**Supplementary materials**

**Supplementary Figures**

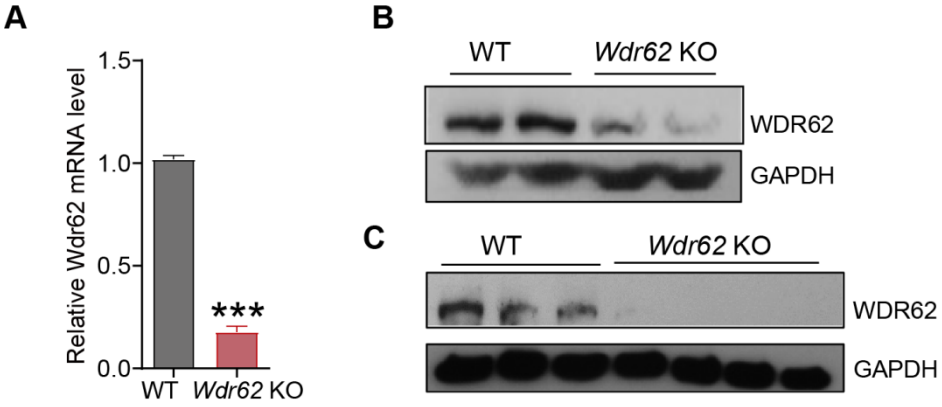


**Figure S1.** The knock out efficiency of *Wdr62* KO mice (related to Figure 1)

**(A)** Real-time PCR analysis of *Wdr62* mRNA levels in E18.5 mice brains. WT n = 2, KO n = 4. Data are presented as means ± SEM, ***$p < 0.001$, two-tailed unpaired t-test. **(B)** Immunoblot analysis of WDR62 protein levels in P6 WT and *Wdr62* KO mouse brains. **(C)** Western blot analyses of WDR62 protein expression in 2DIV primary cultured MEF cells. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein level was loaded as a control.

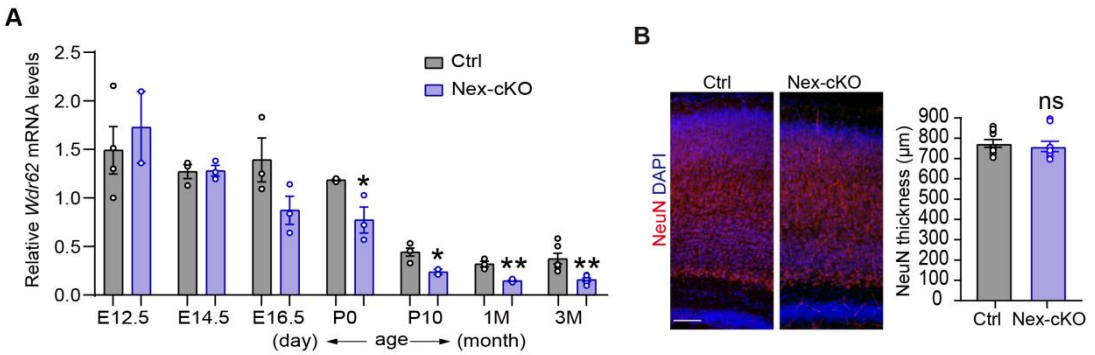


**Figure S2.** *Wdr62* expression in Nex-cKO mice (related to Figure 3)

**(A)** Relative *Wdr62* mRNA levels during different developmental stages of *Wdr62*$^{folx/flox}$; Nex$^{+/+}$ (control, ctrl) and *Wdr62*$^{folx/flox}$; Nex $^{Cre/+}$ (*Wdr62* Nex-cKO) littermates mice brains. E12.5: Ctrl n = 4, *Wdr62* Nex-cKO n = 2; E14.5, E16.5, P0 and 1 month old mice: Ctrl n = 3, *Wdr62* Nex-cKO n = 3; P10 mice: Control n = 4, *Wdr62* Nex-cKO n = 2; 3 months old mice: Ctrl and *Wdr62*

Nex-cKO n = 6. **(B)** Coronal sections of Ctrl and Nex-cKO mice at P3 were immunostained for newborn neuronal markers, NeuN, and nuclear using 4',6-diamidino-2-phenylindole (DAPI). Bar graph shows the thickness of NeuN+ cells in the cortical plate. Ctrl n = 10, *Wdr62* Nex-cKO n = 9. n = brain slices from at least three independent brains. Data are presented as means ± SEM; $**p < 0.01$, $*p < 0.05$, ns: $p > 0.05$; multiple unpaired t-tests (A); mann-Whitney test (B). Scale bar: 100 μm.

