

## Figure S1

Igni_0397	-----M	1
APE_1655	-----MRGRWPYEEKPLI	13
Hbut_0035	-----MKVGSEHSGYPLHRTWPFERNPLL	24
ST0127	-----MPFEDAPHL	9
M1425_1048	-----MPFENAPHL	9
SSO1631	-----MPFENAPHL	9
SSO1840	-----MPFENAPHL	9
Cmaq_1900	-----MPLDYSQRPLL	11
Pisl_0113	-----MREVKKLIERFHSAPLI	17
Tneu_1901	-----MRGIRELIERFHSAPLI	17
Pcal_1716	-----MRSVEELIRRFHEAPLL	17
PAE0596	-----MRNVQELIRRFHEAPLL	17
Pars_2255	-----MRNVQELIRRFHEAPLL	17
Msed_0512	-----MVAPYV	6
AF_2413	-----MDTPFI	6
NP_1546A	-----MSTGAPIPSPRDIDTSQRPFV	21
VNG_1185G	-----MTPVDTSERPVV	12
Hlac_1215	-----MFDNLDTDRRPLV	13
rrnAC3489	-----MRGKLDLGEQPLV	13
Hmuk_1679	-----MRGVDVEERPLV	12
LRZ99_07755	-----MI	2
Memar_0879	-----MRNSIGDSGKSEPTL	15
Mboo_0958	-----MNDSGAPRI	10
Mpal_2626	-----MI-----ENLTKADKELGECGIRTDVVKGIPGGAPPRI	33
Mthe_1135	-----MLL	3
C0624_00890	-----MSTSQEEFIPKW	12
Dace_3005	-----MADQEEKFIPKW	12
CSA32_02305	-----MNFIPKW	7
DSY57_04415	-----MDFEPKW	7
B5M56_04105	-----MSQ-----KHPA-----GEHNGKKAELRL	19
DSCW_66730	-----MNPHPMYQGSPPHG-----RGSGKNDTLRL	26
DEB50_09370	-----MAHPH-----KTHPPGHGG-----PHAAGKNNTLRL	26
CSA25_02885	-----MAHPH-----KTPPHGHAA-----PHAGGKKDILRL	26
DVU_0855	-----MGAHPTAHGPRTLEDGSPTCKL	22
Ddes_0287	MSKHMHHAGGHPGGMPGSAGNPISGHPGAEDGKHRGHPGGHGMSAPLRTLEDGSPACRL	60
Mbur_1233	-----MVKPPRL	7
<b>Mbar_A1458</b>	----- <b>MIAMTNAPRL</b>	<b>10</b>
MA_0573	-----MIAMTNPPRL	10
MM_1737	-----MIAMTNPPRL	10

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Igni_0397	VFWETTKACKLVCKHCRAEAIEEPL-PDELTHEESLALIDQITEFGRPYPHLIMTGGDVL	60
APE_1655	VFWESTKACLLACKHCRAEALTEPQ-PGELSTREALALVDQVVEFGRPYPILVITGGDPL	72
Hbut_0035	VFWETTKACMLACKHCRAAILKSL-PGELTTEEAYKLIDDVAAFGQPYPIVLVTGGDPL	83
ST0127	VFWEVTKACPLTCKHCRAAIDKPL-PGELTTEEGKKLLEEISQFGK--VVVFTGGDPL	66
M1425_1048	VFWEVTKACPLTCKHCRAAIDKPL-PSELNTEESRKLLIEDIARFGK--VVIVFTGGDPL	66
SSO1631	VFWEVTKACPLTCKHCRAAIDKPL-PGELNTEESRKLLIEDIARFGK--VVIVFTGGDPL	66
SSO1840	VFWEVTKACPLSCKHCRAAIDKPL-PGELSTEESSKKLLIEDIARFGK--VVIVFTGGDPL	66
