Supporting Information

Jang et al. 10.1073/pnas.0913572107

SI Materials and Methods

7,8-Dihydroxyflavone Activates Tyrosine Kinase Receptor B in Mouse Brain. To assess whether 7,8-dihydroxyflavone can provoke tyrosine kinase receptor B (TrkB) activation in the brain, we injected mice i.p. with a dose of 5 mg/kg at various time points. TrkB but not TrkA was selectively phosphorylated in the brain 2 h after injection, indicating that 7,8-dihydroxyflavone can penetrate the brain–blood barrier and provoke TrkB activation. Different dosages, from 2 to 10 mg/kg, exhibited similar results with no detectable toxicity. The protein and mRNA levels of neurotrophic receptors were not altered after drug treatment (Fig. S4), indicating that 7,8-dihydroxyflavone does not induce Trk receptor transcription or translation.

Structure–Activity Relationship Study. 7,8-Dihydroxyflavone, but not other tested flavone derivatives, possesses neurotrophic activities. To explore the structure–activity relationship of flavonoids in promoting neuronal survival and TrkB activation, we tested numerous flavone derivatives and found that the 7,8-dihydroxy catechol moiety is essential for activating TrkB and protecting neurons from apoptosis (Fig. S6).

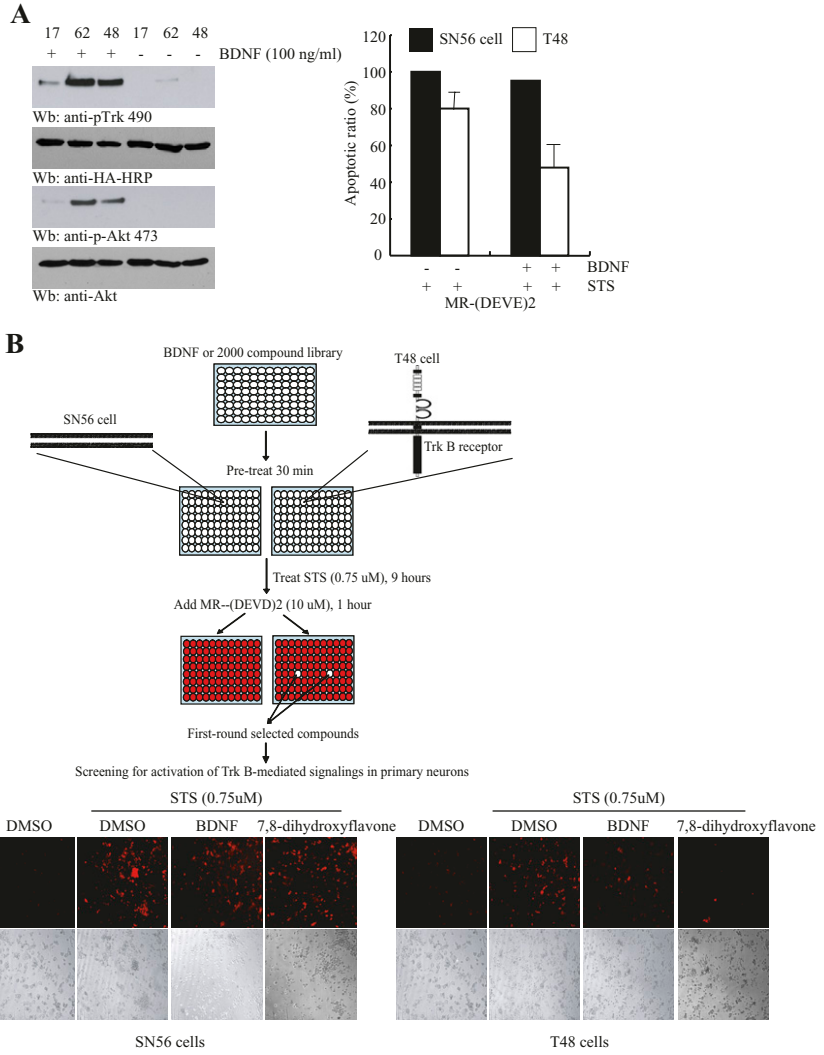

Fig. S1. Screening of TrkB receptor agonists. (*A*) TrkB stably transfected cells are resistant against apoptosis. SN56 cells were stably transfected with rat TrkB full-length receptor. Both T48 and T62 stably transfected clones were responsive to BDNF treatment and activated TrkB and Akt (*Left*). Quantitative analysis of apoptosis in a stable T48 cell line (*Right*). Data are expressed as mean ± SEM. (*B*) Chemical-genetic screening for TrkB agonists. The schematic flowchart of the scanning strategy for TrkB agonists (*Upper*). The microscopic pictures show representative SN56 and T48 cells pretreated with BDNF or 7,8-dihydroxyflavone, followed by staurosporine. The apoptotic cells were stained with red fluorescent activated by caspase-3 (*Lower*).

