Supplemental Information

Supplemental Figures

Figure S1, related to Figure 1

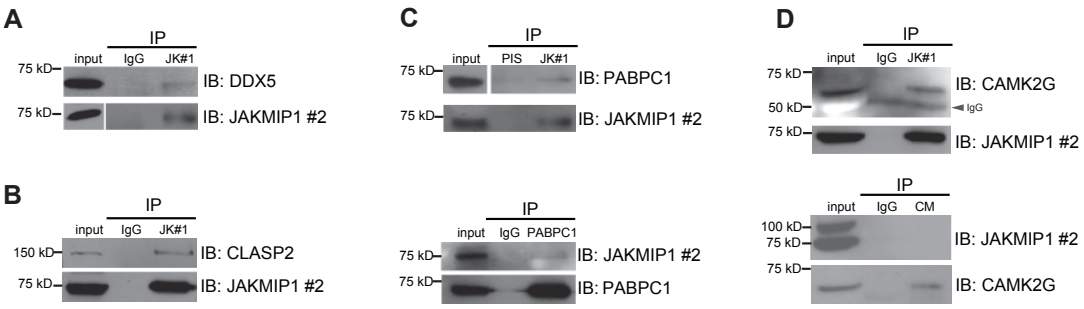


Figure S2, related to Figure 2

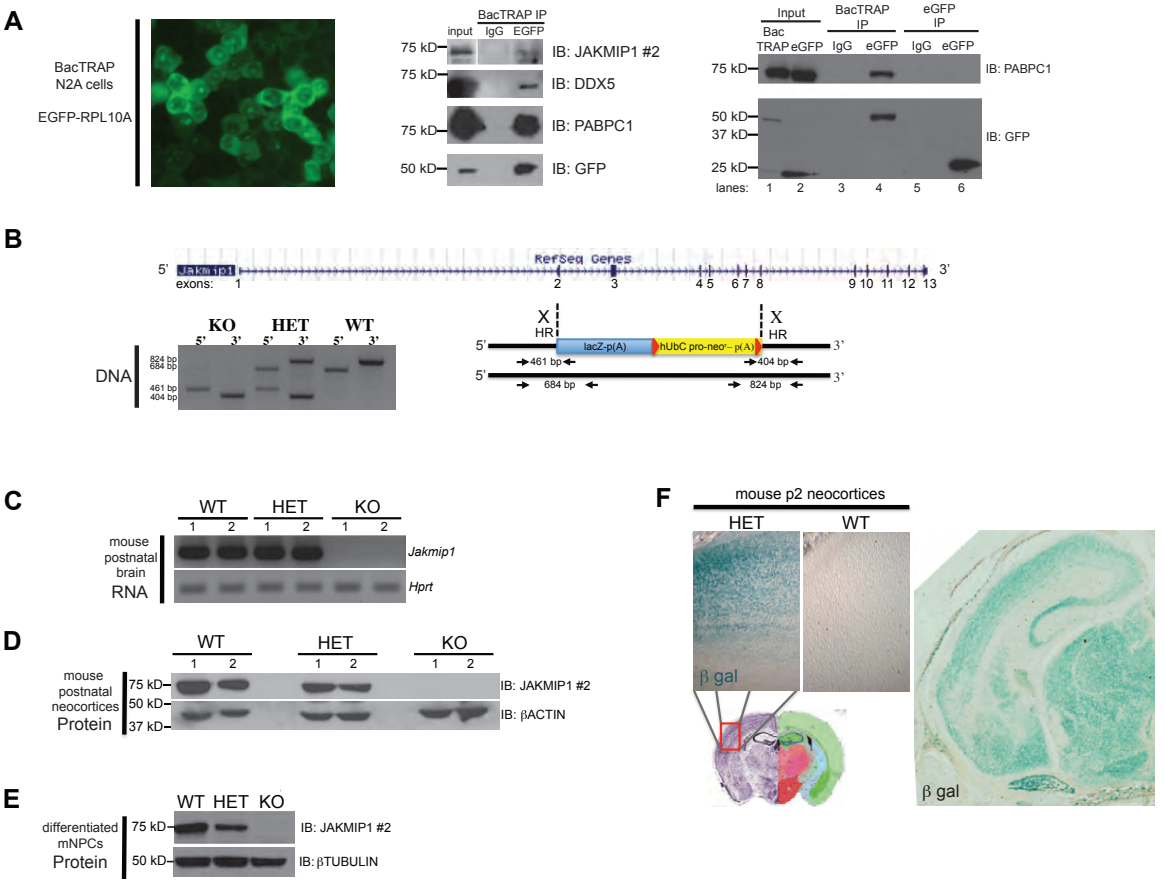


Figure S3, related to Table 2

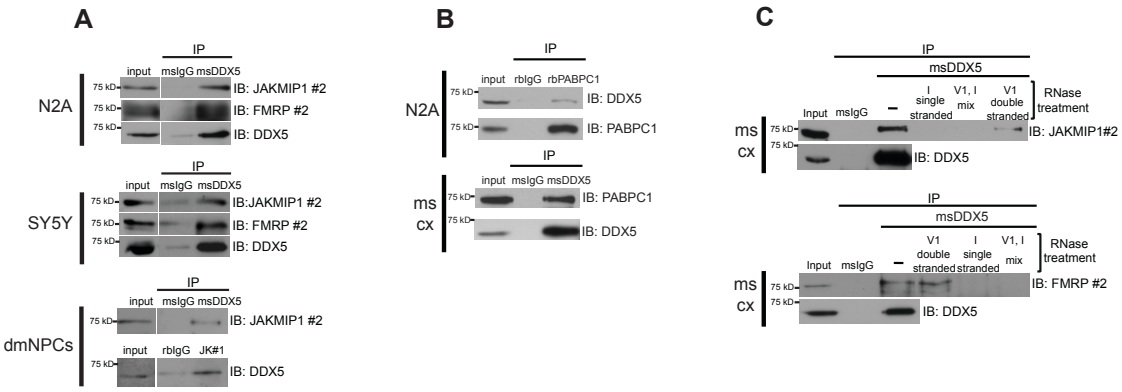


