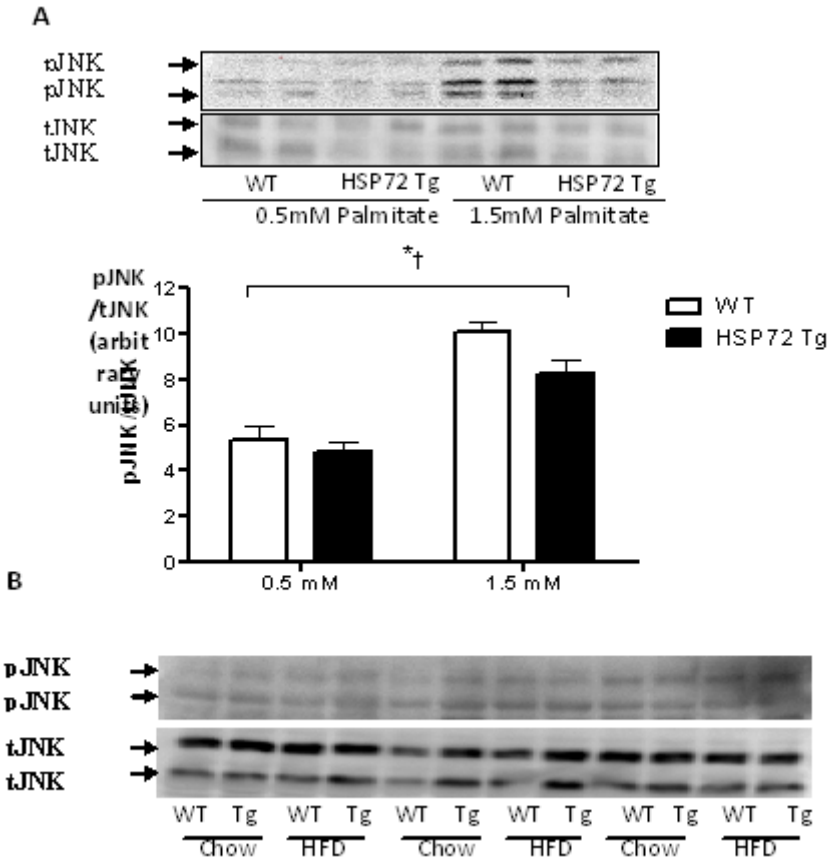
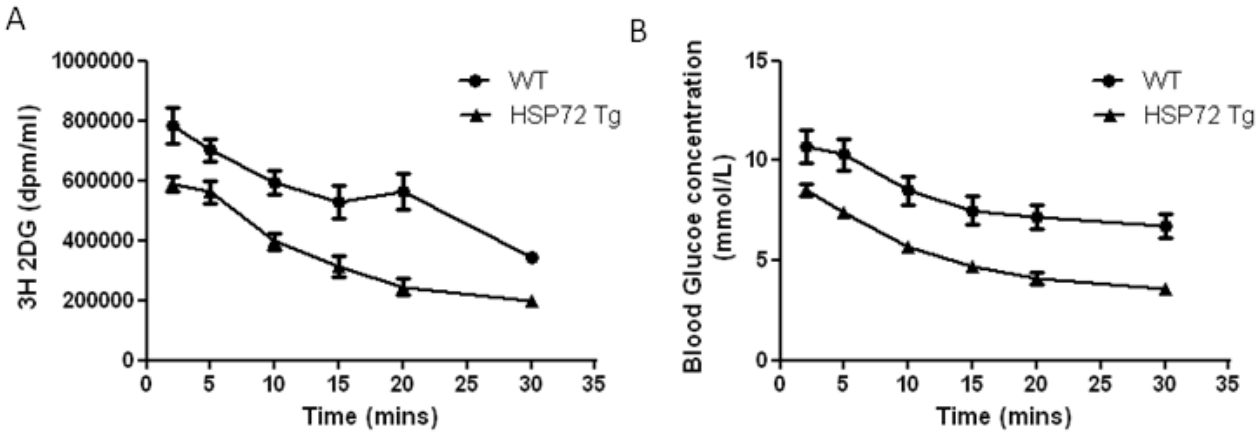
SUPPLEMENTARY DATA

Supplementary Figure 1. A) Levels of phosphorylated and total JNK in soleus muscle from WT and HSP72 Tg mice incubated with low (0.5 mM) or high (1.5 mM) palmitate for 6 h and (B) JNK expression in quadriceps muscle from WT and HSP72 Tg mice fed a normal chow (NC) or high fat diet (HFD) for 10 wk. Data are mean ± SEM; n = 6. * P<0.05 main effect for palmitate treatment, † P<0.05 main effect for genotype.

