Supporting Information

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SI Materials and Methods

Materials. Reagents were obtained as follows: Polyfect transfection reagent from Qiagen; high-glucose Dulbecco's modified Eagle's medium (DMEM) from Biowest; FBS from Atlas Biologicals; penicillin/streptomycin, blasticidin, 0.25% trypsin-EDTA, and tetracyclin from Invitrogen; and SYBR Green master mix from Applied Biosystems.

Antibodies. Antibodies against c-Fos, c-Jun, phospho-Elk-1 (S383), Elk-1, GAPDH, and normal rabbit IgG were purchased from Santa Cruz Biotechnology; anti-SP1 from Millipore; anti-hemagglutinin (HA) antibody from Covance; Flag, and alpha-tubulin from Sigma Aldrich; and phospho-ERK (T202/Y204), GST, and serum response factor (SRF) from Cell Signaling. Anti-rabbit inositol polyphosphate kinase (IPMK) antibody was raised against a peptide starting with Cys followed by mouse IPMK amino acids 295–311 (SKAYSTHTKLYAKKHQS) from Covance. Horseradish peroxidase-conjugated secondary antibodies were purchased from Thermo Scientific.

Plasmids. The cDNAs for human (NCBI Gene ID 253430) and mouse (NCBI Gene ID 69718) were obtained from Open Biosystems. IPMK and SRF cDNA constructs were amplified by PCR, and the products were cloned into pCMV-GST and pcDNA3.1-Flag, respectively. All constructs were confirmed by DNA sequencing. Plasmids encoding HA-human SRF (cat. no. 11977) were purchased from Addgene.

Cell Culture, Transfection, and Cell Viability Test. Human embryonic kidney HEK293 cells, HEK293T cells, and mouse embryonic fibroblasts (MEFs) were maintained in a humid atmosphere of 95% air and 5% CO_2 at 37 °C in high-glucose DMEM supplemented with 10% FBS, L-glutamine (2 mM), and penicillin/streptomycin (100 μg/mL). For transient transfection, we used Polyfect reagent for HEK293 and HEK293T cells according to the manufacturer's protocol. Stable IPMK-null MEF cell lines expressing WT-IPMK or catalytically inactive, mutant K129A IPMK (KA) were generated and maintained as described previously (1). For cell-viability assay, we first stained cells with trypan blue and quantitated live and dead cells by using an automated cell counter (Invitrogen).

Immunoprecipitation and GST Pull-Down. Cells were lysed in 40 mM Tris at pH 7.4, 120 mM NaCl, 1% Nonidet P-40, 1 mM EDTA, 50 mM NaF, 10 mM $Na_4O_7P_2$, 1.5 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitor mixtures (Roche). Cell lysates were incubated at 4 °C for 10 min, and the supernatant was collected by centrifuging lysates at 15,814 × *g* for 10 min. Two milligrams of total protein was incubated with 1 μg of primary antibody for 16 h with rotation at 4 °C. Fifteen microliters of TrueBlot bead (Rockland Immunochemicals) were added, and the incubation was continued for an additional 1 h. Samples were washed three times with the same lysis buffer. Proteins were separated by using SDS/PAGE and analyzed by immunoblotting. Detection of proteins was done with an enhanced chemiluminescence system (Thermo Scientific). For GST pull-down assay, 40 μL of 50% slurry glutathione agarose beads (Incospharm) were added to 2 mg of total cell lysates and incubated for 2 h with rotation at 4 °C. Then, samples were washed three times with the same lysis buffer.

Luciferase Assay. The Cignal serum response element (SRE) Reporter (2) kit (Qiagen, CCS-010L) was used to monitor the activity of SRF in cultured HEK293 cells. HEK293 cells were transiently transfected with SRE reporter construct along with empty vector, WT-IPMK, or catalytically inactive, mutant K129A IPMK (KA) expression plasmids. Posttransfection, cells were incubated in DMEM supplemented with 10% FBS for 36 h. Thereafter, cells were incubated in serum-free DMEM overnight and then treated with media containing 10% FBS for 6 h. Subsequently, cells were lysed, and the luciferase activity was measured with *SpectraMax M3* Microplate Reader (Molecular Device) using a dual luciferase assay kit (Promega). Constitutively expressing Renilla luciferase was used as an internal control for normalizing transfection efficiency.

