Supplementary information (Sasabe *et al.*),

Methods

Antibodies Rabbit polyclonal antibodies to D-Ser and NR1, mouse monoclonal anti-neuronal nuclei (NeuN) antibody, and goat polyclonal anti-ChAT antibody were purchased from Chemicon. Rabbit polyclonal antibodies to phospho-JNK (for immunoblotting), phospho-p38, total-p38, and mouse monoclonal antibodies to phospho-ERK 1/2 and GFAP were from Cell Signaling. Rabbit polyclonal antibodies to JNK1, ERK1, actin; goat polyclonal antibodies to NR2C, NR2D, human DAO; and mouse monoclonal anti-phospho JNK (for immunofluorescence) were obtained from Santa Cruz. Additional purchases included mouse monoclonal antibody to non-phosphorylated neurofilaments (SMI-32), Sternberger Monoclonals; mouse monoclonal antibody to SRR, BD; rabbit polyclonal anti-GFAP antibody, Dako; sheep polyclonal antibody to DAO, Abcam; rabbit polyclonal anti-Iba1 antibody, Wako; mouse monoclonal anti-hSOD1 antibody, Roche; mouse monoclonal antibody to tubulin, Oncogene; rabbit polyclonal anti-NR2A antibody, Upstate; and mouse monoclonal antibody to NR2B, Zymed.

Histological analysis Mice were anesthetized with diethyl ether and transcardially perfused with ice-cold phosphate buffer (PB) (pH 7.4). Spinal cords, removed on ice, were incubated in a 4% paraformaldehyde solution in PB overnight under constant rotation at 4 °C and then were transferred to a 20% sucrose solution in PB until they sank and were finally frozen in Tissue-Tek O.C.T. Compound (Sakura Finetechnical Co., Ltd.). Sections were sliced on a cryostat at -19 °C through the lumbar spinal cord at the L2-L4 level and stored at -80 °C until they were used. Spinal cord sections were incubated at room temperature for 1 h with a blocking solution containing appropriate normal serum (1:60, Vector Laboratories, Burlingame, CA) and 0.3% Triton X-100 in tris-buffered saline (TBS). Subsequently, they were incubated at 4°C overnight with appropriate primary antibody. For D-Ser immunofluorescence analysis, sections were incubated with rabbit polyclonal anti-D-Ser antibody (1:300) in the blocking solution. This antibody was raised in rabbits against a glutaraldehyde conjugate of D-Ser and bovine serum albumin, and negatively purified against that of L-serine or D-alanine. In some experiments, anti-D-Ser antibody was preincubated with D-Ser-glutaraldehyde (GA)-BSA, L-Ser-GA-BSA, or GA-BSA conjugate (100 mg/ml, 4ºC overnight)

(*Current Protocols in Immunology,* vol. 9, chap. 2.), and anti-DAO antibody was preabsorbed with porcine DAO (3 mg/ml, 4ºC overnight). The sections were then incubated for 1 h at room temperature with biotin-labeled appropriate secondary antibody (all 1:200, Vector Laboratories) dissolved in the blocking solution. The secondary antibody was visualized with a TSA kit (NEN-Perkin-Elmer, NEL 701A). For double staining, sections were incubated also with SMI-32 (1:200), anti-NeuN antibody (1:50), rabbit anti-GFAP antibody (1:500, Dako), mouse anti-GFAP antibody (1:100, Cell signaling), or rabbit anti-Iba-1 antibody (1:50) in addition to the primary antibody and then visualized with anti-rabbit-Tex-Red (1:500, Jackson Laboratories). For fluorescent Nissl co-staining, sections were finally incubated with NeuroTrace 530/615 (1:50, Molecular Probes) at room temperature for 20 min and then washed with TBS. For the staining of microglia with isolectin B4, sections were co-incubated with anti-D-Ser antibody and Alexa Fluor 594 conjugated-Isolectin B4 from *Griffonia simplicifolia* (10 μg / ml, Molecular Probes) overnight; further processes were the same as above. Processed sections were mounted on slides and coverslipped with VECTASHIELD (Vector Laboratories).

