This article contains the following supplemental material.

Supplementary Table S1: Breeding outcome of Het x Het mating of Prkaa1T183A and Prkaa2T172A mice.

Supplementary Table S2: RT-PCR primers used for this study.

Supplementary Figure S1. Gene targeting strategy to generate whole body AMPK-αα1KI and AMPK-αα2KI mice.

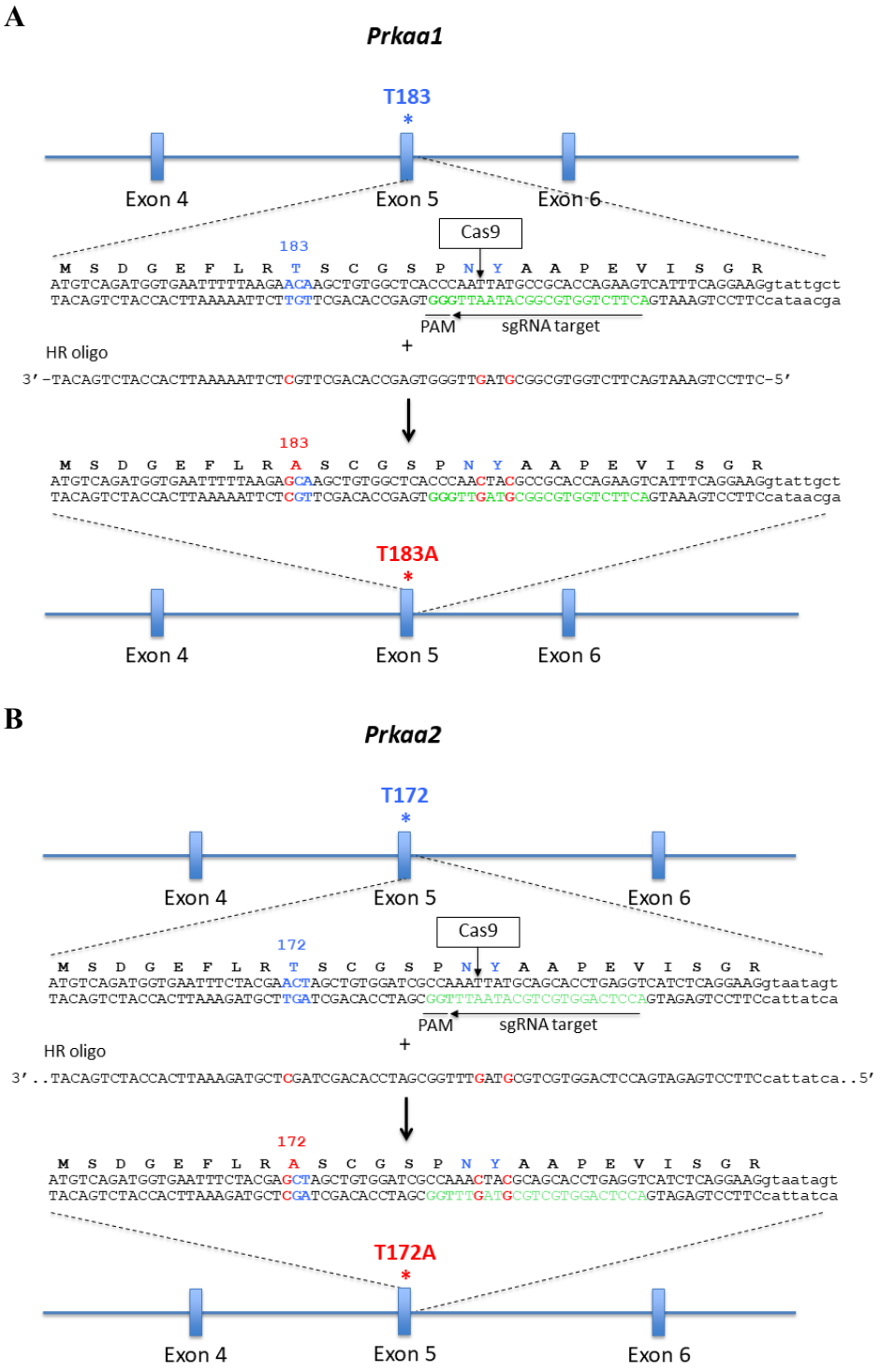
Supplementary Figure S2. Uncropped Western Blots used for Figures 1, 3, and 4.

