Supplemental Information for:

Activation of Crtc2/Creb1 in skeletal muscle enhances weight loss during intermittent fasting

Nelson E. Bruno[1]*, Jerome C. Nwachukwu[1], David C. Hughes[2], Sathish Srinivasan[1], Richard Hawkins[3], David Sturgill[4], Gordon L. Hager[4], Stephen Hurst[5], Shey-Shing Sheu[5], Sue C. Bodine[2], Michael D. Conkright[3], Kendall W. Nettles[1]*

[1]Department of Integrative Structural and Computational Biology, The Scripps Research Institute, 130 Scripps Way, Jupiter FL 33458, USA

[2]Section for Endocrinology and Metabolism, Department of Internal Medicine, University of Iowa, 285 Newton Rd, Iowa City, IA 52242

[3]Department of Cancer Biology, The Scripps Research Institute, 130 Scripps Way, Jupiter FL 33458, USA

[4]Laboratory of Receptor Biology and Gene Expression, National Cancer Institute, NIH, Bethesda, MD 20892, USA

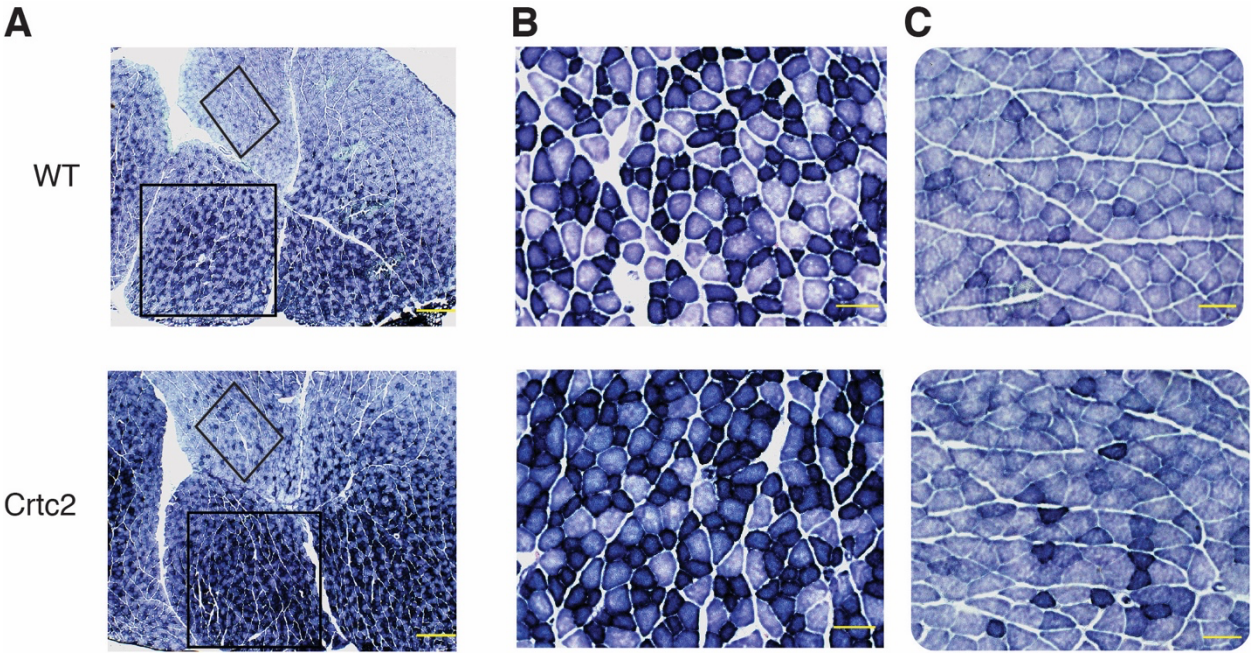
[5]Center for Translational Medicine, Department of Medicine, Thomas Jefferson University, Philadelphia, PA, 19107, USA.

