Science Signaling
AAAS

www.sciencesignaling.org/cgi/content/full/4/158/ra6/DC1

Supplementary Materials for

Wnt Signaling Regulates Hepatic Metabolism

Hongjun Liu, Maria M. Fergusson, J. Julie Wu, Ilsa I. Rovira, Jie Liu, Oksana Gavrilova, Teng Lu, Jianjun Bao, Donghe Han, Michael N. Sack, Toren Finkel*

*To whom correspondence should be addressed. E-mail: finkelt@nih.gov

Published 1 February 2011, *Sci. Signal.* **4**, ra6 (2011)
DOI: 10.1126/scisignal.2001249

The PDF file includes:

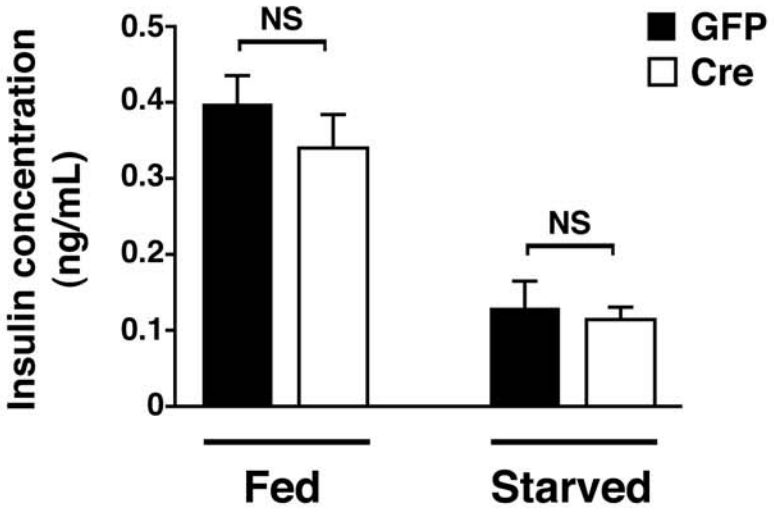
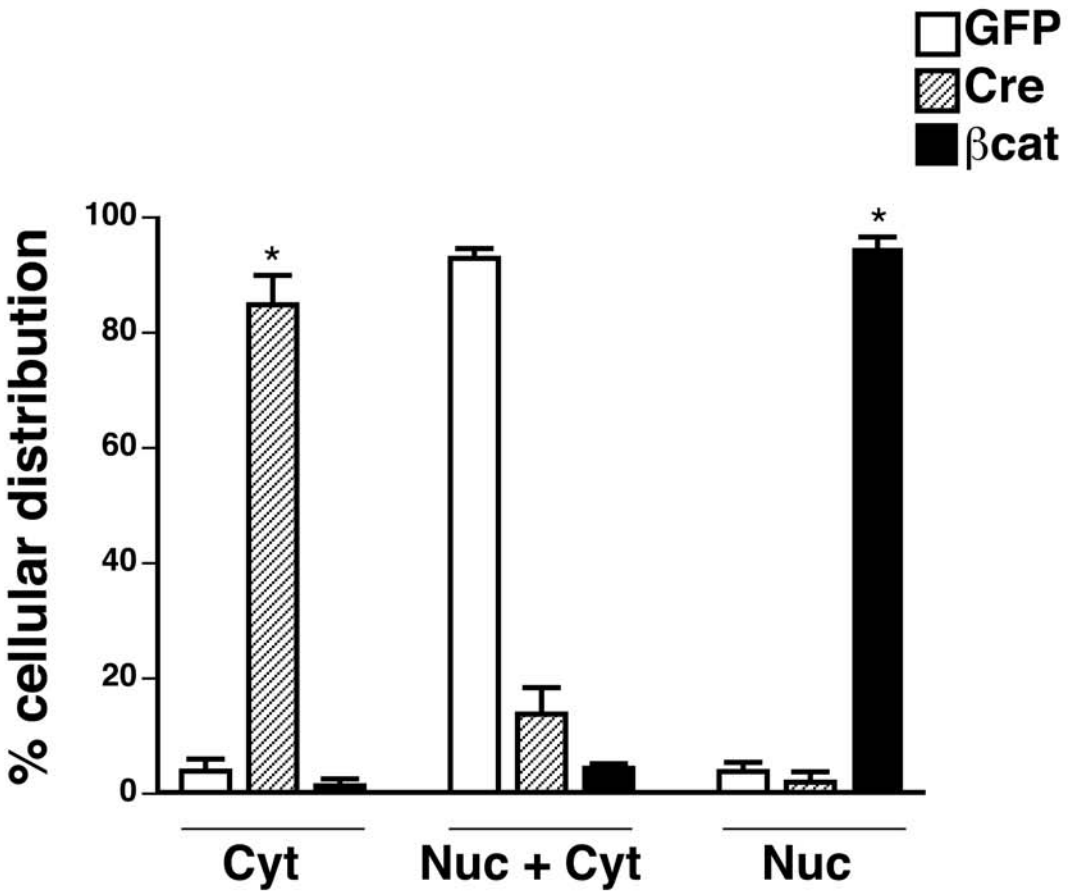


