

Supplementary information

Figure S1

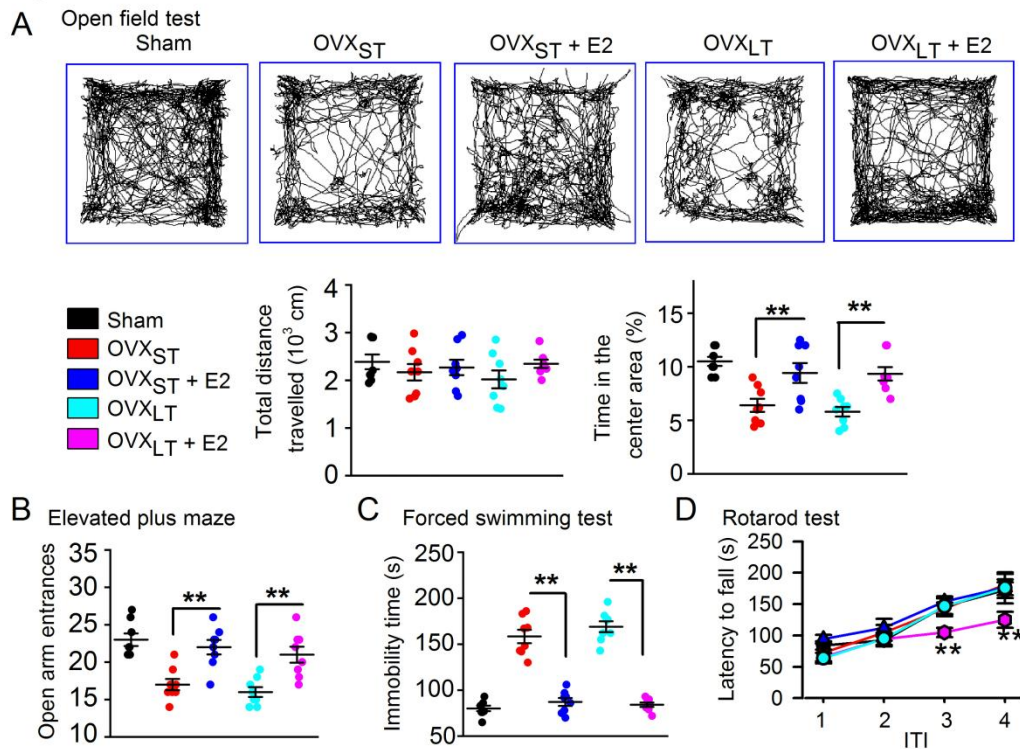


Figure S1. E2 replacement on the emotional disorders and motor learning

(A) Upper: sample traces of locomotor activity in the open field test. Lower: Summary of total distance travelled and time spent in the center area from OVX_{ST} and OVX_{LT} mice. Data are represented as mean \pm SEM, n = 8 mice per group. 2-way ANOVA followed by Bonferroni's *post hoc* test; $**p < 0.01$ between the marked groups. (B-D) Summary data in elevated plus maze, forced swimming test, and rotarod test from OVX_{ST} and OVX_{LT} mice with E2 treatment. Data are represented as mean \pm SEM, n = 8 mice in each group. 2-way ANOVA followed by Bonferroni's *post hoc* test; $**p < 0.01$ between the marked groups. In rotarod test, $**p < 0.01$ compared to sham.

