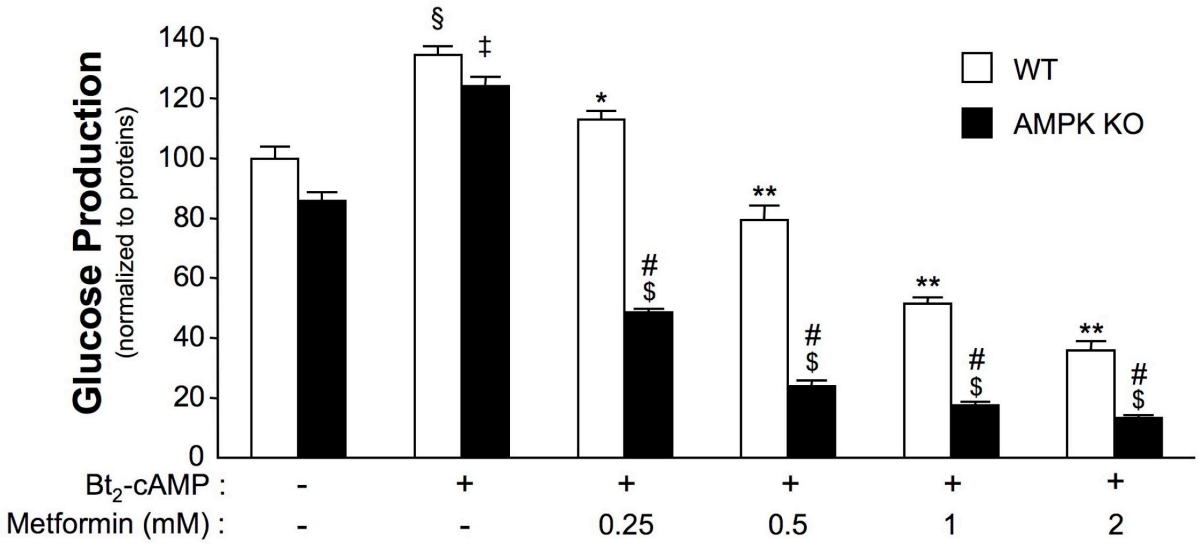

Figure S1 : Effets of metformin on glucose production from dihydroxyacetone in WT and AMPK KO hepatocytes. After attachment, WT and AMPK-deficient primary hepatocytes were cultured for 16h in M199 medium containing 100 nM dex. Hepatocytes were then incubated in glucose-free DMEM containing 10 mM dihydroxyacetone and 100 nM dex alone or with 100 µM Bt_2-cAMP and with or without 0.25, 0.5, 1 or 2 mM metformin. After 8h, medium was collected for glucose measurement. Glucose production was normalized to protein content and expressed as a percentage of glucose produced by WT hepatocytes incubated in the absence of both Bt_2-cAMP and metformin. Data are means ± SEM of triplicates. $^{\S}P<0.001$ and $^{\ddagger}P<0.001$ compared with WT and AMPK KO hepatocytes incubated without Bt_2-cAMP respectively, $^{*}P<0.05$ and $^{**}P<0.001$ compared with WT hepatocytes incubated with Bt_2-cAMP alone, $^{\$}P<0.001$ compared with AMPK KO hepatocytes incubated with Bt_2-cAMP alone, $^{\#}P<0.01$ compared with WT hepatocytes incubated under the same conditions.

