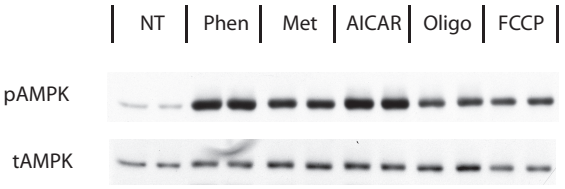
Supplemental Figure 1

A



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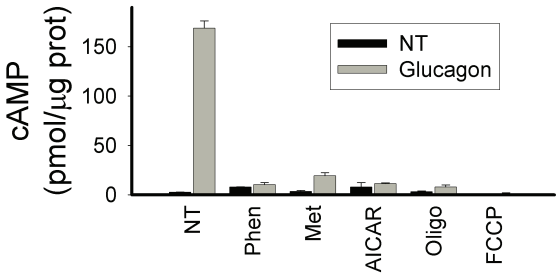
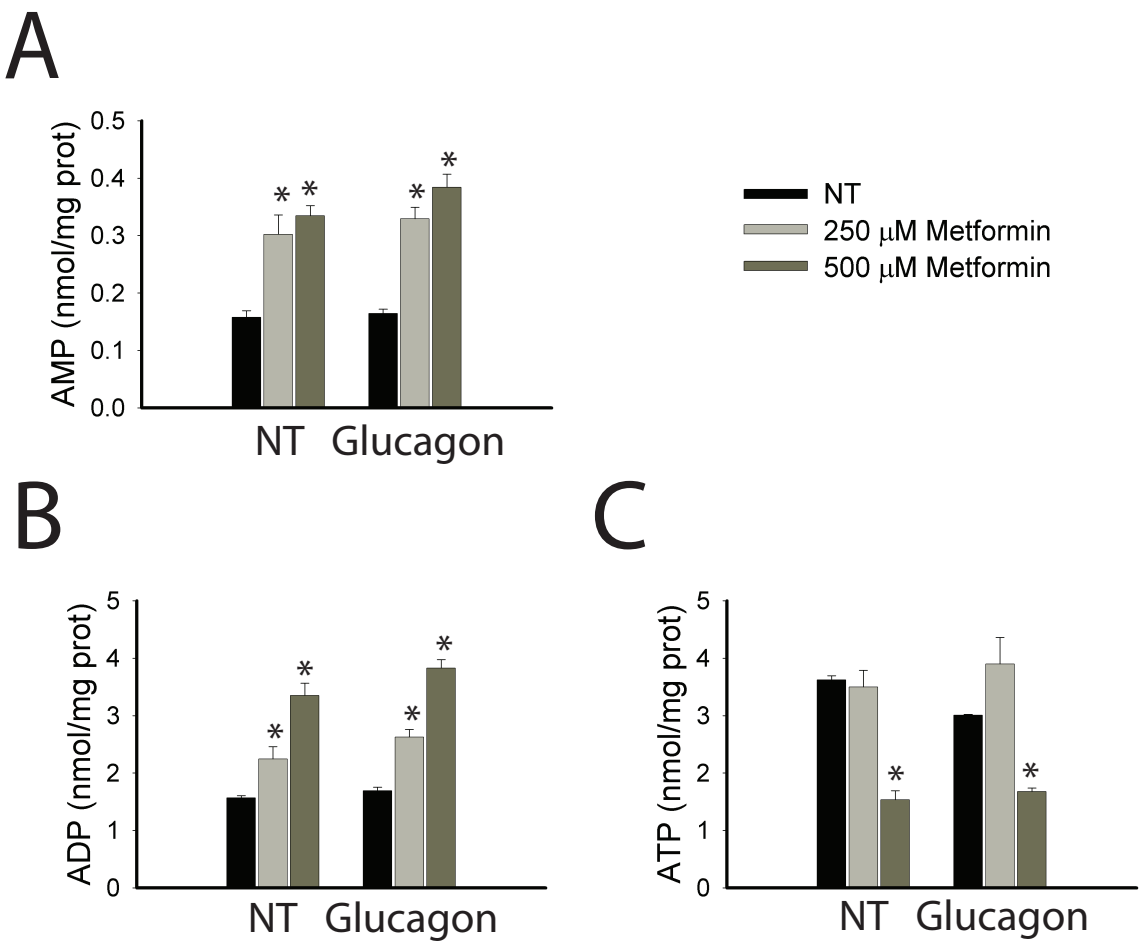
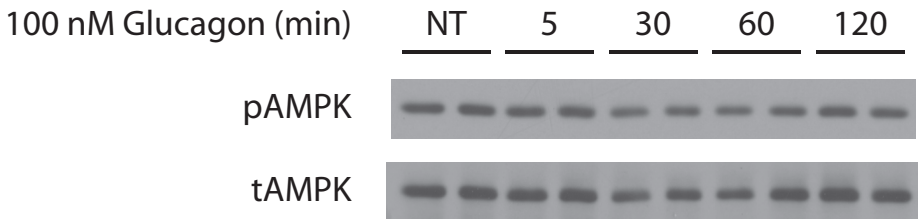


Supplemental figure 1. A-B. Primary hepatocytes were treated with 250 uM Phenformin, 1 mM Metformin 250 uM AICAR, 100 nM Oligomycin, or 500 nM FCCP for 2 hours, and assayed for total and phospho T172 AMPK **(A)** or treated with 5 nM glucagon for 15 minutes and subjected to assay for cAMP **(B).**

