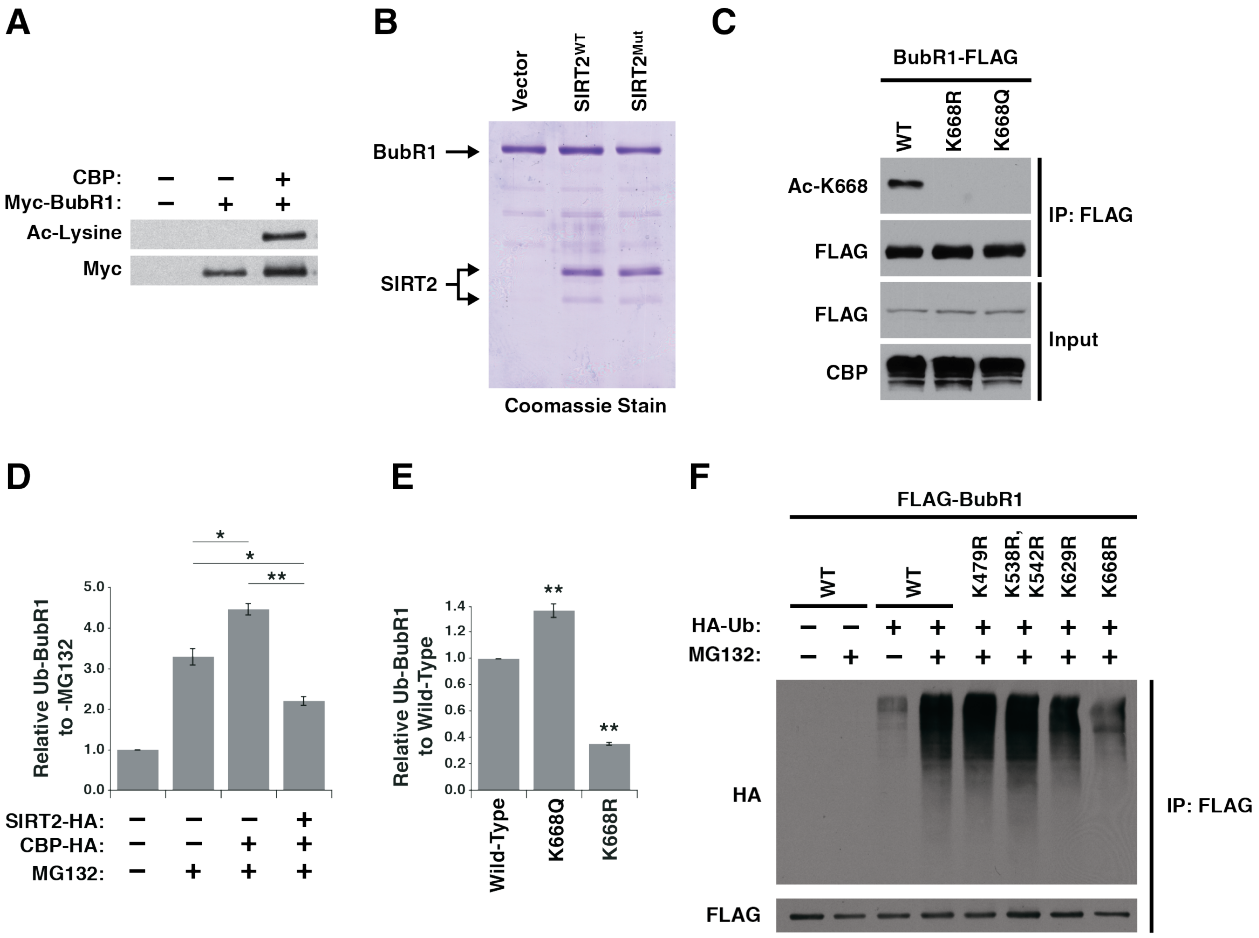

Figure S3. *In vitro* deacetylation of BubR1 by SIRT2 for Mass Spectrometry analysis. (A) 293T cells were transfected with Myc-BubR1 with or without CBP-HA. Cell extracts were immunoprecipitated with anti-Myc antibodies, and western blotted for Ac-lysine, Myc, and FLAG. (B) Reactions were separated on SDS-PAGE and stained with Coomassie Brilliant Blue, and Myc-BubR1 was excised and analyzed by Mass Spectrometry. (C) Confirmation of anti-BubR1 K668 specificity. 293T cells were transfected with CBP-HA, and either wild-type, K668R, or K668Q BubR1-FLAG. Cell extracts were immunoprecipitated with anti-FLAG antibodies, and western blotted for Ac-BubR1 K668, FLAG, and HA. (D) Quantification of ubiquitinated BubR1 from experiment shown in Fig. 3E. (E) Quantification of ubiquitinated BubR1 from experiment shown in Fig. 3F. (F) 293T cells were co-transfected with HA-

Ubiquitin, and wild-type, K479R, K538,542R, K629R, or K668R BubR1-FLAG. Cells were treated with MG132, and cell extracts were immunoprecipitated with anti-FLAG antibodies and western blotted for HA and FLAG. Error bars represent SEM. *P* values calculated using Student's *t*-test ($n = 3$), * $P < 0.05$, ** $P < 0.005$.