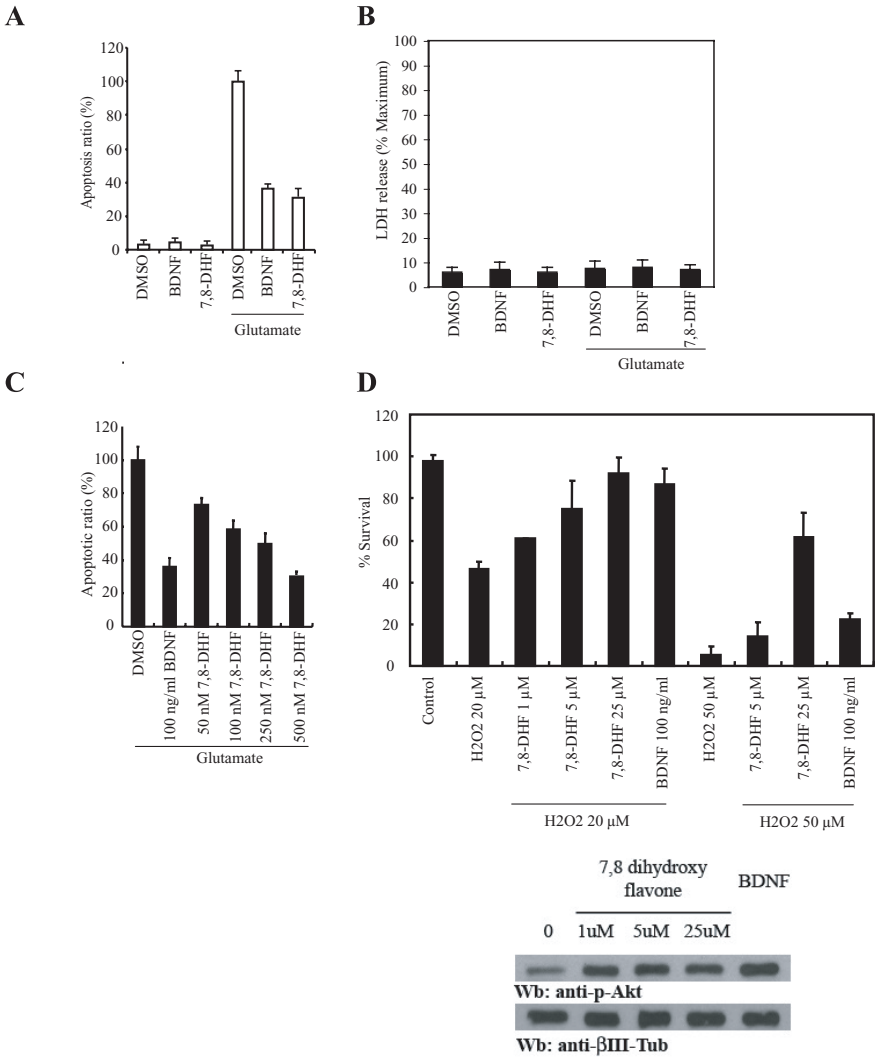

Fig. S2. 7,8-Dihydroxyflavone effectively protects neurons from apoptosis. (*A*) 7,8-Dihydroxyflavone protects rodent neurons from glutamate-provoked apoptosis. Primary cortical neurons were pretreated with 100 ng/mL BDNF or 0.5 μM 7,8-dihydroxyflavone for 30 min, followed by 50 μM glutamate for 16 h. Neurons were stained with MR(DEVD)2 and DAPI. Only the neurons with both active caspase (red) and nuclear condensation were counted as apoptotic cells. Approximately 500 neurons were counted in total. (*B*) LDH release assay. Neurons treated as in *A* were lysed, and the cell lysates were analyzed with the LDH release kit as described by the manufacture. (*C*) 7,8-Dihydroxyflavone prevents glutamate-elicited neuronal cell death in a dose-dependent manner. 7,8-Dihydroxyflavone protects cortical neurons from apoptosis at 50 nM, and has an even more potent prosurvival effect than BDNF at 500 nM. (*D*) 7,8-Dihydroxyflavone protects human neurons made from ES cells from H_2O_2-induced apoptosis. Neurons from human ES cells were preincubated with various concentrations of indicated 7,8-dihydroxyflavone for 40 min, and then 20 or 50 μM H_2O_2 was added. In 3 h, the apoptotic cells were quantitatively analyzed. 7,8-Dihydroxyflavone exhibited a dose-dependent protective effect against H_2O_2. At 25 μM, 7,8-dihydroxyflavone even displayed stronger protective effect than BDNF (*Upper*). 7,8-Dihydroxyflavone induced Akt activation in human neurons (*Lower*).

