Published in final edited form as:

Neuroreport. 2019 May 22; 30(8): 556–561. doi:10.1097/WNR.0000000000001241.

Neuronal deletion of *Pten* results in cerebellar motor learning dysfunction and alterations in intracellular signaling

Suzanne O. Nolan¹, Taylor S. Jefferson², Conner D. Reynolds³, Gregory D. Smith⁴, Andrew J. Holley¹, Samantha L. Hodges⁵, and Joaquin N. Lugo^{1,5}

¹Department of Psychology and Neuroscience, Baylor University, Waco, TX 76798, USA,

²Department of Physiology, Feinberg School of Medicine, Northwestern University, Chicago, IL, 60611,

³Texas College of Osteopathic Medicine, University of North Texas Health Science Center, Fort Worth, TX 767107, USA,

⁴David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, 90024, USA,

⁵Institute of Biomedical Studies, Baylor University, Waco, TX 76798, USA

Abstract

The purpose of this investigation was to examine cerebellar levels of several molecular signaling pathways, including PI3K/AKT/mTOR signaling and markers of neuronal migration, following loss of the *Pten* (phosphatase and tensin homolog) gene in a subset of neurons, as well as the accompanying behavior phenotype in mice. Motor coordination and learning were measured by the sticker removal task and the accelerating rotarod. Western blots were conducted on cerebellar tissue samples. We demonstrated that neuron-specific deletion of *Pten* (NS-*Pten*) in mice led to deficits in motor coordination. These changes were accompanied by alterations in many different proteins, including the PI3K/AKT/mTOR signaling pathway, FMRP, glutamate receptors and neuronal migration markers. These data firstly support a role for hyperactivation of mTOR in the cerebellum following loss of *Pten*, accompanied by behavioral deficits. Moreover, the results of the current study support a broader role for *Pten* signaling in early neuronal migration and organization of the cerebellum, and point to a putative role for *Pten* for many neuropsychiatric conditions.

Keywords

Motor coordination; Mouse genetics; Focal cortical dysplasia; mTOR signaling

*Corresponding author: Joaquin N. Lugo, PhD, Baylor University, Department of Psychology and Neuroscience, One Bear Place # 97334, Waco, TX 76798, Phone: 254-710-2389; Fax: 254-710-3033, joaquin_lugo@baylor.edu.

INTRODUCTION

The mammalian target of rapamycin (mTOR) signaling pathway has been demonstrated as a critical regulator of many cell biological processes, including transcription and actin dynamics, thereby influencing many broad biological processes [1]. Dysregulation of the mTOR signaling pathway in the central nervous system has been implicated as a disease mechanism underlying a variety of neurological conditions including tuberous sclerosis complex (*TSC1/2*), Fragile X syndrome (*FMR1*), and schizophrenia [2]. Among the behavioral phenotypes linked to dysregulation of different components of mTOR signaling are deficits in cerebellar functioning. For example, loss of ubiquitin-protein ligase E3A (UBE3A) results in overactivation of mTOR signaling, and is accompanied by deficits in accelerated rotarod learning [3]. Similarly, loss of *TSC1*, a negative regulator of mTOR signaling, in cerebellar Purkinje cells results in hyperactivation of the mTOR pathway and accompanying behavioral dysfunction [4]. However, it seems that a normal baseline of mTOR pathway is necessary since reduction in the mTOR pathway by pharmacological and genetic inhibition also results in diminished cerebellar-dependent motor learning [5]. Overall, these findings support a role for homeostatic mTOR signaling in proper functioning of the cerebellum.