RNA Isolation and Quantitative Real-Time PCR. Total RNA was isolated from cells using Tri reagent (Molecular Research Center) according to the manufacturer's protocol. Three micrograms of total RNA was used for the synthesis of first-strand cDNA by using SuperScript III reverse transcriptase (Invitrogen). Quantitative PCR analysis was performed with SYBR Green master mix and the Step One Plus Real-Time PCR system (Applied Biosystems). Genes of interest were normalized to attachment region binding protein (ARBP) gene and presented as fold changes over baseline using the $\Delta\Delta Ct$ method. Primer sequences for qPCR were as follows: Mouse *c-fos* primers, 5′-GGGGACAGCCTTTCCTACTA-3′, 5′-CTGTCACCGTGGGGATAAAG-3′; Mouse *c-jun* primers, 5′-ACGACCTTCTACGACGATGC-3′, 5′-CCAGGTTCAAGGTCATGCTC-3′; Human *c-fos* primers, 5′-CTGGCGTTGTGAAGACCAT-3′ 5′-TCCCTTCGGATTCTCCTTTT-3′; Human *c-jun* primers, 5′-ATCAAGGCGGAGAGGAAGCG-3′, 5′-TGAGCATGTTGGCCGTGGAC-3′; Mouse *cyr61* primers, 5′-CAGCTCACTGAAGAGGCTTC-3′, 5′-GCGTGCAGAGGGTTGAAAAG-3′; Human *cyr61* primers, 5′-CCTCGGCTGGTCAAAGTTAC-3′, 5′-TTTCTCGTCAACTCCACCTC-3′; Mouse *Arbp* primers, 5′-TCACTGTGCCAGCTCAGAAC-3′, 5′-AATTTCAATGGTGCCTCTGG-3′; Human *Arbp* primers, 5′-AATAAGGTGCCAGCTGCTGCC-3′; 5′-CATGTTCAGCAGCGTGGCTTC-3′.

1. Kim S, et al. (2011) Amino acid signaling to mTOR mediated by inositol polyphosphate multikinase. *Cell Metab* 13(2):215–221.

2. Nichols M, et al. (1992) Phosphorylation of CREB affects its binding to high and low affinity sites: Implications for cAMP induced gene transcription. *EMBO J* 11(9):3337–3346.

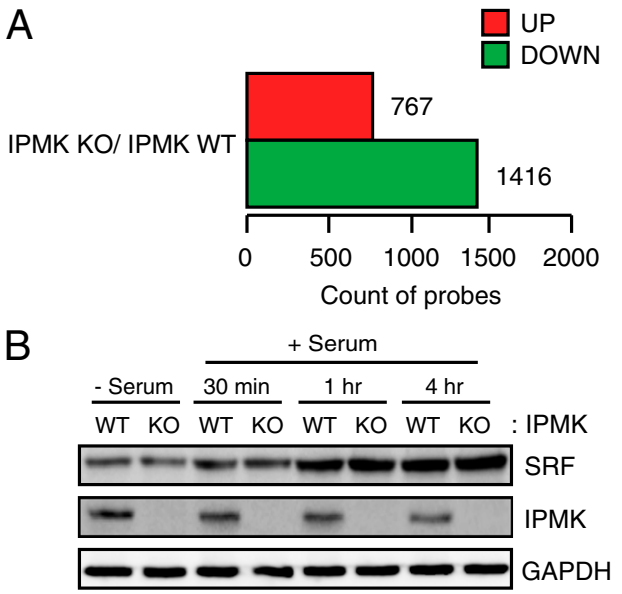

Fig. S1. IPMK influences gene expression profiles in response to serum stimulation. (*A*) For microarray analysis, wild-type and IPMK-deleted MEFs were deprived of serum for 12 h and stimulated with 10% FBS for 30 min. The total number of probe sets showing >twofold change was 2,183. In IPMK-deleted MEFs, 1,416 genes are down-regulated whereas 767 genes are up-regulated. (*B*) Immunoblot analysis of SRF protein level. Proteins were prepared from WT and IPMK-deleted MEFs that were stimulated with 10% FBS for 0.5, 1, and 4 h.

