SUPPLEMENTAL MATERIALS

MATERIALS AND METHODS

Neuropathology

Following deparaffinization, mounted sections were incubated in 88% formic acid for 5 minutes for antigen retrieval prior to immunostaining. Primary antibodies were visualized using Vectastain ABC-Elite reagents (Vector Labs, Burlingame, California) and the chromagen 3, 3'-diaminobenzidine (Sigma-Aldrich, St. Louis, Missouri).

Cell culture

LA-N-1 cells were cultured in RPMI-1640 medium supplemented with 2 mM L-glutamine 10% fetal bovine serum and 1% Penicillin-Streptomycin (Invitrogen, Carlsbad, California). N2a and SK-N-AS cell lines were maintained according to instruction from American Type Culture Collection. Primary cortical neurons for reporter assays were prepared from fetal rats at 16 days of gestation. After dissociation, neurons were maintained in Neurobasal media with B27 supplement and 2 mM glutamine, with 25 μM glutamate added only during initial plating. All cells were maintained in 5% CO_2 at 37ºC.

Primary rat cortical neurons were maintained on laminin and polyD-lysine pre-coated cover slips (Fisher Scientific, Pittsburgh, Pennsylvania). Under these conditions, >85% of cells are neurons.

Reporter assay

Cells were transfected using Lipofectamine Plus and 2000 (Invitrogen) for cell lines and neurons, respectively. 48 hours after transfection, cells were harvested, lysed and assayed using the Dual-Luciferase Reporter Assay System (Promega).

Transcript expression

Total RNA was extracted from transfected LA-N-1 cells (RNAgents Total RNA Isolation System, Promega). The extraction was repeated and the resulting sample treated with DNase I (Invitrogen). Single strand cDNA was synthesized from isolated RNA (SuperScript First-Strand Synthesis System, Invitrogen) using random hexamers.