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Supplemental Information

AMPK Is a Negative Regulator of the Warburg Effect and Suppresses Tumor Growth In Vivo

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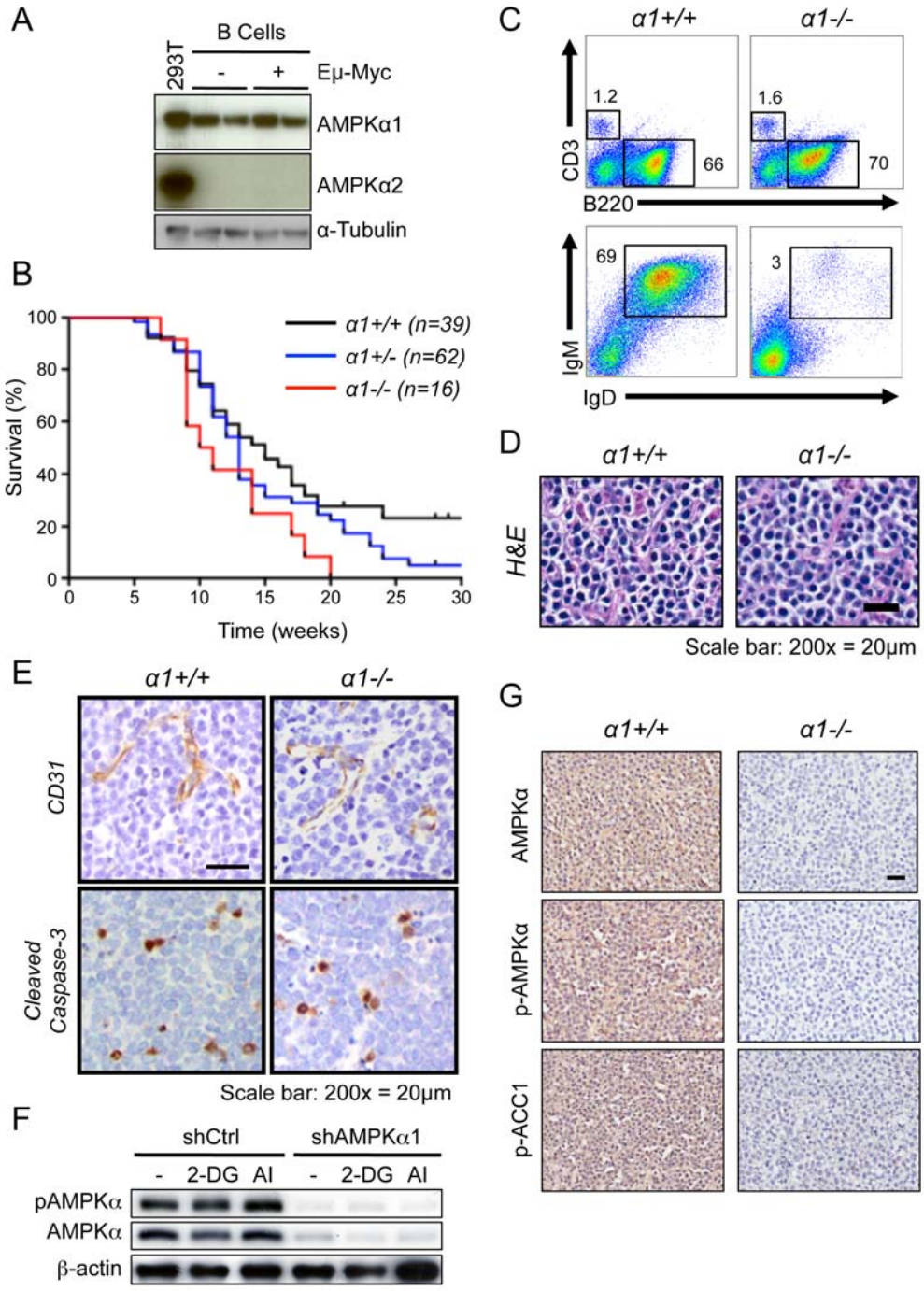

