Supplementary Materials: The Neuroprotective Properties of *Hericium erinaceus* in Glutamate-Damaged Differentiated PC12 Cells and an Alzheimer's Disease Mouse Model

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2. Results

The Effects of HE on L-Glu-Induced Intracellular ROS Accumulation

Three-hour HE preincubation followed by another 12 h coexposure to L-Glu strongly reduced high green fluorescence in DCFH-DA staining, suggesting its inhibition of ROS accumulation (Figure S1).

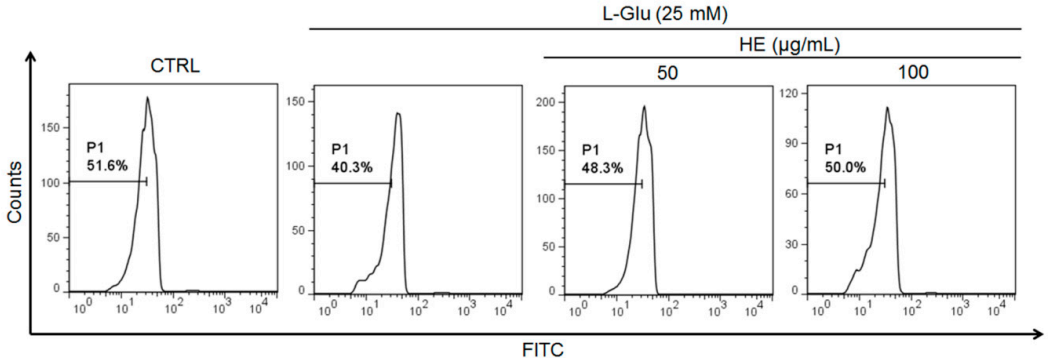

Figure S1. The overaccumulation of ROS caused by 12 h L-Glu incubation was significantly reduced by 3 h HE pretreatment, detected via DCFH-DA staining (n = 3).

4. Methods and Materials

Assessment of ROS

The intracellular ROS level was measured by 2′,7′-Dichlorodihydrofluorescein diacetate (DCFH-DA, Sigma-Aldrich, St. Louis, Missouri, USA) staining. Cells were seeded into 6-well plates at 2 × 10^5 cells per well. DPC12 cells were treated with HE (50 and 100 μg/mL) for 3 h, and then coincubated with 25 mM of L-Glu for another 12 h. After three washes with phosphate-buffered saline (PBS), the changes of intracellular ROS level were analyzed by flow cytometry (FC500, Beckman Coulter, Brea, CA, USA). The experiment was repeated three times.