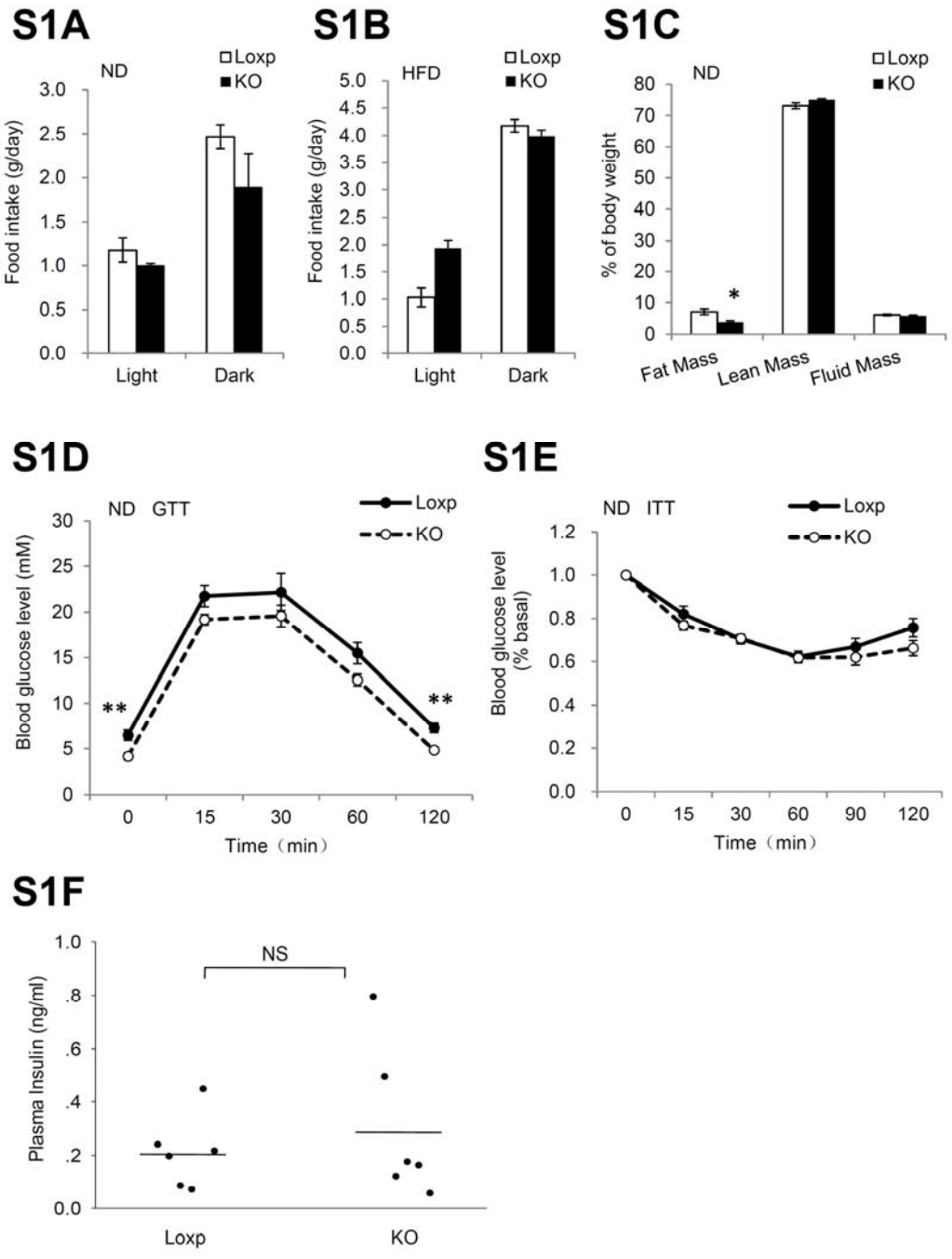
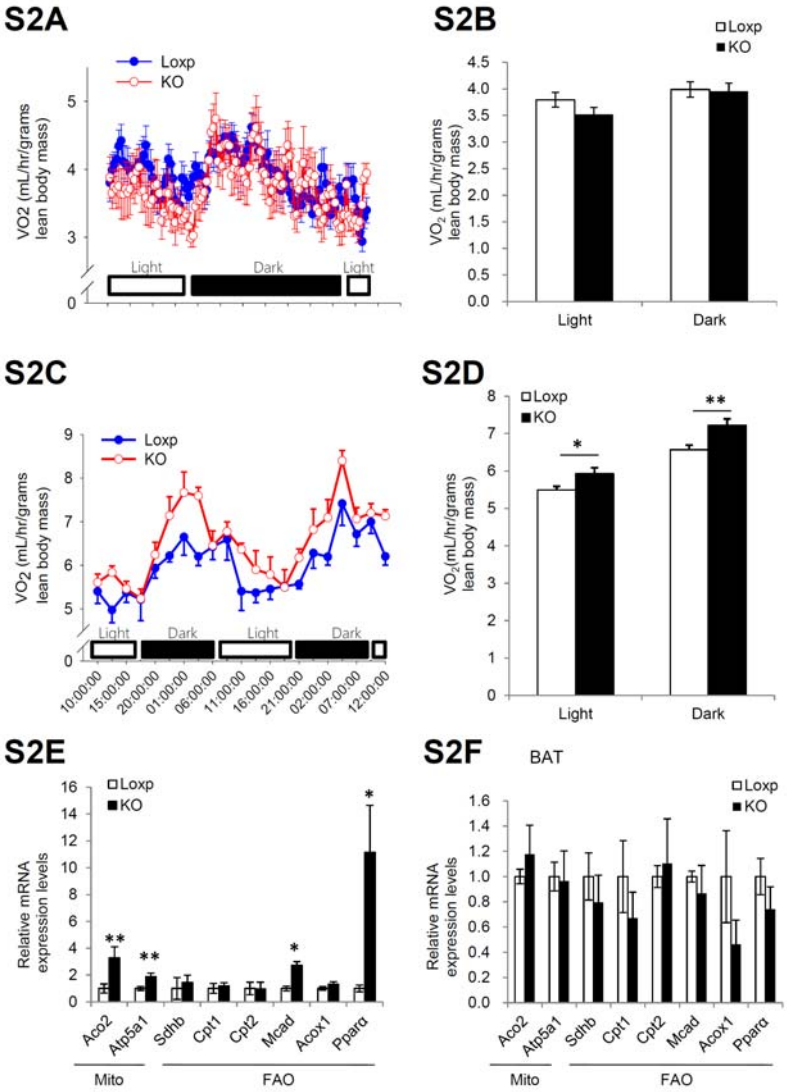
SUPPLEMENTARY DATA

