

## Supplementary Figure Legends

### **Figure S1. PAG *Tac2*-expressing neurons are mainly located in the dmPAG** (related to Figure 1).

(A) Representative fluorescent images showing absence of tdTomato-labeled  $Tac2^+$  cells in control mice (*Tac2*-Cre, Ai9, or WT C57BL/6 mice). Scale bars, 500  $\mu\text{m}$ . (B) *Tac2* mRNA expression pattern in ABA ISH database (left), with an enlarged view (right) from the left panel. (C) Distribution pattern of  $Tac2^+$  cells in PAG subregions examined fluorescence ISH. Scale bars, 50  $\mu\text{m}$ . (D) Pie chart showing numbers of  $Tac2^{\text{tdTomato}}$  cells in PAG subregions. N = 3 mice. Data were expressed as mean  $\pm$  S.E.M.

### **Figure S2. Mapping of c-Fos expression in WT mice subjected to the RI assay** (related to Figure 2)

(A) Brain areas with high-level c-Fos expression in WT mice subjected to the RI assay. Scale bar, 1,000  $\mu\text{m}$ . (B) Representative fluorescent image showing multiple hypothalamic subnuclei were activated by the RI assay, including the PVT, SBPV, PVZ, AHN, LHA, BLA, DMH, VMH, MRN, and PAG. Scale bar, 100  $\mu\text{m}$ . (C) Quantification of number of Fos<sup>+</sup> cells in selected brain areas in WT mice subjected to the RI assay. Data are presented as total number of Fos<sup>+</sup> cells in five brain sections separated by 200  $\mu\text{m}$  (N = 3 mice per condition). Data were expressed as mean  $\pm$  S.E.M. *Abbreviations:* PVT, Paraventricular nucleus of the thalamus; SBPV, Subparaventricular zone hypothalamus; PVZ, Periventricular zone; AHN, Anterior hypothalamic nucleus; LHA, Lateral hypothalamic area; BLA, Basolateral amygdala nucleus, DMH, Dorsomedial nucleus of the hypothalamus; VMH, Ventromedial hypothalamic nucleus; MRN, Midbrain reticular nucleus; PAG, Periaqueductal gray.

### **Figure S3. Using a Cre-out strategy to test the role of dmPAG<sup>Tac2</sup> negative neurons in aggression** (related to Figure 3)

(A) Schematic showing a Cre-out strategy. (B-D)  $\text{Ca}^{2+}$  activity time-locked to each attack episode. The panels showing (B) Distribution of attack episode, (C) Heatmap of GCaMP6m, and (D)  $\Delta F/F\%$ . (E) Analysis of peak  $\Delta F/F$  before (Pre) and after (Post) each attack episode. Data represent mean  $\pm$  S.E.M. Significance was calculated by means of paired *t*-test.  $*P < 0.05$ . (F) Analysis of peak  $\Delta F/F$  in dmPAG<sup>Tac2</sup> neurons (N = 10 trails, in Figure 3H) versus dmPAG<sup>Tac2</sup> negative neurons (N = 46 trails) during each attack episode. Data represent mean  $\pm$  S.E.M. unpaired *t*-test,  $****P < 0.0001$ .

### **Figure S4. *Tac2*-expressing neurons in dmPAG respond to aggression** (related to Figure 3)

(A-F) Ca<sup>2+</sup> activity time-locked to mice encounter (A-B), male-female encounter (C-D), and male-female interaction (E-F). (A) Heatmap of GCaMP6m  $\Delta F/F$  signals (top) and  $\Delta F/F\%$  (bottom). (B) Analysis of peak  $\Delta F/F$  before (Pre) and after (Post) each male-male encounter episode. The other panels present the same manner but with different behavioral events, including male-female encounter (C-D) and male-female interaction (E-F). (G-H) Ca<sup>2+</sup> activity time-locked to motor activity in the rotarod test. (G) Heatmaps of GCaMP6m  $\Delta F/F$  signals (top) and  $\Delta F/F\%$  (bottom). (H) Analysis of peak  $\Delta F/F$  before (Pre) and after (Post) the start of rotarod test. (I-P) Ca<sup>2+</sup> activity time-locked to object exploration (I-J), fox odor sniffing (K-L), saline odor sniffing (M-N), and pain stimulation (O-P). (I) Distribution of object exploration (top), heatmaps of GCaMP6m  $\Delta F/F$  signals (middle) and  $\Delta F/F\%$  (bottom). (J) Analysis of peak  $\Delta F/F$  before (Pre) and after (Post) each object exploration episode. The other panels present the same manner but with different behavioral events, including fox odor sniffing (K-L), saline odor sniffing (M-N), and pain stimulation (O-P). Data represent mean  $\pm$  S.E.M. Significance was calculated by means of paired *t*-test.

**Figure S5. Behavioral phenotypes (locomotion, anxiety, social behaviors) in *Tac2*-Cre mice** (related to Figure 4).

(A) Schematic of the open-field test. (B-E) Locomotor performance of WT and *Tac2*-Cre mice on (B) total distance, (C) average speed, (D) time spent in central and peripheral zones, and (E) entries into the central and peripheral zones. (F) Schematic of the elevated-plus maze test. (G-J) Anxiety-like behaviors of WT and *Tac2*-Cre mice on (G) numbers of entries in closed arm, (H) time spent in closed arm, (I) numbers of entries in open arm, (J) time spent in open arm. (K) Schematic of the social interaction test (sociability session). (L-M) Social preference of WT and *Tac2*-Cre mice on (L) interaction time and (M) social preference index. (N) Schematic of the social interaction test (social novelty session). (O-P) Social preference of WT and *Tac2*-Cre mice on (O) interaction time and (P) social preference index. (Q) Schematic of the RI assay. (R-T) Behavioral performance of WT and *Tac2*-Cre mice on (R) latency to attack, (S) number of attack and (T) duration of attack. Data represent mean  $\pm$  S.E.M. N = 24 in WT mice and N = 25 in *Tac2*-Cre mice. Unpaired *t*-test, \**P* < 0.05, \*\*\**P* < 0.001, \*\*\*\**P* < 0.0001.

**Figure S6. Percentage of *Tac2*-Cre mice exhibit attack behaviors** (related to Figure 4-5).

(A) Percent of mice exhibiting attack before and after genetic ablation with DTA or mCherry (control). (B) Number (left) and duration (right) of investigation before and after ablation of dmPAG<sup>Tac2</sup> neurons with DTA in *Tac2*-cre mice. N = 7 mice. Data represent mean  $\pm$  S.E.M. Significance was calculated by means of paired *t*-test. (C) Percent of mice exhibiting attack before and after receiving hM4Di or mCherry (control). N = 10 in *Tac2*<sup>hM3Dq</sup> and N = 7 in *Tac2*<sup>mCherry</sup> groups. (D) Percent of animals exhibiting attack before and after hM3Dq or mCherry (control). N = 10 in *Tac2*<sup>hM4Di</sup>

and  $N = 7$  in  $Tac2^{mCherry}$  group. Data represent mean  $\pm$  S.E.M. Significance was calculated by One-way ANOVA and Tukey's multiple comparisons test,

**Figure S7. Behavioral phenotypes (locomotion and anxiety behaviors) following chemogenetic inhibition** (related to Figure 4).

