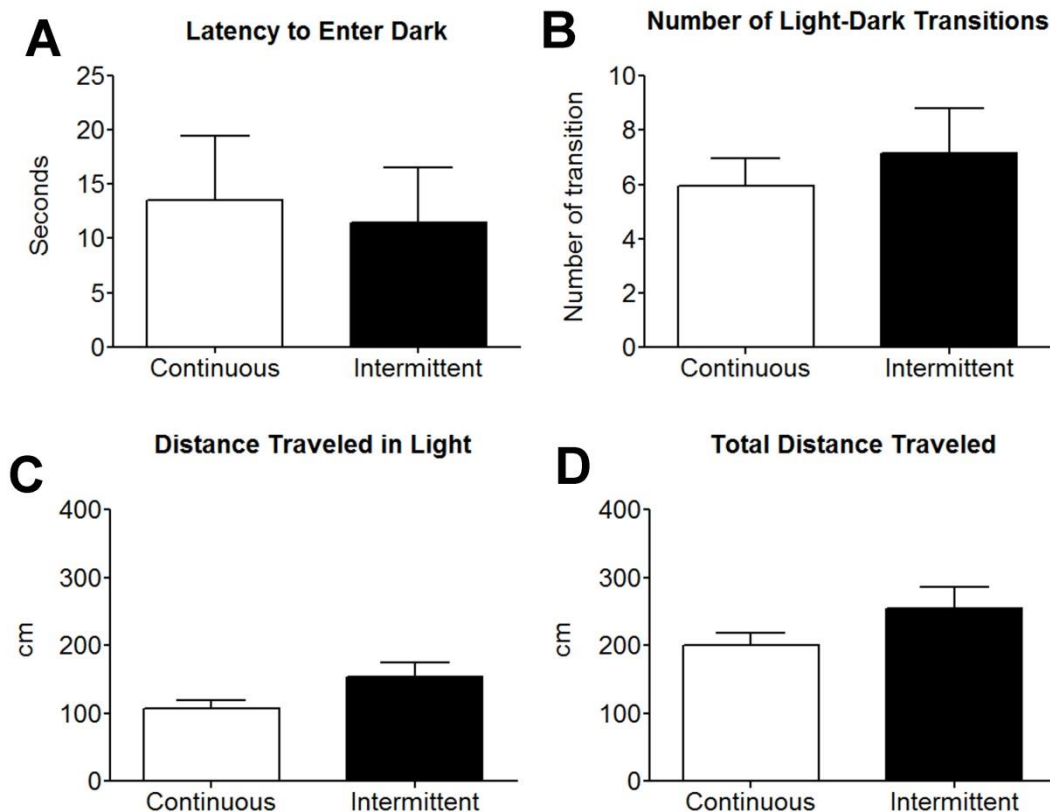


## Estrogens stimulate serotonin neurons to inhibit binge-like eating in mice

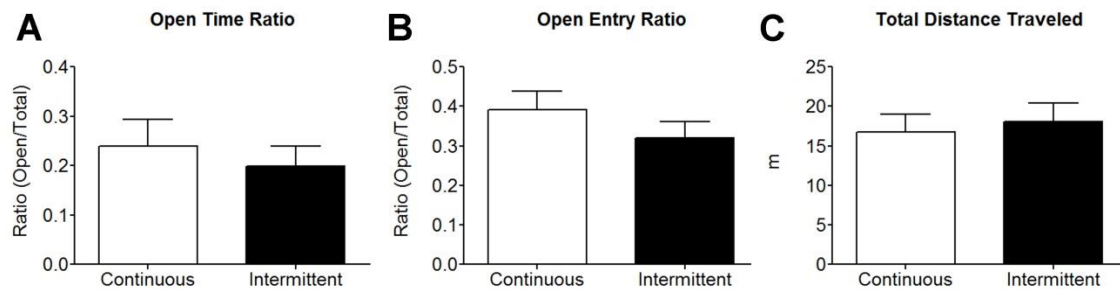
### Supplemental Figure 1. Light-dark tests in mice with binge-like eating behavior.

