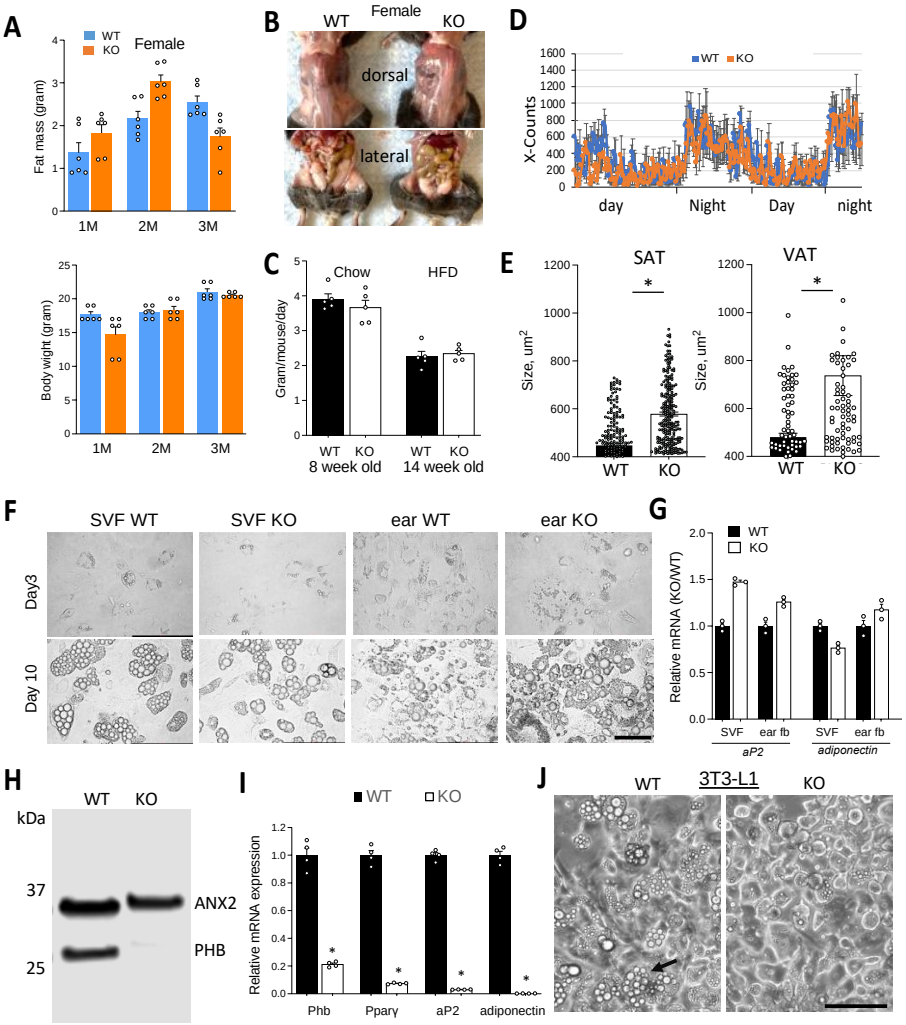

Figure S1- Adipocyte PHB KO mice display adipocyte abnormality.
A: EchoMRI measurements on female mice raised on chow reveal a trend for reduced fat mass accumulation in PHB Ad-KO littermates detectable at 3 months. N=6. *B:* Images demonstrating less subcutaneous and visceral AT in PHB Ad-KO female littermates. *C:* Food consumption measured over 2 days in PHB Ad-KO and WT littermates (N=5). *D:* Spontaneous locomotor activity measured over 2 days in PHB Ad-KO and WT littermates (N=5). *E:* Adipocyte size in males quantified. *F:* Brightfield microscopy on stromal cells derived from ears and SAT of WT and PHB Ad-KO subjected to adipogenesis. Arrows: lipid droplets, accumulating comparably in cells from WT and PHB-KO mice. *G:* RT-PCR for adipogenic genes reveals their similar expression in fibroblasts (fb) derived from SAT and ears of WT and PHB-KO mice 3 days post-induction. *H:* Immunoblotting demonstrating *Phb* knockout in 3T3-L1 preadipocytes, ANX2 antibody was probed with for loading control. For CRISPR/Cas9 guide RNA expression, the 20-bp target sequence 5'-TCAATAAGCATGTCTCCGACTGG-3' containing the PAM sequence (underlined) was ligated into *Lenti-CRISPR v2* plasmid (Addgene #52961). *I:* RT-PCR for adipogenic genes reveals their reduced expression in *Phb*-KO 3T3-L1 adipocytes. Primers were as follows: *Pparg*: 5'- TTTAAAAACAAGACTACCCTTTACTGAAATT-3' and 5'-AGAGGTCCACAGAGCTGATTCC-3'; *Apn*: 5'-TGTTCCTCTTAATCCTGCCCA-3' and 5'-CCAACCTGCACAAGTTCCCTT-3'; *aP2*: 5'-GATGAAATCACCGCAGACGAC-3' and 5'-ATTCCACCACCAGCTTGTCAC -3'. *J:* Lipid droplet formation (arrow) efficient in parental 3T3-L1 adipocytes but not in *Phb*-KO 3T3-L1 adipocytes. In all panels, plotted are mean ± SEM. **P*<0.05 (Student's t-test). Scale bar: 50 µm.

