SIRT1 regulates the function of the Nijmegen breakage syndrome protein

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Supplementary Experimental Procedures

Plasmids, Antibodies, and Viruses

The following expression plasmids used in these experiments have been described previously: pcDNA3-Myc-NBS1 (Maser et al., 2001), HA-NBS1 (Lim et al., 2000), Flag-ATM (Lim et al., 2000), Flag-PCAF (Yang et al., 1996), HA-p300 (Aizawa et al., 2004), pRc/RSV-HA-CBP (Zhang et al., 2000), Flag-SIRT1-7 (Michishita et al., 2005; North et al., 2003), Myc-SIRT1 (Langley et al., 2002), GST-SIRT1 (Langley et al., 2002), and Myc-SIRT1(H363Y) (Langley et al., 2002). The plasmid encoding Myc-NBS1mt was generated using the QuickChange Site-Directed Mutagenesis kit following the manufacturer's protocol (Stratagene). Plasmids encoding GST-NBS1 deletion mutants were created by inserting PCR products of NBS1 fragments into Bam H1/Not 1 digested pGEX-5X-1 vectors (Amersham). pBS/U6-SIRT1 was constructed by inserting oligodeoxynucleotides, which targeted the sequence 5'GAAGTTGACCTCCTCATTGT3' into the pBS/U6 vector (Sui et al., 2002). SIRT1 siRNA and control siRNA adenoviruses were described previously (Rodgers et al., 2005). Plasmids that express Myc-tagged NBS1 lysine to glutamine mutants (5KQ: K544Q/K665Q/K690Q/K698Q/K715Q; 7KQ: K441Q/K504Q/K544Q/K665Q/K690Q/K698Q/K715Q; 9KQ: K233Q/K334Q/K441Q/K504Q/K544Q/K665Q/K690Q/K698Q/K715Q; 10KQ: K208Q/K233Q/K334Q/K441Q/K504Q/K544Q/K665Q/K690Q/K698Q/K715Q) were generated by standard PCR and subcloning.

Mouse affinity purified monoclonal anti-Flag M2, rabbit affinity purified polyclonal anti-HA, and mouse monoclonal anti-acetylated-tubulin (clone 6-11B-1) antibodies were purchased from Sigma. Mouse monoclonal anti-c-Myc (clone 9E10) and mouse monoclonal anti-p53 (clone DO-1) antibodies were purchased from Santa Cruz Biotechnology. Rabbit polyclonal anti-hNBS1, rabbit polyclonal anti-phosphorylated-Ser343-hNBS1, rabbit polyclonal anti-phosphorylated-Ser343-mNbs1, and rabbit polyclonal anti-ATM were purchased from Novus Biologicals. Mouse monoclonal anti-hNBS1 (clone 34) was purchased from BD Biosciences. Protein A purified mouse monoclonal anti-phosphorylated-Ser1981-ATM (clone 10H11.E12) was purchased from Rockland Immunochemicals. Mouse monoclonal anti-hNBS1 (clone 1C3), mouse monoclonal anti-MRE11 (clone 12D7), and mouse monoclonal anti-RAD50 (clone 13B3) were purchased from GeneTex. Rabbit polyclonal anti-acetyl-lysine, rabbit polyclonal anti-SIRT1, and mouse monoclonal anti-GST (clone DG122-2A7) antibodies were purchased from Upstate (Millipore). Mouse monoclonal anti-BrdU (clone BMC9318) was purchased from Roche. Rabbit polyclonal anti-acetylated-Lys382-p53 was purchased from Cell Signaling Technology.

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Supplemental Figure

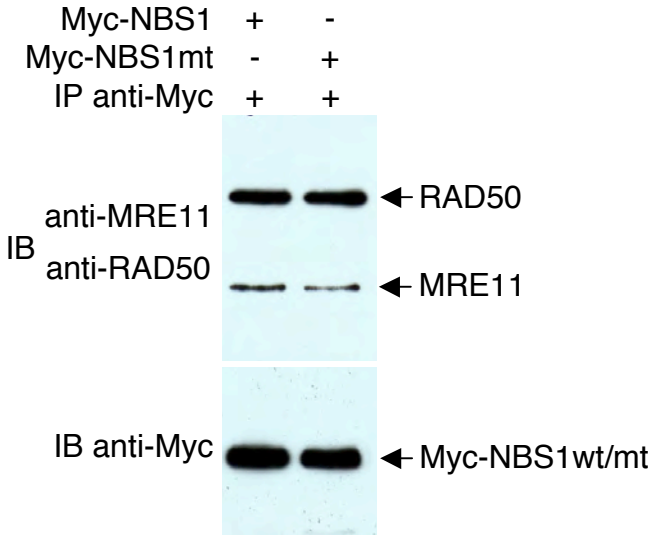

Figure S1. NBS1 acetylation does not affect its association with MRE11 and RAD50