(**A-D**) Performance of the open-field test in  $Tac2^{hM4Di}$  mice receiving saline or CNO (1 mg/kg, *i.p.*). Analysis showing results of total distance (**A**), average speed (**B**), time spent in the central or peripheral zones (**C**), and entries into the central or peripheral zones (**D**). (**E-H**) Performance of the elevated-plus maze in  $Tac2^{hM4Di}$  mice receiving saline or CNO (1 mg/kg, *i.p.*). Analysis showing results of entries in the open arm (**E**), total time spent in the open arm (**F**), entries in the closed arm (**G**), and total time spent in the closed arm (**H**).  $N = 10$  mice. Data represent mean  $\pm$  S.E.M. Significance was calculated by means of paired *t*-test,  $*P < 0.05$ .

**Figure S8. Detailed analysis of specific components of fighting behaviors following chemogenetic inhibition experiments** (related to Figure 4).

(**A-C**) Analysis of chasing behaviors, showing the latency to exhibit chasing (**A**), number of chasing (**B**), and duration of chasing (**C**). (**D-F**) Analysis of wrestling behaviors, showing latency to exhibit wrestling (**D**), number of wrestling (**E**), and duration of wrestling (**F**). (**G-I**) Analysis of biting behaviors, showing latency to exhibit biting (**G**), number of biting (**H**), and duration of biting (**I**).  $N = 10$  in  $Tac2^{hM4Di}$  group and  $N = 7$  in  $Tac2^{mCherry}$  group. Data represent mean  $\pm$  S.E.M. Significance was calculated by means of paired *t*-test.  $**P < 0.01$ ,  $***P < 0.001$ ,  $****P < 0.0001$ .

**Figure S9. Behavioral phenotypes (locomotion and anxiety behaviors) following chemogenetic activation** (related to Figure 5).

(**A-D**) Performance of the open-field test of  $Tac2^{hM3Dq}$  mice receiving saline or CNO (0.5 mg/kg, *i.p.*). Analysis showing results of total distance (**A**), average speed (**B**), total time in the central or peripheral zones (**C**), and entries in the central or peripheral zones (**D**). (**E-H**) Performance of elevated-plus maze of  $Tac2^{hM3Dq}$  mice receiving saline or CNO (0.5 mg/kg, *i.p.*). Analysis showing results of entries in the open arm (**E**), total time spent in the open arm (**F**), entries in the closed arm (**G**), and total time spent in the closed arm (**H**).  $N = 8$  mice. Data represent mean  $\pm$  S.E.M. Significance was calculated by means of paired *t*-test.

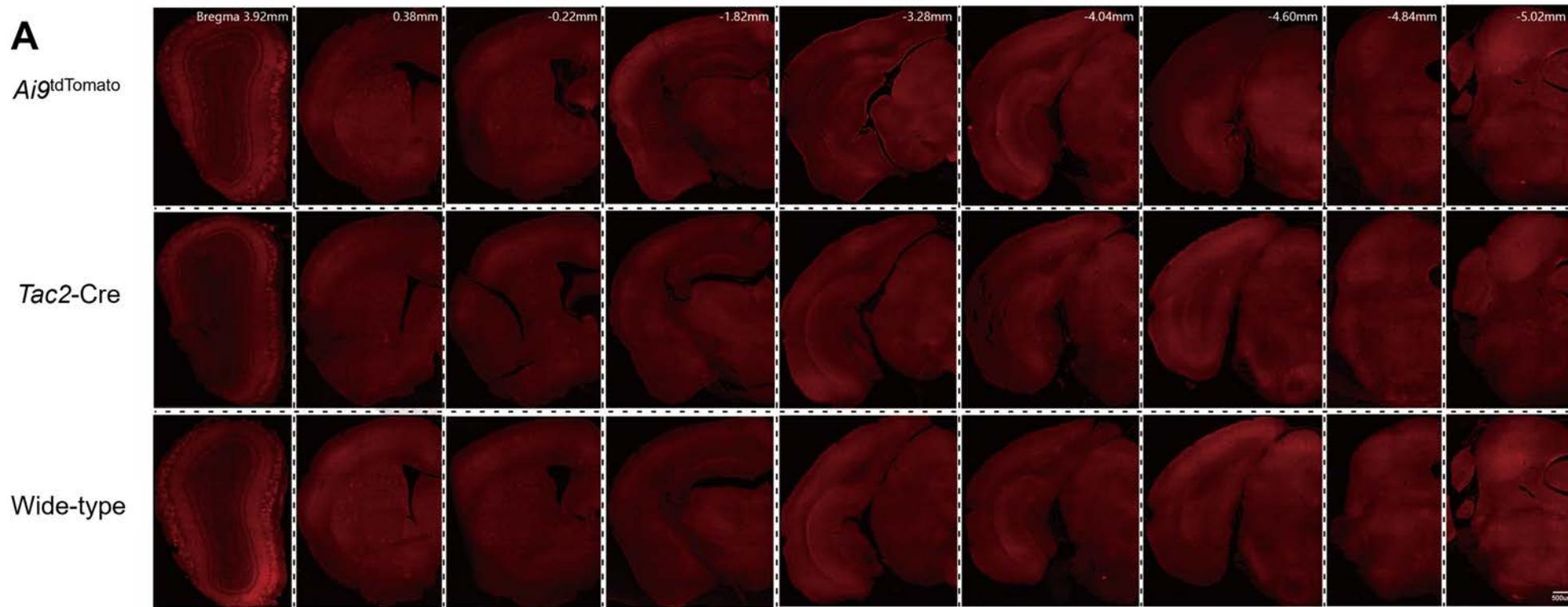
**Figure S10. Detailed analysis of specific components of fighting behaviors following chemogenetic activation experiments** (related to Figure 5).

(A-C) Analysis of chasing behaviors, showing the latency to exhibit chasing (A), number of chasing (B), and duration of chasing (C). (D-F) Analysis of wrestling behaviors, showing latency to exhibit wrestling (D), number of wrestling (E), and duration of wrestling (F). (G-I) Analysis of biting behaviors, showing latency to exhibit biting (G), number of biting (H), and duration of biting (I). N = 10 in *Tac2*<sup>hM3Dq</sup> group and N = 7 in *Tac2*<sup>mCherry</sup> group. Data represent mean ± S.E.M. Significance was calculated by means of paired *t*-test. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.

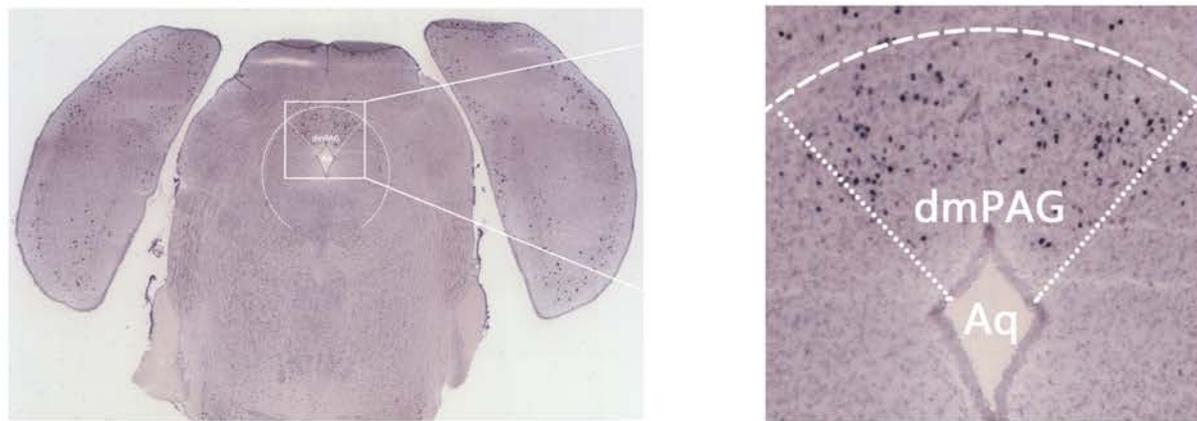
**Figure S11: Pharmacological validation of molecular targets identified by TRAP-seq** (related to Figure 7)

