# Supporting Information

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### SI Materials and Methods

**Antibody Production and Immunocytochemistry.** For antibody production, the full-length coding sequence of ApAUF1 was expressed in *Escherichia coli* and ApAUF1 protein was purified and used to immunize BALB/c mice. For immunostaining of endogenous ApAUF, cultured *Aplysia* sensory neurons were fixed by using 4% (wt/vol) paraformaldehyde, stained using an ApAUF1 antibody (1:250), and subsequently incubated with an Alexa594-conjugated secondary antibody. For immunostaining of overexpressed ApAUF1, cultured sensory neurons injected with pNEXδ-3xflag-ApAUF1 and pNEXδ-EGFP were fixed by using the fixative solution containing 4% (wt/vol) paraformaldehyde and 30% (wt/vol) sucrose in PBS.

**RNA Gel Mobility Shift Assay.** To generate pGEX-KG-ApAUF1, ApAUF1 was subcloned into a SmaI-EcoRI site of pGEX-KG vector (Pharmacia). The ApAUF1 GST-fusion protein was purified as described (1). To make an RNA probe for gel mobility shift assays, the human c-fos (accession number V01512) 3′ UTR (214 bp) and *A. kurodai* C/EBP 3′ UTR (448 bp) were subcloned into a HinDIII-EcoRI site of the pLitmus28i vector (New England BioLabs) to generate pLitmus28i-cfos and pLitmus28i-ApC/EBP, respectively. A 389-bp region of *Aplysia* 18S rRNA (accession number AY 039804) was TA cloned into pGEM-T vector (Promega) to generate pGEM-T-18S rRNA. After linearization of pLitmus28i-cfos, pLitmus28i-ApC/EBP, and pGEM-T-18S rRNA, in vitro transcription was performed by using an Ambion T7 MEGAscript kit as specified by the manufacturer's instructions. RNA probe labeling and gel mobility shift assays were performed as described (1). In the assay, 10, 100, or 500 nM of purified proteins were added to the reaction. Because supershift of RNA probe was clearly detected only with 500 nM, the lanes loaded with 10 and 100 nM proteins were omitted in Fig. 2.

**RNA-Protein Pull-Down Assay.** mRNA-protein pull-down assay was performed as described (2) with slight modifications. Biotin-labeled RNA was prepared by in vitro transcription with T7 RNA polymerase (Promega). A 3× Flag-tagged ApAUF1-overexpressing 293T cell was lysed by using lysis/binding buffer containing 50 mM Tris·HCl at pH 7.6, 150 mM NaCl, 5% (vol/vol) glycerol, 0.1% (vol/vol) Triton X-100, 1 mM DTT, 0.2 mg/mL heparin, 0.2 mg/mL yease tRNA, 0.25% (wt/vol) BSA, protease inhibitor cocktail, protein phosphatase inhibitor cocktail (Roche), and 40 U/mL RNasin (Promega). Eight micrograms of biotinylated RNA were mixed with 200 μg (0.2 mg/mL) of precleared cell lysate and incubated for 1 h at 4 °C. After pulling down with NeutraAvidin Agarose Resin (Thermo Scientific), Western blot was performed by using an mFlag-m2 antibody (1:2,000; Sigma).

**RT-PCR Analysis.** For mRNA decay assays, the reporter construct was designed to express firefly luciferase containing an AU-rich element (luci-ARE), or 18s rRNA (luci-18S), in the pcDNA3.1 (+) vector. HEK293T cells were transfected with 1.25 μg of reporter construct and 0.25 μg of ApAUF1 or EGFP expression constructs under the control of the CMV promoter. Eighteen hours after transfection, Actinomycin D (Sigma) was added to the media in a final concentration of 5 μg/mL for 6 h. Cells were washed with 1× PBS once, and total RNAs were extracted by using TRIzol Reagent (Invitrogen). The primer sequences for detection of firefly luciferase, ApAUF1, and GAPDH are as follows: firefly luciferase (sense, 5′-ATCCATCTTGCTCCAACACC-3′; antisense, 5′-TCGCGGTTGTTACTTGACTG-3′), ApAUF1 (sense, 5′-TGGATCCCAAGAGGTTGAAG -3′; antisense, 5′-GGATGATAGCCTCCATGAGC-3′), and GAPDH (sense, 5′-GACCCTCTGGTGAAGGTGAA-3′; antisense, 5′-TGGACAGCTTCACACCTTTG-3′). PCR products were visualized on a 2% agarose gel, and intensities were quantified by using ImageJ software.

**In Situ Hybridization in Cultured *Aplysia* Sensory Neuron.** In situ hybridizations were performed as described by using an ApC/EBP mRNA specific probe (3). Signal intensity was measured by using Photoshop (Adobe).

