

Supplemental Information

Endogenous Fatty Acid Synthesis

Drives Brown Adipose Tissue Involution

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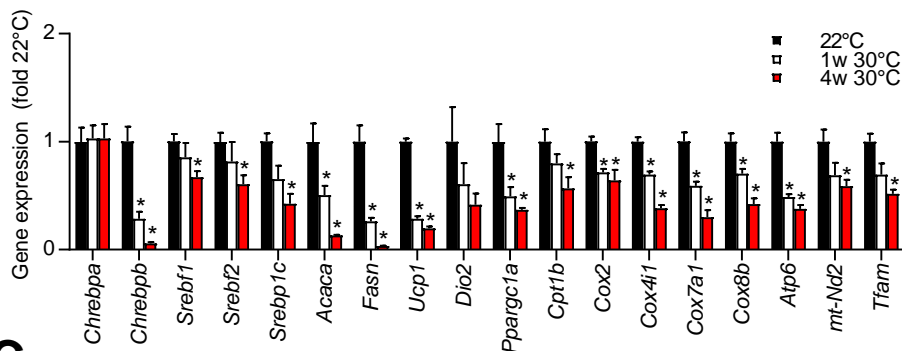
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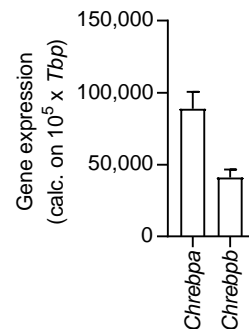
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FigureS1

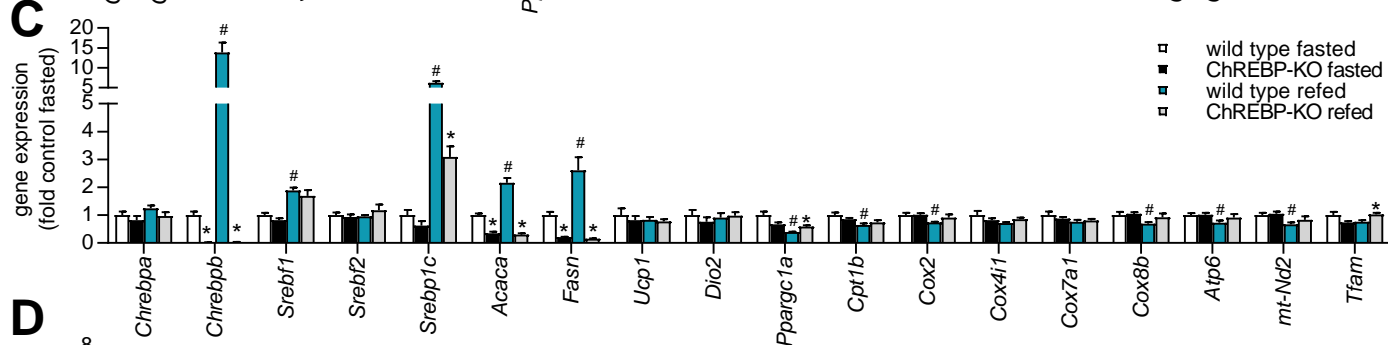
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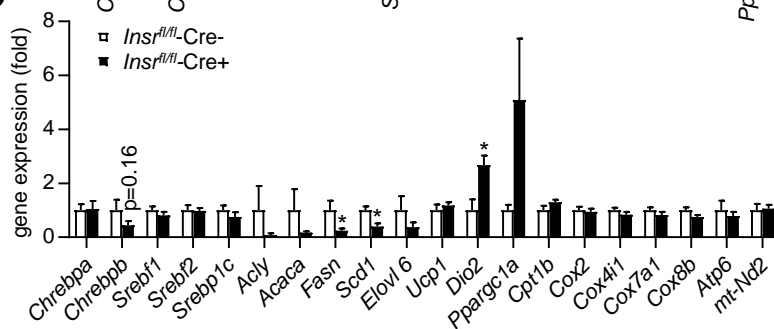
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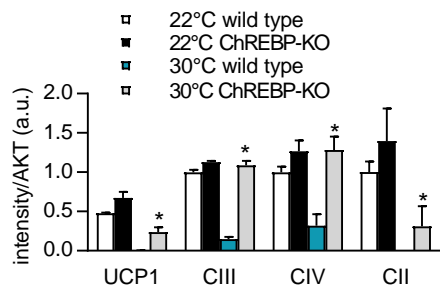
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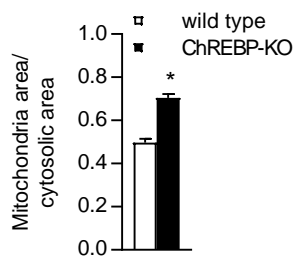
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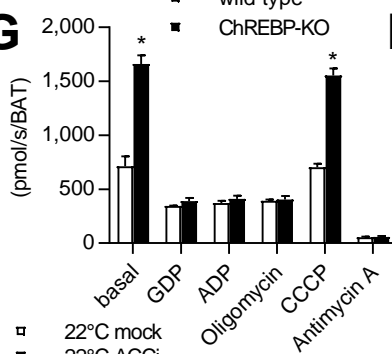
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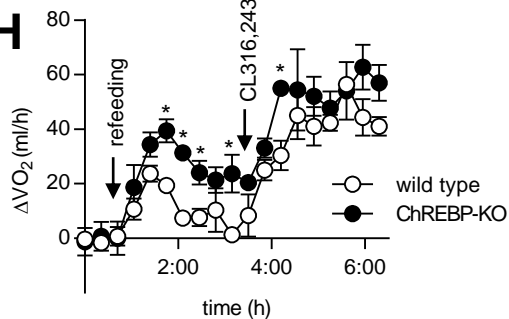
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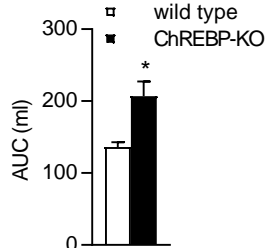
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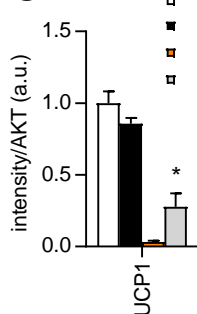
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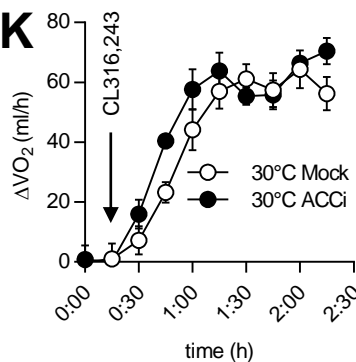
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J