(A-C) Analysis of latency to attack (A) number of attack (B) and duration of attack (C) in *Tac2*<sup>hM4di</sup> mice injected with SB-224289/vehicle and CNO/saline in the dmPAG. N = 9 mice/group. (D). Heatmap showing counts of *Tph2* from TRAP-seq raw results. (E) Heatmap showing Log<sub>2</sub>FC value of *Tph2* transcript counts in individual sample pairs. (F) Counts of 5-HTR related genes detected in the IP samples from RI mice and home-cage controls. (G) Log<sub>2</sub>FC value in the counts of 5-HTR related genes in the IP samples from RI mice normalized to that of the home-cage control mice. Data represent mean ± SEM. Significance was calculated by means of paired *t*-test. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.

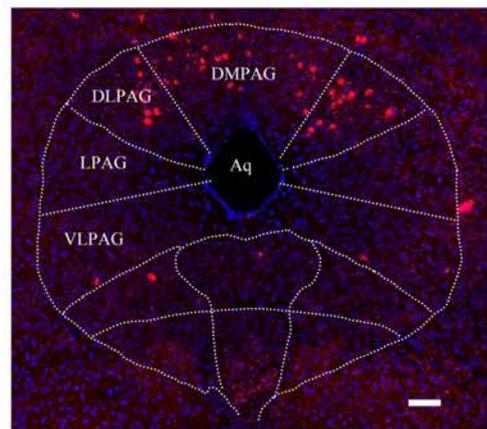
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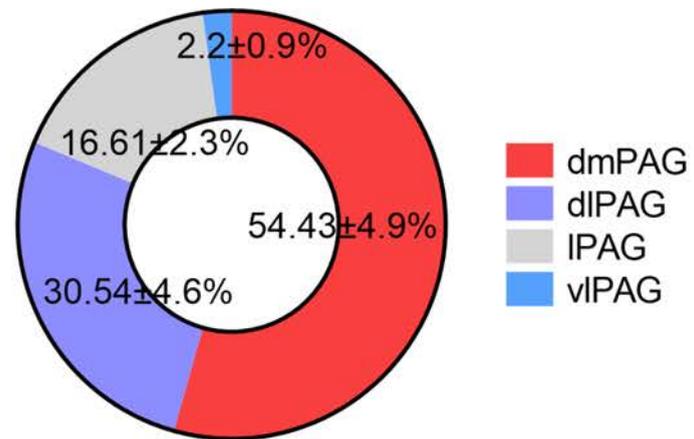
**B** Control



