Restoring Social Deficits in IRSp53-Deleted Mice: Chemogenetic Inhibition of Ventral Dentate Gyrus Emx1-Expressing Cells

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Abstract

IRSp53 is a synaptic scaffold protein reported to be involved in schizophrenia, autism spectrum disorders, and social deficits in knockout mice. Identifying critical brain regions and cells related to IRSp53 deletion is expected to be of great help in the treatment of psychiatric problems. In this study, we performed chemogenetic inhibition within the ventral dentate gyrus (vDG) of mice with IRSp53 deletion in Emx1-expressing cells (Emx1-Cre;IRSp53 flox/flox). We observed the recovery of social deficits after chemogenetic inhibition within vDG of Emx1-Cre;IRSp53 flox/flox mice. Additionally, chemogenetic activation induced social deficits in Emx1-Cre mice. CRHR1 expression increased in the hippocampus of Emx1-Cre;IRSp53 flox/flox mice, and CRHR1 was reduced by chemogenetic inhibition. Htd2, Ccn1, and Atp61l were decreased in bulk RNA sequencing, and Eya1 and Ecrg4 were decreased in single-cell RNA sequencing of the hippocampus in Emx1-Cre;IRSp53 flox/flox mice compared to control mice. This study determined that the vDG is a critical brain region for social deficits caused by IRSp53 deletion. Social deficits in Emx1-Cre;IRSp53 flox/flox mice were recovered through chemogenetic inhibition, providing clues for new treatment methods for psychiatric disorders accompanied by social deficits.

Introduction

IRSp53, also known as BAIAP2, is a synaptic scaffold protein that affects actin polymerization and synaptic transmission[1–5]. Mice lacking IRSp53 in Emx1-expressing dorsal telencephalic glutamatergic neurons display social deficits, hyperactivity, and decreased prepulse inhibition [6–8]. Furthermore, the re-expression of IRSp53 in adult mice restores social deficits without changes in hyperactivity[9]. IRSp53 in the cerebral cortex and hippocampus has been suggested to be important for synaptic functions and various behaviors, including social behavior; thus, further research is needed to identify the brain regions involved in social deficits.

Empty spiracles homeobox 1 (Emx1) is expressed in neural stem cells, progenitor cells, differentiated neurons, and glial cells [10,11]. Emx1-expressing neural stem cells develop and differentiate into neurons and glial cells in the subventricular zone (SVZ) and subgranular zone (SGZ), where neurogenesis occurs from early E9.5 embryos to adulthood. Adult neurogenesis occurs in the SVZ, which differentiates into inhibitory granule neurons in the olfactory bulb, and in the SGZ, which differentiates into excitatory granule neurons in the dentate gyrus. Dysregulated adult neurogenesis is associated with psychiatric disorders, including cognitive decline and mood disorders [12–16]. In this study, we attempted to restore social deficits by regulating Emx1-expressing cells in the SGZ, which contributes to adult neurogenesis.

The ventral DG (vDG) is associated with mood disorders, such as major depressive disorder, and the action of antidepressants is associated with the restoration of neurogenesis in the vDG via 5HT1 and TrkB receptors.[14,17–24] A study by Anacker et al. using Cre-induced chemogenetic modulation and apoptosis in Nestin-expressing cells in the vDG showed that chemogenetic inhibition and Cre-induced apoptosis resulted in reduced social behavior and locomotion [25]. In this study, we investigated the

recovery of social deficits through chemogenetic modulation of Emx1-expressing cells in the vDG of IRSp53 deleted mice.

The present study was conducted to identify brain regions and cellular/molecular changes related to social deficits and their recovery in Emx1-Cre conditional IRSp53 knockout mice (Emx1-Cre;IRSp53 flox/flox). Recovery from social deficits via chemogenetic modulation has been attempted in Emx1-expressing cells in the vDG. In addition, protein and transcriptomic changes were explored at the tissue and single-cell levels in the hippocampi of control and Emx1-Cre;IRSp53 flox/flox mice.

Methods and Materials

Animals and Experimental Design

C57BL/6J background mice had ad libitum access to food and water and were housed with 4–5 animals per cage under a 12 h light/dark cycle. We included only male mice in this study because previous studies have shown that only male mice exhibit social deficits. All mice were bred and maintained in accordance with the requirements of the National Center for Mental Health (NCMH) Animal Research. All procedures and methods were approved by the NCMH Animal Research Committee (IACUC No: NCMH-2005-001-003-01). Mice were identified by polymerase chain reaction (PCR) genotyping using the following PCR primers: IRSp53 flox AGGAGGTGTTTCTGCTCTGG/AATAGCAGTCTGGGGTCTGG and Cre CGTACTGACGGTGGGAGAAT/TGCATGATCTCCGGTATTGA. Emx1-Cre;IRSp53 flox/flox mice was a gift from Eunjoon Kim, KAIST (Daejeon, South Korea)[26].

Quantification and statistical analysis

Statistical analyses were performed using Prism 9 software (GraphPad Software, La Jolla, CA, USA). Data normality was determined using the Shapiro-Wilk normality test. Normally distributed data were analyzed using Student's t-test and analysis of variance (ANOVA), followed by post hoc tests. Data that failed the normality test were analyzed using the Mann-Whitney U test.

Further detailed information on methods is available in **Supplementary Information.**

Results

Chemical inhibition of Emx1-expressing cells in the ventral dentate gyrus restores social deficits in Emx1-Cre;IRSp53 flox/flox mice

In previous studies, we observed an increase in the intrinsic excitability of pyramidal neurons in layers V and VI of the medial prefrontal cortex with the deletion of IRSp53 [7, 8]. In this study, chemogenetic modulation was used to induce an increase in excitability in the vDG of control mice and suppress excitability in the vDG of Emx1-Cre;IRSp53 flox/flox mice to examine whether each affected social behavior.

First, AAV9-hSyn-DIO-hM3Dq-mCherry chemogenetic activation was performed in the vDG of Emx1-Cre mice as a control. Because we used an AAV vector with the human Synapsin I (hSyn) promoter, it was expected to be expressed mainly in neurons[27]. A small dose of clozapine (0.5 mg/kg) induced chemogenetic activation, and there was no difference in locomotion between the vehicle and clozapine groups. However, mice subjected to chemogenetic activation showed decreased exploration of a stranger mouse compared to that of an object (Fig. 1a and b).

In Emx1-Cre;IRSp53 flox/flox mice, chemogenetic inhibition was conducted using AAV-hSyn-DIO-hM4Di-mCherry. There was decreased locomotion in the clozapine group compared to the control group, and also social deficits were improved (Fig. 1c and d).

Further experiments investigated whether fluoxetine improves social deficits in Emx1-Cre;IRSp53 flox/flox mice. Instead, social deficits were induced in control (IRSp53 flox/flox) mice, and social deficits did not improve in Emx1-Cre;IRSp53 flox/flox mice (Supplementary Fig. 1a and b).

Behavioral experiments showed that chemogenetic activation of Emx1-expressing cells in the vDG of Emx1-Cre mice reduced social behavior, and chemical inhibition of Emx1-expressing cells in the vDG of Emx1-Cre;IRSp53 flox/flox mice helped ameliorate social deficits.

Increased CRHR1 expression in the hippocampus in Emx1-Cre;IRSp53 flox/flox mice is restored by chemogenetic inhibition

To observe the changes caused by IRSp53 deletion, we examined protein expression in the hippocampus of the mice (Fig. 2a-h). Compared with control (IRSp53 flox/flox) mice, Emx1-Cre;IRSp53 flox/flox mice showed increased levels of CRHR1, a corticotropin-releasing hormone receptor on the stress-related HPA axis, and SOX2, a neural stem cell marker. Additionally, the deletion of IRSp53/BAIAP2 in Emx1-Cre;IRSp53 flox/flox mice was confirmed.

Regarding hippocampal protein expression before and after chemogenetic inhibition of Emx1-expressing cells in the vDG of Emx1-Cre;IRSp53 flox/flox mice (Fig. 2i–o), CRHR1 decreased after chemogenetic inhibition, and SOX2 did not differ between the control and cKO groups.

