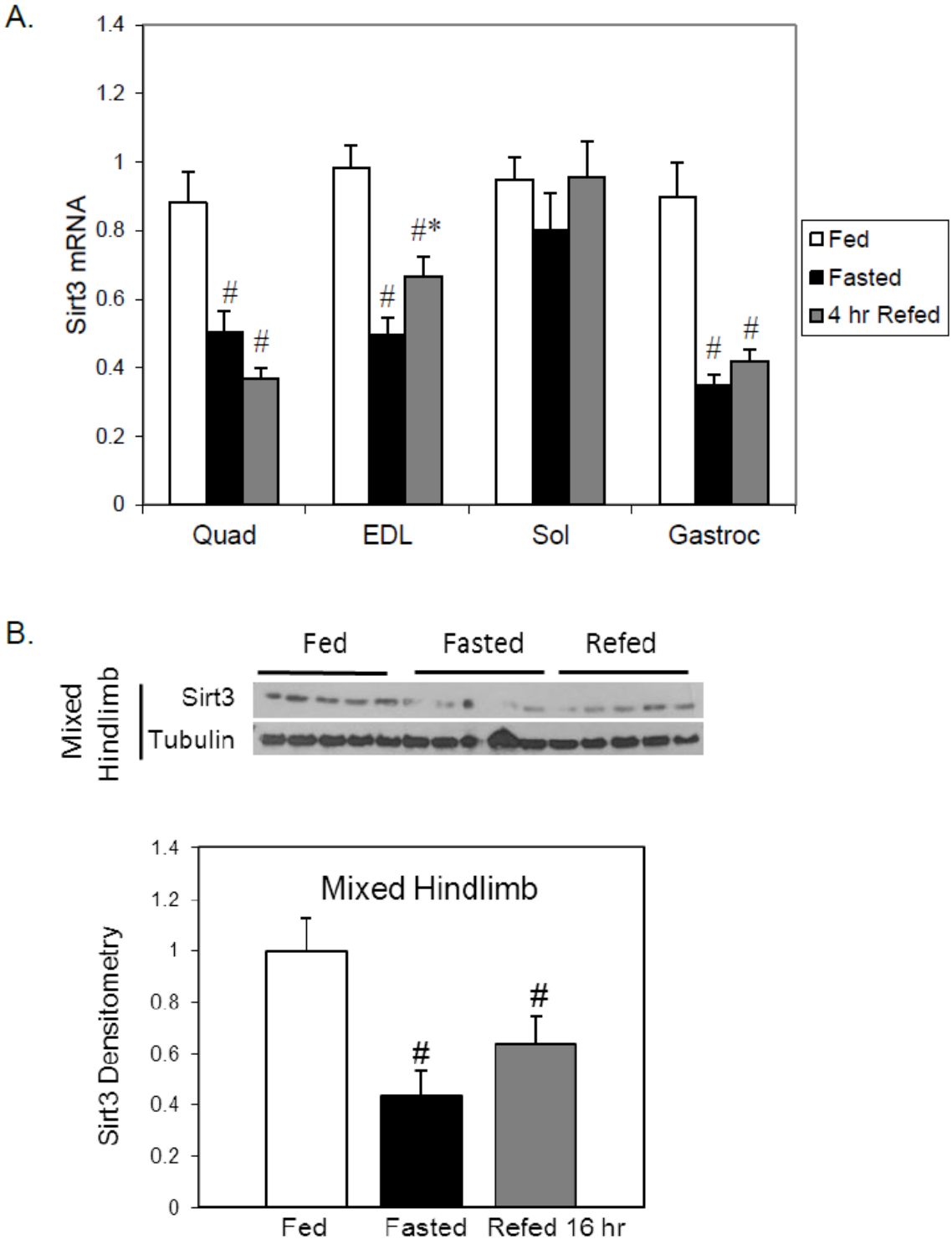
SUPPLEMENTARY DATA

Supplementary Figure 1. Sirt3 levels decrease in skeletal muscle with fasting and tend to recover with refeeding. (A) Sirt3 mRNA levels in C57Bl/6 mice fasted for 24 hours or refed for 4 hours (n=6, #-p<0.05 vs Fed, *-p<0.05 vs. Fasted, ANOVA). (B) Sirt3 protein levels by Western blot in mixed hindlimb muscle from fed, 24 hour fasted, and 16 hour refed C57Bl/6 mice (n=5, #-p<0.05 vs Fed). (C) Sirt3 densitometry from Figure 2B. EDL muscles were collected from WT controls in either the fed or fasted state.