Correspondence: Nelson E Bruno, nbruno@scripps.edu; Kendall Nettles, knettles@scripps.edu

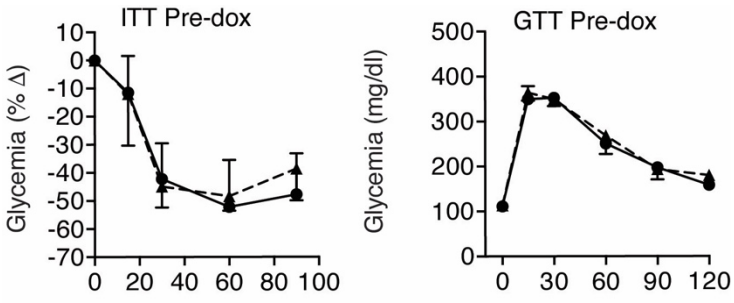
Pathway name	# Entities found	# Entities total	FDR
Extracellular matrix organization	45	329	3.98E-14
Assembly of collagen fibrils and other multimeric structures	17	67	2.12E-14
MET promotes cell motility	10	45	5.98E-07
Elastic fibre formation	12	46	6.13E-11
Regulation of Insulin-like Growth Factor (IGF) transport and uptake	13	127	2.47E-05
Neutrophil degranulation	26	480	2.35E-05
Immune System	79	2823	7.75E-06
Signaling by Receptor Tyrosine Kinases	23	622	7.13E-04
Signaling by Interleukins	25	639	0.049

Supplemental Table 1. Gene pathway enrichment for secreted proteins among DEGs regulated by Crtc2 during AD

