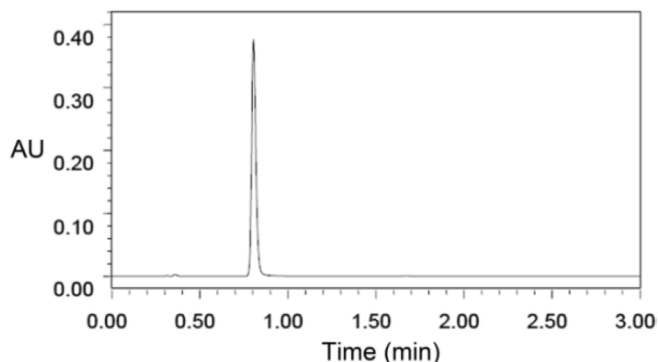
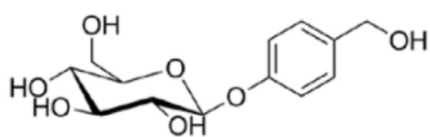


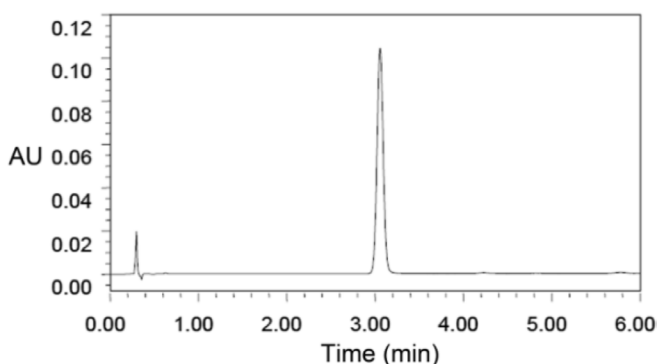
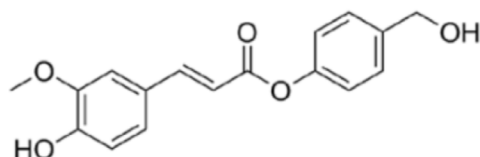
Neuroprotective effect of a novel gastrodin derivative against ischemic brain injury: involvement of peroxiredoxin and TLR4 signaling inhibition

Supplementary Materials

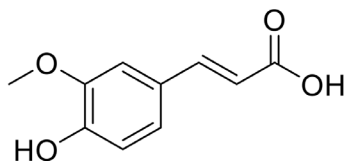
A Gastrodin (Gas)



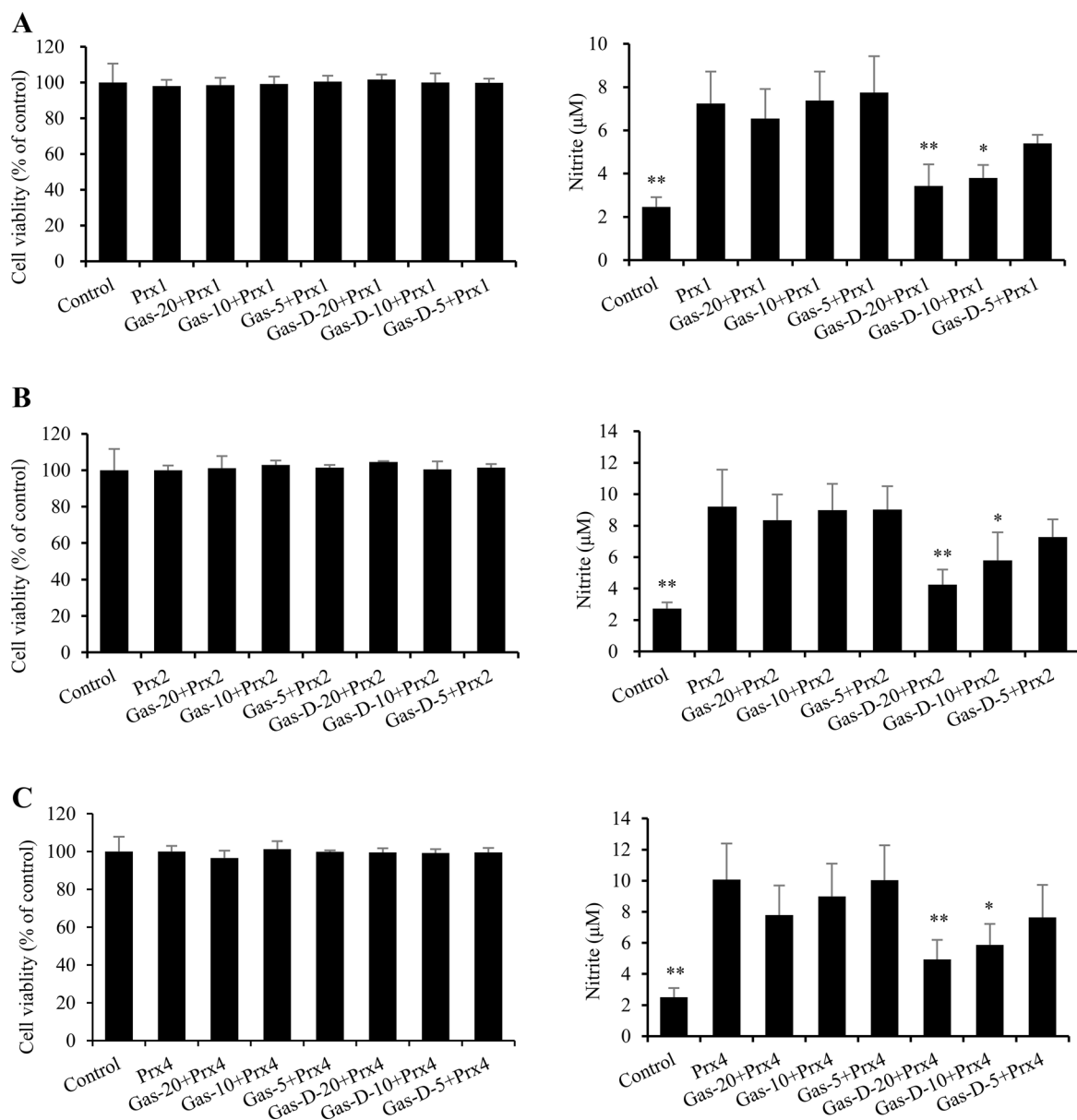
B Gastrodin derivative (Gas-D)



