

SUPPLEMENTARY INFORMATION

Distribution and diversity of classical deacylases in bacteria

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Supplementary Figure 1-28

Supplementary Table 1-10

a

β1 →

1a_Arabidopsis_thaliana_HDA14

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1
1a_Arabidopsis_thaliana_HDA14 MSMALI.....VRP
1b_Klebsiella_pneumoniae_HdaH MKRKTG.....FFFDE
1c_Shinella_sp_DD12_DAC MTTCFLF.....ENP
1d_Neisseria_zoodegmatidis_DAC MNKIVRYM.....RLQLRRMLGRQ...A.RAAWISHP
1e_Stentor_coeruleus_HdaH MSKGINEI.EVDIHEICCLGRHSDLEEIL.....NSIDFYADIMLERDEEGFIPYH..LSLIYCRHKCVDLILSL
1f_S_cerevisiae_HOS3 MSSKHSDP.L.ER.....FYKQFQA...FVQNNPNVISAA..RAAAQIPE.....SAKAVVVLSP
1g_Homo_sapiens_HDAC4 MSSQSHPDGL.SG.....RDQPVELLNPARVNHPSTVDVATAL.....PLQVAP
2a_Homo_sapiens_HDAC2 MAYSQGGG.KK.....KVCYYYDG
2b_S_cerevisiae_HOS1 MSKLVIST.S.....IFQ
2c_Bacillus_subtilis_AcuC MRDSV.....FIYSP
2d_Chromatiales_bacterium_Acu MFVAK.....DE
3_Legionella_cherrii_ApaH MRHKYFF.....KE
4_Pseudomonas_sp_M30-35_ApaH MLTIYS.....D
5a_Acinetobacter_wuhouensis_H MLNVCF.....SP
5b_Vibrio_cholerae_HdaH MIPLIYHP.IY.....
5c_Moraxella_bovis_HDAC MFFKIAHC.HE.....
5d_Turneriella_parva_HdaH MSRKSAEL.....VYSP
5e_Nannocystis_exedens_AcuC MTTGEKIF.R.QL.....RRRANQMFAFLRP...P.PAAFYVGP
5f_Homo_sapiens_HDAC11 MLHTTQLY.Q.H.....VPE...T.RWPIVYSP
5g_Bacteriovorax_stolpii_AcuC MFFYHPDC.DLR.....

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1a_Arabidopsis_thaliana_HDA14

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10 20
1a_Arabidopsis_thaliana_HDA14 ..F.....F...VPGSAGISGSRN.....
1b_Klebsiella_pneumoniae_HdaH ..R.....C...FWHSTG.LHAVTLPVGG
1c_Shinella_sp_DD12_DAC ..I.....F...LE.....
1d_Neisseria_zoodegmatidis_DAC ..L.....F...LHYGC.....
1e_Stentor_coeruleus_HdaH SIDFQVPYQGFMPHILAMACAGFPPEC...HEDL...I...Q
1f_S_cerevisiae_HOS3 ..Y.....S.....
1g_Homo_sapiens_HDAC4 ..S.....A...VPMDLRLDHQFSLPVAEPALREQQL.....QOELLALKKQKQQRQILIAEFQRQH
2a_Homo_sapiens_HDAC2 ..DI.....GNYYYGGG...HPMK...P
2b_S_cerevisiae_HOS1 ..S.....QVA..D.L...LPCN...N
2c_Bacillus_subtilis_AcuC ..SY.....QTYMFHQE...HPFN...Q
2d_Chromatiales_bacterium_Acu ..RI.....AAYGFGE...HPFG...L
3_Legionella_cherrii_ApaH ..K.....VSPES...HKEK...NQECLIQIPSA
4_Pseudomonas_sp_M30-35_ApaH ..D.....H...HLHHG...SCELID
5a_Acinetobacter_wuhouensis_H ..R.....YFAQT...HTNS...ME
5b_Vibrio_cholerae_HdaH ..F.....SQLDLPVG...HRYP...I
5c_Moraxella_bovis_HDAC ..F.....ALPLPDG...HRFP...M
5d_Turneriella_parva_HdaH ..IY.....DLS..DYT...HVIS...A
5e_Nannocystis_exedens_AcuC ..H.....Y...ECSW
5f_Homo_sapiens_HDAC11 ..R.....Y...NITFM
5g_Bacteriovorax_stolpii_AcuC ..FSEYGIE...IPIV...D

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1a_Arabidopsis_thaliana_HDA14

```

1a_Arabidopsis_thaliana_HDA14 .....
1b_Klebsiella_pneumoniae_HdaH .....
1c_Shinella_sp_DD12_DAC .....
1d_Neisseria_zoodegmatidis_DAC .....
1e_Stentor_coeruleus_HdaH .....TVNCL.LKHKQDVTSRDR
1f_S_cerevisiae_HOS3 .....
1g_Homo_sapiens_HDAC4 EQLSRQHEAQLHEHIKQQQEMLAMKHQQELLEHQKLERHRQEQELEKQHREQKLQQLKNKEKKGESAVASTEVKMKL
2a_Homo_sapiens_HDAC2 .....
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 .....
5e_Nannocystis_exedens_AcuC .....
5f_Homo_sapiens_HDAC11 .....
5g_Bacteriovorax_stolpii_AcuC .....

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1a_Arabidopsis_thaliana_HDA14

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30 40
1a_Arabidopsis_thaliana_HDA14 .....ICKKNQWRKYLKPSGSSINC.....SFS
1b_Klebsiella_pneumoniae_HdaH .....WVQ
1c_Shinella_sp_DD12_DAC .....
1d_Neisseria_zoodegmatidis_DAC .....
1e_Stentor_coeruleus_HdaH .....LGRT.....ALH.IVCAYGLADLIPILVQAGVKPELKD
1f_S_cerevisiae_HOS3 .....
1g_Homo_sapiens_HDAC4 QEFVLNKKKALAHNRNLNHCISSDPRYWGKTQHSSLDQSSPPQSGVSTSYNHPVLGMYDAKDDFPLRK.TASEPNLK
2a_Homo_sapiens_HDAC2 .....
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 .....
5e_Nannocystis_exedens_AcuC .....
5f_Homo_sapiens_HDAC11 .....
5g_Bacteriovorax_stolpii_AcuC .....

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1a_Arabidopsis_thaliana_HDA14

1a_Arabidopsis_thaliana_HDA14
1b_Klebsiella_pneumoniae_HdaH
1c_Shinella_sp_DD12_DAC
1d_Neisseria_zoodegmatis_DAC
1e_Stentor_coeruleus_HdaH FSGKMPIHYA IENYQAEFCFKQLLOEMG.....SDMFFCT
1f_S._cerevisiae_HOS3
1g_Homo_sapiens_HDAC4LRS.....RLKQKVAERRSSPLLRKDGPPVVTALKKRPDLVTDTSACSSAPGSGFPSSPNSSG.SVS
2a_Homo_sapiens_HDAC2
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
5e_Nannocystis_exedens_AcuC
5f_Homo_sapiens_HDAC11
5g_Bacteriovorax_stolpii_AcuC

1a_Arabidopsis_thaliana_HDA14

1a_Arabidopsis_thaliana_HDA14
1b_Klebsiella_pneumoniae_HdaHPPA.....
1c_Shinella_sp_DD12_DAC
1d_Neisseria_zoodegmatis_DAC
1e_Stentor_coeruleus_HdaH DN.....
1f_S._cerevisiae_HOS3
1g_Homo_sapiens_HDAC4 AENGIAPAVPSIPAETS LAHRLVAREGSAAPLPLYTSPSLPNITLGLPATGPSAGTAGQQDAERLTLPALQQRSLFPP
2a_Homo_sapiens_HDAC2
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
5e_Nannocystis_exedens_AcuC
5f_Homo_sapiens_HDAC11
5g_Bacteriovorax_stolpii_AcuC

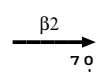
1a_Arabidopsis_thaliana_HDA14

1a_Arabidopsis_thaliana_HDA14
1b_Klebsiella_pneumoniae_HdaH
1c_Shinella_sp_DD12_DAC
1d_Neisseria_zoodegmatis_DAC
1e_Stentor_coeruleus_HdaHRGDKPIHTSVRAGSWEC.....
1f_S._cerevisiae_HOS3
1g_Homo_sapiens_HDAC4 GTHLTPYLSTSPLE RDGGAHSP LQH MV LLEQPPAQAPLVTGLGALPLH.....AQSLVGADRVSPSIHKLRQHR
2a_Homo_sapiens_HDAC2
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
5e_Nannocystis_exedens_AcuC
5f_Homo_sapiens_HDAC11
5g_Bacteriovorax_stolpii_AcuC



1a_Arabidopsis_thaliana_HDA14

1a_Arabidopsis_thaliana_HDA14
1b_Klebsiella_pneumoniae_HdaHLIYSVSA.....
1c_Shinella_sp_DD12_DAC
1d_Neisseria_zoodegmatis_DAC
1e_Stentor_coeruleus_HdaH
1f_S._cerevisiae_HOS3FNILITFGSEEML.....
1g_Homo_sapiens_HDAC4 PLGRTQSAPLPQNAQALQHLVLIQQHQHFLEKHKQFQQQQQMQMNIIPKPSAPARQFESHPEETEELREHQALLDE
2a_Homo_sapiens_HDAC2
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
5e_Nannocystis_exedens_AcuC
5f_Homo_sapiens_HDAC11
5g_Bacteriovorax_stolpii_AcuC



1a_Arabidopsis_thaliana_HDA14

1a_Arabidopsis_thaliana_HDA14
 1b_Klebsiella_pneumoniae_HdaH
 1c_Shinella_sp_DD12_DAC
 1d_Neisseria_zoodegmatis_DAC
 1e_Stentor_coeruleus_HdaH
 1f_S_cerevisiae_HOS3
 1g_Homo_sapiens_HDAC4
 2a_Homo_sapiens_HDAC2
 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
 5e_Nannocystis_exedens_AcuC
 5f_Homo_sapiens_HDAC11
 5g_Bacteriovorax_stolpii_AcuC

1a_Arabidopsis_thaliana_HDA14

T . T 00000
 80 90

1a_Arabidopsis_thaliana_HDA14
 1b_Klebsiella_pneumoniae_HdaH
 1c_Shinella_sp_DD12_DAC
 1d_Neisseria_zoodegmatis_DAC
 1e_Stentor_coeruleus_HdaH
 1f_S_cerevisiae_HOS3
 1g_Homo_sapiens_HDAC4
 2a_Homo_sapiens_HDAC2
 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
 5e_Nannocystis_exedens_AcuC
 5f_Homo_sapiens_HDAC11
 5g_Bacteriovorax_stolpii_AcuC

1a_Arabidopsis_thaliana_HDA14

α2 000000

1a_Arabidopsis_thaliana_HDA14
 1b_Klebsiella_pneumoniae_HdaH
 1c_Shinella_sp_DD12_DAC
 1d_Neisseria_zoodegmatis_DAC
 1e_Stentor_coeruleus_HdaH
 1f_S_cerevisiae_HOS3
 1g_Homo_sapiens_HDAC4
 2a_Homo_sapiens_HDAC2
 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
 5e_Nannocystis_exedens_AcuC
 5f_Homo_sapiens_HDAC11
 5g_Bacteriovorax_stolpii_AcuC

1a_Arabidopsis_thaliana_HDA14

α3 η1 β3 α4
 100 110 120

1a_Arabidopsis_thaliana_HDA14
 1b_Klebsiella_pneumoniae_HdaH
 1c_Shinella_sp_DD12_DAC
 1d_Neisseria_zoodegmatis_DAC
 1e_Stentor_coeruleus_HdaH
 1f_S_cerevisiae_HOS3
 1g_Homo_sapiens_HDAC4
 2a_Homo_sapiens_HDAC2
 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
 5e_Nannocystis_exedens_AcuC
 5f_Homo_sapiens_HDAC11
 5g_Bacteriovorax_stolpii_AcuC

1a_Arabidopsis_thaliana_HDA14 00000000.00.....00.00.....0 η2

130 140

1a_Arabidopsis_thaliana_HDA14 VHDKAYVFGLE.KA.....MD.EA.....S
 1b_Klebsiella_pneumoniae_HdaH IHPDSYLERFK.AI.....SD.NG.....G
 1c_Shinella_sp_DD12_DAC AHPERHLLTAVM.SA.....MP.E.....E
 1d_Neisseria_zoodegmatis_DAC IHPRKYLRFLE.SV.....QP.QP.....G
 1e_Stentor_coeruleus_HdaH VHEYSYILGLK.KA.....INKIP.....E
 1f_S_cerevisiae_HOS3 VHGSSWPAELI.EL.....CQ.MADAKLLKG.....
 1g_Homo_sapiens_HDAC4 VHESEHTLLYGTNPLNRQKLD SKKLLGSLASVFRLP.C.....G
 2a_Homo_sapiens_HDAC2 YHSDEYIKFLR.SI.....RP.D.....NM
 2b_S_cerevisiae_HOS1 FHSKSYIDYLI.NG.....RF.NK.....MM.AQDVNNP MVESKWSLSELADNWNKIDY
 2c_Bacillus_subtilis_AcuC VHTDDYIQAVK.LA.....GA.G.....KL
 2d_Chromatiales_bacterium_Acu FHTPRYLDFVA.DT.....CS.GE.....GM
 3_Legionella_cherrii_ApaH VHTSEYLQII.RD.....CI.QA.....Q
 4_Pseudomonas_sp_M30-35_ApaH IHTAEYLNFFE.GA.....WA.RW.....QEODGTG DLLPYTWPARTLSQ
 5a_Acinetobacter_wuhouensis_H LHSPKYVEAFM.TG.....QP.NK.....
 5b_Vibrio_cholerae_HdaH LHPDPYVQALL.EG.....RL.PA.....A
 5c_Moraxella_bovis_HDAC THTADYWHALD.NL.....TL.SP.....K
 5d_Turneriella_parva_HdaH VHTERYLKDFL.GA.....RL.TE.....Q.TQR
 5e_Nannocystis_exedens_AcuC VEDDAYLDACQ.RP.E.....V.M.....
 5f_Homo_sapiens_HDAC11 VHTRRYLNLK.WS.F.....A.VA.....T
 5g_Bacteriovorax_stolpii_AcuC VHNKDEIHRLL.GT.....AG.ER.....Q

1a_Arabidopsis_thaliana_HDA140 β4 → TT TT → β5

150

1a_Arabidopsis_thaliana_HDA14DS.....GLI..FIEGSGPTYAT.....
 1b_Klebsiella_pneumoniae_HdaHG...ML..GKEAPLG.....
 1c_Shinella_sp_DD12_DACDIA..QV..EADTYAS.....
 1d_Neisseria_zoodegmatis_DACK.IY..RI..DDTVMG.....
 1e_Stentor_coeruleus_HdaHL...S.FPQ.KY..DITLIT.....
 1f_S_cerevisiae_HOS3EI.....EVPDTWN..SGDIYLS.....
 1g_Homo_sapiens_HDAC4CV..GV..DSDTIWNE.....
 2a_Homo_sapiens_HDAC2SEYSKQ.....MQRFN..V..GEDCPVF.....
 2b_S_cerevisiae_HOS1 NPSQDLQRFRTTRENLYNYLNSQALENNMD.C.INNSEVPTNDKPTDYILNSETKQY.NL.EGDCPIF.....
 2c_Bacillus_subtilis_AcuCP.AEE.....GESY.G.LD.GGDTPAF.....
 2d_Chromatiales_bacterium_AcuA.....KGWK..QL..NSDILIT.....
 3_Legionella_cherrii_ApaHRL.....PTSLHGQL.....GYY..SF..DAGAPIT.....
 4_Pseudomonas_sp_M30-35_ApaHK.....M.....R.RIGFPWS.....
 5a_Acinetobacter_wuhouensis_HD.....C.....R..KIGLPMS.....
 5b_Vibrio_cholerae_HdaHK.....M.....R..RIGFPWS.....
 5c_Moraxella_bovis_HDACD.....C.....R..KIGLPMS.....
 5d_Turneriella_parva_HdaHS.....DILG.GL.AVSE.VDT.....
 5e_Nannocystis_exedens_AcuCIT.....ERG.WAINVGGGF.HHCS.....SDRGGFCVA
 5f_Homo_sapiens_HDAC11GE..I.....Y.....Q..CYELVNDQGGKFER
 5g_Bacteriovorax_stolpii_AcuC

1a_Arabidopsis_thaliana_HDA14α6 β6 TT TT TT

160 170 180 190 200 210

1a_Arabidopsis_thaliana_HDA14STTFQDSLIAAGAGMALVDSVIAASRNSVDPPIG.FAL.IRPPGHHAV.....PKGPMGFCV