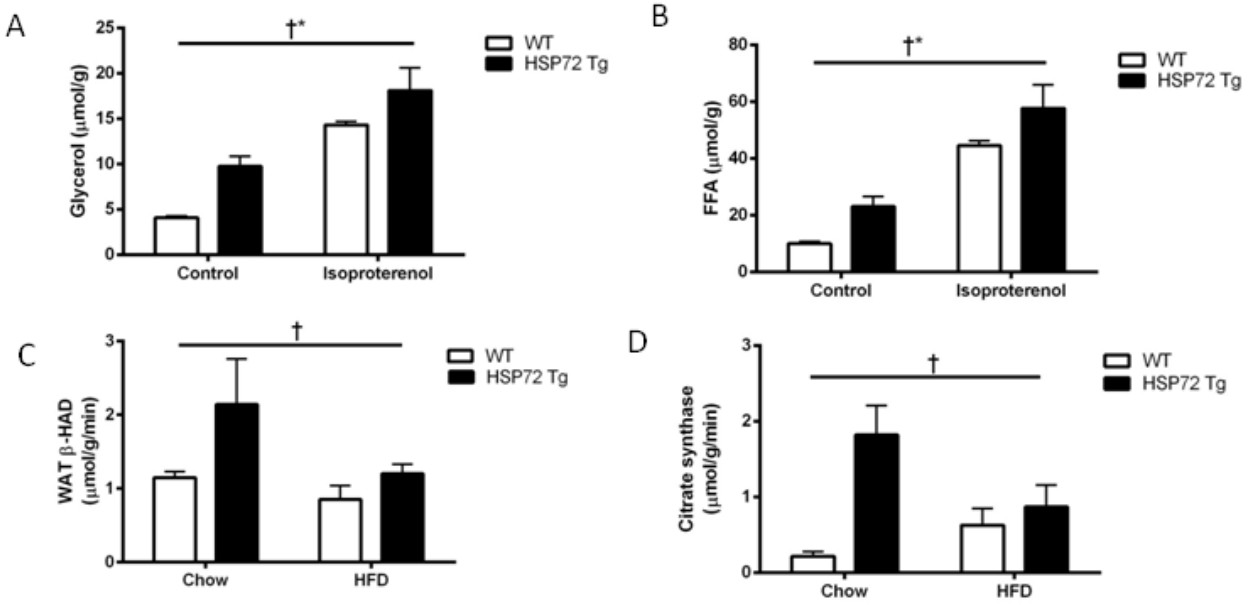


SUPPLEMENTARY DATA

Supplementary Figure 2. Tracer kinetics and plasma glucose concentrations during Insulin-stimulated glucose clearance studies: (A) tritiated 2-deoxyglucose counts and (B) Blood glucose concentrations measured over 30 minutes after insulin stimulation in WT and HSP72Tg mice. n = 7-8. Data are mean ± SEM.