Located on chromosome ten, the human phosphatase and tensin homolog (*PTEN*) gene codes for a tumor suppressor protein that acts as a repressor of the PI3K/AKT/mTOR pathway by promoting the dephosphorylation of PIP₃. Even though *Pten*, *TSC1*, and UBE3A function differently within the pathway, loss of function mutations to these genes result in similar net hyperactivation of mTOR signaling across several regions of the brain [6–8]. For example, loss of *Pten* in a subset of neurons results in hyperactivation of mTOR in the hippocampal and cortex [6]. Following this conditional loss of *Pten*, adult mice display behavioral alterations in sociability, repetitive behavior, and hyperactivity [6]. However, despite what is known about the role of mTOR signaling and the cerebellar functioning, the loss of *Pten* on key signaling markers in the cerebellum and the accompanying motor behavior phenotype in this model is not well understood.

The purpose of this investigation is to examine cerebellar-dependent behavioral abnormalities and accompanying molecular signaling pathways, following the loss of *Pten*. The neuronal subset-specific knockout of *Pten* (NS-*Pten*^{-/-} model) used in this experiment eliminates *Pten* in a select subset of post-mitotic neurons in the cortex, cerebellum and hippocampus. Here we expand on previous work by demonstrating that neuron-specific deletion of *Pten* in mice led to specific deficits in motor coordination. These changes were accompanied by alterations in protein levels associated with the PI3K/AKT/mTOR signaling pathway, synaptic proteins, and neuronal migration. This study presents further evidence supporting a role for *Pten* and constitutive mTOR signaling in the proper development and functionality of the cerebellum.

METHODS

Animals

Neuron subset-specific *Pten*^{+/-} (NS-*Pten*) parents were bred to produce NS- *Pten*^{+/+} mice and NS- *Pten*^{-/-} mice as previously described [9], at Baylor University. All pups were housed with parents and littermates until weaning on postnatal day (PD) 21. The light cycle was kept at 14 hr. light/ 10 hr. dark cycle (lights on/off at 6:00/20:00), and the colony room was kept at an ambient temperature of 22° C. Animals had *ad libitum* access to food and water. All procedures performed were in accordance with Baylor University Institutional Animal Care and Use Committee, as well as the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. All testing was conducted during the light cycle, specifically between 9 am and 2 pm, at 6–8 weeks of age. Two different cohorts of animals were used for behavioral testing: one for accelerating rotarod and one for sticker removal.

Accelerating Rotarod

To assess motor learning and coordination, performance on the accelerating rotarod task was measured, according to previous methods [10] with a sample size of WT = 29 and NS-*Pten*^{-/-} = 21. Briefly, subjects were tested on two trials per day, 60 minutes apart, for four days, on the gradually accelerating apparatus (Series 8 Rotorod; IITC Inc., Woodland Hills, CA, USA). The inter-trial interval was 60 minutes. An experimenter blinded to subject identity scored the latency for the animal to fall off the rotating rod. Between trials and prior to the first trial, the apparatus was cleaned thoroughly with a 30% isopropanol.

Sticker Removal Task

A separate cohort was used to evaluate sticker removal latency according to previous methods, which has been shown to measure forelimb coordination [11] with a sample size of WT = 17 and NS-*Pten*^{-/-} = 13. Briefly, animals were rendered immobile by scruffing and a 0.635 cm round adhesive sticker (Avery Dennison, Glendale, CA, USA) was placed on the nose using small forceps. The mouse was then placed into the home cage with a lid. An experimenter recorded the amount of time to the first attempt to remove the label with forepaws, as well as the latency to completely remove the label. If the mouse did not remove the sticker in 60 seconds, the mouse was removed from the testing area and the sticker was removed by the experimenter. Each mouse was given three non-consecutive trials. There was an inter-trial interval of 5 minutes.