In addition, changes in hippocampal protein expression before and after chemogenetic activation of Emx1-expressing cells in the vDG of Emx1-Cre mice (Supplementary Fig. 2) showed a decrease in doublecortin (DCX), a neural progenitor marker, without changes in CRHR1.

Taken together, we observed increased CRHR1 and SOX2 protein expression in the hippocampus of Emx1-Cre;IRSp53 flox/flox mice compared to that in control mice, decreased CRHR1 expression after chemogenetic inhibition of Emx1-Cre;IRSp53 flox/flox mice, and decreased DCX expression due to chemogenetic activation of Emx1-Cre mice.

Increased Htd2, Ccn1 and Atp6ap1l expression and altered biological pathways in Emx1-Cre;IRSp53 flox/flox mice are revealed in hippocampal transcriptome analysis

Bulk RNA sequencing was performed for hippocampal transcriptome analysis of the control and Emx1-Cre;IRSp53 flox/flox mice (Fig. 3). RNA expression in control (flox1-3) and Emx1-Cre;IRSp53 flox/flox (cko1-3) mice was relatively well-differentiated (Fig. 3a). In gene ontology (GO) terms, the regulation of multicellular organismal processes and vitamin binding had the greatest influence on biological processes and molecular function, respectively.

Among the differentially expressed genes (DEG) between groups (Fig. 3d, e), Htd2, Ccn1, and Atp6ap1l showed significant differences, and the results of Emx1-Cre;IRSp53 flox/flox mice showed fold-changes of 3.07, 2.14, and 2.66, respectively, compared to control mice.

Through bulk RNA sequencing of the hippocampus, changes in GO terms, such as the regulation of multicellular organism processes and vitamin binding, were revealed in Emx1-Cre;IRSp53 flox/flox mice, and differences in Htd2, Ccn1, and Atp6ap1l were identified.

Eya1 in astrocytes and Ecrg4 in microglial cells and perivascular macrophages in the hippocampus of Emx1-Cre;IRSp53 flox/flox mice are showed in single cell transcriptomic analysis

Fresh hippocampal tissue was isolated and dissociated to investigate the effects of IRSp53 deletion in Emx1-expressing cells, and single-cell RNA sequencing was performed (Fig. 4). Cell distribution was as follows: oligodendrocytes (33.4%), microglia (32.5%), vascular endothelial cells (13.5%), and choroidal epithelial cells (7.9% (Fig. 4a-c).

The most obvious difference between the groups was a decrease in Eya1 expression in astrocytes and an increase in Ecrg4 expression in perivascular macrophages, vascular smooth muscle cells, microglia, and vascular endothelial cells in Emx1-Cre;IRSp53 flox/flox mice compared to control mice.

Discussion

In this study, social deficits were recovered through chemogenetic inhibition of Emx1-expressing cells in the vDG of Emx1-Cre;IRSp53 flox/flox mice, and protein and transcriptome experiments were conducted to investigate the mechanism of social deficits and their recovery. Behavioral experiments confirmed that chemogenetic activation of Emx1-expressing vDG cells in Emx1-Cre mice reduced social behavior, and chemical inhibition of Emx1-expressing vDG cells in Emx1-Cre;IRSp53 flox/flox mice improved social deficits. At the protein level, increased protein expression of CRHR1 and SOX2 was observed in the hippocampus of Emx1-Cre;IRSp53 flox/flox mice compared to control mice, and a decrease in CRHR1 was observed after chemogenetic inhibition of Emx1-Cre;IRSp53 flox/flox mice. Through bulk RNA sequencing of the hippocampus, changes in the regulation of multicellular organism processes and vitamin binding were revealed in Emx1-Cre;IRSp53 flox/flox mice, and differences in Htd2, Ccn1, and Atp6ap1l were identified. Single-cell RNA sequencing of the hippocampus showed that Eya1 expression

was decreased in astrocytes, and Ecrg4 expression was increased in the microglia of Emx1-Cre;IRSp53 flox/flox mice.

In this study, we demonstrated the rescue of social deficits by chemogenetic inhibition of the vDG in Emx1-Cre;IRSp53 flox/flox mice, and we suggest that chemogenetic inhibition of Emx1-expressing neural stem cells, neurons, and glial cells is related to the recovery of sociability. A difference from previous studies showing social deficits through chemogenetic inhibition in Nestin-Cre WT mice is that nestin expression was restricted to early-stage neural stem and progenitor cells [28]. Since Emx1 expression is present in the neural stem cells of newly born post-mitotic neurons, Cre-selective chemogenetic modulation may have affected Emx1-expressing cells with a wider cell growth cycle [10, 11, 14, 25, 29]. Because we used an AAV vector from the hSyn promoter, the effects of neuron-specific chemical modulation may differ [27, 30–32]. In particular, according to previous IRSp53 studies [6, 7], it can be inferred that the social deficits due to the increased excitability of newborn neurons in the vDG of IRSp53 knockout mice are corrected by chemogenetic inhibition. In addition, we found that increased neuronal excitability caused by chemogenetic activation induced social deficits in Emx1-Cre mice.

Stress-vulnerable individuals exhibit stronger activation of the HPA axis, including CRHR1, and fail to adapt to stress caused by relatively weak external environmental changes. Genome-wide association studies have shown that genes associated with stress vulnerability are also associated with HPA axis activation [33–37]. Furthermore, stress vulnerability is studied in clinical areas such as PTSD and mood disorders [38–40]. Psychiatric risk genes appear not only in one diagnostic category of mental disorders, such as schizophrenia, mood disorders, and autism spectrum disorders but also in vulnerable factors across multiple diagnostic categories. Genes known to be susceptible to stress include serotonin transporter, brain-derived neurotrophic factor, bromodomain 1, Cacna1a, Catechol-O-methyltransferase, Disrupted-in-schizophrenia-1, Estrogen receptor alpha, Fkbp5, Glutamate decarboxylase1, and Neuregulin1 [41–46]. In this study, we observed that the deletion of IRSp53 can lead to stress vulnerability phenomena associated with HPA axis activation, including increased CRHR1 and social deficits. We attempted to restore CRHR1 and social deficits through chemogenetic modulation of vDG.

CRHR activation is expected to be affected by environmental changes such as stress, but genetic factors also affect vulnerability [47, 48]. CRHR activation in the hippocampus, especially in the dentate gyrus, improves synaptic function and structure when it acts for a short period; however, deteriorating effects, such as decreased numbers of synapses, appear when it acts for a long time [49]. In particular, CRHR1 activation has been reported to increase the excitability of hippocampal CA1 glutamatergic neurons [50]. The increased excitability of hippocampal neurons has been actively studied for its role in stress in epileptic disorders [51–55]. Furthermore, the deletion of CRHR1 in telencephalon glutamatergic neurons results in decreased cognitive function, including memory impairment [56, 57]. CRHR1 overexpression is associated with vulnerability to stress and increased alcohol consumption during stressful situations [58, 59]. The mutant mice in this study were expected to have genetic susceptibilities due to IRSp53 deletion in telencephalic glutamatergic neurons without prominent stress triggers. We attempted to normalize

changes in CRHR1 and social behavior using chemogenetic modulation in the vDG. Therefore, this study suggests that social abnormalities can be restored by correcting the increased excitability of the vDG caused by genetic or environmental problems.

Among the results of bulk RNA sequencing analyses, Htd2 (Hydroxyacyl-Thioester Dehydratase Type 2) is known to exist in adipocytes as a key enzyme in mitochondrial fatty acid synthesis (mtFAS) [60–63]. Previous reports have suggested that abnormalities in mtFAS can impact CNS function and are associated with neurodegeneration [64]. The extracellular matrix protein Ccn1 (a cystine-rich inducer of angiogenesis 61) is involved in repair, fibroblast senescence, and DNA damage response pathways [65]. Ccn1 mutant mice showed exacerbated fibrosis, which was reversed by topical application of CCN1. In addition, increased CCN1 secretion in glioblastoma cells results in macrophage infiltration into the tumor tissue [66]. Studies of psychedelics and stimulants that induced Ccn1 expression in the rodent neocortex suggest that Ccn1 is associated with the age of onset of schizophrenia [67]. Atp6apl1 (ATPase proton transporting accessory protein 1 like) was found to be decreased in the PTSD patient group compared to the control group, and ATP6AP1l, a paralog of the vesicular ATPase whose function has not yet been identified, was considered one of the stress-related factors in a previous study [68–70]. A comprehensive interpretation of the results of the bulk RNA sequencing study showed that, although no direct association with CRHR1 was found, factors that could have a wide range of effects on brain function were identified, particularly those clinically associated with schizophrenia, neurodegeneration, and stress-related disorders, such as PTSD.

