Supplementary Information

Supplementary information includes 7 supplementary figures and 4 supplementary tables.

Supplementary Figure 1. Related to Figure 1. (**A**) Immunoblots of Lrpprc and Hsp90 in various tissues from ~8-week-old male CON and Lrpprc^{BKO} mice at RT. (**B**) Q-PCR analysis of mRNA levels of *Lrpprc* in BAT, iWAT and eWAT from ~8-week-old CON and Lrpprc^{BKO} mice at RT. (**C**) Q-PCR analysis of relative mRNA levels of *Lrpprc, Ucp1, Pgc1α, Cidea, Cox8b*, *Dio2* and Pgc1α in BAT from ~8-week-old male CON and Lrpprc^{BKO} mice at RT. Sample size: CON (n=4) and Lrpprc^{BKO} (n=4). (**D**) Left: Immunoblots of phosphor-PKA substrates, Lrpprc, Ucp1 and Hsp90 in BAT from ~8-week-old CON and Lrpprc^{BKO} mice at RT. Right: Relative abundance (to Hsp90) shown. Significance between genotypes indicated. (**E**) Relative mtDNA copy numbers in BAT from ~8-week-old CON and Lrpprc^{BKO} mice at RT. Sample size: CON (n=5) and Lrpprc^{BKO} (n=5). **(F)** Representative H&E staining of BAT from ~8-12-week of male CON (*Lrpprc$^{f/f}$*) and Lrpprc^{BKO} (*Ucp1-Cre;Lrpprc$^{f/f}$*) mice housed at room temperature (RT) and thermoneutrality (30°C). Scale bar: 50μm. (**G**) Frequency of mitochondrial and non-mitochondrial proteins identified by mass spectrometry from isolated mitochondria of ~8-10-week-old male CON and Lrpprc^{BKO} mice housed at RT and 30°C. Sample size: n=3 for each condition (CON/RT, CON/30°C, Lrpprc^{BKO}/RT and Lrpprc^{BKO}/30°C). (**H**) Volcano plots showing significantly ($p<0.1$) down- or up-regulated mitochondrial proteins (over 1.5-fold) in Lrpprc^{BKO} mice at RT and 30°C. (**I**) Principal component analysis of mitochondrial proteome in the four genotype/conditions. (**J**) Heatmap of Log2 FC values of the complex IV protein abundances measured by mass spectrometry in BAT mitochondria from CON and Lrpprc^{BKO} mice housed at RT and 30°C. mt-unit: mitochondrion-encoded subunit, n-unit: nucleus-encoded subunit, n-af: nucleus-encoded assembly factor. (**K**) Gene Ontology (GO) analysis showing enriched cellular components and molecular functions in mitochondrial proteome. (**L**) Clustering analysis of mitochondrial proteome using fold changes of BAT mitochondrial protein abundance from ~8-12-week-old male Lkb1^{BKO}, Tfam^{BKO} and Lrpprc^{BKO} (to their relative controls) on normal chow at both RT and 30ºC. (**M**) Lists of proteins that are both upregulated or downregulated in the BAT from Tfam^{BKO} and Lrpprc^{BKO} mice. (**N**) Recordings of respiratory exchange ratio (RER) in ~8-12-week-old male CON and Lrpprc^{BKO} mice at RT and 30°C. Red arrowhead: time of CL injection. Average night and day EE (**O**), RER (**P**) and physical activity (**Q**) in the aforementioned mice. Average night and day food intake (**R**), 3-day cumulative food intake curve (**S**) and

total cumulative food intake (**T**) in the aforementioned mice. Sample size: CON/RT (n=6), Lrpprc^{BKO}/RT (n=6), CON/30°C (n=6) and Lrpprc^{BKO}/30°C (n=5). Data were presented as average ± SEM. Unpaired t-test. *: $p<0.05$ and **: $p<0.01$.

Supplementary Figure 2. Related to Figure 1. (**A**) The mRNA levels of thermogenic genes (*Ucp1, Cidea* and *Cox8b*) in iWAT from ~8-week- and ~8-month-old male CON and Lrpprc^{BKO} mice at normal chow at RT. Sample size: CON/8w (n=8), Lrpprc1^{BKO}/8w (n=8), CON/8m (n=6), and Lrpprc1^{BKO}/8m (n=6). (**B**) Left: Immunoblots showing phospho-PKA substates, Ucp1 and Hsp90 levels in ~8-week-old male CON and Lrpprc^{BKO} mice at RT. Right: Relative Upc1 protein abundance (to Hsp90) shown. Significance between genotypes indicated. (**C**) Representative H&E staining of iWAT from male CON and Lrpprc^{BKO} mice at ~8-week- and ~8-month of age. Red arrows: multilocular beige adipocytes. Scale bar: 100μm. (**D**) The mRNA levels of thermogenic genes (*Ucp1, Cidea* and *Cox8b*) in iWAT from ~10-week-old male CON and Lrpprc^{BKO} mice at normal chow at 30ºC. Sample size: CON/30ºC (n=7) and Lrpprc1^{BKO}/30ºC (n=6). (**E**) Representative H&E staining of iWAT from male CON and Lrpprc^{BKO} mice at ~10-week of age at 30ºC. Scale bar: 100μm. (**F**) Q-PCR analysis of *Ucp1, Ryr2* and *Atp2a2* (calcium cycle), *Slc6a8, Ckmt1, Ckmt3, Gatm* and *Gatmt* (Creatine cycle) in the iWAT of ~8-week-old male CON and Lrpprc^{BKO} mice at RT. Sample size: CON (n=6) and Lrpprc1^{BKO} (n=6). (**G**) Representative H&E staining of eWAT from male CON and Lrpprc^{BKO} mice at ~8-week of age at RT. Scale bar: 50μm. (**H**) Q-PCR analysis of *Ucp1, Ryr2* and *Atp2a2* (calcium cycle), *Slc6a8, Ckmt1, Ckmt3, Gatm* and *Gatmt* (Creatine cycle) in the eWAT of ~8-week-old male CON and Lrpprc^{BKO} mice at RT. Sample size: CON (n=4) and Lrpprc1^{BKO} (n=4). (**I**) Diagram of experimental design for beige adipocyte ablation. All mice were injected with diphtheria toxin (DT) at 3-week of age. Genotypes of CON, Lrpprc^{BKO} and Lrpprc^{BKO};iDTR mice shown. Q-PCR analysis of *Ucp1* in the iWAT (**J**) and *Lrpprc* and *mt-Co1* in the BAT (**K**) from ~8-10-week-old male and female CON, Lrpprc^{BKO} and Lrpprc^{BKO};iDTR mice. (**L**) CTT of ~8-10-week-old male and female CON, Lrpprc^{BKO} and Lrpprc^{BKO};iDTR mice. Sample size: CON (n=4), Lrpprc^{BKO} (n=3) and Lrpprc^{BKO};iDTR (n=3). (**M**) Two areas of interest were selected for temperature measurement by Infrared (IR) thermal imaging; one was the neck (overlaying the BAT) and other one was the back (not overlaying the BAT). (**N**) Experimental design. IR imaging of mice before and after they were transferred from thermoneutrality to RT for 15 minutes. (**O**) Representative IR images of control mice before and after transferring from 30°C to RT under feeding condition. White cycle: area of interest. (**P**) Before-after plot of core body temperature

(measured by probe), neck and back temperatures (measured by IR) of ~10-week-old male wild-type mice at either feeding (with or without Propranolol injection) or after 4-hour fasting. (**Q**) Calculation of Temp (Neck-Back), the difference between neck and back surface temperatures by IR under aforementioned conditions. Sample size: n=4. (**R**) Temp (Neck-Back) of ~10-week-old wild-type mice 15 minutes after 1 mg kg^{-1} CL (or vehicle PBS) injection under feeding or after 4-hour fasting at thermoneutrality. Sample size: n=7 at feeding and n=5 after fasting. (**S**) Temp (Neck-Back) of ~10-week-old male and female CON and $Lrpprc^{BKO}$ mice before and after 15-minute RT stimulation from thermoneutrality under feeding condition. (**T**) Temp (Neck-Back) of ~10-week-old male and female CON and $Lrpprc^{BKO}$ mice after 4-hour fasting at thermoneutrality. Sample size: CON (n=6) and $Lrpprc^{BKO}$ (n=9). (**U**) Temp (Neck-Back) of ~8-week-old male and female CON and $Lrpprc^{BKO}$ mice housed at RT. Sample size: CON (n=7) and $Lrpprc^{BKO}$ (n=11). (**V**) Left: Representative sagittal views of fused PET and CT, and PET-only showing ^{18}F-FDG uptake in BAT from CON and $Lrpprc^{BKO}$ mice. Color map showed at the bottom. Right: Average BAT ^{18}F-FDG uptake in CON and $Lrpprc^{BKO}$ mice. Sample size: CON (n=9) and $Lrpprc^{BKO}$ (n=8). (**W**) The rate of serum glycerol increases in response to 1mg kg^{-1} CL316,423 injection (indicator of *in vivo* lipolytic activity) in ~8-12-week-old male CON and $Lrpprc^{BKO}$ mice under normal chow at RT. Sample size: CON (n=5) and $Lrpprc^{BKO}$ (n=5). (**X**) The rate of glycerol release in the media in response to 10mM Forskolin (indicator of *ex vivo* lipolytic activity) from BAT tissue of ~8-12-week-old male CON and $Lrpprc^{BKO}$ mice under normal chow at RT and 30°C. (**Y**) Left: Immunoblots showing amounts of Hsl, Atgl and Hsp90 in the BAT of ~8-12-week-old male CON and $Lrpprc^{BKO}$ mice under normal chow at both RT and 30ºC. Right: Relative abundance (to Hsp90) shown. (**Z**) Serum triglyceride levels in ~12-week-old male and female wild-type mice 2 hours after DMSO or 25 mg kg^{-1} Orlistat injection at thermoneutrality. Sample size: DMSO (n=6) and Orlistat (n=6). (**AA**) Blood glucose levels in ~12-week-old male and female wild-type mice 2 hours after DMSO or 10 mg kg^{-1} Sotagliflozin injection at thermoneutrality. Sample size: DMSO (n=8) and Sotagliflozin (n=8). Data were presented as average ± SEM. Unpaired t-test. *: $p<0.05$ and **: $p<0.01$.

Supplementary Figure 3. Related to Figure 2. (**A**) Heatmap showing cluster analysis of DEGs in the BAT of male CON and Lrpprc^{BKO} mice at RT and 30°C. (**B**) Volcano plots showing significantly ($p<0.05$) down- or up-regulated genes in the BAT of Lrpprc^{BKO} mice at RT and 30°C. (**C**) Venn diagram showing down-regulated DEGs in the BAT of Lrpprc^{BKO} mice at RT and 30°C. (**D**) GO analysis of shared down-regulated DEGs. (**E**) KEGG mmu00190 Oxidative phosphorylation pathway. Down-regulated genes were highlighted in blue. The mtDNA-encoded ETC subunits were labeled by a red asterisk. (**F**) Venn diagram showing up-regulated DEGs in the BAT of Lrpprc^{BKO} mice at RT and thermoneutrality. (**G**) GO analysis of shared up-regulated DEGs. (**H**) Left: Immunoblots showing amounts of p-eIF2α, total eIF2α, and Hsp90 in the BAT of ~8-12-week-old male Tfam^{BKO} mice compared with their relative controls at normal chow at both RT and 30ºC. Right: Relative phosphorylation (to total protein) shown. Significance between genotypes indicated. (**I**) Immunoblots showing amounts of p-eIF2α, total eIF2α, and Hsp90 in the BAT of ~8-12-week-old male Lrpprc^{BKO} and betaless mice compared with their relative controls at normal chow at both RT and 30ºC. (**J**) Circulating Fgf21 levels in ~8-12-week-old male CON and Lrpprc^{BKO} mice at RT and 30ºC. Sample size: CON/RT (n=4), Lrpprc^{BKO}/RT (n=6), CON/30°C (n=5) and Lrpprc^{BKO}/30°C (n=5). (**K**) Representative H&E staining of BAT from ~8-12-week of male CON and Atf4^{BKO} mice housed at RT and 30°C. Scale bar: 50μm. (**L**) Heatmap showing log2 fold changes of mtDNA- and nuclear-encoded ETC genes, known ATF4 target genes in the BAT of CON and Atf4^{BKO} mice housed at RT and 30°C. Hourly CL-induced VO2 (**M**) and EE (**N**) in ~8-12-week old male CON and ATF4^{BOX} mice at RT and 30°C. Average night and day EE (**O**), RER (**P**) and physical activity (**Q**) in the aforementioned mice. Average night and day food intake (**R**), 3-day cumulative food intake curve (**S**) and total cumulative food intake (**T**) in the aforementioned mice. Sample size: CON/RT (n=4), Atf4^{BKO}/RT (n=3), CON/30°C (n=6) and Atf4^{BKO}/30°C (n=6). Average night and day EE (**U**), RER (**V**) and physical activity (**W**) in ~10-week old male CON, Lrpprc^{BKO} and Lrpprc;Atf4^{BKO} mice at RT and 30°C. Average night and day food intake (**X**), 3-day cumulative food intake curve (**Y**) and total cumulative food intake (**Z**) in the aforementioned mice. Sample size: CON/RT(n=8), Lrpprc^{BKO}/RT (n=3), Lrpprc;Atf4^{BKO}/RT(n=6), CON/30°C(n=11), Lrpprc^{BKO}/30°C (n=6) and

1751 Lrpprc;Atf4^{BKO}/30°C (n=6). Data were presented as average ± SEM. Unpaired t-test. n.s.:
1752 not significant, *: $p<0.05$, and **: $p<0.01$.
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Supplementary Figure 4. Related to Figure 3. Average night and day EE (**A**) and hourly CL-induced EE (**B**) and CL-induced VO2 (**C**) in ~8-10-week-old male CON and ATF4^{BOX} mice at RT and 30°C. Average night and day RER (**D**) and physical activity (**E**) in ~8-10-week-old male CON and ATF4^{BOX} mice for three days at RT and 30°C. Average night and day food intake (**F**), 3-day cumulative food intake curve (**G**) and total cumulative food intake (**H**) in above mice. Sample size: CON/RT (n=10), ATF4^{BOX}/RT (n=4), CON/30°C (n=10) and ATF4^{BOX}/30°C (n=5). Average night and day EE (**I**) and physical activity (**J**) in ~8-10-week-old male Ucp1 KO and ATF4^{BOX};Ucp1 KO mice at RT. Sample size: Ucp1 KO/RT (n=5) and ATF4^{BOX};Ucp1 KO/RT (n=4). Average night and day food intake (**K**), 3-day cumulative food intake curve (**L**) and total cumulative food intake (**M**) in ~8-10-week-old male Ucp1 KO and ATF4^{BOX};Ucp1 KO mice at RT. Sample size: Ucp1 KO/RT (n=5) and ATF4^{BOX};Ucp1 KO/RT (n=3). Average night and day EE (**N**) and physical activity (**O**) in ~12-week-old female Ucp1 KO and ATF4^{BOX};Ucp1 KO mice at 30°C. Sample size: Ucp1 KO/30°C (n=5) and ATF4^{BOX};Ucp1 KO/30°C (n=5). Average night and day food intake (**P**), 3-day cumulative food intake curve (**Q**) and total cumulative food intake (**R**) in ~12-week-old female Ucp1 KO and ATF4^{BOX};Ucp1 KO mice at 30°C. Sample size: Ucp1 KO/30°C (n=4) and ATF4^{BOX};Ucp1 KO/30°C (n=5). Data were presented as average ± SEM.

Supplementary Figure 5. Related to Figure 4. (**A**) Q-PCR analysis of *Slc3a2* mRNA levels in the BAT of ~10-week-old male CON and Lrpprc^{BKO} mice housed at RT and 30ºC. Sample size: n=5 for each genotype/condition. (**B**) Left: Immunoblots of Slc3a2 and Actin in the BAT of ~10-week-old male CON and Lrpprc^{BKO} mice at *ad libitum* feeding at RT and 30ºC. Right: Relative abundance (to Actin) shown. Significance between genotypes indicated. (**C**) Heatmap showing log2 fold changes of mRNA levels of *Slc3a2, Slc7a1, Slc7a11* and *Slc38a2* in the BAT of Lrpprc^{BKO}, Tfam^{BKO}, ATF4^{BOX} and ATF4^{BOX};Ucp1KO mice. Sample size: n=5 for each genotype/condition. Left: Immunoblots of Slc3a2 and Actin proteins in the BAT of Tfam^{BKO} (**D**), ATF4^{BOX} (**F**) and ATF4^{BOX};Ucp1KO (**F**) mice and their relative controls at different ambient temperatures. Right: Relative abundance (to Actin) shown. Significance between genotypes indicated. (**G**) Top: Representative sagittal views of fused PET and CT, and PET-only showing ^{18}F-Fluciclovine uptake in BAT from12-week-old male CON and Lrpprc^{BKO} mice. Color map showed at the right. Bottom: Uptake curves in BAT from CON and Lrpprc^{BKO} mice over time. Sample size: CON (n=9) and Lrpprc^{BKO} (n=8). (**H**) Average values of 18F-Fluciclovine uptake in ~10-12-week-old male CON and Lrpprc;Atf4^{BKO} mice. Sample size: CON (n=5) and Lrpprc;Atf4^{BKO} (n=4). (**I**) Left: Immunoblots of p-eIF2α, total eIF2α, p-S6, total S6, p-4Ebp1, total 4Ebp1, puromycylated protein, ubiquitinated protein and Hsp90 in the BAT of ~10-week-old male CON and Lrpprc^{BKO} mice at *ad libitum* feeding at RT and 30°C. Right: Relative abundance (to Hsp90) or phosphorylation (to total protein) shown. Significance between genotypes indicated. (**J**) Left: Immunoblots of Lrpprc, p-eIF2α, total eIF2α, Slc3a2, p-S6, total S6, p-4Ebp1, total 4Ebp1, puromycylated protein, ubiquitinated protein and Hsp90 in the BAT of ~10-week-old male CON, Lrpprc^{BKO} and Lrpprc;Atf4^{BKO} mice at *ad libitum* feeding at RT. Right: Relative abundance (to Hsp90) or phosphorylation (to total protein) shown. Significance between genotypes indicated. (**K**) Left: Immunoblots of Lrpprc, p-eIF2α, total eIF2α, Slc3a2, p-S6, total S6, p-4Ebp1, total 4Ebp1, puromycylated protein, ubiquitinated protein and Hsp90 in the BAT of ~10-week-old male CON, Lrpprc^{BKO} and Lrpprc;Atf4^{BKO} mice at *ad libitum* feeding at 30ºC. Right: Relative abundance (to Hsp90) or phosphorylation (to total protein) shown. Significance between genotypes indicated. (**L**) Left: Immunoblots of p-S6, total S6, p-4Ebp1, total 4Ebp1, puromycylated protein, ubiquitinated protein and Hsp90 in the BAT of ~10-week-old male

Tfam^{BKO} mice at *ad libitum* feeding at RT and 30ºC. Right: Relative abundance (to Hsp90) or phosphorylation (to total protein) shown. Significance between genotypes indicated. (**M**) Left: Immunoblots of Ucp1, p-S6, total S6, p-4Ebp1, total 4Ebp1, puromycylated protein, ubiquitinated protein and Hsp90 in the BAT of ~10-week-old male CON, ATF4^{BOX}, Ucp1 KO, ATF4^{BOX};Ucp1 KO mice at *ad libitum* feeding at RT. Right: Relative abundance (to Hsp90) or phosphorylation (to total protein) shown. Significance between genotypes indicated. (**N**) Left: Immunoblots of p-S6, total S6, p-4Ebp1, total 4Ebp1, puromycylated protein, ubiquitinated protein and Hsp90 in the BAT of ~10-week-old male CON and Lrpprc^{BKO} mice at *ad libitum* feeding at RT with DMSO or BCH treatment. Right: Relative abundance (to Hsp90) or phosphorylation (to total protein) shown. Significance between genotypes and treatment indicated. (**O**) Left: Immunoblots of p-S6, total S6, p-4Ebp1, total 4Ebp1, puromycylated protein, ubiquitinated protein and Hsp90 in the BAT of ~10-week-old male CON and ATF4^{BOX} mice at *ad libitum* feeding at 30ºC with DMSO or RAPA treatment. Right: Relative abundance (to Hsp90) or phosphorylation (to total protein) shown. Significance between genotypes and treatment indicated. (**P**) Left: Immunoblots of p-S6, total S6, p-4Ebp1, total 4Ebp1, puromycylated protein, ubiquitinated protein and Hsp90 in the muscle of ~10-week-old male CON and ATF4^{BOX} mice at *ad libitum* feeding at 30ºC with DMSO or RAPA treatment. Right: Relative abundance (to Hsp90) or phosphorylation (to total protein) shown. (**Q**) Left: Immunoblots of Lrpprc, Lc3b (I and II) and Hsp90 in the BAT from ~8-12-week-old male CON and Lrpprc^{BKO} mice at *ad libitum* feeding at RT and 30°C. Right: Relative abundance (to Hsp90) or Lc3b processing (ratio of Lc3b II and Lc3b I) shown. Significance between genotypes indicated. (**R**) Heatmap showing log2 fold changes of mRNA levels of *Nfe2l1, Psma1, Psmb1, Psmd1, Atg5, Atg7, Beclin, Lc3b* and *Ulk1* in the BAT of Lrpprc^{BKO} and ATF4^{BOX} mice at RT and 30°C. Sample size: n=5 for each genotype/condition. (**S**) Core temperature drop of ~8-week-old male and female CON and Lrpprc^{BKO} mice after 8-hour CTT from RT. DMSO or αMT was injected 1 hour prior to CTT. Sample size: CON/DMSO (n=5), Lrpprc^{BKO}/DMSO (n=6), CON/αMT (n=5) and Lrpprc^{BKO}/αMT (n=5). (**T**) Core temperature drop of ~10-week-old male and female Tfam^{BKO} mice and their relative controls with pretreatment of DMSO or RAPA or BORT or BCH after 8 hours 4ºC CTT from RT. Sample size: CON/RT/DMSO (n=4), Tfam^{BKO}/RT/DMSO (n=4), CON/RT/RAPA (n=4), Tfam^{BKO}/RT/RAPA (n=5),