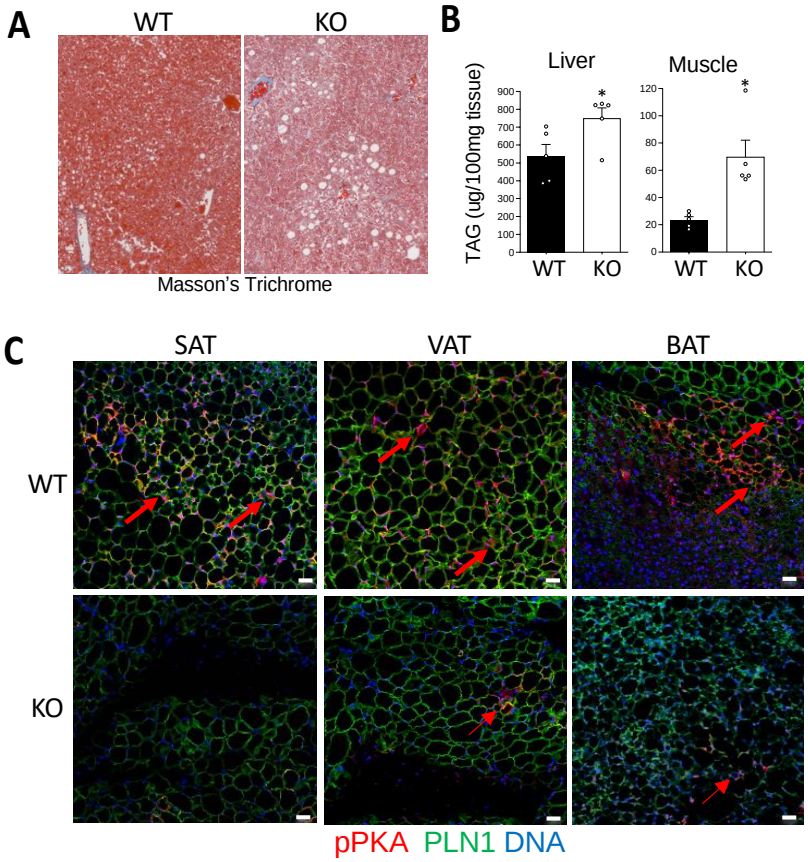

Figure S2- Lipid metabolism disbalance in PHB Ad-KO mice.
A: Masson's trichrome staining reveals lipid droplet deposition in livers of PHB Ad-KO mice. *B:* Measurement of extracted triglycerides used for quantification of ectopic lipid deposition in livers and gastrocnemius muscle of Ad-KO mice. EnzyChrom™ Triglyceride Assay Kit from BioAssay System (ETGA-200) was used. *C:* IF analysis of PKA substrate phosphorylation (antibody from CellSignaling, Cat. #9621, 1:100) in adipocytes of SAT, VAT and BAT identified based on perilipin-1 (PLN1) expression. Phospho-PKA (arrows) is lower in Ad-KO tissues. In all panels, plotted are mean ± SEM. **P*<0.05 (Student's t-test). Scale bar: 50 µm.