Western Blotting

Adult mice (> PD60) were sacrificed by rapid decapitation with surgical scissors. The brain was removed and rinsed in 1X phosphate buffer (PBS) solution. Using previously described methods [6], cerebellar hemispheres were then rapidly dissected from each hemisphere and rapidly rinsed in ice cold 1X PBS then frozen on dry ice before stored in microcentrifuge tubes at -80 °C until processing. Using the procedure described previously, total homogenate and crude synaptosome (P2) samples were produced [12]. Cerebellar synaptosomal samples were used for the following targets: FMRP, phosphorylated-FMRP, Kv4.2, mGluR1/5, nR2A, pan-shank, PSD-95, Sap102, Sapap1. Cerebellar homogenate

samples were used for the following targets: AKT, phosphorylated-AKT (s473), DAB1, ERK, phosphorylated-ERK, s6 ribosomal protein, phosphorylated-s6 (s235/236), and phosphorylated-s6 (s240/244). The samples were diluted in Laemmli buffer, loaded and run through SDS – Page then transferred to Hybond-P polyvinyl difluoride membranes overnight (GE Healthcare, Piscataway, NJ, USA) [12]. Membranes were incubated at 1 hour at room temperature in blocking solution (5% nonfat milk diluted in 1 X Tris buffered saline (50 mM Tris-HCL, pH 7.4, 150 mM NaCl, 2.7 mM KCl) with 0.1 % Tween (1 X Tris Buffered Saline (TTBS)). Following blocking, membranes were incubated overnight at 4° C with primary antibodies diluted in the 5% milk solution. Following the overnight incubation, membranes were rinsed in 1X TTBS 3 times for 5 minutes each wash. Membranes were then incubated with horseradish peroxidase-labeled secondary antibody immunoglobulin G for 1 hour. Following the final incubation, membranes were washed in 1X TTBS 3 times for 5 minutes before being incubated with GE ECL Prime (GE Healthcare). Following all incubation and rinsing steps, immunoreactive bands were captured using the ProteinSimple western blot imaging system (ProteinSimple, Santa Clara, CA, USA). Information regarding primary and secondary antibodies and their concentrations, as well as the sample type used can be found in Supplemental Digital Content 1. Following image capture, ProteinSimple AlphaView software was used to quantify the optical density of the bands on the blot. Measurements from all bands were normalized to each subject's actin level. All experimental data points represent a single mouse ($n = 1$). All groups were normalized to the average of the WT group per blot.

Statistical Analysis

All data were analyzed using GraphPad Software 7.0 (San Diego, CA) or IBM SPSS Statistics 23.0 (Aramonk, NY). Results were evaluated using a repeated-measures two-way (Genotype [wildtype, heterozygote, knockout]) analysis of variance (ANOVA) on each variable for the specific test. Independent samples *t*-tests were then run to examine genotype effects at each time point measured. The analyses for the accelerating rotarod and the sticker removal task also included a repeated measure (trial). For western blotting, optical densities were evaluated using independent samples *t*-tests. Any violations of the assumption of homogeneity of variance resulted in the use of the non-parametric Mann-Whitney *U* test.

RESULTS

Deletion of *Pten* in neurons results in diminished performance on the accelerating rotarod task and the sticker removal task.

Performance on the accelerating rotarod task was analyzed using a repeated measures two-way ANOVA for genotype, with a within-subjects variable of trial (8 trials). Results indicated a main effect of genotype, such that NS-*Pten*^{-/-} mice performed worse across all of the trials of the accelerating rotarod, $F_{\text{genotype}(1,46)} = 11.07$, $P < 0.01$ (Figure 1a). There was a main effect of time $F_{\text{genotype}(7,336)} = 10.6$, $P < 0.001$. There was a marginal group $\times$ trial interaction $F_{\text{genotype} \times \text{trial}(7, 336)} = 1.92$, $P = 0.06$. We then ran separate *t*-tests across each trial and found statistically significant differences at many of the trials: trial 1 $t(1,48) = 6.7$, $P > 0.05$, trial 2 $t(1,48) = 7.11$, $P < 0.05$, trial 3 $t(1,48) = 29.3$, $P < 0.01$, trial 4 $t(1,48) =$

15.1, $P > 0.05$, trial 5 $t(1,48) = 28.4$, $P < 0.01$, trial 6 $t(1,48) = 36.6$, $P < 0.001$, trial 7 $t(1,48) = 32.7$, $P < 0.05$, and trial 8 $t(1,48) = 23.2$, $P < 0.05$.