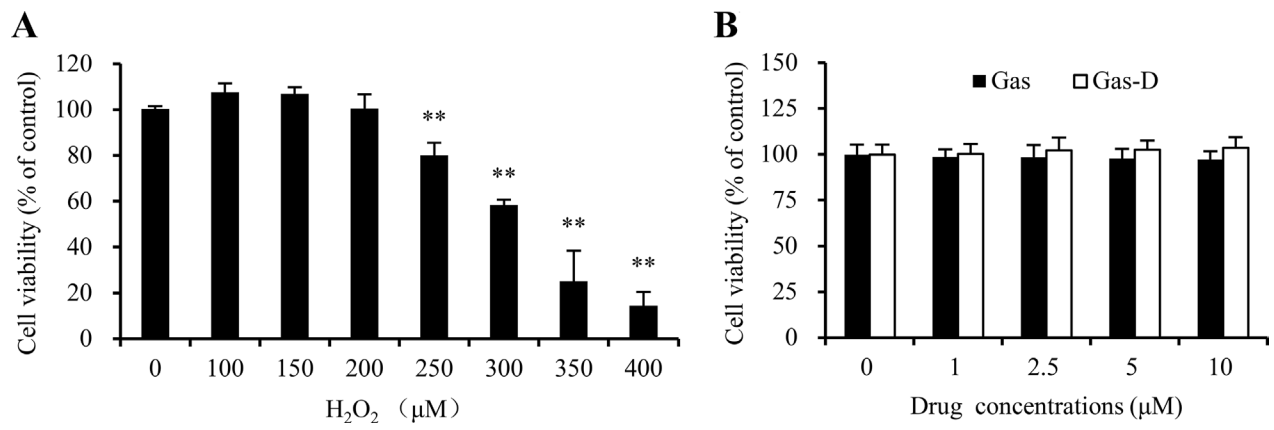
C Ferulic acid



Supplementary Figure 1: Chemical structures and UPLC chromatograms of compounds. The separations were performed on a Waters BEH C18 (50 × 2.1 mm i.d., 1.7 μm) column. The mobile phase consisted of acetonitrile and 0.1% aqueous phosphoric acid (v/v, B) using isocratic programs of 3% A for gastrodin (A) and 25% A for gastrodin derivative (B) respectively. The flow rate was 0.4 ml/min, and the column temperature was maintained at 30°C. The detective wavelengths for Gas and Gas-D were set at 220 nm and 330 nm, respectively. As a result, the respective purities of the tested compounds were more than 98.0% based on peak area normalization. (C) Chemical structure of ferulic acid.



Supplementary Figure 2: Dose-dependent effects of Gas and Gas-D on cellular viability and NO production in macrophages treated with Prxs. RAW264.7 cells were pretreated for 1 h with vehicle, Gas, or Gas-D at doses of 5, 10, or 20 µM and then incubated with vehicle (Control) or the indicated Prx subtype (Prx1, Prx2, or Prx4) at a dose of 20 nM for 24 h. Cell viability was examined by the MTT assay, and NO content was measured by a NO kit. (A–C) Effects of Gas and Gas-D on Prx1-, Prx2-, and Prx4-stimulated cells. The results are expressed as a percentage of the control group. The data are representative of three independent experiments with 8 or 6 replicates for the MTT and NO assays, respectively.



Supplementary Figure 3: Cytotoxic effects of H₂O₂, gastrodin (Gas), and its derivative (Gas-D) in SH-SY5Y cells. Cells were incubated with H₂O₂ at the indicated concentrations for 24 h, and cell viability was examined by the MTT assay. The results are representative of three independent experiments. The data are expressed as mean ± SEM (*n* = 6). **p* < 0.05, ***p* < 0.01, vs. vehicle-treated control group.