Supporting Information

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SI Materials and Methods

Reagents and Antibodies. Anti-rabbit and anti-mouse IgG agarose were purchased from eBiosciences. Anti-myc agarose was obtained from Sigma. Antibodies against CBP (A-22), c-fos (sc-52), were obtained from Santa Cruz Biotechnology. Antibody against c-jun was purchased from Cell Signaling Technologies. Antibody against Arc was obtained as a gift from Paul F. Worley's laboratory (Johns Hopkins University School of Medicine, Baltimore). Antibodies against acetyl-histone H3 and acetyl-histone H4 were purchased from Millipore. Anti-rabbit antibody against inositol polyphosphate multikinase (IPMK) was produced in-house.

RNA Isolation and RT-PCR. Total RNA was isolated from dissected hippocampi of nestin-Cre $IPMK^{floxed/floxed}$ or synapsin-Cre $IPMK^{fl/fl}$ mutants and control littermates, using Qiazol reagent (Qiagen), according to the manufacturer's protocol. A single microgram of total RNA was used in first-strand cDNA synthesis, using the first strand synthesis kit (Applied Biosystems). Quantitative PCR (qPCR) was set up according to Applied Biosystems guidelines. qPCR primers were selected from Applied Biosystems TaqMan probes. Genes of interest were normalized to GAPDH and presented as fold changes over baseline, using the delta–delta Ct method, wherein untreated $IPMK^{fl/fl}$ served as control baseline.

Continuous Cell Culture and Transfection. HEK 293 and Neuro2A cells were maintained in Dulbecco's modified Eagle medium (DMEM) with 10% (vol/vol) FBS (FBS) and 2 mM L-glutamine at 37 °C with a 5% CO_2 atmosphere in a humidified incubator. PC12 cells were maintained in DMEM supplemented with 10% (vol/vol) horse serum, 5% (vol/vol) FBS, and 2 mM L-glutamine. For transient transfection of cells with expression constructs, we used Polyfect reagent (Qiagen) for HEK 293 cells, Lipofectamine 2000 (Invitrogen) for PC12 cells, and SignaGen transfection reagent for Neuro2A cells, according to the manufacturers' protocols. For stable PC12 cell lines, control myc, wild-type mycIPMK, kinase-dead mycIPMK, and myc-*Arabidopsis thaliana* IPMK (atIpk2) constructs were cloned into pMXS vector containing blasticidin resistance. These plasmids were transfected into PC12 cells and selected with 5 μg/mL blasticidin resistance for 2 wk. Transient knockdown of rat IPMK in PC12 cells was achieved by shRNA from Origene. For both KCl and NGF treatment of PC12 cells, cells were starved overnight in starvation media (0.1% horse serum, 2 mM L-glutamine in DMEM). NGF was added the following day at 100 ng/mL. KCl was added to a final concentration of 50–75 mM. Cells were harvested at 25 min for mRNA processing and 1 h for Western blotting.

Virus Production. GFP and GFP-Cre lentivirus was purchased from KeraFAST. Control myc, wild-type mycIPMK, kinase-dead mycIPMK, and myc-atIpk2 lentiviruses were made as described previously (1).

Protein Interaction Immunoprecipitation Assays. pCMV mycIPMK or pCMV myc plasmid was transfected into HEK 293 cells or PC12 cells. Forty-eight hours after transfection, immunoprecipitation of the myc tag was performed with 250–500 μg of protein lysates in lysis buffer (150 mM NaCl, 0.5% CHAPS, 0.1% Triton, 0.1% BSA, 1 mM EDTA, protease inhibitors, phosphatase inhibitors) incubated overnight at 4 °C with primary antibody. On the second day, anti-mouse or anti-rabbit IgG agarose beads were added and allowed to incubate for 1–2 h at 4 °C. Beads were pelleted and washed with wash buffer containing 250–500 mM NaCl three times, and SDS sample buffer loading dye was added. Immunoprecipitated samples were resolved by polyacrylamide gel electrophoresis (PAGE), and proteins were detected by Western blotting.

