

Archival Report

CYFIP1 Dosages Exhibit Divergent Behavioral Impact via Diametric Regulation of NMDA Receptor Complex Translation in Mouse Models of Psychiatric Disorders

Nam-Shik Kim, Francisca Rojas Ringeling, Ying Zhou, Ha Nam Nguyen, Stephanie J. Temme, Yu-Ting Lin, Stephen Eacker, Valina L. Dawson, Ted M. Dawson, Bo Xiao, Kuei-sen Hsu, Stefan Canzar, Weidong Li, Paul Worley, Kimberly M. Christian, Ki-Jun Yoon, Hongjun Song, and Guo-li Ming

ABSTRACT

BACKGROUND: Gene dosage imbalance caused by copy number variations (CNVs) is a prominent contributor to brain disorders. In particular, 15q11.2 CNV duplications and deletions have been associated with autism spectrum disorder and schizophrenia, respectively. The mechanism underlying these diametric contributions remains unclear.

METHODS: We established both loss-of-function and gain-of-function mouse models of *Cyfp1*, one of four genes within 15q11.2 CNVs. To assess the functional consequences of altered CYFIP1 levels, we performed systematic investigations on behavioral, electrophysiological, and biochemical phenotypes in both mouse models. In addition, we utilized RNA immunoprecipitation sequencing (RIP-seq) analysis to reveal molecular targets of CYFIP1 in vivo.

RESULTS: *Cyfp1* loss-of-function and gain-of-function mouse models exhibited distinct and shared behavioral abnormalities related to autism spectrum disorder and schizophrenia. RIP-seq analysis identified messenger RNA targets of CYFIP1 in vivo, including postsynaptic NMDA receptor (NMDAR) complex components. In addition, these mouse models showed diametric changes in levels of postsynaptic NMDAR complex components at synapses because of dysregulated protein translation, resulting in bidirectional alteration of NMDAR-mediated signaling. Importantly, pharmacological balancing of NMDAR signaling in these mouse models with diametric *Cyfp1* dosages rescues behavioral abnormalities.

CONCLUSIONS: CYFIP1 regulates protein translation of NMDAR and associated complex components at synapses to maintain normal synaptic functions and behaviors. Our integrated analyses provide insight into how gene dosage imbalance caused by CNVs may contribute to divergent neuropsychiatric disorders.

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Mental disorders such as schizophrenia (SCZ) and autism spectrum disorder (ASD) are chronic and disabling. A large number of susceptibility genes for ASD and SCZ have been identified from human genetic studies, and various experimental models are being developed to investigate how these susceptibility genes regulate behavior (1,2). In addition to single nucleotide polymorphisms or mutations in individual genes, submicroscopic variations in DNA copy number variation (CNV) are also widespread in human genomes, and specific CNVs have been identified as significant risk factors for ASD and SCZ (3). Moreover, aggregate data have provided support for the polygenic inheritance and genetic overlap between SCZ and ASD (4–7). Intriguingly, diametric dosages of the same mutations (deletion vs. duplication), including both individual genes (e.g., *MeCP2*, *SHANK3*) and CNVs (e.g., 15q11.2, 16p11.2, 22q11.2), lead to divergent brain disorders (8). The underlying mechanism is unknown.

In particular, 15q11.2 CNVs have emerged as prominent genetic risk factors for various neuropsychiatric disorders, including ASD, SCZ, and intellectual disability (9–12). Specifically, 15q11.2 microduplications have been associated with ASD (13,14), whereas microdeletions of the same region have been identified as one of the three most frequent CNV risk factors for SCZ (15). Even nondiagnosed carriers of 15q11.2 microdeletions showed cognitive function that was in between neurotypical control subjects and patients with SCZ (16–18). The 15q11.2 microdeletion and microduplication also result in reciprocal effects on the volume of some human brain regions, including gray matter in the perigenual anterior cingulate cortex and white matter in the temporal lobe (16,18–20). It remains unclear how different doses of genes within the 15q11.2 region may contribute to the etiopathology underlying divergent neuropsychiatric disorders.

CYFIP1 is 1 of 4 genes within 15q11.2 and encodes a protein that interacts with FMRP (fragile X mental retardation protein) and eIF4E (eukaryotic translation initiation factor 4E) and negatively regulates messenger RNA (mRNA) translation at synapses in an activity-dependent manner (21,22). An altered level of *CYFIP1* leads to abnormalities in dendrite complexity and spine morphology (23–25), synaptic function (26,27), and behavior (25,28–32). Moreover, common single nucleotide polymorphisms in *CYFIP1* have been associated with SCZ (33) and ASD (34), and *CYFIP1* mRNA expression is increased in patients with ASD (35,36). These findings highlight *CYFIP1* as the most compelling genetic risk factor for neuropsychiatric disorders within the 15q11.2 region. Thus, an increase or decrease in *CYFIP1* levels may lead to diametric alterations in common signaling pathways, which could be the underlying mechanism for the pathogenesis of the 15q11.2 CNV-mediated risk for neuropsychiatric disorders. However, mRNA targets of *CYFIP1* and effects of the *CYFIP1* dosage on the protein translation of those targets have not been examined in a nonbiased manner.

To understand how different dosages of *CYFIP1* may lead to divergent brain disorders, we established both loss-of-function and gain-of-function mouse models of *Cyfp1*. The loss-of-*Cyfp1* model exhibited distinct behavioral abnormalities related to SCZ, whereas the gain-of-*Cyfp1* model exhibited ASD-related behavioral phenotypes. Mechanistically, genome-wide RNA immunoprecipitation sequencing (RIP-seq) identified novel *CYFIP1*-associated mRNA targets related to synaptic function, postsynaptic density, and the NMDA receptor (NMDAR) complex. Furthermore, these mouse models showed dysregulation of postsynaptic protein translation on specific targets of *CYFIP1* and diametric changes in the levels of postsynaptic NMDAR complex components and signaling at synapses. Importantly, bidirectional pharmacological manipulations to rebalance NMDAR signaling largely rescue the behavioral abnormalities of these mouse models. Our integrated study provides insight into how 15q11.2 CNVs may contribute to divergent neuropsychiatric disorders.

METHODS AND MATERIALS

See [Supplemental Methods and Materials](#) for a detailed description of experimental methods, and see [Key Resources Table](#) for materials and suppliers.

Animals

The loss-of-function (cKO) and gain-of-function (cOE) mice were generated by the Transgenic Core Laboratory at Johns Hopkins School of Medicine ([Figure S1](#)). Both cKO and cOE mice were crossed with *Nestin-Cre* mice, and they were backcrossed to C57BL/6J at least 6 times before all experiments. For most behavioral experiments, 3- to 4-month-old male mice were used. In the marble burying assay, nest building assay, and pup retrieval assay, 3- to 4-month-old female mice were used. All mouse work was performed with protocols approved by the Animal Care and Use Committee of Johns Hopkins University School of Medicine and University of Pennsylvania.

Data and Code Availability

The GEO accession number for the RIP-seq dataset is GSE166939.

RESULTS

Loss of *CYFIP1* Function Results in Behavioral Abnormalities Related to Schizophrenia

To investigate the *in vivo* effects of differential *Cyfp1* dosages on animal behavior under the same conditions, we generated a conditional knockout mouse model (*Nestin-Cre: Cyfp1^{flxed/flxed}*, named cKO) and a conditional over-expression mouse model of *Cyfp1* (*Nestin-Cre: ROSA26^{Cyfp1} KI/KI*, named cOE). cKO showed complete ablation of the *CYFIP1* protein in forebrain lysates after E17.5 ([Figure S1C](#)). On the other hand, the homozygote cOE mice showed about 1.5- to 2-fold increase of *CYFIP1* protein levels in the adult hippocampus and cortex ([Figure S1F](#)), similar to patients with ASD with 15q11.2 duplication (14). We used the homozygote cOE mice for all analyses. The cKO and cOE mice were fertile and displayed a normal appearance and expected Mendelian ratio of genotypes in adulthood (data not shown).

