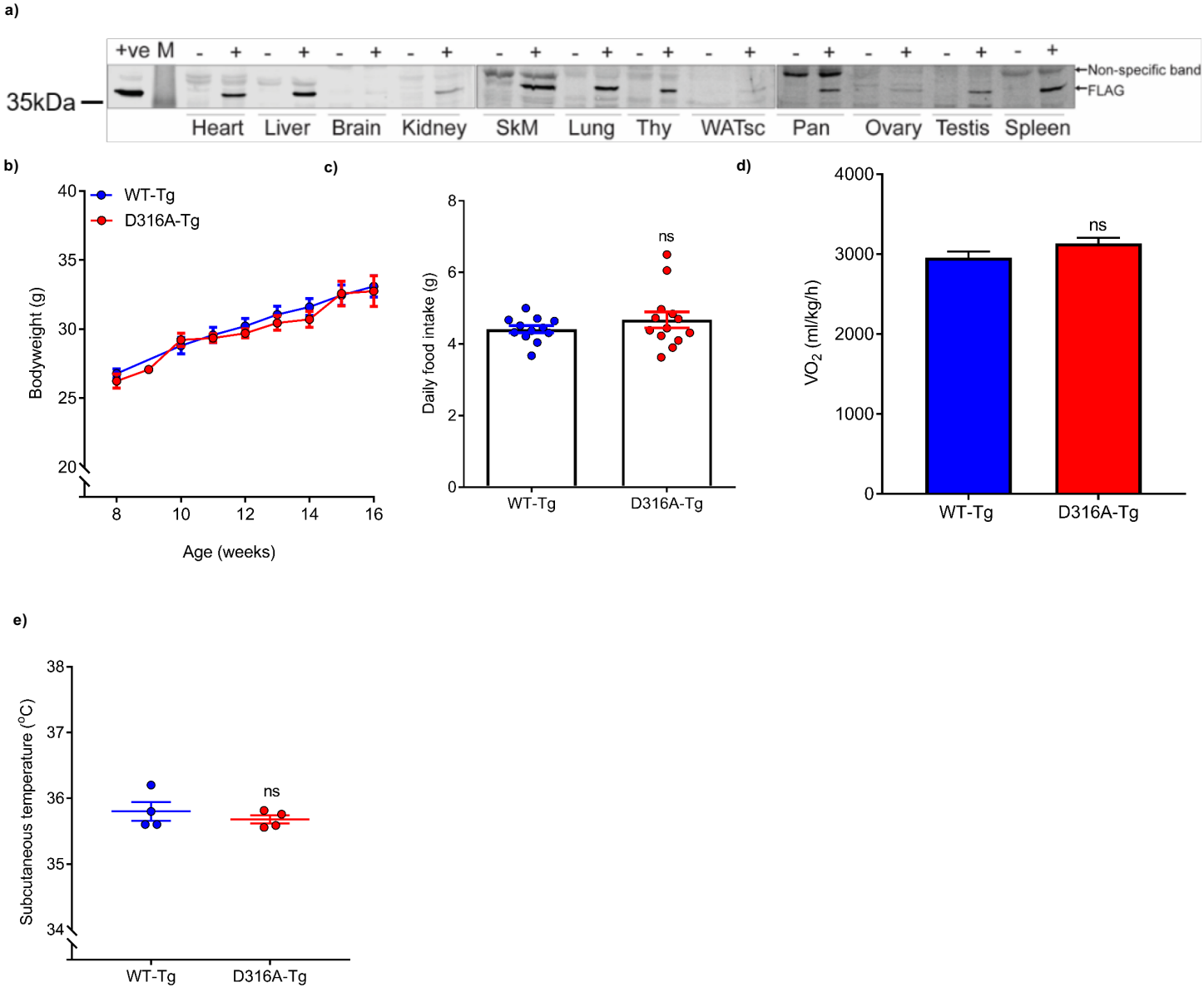