Chromatin Immunoprecipitation (ChIP) Assays. In brief, intact cells were treated with 2 mM disuccinimidyl glutarate (Pierce) to crosslink protein complexes, followed by formaldehyde to link protein to DNA covalently. For in vitro primary cortical neuron experiments, eight million cortical neurons were used per ChIP. Cells were lysed, the nucleoprotein complexes were sonicated, and the crosslinked DNA–protein complexes were enriched by IP. The retrieved complexes were analyzed by PCR amplification to detect and quantify specific DNA targets. For real-time PCR, we used Brilliant SYBR Green master mix (Stratagene), according to the manufacturer's protocol. For in vivo experiments, bilateral hippocampi were used per ChIP. Samples were fixed in 1% formaldehyde, quenched with 2 M glycine, and washed with cold PBS + mixture protease inhibitor tablets. Samples were lysed in 10% SDS lysis buffer, then sonicated. The supernatant was incubated with primary antibody overnight at 4 °C. The next day, samples were incubated with agarose beads for 1 h at 4 °C. Beads were washed two times each with low salt, high salt, LiCl, and Tris-EDTA (TE) solutions. Samples were eluted in Elution Buffer and then reverse crosslinked at 65 °C for at least 6 h. Samples were purified using a PCR Purification Kit (Qiagen) and processed by qPCR. Each qPCR was conducted in triplicate and normalized to a negative control region. ChIP primers were mouse c-fos promoter, 5-TCCATATTAGGACATCTGCGTCA-3, 5-CGGCTCTATCCAGTCTTCTCAGT-3; rat c-fos promoter: 5-TTCTCTGTTCCGCTCATGACG-3, 5-CTTCTCAGTTGCTAGCTGCAATCG-3; negative control region: forward 5′-GGACAATTCAACCGAGGAAA-3′, reverse 5′-TGAACTGGTTTGGTGTGCTC-3′.

Image Quantification and Statistical Analysis. Images were quantified with ImageJ software. Data are presented as means ± SEM from at least three independent experiments. Data were analyzed with one- or two-way ANOVA followed by Holm-Sidak post hoc or Dunnett's post hoc tests. Student *t* test was used when two groups were compared.