Anxiety-related responses in light-dark tests in C57Bl6 female (14 weeks) mice subjected to the continuous or intermittent HFD exposure. Note that the light-dark tests were performed around the same time (11am) when these mice would have been tested for binge-like eating behavior. (A) Latency to enter the dark chamber. (B) The total number of transitions between the light and dark chambers. (C) Distance traveled in the light chamber. (D) Total distance traveled. N=6/group. Results are shown as MEAN  $\pm$  SEM. No significant difference was detected between two groups in t test.



**Supplemental Figure 2. Elevated plus maze tests in mice with binge-like eating**

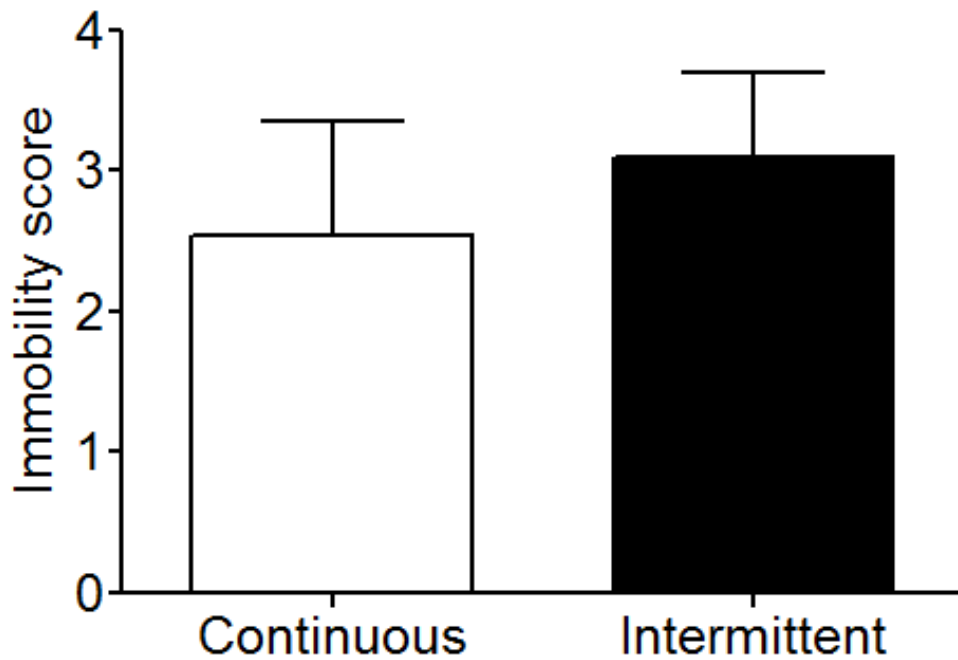
**behavior.** Anxiety-like behavior measured on the EPM in C57Bl6 female (14 weeks) mice subjected to the continuous or intermittent HFD exposure. Note that the elevated plus maze tests were performed around the same time (11am) when these mice would have been tested for binge-like eating behavior. (A) Open time ratio. (B) Open entry ratio. (C) Total distance traveled. N=6/group. Results are shown as MEAN  $\pm$  SEM. No significant difference was detected between two groups in t test.



