Expanded View Figures

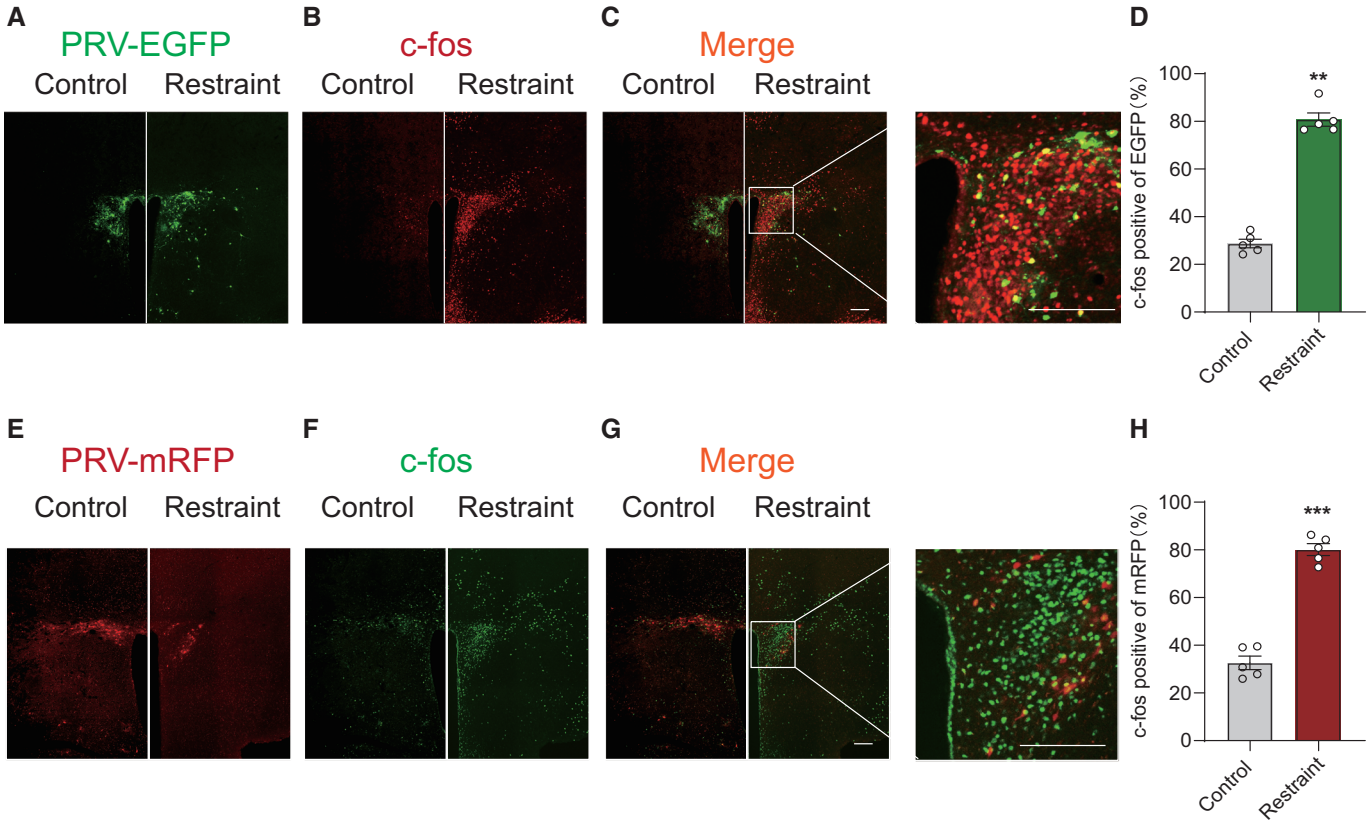

Figure EV1. PVN neurons traced by pseudorabies virus are activated under restraint stress.