Figure S1: Deletion of hepatic β-catenin does not alter serum insulin concentrations. Insulin concentrations were measured in floxed β-catenin mice injected one week earlier with an adenovirus encoding either GFP or Cre recombinase under fed conditions and after five hours of fasting. n=8 mice per condition. NS=not significant by Student's t-test.


GFP
Cre
βcat
% cellular distribution
100
80
60
40
20
0
*
*
Cyt
Nuc + Cyt
Nuc

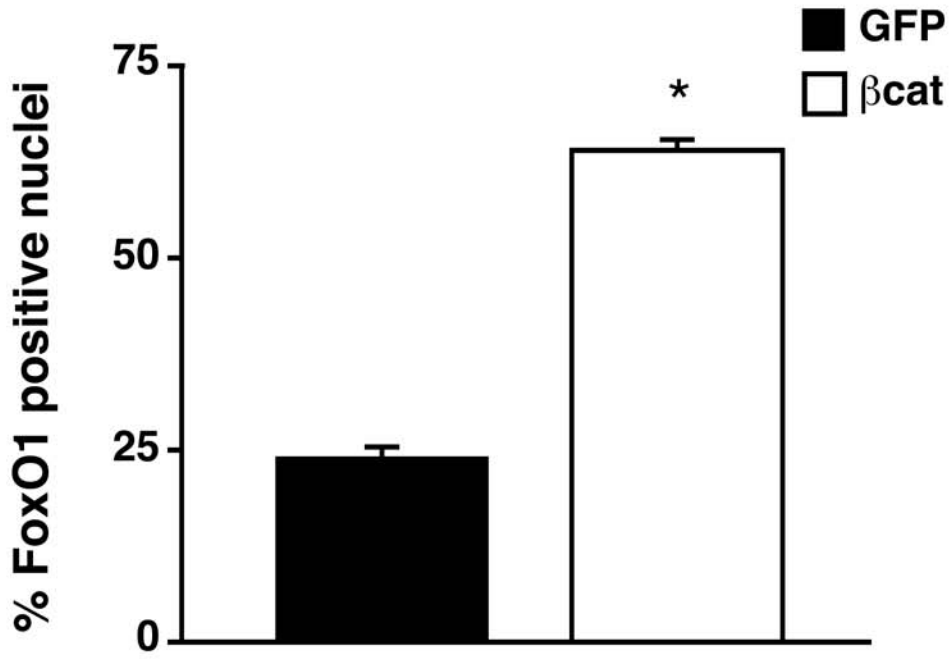

GFP
βcat
% FoxO1 positive nuclei
75
50
25
0
*

Figure S2: Quantification of FoxO1 subcellular localization. (**A**) Confocal images were obtained for FoxO1 subcellular localization in control (GFP), Cre-recombinase (Cre), or β-catenin infected (βcat) hepatocytes. Localization was categorized as cytosolic (Cyt), a mixture of nuclear and cytosolic (Nuc + Cyt), or predominantly nuclear (Nuc). Over 100 cells per condition in multiple random fields were assessed. *$p<0.05$ by non-parametric ANOVA Kruskal-Walis test with Dunn's multiple comparison. (**B**) In vivo nuclear localization of FoxO1 in control (GFP) infected or Ad-βcat infected mice. Livers were harvested three days after tail vein injection and FoxO1 positive nuclei assessed. Over 300 nuclei per condition were counted. *$p<0.05$ by Student's t-test.

