SUPPLEMENTAL FIGURES

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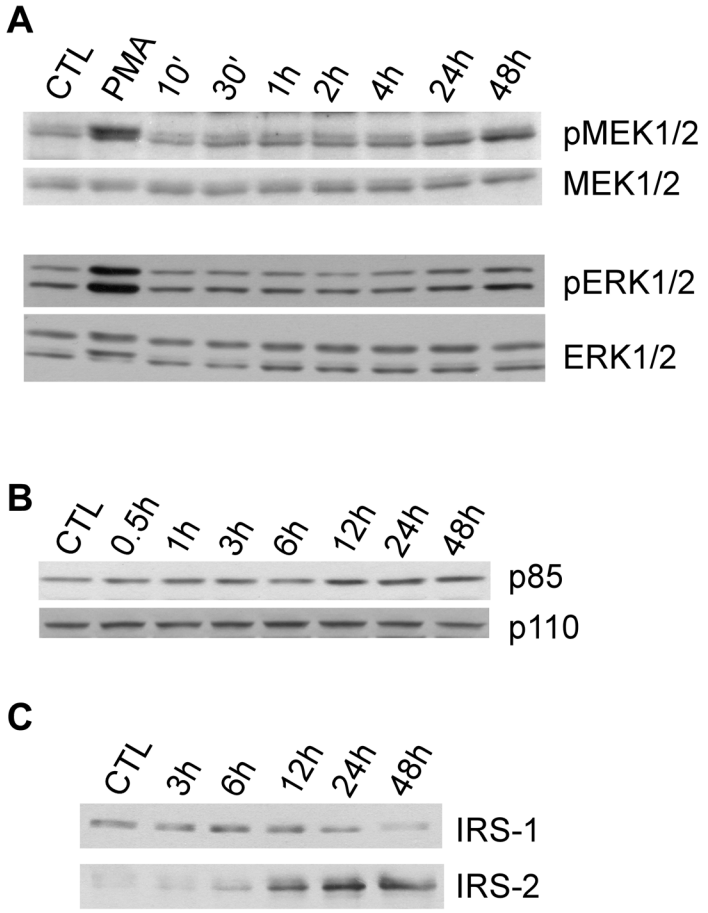

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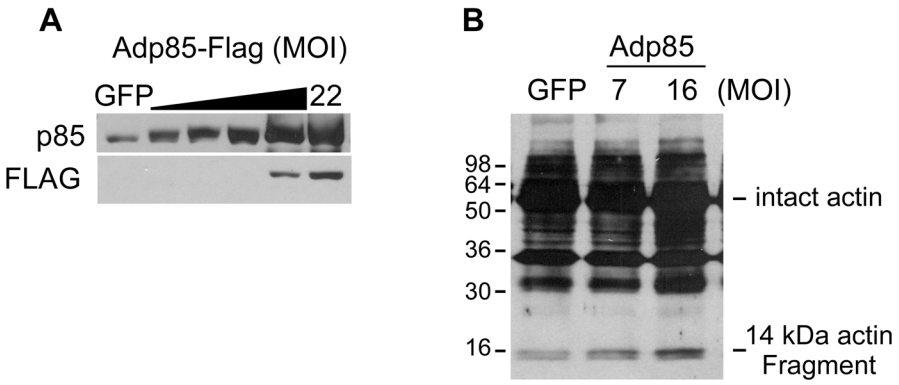

SUPPLEMENTAL FIGURES

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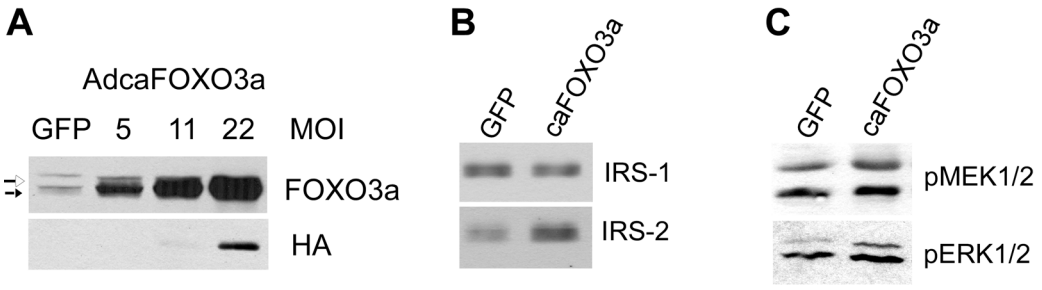