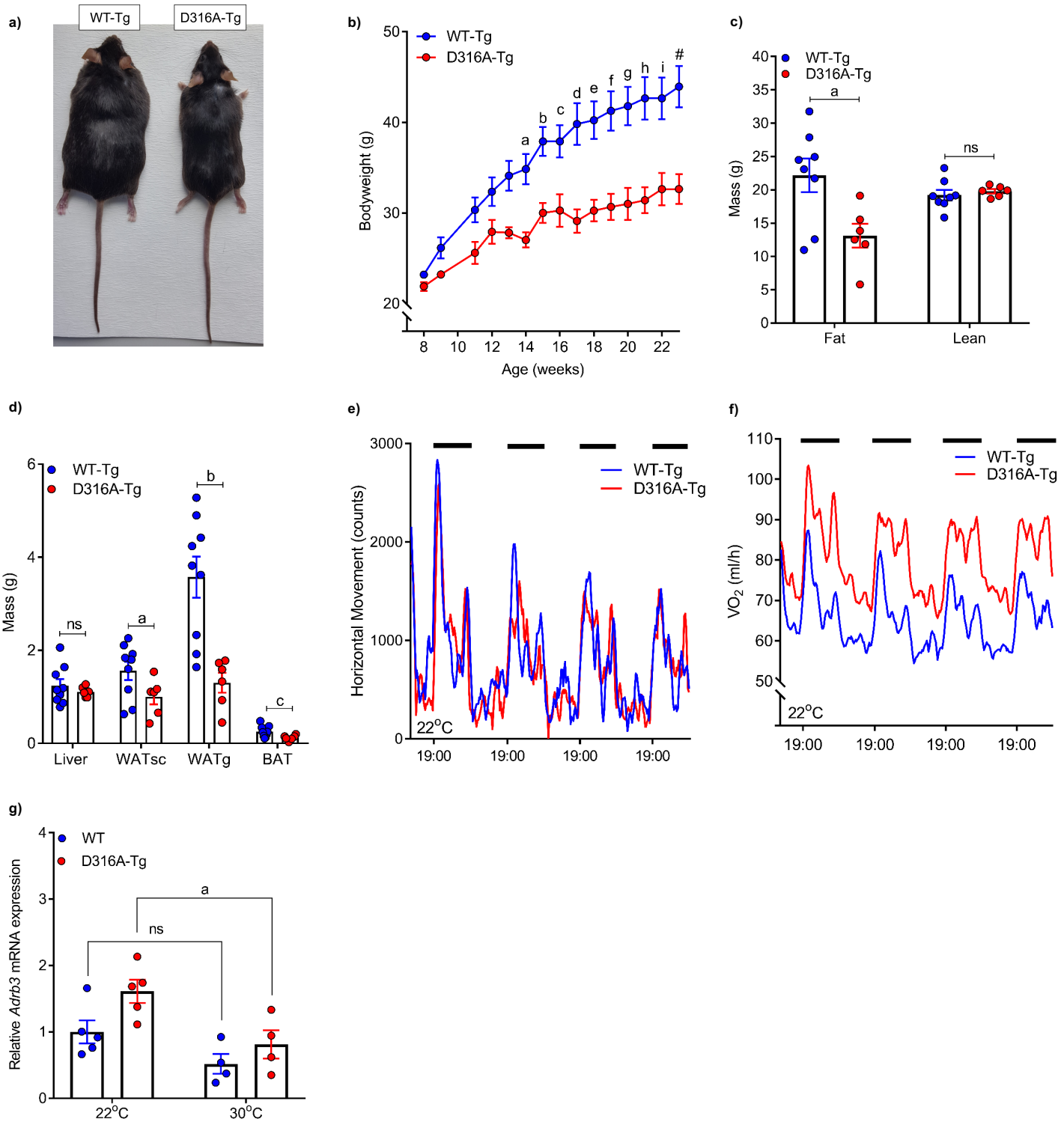
Supplementary Figure 1



