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Priority Communication

Persistently Elevated mTOR Complex 1-S6 Kinase 1 Disrupts DARPP-32–Dependent D_1 Dopamine Receptor Signaling and Behaviors

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ABSTRACT

BACKGROUND: The serine-threonine kinase mTORC1 (mechanistic target of rapamycin complex 1) is essential for normal cell function but is aberrantly activated in the brain in both genetic-developmental and sporadic diseases and is associated with a spectrum of neuropsychiatric symptoms. The underlying molecular mechanisms of cognitive and neuropsychiatric symptoms remain controversial.

METHODS: The present study examines behaviors in transgenic models that express Rheb, the most proximal known activator of mTORC1, and profiles striatal phosphoproteomics in a model with persistently elevated mTORC1 signaling. Biochemistry, immunohistochemistry, electrophysiology, and behavior approaches are used to examine the impact of persistently elevated mTORC1 on D_1 dopamine receptor (D1R) signaling. The effect of persistently elevated mTORC1 was confirmed using D1-Cre to elevate mTORC1 activity in D1R neurons.

RESULTS: We report that persistently elevated mTORC1 signaling blocks canonical D1R signaling that is dependent on DARPP-32 (dopamine- and cAMP-regulated neuronal phosphoprotein). The immediate downstream effector of mTORC1, ribosomal S6 kinase 1 (S6K1), phosphorylates and activates DARPP-32. Persistent elevation of mTORC1-S6K1 occludes dynamic D1R signaling downstream of DARPP-32 and blocks multiple D1R responses, including dynamic gene expression, D1R-dependent corticostriatal plasticity, and D1R behavioral responses including sociability. Candidate biomarkers of mTORC1–DARPP-32 occlusion are increased in the brain of human disease subjects in association with elevated mTORC1-S6K1, supporting a role for this mechanism in cognitive disease.

CONCLUSIONS: The mTORC1-S6K1 intersection with D1R signaling provides a molecular framework to understand the effects of pathological mTORC1 activation on behavioral symptoms in neuropsychiatric disease.

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The serine-threonine kinase mTOR (mechanistic target of rapamycin) is a conserved signaling hub that integrates extracellular and environmental inputs to coordinate cell growth and metabolism (1). Aberrant mTOR complex 1 (mTORC1) signaling is implicated in human brain diseases (2,3). Hyperactive mTORC1 caused by loss-of-function mutations of mTOR upstream suppressor genes, such as *TSC1/2* and *PTEN*, manifests as a high rate of epilepsy, cognitive impairment, and autism spectrum disorder (4,5). Reportedly mTORC1 signaling is also dysregulated in sporadic diseases associated with cognitive and behavioral symptoms, including schizophrenia and neurodegenerative diseases (6–8).

Genetic deletion of mTORC1 signaling suppressor genes *Tsc1*, *Tsc2*, or *Pten* in the mouse model results in behavioral deficits that can be related to human disease; an mTORC1 inhibitor, rapamycin, can ameliorate certain developmental and behavioral deficits in these models (9–11). Studies have suggested that elevated mTORC1 causes "exaggerated" protein synthesis that alters the composition of synapses, resulting in "hyperconnectivity" and reduced magnitude of mGluR-LTD (metabotropic glutamate receptor-mediated long-term depression) (12–14). These effects are thought to be causal for behavioral deficits because they can be mitigated by manipulations that counter mTORC1 actions on cap-dependent protein synthesis, or by treatment with a positive allosteric modulator of mGluR5 (13–15). However, the notion that phenotypes are directly linked to mTORC1 activation is challenged by the observation that deletion of *Rptor*, which encodes Raptor, an essential component of mTORC1, fails to restore sociability in *Pten* knockout mice despite restoration of mTORC1 activity (16). Moreover, the notion that elevated mTORC1 causes behavioral deficits by exaggerated protein translation is challenged by the observation that mice with neuronal overexpression of eIF4E, an effector of mTORC1 translation initiation, do not exhibit social behavior deficits (17). Further, deletion of *Rictor*, an

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essential component of mTORC2, disrupts mGluR-LTD, while deletion of *Rptor*, essential for mTORC1, does not (18). Finally, knockout of *Rictor* mitigates behavioral deficits in *Pten* knockout mice (16).

To examine how mTORC1 signaling impacts behavior we considered the possibility that genetic deletion of *Tsc1*, *Tsc2*, or *Pten* may impact cellular functions in addition to mTORC1 or cause gene-specific adaptations. Accordingly, we examined transgenic models that express Rheb, the most proximal known activator of mTORC1. Rheb is a small GTPase that directly binds mTORC1 and is obligate for mTORC1 activation (19). We compared behaviors in identically designed mouse models that express either wild-type Rheb [Rheb(WT)], which does not result in significant elevation of mTORC1 owing to preservation of regulatory mechanisms that control its GTP state, or a point mutant of Rheb [Rheb(S16H)], which causes a persistent GTP-bound state and increases activation of mTORC1 (20,21).

This analysis revealed cognitive and behavioral deficits consequent to elevated mTORC1 activity in the brain. An unbiased phosphoproteomic analysis of striatum from mice with elevated mTORC1 activity revealed increased D_1 dopamine receptor (D1R) signaling with elevated basal DARPP-32(pT34) (dopamine- and cAMP-regulated neuronal phosphoprotein). Further analysis revealed that mTORC1-S6K1 directly phosphorylates DARPP-32(T34) and activates its downstream signaling. However, dynamic D1R signaling downstream of DARPP-32(pT34) was blocked, including D1R-dependent gene expression, synaptic plasticity, and D1R-induced locomotor activation. Building on the observation that D1R-expressing neurons encode and mediate sociability (22), we confirmed that persistently elevated mTORC1 in D1R neurons produces profound sociability deficits. Finally, we examined human postmortem brain from subjects with Alzheimer's disease (AD) or tuberous sclerosis (TS) and found correlations between elevated mTORC1 activity and DARPP-32 signaling, suggesting that disruption of DARPP-32–dependent D1R signaling may contribute to behavioral symptoms in these diseases.

METHODS AND MATERIALS

Detailed methods and materials are available in Supplement 1. Rosa-Myc-Rheb(S16H) and Rosa-Myc-Rheb(WT) mice were generated as described elsewhere (21). In this study, Rheb(S16H) refers to *Rosa-Myc-Rheb(S16H)$^{f/f}$;Nestin-Cre* and D1-Rheb(S16H) refers to *Myc-Rheb(S16H)$^{f/f}$;D1-Cre*. Littermate controls (Ctrl) for Rheb(S16H) and D1-Rheb(S16H) are *Rosa-Myc-Rheb(S16H)$^{f/f}$*. *Rosa-Myc-Rheb(WT)$^{f/f}$;Nestin-Cre* is referred to as Rheb(WT) and *Rosa-Myc-Rheb(WT)$^{f/f}$* is referred to as Ctrl.

Mice at 6 weeks of age were sacrificed, and their brains were quickly removed. The brains were mounted and frozen at −80°C until sectioned for immunohistochemistry (IHC), or the striatum was dissected for Western blot (WB) analysis or phosphoproteomics. All procedures were approved by the Animal Care and Use Committee of Johns Hopkins University School of Medicine. Data are shown as mean ± standard error of the mean and were analyzed using GraphPad Prism (GraphPad Software, San Diego, CA) or Statistica 13.3 (TIBCO Software, Palo Alto, CA). The statistical methods are detailed in the figure legends.

RESULTS

Mouse Model With Behavioral Deficits Resulting From Persistent Elevation of mTORC1 Activity

Transgenic mice expressing Myc-Rheb(S16H) in the brain exhibit elevation of mTORC1, as revealed by increased phosphorylated 4E-BP1(T37/46), S6K1(T389), and S6(S240/244) in striatal lysate (Figure 1A–D). In Rheb(WT) mice, mTORC1 activity is not robustly elevated (Figure 1C, D). IHC staining confirmed an increase in mTORC1-S6K1 activity in the striatum of Rheb(S16H) mice, as indicated by increased S6(pS240/244). This was evident both in D1R-expressing neurons, identified by colabeling with substance P (positive), and in presumptive D2R-expressing neurons (substance P negative) (Figure S1A, B in Supplement 1).

We examined the impact of mTORC1 activation on sociability using a three-chamber social-motivation task. Rheb(S16H) and Ctrl littermates preferred investigating the social object; however, Rheb(S16H) mice investigated the social object significantly less than their Ctrl littermates (Figure 1E). To ascertain the reduced social investigation results from hyperactive mTORC1, we tested Rheb(WT) in the same task and found that these animals exhibited a preference for social object similar to their Ctrl littermates (Figure 1F).

The sociability deficit in Rheb(S16H) mice was further confirmed in a four-trial social recognition task. Rheb(S16H) mice spent less time interacting with juveniles during the habituation stage (trials 1–3). During the dishabituation stage (trial 4), Rheb(S16H) mice exhibited reduced interaction to new juveniles (Figure 1G). In the same task, Rheb(WT) and their Ctrl mice showed expected and similar habituation and dishabituation performances (Figure 1H). Although Rheb(S16H) exhibited higher exploratory activity when compared with Rheb(WT), Rheb(S16H) or Rheb(WT) mice exhibited similar levels of novelty-induced exploration in an open field as their littermate controls, suggesting that the impaired sociability in Rheb(S16H) was not caused by depressed explorative activity (Figure S1C, D in Supplement 1).

In a novel object recognition task, Rheb(S16H) and Ctrl mice spent a similar amount of time exploring objects during the learning session when two identical objects were presented (Figure 1I). However, in the testing session after a novel object replaced one of the familiar objects, Rheb(S16H) failed to distinguish between these objects (Figure 1J). These data validate the Rheb(S16H) mouse as a model of sociability and cognitive deficits resulting from persistent elevation of mTORC1 activity.