According to single-cell RNA sequencing results, Eya1 plays an important role in cranial placode neurogenesis by acting as a cofactor for the homeobox transcription factor Six1 [71, 72]. Deletion of Eya1 (eye defects) causes BOR syndrome (branchio-oto-renal syndrome) and branchio-oto syndrome [73]. Cases of autism spectrum disorder with BOR syndrome have been reported [74], and Eya1 is widely expressed throughout the brain, as well as in neurogenesis-related regions of the central nervous system related to vision and hearing [75]. Ecrg4 (esophageal cancer-associated gene 4), also known as C2orf40, is a tumor suppressor gene identified in esophageal, breast, and colorectal carcinomas and gliomas [76, 77]. It is also highly expressed in leukocytes, and ECRG4 knockout mice showed abnormal wound healing [76]. Ecrg4 is expressed in the choroid plexus to produce augurin and functions in fetal brain development, cerebrospinal fluid homeostasis, and neural progenitor cell response to CNS injury [78]. A single-nucleotide variant in rs34487851 upstream of Ecrg4 has been reported in Alzheimer's disease [79, 80]. Studies using oligodendrocyte progenitor cells have shown that this gene is associated with neural aging [81].

Further studies are needed to determine the mechanism by which the deletion of IRSp53 and CRHR1 increases, the interaction between genetic susceptibility and different levels of environmental stress, and the key steps in treating social deficits during neural stem cell development, even in adults. One limitation of this study is that chemogenetic modulation was restricted to Emx1-expressing cells in the vDG. We focused on the expression of restricted proteins such as CRHR1. It is difficult to explain and connect directly the results from protein expression, such as CRHR1 and transcriptomics. Additionally, owing to

the fragility of adult neurons, many neuronal losses are considered to occur during hippocampal dissociation for single-cell RNA sequencing. Thus, more meticulous protocols and sufficient experience are needed for successful hippocampal dissociation [82–85].

Chemogenetic inhibition of vDG significantly affected the recovery of social deficits in Emx1-Cre;IRSp53 flox/flox mice, and the restoration of CRHR1 reduction in the hippocampus was observed as a mechanism. Further studies are needed to determine whether regulating excitability in Emx1-expressing cells of the vDG helps mice with genetic susceptibility to stress or prominent environmental stress to recover from social abnormalities. If this approach helps adult mice with stress vulnerability and social deficits recover, it may provide clues for the treatment of psychiatric disorders in the future.

Declarations

Conflict of interest

There is no conflict of interest.

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References

1. Chung W, Choi SY, Lee E, Park H, Kang J, Park H *et al.* Social deficits in IRSp53 mutant mice improved by NMDAR and mGluR5 suppression. Nature neuroscience 2015; 18(3): 435–443.
2. Kim M-H, Choi J, Yang J, Chung W, Kim J-H, Paik SK *et al.* Enhanced NMDA receptor-mediated synaptic transmission, enhanced long-term potentiation, and impaired learning and memory in mice lacking IRSp53. Journal of Neuroscience 2009; 29(5): 1586–1595.
3. Choi J, Ko J, Racz B, Burette A, Lee J-R, Kim S *et al.* Regulation of dendritic spine morphogenesis by insulin receptor substrate 53, a downstream effector of Rac1 and Cdc42 small GTPases. Journal of Neuroscience 2005; 25(4): 869–879.
4. Kang J, Park H, Kim E. IRSp53/BAIAP2 in dendritic spine development, NMDA receptor regulation, and psychiatric disorders. Neuropharmacology 2016; 100: 27–39.

5. Feng Z, Lee S, Jia B, Jian T, Kim E, Zhang M. IRSp53 promotes postsynaptic density formation and actin filament bundling. Journal of Cell Biology 2022; 221(8): e202105035.
6. Kim Y, Noh YW, Kim K, Kim E. Hyperactive ACC-MDT pathway suppresses prepulse inhibition in mice. Schizophrenia bulletin 2020.
7. Kim Y, Noh YW, Kim K, Yang E, Kim H, Kim E. IRSp53 Deletion in Glutamatergic and GABAergic Neurons and in Male and Female Mice Leads to Distinct Electrophysiological and Behavioral Phenotypes. Frontiers in cellular neuroscience 2020; 14: 23.
8. Kim Y, Noh YW, Kim K, Kim E. Hyperactive ACC-MDT pathway suppresses prepulse inhibition in mice. Schizophrenia Bulletin 2021; 47(1): 31–43.
9. Noh YW, Yook C, Kang J, Lee S, Kim Y, Yang E *et al.* Adult re-expression of IRSp53 rescues NMDA receptor function and social behavior in IRSp53-mutant mice. Communications Biology 2022; 5(1): 838.
10. Briata P, Di Blas E, Gulisano M, Mallamaci A, Iannone R, Boncinelli E, Corte G. EMX1 homeoprotein is expressed in cell nuclei of the developing cerebral cortex and in the axons of the olfactory sensory neurons. Mechanisms of development 1996; 57(2): 169–180.
11. Chan C-H, Godinho L, Thomaidou D, Tan S-S, Gulisano M, Parnavelas J. Emx1 is a marker for pyramidal neurons of the cerebral cortex. Cerebral cortex 2001; 11(12): 1191–1198.
12. Kobeissy FH, Hansen K, Neumann M, Fu S, Jin K, Liu J. Deciphering the role of Emx1 in neurogenesis: a neuroproteomics approach. Frontiers in molecular neuroscience 2016; 9: 98.
13. Lledo P-M, Alonso M, Grubb MS. Adult neurogenesis and functional plasticity in neuronal circuits. Nature Reviews Neuroscience 2006; 7(3): 179–193.
14. Anacker C, Hen R. Adult hippocampal neurogenesis and cognitive flexibility—linking memory and mood. Nature Reviews Neuroscience 2017; 18(6): 335–346.
15. Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, Rábano A, Cafini F, Pallas-Bazarra N *et al.* Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. Nature medicine 2019; 25(4): 554–560.
16. Surget A, Belzung C. Adult hippocampal neurogenesis shapes adaptation and improves stress response: a mechanistic and integrative perspective. Molecular Psychiatry 2022; 27(1): 403–421.
17. Kheirbek MA, Hen R. Dorsal vs ventral hippocampal neurogenesis: implications for cognition and mood. Neuropsychopharmacology 2011; 36(1): 373.
18. Eadie B, Zhang W, Boehme F, Gil-Mohapel J, Kainer L, Simpson J, Christie B. Fmr1 knockout mice show reduced anxiety and alterations in neurogenesis that are specific to the ventral dentate gyrus. Neurobiology of disease 2009; 36(2): 361–373.
19. Samuels BA, Anacker C, Hu A, Levinstein MR, Pickenhagen A, Tsetsenis T *et al.* 5-HT1A receptors on mature dentate gyrus granule cells are critical for the antidepressant response. Nature neuroscience 2015; 18(11): 1606–1616.