Cmaq_1900	VFWETTKACPLSCRHCRAAAILKPL-PGELSTDEGKRLIEQLPEFGKPTPVLIITGGDPL	70
Pisl_0113	VFWESTKACPLACKHCRAADAILKPL-PGELNTEEGKRLIEQVASFGDPKPLLITGGDPL	76
Tneu_1901	VFWESTKACPLACKHCRAADAILRPL-PGELNTEEGKRLIEQVASFGDPKPLLITGGDPL	76
Pcal_1716	VFWESTKACPLACKHCRAADAILKPL-PGELSTEEGKRLIEQVAEFGDPKPLLIITGGDPL	76
PAE0596	VFWESTKACPLACKHCRAADAILKPL-PGELTTQEGKRLIEQVAEFGDPKPLLIITGGDPL	76
Pars_2255	VFWESTKACPLACKHCRAADAILKPL-PGELTTQEGKRLIEQVAQFGDPKPLLIITGGDPL	76
Msed_0512	VVLESTKACDLACKHCRAKAIPNRL-PGELTTEEVKSLVDDLASSGV--KLFVISGGDAL	63
AF_2413	VFWELTRACMLACKHCRAKAIRKRH-PDELTTTEECFNVIDQLSEFNP-KPLLIITGGDPL	64
NP_1546A	LIWEVTQACELACDHCRADAKPSRH-PDELTTAEGKRLLDSSAAEFGD-GQLVVLSSGGDPM	79
VNG_1185G	LVWEVTQACALACDHCRASARQHRH-PSELSTAEGKQLLTDAAAFGD-GQLVVLSSGGDPL	70
Hlac_1215	LIWEVTQACGLACRHCRAADAKPARH-PDELSTEEGKRLLDNAATFGD-GQLVVLSSGGDPL	71
rrnAC3489	LIWEVTQACELACKHCRADAQPRRH-PDELSTAEGKALLDQASEFGD-GQLVVLSSGGDPL	71
Hmuk_1679	LIWELTQACGLTCKHCRADARPERH-PDELTTAEGKRLLDQAADFGD-GQLVVLSSGGDPL	70
LRZ99_07755	VSWNTTNACNMYCQHCRYDAGAKA--DEELNTEVEGKALITEIAKAGF--KIMIFSGGEPL	58
Memar_0879	ISWNVTYRCLNLRCAHCYMDAGDGGG-ALELSTSEAKMLIDQVKQAGS--PVLILSGGEPL	72
Mboo_0958	ISWNLTLRCLPLKCAHCVYDAGEKEA-DRVLSTQEALSVIDQIRAIGK--PVVVLSSGEPL	67
Mpal_2626	ISWNLTLRCLPLKCAHCVYDAGEKEA-DRVLSTQEALSVIDQIRAIGK--PVVVLSSGEPL	90
Mthe_1135	VAWESTGACNLSCSYCRASAGPAPS-GDELSTGEIKGLIKEIAPMGA---MLIISGGEPL	59
C0624_00890	IAWETTQRCLNLCVHCRCSSDMDAA-EGDFNTEEAFLKIDDICEVSK--PVMVLSSGEPL	69
Dace_3005	IAWETTRRCLNLCVHCRCSSDMEAA-SGDFNTEEAFLKIDDICEVSK--PVMVLSSGEPL	69
CSA32_02305	IAWEITRRCLNLCVHCRSSSQIEVVEHPDFSYEEAKRVLRDIHSYAN--PVIVLSSGEPL	65
DSY57_04415	IAWETTRRCLNLCVHCRSSSKLEIKGHPDFSLDEAKRVLDDIHSYAD--PVIVLSSGEPL	65
B5M56_04105	VAWETTRNCLNLSRHCRAATMGPY-SGELDTDASFRLLDQIAETGS--PIVILTGGGEPL	76
DSCW_66730	VAWETTRNCLNLSRHCRAAATKGPY-DGELDTDQSFALLDQIAEVGR--PIIILTGGGEPL	83
DEB50_09370	VAWETTRRCLNLTCKHCRAAAEDHVY-NDELTTTEESFRLLDQIREVGQ--PIIILTGGGEPL	83
CSA25_02885	VAWETTRRCLNLTCKHCRAAAQDHVY-KDELTTQESFTLLDQIREVGQ--PIIILTGGGEPL	83
