SUPPLEMENTAL FIGURE LEGENDS

Figure S1: NCL represses p53 translation. A. Nucleolin protein represses p53 translation *in vitro*. Native nucleolin protein (Vaxron) was added to rabbit reticulocyte lysate together with p53 cDNA (75 base 5′-UTR, p53 coding sequence, full-length 3′-UTR). The expression of [^{35}S] methionine-labeled newly synthesized protein was assessed by autoradiography. **B.** NCL repression of p53 translation is specific. p53 cDNA (75 base 5′-UTR, full-length coding sequence, 3′-UTR), luciferase coding sequence, and/or nucleolin coding sequence were co-expressed in rabbit reticulocyte lysate. The expression of [^{35}S] methionine-labeled newly synthesized protein was assessed by autoradiography. **C.** Nucleolin represses the translation of 5′-m^{7}GpppN capped and 3′-polyadenylated p53 mRNA *in vitro*. Pre-made 5′-capped and 3′-poly(A) tailed p53 mRNA (75 base 5′-UTR, full-length coding sequence, 3′-UTR) was expressed with increasing amount of nucleolin protein (Vaxron) in rabbit reticulocyte lysate and the expression of [^{35}S] methionine-labeled newly synthesized protein was assessed by autoradiography.

Figure S2: Nucleolin (NCL) fails to repress expression of human p53 mRNA containing only a 3′-UTR sequence. A. Overexpression of NCL fails to repress luciferase expression in a cell line stably expressing a human p53 mRNA with no 5’-UTR, but with only a 3′-UTR. MCF-7 stable cell lines were established to constitutively express a firefly luciferase reporter gene without any UTR sequences of human p53 mRNA (LUC) or containing only full-length p53 3′-UTR (LUC3′). Flag-tagged nucleolin (NCL) or control vector (Flag) were co-transfected with a renilla luciferase (19) expression vector (internal control) into these stable cell lines. 24 hrs post transfection, modulation of the reporter gene expression was assessed by comparing LUC/RL ratio of each samples to that of LUC samples. Error bars represent average ± standard deviation for three independent experiments. **B.** Knocking down NCL in cells has no effect on luciferase expression in cells stably expressing a human p53 mRNA with only a 3′-UTR. NCL siRNA duplex (NCLsi) (1) or control siRNA (ctrl si) was introduced into the above MCF-7 stable reporter cell line. Modulation of the reporter gene expression was assessed by comparing firefly luciferase reading of each sample to that of LUC samples. Error bars represent average ± standard deviation for three independent experiments.

Figure S3: Manipulations of NCL levels have no impact on the mRNA levels of the luciferase reporter gene. Luciferase reporter stable lines with either 5′-UTR or 3′-UTR or both were transiently transfected with Flag-NCL (NCL, left panel) or NCLsi (right panel) as explained in Figure 2, S2. The relative LUC/RL mRNA ratio (left panel) or LUC/GAPDH (right panel) mRNA ratio was calculated by normalizing the LUC/RL or LUC/GAPDH mRNA ratio of each sample to the ratio of cells transfected with empty vector or control siRNA. Data shown are average ±SD for three independent experiments.

Figure S4: NCL binds human p53 mRNA in cells. GFP-tagged NCL or its deletion mutants were immunoprecipitated from MCF-7 cells either stably or transiently (labeled by asterisks in upper panel) expressing these proteins by using anti-GFP antibody. The bound proteins were detected by western blot analysis using anti-GFP antibody (lower panel) and labeled by asterisks and the bound human p53 mRNA was amplified by RT-PCR (upper panel). PCR products were separated on 1% agarose gel and visualized by ethidium bromide staining. Bar graph (middle panel) was plotted for the band intensity of each PCR product relative to that in lane 1. M: DNA ladder. Lane 1: GFP. Lane2: GFP-NCL. Lane 3: NCL with mutation of NLS. Lane4: NCL with mutation of NES. Lane 11: PCR positive control using MCF-7 cDNA as template to amplify p53 mRNA.

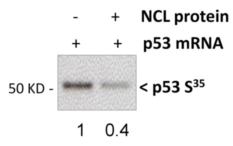
Figure S5: NCL has no effect on the levels of mutant luciferase reporter gene mRNAs. MCF-7 cells were transiently transfected with empty vector (vector) or Flag-NCL (NCL) together with wild-type or mutant firefly luciferase constructs (LUC) as explained in the Figure 3C, plus a control Renilla luciferase expression construct. The relative LUC/RL mRNA ratio was calculated by normalizing the LUC/RL mRNA ratio of each sample to the ratio of cells transfected with empty vector and firefly luciferase construct containing wild-type 145 base 5′-UTR and full length 3′-UTR of human p53. Data shown are average ± SD for three independent experiments.

Figure S6: NCL and its deletion mutants have no impact on the levels of luciferase reporter gene mRNAs. MCF-7 cells were transiently transfected with empty vector (vector) or Flag-NCL (NCL) or its deletion mutants together with firefly luciferase constructs (LUC) as explained in the Figure 5, plus a control Renilla luciferase expression construct. The relative LUC/RL mRNA ratio was calculated by normalizing the LUC/RL mRNA ratio of each sample to the ratio of cells transfected with empty vector and firefly luciferase construct containing a 145 base 5′-UTR and full length 3′-UTR of human p53. Data shown are average ± SD for three independent experiments.