A–H Representative images showing PVN neurons traced by PRV-EGFP from iWAT (A, C, C′, green) were activated by restraint stress (B, C, C′, red) on the 4th day after injection. (D) The percentage of colocalization of PRV-EGFP and c-Fos, Mann–Whitney *U*-test, $P = 0.0079$. Representative images showing PVN neurons traced by PRV-mRFP from eWAT (E, G, G′, red) were activated by restraint stress (F, G, G′, green). (H) The percentage of colocalization of PRV-mRFP and c-Fos, two-tailed unpaired *t*-test, $t_{(8)} = 12.61$, $P < 0.0001$. Control group $n = 5$, restraint group $n = 5$ in (D) and (H). Shapiro–Wilk test and *F*-test were used to test the normality and equal variance assumptions, respectively. Two-tailed *t*-tests were performed to assess differences between two experimental groups with normally distributed data and equal variance. Two-tailed *t*-tests with Welch's correction were used when a two-sample comparison of means with unequal variances. For non-normally distributed data, Mann–Whitney *U*-tests were performed to compare two groups. $P < 0.05$ was considered statistically significant. $^{**}P < 0.01$, $^{***}P < 0.001$. Data are presented as means ± SEM. Scale bar: A–C and E–G, 200 μm.

Source data are available online for this figure.