 1b_Klebsiella_pneumoniae_HdaHPGSYEIACLSAGLACA AAVEAVLKGE.....LDNA.YSL.SRPPGHHACL.....PDQSMGFCF
 1c_Shinella_sp_DD12_DACPATLQAALGTGGATAAVDAVFTGK.....ADNA.FVA.ARPPGHHAE.....RDKAMGFCF
 1d_Neisseria_zoodegmatis_DACSGALEAARYAAGAVAAVDMVMKGE.....AFHA.FCA.IRPPGHHAK.....SDQAGGFCFL
 1e_Stentor_coeruleus_HdaHKESYNAAALIAANCVIEAIDEVVOGK.....FKNA.FCVI.RPPGHHVGGPFGAVTA EEDPNSKSTGFCFL
 1f_S_cerevisiae_HOS3SKTIKALQGTIGAIETGVDSIFKGPSAEHISNRA.FVA.IRPPGHHCH.....YGTSPGFCFL
 1g_Homo_sapiens_HDAC4VHSAGAARLAVGCVVLELVFKVATGE.....LKNQ.FAV.VRPPGHHAE.....ESTPMGFCY
 2a_Homo_sapiens_HDAC2DGLFEPFCQLSTGGSVAGAVKLN.R.....QQT.D.MAVNWAGGLHHAK.....KSEASGFCY
 2b_S_cerevisiae_HOS1SYLPMYCCQVITGATLNLLDHLSP.....TERL.IGINWDGGRHHAF.....KQRASGFCY
 2c_Bacillus_subtilis_AcuCAGMHEAASLLVGGTLTAADWVMS.....GQAL.HAANLGGGLHHGF.....RGRASGFCI
 2d_Chromatiales_bacterium_AcuPGLYEASADIA GTTLALVDLVMG.....GRAR.RAF.TPIGGLHHAG.....RDHAA GFCV
 3_Legionella_cherrii_ApaHPGTFEVLK.....DISMTLF.....HSKK.VHFSFGLPTHHAF.....ADEGSGFCI
 4_Pseudomonas_sp_M30-35_ApaHAGTWAAVYSAAQVALTAQHEITNGA.....HSA.FAL.CRPPGHHAR.....SDVMSGFCY
 5a_Acinetobacter_wuhouensis_HEQLRDVLA INAGQALMATELAF.E.....H..G.IAANTAQGFHHAR.....PEYGGFCY
 5b_Vibrio_cholerae_HdaHKTLIERTLHSGVGTCLTVEQA.....LQSG.VAIHLSGGYHHAH.....ADFGSGFCFL
 5c_Moraxella_bovis_HDACKELIDRERLVV GATIECAKFA.....LNDG.ISLSTSGGTHHAF.....ADSGEGFCI
 5d_Turneriella_parva_HdaHERIVNAVCTAAGGTTLAAELAL.....KHG.VASNLSGGFHHAF.....ADHAE GFCF
 5e_Nannocystis_exedens_AcuCARILDQRAMTGGTLMATDIAL.....GTGKIAINLGGGFHHCH.....RQIGRGFCVA
 5f_Homo_sapiens_HDAC11RKVLRPLRTQTGGTIMAGKLV.....ERG.WAINVGGGFHHCS.....SDRGGFCVA
 5g_Bacteriovorax_stolpii_AcuC YNPKNQKRDWKELVETILMQVAMTYNSTKYA.....LKNQ.FSYHLGGGMHHS.....SFAGRGFCFL

1a_Arabidopsis_thaliana_HDA14α7 β7 α8

220 230 240 250

1a_Arabidopsis_thaliana_HDA14 FGNVAIAARHAQR.T..HGLKRIFIIDVHHGNGTNDAFTE.D
 1b_Klebsiella_pneumoniae_HdaH LANIPIAVERAKA.Q..LGLGKVAIIDVDVHHGNGTQHIYLO.R
 1c_Shinella_sp_DD12_DAC FNNAAIAARHAQK.A..HGAERVAIVDWDVHHGNGTQDIFED.D
 1d_Neisseria_zoodegmatis_DAC VNNVAVGVMAHIA.R..FRLERIAVVDVHHGNGTAEIFKD.D
 1e_Stentor_coeruleus_HdaH FNNIAIGAGYAKY.KYSKVIKVAIIDVHHGNGTEAMVRN..LIPNQVTHEITG
 1f_S_cerevisiae_HOS3 LNNHVAIEIYAYD.T..YNVTHVLDVLDLHHGNGTQDICWKRA GFKPEEPEPSSYDDFGKKFAEF.D
 1g_Homo_sapiens_HDAC4 FNSVAVAAKLLQQR..LSVSKILLVLDVHHGNGTQQA FYS.D
 2a_Homo_sapiens_HDAC2 VNDIVLAILLELLK...YHQRVLYIDIDHHGNGVEEAFYT..T
 2b_S_cerevisiae_HOS1 INDVLLIQRLRK.A..KLNKITVVDVLDLHHGNGVEKAFOY..S
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 3_Legionella_cherrii_ApaH LNKSAVLLKHMQR.N..TKPLKHIIVGTDVNRDNGLCDILMN..S
 4_Pseudomonas_sp_M30-35_ApaH LNNAAIAAQAFID.Q..GHKRVAILVDVHHGNGTQSIFY.S..Y
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 5b_Vibrio_cholerae_HdaH FNDLIAIAHFALS.L..PSVDKVIDSDVHHGNGTATLCAE..R
 5c_Moraxella_bovis_HDAC LNDICVASNVLLN.Q..GLAKRILLDLVDVHHGNGNASIMAD..N
 5d_Turneriella_parva_HdaH VNDTVLAI RALRK.T..RPGLKVAVIDLDVHHGNGTAKLLQD..D
 5e_Nannocystis_exedens_AcuC FHDVAIAIEAQR.L..GFHERILVVDLQHDGNGTRALFAD..D
 5f_Homo_sapiens_HDAC11 YADITLAIKFLFERV..EGISRATIIDLDAGHNGHERDFMD..D
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1a_Arabidopsis_thaliana_HDA14

390

1a_Arabidopsis_thaliana_HDA14
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1f_S_cerevisiae_HOS3
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
5e_Nannocystis_exedens_AcuC
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3_Legionella_cherrii_ApaH
4_Pseudomonas_sp_M30-35_ApaH
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5b_Vibrio_cholerae_HdaH
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5d_Turneriella_parva_HdaH
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1a_Arabidopsis_thaliana_HDA14

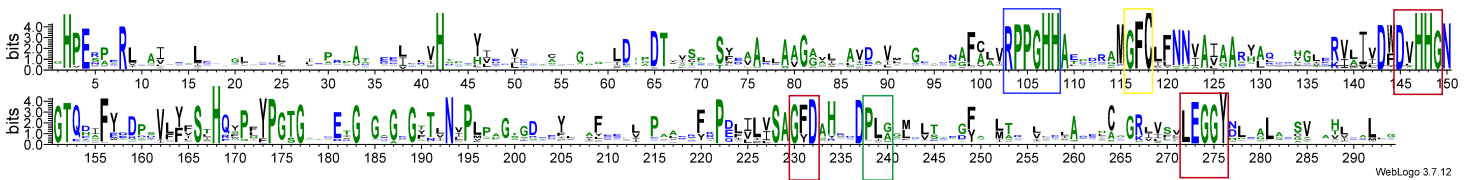
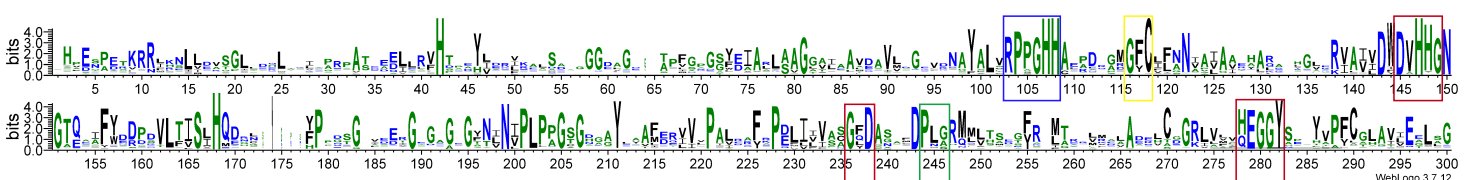
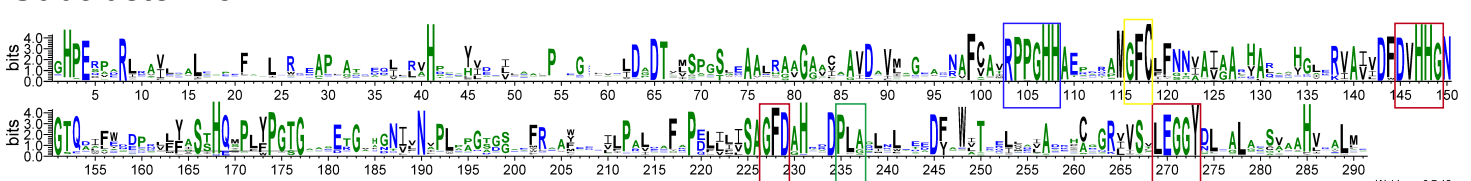
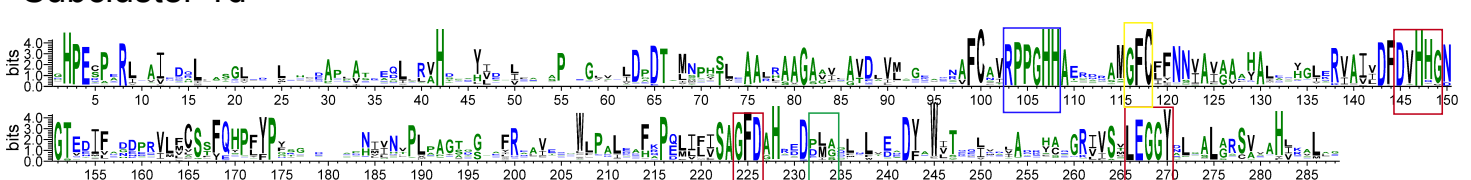
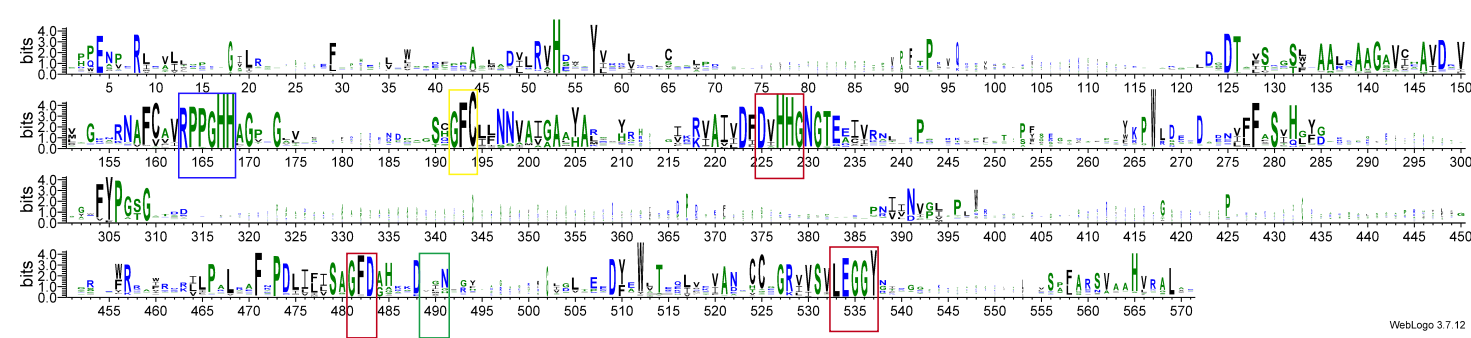
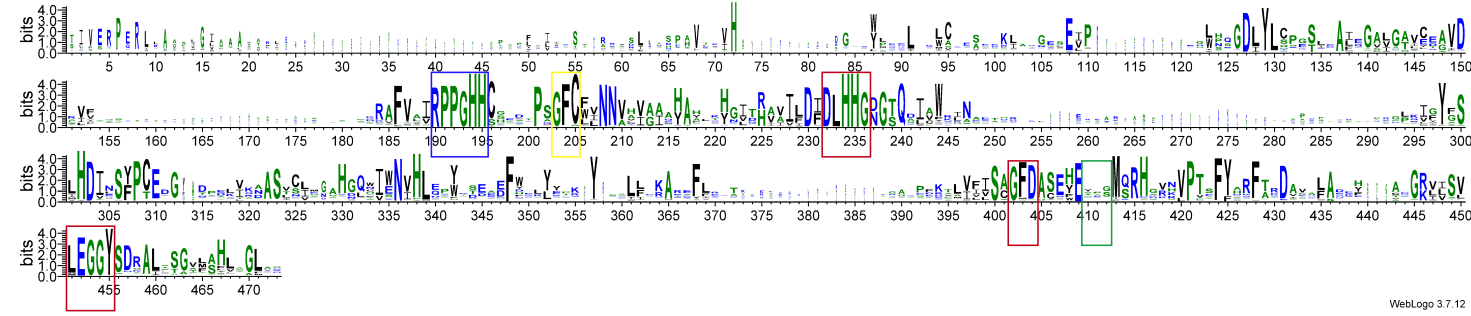
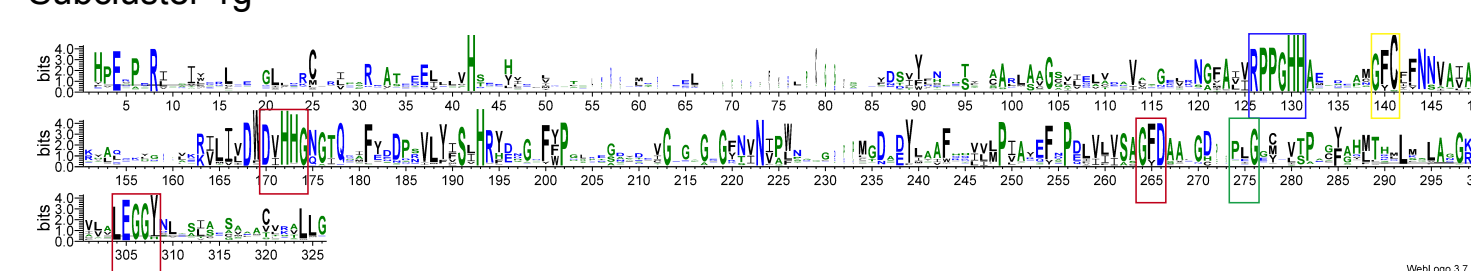
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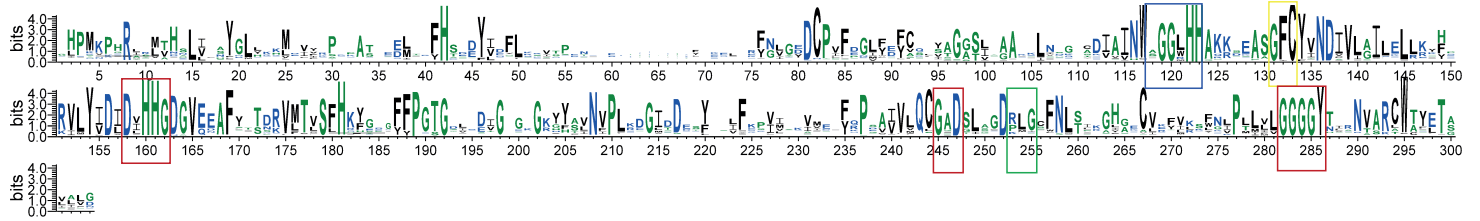
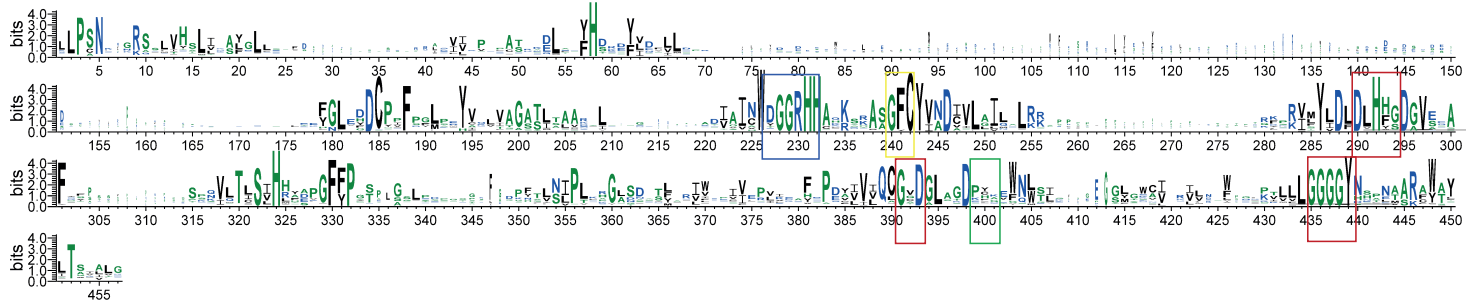
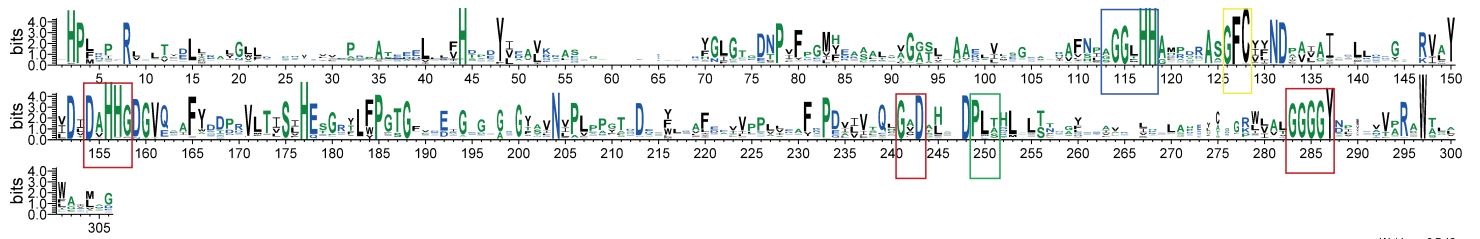
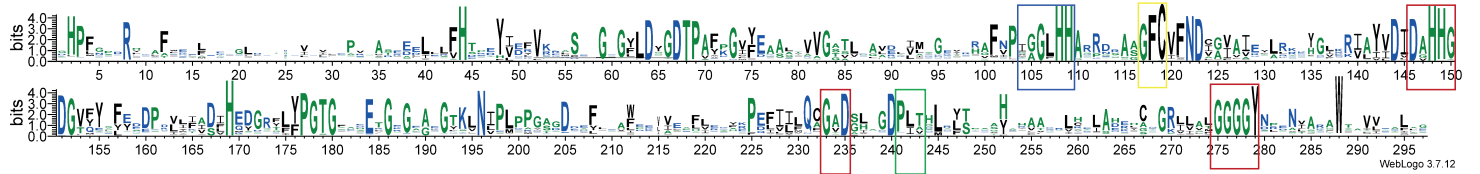
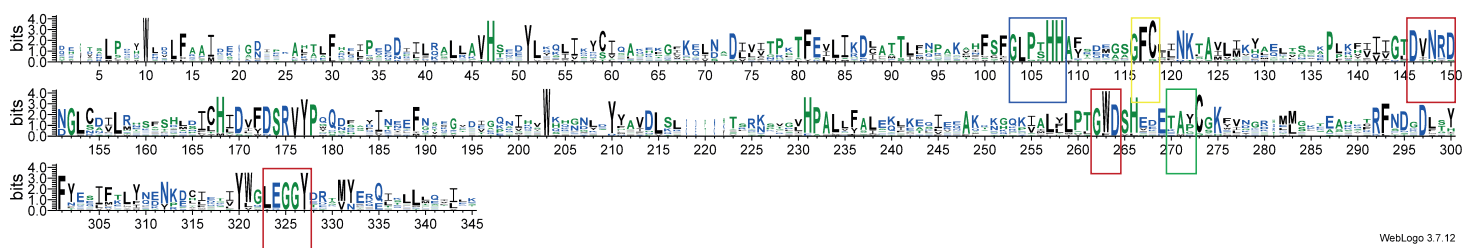
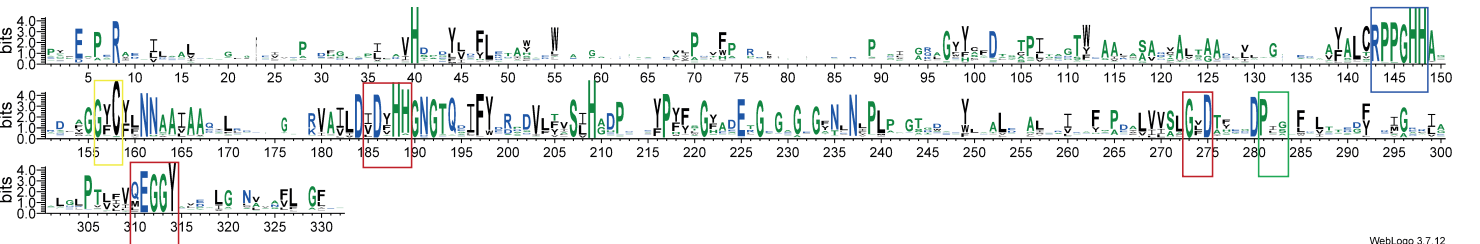
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1d_Neisseria_zoodegmatis_DAC
1e_Stentor_coeruleus_HdaH
1f_S_cerevisiae_HOS3
1g_Homo_sapiens_HDAC4
2a_Homo_sapiens_HDAC2
2b_S_cerevisiae_HOS1
2c_Bacillus_subtilis_AcuC
2d_Chromatiales_bacterium_Acu
3_Legionella_cherrii_ApaH
4_Pseudomonas_sp_M30-35_ApaH
5a_Acinetobacter_wuhouensis_H
5b_Vibrio_cholerae_HdaH
5c_Moraxella_bovis_HDAC
5d_Turneriella_parva_HdaH
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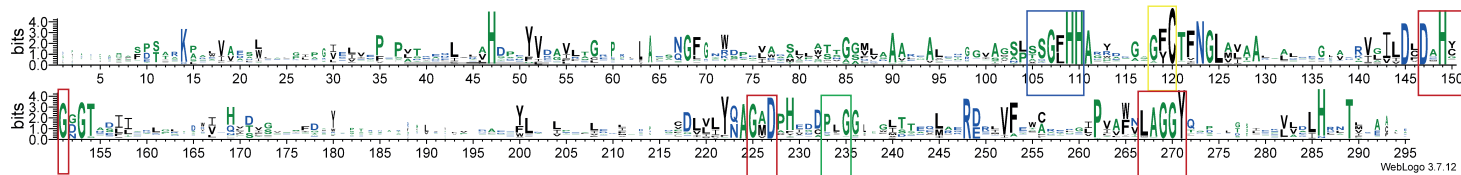
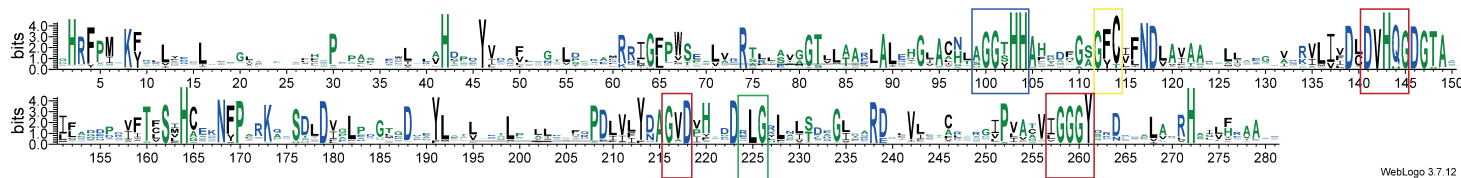
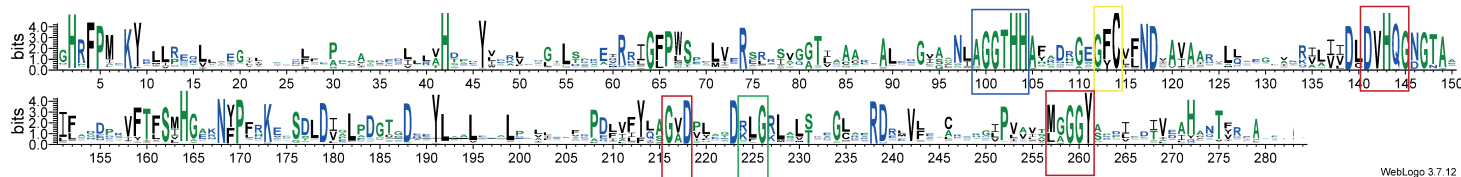
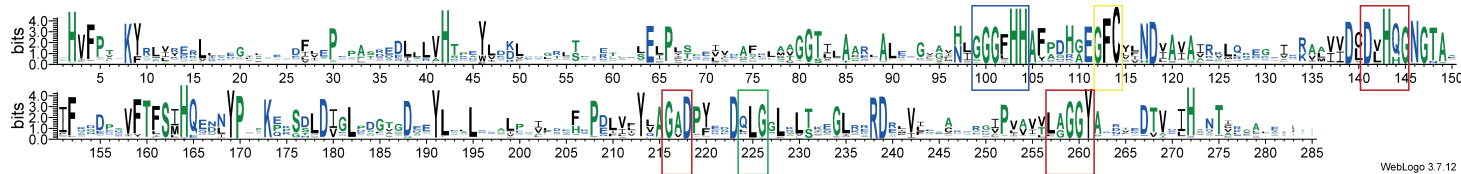
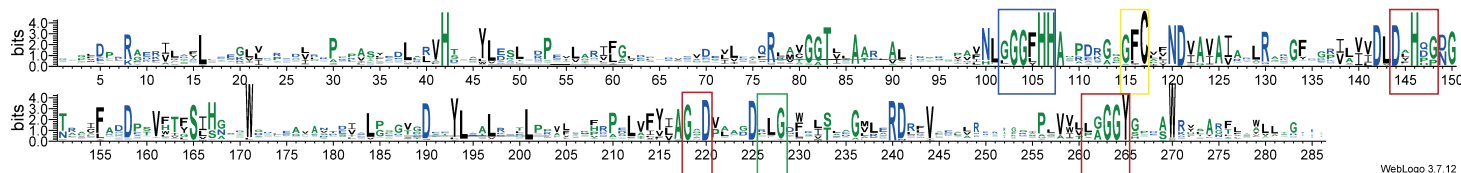
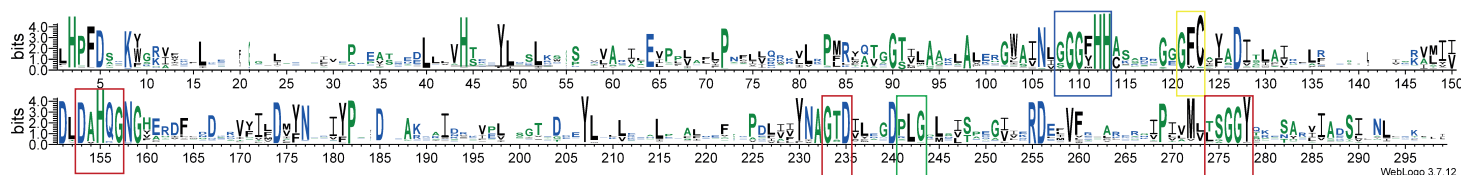
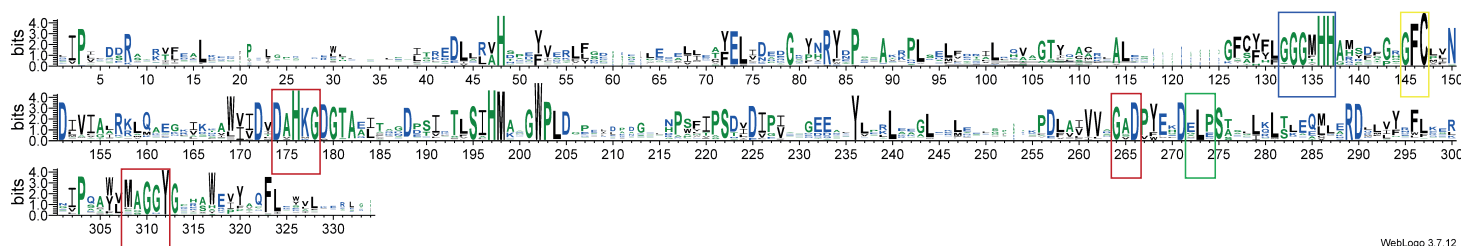
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1c_Shinella_sp_DD12_DAC
1d_Neisseria_zoodegmatis_DAC ..IYDKFLK.....SEH.....
1e_Stentor_coeruleus_HdaH GEEGEEREDNEEDDEE.....ESEEDSQUESKTKDKANN.....GE.....PED..N...
1f_S_cerevisiae_HOS3 ISRHLETLE..IEKKGDESDHELKEKNWK.....NSHQRRLLQGNGMYKIPSNTKPHRIRQPQNANTPTYDDSDIS
1g_Homo_sapiens_HDAC4 ..IHSKYWR.....CLQRTT.STAGR.....SLIEAQTC.....ENEAE
2a_Homo_sapiens_HDAC2 HKKGAKKARIEEDKKETEDKKTDVKEEDK.....SKDNS.....GE.....KTD..T...
2b_S_cerevisiae_HOS1 ..ENYQYWI.....YEME.....GS.....SRMKML.....
2c_Bacillus_subtilis_AcuC PSSWSDPADLYPP...IPRKPEITEKNAQTVSKALYAIR.....SE.....Q...
2d_Chromatiales_bacterium_Acu
3_Legionella_cherrii_ApaHHQM.....GK.....
4_Pseudomonas_sp_M30-35_ApaH
5a_Acinetobacter_wuhouensis_HGY.....
5b_Vibrio_cholerae_HdaH
5c_Moraxella_bovis_HDAC
5d_Turneriella_parva_HdaH
5e_Nannocystis_exedens_AcuC FPI SDE.LK.....AEL.....D...
5f_Homo_sapiens_HDAC11
5g_Bacteriovorax_stolpii_AcuC

1a_Arabidopsis_thaliana_HDA14

1a_Arabidopsis_thaliana_HDA14L
1b_Klebsiella_pneumoniae_HdaH
1c_Shinella_sp_DD12_DAC
1d_Neisseria_zoodegmatis_DACA
1e_Stentor_coeruleus_HdaH
1f_S_cerevisiae_HOS3 MISHVSRKHTRSGG.RW.....
1g_Homo_sapiens_HDAC4 TVTAMASL...SVG.VKPAEKRPE.EPMEEPP.L
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2c_Bacillus_subtilis_AcuCQR.....T..K
2d_Chromatiales_bacterium_Acu
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4_Pseudomonas_sp_M30-35_ApaH
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5c_Moraxella_bovis_HDAC
5d_Turneriella_parva_HdaHIKIMH...GRQ
5e_Nannocystis_exedens_AcuCPENYLRAIDEAADE.YRFQLSSP.R
5f_Homo_sapiens_HDAC11AVP..
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b**Subcluster 1a****Subcluster 1b****Subcluster 1c****Subcluster 1d****Subcluster 1e****Subcluster 1f****Subcluster 1g**

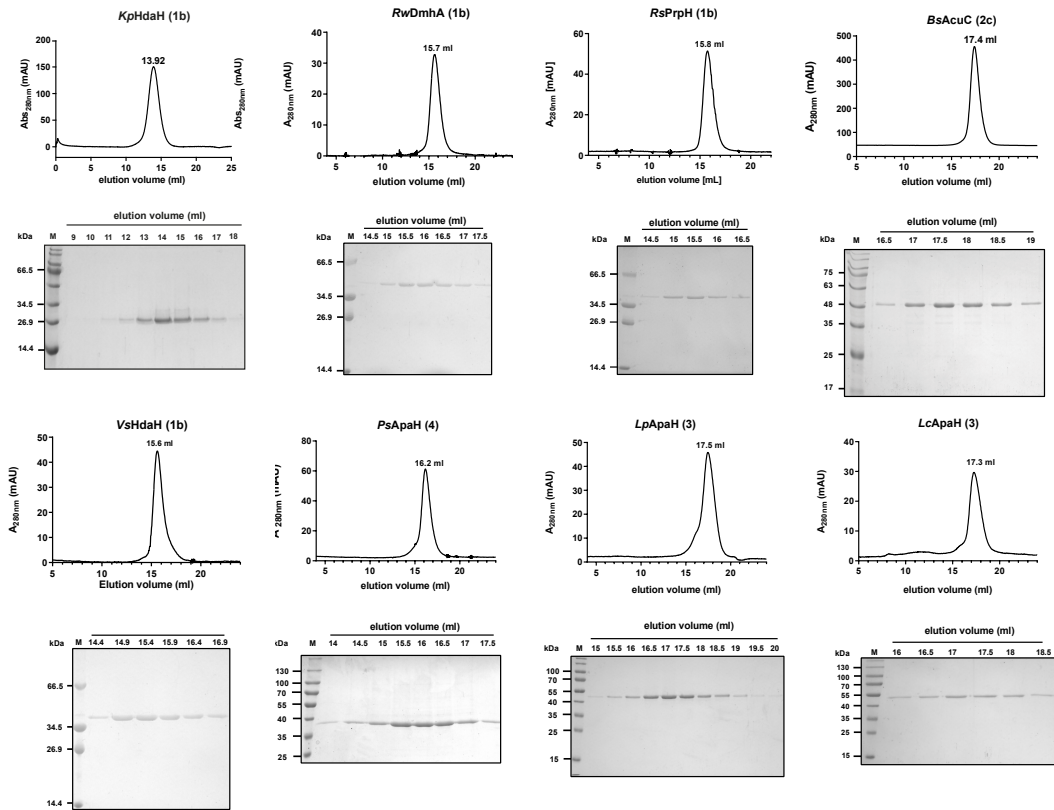
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d**Subcluster 5a****Subcluster 5b****Subcluster 5c****Subcluster 5d****Subcluster 5e****Subcluster 5f****Subcluster 5g**

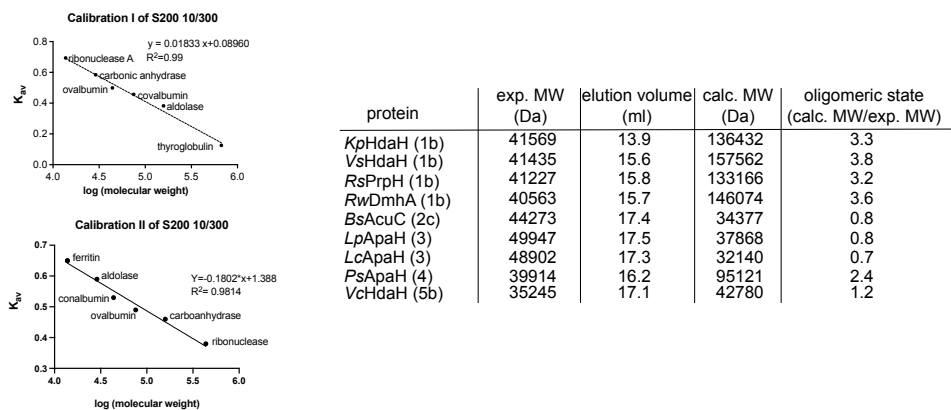
Supplementary Figure 1: Primary sequence alignment and sequence logo representations of selected deacylases representing all clusters 1-5 and the sub-clusters.

- 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/species): 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 server¹. 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 3.0².
- 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 (*KpHdaH*: H143-H144); yellow box: conserved GFC-motif lining the substrate binding channel; brown boxes: Asp-His-Asp for coordination of the catalytic Zn²⁺-ion and the XGGY-motif lining the foot pocket and the catalytic Tyr (*KpHdaH*: 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

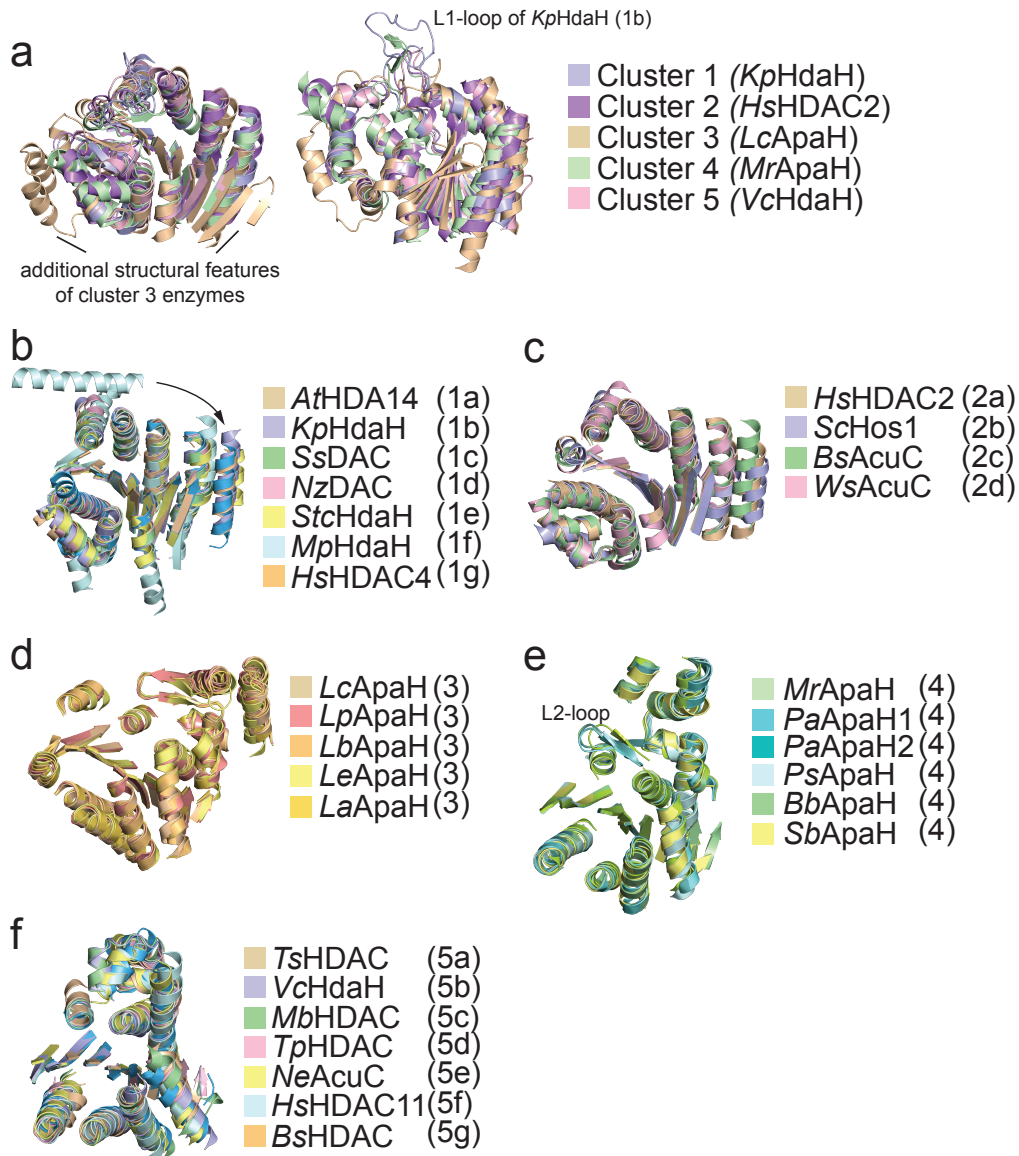


b



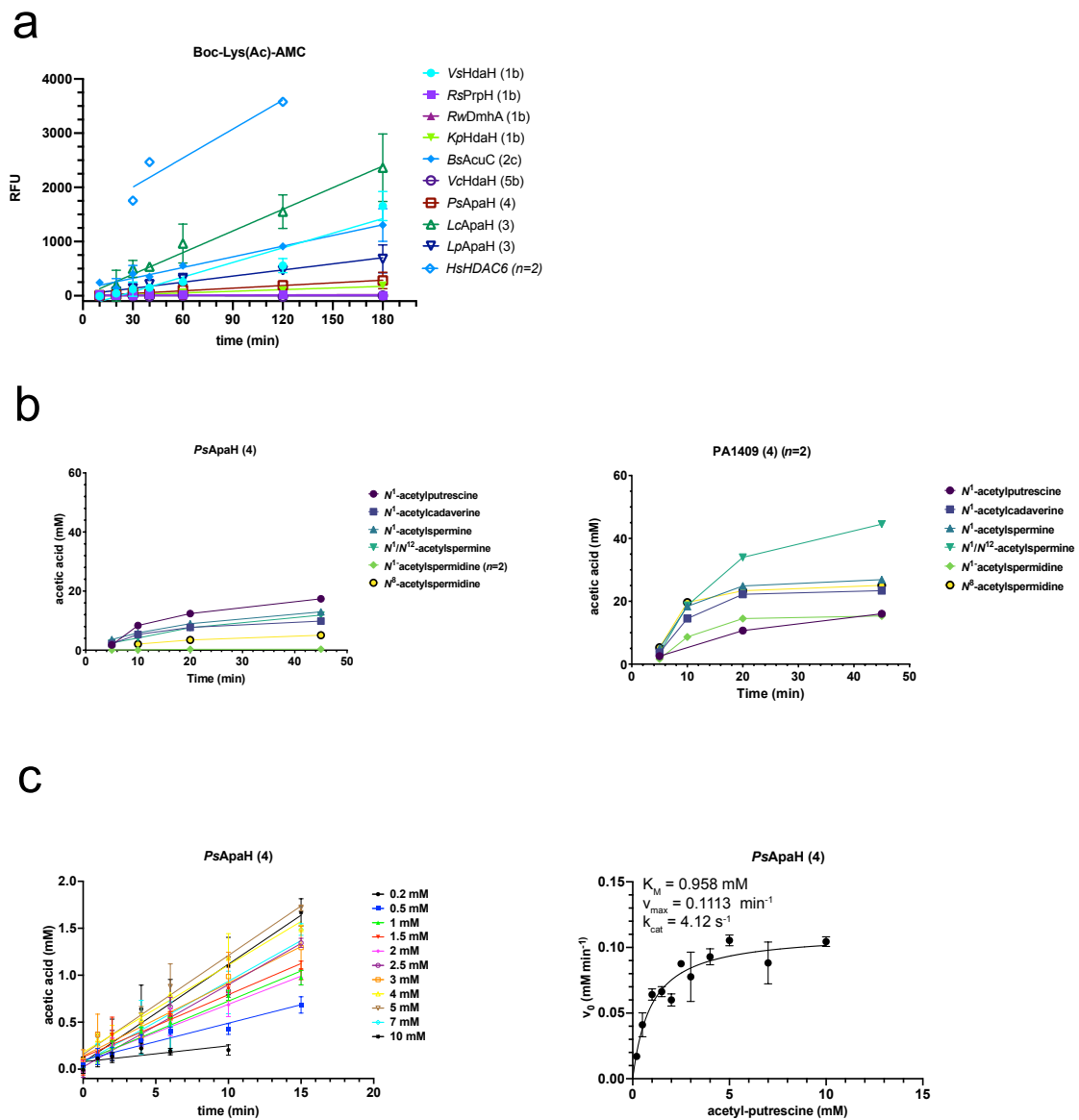
Supplementary Figure 2: Purification and analytical size-exclusion chromatography (SEC) of all purified bacterial deacylases.

- 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 KpHdaH (1b) and the mutants thereof and calibration curve II for the other enzymes. Source data are provided as Source Data file.



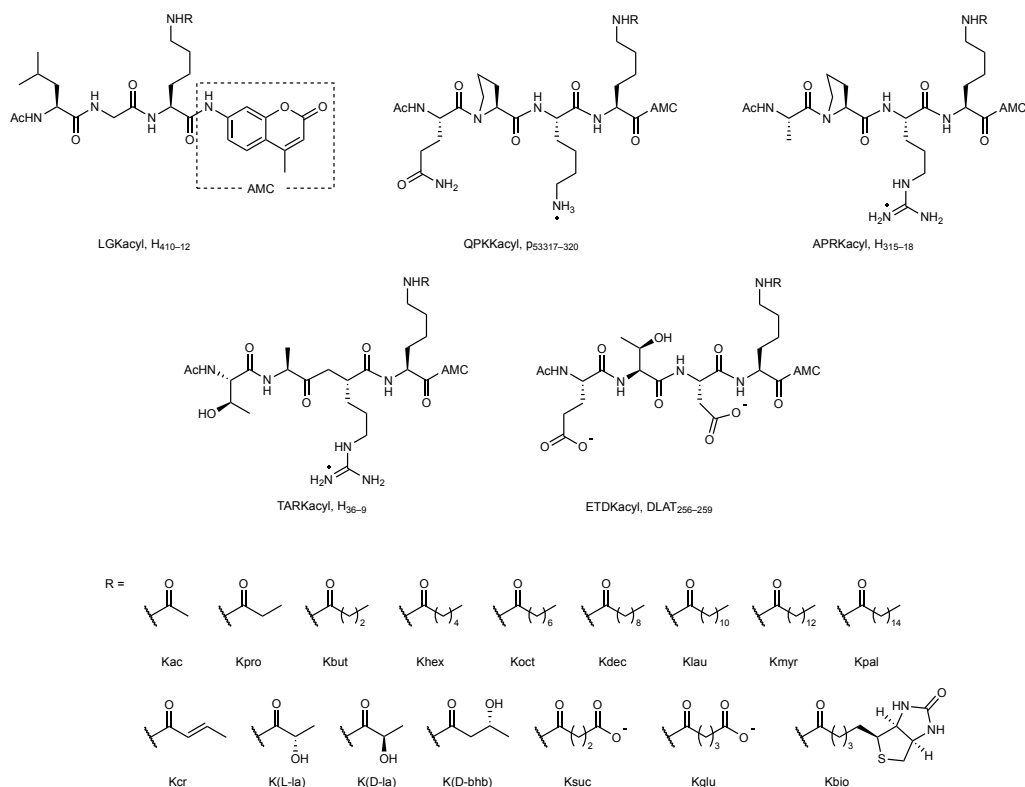
Supplementary Fig. 3: Structural alignments of core domains of deacylases reveal high structural similarity of enzymes within each main cluster and between the clusters.

- 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 *KpHdaH* (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 *KpHdaH* (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 α -helix usually lining the central β -sheet is displaced by approximately 90°. Moreover, this 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 *KpHdaH* (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).



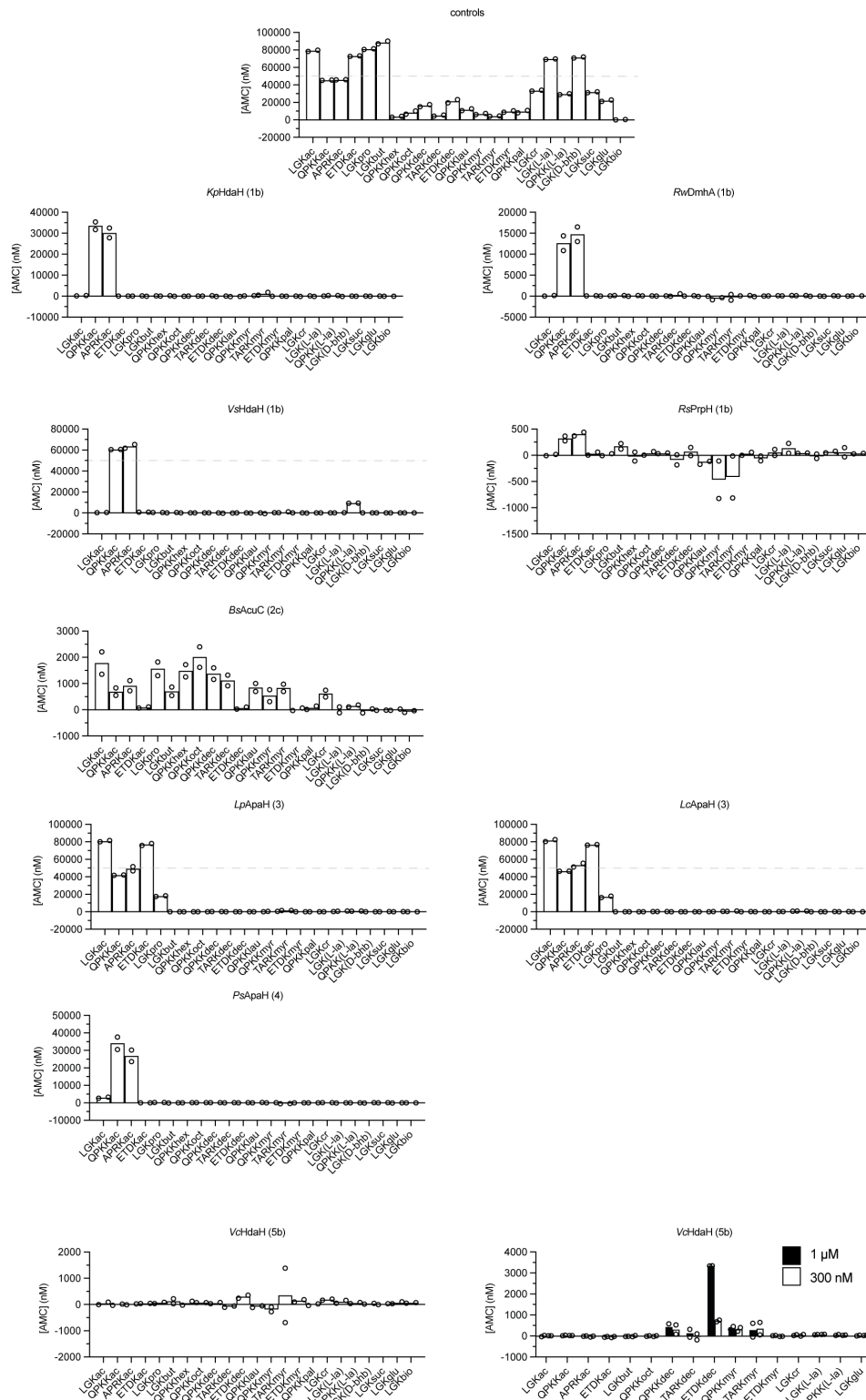
Supplementary Figure 4: Determination of the dynamic ranges for the pre-screening assays.

- 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 μ M 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 enzymes 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 *HsHDAC6* (1g) ($n=2$). Shown are means \pm SD. For *HsHDAC6* (1g) means are shown. Source data are provided as Source Data file.
- b** Determination of the dynamic range for the screening of *PsApaH* (4) on its capacity to deacetylate acetylated polyamines as substrates. 200 nM *PsApaH* (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 enzymes 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 *PsApaH* (4) and PA1409, respectively. Shown are means \pm SD for *PsApaH* (4) and means for PA1409. Source data are provided as Source Data file.
- c** Michaelis-Menten kinetics for the acetyl-putrescine deacetylation by *PsApaH* (4). Initial reaction rates were (v_0) determined using 200 nM *PsApaH* (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 function of the acetylputrescine concentration resulting in a hyperbolic curve that was fitted by a Michaelis-Menten equation resulting K_M , v_{max} and k_{cat} . The experiments were conducted in three biologically independent replicates ($n=3$). Shown are means \pm SD. Source data are provided as Source Data file.

