Journal Pre-proofs

Research article

Vorinostat, a histone deacetylase inhibitor, ameliorates the sociability and cognitive memory in an *Ash1L*-deletion-induced ASD/ID mouse model

Yuen Gao, Mohammad B. Aljazi, Yan Wu, Jin He

PII: S0304-3940(21)00620-0
DOI: https://doi.org/10.1016/j.neulet.2021.136241
Reference: NSL 136241

To appear in: *Neuroscience Letters*

Received Date: 12 July 2021
Revised Date: 6 September 2021
Accepted Date: 7 September 2021

Please cite this article as: Y. Gao, M.B. Aljazi, Y. Wu, J. He, Vorinostat, a histone deacetylase inhibitor, ameliorates the sociability and cognitive memory in an *Ash1L*-deletion-induced ASD/ID mouse model, *Neuroscience Letters* (2021), doi: https://doi.org/10.1016/j.neulet.2021.136241

Vorinostat, a histone deacetylase inhibitor, ameliorates the sociability and cognitive memory in an *Ash1L*-deletion-induced ASD/ID mouse model

Yuen Gao[1], Mohammad B Aljazi[1], Yan Wu[1], Jin He[1*]

[1]Department of Biochemistry and Molecular Biology, College of Natural Science, Michigan State University, East Lansing, MI 48824

*Correspondence: hejin1@msu.edu

Running title: Vorinostat ameliorates *Ash1L*-deletion-induced ASD/ID-like behavioral deficits

Highlights

- Postnatal administration of vorinostat (SAHA) ameliorates the core ASD-like behaviors in the *Ash1L*-deletion-induced ASD/ID mouse model.
- Postnatal administration of vorinostat (SAHA) ameliorates the cognitive memory in the *Ash1L*-deletion-induced ASD/ID mouse model.
- Different behavioral deficits have distinct responses to vorinostat (SAHA) treatment.
- No obvious drug toxicity was observed during low-dose vorinostat (SAHA) treatment.

Vorinostat, a histone deacetylase inhibitor, ameliorates the sociability and cognitive memory in an *Ash1L*-deletion-induced ASD/ID mouse model

Yuen Gao[1], Mohammad B Aljazi[1], Yan Wu[1], Jin He[1*]

[1]Department of Biochemistry and Molecular Biology, College of Natural Science, Michigan State University, East Lansing, MI 48824

*Correspondence: hejin1@msu.edu

Running title: Vorinostat ameliorates *Ash1L*-deletion-induced ASD/ID-like behavioral deficits

Abstract

Autism spectrum disorder (ASD) and intellectual disability (ID) are neurodevelopmental diseases associated with various gene mutations. Previous genetic and clinical studies reported that *ASH1L* is a high ASD risk gene identified in human patients. Our recent study used a mouse model to demonstrate that loss of ASH1L in the developing mouse brain was sufficient to cause multiple developmental defects, core autistic-like behaviors, and impaired cognitive memory, suggesting that the disruptive *ASH1L* mutations are the causative drivers leading the human ASD/ID genesis. Using this *Ash1L*-deletion-induced ASD/ID mouse model, here we showed that postnatal administration of vorinostat (SAHA), a histone deacetylase inhibitor (HDACi), significantly ameliorated both ASD-like behaviors and ID-like cognitive memory deficit. Thus, our study demonstrates that SAHA is a promising reagent for the pharmacological treatment of core ASD/ID behavioral and memory deficits caused by disruptive *ASH1L* mutations.

Key words: Vorinostat, SAHA, histone deacetylase inhibitor, ASH1L, autism spectrum disorder, intellectual disability

1. Introduction

Autism spectrum disorder is one of most prevalent neurodevelopmental disorders that have a strong genetic basis[1]. Recent whole exome sequencing analyses on large cohorts of ASD patients identified hundreds of ASD risk genes that encode proteins mainly involved in either

epigenetically transcriptional regulation or neuronal communication[2-6]. Among the ASD risk genes encoding epigenetic factors, *ASH1L* (*A*bsent, *S*mall, or *H*omeotic discs *1*-*L*ike) is identified by multiple studies as a high ASD risk gene[2-5]. The genetic findings are further supported by multiple clinical cases reporting that some children diagnosed with ASD and/or intellectual disability (ID) acquire *de novo* disruptive or missense mutations of *ASH1L*[7-12]. In addition to ASD and ID, patients also display various developmental abnormalities including delayed myelination, microcephaly, craniofacial deformity, and skeletal abnormality[7, 9, 10], suggesting critical roles of *ASH1L* in normal embryonic and postnatal development .

To examine the pathogenic role of disruptive *ASH1L* mutations in the ASD/ID genesis, recently we used a conditional *Ash1L* knockout mouse model to show that deletion of *Ash1L* in the developing mouse brain (*Ash1L*-Nes-cKO) was sufficient to cause multiple developmental defects, core autistic-like behaviors, and ID-like memory deficits, suggesting that the disruptive *ASH1L* mutations are likely to be the causative drivers leading to the human ASD/ID development[13]. The *Ash1L*-deletion-indcued ASD/ID mouse model has high penetrance of ASD/ID-like phenotypes that recapitulate most clinical manifestations found in human patients, thus providing us an ideal *in vivo* system to evaluate the therapeutic effects of pharmacological treatment for the disruptive *Ash1L*-mutation-induced ASD/ID.

Functionally, ASH1L is a member of Trithorax-group (TrxG) proteins that facilitate gene expression during normal development[14]. Consistent with its function in transcriptional activation, our previous study showed that deletion of ASH1L in the differentiating neural progenitor cells (NPCs) reduced the expression of genes involved in brain development, suggesting that the impaired neurodevelopment-related gene expression is likely to be a key molecular mechanism linking *ASH1L* disruptive mutations to abnormal brain development and pathogenesis of ASD/ID. Biochemically, ASH1L is a histone methyltransferase that mediates di-methylation of histone H3 lysine 36 (H3K36me2)[15]. Although the mechanistic link between ASH1L-mediated histone H3K36me2 modification to its function in transcriptional activation is not fully elucidated, previous studies reported that histone H3K36me2 facilitates transcriptional activation by antagonizing Polycomb repressive complex 2 (PRC2)-catalyzed histone H3K27me3 modification and its-mediated gene silencing[15].