We first tested cKO mice with a battery of behavioral analyses related to human mental disorders. cKO mice displayed normal locomotor activity, motor coordination, nociception response, novel object recognition, and repetitive behaviors ([Figure S2A–E](#) and [Table S1](#)). To test whether cKO mice exhibit behavioral abnormalities related to negative symptoms in SCZ, we first performed a 3-chamber social interaction assay (37). cKO mice showed a similar preference for a mouse (stranger 1) over an empty cage in comparison with littermate wild-type (WT) mice (*Cyfp1^{flxed/flxed}*, named WT1) ([Figure 1A](#)). However, when the empty cage was replaced by a novel mouse (stranger 2), cKO mice did not show a significant preference for stranger 2 over stranger 1, while WT1 mice preferred to interact with stranger 2 ([Figure 1B](#)), indicating impaired social novelty recognition. Moreover, cKO mice exhibited impaired prepulse inhibition (PPI) and an elevated startle response ([Figure 1C, D](#)), suggesting deficits in sensorimotor gating commonly found in patients with SCZ. In addition, cKO mice showed elevated behavioral despair with increased immobility in both the tail suspension test (TST) and forced swim test (FST) compared with WT1 mice ([Figure 1E, F](#)).

We also measured amphetamine-induced hyperactivity, which is widely used in animals to model neuropsychiatric disorders and positive symptoms of SCZ (38). cKO mice showed increased locomotor activity after an acute injection of amphetamine compared with the WT1 mice ([Figure 1G](#)). The *Nestin-Cre* transgene did not affect social interaction, amphetamine-induced hyperactivity, or prepulse inhibition ([Figure S2F–I](#)).

In summary, these results suggest that *Cyfp1* loss-of-function in mice leads to multiple behavioral abnormalities related to negative and positive symptoms of patients with SCZ.

Increased *Cyfp1* Dosage Leads to Behavioral Abnormalities Related to ASD

Given that *CYFIP1* mRNA expression is significantly upregulated in patients with ASD (14,24,36), we examined whether

Altered CYFIP1 Doses Imbalance NMDAR in Mental Disorder

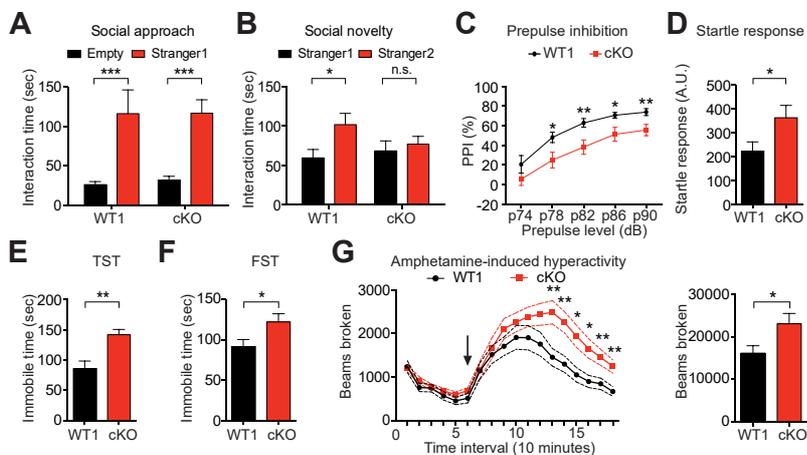


Figure 1. *Cyfip1* cKO mice displayed impaired social novelty recognition, abnormal sensorimotor gating, elevated despair, and altered psychostimulant response. **(A, B)** Normal social approach and impaired social novelty recognition of cKO mice in the three-chamber assay. **(A)** In the first stage, both WT1 mice and cKO mice showed a significant preference for a mouse (stranger 1) over an empty cage, as measured by time spent in close interaction. **(B)** In the second stage, cKO mice showed no preference for a novel mouse (stranger 2) over a familiar mouse (stranger 1), while WT1 mice interacted significantly more with stranger 2 than stranger 1. Values represent mean \pm SEM ($n = 10$ WT1, 10 cKO; *** $p < .001$; * $p < .05$; n.s.: $p > .05$; Student's t test). **(C)** Abnormal prepulse inhibition and **(D)** increased basal acoustic startle response (120 dB) of cKO mice compared with WT1 mice. Values represent mean \pm SEM ($n = 19$ WT1, 15 cKO; * $p < .05$; ** $p < .01$; Student's t test). **(E, F)** Elevated behavioral despair in cKO mice. The immobile time

in both **(E)** TST and **(F)** FST was increased in cKO mice compared with WT1 mice. Values represent mean \pm SEM ($n = 19$ WT1, 15 cKO; * $p < .05$; ** $p < .01$; Student's t test). **(G)** Enhanced amphetamine-induced hyperactivity in cKO mice compared with WT1 mice. Amphetamine (2.5 mg/kg, i.p.) was injected after 1-hour habituation in an open field. Shown on the left is the trace of the locomotor activity presented as the number of beams broken every 10 minutes. An arrow represents the time of the amphetamine injection. Shown on the right is the total number of beams broken after amphetamine injection. Values represent mean \pm SEM ($n = 11$ WT1, 14 cKO; ** $p < .01$; * $p < .05$; n.s.: $p > .05$; Student's t test). cKO, conditional knockout; FST, forced swim test; i.p., intraperitoneal; n.s., not significant; TST, tail suspension test; WT, wild-type.

overexpression of CYFIP1 may result in ASD-related behavioral abnormalities. cOE mice displayed normal locomotor activity and novel object recognition (Figure S3A, B; Table S1). In the first stage of the 3-chamber social interaction assay, cOE mice exhibited decreased interaction with stranger 1 compared with littermate WT mice (*ROSA26^{Cyfip1} K1/K1*, named WT2) (Figure 2A). cOE mice also did not prefer to interact with a novel mouse (stranger 2), whereas the WT2 mice interacted significantly more with stranger 2 than with stranger 1 (Figure 2B). These results suggest that cOE mice have an impaired social approach and social novelty recognition. Repetitive and stereotyped patterns of behavior are one of the core behavioral domains for the ASD diagnosis (39). We assessed repetitive behaviors of cOE mice with the marble burying assay and measuring digging behavior (40). cOE mice buried significantly more marbles than the WT2 mice (Figure 2C). Moreover, cOE mice also spent more time engaged in digging behavior than the WT2 mice (Figure 2D).

Maternal behaviors are frequently impaired in mouse models of ASD (41–43). The survival rate of pups from cOE dams was markedly lower than that from WT2 dams (Figure 2E). At postnatal day 3, some of the pups from cOE dams did not show milk in their stomachs, whereas milk was observed in the stomachs of all pups from WT2 dams (Figure S3C). As a result, the number of surviving pups at postnatal day 7 from cOE dams was significantly smaller compared with that from WT2 dams (Figure 2E; Figure S3D). Among surviving pups from cOE dams, the number of cOE and WT pups was similar, suggesting that impaired maternal care is not dependent on genotypes of the pups (data not shown). Moreover, cOE females showed impaired nest building behaviors (Figure 2F) and less efficient pup retrieval compared with WT2 females (Figure 2G), suggesting that multiple traits of maternal behaviors are impaired with an increased *Cyfip1*

dosage in a mouse model. In contrast, cKO dams showed similar levels in the pup survival rate with the WT1 dams (data not shown). In addition, similar to cKO mice, cOE mice displayed increased amphetamine-induced hyperactivity compared with the WT2 mice, indicating dysfunction in the dopaminergic system of the cOE mice (Figure 2H). Unlike cKO mice, cOE mice did not display elevated despair in the TST (Figure S3E) or abnormal sensorimotor gating in the PPI (Figure S3F; Table S1). Taken together, our behavioral analyses of cOE mice showed that increased *Cyfip1* dosage results in several behavioral abnormalities related to ASD, including social impairment, increased repetitive behaviors, and abnormal maternal behaviors.