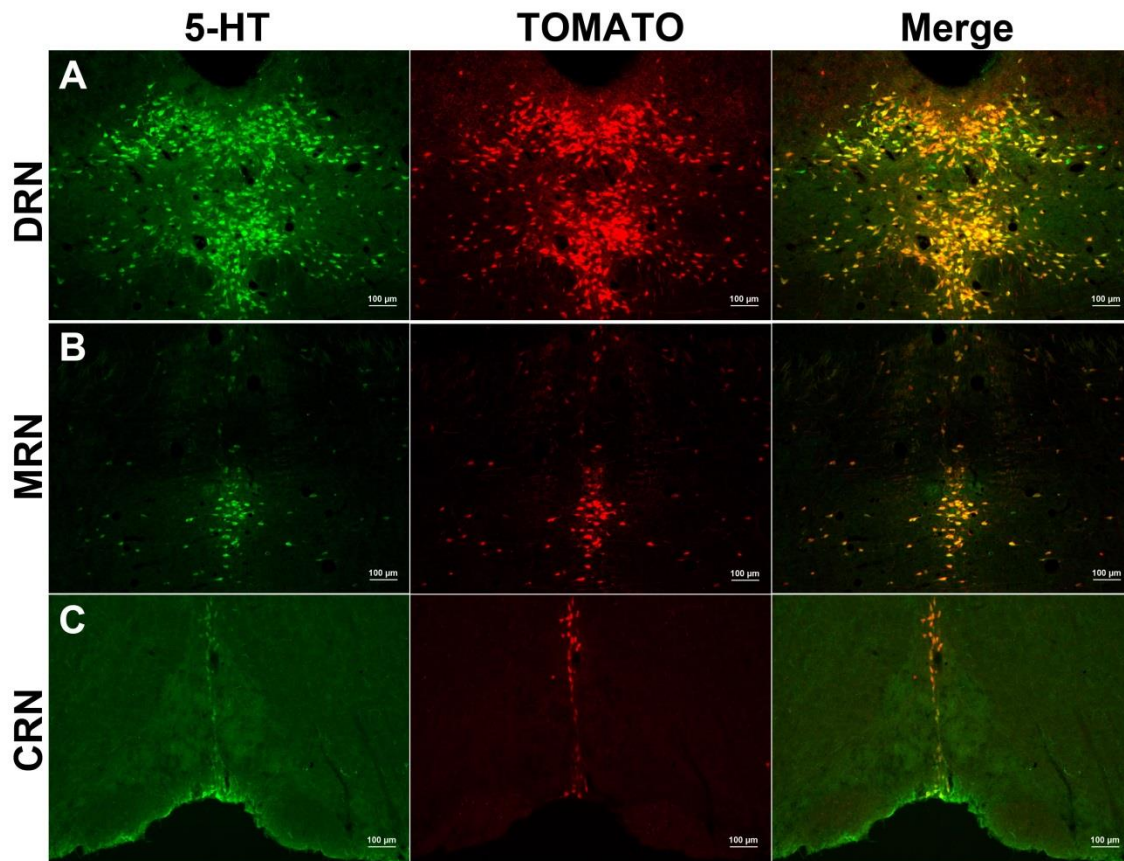
**Supplemental Figure 3. Forced swim tests in mice with binge-like eating behavior.**

Immobility score in the forced swim test in C57Bl6 mice subjected to the continuous or intermittent HFD exposure. Note that the forced swim tests were performed at the same time (11am) when these mice would have been tested for binge-like eating behavior.