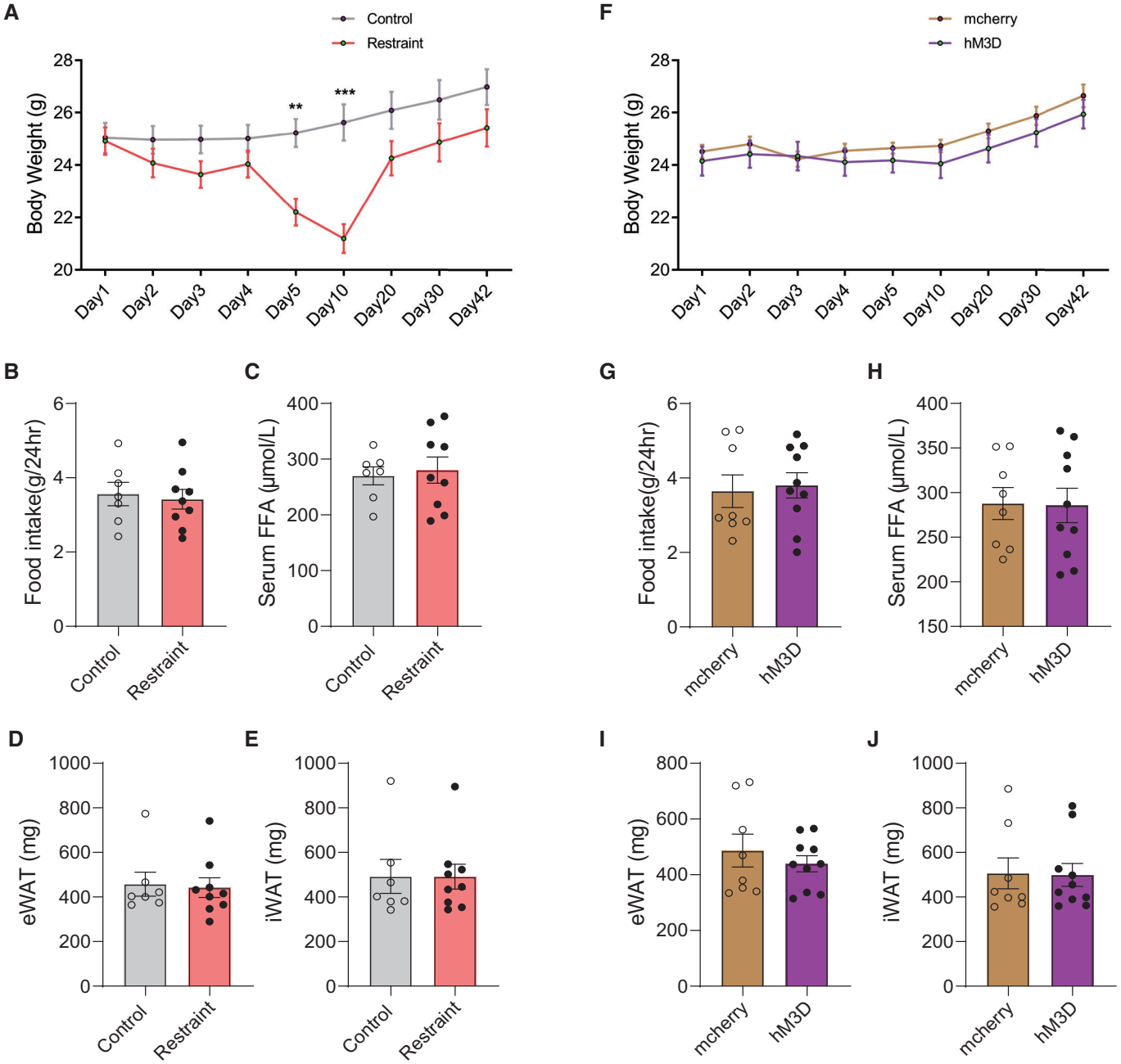

Figure EV2.

Figure EV2. Body weight, fat weight, food intake and serum FFA level under chronic restraint stress and PVN activation paradigms.

A The body weight of mice during chronic restraint paradigms. subgroups, $F_{(1, 14)} = 4.414$, $P = 0.0542$; time, $F_{(8, 112)} = 52.15$, $P < 0.0001$; subgroups × time, $F_{(8, 112)} = 26.58$, $P < 0.0001$.
B The amount of accumulative of 24-h food intake after chronic restraint treatment. two-tailed unpaired *t*-test, $t_{(14)} = 0.3420$, $P = 0.7374$.
C The serum FFA level after chronic restraint treatment. two-tailed unpaired *t*-test, $t_{(14)} = 0.3358$, $P = 0.7420$.
D–F The mass of eWAT (D, Mann–Whitney *U*-test, $P = 0.9182$) and iWAT (E, Mann–Whitney *U*-test, $P > 0.9999$) after chronic restraint treatment. F. the body weight of mice during chronic PVN activation. subgroups, $F_{(1, 16)} = 0.5661$, $P = 0.4627$; time, $F_{(8, 128)} = 51.72$, $P < 0.0001$; subgroups × time, $F_{(8, 128)} = 1.772$, $P = 0.0883$.
G The amount of accumulative of 24-h food intake after chronically reactivating PVN neurons. Mann–Whitney *U*-test, $P = 0.7618$.
H The serum FFA level after chronically reactivating PVN neurons. two-tailed unpaired *t*-test, $t_{(16)} = 0.08091$, $P = 0.9365$.
I, J The mass of eWAT (I, two-tailed unpaired *t*-test, $t_{(16)} = 0.7674$, $P = 0.4540$) and iWAT (J, Mann–Whitney *U*-test, $P = 0.8968$) after chronic restraint treatment.

Data information: Control group $n = 7$, restraint group $n = 9$ in (A–E); mcherry group $n = 8$, hM3D group $n = 10$ in (F–J). Shapiro–Wilk test and *F*-test were used to test the normality and equal variance assumptions, respectively. Two-tailed *t*-tests were performed to assess differences between two experimental groups with normally distributed data and equal variance. Two-tailed *t*-tests with Welch's correction were used when a two-sample comparison of means with unequal variances. For non-normally distributed data, Mann–Whitney *U*-tests were performed to compare two groups. For multiple groups, two-way ANOVAs followed by Sidak multiple comparisons test were used. $P < 0.05$ was considered statistically significant. $^{**}P < 0.01$, $^{***}P < 0.001$. Data are presented as means ± SEM.
Source data are available online for this figure.

Figure EV3. Chronic activation of the PV neurons responding to restraint stress did not affect sympathetic innervation in WAT.

A Photomicrograph illustrating the PRV-mRFP labelling in the hypothalamus 6 days after PRV-mRFP injected into eWAT; arrow pointed to the DMH traced by PRV-mRFP.
B c-Fos staining after 2 h restraint in the same brain slice.
C, C′ Low and high magnification of the photomicrograph illustrating single c-Fos immunolabelling (green) without PRV-mRFP labelling (red) in the paraventricular thalamic nucleus (PV).
D Photomicrograph illustrating the PRV-EGFP labelling in the hypothalamus 6 days after PRV-EGFP injected into iWAT; arrow pointed to the DMH traced by PRV-EGFP.
E c-Fos staining after 2 h restraint in the same brain slice.
F, F′ Low and high magnification of the photomicrograph illustrating single c-Fos immunolabelling (red) without PRV-EGFP labelling (green) in the PV.
G, H Mcherry and hM3D-mcherry expression in PV.
I–L Immunohistochemical staining of TH in iWAT and eWAT (I and K). Statistical analysis of the histological data of iWAT and eWAT (J and L).

