Supporting Information

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SI Materials and Methods

Plasmids. The mammalian expression vector encoding Src homology region 2-containing protein tyrosine phosphatase-2 (SHP-2) has been described elsewhere (1). For purification of recombinant proteins from *Escherichia coli*, SHP-2 cDNA was inserted in-frame with the GST sequence in a pGEX bacterial expression vector. Introduction of the Cys-459 to Ser mutation was accomplished via oligonucleotide-mediated site-directed mutagenesis (QuikChange; Stratagene). All sequences were verified by direct DNA sequencing.

Cell Culture. HEK cells stably expressing neuronal nitric oxide (NO) synthase (nNOS; HEK-nNOS) were cultured as described previously (2, 3). To trigger NO production from nNOS, HEK-nNOS cells were exposed to the calcium ionophore A23187 (10 μM); in some experiments, the NO synthase inhibitor *N*-nitro-L-arginine (1 mM) was included to block NO production. Mixed cortical neuronal/glial cultures were obtained from embryonic day 17 rats as previously described (4). Cells were grown on glass coverslips coated with poly-L-lysine and typically cultured 14 to 16 d in vitro. Cultures were transfected by using Lipofectamine 2000 (Invitrogen) and exposed to NMDA (Sigma) 2 d after transfection. NMDA exposure (50 μM) for 20 min in Earle balanced salt solution (5) was followed by replacement of the buffer with the saved conditioned medium and incubation for an additional 1 h for biotin-switch assays or 16 h for cell death assays.

Western Blot Analysis. Proteins were resolved on 4% to 12% polyacrylamide gels and then transferred to nitrocellulose membranes. The membranes were blocked in 5% nonfat skim milk in Tris-buffered saline solution containing 0.1% Tween 20 and incubated with anti–SHP-2 antibody (C-18 or B-1; Santa Cruz Biotechnology), followed by incubation with secondary antibody. Proteins were visualized by using ECL Plus (GE Healthcare) as directed by the manufacturer.

RNAi-Mediated Knockdown of SHP-2. For expression of small hairpin SHP-2 RNAs, GATCCAAGAAGCAGAGAAGCTGCTTTCAAGAGAAGCAGCTTCTCTGCTTCTTTTA and AGCTTAAAAGAAGCAGAGAAGCTGCTTCTCTTGAAAGCAGCTTCTCTGCTTCTTG (SHP-2 shRNA1), or GATCCCACTGGGGACTACTATGACTTCAAGAGAGTCATAGTAGTCCCCAGTGTTA and AGCTTAACACTGGGGACTACTATGACTCTCTTGAAGTCATAGTAGTCCCCAGTGG (SHP-2 shRNA2) (6), were annealed and ligated into the HindIII and BamHI sites of p*Silencer* 4.1-CMV neo (Ambion). p*Silencer*-mediated knockdown of SHP-2 in culture was compared with cells transfected with p*Silencer* 4.1-CMV neo Negative Control, which was supplied with the p*Silencer* 4.1-CMV neo kit (Ambion). The p*Silencer* 4.1-CMV neo Negative Control plasmid encodes a hairpin RNA whose sequence was not found in the mouse, human, or rat genome databases.

1. Chen Y, et al. (2003) Identification of Shp-2 as a Stat5A phosphatase. *J Biol Chem* 278(19):16520–16527.
2. Bredt DS, et al. (1991) Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature* 351(6329):714–718.
3. Uehara T, et al. (2006) S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. *Nature* 441(7092):513–517.
4. Lei SZ, et al. (1992) Effect of nitric oxide production on the redox modulatory site of the NMDA receptor-channel complex. *Neuron* 8(6):1087–1099.
5. Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, Lipton SA (1995) Apoptosis and necrosis: Two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proc Natl Acad Sci USA* 92(16):7162–7166.
6. Ivins Zito C, Kontaridis MI, Fornaro M, Feng GS, Bennett AM (2004) SHP-2 regulates the phosphatidylinositide 3′-kinase/Akt pathway and suppresses caspase 3-mediated apoptosis. *J Cell Physiol* 199(2):227–236.

