
The Supplementary Information (SI)

Methods

Cell culture and transfections

H1299, U2OS, 293, HeLa cells were maintained in DMEM medium supplemented with 10% fetal bovine serum. H1299 and 293 cells were transfected with the plasmid DNA using the calcium phosphate protocol. U2OS cells were transfected with siRNA duplexes by Lipofectamine2000 (Invitrogen) according to the manufacturer's protocol.

In Vitro Deacetylation Assays

In Vitro Deacetylation Assays were performed as previously described. Purified acetylated p53 was incubated with purified Sirt1 and DBC-1 as indicated at 30°C for 1 hour in the presence of 50 µM NAD. Reactions were performed in a buffer containing 50 mM TrisHCl (pH 9.0), 50mM NaCl, 4 mM MgCl₂, 0.5 mM DTT, 0.2 mM PMSF, 0.02% NP-40, and 5% glycerol. Nicotinamide (Sigma) was added to 5mM as indicated to inhibit all deacetylase activity of the Sirtuins. The reactions were resolved on SDS-PAGE and analyzed by Western blot using antibodies specific for acetylated p53, total p53 (DO-1, sc-126, Santa Cruz), Sir2-CT and DBC-1 (Bethyl, BL1924).

Immunoprecipitation and Immunoblot

Cells were transfected with Flag-tagged expression constructs for p53, Sirt1 and DBC1 using the Calcium Phosphate Method as previously described. To immunoprecipitate the ectopically expressed FLAG-tagged proteins, transfected cells were lysed 24 hours post transfection in Flag-lysis buffer (50 mM Tris-HCl pH 7.9, 137 mM NaCl, 10 mM NaF, 1mM EDTA, 1% Triton X-100, 0.2% Sarkosyl, 10% glycerol, and fresh proteinase inhibitor cocktail(SIGMA)) or for high stringency in BC500 (20 mM Tris pH7.9, 500 mM NaCl, 10% glycerol, 0.2 mM EDTA, 0.5% Triton X-100, and fresh proteinase inhibitor cocktail). The whole cell extracts were immunoprecipitated with the monoclonal anti-Flag antibody-conjugated M2 agarose beads (Sigma) at 4°C overnight. After three washes with either BC500 or Flag-lysis buffer, followed by two washes with BC100 (20 mM Tris pH7.9, 100 mM NaCl, 10% glycerol, 0.2 mM EDTA, 0.1% Triton X-100), the bound proteins are eluted using Flag-Peptide (Sigma)/BC100 for 3 hours at 4°C. The eluted material was resolved by SDS-PAGE and detected by antibodies as indicated. The endogenous Sirt1 complex was purified from HeLa cells using the Sirt1-CT specific antibody for immunoprecipitation. Interacting proteins were identified using Mass-Spectrometry. For analysis of the Sirt1 complex, 50µl of M2-eluted F-Sirt1 containing approximately 12.5µg of total purified F-Sirt1 were fractionated by size exclusion chromatography on a Sepherose 12 Column on the SMART System (GE Healthcare) according to manufacturer's protocol. To coimmunoprecipitate Sirt1 and DBC1 using specific antibodies, cells were lysed in BC100 and cell lysates were first incubated with 20ul protein G agarose beads for 2 hours with gentle rotation. The cleared supernatants were incubated with DBC1 or Sirt1 specific antibodies overnight before addition of 20ul of protein G agarose beads for 4 hours. After four washes with the lysis buffer, the immunoprecipitated materials were eluted with the SDS sample buffer with boiling, resolved by SDS-PAGE and detected with antibodies as indicated.

GST-Pull-down

pCIN4-DBC1 or pCIN4-Sirt1 were labeled by incorporation of ³⁵S-Methionine during in vitro translation (TNT Coupled Reticulocyte Lysate System, Promega Corporation). 5ul of ³⁵S-labeled protein was incubated with 3µg of the purified GST protein fragments as indicated in the presence of 0.2% BSA in BC100 on a rotator overnight at 4°C. The proteins were

pulled down using GST beads and the beads were washed five times with BC100 before elution with 50ul of BC100 plus 20mM reduced glutathione for 2 hours with gentle rotation. Eluted materials were resolved on SDS-PAGE and the presence of ³⁵S-labeled protein was detected by autoradiography and the levels of the GST proteins by Coomassie stain.

siRNA-mediated ablation of DBC1, Sirt1 and p53

The ablation of DBC1 was performed by transfection of the U2OS cells with either of two siRNA duplex oligos (DBC1-RNAi#1: 5' CAGCGGGUCUUCACUGGUAUU 3' or DBC1-RNAi#2: 5' CUACUGAGCCUUCUGAAUU 3' (synthesized by Dharmacon)), which covered mRNA regions 582-602nt (55-61aa) and 2097-2115nt (560-565aa) of DBC1 respectively, by using Lipofectamine2000 according to the manufacturer's protocol. Sirt1 RNAi (SiGenome Smartpool M-003540-01 (Dharmacon)), p53 RNAi (SiGenome Smartpool M-003329-01-0010 (Dharmacon)) and Control RNAi (On_target plus siControl non_targeting pool D-001810-10-20 (Dharmacon)) were used and transfected according to the manufacturers guidelines.

Luciferase reporter gene assay

H1299 cells were transfected at 70% confluence in 6-well plates with plasmid DNA as indicated in the relevant figures. After 24 hours of incubation, cells were then harvested and the luciferase activity was measured using the Dual Luciferase Reporter Assay System Kit from Promega according the manufacturer's protocol. Activity was assayed in three separate experiments and shown as the average mean \pm standard deviation (s.d.)

In vitro acetylation assay.

In vitro acetylation assays were performed as described previously (Gu et al., 1997, Cell 90: 595-606). Recombinant GST-p53 was incubated with Flag-purified Sirt1 and DBC1 as indicated in the presence of ¹⁴C-labelled Acetyl-CoA in reaction buffer containing 50mM HEPES (pH8.0), 10% glycerol, 1mM DTT, 1mM PMSF and 10mM sodium butyrate. Reactions were incubated at 30°C for 1 hour and resolved on SDS-PAGE. Protein levels were detected by Coomassie-Blue staining and in vitro acetylation was detected by autoradiography.

Immunofluorescent Staining

Cells were fixed with 4% paraformaldehyde for 20 min on ice, rehydrated for 5 min in serum-free DMEM, and permeabilized with 0.2% Triton X-100 (Fisher) for 10 min on ice. Cells were incubated in 1% bovine serum albumin (BSA) (Sigma)/phosphate buffered salt solution (PBS) (Cellgro) for 30 min. Primary antibodies (as indicated) were added in 1% BSA/PBS for 45 min at room temperature. After washing with 1% BSA/PBS, secondary antibodies were added and incubated for 30 min at room temperature. Finally, cells were counterstained with DAPI to visualize the nuclei essentially as described before.