**C**

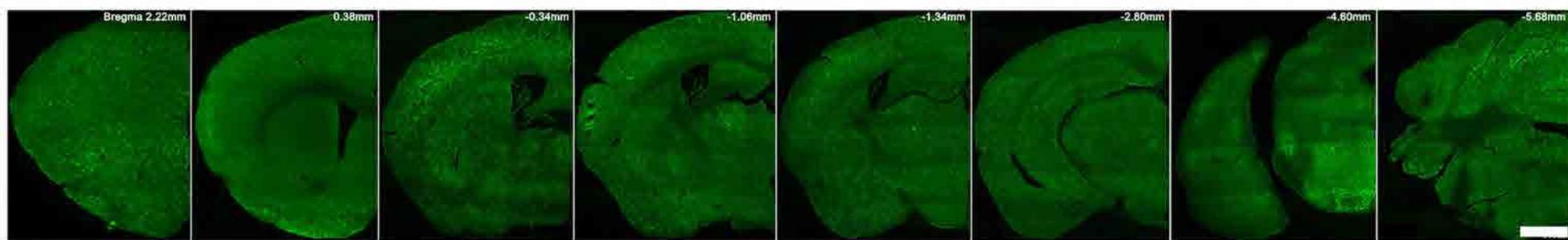


**D**

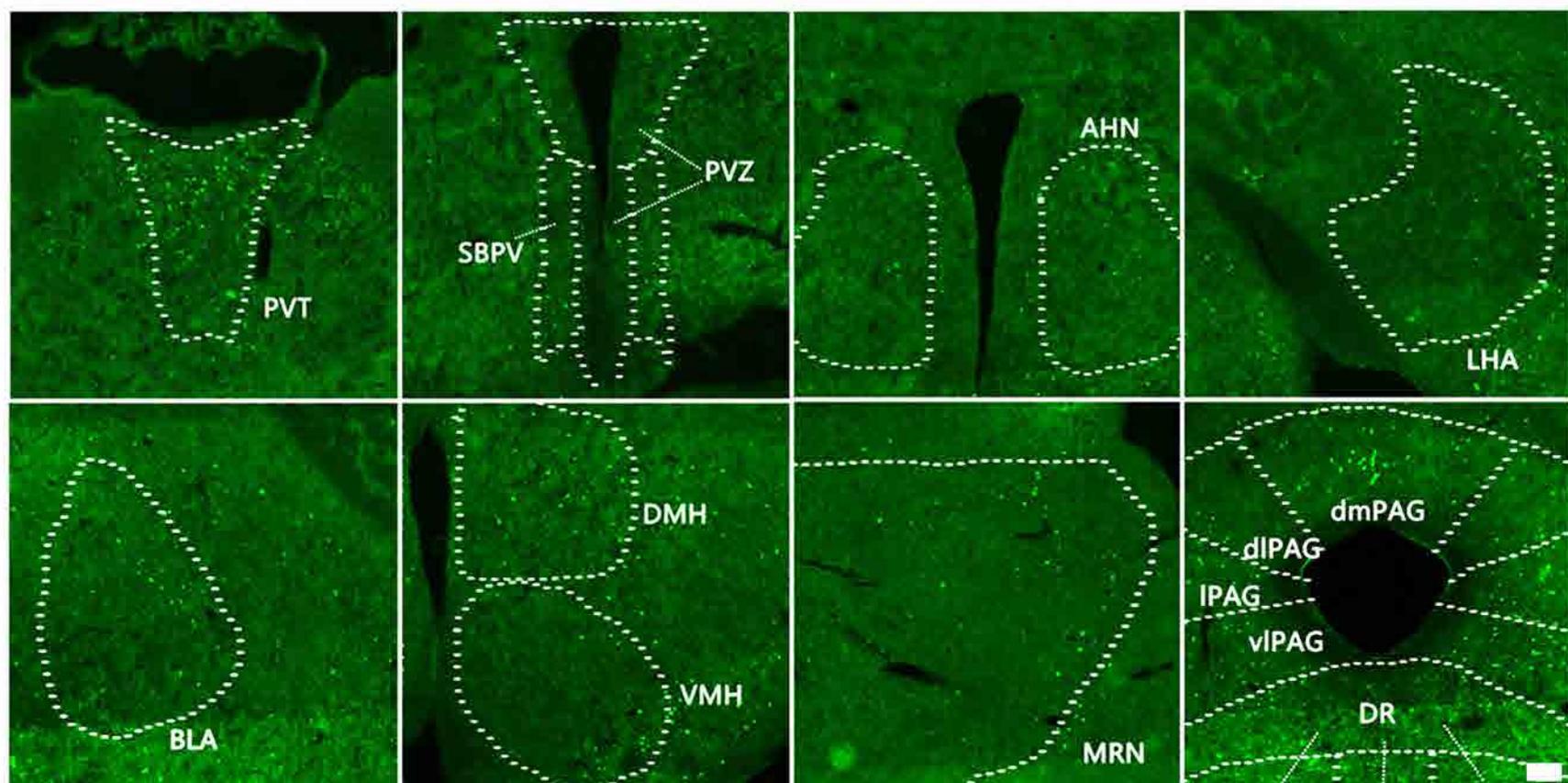


# Figure S2

A



B



C

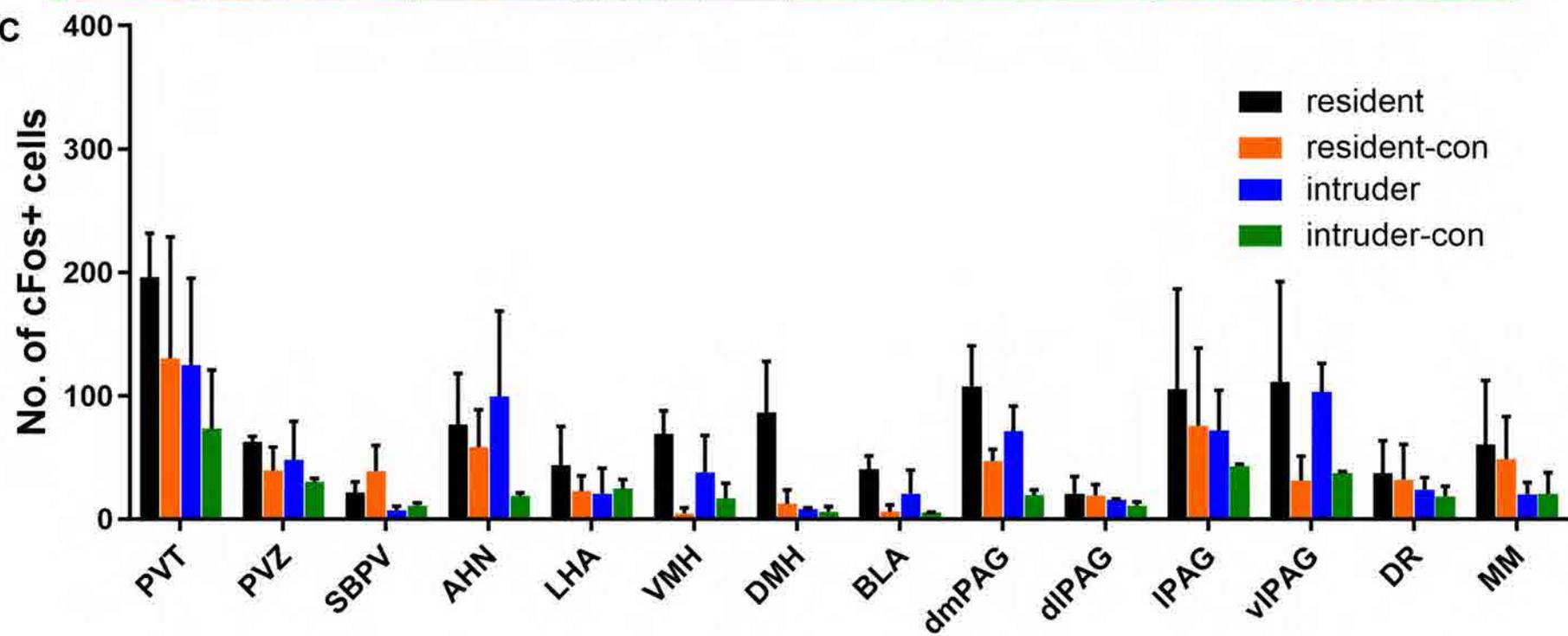
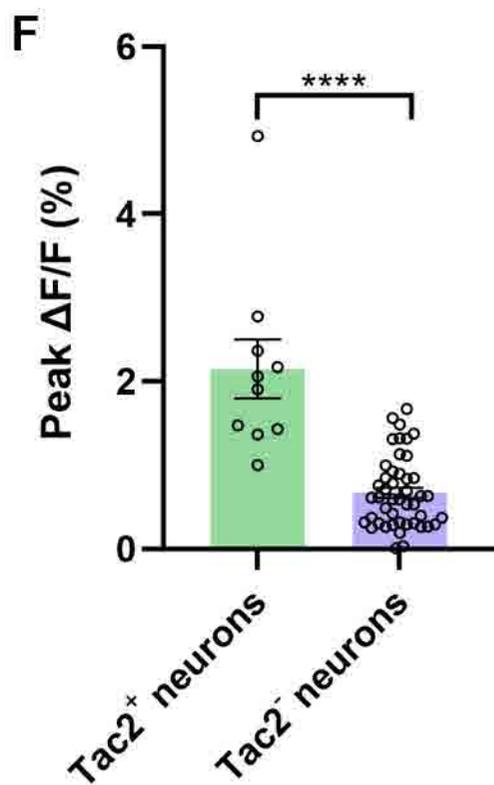