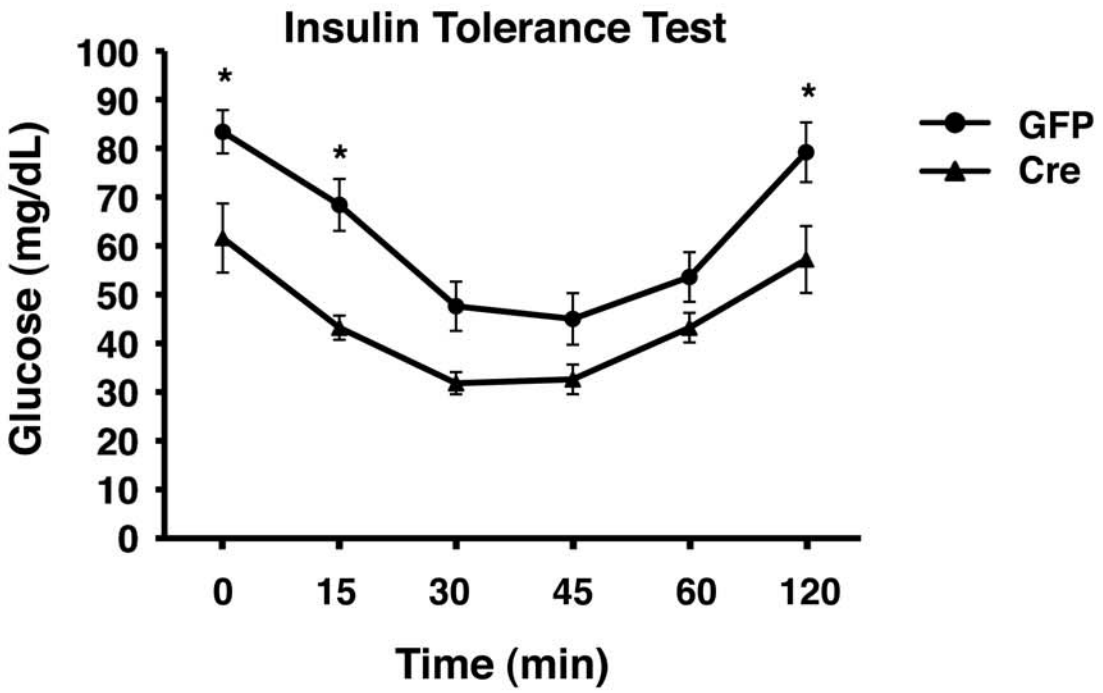


Figure S3: Insulin tolerance tests (ITTs) in flox/flox β-catenin mice after injection with adenoviruses encoding GFP or Cre recombinase. Glucose measurements were made at the indicated times following an initial injection of insulin at 0.5 units/kg of body weight. n=5 mice per condition. Alterations in insulin tolerance in β-catenin deleted mice presumably reflects the direct effects of increased hepatic insulin sensitivity, or the secretion of hepatic factors in β-catenin deleted mice that modulates peripheral tissues.*$p<0.05$ by ANOVA with Bonferroni correction.