Data information: mcherry group $n = 6$, hM3D group $n = 6$ in (I–L). Shapiro–Wilk test and *F*-test were used to test the normality and equal variance assumptions, respectively. Two-tailed *t*-tests were performed to assess differences between two experimental groups with normally distributed data and equal variance. Two-tailed *t*-tests with Welch's correction were used when a two-sample comparison of means with unequal variances. For non-normally distributed data, Mann–Whitney *U*-tests were performed to compare two groups. $P < 0.05$ was considered statistically significant. Data are presented as means ± SEM. Scale bar: A–H, 200 μm; I and K, 2,000 μm.
Source data are available online for this figure.

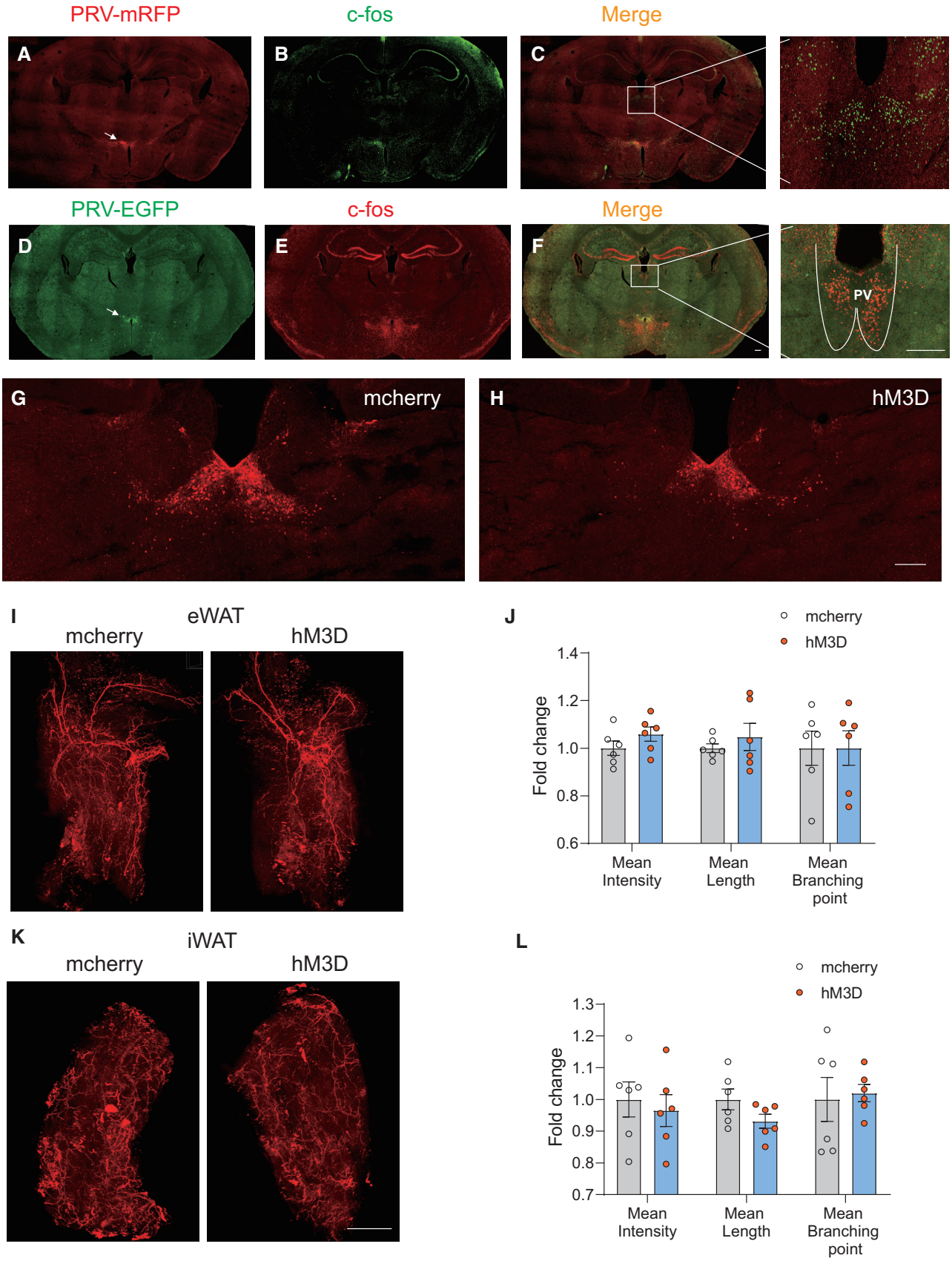

Figure EV3.

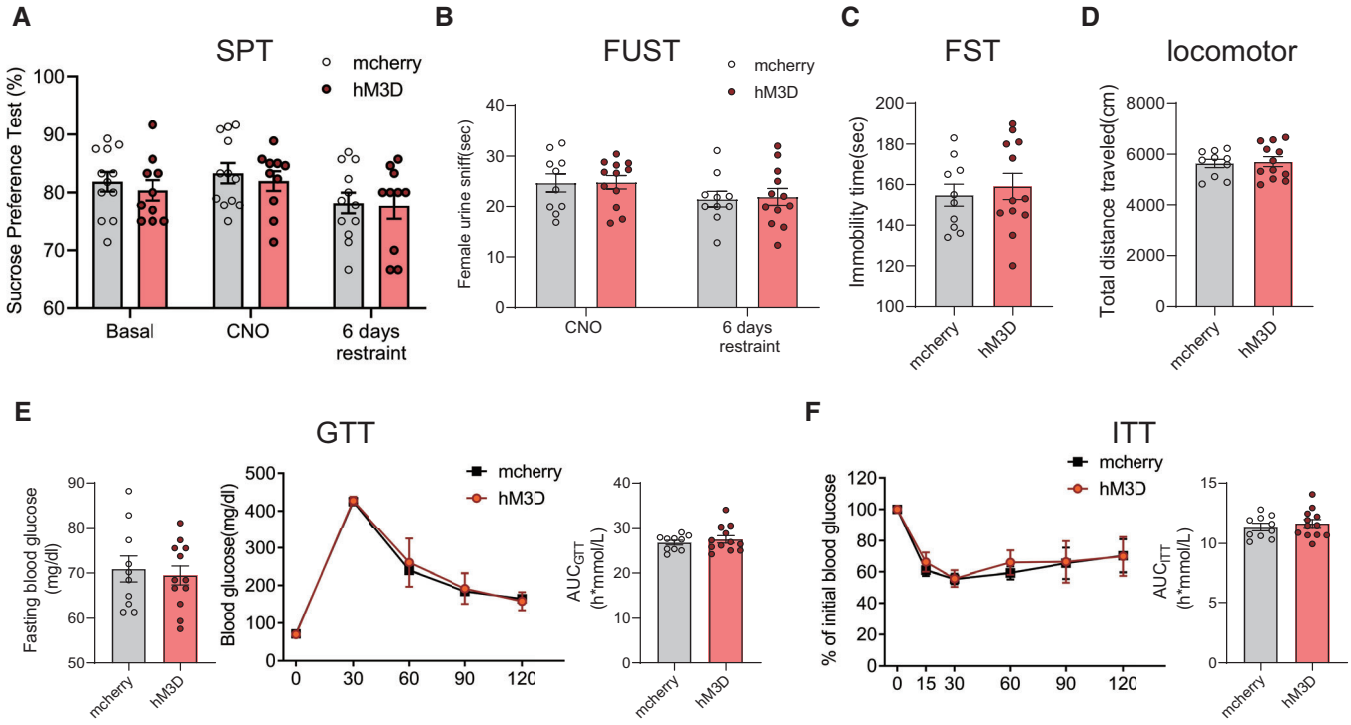

Figure EV4. Chronic activation of the PV neurons responding to restraint stress did not affect depressive-like behaviours or insulin resistance.