Supplementary Figure 1. Phenotype of mice maintained on a chow diet.

a, Representative western blots of AMPKγ1 (containing a C-terminal Flag tag epitope) transgene expression in tissue lysates (SkM, gastrocnemius skeletal muscle; Thy, thymus; WATsc, subcutaneous white adipose tissue; Pan, pancreas) isolated from non-transgenic control mice (-) and D316A-Tg mice crossed with β-actin-cre (+). A sample of HEK293 cells transfected with Flag-tagged AMPKγ1 is included as a positive control. A non-specific cross-reacting band is present in a number of the tissue lysates in both the non-transgenic control mice and D316A-Tg mice. Identical results were obtained on tissue samples from animals isolated from multiple independent experimental cohorts. **b**, Bodyweight of mice maintained on a chow diet (mean ±sem, n=20 for WT-Tg and 19 for D316A-Tg) and (**c**) daily food intake on chow measured over a 3-week period (mean ±sem, n=12 for WT-Tg and 13 for D316A-Tg). **d**, Oxygen consumption (VO_2; mean ±sem, n=7 mice per genotype). Data points from individual mice were omitted in the graph to more clearly show mean values and error bar sizes. (**e**) subcutaneous body temperature of mice on a chow diet (n=4 mice per genotype). Statistical analysis for panel b was performed by multiple t-test adjusted for multiple comparisons and for panels c, d and e by Student's t-test. In all cases ns=non-significant.