Annexin V-FITC Staining

The apoptosis assay was performed using the BD-Bioscience Annexin V-FITC staining kit according to the manufacturer's protocol. After siRNA mediated knockdown, U2OS cells were treated with Etoposide (30 hours) before fixing and TUNEL staining or Annexin V assays. Apoptosis observed in TUNEL and Annexin V assays were quantified for three separate experiments and presented as the average mean \pm standard deviation (s.d.).

Supplementary Figure Legends

S1. Identification of DBC1 as a key component of the Sirt1 complexes in human cells. (a) Colloidal-blue staining of affinity-purified Sirt1 complexes from HeLa extracts with the α -Sirt1 antibody column (lane 2) and a control elute from the same extract with a control antibody column (lane 3). Specific Sirt1 interacting proteins were analyzed by mass spectrometry. (b) Schematic representation of the DBC1 protein.

S2. Endogenous Sirt1 is coimmunoprecipitated by DBC1. Coimmunoprecipitation of Sirt1 with DBC1 from U2OS cells. Western Blot analysis of whole-cell extract (input, lane 1) and immunoprecipitates with a DBC1-specific antibody (lane 3) or control IgG (lane 2) or mock IP with DBC1 antibody alone (lane 4) by anti DBC1 antibody (lower panel) and Sirt1 (upper panel).

S3. Sirt1 interacts with DBC1 *in vitro*. Full-length DBC1 (lane 3) as well as GST-NT-DBC1 (lane 4), GST-M-DBC1 (lane 5), GST-CT-DBC1 (lane 6) and GST alone were used in a GST pull-down assay with *in vitro* translated ^{35}S -labelled Sirt1. ^{35}S -Sirt1 was detected by autoradiography (upper panel), while the GST-proteins were detected by Coomassie-Stain (lower panel).

S4. Effect of DBC1 on HDAC1 mediated p53 deacetylation *in vitro*. Purified HDAC1 complex was used to deacetylate p53 *in vitro* (lane 2). Increasing amounts of DBC1 were added in addition to HDAC1 (lanes 3-4) to investigate whether DBC1 can inhibit p53 deacetylation by HDAC1. The samples were resolved by SDS-PAGE and analyzed by Western Blot using antibodies for acetylated p53, total p53 (DO-1), HDAC1 and DBC1.

S5. DBC1 inhibits deacetylation of p53 *in vivo*. H1299 cells were transfected with expression vectors for Flag-p53, Sirt1 and DBC1 as indicated. Flag-p53 was immunoprecipitated and whole cell lysates (lower four panels) and immunoprecipitates (top panel) were resolved by SDS-PAGE and analyzed by Western Blot using antibodies as indicated.

S6. siRNA-mediated ablation of the endogenous DBC1 protein. Western Blot analysis of the whole cell extracts of U2OS cells treated with either DBC1 siRNA or control siRNA using antibodies against DBC1, p53 (DO-1), PUMA, p21, and actin (upper 5 panels). Western blot analysis of the immunoprecipitates by anti-acetylated p53 antibody and normal IgG from U2OS cells using anti-p53 (DO-1) antibody (lower panel).

S7. Effect of DBC1 on p300 mediated p53 acetylation *in vitro*. Purified GST-p53 was used as a substrate for acetylation by p300 *in vitro* (lane 2). Increasing amounts of purified DBC1 were added (lanes 3-5) to the reactions. The reactions were resolved by SDS-PAGE and acetylated GST-p53 and self-acetylated F-p300 were visualized by autoradiation (labeling by ^{14}C -labeled Ac-CoA) (upper panel). All protein levels were visualized by Coomassie Staining (lower panel).

S8. p300 mediated acetylation of p53 and DBC1 *in vivo*. H1299 cells were transfected with expression vectors for either Flag-p53 or F-DBC1 with or without F-p300 as indicated. Flag-tagged proteins were M2-immunoprecipitated, resolved by SDS-PAGE and analyzed by Western Blot using an acetylated-Lysine antibody (upper panel), or Flag Antibody (middle and lower panel).

S9. Interaction of DBC1 with Sirt1 or HATs *in vivo*. H1299 cells were transfected with expression vectors for Flag-Sirt1 (lane 1), Flag-MOF (lane 2), Flag-HA-TIP60 (lane 3) and Flag-p300 (lane 4) and control vector (lane 5). The tagged proteins were M2-immunoprecipitated and inputs and immunoprecipitates were resolved by SDS-PAGE and analyzed by Western Blot using DBC1 specific antibody for the input (upper panel), DBC1 after immunoprecipitation (middle panel) and Flag-Antibody (bottom panel).

