

## Supplementary tables

**Supplementary table 1.** The number of spines analyzed in each experiment.

|           |                          |              |   |
|-----------|--------------------------|--------------|---|
| Figure 1D | Fixed brain slice        | B6           | 3436 spines from 25 dendrites of 3 mice     |
|           |                          | BTBR         | 1787 spines from 11 dendrites of 3 mice     |
| Figure 1F | Primary neuronal culture | B6           | 7544 spines from 63 dendrites of 20 neurons |
|           |                          | BTBR         | 5767 spines from 65 dendrites of 20 neurons |
| Figure 3  | Fixed brain slice        | Vehicle      | 1570 spines from 17 dendrites of 3 mice     |
|           |                          | Vortioxetine | 1937 spines from 20 dendrites of 3 mice     |

**Supplementary table 2.** List of all compounds for phenotypic screening.

| Target molecule                     | Compound name                 | CAS No.      |
|-------------------------------------|-------------------------------|--------------|
| 5-Hydroxytryptamine (5-HT) receptor | Ferulic acid (sodium)         | 24276-84-4   |
|                                     | Azasetron (hydrochloride)     | 123040-16-4  |
|                                     | Rupatadine (Fumarate)         | 182349-12-8  |
|                                     | LY310762                      | 192927-92-7  |
|                                     | Pimavanserin                  | 706779-91-1  |
|                                     | Eletriptan (hydrobromide)     | 177834-92-3  |
|                                     | Granisetron (Hydrochloride)   | 107007-99-8  |
|                                     | Vortioxetine                  | 508233-74-7  |
|                                     | CP-809101 (hydrochloride)     | 1215721-40-6 |
|                                     | Cisapride                     | 81098-60-4   |
|                                     | Tandospirone                  | 87760-53-0   |
|                                     | Setiptiline                   | 57262-94-9   |
|                                     | Sarpogrelate (hydrochloride)  | 135159-51-2  |
|                                     | RS 127445                     | 199864-87-4  |
|                                     | Flibanserin                   | 167933-07-5  |
|                                     | SB-742457                     | 607742-69-8  |
|                                     | Loxapine                      | 1977-10-2    |
|                                     | LY 344864                     | 186544-26-3  |
|                                     | Aripiprazole                  | 129722-12-9  |
|                                     | Zolmitriptan                  | 139264-17-8  |
|                                     | Prucalopride (succinate)      | 179474-85-2  |
|                                     | BRL 54443                     | 57477-39-1   |
|                                     | Clozapine (N-oxide)           | 34233-69-7   |
|                                     | Mosapride (citrate)           | 112885-42-4  |
|                                     | Ramosetron (Hydrochloride)    | 132907-72-3  |
|                                     | Piboserod                     | 152811-62-6  |
|                                     | Tropisetron (Hydrochloride)   | 105826-92-4  |
|                                     | Rizatriptan (benzoate)        | 145202-66-0  |
|                                     | Dalasetron (Mesylate hydrate) | 878143-33-0  |
|                                     | Palonosetron (Hydrochloride)  | 135729-62-3  |
|                                     | Ondansetron                   | 99614-02-5   |
| Tianeptine (sodium salt)            | 30123-17-2                    |              |
| Mirtazapine                         | 85650-52-8                    |              |
| SB 242084                           | 181632-25-7                   |              |
| Adoprazine                          | 222551-17-9                   |              |

