

Figure S1 Lingjun Meng

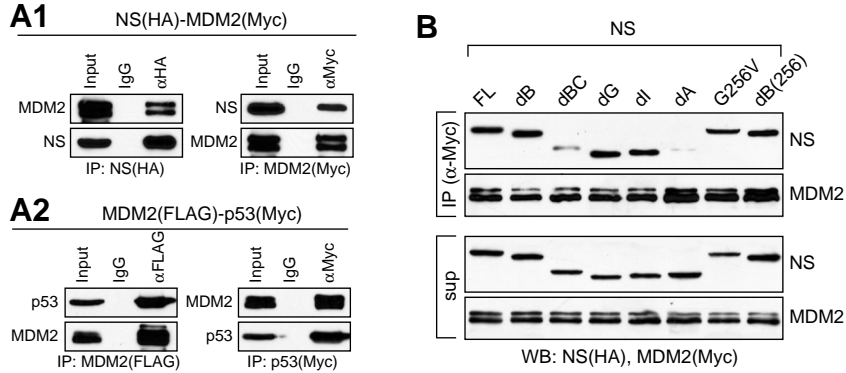


Figure S1. Coimmunoprecipitation of MDM2 and NS, MDM2 and p53, and MDM2 and NS mutants.

HEK293 cells were double-transfected with HA-tagged NS and Myc-tagged MDM2 (**A1**) or with Myc-tagged p53 and FLAG-tagged MDM2 (**A2**). Protein complexes were immunoprecipitated (IP) and western detected by anti-tag antibodies. Judged by their amounts relative to the input and compared to the triple-coIP results (Fig. 1A), we concluded that p53 coexpression did not affect the MDM2-NS binding, nor did NS coexpression affect the interaction between MDM2 and p53. (**B**) To confirm the MDM2-interactive domains of NS, CoIP assays were performed by immunoprecipitating the MDM2 proteins (anti-Myc) and western detecting the copurified NS mutants (anti-HA). Our results again confirmed the importance of the A and BC-domains of NS in MDM2 binding.

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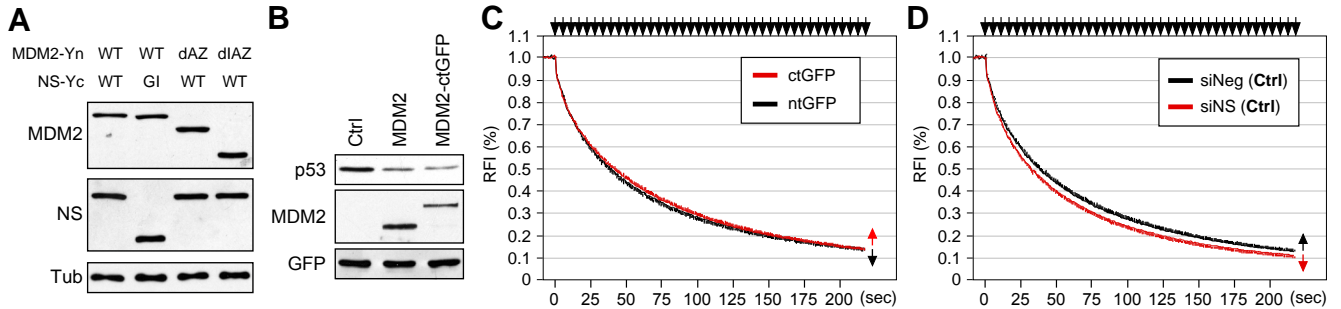


Figure S2. Control experiments for BiFC and FLIP assays.

(A) Western blots confirmed that wild-type (WT) and mutant MDM2-Yn and NS-Yc proteins were expressed at the same level in each BiFC sample, therefore excluding the possibility that the observed differences were caused by different expression levels of the fusion proteins. (B) When coexpressed with p53, the C-terminal MDM2-GFP fusion was able to reduce the p53 protein as did the wild-type MDM2. GFP proteins were coexpressed as internal controls. (C) FLIP experiments were designed where a 2- μ m-diameter circle in the nucleolus was repeatedly bleached and the loss of fluorescence signal in the nucleoplasm was monitored over time. Both the N- (ntGFP) and C-terminally GFP-fused MDM2 proteins (ctGFP) exhibited the same FLIP rate ($p = 0.95$ by Repeated Measures ANOVA, $n = 20$). Error bars (s.e.m.) were shown on one side of the curve for clarity (indicated by arrows). Top arrows represent bleaching pulses. (D) The role of NS in regulating the dynamic distribution of MDM2 was examined by knockdown experiments. Knocking down the endogenous expression of NS (siNS) in normal growing interphase cells did not significantly affect the nucleoplasmic retention of MDM2 (red lines, $p = 0.97$, $n = 30$).

