Supplemental Material

Materials and Methods

Expression and purification of recombinant hnRNP A2.

The pET 28a (+)/hnRNP A2 plasmid with an N-terminal His-tag was a kind gift from Dr. G. Dreyfuss (University of Pennsylvania, Philadelphia, PA). Phosphorylation mutants of hnRNPA2 were made using a Site-Directed Mutagenesis kit from Stratagene in a pET28a(+) vector. The proteins were expressed in *E. coli BL21(pLys)* strain by growing the cultures for 24 h at 30°C with 3 h isopropyl β-D-1-thiogalactopyranoside (1 mM) induction. Protein purification was done using PrepEase® Ni-TED column (USB Corporation, OH, USA) according to the manufacturer's suggested protocol. Eluates were collected in 1-ml fractions. Fractions were run on 12% SDS-PAGE and were Coomassie stained. Eluates showing a single band were concentrated further, confirmed by Western blotting, and used for kinase assays.

In vitro Transcription Translation

The wild type and mutant (T98A, S219A) hnRNPA2 cDNAs were transcribed *in vitro* and translated using Quick Coupled T7 RRL kit obtained from Promega Corp.

Supplementary Figure Legends

Figure S1. Estimation of mitochondrial DNA content : Relative quantitation of mtDNA in C2C12 control cells, cells treated with ethidium bromide or 2,3'-dideoxycytidine (as described in materials and methods) and reverted cells.

Figure S2. Western blot of nuclear extracts (50 µg protein) from untreated or treated mtDNA-depleted cells: (panel i) extracts from cells treated with the Ser473 phosphorylation inhibitor ML9 and untreated cells probed with pAkt (Ser473) antibody;

(panel ii) extracts from cells treated with the Thr308 phosphorylation inhibitor UCN01 and untreated cells probed with pAkt (Thr308) antibody; (panel iii) total Akt levels in mtDNA-depleted cells treated with either ML9 or UCN01. UT = untreated.

Figure S3. Calcineurin Aα and IGF1R mRNA silencing by siRNA expression. (A) Real-time PCR showing calcineurin Aα mRNA levels in mtDNA-depleted cells expressing either mock (scrambled) or calcineurin siRNA. (B) Real-time PCR showing IGF1R mRNA levels in mtDNA-depleted cells with mock (scrambled) or IGF1R siRNA.

Figure S4. Effects of hnRNPA2 silencing on Akt protein levels in C2C12 cells. Immunoblot analysis of total cell lysate (50 µg each) and nuclear protein extracts (30 µg each) from control, mtDNA-depleted, and mtDNA-depleted/hnRNPA2 silenced cells is shown. The blots were developed with Akt antibody and companion blots were probed with beta actin antibody (total extract) or antibody to nuclear protein p97 (nuclear extract) for assessing loading levels.

Figure S5. Patterns of nuclear proteins pulled down by Akt antibody. Nuclear extracts (1 mg protein) from control, mtDNA-depleted, and mtDNA-depleted/hnRNPA2-silenced cells were immunoprecipitated with Akt antibody. The immunoprecipitates were run on an 8-20% gradient SDS-PAGE and stained with Coomassie Blue. The arrows indicate the distinctive protein bands immunoprecipitated from mtDNA-depleted nuclear extract that were either absent, or present in very low levels, in control and mtDNA-depleted/hnRNPA2-silenced cell extracts. These protein bands were excised and subjected to nano LC/MS analysis.

Figure S6. Purification and characteristics of WT and Akt1 site mutated hnRNPA2. (A) Autoradiogram of wild type, T98A mutant, and S219A mutant hnRNPA2 translated *in*

vitro in RRL in the presence of added ^{35}S-Met. (B). Coomassie blue-stained SDS–PAGE pattern of purified recombinant hnRNPA2 proteins. The patterns of WT (hnRNPA2) and mutant forms (T98A, S219A) (1.0µg each) are shown.

Figure S7. Activation of stress signaling markers and Akt in ddC mtDNA depleted cells. (A). Total Akt levels from nuclear extract (50µg protein) in control and 2,3'-dideoxycytidine treated cells (ddC mtDNA depleted cells). (B) Real Time PCR quantitation of the mRNA levels of mitochondrial stress target genes in control and 2,3'-dideoxycytidine treated mtDNA-depleted cells. Beta actin was used as an internal control.