|                     |                                  |              |
|---------------------|----------------------------------|--------------|
|                     | TG6-10-1                         | 1415716-58-3 |
|                     | Risperidone                      | 106266-06-2  |
|                     | 8-OH-DPAT                        | 78950-78-4   |
| Adrenergic receptor | Silodosin                        | 160970-54-7  |
|                     | Deoxycorticosterone (acetate)    | 56-47-3      |
|                     | Indacaterol (maleate)            | 753498-25-8  |
|                     | Nebivolol (hydrochloride)        | 152520-56-4  |
|                     | Phentolamine (mesylate)          | 65-28-1      |
|                     | Scopine (hydrochloride)          | 85700-55-6   |
|                     | ICI 118,551 (hydrochloride)      | 72795-01-8   |
|                     | (S)-Timolol (Maleate)            | 26921-17-5   |
|                     | Esmolol (hydrochloride)          | 81161-17-3   |
|                     | Methoxyphenamine (Hydrochloride) | 5588-10-3    |
|                     | Atipamezole (hydrochloride)      | 104075-48-1  |
|                     | Levobetaxolol (hydrochloride)    | 116209-55-3  |
|                     | Ritodrine (hydrochloride)        | 23239-51-2   |
|                     | Carvedilol                       | 72956-09-3   |
|                     | Tamsulosin                       | 106133-20-4  |
|                     | Tizanidine (hydrochloride)       | 64461-82-1   |
|                     | Formoterol (Fumarate)            | 43229-80-7   |
|                     | Salmeterol (xinafoate)           | 94749-08-3   |
|                     | Ivabradine (hydrochloride)       | 148849-67-6  |
|                     | Naftopidil                       | 57149-07-2   |
|                     | UK 14,304 (tartrate)             | 70359-46-5   |
|                     | Detomidine (hydrochloride)       | 90038-01-0   |
|                     | Asenapine (maleate)              | 85650-56-2   |
| Dopamine receptor   | Lurasidone (Hydrochloride)       | 367514-88-3  |
|                     | Clebopride (malate)              | 57645-91-7   |
|                     | Amisulpride                      | 71675-85-9   |
|                     | rac-Rotigotine (Hydrochloride)   | 102120-99-0  |
|                     | Cabergoline                      | 81409-90-7   |
|                     | ST-836 (hydrochloride)           | 1415564-68-9 |
|                     | Azapaperone                      | 1649-18-9    |
|                     | Levosulpiride                    | 23672-07-3   |
|                     | Rotigotine (Hydrochloride)       | 125572-93-2  |
|                     | Chlorprothixene                  | 113-59-7     |
|                     | Ropinirole (hydrochloride)       | 91374-20-8   |

|   |  |              |
|---|--|--------------|
|   | Paliperidone                           | 144598-75-4  |
|   | SKF 38393 (hydrochloride)              | 62717-42-4   |
|   | Iloperidone                            | 133454-47-4  |
|   | B-HT 920                               | 36085-73-1   |
|   | GBR 12935 (dihydrochloride)            | 67469-81-2   |
|   | Vanoxerine (dihydrochloride)           | 67469-78-7   |
| Muscarinic acetylcholine (mACh) receptor    | Benztropine (mesylate)                 | 132-17-2     |
|   | Benzetimide (hydrochloride)            | 5633-14-7    |
|   | Aclidinium (Bromide)                   | 320345-99-1  |
|   | Tolterodine (Tartrate)                 | 124937-52-6  |
|   | (R)-5-Hydroxymethyl Tolterodine        | 207679-81-0  |
|   | Tiotropium (Bromide)                   | 136310-93-5  |
|   | Tarafenacin (D-tartrate)               | 1159101-48-0 |
|   | TBPB                                   | 634616-95-8  |
|   | Imidafenacin                           | 170105-16-5  |
|   | Cevimeline (hydrochloride hemihydrate) | 153504-70-2  |
|   | Fesoterodine (fumarate)                | 286930-03-8  |
|   | Otilonium (bromide)                    | 26095-59-0   |
|   | Diphenmanil (methylsulfate)            | 62-97-5      |
|   | VU0152100                              | 409351-28-6  |
|   | Xanomeline (oxalate)                   | 141064-23-5  |
|   | Tropium (chloride)                     | 10405-02-4   |
| Darifenacin (hydrobromide)                  | 133099-07-7                            |              |
| $\gamma$ -Aminobutyric acid (GABA) receptor | Indiplon                               | 325715-02-4  |
|   | Afloqualone                            | 56287-74-2   |
|   | Bemegride                              | 64-65-3      |
|   | Phenylpiracetam                        | 77472-70-9   |
|   | Etifoxine                              | 21715-46-8   |
|   | NS11394                                | 951650-22-9  |
|   | Lorediplon                             | 917393-39-6  |
|   | AWD 131-138                            | 188116-07-6  |
|   | Ginkgolide A                           | 15291-75-5   |
|   | (R)-Baclofen                           | 69308-37-8   |
|   | Dihydroergotoxine (mesylate)           | 8067-24-1    |
| $\gamma$ -Secretase                         | PF-3084014                             | 1290543-63-3 |
|   | MK-0752                                | 471905-41-6  |
|   | DAPT                                   | 208255-80-5  |

