Supplement

Purification of metal-dependent lysine deacetylases with consistently high activity

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Figures S1-S4

Table S1

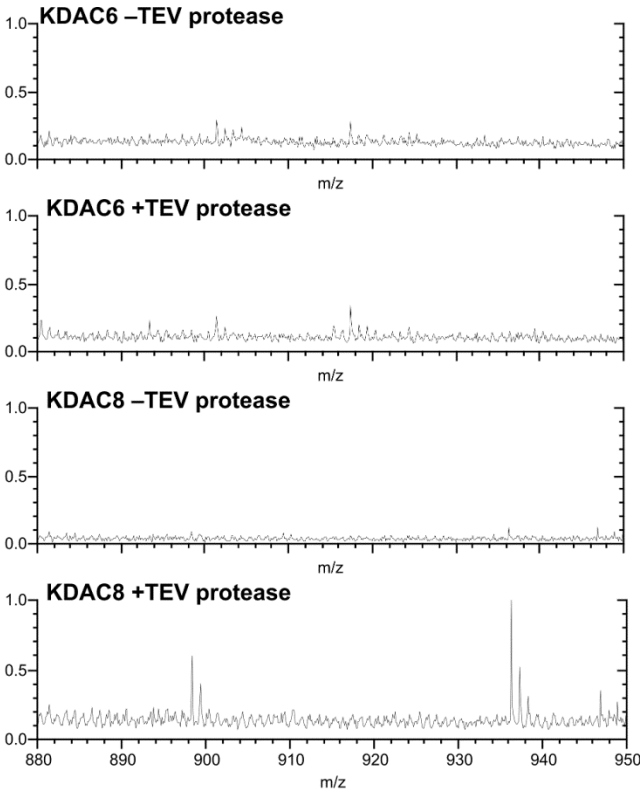

Figure S1. Detection of the cleaved His_6 tag. An equal amount of His_6 tagged KDAC6 and KDAC8 were incubated with TEV protease and the samples were subjected to MALDI-TOF analysis. A peak at the mass corresponding to the cleaved His_6 tag (898 m/z) as well as the tag containing potassium (936 m/z) was detected only in the KDAC8 sample. All spectra are normalized to the intensity of the 936 m/z peak in the KDAC8+TEV spectrum.

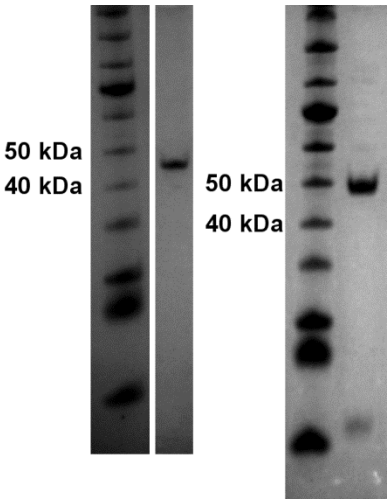

Figure S2. Purified KDAC variants. KDAC8HA (left) and KDAC4HY (right) were purified as described and subjected to SDS-PAGE. Both variants were obtained at similar purity to their wild-type counterparts (Figure 1).

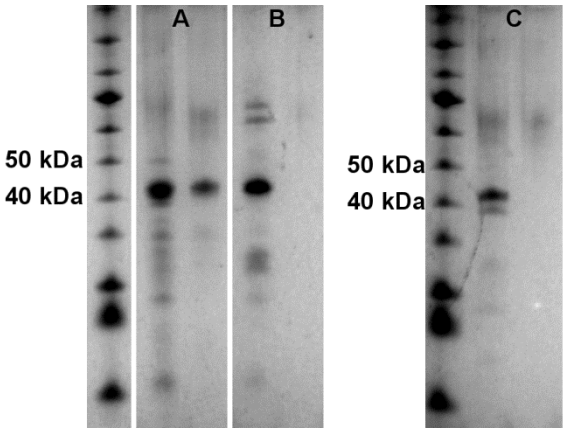

Figure S3. Cobalt resin is critical for secondary purification. KDAC8 was purified using cobalt- (A), nickel- (B) or zinc- (C) containing resin. The resulting protein was subjected to SDS-PAGE to determine whether KDAC8 was successfully recovered. In each gel, lane 1 is the protein after the first column and dialysis with TEV protease, and lane 2 is the protein that flowed through the second column (i.e., the desired final product). The first column resulted in partially purified protein in all three cases; however, KDAC8 was only recovered in the flow-through from the second column when cobalt resin was used.

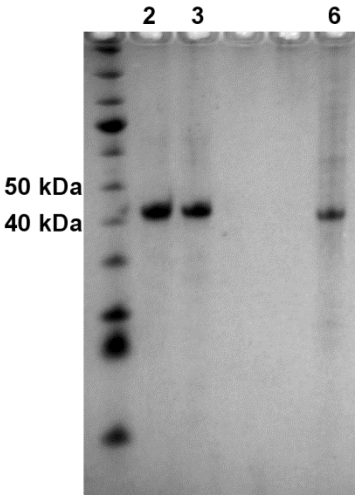

Figure S4. Comparison of purified KDAC8 to commercial KDAC8. Approximately 1 µg of total protein from KDAC8 purified using our purification protocol (lane 2), BPS Bioscience (lane 3), and Novus Biologicals (lane 6) were subjected to SDS-PAGE.

Table S1. Additional cobalt does not increase KDAC8 activity.

Co^{2+} (fold excess)	Activity[a] (pmol min^{-1} μg^{-1})
0	2.12 ± 0.10
0.5	1.96 ± 0.17
1	2.08 ± 0.11
2	2.13 ± 0.03
5	2.72 ± 0.09
10	2.64 ± 0.10
100	2.20 ± 0.19

[a]Activity measured against Fluor-de-Lys substrate {K-ac}-AMC. Error represents standard deviation for technical triplicates.