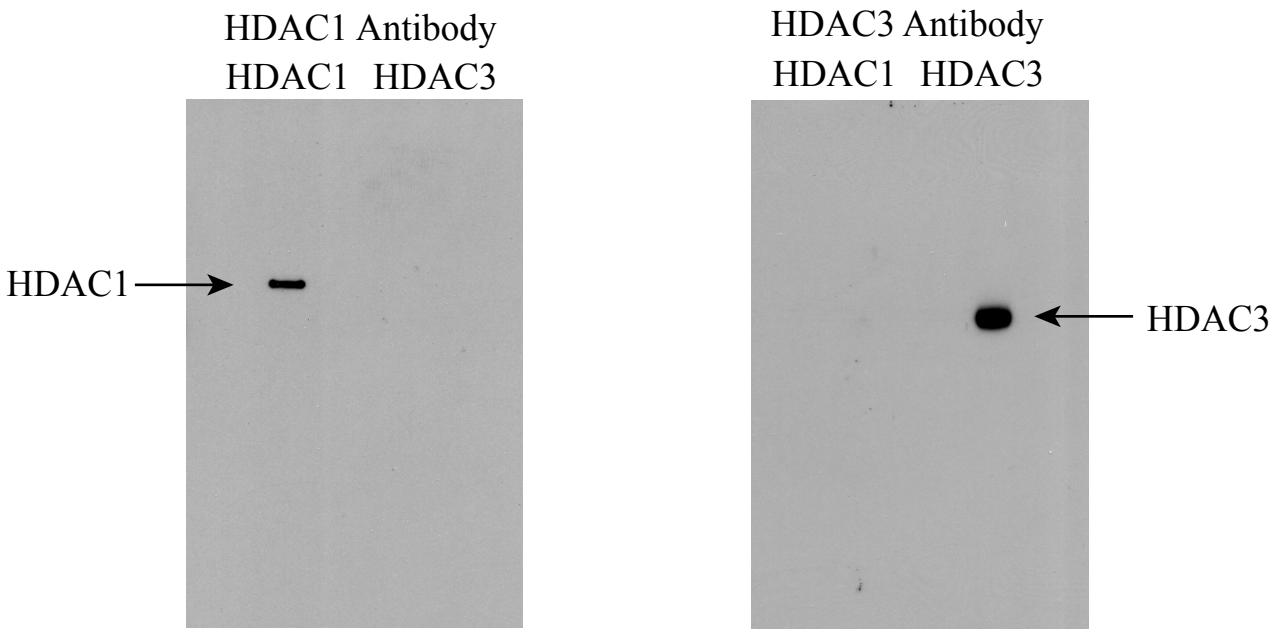
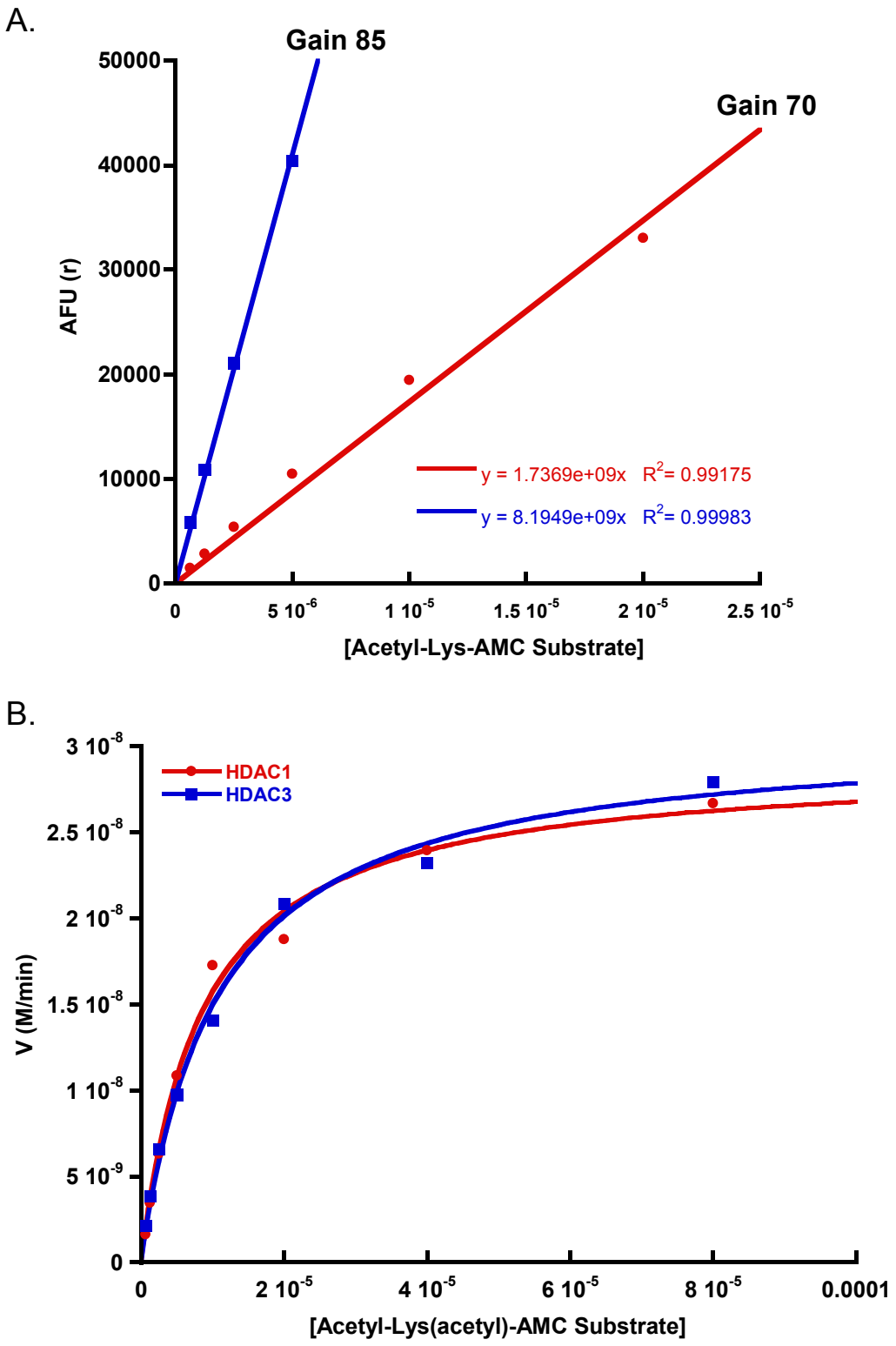
Supplementary Figure 1