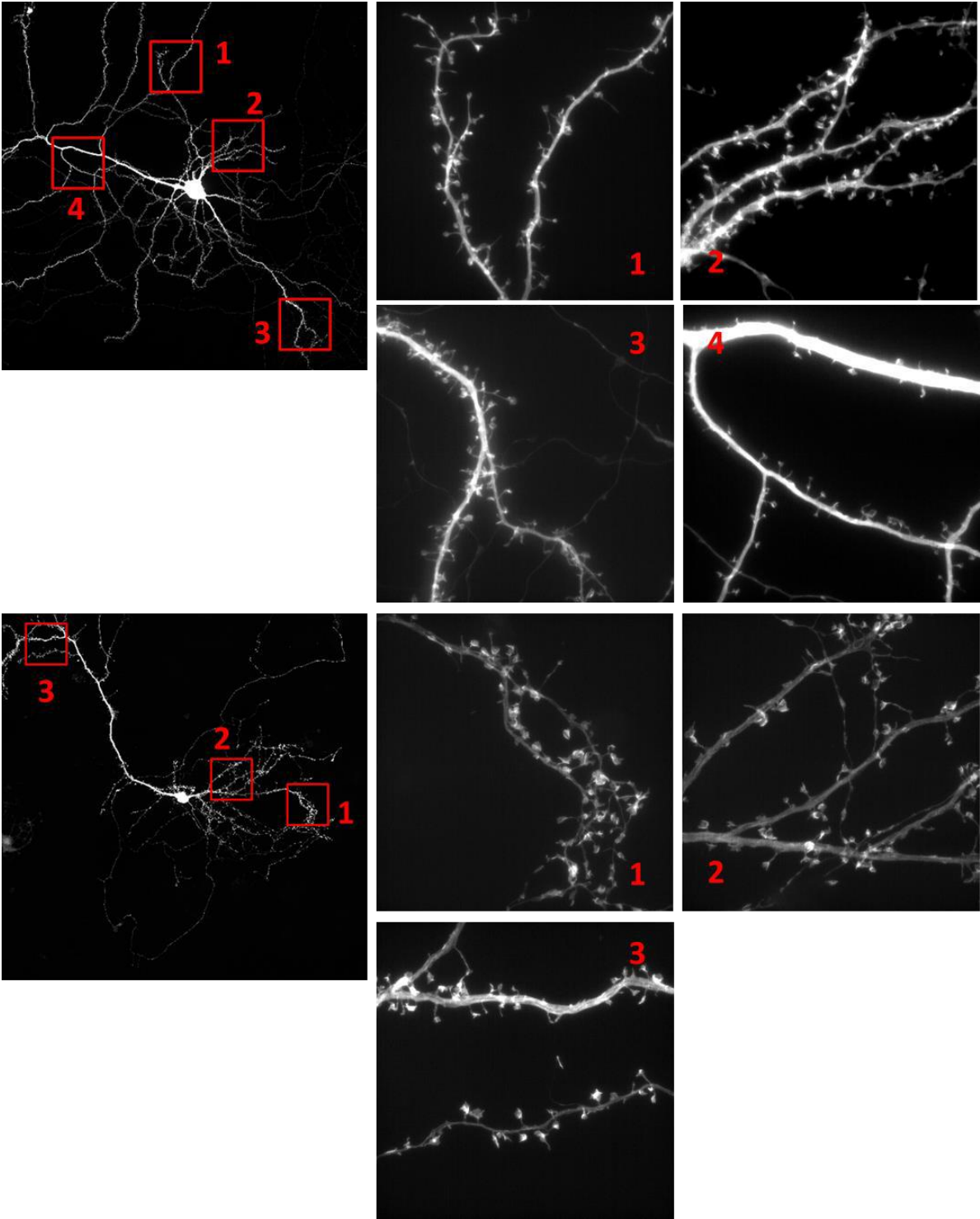
|  |                             |              |
|--|-----------------------------|--------------|
|  | RO4929097                   | 847925-91-1  |
|  | LY-411575                   | 209984-57-6  |
|  | BMS-708163                  | 1146699-66-2 |
|  | gamma-secretase modulator 3 | 1431697-84-5 |
|  | Semagacestat                | 425386-60-3  |
| $\alpha$ -Amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor | Perampanel                  | 380917-97-5  |
|  | Talampanel                  | 161832-65-1  |
|  | ZK200775                    | 161605-73-8  |
|  | CX546                       | 215923-54-9  |
|  | Naspm                       | 122306-11-0  |
|  | CFM-2                       | 178616-26-7  |
|  | PEPA                        | 141286-78-4  |
|  | Noopept                     | 157115-85-0  |
| Nicotinic acetylcholine (nACh) receptor                                  | PNU-120596                  | 501925-31-1  |
|  | A-867744                    | 1000279-69-5 |
|  | Cisatracurium (besylate)    | 96946-42-8   |
|  | PNU-282987                  | 123464-89-1  |
|  | Catharanthine               | 2468-21-5    |
|  | Varenicline                 | 249296-44-4  |
|  | Vecuronium (bromide)        | 50700-72-6   |
| Leucine rich-repeat kinase 2 (LRRK2)                                     | PF-06447475                 | 1527473-33-1 |
|  | LRRK2-IN-1                  | 1234480-84-2 |
|  | GNE-7915                    | 1351761-44-8 |
|  | HG-10-102-01                | 1351758-81-0 |
|  | JH-II-127                   | 1700693-08-8 |
|  | GSK2578215A                 | 1285515-21-0 |
|  | CZC-54252                   | 1191911-27-9 |
|  | CZC-25146                   | 1191911-26-8 |
| N-methyl-D-aspartate (NMDA) receptor                                     | Mephenesin                  | 59-47-2      |
|  | Eliprodil                   | 119431-25-3  |
|  | (+)-MK 801 (Maleate)        | 77086-22-7   |
|  | PEAQX (tetrasodium hydrate) |              |
|  | QNZ46                       | 1237744-13-6 |
|  | Memantine (hydrochloride)   | 41100-52-1   |
|  | SDZ 220-581                 | 174575-17-8  |
| Opioid receptor  | JDTic (dihydrochloride)     | 785835-79-2  |
|  | Trimebutine (maleate)       | 34140-59-5   |