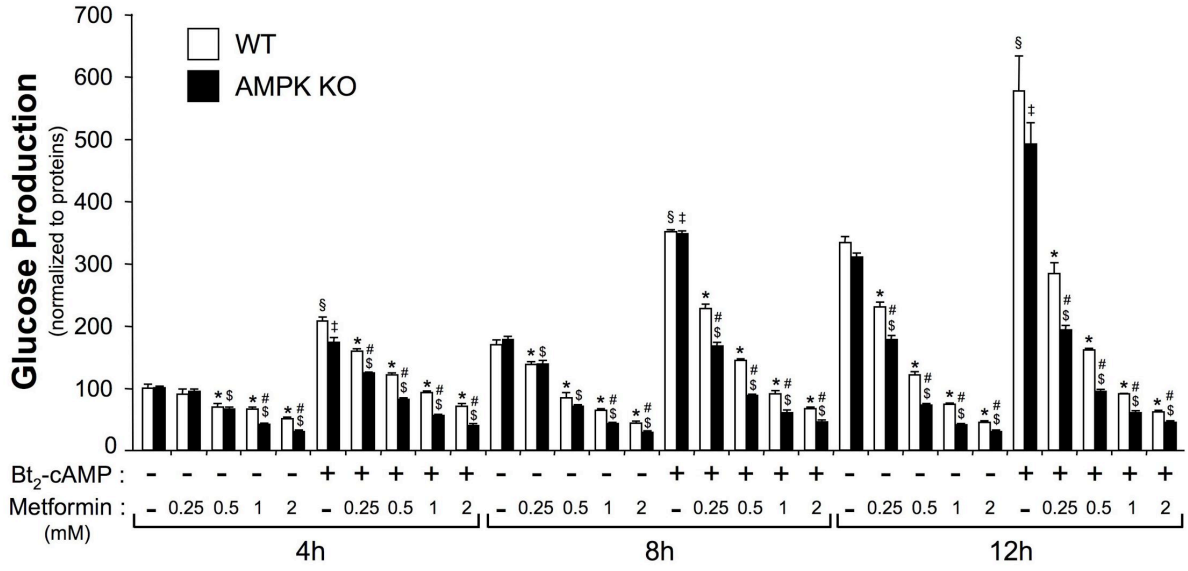

Figure S2 : Dose- and time-response analysis of the effect of metformin on glucose production in WT and AMPK KO hepatocytes.

After attachment, WT and AMPK-deficient primary hepatocytes were cultured for 16h in M199 medium containing 100 nM dex. Hepatocytes were then incubated in glucose-free DMEM containing lactate/pyrurate (10/1 mM) and 100 nM dex alone or with 100 μM Bt_2-cAMP and with or without 0.25, 0.5 or 1 mM metformin. After 4, 8 or 12h, medium was collected for glucose measurement. Glucose production was normalized to protein content and expressed as a percentage of glucose production by WT hepatocytes incubated for 4h in the absence of both Bt_2-cAMP and metformin. Data are means ± SEM of triplicates. $^{\S}P<0.001$ and $^{\ddagger}P<0.001$ compared with WT and AMPK KO hepatocytes incubated without Bt_2-cAMP respectively, for 4, 8 or 12 hours. $^{*}P<0.001$ and $^{\$}P<0.001$ as compared with WT and AMPK KO hepatocytes incubated with or without Bt_2-cAMP in the absence of metformin, respectively, for 4, 8 or 12 hours. $^{\#}P<0.05$ compared with WT hepatocytes incubated under the same conditions.