Persistent Elevation of mTORC1 Activity Causes Overrepresentation of D1R Signaling

To assess the molecular basis of behavioral deficits linked to mTORC1 activation, we performed an unbiased quantitative phosphoproteomic analysis of Rheb(S16H) and focused on striatum because of its prominent role in social behaviors (22,23). Among >7000 phosphopeptides quantified, ~1600 phosphopeptides were significantly altered (Figure 2A; Table S1 in Supplement 2). The motif-X analysis (https://motif-

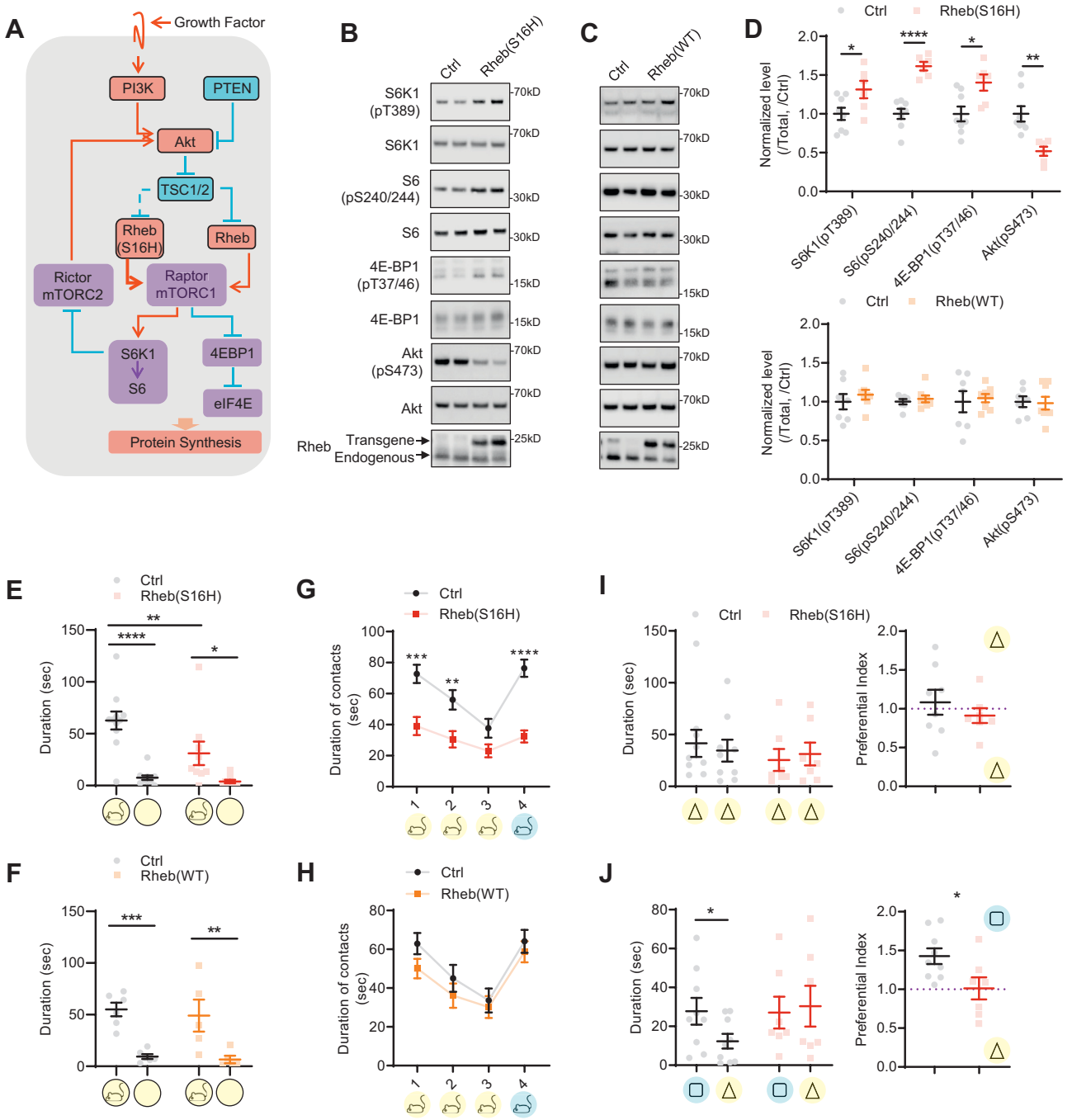


Figure 1. Persistent activation of mTORC1 impairs social and cognitive behavioral domains. **(A)** Schematic figure of canonical growth factor signaling activating mTORC1. Rheb acts as a direct and most proximal activator for mTORC1. **(B, C)** Representative blots and **(D)** quantification of the Western blot for the phosphorylation levels of S6K1, S6, 4E-BP1, and Akt in the striatum lysate of **(B)** Rheb(S16H), **(C)** Rheb(WT), and their Ctrl littermates, respectively. $^{*}p < .05$, $^{**}p < .05$, and $^{****}p < .0001$ by unpaired *t* test; $n = 8$ for Ctrl and $n = 6$ for Rheb(S16H). $n = 7$ for Ctrl, and $n = 8$ for Rheb(WT). **(E)** The total time spent investigating a social or nonsocial object by Rheb(S16H) mice and their Ctrl littermates during a social motivation task. Statistical significance was determined by two-way mixed-design ANOVA: genotype, $^{*}p = .0229$; objects, $^{****}p < .0001$; Fisher's post hoc test: Ctrl, social vs. empty, $^{****}p < .0001$; Rheb(S16H), social vs. empty, $^{*}p = .0213$; social, Ctrl vs. Rheb(S16H), $^{**}p = .0035$; $n = 11$ for Ctrl, and $n = 9$ for Rheb(S16H). **(F)** The total time spent investigating the social or nonsocial object by Rheb(WT) mice and their Ctrl littermates during the social motivation task. Statistical significance was determined by two-way mixed-design ANOVA: objects, $^{****}p < .0001$; genotype, $p = .6622$; and interaction, $p = .8124$; Fisher's post hoc test: Ctrl, social vs. empty, $^{***}p = .0006$; Rheb(S16H), social vs. empty, $^{*}p = .0024$; $n = 6$ for Ctrl, and $n = 5$ for Rheb(WT). **(G)** The duration of social investigation spent by the Rheb(S16H) mice and the Ctrl littermates during the social recognition task. Two-way mixed-design ANOVA followed by Fisher's post hoc test: time, $^{***}p = .0002$; trials, $^{**}p = .0014$; Ctrl vs. Rheb(S16H), trial 1, $^{***}p = .0001$; trial 2, $^{**}p = .0023$; and trial 4, $^{****}p < .0001$; Ctrl, trial 3 vs. trial 4, $^{****}p < .0001$; Rheb(S16H), trial 3 vs. trial 4, $p = .0814$. Data

x.med.harvard.edu/motif-x.html) of upregulated phosphosites revealed that the most highly enriched motifs corresponded to the phosphorylation target sites of mTOR kinase, TP(pT), and S6K1, RxxS(pS) (Figure 2B) (24,25). We verified the enrichment of phosphorylated motifs with antibodies selective for RxxS/T(pS/T), RRxS/T(pS/T), and LxRxxS/T(pS/T) in striatal lysate of Rheb(S16H) (Figure S2A–D in Supplement 1).

Among the overrepresented motifs, we noted that the RRxS(pS) epitope is shared with protein kinase A (PKA). D1R-activated PKA phosphorylates a panel of substrates, including DARPP-32, to amplify and sustain its effect (26). Putative D1R-targeted phosphopeptides (27) from Rheb(S16H) striatum exhibited enrichment in the group of globally upregulated sequences (Figure 2C). The cumulative distribution of the fold change of these D1R-targeted phosphopeptides exhibited a significant shift toward upregulation when compared with overall phosphopeptides at the same level of abundance (Figure 2D). We then extracted the differentially expressed phosphopeptides, and we input their annotated genes into ingenuity pathway analysis and Kyoto Encyclopedia of Genes and Genome (KEGG) pathway analysis. Both analyses highlighted a set of enriched pathways that are notable for their role in dopamine receptor signaling (Figure 2E, F; Table S2 in Supplement 2).

Persistent Elevation of mTORC1 Increases DARPP-32(pT34) and Its Nuclear Localization

Among the enriched pathways, DARPP-32 signaling was highlighted. D1R-activated PKA phosphorylates DARPP-32 at T34 and indirectly causes dephosphorylation of DARPP-32(T75) via B56/PP2A. In response to psychostimulants, levels of DARPP-32 phosphorylation at T34 and T75 are reciprocally dynamic (28). DARPP-32(pT34) potently inhibits PP1 (protein phosphatase 1), which increases MAPK (mitogen-activated protein kinase) signaling to amplify D1R signaling (Figure 3A) (28–31). We observed reductions of two phosphopeptides corresponding to DARPP-32(pT75) in the Rheb(S16H) striatal phosphoproteomic dataset (Figure S2E, F in Supplement 1), and we confirmed this finding together with the elevation of DARPP-32(pT34) in Rheb(S16H) striatum by WB (Figure 3B, C). This elevation did not appear to be due to hyperactive PKA because Rheb(S16H) did not alter basal cAMP (cyclic adenosine monophosphate) or the PKA substrate GluA1(pS845) (Figure 3B, C). DARPP-32(pT34) was not increased in the striatum of Rheb(WT) (Figure 3B, C).

To evaluate the prediction that elevated mTORC1 increases DARPP-32 activation, we examined DARPP-32(pT34) nuclear entry (32). Biochemical fractionation confirmed that DARPP-32 is present in both cytoplasmic and nuclear fractions (Figure S3D in Supplement 1) and further demonstrated that both total and phosphorylated forms of DARPP-32 are increased in the soluble nuclear fraction of Rheb(S16H) striatum compared with Ctrl littermates (Figure 3D, E). The nuclear enrichment of DARPP-32(pT34) was further confirmed by IHC, which demonstrated elevated DARPP-32(pT34) in both substance P–positive and –negative neurons (Figure 3F, G). IHC revealed an increase in foci number and intensity of histone H3(pS10) in the nucleus accumbens (NAc) of Rheb(S16H) mice and was confirmed by WB of histone H3(pS10) in the chromatin-bound biochemical fraction of Rheb(S16H) striatum (Figure 3H–L).

