

SUPPLEMENTARY FIGURE LEGENDS

Supplementary figure 1. Positive regulation of PML-NBs by SIRT1. MEFs derived from mice with different SIRT1 gene dosage were grown on coverslips and at 24 h cells were subjected to immunostaining using anti-PML antibody followed by confocal microscopy. To facilitate comparison, photomicrographs of each cell type were prepared using identical microscope settings and exposure times. PML staining in a representative image of cells expressing different SIRT1 levels are shown.

Supplementary figure 2. SIRT1 promotes the sumoylation of HA-PML in a deacetylase-independent manner. HEK-293 cells were transfected with the indicated plasmids. 48 h after transfection whole cell lysate was recovered and purification of His-SUMO1 conjugated proteins by nickel affinity chromatography was performed. Purified His-SUMO1-conjugated HA-PML was probed with anti-SUMO1 and anti-HA antibodies. Input extracts were probed with anti-HA (Roche), anti-SIRT1, anti β -galactosidase and anti-tubulin antibodies as indicated.

Supplementary figure 3. Total HA-Sp100 or SUMO1-conjugated Sp100 protein levels are not affected by the expression of SIRT1. HEK-293 cells were transfected with the indicated plasmids. 48 h after transfection whole cell lysates were recovered and purification of His-SUMO1 conjugated proteins by nickel affinity chromatography was performed. Purified His-SUMO1-conjugated HA-Sp100 was probed with anti-HA antibody (upper panel). Input extracts were probed with anti-HA, anti-FLAG, anti β -galactosidase and anti-tubulin antibodies as indicated.

Supplementary figure 4. Analysis of VSV protein synthesis in cells with different SIRT1 gene dosage. SIRT1^{-/-}, WT or SIRT1-tg MEFs were infected with VSV at MOI of 5 and at the indicated times, Western-blot analysis, using antibodies against both the M and N protein from VSV, was performed.

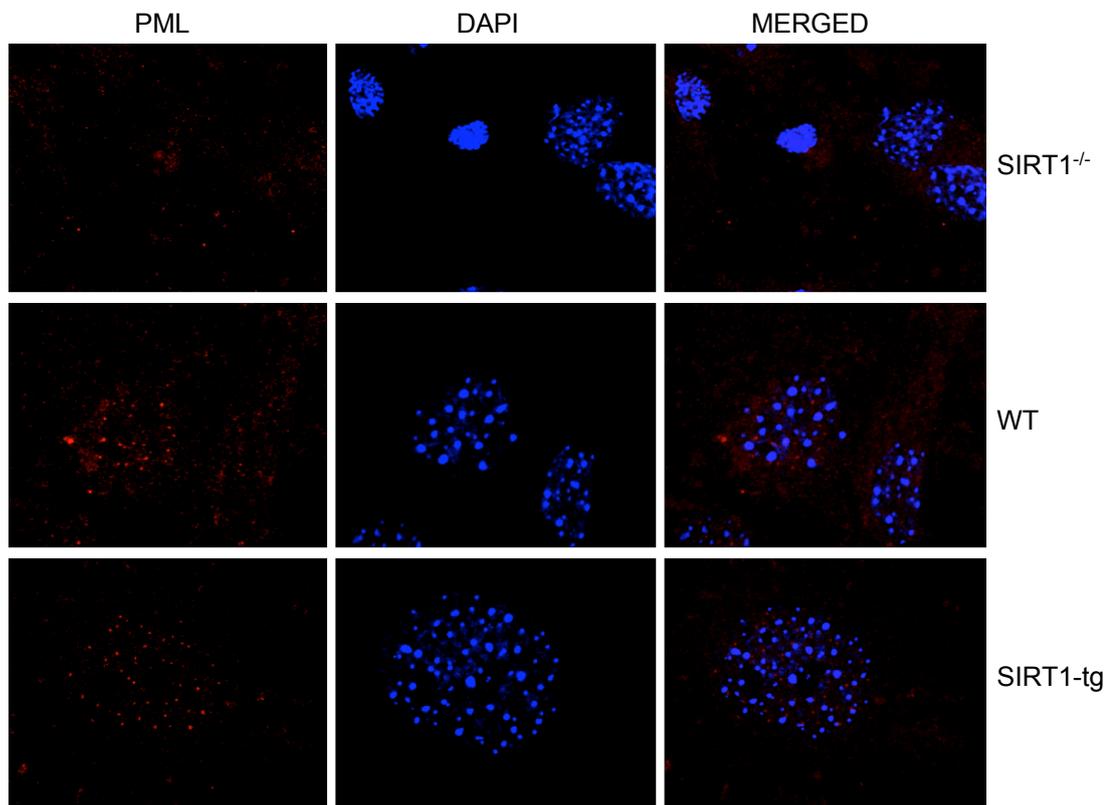
Supplementary figure 5. Both SIRT1-WT and the acetylation mutant SIRT1 H363Y coimmunoprecipitate with PML. HEK-293 cells were transfected with the indicated plasmids, and immunoprecipitations using anti-FLAG, anti-HA and a control antibody (C) in cell lysates prepared 48h after transfection were performed. Input extracts were probed with anti-HA and anti-FLAG antibodies as indicated (left panel).