CYFIP1 Interacts With mRNAs Encoding Synaptic and NMDAR Complex-Related Proteins in the Mouse Hippocampus and Human Cortical Tissue

CYFIP1 regulates mRNA translation in neurons (21,22). To identify which mRNAs are the potential targets of CYFIP1 for translation regulation, we performed RIP-seq (44) of the adult mouse hippocampus. We took advantage of our cOE mouse model, which expresses a hemagglutinin (HA)-tagged CYFIP1 (Figure S1D), enabling us to immunoprecipitate CYFIP1 and associated mRNA using an anti-HA antibody with high specificity (Figure 3A, B). We identified 1721 transcripts that were found to be enriched in 3 out of 4 comparisons with control subjects (Table S2). Gene Ontology analysis showed significant enrichment of terms related to neuronal and synaptic components and processes and more specifically to the NMDAR complex (Figure 3C, D; Table S3). Disease ontology analysis revealed enrichment for neuropsychiatric diseases, including SCZ and ASD (Figure 3D; Table S3).

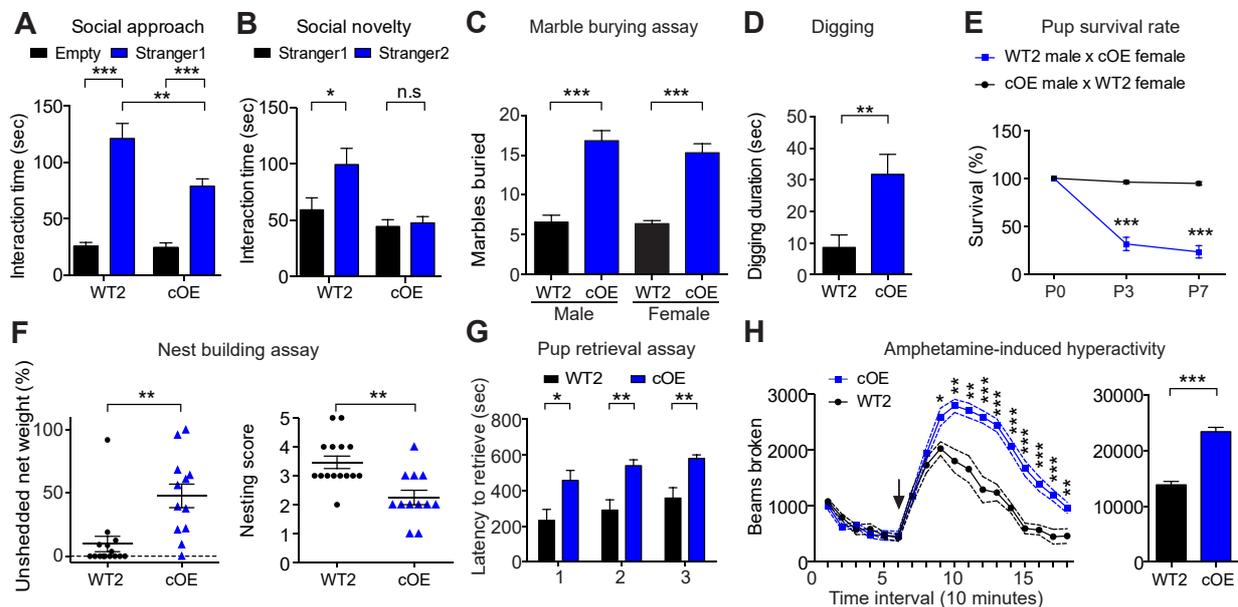


Figure 2. *Cyfip1* cOE mice displayed impaired social interaction, increased repetitive behaviors, abnormal maternal behaviors, and altered psychostimulant response. **(A, B)** Reduced social approach and impaired social novelty recognition of cOE mice in the three-chamber assay. **(A)** In the first stage, cOE mice showed a significantly lower preference for a mouse (stranger 1) over an empty cage compared with WT2 mice. **(B)** In the second stage, cOE mice showed no preference for a novel mouse (stranger 2) over a familiar mouse (stranger 1), while WT2 mice interacted significantly more with stranger 2 than stranger 1. Values represent mean \pm SEM ($n = 13$ WT2, 13 cOE; *** $p < .001$; ** $p < .01$; * $p < .05$; n.s.: $p > .05$; one-way analysis of variance). **(C)** Increased repetitive behavior of cOE mice in the marble burying test. Number of marbles buried during a 30-minute test period was counted. Values represent mean \pm SEM ($n = 16$ male WT2, 11 male cOE, 8 female WT2, 13 female cOE; *** $p < .001$; Student's t test). **(D)** Increased digging behavior of cOE mice. Time spent in digging was measured during a 10-minute test period. Values represent mean \pm SEM ($n = 11$ WT2, 10 cOE; ** $p < .01$; Student's t test). **(E)** Pups from cOE dams showed reduced survival rate in the early postnatal period. Survival rate of pups from each litter was quantified according to genotypes of the dams. Values represent mean \pm SD ($n = 10$ WT2, 14 cOE litters; *** $p < .001$; Student's t test). **(F)** cOE females displayed abnormal nest building behavior. Shown on the left is the percentage weight of the unshedded nest and shown on the right is the nest building score 24 hours after a new nestlet presentation. Values represent mean \pm SEM ($n = 15$ WT2, 12 cOE; ** $p < .01$; Student's t test). **(G)** cOE females showed impaired pup retrieval behavior. The retrieval latencies of 3 pups in the pup retrieval test are shown. Values represent mean \pm SEM ($n = 13$ WT2, 11 cOE; ** $p < .01$; * $p < .05$; Student's t test). **(H)** Enhanced amphetamine-induced hyperactivity in cOE mice compared with WT2 mice. Shown on the left is the trace of the locomotor activity presented as the number of beams broken every 10 minutes. An arrow represents the time of the amphetamine injection. Shown on the right is the total number of beams broken after the amphetamine injection. Values represent mean \pm SEM ($n = 11$ WT1, 14 cOE; *** $p < .001$; ** $p < .01$; * $p < .05$; Student's t test). cOE, conditional overexpression; n.s., not significant; WT, wild-type.

To confirm the interaction between the CYFIP1 protein and some of the targets identified by our genome-wide analysis, we performed immunoprecipitation of endogenous CYFIP1 with an anti-CYFIP1 antibody using hippocampal tissue from the WT mice, followed by quantitative polymerase chain reaction (RIP-qPCR). We chose several mRNAs related to the NMDAR complex for further validation and indeed found that their mRNAs were enriched in the anti-CYFIP1 pulled-down samples compared with the IgG pulled-down samples, including *Shank1*, *Shank2*, *Grin2a*, *Grin2b*, and *Gabbr2* (Figure 3E). To explore whether these interactions are conserved in the human brain, we performed RIP-qPCR experiments using surgical adult human cortical tissues. We found that indeed they were also significantly enriched in the cerebral cortex samples pulled down with an anti-CYFIP1 antibody compared with the IgG pull down (Figure 3F).