In mammalian cells, histone hyperacetylation functions as another important epigenetic modification to antagonizing Polycomb-mediated transcriptional repression[16]. The histone

acetylation and deacetylation are catalyzed by histone acetylases (HATs) and deacetylases (HDACs)[17], respectively. The HDACs are broadly grouped into four classes that mediate deacetylation of histone and non-histone substrates. The HDAC-mediated histone deacetylation at transcriptional regulatory regions induces chromatin compaction and blocks transcriptional activation[18]. Conversely, HDAC inhibitors (HDACi) repress HDACs' enzymatic activity and shift the equilibrium of histone acetylation/deacetylation to hyperacetylation, which increases the accessibility to transcription factors and facilitates gene expression[19]. Based on these molecular mechanisms revealed by previous studies as well as our previous study in the *Ash1L*-deletion-induced ASD/ID mouse model, we propose to use HDACs inhibitors to rescue the *Ash1L*-deletion-induced impaired gene expression during brain development, and consequently ameliorate the ASD/ID-like behavioral deficits in the *Ash1L*-deletion-induced ASD/ID mouse model.

In this study, we investigate the effects of vorinostat (suberoylanilide hydroxamic acid, SAHA)[20], a pan-HDAC inhibitor that inhibits the enzymatic activity of all HDAC classes, in ameliorating the ASD/ID-like behaviors in the *Ash1L*-deletion-induced ASD/ID mouse model. The results showed that postnatal administration of low-dose SAHA significantly ameliorated the sociability, repetitive behaviors, and cognitive memory of the *Ash1L*-deficient mice, suggesting SAHA is a potential promising reagent for the treatment of ASD/ID behavioral and memory deficits caused by disruptive *ASH1L* mutations.

2. Materials and methods

2.1. Mice

The *Ash1L* conditional knockout mice were described in our previous report[13]. All mice were backcrossed to C57BL/6 mice for at least five generations to reach a pure C57BL/6 background before experiments. Mice were housed under standard conditions (12h light: 12h dark cycles) with food and water *ad libitum*. All mouse experiments were performed with the approval of the Michigan State University Institutional Animal Care & Use Committee.

2.2. Genotyping

Genomic DNA was extracted from mouse tails with lysis buffer of 0.01M NaOH. After neutralization with Tris-HCl (PH 7.6), the extracted genomic DNA was used for genotyping PCR assays.

2.3. SAHA administration

Suberoylanilide hydroxamic acid (SAHA) was purchased for Selleckchem (Catalog No.S1047). SAHA was administered i.p. at 5 mg/kg/day in a 5 ml/kg dose volume of using 2-hydroxypropyl-beta-cyclodextrin as vehicle (9 g/L). The controlled mice were injected with vehicle only.

2.4. Open field test

Mice were acclimated for 60 min to the behavioral testing room before assessment. All testing was performed under white lights (~130 Lux). The open-field apparatus consisted of a custom-made, square white polyvinylchloride foam box (40 x 40 x 40cm). Their behavior was recorded for the first 5 min of habituation to trace and measure time spent in open field with a digital CCD camera connected to a computer running an automated video tracking software package (ANY-maze, Stoelting Co). Throughout testing, objects and apparatus were cleaned with 70% ethanol between trials.

2.5. Novel object recognition test (NOR)

NOR was assessed using a 3-day paradigm that included habituation, training, and testing as described previously[13, 21-24]. Each day, mice were acclimated for 60 min to the behavioral testing room before assessment. All testing was performed under red lights, and behaviors were video recorded and automatically scored using ANY-maze. During habituation (day1), mice were placed into the open field apparatus, a square white polyvinylchloride foam 40x40x40 cm box, for 30 min. For training (day 2), two identical objects consisting of blocks were placed in opposite corners of the open field apparatus, and the animals were allowed to explore the objects for 30 min. The object pairs used were counterbalanced across treatments. For testing (day 3), mice were placed in the same apparatus, but this time one object of the pair was replaced with another dissimilar object (novel object), and they were allowed to freely explore for 5 minutes. Their behavior was recorded, and the time the mice spent with their nose oriented towards the object within 3.5cm of the object edge was considered exploration time. Throughout testing, objects and apparatus were cleaned with 70% ethanol between trials. Discrimination index was calculated as:

$$DI = \frac{(\text{time investigating novel} - \text{time investigating familiar})}{(\text{time investigating novel} + \text{time investigating familiar})} \quad (1)$$

2.6. Sociability test

This test was adapted from Crawley's sociability and preference for social novelty protocol[13, 25, 26], which consists of three phases. Mice were acclimated for 60 min to the behavioral testing room under red lights before testing. The behaviors during all three phases were video recorded and automatically scored using ANY-maze. In phase 1, the experimental mouse was placed in the center of a three-chamber apparatus (polyvinylchloride, 60x40x40 cm) and allowed to freely explore for 5 minutes. During this time, the mouse had free access to all three chambers, which are connected by small openings at the bottom of the dividers. In phase 2, two identical, wire cup-like containers were placed one in each of the side chambers. The experimental mouse was allowed to freely explore the three chambers again for 5 minutes. In phase 3 (sociability), an unfamiliar same-sex mouse was placed in one of the containers ("social stimulus"), while the other remained empty ("object"). The experimental mouse was allowed to freely explore the three chambers for 5 minutes. Throughout testing, objects and apparatus were cleaned with 70% ethanol between trials. For analysis, the time with total body spent in each of the three chambers was recorded. Discrimination index was calculated as:

$$DI = \frac{(\text{time interactig with social stimulus} - \text{time interacting with object})}{(\text{time interacting with social stimulus} + \text{time interacting with object})} \quad (2)$$

2.7. Quantitative self-grooming measurement

Mice were acclimated for 60 minutes to the behavioral testing room before assessment. After the mice were placed into an open field apparatus (40 x 40 x 40 cm) and habituated for 30 minutes, the mouse grooming behavior was video recorded for 10 minutes. The total grooming time and number of grooming episodes were manually measured.

2.8. Paw clasping scoring

The paw clasping scoring method was adopted from the reported protocol[27]. Briefly, the mice were suspended by tail for 10 seconds. If the hindlimbs are consistently splayed outward, away from the abdomen, it was assigned a score of 0. If both hindlimbs were partially retracted toward the abdomen for more than 50% of the time suspended, it was designated as mild paw clasping

and received a score of 1. If its hindlimbs were entirely retracted and touching the abdomen for more than 50% of the time suspended, it was designated as severe paw clasping and received a score of 2.

2.9. Statistics and reproducibility

All statistical analyses were performed using GraphPad Prism 8 (GraphPad Software). Parametric data were analyzed by a two-tailed *t* test or two-way ANOVA test for comparisons of multiple samples. *P* values < 0.05 were considered statistically significant. Planned comparisons (Šídák's multiple comparisons test) were used if ANOVAS showed significant main or interaction effects. Data are presented as mean ± SEM.