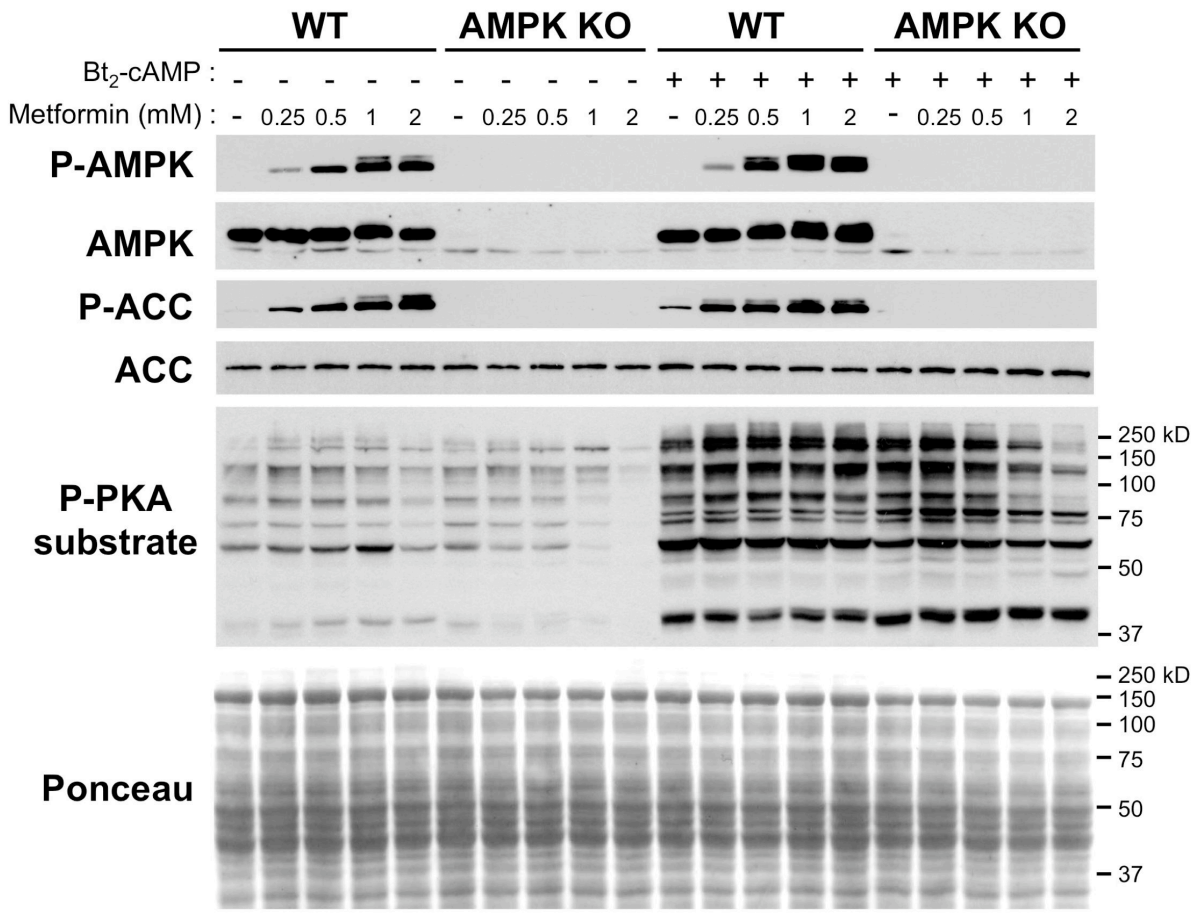

Figure S3 : Effect of metformin on Bt_2-cAMP-induced PKA activation in WT and AMPK KO hepatocytes.
After attachment, WT and AMPK-deficient primary hepatocytes were cultured for 16h in M199 medium containing 100 nM dex. Hepatocytes were then incubated in glucose-free DMEM containing lactate/pyrurate (10/1 mM) and 100 nM dex alone or with 100 µM Bt_2-cAMP and with or without 0.25, 0.5, 1 or 2 mM metformin. After 8h, cells were harvested for western-blot analysis. Immunoblots were performed against phospho-AMPKα (Thr172), AMPKα, phospho-ACC (Ser79), ACC and phospho-PKA substrate. Ponceau staining is shown as a loading control. Blots presented are representative of three independent experiments.