Figure S2

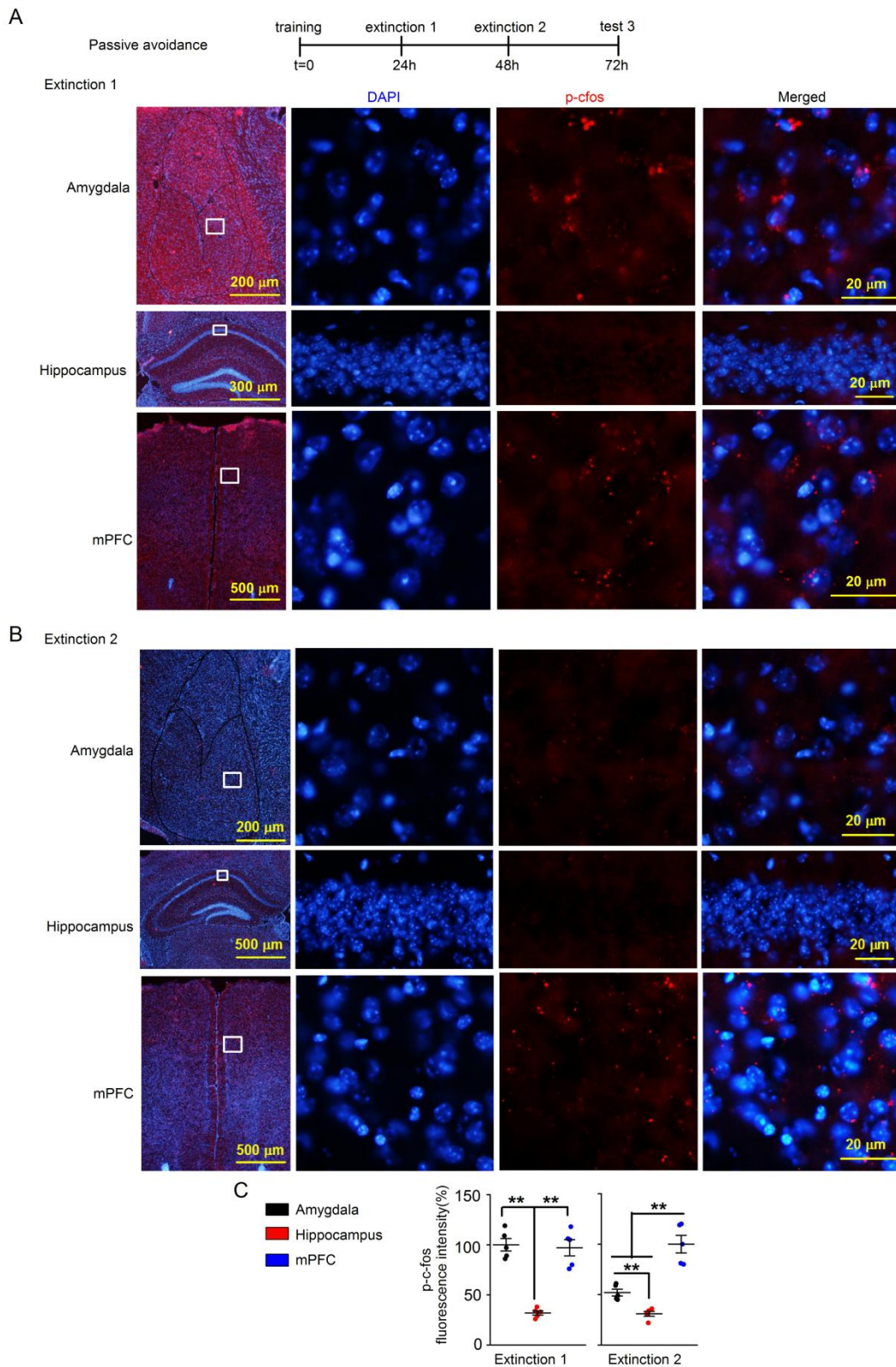


Figure S2. Neuronal activation in brain regions involved in fear extinction

(A, B) Representative images for p-c-fos (red) and nucleus (blue) from mice 90 min after the fear extinction 1 or extinction 2 in amygdala, hippocampus, and mPFC. (C) Integrated intensity of p-c-fos

measured across 400 neurons (20 neurons per slice, 4 slices/mouse, n = 5 mice in each group) in brain regions. Data are represented as mean \pm SEM, 1-way ANOVA followed by Bonferroni's *post hoc* test; ** $p < 0.01$ between the marked groups.

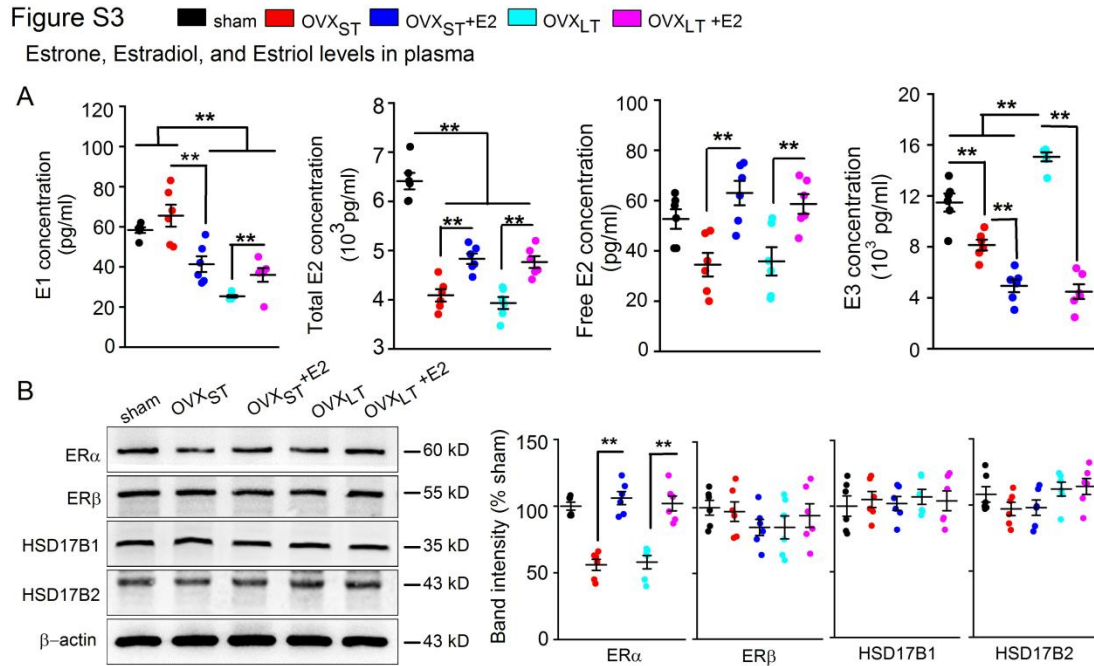


Figure S3. Levels of estrogen and its receptors in OVX_{ST} mice and OVX_{LT} mice with E2 replacement

(A) Levels of estrone (E1), estradiol (E2, total and free) and estriol (E3) in plasma. Levels of E2 was increased in plasma by exogenous injection, but exogenous E2 affected the levels of E1 and E3. Data are represented as mean \pm SEM, n = 6 mice per group. 2-way ANOVA followed by Bonferroni's *post hoc* test; ** $p < 0.01$ between the marked groups. (B) Left: western-blot samples showing the levels of ER α , ER β , and enzymes HSD17B1 and HSD17B2 responsible for estrogen conversion in mPFC. Right: Summary of relative levels of ER α , ER β , HSD17B1, and HSD17B2 in mPFC. Data are represented as mean \pm SEM, n = 6 mice per group. 2-way ANOVA followed by Bonferroni's *post hoc* test; ** $p < 0.01$ between the marked groups.

