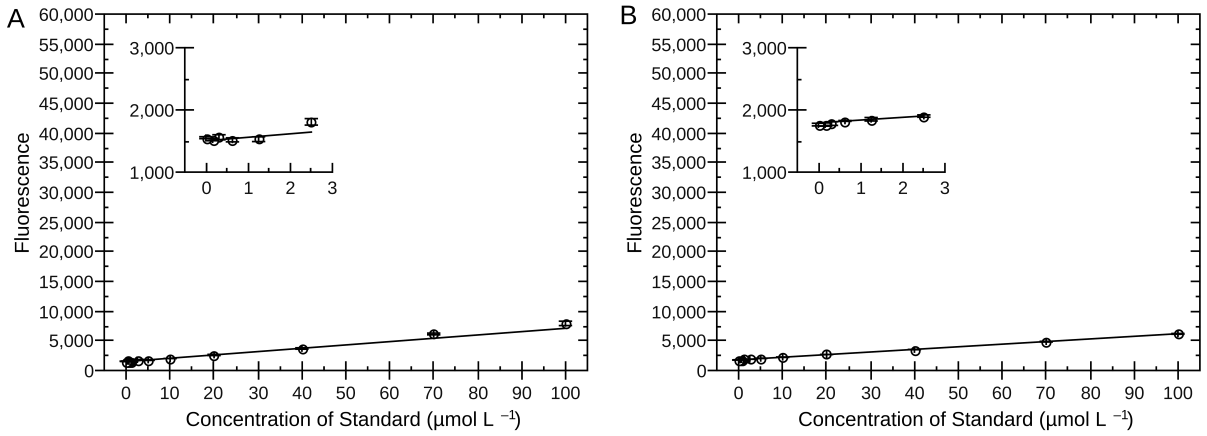

Figure S1. MOPS and HEPES buffers result in loss of sensitivity. Several concentrations of unacetylated lysine (ac-K-NH_2) in MOPS buffer (30 mM MOPS pH 8.0, 150 mM KCl, 5% glycerol; A) or HEPES buffer (50 mM HEPES pH 8.0, 137 mM NaCl, 2.7 mM KCl; B) were treated in the same manner as reactions. Error bars represent the standard deviation of triplicate measurements. Line represents a weighted linear fit to the data. r^2 = 0.9969 (MOPS) and 0.9998 (HEPES). Insets show the same data zoomed to only the low concentrations. At least 2 µM standard is required to obtain a signal above background, and the maximum signal intensity is far lower than in the phosphate buffer (Figure 2).

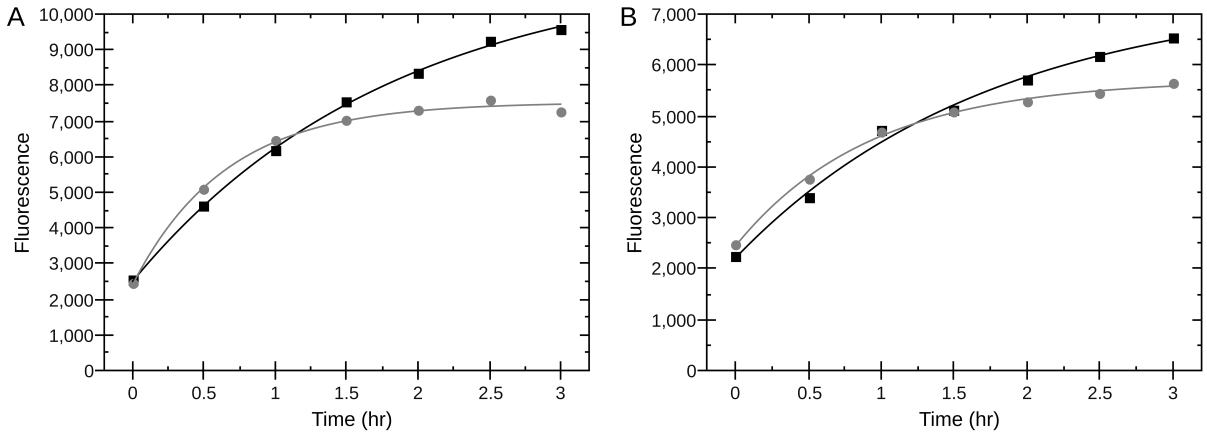

Figure S2. KDAC8 activity at 25 °C versus 37 °C. KDAC8 was incubated with ac-SL{K-ac}FG-am (A) or ac-FA{K-ac}WR-am (B) at either 25 °C (black squares) or 37 °C (gray circles). Aliquots were taken at indicated timepoints and processed as described in the materials and methods. Fluorescence, corresponding to deacetylation, was recorded at each timepoint. At 37 °C, KDAC8 exhibited significant loss of activity after ~1 hr and very little activity after ~1.5 hr; however, the enzyme remained active at 25 °C for the duration of the experiment. Lines represent theoretical fits to emphasize the change in activity over time, illustrating that although the 37 °C reactions begin at faster rates, by 1 hr the total product formation is similar at the two temperatures as indicated by the intersection of the lines. The 37 °C reactions have nearly ceased after 2 hours, as illustrated by the nearly horizontal lines (gray) between 2 and 3 hours and the continued increase in product formation by the 25 °C reactions (black).

