Supplementary Figure Legends

Supplementary Figure S1. The effects of RASSF1C on cell-cycle distribution and p53 activity

(**A**) HCT116 cells ($p53^{+/+}$, $p53^{-/-}$ and $p53^{-/-}$ complemented with HA-p53) were transfected with pcDNA-CD4 and HA-RASSF1C. The cell-cycle profile of CD4-positive cells was determined 36 h after transfection. Data are expressed as means ± standard errors of values from three independent experiments. (**B)** Lysates of cells in (**A**) were analyzed by immunoblotting for the indicated proteins. (**C**) U2OS cells were co-transfected with pGL3-p53-responsive reporter and pcDNA3-β-Gal reporter constructs and the indicated combinations of pcDNA3-HA-p53 and HA-RASSF1A or HA-RASSF1C. Cell lysates were subjected to immunoblot and luciferase assays. Luciferase activity was normalized to β-galactosidase activity and expressed relative to that in cells transfected with the empty vector. Data are expressed as means ± standard errors of values from three independent experiments. Asterisks indicate nonspecific bands.

Supplementary Figure S2. The effects of RASSF1C on p53 stability

(**A**) U2OS cells transfected with HA-RASSF1C were incubated for the indicated times with cycloheximide (50 μg/ml) and analyzed by immunoblotting. (**B**) U2OS cells transfected with His_6-tagged ubiquitin were co-transfected with a combination of Flag-MDM2, HA-p53, and HA-RASSF1C, as indicated. Cells were treated with MG132 (20 μ M) for 6 h prior to harvesting. The His_6-purified fractions were analyzed for ubiquitinated p53. Molecular weights are in kDa.

Supplementary Figure S3. Regulation of p53 stability by RASSF1A and interaction of RASSF1A with MDM2

(**A**) *Mdm2*$^{+/+}$*p53*$^{-/-}$ and *Mdm2*$^{-/-}$*p53*$^{-/-}$ MEFs transiently transfected with HA-p53 and HA-RASSF1A, or the corresponding empty vector (control), were incubated for the indicated times with cycloheximide (50 μg/ml) and then analyzed by immunoblotting for the indicated proteins. (**B**) RASSF1A directly interacts with MDM2 but not with p53 *in vitro*. RASSF1A, MDM2, and p53 proteins were *in vitro* translated (IVT) in the presence of [^{35}S] methionine and mixed. Cell lysates were immunoprecipitated with anti-RASSF1A and analyzed by SDS-PAGE and autoradiography. (**C**) *Mdm2*$^{-/-}$*p53*$^{-/-}$ MEFs transiently transfected with the indicated expression plasmids were immunoprecipitated with an anti-RASSF1A antibody and analyzed by immunoblotting.

Supplementary Figure S4. Interaction of RASSF1A with MDM2 and DAXX in the nucleus

(**A**) 0.2% (w/v) Triton X-100-permeabilized U2OS cells were fixed and stained with anti-RASSF1A (*green*) or anti-MDM2 (*red*) antibodies. Scale bar, 10 μm. (**B**) U2OS cell lysates were fractionated into nuclear and cytoplasmic fractions and analyzed by immunoblotting for the indicated proteins. β-tubulin and HDAC1 were used as cytoplasmic and nuclear markers, respectively (*left*). Both nuclear and cytoplasmic extracts were immunoprecipitated (IP) with an anti-RASSF1A antibody and the resulting precipitates were analyzed by immunoblotting for the indicated proteins (*right*). Asterisks indicate nonspecific bands. (**C**) 0.2% (w/v) Triton X-100-permeabilized U2OS cells were fixed and stained with anti-RASSF1A (*green*) or anti-DAXX (*red*) antibodies. Scale bar, 10 μm.