Given that CYFIP1 interacts with FMRP, we compared mRNA targets of these two proteins. Between 1721 CYFIP1 targets we identified and 842 FMRP targets previously

reported (45), only 130 mRNA targets were shared (Table S4), suggesting independent direct regulation of many mRNA targets by CYFIP1 and FMRP.

CYFIP1 Regulates mRNA Translation of NMDAR Subunits and Associated Complex

CYFIP1 represses the translation of its target mRNAs by inhibiting the interaction between eIF4E and eIF4G at the 5' cap structure (21,22,46). The mRNA levels encoding the NMDAR subunits and associated complex proteins were not changed in either cKO or cOE mice (Figure S4A, B), indicating that CYFIP1 may not regulate transcription or the stability of these mRNA targets. To explore whether altered CYFIP1 dosages lead to dysregulated protein translation of CYFIP1 target mRNAs, we applied the puromycin-associated nascent chain proteomics (PUNCH-P) technique (47) to monitor the amount of nascent peptides from cKO and cOE hippocampi (Figure 4A). To determine effects of CYFIP1 ablation on the general translation rate, the amount of total

biotin-puromycin-labeled nascent peptides were accessed by streptavidin-horseradish peroxidase immunoblotting. Similar amounts of peptides were being synthesized in cKO mice compared with WT1 mice, suggesting that the loss of CYFIP1 function does not change the general translation rate (Figure 4B). To monitor the protein translation rate of specific target mRNAs, biotin-puromycin-labeled peptides were captured and purified by streptavidin beads and examined by specific antibodies. The protein synthesis of postsynaptic NMDAR subunits and associated complex components, but not presynaptic protein SYN1, was significantly increased in the cKO mice compared with the WT1 mice (Figure 4C). Conversely, the amount of protein synthesis from those transcripts was substantially lower in cOE mice compared with WT2 mice (Figure 4D).

To confirm our results, we performed polysome profile analysis with hippocampal lysates. The general polysome profile was not significantly altered in cKO mice compared with WT1 mice (Figure S4C), consistent with the unchanged general translation rate revealed by PUNCH-P (Figure 4B). Notably, *Grin2b* and *Shank2* mRNA distributions were shifted to heavier polysome fractions in cKO mice compared with WT1 mice (Figure S4C, E), indicating an enhanced translation efficiency. In contrast, *Grin2b* and *Shank2* mRNA distributions were shifted to the lighter fractions in cOE mice compared with WT2 mice (Figure S4D, F), indicating repressed translation of those mRNAs. These results suggest that the balanced level of CYFIP1 protein is important for translational regulation of its target mRNAs.

Protein Expression of the NMDAR-Associated Complex and Postsynaptic Scaffolding Are Altered in the Synapse Depending on CYFIP1 Dosages

To examine changes in protein expression at synapses of cKO and cOE mice, including the CYFIP1-interacting mRNA targets identified by RIP-seq analysis, synaptosomes were isolated from the hippocampi for Western blotting analysis (Figure S5A). As expected, the expression of CYFIP1 was completely abolished in the cKO hippocampus and increased in the cOE hippocampus (Figure 5A, B). The expression levels of the NMDAR subunits (GRIN1 and GRIN2B) and NMDAR-associated postsynaptic scaffolding proteins (SHANK2 and PSD95) (48) were significantly increased in the cKO synaptosomes (Figure 5A). In contrast, expression levels of NMDAR subunits (GRIN1, GRIN2A, and GRIN2B), SHANK2, and PSD95 were reduced in the cOE synaptosome compared with the WT2 synaptosome (Figure 5B), suggesting a reciprocal regulation of postsynaptic proteins dependent on different CYFIP1 dosages. The expression levels of the AMPA receptor subunits, presynaptic proteins, and Homer1 were unchanged in both the cKO and cOE hippocampi. Different from previous reports (21,22), we did not observe any significant changes in the expression of ARC (activity-regulated cytoskeleton-associated protein) in our models (Figure 5A, B).

To examine whether similar regulation occurs in human models, we used patient induced pluripotent stem cell (iPSC) lines we previously generated from subjects with 15q11.2del CNVs and control subjects (49). Western blotting analyses of

cortical neurons differentiated from these iPSC lines also showed increased levels of GRIN2B proteins in both the whole cell lysate and synaptosomes (Figure S5B, C).

Imbalanced CYFIP1 Expression Results in Reciprocal Alterations in NMDAR Functions

Dysfunction in NMDARs at synapses is associated with various neuropsychiatric disorders (43,50–52). To examine whether the altered molecular composition of synapses at the postsynaptic site influences the function of NMDAR in cKO and cOE mice, we compared the relative contribution of the NMDA versus AMPA receptors to evoke excitatory postsynaptic currents using whole-cell patch-clamp recordings from dentate gyrus granule cells in acute hippocampal slices. Consistent with the reciprocal protein expression of the NMDAR subunits in the hippocampi of cKO and cOE mice (Figure 5A, B), we found an increased NMDA/AMPA ratio in cKO mice but a decreased NMDA/AMPA ratio in cOE mice compared with their WT littermates (Figure 6A, B).

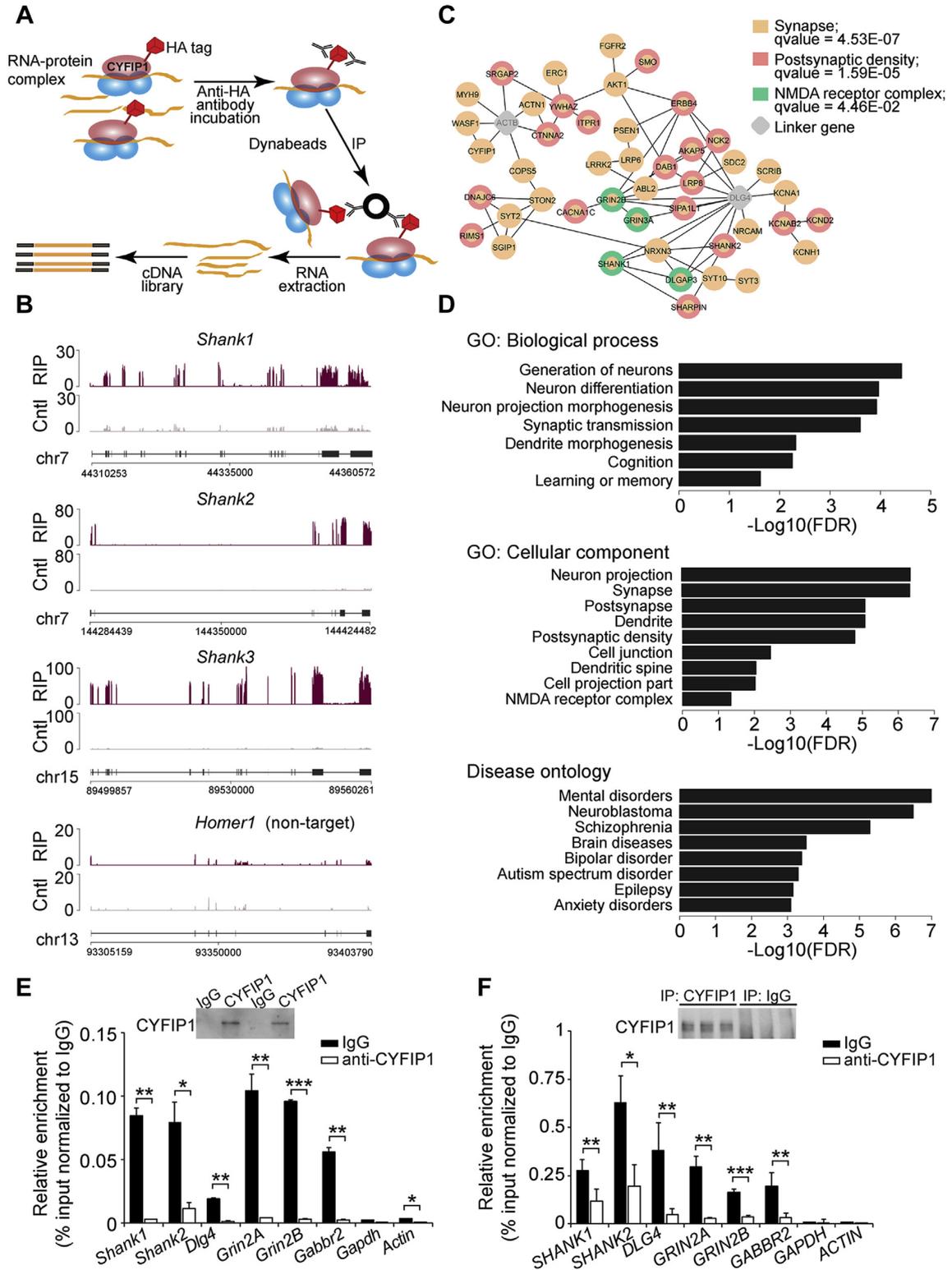
Locomotor hyperactivity induced by systemic administration of an NMDAR antagonist has been used as an *in vivo* measurement of central neurotransmitter system activity (38,53). Systemic injection of MK-801, a noncompetitive NMDAR antagonist, increased locomotor activity in both genotypes, but the levels of hyperactivity were significantly higher in cKO mice compared with WT1 mice (Figure 6C, top panel). Conversely, cOE mice showed a decreased level of hyperactivity compared with WT2 mice (Figure 6C, lower panel), suggesting reciprocal sensitivities for MK-801 in cKO and cOE mice.