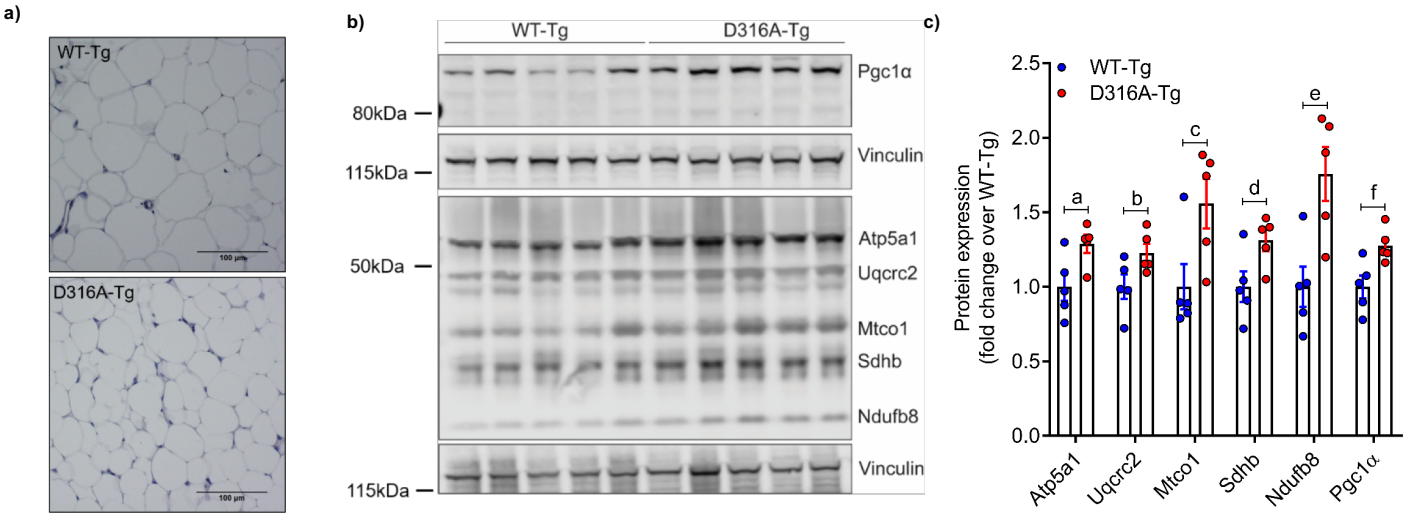
Supplementary Figure 2



Supplementary Figure 2. Protection against diet-induced obesity in female mice and oxygen consumption measured per mouse (not corrected for bodyweight).

a, An image showing a representative WT-Tg and D316A-Tg mouse after 16 weeks on HFD diet. Similar results were obtained from >100 mice per genotype from multiple independent experimental cohorts. **b,** Bodyweight of female mice maintained on a HFD from 8 weeks of age. Results shown are the mean ±sem (n=9 per genotype). *P* values indicate means that are significantly different from WT-Tg mice; ^{a}P=0.0157, ^{b}P=0.0142, ^{c}P=0.0207, ^{d}P=0.0001, ^{e}P=0.0005, ^{f}P=0.0002, ^{g}P=0.0001, ^{h}P=0.0001, ^{i}P=0.0005, $^{\#}P$<0.0001. **c**, Total body fat and lean mass measured by EchoMRI body composition analyser (n=8 for WT-Tg and n=6 for D316A-Tg). ^{a}P=0.0022, ns=non-significant. (**d**) tissue weights after 14 weeks on a HFD (n=9 for WT-Tg and n=6 for D316A mice). ^{a}P=0.0199, ^{b}P=0.0007, ^{c}P=0.0345, ns=non-significant. **e,** Movement measured by horizontal beam-breaks over a 84-hour period in male mice fed a HFD for 6 weeks (n=8 for WT-Tg and 6 for D316A-Tg mice). **f**, Whole body oxygen consumption (VO_2) monitored continuously over an 84-hour period, not corrected for bodyweight (i.e. VO_2 per mouse). n=8 for WT-Tg and 6 for D316A-Tg mice. **g**, mRNA expression of *Adrb3* in BAT from mice housed at 22°C (n=5 per genotype) or 30°C (n=4 per genotype) and fed a HFD for 11 weeks. ^{a}P=0.0383, ns=non-significant. Statistical analysis for panels b and c was performed by two-way ANOVA followed by Bonferroni's multiple comparisons test and for panels d and g by Student's t-test (unpaired, 2-tailed).