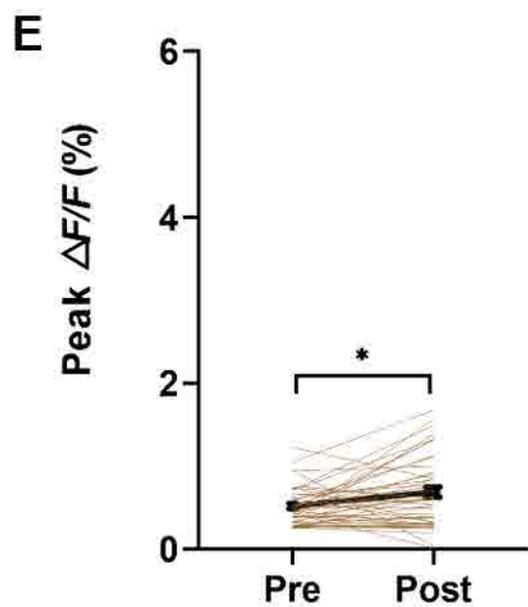
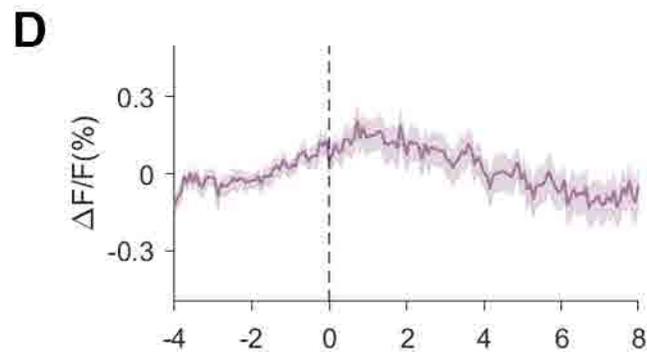
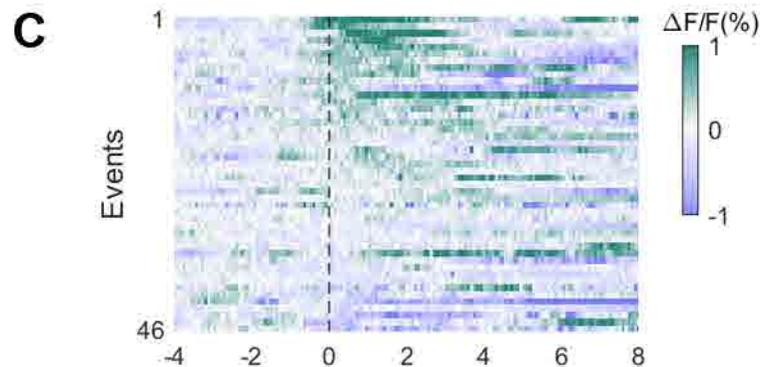
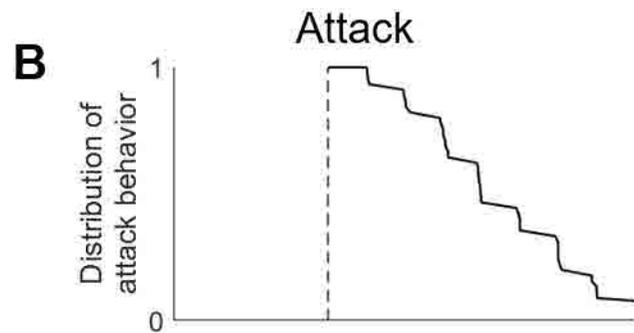
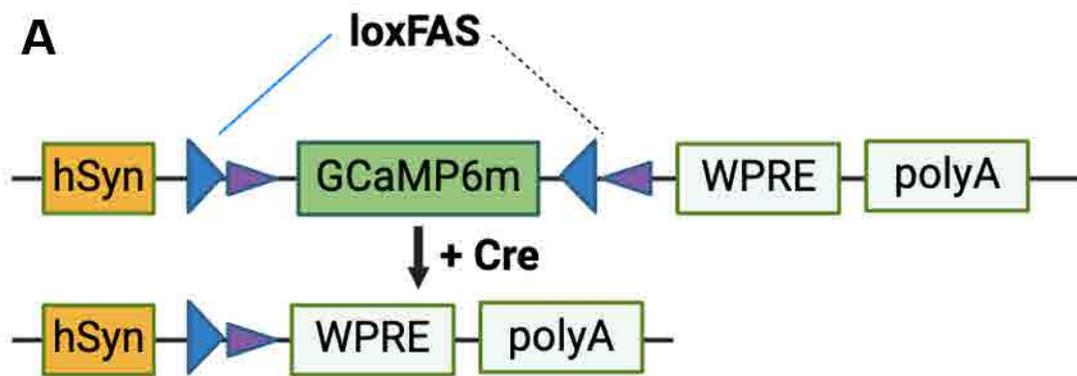
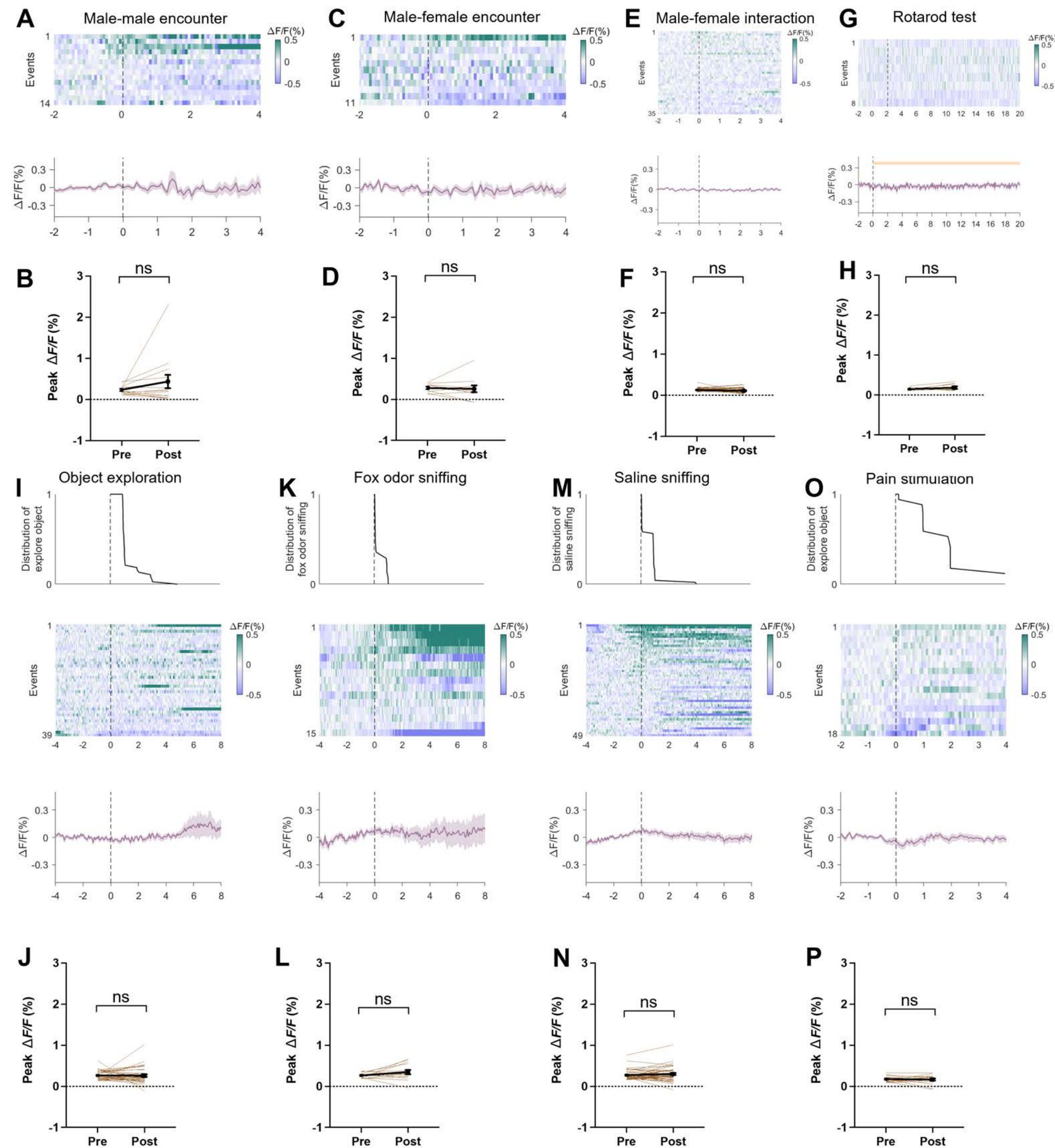