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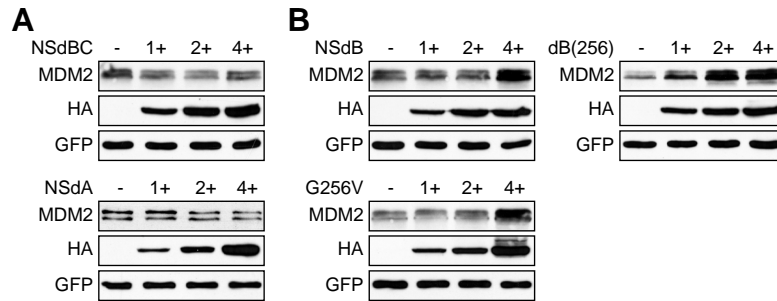


Figure S3. The NS's ability to increase MDM2 protein requires its BC and A-domains, but does not depend on its nucleolar localization or GTP binding.

(A) Overexpression of the non-MDM2-binding NSdBC and NSdA mutants failed to increase the protein level of exogenously expressed MDM2 in H1299 cells. (B) Coexpression of the NSdB deletion mutant, the G256V point-mutation mutant, and a double mutant of dB and G256V (dB(256)) increased MDM2 protein in a dose-dependent manner.

Figure S4 Lingjun Meng

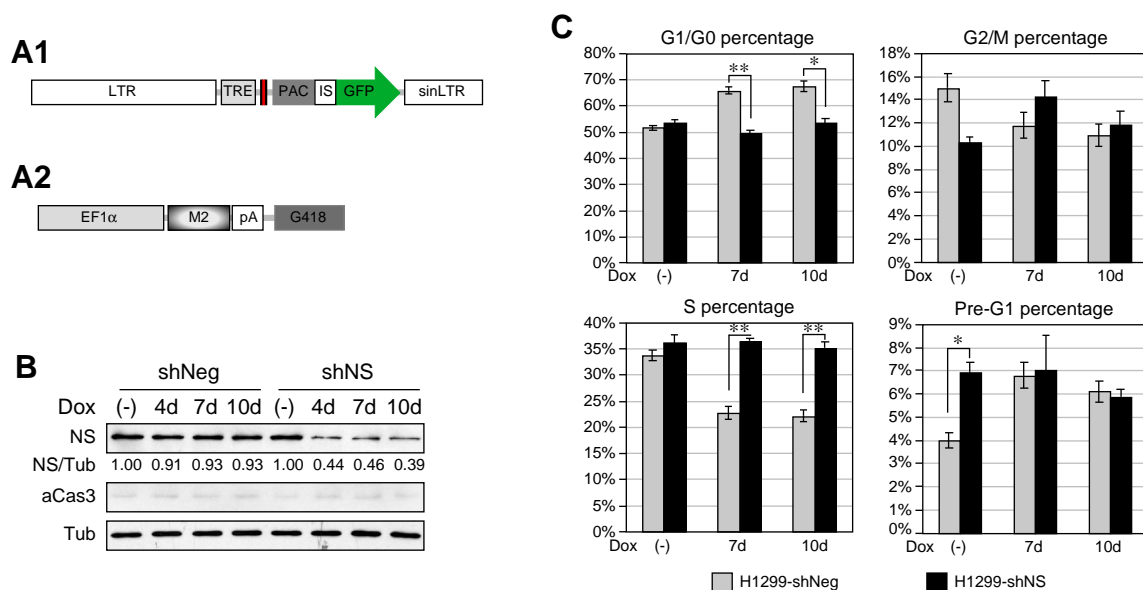


Figure S4. Generation of U2OS and H1299 cell models with inducible NS-knockdown capabilities.

(A1) An inducible version of shNS-2 (red box) was created in the TMP vector for making stable cell lines with Dox-inducible NS-knockdown capabilities. The TRE promoter contains seven copies of the tetracycline operator and a CMV minimal promoter. An internal ribosomal entry site (IS)-green fluorescent protein (GFP) cassette was engineered to facilitate transgene detection. (A2) A second transgene contains a reverse Tet-transactivator (rtTA-M2 or M2) expressed from the EF1 α promoter and a G418-selection cassette. Together, these two transgenes allow NS knockdown in a Dox-inducible manner. (B) H1299 cells were sequentially transfected with the EF1 α promoter-driven M2 construct and the TMP-shNeg or TMP-shNS-2 vector. Clones were selected and maintained by G418 for the EF1 α ::M2 construct and by puromycin for the TMP-based plasmids. After Dox treatment for 4d, 7d, and 10d, the H1299-shNS cell model displays a 56%, 54%, and 41% knockdown efficiency of NS proteins, respectively. (C) Cell-cycle profile analyses showed no significant differences in the pre-G1 and G2/M percentages between the control and NS-knockdown H1299 cells after 7 and 10d-Dox treatment. A Dox-dependent G1-phase increase and S-phase decrease was seen in the H1299-shNeg cells.