

## **SUPPORTING INFORMATION**

### **Characterizing SIRT3 Deacetylase Affinity for Aldehyde Dehydrogenase 2**

Peter S. Harris, Jose D. Gomez, Donald S. Backos and Kristofer S. Fritz\*

Graduate Program in Toxicology, Skaggs School of Pharmacy and Pharmaceutical Sciences,  
University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA

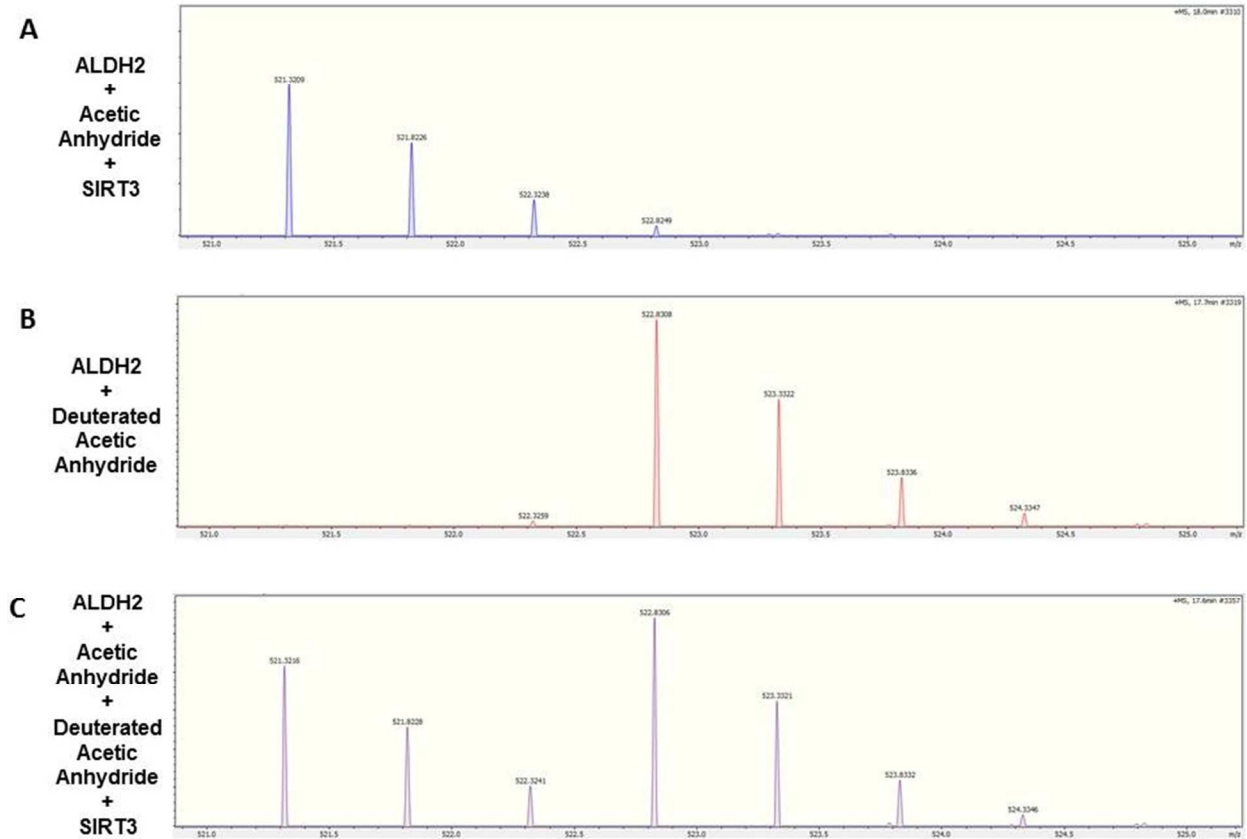
\*Contact: Kristofer S. Fritz, Graduate Program in Toxicology, Skaggs School of Pharmacy and  
Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, 12850 E.  
Montview Blvd., Box C238, Aurora, CO, 80045, USA

Email: [kristofer.fritz@ucdenver.edu](mailto:kristofer.fritz@ucdenver.edu)