CON/RT/BORT (n=3), Tfam^{BKO}/RT/BORT (n=6), CON/RT/BCH (n=5) and Tfam^{BKO}/RT/BCH (n=5). (**U**) Core temperature drop of ~8-week-old male and female CON and Gnas^{BKO} mice after 8-hour CTT from RT. DMSO or RAPA or BORT was injected 1 hour prior to CTT. Sample size: CON/DMSO (n=5), Gnas^{BKO}/DMSO (n=7), CON/RAPA (n=3), Gnas^{BKO}/RAPA (n=6), CON/BORT (n=5) and Gnas^{BKO}/BORT (n=5). (**V**) Lactate concentration in the media from BAT of CON and Lrpprc^{BKO} mice after DMSO or 20mM BCH treatment for 24 hours. Sample size: CON (n=5) and Lrpprc^{BKO} (n=5). (**W**) Lactate concentration in the media from BAT of CON and Lrpprc;Atf4^{BKO} mice. Sample size: CON (n=6) and Lrpprc;Atf4^{BKO} (n=5). (**X**) Lactate concentration in the media from BAT of CON and ATF4^{BOX} mice after DMSO or 20mM BCH treatment for 24 hours. Sample size: CON (n=5) and ATF4^{BOX} (n=5). Data were presented as average ± SEM. Unpaired t-test. n.s.: non-significant; **: $p<0.01$. (**Y**) Heatmaps showing log2 FC of ATF4 targets (top), mt ETC (middle) and nuclear ETC (bottom) caused by ATF4 overexpression in WT and Ucp1 KO BAs. (**Z**) Volcano plots showing the changes of H/L ratio between Cre- and GFP-infected BAs (X axis) and -log10 p value (Y axis) after 24h, 48h and 72h of heavy labeling. Grey box: proteins with higher turnover after ATF4-overexpression. (**AA**) Network analysis of 92 proteins with increased turnover rate at 24h, 48h and 72h timepoints in https://www.networkanalyst.ca/. Most enriched biological processes, numbers of hits and adjusted p values (AdjP) shown.

Supplementary Figure 6. Related to Figure 5. Average night and day EE (**A**) and regression plot of average EE as a function of body weight (**B**) in CP or LP-fed mice at 30°C. Red arrow: EE increase. Average night and day RER (**C**) and physical activity (**D**) in ~12-week-old male C57bl/6J mice after 4-week CP or LP feeding at 30°C. Sample size: CP (n=9) and LP (n=9). Average night and day food intake (**E**), 3-day cumulative food intake curve (**F**) and total cumulative food intake (**G**) in ~12-week-old male C57bl/6J mice after 4-week CP or LP feeding at 30°C. Sample size: CP (n=8) and LP (n=8). Q-PCR analysis of mt-encoded ETC (**H**) and nuclear-encoded ETC genes (**I**) in the BAT of ~12-week-old male C57bl/6J mice after 4-week CP or LP feeding at 30°C. Sample size: CP (n=5) and LP (n=5). (**J**) Q-PCR analysis of ATF4 target genes in the muscle of ~12-week-old male C57bl/6J mice after 4-week CP or LP feeding at 30°C. Sample size: CP (n=5) and LP (n=5). (**K**) Immunoblots of puromycylated protein, ubiquitinated protein and Hsp90 in the muscle of ~12-week-old male C57bl/6J mice at *ad libitum* CP or LP feeding at 30°C. Average night and day EE (**L**) and regression plot of average EE as a function of body weight (**M**) in CP or LP-fed ~12-week-old male CON and $Atf4^{BKO}$ mice at 30°C. Red arrow: EE increase. Average night and day RER (**N**) and physical activity (**O**) in CP or LP-fed ~12-week-old male CON and $Atf4^{BKO}$ mice at 30°C. Sample size: CON-CP (n=7), CON-LP (n=8), $Atf4^{BKO}$-CP (n=8) and $Atf4^{BKO}$-LP (n=7). Average night and day food intake (**P**), 3-day cumulative food intake curve (**Q**) and total cumulative food intake (**R**) in ~12-week-old male C57bl/6J mice after 4-week CP or LP feeding at 30°C. Sample size: CON-CP (n=7), CON-LP (n=8), $Atf4^{BKO}$-CP (n=7) and $Atf4^{BKO}$-LP (n=6). Q-PCR analysis of mtDNA-encoded ETC genes (**S**) and nuclear-encoded ETC genes (**T**) in the BAT of the aforementioned mice. Sample size: CON-CP (n=6), CON-LP (n=6), $Atf4^{BKO}$-CP (n=5) and $Atf4^{BKO}$-LP (n=5). (**U**) Circulating Fgf21 levels in CP or LP-fed ~12-week-old male CON and $Atf4^{BKO}$ mice at 30°C. Sample size: CON-CP (n=4), CON-LP (n=4), $Atf4^{BKO}$-CP (n=5) and $Atf4^{BKO}$-LP (n=5). Data were presented as average ± SEM. Unpaired t-test. *: $p<0.05$ and **: $p<0.01$.

Supplementary Figure 7. Related to Figure 6. (**A**) Body weight of male CON and Lrpprc^{BKO} mice at RT and 30°C fed with normal chow (NC). (**B**) Body weight, lean mass, fat mass, and fat percentage of ~8-month-old male CON and Lrpprc^{BKO} mice at RT and 30°C. (**C**) Tissue mass of BAT, iWAT, and eWAT of ~8-month-old male CON and Lrpprc^{BKO} mice. Sample size: CON/RT (n=8), Lrpprc^{BKO}/RT (n=10), CON/30ºC (n=7) and Lrpprc^{BKO}/30ºC (n=5). (**D**) Heatmap showing log2 fold changes of known ATF4 target genes in the BAT of Lrpprc^{BKO} mice after HFD at both RT and 30ºC. Sample size: CON/RT/HFD (n=8), Lrpprc^{BKO}/RT/HFD (n=7), CON/30°C/HFD (n=4) and Lrpprc^{BKO}/30°C/HFD (n=6). (**E**) Tissue mass of eWAT, iWAT, and BAT of male CON and Lrpprc^{BKO} mice at normal chow (NC) and after HFD. Sample size: male CON/NC/RT (n=8), Lrpprc^{BKO}/NC/RT (n=10), CON/4w-HFD/RT (n=8), Lrpprc^{BKO}/4w-HFD/RT (n=6), CON/12w-HFD/RT (n=5), Lrpprc^{BKO}/12w-HFD/RT (n=5), CON/8w-HFD/30°C (n=4), Lrpprc^{BKO}/8w-HFD/30°C (n=6), CON/12w-HFD/30°C (n=6) and Lrpprc^{BKO}/12w-HFD/30°C (n=13). (**F**) Fat percentage, lean and fat mass of male CON and Lrpprc^{BKO} mice before and after 4-week and 12-week HFD at RT and 30ºC. Sample size: CON/NC/RT (n=3), Lrpprc^{BKO}/NC/RT (n=7), CON/4w-HFD/RT (n=10), Lrpprc^{BKO}/4w-HFD/RT (n=9), CON/12w-HFD/RT (n=6), Lrpprc^{BKO}/12w-HFD/RT (n=5), CON/NC/30ºC (n=7), Lrpprc^{BKO}/NC/30ºC (n=6), CON/4w-HFD/30ºC (n=6), Lrpprc^{BKO}/4w-HFD/30ºC (n=8), CON/12w-HFD/30ºC (n=6) and Lrpprc^{BKO}/12w-HFD/30ºC (n=13). (**G**) Representative H&E staining of eWAT from male CON and Lrpprc^{BKO} mice after 12-week HFD. Scale bar: 100 μm. (**H**) Adipocyte size distribution in eWAT from male CON and Lrpprc^{BKO} mice after 12-week HFD. Total adipocytes counted: CON/RT (n=200), Lrpprc^{BKO}/RT (n=519), CON/30ºC (n=347) and Lrpprc^{BKO}/30ºC (n=666). (**I**) Serum leptin levels in male CON and Lrpprc^{BKO} mice after 12-week. Sample size: CON/RT (n=5), Lrpprc^{BKO}/RT (n=5), CON/30ºC (n=4) and Lrpprc^{BKO}/30ºC (n=8). (**J**) Q-PCR analysis of mRNA levels of macrophage markers (*Cd68, Adgre1* and *Cd11c*) and pro-inflammatory cytokine (*Ccl2*) in eWAT of male CON and Lrpprc^{BKO} mice after 12-week HFD at RT and 30°C. Sample size: CON/RT (n=7), Lrpprc^{BKO}/RT (n=7), CON/30ºC (n=8) and Lrpprc^{BKO}/30ºC (n=8). (**K**) Body weight of female CON and Lrpprc^{BKO} mice after 12-week HFD at RT and 30°C. Sample size: CON/RT (n=8), Lrpprc^{BKO}/RT (n=7), CON/30°C (n=11) and Lrpprc^{BKO}/30°C (n=17). (**L**) Body weight, lean mass, fat mass, and fat percentage of female CON and

Lrpprc^{BKO} mice after 12-week HFD. Sample size: CON/RT (n=8), Lrpprc^{BKO}/RT (n=7), CON/30°C (n=8) and Lrpprc^{BKO}/30°C (n=11). (**M**) Tissue mass of eWAT, iWAT, and BAT of female CON and Lrpprc^{BKO} mice before and after HFD**.** Sample size: CON/RT (n=8), Lrpprc^{BKO}/RT (n=7), CON/30°C (n=18) and Lrpprc^{BKO}/30°C (n=17). (**N**) Liver triglyceride contents of female CON and Lrpprc^{BKO} mice after 12-week HFD. Sample size: CON/RT (n=8), Lrpprc^{BKO}/RT (n=7), CON/30°C (n=8) and Lrpprc^{BKO}/30°C (n=11). Average night and day EE (**O**) and regression plots of average EE (**P**) as a function of body weight in ~12-week-old male CON and Lrpprc^{BKO} mice after 4-week HFD at 30°C. Average night and day RER (**Q**) and regression plots of average RER (**R**) as a function of body weight in the aforementioned mice. Average night and day physical activity (**S**) and regression plots of average physical activity (**T**) as a function of body weight in the aforementioned mice. Average night and day food intake (**U**) and regression plots of average food intake (**V**) as a function of body weight in the aforementioned mice. Sample size: CON/30°C (n=4) and Lrpprc^{BKO}/3°0C (n=7). (**W**) Body weight of male CON and Atf4^{BKO} mice under 12-week HFD. Sample size: CON/RT (n=10), Atf4^{BKO}/RT (n=10), CON/30°C (n=13) and Atf4^{BKO}/30°C (n=13). (**X**) Body weight, lean mass, fat mass, and fat percentage of male CON and Atf4^{BKO} mice under 12-week HFD. (**Y**) Tissue mass of BAT, iWAT, and eWAT of male CON and Atf4^{BKO} mice under 12-week HFD. (**Z**) Serum glucose levels during ITT in male CON and Atf4^{BKO} mice after 12-week HFD. (**AA**) Area under the curve (AUC) values of glucose levels in ITTs showed. Sample size: CON/RT (n=5), Atf4^{BKO}/RT (n=8), CON/30°C (n=16) and Atf4^{BKO}/30°C (n=8). Serum insulin (**AB**)**,** serum triglyceride contents (**AC**) and liver triglyceride contents (**AD**) of male CON and Atf4^{BKO} mice after HFD**.** Sample size: CON/RT (n=5), Atf4^{BKO}/RT (n=8), CON/30°C (n=8) and Atf4^{BKO}/30°C (n=8). (**AE**) Fat tissue mass of male CON, Lrpprc^{BKO} and Lrpprc;Atf4^{BKO} mice after 12-week HFD at RT. Sample size: CON (n=10), Lrpprc^{BKO} (n=5), and Lrpprc;Atf4^{BKO} (n=5). (**AF**) Representative H&E staining of eWAT from male CON, Lrpprc^{BKO} and Lrpprc;Atf4^{BKO} mice after 12-week HFD. Scale bar: 50μm. (**AG**) Adipocyte size distribution in eWAT from male CON, Lrpprc^{BKO} and Lrpprc;Atf4^{BKO} mice after 12-week HFD. Total adipocytes counted: CON (n=1020), Lrpprc^{BKO} (n=935), and Lrpprc;Atf4^{BKO} (n=1133). (**AH**) Fat tissue mass of male CON, Lrpprc^{BKO} and Lrpprc;Atf4^{BKO} mice after 12-week HFD at 30°C. Sample size: CON (n=9), Lrpprc^{BKO} (n=6), and Lrpprc;Atf4^{BKO}

(n=5). (**AI**) Representative H&E staining of eWAT from male CON, Lrpprc^{BKO} and Lrpprc;Atf4^{BKO} mice after 12-week HFD. Scale bar: 50μm. (**AJ**) Adipocyte size distribution in eWAT from male CON, Lrpprc^{BKO} and Lrpprc;Atf4^{BKO} mice after 12-week HFD. Total adipocytes counted: CON (n=681), Lrpprc^{BKO} (n=983), and Lrpprc;Atf4^{BKO} (n=945). (**AK**) Heatmap showing log2 fold changes of known ATF4 target genes in the BAT of ATF4^{BOX} mice after 12-week HFD at both RT and 30ºC. Sample size: CON/RT (n=8), ATF4^{BOX}/RT (n=7), CON/30°C (n=4) and ATF4^{BOX}/30°C (n=6). (**AL**) Adipocyte size distribution in eWAT from male CON and ATF4^{BOX} mice after 12-week HFD. Total adipocytes counted: CON/RT (n=2647), ATF4^{BOX}/RT (n=2312), CON/30ºC (n=1868) and ATF4^{BOX}/30ºC (n=2650). (**AM**) Q-PCR analysis of mRNA levels of macrophage markers (*Cd68, Adgre1* and *Cd11c*) and pro-inflammatory cytokine (*Ccl2*) in eWAT of male CON and ATF4^{BOX} mice after 12-week HFD. Sample size: CON/RT (n=8), ATF4^{BOX}/RT (n=8), CON/30°C (n=7) and ATF4^{BOX}/30°C (n=5). (**AN**) Body weight of female CON and ATF4^{BOX} mice after 12-week HFD at RT and 30°C. Sample size: CON/RT (n=7), ATF4^{BOX}/RT (n=8), CON/30°C (n=5) and ATF4^{BOX} /30°C (n=6). (**AO**) Tissue mass of eWAT, iWAT, and BAT of female CON and ATF4^{BOX} mice before and after HFD**.** Sample size: CON/RT (n=6), ATF4^{BOX}/RT (n=8), CON/30°C (n=5) and ATF4^{BOX}/30°C (n=5). (**AP**) Liver triglyceride contents of female CON and ATF4^{BOX} mice after 12-week HFD. Sample size: CON/RT (n=7), ATF4^{BOX}/RT (n=8), CON/30°C (n=5) and ATF4^{BOX} /30°C (n=5). (**AQ**) Representative H&E staining of iWAT from male CON, Lrpprc^{BKO}, Atf4^{BKO}, Lrpprc;Atf4^{BKO} and ATF4^{BOX} mice after 12-week HFD at RT and 30°C. Red arrows: multilocular beige adipocytes. Scale bar: 50μm. (**AR**) Heatmap showing fold change values (Log2FC) of thermogenic genes in iWAT of Lrppc;Atf4^{BKO}, ATF4^{BOX} and Atf4^{BKO} mice after 12-week HFD at RT. Data were presented as average ± SEM. Unpaired t-test. *: $p<0.05$ and **: $p<0.01$.

Supplementary Table 1. (separate EXCEL file)

Excel table of mass spectrometry data of mitochondrial proteome from CON and Lrpprc^{BKO} mice at RT and 30ºC.

Supplementary Table 2. (separate EXCEL file)

Excel table of mass spectrometry data of pSILAC in GFP- and Cre-infected brown adipocytes in wild-type and Ucp1 deficient background.

Supplementary Table 3. Composition of diets

	CP (control diet)		LP (low-protein diet)	
	g%	**kcal%**	**g%**	**kcal%**
Protein	23.4	24.9	4.7	5
Carbohydrate	59.9	62.1	78.6	82
Fat	5.4	13	5.4	13
Total kcal/g	3.76		3.76	
Ingredient	**g**	**kcal**	**g**	**kcal**
Casein	250	1000	50	200
L-Cystine	3.8	15.2	1.05	4.2
Corn Starch	261	1044	463.75	1855
Maltodextrin 10	110	440	110	440
Dextrose	244	976	244	976
Cellulose, BW200	75	0	75	0
Inulin	25	37.5	25	37.5
Soybean Oil	59	531	59	531
Mineral Mix S10026	10	0	10	0
Dicalcium Phosphate	13	0	13	0
Calcium Carbonate	5.5	0	5.5	0
Potassium Citrate	16.5	0	16.5	0
Vitamin Mix V10001	10	40	10	40
Choline Bitartrate	2	0	2	0
Total	1084.8	4084	1084.8	4084

Supplementary Table 4: List of primer sequences for q-PCR

36B4-F	TTTGGGCATCACCACGAAAA
36B4-R	GGACACCCTCCAGAAAGCGA
Lrpprc-F	AGCCTGCTCCTGTGAGAAAG
Lrpprc-R	TCCCAGATCTTGTGAGCAAA
mt-Nd4-F	CTAATAATCGCACATGGCCTC
mt-Nd4-R	CGTAGTTGGAGTTTGCTAGG
mt-Nd5-F	CATCCTTCTCAACTTTACTGGG
mt-Nd5-R	TTTATGGGTGTAATGCGGT
mt-Cyb-F	CCATTCTACGCTCAATCCCCA
mt-Cyb-R	AGGCTTCGTTGCTTTGAGGTA
mt-Co1-F	ACACAACTTTCTTTGATCCCG
mt-Co1-R	AGAATCAGAACAGATGCTGG
mt-Co2-F	ATAATCCCAACAAACGACCT
mt-Co2-R	CTCGGTTATCAACTTCTAGCA
mt-Co3-F	GGTATAATTCTATTCATCGTCTCGG
mt-Co3-R	AGAACGCTCAGAAGAATCCT
mt-Atp8-F	GGCACCTTCACCAAAATCACT
mt-Atp8-R	GGGGTAATGAATGAGGCAAATAGA
mt-Atp6-F	CCTTCAATCCTATTCCCATCC
mt-Atp6-R	GTTGGAAAGAATGGAGACGG
Ndufs1-F	AGGATATGTTCGCACAACTGG
Ndufs1-R	TCATGGTAACAGAATCGAGGGA
Ndufs4-F	CTGCCGTTTCCGTCTGTAGAG
Ndufs4-R	TGTTATTGCGAGCAGGAACAAA
Ndufs8-F	AGTGGCGGCAACGTACAAG
Ndufs8-R	TCGAAAGAGGTAACTTAGGGTCA
Sdha-F	GGAACACTCCAAAAACAGACCT
Sdha-R	CCACCACTGGGTATTGAGTAGAA
Sdhb-F	AATTTGCCATTTACCGATGGGA
Sdhb-R	AGCATCCAACACCATAGGTCC

