

Figure S1. Trehalose induces cell cycle block in SH-SY5Y cells. SH-SY5Y cells were incubated with or without trehalose (50 or 100 mM) for 48 h, and the cell cycle phases in propidium iodide-stained cells were analyzed by flow cytometry. The results from a representative of three independent experiments are presented.

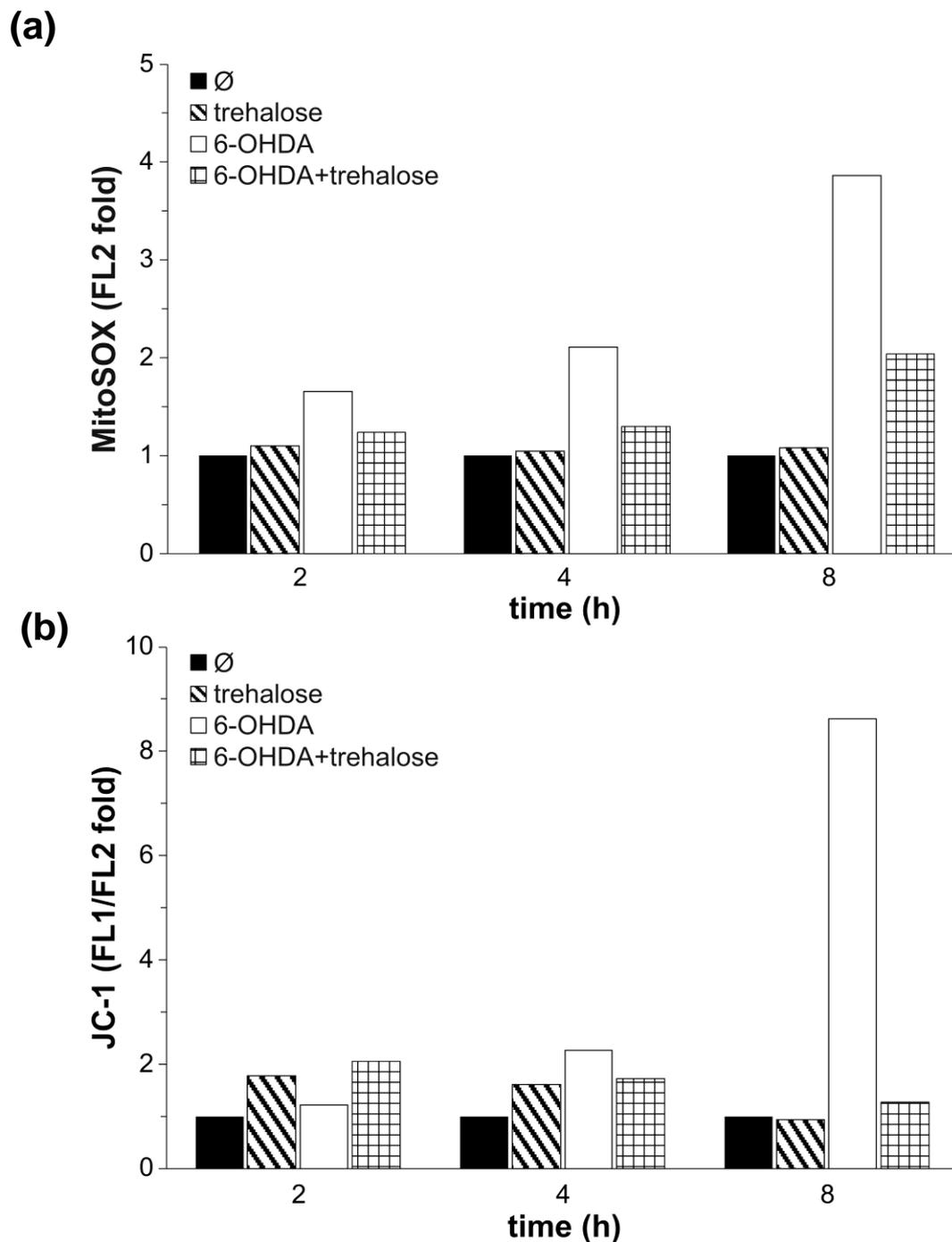


Figure S2. Trehalose prevents 6-OHDA-induced mitochondrial superoxide production and depolarization in SH-SY5Y cells. SH-SY5Y cells were incubated with or without trehalose (TRE; 100 mM) for 24 h and then exposed or not to 6-OHDA (60 μ M) for the indicated times. Mitochondrial superoxide production **(a)**, and mitochondrial depolarization **(b)** were determined by flow cytometric analysis of MitoSOX (FL2) and JC-1 (FL1/FL2) median fluorescence, respectively. The data from a representative of two independent experiments are shown.