Figure S3



# Figure S4



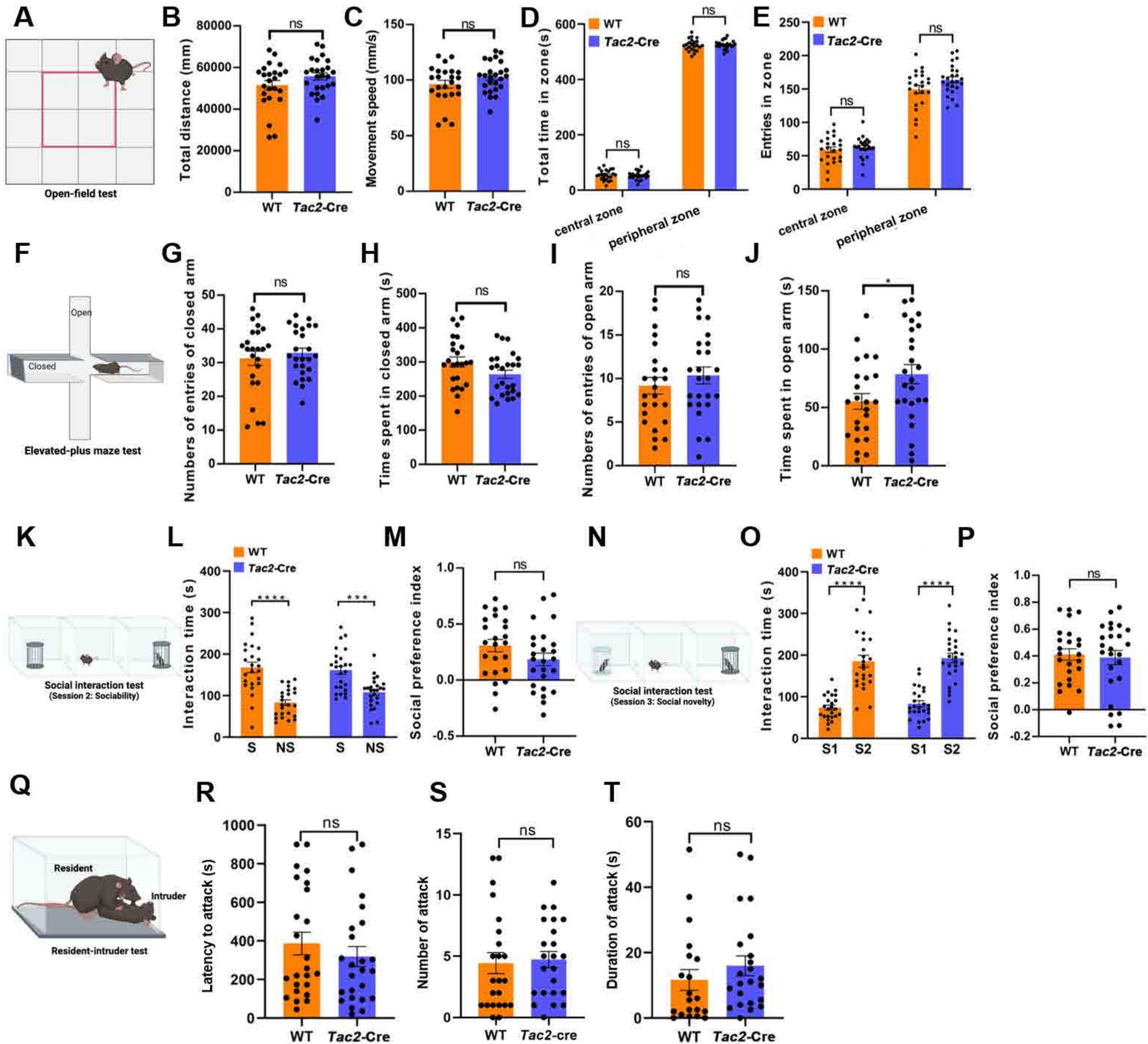
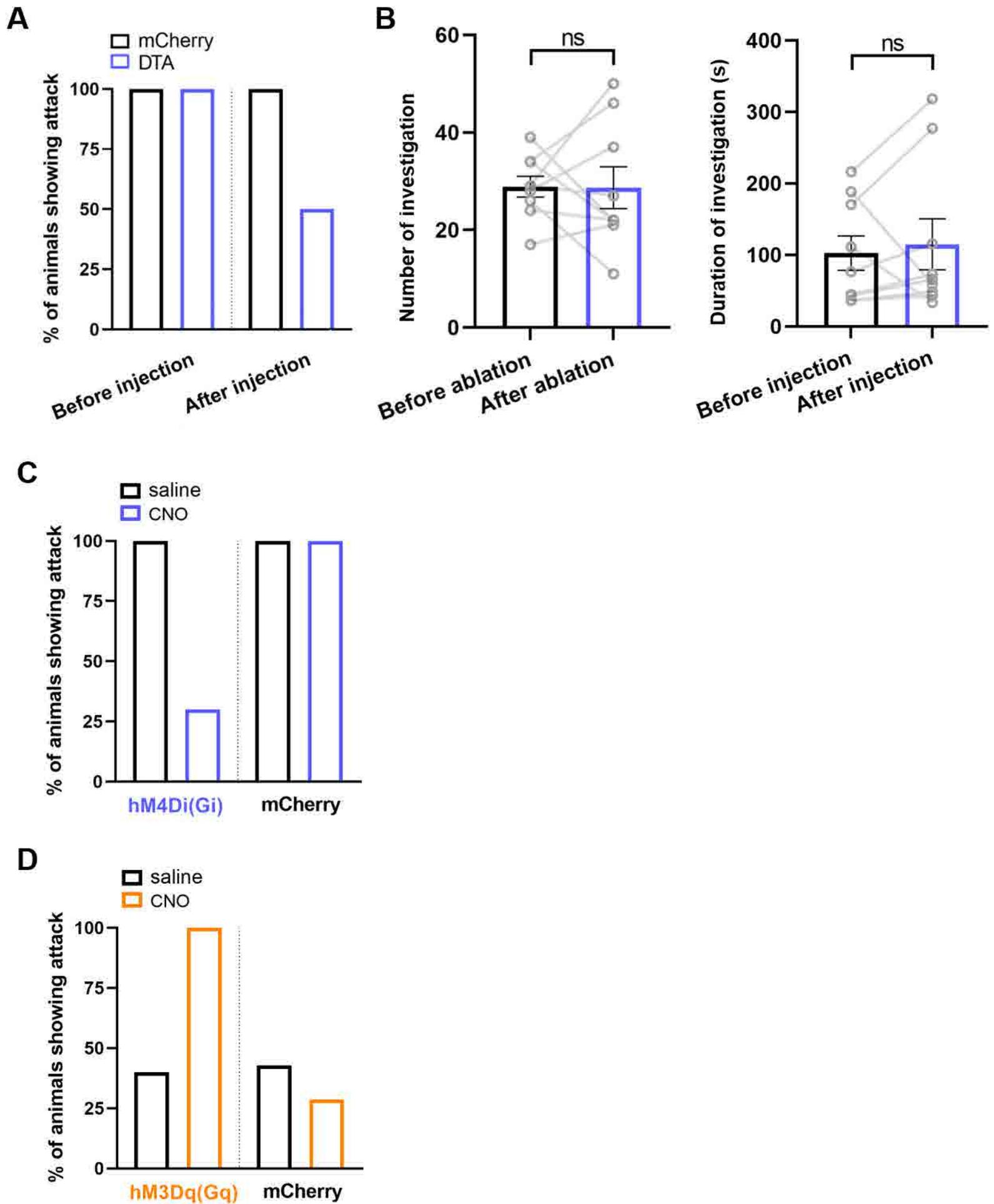
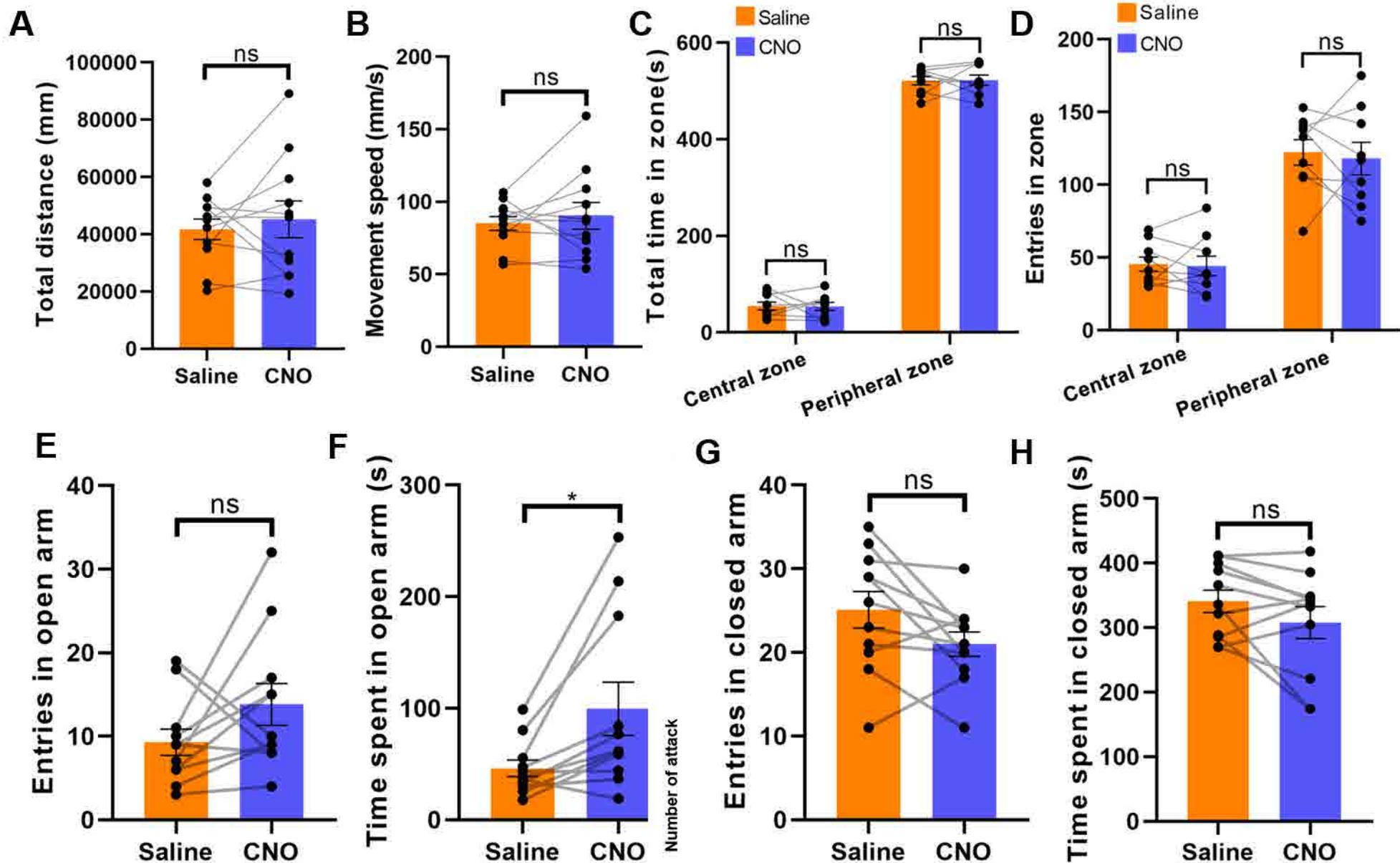
**Figure S5**