Figure S1

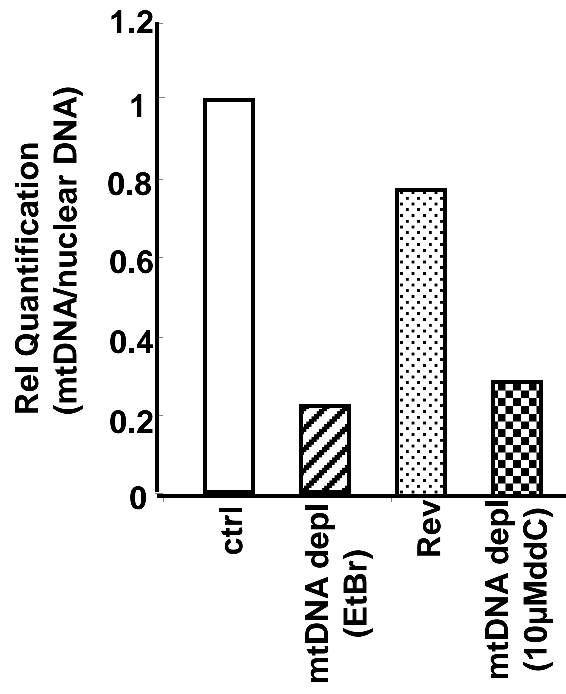


Figure S2

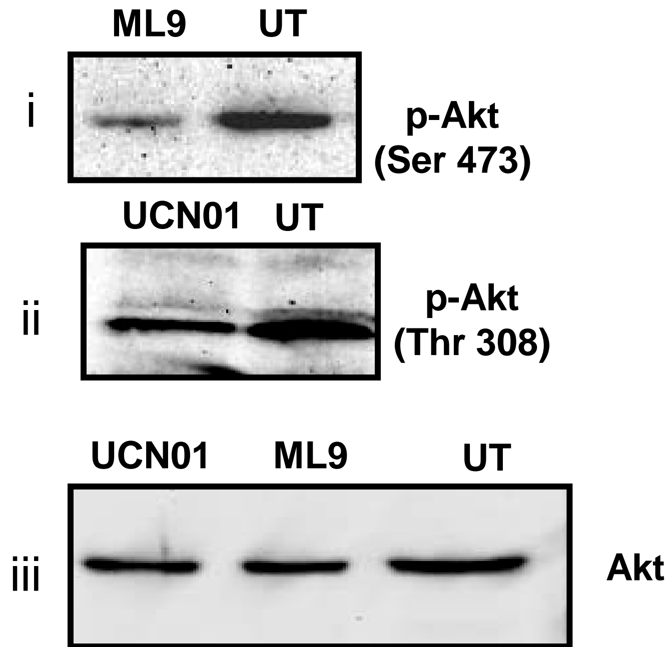
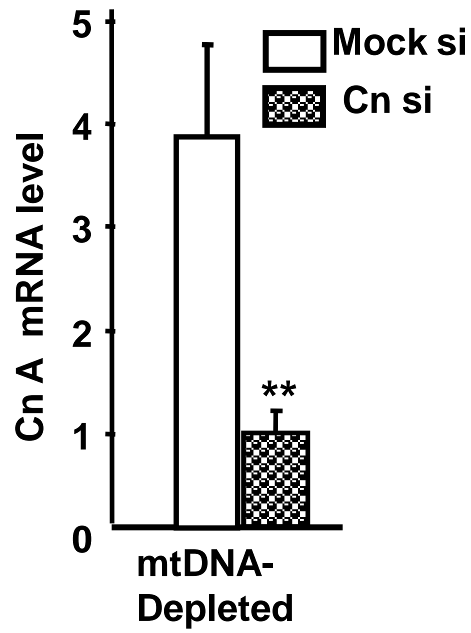
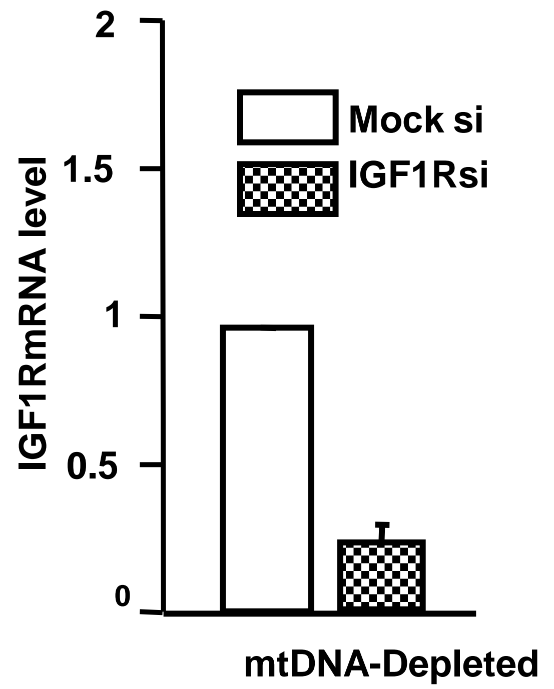


