Supplementary method

Phosphorylation assay

For phosphorylation, HEK 293T cells were cotransfected with GFP-Dyn2 and Dyrk1A, Dyrk1A-2YF (kinase-dead mutant), or pcDNA-His (Cont) vector. 48 h after transfection, the cells were lysed with lysis buffer (1% Triton X-100, 0.5% sodium deoxycholate, 20 mM Tris, pH 8.0, 150 mM NaCl, 1 mM $MgCl_2$, 1 mM EGTA, 1 mM PMSF) at 4°C for 1 h and centrifuged at 15,000 g for 15 min. The supernatants were incubated at 30°C for 30 min in the presence of 2 mM Mg^{2+}-ATP, a mixture of phosphatase inhibitors. Incubations were terminated by addition of 10 mM EDTA and samples were immunoprecipitated 4 h with anti-GFP antibody, followed by an additional 2 h of incubation at 4°C with protein A-Sepharose beads (Amersham Biosciences, Piscataway, NJ). The immunoprecipitates were extensively washed with lysis buffer and subjected to SDS-PAGE and immunoblotting with *p*-Threonine antibody (Cell Signaling, Danver, MA).

Immunoblotting

For checking GFP-Dyn2, -Amp1, -Endo2, His-Dyrk1A, and His-Dyrk1A-2YF expression levels, COS-7 cells were triply transfected with GFP-Amp1 (A) or -Endo2 (B), RFP-LCa, and Dyrk1A or pcDNA-His (Cont) vector using Liopofectamine 2000 (Invitrogen). 48 h after transfection, the cells were washed twice with cold PBS and extracted at 4°C for 1 h in a lysis buffer. They were then clarified by centrifugation at 15,000 g for 15 min, and protein concentrations were determined with a Bradford protein assay reagent kit (Bio-Rad, Hercules, CA). Samples containing 20 μg of total protein were subjected to SDS-PAGE and transferred to a polyvinylidine diflouride membrane (Bio-Rad). The membrane was blocked with 5% skim milk TBST (10 mM Tris–HCl, 100 mM NaCl, and 0.1% Tween-10, pH 7.5) for 1 h,

washed, and probed with anti-GFP antibody (Abcam, Cambridge, UK) or anti-Dyrk1A antibody (Abcam) 1 h at room temperature. After extensive washing in TBST, the membrane was incubated with Horseradish peroxidase-conjugated secondary antibody (Jackson ImmunoResearch, West Grove, PA). Proteins were visualized with enhanced chemiluminescence reagent (Amersham Biosciences).

RNA interference

Dyrk1A shRNAs (rat, mouse) were designed from nucleotide 838-858 and 2087-2107. Complementary oligonucleotides were synthesized separately, with the addition of an *ApaI* site at the 5' end and an *EcoRI* site at the 3' end. The target sequence of shRNA #1 was 5'-GAACT TAGTA TCATT CACTG T-3' and that of shRNA #2 was 5'-GAGCT ATGGA CGTTA ATTTG A-3'. The sequence of control shRNA was 5'-GCCAT TCCTC ATATA CTATA G-3'. The annealed cDNA fragment was cloned into the *ApaI-EcoRI* sites of the vector pU6-mRFP vector (insert DsRed sequences to pSilencer.U.1.0 vector, Ambion). The fidelity of all constructs was verified by sequencing.