1. Tiscornia G, Singer O, Verma IM (2006) Production and purification of lentiviral vectors. *Nat Protoc* 1(1):241–245.

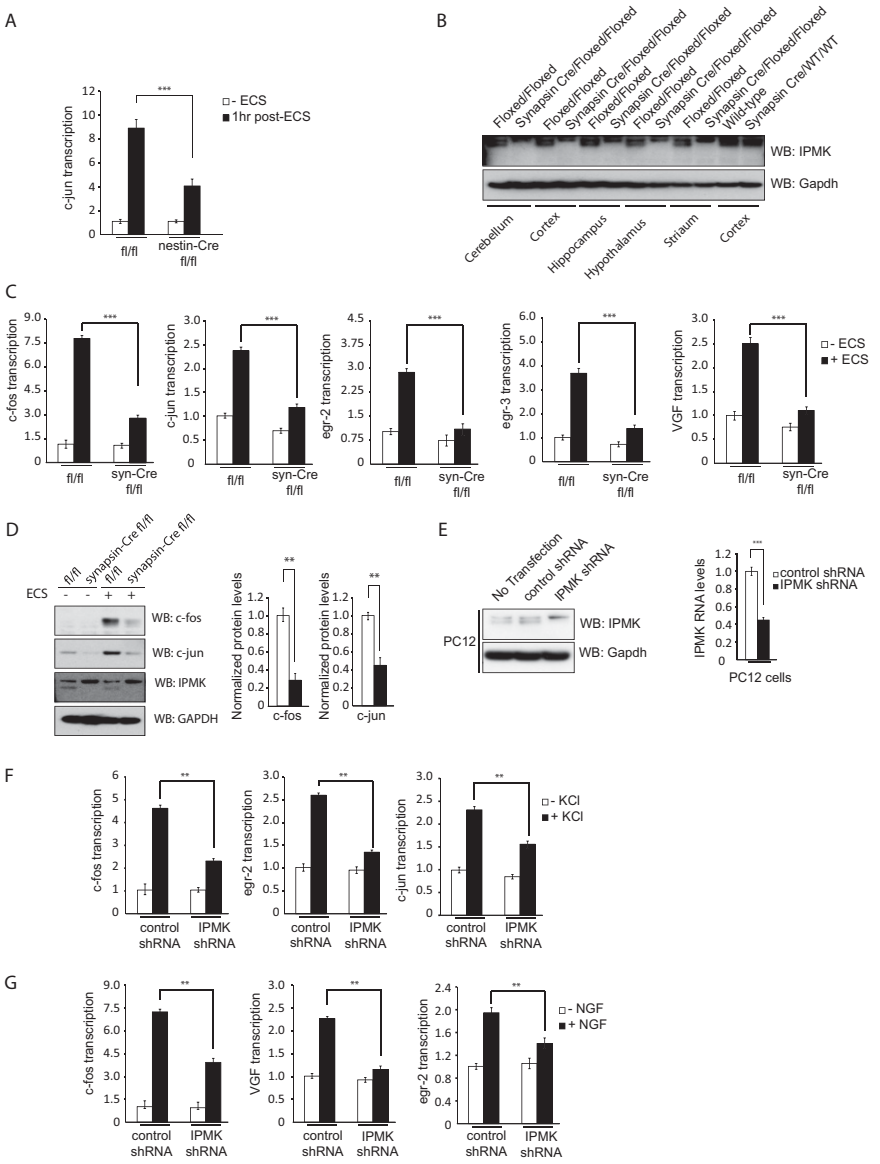

Fig. S1. IPMK is expressed primarily in neurons and affects neuronal immediate early gene (IEG) induction. (*A*) Nestin-Cre IPMK$^{fl/fl}$ mice exhibit significantly impaired c-jun mRNA induction 1 h after ECS treatment. (*B*) Synapsin-Cre IPMK$^{fl/fl}$ mice display essentially complete depletion of IPMK in cerebellum, cortex, hippocampus, hypothalamus, and striatum of the brain compared with wild-type mice or synapsin-Cre IPMK WT/WT mice. (*C*) Levels of IEG expression as assessed via qPCR are increased in the hippocampus of IPMK floxed/floxed mice after electroconvulsive stimulation. In synapsin-Cre IPMK$^{fl/fl}$ mice, levels of IEG expression are decreased 60%–80%. ***$P < 0.001$, Student *t* test. Data are means ± SEM from three experiments. (*D*) Western blotting of hippocampal tissue in IPMK$^{fl/fl}$ mice after electroconvulsive stimulation show robust enhancement of c-fos and c-jun protein levels. In synapsin-Cre IPMK floxed/floxed mice, levels of IEG expression are decreased 50%–60%. ***$P < 0.01$, Student *t* test. Data are means ± SEM from three experiments. (*E*) shRNA-mediated knockdown of IPMK in PC12 cells show a 60% decrease in IPMK mRNA levels. ***$P < 0.001$. Data are means ± SEM from three experiments. (*F*) PC12 cells are transfected with either control shRNA or shRNA targeted against IPMK. Cells are depolarized with KCl, and c-fos, egr-2, and c-jun transcript levels are assessed with qPCR. IPMK knockdown in PC12 cells demonstrate a 50%–60% decrease in transcript levels. ***$P < 0.001$. Data are means ± SEM from three experiments. (*G*) Knockdown of IPMK in PC12 cells decreases c-fos, VGF, and egr-2 mRNA levels after treatment with NGF. ***$P < 0.001$. Data are means ± SEM from three experiments.