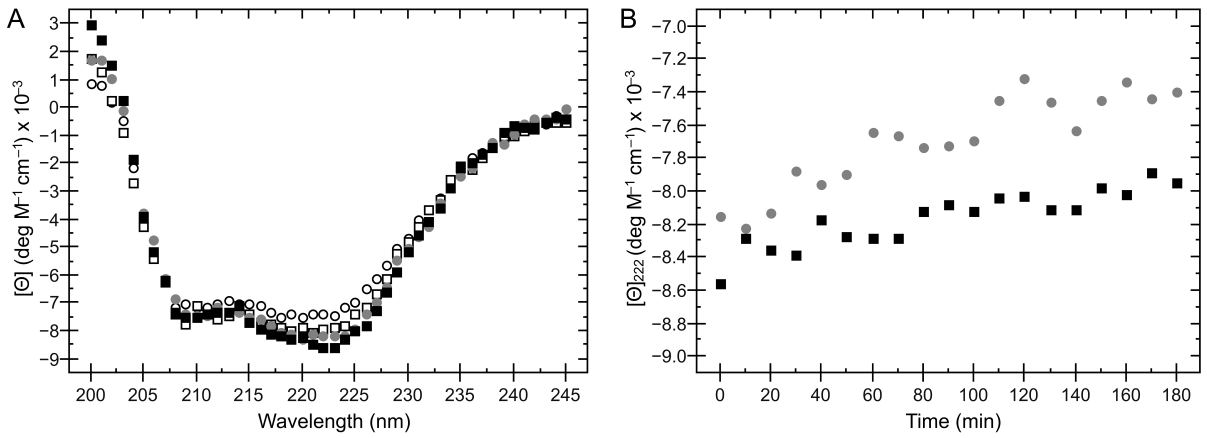

Figure S3. KDAC8 stability at 25 °C versus 37 °C. KDAC8 was incubated in reaction buffer and monitored by circular dichroism spectroscopy as described in materials and methods. (A) Comparison of 25 °C (squares) and 37 °C (circles) for initial measurement (shaded) or after 3 hr (open). The 37 °C sample exhibits significantly more change during the 3 hrs, indicated most prominently by the reduction of signal intensity around 222 nm but also accompanied by some changes in the overall spectrum. (B) The same samples monitored at 222 nm (average of 221-223 nm data points) every 10 minutes at 25 °C (black squares) or 37 °C (gray circles). By 1 hr, the 37 °C sample exhibited a significant decrease in signal intensity, consistent with the loss of activity (Figure S2). At 3 hr, the 25 °C sample has changed little whereas the 37 °C sample has continuously lost signal, indicating ongoing structural effects throughout the experiment.

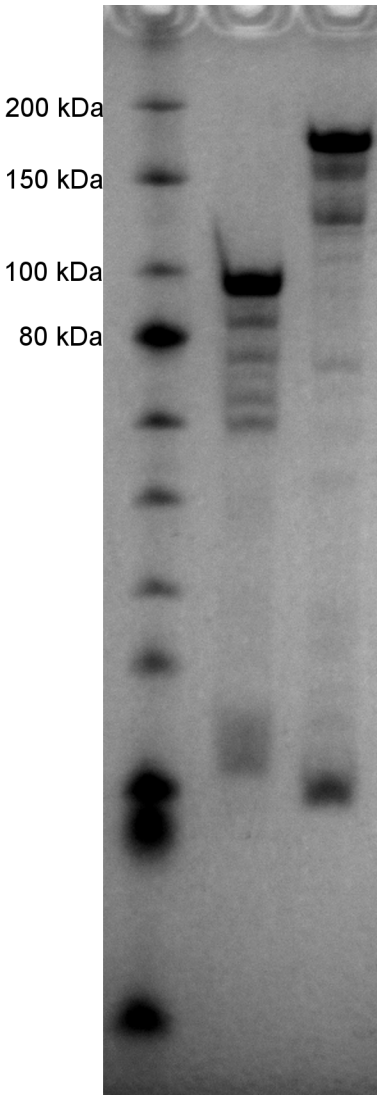

Figure S4. Purity of commercial KDACs. Approximately 2 μg Sirtuin1 (193-741)-GST (lane 2) and HDAC6-GST (lane 3) were subjected to SDS-PAGE and stained with GelCode Blue. The expected mass of the proteins are 87.2 kDa and 159 kDa, respectively. Under the staining conditions, all bands of at least 10 ng are visible. The proteins are substantially less pure than our preparation of KDAC8, consistent with the company estimates of 60-80%.

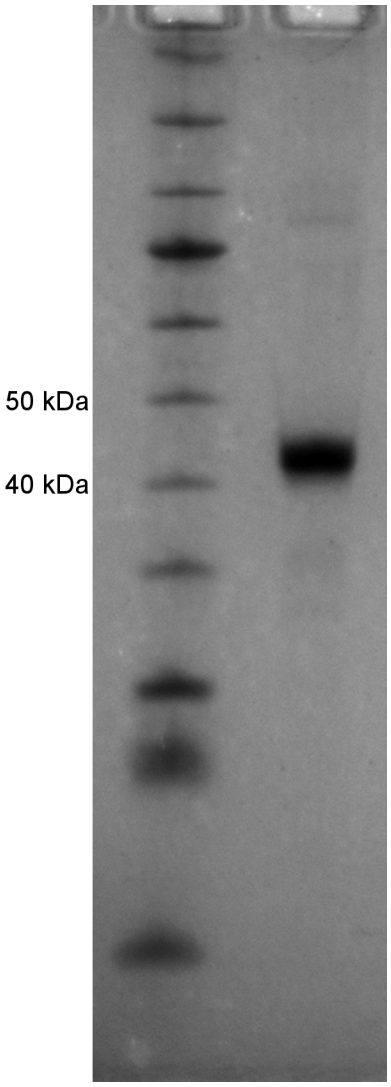

Figure S5. Purified KDAC8. 2 µg KDAC8 purified as described in materials and methods was subjected to SDS-PAGE and stained with GelCode Blue. The expected mass of the protein is 42.5 kDa following cleavage with TEV protease. Under the staining conditions, all bands of at least 10 ng would appear. Resulting protein was ≥95% pure and used for all subsequent activity assays.