DVU_0855	IAWEVTRSCNLACKHCRAEAHMEPY-PGEFSTDEAKALIDTFPDVGN--PIIIFTGGDPM	79
Ddes_0287	IAWEVTRSCNLACKHCRAEAHPEPY-PGELSTAEAKALIDTFTEVGK--PIIIFTGGDPM	117
Mbur_1233	IAWELTWGONLACVHCRGSSTSEIP-EGELSTSEAKHFVDEIVEMGD--PILILTGGGEPL	64
<b>Mbar_A1458</b>	<b>IAWELTAGCNLNCVHCRGASTSSVP-AGELTTDEAKHFIDEVASIGK--PILILSGGEPL</b>	<b>67</b>
MA_0573	IAWELTAGCNLNCVHCRGASTSSVP-EGELTTEEAKHFIDEVVELGK--PILILSGGEPL	67
MM_1737	IAWELTAGCNLNCVHCRGASTSSVP-EGELTTEEAKHFIDVVELGK--PILILSGGEPL	67
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Igni_0397	MRKDFWDLARYSISKGIRTLVAPSVTPLLTEEKIKKKMKEIGIIGMSLSLDGARAETHDSI	120
APE_1655	MRRDFWRILEYAVSQGLRVAVAPSVTPLLTREVVRMMARMGVARISISIDSGLPVHDAI	132
Hbut_0035	LRSDIWDIIAYAKGKGLRLAVAPAVSPNLTEDEKVKKLAELGVDGVSISLDGSRPEIHDGI	143
ST0127	SRDDIFELMDYAKSLGLIVSIAPSPSHRLDDETMKII SN-SALYMSISLDGYPETHDWL	125
M1425_1048	SRSDIFELMEYAKSLGLVVSIAAPSPSHRLDDETMKII SN-YARYMSISLDGATPQTHDWL	125
SSO1631	SRSDIFELMEYAKSLGLVVSIAAPSPSHRLDDETMKII SN-YARYMSISLDGATSQTHDWL	125
SSO1840	SRSDIFEIMEYAKSLGLIVSIAPSPSYRLRDETMKMISN-YARYMSISIDGATSQTHDWL	125
Cmaq_1900	MREDIFELIDYAKSLNVPVAVSPTVSEKLLSDNVID-ELRRVSSVSVSLDGASPTTHEYI	129
Pisl_0113	MRNDLFDLIDYANQLGVPTSLAPAVSPNLNQEALKAVREHGVKSISISLDGAREETHDEL	136
Tneu_1901	MRNDLFDLVDYAVQLGVPTSLAPAVSPNLSPETLKAIREHGVKAISISLDGAREETHDEI	136
Pcal_1716	MRADLFELVDYANSLGVPVSLAPAVSKSLDDEALRRIKSSGVKSISISLDGATAETHDEL	136
PAE0596	MRSDFELIDYANSLSVPVSLAPAVSPNLNTETLKLKESGVKSISISLDGARPETHDEI	136
Pars_2255	MRADLFELVDYANSLSVPVSLAPAVSPNLTPPEVMKEMKQAGVKSISISLDGAFPETHDEL	136
Msed_0512	KRDDIFEILEYSSAK-ITTALSPSGSR-INVEVAKRIKDTGVSMVSI SV DGP-EEIHDEF	120
AF_2413	MRDDVTEIIISHAAEKGFRVAIAFSGTEKATEEKLRELKEAGVARVAVSIDGSDEEKHDSF	124
NP_1546A	KRDDLVELVRYGTKQGLRMTLTPSGTEALTPDNIAALVDAGLQRMALSV DAP-ATAHDF	138
VNG_1185G	ARPDTVELVEHGTD CGLRMTVTPSGTASLTPTAIEALADAGVAQFAVSI DGP TTHDEF	130
Hlac_1215	ARDDLLELVSYGDDQGLRMTITPSGTQSLTADRIEDLADAGIRRMALSLDGATRESHDRF	131
rrnAC3489	ARGDLPELVEYGTQGLRMTLTPSGTNSITPERLAELDDAGLRLALSIDGGSDAHDTF	131
Hmuk_1679	VRDDVTELVEYGTQGLGMLTTPSGTESLTPERIEALQDAGLRRMALSLDGGDADSHDAF	130
