



**Figure S2** Effect of Mediator MED23 deletion on signaling and expression of genes involved in glucose and lipid metabolism. **(A)** Immunoblot of AKT, FOXO1 and GSK3α/β activation with insulin (Ins) stimulation in primary hepatocytes. **(B)** Immunoblot of CREB activation upon glucagon treatment in primary hepatocytes. Both experiments and immunoblot assays were repeated independently two times **(A and B)** and the results were consistent. **(C)** Quantitative PCR (Q-PCR) revealed the reduced gene expression from RNA-seq analysis in

livers of LMKO compared to control mice under fasting state. n = 5-7 for each group.

**(D)** Venn diagram of downregulated genes (fold change  $\geq 1.5$ ) in LMKO to control and FOXO1 target genes. **(E)** Glucose output of primary hepatocytes isolated from control and LMKO mice. **(F)** Immunoblot of MED23 expression in Ad-ctrl or Ad-cre infected primary hepatocytes isolated from control mice. **(G-H)** Q-PCR analysis of genes expression responding to forskolin (FSK) in control (*Med23<sup>fl/fl</sup>*) primary hepatocytes infected with Ad-ctrl or Ad-cre. **(I)** Glucose output of Ad-ctrl or Ad-cre infected primary hepatocytes isolated from control mice. For panel **C**, **E**, **G** to **I**, data are presented as mean  $\pm$  s.e.m. *NS*, not significant, \**P* <0.05, \*\**P* <0.01, \*\*\**P* <0.001 vs control by Student's two-tailed *t*- test.