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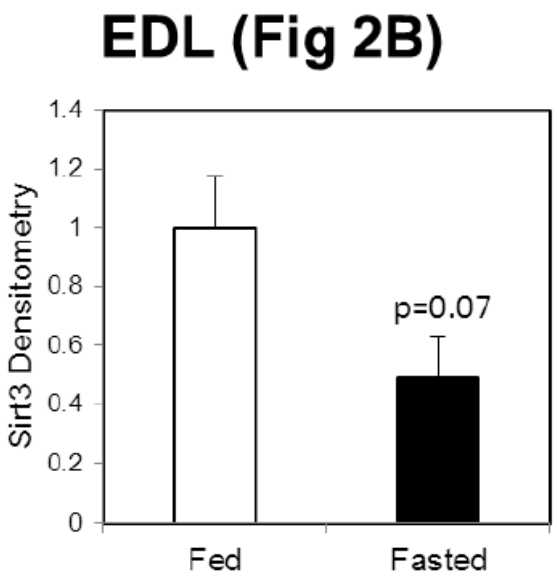
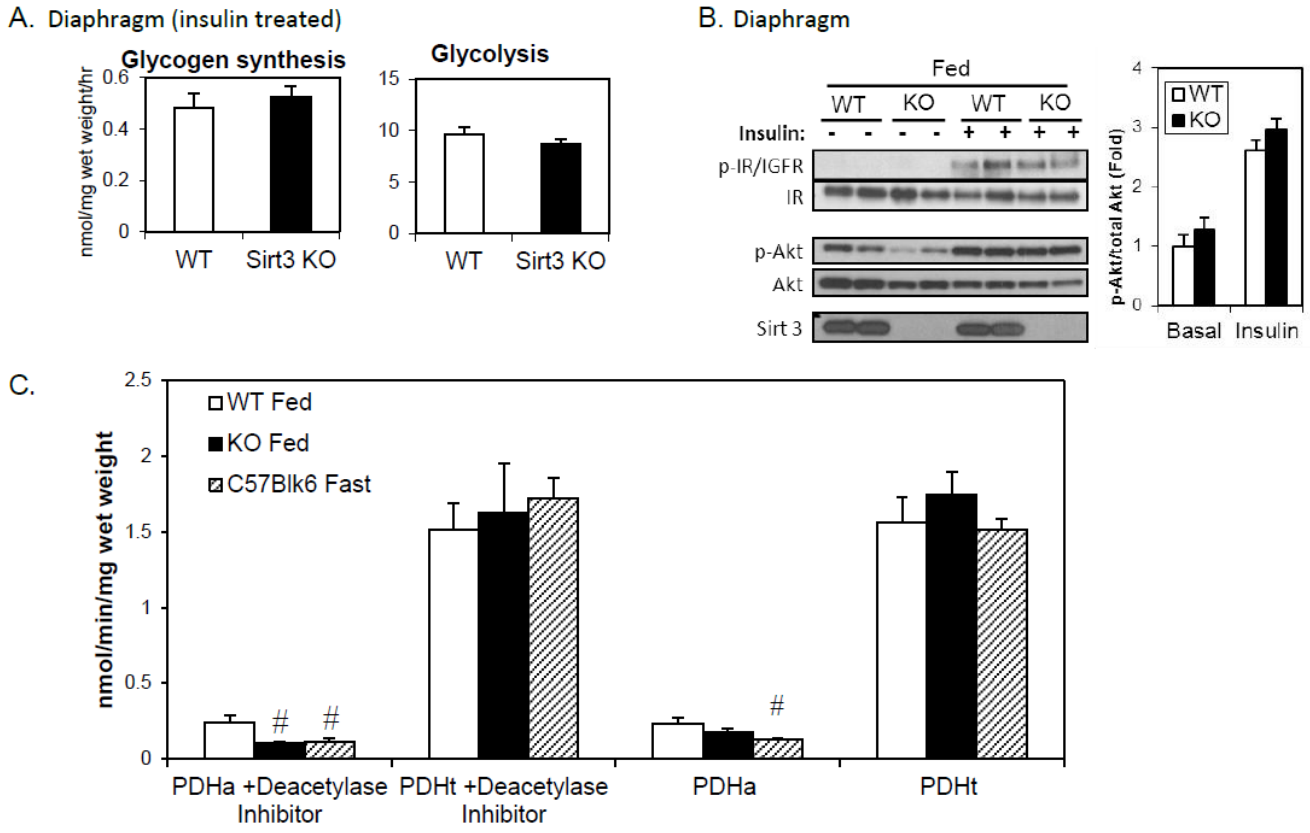