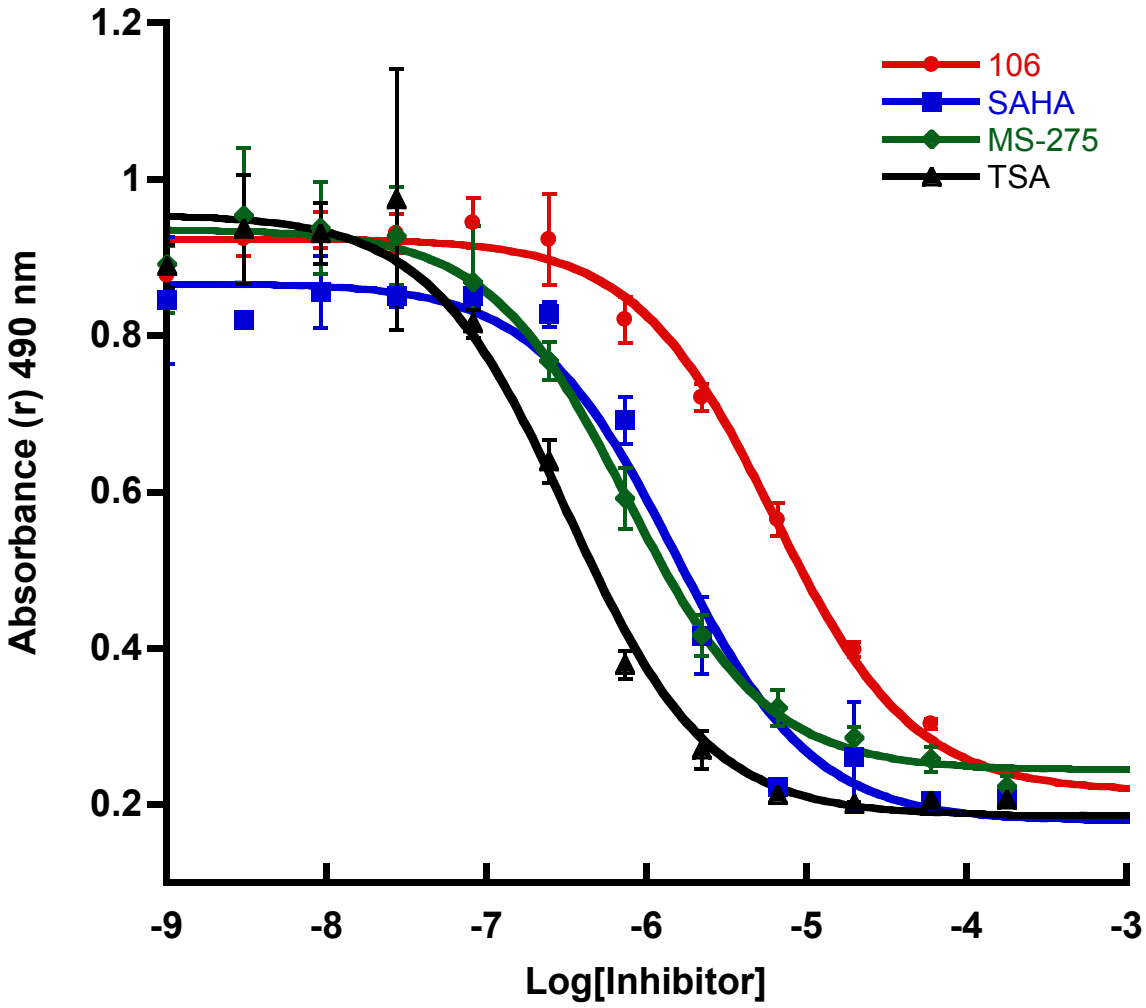
Supplementary Figure 1. Recombinant human HDAC1 and HDAC3 were analyzed by western blotting. No cross contamination of HDAC1 and HDAC3 was detected. The recombinant human HDAC1 and HDAC3 were also tested against HDAC2 and HDAC8 antibodies, and there is no cross contamination (data not shown).

