

Supplementary Information for

The phosphorylation of AMPK β 1 is critical for increasing autophagy and maintaining mitochondrial homeostasis in response to fatty acids

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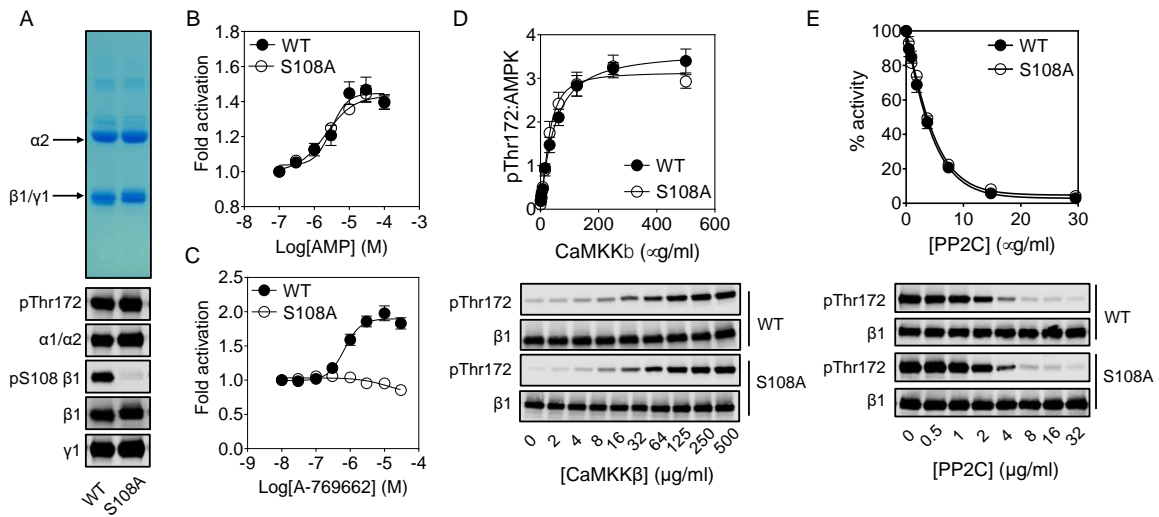
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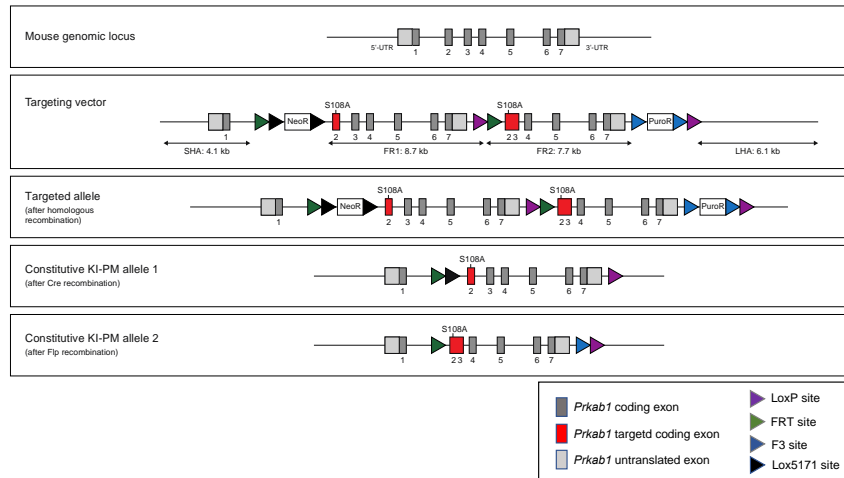
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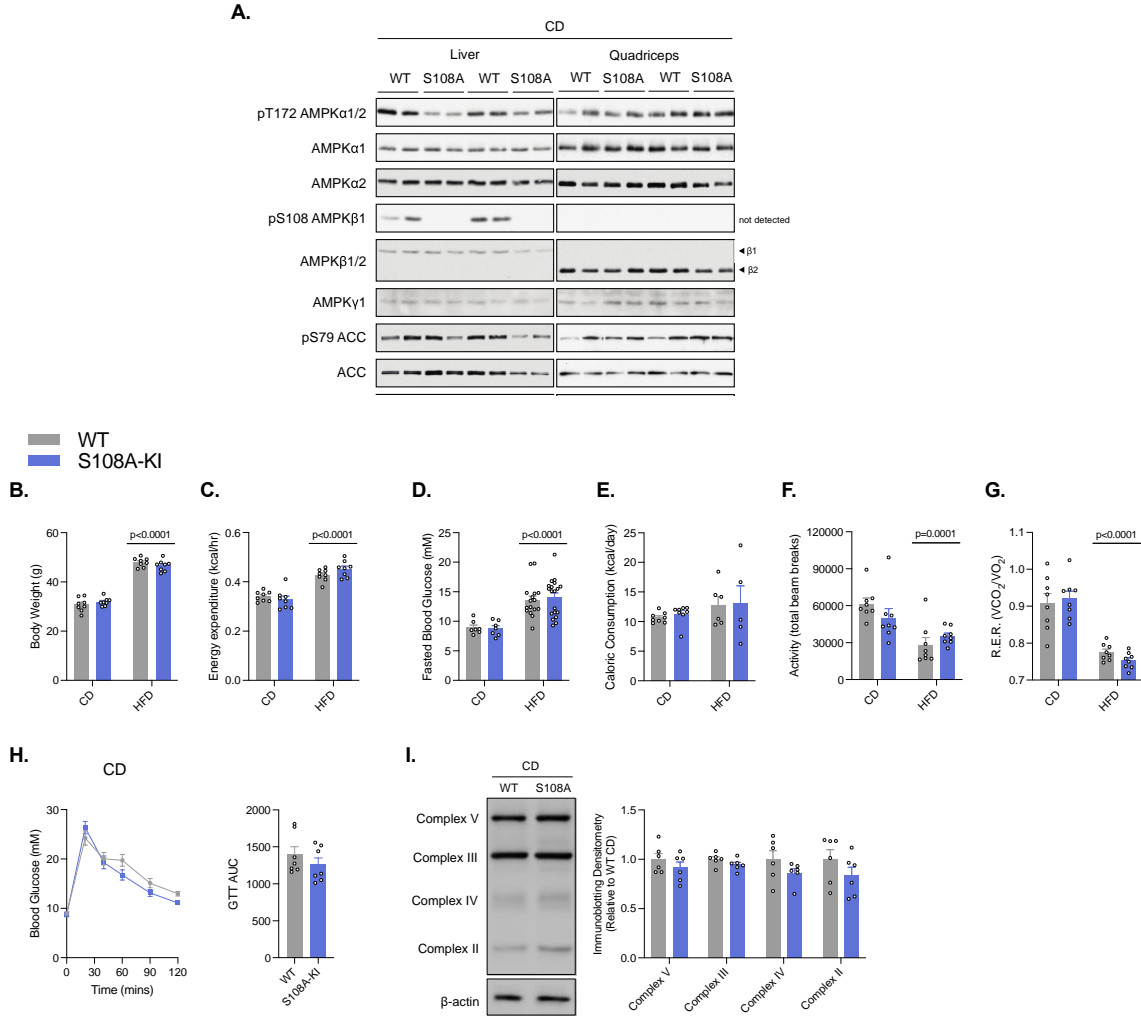
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SI References