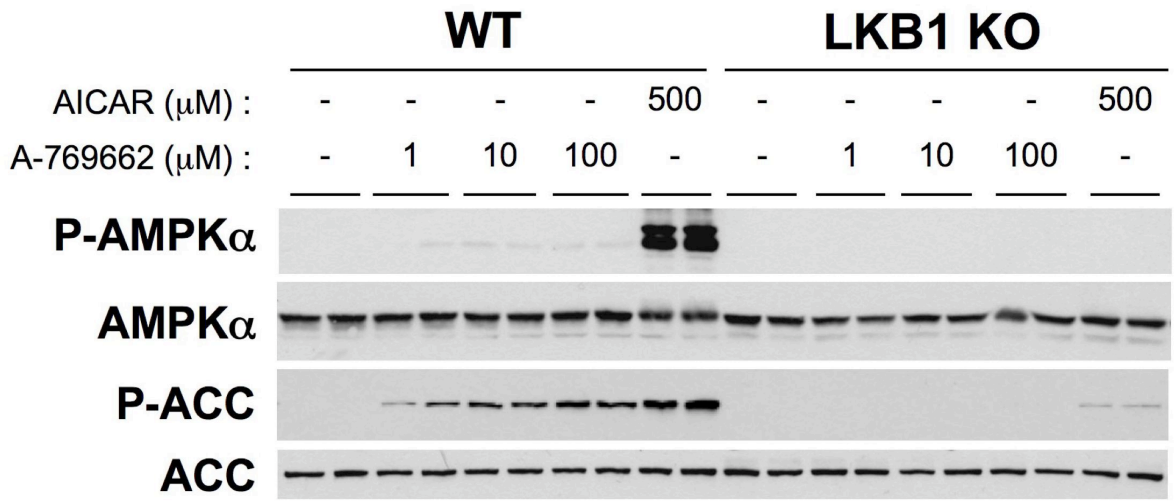

Figure S4 : Activation of AMPK by A-762669 is dependent on LKB1 in hepatocytes.
After attachment, WT and LKB1-deficient primary hepatocytes were cultured for 16h in M199 medium containing 100 nM dex. Hepatocytes were then incubated with or without either 1, 10 or 100 µM A-769662 or 500 µM AICAR. After 8h, cells were harvested for western-blot analysis. Immunoblots were performed against phospho-AMPKα (Thr172), AMPKα, phospho-ACC (Ser79) and ACC. Blots presented are representative of three independent experiments.

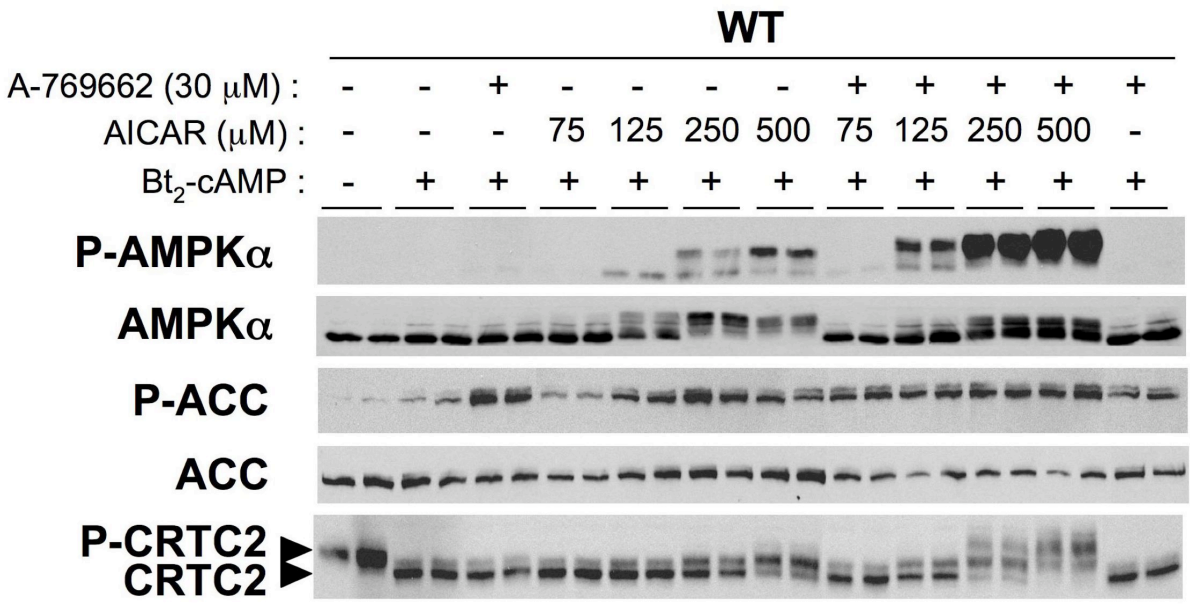

Figure S5 : Synergic activation of AMPK by AICAR and A769662 in WT hepatocytes.
After attachment, WT primary hepatocytes were cultured for 16h in M199 medium containing 100 nM dex. Hepatocytes were then incubated either with or without 100 μM Bt_2-cAMP and with or without increasing concentrations of AICAR (75, 125, 250 or 500 μM) or 30 μM A-769662 or both. After 8h, cells were harvested for western-blot analysis. Immunoblots were performed against phospho-AMPKα (Thr172), AMPKα, phospho-ACC (Ser79), ACC and CRTC2. Blots presented are representative of two independent experiments.

