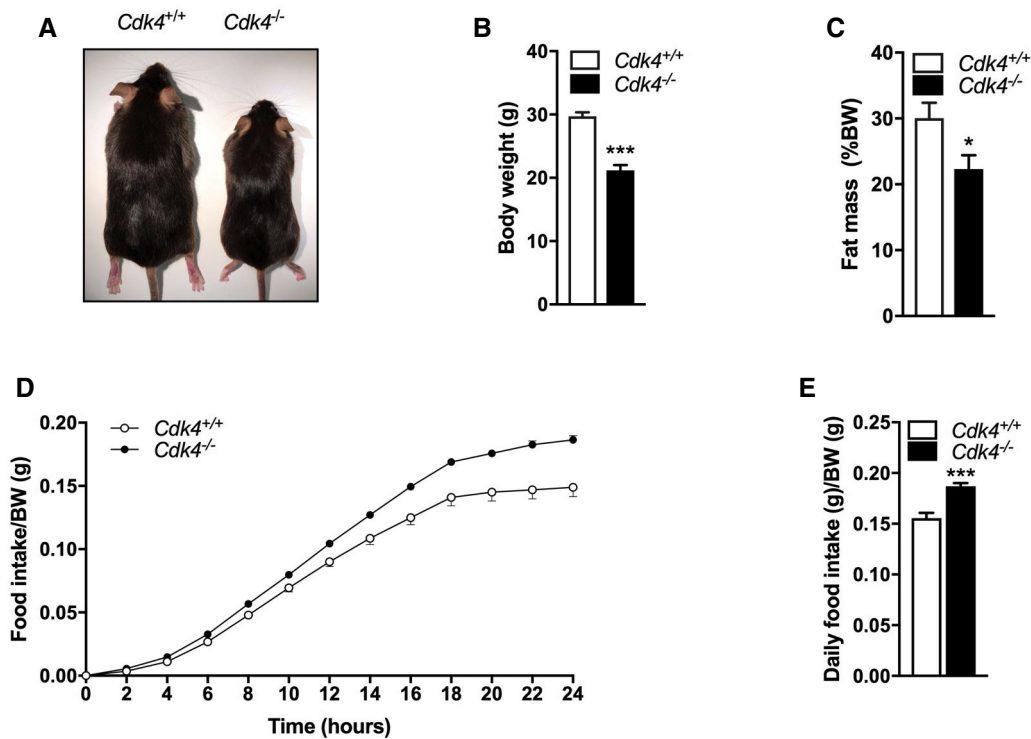# Expanded View Figures

Figure EV1. **Cdk4$^{-/-}$ mice are smaller and have increased food intake compared to their wild-type littermates (Cdk4$^{+/+}$).**

A Whole-body image of Cdk4$^{+/+}$ and Cdk4$^{-/-}$ mice.
B, C Body weight (B) and fat mass percentage (C) of Cdk4$^{+/+}$ (n = 5) and Cdk4$^{-/-}$ (n = 6) mice.
D, E Food intake was measured using the Phenomaster device (TSE system) and is shown as cumulative intake over 24 h (D) and daily cumulative food intake (E) of 14-week-old Cdk4$^{+/+}$ (n = 7) and Cdk4$^{-/-}$ (n = 8) mice.