Figure S4, related to Figure 2

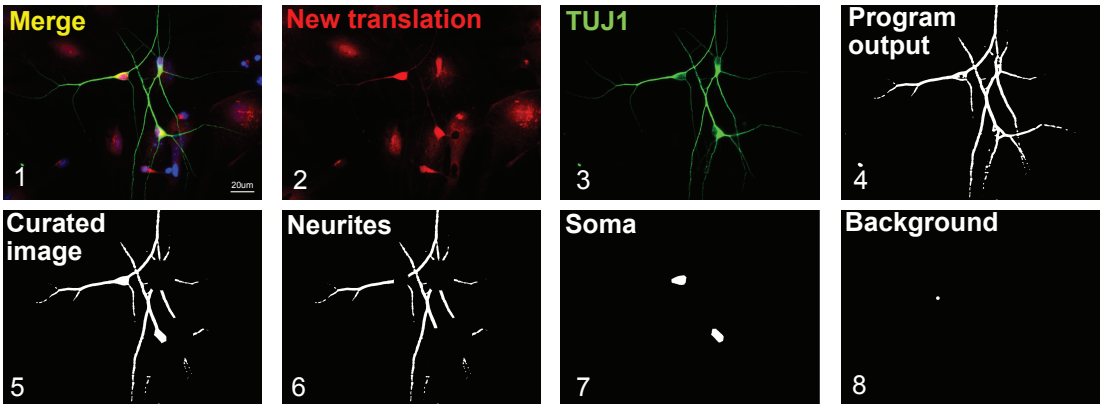


Figure S5, related to Figure 3

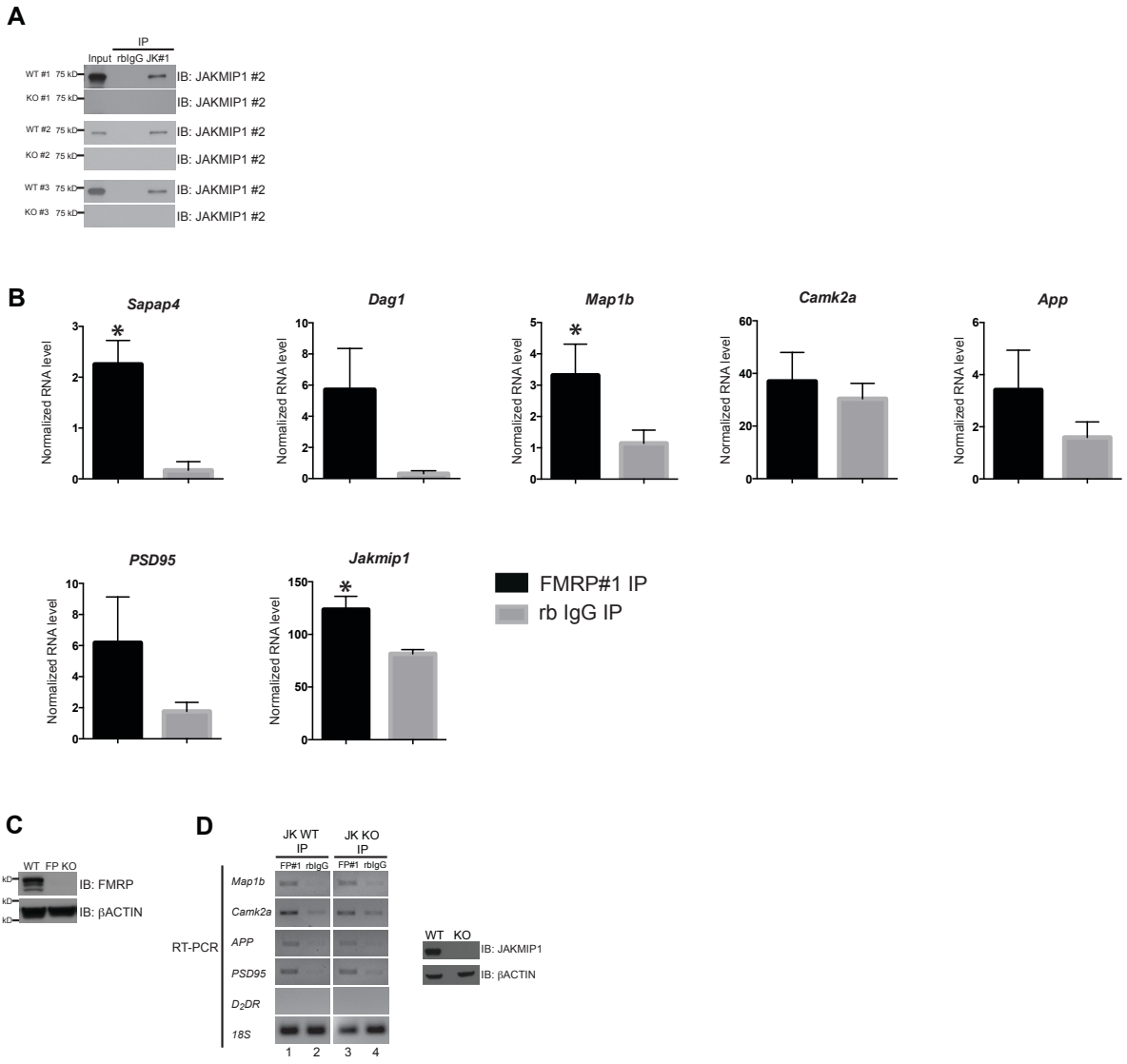


Figure S6, related to Figure 3

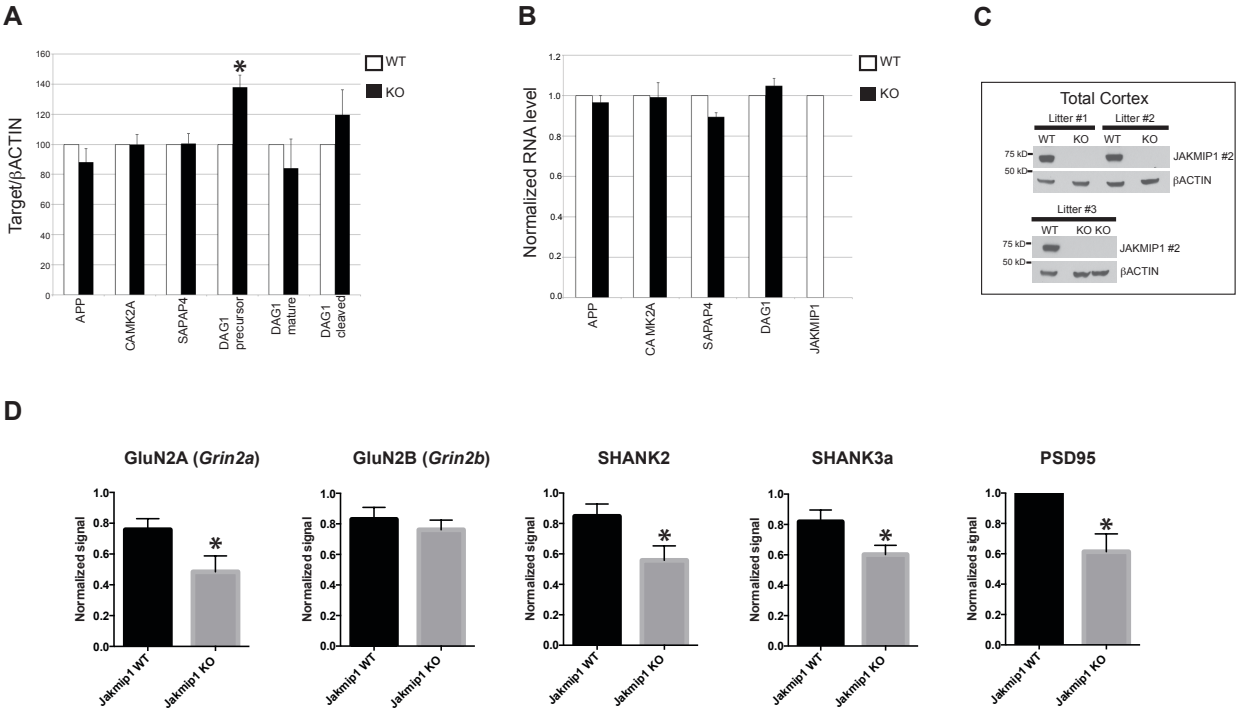
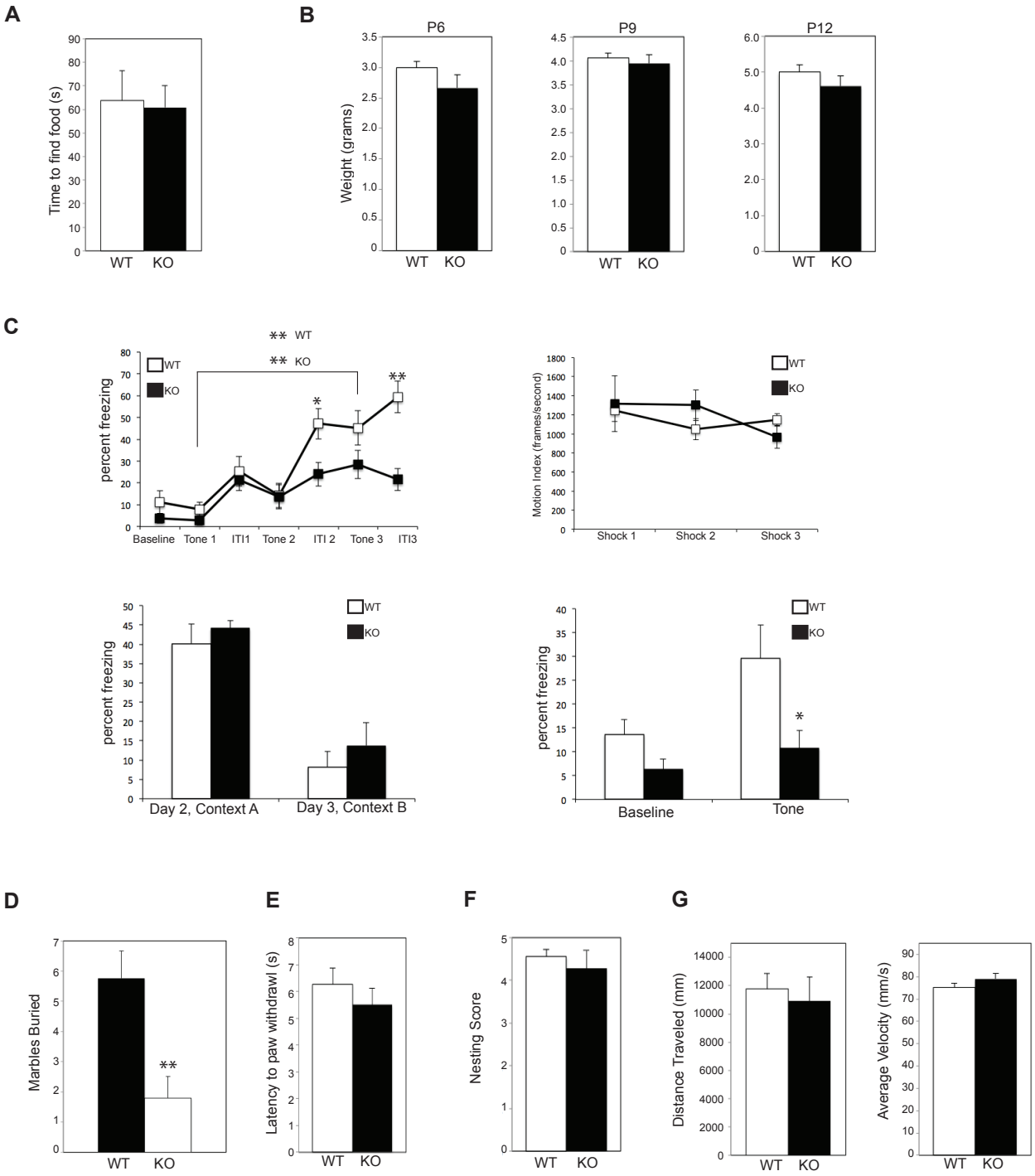