SUPPLEMENTARY DATA

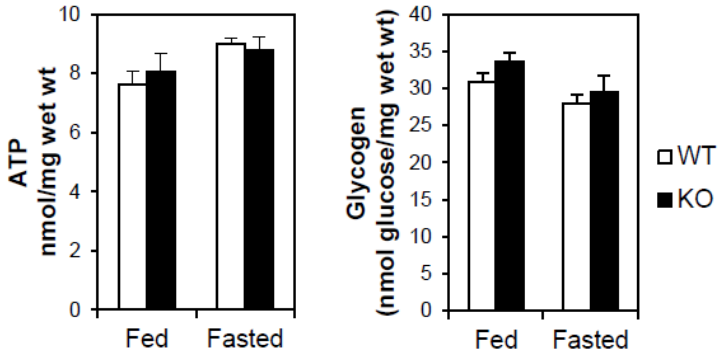
Supplementary Figure 2. Sirt3 deletion leads to decreased PDH activity in the absence of insulin resistance or AMPK activation. (A) Glycogen synthesis and glycolysis rates were measured in the presence of glucose and palmitate in hemi-diaphragms from Sirt3 KO and WT fed animals. (B) Insulin (1mU/ml) signaling in Hemi-diaphragms from fed Sirt3 KO and WT controls. Densitometry was performed on phosphor-Akt and presented as ratio of phospho:total Akt. (C) PDHa and PDHt activity was measured in gastrocnemius homogenates from 8-16 week old male mice incubated in the presence or absence of deacetylase inhibitors as described in Methods (n=6-7, #-p<0.05 vs WT Fed + deacetylase inhibitors ANOVA). (D) Phospho AMPK T-172 levels were measured in gastrocnemius from fed and 24-hour fasted Sirt3KO and WT. (E) ATP and Glycogen levels were measured by enzymatic coupling assay in gastrocnemius from fed or 24-hour fasted Sirt3KO and WT mice (n=4-6). (F) Densitometric quantification of pS232, pS293, and pS300 phosphorylation sites in WT and Sirt3KO in Figure 2D (n=4-6 from 2-3 separate experiments).



