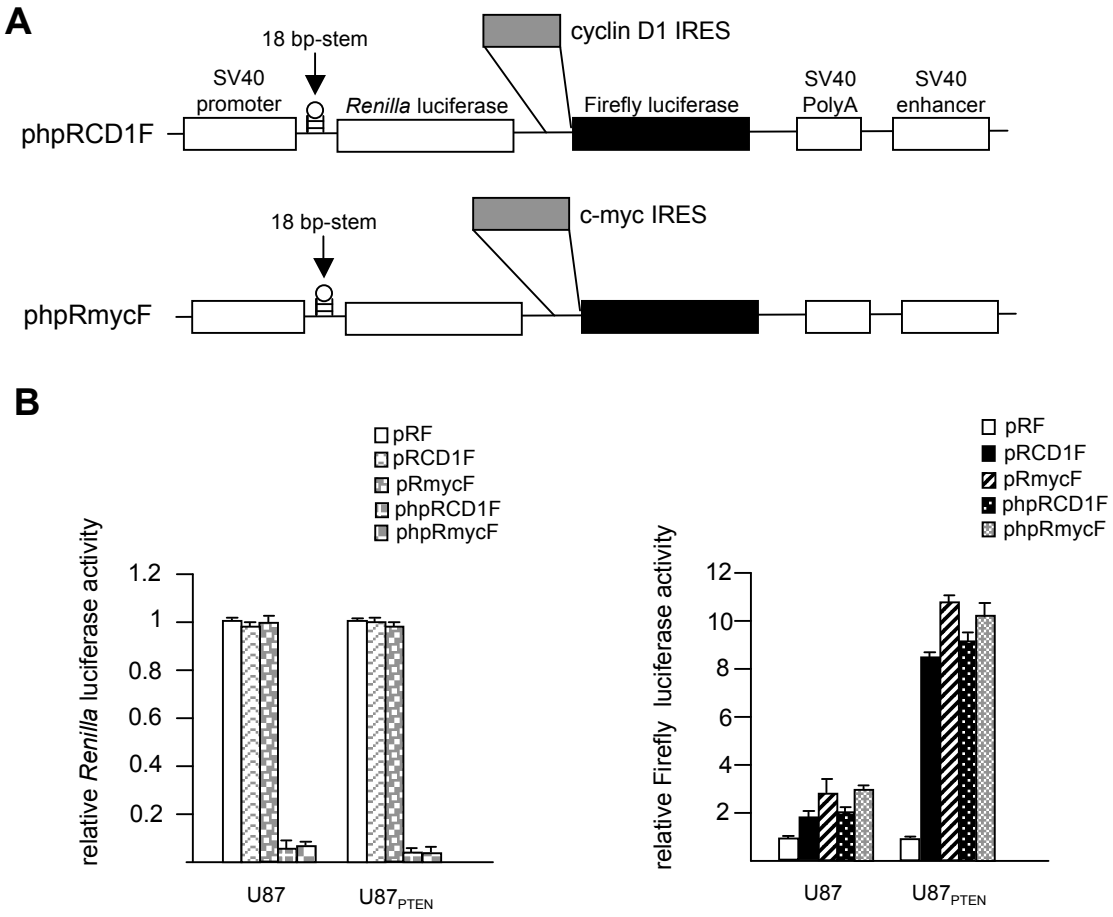
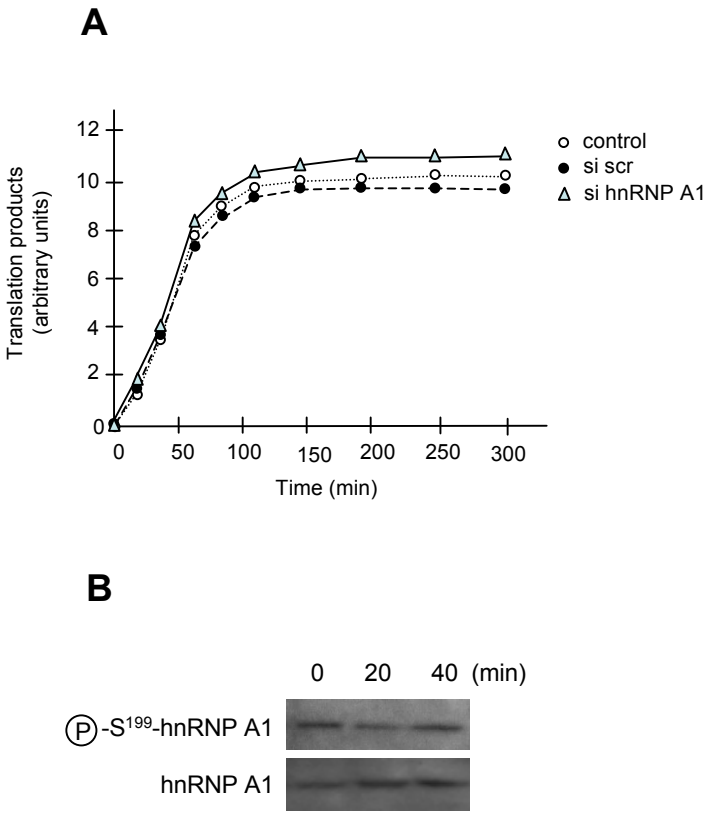
Supplemental figure 1. Cyclin D1 and c-*myc* IRES sequences facilitate IRES-mediated translation in dicistronic plasmids when cap-dependent translation is inhibited in *cis*. *A*, schematic diagram of plasmids used in transient transfections. A stable hairpin structure was introduced upstream of the *Renilla* start to generate phpRCD1F and phpRmycF as described (Sharma *et al*.). These constructs contain nucleotides -209 through -44 of the cyclin D1 and -398 through -165 of the c-*myc* 5' UTRs, respectively, inserted within the intercistronic region of the dicistronic reporters. *B*, relative firefly and *Renilla* luciferase activities in extracts of the indicated transiently transfected cells with the plasmids shown. pSVβGal was used as a control to normalize for transfection efficiencies. The relative firefly and *Renilla* luciferase activities conferred by the pRF plasmid was normalized to 1 for each cell line. The results show the mean and S.D. from four independent transfections. These data suggest that the cyclin D1 and c-*myc* IRES sequences can promote cap-independent translation in an Akt-dependent manner. These results are also consistent with the notion that these sequences do not allow ribosomal readthrough of the *Renilla* translation termination codon, as a proportional decrease in IRES-mediated translation was not observed relative to the degree of cap-dependent inhibition induced by the hairpin structure.