LRZ99_07755	MRPDIIELVAHAKSKGLRPVFGTNGTL-ITLEMAKRLKTAGALAIGISLDSVDVAKHDF	117
Memar_0879	LRDDIFEIAEYGTQGLRMAIGTNGTL-IDDRTAVRLAGAGVRKAAISLDSADPGVHDF	131
Mboo_0958	MRDDLCTIARYGTDRLRMVMGTSGYF-LDRPMAARLKEAGIRAAAI SLDSADPAVHDSF	126
Mpal_2626	LREDMYDIARYGTEQGLRMVMGTSGYL-IDQETAAKLKEAGIRAVAI SLDSKDPATHDAF	149
Mthe_1135	LRQDVFEVARYAAGSGVRVSLASNGTL-ITPEIVDRILLSGISRVSI SLDGASAKTNDAT	118
C0624_00890	LRKDIWEIAQYGTDKGLRMCMTATNGTL-ITDEICQKMKEVDLKMVSLSLDGSTAAIHDDF	128
Dace_3005	MRPDIFEIAEYGTSGGLRMCMTATNGTL-ITDEVCAKMNKADIKMVSLSLDGSTAEIHDDF	128
CSA32_02305	LREDVFDIAAYGTNLGLRMCCLATNGTL-VTEDTCRLIKESGIKMVSLSIDGATAAVHDF	124
DSY57_04415	LREDIFDIATYGAGLGLRMCMTATNGTL-VNQEVCRGMKESGIKMVSLSLDGASAAIHDDF	124
B5M56_04105	LRPDIFDIAAHGTSNGLKMVMAPNGTL-ITEESAKKMADAGISIRISISIDGATREDHDSF	135
DSCW_66730	LRADIFDIAKYGDNLGLRMVMAPNGTL-ITPQIAEKMAASGIRIRISASIDGATKEFHDKF	142
DEB50_09370	LRDDIFDIAAYGDKIGLRMVMAPNGTL-LNEDNVKRLIKSGIKRISVSLDGSTAASHDAF	142
CSA25_02885	LRDDIFDIAAYGDKIGLRMVMAPNGTL-LDEESVTRLMKSGIKRISVSLDGATAASHDAF	142
DVU_0855	MRGDVYELIAYATDKGLRCVMSPNGTL-ITPEHAQRMKASGVQRCSISIDGPDAASHDAF	138
Ddes_0287	IRPDVYELVAYAHSKGLPCAFSPNGTL-ITPETAQKIKNAGVNRCSISIDGADAASHDSF	176
Mbur_1233	VRKDVYEIARYATDKGLRVALATNGTL-LNDGVVKKLKDAGVQRVSI SLDGSTAQTHDDF	123
<b>Mbar_A1458</b>	<b>TRPDVFEIARYGTDAGLRVVLATNGTL-LTPEIVEKLRAAGVQRLSVSI DGGANAETHDNF</b>	<b>126</b>
MA_0573	TRSDVFEIARYATDAGLRVVLATNGTL-LTPELVEKLRAAGVQRLSVSI DGGATAKTHDEF	126
MM_1737	TRTDVFEIARYGTDAGLRVVLATNGTL-LTPEIVKKLRDAGVQRLSI SLDGATAKTHDEF	126
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Igni_0397	RGINGIFERTVELMNFVKKDVGMLQINTAVMRDNVEELPEVFKLITDAGVDAWEVFFYLI	180
APE_1655	RGVPGTFKASVNIVREA-LAAGLPVQINTTVMKPTVDSLPETLKLLLDLGVVDVWEVFFYV	191
Hbut_0035	RGTSGVFEKTLWAIKTF-QEYGVRVQVNTAVMRDNVHDLADIAALLLKLGVKVWEVFFYLV	202
ST0127	RGF-GNYRYAINGIKLG-LKYGIQVQVNTLVWKKSYEELPYIAKLLKDLGVKVWEVFFLI	183
M1425_1048	RGL-GSYKYALRGIELG-LKYGIQVQVNTLVWKKSYSELPFVVKLLKEMGVKIWEVFFLI	183