Data information: All data are shown as the mean ± SEM. Student's t-test was used for statistical analysis *P < 0.05; ***P < 0.001.
Source data are available online for this figure.
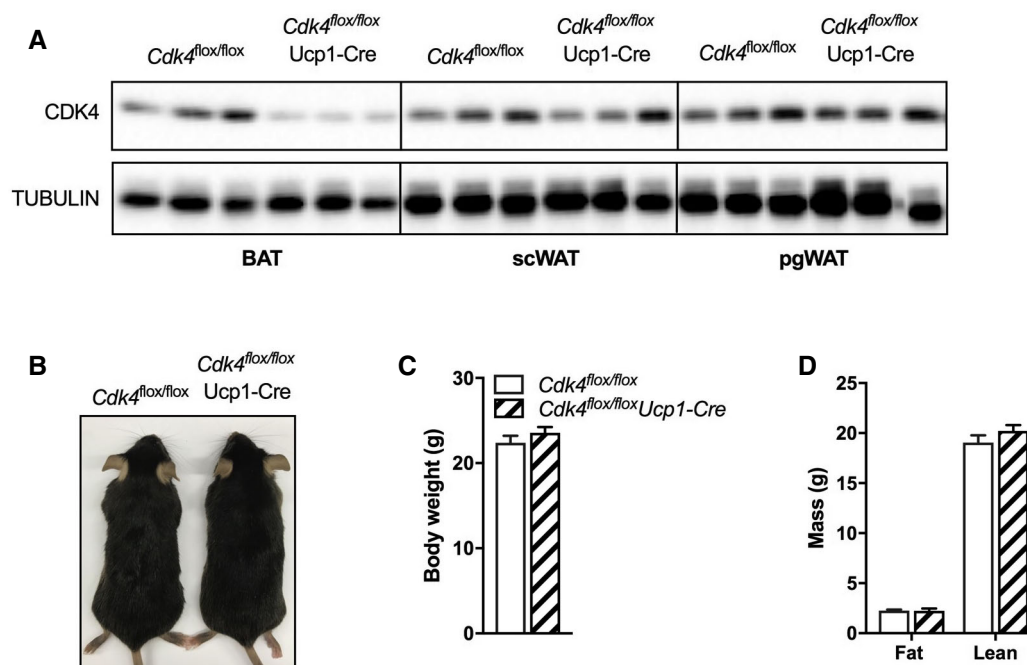
Figure EV2. _Cdk4_ BAT-specific knockout mice have normal body weight, fat mass, and lean mass.

A   Western blot for CDK4 expression in iBAT, scWAT, and pgWAT from _Cdk4$^{flox/flox}$_ (_n_ = 3 biological replicates) and _Cdk4$^{flox/flox}$_ Ucp1-Cre (_n_ = 3) mice. Tubulin was used
          as loading control. Please notice that upon amplification, the western blot images are abnormally pixelated. This is because the saturation of the images is shown
          in FigEV2A_Exposure_3.0sec.scn, which is a direct acquisition file from the Imager, and is found in the data source folder. A JPEG image is also available in this
          folder.
B   Whole-body image of _Cdk4$^{flox/flox}$_ and _Cdk4$^{flox/flox}$_ Ucp1-Cre mice.
C, D   Body weight (C) and body composition (fat and lean mass) (D) of _Cdk4$^{flox/flox}$_ (_n_ = 8) and _Cdk4$^{flox/flox}$_ Ucp1-Cre (_n_ = 8) mice.

Data information: All data are shown as the mean $\pm$ SEM.
Source data are available online for this figure.
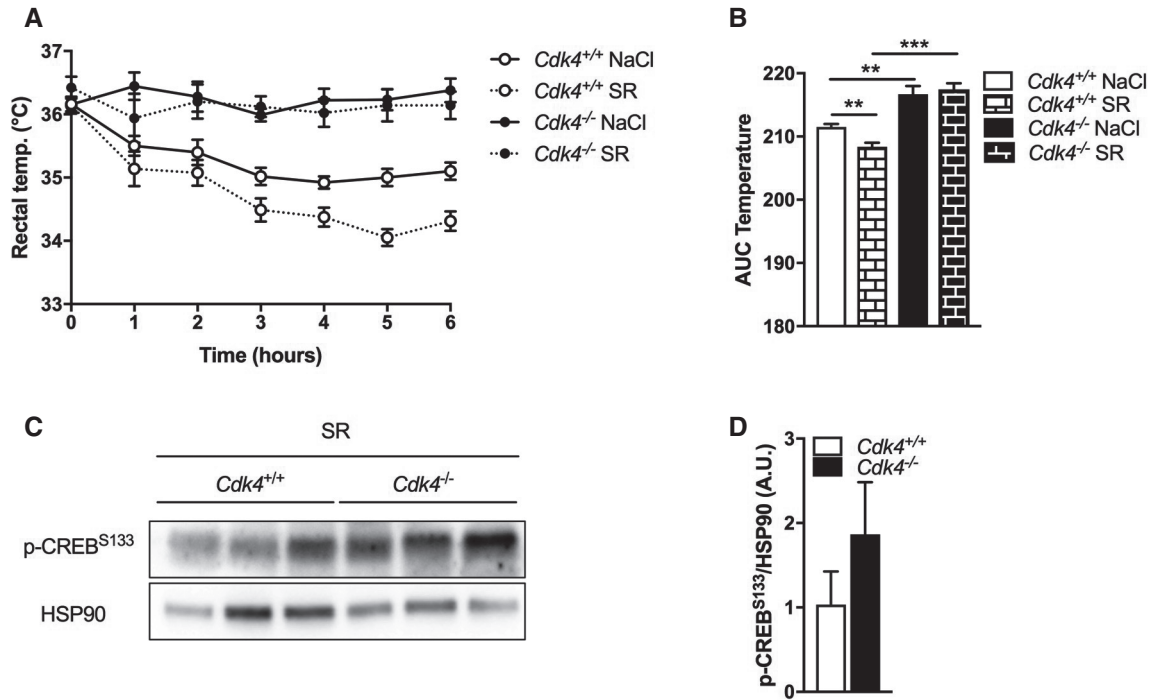
Figure EV3. β3-receptor inhibition does not blunt the increased thermogenic response of *Cdk4*$^{-/-}$ animals.

