Expanded View Figures

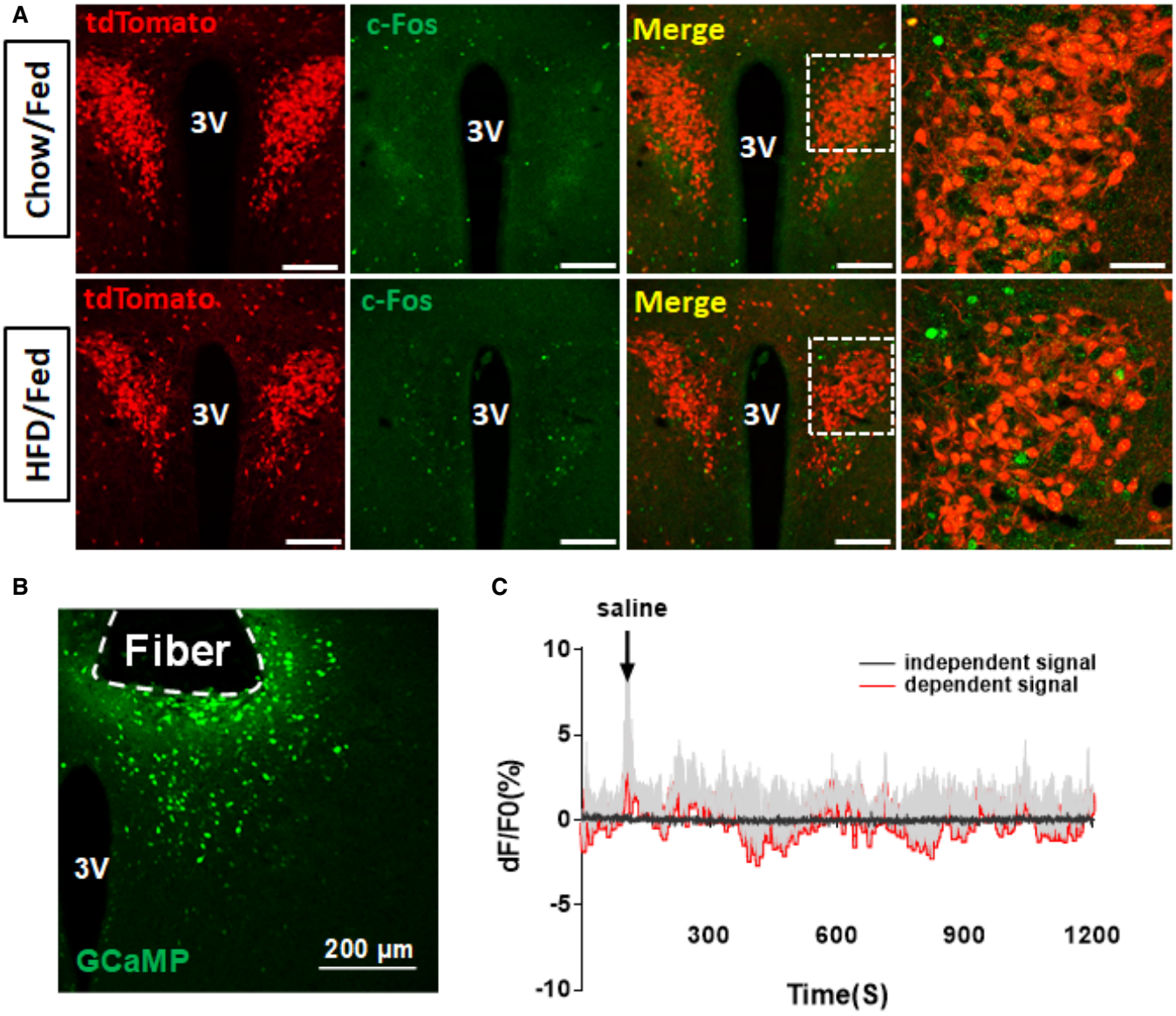

Figure EV1. Response of PVH CRH neurons in chow/HFD-fed conditions and saline treatments.