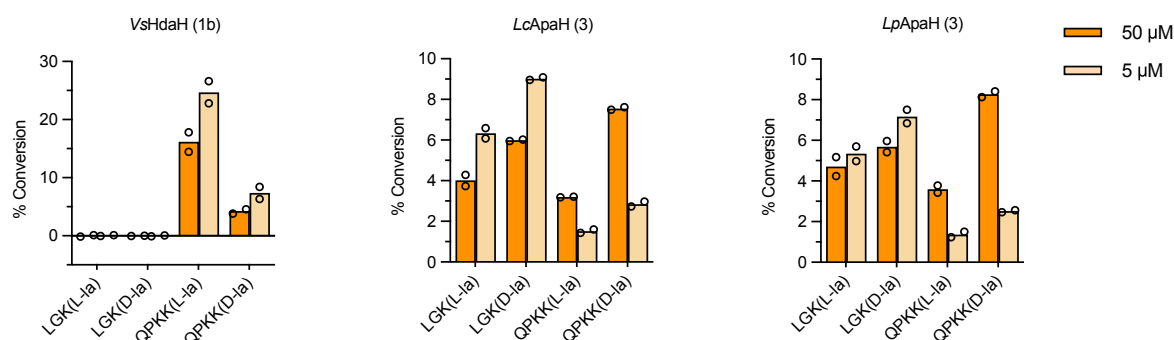


Supplementary Figure 5: Chemical structures of peptides and acyl-chain types used as substrates for deacylation by selected bacterial deacylases.

- 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}, H₃₁₅₋₁₈ or TARK_{acyl}, H₃₆₋₉), histone H4 (LGK_{acyl}, H₄₁₀₋₁₂) tumor suppressor protein p53 (QPKK_{acyl}, p53₃₁₇₋₃₂₀) and DLAT (Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex; ETDK_{acyl}, DLAT₂₅₆₋₂₅₉). Charges were shown as expected at physiological pH.
- 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. hexanoyl (C-6; K_{hex}), octanoyl (C-8; K_{oct}), decanoyl (C-10; K_{dec}), to longer-chain aliphatic acylations, i.e. lauroyl (C-12; K_{lau}), myristoyl (C-14; K_{myr}) and palmitoyl (C-16; K_{pal}). Moreover, we also analyzed acylations such as the unsaturated crotonyl (K_{cro}), the branched acylations L-lactyl (K_{L-la}), D-β-hydroxybutyryl (K_{D-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-la}, K_{D-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).

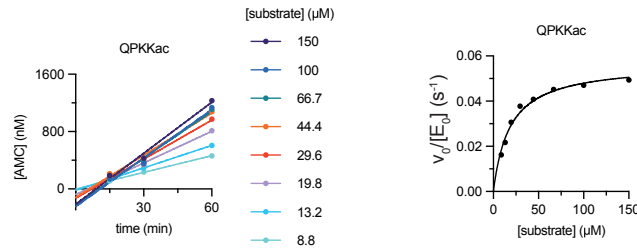


Supplementary Figure 6: Bar graphs obtained for the pre-screening to uncover acyl-chain preferences of selected bacterial deacylases. A Fluor-de-Lys assay-based screening was performed using histone H3 (APRK_{acyl}, H3₁₅₋₁₈ or TARK_{acyl}, H3₆₋₉), histone H4 (LGK_{acyl}, H4₁₀₋₁₂), p53 (QPKK_{acyl}, p53₃₁₇₋₃₂₀) 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 $50 \mu\text{M}$ AMC (dashed grey line). Positive controls were: HDAC3/NCOR2 (K_{ac}, K_{pro}, K_{but}, K_{cr}, K_(L-la), K_(D-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 control was available. Enzyme concentration was set to 100 nM . As we did not observe a strong conversion for VcHdaH (5b), follow-up screening was performed at higher enzyme concentrations of 300 nM and $1 \mu\text{M}$. 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.

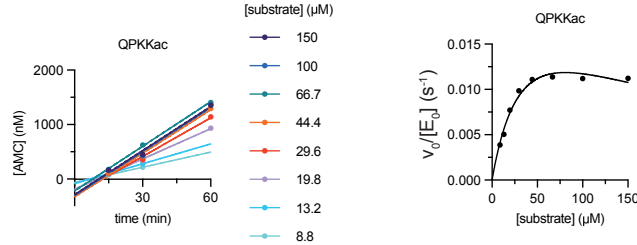


Supplementary Figure 7: Bar graphs obtained for the follow-up screening to uncover de-D/L-lactylase activity for VsHdaH (1b), LcApaH (3) and LpApaH (3). A Fluor-de-Lys assay-based screening was performed using histone H4 (LGK_{L-Ia}/LGK_{D-Ia}, H4₁₀₋₁₂) and p53 (QPKK_{L-Ia}/QPKK_{D-Ia}, p53₃₁₇₋₃₂₀) tri- 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 (VsHdaH (1b)) or 300 nM (LcApaH (3), LpApaH (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.

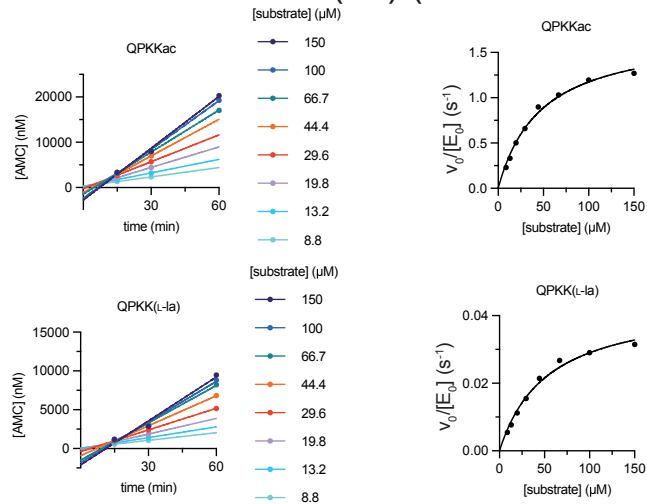
KpHdaH (1b) (discontinuous)



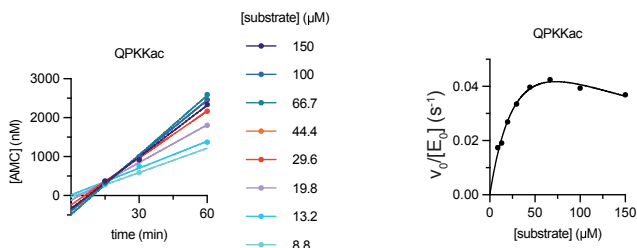
RwDmhA (1b) (discontinuous)



VsHdaH (1b) (discontinuous)

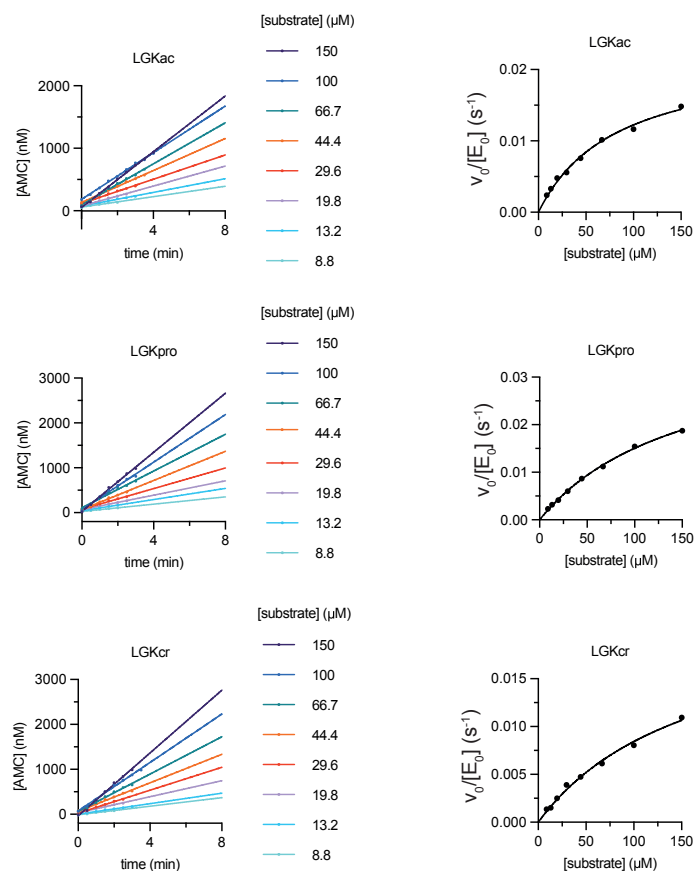


PsApaH (4) (discontinuous)



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 *KpHdaH* (1b), *PsApaH* (4), *RwDmhA* (1b) and *VsHdaH* (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 (p53₃₁₇₋₃₂₀; QPKK_{ac}/ QPKK_{L-Ia}). 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.

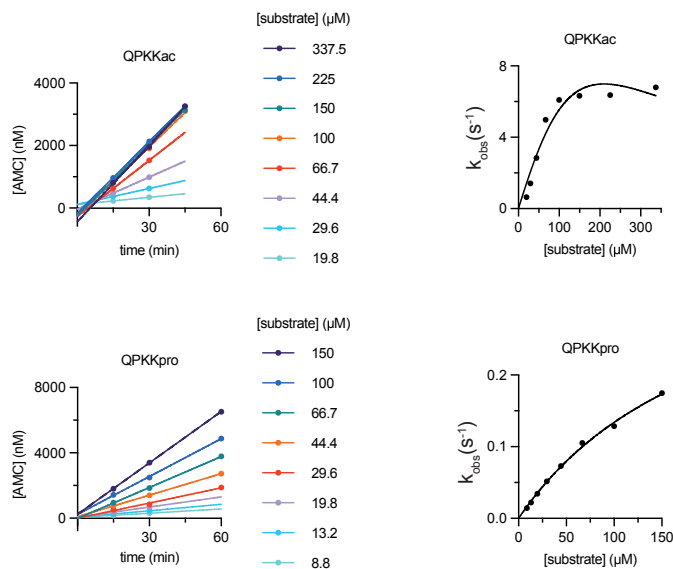
BsAcuC (2c) (continuous)



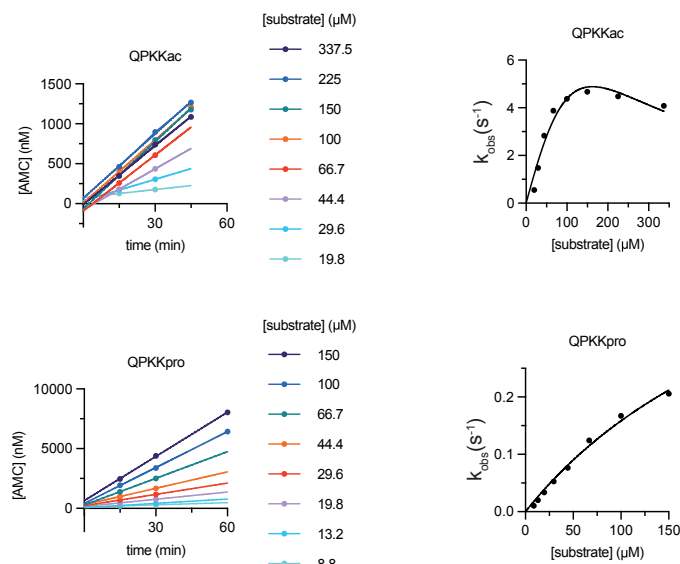
Supplementary Figure 9: Michaelis-Menten kinetics obtained for deacylation of the LGK-based peptides and acyl-chain types by a continuous assay format for bacterial deacylase *BsAcuC* (2c)

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 *BsAcuC* (2c). The peptide sequences and acyl-chain types are indicated (histone H4₁₀₋₁₂; 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: $\text{nM}\cdot\text{s}^{-1}$). 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. $v_0/[E_0] = [\text{AMC}]\text{s}^{-1}/[E_0]$). 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 ($k_{\text{cat}}=v_{\text{max}}/[E_0]$; 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.

LpApaH (3) (discontinuous)

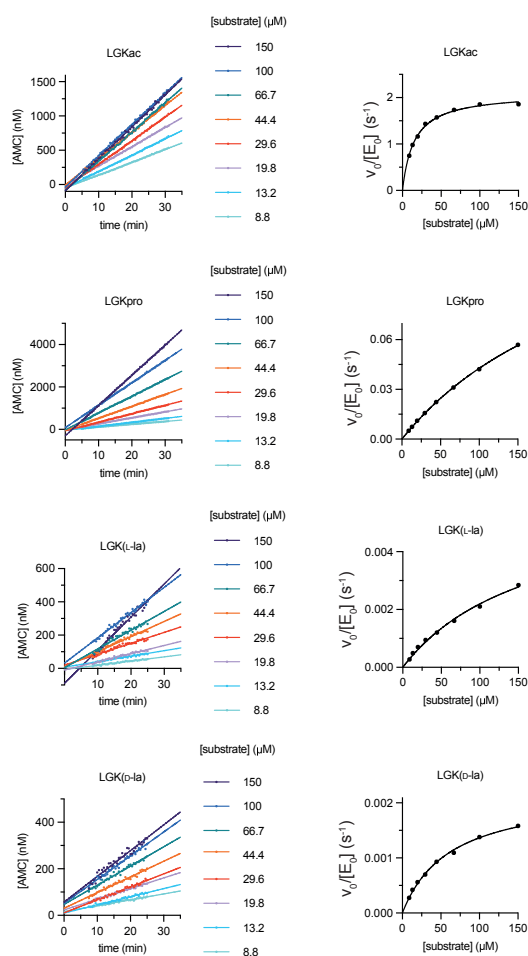


LcApaH (3) (discontinuous)

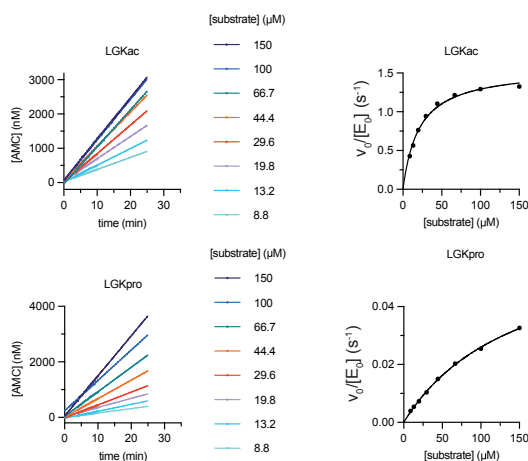


Supplementary Figure 10: Michaelis-Menten kinetics obtained for deacetylation and depropylation of the p53-derived QPKK-based peptides and acyl-chain types by a discontinuous assay format for bacterial deacylases LpApaH (3) and LcApaH (3). For the QPKK-based substrates the discontinuous method was used 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 (p53₃₁₇₋₃₂₀; 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: $nM \cdot s^{-1}$). 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.

LcApaH (3) (continuous)

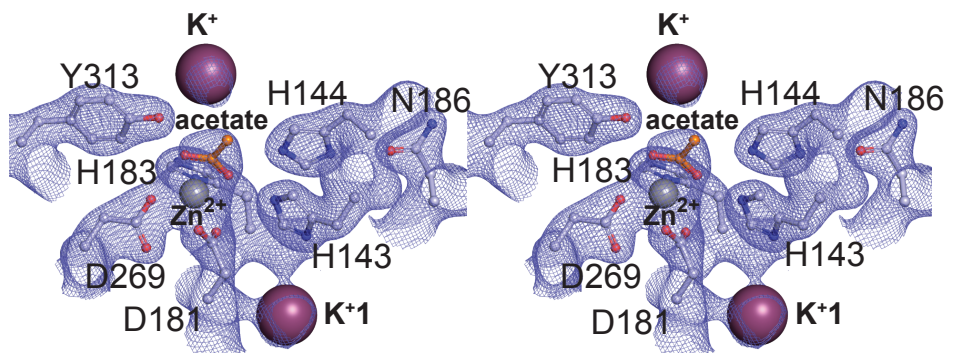
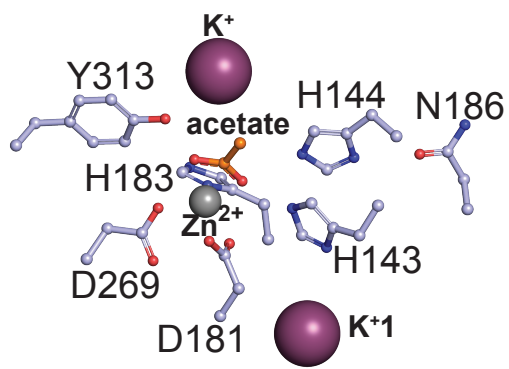


LpApaH (3) (continuous)

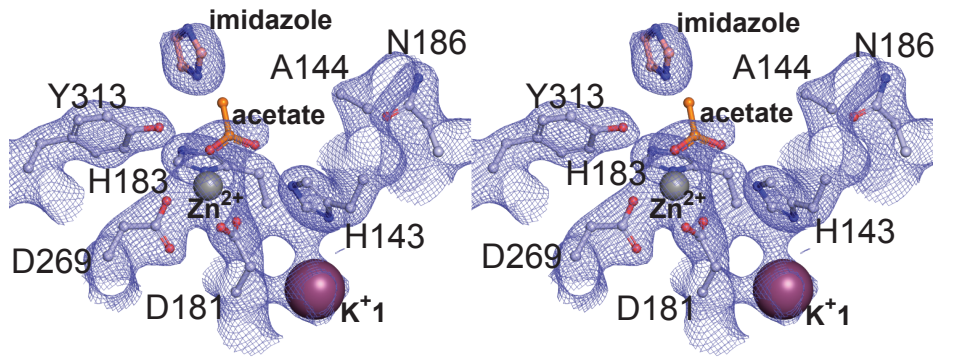
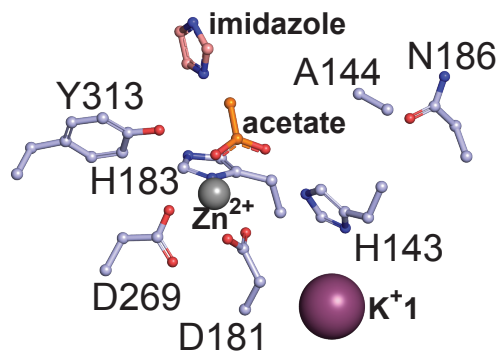


Supplementary Figure 11: Michaelis-Menten kinetics obtained for deacylation of the LGK-based peptides and acyl-chain types for bacterial deacylases LcApaH (3) and LpApaH (3). For the histone H4-derived LGK_{ac}-based substrate peptide the continuous method was used for enzyme kinetics. Michaelis-Menten kinetics were performed to reveal the acyl-chain preference of LcApaH (3) and LpApaH (3). The peptide sequences and acyl-chain types are indicated (histone H4₁₀₋₁₂; LGK_{ac}/LGK_{pro}/LGK_{L-la}/LGK_{D-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. $v_0/[E_0] = [\text{AMC}]s^{-1}/[E_0]$). 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 ($k_{\text{cat}} = v_{\text{max}}/[E_0]$; 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.

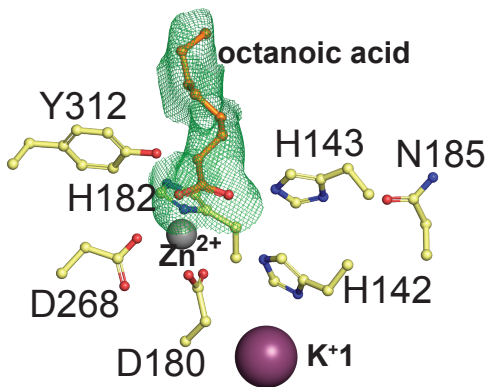
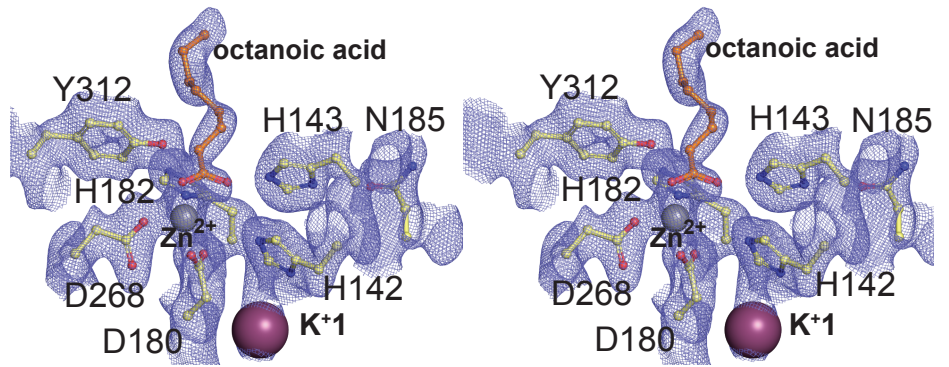
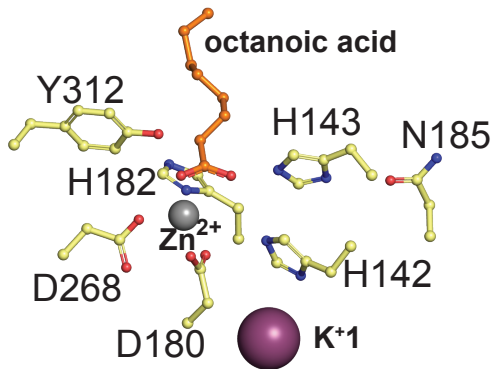
KpHdaH (1b)



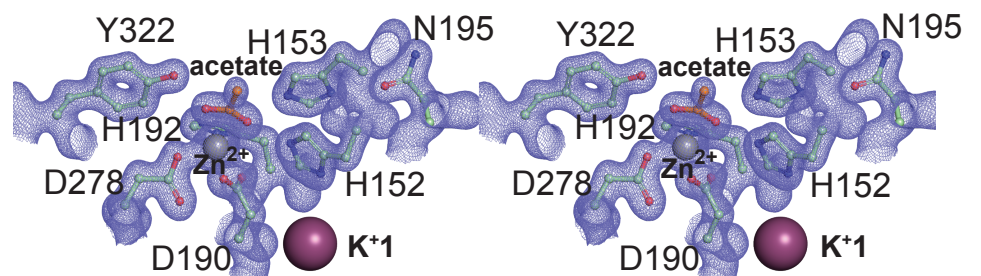
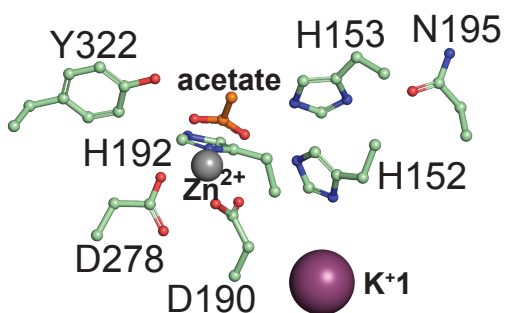
KpHdaH (1b) H144A



RwDmhA (1b)

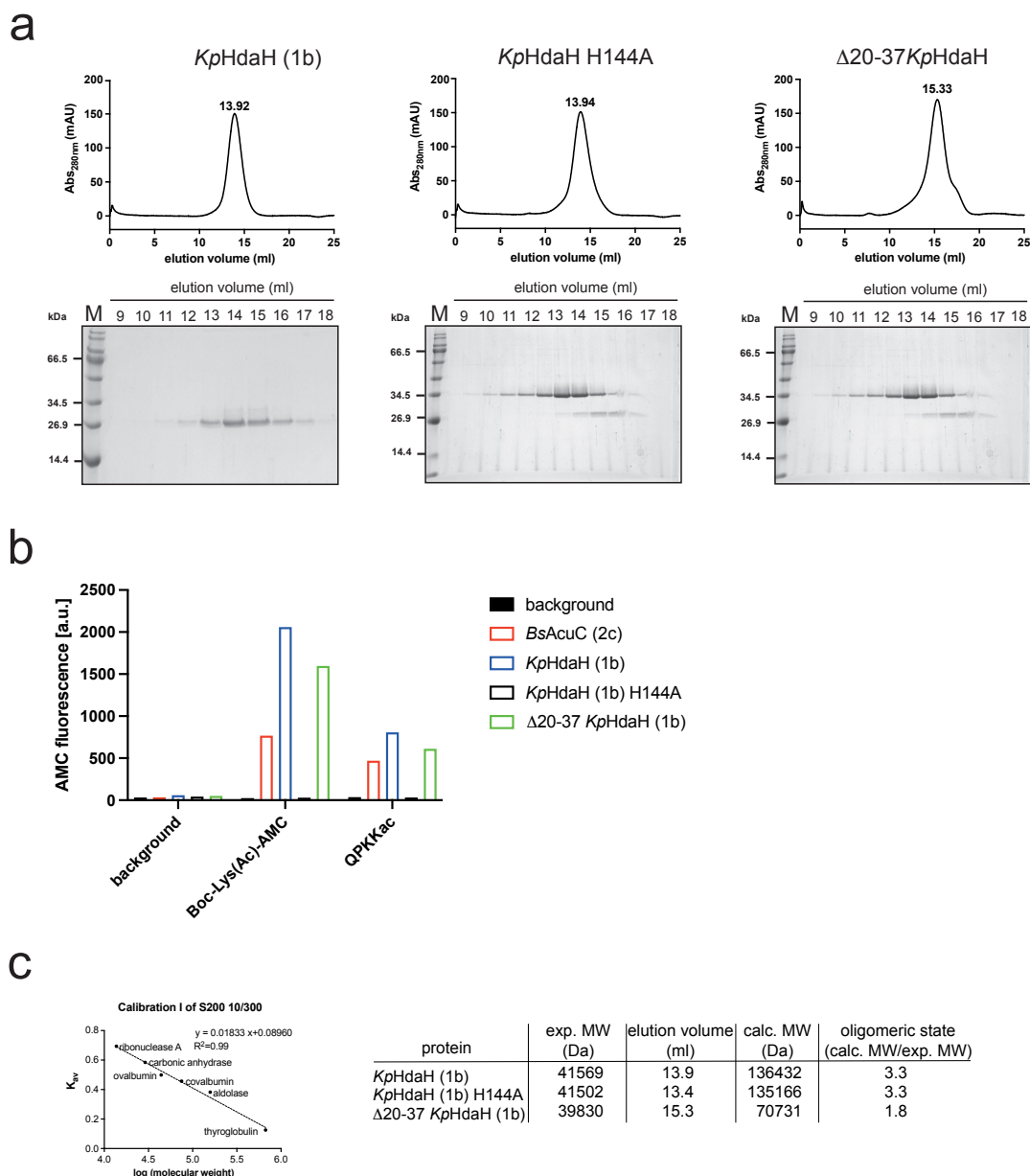


RsPrpH (1b)



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: *KpHdaH*, *KpHdaH* H144A, *RwDmhA* and *RsPrpH* of subcluster 1b. For all structures a representative closeup of the active site is shown on the left, including the catalytic Zn²⁺-ion, the coordinating His and the two Asp residues, the catalytic base His, except for the inactivated *KpHdaH* 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 electron density of the 2F_o-F_c map contoured at 1σ in blue. For *RwDmhA* (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 Zn²⁺-ion is shown as grey sphere. The stereo diagrams were prepared in wall-eyed viewing mode with PyMOL version 2.3.4³.



Supplementary Figure 13: Analytical size exclusion chromatography of the L1-loop deleted mutant $\Delta 20-37KpHdaH$ shows formation of a dimer and the $\Delta 20-37KpHdaH$ (1b) is still catalytically active albeit with a lower activity compared to wildtype *KpHdaH* (1b).

- a** The analytical SEC experiment on a calibrated S200 10/300 GL column reveals the deletion of the loop abolishes tetramer formation. Instead, $\Delta 20-37KpHdaH$ (1b) elutes as an apparent dimer. Analyses of the crystal structure of *KpHdaH* (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 $\Delta 20-37KpHdaH$ being active as lysine deacylase, however, the activity is reduced compared to wildtype *KpHdaH*. *BsAcuC* (2c) shows lower activity compared to $\Delta 20-37KpHdaH$ and wildtype *KpHdaH*. The experiment was conducted in assay buffer (50 mM Tris/HCl, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 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 *KpHdaH* (1b) and $\Delta 20-37KpHdaH$ as determined by analytical size exclusion chromatography. Source data are provided as Source Data file.

β1

1a_Arabidopsis_thaliana_HDA14

	1		10
1a_Arabidopsis_thaliana_HDA14	MSMALI...	VRP.....	FFVP.....
1a_Candidatus_Hinthialibacter	MSFGLI...	VD.....	SCF.....
1a_Actinobacteria_bacterium_R	MCESCGVRL	LLLY.....	
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.....SFPLHDAQA..GDAELIHQ....
1e_Babesia_microti_strain_RI	MASGSQ...	VGT.....	CYR..KFO.P.....PLHKHILR..NDYDAIRYLSS
1f_Pneumycystis_carinii_HdaH	MANPNY...	SLN.....	LSDSFYDE.TK.....EASYLTSHETDSL..LDA.....
1f_Malassezia_pachydermatis_H	MVFLVK...	LPARKPADGR	PPTNE..QES.....
1f_Saccharomyces_cerevisiae_H	MSSKHS...	D.....	PLER.....
1g_Homo_sapiens_HDAC4	MSSQ...	SH.PD.....	GLSG..RDQ.PVELLNPARVNHMPSTVDVATALPLQVAPSAVPMDLRLDHO...
1g_Saccharomyces_cerevisiae_H	MDSVMV...	K.....	KEVL..EN.....
1g_Arabidopsis_thaliana_HDA5	MAMAGE...	SS.....	GKKI..GD CDGKV.....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_HdaHLMTPPPDPAKPGPPSDDLVLGDDI.LARYVAEASAPQ.LPSINRRDHHE
1e_Babesia_microti_strain_RI	H..HRDCINDMDHdGRNAFHLLALQTANSKALYL..LLYYPFVHTNQVF..ATLDWTCDSDDEIDRDIHVHG.....
1f_Pneumycystis_carinii_HdaH
1f_Malassezia_pachydermatis_H
1f_Saccharomyces_cerevisiae_H
1g_Homo_sapiens_HDAC4FSL..PVAEPALREQQLO..QEL.LALKKQKQIQRQILIAEFQRQHEQ
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_HDA8HDAVEGV.....
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_ApHNT.....
1d_Thioploca_ingrica_DACHPL.....
1d_Neisseria_zoodegmatis_DAC
1e_Stentor_coeruleus_HdaH
1e_Chlorella_variabilis_HdaH	CTPLHVALLHGQLE.....AARA.....LLEHGA.....
1e_Babesia_microti_strain_RINAKPAYQAGDRILMKWNETPDLYKKAAFSALEGYSIRGCGLRIVEDLYEQHYKYLDPKN.....
1f_Pneumycystis_carinii_HdaHILNISL.....KDEETK.....
1f_Malassezia_pachydermatis_H
1f_Saccharomyces_cerevisiae_HQQQEMLAMKHQELLEHOR.....
1g_Homo_sapiens_HDAC4	LSRQHEAQLHEHIK.....P.....DHDLLKR.....
1g_Saccharomyces_cerevisiae_H
1g_Arabidopsis_thaliana_HDA5	...RKV.....

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

SLE.....TAKYARLYSKEINFPEKIETID..ELKEKNYISTIITSEIVMEKDD...NTRVSFASDFI
TLERSAREY.....
KLSDAVDEQ.....
.VP.....
KLERHRQEQELEKQHREKQ.....LQQLKNKEKESAVASTEVKMKLQEFVNLNKKKALAHRNL
KLEENKEE.....NSLSTTSKSRQVIV.....

1a_Arabidopsis_thaliana_HDA14

20

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

GISGSRNICKK
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SGVHAVRPVTIVPATAASTTTETPAH.....
NHCISSDPR..YWYGKTKHSSLDQSSPPQSGV.....
GL

1a_Arabidopsis_thaliana_HDA14

30

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

NQWRKYL.....
FTH.....
WAA.....QMDFSAAAEAVLA.....FPECHEDLIQTVNCL...LK.....AAE
IESYREKILDCLKILITFFRVFDHKE...TVDSVKCMHLKNI.....
MMPAAEDTDGAAFQEAVPA.....PAL
FYK.....STSYNHPVL.....GMYD.....AKDDFPLRKTAS
PV.....CMPKI
IYD.....

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
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 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_zoodegmatidis_DAC
1e_Stentor_coeruleus_HdaH FFCTDN R
1e_Chlorella_variabilis_HdaH CRTRSKA
1e_Babesia_microti_strain_RI LTCDNNSKVRDGSRAEMDYLNNSQKDKIFNNKLILKEDHTFLKQVPGEVIFERLFSKYGECFITTISSELLLF
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_zoodegmatidis_DAC
1e_Stentor_coeruleus_HdaH GDKPIHTSVRAGSWEFCNILITF GSEMLNENNEF
1e_Chlorella_variabilis_HdaH GHNALHLAALAGSSRCAALLAAA AL
1e_Babesia_microti_strain_RI FEQLFYRCIHRNSWRVFLALASFNNLTTFHMFSSPLCVHRFKFAHSMGNVYAFFSILNYSVRLLYPNETKCVENMDF
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_zoodegmatidis_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 HRP LGR TQSAPLPQNAQA I QHLVIQQHQHFLEKHKQFQQQLQMNKIIPKPSEPARQFESH P
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
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

$\alpha 1$ $\beta 2$ $\alpha 2$
 1a_Arabidopsis_thaliana_HDA14 00 $\xrightarrow{70}$ TT 80 000000 90 $\xrightarrow{100}$ 00 00

1a_Arabidopsis_thaliana_HDA14 ADARLIYSVSAALGHNKES...HP**E**CSA**R**VPAIVN...AL...EM..N.....E..LT.
 1a_Candidatus_Hinthialibacter G.....R.....HP**E**RP**E**RLDSIDS...AF...KE..H.....
 1a_Actinobacteria_bacterium_R TG.....G.....W...HP**E**RAA**R**LDAAIE...GA...RR...S...S..L.
 1b_Arabidopsis_thaliana_HDA8 HPENADRVRN...ML.SILRR.GP.....I.
