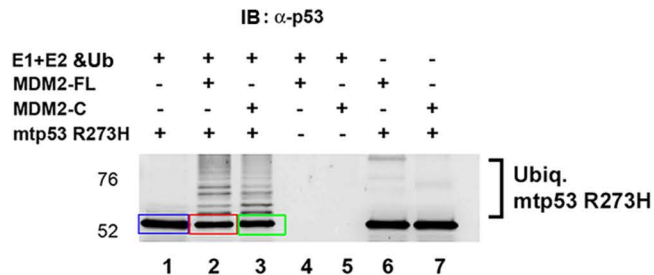
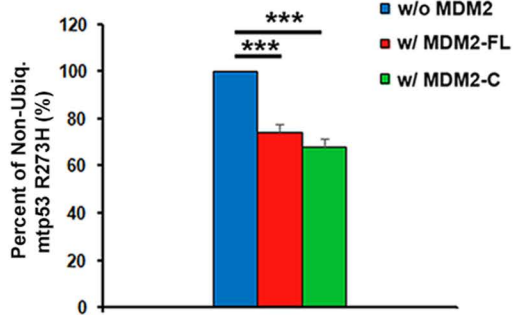


Supplementary Figure 2

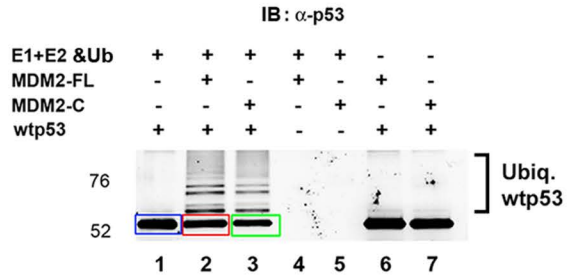
A



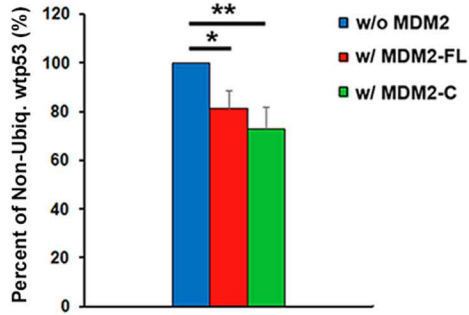
B



C



D



Supplementary Figure S1. Absence of MDM2 protein in *E. coli* cell lysate

(A) 2µg of bacterially purified MDM2-FL and MDM2-C, lanes 2 and 3, respectively, were loaded into 10% SDS-PAGE gel. Equal volume of untransformed *E. coli* lysate was loaded into lane 1. MDM2 was detected using 4B11 MDM2 antibody recognizing both MDM2 isoforms.

(B) Duplicate SDS-PAGE gel ran as described in (A). Proteins were stained by Coomassie Staining to verify purification. Arrows indicate the bands for MDM2-FL and MDM2-C.

Supplementary Figure S2. Total p53 protein levels decreases with MDM2-C ubiquitination

(A) *In vitro* ubiquitination assay was performed with 2 µL of baculovirus-expressed mtp53 R273H in insect cells and 1µg of recombinant MDM2-FL and MDM2-C translated in *E. coli* system. Samples were run on a 4-12% SDS-PAGE gradient gel, and the proteins were transferred to PVDF membrane. Mtp53 was probed by western blot using Typhoon to scan fluorescence on Cy-3 antibody (anti-mouse). Red rectangle represents non-ubiquitinated mtp53 in the presence of MDM2-FL, and green rectangle represents non-ubiquitinated mtp53 in the presence of MDM2-C. Blue rectangle represents non-ubiquitinated mtp53 in the absence of MDM2. For graph construction, rectangular bands from lanes 2 and 3 were normalized to rectangular band from lane 1.

(B) Bar graph shows the percent of non-ubiquitinated mtp53 R273H from Fig 2A comparing non-ubiquitinated mtp53 in the presence of MDM2-FL or MDM2-C (red bar and green bar from rectangular bands in lane 2 & 3, respectively) to non-ubiquitinated mtp53 in the absence of MDM2 (blue bar from rectangular band in lane 1). Student T-test was performed for statistical significance with the following p-value representation: * = p-value ≤ 0.05, ** = p-value ≤ 0.01, *** = p-value ≤ 0.005.

(C) *In vitro* ubiquitination assay with 2 µL of baculovirus-expressed wtp53 in insect cells was performed as described in (A). Wtp53 ubiquitination was probed based on description from (A). Red rectangle represents non-ubiquitinated wtp53 in the presence of MDM2-FL, and green rectangle represents non-ubiquitinated wtp53 in the presence of MDM2-C. Blue rectangle represents non-ubiquitinated wtp53 in the absence of MDM2. For graph construction, rectangular bands from lanes 2 and 3 were normalized to rectangular band from lane 1.

(D) Bar graph shows the percent of non-ubiquitinated mtp53 R273H from Fig 2C comparing non-ubiquitinated wtp53 in the presence of MDM2-FL or MDM2-C (red bar and green bar from rectangular bands in lane 2 & 3, respectively) to non-ubiquitinated wtp53 in the absence of MDM2 (blue bar from rectangular band in lane 1). Student T-test was performed for statistical significance with the following p-value representation: * = p-value ≤ 0.05, ** = p-value ≤ 0.01, *** = p-value ≤ 0.005.