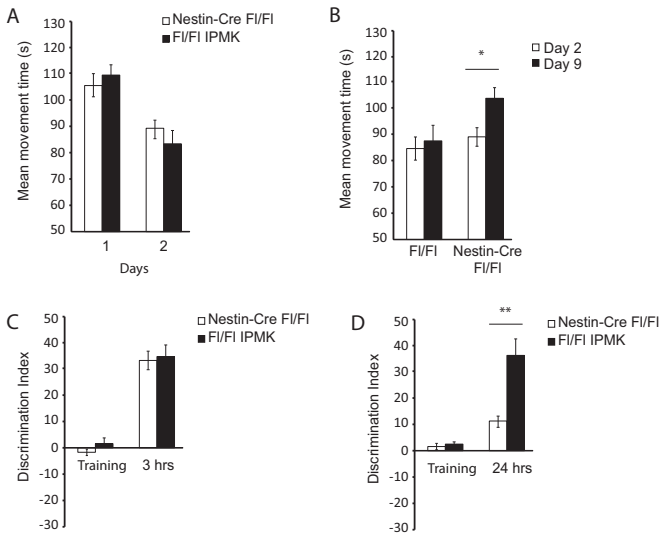

Fig. S2. Nestin-Cre IPMK$^{fl/fl}$ mice exhibit impaired memory for context and deficits in the novel object recognition test. (*A*) To examine memory for a context, mice are allowed to freely explore an open field chamber for two 5-min sessions on each of 2 consecutive days (day 1 and day 2). Both groups show similar levels of activity, as measured by movement time in the field, and both show significant habituation from day 1 to day 2. $P < 0.05$ for IPMK$^{fl/fl}$ mice ($n = 15$), and $P < 0.05$ for Nestin-Cre IPMK$^{fl/fl}$ mice ($n = 14$). (*B*) Mice are then returned to the same chamber 7 d later for two additional 5-min sessions, and on this day (day 9), Nestin-Cre IPMK$^{fl/fl}$ mice show significantly less retention for the context. $*P < 0.05$. (*C*) Normal short-term memory for object recognition 3 h after training [IPMK$^{fl/fl}$ mice ($n = 12$); Nestin-Cre IPMK$^{fl/fl}$ mice [$n = 14$]). (*D*) Nestin-Cre IPMK$^{fl/fl}$ mice demonstrate impaired long-term memory for object recognition when tested 24 h after training. $**P < 0.01$, IPMK$^{fl/fl}$ mice ($n = 14$); Nestin-Cre IPMK$^{fl/fl}$ mice ($n = 13$).