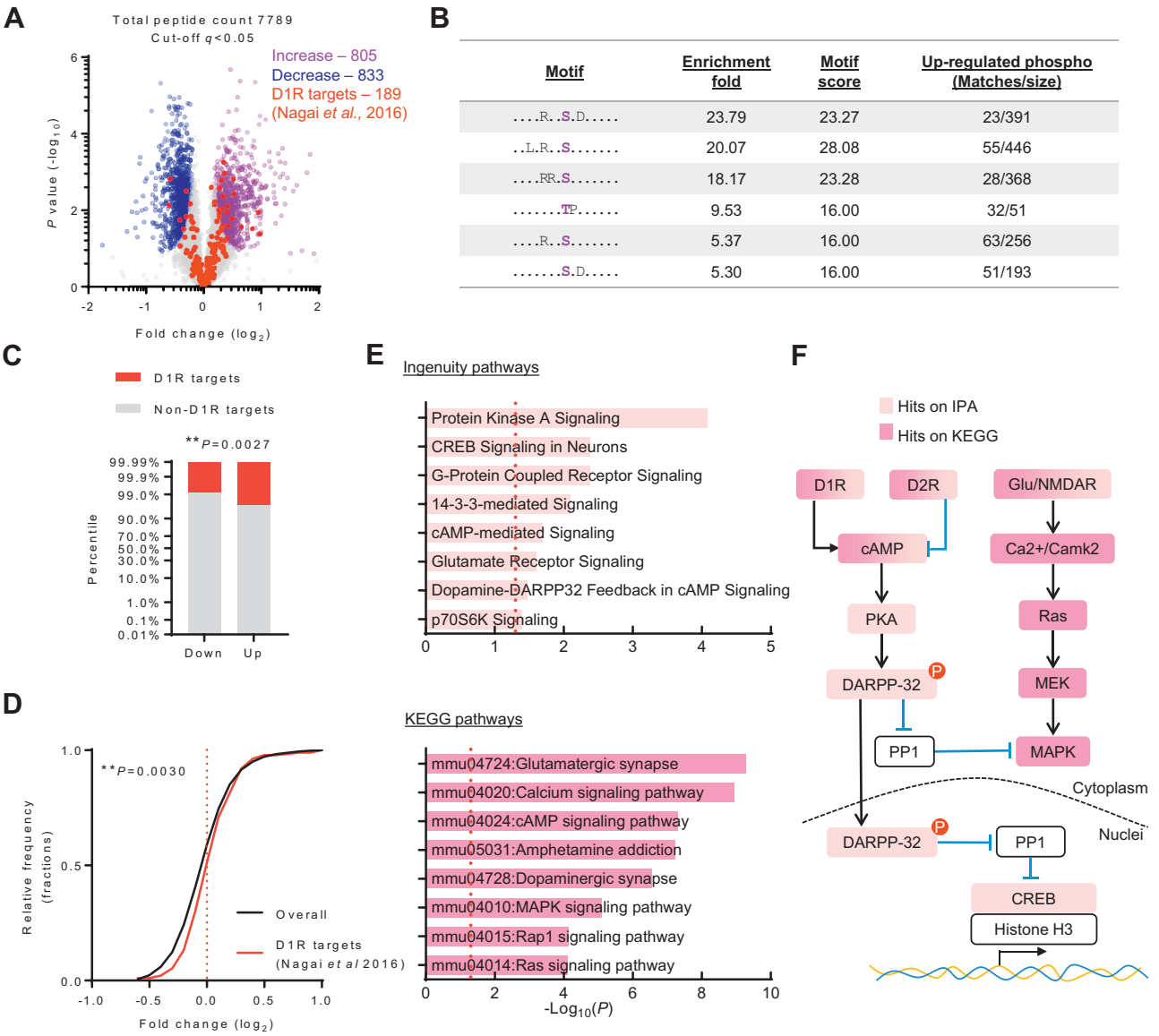
S6K1 Directly Phosphorylates DARPP-32

DARPP-32(T34) conforms to the conserved S6K1 phosphorylation consensus motif RxRxxT (Figure 4A) (25). Using an in vitro kinase assay, we found that constitutively active S6K1 phosphorylates recombinant GST-DARPP-32(T34), as revealed by WB with antibodies specific to DARPP-32(pT34) and to phospho-motif RxRxxS/T(pS/T). Multiple negative controls included deletion of amino acids 1–80 and point mutant T34A (Figure 4B). Additionally, S6K1 inhibitors PF-4708671 or LY-2584702 completely blocked phosphorylation, as did an inactive analogue of ATP, AMP-PNP (Figure S4A in Supplement 1).

To test whether S6K1 can phosphorylate DARPP-32 in cells, we cotransfected DARPP-32 with S6K1 in HEK293FT cells. DARPP-32(pT34) was robustly elevated when coexpressed with constitutively active S6K1(E389ΔCT), compared with coexpression with either WT or inactive mutant S6K1(F5A). The activity of S6K1 WT and mutants was confirmed by the phosphorylation of its immediate substrate S6(S240/244). The efficacy of S6K1(WT) to phosphorylate DARPP-32(pT34) was markedly enhanced by coexpression of Rheb(S16H) (Figure 4C, D). Rheb(S16H)-driven hyperphosphorylation of DARPP-32(T34) was reduced by PF-4708671 or LY-2584702, as well as by an AGC kinase inhibitor H89, but was not reduced by the p90RSK inhibitor SL0101-1 (Figure S4B, C in Supplement 1).

To test whether elevated basal DARPP-32(pT34) in Rheb(S16H) striatum is caused by hyperactive mTORC1-S6K1, we treated Rheb(S16H) mice with PF-4708671 (50 mg/kg, intraperitoneal [i.p.]). We confirmed a previous report that a single dose of PF-4708671 acutely inhibits S6K1 activity (33), as indicated by a reduction of S6(pS240/244) and concurrent increase of S6K1(pT389) in the striatum of Rheb(S16H) mice. Elevated DARPP-32(pT34) in Rheb(S16H) was reduced by acute PF-4708671 treatment, while

are presented as mean ± SEM; $n = 11$ for Ctrl, and $n = 10$ for Rheb(S16H). **(H)** The duration of social investigation spent by the Rheb(WT) and Ctrl littermates during the social recognition task. Two-way mixed-design ANOVA, trial, $^{****}p < .0001$; genotype, $p = .3092$; and interaction, $p = .4736$. Data are presented as mean ± SEM; $n = 10$ for Ctrl, and $n = 12$ for Rheb(WT). **(I, J)** a Novel object recognition task. (Left) Time spent on exploring each object, and (right) preferential index are shown during the **(I)** learning session and **(J)** testing session by Rheb(S16H) mice and the Ctrl littermates. Preferential index is calculated as the ratio for time spent investigating **(I)** object 1 or **(J)** a novel object to total spent investigating. Statistical significance was determined by two-way ANOVA followed by Bonferroni's post hoc test: **(J)** left, interaction, $^{*}p = .0358$; Ctrl, novel vs. familiar, $^{*}p = .0199$; right, unpaired t test, $^{*}p = .0208$; $n = 7$ for Rheb(S16H), and $n = 9$ for Ctrl littermates. Mean ± SEM and/or individual data are presented. ANOVA, analysis of variance; Ctrl, control; mTORC1, mTOR complex 1; WT, wild-type.



Motif	Enrichment fold	Motif score	Up-regulated phospho (Matches/size)
....R..S.D.....	23.79	23.27	23/391
..L.R..S.....	20.07	28.08	55/446
....RR.S.....	18.17	23.28	28/368
.....TP.....	9.53	16.00	32/51
....R..S.....	5.37	16.00	63/256
.....S.D.....	5.30	16.00	51/193

Figure 2. Phosphoproteomic analysis reveals overrepresentation of D1R-DARPP-32 signaling in the Rheb(S16H) striatum. **(A)** Volcano plot depicting the relative phosphopeptide abundance in the Rheb(S16H) striatum compared to Ctrl littermates. **(B)** Motif-X analysis was used to reveal the enriched phosphorylation motifs in the identified phosphopeptides that were significantly ($q < .05$) increased in the striatum of Rheb(S16H) mice compared with Ctrl littermates. **(C)** Comparison of phosphopeptides regulated by D1R activation with robustly altered ($q < .05$) expression in Rheb(S16H) striatum vs. of Ctrl littermates: 833 phosphopeptides were downregulated in the Rheb(S16H) striatum, 7 of which were D1R targets, and 805 phosphopeptides were upregulated in the Rheb(S16H) striatum, 24 of which were D1R targets. There were significantly more D1R targets in the upregulation group than in the downregulation group. $^{**}p = .0027$ by χ^2 test with Yate's correction. **(D)** The cumulative distribution of D1R targets compared with the distribution of total differentially altered phosphopeptides with the same level of abundance in the Rheb(S16H) striatum phosphoproteomic dataset. $^{**}p = .0030$ value was determined by Kolmogorov–Smirnov test. **(E)** (Upper) Shortlisted IPA and (lower) KEGG pathway analysis on genes with robustly altered phosphopeptide levels ($q < .05$). **(F)** Schematic signaling diagram showing the positive hits in IPA and KEGG analysis in D1/2R- and NMDAR- mediated dopamine signaling. cAMP, cyclic adenosine monophosphate; Ctrl, control; D1R, D_1 receptor; IPA, Ingenuity Pathway Analysis; KEGG, Kyoto Encyclopedia of Genes and Genome; NMDAR, NMDA receptor.