K



L

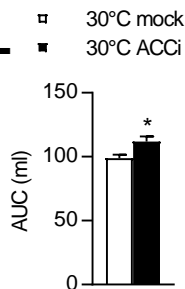


Figure S1. ChREBP controls lipogenesis and thermoneutrality-induced involution of brown adipose tissue (BAT). Related to Figure 1.

(A) Expression *de novo* lipogenesis and thermogenic genes in wild type mice acclimated to 22°C or 30°C for 1 or 4 weeks (n=6).

(B) Absolute gene expression levels per *Tbp* for *Chrebp-alpha* and *Chrebp-beta* in BAT of wildtype mice housed at 22 °C (n=4)

(C) Gene expression in BAT of wild type and ChREBP-KO mice housed at 30°C for 1 week after overnight fasting or 4 hours of refeeding (n=5).

(D) Gene expression in BAT of *Ucp1-ERT2-Cre-IR^{flox/flox}* (*Ins^{fl/fl}*-Cre+) mice and Cre-negative littermates (*Ins^{fl/fl}*-Cre-) housed at 22°C (n=4-6).

(E) Quantification of UCP1/AKT protein levels in BAT of wild type and ChREBP-KO mice from Fig. 1L (n=3).

(F) Quantification of mitochondrial content in thermoneutral BAT of wild type and ChREBP-KO mice, based on EM images in Fig. 1M (n=6).

(G) Oroboros oximetry measurement of mitochondria isolated from thermoneutral BAT of wild type and ChREBP-KO mice (n=3).

(H, I) Indirect calorimetry and quantification of oxygen consumption of wild type and ChREBP-KO mice adapted to thermoneutrality in response to refeeding and subsequent CL316,243 injection (n=3).

(J) Quantification of UCP1/AKT in mice treated with vehicle or ACC inhibitor at 22°C or 30°C as shown in Fig. 1P (n=5).

(K, L) Indirect calorimetry and quantification of oxygen consumption in response to CL316,243 injection in wild type mice treated with vehicle or ACC inhibitor at thermoneutrality (n=3).

Data are presented as means \pm SEM. * indicates $p < 0.05$ by Student's t-test or one-way ANOVA.