Figure S7, related to Figure 4



Supplemental Images for Table 3 and Table 4

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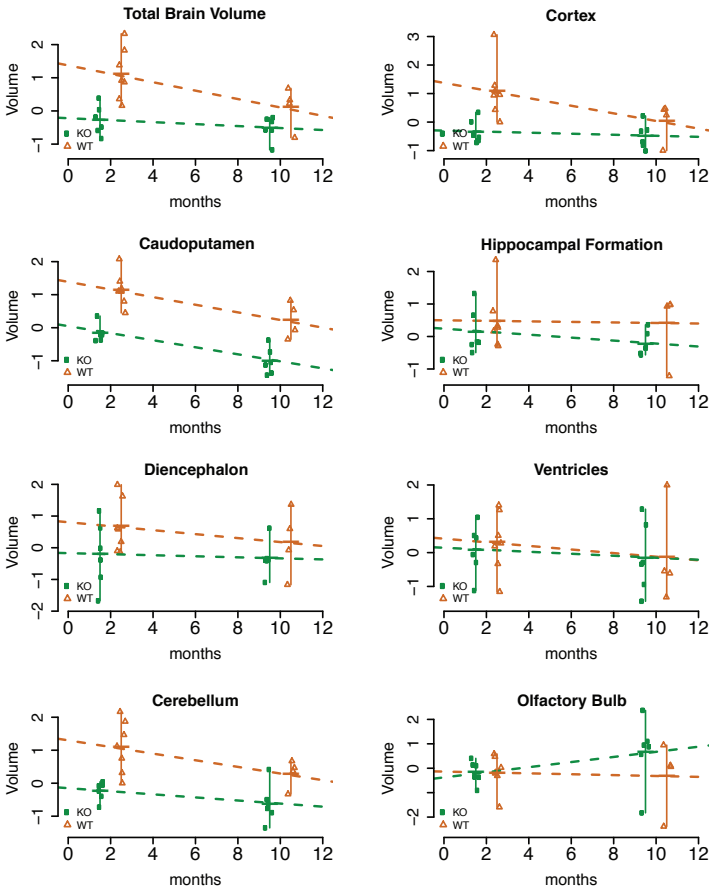

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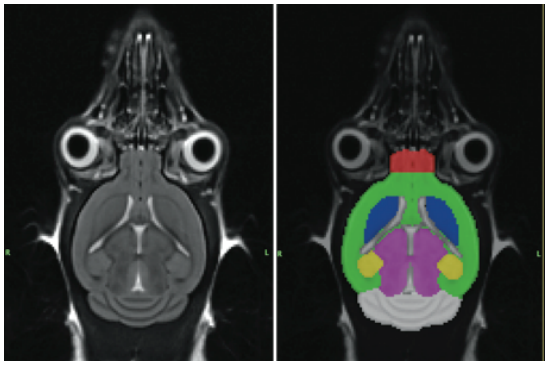

Supplemental Figure Legends

Figure S1, related to Figure 1. JAKMIP1 co-immunoprecipitates with proteins involved in translation. JAKMIP1 (A, B) or JAKMIP1 and target proteins (C, D) were immunoprecipitated from mouse postnatal neocortex followed by immunoblotting with target protein or JAKMIP1. CM is rabbit anti CAMK2G.

Figure S2, related to Figure 2. JAKMIP1 immunoprecipitates with ribosomal complexes. (A) JAKMIP1, DDX5 and PABCP1 coimmunoprecipitate with EGFP-RPL10A complexes in BacTRAP N2A cells. Left: Representative image of BacTRAP N2A cells. Middle: Co-IP of translational complexes with JAKMIP1, DDX5 and PABPC1. Right: The EGFP protein of the BacTRAP fusion protein does not show non-specific binding to PABPC1-containing translational complexes. Top panel: PABPC1 associates with BacTRAP EGFP/RPL10A fusion proteins (lane 4), but not EGFP alone (lane 6). Bottom panel: Confirmation of EGFP/RPL10A IP from BacTRAP cell lines (lane 4) and cell lines expressing EGFP alone (lane 6). **Generation of the *Jakmip1* knockout mouse** (B) Schematic representation of gene ablation strategy. *Jakmip1* exons 2 through 8 were replaced with a LacZ/neo cassette. Genotyping strategy is outlined along with representative PCR amplicons from KO, HET, and WT mice. Depiction of *Jakmip1* is from http://www.genome.ucsc.edu. (C) *Jakmip1* RNA is absent from KO, but not HET or WT, mouse postnatal brain by qRT-PCR using primers that amplify exons 3 and 4, within the region removed by the LacZ/neo cassette. Signal from the plateau phase (45 PCR cycles) is shown. (D and E) Immunoblots demonstrating JAKMIP1 protein loss in KO postnatal neocortex (D) and differentiated mouse neural progenitor cells (E). (F) β-galactosidase is expressed in *Jakmip1* HET, but not WT, littermate by X-gal staining. Coronal atlas image is from © 2012 Allen Institute for Brain Science. Allen Mouse Brain Atlas [Internet]. Available from: http://mouse.brain-map.org/.

Figure S3, related to Table 2. DDX5 binds to PABPC1, JAKMIP1 and FMRP with relationships dependent on single stranded RNA. (A) DDX5, JAKMIP1, and FMRP Co-IP in N2A, SY5Y, and differentiated mouse neural progenitor cells (dmNPCs). DDX5 was immunoprecipitated from mouse N2A cells (top panels) or human SY5Y cells (middle panels) and immunoblotted for JAKMIP1, FMRP, and DDX5. Bottom panels: DDX5 and JAKMIP1 associate in differentiated mouse neural progenitor cells. DDX5 or JAKMIP1 was immunoprecipitated from two week differentiated mouse neural progenitor cells and immunoblotted with JAKMIP1 and DDX5, respectively. (B) DDX5 interacts with PABPC1 by IP in mouse N2A cells by PABPC1 pull down (top panels) and mouse postnatal neocortex by DDX5 pull down (bottom panels). (C) DDX5 association with JAKMIP1 (top panels) and FMRP (bottom panels) is dependent on single-stranded, but not double-stranded RNA. DDX5 IP reactions were conducted in the presence of RNases as described in Figure 3A.

Figure S4, related to Figure 2. Representative images used for FUNCAT analysis.
Merged TUJ1/new translation images from Click-IT translational analysis (box 1). Blue is a DAPI nuclear counter stain. New translation is determined by red pixels units (box 2) within the TUJ1-positive space (box 3). Representative mask images from our customized computer analysis (box 4). Images were curated to exclude artifacts and overlapping glia signal (box 5), soma (box 6), or neurites (box 7). Background fluorescence was calculated from a manually placed 19 pixel diameter circle adjacent to the neuronal space and not overlapping glia occupied space (box 8). Brightness and contrast on top row box 2 was slightly adjusted to highlight neuronal and glial translation.

Figure S5, related to Figure 3. JAKMIP1 shows successful immunoprecipitation; FMRP binds its mRNA targets in WT and *Jakmip1* KO mice. (A) Immunoblot confirmation of JAKMIP1 IP from *Jakmip1* WT and KO mouse brains carried out in Figure 3D. (B) Positive control RNA-immunoprecipitation reactions using FMRP #1 antibody and rb IgG (WT, n=4 or 5). *D_2DR* RNA was

not present in immunoprecipitation reactions. Statistical significance was determined using one-tailed paired t-tests. Data are shown as mean +/- SEM. (C) Immunoblots demonstrate loss of FMRP in *Fmr1* KO postnatal brain used in Figure 3C. (D) FMRP binds its targets independently of JAKMIP1 in postnatal mouse neocortex. From left to right: RNA immunoprecipitating with FMRP#1 (lane 1, FP#1) or rabbit IgG (lane 2, rbIgG) in Jakmip1 WT (JK WT) mouse neocortex, and FMRP#1 (lane 3) or rabbit IgG (lane 4) in *Jakmip1* KO (JK KO) mouse neocortex. Confirmation of JAKMIP1 KO by Western Blotting is shown to the right. RT-PCR targets were analyzed at 30 or 35 cycles. D_2DR signal is shown as a negative control, and 18S as shown as a loading control.

Figure S6, related to Figure 3. Expression of JAKMIP1/FMRP shared mRNA targets in postnatal neocortex of *Jakmip1* KO mice. (A and B) Protein (A) and corresponding RNA (B) of JAKMIP1/FMRP mRNA targets from cortex of postnatal *Jakmip1* KO and WT mice. (A) Protein is shown as a percentage of WT littermate measured by image densitometry (WT, n=3; KO, n=4). Statistical significance was determined using a two-tailed, one sample t-test (DAG1 precursor, p=0.019). RNA signal (B) is displayed as a fold change from WT littermate and was measured by qRT-PCR. (C) Loss of JAKMIP1 protein was confirmed in the neocortex of samples used in (A) and (B) and Figure 3E. (D) Protein expression levels of JAKMIP1 mRNA targets in postnatal neocortex synaptosomal membranes of *Jakmip1* KO mice and WT controls. GluN2A (*Grin2a*) (*Jakmip1* KO, n=7 : WT, n=7, P=0.05), GluN2B (*Grin2b*) (*Jakmip1* KO, n=6 : WT, n=7), SHANK2 (*Jakmip1* KO, n=7 : WT, n=7, P=0.039), SHANK3a (*Jakmip1* KO, n=7 : WT, n=7, P=0.05), PSD95 (*Jakmip1* KO, n=3: WT, n=3, P=0.031). Statistical significance was determined using two tailed unpaired t-tests. Signal was normalized to corresponding βactin loading control and then to 1 within blot. All data are shown as mean +/- SEM.