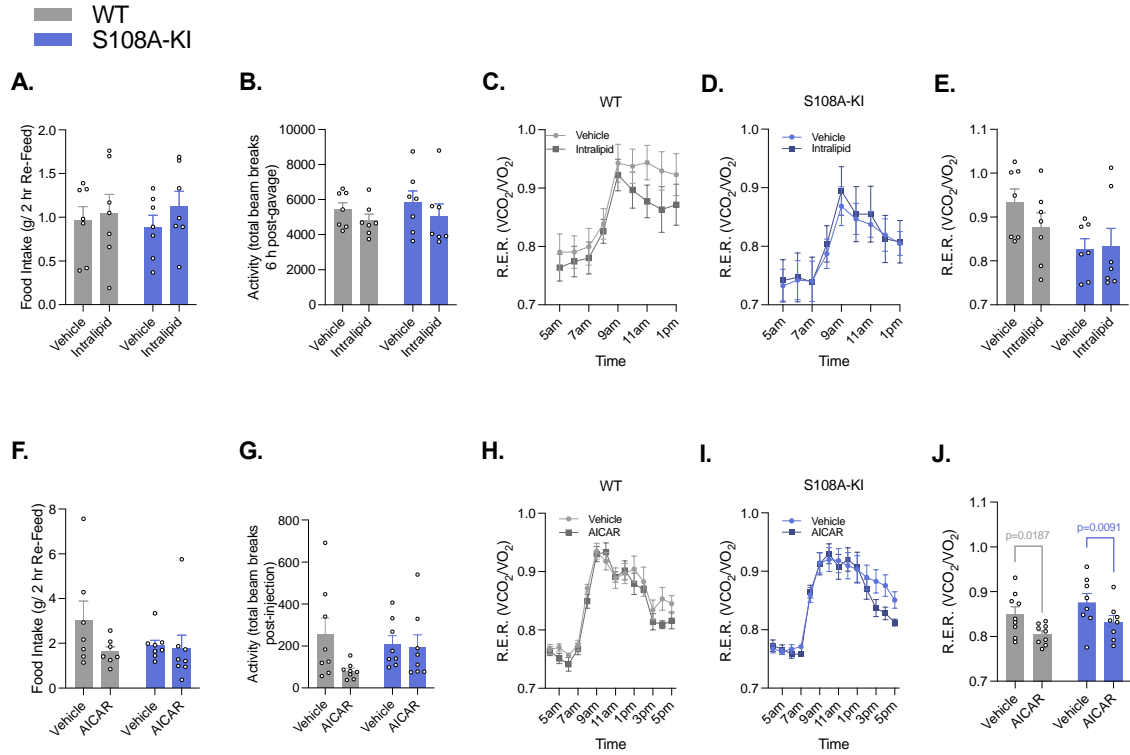
Supplemental Figure 1. Regulation of recombinant AMPK α 2 β 1 γ 1 WT and S108A mutant. (A) Recombinant human AMPK α 2 β 1 γ 1 wild-type (WT) and AMPK α 2 β 1(S108A) γ 1 mutant was expressed and purified as described previously (1), resolved by SDS-PAGE and stained with Coomassie blue (upper image) or subjected to immunoblot analysis (lower images) with the indicated antibodies. (B-C) AMPK activity of bacterially expressed recombinant α 2 β 1 γ 1 WT or S108A mutant in the presence of increasing concentrations of AMP (B) or A769662 (C) was measured as described (1). Results are presented as fold activation compared to no activator control (average \pm SEM of at least 3 independent experiments). (D) Unphosphorylated AMPK α 2 β 1 γ 1 WT and S108A was incubated with varying concentrations of recombinant CaMKK β and the phosphorylation of AMPK α Thr172 was determined by immunoblot analysis. The bands were quantified and normalized to AMPK β 1 expression and displayed in the graph above a representative blot (average \pm SEM of at least 3 independent experiments). (E) Phosphorylated AMPK α 2 β 1 γ 1 WT and S108A was incubated with varying concentrations of recombinant protein phosphatase 2C α (PP2C) and phosphorylation of AMPK α Thr172 was determined by immunoblot analysis. The percentage of pThr172 was determined relative to phosphorylated AMPK α 2 β 1 γ 1 WT or S108A in the absence of PP2C α and a graph is displayed above a representative blot (average \pm SEM of at least 3 independent experiments).



