SUPPLEMENTARY INFORMATION

Distribution and diversity of classical deacylases in bacteria

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[6]Current address: Institute of Chemical Sciences and Engineering (ISIC), School of Basic Sciences (SB), EPFL, 1015 Lausanne, Switzerland

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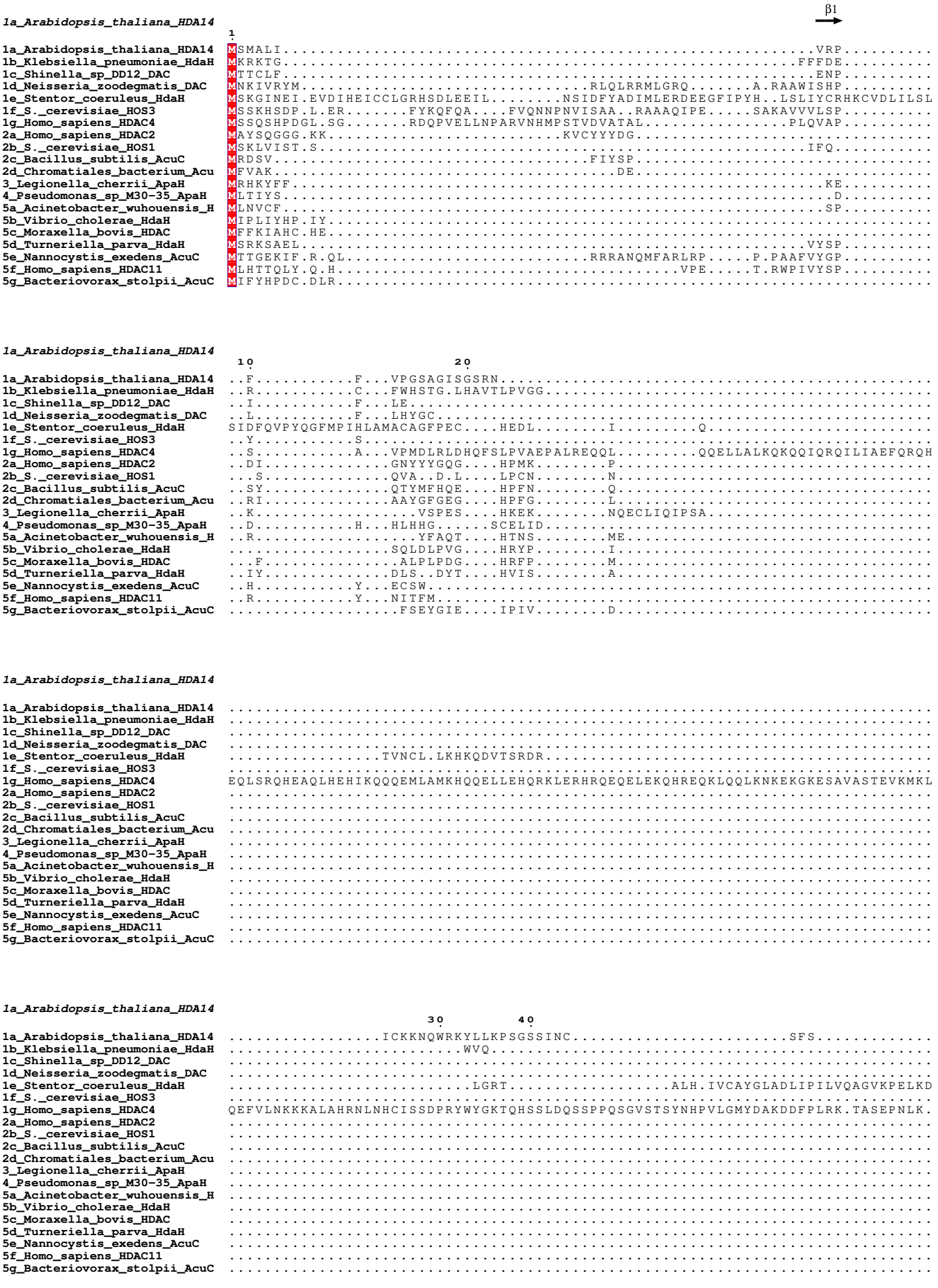
Supplementary Figure 1-28

Supplementary Table 1-10

a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1g_Homo_sapiens_HDAC4
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2b_S._cerevisiae_HOS1
2c_Bacillus_subtilis_AcuC
2d_Chromatiales_bacterium_Acu
3_Legionella_cherrii_ApaH
4_Pseudomonas_sp_M30-35_ApaH
5a_Acinetobacter_wuhouensis_H
5b_Vibrio_cholerae_HdaH
5c_Moraxella_bovis_HDAC
5d_Turneriella_parva_HdaH
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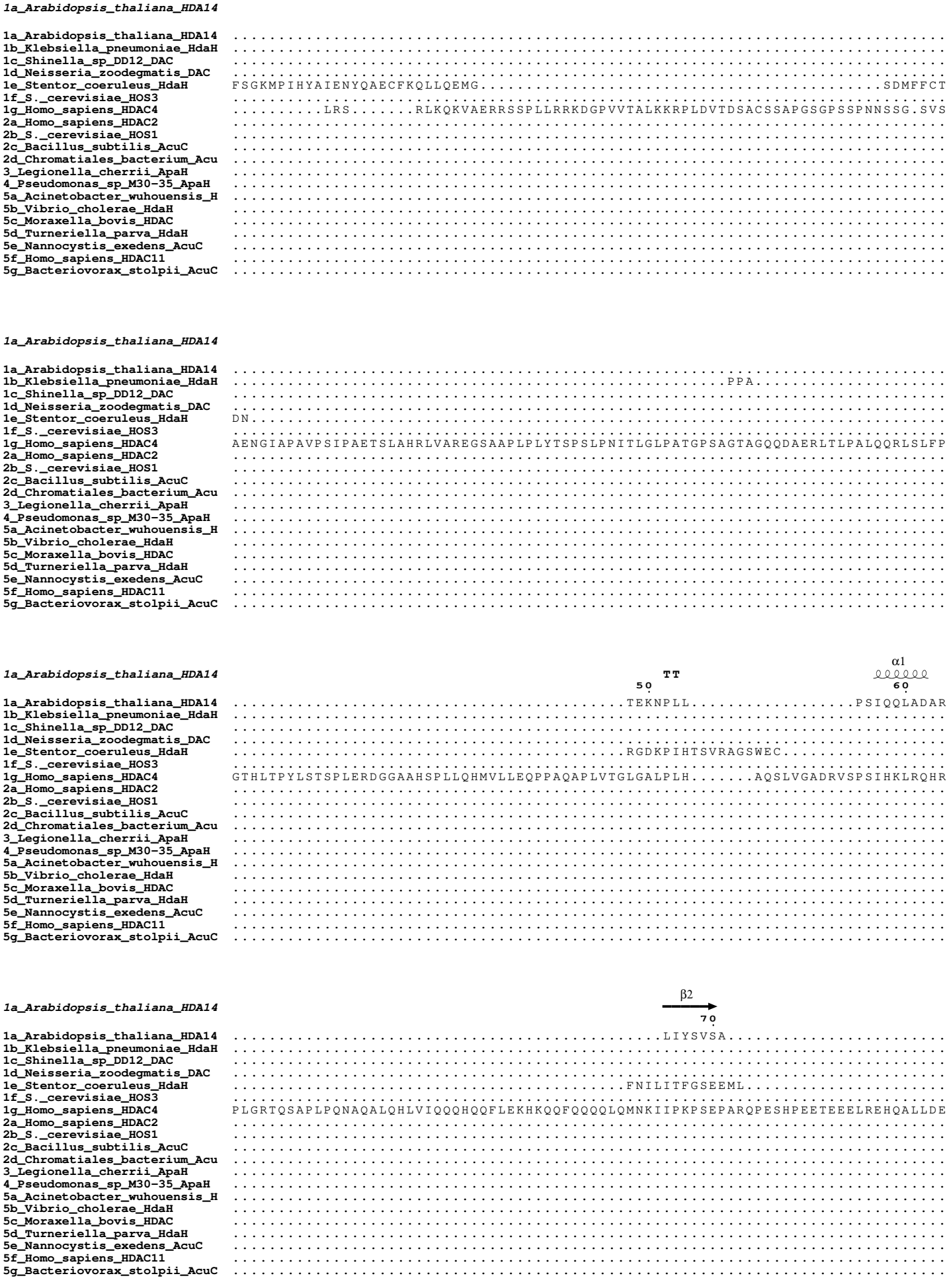
5g_Bacteriovorax_stolpii_AcuC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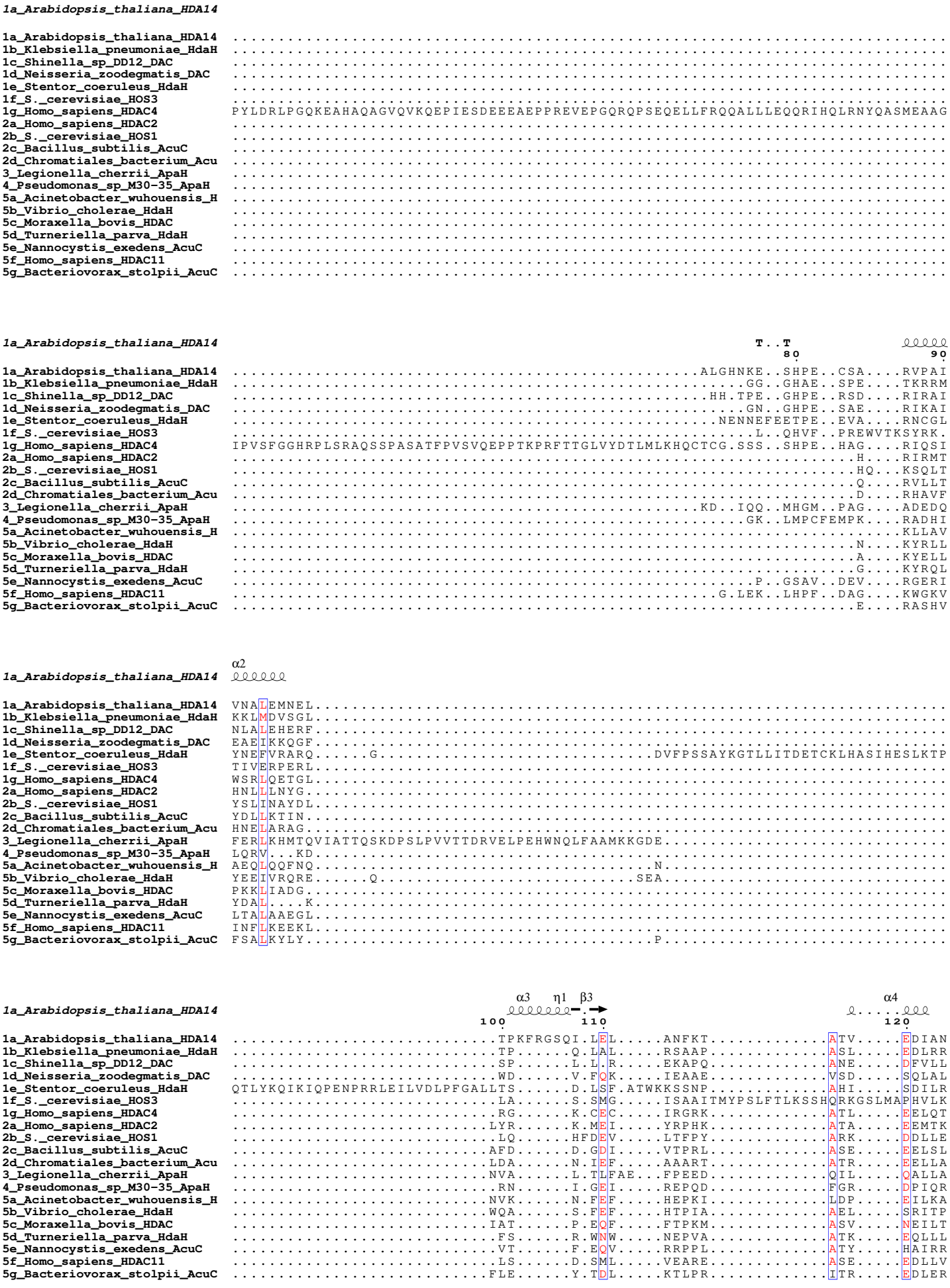
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β3
α4
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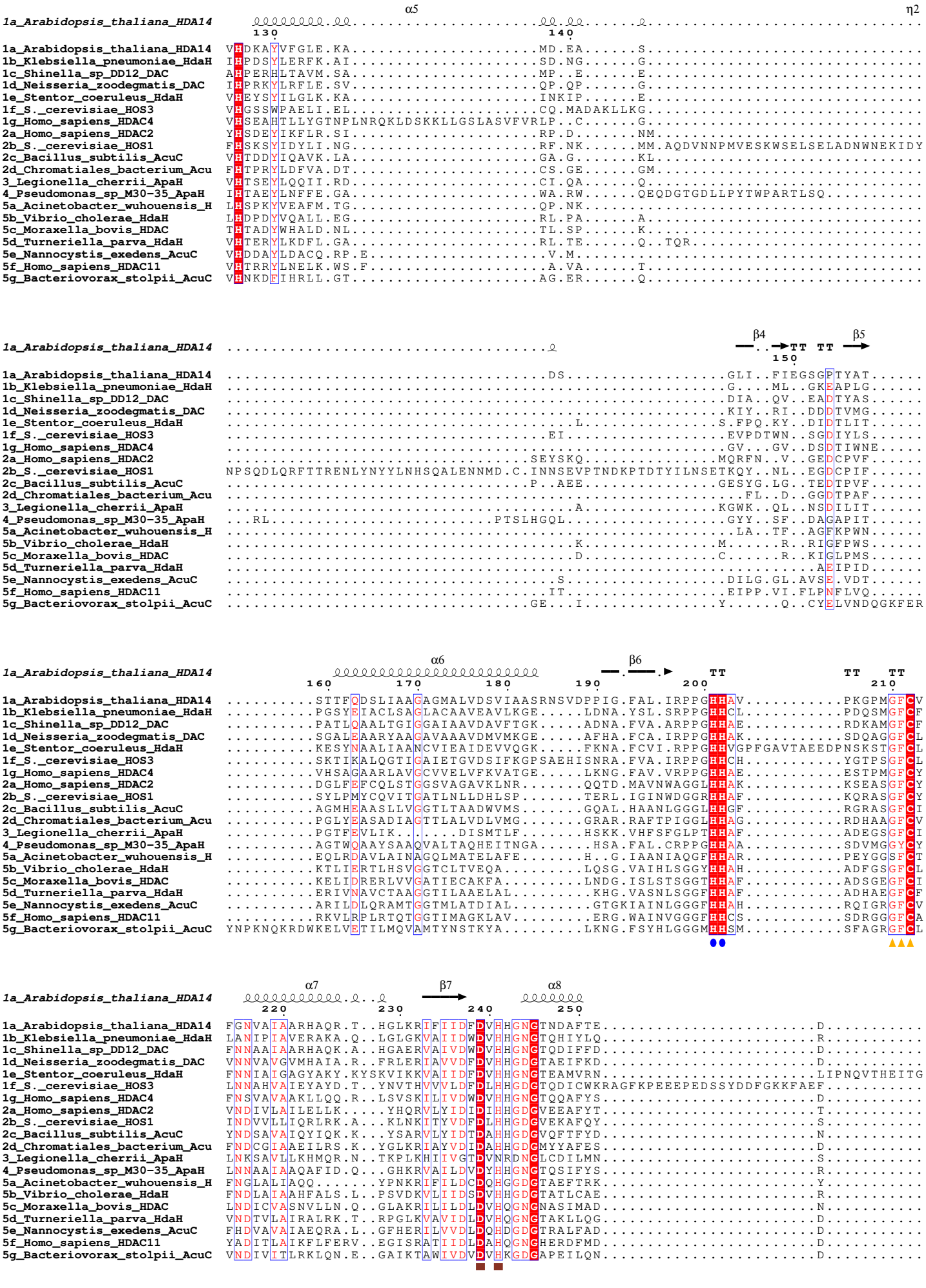

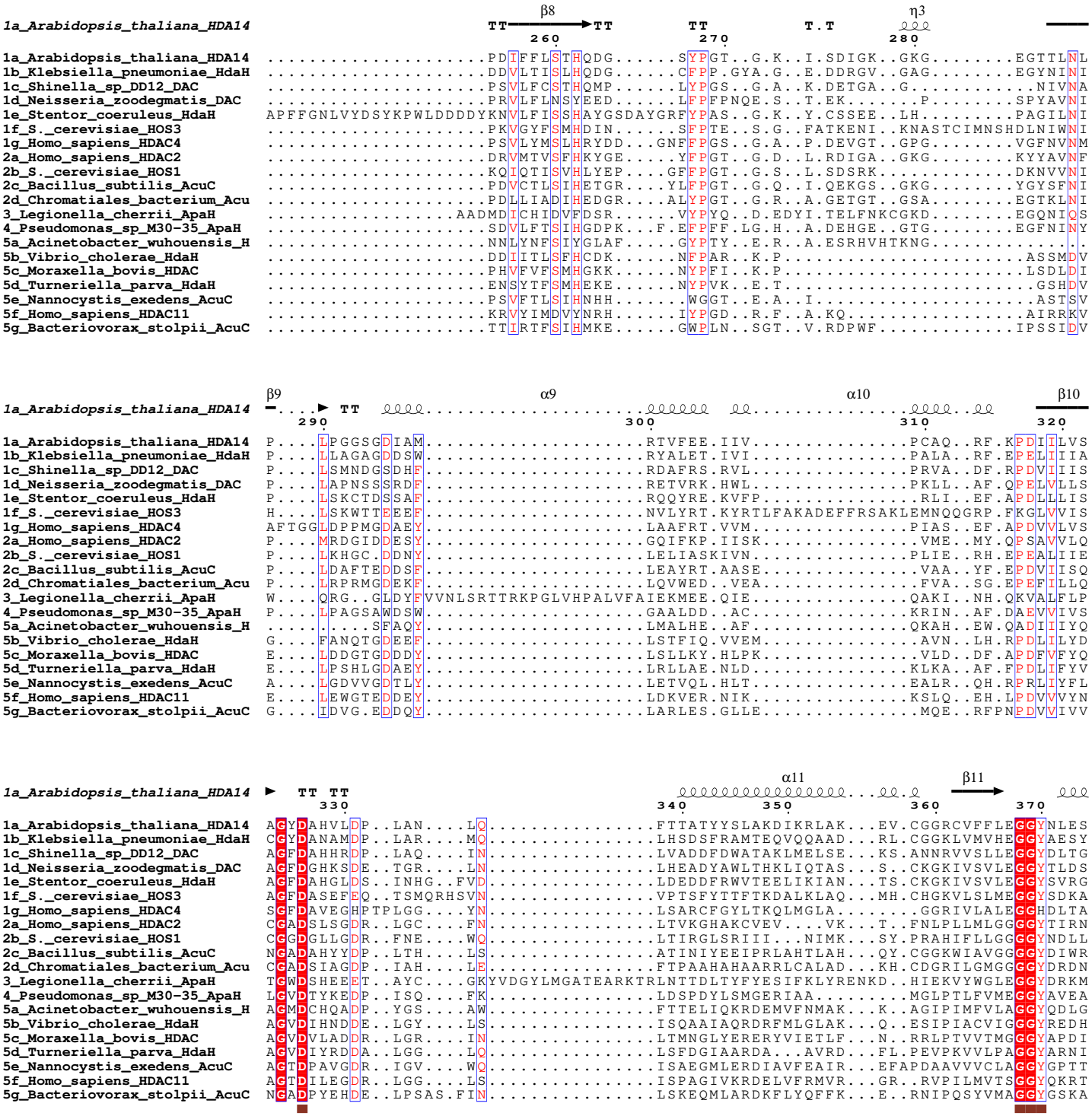
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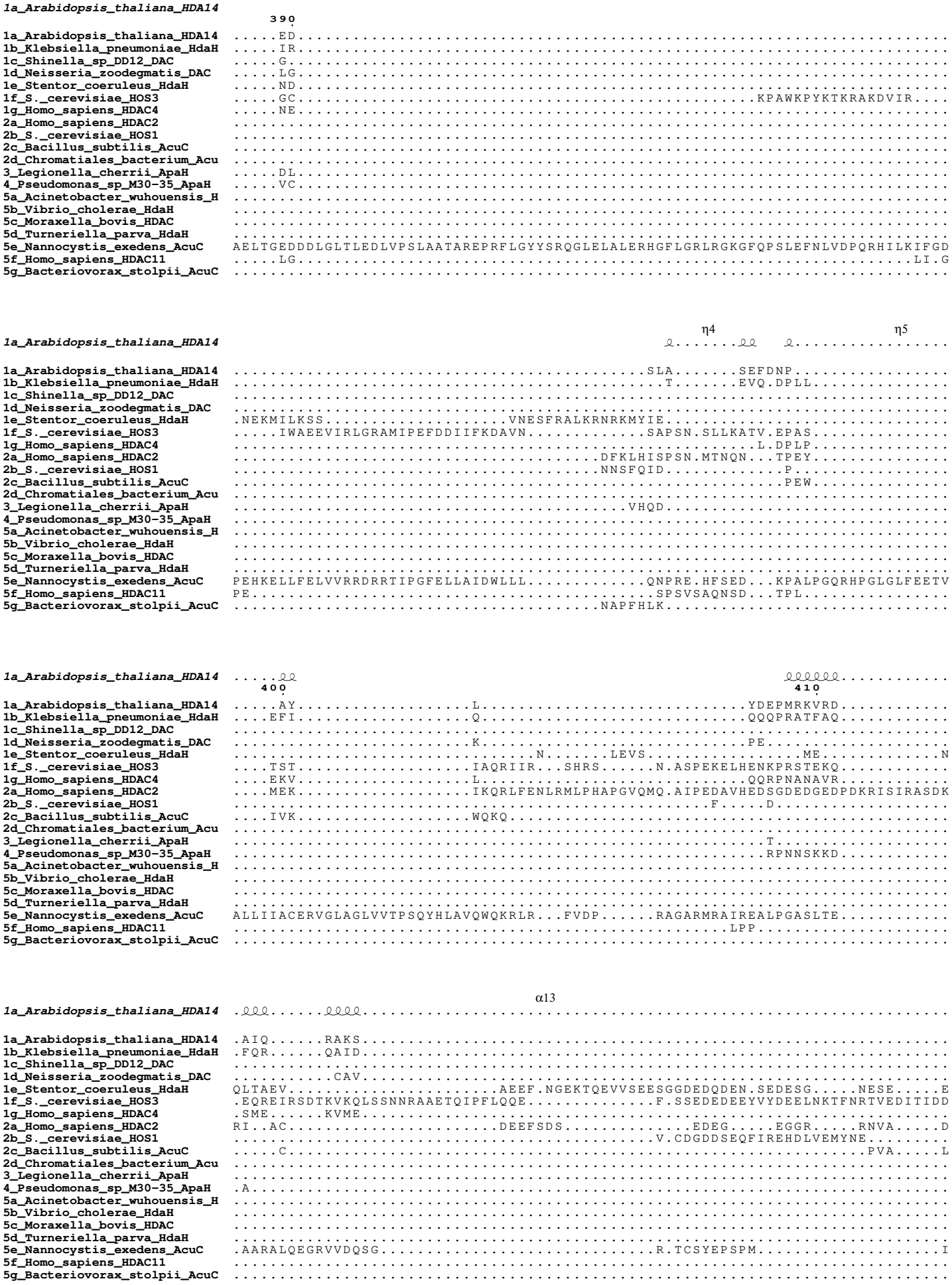
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η5
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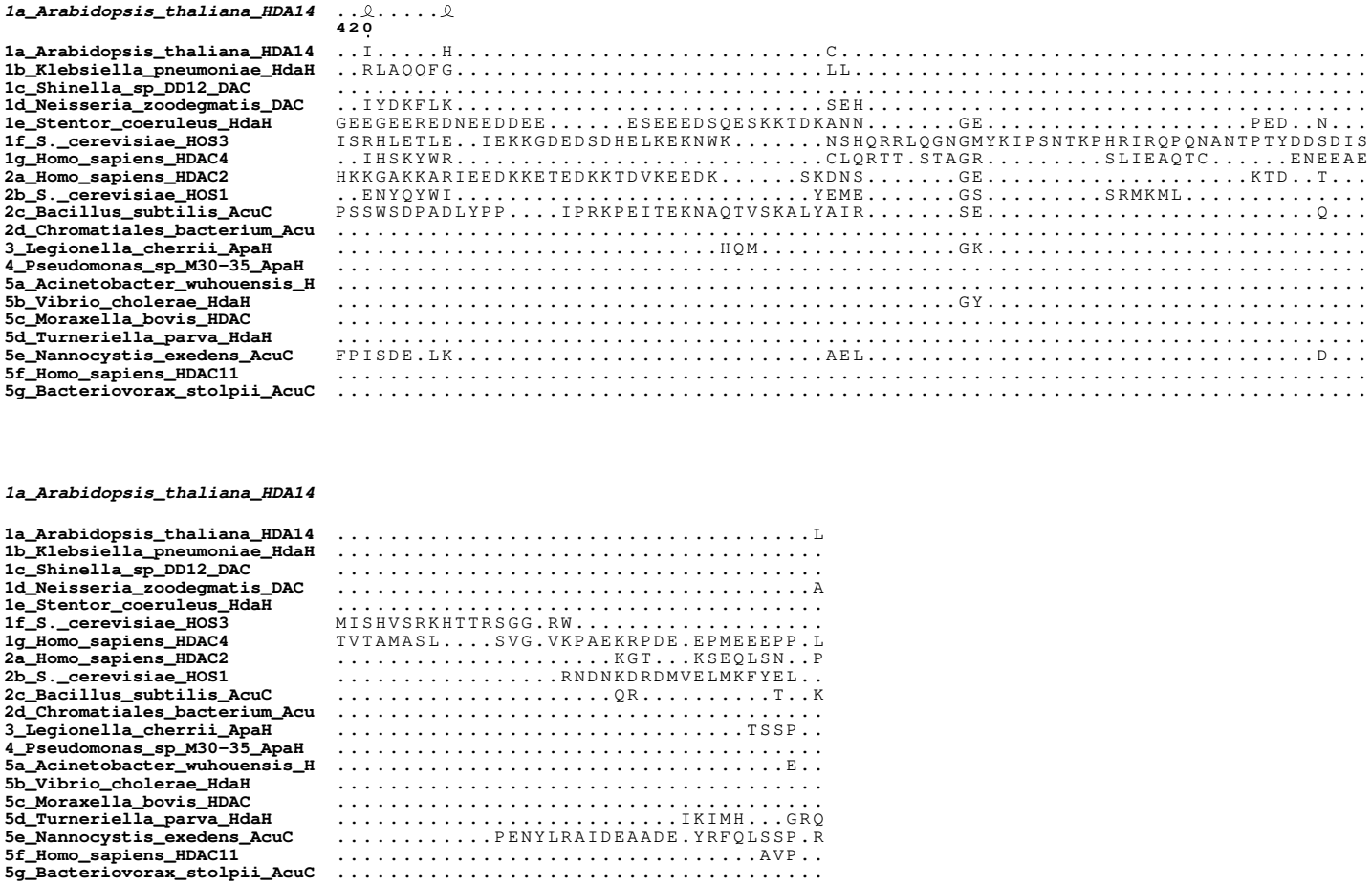
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b

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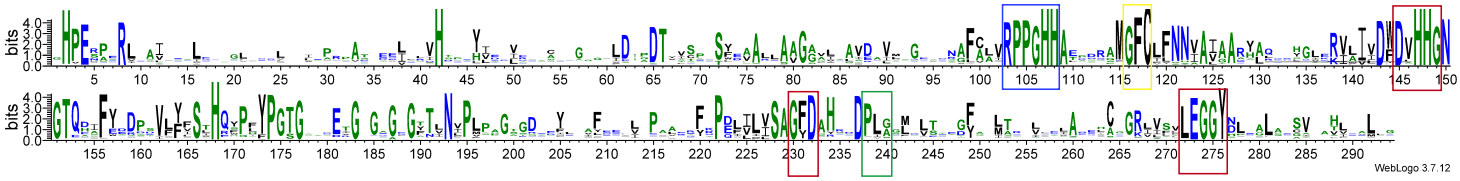
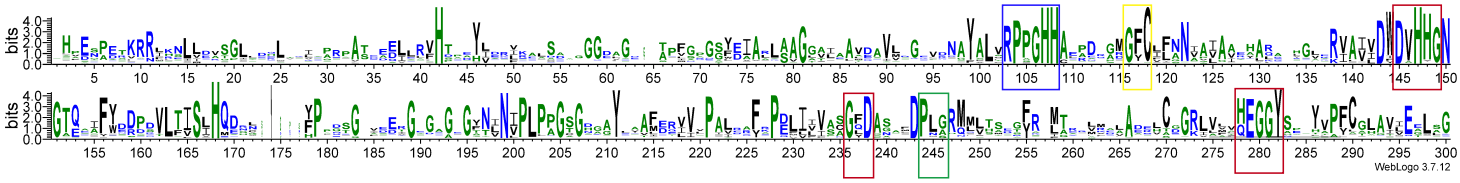


Subcluster 1b



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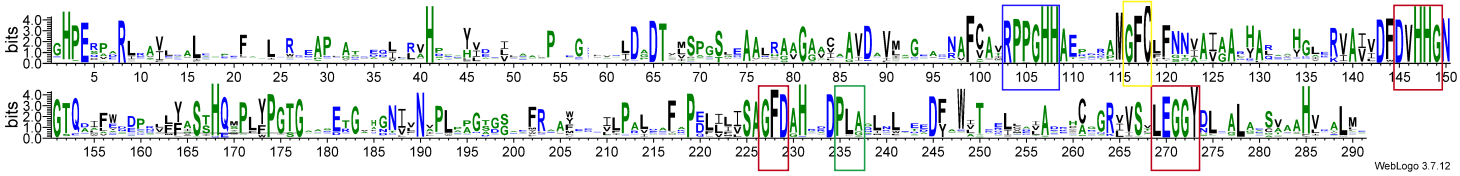
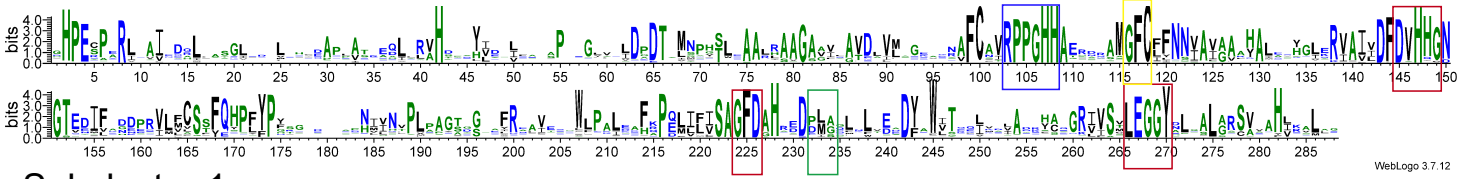


Subcluster 1d



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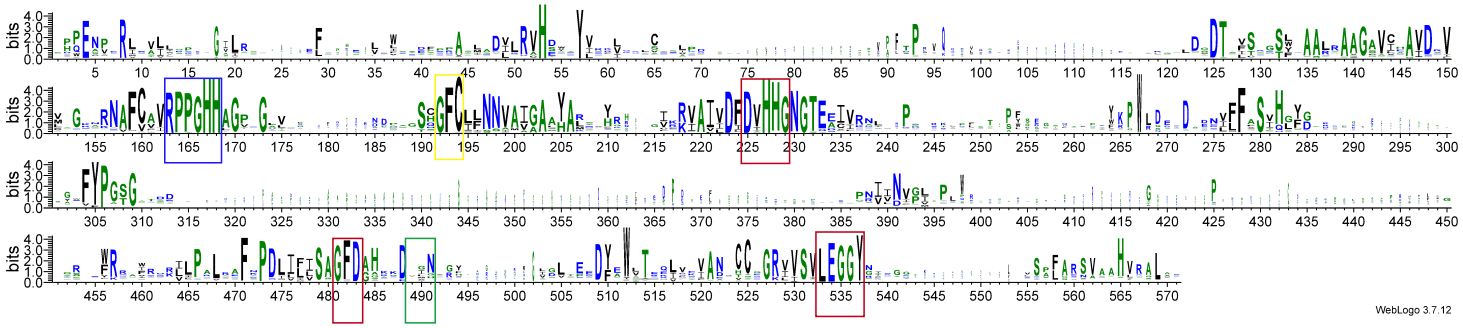

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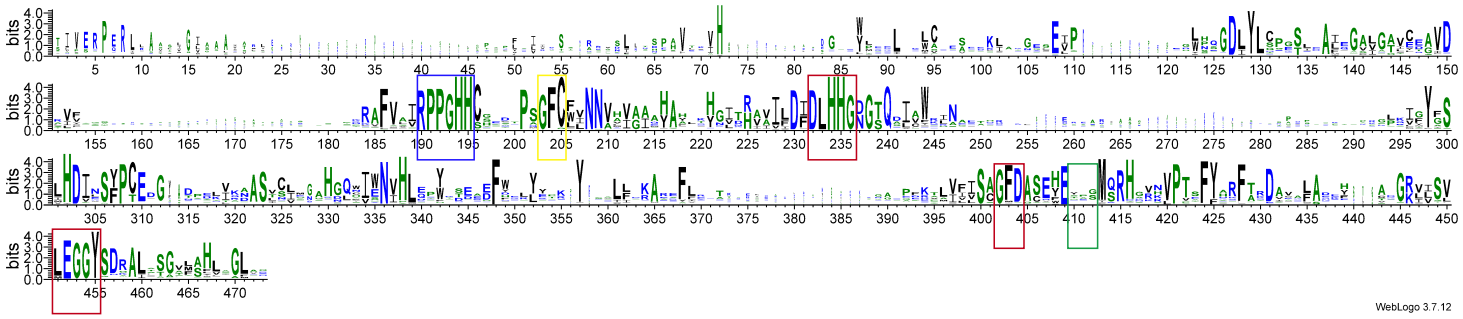

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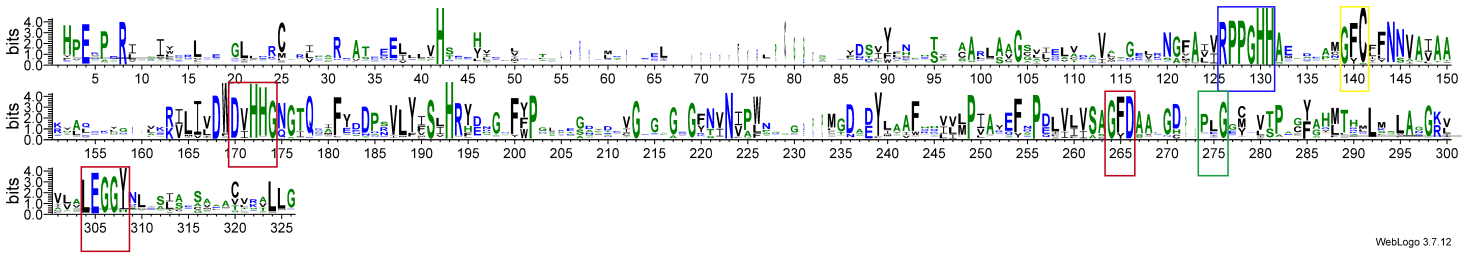

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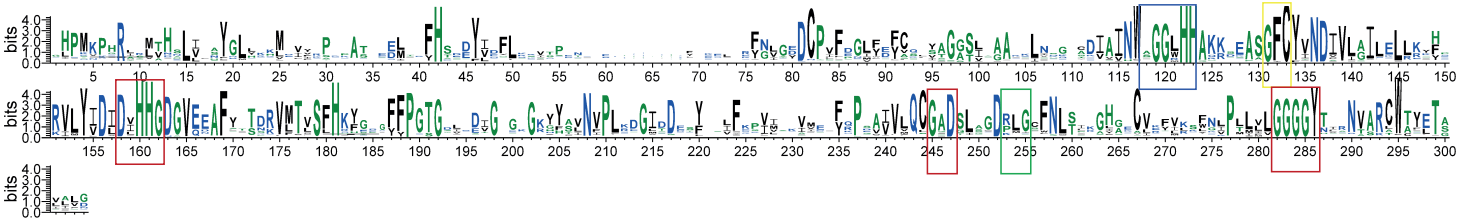
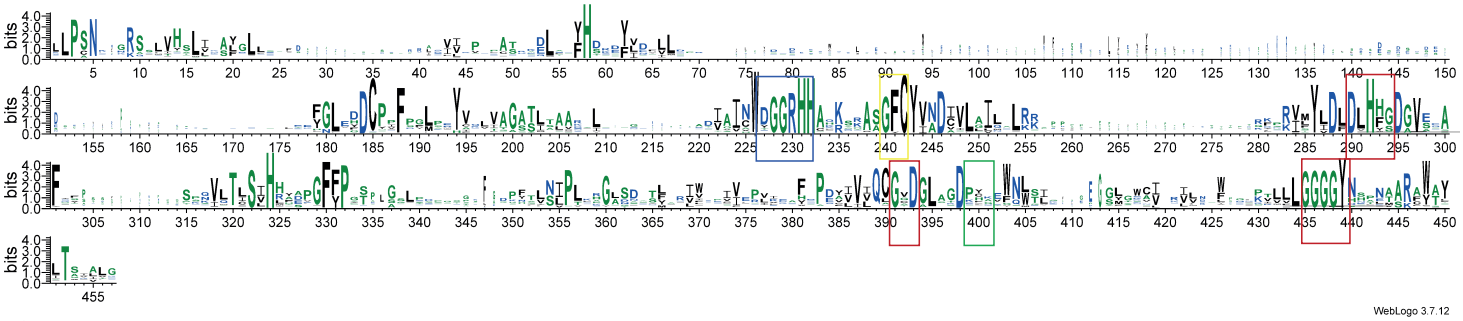
Subcluster 2a



Subcluster 2b



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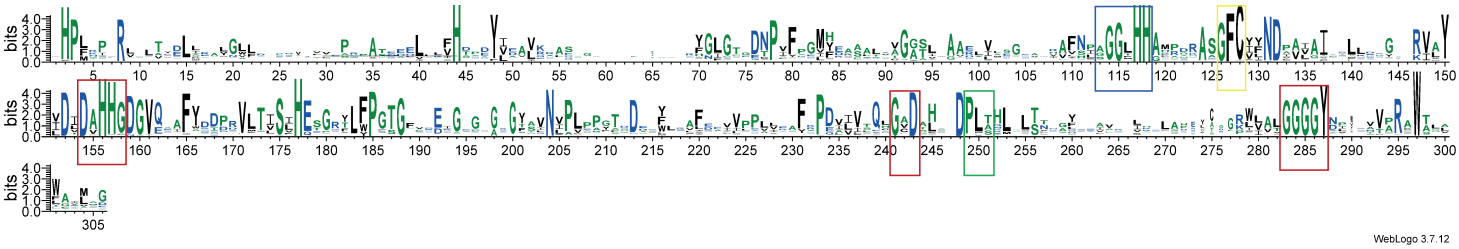

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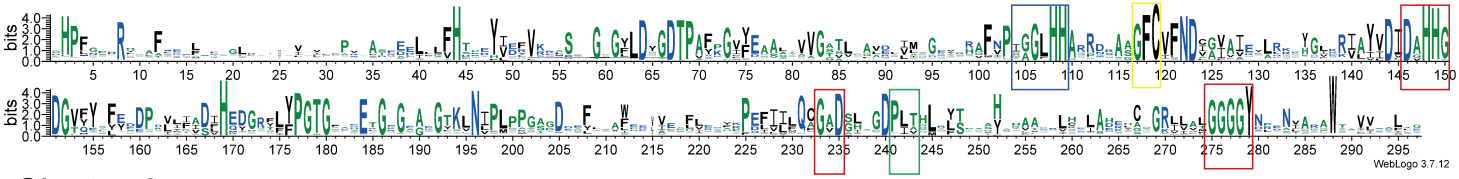

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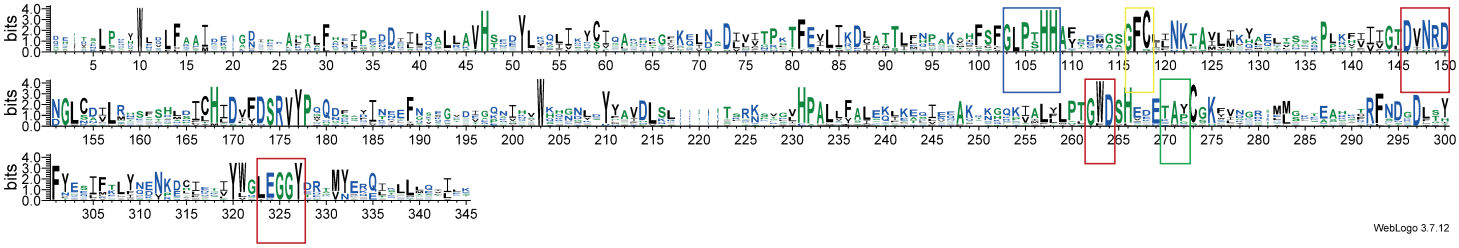

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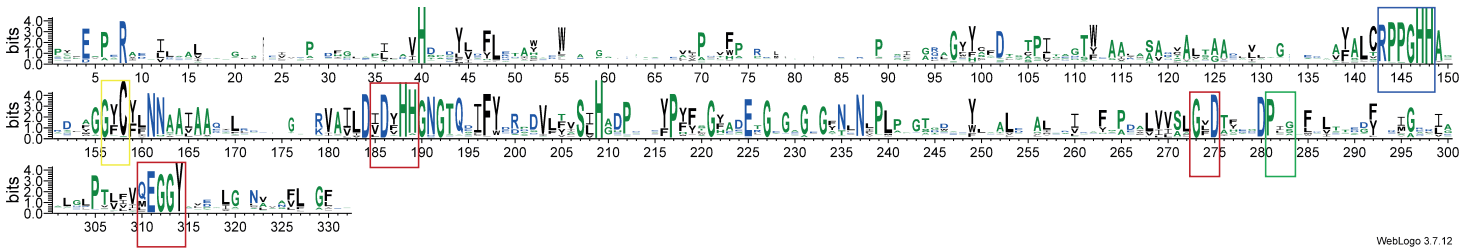