GluA1(pS845) was not altered, suggesting that the reduction of DARPP-32(pT34) was caused by PF-4708671 inhibition of S6K1, instead of off-target inhibition of PKA (Figure 4E, F). Taken together, these data indicate that DARPP-32 is a substrate of mTORC1-S6K1 in vivo.

mTORC1-S6K1 Occludes Dynamic DARPP-32 Phosphorylation In Vitro and In Vivo

To model the impact of persistently elevated mTORC1-S6K1 on DARPP-32 phosphorylation, we reconstituted S6K1–

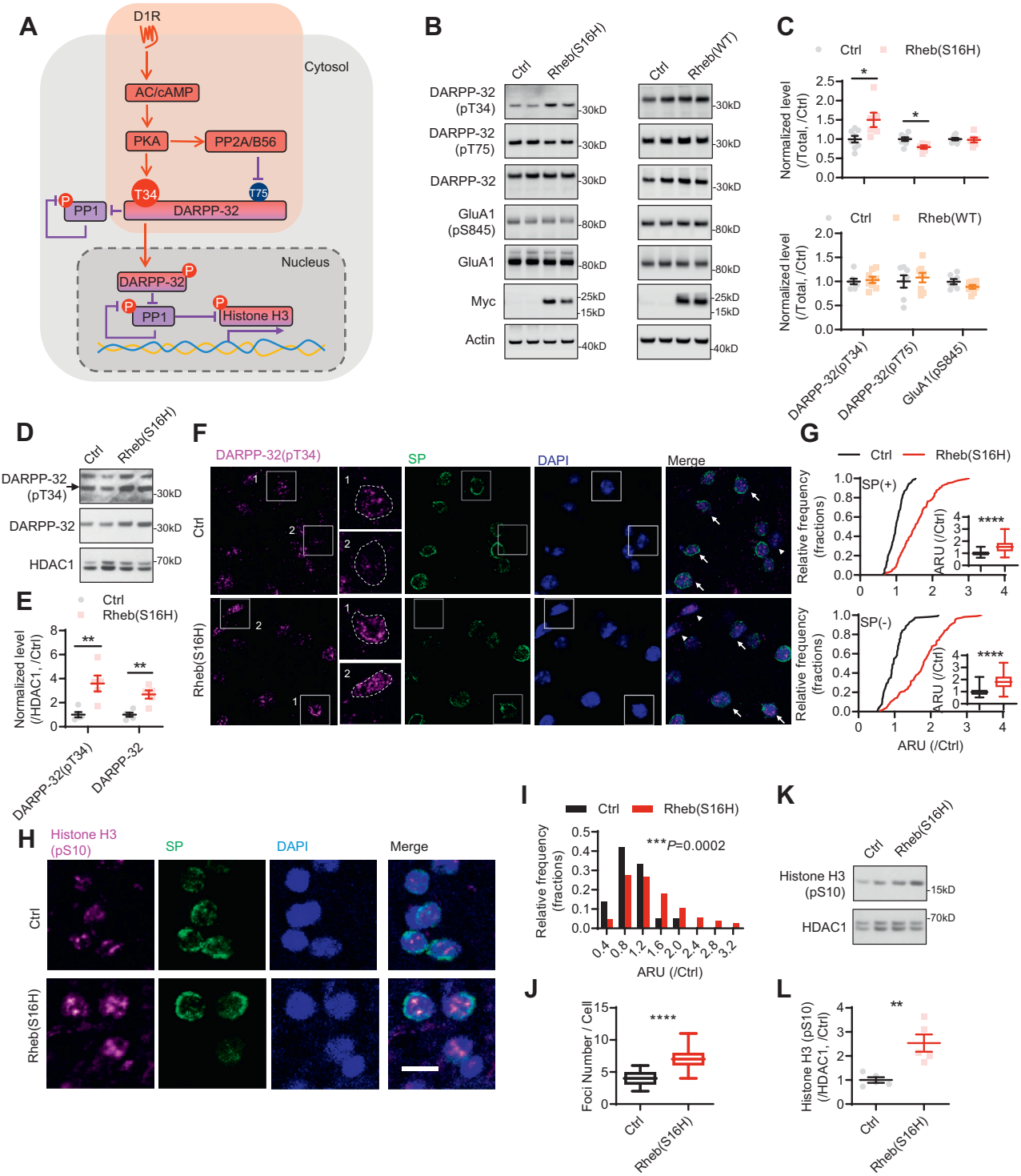


Figure 3. Elevated basal DARPP-32 signaling in the Rheb(S16H) striatum. **(A)** Schematic figure of canonical D1R–DARPP-32 signaling and its action on nucleosome response. **(B)** Representative blots and **(C)** quantification of the phosphorylation levels of DARPP-32(pT34/pT75) and GluA1(pS845) in the striatum lysate of Rheb(S16H) mice or Rheb(WT) mice, and their Ctrl littermates, respectively. $n = 8$ for Ctrl, and $n = 6$ for Rheb(S16H); $n = 7$ for Ctrl, and $n = 8$ for Rheb(WT). $^{*}p < .05$ was determined by unpaired t test. **(D)** Representative blots and **(E)** quantification of the phosphorylated and total form of DARPP-32 in the nuclear soluble fraction of the striatum of Rheb(S16H) mice and Ctrl littermates. $^{**}p < .01$ by unpaired t test; $n = 5$ for each genotype. **(F)** Representative images and **(G)** quantification of the immunohistochemistry of DARPP-32(pT34) in NAc of Rheb(S16H) mice and Ctrl littermates. Magenta channel shows DARPP-32(pT34), green channel shows SP, and blue channel shows DAPI-stained nuclei. SP-positive cells are indicated by white arrows, and SP-negative cells

DARPP-32 in HEK293FT cells and treated the cells with forskolin, which activates adenylyl cyclase to produce cAMP, or isoproterenol, which activates endogenous Gαs/adenylyl cyclase/PKA signaling. Cells transfected with DARPP-32 and S6K1 showed marked increases of DARPP-32(pT34) and PP1α(pT320) upon stimulation with forskolin. By contrast, cells transfected with Rheb(S16H), DARPP-32, and S6K1 showed elevated basal DARPP-32(pT34) and PP1α(pT320) but diminished responses of these phosphosites upon forskolin stimulation (Figure S5A, B in Supplement 1). Cotransfection of constitutively active S6K1(E389ΔCT) with DARPP-32 similarly reduced dynamic phosphorylation of DARPP-32(T34) by isoproterenol. The observed effect of elevated mTORC1-S6K1 to mimic Gαs G protein–coupled receptor or adenylyl cyclase activation and increase DARPP-32 phosphorylation yet block dynamic increases indicates occlusion of DARPP-32 signaling (Figure S5C, D).

To examine the effect of persistently elevated mTORC1 on DARPP-32 signaling in vivo, we treated Rheb(S16H) mice and their Ctrl littermates with amphetamine (3 mg/kg, i.p.). Striatum tissues were collected 20 minutes after treatment to capture the immediate activation of D1R-DARPP-32 signaling (Figure 5A) (28,29). Amphetamine induced similar levels of dopamine release and cAMP production in Rheb(S16H) striatum when compared with their Ctrl littermates, suggesting that D1R-cAMP signaling axis is not diminished by persistently elevated mTORC1 activity (Figure 5B and Figure S5E in Supplement 1). Basal levels of DARPP-32(pT34) and PP1α(pT320) were increased in Rheb(S16H) striatum; however, neither phosphosite changed in response to amphetamine. In the striatum, D1R-DARPP32(pT34)-mediated inhibition of PP1 increases the phosphorylation of extracellular signal-regulated kinase (Erk), which phosphorylates mGluR5 and contributes to dopamine's action at the synapse (30,34). Consistent with occlusion of this pathway in Rheb(S16H) striatum, basal levels of Erk1/2(pT202/Y204) and mGluR5(pS1126) were increased and were not further increased by amphetamine (Figure 5C, D).

Persistent Elevation of mTORC1-S6K1 Disrupts D1R Responses In Vivo

We asked whether physiological responses to D1R activation would correspond to the elevated basal DARPP-32. We first examined the nucleosome response and found that histone H3(pS10) was elevated at 60 minutes after amphetamine treatment (5 mg/kg, i.p.) in Ctrl mice but not in Rheb(S16H) mice (Figure 6A, B). Consistent with the notion that the nucleosome response is critical to regulate dynamic gene expression (32), the induction of *Homer1a* and *Npas4* were disrupted in Rheb(S16H) mice (Figure 6C).

We next examined D1R-mediated plasticity of corticostriatal synapses. We confirmed that high-frequency stimulation induced corticostriatal long-term potentiation (LTP), and that subsequent low-frequency stimulation induced depotentiation in field recordings of acute striatal slices from Ctrl and Rheb(S16H) mice (Figure 6D, E). The low-frequency stimulation–induced depotentiation is prevented by prior activation of D1R (35) and is mediated by MAPK-dependent phosphorylation of mGluR5 in combination with induction of *Homer1a* (34). In Rheb(S16H) slices, SKF38393 failed to block depotentiation, indicating the failure of this D1R-mediated synaptic mechanism (Figure 6F, G). Control experiments provided further evidence of specificity of D1R deficits by demonstrating preservation of plasticity of the hippocampus Schaffer-CA1 synapse, including mGluR-dependent LTD, low-frequency LTD, theta burst LTP, and high-frequency tetanus LTP in Rheb(S16H) mice (Figure S6A–D in Supplement 1).

Finally, we monitored locomotor activity in Rheb(S16H) mice. Amphetamine (2 mg/kg, i.p.) induced robust locomotor activation in Ctrl mice but not in Rheb(S16H) mice beginning at 20 minutes after administration. This time course suggests a physiological role for mTORC1–S6K1–DARPP-32 signaling that is normally manifest later in the response and is disrupted in Rheb(S16H) mice (Figure 6H). Consistent with this premise, rapamycin pretreatment (10 mg/kg, i.p.) reduced locomotor responses to amphetamine in Ctrl mice but did not alter the response in Rheb(S16H) mice (Figure S6E). To assess mTORC1's impact on D1R signaling, we administered the D1R selective agonist SKF-81297 (2.5 mg/kg, i.p.) and found a reduced locomotor response in Rheb(S16H) mice (Figure 6I). Rheb(S16H) and Ctrl mice showed similar locomotor activation in response to the NMDA receptor antagonist MK-801 (0.3 mg/kg, i.p.), indicating a selective impact of persistent elevation of mTORC1 activity on D1R-dependent locomotion (Figure S6F in Supplement 1).