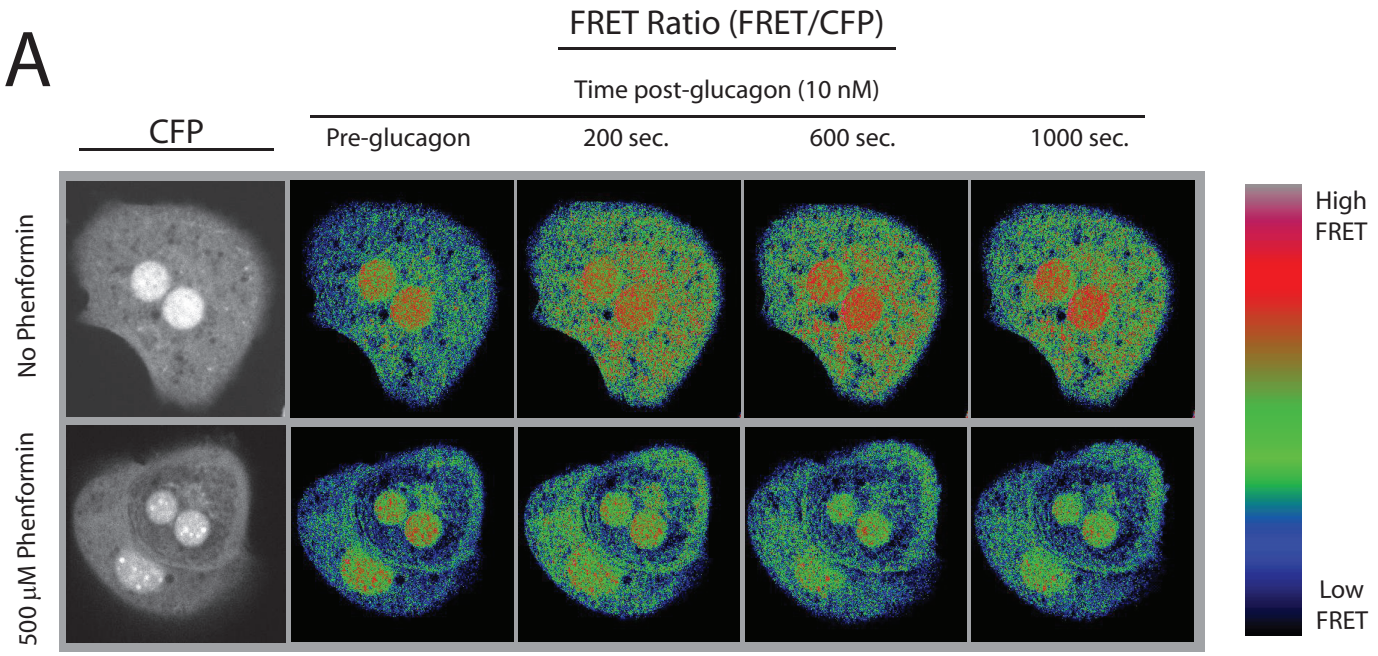
Supplemental figure 2

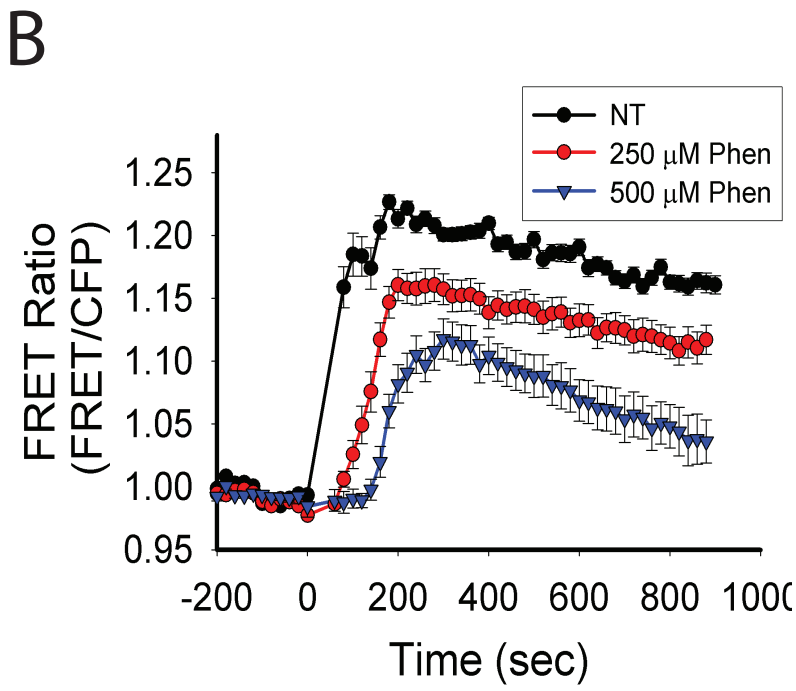




Supplemental Figure 2. **A-C.** Primary hepatocytes isolated from fasted mice were plated in M199 media and treated with the indicated concentrations of metformin and pbs or 100 nM glucagon for 18 hours. Metabolites were extracted with perchloric acid and analyzed by HPLC. **D.** Primary hepatocytes plated overnight in M199 media were treated with 100 nM glucagon for the indicated times and extracts were probed by western blot for the phosphorylation status of AMPK.