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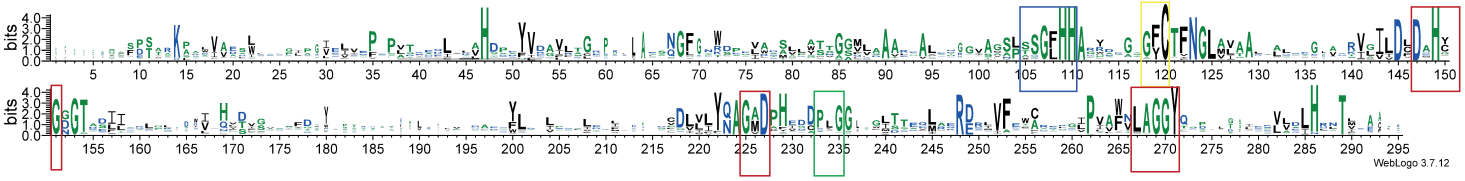
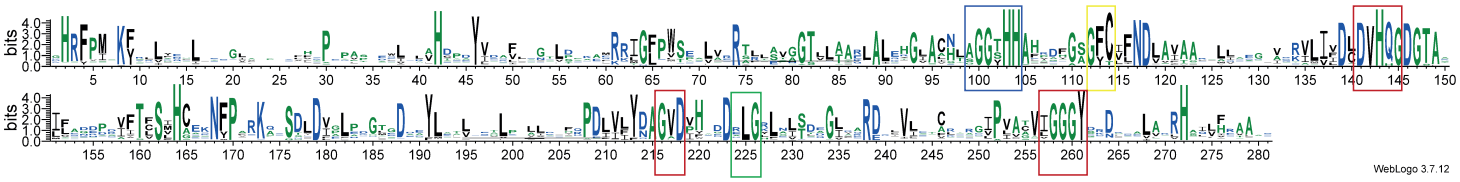
Subcluster 5a



Subcluster 5b



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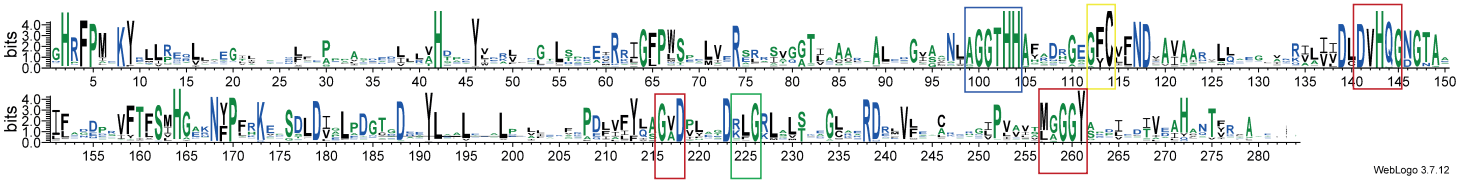
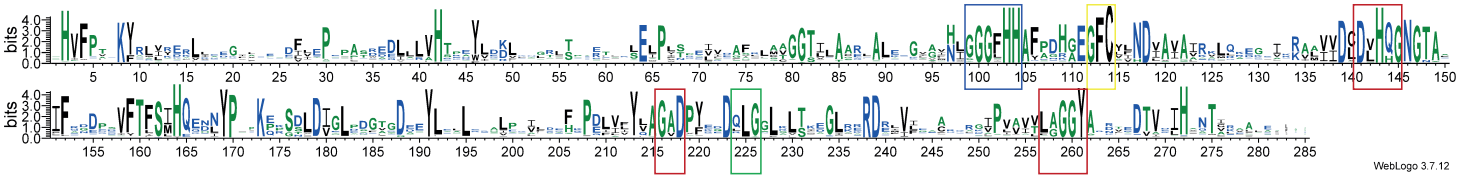


Subcluster 5d



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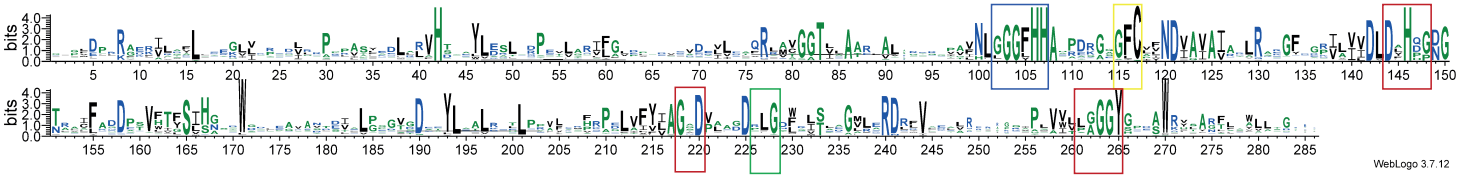

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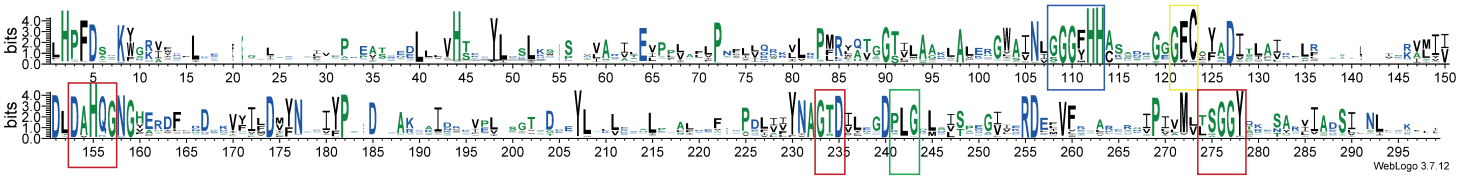

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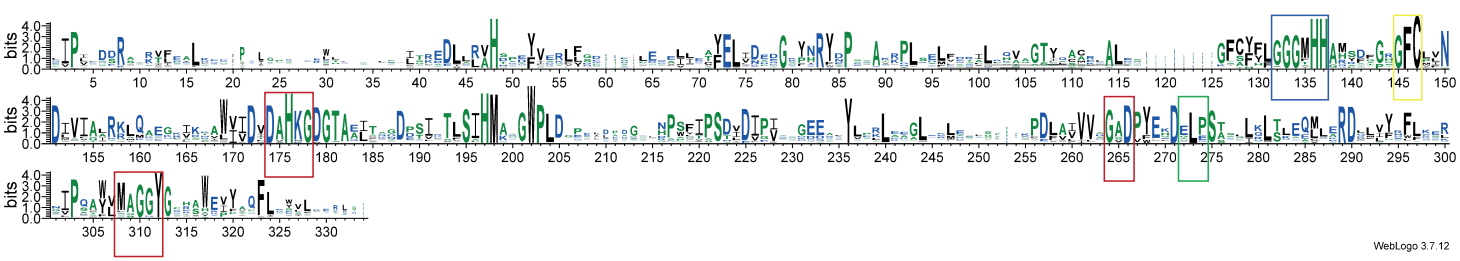


Supplementary Figure 1: Primary sequence alignment and sequence logo representations of selected deacylases representing all clusters 1-5 and the sub-clusters.

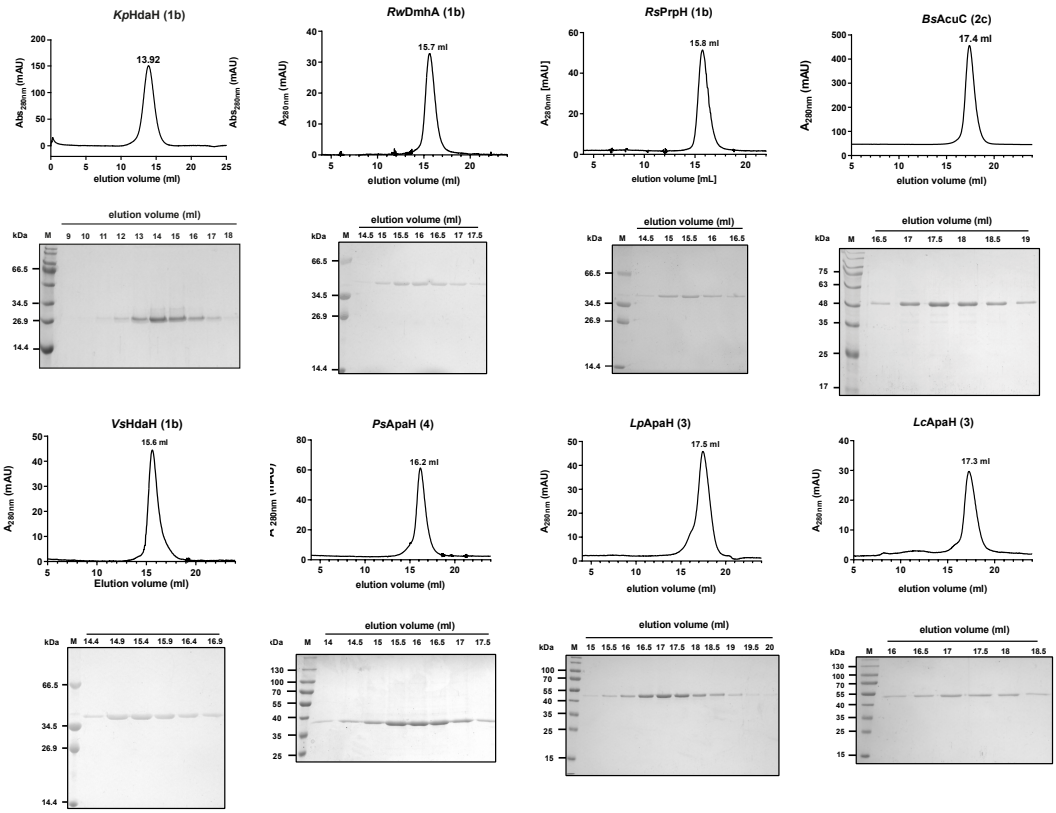
a Show are the following sequences of the following deacylases representing the five clusters and the sub-clusters (code: sub-cluster, accession number/protein name/spcies): 1a, AT4G33470.1/HDA14/*Arabidopsis thaliana*; 1b, A0A378F8Q8/HdaH/*Klebsiella pneumoniae*; 1c, A0A021X4K7/Deacetylase (DAC)/*Shinella sp.* DD12;1d, A0A1X3CVE4/Deacetylase (DAC)/*Neisseria zoodegmatis*; 1e, A0A1R2CWG0/HdaH/*Stentor coeruleus*; 1f, Q02959/HOS3/Saccharomyces cerevisiae (strain ATCC 204508 / S288c); 1g, P56524/HDAC4/*Homo sapiens*; 2a, Q92769/HDAC2/*Homo sapiens*; 2b, Q12214/HOS1/*Saccharomyces cerevisiae* (strain ATCC 204508 / S288c); 2c,P39067/AcuC/*Bacillus subtilis* (strain 168); 2d, A0A2E9D287/AcuC/*Chromatiales bacterium*; 3, A0A0W0SGS1/ApaH/*Legionella cherrii*; 4, A0A1Y0KY79/ApaH/*Pseudomonas sp.* M30-35; 5a, A0A385C7X8/HDAC/*Acinetobacter wuhouensis*; 5b, A0A395TF31/HDAC/*Vibrio cholerae*; 5c, A0A2Z4RAM2/HDAC/*Moraxella bovis*; 5d, I4B797HdaH/*Turneriella parva* (strain ATCC BAA-1111 / DSM 21527 / NCTC 11395 / H) (*Leptospira parva*); 5e, A0A1I2D6R7/AcuC/*Nannocystis exedens*; 5f, Q96DB2/HDAC11/*Homo sapiens*; 5g, A0A2K9NTX8/AcuC/*Bacteriovorax stolpii* (*Bdellovibrio stolpii*). All sequences were aligned using the T-COFFEE multiple sequence alignment server[1]. The important catalytic residues are highlighted. The secondary structure elements and numbering is shown for the deacylase shown at the top of the amino acid sequences above the sequence alignment. The alignment was created by ESPript version 3.0[2].

b Logo representation of enzymes of cluster 1. For each subcluster a sequence logo representation is shown. The important residues are highlighted. Blue box: double-His motif with the second His acting as catalytic base/acid (*Kp*HdaH: H143-H144); yellow box: conserved GFC-motif lining the substrate binding channel; brown boxes: Asp-His-Asp for coordination of the catalytic Zn^{2+}-ion and the XGGY-motif lining the foot pocket and the catalytic Tyr (*Kp*HdaH: Y313); green box: RPP-motif lining the foot pocket.

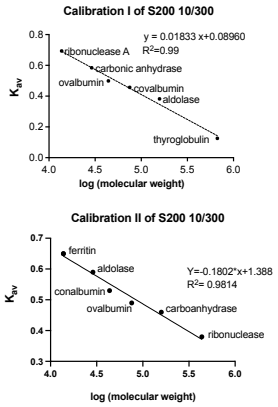
c Logo representation of enzymes of cluster 2, 3 and 4. For each cluster 2 a sequence logo representation is shown for each subcluster. The important residues are highlighted. Colour coding as described in b.

d Logo representation of enzymes of cluster 5. For each subcluster a sequence logo representation is shown. The important residues are highlighted. Colour coding as described in b.

a



b

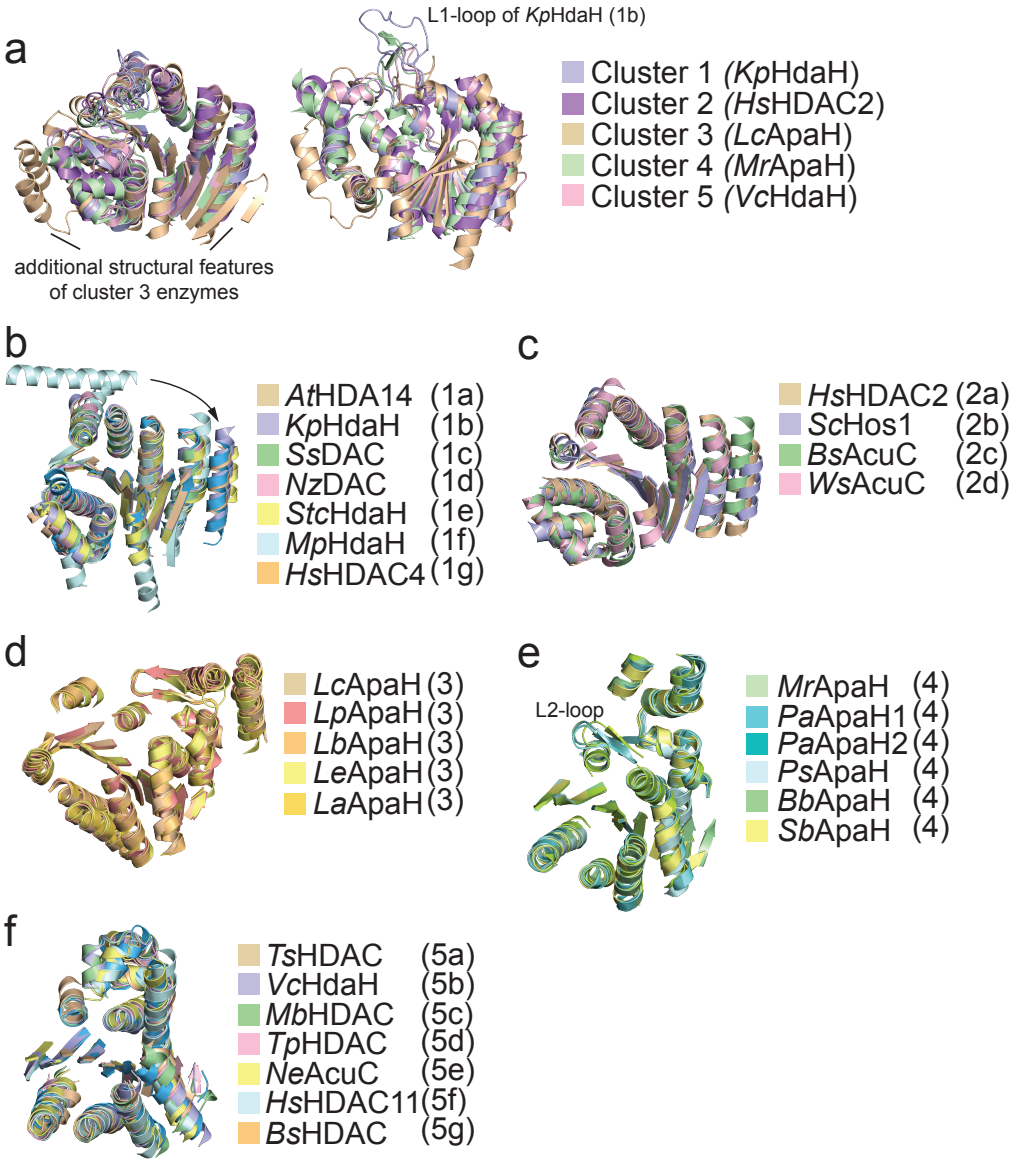


protein	exp. MW (Da)	elution volume (ml)	calc. MW (Da)	oligomeric state (calc. MW/exp. MW)
*Kp*HdaH (1b)	41569	13.9	136432	3.3
*Vs*HdaH (1b)	41435	15.6	157562	3.8
*Rs*PrpH (1b)	41227	15.8	133166	3.2
*Rw*DmhA (1b)	40563	15.7	146074	3.6
*Bs*AcuC (2c)	44273	17.4	34377	0.8
*Lp*ApaH (3)	49947	17.5	37868	0.8
*Lc*ApaH (3)	48902	17.3	32140	0.7
*Ps*ApaH (4)	39914	16.2	95121	2.4
*Vc*HdaH (5b)	35245	17.1	42780	1.2

Supplementary Figure 2: Purification and analytical size-exclusion chromatography (SEC) of all purified bacterial deacylases.

a Analytical size-exclusion chromatography (SEC) of all bacterial deacylases. 300 µg of bacterial deacylases were analysed on a Superdex 200 Increase 10/300 GL column. The elution volumes and calculated molecular weights in comparison to expected molecular weights are used to calculate an oligomeric state. The calculated molecular weights were obtained based on the elution volume using a calibration curve. Below the SEC chromatograms, the SDS-PAGE gels were shown analysing fractions of the observed absorption peak at 280 nm (A_{280nm}). SDS-PAGE was stained using Coomassie Brilliant Blue (CBB). Source data are provided as Source Data file.

b The elution volumes and calculated molecular weights (calc. MW) in comparison to expected molecular weights (exp. MW) are used to calculate the apparent oligomeric states of the enzymes. The calculated molecular weights were obtained based on the elution volume using a calibration curve. Calibration curve I was used for *Kp*HdaH (1b) and the mutants thereof and calibration curve II for the other enzymes. Source data are provided as Source Data file.



Supplementary Fig. 3: Structural alignments of core domains of deacylases reveal high structural similarity of enzymes within each main cluster and between the clusters.

a AlphaFold2 structure predictions were performed for selected enzymes representing the sub-clusters. a Superposition of representative enzymes representing the five main clusters (cluster 1-5). All structures show r.m.s.d. values close to 1 Å or lower to the reference structure (Supplementary Data 4). L1-loop of *Kp*HdaH (1b) and the additional structural features of cluster 3 enzymes, i.e. the extended β-sheet and the additional α-helices, enzymes are highlighted.

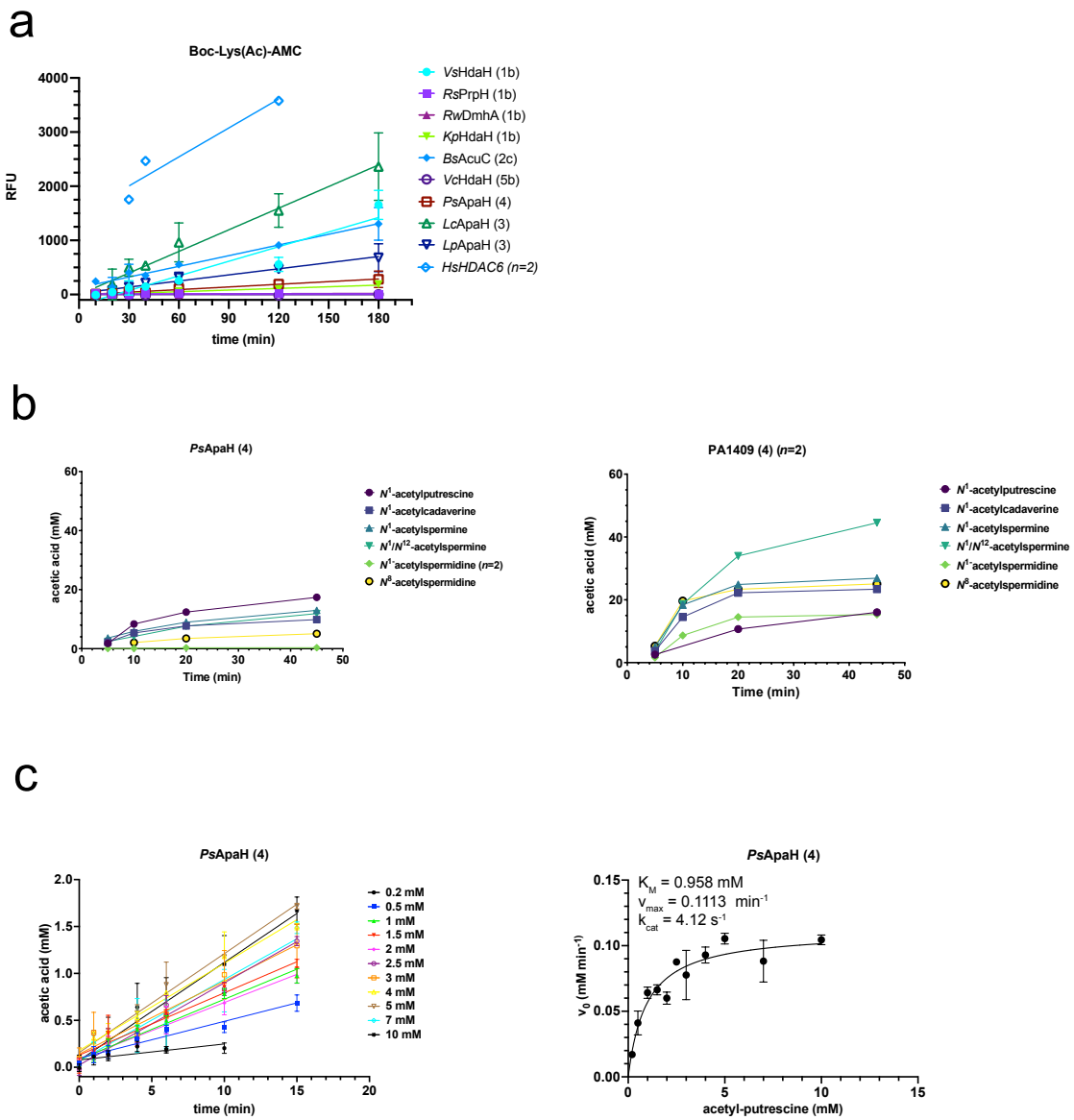
b Superposition of representative enzymes of cluster 1 (sub-clusters 1a-1g). All structures show r.m.s.d. values close to 1 Å or lower to the reference structure (Supplementary Data 4). We observed two exceptions: Firstly, the eukaryotic enzyme HdaH of *Malassezia pachydermatis* (1f) compared to the bacterial *Kp*HdaH (1b) with an r.m.s.d value of 7 Å in the comparison of cluster 1 enzymes. *M. pachydermatis* (1f) shows a reorganization of the catalytic core domain, i.e. the a-helix usually lining the central β-sheet is displaced by approximatly 90°. Moreover, thos enzyme shows an extended α-helical N-terminal domain preceding the catalytic domain.

c Superposition of representative enzymes of cluster 2 (sub-clusters 2a-2d). All structures show r.m.s.d. values close to 1 Å or lower to the reference structure (Supplementary Data 4).

d Superposition of representative enzymes of cluster 3. All structures show r.m.s.d. values close to 1 Å or lower to the reference structure (Supplementary Data 4). The cluster 3 enzyme of *L. cherrii* shows an r.m.s.d to the structure of *Kp*HdaH (1b) of 5.5 Å in the comparison of enzymes representing the main-clusters. The structural dissimilarity of the cluster 3 enzymes is due to the additional structural features, i.e. the extended β-sheet and the additional α-helices compared in the catalytic core.

e Superposition of representative enzymes of cluster 4. All structures show r.m.s.d. values close to 1 Å or lower to the reference structure (Supplementary Data 4).

f Superposition of representative enzymes of cluster 5 (sub-clusters 5a-5g). All structures show r.m.s.d. values close to 1 Å or lower to the reference structure (Supplementary Data 4).

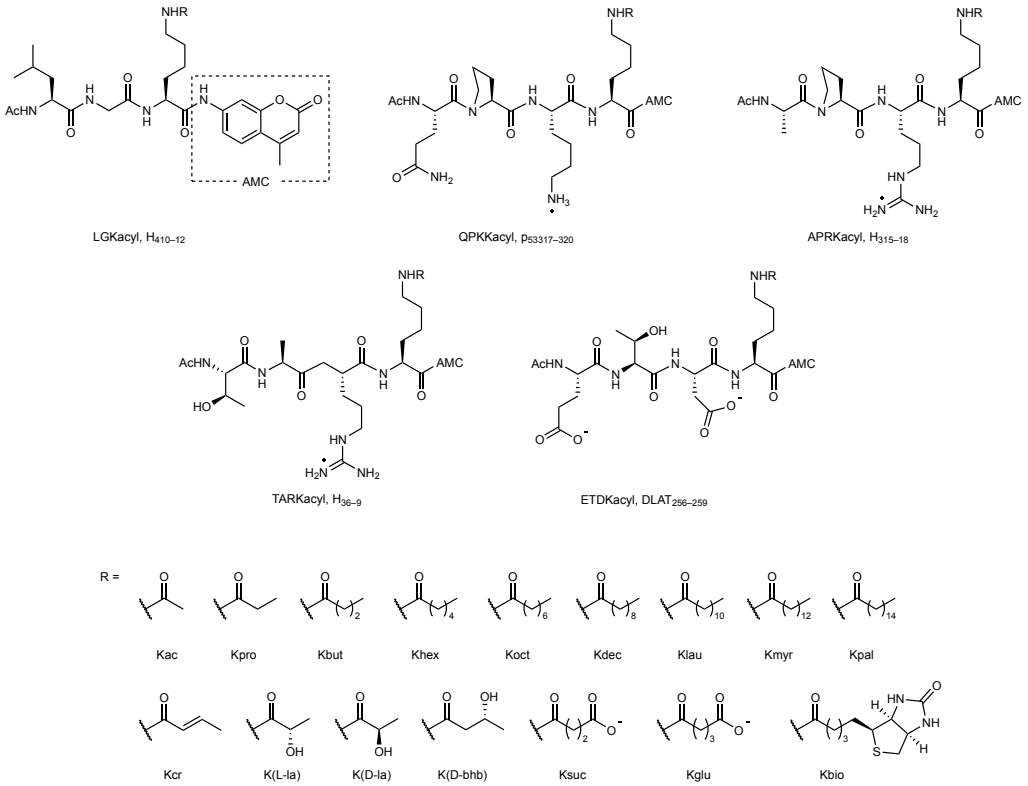


Supplementary Figure 4: Determination of the dynamic ranges for the pre-screening assays.

a Determination of the dynamic range for the pre-screening of the bacterial deacylases to use Boc-Lys(Ac)-AMC as a substrate. 20 nM enzyme and 20 µM Boc-Lys(Ac)-AMC substrate was used and the fluorescence of the released AMC-fluorophore was measured. An assay-incubation time of 30 min was selected as enyzmes show a linear correlation of the fluorescence signal and assay incubation time. The experiments were conducted in three biologically independent replicates (n=3) except for *Hs*HDAC6 (1g) (n=2). Shown are means±SD. For *Hs*HDAC6 (1g) means are shown. Source data are provided as Source Data file.

b Determination of the dynamic range for the screening of *Ps*ApaH (4) on its capacity to deacetylate acetylated polyamines as substrates. 200 nM *Ps*ApaH (4) (left panel) and 100 nM PA1409 enzyme (right panel) and 3 mM acetylated polyamines as indicated. Acetylated polyamines were used as substrates and the released acetate was detected by a coupled enzymatic assay by formation of $NADH+H^+$ from NAD^+ (K-ACET, Megazyme). An assay-incubation time of 20 min was selected as enyzmes show a linear correlation of the absorption at 340 nM and assay incubation time. The release of acetate was normalized to the enzyme concentration used. The experiments were conducted in three or two biologically independent replicates (n=3 and n=2) for *Ps*ApaH (4) and PA1409, respectively. Shown are means±SD for *Ps*ApaH (4) and means for PA1409. Source data are provided as Source Data file.

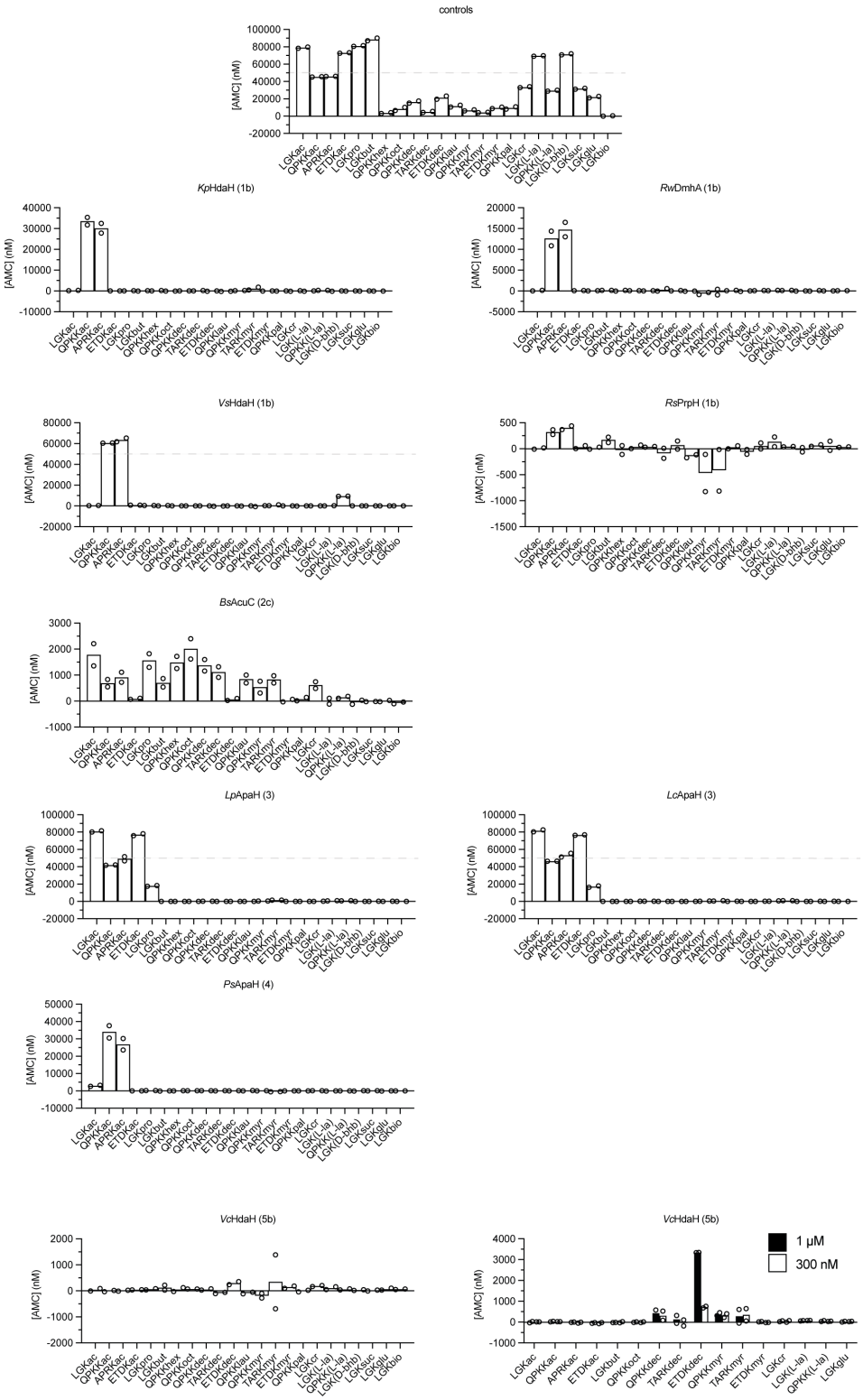
c Michaelis-Menten kinetics for the acetyl-putrescine deacetylation by *Ps*ApaH (4). Initial reaction rates were (v_0) determined using 200 nM *Ps*ApaH (4) and increasing concentrations of acetyl-putrescine as indicated and measuring the released acetate. Linearity was present up to 15 min assay time. The reaction rates (v_0) were plotted as a funtion of the acetylputrescine concentration resulting in a hyperbolic curve that was fitted by a Michelis-Menten equation resulting K_M, v_{max} and k_{cat}. The experiments were conducted in three biologically independent replicates (n=3). Shown are means±SD. Source data are provided as Source Data file.



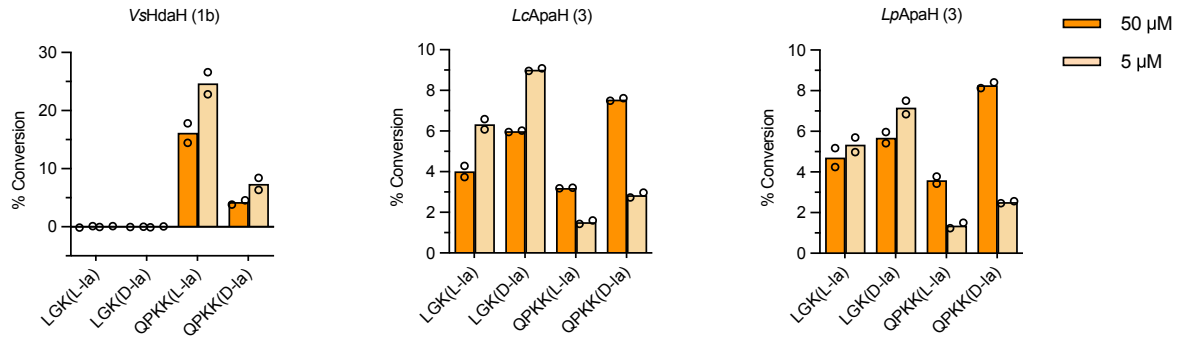
Supplementary Figure 5: Chemical structures of peptides and acyl-chain types used as substrates for deacylation by selected bacterial deacylases.

a. Peptides with varying sequences used to study substrate preference for various bacterial deacylases. The peptides suitable to detect a successful lysine deacylation by release of an C-terminally attached AMC (7-amino-4-methylcoumarin) fluorophore upon trypsin cleavage (Fluor-deLys-assay). The AMC fluorophore is quenched in the peptide but is fluorescent in its free form. The peptides used were derived from histone H3 ($APRK_{acyl}$, $H3_{15-18}$ or $TARK_{acyl}$, $H3_{6-9}$), histone H4 (LGK_{acyl}, $H4_{10-12}$) tumor suppressor protein p53 ($QPKK_{acyl}$, $p53_{317-320}$) and DLAT **(**Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex; $ETDK_{acyl}$; $DLAT_{256-259}$). Charges were shown as expected at physiological pH.

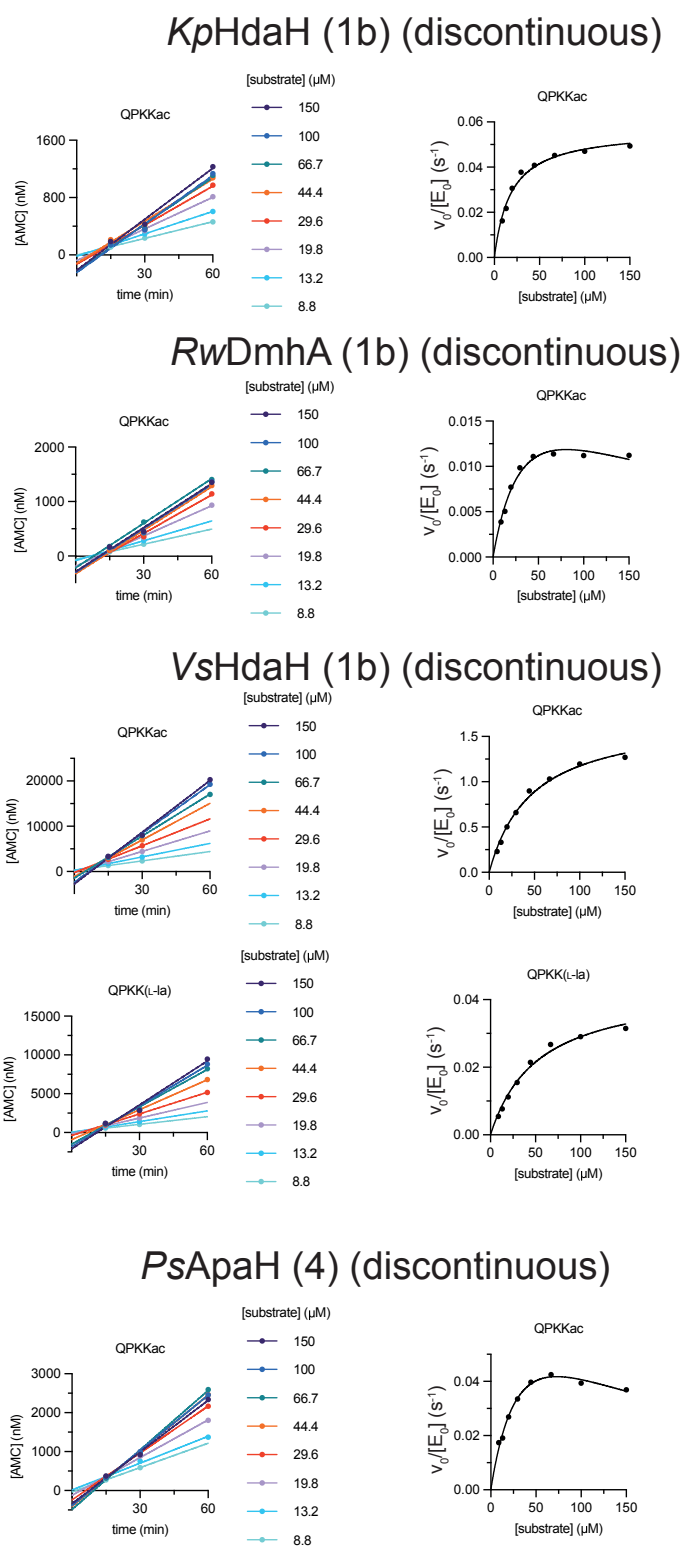
b. The tri- or tetrapeptide sequences were modified at the C-terminal lysine side chain by various acyl-chain types . These acyl-chains range from short aliphatic acylations, i.e. acetyl (C-2; K_{ac}), propionyl (C-3; K_{pro}), butyryl (C-4; K_{but}), over medium-sized aliphatic acylations, i.e. hexanoly (C-6; K_{hex}), octanoyl (C-8; K_{oct}), decanoyl (C-10; K_{dec}), to longer-chain aliphatic acylations, i.e. lauroyl (C-10; K_{lau}), myristoyl (C-12; K_{myr}) and palmitoyl (C-14; K_{pal}). Moreover, we also analyzed acylations such as the unsaturated crotonyl (K_{cro}), the branched acylations L-lactyl ($K_{L\text{-}la}$), D-β-hydroxybutyryl $K_{D\text{-}bhb}$), charged-acylations, such as succinyl (K_{suc}) and glutaryl (K_{glu}) and biotinyl (K_{bio}) (Fig. 3a; Supplementary Fig. 5; Supplementary Fig. 6). As positive controls, we analyzed the conversion of these lysine acylations by human HDAC3/NCoR2 (K_{ac}, K_{pro}, K_{but}, K_{cro}, $K_{L\text{-}la}$, $K_{D\text{-}bhb}$), human SIRT2 (K_{hex}, K_{oct}, K_{dec}, K_{lau}, K_{myr}, K_{pal}) and human SIRT5 (K_{suc}, K_{glu}). For deacylation of K_{bio}, we did not have a positive control (Fig. 3a; Supplementary Fig. 5). Charges were shown as expected at physiological pH. As AMC fluorescence does not follow a linear trend at high concentrations, we obtained a calculated conversions >100% for some enzyme-peptide combinations. To this end, we categorized these values in a single category to all conversions >48% on the heat map (Fig. 3a; Supplementary Fig. 6).



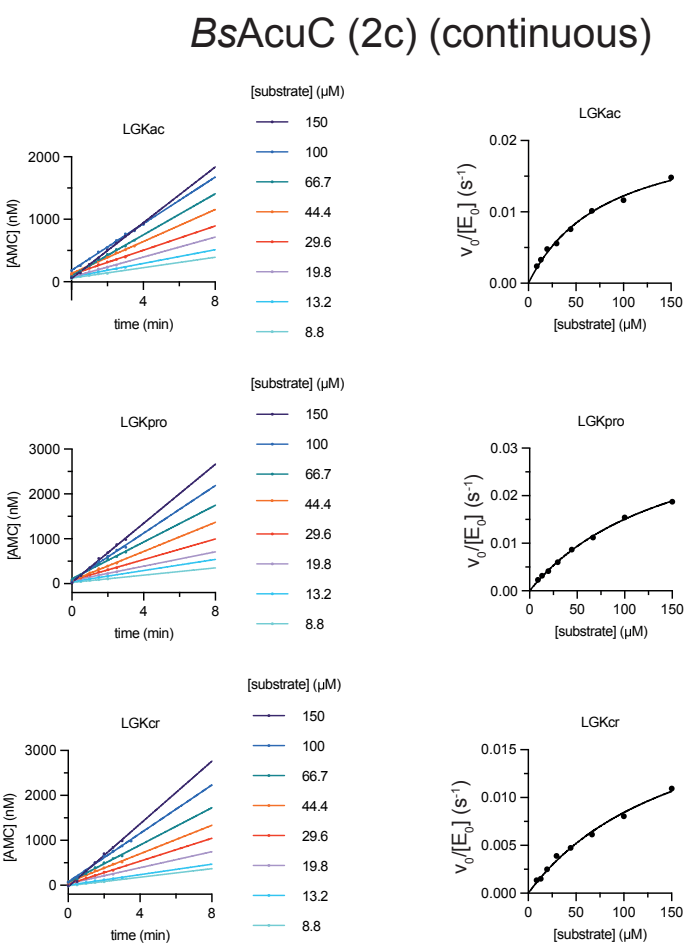
Supplementary Figure 6: Bar graphs obtained for the pre-screening to uncover acyl-chain preferences of selected bacterial deacylases. A Fluor-de-Lys assay-based screening was performed using histone H3 ($APRK_{acyl}$, $H3_{15\text{-}18}$ or $TARK_{acyl}$, $H3_{6\text{-}9}$), histone H4 (LGK_{acyl}, $H4_{10\text{-}12}$), p53 ($QPKK_{acyl}$, $p53_{317\text{-}320}$) and DLAT derived tri- or tetrapeptide sequences. Depicted is the conversion of peptide in released [AMC] in µM. AMC fluorescence does not follow a linear trend at high concentrations, which explains calculated conversions >100%. To this end, a single category is given to all conversions >48% and the bar graphs show a maximal conversion of 50 µM AMC (dashed grey line). Positive controls were: HDAC3/NCoR2 (K_{ac}, K_{pro}, K_{but}, K_{cr}, $K_{(L\text{-}la)}$, $K_{(D\text{-}bhb)}$, SIRT2 (K_{hex}, K_{oct}, K_{dec}, K_{lau}, K_{myr}, K_{pal}) and SIRT5 (K_{suc}, K_{glu}). For the H4-derived peptide LGK_{bio} no positive contral was available. Enzyme concentration was set to 100 nM. As we did not observe a strong conversion for *Vc*HdaH (5b), follow-up screening was performed at higher enzyme concentrations of 300 nM and 1 µM. This data is the basis for the heatmap shown in Fig. 3a. The experiment was performed in two independent replicates (n=2). Bars depict means. Source data are provided as Source Data file.