Figure S1. Characterization of AMPKα1-deficient Eμ-Myc lymphomas, Related to Figure 1

A) Whole cell lysates from mouse B cells (+/- Eμ-Myc expression) and 293T cells were analyzed by immunoblot using antibodies against AMPKα1, AMPKα2 or actin. B) Kaplan-Meier curve showing overall survival of Eμ-Myc mice deficient ($\alpha 1^{-/-}$, red, n=16), heterozygous ($\alpha 1^{+/-}$, blue, n=62) or wild type ($\alpha 1^{+/+}$, black, n=39) for AMPKα1. C) Flow cytometry analysis of isolated lymph node tumors from Eμ-Myc/$\alpha^{+/+}$ and Eμ-Myc/$\alpha 1^{-/-}$ mice. (*Top*) Representative dot plots of CD3 versus B220 staining of lymph node cells isolated from Eμ-Myc/$\alpha^{+/+}$ and Eμ-Myc/$\alpha 1^{-/-}$ animals. (*Bottom*) Representative sIg^+ (IgM and IgD) surface staining on $B220^+$ cells. These data are representative of tumors from 4 Eμ-Myc/$\alpha^{+/+}$ and 5 Eμ-Myc/$\alpha 1^{-/-}$ mice. D) H&E staining of Eμ-Myc/$\alpha^{+/+}$ and Eμ-Myc/$\alpha 1^{-/-}$ lymphoma sections. E) Representative histological sections of Eμ-Myc/$\alpha^{+/+}$ and Eμ-Myc/$\alpha 1^{-/-}$ lymphomas stained for CD31 and cleaved Caspase-3. F) Eμ-Myc lymphoma cells expressing control (shCtrl) or AMPKα1-specific (shAMPKα1) shRNAs were treated with 2-deoxyglucose (2-DG, 10 mM) or AICAR (AI, 1 mM) for 1 hour, and whole cell lysates analyzed for total and phospho-T172-AMPKα by immunoblot. G) Histological analysis of cell signaling in Eμ-Myc/$\alpha 1^{-/-}$ lymphomas. Representative sections from Eμ-Myc/$\alpha^{+/+}$ and Eμ-Myc/$\alpha 1^{-/-}$ lymphomas were stained with antibodies detect AMPK activity (phosphorylated (pAMPKα, T172) and total AMPKα, and phospho-ACC1 (S79).

