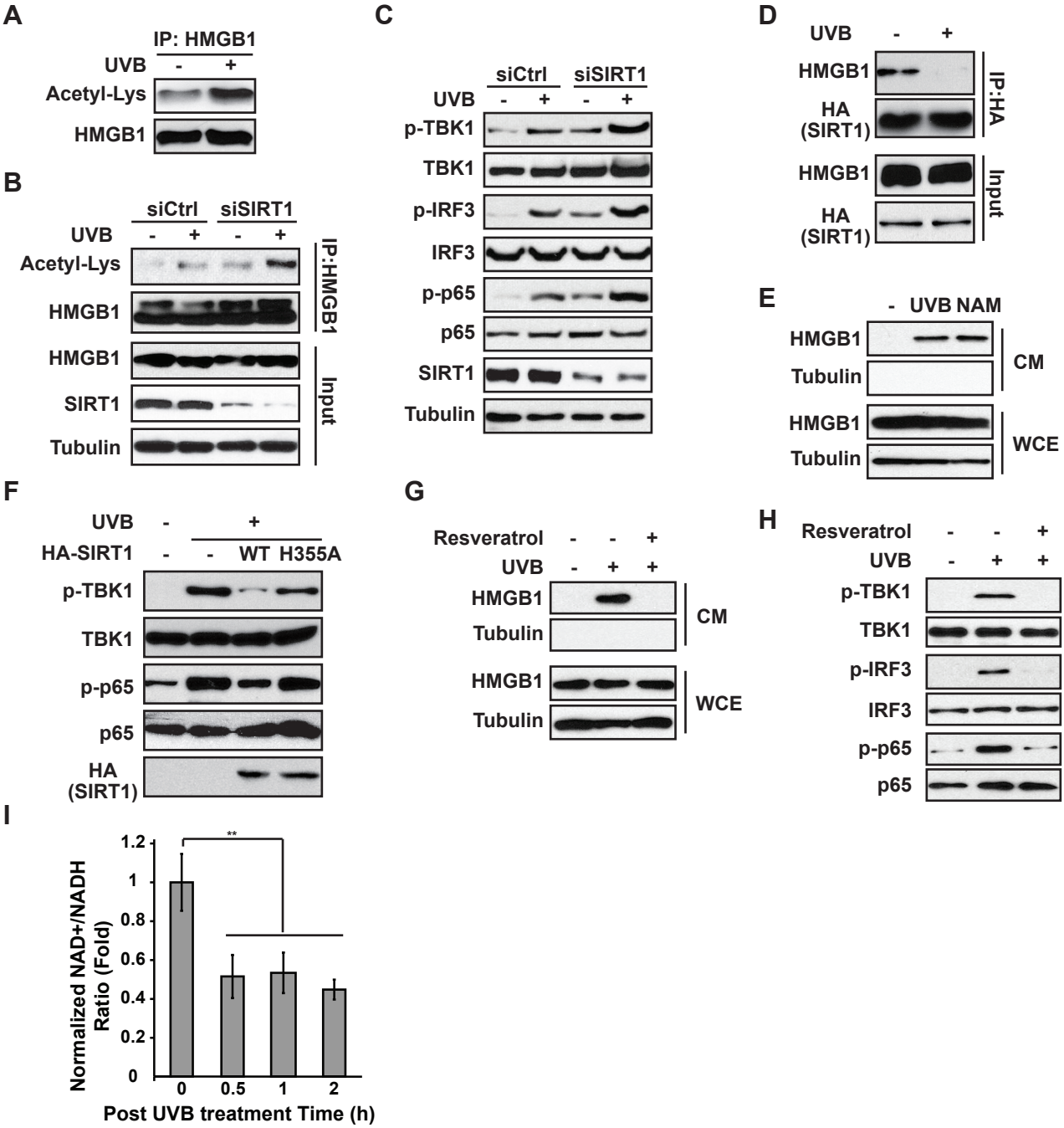
s-Figure 3



Supplemental Figure 3. (A) SK-Mel-28 cells were treated with UVB (50 mJ/cm2). Cells were harvested at 4 h after the treatment; immunoprecipitated (IP) HMGB1 was probed with anti-acetylated Lys. (B) SK-Mel-28 cells were transfected with control (siCtrl) or SIRT1 siRNA (siSIRT1) as shown. Cells were harvested at 4 h after UVB (50 mJ/cm2) exposure and acetylation of HMGB1 was analyzed as in (A). (C) SK-Mel-28 cells were treated as in (B). Whole-cell extracts were analyzed by indicated antibodies. (D) SK-Mel-28 cells were transfected with HA-SIRT1 and treated with UVB (50 mJ/cm2). Cells were harvested at 4 h after treatment and the interaction of HMGB1 and SIRT1 was analyzed with Co-IP. (E) SK-Mel-28 cells were treated with UVB (50 mJ/cm2) or nicotinamide (10mM, NAM). Conditioned media (CM) and whole-cell extracts were collected at 4 h after treatment and analyzed by western blots. (F) SK-Mel-28 cells were transfected with control, wild-type (WT) SIRT1 or dominant-negative H355A mutant of SIRT1, and treated with UVB (50 mJ/cm2). Whole-cell lysates were analyzed by western blot with the indicated antibodies. (G) SK-Mel-28 cells were pre-incubated with resveratrol (50 μM, 4 h) or DMSO. Cells were then exposed to UVB (50 mJ/cm2). CM and whole-cell extracts were collected and analyzed as in (D). (H) Whole-cell lysates from SK-Mel-28 cells treated as in (F) were analyzed by indicated antibodies. (I) SK-Mel-28 cells were exposed to UVB (50 mJ/cm2). Cells were harvested at indicated times after treatment and the ratio of NAD+/NADH was analyzed. **: $p<0.01$.