

AWARD NUMBER: W81XWH-13-1-0380

TITLE: The Role of the New mTOR Complex, mTORC2, in Autism Spectrum Disorders

PRINCIPAL INVESTIGATOR: Mauro Costa-Mattioli

CONTRACTING ORGANIZATION: Baylor College of Medicine, Houston, TX 77584

REPORT DATE: December 2017

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE December 2017		2. REPORT TYPE Final		3. DATES COVERED 30 Sept 2013 - 29 Set 2017	
4. TITLE AND SUBTITLE SUBTITLE The Role of the New mTOR Complex, mTORC2, in Autism Spectrum Disorders				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-13-1-0380	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Mauro Costa-Mattioli E-Mail: costamat@bcm.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Baylor College of Medicine One Baylor Plaza Houston, TX 77030-3498				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The goal of my DOD-supported research is to determine the role of the new mTOR complexes (mTORC1 and mTORC2) in Autism Spectrum Disorder (ASD). Individuals with ASD exhibit impaired social interactions, repetitive abnormal repetitive behavior as well as cognitive problems. In addition, a large proportion of ASD individual suffer from seizures. Autism is a heritable genetically heterogeneous disorder and mutations in the negative regulator of the mammalian target of rapamycin (mTOR) signaling pathway PTEN were associated with ASD. However, little is known about the mechanism underlying Pten-induced pathology. Here, we show that in the hippocampus of <i>pten</i> fb-KO mice, where Pten is conditionally deleted in the murine forebrain, the activity of both mTORC1 and mTORC2 is increased. In addition, we found that <i>pten</i> fb-KO mice exhibit seizures, learning and memory and ASD-like behaviors. Interestingly, genetic dissection of mTOR complexes function <i>in vivo</i> reveals that mTORC1 is responsible for the enlarged brain phenotype whereas mTORC2 regulates EEG seizures, learning and memory as well ASD-like phenotypes in <i>pten</i> -deficient mice. Moreover, we found that mTORC2 regulates these processes by controlling glucose metabolism. We also found that mTORC2, but not mTORC1, is crucially required for mGluR-LTD, a major form of synaptic plasticity involved mnemonic processes. Our new insights hold promise for new specific mTORC2-based treatments for ASD and related mTORopathies.					
15. SUBJECT TERMS None provided					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	16	19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Accomplishments.....	N/A
4. Impact.....	N/A
5.Changes/Problems.....	N/A
6. Products.....	N/A
7.Participants & Other Collaborating Organizations.....	N/A
8.Special Reporting Requirements.....	N/A
9. Appendices.....	N/A

1. Introduction: Autism Spectrum Disorder (ASD) is one of the most common neurological disorders worldwide, characterized by abnormal social interaction, deficits in communication and restricted/repetitive stereotypic behaviors. In addition, a significant number of autistic individuals suffer from intellectual disability and seizures¹⁻⁴. Single gene mutations are linked to ASD and dysregulation of Mechanistic Target Of Rapamycin (mTOR) signaling cascade has been associated with ASD⁵⁻⁷. More specifically, loss-of function mutations of the phosphatase and tensin homolog (PTEN), a negative regulator of mTOR signaling, were associated with syndromic ASD⁸. While in PTEN-deficient individuals (or mouse models), the activity of both mTOR complexes (mTORC1 and mTORC2) is up-regulated, it is generally believed that persistent increased mTORC1 activity leads to ASD-like symptoms⁹. However, most of the evidence supporting a role for mTORC1 in ASD relies heavily on the chronic pharmacological inhibition of mTOR by rapamycin, which blocks the activity of both mTORC1 and mTORC2 complexes in brain. Currently, there are no drugs to specifically target mTORC1 or mTORC2. Thus, the scope of this research was to determine the role of mTOR complexes in a pten-deficient mouse model of ASD using molecular genetics. To investigate the selective involvement of mTORC1 and/or mTORC2 in pten-associated ASD, we used the Cre-loxP system and generated mice lacking a) pten (pten fb-KO mice), b) pten and raptor (a defining component of mTORC1; pten-raptor fb-KO mice) and c) pten and rictor (a defining component of mTORC2) in the murine forebrain.

2. Keywords: Autism Spectrum Disorder (ASD), mTORC2, mTORC1, protein synthesis, actin polymerization, metabolism, long-term memory, social behaviors, repetitive behaviors, seizures.

3. Overall Project Summary.

The major goal of our grant application was to elucidate the molecular and cellular mechanisms underlying ASD, with a special emphasis on the mTOR signaling pathway and its two major complexes. We believe that in the three years of funding, we have made remarkable progress and generated data that represent paradigm shift in the field. Specifically, we have discovered that each mTOR complex differentially contributes to different aspects of ASD. While the activity of both mTOR complexes (mTORC1 and mTORC2) is increased in pten-deficient individuals and mice⁸, the individual contribution of mTOR complexes to the molecular, behavioral and electrophysiological abnormalities associated with pten deficiency remains unknown. We found that mice lacking pten in forebrain neurons (pten fb-KO) exhibit increased mTORC1 and mTORC2. In addition, pten fb-KO mice show enhanced brain size, ASD-like behaviors (including social and cognitive deficits as well as repetitive behaviors), seizures and early mortality. We found that genetic deletion of mTORC1 (raptor), but not mTORC2 (rictor), only restores normal brain size. Surprisingly, genetic silencing of mTORC2, but not mTORC1, in pten-deficient mice, prolonged lifespan, suppressed seizures, rescued the cognitive and ASD-like behaviors as well as the seizure phenotype. These insights hold promise for new specific mTORC2-based treatments for ASD and related mTORopathies.

Finally, we have also discovered that mTORC2, but not mTORC1, is crucially required for metabotropic glutamate receptor-mediated long-term depression (mGluR-LTD), a major form of synaptic plasticity that is usually dysregulated in ASD and other neurological disorders.

Selective genetic silencing of mTORC2 or mTORC1 in *pten*-deficient mice. As previously reported, we found that mTORC2-mediated phosphorylation of Akt at Ser473 (an established readout of mTORC2 activity⁶) and mTORC1-mediated phosphorylation of ribosome protein S6 (an established readout of mTORC1 activity)¹⁰ were both increased in the hippocampus of *pten* fb-KO mice (**Fig. 1e-g**). We found that while in the hippocampus of *pten-rictor* fb-DKO mice mTORC1 activity remained abnormally up-regulated, but mTORC2 activity was restored. By contrast, in *pten-raptor* fb-DKO mice, the opposite is true, namely mTORC2 activity remains up-regulated by mTORC1 activity is normalized (**Fig. 1**). Hence, conditional deletion of *rictor* selectively blocks mTORC2 activity in *pten*-deficient neurons and conditional deletion of *raptor* selectively block mTORC1 activity in *pten*-deficient neurons. These loss-of-function molecular genetic manipulations allow us to dissect the functional role of each mTOR complex in *pten*-deficient-mediated pathology.