20. O'Leary OF, Cryan JF. A ventral view on antidepressant action: roles for adult hippocampal neurogenesis along the dorsoventral axis. Trends in pharmacological sciences 2014; 35(12): 675–687.
21. Wu MV, Hen R. Functional dissociation of adult-born neurons along the dorsoventral axis of the dentate gyrus. Hippocampus 2014; 24(7): 751–761.
22. Banasr M, Soumier A, Hery M, Mocaër E, Daszuta A. Agomelatine, a new antidepressant, induces regional changes in hippocampal neurogenesis. Biological psychiatry 2006; 59(11): 1087–1096.
23. Casarotto PC, Girych M, Fred SM, Kovaleva V, Moliner R, Enkavi G *et al.* Antidepressant drugs act by directly binding to TRKB neurotrophin receptors. Cell 2021; 184(5): 1299–1313. e1219.
24. Saarelainen T, Hendolin P, Lucas G, Koponen E, Sairanen M, MacDonald E *et al.* Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. Journal of Neuroscience 2003; 23(1): 349–357.
25. Anacker C, Luna VM, Stevens GS, Millette A, Shores R, Jimenez JC *et al.* Hippocampal neurogenesis confers stress resilience by inhibiting the ventral dentate gyrus. Nature 2018; 559(7712): 98–102.
26. Noh YW, Kim Y, Lee S, Kim Y, Shin JJ, Kang H *et al.* The PFC-LH-VTA pathway contributes to social deficits in IRSp53-mutant mice. Molecular Psychiatry 2023: 1–13.
27. Kügler S, Kilic E, Bähr M. Human synapsin 1 gene promoter confers highly neuron-specific long-term transgene expression from an adenoviral vector in the adult rat brain depending on the transduced area. Gene Ther 2003; 10(4): 337–347.
28. Bernal A, Arranz L. Nestin-expressing progenitor cells: function, identity and therapeutic implications. Cell Mol Life Sci 2018; 75(12): 2177–2195.
29. Gorski JA, Talley T, Qiu M, Puelles L, Rubenstein JL, Jones KR. Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. Journal of Neuroscience 2002; 22(15): 6309–6314.
30. Barbieri R, Contestabile A, Ciardo MG, Forte N, Marte A, Baldelli P *et al.* Synapsin I and Synapsin II regulate neurogenesis in the dentate gyrus of adult mice. Oncotarget 2018; 9(27): 18760–18774.
31. Fornasiero EF, Bonanomi D, Benfenati F, Valtorta F. The role of synapsins in neuronal development. Cellular and Molecular Life Sciences 2010; 67: 1383–1396.
32. Cesca F, Baldelli P, Valtorta F, Benfenati F. The synapsins: key actors of synapse function and plasticity. Prog Neurobiol 2010; 91(4): 313–348.
33. Francis T, Chandra R, Gaynor A, Konkalmatt P, Metzbower S, Evans B *et al.* Molecular basis of dendritic atrophy and activity in stress susceptibility. Molecular psychiatry 2017; 22(11): 1512–1519.
34. Carlsson P-I, Van Laer L, Borg E, Bondeson M-L, Thys M, Fransen E, Van Camp G. The influence of genetic variation in oxidative stress genes on human noise susceptibility. Hearing Research 2005; 202(1–2): 87–96.
35. Firk C, Markus CR. Serotonin by stress interaction: a susceptibility factor for the development of depression? Journal of Psychopharmacology 2007; 21(5): 538–544.

36. Ebner K, Singewald N. Individual differences in stress susceptibility and stress inhibitory mechanisms. Current Opinion in Behavioral Sciences 2017; 14: 54–64.
37. DeRijk RH. Single Nucleotide Polymorphisms Related to HPA Axis Reactivity. Neuroimmunomodulation 2009; 16(5): 340–352.
38. Hankin BL, Young JF, Abela JR, Smolen A, Jenness JL, Gulley LD *et al.* Depression from childhood into late adolescence: Influence of gender, development, genetic susceptibility, and peer stress. Journal of abnormal psychology 2015; 124(4): 803.
39. Lee HJ, Lee MS, Kang RH, Kim H, Kim SD, Kee BS *et al.* Influence of the serotonin transporter promoter gene polymorphism on susceptibility to posttraumatic stress disorder. Depression and anxiety 2005; 21(3): 135–139.
40. Xie P, Kranzler HR, Yang C, Zhao H, Farrer LA, Gelernter J. Genome-wide association study identifies new susceptibility loci for posttraumatic stress disorder. Biological psychiatry 2013; 74(9): 656–663.
41. Michels S, Ganjam GK, Martins H, Schratt GM, Wöhr M, Schwarting RK, Culmsee C. Downregulation of the psychiatric susceptibility gene Cacna1c promotes mitochondrial resilience to oxidative stress in neuronal cells. Cell death discovery 2018; 4(1): 54.
42. van Doeselaar L, Stark T, Mitra S, Yang H, Bordes J, Stolwijk L *et al.* Sex-specific and opposed effects of FKBP51 in glutamatergic and GABAergic neurons: Implications for stress susceptibility and resilience. *Proceedings of the National Academy of Sciences* 2023; 120(23): e2300722120.
43. Clark MB, Wrzesinski T, Garcia AB, Hall NA, Kleinman JE, Hyde T *et al.* Long-read sequencing reveals the complex splicing profile of the psychiatric risk gene CACNA1C in human brain. Molecular psychiatry 2020; 25(1): 37–47.
44. de Kloet ER, Joëls M, Holsboer F. Stress and the brain: from adaptation to disease. Nature Reviews Neuroscience 2005; 6(6): 463–475.
45. Meyer-Lindenberg A, Weinberger DR. Intermediate phenotypes and genetic mechanisms of psychiatric disorders. Nature Reviews Neuroscience 2006; 7(10): 818–827.
46. Knickmeyer RC, Wang J, Zhu H, Geng X, Woolson S, Hamer RM *et al.* Common Variants in Psychiatric Risk Genes Predict Brain Structure at Birth. Cerebral Cortex 2013; 24(5): 1230–1246.
47. Claes SJ. CRH, stress, and major depression: a psychobiological interplay. Vitamins and hormones 2004; 69: 117–150.
48. Teicher MH, Anderson CM, Polcari A. Childhood maltreatment is associated with reduced volume in the hippocampal subfields CA3, dentate gyrus, and subiculum. *Proceedings of the National Academy of Sciences* 2012; 109(9): E563-E572.
49. Maras PM, Baram TZ. Sculpting the hippocampus from within: stress, spines, and CRH. Trends in Neurosciences 2012; 35(5): 315–324.
50. Kratzer S, Mattusch C, Metzger MW, Dedic N, Noll-Hussong M, Kafitz KW *et al.* Activation of CRH receptor type 1 expressed on glutamatergic neurons increases excitability of CA1 pyramidal neurons by the modulation of voltage-gated ion channels. Front Cell Neurosci 2013; 7: 91.