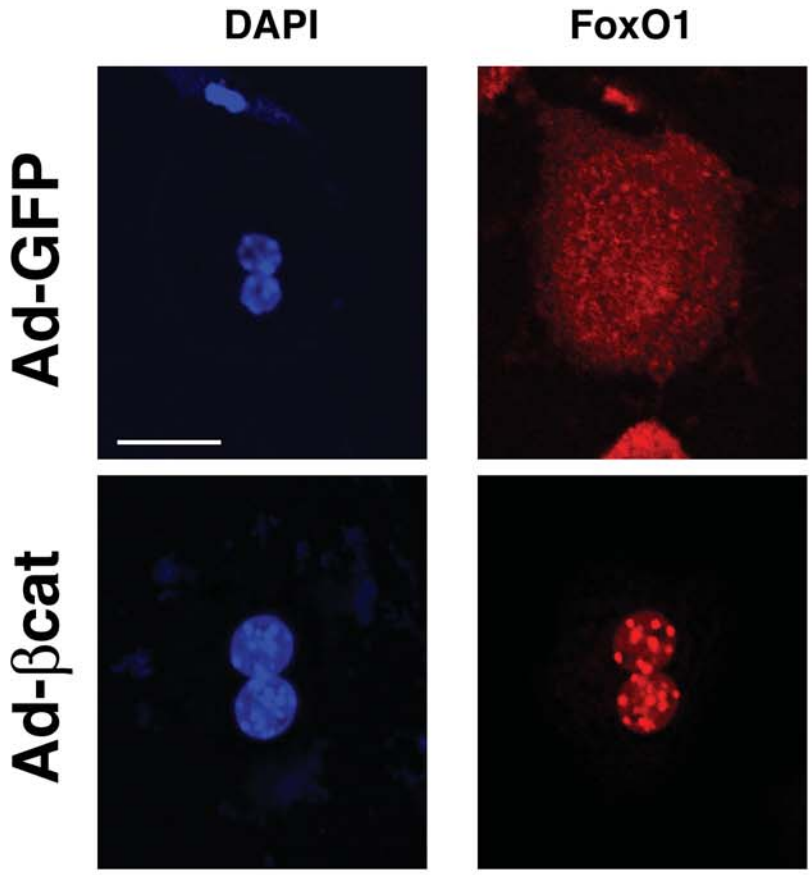


Figure S4: Subcellular localization of FoxO1 in primary hepatocytes after insulin stimulation. In control (GFP) infected cells (top panel), insulin stimulation leads to the apparent cytosolic localization of FoxO1 (53 out of 70 cells analyzed; compare also to Fig. 2A). In contrast, overexpression of β-catenin (bottom panels) appears to override the insulin signal and maintains FoxO1 in the nucleus (52 out of 60 cells showed persistent nuclear localization). Scale bar represents 20 microns.

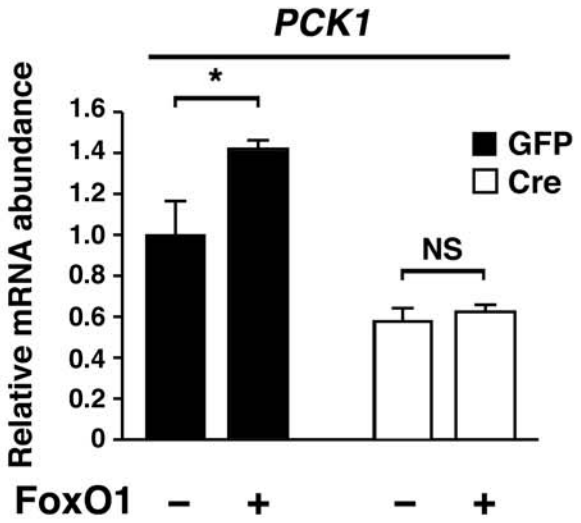


Figure S5: Deletion of β-catenin decreases the mRNA abundance of FoxO1 transcriptional targets. Primary hepatocytes from β-catenin flox/flox mice were infected with Ad-Cre to delete β-catenin or with a GFP control virus. Cells were then re-infected with an adenovirus encoding full-length FoxO1 or again with the GFP control virus. *PCK1* mRNA abundance was assessed 24 hours later. These results support a requirement for β-catenin in FoxO1-mediated increases in gluconeogenic mRNA abundance. n=6 sets of cells per condition; *$p<0.05$ by Student's t-test.