Figure S4

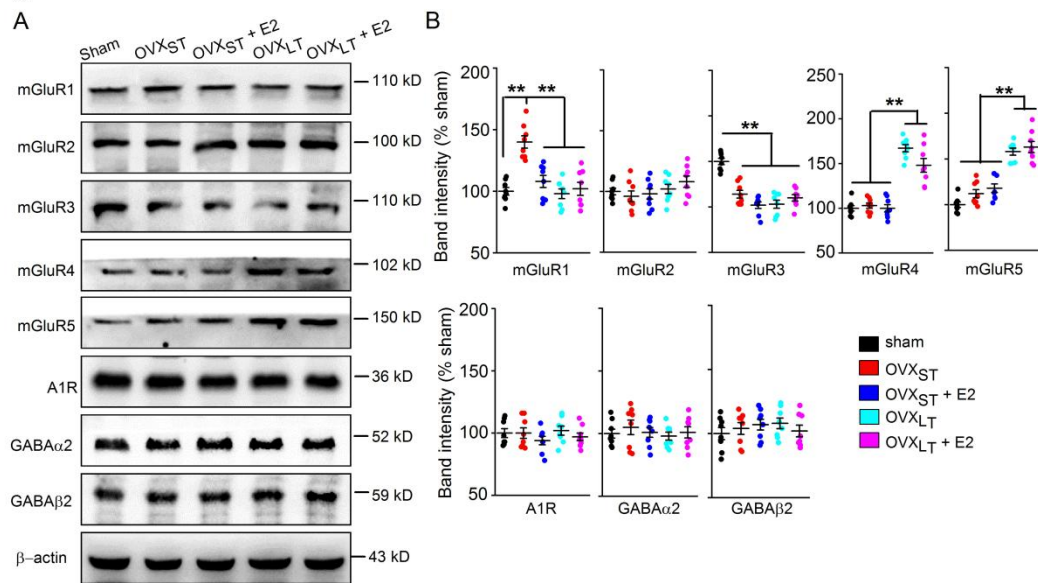


Figure S4. Synaptic plasticity-related proteins in OVX mice

(A) Western-blot samples showing the proteins in mPFC. (B) Summary of levels of mGluR1, mGluR2, mGluR3, mGluR4, mGluR5, A1R, GABA_{α2}, and GABA_{β2} in mPFC. Data are represented as mean ± SEM, n = 8 mice in each group. 2-way ANOVA followed by Bonferroni's *post hoc* test. ***p* < 0.01 between the marked groups.

Figure S5

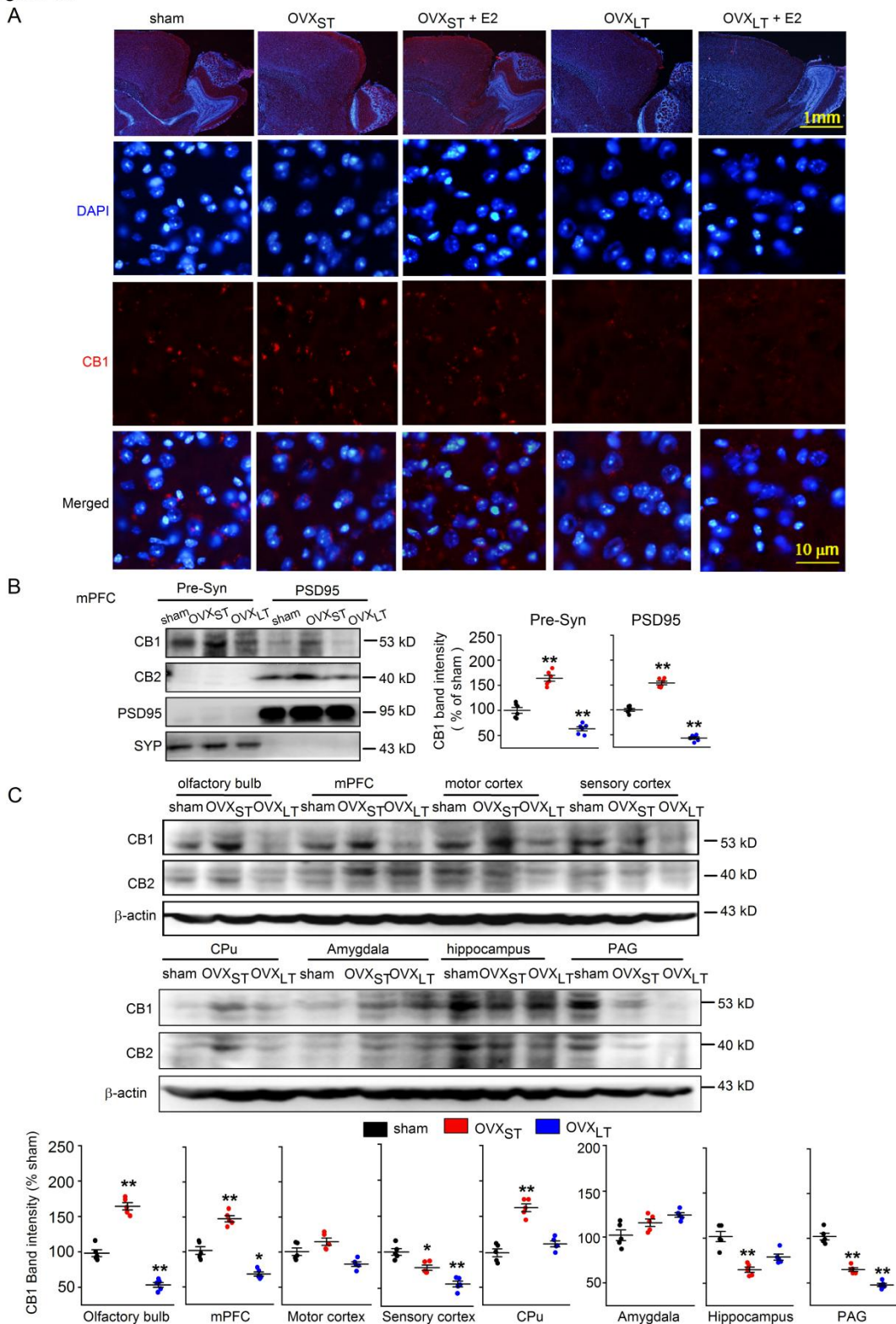


Figure S5. Decreased CB1 in both presynaptic and postsynaptic fraction

(A) Representative images of mPFC sections stained for CB1 (red) and nucleus (blue). (B) Left: Samples of Western blot for CB1 and CB2 in presynaptic and postsynaptic fraction of mPFC. Right: Band intensities were quantified as percentage of values from sham mice. PSD, postsynaptic density;

Syn, synaptosome; Pre-syn, presynaptic fraction; SYP, synaptophysin. PSD95 was used as loading control for postsynaptic fraction. SYP was used as loading control for presynaptic fraction. Data are represented as mean \pm SEM, n = 6 mice per group. 2-way ANOVA followed by Bonferroni's *post hoc* test; $**p < 0.01$ vs sham. (C) Samples of Western-blot for CB1 and CB2 in olfactory bulb, mPFC, motor cortex, sensory cortex, caudate putamen striatum (CPu), amygdala, hippocampus, and periaqueductal gray (PAG). Data are represented as mean \pm SEM, n = 6 mice per group. 1-way ANOVA followed by Bonferroni's *post hoc* test; $*p < 0.05$, $**p < 0.01$ vs sham.