51. Joëls M. Functional actions of corticosteroids in the hippocampus. European journal of pharmacology 2008; 583(2–3): 312–321.
52. McEwen BS. Plasticity of the hippocampus: adaptation to chronic stress and allostatic load. Annals of the New York Academy of Sciences 2001; 933(1): 265–277.
53. Gunn B, Baram T. Stress and seizures: space, time and hippocampal circuits. Trends in neurosciences 2017; 40(11): 667–679.
54. Narayanan R, Chattarji S. Computational analysis of the impact of chronic stress on intrinsic and synaptic excitability in the hippocampus. Journal of neurophysiology 2010; 103(6): 3070–3083.
55. Williams ES, Manning CE, Eagle AL, Swift-Gallant A, Duque-Wilckens N, Chinnusamy S *et al.* Androgen-dependent excitability of mouse ventral hippocampal afferents to nucleus accumbens underlies sex-specific susceptibility to stress. Biological psychiatry 2020; 87(6): 492–501.
56. Refojo D, Schweizer M, Kuehne C, Ehrenberg S, Thoeringer C, Vogl AM *et al.* Glutamatergic and Dopaminergic Neurons Mediate Anxiogenic and Anxiolytic Effects of CRHR1. Science 2011; 333(6051): 1903–1907.
57. Wang X-D, Chen Y, Wolf M, Wagner KV, Liebl C, Scharf SH *et al.* Forebrain CRHR1 deficiency attenuates chronic stress-induced cognitive deficits and dendritic remodeling. Neurobiology of Disease 2011; 42(3): 300–310.
58. Kwon M, Lee JH, Yoon Y, Pleasure SJ, Yoon K. The CRHR1/CREB/REST signaling cascade regulates mammalian embryonic neural stem cell properties. EMBO reports 2023; 24(2): e55313.
59. Broccoli L, Uhrig S, von Jonquieres G, Schönig K, Bartsch D, Justice NJ *et al.* Targeted overexpression of CRH receptor subtype 1 in central amygdala neurons: effect on alcohol-seeking behavior. Psychopharmacology 2018; 235(6): 1821–1833.
60. Zeng M, Xu J, Zhang Z, Zou X, Wang X, Cao K *et al.* Htd2 deficiency-associated suppression of α-lipoic acid production provokes mitochondrial dysfunction and insulin resistance in adipocytes. Redox Biology 2021; 41: 101948.
61. Hiltunen JK, Chen Z, Haapalainen AM, Wierenga RK, Kastaniotis AJ. Mitochondrial fatty acid synthesis – An adopted set of enzymes making a pathway of major importance for the cellular metabolism. Progress in Lipid Research 2010; 49(1): 27–45.
62. Autio KJ, Guler JL, Kastaniotis AJ, Englund PT, Hiltunen JK. The 3-hydroxyacyl-ACP dehydratase of mitochondrial fatty acid synthesis in Trypanosoma brucei. FEBS Letters 2008; 582(5): 729–733.
63. Autio KJ, Kastaniotis AJ, Pospiech H, Miinalainen IJ, Schonauer MS, Dieckmann CL, Hiltunen JK. An ancient genetic link between vertebrate mitochondrial fatty acid synthesis and RNA processing. The FASEB Journal 2008; 22(2): 569–578.
64. Kastaniotis AJ, Autio KJ, R. Nair R. Mitochondrial Fatty Acids and Neurodegenerative Disorders. The Neuroscientist 2021; 27(2): 143–158.
65. Jun J-I, Lau LF. The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. Nature Cell Biology 2010; 12(7): 676–685.

66. Uneda A, Kurozumi K, Fujimura A, Fujii K, Ishida J, Shimazu Y *et al.* Differentiated glioblastoma cells accelerate tumor progression by shaping the tumor microenvironment via CCN1-mediated macrophage infiltration. Acta Neuropathologica Communications 2021; 9(1): 29.
67. Ito T, Hiraoka S, Kuroda Y, Ishii S, Umino A, Kashiwa A *et al.* Effects of schizophrenomimetics on the expression of the CCN1 (CYR 61) gene encoding a matricellular protein in the infant and adult neocortex of the mouse and rat. International Journal of Neuropsychopharmacology 2007; 10(6): 717–725.
68. Logue MW, Smith AK, Baldwin C, Wolf EJ, Guffanti G, Ratanatharathorn A *et al.* An analysis of gene expression in PTSD implicates genes involved in the glucocorticoid receptor pathway and neural responses to stress. Psychoneuroendocrinology 2015; 57: 1–13.
69. Abbas YM, Wu D, Bueler SA, Robinson CV, Rubinstein JL. Structure of V-ATPase from the mammalian brain. Science 2020; 367(6483): 1240–1246.
70. Supek F, Supekova L, Mandiyan S, Pan YC, Nelson H, Nelson N. A novel accessory subunit for vacuolar H(+)-ATPase from chromaffin granules. J Biol Chem 1994; 269(39): 24102–24106.
71. Riddiford N, Schlosser G. Six1 and Eya1 both promote and arrest neuronal differentiation by activating multiple Notch pathway genes. Developmental Biology 2017; 431(2): 152–167.
72. Ahmed M, Xu J, Xu P-X. EYA1 and SIX1 drive the neuronal developmental program in cooperation with the SWI/SNF chromatin-remodeling complex and SOX2 in the mammalian inner ear. Development 2012; 139(11): 1965–1977.
73. Xu P-X, Adams J, Peters H, Brown MC, Heaney S, Maas R. Eya1-deficient mice lack ears and kidneys and show abnormal apoptosis of organ primordia. Nature Genetics 1999; 23(1): 113–117.
74. Yaylaci F, Sahbudak B, Citli S, Kilit N. ASD with the Bor Syndrome: A Case Report. Psychopharmacol Bull 2020; 50(2): 45–50.
75. Ishihara T, Sato S, Ikeda K, Yajima H, Kawakami K. Multiple evolutionarily conserved enhancers control expression of Eya1. Developmental Dynamics 2008; 237(11): 3142–3156.
76. Götze S, Feldhaus V, Traska T, Wolter M, Reifenberger G, Tannapfel A *et al.* ECRG4 is a candidate tumor suppressor gene frequently hypermethylated in colorectal carcinoma and glioma. BMC Cancer 2009; 9(1): 447.
77. Sabatier R, Finetti P, Adelaide J, Guille A, Borg J-P, Chaffanet M *et al.* Down-Regulation of ECRG4, a Candidate Tumor Suppressor Gene, in Human Breast Cancer. PLoS ONE 2011; 6(11): e27656.
78. Gonzalez AM, Podvin S, Lin S-Y, Miller MC, Botfield H, Leadbeater WE *et al.* Ecrg4 expression and its product augurin in the choroid plexus: impact on fetal brain development, cerebrospinal fluid homeostasis and neuroprogenitor cell response to CNS injury. Fluids and Barriers of the CNS 2011; 8(1): 6.
79. Woo J-M, Park SJ, Kang HI, Kim BG, Shim SB, Jee SW *et al.* Characterization of changes in global gene expression in the brain of neuron-specific enolase/human Tau23 transgenic mice in response to overexpression of Tau protein. Int J Mol Med 2010; 25(5): 667–675.

80. Chung J, Zhang X, Allen M, Wang X, Ma Y, Beecham G *et al.* Genome-wide pleiotropy analysis of neuropathological traits related to Alzheimer's disease. Alzheimer's Research & Therapy 2018; 10(1): 22.
81. Kujuro Y, Suzuki N, Kondo T. Esophageal cancer-related gene 4 is a secreted inducer of cell senescence expressed by aged CNS precursor cells. *Proceedings of the National Academy of Sciences* 2010; 107(18): 8259–8264.
82. Brewer GJ, Torricelli JR. Isolation and culture of adult neurons and neurospheres. Nat Protoc 2007; 2(6): 1490–1498.
83. Varghese K, Das M, Bhargava N, Stancescu M, Molnar P, Kindy MS, Hickman JJ. Regeneration and characterization of adult mouse hippocampal neurons in a defined in vitro system. Journal of neuroscience methods 2009; 177(1): 51–59.
84. Mynlieff M. Dissociation of postnatal hippocampal neurons for short term culture. Journal of neuroscience methods 1997; 73(1): 35–44.
85. Sheldon C, Church J. Intracellular pH response to anoxia in acutely dissociated adult rat hippocampal CA1 neurons. Journal of Neurophysiology 2002; 87(5): 2209–2224.