Sdhc-F	GCTGCGTTCTTGCTGAGACA
Sdhc-R	ATCTCCTCCTTAGCTGTGGTT
Sdhd-F	TGGTCAGACCCGCTTATGTG
Sdhd-R	GGTCCAGTGGAGAGATGCAG
Cyc1-F	CAGCTTCCATTGCGGACAC
Cyc1-R	GGCACTCACGGCAGAATGAA
Cox4-F	ATGTCACGATGCTGTCTGCC
Cox4-R	GTGCCCCTGTTCATCTCGGC
Cox4i1-F	ATTGGCAAGAGAGCCATTTCTAC
Cox4i1-R	CACGCCGATCAGCGTAAGT
Cox5a-F	GGGTCACACGAGACAGATGA
Cox5a-R	CCAAGATGCGAACAGCACTA
Cox5b-F	GATGAGGAGCAGGCTACTGG
Cox5b-R	TGCAGCCCACTATTCTCTTG
Cox6b1-F	CCCCAACCAGAACCAGACTA
Cox6b1-R	GATCTTCCCAGGAAATGTGC
Atp5a1-F	TCTCCATGCCTCTAACACTCG
Atp5a1-R	CCAGGTCAACAGACGTGTCAG
Atp5j2-F	TGCCGAGCTGGATAATGATGC
Atp5j2-R	ACCATGCTAATCCCCGAGATG
Atp5b-F	GCAAGGCAGGGACAGCAGA
Atp5b-R	CCCAAGGTCTCAGGACCAACA
Gdf15-F	CTGGCAATGCCTGAACAACG
Gdf15-R	GGTCGGGACTTGGTTCTGAG
Fgf21-F	GTGTCAAAGCCTCTAGGTTTCTT
Fgf21-R	GGTACACATTGTAACCGTCCTC
Ddit3-F	CTGCCTTTCACCTTGGAGAC
Ddit3-R	GGACGCAGGGTCAAGAGTTAG
Trib3-F	GGGGCCTTATATCCTTTTGG
Trib3-R	GCAGGGTACACCTTGCAG
Atf5-F	CCTTGCCCTTGCCACCTTTGAC

Atf5-R	CCAGAGGAGGAGGCTGCTGT
Atf3-F	GCTGCCAAGTGTCGAAACAAG
Atf3-R	CAGTTTTCCAATGGCTTCAGG
Lonp1-F	TGAGCTGCAAGATGTTCTGG
Lonp1-R	AGCCCCAATTCCTTCTTGAT
Cyb5r1-F	CTACCTCTCTGCCCGAATTG
Cyb5r1-R	CCCAATCTTCAGGCTATCCA
Aldh1l2-F	CACCCCTGTGATTGAGGACT
Aldh1l2-R	GCCTCTTCGTCCTCTCCTCT
Psat1-F	TGCTCGAAATGACTCACAGG
Psat1-R	CAGCACTCCTTCCAGCTTTC
Atf4-F	AAGGAGGAAGACACTCCCTCT
Atf4-R	CAGGTGGGTCATAAGGTTTGG
Mthdf2-F	AGGTCCCAAGCCTTTGAGTT
Mthfd2-R	GTAAGGGAGTGCCGTTGAAA
Pck2-F	ATGGCTGCTATGTACCTCCC
Pck2-R	GCGCCACAAAGTCTCGAAC
Dhrs9-F	TACCTCCTCGGTGAACTTGG
Dhrs9-R	TGGGATTTGCCAGCTCTACT
Mmp28-F	GAGGCGTAAGAAACGCTTTG
Mmp28-R	CCAGAACTCCAGTGCTGACA
Bmal1-F	TGACCCTCATGGAAGGTTAGAA
Bmal1-R	GGACATTGCATTGCATGTTGG
Ucp1-F	ACTGCCACACCTCCAGTCATT
Ucp1-R	CTTTGCCTCACTCAGGATTGG
Cox8b-F	GAACCATGAAGCCAACGACT
Cox8b-R	GCGAAGTTCACAGTGGTTCC
Cidea-F	TGCTCTTCTGTATCGCCCAGT
Cidea-R	GCCGTGTTAAGGAATCTGCTG
Dio2-F	CAGTGTGGTGCACGTCTCCAATC
Dio2-R	TGAACCAAAGTTGACCACCAG

Pgc1a-F	AGCCGTGACCACTGACAACGAG
Pgc1a-R	GCTGCATGGTTCTGAGTGCTAAG
Cd68-F	GCAGCACAGTGGACATTCAT
Cd68-R	TTGCATTTCCACAGCAGAAG
Adgre1-F	TTTCCTCGCCTGCTTCTTC
Adgre1-R	CCCCGTCTCTGTATTCAACC
Cd11c-F	CAGAACTTCCCAACTGCACA
Cd11c-R	TCTCTGAAGCTGGCTCATCA
Ccl2-F	CTTCTGGGCCTGCTGTTCA
Ccl2-R	CCAGCCTACTCATTGGGATCA
Gyk-F	TTCCAGGAAATAATAACTTTGTCAAG
Gyk-R	CACTGCACTGAAATACGTGCT
Ldhb-F	ACAAGTGGGTATGGCATGTG
Ldhb-R	ACATCCACCAGGGCAAGTT
Acaa2-F	CCTCAGTTCTTGTCTGTTCAG
Acaa2-R	AGGTGTGCGGTGATTCTG
Plin5-F	GTGAATCGAGTGGTGGCTTT
Plin5-R	CAGGGCACAGGTAGTCACAC
Hoxc9-F	GCAGCAAGCACAAAGAGGAGAAG
Hoxc9-R	GCGTCTGGTACTTGGTGTAGGG
Prdm16-F	TGGCCTTCATCACCTCTCTGAA
Prdm16-R	TTTCTGATCCACGGCTCCTGTGA
Ryr2-F	ATGGCTTTAAGGCAGAGCG
Ryr2-R	CAGAGCCCGAATCATCCAGC
Atp2a2-F	ACCTTTGCCGCTCATTTTCCAG
Atp2a2-R	AGGCTGCACACACTCTTTACC
Slc6a8-F	TGCATATCTCCAAGGTGGCAG
Slc6a8-F	CTACAAACTGGCTGTCCAGA
Ckmt1-F	TGAGGAGACCTATGAGGTATTTGC
Ckmt1-R	TCATCAAAGTAGCCAGAACGG
Ckmt2-F	GCATGGTGGCTGGTGATGAG

Ckmt2-R	AAACTGCCCGTGAGTAATCTTG
Gatm-F	GACCTGGTCTTGTGCTCTCC
Gatm-R	GGGATGACTGGTGTTGGAGG
Gatmt-F	GCAGCCACATAAGGTTGTTCC
Gatmt-R	CTCTTCAGACAGCGGGTACG
Slc3a2-F	TGCTCAGGCTGACATTGTAGC
Slc3a2-R	TCAGCCAAGTACAAGGGTGC
Slc7a1-F	AATTATCATCTTAACAGGACTG
Slc7a1-R	GACCAGGACATTGATACA
Slc7a11-F	TGGAACTGCTCGTAATAC
Slc7a11-R	GTTCAGGAATTTCACATTGA
Slc38a2-F	GACACAGTAAGTGAGTGACG
Slc38a2-R	CTCTCTTTGGATACCTGACC
Nfe2l1-F	GACAAGATCATCAACCTGCCTGTAG
Nfe2l1-R	GCTCACTTCCTCCGGTCCTTTG
Psma1-F	TGCGTGCGTTTTTGATTTTAGAC
Psma1-R	CCCTCAGGGCAGGATTCATC
Psmb1-F	CGTTGAAGGCATAAGGCGAAAA
Psmb1-R	TTCCACTGCTGCTTACCGAG
Psmd1-F	GTGATAAAACACTTTCGAGGCCA
Psmd1-R	TGAATGCAGTCGTGAATGACTT
Atg5-F	TAGAATATATCAGACCACGACG
Atg5-R	CTCCTCTTCTCTCCATCTTC
Atg7-F	TCCGTTGAAGTCCTCTGCTT
Atg7-R	CCACTGAGGTTCACCATCCT
Beclin-F	GGCCAATAAGATGGGTCTGA
Beclin-R	GCTGCACACAGTCCAGAAAA
Lc3b-F	ACAAAGAGTGGAAGATGTCCGGCT
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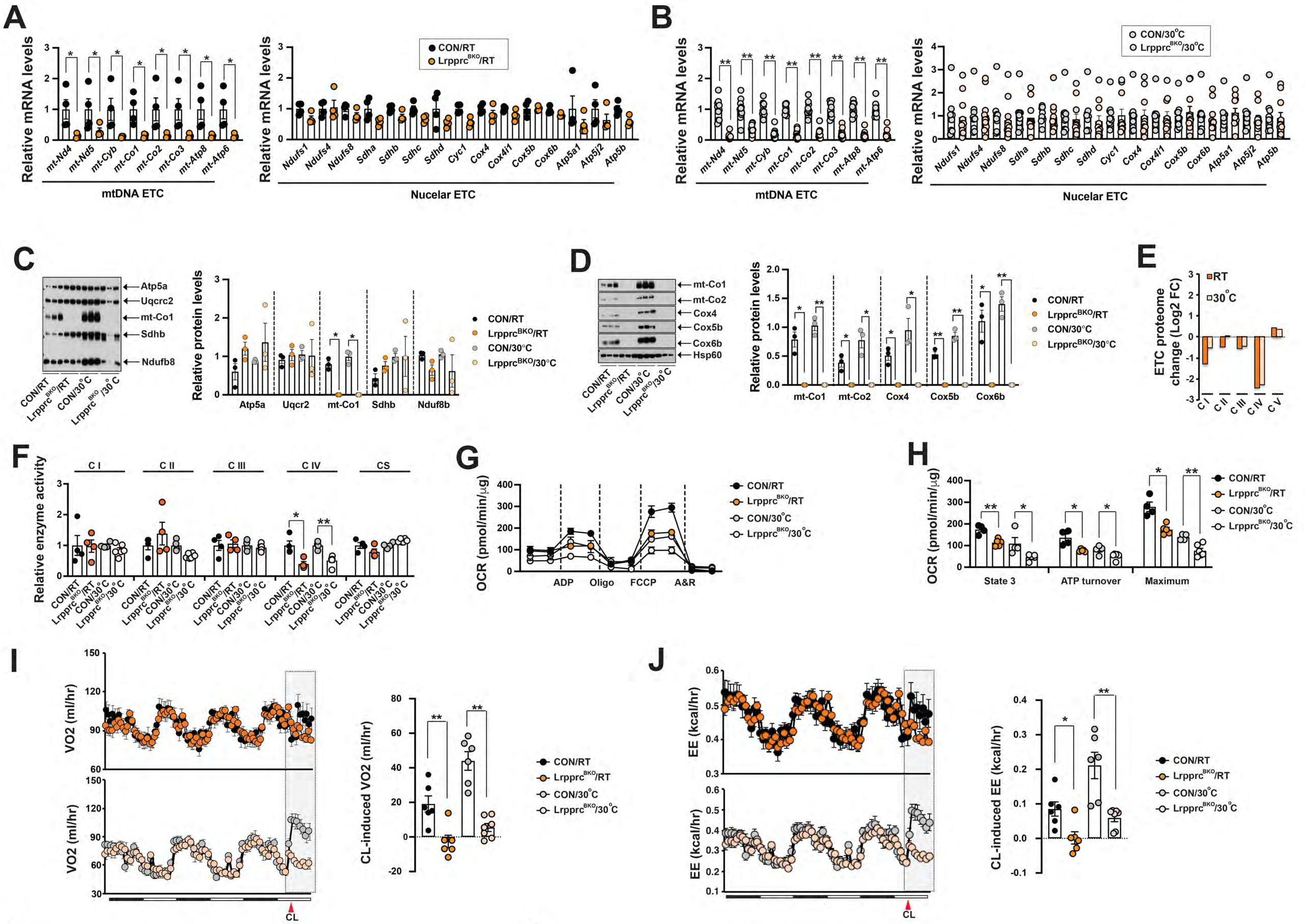
Ulk-R	AGCCATGTACATAGGAGAAC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Lrpprc BKO/RT
B
CON/30°C
Lrpprc BKO/30°C
C
Relative protein levels
CON/RT
Lrpprc BKO/RT
CON/30°C
Lrpprc BKO/30°C
D
E
ETC proteome change (Log2 FC)
RT
30°C
F
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C II
C III
C IV
CS
G
OCR (pmol/min/μg)
ADP
Oligo
FCCP
A&R
H
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Maximum
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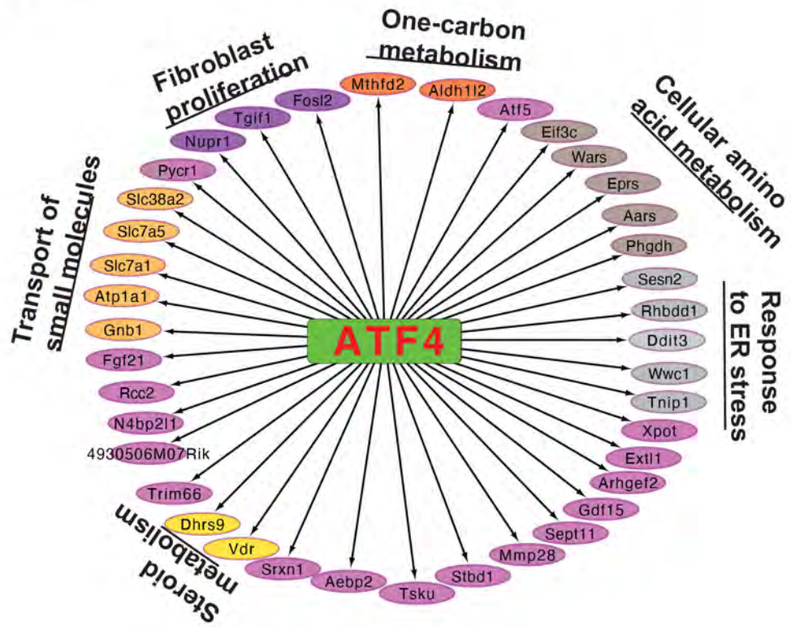
CL-induced VO2 (ml/hr)
CL
J
EE (kcal/hr)
CL-induced EE (kcal/hr)
K
CTT with food
CTT without food
Core temp (°C)
(Hours under 4°C)
Lrpprc f/f (CON)
Lrpprc BKO
Tfam f/f (CON)
Tfam BKO
Gnas f/f (CON)
Gnas BKO
L
Temp (Neck-Back) (°C)
PBS
Propranolol (-- βAR)
DMSO
Sotagliflozin (-- glucose)
Orlistat (-- lipid)
BCH (-- AA)
αMT (-- AA)
n.s.
CON
Lrpprc BKO