Figure S6

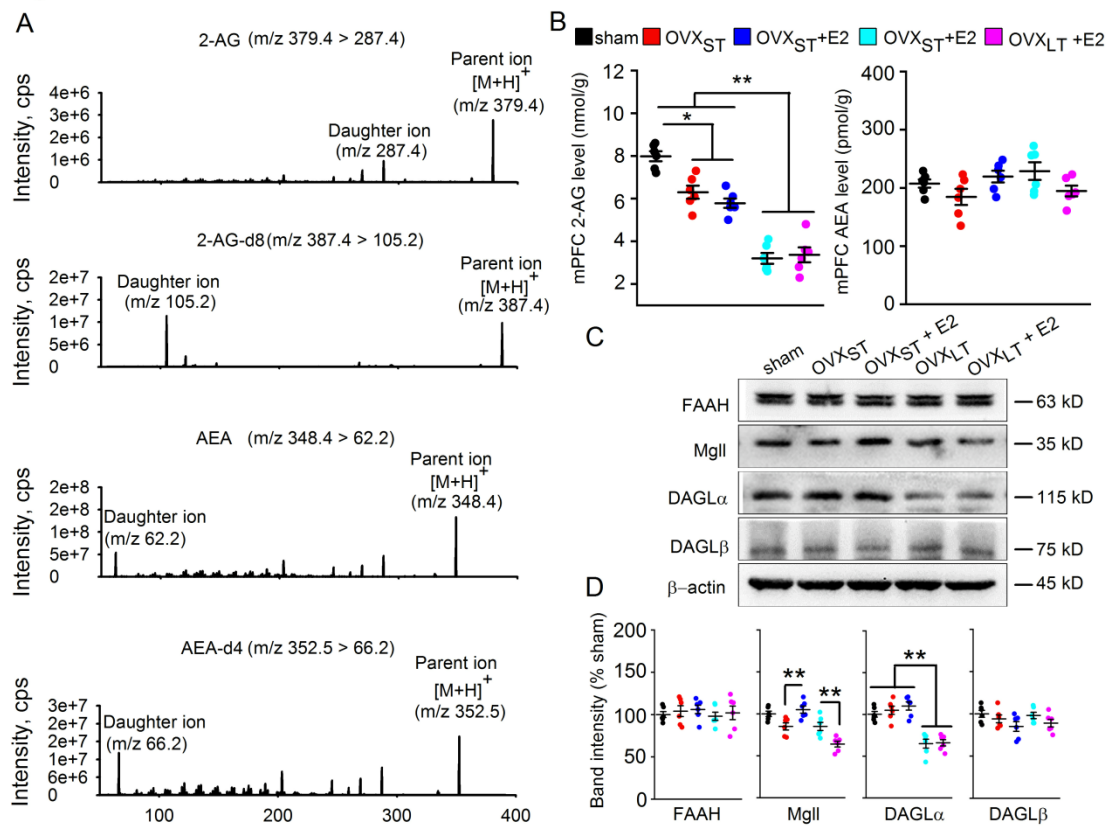


Figure S6. Decreased 2-AG but normal AEA in OVX_{LT} mice

(A) Representative mass spectrograms showing parent and daughter ion for 2-AG, 2-AG-d8, AEA, and AEA-d4. (B) Levels of 2-AG and AEA in mPFC were normalized to sham group according to quantitative LC-MS/MS results. Data are represented as mean \pm SEM, n = 6 mice per group. 2-way ANOVA followed by Bonferroni's *post hoc* test; $**p < 0.01$ between the marked groups. (C) Samples of Western-blot for FAAH (AEA degradative enzyme), MGLL (2-AG degradative enzyme), DAGL α , and DAGL β (Catalyzes the hydrolysis of diacylglycerol to 2-AG) in mPFC. (D) Relative immunoreactivities of MGLL, FAAH, DAGL α , and DAGL β were normalized to sham group. Data are

represented as mean \pm SEM, n = 6 mice per group. 2-way ANOVA followed by Bonferroni's *post hoc* test; ** $p < 0.01$ between the marked groups.

