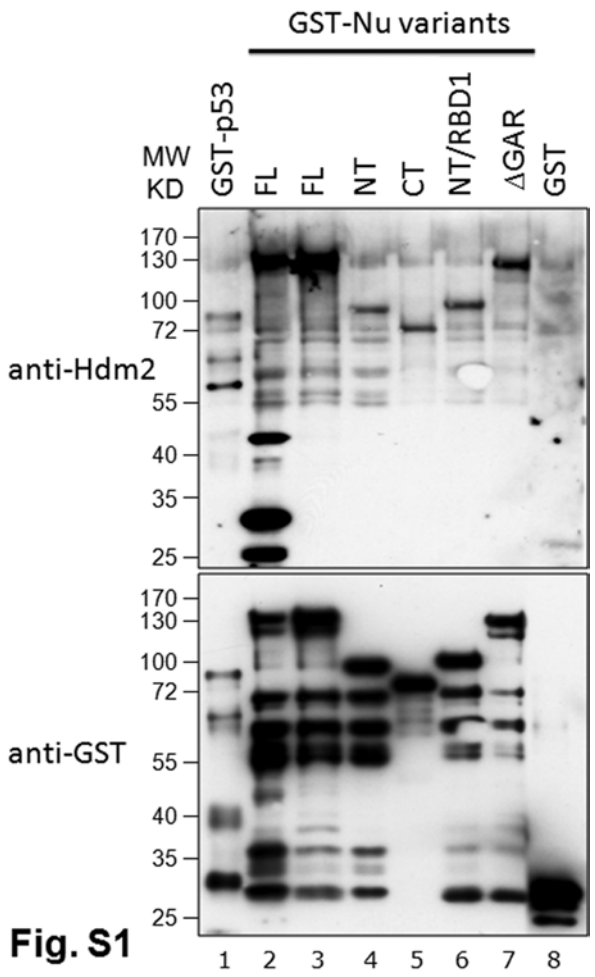

Fig. S1

Fig. S1, Far-Western analyses of direct nucleolin-Hdm2 interactions. Upper panel, Equivalent amounts (400 ng) of GST-tagged nucleolin FL (lanes 2 and 3) or nucleolin domains (lanes 4 to 7) were subjected to SDS-PAGE. As negative control for the nucleolin-Hdm2 interactions, GST alone (lane 8) was used. GST-tagged p53 (lane 1) served as a positive control. The separated proteins were then electrotransferred to a nitrocellulose membrane for Far-Western analysis. The membrane was probed with purified Hdm2 protein (0.2 µg/ml), as described in Materials and Methods, and the bound Hdm2 detected by a monoclonal anti-Hdm2 antibody (SMP14). This figure, along with Fig. 2, indicates that both cleaved p53 and GST-

tagged p53 associate with Hdm2 in our Far-Western analyses. **Lower panel,** Equivalent loading of the GST-nucleolin fusion proteins was confirmed by running a parallel blot and probing directly with anti-GST antibodies. The multiple lower molecule bands indicate degraded protein fragments. These data indicate that both the nucleolin NT and RBD domains can independently bind Hdm2.