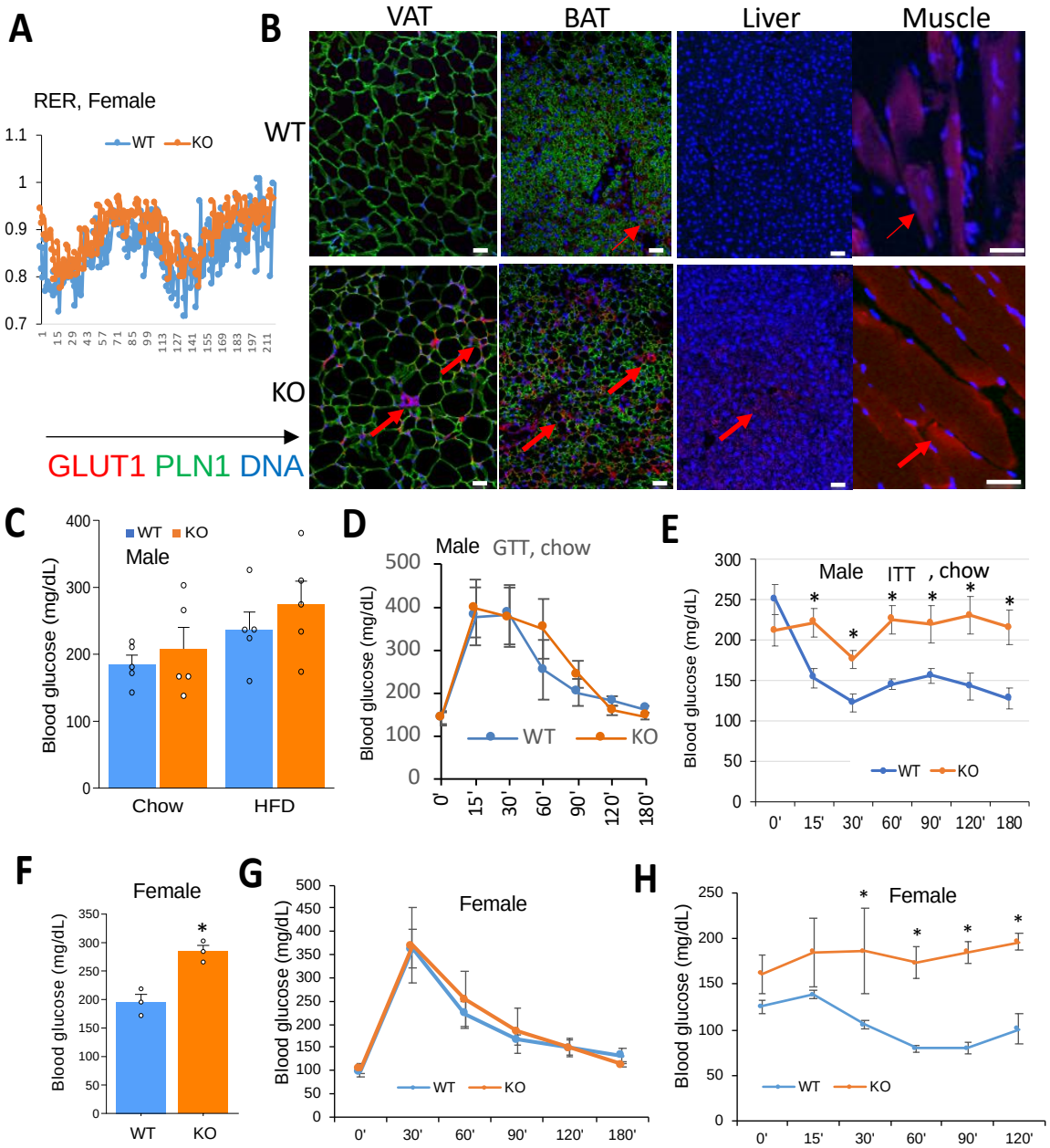

Figure S3- Metabolic phenotype of PHB Ad-KO males and females.
A: Respiratory exchange ratio (RER) shows an increase in PHB Ad-KO female mice. *B:* IF for GLUT1 (Antibody from Mybiosourse MBS179154, 1:100) demonstrates its increased expression in VAT, BAT, liver and muscle of PHB Ad-KO mice. *C:* Steady-state blood glucose levels in PHB Ad-KO and WT males. *D:* Glucose tolerance test in male mice raised on chow (N=5). *E:* Insulin tolerance test (ITT) in male mice raised on chow (N=5). *F:* Steady-state blood glucose levels in PHB Ad-KO and WT female littermates raised on chow. *G:* Glucose tolerance test in PHB Ad-KO and WT female littermates raised on chow (N=5). *H:* Insulin tolerance test (ITT) in PHB Ad-KO and WT female littermates raised on chow (N=5). In all panels, plotted are mean ± SEM; *P<0.05 (Student's t-test). Scale bar: 50 µm.