Figure S7, related to Figure 4. *Jakmip1* KO mice performance on additional behavioral tests. (A) Olfaction test. Latency to find buried food (WT, n=8; KO, n=8). (B) Weights of mice used for USV analysis (WT, n=15; KO, n=15 for P6 and P12. WT, n=17; KO, n=17 for P9). (C) Auditory Fear Conditioning. Top: Acquisition of Fear Conditioning. Top left: Day 1, context A. Percent freezing to tone/foot shock pairing and intertone interval (ITI). (WT vs. KO: ITI2 ; P=0.027; IT3, P=0.001, two sample, two tailed, unpaired t-test : Tone 1 vs. Tone 3; WT learning, P=0.002; KO learning, P=0.009, two sample, two tailed, paired t-test). Top right: Motion to foot shock, Day 1, context A. Bottom left: Contextual fear and generalized fear assessment. Percent freezing of mice placed in context A one day after acquisition of fear conditioning or context B two days after conditioning. Bottom right: Percent freezing of mice placed in a context B three days after acquisition of fear conditioning after a tone was given. (P=0.049, WT, n=9; KO, n=7). (D) Marble Burying Test. The number of marbles buried during a 30 minute trial (12 marbles maximum). (WT, n=17; KO, n=15, P=0.0019). (E) Hot plate startle. Time before paw is withdrawn from a 55 degrees hot plate (max time is 15 seconds) (WT, n=8; KO, n=7). (F) Nesting behavior. The nesting score represents the nest architecture and amount of material used after an overnight period (1, poor; 5, good). (WT, n=9; KO, n=7). (G) Open field test. Distance traveled and average velocity over a 20 minute recording period (WT, n=15; KO, n=13). P values were calculated using a two sample, two tailed t-test. All data are shown as mean +/- SEM.

Supplemental Images for Table 3 and Table 4; Results of structural neuroimaging analysis. (A) Structural volumes for wild type (orange triangles) and KO mice (green circles) from both age cohorts are shown. Prior to analysis, all structural volumes were adjusted for individual differences in intracranial volume using linear regression. The thin vertical lines show the data range and the thicker horizontal lines represent the mean for each time point and strain. ANCOVA results testing for the effect of strain, age, and strain X age interaction are shown in Table 3. Summary of the results for t-tests in the

2 month age cohort are shown in Table 4. (B) Sample atlas (left) and segmentation template (right) are shown. See supplementary experimental methods for details.

Movie S1, related to Figure 4. *Jakmip1* male KO mice display repetitive behaviors.

Jakmip1 KO mice display jumping behavior in the home cage.

Supplemental Experimental Procedures

Immunoprecipitation

IPs were performed using protein A (rabbit IgG) or protein G (mouse IgG) Dynabeads (Invitrogen, Carlsbad, CA). JAKMIP1 was immunoprecipitated from the S2 cellular fraction (Vidal et al., 2007) from duplicate pools of independent C57BL/6 mouse neocortex (n=8) during the postnatal peak of JAKMIP1 protein expression using protein A Dynabeads (Invitrogen, Carlsbad, CA) and JAKMIP1 #1 or rabbit preimmune serum (control IP). IPs were performed in lysis buffer (0.32M sucrose, 5mM Tris-HCl) and primary antibody incubations were conducted overnight at 4 degrees Celsius. IPs were conducted in a ratio of 60:4:1 [lysate (ug): Dynabeads (ul): antibody (ug)]. For MudPIT runs, immunoprecipitates were washed in 200mM NaCl, 0.1% NP40, 0.32M sucrose, 5mM Tris-HCl (pH 8.0) and eluted in 100mM Tris-HCl, 8M Urea for 30 minutes at room temperature. We defined a JAKMIP1 interactor as: a) being identified in both the initial and the replication MudPIT run, b) having both its normalized spectra abundance factor (NSAfe5), which controls for the size and abundance of the protein (Florens et al., 2006) and its spectral counts above conservative threshold values in the JAKMIP1 IP versus control IP condition in at least one run, and c) both the NSAfe5 and spectral counts enriched in the JAKMIP1 IP over the control IP in the remaining run. PatternLab ACFold analysis (Carvalho et al., 2008) was additionally conducted to identify statistically significantly enriched proteins in the JAKMIP1 immunoprecipitated versus control conditions at a false discovery rate of 15% (Table 1). For IP/Western blot experiments, immunoprecipitates were washed in 200mM NaCl or 50mM (low salt;

JAKMIP1/FMRP and a subset of JAKMIP1/DDX5 IPs), 0.1% NP40, 0.32M sucrose, 5mM Tris-HCl (pH 8.0) and eluted in 100mM Tris-HCl, 8M Urea for 30 minutes at room temperature. The cellular S2 fraction or S1 fraction was obtained as previously described (Vidal et al., 2007). A Bradford assay (Bio-Rad, Hercules, CA) was used to determine protein concentration. IP reactions from BacTRAP N2A cell lines were carried out using the S2 cellular fraction and an equal amount of the bioreactor supernatants of mouse anti eGFP antibody clones 19C8 and 19F7 from the Monoclonal Antibody Core Facility at Memorial Sloan-Kettering. IPs for BacTRAP experiments were performed in 0.15M KCl IP wash buffer [20mM HEPES-KOH (pH 7.4), 5mM $MgCl_2$, 150mM KCl, 1% NP40]. Protein was precleared for 2 hours in mouse IgG bound Protein G Dynabeads (Invitrogen) prior to 4 degrees Celsius overnight IP reactions. Final washes were conducted in 0.35M KCl IP wash buffer [20mM HEPES-KOH (pH 7.4), 5mM $MgCl_2$, 350mM KCl, 1% NP40], and protein was eluted in 100mM Tris-HCl, 8M Urea for 30 minutes at room temperature.

To test the RNA dependency of DDX5-FMRP, we digested S2 cellular fractions of C57BL/6 mouse brain with 2U RnaseV1 per mg of protein, which digests double stranded RNA, and/or 100U RNase I per mg of protein, which digests single stranded RNA, with final IP wash in 200mM NaCl, 0.1% NP40, 0.32M sucrose, 5mM Tris-Cl (pH 8.0). For DDX5-JAKMIP1 and FMRP-JAKMIP1 reactions, S2 cellular fractions were digested with 4U RNase V1 /mg of protein and/or 1000 U RNase I /mg of protein. For these reactions, the final IP wash was conducted in 50mM NaCl, 0.1% NP40, 0.32M sucrose, 5mM Tris-Cl (pH 8.0).

Samples were boiled in a Tris-glycine SDS loading buffer prior to western blot analysis. In some Co-IP reactions, lysate was precleared with Dynabeads at 4 degrees Celsius. A Reliablot WesternBlot blot blocking kit (Bethyl laboratories, Montgomery, TX) was used to decrease rabbit IgG signal in some cases when the species of antibody used for both IP and blotting were both rabbit. 1%-100% of input protein was run alongside immunoprecipitation reactions during Western Blotting.

Immunoblotting

Protein lysates were boiled in a Tris-glycine SDS loading buffer containing DTT prior to western blot analysis. Samples were run out on Tris-glycine SDS page gels and transferred to a polyvinylidene fluoride (PVDF) membrane. Immunoblots were pre-blocked in 5% milk in TBST for one hour. Primary antibodies were diluted in blocking buffer and incubated with immunoblots overnight at 4 degrees Celsius. Secondary antibodies were diluted in blocking buffer and incubated with immunoblots for 1-2 hours at room temperature. Immunoblots were incubated with SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, Waltham, MA) or Luminata Forte Western HRP Substrate (Millipore, Billerica, MA) and exposed to Hyblot CL autoradiography film (Denville Scientific, Metuchen, NJ) prior to development. Synaptosomal membrane proteins (LP1) were prepared as previously described (Hallett et al., 2008).

Generation of a *Jakmip1* knockout KO mouse

We confirmed absence of JAKMIP1 protein in *Jakmip1* KO mice in C57BL/6 postnatal mouse neocortex and differentiated mouse neural progenitor cells (Figure S2). qRT-PCR analysis within the LacZ-Neo cassette from *Jakmip1* KO mouse postnatal brain showed a complete loss of *Jakmip1* RNA signal. RNA was extracted using a miRNeasy kit (Qiagen, Germantown, MD) according to manufacturer's instructions. RT-PCR was performed as described (Spiteri et al., 2007) with the exception that random hexamers were used to synthesize cDNA. β-galactosidase staining was conducted in coronal sections of postnatal *Jakmip1* HET and WT (control) mouse brains to test for proper *Jakmip1* promoter activity. Staining recapitulated known *Jakmip1* RNA expression. Mouse brains were dissected and sectioned at 10um onto Superfrost+ glass slides (Fisher Scientific, Waltham, MA) and post-fixed in 4% PFA. Sections were incubated in a LacZ staining solution containing 2mM MgCl2, 0.01% Deoxycholic acid, 0.02% IGEPAL CA-630, 0.1% X-Gal in dimethylformamide, 5mM Potassium Ferrocyanide, 5mM Potassium Ferricyanide in 1X PBS overnight at 37 degrees Celsius. Sections were

fixed for 1 hour in 10% neutral buffered formalin and dehydrated in an ethanol gradient followed by incubation in Xylene. Permount (Fisher Scientific, Waltham, MA) was used to mount slides onto coverslips.