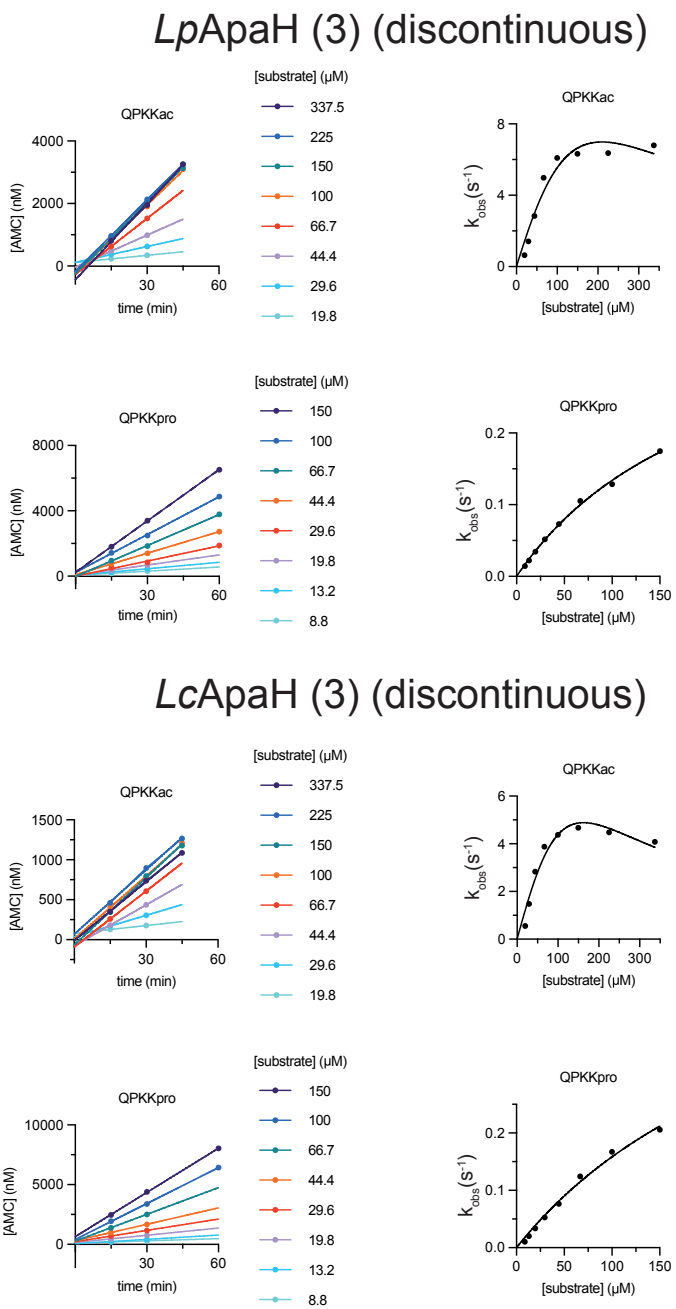
Supplementary Figure 7: Bar graphs obtained for the follow-up screening to uncover de-D/L-lactylase activity for *Vs*HdaH (1b), *Lc*ApaH (3) and *Lp*ApaH (3). A Fluor-de-Lys assay-based screening was performed using histone H4 ($LGK_{L\text{-}la}$/$LGK_{D\text{-}la}$, $H4_{10\text{-}12}$) and p53 ($QPKK_{L\text{-}la}$/$QPKK_{D\text{-}la}$, $p53_{317\text{-}320}$) tri- and tetrapeptide sequences. Depicted is the conversion of peptide in released [AMC] in µM. The de-D/L-lactylase selectivity assays were performed at 100 nM (*Vs*HdaH (1b) or 300 nM (*Lc*ApaH (3), *Lp*ApaH (3)), and at 50 µM and 5 µM substrate concentration as indicated. This data is the basis for the heatmap shown in Fig. 3a. The experiment was performed in two independent replicates (*n*=2). Bars depict means. Source data are provided as Source Data file.



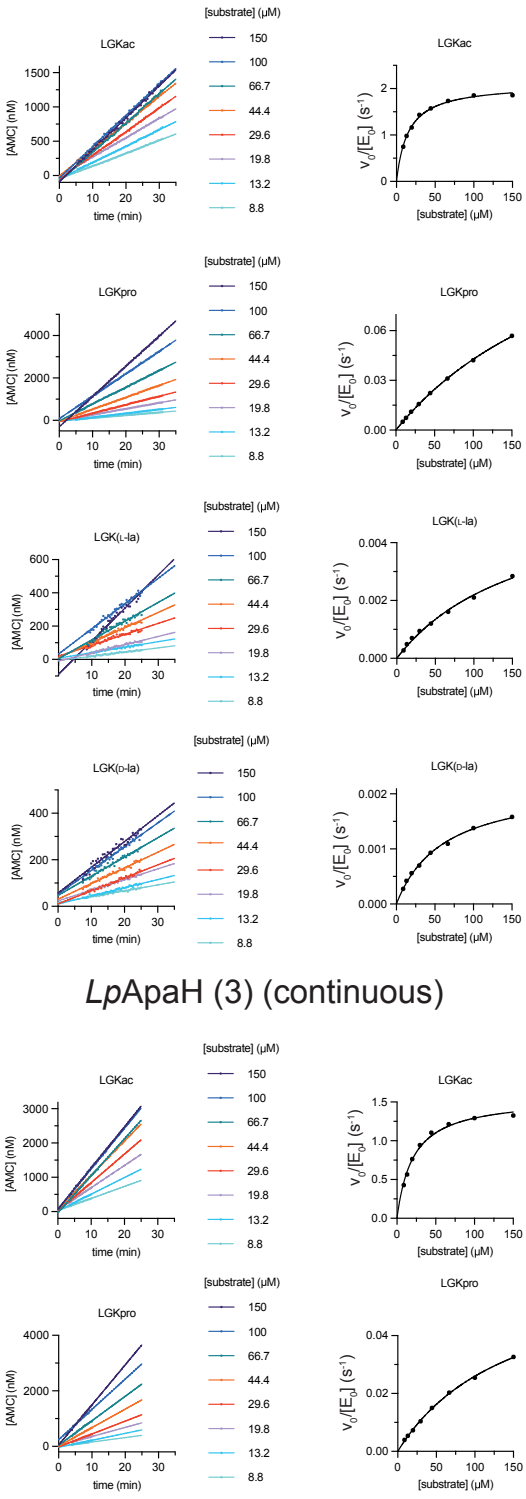
Supplementary Figure 8: Michaelis-Menten kinetics obtained for deacylation of the p53-derived QPKK-based peptides and acyl-chain types by a discontinuous assay format for bacterial deacylases *Kp*HdaH (1b), *Ps*ApaH (4), *Rw*DmhA (1b) and *Vs*HdaH (1b). For the QPKK-based substrates the discontinuous method was used for enzyme kinetics. Michaelis-Menten kinetics were performed to reveal the peptide substrate and acyl-chain preference of bacterial deacylases. The peptide sequences and acyl-chain types are indicated ($p53_{317-320}$; $QPKK_{ac}$/ $QPKK_{L-la}$). Left panels: Conversion of the AMC fluorophore as a function of time results in the initial reaction rates, v_0, (unit: $nM*s^{-1}$). Right panels: The initial reaction rates, v_0, were divided by the total enzyme concentration, $[E_0]$ (unit: nM) to yield the observed rate constants, $v_0/[E_0]$, (units: s^{-1}, i.e. $v_0/[E_0] = [AMC]s^{-1}/[E_0]$). The values obtained for $v_0/[E_0]$ were plotted as a function of the substrate concentration. The data was fitted to the hyperbolic Michaelis-Menten equation resulting the k_{cat}, i.e. the first-order rate constant under conditions of substrate saturation ($k_{cat}=v_{max}/[E_0]$; unit: s^{-1}), and K_M value (unit: nM). The experiment was performed in duplicates and the graph depicts the means of both recorded independent replicates (n=2). Source data are provided as Source Data file.



Supplementary Figure 9: Michaelis-Menten kinetics obtained for deacylation of the LGK-based peptides and acyl-chain types by a continuous assay format for bacterial deacylase *Bs*AcuC (2c) For the LGK-based substrates the continuous method was used for enzyme kinetics. Michaelis-Menten kinetics were performed to reveal the acyl-chain preference of *Bs*AcuC (2c). The peptide sequences and acyl-chain types are indicated (histone $H4_{10-12}$; LGK_{ac}/LGK_{pro}/LGK_{cro}). Left panels: Conversion of the AMC fluorophore as a function of time results in the initial reaction rates, v_0, (unit: $nM*s^{-1}$). Right panels: The initial reaction rates, v_0, were divided by the total enzyme concentration, $[E_0]$ (unit: nM) to yield the observed rate constants, $v_0/[E_0]$, (units: s^{-1}, i.e. $v_0/[E_0] = [AMC]s^{-1}/[E_0]$). The values obtained for $v_0/[E_0]$ were plotted as a function of the substrate concentration. The data was fitted to the hyperbolic Michaelis-Menten equation resulting the k_{cat}, i.e. the first-order rate constant under conditions of substrate saturation ($k_{cat}=v_{max}/[E_0]$; unit: s^{-1}), and K_M value (unit: nM). The experiment was performed in duplicates and the graph depicts the means of both recorded independent replicates (n=2). Source data are provided as Source Data file.

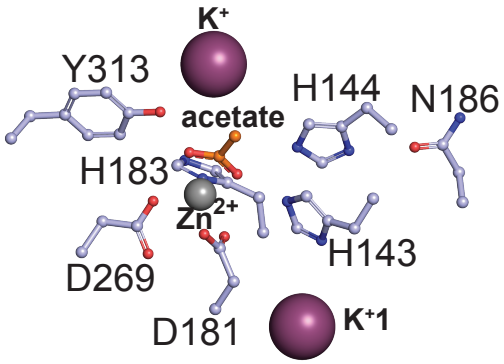


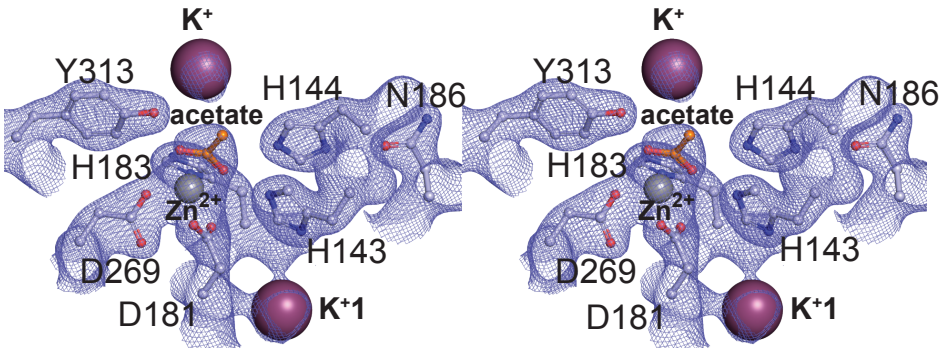
Supplementary Figure 10: Michaelis-Menten kinetics obtained for deacetylation and depropionylation of the p53-derived QPKK-based peptides and acyl-chain types by a discontinuous assay format for bacterial deacylases *Lp*ApaH (3) and *Lc*ApaH (3). For the QPKK-based substrates the discontinuous method was used for enzyme kinetics. Michaelis-Menten kinetics were performed to reveal the peptide substrate and acyl-chain preference of bacterial deacylases. The peptide sequences and acyl-chain types are indicated ($p53_{317-320}$; $QPKK_{ac}$/$QPKK_{pro}$). Left panels: Conversion of the AMC fluorophore as a function of time results in the initial reaction rates, v_0, (unit: $nM*s^{-1}$). Right panels: The initial reaction rates, v_0, were divided by the total enzyme concentration, $[E_0]$ (unit: nM) to yield the observed rate constants, $v_0/[E_0]$, (units: s^{-1}, i.e. $v_0/[E_0] = [AMC]s^{-1}/[E_0]$). The values obtained for $v_0/[E_0]$ were plotted as a function of the substrate concentration. The data was fitted to the hyperbolic Michaelis-Menten equation resulting the k_{cat}, i.e. the first-order rate constant under conditions of substrate saturation ($k_{cat}=v_{max}/[E_0]$; unit: s^{-1}), and K_M value (unit: nM). The experiment was performed in duplicates and the graph depicts the means of both recorded independent replicates (n=2). Source data are provided as Source Data file.



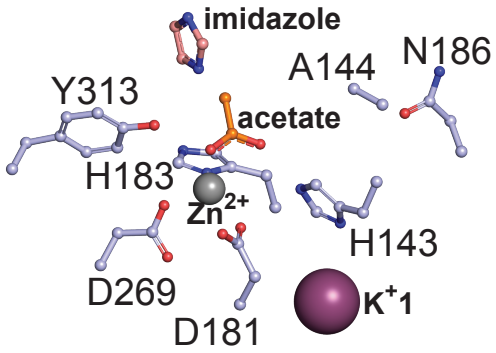
Supplementary Figure 11: Michaelis-Menten kinetics obtained for deacylation of the LGK-based peptides and acyl-chain types for bacterial deacylases *Lc*ApaH (3) and *Lp*ApaH (3). For the histone H4-derived LGK_{ac}-based substrate peptide the continuous method was used for enzyme kinetics. Michaelis-Menten kinetics were performed to reveal the acyl-chain preference of *Lc*ApaH (3) *and* LpApaH (3). The peptide sequences and acyl-chain types are indicated (histone $H4_{10\text{-}12}$; LGK_{ac}/LGK_{pro}/$LGK_{L\text{-}la}$/$LGK_{D\text{-}la}$). Left panels: Conversion of the AMC fluorophore as a function of time results in the initial reaction rates, v_0, (unit: nM*s^{-1}). Right panels: The initial reaction rates, v_0, were divided by the total enzyme concentration, $[E_0]$ (unit: nM) to yield the observed rate constants, $v_0/[E_0]$, (units: s^{-1}, i.e. $v_0/[E_0] = [AMC]s^{-1}/[E_0]$). The values obtained for $v_0/[E_0]$ were plotted as a function of the substrate concentration. The data was fitted to the hyperbolic Michaelis-Menten equation resulting the k_{cat}, i.e. the first-order rate constant under conditions of substrate saturation ($k_{cat}=v_{max}/[E_0]$; unit: s^{-1}), and K_M value (unit: nM). The experiment was performed in duplicates and the graph depicts the means of both recorded independent replicates (n=2). Source data are provided as Source Data file.

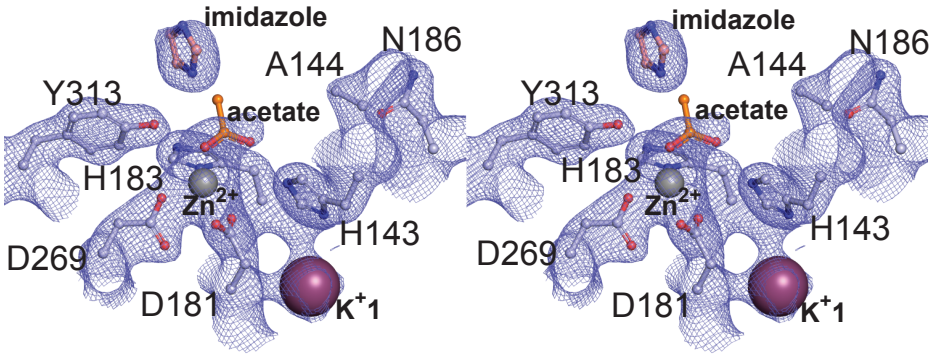
*Kp*HdaH (1b)



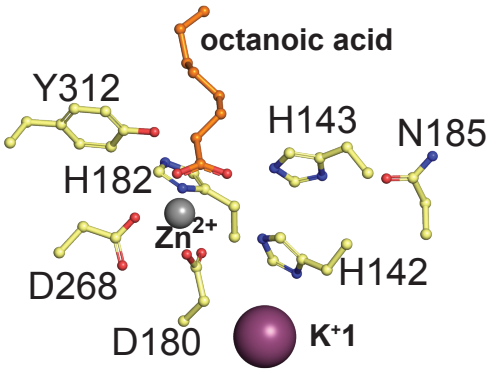


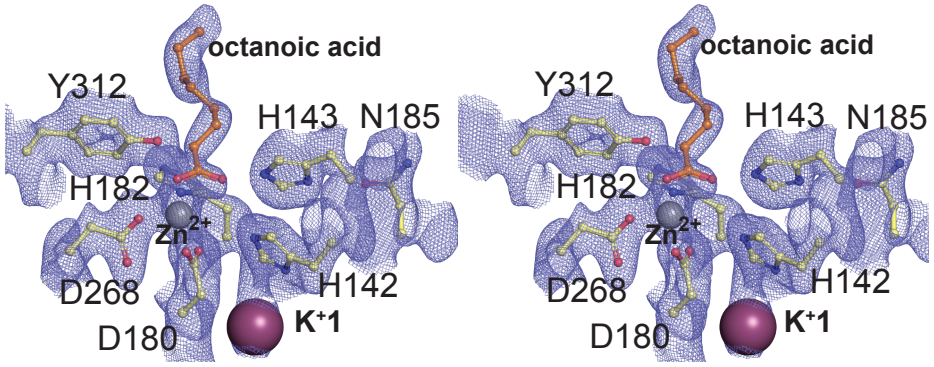
*Kp*HdaH (1b) H144A

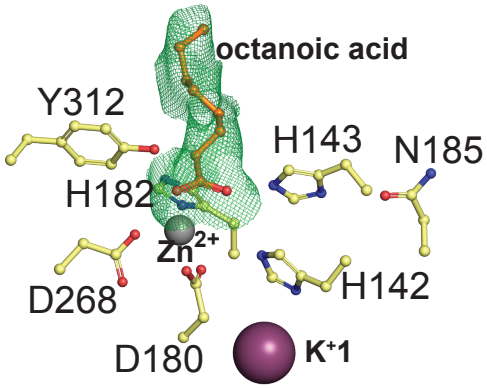




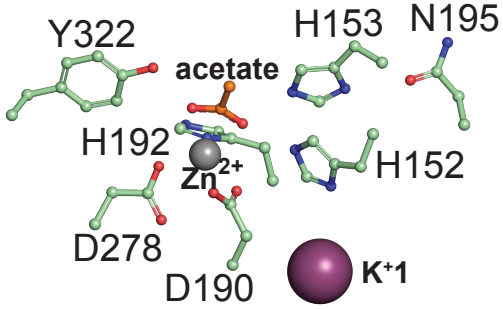
*Rw*DmhA (1b)

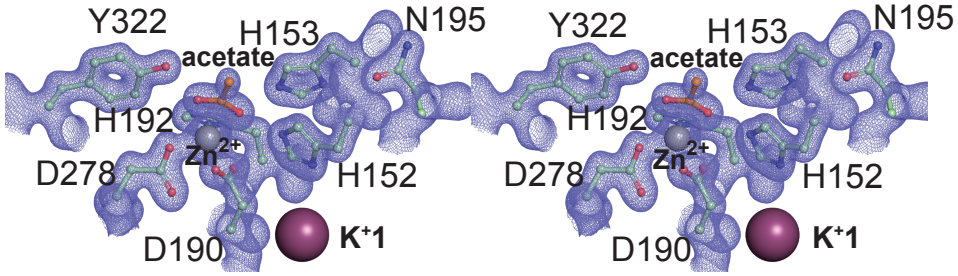




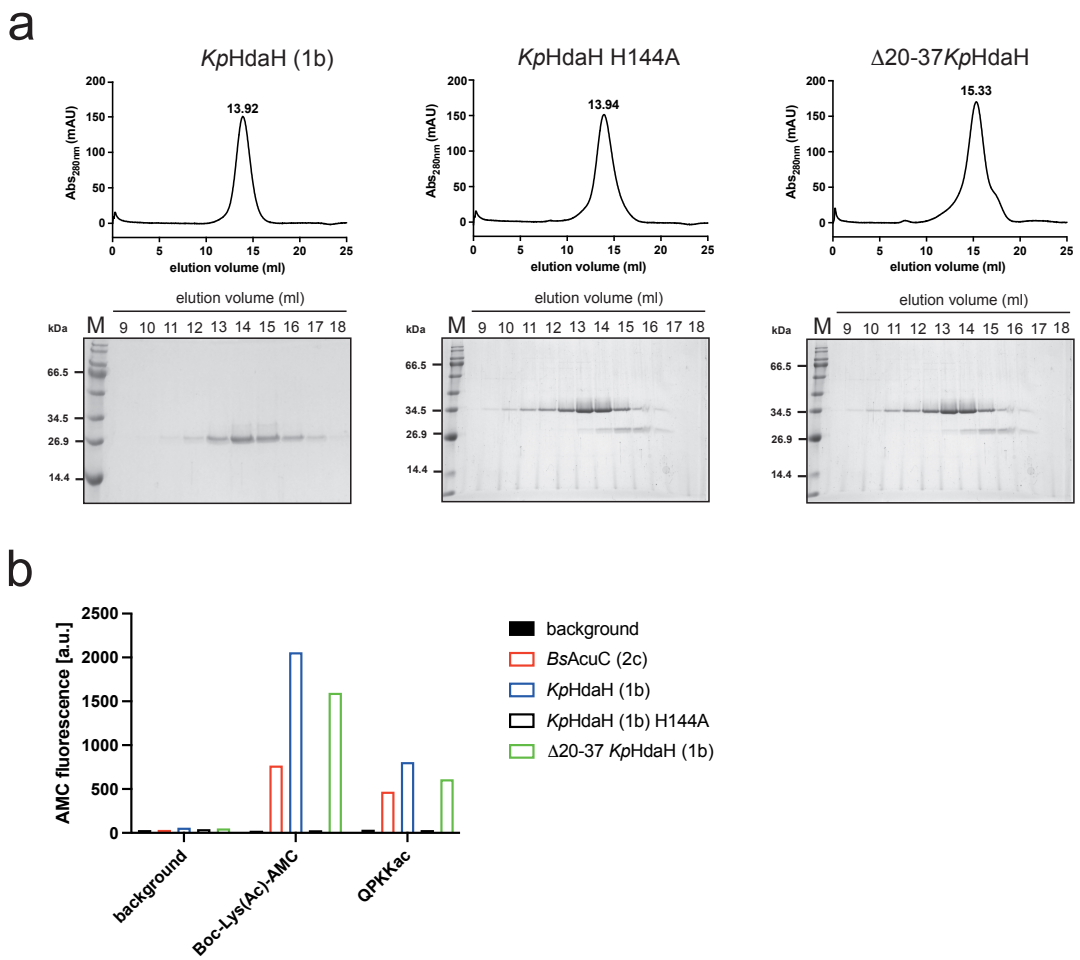


*Rs*PrpH (1b)





Supplementary Figure 12: Stereo images of representative electron density for structures of bacterial deacylases solved in this study.
Stereo figures for the structures of bacterial deacylases of sub-cluster 1b: *Kp*HdaH, *Kp*HdaH H144A, *Rw*DmhA and *Rs*PrpH of subcluster 1b. For all structures a representative closeup of the active site is shown on the left, including the catalytic Zn^{2+}-ion, the coordinating His and the two Asp residues, the catalytic base His, except for the inactivated *Kp*HdaH mutant here the His is mutated to an Ala, and the active site Tyr important to orient and polarize the carbonyl-group of the substrate and to stabilize the tetrahedral intermediate. As stereo images the closeup of the active site is shown with the is the electron density of the $2F_o$-F_c map contoured at 1σ in blue. For *Rw*DmhA (1b) the F_o-F_c omit map is shown in green for the bound octanoic acid (contour level 3σ). In violet spheres the potassium ions are shown, the catalytic Zn^{2+}-ion is shown as grey sphere. The stereo diagrams were prepared in wall-eyed viewing mode with PyMOL version 2.3.4[3].



protein	exp. MW (Da)	elution volume (ml)	calc. MW (Da)	oligomeric state (calc. MW/exp. MW)
*Kp*HdaH (1b)	41569	13.9	136432	3.3
*Kp*HdaH (1b) H144A	41502	13.4	135166	3.3
Δ20-37 *Kp*HdaH (1b)	39830	15.3	70731	1.8

Supplementary Figure 13: Analytical size exclusion chromatography of the L1-loop deleted mutant Δ20-37*Kp*HdaH shows formation of a dimer and the Δ20-37*Kp*HdaH (1b) is still catalytically active albeit with a lower activity compared to wildtype *Kp*HdaH (1b).

a The analytical SEC experiment on a calibrated S200 10/300 GL column reveals the deletion of the loop abolishes tetramer formation. Instead, Δ20-37*Kp*HdaH (1b) elutes as an apparent dimer. Analyses of the crystal structure of *Kp*HdaH (1b) and interface areas suggests the side-by-side dimer being abolished upon L1-loop deletion while the head-to-head dimer stays intact.

b A Fluor-de-Lys assay shows Δ20-37*Kp*HdaH being active as lysine deacylase, however, the activity is reduced compared to wildtype *Kp*HdaH. *Bs*AcuC (2c) shows lower activity compared to Δ20-37*Kp*HdaH and wildtype *Kp*HdaH. The experiment was conducted in assay buffer (50 mM Tris/HCl, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 0.5 mg/mL BSA, pH 8.0). The experiment was performed once (n=1). Source data are provided as Source Data file.

c Evaluation of the oligomeric state of *Kp*HdaH (1b) and Δ20-37*Kp*HdaH as determined by analytical size exclusion chromatography. Source data are provided as Source Data file.

```
1a_Arabidopsis_thaliana_HDA14
                               β1
                              -->
                              1            10
                              .            .
1a_Arabidopsis_thaliana_HDA14  MSMALI....VRP..........FFVP...................................................
1a_Candidatus_Hinthialibacter  MSFGLI.....VD..........SCF....................................................
1a_Actinobacteria_bacterium_R  MCESCGVRLLLY..................................................................
1b_Arabidopsis_thaliana_HDA8   MVTNRV......DV...............................................................F
1b_Klebsiella_pneumoniae_HdaH  MKRKTG........................................................................
1b_Vibrio_sp_vnigr-6D03_HdaH   MSKRTG........................................................................
1c_Shinella_sp_DD12_HdaH       MTTC....LF.E..................................................................
1c_Haematospirillum_jordaniae  MPT....LLV.S..................................................................
1c_Micavibrio_sp_TMED2_Acu     MSL...........................................................................
1d_Legionella_londiniensis_Ap  MAI........................................................................AFI
1d_Thioploca_ingrica_DAC       MPIA....YF.H..................................................................
1d_Neisseria_zoodegmatis_DAC   MNKIVR.....YMR...................L......................QLRRM.............LGRQ
1e_Stentor_coeruleus_HdaH      MSKGIN.....EIE...................V......................DIHEICCL..GRHSDLEEILNS
1e_Chlorella_variabilis_HdaH   MSADGM.....SG..........GSES..EDE.GPS..................SFPLHDAAQA..GDAELIHQ....
1e_Babesia_microti__strain_RI  MASGSQ.....VGT..........CYR..KFQ.P......................PLHKHILR..NDYDAIRYILSS
1f_Pneumycystis_carinii_HdaH   MANPNY.....SLN..........LSDSFYDE.TK................EASYLTSHETDSL..LDA.........
1f_Malassezia_pachydermatis_H  MVFLVK.....LPARKPADGRPPTTNE..QES..............................................
1f_Saccharomyces_cerevisiae_H  MSSKHS.....D...........PLER...................................................
1g_Homo_sapiens_HDAC4          MSSQ....SH.PD..........GLSG..RDQ.PVELLNPARVNHMPSTVDVATALPLQVAPSAVPMDLRLDHQ....
1g_Saccharomyces_cerevisiae_H  MDSVMV.....K................................................KEVL..EN..........
1g_Arabidopsis_thaliana_HDA5   MAMAGE.....SS..........GKKI..GDCDGKV..................A...........GNRQ........

1a_Arabidopsis_thaliana_HDA14

1a_Arabidopsis_thaliana_HDA14  ..............................................................................
1a_Candidatus_Hinthialibacter  ..............................................................................
1a_Actinobacteria_bacterium_R  ..............................................................................
1b_Arabidopsis_thaliana_HDA8   W..HEGMLR.....................................................................
1b_Klebsiella_pneumoniae_HdaH  ..............................................................................
1b_Vibrio_sp_vnigr-6D03_HdaH   ..............................................................................
1c_Shinella_sp_DD12_HdaH       ..............................................................................
1c_Haematospirillum_jordaniae  ..............................................................................
1c_Micavibrio_sp_TMED2_Acu     ..............................................................................
1d_Legionella_londiniensis_Ap  T..HKDCLL.....................................................................
1d_Thioploca_ingrica_DAC       ..............................................................................
1d_Neisseria_zoodegmatis_DAC   A..RAAWIS.....................................................................
1e_Stentor_coeruleus_HdaH      IDFYADIMLERDEEGFIPYHLSLIYCRHKCVDL..ILS........................................
1e_Chlorella_variabilis_HdaH   ..............................LMTPPPDAKPGPPSDDLVGLDDI.LARYVAEASAPQ.LPSINRRDHHE
1e_Babesia_microti__strain_RI  H..HRDCINDMDHDGRNAFHLALQTANSKALYL..LLYYPFVHTNQVF..ATLDWTCDSDDEIDRDIVHVG.......
1f_Pneumycystis_carinii_HdaH   ..............................................................................
1f_Malassezia_pachydermatis_H  ..............................................................................
1f_Saccharomyces_cerevisiae_H  ..............................................................................
1g_Homo_sapiens_HDAC4          ..............................FSL..PVAEPALREQQLQ..QEL.LALKQKQQIQRQILIAEFQRQHEQ
1g_Saccharomyces_cerevisiae_H  ..............................................................................
1g_Arabidopsis_thaliana_HDA5   ..............................................................................

1a_Arabidopsis_thaliana_HDA14

1a_Arabidopsis_thaliana_HDA14  ...GSA........................................................................
1a_Candidatus_Hinthialibacter  ..............................................................................
1a_Actinobacteria_bacterium_R  ..............................................................................
1b_Arabidopsis_thaliana_HDA8   ..............................................................HDAVEGV.........
1b_Klebsiella_pneumoniae_HdaH  ..............................................................................
1b_Vibrio_sp_vnigr-6D03_HdaH   ..............................................................................
1c_Shinella_sp_DD12_HdaH       ..............................................................................
1c_Haematospirillum_jordaniae  ..............................................................................
1c_Micavibrio_sp_TMED2_Acu     ..............................................................................
1d_Legionella_londiniensis_Ap  ..............................................................HNT.............
1d_Thioploca_ingrica_DAC       ..............................................................................
1d_Neisseria_zoodegmatis_DAC   ..............................................................HPL.............
1e_Stentor_coeruleus_HdaH      ..............................................................................
1e_Chlorella_variabilis_HdaH   CTPLHVALLHGQLE........................................AARA.........LLEHGA.....
1e_Babesia_microti__strain_RI  .............NAKPAYQAGDRLLMKWNETPDLYKKAAFSALEGYSIRGCGLRIVEDLYEQHYKYILDKPN.....
1f_Pneumycystis_carinii_HdaH   .......ILNISL...........................................................KDEETK
1f_Malassezia_pachydermatis_H  ..............................................................................
1f_Saccharomyces_cerevisiae_H  ..............................................................................
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1g_Saccharomyces_cerevisiae_H  ............P...........................................................DHDLKR
1g_Arabidopsis_thaliana_HDA5   ...RKV........................................................................
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1a_Arabidopsis_thaliana_HDA14

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1a_Arabidopsis_thaliana_HDA14  ..........................................................................
1a_Candidatus_Hinthialibacter  ..........................................................................
1a_Actinobacteria_bacterium_R  ..........................................................................
1b_Arabidopsis_thaliana_HDA8   ..........................................................................
1b_Klebsiella_pneumoniae_HdaH  ..........................................................................
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1c_Shinella_sp_DD12_HdaH       ..........................................................................
1c_Haematospirillum_jordaniae  ..........................................................................
1c_Micavibrio_sp_TMED2_Acu     ..........................................................................
1d_Legionella_londiniensis_Ap   ..........................................................................
1d_Thioploca_ingrica_DAC       ..........................................................................
1d_Neisseria_zoodegmatis_DAC   ..........................................................................
1e_Stentor_coeruleus_HdaH      ..........................................................................
1e_Chlorella_variabilis_HdaH   SLE.......................................................................
1e_Babesia_microti__strain_RI  TLERSAREY.........TAKYARLYSKEINFPEKIETID..ELKEKNYISTIITSEIVMEKDD...NTRVSFASDFI
1f_Pneumycystis_carinii_HdaH   KLSDAVDEQ.....................................................................
1f_Malassezia_pachydermatis_H  .VP.....................................................................
1f_Saccharomyces_cerevisiae_H  ..........................................................................
1g_Homo_sapiens_HDAC4          KLERHRQEQELEKQHREQK.....................LQQLKNKEKGKESAVASTEVKMKLQEFVLNKKKALAHRNL
1g_Saccharomyces_cerevisiae_H  KLEENKEEE.....................................NSLSTTSKSKRQVIV.................
1g_Arabidopsis_thaliana_HDA5   ..........................................................................
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1a_Arabidopsis_thaliana_HDA14

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                                                                                                  20
                                                                                                  .
1a_Arabidopsis_thaliana_HDA14  ..........................................................GISGSRNICKK
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1b_Klebsiella_pneumoniae_HdaH  ..........................................................................
1b_Vibrio_sp_vnigr-6D03_HdaH   ..........................................................................
1c_Shinella_sp_DD12_HdaH       ..........................................................................
1c_Haematospirillum_jordaniae  ..........................................................................
1c_Micavibrio_sp_TMED2_Acu     ..........................................................SI........
1d_Legionella_londiniensis_Ap   ..........................................................................
1d_Thioploca_ingrica_DAC       ..........................................................................
1d_Neisseria_zoodegmatis_DAC   ..........................................................................
1e_Stentor_coeruleus_HdaH      ............LSIDFQVPYQGFMPIHLAMACAG..................................................
1e_Chlorella_variabilis_HdaH   ..................LGCEGSPPLHVAVCVAAHGAMQEFALAAMALLLQFGADPYERNNNGRTAL.....H...
1e_Babesia_microti__strain_RI  DSCDNFELQPIPLETVDLLVSFDMTSPIHLLFSNIY.....................................................
1f_Pneumycystis_carinii_HdaH   ..........................................................................
1f_Malassezia_pachydermatis_H  ................SGVHAVRPVTIVPATAASTTTTETPAH.....................................
1f_Saccharomyces_cerevisiae_H  ..........................................................................
1g_Homo_sapiens_HDAC4          NHCISSDPR..YWYGKTQHSSLDQSSPPQSGV.....................................................
1g_Saccharomyces_cerevisiae_H  ..........................................................................
1g_Arabidopsis_thaliana_HDA5   ..........................................................GL........
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1a_Arabidopsis_thaliana_HDA14

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                30
                .
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1a_Actinobacteria_bacterium_R  ..........................................................................
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1b_Klebsiella_pneumoniae_HdaH  ..........................................................................
1b_Vibrio_sp_vnigr-6D03_HdaH   ..........................................................................
1c_Shinella_sp_DD12_HdaH       ..........................................................................
1c_Haematospirillum_jordaniae  ..........................................................................
1c_Micavibrio_sp_TMED2_Acu     ....FTH.....................................................................
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1d_Thioploca_ingrica_DAC       ..........................................................................
1d_Neisseria_zoodegmatis_DAC   ..........................................................................
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1e_Chlorella_variabilis_HdaH   .....WAA.....QMDFSAAAEAVLA.....................................................AAE
1e_Babesia_microti__strain_RI  ........................IESYREKILDCLKILITFFRVFDHKE....TVDSVKCMHLKNI..........
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1f_Malassezia_pachydermatis_H  .......MMPAAEDTDGAAFQEAVPA.....................................................PAL
1f_Saccharomyces_cerevisiae_H  ....FYK.....................................................................
1g_Homo_sapiens_HDAC4          ........................STSYNHPVL.........GMYD..................AKDDFPLRKTAS
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1g_Arabidopsis_thaliana_HDA5   ....IYD.....................................................................
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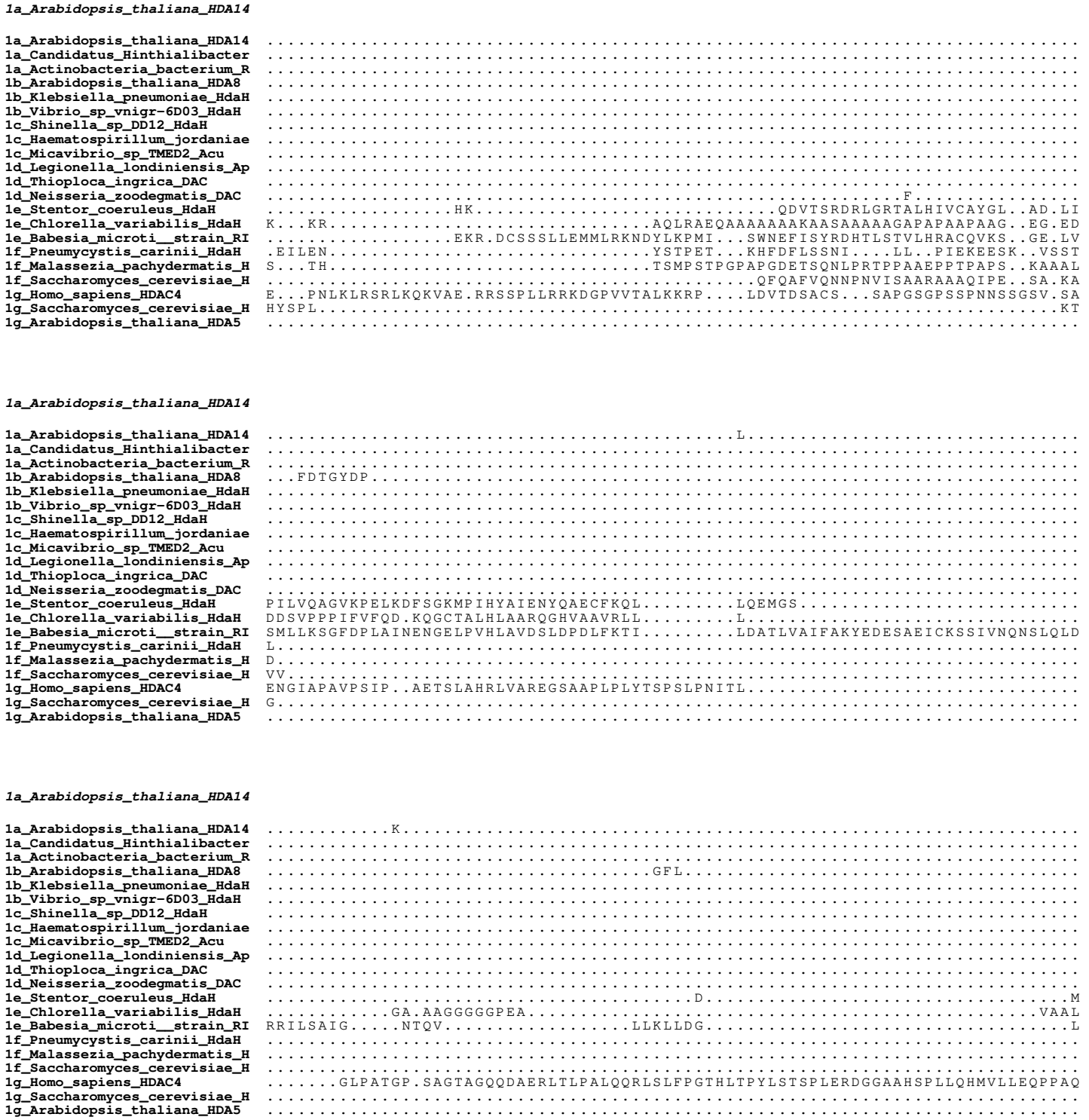
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1a_Arabidopsis_thaliana_HDA14
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1a_Actinobacteria_bacterium_R
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1b_Klebsiella_pneumoniae_HdaH
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1c_Shinella_sp_DD12_HdaH
1c_Haematospirillum_jordaniae
1c_Micavibrio_sp_TMED2_Acu
1d_Legionella_londiniensis_Ap
1d_Thioploca_ingrica_DAC
1d_Neisseria_zoodegmatis_DAC
1e_Stentor_coeruleus_HdaH
1e_Chlorella_variabilis_HdaH
1e_Babesia_microti__strain_RI
1f_Pneumycystis_carinii_HdaH
1f_Malassezia_pachydermatis_H
1f_Saccharomyces_cerevisiae_H
1g_Homo_sapiens_HDAC4
1g_Saccharomyces_cerevisiae_H
1g_Arabidopsis_thaliana_HDA5
1a_Arabidopsis_thaliana_HDA14
1a_Arabidopsis_thaliana_HDA14
1a_Candidatus_Hinthialibacter
1a_Actinobacteria_bacterium_R
1b_Arabidopsis_thaliana_HDA8
1b_Klebsiella_pneumoniae_HdaH
1b_Vibrio_sp_vnigr-6D03_HdaH
1c_Shinella_sp_DD12_HdaH
1c_Haematospirillum_jordaniae
1c_Micavibrio_sp_TMED2_Acu
1d_Legionella_londiniensis_Ap
1d_Thioploca_ingrica_DAC
1d_Neisseria_zoodegmatis_DAC
1e_Stentor_coeruleus_HdaH
1e_Chlorella_variabilis_HdaH
1e_Babesia_microti__strain_RI
1f_Pneumycystis_carinii_HdaH
1f_Malassezia_pachydermatis_H
1f_Saccharomyces_cerevisiae_H
1g_Homo_sapiens_HDAC4
1g_Saccharomyces_cerevisiae_H
1g_Arabidopsis_thaliana_HDA5
1a_Arabidopsis_thaliana_HDA14
1a_Arabidopsis_thaliana_HDA14
1a_Candidatus_Hinthialibacter
1a_Actinobacteria_bacterium_R
1b_Arabidopsis_thaliana_HDA8
1b_Klebsiella_pneumoniae_HdaH
1b_Vibrio_sp_vnigr-6D03_HdaH
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1c_Haematospirillum_jordaniae
1c_Micavibrio_sp_TMED2_Acu
1d_Legionella_londiniensis_Ap
1d_Thioploca_ingrica_DAC
1d_Neisseria_zoodegmatis_DAC
1e_Stentor_coeruleus_HdaH
1e_Chlorella_variabilis_HdaH
1e_Babesia_microti__strain_RI
1f_Pneumycystis_carinii_HdaH
1f_Malassezia_pachydermatis_H
1f_Saccharomyces_cerevisiae_H
1g_Homo_sapiens_HDAC4
1g_Saccharomyces_cerevisiae_H
1g_Arabidopsis_thaliana_HDA5

```
1a_Arabidopsis_thaliana_HDA14

1a_Arabidopsis_thaliana_HDA14    ..............................................................................
1a_Candidatus_Hinthialibacter    ..............................................................................
1a_Actinobacteria_bacterium_R    ..............................................................................
1b_Arabidopsis_thaliana_HDA8     ..................DVLEK.......................................................
1b_Klebsiella_pneumoniae_HdaH    ..............................................................................
1b_Vibrio_sp_vnigr-6D03_HdaH     ..............................................................................
1c_Shinella_sp_DD12_HdaH         ..............................................................................
1c_Haematospirillum_jordaniae    ..............................................................................
1c_Micavibrio_sp_TMED2_Acu       ..............................................................................
1d_Legionella_londiniensis_Ap    ..............................................................................
1d_Thioploca_ingrica_DAC         ..............................................................................
1d_Neisseria_zoodegmatis_DAC     ..............................................................................
1e_Stentor_coeruleus_HdaH        FFCTDN.......................................................................R
1e_Chlorella_variabilis_HdaH     CRTRSKA.......................................................................
1e_Babesia_microti__strain_RI    LTCDNNSSKVRDGSRAEMDYLKNNSQKDKIFNNKLILKEDHTFLKQVPGEVIFERLFSKYGECFITTISSLFSELLLF
1f_Pneumycystis_carinii_HdaH     ..............................................................................
1f_Malassezia_pachydermatis_H    ..............................................................................
1f_Saccharomyces_cerevisiae_H    ..............................................................................
1g_Homo_sapiens_HDAC4            APLVTGL.......................................................................
1g_Saccharomyces_cerevisiae_H    ..............................................................................
1g_Arabidopsis_thaliana_HDA5     ..............................................................................