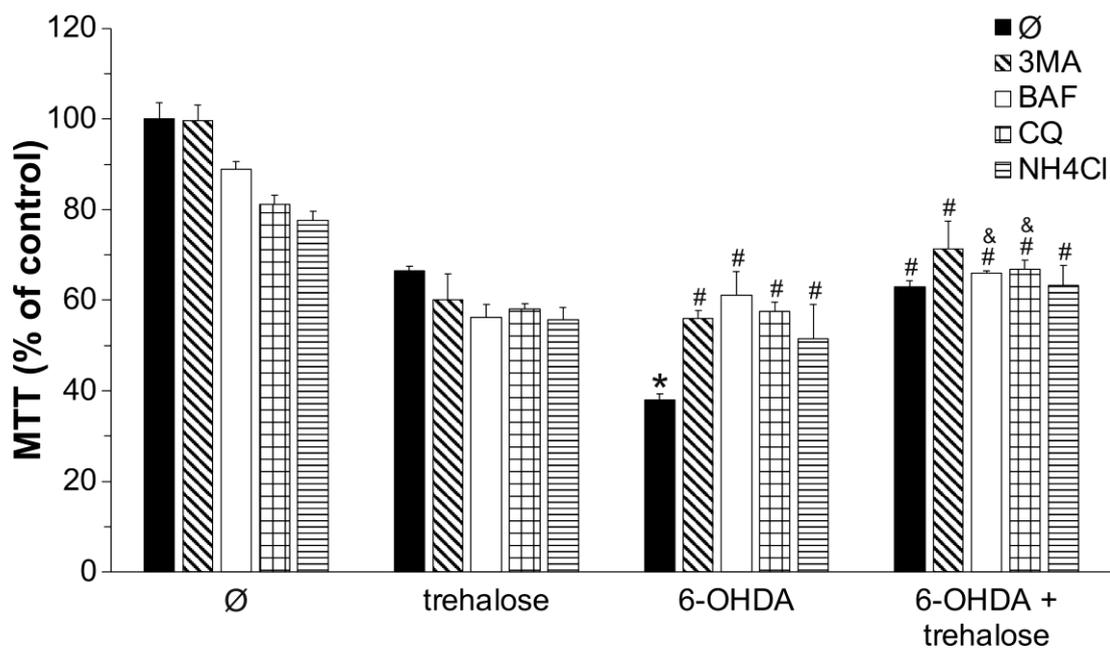
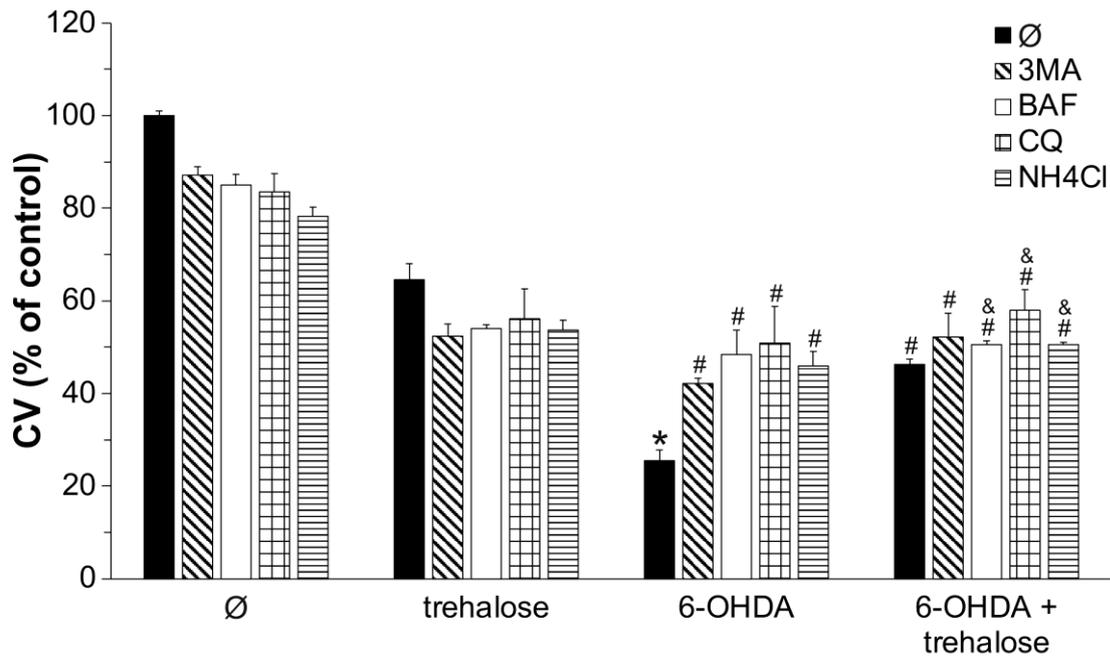