Breeding Strain	**WT**	**Het (WT/KI)**	**KI (KI/KI)**	***P* (chi-squared)**
Prkaa1^{T183A} (Het/WT)	85	166	10	<0.0001
Prkaa1^{T183A} *Prkaa2*^{T172A} (Het/Het)	20	66	2	<0.0001
Prkaa2^{T172A} (Het/WT)	27	40	21	0.4618
Prkaa2^{T172A} *Prkaa1*^{T183A} (Het/Het)	23	44	22	0.8825

Supplementary Table 1: Breeding outcome of Het x Het mating of *Prkaa1*^{T183A} and *Prkaa2*^{T172A} mice.

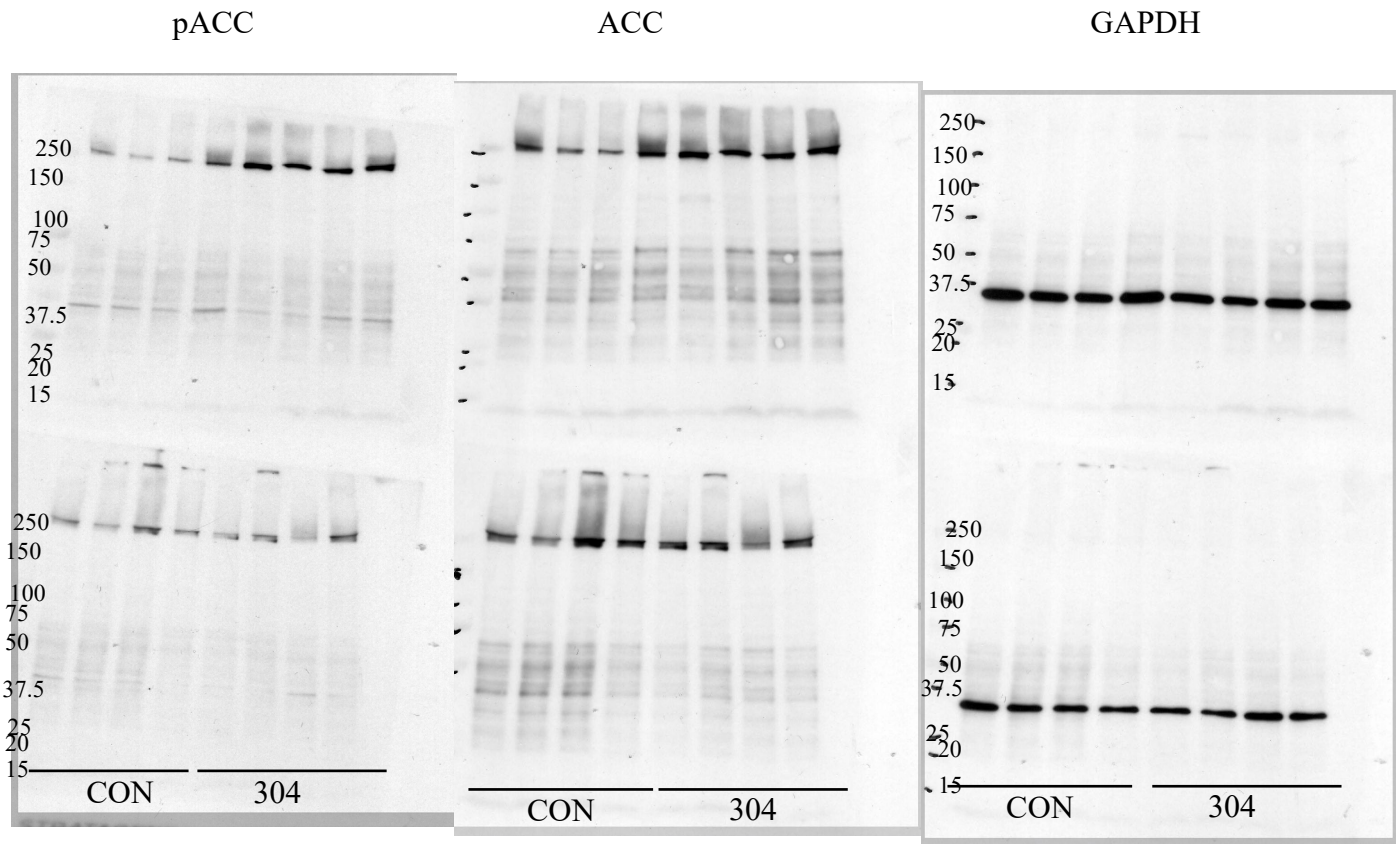
Gene	**Forward**	**Reverse**
NGAL	GCCCAGGACTCAACTCAGAA	GACCAGGATGGAGGTGACAT
KIM-1	GGAAGTAAAGGGGGTAGTGGG	AAGCAGAAGATGGGCATTGC
IL-6	CTCTGGGAAATCGTGGAAAT	CCAGTTTGGTAGCATCCATC
MCP-1	CAAGAAGGAATGGGTCCAGA	GTGCTGAAGACCTTAGGGCA
RIP1	CGTGAGAATATTAAGAGTGC	TGTACCTGTAGTTCCAAATC
RIP3	CACATACTTTACCCTTCAGA	TCAGAACAGTTGTTGAAGAC
Pgc1a	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
ACox1	CTTGGATGGTAGTCCGGAGA	TGGCTTCGAGTGAGGAAGTT
SIRT1	AGAACCACCAAAGCGGAAA	TCCCACAGGAGACAGAAACC
SIRT3	GGATTCGGATGGCGCTTGA	CACCTGTAACACTCCCGGAC
GAPDH	AAGGTCATCCCAGAGCTGAA	CTGCTTCACCACCTTCTTGA