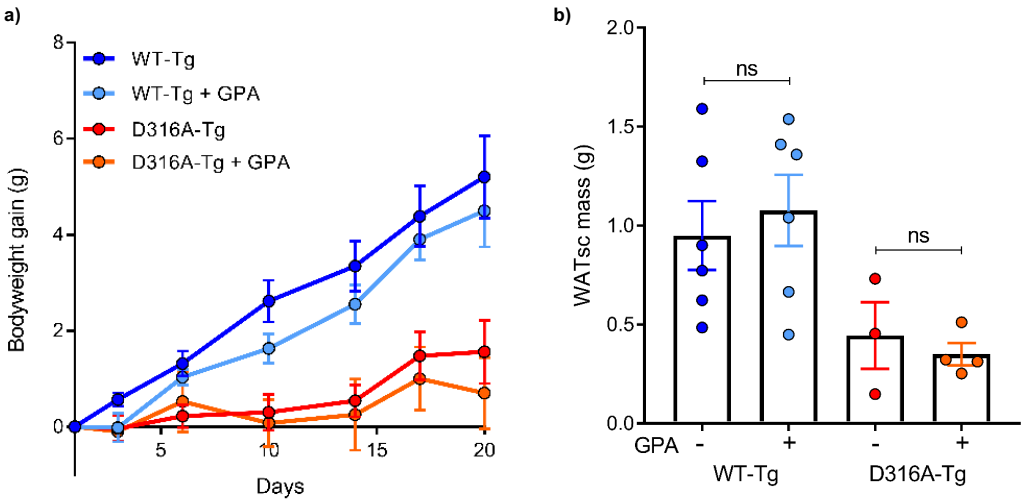
Supplementary Figure 3



Supplementary Figure 3. No gross morphological changes in gonadal WAT and Pgc1α and mitochondrial protein expression on chow diet.

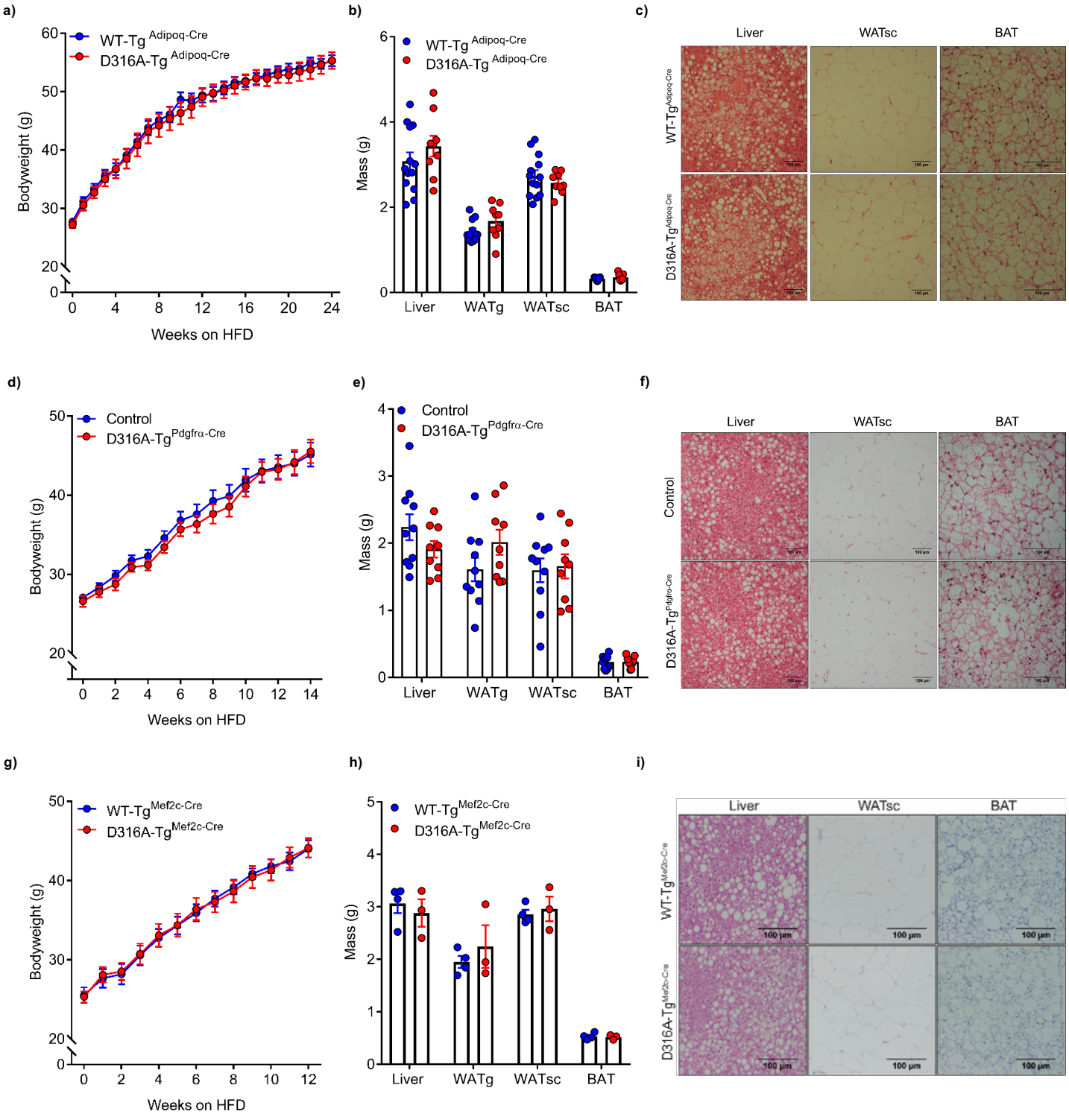
a, Representative images (from 6 mice per genotype from a single cohort) of haematoxylin staining of WATg from WT-Tg and D316A-Tg mice fed a HFD for 16 weeks. Similar results were obtained from mice from multiple (>10) independent experimental cohorts. **b**, Western blot analysis of Pgc1α and mitochondrial electron transport chain proteins in WATsc from mice maintained on a chow diet. In each case, samples from 5 mice per genotype were analysed and vinculin used as a loading control. **c,** Quantification of protein expression data shown in (**b**). *P* values indicate means that are significantly different from WT-Tg mice; **P*< 0.05, ***P*<0.01. Statistical analysis for panel c was performed by Student's t-test (unpaired, 2-tailed).