1a_Arabidopsis_thaliana_HDA14

1a_Arabidopsis_thaliana_HDA14    ..............................................................................
1a_Candidatus_Hinthialibacter    ..............................................................................
1a_Actinobacteria_bacterium_R    ..............................................................................
1b_Arabidopsis_thaliana_HDA8     ..............................................................................
1b_Klebsiella_pneumoniae_HdaH    ..............................................................................
1b_Vibrio_sp_vnigr-6D03_HdaH     ..............................................................................
1c_Shinella_sp_DD12_HdaH         ..............................................................................
1c_Haematospirillum_jordaniae    ..............................................................................
1c_Micavibrio_sp_TMED2_Acu       ..............................................................................
1d_Legionella_londiniensis_Ap    ..............................................................................
1d_Thioploca_ingrica_DAC         ..............................................................................
1d_Neisseria_zoodegmatis_DAC     ..............................................................................
1e_Stentor_coeruleus_HdaH        GDKPIHTSVRAGSWECFNILITF...........................................GSEEMLNENNEF
1e_Chlorella_variabilis_HdaH     GHNALHLAALAGSSRCAALLAAA............................................AL.........
1e_Babesia_microti__strain_RI    FEQLFYRCIHRNSWRVFLALASFNNTLTFHMFSSPLCVHRFIKFAHSMGNVYAFFSILNYSVRLLYPNETKCVENMDF
1f_Pneumycystis_carinii_HdaH     ..............................................................................
1f_Malassezia_pachydermatis_H    ..............................................................................
1f_Saccharomyces_cerevisiae_H    ..............................................................................
1g_Homo_sapiens_HDAC4            GALPLHAQSLVGADRVSPSIHKL............................................RQ.........
1g_Saccharomyces_cerevisiae_H    ..............................................................................
1g_Arabidopsis_thaliana_HDA5     ..............................................................................


1a_Arabidopsis_thaliana_HDA14

1a_Arabidopsis_thaliana_HDA14    ..............................................................................
1a_Candidatus_Hinthialibacter    ..............................................................................
1a_Actinobacteria_bacterium_R    ..............................................................................
1b_Arabidopsis_thaliana_HDA8     ..............................................................................
1b_Klebsiella_pneumoniae_HdaH    ..............................................................................
1b_Vibrio_sp_vnigr-6D03_HdaH     ..............................................................................
1c_Shinella_sp_DD12_HdaH         ..............................................................................
1c_Haematospirillum_jordaniae    ..............................................................................
1c_Micavibrio_sp_TMED2_Acu       ..............................................................................
1d_Legionella_londiniensis_Ap    ..............................................................................
1d_Thioploca_ingrica_DAC         ..............................................................................
1d_Neisseria_zoodegmatis_DAC     ..............................................................................
1e_Stentor_coeruleus_HdaH        E.......................ETP........................................EEVA.......
1e_Chlorella_variabilis_HdaH     ..............DAAAGKSKLGLTP........................................ADLAAR.....
1e_Babesia_microti__strain_RI    ELGDMSMTFNRRNVKVEKGQDKITPAP........................................TNFAQY.....
1f_Pneumycystis_carinii_HdaH     ..............................................................................
1f_Malassezia_pachydermatis_H    ..............................................................................
1f_Saccharomyces_cerevisiae_H    ..............................................................................
1g_Homo_sapiens_HDAC4            ..............HRPLGRTQSAPLPQNAQALQHLVIQQQHQQFLEKHKQQFQQQQLQMNKIIPKPSEPARQPESHP
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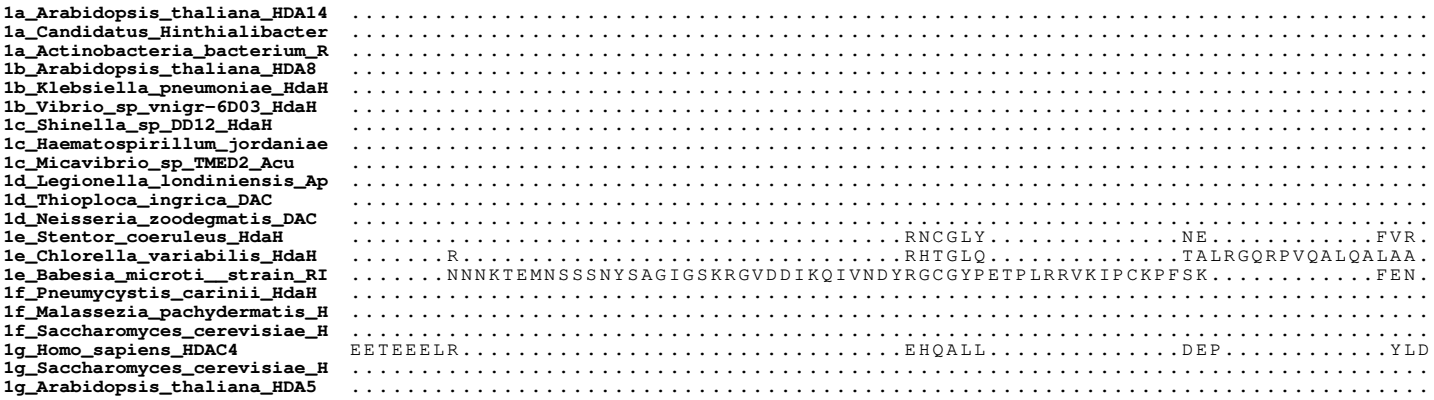
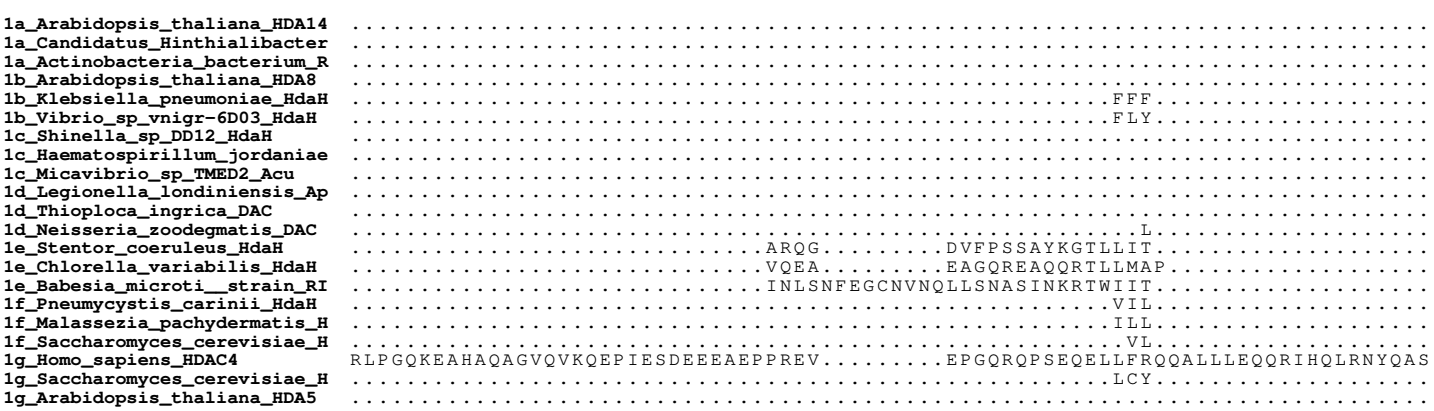
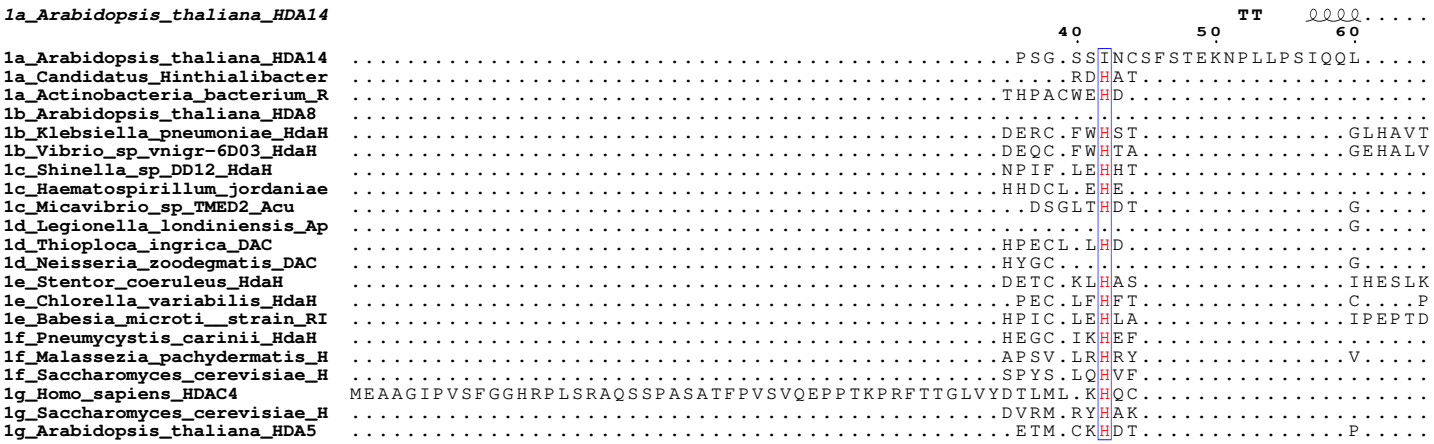
1a_Arabidopsis_thaliana_HDA14

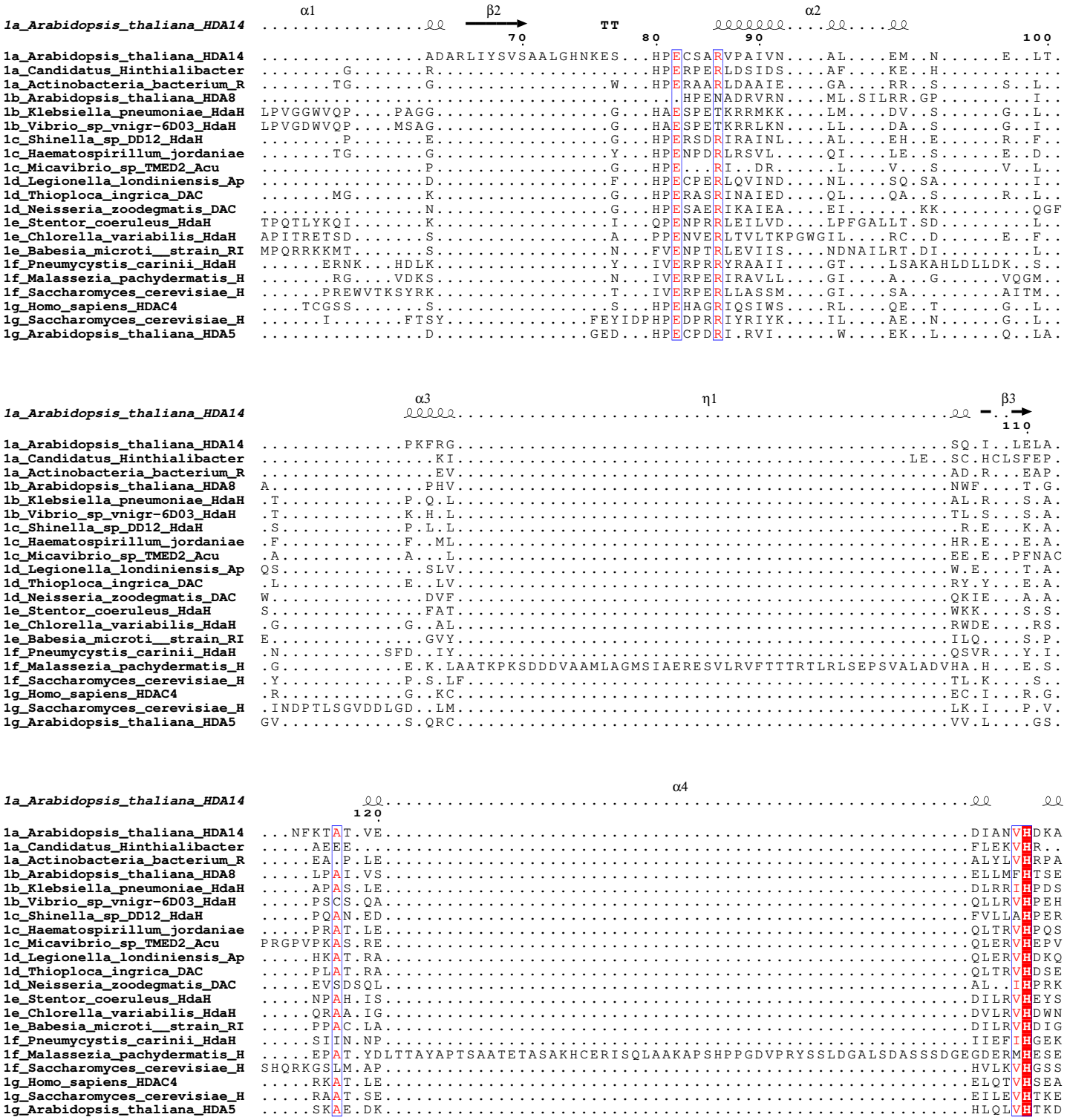


1a_Arabidopsis_thaliana_HDA14



1a_Arabidopsis_thaliana_HDA14




α1
β2
TT
α2
70
80
90
100
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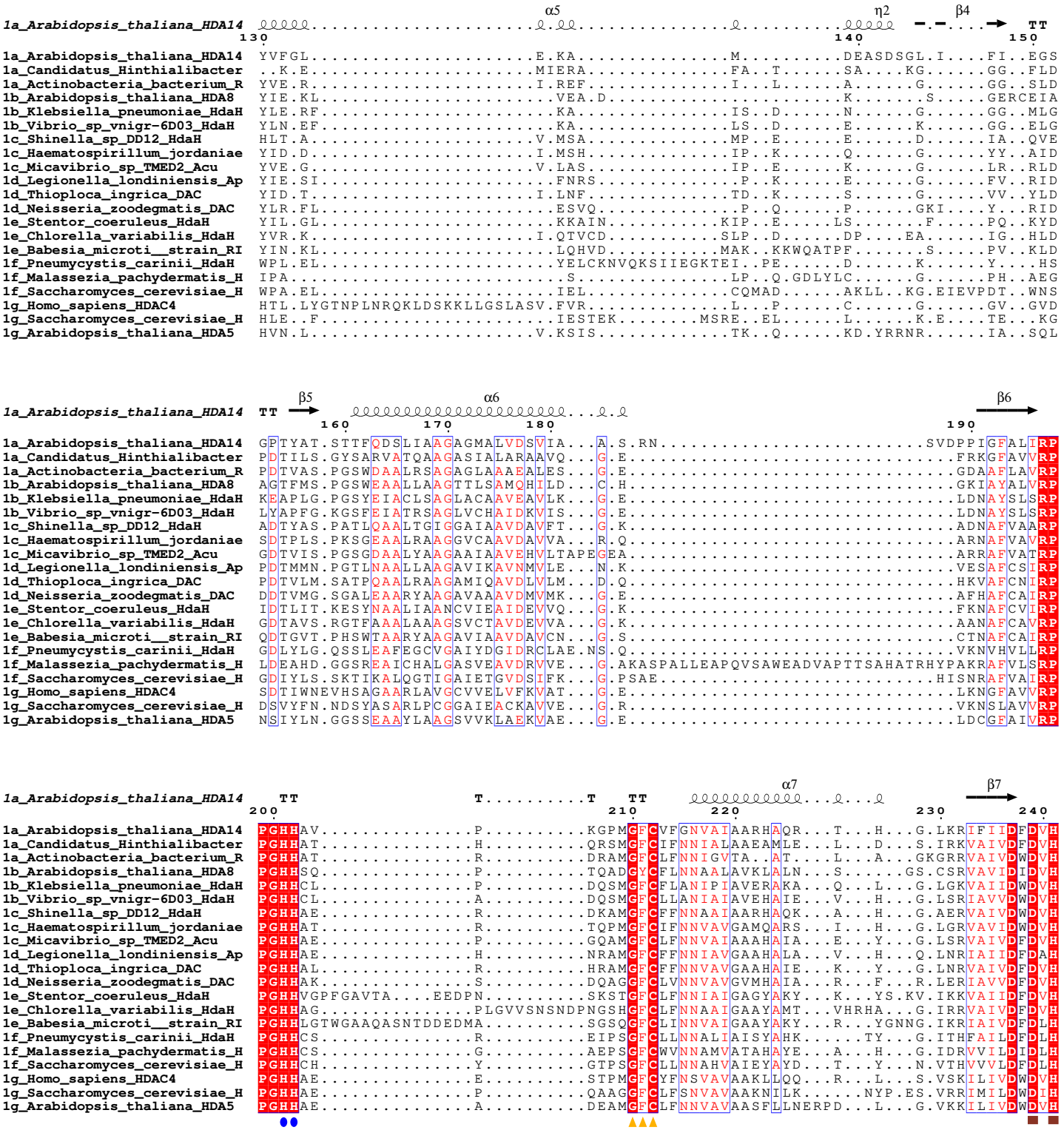
1a_Candidatus_Hinthialibacter
1a_Actinobacteria_bacterium_R
1b_Arabidopsis_thaliana_HDA8
1b_Klebsiella_pneumoniae_HdaH
1b_Vibrio_sp_vnigr-6D03_HdaH
1c_Shinella_sp_DD12_HdaH
1c_Haematospirillum_jordaniae
1c_Micavibrio_sp_TMED2_Acu
1d_Legionella_londiniensis_Ap
1d_Thioploca_ingrica_DAC
1d_Neisseria_zoodegmatis_DAC
1e_Stentor_coeruleus_HdaH
1e_Chlorella_variabilis_HdaH
1e_Babesia_microti__strain_RI
1f_Pneumycystis_carinii_HdaH
1f_Malassezia_pachydermatis_H
1f_Saccharomyces_cerevisiae_H
1g_Homo_sapiens_HDAC4
1g_Saccharomyces_cerevisiae_H
1g_Arabidopsis_thaliana_HDA5
α3
η1
β3
110
α4
120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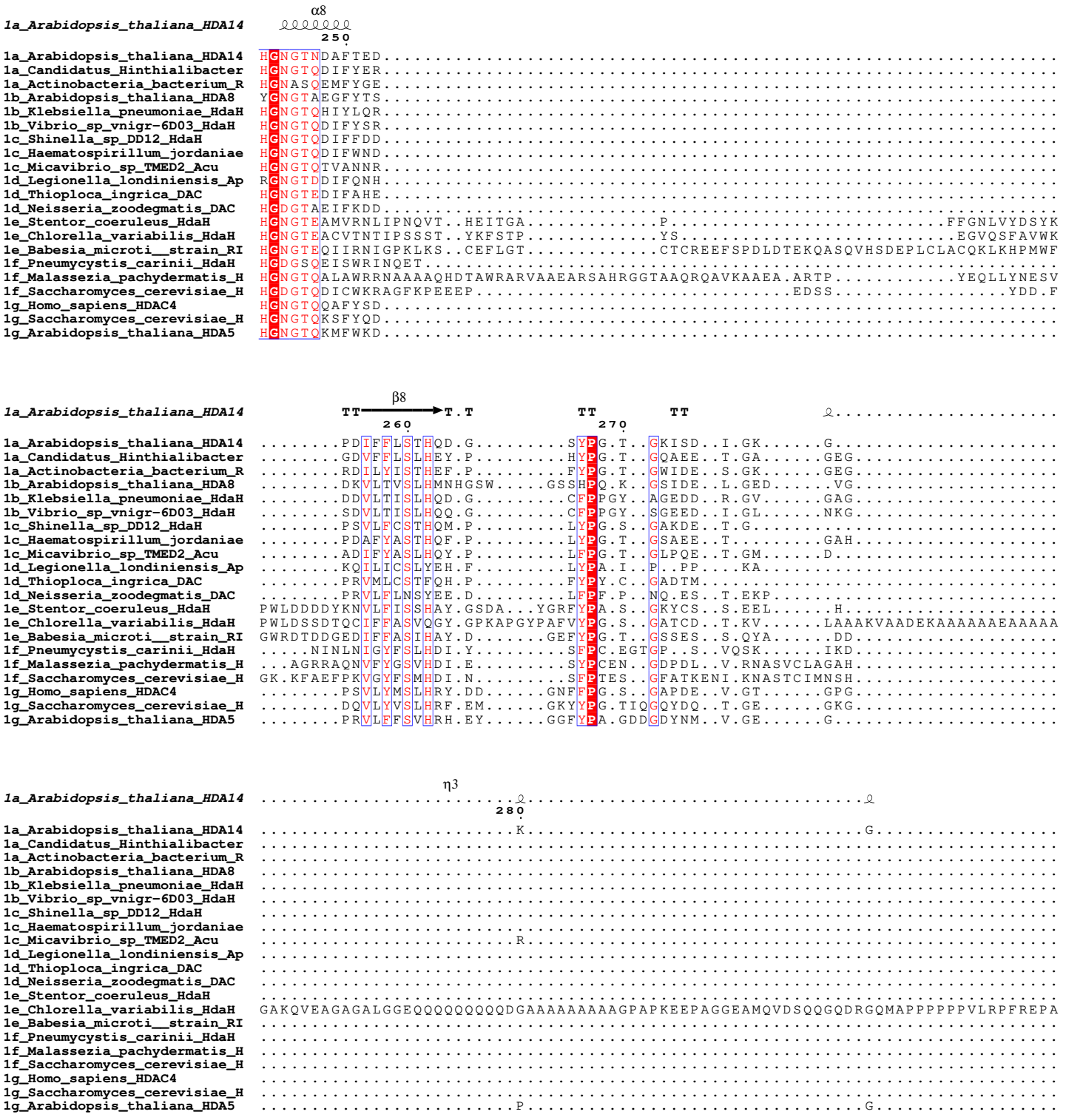
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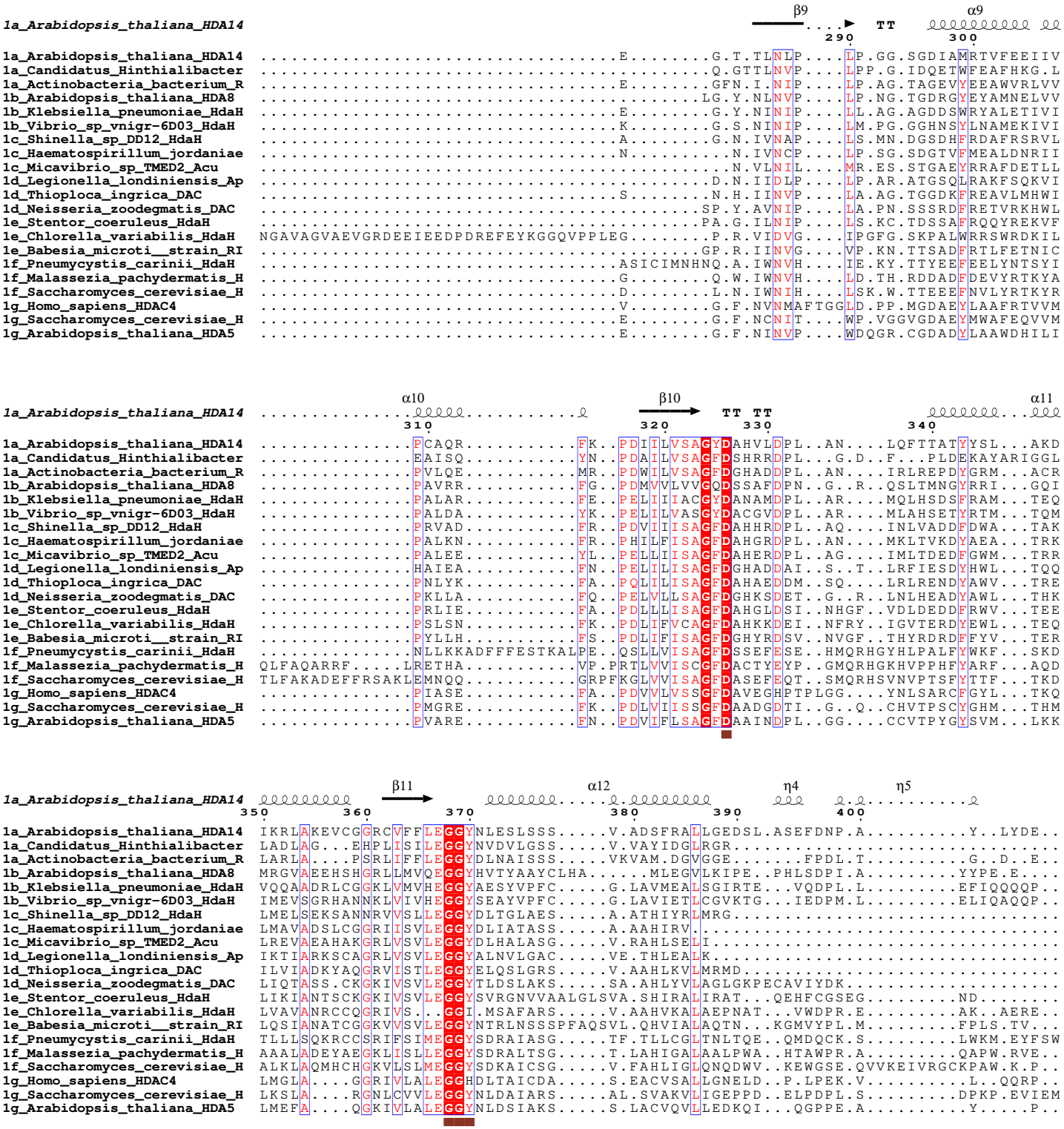
260
270
η3
280


β9
α9
TT
290
300
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1a_Actinobacteria_bacterium_R
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1b_Klebsiella_pneumoniae_HdaH
1b_Vibrio_sp_vnigr-6D03_HdaH
1c_Shinella_sp_DD12_HdaH
1c_Haematospirillum_jordaniae
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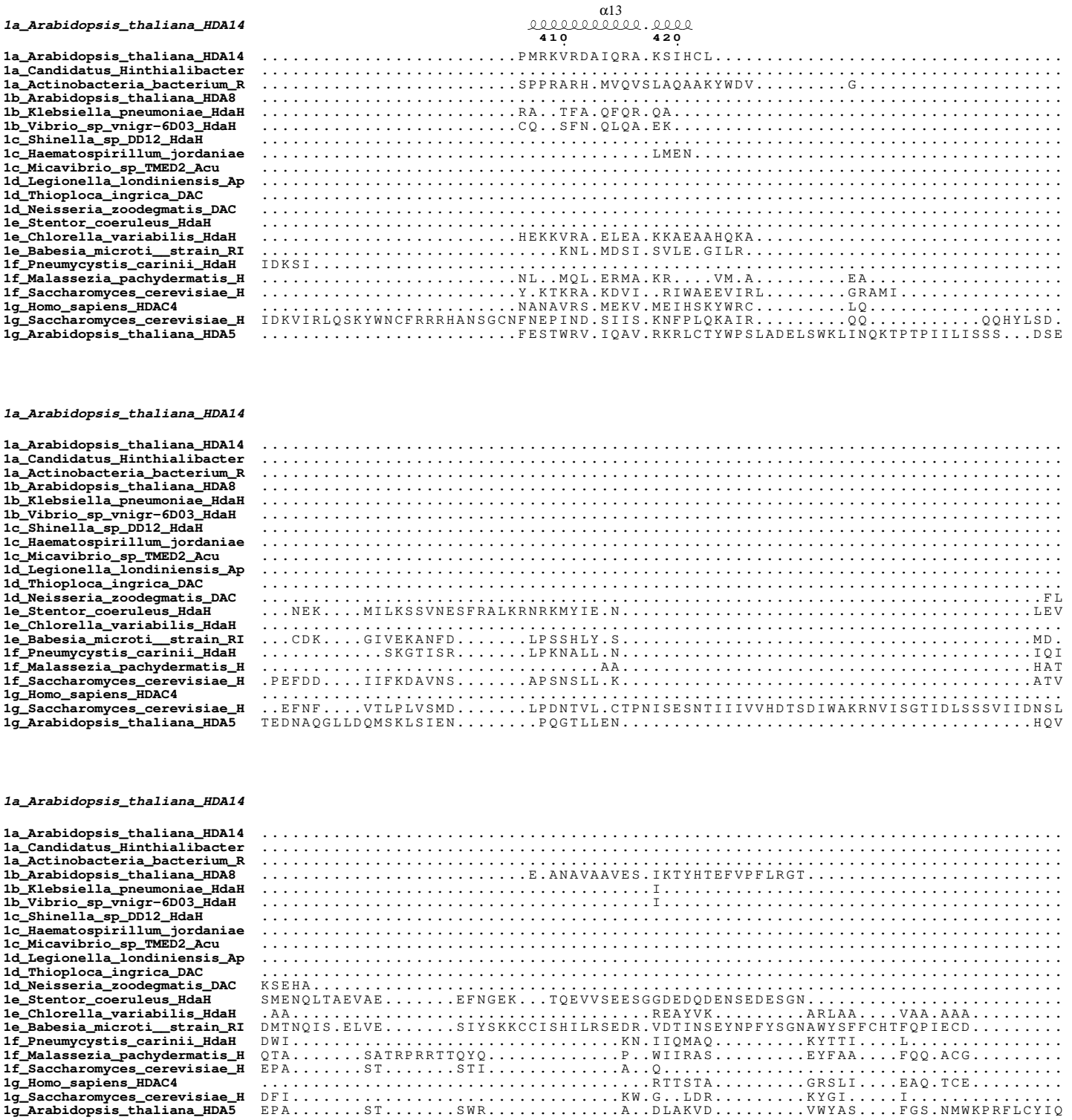
1d_Legionella_londiniensis_Ap
1d_Thioploca_ingrica_DAC
1d_Neisseria_zoodegmatis_DAC
1e_Stentor_coeruleus_HdaH
1e_Chlorella_variabilis_HdaH
1e_Babesia_microti__strain_RI
1f_Pneumycystis_carinii_HdaH
1f_Malassezia_pachydermatis_H
1f_Saccharomyces_cerevisiae_H
1g_Homo_sapiens_HDAC4
1g_Saccharomyces_cerevisiae_H
1g_Arabidopsis_thaliana_HDA5
α10
β10
TT TT
α11
310
320
330
340
β11
α12
η4
η5
350
360
370
380
390
400


α13
410
420
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1b_Arabidopsis_thaliana_HDA8 .....
1b_Klebsiella_pneumoniae_HdaH .....RA..TFA.QFQR.QA.....
1b_Vibrio_sp_vnigr-6D03_HdaH .....CQ..SFN.QLQA.EK.....
1c_Shinella_sp_DD12_HdaH .....
1c_Haematospirillum_jordaniae .....LMEN.....
1c_Micavibrio_sp_TMED2_Acu .....
1d_Legionella_londiniensis_Ap .....
1d_Thioploca_ingrica_DAC .....
1d_Neisseria_zoodegmatis_DAC .....
1e_Stentor_coeruleus_HdaH .....
1e_Chlorella_variabilis_HdaH .....HEKKVRA.ELEA.KKAEAAHQKA.....
1e_Babesia_microti__strain_RI .....KNL.MDSI.SVLE.GILR.....
1f_Pneumycystis_carinii_HdaH IDKSI.....
1f_Malassezia_pachydermatis_H .....NL..MQL.ERMA.KR....VM.A.....EA.....
1f_Saccharomyces_cerevisiae_H .....Y.KTKRA.KDVI..RIWAEEVIRL.....GRAMI.....
1g_Homo_sapiens_HDAC4 .....NANAVRS.MEKV.MEIHSKYWRC.....LQ.....
1g_Saccharomyces_cerevisiae_H IDKVIRLQSKYWNCFRRRHANSGCNFNEPIND.SIIS.KNFPLQKAIR.....QQ.....QQHYLSD.
1g_Arabidopsis_thaliana_HDA5 .....FESTWRV.IQAV.RKRLCTYWPSLADELSWKLINQKTPTPIILISSS...DSE
1a_Arabidopsis_thaliana_HDA14
1a_Arabidopsis_thaliana_HDA14 .....
1a_Candidatus_Hinthialibacter .....
1a_Actinobacteria_bacterium_R .....
1b_Arabidopsis_thaliana_HDA8 .....
1b_Klebsiella_pneumoniae_HdaH .....
1b_Vibrio_sp_vnigr-6D03_HdaH .....
1c_Shinella_sp_DD12_HdaH .....
1c_Haematospirillum_jordaniae .....
1c_Micavibrio_sp_TMED2_Acu .....
1d_Legionella_londiniensis_Ap .....
1d_Thioploca_ingrica_DAC .....
1d_Neisseria_zoodegmatis_DAC .....FL
1e_Stentor_coeruleus_HdaH ...NEK....MILKSSVNESFRALKRNRKMYIE.N.....LEV
1e_Chlorella_variabilis_HdaH .....
1e_Babesia_microti__strain_RI ...CDK....GIVEKANFD.....LPSSHLY.S.....MD.
1f_Pneumycystis_carinii_HdaH .....SKGTISR.....LPKNALL.N.....IQI
1f_Malassezia_pachydermatis_H .....AA.....HAT
1f_Saccharomyces_cerevisiae_H .PEFDD....IIFKDAVNS.....APSNSLL.K.....ATV
1g_Homo_sapiens_HDAC4 .....
1g_Saccharomyces_cerevisiae_H ..EFNF....VTLPLVSMD.....LPDNTVL.CTPNISESNTIIIVVHDTSDIWAKRNVISGTIDLSSSVIIDNSL
1g_Arabidopsis_thaliana_HDA5 TEDNAQGLLDQMSKLSIEN.....PQGTLLEN.....HQV
1a_Arabidopsis_thaliana_HDA14
1a_Arabidopsis_thaliana_HDA14 .....
1a_Candidatus_Hinthialibacter .....
1a_Actinobacteria_bacterium_R .....
1b_Arabidopsis_thaliana_HDA8 .....E.ANAVAAVES.IKTYHTEFVPFLRGT.....
1b_Klebsiella_pneumoniae_HdaH .....I.....
1b_Vibrio_sp_vnigr-6D03_HdaH .....I.....
1c_Shinella_sp_DD12_HdaH .....
1c_Haematospirillum_jordaniae .....
1c_Micavibrio_sp_TMED2_Acu .....
1d_Legionella_londiniensis_Ap .....
1d_Thioploca_ingrica_DAC .....
1d_Neisseria_zoodegmatis_DAC KSEHA.....
1e_Stentor_coeruleus_HdaH SMENQLTAEVAE.....EFNGEK...TQEVVSEESGGDEDQDENSEDESGN.....
1e_Chlorella_variabilis_HdaH .AA.....REAYVK.....ARLAA....VAA.AAA.....
1e_Babesia_microti__strain_RI DMTNQIS.ELVE.....SIYSKKCCISHILRSEDR.VDTINSEYNPFYSGNAWYSFFCHTFQPIECD.....
1f_Pneumycystis_carinii_HdaH DWI.....KN.IIQMAQ.....KYTTI....L.....
1f_Malassezia_pachydermatis_H QTA.....SATRPRRTTQYQ.....P..WIIRAS.....EYFAA....FQQ.ACG.....
1f_Saccharomyces_cerevisiae_H EPA.....ST.....STI.....A..Q.....
1g_Homo_sapiens_HDAC4 .....RTTSTA.....GRSLI....EAQ.TCE.....
1g_Saccharomyces_cerevisiae_H DFI.....KW.G..LDR.....KYGI.....I.....
1g_Arabidopsis_thaliana_HDA5 EPA.....ST.....SWR.....A..DLAKVD.....VWYAS....FGS.NMWKPRFLCYIQ

1a_Arabidopsis_thaliana_HDA14

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1a_Arabidopsis_thaliana_HDA14  ..............................................................................
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1a_Actinobacteria_bacterium_R  ..............................................................................
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1b_Klebsiella_pneumoniae_HdaH  ..............................................................................
1b_Vibrio_sp_vnigr-6D03_HdaH   ..............................................................................
1c_Shinella_sp_DD12_HdaH       ..............................................................................
1c_Haematospirillum_jordaniae  ..............................................................................
1c_Micavibrio_sp_TMED2_Acu     ..............................................................................
1d_Legionella_londiniensis_Ap  ..............................................................................
1d_Thioploca_ingrica_DAC       ..............................................................................
1d_Neisseria_zoodegmatis_DAC   ..............................................................................
1e_Stentor_coeruleus_HdaH      ..............................................................................
1e_Chlorella_variabilis_HdaH   ...APAAPAGTAPAAAGTSAPA.ASPPT..................................................
1e_Babesia_microti__strain_RI  ...DDIRQNNN......SPSPM.CI..........P..........................................
1f_Pneumycystis_carinii_HdaH   .........ET......KTDLI.L.........S............................................
1f_Malassezia_pachydermatis_H  ...IDARPLTL......TPSAT.TT..........PRRAVL.....................................
1f_Saccharomyces_cerevisiae_H  ........RII......RSHRSNASPEK.ELHENKPRSTE........................KQEQREIR....SD
1g_Homo_sapiens_HDAC4          ..............................................................................
1g_Saccharomyces_cerevisiae_H  .........DV......NIPLT.L.........F....................................EPDNYSGM
1g_Arabidopsis_thaliana_HDA5   GGQVDGLKKVC......VGSMD.KSPPKETVWETFPHRLFFGRESSVGWGVGGVAFTNPLANLID.QTHMCLYRITLE
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1a_Arabidopsis_thaliana_HDA14