Figure S7

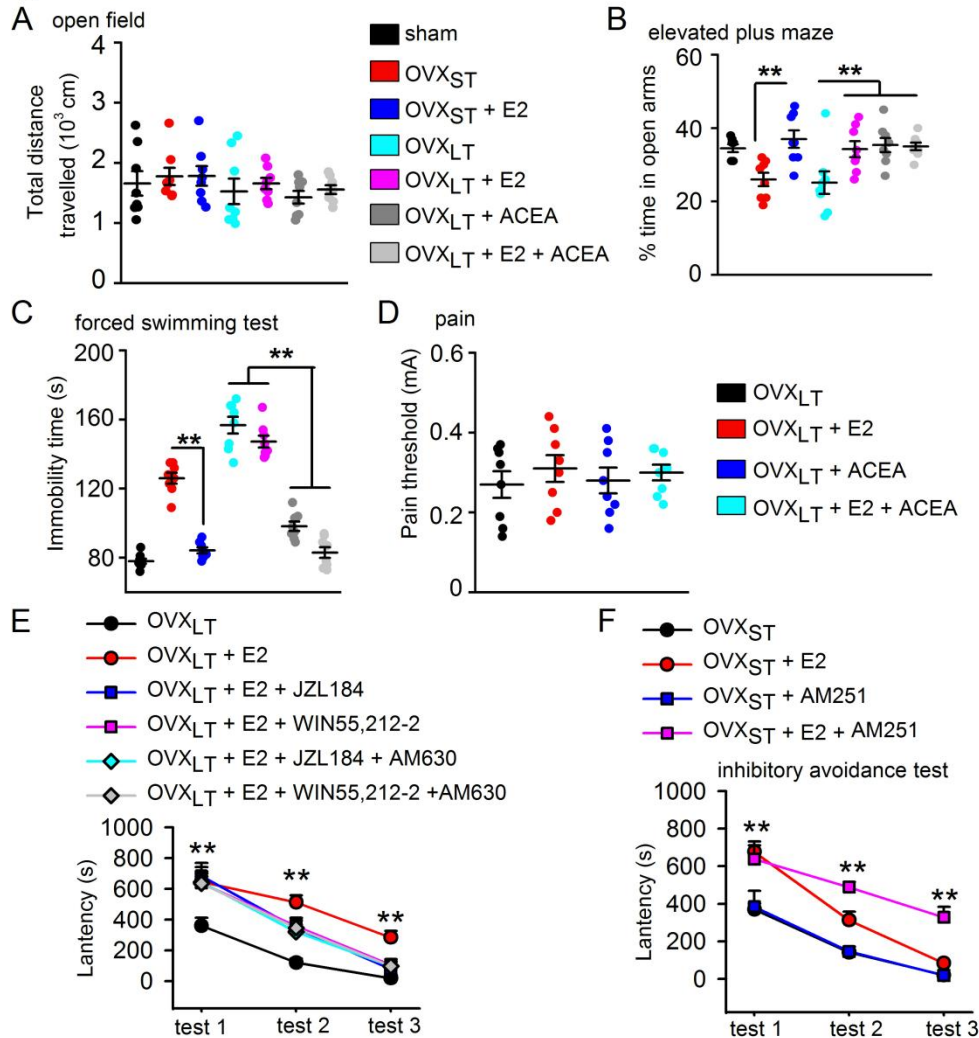


Figure S7. ACEA rescued fear memory extinction in E2 treated OVX_{LT} mice

(A) ACEA (a CB1 selective agonist, 0.5 mg/kg, 1-week once daily, s.c.) did not affect locomotor activity of OVX_{ST} and OVX_{LT} mice. Data are represented as mean \pm SEM, n = 8 mice per group. (B) ACEA and E2 (0.1 mg/kg, s.c.) induced anxiolytic effects in OVX_{ST} and OVX_{LT} mice. Data are represented as mean \pm SEM, n = 8 mice per group. 2-way ANOVA followed by Bonferroni's *post hoc* test; ** $p < 0.01$ between the marked groups. (C) ACEA and E2 showed anti-depressive effects in OVX_{ST} and OVX_{LT} mice. Data are represented as mean \pm SEM, n = 8 mice per group. 2-way ANOVA followed by Bonferroni's *post hoc* test; ** $p < 0.01$ between the marked groups. (D) ACEA and E2 did not affect pain sensitivity of mice. (E) ZL184 (an MGLL inhibitor, 8 mg/kg, s.c.) and WIN55,212-2

(a non-selective cannabinoid receptor agonist, 1 mg/kg, s.c.) recovered the E2 function in memory extinction. AM630 (a CB2 selective antagonist, 1 mg/kg, 1-week once daily, s.c.) could not blocked these effects. Data are represented as mean \pm SEM, n = 8 mice per group. 1-way ANOVA followed by Bonferroni's *post hoc* test; $**p < 0.01$ vs OVX_{LT} control mice. (F) E2-treated OVX_{ST} mice also showed impaired fear memory extinction after blocking CB1 by AM251 (CB1 antagonist, 3 mg/kg, s.c.). Data are represented as mean \pm SEM, n = 8 mice per group. 2-way ANOVA followed by Bonferroni's *post hoc* test; $**p < 0.01$ vs OVX_{LT} control mice.

Figure S8

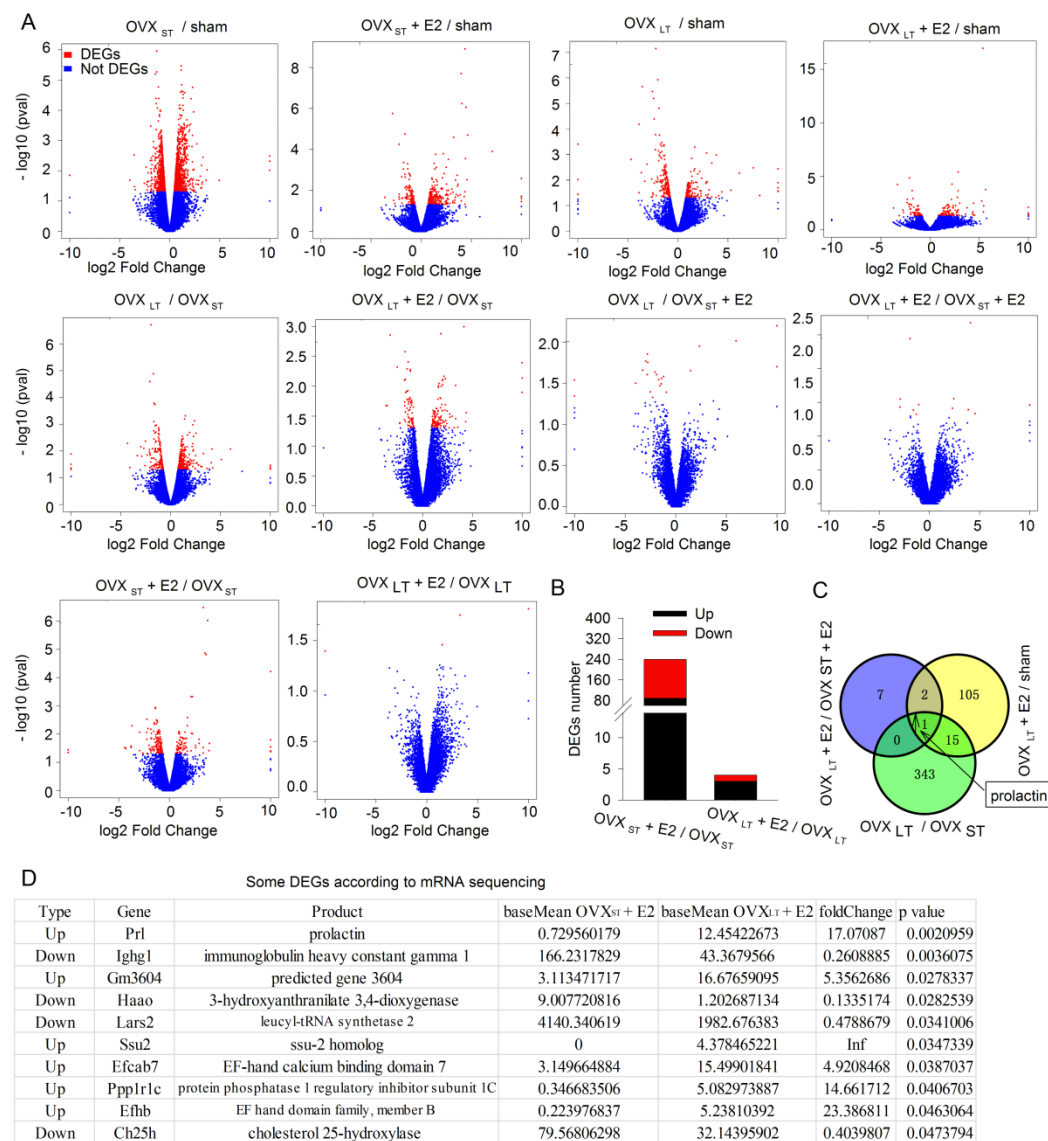


Figure S8. Prolactin was predicted as a candidate for impaired fear extinction in E2 treated OVX_{LT} mice