Figure 1

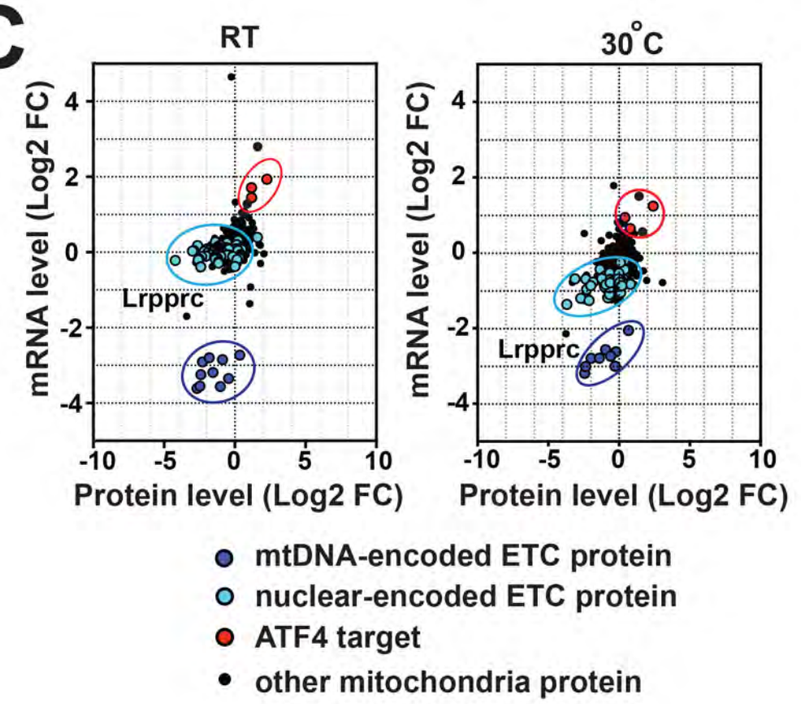
A

TF	Motif	# Targets
ATF4		39
ATF3/CREG1		46
CEBPα/DDIT3		26
NFKB1		14
SRF		14

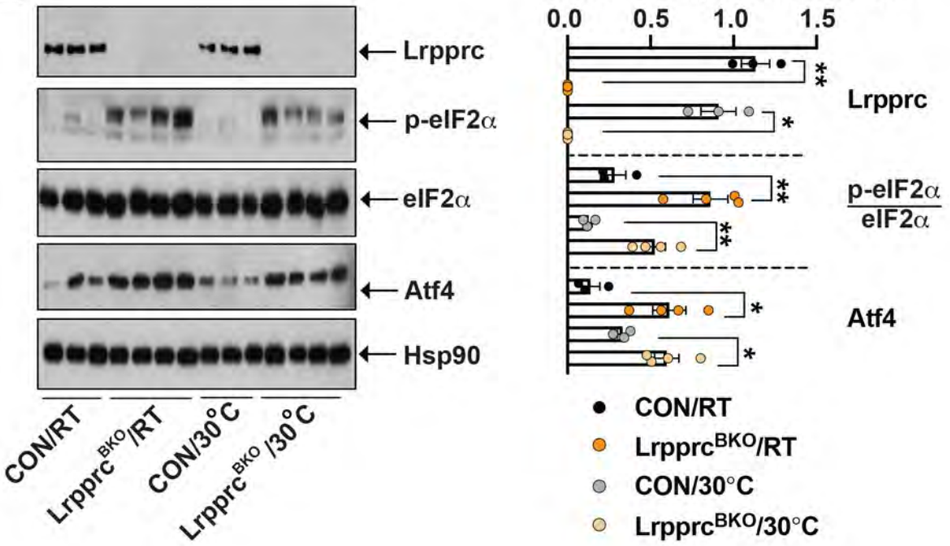
B



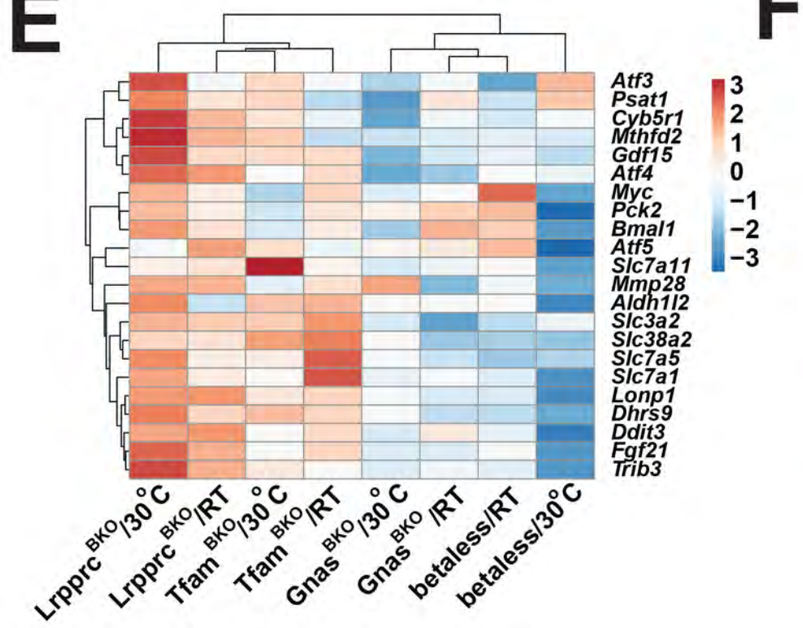
C



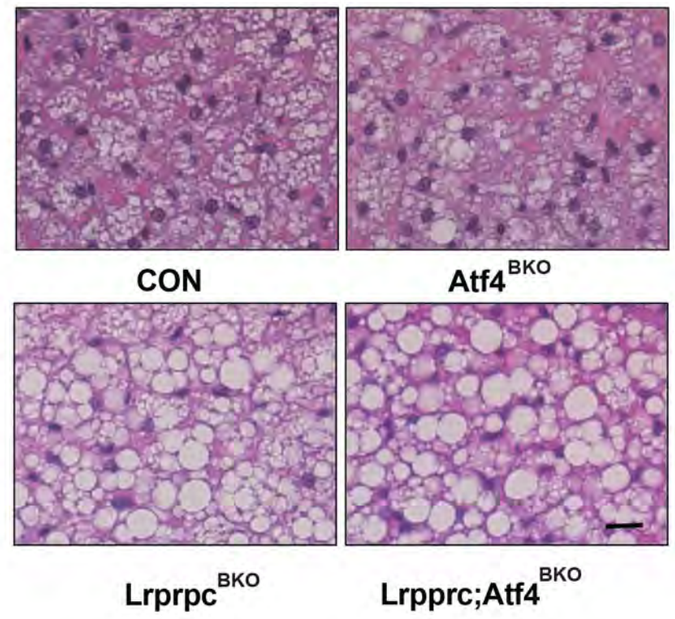
D



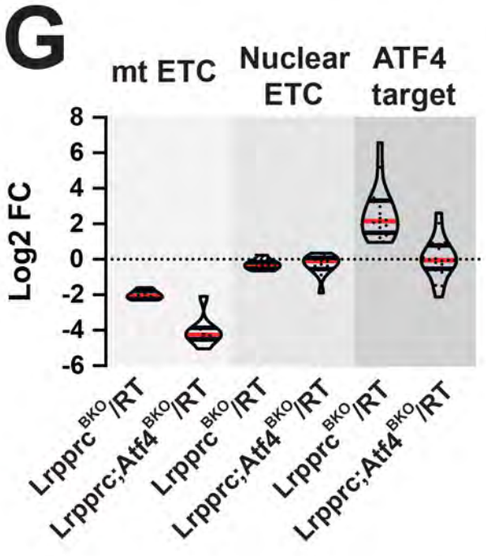
E



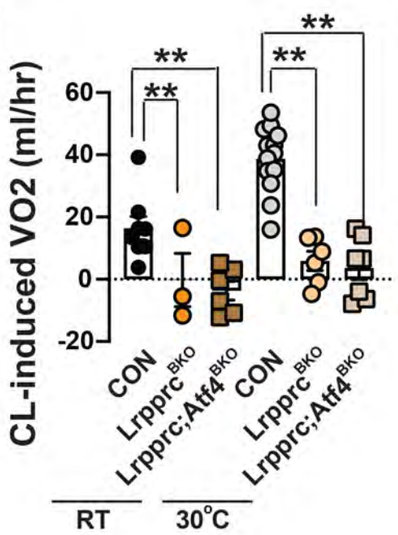
F



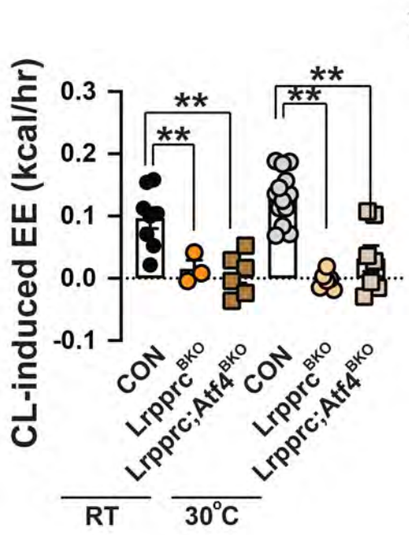
G



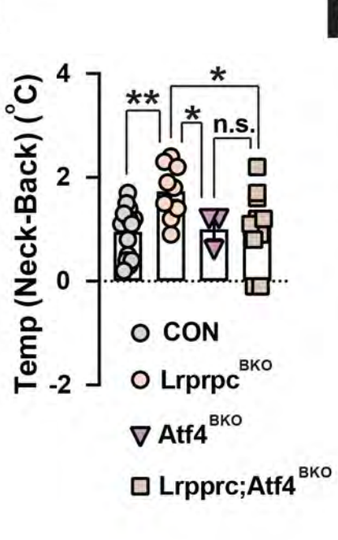
H



I



J



K

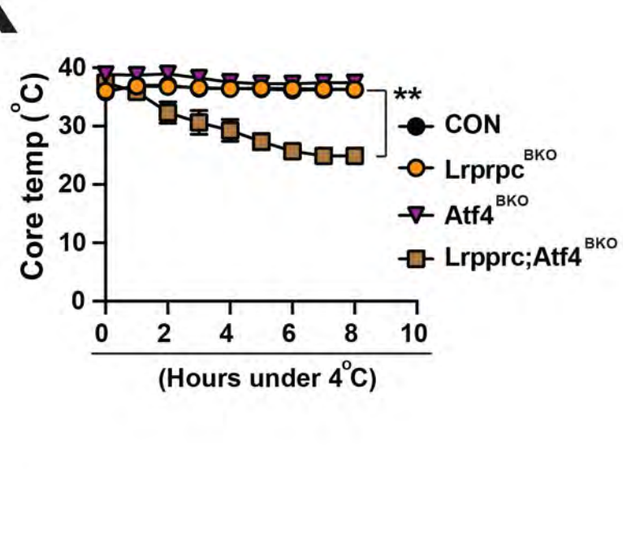


Figure 2

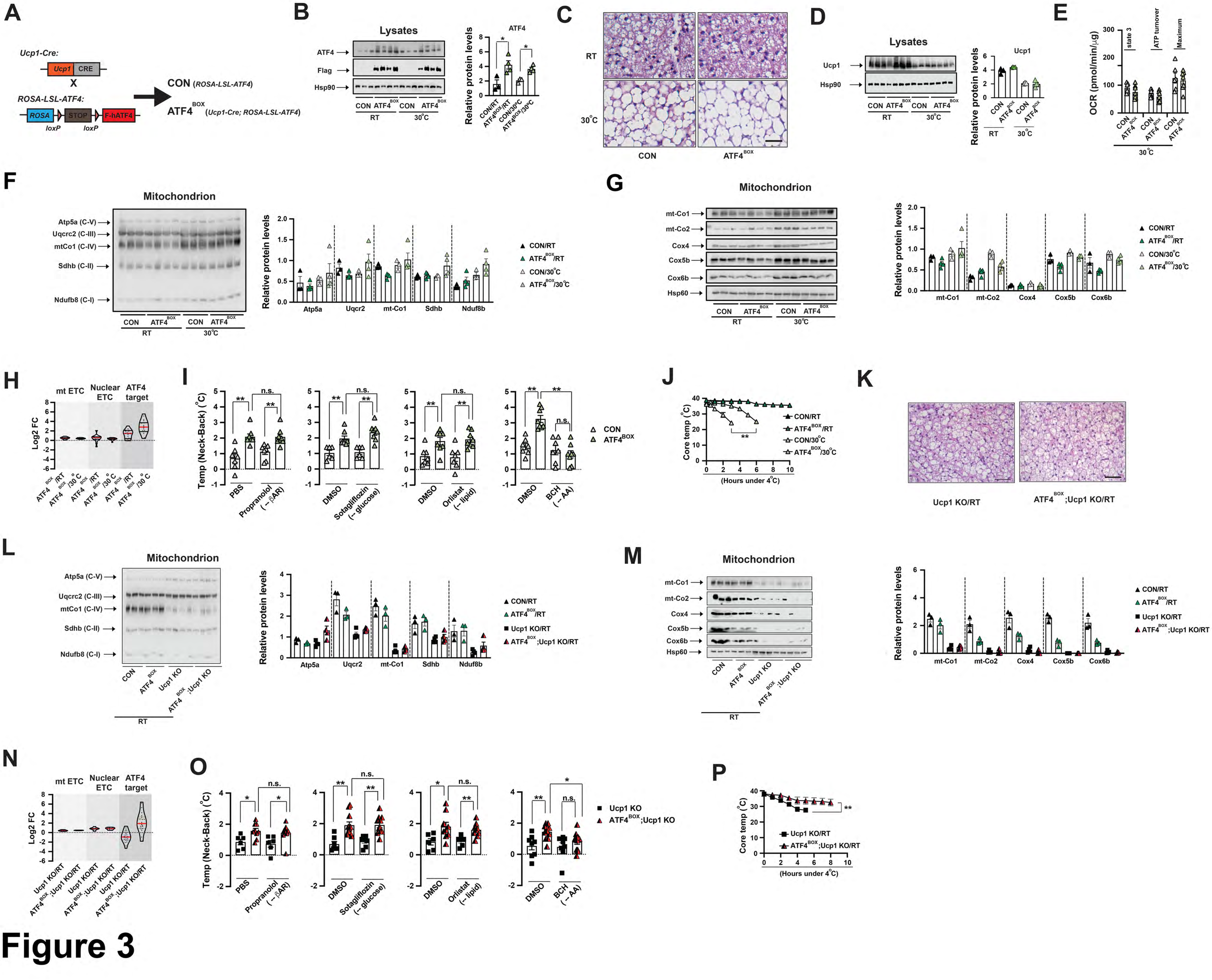

A
Ucp1-Cre:
Ucp1
CRE
X
ROSA-LSL-ATF4:
ROSA
STOP
F-hATF4
loxP
loxP
CON (ROSA-LSL-ATF4)
ATF4BOX (Ucp1-Cre; ROSA-LSL-ATF4)
B
Lysates
ATF4
Flag
Hsp90
CON ATF4BOX CON ATF4BOX
RT
30°C
Relative protein levels
ATF4
CON/RT
ATF4BOX/RT
CON/30°C
ATF4BOX/30°C
C
RT
30°C
CON
ATF4BOX
D
Lysates
Ucp1
Hsp90
CON ATF4BOX CON ATF4BOX
RT
30°C
Relative protein levels
Ucp1
E
OCR (pmol/min/μg)
state 3
ATP turnover
Maximum
CON ATF4BOX CON ATF4BOX CON ATF4BOX
30°C
F
Mitochondrion
Atp5a (C-V)
Uqcrc2 (C-III)
mtCo1 (C-IV)
Sdhb (C-II)
Ndufb8 (C-I)
CON ATF4BOX CON ATF4BOX
RT
30°C
Relative protein levels
Atp5a
Uqcr2
mt-Co1
Sdhb
Nduf8b
CON/RT
ATF4BOX/RT
CON/30°C
ATF4BOX/30°C
G
Mitochondrion
mt-Co1
mt-Co2
Cox4
Cox5b
Cox6b
Hsp60
CON ATF4BOX CON ATF4BOX
RT
30°C
Relative protein levels
mt-Co1
mt-Co2
Cox4
Cox5b
Cox6b
CON/RT
ATF4BOX/RT
CON/30°C
ATF4BOX/30°C
H
mt ETC
Nuclear ETC
ATF4 target
Log2 FC
ATF4BOX /RT
ATF4BOX /30°C
I
Temp (Neck-Back) (°C)
n.s.
PBS
Propranolol (-- βAR)
DMSO
Sotagliflozin (-- glucose)
DMSO
Orlistat (-- lipid)
DMSO
BCH (-- AA)
CON
ATF4BOX
J
Core temp (°C)
(Hours under 4°C)
CON/RT
ATF4BOX/RT
CON/30°C
ATF4BOX/30°C
K
Ucp1 KO/RT
ATF4BOX;Ucp1 KO/RT
L
Mitochondrion
Atp5a (C-V)
Uqcrc2 (C-III)
mtCo1 (C-IV)
Sdhb (C-II)
Ndufb8 (C-I)
CON
ATF4BOX
Ucp1 KO
ATF4BOX;Ucp1 KO
RT
Relative protein levels
Atp5a
Uqcr2
mt-Co1
Sdhb
Nduf8b
CON/RT
ATF4BOX/RT
Ucp1 KO/RT
ATF4BOX;Ucp1 KO/RT
M
Mitochondrion
mt-Co1
mt-Co2
Cox4
Cox5b
Cox6b
Hsp60
CON
ATF4BOX
Ucp1 KO
ATF4BOX;Ucp1 KO
RT
Relative protein levels
mt-Co1
mt-Co2
Cox4
Cox5b
Cox6b
CON/RT
ATF4BOX/RT
Ucp1 KO/RT
ATF4BOX;Ucp1 KO/RT
N
mt ETC
Nuclear ETC
ATF4 target
Log2 FC
Ucp1 KO/RT
ATF4BOX;Ucp1 KO/RT
O
Temp (Neck-Back) (°C)
n.s.
PBS
Propranolol (-- βAR)
DMSO
Sotagliflozin (-- glucose)
DMSO
Orlistat (-- lipid)
DMSO
BCH (-- AA)
Ucp1 KO
ATF4BOX;Ucp1 KO
P
Core temp (°C)
(Hours under 4°C)
Ucp1 KO/RT
ATF4BOX;Ucp1 KO/RT

Figure 3

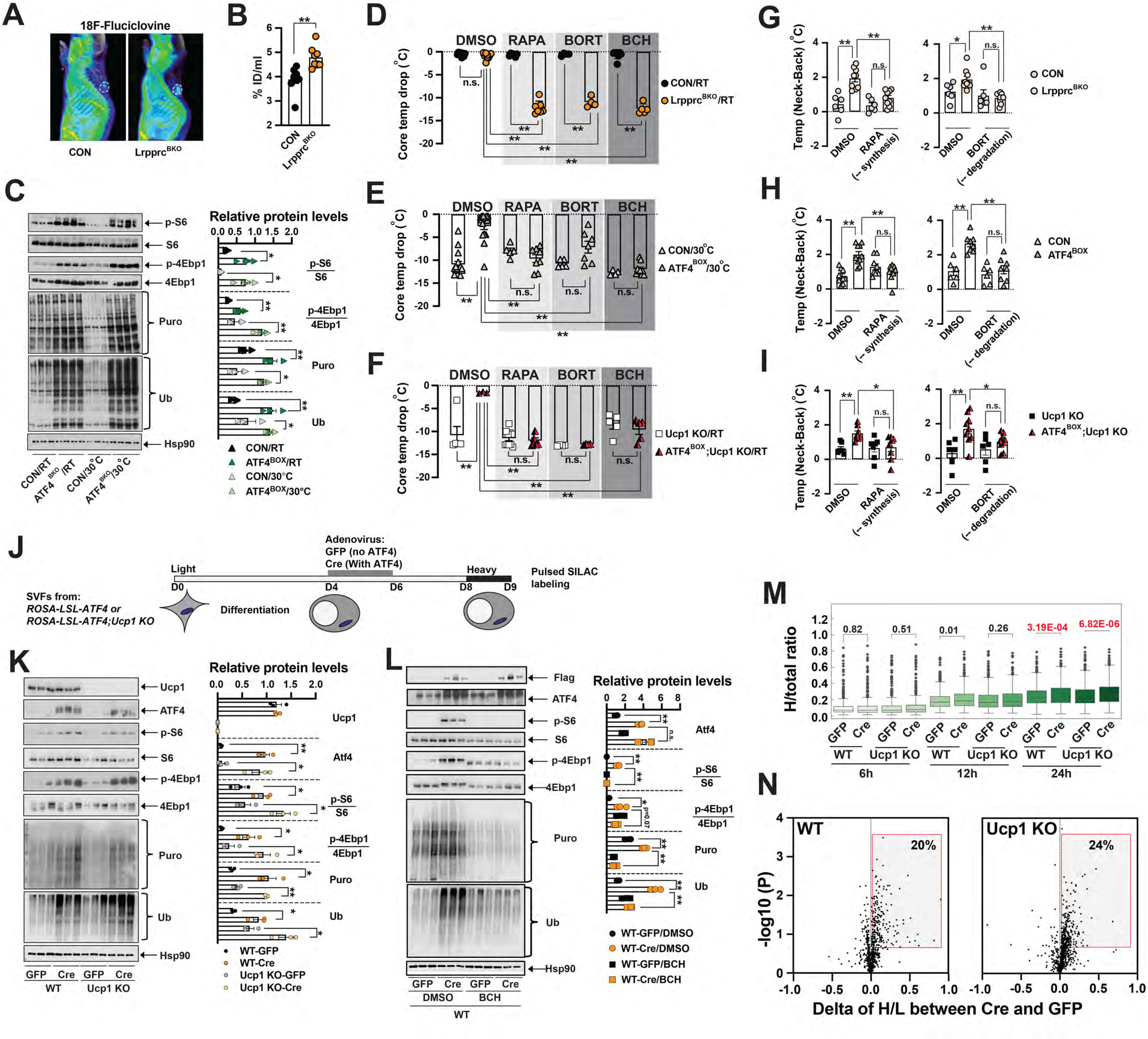
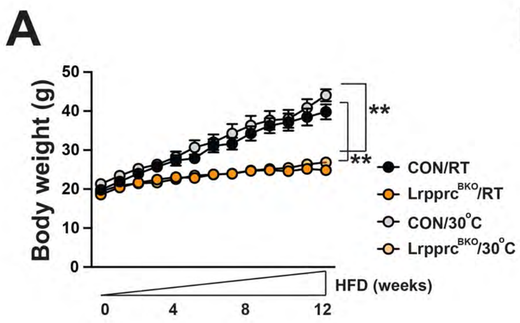
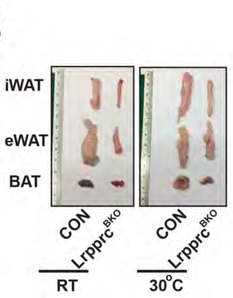
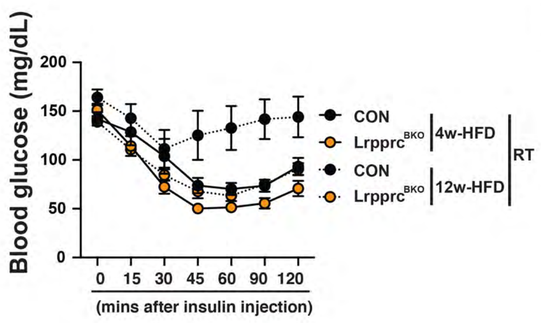

A
18F-Fluciclovine
CON
Lrpprc^BKO
B
% ID/ml
CON
Lrpprc^BKO
C
p-S6
S6
p-4Ebp1
4Ebp1
Puro
Ub
Hsp90
CON/RT
ATF4^BKO/RT
CON/30°C
ATF4^BKO/30°C
Relative protein levels
p-S6 / S6
p-4Ebp1 / 4Ebp1
Puro
Ub
CON/RT
ATF4^BOX/RT
CON/30°C
ATF4^BOX/30°C
D
DMSO RAPA BORT BCH
Core temp drop (°C)
CON/RT
Lrpprc^BKO/RT
E
CON/30°C
ATF4^BOX/30°C
F
Ucp1 KO/RT
ATF4^BOX;Ucp1 KO/RT
G
Temp (Neck-Back) (°C)
DMSO
RAPA (-- synthesis)
BORT (-- degradation)
CON
Lrpprc^BKO
H
CON
ATF4^BOX
I
Ucp1 KO
ATF4^BOX;Ucp1 KO
J
Adenovirus:
GFP (no ATF4)
Cre (With ATF4)
Light
Heavy
Pulsed SILAC labeling
D0 D4 D6 D8 D9
SVFs from:
ROSA-LSL-ATF4 or
ROSA-LSL-ATF4;Ucp1 KO
Differentiation
K
Ucp1
ATF4
p-S6
S6
p-4Ebp1
4Ebp1
Puro
Ub
Hsp90
GFP Cre GFP Cre
WT Ucp1 KO
Relative protein levels
Ucp1
Atf4
p-S6 / S6
p-4Ebp1 / 4Ebp1
Puro
Ub
WT-GFP
WT-Cre
Ucp1 KO-GFP
Ucp1 KO-Cre
L
Flag
ATF4
p-S6
S6
p-4Ebp1
4Ebp1
Puro
Ub
Hsp90
GFP Cre GFP Cre
DMSO BCH
WT
Relative protein levels
Atf4
p-S6 / S6
p-4Ebp1 / 4Ebp1
p=0.07
Puro
Ub
WT-GFP/DMSO
WT-Cre/DMSO
WT-GFP/BCH
WT-Cre/BCH
M
H/total ratio
0.82
0.51
0.01
0.26
3.19E-04
6.82E-06
GFP Cre GFP Cre GFP Cre GFP Cre GFP Cre GFP Cre
WT Ucp1 KO WT Ucp1 KO WT Ucp1 KO
6h 12h 24h
N
WT
20%
Ucp1 KO
24%
-log10 (P)
Delta of H/L between Cre and GFP

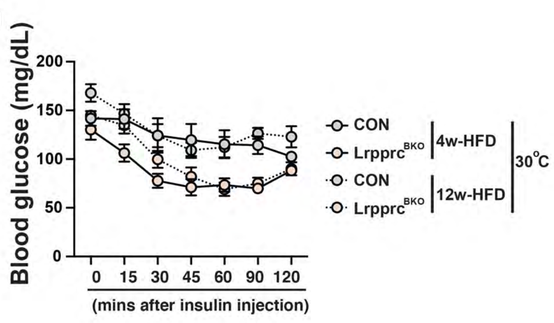
Figure 4

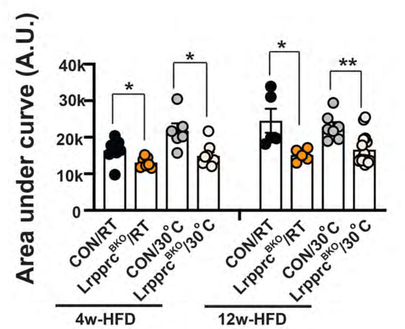
Figure 5

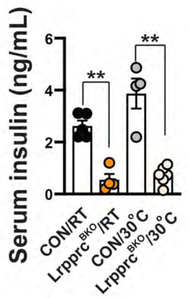

A
Body weight (g)
HFD (weeks)
CON/RT
Lrpprc^BKO/RT
CON/30°C
Lrpprc^BKO/30°C
RT

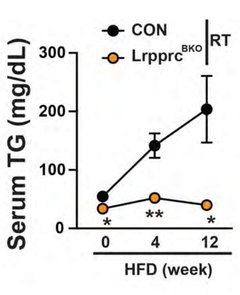

B
iWAT
eWAT
BAT
CON
Lrpprc^BKO
RT
30°C

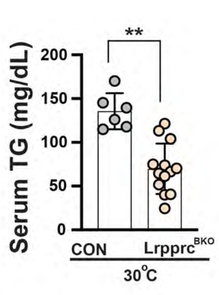

C
Blood glucose (mg/dL)
(mins after insulin injection)
CON
Lrpprc^BKO
4w-HFD
12w-HFD
RT