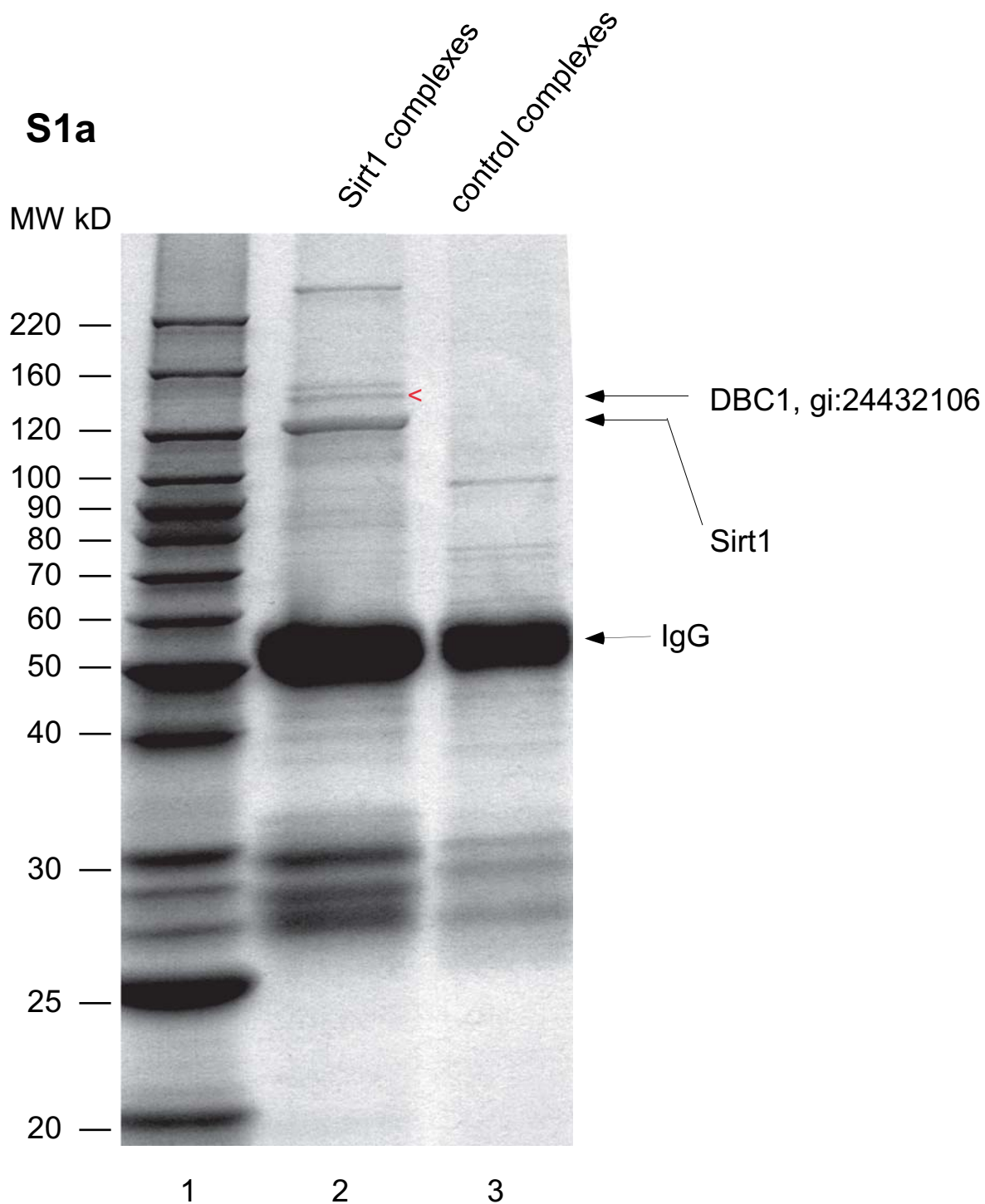
S10. Effect of DNA-Damage on sub-cellular localization of Sirt1 and DBC1. (S10A) The localization of DBC1 is not regulated upon DNA damage. A stable cell line of H1299 cells expressing F-HA-Sirt1 was used to analyze the sub-cellular localization of endogenous DBC1 (red) and Sirt1 (green) either untreated (upper row) or after treatment with either Actinomycin D (middle Panel) or Etoposide (lower Panel) for 6 hours. In all cases DBC1 and Sirt1 co-localize to the nucleus and are not affected by the treatment. (S10B) No truncation forms of DBC1 are induced upon DNA damage. Western blot analysis of endogenous DBC1 from U2OS cells harvested at different time points after treated with Etoposide.

S11. DBC1 inactivation abrogates p53-mediated apoptosis. 72 hours after transfection with DBC1 or control siRNA duplexes, U2OS cells were treated with either the control dissolvent DMSO or the DNA damaging reagent Etoposide (20 μ M) for 30 hours. The cells were fixed and stained by the TUNEL assay for apoptosis (Green). Nuclei were visualized by DAPI staining (Blue).

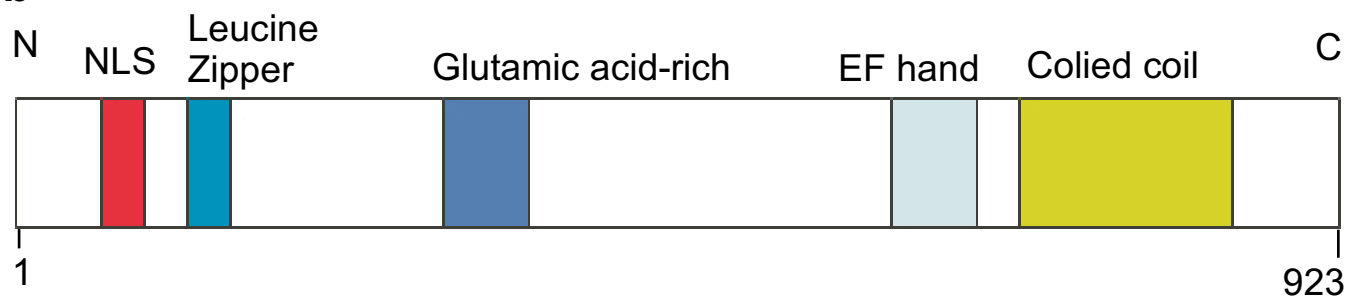
S12. DBC1 inactivation abrogates p53-mediated apoptosis through Sirt1. U2OS cells were treated with either control siRNA (I), DBC1 siRNA alone (II) or in combination with siRNA for Sirt1 (III) or p53 (IV). After transfection the cells were treated as indicated and apoptosis was quantitated by annexin V staining followed by FacScan.