Performance on the sticker removal task was analyzed using a repeated measures two-way ANOVA for genotype, with a within-subjects variable of trial (3 trials). Results for between-subjects effects indicated NS-*Pten*^{-/-} mice displayed a trending significance for a main effect of genotype, but this effect did not quite reach statistical significance, $F_{\text{genotype}}(1, 27) = 3.46$, $P = 0.07$ (Figure 1b). Results for tests of within-subjects indicated no significant effects, $F_{\text{trial}}(2, 54) = 2.32$, $P = 0.11$, $F_{\text{trial} \times \text{genotype}}(2, 54) = 0.83$, $P = 0.44$. We then ran separate *t*-tests across each trial and found no difference between the WT and NS-*Pten*^{-/-} mice at the first trial, $t(1,29) = 1.45$, $P > 0.05$. We did find differences between the group at the second trial $t(1,29) = 15.15$, $P < 0.05$ and during the third trial $t(1,29) = 16.62$, $P < 0.05$.

Deletion of *Pten* in neurons results in aberrant changes in several molecular signaling cascades.

Many perturbations across different molecular signaling cascades, such as mTOR and DAB1, were detected in cerebellar synaptosomal and total homogenate preparations. Overall, results indicated hyperactivation of the PI3K/AKT/mTOR signaling pathway. Specifically, significant increases were noted in the ratio of phosphorylated AKT (s473) to total AKT, $U = 0$, $P < 0.0001$, and the ratio of phosphorylated s6 at the serine 235/236 site to total s6 in total preparations, $t(16) = 3.61$, $P = 0.002$ (Figures 2a–b). Similar to previous findings, we also detected increases in both FMRP, $U = 37$, $P = 0.001$, and the ratio of phosphorylated FMRP to total FMRP, $U = 48$, $P = 0.007$ (Figures 2c–d). There were increased protein levels of the potassium voltage-gated ion channel, Kv4.2, $t(16) = 4.34$, $P = 0.0005$, (Figure 2e) and increased levels of different glutamate receptor related proteins, such as mGluR1/5, $U = 4$, $P < 0.0001$, (Figure 2f) and NR2A, $U = 54$, $P = 0.015$ (Figure 2g). Increased levels of synapse-associated protein 102 (SAP-102), $U = 3$, $P = 0.0003$ (Figure 2h) was also detected. Finally, we detected increased DAB1 protein levels in total preparations, $t(28) = 3.02$, $P = 0.005$ (Figure 2i). No changes were detected in total preparations of AKT, $t(27) = 0.19$, $P = 0.85$. No changes were detected in synaptosomal preparations of the following proteins: Pan-shank, $t(28) = 1.34$, $P = 0.19$; PSD-95, $t(27) = 1.18$, $P = 0.10$; Sapap1, $t(16) = 1.48$, $P = 0.16$; ERK, $t(10) = 0.11$, $P = 0.91$; the ratio of phosphorylated ERK to total ERK, $U = 18$, $P = 0.99$; s6 kinase, $t(28) = 0.22$, $P = 0.82$; or the ratio of phosphorylated s6 at the serine 240 site to total s6, $t(28) = 0.77$, $P = 0.47$.