SUPPLEMENTARY DATA

D. Gastrocnemius

E. Gastrocnemius



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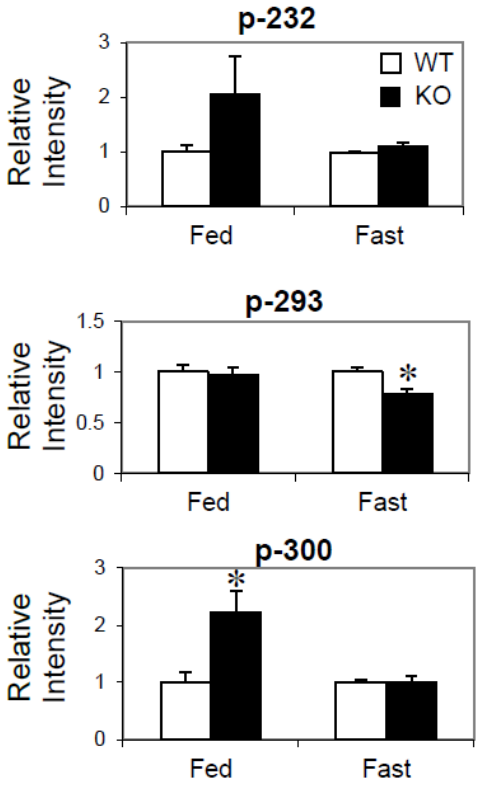
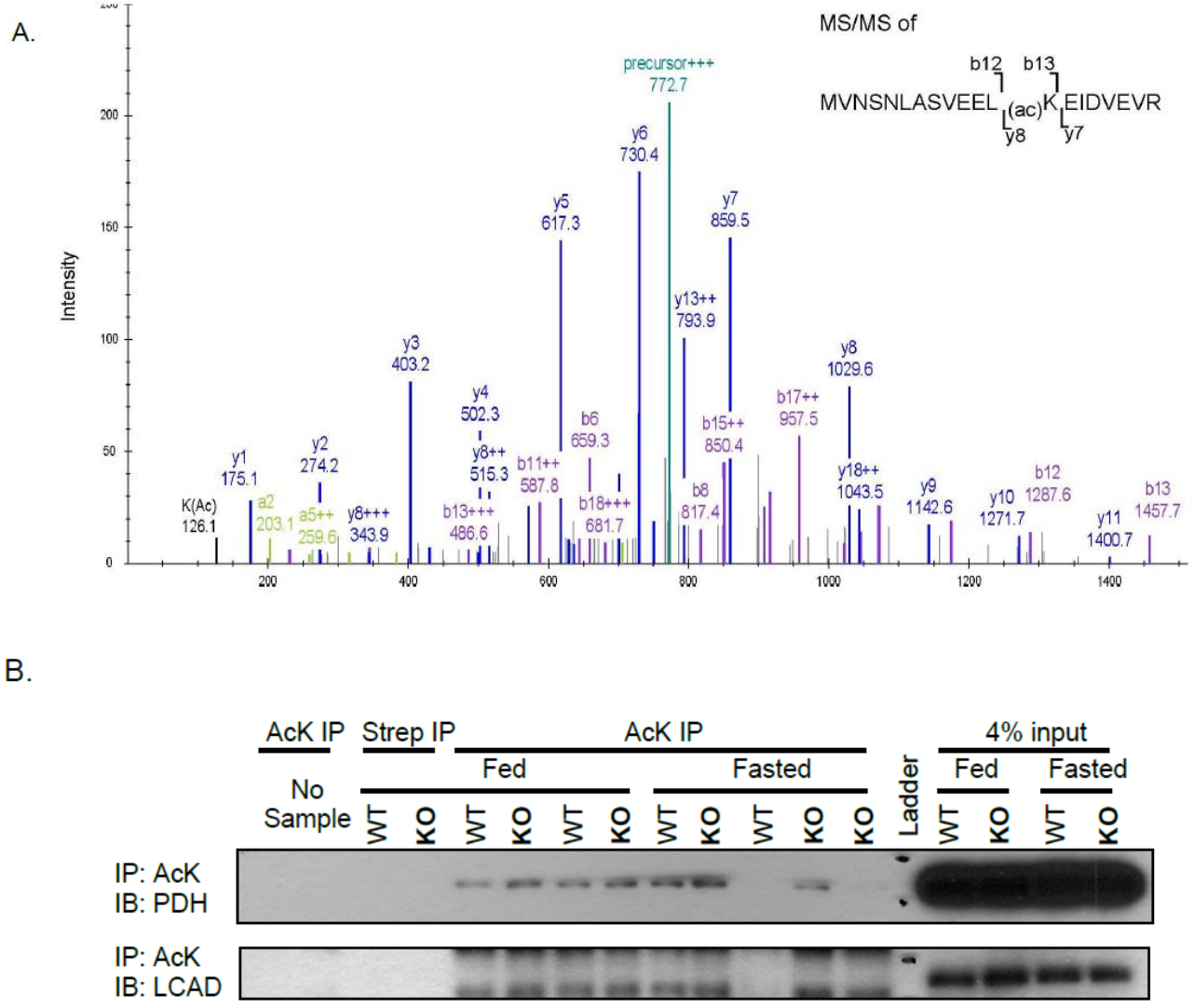