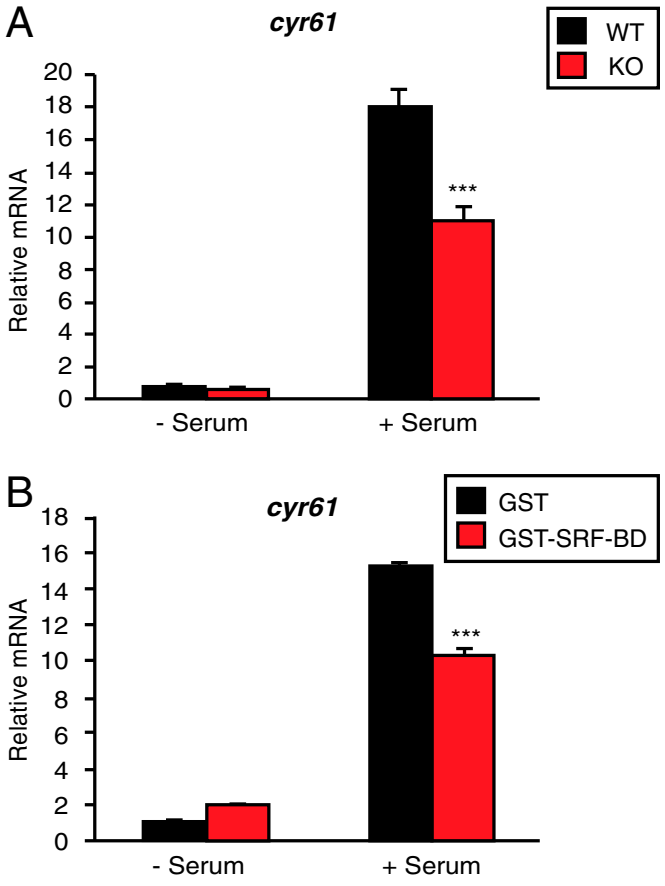

Fig. S2. IPMK promotes SRF-induced *cyr61* expression. (*A*) WT and IPMK KO MEFs were deprived of serum for 12 h and stimulated with 10% FBS for 30 min. The mRNA levels of the SRF target gene *cyr61* as measured via qPCR. (*B*) GST or GST-SRF-BD was transfected in HEK293 cells. Expression of *cyr61* was analyzed as in *A*. Bars represent mean ± SE ($n = 3$). ***$P < 0.001$.