3. Results

3.1. SAHA administration does not ameliorate the postnatal growth retardation of *Ash1L*-Nes-cKO mice

To evaluate the therapeutic effects of SAHA on the behavioral defects caused by loss of ASH1L in the developing mouse brain, we included 2 groups of wild-type and *Ash1L*-Nes-cKO mice that were received SAHA or vehicle administration, respectively. Each group had 10 mice with matched ages and sexes (5 males and 5 females per group). The SAHA was dissolved in PBS with 2-hydroxypropyl-β-cyclodextrin as vehicle and administrated at the dose of 5mg/kg once daily via intraperitoneal injection from postnatal day (P) 10 to 60. The three-chamber, novel object recognition (NOR), and open field tests were performed after the SAHA administration was completed (Figure 1A).

Our previous study reported that the *Ash1L*-Nes-cKO pups displayed significant postnatal growth retardation before weaning, and the adult *Ash1L*-Nes-cKO mice had relatively lower body weight than that of wild-type mice[13]. Here we observed that both vehicle- and SAHA-treated *Ash1L*-Nes-cKO mice showed significant lower body weight compared to the wild-type controls before weaning and in their adulthood (Fig. 1B-D), suggesting that the postnatal SAHA administration did not ameliorate the postnatal growth retardation of *Ash1L*-Nes-cKO mice. Except for the growth retardation, we did not observe other significant gross developmental defects in the SAHA-treated mice. Laboratory tests after SAHA treatment did not reveal obvious abnormalities in peripheral blood cell counts as well as liver and renal functions in the SAHA-

treated *Ash1L*-Nes-cKO mice (Tables S1, S2), suggesting the SAHA administration at 5mg/kg for around two months had low toxicity and was well tolerated by the animals.

3.2. SAHA administration ameliorates the core ASD-like behaviors of *Ash1L*-Nes-cKO mice

Impaired social interaction is one of main clinical manifestations of ASD patients. Our previous study demonstrated that loss of ASH1L in the developing mouse brain was sufficient to induce the autistic-like deficit in sociability, suggesting *ASH1L* disruptive mutations was likely to be the causative drivers leading to the deficit of social interaction in human patients[13]. To examine whether the postnatal SAHA administration could ameliorate the autistic-like social behaviors, we performed three-chamber tests to examine the voluntary exploration of a social vs. a non-social stimulus (sociability) after the SAHA administration was completed at P60 (Figure 1A)[25, 26]. Consistent with our previous report, the results showed there existed a main effect of stimulus type ($F1,72 = 93.87$, $p < 0.0001$). Planned comparisons revealed that for the vehicle-treated groups, the wild-type mice spent more time with the social stimulus than with the object ($p < 0.001$), representing normal sociability. In contrast, the *Ash1L*-Nes-cKO mice spent significant less time with the social stimulus ($p < 0.001$) and had lower discrimination index towards the social stimulus ($p = 0.0061$) (Figure 2A-C), suggesting impaired sociability. Compared to the vehicle-treated controls, the SAHA-treated wild-type mice showed a similar preference to the social partner (Figure 2B, C), indicating the SAHA treatment did not affect the normal sociability of wild-type mice. Different from the vehicle-treated *Ash1L*-Nes-cKO mice, both male and female SAHA-treated *Ash1L*-Nes-cKO mice spent more time with the social stimulus than with the object, which led to a higher discrimination index favoring the social stimulus ($p = 0.0412$) (Figures 2B, C and S1A, B), suggesting the SAHA treatment significantly ameliorated the sociability of *Ash1L*-Nes-cKO mice.

Repetitive behavior is another main clinical manifestation of ASD patients. Our earlier study showed that loss of ASH1L in the developing brain caused over-grooming, an ASD-like repetitive behavior, in the *Ash1L*-Nes-cKO mice[13]. To examine whether the SAHA treatment could ameliorate over-grooming, we compared the grooming episodes and time between different mouse groups. Consistent with our previous report[13], the *Ash1L*-Nes-cKO mice had increased overall grooming episodes ($t = 3.915$, $df = 18$, $p = 0.001$) and time ($t = 4.05$, $df = 18$, $p = 0.0008$) within

the vehicle-treated groups. Compared to the vehicle-treated controls, the SAHA-treated wild-type mice had similar grooming episodes and time, suggesting that the SAHA treatment did not induce over-grooming in the wild-type mice. Different from the vehicle-treated controls, both grooming episodes ($t = 3.733$, $df = 18$, $p = 0.0015$) and time ($t = 3.20$, $df = 18$, $p = 0.005$) were largely reduced in both male and female SAHA-treated *Ash1L*-Nes-cKO mice (Figures 2D, E and S1C-F), suggesting the SAHA treatment ameliorated the over-grooming in the *Ash1L*-Nes-cKO mice.

Taken together, these results suggested that the postnatal SAHA treatment ameliorated the core ASD-like symptoms including impaired sociability and repetitive behaviors in the *Ash1L*-deletion-induced ASD mouse model.

3.3. SAHA administration ameliorates the cognitive memory of *Ash1L*-Nes-cKO mice

Some patients with *ASH1L* mutations display various degrees of intellectual disability. Consistent with the clinical observations, our previous study showed that the loss of ASH1L in the developing mouse brain induced the ID-like cognitive memory deficit[13]. To examine whether the postnatal SAHA administration could ameliorate the cognitive memory deficit, we performed the novel object recognition test to examine the long-term memory after the SAHA administration was completed (Figure 1A). The results showed that consistent with our previous report, there existed a main effect of interactive type ($F_{1,36} = 10.16$, $p = 0.003$). Compared to the vehicle-treated wild-type mice that had a positive discrimination index favoring the novel object, the *Ash1L*-Nes-cKO mice had a significant reduced discrimination index ($p = 0.045$), suggesting impaired cognitive memory (Figure 3). Same as the vehicle-treated controls, the SAHA-treated wild-type mice had a similar positive discrimination index favoring the novel object, suggesting the SAHA treatment did not affect the normal cognitive memory of wild-type mice. In contrast, compared to the vehicle-treated *Ash1L*-Nes-cKO mice, both male and female SAHA-treated *Ash1L*-Nes-cKO mice had a significant increased discrimination index towards to the novel object ($p < 0.0001$) (Figures 3 and S2A, B), suggesting that the postnatal SAHA treatment significantly ameliorated the cognitive memory of *Ash1L*-Nes-cKO mice.