Figure S3





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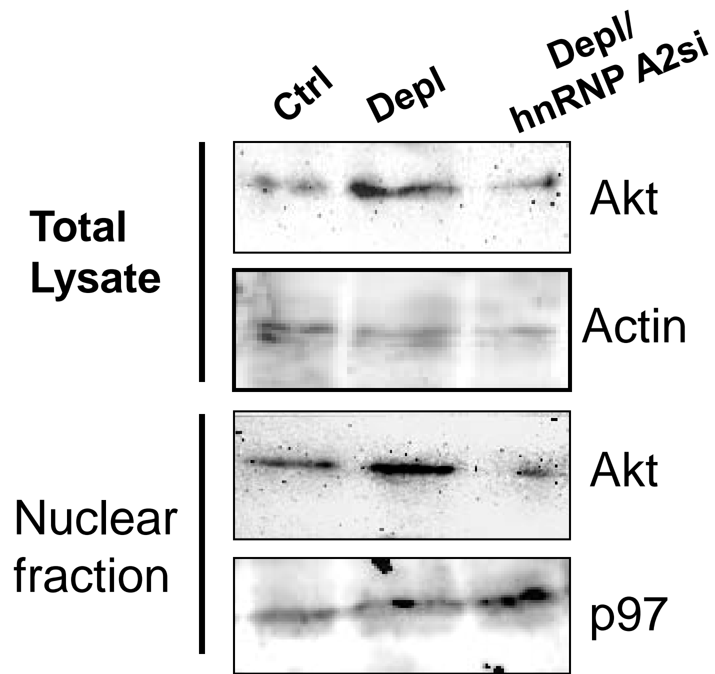
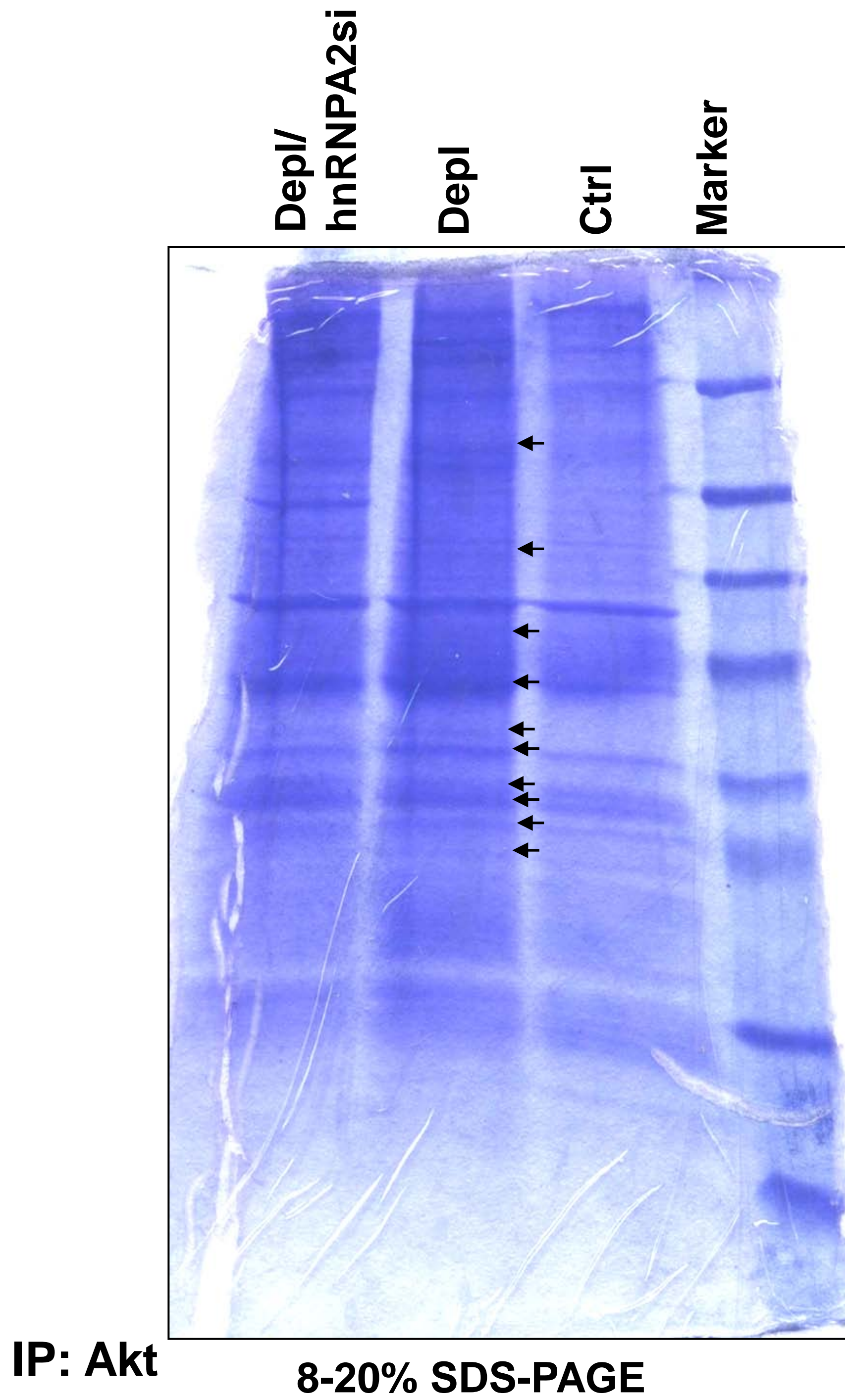


Figure S5



IP: Akt