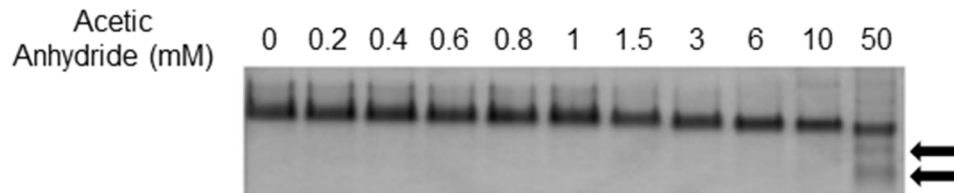
Phone: 303-724-7932

<b>Contents</b>		<b>Page</b>
Figure S1	MS spectra demonstrating SIRT3 deacetylation at Lys511 of ALDH2	S-3
Figure S2	Migration of acetylated ALDH2 in a native gel	S-4
Figure S3	Acetylation of ALDH2 by sulfo-NHS acetate affects migration and activity	S-5
Figure S4	Dose response curve for ALDH2 and acetic anhydride	S-6
Table S1	MS data for STAR values	S-7
Table S2	MS/MS data for acetylated ALDH2 peptides	S-7

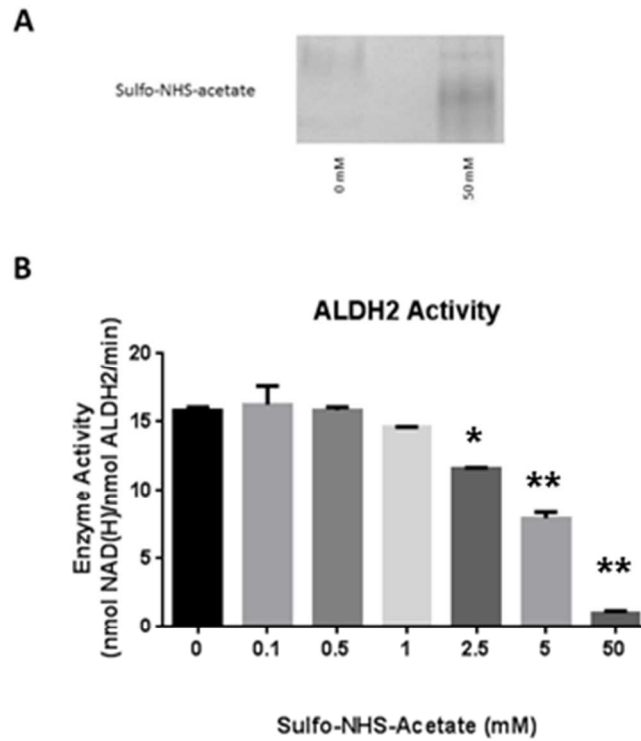
**Figure S1. Stable isotope dilution mass spectrometry for the determination of Lys511 deacetylation by SIRT3.** (A) MS spectra of a peptide encompassing amino acids 507 to 515 of ALDH2 dosed with acetic anhydride followed by SIRT3 treatment, (B) deuterated acetic anhydride, and (C) a 1:1 mixture of sample A and B.



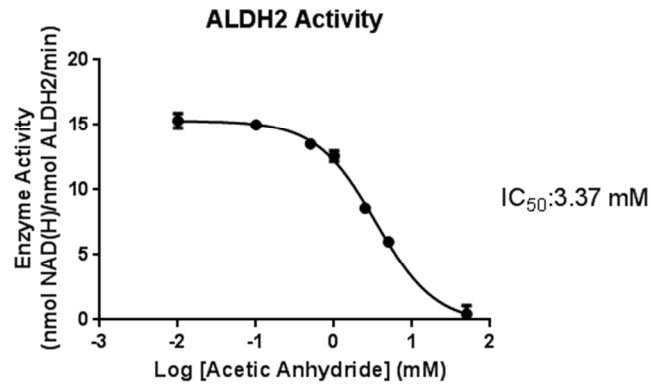
**Figure S2. Acetylation of recombinant ALDH2 alters migration through a non-reducing, non-denaturing native gel.** The arrows depict laddering of the protein at the highest concentration of acetic anhydride suggesting destabilization of the homotetramer in to dimers and monomers. Protein was visualized 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.**