Supplementary Figure S5. Effects of the N-terminal region of RASSF1A on MDM2 and p53 stability. (A) *Mdm2*$^{-/-}$*p53*$^{-/-}$ MEFs were transfected with MDM2 and the cell lysates were analyzed by immunoblotting for the indicated proteins. Fold induction of Rassf1a level was calculated using the densities of Rassf1a/Actin. **(B)** U2OS cells were co-transfected with expression vectors for HA-p53, pCMV-MDM2, or HA-RASSF1A-N, as indicated. The cell lysates were analyzed by immunoblotting for the indicated proteins. **(C)** U2OS cells were co-transfected with expression vectors for HA-p53, pCMV-MDM2, or HA-RASSF1A-C, as indicated. The cell lysates were analyzed by immunoblotting for the indicated proteins.

Supplementary Figure S6. RASSF1A disrupts MDM-DAXX-HAUSP interactions *in vivo*. *Mdm2*$^{-/-}$*p53*$^{-/-}$ MEFs co-transfected with MDM2 and Flag-DAXX or Myc-HAUSP and increasing amounts of HA-RASSF1A were treated with MG132 for 6 h and Cell lysates were analyzed by immunoblotting as in **Figure 6A**.

Supplementary Figure S7. Effects of RASSF1C on the MDM2-DAXX-HAUSP interactions *in vivo*

(A, B, C) *Mdm2*$^{-/-}$*p53*$^{-/-}$ MEFs transfected with MDM2, Flag-DAXX, Myc-HAUSP or HA-RASSF1C as indiacted were treated with MG132 for 6 h and immunoprecipitated (IP) with anti-MDM2 or anti-Flag antibodies. The resulting precipitates were analyzed by immunoblotting.

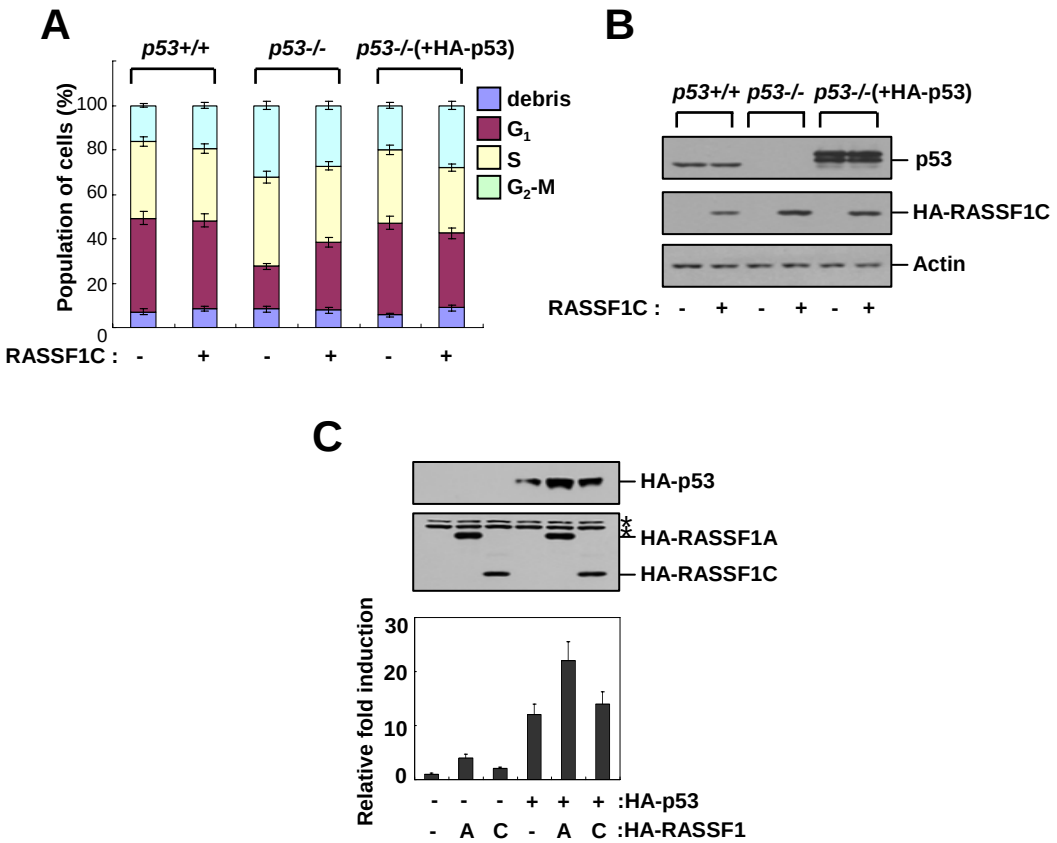
Supplementary Figure S8. Inhibition of HAUSP-mediated MDM2 deubiquitination by RASSF1A