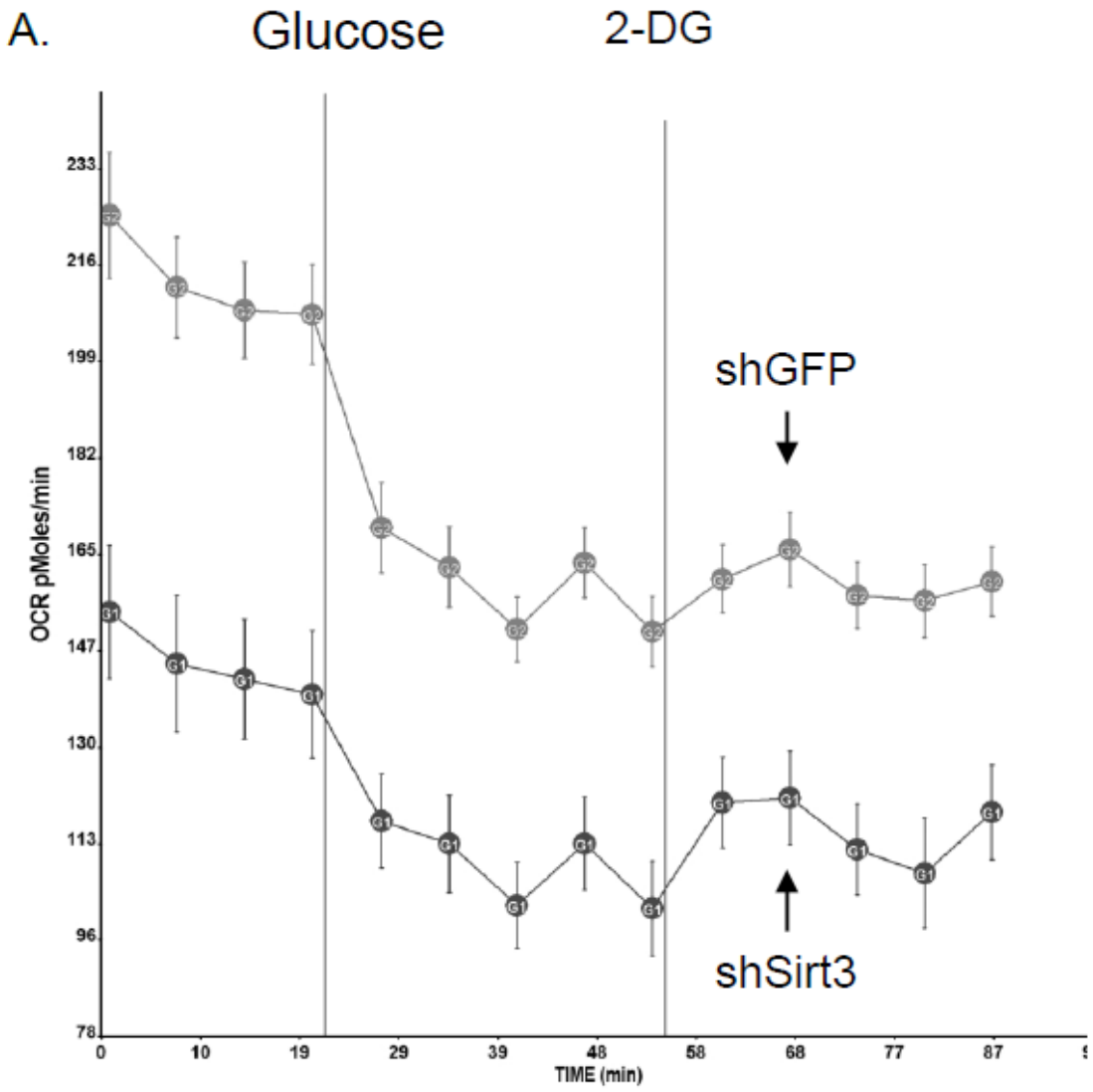
SUPPLEMENTARY DATA

Supplementary Figure 3. (A) Tandem mass spectrum of the acetylated PDH E1α lysine 336 containing peptide ^{324}MVNSNLASVEELacKEIDVEVR^{343} (m/z - 772.73^{+++}), with a mass of 2315.16 Da. Ions used to determine the site of acetylation are indicated. (B) Immunoprecipitation of muscle mitochondrial lysates from fed and fasted WT and Sirt3KO was done with Acetyl-lysine (AcK) beads or streptavidin beads as controls. Immunoprecipitates were subjected to SDS-PAGE along with 4% of the IP mitochondrial lysate input and western blotting was done with either PDH E1α or LCAD antibody.



SUPPLEMENTARY DATA

Supplementary Figure 4. Oxygen consumption rate (OCR) is reduced in shSirt3 cells. (A) OCR under basal, glucose-stimulated (25 mM), or 2-deoxyglucose (2-DG)-stimulated conditions is decreased in shSirt3 compared to controls as previously reported (30). (B) OCR as a percentage of basal (no glucose) respiration is unchanged in shSirt3 myoblasts.