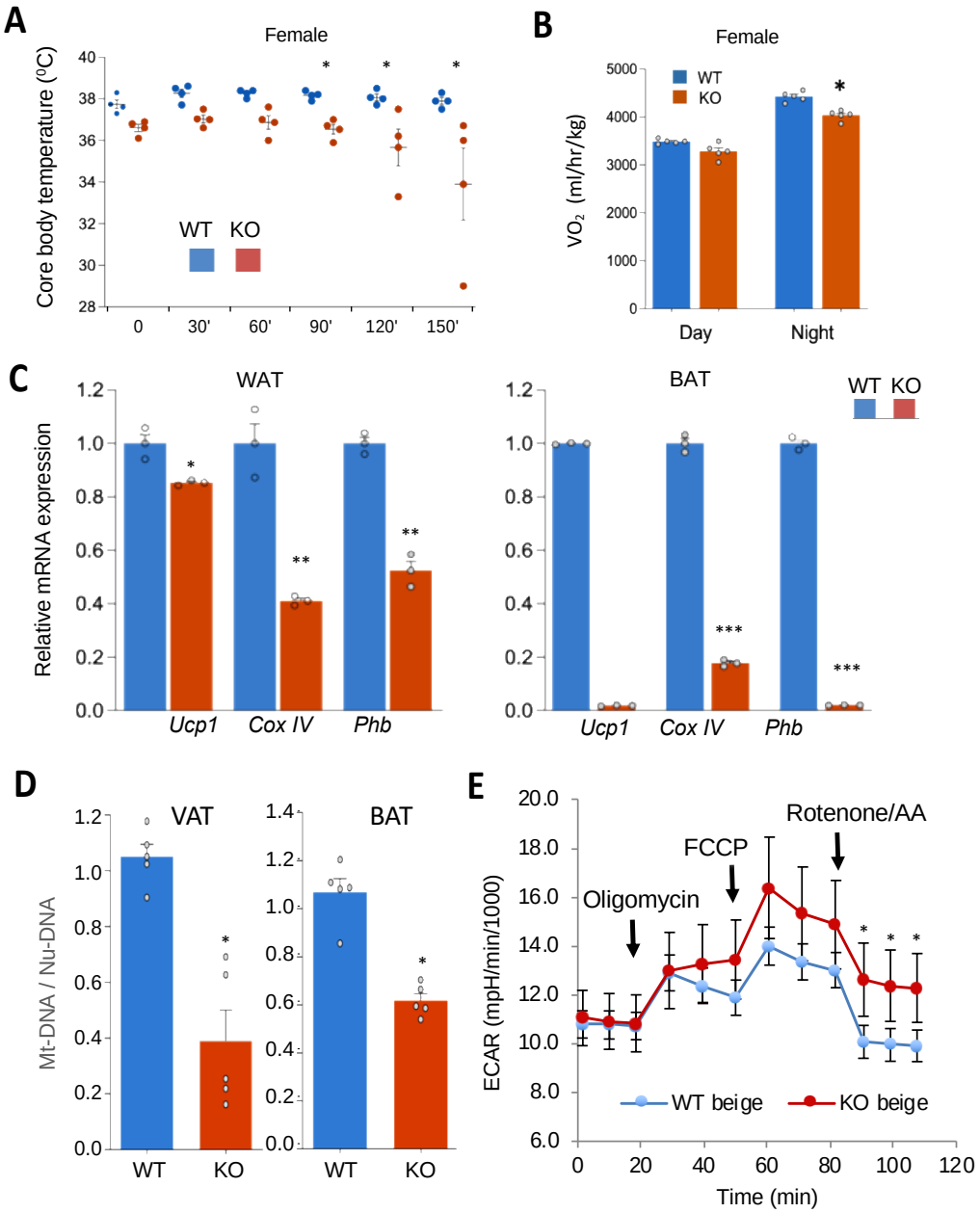

Figure S4- Mitochondrial dysfunction in BAT of PHB Ad-KO mice.
A: Reduced cold tolerance of female PHB Ad-KO mice at 4ºC (N=4). *B:* Indirect calorimetry data over 2 days showing reduced oxygen consumption by PHB Ad-KO female mice (N=4). *C:* Quantitative RT-PCR analysis of *Ucp1*, *Cox IV* and *Phb* mRNA expression, normalized to 18S RNA, in WAT and BAT. Shown is a drastic decrease of *Ucp1* and *Cox IV* in BAT of PHB Ad-KO littermates. *D:* RT-PCR with primer pairs specific for mitochondrial DNA and genomic DNA reveals a relatively low mitochondrial DNA content in both WAT and BAT of PHB Ad-KO mice (N=5). Mouse mt-DNA 5'-GCC AGC CTG ACC CAT AGC CAT AAT AT-3' and 5'-GAG AGA TTT TAT GGG TGT AAT GCG G-3'; Nuc-DNA forward: 5'-TTG AGA CTG TGA TTG GCA ATG CCT-3' and 5'-CCT TTA ATG CCC ATC CCG GAC T-3'. *E:* Analysis of data from Fig. 3H on cells from PHB Ad-KO and WT mice. Extracellular acidification rate (ECAR), measured upon successive treatment with oligomycin, FCCP (Carbonyl cyanide-p-trifluoromethoxyphenyl-hydrazone) and rotenone / antimycin A, demonstrates an increase in PHB-KO adipocytes. Plotted are mean ± SEM; *P<0.05 (Student's t-test).