Persistent Elevation of mTORC1 Activity in D1R Expressing Neurons Causes Social Interaction Deficits

D1R neurons of the mesocorticolimbic circuitry mediate reward processing and integrate neuromodulatory inputs that influence social behaviors (22,23); selective disruption of D1R has been linked with social deficits (36–39). We asked whether selective increase of mTORC1 in D1R neurons is sufficient to re-create this phenotype. We used D1-Cre to drive Rheb(S16H) expression in D1R-expressing neurons (40) and

are indicated by white triangles in the merge channel. $n = 113$ cells for Ctrl, and $n = 118$ cells for Rheb(S16H); box plot, interquartile ± min–max; 3 independent experiments were performed, 4 views were collected per mouse, and at least 8 cells were analyzed per view. Scale bar, solid white = 10 μm. **(H)** Representative images and **(I, J)** quantification of the histone H3(pS10) foci in the NAc of Rheb(S16H) mice and Ctrl littermates. **(I)** Relative foci intensity, $n = 57$ for Ctrl, and $n = 105$ for Rheb(S16H), ***$p = .0002$ by Kolmogorov-Smirnov test. **(J)** Foci number per nuclei, box plot, interquartile ± min–max, $n = 14$ neurons for Ctrl, $n = 15$ neurons for Rheb(S16H), ****$p < .0001$ by Mann-Whitney U test; data collected from at least 3 mice per genotype. **(K)** Representative blots and **(L)** quantification of phosphorylated histone H3 at Ser-10 in the chromatin-bound fraction of the striatum from Rheb(S16H) mice compared with Ctrl littermates, $n = 5$ for both genotypes. AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; Ctrl, control; D1R, D_1 receptor; NAc, nucleus accumbens; SP, substance P; WT, wild-type.

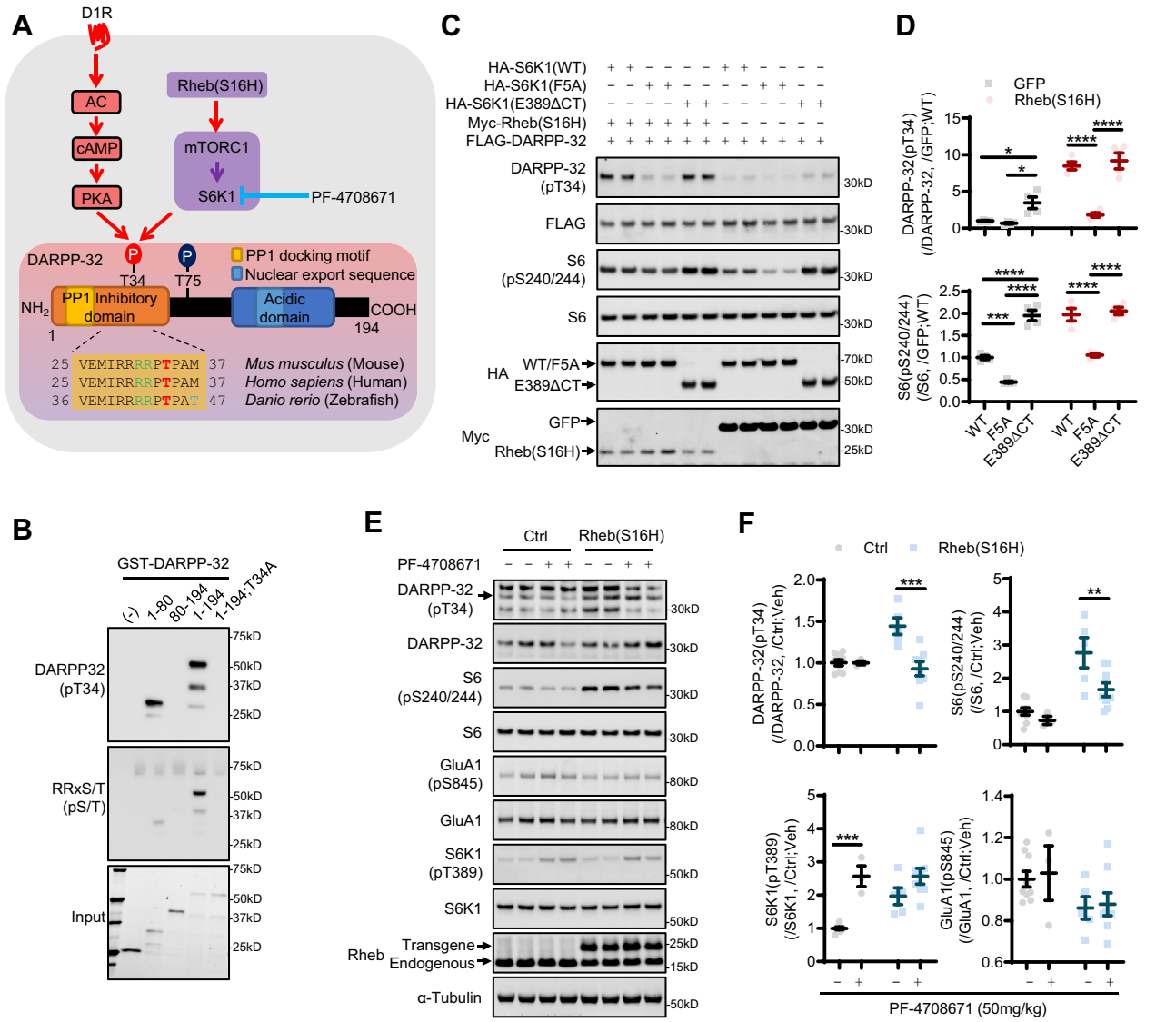


Figure 4. S6K1 directly phosphorylates DARPP-32 and causes elevated basal DARPP-32 signaling in the Rheb(S16H) striatum. **(A)** Schematic diagram of the canonical D1R–DARPP-32 signaling pathway. **(B)** Representative blots of recombinant GST-tagged WT and mutant DARPP-32 incubated with constitutively active S6K1 in a cell-free system. **(C)** Representative blots and **(D)** quantification for the level of DARPP-32(pT34) and S6(pS240/244) from the lysate of HEK293FT cells transfected with various combinations of FLAG-DARPP-32, Myc-Rheb(S16H) or Myc-GFP, and WT or mutant HA-S6K1 (F5A, inactive; E389ΔCT, constitutively active). Statistical significance was determined by two-way ANOVA followed by Bonferroni's post hoc test: DARPP-32(pT34), interaction, $^{***}p = .0002$; GFP/S6K1(WT) vs. GFP/S6K1(F5A), $^{*}p = .0304$; GFP/S6K1(F5A) vs. GFP/S6K1(E389ΔCT), $^{*}p = .0135$; Rheb(S16H)/S6K1(WT) vs. Rheb(S16H)/S6K1(F5A), $^{****}p < .0001$; Rheb(S16H)/S6K1(E389ΔCT) vs. Rheb(S16H)/S6K1(F5A), $^{****}p < .0001$; S6(pS240/244), interaction, $^{***}p = .0003$; GFP/S6K1(WT) vs. GFP/S6K1(F5A), $^{***}p = .0007$; GFP/S6K1(WT) vs. GFP/S6K1(E389ΔCT), $^{****}p < .0001$; GFP/S6K1(F5A) vs. GFP/S6K1(E389ΔCT), $^{****}p < .0001$; Rheb(S16H)/S6K1(WT) vs. Rheb(S16H)/S6K1(F5A), $^{****}p < .0001$; Rheb(S16H)/S6K1(E389ΔCT) vs. Rheb(S16H)/S6K1(F5A), $^{****}p < .0001$; $n = 4$ per group. **(E)** Representative blots and **(F)** quantification of DARPP-32(pT34), S6(pS240/244), S6K1(pT389), and GluA1(pS845) from the striatal lysate of Rheb(S16H) mice and Ctrl littermates treated with vehicle or PF-4708671. Mice were sacrificed 2 hours after intraperitoneal injection of PF-4708671 or vehicle, and the striatum was collected and lysed for WB. Statistical significance was determined by two-way ANOVA followed by Bonferroni's post hoc test: DARPP-32(pT34), interaction, $^{**}p = .0046$; Rheb(S16H)/vehicle vs. Rheb(S16H)/PF-4708671, $^{***}p = .0002$; S6(pS240/244), treatment, $^{*}p = .0155$; Rheb(S16H)/vehicle vs. Rheb(S16H)/PF-4708671, $^{**}p = .0074$; S6K1(pT389), interaction, $^{*}p = .0444$; Ctrl/vehicle vs. Ctrl/PF-4708671, $^{***}p = .0004$; $n = 9$ for Ctrl/vehicle, $n = 3$ for Ctrl/PF-4708671, $n = 5$ for Rheb1(S16H)/vehicle, and $n = 8$ for Rheb1(S16H)/PF-4708671. Individual data and mean ± SEM are presented. AC, adenylyl cyclase; ANOVA, analysis of variance; cAMP, cyclic adenosine monophosphate; Ctrl, control; D1R, D_1 receptor; mTORC1, mTOR complex 1; WB, Western blot; WT, wild-type.