3.4. SAHA administration does not alleviate anxiety-like or ataxia-like behaviors

Anxiety is one of common clinical manifestations of ASD patients. Our previous study showed that compared to the wild-type mice, the *Ash1L*-Nes-cKO mice spent less time in exploring the

central arena in open field tests, suggesting the loss of ASH1L in the developing mouse brain caused anxiety-like behaviors[13]. To examine whether the SAHA treatment could alleviate the anxiety-like behavior, we performed the open field test after the SAHA administration was completed (Figure 1A). Consistent with our previous report[13], the results showed that compared to the vehicle-treated wild-type mice, the vehicle-treated *Ash1L*-Nes-cKO mice spent less time in exploring the central arena ($t = 5.212$, $df = 18$, $p = 0.0058$), indicating anxiety-like behaviors. However, compared the vehicle-treated *Ash1L*-Nes-cKO mice, the SAHA-treated *Ash1L*-Nes-cKO mice did not show significant changes in time spent in exploring the central arena (Figure 4A), indicating that SAHA treatment did not ameliorates the anxiety-like behavior of *Ash1L*-Nes-cKO mice. The difference in anxiety-like behaviors were unlikely to be caused by the altered locomotor activities among different groups since all groups of mice displayed comparable running distances in the open field (Figure 4B).

Our previous study showed that the *Ash1L*-Nes-cKO mice demonstrated a high incidence of ataxia-like behaviors such as hind paw clasping when suspended by tail[13]. In this study, both vehicle- and SAHA-treated *Ash1L*-Nes-cKO mice had similar higher incidences of paw clasping compared to the wild-type controls ($p < 0.01$) (Figure 4C), indicating the SAHA treatment could not ameliorate the ataxia-like behavior.

Discussion

In this study, we used the *Ash1L* conditional knockout mice to investigate the therapeutic effects of SAHA, a pan-HDAC inhibitor, in treating ASD/ID-like behavioral deficits caused by loss of ASH1L in the developing mouse brain. The results demonstrated that the early postnatal treatment of SAHA for around two months ameliorated the core ASD-like deficit in sociability, repetitive behaviors, and the ID-like deficit in cognitive memory (Figures 2, 3, S1 and S2). Compared to its effect in ameliorating the sociability, the effect of SAHA in improving cognitive memory appeared to be more prominent since all conditional *Ash1L*-KO animals received SAHA treatment showed enhanced object recognition memory (Figures 3, S2). Consistent with our observation that SAHA improves the cognitive memory in this *Ash1L*-deletion-induced ASD/ID mouse model, previous studies have showed that HDAC inhibitors improve cognitive function under a variety of normal and pathological conditions[28-31], suggesting SAHA might improve the cognitive memory through a general mechanism in enhancing hippocampal function.

In contrast to the SAHA treatment ameliorating the social behavior and cognitive memory in the conditional *Ash1L*-KO mice, the same treatment did not show obvious effects in ameliorating the postnatal growth retardation, the anxiety-like behavior, or the ataxia-like bodily movement (Figures 1, 4). Although the mechanisms underlying the different responses of individual developmental and behavioral deficits to the same SAHA treatment are unclear, our previous study suggests that impaired expression of genes during brain development is one of the key mechanisms leading to the disruptive *Ash1L*-mutation-induced brain functional abnormality[13]. Therefore, we speculate that the development of neural circuits for regulating postnatal growth, emotions, and bodily movement, might be largely completed during the embryonic brain development, which makes them less responsive to the postnatal pharmacological intervention. In contrast, the neural circuits mediating cognitive memory formation and sociality might still contain certain degree of plasticity that is more readily subjected to the postnatal SAHA treatment-induced functional enhancement.

SAHA is a pan-HDACs inhibitor approved by the U.S. Food and Drug Administration (FDA) to treat human cutaneous T-cell lymphoma (CTCL)[32]. The recommended dose for the CTCL treatment is 400 mg/day or 5-8 mg/kg/day (based on 50-80 kg body weight) for human patients, which is equivalent to 60~100 mg/kg/day for mice[33]. To reduce the possible drug toxicity caused by SAHA treatment, we use a low dose of SAHA (5 mg/kg/day) in this study, which was 12 ~ 20 times less than the recommended dose for the human CTCL treatment. During two months of SAHA treatment, we do not observe obvious gross and laboratory abnormalities in the SAHA-treated groups (Tables S1, S2), suggesting the low dose of SAHA used in this study is well tolerated and has low toxicity to mice. However, its safety and efficacy in treating human patients merit further investigation.

HDACs and their mediated epigenetic modifications have been reported to play critical roles in regulating gene expression during normal brain development[34-36]. However, the underlying mechanisms linking their functions to the pathogenesis of *ASH1L*-mutation-induced ASD/ID remain unknown. Further genome-wide transcriptome and epigenome analysis of HDACs and ASH1L during brain development will help to elucidate the molecular mechanisms underlying their functions in the pathogenesis and treatment of *ASH1L*-deletion-induced ASD/ID .

In summary, our current study provides experimental evidence to show that the postnatal administration of low-dose SAHA significantly ameliorates the sociability, repetitive behaviors,

and cognitive memory in the *Ash1L*-deletion-induced ASD/ID mouse model, indicating that SAHA is a promising reagent for the pharmacological treatment of core ASD/ID behavioral and memory deficits caused by disruptive *ASH1L* mutations.

References

1. Lord, C., et al., *Autism spectrum disorder.* Nat Rev Dis Primers, 2020. **6**(1): p. 5.
2. Satterstrom, F.K., et al., *Large-Scale Exome Sequencing Study Implicates Both Developmental and Functional Changes in the Neurobiology of Autism.* Cell, 2020. **180**(3): p. 568-584 e23.
3. Ruzzo, E.K., et al., *Inherited and De Novo Genetic Risk for Autism Impacts Shared Networks.* Cell, 2019. **178**(4): p. 850-866 e26.
4. Stessman, H.A., et al., *Targeted sequencing identifies 91 neurodevelopmental-disorder risk genes with autism and developmental-disability biases.* Nat Genet, 2017. **49**(4): p. 515-526.
5. Lalli, M.A., et al., *High-throughput single-cell functional elucidation of neurodevelopmental disease-associated genes reveals convergent mechanisms altering neuronal differentiation.* Genome Res, 2020. **30**(9): p. 1317-1331.
6. Grove, J., et al., *Identification of common genetic risk variants for autism spectrum disorder.* Nat Genet, 2019. **51**(3): p. 431-444.
7. Shen, W., et al., *De novo loss-of-function variants of ASH1L are associated with an emergent neurodevelopmental disorder.* Eur J Med Genet, 2019. **62**(1): p. 55-60.
8. Xi, H., et al., *A chromosome 1q22 microdeletion including ASH1L is associated with intellectual disability in a Chinese family.* Mol Cytogenet, 2020. **13**: p. 20.
9. Okamoto, N., et al., *Novel MCA/ID syndrome with ASH1L mutation.* Am J Med Genet A, 2017. **173**(6): p. 1644-1648.
10. de Ligt, J., et al., *Diagnostic exome sequencing in persons with severe intellectual disability.* N Engl J Med, 2012. **367**(20): p. 1921-9.
11. Faundes, V., et al., *Histone Lysine Methylases and Demethylases in the Landscape of Human Developmental Disorders.* Am J Hum Genet, 2018. **102**(1): p. 175-187.
12. Wang, T., et al., *De novo genic mutations among a Chinese autism spectrum disorder cohort.* Nat Commun, 2016. **7**: p. 13316.
13. Gao, Y., et al., *Loss of histone methyltransferase ASH1L in the developing mouse brain causes autistic-like behaviors.* Commun Biol, 2021. **4**(1): p. 756.
14. Schuettengruber, B., et al., *Trithorax group proteins: switching genes on and keeping them active.* Nat Rev Mol Cell Biol, 2011. **12**(12): p. 799-814.
15. Yuan, W., et al., *H3K36 methylation antagonizes PRC2-mediated H3K27 methylation.* J Biol Chem, 2011. **286**(10): p. 7983-9.
16. Tie, F., et al., *CBP-mediated acetylation of histone H3 lysine 27 antagonizes Drosophila Polycomb silencing.* Development, 2009. **136**(18): p. 3131-41.
17. Shahbazian, M.D. and M. Grunstein, *Functions of site-specific histone acetylation and deacetylation.* Annu Rev Biochem, 2007. **76**: p. 75-100.
18. Seto, E. and M. Yoshida, *Erasers of histone acetylation: the histone deacetylase enzymes.* Cold Spring Harb Perspect Biol, 2014. **6**(4): p. a018713.

