Supporting Information

Mutagenesis Studies of the 14 Å Internal Cavity of Histone Deacetylase 1: Insights towards the Acetate Escape Hypothesis and Selective Inhibitor Design

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I. Primer sequences used in HDAC1 mutagenesis

HDAC1 mutants were created in pBJ5HDAC1(*1, 2*) using the Not1 and ECOR1 restriction sites via a two step PCR using the following primer sequences (mutated codon is underlined, if present):

pBJ5 Not1 start, GCCAGAATGCGGCCGCATGGCGCAG**;**

pBJ5 153 rev, TATCATGTCTGGATCCGG;

HDAC1 V19A rev, CATCCCCGTCGTAGTAGTAACAGAC;

HDAC1 V19A for, GTTACTACTACGACGGGGATGCTGGAAATTACTATTATGG**;**

HDAC1 Y23/Y24 rev, GTAATTTCCAACATCCCCG;

HDAC1 Y23A for, CGGGGATGTTGGAAATTACGCATATGGACAAGGCCACCCAATGAAGCC;

HDAC1 Y23F for, CGGGGATGTTGGAAATTACTTCTATGGACAAGGCCACCCAATGAAGCC;

HDAC1 Y23C for, CGGGGAT GTTGGAAATTACTGTTATGGACAAGGCCACCCAATGAAGCC;

HDAC1 Y23H for, CGGG GATGTTGGAAATTACCATTATGGACAAGGCCACCCAATGAAGCC;

HDAC1 Y23R for, CGGGGATGTTGGA AATTACCGATATGGACAAGGCCACCCAATGAAGCC;

HDAC1 Y24A for, CGGGGATGTTGGAAATTACTATGCAGGACAAGGCCACCCAATGAAGCC;

HDAC1 Y24F for, CGGGGATGTTGGAAATTACTATTTCGGACAAGGCCACCCAATGAAGCC;

HDAC1 M30A rev, GGCTTCATTGGGTGGCCTTGTCC;

HDAC1 M30A for, GGACAAGGCCACCCAGCGAAGCCTCACCGAATC;

HDAC1 R34A rev, GGCTTCATTGGGTGGCCTTGTCC;

HDAC1 R34A for, GGCCACCCAATGAAGCCTCACGCAATCCGCATGACTCATAATTTGCTG;

HDAC1 R34K for, GGCCACCCAATGAAGCCTCACAAGATCCGCATGACTCATAATTTGCTG;

HDAC1 I35A rev: CGGTGAGGCTTCATTGGGTGG;

HDAC1 I35A for, CCCAATGAAGCCTCACCGAGCCCGCATGACTCATAATTTGCTGC;

HDAC1 R36A for**,** CCCAATGAAGCCTCACCGAATCGCCATGACTCATAATTTGCTGC;

HDAC1 R36K for, CCCAATGAAGCCTCACCGAATCAAAATGACTCATAATTTGCTGC;

HDAC1 F109A rev, CTCAAACAGGCCATCGAATACTGG

HDAC1 F109A for, CCAGTATTCGATGGCCTGTTTGAGGCCTGTCAGTTGTCTACTGGTGG

HDAC1 S113A rev, CAACTGACAGAACTCAAACAGGCC;

HDAC1 S113A for, GGCCTGTTTGAGTTCTGTCAGTTGGCAACTGGTGGTTCTGTGGC;

HDAC1 L139A rev, GCCCCCAGCCCAATTCACAGCG;

HDAC1 L139A for, GGGCTGGGGGCGCGCACCATGCAAAGAAGTCCGAGGC;

HDAC1 C151A rev, GAAGCCAGATGCCTCGGACTTC;

HDAC1 C151A for, GTCCGAGGCATCTGGCTTCGCTTACGTCAATGATATC;

HDAC1 C151S for, GTCCGAGGCATCTGGCTTCTCTTACGTCAATGATATC;

HDAC1 Y303A rev, CCACCGCCTCCCAGCATCAGC;

HDAC1 Y303A for, GGGAGGCGGTGGGCTCACCATTCGTAACGTTGCCCGG;

HDAC1 Y303F for, GGGAGGCGGTGGTTTCACCATTCGTAACGTTGCCCGG

II. Deacetylase activity data for all HDAC1 mutants

Table S1. Percent deacetylase activities for HDAC1 Y23A, Y24A, R34A and C151A mutants[a]

Sample	Mean ± Error
No protein	16 ± 4.4%
HDAC1 H141A	20 ± 1.6%
Wild Type HDAC1	100%
HDAC1 Y23A	35 ± 7.3%
HDAC1 Y24A	26 ± 5.8%
HDAC1 R34A	31 ± 6.6%
HDAC1 C151A	21 ± 4.6%

[a]The mean deacetylase activities for each mutant is a percent of the wild type (see to 100%) and the standard error of at least four independent trials is shown (Data displayed as a histograms in Figure 3A of the manuscript).