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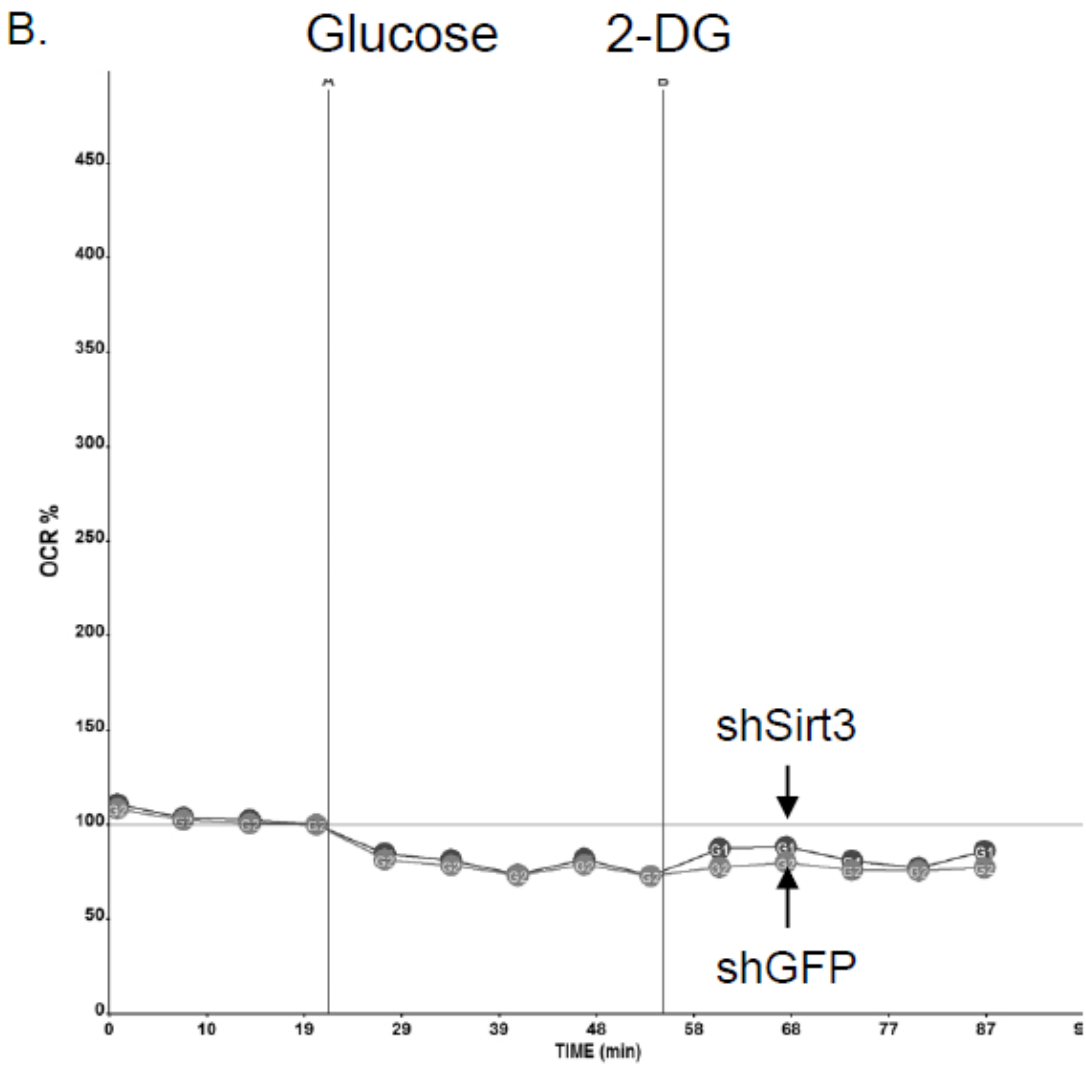

SUPPLEMENTARY DATA

Supplementary Figure 5. Dephosphorylation of PDH E1α achieved by treatment with dichloroacetate (DCA) prior to PDH activity assay. (A) WT, K336Q, and K336R myoblasts were grown to confluence then treated for 2 hours with vehicle or increasing concentrations of DCA prior to lysis and protein isolation. Western blot analysis was performed on whole cell lysates using phosphor-specific antibodies to PDH E1α. (B) WT, K336Q, and K336R myoblasts were grown to confluence and treated for 2 hours with 20 mM DCA prior to harvest for PDH activity assay performed as in Methods. After harvest, an aliquot of cell lysate was immediately frozen (t=0 hr) and compared to an aliquot of cell lysate taken just prior to spectrophotometric analysis of PDH activity (t=3 hr).