Supplementary figure 6. SIRT1 sumoylation *in vitro*. *In vitro* sumoylation assay was performed with [³⁵S]-labeled *in vitro*-translated SIRT1 and incubated in a sumoylation mix containing (+) or not (-) SUMO1 as indicated.

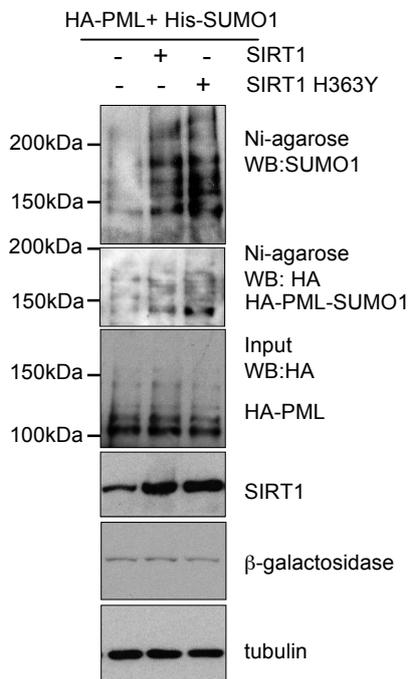
Supplementary figure 7. SIRT1 does not affect the desumoylation of PML by SENP1. [³⁵S]-labeled SUMO1-PML protein obtained by *in vitro* sumoylation assay was incubated with 2 µg recombinant SENP1 in 30 µl reaction buffer containing 50 mM Tris (pH 7.5), 2 mM MgCl₂ and 5 mM β-mercaptoethanol in the absence (-) or presence (+) of *in vitro* sumoylated SIRT1 as indicated. Reactions were incubated at 37⁰C for 1 h, and terminated with SDS sample buffer.

Supplementary figure 8. The levels of free non-conjugated SUMO1 are not affected by overexpression of SIRT1. MCF7 cells were transfected with SUMO1 and increasing doses of FLAG-SIRT1 as indicated and cellular extracts obtained 48 h after transfection

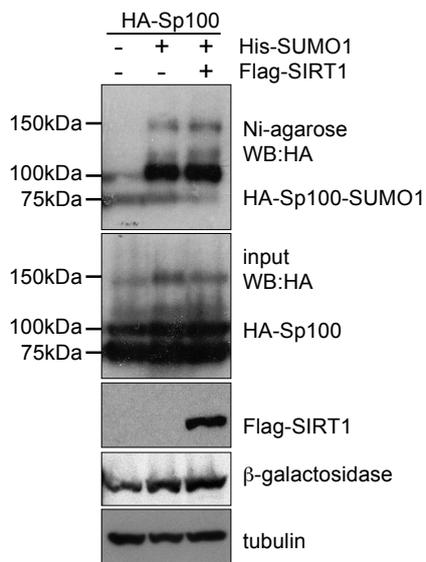
were analyzed by Western-blot with anti-SUMO1 antibody. Asterisk indicates a nonspecific band.



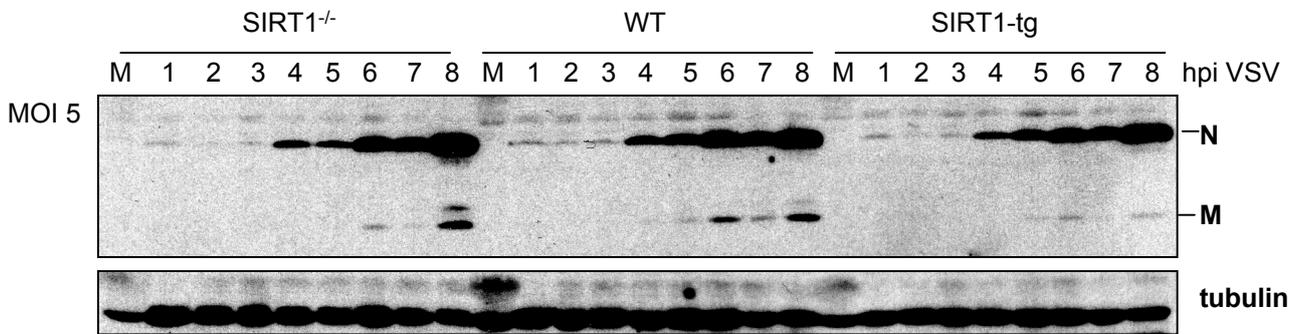
Supplementary Figure 1. Campagna et al., 2009