Supplementary Figure 1. Fat-specific knockout of Rheb protects mice against high fat diet-induced obesity. Food intake of Rheb^{fKO} (KO) and Loxp control (L) mice were fed a ND (A) or HFD (B) for 17 weeks. (C) Body composition (n=6/group), glucose tolerance tests (GTT) (D), insulin tolerance tests (ITT) (E) and fasting insulin (F) were performed in KO and Loxp control mice (n=6/group) under ND feeding condition. Data were mean ± S.E.M.



SUPPLEMENTARY DATA

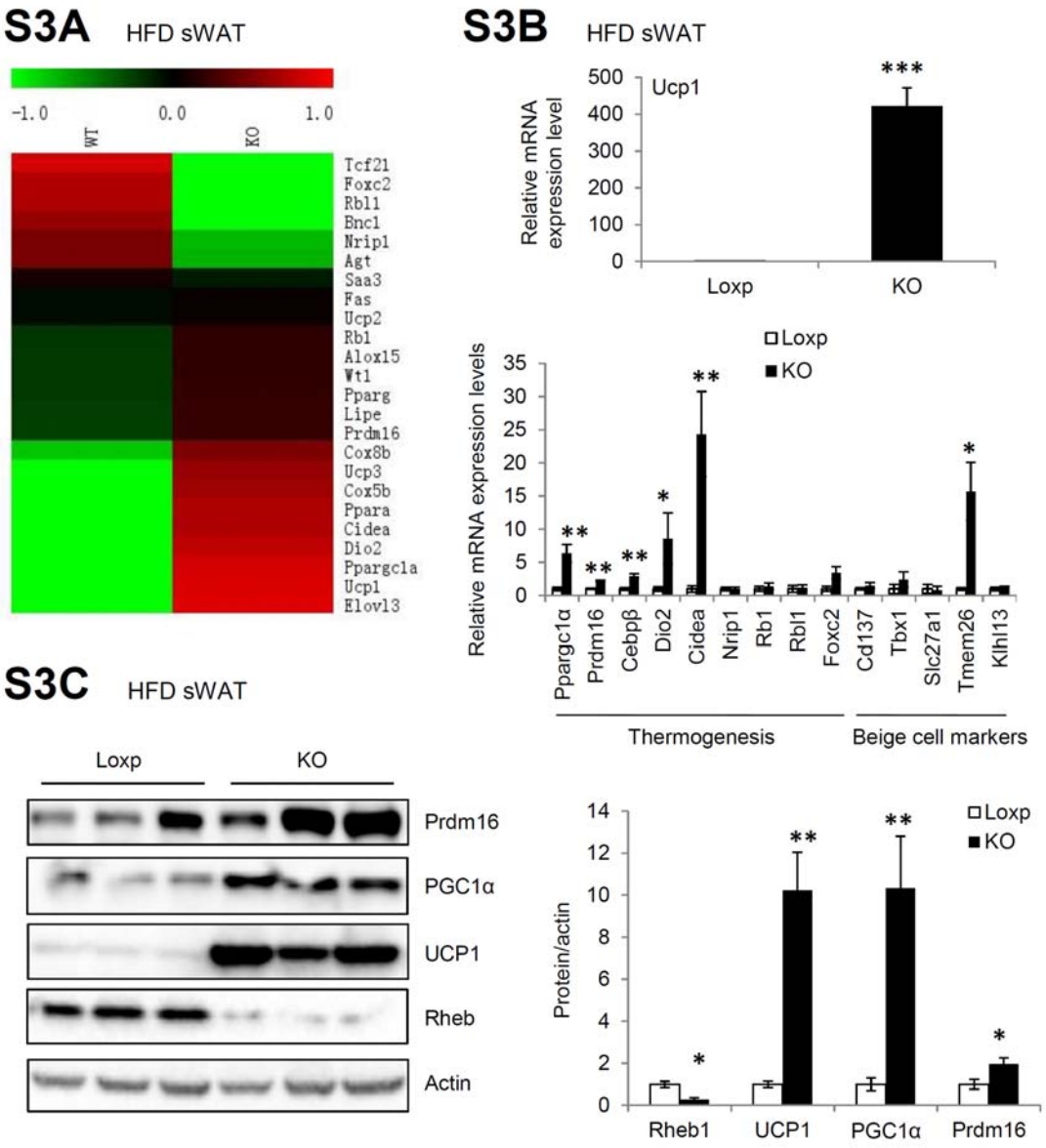
Supplementary Figure 2. Fat-specific knockout of Rheb increases energy expenditure and enhances mitochondrial respiratory chain activity in mice. (A) O_2 consumption of Rheb^{fKO} (KO) and Loxp control mice fed ND at thermoneutrality (~30°C) was measured by indirect calorimetry using CLAMS. (B) Average of O_2 consumption was normalized to lean body mass and analyzed by t test. (C) O2 consumption of Rheb^{fKO} (KO) and Loxp control mice after a 9-week HFD beginning at 8 weeks old(n=6/group) were measured by indirect calorimetry using CLAMS. (D) Average of O_2 consumption was normalized to lean body mass and analyzed by t test. (E) mRNA levels of genes involved in mitochondrial (Mito) and fatty acid metabolism in sWAT of KO and control littermates after a 9-week HFD began at 8 weeks old (n=6/group). Data were normalized to actin and expressed as mean ± S.E.M. *P < 0.05; **p < 0.01 versus control. (F) qRT-PCR analyses of mRNA levels of genes involved in mitochondrial (Mito) and fatty acid metabolism in BAT of 4-month-old KO and Loxp control littermates (n=6/group) fed a normal chow diet (FAO: fatty acid oxidation). Data were normalized to actin and expressed as mean ± S.E.M.