Figures

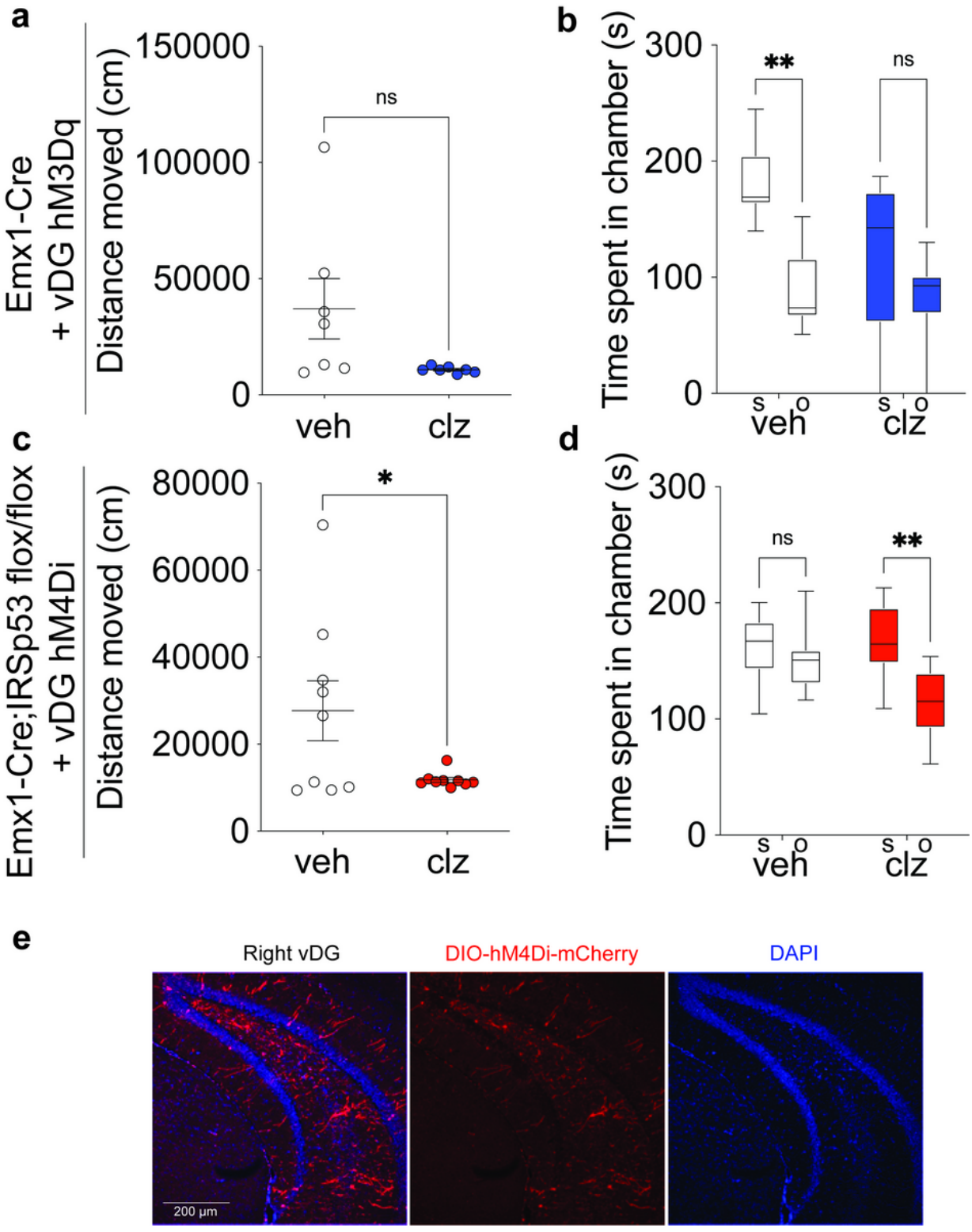


Figure 1

Behavioral experiments showed that chemical inhibition of Emx1-expressing cells in the ventral dentate gyrus restored social deficits in Emx1-Cre;IRSp53 flox/flox mice.

(a) Normal locomotor activities after (clozapine, clz) chemogenetic activation of Emx1-Cre wild-type mice compared to before (vehicle, veh) chemogenetic activation in the open-field test. n = 7 mice, ns, not

significant, one-way ANOVA

(b) Decreased sociability after Emx1-Cre wild-type mice compared to before chemogenetic activation in the three-chambered social interaction test. n = 7, **p < 0.01, ns, not significant, two-way ANOVA with Tukey's test

(c) Decreased locomotor activities after chemogenetic activation of Emx1-Cre;IRSp53 flox/flox mice compared to before chemogenetic activation in the three-chambered social interaction test. n = 9 mice, ns, not significant, one-way ANOVA

(d) Decreased sociability after Emx1-Cre;IRSp53 flox/flox mice compared to before chemogenetic activation in the open-field test. n = 9, **p < 0.01, ns, not significant, two-way ANOVA with Tukey's test

(e) Cre-selective DIO-hM4Di-mCherry expression was observed in the right ventral dentate gyrus: scale bar, 200 mm.

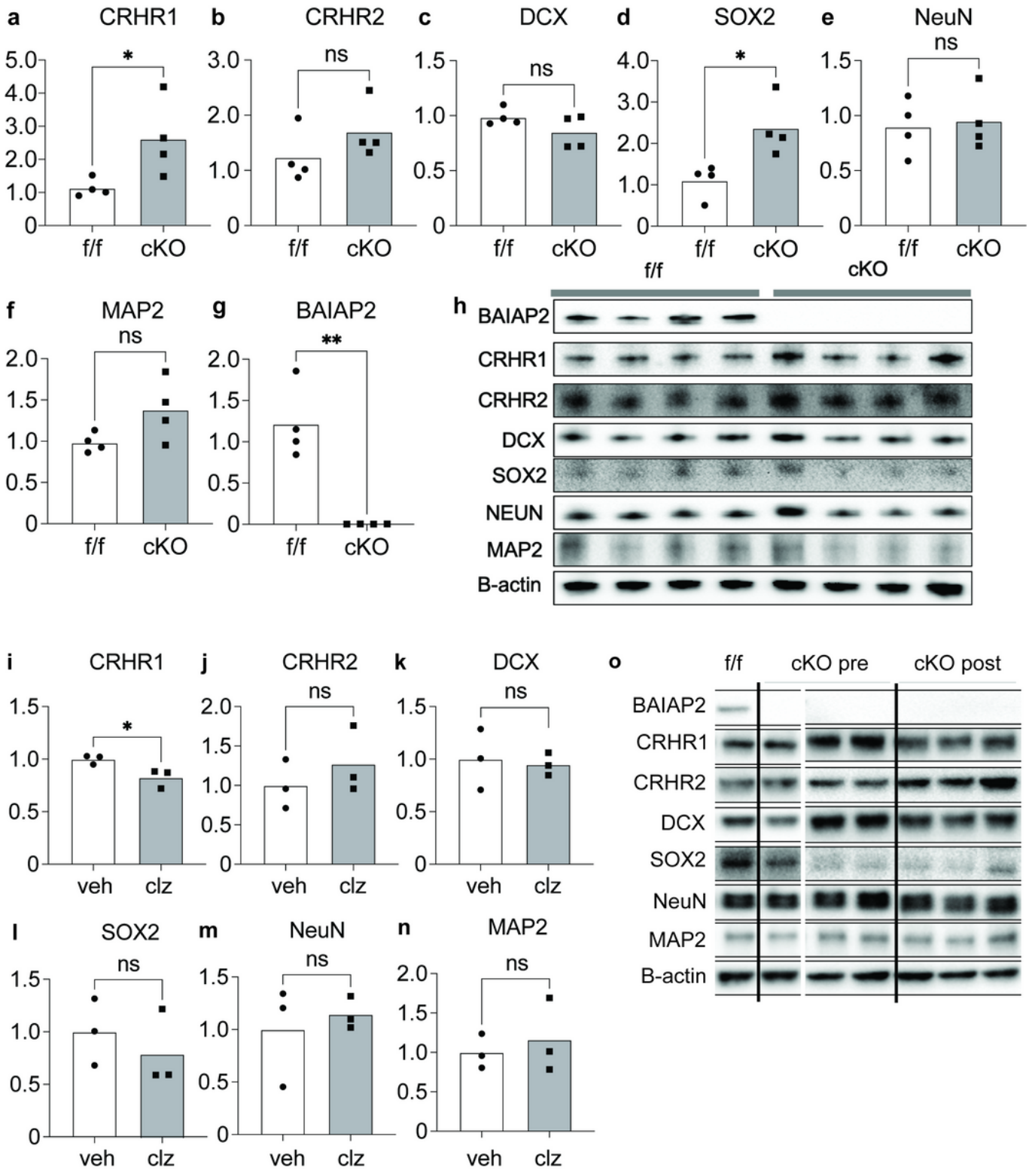


Figure 2

Protein expression of the hippocampus showed increased CRHR1 in Emx1-Cre;IRSp53 flox/flox mice is restored by chemogenetic inhibition.