Supplementary Figure 2



Supplementary Figure 2. *A*. Acetyl-Lys-AMC substrate has a direct linear relationship with V (M/min), at both gain 70 and gain 85 of the Tecan plate reader instrument. *B*. The substrate K_m for both HDAC1 and HDAC3 was determined to be ~ 10 μM.

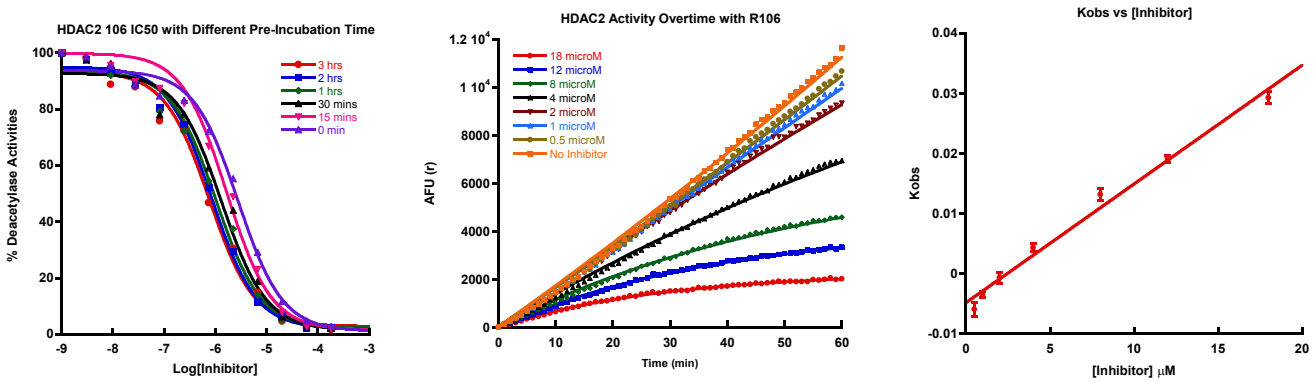
Supplementary Figure 3



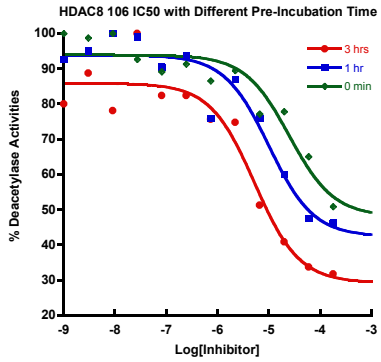
Supplementary Figure 3. MTS assays were used to assess the relative toxicities of HDAC inhibitors in GM15850 cells. Inhibitor **106** has the highest EC_{50} at 6.3 (± 0.9) μM (lowest toxicity) in comparison to MS-275 (a benzamide) at 768 (± 74) nM, and two hydroxamates TSA at 328 (± 57) nM, and SAHA at 1.5 (± 0.3) μM.

Supplementary Figure 4

A.