SUPPLEMENTARY DATA

Supplementary Figure 3. Fat-specific knockout of Rheb promotes thermogenic gene expression and beige fat development in mice. (A) Heat map of gene chip assay showing the relative expression of thermogenic genes and randomly selected genes in sWAT from 4-month-old $Rheb^{fKO}$ (KO) and control littermates fed a normal chow diet. Each row depicts an individual gene. Transcript enrichment is encoded in the heat map from low (green) to high (red). (B) mRNA levels of thermogenic and beige marker genes in sWAT of KO and control mice after a 9-week HFD began at 8 weeks old (n = 6/group) were quantified by qRT-PCR, and normalized with actin. Data were mean ± S.E.M. *P < 0.05; **P < 0.01. (C) Western blot analyses of thermogenic proteins in sWAT. (D) Body temperature of 12-week-old KO and Loxp control mice exposed to room temperature (25 °C) on the ND feeding condition (n = 4). (E) Protein levels of phosphorylated S6K and S6 in sWAT of $Rheb^{fKO}$ (KO) and Loxp control mice at 4 °C for 4 hours every day continuously for 4 days. (F) UCP1 protein expression and mTORC1 signaling (P-S6K, P-S6 and P-4EBP1) in sWAT of C57BL/6 mice from cold challenge experiment as indicated time. Data were mean ± S.E.M.