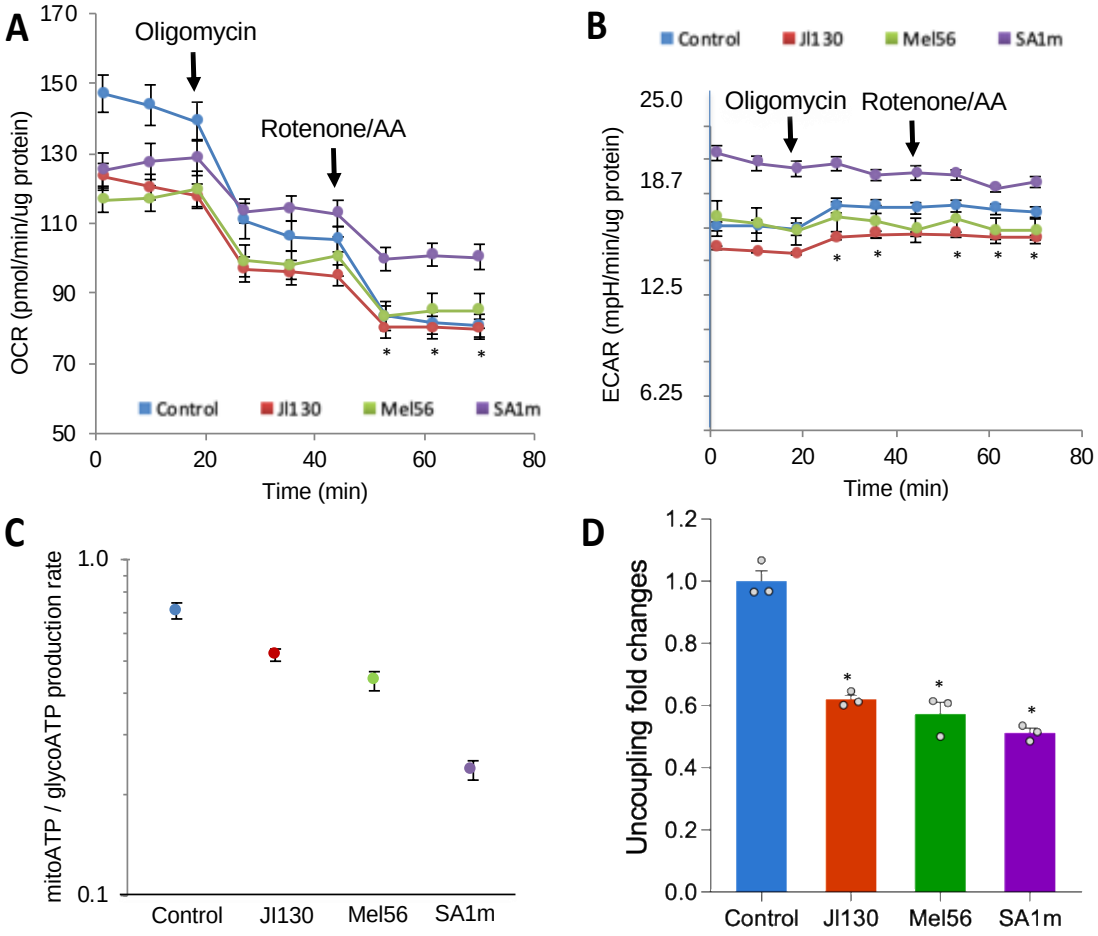

Figure S5- PHB modulators suppress mitochondrial respiration and uncoupling.
Mouse brown preadipocytes were treated with indicated PHB ligands during brown adipogenesis induction and subjected to Seahorse Real-time ATP Rate Assay on day 7. *A*: Decreased mitochondrial respiration in treated cells. *B:* Decreased extracellular acidification in treated cells. *C:* XF ATP Rate Index indicating a shift to glycolytic ATP production in treated cells. *D:* Decreased ATP-uncoupled respiration in treated cells. Plotted are mean ± SEM; *$P<0.05$ (Student's t-test).