(A) Volcano plots of mRNA sequencing data from the mPFC of OVX_{ST} versus sham, OVX_{ST} + E2 versus sham, OVX_{LT} versus sham, OVX_{LT} + E2 versus sham, OVX_{LT} mice versus OVX_{ST} mice, OVX_{LT} + E2 mice versus OVX_{ST} mice, OVX_{LT} mice versus OVX_{ST} + E2 mice, OVX_{LT} + E2 mice versus OVX_{ST} + E2 mice, OVX_{ST} + E2 mice versus OVX_{ST} mice, and OVX_{LT} + E2 mice versus OVX_{LT} mice. (B) Number of differently expressed mRNAs (DEGs) in OVX_{ST} + E2 mice versus OVX_{ST} mice and OVX_{LT} + E2 mice versus OVX_{LT} mice. (C) Venn diagram showing different mRNAs in OVX_{LT} + E2 mice overlap with different mRNAs in OVX_{LT} + E2 mice versus sham mice and different mRNAs in OVX_{LT} mice versus OVX_{ST} mice. Prolactin was predicted as the only candidate for the impaired fear extinction. (D) List of part differently expressed mRNAs in OVX_{ST} + E2 mice versus OVX_{LT} + E2. Data are represented as mean ± SEM, n = 3 mice per group. Unpaired Student's *t*-test between the groups.

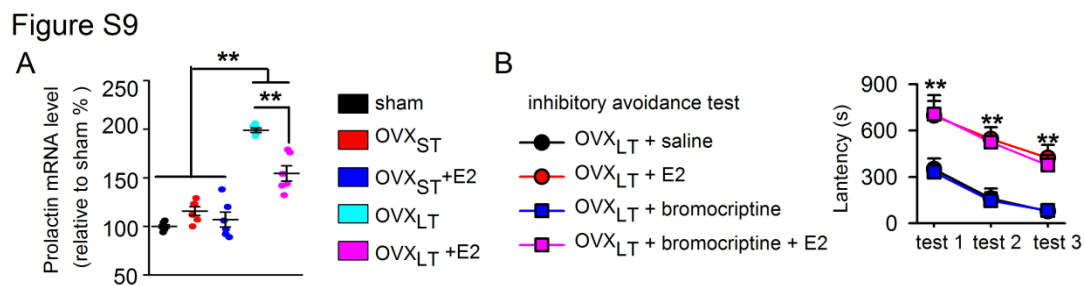


Figure S9. Targeting prolactin did not rescue impairment of fear extinction of E2 treated OVX_{LT} mice

(A) Levels of prolactin mRNA in mPFC of different groups confirmed by qPCR. Data are represented as mean ± SEM, n = 6 mice per group. 2-way ANOVA followed by Bonferroni's *post hoc* test; ***p* < 0.01 between the marked groups. (B) Bromocriptine (a prolactin inhibitor, 3 mg/kg, s.c.) did not affect E2 induced fear memory extinction impairment in OVX_{LT} mice. Data are represented as mean ± SEM, n = 8 mice per group. 2-way ANOVA followed by Bonferroni's *post hoc* test; ***p* < 0.01 vs saline control.