|   |                               |              |
|---|-------------------------------|--------------|
|   | ADL-5859                      | 850173-95-4  |
|   | JTC-801                       | 244218-51-7  |
|   | Matrine                       | 519-02-8     |
|   | Meptazinol (hydrochloride)    | 59263-76-2   |
|   | BAN ORL 24                    | 475150-69-7  |
| Monoamine oxidase                                 | TVP1022                       | 185517-74-2  |
|   | Lazabemide                    | 103878-84-8  |
|   | Tedizolid (phosphate)         | 856867-55-5  |
|   | Rasagiline (mesylate)         | 161735-79-1  |
|   | Safinamide                    | 133865-89-1  |
|   | Tranlycypromine (hemisulfate) | 13492-01-8   |
| Neurokinin receptor                               | Talnetant                     | 174636-32-9  |
|   | Fosaprepitant (dimeglumine)   | 265121-04-8  |
|   | SB-222200                     | 174635-69-9  |
|   | NKP608                        | 177707-12-9  |
|   | Maropitant                    | 147116-67-4  |
|   | Aprepitant                    | 170729-80-3  |
| Fatty acid amide hydrolase (FAAH)                 | PF-3845                       | 1196109-52-0 |
|   | FAAH-IN-2                     | 184475-71-6  |
|   | URB-597                       | 546141-08-6  |
|   | LY2183240                     | 874902-19-9  |
| Selective serotonin reuptake inhibitor (SSRI)     | Fluvoxamine (maleate)         | 61718-82-9   |
|   | Dapoxetine (hydrochloride)    | 129938-20-1  |
|   | Sertraline (hydrochloride)    | 79559-97-0   |
|   | Milnacipran (hydrochloride)   | 101152-94-7  |
| Amyloid- $\beta$                                  | Frentizole                    | 26130-02-9   |
|   | FPS-ZM1                       | 945714-67-0  |
|   | ARN2966                       | 102212-26-0  |
| Acetylcholinesterase (AChE)                       | Galanthaminone                | 510-77-0     |
|   | ( $\pm$ )-Huperzine A         | 120786-18-7  |
| Calcitonin receptor-like receptor (CGRP) receptor | MK-3207 (Hydrochloride)       | 957116-20-0  |
|   | MK-0974                       | 781649-09-0  |
| $\beta$ -Secretase                                | LY2811376                     | 1194044-20-6 |
| Monoacylglycerol lipase (MAGL)                    | JZL195                        | 1210004-12-8 |