DISCUSSION

In the present study, we first confirmed that deletion of *Pten* in the cerebellum results in hyperactivation of mTOR. We found increased levels of several key components in the mTOR signaling cascade, including AKT and s6. These results support the hypothesis that loss of *Pten* signaling results in hyperactivation of mTOR signaling in the cerebellum, similar to previous work in the hippocampus and cortex [6]. We also found increased levels of both FMRP and phosphorylated FMRP, within the cerebellum. Previous evidence from our lab has demonstrated that FMRP is increased in the *Pten* knockout mice in the hippocampus [6]. Given that FMRP is hypothesized to be involved in the inhibition of

translation of small proteins, particularly several ion channel proteins [13–15] it follows that we found increased levels of Kv4.2, similar to our previous finding in the hippocampus [6]. Kv4.2 channels are important for A-type current and play an important role in neuronal excitability [16]. We also found an increase in the postsynaptic protein Sap102. Many of the western blotting findings in the cerebellum are similar to those we reported in the hippocampus in a previous study [6]. However, one difference we found was that in the cerebellum we found an increase in protein levels of glutamate receptor subunit NR2A and mGluR1/5. Previously our lab demonstrated decreases in these proteins in the hippocampus, suggesting a differential role for glutamate signaling across the different brain regions of the NS-*Pten*^{-/-} mouse.

Similar to westerns, the behavioral findings in our study are in line with findings using another regulator of the mTOR pathway, *Tsc1*, which when deleted results in hyperactivation of the mTOR pathway. Mice with deletion of *Tsc1* have a learning impairment in the accelerating rotarod task compared to wildtype mice [4]. However, reduction in mTOR can also adversely affect motor behavior. Inhibition of mTOR through the pharmacological inhibitor rapamycin and genetic knockdown of mTOR by siRNA results in impaired motor learning in the accelerating rotarod test [5]. Moreover, this impaired motor learning phenotype may be due to impairments in AMPA receptor trafficking related to altered mTOR signaling, which is required for stable memory formation [17, 18]. Overall, our results add to the growing body of literature that mTOR signaling is necessary for proper motor learning.

However, while in line with previous evidence concerning mTOR signaling and cerebellar learning, it is worth noting that our results in the behavioral tasks are different from previous studies examining the impact of deletion of *Pten* on performance in the accelerating rotarod task, which did not find evidence of impaired rotarod performance [9]. However, these conflicting results could be explained by methodological differences and the use of a different mouse strain. In the Kwon study, they used a C57BL6 strain and not the mixed FVN background strain we used in our experiment. The background genetics of the mouse could impact the behavioral phenotype in the cerebellar tasks. In addition, trials were run with three sets of three trials per day for 5 days, with 10 minutes in between. In our study, the paradigm was two trials per day, separated by 60 minutes, for a total of 4 days. It is possible that in order to detect this subtle motor coordination deficit, a longer period of rest for consolidation between trials is necessary, with less trials in total [19]. Accordingly, we hypothesize that NS-*Pten*^{-/-} mice may have impairments in the types of protein synthesis that occurs during this consolidation period. This hypothesis is further supported by acknowledging the role of mTOR signaling in the cellular mechanisms of motor learning consolidation, [5] and the dysregulation of this pathway demonstrated in the current study.

A final important finding of the present study is the increase in DAB1 protein levels in the cerebellum. A previous study using the NEX-*Pten* knockout line, a mouse line with deletion of *Pten* in excitatory neurons in the forebrain, reported aberrant migration of neurons in the forebrain concurrent with a significant increase in DAB1 levels [7]. DAB1 and the Reelin signaling pathway are key players in neuronal positioning [20]. In addition to forebrain organization, it is also hypothesized that *Pten* plays a key role in cerebellar lamination

during early development. When *Pten* is deleted from Purkinje neurons, histological analyses demonstrate loss of cerebellar foliation [21]. Aside from the role of Purkinje cells, analyses in *Pten* mutants also reveal that granule cells fail to migrate to the appropriate location, instead proliferating near the pial surface in the external germinal layer of the cerebellum, maturing in this location [22]. Recent evidence coming to light supports an expanded role for cerebellar granule cells, particularly in the context of motor learning [23]. Thus, it is possible that altered migration of these granule cells may contribute to the aberrant motor phenotype demonstrated in the current studies, and future studies should expand on this relationship, to examine the role aberrant migration of these granule cells in other aspects of the *Pten* phenotype.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

SN analyzed the data and wrote the manuscript. JNL planned and organized the study and reviewed the manuscript. TJ, CR, GS and AH collected and organized the data and reviewed the manuscript. The authors would like to thank Matthew Binder for his helpful review and other members of the Lugo lab for their contributions to the lab.