A–D Chronically reactivating hM3D-labelled PV neurons did not affect the depressive-like behaviours, as evaluated by SPT (A, subgroups, $F_{(1, 20)} = 0.8020$, $P = 0.3812$; treatment, $F_{(2, 40)} = 2.940$, $P = 0.0644$; subgroups × treatment, $F_{(2, 40)} = 0.03915$, $P = 0.9616$), FUST (B, subgroups, $F_{(1, 20)} = 0.04271$, $P = 0.8384$; treatment, $F_{(1, 20)} = 2.907$, $P = 0.1037$; subgroups × treatment, $F_{(1, 20)} = 0.007554$, $P = 0.9316$) and FST (C, two-tailed unpaired *t*-test, $t_{(20)} = 0.4940$, $P = 0.6267$) and the locomotor activity (D, two-tailed unpaired *t*-test, $t_{(20)} = 0.2734$, $P = 0.7874$).

E, F Chronically reactivating hM3D-labelled PV neurons did not induce insulin resistance evaluated by GTT (E, left, fasting blood glucose level, two-tailed unpaired *t*-test, $t_{(20)} = 0.4146$, $P = 0.6829$; middle, glucose tolerance test, subgroups, $F_{(1, 20)} = 0.2836$, $P = 0.6002$; time, $F_{(4, 80)} = 497.7$, $P < 0.0001$; subgroups × treatment, $F_{(4, 80)} = 0.7470$, $P = 0.5629$; right, the area under the curve of GTT, two-tailed unpaired *t*-test, $t_{(20)} = 0.7050$, $P = 0.4889$) and ITT (F, left, subgroups, ?$F_{(1, 20)} = 0.8558$, $P = 0.3659$; time, $F_{(5, 100)} = 131.8$, $P < 0.0001$; subgroups × treatment, $F_{(5, 100)} = 1.249$, $P = 0.2923$; right, the area under the curve of ITT, two-tailed unpaired *t*-test with Welch's correction, $t_{(20)} = 0.5915$, $P = 0.5608$).

Data information: mcherry group $n = 10$, hM3D group $n = 12$ in (A–F). Shapiro–Wilk test and *F*-test were used to test the normality and equal variance assumptions, respectively. Two-tailed *t*-tests were performed to assess differences between two experimental groups with normally distributed data and equal variance. Two-tailed *t*-tests with Welch's correction were used when a two-sample comparison of means with unequal variances. For non-normally distributed data, Mann–Whitney *U*-tests were performed to compare two groups. For multiple groups, two-way ANOVAs followed by Sidak multiple comparisons test were used. $P < 0.05$ was considered statistically significant. Data are presented as means ± SEM.
Source data are available online for this figure.

