

Supplementary Online Material for

**Metformin stimulates IGFBP-2 gene expression through
PPARalpha in diabetic states**

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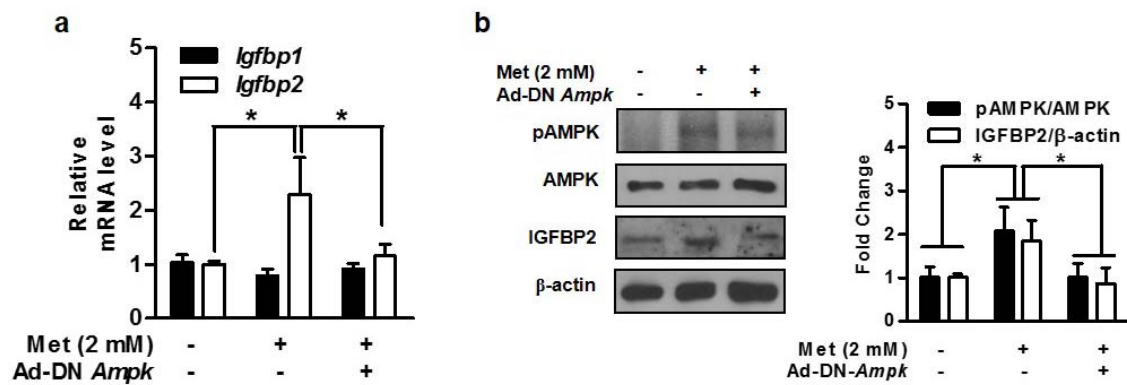
Supplementary material and method

Recombinant adenovirus. Adenoviruses overexpressing dominant negative (DN)-*Ampk α 1* has been described previously¹.

Supplementary references

1. Woods, A., *et al.* Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. *Molecular and cellular biology* **20**, 6704-6711 (2000).

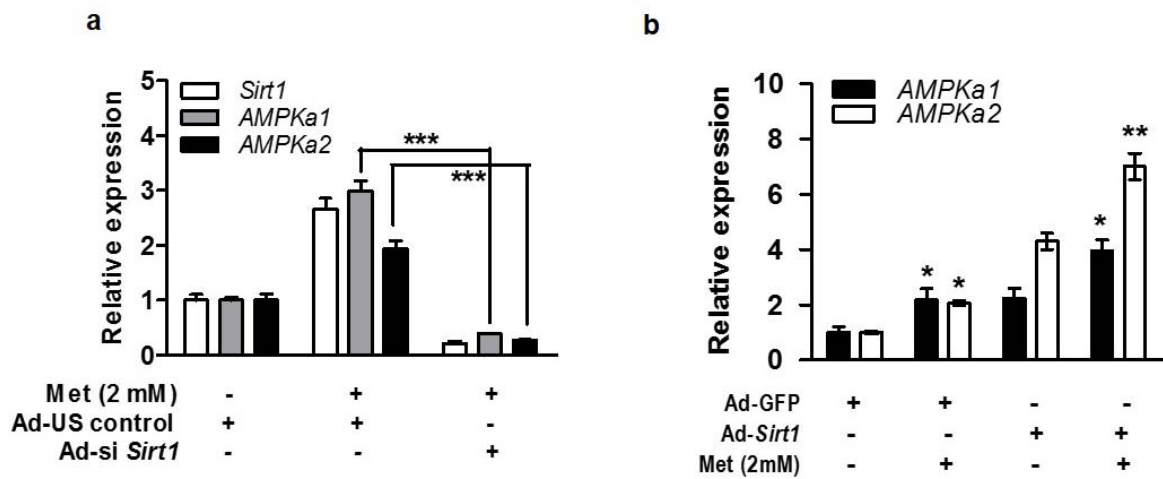
Supplementary Figure 1. Metformin-induced *Igfbp-2* gene expression is mediated by AMPK α 1. (a) Expression of hepatic *Igfbp-2* in mouse primary hepatocytes. Mouse primary hepatocytes were infected with Ad-DN-*Ampk α 1* for 36 h and then treated with metformin for 12 h. (b) IGFBP2 protein level and AMPK phosphorylation in mouse primary hepatocytes for the indicated conditions. * $p < 0.05$ vs. untreated control and/or metformin-treated cells.



Supplementary Figure 2. AMPK α expression is affected by Sirt1 expression level. (a)

Effect of *Sirt1* depletion on expression of *AMPK α 1* and *AMPK α 2*. AML12 cells were infected with Ad-US or Ad-si *Sirt1* for 36 h and then treated with metformin for 12 h. (b)

Effect of *Sirt1* overexpression on *AMPK α 1* and *AMPK α 2* expression. Mouse primary hepatocytes were infected with Ad-GFP or Ad- *Sirt1* for 24 h and then treated with metformin for 12 h. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. untreated control.



Supplementary Table 1. List of primers employed for gene expression analysis by real-time PCR.

	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Igfbp-1</i>	ATCAGCCCATCCTGTGGAAC	TGCAGCTAATCTCTCTAGCACTT
<i>Igfbp-2</i>	ACCCCTTGCCAGCAGGAGTTGGA	TCCCTGGATGGGCTTCCCGGT
<i>Acc1</i>	TGACAGACTGATCGCAGAGAAAG	TGGAGAGCCCCACACACA
<i>Gpat</i>	CAACACCATCCCCGACATC	GTGACCTTCGATTATGCGATCA
<i>Ppara</i>	AGAGCCCCATCTGTCCTCTC	ACTGGTAGTCTGCAAAACCAA
<i>Sirt1</i>	GCAGATTAGTAGGCGGCTTG	TCTCCATCAGTCCCAAATCC
<i>AMPKa1</i>	GTCAAAGCCGACCCAATGATA	CGTACACGAAATAATAGGGGTT
<i>AMPKa2</i>	TCCTGAAGACCCCTCCTACG	GAGTGGTTCTCAGCTGTGCT
<i>L32</i>	ACATTTGCCCTGAATGTGGT	ATCCTCTTGCCCTGATCCTT