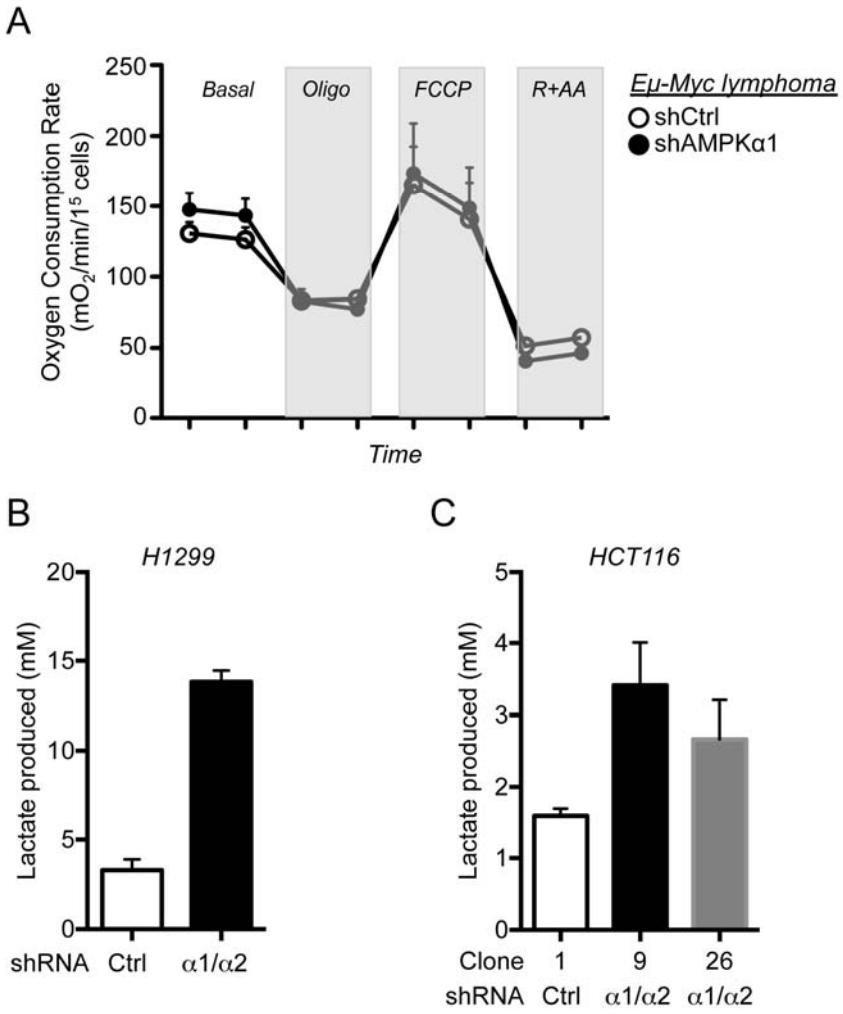

Figure S2. Silencing AMPKα1 in Eμ-Myc lymphoma cells promotes increased glucose consumption and lactate production, Related to Figure 2

A) Eμ-Myc lymphoma cells expressing control (Ctrl, open circle) or AMPKα1-specific (α1, closed circle) shRNAs were analyzed for oxygen consumption using the Seahorse XF-24 analyzer. A representative oxygen consumption trace is shown following treatment with oligomycin (ATP synthase inhibitor), FCCP (uncoupling agent), or Rotenone and Antimycin-A (Complex I and III inhibitors, respectively). B) H1299 cells expressing control (open bar) or AMPKα1/α2 (closed bar) shRNAs were cultured for 48 hours, and extracellular lactate produced was determined by enzymatic assay (n= 3 per sample). C) HCT116 cells expressing control (open bar) or AMPKα1/α2 (closed bar) shRNAs were cultured for 48 hours and extracellular lactate produced was determined by enzymatic assay (n= 3 per sample).