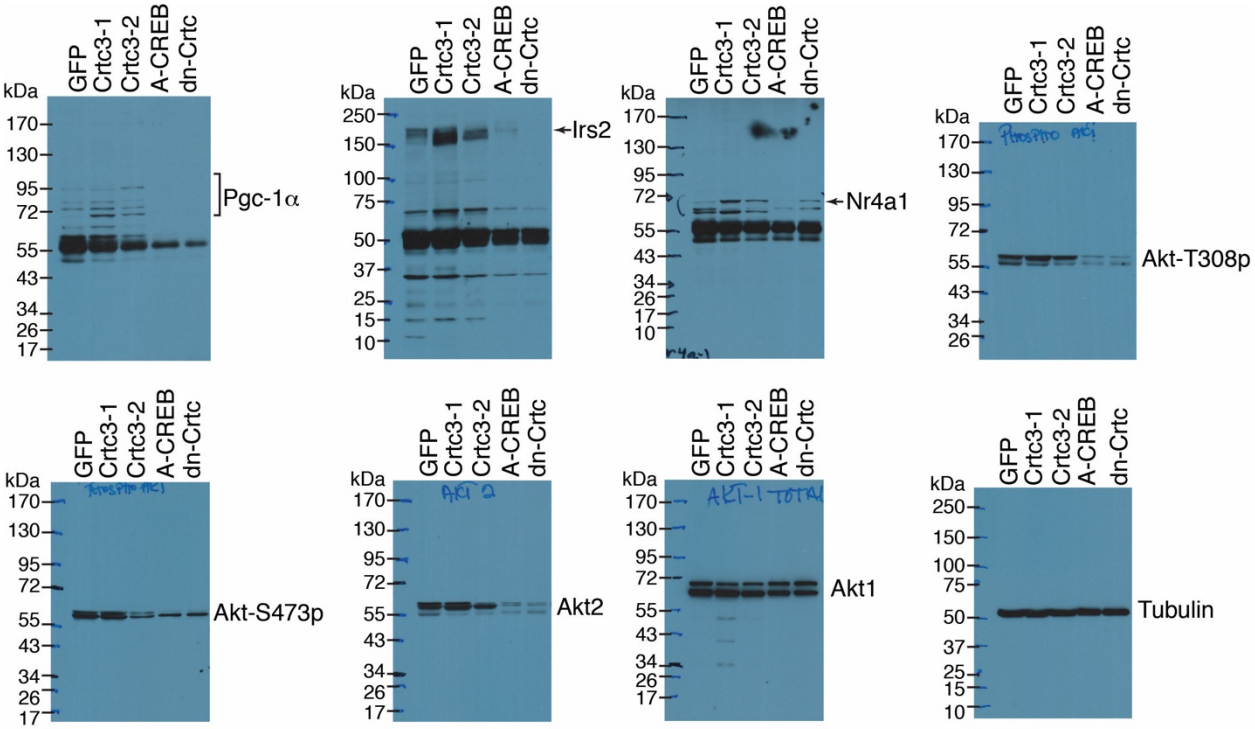
Supplemental Figures



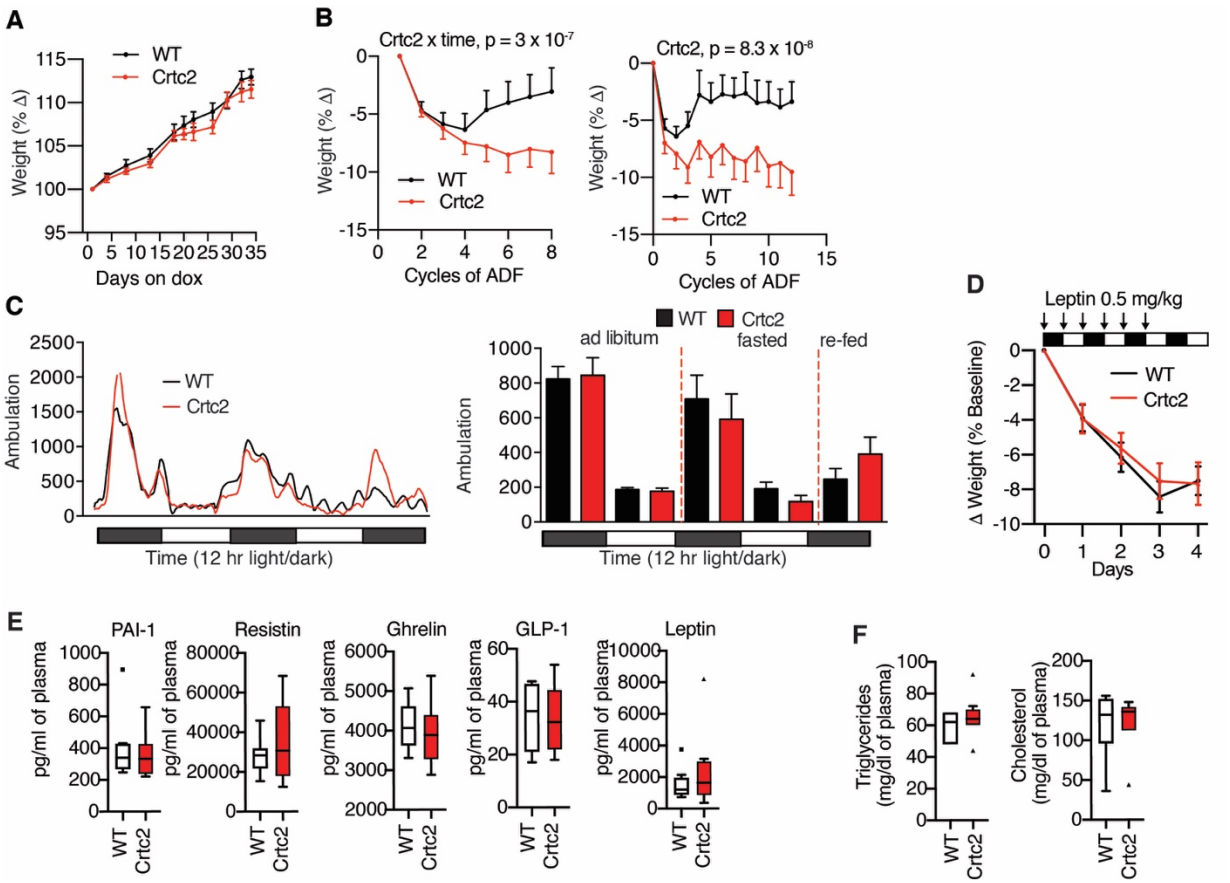
Supplemental Fig 1. Analysis of succinate dehydrogenase in skeletal muscle fibers.
(**A–C**) Histological analysis of succinate dehydrogenase in myofibers from gastrocnemius muscle sections of WT and Crtc2 mice after Dox treatment. Boxes highlight areas of lighter and darker staining and were selected by visual inspection. **B**) Scale bar = 2 mm. **C**) Scale bar = 100 μm. Close up from boxed areas in **B**)