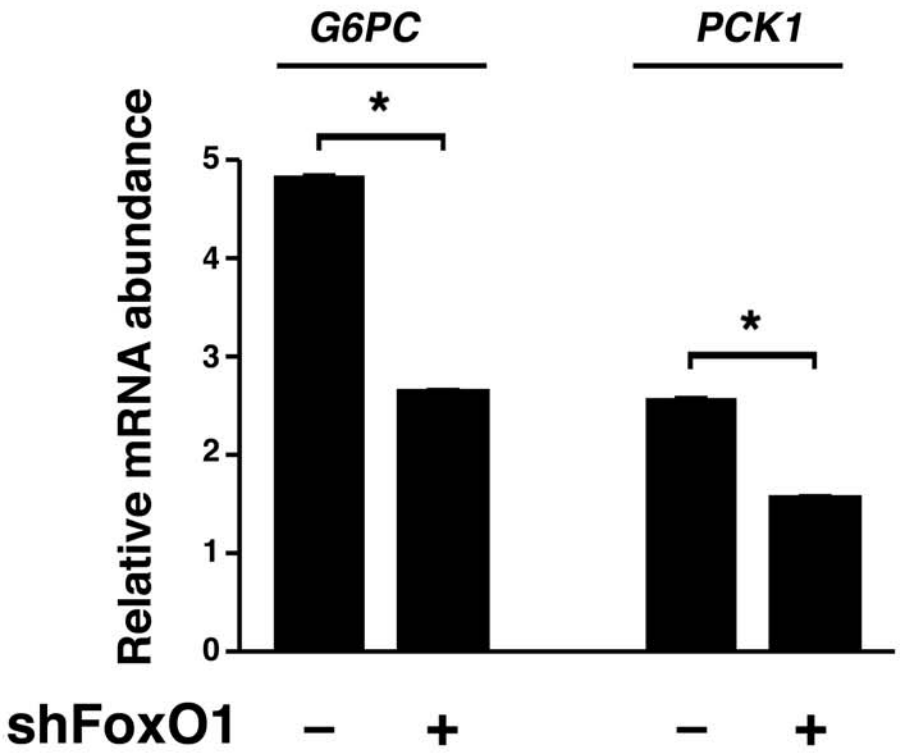


Figure S6: Knockdown of FoxO1 decreases the mRNA abundance of β-catenin transcriptional targets. Hep1-6 cells were stably infected with a scrambled control lentivirus or a lentivirus encoding an shRNA directed against FoxO1. Puromycin resistant Hep1-6 cells were then infected with an adenovirus expressing β-catenin. Following Ad-β-catenin infection, mRNA abundance of *G6PC* and *PCK1* was measured. Values are normalized to mRNA abundance observed in the scrambled control cell line infected with Ad-GFP. n=6 sets of cells per group; *$p<0.05$ by Student's t-test.