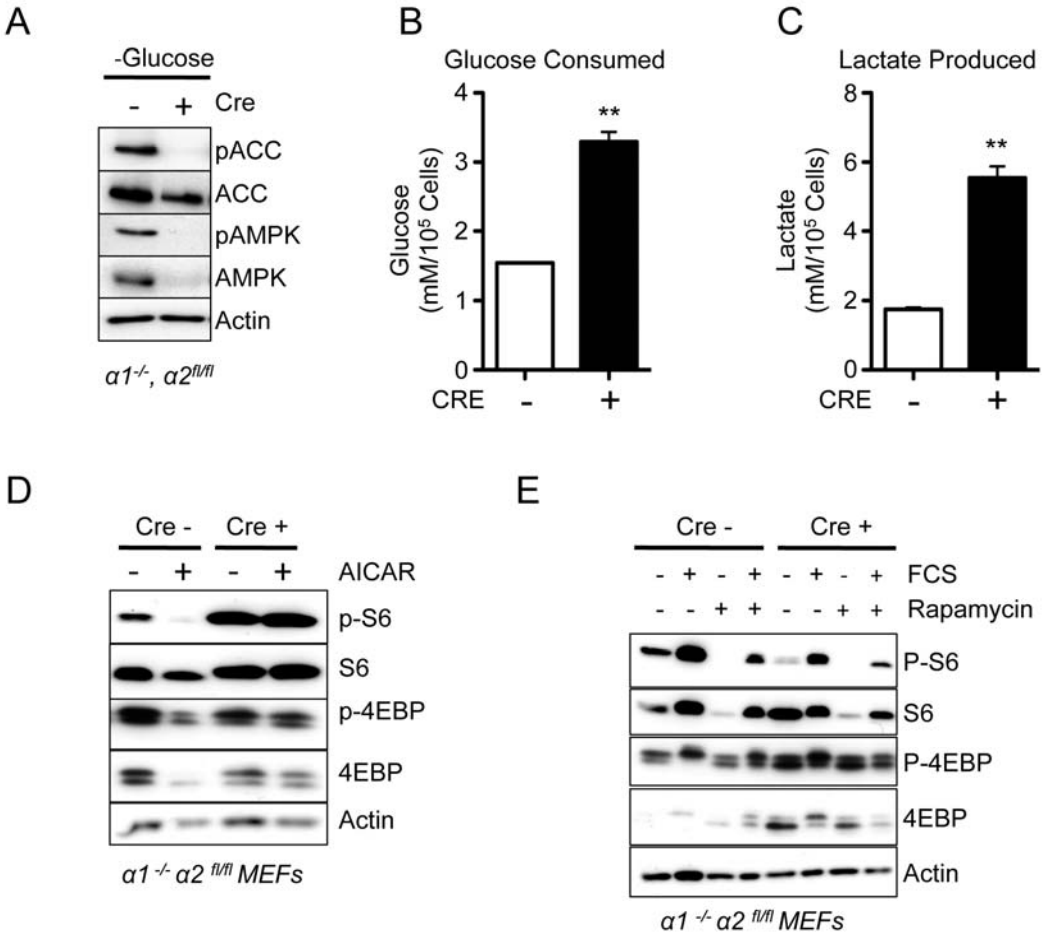

Figure S3. AMPKα-deficient cells display enhanced glycolytic metabolism and sustained mTORC1 activity, Related to Figure 3

A) Immunoblot of whole cell lysates from control (Cre-) or AMPKα-deficient (Cre+) MEFs cultured in glucose-free medium for 24 hours using antibodies against phospho-T172 and total AMPKα, phospho-S79 and total ACC, and actin. B-C) Control (Cre-, open bar) or AMPKα-deficient (Cre+, closed bar) MEFs were analyzed for glucose consumption (B) and lactate production (C). Data are expressed as the mean ± SD (n=3 per sample). D) Control (Cre-) or AMPKα-deficient (Cre+) MEFs were treated with 1mM AICAR for 1 hour, and whole cell lysates analyzed by immunoblot using antibodies against total and phospho-ribosomal S6 (pS6, S240/244), total and phospho-4EBP1 (p4EBP1, S37/46), and actin. E) Serum-starved control (Cre-) or AMPKα-deficient (Cre+) MEFs were treated with 25nM rapamycin (25nM, 2hours), followed by re-addition of media containing 0% or 10% FCS for 15min. Immunoblot of whole cell lysates was conducted as in (a). **, $p<0.01$; ***, $p<0.001$.

