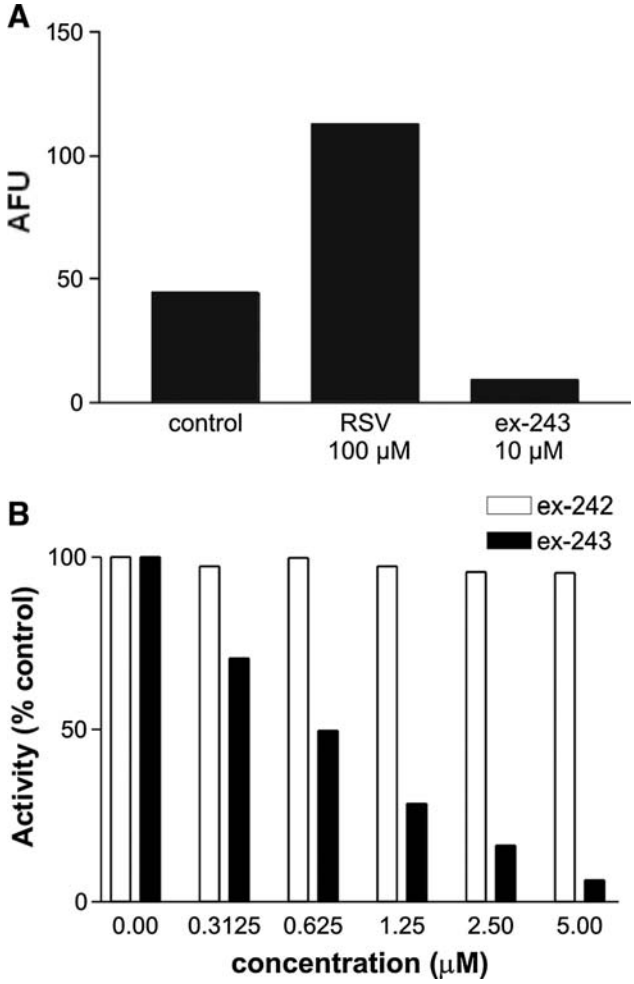
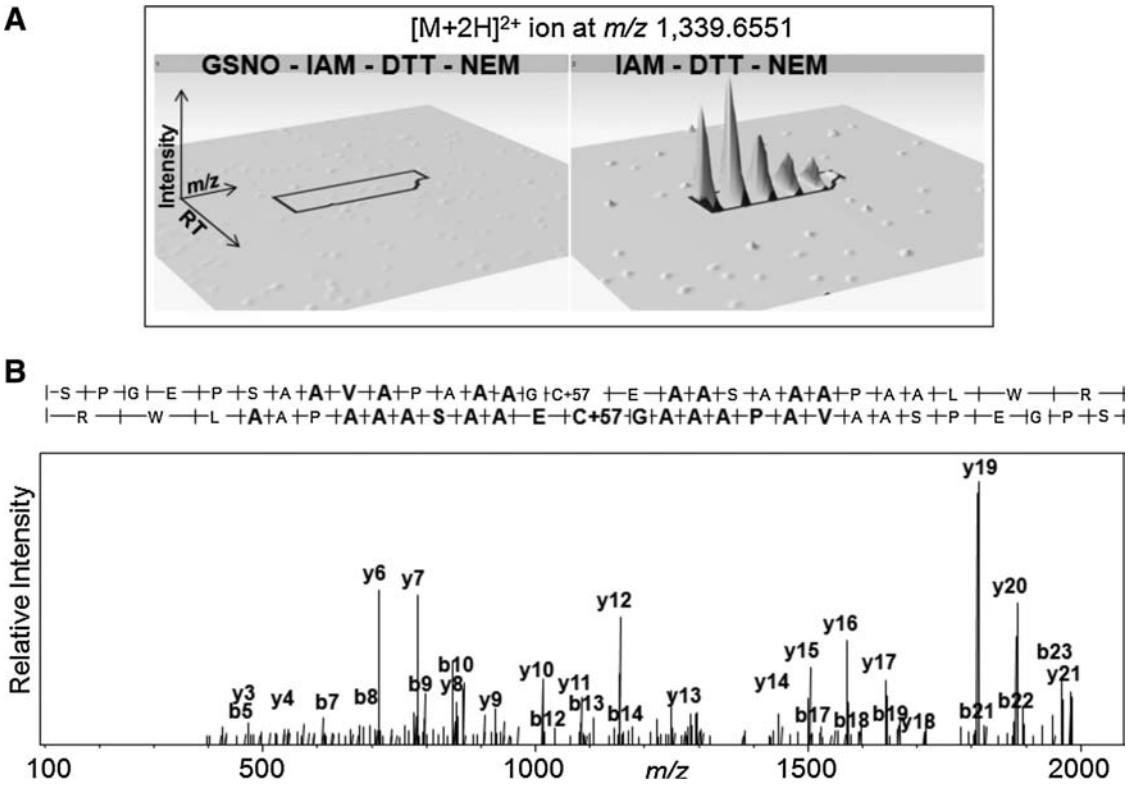


SUPPLEMENTAL FIG. 1. (**A**) SIRT1-Flag activity was tested for specificity by using a SIRT1 activator, RSV (100 μM) and a specific SIRT1 inhibitor ((S)-6-chloro-2,3,4,9-tetrahydro-1-*H*-carbazole-1-carboxamide), Ex-243, or the inactive stereoisomer ((R)-6-chloro-2,3,4,9-tetrahydro-1-*H*-carbazole-1-carboxamide), Ex-242. SIRT1 is stimulated 2-fold by RSV, whereas Ex-243 results in a step-wise inhibition of SIRT1 activity with complete inhibition at 5 μM Ex-243 (**B**). The stereoisomer Ex-242 had no effect on SIRT1-Flag activity.



SUPPLEMENTAL FIG. 2. Reactive SIRT1 Cysteine 61 is *S*-glutathiolated *in vitro*, blocking the carbamidomethylation of this residue under nonreducing conditions. Label-free quantitation and sequencing of mouse SIRT1 (mSIRT1) peptide 46–74 bearing a carbamidomethyl-modified cysteine. Purified recombinant mSIRT1 was treated *in vitro* with GSNO to label redox-sensitive cysteines amenable to *S*-glutathiolation, followed by in-gel IAM labeling of unreacted cysteines under nonreducing conditions, reduction of disulfides with DTT, and in-gel alkylation with NEM, followed by trypsin digestion. Eluted