

Supporting Information

Lysine deacetylases exhibit distinct changes in activity profiles due to fluorophore-conjugation of substrates

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Table S1. NMR peaks for {K-ac} in d₆-DMSO.

| Proton(s) | δ (ppm) |
|---|----------------|
| N α (NH) | 7.35 (s, 1H) |
| N α acetyl methyl (CH ₃) | 1.84 (s, 3H) |
| C α (CH) | 4.13 (m, 1H) |
| C α amide (NH ₂) | 7.86 (d, 2H) |
| C β (CH ₂) | 1.68 (m, 2H) |
| C γ (CH ₂) | 1.30 (m, 2H) |
| C δ (CH ₂) | 1.55 (m, 2H) |
| C ϵ (CH ₂) | 2.98 (q, 2H) |
| N ϵ (NH) | 6.94 (s, 1H) |
| N ϵ acetyl methyl (CH ₃) | 1.78 (s, 3H) |

Table S2. Endpoint specific activity ($\times 10^{-3} \text{ s}^{-1}$) for KDACs with 100 μM peptide substrates

| Substrate | KDAC8 | KDAC6 | KDAC4HY | Sirt1 |
|--------------------|---------------|---------------|----------------|----------------|
| {K-ac} | < 0.1 | 1.1 \pm 0.2 | 1.1 \pm 0.9 | 1.0 \pm 2.0 |
| {K-ac}W | 1.5 \pm 0.9 | 70 \pm 30 | 1.8 \pm 0.2 | 14 \pm 10 |
| {K-ac}-AMC | 1.8 \pm 0.5 | 190 \pm 30 | 510 \pm 60 | 0.6 \pm 0.1 |
| RG{K-ac} | < 0.1 | 22 \pm 11 | 2.2 \pm 1.4 | 3.5 \pm 1.0 |
| RG{K-ac}W | 1.4 \pm 1.1 | 100 \pm 30 | 3.4 \pm 0.7 | 30.5 \pm 1.3 |
| RG{K-ac}-AMC | 3.8 \pm 1.7 | 190 \pm 50 | 460 \pm 60 | 1.0 \pm 0.3 |
| RH{K-ac}{K-ac} | 4.3 \pm 1.8 | 25 \pm 9 | 16 \pm 4 | 27.6 \pm 1.6 |
| RH{K-ac}{K-ac}W | 9.2 \pm 1.4 | 90 \pm 40 | 26.4 \pm 1.6 | 57.3 \pm 0.9 |
| RH{K-ac}{K-ac}-AMC | 24 \pm 4 | 230 \pm 40 | 430 \pm 90 | 1.0 \pm 0.1 |

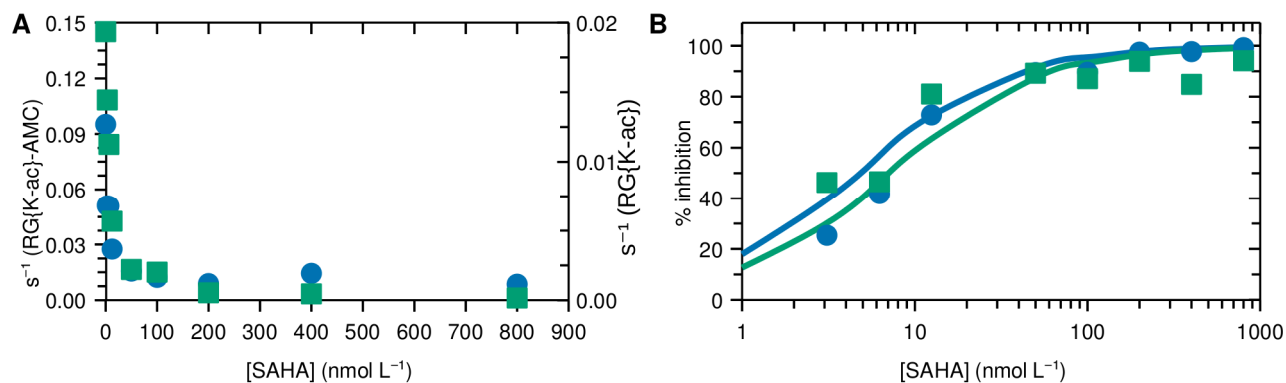


Figure S1. SAHA titrations of KDAC6. Endpoint assays of KDAC6 with RG{K-ac} (blue circles) or RG{K-ac}-AMC (green squares) in the presence of variable amounts of SAHA, performed as described in the methods where reactions were supplemented by 0-800 nmol L⁻¹ SAHA. (A) Specific activity. Note the use of two vertical axis scales. (B) Data plotted as percent inhibition, where lines represent best-fit to the data. Calculated IC₅₀ values are 4.7 ± 0.9 nmol L⁻¹ and 7.2 ± 0.7 nmol L⁻¹, respectively.