Supplemental Fig. 2. Glucose tolerance test controls
GTT on WT and Crtc2 transgenic animals before dox treatment. Mice were fasted for 16 hrs before i.p. injection of 20% glucose. n =10 mice per group.



Supplemental Fig. 3. Uncropped immunoblots from Fig 2G



Supplemental Fig. 4. Effects of skeletal muscle overexpression of Crtc2 on the response to ADF and plasma feeding hormones in Crtc2 mice fed ad libitum.

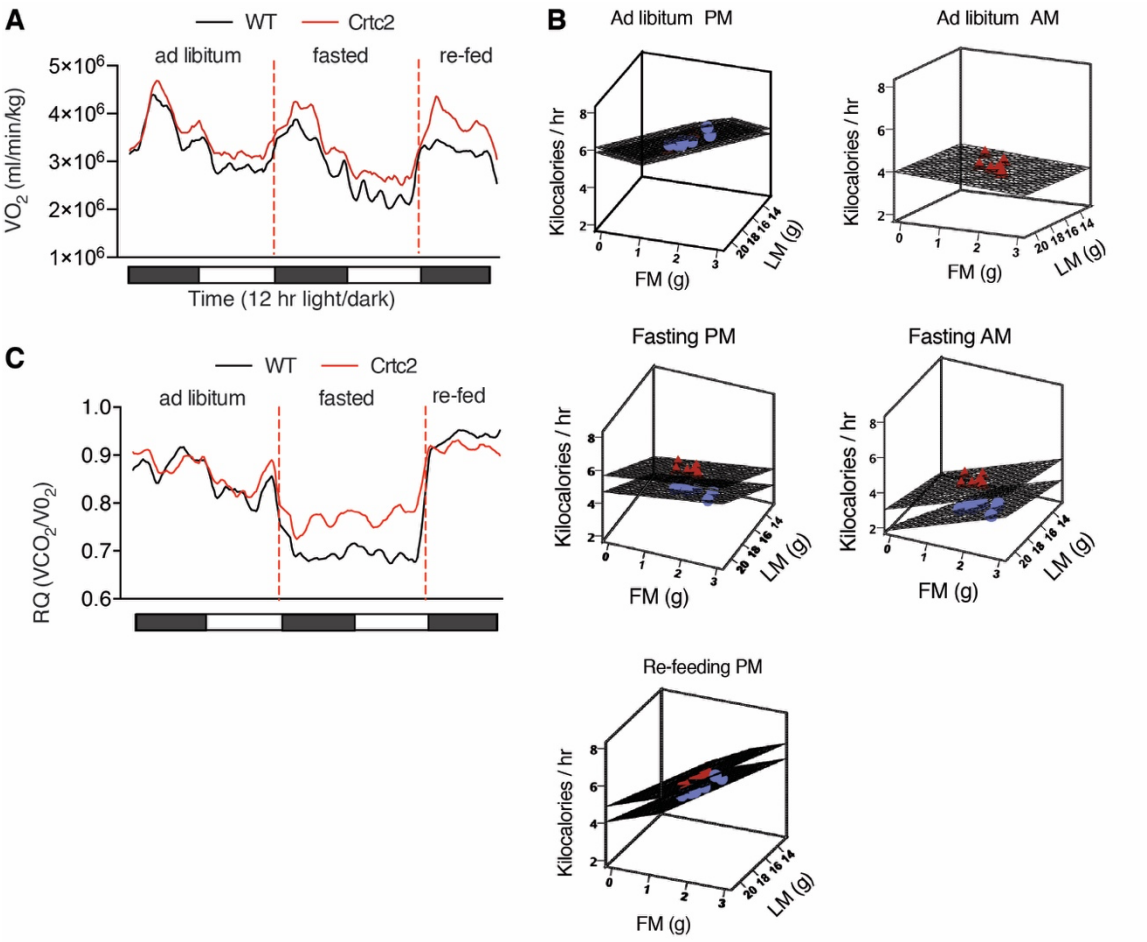
(**A**) Weight gain after treatment of 18-week old WT and Crtc2 transgenic mice with doxycycline, n=8 per group