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1a_Arabidopsis_thaliana_HDA14  ..............................................................................
1a_Candidatus_Hinthialibacter  ..............................................................................
1a_Actinobacteria_bacterium_R  ..............................................................................
1b_Arabidopsis_thaliana_HDA8   ..............................................................................
1b_Klebsiella_pneumoniae_HdaH  ..............................................................................
1b_Vibrio_sp_vnigr-6D03_HdaH   ..............................................................................
1c_Shinella_sp_DD12_HdaH       ..............................................................................
1c_Haematospirillum_jordaniae  ..............................................................................
1c_Micavibrio_sp_TMED2_Acu     ..............................................................................
1d_Legionella_londiniensis_Ap  ..............................................................................
1d_Thioploca_ingrica_DAC       ..............................................................................
1d_Neisseria_zoodegmatis_DAC   ..............................................................................
1e_Stentor_coeruleus_HdaH      .............................................................................E
1e_Chlorella_variabilis_HdaH   ..............................................................................
1e_Babesia_microti__strain_RI  ............................................FD.........................IDSSTIN
1f_Pneumycystis_carinii_HdaH   ................................EDR.........MTL...RERK...............KPLDSSLIS
1f_Malassezia_pachydermatis_H  ..........................................RGLDL...DLAT...............PSRVTAGGH
1f_Saccharomyces_cerevisiae_H  T.................................KVKQLSSNNR.AA...ETQIPFLQQEFSSEDEDEEYVYDEELNK
1g_Homo_sapiens_HDAC4          ..................................................NE..........................
1g_Saccharomyces_cerevisiae_H  ITSQEVLIYLWDNYIKYFPSVAKIAFIGIGDSYSG.........IVHLLGHRDT...............RAVTKTVIN
1g_Arabidopsis_thaliana_HDA5   Q.................................FNDVLSQENGLNV...DSDS...............PVFDLAALQ
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1a_Arabidopsis_thaliana_HDA14

```
1a_Arabidopsis_thaliana_HDA14  ..............................................................................
1a_Candidatus_Hinthialibacter  ..............................................................................
1a_Actinobacteria_bacterium_R  ..............................................................................
1b_Arabidopsis_thaliana_HDA8   ..............................................................................
1b_Klebsiella_pneumoniae_HdaH  ..............................................................................
1b_Vibrio_sp_vnigr-6D03_HdaH   ..............................................................................
1c_Shinella_sp_DD12_HdaH       ..............................................................................
1c_Haematospirillum_jordaniae  ..............................................................................
1c_Micavibrio_sp_TMED2_Acu     ..............................................................................
1d_Legionella_londiniensis_Ap  ..............................................................................
1d_Thioploca_ingrica_DAC       ..............................................................................
1d_Neisseria_zoodegmatis_DAC   ..............................................................................
1e_Stentor_coeruleus_HdaH      SEEG...........EEGEEREDNE.....................................................
1e_Chlorella_variabilis_HdaH   ..............................................................................
1e_Babesia_microti__strain_RI  L.DGNNMHGKFIPLFTDPGYSESQELTHGHDVDWIDNIKFEIM.....KANYTCALLLKEFF..........TRYVHL
1f_Pneumycystis_carinii_HdaH   L..............................IDQIQKIKI......................................
1f_Malassezia_pachydermatis_H  ..................................AL.................................RD..RSV..
1f_Saccharomyces_cerevisiae_H  ............................................................TFNRTV.......EDITI
1g_Homo_sapiens_HDAC4          ..........................................................................EAET
1g_Saccharomyces_cerevisiae_H  F..............................LGDKQLKPLVPLVDETLS..........EW........YFKNSLI..
1g_Arabidopsis_thaliana_HDA5   L..............................VDNKGSILEAPL.....N..........SWYGNVVCLGKE..RDIPI
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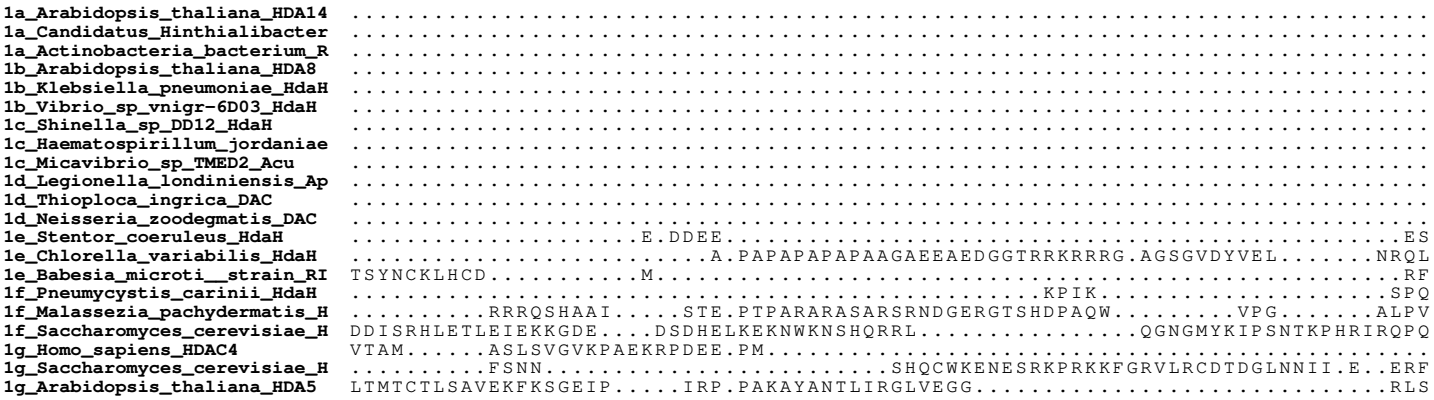
1a_Arabidopsis_thaliana_HDA14



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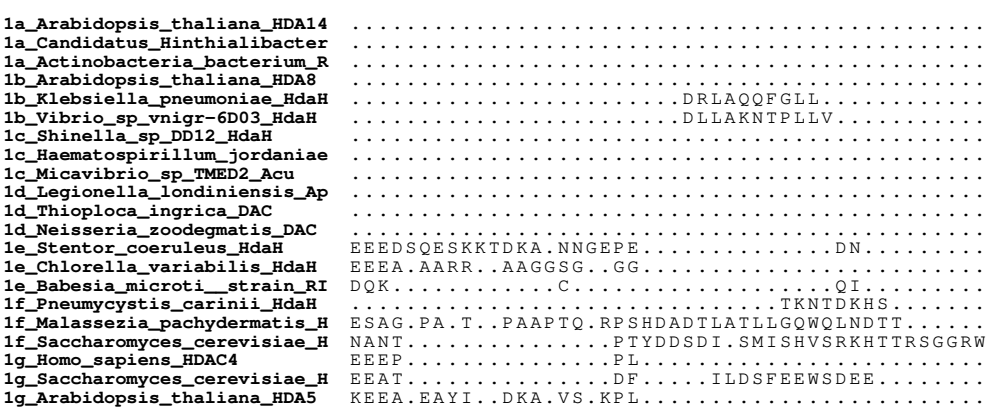
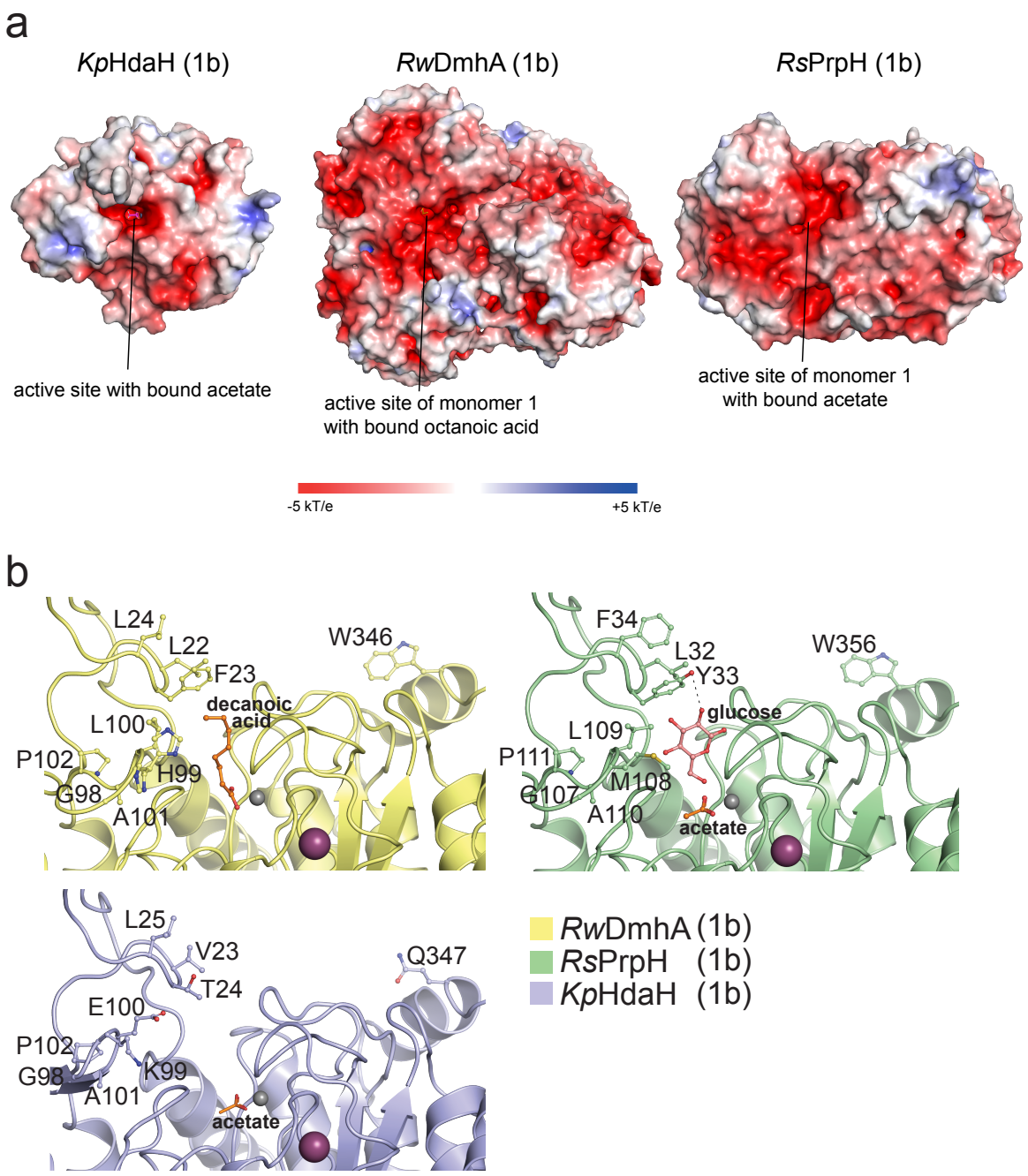


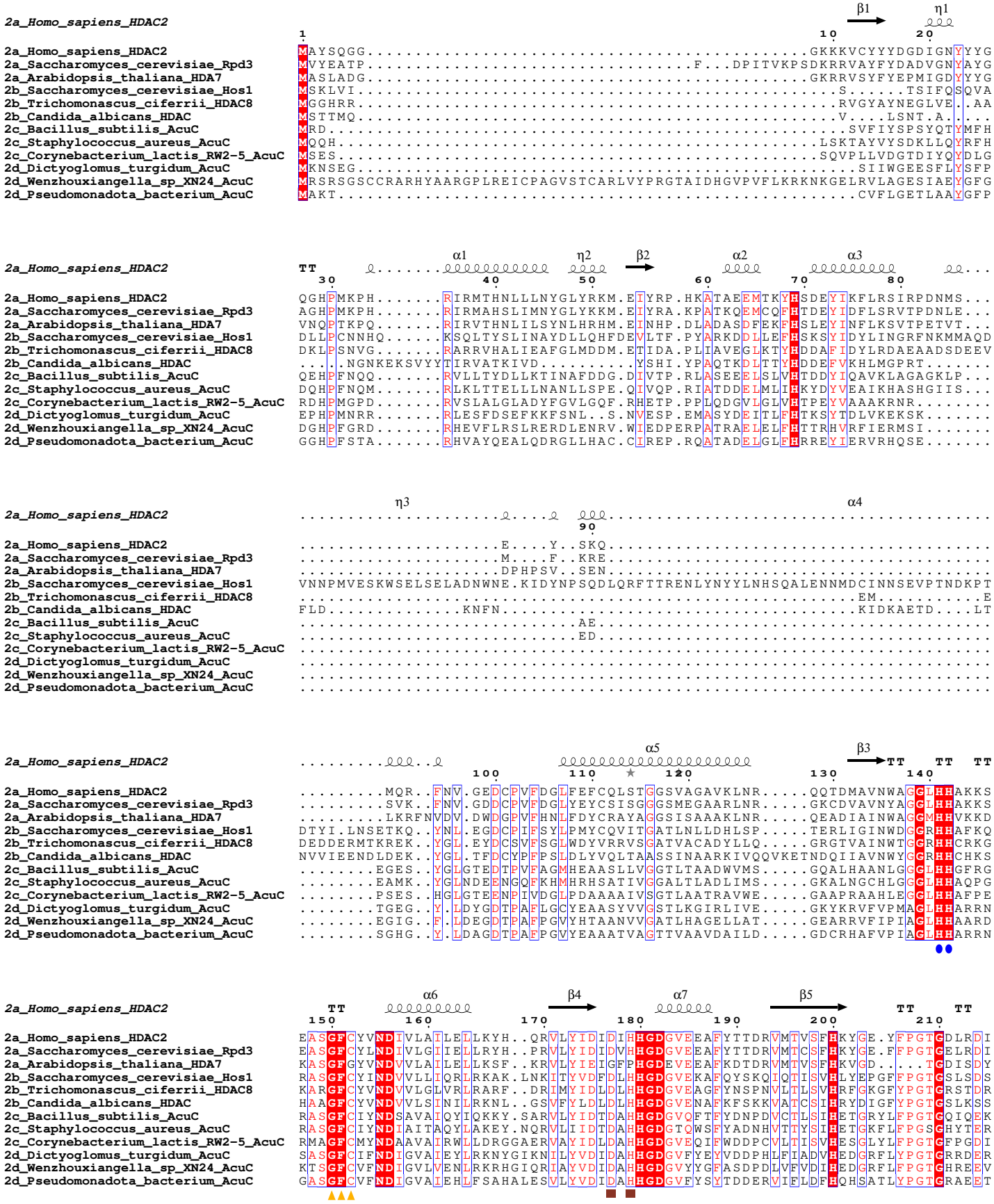
Supplementary Figure 14: Primary sequence alignment of selected bacterial deacylases of cluster 1 (UniProt accession numbers are shown).
Show are the following sequences of the following deacylases representing enzymes of cluster 1 and the sub-clusters (code: sub-cluster, accession number/protein name/spcies): 1a, AT4G33470.1/HDA14/*Arabidopsis thaliana*; 1a, A0A136MHB6/AphA/*Candidatus Hinthialibacteria bacterium* OLB16; 1a, A0A1F2W7Q1/HdaH/*Actinobacteria bacterium* RBG_16_70_17; 1b, Q94EJ2/HDA8/*Arabidopsis thaliana*; 1b, A0A378F8Q8/HdaH/*Klebsiella pneumoniae*; 1b, A0A2N0XRJ0/HdaH/*Vibrio sp.* vnigr-6D03; 1c, A0A021X4K7/Deacetylase (DAC)/*Shinella sp.* DD12; 1c, A0A143DCS4/AcuC/*Haematospirillum jordaniae*; 1c, A0A1Z8KYS1/Acu protein/*Micavibrio sp.* TMED2; 1d, A0A0W0VIH3/ApaH/*Legionella londiniensis*; 1d, A0A090BV44/Deacetylase/*Thioploca ingrica*; 1d, A0A1X3CVE4/Deacetylase/*Neisseria zoodegmatis*; 1e, A0A1R2CWG0/HdaH/*Stentor coeruleus*; 1e, E1ZJU5/HdaH/*Chlorella variabilis* (green alga); 1e, A0A1N6LWP9/HOS3/*Babesia microti* (strain RI); 1f, A0A0W4ZQY2/HdaH/*Pneumocystis carinii* (strain B80); 1f, A0A0M9VQN5/HdaH/*Malassezia pachydermatis*; 1f, Q02959/HOS3/*Saccharomyces cerevisiae* (strain ATCC 204508 / S288c); 1g, P56524/HDAC4/*Homo sapiens*; 1g, P53973/Hda1/*Saccharomyces cerevisiae* (strain ATCC 204508 / S288c); 1g, AT5G61060.1 (Q8RX28)/HDA5/*Arabidopsis thaliana*. All sequences were aligned using the T-COFFEE multiple sequence alignment server[1]. The important catalytic residues are highlighted. The secondary structure elements and numbering is shown for the deacylase shown at the top of the amino acid sequences above the sequence alignment. The alignment was created by ESPript version 3.0[2].



Supplementary Figure 15: Molecular determinant of substrate specificity for cluster 1 enzymes *Kp*HdaH (1b), *Rw*DmhA (1b) and *Rs*PrpH (1b).

a To show the electrostatics at the active site the electrostatic surface potential was plotted onto the surface of the structures of *Kp*HdaH (1b), *Rw*DmhA (1b) and *Rs*PrpH (1b). As visible, all enzymes show a highly negatively-charged surface surrounding the entry to the active site suggesting to play a role in substrate specificity towards proteins/molecules with neutral/positively charged electrostatic potential. All enzymes show a preference for peptides with a positively-charged residue at -1 position from the acetylated lysine side chain. For *Kp*HdaH (1b) a monomer is shown, for *Rw*DmhA (1b) a tetramer and for *Rs*PrpH (1b) a head-to-head dimer is shown. The electrostatics was calculated using the APBS plugin in PyMOL[4].

b Residues lining the substrate binding tunnel mediate substrate specificity of sub-cluster 1b enzymes. For *Rw*DmhA (1b) and *Rs*PrpH (1b) we observed differences in sequence motifs lining the substrate binding pocket in the L1-loop (22-LFL-24/32-LYF-34 motif, and the motif 98-GHLAP-102/107-GMLAP-111 as well as W346/W356 in the C-terminal α-helix lining the active site rim. This is substituted by polar motifs in *Kp*HdaH (1b) (23-VTL-25; *Kp*HdaH: 99-GKEAP-102; 343-FIOOQ-347; Q347 corresponding to W346/W356 in *Rw*DmhA (1b) and *Rs*PrpH (1b). This is supported by the observation in *Rw*DmhA (1b) a hydrophobic octanoic acid and in *Rs*PrpH (1b) a glucose molecule contacted by Y33 of the 32-LYF-34 motif is bound in the substrate binding channel. The substrate access to the active site is determined by the oligomerization of cluster 1b enzymes forming tetramers, the sequence of motifs lining the substrate binding tunnel affecting substrate binding sterically and electrostatically.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2a_Saccharomyces_cerevisiae_Rpd3
2a_Arabidopsis_thaliana_HDA7
2b_Saccharomyces_cerevisiae_Hos1
2b_Trichomonascus_ciferrii_HDAC8
2b_Candida_albicans_HDAC
2c_Bacillus_subtilis_AcuC
2c_Staphylococcus_aureus_AcuC
2c_Corynebacterium_lactis_RW2-5_AcuC
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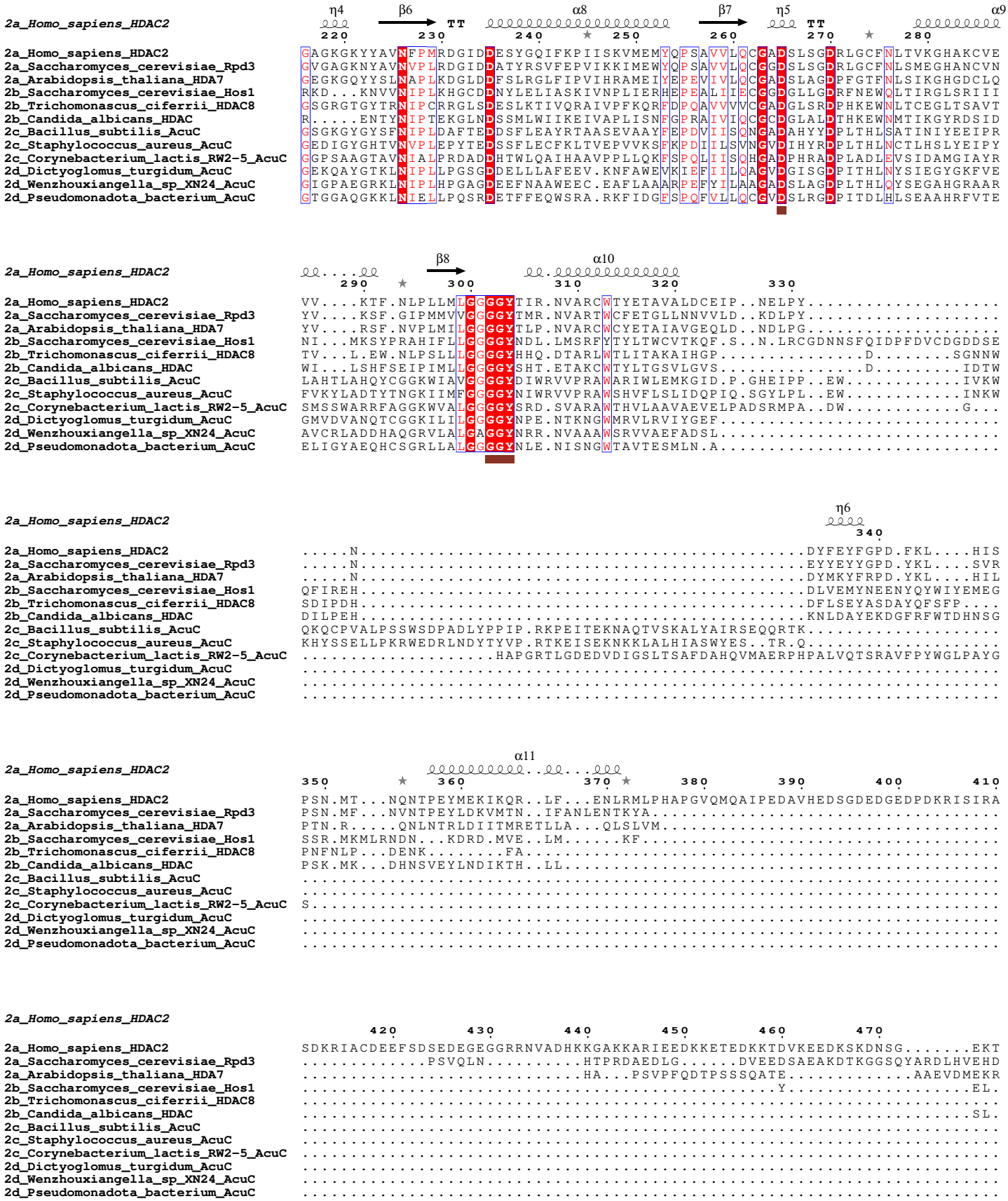
2d_Pseudomonadota_bacterium_AcuC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220 230 240 250 260 270 280
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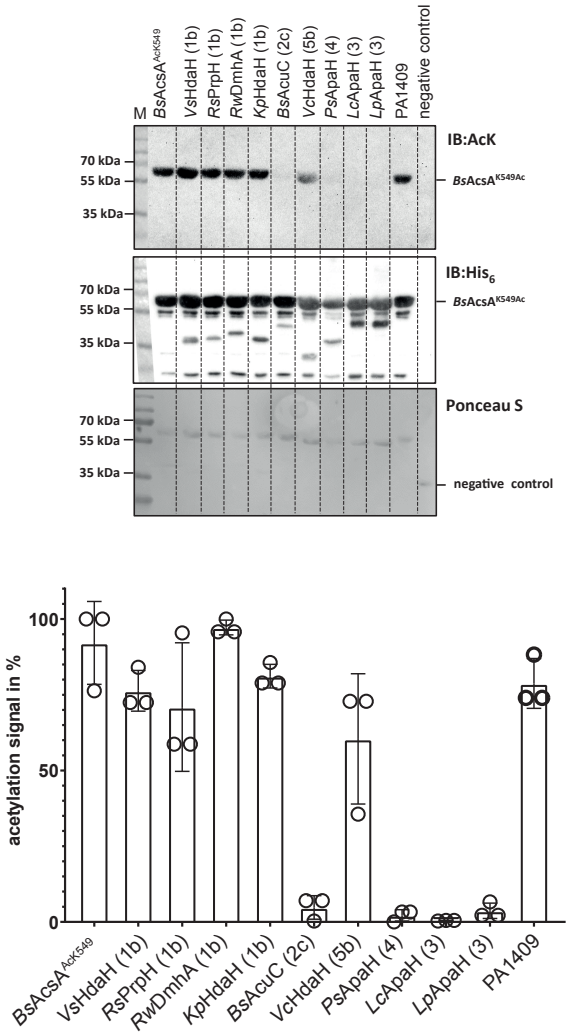
2a_Saccharomyces_cerevisiae_Rpd3 GVGAGKNYAVNVPLRDGIDDATYRSVFEPVIKKIMEWYQPSAVVLQCGDSLSGDRLGCFNLSMEGHANCVN
2a_Arabidopsis_thaliana_HDA7 GEGKGQYYSLNAPLKDGLDDFSLRGLFIPVIHRAMEIYEPEVIVLQCGADSLAGDPFGTFNLSIKGHGDCLQ
2b_Saccharomyces_cerevisiae_Hos1 RKD...KNVVNIPLKHGCDDNYLELIASKIVNPLIERHEPEALIIECGGDGLLGDRFNEWQLTIRGLSRIII
2b_Trichomonascus_ciferrii_HDAC8 GSGRGTGYTRNIPCRRGLSDESLKTIVQRAIVPFKQRFDPQAVVVCCADGLSRDPHKEWNLTCEGLTSAVT
2b_Candida_albicans_HDAC R.....ENTYNIPTEKGLNDSSMLWIIKEIVAPLISNFGPRAIVIQCGCDGLALDTHKEWNMTIKGYRDSID
2c_Bacillus_subtilis_AcuC GSGKGYGYSFNIPLDAFTEDDSFLEAYRTAASEVAAYFEPDVIISQNGADAHYYDPLTHLSATINIYEEIPR
2c_Staphylococcus_aureus_AcuC GEDIGYGHTVNVPLEPYTEDSSFLECFKLTVEPVVKSFKPDIILSVNGVDIHYRDPLTHLNCTLHSLYEIPY
2c_Corynebacterium_lactis_RW2-5_AcuC GGPSAAGTAVNIALPRDADDHTWLQAIHAAVPPLLQKFSPQLIISQHGADPHRADPLADLEVSIDAMGIAYR
2d_Dictyoglomus_turgidum_AcuC GEKQAYGTKLNIPLLPGSGDDELLLAFEEV.KNFAWEVKIEFIILQAGVDGISGDPITHLNYSIEGYGKFVE
2d_Wenzhouxiangella_sp_XN24_AcuC GIGPAEGRKLNIPLHPGAGDEEFNAAWEEC.EAFLAAARPEFYILAACADSLAGDPLTHLQYSEGAHGRAAR
2d_Pseudomonadota_bacterium_AcuC GTGGAQGKKLNIELLPQSRDETFFEQWSRA.RKFIDGFSPQFVLLQCGVDSLRGDPITDLHLSEAAHRFVTE
β8 α10
2a_Homo_sapiens_HDAC2
290 300 310 320 330
2a_Homo_sapiens_HDAC2 VV....KTF.NLPLLMLGGGGYTIR.NVARCWTYETAVALDCEIP..NELPY.....
2a_Saccharomyces_cerevisiae_Rpd3 YV....KSF.GIPMMVVGGGGYTMR.NVARTWCFETGLLNNVVLD..KDLPY.....
2a_Arabidopsis_thaliana_HDA7 YV....RSF.NVPLMILGGGGYTLP.NVARCWCYETAIAVGEQLD..NDLPG.....
2b_Saccharomyces_cerevisiae_Hos1 NI...MKSYPRAHIFLLGGGGYNDL.LMSRFYTYLTWCVTKQF.S..N.LRCGDNNSFQIDPFDVCDGDDSE
2b_Trichomonascus_ciferrii_HDAC8 TV...L.EW.NLPSLLLGGGGYHHQ.DTARLWTLITAKAIHGP.....D.....SGNNW
2b_Candida_albicans_HDAC WI...LSHFSEIPIMLLGGGGYSHT.ETAKCWTYLTGSVLGVS.....D.....IDTW
2c_Bacillus_subtilis_AcuC LAHTLAHQYCGGKWIAVGGGGYDIWRVVPRAWARIWLEMKGID.P.GHEIPP..EW.....IVKW
2c_Staphylococcus_aureus_AcuC FVKYLADTYTNGKIIMFGGGGYNIWRVVPRAWSHVFLSLIDQPIQ.SGYLPL..EW.....INKW
2c_Corynebacterium_lactis_RW2-5_AcuC SMSSWARRFAGGKWVALGGGGYSRD.SVARAWTHVLAAVAEVELPADSRMPA..DW.....G...
2d_Dictyoglomus_turgidum_AcuC GMVDVANQTCGGKILILGGGGYNPE.NTKNGWMRVLRVIYGEF.....
2d_Wenzhouxiangella_sp_XN24_AcuC AVCRLADDHAQGRVLALGAGGYNRR.NVAAAWSRVVAEFADSL.....
2d_Pseudomonadota_bacterium_AcuC ELIGYAEQHCSGRLLALGGGGYNLE.NISNGWTAVTESMLN.A.....
η6
2a_Homo_sapiens_HDAC2
340
2a_Homo_sapiens_HDAC2 .....N.....DYFEYFGPD.FKL....HIS
2a_Saccharomyces_cerevisiae_Rpd3 .....N.....EYYEYYGPD.YKL....SVR
2a_Arabidopsis_thaliana_HDA7 .....N.....DYMKYFRPD.YKL....HIL
2b_Saccharomyces_cerevisiae_Hos1 QFIREH.....DLVEMYNEENYQYWIYEMEG
2b_Trichomonascus_ciferrii_HDAC8 SDIPDH.....DFLSEYASDAYQFSFP....
2b_Candida_albicans_HDAC DILPEH.....KNLDAYEKDGFRFWTDHNSG
2c_Bacillus_subtilis_AcuC QKQCPVALPSSWSDPADLYPPIP.RKPEITEKNAQTVSKALYAIRSEQQRTK.....
2c_Staphylococcus_aureus_AcuC KHYSSELLPKRWEDRLNDYTYVP.RTKEISEKNKKLALHIASWYES..TR.Q.....
2c_Corynebacterium_lactis_RW2-5_AcuC .....HAPGRTLGDEDVDIGSLTSAFDAHQVMAERPHPALVQTSRAVFPYWGLPAYG
2d_Dictyoglomus_turgidum_AcuC .....
2d_Wenzhouxiangella_sp_XN24_AcuC .....
2d_Pseudomonadota_bacterium_AcuC .....
α11
2a_Homo_sapiens_HDAC2
350 360 370 380 390 400 410
2a_Homo_sapiens_HDAC2 PSN.MT...NQNTPEYMEKIKQR..LF...ENLRMLPHAPGVQMQAIPEDAVHEDSGDEDGEDPDKRISIRA
2a_Saccharomyces_cerevisiae_Rpd3 PSN.MF...NVNTPEYLDKVMTN..IFANLENTKYA.....
2a_Arabidopsis_thaliana_HDA7 PTN.R.....QNLNTRLDIITMRETLLA...QLSLVM.....
2b_Saccharomyces_cerevisiae_Hos1 SSR.MKMLRNDN...KDRD.MVE..LM.....KF.....
2b_Trichomonascus_ciferrii_HDAC8 PNFNLP...DENK.....FA.....
2b_Candida_albicans_HDAC PSK.MK...DHNSVEYLNDIKTH..LL.....
2c_Bacillus_subtilis_AcuC .....
2c_Staphylococcus_aureus_AcuC .....
2c_Corynebacterium_lactis_RW2-5_AcuC S.....
2d_Dictyoglomus_turgidum_AcuC .....
2d_Wenzhouxiangella_sp_XN24_AcuC .....
2d_Pseudomonadota_bacterium_AcuC .....
2a_Homo_sapiens_HDAC2
420 430 440 450 460 470
2a_Homo_sapiens_HDAC2 SDKRIACDEEFSDSEDEGEGGRRNVADHKKGAKKARIEEDKKETEDKKTDVKEEDKSKDNSG.....EKT
2a_Saccharomyces_cerevisiae_Rpd3 .....PSVQLN.....HTPRDAEDLG.....DVEEDSAEAKDTKGGSQYARDLHVEHD
2a_Arabidopsis_thaliana_HDA7 .....HA...PSVPFQDTPSSSQATE.....AAEVDMEKR
2b_Saccharomyces_cerevisiae_Hos1 .....Y.....EL.
2b_Trichomonascus_ciferrii_HDAC8 .....
2b_Candida_albicans_HDAC .....SL.
2c_Bacillus_subtilis_AcuC .....
2c_Staphylococcus_aureus_AcuC .....
2c_Corynebacterium_lactis_RW2-5_AcuC .....
2d_Dictyoglomus_turgidum_AcuC .....
2d_Wenzhouxiangella_sp_XN24_AcuC .....
2d_Pseudomonadota_bacterium_AcuC .....

2a_Homo_sapiens_HDAC2

