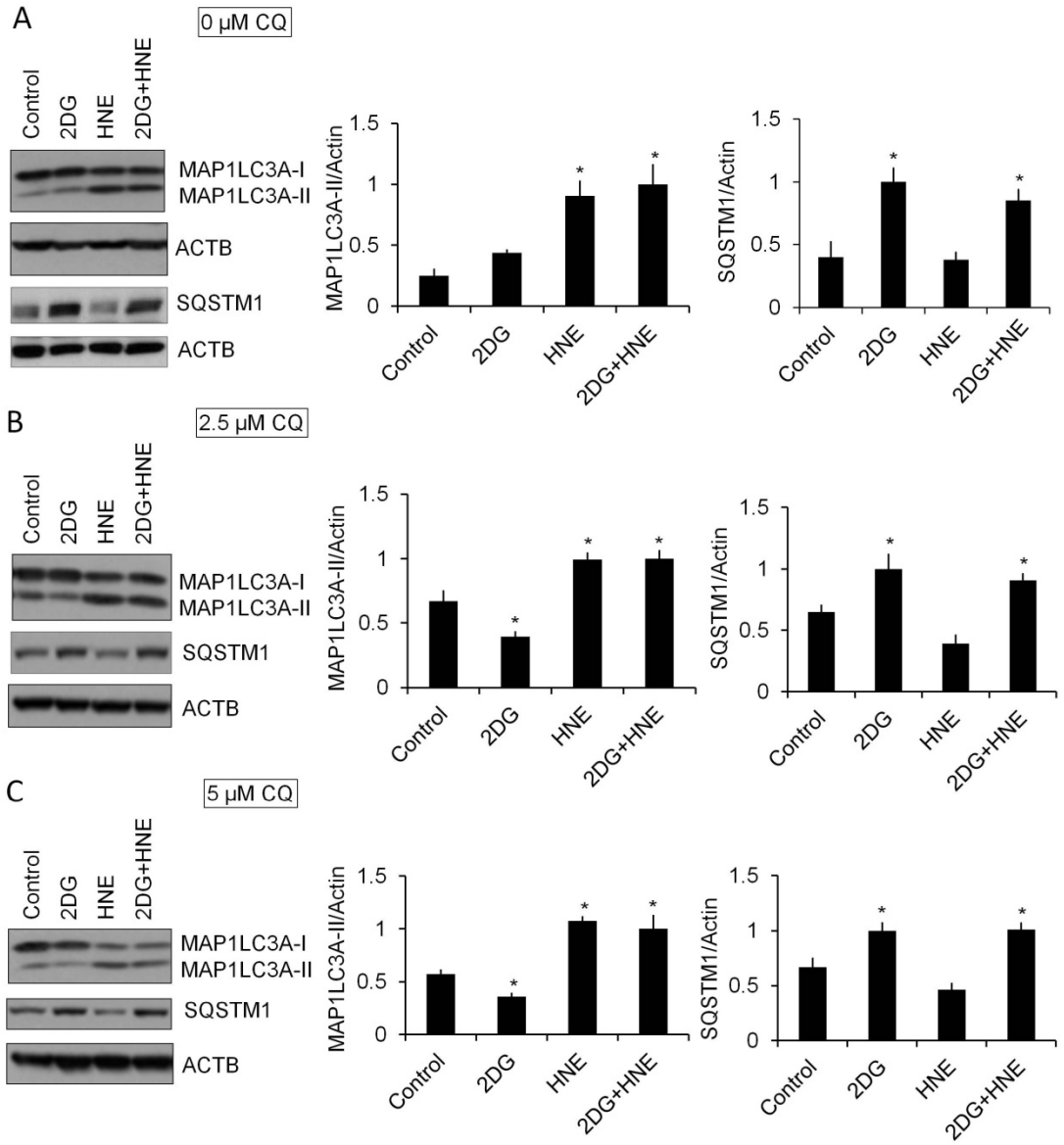

Figure S2. Autophagy assessment in response to HNE in the presence of increasing doses of chloroquine (CQ). Differentiated SH-SY5Y cells were treated with 20 mM 2DG, followed by 30 μM HNE for 2 h in the presence or absence of increasing concentrations of CQ. (**A**) Western blot analyses and quantification of protein extracts from cells treated with 0 μM CQ for SQSTM1, MAP1LC3A-I and MAP1LC3A-II. (**B**) Western blot analyses and quantification of protein extracts from cells treated with 2.5 μM CQ for SQSTM1, MAP1LC3A-I and MAP1LC3A-II. (**C**) Western blot analyses and quantification of protein extracts from cells treated with 5 μM CQ for SQSTM1, MAP1LC3A-I and MAP1LC3A-II. ACTB was used as a protein loading control. Data = mean ± SEM (n=3), normalized to HNE for MAP1LC3A-I and MAP1LC3A-II, or 2DG for SQSTM1. *p<0.05, compared to Control. Student's t-test.