**Supplementary table 3.** Secondary assay results for hit compounds. "↑" indicates that the percentage of Mature Spine Neurons was more than 10% higher than the DMSO treatment group. "↔" denotes that the difference from the DMSO treatment was within ±10%. "↓" denotes that it was more than 10% lower than the DMSO treatment. Each column represents a different compound treatment concentration. Assays are tested at 2-fold (1, 0.5, 0.25, 0.13, 0.06, 0.03 μM) or 3-fold (1, 0.3, 0.1, 0.03, 0.01 μM) lower concentration. Empty columns mean that it is "not evaluated".

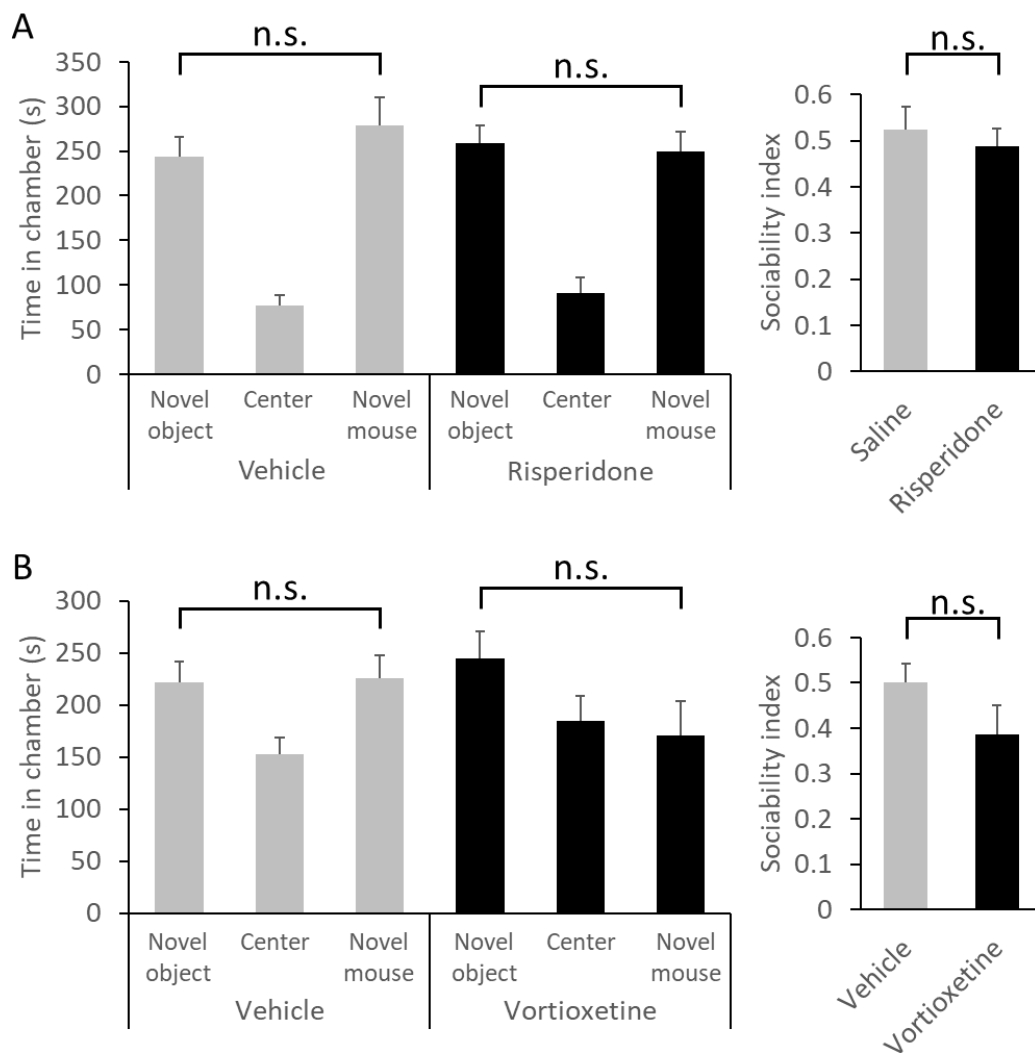
|               | Compound concentration (μM) |      |      |     |      |      |     |     |   |
|---------------|-----------------------------|------|------|-----|------|------|-----|-----|---|
|               | 0.01                        | 0.03 | 0.06 | 0.1 | 0.13 | 0.25 | 0.3 | 0.5 | 1 |
| Vortioxetine  |                             | ↑    | ↑    |     | ↔    | ↔    |     | ↑   | ↑ |
| 8-OH-DPAT     | ↑                           | ↔    |      | ↔   |      |      | ↔   |     | ↔ |
| Risperidone   | ↔                           | ↑    |      | ↔   |      |      | ↑   |     | ↑ |
| Indacaterol   |                             |      | ↑    |     | ↑    | ↑    |     | ↑   | ↔ |
| Tandospirone  |                             |      |      | ↑   |      |      | ↑   |     | ↑ |
| Palonosetron  |                             |      |      | ↑   |      |      | ↑   |     | ↔ |
| Rotigotine    |                             |      |      | ↔   |      |      | ↔   |     | ↑ |
| Diphenmanil   |                             |      |      | ↔   |      |      | ↑   |     | ↑ |