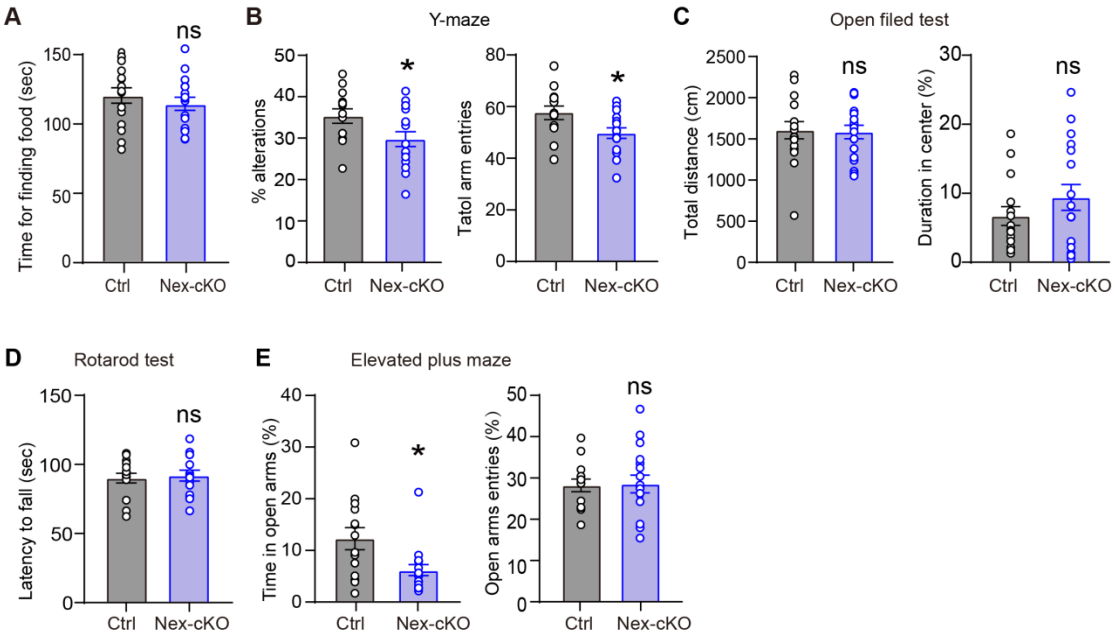


**Figure S3.** *Wdr62* Nex-cKO mice had normal acoustic startle response, locomotor activity, and anxiety behavior (related to Figure 3)

**(A)** *Wdr62* Nex-cKO mice showed no significant difference from control littermates in time taken to find food. **(B)** The OFT indicated normal locomotor activity. Ctrl n = 15, *Wdr62* Nex-cKO n = 17. **(C)** The rotarod test showed normal ability and motor learning abilities in *Wdr62* Nex-cKO mice. Ctrl n = 15, *Wdr62* Nex-cKO n = 17. **(D)** In the elevated plus-maze test, *Wdr62* Nex-cKO mice spent less time in the open arm, which indicated an anxiety tendency. Ctrl n = 15, *Wdr62* Nex-cKO n = 17. **(E)** Left: Y maze spontaneous alternation test showed fewer spontaneous alternations in *Wdr62* Nex-KO mice than those in control mice. Right: Y maze total arm entries showed cKO *Wdr62* Nex-KO mice had fewer total arm entries than those in control mice. Ctrl n = 15, *Wdr62* Nex-cKO n = 17. All data are presented as means ± SEM. $***p < 0.001$; $**p < 0.01$; $*p < 0.05$; ns: $p > 0.05$; two-tailed unpaired t-tests (A–C and E); Mann-Whitney test (D).