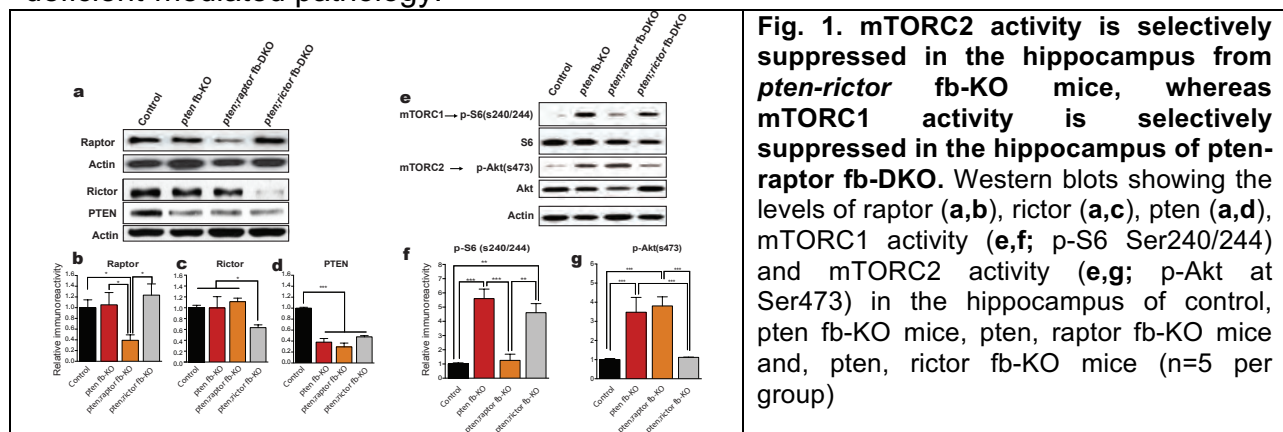
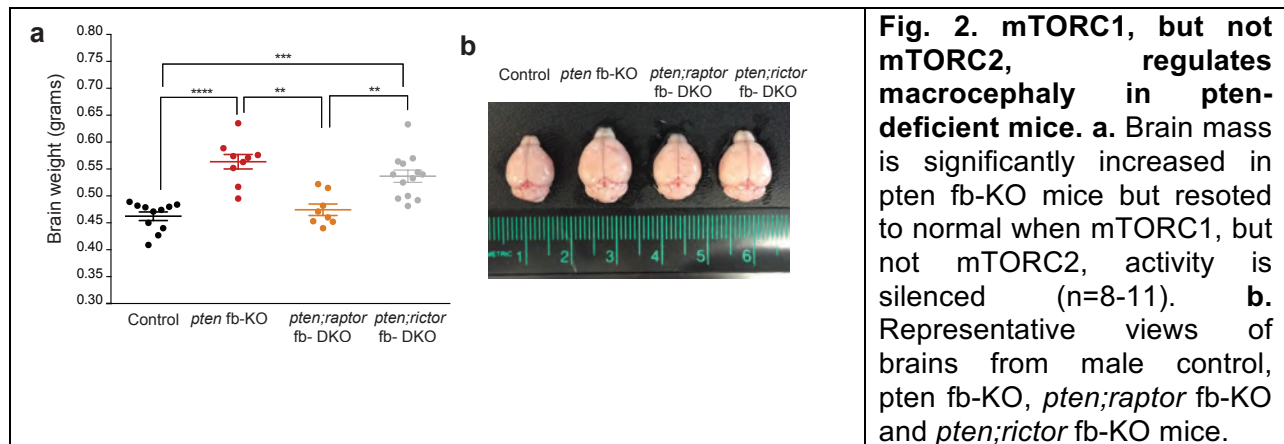


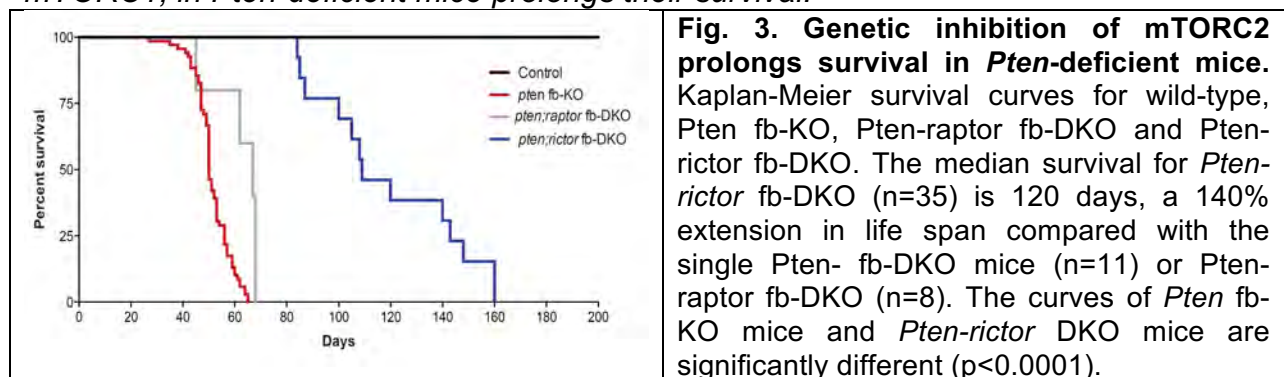
Fig. 1. mTORC2 activity is selectively suppressed in the hippocampus from *pten-rictor* fb-KO mice, whereas mTORC1 activity is selectively suppressed in the hippocampus of *pten-raptor* fb-DKO. Western blots showing the levels of raptor (a,b), rictor (a,c), pten (a,d), mTORC1 activity (e,f; p-S6 Ser240/244) and mTORC2 activity (e,g; p-Akt at Ser473) in the hippocampus of control, *pten* fb-KO mice, *pten*, *raptor* fb-KO mice and, *pten*, *rictor* fb-KO mice (n=5 per group)

Genetic silencing of mTORC1, but not mTORC2, restore brain size in *pten*-deficient mice. A significant percentage of children with ASD exhibit brain enlargement¹, which becomes noticeable a few months after birth and persists until early adolescence. However, little is not about the molecular mechanism underlying the early brain overgrowth^{11,12}. Moreover, it is currently unknown whether the same mechanism regulating brain size also controls the other core features of ASD, including the seizures and behavioral and electrophysiological abnormalities.

To measure the contribution of each mTOR complex to the brain size in *pten*-deficient mice, we measured the size of the brain at 4 weeks postnatal. Deletion of *pten* in the postnatal forebrain leads to an increased brain size, compared to control littermates (**Fig. 2a-b**). Interestingly, genetic silencing of mTORC1, but not mTORC2, restores brain size in *pten*-deficient mice (**Fig. 2a-b**). Thus, the exacerbated increased in mTORC1 (but not mTORC2) activity accounts for the increased brain size in *pten*-deficient mice.