```
                                    480
2a_Homo_sapiens_HDAC2               DTKGTKSEQLSNP
2a_Saccharomyces_cerevisiae_Rpd3    .....N.EFY...
2a_Arabidopsis_thaliana_HDA7        N....D.PR...I
2b_Saccharomyces_cerevisiae_Hos1    .............
2b_Trichomonascus_ciferrii_HDAC8    .............
2b_Candida_albicans_HDAC            .............
2c_Bacillus_subtilis_AcuC           .............
2c_Staphylococcus_aureus_AcuC       .............
2c_Corynebacterium_lactis_RW2-5_AcuC .............
2d_Dictyoglomus_turgidum_AcuC       .............
2d_Wenzhouxiangella_sp_XN24_AcuC    .............
2d_Pseudomonadota_bacterium_AcuC    .............
```

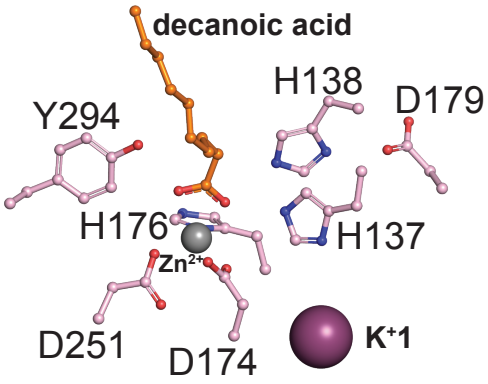
Supplementary Figure 16: Primary sequence alignment of selected bacterial deacylases of cluster 2 (UniProt accession numbers are shown).
Show are the following sequences of the following deacylases representing enzymes of cluster 2 and the sub-clusters (code: sub-cluster, accession number/protein name/spcies): 2a, Q92769/HDAC2/*Homo sapiens*; 2a, P32561/Rpd3/*Saccharomyces cerevisiae* (strain ATCC 204508 / S288c) (Baker's yeast); 2a, Q9FH09/Hda7/*Arabidopsis thaliana*; 2, bQ12214/Hos1/*Saccharomyces cerevisiae* (strain ATCC 204508 / S288c) (Baker's yeast); 2b, A0A642V618/HDAC8/*Trichomonascus ciferrii*; 2b, A0A8H6F6H7/HDAC/*Candida albicans*; 2c, P39067/AcuC/*Bacillus subtilis* (strain 168); 2c, Q6GFX3/AcuC/*Staphylococcus aureus* (strain MRSA252); 2c, A0A0K2H387/AcuC/*Corynebacterium lactis* RW2-5; 2d, B8DYU0/AcuC/*Dictyoglomus turgidum* (strain DSM 6724 / Z-1310); 2d, A0A6M1Y8Z6/AcuC/*Wenzhouxiangella sp.* XN24; 2d, A0A6L7MC72/AcuC/*Pseudomonadota bacterium*. All sequences were aligned using the T-COFFEE multiple sequence alignment server[1]. The important catalytic residues are highlighted. The secondary structure elements and numbering is shown for the deacylase shown at the top of the amino acid sequences above the sequence alignment. The alignment was created by ESPript version 3.0[2].

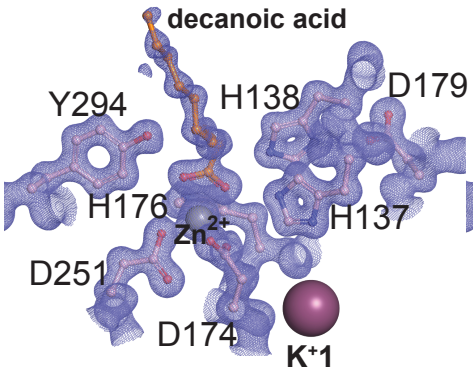


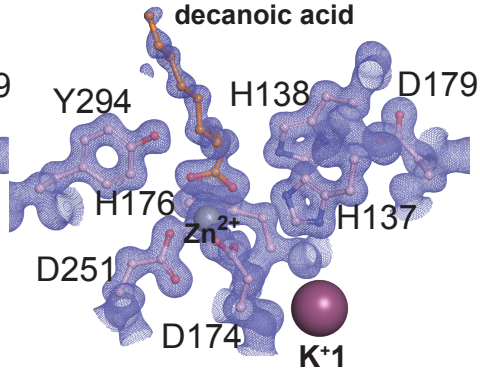
Supplementary Figure 17: Deacetylation of acetylated *Bacillus subtilis* deacetylase AcsA by bacterial deacylases.

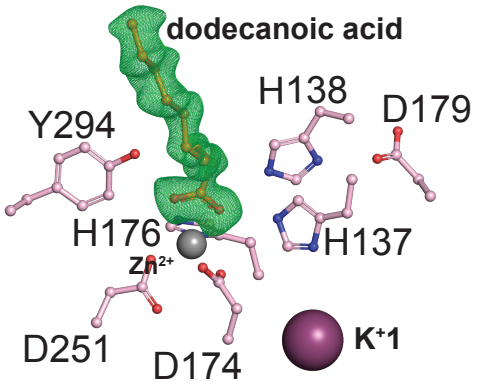
Indicated bacterial deacylases were analysed for their capacity to deacetylate K549-acetylated *B. subtilis* AcsA. As a readout an immunoblot was performed and stained with an anti-acetyl-lysine antibody (IB: AcK). Lys549-acetylated *B. subtilis* AMP-forming acetyl-CoA synthetase (BsAcsA^{AcK549}) was used as positive control and non-acetylated His_6-Ran as negative control. Ponceau S-red staining was performed as loading control (LC: PoS). The quantification was done using ImageJ. The signal of the anti-AcK-blot was normalized to the anti-His_6-blot (IB: His_6) used as loading control. The highest signal of each blot was set to 100%. One representative immunoblot is shown. Ponceau S-red staining of the membrane was done as second loading control. Three biologically independent replicates were performed (*n*=3). The bar graph shows the values mean±SD. Source data are provided as Source Data file.

*Vc*HdaH (5b)

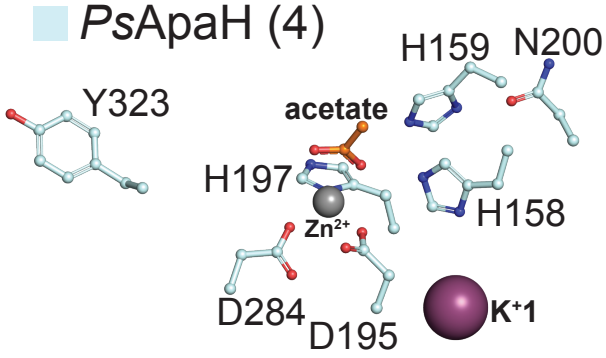


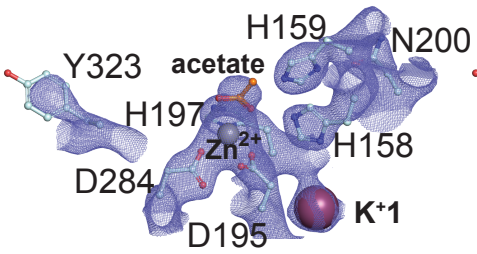


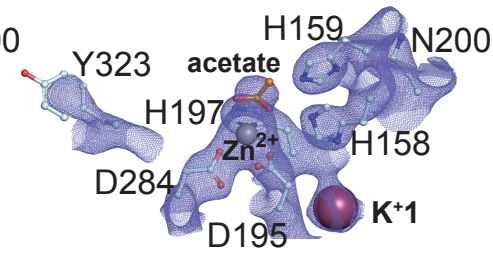




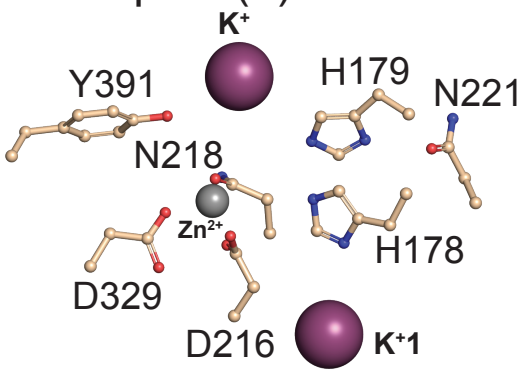
*Ps*ApaH (4)

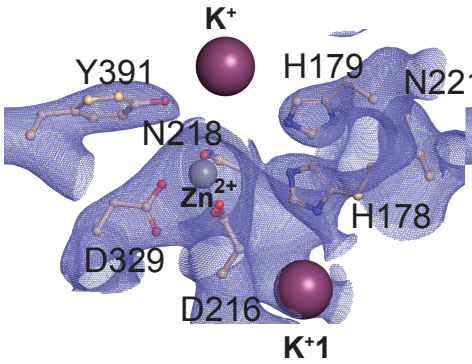


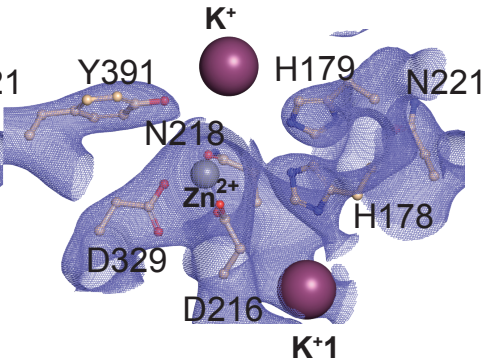




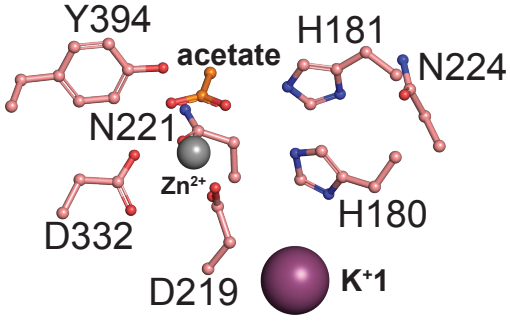
*Lc*ApaH (3)

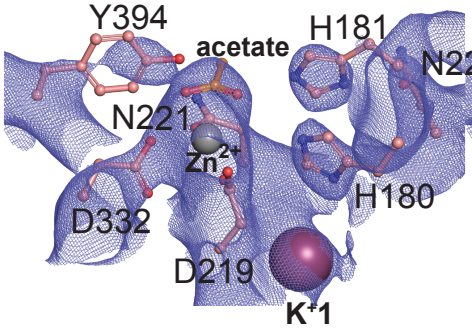


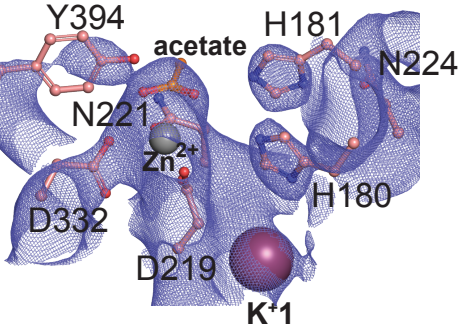




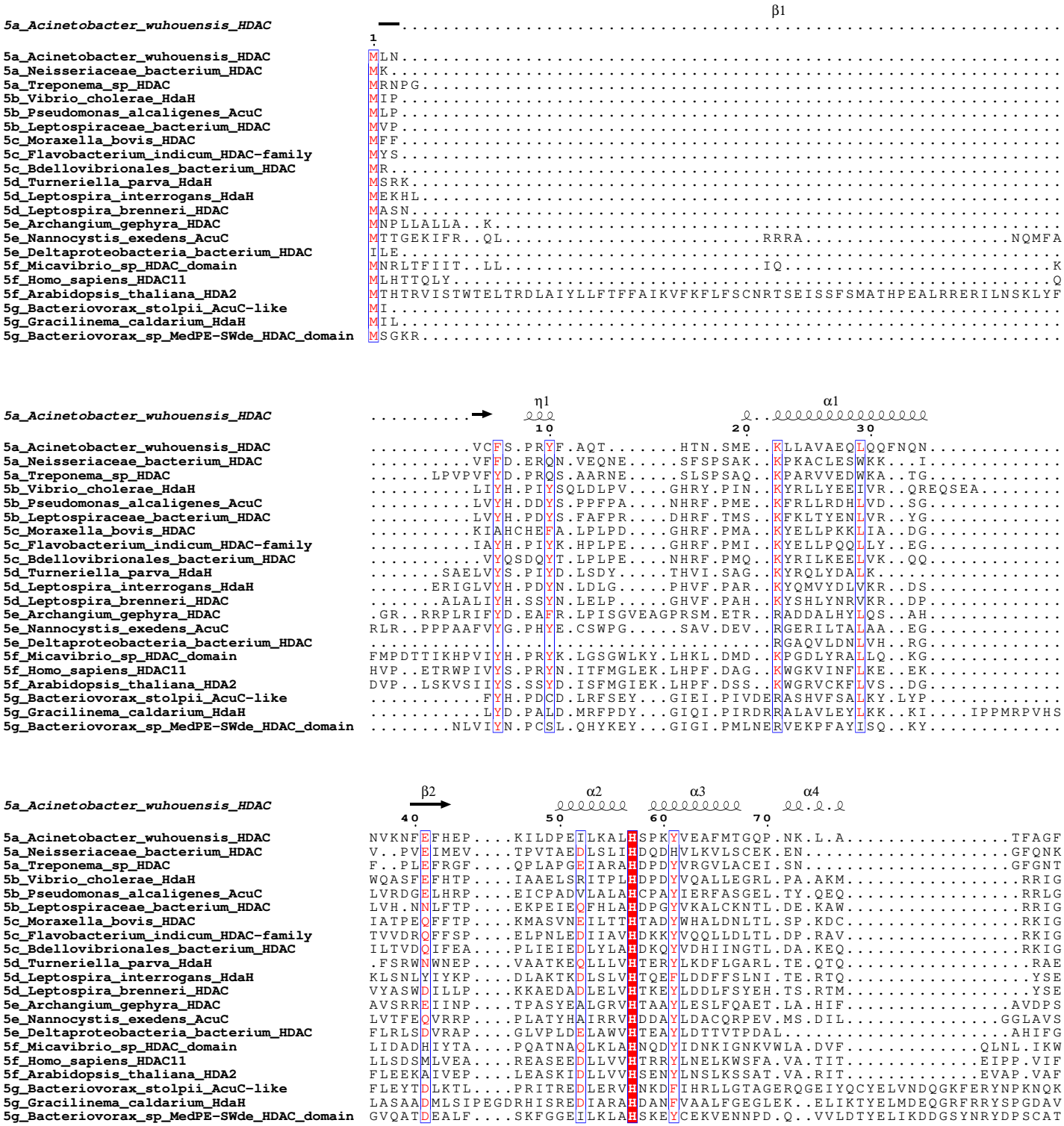
*Lp*ApaH (3)

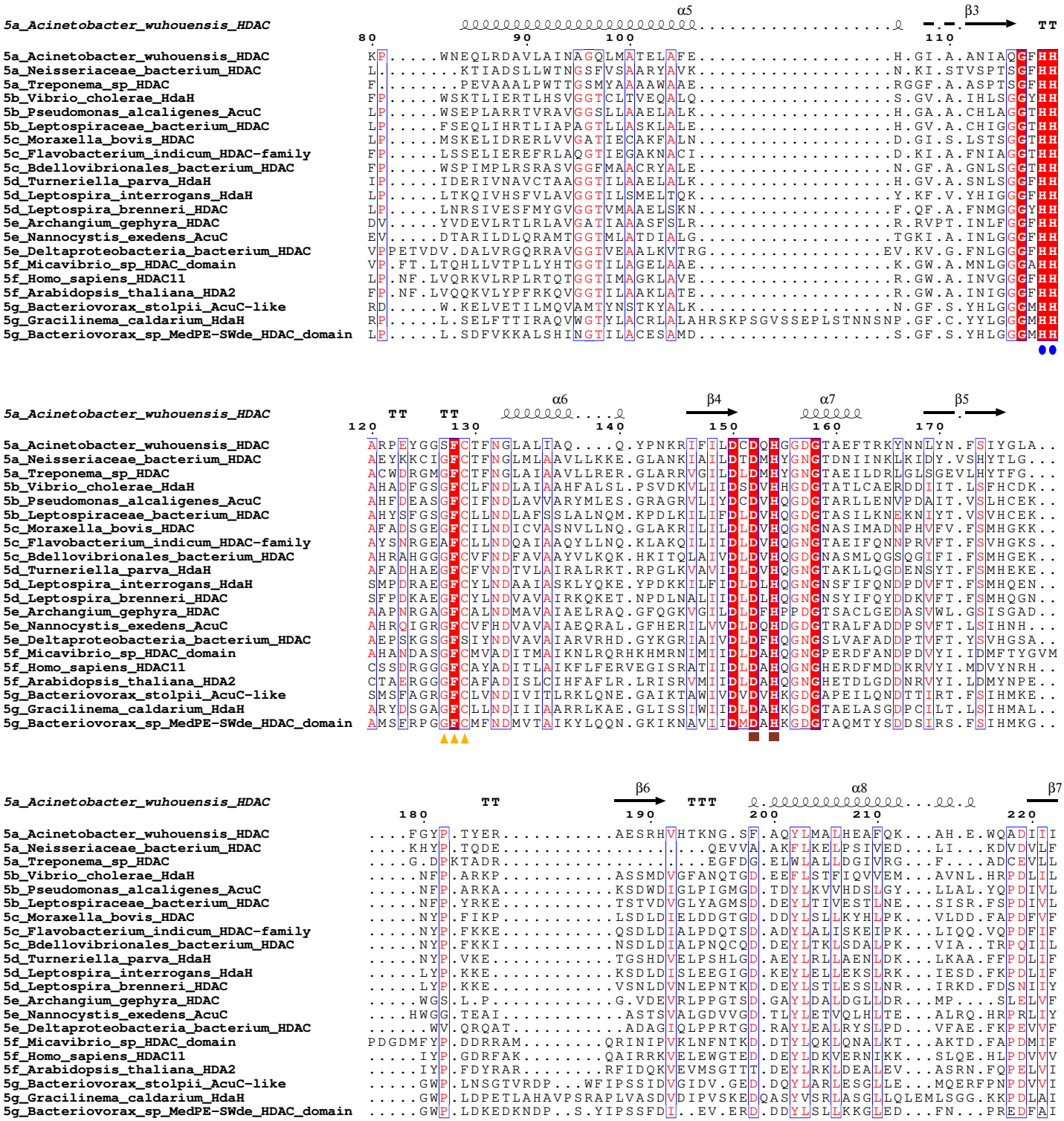






Supplementary Figure 18: Stereo images of representative electron density for structures of bacterial deacylases solved in this study.
Stereo figures for the structures of bacterial deacylases: *Vc*HdaH of sub-cluster 5b, *Lc*ApaH as well as *Lp*ApaH of cluster 3 and *Ps*ApaH of cluster 4. For all structures a representative closeup of the active site is shown on the left, including the catalytic Zn^{2+}-ion, including the coordinating His and the two Asp residues, the catalytic base His and the active site Tyr important to orient and polarize the carbonyl-group of the substrate and to stabilize the tetrahedral intermediate. As stereo images the closeup of the active site is shown with the is the electron density of the $2F_o$-F_c map contoured at 1σ in blue. For *Vc*HdaH (5b) the F_o-F_c omit map is shown in green for the bound decanoic acid (contour level 3σ). In violet spheres the potassium ions are shown, the catalytic Zn^{2+}-ion is shown as grey sphere. The stereo diagrams were prepared in wall-eyed viewing mode with PyMOL version 2.3.4[3].


β1
5a_Acinetobacter_wuhouensis_HDAC
5a_Acinetobacter_wuhouensis_HDAC
5a_Neisseriaceae_bacterium_HDAC
5a_Treponema_sp_HDAC
5b_Vibrio_cholerae_HdaH
5b_Pseudomonas_alcaligenes_AcuC
5b_Leptospiraceae_bacterium_HDAC
5c_Moraxella_bovis_HDAC
5c_Flavobacterium_indicum_HDAC-family
5c_Bdellovibrionales_bacterium_HDAC
5d_Turneriella_parva_HdaH
5d_Leptospira_interrogans_HdaH
5d_Leptospira_brenneri_HDAC
5e_Archangium_gephyra_HDAC
5e_Nannocystis_exedens_AcuC
5e_Deltaproteobacteria_bacterium_HDAC
5f_Micavibrio_sp_HDAC_domain
5f_Homo_sapiens_HDAC11
5f_Arabidopsis_thaliana_HDA2
5g_Bacteriovorax_stolpii_AcuC-like
5g_Gracilinema_caldarium_HdaH
5g_Bacteriovorax_sp_MedPE-SWde_HDAC_domain
η1
α1
10
20
30
β2
α2
α3
α4
40
50
60
70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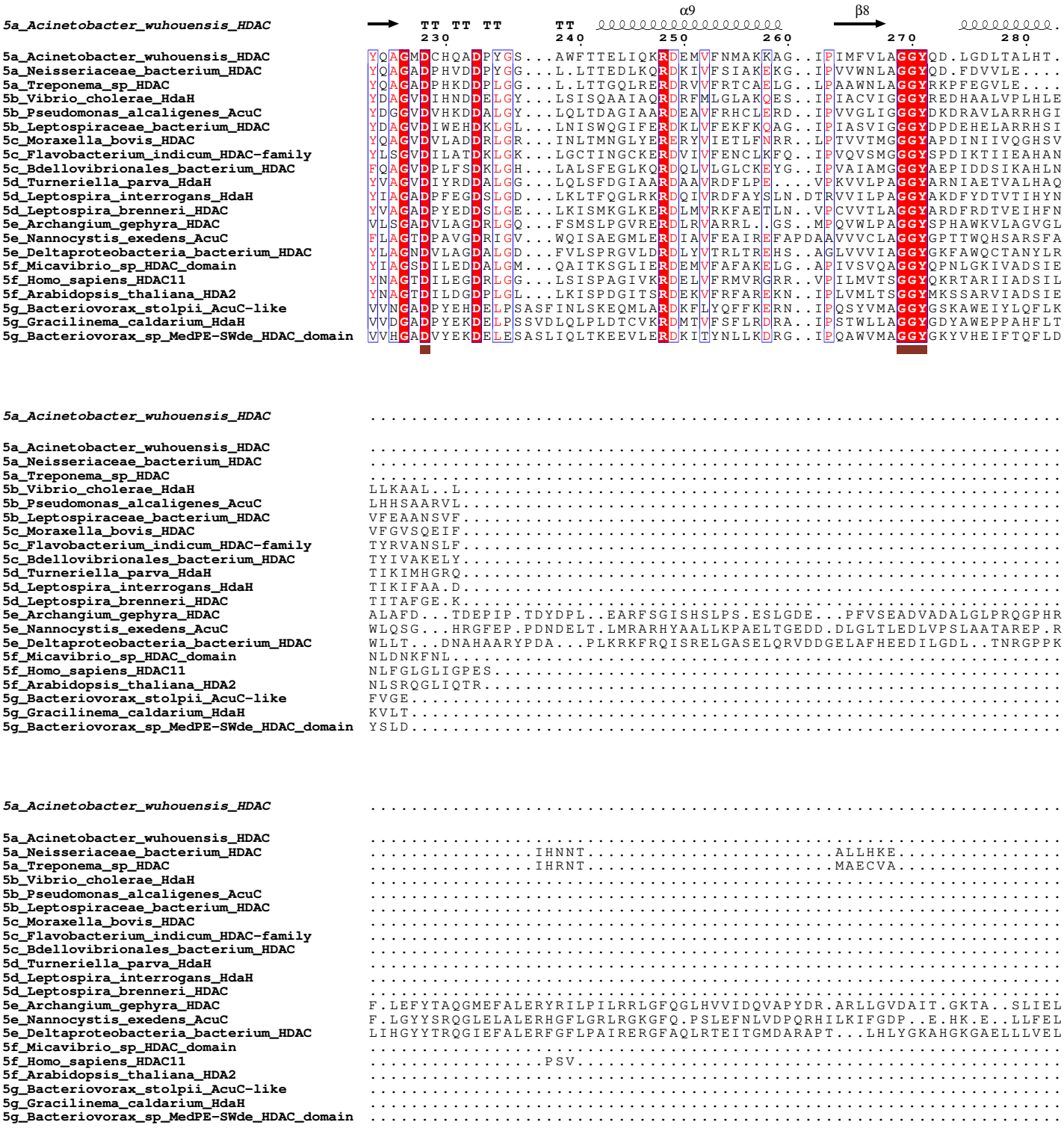
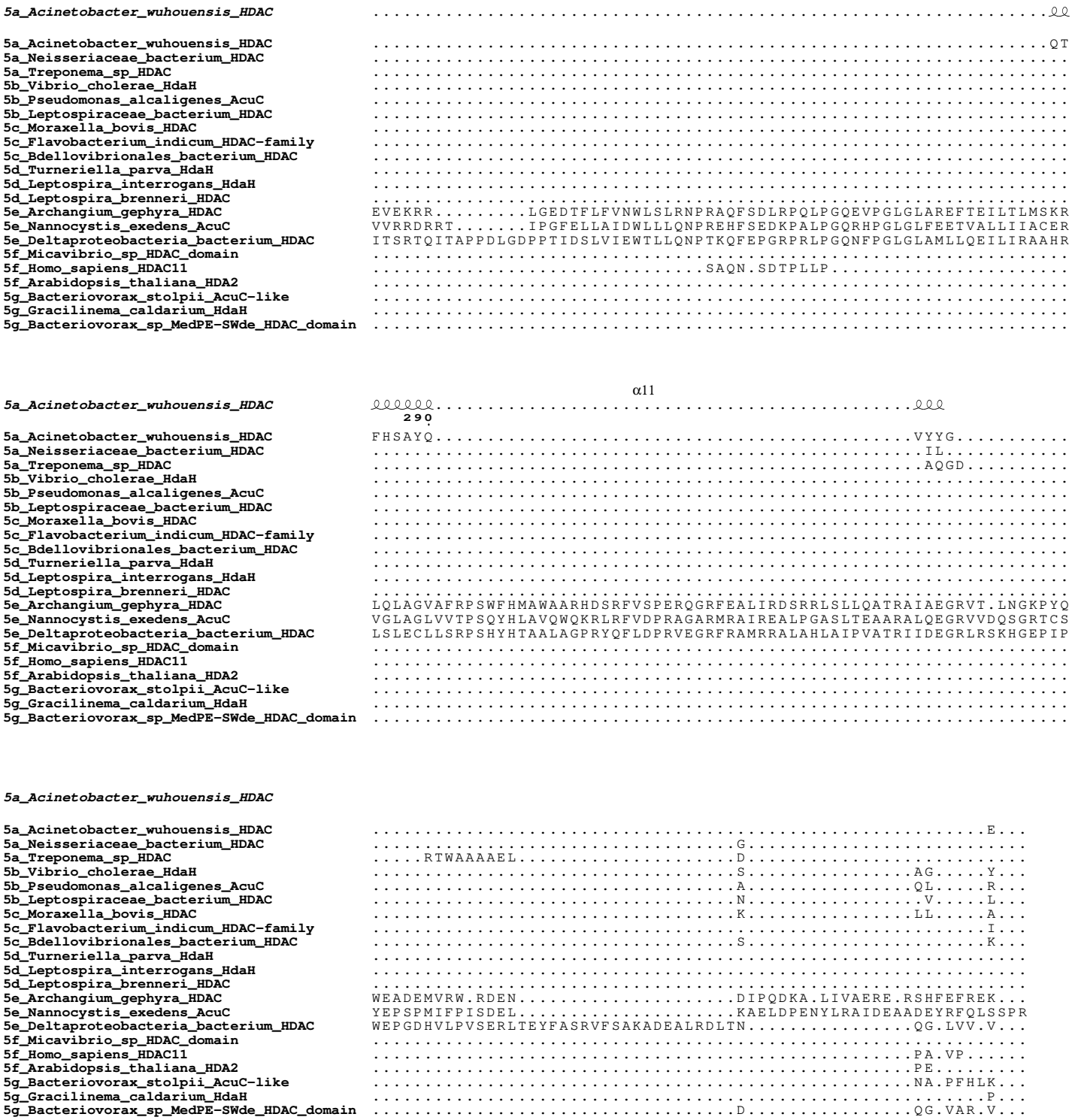
5a_Acinetobacter_wuhouensis_HDAC
5a_Neisseriaceae_bacterium_HDAC
5a_Treponema_sp_HDAC
5b_Vibrio_cholerae_HdaH
5b_Pseudomonas_alcaligenes_AcuC
5b_Leptospiraceae_bacterium_HDAC
5c_Moraxella_bovis_HDAC
5c_Flavobacterium_indicum_HDAC-family
5c_Bdellovibrionales_bacterium_HDAC
5d_Turneriella_parva_HdaH
5d_Leptospira_interrogans_HdaH
5d_Leptospira_brenneri_HDAC
5e_Archangium_gephyra_HDAC
5e_Nannocystis_exedens_AcuC
5e_Deltaproteobacteria_bacterium_HDAC
5f_Micavibrio_sp_HDAC_domain
5f_Homo_sapiens_HDAC11
5f_Arabidopsis_thaliana_HDA2
5g_Bacteriovorax_stolpii_AcuC-like
5g_Gracilinema_caldarium_HdaH
5g_Bacteriovorax_sp_MedPE-SWde_HDAC_domain


α9
β8
5a_Acinetobacter_wuhouensis_HDAC
230
240
250
260
270
280
5a_Acinetobacter_wuhouensis_HDAC
5a_Neisseriaceae_bacterium_HDAC
5a_Treponema_sp_HDAC
5b_Vibrio_cholerae_HdaH
5b_Pseudomonas_alcaligenes_AcuC
5b_Leptospiraceae_bacterium_HDAC
5c_Moraxella_bovis_HDAC
5c_Flavobacterium_indicum_HDAC-family
5c_Bdellovibrionales_bacterium_HDAC
5d_Turneriella_parva_HdaH
5d_Leptospira_interrogans_HdaH
5d_Leptospira_brenneri_HDAC
5e_Archangium_gephyra_HDAC
5e_Nannocystis_exedens_AcuC
5e_Deltaproteobacteria_bacterium_HDAC
5f_Micavibrio_sp_HDAC_domain
5f_Homo_sapiens_HDAC11
5f_Arabidopsis_thaliana_HDA2
5g_Bacteriovorax_stolpii_AcuC-like
5g_Gracilinema_caldarium_HdaH
5g_Bacteriovorax_sp_MedPE-SWde_HDAC_domain
5a_Acinetobacter_wuhouensis_HDAC
5a_Acinetobacter_wuhouensis_HDAC
5a_Neisseriaceae_bacterium_HDAC
5a_Treponema_sp_HDAC
5b_Vibrio_cholerae_HdaH
5b_Pseudomonas_alcaligenes_AcuC
5b_Leptospiraceae_bacterium_HDAC
5c_Moraxella_bovis_HDAC
5c_Flavobacterium_indicum_HDAC-family
5c_Bdellovibrionales_bacterium_HDAC
5d_Turneriella_parva_HdaH
5d_Leptospira_interrogans_HdaH
5d_Leptospira_brenneri_HDAC
5e_Archangium_gephyra_HDAC
5e_Nannocystis_exedens_AcuC
5e_Deltaproteobacteria_bacterium_HDAC
5f_Micavibrio_sp_HDAC_domain
5f_Homo_sapiens_HDAC11
5f_Arabidopsis_thaliana_HDA2
5g_Bacteriovorax_stolpii_AcuC-like
5g_Gracilinema_caldarium_HdaH
5g_Bacteriovorax_sp_MedPE-SWde_HDAC_domain
5a_Acinetobacter_wuhouensis_HDAC
5a_Acinetobacter_wuhouensis_HDAC
5a_Neisseriaceae_bacterium_HDAC
5a_Treponema_sp_HDAC
5b_Vibrio_cholerae_HdaH
5b_Pseudomonas_alcaligenes_AcuC
5b_Leptospiraceae_bacterium_HDAC
5c_Moraxella_bovis_HDAC
5c_Flavobacterium_indicum_HDAC-family
5c_Bdellovibrionales_bacterium_HDAC
5d_Turneriella_parva_HdaH
5d_Leptospira_interrogans_HdaH
5d_Leptospira_brenneri_HDAC
5e_Archangium_gephyra_HDAC
5e_Nannocystis_exedens_AcuC
5e_Deltaproteobacteria_bacterium_HDAC
5f_Micavibrio_sp_HDAC_domain
5f_Homo_sapiens_HDAC11
5f_Arabidopsis_thaliana_HDA2
5g_Bacteriovorax_stolpii_AcuC-like
5g_Gracilinema_caldarium_HdaH
5g_Bacteriovorax_sp_MedPE-SWde_HDAC_domain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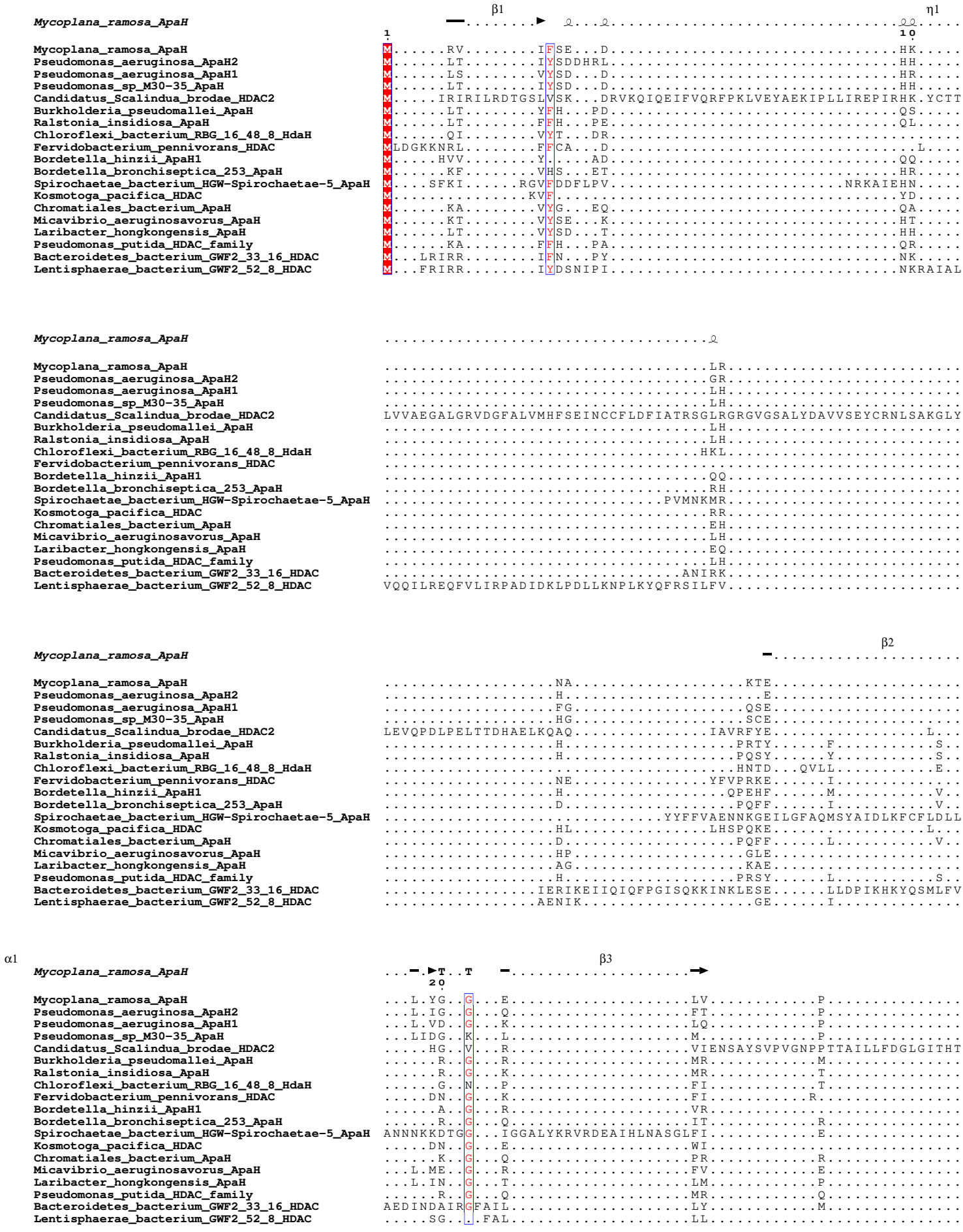
5a_Acinetobacter_wuhouensis_HDAC
5a_Neisseriaceae_bacterium_HDAC
5a_Treponema_sp_HDAC
5b_Vibrio_cholerae_HdaH
5b_Pseudomonas_alcaligenes_AcuC
5b_Leptospiraceae_bacterium_HDAC
5c_Moraxella_bovis_HDAC
5c_Flavobacterium_indicum_HDAC-family
5c_Bdellovibrionales_bacterium_HDAC
5d_Turneriella_parva_HdaH
5d_Leptospira_interrogans_HdaH
5d_Leptospira_brenneri_HDAC
5e_Archangium_gephyra_HDAC
5e_Nannocystis_exedens_AcuC
5e_Deltaproteobacteria_bacterium_HDAC
5f_Micavibrio_sp_HDAC_domain
5f_Homo_sapiens_HDAC11
5f_Arabidopsis_thaliana_HDA2
5g_Bacteriovorax_stolpii_AcuC-like
5g_Gracilinema_caldarium_HdaH
5g_Bacteriovorax_sp_MedPE-SWde_HDAC_domain
α11
290

Supplementary Figure 19: Primary sequence alignment of selected bacterial deacylases of cluster 5 (UniProt accession numbers are shown).
Show are the following sequences of the following deacylases representing enzymes of cluster 5 and the sub-clusters (code: sub-cluster, accession number/protein name/spcies): 5a, A0A385C7X8/HDAC/*Acinetobacter wuhouensis*; 5a, A0A3S0JDS2/HDAC/*Neisseriaceae bacterium*; 5a, A0A7X8CU14/HDAC/*Treponema sp.*; 5b, A0A395TF317HDAC/*Vibrio cholerae*; 5b, U2ZNB5/AcuC/*Pseudomonas alcaligenes* (strain ATCC 14909 / DSM 50342 / JCM 20561 / NBRC 14159 / NCIMB 9945 / NCTC 10367 / 1577); 5b, A0A960ZHD4/HDAC/*Leptospiraceae bacterium*; 5c, A0A2Z4RAM2/HDAC/*Moraxella bovis*; 5c, H8XRQ2/HDAC family/*Flavobacterium indicum* (strain DSM 17447 / CIP 109464 / GPTSA100-9); 5c, A0A2H0QAI6/HDAC/*Bdellovibrionales bacterium* CG11_big_fil_rev_8_21_14_0_20_38_13; 5d, I4B797/HDAC Superfamily/*Turneriella parva* (strain ATCC BAA-1111 / DSM 21527 / NCTC 11395 / H) (*Leptospira parva*); 5d, A0A098MZK6/HdaH/*Leptospira interrogans*; 5d, A0A2M9Y5D1/HDAC/*Leptospira brenneri*; 5e, A0A2W5V5Z1/HDAC/*Archangium gephyra*; 5e, A0A1I2D6R7/AcuC/*Nannocystis exedens*; 5e, A0A925AY58/HDAC/*Deltaproteobacteria bacterium*; 5f, A0A2D5Y199/HDAC domain/*Micavibrio sp.*; 5f, Q96DB2/HDAC11/*Homo sapiens*; 5f, Q944K3/HDA2/*Arabidopsis thaliana*; 5g, A0A2K9NTX8/AcuC like/*Bacteriovorax stolpii* (*Bdellovibrio stolpii*); 5g, F8F277/HdaH/*Gracilinema caldarium* (strain ATCC 51460 / DSM 7334 / H1) (*Treponema caldarium*); 5g, A0A1J5KZ05/HDAC domain/*Bacteriovorax sp.* MedPE-SWde All sequences were aligned using the T-COFFEE multiple sequence alignment server[1]. The important catalytic residues are highlighted. The secondary structure elements and numbering is shown for the deacylase shown at the top of the amino acid sequences above the sequence alignment. The alignment was created by ESPript version 3.0[2].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Pseudomonas_putida_HDAC_family
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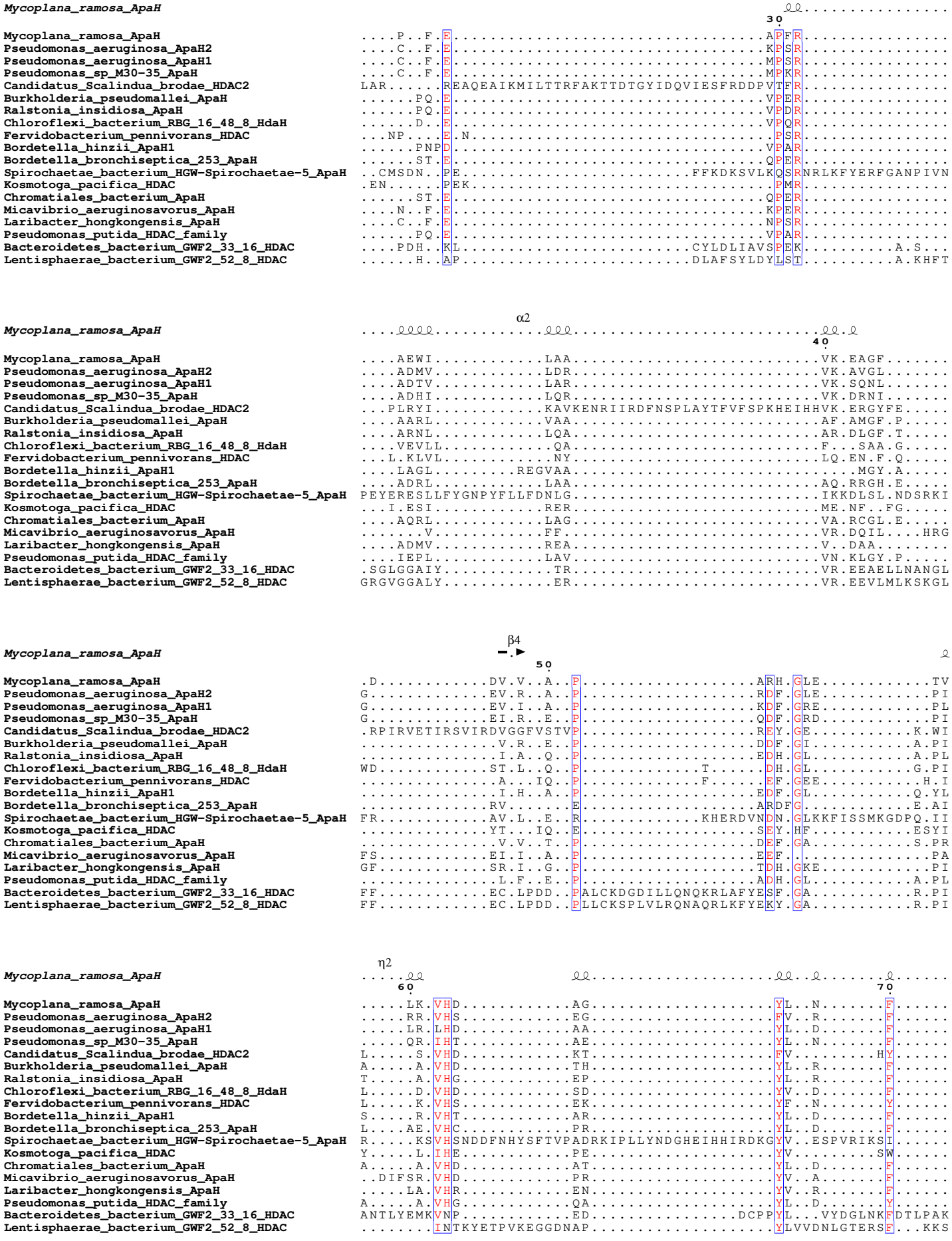
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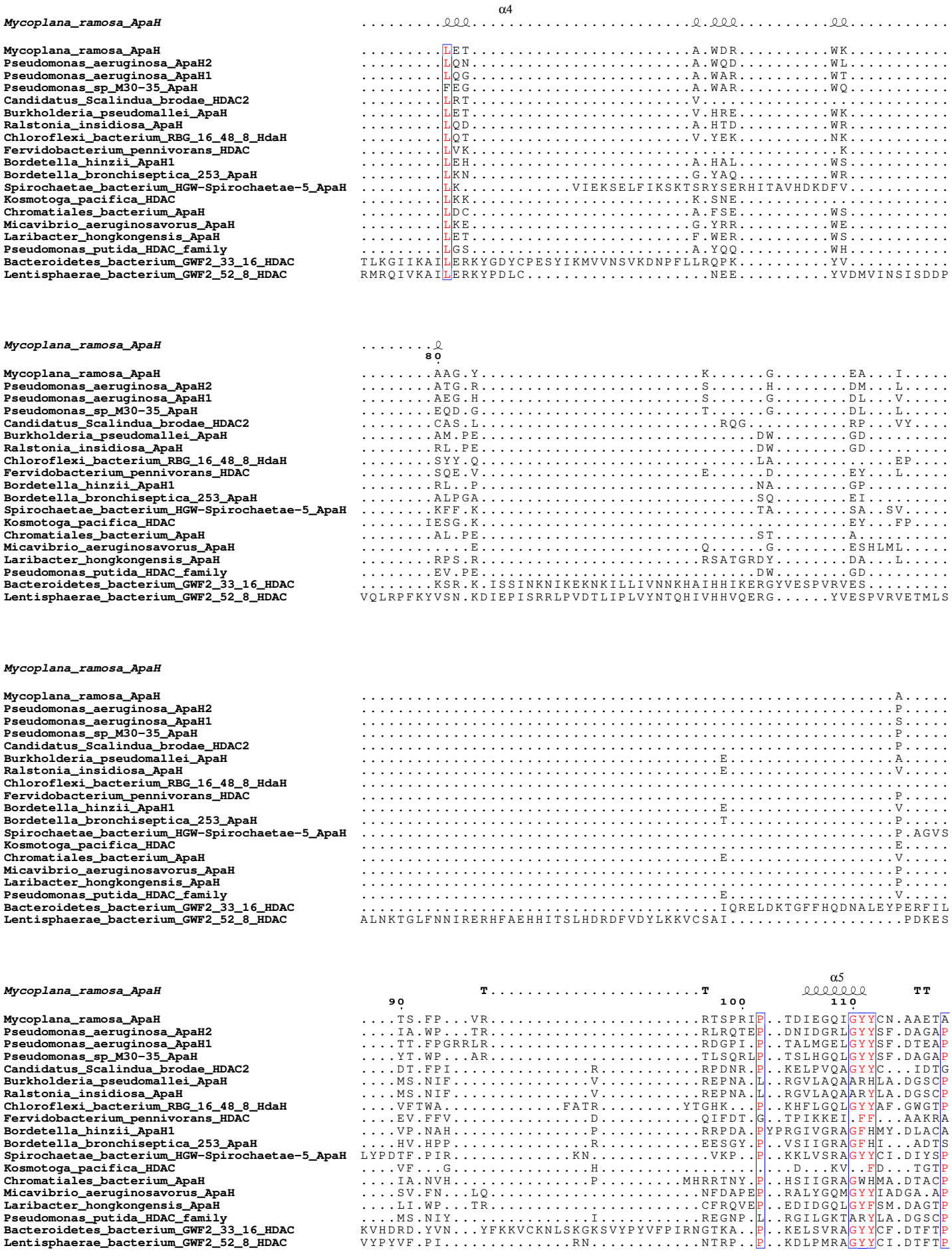
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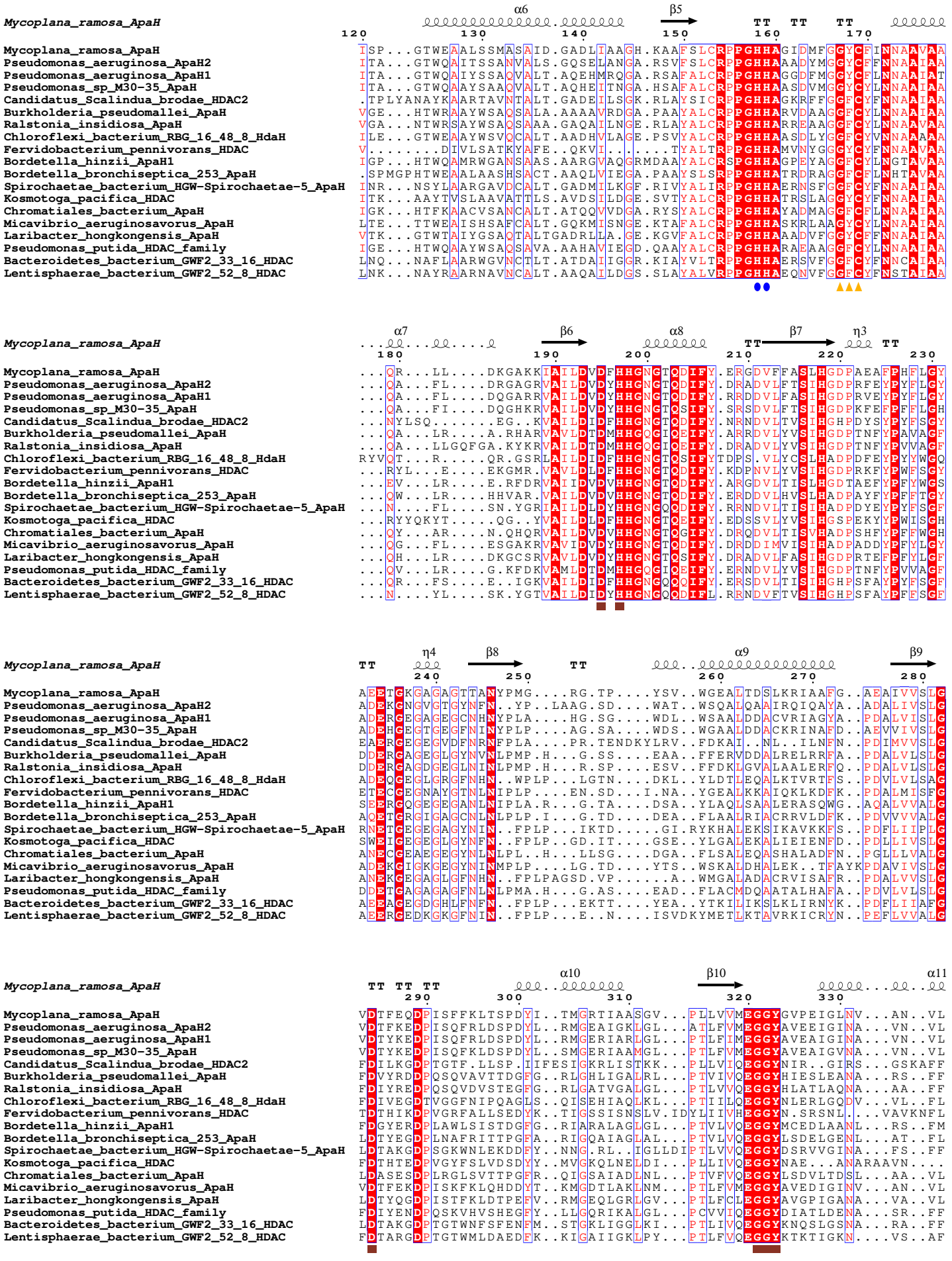
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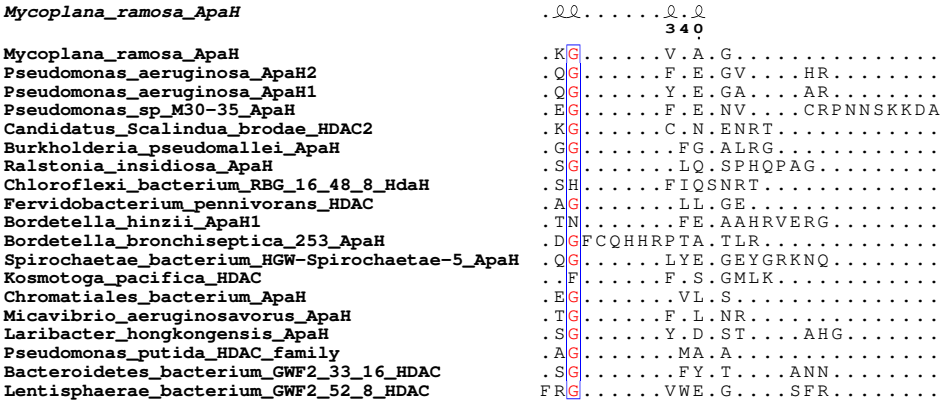
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Pseudomonas_putida_HDAC_family
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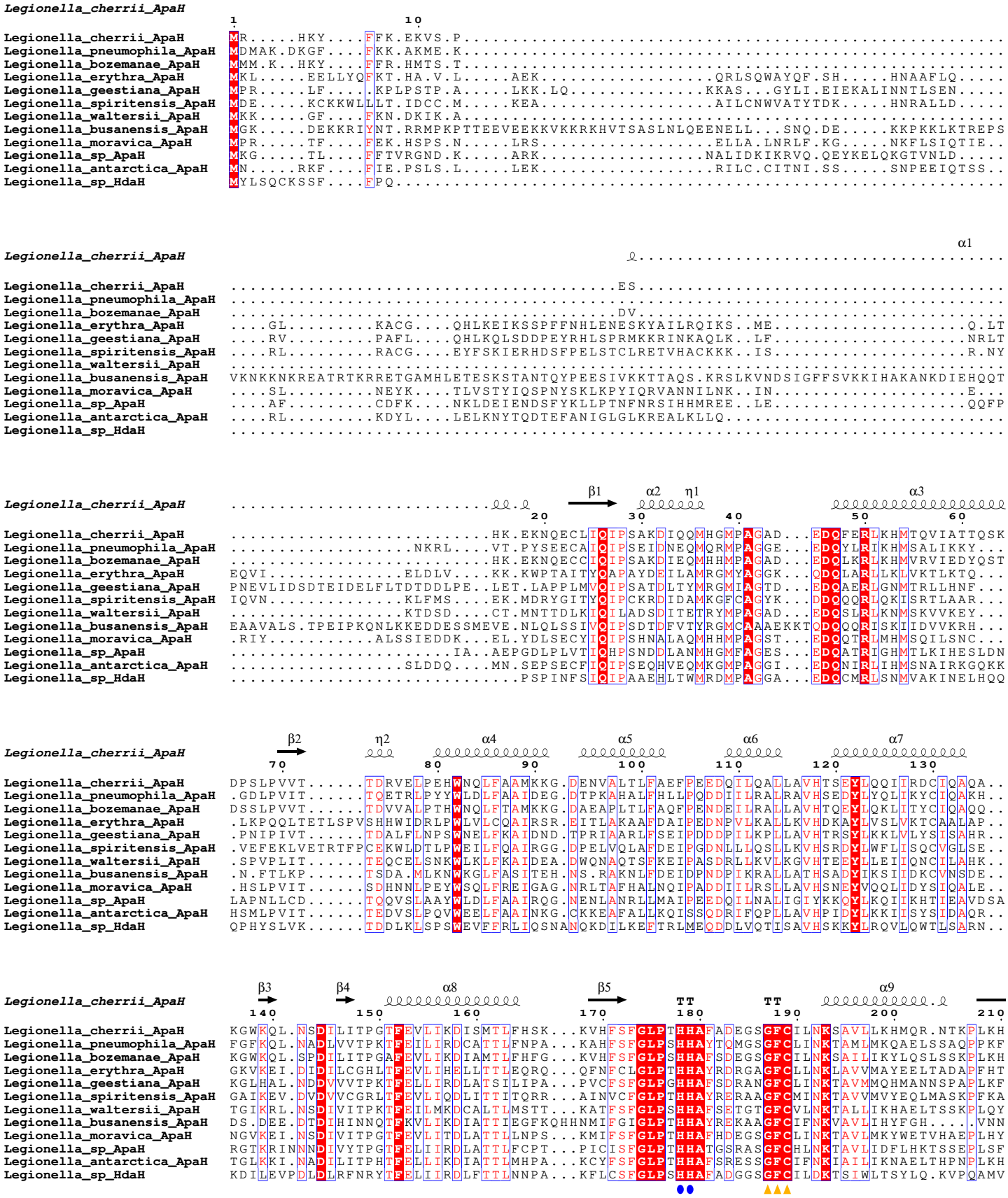
Supplementary Figure 20: Primary sequence alignment of selected bacterial deacylases of cluster 4 (UniProt accession numbers are shown).
Show are the following sequences of the following deacylases representing enzymes of cluster 4 and the sub-clusters (code: sub-cluster, accession number/protein name/spcies): 4, Q48935/ApaH/*Mycoplana ramose* (*Mycoplana bullata*); 4, Q9I6H0/ApaH2/*Pseudomonas aeruginosa* (strain ATCC 15692 / DSM 22644 / CIP 104116 / JCM 14847 / LMG 12228 / 1C / PRS101 / PAO1); 4, Q9I3T5/ApaH1/*Pseudomonas aeruginosa* (strain ATCC 15692 / DSM 22644 / CIP 104116 / JCM 14847 / LMG 12228 / 1C / PRS 101 / PAO1); 4, A0A1Y0KY79/ApaH/*Pseudomonas sp.* M30-35; 4, A0A0B0EJX1/HdaC2/*Candidatus Scalindua brodae*; 4, Q3JUN4/ApaH/*Burkholderia pseudomallei* (strain 1710b); 4, A0A191ZTI7/ApaH/*Ralstonia insidiosa*; 4, A0A1F8P1D9/HdaH/*Chloroflexi bacterium* RBG_16_48_8; 4, A0A172T1R4/HDAC/*Fervidobacterium pennivorans*; 4, A0A0H4W2H7/ApaH1/*Bordetella hinzii*; 4, A0A0C6P6V8/ApaH/*Bordetella bronchiseptica* 253; 4, A0A2N1RQT6/ApaH/*Spirochaetae bacterium* HGW-Spirochaetae-5; 4, A0A0G2Z8U2/HDAC/*Kosmotoga pacifica*; 4, A0A1E5A0H0/ApaH/*Chromatiales bacterium* (ex *Bugula neritina* AB1); 4, A0A2W5MSH4/ApaH/*Micavibrio aeruginosavorus*; 4, A0A248LJH0/ApaH/*Laribacter hongkongensis*; 4, A0A1L7NGC7/HDAC family/*Pseudomonas putida* (*Arthrobacter siderocapsulatus*); 4, A0A1F3IP43/HDAC/*Bacteroidetes bacterium* GWF2_33_16; 4, A0A1G0YY82/HDAC/*Lentisphaerae bacterium* GWF2_52_8All sequences were aligned using the T-COFFEE multiple sequence alignment server[1]. The important catalytic residues are highlighted. The secondary structure elements and numbering is shown for the deacylase shown at the top of the amino acid sequences above the sequence alignment. The alignment was created by ESPript version 3.0[2].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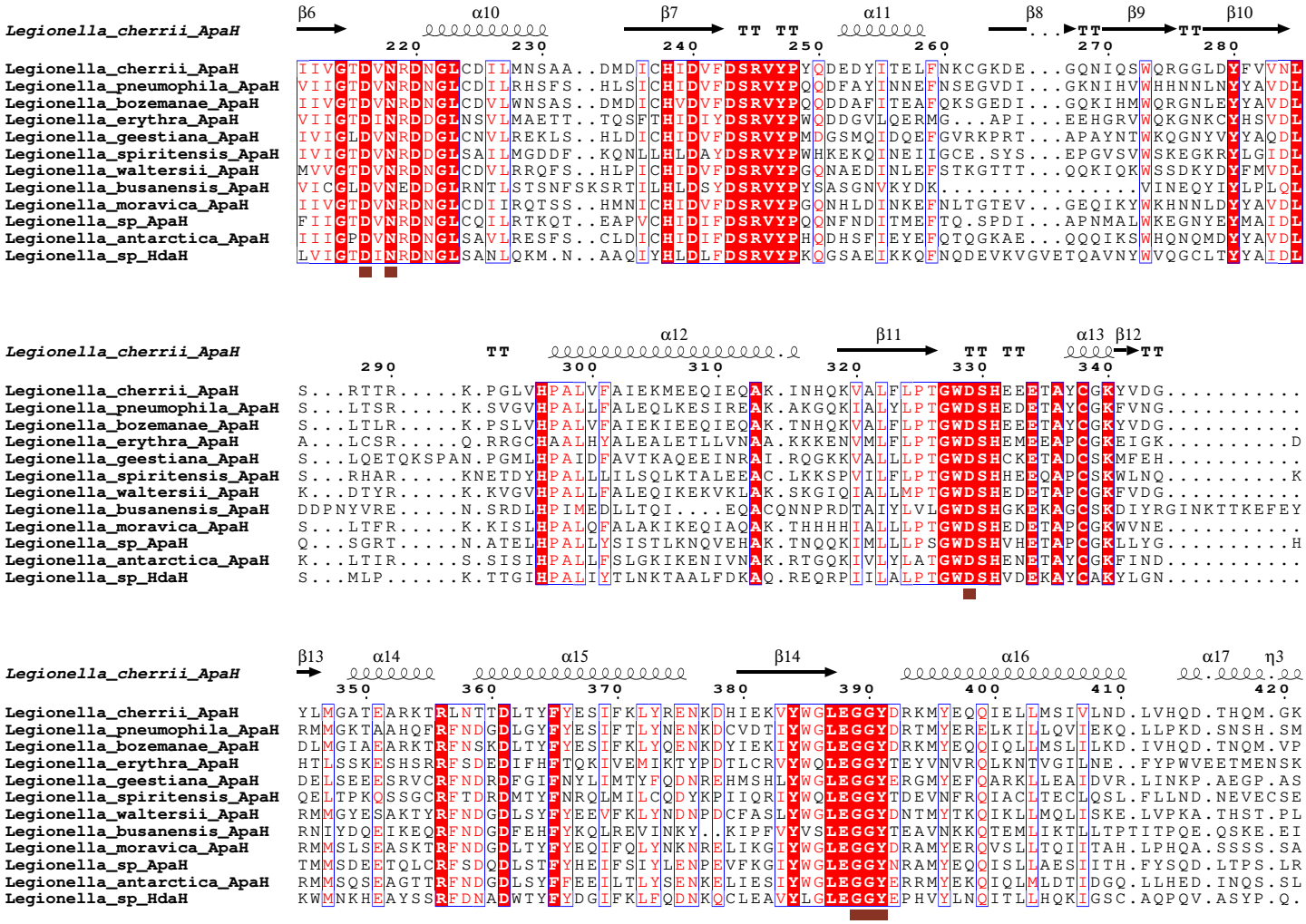
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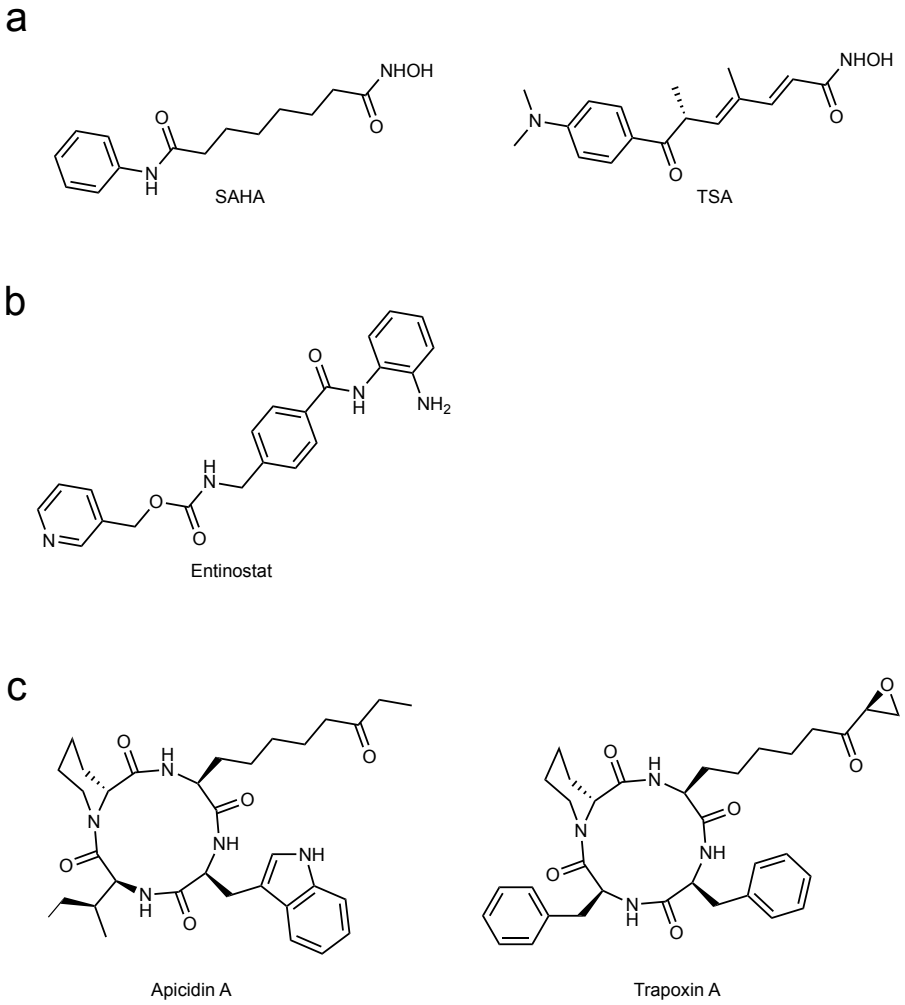
Legionella_pneumophila_ApaH S...LTSR.....K.SVGVHPALLFALEQLKESIREAK.AKGQKIALYLPTGWDSHEDETAYCGKFVNG..........
Legionella_bozemanae_ApaH S...LTLR.....K.PSLVHPALVFAIEKIEEQIEQAK.TNHQKVALFLPTGWDSHEEETAYCGKYVDG..........
Legionella_erythra_ApaH A...LCSR.....Q.RRGCHAALHYALEALETLLVNAA.KKKENVMLFLPTGWDSHEMEEAPCGKEIGK.........D
Legionella_geestiana_ApaH S...LQETQKSPAN.PGMLHPAIDFAVTKAQEEINRAI.RQGKKVALLLPTGWDSHCKETADCSKMFEH..........
Legionella_spiritensis_ApaH S...RHAR.....KNETDYHPALLLILSQLKTALEEAC.LKKSPVILFLPTGWDSHHEEQAPCSKWLNQ.........K
Legionella_waltersii_ApaH K...DTYR.....K.KVGVHPALLFALEQIKEKVKLAK.SKGIQIALLMPTGWDSHEDETAPCGKFVDG..........
Legionella_busanensis_ApaH DDPNYVRE.....N.SRDLHPIMEDLLTQI....EQACQNNPRDTAIYLVLGWDSHGKEKAGCSKDIYRGINKTTKEFEY
Legionella_moravica_ApaH S...LTFR.....K.KISLHPALQFALAKIKEQIAQAK.THHHHIALLLPTGWDSHEDETAPCGKWVNE..........
Legionella_sp_ApaH Q...SGRT.....N.ATELHPALLYSISTLKNQVEHAK.TNQQKIMLLLPSGWDSHVHETAPCGKLLYG.........H
Legionella_antarctica_ApaH K...LTIR.....S.SISIHPALLFSLGKIKENIVNAK.RTGQKIVLYLATGWDSHENETAYCGKFIND..........
Legionella_sp_HdaH S...MLP.....K.TTGIHPALIYTLNKTAALFDKAQ.REQRPIILALPTGWDSHVDEKAYCAKYLGN..........
Legionella_cherrii_ApaH
β13 α14 α15 β14 α16 α17 η3
350 360 370 380 390 400 410 420
Legionella_cherrii_ApaH YLMGATEARKTRLNTTDLTYFYESIFKLYRENKDHIEKVYWGLEGGYDRKMYEQQIELLMSIVLND.LVHQD.THQM.GK
Legionella_pneumophila_ApaH RMMGKTAAHQFRFNDGDLGYFYESIFTLYNENKDCVDTIYWGLEGGYDRTMYERELKILLQVIEKQ.LLPKD.SNSH.SM
Legionella_bozemanae_ApaH DLMGIAEARKTRFNSKDLTYFYESIFKLYQENKDYIEKIYWGLEGGYDRKMYEQQIQLLMSLILKD.IVHQD.TNQM.VP
Legionella_erythra_ApaH HTLSSKESHSRRFSDEDIFHFTQKIVEMIKTYPDTLCRVYWQLEGGYTEYVNVRQLKNTVGILNE..FYPWVEETMENSK
Legionella_geestiana_ApaH DELSEEESRVCRFNDRDFGIFNYLIMTYFQDNREHMSHLYWGLEGGYERGMYEFQARKLLEAIDVR.LINKP.AEGP.AS
Legionella_spiritensis_ApaH QELTPKQSSGCRFTDRDMTYFNRQLMILCQDYKPIIQRIYWQLEGGYTDEVNFRQIACLTECLQSL.FLLND.NEVECSE
Legionella_waltersii_ApaH RMMGYESAKTYRFNDGDLSYFYEEVFKLYNDNPDCFASLYWGLEGGYDNTMYTKQIKLLMQLISKE.LVPKA.THST.PL
Legionella_busanensis_ApaH RNIYDQEIKEQRFNDGDFEHFYKQLREVINKY..KIPFVYVSLEGGYTEAVNKKQTEMLIKTLLTPTITPQE.QSKE.EI
Legionella_moravica_ApaH RMMSLSEASKTRFNDGDLTYFYEQIFQLYNKNRELIKGIYWGLEGGYDRAMYERQVSLLTQIITAH.LPHQA.SSSS.SA
Legionella_sp_ApaH TMMSDEETQLCRFSDQDLSTFYHEIFSIYLENPEVFKGIYWGLEGGYNRAMYEQQISLLAESIITH.FYSQD.LTPS.LR
Legionella_antarctica_ApaH RMMSQSEAGTTRFNDGDLSYFFEEILTLYSENKELIESIYWGLEGGYERRMYEKQIQLMLDTIDGQ.LLHED.INQS.SL
Legionella_sp_HdaH KWMNKHEAYSSRFDNADWTYFYDGIFKLFQDNKQCLEAVYLGLEGGYEPHVYLNQITLLHQKIGSC.AQPQV.ASYP.Q.
Legionella_cherrii_ApaH
Legionella_cherrii_ApaH TSS.........P
Legionella_pneumophila_ApaH S...........Y
Legionella_bozemanae_ApaH DSS........KKI
Legionella_erythra_ApaH ALA.........T
Legionella_geestiana_ApaH SSLVEDDAPSGPSL
Legionella_spiritensis_ApaH KME.I.......TG
Legionella_waltersii_ApaH NQ..........L
Legionella_busanensis_ApaH NNY........SFV
Legionella_moravica_ApaH GMS.........Y
Legionella_sp_ApaH ....R
Legionella_antarctica_ApaH GIN.........C
Legionella_sp_HdaH ....P

Supplementary Figure 21: Primary sequence alignment of selected bacterial deacylases of cluster 3 (UniProt accession numbers are shown).
Show are the following sequences of the following deacylases representing enzymes of cluster 3 and the sub-clusters (code: sub-cluster, accession number/protein name/spcies): 3, A0A0W0SGS1/ApaH/*Legionella cherrii*; 3, A0A2S6EWV0/ApaH/*Legionella pneumop*hila; 3, A0A0W0RV07/ApaH/*Legionella bozemana*e (*Fluoribacter bozemanae*); 3, A0A0W0TS12/ApaH/*Legionella erythra*; 3, A0A0W0TY70/ApaH/*Legionella geestiana*; 3, A0A0W0YXX3/ApaH/*Legionella spiritensis*; 3, A0A0W1A0K6/ApaH/*Legionella waltersii*; 3, A0A378JU93/ApaH/*Legionella busanensis*; 3, A0A378K274/ApaH/*Legionella moravica*; 3, A0A522FB57/ApaH/*Legionella sp.*; 3, A0A6F8T350/ApaH/*Legionella antarctica*; 3, A0A8I1Q8A3/HdaH/*Legionella sp.* All sequences were aligned using the T-COFFEE multiple sequence alignment server[1]. The important catalytic residues are highlighted. The secondary structure elements and numbering is shown for the deacylase shown at the top of the amino acid sequences above the sequence alignment. The alignment was created by ESPript version 3.0[2].

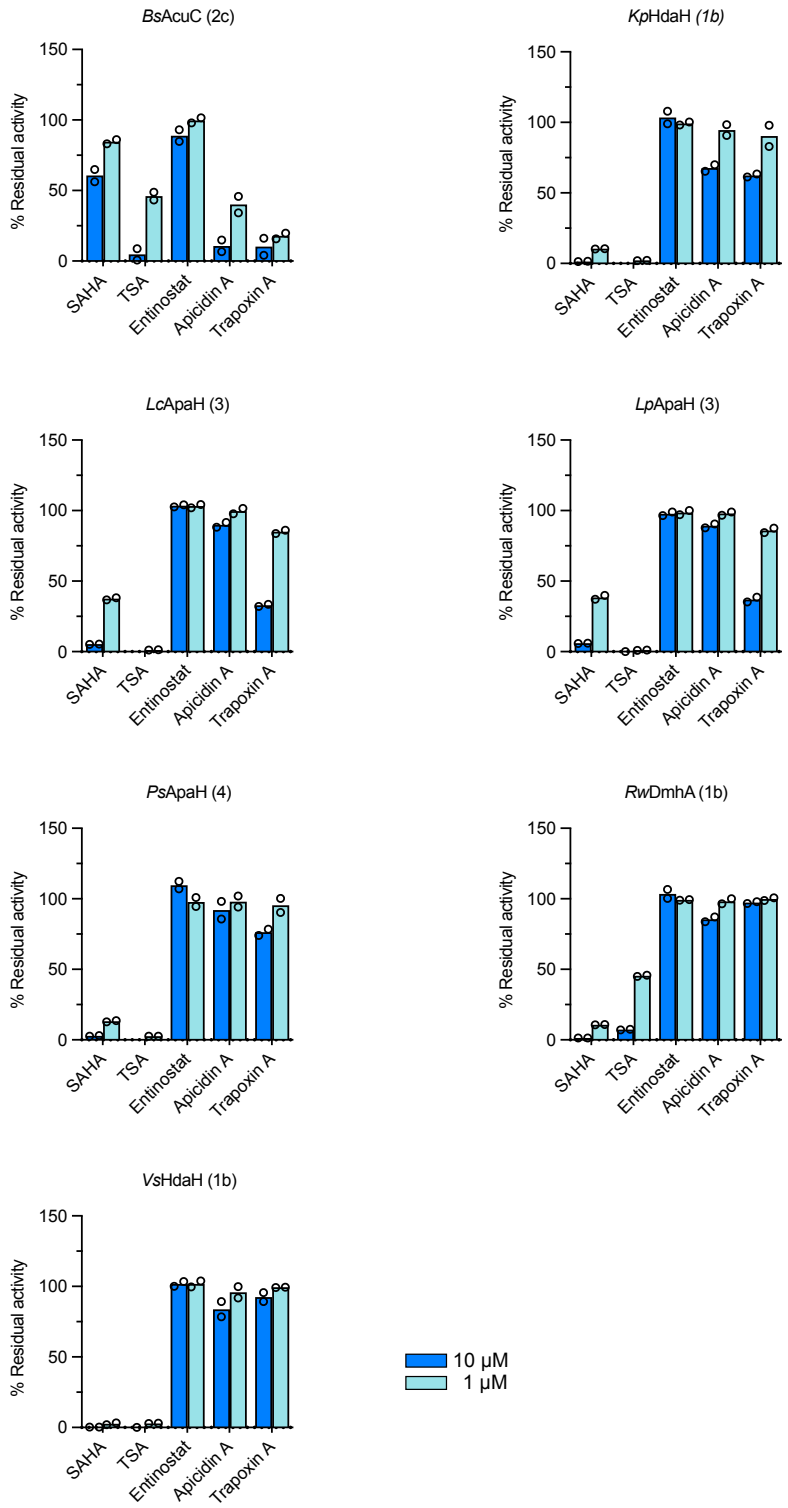


Supplementary Figure 22: Overview of structures of deacylase hydroxamate inhibitors SAHA, trichostatin A (TSA), benzamide etinostat (MS-275) and the cyclic peptides apicidin A and trapoxin A.

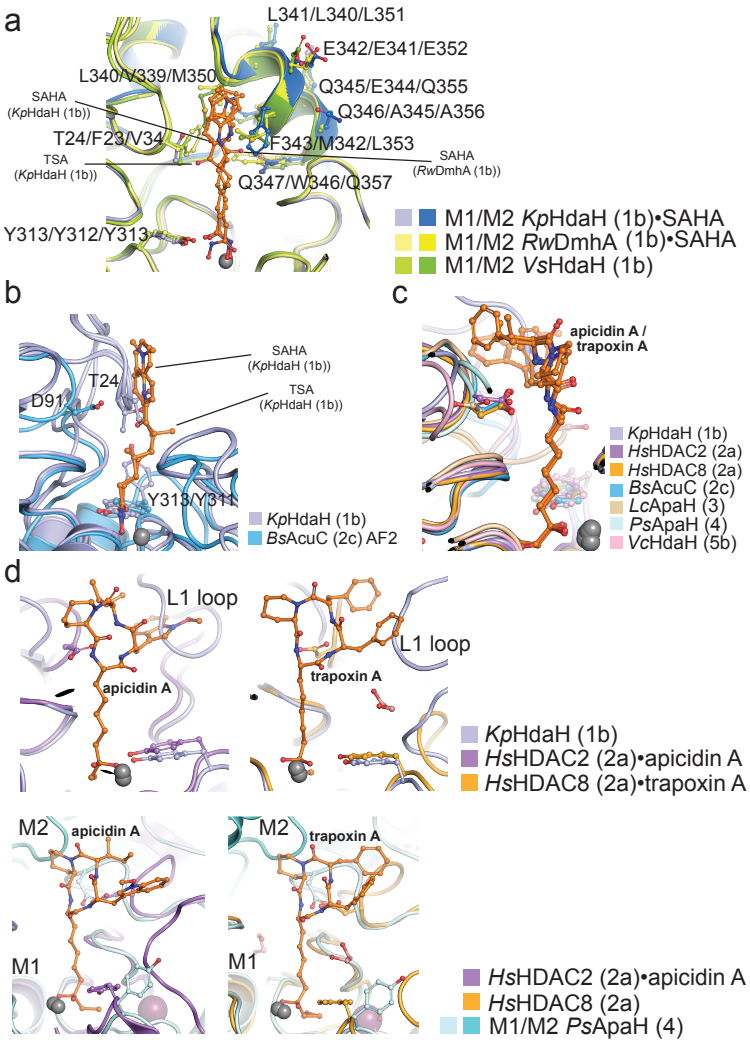
a. The hydroxamate inhibitors trichostatin A (TSA) and SAHA (vorinostat, suberoylanilide hydroxamic acid (SAHA)) are potent class I and class II classical HDAC pan-inhibitors. The hydroxamate moiety is used to complex the catalytic Zn^{2+}-ion. Bacterial deacylases of all clusters, except from cluster B, are potently inhibited by these hydroxamate inhibitors.