19. Xu, W.S., R.B. Parmigiani, and P.A. Marks, *Histone deacetylase inhibitors: molecular mechanisms of action.* Oncogene, 2007. **26**(37): p. 5541-52.
20. Bubna, A.K., *Vorinostat-An Overview.* Indian J Dermatol, 2015. **60**(4): p. 419.
21. Ennaceur, A. and J. Delacour, *A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data.* Behav Brain Res, 1988. **31**(1): p. 47-59.
22. Ennaceur, A., et al., *A new one-trial test for neurobiological studies of memory in rats. II: Effects of piracetam and pramiracetam.* Behav Brain Res, 1989. **33**(2): p. 197-207.
23. Ennaceur, A. and K. Meliani, *A new one-trial test for neurobiological studies of memory in rats. III. Spatial vs. non-spatial working memory.* Behav Brain Res, 1992. **51**(1): p. 83-92.
24. Benice, T.S. and J. Raber, *Object recognition analysis in mice using nose-point digital video tracking.* J Neurosci Methods, 2008. **168**(2): p. 422-30.
25. Kaidanovich-Beilin, O., et al., *Assessment of social interaction behaviors.* J Vis Exp, 2011(48).
26. Moy, S.S., et al., *Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice.* Genes Brain Behav, 2004. **3**(5): p. 287-302.
27. Guyenet, S.J., et al., *A simple composite phenotype scoring system for evaluating mouse models of cerebellar ataxia.* J Vis Exp, 2010(39).
28. Guan, J.S., et al., *HDAC2 negatively regulates memory formation and synaptic plasticity.* Nature, 2009. **459**(7243): p. 55-60.
29. McQuown, S.C., et al., *HDAC3 is a critical negative regulator of long-term memory formation.* J Neurosci, 2011. **31**(2): p. 764-74.
30. Graff, J., et al., *An epigenetic blockade of cognitive functions in the neurodegenerating brain.* Nature, 2012. **483**(7388): p. 222-6.
31. Kilgore, M., et al., *Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease.* Neuropsychopharmacology, 2010. **35**(4): p. 870-80.
32. *Vorinostat (Zolinza) for cutaneous T-Cell lymphoma.* Med Lett Drugs Ther, 2007. **49**(1256): p. 23-4.
33. Nair, A.B. and S. Jacob, *A simple practice guide for dose conversion between animals and human.* J Basic Clin Pharm, 2016. **7**(2): p. 27-31.
34. Fischer, A., et al., *Targeting the correct HDAC(s) to treat cognitive disorders.* Trends Pharmacol Sci, 2010. **31**(12): p. 605-17.
35. Abel, T. and R.S. Zukin, *Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders.* Curr Opin Pharmacol, 2008. **8**(1): p. 57-64.
36. Cho, Y. and V. Cavalli, *HDAC signaling in neuronal development and axon regeneration.* Curr Opin Neurobiol, 2014. **27**: p. 118-26.

Acknowledgements

This work was supported by the National Institutes of Health (grant R01GM127431).

Author Contributions

J.H. conceived and oversaw the project. Y.G. performed the experiments. Y.G., M.B.A. and Y.W. maintained the mouse colonies. Y.G. and J.H. interpreted the data. Y.G and J.H. wrote the manuscript.

Competing Interest Statement

Authors declare no competing interests.