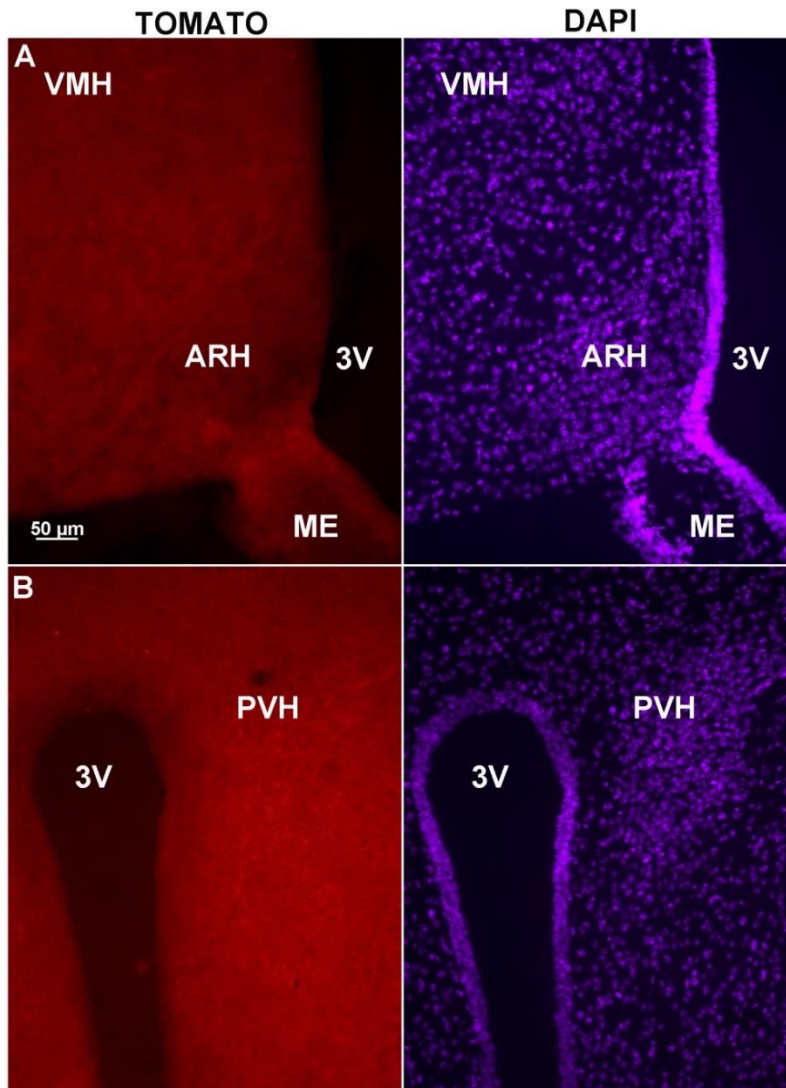
N=6/group. Results are shown as MEAN  $\pm$  SEM. No significant difference was detected between two groups in t test.



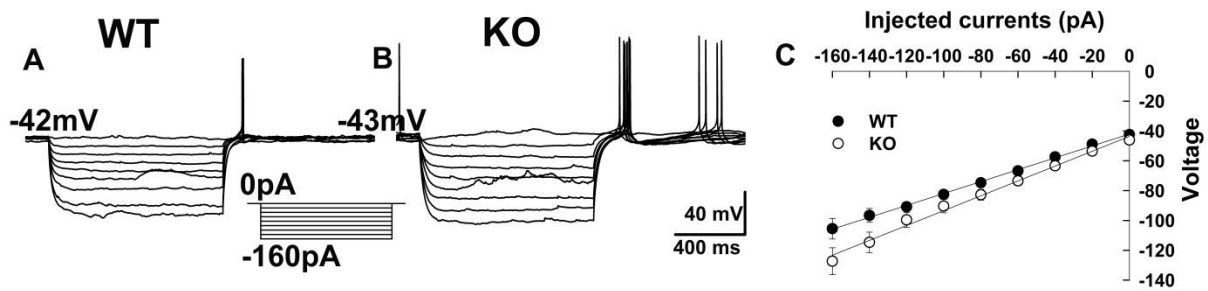
**Supplemental Figure 4. Tamoxifen-induced Cre activity in 5-HT neurons in TPH2-CreER mice.** (A-C) Representative immunofluorescent images for 5-HT (left, green), TOMATO (middle, red) and merge (right, yellow) in coronal mouse brain sections containing the DRN (A), MRN (B) and CRN (C) from TPH2-CreER/Rosa26-tdTOMATO mice after tamoxifen inductions. Yellow neurons indicate 5-HT neurons that co-express TOMATO. Scale bars=100  $\mu$ m.



**Supplemental Figure 5. Lack of Cre activity in non-5-HT regions in TPH2-CreER mice.** (A-B) Representative immunofluorescent images for TOMATO (left) and DAPI (right) in coronal mouse brain sections that do not contain 5-HT neurons, including the ARH and VMH (A) and PVH (B) from TPH2-CreER/Rosa26-tdTOMATO mice after tamoxifen inductions. Scale bars= 50  $\mu$ m. 3V, third ventricle; ARH, arcuate nucleus of the hypothalamus; ME, median eminence; PVH, paraventricular nucleus of the hypothalamus; VMH, ventromedial hypothalamic nucleus.