Figure S10

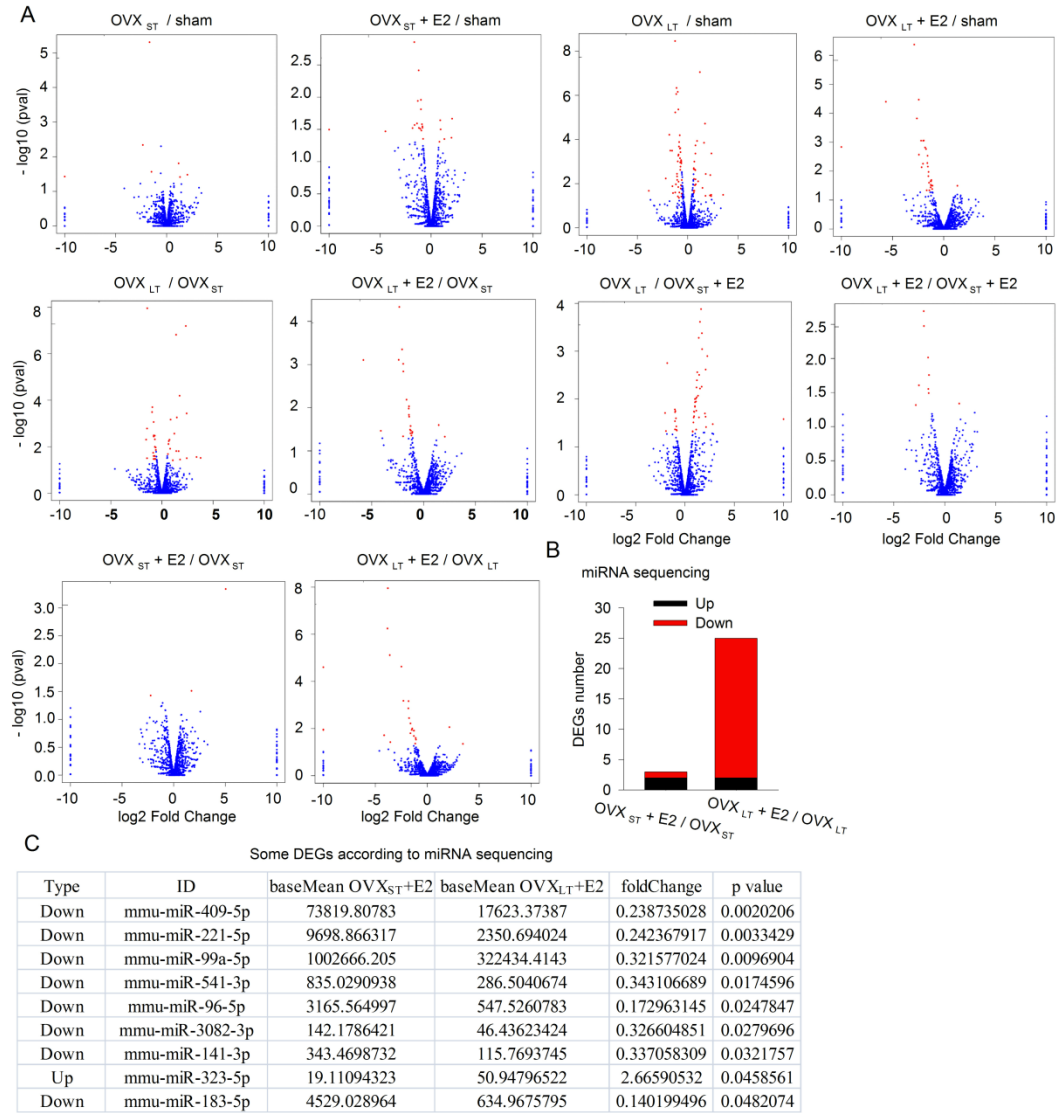


Figure S10. Differently expressed miRNAs between E2 treated OVX_{ST} mice and E2 treated OVX_{LT} mice

(A) Volcano plots of miRNA sequencing from the mPFC of OVX_{ST} versus sham, OVX_{ST} + E2 versus sham, OVX_{LT} versus sham, OVX_{LT} + E2 versus sham, OVX_{LT} versus OVX_{ST} mice, OVX_{LT} + E2 versus OVX_{ST} mice, OVX_{LT} versus OVX_{ST} + E2 mice, OVX_{LT} + E2 versus OVX_{ST} + E2 mice, OVX_{ST} + E2 versus OVX_{ST} mice, and OVX_{LT} + E2 versus OVX_{LT} mice. (B) Number of differently expressed miRNAs (DEGs) in OVX_{ST} + E2 mice versus OVX_{ST} and OVX_{LT} + E2 versus OVX_{LT} mice. (C) List of some differently expressed miRNAs in OVX_{ST} + E2 mice versus OVX_{LT} + E2. Data are represented as mean ± SEM, n = 3 mice per group. Unpaired Student's *t*-test between the groups.

Figure S11

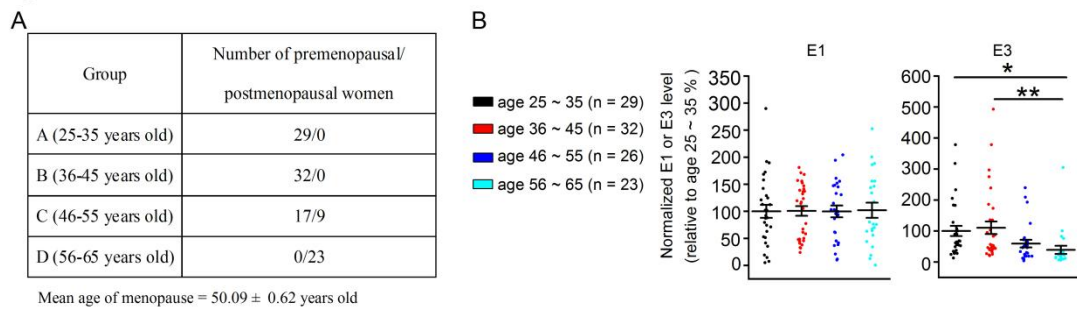


Figure S11. Information from women

(A) Counting of premenopausal and postmenopausal women enrolled in human research. (B)

Normalized E1, total E2, and E3 in plasma from different ages. Data are represented as mean ± SEM.

1-way ANOVA followed by Bonferroni's *post hoc* test; * $p < 0.05$. ** $p < 0.01$ between the marked groups.