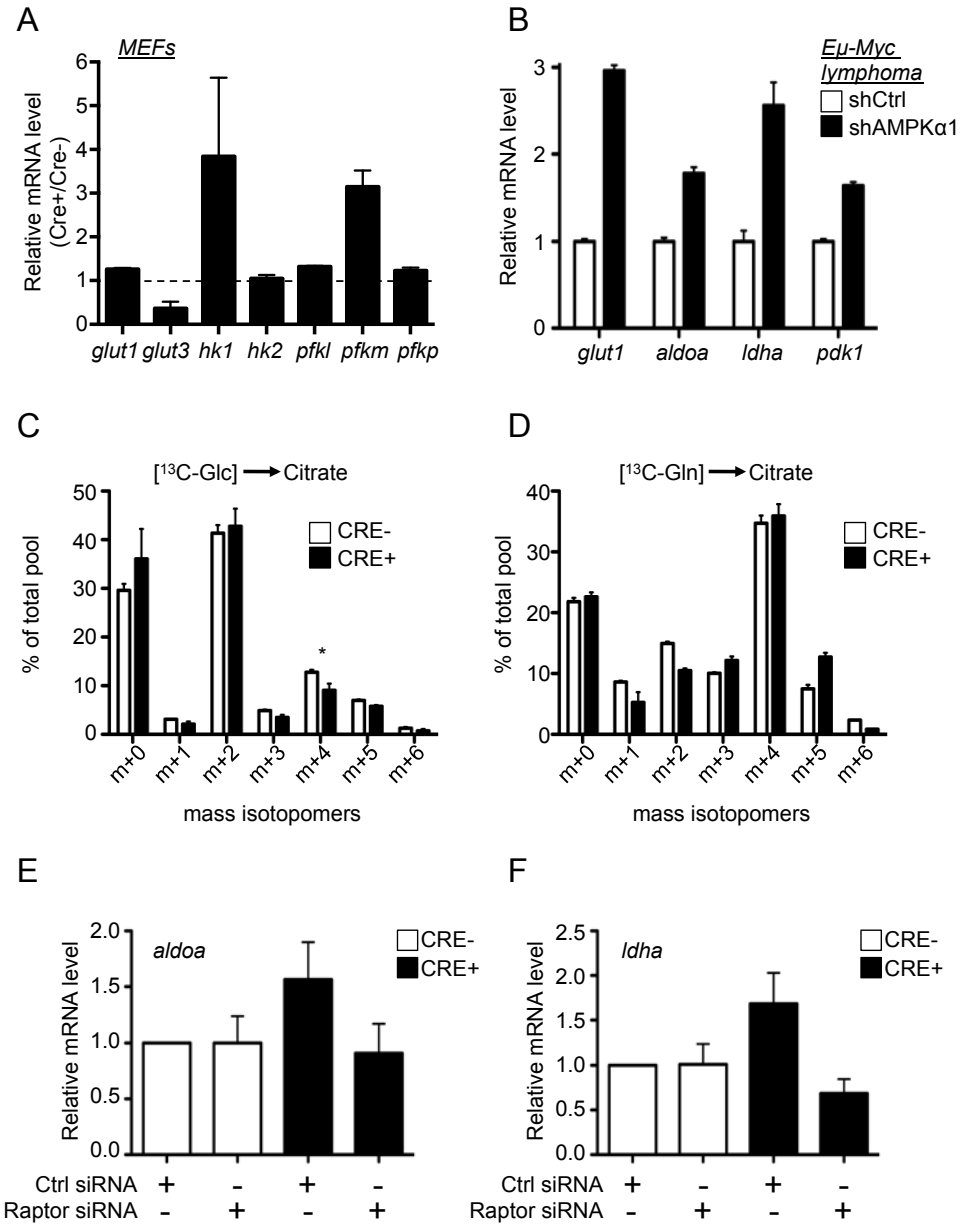

Figure S4. Glycolytic gene expression and carbon flux analysis of AMPKα-deficient cells, Related to Figure 4

A) Relative expression of *glut1, glut3, hk1, hk2, pfkl, pfkm* and *pfkp* mRNA by AMPKα-deficient MEFs. Expression of mRNA transcripts was determined relative to *actin* mRNA levels, and normalized relative to isogenic controls (Cre-). B) Relative expression of *glut1, aldoa*, *ldha*, and *pdk1* mRNA by Eμ-Myc lymphoma cells expressing control (shCtrl) or AMPKα1-specific (shRNA). Expression of mRNA transcripts was determined relative to *actin* mRNA levels, and normalized relative to parental lymphoma cells. C) Control (Cre-, open bars) or AMPKα-deficient (Cre+, closed bars) MEFs were cultured for 2 hours with medium containing uniformly labeled ^{13}C-glucose, metabolites extracted from cells, and enrichment of intracellular citrate was measured by GC-MS. Heavy-labeled mass isotopomers (m+) of citrate are indicated. D) Cells as in (C) were cultured for 2 hours with medium containing uniformly labeled ^{13}C-glutamine, and enrichment of glutamine-derived mass isotopomers in intracellular citrate was measured by GC-MS. E-F) Control (Cre-) or AMPKα-null (Cre+) MEFs were transfected with siRNA targeting Raptor (20 nM), and cells harvested for analysis 72 hours later. Relative expression of *aldoa* mRNA (E) and ldha mRNA (F) were determined by qPCR for triplicate samples. *, $p<0.05$.