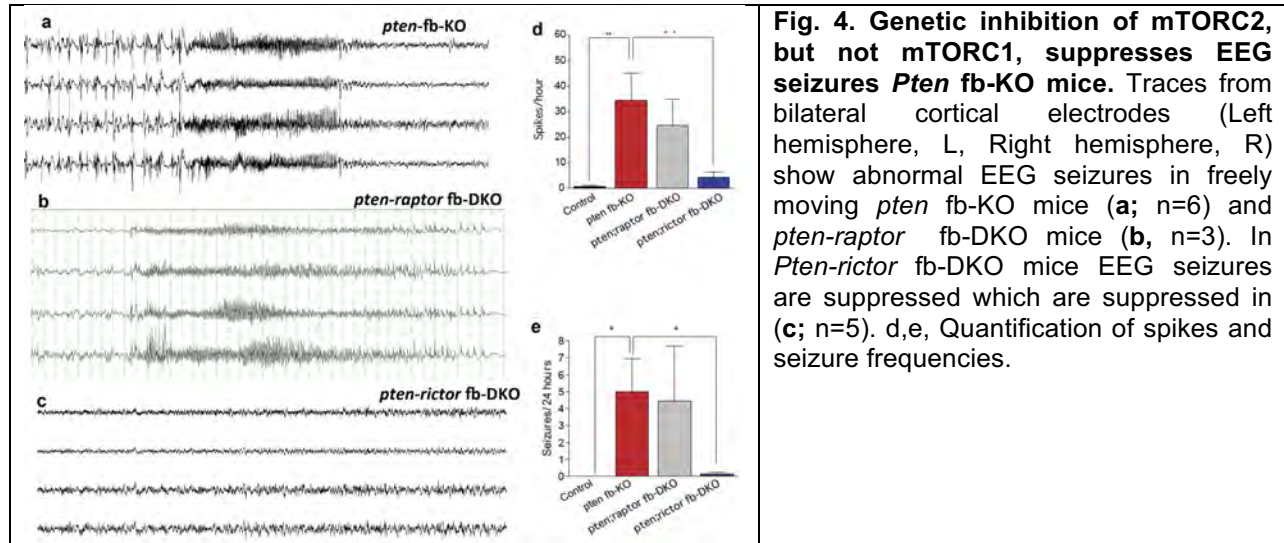


Genetic silencing of mTORC2, but not mTORC1, prolonged survival in pten-deficient mice. Not unexpectedly, Kaplan-Meier analysis of animal survival revealed a dramatic decreased in survival in *pten* fb-KO mice compared to control mice (**Fig. 3**). The majority of the *pten* fb-KO mice die a few weeks postnatal. Interestingly, genetic suppression of mTORC1 had no a major effect on *pten* fb-KO mice survival (compare *pten* fb-KO mice vs. *pten;raptor* DKO survival curves). Remarkably, genetic suppression of mTORC2 significantly extended survival (almost three times) in pten-deficient mice (compare *pten* fb-KO mice vs. *pten;rictor* DKO survival curves; **Fig. 3**; *pten;rictor* fb-DKO die at an age of 119.4 +/- 25). Hence, selective inhibition of mTORC2, but not mTORC1, in *Pten*-deficient mice prolongs their survival.

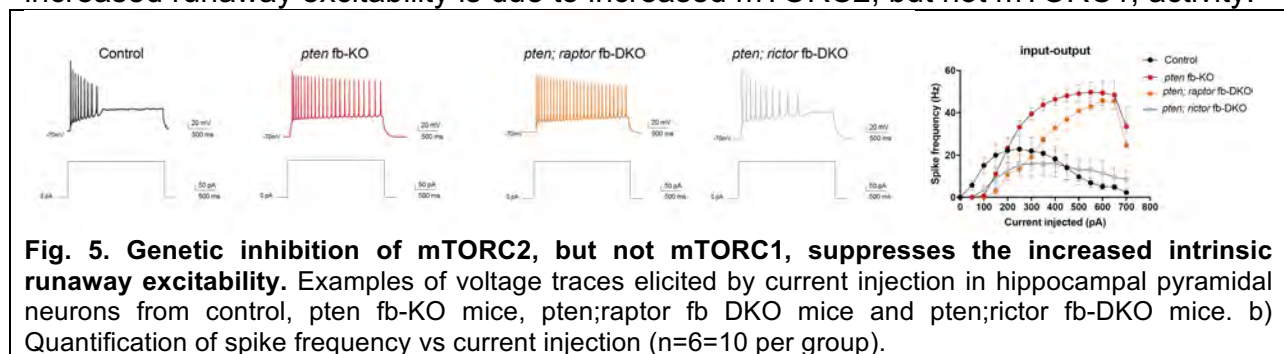


Genetic silencing of mTORC2, but not mTORC1, suppressed seizures and runaway hyperexcitability in pten-deficient mice.

Because both human individuals and mouse models with *Pten* mutations exhibit epilepsy⁸, we next analyzed spontaneous seizures and abnormal electroencephalogram (EEG) activity. We found that *pten* fb-KO mice show abnormal interictal spikes and EEG seizures (**Fig. 4**). Consistent with their survival rates, *pten;raptor* fb-DKO mice also showed tonic-clonic and EEG seizures. By contrast, *pten-rictor* fb-DKO mice showed only some abnormal interictal spikes in the EEG pattern but not EEG or behavioral seizures (**Fig. 4**). Thus, inhibition of mTORC2, but not mTORC1, suppresses the seizures EEG phenotype in *Pten*-deficient mice.



Given the increased excitability in the brain network of *pten*-deficient mice, we next examined whether intrinsic excitability is altered in hippocampal pyramidal neurons. To this end, we performed whole-cell recordings in slices, as we previously described¹³. Neurons fired significantly more action potentials to equivalent step injections. Interestingly, such an increased runaway excitability is restored in *pten*-deficient neurons lacking *riCTOR*, but not *raptor* (Fig. 5). These data indicate that the increased runaway excitability is due to increased mTORC2, but not mTORC1, activity.