Figure S12

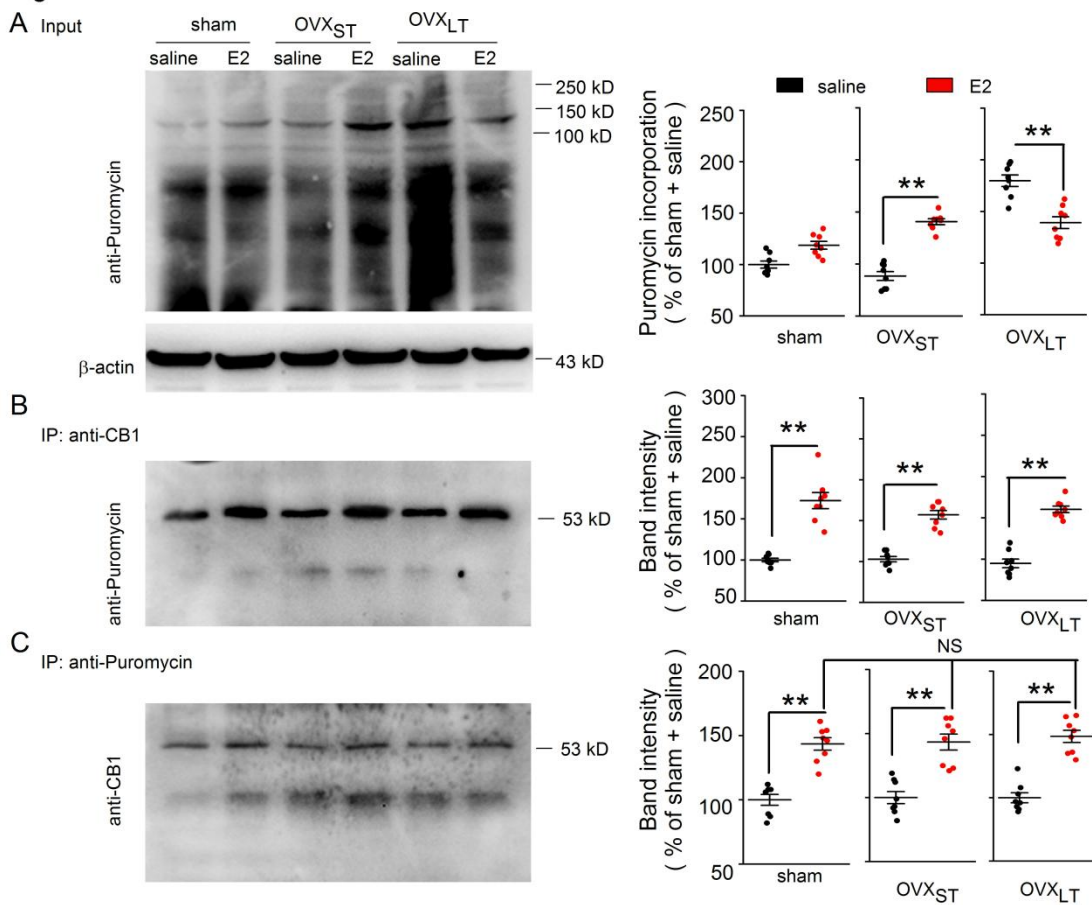


Figure S12. Increase of CB1 synthesis by E2 in sham, OVX_{ST} and OVX_{LT} mice

(A) Representative western blots of lysates from mPFC slices incubated with puromycin to measure

basal rates of CB1 protein synthesis. (B) Co-IP showing newly synthesized puromycin inserted protein CO-IP by anti-CB1 antibody before and after E2 treatment. (C) Co-IP showing newly synthesized CB1 protein level by puromycin inserted protein CO-IP by anti-puromycin antibody before and after E2 treatment. A-C: Data are represented as mean \pm SEM, n = 8 mice per group. 2-way ANOVA followed by Bonferroni's *post hoc* test; $**p < 0.01$ between the marked groups. ns: no significance.

Figure S13
A

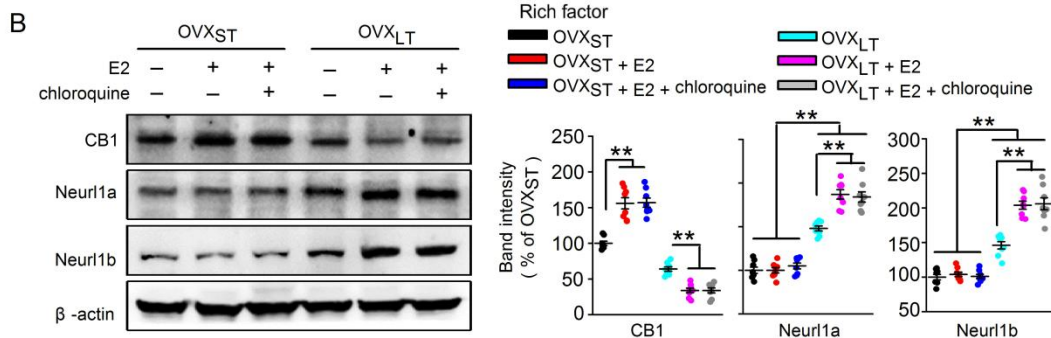
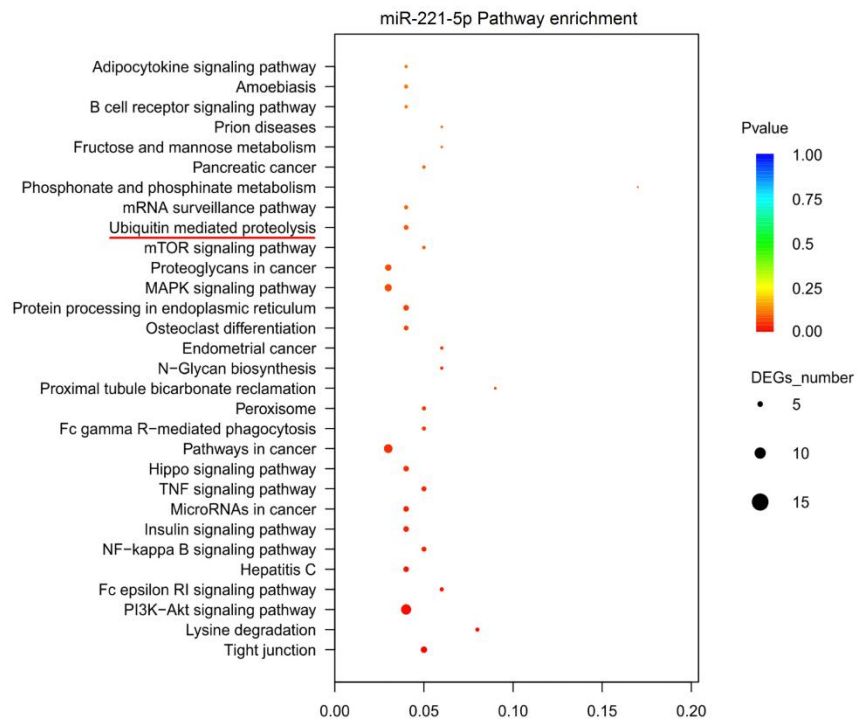


Figure S13. MiR-221-5p regulated ubiquitin mediated proteolysis pathway

(A) MiR-221-5p predicted target genes were analyzed for different cellular pathways using Functional annotation clustering with DAVID functional annotation clustering tool (<http://david.abcc.ncifcrf.gov/>). Enriched by KEGG pathways, genes displayed a significant enrichment for ubiquitin mediated proteolysis. (B) Left: Samples of western-blot showing the levels of CB1, Neur11a, and Neur11a in

OVX_{ST} and OVX_{LT} mice mPFC after E2 (0.1 mg/kg, 1-week once daily, s.c.) or chloroquine (a autophagy–lysosome pathway inhibitor, 20 mg/kg, i.p.). Right: Relative immunoreactivities of CB1, NeuN1a, and NeuN1a were normalized to OVX_{ST} untreated group. Data are represented as mean \pm SEM, n = 8 mice per group. 2-way ANOVA followed by Bonferroni's *post hoc* test; $**p < 0.01$ between marked groups.