 1b_Klebsiella_pneumoniae_HdaH LPVGGWVQP...PAGG...G...HA**E**SP**E**TKRRMKK...LM...DV...S...G..L.
 1b_Vibrio_sp_vnigr-6D03_HdaH LPVGDWVQP...MSAG...G...HA**E**SP**E**TKRRLKN...LL...DA...S...G..I..
 1c_Shinella_sp_DD12_HdaH P.....E.....G...HP**E**RS**D**RIRAINL...AL...EH..E...R..F.
 1c_Haematospirillum_jordaniae TG.....G.....Y...HP**E**NP**D**RLRSVL...QT...LE..S...E..D.
 1c_Micavibrio_sp_TMED2_Acu P.....G.....G...HP**E**RI...DR...L...V...S...V..L.
 1d_Legionella_londiniensis_Ap D.....D.....F...HP**E**CP**E**RLQVIND...NL...SQ.SA...S...I.
 1d_Thioploca_ingrica_DAC MG.....K.....G...HP**E**RS**R**INAIED...QL...QA...S...R..L.
 1d_Neisseria_zoodegmatis_DAC N.....K.....G...HP**E**SA**E**RIKAIEA...EI...A...G...QGF
 1e_Stentor_coeruleus_HdaH TPQTLTKQI...N.....I...QP**E**NR**R**LEILVD...LPFGALLT.SD.....L.
 1e_Chlorella_variabilis_HdaH APITRETS...S.....A...PP**E**N**V**E**R**LTIVLTKPGWGIL...RC..D...E..F.
 1e_Babesia_microti_strain_RI MPQRRKKMT...S.....N...F**V**EN**P**T**R**LEVIIS...NDNAILRT.DI...L.
 1f_Pneumocystis_carinii_HdaH ERNK...HDLK...Y...I**V**ER**P**RYRAII...GT...LSAKAHLDDLK...S.
 1f_Malassezia_pachydermatis_H RG...VDKS...N...I**V**ER**P**ERIRAVLL...GI...A...G...VQGM
 1g_Saccharomyces_cerevisiae_H PREWVTKSYRK...T...I**V**ER**P**ERLLASSM...GI...SA...AITM.
 1g_Homo_sapiens_HDAC4 TCGSS...S.....S...HP**E**HAG**R**IQSIWS...RL...QE..T...G..L.
 1g_Saccharomyces_cerevisiae_H I.....FTSY.....FEYIDP**H**EP**D**PR**I**YRIYK...IL...AE..N...G..L.
 1g_Arabidopsis_thaliana_HDA5 D.....GED...HP**E**CP**D**RI.RVI...W...EK..L...Q..LA

$\alpha 3$ $\eta 1$ $\beta 3$
 1a_Arabidopsis_thaliana_HDA14 00000 00 $\xrightarrow{110}$

1a_Arabidopsis_thaliana_HDA14 PKFRG.....SQ.I...LELA.
 1a_Candidatus_Hinthialibacter K.I.....LE.SC.HCLSFEP.
 1a_Actinobacteria_bacterium_R EV.....AD.R...EAP.
 1b_Arabidopsis_thaliana_HDA8 A.....PHV.....NWF...T.G.
 1b_Klebsiella_pneumoniae_HdaH P.Q.L.....AL.R...S.A.
 1b_Vibrio_sp_vnigr-6D03_HdaH T.....K.H.L.....TL.S...S.A.
 1c_Shinella_sp_DD12_HdaH S.....P.L.L.....R.E...K.A.
 1c_Haematospirillum_jordaniae F.....F.ML.....HR.E...E.A.
 1c_Micavibrio_sp_TMED2_Acu A.....A...L.....EE.E...PFNAC
 1d_Legionella_londiniensis_Ap QS.....SLV.....W.E...T.A.
 1d_Thioploca_ingrica_DAC L.....E..LV.....RY.Y...E.A.
 1d_Neisseria_zoodegmatis_DAC W.....DVF.....QKIE...A.A.
 1e_Stentor_coeruleus_HdaH S.....FAT.....WKK...S.S.
 1e_Chlorella_variabilis_HdaH G.....G..AL.....RWDE...RS.
 1e_Babesia_microti_strain_RI E.....GVY.....ILO...S.P.
 1f_Pneumocystis_carinii_HdaH N.....SFD..IY.....QSVR...Y.I.
 1f_Pneumocystis_carinii_HdaH G.....E.K.LAATKPKSDDDDVAAMLGMSIAERESVLRVFTTTRTLRLSEPSVALADVHA.H...E.S.
 1f_Malassezia_pachydermatis_H Y.....P.S.LF.....TL.K...S.
 1g_Homo_sapiens_HDAC4 R.....G..KC.....EC.I...R.G.
 1g_Saccharomyces_cerevisiae_H INDPTLSGVDDLGD...LM...LK.I...P.V.
 1g_Arabidopsis_thaliana_HDA5 GV.....S.QRC.....VV.L...GS.

$\alpha 4$
 1a_Arabidopsis_thaliana_HDA14 00 00
 120

1a_Arabidopsis_thaliana_HDA14 ...NFKT**A**T.VE.....DIAN**V**H**D**KA
 1a_Candidatus_Hinthialibacter AE**E**E.....FL**E**K**V**HR..
 1a_Actinobacteria_bacterium_R EA.P.LE.....AL**Y**L**V**HR**P**A
 1b_Arabidopsis_thaliana_HDA8 LP**A**I.VS.....ELL**M**F**T**SE
 1b_Klebsiella_pneumoniae_HdaH AP**A**S.LE.....DL**R**R**I**HP**D**S
 1b_Vibrio_sp_vnigr-6D03_HdaH PS**C**S.QA.....QL**L**R**V**HP**E**H
 1c_Shinella_sp_DD12_HdaH PQ**A**N.ED.....FV**L**L**A**HP**E**R
 1c_Haematospirillum_jordaniae PR**A**T.LE.....QL**T**R**V**HP**Q**S
 1c_Micavibrio_sp_TMED2_Acu PR**G**P**V**PK**A**S.RE.....QL**E**R**V**HP**E**V
 1d_Legionella_londiniensis_Ap HK**A**T.RA.....QL**E**R**V**HP**D**K**Q**
 1d_Thioploca_ingrica_DAC PL**A**T.RA.....QL**T**R**V**HP**D**SE
 1d_Neisseria_zoodegmatis_DAC EV**S**DS**Q**L.....AL...I**H**PR**K**
 1e_Stentor_coeruleus_HdaH NP**A**H.IS.....DIL**R**V**H**E**Y**S
 1e_Chlorella_variabilis_HdaH QR**A**A.IG.....DVL**R**V**H**D**W**N
 1e_Babesia_microti_strain_RI PP**A**C.LA.....DIL**R**V**H**D**I**G
 1f_Pneumocystis_carinii_HdaH SI**I**N.NP.....IIE**F**I**H**GE**K**
 1f_Malassezia_pachydermatis_H EP**A**T.YDLTTAYAPTSAATETASAKHCERISQLAAKAPSHPPGDVPRYSSLDGALSASSDGDGEGDERM**H**ESE
 1f_Saccharomyces_cerevisiae_H SH**Q**R**K**GS**L**M.AP.....HVL**K**V**H**GS**S**
 1g_Homo_sapiens_HDAC4 RK**A**T.LE.....EL**Q**T**V**H**S**E**A**
 1g_Saccharomyces_cerevisiae_H RA**A**T.SE.....EIL**E**V**H**T**K**E
 1g_Arabidopsis_thaliana_HDA5 SK**A**E.DK.....HL**Q**L**V**H**T**K**D**

α13
0000000000.0000
410 420

1a_Arabidopsis_thaliana_HDA14 PMRKVRDAIQRA.KSIHCL.....

1a_Candidatus_Hinthialibacter SPPRARH.MVQVSLAQAAKYWDV.....G.....

1a_Actinobacteria_bacterium_R RA..TFA.QFQR.QA.....

1b_Arabidopsis_thaliana_HDA8 CQ..SFN.QLQA.EK.....

1b_Klebsiella_pneumoniae_HdaH LMEN.....

1b_Vibrio_sp_vnigr-6D03_HdaH HEKKVRA.ELEA.KKAEAAHQKA.....

1c_Shinella_sp_DD12_HdaH KNL.MDSI.SVLE.GILR.....

1c_Haematospirillum_jordaniae IDKSI.....

1c_Micavibrio_sp_TMED2_Acu NL..MQL.ERMA.KR...VM.A...EA.....

1d_Legionella_londiniensis_Ap Y.KTKRA.KDVI..RIWAEVIRL.....GRAMI.....

1d_Thioploca_ingrica_DAC NANAVRS.MEKV.MEIHSHKYWRC.....LQ.....

1d_Neisseria_zoodegmatidis_DAC IDKVIRLQSKYWNCFRRRHANSNGCNFNPEIND.SIIS.KNFPLQKAIR.....QQ.....QQHYLSD.....

1e_Stentor_coeruleus_HdaH FESTWRV.IQAV.RKRLCTYWPSLADELSWKLINQKTPPIILISS...DSE

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_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_zoodegmatidis_DAC FL

1e_Stentor_coeruleus_HdaH NEK...MILKSSVNESFRALKRNRKMYIE.N...NEK...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...VTLPVLSMD...LPDNTVL.CTPNISESNTIIIVVHDTSDIWAQRNVISGTDLSSSVIIDNSL

1g_Arabidopsis_thaliana_HDA5 TEDNAQGLLDQMSKLSIEN...PQGTLLN...HQV

1a_Arabidopsis_thaliana_HDA14

1a_Candidatus_Hinthialibacter

1a_Actinobacteria_bacterium_R E.ANAVAAVES.IKTYHTEFVPLRGT.....

1b_Arabidopsis_thaliana_HDA8 I.....

1b_Klebsiella_pneumoniae_HdaH I.....

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_zoodegmatidis_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

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
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
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1a_Arabidopsis_thaliana_HDA14

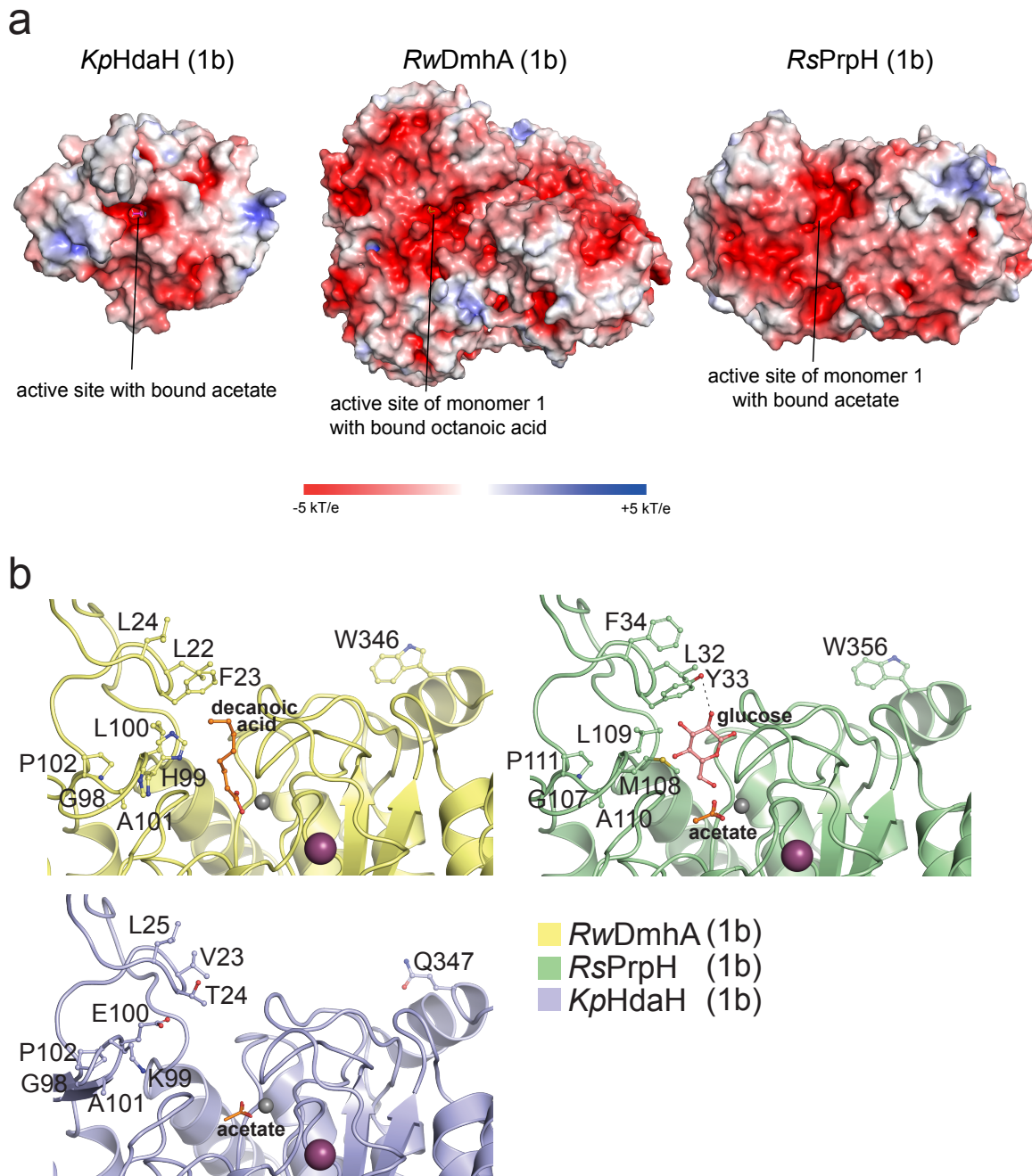
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1a_Candidatus_Hinthialibacter
1a_Actinobacteria_bacterium_R
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1b_Vibrio_sp_vnigr-6D03_HdaH
1c_Shinella_sp_DD12_HdaH
1c_Haematospirillum_jordaniae
1c_Micavibrio_sp_TMED2_Acu
1d_Legionella_londiniensis_Ap
1d_Thioploca_ingrica_DAC
1d_Neisseria_zoodegmatidis_DAC
1e_Stentor_coeruleus_HdaH E.DDEE ES
1e_Chlorella_variabilis_HdaH A.PAPAPAPAPAAGAEAAEDGGTRKRKRRRG.AGSGVDYVEL NRQL
1e_Babesia_microti_strain_RI TSYNCKLHCD M RF
1f_Pneumocystis_carinii_HdaH KPIK SPQ
1f_Malassezia_pachydermatis_H RRRQSHAAI STE.PTPARARASARSRNDGERGTSHPAQW VPG ALPV
1f_Saccharomyces_cerevisiae_H DDISRHLELEIEKKGDE DSDHELKEKNWKNSHQRRL QGNGMYKIPSNTKPHRIRQPQ
1g_Homo_sapiens_HDAC4 VTAM ASLSVGVKPAEKRPDEE.PM
1g_Saccharomyces_cerevisiae_H FSNN SHQCWKENESRKPRKFKFGRVLRCDTDGLNNII.E.ERF
1g_Arabidopsis_thaliana_HDA5 LTMTCTLSAVEKFKSGEIP IRP.PAKAYANTLIRGLVEGG RLS

1a_Arabidopsis_thaliana_HDA14

1a_Arabidopsis_thaliana_HDA14
1a_Candidatus_Hinthialibacter
1a_Actinobacteria_bacterium_R
1b_Arabidopsis_thaliana_HDA8
1b_Klebsiella_pneumoniae_HdaH DRLAQQFGLL
1b_Vibrio_sp_vnigr-6D03_HdaH DLLAKNTPLL
1c_Shinella_sp_DD12_HdaH
1c_Haematospirillum_jordaniae
1c_Micavibrio_sp_TMED2_Acu
1d_Legionella_londiniensis_Ap
1d_Thioploca_ingrica_DAC
1d_Neisseria_zoodegmatidis_DAC
1e_Stentor_coeruleus_HdaH EEEDSQESKKTDKA.NNGEPE DN
1e_Chlorella_variabilis_HdaH EEEA.AARR..AAGGSG..GG
1e_Babesia_microti_strain_RI DQK C QI
1f_Pneumocystis_carinii_HdaH TKNTDKHS
1f_Malassezia_pachydermatis_H ESAG.PA.T..PAAPTQ.RPSHDADTLATLLGQWQLNDTT
1f_Saccharomyces_cerevisiae_H NANT PTYDDSDI.SMISHVSRKHTTRSGGRW
1g_Homo_sapiens_HDAC4 EEEP PL
1g_Saccharomyces_cerevisiae_H EEAT DF ILDSFEEWSDEE
1g_Arabidopsis_thaliana_HDA5 KEEA.EAYI..DKA.VS.KPL RLS

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/species): 1a, AT4G33470.1/HDA14/*Arabidopsis thaliana*; 1a, A0A136MHB6/AphA/*Candidatus Hintialibacteria 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 server¹. 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 3.0².



Supplementary Figure 15: Molecular determinant of substrate specificity for cluster 1 enzymes *KpHdaH* (1b), *RwDmhA* (1b) and *RsPrpH* (1b).

- a** To show the electrostatics at the active site the electrostatic surface potential was plotted onto the surface of the structures of *KpHdaH* (1b), *RwDmhA* (1b) and *RsPrpH* (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 *KpHdaH* (1b) a monomer is shown, for *RwDmhA* (1b) a tetramer and for *RsPrpH* (1b) a head-to-head dimer is shown. The electrostatics was calculated using the APBS plugin in PyMOL⁴.
- b** Residues lining the substrate binding tunnel mediate substrate specificity of sub-cluster 1b enzymes. For *RwDmhA* (1b) and *RsPrpH* (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 *KpHdaH* (1b) (23-VTL-25; *KpHdaH*: 99-GKEAP-102; 343-FIOOQ-347; Q347 corresponding to W346/W356 in *RwDmhA* (1b) and *RsPrpH* (1b). This is supported by the observation in *RwDmhA* (1b) a hydrophobic octanoic acid and in *RsPrpH* (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.

2a_Homo_sapiens_HDAC2

2a_Homo_sapiens_HDAC2

2a_Saccharomyces_cerevisiae_Rpd3

2a_Arabidopsis_thaliana_HDA7

2b_Saccharomyces_cerevisiae_Hos1

2b_Trichomonascus_ciferrii_HDAC8

2b_Candida_albicans_HDAC

2c_Bacillus_subtilis_AcuC

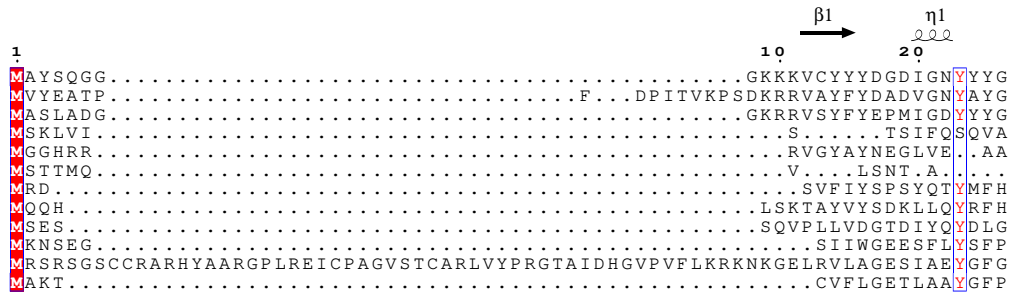
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2c_Corynebacterium_lactis_RW2-5_AcuC

2d_Dictyoglomus_turgidum_AcuC

2d_Wenzhouxiangella_sp_XN24_AcuC

2d_Pseudomonadota_bacterium_AcuC



2a_Homo_sapiens_HDAC2

2a_Homo_sapiens_HDAC2

2a_Saccharomyces_cerevisiae_Rpd3

2a_Arabidopsis_thaliana_HDA7

2b_Saccharomyces_cerevisiae_Hos1

2b_Trichomonascus_ciferrii_HDAC8

2b_Candida_albicans_HDAC

2c_Bacillus_subtilis_AcuC

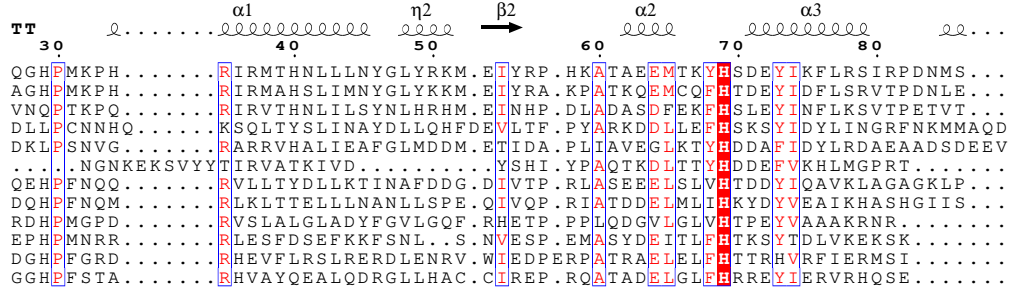
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2c_Corynebacterium_lactis_RW2-5_AcuC

2d_Dictyoglomus_turgidum_AcuC

2d_Wenzhouxiangella_sp_XN24_AcuC

2d_Pseudomonadota_bacterium_AcuC



2a_Homo_sapiens_HDAC2

2a_Homo_sapiens_HDAC2

2a_Saccharomyces_cerevisiae_Rpd3

2a_Arabidopsis_thaliana_HDA7

2b_Saccharomyces_cerevisiae_Hos1

2b_Trichomonascus_ciferrii_HDAC8

2b_Candida_albicans_HDAC

2c_Bacillus_subtilis_AcuC

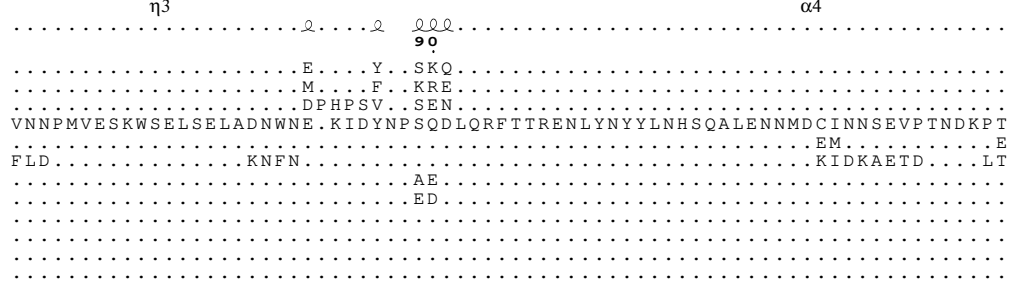
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2d_Dictyoglomus_turgidum_AcuC

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2d_Pseudomonadota_bacterium_AcuC



2a_Homo_sapiens_HDAC2

2a_Homo_sapiens_HDAC2

2a_Saccharomyces_cerevisiae_Rpd3

2a_Arabidopsis_thaliana_HDA7

2b_Saccharomyces_cerevisiae_Hos1

2b_Trichomonascus_ciferrii_HDAC8

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2c_Bacillus_subtilis_AcuC

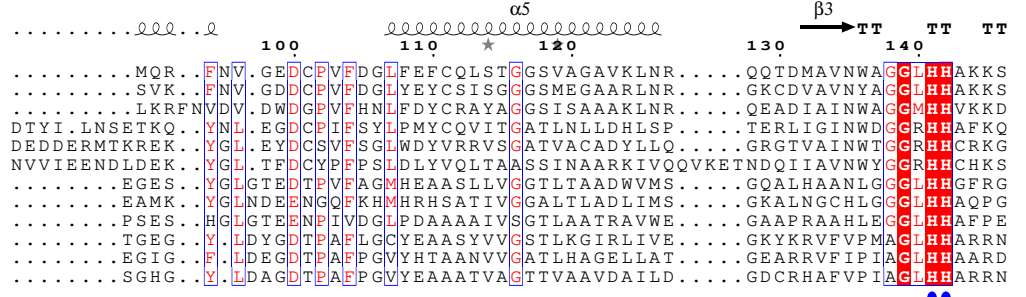
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2d_Dictyoglomus_turgidum_AcuC

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2d_Pseudomonadota_bacterium_AcuC



2a_Homo_sapiens_HDAC2

2a_Homo_sapiens_HDAC2

2a_Saccharomyces_cerevisiae_Rpd3

2a_Arabidopsis_thaliana_HDA7

2b_Saccharomyces_cerevisiae_Hos1

2b_Trichomonascus_ciferrii_HDAC8

2b_Candida_albicans_HDAC

2c_Bacillus_subtilis_AcuC

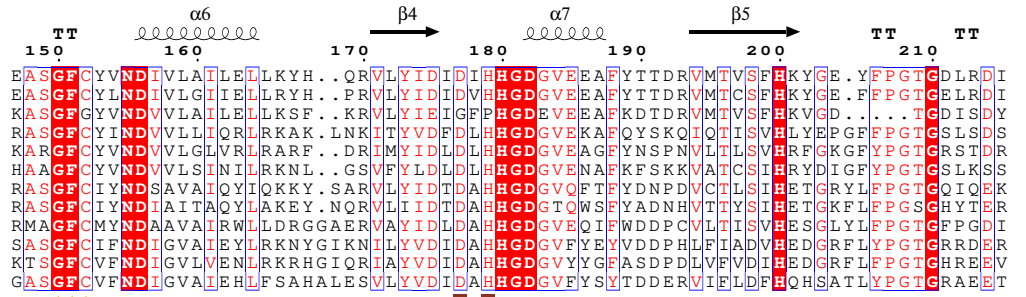
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2d_Dictyoglomus_turgidum_AcuC

2d_Wenzhouxiangella_sp_XN24_AcuC

2d_Pseudomonadota_bacterium_AcuC



2a_Homo_sapiens_HDAC2

η4 β6 α8 β7 η5 α9

220 230 240 250 260 270 280

2a_Homo_sapiens_HDAC2
 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
 2d_Dictyoglomus_turgidum_AcuC
 2d_Wenzhouxiangella_sp_XN24_AcuC
 2d_Pseudomonadota_bacterium_AcuC

2a_Homo_sapiens_HDAC2

β8 α10

290 300 310 320 330

2a_Homo_sapiens_HDAC2
 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
 2d_Dictyoglomus_turgidum_AcuC
 2d_Wenzhouxiangella_sp_XN24_AcuC
 2d_Pseudomonadota_bacterium_AcuC

2a_Homo_sapiens_HDAC2

η6

340

2a_Homo_sapiens_HDAC2
 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
 2d_Dictyoglomus_turgidum_AcuC
 2d_Wenzhouxiangella_sp_XN24_AcuC
 2d_Pseudomonadota_bacterium_AcuC

2a_Homo_sapiens_HDAC2

α11

350 360 370 380 390 400 410

2a_Homo_sapiens_HDAC2
 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
 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
 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_Wenzhouxiangella_sp_XN24_AcuC
 2d_Pseudomonadota_bacterium_AcuC

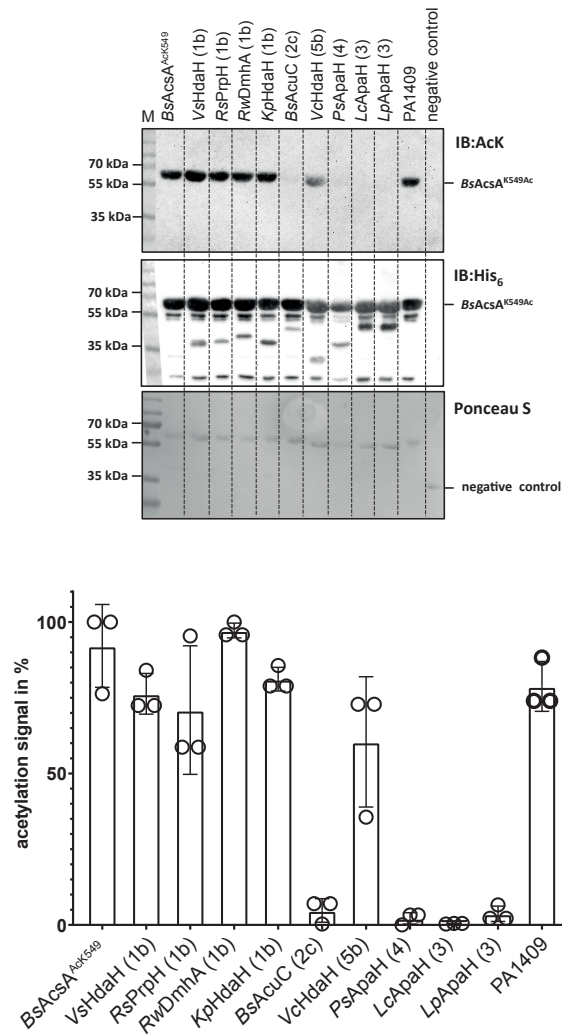
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480

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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 .....
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Supplementary Figure 16: Primary sequence alignment of selected bacterial deacylases of cluster 2 (UniProt accession numbers are shown).

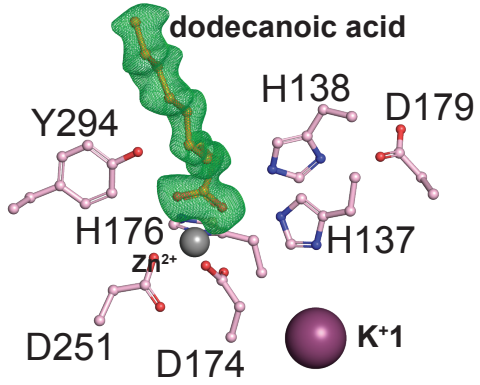
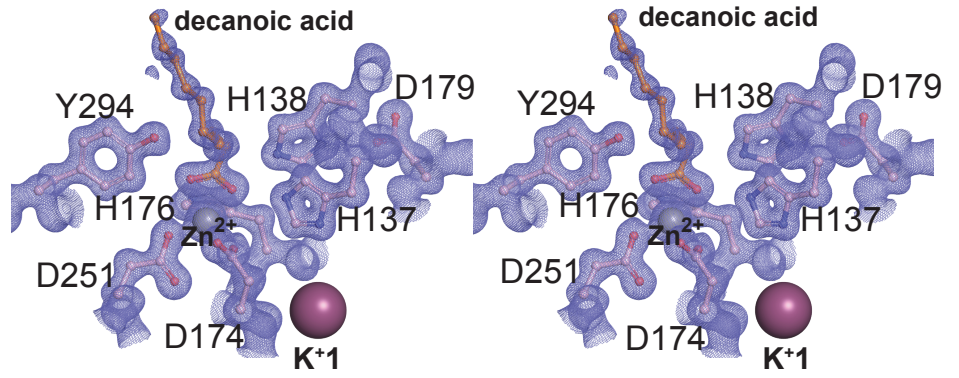
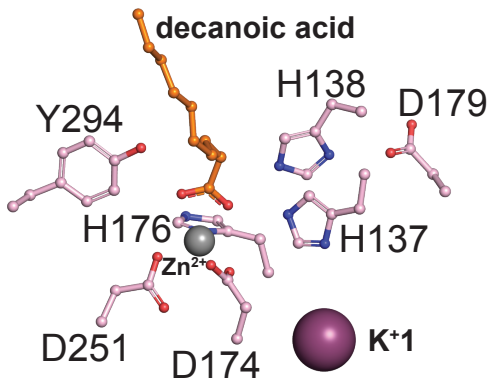
Show are the following sequences of the following deacylases representing enzymes of cluster 2 and the sub-clusters (code: sub-cluster, accession number/protein name/species): 2a, Q92769/HDAC2/*Homo sapiens*; 2a, P32561/Rpd3/*Saccharomyces cerevisiae* (strain ATCC 204508 / S288c) (Baker's yeast); 2a, 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 server¹. 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 3.0².



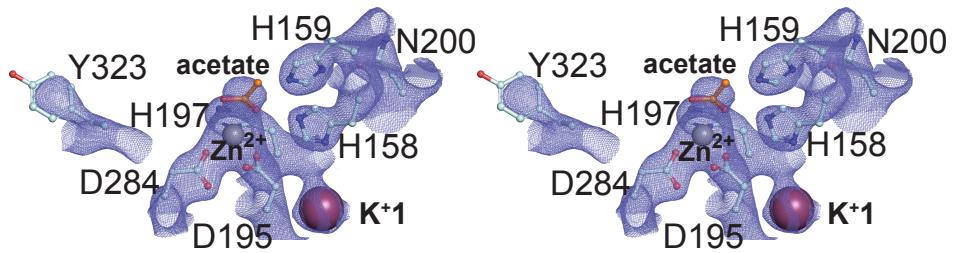
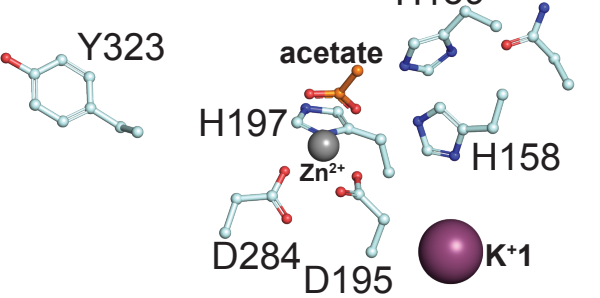
Supplementary Figure 17: Deacetylation of acetylated *Bacillus subtilis* deacetylase AcsA by bacterial deacylases.

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 His₆-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-His₆-blot (IB: His₆) 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 \pm SD. Source data are provided as Source Data file.