Supplementary Figure 4



Supplementary Figure 4. Treatment with β-guanidinopropionic acid does not antagonize the effect of AMPK activation to protect against diet-induced obesity.
a, Eight-week old mice were fed a HFD and after 1 week mice were given access to either distilled water or water containing 0.5% (w/v) β-guanidinopropionic acid (β-GPA). Bodyweights were measured over the next 3 weeks. Results are plotted as the mean ±sem (n=6 for WT-Tg mice and n=4-5 for D316A-Tg mice). **b**, Following this 3-week period, WATsc weight was determined (n=6 for WT-Tg mice and n=3-4 for D316A-Tg mice). ns, no significant difference determined by Student's t-test (unpaired, 2-tailed) between β-GPA treated and non-treated animals.

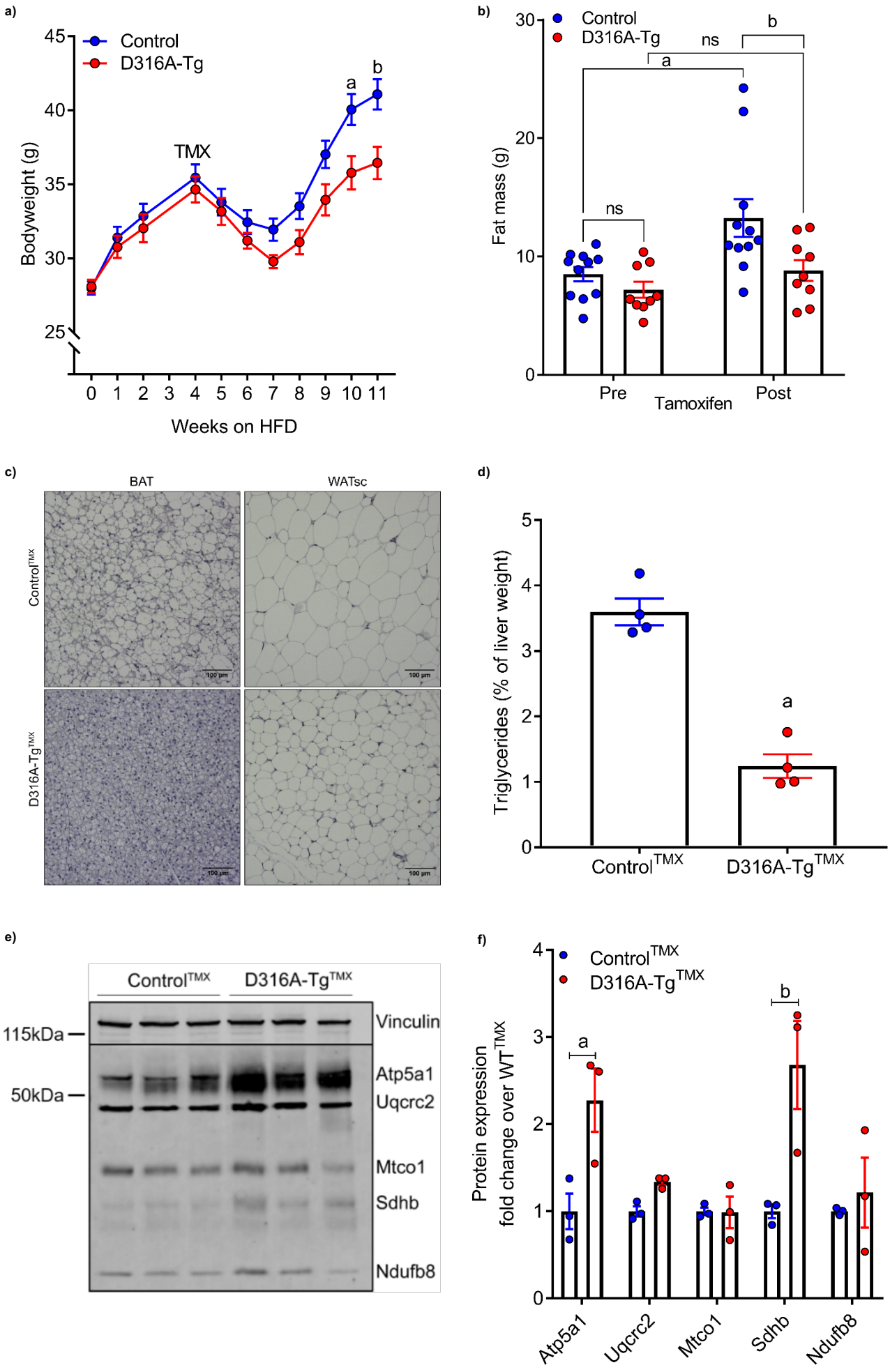
Supplementary Figure 5



Supplementary Figure 5. Cell lineage-specific activation of AMPK does not phenocopy global AMPK activation.

a-c, AMPKγ1 floxed mice were crossed with mice expressing Cre-recombinase under the control of (a-c) the adiponectin promoter (Adipoq-Cre, n=13 WT-Tg and n=9 D316A-Tg) to generate mature adipose-specific expression, (**d-f**) the Pdgfrα promoter (Pdgfrα-Cre, n=10 WT-Tg and n=9 D316A-Tg) or (**g-i**) the Mef2c promoter (Mef2c-Cre, n=8 WT-Tg and n=5 D316A-Tg) to generate skeletal muscle-specific expression. **a**, **d** and **g**, Bodyweight was measured in mice maintained on HFD. **b, e and h,** Tissue weights at termination (Adipoq-Cre, n=13 WT-Tg and n=9 D316A-Tg; Pdgfrα-Cre, n=10 WT-Tg and n=9 D316A-Tg and Mef2c-Cre, n=4 WT-Tg and n=3 D316A-Tg). **c, f** and **i,** representative images (from 3-6 mice per group per experiment) of H&E stained tissue sections. Similar results were obtained from an independent cohort of the Adipoq-Cre mice. Results of Pdgfrα-Cre and Mef2c-Cre are from a single cohort of mice.