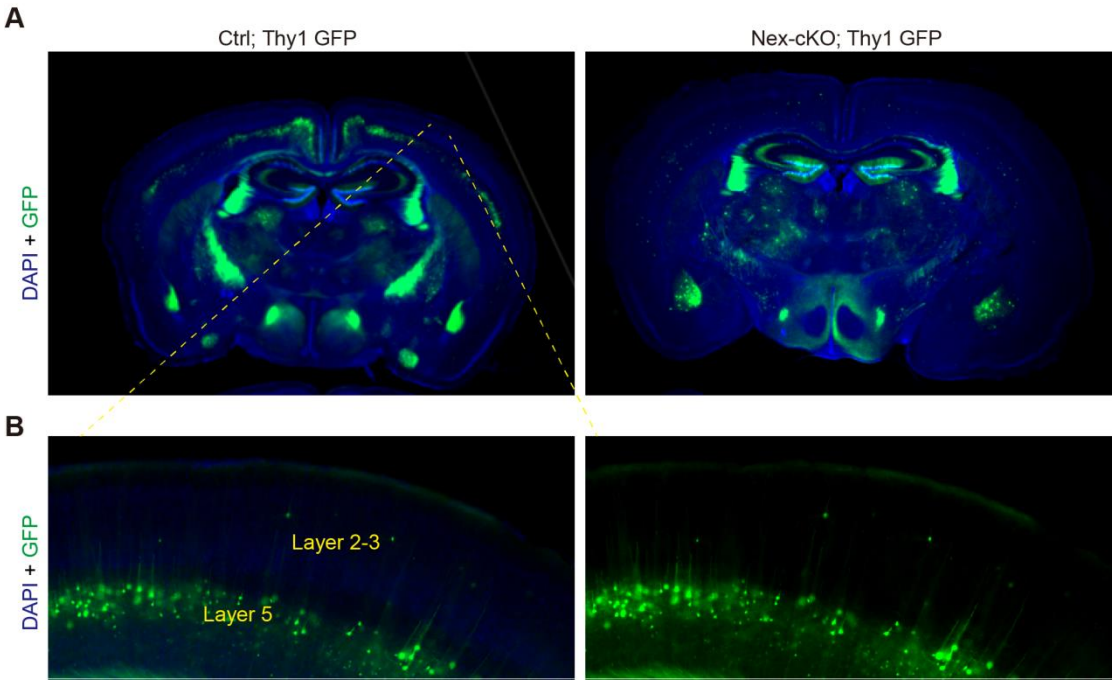


**Figure S4.** Thy1-GFPm-labeled pyramidal neurons in the brain (related to Figure 4)

**(A)** Large scale image of Thy1-GFPm-labeled control and *Wdr62* Nex-cKO mouse brain captured by OLYMPUS slideview VS200. **(B)** Image showing the region (layers 2/3 and 5) of analysis in the brain.