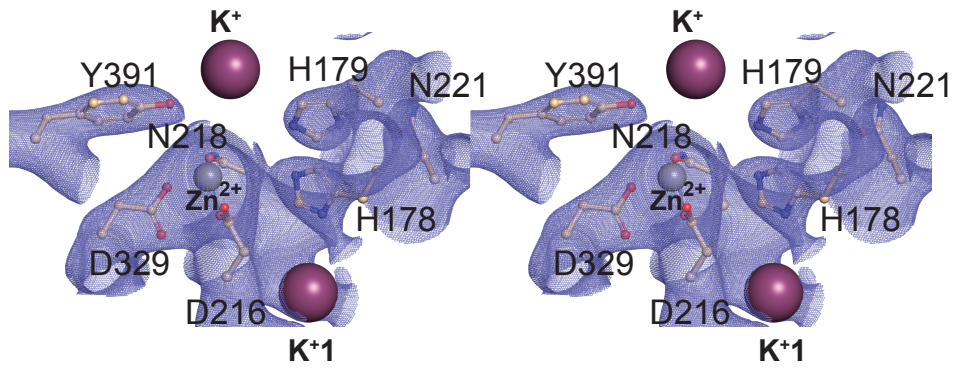
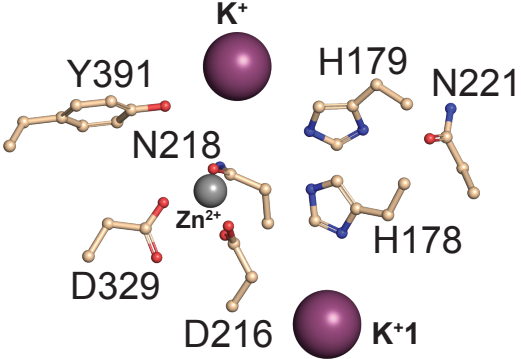
VcHdaH (5b)



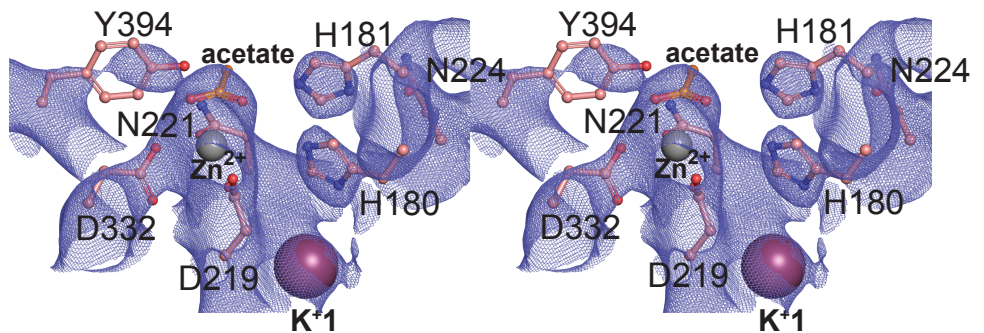
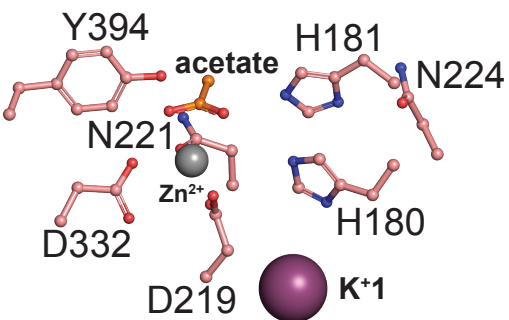
PsApaH (4)



LcApaH (3)



LpApaH (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: *VcHdaH* of sub-cluster 5b, *LcApaH* as well as *LpApaH* of cluster 3 and *PsApaH* 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 *VcHdaH* (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 2.3.4³.

5a_Acinetobacter_wuhouensis_HDAC

80 90 100 110 α5 β3 TT

5a_Acinetobacter_wuhouensis_HDAC KP...WNEQLRDAVLAINAGQLMATELAFE...H.GI.A.ANIAQCFHH

5a_Neisseriaceae_bacterium_HDAC L...KTADSLWINGSFVSAARYAVK...N.KI.STVSPSTSGFHH

5a_Treponema_sp_HDAC F...PEVAAALPWTGSMYAAAWAAE...RGGF.A.ASPTSAGFHH

5b_Vibrio_cholerae_HdaH FP...WSKTLIERTLHSVGGTCLTVEQALQ...S.GV.A.IHLSGGYHH

5b_Pseudomonas_alcaligenes_AcuC LP...WSEPLARRTRVAVGGSLLAELALK...H.GA.A.CHLAGGTHH

5b_Leptospiraceae_bacterium_HDAC LP...FSEQLIHRTLIAPAGTLLASKLAL...H.GV.A.CHIGGTHH

5c_Moraxella_bovis_HDAC LP...MSKELIDRERLVVGTIECAKFLN...D.GI.S.LSTSGCTHH

5c_Flavobacterium_indicum_HDAC-family FP...LSSELIEREFRLAQGTIEGAKNA...D.KI.A.FNIAAGTTHH

5c_Bdellovibrionales_bacterium_HDAC FP...WSPIMPLRSRASVGGFMACRYALE...N.GF.A.GNLSGCTHH

5d_Turneriella_parva_HdaH IP...IDERIVNAVCTAAGGTILAEALALK...H.GV.A.SNLSGGFHH

5d_Leptospira_interrogans_HdaH LP...LTKQIVHSFVLAVGGTILSMELTQK...Y.KF.V.YHIGGGFHH

5d_Leptospira_brenneri_HDAC LP...LNRSIVESFMYGVGGTVMAEELSKN...F.QF.A.FNMGGYHH

5e_Archangium_gephyra_HDAC DV...YVDEVLRTLRLAVGATIAASFSLR...R.RVPT.INLFGCFHH

5e_Nannocystis_exedens_AcuC EV...DTARILDQRAMTGGTMLATDIALG...TGKI.A.INLGGCFHH

5e_Deltaproteobacteria_bacterium_HDAC VP...PETVDV.DALVRGQRRVGGTVEAALKVTRG...EV.KV.G.FNLGGFHH

5f_Micavibrio_sp_HDAC_domain VP...FT.LTQHLLVTPLLYHTGGTILAGELAAE...K.GW.A.MNLGGAAHH

5f_Homo_sapiens_HDAC11 LP...NF.LVQKVLRLPRTQGGTILMAGKLA...R.GW.A.INVGGFHH

5f_Arabidopsis_thaliana_HDA2 FP...NF.LVQKVLRLPRTQGGTILMAGKLA...R.GW.A.INVGGFHH

5g_Bacteriovorax_stolpii_AcuC-like RD...W.KELVETILMQVAMTYNSTKYALK...N.GF.S.YHLGCMHH

5g_Gracilinema_caldarium_HdaH RP...L.SELFTTIRAQVGGTYLAARLALAHRSKPSGVSSEPLSTNNSNP.GF.C.YYLGGMHH

5g_Bacteriovorax_sp_MedPE-SWde_HDAC_domain LP...L.SDFVKKALSHINGTILACESAMD...S.GF.S.YHLGGMHH

5a_Acinetobacter_wuhouensis_HDAC

120 130 140 α6 β4 α7 β5 TT TT

5a_Acinetobacter_wuhouensis_HDAC ARP...EYGGSFCTFNGLALIAQ...Q.YPNKRIFTLDCQHGDDTAEFTRKYNNLYN.FSIYGLA...

5a_Neisseriaceae_bacterium_HDAC AEYK...KICGFCTFNGLMLAVLLKKE.GLANKIAILD...DMHYNGT...TDNIINKLKIDY.VSHYTLG...

5a_Treponema_sp_HDAC ACW...DRGMGFCFTFNGLAIAAVLLRER.GLARRV...GILDLDMHYNGTAEILDRGLSSEVHLHYTFG...

5b_Vibrio_cholerae_HdaH AHAD...DFSGFCFLNDAIAAHFALSRL.PSVDKV...LIDSDVHHGGDTATLCAERDDIIT.VSFHCDK...

5b_Pseudomonas_alcaligenes_AcuC AHF...DEASGFCFLNDLAVVARYMLES.GRAGRV...LIDSDVHHGGDTARLLENVPDAIT.VSLHCEK...

5b_Leptospiraceae_bacterium_HDAC AHY...SFGSGFCFLNDLAFSSALNQM.KPDLKIL...FIDHDVHGGDTASILKNEKNIYT.VSVHCEK...

5c_Moraxella_bovis_HDAC AFAD...SSEGGFCFLNDICVANSVLLNQ.GLAKRIL...LIDHDVHGGGNASIMADNPHVVF.VSMHGKK...

5c_Flavobacterium_indicum_HDAC-family AYS...NRGEAFCLNDQAIQAQYLLNQ.KLAKQIL...LIDHDVHGGNGTAEIFQNNRPFVTF.VSVHGKS...

5c_Bdellovibrionales_bacterium_HDAC AFAD...HAEAFCLNDFAVAAYVLKQK.HKITQLA...IVDLDVHGGGNASMLQSGQGIIFI.VSMHGEK...

5d_Turneriella_parva_HdaH AFAD...HAEAFCLNDTVLAIARLRKT.RPGLKV...AVDLDVHGGNGTAKLLQGDENSYTF.VSMHEKE...

5d_Leptospira_interrogans_HdaH SMP...DRAEGFCYLNDAAIASKLYQKE.YPDKKIL...FIDLDLHGGNGNSYIFQNDKPVFT.VSMHQEN...

5d_Leptospira_brenneri_HDAC SFP...DKAEGFCYLNDAVAIRKQKET.NPDLNAL...IIDLHGGNGNSYIFQYDDKPVFT.VSMHQEN...

5e_Archangium_gephyra_HDAC AAP...NRGAFCALNDMAVAELRAQ.GFQGV...LIDDFHPDGTASCLGDDASVWL.GSISGAD...

5e_Nannocystis_exedens_AcuC AHR...AIGRGGFCVHDDVAVAIAEORAL.GFHERI...LVVDLDHGGDTRALFADDPVFT.VSIIHNH...

5e_Deltaproteobacteria_bacterium_HDAC AEP...SKGSGFCYLNDAVAIARVRHD.GYKGRIL...AVDLDHGGNGSLVAFADDPVFT.VSVHGS...

5f_Micavibrio_sp_HDAC_domain AHAN...DASGFCMVADITMAIKNLRQRHKHMRN...IMIDLDAHGGNGRPFADNDPVYTI.IDMFTYGV...

5f_Homo_sapiens_HDAC11 CSS...DRGGGFCAYADITLAIKFLFERVEGISRA...TIDLDAHGGNGHERDFMDKRVYI.MDVYNRH...

5f_Arabidopsis_thaliana_HDA2 CTA...ERGGGFCAFADISLCHFAFLR.LRISRVM...TIDLDAHGGNGHETDLDGDNRVYI.LDMYNPE...

5g_Bacteriovorax_stolpii_AcuC-like SMS...FAGRGFCVNDIVITLRKLQNE.GAIKTAW...VVDVHGGDGAPEILQNDTIRT.FSITHMKE...

5g_Gracilinema_caldarium_HdaH ARY...DSGAGFCFLNDIITIAARRLKAE.GLISSI...WIDLDAHGGDTAELASGDPICILT.VSITHMAL...

5g_Bacteriovorax_sp_MedPE-SWde_HDAC_domain AMS...ERPGGFCFLNDMVTAIKYLQQN.GKIKNAV...IDLDAHGGDTAQMTYSDDSIIRS.VSITHMKG...

5a_Acinetobacter_wuhouensis_HDAC

180 190 200 210 220 β6 α8 β7 TT TTT

5a_Acinetobacter_wuhouensis_HDAC ...FGY...TYER...AESRH...VHTKNG.SF.AQ...YMLA...LHEAFQK...AH.E.WQAD...I...I

5a_Neisseriaceae_bacterium_HDAC ...KHY...TQDE...QEVVA...AK...FLKEL...LPSIVED...LI...KDV...V...L...F

5a_Treponema_sp_HDAC ...G.D...PKTADR...EGFDG...ELWLAL...LDGIVERG...F...ADCE...V...L

5b_Vibrio_cholerae_HdaH ...NF...ARKP...ASSMD...VGFANQ...T...D...E...F...L...S...T...F...I...Q...V...M...E...S...A...V...N...L...Y...R...P...D...I...L

5b_Pseudomonas_alcaligenes_AcuC ...NF...ARKA...KSDWD...IGLPIGM...D...T...D...Y...L...K...V...H...D...S...L...G...Y...L...L...L...A...L...Y...P...D...I...V...L

5b_Leptospiraceae_bacterium_HDAC ...NF...YRKE...TSTVD...VGLYAGMS...D...D...E...Y...L...T...I...V...E...S...T...L...N...E...S...I...S...R...F...S...P...D...I...V...L

5c_Moraxella_bovis_HDAC ...NY...FIKP...LSDLD...IELDDGTG...D...D...D...Y...L...S...L...K...Y...H...L...P...K...V...L...D...D...F...A...P...D...F...V...F

5c_Flavobacterium_indicum_HDAC-family ...NY...FKKE...QSDLD...IALPDQTS...D...A...D...Y...L...A...L...I...S...K...E...I...P...K...L...I...Q...Q...V...Q...P...D...F...H...F

5c_Bdellovibrionales_bacterium_HDAC ...NY...FKKI...NSDLD...IALPNQ...C...D...D...E...Y...L...T...K...L...S...D...A...L...P...K...V...I...A...T...R...P...Q...I...L

5d_Turneriella_parva_HdaH ...NY...VKE...TGSHD...VELPSHLG...D...A...E...Y...L...R...L...A...E...N...L...D...K...L...K...A...F...F...P...D...L...I...F

5d_Leptospira_interrogans_HdaH ...LY...KKE...KSDLD...ISLEEG...I...G...D...K...E...Y...L...E...L...L...E...K...S...L...R...K...I...E...S...D...F...K...P...D...L...I...F

5d_Leptospira_brenneri_HDAC ...LY...KKE...VSNLD...VNLEPNTK...D...D...E...Y...L...S...T...L...E...S...S...L...N...R...N...I...R...K...D...F...D...S...N...I...Y

5e_Archangium_gephyra_HDAC ...WGS...L.P...G.VDE...VRLPPGTS...D...G...A...Y...L...D...A...L...D...G...L...D...R...M...P...S...L...E...L...V...F

5e_Nannocystis_exedens_AcuC ...HWG...TEAI...ASTS...VALGDVVG...D...T...L...Y...L...E...T...V...Q...L...H...T...E...A...L...R...Q...H...R...P...R...L...Y

5e_Deltaproteobacteria_bacterium_HDAC ...WV...QRQAT...ADAG...IQLPRTG...D...R...A...Y...L...E...A...L...R...Y...S...L...P...D...V...F...A...E...F...K...P...E...V...V...F

5f_Micavibrio_sp_HDAC_domain PDGDMFY...P.DDRRAM...QRINIP...VKLNFNTK...D...D...T...Y...L...Q...L...Q...N...A...L...K...T...A...K...T...D...F...A...P...D...M...I...F

5f_Homo_sapiens_HDAC11 ...IY...P.GDRFAK...QAIRK...VELEWGT...D...D...E...Y...L...D...K...V...E...R...N...I...K...K...S...L...Q...E...H...L...P...D...V...V...F

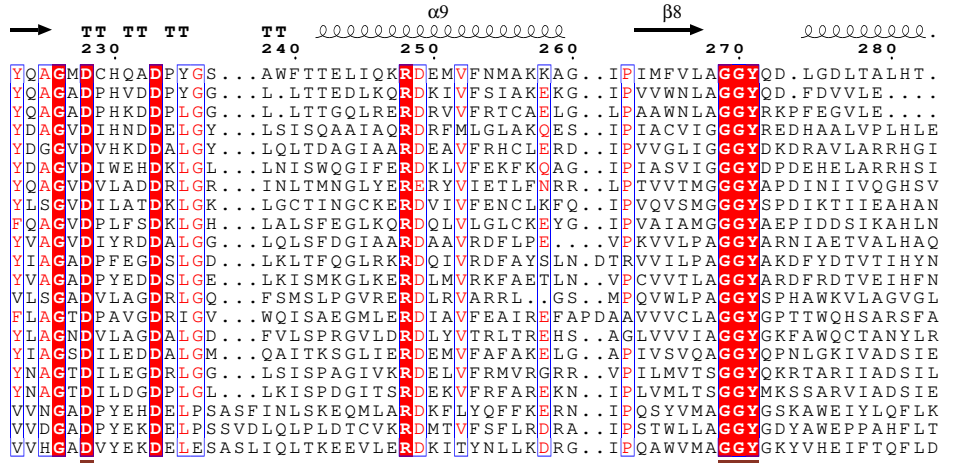
5f_Arabidopsis_thaliana_HDA2 ...IY...P.FDYRAR...RFIDQK...VEVMSGTT...D...D...E...Y...L...R...K...L...D...E...A...L...E...V...A...S...R...N...F...Q...P...E...L...V...I

5g_Bacteriovorax_stolpii_AcuC-like ...GW...P.LNSGTVRDP...WFIPSSID...VGIDV...G...E...D...D...Q...Y...L...A...R...L...E...S...L...L...E...L...M...Q...E...R...F...P...D...V...I

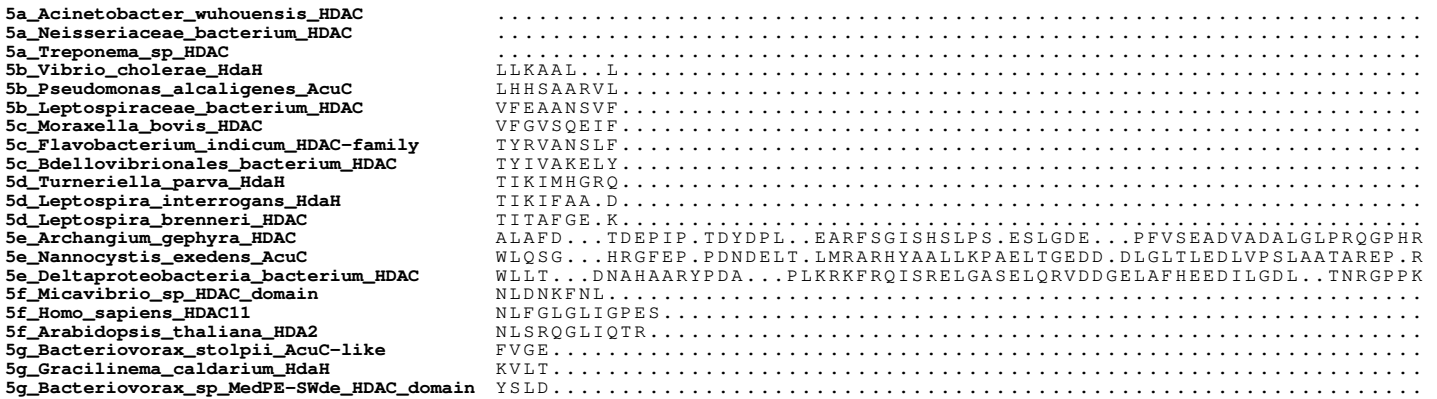
5g_Gracilinema_caldarium_HdaH ...GW...P.LDPETLAHAVPSRAPLVASD...DIPVSKED...QAS...Y...V...S...R...L...A...S...G...L...Q...L...E...M...L...S...G...G...K...P...D...L...A...I

5g_Bacteriovorax_sp_MedPE-SWde_HDAC_domain ...GW...P.LDKEDKNDP...S.YIPSSFD...I...E...V...E...R...D...D...D...Y...L...S...L...K...K...G...L...E...D...F...N...P...R...E...D...F...A...I

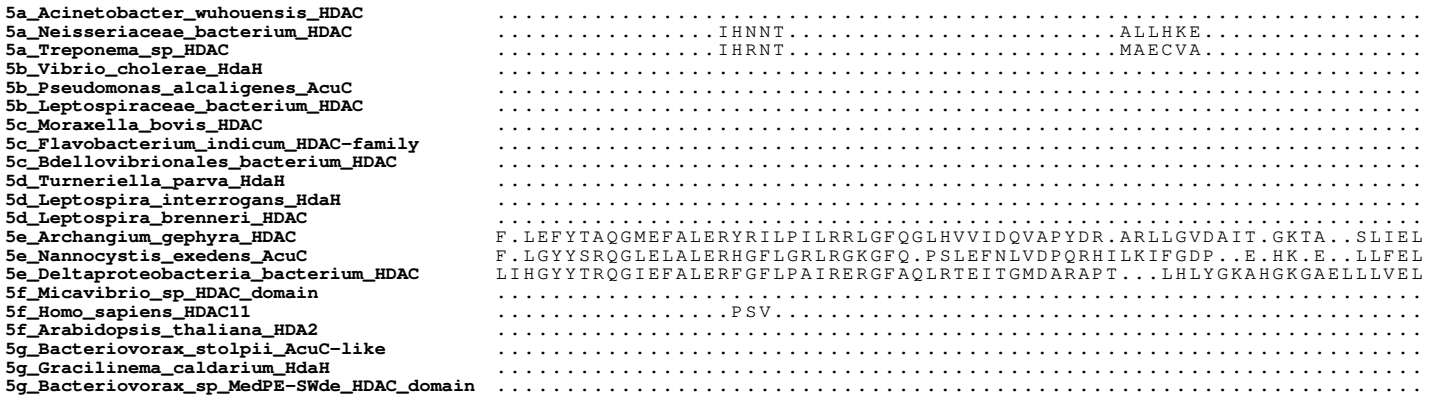
5a_Acinetobacter_wuhouensis_HDAC



5a_Acinetobacter_wuhouensis_HDAC



5a_Acinetobacter_wuhouensis_HDAC



5a_Acinetobacter_wuhouensis_HDAC QQ

5a_Acinetobacter_wuhouensis_HDAC QT

5a_Neisseriaceae_bacterium_HDAC IL

5a_Treponema_sp_HDAC AQGD

5b_Vibrio_cholerae_HdaH VYYG

5b_Pseudomonas_alcaligenes_AcuC IL

5b_Leptospiraceae_bacterium_HDAC AQGD

5c_Moraxella_bovis_HDAC VYYG

5c_Flavobacterium_indicum_HDAC-family IL

5c_Bdellovibrionales_bacterium_HDAC AQGD

5d_Turneriella_parva_HdaH VYYG

5d_Leptospira_interrogans_HdaH IL

5d_Leptospira_brenneri_HDAC AQGD

5e_Archangium_gephyra_HDAC VYYG

5e_Nannocystis_exedens_AcuC IL

5e_Deltaproteobacteria_bacterium_HDAC AQGD

5f_Micavibrio_sp_HDAC_domain VYYG

5f_Homo_sapiens_HDAC11 IL

5f_Arabidopsis_thaliana_HDA2 AQGD

5g_Bacteriovorax_stolpii_AcuC-like VYYG

5g_Gracilinema_caldarium_HdaH IL

5g_Bacteriovorax_sp_MedPE-SWde_HDAC_domain AQGD

5a_Acinetobacter_wuhouensis_HDAC QQ

5a_Acinetobacter_wuhouensis_HDAC 290

5a_Neisseriaceae_bacterium_HDAC VYYG

5a_Treponema_sp_HDAC IL

5b_Vibrio_cholerae_HdaH AQGD

5b_Pseudomonas_alcaligenes_AcuC VYYG

5b_Leptospiraceae_bacterium_HDAC IL

5c_Moraxella_bovis_HDAC AQGD

5c_Flavobacterium_indicum_HDAC-family VYYG

5c_Bdellovibrionales_bacterium_HDAC IL

5d_Turneriella_parva_HdaH AQGD

5d_Leptospira_interrogans_HdaH VYYG

5d_Leptospira_brenneri_HDAC IL

5e_Archangium_gephyra_HDAC AQGD

5e_Nannocystis_exedens_AcuC VYYG

5e_Deltaproteobacteria_bacterium_HDAC IL

5f_Micavibrio_sp_HDAC_domain AQGD

5f_Homo_sapiens_HDAC11 VYYG

5f_Arabidopsis_thaliana_HDA2 IL

5g_Bacteriovorax_stolpii_AcuC-like AQGD

5g_Gracilinema_caldarium_HdaH VYYG

5g_Bacteriovorax_sp_MedPE-SWde_HDAC_domain IL

5a_Acinetobacter_wuhouensis_HDAC E

5a_Neisseriaceae_bacterium_HDAC G

5a_Treponema_sp_HDAC RTWAAAEL D

5b_Vibrio_cholerae_HdaH S AG Y

5b_Pseudomonas_alcaligenes_AcuC A QL R

5b_Leptospiraceae_bacterium_HDAC N V L

5c_Moraxella_bovis_HDAC K LL A

5c_Flavobacterium_indicum_HDAC-family S I

5c_Bdellovibrionales_bacterium_HDAC S K

5d_Turneriella_parva_HdaH WEADEMVRW.RDEN DIPQDKA.LIVAERE.RSHFEFREK...

5d_Leptospira_interrogans_HdaH YEPSPMIFPISDEL KAELDPENYLRAIDEAADEYRFQLSSPR

5d_Leptospira_brenneri_HDAC WEPGDHVLVPSERLTEYFASRVFSAKADEALRDLTN QG.LVV.V

5e_Archangium_gephyra_HDAC WEPGDHVLVPSERLTEYFASRVFSAKADEALRDLTN QG.LVV.V

5e_Nannocystis_exedens_AcuC WEPGDHVLVPSERLTEYFASRVFSAKADEALRDLTN QG.LVV.V

5e_Deltaproteobacteria_bacterium_HDAC WEPGDHVLVPSERLTEYFASRVFSAKADEALRDLTN QG.LVV.V

5f_Micavibrio_sp_HDAC_domain WEPGDHVLVPSERLTEYFASRVFSAKADEALRDLTN QG.LVV.V

5f_Homo_sapiens_HDAC11 WEPGDHVLVPSERLTEYFASRVFSAKADEALRDLTN QG.LVV.V

5f_Arabidopsis_thaliana_HDA2 WEPGDHVLVPSERLTEYFASRVFSAKADEALRDLTN QG.LVV.V

5g_Bacteriovorax_stolpii_AcuC-like WEPGDHVLVPSERLTEYFASRVFSAKADEALRDLTN QG.LVV.V

5g_Gracilinema_caldarium_HdaH WEPGDHVLVPSERLTEYFASRVFSAKADEALRDLTN QG.LVV.V

5g_Bacteriovorax_sp_MedPE-SWde_HDAC_domain WEPGDHVLVPSERLTEYFASRVFSAKADEALRDLTN QG.LVV.V

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/species): 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 server¹. 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 3.0².

Mycoplana_ramosa_ApaH

Mycoplana_ramosa_ApaH P F E
Pseudomonas_aeruginosa_ApaH2 C F E
Pseudomonas_aeruginosa_ApaH1 C F E
Pseudomonas_sp_M30-35_ApaH C F E
Candidatus_Scalindua_brodac HDAC2 LAR... REAQEAIKMLTTRFAKTDTDTGYIDQVIESFRDDPVTF
Burkholderia_pseudomallei_ApaH PQ E
Ralstonia_insidiola_ApaH PQ E
Chloroflexi_bacterium_RBG_16_48_8_HdaH D E
Fervidobacterium_pennivorans_HDAC NP E N
Bordetella_hinzii_ApaH1 PNP D
Bordetella_bronchiseptica_253_ApaH ST E
Spirochaetae_bacterium_HGW-Spirochaetae-5_ApaH CMSDN E
Kosmotoga_pacifica_HDAC EN E
Chromatiales_bacterium_ApaH ST E
Micavibrio_aeruginosavorus_ApaH N F E
Laribacter_hongkongensis_ApaH C F E
Pseudomonas_putida_HDAC_family PQ E
Bacteroidetes_bacterium_GWF2_33_16_HDAC PDH KL
Lentisphaerae_bacterium_GWF2_52_8_HDAC H AP

Mycoplana_ramosa_ApaH

Mycoplana_ramosa_ApaH AEWI LAA VK.EAGF
Pseudomonas_aeruginosa_ApaH2 ADMV LDR VK.AVGL
Pseudomonas_aeruginosa_ApaH1 ADTV LAR VK.SQNL
Pseudomonas_sp_M30-35_ApaH ADHI LQR VK.DRNI
Candidatus_Scalindua_brodac HDAC2 PLRYI KAVKENRIIRDFNSFLAYTFVSPKHEIHVKE.ERGYFE
Burkholderia_pseudomallei_ApaH AARL VAA AF.AMGF.P
Ralstonia_insidiola_ApaH ARNL LQA AR.DLGF.T
Chloroflexi_bacterium_RBG_16_48_8_HdaH VEVLL QA F.SAA.G
Fervidobacterium_pennivorans_HDAC L.KLVL NY LQ.EN.F.Q
Bordetella_hinzii_ApaH1 LAGL REGVAA MG.Y.A
Bordetella_bronchiseptica_253_ApaH ADRL LAA AQ.RRGH.E
Spirochaetae_bacterium_HGW-Spirochaetae-5_ApaH PEYERESLLFYGNPYFLFDNLG IKKDLSSL.NDSRKI
Kosmotoga_pacifica_HDAC I.ESI RER ME.NF.FG
Chromatiales_bacterium_ApaH AQRL LAG VA.RCGL.E
Micavibrio_aeruginosavorus_ApaH V FF VR.DQIL.HRG
Laribacter_hongkongensis_ApaH ADMV REA V.DAA
Pseudomonas_putida_HDAC_family IEPL LAV VN.KLGY.P
Bacteroidetes_bacterium_GWF2_33_16_HDAC SGLGGAIY TR VR.EEAELLNANGL
Lentisphaerae_bacterium_GWF2_52_8_HDAC GRGVGGALY ER VR.EEVLMKSKGL

Mycoplana_ramosa_ApaH

Mycoplana_ramosa_ApaH D DV.V.A.P ARH.GLE.TV
Pseudomonas_aeruginosa_ApaH2 G AV.R.A.P RDF.GLE.PI
Pseudomonas_aeruginosa_ApaH1 G EV.I.A.P KDF.GRE.PL
Pseudomonas_sp_M30-35_ApaH G EI.R.E.P QDF.GRD.PI
Candidatus_Scalindua_brodac HDAC2 RPIRVETIRSVIRDVGGFVSTV REY.GE.K.WI
Burkholderia_pseudomallei_ApaH V.R.E.P DDF.GI.A.PI
Ralstonia_insidiola_ApaH I.A.Q.P EDH.GL.A.PL
Chloroflexi_bacterium_RBG_16_48_8_HdaH WD ST.L.Q.P T.DH.GL.G.PI
Fervidobacterium_pennivorans_HDAC A.A.I.Q.P F.EE.H.I
Bordetella_hinzii_ApaH1 I.H.A.P EDF.GL.Q.YL
Bordetella_bronchiseptica_253_ApaH RV E ARDF.G.E.AI
Spirochaetae_bacterium_HGW-Spirochaetae-5_ApaH FR AV.L.E.R KHERDVND.GLKKFISSMKGDPO.II
Kosmotoga_pacifica_HDAC YT IQ E SEY.HF.ESYI
Chromatiales_bacterium_ApaH V.V.T.P DEF.GA.S.PR
Micavibrio_aeruginosavorus_ApaH FS EI.I.A.P EEF.PA
Laribacter_hongkongensis_ApaH GF TDH.GKE.PI
Pseudomonas_putida_HDAC_family L.F.E.P ADH.GL.A.PL
Bacteroidetes_bacterium_GWF2_33_16_HDAC FF EC.LPDD.P ALCKDGDILLQNKRLAFYSEF.GA.R.PI
Lentisphaerae_bacterium_GWF2_52_8_HDAC FF EC.LPDD.P LLCKSPLVLRQNAQRLKFYEKY.GA.R.PI

Mycoplana_ramosa_ApaH

Mycoplana_ramosa_ApaH LK.VHD.AG.YL.N.F
Pseudomonas_aeruginosa_ApaH2 RR.VHS.EG.FV.R.F
Pseudomonas_aeruginosa_ApaH1 LR.LHD.AA.YL.D.F
Pseudomonas_sp_M30-35_ApaH QR.IHT.AE.YL.N.F
Candidatus_Scalindua_brodac HDAC2 L.S.VHD.KT.FV.H.Y
Burkholderia_pseudomallei_ApaH A.A.VHD.TH.YL.R.F
Ralstonia_insidiola_ApaH T.A.VHG.EP.YL.R.F
Chloroflexi_bacterium_RBG_16_48_8_HdaH L.D.VHD.SD.YV.D.F
Fervidobacterium_pennivorans_HDAC L.K.VHS.EK.YF.N.Y
Bordetella_hinzii_ApaH1 S.R.VHT.AR.YL.D.F
Bordetella_bronchiseptica_253_ApaH L.AE.VHC.PR.YL.D.F
Spirochaetae_bacterium_HGW-Spirochaetae-5_ApaH R.KSVHSNDDFNHYSFTVPADRKIPLLYNDGHEIHHIRDKG.YV.ESPVRIKSI
Kosmotoga_pacifica_HDAC Y.L.IHE.PE.YV.SW
Chromatiales_bacterium_ApaH A.A.VHD.AT.YL.D.F
Micavibrio_aeruginosavorus_ApaH DIFSR.VHD.PR.YV.A.F
Laribacter_hongkongensis_ApaH LA.VHR.EN.YV.R.F
Pseudomonas_putida_HDAC_family A.A.VHG.QA.YL.D.F
Bacteroidetes_bacterium_GWF2_33_16_HDAC ANTLYEMK.VNP.ED.DCPP.YL.VYDGLNKEFTLPAK
Lentisphaerae_bacterium_GWF2_52_8_HDAC I.NTKYETPVKEGGDNAP.YLVVDNLGTERS.F.KKS

Mycoplana_ramosa_ApaH 000 0.000 00.....

Mycoplana_ramosa_ApaH IET A.WDR WK.....

Pseudomonas_aeruginosa_ApaH2 IQN A.WQD WL.....

Pseudomonas_aeruginosa_ApaH1 IQG A.WAR WT.....

Pseudomonas_sp_M30-35_ApaH FEG A.WAR WQ.....

Candidatus_Scalindua_brodac_HDAC2 LRT V

Burkholderia_pseudomallei_ApaH LET V.HRE WK.....