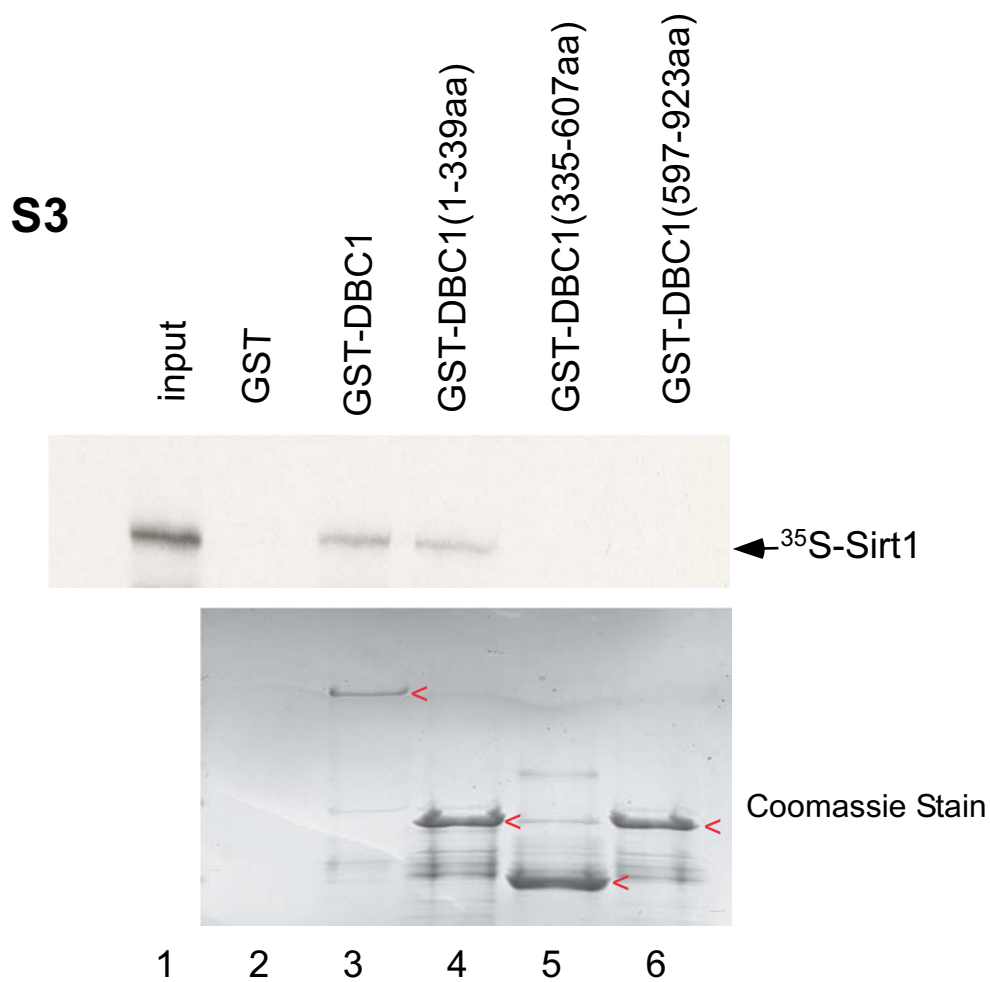
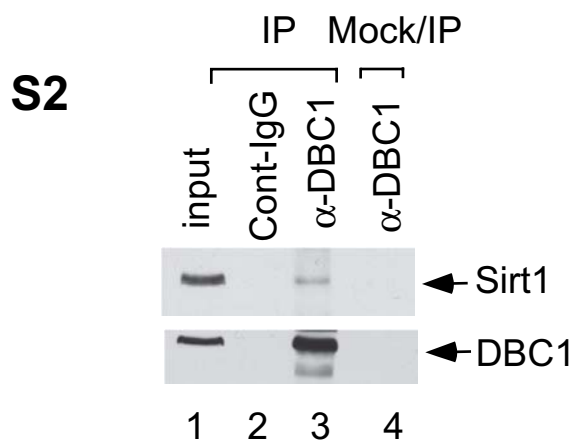
S13. Sirt1 depletion induces p53 acetylation. Western Blot analysis of the whole cell extracts of U2OS cells treated with either Sirt1 siRNA or control siRNA using antibodies against DBC1, p53 (DO-1), PUMA, Bax, and actin (upper 5 panels). Western blot analysis of the immunoprecipitates by anti-acetylated p53 antibody and normal IgG from U2OS cells using anti-p53 (DO-1) antibody (lower panel).

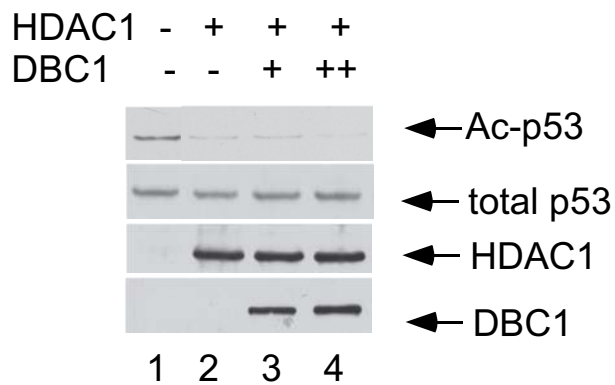
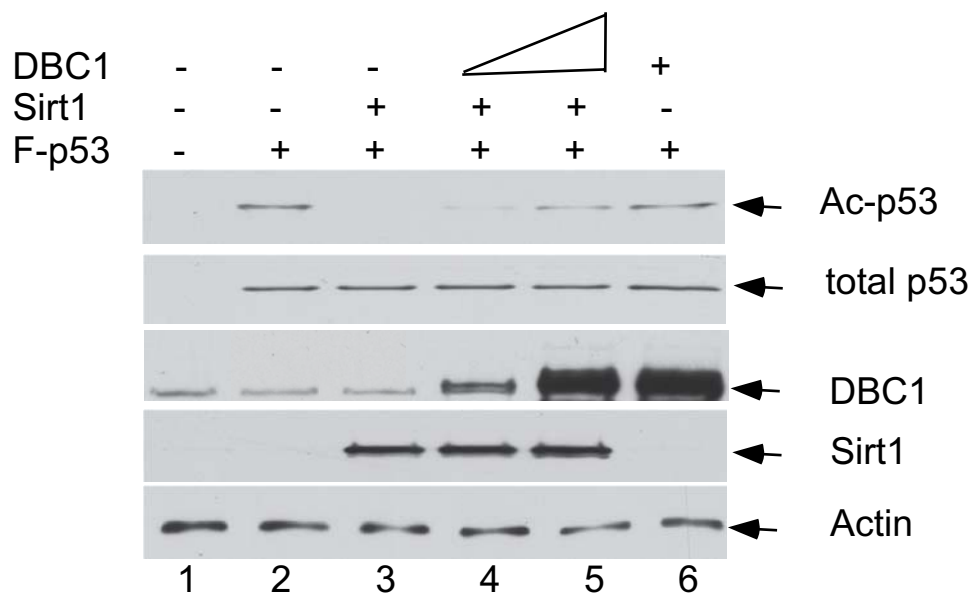
S14. Endogenous HIC1 failed to co-immunoprecipitate with Sirt1 in human U2OS cells. Coimmunoprecipitation of Sirt1 with HIC1 from U2OS cells. Western Blot analysis of whole-cell extract (input, lane 1, lane 4) and immunoprecipitates with a HIC1-specific antibody (lane 3) or control IgG (lane 2, lane 5) or IP with Sirt1 antibody (lane 6) by anti HIC1 antibody and the Sirt1 antibody as indicated.