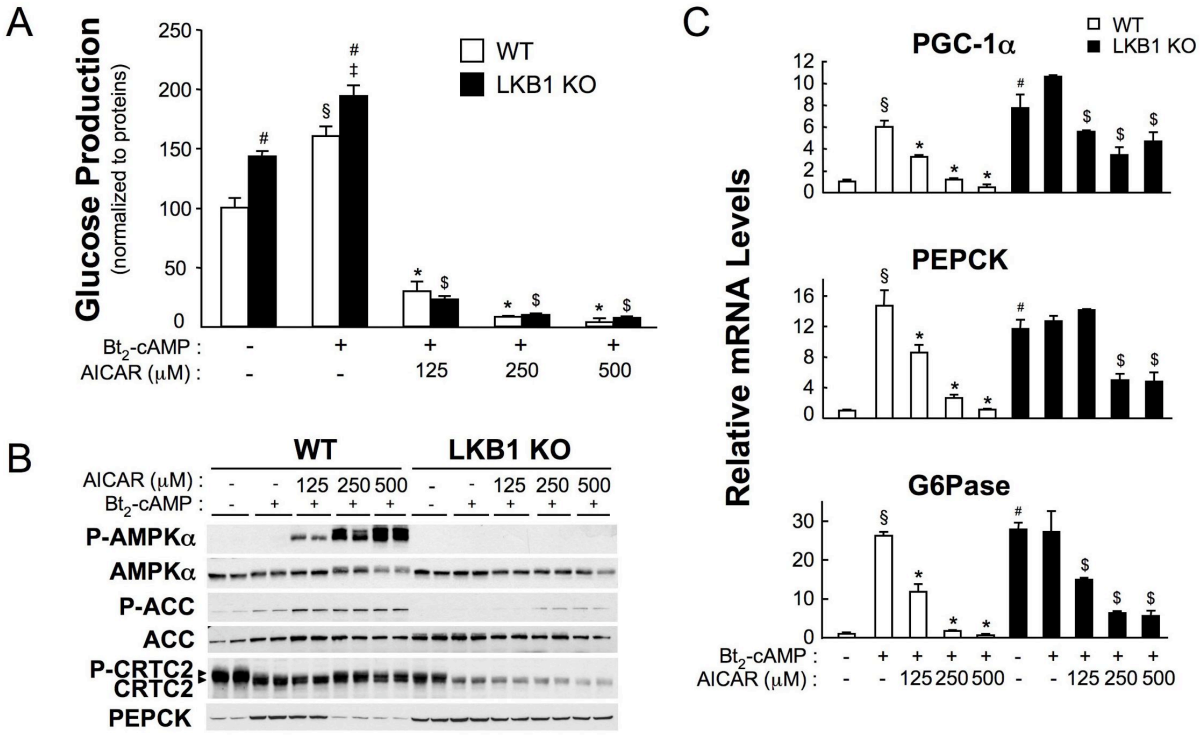

Figure S6 : Effects of AICAR on gluconeogenesis in WT and LKB1 KO hepatocytes.
After attachment, WT and LKB1-deleted primary hepatocytes were cultured for 16h in M199 medium containing 100 nM dex. Hepatocytes were then incubated in glucose-free DMEM containing lactate/pyrurate (10/1 mM) and 100 nM dex alone or with 100 µM Bt_2-cAMP and with and without 125, 250 or 500 µM AICAR. After 8h, medium was collected for glucose production measurement and cells were harvested for western-blot and gluconeogenic gene expression analysis. (**A**) Glucose production was normalized to protein content and expressed as a percentage of glucose produced by WT hepatocytes incubated in the absence of both Bt_2-cAMP and AICAR. Results representative of three independent experiments are shown. (**B**) Immunoblots were performed against phospho-AMPKα (Thr172), AMPKα, phospho-ACC (Ser79), ACC, CRTC2 and PEPCK. Blots presented are representative of three independent experiments. (**C**) Relative mRNA levels of PGC-1α, PEPCK and G6Pase expressed as fold activation relative to levels in WT hepatocytes incubated in the absence of both Bt_2-cAMP and AICAR. Results representative of three independent experiments are shown. Data are means ± SEM. $^{§}P<0.001$ and $^{‡}P<0.001$ compared with WT and AMPK KO hepatocytes incubated without Bt_2-cAMP respectively, $^{*}P<0.001$ and $^{\$}P<0.001$ compared with WT and LKB1 KO hepatocytes incubated with Bt_2-cAMP alone respectively, $^{\#}P<0.001$ compared with WT hepatocytes incubated under the same conditions.