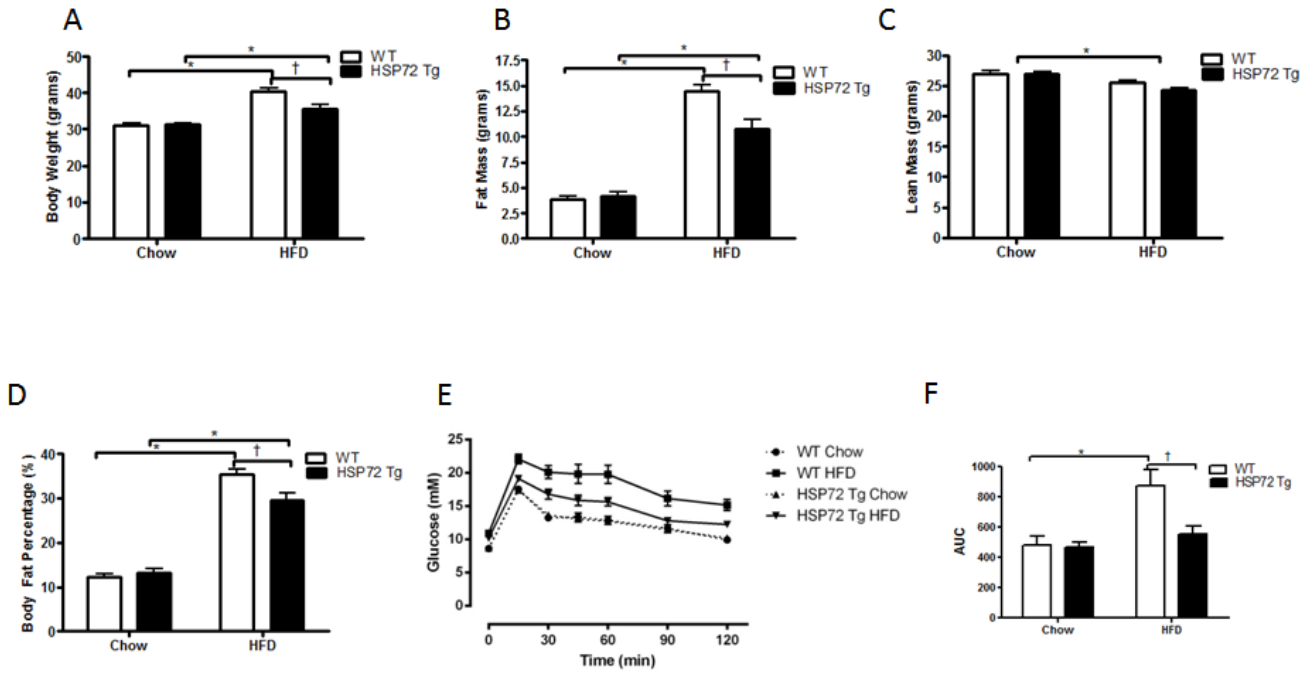


Supplementary Figure 3. Lipolysis assay on white adipose tissue (epididymal fat pad) explants from WT and HSP72Tg mice. Glycerol (A) and free fatty acid release was measured using manufacturers instructions in to the medium in response to basal (control) and isoproterenol stimulated conditions. † P<0.05 main effect for genotype * P<0.05 treatment effect by isoproterenol. Oxidative enzyme activities in white adipose tissue. β-HAD activity (C) and citrate synthase activity (D) † P<0.05 main effect for genotype, Data are mean ± SEM, n=4-9 per group.



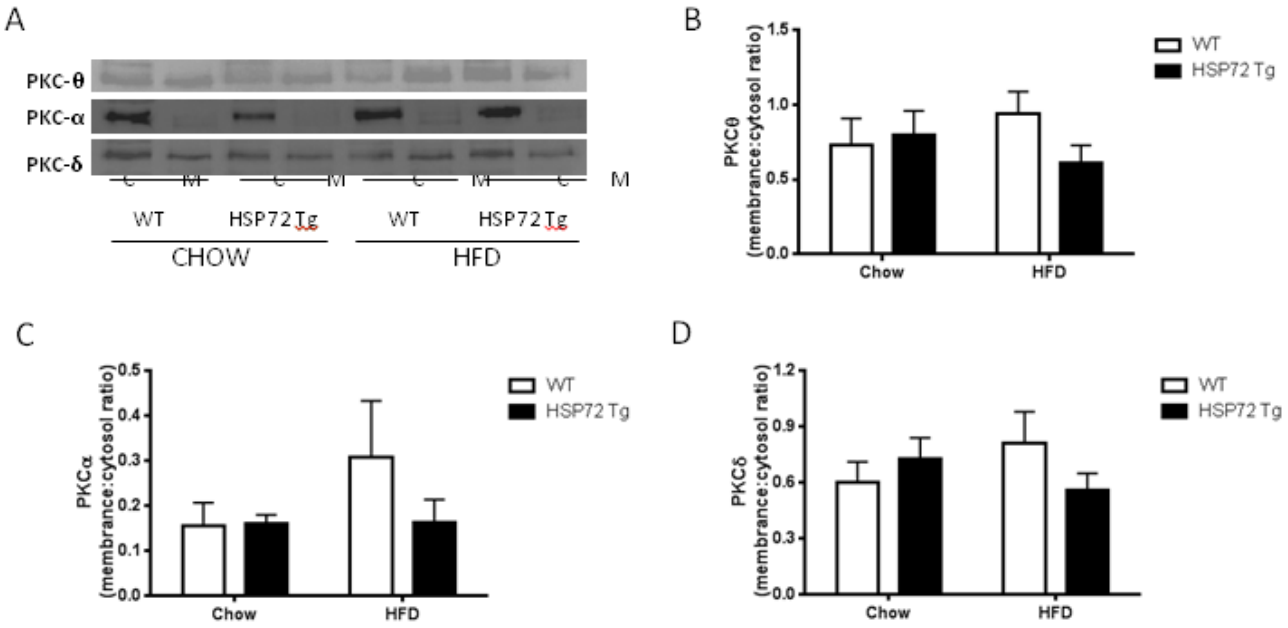
SUPPLEMENTARY DATA

Supplementary Figure 4. Characteristics of heterozygous HSP72Tg and littermate control mice on a C57Bl/6 background fed a normal chow or high fat diet for 12 wk. Body weight (A), fat mass (B), lean mass (C), percent body fat (D), glucose levels during an oral glucose tolerance test (OGTT) (E) and area under the curve for the OGTT (F). * P<0.05 main effect for diet within genotype † P<0.05 genotype effect on HFD. Data are mean ± SEM, n=17-23 per group.