Activation of NMDAR results in the upregulation of diverse downstream signaling events that are critical for synaptic function, such as phosphorylation of CaMKII α (calcium/calmodulin-dependent protein kinase II α) (54) and p38 MAPK (mitogen-activated protein kinase) (55). We examined the perturbation of NMDAR downstream signaling owing to alterations of NMDAR activity in cKO and cOE mice. The levels of phosphorylation for CaMKII α and p38 MAPK were higher in synaptosomes from hippocampi of cKO versus WT1 mice, but lower in cOE versus WT2 mice (Figure 6D).

Together, these results show that synaptic NMDAR function and downstream signaling are reciprocally impaired in opposite directions, depending on CYFIP1 levels in each mouse model.

Bidirectional Modulation of NMDAR Signaling Rescues Behavioral Abnormalities in cKO and cOE Mice

Imbalance in NMDAR signaling has been implicated in multiple neuropsychiatric disorders (51,52). Because our mouse models showed bidirectional dysfunction in NMDAR signaling on different *Cyfp1* dosages, we hypothesized that rebalancing NMDAR activity by reducing it in cKO mice and enhancing it in cOE mice may rescue the behavioral abnormalities in these models (Figure 7A). First, we treated cKO mice with the NMDAR antagonist memantine (56–59), which effectively normalized the augmented synaptic NMDAR signaling in cKO mice (Figure S6A). Indeed, memantine treatment rescued the



Altered CYFIP1 Doses Imbalance NMDAR in Mental Disorder

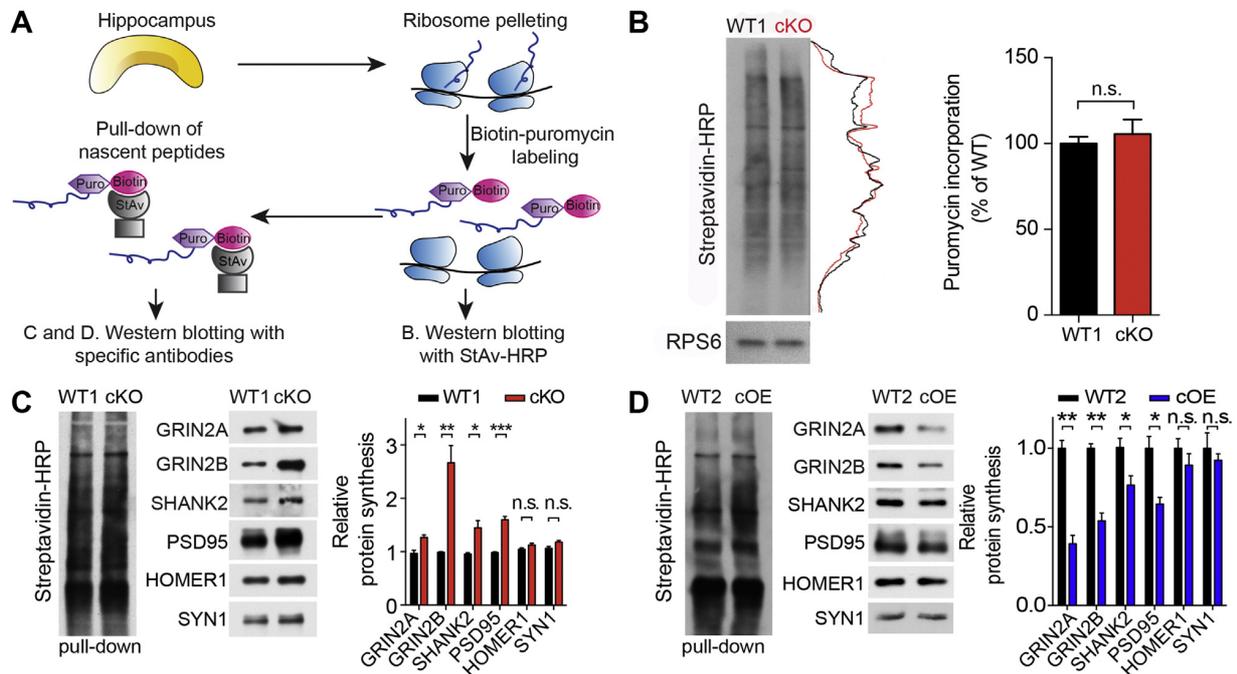


Figure 4. Translational regulation of messenger RNAs encoding the NMDA receptor complex by CYFIP1. **(A)** A schematic diagram of the experimental design. Ribosomal fractions from hippocampal lysates were pelleted by ultracentrifugation and then labeled with biotin-puromycin to release newly synthesizing peptides. Released biotin-puromycin-tagged nascent peptides were **(B)** directly applied to Western blotting with StAv-HRP to monitor the general translation rate or **(C, D)** pulled down with magnetic beads conjugated with streptavidin and applied to Western blotting with specific antibodies. **(B)** Normal general translation rate in cKO mice. Shown on the left is a representative immunoblot of total biotin-puromycin-labeled peptide detected by StAv-HRP. Vertical line traces of the HRP signal on each lane are shown on the right of the immunoblot. HRP signals were normalized with RPS6 signals and quantified. Values represent mean \pm SEM ($n = 3$ independent experiments, 3 animals per group in each experiment; n.s.: $p > .05$; Student's t test). **(C)** Protein synthesis for the NMDA receptor complex was increased in cKO mice and **(D)** reduced in cOE mice. Shown on the left are representative immunoblots of the total pulled-down nascent peptides detected by StAv-HRP. The same samples were applied to Western blotting with specific antibodies shown in the middle. Relative protein synthesis in cKO and cOE mice were normalized by StAv-HRP signals, quantified, and compared with WT1 and WT2 mice, respectively, shown on the right. Values represent mean \pm SEM ($n = 3$ independent experiments, 3 animals per group in each experiment; *** $p < .001$, ** $p < .01$; * $p < .05$; n.s.: $p > .05$; Student's t test). cKO, conditional knockout; cOE, conditional overexpression; HRP, horseradish peroxidase; n.s., not significant; StAv-HRP, HRP-conjugated streptavidin; WT, wild-type.

elevated behavioral despair of cKO mice in both the TST and FST (Figure 7B, C). In addition, the increased amphetamine-induced hyperactivity of cKO mice was also normalized with memantine treatment (Figure 7D). In contrast, impaired sensorimotor gating assessed by PPI in cKO mice was not improved with memantine treatment (Figure S6B).