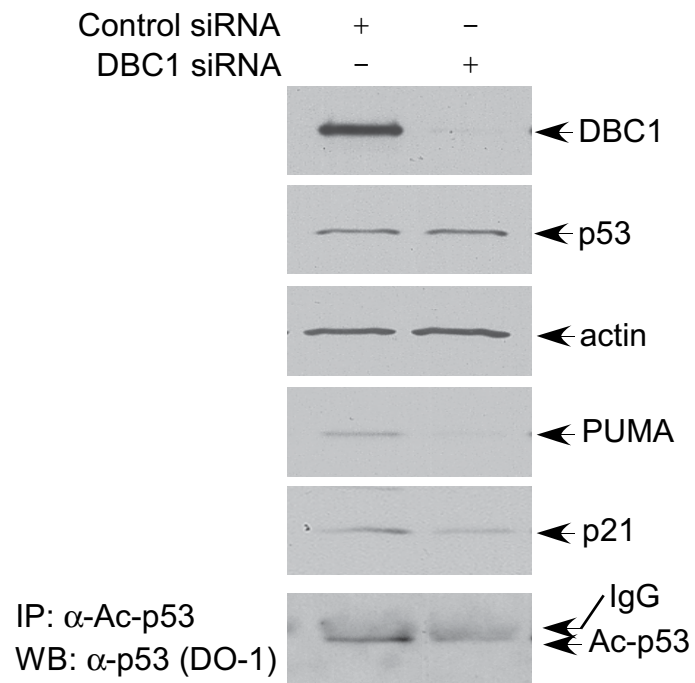
S1b

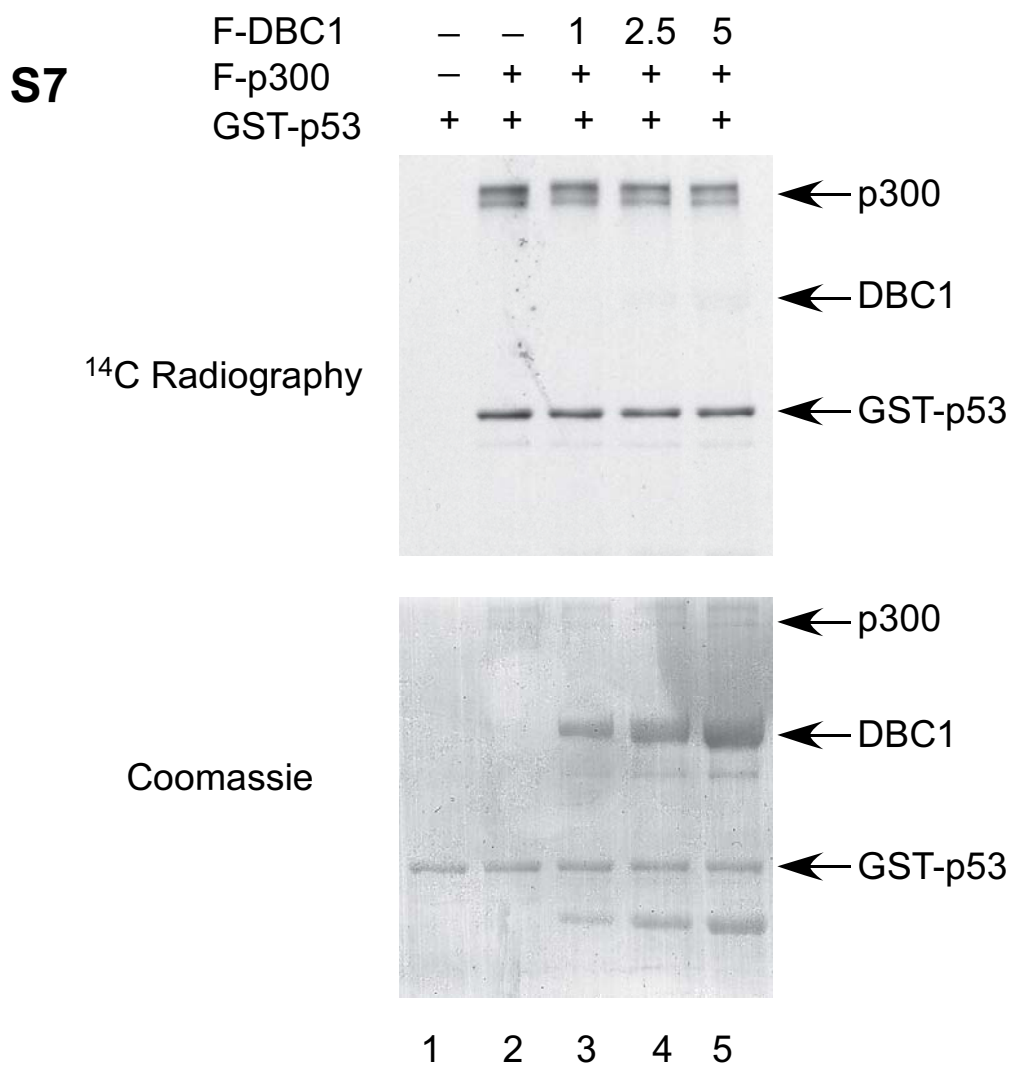


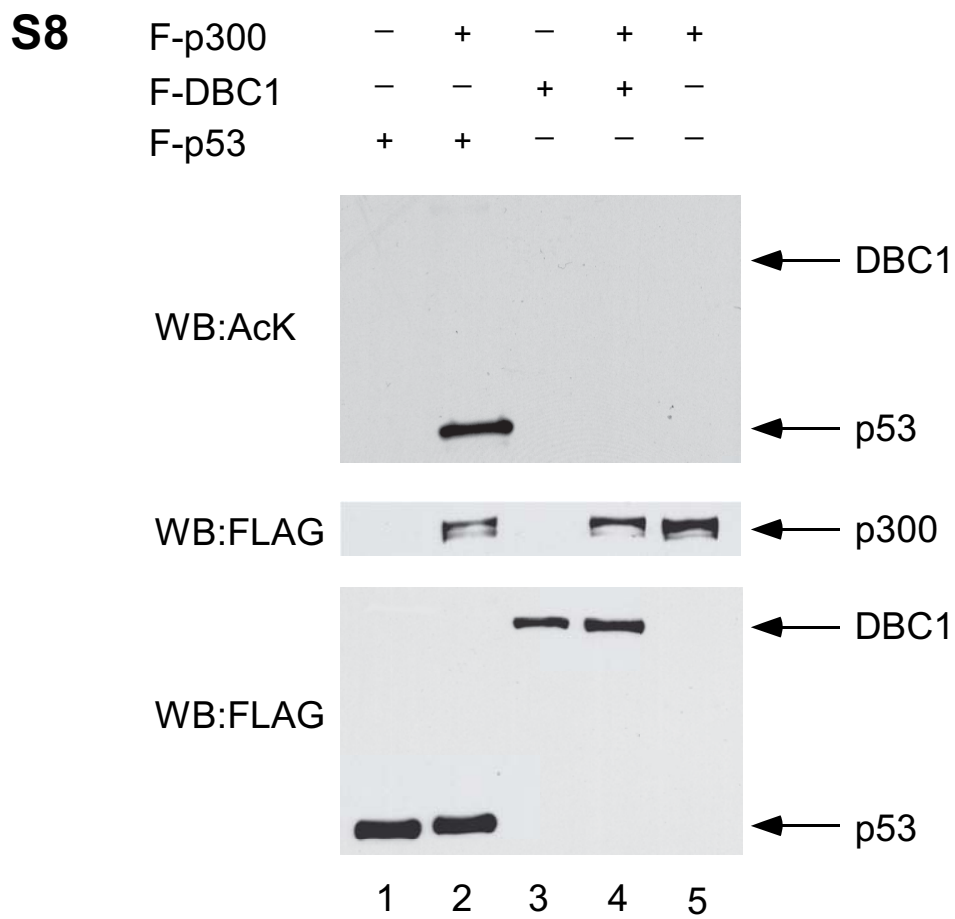


S4**S5**

S6

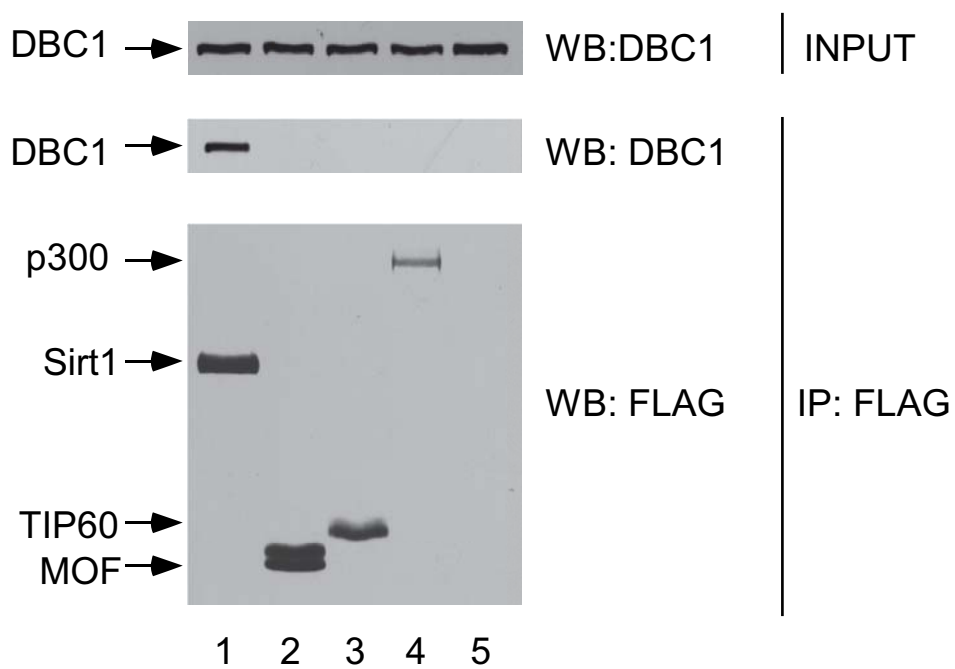