Ralstonia_insidiosa_ApaH IQD A.HTD WR.....

Chloroflexi_bacterium_RBG_16_48_8_HdaH IQT V.YEK NK.....

Fervidobacterium_pennivorans_HDAC IQV V K.....

Bordetella_hinzii_ApaH1 IEL A.HAL WS.....

Bordetella_bronchiseptica_253_ApaH LKN G.YAQ WR.....

Spirochaetae_bacterium_HGW-Spirochaetae-5_ApaH LK VIEKSELFIKSKTSRYSERHITAVHDKDFV.....

Kosmotoga_pacifica_HDAC LKK K.SNE

Chromatiales_bacterium_ApaH LDC A.FSE WS.....

Micavibrio_aeruginosavorus_ApaH LKE G.YRR WE.....

Laribacter_hongkongensis_ApaH LET F.WER WS.....

Pseudomonas_putida_HDAC_family LGS A.YQQ WH.....

Bacteroidetes_bacterium_GWF2_33_16_HDAC TLKGIKAI LERKYGDYCPESYIKMVVNSVKDNPFLLRQPK..... YV.....

Lentisphaerae_bacterium_GWF2_52_8_HDAC RMRQIVKAI LERKYPDL NEE YVDMVINSISDDP

Mycoplana_ramosa_ApaH 0
80

Mycoplana_ramosa_ApaH AAG.Y K G EA I.....

Pseudomonas_aeruginosa_ApaH2 ATG.R S H DM L.....

Pseudomonas_aeruginosa_ApaH1 AEG.H S G DL V.....

Pseudomonas_sp_M30-35_ApaH EQD.G T G DL L.....

Candidatus_Scalindua_brodac_HDAC2 CAS.L RQG RP VY.....

Burkholderia_pseudomallei_ApaH AM.PE DW GD

Ralstonia_insidiosa_ApaH RL.PE DW GD

Chloroflexi_bacterium_RBG_16_48_8_HdaH SY.Q LA

Fervidobacterium_pennivorans_HDAC SQE.V E D EY L.....

Bordetella_hinzii_ApaH1 RL.P NA GP

Bordetella_bronchiseptica_253_ApaH ALPGA SQ EI

Spirochaetae_bacterium_HGW-Spirochaetae-5_ApaH KFF.K TA SA SV.....

Kosmotoga_pacifica_HDAC IESG.K

Chromatiales_bacterium_ApaH AL.PE ST PE A.....

Micavibrio_aeruginosavorus_ApaH E Q G ESHML.....

Laribacter_hongkongensis_ApaH RPS.R RSATGRDY DA L.....

Pseudomonas_putida_HDAC_family EV.PE DW GD

Bacteroidetes_bacterium_GWF2_33_16_HDAC KSR.K.ISSINKNIKEKNKILLIVNNKHAIHHIKERGYVESPVRVES.....

Lentisphaerae_bacterium_GWF2_52_8_HDAC VQLRPFKYVSN.KDIEPISRRLPVDTLIPLVYNTQIHVHHVQERG..... YVESPVVETMLS

Mycoplana_ramosa_ApaH

Mycoplana_ramosa_ApaH A.....

Pseudomonas_aeruginosa_ApaH2 P.....

Pseudomonas_aeruginosa_ApaH1 S.....

Pseudomonas_sp_M30-35_ApaH P.....

Candidatus_Scalindua_brodac_HDAC2 P.....

Burkholderia_pseudomallei_ApaH E A.....

Ralstonia_insidiosa_ApaH E V.....

Chloroflexi_bacterium_RBG_16_48_8_HdaH

Fervidobacterium_pennivorans_HDAC P.....

Bordetella_hinzii_ApaH1 E V.....

Bordetella_bronchiseptica_253_ApaH T P.....

Spirochaetae_bacterium_HGW-Spirochaetae-5_ApaH P.AGV5

Kosmotoga_pacifica_HDAC E.....

Chromatiales_bacterium_ApaH V.....

Micavibrio_aeruginosavorus_ApaH P.....

Laribacter_hongkongensis_ApaH P.....

Pseudomonas_putida_HDAC_family V.....

Bacteroidetes_bacterium_GWF2_33_16_HDAC IQRELDKTFGFHQDNALEYPERFIL

Lentisphaerae_bacterium_GWF2_52_8_HDAC ALNKTGLFNNIRERHFAEHHTSLHDRDFVDYLLKVCSAI PKES

Mycoplana_ramosa_ApaH

90 T T 100 000000 TT
110

Mycoplana_ramosa_ApaH TS.FP...VR RTSPRI P TDIEGQI GYYCN.AAETA

Pseudomonas_aeruginosa_ApaH2 IA.WP...TR RLRQTE P DNIDGRL GYYSF.DAGA P

Pseudomonas_aeruginosa_ApaH1 TT.FPGRRLR RDGPI P TALMGEL GYYSF.DTEA P

Pseudomonas_sp_M30-35_ApaH YT.WP...AR TSLQRL P TSLHGQL GYYSF.DAGA P

Candidatus_Scalindua_brodac_HDAC2 DT.FPI R RPDNR P KELPQA GYYC...IDTG

Burkholderia_pseudomallei_ApaH MS.NIF V REPNA.L RGVLAQAARHLA.DGSC P

Ralstonia_insidiosa_ApaH MS.NIF V REPNA.L RGVLAQAARYLA.DGSC P

Chloroflexi_bacterium_RBG_16_48_8_HdaH VFTWA FATR YTGKH P KHFLGQL GYYAF.GWGT P

Fervidobacterium_pennivorans_HDAC EV.FV D QIFDT.G TPIKKEI.FF...AAKRA

Bordetella_hinzii_ApaH1 VP.NAH P RRPDA.P PYPRGIVGRA GFHMY.DLACA

Bordetella_bronchiseptica_253_ApaH HV.HPP R EESGY.P VSIIGRA GFHT...ADTS

Spirochaetae_bacterium_HGW-Spirochaetae-5_ApaH LYPDTF.PIR KN VKP P KKLVSRA GYYCI.DIYS P

Kosmotoga_pacifica_HDAC VF...G H

Chromatiales_bacterium_ApaH IA.NVH P MHRRTNY.P HSIIGRA GWHMA.DTAC P

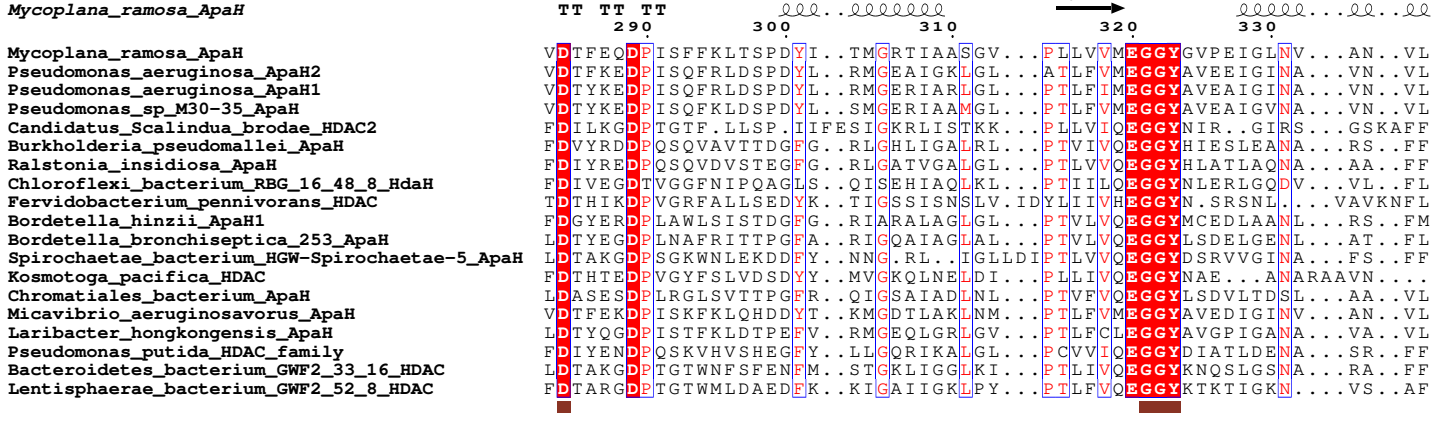
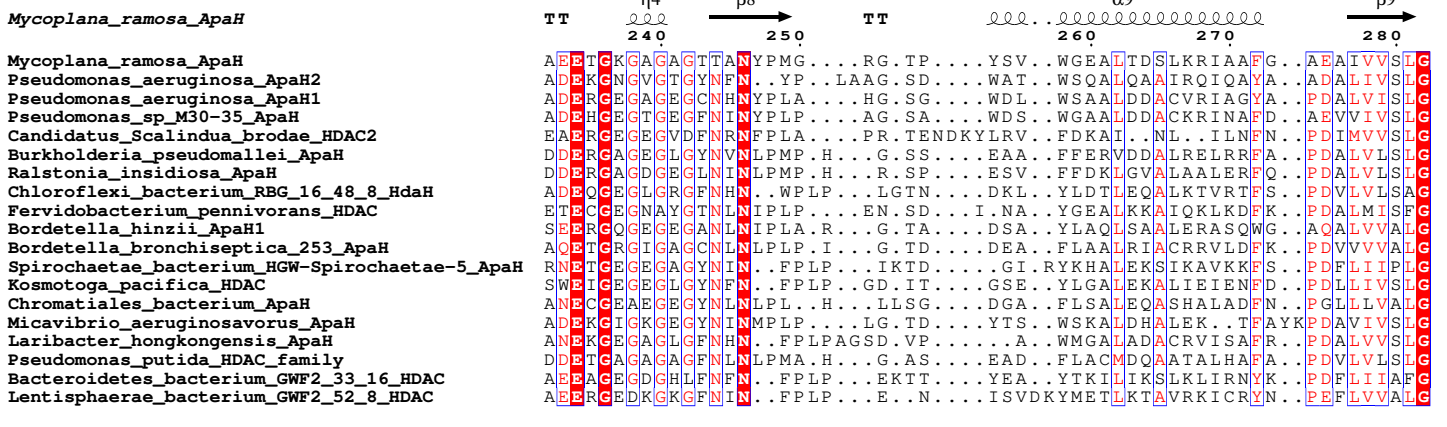
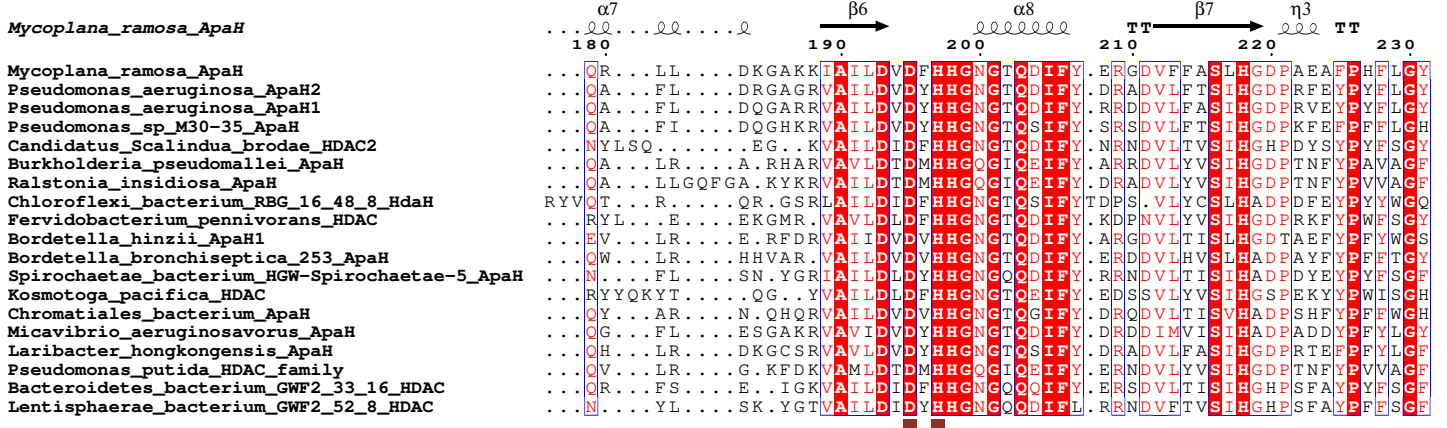
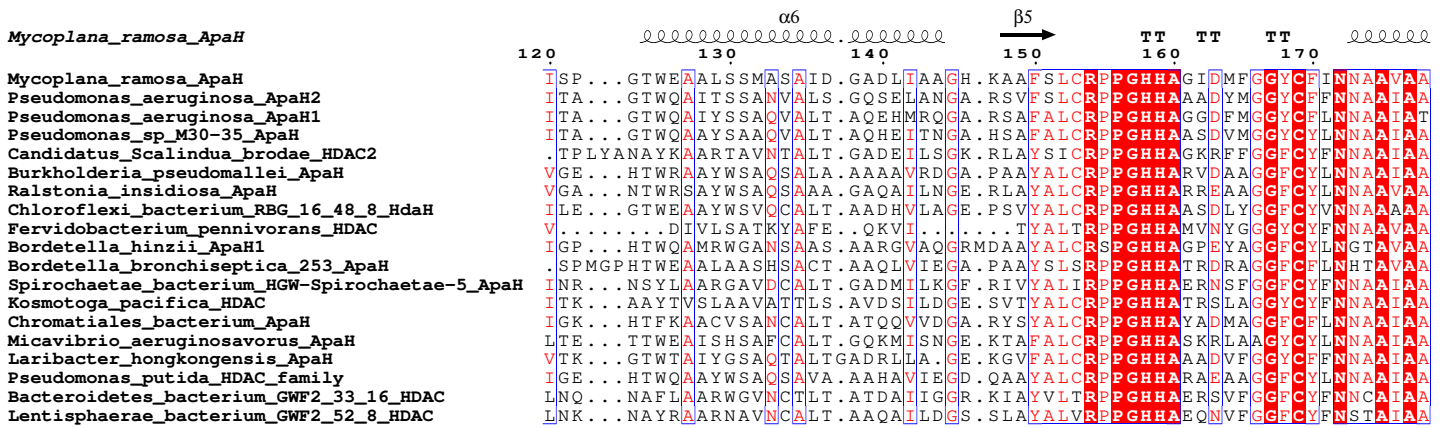
Micavibrio_aeruginosavorus_ApaH SV.FN...LQ

Laribacter_hongkongensis_ApaH LI.WP...TR CFRQVE P EDIDGQL GYFSM.DAGT P

Pseudomonas_putida_HDAC_family MS.NIY I MS.NIY REGNP.L RGLGKTARYLA.DGSC P

Bacteroidetes_bacterium_GWF2_33_16_HDAC KVHDRD.YVN...YFKVKCKNLSKGGKSVYPYVFP.IRNGTKA...P KELSVA GYYCF.DTFT P

Lentisphaerae_bacterium_GWF2_52_8_HDAC VYPYVF.PI RN NTRP P KDLPMA GYYCI.DTFT P



<i>Mycoplana_ramosa_ApaH</i>	. Q Q Q . Q 3 4 0
<i>Mycoplana_ramosa_ApaH</i>	. K G V . A . G
<i>Pseudomonas_aeruginosa_ApaH2</i>	. Q G F . E . G V H R
<i>Pseudomonas_aeruginosa_ApaH1</i>	. Q G Y . E . G A A R
<i>Pseudomonas_sp_M30-35_ApaH</i>	. E G F . E . N V C R P N N S K K D A
<i>Candidatus_Scalindua_brodae_HDAC2</i>	. K G C . N . E N R T
<i>Burkholderia_pseudomallei_ApaH</i>	. G G F G . A L R G
<i>Ralstonia_insidiosa_ApaH</i>	. S G L Q . S P H Q P A G
<i>Chloroflexi_bacterium_RBG_16_48_8_HdaH</i>	. S H F I Q S N R T
<i>Fervidobacterium_pennivorans_HDAC</i>	. A G L L . G E
<i>Bordetella_hinzii_ApaH1</i>	. T N F E . A A H R V E R G
<i>Bordetella_bronchiseptica_253_ApaH</i>	. D G F C Q H H R P T A . T L R
<i>Spirochaetae_bacterium_HGW-Spirochaetae-5_ApaH</i>	. Q G L Y E . G E Y G R K N Q
<i>Kosmotoga_pacifica_HDAC</i>	. F F . S . G M L K
<i>Chromatiales_bacterium_ApaH</i>	. E G V L . S
<i>Micavibrio_aeruginosavorus_ApaH</i>	. T G F . L . N R
<i>Laribacter_hongkongensis_ApaH</i>	. S G Y . D . S T A H G
<i>Pseudomonas_putida_HDAC_family</i>	. A G M A . A
<i>Bacteroidetes_bacterium_GWF2_33_16_HDAC</i>	. S G F Y . T A N N
<i>Lentisphaerae_bacterium_GWF2_52_8_HDAC</i>	F R G V W E . G S F R

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/species): 4, Q48935/ApaH/*Mycoplana ramosa* (*Mycoplana bullata*); 4, Q916H0/ApaH2/*Pseudomonas aeruginosa* (strain ATCC 15692 / DSM 22644 / CIP 104116 / JCM 14847 / LMG 12228 / 1C / PRS101 / PAO1); 4, Q913T5/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 server¹. 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 3.0².

Legionella_cherrii_ApaH

1 10
MR...HKY...FKK.EKVS.P
MDMAK.DKGF...FKK.AKME.K
MMM.K...HKY...FFR.HMTS.T
MKL...EELLYQ...FKT.HA.V.L...AEK...
MPR...LF...KPLPSTP.A...LKK.LQ...
MDE...KCKKWL...LT.IDCC.M...KEA...
MKN...GF...FKN.DKIK.A...
MGR...DEKKRI...YNT.RRMPKPTTEEVEEKVKRKHVTSASLNLQEEENELL...SNQ.DE...KPKPKLKTREPS
MPR...TF...FEK.HSPS.N...LRS...ELLA.LNRLF.KG...NKFLSIQTIE...E...
MKG...TL...FTVVRGND.K...ARK...NALIDKIKRVQ.QEYKELQKGTVNLD...
MN...RKF...FIE.PSL.S.L...LEK...RILC.CITNI.SS...SNPEEIQTSS...
MGLSQCXSSSF...EPQ

Legionella_cherrii_ApaH

ES
DV
Q.LT
NRLT
R.NY
VKNKKNKREATRTRKRRETTGAMHLETESKSTANTQYPEESIVKKTQAQS.KRSLKVNDSIGFFSVKKIHKAKNKDIEHQQT
SL...TF...NEYK...TLVSTYIQSPNYSKLKPVIQRVANNILNK...IN...E...
AF...CDFK...NKLEDEIENDSFYKLLPTNFNRSIHHMREE...LE...QQFP
RL...KDYL...LELKNYTQDTEFANIGLGLKREALKLLQ

Legionella_cherrii_ApaH

20 30 40 50 60
HK.EKNQECLIQIPSAKD...IQQM...HGM...AGAD...EDQFERL...KHM...TQVIATTQSK
NKRL...VT.PYSEECALIQIPSEIDNEQMQRMPAGGE...EDQYLR...KHM...SALIKKY...
HK.EKNQECCIQIPSAKDIEQM...HGM...AGAD...EDQLK...KHM...VVRVIEDYQST
ELDLV...KK.KWPTAITYQAPAYDEILAMRGM...YAGGK...QDLAR...LKL...LVKTLKTQ...
LET.LAPPLM...VOIP...SATD...LTYM...RGM...IAGTD...EDQAE...RL...GNM...TRLLHNF...
KLFMS...EK.MDRYGITY...VOIP...CKRD...IDAM...KGF...CAGYK...EDQQR...RL...QKI...SRTLAAR...
KTSDS...CT.MNTDDLK...IQIL...LADSD...ITET...RYM...PAGAD...EDQSL...RL...KNM...SKVVKEY...
CT.MNTDDLK...IQIL...LADSD...ITET...RYM...PAGAD...EDQSL...RL...KNM...SKVVKEY...
ALSSIEDDK...EL.YDLSECY...VOIP...SDTD...FVTY...RGM...CAAAEAKKT...EDQQR...RL...SKI...IDVVRKH...
ALSSIEDDK...EL.YDLSECY...VOIP...SHNALAQ...MHM...PAGST...EDQQT...RL...MHM...SQVLGNC...
IA.AEPGDLPVLT...IQHP...SND...DLAN...MHG...M...FAGES...EDQAT...RL...GHM...TLKIHESLDN
MN.SEPSECF...IQIP...SEQH...VEQM...KGM...PAGGI...EDQNI...RL...LIH...MNSAIRKQKK
PSPINFS...IQIP...AAE...H...LTW...MRD...M...FAGGA...EDQCM...RL...SNM...VAKINELHQQ

Legionella_cherrii_ApaH

70 80 90 100 110 120 130
DPSLPVVT...TD...RV...EL...PE...HW...NQ...LFA...AAM...KKG...DEN...V...ALT...IF...AE...F...PE...ED...Q...IL...QA...LL...AV...H...TS...E...Y...L...L...Q...Q...I...IR...DC...IQ...QA...
TD...Q...ET...RL...PY...Y...W...LD...LFA...A...I...DE...G...D...TP...KA...HA...LF...H...LL...P...OD...DI...LR...AL...RA...V...H...SE...D...Y...L...Y...Q...L...IK...YC...IQ...AK...H...
TD...V...VA...L...P...TH...W...NQ...LFA...A...M...K...K...G...D...AE...AP...LT...LF...AQ...F...PE...ND...E...IL...RA...LL...AV...H...TS...E...Y...L...L...Q...Q...I...IR...DC...IQ...QA...
SH...H...W...ID...RL...P...W...LV...L...C...Q...AI...R...SR...E...IT...LA...KA...AF...DA...I...PE...DN...P...VL...K...AL...L...V...H...TK...AY...L...V...SL...V...K...TC...A...A...L...AP...
TD...D...AL...F...L...NP...S...W...NE...LFA...A...I...R...R...G...D...TP...RI...A...AR...L...F...SE...I...P...DD...P...IL...K...P...LL...AV...H...TR...S...Y...L...L...Q...Q...I...IR...DC...IQ...QA...
CE...K...W...LD...TL...P...WE...I...LFA...A...I...R...R...G...D...TP...RI...A...AR...L...F...SE...I...P...DD...P...IL...K...P...LL...AV...H...TR...S...Y...L...L...Q...Q...I...IR...DC...IQ...QA...
TE...Q...CE...L...SN...K...W...LK...LFA...A...I...R...R...G...D...TP...RI...A...AR...L...F...SE...I...P...DD...P...IL...K...P...LL...AV...H...TR...S...Y...L...L...Q...Q...I...IR...DC...IQ...QA...
SD...H...NN...L...PE...Y...W...SO...LFA...A...I...R...R...G...D...TP...RI...A...AR...L...F...SE...I...P...DD...P...IL...K...P...LL...AV...H...TR...S...Y...L...L...Q...Q...I...IR...DC...IQ...QA...
TQ...Q...V...S...LA...Y...W...LD...LFA...A...I...R...R...G...D...TP...RI...A...AR...L...F...SE...I...P...DD...P...IL...K...P...LL...AV...H...TR...S...Y...L...L...Q...Q...I...IR...DC...IQ...QA...
TED...V...S...L...P...V...IT...TD...ED...VS...LA...Y...W...LD...LFA...A...I...R...R...G...D...TP...RI...A...AR...L...F...SE...I...P...DD...P...IL...K...P...LL...AV...H...TR...S...Y...L...L...Q...Q...I...IR...DC...IQ...QA...
TD...D...DL...K...L...SP...S...W...EV...E...F...ER...LI...Q...S...NA...N...Q...KD...IL...KE...E...TR...L...ME...Q...DD...LV...QT...I...SA...V...H...SK...K...Y...L...R...Q...V...L...Q...WT...L...S...A...R...N...

Legionella_cherrii_ApaH

140 150 160 170 180 190 200 210
KGW...K...Q...L...NS...D...I...L...I...TP...G...TF...E...V...L...I...K...D...I...S...M...T...L...F...H...S...K...K...V...H...F...S...F...G...L...P...T...H...H...A...F...A...D...E...G...S...G...F...C...I...L...N...K...S...A...V...L...K...H...M...Q...R...N...T...K...P...L...K...H...
FG...F...K...Q...L...NA...D...L...V...V...TP...K...TF...E...I...L...I...R...D...C...A...T...L...F...N...P...A...K...A...H...F...S...F...G...L...P...S...H...H...A...Y...T...O...M...G...S...G...F...C...I...L...N...K...T...A...M...L...M...K...Q...A...E...L...S...S...A...Q...E...P...K...F...
KG...W...K...Q...L...SP...D...I...L...I...TP...G...TF...E...V...L...I...K...D...I...A...M...T...L...F...H...F...G...K...V...H...F...S...F...G...L...P...S...H...H...A...Y...S...D...E...G...S...G...F...C...I...L...N...K...S...A...I...L...I...K...Y...L...Q...S...L...S...S...K...P...L...K...H...
D...I...D...I...L...C...G...H...L...TF...E...V...L...I...H...E...L...L...T...I...E...Q...R...Q...D...Q...F...N...F...C...F...G...L...P...T...H...H...A...Y...R...D...R...G...A...G...F...C...I...L...N...K...L...A...V...M...A...Y...E...E...L...T...A...D...A...F...H...T...
ND...D...V...V...V...TP...K...TF...E...L...I...R...D...L...A...T...S...I...L...I...P...A...P...V...C...F...S...F...G...L...P...G...H...H...A...F...S...D...R...A...N...G...F...C...I...L...N...K...I...A...V...M...M...Q...H...M...A...N...N...S...P...A...L...K...F...
D...V...V...C...G...R...L...TF...E...V...L...I...Q...D...L...I...T...I...T...Q...R...R...A...I...N...V...C...F...G...L...P...T...H...H...A...Y...R...E...A...A...G...F...C...I...N...K...T...A...V...M...V...Y...E...Q...L...M...A...S...K...P...F...K...A...
NS...D...I...V...I...TP...K...TF...E...I...L...M...K...D...C...A...L...T...M...S...T...K...A...T...F...S...F...G...L...P...T...H...H...A...Y...S...E...T...G...T...G...F...C...V...L...N...K...T...A...L...L...I...K...H...A...E...L...T...S...S...K...P...L...Q...Y...D...
D...T...D...I...H...I...N...Q...TF...E...V...L...I...K...D...I...A...T...I...E...G...F...K...Q...H...N...M...I...F...G...I...G...L...P...T...H...H...A...Y...R...E...K...A...A...G...F...C...I...N...K...V...A...V...L...I...H...Y...F...G...H...V...N...N...
NS...D...I...V...I...TP...G...TF...E...V...L...I...D...L...A...T...T...L...N...P...S...K...M...I...F...S...F...G...L...P...T...H...H...A...Y...H...D...E...G...S...G...F...C...I...N...K...T...A...V...L...M...K...Y...E...T...V...H...A...E...P...L...H...Y...
ND...I...V...Y...TP...G...TF...E...L...I...R...D...L...A...T...T...L...F...C...P...T...P...I...C...I...S...F...G...L...P...T...H...H...A...T...G...S...R...A...S...G...F...C...H...L...N...K...T...A...V...L...I...D...F...L...H...K...T...S...S...E...P...L...S...F...
NA...D...I...L...I...TP...H...TF...E...L...I...K...D...I...A...T...T...L...M...H...P...A...K...C...Y...F...S...F...G...L...P...T...H...H...A...F...S...R...E...S...S...G...F...C...I...N...K...I...A...I...L...I...K...N...A...E...L...T...H...P...N...P...L...K...H...
D...L...R...F...N...R...Y...TF...E...L...I...R...D...L...F...T...T...L...N...N...P...A...K...F...L...C...S...F...G...L...P...S...H...H...A...F...A...D...G...G...S...G...F...C...I...L...D...K...T...S...I...W...L...T...S...Y...L...Q...V...K...P...Q...A...M...V...

Legionella_cherrii_ApaH $\beta 6$ $\alpha 10$ $\beta 7$ $\alpha 11$ $\beta 8$ $\beta 9$ $\beta 10$
 220 230 240 250 260 270 280
Legionella_cherrii_ApaH IIVCGHDVNRDNGICDILMNSAA..DMDICHDVFD²⁴⁰DSRVYFYQDEDEYITELFNKCGKDE...GQNIQS²⁶⁰QRGGLDYFV²⁸⁰VNI
Legionella_pneumophila_ApaH VIIGTDVNRDNGICDILRHSFS..HLSICHDVFD²⁴⁰DSRVYFYQDFAYINNEFNSEGVDI...GKNIHV²⁶⁰WHHNNLN²⁸⁰YAV²⁸⁰VDL
Legionella_bozemaniae_ApaH IIVGTDVNRDNGICDVLWNSAS..DMDICHDVFD²⁴⁰DSRVYFYQDDAFITEAFQKSGEDI...GQKIHM²⁶⁰WQRGNLE²⁸⁰YAV²⁸⁰VDL
Legionella_erythra_ApaH VIIGTDINRDNGICNSVLM²³⁰MAETT..TQSFTHIDIY²⁴⁰DSRVYFYQDDGVLQ²⁶⁰ERMG...API...EEHGRV²⁸⁰WQKGNKC²⁸⁰YHS²⁸⁰VDL
Legionella_geestiana_ApaH IIVIGTDVNRDNGICNVLR²³⁰REKLS..HLDICHDVFD²⁴⁰DSRVYFYQD²⁶⁰GSMQID²⁶⁰QEF²⁶⁰GVRK²⁶⁰PT...APAYNT²⁸⁰WKQGN²⁸⁰YV²⁸⁰YAD²⁸⁰DL
Legionella_spiritensis_ApaH IIVIGTDVNRDNGISAILMGDDF..KQNLHL²⁴⁰DAY²⁴⁰DSRVYFYWH²⁶⁰HEKQ²⁶⁰INEI²⁶⁰IGCE²⁶⁰.SYS...EPGVSV²⁸⁰W²⁸⁰SKGKR²⁸⁰Y²⁸⁰LG²⁸⁰IDL
Legionella_waltersii_ApaH MVVGTDDVNRDNGICDVLRRQFS..HLPICHDVFD²⁴⁰DSRVYFYQNAEDINLEFSTKGT²⁶⁰TTT...QQKI²⁸⁰QKW²⁸⁰SSDK²⁸⁰YD²⁸⁰YFM²⁸⁰VDL
Legionella_busanensis_ApaH VICGTDVNEEDNGICRNTLSTSNF²³⁰S²³⁰KSR²³⁰TI²³⁰LH²³⁰LS²³⁰Y²³⁰DSRVYFY²⁴⁰Y²⁴⁰ASGN²⁴⁰V²⁴⁰KYD²⁴⁰K...VINE²⁶⁰EQ²⁶⁰Y²⁶⁰I²⁶⁰L²⁶⁰FL²⁶⁰Q²⁶⁰L
Legionella_moravica_ApaH IIVGTDVNRDNGICDILRQTSS..HMNICHDVFD²⁴⁰DSRVYFYQNHLDINKEFNLTGTEV...GEQIK²⁶⁰Y²⁶⁰W²⁶⁰KHNN²⁶⁰LD²⁶⁰Y²⁶⁰AV²⁶⁰VDL
Legionella_sp_ApaH FIIIGTDVNRDNGICQIL²³⁰RTKQT..EAPVCHIDI²⁴⁰F²⁴⁰DSRVYFYQNFNDITMEFTQ.SPDI...APNMAL²⁶⁰W²⁶⁰KEGN²⁶⁰Y²⁶⁰E²⁶⁰Y²⁶⁰MA²⁶⁰IDL
Legionella_antarctica_ApaH IIVIGTDVNRDNGISAVLRESFS..CLD²³⁰ICHD²⁴⁰IF²⁴⁰DSRVYFYQH²⁶⁰DHSF²⁶⁰I²⁶⁰EY²⁶⁰E²⁶⁰F²⁶⁰QTQ²⁶⁰GKAE...QQIK²⁸⁰S²⁸⁰W²⁸⁰HQ²⁸⁰NQ²⁸⁰MD²⁸⁰Y²⁸⁰AV²⁸⁰VDL
Legionella_sp_HdaH LVIIGTDINRDNGISANLQ²³⁰KN..AAQ²³⁰Y²³⁰HL²³⁰DL²³⁰F²³⁰DSRVYFYQ²⁴⁰GS²⁴⁰AE²⁴⁰IK²⁴⁰KF²⁴⁰NQ²⁴⁰DEVK²⁶⁰V²⁶⁰GV²⁶⁰ET²⁶⁰QAVN²⁶⁰V²⁶⁰Y²⁶⁰VQ²⁶⁰GCLT²⁸⁰Y²⁸⁰AV²⁸⁰IDL

Legionella_cherrii_ApaH $\alpha 12$ $\beta 11$ $\alpha 13$ $\beta 12$
 290 300 310 320 330 340
Legionella_cherrii_ApaH S...RTR...K.PGLVHPALVFAIEK³⁰⁰MEEQIEQAK.INHQV³¹⁰AL³¹⁰LP³¹⁰T³¹⁰GW³¹⁰DS³¹⁰HEE³¹⁰ET³¹⁰AY³¹⁰CG³¹⁰K³¹⁰Y³¹⁰VDG...
Legionella_pneumophila_ApaH S...LTSR...K.SVGVHPALVFALEQL³⁰⁰KESIREAK.AKGQ³¹⁰IAL³¹⁰LP³¹⁰T³¹⁰GW³¹⁰DS³¹⁰HEE³¹⁰ET³¹⁰AY³¹⁰CG³¹⁰K³¹⁰F³¹⁰VNG...
Legionella_bozemaniae_ApaH S...LTLR...K.PSLVHPALVFAIEK³⁰⁰IEEQIEQAK.TNHQV³¹⁰AL³¹⁰LP³¹⁰T³¹⁰GW³¹⁰DS³¹⁰HEE³¹⁰ET³¹⁰AY³¹⁰CG³¹⁰K³¹⁰Y³¹⁰VDG...