Next, we took advantage of a partial agonist of NMDAR, D-cycloserine (DCS), which has been shown to rescue ASD-related behaviors in animal models with reduced NMDAR function (43,60). In the 3-chamber social interaction assay, reduced social interaction and impaired social novelty recognition of cOE mice was improved by the DCS treatment

Figure 3. RIP-seq experiments reveal mRNA targets of CYFIP1. **(A)** An experimental scheme for RIP-seq. **(B)** Protein-protein interaction network showing interactions between mRNA targets of CYFIP1 related to synapse, postsynaptic density, and NMDA receptor complex. q values: p value adjusted for false discovery rate using Benjamini-Hochberg procedure. **(C)** Representative coverage plots for mRNA targets of CYFIP1 (*Shank1*, *Shank2*, *Shank3*). Coverage plot for Homer1 (control, nontarget) is also shown for comparison. Read coverage is normalized by library size. Top panel shows coverage in the RIP library, middle panel shows the control library, and lower panel shows a representation of the genetic features of each mRNA. For genes with multiple transcripts, the longest one is shown. **(D)** GO analysis of CYFIP1 mRNA targets reveals enrichment for terms related to neuronal function and postsynaptic density. Disease ontology analysis shows enrichment for genes associated with mental disorders, including schizophrenia and autism spectrum disorder. **(E)** Association of the CYFIP1 protein with mRNAs encoding the NMDA receptor complex. Shown on the top is a representative immunoblot of the CYFIP1 protein pulled down by IP from hippocampal lysates. Shown on the bottom are quantitative PCR results from co-IPed mRNAs by anti-CYFIP1 antibody compared with co-IPed mRNAs by control IgG. Values represent mean \pm SEM ($n = 4$ independent experiments; *** $p < .001$; ** $p < .01$; * $p < .05$; Student's t test). **(F)** RIP-qPCR of human surgical cerebral cortex samples confirms that CYFIP1 binds its targets in the human brain. Values represent mean \pm SEM ($n = 3$ independent experiments; *** $p < .001$; ** $p < .01$; * $p < .05$; Student's t test). cDNA, complementary DNA; chr, chromosome; Cntl, control; FDR, false discovery rate; GO, Gene Ontology; HA, hemagglutinin; IP, immunoprecipitation; mRNA, messenger RNA; PCR, polymerase chain reaction; qPCR, quantitative PCR; RIP-seq, RNA immunoprecipitation sequencing.

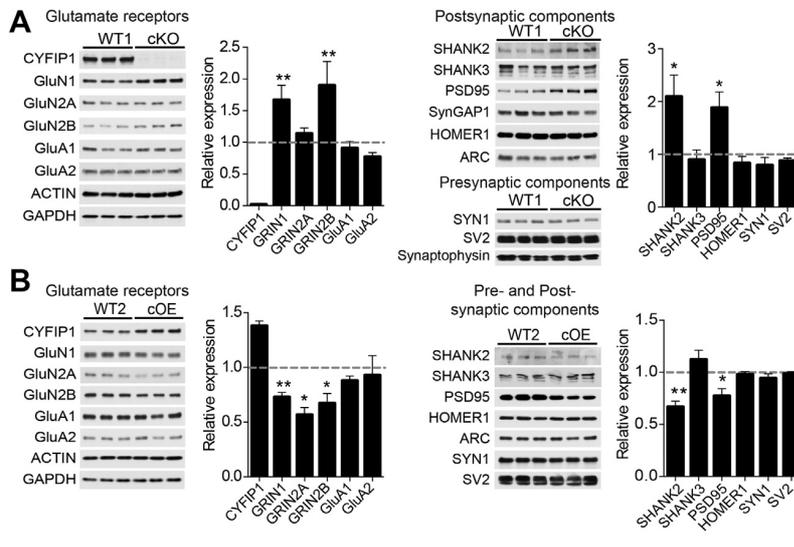


Figure 5. Altered postsynaptic protein expression in synapses with differential *Cyfip1* dosages. **(A)** Increased expression of the NMDA receptor subunits and associated complex in cKO mice. Shown are representative immunoblots of synaptosomal fractions from the hippocampal lysates of 3-month-old littermate WT1 and cKO mice. All data were normalized to ACTIN levels for the loading control, and then the protein expression levels of cKO mice were normalized to that of WT1 mice and plotted as relative changes of the expression level. Values represent mean \pm SEM ($n = 3-7$ animals; $**p < .01$; $*p < .05$; Student's *t* test). **(B)** Decreased expression of the NMDA receptor subunits and associated complex in cKO mice. Shown are representative immunoblots of synaptosomal fractions from the hippocampal lysates of 3-month-old littermate WT2 and cOE mice. All data were normalized to ACTIN levels for the loading control, and then the protein expression levels of cOE mice were normalized to that of WT2 mice and plotted as relative changes of the expression level. Values represent mean \pm SEM ($n = 3-6$ animals; $**p < .01$; $*p < .05$; Student's *t* test). cKO, conditional knockout; cOE, conditional overexpression; WT, wild-type.

(Figure 7E, F). In addition, the increased amphetamine-induced hyperactivity of cOE was restored by DCS treatment (Figure 7G). The repetitive behaviors of cOE mice assessed by the marble burying assay were not rescued by DCS treatment (data not shown).

In summary, bidirectional modulation to rebalance NMDAR function in cKO and cOE mice successfully rescued some behavioral abnormalities related to psychiatric disorders,

implying that dysregulation of the NMDAR complex by abnormal levels of CYFIP1 is responsible for the abnormal behaviors in our animal models.

DISCUSSION

Balanced action of molecular regulators is essential to maintain the homeostatic control of the brain. Hence, either loss or gain of molecular functions can be deleterious to the nervous

