Figure EV1. Chronic cold exposure induces glucose metabolism in BAT.
Mice, housed at 30°C or 6°C for 10 days, were administered with [U-13C]glucose (2 g/kg, IP). 15 minutes after injection, BAT was harvested for metabolic enrichment assay.

A. To directly compare the change of enrichment between different metabolites, the relative metabolic 13C enrichments in BAT of male mice are normalized to the average enrichment of each metabolite in 30°C group.
B. Relative abundance of glycolytic and TCA cycle intermediates in BAT.
C. The enrichment and relative abundance of glyceraldehyde 3-phosphate (G3P).
D. The enrichment of palmitate.

Data information: n = 10, data are represented as the mean ± SD. Statistical analysis was performed using two-tailed Student’s t-test, *P < 0.05.
Source data are available online for this figure.
Figure EV2. Chronic cold exposure does not induce oxidative metabolism in liver or muscle.
Mice, housed at 30°C or 6°C for 10 days, were administered with [U-13C]glucose (2 g/kg, IP). 15 minutes after injection, liver and muscle were harvested for metabolic enrichment assay.

A m+6 glucose enrichment in liver.

B Metabolic 13C enrichments in liver are shown as m+3 glycolysis intermediates, m+2 TCA cycle intermediates. GAP, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate.

C After normalizing to the glucose enrichment in the liver of each mouse, the relative metabolic 13C enrichments were shown as m+3 glycolysis intermediates and m+2 TCA cycle intermediates.

D m+6 glucose enrichment in muscle.

E Metabolic 13C enrichments in muscle are shown as m+3 glycolysis intermediates, m+2 TCA cycle intermediates.

Data information: n = 10, data are represented as the mean ± SD. Statistical analysis was performed using two-tailed Student’s t-test, *P < 0.05.

Source data are available online for this figure.
Figure EV3. Chronic cold exposure induces oxidative metabolism in BAT and sWAT of female mice.

Mice, housed at 30 or 6°C for 10 days, were administered with [U-13C]glucose (2 g/kg, IP). 15 minutes after injection, BAT, sWAT, and gWAT were harvested for metabolic enrichment assay.

A–C Metabolic 13C enrichments in BAT of female mice are shown as m+6 glucose and m+3 glycolytic intermediates (A), m+2 TCA cycle intermediates (B), and the enrichment of G3P (C).

D, E Metabolic 13C enrichments in sWAT of female mice are shown as m+6 glucose and m+3 glycolysis intermediates (D), m+2 TCA cycle intermediates (E).

F The m+3 enrichment of G3P in sWAT of both female and male mice.

G, H Metabolic 13C enrichments in gWAT of female mice are shown as m+6 glucose and m+3 glycolysis intermediates (G), m+2 TCA cycle intermediates (H).

I The m+3 enrichment of G3P in gWAT of both female and male mice.

Data information: n = 6-8 female mice, and n = 10 male mice, data are represented as the mean ± SD. Statistical analysis was performed using two-tailed Student's t-test, *P < 0.05.

Source data are available online for this figure.
Figure EV4. β3-AR agonist activates glucose oxidation in differentiated primary brown adipocytes.

A–C In the sample [U-13C]glucose experiment as shown in Fig 5, the enrichments of other metabolites were used for MFA modeling. n = 3 biological repeats, data are represented as the mean ± SD. Statistical analysis was performed using two-way ANOVA followed by Tukey's multiple comparisons test, *P < 0.05.

Source data are available online for this figure.
Figure EV5. CHC represses glucose metabolism in multiple adipose tissues.

A Relative mRNA levels of Mpc1/2 expression were measured by qPCR in BAT of the mice, housed at 30 or 6°C for 10 days. n = 5–6 biological replicates.

B Relative mRNA levels of Mpc1/2 expression were measured by qPCR in the pre-differentiated day 0 and fully differentiated brown adipocytes day 6. n = 4 biological replicates.

C Oxygen consumption rate (OCR) of mouse brown adipocytes treated with MPC inhibitor CHC (2 mM) or UK5099 (2 μM), n = 6–7 biological repeats. CL, CL316,243.

D, E Mice were housed at 6°C for 10 days, and mice were IP injected with PBS or CHC (500 mg/kg). 30 minutes after CHC treatment, mice were administered with [U-13C]glucose (2 g/kg, IP). Metabolic 13C enrichments in sWAT (D) and gWAT (E) of male mice are shown as m+2 and m+3 TCA cycle intermediates. n = 7 biological replicates.

Data information: data are represented as the mean ± SD, except that (C) is represented as the mean ± SEM. Statistical analysis was performed using two-tailed Student’s t-test. *P < 0.05.

Source data are available online for this figure.