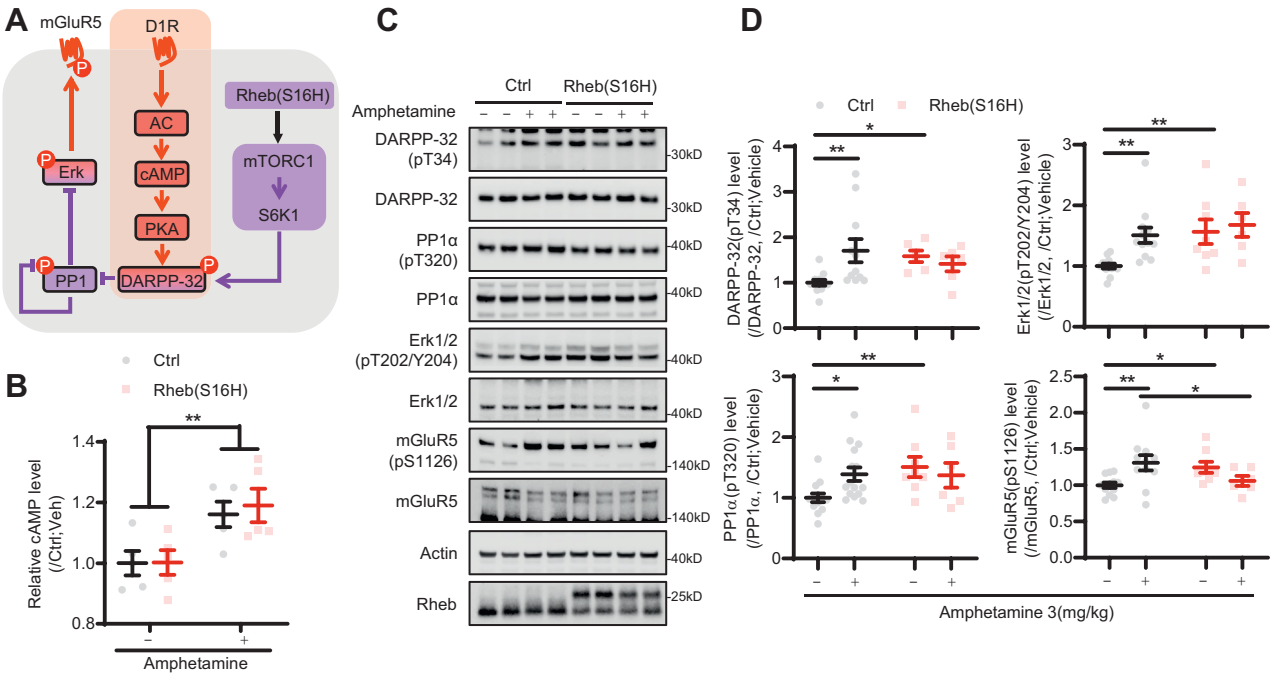


Figure 5. Dynamic DARPP-32 signaling is occluded in the Rheb(S16H) striatum. **(A)** Schematic of canonical D1R–DARPP-32 signaling dynamic and its action on mGluR5. **(B)** Production of cAMP in the striatum of the amphetamine treated Rheb(S16H) mice and Ctrl littermates. Amphetamine (5 mg/kg, intraperitoneal) induced robust cAMP production in both Ctrl and Rheb1(S16H) mice, but no difference was found between genotypes (two-way mixed-design ANOVA, treatment, $^{**}p = .0013$; interaction, $p = .2820$); $n = 5$ for each group. **(C)** Representative blots and **(D)** quantification of blots for the phosphorylation levels of DARPP-32, PP1α, Erk1/2, and mGluR5 in the striatum lysate of Ctrl and Rheb(S16H) mice treated with amphetamine (3 mg/kg, intraperitoneal) or vehicle. Striatum was collected 15 minutes after injection. Statistical significance was determined by two-way ANOVA followed by Fisher's least significant difference post hoc test: DARPP-32(pT34), interaction, $^{*}p = .0307$; Ctrl/vehicle vs. Rheb(S16H)/vehicle, $^{*}p = .0389$; Ctrl/vehicle vs. Ctrl/amphetamine, $^{**}p = .0038$; PP1α(pT320), interaction, $p = .0561$; Ctrl/vehicle vs. Rheb(S16H)/vehicle, $^{**}p = .0080$; Ctrl/vehicle vs. Ctrl/amphetamine, $^{*}p = .0151$; Erk1/2(pT202/Y204), interaction, $p = .1652$; Ctrl/vehicle vs. Rheb(S16H)/vehicle, $^{**}p = .0048$; Ctrl/vehicle vs. Ctrl/amphetamine, $^{**}p = .0047$; mGluR5(pS1126), interaction, $^{**}p = .0047$; Ctrl/vehicle vs. Rheb(S16H)/vehicle, $^{*}p = .0309$; Ctrl/vehicle vs. Ctrl/amphetamine, $^{**}p = .0040$; Ctrl/amphetamine vs. Rheb(S16H)/amphetamine, $^{*}p = .0485$; at least $n = 6$ mice per group. Individual data and/or mean ± SEM are presented. AC, adenylyl cyclase; ANOVA, analysis of variance; cAMP, cyclic adenosine monophosphate; Ctrl, control; D1R, D_1 receptor; mGluR5, metabotropic glutamate receptor 5; mTORC1, mTOR complex 1.

confirmed the elevation of mTORC1, as shown by the increase of S6(pS240/244), and the suppression of mTORC2, as shown by the reduction of Akt(pS473) in the striatum of D1-Rheb(S16H) mice. D1-Rheb(S16H) exhibited robust elevation of basal DARPP-32(pT34) while GluA1(pS845) was not altered (Figure 7A, B).

D1-Rheb(S16H) mice exhibited normal novelty-induced locomotor activation in an open field as well as in the testing chamber during habituation but exhibited reduced amphetamine (2 mg/kg, i.p.)-induced locomotor activation (Figure 7C and Figure S7A, B in Supplement 1). In the social motivation test, D1-Rheb(S16H) mice failed to distinguish between social and nonsocial objects (Figure 7D, E). In the social novelty test, D1-Rheb(S16H) mice failed to distinguish between familiar and stranger social objects (Figure 7F, G). D1-Rheb(S16H) mice exhibited normal recognition of novel objects (Figure S7C, D in Supplement 1). Accordingly, social interaction deficits in D1-Rheb(S16H) mice are likely not attributable to a failure to recognize familiar and stranger mice. These data demonstrate that persistent elevation of mTORC1 in D1R expressing neurons is sufficient to cause social interaction deficits.

Hyperactive mTORC1–S6K1–DARPP-32 Signaling in Human Brain Diseases

A primary goal of our analysis was to identify signaling pathways and biomarkers that can be linked to human cognitive disease. In the human TS brain samples (Brodmann area [BA] 46), the levels of S6(pS240/244) and DARPP-32(pT34) were significantly elevated and were positively correlated within the TS cohort but not the control cohort (Figure 8A–C). In the human AD brain samples, S6(pSer-240/244) was elevated in the middle frontal gyrus (BA 46) and middle temporal gyrus (BA 21); PP1α(pT320) was increased in the AD cohort (Figure 8D and Figure S8A, C in Supplement 1); and the level of DARPP-32(pT34) correlated with S6(pS240/244) in the AD group but not in the age-matched controls (Figure S8B, D in Supplement 1). Combining brain samples from the middle frontal gyrus and middle temporal gyrus revealed an increase of DARPP-32(pT34) in AD and strong