| AWD 131-138   |                             |      |      | ↑   |      |      | ↑   |     | ↑ |
| A-867744      |                             | ↔    | ↑    |     | ↔    | ↑    |     | ↑   | ↔ |
| Cisatracurium |                             | ↑    |      | ↑   |      |      | ↑   |     | ↑ |
| HG-10-102-01  |                             | ↑    |      | ↑   |      |      | ↔   |     | ↓ |
| Mephenesin    |                             |      |      | ↔   |      |      | ↔   |     | ↑ |
| JTC-801       |                             |      |      | ↑   |      |      | ↑   |     | ↑ |
| Maropitant    |                             |      |      | ↑   |      |      | ↑   |     | ↔ |

**Supplementary figures**



**Supplementary figure 1. Visualization of Neuronal Structure** Fluorescent images of two cultured neurons from B6 mice that were labeled with the combination of streptavidin-GFP and intracellularly injected biotin using the patch clamp technique. Multiple enlarged images in the red frame are shown in the right panel. The length of one side of the red frame is 40  $\mu\text{m}$ .





**Supplementary figure 2. Efficacy of risperidone and vortioxetine on social behavior in BTBR mice**  
 Comparison of social behavior using the 3-chamber social interaction test. Left graph shows time spent in each chamber. Right graph sociability index. (A) Risperidone was administered at a dose of 0.1 mg/kg (i.p) once daily for 7 days. Risperidone was dissolved in saline (0.9% NaCl solution) containing 0.01% v/v acetic acid. (B) Vortioxetine was administered as a single dose at 10 mg/kg (i.p). All bar graph data represent mean  $\pm$  SEM, \* $P < 0.05$ , \*\* $P < 0.01$ , Kruskal-Wallis test with Dunn's post hoc test and Mann-Whitney U test. N = 13 - 15 /group.