Supplementary Figure 6

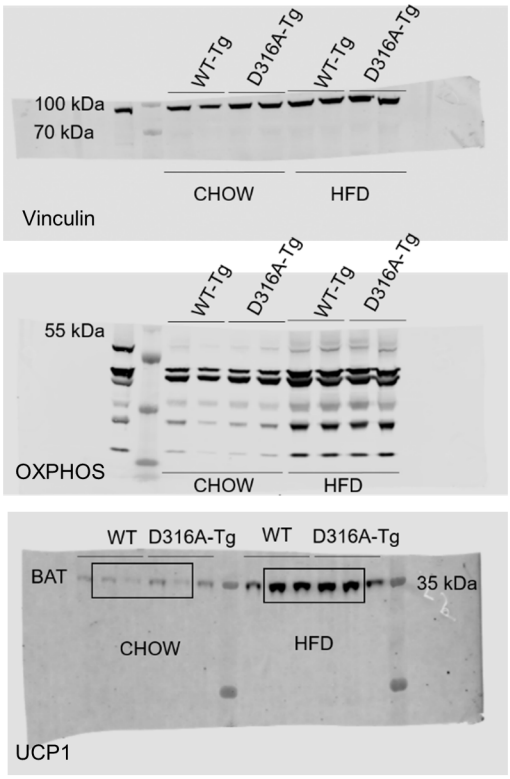


Supplementary Figure 6. AMPK activation in adult mice protects against diet-induced obesity

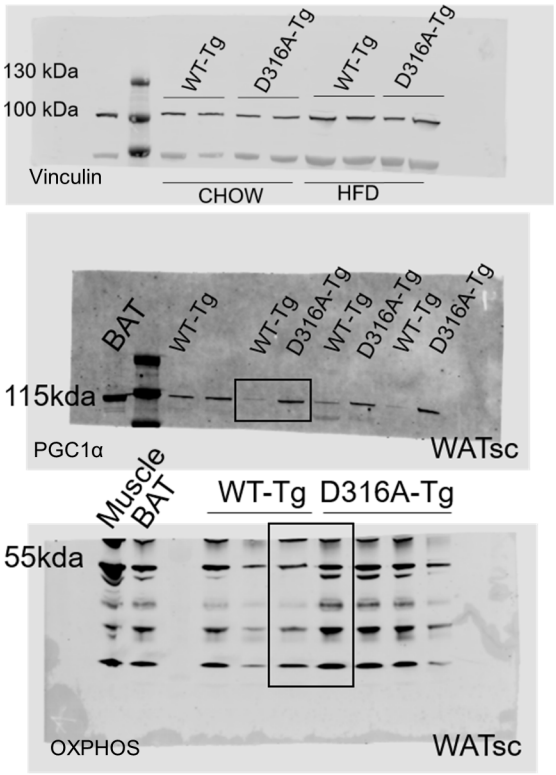
Male mice expressing both the D316A γ1 transgene (D316A-Tg, n=9) and an inducible CAGGCre-ER^{TM} and either D316A-Tg or CAGGCre-ER^{TM} alone (Control, n=10) were fed a high fat diet from 8 weeks of age. After 4 weeks, mice were injected intraperitoneally with tamoxifen (TMX; 3 mg in 0.15 ml corn oil) for 4 consecutive days. Mice were maintained on a high fat diet for a further 7 weeks. **a**, bodyweight of mice maintained on a high fat diet. ^{a}P=0.0055, ^{b}P=0.0018. **b,** Total body fat before, and 6 weeks after, tamoxifen induction. ^{a}P=0.0136, ^{b}P=0.0359. **c**, Representative images (from 6 mice per group, from a single experimental cohort) of haematoxylin staining of BAT and WATsc (scale bar = 100 μm). **d**, Liver triglyceride measured as percentage of total liver weight (n=4 per group). ^{a}P=0.0001. **e,** Western blot analysis of mitochondrial electron transport chain proteins in WATsc from tamoxifen treated Control and D316A-Tg mice. Samples from three mice per group are shown and vinculin is used as a loading control. **f**, quantification of protein expression (n=6 mice per group). ^{a}P=0.0095, ^{b}P=0.0007. In all cases, results are the mean ±sem. Statistical analysis for panels a, b and f was performed by two-way ANOVA followed by Bonferroni's multiple comparisons test and for panel d by Student's t-test (unpaired, 2-tailed).