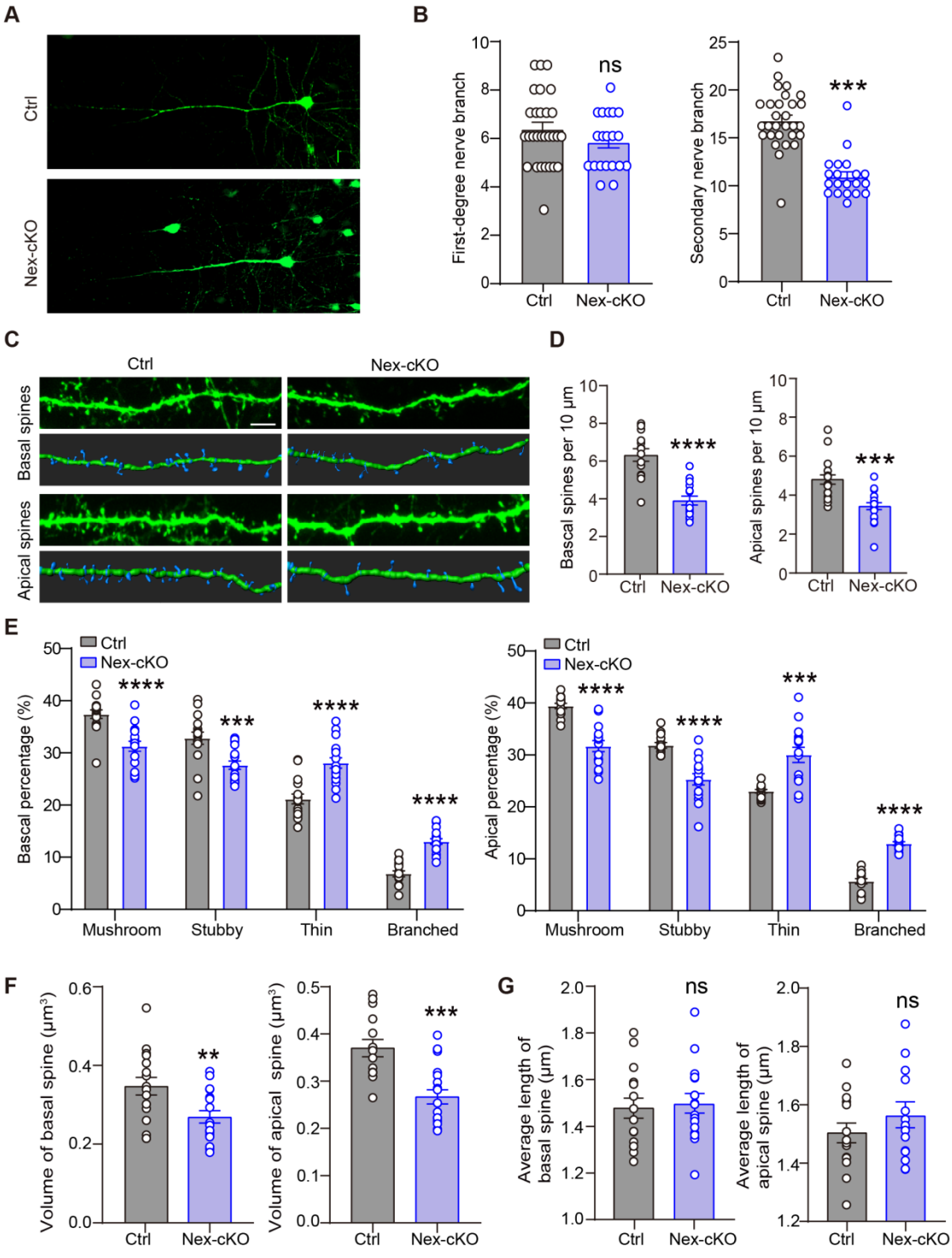


**Figure S5.** Morphology of layer 5 pyramidal neurons in the cortex (related to Figure 5)

**(A)** Representative images of Thy1-GFPm-labeled layer 5 pyramidal neurons of the cortex. **(B)** Quantification indicates reduced secondary dendritic arborization in *Wdr62* deficient neurons. **(C)** Higher-magnification images of apical/basal dendritic spines from Thy1-GFPm-labeled secondary dendrites of layer 5 pyramidal neurons in the cortex. **(D)** Quantification of total apical/basal spine

density in C. **(E)** The proportion of apical/basal dendritic spines that are mushroom (mature), stubby (mature), thin (immature), and branched (immature) subtypes; Ctrl n = 16 cells from three mice, *Wdr62* Nex-cKO n = 17 from three mice. **(F, G)** Quantification showed that the volume, but not the length, of apical/basal dendritic spines was significantly reduced. All data are presented as means ± SEM; ****$p < 0.0001$, ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; ns: $p > 0.05$; two-way ANOVA with Bonferroni's multiple comparisons test (E), two-tailed unpaired t-tests (D, F, and G). Scale bar: 5 μm.

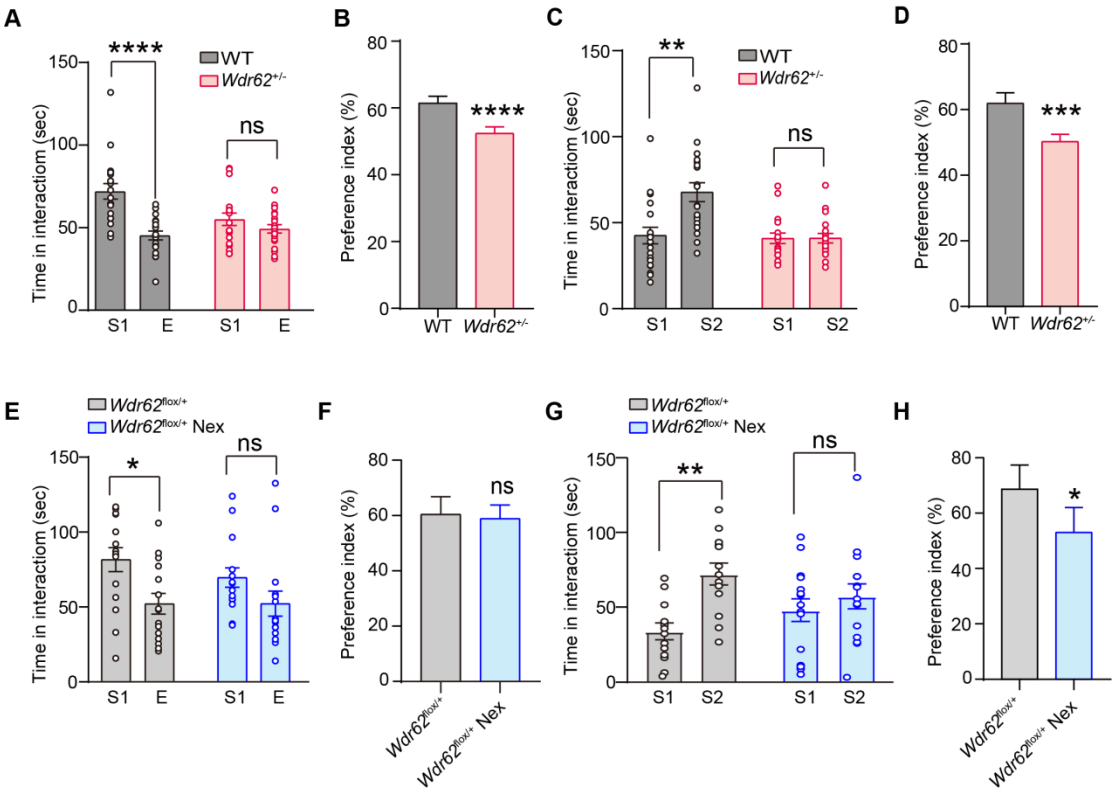


**Figure S6.** WDR62 haploinsufficiency results in social interaction defects (related to Figure 6)

**(A)** *Wdr62*$^{+/-}$ mice showed shorter social interaction time with (stranger 1) S1 than that of Ctrl mice. **(B)** Graph of social preference index (% time spent investigating social or empty cage out of total object investigation time) showing a significantly reduced preference index of *Wdr62*$^{+/-}$ mice during the social interaction test. **(C)** In the social novelty test, *Wdr62*$^{+/-}$ mice displayed no preference for interacting with the (stranger 2) S2 or S1 mouse. **(D)** The preference index for social novelty was significantly reduced in *Wdr62*$^{+/-}$ mice. **(E)** *Wdr62*$^{flox/+}$; Nex-Cre mice showed a shorter social interaction time with S1 than that of control mice. **(F)** Graph of social preference index showing normal preference index during the social interaction test. **(G)** In the social novelty