(**A**) U2OS cells transfected with Myc-HAUSP with or without HA-RASSF1A were incubated for the indicated times with cycloheximide (50 μg/ml) and analyzed by immunoblotting. (**B**) U2OS cells co-transfected with His_6-tagged ubiquitin, Myc-HAUSP, and HA-RASSF1A were treated with MG132 (20 μM) for 6 h and then harvested. The His_6-purified fractions were analyzed for ubiquitinated MDM2. Molecular weights are in kDa.

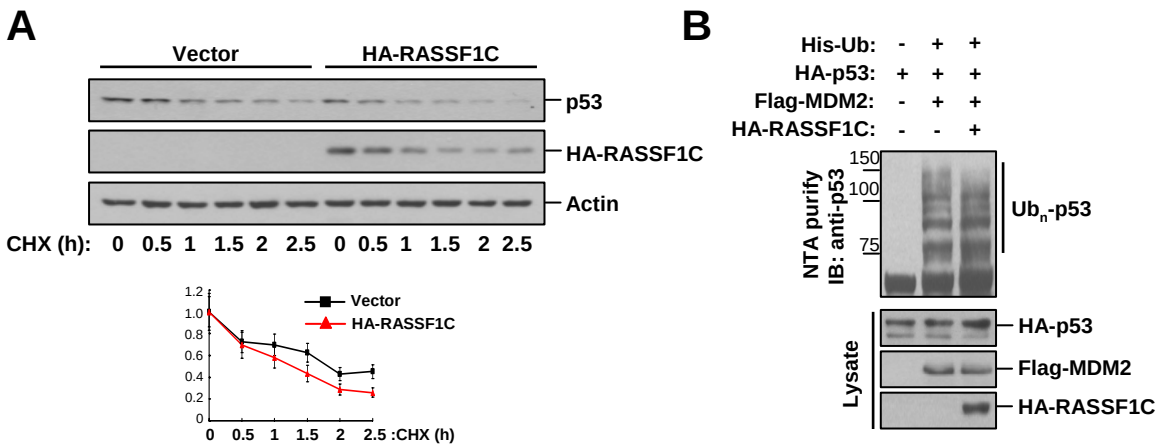
Supplementary Figure S9. RASSF1A promotes p53 activation by decreasing MDM2 levels upon γ-irradiation treatment.

U2OS cells stably expressing pcDNA4/TO-HA-RASSF1A were incubated without (mock-treated) or with tetracycline (1 μM) for 24 h to induce RASSF1A expression. The cells were subsequently exposed to 10 Gy of γ-irradiation, incubated for the indicated times, and then analyzed by immunoblotting for the indicated proteins.