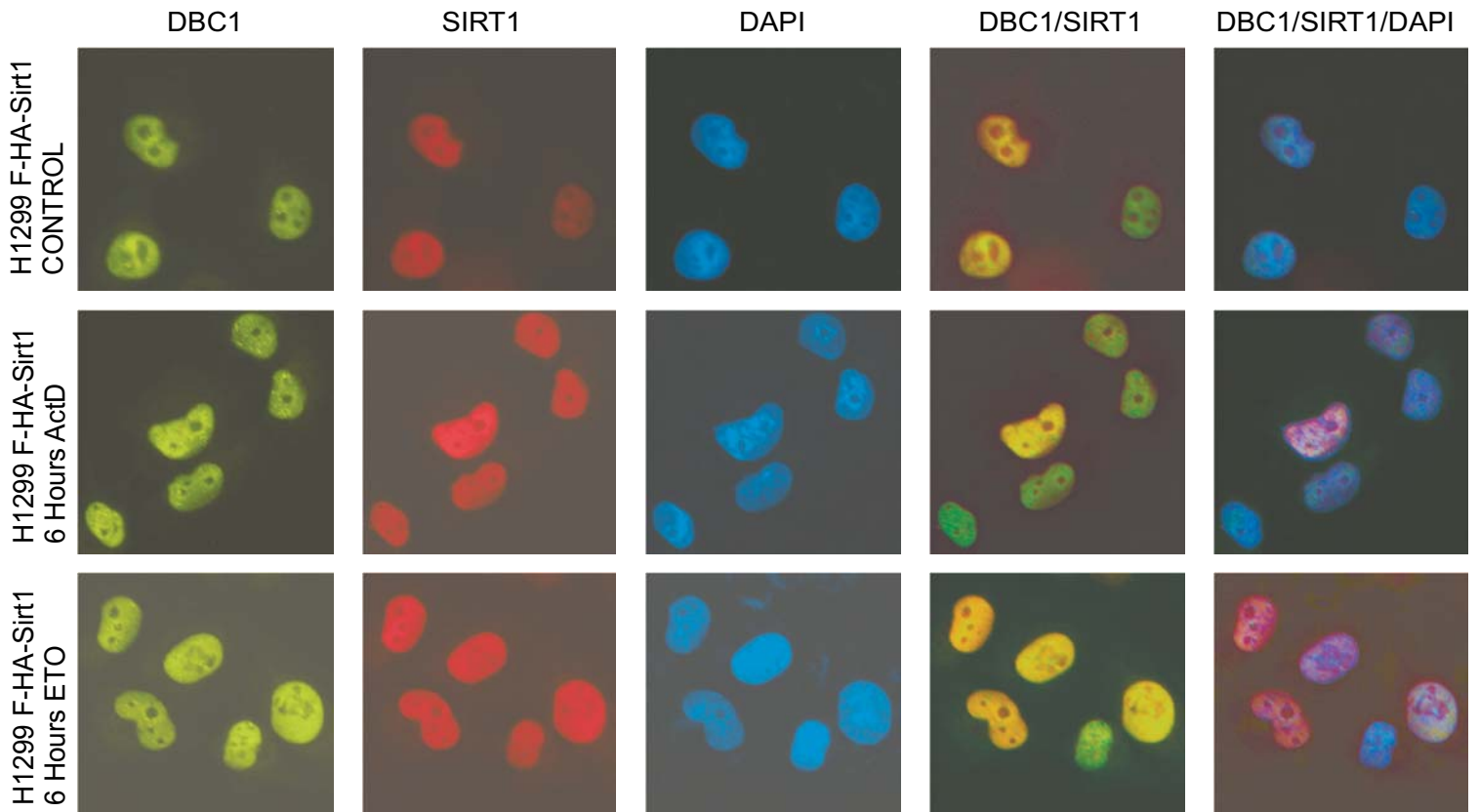


S9

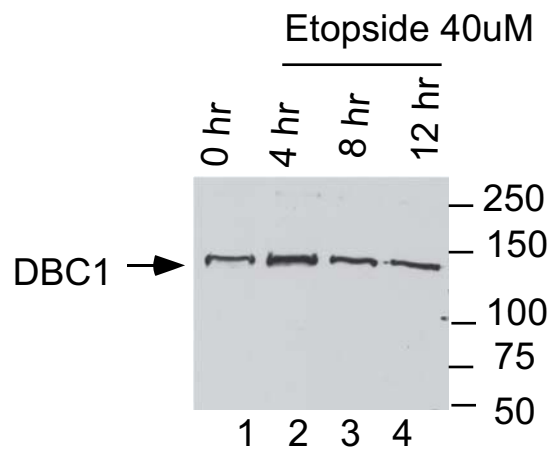
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F-HA-TIP60	-	-	+	-	-
F-MOF	-	+	-	-	-
F-Sirt1	+	-	-	-	-



