SUPPORTING INFORMATION

Characterizing SIRT3 Deacetylase Affinity for Aldehyde Dehydrogenase 2

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Figure S1. Stable isotope dilution mass spectrometry for the determination of Lys511 deacetylation by SIRT3. (A) MS spectra of a peptide encompassing amino acids 507 to 515 of ALDH2 dosed with acetic anhydride followed by SIRT3 treatment, (B) deuterated acetic anhydride, and (C) a 1:1 mixture of sample A and B.
Figure S2. Acetylation of recombinant ALDH2 alters migration through a non-reducing, non-denaturing native gel. The arrows depict laddering of the protein at the highest concentration of acetic anhydride suggesting destabilization of the homotetramer into dimers and monomers. Protein was visualized following silver staining.
**Figure S3. Decrease of ALDH2 activity following sulfo-NHS-acetate treatment.** (A) The hyperacetylation of ALDH2 by sulfo-NHS-acetate results in a change in migration when the protein is run on a non-reducing native gel and visualized using Imperial Protein Stain. (B) Sulfo-NHS-acetate acetylation was utilized to confirm our findings with acetic anhydride. Here, ALDH2 activity is decreased upon acetylation by sulfo-NHS-acetate. (*p<0.05, **p<0.01)
Figure S4 Sigmoidal dose response curve of ALDH2 activity following acetic anhydride treatment. Plotted are the averages for each concentration with the error bars depicting the standard deviation (n=3).
Table S1. Mass to charge and integral values used to calculate the STAR values.

Table S2. MS/MS identification of acetylated ALDH2 peptides.