Akt3 Inhibits Adipogenesis and Protects from Diet-Induced Obesity via Novel Signaling Pathway

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SUPPLEMENTAL FIGURES

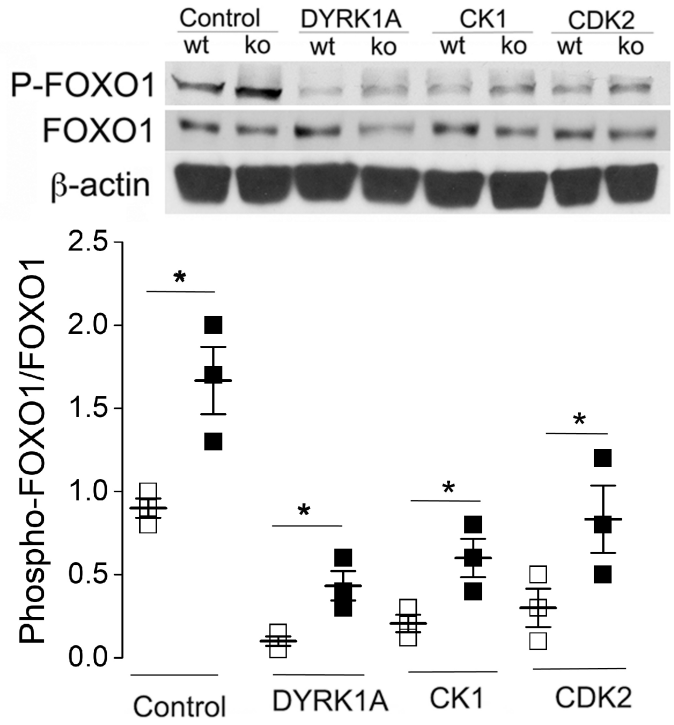

Figure S1. Dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A), casein kinase 1 (CK1), and cyclin-dependent kinase 2 (CDK2) are not involved in enhanced phosphorylation of FOXO1 in Akt3$^{-/-}$ MEFs. WT and Akt3$^{-/-}$ cells were treated with DYRK1A inhibitor (harmine, 1 μM), CK1 inhibitor (D4476, 50 μM) or Cdk2 Inhibitor II (1 μM) overnight. Phosphorylation and expression of FOXO1 were assessed by Western blotting analysis using protein samples extracted from these cells. Data represent means ± SEM. **P*<0.05.