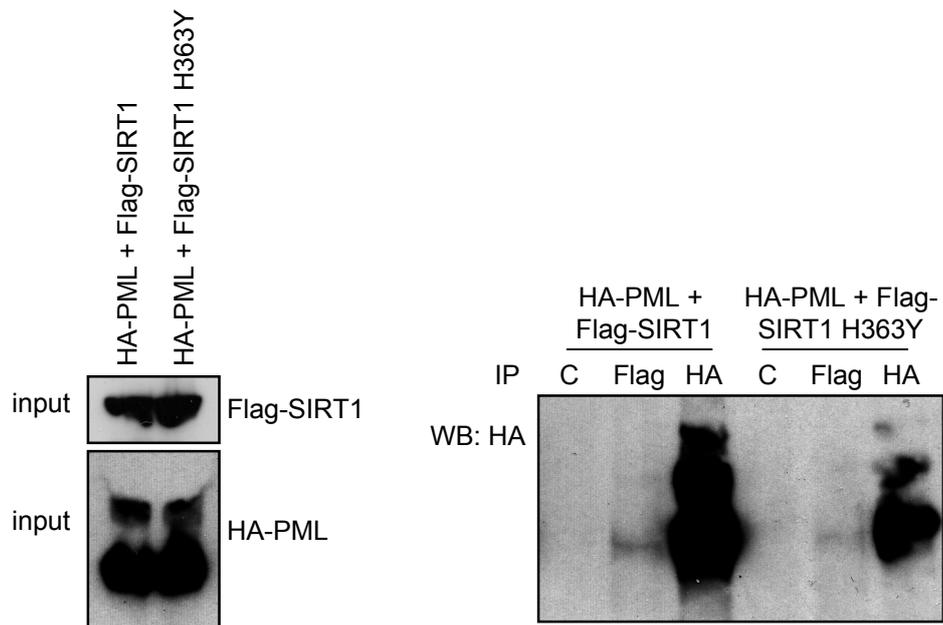
Supplementary Figure 2. Campagna et al., 2009



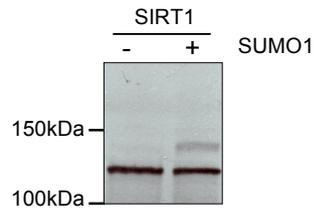
Supplementary Figure 3. Campagna et al., 2009



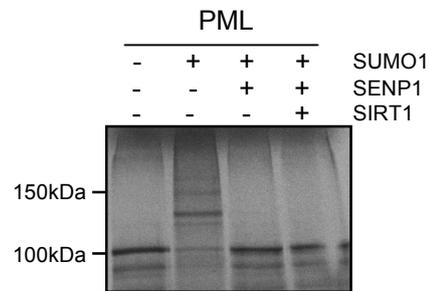
Supplementary Figure 4. Campagna et al., 2009



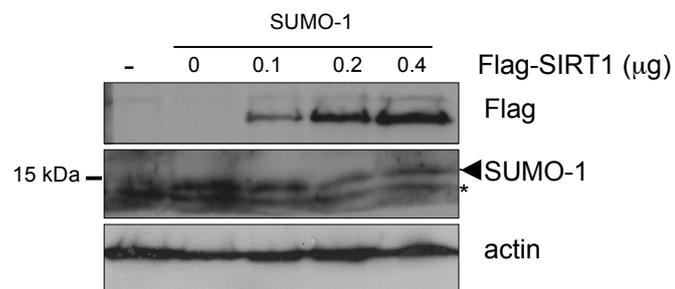
Supplementary Figure 5. Campagna et al., 2009



Supplementary Figure 6. Campagna et al., 2009



Supplementary Figure 7. Campagna et al., 2009



Supplementary Figure 8. Campagna et al., 2009