Figure S3. Autophagy is not involved in trehalose-mediated protection from 6-OHDA. SH-SY5Y cells were incubated with or without trehalose (100 mM) for 24 h and then treated or not with 6-OHDA (60 μ M) for 24 h, in the presence or absence of 3-methyladenine (3MA; 4 mM), bafilomycin A1 (BAF; 10 nM), chloroquine (CQ; 20 μ M), or NH₄Cl (10 mM). Cell viability was determined by crystal violet (CV) and MTT assays, and the cytotoxicity was evaluated by LDH release. The data are presented as mean \pm SD values of triplicates from a representative of three independent experiments (* p < 0.05 vs. no treatment; # p < 0.05 vs. 6-OHDA; & p < 0.05 vs. 6-OHDA + trehalose).

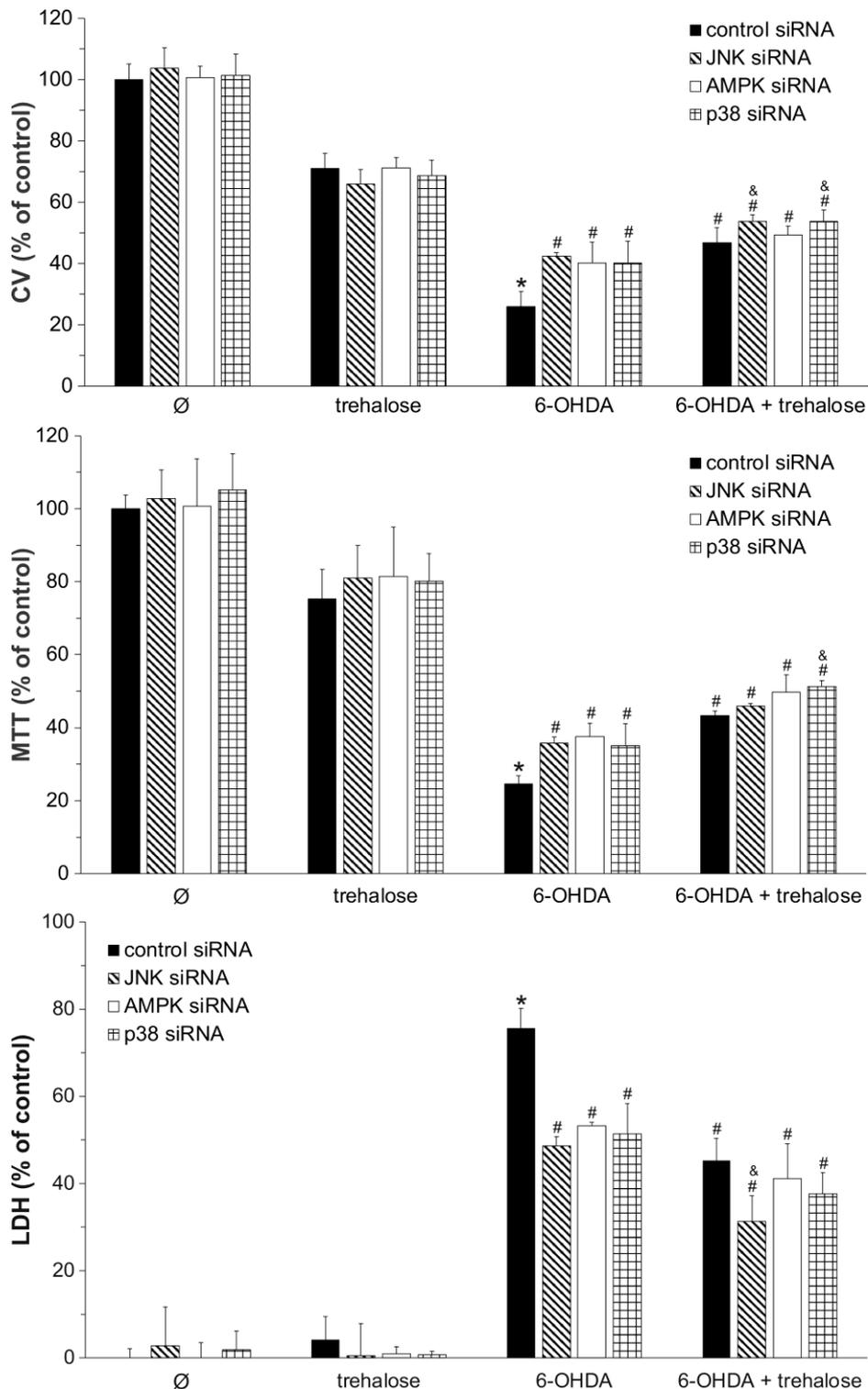