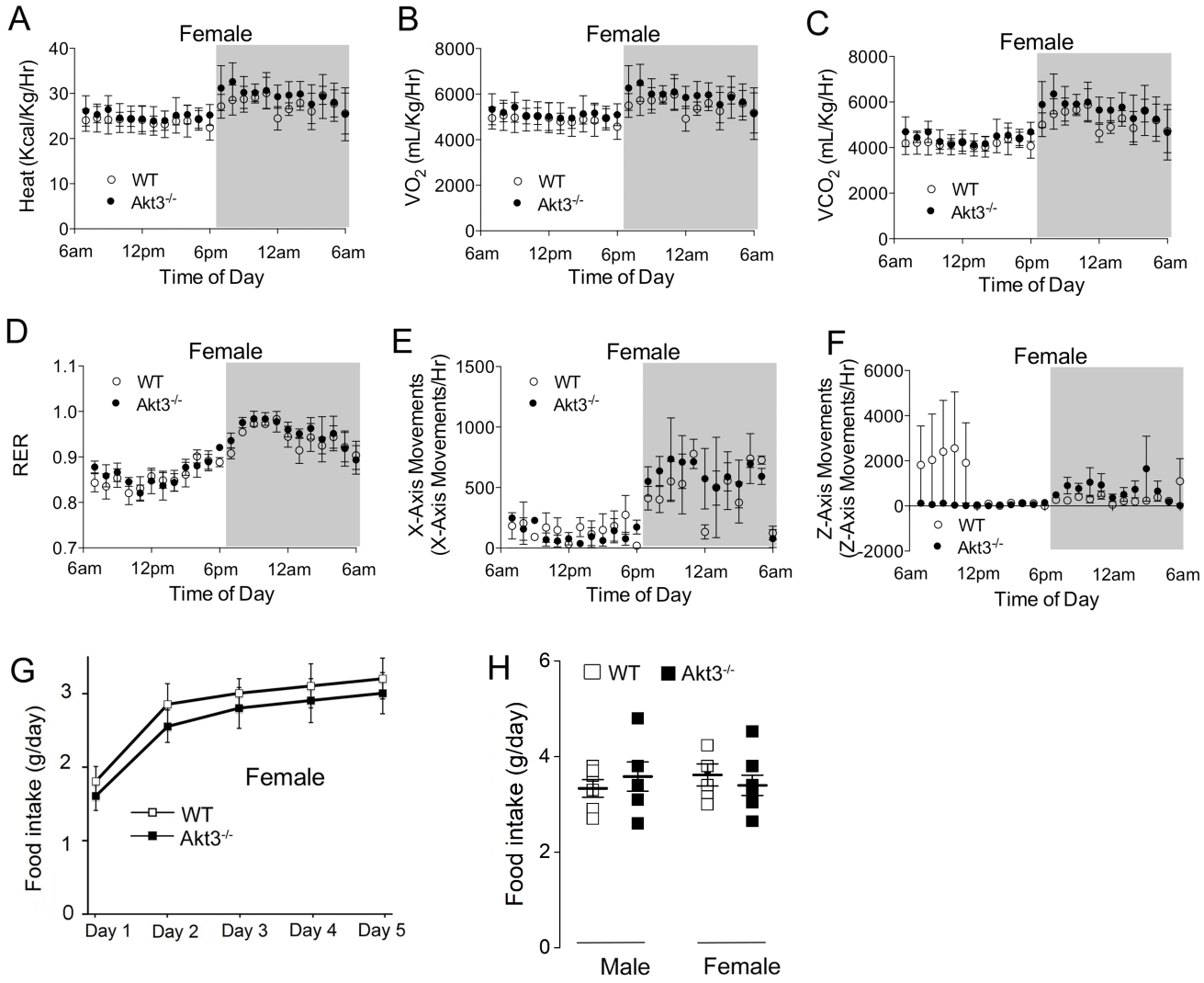

Figure S2. Energy expenditure and Food intake are not significantly changed in *female Akt3*$^{-/-}$ mice. Energy expenditure (A-D), locomotor activity (E-F) and food intake (G-H) were analyzed in 8-week-old *WT* and *Akt3*$^{-/-}$ mice fed a chow diet using CLAMS system (comprehensive laboratory animal monitoring system). Mice were allowed to equilibrate in cages at room temperature (22°C) for 72 hours before 24 hours of continuous data collection. (A) Heat production, VO2 (B), VCO2 (C) and respiratory exchange ratio (RER, D), X-axis (E) and Z-axis (F) movements in female WT and Akt3-/- mice during day and night cycles. n=3 per genotype. (G) Food intake in female *WT* and *Akt3*$^{-/-}$ mice fed a HFD during the first 5-days of transfer to HFD. n=6. (H) Food consumption of *WT* and *Akt3*$^{-/-}$ mice fed on HFD for 20 weeks (n=8). The food consumption of each animal was measured for 5 consecutive days and the average daily food consumption was calculated. Data represent means ± SEM.