Figure S2

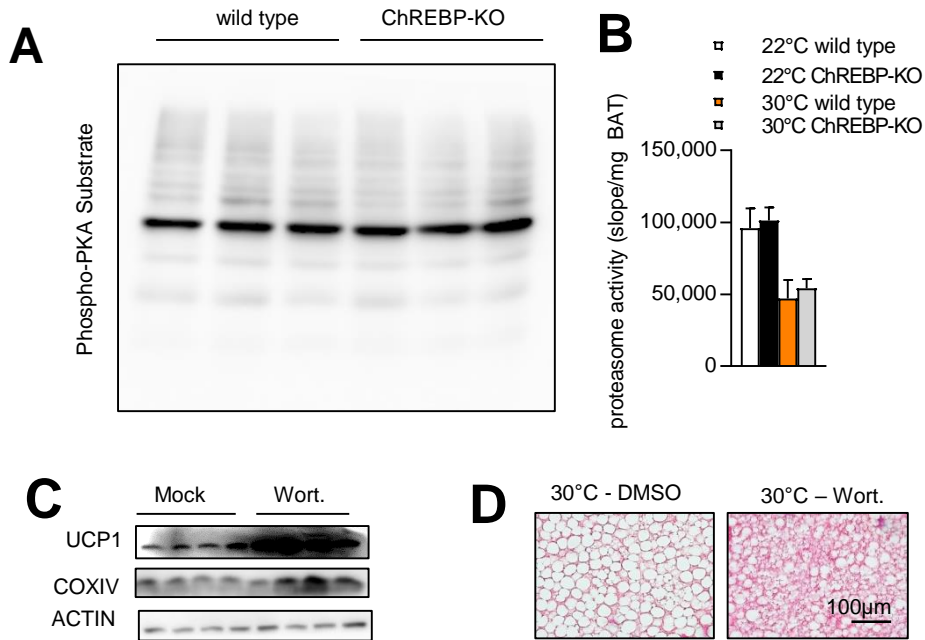


Figure S2. ChREBP deficiency does not alter sympathetic tone or proteasomal activity, while wortmannin prevents BAT whitening. Related to Figure 3.

(A) Western Blot analysis for PKA substrate phosphorylation in BAT of ChREBP-KO mice adapted to 30°C.

(B) Trypsin-like proteasome activity measurement of BAT of wild type or ChREBP-KO mice housed at 22°C or 30°C (n=3-4)

(C) Western Blot analysis for UCP1 and COXIV in thermoneutral BAT of mice treated with vehicle or wortmannin for 1 week (n=4).

(D) Representative H.E. images of BAT isolated from mice treated with vehicle or wortmannin for 1 week (n=4).