Figures and figure legend

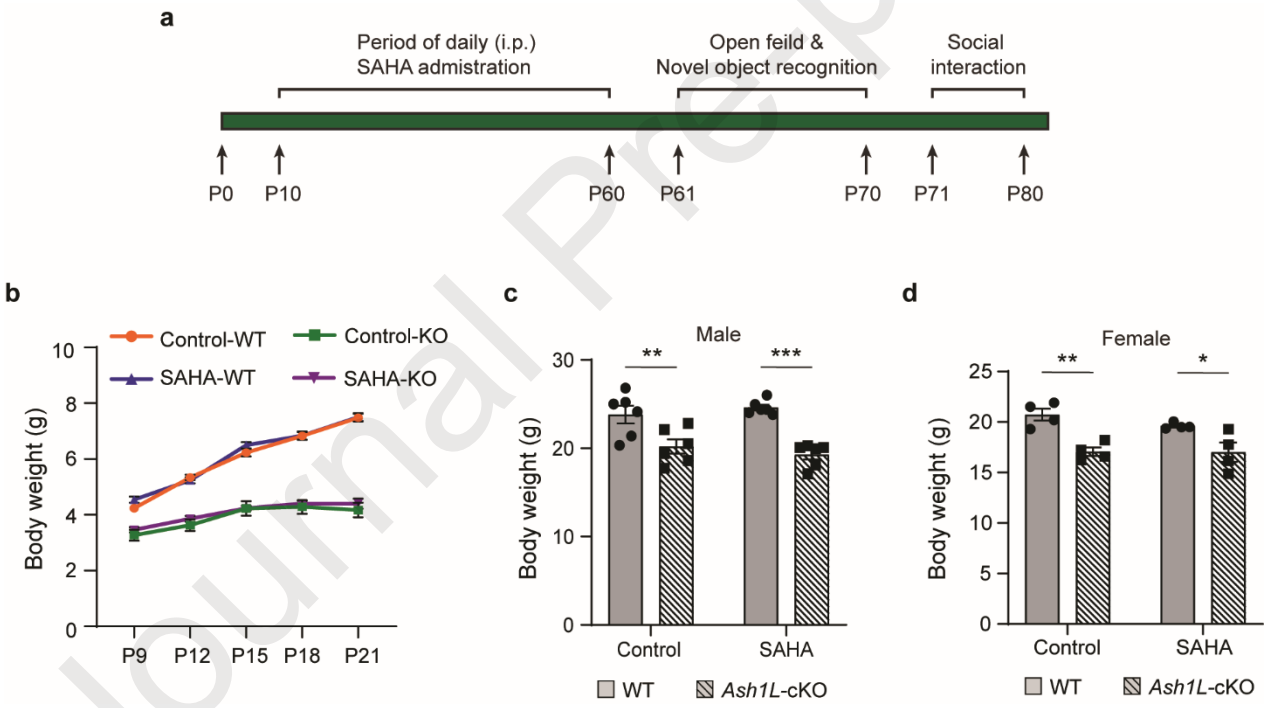

Figure 1. SAHA administration does not ameliorate the postnatal growth retardation of *Ash1L*-Nes-cKO mice. (A) Diagram showing the experimental design for SAHA administration and behavioral tests. P, postnatal day. (B) Postnatal growth curve before weaning. Mixed gender body weight was plotted. For each group, n = 10 mice. *P*-values calculated using a two-way ANOVA test. Error bars in graphs represent mean ± SEM. (C) The body weight of *Ash1L*-Nes-cKO male mice after 50 days SAHA treatment. n = 6 per group. *P* value calculated using two-way

ANOVA test and Sidak's multiple comparisons test. $^{**}p < 0.01$; $^{***}p < 0.001$. Error bars in graphs represent mean ± SEM. (D) The body weight of *Ash1L*-Nes-cKO female mice after 50 days SAHA treatment. n = 4 per group. *P* value calculated using two-way ANOVA test and Sidak's multiple comparisons test. $^{*}p < 0.05$; $^{**}p < 0.01$. Error bars in graphs represent mean ± SEM.

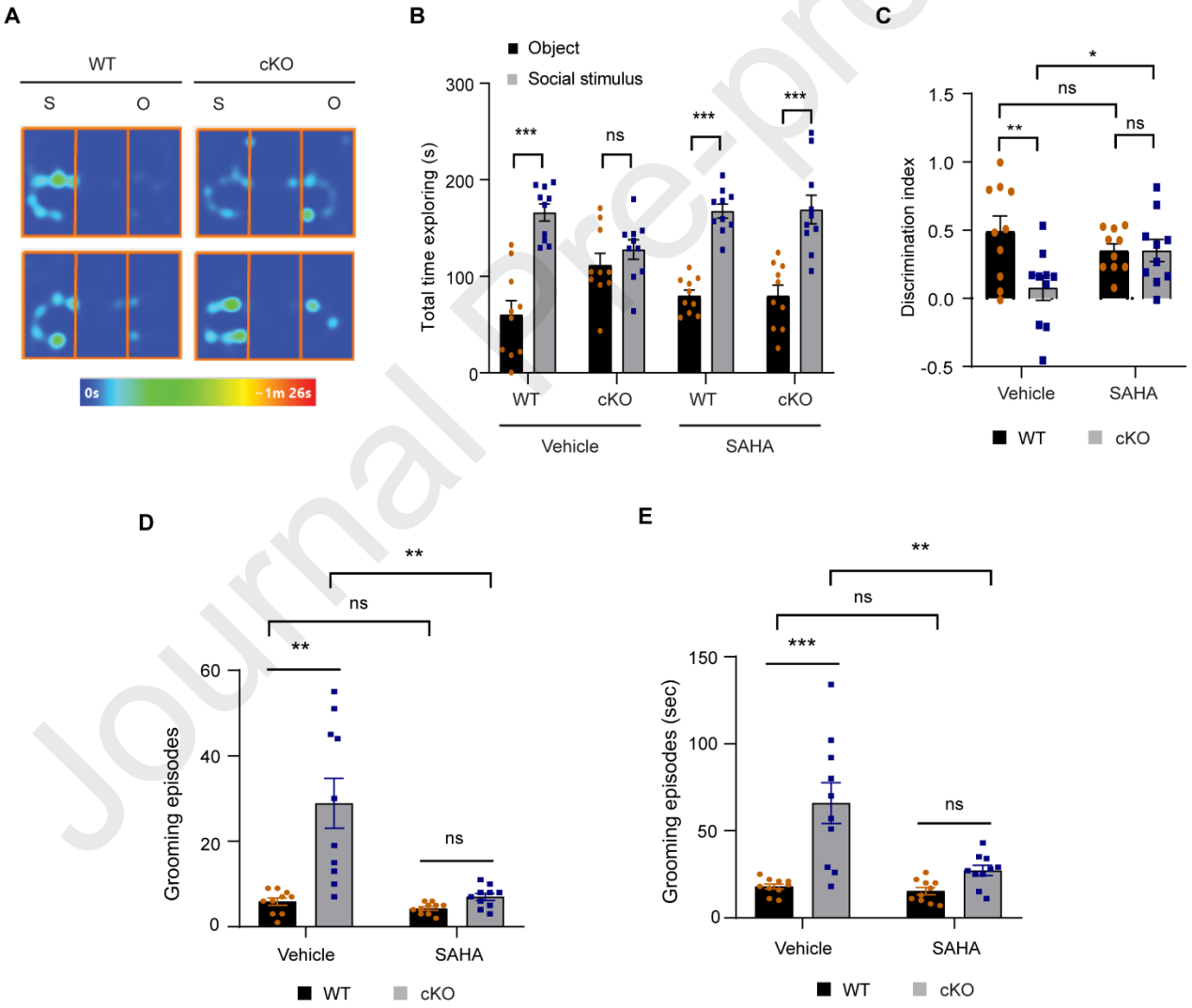

Figure 2. SAHA administration ameliorates the sociability of *Ash1L*-Nes-cKO mice. (A) Representative heatmaps showing the time spent in exploring social stimulus or object. S: social

partner, O: object. (B) Quantitative results showing the time spent in exploring the social stimulus. n = 10 per group. *P* value calculated using two-way ANOVA test and Sidak's multiple comparisons test. ***$P < 0.001$; ns, not significant. Error bars in graphs represent mean ± SEM. (C) The quantitative discrimination ratio of sociability tests. The discrimination ratio was calculated as: (time interacting with the social stimulus-time interacting with the object)/total time. n = 10 per group. *P* value calculated using two-way ANOVA test and Sidak's multiple comparisons test.*$p < 0.05$; **$P < 0.01$; ns, not significant. (D) Total grooming episodes in 10 minutes. (E) Total grooming time in 10 minutes. For the quantitative grooming measurement (D, E), n=10 per group, *P* value calculated using a two-tailed *t* test. Error bars in graphs represent mean ± SEM. Note: **$P < 0.01$, ***$P < 0.001$; ns, not significant. For panel B-E, the data from mixed gender were plotted.