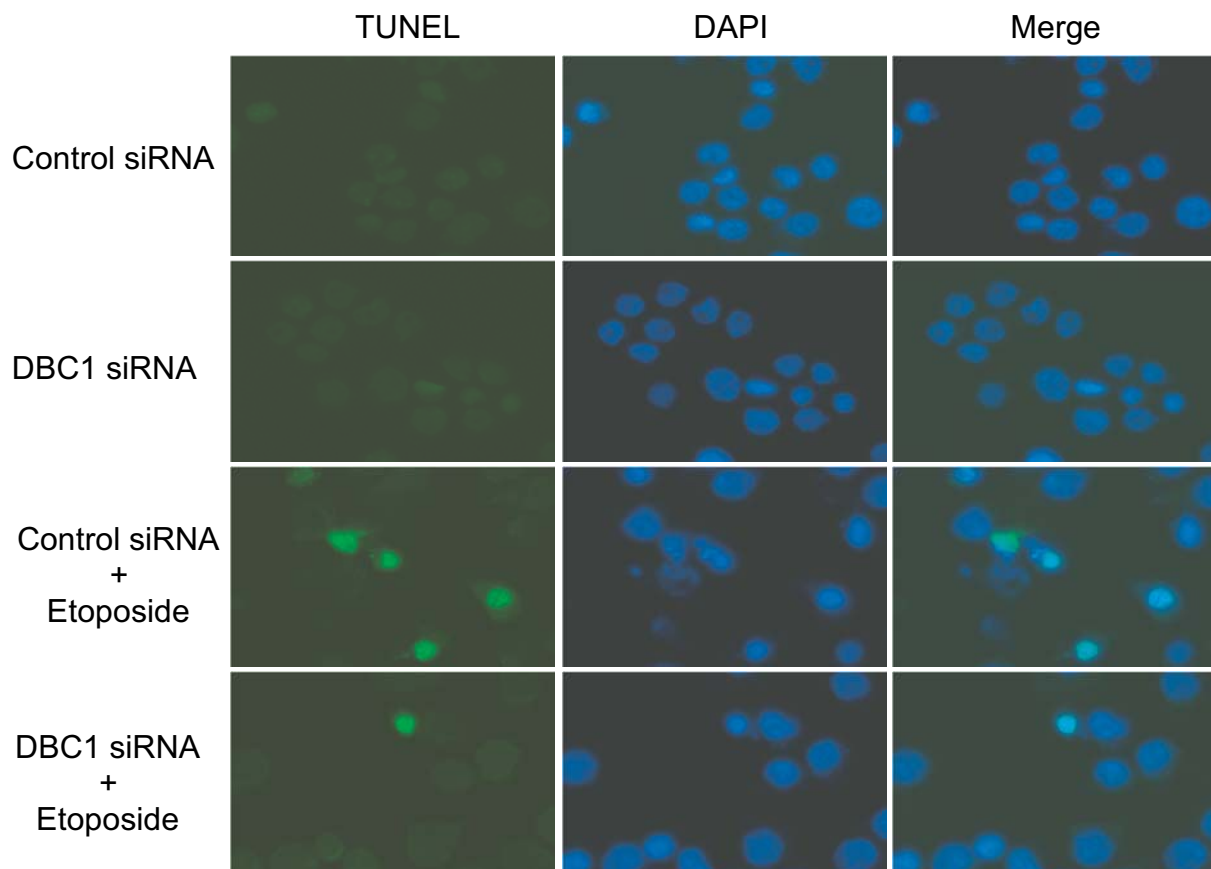
S10A



S10B



S11



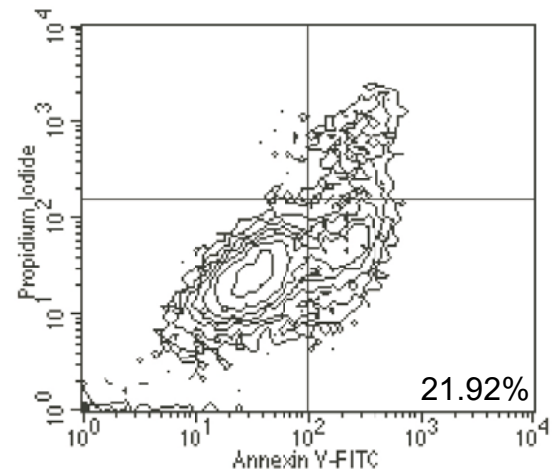
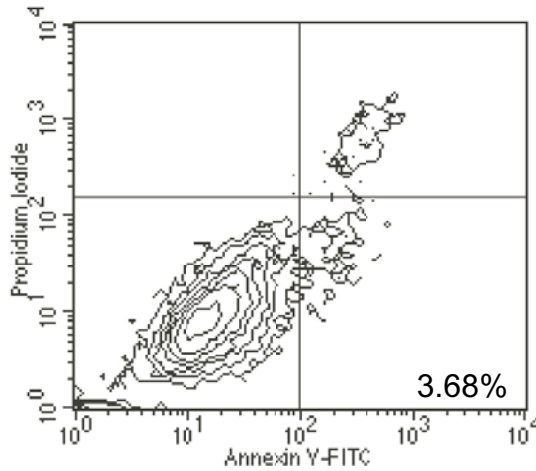
S12

No treatment

Etoposide

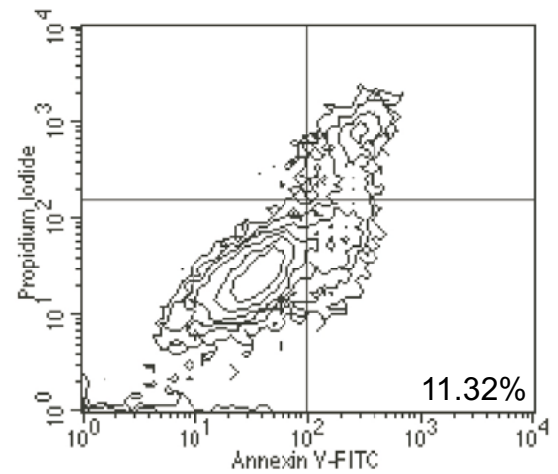
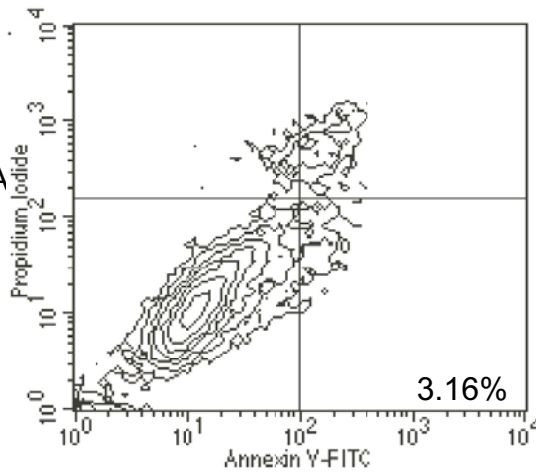
Control siRNA