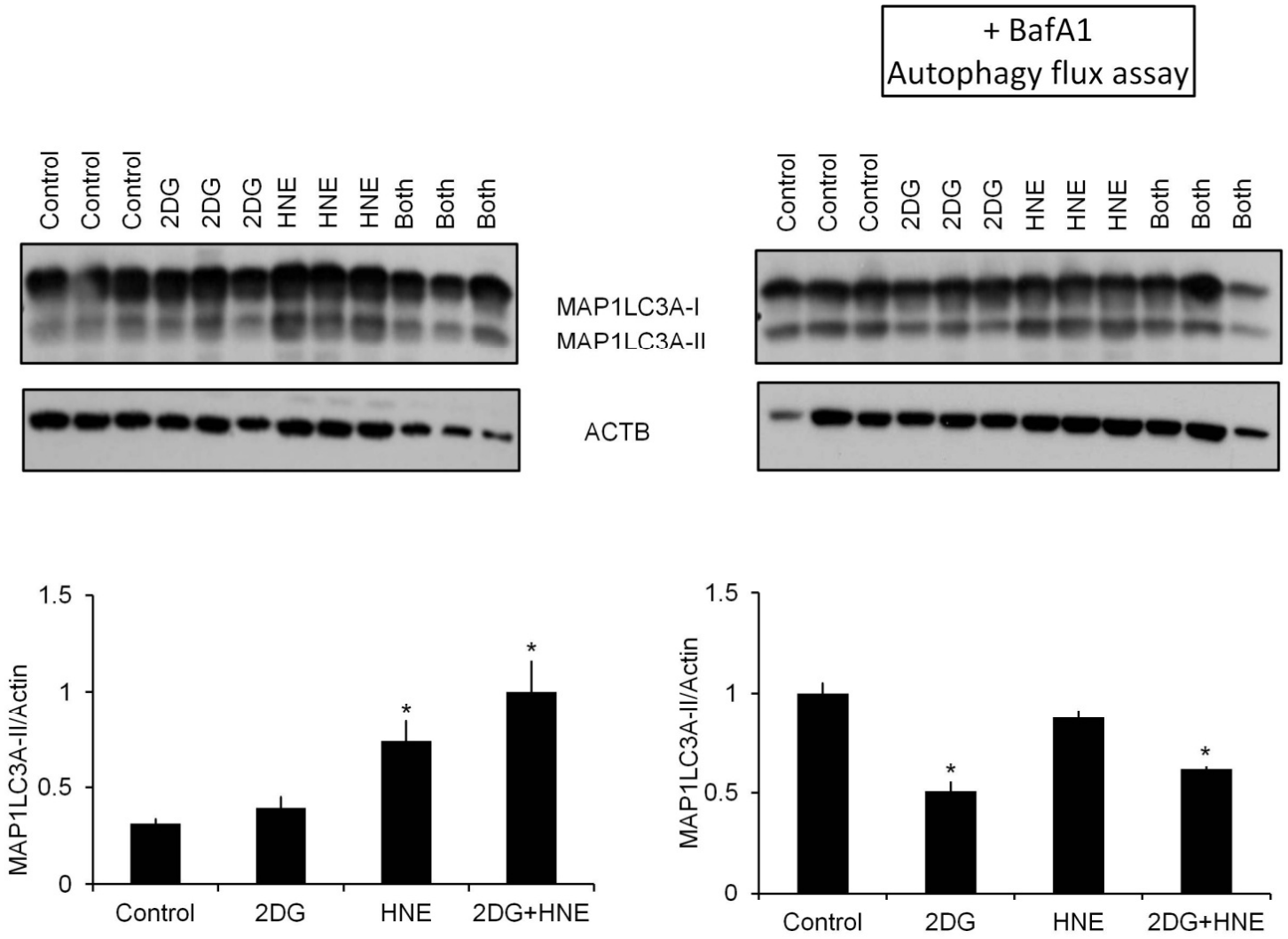

Figure S3. HNE induces and 2DG inhibits autophagic flux in rat primary cortical neurons. Primary cortical neurons were cultured from E18 rat embryos, in neurobasal media + B27 for 7 days. Half of the medium was changed every 3 days. Western blot analyses were performed with protein extracts from cells after exposure to 20 mM 2DG for 24 h, followed by 30 µM HNE for 2 in the presence or absence of 10 nM bafilomycin A_1 (BafA1) for MAP1LC3A. ACTB was used as a protein loading control. Data = mean ± SEM (n=3), normalized to lane with the highest signal. *p<0.05 compared to Control; Student's t-test.