b. The benzamide inhibitor entinostat (MS-275) shows selectivity towards class I HDACs. Our data show no bacterual deacylase being potently inhibited by this benzamide inhibitor.

c. The cyclic peptide inhibitors trapoxin A and apicidin A were shown to selectively inhibit class I HDACs. This is in agreement with our data revealing only cluster B enzyme *Bs*AcuC being selectively and potently inhibited by both cyclic peptide inhibitors.



Supplementary Figure 23: Screening of hydroxamate, benzamide and cyclic peptide inhibitors to inhibit selected bacterial deacylases.
The hydroxamate inhibitors trichostatin A (TSA) and SAHA (vorinostat), the benzamide inhibitor entinostat (MS-275) and the cyclic peptide inhibitors trapoxin A and apicidin A were used at concentrations of 1 µM and 10 µM and varying enzyme concentrations (cluster A: 15 nM *Kp*HdaH, 30 nM *Rw*DmhA 1.5 nM *Vs*HdaH; cluster B: 200 nM *Bs*AcuC; cluster D: 40 nM *Ps*ApaH; cluster E: 30.5 nM *Lc*ApaH, 0.5 nM *Lp*ApaH) to screen selected bacterial deacylases. The assay was conducted for 1h at 37°C before stopping the reaction. The measured residual activity was determined in % based on the AMC fluorescence obtained defining the 100% value by measuring the fluorescence of the free AMC-fluorophore at the substrate concentrations (40 µM LGKac *Bs*AcuC (2c), *Lc*ApaH (3), *Lp*ApaH (3); 60 µM QPKKac: *Kp*HdaH (1b), *Rw*DmhA (1b), *Vs*HdaH (1b), *Ps*ApaH (1b)). The data obtained for 1 µM inhibitor was used to create the heat map shown in Fig. 8a. The experiment was performed in two independent replicates (*n*=2). Bars depict means. Source data are provided as Source Data file.



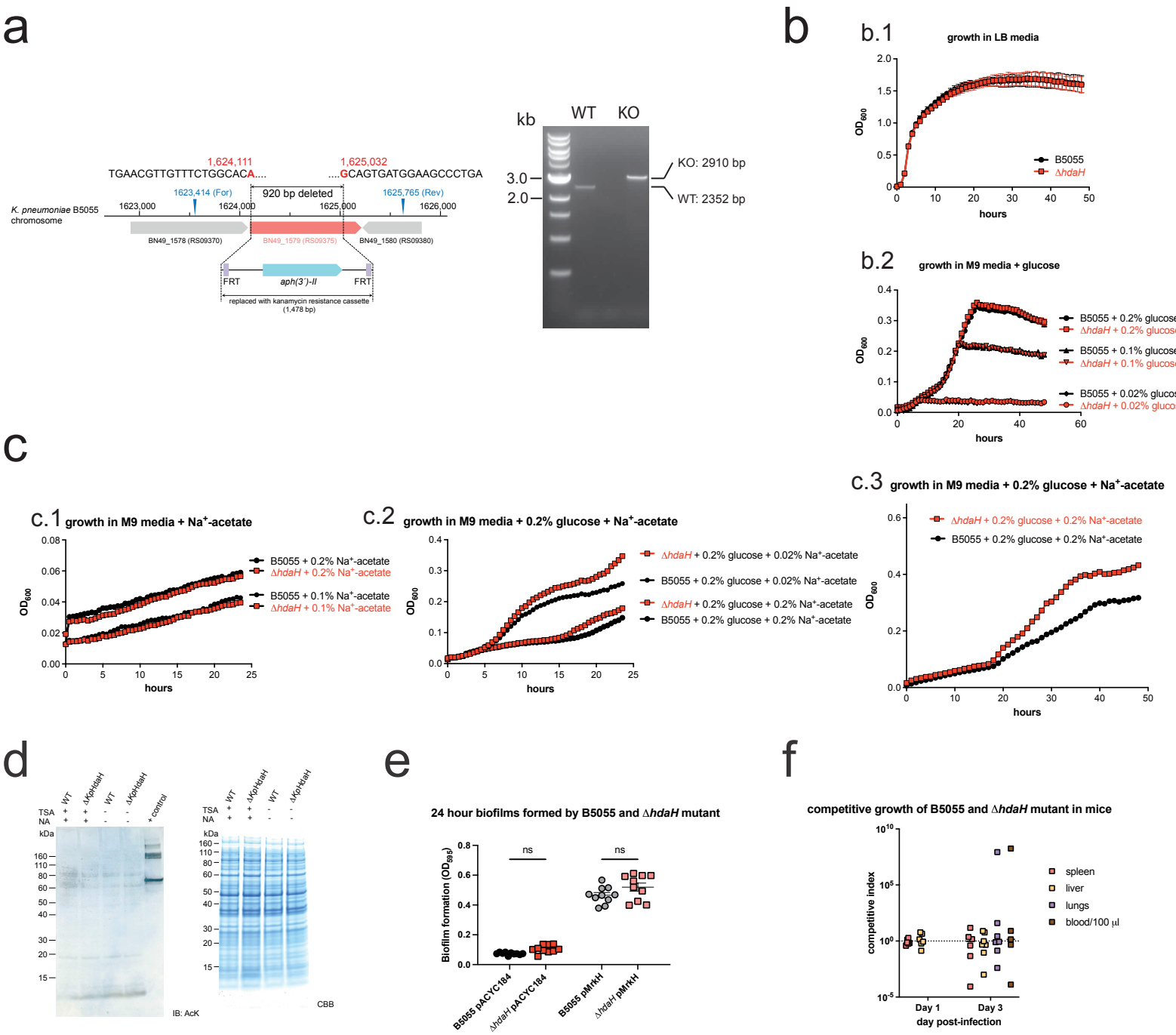
Supplementary Figure 24: Structural determinants for selectivity of hydroxamate inhibitors SAHA and TSA and of trapoxin A and apicidin A in inhibition of bacterial deacylases.

a *Rw*DmhA (1b) is the only enzyme showing better inhibition by SAHA compared to TSA and *Vs*HdaH (1b) is equally well inhibited by SAHA and TSA. To reveal the underlying mechanism, the structures of *Rw*DmhA (1b) and *Vs*HdaH (1b) (AF2) were superimposed with *Kp*HdaH (1b)•TSA and *Kp*HdaH (1b)•SAHA. Shown is a closeup to provide details on binding of the inhibitors.

b To reveal molecular determinants underlying the low potency of inhibition of *Bs*AcuC (2c) activity observed for SAHA and TSA, the AlphaFold2 model of *Bs*AcuC (2c) was superimposed with the structures of *Kp*HdaH (1b)•TSA and *Kp*HdaH (1b)•SAHA.

c D101/D100 in *Hs*HDAC8/*Hs*HDAC2 is needed for inhibition by trapoxin A and apicidin A. Superposition of the structures of the core domains of bacterial deacylases *Kp*HdaH (1b), *Rw*DmhA (1b), *Rs*PrpH (1b) *Bs*AcuC (2c), *Lp*ApaH (3), *Lc*ApaH (3), *Ps*ApaH (4) VcHdaH (5b) and *Hs*HDAC2 (2a) and *Hs*HDAC8 (2a) and modelling of trapoxin A and apicidin A reveals, except from *Ps*ApaH (4) and *Vc*HdaH (5b), either a negatively charged D or E is present at analogue localization as in *Hs*HDAC2 (2a) and *Hs*HDAC8 (2a). The observed lack of inhibition of *Kp*HdaH (1b), *Rw*DmhA (1b), *Rs*PrpH (1b), *Lp*ApaH (3), *Lc*ApaH (3), *Ps*ApaH (4) *Vc*HdaH (5b) is due to other determinants in structure or sequence.

d Except from *Bs*AcuC (2c) none of the bacterial enzymes is inhibited by apicidin A or trapoxin A. Superposition of structures of bacterial deacylases with apicidin A and trapoxin A were done to reveal molecular mechanisms explaining the lack of inhibition of bacterial deacylases *Kp*HdaH (1b), and *Ps*ApaH (4) by apicidin A and trapoxin A. *Ps*ApaH (4) and *Vc*HdaH (5b) do not contain an D/E at the analogous positiion to D101/D100 in *Hs*HDAC8/*Hs*HDAC2 explaining their lack in inhibition by cyclic peptides (*Kp*HDAH (1b): E101; *Bs*AcuC (2c): D91 ; *Lp*ApaH (3): D146; *Lc*ApaH (3): D144; *Ps*ApaH (4): G117; *Vc*HdaH (5b): G97; *Hs*HDAC2: D100; *Hs*HDAC8: D101). For the complexes the crystal structures of the indicated enyzmes were superimposed with the structures of HDAC2•apicidinA (PDB: 7LTG [https://doi.org/10.2210/pdb7LTG/pdb]) and HDAC8•trapoxin A (PDB: 5VI6 [https://doi.org/10.2210/pdb5VI6/pdb]) to model the localization of apicidin A and trapoxin A. For *Bs*AcuC (2c) the AlphaFold2 model was used.


a
1,624,111
TGAACGTTGTTTCTGGCACA....
....GCAGTGATGGAAGCCCTGA
1,625,032
1623,414 (For)
920 bp deleted
1625,765 (Rev)
K. pneumoniae B5055 chromosome
1623,000
1624,000
1625,000
1626,000
BN49_1578 (RS09370)
BN49_1579 (RS09375)
BN49_1580 (RS09380)
FRT
aph(3')-II
FRT
replaced with kanamycin resistance cassette (1,478 bp)
kb
WT
KO
3.0
2.0
KO: 2910 bp
WT: 2352 bp
b
b.1
growth in LB media
OD600
hours
B5055
ΔhdaH
b.2
growth in M9 media + glucose
B5055 + 0.2% glucose
ΔhdaH + 0.2% glucose
B5055 + 0.1% glucose
ΔhdaH + 0.1% glucose
B5055 + 0.02% glucose
ΔhdaH + 0.02% glucose
c
c.1 growth in M9 media + Na+-acetate
B5055 + 0.2% Na+-acetate
ΔhdaH + 0.2% Na+-acetate
B5055 + 0.1% Na+-acetate
ΔhdaH + 0.1% Na+-acetate
c.2 growth in M9 media + 0.2% glucose + Na+-acetate
ΔhdaH + 0.2% glucose + 0.02% Na+-acetate
B5055 + 0.2% glucose + 0.02% Na+-acetate
ΔhdaH + 0.2% glucose + 0.2% Na+-acetate
B5055 + 0.2% glucose + 0.2% Na+-acetate
c.3 growth in M9 media + 0.2% glucose + Na+-acetate
ΔhdaH + 0.2% glucose + 0.2% Na+-acetate
B5055 + 0.2% glucose + 0.2% Na+-acetate
d
TSA
NA
WT
ΔKpHdaH
+ control
kDa
IB: AcK
CBB
e
24 hour biofilms formed by B5055 and ΔhdaH mutant
Biofilm formation (OD595)
ns
B5055 pACYC184
ΔhdaH pACYC184
B5055 pMrkH
ΔhdaH pMrkH
f
competitive growth of B5055 and ΔhdaH mutant in mice
competitive index
spleen
liver
lungs
blood/100 µl
Day 1
Day 3
day post-infection

Supplementary Figure 25: Impact of genomic deletion of *hdaH* in *K. pneumoniae* on growth, biofilm formation and virulence.

a Genomic deletion of the gene *hdaH* encoding for *Kp*HdaH (1b) in the genome of *K. pneumoniae* B5055 (B5055). To determine whether the gene encoded a product that was functional in the metabolism and pathogenesis of site-specific mutagenesis deleting the *hdaH* gene in B5055 was conducted. The *Kp*HdaH enzyme is annotated as a putative histone deacetylase encoded by BN49_1579 (BN49_RS09375) on the chromosome of *Klebsiella pneumoniae*. The BN49_1579 gene was disrupted by replacing a 920 bp coding region with a 1,478 bp kanamycin resistance cassette (flanked by FRT sites) using λ-red recombinase-mediated gene gorging method[5]. The location of BN49_1579 and key sites for mutagenesis are shown, with genomic coordinates obtained from www.biocyc.org. Block arrows depict annotated transcription units in the direction of transcription. Dotted lines indicate the excision points immediately adjacent to the deleted sequence. The up- and downstream sequences flanking the deletion site are shown above, with the last bp retained and their coordinates shown in red. Blue triangles show primer binding sites (For, forward primer; Rev, reverse primer) for PCR that confirms gene deletion. The agarose gel electrophoresis image shows PCR products of expected size for wildtype (WT) and knockout (KO) *K. pneumoniae*. The B5055 *hdaH* mutant (Δ*hdaH*) and wildtype (WT) B5055 were analysed by whole-genome sequencing using the Illumina platform. No secondary mutation outside of BN49_1579 was detected, thus validating site-specific mutation in the mutant strain. Source data are provided as Source Data file.

b Growth of B5055 in LB-medium and M9-medium supplemented with glucose is not affected by the absence of HdaH. Top panel, b.1: The B5055 WT and B5055 Δ*hdaH* were grown in Luria-Bertani broth, a rich medium containing multiple carbon sources. The growth curves of the WT and Δ*hdaH* were determined using a CLARIOstar Plus plate reader over 48 hours. There was no difference in the fundamental growth kinetics. Both isolates demonstrated similarities in lag, log and stationary phases. Lower panel, b.2: To determine whether the multiple carbon sources and high glucose concentrations of LB obscured differences in growth kinetics, the B5055 WT and B5055 Δ*hdaH* were grown in minimal medium (M9) with varying concentrations of glucose. Terminal OD_{600} was determined by glucose concentration and were approximately 25% of OD_{600} generated by growth in LB broth. Again, there were no differences in the growth kinetics of the B5055 WT and B5055 Δ*hdaH*. The analysis of growth in rich and simple media comparing B5055 WT and B5055 Δ*hdaH* in central glucose metabolism of B5055 suggested that *Kp*HdaH (1b) had no role in determining the kinetics of growth when glucose was provided as the sole source of carbon. The experiment was performed with three biological replicates (n=3) each containing 4 technical replicates. Data are presented as means±SD of the three biological replicates. Source data are provided as Source Data file.

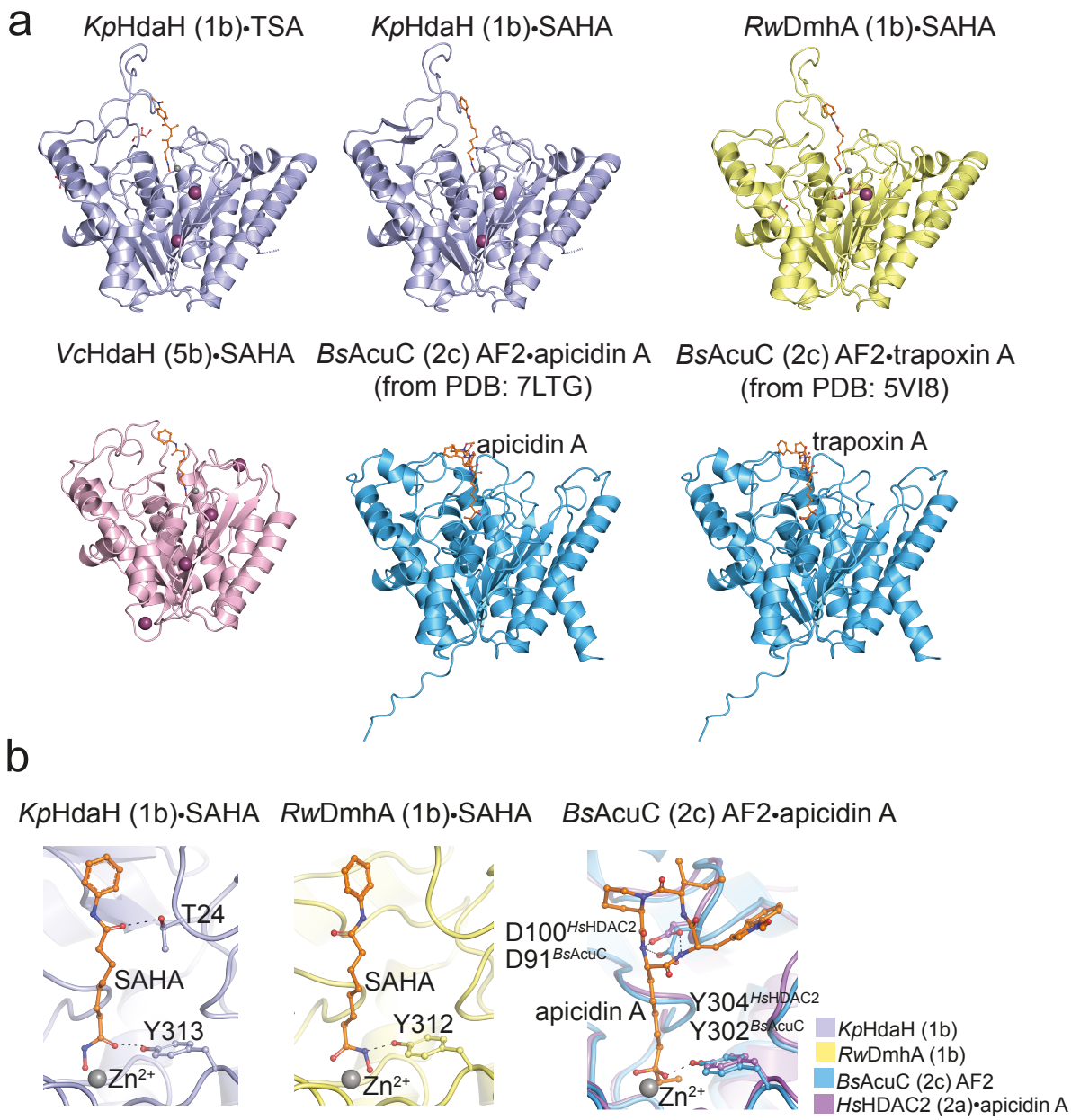
c We next explored whether B5055 Δ*hdaH* increased the rate or level of growth when sodium acetate was provided as the sole carbon source. Left pane, c.1: Growth of B5055 in sodium acetate was very limited albeit the level of growth was determined by the concentration by the amount of sodium acetate added. Again there was no difference in growth rate when the WT was compared with the Δ*hdaH* mutant. Middle panel, c.2: When glucose was added to acetate in M9 medium, differences in growth rate were observed between the B5055 WT and the B5055 Δ*hdaH* mutant. The level of growth for the B5055 Δ*hdaH* was increased over that of the B5055 WT for the first 24 hours, suggesting that the presence of an intact *hdaH* locus, together with sodium acetate reduced growth of *K. pneumoniae* B5055. Right panel,c.3: When the analysis was extended by a further 24 hours, a second lag and renewed exponential growth was seen in an additional phase of growth (diauxic growth). We hypothesized that once glucose was consumed B5055 swiched to using acetate as the carbon source. The experiment was performed with two biological replicates (n=2), each containing 4 technical replicates. Data are represented as means. Source data are provided as Source Data file.

d Genomic deletion *of K. pneumoniae hdaH* does not result in a generalised alteration of the acetylation pattern in cells grown in M9 medium. To determine whether any deacylation activity of *Kp*HdaH could be identified in cultured *K. pneumoniae* B5055, the B5055 WT and B50555 Δ*hdaH* mutant from stationary phase cultures of bacteria grown in M9 medium were analysed by Western immunoblotting using an acetyl-group specific antibody (IB: AcK). The positive control was acetylated bovine serum albumin (BSA), which was clearly positive in the Western blot, with a MW consistent with that of BSA (67 kDa). Different additives including the classical deacetylase inhibitor trichostatin A (TSA; 200 nM) and nicotinamide (NA; 2 mM) were supplied but there was no difference in the Western blot profile of B5055 WT and B5055 Δ*hdaH*. Source data are provided as Source Data file.

e *K. pneumoniae* HdaH does not affect biofilm formation. Two further analyses were undertaken of the B5055 WT and B5055 Δ*hdaH*. Firstly, the impact of the putative de-acetylase on biofilm formation was determined. *K. pneumoniae* B5055 is a poor biofilm former because it lacks an intact

copy of MrkH, the transcriptional activator of Mrk fimbriae expression. The Mrk fimbria is a key mediator of *K. pneumoniae* biofilm formation[6]. To address this issue, B5055 and the Δ*hdaH* mutant of B5055 were transfected with a functional plasmid copy of MrkH (pMrkH). The supply of *mrkH* in trans resulted in an increase in biofilm formation, however there was no significant difference in the level of biofilm produced by the WT complemented with pMrkH) and the Δ*hdaH* mutant. Ten independent biological experiments were conducted and the data shown are the technical replicates from one representative experiment. Ten individual data points for each isolate (B5055 WT and B5055 Δ*hdaH*) were collected from each of two independent experiments. A representative experiment is presented (technical replicates, n=10). There was no statistical difference (ns) in the level of biofilm produced by B5055 and B5055 Δ*hdaH* using an ordinary one-way ANOVA test (Tukey's multiple comparison test) with a significance level of $p<0.05$. Exact values can be found in the Source Data. Source data are provided as Source Data file.

f *K. pneumoniae* HdaH has no direct effect on *K. pneumoniae* virulence. Lastly, the virulence of the WT and the Δ*hdaH* mutant was tested in C57BL/6 (BL/6) mice. B5055 is a known virulent isolate in BL/6 mice[7,8]. Animals were infected with 3 different doses of WT and Δ*hdaH* mutant. *In toto*, these studies failed to reveal a dose-dependent phenotype associated with loss of the *hdaH* locus. While in some experiments, it appeared that there may have been some growth attenuation of Δ*hdaH*, the effects were not consistent. At low doses (10^3 colony forming units (CFU) injected intravenously (i.v.)), there may have been some growth deficit in Δ*hdaH* but this was not seen when doses were increased 10-fold, where survival and weight loss post-infection were not increased by loss of the locus. Competitive infections were also assessed, where C57BL/6 mice were infected with equal numbers of the WT and *Kp*HdaH Δ*hdaH* mutant and then killed at day 1 or day 3 post-infection, and the number of *K. pneumoniae* in various tissues analysed by viable counts (right panel). However, again no significant effect was observed and the data shows quite huge variation. Data points represent individual mice (n=6) in one experiment. The result is representative of two independent experiments. Source data are provided as Source Data file.

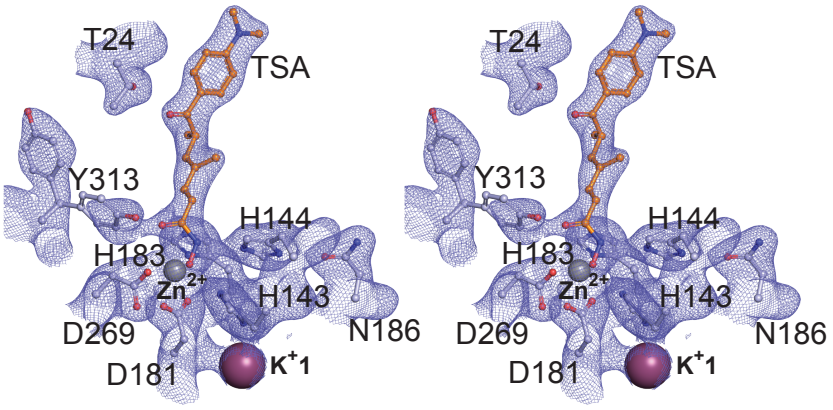


Supplementary Figure 26: Overview of structures of bacterial deacylases in complexes with the hydroxamate inhibitors SAHA, and trichostatin A (TSA).

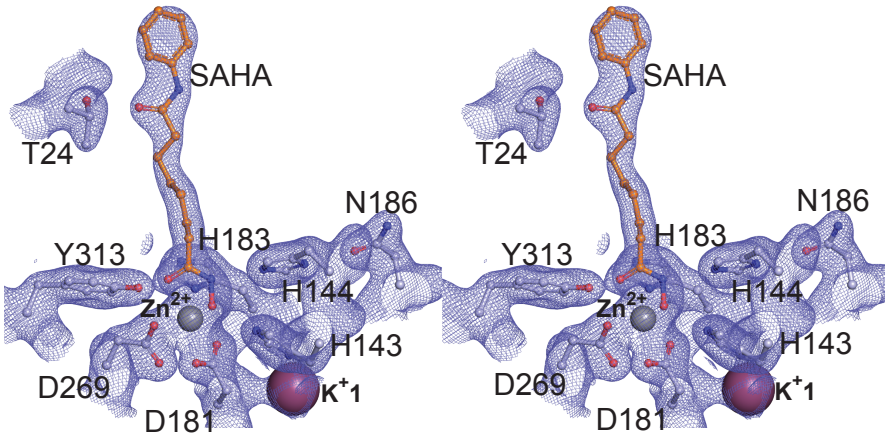
a. Overview of all inhibitor bound-structures of bacterial deacylases of all sub-clusters. Shown are the following complexes of the deacylases in complexes with the hydroxamates SAHA and/or TSA: *Kp*HdaH•TSA, *Kp*HdaH•SAHA and *Rw*DmhA•SAHA of sub-cluster 1b and *Vc*HdaH•SAHA of cluster 5b. For the complexes of *Bs*AcuC of sub-cluster 2c with apixidin A and trapoxin A, the AlphaFold2 model of *Bs*AcuC was superimposed with the structures of HDAC2•apicidinA (PDB: 7LTG [https://doi.org/10.2210/pdb7LTG/pdb]) and HDAC8•trapoxin A (PDB: 5VI6 [https://doi.org/10.2210/pdb5VI6/pdb]).

b. Closeup of the structures of *Kp*HdaH•TSA, *Rw*DmhA•SAHA and of the AlphaFold2 model of *Bs*AcuC in complex with apicidin A (from PDB: 7LTG [https://doi.org/10.2210/pdb7LTG/pdb]). The conserved Tyr (KpHdaH: Tyr313) directly contacts the oxygen /nitrogen of the hydroxamate groups of SAHA or TSA, respectively, or the gemdiol(ate) function of the apicidin A.. This is at the analogous position of the substrates' carbonyl oxygen of the acyl group. The hydroxamate and gemdiol(ate) function furthermore directyl coordinates the catalytic Zn^{2+}-ion in a bidentate fashion. As dexcribed for the interaction with SAHA, Thr24 in *Kp*HdaH form an interaction to the TSA carbonyl oxygen. The interaction of the cyclic peptide inhibitor apicidin A is created by formation of a hydrogen bound with the conserved Asp91 in *Bs*AcuC (2c) towards the peptide bonds of the cyclic peptide inhibitor. This Asp contributes to the selectively to inhibit class I HDACs.

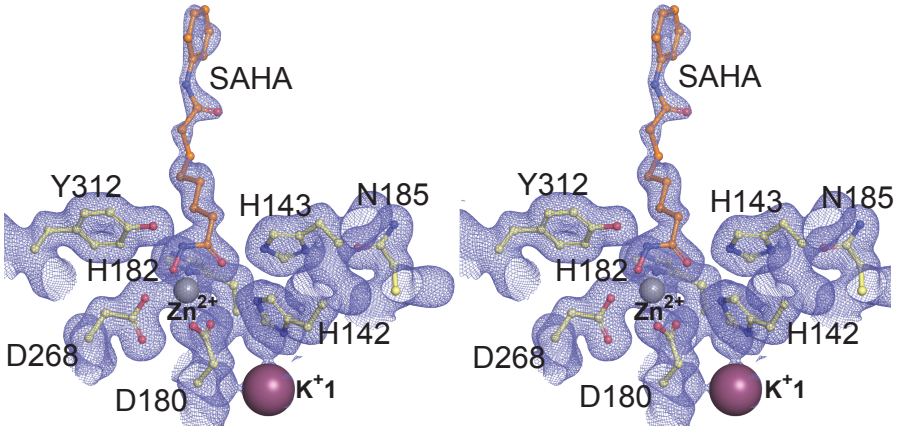
*Kp*HdaH (1b)•TSA



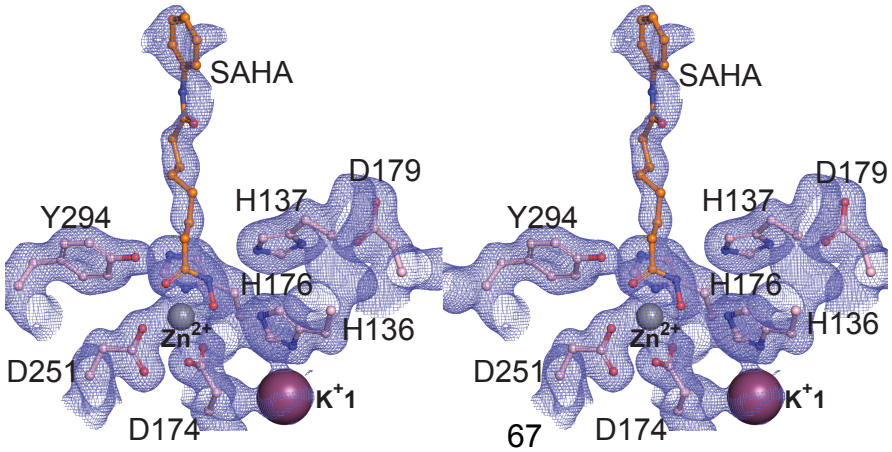
*Kp*HdaH (1b)•SAHA



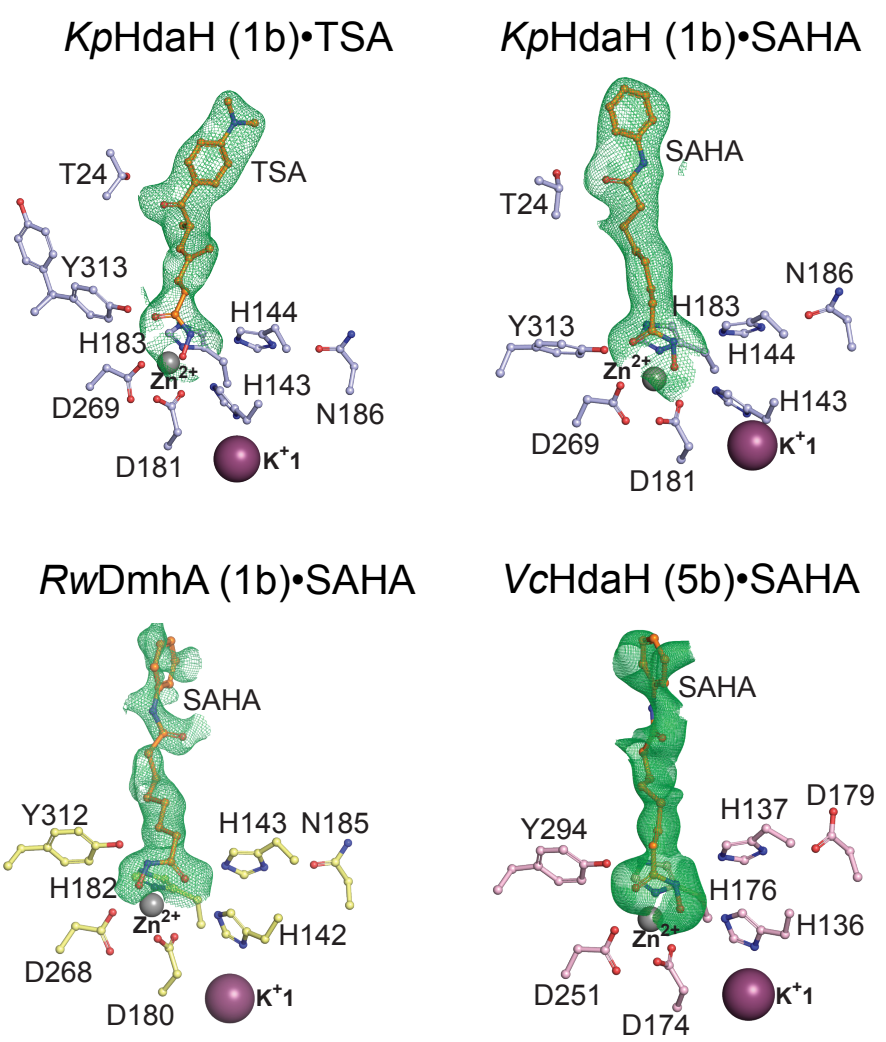
*Rw*DmhA (1b)•SAHA



*Vc*HdaH (5b)•SAHA



Supplementary Figure 27: Stereo images of representative electron density for inhibitor-bound structures of bacterial deacylases of sub-cluster 1b solved in this study.
Stereo figures for the inhibitor bound-structures of bacterial deacylases of all sub-clusters. Shown are closeups of the following complexes: *Kp*HdaH (1b)•SAHA, *Kp*HdaH (1b)•TSA and *Rw*DmhA (1b)•SAHA and *Vc*HdaH (5b)•SAHA of subcluster 1b. For all structures a representative closeup of the active site is shown, including the bidentate Zn^{2+}-binding of the hydroxamate inhibitor, the coordinating His and the two Asp residues, the catalytic base His and the active site Tyr important to orient and polarize the carbonyl-group of the substrate and to stabilize the tetrahedral intermediate. Shown in blue is the electron density of the $2F_o$-F_c map contoured at 1σ. The stereo diagrams were prepared in wall-eyed viewing mode with PyMOL version 2.3.4[3].



Supplementary Figure 28: F_o-F_c omit maps for the electron density of the hydroxamates TSA and SAHA for all inhibitor-bound structures of bacterial deacylases solved in this study.
Shown are the F_o-F_c omit maps (contour level 3σ) for the inhibitors TSA and SAHA for the structures of the deacylases in complexes with the hydroxamate inhibitors: *Kp*HdaH (1b)•SAHA, *Kp*HdaH (1b)•TSA, *Rs*PrpH (1b)•SAHA, *Rw*DmhA (1b)•SAHA and *Vc*HdaH (5b)•SAHA. For all structures a representative closeup of the active site is shown, including the bidentate Zn^{2+}-binding of the hydroxamate inhibitor, the coordinating His and the two Asp residues, the catalytic base His and the active site Tyr important to orient and polarize the carbonyl-group of the substrate and to stabilize the tetrahedral intermediate. Shown in green is the electron density of the F_o-F_c map contoured at 3σ. The figures were prepared with PyMOL version 2.3.4[3].
and the XGGY-motif lining the foot pocket and the catalytic Tyr (*Kp*HdaH: Y313); green box: RPP-motif lining the foot pocket.

Supplementary Table 1: *Escherichia coli* strains used in this study.

Strain	Genotype	Reference/construction
BL21 (DE3)	*B F^- ompT gal dcm lon $hsdS_B(r_B^-m_B^-)$ λ(DE3 [lacI lacUV5-T7p07 ind1 sam7 nin5]) $[malB^+]_{K-12}(\lambda^S)$*	[9]
DH5α	*F^- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20 φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17($r_K^-m_K^+$), λ^-*	[10]

Supplementary Table 2: Oligonucleotides used in this study. All oligonucleotides ordered from Sigma/Merck (https://www.sigmaaldrich.com/DE/de/configurators/tube?product=standard).