Figure S4. Inhibition of JNK, AMPK, and p38 MAPK is involved in trehalose-mediated protection from 6-OHDA. After transfection with control siRNA or siRNA against JNK, AMPK, or p38 MAPK, SH-SY5Y cells were incubated with or without trehalose (100 mM) for 24 h and then treated or not with 6-OHDA (60 μ M) for another 24 h. Cell numbers were determined by crystal violet (CV) and MTT assays, and the data are presented as mean \pm SD values of triplicates from a representative of three independent experiments (* $p < 0.05$ vs. control siRNA/no treatment; # $p < 0.05$ vs. control siRNA/6-OHDA; & $p < 0.05$ vs. control siRNA/6-OHDA + trehalose).

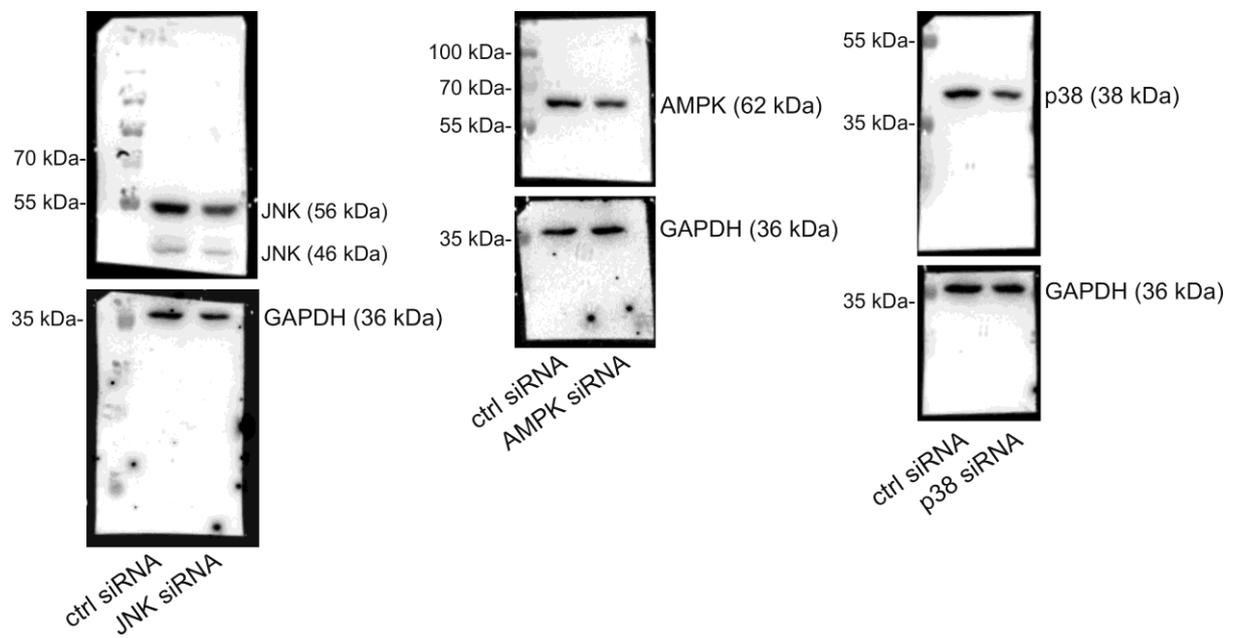


Figure S5. Original immunoblots confirming JNK, AMPK, and p38 MAPK downregulation by siRNA.