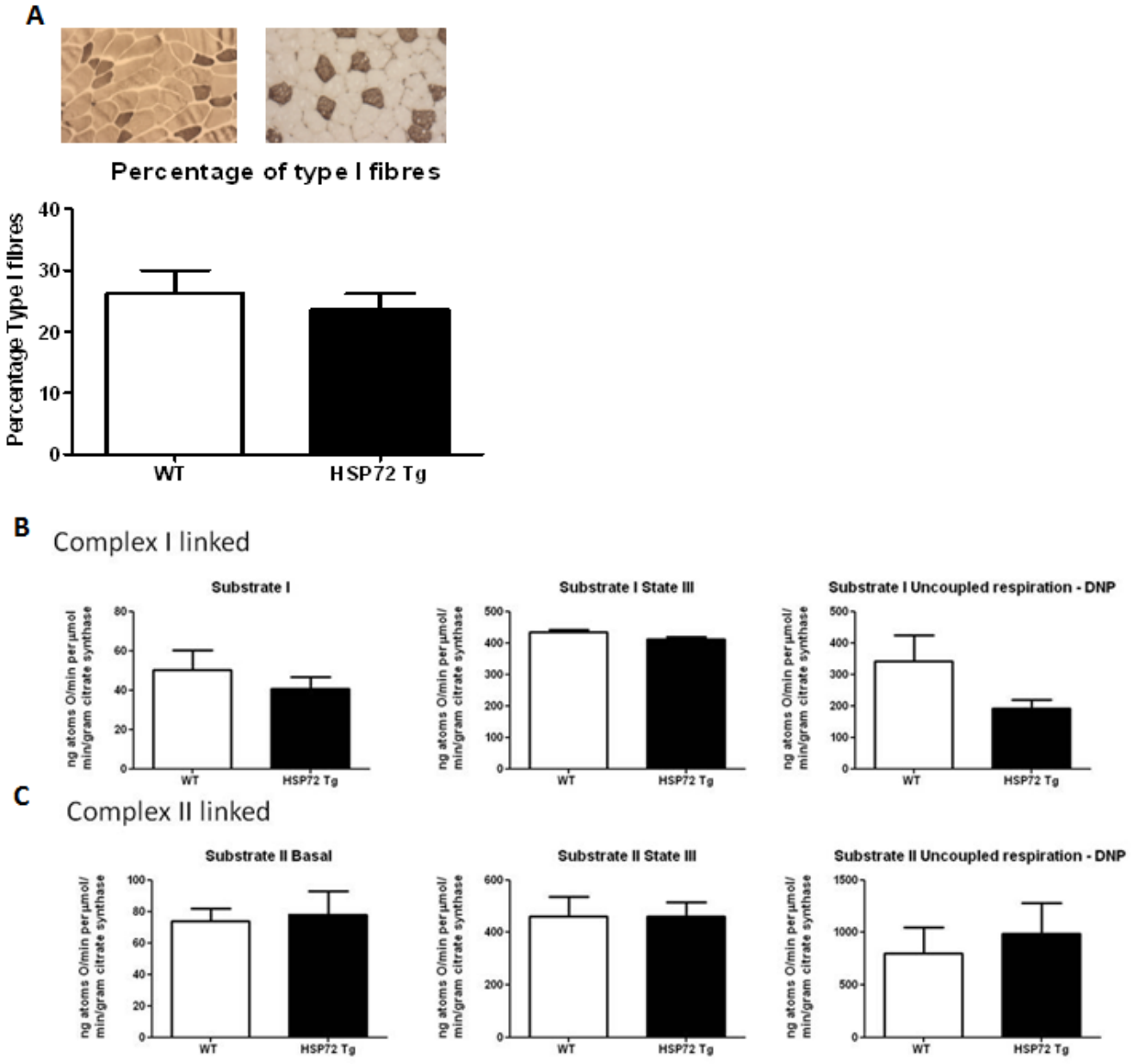
SUPPLEMENTARY DATA

Supplementary Figure 5. A) Western blots of PKC isoforms in the cytoplasmic (C) and membrane (M) fractions of skeletal muscle form WT and HSP72Tg mice. (B-D) Quantification of westerns blots for PKC isoforms (membrane to cytosol ratio). Data are mean ± SEM, n=6 per group.