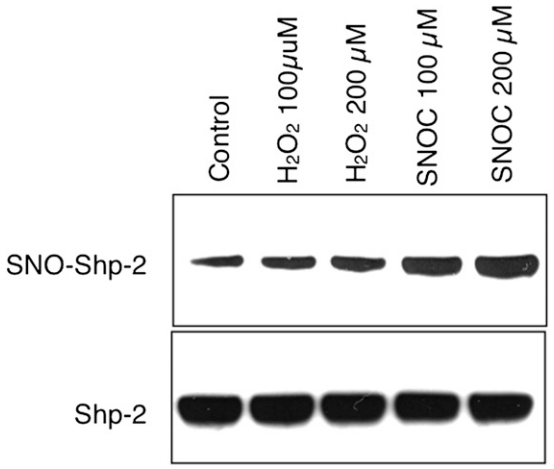

Fig. S1. S-nitrosocysteine (SNOC), but not H_2O_2, induces S-nitrosylation of SHP-2. The biotin-switch assay was used to detect SNO–SHP-2 formation in cerebrocortical neurons 15 min after exposure to SNOC or H_2O_2.

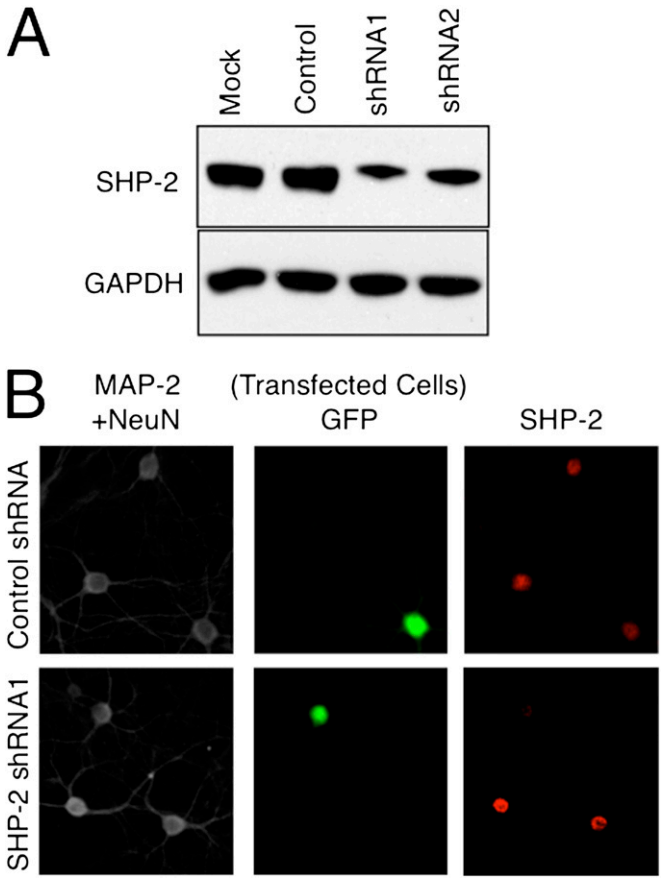

Fig. S2. RNAi-mediated knockdown of endogenous SHP-2. (*A*) Mouse embryonic fibroblast cells were transfected with plasmid constructs expressing shRNAs directed against mouse/rat SHP-2 (shRNA1 and shRNA2). Total cell lysates were analyzed by Western blotting for SHP-2 (*Upper*) and GAPDH (*Lower*). Control shRNA encodes a sequence that was not found in the mouse, human, or rat genome databases. (*B*) Reduction of SHP-2 expression by shRNA in primary cortical neurons by immunocytochemistry. Rat cortical neurons seeded at low density were cotransfected with GFP and control shRNA or SHP-2 shRNA1. Two days after transfection, cells were fixed and immunostained with MAP-2 and NeuN antibodies (Cy5; white) to identify neuronal cells, and with SHP-2 antibody (Cy3; red). Transfected cells were identified by expression of GFP (green).