test, *Wdr62*$^{flox/+}$; Nex-Cre mice displayed no significant preference for interacting with the S2 or S1 mouse. (H) The preference index of social novelty was reduced in *Wdr62*$^{flox/+}$; Nex-Cre mice. All data are presented as means ± SEM; ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; ns: $p > 0.05$; WT and *Wdr62*$^{+/-}$ n = 19 (A–D). Ctrl and *Wdr62*$^{flox/+}$; Nex-Cre n = 15 (E–H). two-way ANOVAs with Bonferroni's multiple comparisons test (A, C, E, and G), two-tail unpaired t-tests (B, D, F, and H).

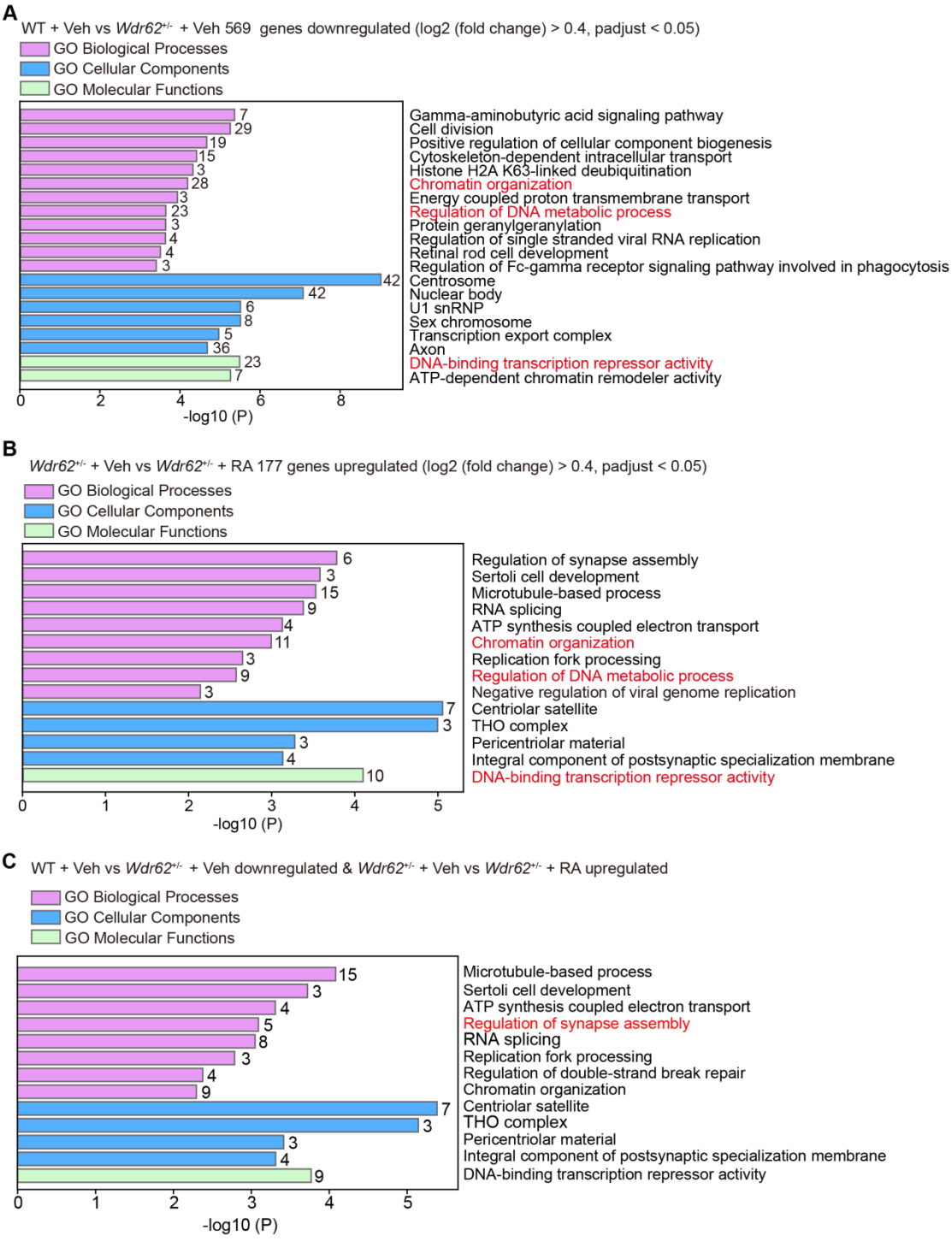

**Figure S7** (related to Figure 7)

**(A)** Gene ontology (GO) analysis revealed the GO terms indicating the biological processes, cellular components and molecular functions enriched in downregulated genes between WT mice reciveing oral administration of vehicle (WT + Veh) and *Wdr62*$^{+/-}$ mice reciveing oral administration of vehicle (*Wdr62*$^{+/-}$ + Veh). **(B)** GO analysis revealed the GO terms indicating the biological processes, cellular components and molecular functions enriched in upregulated genes between *Wdr62*$^{+/-}$ mice reciveing oral administration of vehicle (*Wdr62*$^{+/-}$ + Veh) and *Wdr62*$^{+/-}$ mice reciveing oral administration of ATRA (*Wdr62*$^{+/-}$ + RA). (C) Gene ontology (GO) analysis for the common regulate gene in Figure 7B. Terms indicating the biological processes, cellular components and molecular functions enriched in downregulated genes between WT mice receiving oral administration of vehicle (WT + Veh) and *Wdr62*$^{+/-}$ mice receiving oral administration of vehicle (*Wdr62*$^{+/-}$ + Veh).

## Supplemental methods

### Behavioral study

### Open-field test

The test mouse was first allowed to explore the open-field test (OFT) apparatus for 5 min. After washing the apparatus with 75% ethanol, the test mouse was introduced into the apparatus again and allowed to explore. The time spent in the center region (60%) of the apparatus and the distance traveled in the apparatus was calculated.

### Y maze test

The Y maze test is a rapid and easily administered test to assess spatial working memory. A Y maze with a three-armed runway (36 × 6 × 12 cm; 120 ° apart) was placed in the test room. Different visual cues were located on the wall at the end of each arm. Activity was recorded using a camera, and behavior was assessed using automatic video tracking software, Smart 3.0 (Panlab

company). Each mouse was randomly introduced into the end of one arm, facing the center, and allowed to freely explore the apparatus for 5 min. The equipment was cleaned with 70% ethanol and dried before a new trial started. Mice entered a different arm when they remembered which arm it had entered on a previous trial. A point was given when the mouse entered each of the three arms without repetition (e.g., CAB, BCA, and ABC). The number of points was then divided by the total arm entries minus one.