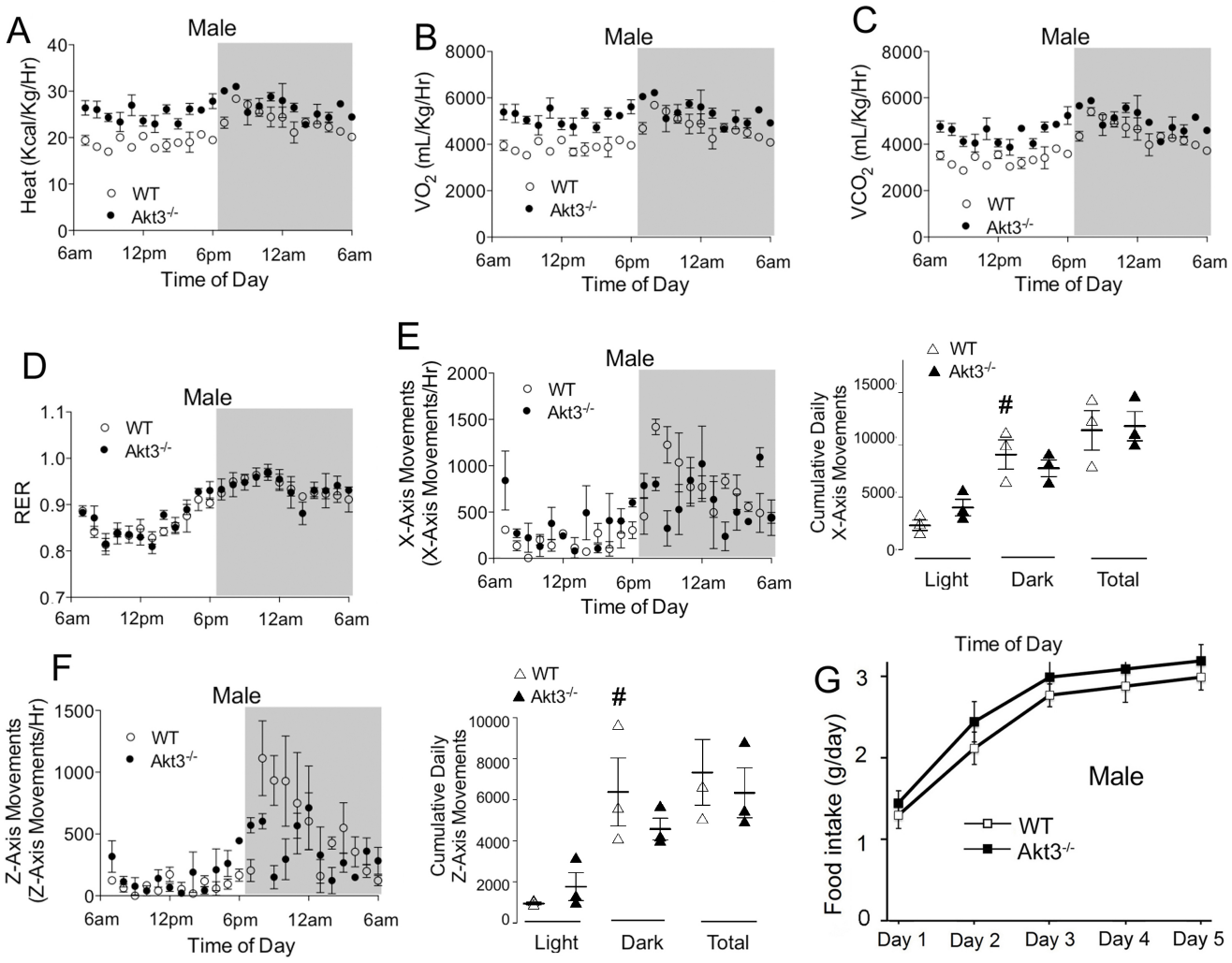

Figure S3. Increased energy expenditure in male *Akt3*$^{-/-}$ mice. Energy expenditure (A-C), RER (D), locomotor activity (E-F), and food intake (G) were analyzed in 8-week-old *WT* and *Akt3*$^{-/-}$ male mice fed a chow diet for five days using CLAMS system. Mice were allowed to equilibrate in cages at room temperature (22°C) for 72 hours before 24 hours of continuous data collection. Total X-axis movements (E, right panel) and total Z-axis movements (F, right panel) during the light periods, dark periods and 24-hour periods. n=3 mice per genotype. (G) Food intake in male *WT* and *Akt3*$^{-/-}$ mice fed a HFD during the first 5-days of transfer to HFD. n=6. Data represent means ± SEM.

(E, F) Cumulative data graphs were analyzed using two-way ANOVA. #, $p<0.05$ vs light, same genotype.