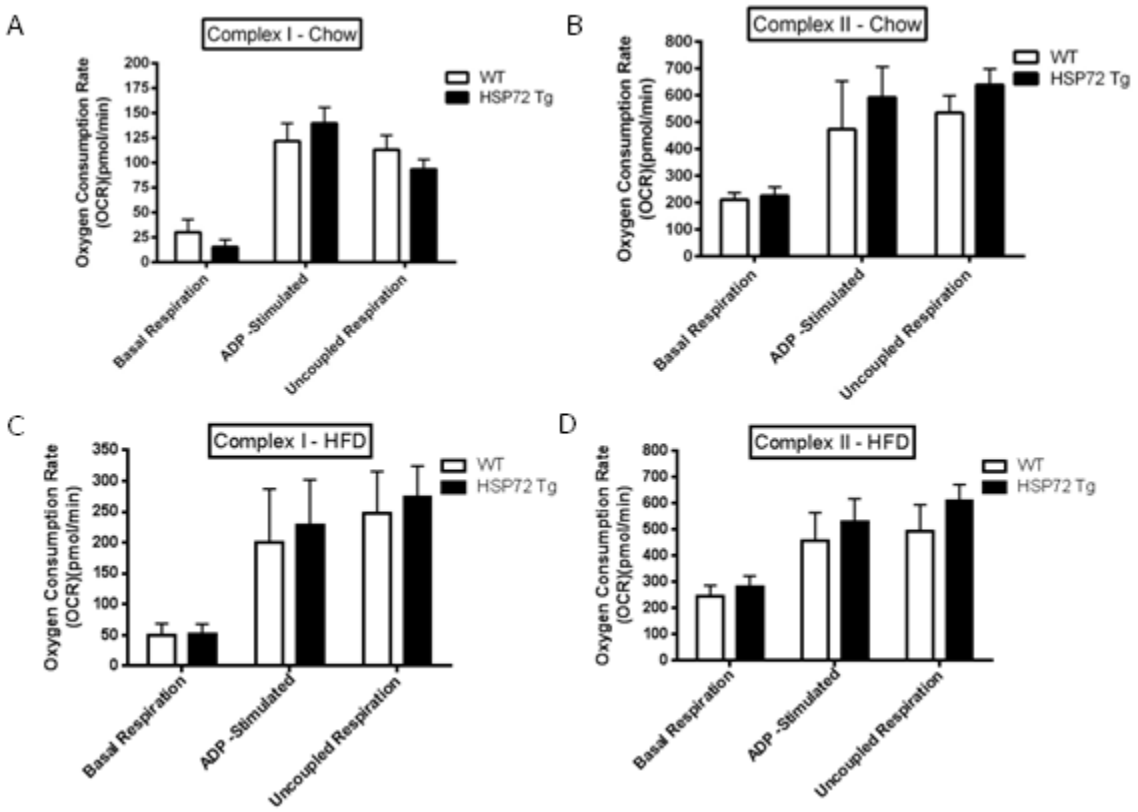
SUPPLEMENTARY DATA

Supplementary Figure 6. Skeletal muscle fibre typing in WT and HSP72 Tg mice fed a chow diet. (A) Representative cross sectional images from quadriceps muscle from WT and Hsp72 Tg mice and quantification of the slow twitch fibre number. Dark staining represents slow twitch fibres. Data are mean ± SEM; n = 5-9. Oxygen consumption rates in isolated mitochondria from WT and Hsp72 Tg mice fed a normal chow diet. Basal, ADP-stimulated State III (2.4mM), and DNP stimulated uncoupled respiration (0.1mM) were measured in isolated mitochondria preps in the presence of (B) complex 1 substrates (5mM pyruvate, 2mM malate) and (C) complex II substrates(10mM succinate, 4µM rotenone) Data are mean ± SEM; n = 3-5.



