Supplemental Figure 1: Readily available fuel sources in the plasma of mice during Room Temp and Cold exposure vary with age (A) Plasma acylcarnitine levels from 3-month old and (B) 24-month old mice at room temperature (RT) or Cold (4°C) as measured by UPLC-MS/MS. (C) Free fatty acid (FFA) and (D) glucose levels in the plasma of 3-month, 6-month, 12-month, and 24-month old C57BL6 male mice at room temperature and Cold. Data are presented as mean ± SEM. *p ≤ 0.05. N = 4-5/group.

Supplemental Figure 2: Impaired thermogenesis with aging and acylcarnitine levels in BATless mice. (A) Core body temperature in 3-month, 6-month, 12-month and 24-month old mice placed in the cold room. Core temperature was measured by telemetric transmitter implanted in the peritoneal cavity 2 weeks prior to cold exposure (ETA-F10, Data Sciences International, St. Paul, MN, USA). (B) H&E sections of BAT in 3-month, 6-month, 12-month and 24-month old mice sacrificed after 5 hours of cold exposure. (D) Serum acylcarnitine levels as measured by UPLC-MS/MS from 12-month old littermate controls and BATless mice that express Diphtheria Toxin using the UCP1 promoter. Data are presented as mean ± SEM.

Supplemental Figure 3: The effects of CL-316,243 treatment on brown adipose tissue gene expression and tissue acylcarnitine profiles. (A) Gene expression changes by real-time PCR of enzymes involved in acylcarnitine metabolism in brown adipose tissue (BAT) of mice treated with PBS or CL-316,243. N= 5-7/group. (B) LC-MS/MS measurement of acylcarnitine levels of mice treated with PBS control or CL-316,243 in the BAT and (C) Liver. N = 5-7/group. Data are presented as mean ± SEM. *p ≤ 0.05, **p ≤ 0.01, ***p≤0.001. See also Figure S1.

Supplemental Figure 4: Assessment of HNF4αF/F mice treated with AAV8-TBG-eGFP or AAV8-TBG-Cre during cold exposure. (A) Blood glucose levels in AAV8-TBG-eGFP or AAV8-TBG-Cre infected mice one week after adenoviral administration monitored by glucometer measurement from tail vein bleed. N = 6. (B) RT-PCR of BAT cDNA for thermogenic transcripts and (C) acylcarnitine metabolism transcripts. N = 6. Data are presented as mean ± SEM.

Supplemental Figure 5: Increased acylcarnitine levels in response to the cold. (A) Time course of serum acylcarnitine levels of 3-month old C57BL6J male. Blood was taken by tail vein, serum was extracted by centrifugation, and acylcarnitine levels were measured by LC-MS/MS.

Supplemental Figure 6: Conditional deletion of HNF4α in hepatocytes. (A) Gene expression by real-time PCR in hepatocytes from HNF4αF/F Rosa26LSL-tdTomato mice infected with CMV-eGFP (eGFP) or CMV-eGFP-Cre (Cre) 48 hours post infection. N = 3.

Supplemental Figure 7: Assessment of acylcarnitine levels and thermogenic markers in mice treated with a single bolus of L-carnitine. (A) LC-MS/MS measurement of serum acylcarnitine in 5 month old C57BL6J male mice treated with PBS, 100mg/kg, 250 mg/kg, or 500mg/kg L-carnitine, singly housed with no bedding, and then placed in the
cold for 5 hours. (B) Real-time PCR of brown adipose tissue thermogenic transcripts in 24-month old mice treated with 100mg/kg body weight L-carnitine. N = 5/group.