A Immunostaining of c-Fos in 8- to 10-week-old CRH-Cre::Ai9 reporter male mice when they were fed chow (top row) or fed HFD for 4 weeks (bottom row). Red: tdTomato for CRH-Cre neurons in the PVH and green for c-Fos. 3V: the 3rd ventricle. The picture on the right is an amplified picture for the boxed area shown in the merged picture.

B A representative picture showing GCaMP6m expression in PVH CRH neurons with tract indicating optic fiber implantation.

C Traces showing GCaMP6m signaling with saline administration.

Data information: Scale bar = 200 μm in all pictures except the amplified ones (= 50 μm).
Source data are available online for this figure.

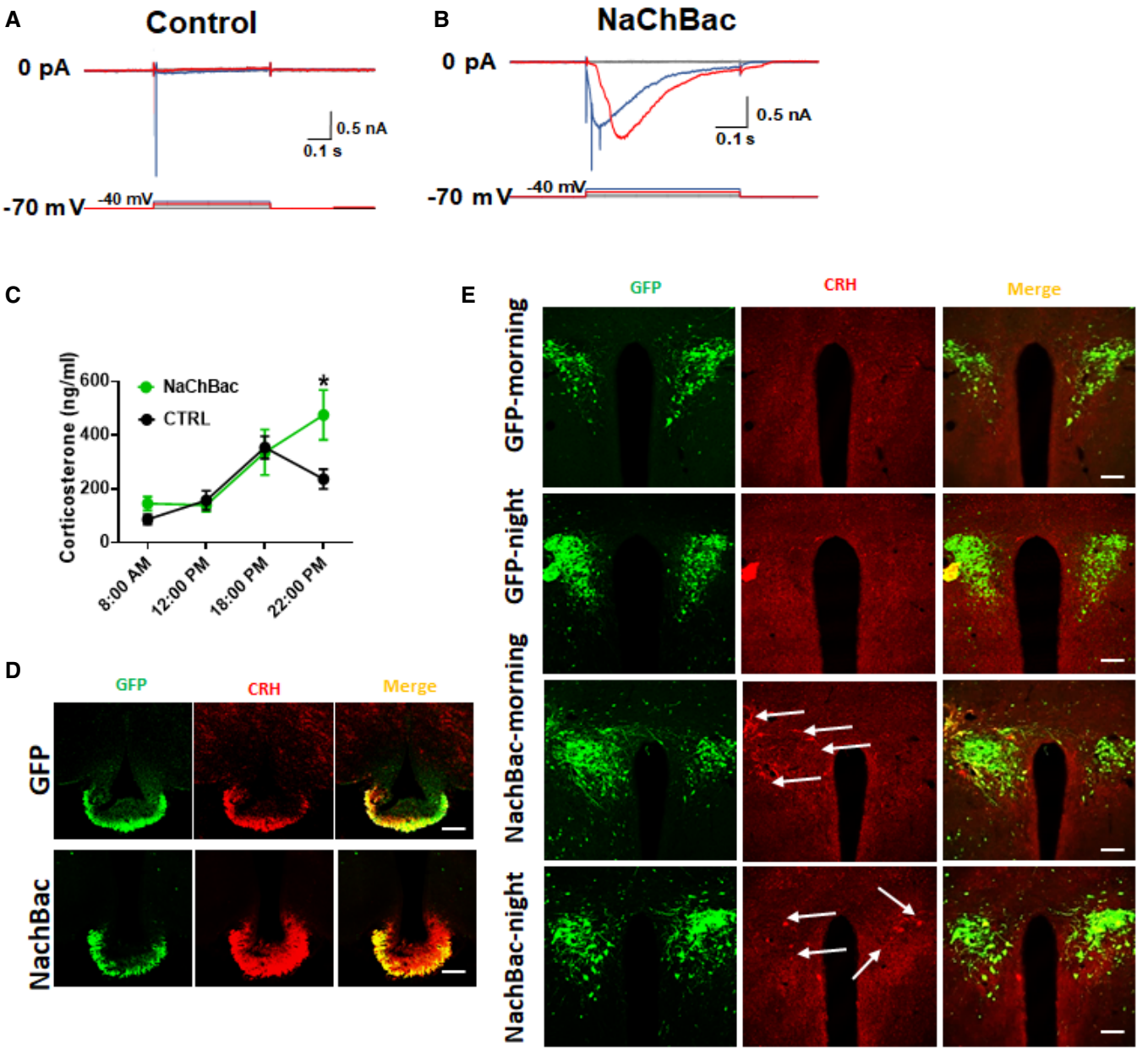

Figure EV2. NachBac expression increased the activity level of PVH CRH neurons.