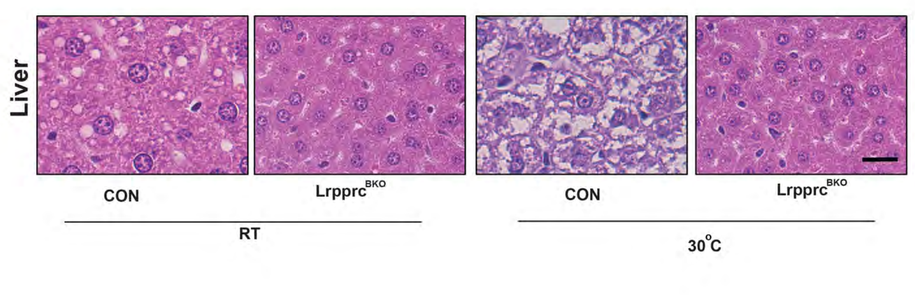

D
Blood glucose (mg/dL)
(mins after insulin injection)
CON
Lrpprc^BKO
4w-HFD
12w-HFD
30°C

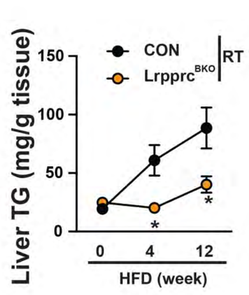

E
Area under curve (A.U.)
CON/RT
Lrpprc^BKO/RT
CON/30°C
Lrpprc^BKO/30°C
4w-HFD
12w-HFD

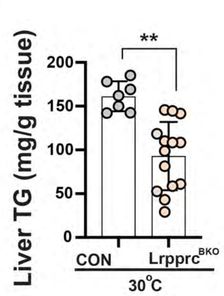

F
Serum insulin (ng/mL)
CON/RT
Lrpprc^BKO/RT
CON/30°C
Lrpprc^BKO/30°C

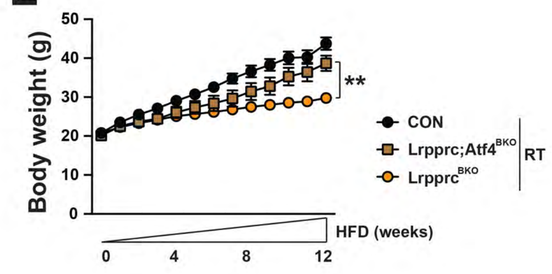

G
Serum TG (mg/dL)
HFD (week)
CON
Lrpprc^BKO
RT

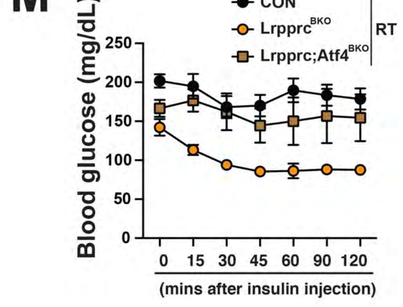

H
Serum TG (mg/dL)
CON
Lrpprc^BKO
30°C

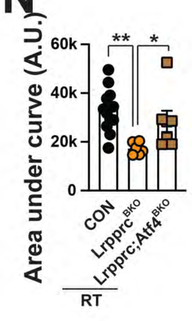

I
Liver
CON
Lrpprc^BKO
RT
CON
Lrpprc^BKO
30°C

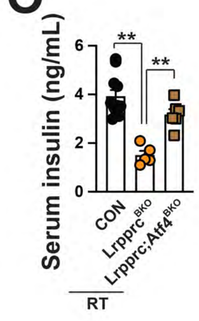

J
Liver TG (mg/g tissue)
HFD (week)
CON
Lrpprc^BKO
RT

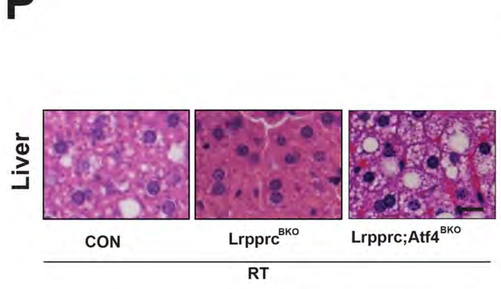

K
Liver TG (mg/g tissue)
CON
Lrpprc^BKO
30°C

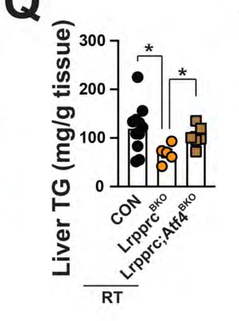

L
Body weight (g)
HFD (weeks)
CON
Lrpprc;Atf4^BKO
Lrpprc^BKO
RT

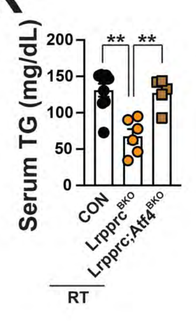

M
Blood glucose (mg/dL)
(mins after insulin injection)
CON
Lrpprc^BKO
Lrpprc;Atf4^BKO
RT

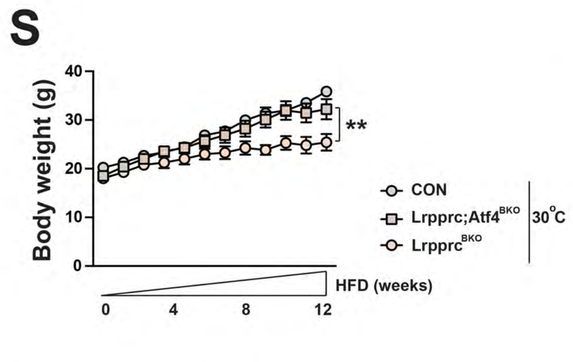

N
Area under curve (A.U.)
CON
Lrpprc^BKO
Lrpprc;Atf4^BKO
RT

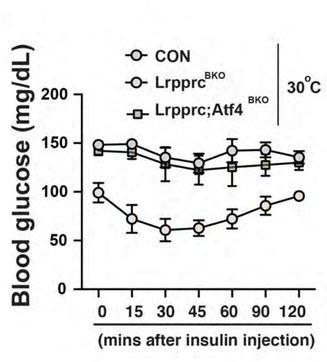

O
Serum insulin (ng/mL)
CON
Lrpprc^BKO
Lrpprc;Atf4^BKO
RT

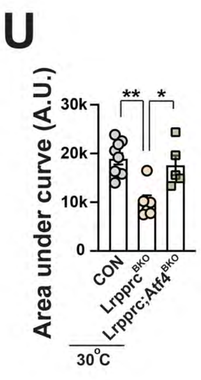

P
Liver
CON
Lrpprc^BKO
Lrpprc;Atf4^BKO
RT

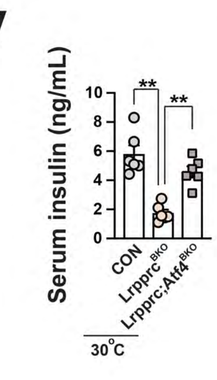

Q
Liver TG (mg/g tissue)
CON
Lrpprc^BKO
Lrpprc;Atf4^BKO
RT

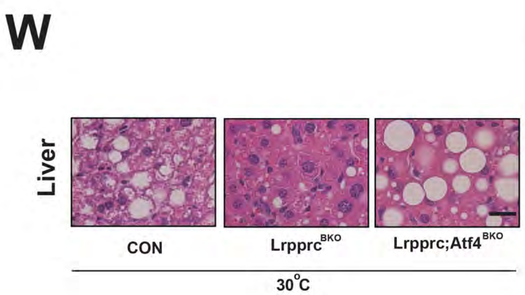

R
Serum TG (mg/dL)
CON
Lrpprc^BKO
Lrpprc;Atf4^BKO
RT

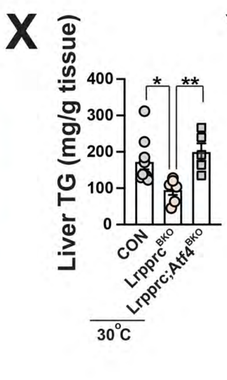

S
Body weight (g)
HFD (weeks)
CON
Lrpprc;Atf4^BKO
Lrpprc^BKO
30°C


T
Blood glucose (mg/dL)
(mins after insulin injection)
CON
Lrpprc^BKO
Lrpprc;Atf4^BKO
30°C


U
Area under curve (A.U.)
CON
Lrpprc^BKO
Lrpprc;Atf4^BKO
30°C


V
Serum insulin (ng/mL)
CON
Lrpprc^BKO
Lrpprc;Atf4^BKO
30°C


W
Liver
CON
Lrpprc^BKO
Lrpprc;Atf4^BKO
30°C


X
Liver TG (mg/g tissue)
CON
Lrpprc^BKO
Lrpprc;Atf4^BKO
30°C

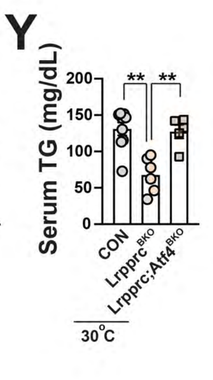
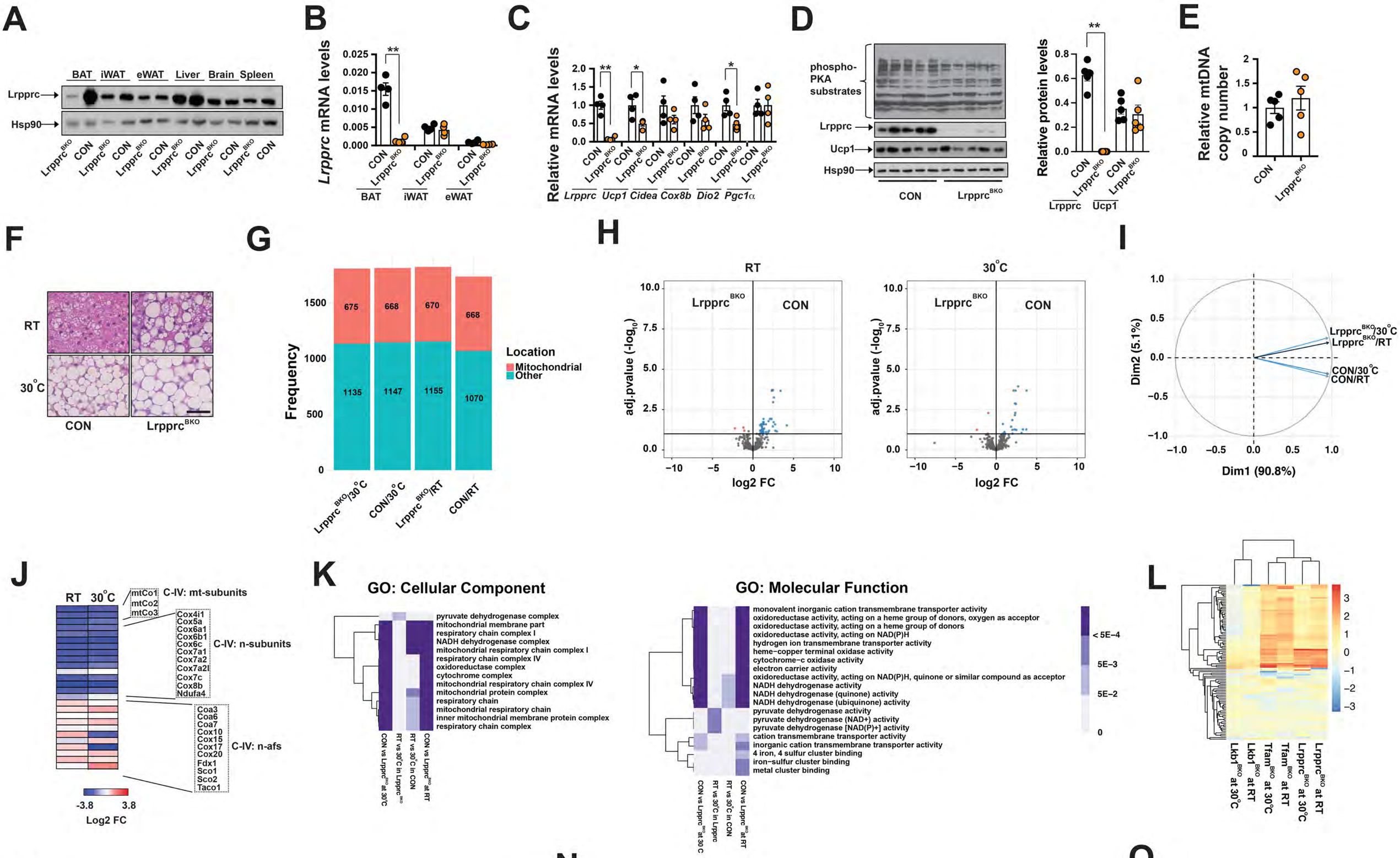

Y
Serum TG (mg/dL)
CON
Lrpprc^BKO
Lrpprc;Atf4^BKO
30°C

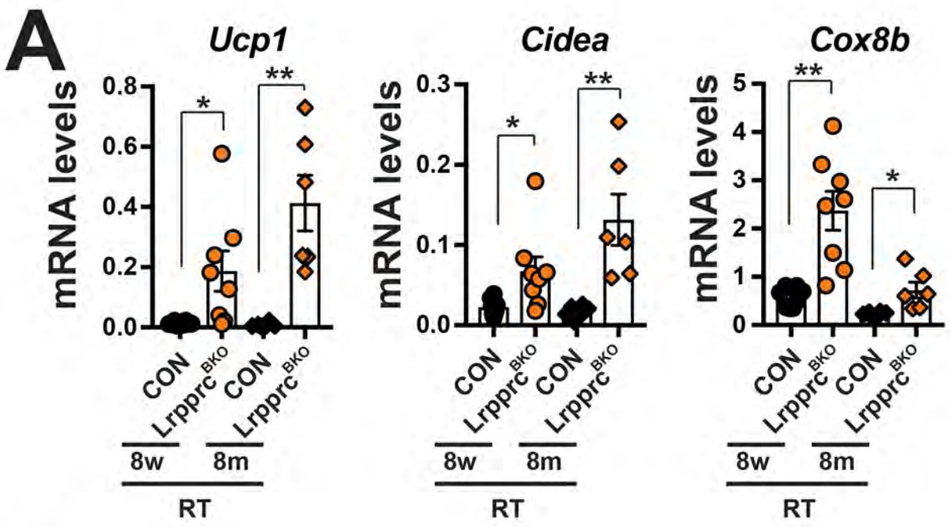
Figure 6

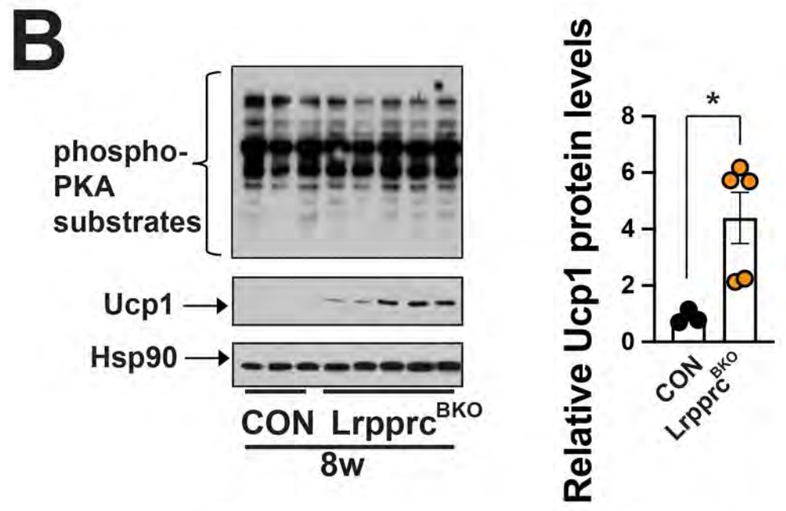
A
Body weight (g)
HFD (weeks)
CON/RT
ATF4BOX/RT
CON/30°C
ATF4BOX/30°C
B
Tissue mass (g)
BAT iWAT eWAT
RT
30°C
C
Weight (g) or Fat %
Lean mass
Fat mass
Fat %
D
eWAT
RT
30°C
CON
ATF4BOX
E
Blood glucose (mg/dL)
(mins after insulin injection)
F
Area under curve (A.U.)
G
Serum insulin (ng/mL)
H
Serum TG (mg/dL)
I
Liver
J
Liver TG (mg/g tissue)
K
Body weight (g)
Ucp1 KO/RT
ATF4BOX;Ucp1 KO/RT
Ucp1 KO/30°C
ATF4BOX;Ucp1 KO/30°C
HFD (weeks)
L
Tissue mass (g)
BAT
iWAT
eWAT
M
Weight (g) or Fat %
Lean mass
Fat mass
Fat %
N
eWAT
Ucp1 KO
ATF4BOX;Ucp1 KO
O
Blood glucose (mg/dL)
(mins after insulin injection)
P
Area under curve (A.U.)
Q
Serum TG (mg/dL)
R
Liver
S
Liver TG (mg/g tissue)
T
Index of ATF4 target gene expression in BAT
Index of HFD-induced obesity
Wild-type
GnasBKO
TfamBKO
LrpprcBKO
Atf4BKO
Lrpprc;Atf4BKO
ATF4BOX
ATF4BOX;Ucp1 KO