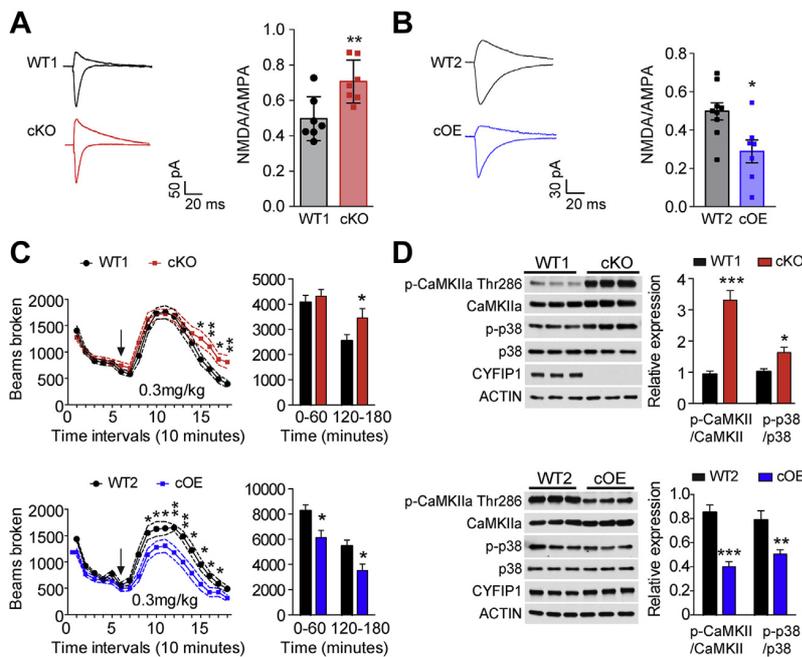


Figure 6. Reciprocal changes in NMDA receptor function in the *Cyfip1* cKO and cOE mice. **(A-B)** NMDA/AMPA ratio recorded from acute hippocampal slices of cKO and cOE mice. Shown on the left are sample recording traces. Shown on the right are summary of NMDA- or AMPA-mediated evoked synaptic current ratio. Values represent mean \pm SEM ($n = 7$ WT1, 7 cKO, 9 WT2, 7 cOE; $**p < .01$; $*p < .05$; Student's *t* test). **(C)** Reciprocal sensitivity on MK-801 administration in cKO (upper panel) and cOE (lower panel) mice. Shown on the left, MK-801 (0.3 mg/kg, i.p.) was injected after a 1-hour habituation, and the locomotor activity in an open field was presented by the number of beams broken every 10 minutes. Arrows represent the time of the MK-801 injection. Shown on the right, locomotor activity was quantified during the first hour and the last hour after MK-801 injection. Values represent mean \pm SEM ($n = 15$ WT1, 14 cKO, 9 WT2, 12 cOE; $**p < .01$; $*p < .05$; Student's *t* test). **(D)** Reciprocal alteration in NMDA receptor-mediated signaling in cKO and cOE mice. Shown on the left are representative immunoblots of the synaptosomal fraction from the hippocampal lysate of 3-month-old mice. Levels of phosphorylated CaMKII α and p38 were increased in cKO mice compared with WT1 mice but decreased in cOE mice compared with WT2 mice. Shown on the right are quantified levels of phosphorylated protein normalized to the amount of total protein.

Values represent mean \pm SEM ($n = 6$ animals; $***p < .001$; $**p < .01$; $*p < .05$; Student's *t* test). CaMKII α , calcium/calmodulin-dependent protein kinase II α ; cKO, conditional knockout; cOE, conditional overexpression; WT, wild-type.

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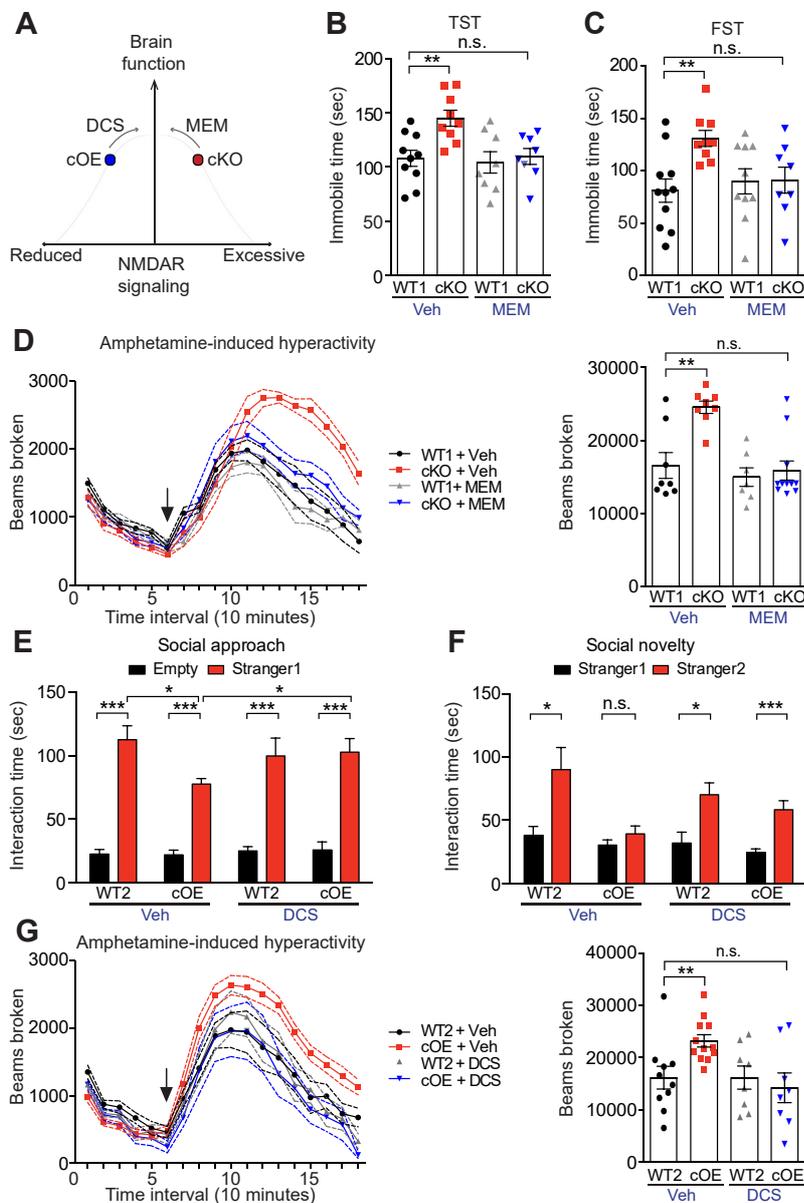


Figure 7. Treatment with memantine in cKO and D-cycloserine in cOE rescued behavioral abnormalities. **(A)** A model of NMDAR dysfunction with different levels of *Cyfp1* and pharmacological approaches to rebalance NMDAR signaling. **(B–C)** Behavioral despair in cKO mice was rescued by MEM but not VEH treatment. Immobile time in both the **(B)** TST and **(C)** FST was restored in cKO mice after memantine treatment to the level of WT1 mice. Values represent mean \pm SEM (TST: $n = 10$ WT1 + VEH, 9 cKO + VEH, 8 WT1 + MEM, 8 cKO + MEM; FST: $n = 11$ WT1 + VEH, 9 cKO + VEH, 10 WT1 + MEM, 8 cKO + MEM; ** $p < .01$; n.s.: $p > .05$; one-way analysis of variance). **(D)** The level of amphetamine-induced hyperactivity in cKO mice was restored to the level of WT1 mice after MEM treatment. Shown on the left is the trace of locomotor activity. An arrow represents the time of amphetamine injection. Shown on the right is the total number of beams broken after the amphetamine injection. Values represent mean \pm SEM. ($n = 8$ WT1 + VEH, 8 cKO + VEH, 7 WT1 + MEM, 11 cKO + MEM; ** $p < .01$; n.s.: $p > .05$; Student's t test). **(E–F)** Social impairments of cOE mice were improved after DCS treatment. **(E)** In the first stage, interaction time with stranger 1 by cOE mice after DCS treatment was significantly increased compared with cOE mice without treatment, similar to WT1 mice with or without treatment. **(F)** In the second stage, cOE mice showed a significant preference for stranger 2 after DCS treatment, whereas the nontreated cOE mice showed no preference for stranger 2. Values represent mean \pm SEM ($n = 7$ WT2 + VEH, 7 cOE + VEH, 7 WT2 + DCS, 7 cOE + DCS; *** $p < .001$; * $p < .05$; n.s.: $p > .05$; one-way analysis of variance). **(G)** The level of amphetamine-induced hyperactivity in cOE mice was restored to the level of WT2 mice after DCS treatment. Shown on the left is the trace of locomotor activity. An arrow represents the time of amphetamine injection. Shown on the right is the total number of beams broken after the amphetamine injection. Values represent mean \pm SEM ($n = 10$ WT2 + VEH, 12 cOE + VEH, 8 WT2 + DCS, 8 cOE + DCS; ** $p < .01$; n.s.: $p > .05$; one-way analysis of variance). cKO, conditional knockout; cOE, conditional overexpression; DCS, D-cycloserine; FST, forced swim test; MEM, memantine; NMDAR, NMDA receptor; n.s., not significant; TST, tail suspension test; Veh, vehicle; WT, wild-type.