(**B**) Changes in body weight during ADF.

(**C**) Average ambulatory activity during ad libitum feeding, fasting and re-feeding, n = 8 mice per group. 12-hr averages of the data. There were no significant effects of Crtc2. **A–C)** Data shown as mean ± SEM.

(**D**) WT or Crtc2 mice were injected twice daily with 0.5 mg/kg leptin and weight was monitored, n=8 mice per group. Mean ± SEM

(**E–F**) Analysis of plasma proteins regulating feeding and triglycerides and cholesterol in Crtc2 expressing and WT mice. Whisker box plots

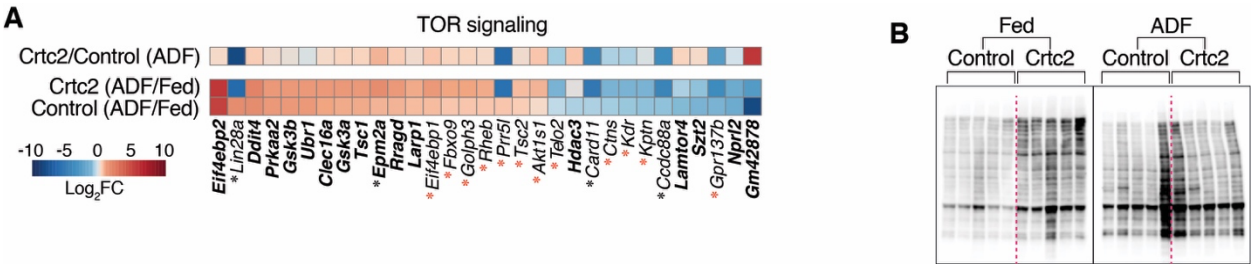


Supplemental Fig. 5. Whole-body energetics.

(**A**) VO_2 (and VCO_2) were measured continuously for 72 hrs. in a CLAMS animal monitoring system during alternate day fasting. N = 8 per group

