

## **AMPK is not required for the effect of metformin on the inhibition of BMP6-induced hepcidin gene expression in hepatocytes.**

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### **Legend of Figures**

#### **Supplemental Figure 1: Effect of metformin, 991 and C13 on basal hepcidin gene expression in WT and AMKPLiv KO mice.**

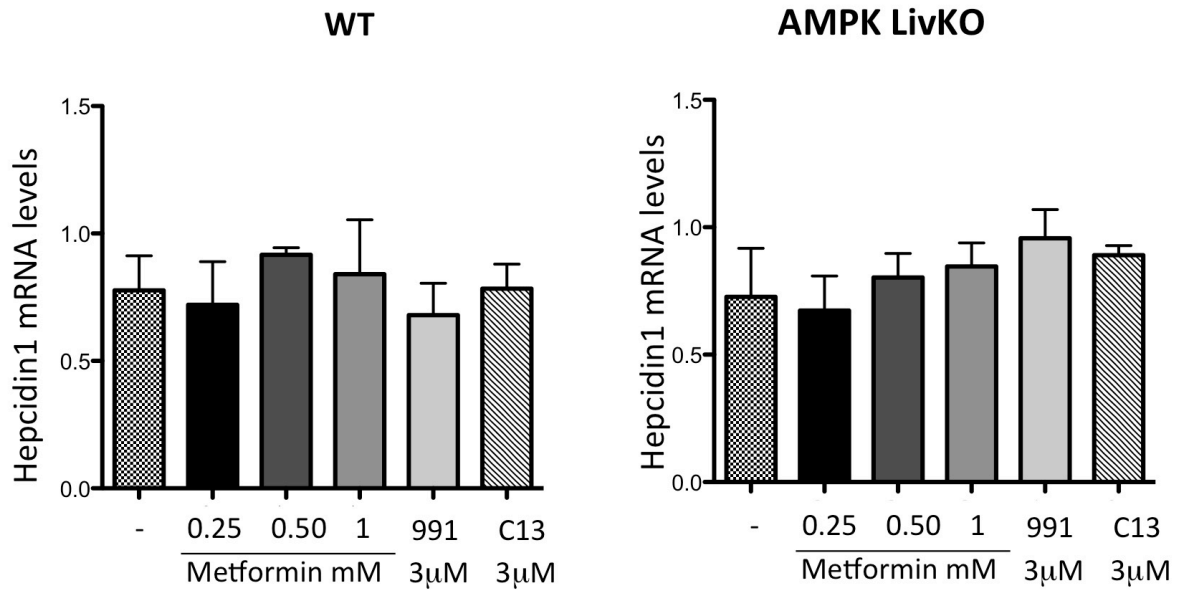
(A) Q-PCR analysis showing hepcidin mRNA levels in mouse primary hepatocytes treated with metformin (from 0.25 to 1mM) for 24 h), 991 (3 $\mu$ M) or C13 (1 $\mu$ M) for 24 h. RNA extraction and real-time quantification of transcripts were performed as described. mRNA expression was calculated using the  $\Delta\Delta$ Ct method and normalized to the expression of cyclophilin. Hepcidin gene expression is expressed as-fold hepcidin expression of that in non-treated hepatocytes. Error bars represent SD for n=3 samples in each group.

#### **Supplemental Figure 2: Effect of metformin on basal SHP gene expression in WT and AMPKPLiv KO mice.**

(A) Q-PCR analysis showing SHP mRNA levels in mouse primary hepatocytes treated with metformin (from 0.25 to 1mM), 991 (3 $\mu$ M) or C13 (3 $\mu$ M) for 24 h. RNA extraction and real-time quantification of transcripts were performed as described. mRNA expression was calculated using the  $\Delta\Delta$ Ct method and normalized to the expression of cyclophilin. SHP gene expression is expressed as-fold SHP expression of that in non-treated hepatocytes.

Error bars represent SD for n=3 samples in each group. Statistical significance is indicated by \* symbols (\*p < 0.05, \*\*p < 0.005, \*\*\*p < 0.0005 as compared to that of non-treated hepatocytes).

SFig 1



SFig 2

