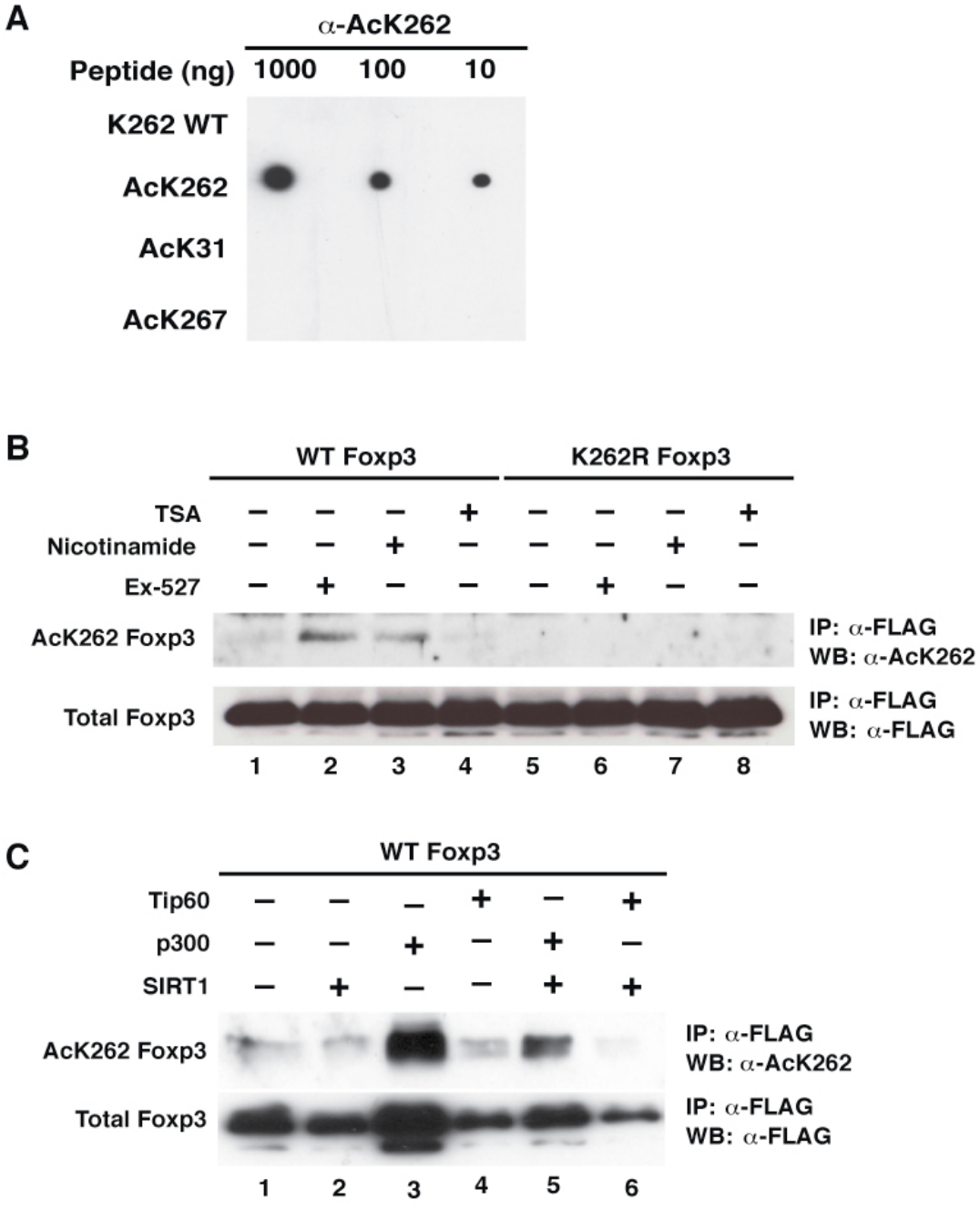
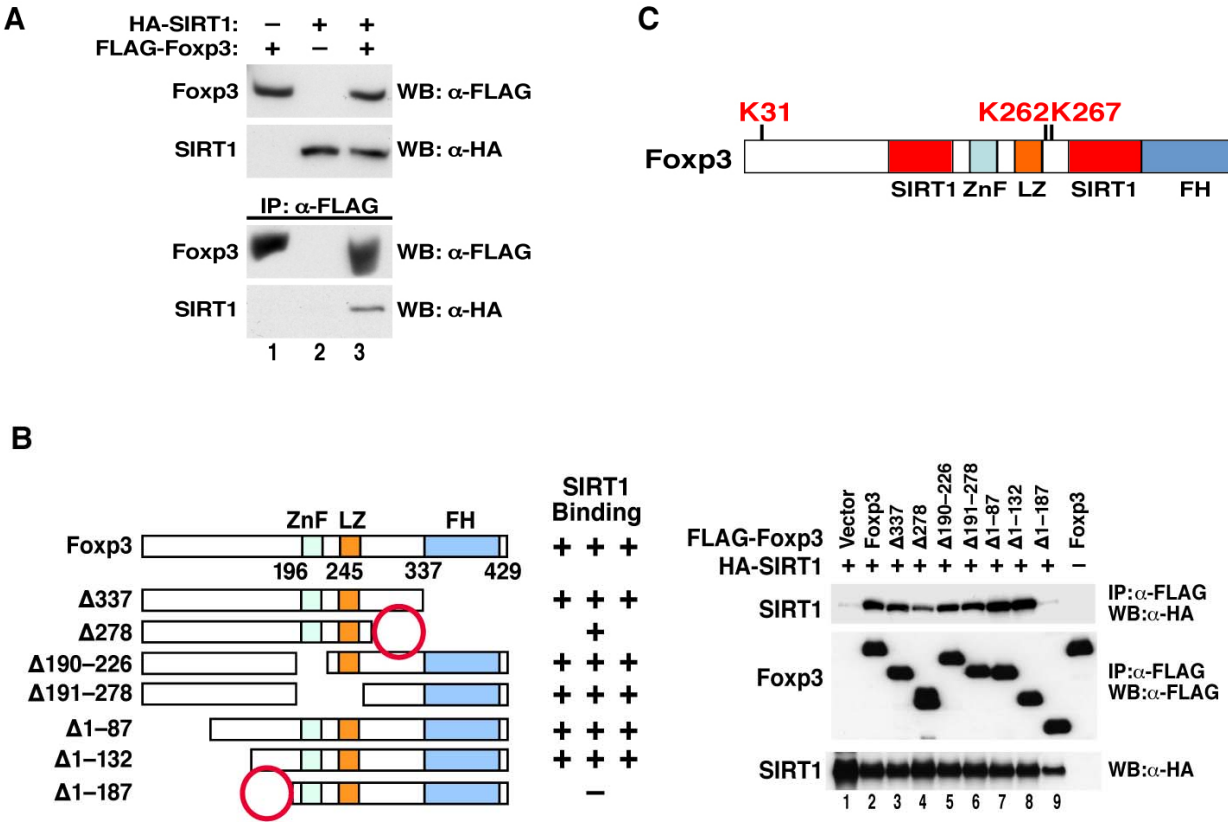
Supplemental Material