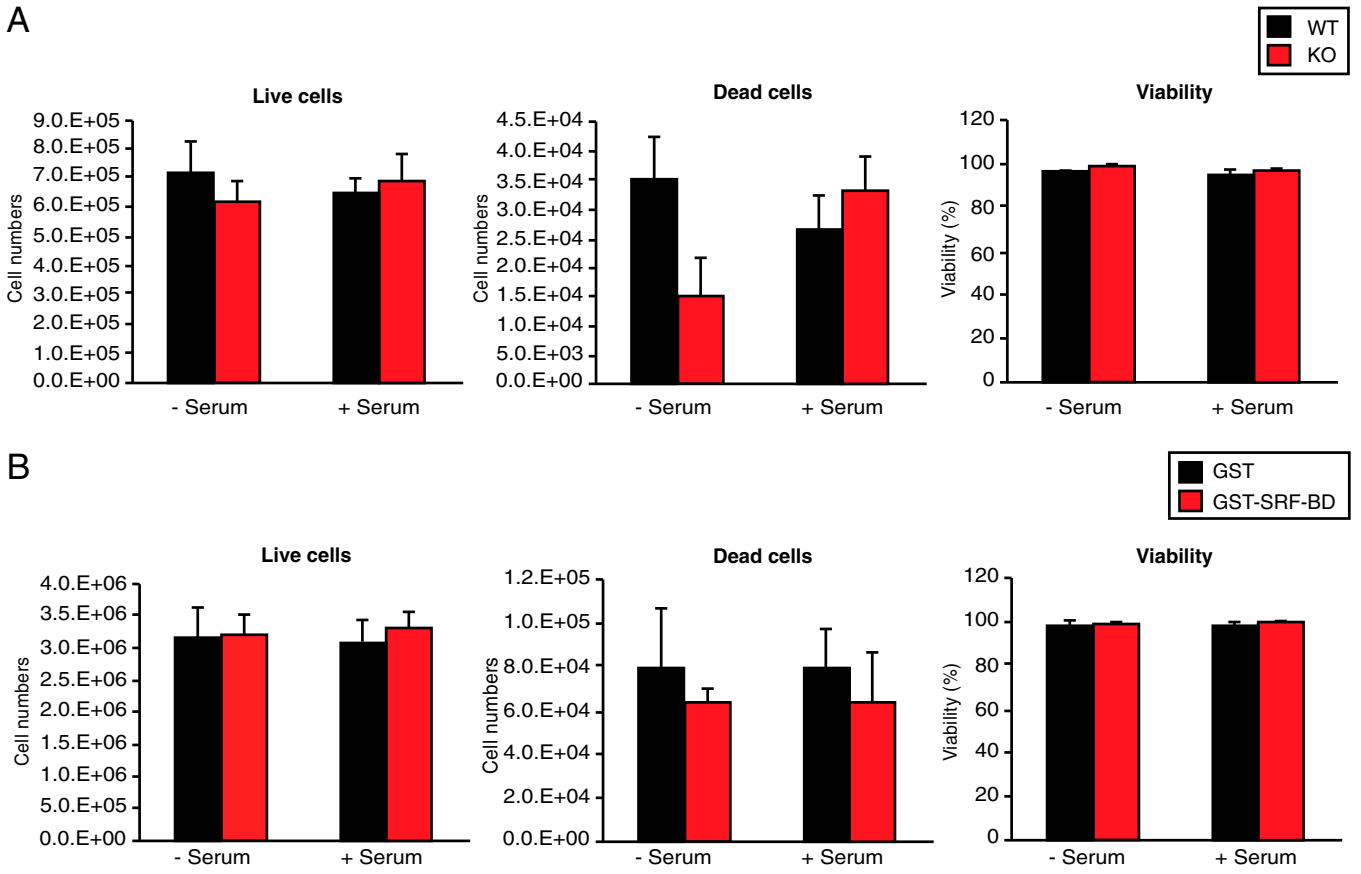

Fig. S3. Cell viability is not altered by IPMK. (*A*) WT and IPMK KO MEFs were deprived of serum for 12 h and stimulated with 10% FBS for 30 min. Cell numbers and viability were measured by trypan blue assay. (*B*) GST or GST-SRF-BD was transfected in HEK293 cells. Cell numbers and viability were analyzed as in *A*. Bars represent mean ± SD ($n = 3$).

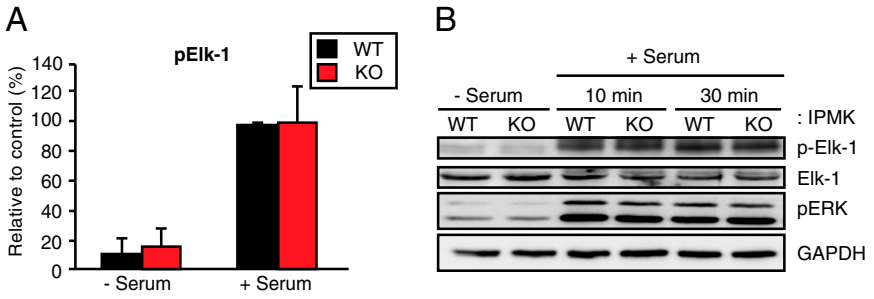

Fig. S4. IPMK deletion does not affect Elk-1 phosphorylation. (*A*) Quantification analysis of data for Fig. 2*A*. Bars represent mean ± SD ($n = 3$). (*B*) WT and IPMK KO MEFs were deprived of serum for 12 h and stimulated with 10% FBS for 10 and 30 min.