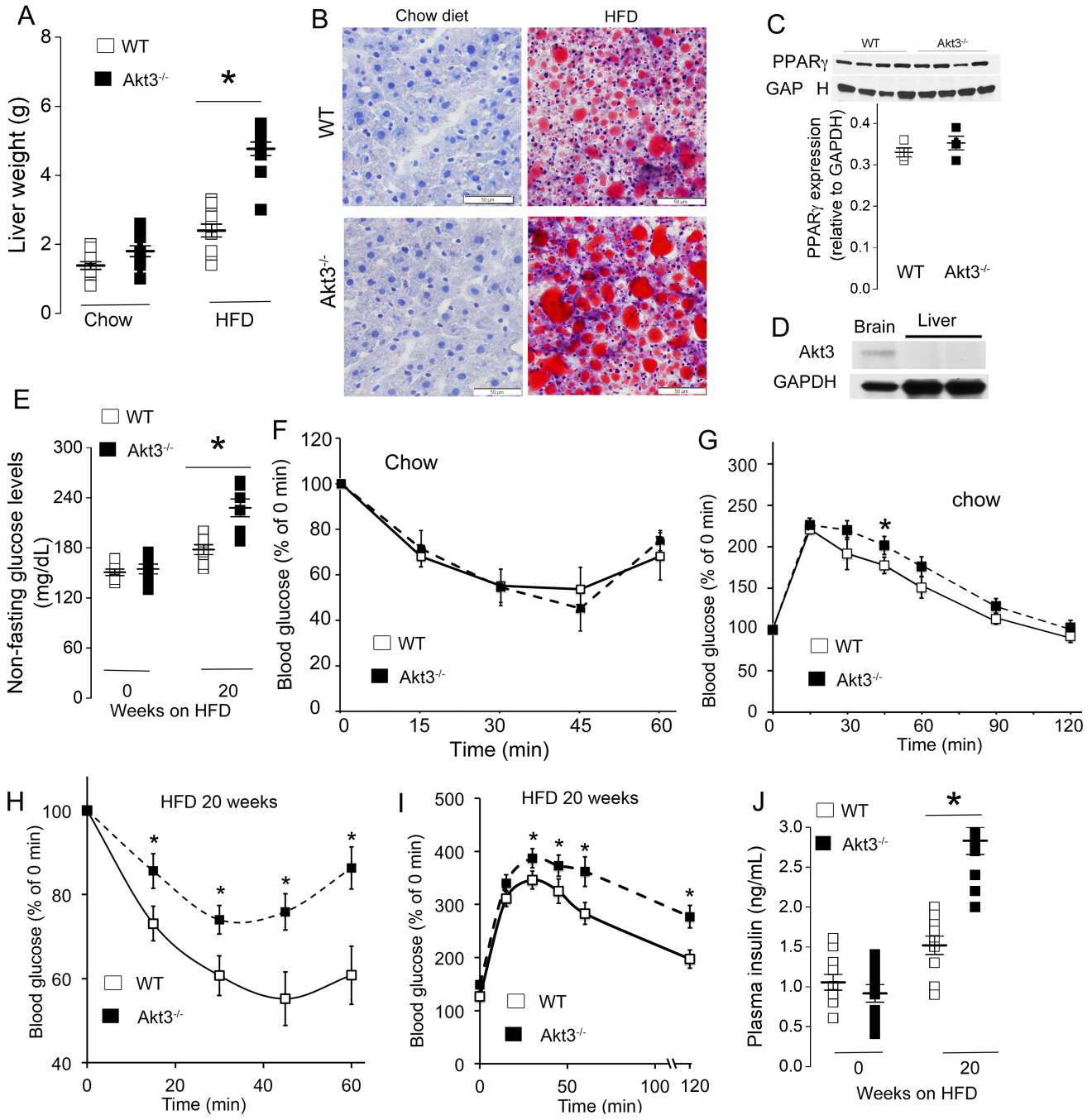

Figure S4. Increased liver steatosis in *Akt3*$^{-/-}$ mice fed HFD and glucose tolerance and systemic insulin sensitivity are impaired in obese *Akt3*$^{-/-}$ mice. (A) Liver weights of *WT* and *Akt3*$^{-/-}$ mice on a chow diet (n=12) or a HF diet (n=12). (B) Oil Red O staining of liver of *WT* and *Akt3*$^{-/-}$ mice fed a chow diet or high fat diet. The result shows pronounced liver steatosis in obese female *Akt3*$^{-/-}$ mice. Scale bar= 50 µm. (C) Expression of PPARγ in liver of WT and Akt3$^{-/-}$ mice fed a chow diet. (D) Expression of Akt3 in brain and liver of Akt3$^{-/-}$ mice. Brain protein was used as a positive control. (E) Non-fasting blood glucose levels in age-matched, sex-matched *WT* and *Akt3*$^{-/-}$ mice before (0 week) and after they were fed a high fat diet (HFD) for 20 weeks. n=8. (F) Insulin tolerance test. Blood glucose levels in age-matched, sex-matched *WT* and *Akt3*$^{-/-}$ mice on a chow diet were determined at the indicated times after intraperitoneal injection with a bolus of insulin. n=10 (5 male, 5 female). (G)