A, B   Acute cold test (4°C) after 5 days of treatment with a β3-adrenergic antagonist (SR; *Cdk4*$^{+/+}$ [*n* = 5] and *Cdk4*$^{-/-}$ [*n* = 5]) or vehicle (NaCl; *Cdk4*$^{+/+}$ [*n* = 8] and *Cdk4*$^{-/-}$ [*n* = 9]) (A) and corresponding quantification of the area under the curve (AUC) (B).

C, D   Western blot analysis (C) and quantification of p-CREB S133 protein expression after 7 days of SR treatment (D) (*n* = 3 biological replicates). HSP90 was used as loading control.

Data information: All data are shown as the mean ± SEM. Student's *t*-test was used for statistical analyses. **$P < 0.01$, ***$P < 0.001$.
Source data are available online for this figure.

Figure EV4. *Cdk4* deficiency in Sf1 neurons does not affect body weight, body composition, or indirect calorimetry measures.

A, B   Body weight (A) and body composition (fat mass and lean mass) (B) of *Cdk4*$^{flox/flox}$ (*n* = 17) and *Cdk4*$^{flox/flox}$ Sf1-Cre (*n* = 11) mice.

C–H   Indirect calorimetry was performed using the Oxymax apparatus (Columbus Instruments) in *Cdk4*$^{flox/flox}$ (*n* = 6) and *Cdk4*$^{flox/flox}$ Sf1-Cre (*n* = 5) mice. Whole-body oxygen consumption rate (VO$_2$) (C, D), respiratory exchange ratio (RER) (E, F), and energy expenditure (G, H) were measured at 24°C during the light phase (white rectangle) and dark phase (black rectangle).

I, J   CalR was used to implement GLM-regression plot for each group corresponding to the association between energy balance and total body mass at 24°C (I). The CalR interface displays each mouse as a dot, and the standard error of mean for each group in gray. The "mass effect" and "group effect" were analyzed using a generalized linear model (GLM) using body weight as a covariate. The results of this analysis are shown in table (J) for *Cdk4*$^{flox/flox}$ (*n* = 6) and *Cdk4*$^{flox/flox}$ Sf1-Cre (*n* = 5) mice.