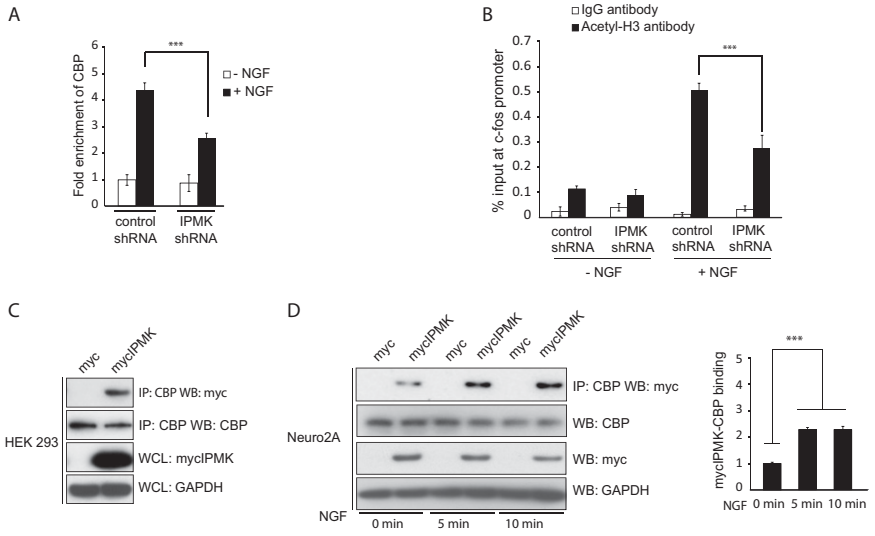

Fig. S3. IPMK knockdown in PC12 cells impairs recruitment of CBP to the c-fos promoter. (*A*) KCl-mediated depolarization of PC12 cells causes an increase in CBP recruitment to the c-fos promoter in control shRNA transfected cells. In cells transfected with IPMK shRNA, CBP recruitment to the c-fos promoter is decreased by ~40%. (*B*) PC12 cells with knockdown of IPMK exhibit decreased H3 acetylation at the c-fos promoter after KCl treatment. (*C*) Overexpressed myc-IPMK interacts with CBP. myc-IPMK is overexpressed in HEK293 cells. Anti-CBP antibody is used to immunoprecipitate lysates, and coimmunoprecipitates are resolved via gel electrophoresis. Anti-myc antibody is used to probe for myc-IPMK. (*D*) myc-IPMK is transfected into Neuro2A cells. Cells are treated with NGF, and lysates are immunoprecipitated with anti-CBP antibody. Coimmunoprecipitates are separated via gel electrophoresis, and myc-IPMK is identified with anti-myc antibody.

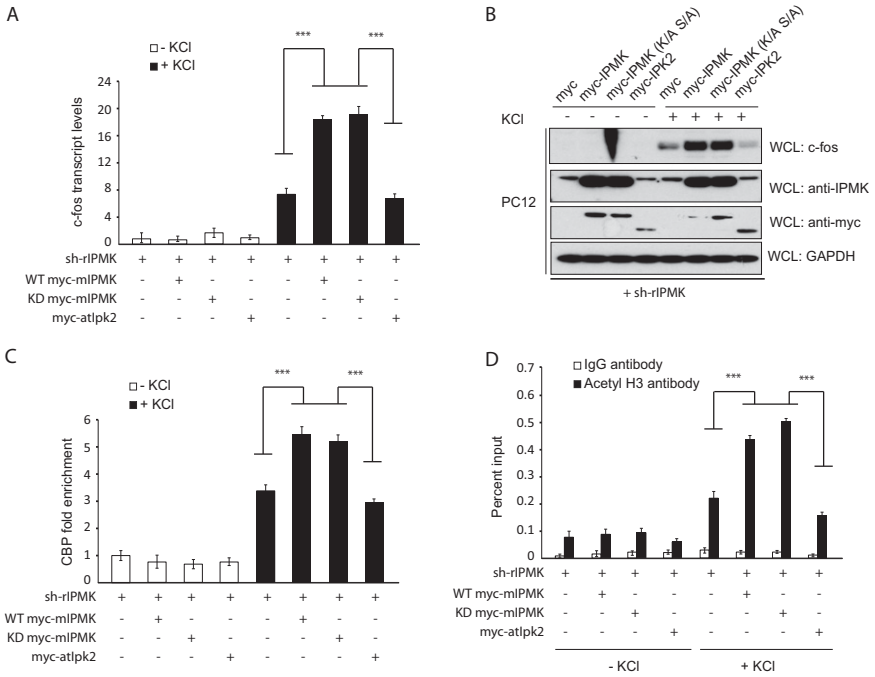

Fig. S4. Mutant-IPMK complementation of PC12 cells with knockdown of endogenous IPMK demonstrates that IPMK kinase activity is not required to affect CBP function. (*A*) Myc-tagged IPMK constructs are stably overexpressed in PC12 cells and endogenous IPMK knocked down with shRNA. PC12 cells are then treated with KCl. Wild-type and kinase-dead IPMK constructs demonstrate a corresponding increase in c-fos transcription levels. PC12 cells with overexpressed atIpk2 do not show this increase. ***$P < 0.001$. Data are means ± SEM from three experiments. (*B*) Cells with overexpression of atIpk2 show a diminished increase in c-fos protein levels compared with those with wild-type and kinase-dead IPMK. ***$P < 0.001$. Data are means ± SEM from three experiments. (*C*) The ChIP assay is used to assess CBP binding to the c-fos promoter in PC12 cells. Although wild-type and kinase-dead constructs enhance CBP recruitment to the c-fos promoter, atIpk2 does not. ***$P < 0.001$. Data are means ± SEM from three experiments. (*D*) Wild-type and kinase-dead constructs of IPMK enhance histone acetylation in PC12 cells treated with KCl, whereas cells transfected with atIpk2 do not. ***$P < 0.001$. Data are means ± SEM from three experiments.