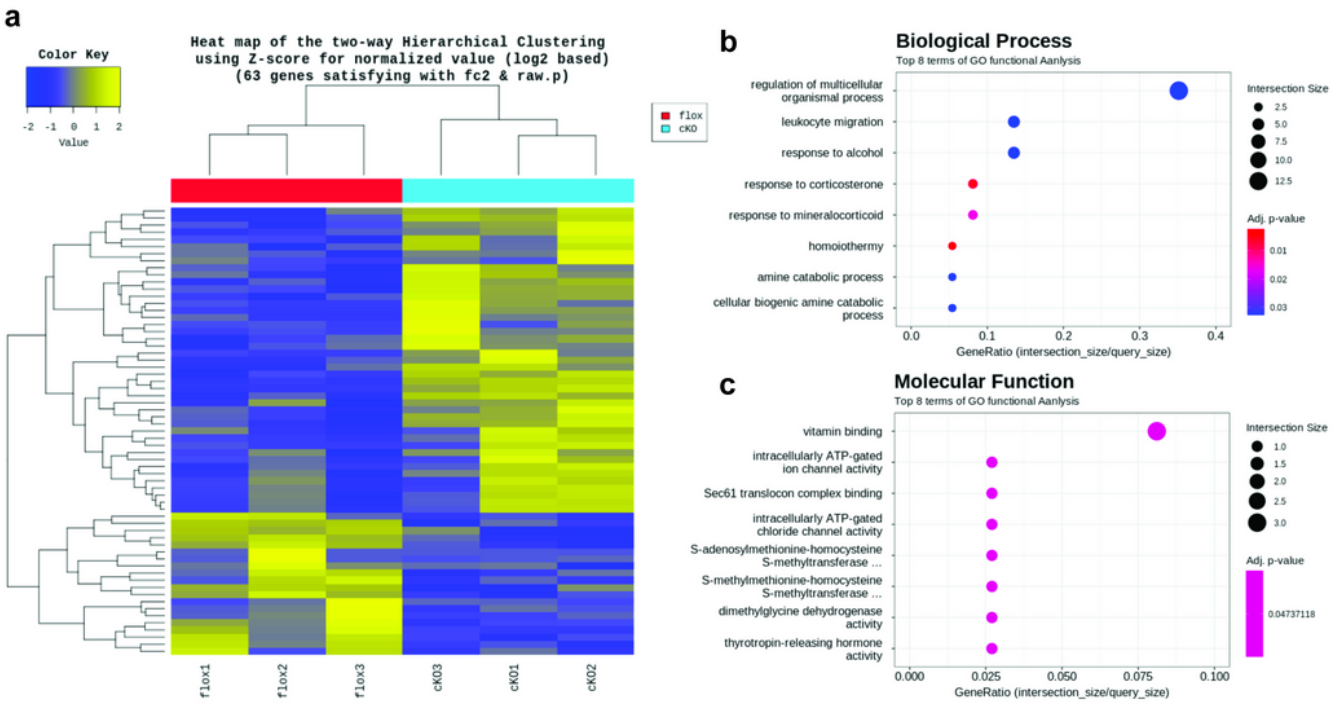
(a-g) CRHR1 (a) and SOX2 (d) were increased in Emx1-Cre;IRSp53 flox/flox mice, but BAIAP2 was not expressed in Emx1-Cre;IRSp53 flox/flox (*Emx1-Cre;* IRSp53 flox/flox) mice compared to control (IRSp53

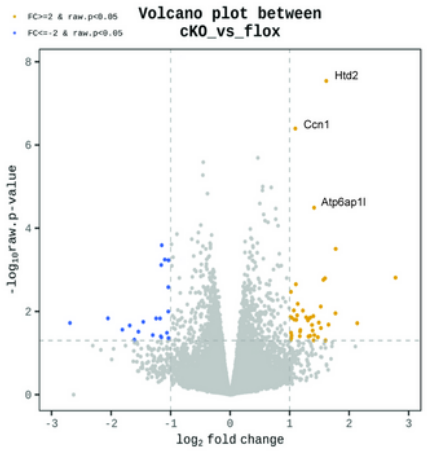
flox/flox) mice (g). n=4 hippocampi in each group. *p < 0.05, ns, not significant, Student's t-test.

(e) An image of immunoblotting results between control and Emx1-Cre;IRSp53 flox/flox mice.

(i-n) CRHR1 (A) were decreased after (clozapine, clz) chemogenetic inhibition in vDG of Emx1-Cre;IRSp53 flox/flox mice compared to before (vehicle, veh) chemogenetic inhibition, but SOX2 showed no difference before and after chemogenetic inhibition in vDG of Emx1-Cre;IRSp53 flox/flox. n=3 hippocampi in each group. *p < 0.05, ns, not significant, Student's t-test.

(o) An image of immunoblotting results before and after chemogenetic inhibition in vDG of Emx1-Cre;IRSp53 flox/flox mice.





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Gene Symbol	gene biotype	cKO/flox.fc	cKO/flox baseMean	cKO/flox.lfcSE	cKO/flox.stat	cKO/flox raw.pval	cKO/flox bh.pval	cKO.mean	flox.mean
Htd2	protein_coding	3.066896	185.264001	0.29142652	5.547811069	2.8927E-08	0.00045305	8.125855	6.510843
Ccn1	protein_coding	2.140137	307.112572	0.21661961	5.067422716	4.0324E-07	0.00315776	8.709218	7.612399
Atp6ap1l	protein_coding	2.661448	124.926894	0.33973016	4.15686186	3.2265E-05	0.04412823	7.505142	6.091449
Inafm1	protein_coding	-2.217044	189.762524	0.31421853	-3.655537191	0.00025664	0.12439379	6.879083	8.032409
Ttn	protein_coding	-2.051861	89.613943	0.3017399	-3.436511662	0.00058926	0.17036036	5.874830	6.913447
Pcdha1	protein_coding	-2.139688	145.409328	0.31841842	-3.446410689	0.00056809	0.17036036	6.534151	7.630383
Gm5784	lncRNA	-2.230967	73.832283	0.34412747	-3.364071748	0.00076802	0.1979533	5.519751	6.669394
Gdf1	protein_coding	6.866050	599.941516	0.87782077	3.166341594	0.00154369	0.27539041	10.032505	7.253138
Sp8	protein_coding	2.153189	97.401634	0.36175597	3.058623	0.00222357	0.32547214	7.056187	5.947663
Gm46277	lncRNA	-2.055515	93.427620	0.34532082	-3.01024386	0.00261038	0.34356113	5.935966	6.973081
Ecel1	protein_coding	2.030979	203.692972	0.34880457	2.930509635	0.00338406	0.36057168	8.093321	7.069093
Fos	protein_coding	2.199992	1198.547270	0.41854863	2.717720665	0.00657333	0.4757418	10.686439	9.549140
Gm30512	lncRNA	2.335144	84.869541	0.47200029	2.5921832	0.0095369	0.52947067	6.892571	5.670281
Acnat1	protein_coding	2.183881	87.409196	0.4509307	2.499040642	0.012453	0.56568331	6.906123	5.778266
Fndc9	protein_coding	2.048650	71.370383	0.42164212	2.453913293	0.01413111	0.58111058	6.584751	5.547043
Fosb	protein_coding	2.033540	366.349832	0.47895887	2.137956577	0.03252027	0.75741738	8.939897	7.916426
LOC101056144	protein_coding	-2.462642	57.869910	0.62364761	-2.084842409	0.03708361	0.77131934	5.063059	6.362998
Magel2	protein_coding	2.039686	297.732696	0.5094735	2.0184507	0.04354434	0.80324918	8.642514	7.613513

