Supporting Information for "Phosphorylation of Histone Deacetylase 8: Structural and Mechanistic Analysis of Phosphomimetic S39E Mutant"

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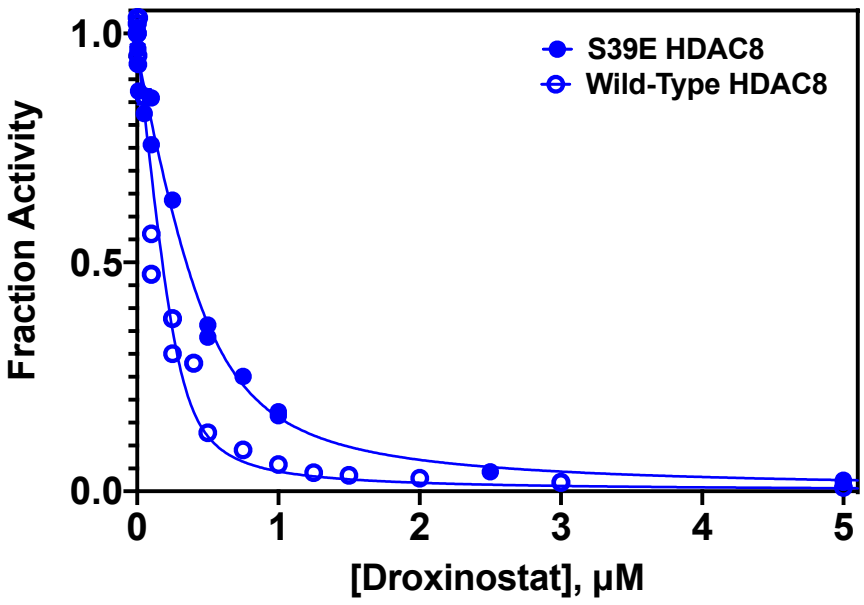

Figure S1. Inhibition of wild-type and S39E HDAC8 by Droxinostat. Cobalt(II)-bound wild-type (0.3 μM) or S39E HDAC8 (0.5 μM) was added to reactions containing 100 μM HDAC8 Fluor de Lys substrate, HDAC8 reaction buffer (25 mM HEPES, pH 8, 127 mM NaCl, 3 mM KCl), and 0-10 mM Droxinostat at 30°C. Reaction time points were quenched into a solution containing HDAC inhibitor Trichostatin A. The fluorescence of product (ex. 340 nm, em. 450 nm) and substrate (ex. 340 nm, em. 380 nm) was measured and product formation was calculated using a standard curve. The initial velocities of each reaction were determined from a linear fit to the change in product formation over time. Reaction velocities were normalized to a 0 μM Droxinostat reaction, and each reaction contained 0.1% DMSO. The half maximal inhibitory concentration, IC_{50}, was determined by globally fitting equation S1 to the dependence of relative activity (E_{tot}=0.3 μM for wild-type, 0.5 μM for S39E) on [Droxinostat] (I_{tot}=0-10 μM) at 100 μM substrate from two separate experiments. For wild-type and S39E HDAC8, the IC_{50} was 33 ± 11 nM and 119 ± 14 nM, respectively.

Equation S1. $$\frac{v}{v_0} = M\frac{E_{tot}-I_{tot}-IC_{50}+\sqrt{(E_{tot}-I_{tot}-IC_{50})^2+4E_{tot}IC_{50}}}{2E_{tot}}$$

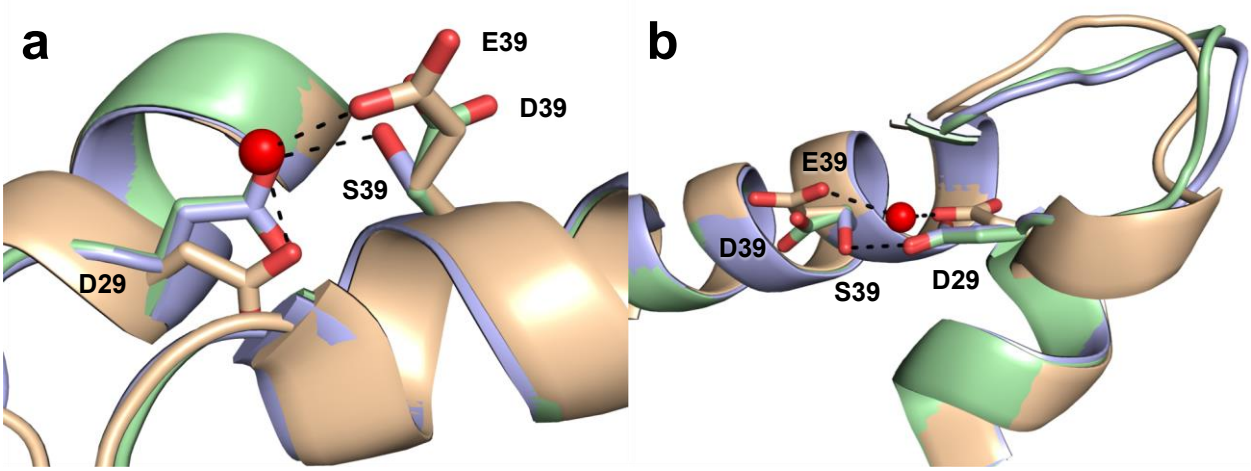

Figure S2. Structural comparison of wild-type, S39D, and S39E HDAC8. (a) Close-up view of a superposition of wild-type HDAC8 (PDB 2V5W, monomer A; C = light blue, O = red), S39D HDAC8 (PDB 2V5X, monomer A; C = light green, O = red), and S39E HDAC8 (PDB 5BWZ, monomer A; C = wheat, O = red, water = red sphere). The hydrogen bond between S39 and D29 is lost in S39D and S39E HDAC8. D29 and its associated helix adopt similar conformations in wild-type and S39D HDAC8; however, D29 undergoes a significant conformational change in S39E HDAC8, which in turn causes a slight twist of the helix containing D29. These structural changes accommodate a hydrogen bonded water molecule that bridges D29 and E39. (b) Superposition of wild-type, S39D, and S39E HDAC8, revealing conformational changes in the L1 loop that are more pronounced in S39E HDAC8 than S39D HDAC8 in comparison with wild-type HDAC8.