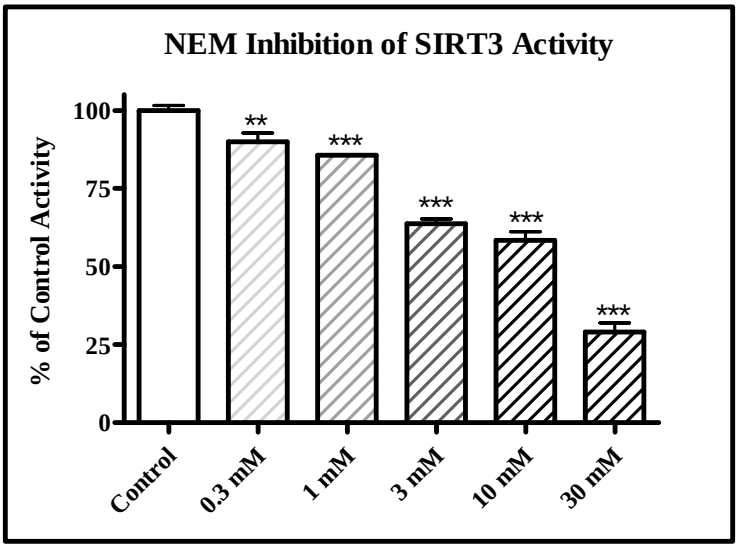
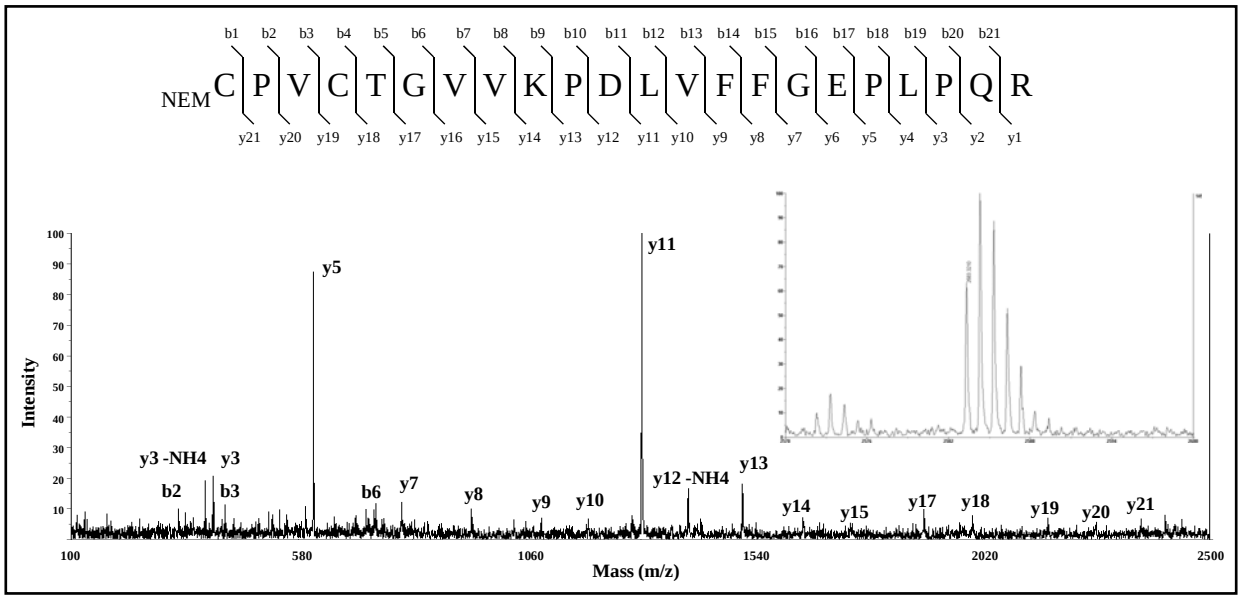
Supplemental Figure 1. NEM Inhibits SIRT3 Activity. The cysteine-specific inhibition of SIRT3 by 0.3 to 30 mM N-Ethylmaleimide (NEM) was identified using the SIRT3 activity assay as previously described (Cayman Chem). Error bars represent SEM (n=3).



Supplemental Figure 2. MALDI-TOF/TOF analysis of NEM modified rSIRT3, Cys^{280} peptide. The mass spectra details MS and MS/MS analysis of a tryptic peptide containing Cys^{280} from NEM modified rSIRT3 and contains one NEM (Cys^{280}) and one Carbamidomethyl (Cys^{283}) modified cysteine (100-fold molar excess NEM). MS analysis of NEM modified rSIRT3 identified the parent ion (2583.32 m/z) (MS insert) and MS/MS analysis confirmed the precise location of the NEM adduct (Cys^{280}), as shown by the b/y ion fragmentation spectra. Specifically, the fragmentation ions b2, b3, y19, y20 and y21 confirm the location of NEM.



Supplemental Figure 3. A SIRT3 activity assay from MBL Intl. was utilized to verify the inhibitory effects of 4-HNE on SIRT3 activity. The assay was performed as instructed by the manufacturer. Increasing concentrations of 4-HNE were incubated with rSIRT3 for 30 min at 37°C. The assay was then performed for 30 min at 25°C and fluorescence was quantified using a SpectraMAX GeminiEM fluorimeter (Ex. 355, Em.460) (Molecular Devices). This particular assay employs a substrate/quencher/fluorophor target for SIRT3 activity, which prevents excitation of the fluorophore until it is released from the substrate, post-deacetylation. SIRT3 activity was significantly inhibited by 4-HNE by 17% at 10 μM and 49% at 100 μM ($p < 0.001$). Error bars represent SEM.