SUPPLEMENTARY DATA



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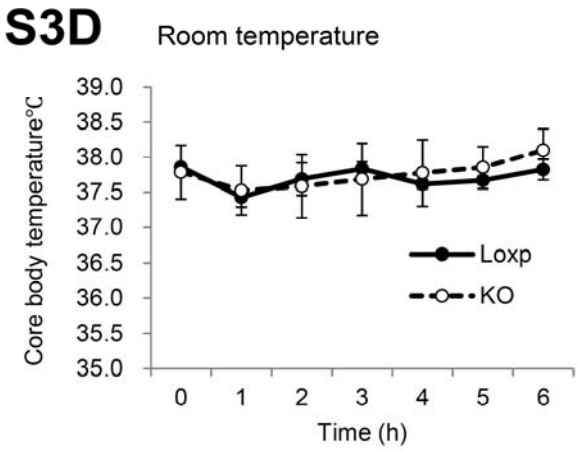
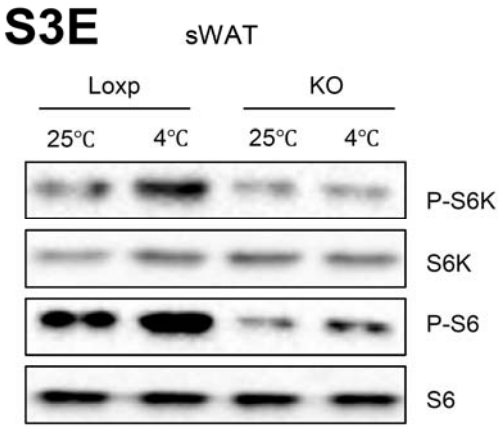
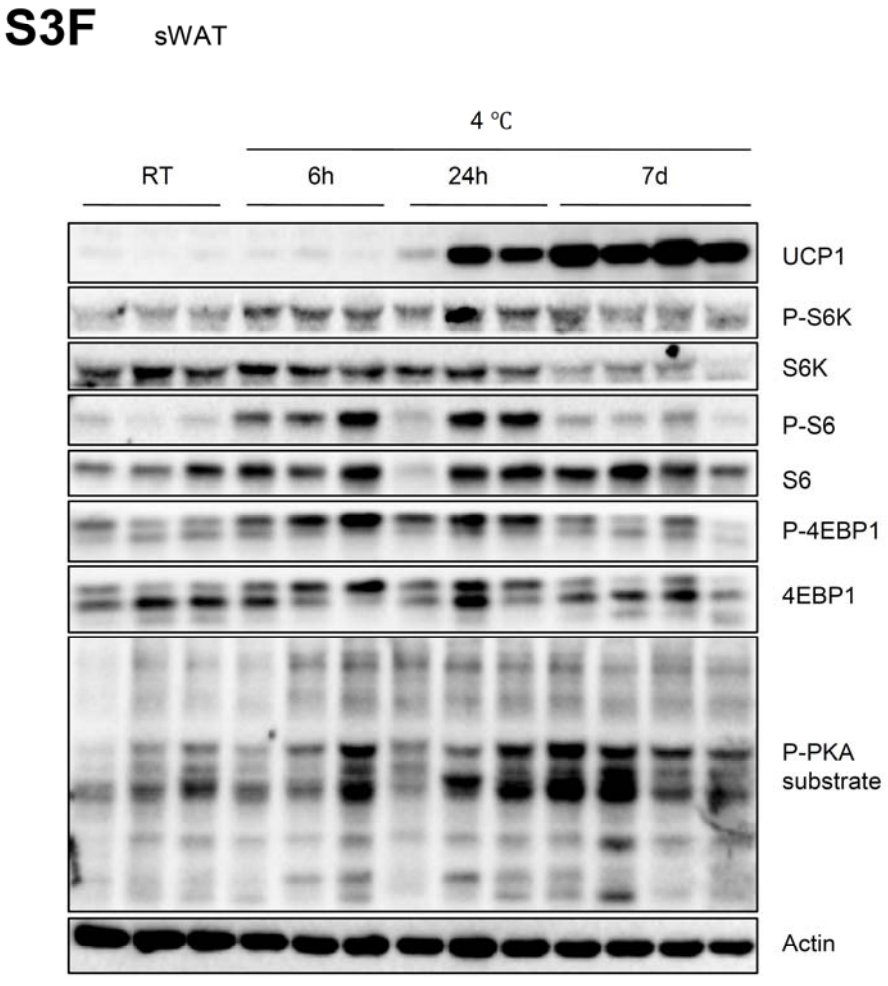
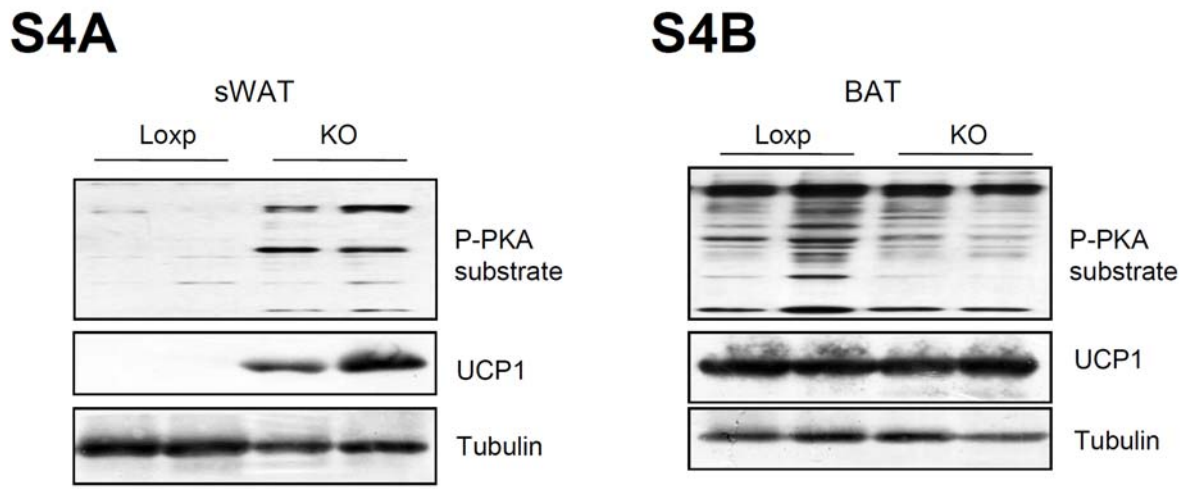






SUPPLEMENTARY DATA

Supplementary Figure 4. Rheb deficiency promotes UCP1 expression via activation of protein kinase A (PKA) pathway in white adipocytes. Protein levels of phosphorylated PKA substrate and UCP1 in sWAT (A) and BAT (B) of Rheb^{fKO} (KO) and Loxp control mice after 17 weeks of normal chow diet.



SUPPLEMENTARY DATA

Supplementary Figure 5. The farnesyl transferase inhibitor FTI-276 inhibits the interaction between Rheb and PDE4D5. (A)The immunoprecipitation (IP) of Rheb and coimmunoprecipitation (coIP) of PDE4D5 in primary subcutaneous white adipocytes were treat with or without the farnesyl transferase inhibitor FTI-276 (FTI) (5μM) for 24 hrs. Data were representative of three independent experiments each with a similar result.