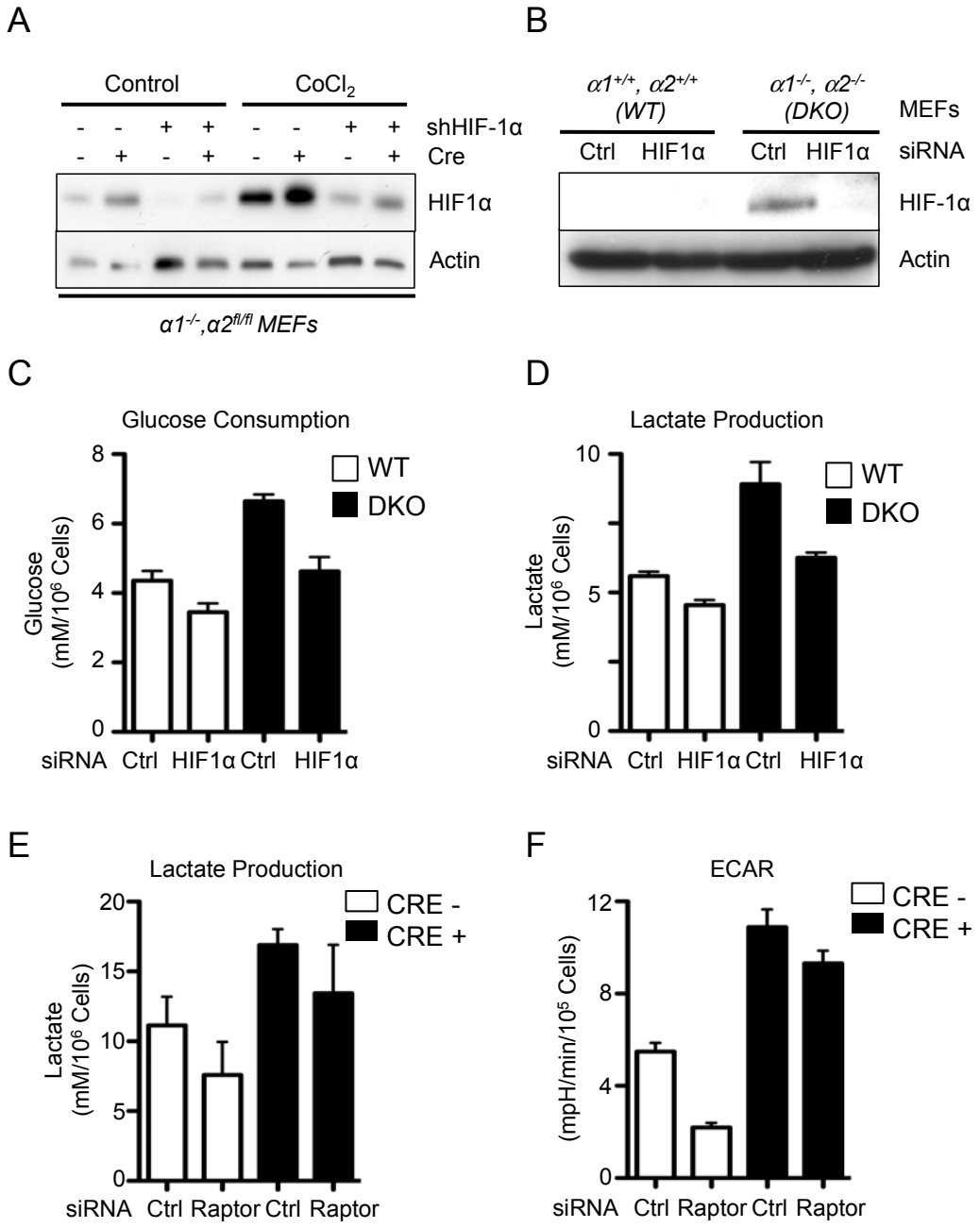

Figure S5. HIF-1α knockdown in AMPKα-deficient MEFs promotes increased aerobic glycolysis, Related to Figure 5