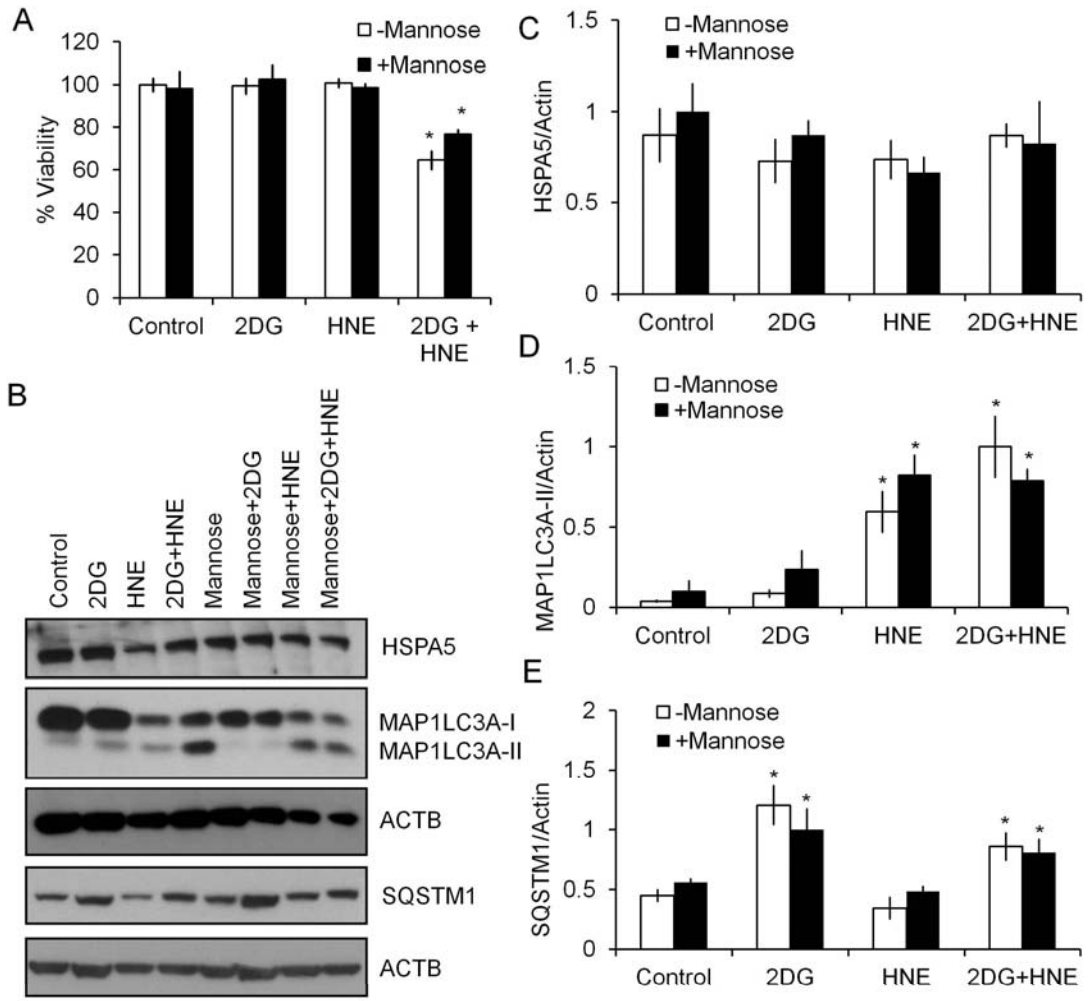

Figure S4. 2DG did not increase ER stress, and mannose did not change cell viability or autophagy. Differentiated SH-SY5Y cells were treated with 20 mM 2DG in the presence or absence of 10 mM mannose, followed by 30 µM HNE for 2 h. (**A**) Cell viability following exposure to 2DG in the presence or absence of mannose and HNE. Data = mean ± SEM (n=3), normalized to Control. (**B**) Western blot analyses of protein extracts from cells treated with 2DG, mannose and HNE. ACTB was used as a protein loading control. (**C**) Quantification of HSPA5 from panel B. Data = mean ± SEM (n=3), normalized to mannose. (**D**) Quantification of MAP1LC3II-A from (**B**). Data = mean ± SEM (n=3), normalized to 2DG+HNE. *p<0.05, compared to Control. Student's t-test. (**E**) Quantification of SQSTM1 from (**B**). Data = mean ± SEM (n=3), normalized to mannose. *p<0.05, compared to Control; Student's t-test.