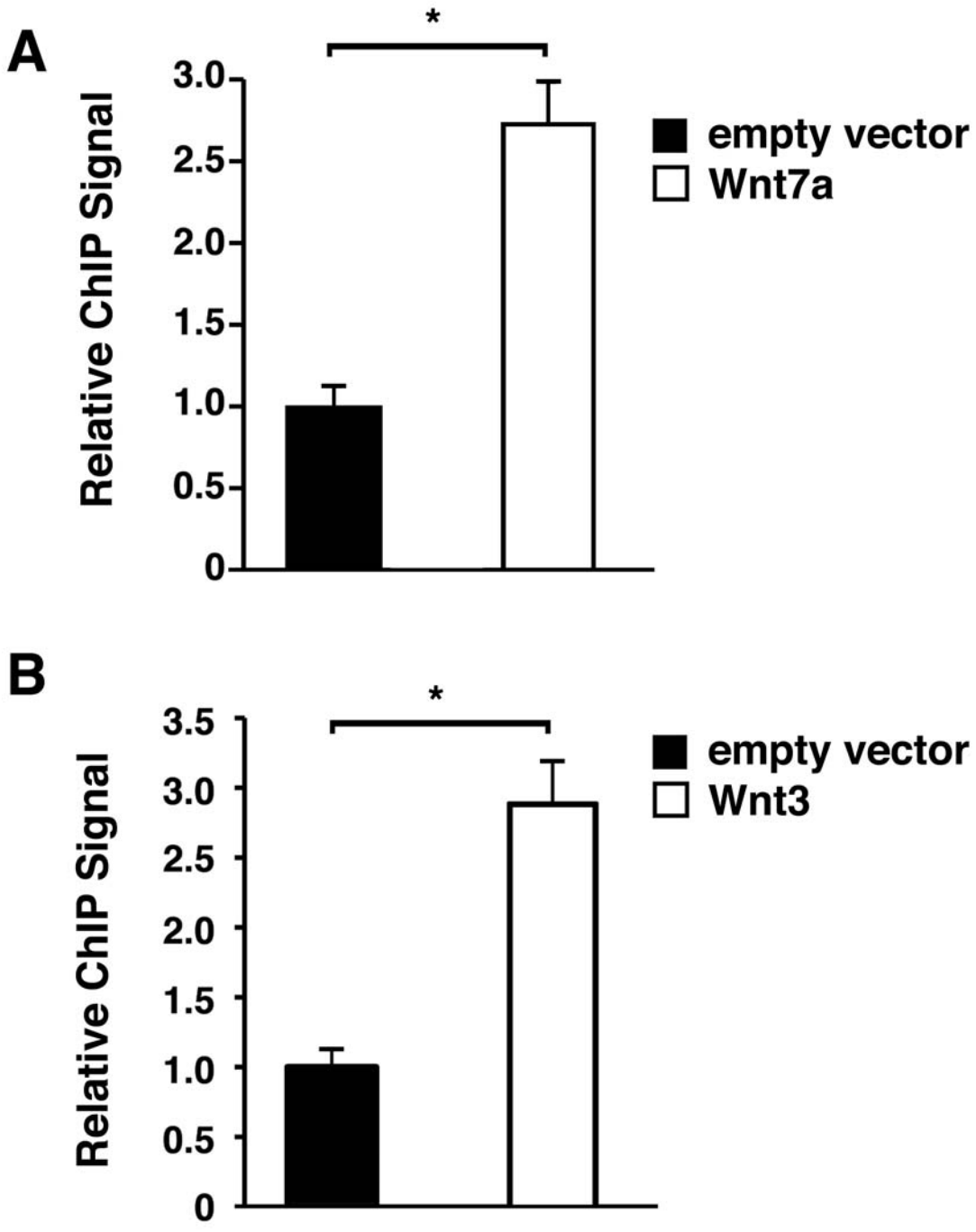


Figure S7: Wnt stimulation increases binding of β-catenin to the *G6PC* promoter. (**A**) Hepa1-6 cells were transiently transfected with an expression vector encoding Wnt7a (20 μg) or with an equal amount of an empty vector control. After 24 hours, chromatin immunoprecipitations were performed using an antibody directed at β-catenin. n=6 sets of cells per condition. (**B**) A similar experiment to (A) was performed using a plasmid encoding Wnt3. n=3 sets of cells per condition; *$p<0.05$ by Student's t-test.

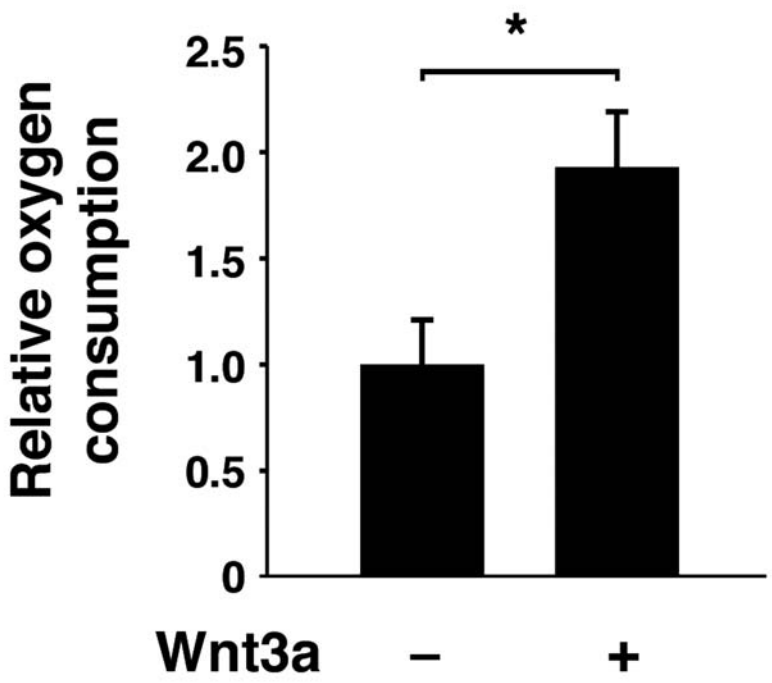


Figure S8: Wnt3a stimulates hepatocyte oxygen consumption. Basal metabolism as assessed by oxygen consumption in primary hepatocytes cultured overnight in the presence or absence of recombinant Wnt3a protein (50 ng/ml). Oxygen consumption is calculated relative to the untreated controls and was measured using a Seahorse XF24 analyzer. Shown is one experiment performed in quadruplicate, representative of two similar experiments. *$p<0.05$ by unpaired t-test.