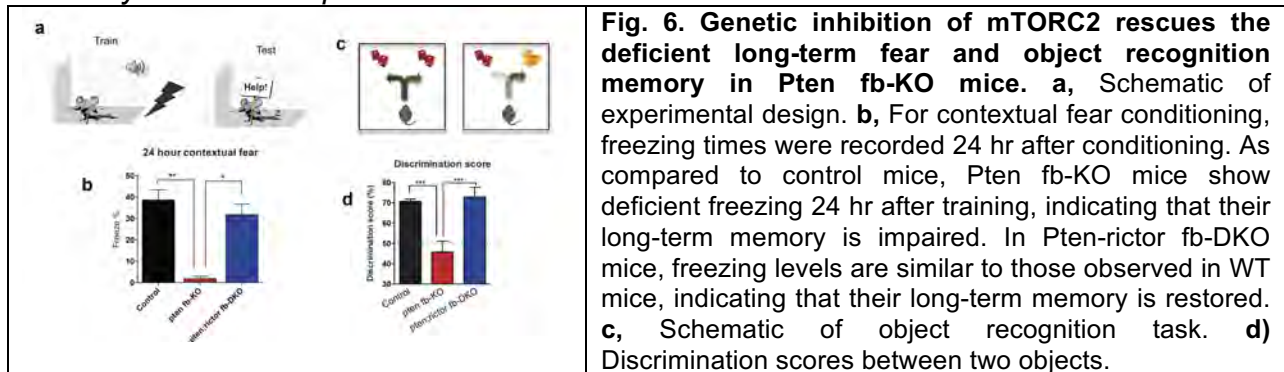


Because in *pten*; *raptor* fb-DKO mice genetic inhibition of mTORC1 had not effect on seizures onset and duration, hyperexcitability and animal survival, we focused on the behavioral characterization of *pten-riCTOR* deficient mice.

Genetic silencing of mTORC2 rescues long-term memory deficits in *pten*-deficient mice. Emotions have a powerful impact on memory; most vivid autographical memories tend to be of emotional effects. In addition, 70-80% of autistic individuals suffer from mental retardation¹⁻³. Thus, we first tested emotional memory, as we previously described^{13,14}. To this end, mice were studied in contextual Pavlovian fear conditioning. Contextual fear conditioning, and hippocampus-dependent task, was induced by pairing a context (conditioned stimulus; CS) with a foot shock (the unconditioned stimulus; US). Mice were subsequently exposed to the auditory tone or visual stimulus (CS) and fear responses [mouse stop moving (“freezes”)] were taken as an index of the strength of

memory (**Fig. 6a**). As expected, compared to control mice, *pten* fb-KO mice showed a dramatic reduction in freezing behavior 24 hr post-training, indicating that their long-term fear memory is impaired (**Fig. 6b**). Strikingly, long-term memory is significantly improved once *ric* is deleted in *pten*-deficient mice. Thus, silencing *mTORC2* activity restores LTM in mice lacking *pten*.

We next studied object recognition memory, another hippocampal dependent task. In this task, an object is presented to the subject mouse. After a 24 hr delay, the object is presented again with a new object (**Fig. 6c**). The time spent exploring each object is tracked via by a computer-operated optical animal activity system (ANIMAZE). We found that *Pten*-deficient mice failed to discriminate between and old and a new object (**Fig. 6d**). However, genetic deletion of *ric* restore the object recognition long-term memory deficits in *Pten*-deficient mice. Taken together, these data indicate that inhibition of *mTORC2*, but not *mTORC1*, restore hippocampal-dependent long-term memory formation in *pten*-deficient mice.



Genetic silencing of mTORC2 rescues social behaviors in *pten*-deficient mice.

Given that social interaction deficits are salient features of ASD individuals², we next studied social behaviors. First, we assessed reciprocal social interactions by recording the amount of time a pair of mice spent interacting in a neutral arena¹⁵ (**Fig. 7a**), as we previously described⁵. We found that compared to control mice, *pten*-deficient mice showed reduced reciprocal interaction (**Fig. 7b**). Interestingly, in *pten-ric* fb-KO mice, reciprocal social interaction is normal. Next, we measured sociability and preference for social novelty using the Crawley 3-chamber test¹⁵ (**Fig. 7c, 7e**). In the sociability task, we compared the time a mouse spends interacting with an empty wired cage and one containing a mouse (**Fig. 5c**); whereas in the social novelty test, we measured the time a mouse spends interacting with a familiar or a stranger mouse (**Fig. 7e**). Consistent with the direct social interaction results, we found that *pten* fb-KO mice had normal sociability (**Fig. 7d**), but showed no preference for interaction with a stranger versus a familiar mouse in the social novelty test (**Fig. 7f**). Strikingly, deletion of *mTORC2* rescues the social novelty deficits in *pten*-deficient mice (**Fig. 7f**). Taken together these data indicate that *pten*-fb KO mice display social deficits and inhibition of *mTORC2* restored their phenotypes.

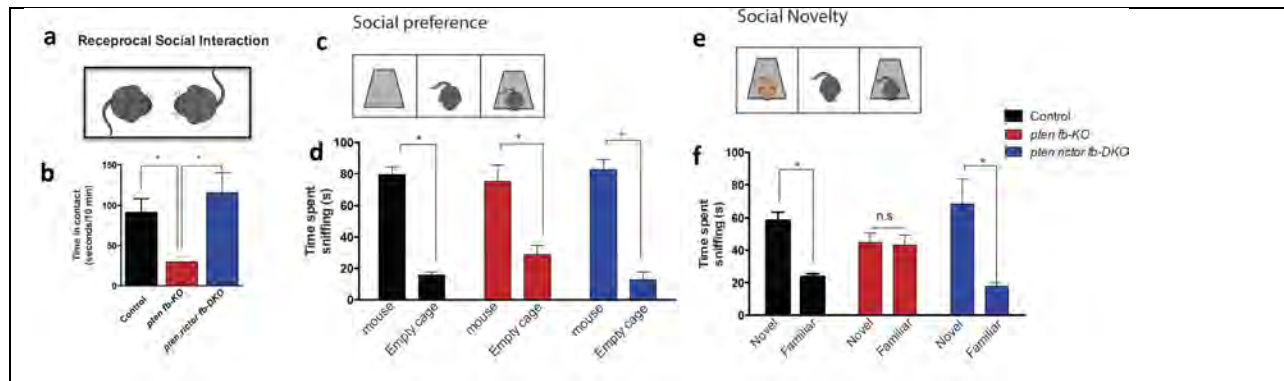


Fig. 7. Social behaviors are normal in *Pten-ric1or fb-DKO* mice. **a)** Schematic of reciprocal social interaction task. **b,** Compared to control or *pten-ric1or fb-DKO* mice, *pten-fb KO* mice showed reduced reciprocal interaction. **c, e,** Schematic of three chamber social interaction task. **f,** Control mice spent most of the time interacting with the stranger mouse but *Pten fb-KO* mice spent equal time interacting with the familiar or strange mouse, indicating that social behavior is impaired in these mice. Like WT mice, *Pten-ric1or fb-KO* mice spent more time interacting with the stranger mouse, indicating that social behavior is restored.

Genetic silencing of mTORC2 rescues repetitive behaviors in *pten*-deficient mice.