Legionella_erythra_ApaH A...LCSR...Q.RRGC³⁰⁰HALHYALEALE³¹⁰TLLVNA.KKKEN³¹⁰VML³¹⁰LP³¹⁰T³¹⁰GW³¹⁰DS³¹⁰HEE³¹⁰ET³¹⁰AY³¹⁰CG³¹⁰KE³¹⁰IGK...D
Legionella_geestiana_ApaH S...LQETQKSPAN.PGMLHPALIDFAV³⁰⁰TKAQEEINRAI.RQGK³¹⁰VALL³¹⁰LP³¹⁰T³¹⁰GW³¹⁰DS³¹⁰SH³¹⁰CK³¹⁰ET³¹⁰AD³¹⁰CS³¹⁰K³¹⁰M³¹⁰FEH...
Legionella_spiritensis_ApaH S...RHAR...KNETDYHPALLLIL³⁰⁰LQK³⁰⁰TAL³⁰⁰EEAC.LK³⁰⁰SPV³⁰⁰VALL³⁰⁰LP³⁰⁰T³⁰⁰GW³⁰⁰DS³⁰⁰HEE³⁰⁰ET³⁰⁰AY³⁰⁰CG³⁰⁰K³⁰⁰Y³⁰⁰VDG...K
Legionella_waltersii_ApaH K...DTYR...K.KVGVHPALLFALEQ³⁰⁰IKEK³⁰⁰VKLAK.SK³⁰⁰GI³⁰⁰IAL³⁰⁰LP³⁰⁰T³⁰⁰GW³⁰⁰DS³⁰⁰HEE³⁰⁰ET³⁰⁰AY³⁰⁰CG³⁰⁰K³⁰⁰F³⁰⁰VDG...
Legionella_busanensis_ApaH DDPNYVRE...N.SRDLHPIMEDLL³⁰⁰TQI...EQAC³⁰⁰NNPRD³⁰⁰TAI³⁰⁰Y³⁰⁰LV³⁰⁰IG³⁰⁰W³⁰⁰DS³⁰⁰SH³⁰⁰GK³⁰⁰ER³⁰⁰AG³⁰⁰CS³⁰⁰K³⁰⁰DI³⁰⁰Y³⁰⁰RG³⁰⁰INK³⁰⁰TT³⁰⁰K³⁰⁰FE³⁰⁰Y
Legionella_moravica_ApaH S...LTFR...K.KISLHPALQFALAK³⁰⁰IKEQIAQAK.THHH³¹⁰IALL³¹⁰LP³¹⁰T³¹⁰GW³¹⁰DS³¹⁰HEE³¹⁰ET³¹⁰AY³¹⁰CG³¹⁰K³¹⁰W³¹⁰VNE...
Legionella_sp_ApaH Q...SGRT...N.ATELHPALLYSIST³⁰⁰LKNQ³⁰⁰VEHAK.TN³⁰⁰QK³⁰⁰IMLL³⁰⁰LP³⁰⁰S³⁰⁰GW³⁰⁰DS³⁰⁰SH³⁰⁰VH³⁰⁰ET³⁰⁰AP³⁰⁰CG³⁰⁰K³⁰⁰L³⁰⁰LYG...H
Legionella_antarctica_ApaH K...L²⁹⁰TR...S.SISLHPALLFSLG³⁰⁰KIKENIVNAK.RT³⁰⁰GK³⁰⁰I³⁰⁰VLY³⁰⁰LA³⁰⁰T³⁰⁰GW³⁰⁰DS³⁰⁰HEE³⁰⁰ET³⁰⁰AY³⁰⁰CG³⁰⁰K³⁰⁰F³⁰⁰IND...
Legionella_sp_HdaH S...MLP...K.TTGIHPALIY³⁰⁰TLNK³⁰⁰TAA³⁰⁰LFD³⁰⁰KAQ.RE³⁰⁰QRP³⁰⁰I³⁰⁰LAL³⁰⁰LP³⁰⁰T³⁰⁰GW³⁰⁰DS³⁰⁰SH³⁰⁰VD³⁰⁰ER³⁰⁰AY³⁰⁰CA³⁰⁰K³⁰⁰Y³⁰⁰LG³⁰⁰N...

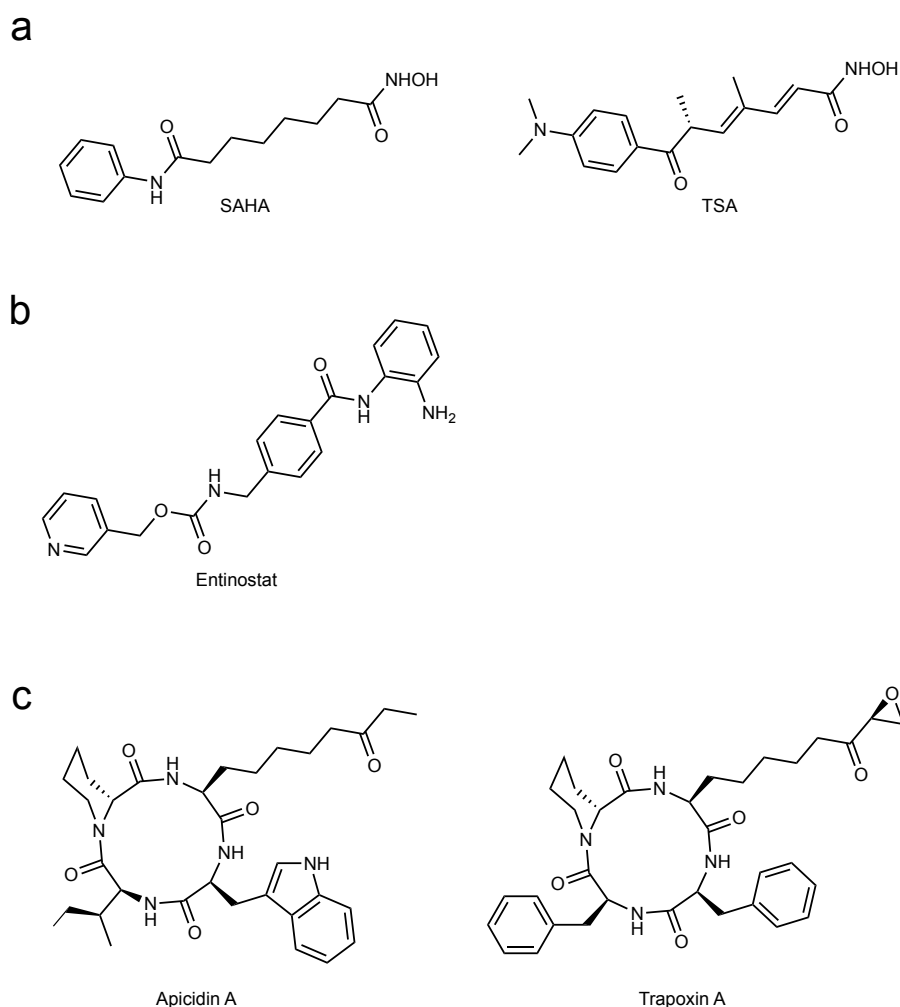
Legionella_cherrii_ApaH $\beta 13$ $\alpha 14$ $\alpha 15$ $\beta 14$ $\alpha 16$ $\alpha 17$ $\eta 3$
 350 360 370 380 390 400 410 420
Legionella_cherrii_ApaH YL³⁵⁰M³⁵⁰GAT³⁵⁰E³⁵⁰ARK³⁵⁰T³⁵⁰R³⁵⁰LNT³⁵⁰TD³⁵⁰LY³⁵⁰F³⁵⁰Y³⁵⁰ES³⁵⁰IF³⁵⁰K³⁵⁰LY³⁵⁰REN³⁵⁰KD³⁵⁰HIEK³⁵⁰VY³⁵⁰WG³⁵⁰LEGGY³⁵⁰DR³⁵⁰KM³⁵⁰Y³⁵⁰EQ³⁵⁰Q³⁵⁰IEL³⁵⁰MS³⁵⁰I³⁵⁰V³⁵⁰LND³⁵⁰.LVH³⁵⁰QD³⁵⁰.TH³⁵⁰QM³⁵⁰.GK
Legionella_pneumophila_ApaH RM³⁵⁰M³⁵⁰GK³⁵⁰TA³⁵⁰AHQ³⁵⁰R³⁵⁰FND³⁵⁰GD³⁵⁰LY³⁵⁰F³⁵⁰Y³⁵⁰ES³⁵⁰IF³⁵⁰FLY³⁵⁰NEN³⁵⁰KD³⁵⁰CV³⁵⁰DT³⁵⁰IY³⁵⁰WG³⁵⁰LEGGY³⁵⁰DR³⁵⁰TM³⁵⁰Y³⁵⁰ER³⁵⁰EL³⁵⁰KI³⁵⁰LL³⁵⁰QV³⁵⁰IE³⁵⁰KQ³⁵⁰.LL³⁵⁰PK³⁵⁰D³⁵⁰.SN³⁵⁰SH³⁵⁰.SM
Legionella_bozemaniae_ApaH DL³⁵⁰M³⁵⁰GIA³⁵⁰E³⁵⁰ARK³⁵⁰T³⁵⁰R³⁵⁰FNS³⁵⁰KD³⁵⁰LY³⁵⁰F³⁵⁰Y³⁵⁰ES³⁵⁰IF³⁵⁰K³⁵⁰LY³⁵⁰QEN³⁵⁰KD³⁵⁰YIEK³⁵⁰IY³⁵⁰WG³⁵⁰LEGGY³⁵⁰DR³⁵⁰KM³⁵⁰Y³⁵⁰EQ³⁵⁰Q³⁵⁰IQL³⁵⁰MS³⁵⁰L³⁵⁰IL³⁵⁰KD³⁵⁰.IV³⁵⁰H³⁵⁰QD³⁵⁰.TN³⁵⁰QM³⁵⁰.VP
Legionella_erythra_ApaH HT³⁵⁰L³⁵⁰SSK³⁵⁰ESH³⁵⁰SR³⁵⁰F³⁵⁰SD³⁵⁰E³⁵⁰IF³⁵⁰HE³⁵⁰TQ³⁵⁰K³⁵⁰IV³⁵⁰EM³⁵⁰IK³⁵⁰TY³⁵⁰PD³⁵⁰LCR³⁵⁰VY³⁵⁰WG³⁵⁰LEGGY³⁵⁰TE³⁵⁰Y³⁵⁰VN³⁵⁰V³⁵⁰R³⁵⁰QL³⁵⁰KN³⁵⁰TV³⁵⁰GIL³⁵⁰NE...FYP³⁵⁰W³⁵⁰VEE³⁵⁰T³⁵⁰M³⁵⁰EN³⁵⁰SK
Legionella_geestiana_ApaH DE³⁵⁰L³⁵⁰SEE³⁵⁰ES³⁵⁰RV³⁵⁰CR³⁵⁰FND³⁵⁰DF³⁵⁰GI³⁵⁰F³⁵⁰N³⁵⁰YL³⁵⁰IM³⁵⁰TY³⁵⁰QDN³⁵⁰RE³⁵⁰HMS³⁵⁰HL³⁵⁰Y³⁵⁰WG³⁵⁰LEGGY³⁵⁰ER³⁵⁰GM³⁵⁰Y³⁵⁰EF³⁵⁰Q³⁵⁰ARK³⁵⁰LLEA³⁵⁰ID³⁵⁰VR³⁵⁰.L³⁵⁰INK³⁵⁰P³⁵⁰.AEG³⁵⁰P³⁵⁰.AS
Legionella_spiritensis_ApaH QE³⁵⁰L³⁵⁰TP³⁵⁰K³⁵⁰Q³⁵⁰SSG³⁵⁰CR³⁵⁰F³⁵⁰TD³⁵⁰RD³⁵⁰MT³⁵⁰Y³⁵⁰FN³⁵⁰R³⁵⁰QL³⁵⁰ML³⁵⁰LC³⁵⁰Q³⁵⁰D³⁵⁰Y³⁵⁰K³⁵⁰PI³⁵⁰I³⁵⁰QR³⁵⁰IY³⁵⁰WG³⁵⁰LEGGY³⁵⁰T³⁵⁰DE³⁵⁰VN³⁵⁰FR³⁵⁰Q³⁵⁰IAC³⁵⁰L³⁵⁰TE³⁵⁰CL³⁵⁰Q³⁵⁰SL³⁵⁰.FL³⁵⁰ND³⁵⁰.NE³⁵⁰VE³⁵⁰CE
Legionella_waltersii_ApaH RM³⁵⁰MG³⁵⁰Y³⁵⁰ESA³⁵⁰KTY³⁵⁰R³⁵⁰FND³⁵⁰GD³⁵⁰LY³⁵⁰F³⁵⁰Y³⁵⁰EV³⁵⁰F³⁵⁰K³⁵⁰LY³⁵⁰N³⁵⁰ND³⁵⁰PC³⁵⁰FAS³⁵⁰LY³⁵⁰WG³⁵⁰LEGGY³⁵⁰D³⁵⁰NT³⁵⁰MY³⁵⁰TK³⁵⁰Q³⁵⁰IKL³⁵⁰LM³⁵⁰QL³⁵⁰IS³⁵⁰KE³⁵⁰.L³⁵⁰VP³⁵⁰KA³⁵⁰.TH³⁵⁰ST³⁵⁰.PL
Legionella_busanensis_ApaH RN³⁵⁰IY³⁵⁰DQ³⁵⁰E³⁵⁰IKE³⁵⁰OR³⁵⁰FND³⁵⁰GD³⁵⁰FE³⁵⁰H³⁵⁰Y³⁵⁰K³⁵⁰QL³⁵⁰RE³⁵⁰V³⁵⁰INK³⁵⁰Y...K³⁵⁰IP³⁵⁰F³⁵⁰VY³⁵⁰VS³⁵⁰LEGGY³⁵⁰TE³⁵⁰AV³⁵⁰N³⁵⁰K³⁵⁰Q³⁵⁰TEM³⁵⁰L³⁵⁰IK³⁵⁰L³⁵⁰LT³⁵⁰PT³⁵⁰IT³⁵⁰P³⁵⁰QE³⁵⁰.QS³⁵⁰KE³⁵⁰.E³⁵⁰I
Legionella_moravica_ApaH RM³⁵⁰MS³⁵⁰LS³⁵⁰E³⁵⁰ASK³⁵⁰T³⁵⁰R³⁵⁰FND³⁵⁰GD³⁵⁰LY³⁵⁰F³⁵⁰Y³⁵⁰EQ³⁵⁰IF³⁵⁰FLY³⁵⁰N³⁵⁰KN³⁵⁰RE³⁵⁰L³⁵⁰IKG³⁵⁰IY³⁵⁰WG³⁵⁰LEGGY³⁵⁰DR³⁵⁰AM³⁵⁰Y³⁵⁰ER³⁵⁰Q³⁵⁰V³⁵⁰SL³⁵⁰IT³⁵⁰QI³⁵⁰IT³⁵⁰AH³⁵⁰.L³⁵⁰PH³⁵⁰QA³⁵⁰.SS³⁵⁰SS³⁵⁰.SA
Legionella_sp_ApaH TM³⁵⁰MS³⁵⁰DE³⁵⁰ET³⁵⁰QL³⁵⁰CR³⁵⁰FND³⁵⁰Q³⁵⁰DL³⁵⁰S³⁵⁰Y³⁵⁰HE³⁵⁰IF³⁵⁰FLY³⁵⁰LEN³⁵⁰PE³⁵⁰V³⁵⁰FKG³⁵⁰IY³⁵⁰WG³⁵⁰LEGGY³⁵⁰NR³⁵⁰AM³⁵⁰Y³⁵⁰EQ³⁵⁰IS³⁵⁰L³⁵⁰AE³⁵⁰SI³⁵⁰ITH³⁵⁰.F³⁵⁰YS³⁵⁰QD³⁵⁰.L³⁵⁰TP³⁵⁰S³⁵⁰.LR
Legionella_antarctica_ApaH RM³⁵⁰MS³⁵⁰QS³⁵⁰E³⁵⁰AGT³⁵⁰R³⁵⁰FND³⁵⁰GD³⁵⁰LY³⁵⁰F³⁵⁰FE³⁵⁰IL³⁵⁰TY³⁵⁰SEN³⁵⁰KE³⁵⁰L³⁵⁰IES³⁵⁰IY³⁵⁰WG³⁵⁰LEGGY³⁵⁰ER³⁵⁰MY³⁵⁰E³⁵⁰K³⁵⁰Q³⁵⁰IQL³⁵⁰ML³⁵⁰DT³⁵⁰ID³⁵⁰GO³⁵⁰.L³⁵⁰L³⁵⁰HE³⁵⁰D³⁵⁰.IN³⁵⁰QS³⁵⁰.SL
Legionella_sp_HdaH KW³⁵⁰M³⁵⁰NK³⁵⁰H³⁵⁰E³⁵⁰AY³⁵⁰SS³⁵⁰R³⁵⁰FD³⁵⁰NAD³⁵⁰WT³⁵⁰Y³⁵⁰DG³⁵⁰IF³⁵⁰K³⁵⁰LF³⁵⁰QDN³⁵⁰K³⁵⁰CLEA³⁵⁰VY³⁵⁰LG³⁵⁰LEGGY³⁵⁰EP³⁵⁰HV³⁵⁰Y³⁵⁰LN³⁵⁰Q³⁵⁰IT³⁵⁰LL³⁵⁰H³⁵⁰Q³⁵⁰K³⁵⁰IG³⁵⁰SC³⁵⁰.AQ³⁵⁰P³⁵⁰Q³⁵⁰V³⁵⁰.AS³⁵⁰Y³⁵⁰P³⁵⁰.Q.

Legionella_cherrii_ApaH

Legionella_cherrii_ApaH TSS.....P
Legionella_pneumophila_ApaH S.....Y
Legionella_bozemaniae_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_ApaHR
Legionella_antarctica_ApaH GIN.....C
Legionella_sp_HdaHP

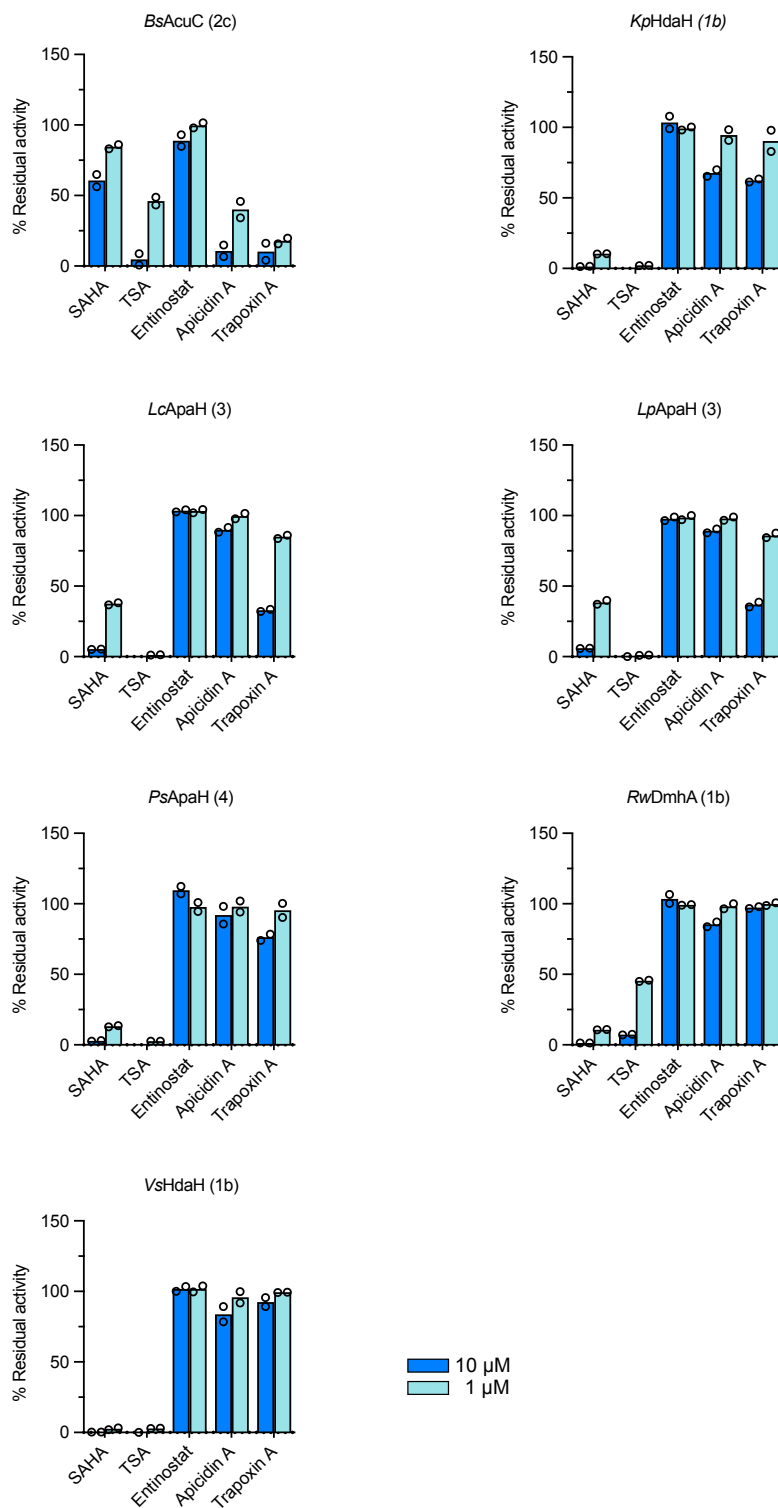
Supplementary Figure 21: Primary sequence alignment of selected bacterial deacylases of cluster 3 (UniProt accession numbers are shown).

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/species): 3, A0A0W0SGS1/ApaH/*Legionella cherii*; 3, A0A2S6EWW0/ApaH/*Legionella pneumophila*; 3, A0A0W0RV07/ApaH/*Legionella bozemanæ* (*Fluoribacter bozemanæ*); 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, A0A811Q8A3/HdaH/*Legionella sp.* All sequences were aligned using the T-COFFEE multiple sequence alignment server¹. 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 3.0².



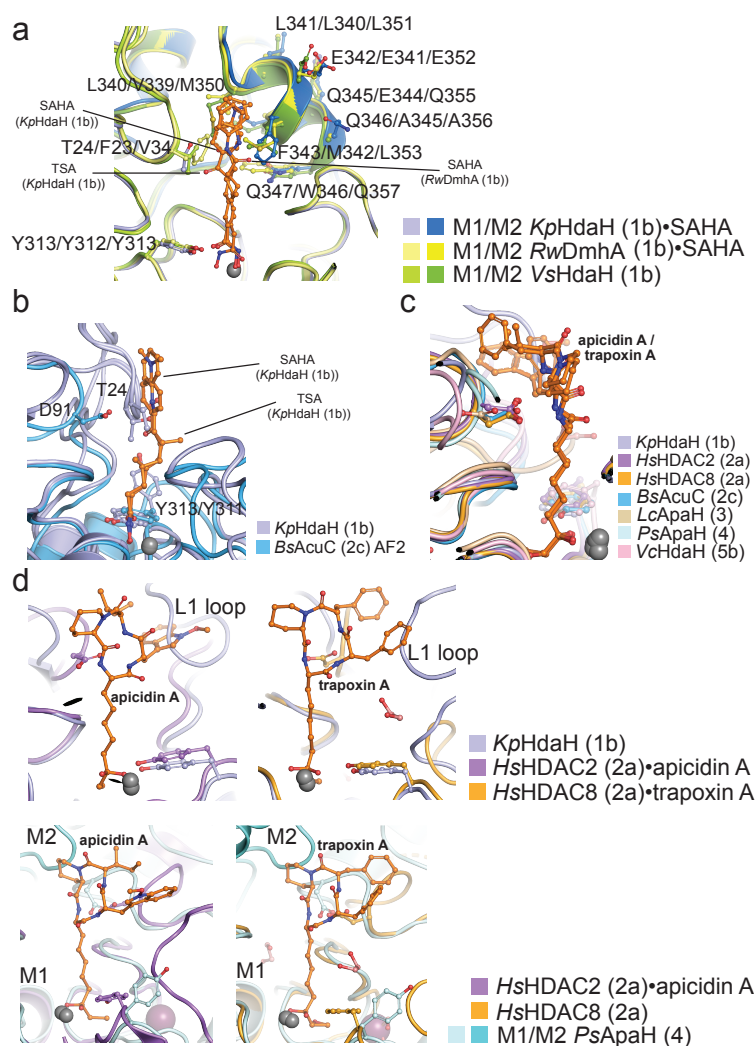
Supplementary Figure 22: Overview of structures of deacylase hydroxamate inhibitors SAHA, trichostatin A (TSA), benzamide entinostat (MS-275) and the cyclic peptides apicidin A and trapoxin A.

- 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.
- The benzamide inhibitor entinostat (MS-275) shows selectivity towards class I HDACs. Our data show no bacterial deacylase being potently inhibited by this benzamide inhibitor.
- 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 *BsAcuC* being selectively and potently inhibited by both cyclic peptide inhibitors.



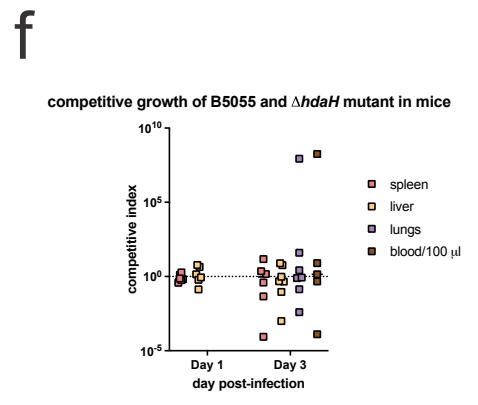
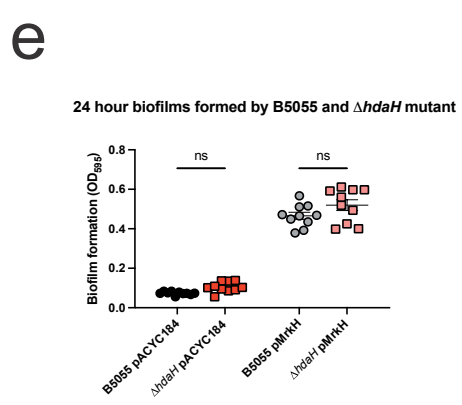
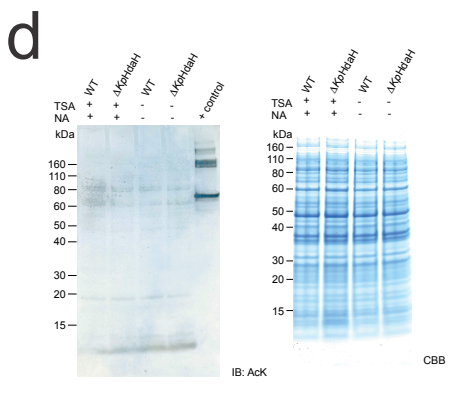
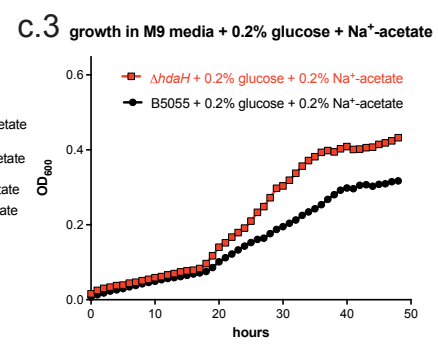
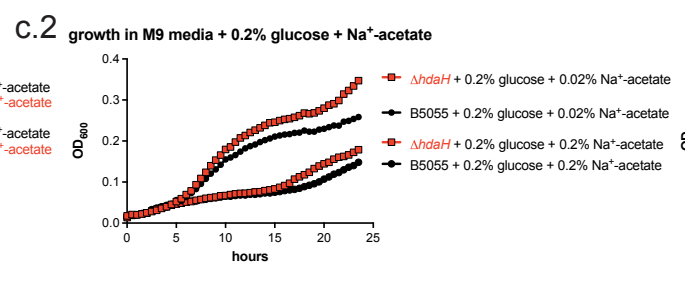
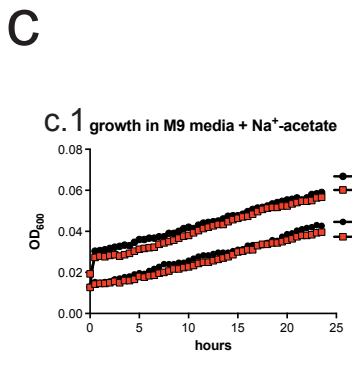
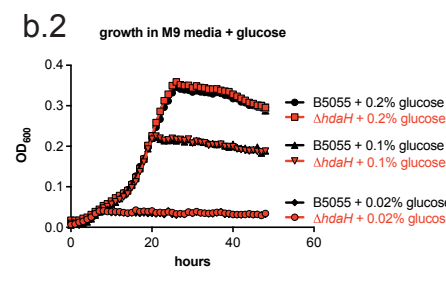
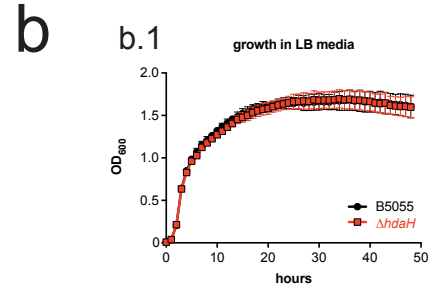
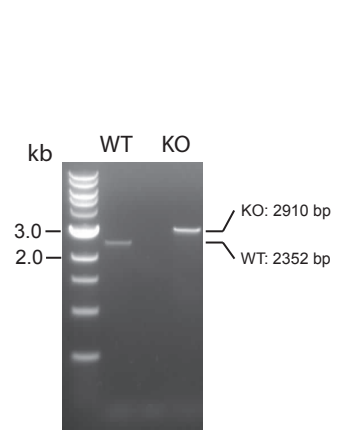
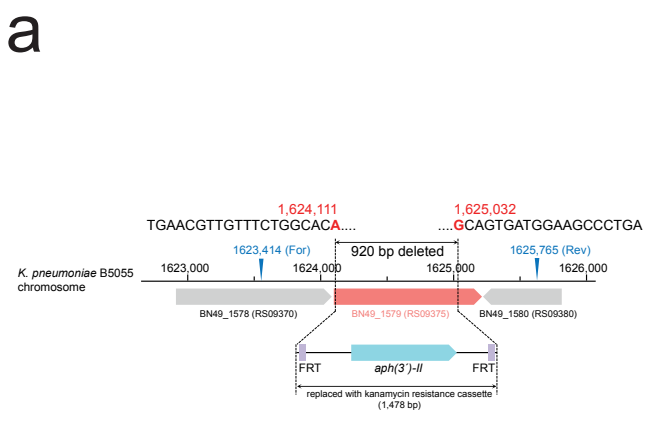
Supplementary Figure 23: Screening of hydroxamate, benzamide and cyclic peptide inhibitors to inhibit selected bacterial deacylases.

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 μ M and 10 μ M and varying enzyme concentrations (cluster A: 15 nM *KpHdaH*, 30 nM *RwDmhA* 1.5 nM *VsHdaH*; cluster B: 200 nM *BsAcuC*; cluster D: 40 nM *PsApaH*; cluster E: 30.5 nM *LcApaH*, 0.5 nM *LpApaH*) 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 μ M LGKac *BsAcuC* (2c), *LcApaH* (3), *LpApaH* (3); 60 μ M QPKKac: *KpHdaH* (1b), *RwDmhA* (1b), *VsHdaH* (1b), *PsApaH* (1b)). The data obtained for 1 μ M 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** *RwDmhA* (1b) is the only enzyme showing better inhibition by SAHA compared to TSA and *VsHdaH* (1b) is equally well inhibited by SAHA and TSA. To reveal the underlying mechanism, the structures of *RwDmhA* (1b) and *VsHdaH* (1b) (AF2) were superimposed with *KpHdaH* (1b)•TSA and *KpHdaH* (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 *BsAcuC* (2c) activity observed for SAHA and TSA, the AlphaFold2 model of *BsAcuC* (2c) was superimposed with the structures of *KpHdaH* (1b)•TSA and *KpHdaH* (1b)•SAHA.
- c** D101/D100 in *HsHDAC8/HsHDAC2* is needed for inhibition by trapoxin A and apicidin A. Superposition of the structures of the core domains of bacterial deacylases *KpHdaH* (1b), *RwDmhA* (1b), *RsPrpH* (1b) *BsAcuC* (2c), *LpApaH* (3), *LcApaH* (3), *PsApaH* (4) *VcHdaH* (5b) and *HsHDAC2* (2a) and *HsHDAC8* (2a) and modelling of trapoxin A and apicidin A reveals, except from *PsApaH* (4) and *VcHdaH* (5b), either a negatively charged D or E is present at analogue localization as in *HsHDAC2* (2a) and *HsHDAC8* (2a). The observed lack of inhibition of *KpHdaH* (1b), *RwDmhA* (1b), *RsPrpH* (1b), *LpApaH* (3), *LcApaH* (3), *PsApaH* (4) *VcHdaH* (5b) is due to other determinants in structure or sequence.
- d** Except from *BsAcuC* (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 *KpHdaH* (1b), and *PsApaH* (4) by apicidin A and trapoxin A. *PsApaH* (4) and *VcHdaH* (5b) do not contain an D/E at the analogous position to D101/D100 in *HsHDAC8/HsHDAC2* explaining their lack in inhibition by cyclic peptides (*KpHdaH* (1b): E101; *BsAcuC* (2c): D91; *LpApaH* (3): D146; *LcApaH* (3): D144; *PsApaH* (4): G117; *VcHdaH* (5b): G97; *HsHDAC2*: D100; *HsHDAC8*: D101). For the complexes the crystal structures of the indicated enzymes were superimposed with the structures of HDAC2•apicidinA (PDB: [7LTG](https://doi.org/10.2210/pdb7LTG/pdb) [<https://doi.org/10.2210/pdb7LTG/pdb>]) and HDAC8•trapoxin A (PDB: [5VI6](https://doi.org/10.2210/pdb5VI6/pdb) [<https://doi.org/10.2210/pdb5VI6/pdb>]) to model the localization of apicidin A and trapoxin A. For *BsAcuC* (2c) the AlphaFold2 model was used.

