

## SUPPLEMENTARY FIGURE LEGENDS

### Supplementary Figure 1

**(A,B)** Histograms show mean the time spent exploring an object (O) and an age- and sex-matched mouse (S1) during the sociability phase of the TC test in VEH- or CNO-injected PV\_A **(A)** and PV\_Wt **(B)** males. All groups explored significantly more the mouse than the object [S1 vs O, PV\_A/VEH:  $t_{(6)} = 5.91$ ,  $P = 0.01$ ; PV\_A/CNO:  $t_{(9)} = 3.3$ ,  $P = 0.009$ ; PV\_Wt/VEH:  $t_{(14)} = 5.61$ ,  $P = 0.001$ ; PV\_Wt/CNO:  $t_{(11)} = 4.63$ ,  $P = 0.001$ ]. **(C,D)** Histograms show the mean time spent exploring the familiar (S1) and the novel (S2) mice during the social novelty phase of the TC test. Impairments in reactivity to social novelty, revealed by the absence of a significant difference in the time spent exploring S1 and S2, were found in CNO-injected mice regardless of genotype [PV\_A/VEH:  $t_{(6)} = 3.63$ ,  $P = 0.01$ ; PV\_A/CNO: S1 vs S2:  $t_{(9)} = 1$ ,  $P = 0.3$ ; PV\_Wt/VEH:  $t_{(14)} = 3.85$ ,  $P = 0.001$ ; PV\_Wt/CNO:  $t_{(11)} = 1.43$ ,  $P = 0.18$ ]. **(E)** Table showing the velocity (cm/s) and distance (cm) traveled by VEH- or CNO-injected PV\_A and PV\_Wt males during the habituation phase of NOR test. No significant difference was detected among the four groups [2-way ANOVA, Velocity: interaction,  $F_{(1, 42)} = 0.63$ ,  $P = 0.43$ ; treatment,  $F_{(1,42)} = 0.043$ ,  $P = 0.83$ ; genotype,  $F_{(1,42)} = 3.93$ ,  $P = 0.99$ . Distance traveled: interaction,  $F_{(1,42)} = 0.64$ ,  $P = 0.42$ ; treatment,  $F_{(1,42)} = 0.030$ ,  $p = 0.86$ ; genotype:  $F_{(1,42)} = 7.63$ ,  $P = 0.997$ ]. **(F-G)** Histograms show the mean time spent exploring the two identical objects (O1 and O2) during the training phase of the NOR test in the same groups. No significant difference was found in the time spent exploring O1 and O2 in any group (PV\_A/VEH:  $t_{(7)} = 0.43$ ,  $P = 0.6$ ; PV\_A/CNO:  $t_{(9)} = 0.1$ ,  $P = 0.9$ ; PV\_Wt/Veh:  $t_{(13)} = 0.18$ ,  $P = 0.8$ ; PV\_Wt/CNO:  $t_{(13)} = 0.64$ ,  $P = 0.53$ ). **(H-I)** Histograms show the mean time spent exploring the familiar (FO) and the novel (NO) objects during the NOR test phase. The CNO-injected mice exhibit a reduced preference for the novel object over the familiar one compared to VEH-treated controls [FO vs NO, PV\_A/VEH:  $t_{(7)} = 2.43$ ,  $P = 0.04$ ; PV\_A/CNO:  $t_{(9)} = 0.53$ ,  $P = 0.6$ ; PV\_Wt/VEH:  $t_{(13)} = 2.73$ ,  $P = 0.01$ ; PV\_Wt/CNO:  $t_{(13)} = 2.3$ ,  $P = 0.04$ ]. Data are expressed as mean  $\pm$  s.e.m. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

### Supplementary Figure 2

**(A)** Histograms show the mean time spent exploring the object (O) and an age- and sex-matched mouse (S1) during the sociability phase of the TC test in VEH- or CNO- injected PV\_Wt females. All groups explored significantly more the mouse than the object [S1 vs O, PV\_Wt/VEH:  $t_{(7)} =$

3.36,  $P = 0.01$  ; PV\_Wt/CNO:  $t_{(5)} = 14.71$ ,  $P = 0.001$ ]. **(B)** Histograms show the mean time spent exploring the familiar (S1) and the novel (S2) mice during the social novelty phase of the TC test. Impairment in reactivity to social novelty, revealed by the absence of a significant difference in the time spent exploring S1 and S2 was found in CNO-injected PV\_Wt females [S1 vs S2, PV\_Wt/VEH:  $t_{(7)} = 3.51$ ,  $P = 0.009$ ; PV\_Wt/CNO:  $t_{(5)} = 2.13$ ,  $P = 0.09$ ]. **(C)** Table showing the velocity (cm/s) and distance (cm) traveled by VEH- or CNO-injected PV\_Wt females during the habituation phase of the NOR test. No difference was detected between the two groups and no effect of sex was reported [Two-way ANOVA, treatment: Velocity:  $F_{(1, 37)} = 1.15$ ,  $P = 0.3$ ; Distance traveled:  $F_{(1, 37)} = 0.44$ ,  $P = 0.51$ . Sex: Velocity:  $F_{(1, 37)} = 0.06$ ,  $P = 0.8$ ; Distance traveled:  $F_{(1, 37)} = 0.34$ ,  $P = 0.56$ . Interaction: Velocity:  $F_{(1, 37)} = 0.0004$ ,  $P = 0.98$ ; Distance traveled:  $F_{(1, 37)} = 0.09$ ,  $P = 0.76$ ]. **(D)** Histograms show the mean time spent exploring the two identical objects (O1 and O2) during the training phase of the NOR test in the same groups. No significant difference was found in the time spent exploring O1 and O2 in any group [PV\_Wt/VEH:  $t_{(6)} = 0.68$ ,  $P = 0.5$  ; PV\_Wt/CNO:  $t_{(5)} = 0.13$ ,  $P = 0.9$ ]. **(E)** Histograms show the mean time spent exploring the familiar object (FO) and the novel object (NO) during the NOR test phase in the same groups. NOR impairments, revealed by the absence of a significant difference in the time spent exploring FO and NO were found only in CNO-PV\_Wt females [FO vs NO, PV\_Wt/VEH,  $t_{(6)} = 4.62$ ,  $P = 0.004$ ; PV\_Wt/CNO  $t_{(5)} = 0.98$ ,  $P = 0.3$ ]. Data are expressed as mean  $\pm$  s.e.m. \* $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

