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Supplemental information

Olanzapine enhances early brain maturation through activation of the NODAL/FOXH1 axis

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Supplemental figures

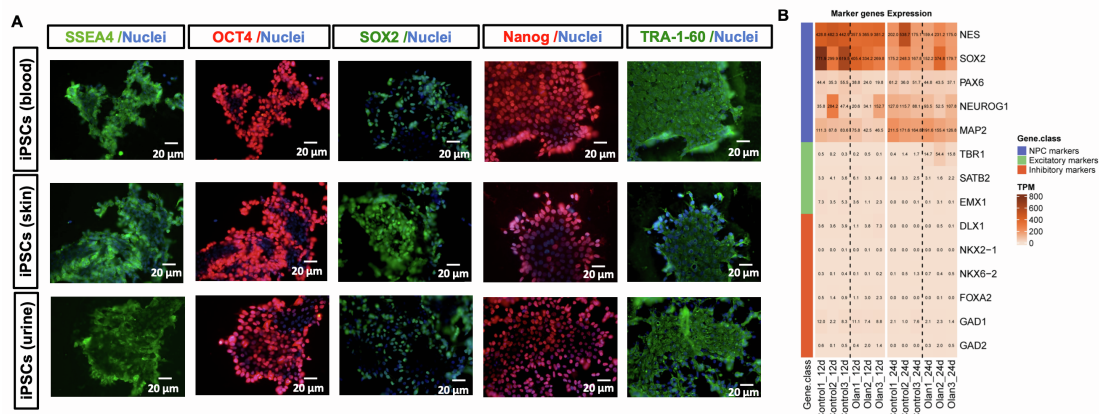


Figure S1. Validation of the expression of induced pluripotent stem cell lines (iPSCs) and cerebral organoids (CO). (A) The stemness markers SSEA4, OCT4, SOX2, NANOG, and TRA-1-60 were validated in the three cultured iPSC cell lines. (B) The expression levels of neural marker genes spanning the neural cell differentiation were validated using RNA-seq. The expression of each marker gene were presented as transcripts per million (TPM). Note that neural progenitor cell (NPC) markers includes NES, SOX2, PAX6, NEUROG1, and MAP2, and cortical markers including excitatory markers TBR1, SATB2, EMX1, and inhibitory markers DLX1, NKX2-1, NKX6-2, FOXA2, GAD1, and GAD2. Both 12-day and 24-day CO mainly consisted of NPC, while 24-day CO expressed more genes regulating neural maturation (e.g., MAP2 in both control and olanzapine-treated group, and TBR1 in olanzapine-treated group). Scale bar=20 μ m. Related to STAR Methods and Figure 1.