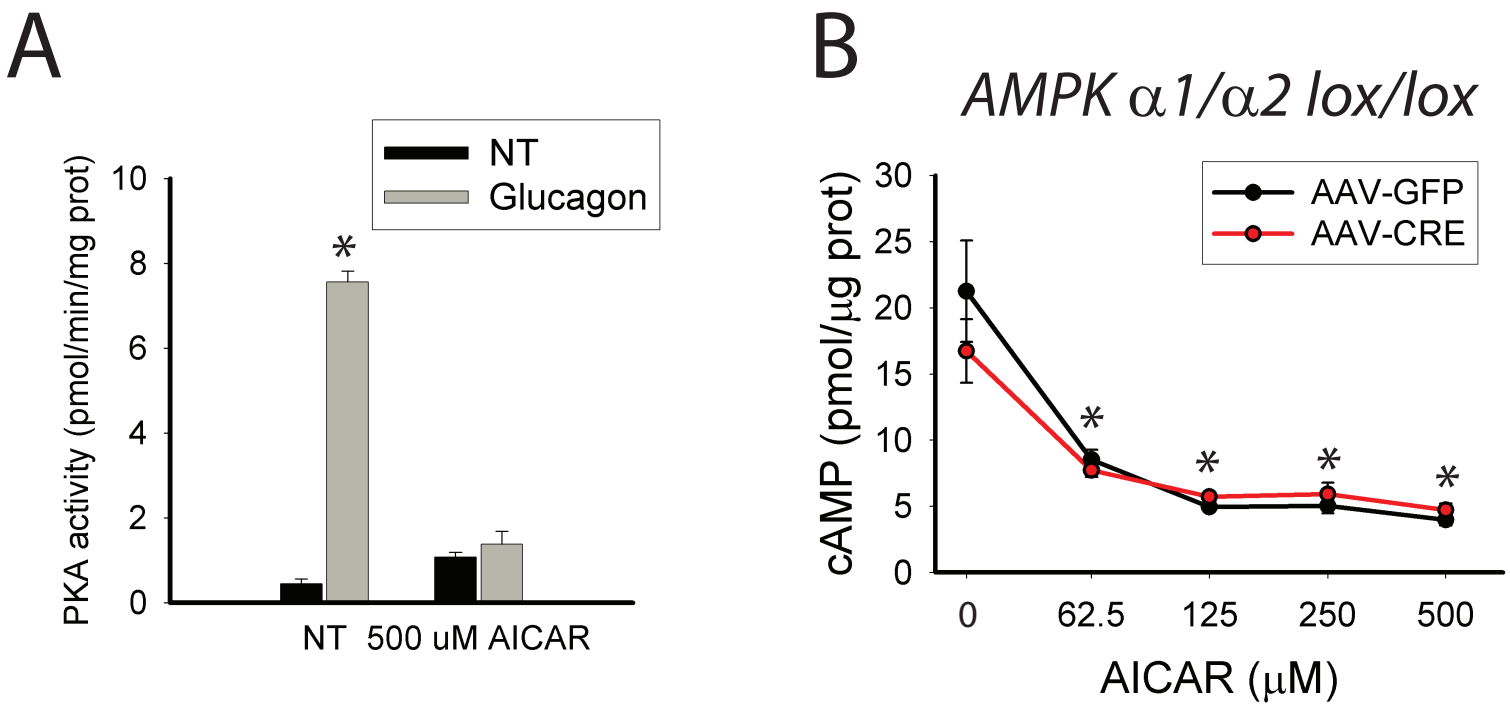
Supplemental figure 3

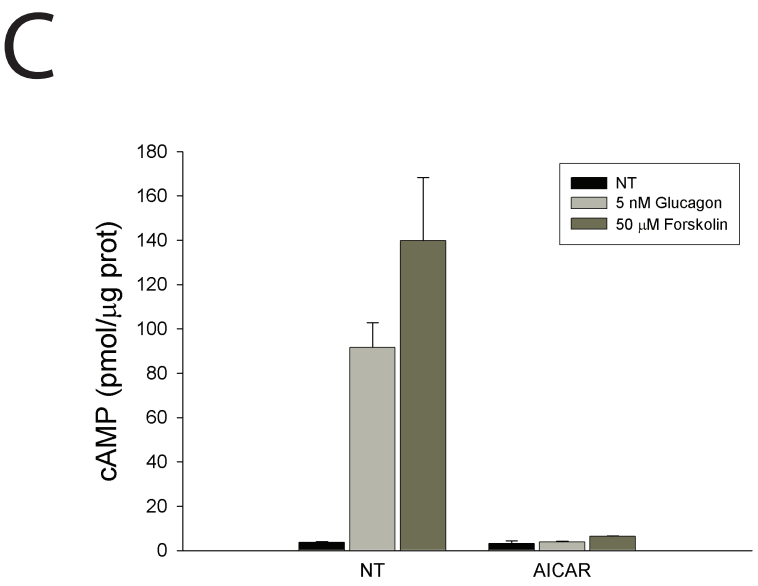




Supplemental figure 3. A-B. Primary hepatocytes were isolated and infected with adenovirus expressing the AKAR3 FRET-based activity probe.Cells were treated with the indicated concentrations of phenformin for 2 hours and images were aquired starting 5 minutes before addition of 10 nM glucaogn. **A.** Representative cells from untreated and phenformin treated cells, pseudocolored to reflect the FRET/CFP ratio. **B.** The calculated FRET/CFP ratios from 30-50 cytoplasmic ROI (one ROI per cell) for untreated, 250 uM, and 500 uM phenformin treated cells.