Figure 3

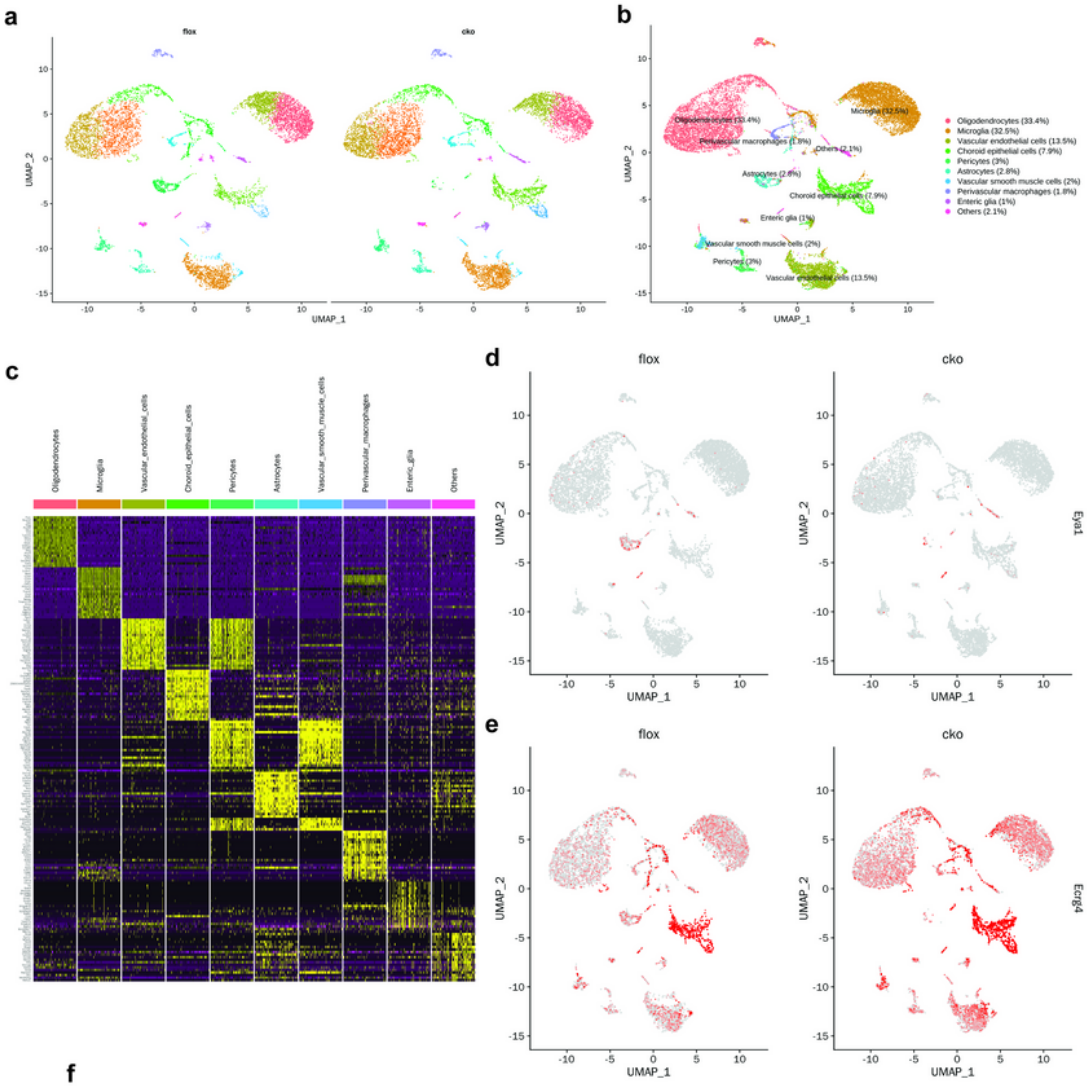
Transcriptomic analyses of the hippocampus showed increased Htd2, Ccn1 and Atp6ap1l expression and changed biological and molecular pathways in Emx1-Cre;IRSp53 flox/flox mice.

(a) A heat map of the two-way hierarchical clustering to differentiate hippocampal transcriptomic expression between Emx1-Cre;IRSp53 flox/flox (cko) and control (IRSp53 flox/flox, flox) mice.

(b-c) Gene ontology (GO) term analyses showed the most prominent changes in regulating the multicellular organismal process in the biological process and vitamin binding in molecular function.

(d) A volcano plot showed decreased transcriptomic expression of Htd2, Ccn1, and Atp6ap1l in Emx1-Cre;IRSp53 flox/flox mice compared to control mice.

(e) Detailed list of differentially expressed genes of bulk RNA sequencing of the hippocampus between Emx1-Cre;IRSp53 flox/flox and control mice.



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gene_name	Entrez_Gene_ID	p_val	avg_log2FC	pct.1	pct.2	p_val_adj	cluster	fc
Ecrg4	78896	0.000001	1.342907	0.51	0.24	0.0133708	Perivascular_macrophages	2.53661996
Ttr	22139	3.179E-24	1.303031	1	1	7.485E-20	Vascular_smooth_muscle_cells	2.46746667
Ttr	22139	<1E-140	1.281694	1	1	<1E-140	Microglia	2.43124201
Eya1	14048	4.682E-07	1.215208	0.52	0.15	0.0110219	Astrocytes	2.32174244
Ttr	22139	3.36E-136	1.177927	1	1	7.9E-132	Vascular_endothelial_cells	2.26251428
Ecrg4	78896	6.859E-07	1.11506	0.49	0.24	0.0161471	Vascular_smooth_muscle_cells	2.16604006
Ecrg4	78896	6.41E-07	0.98397	0.64	0.38	0.0150886	Others	1.97790096
Ttr	22139	<1E-140	0.980708	1	1	<1E-140	Oligodendrocytes	1.9734336
Ttr	22139	2.249E-09	0.898055	1	1	5.294E-05	Perivascular_macrophages	1.86355244
Pcsk6	18553	7.138E-07	0.876975	0.52	0.14	0.016803	Astrocytes	1.83652095
Ttr	22139	2.771E-21	0.84966	1	1	6.524E-17	Pericytes	1.80207659
Tmem132b	208151	1.054E-08	0.831598	0.22	0.02	0.000248	Astrocytes	1.77965605
Enpp2	18606	4.531E-07	0.810963	0.75	0.51	0.0106662	Vascular_smooth_muscle_cells	1.75438211
Apoe	11816	8.788E-27	0.681304	0.82	0.74	2.069E-22	Microglia	1.60358833
Ecrg4	78896	3.915E-75	0.624308	0.51	0.28	9.216E-71	Microglia	1.54147139
Enpp2	18606	1.114E-73	0.611759	0.75	0.58	2.622E-69	Microglia	1.52812157
Ecrg4	78896	6.411E-24	0.538828	0.48	0.28	1.509E-19	Vascular_endothelial_cells	1.4527915
Ptgds	19215	9.688E-14	0.521327	0.7	0.58	2.28E-09	Vascular_endothelial_cells	1.43527495
Ecrg4	78896	3.567E-79	0.507455	0.51	0.26	8.398E-75	Oligodendrocytes	1.42153995
Igfbp2	16008	5.321E-36	0.495138	0.28	0.15	1.253E-31	Microglia	1.4094558

Figure 4

Single cell transcriptomic results show changes in molecules of non-neuronal cells such as Eya1 in astrocytes and Ecrg4 in microglial cells and perivascular macrophages in Emx1-Cre;IRSp53 flox/flox mice.

(a-b) Identified cell types by Seurat analysis from single nucleus RNA sequencing in mouse hippocampi and cell type distribution between Emx1-Cre;IRSp53 flox/flox (cko) and control (IRSp53 flox/flox, flox) mice. Graph theory-based cell type clustering of **b** is presented.

(c) Heatmap showing the gene expression patterns for the top 20 genes in each cluster.

(d-e) FeaturePlot showing differentially expressed genes between Emx1-Cre;IRSp53 flox/flox and control mice at the cell level for each cluster. The expression level of eyes absent (*Eya1)* decreased more than twice in astrocytes of Emx1-Cre;IRSp53 flox/flox mice as much as that of control mice (d). The expression level of Ecrg4 decreased more than twice in microglia, perivascular macrophage, vascular smooth cell, and vascular endothelial cell of Emx1-Cre;IRSp53 flox/flox mice compared to control mice (e).

(f) Detailed list of differentially expressed genes of single-cell RNA sequencing of the hippocampus between Emx1-Cre;IRSp53 flox/flox and control mice.

Supplementary Files

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