**Luciferase Assay.** To construct luciferase reporters, we transferred the ApC/EBP promoter region into the 5′ region of firefly luciferase gene (C/EBP-luci). HEK293T cells were transfected with 1 μg of the firefly luciferase reporter, 200 ng of ApAUF1, and 200 ng of renilla luciferase for normalization. As a control, we transfected an equivalent amount of pEGFP-N1. Twenty-four hours after transfection, we treated the cells with 1 μM or 10 μM forskolin (Sigma), or DMSO, and we performed luciferase assays by using Dual Glo luciferase assay systems (Promega). Luciferase activity was measured by using a luminometer (Turner; model 20/e).

1. Yim SJ, et al. (2006) Regulation of ApC/EBP mRNA by the Aplysia AU-rich element-binding protein, ApELAV, and its effects on 5-hydroxytryptamine-induced long-term facilitation. *J Neurochem* 98:420–429.
2. Mastushita-Sakai T, White-Grindley E, Samuelson J, Seidel C, Si K (2010) Drosophila Orb2 targets genes involved in neuronal growth, synapse formation, and protein turnover. *Proc Natl Acad Sci USA* 107:11987–11992.
3. Kim H, et al. (2006) A nucleolar protein ApLLP induces ApC/EBP expression required for long-term synaptic facilitation in aplysia neurons. *Neuron* 49: 707–718.

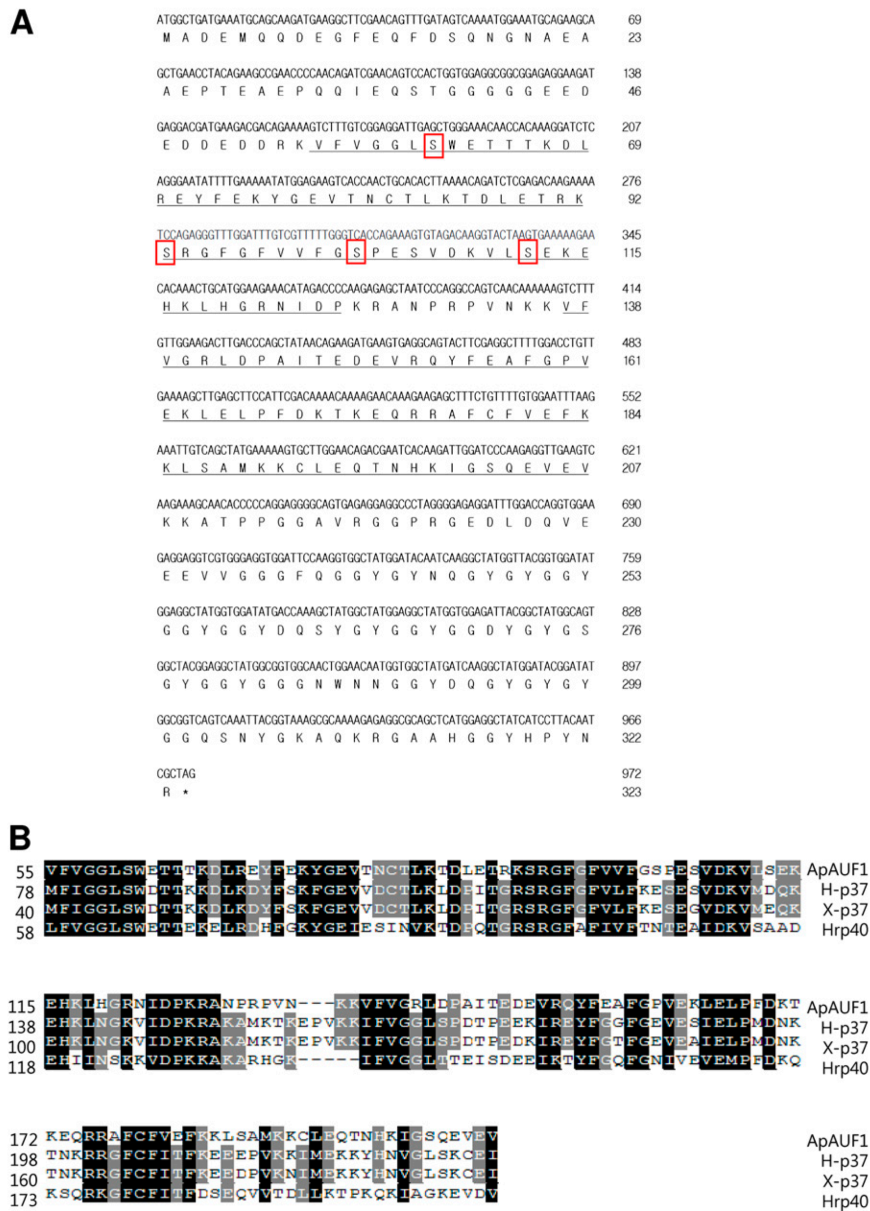

**Fig. S1.** (*A*) DNA and deduced amino acid sequences of the ORF of ApAUF1. Two RNA-recognition motifs (RRM1 and RRM2) are underlined. Broken underline indicates GY-rich domain. Four predicted putative serine phosphorylation sites are boxed. *, stop codon. (*B*) Multiple amino acid alignment of RRMs of ApAUF1 and other homologs from human (H-p37), *Xenopus* (X-p37), and *Drosophila* (Hrp40). Identical (black) and similar (gray) amino acid residues are marked by using software (GeneDoc 2.6.002).