Table S2. Percent deacetylase activities for additional alanine mutants[a]

Sample	Mean± Error
No protein	10 ± 1.9%
HDAC1 H141A	20 ± 1.6%
Wild Type HDAC1	100%
HDAC1 V19A	31 ± 4.7%
HDAC1 M30A	20 ± 6.4%
HDAC1 135A	5.8 ± 2.1%
HDAC1 R36A	25 ± 3.7%
HDAC1 F109A	20 ± 2.7%
HDAC1 S113A	62 ± 4.5%
HDAC1 L139A	39 ± 0.9%
HDAC1 Y303A	6.3 ± 1.7%

[a]The mean deacetylase activities for each mutant is a percent of the wild type (see to 100%) and the standard error for at least four independent trials is shown (see histogram Figure 4A in manuscript).

Table S3. Percent deacetylase activities for substitution mutants[a]

Sample	Mean± Error
No protein	10 ± 1.9%
HDAC1 H141A	20 ± 1.6%
Wild Type	100%
HDAC1 Y23F	12 ± 3.5%
HDAC1 Y23C	36 ± 9.8%
HDAC1 Y23H	12 ± 2.7%
HDAC1 Y23R	26 ± 3.0%
HDAC1 Y24F	50 ± 8.2%
HDAC1 R34K	17 ± 2.8%
HDAC1 R36K	33 ± 3.8%
HDAC1 C151S	30 ± 1.3%
HDAC1 Y303F	38 ± 6.0%

[a]The mean deacetylase activities for each mutant is a percent of the wild type (set at 100%) and the standard error of at least three independent trials is shown (Figure 5A in the manuscript).