Permutation analysis

The R program was used to identify JAKMIP1 protein interactors from gene ontology network 1 that are listed in HPRD (http://www.hprd.org/query; release 9) with at least one protein interaction. Self interactions were filtered out. All 20 network 1 JAKMIP1 interactors met this criterion (ACTC1, CAMK2A, CAMK2G, CLASP1, CLASP2, DDX5, EEF2, EEF1A1, GNB2L1, HNRNPK, MYO5A, PABPC1, PURA, PLEC, PURB, RPS3, GLUD1, RPLP0, YWHAG, and YWHAH). A permutation analysis was conducted by sampling 20 proteins 10,000 times from the pool of proteins in HPRD with at least one interaction (X=9819), where interactions include protein pairs with known direct interactions as well as protein pairs that are part of known protein complexes. Since we never saw more than six PPIs in any permutation, significance was estimated by first fitting the PPI counts with a logarithmic distribution, and then estimating the expected number of permutations required to get 17 PPIs by chance.

Polyribosome gradient preparation and western blot

Mouse neocortex (postnatal day 14) were preincubated at 4 degrees Celsius for 10 minutes after brief homogenization in 250ul lysis buffer (10mM Tris-HCl at pH 8.5, 150mM NaCl, 50mM $MgCl_2$, 1% Nonidet-P40, 0.1U/ul RNase OUT, 40mM dithiothreitol, 1% Sodium deoxycholate, and 1.73mg/ml cycloheximide). After further homogenization for 50 seconds at 4 degrees Celsius, we added 720 ul lysis buffer and centrifuged it for 30 minutes at 12,000 g at 4 degrees Celsius to remove the nuclei. After taking the supernatant, we repeated the centrifuge. The supernatant was supplemented with 500ul of 2X extraction buffer (200mM Tris-HCl at pH 7.5, 300mM NaCl), 1mg/ml heparin, and 10mM phenyl-

methyl-sulfonyl fluoride. It was then centrifuged at 12,000 g for 5 minutes at 4 degrees Celsius to remove mitochondria and membranous debris. The supernatant was centrifuged one more time and was layered onto a linear sucrose gradient (8% – 50% sucrose [w/v], supplemented with 10mM Tris-HCl at pH 7.5, 140mM NaCl, 1.5mM $MgCl_2$, 10mM dithiothreitol, 0.1mg/ml cycloheximide, 0.5mg/ml heparin) and centrifuged in a SW41 rotor for 120 minutes at 38,000 rpm at 4 degrees Celsius. Fractions were collected in 18 fractions and profiled at 254 nm using a UV Isco Fractionator (Teledyne Isco, Thousand Oaks, CA). We measured the average protein concentration of the combined fractions by adding together 30ul from each fraction. We loaded equal volumes of each fraction according to the average protein concentration.

RNA extraction from polyribosome fractions for quantitative RT-PCR

To extract RNA from the fraction, fractions were combined for each mRNP, monosome, and polyribosome fraction based on polyribosome fraction UV profiles. For control RNA, 1ul of pAW109 RNA (Applied Biosystems, Bio-Rad*,* Hercules, CA) was added into 100ul of the combined fractions. Samples were digested in 0.2mg/ml proteinase K in 1% SDS and 10mM EDTA for 60 minutes at 50 degrees Celsius. Using acid phenol chloroform, RNA was extracted. To remove high concentration of heparin, 2.5M LiCl was used for precipitation followed by 70% ethanol.

Nascent synthesis of proteins

Click-iT® L-homopropargylglycine (HPG) was incorporated into newly translated proteins for two hours, as recommended by the manufacturer, followed by conversion to a fluorescently labeled stable triazole conjugate using a 555-fluorophore tagged azide molecule in the presence of copper. The 2 hour time pulse-chase application of HPG was chosen as this allows translation visualization in distal dendritic segments (Dieterich et al., 2010). To determine background fluorescence, a subset of cells were not given HPG or 555-azide. WT and KO cells were analyzed from same-day images. A baseline

exposure time for the red channel (translation) was calculated by sampling random cells. This time interval of exposure was used for all experiments for red channel images. Background fluorescence for each image was calculated from a manually placed 19 pixel diameter circle adjacent but not overlapping with the neuron and glia occupied space. Translation was assessed across imaging days, which represent independent experiments. Individual coverslips, representing distinct reactions were analyzed and outliers within coverslips were manually checked. A strong relationship between day of imaging and 555 fluorescence was observed, so a covariate was used to control for day of imaging. For FUNCAT analysis, three trials were performed: two trials from one littermate mouse set (trial 1: WT, n=74; KO, n=80: trial 2: WT, n=91; KO, n=92) and one trial from an additional littermate set (trial 3: WT, n=62; KO, n=92). Fluorescent images were acquired using a Zeiss Axio Imager, D1 camera and AxioVison software (Zeiss, Oberkochen, Germany).

Harvesting mouse neural progenitor cells and differentiation

Postnatal day 0-2 mouse whole brains were dissected in DMEM-F12 (Gibco, Carlsbad, CA) with removal of the cerebellum and brainstem. Tissue was mechanically dissociated and digested in Papain (Worthington, Columbus, OH) or Trypsin (Invitrogen, Carlsbad, CA) for 30 minutes at 37 degrees Celsius with 5% CO_2. Bovine serum albumin (Invitrogen, Carlsbad, CA) in DMEM was added to stop digestion. Brains were triturated in media and centrifuged at 1,500 rpm. The pellet was reconstituted in DMEM-F12 with 1XB27 without retinoic acid (Invitrogen, Carlsbad, CA). A mixture of epidermal growth factor and fibroblast growth factor was added at 1:5000 (PeproTech, Rocky Hill, NJ), and cells were propagated on polyornithine/fibronectin. Cells were differentiated on polyornithine/laminin in media containing 10ng/ml brain derived neurotrophic factor (PeproTech, Rocky Hill, NJ, 10ng/ml), 10ng/ml NT3 (PeproTech, Rocky Hill, NJ, 10ng/ml), 500ng/ml retinoic acid (Sigma, St. Louis, MO, 500ng/ml), 10uM Forskalin (Sigma, St. Louis, MO, 10uM) and 10mM KCL.

Immunocytochemistry

4% Paraformaldehyde was used to fix cells grown on glass coverslips followed by 0.2% Triton X-100 permeabilization. Cells were blocked for 1 hour at room temperature in 1% bovine serum albumin, 0.2% fish skin gelatin, and 0.1% Triton X-100 in 100mM Tris Buffered Saline. Primary and secondary antibodies were diluted in blocking buffer. Primary antibody was incubated overnight at 4 degrees Celsius while secondary antibody incubations were 2 hours at room temperature. Prolong Gold Antifade reagent (Invitrogen, Carlsbad, CA) was used to mount coverslips to glass slides. Images were obtained with a Zeiss AxioCam.

Colocalization of JAKMIP1 in PABPC1 positive RNP granules

Mouse neural progenitor cells (mNPCs) were grown and differentiated as described above in a 24 well plate. Confluent cells were transfected with 10μg each of *JAKMIP1* and *PABPC1*-containing expression plasmids using Lipofectamine LTX and PLUS Reagents (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. We used a previously published (Steindler et al., 2004) human *JAKMIP1* expression plasmid in which N-ter human *JAKMIP1* (amino acids 1-365) is cloned into pcDNA4/V5-His/zeo (Invitrogen, Carlsbad, CA). PABPC1-dsRED expression constructs were created using Gateway clonase technology (Invitrogen, Carlsbad, CA) with plasmids, pENTR PABPC1 (Open BioSystems/Thermo Scientific, Waltham, MA) and pLVU/dsRED destination vector, Addgene 24178 (Krupka et al., 2010). We validated the correct protein expression from these constructs by conducting immunoblotting for JAKMIP1 and PABPC1 from protein lysates of 293T cells 24 hours post transfection of constructs. We conducted fluorescence immunostaining for PABPC1 and JAKMIP1 24 hours post-transfection of differentiated mNPCS to test for colocalization using anti-RFP and anti-V5 antibodies. Image acquisition was performed using Zeiss AxioCam and all images were taken at 40X/ .75 NA magnification.