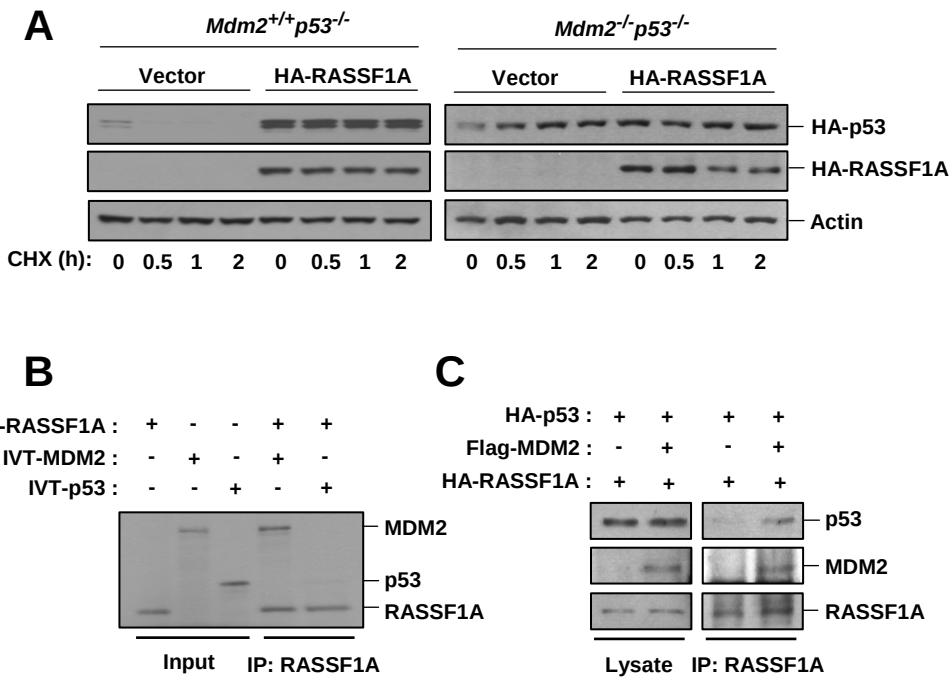
Supplementary Figure S1



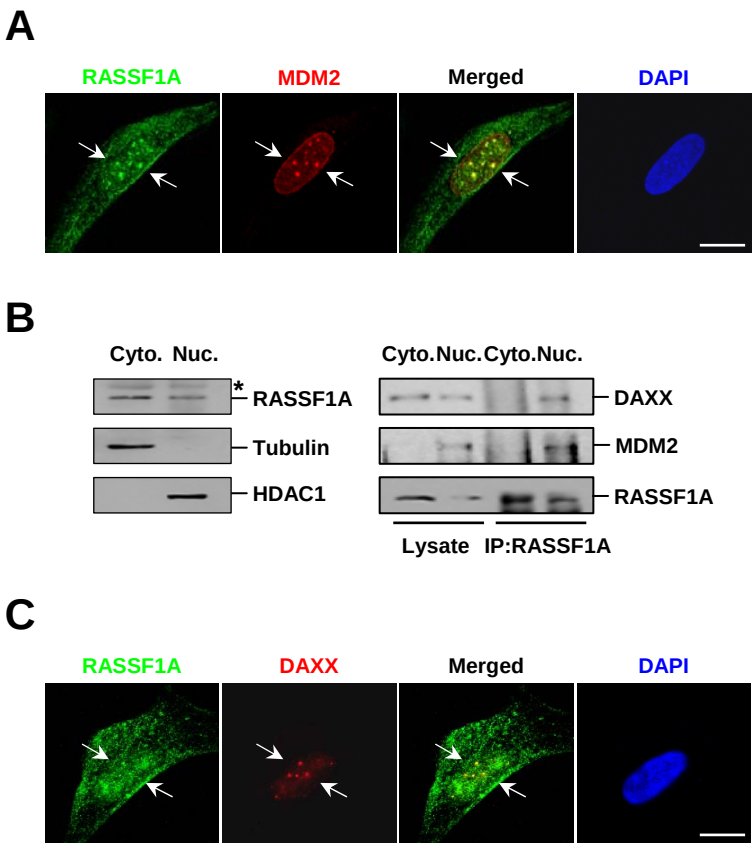
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4