Because ASD patients also exhibit repetitive/stereotyped behaviors, we first assessed marble burying, as we previously described⁵. In this task, mice were individually placed in Plexiglas cages containing 5 cm deep fresh bedding, with 20 black marbles pre-arranged in 5x4 evenly spaced rows (**Fig. 8a**). Testing was conducted for 20 min. After the test period, unburied marbles were counted. We found that *pten*-deficient mice buried more marbles than control mice, indicating repetitive behavior (**Fig. 8b**). Remarkably, deletion of *ric1or* (mTORC2) restored the repetitive behavior in *pten*-deficient mice (**Fig. 8b**). We next assessed behavioral flexibility in the T-maze. In this task, normal animals tend to investigate first one and the other arm of the maze (**Fig. 8c**). Notably, *pten*-deficient mice explored the same arm of the maze, demonstrating and impairment in behavioral flexibility (**Fig. 8d**). Remarkably, in *pten-ric1or fb-DKO* mice the behavioral inflexibility behavior is restored (**Fig. 8d**). In conclusion, *our data demonstrate that inhibition of mTORC2 restores memory, social and repetitive behaviors in pten-deficient mice.*

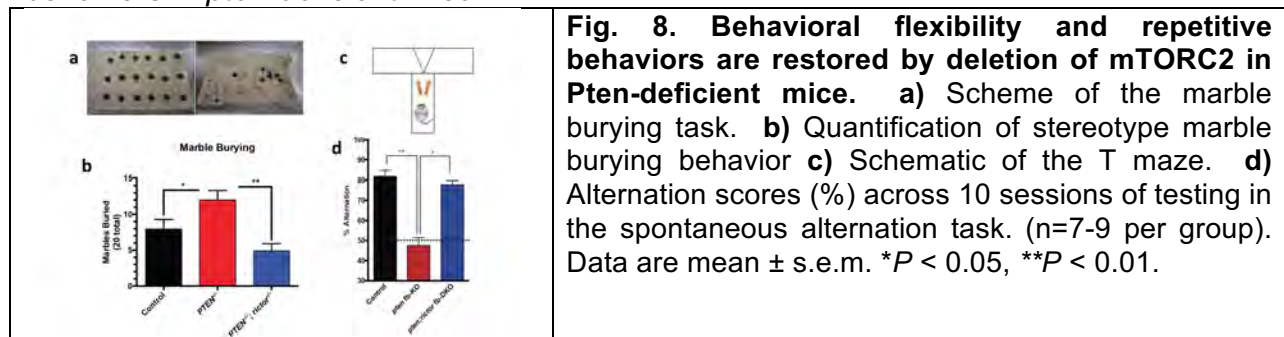
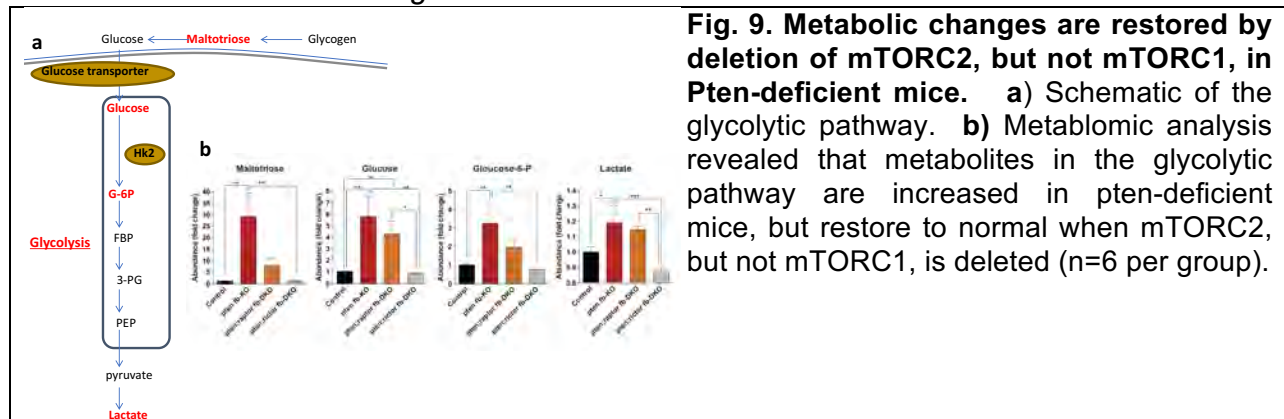


Fig. 8. Behavioral flexibility and repetitive behaviors are restored by deletion of mTORC2 in *Pten*-deficient mice. **a)** Scheme of the marble burying task. **b)** Quantification of stereotype marble burying behavior **c)** Schematic of the T maze. **d)** Alternation scores (%) across 10 sessions of testing in the spontaneous alternation task. (n=7-9 per group). Data are mean $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$.

Genetic inhibition of mTORC2, but not mTORC1, restores key metabolic changes in *pten*-deficient mice.

PTEN regulates changes in metabolism by blocking the Warburg effect^{16,17}, a process in which “cancer” cells catabolize large amounts of glucose through glycolysis. To explore whether the same is true in neurons lacking *pten*, we perform metabolomics from hippocampal samples from control and *pten* deficient mice. Consistent with the idea that PTEN regulates metabolic changes associated with and anti-Warburg effect, we found that *pten*-deficient neurons there is a significant increased in metabolites involved in the glycolytic pathway. Remarkably, suppression of mTORC2, but not mTORC1, activity was sufficient to restore these changes in metabolism associated with the loss of *pten* (**Fig. 9**). Hence, increased mTORC2, but not mTORC1, activity leads to changes in neuronal metabolism associated with the Warburg effect.



Given that a) metabolites in the glycolytic pathway are increased in the brain of *pten* fb-KO mice and b) inhibition of mTORC2 in *pten*-deficient mice reverses not only the metabolic changes, but also the synaptic, network and behavioral abnormalities, we attempted to reduce glycolysis by subjecting *pten*-deficient mice to a ketogenic diet¹⁸. Unfortunately, ketogenic diet failed to reverse the behavioral and synaptic abnormalities in *Pten*-deficient mice. We are currently developing an antisense oligo (ASO) to specifically block mTORC2.