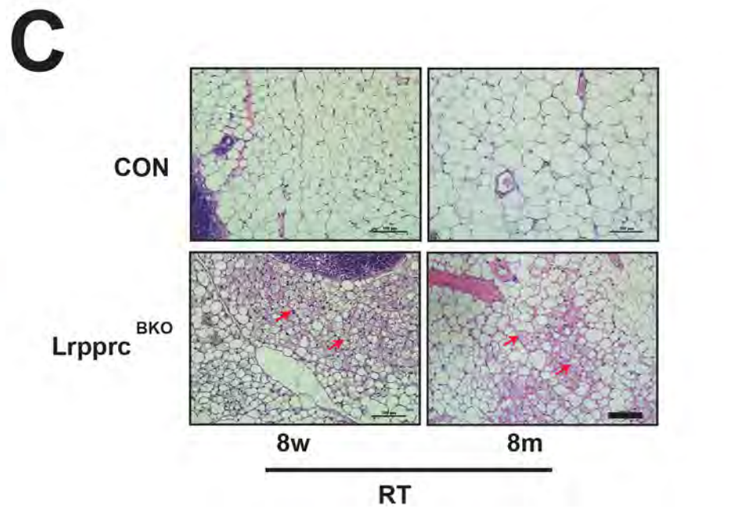
Figure 7

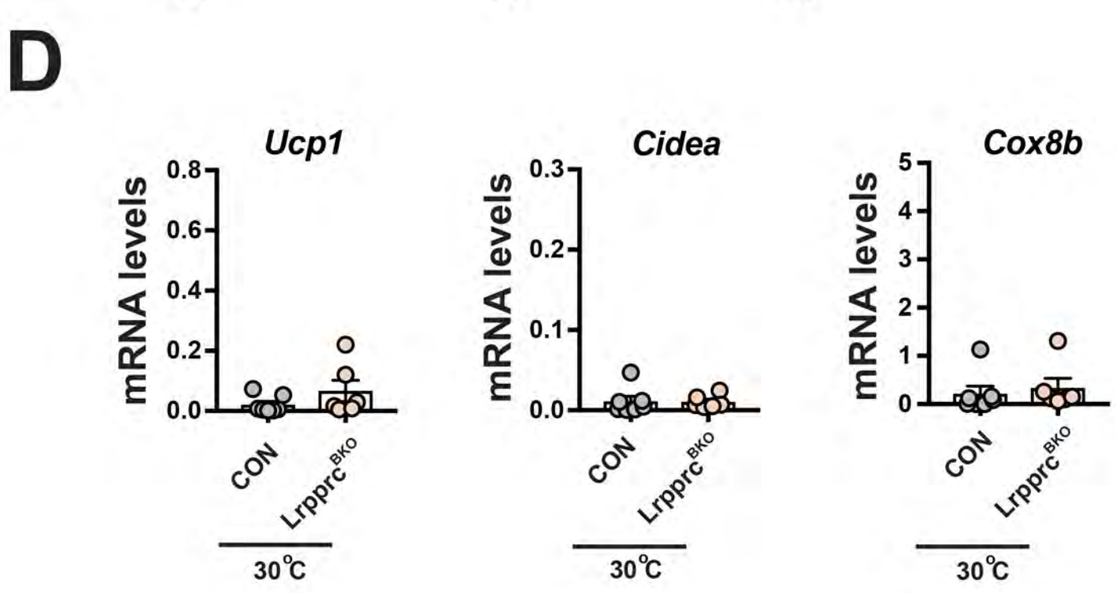


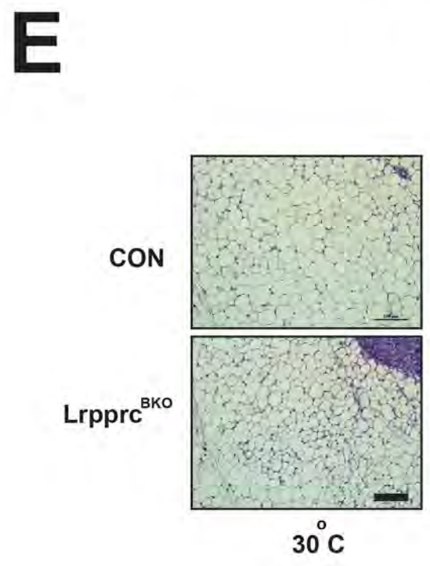
Supplementary Figure 1

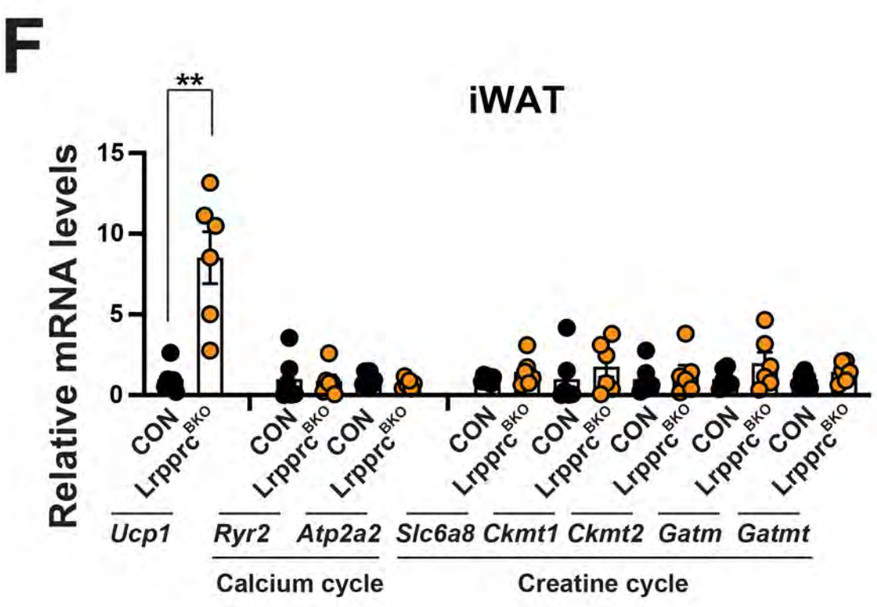

A
Ucp1
Cidea
Cox8b
mRNA levels
CON
Lrpprc BKO
8w
8m
RT

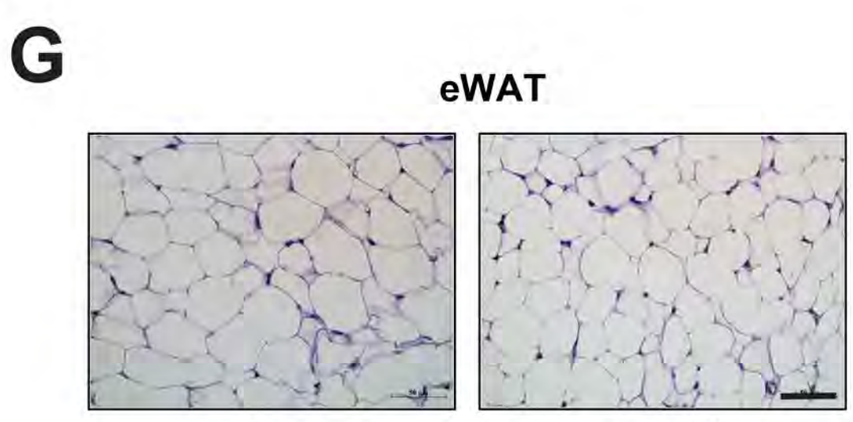

B
phospho-PKA substrates
Ucp1
Hsp90
CON Lrpprc BKO
8w
Relative Ucp1 protein levels

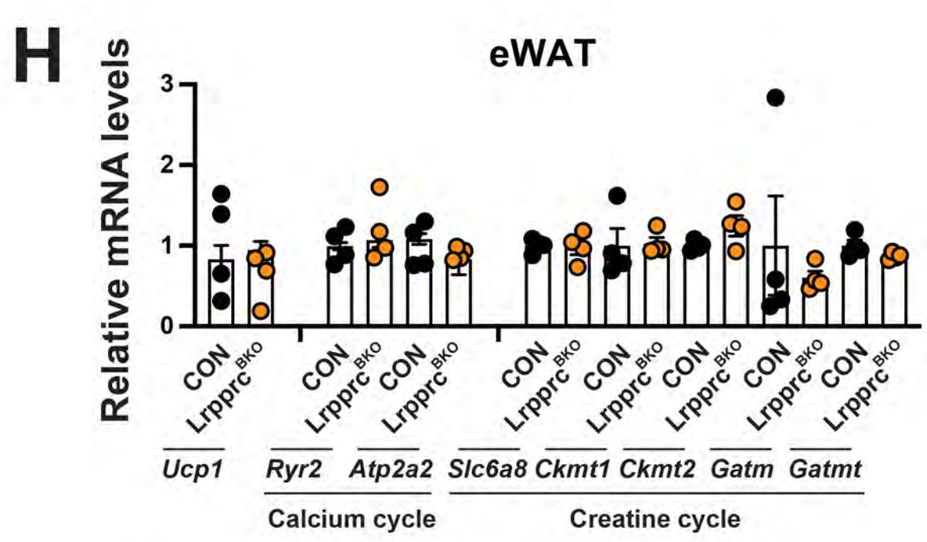

C
CON
Lrpprc BKO
8w
8m
RT

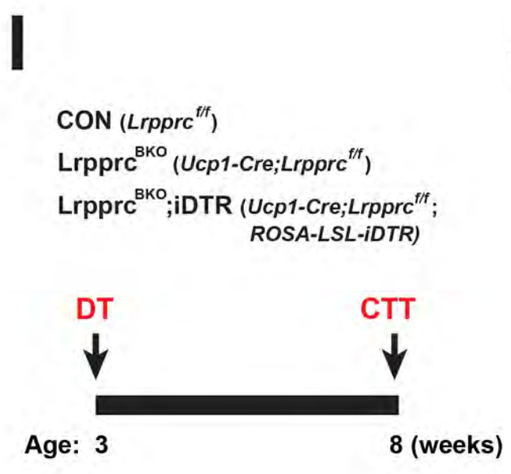

D
Ucp1
Cidea
Cox8b
mRNA levels
CON
Lrpprc BKO
30°C

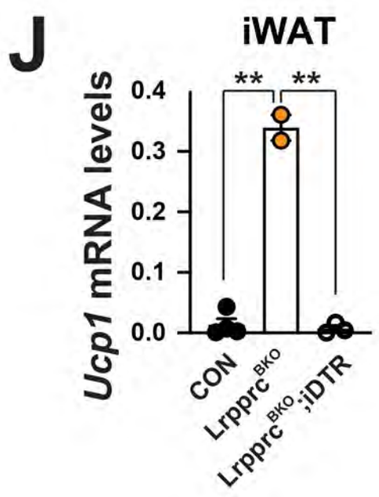

E
CON
Lrpprc BKO
30°C

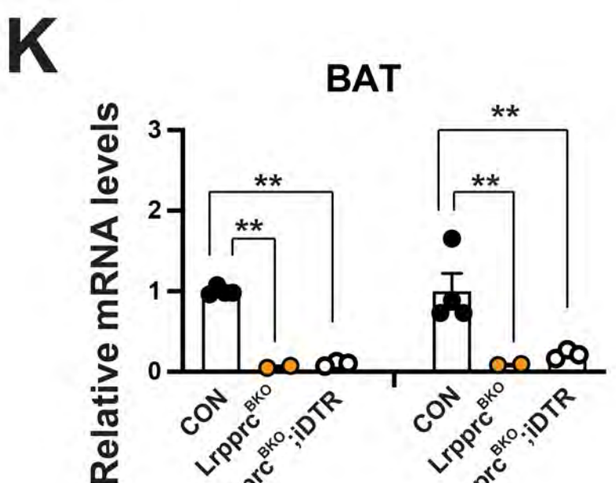

F
iWAT
Relative mRNA levels
Ucp1
Ryr2
Atp2a2
Slc6a8
Ckmt1
Ckmt2
Gatm
Gatmt
Calcium cycle
Creatine cycle

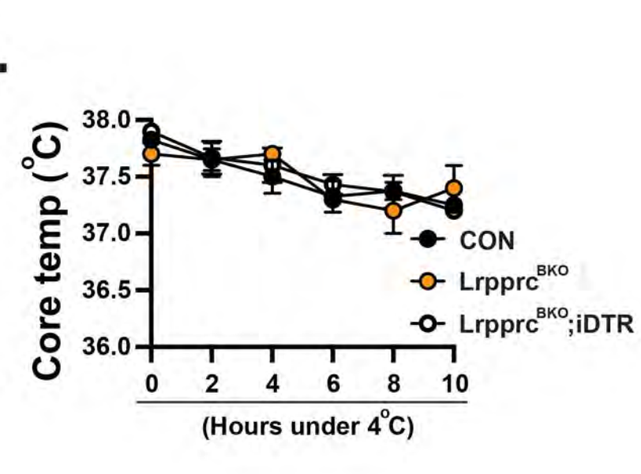

G
eWAT
CON
Lrpprc BKO

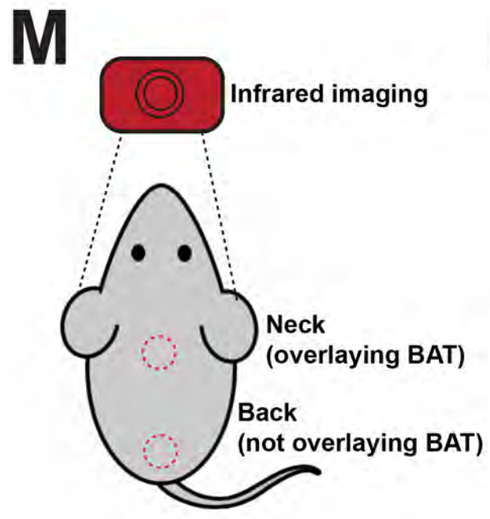

H
eWAT
Relative mRNA levels
Ucp1
Ryr2
Atp2a2
Slc6a8
Ckmt1
Ckmt2
Gatm
Gatmt
Calcium cycle
Creatine cycle

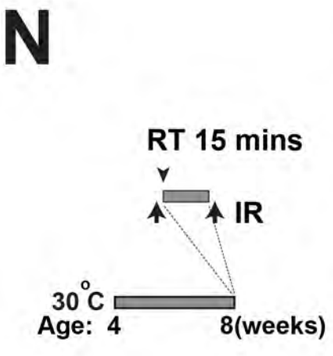

I
CON (Lrpprc f/f)
Lrpprc BKO (Ucp1-Cre;Lrpprc f/f)
Lrpprc BKO;iDTR (Ucp1-Cre;Lrpprc f/f; ROSA-LSL-iDTR)
DT
CTT
Age: 3
8 (weeks)

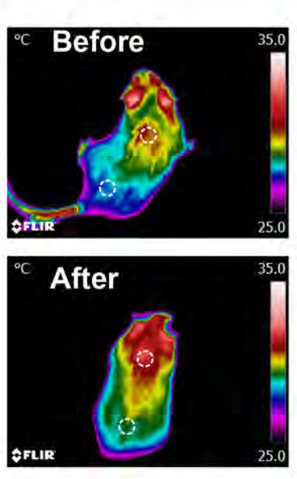

J
iWAT
Ucp1 mRNA levels
CON
Lrpprc BKO
Lrpprc BKO;iDTR

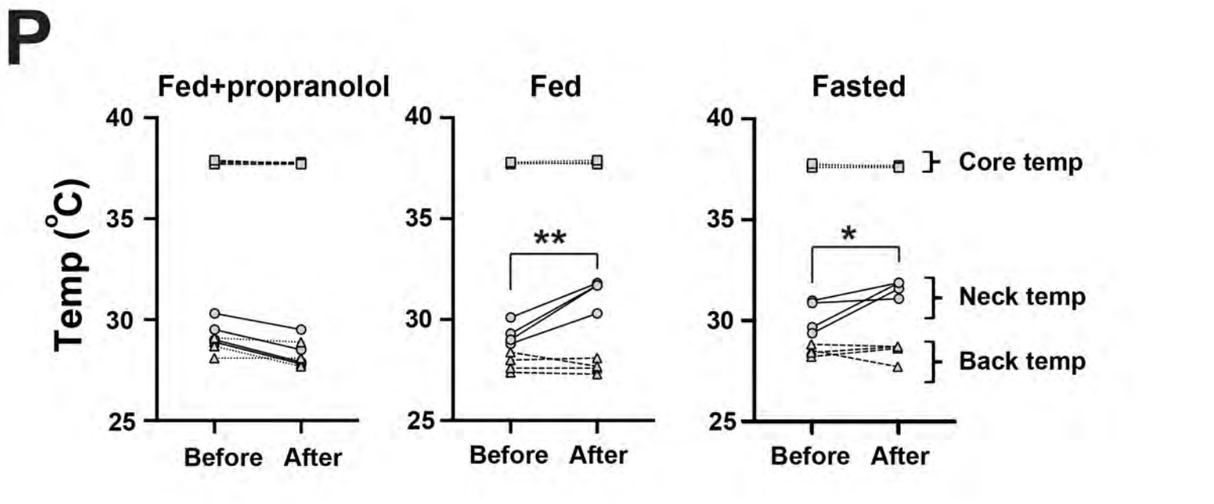

K
BAT
Relative mRNA levels
CON
Lrpprc BKO
Lrpprc BKO;iDTR
Lrpprc
mt-Co1

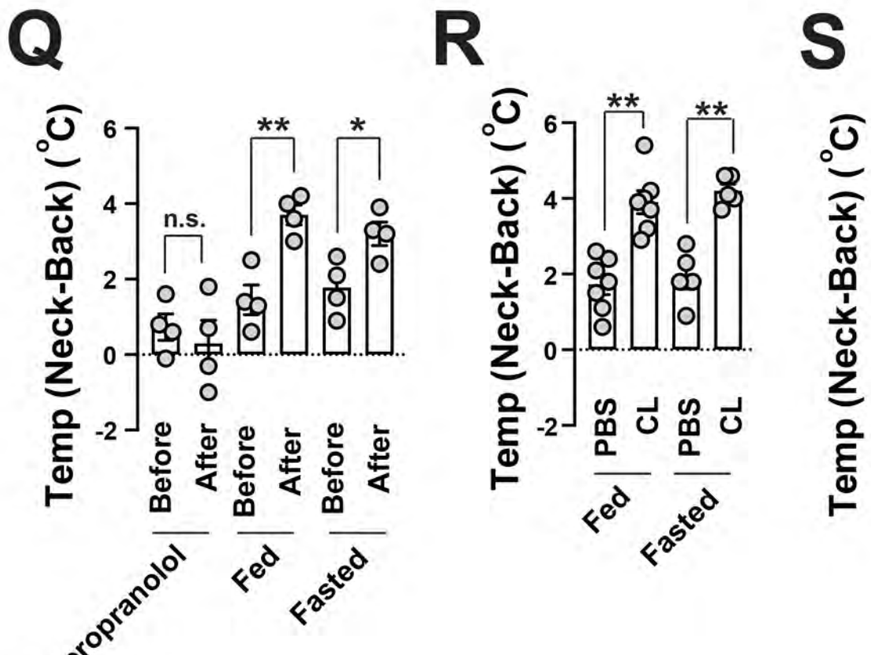

L
Core temp (°C)
CON
Lrpprc BKO
Lrpprc BKO;iDTR
(Hours under 4°C)


M
Infrared imaging
Neck
(overlaying BAT)
Back
(not overlaying BAT)

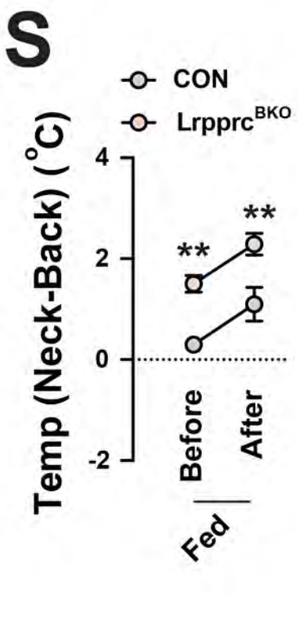

N
RT 15 mins
IR
30°C
Age: 4
8(weeks)

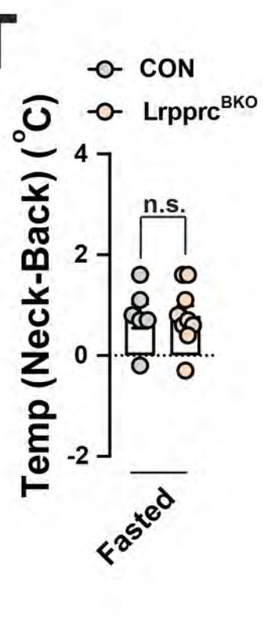

O
Before
After
FLIR

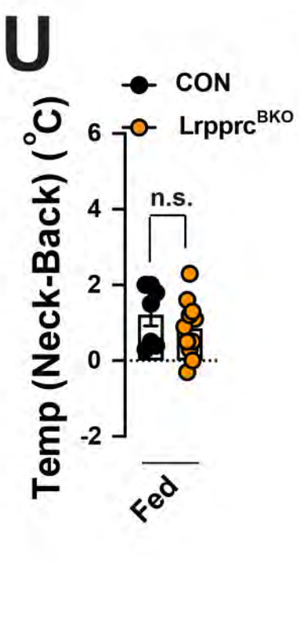

P
Fed+propranolol
Fed
Fasted
Core temp
Neck temp
Back temp
Temp (°C)
Before
After

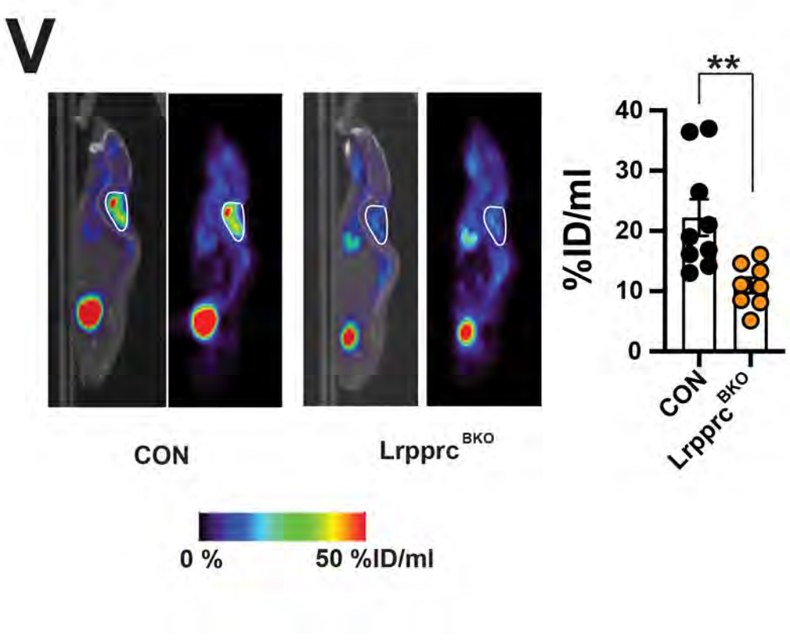

Q
Temp (Neck-Back) (°C)
n.s.
Before
After
Fed+propranolol
Fed
Fasted

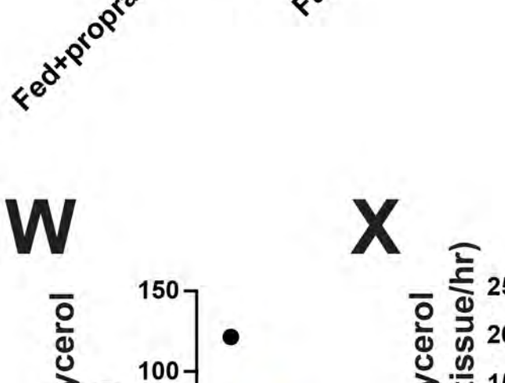
R
Temp (Neck-Back) (°C)
PBS
CL
Fed
Fasted