(I)

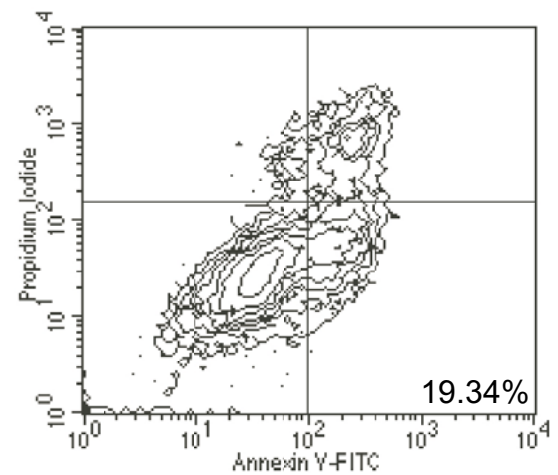
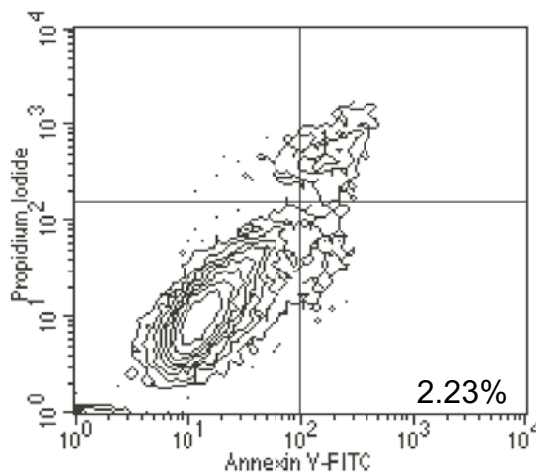


DBC1 siRNA

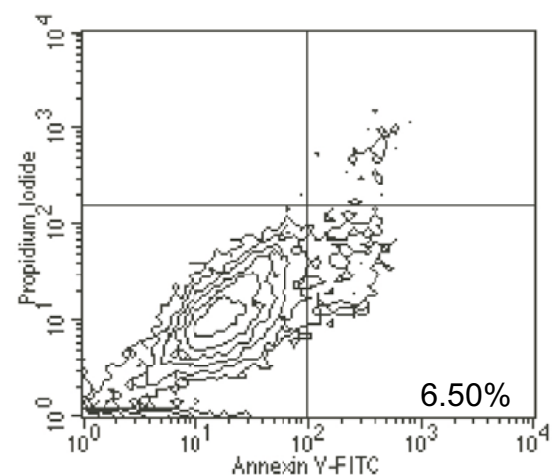
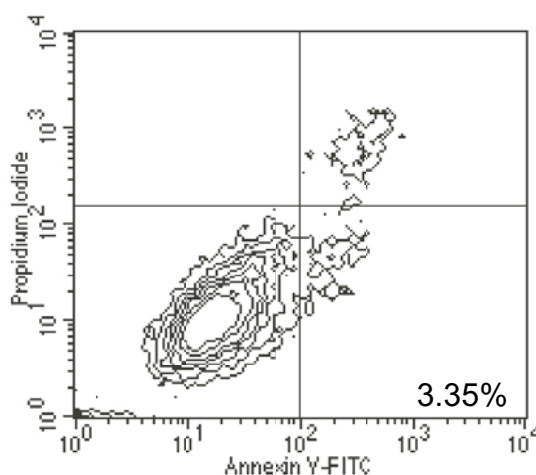
(II)

DBC1 siRNA
Sirt1 siRNA

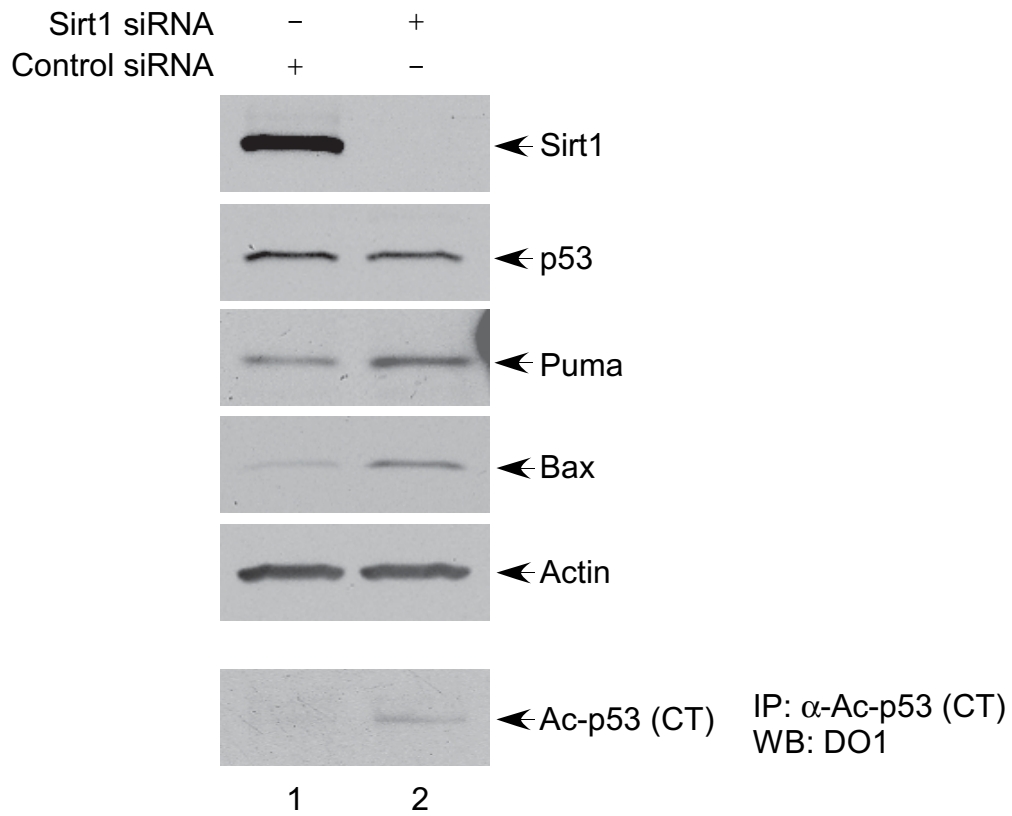
(III)

DBC1 siRNA
p53 siRNA

(IV)



S13



S14.