system (8). CNVs associated with neuropsychiatric disorders are one of the mechanisms by which an altered gene dosage can cause the failure of neuronal homeostasis. Although genetic studies have revealed significant overlap of genetic risk factors among mental disorders, it is not clear how an altered dosage of the same gene may contribute to different brain disorders. In this study, we generated new mouse models with nervous system-specific loss- or gain-of-function of RNA-binding protein CYFIP1, a genetic risk factor both for SCZ and ASD. These mouse models displayed both distinct and shared behavioral abnormalities related to human mental disorders (Table S1). Notably, previous gain- or loss-of-function

CYFIP1 rodent models generated by different strategies showed both similar and distinct behavioral phenotypes compared with our models (25,28–30,32) (Table S1). To investigate the underlying molecular mechanism, we identified novel CYFIP1-associated mRNA targets related to synaptic function, postsynaptic density, and NMDAR complex by genome-wide RIP-seq analysis. Protein translation of CYFIP1 targets, including the NMDAR-associated complex, are diametrically altered on loss or gain of CYFIP1 function. As a result, protein levels of CYFIP1 targets at synapses are dysregulated without a change in their mRNA expression depending on the level of CYFIP1 in the mouse models. Our

study provides one explanation of how the action of an RNA-binding protein can modulate the balance of protein translation and levels at synapses, which is essential to maintain typical behavioral responses.

Abnormal protein synthesis disrupts synaptic function and neuronal networks underlying the pathophysiology of mental disorders. Accordingly, translational machinery and their regulatory components have been frequently identified as risk factors for mental disorders. For example, the mTOR pathway is strongly associated with ASD, and its perturbation in mouse models leads to the core phenotypes of ASD (61–63). Exaggerated cap-dependent protein translation causes synaptic and behavioral abnormalities associated with ASD (64,65), suggesting that aberrant general protein translation is an important molecular process for ASD development. In contrast, recent studies showed that SCZ patient-derived neural cells exhibit dysregulated protein translation and translational machinery, suggesting that translational control may be involved in the disease progression of SCZ (66,67). However, molecular regulators that may explain the dysregulation of protein translation in both SCZ and ASD are unclear. In our study, we identified a set of novel mRNA targets interacting with CYFIP1 that are related to synaptic function. We also found that the protein levels of CYFIP1 targets at synapses are diametrically regulated, depending on the level of CYFIP1. Intriguingly, the impact appears to be specific to CYFIP1 targets as the total protein levels of AMPA receptors and Homer1 do not show compensatory changes. Further pharmacological rescue experiments with NMDAR modulators suggest that the behavioral phenotypes related to SCZ and ASD with loss or gain of CYFIP1 function are, at least in part, caused by the abnormal protein synthesis of CYFIP1 targets, including the NMDAR complex. This is the first piece of evidence suggesting that a regulator of protein translation of specific target genes may diametrically contribute to the pathophysiology of both SCZ and ASD.

NMDARs exhibit a critical role in synaptic transmission and plasticity. Human genetic studies revealed that mutations of NMDARs are strongly implicated in the etiology of both SCZ and ASD (68–71). In addition, the expression of NMDARs and their associated complex are frequently altered in human patients with SCZ (72,73). An imbalance of NMDAR signaling in mouse models results in SCZ- and ASD-like phenotypes that can be rescued by pharmacological manipulation of NMDARs (43,56,74–76). Therefore, protein expression levels of the NMDAR subunits and their associated complex must be tightly controlled to maintain normal synaptic transmission and behavior. In this study, loss or gain of CYFIP1 function in mouse models led to imbalanced NMDAR signaling with aberrant translational control of CYFIP1 target genes. A subset of the behavioral abnormalities related to SCZ and ASD, such as behavioral despair, impaired social interaction, and exaggerated amphetamine-induced hyperactivity, were restored by balancing the altered NMDAR activities pharmacologically in the adult. However, other behavioral abnormalities, including impaired PPI in cKO mice and repetitive behavior in cOE mice, were not rescued (Figure S6B and data not shown). A recent study suggested that CYFIP1 regulates GABAergic (gamma-aminobutyric acidergic) neurotransmission at inhibitory synapses to maintain an excitatory and inhibitory balance (27), implying that these behavioral abnormalities might be caused

by dysregulation of CYFIP1 targets related to GABA receptor signaling, such as *Gabbr2*, *Slc6a1*, and *Slc6a11*. In addition, some behavioral phenotypes might be caused by functions of CYFIP1 in cytoskeleton remodeling (22,49,77). Alternatively, CYFIP1 may regulate neuronal developmental processes that are not restored by the later pharmacological treatments used in this study. It remains to be investigated whether other psychotic drug treatments may rescue some of the behavioral phenotypes in these mouse models.

Although it is not straightforward to directly correlate mouse behavioral phenotypes with patient symptoms, our study provides systematic *in vivo* evidence on how abnormal levels of CYFIP1 lead to common and distinct defects associated with SCZ and ASD at the biochemical, synaptic, and behavioral levels. Future research on other CNV risk factors for various psychiatric disorders will further highlight the homeostatic control of molecular processes governing synaptic function and behavior.

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N-SK and K-JY generated the cKO and cOE mice and performed the behavioral and biochemical analyses. FRR and SC contributed to RIP-seq and analysis. YZ, WL, and KMC contributed to behavioral analysis. HNN contributed to iPSC experiments. SJT, Y-TL, and K-sH contributed to electrophysiological recording. SE, VLD, and TMD contributed to sucrose gradient experiments. BX and PW contributed to cOE mouse line generation. N-SK, K-JY, HS, and G-IM conceived the project and wrote the manuscript with inputs from all authors.

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ARTICLE INFORMATION

From the Department of Neuroscience (N-SK, SJT, KMC, K-JY, HS, G-IM), Mahoney Institute for Neurosciences; Department of Cell and Developmental Biology (HS, G-IM); Institute for Regenerative Medicine (HS, G-IM); Epigenetics Institute (HS); Department of Psychiatry (G-IM), Perelman School for Medicine, University of Pennsylvania, Philadelphia, Pennsylvania; Institute for Cell Engineering (FRR, YZ, HNN, Y-TL, SE, VLD, TMD); Solomon H. Snyder Department of Neuroscience (VLD, TMD, BX, PW), Johns Hopkins University School of Medicine, Baltimore, Maryland; Gene Center (FRR, SC), Ludwig-Maximilians-Universität München, Munich, Germany; Department of Pharmacology (K-sH), College of Medicine, National Cheng Kung University, Tainan, Taiwan; and Bio-X Institutes (WL), Key Laboratory for the Genetics of Development and Neuropsychiatric Disorders, Shanghai Jiao Tong University, Shanghai, China.

N-SK and K-JY are currently affiliated with the Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, South Korea.

BX is currently affiliated with the Department of Biology, Southern University of Science and Technology, Shenzhen, Guangdong, China.

N-SK and FRR contributed equally to this work.

Address correspondence to Guo-li Ming, M.D., Ph.D., at gming@pennmedicine.upenn.edu, or Ki-Jun Yoon, Ph.D., at kijunyoona@kaist.ac.kr.

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