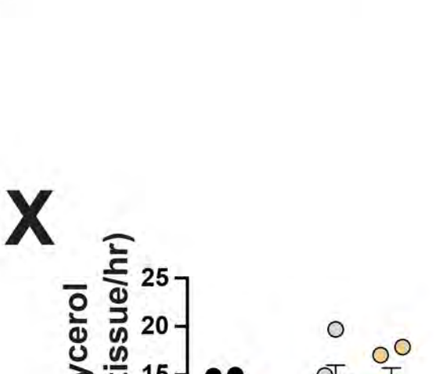

S
CON
Lrpprc BKO
Temp (Neck-Back) (°C)
Before
After
Fed

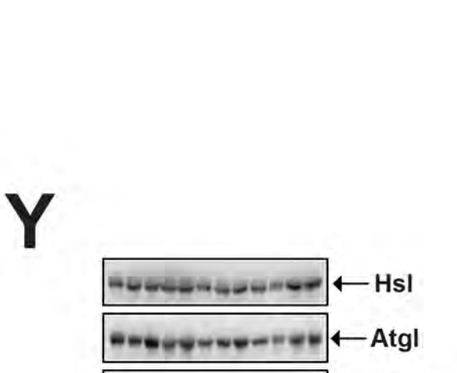

T
CON
Lrpprc BKO
Temp (Neck-Back) (°C)
n.s.
Fasted

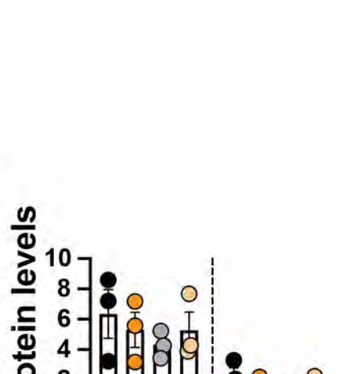

U
CON
Lrpprc BKO
Temp (Neck-Back) (°C)
n.s.
Fed

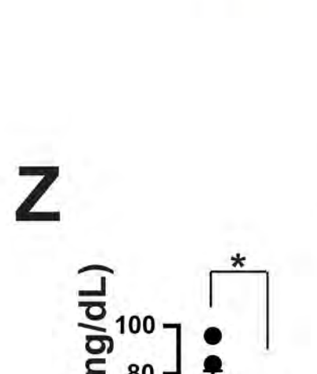

V
CON
Lrpprc BKO
0 %
50 %ID/ml
%ID/ml


W
Serum glycerol (μg/μl/hr)
CON/RT
Lrpprc BKO/RT

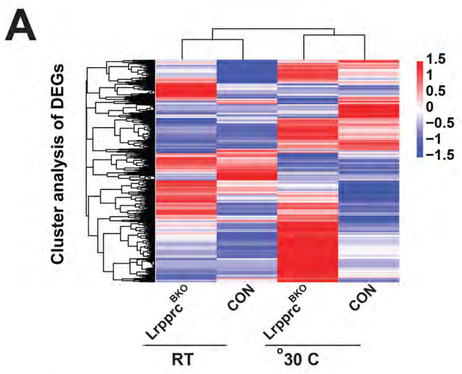

X
Media glycerol (mg/dl/mg tissue/hr)
CON/RT
Lrpprc BKO/RT
CON/30°C
Lrpprc BKO/30°C