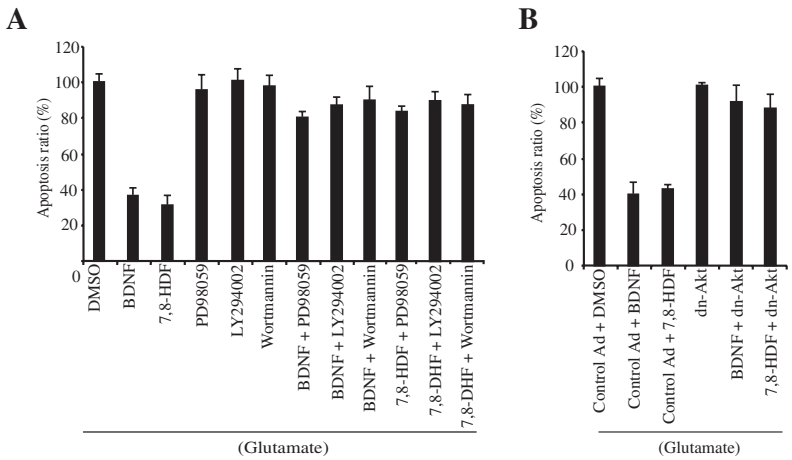

Fig. S3. MAPK and PI3K/Akt signaling cascades are required for the prosurvival action of 7,8-dihydroxyflavone. (*A*) MEK1 or PI3K inhibitors block the prosurvival action of 7,8-dihydroxyflavone. Primary cortical neurons were pretreated with MEK1 inhibitor (PD98059, 10 μM), PI3K inhibitor (wortmannin, 10 nM), and LY294002 (20 μM) for 30 min, followed by 100 ng/mL BDNF or 500 nM 7,8-dihydroxyflavone. After15 min, the neurons were treated with glutamate (50 μM) for 16 h. Apoptosis was quantitatively analyzed. Results are expressed as mean ± SD. (*B*) Dominant-negative Akt blocks the prosurvival action of 7,8-dihydroxyflavone. Cortical neurons were infected with control adenovirus or adenovirus expressing dominant-negative Akt. After 24 h, the neurons were pretreated with BDNF or 7,8-dihydroxyflavone for 15 min, followed by glutamate treatment. Apoptosis was quantitatively analyzed. Results are expressed as mean ± SD.

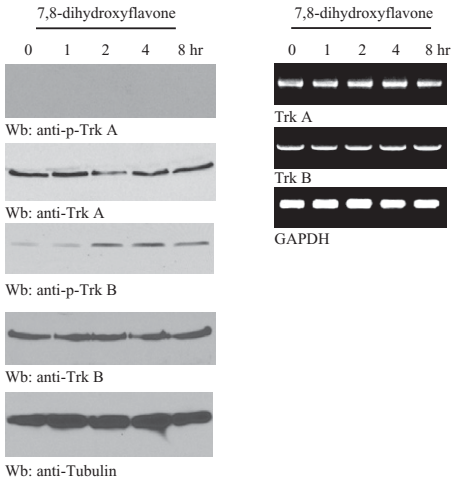

Fig. S4. 7,8-Dihydroxyflavone induces TrkB phosphorylation in mouse brain. 7,8-Dihydroxyflavone (5 mg/kg) was injected i.p. in mice at various time points. Mouse brain lysates were assessed by Western blotting using antibodies against indicated proteins (*Left*). The RT-PCR for TrkA and TrkB remained unchanged upon 7,8-dihydroxyflavone treatment (*Right*).