**Radial arm maze task**

The radial arm maze is a paradigm that is used to assess working and reference memory in rats and mice. Before the task, mice were allowed 1 week to habituate to the testing room and fasted for 24 hours. After every training day, restricted mouse food was provided. For the working memory assessment, all arms contained a reward (food), and the animal was required to visit each arm once only. For assessing reference memory, only some of the arms contained a reward, and the animal was required to visit only the baited arms. Visits to the same or non-baited arms counted as working memory or reference memory errors, respectively.

**Novel object recognition**

Novel object recognition is a relatively sensitive test for assessing cognitively-enhanced activity (40). An open-field box (36 × 36 × 25 cm) was used for this test. During the training period, two identical novel objects were placed into the box, and mice freely explored the box for 5 min. After 1 day, one of the familiar objects used during the training session was replaced with a new object, and the mouse was placed back into the open-field box for 5 min. The time each mouse spent exploring objects during the training and test phases was recorded using automatic video tracking software. The ratio of time spent exploring the novel object to that spent exploring both objects

was used as a measure of recognition memory.

**Three-chamber test**

The three-chamber test is a simple social interaction test, in which behaviors are video-recorded and analyzed to assess active interaction time between test and novel mice (41). It is a sensitive method for examining autism-related behavioral deficits and has been used to measure autism-like behaviors in mutant mouse models (42-44). Briefly, the arena consisted of a transparent box, which was divided into three chambers of the same size with removable doors in each chamber. The test mouse and the age/sex-matched C57BL/6 WT mice (not littermates) were kept in the test room individually 1 hour before the experiment. Two wire cups were placed in the corner of the left and right chambers. The test mouse was introduced into the central chamber and was allowed access to the three chambers for 10 min. During the first stage, a novel age/gender-matched C57BL/6 WT mouse was introduced into the wire cup of the left or right chamber, and an inanimate object was placed into the other wire cup. The test mouse was allowed access to the three chambers and both wire cups for 10 min. The time the test mouse spent interacting with the novel mouse and the inanimate object was recorded. The chamber was cleaned with 75% ethanol after each test. During the second stage, the inanimate object was replaced with another novel age/gender-matched C57BL/6 WT mouse (not a littermate). The test mouse was allowed access to the three chambers and both wire cups for 10 min. The time the test mouse spent interacting with the familiar and novel mice was recorded. The preference index was calculated as: time spent interacting with the novel mouse/ (time spent interacting with the novel mouse + time spent interacting with the object/familiar mouse) × 100%.