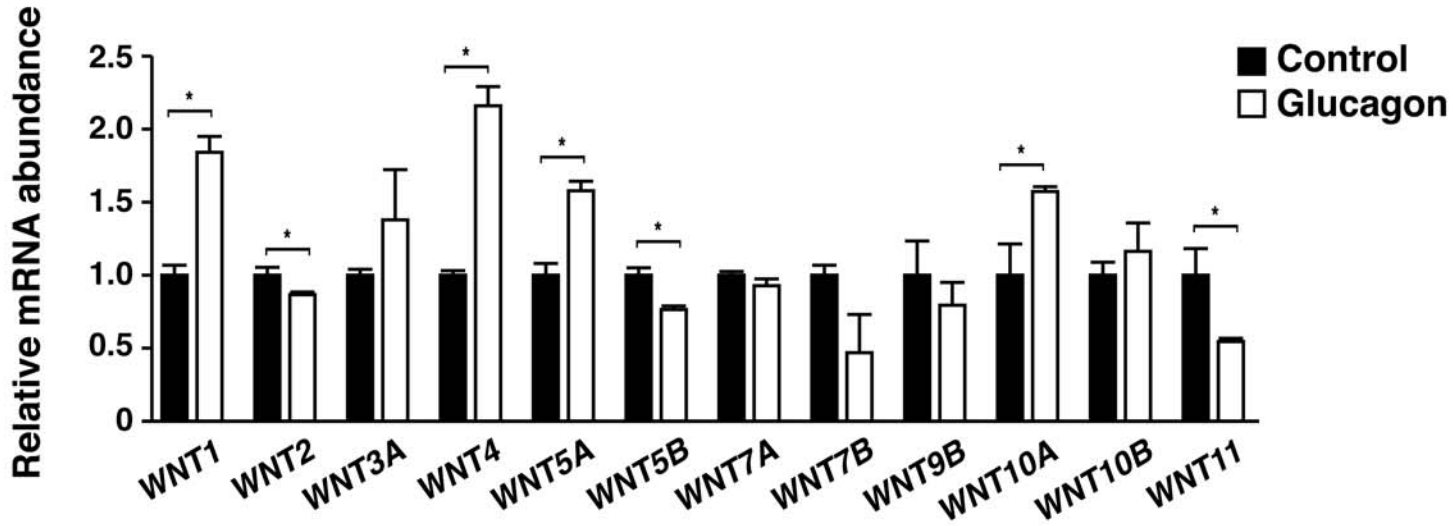


Figure S9: Effects of glucagon stimulation on the abundance of mRNAs encoding Wnt ligands. Primary hepatocytes were stimulated with glucagon and subsequently assessed for changes in the abundance of mRNAs encoding a panel of Wnt ligands. When compared to unstimulated cells, glucagon stimulation did lead to some variations in Wnt ligand mRNA abundance; however, the overall magnitude of differences was small and the pattern did not faithfully reproduce what was observed with in vivo starvation. Shown is one of two similar experiments. n=6 per condition. *$p<0.05$ by Student's t-test.