peptides were subjected to nano-flow reversed-phase chromatography and MS/MS on an LTQ-Orbitrap mass spectrometer. (**A**) Label-free quantitation of relative peak LC-MS peak volume (with dimensions of m/z, retention time, and ion intensity) for the $[M+2H]^{2+}$ ion at m/z 1,339.6551 from the sample treated sequentially with GSNO, IAM, DTT, and NEM (*left panel*) in comparison to that from the sample treated sequentially with only IAM, DTT, and NEM (*right panel*). The normalized volume of the ion feature in the *right panel* was nearly 8 orders of magnitude greater than that of the *left panel* (ArcSinh = 13.6 vs. ArcSinh = 5.8). (**B**) The fragment ion mass spectrum resulting from the collisional dissociation of the $[M+2H]^{2+}$ precursor ion at m/z 1,339.6551. Prominent fragment ions are marked with their assignments to b-type ions (*right*) and y-type ions (*left*) of the SIRT1 peptide ^{46}SPGEPSAAVAPAAAGCEAASAAAPAALWR$_{74}$ bearing a carbamidomethyl moiety on its cysteine residue. Above the spectrum is shown the amino acid sequence that aligns with the b-ion (*forward*) and y-ion (*reverse*) series, containing the cysteine plus an additional nominal mass of 57, corresponding to the carbamidomethyl moiety.