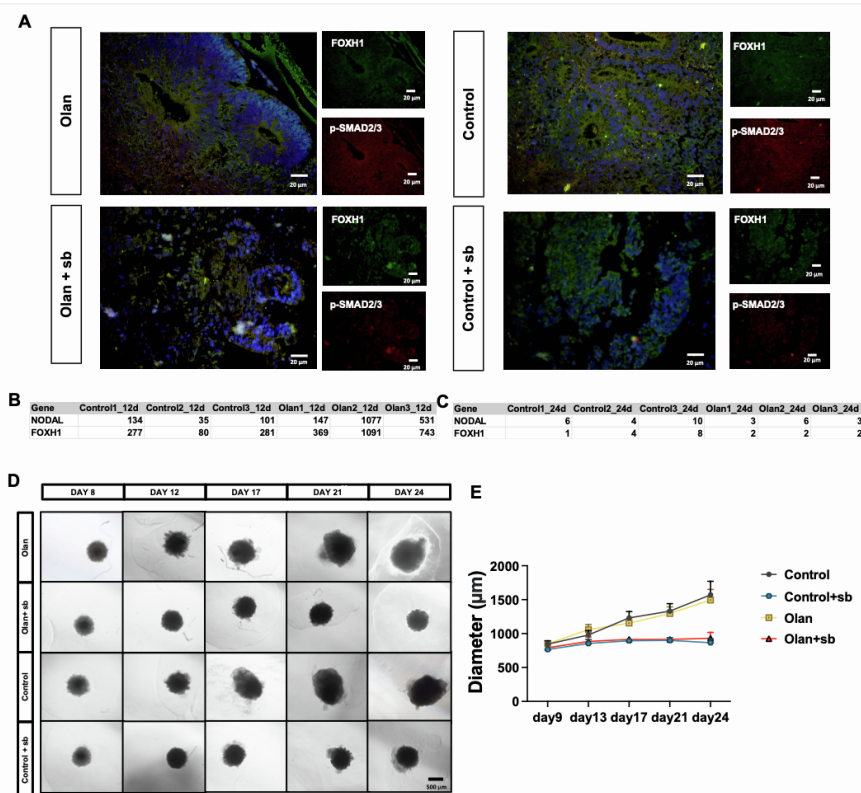


Figure S2. Inhibition of NODAL signaling tackled the development of CO in both olanzapine and control group. (A) Representative figures indicating nearly no expression of FOXH1 and p-SMAD2/3 in 24-day CO regardless of treatment condition. Scale bar=20 μ m. (B-C) Table showing the read count of NODAL and FOXH1 in each RNA-seq sample of 12 days (B) and 24 days (C). Note that reads of NODAL and FOXH1 can barely detected in 24-day CO samples. (D) Phase contrast microscopy showing the somatotype of COs in olanzapine-treated (Olan), olanzapine+SB431542 dual-treated (Olan+sb), control and SB431542 single-treated (Control+sb) groups. Scale bar=500 μ m. (E) Line plot showing the difference of maximal diameter with or without SB431542. Data are represented as mean \pm SD. Related to Figure 3.

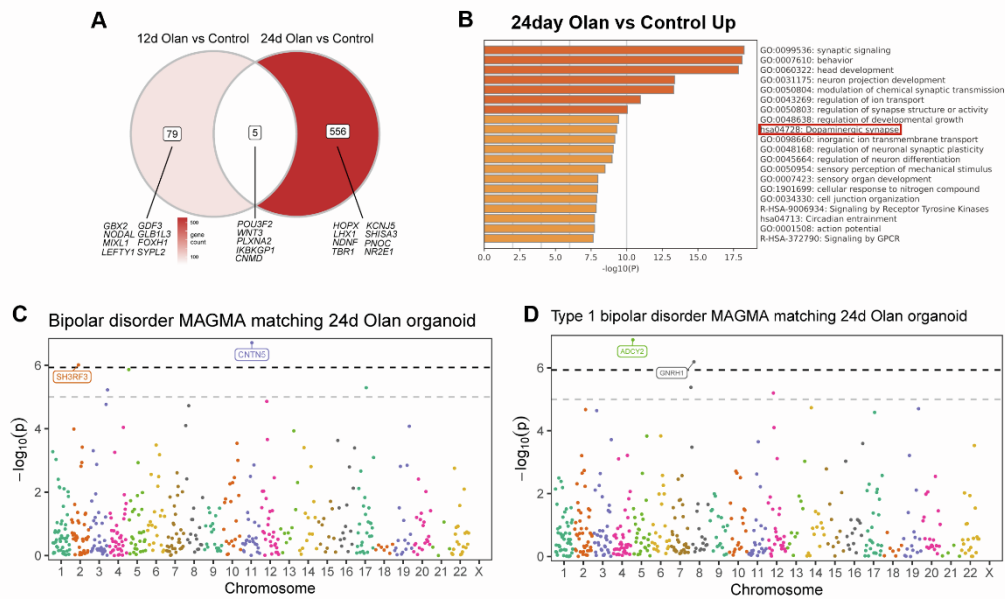


Figure S3. Olanzapine-responsive genes on 24-day CO had correlation with neuropsychiatric conditions. (A) Venn plot showing the difference of transcriptomics between 12-day and 24-day CO. Note that 24-day CO had more perturbed genes which expressed in more mature neuron. (B) Barplot showing the gene annotation terms of upregulated DEGs in 24-day olanzapine-treated CO. (C-D) Manhattan plot showing the olanzapine-responsive genes matched to bipolar disorder (C), and type I bipolar disorder (D). Related to Figure 5.