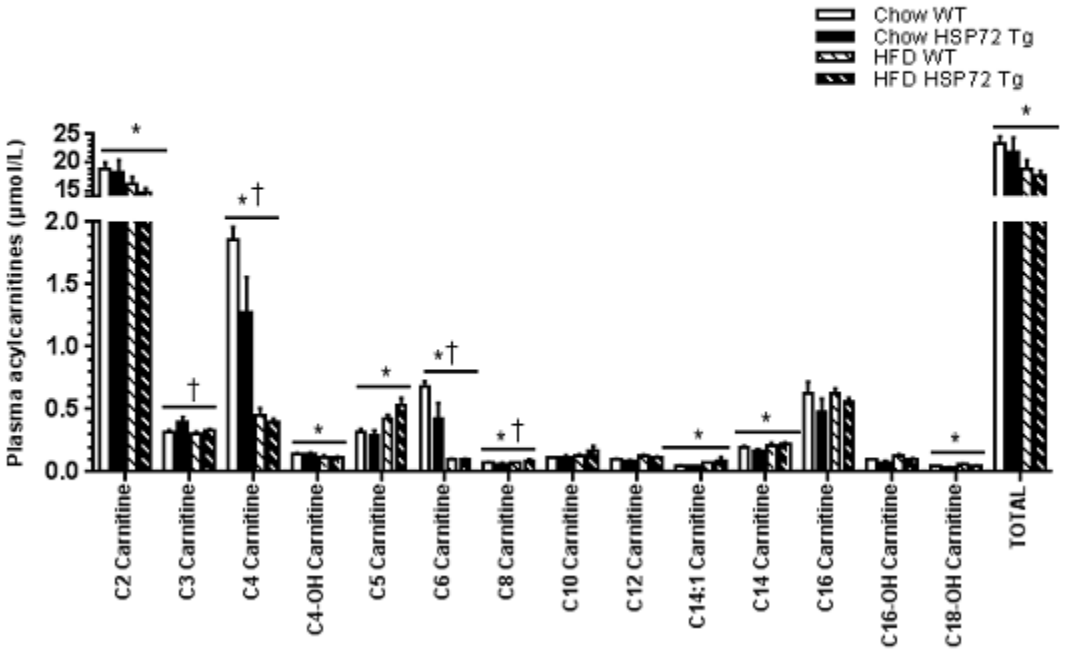
SUPPLEMENTARY DATA

Supplementary Figure 7. Oxygen consumption rates in isolated mitochondria from WT and HSP72 Tg mice backcrossed onto a C57bl/6 background. Mice were fed a normal chow or high fat diet. Basal, ADP-stimulated State III (3mM), and FCCP stimulated uncoupled respiration (1μM) were measured in isolated mitochondria preps in an XF-24 Seahorse Bioanalyser (5ug/well centrifuged at 2000g for 15mins to adhere the mitochondria to the bottom of the plate) in the presence of (A and C) complex 1 substrates (5mM pyruvate, 2mM malate) and (B and D) complex II substrates(10mM succinate, 4μM rotenone) Data are mean ± SEM; n = 5.



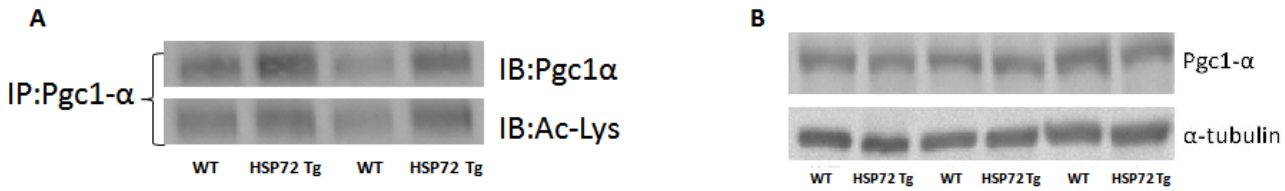
SUPPLEMENTARY DATA

Supplementary Figure 8. Analysis of plasma acylcarnitines from normal chow and high fat fed WT and HSP72Tg mice. Plasma was collected after a 5-6hr fast. n = 4-8 per group, * p =<0.05 for diet effect, † p = <0.05 for genotype effect between WT and HSP72 Tg mice. In the C4, C6 and C8 species there are significant interactions: Diet within WT, Diet within HSP72Tg and Genotype within NC (all p=<0.01). Data are mean ± SEM.