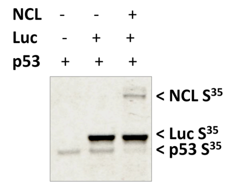
Figure S7: A proposed model for translational control of p53 mRNA by RPL26 and nucleolin. In the absence of stress, rates of p53 mRNA translation are kept low by the binding of nucleolin homodimers to a region of double-strand RNA formed by base-pairing interactions of sequences in the 5' and 3' UTRs. Upon stress stimulation, RPL26 protein becomes available and binds to the nucleolin dimers that are bound to p53 mRNA, disrupts the nucleolin homodimers, and "de-represses" the translation of p53 mRNA. Thus, the binding of RPL26 to p53 mRNA switches p53 mRNA from a repressed state to an active state for translation.

Figure S1

A



B



C

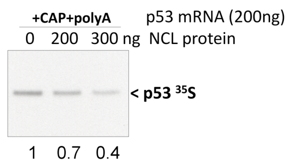
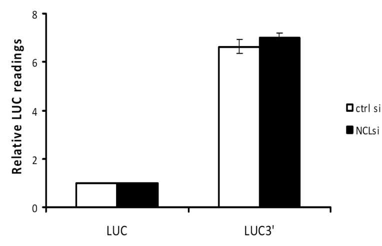


Figure S2

A



B

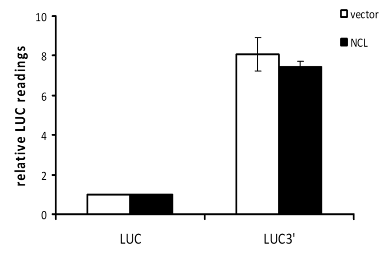


Figure S3

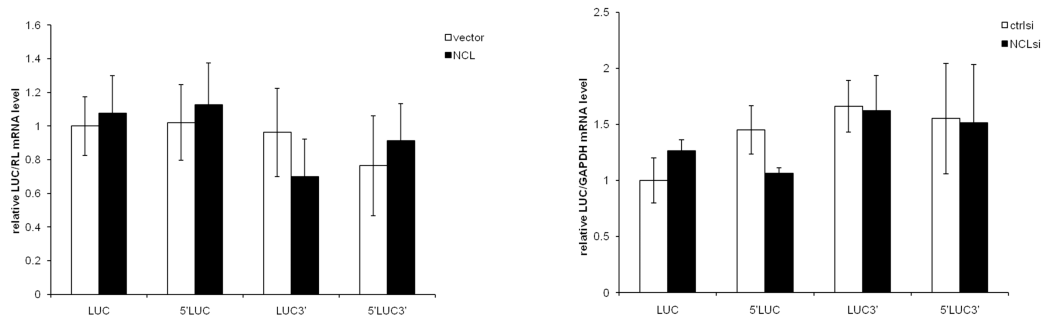


Figure S4

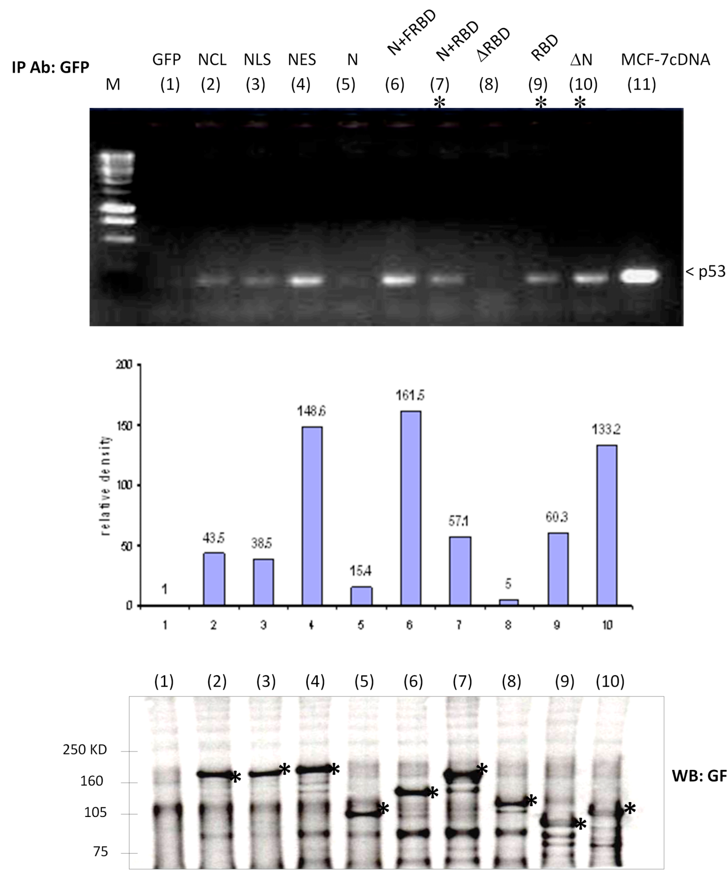


Figure S5

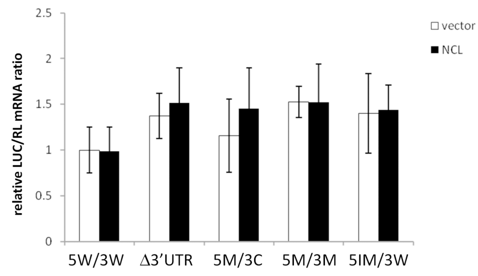


Figure S6

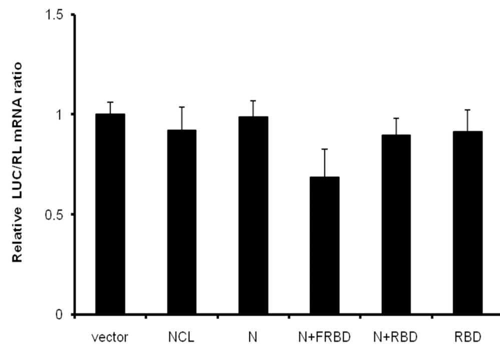


Figure S7