Supplemental figure 4

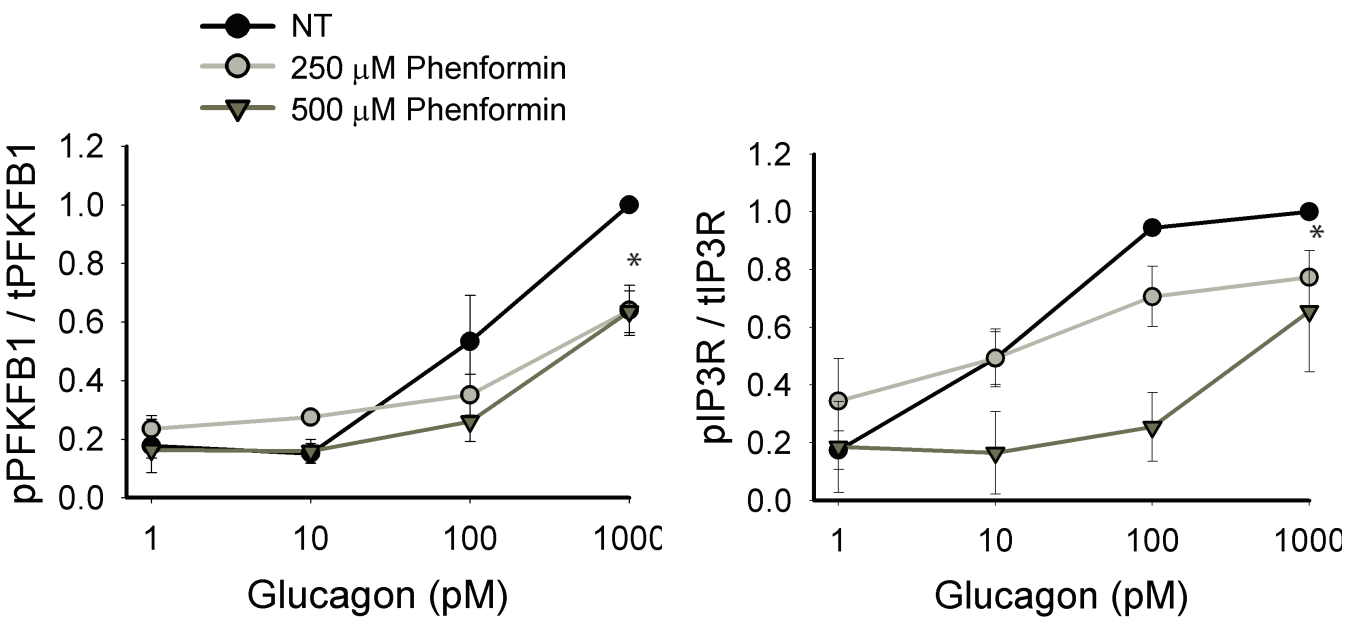




Supplemental figure 4. **A.** Primary hepatocytes were plated overnight in M199 media and treated with pbs or AICAR and stimulated with 5 nM glucagon for 15 minutes. Extracts were assayed for PKA activity (PKI-sensitive kemptide phosphorylation). **B.** Primary hepatocytes isolated from AMPK α1 and α2 floxed mice infected with AAV-TBG-Cre or AAV-TBG-GFP 14 days prior were treated with the indicated AICAR dose for 2 hours and stimulated with 5 nM glucagon for 15 minutes. cAMP levels were quantified by ELISA. **C.** Primary hepatocytes were plated overnight in M199 media and treated with 250 μM AICAR for 2 hours and subsequently stimulated with 5 nM glucagon or 50 μM Forskolin for 15 minutes. cAMP levels were quantified by ELISA.