### Supplementary Figure 3

**(A-C)** Histograms show the mean time spent exploring an object (O) and an age- and sex-matched mouse (S1) during the sociability phase of the TC test in VEH- and CNO- injected Ambra1<sup>+/-</sup> (A) males and Wt mice of both sexes. All groups explored significantly more the mouse than the object [S1 vs O, A/Veh males:  $t_{(5)} = 5.22$ ,  $P = 0.003$ ; A/CNO males:  $t_{(5)} = 3.38$ ,  $P = 0.02$ ; Wt/Veh males:  $t_{(7)} = 4.73$ ,  $P = 0.002$ ; Wt/CNO males:  $t_{(6)} = 2.73$ ,  $P = 0.03$ ; Wt/Veh females:  $t_{(7)} = 7.17$ ,  $P = 0.0002$ ; Wt/CNO females:  $t_{(4)} = 13.3$ ,  $P = 0.0002$ ]. **(D-F)** Histograms show the mean time spent exploring the familiar (S1) and the novel (S2) mice during the social novelty phase of the TC test in the same groups. Impairment in reactivity to social novelty, shown by the absence of a significant difference in the time spent exploring S1 and S2, was found in CNO-injected mice regardless of mutation or sex [A/Veh males:  $t_{(5)} = 6.15$ ,  $P = 0.001$ ; A/CNO males:  $t_{(5)} = 2.62$ ,  $P = 0.05$ ; Wt/Veh males:  $t_{(7)} = 4.88$ ,  $P = 0.002$ ; Wt/CNO males:  $t_{(6)} = 0.31$ ,  $P = 0.76$ ; Wt/Veh females:  $t_{(7)} = 4.92$ ,  $P = 0.002$ ; Wt/CNO females:  $t_{(4)} = 0.3$ ,  $P = 0.8$ ]. **(G-I)** Histograms show the mean time spent

exploring the two identical objects (O1 and O2) during the training phase of the NOR test in the same groups. No significant difference was found in the time spent exploring O1 and O2 in any group [A/Veh:  $t_{(7)} = 0.96$ ,  $P = 0.4$ ; A/CNO:  $t_{(5)} = 1.69$ ,  $P = 0.1$  ; Wt/Veh males:  $t_{(6)} = 0.42$ ,  $P = 0.7$ ; Wt/CNO males:  $t_{(5)} = 1.47$ ,  $P = 0.2$ ; Wt/Veh females:  $t_{(6)} = 0.76$ ,  $P = 0.5$  ; Wt/CNO females:  $t_{(4)} = 1.01$ ,  $P = 0.3$  ]. **(J-L)** Histograms show the mean time spent exploring the familiar object (FO) and the novel object (NO) during the NOR test phase in the same groups. NOR impairment, shown by the absence of a significant difference in the time spent exploring FO and NO were found only CNO-injected mice [FO vs NO, A/Veh:  $t_{(7)} = 4.77$ ,  $P = 0.002$ ; A/CNO:  $t_{(5)} = 0.11$ ,  $P = 0.9$ ; Wt/Veh males:  $t_{(6)} = 4.75$ ,  $P = 0.003$ ; Wt/CNO males:  $t_{(5)} = 0.18$ ,  $P = 0.8$ ; Wt/Veh females:  $t_{(6)} = 3.54$ ,  $P = 0.01$ ; Wt/CNO females:  $t_{(4)} = 0.71$ ,  $P = 0.5$ ]. Data are expressed as mean  $\pm$  s.e.m. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

#### Supplementary Figure 4

**(A)** Histograms show the mean value of the recognition index (RI) calculated in the social novelty phase of the TC test in Wt male mice lacking DREADDs (Wt\_Sham) i.p. injected with vehicle (VEH) or CNO to control for the presence of any off-target effect on behavior. Reactivity to social novelty was the same in CNO- and Veh-injected mice [ $t_{(13)} = 1.183$ ,  $P = 0.257$ ]. **(B)** Histograms show the mean value of RI calculated in the NOR test phase in the same groups. No differences in the recognition are detectable in Wt\_Sham injected with CNO and Veh, suggesting no side effects of CNO on behavior [ $t_{(12)} = 1.183$ ,  $P = 0.259$ ].