Supplementary Table 2: RT-PCR primers used for this study.

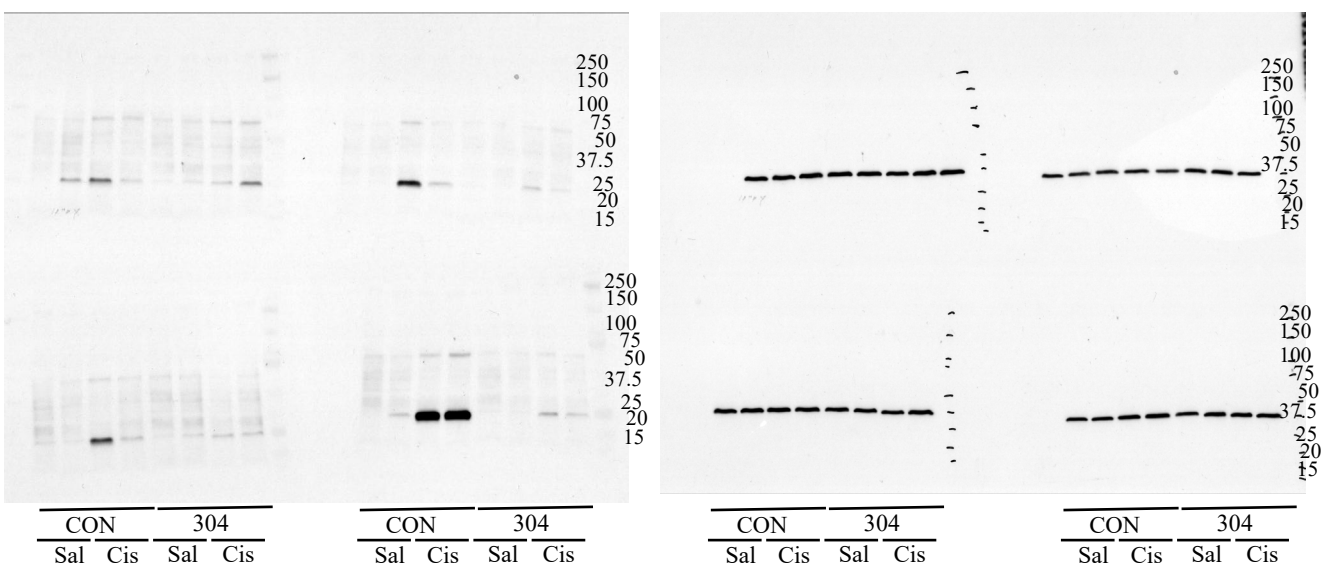


Supplementary Figure S1. Gene targeting strategy to generate whole body AMPK-α1KI and AMPK-α2KI mice. *Prkaa1*^{T183A} and *Prkaa2*^{T172A} mice were produced using CRISPR/Cas9 gene targeting in C57BL/6J mouse embryos. Single guide RNAs (sgRNA) were based on target sites in exon 5 of *Prkaa1* (CTTCTGGTGCGGCATAATT*GGG*) **(A)** and *Prkaa2* (ACCTCAGGTGCTGCATAATT*TGG*) **(B)**. For *Prkaa1* the oligonucleotide encoded the T183A (ACA>GCA) substitution plus sgRNA-inactivating silent mutations in the N189 (AAT>AAC) and Y190 (TAT>TAC) codons **(A)**, whilst for *Prkaa2* the oligonucleotide encoded the T172A (ACT>GCT) substitution plus sgRNA-inactivating silent mutations in the N178 (AAT>AAC) and Y179 (TAT>TAC) codons **(B)**. Founder males heterozygous for alleles that had been successfully modified by homologous recombination were backcrossed with C57BL/6J females to establish the *Prkaa1*^{T183A} and *Prkaa2*^{T172A} lines.