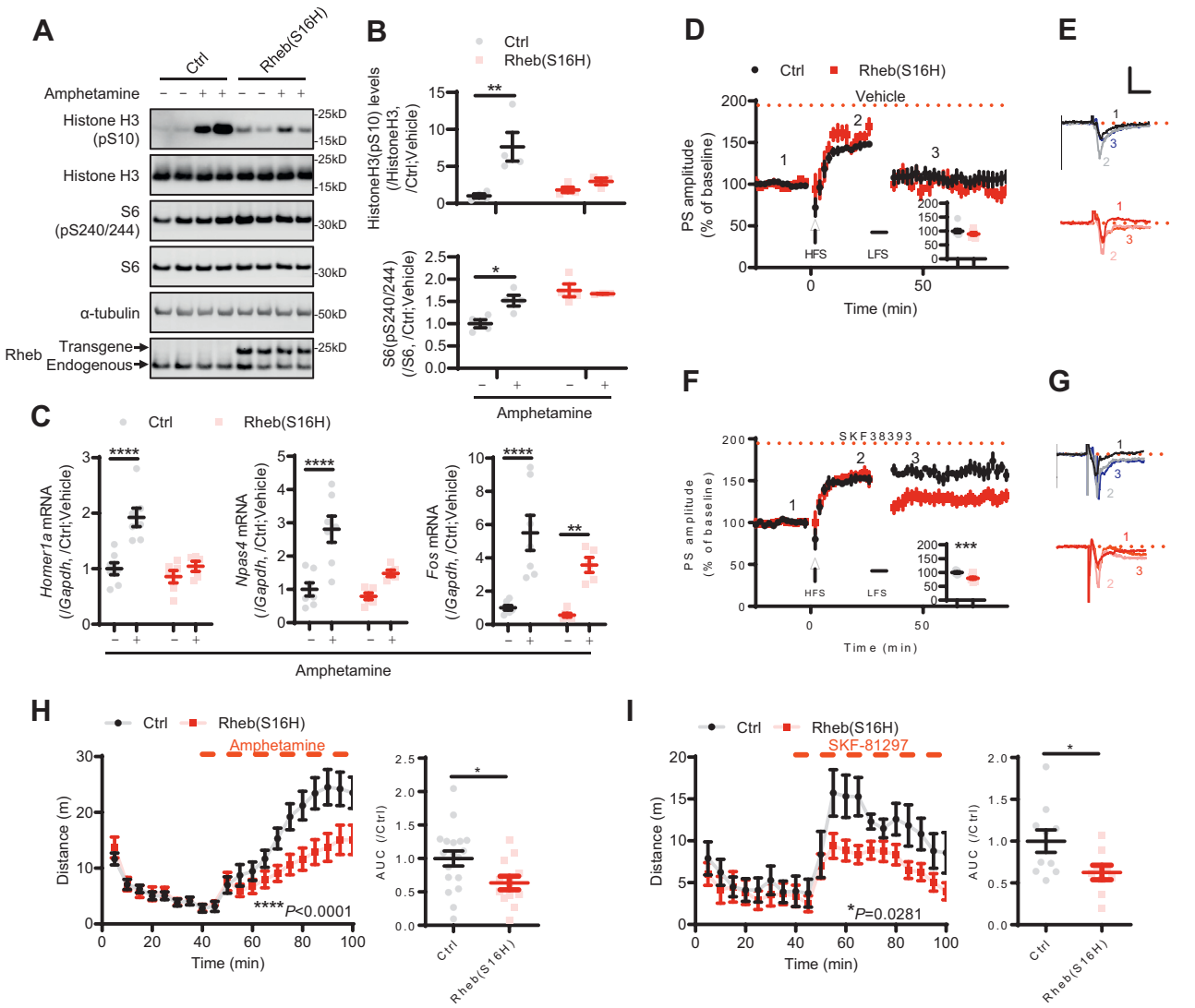


Figure 6. Persistently elevated mTORC1 activity disrupts amphetamine-induced gene expression, and D1R-mediated corticostriatal plasticity and locomotor activation. **(A)** Representative blots and **(B)** quantification of blots for the phosphorylation levels of histone H3 in the striatum lysate of Rheb(S16H) mice and their Ctrl littermates that were treated with amphetamine (5 mg/kg, intraperitoneal [i.p.]) or vehicle (saline, i.p.). Striatum was collected 60 minutes after injection. Statistical significance was determined by two-way ANOVA followed by Bonferroni's post hoc test: histone H3(pS10), interaction, $^{*}p = .0289$; Ctrl/vehicle vs. Ctrl/amphetamine, $^{**}p = .0019$; S6(pS240/244), interaction, $^{*}p = .0237$; genotype, $^{**}p = .022$; Ctrl/vehicle vs. Ctrl/amphetamine, $^{*}p = .0127$; at least $n = 3$ per group. **(C)** The mRNA levels of *Homer1a*, *Npas4*, and *Fos* in Ctrl or Rheb(S16H) striatum with amphetamine (5 mg/kg, 60 min, i.p.) treatment. The levels of genes were normalized to the level of *Gapdh* and to the Ctrl/vehicle group. Statistical significance was determined by two-way ANOVA followed by Bonferroni's post hoc test: *Homer1a*, interaction, $^{*}p = .0104$; Ctrl/vehicle vs. Ctrl/amphetamine, $^{****}p < .0001$; *Npas4*, interaction, $^{*}p = .042$; Ctrl/vehicle vs. Ctrl/amphetamine, $^{****}p < .0001$; *Fos*, interaction, $p = .2531$; treatment, $^{****}p < .0001$; at least $n = 3$ per group. **(D–G)** Corticostriatal LTP and depotentiation in field-potential recordings of brain slices prepared from Rheb(S16H) mice and Ctrl littermates in the presence of **(D, E)** vehicle or **(F, G)** the specific D_1-like receptor agonist, SKF38393 (3 μM; left). Sample traces **(E, G)** and the magnitude (**E, F**, islets) of LFS-induced depotentiation of LTP (63–69 min) in the presence of **(D, E)** vehicle or **(F, G)** SKF38393. **(F)** Islet, $^{***}p = .0003$, was determined by unpaired *t* test; Ctrl/vehicle, $n = 9$ slices from 5 mice; Ctrl/SKF38393, $n = 8$ slices from 4 mice; Rheb(S16H)/vehicle, $n = 6$ slices from 4 mice; Rheb(S16H)/SKF38393, $n = 11$ slices from 6 mice. Scale bar, 1 mV = 10 ms. **(H)** Amphetamine (2 mg/kg, i.p.) and **(I)** SKF-81297 (2.5 mg/kg, i.p.) induced locomotor activity and accumulative distance traveled by Rheb(S16H) mice or Ctrl littermates. Locomotor activation was analyzed in (left) 5-minute bins and (right) the total distance traveled after treatment. Two-way mixed-designed ANOVA: Left, **(H)** amphetamine, interaction, $^{****}p < .0001$; **(I)** SKF-81297, interaction, $^{*}p = .0281$. Right, genotype, **(H)** amphetamine, $^{*}p = .0306$; **(I)** SKF-81297, $^{*}p = .0354$; at least $n = 9$ per group. Individual data and/or mean ± SEM are presented. ANOVA, analysis of variance; AUC, area under the curve; Ctrl, control; D1R, D_1 receptor; LFS, low-frequency stimulation; LTP, long-term potentiation; mRNA, messenger RNA; mTORC1, mTOR complex 1; PS, population spike.

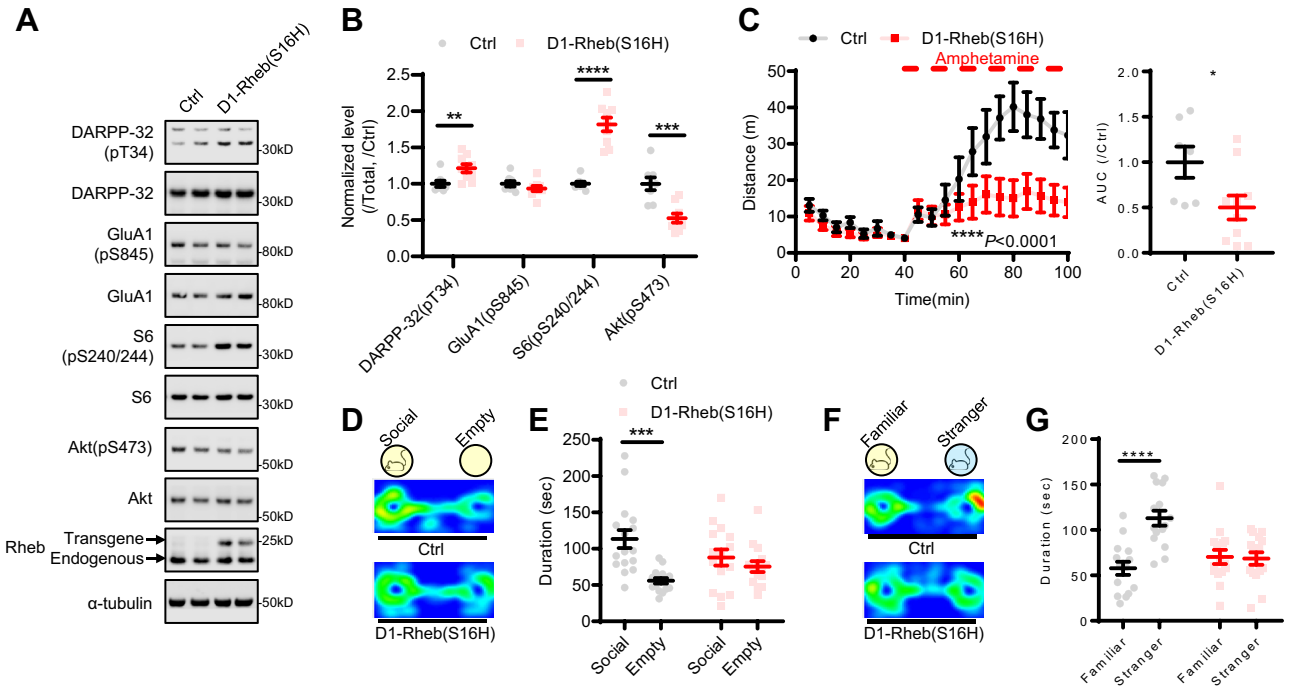


Figure 7. Persistently elevated mTORC1 in D1R neurons evokes social interaction deficit. **(A)** Representative blots and **(B)** quantification of the levels of phosphorylated DARPP-32, GluA1, S6, and Akt in the striatal lysate of the D1-Rheb(S16H) mice and their Ctrl littermates. $^{**}p < .01$, $^{***}p < .001$, and $^{****}p < .0001$ by unpaired *t* test; $n = 9$ for D1-Rheb(S16H) and $n = 8$ for Ctrl. **(C)** Amphetamine (2 mg/kg, intraperitoneal)–induced locomotor activation in D1-Rheb(S16H) mice and Ctrl littermates. Two-way mixed designed ANOVA: Left, genotype, $^{*}p = .0377$; interaction, $^{****}p < .0001$; Right, unpaired *t* test, $^{*}p = .0331$; at least $n = 7$ per group. **(D–G)** Three-chamber sociability task. **(D)** Heat map of the duration spent on the testing chamber of D1-Rheb(S16H) mice and Ctrl littermates on the social motivation task. **(E)** Duration that mice spent investigating a social object and an empty cup in the three-chamber sociability task on D1-Rheb(S16H) mice. Statistical significance was determined by two-way ANOVA followed by Bonferroni's post hoc test: interaction, $^{*}p = .0245$; Ctrl, social vs. empty, $^{***}p = .0003$; D1-Rheb(S16H), social vs. empty, $p = .7492$. **(F)** Heat map of the duration spent on the testing chamber of D1-Rheb(S16H) mice and the Ctrl littermates on a social novelty test. **(G)** Duration that D1-Rheb(S16H) mice and the Ctrl littermates spent investigating familiar and stranger social objects. Statistical significance was determined by two-way ANOVA followed by Bonferroni's post hoc test: interaction, $^{***}p = .0001$; Ctrl, stranger vs. familiar, $^{****}p < .0001$; D1-Rheb(S16H), stranger vs. familiar, $p > .9999$; $n = 15$ for both D1-Rheb(S16H) and Ctrl littermates. Individual data and mean ± SEM are presented. ANOVA, analysis of variance; Ctrl, control; D1R, D_1 receptor; mTORC1, mTOR complex 1.

correlation with S6(pS240/244) in AD but not Ctrl brains (Figure 8E, F). Moreover, *Homer1a* messenger RNA (measured relative to *Homer1c*), which shows a reduced induction in Rheb(S16H) mice, was markedly reduced in the AD brain (Figure 8G). These findings suggest that disruption of DARPP-32-dependent D1R signaling may represent an underlying mechanism that contributes to cognitive and behavioral deficits.

DISCUSSION

The present study identifies a signaling node that couples mTORC1-S6K1 with canonical D1R signaling and acts to occlude DARPP-32 signaling in cells with persistently elevated mTORC1 activity and block D1R responses that are dependent on DARPP-32. In support of this model, we demonstrate that DARPP-32 is a direct substrate of S6K1 in vitro, in heterologous cells, and in vivo. In normal conditions and in response to an acute stimulus such as amphetamine administration, D1R-cAMP-PKA rapidly phosphorylates DARPP-32(T34), which mediates downstream responses in gene expression, corticostriatal plasticity, and behaviors (Figure 8H). But in the condition of persistently elevated mTORC1-S6K1, basal DARPP-32(pT34) is elevated, accompanied by elevated levels of DARPP-32 pathway mediators including PP1α(pT320) and Erk(pT202/Y204) (Figure 3B–I; Figure 5C, D). Persistently elevated mTORC1 does not interrupt D1R-cAMP (Figure 5B), but dynamic DARPP-32(pT34) is occluded, resulting in disruption of multiple D1R-dependent responses (Figure 8H).