Data are presented as means \pm SEM. * indicates $p < 0.05$ by one-way ANOVA

FigureS3

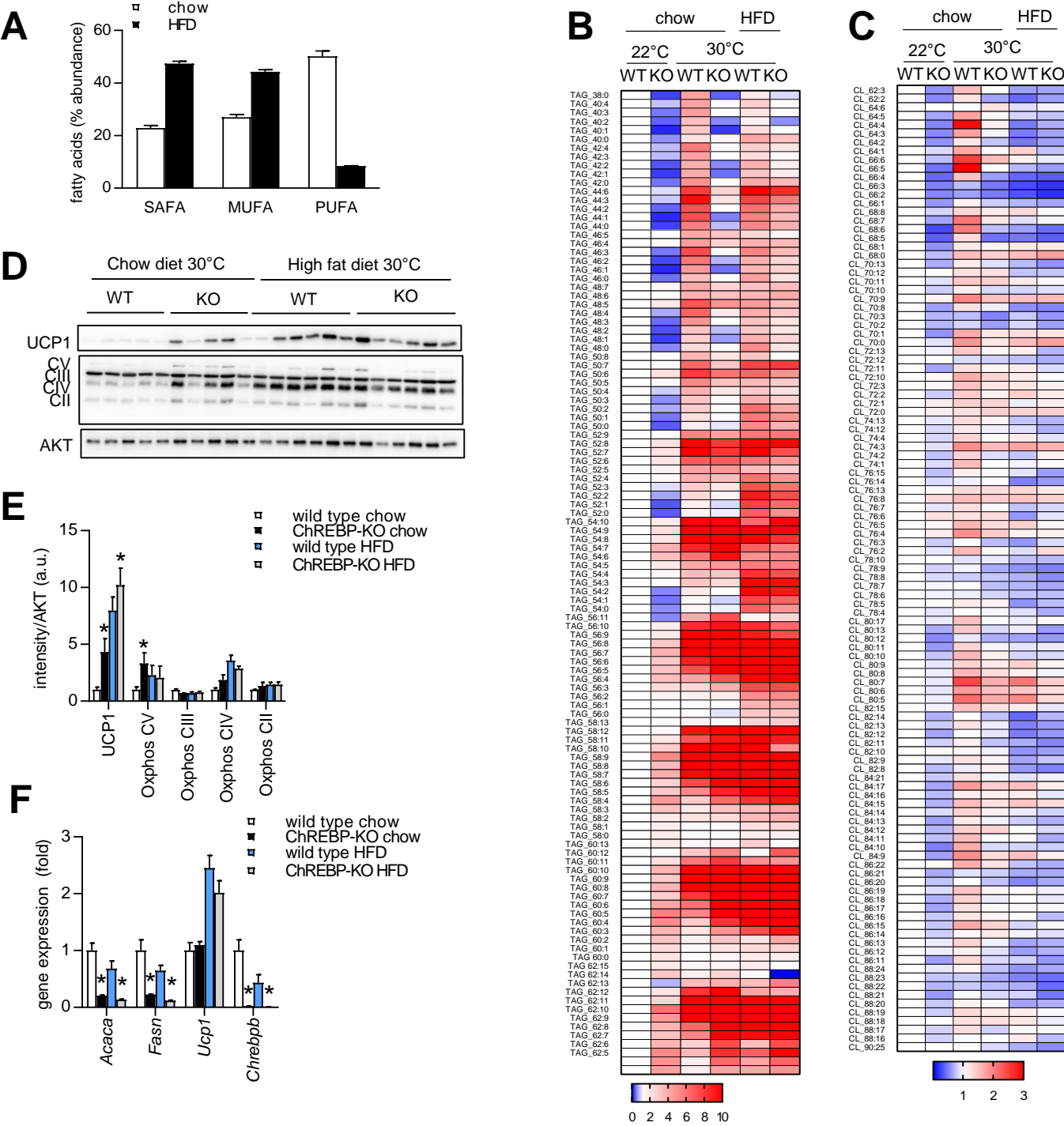


Figure S3. ChREBP-dependent *de novo* lipogenesis mediates remodelling of membrane and storage lipids at thermoneutrality. Related to Figure 4.

(A) Levels of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in diets (n=2).

(B) Heat map of triacylglycerol composition in BAT of wild type and ChREBP-KO mice housed at 22°C (on chow diet) or 30°C (on chow or high fat diet), n=4-7.

(C) Heat map of cardiolipin composition in BAT of wild type and ChREBP-KO mice housed at 22°C (on chow diet) or 30°C (on chow or high fat diet), n=4-7.

(D) Western blot of UCP1 and OXPHOS levels in BAT of wild type and ChREBP-KO mice fed chow or HFD at 30°C (n=5-6).

(E) Quantification of the blot in (D), n=5-6

(F) Gene expression of *Ucp1* and *de novo* lipogenesis genes in BAT of the mice shown in (D, E) (n=4-7).

Data are presented as means \pm SEM. * indicates $p < 0.05$ by two-way ANOVA.