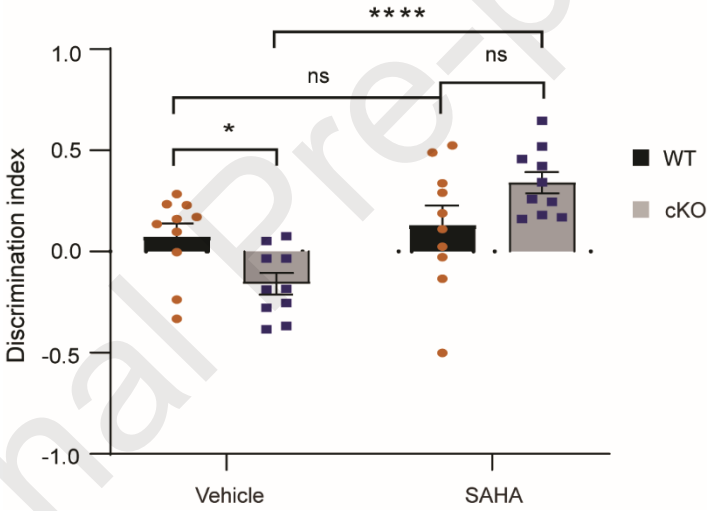

Figure 3. SAHA administration ameliorates the cognitive memory of *Ash1L*-Nes-cKO mice. The quantitative discrimination ratio of NOR tests. The discrimination ratio was calculated as: (time spent on the novel object-time spent on the familiar object)/total time. n = 10 per group. *P* value calculated using two-way ANOVA test and Sidak's multiple comparisons test. *$p < 0.05$; **** $p < 0.0001$; ns, not significant. Error bars in graphs represent mean ± SEM. The data from mixed gender were plotted.

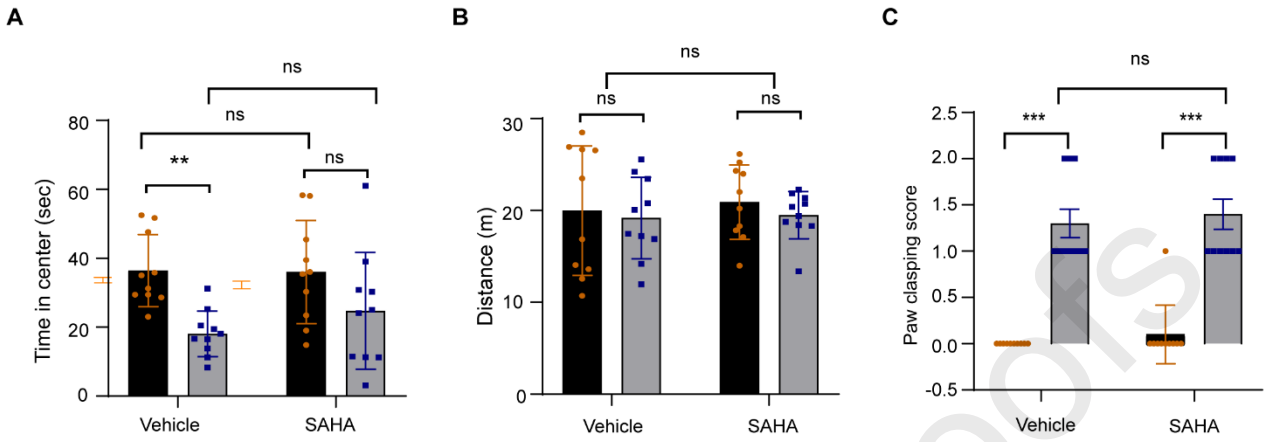

Figure 4. SAHA administration does not alleviate anxiety-like or ataxia-like behaviors. (A) Quantitative time mice spent in the center of arena in the open field tests. n = 10 per group. *P*-values calculated using a two-tailed *t* test. $^{**}p < 0.01$; ns, not significant. Error bars in graphs represent mean ± SEM. (C) Paw clasping scores of mice. n = 10 per group. Error bars in graphs represent mean ± SEM. *P*-values calculated using a two-tailed *t* test. $^{***}p < 0.001$; ns, not significant. For panel A-C, the data from mixed gender were plotted.

Supplementary Information

Vorinostat, a histone deacetylase inhibitor, ameliorates the sociability and cognitive memory in an *Ash1L*-deletion-induced ASD/ID mouse model

Yuen Gao, Mohammad B Aljazi, Yan Wu, Jin He

Correspondence: hejin1@msu.edu

This PDF file includes:

Supplementary Figure 1 and 2

Supplementary Table 1 and 2

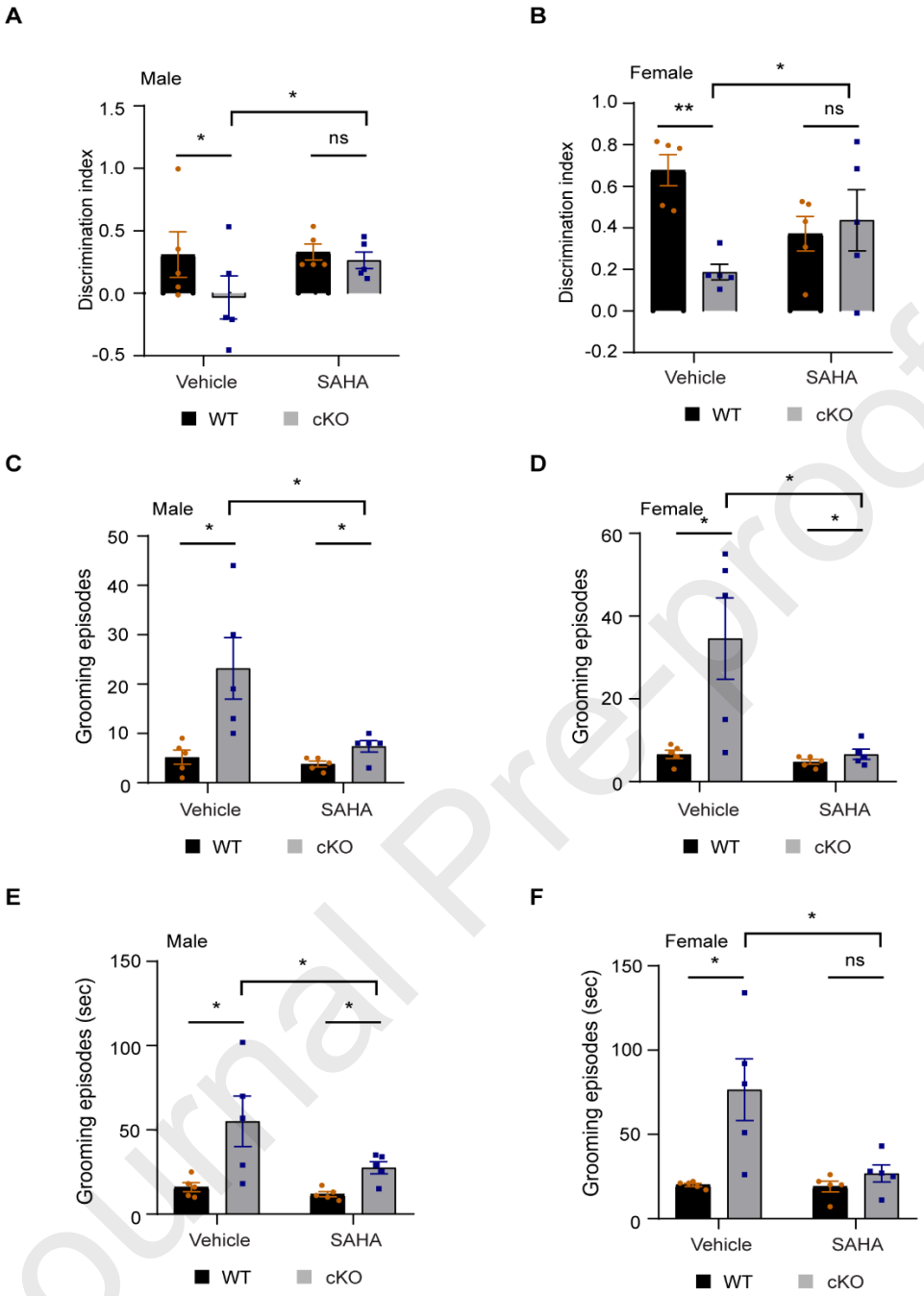

Figure S1. SAHA administration ameliorates the sociability of *Ash1L*-Nes-cKO mice. (A-B) The quantitative discrimination ratio of male (A) and female (B) mice in sociability tests. The discrimination ratio was calculated as: (time interacting with the social stimulus-time interacting with the object)/total time. n = 10 per group. *P* value calculated using two-way ANOVA test and Sidak's multiple comparisons test.*$p < 0.05$; **$P < 0.01$; ns, not significant. **(C-D)** Total grooming episodes of male (C) and female (D) mice in 10 minutes. (**E-F**) Total grooming time of male (E) and female (F) mice in 10 minutes. For the quantitative grooming measurement (C-F), n = 5 per

group, *P* value calculated using a two-tailed *t* test. Error bars in graphs represent mean ± SEM. Note: .*$p < 0.05$; ns, not significant.

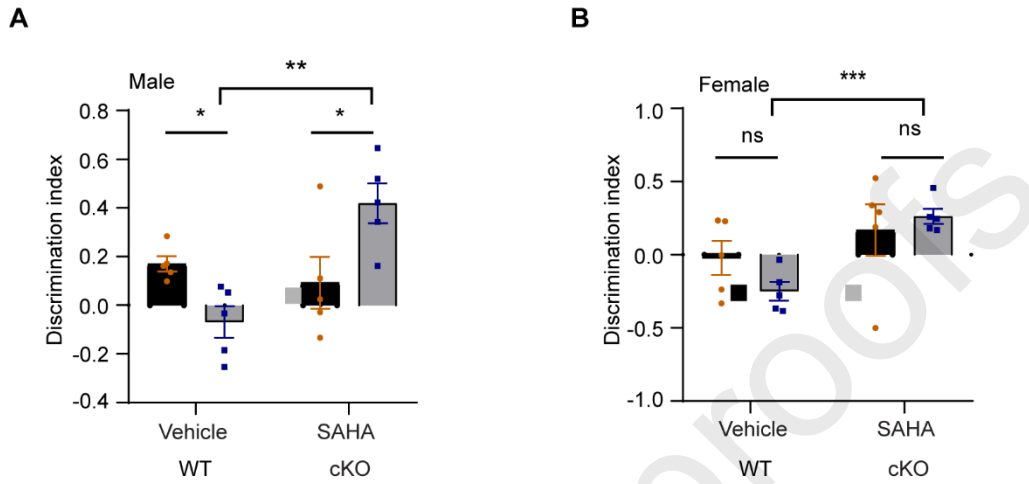

Figure S2. SAHA administration ameliorates the cognitive memory of *Ash1L*-Nes-cKO mice. (A-B) The quantitative discrimination ratio of male (A) and female (B) mice in NOR tests. The discrimination ratio was calculated as: (time spent on the novel object-time spent on the familiar object)/total time. n = 5 per group. *P* value calculated using two-way ANOVA test and Sidak's multiple comparisons test. *$p < 0.05$; **$p < 0.01$; *** $p < 0.001$; ns, not significant. Error bars in graphs represent mean ± SEM.

Supplementary Table 1. Peripheral blood counts of SAHA-treated Ash1L-Nes-cKO mice.

Test Name	Result	Range
Red blood cells (x10.e6/uL)	9.4 ± 0.4	normal
Hemoglobin (g/dL)	13.4 ± 0.6	normal
White blood cells(x10.e3/uL)	5.6 ± 0.4	normal
Platelet (x10.e3/uL)	873 ± 65	normal

Note: The results are shown as mean ± SD, n = 3 mice. The tests are performed by the MSU veterinary diagnostic laboratory.

Supplementary Table 2. Biochemical tests of SAHA-treated Ash1L-Nes-cKO mice.

Test name	Result	Range
Total protein (g/dL)	5.4 ± 0.25	normal
Albumin (g/dL)	3.2 ± 0.4	normal
Total Bilirubin (mg/dL)	0.2 ± 0.1	normal
Alanine aminotransferase (U/L)	24 ± 8	normal
Aspartate aminotransferase (U/L)	87 ± 8	normal
Creatinine (Jaffe) (mg/dL)	0.2 ± 0.1	normal

Note: The results are shown as mean ± SD, n = 3 mice. The tests are performed by the MSU veterinary diagnostic laboratory.

Author Contributions

J.H. conceived and oversaw the project. Y.G. performed the experiments. Y.G., M.B.A. and Y.W. maintained the mouse colonies. Y.G. and J.H. interpreted the data. Y.G and J.H. wrote the manuscript.