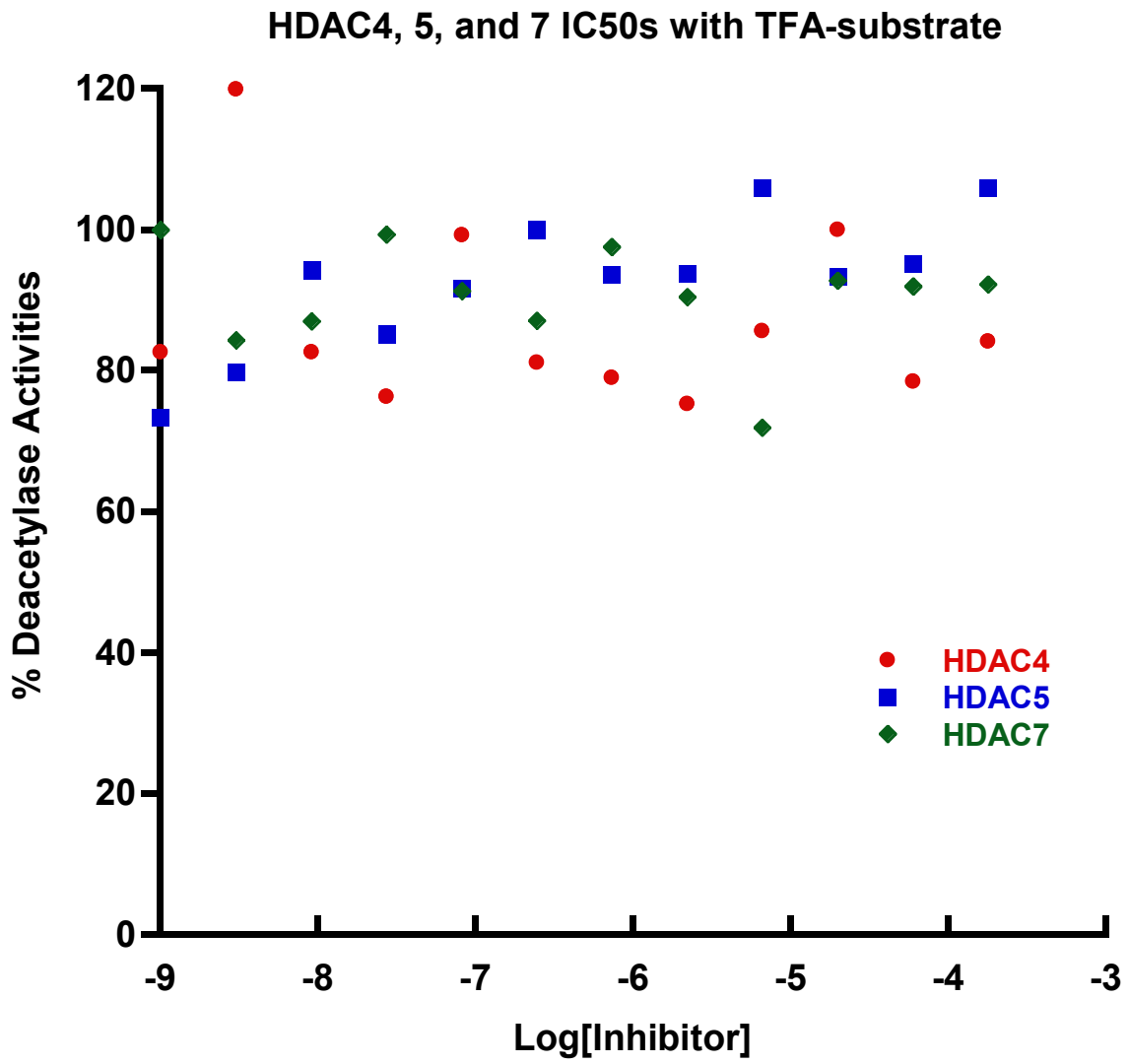


B.