Electrophysiology

Male postnatal (p11-p15) or adult (p43-p69) mice were deeply anesthetized with isoflurane and perfused with a solution of ice-cold low-Ca^{2+}_ artificial CSF (ACSF) containing the following (in mM): 130 NaCl, 3 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 5 $MgCl_2$, 1 $CaCl_2$, and 10 glucose. The brain was rapidly removed and placed in the same solution. Coronal slices were cut (300µm) using a microslicer (Leica VT1000S; Leica Microsystems) and transferred to an incubating chamber containing ACSF (with 2 mM $CaCl_2$ and 2 mM $MgCl_2$) oxygenated with 95%O_2-5%CO_2 (pH 7.2–7.4, 290–310 mOsm). The slices were incubated at 33°C for 30 minutes followed by 30 minutes at room temperature. The microscope (Olympus BX51WI) was equipped with differential interference contrast optics and fluorescence. Whole-cell patch-clamp recordings in voltage clamp mode were obtained from neurons using a MultiClamp 700B Amplifier (Molecular Devices) and pCLAMP 10.5. The patch pipette (3–5 MΩ_resistance) contained a Cs-methanesulfonate-based internal solution (in mM): 130 Csmethanesulfonate, 10 CsCl, 4 NaCl, 1 $MgCl_2$, 5 MgATP, 5 EGTA, 10 HEPES, 5 GTP, 10 phosphocreatine, and 0.1 leupeptin. The $GABA_A$ receptor antagonist, Bicuculline (10µM) was added to the solution to isolate glutamatergic activity (AMPA activity at -70mV and NMDA activity at +40mV). AMPA receptor antagonist NBQX (10µM) was also included in the external solution when recording NMDAR-mediated responses. Data were acquired using Clampfit 10.5 software.

Electrical stimulation: To evoke synaptic currents, a monopolar stimulating electrode (glass-pipette filled with ACSF, impedance 1.5 MΩ) was placed in the corpus callosum 300µm from the recorded cell. Test stimuli (0.5 ms duration) were applied every 60s and responses were averaged over three consecutive trials. Test stimuli were applied at increasing stimulus intensities to assess input–output functions. Intensities were set to evoke responses at 60% of maximal amplitude.

Drugs: Stocks of AP-5, BIC, NBQX (Sigma), and were dissolved in water. During the experiment, stock solutions were added to the perfusate at the desired concentration.

Neuroimaging Analysis

Brain images were acquired using a Rapid Acquisition with Refocused Echoes (RARE) pulse sequence. In order to increase the signal-to-noise we acquired 30 brain images per animal (15/scan x 2 scans) and then averaged all 30 images together to generate a single high-resolution image. We acquired the image slices along the dorsal-ventral axis. The slice thickness was 250um and within each slice the voxel dimensions were 156 um by 156 um. We acquired 32 slices per animal; the brain was represented on between 22 to 25 of the slices. The other RARE parameters were: repetition time (TR) = 4000ms, effective echo time (TE) = 43ms, RARE factor = 8, ip angle = 180, number of excitations = 15, field of view (FOV) = 40mm x 20mm, slices = 32, matrix = 256 x 128, scan time = 16 minutes. After all images were acquired, an atlas was generated by mapping all images into a common space via non-linear warping and then averaging them into a single composite image using the Advanced Normalization Tools (ANTs). The atlas provided yet another level of increased contrast allowing for better recognition of anatomical structures. The atlas was manually segmented to distinguish seven structures; neocortex, olfactory bulb, hippocampus, striatum, diencephalon, ventricles and cerebellum. The anatomic label was mapped back onto each individual image in its native space and manually inspected and corrected each anatomic label. To reduce measurement error, three independent labels were made, which were mapped back to each of the images and then manually corrected. After extracting structure volumes from the labels, the three measures were averaged together to generate the final phenotype. The volume of each of the seven structures was determined and then added all together to generate a seventh measures, total brain volume.

We analyzed 2 month (WT, n=7; KO, n=6) and 10 month old (WT, n=4; KO, n=6) male mice. In order to adjust for individual differences in overall skull size, the determinant of the linear mapping generated from the affine registration to the common template was determined and each structure measure was regressed onto the determinant with a linear model. The residualized measure was then analyzed using ANOVA and t-tests. We did two tests; an ANCOVA to test for significant effects of age,

strain and age times strain interaction and a t-test to test for strain differences in the 2 month olds. The results of the ANCOVA are shown in Table 3. Summary of the t-test for each structure focusing on the 2-month-old group is shown in Table 4.

Behavioral Tests:

Home Cage Behavior

Mice were placed in juxtaposed cages containing fresh bedding. Opaque panels were placed in between cages to prevent mice from observing each other. Mice were allowed to acclimate to the new cage for 10 minutes. Behavior was recorded by the automated system, Top Scan (Clever Sys, Inc., Reston, VA) over a following 10 minute period. Videos were scored blinded to genotype for repetitive hindlimb jumping, digging, and grooming. Repetitive jumping was defined by a period in which the mouse reared against the cage, and then jumped consecutively three times or more. The period ended when the mouse unreared. Grooming was defined by repetitive cleaning of body and face. Digging was defined as the scattering of bedding with forelimbs and/or backlimbs. Age and sex characteristics: 15 WT mice (8 males, 7 females; 2 to 6 months): 13 *Jakmip1* KO mice (8 males, 5 females; 2 to 6 months).

T maze spontaneous alternation test

The T maze spontaneous alternation test was performed as previously described (Penagarikano et al., 2011). A Chi-squared test was performed on the number of overall choices of left or right. No bias in arm choice was observed. Age and sex characteristics: 15 WT mice (8 males, 7 females; 2 to 4 months): 13 *Jakmip1* KO mice (8 males, 5 females; 2 to 4 months).

Three-Chamber Social Interaction Test

This test was conducted as previously described (Silverman et al., 2010). Briefly, each mouse was placed in the center of an interconnected three-chambered box after habituation. The center chamber was empty, while left and right chambers contained an empty wire cup or a sex-matched, novel wild-type mouse in a similar wire cup. Behavior was recorded by the automated system, Top Scan (Clever

Sys, Inc., Reston, VA) over a following 10 minute period. Time spent sniffing the mouse-containing cup or the empty cup and time in each chamber was scored manually, blinded to genotype. Age and sex characteristics: 15 WT mice (8 males, 7 females; 1.7 to 4 months): 13 *Jakmip1* KO mice (8 males, 5 females; 1.5 to 4 months).

Rotarod

Mice were acclimated to the rotarod testing room for 15 minutes. Each mouse was then subjected to two tests, each lasting up to 180 seconds. The first test was the "constant" test, and had an initial rotation rate of 5 rpm and accelerated to 20 rpm in 10 seconds. The second test was the "accelerating" test, and had an initial rotation rate of 5 rpm and accelerated to 60 rpm in 10 seconds. Figure 4G (left) displays the accelerating test. Jakmip1 KO mice performed worse than WTs on the constant test, but data was not significant (data not shown). Latency to fall or to rotate for four times on the bar was recorded. Before each test, each mouse was acclimated to the rotarod initial 5 rpm rotation rate for two minutes before beginning the acceleration. Mice were run in the same order through the ‘constant’ and ‘accelerating’ trials. This test was run blinded to genotype. Age and sex characteristics: 15 WT mice (8 males, 7 females; 2 to 4 months): 13 *Jakmip1* KO mice (8 males, 5 females; 2 to 4 months).

Wire Hang Test

Mice were acclimated to wire cage lid for 30 seconds. The lid was then shaken for 10 seconds for the mice to obtain a grip and then swiftly inverted. Latency to fall was measured over a 60 second maximum session. Mice that fell in less than 10 seconds were given up to two retries. This test was run blinded to genotype. Age and sex characteristics: 9 WT mice (4 males, 5 females; 1.1 to 1.3 years): 7 *Jakmip1* KO mice (4 males, 3 females; 1.1 to 1.3 years).

Light Dark Exploration Test

The light dark exploration test was conducted as previously described (Penagarikano et al., 2011). The latency to enter the illuminated compartment, time in the dark and light chambers, and number of cross overs between light and dark chambers was recorded by the automated system, Top Scan (Clever Sys,

Inc., Reston, VA) over a 10 minute period. Age and sex characteristics: 15 WT mice (8 males, 7 females; 1.6 to 4 months): 13 *Jakmip1* KO mice (8 males, 5 females; 1.4 to 4 months).

Ultrasonic Vocalization

Pups were separated from their mother and placed in a sound proof chamber. The recording room was heated to obviate body temperature-related confounds. Ultrasonic vocalizations (USVs) were recorded for 5 minutes using Avisoft software (Berlin, Germany). Mice were weighed after testing and returned to the home cage. Mice were tested and scored blinded to genotype. Age and sex characteristics: P6; WT, n=15 (6 males, 9 females), KO, n=15 (10 males, 5 females): P9; WT, n=17 (7 males, 10 females), KO, n=17 (12 males, 5 females): P12, WT, n=15 (5 males, 10 females), KO, n=15 (10 males, 5 females).