A, B Recording of GFP-expressing control (A) and NachBac-expressing PVH CRH neurons (B) with a step-wise depolarization protocol to assess least depolarization required to reach threshold for action potential firing (i.e., rheobase).

C Levels of corticosterone measured from blood obtained at the indicated time points in control and NachBac mice (12 weeks old, males, n = 6–7 each, data presented as mean ± SEM, *P = 0.051 control versus NachBac at time 22:00, two-way ANOVA test).

D CRH immunostaining in the median eminence (red, middle) in CRH-Cre mice injected with AAV-Flex-GFP (top panels) or NachBac vectors (bottom panels) to bilateral PVH.

E CRH-Cre mice injected with control AAV-Flex-GFP vectors (GFP) or NachBac vectors (NachBac) to bilateral PVH, and CRH immunostaining in mice perfused at morning (9–10 am) or night (7–8 pm), and brain sections were obtained for CRH immunostaining (red). In both NachBac groups, a number of neurons exhibited CRH-immunoreactive structures (arrows) whereas none was observed in either of GFP groups.

Data information: Scale bar = 200 μm.
Source data are available online for this figure.

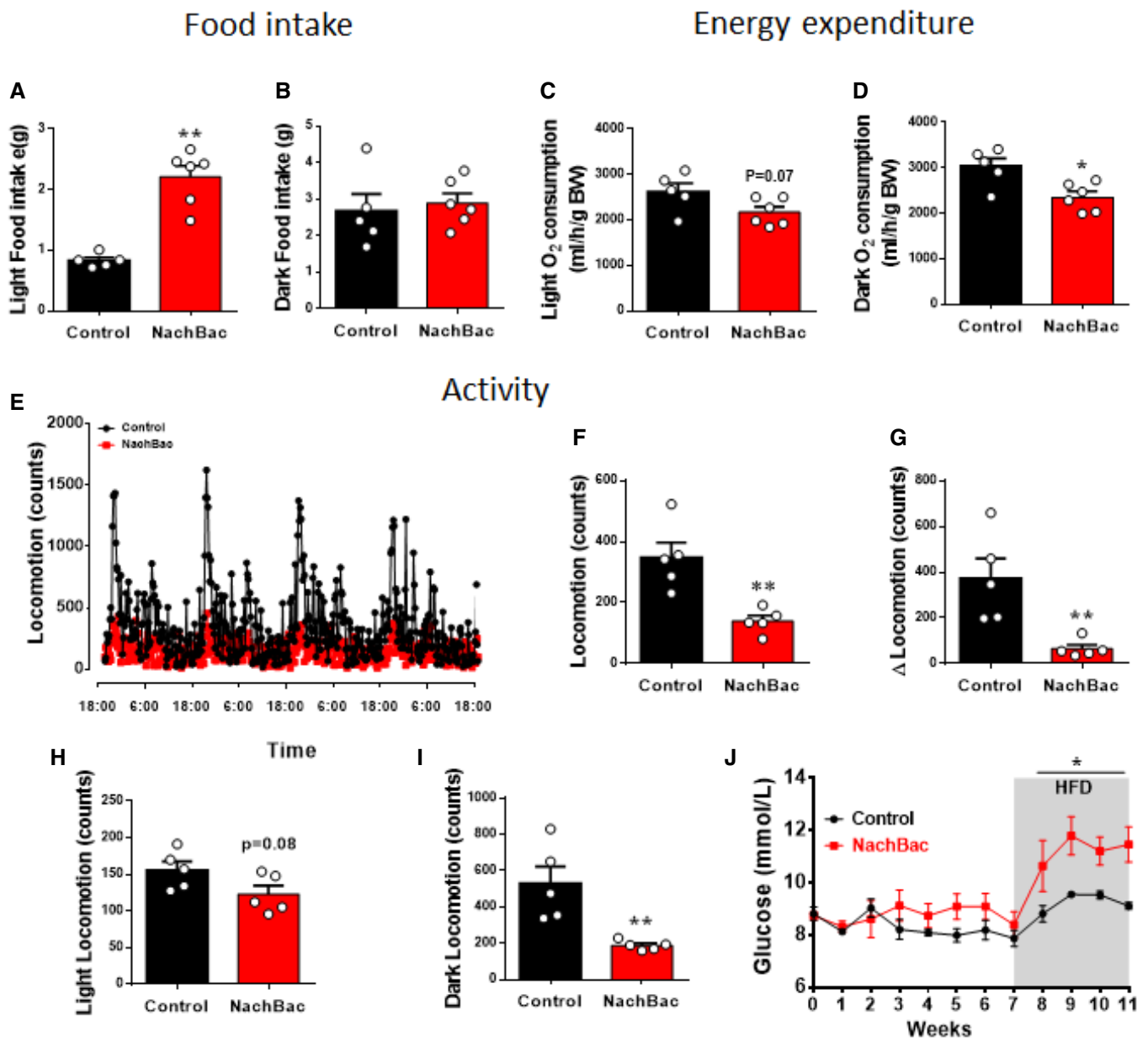

Figure EV3. The effect of NachBac expression in PVH CRH neurons on energy balance.

A–D Comparison between controls and NachBac mice feeding in light (A, $^{**}P < 0.0001$) and dark periods (B, $P = 0.6773$), and energy expenditure in light (C, $P = 0.0676$) and dark periods (D, $^{*}P = 0.0138$), which is from the same data presented in Fig 3D–I.

E–I Comparison between controls and NachBac mice for locomotion patterns as measured in CLAMS cages (E), for average activity (F, $^{**}P = 0.004$), for difference between light and dark periods (G, $^{**}P = 0.0082$), for difference during light periods (H, $P = 0.0813$) and during dark periods (I, $^{**}P = 0.0068$).

J Glucose levels measured during early morning in feeding *ad libitum* mice every week after NachBac vector delivery, which is from the same group of mice presented in Fig 3A ($^{*}P < 0.0001$).

Data information: All comparisons were made with unpaired Student's *t*-tests, $n = 5$ for controls and $n = 6$ for the NachBac group. All data presented as mean ± SEM. Source data are available online for this figure.