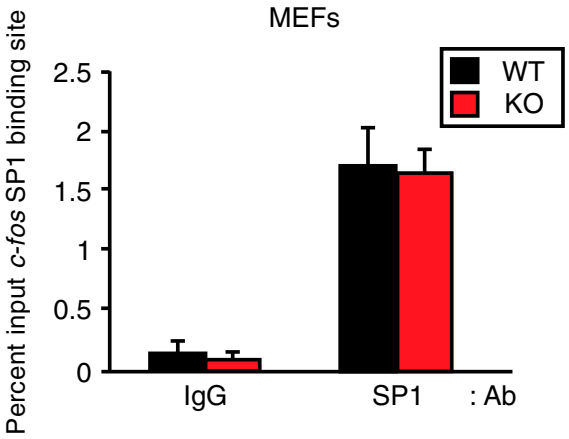

Fig. S5. IPMK deletion does not affect SP1 function. The ChIP assay was used to assess SP1 recruitment to the SP1 binding site of *c-fos* promoter.

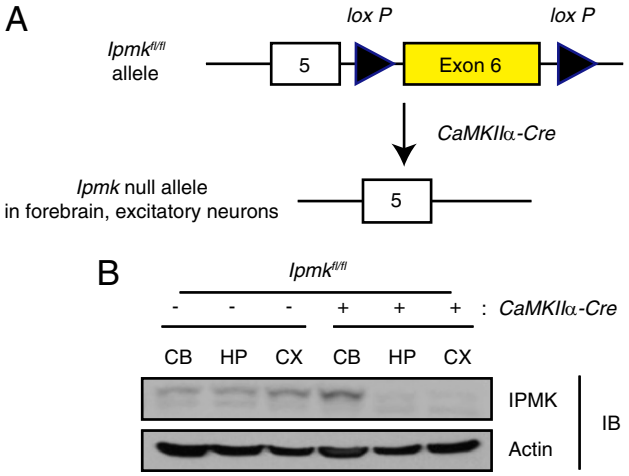

Fig. S6. Generation of forebrain-specific IPMK knockout mice. (*A*) Exon 6 of the *Ipmk* gene was flanked by repeated loxP sites in *Ipmk*$^{fl/fl}$ mice. To conditionally delete IPMK in excitatory neurons of the adult forebrain, we crossed *Ipmk*$^{fl/fl}$ mice with *CaMKIIα-Cre* transgenic mice. Details are available in *SI Materials and Methods*. (*B*) In *CaMKIIα-Cre; Ipmk*$^{fl/fl}$ mice, expression of IPMK was selectively deleted in the hippocampus (HP) and cerebral cortex (CX), but not in cerebellum (CB). Ten-week-old mice were killed for immunoblot analysis.