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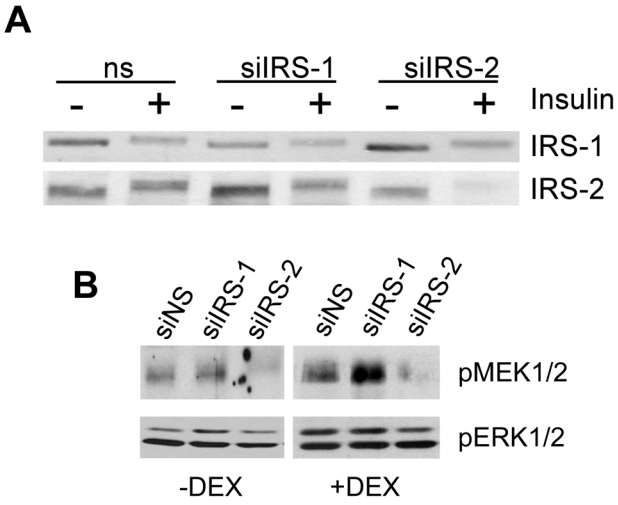

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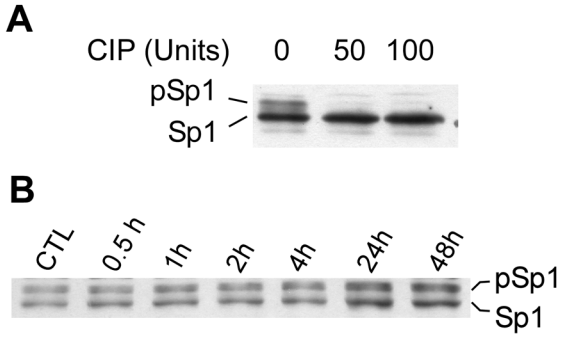
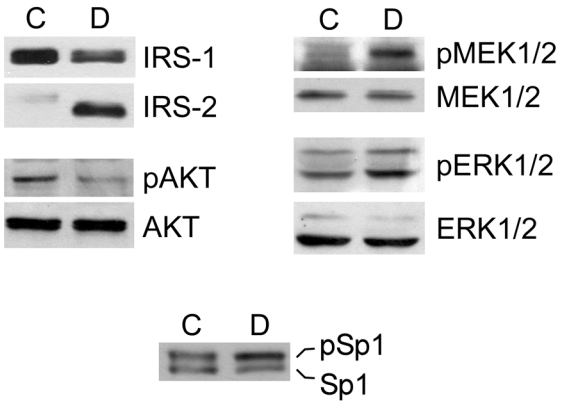


SUPPLEMENTAL FIGURES

S6



SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Time courses of dexamethasone-induced signaling changes. Cells were treated with DEX (100 nM) for the indicated times before harvest. Representative immunoblots for Figures 1B, 1C and 1F are shown in Panels A, B and C, respectively. In Panel A, some cells were treated with for 10 min with 100 nM 12-myristate 13-acetate (PMA) as a positive control for MEK and ERK phosphorylation.

Figure S2. Adenoviral expression of PI3K p85α and constitutively active FOXO3a. L6 myotubes were infected with adenoviruses expressing GFP (control), (**A & B**) Flag-tagged PI3K p85α or (**C**) HA-tagged, constitutively active FOXO3a (caFOXO3a) at various MOI up to 22. Immunoblot analyses were performed with the indicated antibodies. Panel **B** is a representative immunoblot for actin showing the increase in 14-kDa actin cleavage fragment when the p85 subunit is overexpressed. The position of intact actin is also indicated. In Panel **C**, the solid arrow indicates the position of dephosphorylated endogenous FOXO3a plus ectopically expressed HA-tagged, caFOXO3a; the open arrow indicates the position of phosphorylated endogenous FOXO3a protein.

Figure S3. Effect of constitutively active FOXO3a over-expression on IRS-1/2 and MEK/ERK phosphorylation. Cells were infected with an adenovirus encoding EGFP or caFOXO3a at a multiplicity of infection (MOI) of 22 before harvest 48 h later. Representative immunoblots for Figures 3A and 3B are shown in Panels A and B, respectively.

Figure S4. Knockdown of IRS-1 and IRS-2 proteins using pooled siRNA. (**A**) L6 myoblasts were transfected with pooled nonspecific siRNA or pooled siRNA specific for human IRS-1 or IRS-2 as described in Materials and Methods. Some cells were treated with insulin (100 nM) for 10 min prior to harvest. Immunoblot analyses were performed with the indicated antibodies to determine the level of protein expression in transfected cells. Shown in Panel B are representative immunoblots of changes in MEK/ERK phosphorylation associated with IRS-1 andIRS-2 knockdown in the presence and absence of DEX (100 nM; 48h).

Figure S5. DEX induces Sp1 phosphorylation. (**A**) L6 myotubes cells were treated with Dex (100 nM) for 24 h and whole cell lysates were prepared. Calf intestinal phosphatase was added to some lysates before incubation as described in Materials and Methods. An immunoblot analysis of the untreated and CIP-treated lysates was performed using Sp1 antibodies. Shown in Panel **B** is a representative immunoblot for Sp1 showing the time course of changes in phosphorylation after DEX treatment (100 nM).

Figure S6. Changes in IRS protein levels and cell signaling in diabetic rat muscle. Shown are representative immunoblots of IRS-1, IRS-2, pAkt, AKt, pMEK1/2, MEK1/2, pERK1/2, ERK1/2 and Sp1 in gastrconemius muscles of control and acutely diabetic rats.