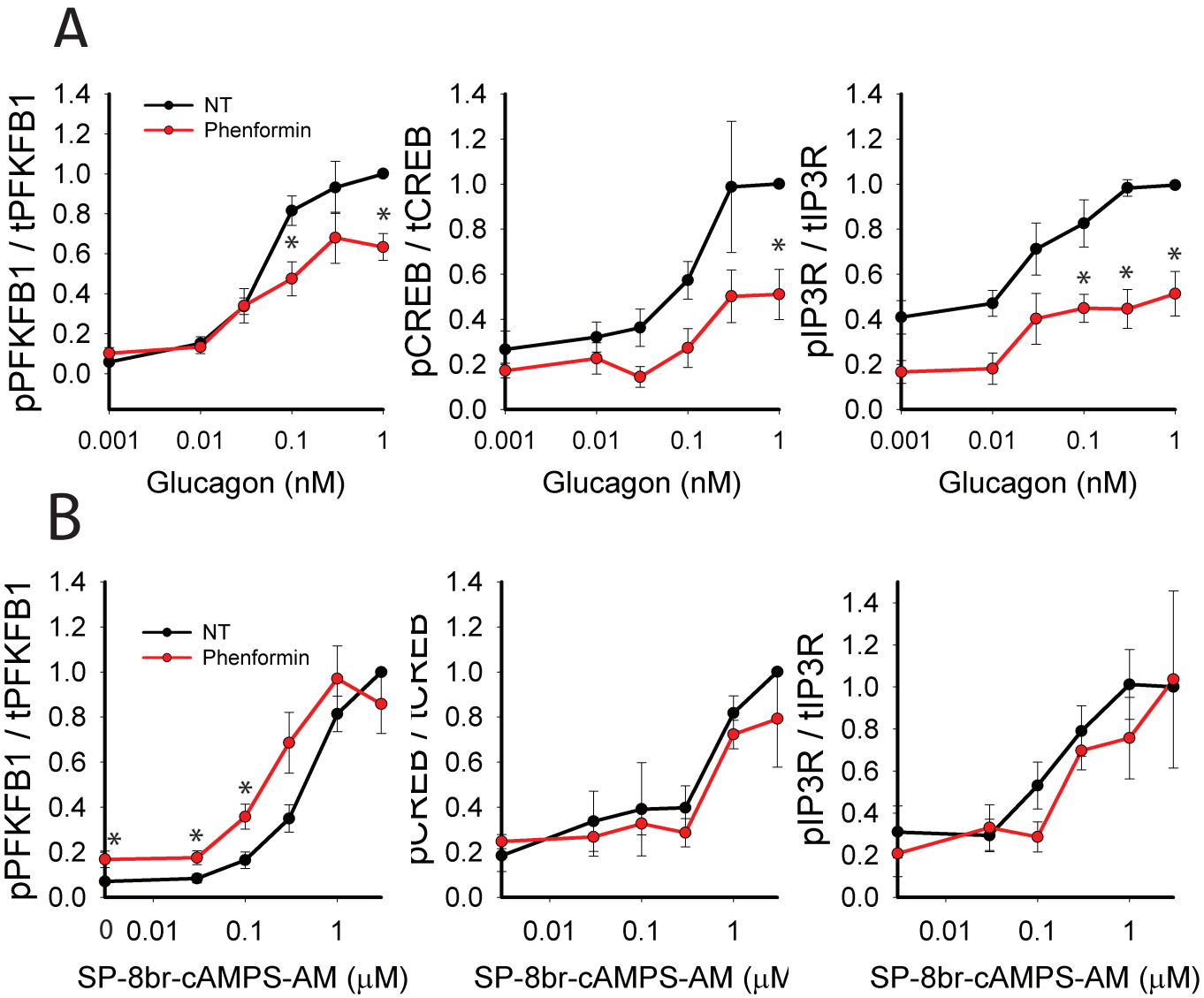
Supplemental figure 5

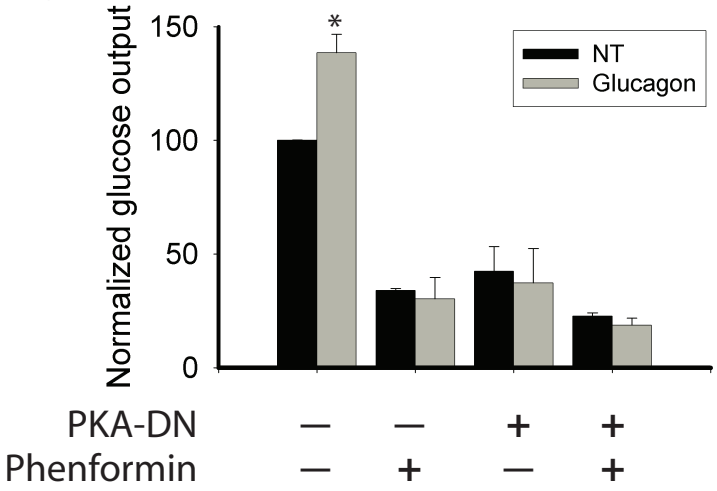
A



Supplemental figure 5. A. Primary hepatocytes were incubated with 250 and 500 uM phenformin for 2 hours and treated with the indicated glucagon concentrations. Western blots were performed, quantified and three representative blots are shown.