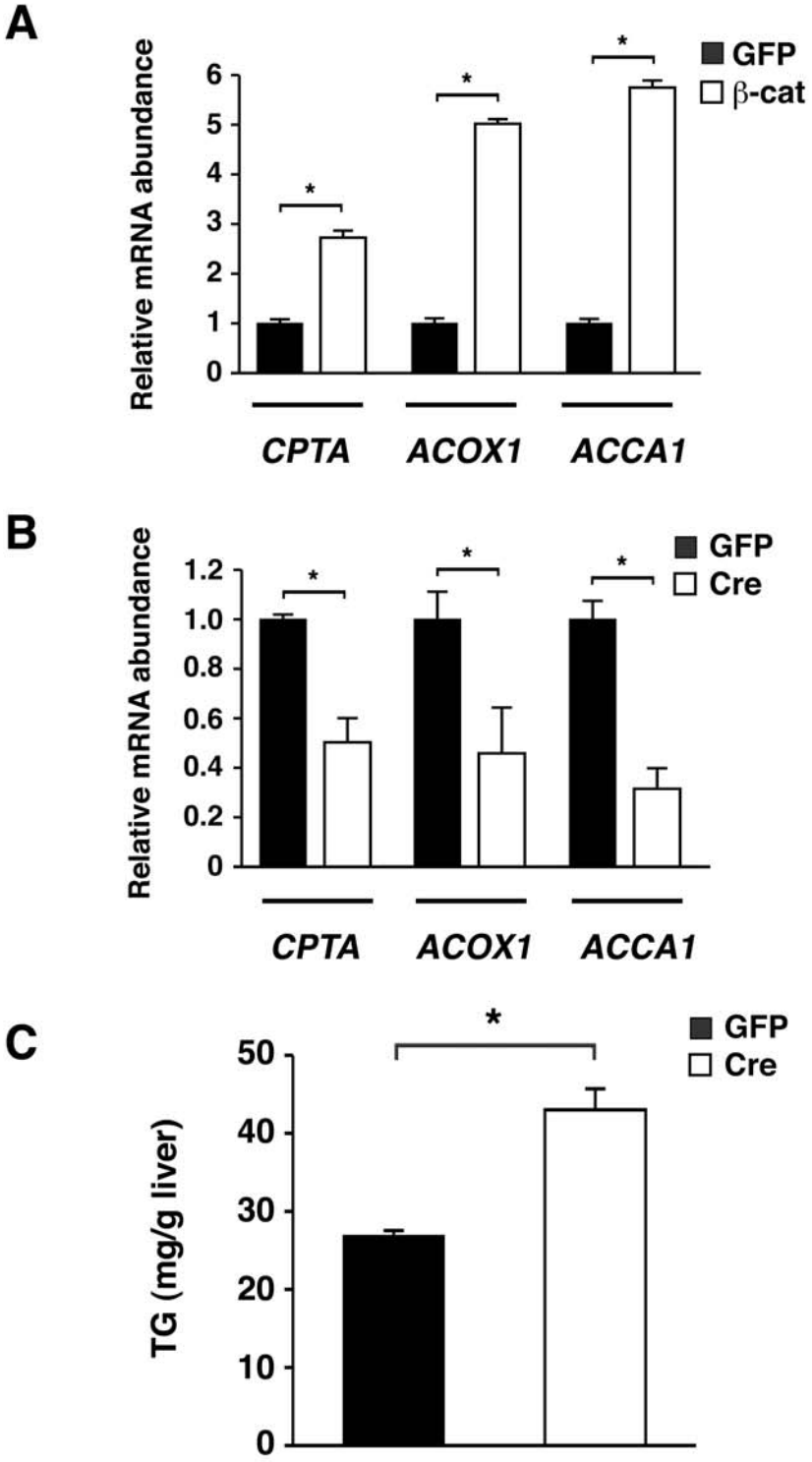


Figure S10: The effect of β-catenin on the abundance of mRNAs encoding lipid enzymes and on triglyceride concentrations. (**A**) After infection of β-catenin flox/flox mice with adenoviruses encoding GFP or β-catenin, the abundance of mRNAs encoding the lipid enzymes carnitine palmitoyltransferase1α (CPT-1α), acyl-coenzyme A (Acox1), and acetyl coenzyme A carboxylase 1β (Acca1β) was analyzed. n=9 per condition. (**B**) Determination of in vivo mRNA abundance for the same lipid enzymes following infection with adenoviruses encoding GFP or Cre recombinase. n=9 per condition. (C) Measurement of hepatic triglyceride concentrations in mice following infection with adenoviruses encoding GFP or Cre recombinase. n=4 per group. Data represent one of two similar experiments. *$p<0.05$ by Student's t-test.