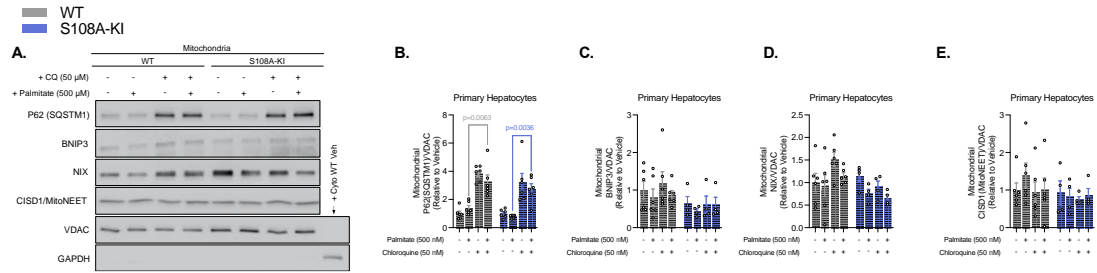
Supplemental Figure 2. S108A knock-in targeting strategy. A schematic illustrating the targeting strategy used to generate C57BL/6NTac *Prkab1*/AMPK β 1 S108A knock-in (KI) mouse model. The targeting strategy is based on NCBI transcript NM_031869.2. Exons and FRT recombination sites are represented by dark grey boxes and triangles, respectively. The amino acid to be exchanged (Ser108) is encoded by nucleotides located at the exon-intron boundary. There was a risk that changes in the nucleotide sequence might interfere with splicing of the transcript (leading to a hypomorphic effect). Therefore, we designed the targeting vector to achieve the S108A mutation with two options (KI-PM allele 1 or KI-PM allele 2). The constitutive KI-PM allele 1 is obtained after Cre-mediated removal of exons 2 to 7 (containing the joint exon 2 and 3) and of the NeoR selection marker. The KI-PM allele 1 strategy resulted in normal expression of the mutant (S108A) protein in tissues studied as demonstrated in Figure 1B and C.