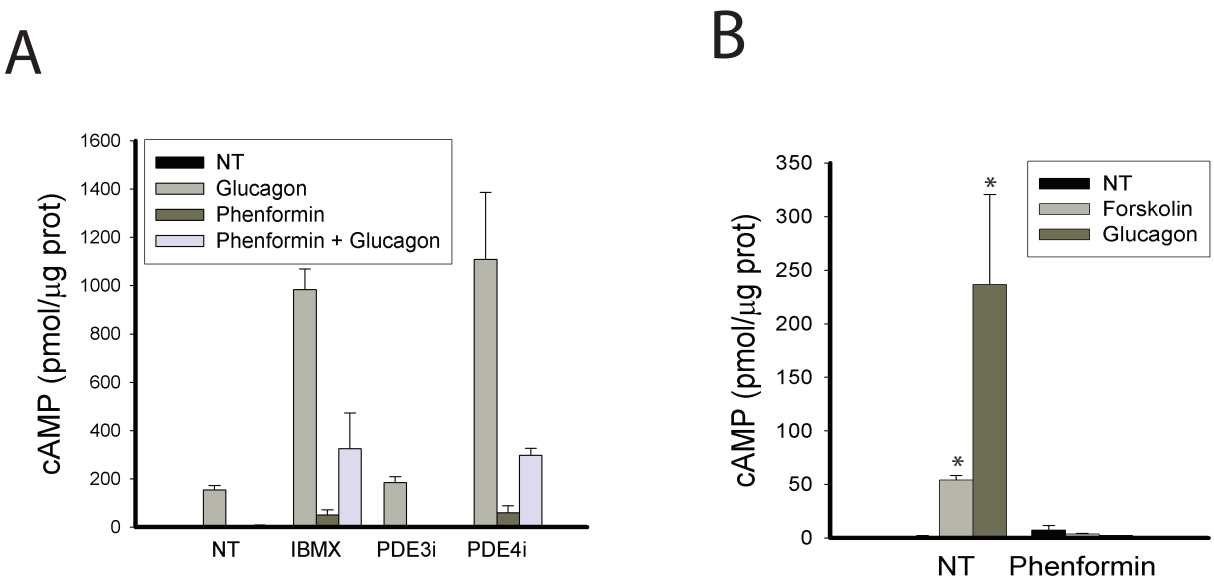
Supplemental figure 6





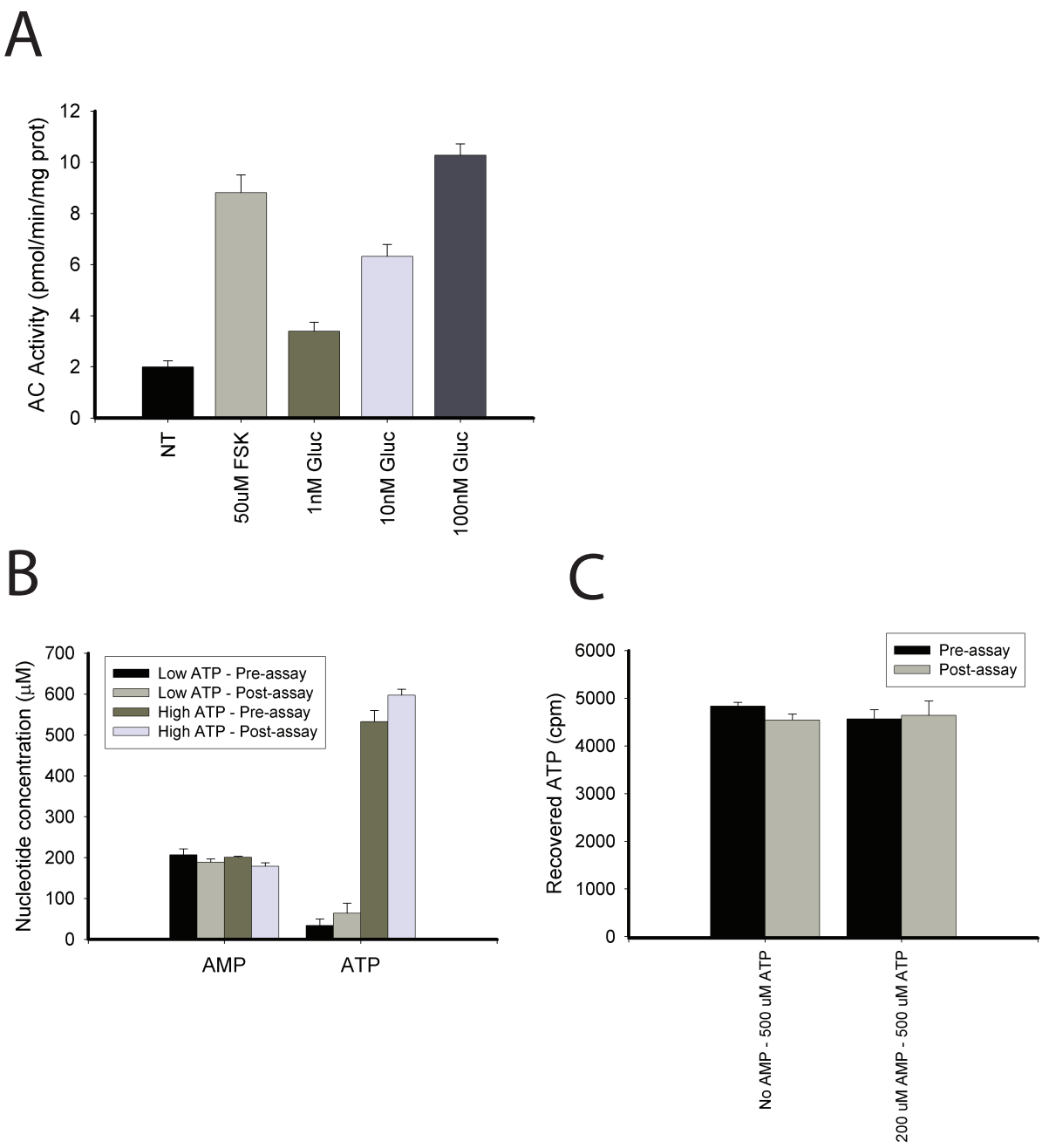
Supplemental figure 6. A-B. Primary hepatocytes were isolated, cultured for 18 hours in the presence or absence of 65 uM phenformin and treated for 15 minutes with the indicated concentrations of glucagon (**A)** or the cell permeable PKA agonist SP-8Br-cAMPS-AM **(B)**. Western blots were performed for phospho and total PFKFB1, CREB, and the IP3R. Three or more western blots were quantified. **C.** Primary hepatocytes isolated from mice infected with AAV-TBG-PKA-DN or AAV-TBG-GFP control virus 7 days earlier were plated and treated for 1 hour with 250 uM phenformin or PBS and then subjected to a glucose production assay for 2 hours with 10 nM glucagon and 250 uM phenformin. Data represents the mean of 3 of these experiments.

Supplemental figure 7



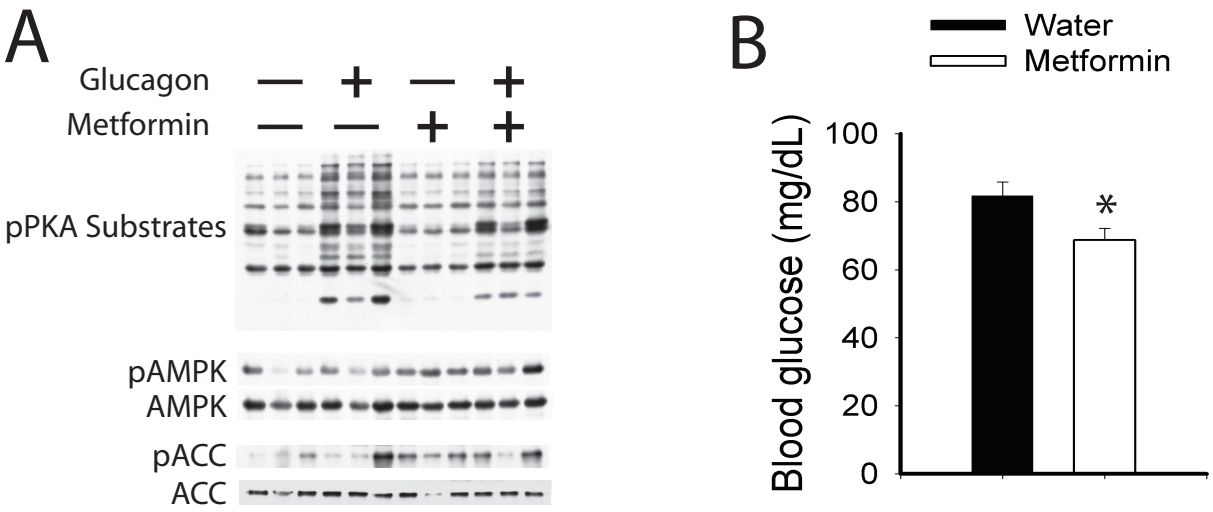
Supplemental figure 7. A. Primary hepatocytes were incubated with 250 uM Phenformin for 2 hours, with or without 20 uM IBMX, 20 uM Cilostamide (PDE3Bi), or 50 uM RO-20-1724 (PDE4i) for the final 30 minutes. Cells were treated with 5 nM glucagon for 15 minutes, lysed, and total cellular cAMP assayed. **B.** Primary hepatocytes were incubated with 250 uM phenformin for 2 hours, treated wiht 5 nM glucagon or 20 uM forskolin for 15 minutes, lysed, and total cellular cAMP was assayed.