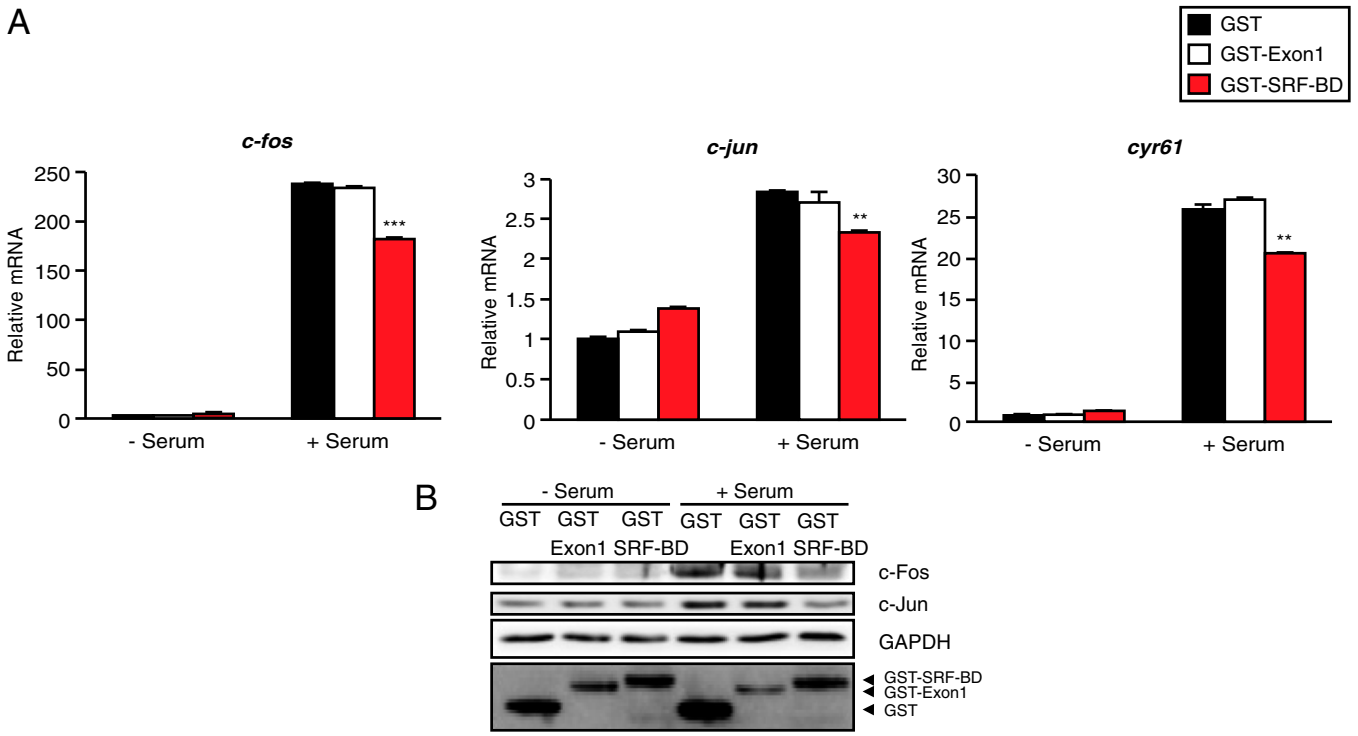

Fig. S7. Dominant-negative IPMK peptide interferes with immediate early gene expression. (*A*) The mRNA levels of the SRF target genes *c-jun*, *c-fos*, and *cyr61* as measured via qPCR. In addition to GST and GST-SRF-BD (in Fig. 4 *A* and *B*), we included GST-exon1 to test specificity. After transfection, HEK293 cells were deprived of serum for 12 h and stimulated with 10% FBS for 30 min. For immunoblot analysis in *B*, cells were stimulated with 10% FBS for 1 h. Bars represent mean ± SE ($n = 3$). $^{**}P < 0.01$; $^{***}P < 0.001$.

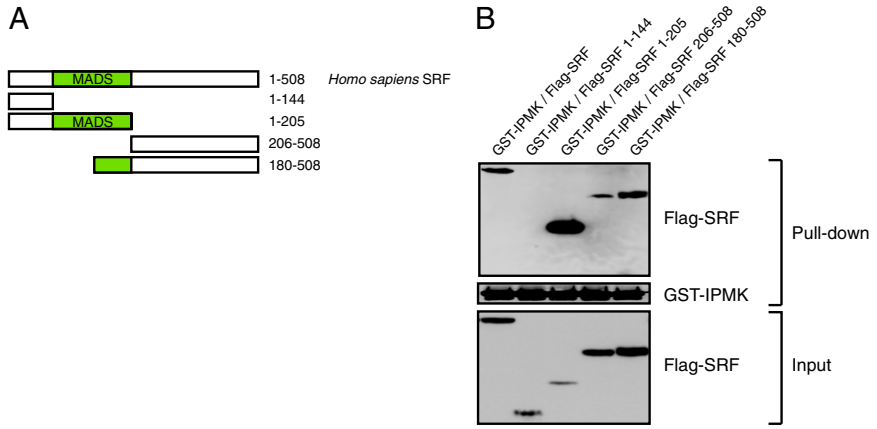

Fig. S8. The MADS (MCM1, Agamous, Deficiens, SRF) domain of SRF is the main binding site for IPMK. (*A*) A schematic diagram of SRF fragments with the numbers of amino acid sequences used for binding studies. (*B*) Mapping of binding region of SRF responsible for IPMK interaction. GST-IPMK was pulled-down from HEK293T cells cotransfected with various Flag-SRF fragments. SRF fragments in IPMK pull-down were determined by immunoblotting.