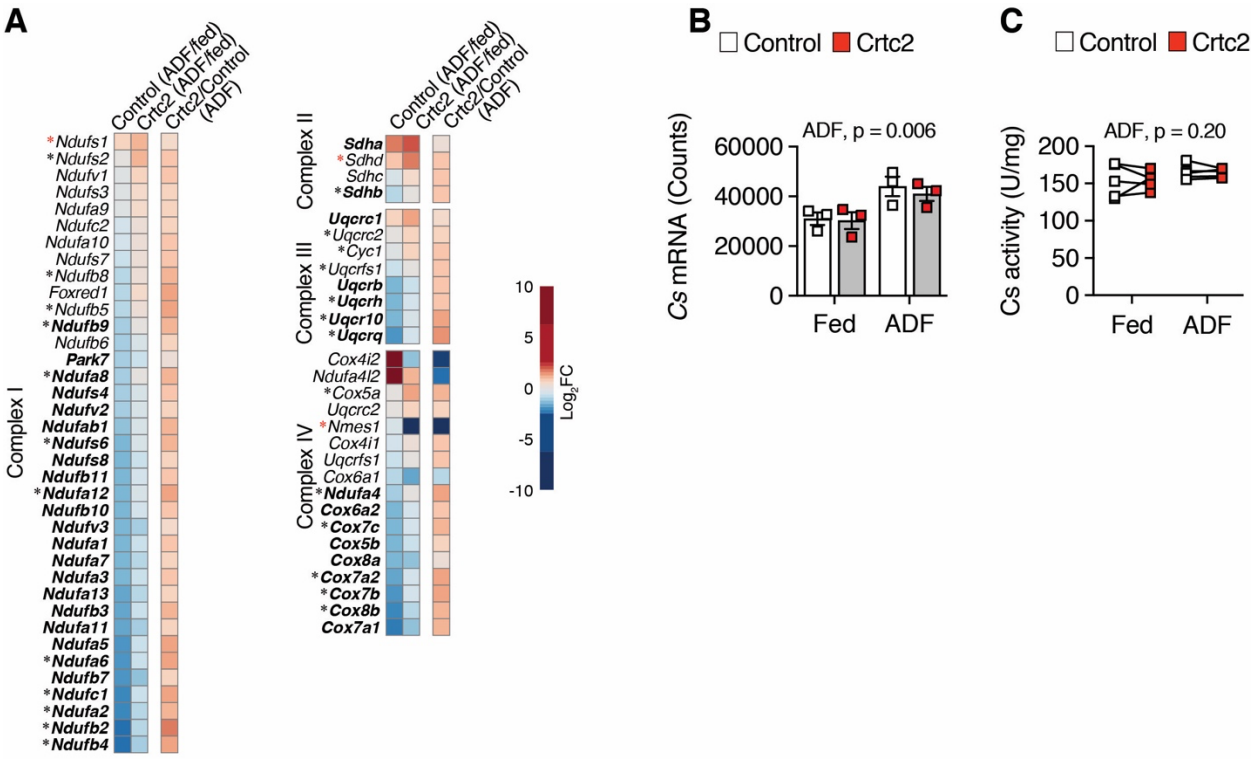
(**B**) Total energy expenditure (EE) for WT and Crtc2 mice was calculated from VO_2 and VCO_2. The data was adjusted by repeated measures ANCOVA in with lean mass (LM) and fat mass (FM) as covariants at the following values: LM = 17.01 g; and FM = 1.62 g. Dot plot graphs shows adjusted means derived from repeated measure general linear model ANCOVA, using 5 consecutive 12-hr time intervals: PM-ad libitum; AM-ad libitum; PM-Fast; AM-Fast; and Re-feed EE as dependent variables, and LM and FM as covariates. The lack of intersection of the planes graphically demonstrates lack of covariation.

(**C**) Respiratory Quotient from CLAMS experiment during alternate day fasting. Data are mean +SEM.