The block of D1R responses consequent to persistent mTORC1 activation poses implications for behaviors and appears relevant for understanding the impact of neuropsychiatric and neurodegenerative diseases that result in elevated mTORC1 signaling. D1R signaling is central to social behavior. Optogenetic activation of the ventral tegmental area projection to NAc or light-evoked D1R signaling in NAc drives social behaviors in rodents (22). Dopamine is also essential for prefrontal cortex function that mediates working memory (41,42), and cortex-dependent working memory and memory flexibility appear vulnerable to disrupted mTORC1 (43–45). Data in the present study suggest that mTORC1 becomes a driver for DARPP-32 signaling in the brain of human subjects with TS and AD. AD often presents with symptoms of mild behavioral

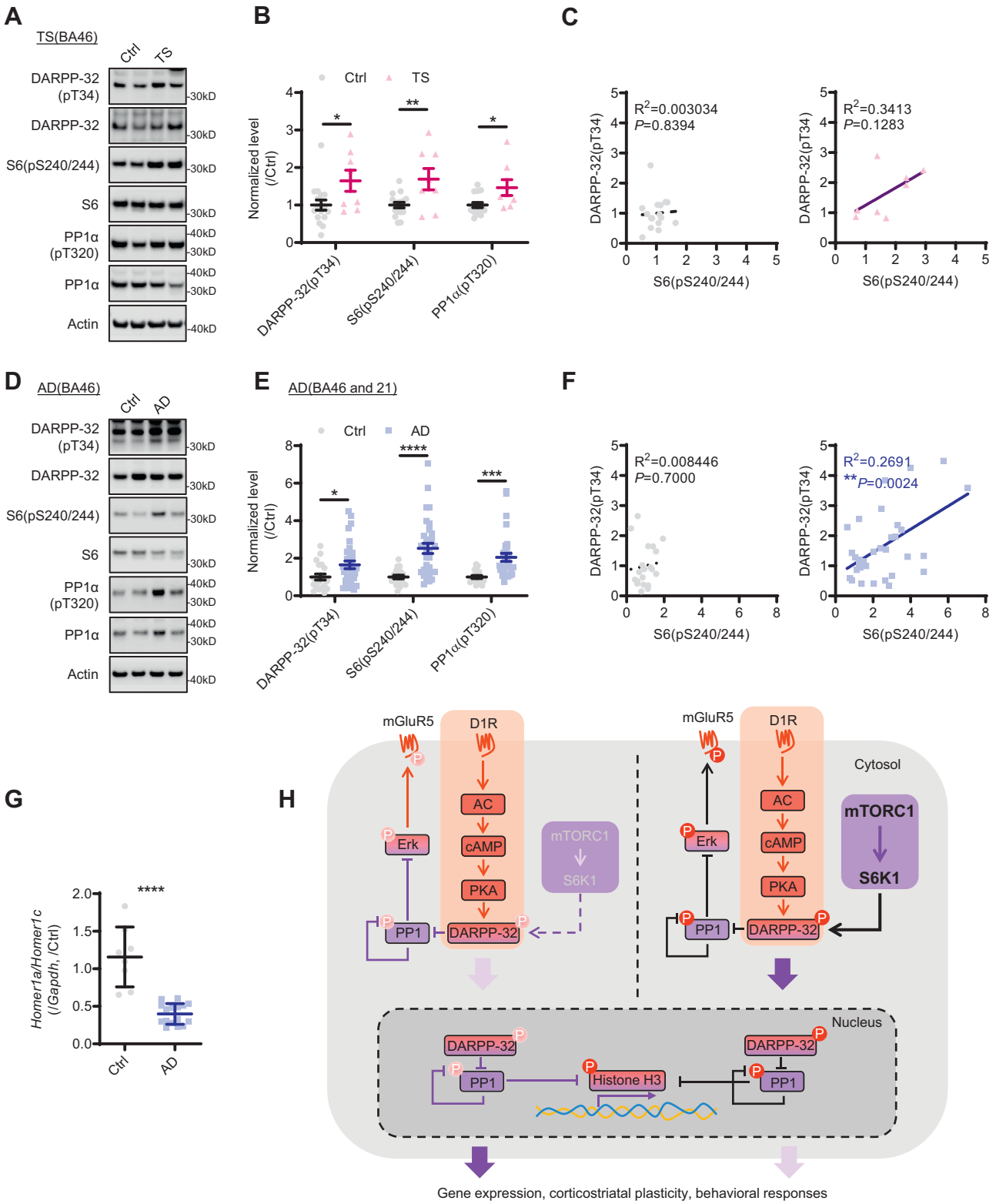


Figure 8. Hyperactive mTORC1–S6K1–DARPP-32 signaling in human brain diseases. **(A)** The representative blots and **(B)** quantification of DARPP-32(pT34), PP1α(pT320), and S6(pS240/244) of the human brain lysate from BA 46 of individuals with TS and age-matched Ctrl. *$p < .05$ and **$p < .01$ were determined by unpaired *t* test. **(C)** The correlation between DARPP-32(pT34) and S6(pS240/244) in Ctrl and TS groups, respectively; $n = 16$ for Ctrl and

impairments that include decreased motivation, emotional dysregulation, and social inappropriateness (46). Our analysis focused on human cortical tissue, and it will be important to also examine mesolimbic structures that are more directly related to these behavioral symptoms. In our studies D1R-Cre was expressed in cortical, striatal, and mesolimbic areas, which precludes a refined analysis of specific circuits and behaviors; however, the conditional Rheb(S16H) model provides an opportunity for this analysis.

The present model may also be relevant to neuropsychiatric symptoms resulting from stress. Chronic stress impacts sociability and is associated with reduction of excitatory input onto D1R-expressing neurons in the NAc (36,37,47–51). Mice that are susceptible to chronic social defeat stress exhibit elevated BDNF (brain-derived neurotrophic factor) signaling as well as increased downstream MAPK signaling in the NAc (50,51). In addition, the BDNF protein level is elevated in depressed human postmortem brain samples (50). Given the important role of neurotrophic factor in mediating mTORC1-S6K1 signaling, it is plausible that stress-induced elevation of BDNF signaling in the NAc elevates mTORC1-S6K1 and consequently occludes D1R-DARPP-32 signaling as a basis for stress-induced disruption of social behaviors. Stress also prominently interacts with drug addiction (52) and schizophrenia (53), diseases in which dopamine signaling plays a central role.

Several compelling questions arise from the current study. What is the mechanism for occlusion of D1R-DARPP-32 signaling? D1R-PKA appears intact, and elevated DARPP-32(pT34) in Rheb(S16H) mice is reduced by S6K1 inhibition (Figure 3). One possibility is an upregulation of phosphatase acting at DARPP-32(pT34) in response to elevated mTORC1-S6K1 that secondarily prevents PKA-indued increases. Heterologous cell reconstitution of mTORC1–DARPP-32 interactions may be useful to define this mechanism. A related question is whether mTORC1 signaling contributes to normal D1R responses. The fact that rapamycin reduces amphetamine-induced locomotion in WT mice (Figure S6E in Supplement 1) suggests this possibility, but further analysis is required. Another question is whether persistently elevated mTORC1 impacts D2R responses and, if so, how this might contribute to behavioral phenotypes. Indeed, DARPP-32 is activated by β-adrenergic and serotonin receptors as well as hormones acting through non-cAMP–dependent pathways (29,54), suggesting a broader role for mTORC1–S6K1–DARPP-32 interaction.

Previous studies have offered alternative models for cognitive deficits related to elevated mTORC1 that include changes to cap-dependent protein synthesis and the composition of synapses (12–14). Our findings are not directly contradictory, but we did not detect changes in LTP or LTD in the hippocampus or corticostriatal synapses (Figure 6D, E and Figure S6A–D in Supplement 1). We note that S6K1 is a component of the protein translation initiation complex (55) and its phosphorylation of DARPP-32 may be sensitive to manipulations used experimentally to implicate translational pathways. mTORC1–S6K1–DARPP-32–PP1 rationalizes increased MAPK (Erk) observed in mouse models with elevated mTORC1 activity, including *Tsc* and *Fmr1* mice (56,57). Our findings do not exclude the possible involvement of 4EBP1-eIF4E in the phenotypes of Rheb(S16H) mice, but it has been reported that a mouse model with neuronal overexpression of eIF4E fails to recapitulate social deficits (17). The requirement for dynamic mTORC1-S6K1 coupling with D1R signaling may rationalize the finding that *Rptor* knockout fails to restore social deficits in *Pten* mice because the disruption of mTORC1 does not restore the dynamic coupling, although it reverses mTORC1-S6K1 hyperactivity (16). The complexity of signaling and adaptation highlights the challenge for developing effective therapeutics to mitigate the effect of persistently elevated mTORC1 activity.

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Data are stored and curated on a secure server located in the Worley laboratory in the Department of Neuroscience and Department of Pathology at Johns Hopkins University. Data will be made available upon request.

The authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

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n = 8 for TS. **(D)** The representative blots of DARPP-32(pT34), PP1α(pT320), and S6(pS240/244) of the human brain lysate from BA 46 of individuals with AD and age-matched Ctrl. **(E)** Quantification of DARPP-32(pT34), PP1α(pT320), and S6(pS240/244) of human brain lysate of AD patients and Ctrl (combining BA 46 and BA 21); $^{*}p < .05$, $^{***}p < .001$, and $^{****}p < .0001$ were determined by unpaired t test. **(F)** The correlation between DARPP-32(pT34) and S6(pS240/244) in AD patients and Ctrl (combining BA 46 and BA 21), respectively; n = 20 for Ctrl and n = 32 for AD. **(G)** Ratio of Homer1a relative to Homer1c in human AD brain samples; n = 8 for Ctrl and n = 16 for AD; $^{****}p < .0001$ were determined by unpaired t test. Individual data and/or mean ± SEM are presented. **(H)** Schematic model of mTORC1-S6K1 negatively impacting D1R signaling under condition of persistent elevated mTORC1 activity. AC, adenylyl cyclase; AD, Alzheimer's disease; BA, Brodmann area; cAMP, cyclic adenosine monophosphate; Ctrl, control; D1R, D_1 receptor; mGluR5, metabotropic glutamate receptor 5; mTORC1, mTOR complex 1; TS, tuberous sclerosis.

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