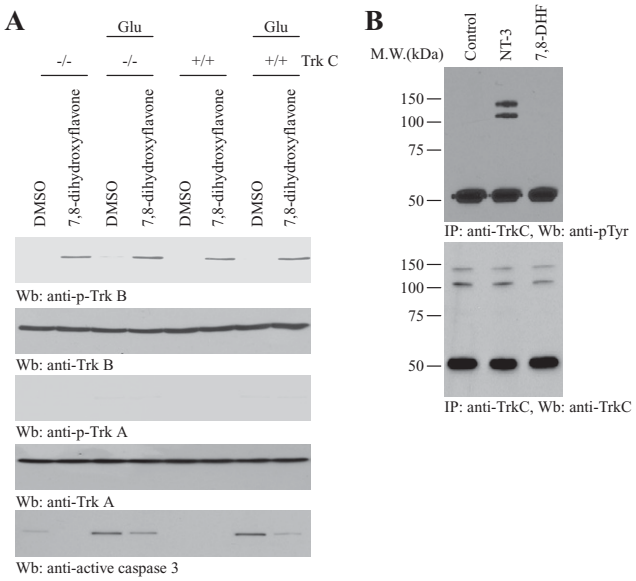

Fig. S5. 7,8-Dihydroxyflavone prevents neuronal apoptosis independent of TrkC receptor. (*A*) 7,8-Dihydroxyflavone diminishes caspase-3 activation regardless of TrkC genotype. (*B*) 7,8-Dihydroxyflavone does not activate TrkC. Cortical neurons were treated with NT-3 or 7,8-dihydroxyflavone for 30 min. TrkC was immunoprecipitated and analyzed by immunoblotting with anti-PY20. NT-3, but not 7,8-dihydroxyflavone, provoked TrkC activation (*Upper*). A similar level of TrkC was immunoprecipitated (*Lower*).

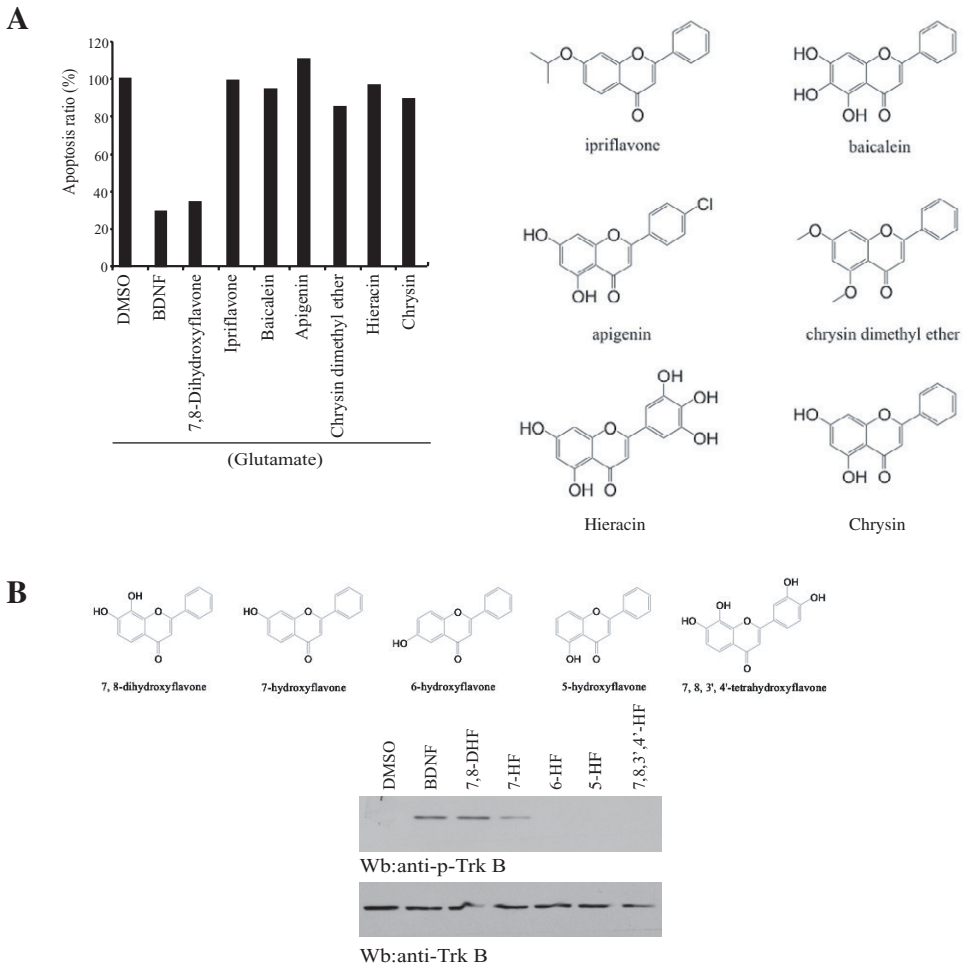

Fig. S6. 7,8-Dihydroxyflavone structure–activity relationship study. (*A*) Apoptotic assay with various flavone derivatives. Cortical neurons (*Right*) were pretreated with various flavone derivatives (0.5 μM) for 30 min, followed by treatment with 50 μM glutamate. Apoptotic neurons were quantitatively analyzed. Compared with 7,8-dihydroxyflavone, 5,7-dihydroxyflavone (chrysin) had no prosurvival activity (*Left*). (*B*) Immunoblotting analysis of p-TrkB with various flavone derivatives (*Upper*). Cortical neurons were pretreated with various flavone derivatives (0.5 μM) for 30 min. Cell lysates were analyzed by immunoblotting with anti-p-TrkB (*Lower*). 7-Hydroxyflavone had a partial agonistic effect.