CONFLICTS OF INTEREST AND SOURCES OF FUNDING

The authors confirm that this article content has no conflict of interest. This work was supported by the National Institutes of Health (NIH) [Grant Number: NS088776] and through a FRIP and URC grant from Baylor University.

REFERENCES

- [1]. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012; 149: 274–93. [PubMed: 22500797]
- [2]. Costa-Mattioli M, Monteggia LM. mTOR complexes in neurodevelopmental and neuropsychiatric disorders. *Nat Neurosci* 2013; 16: 1537–43. [PubMed: 24165680]
- [3]. Sun J, Liu Y, Moreno S, Baudry M, Bi X. Imbalanced mechanistic target of rapamycin C1 and C2 activity in the cerebellum of angelman syndrome mice impairs motor function. *J Neurosci* 2015; 35: 4706–18. [PubMed: 25788687]
- [4]. Tsai PT, Hull C, Chu Y, Greene-Colozzi E, Sadowski AR, Leech JM, et al. Autistic-like behavior and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice. *Nature* 2012; 488: 647–51. [PubMed: 22763451]
- [5]. Bergeron Y, Chagniel L, Bureau G, Massicotte G, Cyr M. mTOR signaling contributes to motor skill learning in mice. *Front Mol Neurosci* 2014; 7: 26. [PubMed: 24772063]
- [6]. Lugo JN, Smith GD, Arbuckle EP, White J, Holley AJ, Floruta CM, et al. Deletion of PTEN produces autism-like behavioral deficits and alterations in synaptic proteins. *Front Mol Neurosci* 2014; 7: 27. [PubMed: 24795561]
- [7]. Kazdoba TM, Sunnen CN, Crowell B, Lee GH, Anderson AE, D’Arcangelo G. Development and characterization of NEX- Pten, a novel forebrain excitatory neuron-specific knockout mouse. *Dev Neurosci* 2012; 34: 198–209. [PubMed: 22572802]
- [8]. Nikolaeva I, Kazdoba TM, Crowell B, D’Arcangelo G. Differential roles for Akt and mTORC1 in the hypertrophy of Pten mutant neurons, a cellular model of brain overgrowth disorders. *Neuroscience* 2017; 354: 196–207. [PubMed: 28457820]
- [9]. Kwon C-H, Luikart BW, Powell CM, Zhou J, Matheny SA, Zhang W, et al. Pten Regulates Neuronal Arborization and Social Interaction in Mice. *Neuron* 2006; 50: 377–88. [PubMed: 16675393]