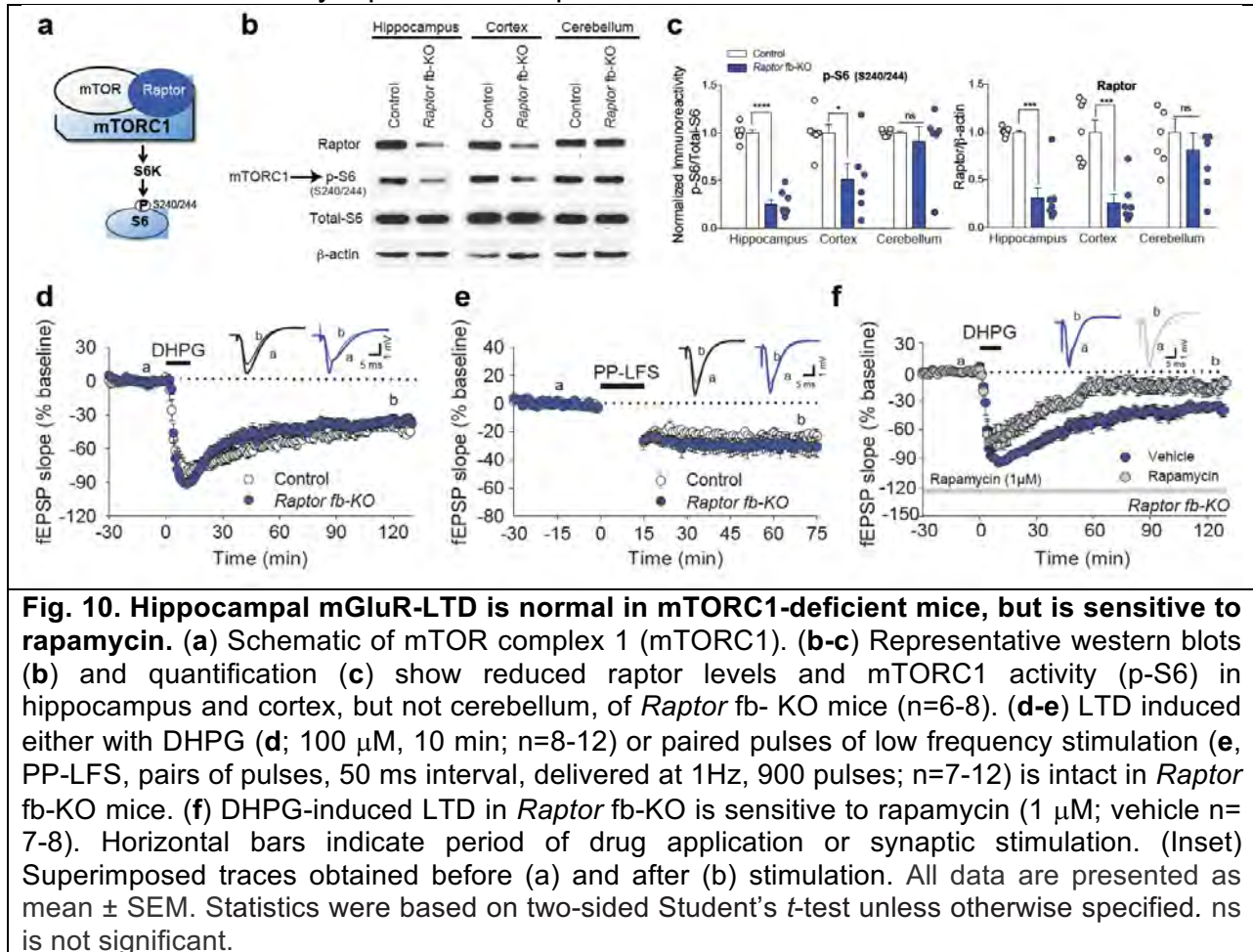
mTORC2, but not mTORC1, is required for mGluR-LTD.

Long-term depression is a major form of synaptic plasticity. Specifically, metabotropic glutamate receptor-mediated long-term depression (mGluR-LTD) is altered in a variety of neurological disorders¹⁹. Thus, the understanding of the molecular mechanisms underlying mGluR-LTD is of crucial relevance because it could lead to the potential development of new treatments for mGluR-LTD-associated cognitive disorders¹⁹. mTORC1 has been postulated to be required for mGluR-LTD. We found that hippocampal mGluR-LTD and associated behaviors are normal in mTORC1-deficient mice.

Deletion of mTORC1's defining component raptor inhibits mTORC1 activity in forebrain structures (**Fig. 10a-c**). Surprisingly, we found that DPHG resulted in normal depression of field excitatory postsynaptic potentials (fEPSPs) in *Raptor* fb-KO slices,

with a magnitude and time course similar to control littermates (**Fig. 10d**). Accordingly, paired pulse stimulation at low frequency (PP-LFS), elicited a similar mGluR-LTD of synaptic transmission in both control and *Raptor* fb-KO slices (**Fig. 10e**). Thus, irrespective of the mGluR-LTD inducing protocol, conditional deletion of mTORC1 in CA1 neurons had no effect on mGluR-induced LTD.

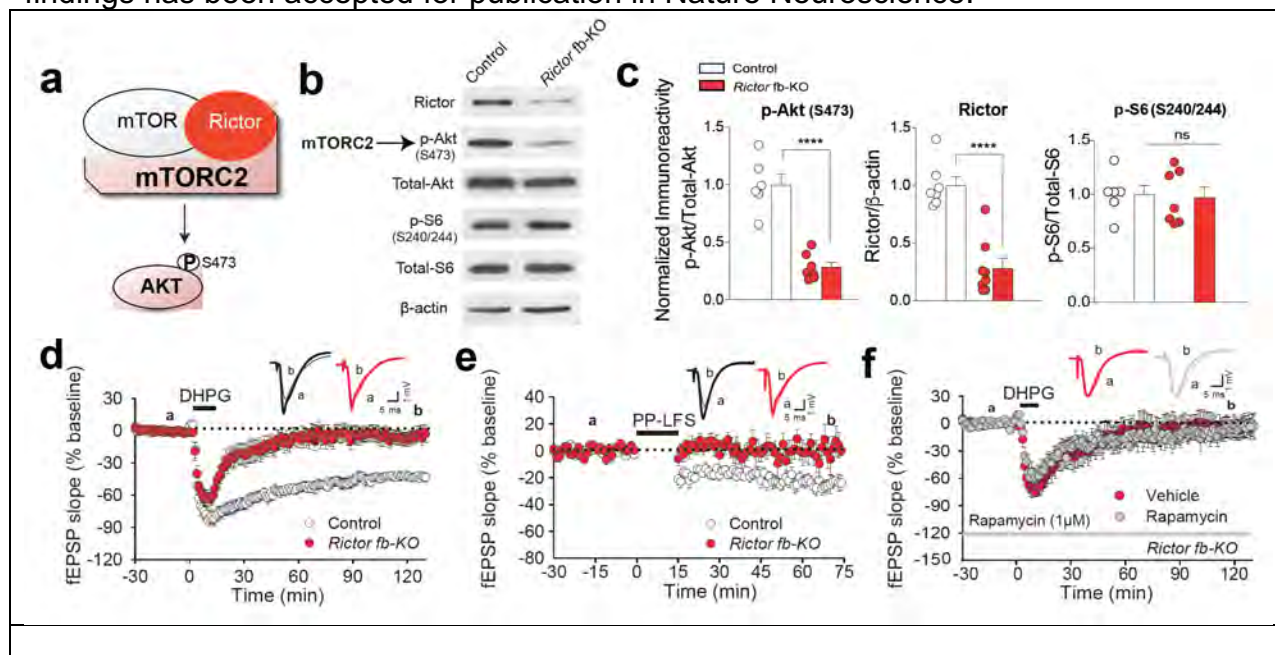
Rapamycin has been shown to inhibit mGluR-LTD^{19,20}. Since rapamycin is reported to be highly specific for mTORC1²¹, it is expected to have no effect on mGluR-LTD in *Raptor* fb-KO mice. However, rapamycin (1 μ M) inhibited mGluR-LTD in *Raptor* fb-KO slices (**Fig. 10f**). Hence, these data support the notion that the effects of rapamycin on mGluR-LTD at CA1 synapses are independent of mTORC1.