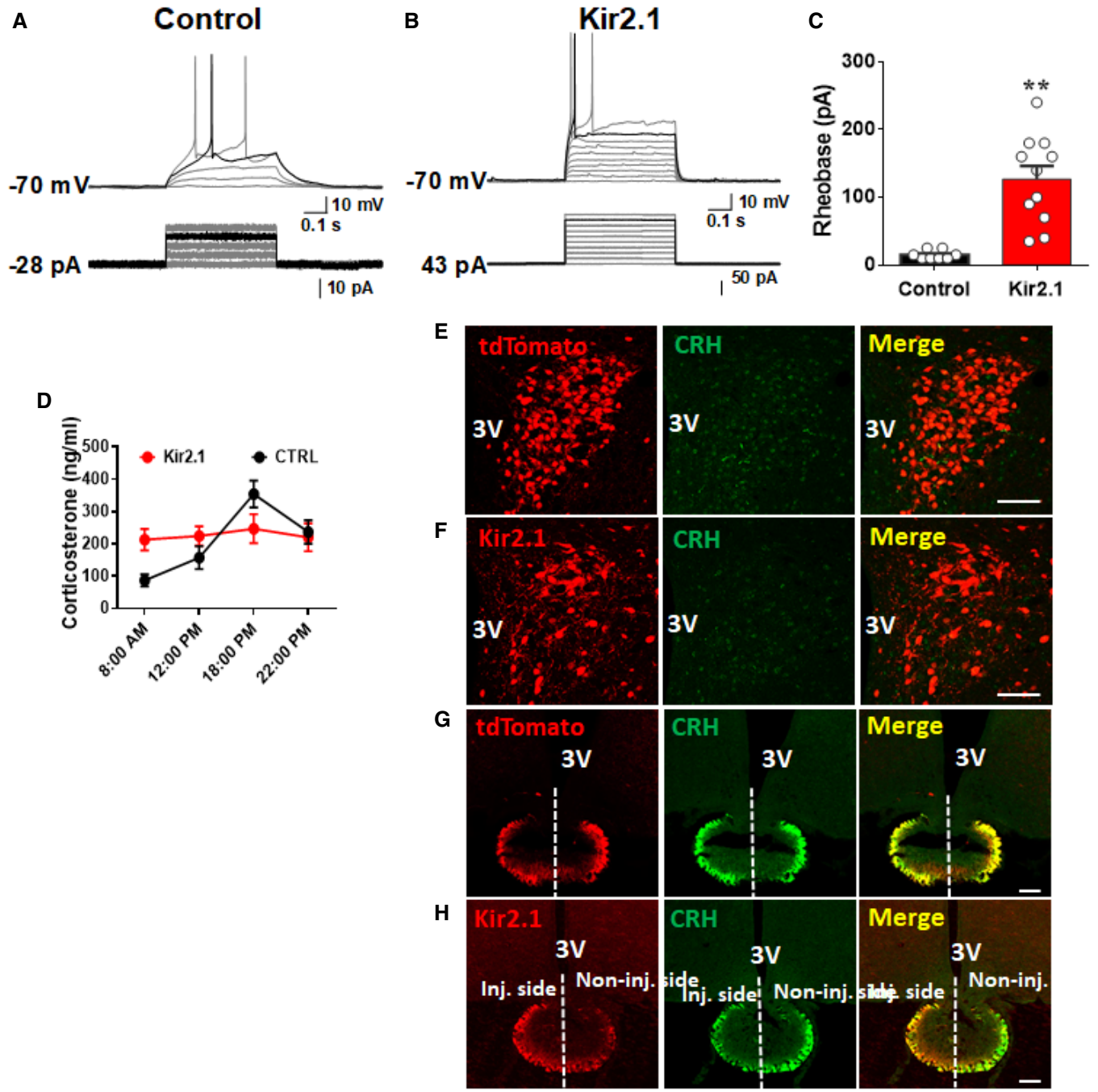

Figure EV4. Kir2.1 expression reduced the activity level of PVH CRH neurons.

A–C Recording of GFP-expressing control (A) and Kir2.1-expressing PVH CRH neurons (B) with a step-wise depolarization protocol to assess least currents required to reach threshold for action potential firing (i.e., rheobase). (C) Comparison of rheobase between GFP- and Kir2.1-expressing PVH CRH neurons, n = 8 for controls and n = 10 for the Kir2.1 group. Data presented as mean ± SEM, $^{**}P$ = 0.0002, unpaired Student's t-tests.

D Levels of corticosterone measured from blood obtained at the indicated time points in control and Kir2.1 mice (12 weeks old, males, n = 6–7 each, the control group is the same as in Fig EV3). Data presented as mean ± SEM.

E–H CRH immunostaining in the PVH (E and F, middle panels) and median eminence (ME, D and E middle panels) in CRH-Cre mice::Ai9 reporter mice (E and G) or with Kir2.1 vector injection to PVH one side only (F and H). Red: tdTomato in the PVH (E, left panel) or ME (G, left panel) in CRH-Cre::Ai9 reporter, or Kir2.1 expression in the PVH (F, left panel) and ME(H, left panel). In contrast to even distribution of CRH between the two sides in control (G, middle panel), in Kir2.1 mice, the Kir2.1 injected side with more Kir2.1 expression but less CRH expression (H, middle panel), demonstrating Kir2.1 expression reduced CRH expression in CRH neuron. Scale bar = 200 μm.

Source data are available online for this figure.