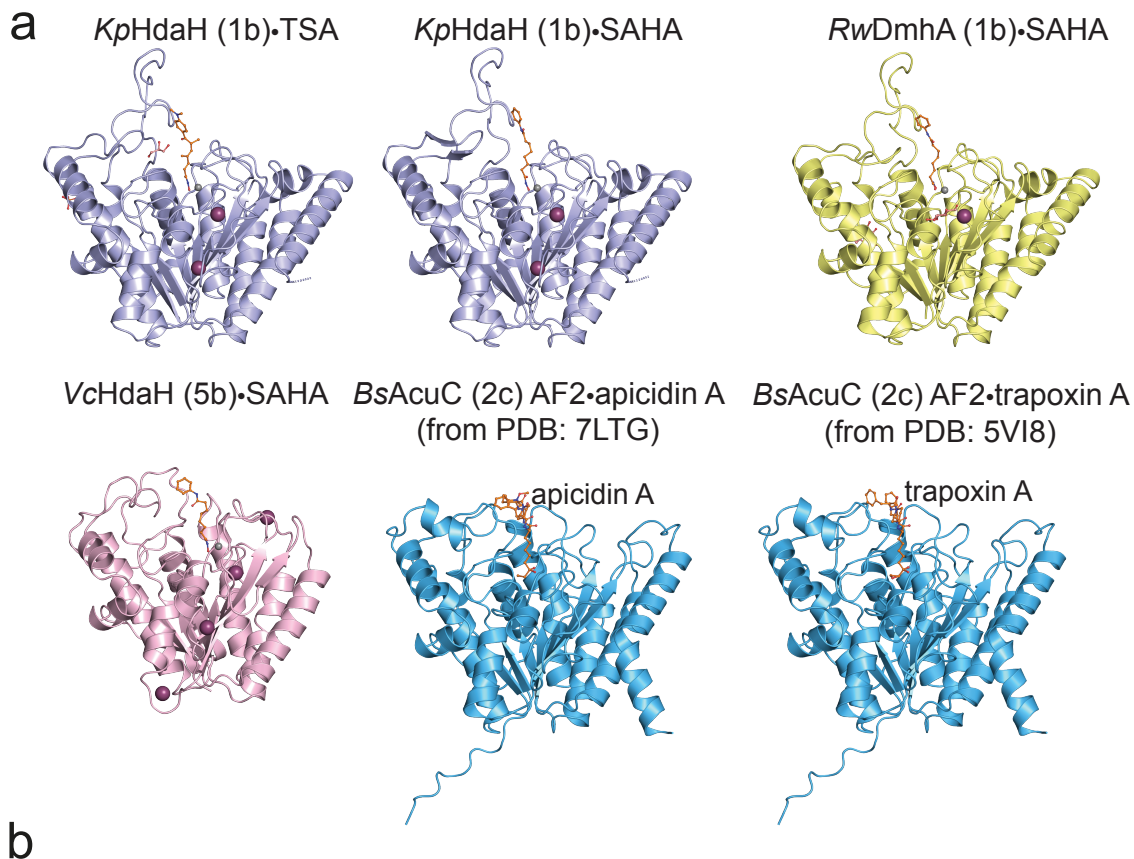


Supplementary Figure 25: Impact of genomic deletion of *hdaH* in *K. pneumoniae* on growth, biofilm formation and virulence.

- a** Genomic deletion of the gene *hdaH* encoding for KpHdaH (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 KpHdaH 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 method⁵. 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 ($\Delta hdaH$) 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 $\Delta hdaH$ were grown in Luria-Bertani broth, a rich medium containing multiple carbon sources. The growth curves of the WT and $\Delta hdaH$ 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 $\Delta hdaH$ were grown in minimal medium (M9) with varying concentrations of glucose. Terminal OD₆₀₀ was determined by glucose concentration and were approximately 25% of OD₆₀₀ generated by growth in LB broth. Again, there were no differences in the growth kinetics of the B5055 WT and B5055 $\Delta hdaH$. The analysis of growth in rich and simple media comparing B5055 WT and B5055 $\Delta hdaH$ in central glucose metabolism of B5055 suggested that KpHdaH (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 \pm SD of the three biological replicates. Source data are provided as Source Data file.
- c** We next explored whether B5055 $\Delta hdaH$ 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 $\Delta hdaH$ 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 $\Delta hdaH$ mutant. The level of growth for the B5055 $\Delta hdaH$ 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 switched 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 KpHdaH could be identified in cultured *K. pneumoniae* B5055, the B5055 WT and B5055 $\Delta hdaH$ 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 $\Delta hdaH$. 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 $\Delta hdaH$. 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

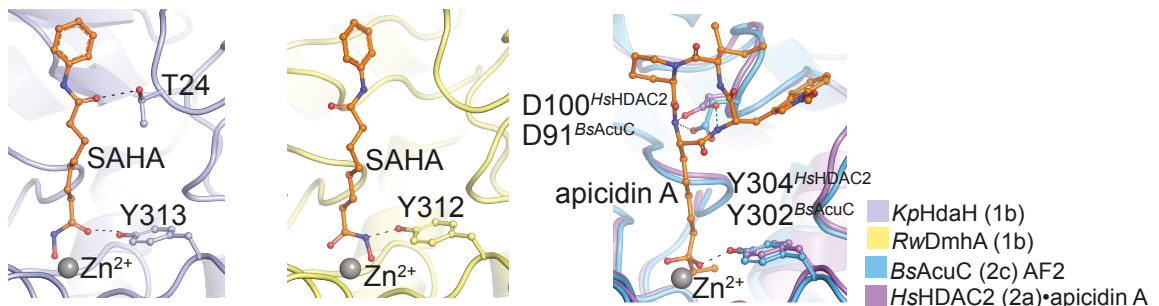
copy of MrkH, the transcriptional activator of Mrk fimbriae expression. The Mrk fimbria is a key mediator of *K. pneumoniae* biofilm formation⁶. To address this issue, B5055 and the $\Delta hdaH$ mutant of B5055 were transfected with a functional plasmid copy of MrkH (pMrkH). The supply of *mrkH* in 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 $\Delta hdaH$ 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 $\Delta hdaH$) 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 $\Delta hdaH$ 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 $\Delta hdaH$ 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 $\Delta hdaH$ 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 $\Delta hdaH$, 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 $\Delta hdaH$ 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 *KpHdaH* $\Delta hdaH$ 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.



b

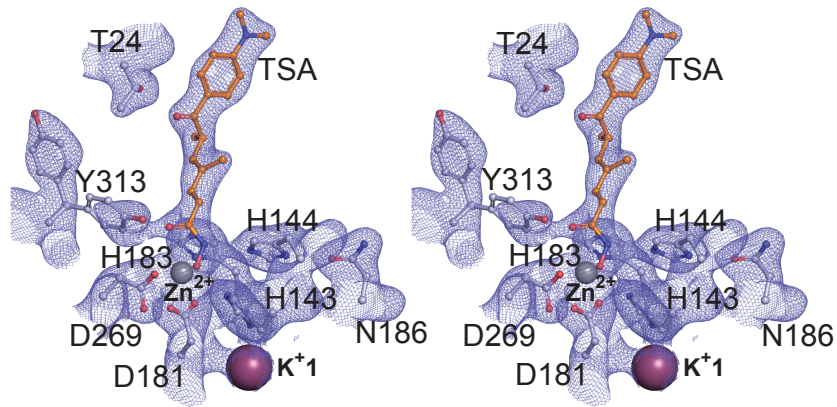
*Kp*HdaH (1b)•SAHA *Rw*DmhA (1b)•SAHA *Bs*AcuC (2c) AF2•apicidin A



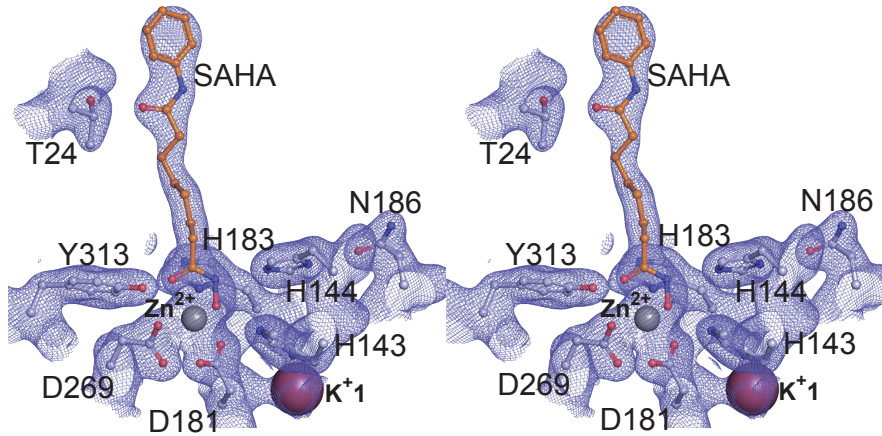
Supplementary Figure 26: Overview of structures of bacterial deacylases in complexes with the hydroxamate inhibitors SAHA, and trichostatin A (TSA).

- 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•apicidin A (PDB: [7LTG](https://doi.org/10.2210/pdb7LTG/pdb) [<https://doi.org/10.2210/pdb7LTG/pdb>]) and HDAC8•trapoxin A (PDB: [5VI6](https://doi.org/10.2210/pdb5VI6/pdb) [<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) [<https://doi.org/10.2210/pdb7LTG/pdb>])). The conserved Tyr (*Kp*HdaH: 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 directly coordinates the catalytic Zn²⁺-ion in a bidentate fashion. As described for the interaction with SAHA, Thr24 in *Kp*HdaH forms an interaction to the TSA carbonyl oxygen. The interaction of the cyclic peptide inhibitor apicidin A is created by formation of a hydrogen bond with the conserved Asp91 in *Bs*AcuC (2c) towards the peptide bonds of the cyclic peptide inhibitor. This Asp contributes to the selective inhibition of class I HDACs.

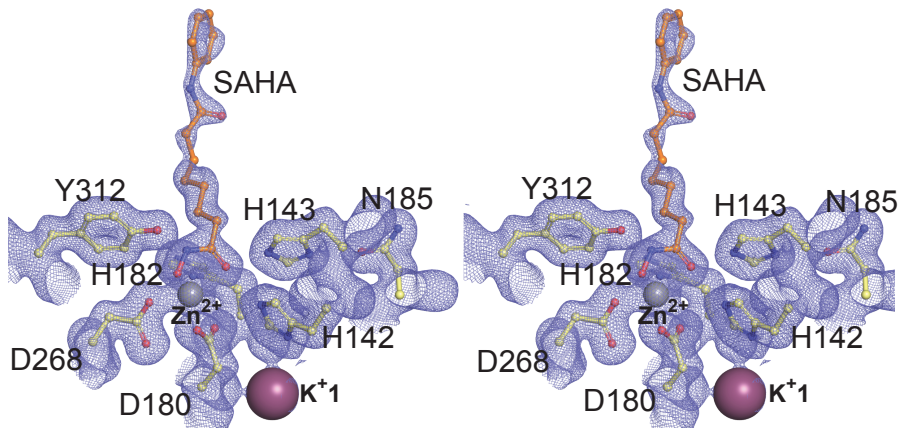
KpHdaH (1b)•TSA



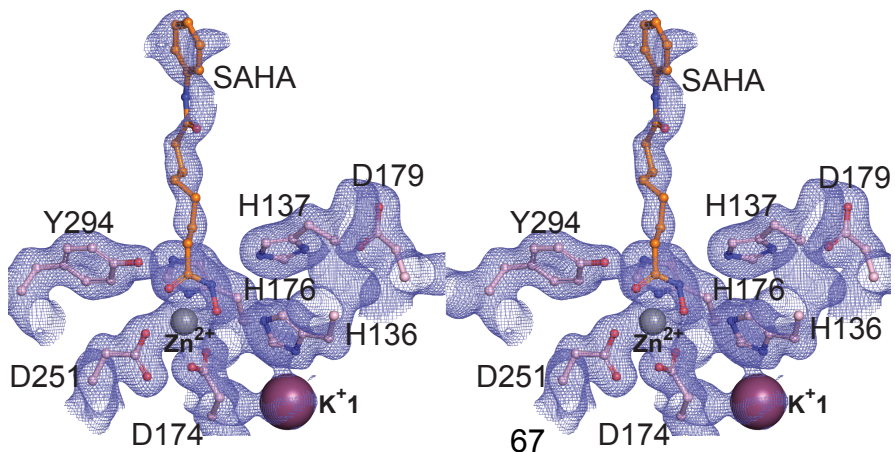
KpHdaH (1b)•SAHA



RwDmhA (1b)•SAHA

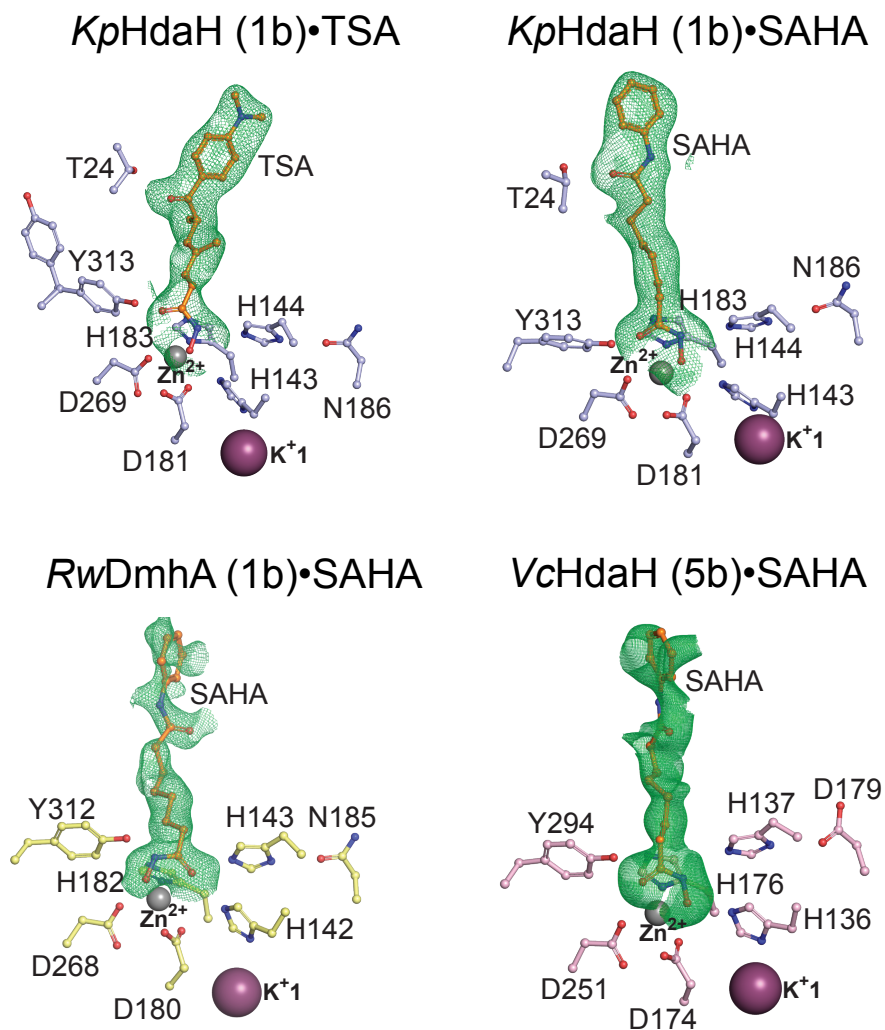


VcHdaH (5b)•SAHA



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: *KpHdaH* (1b)•SAHA, *KpHdaH* (1b)•TSA and *RwDmhA* (1b)•SAHA and *VcHdaH* (5b)•SAHA of subcluster 1b. For all structures a representative closeup of the active site is shown, including the bidentate Zn²⁺-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 2.3.4³.



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: *KpHdaH* (1b)•SAHA, *KpHdaH* (1b)•TSA, *RsPrpH* (1b)•SAHA, *RwDmhA* (1b)•SAHA and *VcHdaH* (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 2.3.4³.

and the XGGY-motif lining the foot pocket and the catalytic Tyr (*KpHdaH*: 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)	<i>B</i> <i>F</i> ⁻ <i>ompT gal dcm lon hsdS_B(r_B⁻m_B⁻) λ(DE3 [lacI lacUV5-T7p07 ind1 sam7 nin5]) [malB⁺]_K-12(λ^S)</i>	⁹
DH5α	<i>F</i> ⁻ <i>endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20 φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17(r_K⁻m_K⁺), λ⁻</i>	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	gtcgtccgccgggtcatgcttgctggcc
QC_VsHdaH_H144A_rev	ggccaggcaagcatgaccggcgacgac
QC_RsPrpH_H143A_fo	gccggccatgctgtctgccggatac
QC_RsPrpH_H143A_rev	gtatccggcagacagggcatggccggc
QC_RwDmhA_H143A_fo	ggcaggatcatgctgtggcagatac
QC_RwDmhA_H143A_rev	gtatctgccagacatgatgacctgcc
QC_VcHdaH_H126A_fo	gtggttatcatgcccccattgacagac
QC_VcHdaH_H126A_rev	gtctgatggggggcatgataaccac
QC_ApaHD3_H159A_fo	ccgggcatgacagcagcaagtgtattatg
QC_ApaHD3_H159A_rev	cataacatcacttctgctgatggcccg
QC_LcApaH_E_H179A_fo	cgacctatgacgacctcgagatgaag
QC_LcApaH_E_H179A_rev	cttcattctgcaaggctgatgggtcg
QC_LpApaH_H181A_fo	gccgagccatgccgctataccc
QC_LpApaH_H181A_rev	gtataggcggcatggctcggc
Gi_pOPIN_fo	gttatgaagggtcagcccgttagaccatttaaacaccac
Gi_pOPIN_rev	ctatacacactcagcataccgtaccaccgatctgttc
Gi_PA1409_fo	cagatcgggtggtaccggatgctgagtgtatagcg
Gi_PA1409_rev	ggtggtgttaaatgtctaacgggctgcaccttc
QC_PsApaH_1-342_Stop_fo	gaaaatgtgtaacgtccgaataatagtaaaaaag
QC_PsApaH_1-342_Stop_rev	ctattattcggacgttacacatttccaag
Insert_LpApaH_28-426_fo	tgtgcaattcagattccgag
Insert_LpApaH_28-426_rev	ggtaccacagctgatggtg
Gi_LpApaH_28-426_fo	catcaccacagccaggatccgtgtgcaattcagattccgag
Gi_LpApaH_28-426_rev	gcggtttctttaccagactcgagggtaccgctcgagtagtaactcatactatgac
Gi_pRSF-Duet_Lp_fo	gtatgagttactaactcgagcggtagccctcgagctggttaaagaac
Gi_pRSF-Duet_Lp_rev	gctcggaatctgaattgcacacggatcctggctgtggtgatg
QC_LpApaH_L414Stop_fo	gtgattgaaaaacagtaactgccgaaag
QC_LpApaH_L414Stop_rev	ctatcttcggcagttactgtttttcaatc
Insert_LcApaH_22-434_fo	tgtctgatccagattccgag
Insert_LcApaH_22-434_rev	ggtaccacagctgatggtg
Gi_LcApaH_22-434_fo	caccacagccaggatccgaattgtctgatccagattccgagtgcc
Gi_LcApaH_22-434_rev	cggtttctttaccagactcgagttacgggctgctggtttgccc
Gi_pRSF-Duet_Lc_fo	gcaaaaccagcagcccgttaactcgagctggttaaagaaccgctg
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}, H3₁₅₋₁₈ or TARK_{acyl}, H3₆₋₉), histone H4 (LGK_{acyl}, H4₁₀₋₁₂) tumor suppressor protein p53 (QPKK_{acyl}, p53₃₁₇₋₃₂₀) or DLAT (Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex; ETDK_{acyl}; DLAT₂₅₆₋₂₅₉). 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 <i>et al.</i> , 2010 ¹¹
QPKKac	Ac-Gln-Pro-Lys-Lys(Ac)-AMC	Madsen <i>et al.</i> , 2016 ¹²
APRKac	Ac-Ala-Pro-Arg-Lys(Ac)-AMC	Moreno-Yruela <i>et al.</i> , 2022 ¹³
ETDKac	Ac-Glu-Thr-Asp-Lys(Ac)-AMC	Galleano <i>et al.</i> , 2016
LGKpro	Ac-Leu-Gly-Lys(Pro)-AMC	Olesen <i>et al.</i> , 2018 ¹⁴
LGKbut	Ac-Leu-Gly-Lys(But)-AMC	Olesen <i>et al.</i> , 2018 ¹⁴
QPKKhex	Ac-Gln-Pro-Lys-Lys(Hex)-AMC	Madsen <i>et al.</i> , 2016 ¹²
QPKKoct	Ac-Gln-Pro-Lys-Lys(Oct)-AMC	Madsen <i>et al.</i> , 2016 ¹²
QPKKdec	Ac-Gln-Pro-Lys-Lys(Dec)-AMC	Madsen <i>et al.</i> , 2016 ¹²
TARKdec	Ac-Thr-Ala-Arg-Lys(Dec)-AMC	Madsen <i>et al.</i> , 2016 ¹²
ETDKdec	Ac-Glu-Thr-Asp-Lys(Dec)-AMC	Galleano <i>et al.</i> , 2016
QPKKlau	Ac-Gln-Pro-Lys-Lys(Lau)-AMC	Madsen <i>et al.</i> , 2016 ¹²
QPKKmyr	Ac-Gln-Pro-Lys-Lys(Myrist)-AMC	Madsen <i>et al.</i> , 2016 ¹²
TARKmyr	Ac-Thr-Ala-Arg-Lys(Myrist)-AMC	Madsen <i>et al.</i> , 2016 ¹²
ETDKmyr	Ac-Glu-Thr-Asp-Lys(Myrist)-AMC	Galleano <i>et al.</i> , 2016
QPKKpal	Ac-Gln-Pro-Lys-Lys(Pal)-AMC	Madsen <i>et al.</i> , 2016 ¹²
LGKcr	Ac-Leu-Gly-Lys(Croton)-AMC	Madsen <i>et al.</i> , 2012a ¹⁵
LGK(L-la)	Ac-Leu-Gly-Lys(L-lactyl)-AMC	Moreno-Yruela <i>et al.</i> , 2022 ¹³
LGK(D-la)	Ac-Leu-Gly-Lys(D-lactyl)-AMC	Moreno-Yruela <i>et al.</i> , 2022 ¹³
QPKK(L-la)	Ac-Gln-Pro-Lys-Lys(L-lactyl)-AMC	Moreno-Yruela <i>et al.</i> , 2022 ¹³
QPKK(D-la)	Ac-Gln-Pro-Lys-Lys(D-lactyl)-AMC	Moreno-Yruela <i>et al.</i> , 2022 ¹³
LGK(D-bhb)	Ac-Leu-Gly-Lys(D-β-hydroxybutyryl)-AMC	Moreno-Yruela <i>et al.</i> , 2022 ¹³
LGKsuc	Ac-Leu-Gly-Lys(Succinyl)-AMC	Madsen <i>et al.</i> , 2012b ¹⁶
LGKglu	Ac-Leu-Gly-Lys(Glutaryl)-AMC	Anderson <i>et al.</i> , 2017 ¹⁷
LGKbio	Ac-Leu-Gly-Lys(Biotinyl)-AMC	Moreno-Yruela <i>et al.</i> , 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
<i>KpHdaH</i> (1b)	12% (w/v) PEG 8000, 10% (v/v) glycerol and 0.5M KCl	with additional 10% glycerol	overnight	overnight	9GLB
<i>KpHdaH</i> H144A (1b)	12% (w/v) PEG 8000, 10% (v/v) glycerol and 0.5M KCl	with additional 10% glycerol	overnight	overnight	9GN1
<i>KpHdaH</i> (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
<i>KpHdaH</i> (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
<i>RsPrpH</i> (1b)	4% tacsimate pH 6 12% (w/v) PEG3350	with saturated glucose	5 days	16 days	9GKU
<i>RwDmhA</i> (1b)	0.2 M KF pH 7.3 and 20% (w/v) PEG3350	with 15% (w/v) PEG400	4 days	7 days	9GKW
<i>RwDmhA</i> (1b)•SAHA	0.2 M NaF pH 7.3 20% (w/v) PEG3350	with saturated glucose	3 days	5 days	9GKX
<i>VcHdaH</i> (5b)	1 M Na/K tartrate, 0.1 M imidazole pH 8 0.2 M NaCl	with saturated glucose	9 days	14 days	9GKY
<i>VcHdaH</i> (5b)•SAHA	1.4 M sodium acetate 0.1 M MES pH 6.5	with saturated glucose	12 days	20 days	9GKV
<i>PsApaH</i> 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
<i>LcApaH</i> ₂₂₋₄₀₉ (3)	1 M (NH ₄) ₂ SO ₄ 5% (w/v) PEG400 0.1 M Na-MES pH 6.5	2 M (NH ₄) ₂ SO ₄ 10% (w/v) PEG400 0.1 M Na-MES pH 6.5 12% (v/v) glycerol	26 days	30 days	9GL0
<i>LpApaH</i> (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 *KpHdaH* (1b) wildtype structure of cluster 1b enzymes and for the catalytically inactive mutant *KpHdaH* (1b) H144A structure.

	<i>KpHdaH</i> (1b) (PDB: 9GLB [https://doi.org/10.2210/pdb9GLB/pdb])	<i>KpHdaH</i> (1b) H144A (PDB: 9GN1 [https://doi.org/10.2210/pdb9GLN1/pdb])
Data collection		
Space group	I 2 3	I 2 3
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (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
<i>R</i> _{merge} (%) ^a	14.14 (24.13)	2.628 (23.49)
<i>I</i> / σ <i>I</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)
<i>R</i> _{work} / <i>R</i> _{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
Additionaly allowed	3.53	4.89
Disfavored	0.82	2.17
Clashscore	4.12	6.09
<i>B</i> -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 *KpHdaH* (1b) WT structure by molecular replacement.

*² One crystal was used to solve the structure of *KpHdaH* (1b) H144A by molecular replacement.

a: $R_{\text{merge}} = \frac{\sum_{\text{hkl}} \sum_{i=1}^n |I_i(\text{hkl}) - \bar{I}(\text{hkl})|}{\sum_{\text{hkl}} \sum_{i=1}^n I_i(\text{hkl})}$ where $I_i(\text{hkl})$ is the measured intensity of the reflection *i* at position *hkl* and $\bar{I}(\text{hkl})$ is the average intensity of the reflection(s) at position *hkl*¹⁹.

b: $R_{\text{work}} = \frac{\sum |F_o - F_c|}{\sum F_o}$ 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 reflections²⁰.

c: MolProbity²¹.

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

	<i>KpHdaH</i> (1b)•SAHA (PDB: 9GN6 [https://doi.org/10.2210/pdb9GN6/pdb])	<i>KpHdaH</i> (1b)•TSA (PDB: 9GN7 [https://doi.org/10.2210/pdb9GN7/pdb])
Data collection		
Space group	I 2 3	I 2 3
Cell dimensions		
<i>a, b, c</i> (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
<i>R</i> _{merge} (%) ^a	11.63 (37.52)	2.781 (28.25)
<i>I</i> / σ <i>I</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)
<i>R</i> _{work} / <i>R</i> _{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
<i>B</i> -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 *KpHdaH* (1b)•SAHA structure by molecular replacement.

*² One crystal was used to solve the *KpHdaH* (1b)•TSA structure by molecular replacement.

a: $R_{\text{merge}} = \frac{\sum_{\text{hkl}} \sum_{i=1}^n |I_i(\text{hkl}) - \bar{I}(\text{hkl})|}{\sum_{\text{hkl}} \sum_{i=1}^n I_i(\text{hkl})}$ where $I_i(\text{hkl})$ is the measured intensity of the reflection *i* at position *hkl* and $\bar{I}(\text{hkl})$ is the average intensity of the reflection(s) at position *hkl*¹⁹.

b: $R_{\text{work}} = \frac{\sum |F_o - F_c|}{\sum F_o}$ 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 reflections²⁰.

c: MolProbity²¹.

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

	<i>RwDmhA</i> (PDB: 9GKW [https://doi.org/10.2210/pdb/9GKW/pdb])	<i>RwDmhA</i> •SAHA (PDB: 9GKX [https://doi.org/10.2210/pdb/9GKX/pdb])
Data collection		
Space group	C 1 2 1	C 1 2 1
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (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
<i>R</i> _{merge} (%) ^a	20.26 (97.36)	19.51 (146.3)
<i>I</i> / σ <i>I</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)
<i>R</i> _{work} / <i>R</i> _{free} ^b	0.1719 (0.239)/	0.1626 (0.3732)/
	0.2173 (0.273)	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
Additionally allowed	2.40	2.75
Disfavored	0.21	0.00
Clashscore	7.45	3.43
<i>B</i> -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 *RwDmhA* structure by molecular replacement.

*² One crystal was used to solve the *RwDmhA*•SAHA structure by molecular replacement.

a: $R_{\text{merge}} = \frac{\sum_{\text{hkl}} \sum_{i=1}^n |I_i(\text{hkl}) - \bar{I}(\text{hkl})|}{\sum_{\text{hkl}} \sum_{i=1}^n I_i(\text{hkl})}$ where $I_i(\text{hkl})$ is the measured intensity of the reflection *i* at position *hkl* and $\bar{I}(\text{hkl})$ is the average intensity of the reflection(s) at position *hkl*¹⁹.

b: $R_{\text{work}} = \frac{\sum |F_o - F_c|}{\sum F_o}$ 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 reflections²⁰.

c: MolProbity²¹.

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

	<i>VcHdaH</i> (5b) (PDB: 9GKY [https://doi.org/10.2210/pdb9GKY/pdb])	<i>VcHdaH</i> (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		
<i>a</i> , <i>b</i> , <i>c</i> (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
<i>R</i> _{merge} (%) ^a	5.977 (188.8)	10.44 (180.9)
<i>I</i> / σ <i>I</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)
<i>R</i> _{work} / <i>R</i> _{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
Additionalily allowed	1.94	2.61
Disfavored	0.00	0.00
Clashscore	5.86	4.57
<i>B</i> -factors		
Protein	14.14	29.85
Ligand/ion	11.71	29.12
Water	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 *VcHdaH* (5b) structure by molecular replacement.

*2 One crystal was used to solve the structure of *VcHdaH* (5b)•SAHA by molecular replacement.

a: $R_{\text{merge}} = \frac{\sum_{\text{hkl}} \sum_{i=1}^n |I_i(\text{hkl}) - \bar{I}(\text{hkl})|}{\sum_{\text{hkl}} \sum_{i=1}^n I_i(\text{hkl})}$ where $I_i(\text{hkl})$ is the measured intensity of the reflection *i* at position *hkl* and $\bar{I}(\text{hkl})$ is the average intensity of the reflection(s) at position *hkl*¹⁹.

b: $R_{\text{work}} = \frac{\sum |F_o - F_c|}{\sum F_o}$ 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 reflections²⁰.

c: MolProbity²¹.

Supplementary Table 9: Data collection and refinement statistics (molecular replacement) for the *RsPrpH* (1b) structure and for the *PsApaH* (4) structure.

	<i>RsPrpH</i> (1b) (PDB: 9GKU [https://doi.org/10.2210/pdb9GKU/pdb])	<i>PsApaH</i> ₁₋₃₄₂ (4) (PDB: 9GKZ [https://doi.org/10.2210/pdb9GKZ/pdb])
Data collection		
Space group	P 1	P 21 21 2
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (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
<i>R</i> _{merge} (%) ^a	7.0 (137.3)	8.563 (49.85)
<i>I</i> / σ <i>I</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)
<i>R</i> _{work} / <i>R</i> _{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
<i>B</i> -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 *RsPrpH* (1b) structure by molecular replacement.

*² One crystal was used to solve the structure of *PsApaH* (4) by molecular replacement.

a: $R_{\text{merge}} = \frac{\sum_{\text{hkl}} \sum_{i=1}^n |I_i(\text{hkl}) - \bar{I}(\text{hkl})|}{\sum_{\text{hkl}} \sum_{i=1}^n I_i(\text{hkl})}$ where $I_i(\text{hkl})$ is the measured intensity of the reflection *i* at position *hkl* and $\bar{I}(\text{hkl})$ is the average intensity of the reflection(s) at position *hkl*¹⁹.

b: $R_{\text{work}} = \frac{\sum |F_o - F_c|}{\sum F_o}$ 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 reflections²⁰.

c: MolProbity²¹.

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

	<i>LcApaH</i> (PDB: 9GL0 [https://doi.org/10.2210/pdb9GL0/pdb])	<i>LpApaH</i> (PDB: 9GL1 [https://doi.org/10.2210/pdb9GL1/pdb])
Data collection		
Space group	P 32 2 1	P 41 2 2
Cell dimensions		
<i>a, b, c</i> (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)
$R_{\text{work}} / R_{\text{free}}$ ^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
Additionalally allowed	10.86	9.87
Disfavored	1.29	2.24
Clashscore	21.44	17.53
<i>B</i> -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 *LcApaH* structure by molecular replacement.

*² One crystal was used to solve the *LpApaH* structure by molecular replacement.

a: $R_{\text{merge}} = \frac{\sum_{\text{hkl}} \sum_{i=1}^n |I_i(\text{hkl}) - \bar{I}(\text{hkl})|}{\sum_{\text{hkl}} \sum_{i=1}^n I_i(\text{hkl})}$ where $I_i(\text{hkl})$ is the measured intensity of the reflection i at position hkl and $\bar{I}(\text{hkl})$ is the average intensity of the reflection(s) at position hkl ¹⁹.

b: $R_{\text{work}} = \frac{\sum |F_o - F_c|}{\sum F_o}$ 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 reflections²⁰.

c: MolProbity²¹.

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