A) Cells were treated with $CoCl_2$ (150 μM, 30 min) and whole cell lysates analyzed by immunoblot using HIF1α and actin antibodies. B) siRNA-mediated knockdown of HIF-1α in AMPK$\alpha^{-/-}$ MEFs. MEFs lacking AMPKα1 and α2 (DKO) were transfected with siRNA targeting HIF-1α (100nM), and cells harvested for analysis after 72 hours. Whole cell lysates were analyzed for HIF-1α and actin expression by immunoblot. C-D) AMPK$\alpha^{-/-}$ MEFs display enhanced aerobic glycolysis. Wild-type (WT, open bars) or AMPK$\alpha 1^{-/-}$, $\alpha 2^{-/-}$ (DKO, closed bar) MEFs were grown for 72 hours, and medium samples were analyzed for glucose (C) and lactate (D) levels by enzymatic assay. Data are expressed as the mean ± SD of total glucose consumed or lactate produced. E-F) siRNA-mediated knockdown of Raptor in AMPK$\alpha^{-/-}$ MEFs. MEFs lacking AMPKα1 and α2 were transfected with siRNA targeting HIF-1α (20nM), and medium samples were analyzed for lactate levels by enzymatic assay (E). F) ECAR was measured for cells treated as in E).

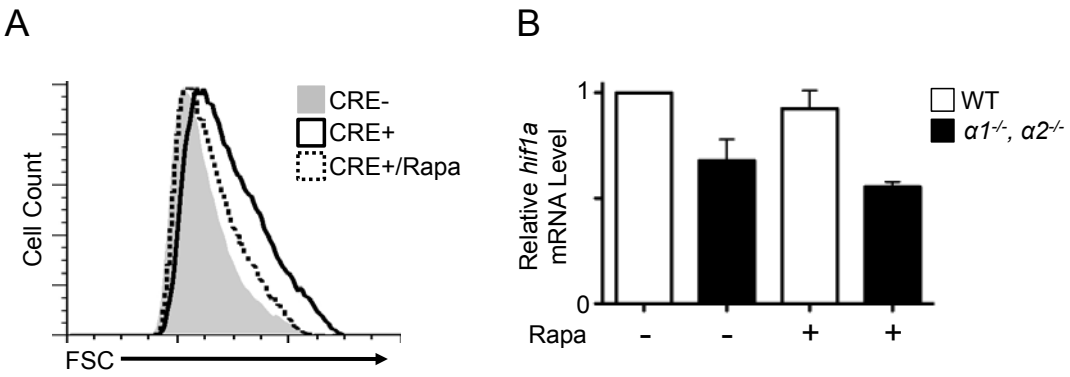

Figure S6. Rapamycin reduces cell size and *hif1a* mRNA levels in AMPKα-deficient MEFs, Related to Figure 6

A) Representative forward scatter (FSC) of control (Cre-) or AMPKα-deficient (Cre+) MEFs cultured with or without 25nM rapamycin for 16 hours. B) Relative expression of *hif1a* mRNA by wild-type (WT) or AMPKα-deficient ($\alpha1^{-/-}$, $\alpha2^{-/-}$) MEFs cultured with or without 25nM rapamycin for 24 hours. Expression of mRNA transcripts was determined relative to *actin* mRNA levels, and normalized relative to wild type cells without rapamycin.

Table S1. List of qPCR primers used in this study, Related to Figure 3

Gene	Direction	Sequence
actb	Forward	atgctccccgggctgtat
	Reverse	cataggagtccttctgacccattc
Aldoa	Forward	gtgggaagaaggagaacctg
	Reverse	ctggagtgttgatggagcag
hif1a	Forward	accttcatcggaaactccaaag
	Reverse	ctgttaggctgggaaaagttagg
ldha	Forward	tgtctccagcaaagactactgt
	Reverse	gactgtacttgacaatgttggga
pdk1	Forward	acaaggagagcttcggggtggatc
	Reverse	ccacgtcgcagtttggatttatgc