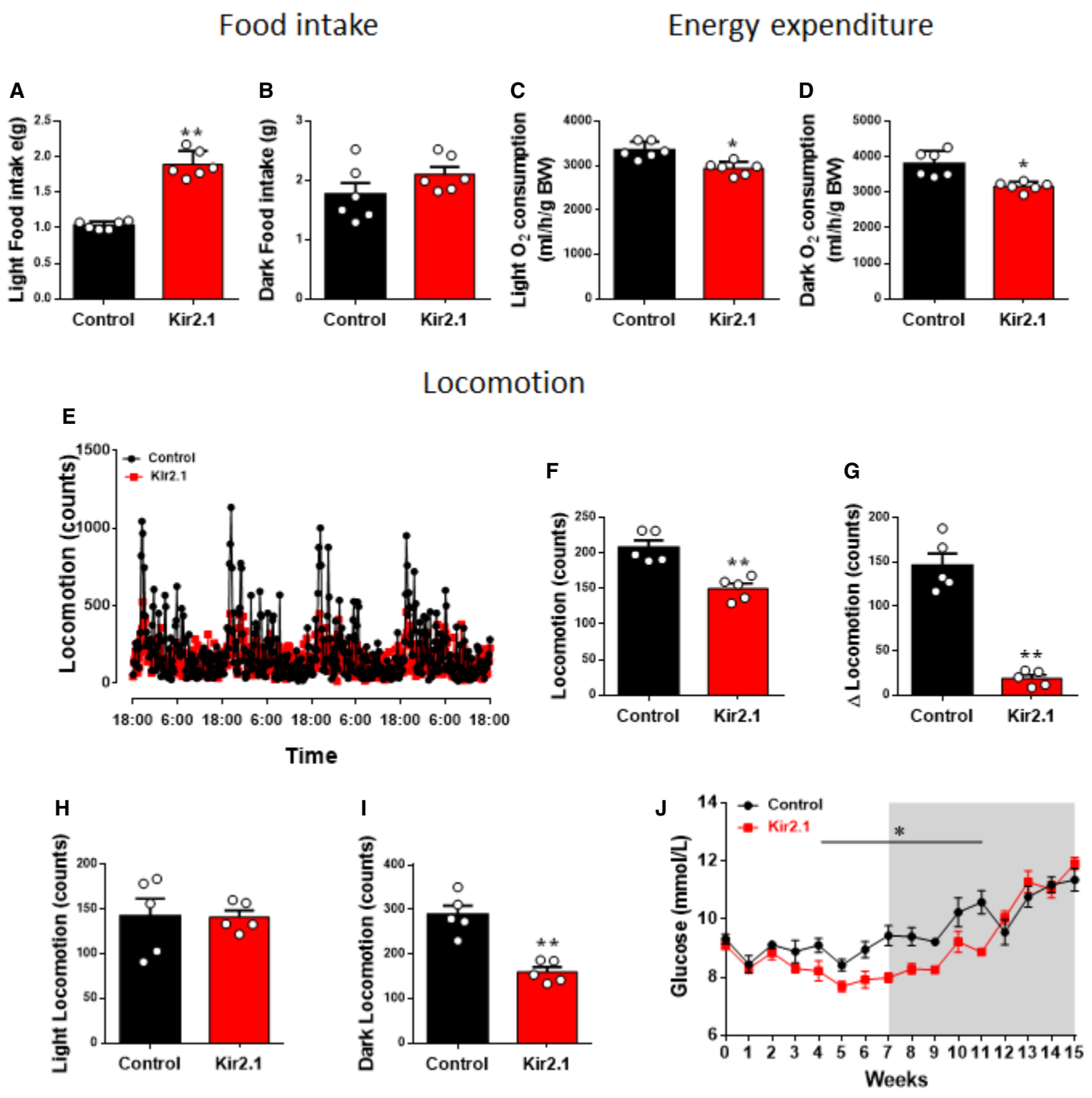

Figure EV5. The effect of Kir2.1 expression in PVH CRH neurons on energy balance.

A–D Comparison between controls and Kir2.1 mice feeding in light (A, $^{**}P < 0.0001$) and dark periods (B, $P = 0.1669$), and energy expenditure in light (C, $^{*}P = 0.0099$) and dark periods (D, $^{*}P = 0.0022$), which is from the same data presented in Fig 5D–I.

E–I Comparison between control and Kir2.1 mice for locomotion patterns as measured in CLAMS cages (E), for average activity (F, $^{**}P = 0.0014$), for difference between light and dark periods (G, $^{**}P < 0.0001$), and for difference during light periods (H, $P = 0.9814$) and during dark periods (I, $^{**}P = 0.0005$).

J lucose levels measured during early morning in feeding *ad libitum* mice every week after Kir2.1 vector delivery, which is from the same group of mice presented in Fig 5A ($^{*}P = 0.0002$).

Data information: $n = 6$ each, all comparisons were made with unpaired Student's *t*-tests. All data presented at mean ± SEM.
Source data are available online for this figure.