**Self-grooming test**

The mice were first habituated in a clean chamber individually for 10 min. Time spent self-grooming was recorded for 10 min. Self-grooming included actions of face-wiping, scratching of the head, neck, or ears, and full-body grooming.

**Stranger-intruder test**

The stranger-intruder test is a simple method to directly assess social ability. Each test mouse was placed into a new cage with clean bedding. A C57 male mouse, matched for weight and age, was placed into the same cage for 10 min. The time the test mouse actively sniffed the C57 mice was recorded.

**Fear-conditioning task**

In this test, a freezing response, which is considered a reliable measure of fear in mice, was elicited by unconditioned electric shock stimuli that were matched with conditioned stimuli of sounds or the surrounding context (45). The apparatus consisted of a conditional shock chamber (25 × 25 × 25 cm), surrounded by a large metal lockable box to minimize noise from the environment and control the recording software, PACKWIN 2.0 (Panlab). The conditional stimulus (CS) was an 80 dB sound at 2000 Hz for 28 s, and the unconditional stimulus (US) was a foot shock at 0.5 mA that lasted 2 s. During the test, the background white noise was 60 dB. On day 1, mice were allowed to freely explore the shock chamber for 2 min. Then, the CS was presented, which was followed by the US. The CS and US were repeated three times. There was a 30 s interval after each CS-US pairing. Mice were introduced into the same contextual chamber 24 h later. The same procedure was performed, except the CS paired with the US was turned off. After 48 h, mice were placed into the same chamber with a novel context (i.e., the chamber was changed to a different color and shape). The same procedure was performed, except the US was

turned off. Animals were observed for freezing behavior every 30 s in each condition. The percentage of freezing bouts was recorded.

**Elevated plus-maze**

The elevated plus-maze was used to investigate the anxiety level of mice and comprises two open arms (30 × 6 cm), two closed arms (30 × 6 cm), and a center section (6 × 6 cm). The same arms are on the opposite sides of the center section. The mouse was placed in the center section of the maze with its head facing the same closed arm at the start of the experiment. The behavior of mice in the elevated plus-maze was recorded and analyzed for 10 min. The time the mouse spent in open or closed arms was exported by the software.

**Morris water maze test**

The water maze test consisted of a circular pool (120 cm in diameter and 50 cm in height), a platform (6 cm in diameter), a camera fixed above the pool, and a computer connected to the camera. The pool was filled with approximately 22 ℃ water to a depth of 25 cm. The platform was placed 1 cm underwater, and a layer of non-toxic titanium dioxide floated on the water surface to prevent animals from seeing the underwater platform. Brightly colored cardboard or plastic panels of different shapes were attached to the walls of the pool as markers for the spatial orientation of the animals. The experiment consisted of two phases: the place navigation and spatial probe experiments. During the training period (5 days), the platform was placed in one quadrant of the pool, and mice were placed into the water facing the wall from four entry points at a fixed time each day. The mice were then quickly dried with towels and placed under a 37 ℃ heat lamp to maintain body temperature. On day 6, the spatial exploration test was conducted by removing the platform after the place navigation test, placing the mouse in the pool in the quadrant opposite to

the previous platform, and recording their swimming trajectory for 60 s to examine the mouse's memory of the original platform. A video tracking system was used to record the swimming path of each mouse in the pool, and the ratio of the time spent within the quadrant where the original platform was located for 60 s to the time spent in the other three quadrants was calculated.

**Nest building test**

The mice were separated kept in single cages 1 h before lights out. A 3.0 g square (10 × 10 cm) of skimmed cotton was placed in the cage. After 12 h, mice were observed for nesting, and their genotypes were photographed and scored according to scoring criteria: 0–1: 90% of the skimmed cotton remained intact; 1–2: a small portion of the skimmed cotton was torn but most remained intact (50%–90%); 2–3: most of the skimmed cotton was torn into pieces (50%–90%) but no nesting; 3–4: more than 90% of the skimmed cotton was torn into pieces and formed a flat nest (less than 50% of the height of the mice); 4–5: more than 90% of the skimmed cotton was torn into pieces, forming a high-quality mouse nest (greater than 50% of the height of the mice). because of the complexity of the experiments, scores could be decimals. For example, if the mouse nest was made perfectly, but 10% of the skimmed cotton remained unshredded, the score could be 4.5 points.

**Rotarod test**

The rotarod apparatus was used to examine motor coordination. The test mouse had was tested over four trials once a day for 2 days, with the rod rotation increasing from 4 to 40 rpm in 5 min. The interval between each trial was 30 min. The rotarod apparatus was stopped when the test mouse fell, and the latency to fall off the rod was determined. The average latency of the four trials on day 2 was calculated.