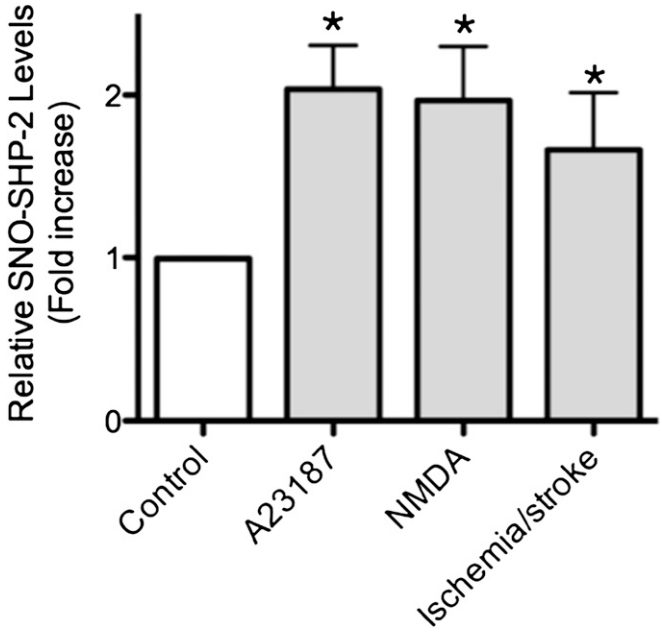

Fig. S3. Ratio of SNO–SHP-2 to total SHP-2 under various conditions. Biotin-switch assays and immunoblot analyses were quantified by densitometry, and the relative ratio of SNO–SHP-2 to total SHP-2 was calculated for the following conditions: control samples, HEK-nNOS cells after nNOS activation by A23187 treatment, cerebrocortical neurons after NMDA exposure in vitro, and mouse brain in vivo after ischemia/stroke. Values are mean ± SEM ($n \geq 3$; $^*P < 0.05$).

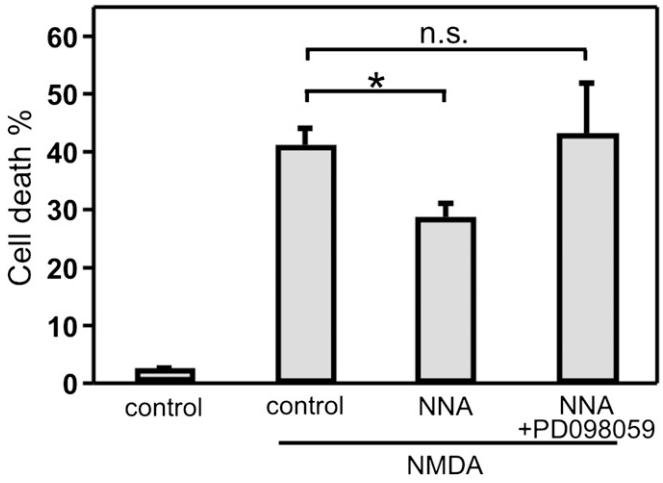

Fig. S4. Inhibition of the ERK pathway prevents the protective effect of nNOS inhibition on NMDA-induced excitotoxicity. Rat cortical neurons were pretreated with the MEK inhibitor PD098059 (50 μM) before exposure to NMDA. Neuronal cell death was measured by TUNEL assay 16 h after NMDA exposure. Values are mean ± SEM ($n \geq 3$; $^*P < 0.01$; n.s., not significant).

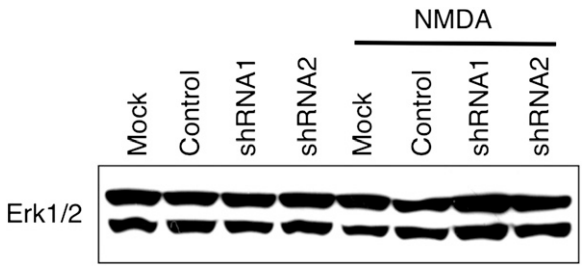

Fig. S5. NMDA exposure per se does not affect the level of ERK1/2 protein expression in cortical neurons in the presence of SHP-2 shRNAs or mock transfection. Cell lysates prepared from cortical neurons were analyzed by immunoblotting with anti-ERK1/2 antibody.