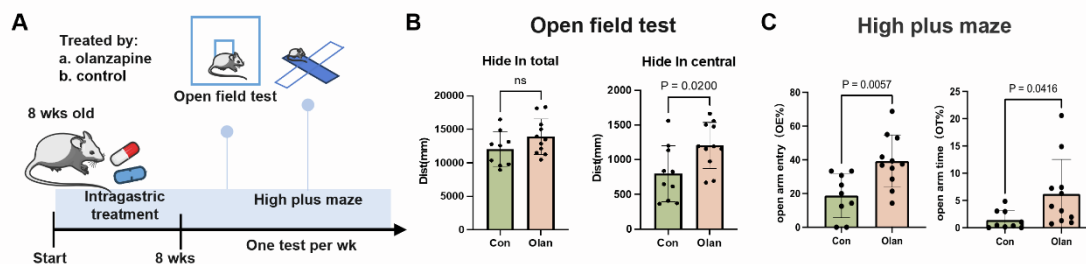


Figure S4. Validation of effective intragastric dosage of olanzapine in mice. (A) The experimental design to investigate the effects of olanzapine on F0 mice. (B) The results of open field test between olanzapine-treated and control F0 mice. (C) The results of high plus maze between olanzapine-treated and control F0 mice. Mean±SD. Student's t test. *P<0.05, **P<0.01. Data are represented as mean ± SD. Related to Figure 7. (Detailed statistical data can be found in Table S11.)

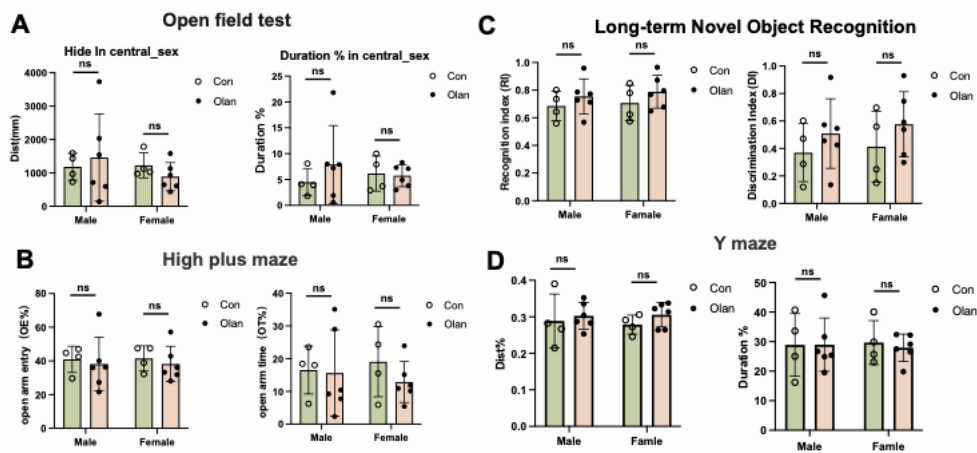


Figure S5. Prenatal exposure to olanzapine will not cause sex differences in cognition and mood in the adult F1 offspring. (A-D) The results of open field test(A), high plus maze(B), long-term novel object recognition(C), and Y maze(D) between olanzapine-treated and control in different sex F1 mice. Mean±SD. Student's t test. ns: P>0.05. Data are represented as mean ± SD. Related to Figure 7. (Detailed statistical data can be found in Table S11.)