**Underground food sniffing test**

The mice were fasted for 24 h the day before the test. During the task, each mouse was placed in a clean rat cage (42.5 × 26.6 × 15.5 cm) with 4-cm-deep bedding that contained a hidden food pellet (0.2 g). The time required to find the buried food pellet was measured (up to 5 min).

**Acute slice preparation**

Mice aged 2–3 weeks were anesthetized with 4% isoflurane and decapitated, and the brain was dissected. The whole brain was sliced into 300 μm slices in cutting solution as described in (46), recovered at 34 ℃ for 30 min, and stored at room temperature. Solutions were continuously gassed with 95% oxygen ($O_2$)/5% carbon dioxide ($CO_2$).

**Whole-cell recordings**

Whole-cell voltage-clamp recordings were acquired from the pyramidal cells of cortex layer 2/3. Pyramidal neurons were identified according to location and morphology. All recordings were made at 20–25 ℃. The internal solution (in mM) was: 135 $CsMeSO_4$, 8 NaCl, 10 HEPES, 5 QX314-Cl, 4 Mg-ATP, 0.3 Na-GTP, 0.3 EGTA, and 0.1 spermine. Osmolarity was adjusted to 290–295 mOm, and pH was buffered at 7.3–7.4. The external solution (mM) was: 119 NaCl, 2.5 KCl, 4 $CaCl_2$, 4 $MgCl_2$, 1 $NaH_2PO_4$, 26.2 $NaHCO_3$, and 11 glucose, bubbled continuously with 95% $O_2$/5% $CO_2$. To record miniature excitatory postsynaptic currents (mEPSCs), picrotoxin (100 μM) was added to the external solution, and the cell membrane was held at -70 mV. Current responses were collected with a Multiclamp 700B amplifier (Axon Instruments), filtered at 2 kHz, and digitized at 10 kHz. Cells with a series resistance larger than 20 MOhm were excluded from the analysis.

**RNA extraction and QPCR analysis**

Total RNA was extracted from brain tissue samples using TRIZol reagent (Invitrogen, Cat# 15596018). First-strand cDNA was generated using the M-MLV reverse transcriptase (Promega, Cat# M1701) according to the manufacturer's protocols. Primers are as listed in Table S3.

**RNA sequencing data analysis**

Library preparation for Transcriptome sequencing a total amount of 3 μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer's recommendations and index codes were added to attribute sequences to each sample. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer (5X). First strand cDNA was synthesized using random hexamer primer and M-MuLV Reverse Transcriptase (RNase H-). Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities. After adenylation of 3' ends of DNA fragments, NEBNext Adaptor with hairpin loop structure were ligated to prepare for hybridization. In order to select cDNA fragments of preferentially 150~200 bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA). Then 3 μl USER Enzyme (NEB, USA) was used with size-selected, adaptor-ligated cDNA at 37℃ for 15 min followed by 5 min at 95 ℃ before PCR. Then PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer. At last, PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system. Clustering and sequencing the clustering of the index-coded samples

was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumia) according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina Hiseq2500/X platform and 125/150 bp paired-end reads were generated. The resulting P values were adjusted using the Benjamini-Hochberg procedure. Differentially expressed genes (DEGs) were defined according to an adjusted P value < 0.05 and Log2 (fold change) > 0.4. Gene Ontology (GO) enrichment analysis of DEGs was performed by Metascape database online analysis. GO terms with a corrected P value < 0.05 were defined as significantly enriched by DEGs.

**Table S3 Primers for genotyping and QPCR analysis**

| **Primer** | **Sequence (5'-3')** |
|---|---|
| WDR62 Floxed mouse line – Forward | GGCAGGTTAAGCTTTGTGAGTT |
| WDR62 Floxed mouse line – Reverse | GGATGCTTGCCTAGTGTGTAC |
| WDR62-KO- Reverse | CGTCTCATCATGAAGCCTAGG |
| Nex-Cre mouse line – Primer1 | GAGTCCTGGAATCAGTCTTTTTC |
| Nex-Cre mouse line – Primer2 | AGAATGTGGAGTAGGGTGAC |
| Nex-Cre mouse line – Primer3 | CCGCATAACCAGTGAAACAG |
| Thy1-YFP- Forward | AAGTTCATCTGCACCACCG |
| Thy1-YFP- Reverse | TCC TTG AAGAAG ATGGTGCG |
| Mouse *Wdr*62 QPCR- Forward | ACCGCAACGTAAGGGTCTACAACA |
| Mouse *Wdr*62 QPCR- Reverse | AAACATCTTGGCAACACACTCGCC |
| Mouse *Gadph* QPCR- Forward | TGATGACATCAAGAAGGTGGTGAAG |
| Mouse *Gadph* QPCR- Reverse | TCCTTGGAGGCCATGTAGGCCAT |