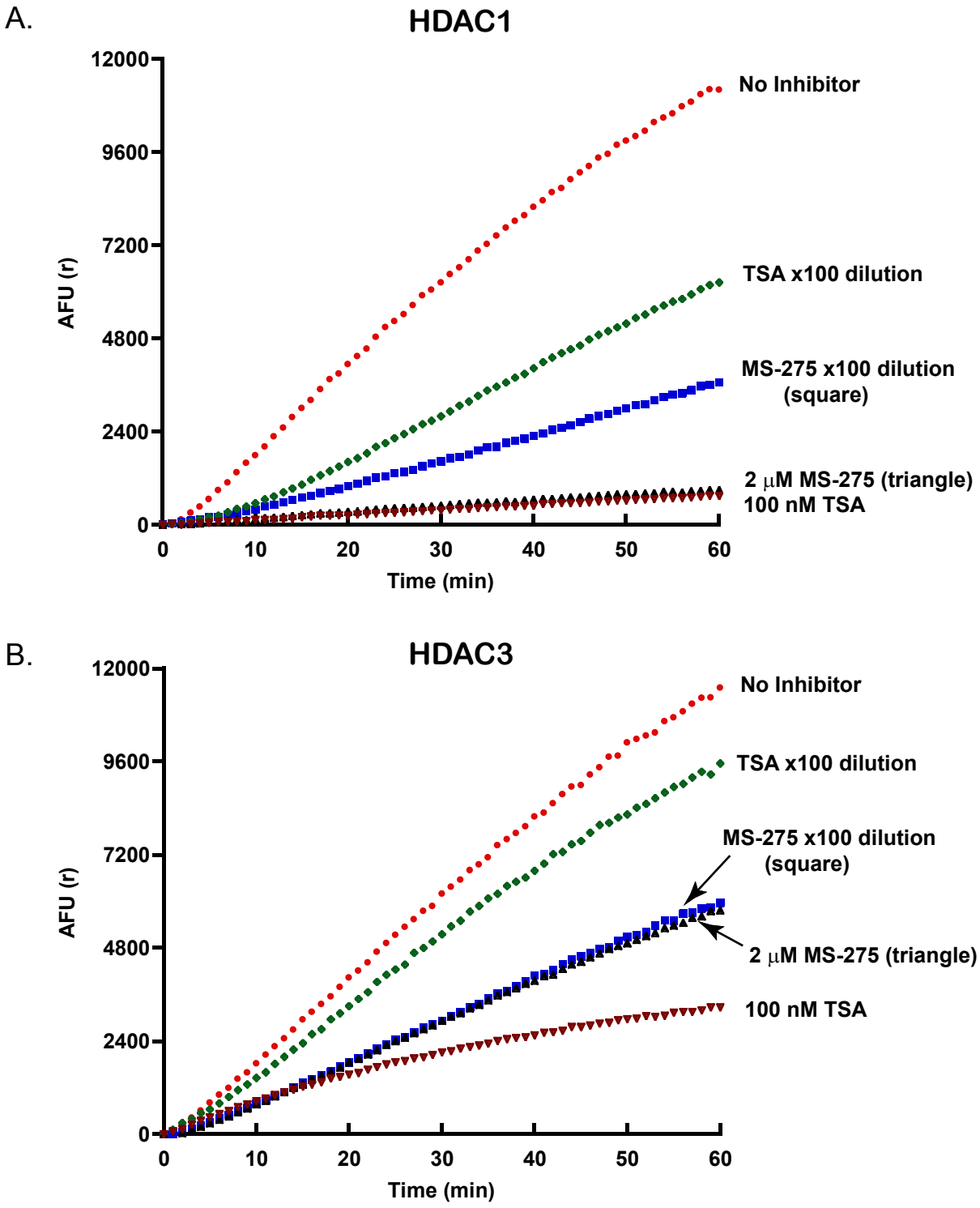
Supplementary Figure 4. *A*. Inhibitor **106** has different HDAC2 IC_{50}s at different pre-incubation times. **106**/HDAC2 IC_{50}s comes to equilibrium after 30 minutes at approximately 800 nM (left). The kinetic curves were determined, and HDAC2 appears to follow mechanism 1 similar to HDAC1 (middle and right). The K_i of HDAC2 was determined using the three highest concentrations. The low concentration points gave a negative k_{-1} values (right). The K_i of **106** for HDAC2 is ~100 nM. *B*. HDAC8 IC_{50}s were determined at three different concentrations. Even after a 3 hr of pre-incubation, the particular IC_{50} is still above 5 μM. The kinetic curves cannot be obtained due to the high sensitivity of HDAC8 to the peptidase.

Supplementary Figure 5



Supplementary Figure 5. Inhibitor 106 has no acitivity against HDAC4, HDAC5, and HDAC7. The titration curves are essentially flat from ~ 1 nM to 180 μM inhibitor concentration.

Supplementary Figure 6



Supplementary Figure 6. *A*. Dilution experiments for TSA and MS-275 with HDAC1. MS-275 behaved similarly to inhibitor **106** with a moderate slow off-rate with HDAC1. TSA, as expected, lost the majority of its inhibition capacity immediately after the 100-fold dilution (from 100 nM to 1 nM). *B*. Dilution experiment for TSA and MS-275 with HDAC3. MS-275 behaved again similarly to inhibitor **106** with a very slow off-rate with HDAC3. TSA, as expected, lost the majority of its inhibition capacity immediately after the 100-fold dilution (from 100 nM to 1 nM).