Supplemental Figure 1: SIRT1 deacetylase regulates acetylation of Foxp3 at K262 in cells. (A) Dot blot analysis of Foxp3 peptides with acetylated K262 Foxp3 antibody (α-AcK262). Different amounts of indicated peptides (1000, 100, and 10 ng) were spotted onto a nitrocellulose membrane and processed by western blotting (WB) with affinity purified α-AcK262 Foxp3 antibodies (B) Immunoprecipitation/Western blot analysis of acetylated FLAG-Foxp3. Expression vectors for FLAG-wt or K262R mutant Foxp3 were transfected into 293T cells that were subsequently treated with Ex-527 (50 μM),

nicotinamide (5 mM), and trichostatin A (TSA, 400 nM) overnight. Immunoprecipitations were performed with α-FLAG agarose and WB with α-AcK262 Foxp3 and α-FLAG antibodies. (C) Expression vectors for FLAG- wt Foxp3 were transfected into 293T cells with p300, SIRT1, and Tip60. Acetylation at K262 in Foxp3 is enhanced by coexpression of p300 but not Tip60. SIRT1 decreases the acetylation of K262 by p300.



Supplemental Figure 2. SIRT1 interacts with Foxp3.
(A) Coimmunoprecipitation/western blot analysis of FLAG-Foxp3 and HA-SIRT1 after transfection of corresponding expression vectors or empty vector controls into 293T cells. (B) Coimmunoprecipitation assay of SIRT1 and Foxp3 deletion mutants. A schematic summary of the binding of Foxp3 mutants to SIRT1 is shown. The forkhead box (FH) indicates the DNA binding domain. ZnF, zinc finger domain; LZ, leucine zipper. (C) The summary of three acetylation sites and SIRT1 binding regions in mouse Foxp3.