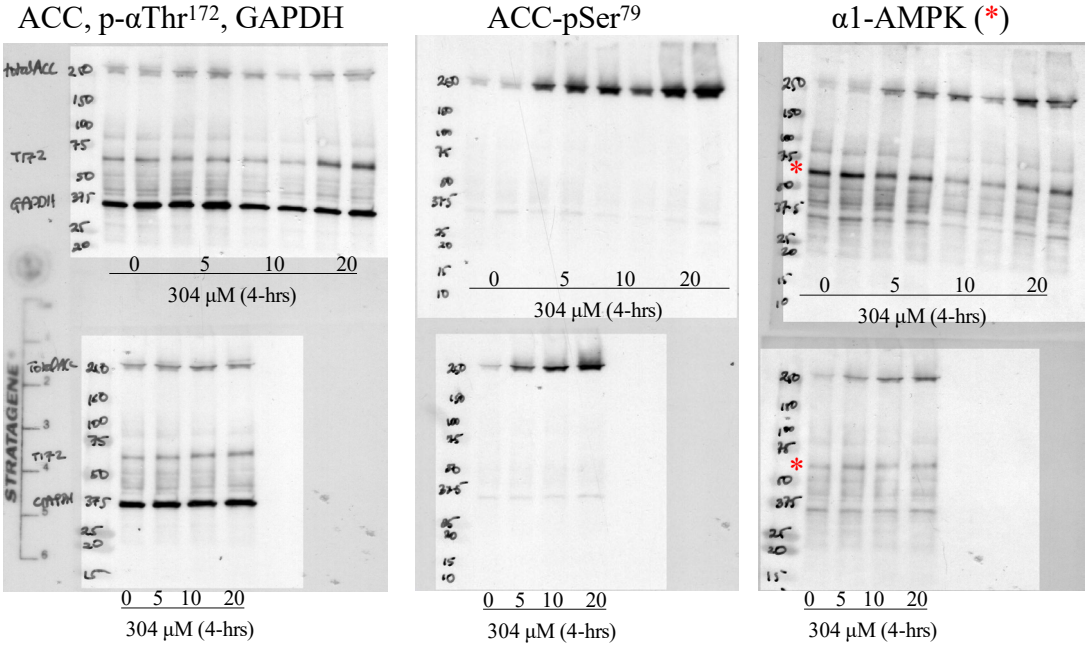
A

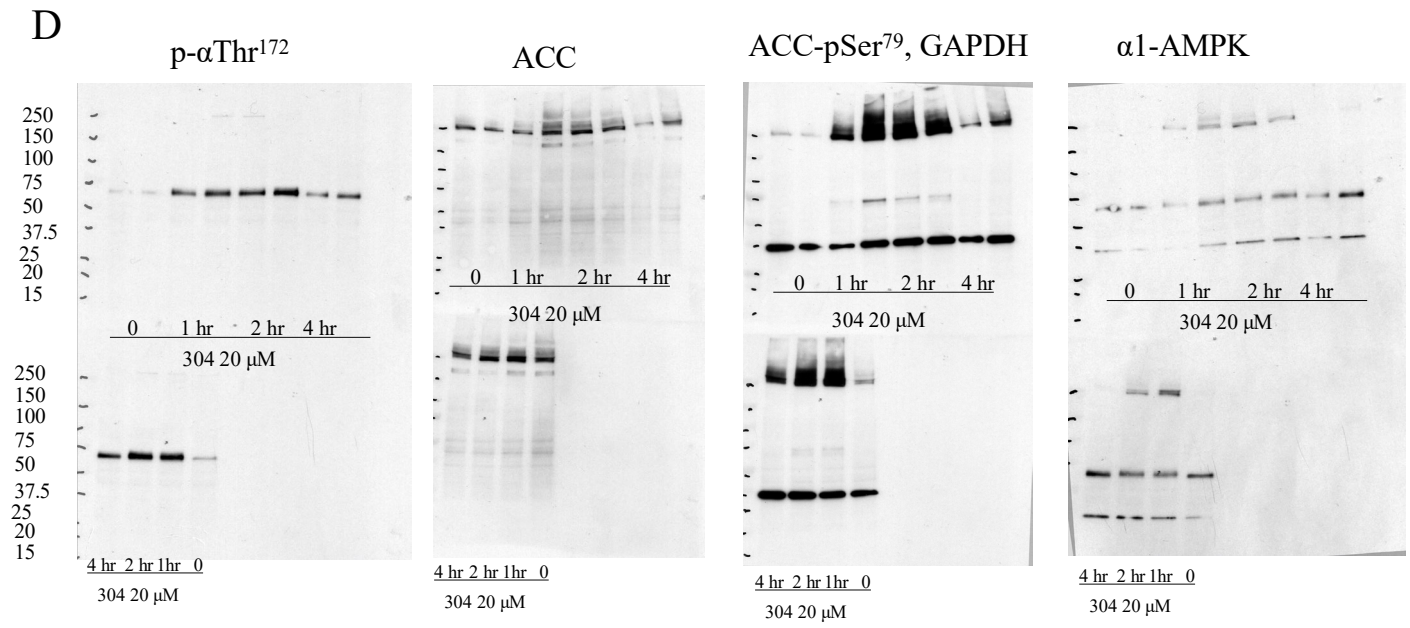

pACC
ACC
GAPDH
250
150
100
75
50
37.5
25
20
15
CON
304

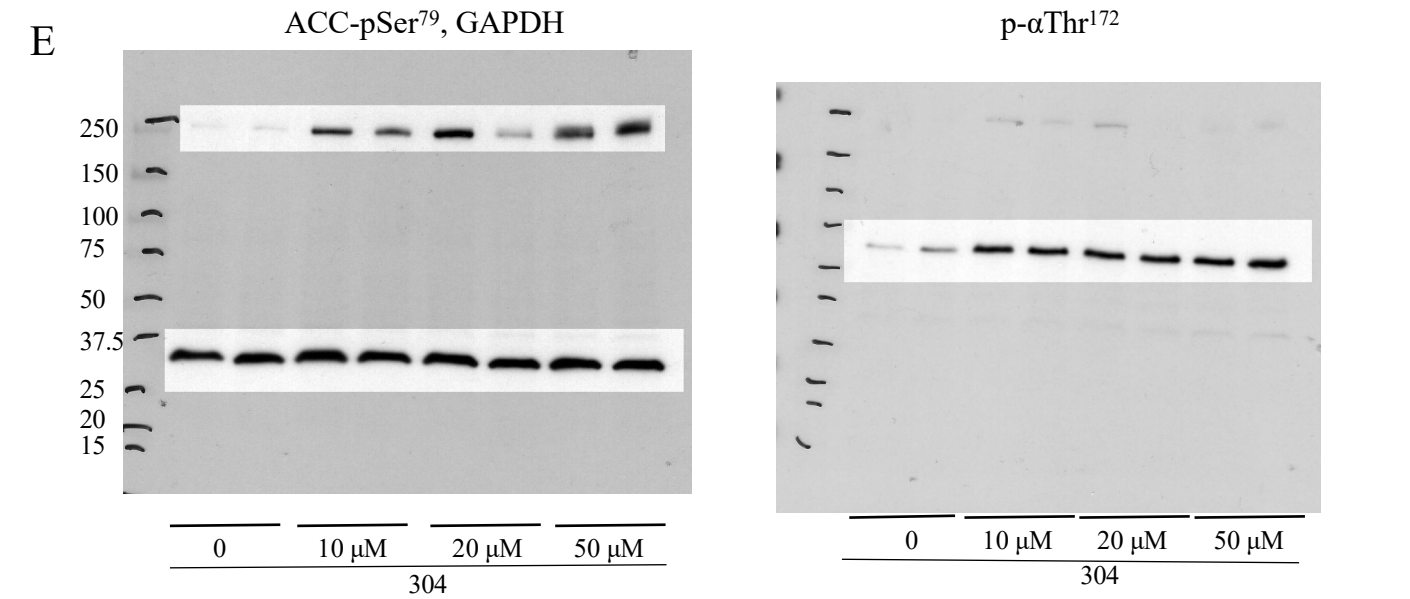
B

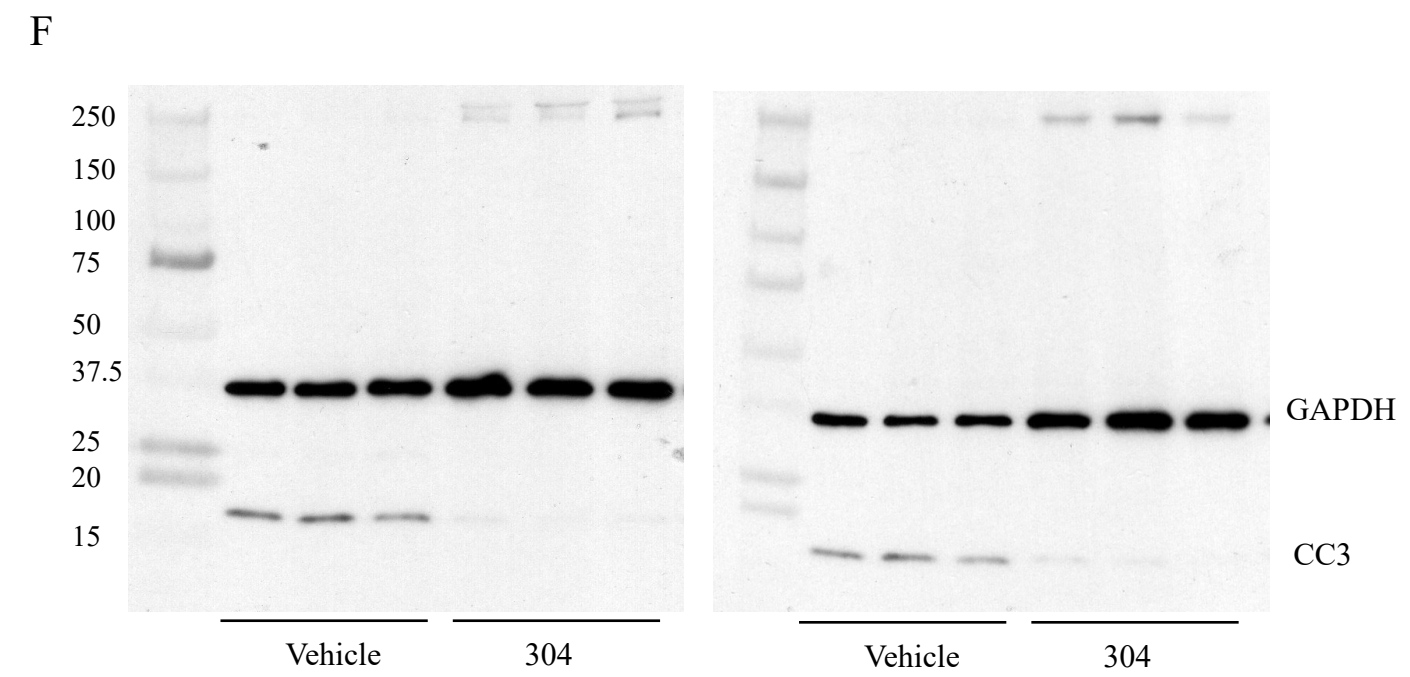

NGAL
GAPDH
250
150
100
75
50
37.5
25
20
15
CON
304
Sal
Cis

C


ACC, p-αThr172, GAPDH
ACC-pSer79
α1-AMPK (*)
0
5
10
20
304 μM (4-hrs)
STRATAGENE







Supplementary Figure S2. Uncropped Western Blots used for Figures 1, 3, and 4. Full length western blot images are shown. **A.** pACC, ACC and GAPDH blots used for Fig 1 A-B. **B**. NGAL and GAPHD blots used for Fig 1 C-D. **C.** pACC, ACC, α-AMPK, p-α-AMPK and GAPDH blots used for Fig 3 A-C. **D.** pACC, ACC, α-AMPK, p-α-AMPK and GAPDH blots used for Fig 3 D-F. **E.** pACC, p-α-AMPK and GAPDH blots used for Fig 3G. **F.** Cleaved caspase 3 (CC3) and GAPDH blots used for Fig 4 C-D.