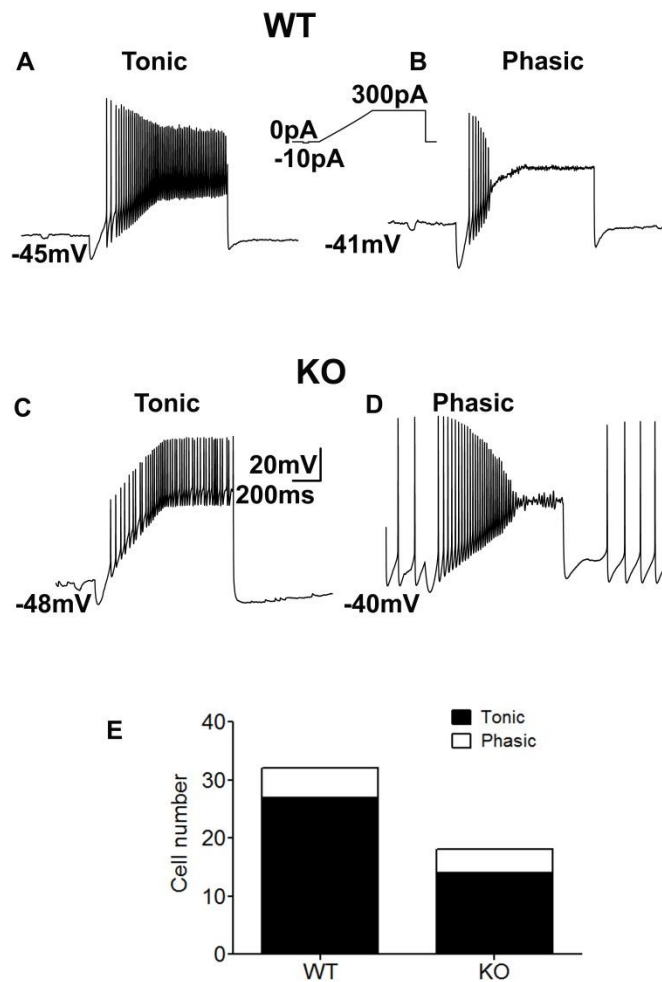


**Supplemental Figure 6. Passive membrane properties of 5-HT neurons from WT or KO mice.** (A-B) Representative traces show membrane voltage responses to series depolarizing steps (from -160 pA to 0 pA in steps of 20 pA) for 1 s in 5-HT neurons from WT (A) and KO (B) mice. (C) Summary data show current-voltage curves between WT and KO mice. Input resistance was determined by the slope of a linear regression fitted line of current-voltage curve. Results are shown as MEAN  $\pm$  SEM.



**Supplemental Figure 7. Firing properties of 5-HT neurons from WT or KO mice.**

(A-B) Representative traces of a tonically (A) or phasically (B) firing 5-HT neuron in WT mice. (C-D) Representative traces of a tonically (C) or phasically (D) firing 5-HT neuron in KO mice. Whether a neuron is tonically firing is determined by the repetitive firing during injected currents for 1 s ranged from 0 pA to 300 pA (as shown in inset). (E) Summary data showing the cell numbers of 5-HT neurons with tonically or phasically firing in WT and KO mice.



**Supplemental Figure 8. Action potentials of 5-HT neurons from WT or KO mice.**

(A) Numbers of 5-HT neurons with or without spontaneous firing in WT and KO mice. \*  $P < 0.05$  between WT and KO by Chi-square test. (B) The basal amplitude of AP in the spontaneous firing 5-HT neurons between WT and KO mice. (C) The basal firing frequency in the spontaneous firing 5-HT neurons between WT and KO mice. (D) The basal APD<sub>50</sub> in the spontaneous firing 5-HT neurons between WT and KO mice. (E) The basal AHP<sub>A</sub> in the spontaneous firing 5-HT neurons between WT and KO mice.