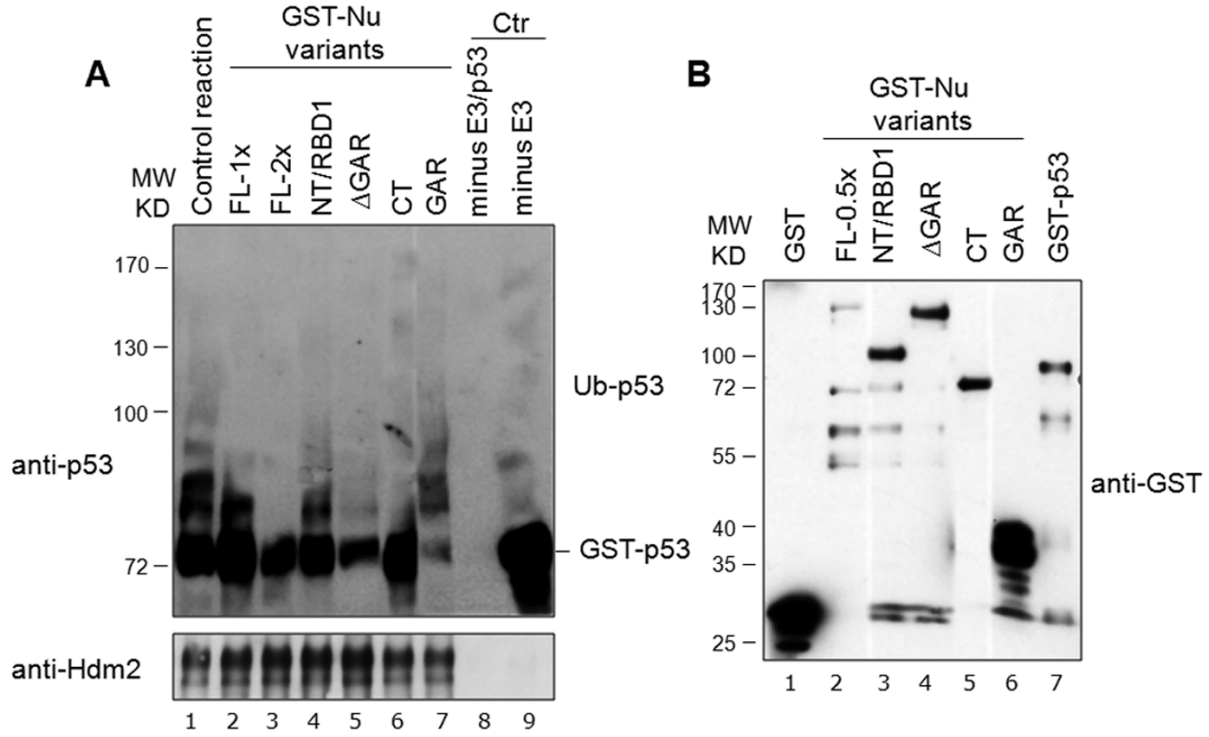

Fig. S2

Fig. S2, p53 ubiquitination using GST-nucleolin domains in vitro. The control reaction contained all the required components for ubiquitination in vitro reactions, including Hdm2-FL as the E3-ligase (lane 1) indicates p53-polyubiquitinated products. Upon increasing amounts of GST-nucleolin FL (lanes 2 and 3; 1x= 200ng), there is a clear decrease in ubiquitinated p53. There is no significant difference in polyubiquitinated-p53 with nucleolin-NT (compare lane 4 vs. 2). The constructs lacking GAR domain (ΔGAR) as well as the CT domain (that contained all

the RBDs and GAR) show less polyubiquitinated-p53 (lane 5, 6) while the presence of GAR-domain leads to a shift to more polyubiquitinated-p53 (lane 7). Controls without p53 and Hdm2 (lane 8) as well as only p53 control (lane 9) are also shown. These data indicates that nucleolin-variants that contained the RBDs or lacked GAR inhibit p53-polyubiquitination. The outcome is unchanged when nucleolin is expressed and purified by multiple approaches (from yeast as GST-tagged or from cells as GFP-tagged, Fig. 7B).

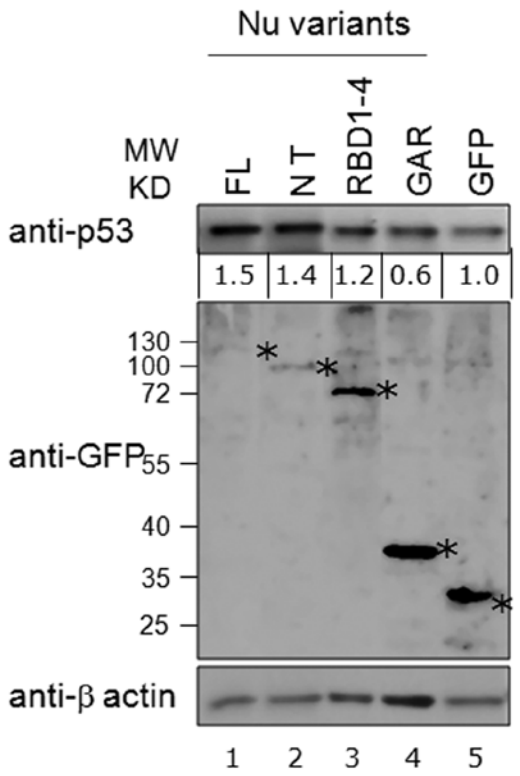

Fig. S3

S3, Effects of nucleolin domain expression on p53 levels. Constructs expressing various GFP-tagged nucleolin domains were transfected into SJSA cells. Post-transfection (36 h), endogenous p53 was detected by Western using an anti-p53 (DO-1) antibody, while the expression level of each nucleolin domain was detected using anti-GFP. β-actin served as a loading control. The level of p53 protein (denoted below the p53 Western blot) was normalized to β-actin expression, after quantitation with Image J software (NIH). Note that when the nucleolin expression is lower, the fold increase in p53 protein level is also reduced.