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p45    : MSEEQFGGDGAAAAATAAVGGSAGEQEGAMVAATQGAAAAAGSAGTGGGTASGGTEGGSAEESEGAKIDASKNEEDEGHSNSSPRHS :  87
p42    : MSEEQFGGDGAAAAATAAVGGSAGEQEGAMVAATQGAAAAAGSAGTGGGTASGGTEGGSAEESEGAKIDASKNEEDEG--------- :  78
p40    : MSEEQFGGDGAAAAATAAVGGSAGEQEGAMVAATQGAAAAAGSAGTGGGTASGGTEGGSAEESEGAKIDASKNEEDEGHSNSSPRHS :  87
p37    : MSEEQFGGDGAAAAATAAVGGSAGEQEGAMVAATQGAAAAAGSAGTGGGTASGGTEGGSAEESEGAKIDASKNEEDEGK-------- :  79
ApAUF1 : ------------------MADEMQQDEGFEQFDSQNGNAEAAEFTEAEPQQIEQSTGGGGGEEDEDDEDDRK--------------- :  54

p45    : EAATAQREEWKMFIGGLSWDTTKKDLKDYFSKFGEVVDCTLKLDPITGRSRGFGFVLFKESESVDKVMDQKEHKLNGKVIDPKRAKA : 174
p42    : ----------KMFIGGLSWDTTKKDLKDYFSKFGEVVDCTLKLDPITGRSRGFGFVLFKESESVDKVMDQKEHKLNGKVIDPKRAKA : 155
p40    : EAATAQREEWKMFIGGLSWDTTKKDLKDYFSKFGEVVDCTLKLDPITGRSRGFGFVLFKESESVDKVMDQKEHKLNGKVIDPKRAKA : 174
p37    : -----------MFIGGLSWDTTKKDLKDYFSKFGEVVDCTLKLDPITGRSRGFGFVLFKESESVDKVMDQKEHKLNGKVIDPKRAKA : 155
ApAUF1 : -----------VFVGGLSWETTTKDLREYFEKYGEVTNCTLKTDLETRKSRGFGFVVFGSPESVDKVLSEKEHKLHGRNIDPKRAN- : 129

p45    : MKTKEPVKKIFVGGLSPDTPEEKIREYFGGFGEVESIELPMDNKTNKRRGFCFITFKEEEPVKKIMEKKYHNVGLSKCEIKVAMSK- : 260
p42    : MKTKEPVKKIFVGGLSPDTPEEKIREYFGGFGEVESIELPMDNKTNKRRGFCFITFKEEEPVKKIMEKKYHNVGLSKCEIKVAMSK- : 241
p40    : MKTKEPVKKIFVGGLSPDTPEEKIREYFGGFGEVESIELPMDNKTNKRRGFCFITFKEEEPVKKIMEKKYHNVGLSKCEIKVAMSK- : 260
p37    : MKTKEPVKKIFVGGLSPDTPEEKIREYFGGFGEVESIELPMDNKTNKRRGFCFITFKEEEPVKKIMEKKYHNVGLSKCEIKVAMSK- : 241
ApAUF1 : --PRPVNKKVFVGRLDEAITEDEVRQYFEAFGPVEKLELPFDKTKEQRRAFCFVEFKKLSAMKKCLEQTNHKIGSQEVEVKKATPPG : 214

                                                          Exon 7
p45    : ---------EQYQQQQQWGSRGGFAG---RARGRGGGPSQNWNQGYSNYWNQGYGNYGYNSQGYGGYGGYDYTGYNNYYGYGDYSNQ : 335
p42    : ---------EQYQQQQQWGSRGGFAG---RARGRGGGPSQNWNQGYSNYWNQGYGNYGYNSQGYGGYGGYDYTGYNNYYGYGDYSNQ : 316
p40    : ---------EQYQQQQQWGSRGGFAG---RARGRGG-------------------------------------------------DQ : 286
p37    : ---------EQYQQQQQWGSRGGFAG---RARGRGG-------------------------------------------------DQ : 267
ApAUF1 : GAVRGGPRGRGFGPGGRGGRGGGFQGGYGYNQGYGYGGYGGYGGYDQSYGYGGYGGDYGYGSGYGGYGGGNWNNGGYDQGYGYGYGG : 301

p45    : QSGYGKVSRRGGHQNSYKPY-- : 355
p42    : QSGYGKVSRRGGHQNSYKPY-- : 336
p40    : QSGYGKVSRRGGHQNSYKPY-- : 306
p37    : QSGYGKVSRRGGHQNSYKPY-- : 287
ApAUF1 : QSNYGKAQKRGAAHGGYHPYNR : 323
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**Fig. S2.** Multiple alignments of mammalian AUF1 isoforms and ApAUF1. Exon 7, which facilitates nuclear export in p42 and p45, is indicated.