Data information: All data are shown as the mean ± SEM.
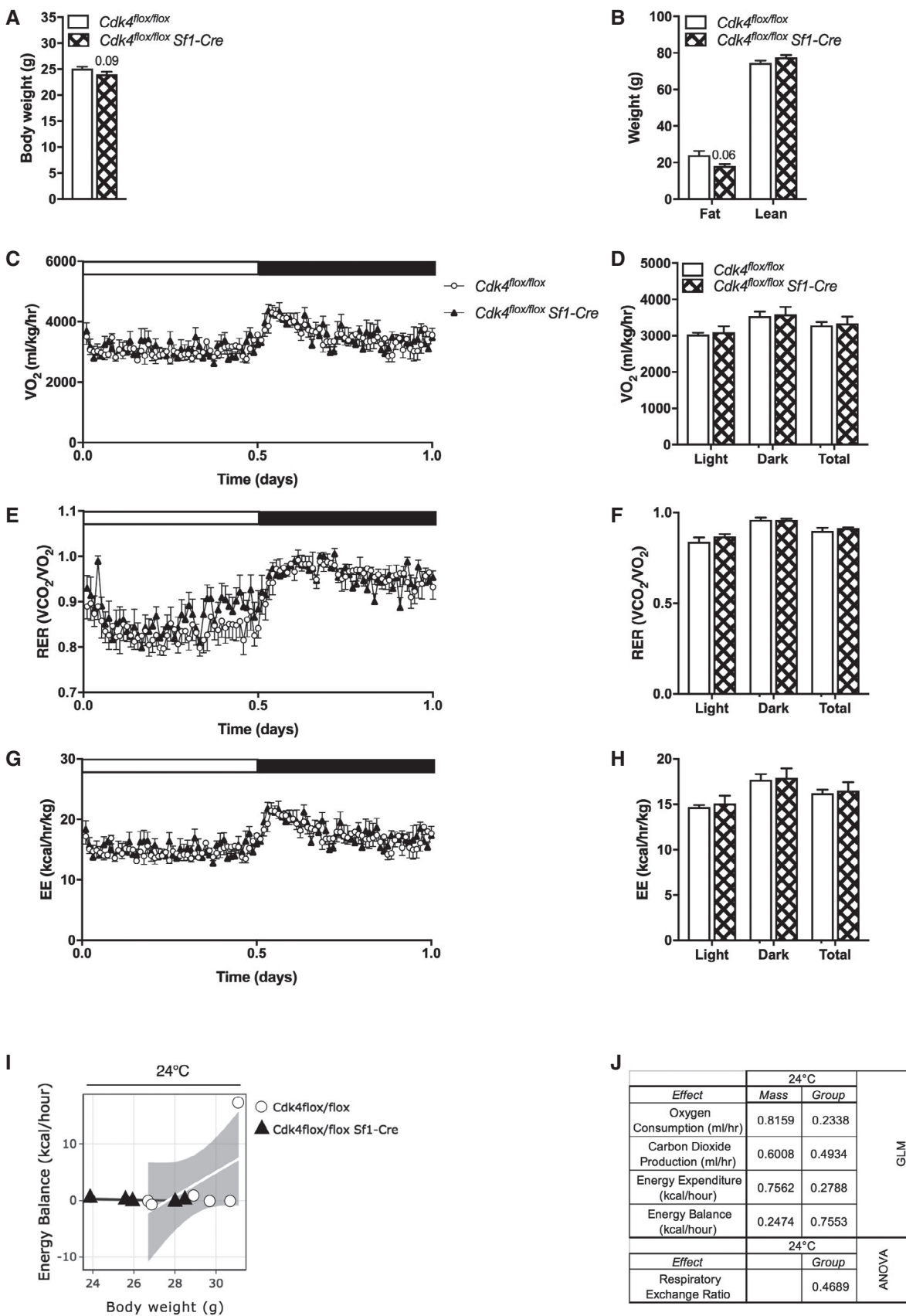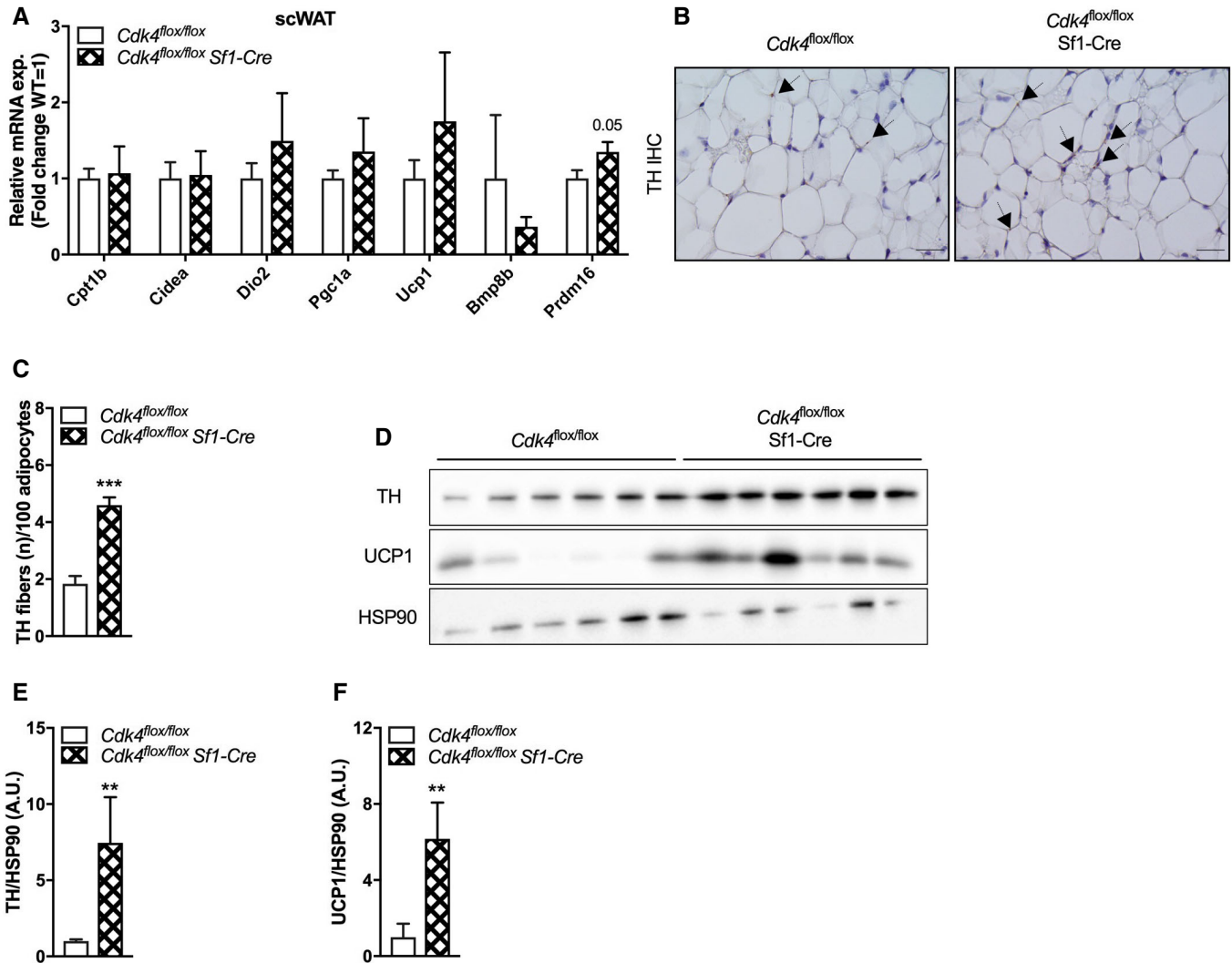
Figure EV4.

Figure EV5. Increased sympathetic innervation and WAT browning in *Cdk4^flox/flox* Sf1-Cre mice.

A Expression of thermogenic genes in scWAT of *Cdk4^flox/flox* (n = 9) and *Cdk4^flox/flox* Sf1-Cre (n = 8) mice as assessed by RT–PCR.

B, C TH immunohistochemical (IHC) staining in iBAT sections (scale bar 20 μm, arrows indicate TH parenchymal fibers) (B) and corresponding quantification of the number of TH fibers relative to 100 adipocytes (C) (*Cdk4^flox/flox* [n = 5] and *Cdk4^flox/flox* Sf1-Cre [n = 6]).

D–F Western blot analysis (D) and quantification of TH (E) and UCP1 protein expression (n = 6 biological replicates) (F). HSP90 was used as loading control.

Data information: All data are shown as the mean ± SEM. Student's *t*-test (C) and Mann–Whitney *U*-test (E, F) were used for statistical analyses. **$P < 0.01$, ***$P < 0.001$.

Source data are available online for this figure.