





GST-A1 hnRNP A1 actin Supplementary figure legends

- Supplementary figure 1. Immunoblot analysis of various proteins in conditionally active AKT cell lines. LN229_{AKT-MER}, LN229_{EV}, MEF_{AKT-MER} and MEF_{EV} cells were treated with either 4OHT, rapamycin or both for 24 h and cell extracts subjected to SDS-PAGE and immunoblotted for the indicated proteins. Similar results were obtained in three independent experiments.
- Supplementary figure 2. Inhibition of hnRNP A1 expression in LN229 cells following siRNAmediated knockdown and ability of resultant cell extracts to support cap-dependent protein synthesis. (A) The relative levels of the indicated proteins were determined by Western analysis of cell lysates prepared from cells treated with the siRNAs shown. A representative immunoblot is shown from multiple experiments displaying similar results. (B) The kinetics of *Renilla* luciferase synthesis were determined in extracts from cells following treatment with the indicated siRNAs. Extracts were programmed with *in vitro* transcribed capped pRF transcripts and translation reactions allowed to proceed at 30°C in the presence of $[^{35}S]$ methionine. Aliguots were removed at the indicated intervals from 0 to 300 min and translation products analyzed by SDS-PAGE. Densitometric quantification of *Renilla* luciferase expression were performed as previously described (Jo et al). Extracts from control or siRNA-treated cells continuously accumulated *Renilla* luciferase for approximately 120 min and were similarly productive. (C) Coomassie-blue stained gels of recombinant native and S199E mutated hnRNP A1 preparations as indicated. Arrow indicates position of hnRNP A1. Single or double asterisks indicate native or S199E mutated proteins, respectively. Molecular weight markers are shown on the left of the figure.

Supplementary figure 3. Expression of native or S199E hnRNP A1 -GFP or -GST fusion

proteins. (A) Whole cell lysates from LN299 cells transfected with empty vector (pGFP-C1), native or S199E mutated hnRNP A1-GFP and treated without or with rapamycin (10 nM, 24 h), were subjected to immunoblotting for the detection of GFP, hnRNP A1-GFP or S199E hnRNP A1-GFP. Single asterisk indicates the position of GFP, while the double asterisks indicate the position of the fusion proteins. Molecular weight markers are shown on the left of the figure. Note that hnRNP A1-GFP or S199E mutated hnRNP A1-GFP proteins were not cleaved. (B) Whole cell lysates from LN229_{AKT-MER}, LN229_{EV} (empty vector control), MEF_{AKT-MER} and MEF_{EV} were analyzed by immunoblotting for the indicated proteins. Transgene expression was assessed using an α -GST antibody.