Figure S6



# Figure S7

hM4Di



# Figure S8

hM3Dq

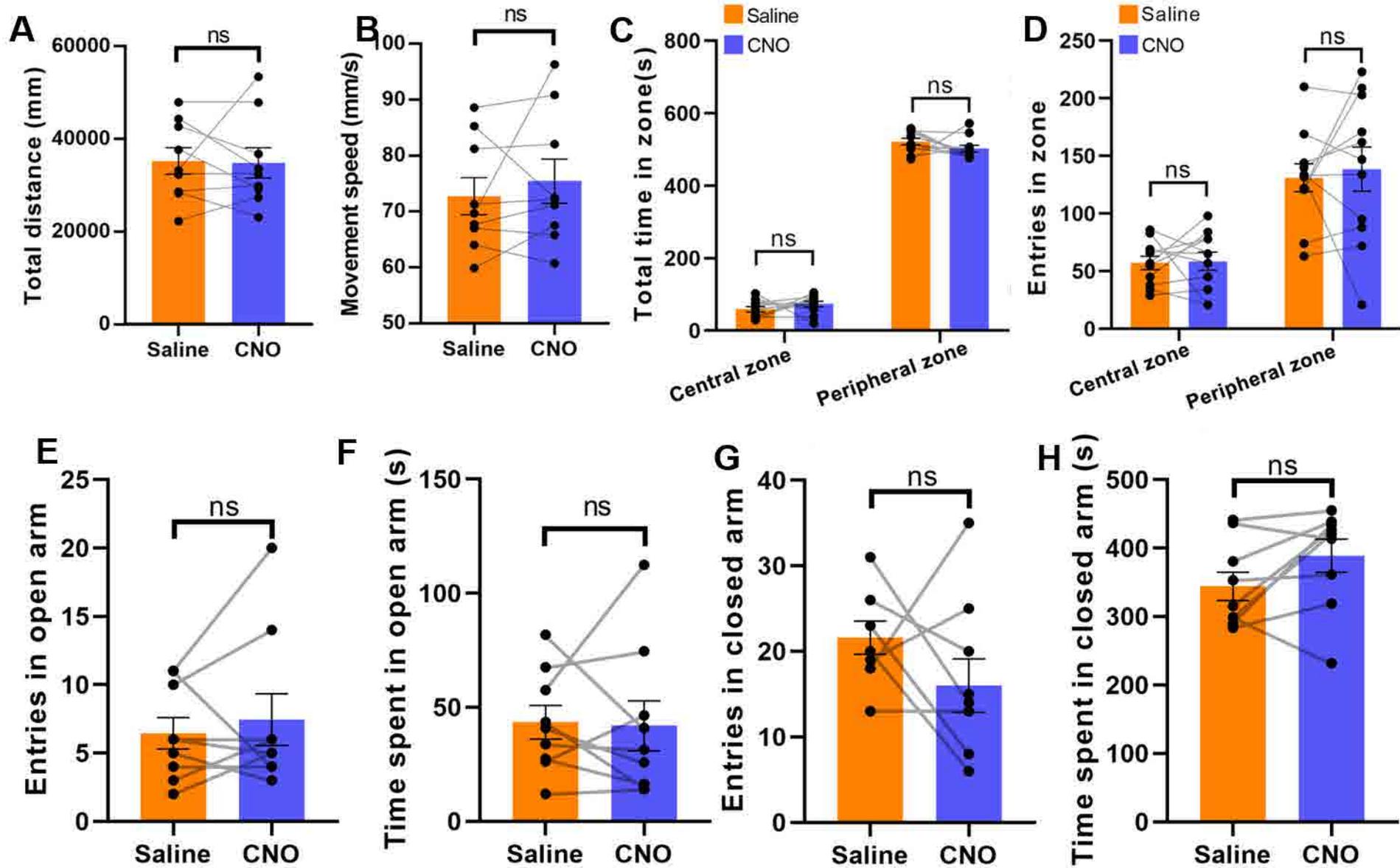


Figure S9

hM4Di