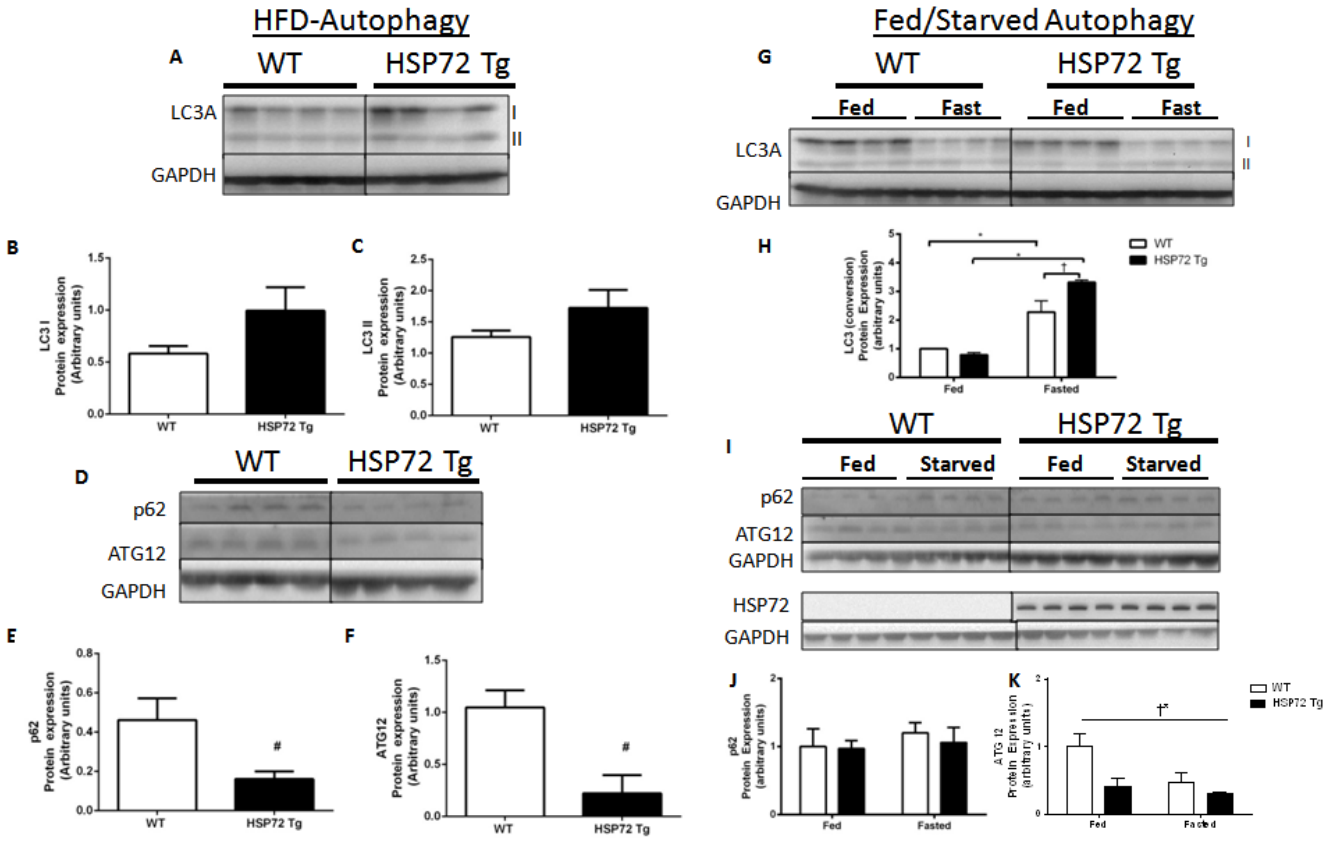
SUPPLEMENTARY DATA

Supplementary Figure 9. A) Ac-Pgc1-α in skeletal muscle. Pgc1-α was immunoprecipitated from nuclear extracts with anti-Pgc1-α and immunoprecipitates were immnuoblotted with anti-acetyl lysine (Ac-Lys) and Pgc1-α (B) Total Pgc1-α in skeletal muscle. Whole cell lysates probed for Pgc1-α: n=9-10 per group.