Return to Huddle

Return to huddle was conducted as previously described (Wang et al., 2011) with some modifications. For each test, one postnatal test mouse aged p14-p16 was placed in a cage similar, but larger (47 x 25.5 cm) than its home cage containing a unfamiliar social huddle of three to six pups within three days the age of the test mouse. The test mouse was habituated to the test cage (3 minutes maximum) and the social huddle (2 minutes) prior to testing. The test mouse was then placed in the opposite corner of the cage from the social huddle and the time it took the mouse to return to the huddle was recorded. If the mouse did not find the huddle within 150 seconds, the test was stopped, and the mouse was given the score of 150 seconds. The test mouse was acclimated to the social huddle for 2 minutes between tests. The test was repeated three times and the last two trials were averaged to generate a single score. The test mouse was weighed after the final trial. The test was run blinded to genotype. Age and sex characteristics: 16 WT mice (7 males, 9 females; p14-p16): 12 *Jakmip1* KO mice (5 males, 7 females; p14-p15).

Hot plate startle

Mice were placed on a 55 degrees Celsius hot plate and time was recorded from time of placement to the

first sign of pain, which included licking or kicking of the paws. The mouse was allowed to stay on the plate for a maximum of 15 seconds. The experiment was performed blinded to genotype. Age and sex characteristics: 8 WT mice (4 males, 4 females; 5 to 7 months): 7 *Jakmip1* KO mice (4 males, 3 females; 5 to 7 months).

Auditory Fear Conditioning

Mice were pre handled four days before the day 1 acquisition trial by being removed from the cage and placed in sterilized beaker for 30 seconds. On day 1, mice were taught the tone-shock pairing. Mice are placed in context A and subjected to the following protocol: two minute wait, 30-second tone with two second shock during the last two seconds of tone, one minute wait, 30-second tone with two second shock during the last two seconds of tone, 1 minute wait, 30-second tone with two second shock during the last two seconds of tone, two minute wait. Freezing was recorded. On the second day (day 2), mice were tested for contextual fear by being placed in context A. Freezing was recorded over an eight minute period with no shock or tone administered. On day 3, mice were tested for generalized contextual fear by being placed in a novel context B. Freezing was recorded over an eight minute period with no shock or tone administered. On day 4, long term memory acquisition of the tone-shock pairing was tested. Mice were placed in context B and subjected to the following protocol: two minute wait, 30-second tone, one minute wait, 30-second tone, one minute wait, 30-second tone, two minute wait. Freezing was recorded. The shock intensity used was 0.5 mA, and the shock duration was two seconds. The tone intensity was 80dB, tone length was 30 seconds, and tone frequency was 2000 hertz (Hz). Mice were allowed 30 minutes to habituate to the room before trials. The experiment was performed blinded to genotype. Age and sex characteristics: 9 WT mice (4 males, 5 females; 9 to 11 months): 7 *Jakmip1* KO mice (4 males, 3 females; 9 to 11 months).

Nesting behavior

On day 1, each mouse was placed into a new, individual cage and provided with one unit of tightly

packed nesting material. Cages with mice were then mounted into the wall bracket and left undisturbed overnight. On the second day, mice were first prodded away from nesting site and then extracted out from the cage and placed back into their respective home cages. Two images of the nesting were taken, one at a close distance and one at a further distance. Nests were scored blinded to genotype according to previously published criteria (Deacon, 2006a). Age and sex characteristics: 9 WT mice (4 males, 5 females; 4 to 6 months): 7 *Jakmip1* KO mice (4 males, 3 females; 3 to 6 months).

Marble Burying Test

The marble burying test was conducted as previously described (Deacon, 2006b). Approximately two inches of extra bedding was placed in two testing cages in a sterile hood. Bedding was then compacted using disinfected and gloved hand. 12 marbles were placed equidistantly in a 3x4 grid. Mice were then placed in the middle of the cage. Lids were placed on the cages shortly after to prevent escape of jumping mice. After a 30 minute trial, mice were extracted and placed back into home cage. The number of marbles that were buried more than two thirds into the bedding were counted and one photograph was taken for each cage to verify results. In between trials, bedding was re-compacted and marbles were cleaned with 1% VIRKON disinfectant spray, dried, and rearranged in a 3x4 grid. This test was run blinded to genotype. Age and sex characteristics: 17 WT mice (12 males, 5 females; 2 to 6 months): 15 *Jakmip1* KO mice (12 males, 3 females; 1.9 to 6 months).

Open field test

General activity of mice inside of a 27.5cm x 27.5cm clear plexiglass arena was recorded by the automated system, Top Scan (Clever Sys, Inc., Reston, VA) over a 20 minute period. Age and sex characteristics: 15 WT mice (8 males, 7 females; 1.4 to 4 months): 13 *Jakmip1* KO mice (8 males, 5 females; 1.3 to 4 months).

Olfaction

Olfaction was measured in mice older than 6 weeks of age by determining latency to find a buried food item after habituation to cage and 24 hour food deprivation according to previously published methods

(Yang and Crawley, 2009). This test was run blinded to genotype. Age and sex characteristics: 8 WT mice (5 males, 3 females; 1.7 to 3 months): 8 *Jakmip1* KO mice (4 males, 4 females; 2 to 3 months).

Hypergeometric Probability Calculations

To calculate the hypergeometric probability we used the R code, 1-phyper (k-1, j, m-j, n). JAKMIP1 interactors were defined as 72 binding partners (n) that were present in both MudPIT runs, had NSAfe5 and spectral counts positive for JAKMIP1 IP versus rabbit preimmune serum IP in both runs, and in at least one run had NSAfe5 differentials greater than 50. There were 4 and 7 JAKMIP1 interactors (k), respectively, also belonging to previously defined FMRP-RNP complexes (j) each containing 10 (Kanai et al., 2004) or 24 (Villace et al., 2004) members, respectively (Table 2). Total brain expressed genes, (m) was defined as 15,132 (Kang et al., 2011).

IP/RT-PCR

IP followed by RT-PCR and qRT-PCR reactions were conducted as previously described (Napoli et al., 2008) with the following modifications. For IP reactions, Protein A Dynabeads (Invitrogen, Carlsbad, CA) were used with primary antibodies made in rabbit and rabbit IgG control, while Protein G Dynabeads (Invitrogen, Carlsbad, CA) were used with primary antibodies made in mouse and mouse IgG control. JAKMIP1 IPs and FMRP IPs were conducted at a ratio of 60:4:0.2 [lysate (ug): Dynabeads (ul): antibody (ug)]. A subset of FMRP IPs were conducted at a ratio of 60:4:2, and a subset of JAKMIP1 IPs (striatum RIP experiments) were conducted at a ratio of 48:4:0.2. Proteins were precipitated with an equal or greater ug amount of rabbit or mouse IgG (Millipore, Billerica, MA) in control conditions. A spike-in RNA, pAW109 (ABI, Foster City, CA), was used to control for loading. RNA was extracted using a miRNeasy kit (Qiagen, Germantown, MD) and reverse transcriptase reactions were carried out using Superscript III and random hexamers (Invitrogen, Carlsbad, CA) according to manufacture's instructions. The following primer sets were used for RT-PCR reactions:

*Sapap4, Camk2a, PSD95_3', Map1b, Fmr1, Ef1a1, Dag1_set1, Dag1_set2, D2DR, BC1, App, 18S, DM151/152 (pAW109)***,** *Taf13, Grin2a, Grin2b, Shank2, Jakmip1.* For Figure 3B and C, PCR thermocycler conditions were the following: 94°C for 2 minutes, 30 or 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute, then 72°C for 2 minutes, and hold at 4°C. qRT-PCR was carried out using the Light Cycler 480 II (Roche Applied Science, Indianapolis, IN) and SensiFAST SYBR No-Rox mix (Bioline, Taunton, MA) (Figure 3D) or LightCycler 480 SYBR Green I (Roche Applied Science, Indianapolis, IN) (Figure 5A). Transcript levels (Normalized RNA) were calculated using the LightCycler 480 SW 1.5 software (Roche Applied Science, Indianapolis, IN) using the formula, 2-(ΔΔCt IP) (where ΔΔCt IP is ΔCtIP -ΔCt Input, and ΔCt equals Ct target - Ct pAW109), and transformed linearly.