Figure S4

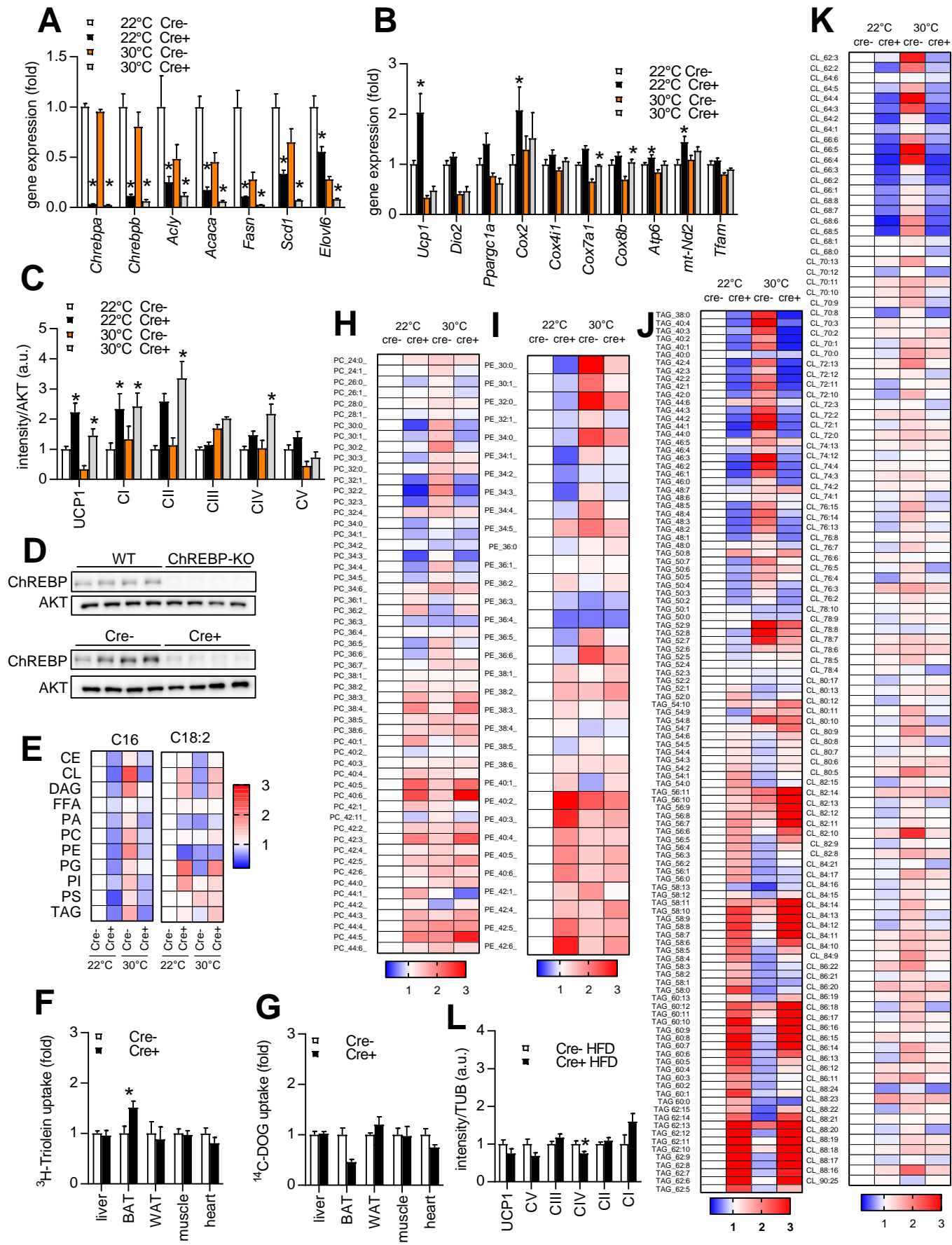


Figure S4. Brown adipocyte-specific knockout reveals cell-autonomous effect of ChREBP on DNL, lipid remodeling and nutrient uptake of BAT. Related to Figure 5.

(A) Expression of ChREBP isoforms and lipogenesis genes in BAT of Cre- and Cre+ mice housed at 22°C or 30°C for 1 week (n=5).

(B) Expression of thermogenesis genes in BAT of Cre- and Cre+ mice housed at 22°C or 30°C for 1 week (n=5).

(C) Quantification of mitochondrial and UCP1 protein levels per AKT in BAT of Cre- and Cre+ mice from Fig. 5E (n=5).

(D) Western blot analysis for ChREBP in BAT of WT and global ChREBP-KO mice (upper panel) as well as Cre- and Cre+ mice (lower panel) acclimated to 30 °C. Note that the observed band represents full length ChREBP-alpha. ChREBP-beta was not detectable.

(E) Heat map of relative levels of lipid classes containing C16 or C18:2 fatty acids in BAT of Cre- or Cre+ mice at 22°C or 30°C. CE, cholesterol esters; CL, cardiolipins; DAG, diacylglycerols; FFA, free fatty acids; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; TAG, triacylglycerol. Data are means of n=4 per group.

(F) ³H-triolein uptake in metabolically active organs of Cre- and Cre+ mice housed at 22°C (n=5-7).

(G) ¹⁴C-DOG uptake in metabolically active organs of the same mice as shown in (E).

(H-K) Heat map of phosphatidylcholine (PC), phosphatidylethanolamine (PE), triacylglycerols (TAG) or cardiolipins (CL) in BAT samples of Cre- or Cre+ mice at 22°C or 30°C. Data are means of n=4.

(L) Protein levels of UCP1 and OXPHOS complexes in BAT of Cre- and Cre+ mice fed HFD at 30°C (n=6).

Data are presented as means ± SEM. * indicates $p < 0.05$ by 2-way ANOVA.