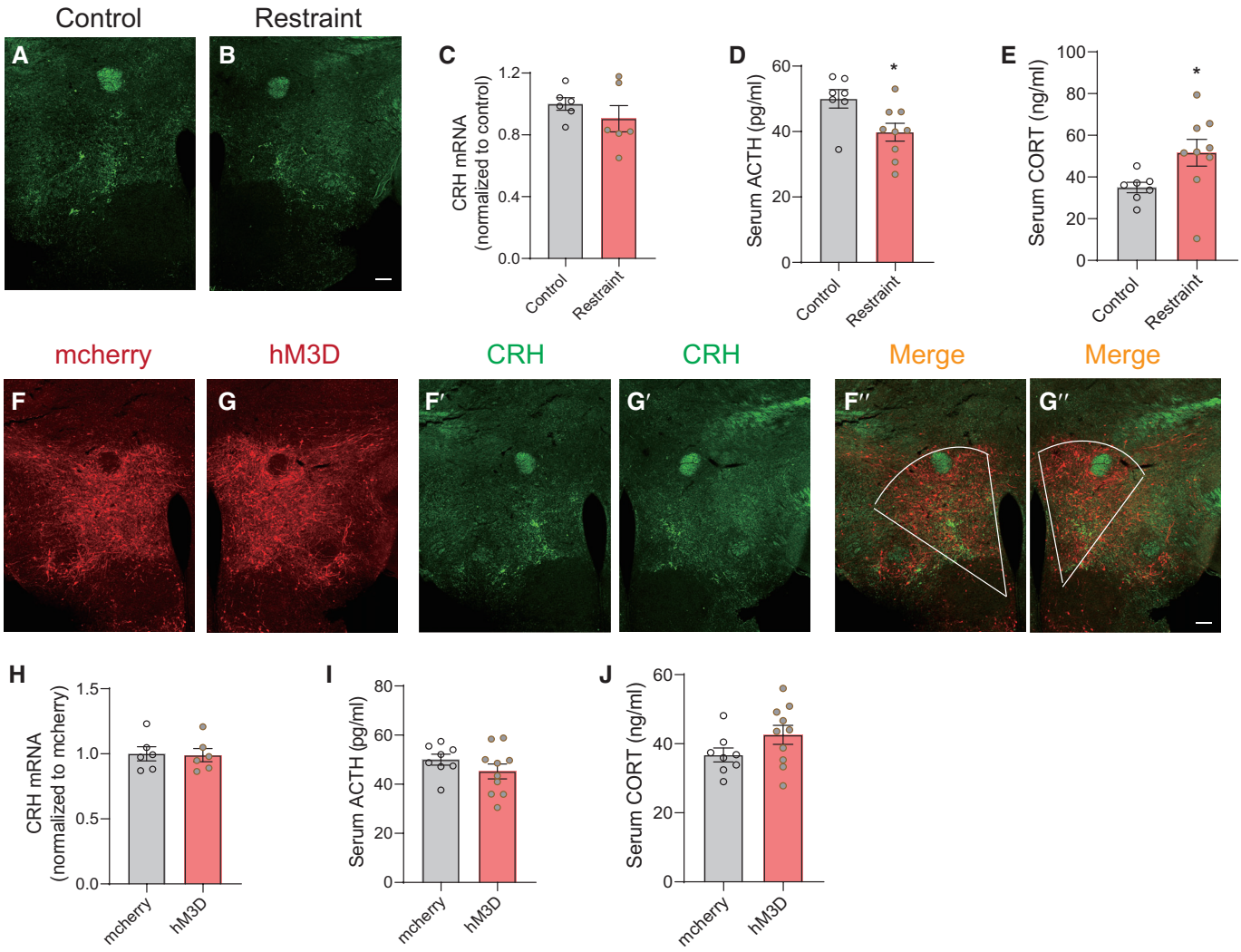

Figure EV5. HPA activity under chronic restraint stress and PVN activation paradigms.

A, B Representative images of immunohistochemical staining of CRH after chronic restraint stress treatment.

C Real-time PCR analysis of CRH in hypothalamus under chronic restraint stress, two-tailed unpaired *t*-test, $t_{(10)} = 1.016$, $P = 0.3336$, control group $n = 6$, restraint group $n = 6$.

D, E ELISA results showing serum ACTH (D, Mann–Whitney *U*-test, $P = 0.0311$) and CORT levels (E, two-tailed unpaired *t*-test, $t_{(14)} = 2.178$, $P = 0.0470$) under chronic restraint stress, control group $n = 7$, restraint group $n = 9$.

F, G Representative images showing mcherry (F, F″) and hM3D-mcherry (G, G") expression in PVN, immunohistochemical staining of CRH (F′ and G′).

H Real-time PCR analysis of CRH in hypothalamus after chronically reactivating PVN neurons, two-tailed unpaired *t*-test, $t_{(10)} = 0.1353$, $P = 0.8951$, mcherry group $n = 6$, hM3D group $n = 6$.

I, J ELISA results showing serum ACTH (I, two-tailed unpaired *t*-test, $t_{(16)} = 1.215$, $P = 0.2420$) and CORT levels (J, two-tailed unpaired *t*-test, $t_{(16)} = 1.616$, $P = 0.1257$) after chronically reactivating PVN neurons, mcherry group $n = 8$, hM3D group $n = 10$.

Data information: Shapiro–Wilk test and *F*-test were used to test the normality and equal variance assumptions, respectively. Two-tailed *t*-tests were performed to assess differences between two experimental groups with normally distributed data and equal variance. Two-tailed *t*-tests with Welch's correction were used when a two-sample comparison of means with unequal variances. For non-normally distributed data, Mann–Whitney *U*-tests were performed to compare two groups. $P < 0.05$ was considered statistically significant. *$P < 0.05$. Data are presented as means ± SEM.
Source data are available online for this figure.