Primer sets

Primers	Forward 5'-3'	Reverse 5'-3'	Reference
Camk2a	AGCCATCCTCACCACTATGCTGG	ACCCTGGCCTGGTCCTTCAATG	(Napoli et al., 2008)
Sapap4	CATCGGGATTCAGGTAGAGG	ATAGGAGAGGTTGCGCTTGA	
PSD95_3'	GGCTTCATTCCCAGCAAACG	CATCAAGGATGCAGTGCTTC	(Zalfa et al., 2007)
Map1b	GGCAAGATGGGGTATAGAGA	CCCACCTGCTTTGGTATTTG	(Napoli et al., 2008)
Jakmip1	GCGGAAGAGGCACTCAGTAA	GTTTGCACACCAAGCTCCTT	
Fmr1	GTGGTTAGCTAAAGTGAGGATGAT	CAGGTTTGTTGGGATTAACAGATC	(Napoli et al., 2008)
Ef1a1	CCAATGGAAGCAGCTGGCTT	CCGTTCTTCCACCACTGATTA	(Zalfa et al., 2007)
Dag1_set2	GTGAGCATTCCAACGGATTT	TGGCTCATTGTGGTCTTCAG	
Dag1_set1	CTGGAAGAACCAGCTTGAGG	GGACAGTCACTGGCTCATCA	
D2DR	GGCCATGCCTATGTTGTATAA	CCCATTCTTTTCTGGTTTGG	(Napoli et al., 2008)
BC1	GTTGGGGATTTAGCTCAGTGG	AGGTTGTGTGTGCCAGTTACC	(Napoli et al., 2008)
App	GGTGGCTGAGGAGATTCAAG	TCACGGTTGCTATGACAACGC	(Napoli et al., 2008)
18S	CATTAAATCAGTTATGGTTCCTTTGG	TCGGCATGTATTAGCTCTAGAATTACC	

DM151/152 (pAW109)	GTCTCTGAATCAGAAATCCTTCTATC	CATGTCAAATTTCACTGCTTCATCC	Applied Biosystems
Taf13	GCCAGGGGAAGAGAAAGAGA	TCCATAGTTGGCTTCGTCAA	
Grin2a	GCCTTGGTCAGCTTGAAAAC	TCCCAATGGTCACCAGTTTA	
Grin2b	GCGGGCGTCTTCTATATGTT	GACAGGTTGGCCATGTTTTT	
Shank2	GCGCCAAGGGATTGCTGTA	CTGCCTTCGCATCGTACCTC	
Hprt	GCTCGAGATGTCATGAAGGAGA	TCAGTGCTTTAATGTAATCCAGC	

Antibodies

We used the following antibodies for IB or immunofluorescence (IF): anti-JAKMIP1 #2 [rabbit polyclonal, Proteintech Group; 1:500-1:1,000 (IB)], anti-JAKMIP1 #1 [$J1_{269-286}$ rabbit polyclonal; 1:200 - 1:2,000 (IB)], anti-DDX5 [mouse monoclonal, Santa Cruz Biotechnology; 1:100 - 1:1000 (IB)], anti-FMRP [mouse monoclonal, Millipore; 1:500 - 1:2000 (IB)], anti-FMRP [rabbit polyclonal, Santa Cruz; 1:1000 (IB)], anti-PABP [rabbit polyclonal, Abcam; 1:500 (IB)], anti-CLASP2 [rabbit polyclonal, Santa Cruz; 1:200 (IB)], anti-CAMK2G [rabbit polyclonal, Proteintech Group; 1:2000 (IB)], anti-CAMK2A [rabbit polyclonal, Abcam; 1:1000 (IB)], anti-PSD95 [rabbit monoclonal, Abcam; 1:1000 (IB)], anti-DAG1 [mouse monoclonal, Santa Cruz; 1:500 (IB)], anti-SAPAP4 [rabbit polyclonal, Santa Cruz; 1:200 (IB)], anti-APP [rabbit polyclonal, Sigma; 1:500 (IB)], anti-RPS6 [mouse monoclonal, Cell Signaling Technologies; 1:1000 (IB)], anti-eIF4E [mouse monoclonal, BD Biosciences; 1:500 (IB)], anti-βtubulin [rabbit polyclonal, Abcam; 1:500 (IB)], anti-βactin [mouse monoclonal, Sigma; 1:1000 - 1:50,000 (IB)], anti-GAPDH [mouse monoclonal, Millipore; 1:10,000 (IB)], anti-GFP [rabbit polyclonal, Abcam; 1:5000 (IB)], anti-RFP [rabbit polyclonal; 1:200 (IF)], anti-V5 [mouse monoclonal, Invitrogen; 1:200 (IF)], anti-TUJ1 [(mouse monoclonal, Covance, 1: 1000 (IF)], anti-GluN2A [(mouse monoclonal, NeuroMab clone N327/95, 1: 500 (IB)], anti-GluN2B [(mouse monoclonal, NeuroMab clone N59/20, 1: 500 (IB)], anti-SHANK2 [(rabbit polyclonal, #1136, gift from Dr. Eunjoon Kim, 1: 10,000 (IB)], anti-SHANK3 [(mouse monoclonal, NeuroMab clone N367/62, 1: 500 (IB)] goat anti-rabbit horseradish

peroxidase (Cell Signaling, 1:10,000), goat anti-mouse horseradish peroxidase (Promega, 1:2,500), goat anti-rabbit horseradish peroxidase (Pierce, 1:10,000), goat anti-mouse horseradish peroxidase (Pierce, 1:10,000), goat anti-mouse Alexa Fluor 488 (Invitrogen, 1:1,000), donkey anti-rabbit Alexa Fluor 555 (Invitrogen, 1:1,000), and donkey anti-rabbit Alexa Fluor 546 (Invitrogen, 1:1,000).

REFERENCES

Carvalho, P.C., Fischer, J.S., Chen, E.I., Yates, J.R., 3rd, and Barbosa, V.C. (2008). PatternLab for proteomics: a tool for differential shotgun proteomics. BMC Bioinformatics *9*, 316.

Deacon, R.M. (2006a). Assessing nest building in mice. Nature protocols *1*, 1117-1119.

Deacon, R.M. (2006b). Digging and marble burying in mice: simple methods for in vivo identification of biological impacts. Nature protocols *1*, 122-124.

Dieterich, D.C., Hodas, J.J., Gouzer, G., Shadrin, I.Y., Ngo, J.T., Triller, A., Tirrell, D.A., and Schuman, E.M. (2010). In situ visualization and dynamics of newly synthesized proteins in rat hippocampal neurons. Nat Neurosci *13*, 897-905.

Florens, L., Carozza, M.J., Swanson, S.K., Fournier, M., Coleman, M.K., Workman, J.L., and Washburn, M.P. (2006). Analyzing chromatin remodeling complexes using shotgun proteomics and normalized spectral abundance factors. Methods *40*, 303-311.

Hallett, P.J., Collins, T.L., Standaert, D.G., and Dunah, A.W. (2008). Biochemical fractionation of brain tissue for studies of receptor distribution and trafficking. Curr Protoc Neurosci *Chapter 1*, Unit 1 16.

Kanai, Y., Dohmae, N., and Hirokawa, N. (2004). Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. Neuron *43*, 513-525.

Kang, H.J., Kawasawa, Y.I., Cheng, F., Zhu, Y., Xu, X., Li, M., Sousa, A.M., Pletikos, M., Meyer, K.A., Sedmak, G.*, et al.* (2011). Spatio-temporal transcriptome of the human brain. Nature *478*, 483-489.

Krupka, N., Strappe, P., Gotz, J., and Ittner, L.M. (2010). Gateway-compatible lentiviral transfer vectors for ubiquitin promoter driven expression of fluorescent fusion proteins. Plasmid *63*, 155-160.

Napoli, I., Mercaldo, V., Boyl, P.P., Eleuteri, B., Zalfa, F., De Rubeis, S., Di Marino, D., Mohr, E., Massimi, M., Falconi, M.*, et al.* (2008). The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. Cell *134*, 1042-1054.

Penagarikano, O., Abrahams, B.S., Herman, E.I., Winden, K.D., Gdalyahu, A., Dong, H., Sonnenblick, L.I., Gruver, R., Almajano, J., Bragin, A.*, et al.* (2011). Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. Cell *147*, 235-246.

Silverman, J.L., Yang, M., Lord, C., and Crawley, J.N. (2010). Behavioural phenotyping assays for mouse models of autism. Nature reviews Neuroscience *11*, 490-502.

Spiteri, E., Konopka, G., Coppola, G., Bomar, J., Oldham, M., Ou, J., Vernes, S.C., Fisher, S.E., Ren, B., and Geschwind, D.H. (2007). Identification of the transcriptional targets of FOXP2, a gene linked to speech and language, in developing human brain. Am J Hum Genet *81*, 1144-1157.

Steindler, C., Li, Z., Algarte, M., Alcover, A., Libri, V., Ragimbeau, J., and Pellegrini, S. (2004). Jamip1 (marlin-1) defines a family of proteins interacting with janus kinases and microtubules. J Biol Chem *279*, 43168-43177.

Vidal, R.L., Ramirez, O.A., Sandoval, L., Koenig-Robert, R., Hartel, S., and Couve, A. (2007). Marlin-1 and conventional kinesin link GABAB receptors to the cytoskeleton and regulate receptor transport. Mol Cell Neurosci *35*, 501-512.

Villace, P., Marion, R.M., and Ortin, J. (2004). The composition of Staufen-containing RNA granules from human cells indicates their role in the regulated transport and translation of messenger RNAs. Nucleic Acids Res *32*, 2411-2420.

Wang, C.C., Held, R.G., Chang, S.C., Yang, L., Delpire, E., Ghosh, A., and Hall, B.J. (2011). A critical role for GluN2B-containing NMDA receptors in cortical development and function. Neuron *72*, 789-805.

Yang, M., and Crawley, J.N. (2009). Simple behavioral assessment of mouse olfaction. Curr Protoc Neurosci *Chapter 8*, Unit 8 24.

Zalfa, F., Eleuteri, B., Dickson, K.S., Mercaldo, V., De Rubeis, S., di Penta, A., Tabolacci, E., Chiurazzi, P., Neri, G., Grant, S.G.*, et al.* (2007). A new function for the fragile X mental retardation protein in regulation of PSD-95 mRNA stability. Nat Neurosci *10*, 578-587.