In addition to mTORC1, mTORC2 has been identified more recently^{22,23}. While little is known regarding its up-stream regulation and downstream effectors, mTORC2 contains *Rictor* (Rapamycin-insensitive companion of mTOR; **Fig. 2a**) as an essential component that is largely insensitive to acute rapamycin treatment^{22,23}. However, in cancer cells, prolonged rapamycin treatment²⁴ or higher concentrations of rapamycin²⁵ suppress mTORC2 activity. Could mTORC2, but not mTORC1, be the major regulator of mGluR-LTD in the mammalian brain? We began addressing this question by examining whether mGluR activation engages mTORC2 function. We found that treatment with DHPG (100 μ M, 10 min) increased the activity of mTORC2, as determined by the

phosphorylation of its downstream target Akt at Ser-473, a reliable readout of mTORC2 activity^{22,23} (data not shown).

To investigate whether mTORC2 is required for mGluR-LTD at CA1 synapses, we studied mTORC2-deficient mice, in which *Rictor* (mTORC2's defining component. **Fig. 11a**) was conditionally deleted in the murine forebrain postnatally (*Rictor* fb-KO mice)¹⁴. As we have previously shown, mTORC2 activity is selectively reduced in the hippocampus from *Rictor* fb-KO mice (**Fig. 11b-c**) and basal synaptic transmission is not altered in these mice¹⁴. As expected, DHPG induced a typical LTD of fEPSPs in control slices (**Fig. 11d**). However, in *Rictor* fb-KO slices, the same stimulation protocol failed to elicit mGluR-LTD (**Fig. 11d**). In agreement with these observations, synaptic induction of mGluR-LTD with PP-LFS was also impaired in *Rictor* fb-KO slices (**Fig. 11e**). Moreover, a high concentration of rapamycin (1 μ M) did not further reduce mGluR-LTD in *Rictor* fb-KO slices (**Fig. 11f**). Taken together, our results indicate that mTORC2, but not mTORC1, is required for mGluR-LTD. A paper describing these findings has been accepted for publication in Nature Neuroscience.



Finally, mGluR-LTD is altered in a variety of neurological disorders including autism spectrum disorders, intellectual disability, Alzheimer's disease, epilepsy and drug addiction¹⁹. In the last few years, the study of the molecular mechanisms implicated in mGluR-LTD has led to the development of "mechanism-based treatments" for some of these disorders. Unexpectedly, our results support the notion that mTORC2, but not mTORC1, is the major mTOR complex driving mGluR-LTD in the adult mammalian brain. Thus, modulation of mTORC2 may emerge as promising new avenues for the treatment of mGluR-LTD-related disorders.

4. Key Research Accomplishment

- We developed a novel way to selectively silence mTORC2 activity in *pten*-deficient mice.
- We developed a novel way to specifically silence mTORC1 activity in *pten*-deficient mice.
- We found that genetic deletion of mTORC1 selectively restores brain size in *pten*-deficient mice.
- We found that genetic deletion of mTORC2 prolongs the survival of *pten*-deficient mice.
- We found that genetic silencing of mTORC2 dramatically suppressed seizures in *pten*-deficient mice.
- We found that genetic deletion of mTORC2 improves cognitive and social phenotypes in *pten*-deficient mice.
- We found that genetic deletion of mTORC2 improves repetitive behaviors in *pten*-deficient mice.
- We found that genetic silencing of mTORC1 failed to restore animal's survival and seizures phenotype.
- We found that genetic silencing of mTORC2, but not mTORC1, restores the brain metabolic changes in *pten*-deficient mice.
- We found that genetic silencing of mTORC2, but not mTORC1, is required for mGluR-LTD.

5. Conclusion

It has been proposed that increased mTORC1 is responsible for the cellular, synaptic and behavioral abnormalities associated with ASD^{8,26-31}. In addition, it is generally believed that a common molecular mechanism regulates both the anatomical (brain size) and core features (behavioral symptoms) of ASD³². Our new data challenge these views by providing causal evidence that mTOR complexes differentially regulate these processes. We found that a) the up-regulation of mTORC1 is only responsible for the enlarged brain size, whereas, b) persistent activation of mTORC2 activity leads to cognitive decline, ASD-like behaviors, seizures and changes in brain metabolism in the *Pten*-ASD mouse model. In addition, we found that mTORC2, but not mTORC1, is crucially required for mGluR-LTD. Hence, we identified a new signaling pathway (mTORC2) crucially involved in ASD and seizure disorders. Our results may lead to the development of new treatments for ASD and seizure disorders.

6. Publications

a. List all manuscripts submitted for publication during the period covered by this report resulting from this project. mTORC2, but not mTORC1, is required for hippocampal mGluR-LTD and associated behaviors. Zhu PJ, Chen C-J, Mays J, Stoica L, Costa-Mattioli M. **Nature Neuroscience**, In press

Some of these results described above were presented in

- "Catastrophic Epilepsy" at the Neurological Research Institute (NRI), Houston, Texas

- Symposium Society for Neuroscience (see 1: Huber KM, Klann E, Costa-Mattioli M, Zukin RS. Dysregulation of Mammalian Target of Rapamycin Signaling in Mouse Models of Autism. *J Neurosci*. 2015 Oct 14;35(41):13836-42. doi: 10.1523/JNEUROSCI.2656-15.2015. PubMed PMID: 26468183; PubMed Central PMCID: PMC4604222.)
- PI3K-mTOR-PTEN Network in Health and Disease, Cold Spring Harbor, 2016.
- Marine Biological Laboratory Course Wood hole, 2016.
- Federation of Latin American and Caribbean Neuroscience, Buenos Aires, Argentina, 2016.
- UT Health Neuroscience symposium, 2016.
- Rush and Helen Record Neuroscience Retreat, 2017.

7. Inventions, Patents and Licenses.

None

8. Reportable Outcome

Nothing to report

9. Other achievements

We developed forebrain-specific Pten double KO mice.

We developed forebrain-specific rictor-Pten double KO mice.

We developed forebrain-specific raptor-Pten double KO mice.