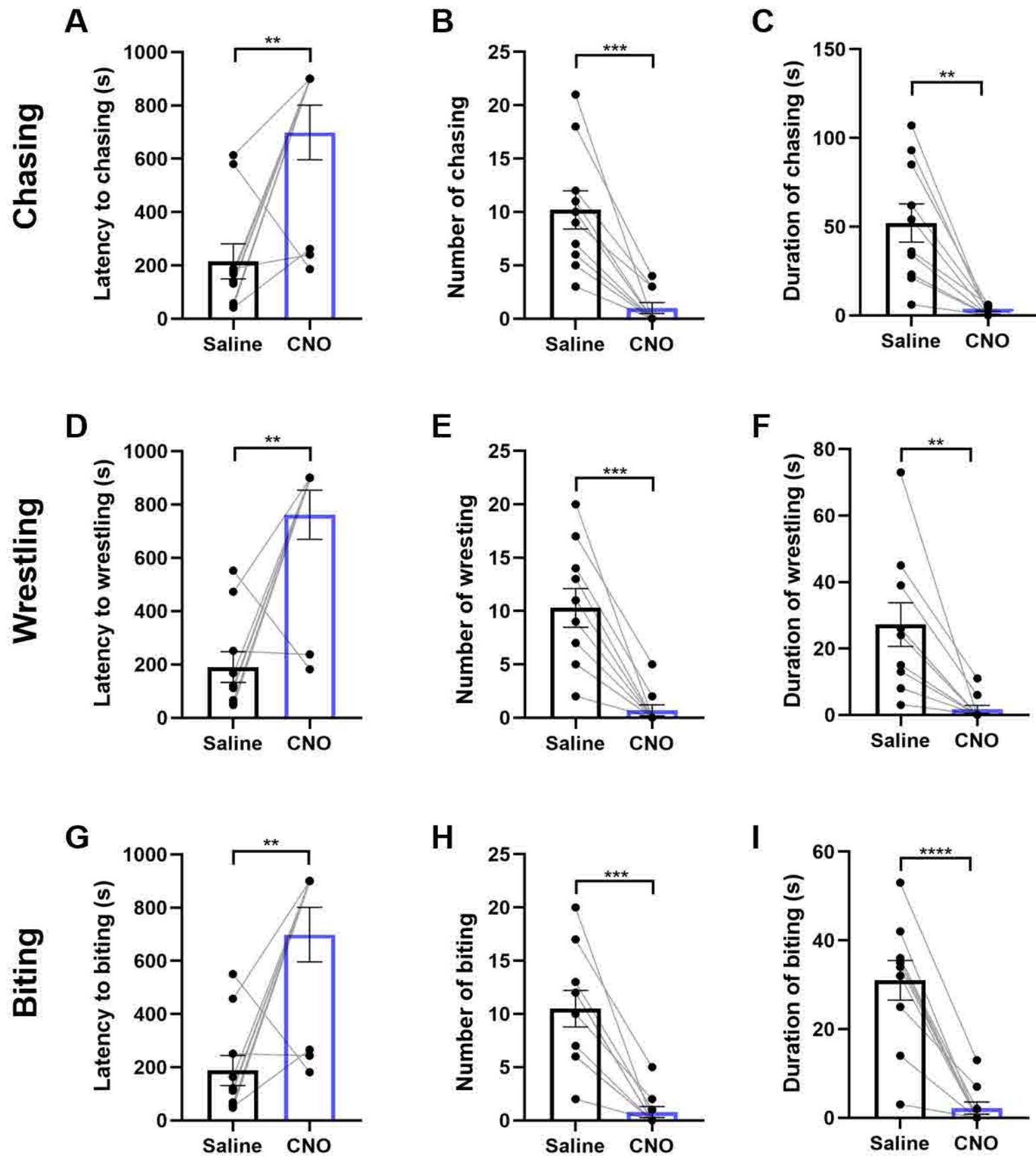
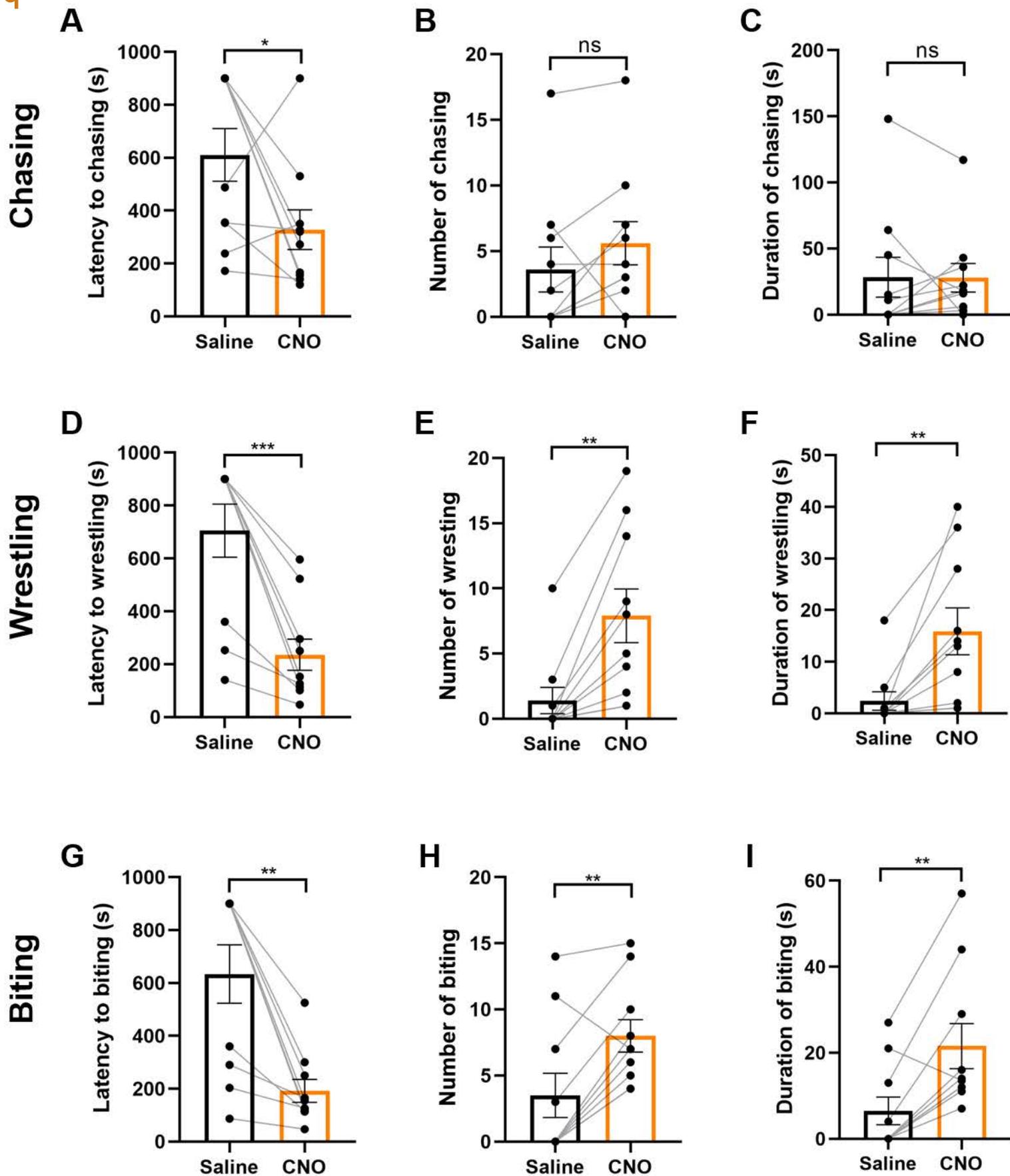


Figure S10

hM43Dq



**Figure S11**