For human samples, sliced sections were deparaffinized, rehydrated to distilled water, and unmasked in ANTIGEN UNMASKING SOLUTION (Vector Laboratories) at 60-70ºC for 15 min. Subsequently, the sections were incubated at room temperature for 1 h in a blocking solution containing goat normal serum and 0.3% Triton X-100 in TBS, and then incubated at 4°C over four nights with anti-D-Ser antibody (1:100). Immunoreactivity was visualized using diaminobenzidine after signal amplification with Vectastain ABC Elite kit (Vector Laboratories), mounted on slides, and coverslipped with Entellan new (MERK, Germany).

For immunocytochemistry, cells were transferred to a 4% paraformaldehyde solution in PB overnight at 4°C. Next, cells were incubated at room temperature for 1 h with a blocking solution containing appropriate normal serum (1:60, Vector Laboratories) and perforated with 0.3% Triton X-100 in TBS for 15 min. Subsequently, they were incubated at room temperature for 1 h with mouse anti-SRR (1:100) or mouse anti-NeuN (1:50) and then were visualized with anti-mouse-FITC. For double staining, rabbit anti-GFAP (1:400, Dako), rabbit anti-Iba1 (1:100), or goat anti-ChAT antibody (1:100) were incubated with the primary antibody and visualized in association with anti-rabbit-Tex-Red or anti-goat-Tex-Red (all 1:200, Jackson Laboratories).

Fluorescence-labeled samples were observed with a confocal laser microscopy (LSM510, Zeiss).

For the quantification of fluorescence intensity of immunostained D-Ser, laser microscopic images of 900-μm-squares containing one spinal ventral horn were converted into gray scale images, and the density of each image was measured by NIH Image Version 1.62.

Immunoblot analysis The tissue lysates (100 μg per lane) or cellular samples (30 μg per lane) were subjected to SDS-PAGE and then electrically blotted to a polyvinylidene difluoride sheet. The sheet was soaked with anti-SRR (1:1000), anti-DAO (1:5000, Abcam), anti-actin (1:5000), anti-ChAT (1:1000), anti-GFAP (1:3000), anti-Iba1 (1:500), anti-hSOD1 (1:1000), anti-phospho-JNK (1:1000), anti-phospho-p38 (1:1000), anti-phospho-ERK1/2 (1:1000), anti-JNK1 (1:1000), anti-p38 (1:1000), anti-ERK1 (1:2000), anti-NR1 (1:200), anti-NR2A (1:1000), anti-NR2B (1:500), anti-NR2C (1:200), or anti-NR2D (1:200) antibody; and subsequently with HRP labeled anti-mouse IgG, anti-rabbit IgG, or anti-goat IgG antibody (Bio-Rad, Hercules, CA). The antigenic bands were visualized with ECL (Amersham Biosciences, Uppsala, Sweden). For the quantification of protein levels, the density of each band was measured by NIH Image Version 1.62.

Purification of recombinant SRR The plasmid encoding the cDNA of mouse *SRR* and *SRR* (K56G) was subcloned into pGEX vector. DH5α-competent cells were transformed with pGEX-SRR plasmid, grown in flasks containing LB medium in the presence of ampicillin at 37ºC overnight. Expression of GST-SRR fusion protein was induced by incubating with 0.1 mM isopropyl-1-thio-β-D-galactopyranoside at 27ºC for 6 h. Bacterial cells, centrifuged and resuspended in phosphate-buffered saline (PBS), were lysed and sonicated in the presence of Triton X-100 (final 0.1%). GST-SRR protein was then precipitated with glutathione-sepharose beads (Amersham Biosciences) at 4ºC for 3 h. The beads were washed three times and stored in 50 mM Tris-HCl (pH 8.3) at 4ºC until use.