10. References

- 1 Fombonne, E. The epidemiology of autism: a review. *Psychological medicine* **29**, 769-786 (1999).
- 2 Mefford, H. C., Batshaw, M. L. & Hoffman, E. P. Genomics, intellectual disability, and autism. *N Engl J Med* **366**, 733-743, doi:10.1056/NEJMra1114194 (2012).
- 3 Toro, R. *et al.* Key role for gene dosage and synaptic homeostasis in autism spectrum disorders. *Trends Genet* **26**, 363-372, doi:S0168-9525(10)00107-1 [pii]10.1016/j.tig.2010.05.007 (2010).
- 4 Ghacibeh, G. A. & Fields, C. Interictal epileptiform activity and autism. *Epilepsy & behavior : E&B* **47**, 158-162, doi:10.1016/j.yebeh.2015.02.025 (2015).
- 5 Buffington, S. A. *et al.* Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. *Cell* **165**, 1762-1775, doi:10.1016/j.cell.2016.06.001 (2016).
- 6 Costa-Mattioli, M. & Monteggia, L. M. mTOR complexes in neurodevelopmental and neuropsychiatric disorders. *Nature neuroscience* **16**, 1537-1543, doi:10.1038/nn.3546 (2013).
- 7 Hoeffler, C. A. & Klann, E. mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends in neurosciences* **33**, 67-75, doi:S0166-2236(09)00187-8 [pii]10.1016/j.tins.2009.11.003 (2010).
- 8 Zhou, J. & Parada, L. F. PTEN signaling in autism spectrum disorders. *Current opinion in neurobiology*, doi:S0959-4388(12)00083-9 [pii]10.1016/j.conb.2012.05.004 (2012).
- 9 Kelleher, R. J., 3rd & Bear, M. F. The autistic neuron: troubled translation? *Cell* **135**, 401-406, doi:S0092-8674(08)01308-1 [pii]10.1016/j.cell.2008.10.017 (2008).
- 10 Ma, X. M. & Blenis, J. Molecular mechanisms of mTOR-mediated translational control. *Nat Rev Mol Cell Biol* **10**, 307-318, doi:nrm2672 [pii]10.1038/nrm2672 (2009).

- 11 Hu, W. F., Chahrour, M. H. & Walsh, C. A. The diverse genetic landscape of neurodevelopmental disorders. *Annual review of genomics and human genetics* **15**, 195-213, doi:10.1146/annurev-genom-090413-025600 (2014).
- 12 Mirzaa, G. M. & Poduri, A. Megalencephaly and hemimegalencephaly: breakthroughs in molecular etiology. *American journal of medical genetics. Part C, Seminars in medical genetics* **166C**, 156-172, doi:10.1002/ajmg.c.31401 (2014).
- 13 Zhu, P. J. *et al.* Suppression of PKR promotes network excitability and enhanced cognition by interferon-gamma-mediated disinhibition. *Cell* **147**, 1384-1396, doi:S0092-8674(11)01375-4 [pii]10.1016/j.cell.2011.11.029 (2011).
- 14 Huang, W. *et al.* mTORC2 controls actin polymerization required for consolidation of long-term memory. *Nature neuroscience* **16**, 441-448, doi:10.1038/nn.3351 (2013).
- 15 Silverman, J. L., Yang, M., Lord, C. & Crawley, J. N. Behavioural phenotyping assays for mouse models of autism. *Nature reviews. Neuroscience* **11**, 490-502, doi:nrn2851 [pii]10.1038/nrn2851 (2010).
- 16 Garcia-Cao, I. *et al.* Systemic elevation of PTEN induces a tumor-suppressive metabolic state. *Cell* **149**, 49-62, doi:10.1016/j.cell.2012.02.030 (2012).
- 17 Ortega-Molina, A. & Serrano, M. PTEN in cancer, metabolism, and aging. *Trends in endocrinology and metabolism: TEM* **24**, 184-189, doi:10.1016/j.tem.2012.11.002 (2013).
- 18 Schwartz, L., Seyfried, T., Alfarouk, K. O., Da Veiga Moreira, J. & Fais, S. Out of Warburg effect: An effective cancer treatment targeting the tumor specific metabolism and dysregulated pH. *Seminars in cancer biology*, doi:10.1016/j.semcancer.2017.01.005 (2017).
- 19 Luscher, C. & Huber, K. M. Group 1 mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease. *Neuron* **65**, 445-459, doi:10.1016/j.neuron.2010.01.016 (2010).
- 20 Hou, L. & Klann, E. Activation of the phosphoinositide 3-kinase-Akt-mammalian target of rapamycin signaling pathway is required for metabotropic glutamate receptor-dependent long-term depression. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **24**, 6352-6361 (2004).
- 21 Benjamin, D., Colombi, M., Moroni, C. & Hall, M. N. Rapamycin passes the torch: a new generation of mTOR inhibitors. *Nat Rev Drug Discov* **10**, 868-880, doi:10.1038/nrd3531 (2011).
- 22 Laplante, M. & Sabatini, D. M. mTOR signaling in growth control and disease. *Cell* **149**, 274-293, doi:S0092-8674(12)00351-0 [pii]10.1016/j.cell.2012.03.017 (2012).
- 23 Wullschleger, S., Loewith, R. & Hall, M. N. TOR signaling in growth and metabolism. *Cell* **124**, 471-484 (2006).
- 24 Sarbassov, D. D. *et al.* Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Molecular cell* **22**, 159-168 (2006).
- 25 Toschi, A. *et al.* Regulation of mTORC1 and mTORC2 complex assembly by phosphatidic acid: competition with rapamycin. *Mol Cell Biol* **29**, 1411-1420, doi:10.1128/MCB.00782-08 (2009).
- 26 Ehninger, D. *et al.* Reversal of learning deficits in a Tsc2(+/-) mouse model of tuberous sclerosis. *Nature medicine* (2008).
- 27 Ehninger, D., de Vries, P. J. & Silva, A. J. From mTOR to cognition: molecular and cellular mechanisms of cognitive impairments in tuberous sclerosis. *Journal of intellectual disability research : JIDR* **53**, 838-851, doi:JIR1208 [pii]10.1111/j.1365-2788.2009.01208.x (2009).
- 28 Ehninger, D. & Silva, A. J. Rapamycin for treating Tuberous sclerosis and Autism spectrum disorders. *Trends in molecular medicine* **17**, 78-87, doi:S1471-4914(10)00148-6 [pii]10.1016/j.molmed.2010.10.002 (2011).

- 29 Carson, R. P., Van Nielen, D. L., Winzenburger, P. A. & Ess, K. C. Neuronal and glia abnormalities in Tsc1-deficient forebrain and partial rescue by rapamycin. *Neurobiology of disease* **45**, 369-380, doi:S0969-9961(11)00290-7 [pii]10.1016/j.nbd.2011.08.024 (2012).
- 30 Tsai, P. T. *et al.* Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice. *Nature* **488**, 647-651, doi:nature11310 [pii]10.1038/nature11310 (2012).
- 31 Wong, M. Mammalian target of rapamycin (mTOR) inhibition as a potential antiepileptogenic therapy: From tuberous sclerosis to common acquired epilepsies. *Epilepsia* **51**, 27-36, doi:EPI2341 [pii]10.1111/j.1528-1167.2009.02341.x (2011).
- 32 Ehninger, D., Li, W., Fox, K., Stryker, M. P. & Silva, A. J. Reversing neurodevelopmental disorders in adults. *Neuron* **60**, 950-960, doi:S0896-6273(08)01055-6 [pii]10.1016/j.neuron.2008.12.007 (2008).

11. Appendices

N/A