Supplemental figure 8



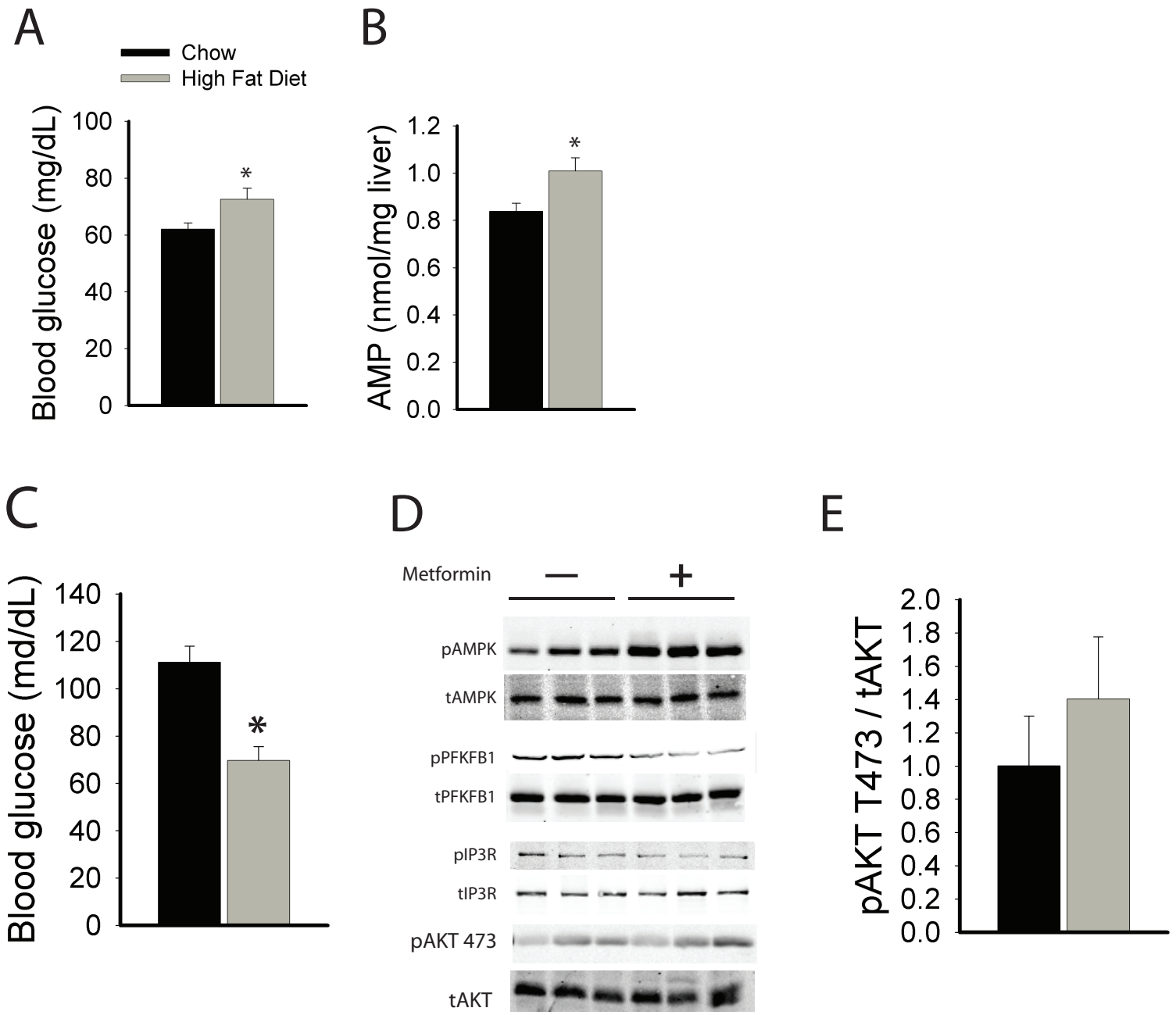
Supplemental figure 8 A. Membranes from primary hepatocytes were isolated and assayed for adenylyl cyclase activity in the presence of the indicated concentrations of forskolin and glucagon. All incubations included 100 uM GTP. **B.** AC assays were performed at low (40 uM) and high (500 uM) ATP concentrations in the presence of 300 uM AMP. Assays were stopped with PCA before or after 10 minutes at 30 C. Nucleotides were quantified by HPLC. **C.** AC assays were perfomed with 500 uM ATP in the presence or absence of 300 uM AMP. Nucleotides were separated by TLC and the radioactivity in the ATP fraction was quantified in scintillation solution after removal from the TLC plates.