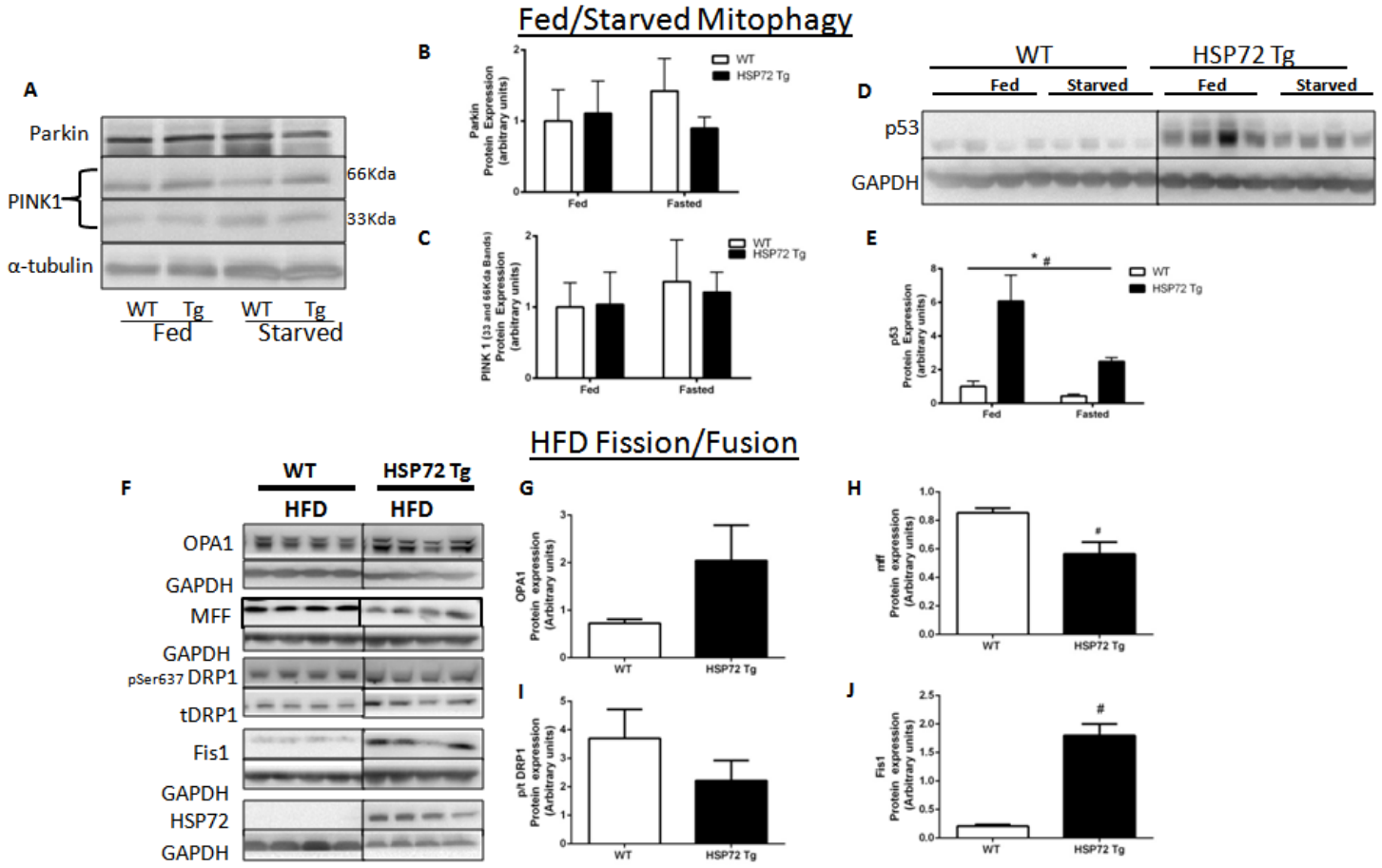
SUPPLEMENTARY DATA

Supplementary Figure 10. (A-F) Analysis of markers of autophagy in skeletal muscle of WT and HSP72Tg mice fed a HFD for 10 weeks. (A) Westerns blots for LC3A I and LCA II (B) Quantification for LC3A I (C) and LC3A II relative to GAPDH. (D) p62 and ATG12 western blots and their respective quantifications (E, F) relative to GAPDH, n = 4, # p =<0.05. (G-K) Analysis of markers of autophagy in skeletal muscle of WT and HSP72Tg fed a chow diet with tissues collected in the Fed state (7am after the night cycle) or following a 24hr period of starvation (Starve). (G) Western blotting for LC3AI and II (H) Analysis of LC3A conversion ratio (I) Western blotting for p62, ATG12 and HSP72 (J,K) relative quantification of p62 and ATG12 relative to GAPDH. n = 4 per group, * p =<0.05 for fasting effect, † p = <0.05 for difference between WT and HSP72 Tg mice.



SUPPLEMENTARY DATA

Supplementary Figure 11. (A-E) Analysis of markers of mitophagy in skeletal muscle of WT and HSP72Tg fed a chow diet with tissues collected in the Fed state (7am after the night cycle) or following a 24hr period of starvation (Starve). (A) Westerns blots for Parkin and PINK1 and their respective quantification (B, C) relative to alpha tubulin. (D) p53 protein expression and respective quantification (E) n = 4 per group, * p =<0.05 for fasting effect, † p = <0.05 for difference between WT and HSP72 Tg mice. (F-J) Analysis of markers of mitochondrial fission and fusion in mice fed a HFD. (F) Western blotting for OPA1, MFF, phosphorylated and total DRP1, Fis 1 and HSP72. (G-J) Quantification of blots for OPA1, MFF, DRP1 and Fis1, n = 4, # p =<0.05 for significant difference between WT and HSP72 Tg mice. Data are mean ± SEM.



SUPPLEMENTARY DATA

Supplementary Figure 12. Extracellular acidification rates (ECAR) and cellular bioenergetics in stably expressing HSP72 or control cells. (A) ECAR as a proxy measure of glycolysis (B) Cellular bioenergetic profile plotting OCR vs ECAR * Denotes statistically significant vs Con for OCR, n=5. Data are mean ± SEM.