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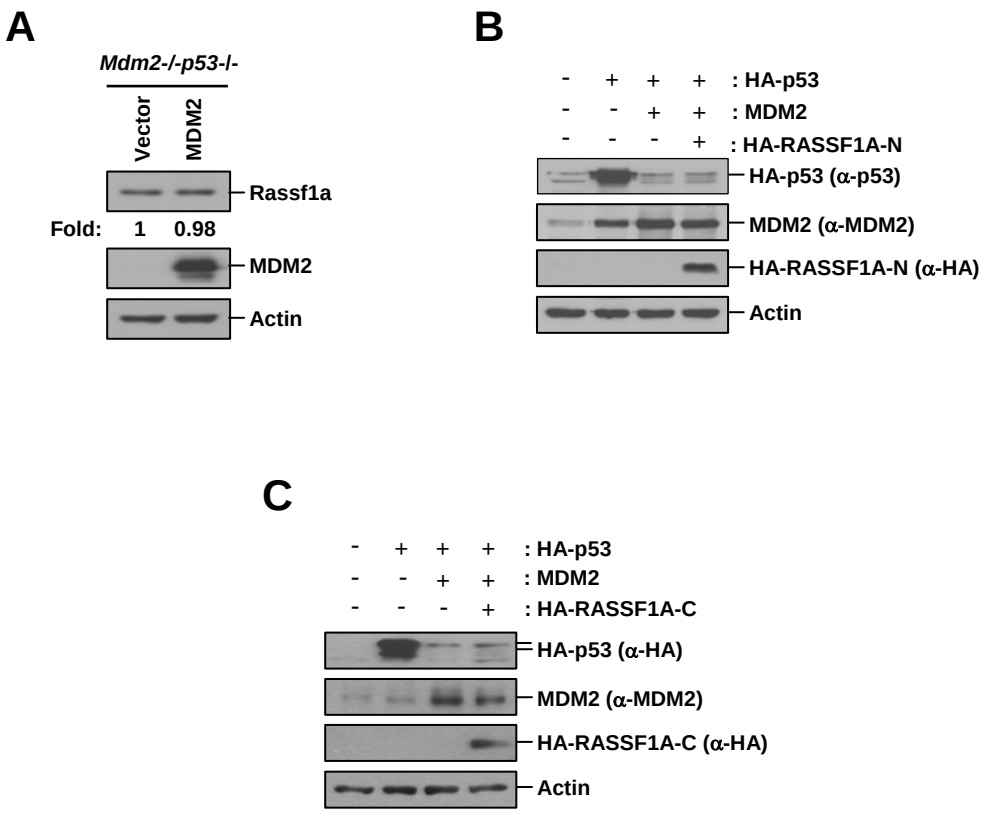