- [10]. Holley AJ, Hodges SL, Nolan SO, Binder M, Okoh JT, Ackerman K, et al. A single seizure selectively impairs hippocampal-dependent memory and is associated with alterations in PI3K/Akt/mTOR and FMRP signaling. A single seizure selectively impairs hippocampal-dependent memory and is associated with alterations in PI3K/Akt/mTOR and FMRP signaling 2018; 3: 511–23.
- [11]. Schrimsher GW, Reier PJ. Forelimb motor performance following cervical spinal cord contusion injury in the rat. *Exp Neurol* 1992; 117: 287–98. [PubMed: 1397165]
- [12]. Lugo JN, Barnwell LF, Ren Y, Lee WL, Johnston LD, Kim R, et al. Altered phosphorylation and localization of the A-type channel, Kv4.2 in status epilepticus. *J Neurochem* 2008; 106: 1929–40. [PubMed: 18513371]
- [13]. Gross C, Yao X, Pong DL, Jeromin A, Bassell GJ. Fragile X mental retardation protein regulates protein expression and mRNA translation of the potassium channel Kv4.2. *J Neurosci* 2011; 31: 5693–8. [PubMed: 21490210]
- [14]. Lee HY, Ge WP, Huang W, He Y, Wang GX, Rowson-Baldwin A, et al. Bidirectional regulation of dendritic voltage-gated potassium channels by the fragile X mental retardation protein. *Neuron* 2011; 72: 630–42. [PubMed: 22099464]
- [15]. Routh BN, Johnston D, Brager DH. Loss of functional A-type potassium channels in the dendrites of CA1 pyramidal neurons from a mouse model of fragile X syndrome. *J Neurosci* 2013; 33: 19442–50. [PubMed: 24336711]
- [16]. Shibata R, Wakazono Y, Nakahira K, Trimmer JS, Ikenaka K. Expression of Kv3.1 and Kv4.2 Genes in Developing Cerebellar Granule Cells. *Dev Neurosci* 1999; 21: 87–93. [PubMed: 10449980]
- [17]. Gleason SD, Kato A, Bui HH, Thompson LK, Valli SN, Stutz PV, et al. Inquiries into the Biological Significance of Transmembrane AMPA Receptor Regulatory Protein (TARP) gamma-8 Through Investigations of TARP gamma-8 Null Mice section sign. *CNS Neurol Disord Drug Targets* 2015; 14: 612–26. [PubMed: 25921737]
- [18]. Wang Y, Barbaro MF, Baraban SC. A role for the mTOR pathway in surface expression of AMPA receptors. *Neurosci Lett* 2006; 401: 35–9. [PubMed: 16677760]
- [19]. Luft AR, Buitrago MM. Stages of motor skill learning. *Mol Neurobiol* 2005; 32: 205–16. [PubMed: 16385137]
- [20]. D'Arcangelo G, Miao GG, Chen SC, Soares HD, Morgan JI, Curran T. A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature* 1995; 374: 719–23. [PubMed: 7715726]
- [21]. Nayeem N, Kerr F, Naumann H, Linehan J, Lovestone S, Brandner S. Hyperphosphorylation of tau and neurofilaments and activation of CDK5 and ERK1/2 in PTEN-deficient cerebella. *Mol Cell Neurosci* 2007; 34: 400–8. [PubMed: 17208451]
- [22]. Kwon CH, Zhu X, Zhang J, Knoop LL, Tharp R, Smeyne RJ, et al. Pten regulates neuronal soma size: a mouse model of Lhermitte-Duclos disease. *Nat Genet* 2001; 29: 404–11. [PubMed: 11726927]
- [23]. Becker MI, Person AL. Cerebellar granule cells expand their talents. *Nat Neurosci* 2017; 20: 633. [PubMed: 28440806]

Figure 1. Motor Coordination and Learning Deficits in NS-*Pten*^{-/-} mice.

(A) NS-*Pten*^{-/-} mice show deficits in cerebellar motor coordination in the accelerating rotarod, compared to WT mice. (B) In a second cohort of animals, cerebellar motor coordination deficits impeded performance on the sticker removal task in NS-*Pten*^{-/-} mice. Data are expressed as mean ± SEM. * = $P < 0.05$.

Figure 2. Deletion of *Pten* in neurons results in alterations in mTOR signaling protein expression, as well as molecular and histological changes in cortical migration patterning.

(A) NS-*Pten*^{-/-} mice show hyperactivation of the mTOR signaling pathway for pAKT/total AKT and (B) pS6(s235/236)/total. (C-I) There was also a significant increase in FMRP, pFMRP/total FMRP, Kv4.2, mGluR1/5, NR2A subunit, and the scaffolding protein SAP102 in the NS-*Pten*^{-/-} mice. (J) One additional observation was a significant increase in DAB1 in the NS-*Pten*^{-/-} mice. Data are expressed as mean ± SEM. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.