Glucose tolerance test. Blood glucose levels in age-matched, sex-matched WT or *Akt3*$^{-/-}$ mice on a chow diet were determined at the indicated times after intraperitoneal injection with a bolus of glucose. n=10 (5 male, 5 female). (H) Insulin tolerance test. Blood glucose levels in *Akt3*$^{-/-}$ or *WT mice*. n=10 (5 male, 5 female) mice with 16 weeks of high fat diet feeding after intraperitoneal injection with a bolus of insulin. (I) Glucose tolerance test. Blood glucose levels in *Akt3*$^{-/-}$ or *WT* mice after 16 weeks of HFD feeding after intraperitoneal injection with a bolus of glucose. n=11(5 male, 6 female) (J) Plasma insulin levels in *WT* and *Akt3*$^{-/-}$ mice on a chow diet (20 weeks old) or a HFD (24 weeks old). n=10 (5 male, 5 female). Data represent means ± SEM. **P*<0.05.

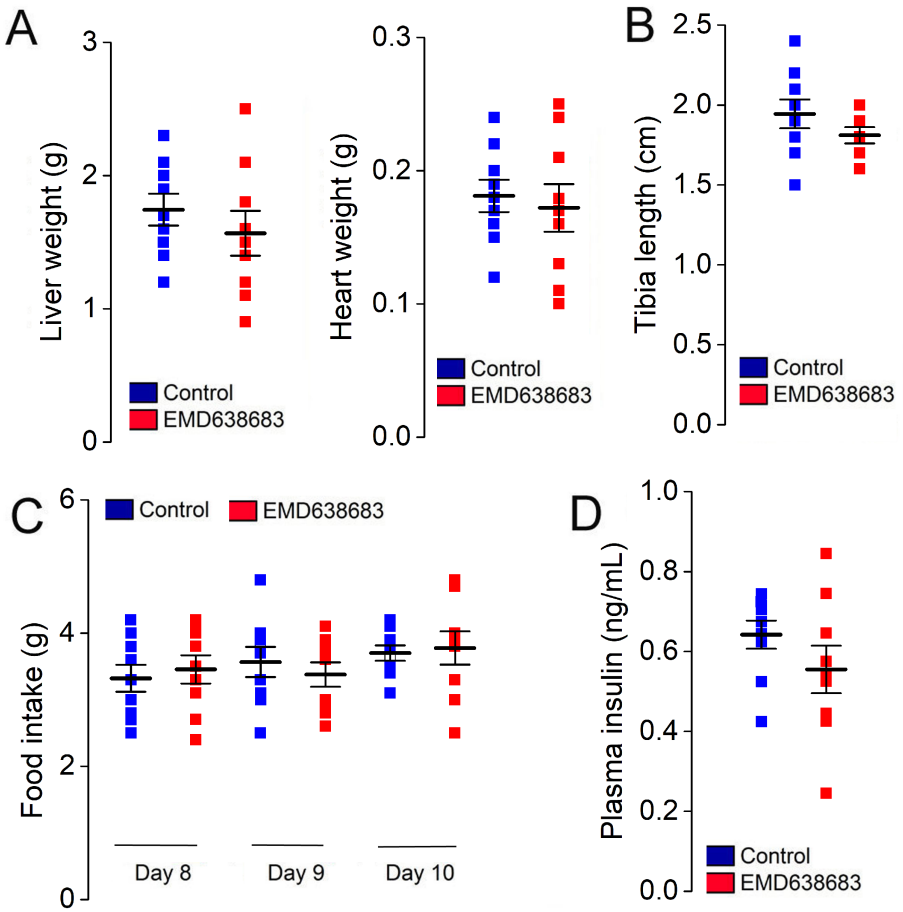

Figure S5. EMD638683 treatment has no effect on liver weight, tibia length and food intake. (A-B) There is no significant change in liver weight, heart weight (A) and tibia length (B) between EMD638683-treated and control mice. (C) Food intake is similar between EMD638683-treated and control mice. (D) Plasma insulin levels are lower in mice treated with EMD638683 compared to control ones, although this difference did not reach statistical significance. Data represent means ± SEM.