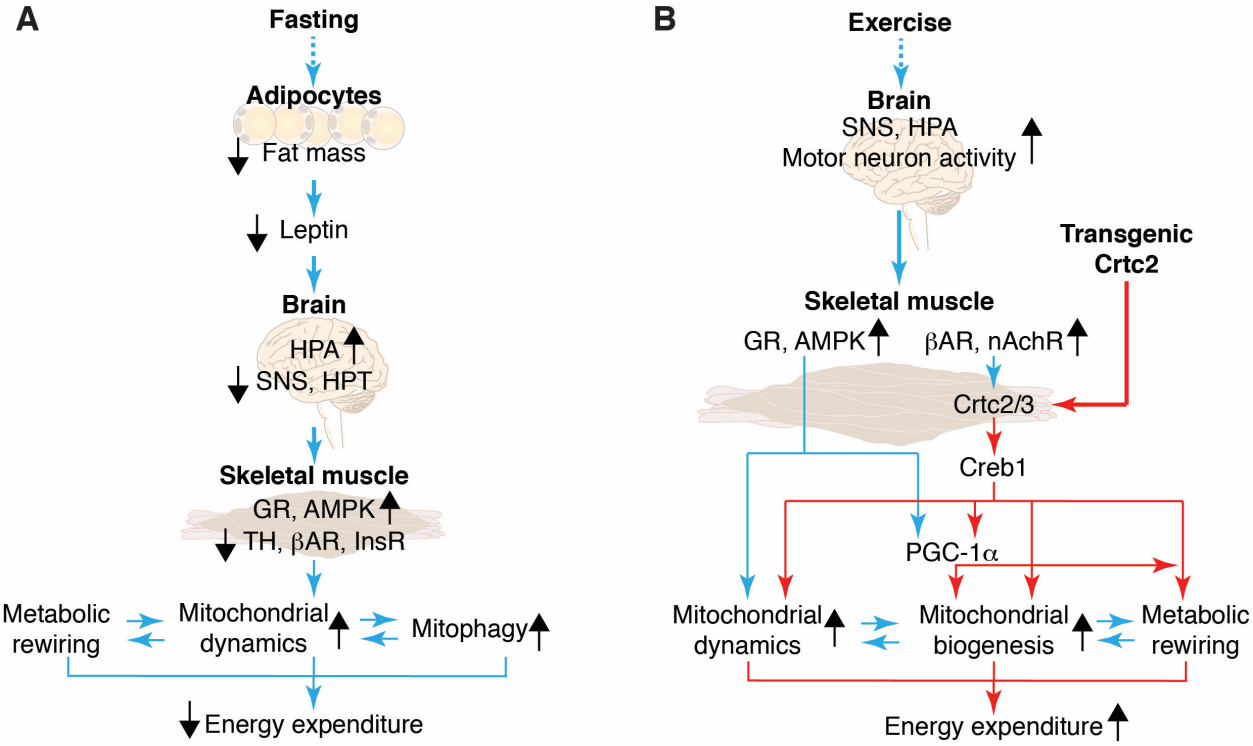


Supplemental Fig. 6. Protein synthesis and mTOR signaling

A) Expression profiles of genes that regulate mTOR. The mRNA levels in control and Crtc2-transduced TA muscles of mice subjected to ADF relative to the ad libitum fed mice (columns 1–2), and effect of Crtc2 transduction relative to control during ADF (column 3) are shown. ADF-regulated genes in control muscle appear in **bold**. *Crtc2-regulated genes in mice subjected to ADF. *ADF-regulated genes in Crtc2-transduced muscle but not control. FC, fold change. **B**) Western blot of in vivo SUnSET assay for new protein synthesis shown in **Figure 7B**.



Supplemental Fig. 7. Electron transport chain gene expression and Citrate Synthase activity **A**) Expression profiles of genes that encode the electron transport chain. The mRNA levels in control and Crtc2-transduced TA muscles of mice subjected to ADF relative to the ad libitum fed mice (columns 1–2), and effect of Crtc2 transduction relative to control during ADF (column 3) are shown. ADF-regulated genes in control muscle appear in **bold**. *Crtc2-regulated genes in mice subjected to ADF. *ADF-regulated genes in Crtc2-transduced muscle but not control. FC, fold change. **B**) Citrate Synthase (*Cs*) mRNA showed a modest increase in expression. **C**) Citrate Synthase enzyme activity was measured from electroporated TA muscles. **B-C**) Statistical analyses were done with 2-way ANOVA.



Supplemental Fig. 8. Hormonal and neural modulation of energy expenditure in skeletal muscle

(**A**) Fasting-induced regulation of mitochondrial dynamics and mitophagy.

(**B**) Exercise-induced regulation of mitochondrial dynamics and mitochondrial biogenesis, which can be constitutively activated by overexpression of Crtc2 in skeletal muscle.

bAR, b-adrenergic receptor; GR, glucocorticoid receptor; HPA, hypothalamic-pituitary-adrenal axis; HPT, hypothalamic-pituitary-thyroid axis; InsR, insulin receptor; nAchR, nicotinic acetylcholine receptor; SNS, sympathetic nervous system; TH, thyroid hormone