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<sup>flox</sup>/flox* (*n* = 3 biological replicates) and *Cdk4<sup>flox</sup>/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.scrn, 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<sup>flox</sup>/flox* and *Cdk4<sup>flox</sup>/flox* *Ucp1-Cre* mice.

C, D. Body weight (C) and body composition (fat and lean mass) (D) of *Cdk4<sup>flox</sup>/flox* (*n* = 8) and *Cdk4<sup>flox</sup>/flox* *Ucp1-Cre* (*n* = 8) mice.

Data information: All data are shown as the mean ± 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 Cdk4fl/fl [n = 17] and Cdk4fl/fl Sf1-Cre [n = 11] mice.
C–H Indirect calorimetry was performed using the Oxymax apparatus (Columbus Instruments) in Cdk4fl/fl [n = 6] and Cdk4fl/fl Sf1-Cre [n = 5] mice. Whole-body oxygen consumption rate (VO2) (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 Cdk4fl/fl [n = 6] and Cdk4fl/fl 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\textsuperscript{flch} Sf1-Cre mice.

A. Expression of thermogenic genes in scWAT of Cdk4\textsuperscript{flch} (n = 9) and Cdk4\textsuperscript{flch} 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\textsuperscript{flch} [n = 5] and Cdk4\textsuperscript{flch} 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.