Primer	**Sequence 5'->3'**
QC_VsHdaH_H144A_fo	gtcgtccgccgggtcatgcttgcctggcc
QC_VsHdaH_H144A_rev	ggccaggcaagcatgacccggcggacgac
QC_RsPrpH_H143A_fo	gccggccatgcctgtctgccggatac
QC_RsPrpH_H143A_rev	gtatccggcagacaggcatggccggc
QC_RwDmhA_H143A_fo	ggcaggtcatgcatgtctggcagatac
QC_RwDmhA_H143A_rev	gtatctgccagacatgcatgacctgcc
QC_VcHdaH_H126A_fo	gtggttatcatgccgcccatgcagac
QC_VcHdaH_H126A_rev	gtctgcatgggcggcatgataaccac
QC_ApaHD3_H159A_fo	ccgggccatgcagcagcaagtgatgttatg
QC_ApaHD3_H159A_rev	cataacatcacttgctgctgcatggcccgg
QC_LcApaH_E_H179A_fo	cgacccatgcagccttcgcagatgaag
QC_LcApaH_E_H179A_rev	cttcatctgcgaaggctgcatgggtcg
QC_LpApaH_H181A_fo	gccgagccatgccgcctataccc
QC_LpApaH_H181A_rev	gtataggcggcatggctcggc
Gi_pOPIN_fo	gttatgaaggtgcagcccgttagaccatttaaacaccac
Gi_pOPIN_rev	ctatacacactcagcataccgtaccaccgatctgttc
Gi_PA1409_fo	cagatcggtggtaccggtatgctgagtgtgtatagcg
Gi_PA1409_rev	ggtggtgtttaaatggtctaacgggctgcaccttc
QC_PsApaH_1-342_Stop_fo	gaaaatgtgtaacgtccgaataatagtaaaaaag
QC_PsApaH_1-342_Stop_rev	ctattattcggacgttacacattttcgaag
Insert_LpApaH_28-426_fo	tgtgcaattcagattccgag
Insert_LpApaH_28-426_fo	ggtacccacgtgatggtg
Gi_LpApaH_28-426_fo	catcaccacagccaggatccgtgtgcaattcagattccgag
Gi_LpApaH_28-426_rev	gcggtttctttaccagactcgagggtaccgctcgagttagtaactcatactatgac
Gi_pRSF-Duet_Lp_fo	gtatgagttactaactcgagcggtaccctcgagtctggtaaagaaac
Gi_pRSF-Duet_Lp_fo	gctcggaatctgaattgcacacggatcctggctgtggtgatg
QC_LpApaH_L414Stop_fo	gtgattgaaaaacagtaactgccgaaag
QC_LpApaH_L414Stop_rev	ctatctttcggcagttactgtttttcaatc
Insert_LcApaH_22-434_fo	tgtctgatccagattccgag
Insert_LcApaH_22-434_rev	ggtacccacgtgatggtg
Gi_LcApaH_22-434_fo	caccacagccaggatccgaattgtctgatccagattccgagtgcc
Gi_LcApaH_22-434_rev	cggtttctttaccagactcgagttacgggctgctggttttgcc
Gi_pRSF-Duet_Lc_fo	gcaaaaccagcagcccgtaactcgagtctggtaaagaaaccgctg
Gi_pRSF-Duet_Lc_rev	ctcggaatctggatcagacaattcggatcctggctgtggtg
QC_LcApaH_N409Stop_fo	gagcattgttctgtaagatctggttc
QC_LcApaH_N409Stop_rev	ctgatgaaccagatcttacagaacaatg

Supplementary Table 3: Peptide substrates with various acyl-chains used in this study. The peptide backbone was derived from histone H3 ($APRK_{acyl}$, $H3_{15-18}$ or $TARK_{acyl}$, $H3_{6-9}$), histone H4 (LGK_{acyl}, $H4_{10-12}$) tumor suppressor protein p53 ($QPKK_{acyl}$, $p53_{317-320}$) or DLAT (Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex; $ETDK_{acyl}$; $DLAT_{256-259}$). The C-terminal lysine side chain carried various acyl-chain types, i.e. acetyl (C-2; K_{ac}), propionyl (C-3; K_{pro}), butyryl (C-4; K_{but}), hexanoly (C-6; K_{hex}), octanoyl (C-8; K_{oct}), decanoyl (C-10; K_{dec}), lauroyl (C-10; K_{lau}), myristoyl (C-12; K_{myr}), palmitoyl (C-14; K_{pal}), crotonyl (K_{cro}), L-lactyl (K_{L-la}), D-β-hydroxybutyryl K_{D-bhb}), succinyl (K_{suc}), glutaryl (K_{glu}) and biotinyl (K_{bio}). The references are given.

Shorthand	Sequence	Source
LGKac	Ac-Leu-Gly-Lys(Ac)-AMC	Bradner *et al.*, 2010[11]
QPKKac	Ac-Gln-Pro-Lys-Lys(Ac)-AMC	Madsen *et al.*, 2016[12]
APRKac	Ac-Ala-Pro-Arg-Lys(Ac)-AMC	Moreno-Yruela *et al*., 2022[13]
ETDKac	Ac-Glu-Thr-Asp-Lys(Ac)-AMC	Galleano *et al.*, 2016
LGKpro	Ac-Leu-Gly-Lys(Pro)-AMC	Olesen *et al*., 2018[14]
LGKbut	Ac-Leu-Gly-Lys(But)-AMC	Olesen *et al*., 2018[14]
QPKKhex	Ac-Gln-Pro-Lys-Lys(Hex)-AMC	Madsen *et al.*, 2016[12]
QPKKoct	Ac-Gln-Pro-Lys-Lys(Oct)-AMC	Madsen *et al.*, 2016[12]
QPKKdec	Ac-Gln-Pro-Lys-Lys(Dec)-AMC	Madsen *et al.*, 2016[12]
TARKdec	Ac-Thr-Ala-Arg-Lys(Dec)-AMC	Madsen *et al.*, 2016[12]
ETDKdec	Ac-Glu-Thr-Asp-Lys(Dec)-AMC	Galleano *et al.*, 2016
QPKKlau	Ac-Gln-Pro-Lys-Lys(Lau)-AMC	Madsen *et al.*, 2016[12]
QPKKmyr	Ac-Gln-Pro-Lys-Lys(Myr)-AMC	Madsen *et al.*, 2016[12]
TARKmyr	Ac-Thr-Ala-Arg-Lys(Myr)-AMC	Madsen *et al.*, 2016[12]
ETDKmyr	Ac-Glu-Thr-Asp-Lys(Myr)-AMC	Galleano *et al.*, 2016
QPKKpal	Ac-Gln-Pro-Lys-Lys(Pal)-AMC	Madsen *et al.*, 2016[12]
LGKcr	Ac-Leu-Gly-Lys(Cr)-AMC	Madsen *et al.*, 2012a[15]
LGK(L-la)	Ac- Leu-Gly-Lys(L-la)-AMC	Moreno-Yruela *et al*., 2022[13]
LGK(D-la)	Ac- Leu-Gly-Lys(D-la)-AMC	Moreno-Yruela *et al*., 2022[13]
QPKK(L-la)	Ac-Gln-Pro-Lys-Lys(L-la)-AMC	Moreno-Yruela *et al*., 2022[13]
QPKK(D-la)	Ac-Gln-Pro-Lys-Lys(D-la)-AMC	Moreno-Yruela *et al*., 2022[13]
LGK(D-bhb)	Ac- Leu-Gly-Lys(D-bhb)-AMC	Moreno-Yruela *et al*., 2022[13]
LGKsuc	Ac-Leu-Gly-Lys(Suc)-AMC	Madsen *et al.*, 2012b[16]
LGKglu	Ac-Leu-Gly-Lys(Glu)-AMC	Anderson *et al*., 2017[17]
LGKbio	Ac-Leu-Gly-Lys(Bio)-AMC	Moreno-Yruela *et al*., 2018[18]

Supplementary Table 4: Crystallization of the enzymes and complexes crystallized in this study. Shown are the enzymes of different sub-clusters, the crystallization conditions, the cryoprotectant used to freeze the crystals in liquid nitrogen, the time needed until crystals were observed (in days) and the time at which the crystals were harvested. The PDB code is shown under which the structures are deposited in the protein data bank (PDB; https://www.rcsb.org/).

Protein	condition	Cryo-protection	Crystals observed	Harvested	PDB entry
*Kp*HdaH (1b)	12% (w/v) PEG 8000, 10% (v/v) glycerol and 0.5M KCl	with additional 10% glycerol	overnight	overnight	9GLB
*Kp*HdaH H144A (1b)	12% (w/v) PEG 8000, 10% (v/v) glycerol and 0.5M KCl	with additional 10% glycerol	overnight	overnight	9GN1
*Kp*HdaH (1b)•SAHA	12% (w/v) PEG 8000, 10% (v/v) glycerol and 0.5M KCl	with additional 10% glycerol	overnight	overnight following soaking (1h)	9GN6
*Kp*HdaH (1b)•TSA	12% (w/v) PEG 8000, 10% (v/v) glycerol and 0.5M KCl	with additional 10% glycerol	overnight	overnight following soaking (1h)	9GN7
*Rs*PrpH (1b)	4% tacsimate pH 6 12% (w/v) PEG3350	with saturated glucose	5 days	16 days	9GKU
*Rw*DmhA (1b)	0.2 M KF pH 7.3 and 20% (w/v) PEG3350	with 15% (w/v) PEG400	4 days	7 days	9GKW
*Rw*DmhA (1b)•SAHA	0.2 M NaF pH 7.3 20% (w/v) PEG3350	with saturated glucose	3 days	5 days	9GKX
*Vc*HdaH (5b)	1 M Na/K tartrate, 0.1 M imidazole pH 8 0.2 M NaCl	with saturated glucose	9 days	14 days	9GKY
*Vc*HdaH (5b)•SAHA	1.4 M sodium acetate 0.1 M MES pH 6.5	with saturated glucose	12 days	20 days	9GKV
*Ps*ApaH$_{1-342}$ (4)	0.1 M sodium acetate pH 4.5 20% (w/v) PEG3000	with 15% (w/v) PEG400	10 days	29 days	9GKZ
*Lc*ApaH$_{22-409}$ (3)	1 M $(NH4)_2SO_4$ 5% (w/v) PEG400 0.1 M Na-MES pH 6.5	2 M $(NH4)_2SO_4$ 10% (w/v) PEG400 0.1 M Na-MES pH 6.5 12% (v/v) glycerol	26 days	30 days	9GL0
*Lp*ApaH (3)	0.2 M calcium acetate 0.1 M HEPES pH 7.5, 18% (w/v) PEG8000	with 12% (w/v) PEG400	6 days	11 days	9GL1

Supplementary Table 5: Data collection and refinement statistics (molecular replacement) for the *Kp*HdaH (1b) wildtype structure of cluster 1b enzymes and for the catalytically inactive mutant *Kp*HdaH (1b) H144A structure.

	*Kp*HdaH (1b) (PDB: 9GLB [https://doi.org/10.2210/pdb9GLB/pdb])	*Kp*HdaH (1b) H144A (PDB: 9GN1 [https://doi.org/10.2210/pdb9GLN1/pdb])
Data collection		
Space group	I 2 3	I 2 3
Cell dimensions		
a, *b*, *c* (Å)	147.163 147.163 147.163	146.117 146.117 146.117
α, β, γ (°)	90.0 90.0 90.0	90.0 90.0 90.0
Resolution (Å)	25.24 - 2.1 (2.175 - 2.1) *[1]	46.21 - 2.35 (2.434 - 2.35) *[2]
R_{merge} (%)[a]	14.14 (24.13)	2.628 (23.49)
$I / \sigma I$	15.77 (1.14)	13.44 (1.46)
Completeness (%)	99.84 (99.94)	99.88 (99.91)
Redundancy	19.9 (20.4)	22.6 (22.5)
Refinement		
Resolution (Å)	2.1	2.35
No. reflections	30973 (3080)	21754 (1152)
R_{work} / R_{free} [b]	0.1496 (0.2466) / 0.1784 (0.2710)	0.1533 (0.2633)/ 0.2042 (0.3170)
No. atoms	3071	2973
Protein	2818	2815
Ligand/ion	25	21
Water	239	137
No. protein residues	370	370
Ramachandran Plot (%)[c]		
Most favored	95.65	92.93
Additionallly allowed	3.53	4.89
Disfavored	0.82	2.17
Clashscore	4.12	6.09
B-factors	51.10	48.21
Protein	50.65	47.80
Ligand/ion	70.88	56.84
Water	55.27	41.93
R.m.s. deviations		
Bond lengths (Å)	0.004	0.013
Bond angles (°)	0.66	2.30

*[1] One crystal was used to solve the *Kp*HdaH (1b) WT structure by molecular replacement.
*[2] One crystal was used to solve the structure of *Kp*HdaH (1b) H144A by molecular replacement.
a: $R_{merge} = \Sigma_{hkl} \Sigma^{n}_{i=1} |I_i(hkl) - \bar{I}(hkl)| / \Sigma_{hkl} \Sigma^{n}_{i=1} I_i(hkl)$ where $I_i(hkl)$ is the measured intensity of the reflection i at position hkl and $\bar{I}(hkl)$ is the average intensity of the reflection(s) at position hkl[19].
b: $R_{work} = \Sigma |F_o - F_c| / \Sigma F_o$ where F_o and F_c are the observed and calculated structure factor amplitudes. R_{free} is calculated similarly to R_{work} using random 5% of working set of reflections[20].
c: MolProbity[21].

Supplementary Table 6: Data collection and refinement statistics (molecular replacement) for the hydroxamate inhibitor complexes *Kp*HdaH (1b)•SAHA and *Kp*HdaH (1b)•TSA.

	*Kp*HdaH (1b)•SAHA (PDB: 9GN6 [https://doi.org/10.2210/pdb9GN6/pdb])	*Kp*HdaH (1b)•TSA (PDB: 9GN7 [https://doi.org/10.2210/pdb9GN7/pdb])
Data collection		
Space group	I 2 3	I 2 3
Cell dimensions		
a, *b*, *c* (Å)	146.206 146.206 146.206	146.57 146.57 146.57
α, β, γ (°)	90.0 90.0 90.0	90.0 90.0 90.0
Resolution (Å)	42.21 - 1.95 (2.02 - 1.95) *[1]	46.35 - 2.18 (2.258 - 2.18) *[2]
R_{merge} (%)[a]	11.63 (37.52)	2.781 (28.25)
$I / \sigma I$	22.62 (1.25)	12.16 (2.58)
Completeness (%)	99.97 (100.00)	99.97 (99.96)
Redundancy	39.5 (41.2)	2.0 (2.0)
Refinement		
Resolution (Å)	1.95	2.18
No. reflections	37871 (2629)	27454 (2367)
R_{work} / R_{free} [b]	0.1373 (0.2950)/ 0.1756 (0.3161)	0.1478 (0.2210)/ 0.1862 (0.2390)
No. atoms	3081	2985
Protein	2827	2822
Ligand/ion	22	44
Water	232	119
No. protein residues	369	369
Ramachandran Plot (%)[c]		
Most favored	95.11	94.29
Additionallly allowed	4.35	5.16
Disfavored	0.54	0.54
Clashscore	2.49	2.65
B-factors	48.60	43.95
Protein	48.38	43.23
Ligand/ion	53.06	59.37
Water	56.12	41.49
R.m.s. deviations		
Bond lengths (Å)	0.012	0.014
Bond angles (°)	1.94	2.24

*[1] One crystal was used to solve the *Kp*HdaH (1b)•SAHA structure by molecular replacement.
*[2] One crystal was used to solve the *Kp*HdaH (1b)•TSA structure by molecular replacement.
a: $R_{merge} = \Sigma_{hkl} \Sigma^{n}_{i=1} |I_i (hkl) - \bar{I} (hkl)| / \Sigma_{hkl} \Sigma^{n}_{i=1} I_i (hkl)$ where I_i (hkl) is the measured intensity of the reflection i at position hkl and $\bar{I}$ (hkl) is the average intensity of the reflection(s) at position hkl[19].
b: $R_{work} = \Sigma |F_o - F_c| / \Sigma F_o$ where F_o and F_c are the observed and calculated structure factor amplitudes. R_{free} is calculated similarly to R_{work} using random 5% of working set of reflections[20].
c: MolProbity[21].

Supplementary Table 7: Data collection and refinement statistics (molecular replacement) for the *Rw*DmhA (1b) octanoic acid structure and for the hydroxamate inhibitor complex *Rw*DmhA (1b)•SAHA.

	*Rw*DmhA (PDB: 9GKW [https://doi.org/10.2210/pdb9GKW/pdb])	*Rw*DmhA•SAHA (PDB: 9GKX [https://doi.org/10.2210/pdb9GKX/pdb])
Data collection		
Space group	C 1 2 1	C 1 2 1
Cell dimensions		
a, *b*, *c* (Å)	131.62 144.03 92.96	132.12 144.96 92.66
α, β, γ (°)	90.0 92.1 90.0	90.0 91.81 90.0
Resolution (Å)	54.64 - 2.1 (2.12 - 2.1) *[1]	46.31 - 1.75 (1.79 - 1.75) *[2]
R_{merge} (%)[a]	20.26 (97.36)	19.51 (146.3)
$I / \sigma I$	7.26 (1.57)	4.90 (0.64)
Completeness (%)	84.54 (85.80)	99.70 (99.05)
Redundancy	4.6 (4.3)	3.5 (3.3)
Refinement		
Resolution (Å)	2.1	1.75
No. reflections	393612 (18427)	174373 (11513)
R_{work} / R_{free} [b]	0.1719 (0.239)/ 0.2173 (0.273)	0.1626 (0.3732)/ 0.1903 (0.4108)
No. atoms	12389	13142
Protein	11187	11200
Ligand/ion	102	162
Water	1100	1780
No. protein residues	1469	1464
Ramachandran Plot (%)[c]		
Most favored	97.40	97.25
Additionallly allowed	2.40	2.75
Disfavored	0.21	0.00
Clashscore	7.45	3.43
B-factors	20.69	21.94
Protein	19.75	19.92
Ligand/ion	52.53	28.69
Water	27.34	34.03
R.m.s. deviations		
Bond lengths (Å)	0.027	0.007
Bond angles (°)	2.21	0.85

*[1] One crystal was used to solve the *Rw*DmhA structure by molecular replacement.
*[2] One crystal was used to solve the *Rw*DmhA•SAHA structure by molecular replacement.
a: $R_{merge} = \Sigma_{hkl} \Sigma^{n}_{i=1} |I_i (hkl) - \bar{I} (hkl)| / \Sigma_{hkl} \Sigma^{n}_{i=1} I_i (hkl)$ where $I_i (hkl)$ is the measured intensity of the reflection i at position hkl and $\bar{I} (hkl)$ is the average intensity of the reflection(s) at position hkl[19].
b: $R_{work} = \Sigma |F_o - F_c| / \Sigma F_o$ where F_o and F_c are the observed and calculated structure factor amplitudes. R_{free} is calculated similarly to R_{work} using random 5% of working set of reflections[20].
c: MolProbity[21].

Supplementary Table 8: Data collection and refinement statistics (molecular replacement) for the apo-structure of *Vc*HdaH (5b) and for the hydroxamate inhibitor complex *Vc*HdaH (5b)•SAHA.

	*Vc*HdaH (5b) (PDB: 9GKY [https://doi.org/10.2210/pdb9GKY/pdb])	*Vc*HdaH (5b)•SAHA (PDB: 9GKV [https://doi.org/10.2210/pdb9GKV/pdb])
Data collection		
Space group	P 1 21 1	P 21 21 21
Cell dimensions		
a, *b*, *c* (Å)	51.11 51.15 108.82	50.92 81.53 133.17
α, β, γ (°)	90.0 102.22 90.0	90.0 90.0 90.0
Resolution (Å)	26.39 - 1.129 (1.14 - 1.13) *[1]	40.45 - 1.643 (1.68 - 1.64) *[2]
R_{merge} (%)[a]	5.977 (188.8)	10.44 (180.9)
$I / \sigma I$	5.56 (0.36)	7.31 (0.23)
Completeness (%)	94.57 (84.52)	77.17 (11.89)
Redundancy	1.9 (1.9)	5.4 (1.6)
Refinement		
Resolution (Å)	1.13	1.64
No. reflections	194387 (5740)	52756 (531)
R_{work} / R_{free} [b]	0.1576 (0.357)/ 0.1789 (0.364)	0.2081 (0.563)/ 0.2130 (0.558)
No. atoms	6006	5335
Protein	5148	4935
Ligand/ion	62	37
Water	796	363
No. protein residues	621	618
Ramachandran Plot (%)[c]		
Most favored	98.06	97.39
Additionallly allowed	1.94	2.61
Disfavored	0.00	0.00
Clashscore	5.86	4.57
B-factors	14.14	29.85
Protein	11.71	29.12
Ligand/ion	16.72	48.57
Water	29.67	37.96
R.m.s. deviations		
Bond lengths (Å)	0.013	0.014
Bond angles (°)	1.66	2.12

*[1] One crystal was used to solve the *Vc*HdaH (5b) structure by molecular replacement.
*[2] One crystal was used to solve the structure of *Vc*HdaH (5b)•SAHA by molecular replacement.
a: $R_{merge} = \Sigma_{hkl} \ \Sigma^{n}_{i=1} |I_i (hkl) - \bar{I} (hkl)| / \Sigma_{hkl} \ \Sigma^{n}_{i=1} I_i (hkl)$ where I_i (hkl) is the measured intensity of the reflection i at position hkl and $\bar{I}$ (hkl) is the average intensity of the reflection(s) at position hkl[19].
b: $R_{work} = \Sigma |F_o - F_c| / \Sigma F_o$ where F_o and F_c are the observed and calculated structure factor amplitudes. R_{free} is calculated similarly to R_{work} using random 5% of working set of reflections[20].
c: MolProbity[21].

Supplementary Table 9: Data collection and refinement statistics (molecular replacement) for the *Rs*PrpH (1b) structure and for the *Ps*ApaH (4) structure.

	*Rs*PrpH (1b) (PDB: 9GKU [https://doi.org/10.2210/pdb9GKU/pdb])	*Ps*ApaH$_{1-342}$ (4) (PDB: 9GKZ [https://doi.org/10.2210/pdb9GKZ/pdb])
Data collection		
Space group	P 1	P 21 21 2
Cell dimensions		
a, *b*, *c* (Å)	103.861 103.698 104.499	147.342 48.858 82.249
α, β, γ (°)	113.6 93 120.61	90.0 90.0 90.0
Resolution (Å)	44.56 - 1.481 (1.5 - 1.48) *1	73.67 - 2.716 (2.89 - 2.72) *2
R_{merge} (%)[a]	7.0 (137.3)	8.563 (49.85)
$I / \sigma I$	9.1 (1.1)	7.04 (1.22)
Completeness (%)	90.8 (64.6)	99.90 (100.00)
Redundancy	1.8 (1.5)	2.0 (2.0)
Refinement		
Resolution (Å)	1.48	2.72
No. reflections	493363 (12890)	16666 (2708)
R_{work} / R_{free} [b]	0.1528 (0.335)/ 0.1834 (0.334)	0.1556 (0.2166)/ 0.2324 (0.2976)
No. atoms	24997	5145
Protein	22739	4986
Ligand/ion	188	30
Water	2070	129
No. protein residues	2960	642
Ramachandran Plot (%)[c]		
Most favored	96.60	94.64
Additionallly allowed	3.12	5.36
Disfavored	0.27	0.00
Clashscore	5.17	7.92
B-factors	28.54	35.79
Protein	27.69	35.82
Ligand/ion	36.43	44.62
Water	37.26	32.87
R.m.s. deviations		
Bond lengths (Å)	0.013	0.008
Bond angles (°)	1.70	0.94

*1 One crystal was used to solve the *Rs*PrpH (1b) structure by molecular replacement.
*2 One crystal was used to solve the structure of *Ps*ApaH (4) by molecular replacement.

a: $R_{merge} = \Sigma_{hkl} \ \Sigma^{n}_{i=1} |I_i (hkl) - \bar{I} (hkl)| / \Sigma_{hkl} \ \Sigma^{n}_{i=1} I_i (hkl)$ where $I_i (hkl)$ is the measured intensity of the reflection i at position hkl and $\bar{I} (hkl)$ is the average intensity of the reflection(s) at position hkl[19].

b: $R_{work} = \Sigma |F_o - F_c| / \Sigma F_o$ where F_o and F_c are the observed and calculated structure factor amplitudes. R_{free} is calculated similarly to R_{work} using random 5% of working set of reflections[20].

c: MolProbity[21].

Supplementary Table 10: Data collection and refinement statistics (molecular replacement) for the structure *Lc*ApaH and for the *Lp*ApaH structure, both enzymes classified into cluster 3.

	*Lc*ApaH (PDB: 9GL0 [https://doi.org/10.2210/pdb 9GL0/pdb])	*Lp*ApaH (PDB: 9GL1 [https://doi.org/10.2210/pdb 9GL1/pdb])
Data collection		
Space group	P 32 2 1	P 41 2 2
Cell dimensions		
a, *b*, *c* (Å)	113.564 113.564 90.426	109.121 109.121 400.511
α, β, γ (°)	90.0 90.0 120.0	90.0 90.0 90.0
Resolution (Å)	43.2 - 2.4 (2.43 - 2.4) *[1]	45.96 - 2.701 (2.73 - 2.7)
R_{merge} (%)[a]	9.656 (137.7)	18.5 (365.9)
$I / \sigma I$	6.22 (0.45)	4.85 (0.27)
Completeness (%)	98.59 (97.53)	94.60 (27.26)
Redundancy	8.2 (1.4)	10.3 (1.1)
Refinement		
Resolution (Å)	2.4	2.7
No. reflections	26336 (867)	64002 (607)
R_{work} / R_{free} [b]	0.2373 (0.5791)/ 0.2633 (0.5272)	0.2403 (0.6215)/ 0.3160 (0.6736)
No. atoms	3141	12721
Protein	3130	12688
Ligand/ion	3	15
Water	8	18
No. protein residues	389	1568
Ramachandran Plot (%)[c]		
Most favored	87.86	87.88
Additionallly allowed	10.86	9.87
Disfavored	1.29	2.24
Clashscore	21.44	17.53
B-factors	123.57	113.81
Protein	123.60	113.83
Ligand/ion	112.79	106.77
Water	114.03	103.69
R.m.s. deviations		
Bond lengths (Å)	0.010	0.009
Bond angles (°)	1.14	1.08

*[1] One crystal was used to solve the *Lc*ApaH structure by molecular replacement.
*[2] One crystal was used to solve the *Lp*ApaH structure by molecular replacement.

a: $R_{merge} = \Sigma_{hkl} \Sigma^{n}_{i=1} |I_i(hkl) - \bar{I}(hkl)| / \Sigma_{hkl} \Sigma^{n}_{i=1} I_i(hkl)$ where $I_i(hkl)$ is the measured intensity of the reflection i at position hkl and $\bar{I}(hkl)$ is the average intensity of the reflection(s) at position hkl[19].

b: $R_{work} = \Sigma |F_o - F_c| / \Sigma F_o$ where F_o and F_c are the observed and calculated structure factor amplitudes. R_{free} is calculated similarly to R_{work} using random 5% of working set of reflections[20].

c: MolProbity[21].

Supplementary References

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