Supplemental figure 2. hnRNP A1 knockdown does not effect *in vitro* protein synthesis of exogenous mRNAs in translation-competent $U87_{PTEN}$ cell extracts. *A*, The kinetics of *Renilla* luciferase synthesis were determined in extracts from cells with the indicated siRNA treatments programmed with exogenous *in vitro* transcribed capped pRF mRNAs. Aliquots were removed at the indicated intervals from 0 to 300 min, and translation products analyzed by SDS-PAGE. Translations were performed at 30˚C in the presence of [^{35}S]methionine. Densitometric quantification of *Renilla* luciferase translation products was performed as described previously (Michel *et al*). Extracts from control or siRNA-treated extracts were similarly productive and continuously accumulated *Renilla* luciferase for approximately 120 min. *B*, Extracts supplemented with *in vitro* phosphorylated hnRNP A1 were subjected to immunoblot analysis for the indicated proteins at various time points during the translation reactions. Experiments in *B* were performed three times with similar results.

Supplemental figure 3. Effects of rapamycin exposure on the cellular distribution of the dominant negative hnRNP A1 mutant or export of the dicistronic reporter mRNAs. *A*, U87 cells expressing the NLS-A1-HA mutant in the absence or presence of rapamycin (10 nM, 24 h) were processed for immunofluorescence microscopy (top panels) as described in figure 8. Below the immunofluorescence images are shown comparison values of positively stained cell numbers for the indicated immunopositive cell compartments without and with rapamycin treatment. Data are mean and S.D. of the percentage of cells in each compartment. Calculated P (> 0.05) values indicated no significant staining differences between control and rapamycin treatment in either compartment. *B*, U87 or $U87_{PTEN}$ cells were transfected with the indicated plasmids and treated without or with rapamycin (10 nM, 24 h). Cells were subsequently fractionated into nuclei and cytoplasm as described (Qiu et al.), and the nuclear (N) and cytoplasmic (C) RNAs were extracted and the indicated dicistronic reporter mRNA levels were quantified by real-time qRT-PCR as in figure 9A. Similar steady-state levels of nuclear

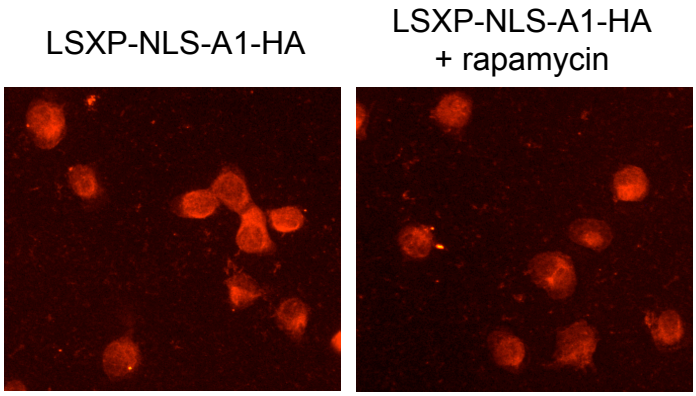


Supplemental figure 1, Jo *et al*.



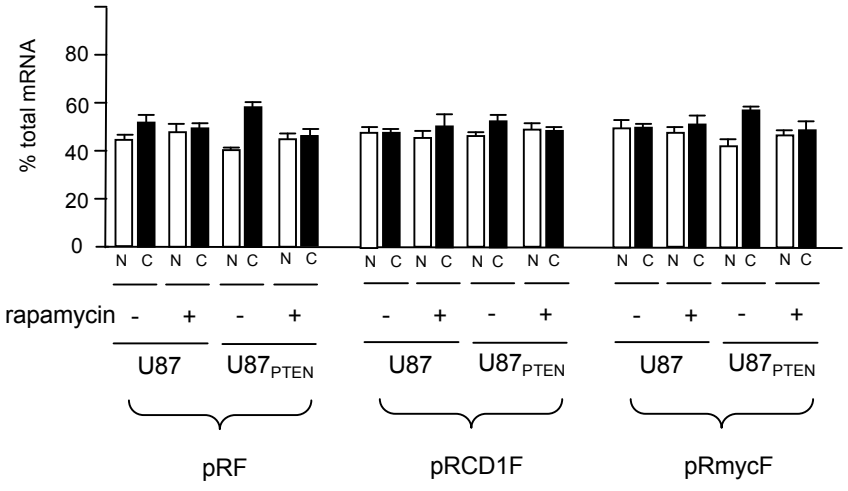
Supplemental figure 2, Jo *et al.*

A



	% cytoplasmic	% nuclear	% both
LSXP-NLS-A1-HA	3.2 (+/- 1.1)	93.8 (+/- 13.1)	3.0 (+/- 2.4)
LSXP-NLS-A1-HA + rapamycin	4.4 (+/- 1.3)	90.3 (+/- 10.6)	5.3 (+/- 2.5)

B



Supplemental figure 3, Jo *et al.*