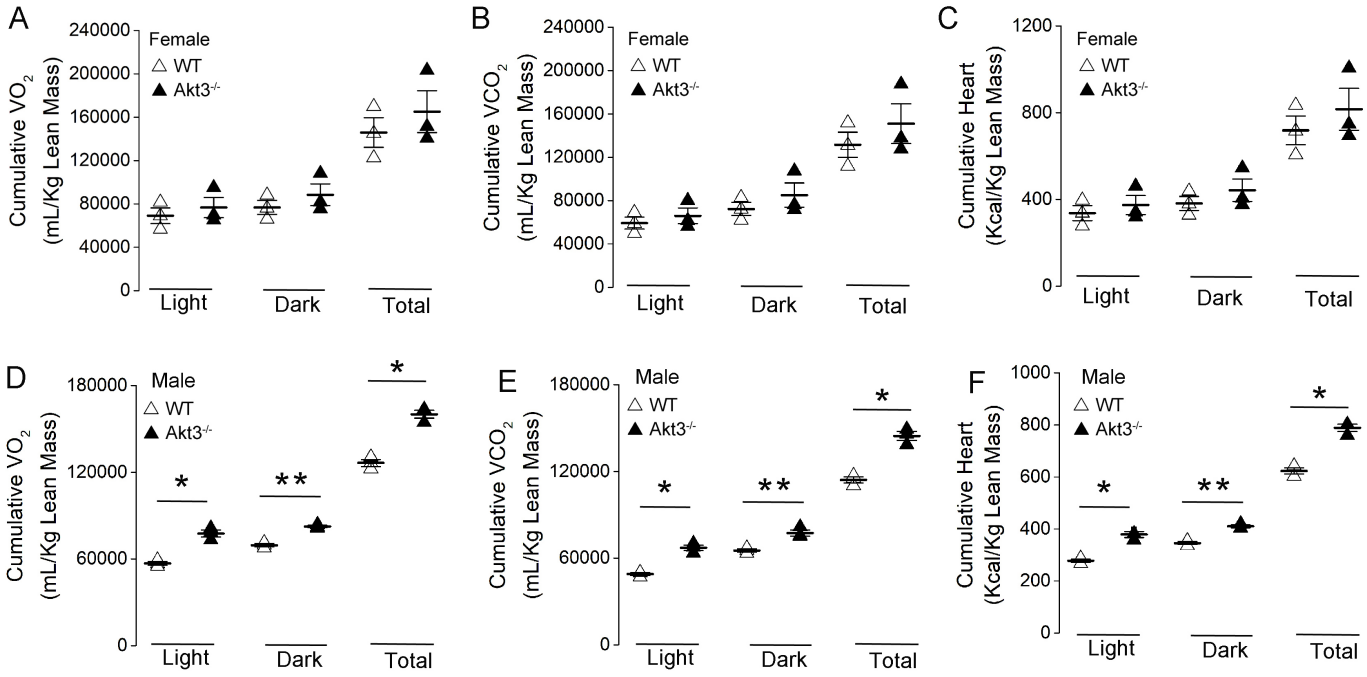

Figure S6. Energy expenditure in male and female *Akt3*$^{-/-}$ mice per lean mass. Energy expenditure was analyzed in 8-week-old *WT* and *Akt3*$^{-/-}$ male mice fed a chow diet for five days using CLAMS system. Mice were allowed to equilibrate in cages at room temperature (22°C) for 72 hours before 24 hours of continuous data collection. Cumulative VO2 (A, D), total VCO2 (B, E), and cumulative heat production (C, F) were calculated during the 12-hour light periods, 12-hour dark periods and 24-hour periods. All measurements were normalized to lean mass. n=3 mice per genotype. Data represent means ± SEM. * $p<0.01$ vs WT, same time of day; **, $p<0.05$ vs WT, same time of day.