III. Acetate inhibition experimental data

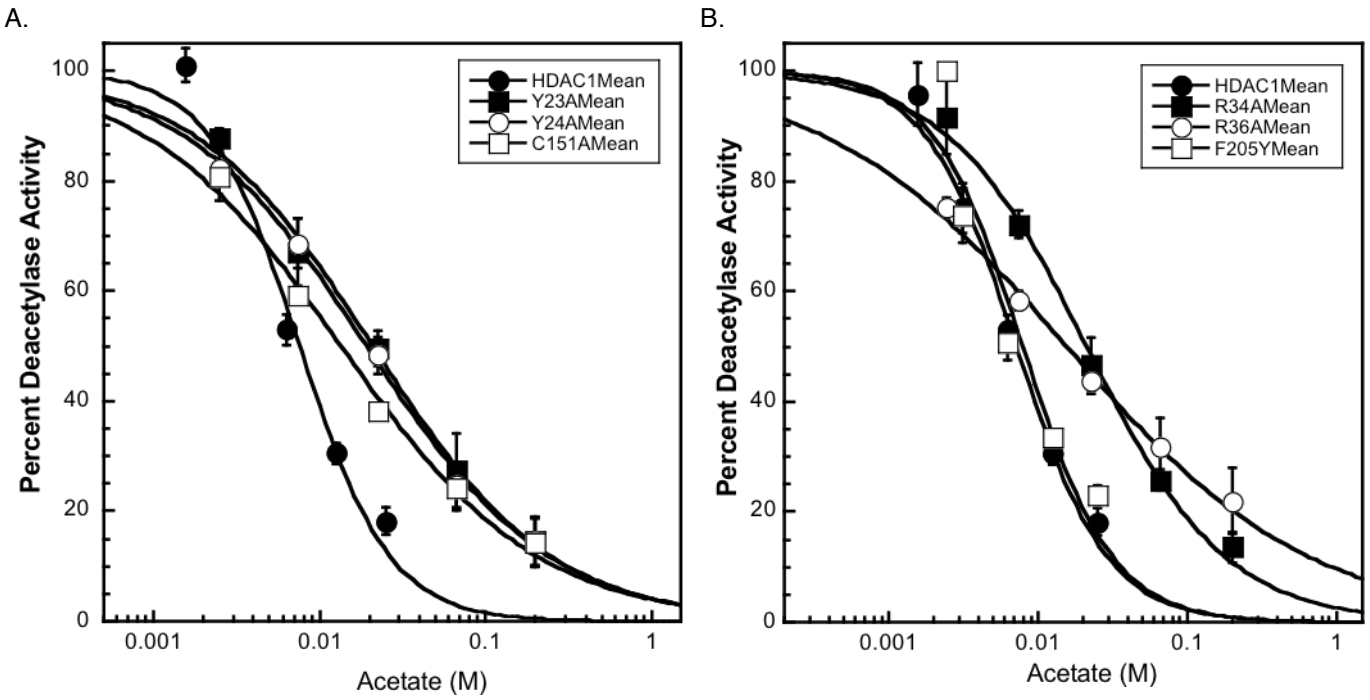

Figure S1. Dose dependent curves for acetate inhibition of wild type and mutant HDAC1. Wild type or mutant proteins were expressed in T-Ag Jurkat cells as FLAG-tagged fusion proteins, immunoprecipitated with anti-FLAG-agarose bead, and tested for catalytic activity in the absence or presence of varying concentrations of acetate (0-200mM). The standard error for at least 4 trials is shown. IC50 values derived from these curves in shown in Table 1.

Table S4. Percentage remaining HDAC activity after incubation of acetate with HDAC1

Concentration (M)	Mean± Error
2.5×10^{-2}	18 ± 2.5 %
1.25×10^{-2}	31 ± 2.0 %
6.25×10^{-3}	53 ± 2.8 %
3.125×10^{-3}	75 ± 4.5 %
1.56×10^{-3}	96 ± 5.7 %

Table S5. Percentage remaining HDAC activity after incubation of acetate with HDAC1F205Y

Concentration (M)	Mean± Error
2.5×10^{-2}	24 ± 1.8 %
1.25×10^{-2}	34 ± 0.7 %
6.25×10^{-3}	51 ± 3.2 %
3.125×10^{-3}	74 ± 5.0 %
1.56×10^{-3}	99 ± 1.2 %

Table S6. Percentage remaining HDAC activity after incubation of acetate with HDAC1Y23A

Concentration (M)	Mean± Error
2.0×10^{-1}	15 ± 4.5 %
6.6×10^{-2}	27 ± 6.7 %
2.25×10^{-2}	50 ± 3.2 %
7.4×10^{-3}	67 ± 6.1 %
2.46×10^{-3}	86 ± 1.9 %

Table S7. Percentage remaining HDAC activity after incubation of acetate with HDAC1 Y24A

Concentration (M)	Mean± Error
2.0×10^{-1}	15 ± 4.4%
6.6×10^{-2}	25 ± 4.3%
2.25×10^{-2}	48 ± 3.4 %
7.4×10^{-3}	69 ± 4.5 %
2.46×10^{-3}	82 ± 1.2 %

Table S8. Percentage remaining HDAC activity after incubation of acetate with HDAC1 R34A

Concentration (M)	Mean± Error
2.0×10^{-1}	14 ± 2.7 %
6.6×10^{-2}	26 ± 2.0 %
2.25×10^{-2}	47 ± 4.8 %
7.4×10^{-3}	73 ± 2.6 %
2.46×10^{-3}	92 ± 6.7 %

Table S9. Percentage remaining HDAC activity after incubation of acetate with HDAC1 R36A

Concentration (M)	Mean± Error
2.0×10^{-1}	22 ± 6.0 %
6.6×10^{-2}	32 ± 5.3 %
2.25×10^{-2}	44 ± 2.3 %
7.4×10^{-3}	58 ± 1.7 %
2.46×10^{-3}	75 ± 1.7 %

Table S10. Percentage remaining HDAC activity after incubation of acetate with HDAC1 C151A

Concentration (M)	Mean± Error
2.0×10^{-1}	14 ± 4.3 %
6.6×10^{-2}	24 ± 4.3 %
2.25×10^{-2}	38 ± 0.3 %
7.4×10^{-3}	59 ± 1.3 %
2.46×10^{-3}	81 ± 4.4 %

IV. Compound 1 experimental data

Table S11. Percentage remaining HDAC activity after incubation of **1** with HDAC1

Concentration (M)	Mean± Error
2.0×10^{-7}	30 ± 3.5 %
1.0×10^{-7}	36 ± 1.1 %
5.0×10^{-8}	44 ± 1.7 %
2.5×10^{-8}	59 ± 5.8 %

Table S12. Percentage remaining HDAC activity after incubation of **1** with HDAC1 S113A

Concentration (M)	Mean± Error
4.0×10^{-7}	36 ± 3.0 %
2.0×10^{-7}	53 ± 1.6 %
1.0×10^{-7}	60 ± 1.9 %
5.0×10^{-8}	78 ± 2.1 %

V. References

1. Taunton, J., Hassig, C. A., and Schreiber, S. L. (1996) A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p, *Science 272*, 408-411.
2. Pflum, M. K., Tong, J. K., Lane, W. S., and Schreiber, S. L. (2001) Histone deacetylase 1 phosphorylation promotes enzymatic activity and complex formation, *The Journal of biological chemistry 276*, 47733-47741.