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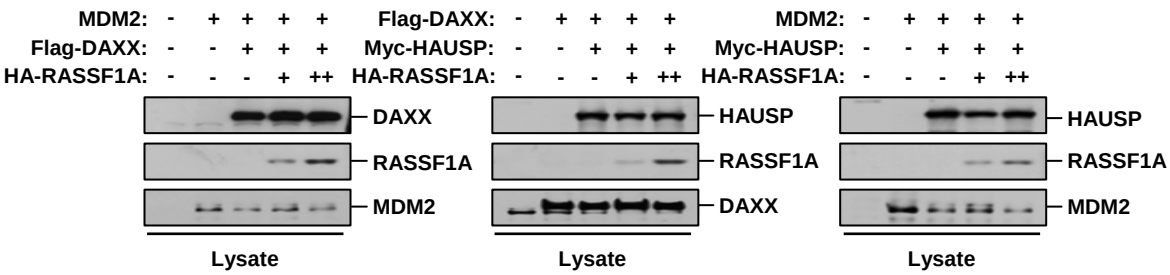
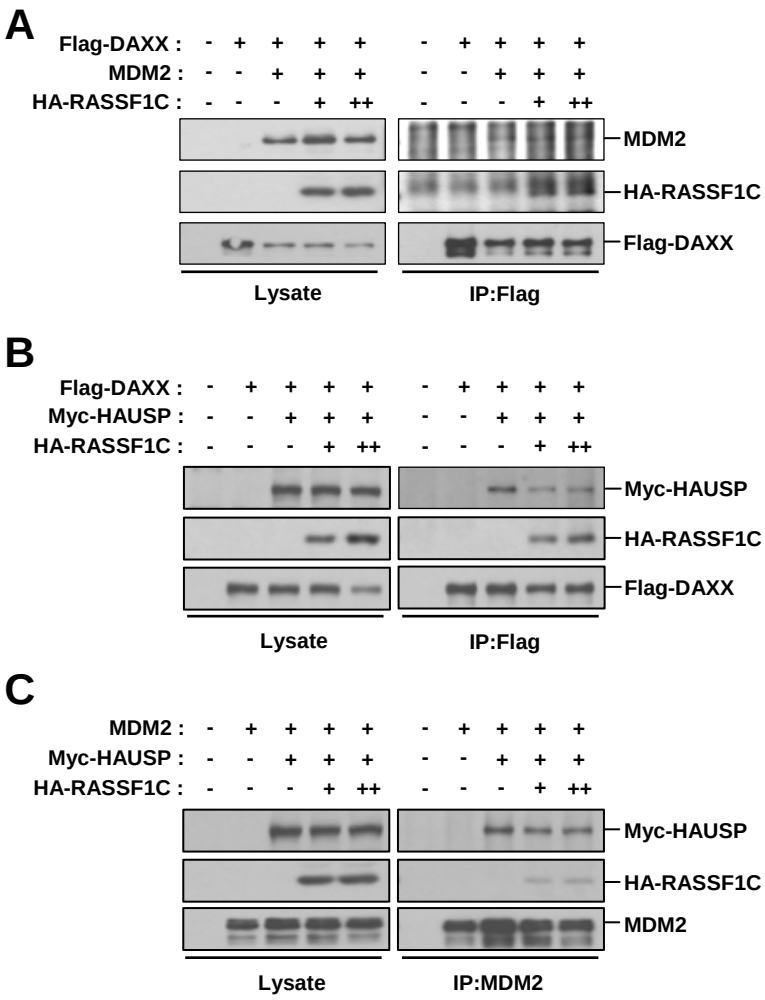
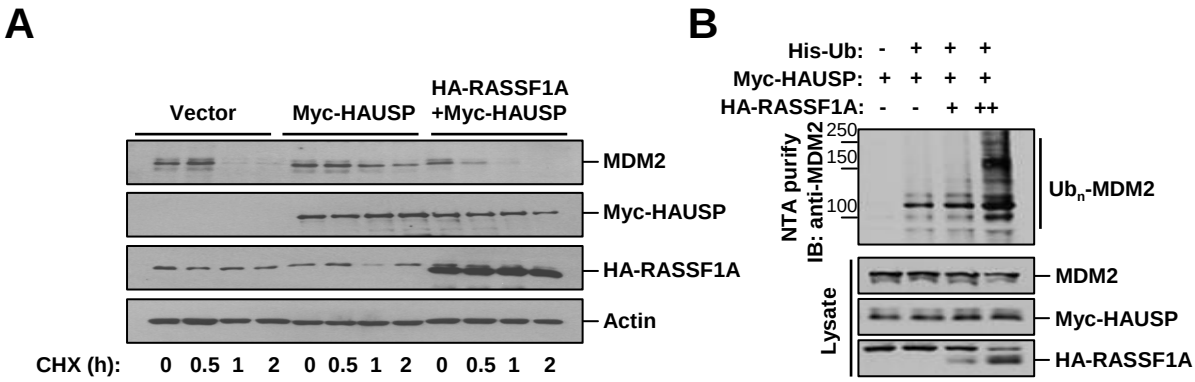


Supplementary Figure S7



Supplementary Figure S8



Supplementary Figure S9