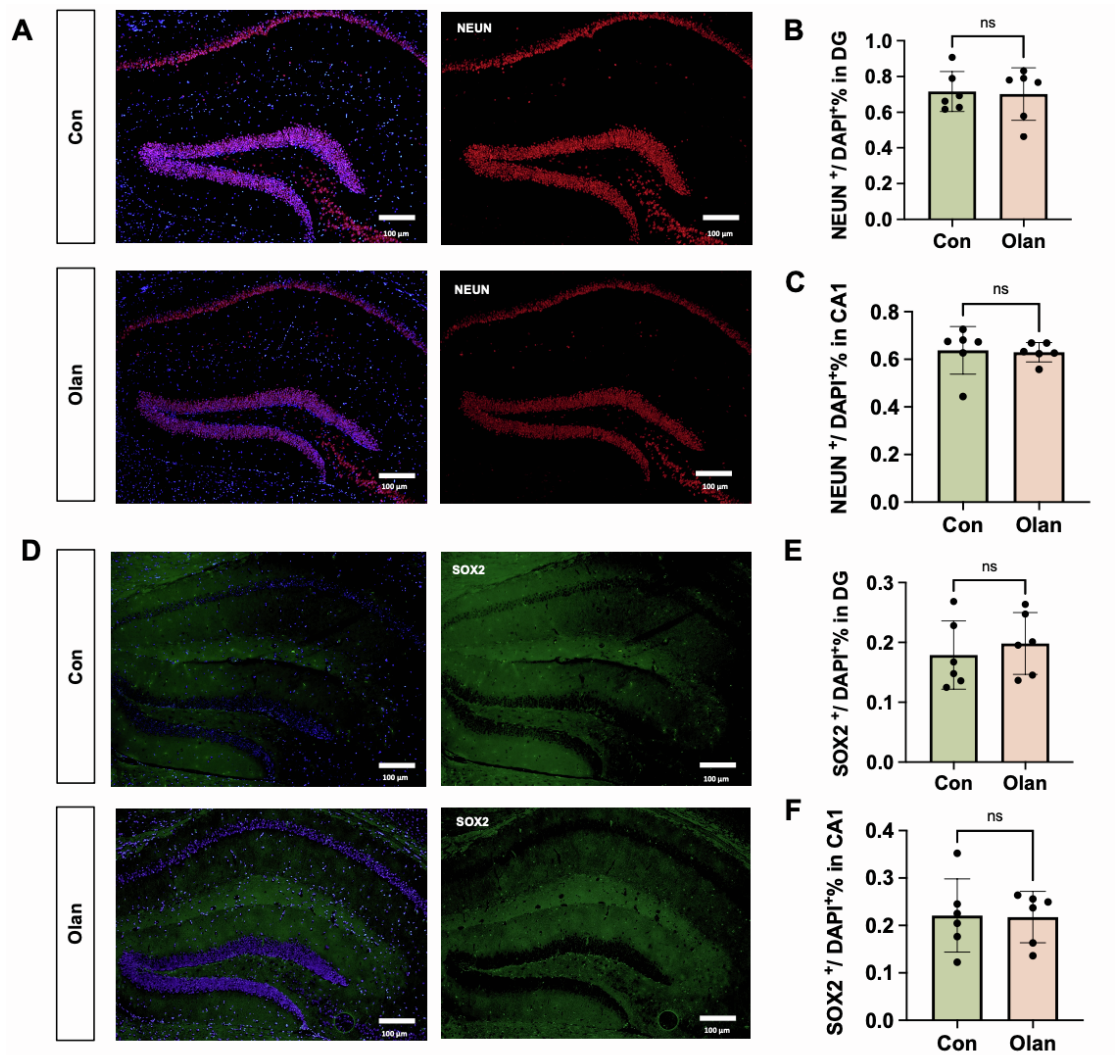


Figure S6. Prenatal exposure to olanzapine will not cause a deficiency in bilateral hippocampal regions in the adult F1 offspring. (A) Images NEUN⁺ for immunostaining of hippocampal regions in different groups. (B-C) The results of the NEUN⁺/DAPI⁺% in DG (B) and CA1 (C). (D) Images SOX2⁺ for immunostaining of hippocampal regions in different groups. The results of SOX2⁺/DAPI⁺% in DG (E) and CA1(F). Mean±SD. Student's t test. ns: P>0.05. Scale bar=100µm. Data are represented as mean ± SD. Related to Figure 7. (Detailed statistical data can be found in Table S11.)