Supplemental figure 9



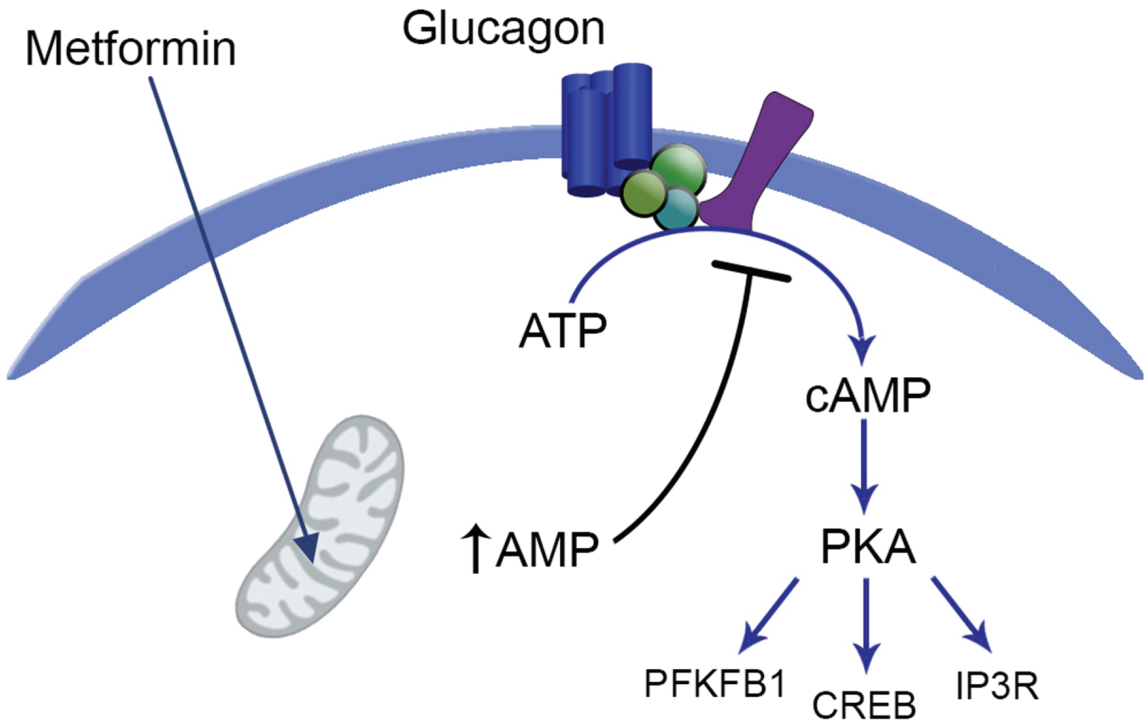
Supplemental figure 9. A. Fed mice were fasted for 1 hour and gavaged with water or 500 mg/kg bw of metformin. One hour later mice were injected intraperitoneally with 2 mg/kg glucagon, and liver tissue was collected 5 minutes later. Western blot analysis was performed, examining reactivity to phospho-PKA substrate antibodies, phosphorylated and total AMPK, and phosphorylated and total ACC. **B.** 18 hour fasted mice were gavaged with 50 mg/kg metformin and 1 hour later blood glucose levels were measured.

Supplemental figure 10



Supplemental figure 10. A. Mice fed normal chow or high fat diet for 10 weeks were fasted overnight and blood glucose was tested. B, and the total hepatic AMP levels were quantified. C-E. Mice fed high fat diet for 10 weeks were fasted overnight and gavaged with 250 mg/kg metformin. One hour later blood glucose was assayed (C) and livers were collected and assayed by western blots for total and phosphorylated AMPK, PFKFB1, IP3R, and AKT (D). pAKT/total AKT is quantified (E).

Supplemental Figure 11



Proposed Model
Metformin enters the cell and acts on the mitochondria, causing increased AMP. Elevated cellular AMP levels inhibit membrane bound Adenylyl Cyclase, causing a reduction in cellular cAMP levels and decreased PKA activation and target phosphorylation.

Supplemental Table 1

Adenine nucleotides in liver

	AMP (mM)	ADP (mM)	ATP (mM)	AMP/ATP
NC – Water	2.39 ± .09	3.28 ± .23	3.53 ± .32	0.75 ± .08
NC – Metformin	2.99 ± .06 *	2.47 ± .14 *	1.70 ± .16 *	1.92 ± .24 *
HFD – Water	2.88 ± .16	3.69 ± .23	3.44 ± .12	0.84 ± .05
HFD – metformin	3.03 ± .21	3.14 ± .25	2.89 ± .31	1.08 ± .09 *

Normal chow (NC) and high fat diet fed mice (HFD) were fasted 18 hours and gavaged with water or 250 mg/kg metformin. 1 hour later, livers were collected, and adenine nucleotides were extracted and quantified by HPLC. Measurements (μmoles/mg protein) were converted to concentrations based on protein recovery and hepatocyte volume[24]. * denotes $p<0.05$ from water control.

Supplemental Table 2

Adenine nucleotides in primary hepatocytes

	AMP (μM)	ADP (mM)	ATP (mM)	AMP/ATP
NT	215 ± 14.8	3.21 ± .195	6.02 ± .376	0.03 ± .001
30 μM	226 ± 27.5	3.08 ± .276	6.59 ± .147	0.03 ± .003
100 μM	297 ± 36.6	4.42 ± .633	7.31 ± .713	0.04 ± .015 *
250 μM	757 ± 185.6 *	5.26 ± .349 *	4.46 ± .140 *	0.16 ± .041 *
300 μM	783 ± 61.0 *	5.98 ± .215 *	4.12 ± .317 *	0.19 ± .026 *
500 μM	1032 ± 90.4 *	5.80 ± .227 *	3.15 ± ,273 *	0.33 ± .043 *

Primary hepatocytes were isolated from fed mice and treated with phenformin for 2 hours. Adenine nucleotides were extracted and quantified by HPLC. Concentration values were calculated as in Table 1. The AMP values and those in figure 1d are derived from the same experiment. * denotes $p<0.05$ compared to controls. NT = Not treated