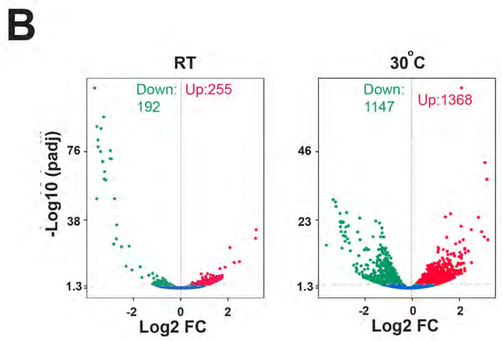

Y
Hsl
Atgl
Hsp90
CON/RT
Lrpprc BKO/RT
CON/30°C
Lrpprc BKO/30°C

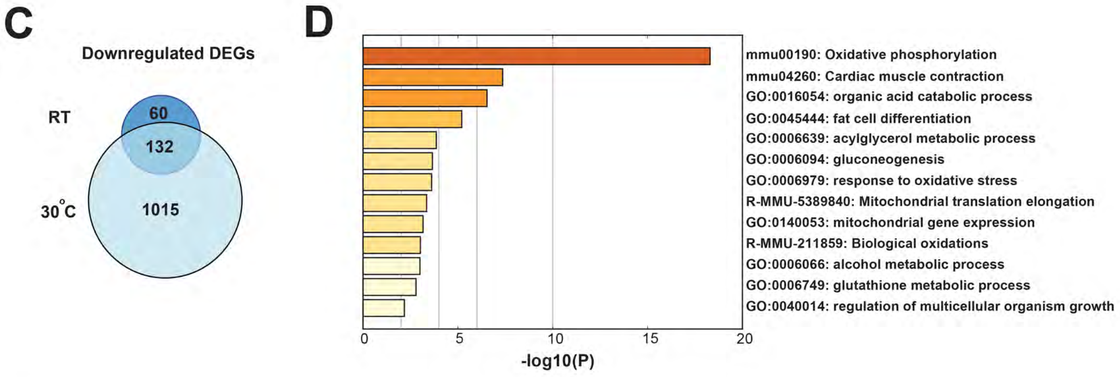

Relative protein levels
Hsl
Atgl
CON/RT
Lrpprc BKO/RT
CON/30°C
Lrpprc BKO/30°C

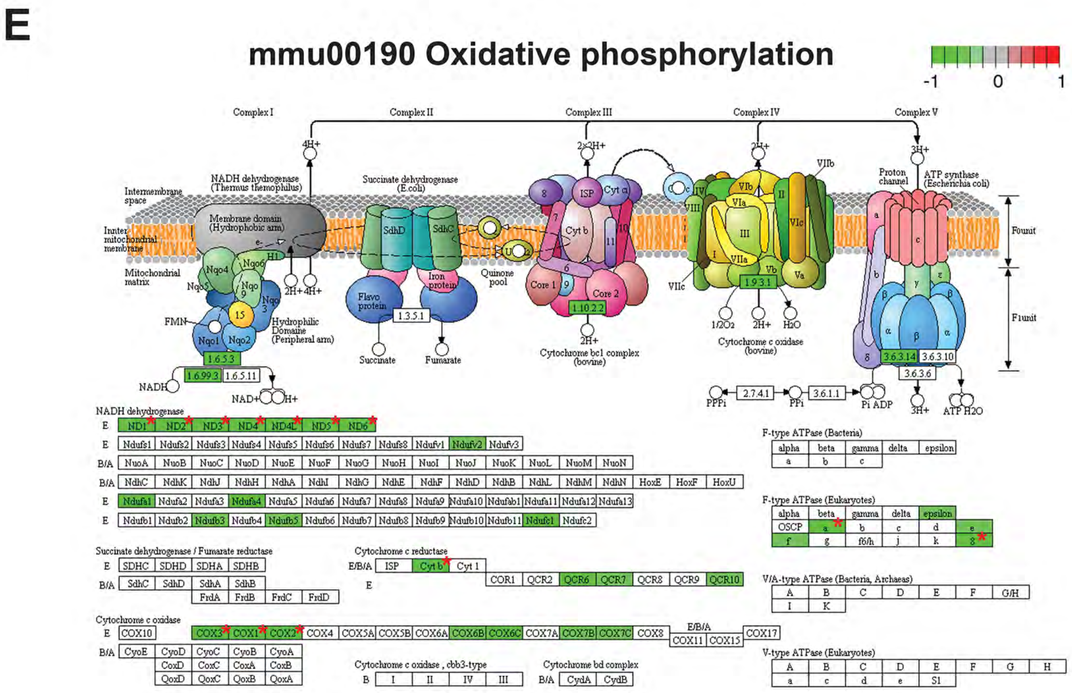

Z
Serum TG (mg/dL)
DMSO
Orlistat

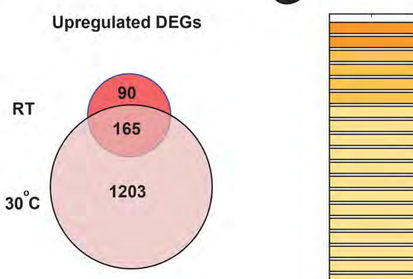

AA
Blood glucose (mg/dL)
DMSO
Sotagliflozin

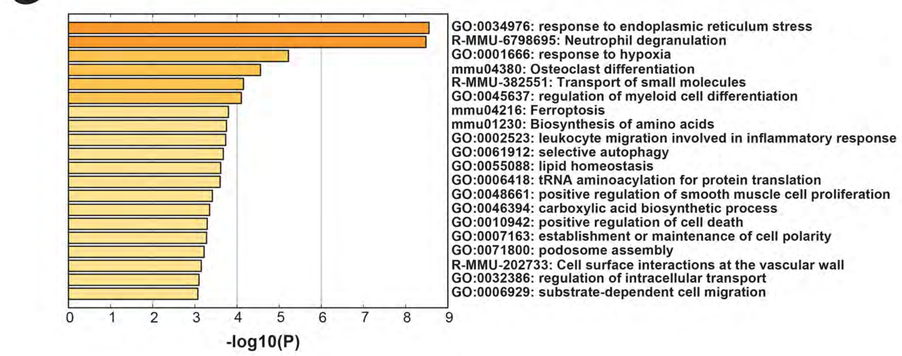


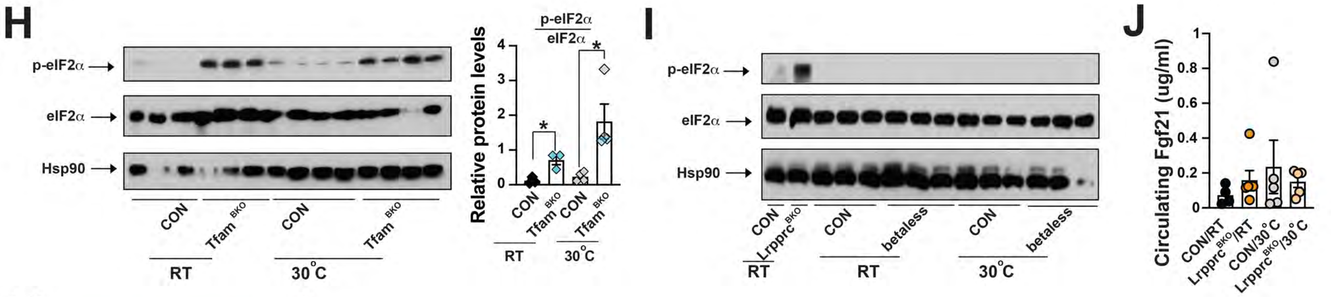


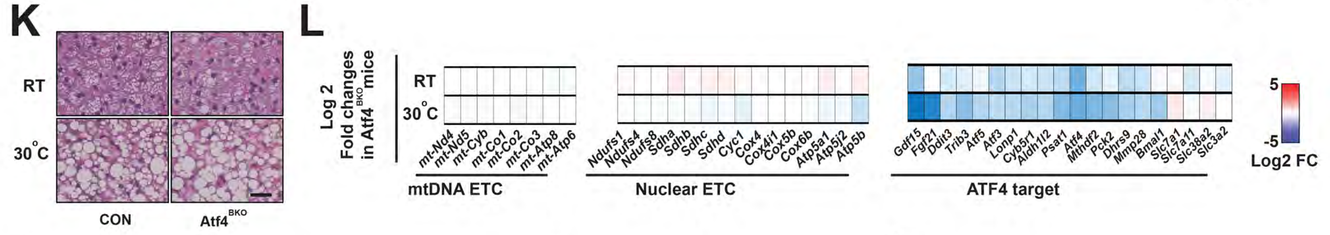


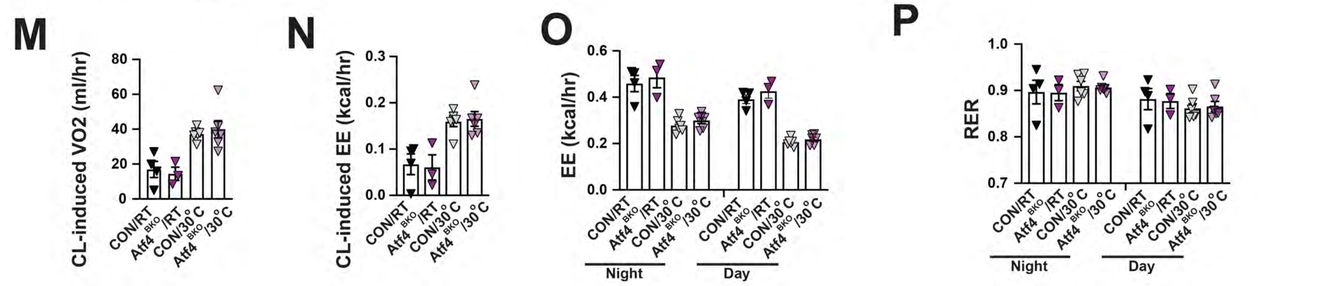


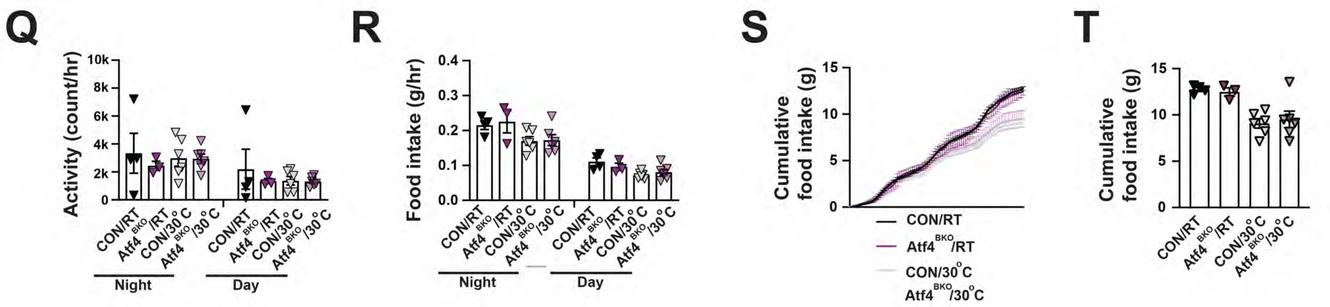


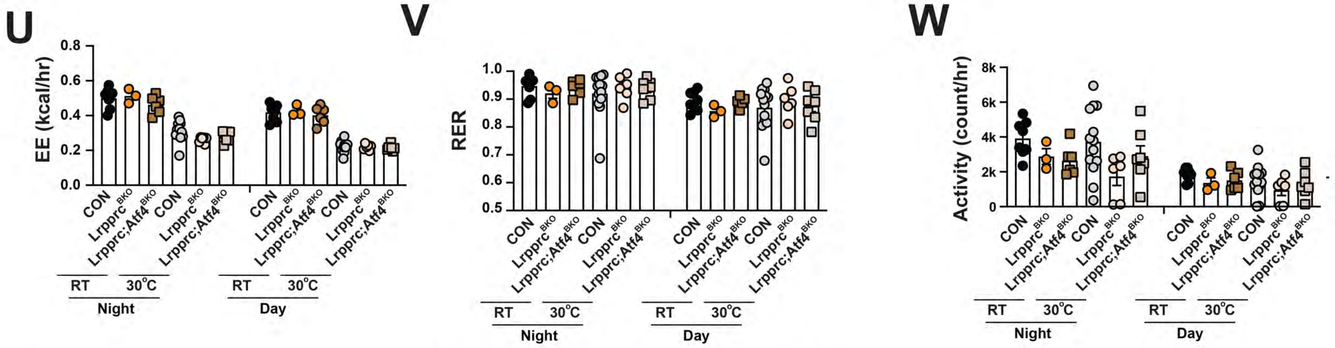


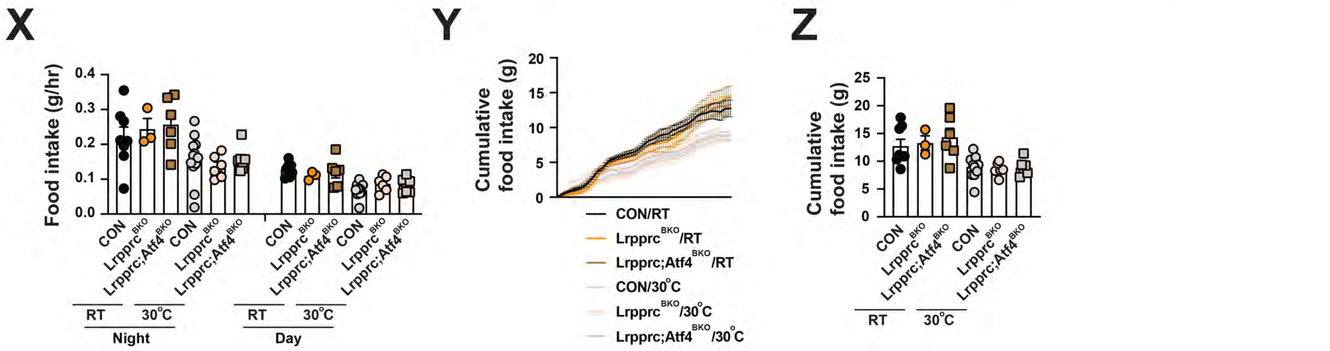




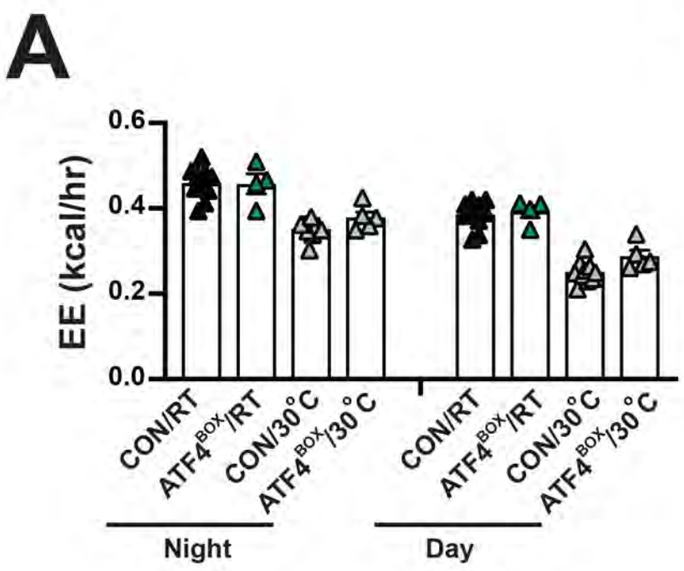


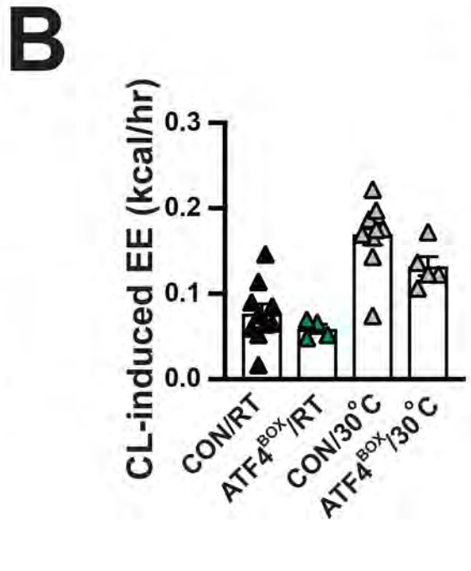


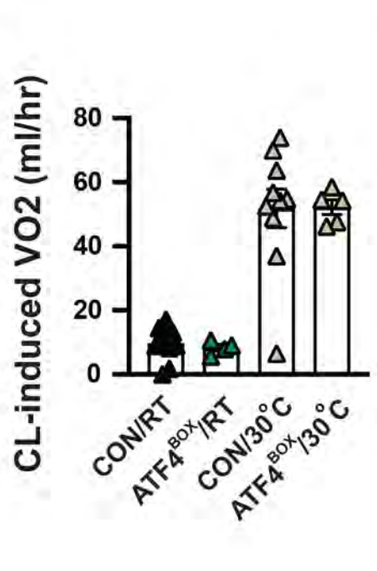


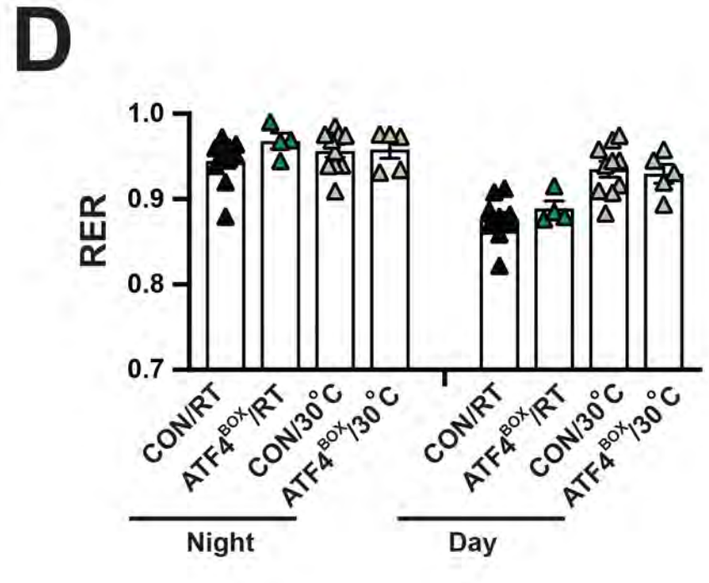


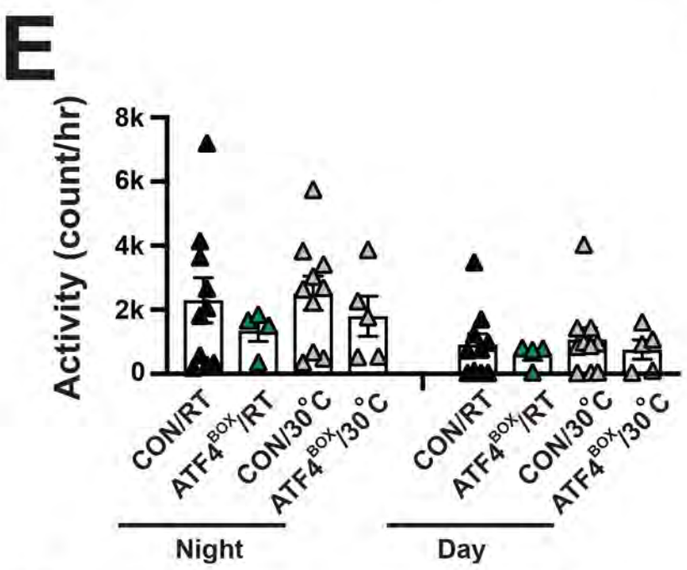
Supplementary Figure 3

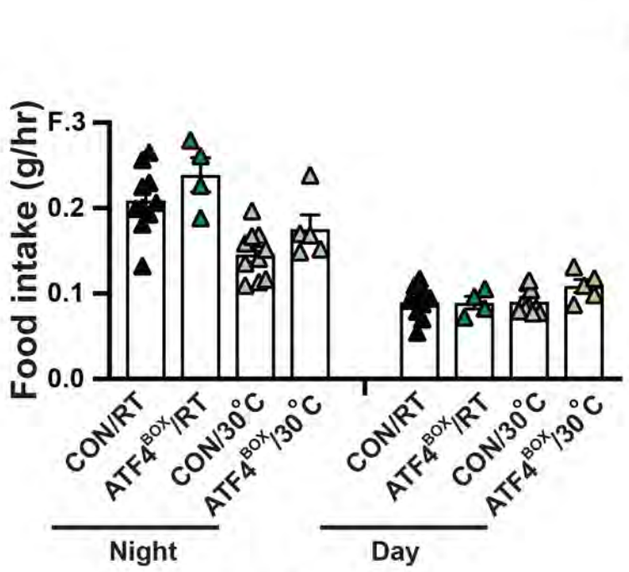


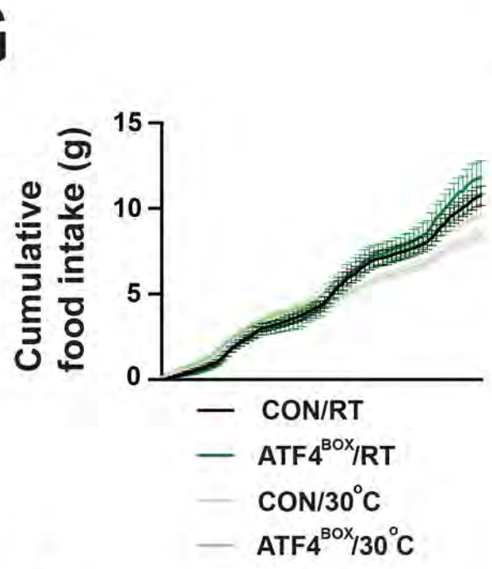


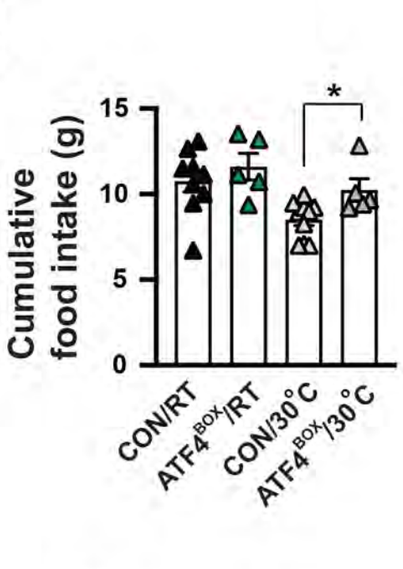


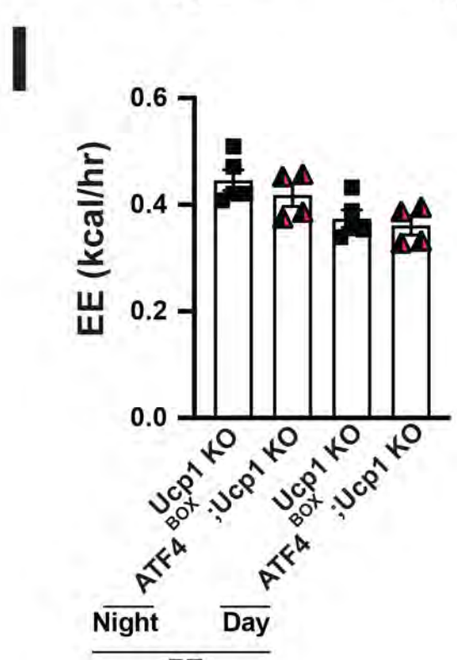


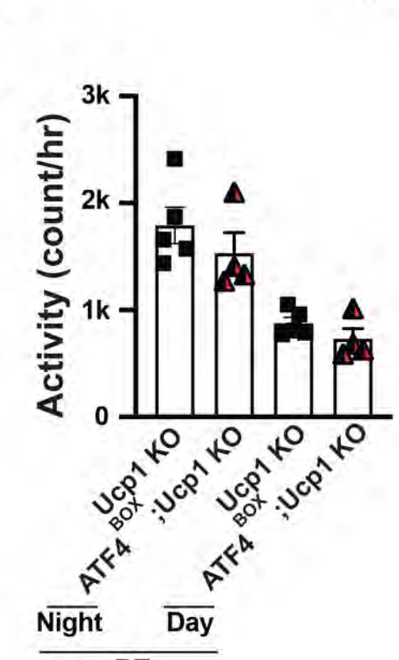


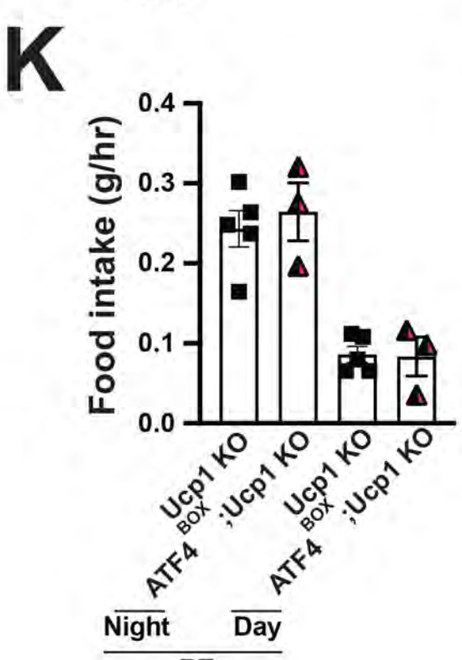


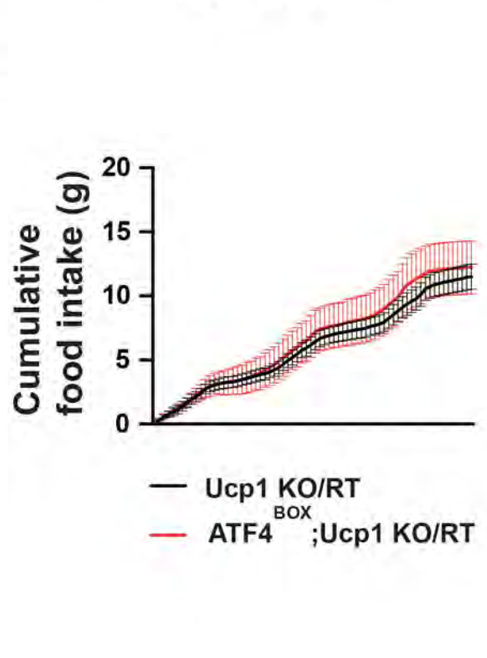


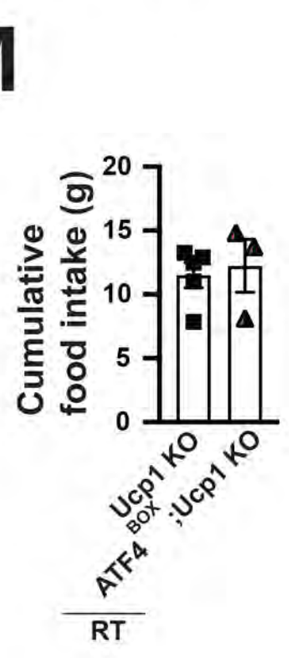


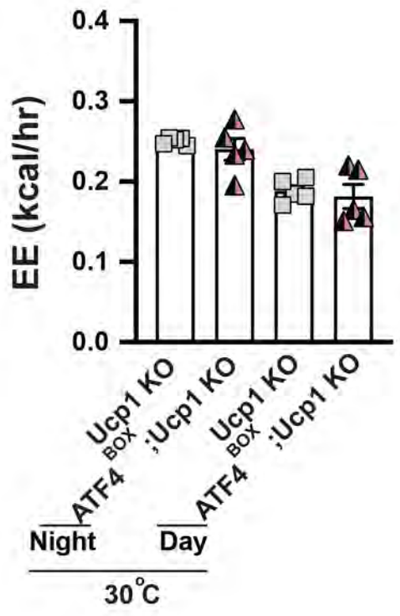


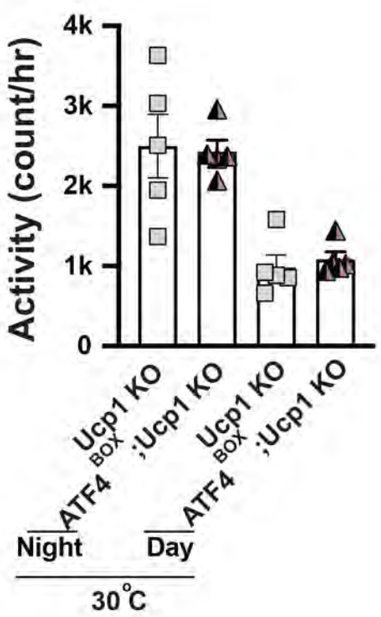


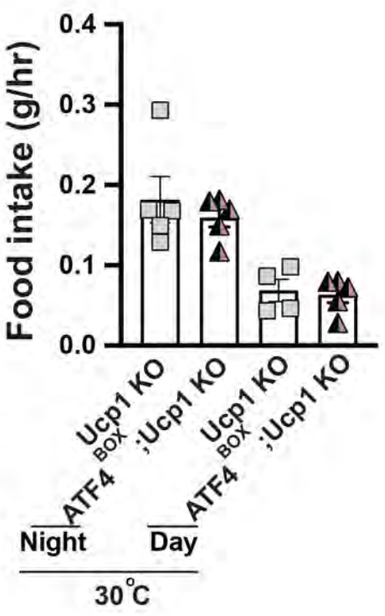


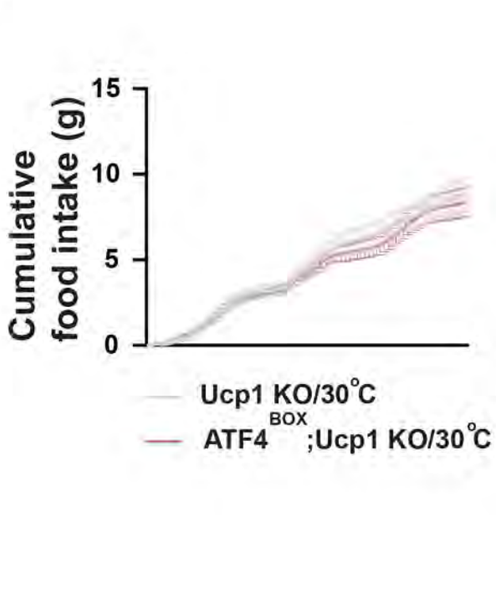


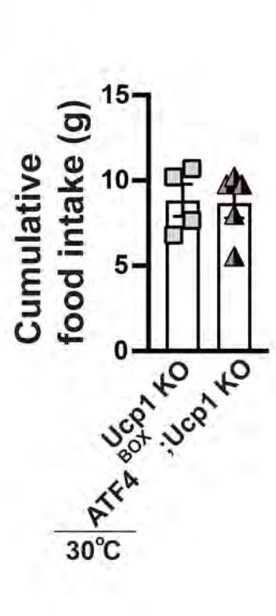








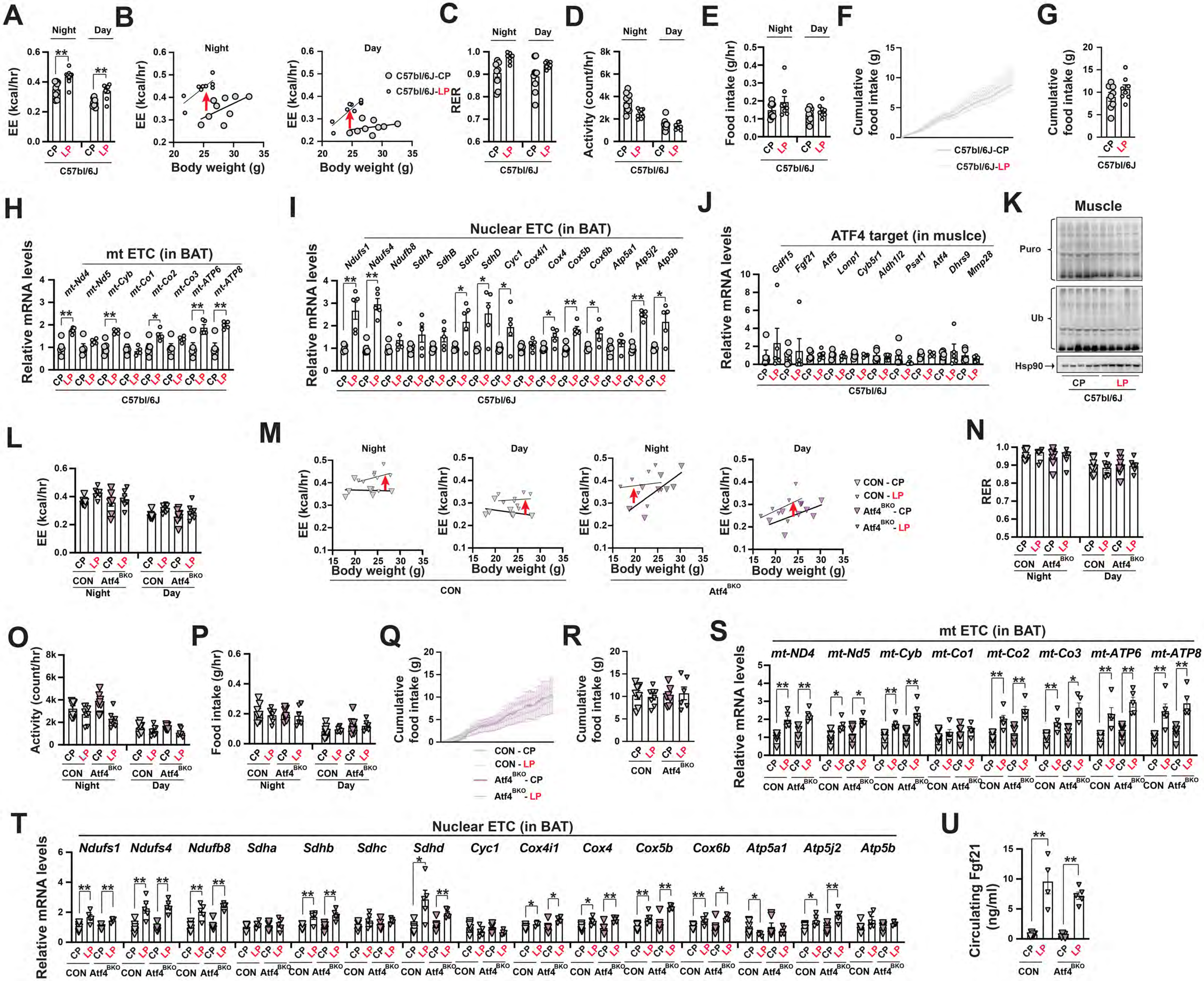




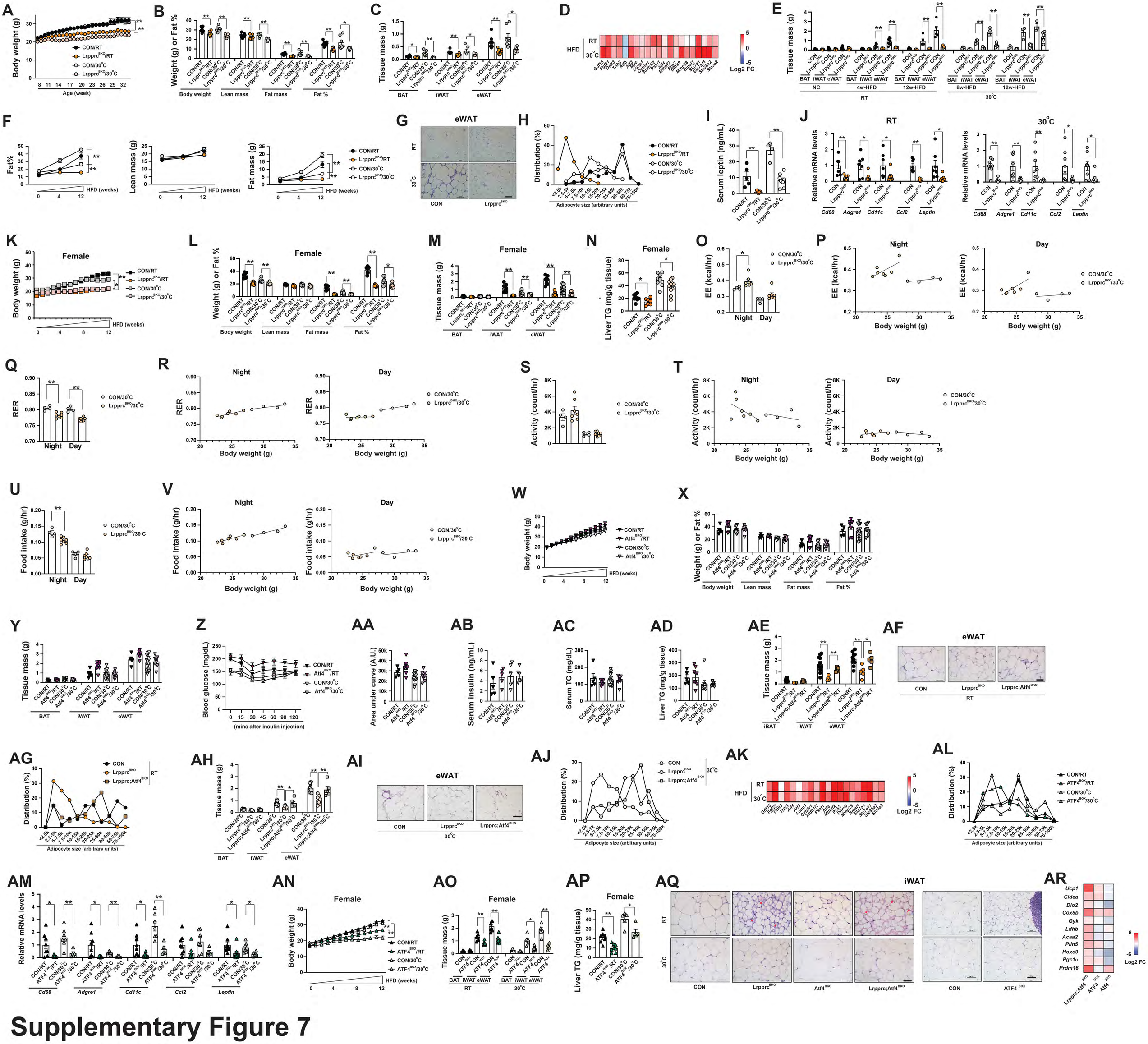
Supplementary Figure 4

Supplementary Figure 5

GO term	Hits	AdjP
Protein folding	44/92	6.96e-40
Protein translation	64/287	3.47e-34
Protein transport	56/580	9.87e-13



Supplementary Figure 6



Supplementary Figure 7