Supplemental Figure 3. S108A-KI mice have no alterations in basal metabolic parameters on a chow or high-fat diet. (A) Immunoblots of AMPK subunits, phosphorylation, and downstream markers of activation in the same liver and Quad samples from Figure 1A (CD: chow diet-fed). (B-G) Body weight (B), energy expenditure (C), 6 hr fasted blood glucose (D), caloric consumption (E), activity levels (F), and respiratory exchange ratio (R.E.R.) (G) in WT and S108A-KI mice fed a control chow diet (CD) or high-fat diet (HFD). (H) Intraperitoneal glucose tolerance test (2.0 g/kg) and calculated area under the curve (GTT AUC) in WT and S108A-KI mice fed a control chow diet. (I) Representative immunoblots and densitometrical analysis of OXPHOS complexes 2-5 of WT and S108A-KI mice fed a control chow diet. Data are means \pm S.E.M. with p-values reported in the graphs. Black bars signify main effects for diet. Statistical significance was accepted at $p < 0.05$ and determined via two-way ANOVA. White circles are individual mice per group.



Supplemental Figure 4. S108A-KI mice have an attenuated response in lipid oxidation to exogenous lipids, but have a normal response to AICAR in vivo. Food intake during 2 hr refeed (A) prior to, and activity levels over 6 hrs (B) following, an oral gavage of saline or Intralipid (10 mL/kg) in chow diet-fed WT and S108A-KI mice. Respiratory exchange ratio (R.E.R.) time plots of chow-fed WT (C) and S108A-KI (D) treated with saline or Intralipid. Average RER (E) over 4 hrs, starting 1 hr post-gavage of saline or Intralipid (10 mL/kg). Food intake during 2 hr refeed (F) prior to, and activity levels over 1 hr, (G) starting 6 hours following, an injection of saline or AICAR (500 mg/kg). Respiratory exchange ratio (R.E.R.) time plots of chow-fed WT (H) and S108A-KI (I) treated with saline or AICAR. Average RER (J) over 1 hr, starting 6 hrs post-injection of saline or AICAR. Data are means \pm S.E.M. White circles are individual mice per group.



Supplemental Figure 5. Other markers of mitophagy are not altered with palmitate treatment or S108A-KI in primary hepatocytes from mice. Representative immunoblotting (A) and densitometrical analysis of P62 (B) BNIP3 (C), NIX (D), CISD1 (E) assessing mitophagy in response to palmitate (500 μ M) in primary hepatocytes from CD-fed WT and S108A-KI mice. Data are means \pm S.E.M. and white circles are technical replicates from experiments from 3 experimental replicates per group. Grey bar equals a WT difference and blue bar equals to a S108A-KI difference. Statistical significance was accepted at $p < 0.05$ and determined via repeated-measures two-way ANOVA with Tukey's posthoc analysis.

SI References

1. M. J. Sanders, *et al.*, Defining the mechanism of activation of AMP-activated protein kinase by the small molecule A-769662, a member of the thienopyridone family. *J. Biol. Chem.* **282**, 32539–32548 (2007).