Supplementary Figure 7

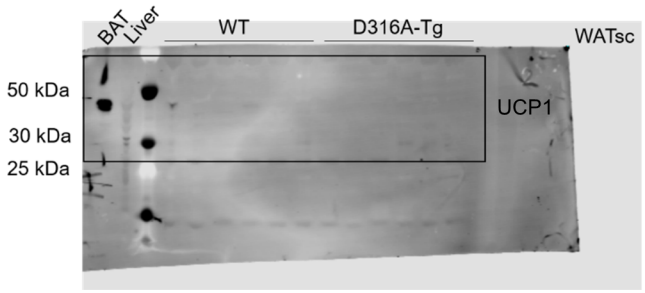
a) Figure 2b



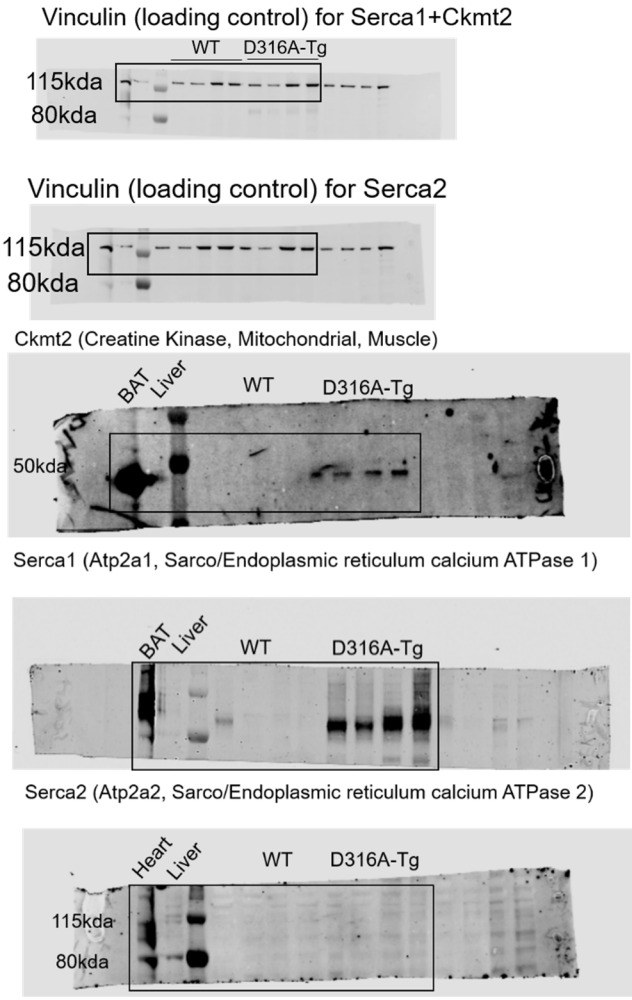
b) Figure 3h



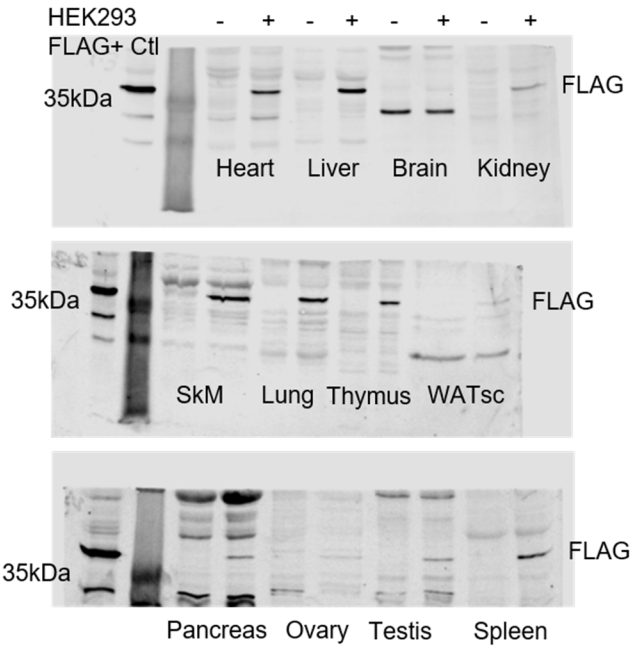
c) Figure 3k



d) Figure 4d



e) Supplementary Figure 1a



Supplementary Figure 7. Uncropped Western blots for data shown in Figures. Individual panels show the uncropped blots for the corresponding Figures, as shown. Where appropriate, black boxes indicate the lanes used for making the Figures.

Supplementary Table 1. Effect of global AMPK activation in heart.

	WT-Tg	D316A-Tg
Heart Rate (bpm)	772 ±21	744 ±26
PR interval (ms)	25.83 ±0.95	25.95 ±0.73
QRS complex duration (ms)	10.03 ±0.28	10.42 ±0.56
Heart weight (% body weight)	0.603±0.026	0.746 ±0.023[a]

Heart rate (beats/minute; bpm), PR interval and QRS duration (millisecond, ms) were measured in conscious male mice (12-13 weeks of age maintained on a chow diet) using an ECGenie System. Values are the average ±SEM from 6 mice per genotype. Heart weight as a percentage of total body weight is the average ±SEM for 6 male mice per group (13-16 weeks of age). a P=0.0021 statistically significant difference between wild type and gain-of-function AMPK.