SSO1631	RGL-GSYKYALRGIELG-LKYGIQVQVNTLVWKKSYSELPFVVKLLKEMGVKIWEVFFLI	183
SSO1840	RGL-GSYNYALRGIELG-LKYGIQVQVNTLVWKKSYSELPFLVKLLKELGVKVWEVFFLI	183
Cmaq_1900	RNRNGVFELTLKAISL-LKAGVKVQVNTTFMKNLVHELPLIVKVLKDLGVYTWEVFFLI	188
Pisl_0113	RGVAGSFRDTVAVIKTA-VDMGIQVQVNTTVWRKSLTELPEVAKLITDLGVRTWEVFFLI	195
Tneu_1901	RGVPGSFRNTLAAIKAA-VDAGVQVQVNTTVWRKSLPELPEVVKLITDLGVKTWEVFFLI	195
Pcal_1716	RGVPGSFAETVSAIKRA-LDLGISVQVNTTVWKKSLSELPDVAYLLRRLGVKVWEVFFLI	195
PAE0596	RGVPGSYKETVNAIKTA-VELGVSQVNTTVWRKSLAELPEVAYLLKNLGVKVWEVFFLI	195
Pars_2255	RGVPGSYKETVTAIKTA-VEIGLPVQVNTTVWKKSLGELPDVAYLLKNLGVKIWEVFFLI	195
Msed_0512	RGVRGAFKMAQAVDSL-HEVKLPVQINSTISRYNVDHLQELRKTVEALRPVYWDVFMFI	179
AF_2413	RGVRGTFRMSMAIENA-KKAGLPFQINTTVTRENIEDLPNIARLCLELGAVMWDVFFV	183
NP_1546A	RGEAGSFERTREAAEAA-RDAGIPLQNTTVCATTVETLPAVREFVAEVGAVLWSVFFLV	197
VNG_1185G	RGEAGSFERTLRAARAI-RELGVPLQVNTTVCADTVEALPAIRDLVAELGVALWSVFFLV	189
Hlac_1215	RGEE-SFESTLEAAEAA-SEAGLPLQINTTVCAETVDELPAIRDRVRDLGAVLWSVFFLV	189
rrnAC3489	RGESGSFEATMAAAEAA-RNLDIPLQINTTVCAETVEQLPAIRDLVADLDAVLWSVFFLV	190
Hmuk_1679	RGESGSFEATLAAAEAA-RETDLPLQINTTVCAETVDQLPAIRELVADLGAVMWSVFFLV	189
LRZ99_07755	RATPGAWQGAQGMRNC-RQAGLPFQIHTTVVDWNYAEVETLTDFAIKEGALAHHTFFLV	176
Memar_0879	RGVAGAWERAVAGIEAC-RDAGIPVQVHTTVTLQNRDLLEGIAEFGESLGVRDQFFFLV	190
Mboo_0958	RGVSGAWERAVAAIKNC-TEEGIGVQINMTAVRPAAGDIESVVALGKNLGVRDYQVFFPV	185
Mpal_2626	RGLDGVWEKATKAIGHC-HDAGIAVQINMSVMRSAISEVEDLIGLGTSLGVHDYQLFFPI	208
Mthe_1135	RG-EGSFDLALRGIRAL-S-GRVEFQINMTITPANIDELDPILDLAEREGAAAHHIFFMV	175
C0624_00890	RSSPGAFEGTIRGAETL-KRNGIKFLVNSSFTRKNQDDIGATFKLAKGLGATAWYMFMI	187
Dace_3005	RQCPGAFEGVKRAAETL-TRNGIKFLINSSFTRKNQHDIANTFKLAKSLGATAWYMFMI	187
CSA32_02305	RNQPAGFTGTMHAIELF-NTHQIDFLVNSSFTRRNKEEAPKIYEMVKTLGATAWYLFMI	183
DSY57_04415	RTQPGAFDGTMNAIKLF-NENNIPFLINSSFTRRNKKEAPKIYQLVKELGATAWYLFMI	183
B5M56_04105	RGVDGAYEGALRGAELA-KEAGIDFQINTTVTRLNMEQLPRIMALAESIGAVAHHIFLLV	194
DSCW_66730	RGVDGAFDAAIRGIEYV-KAAGIEFQINTTITKTNLQIPKILELAEELGAAAHHIFLLV	201
DEB50_09370	RGLDGAFDRAVNGIKTA-KAAGLEFQINTVITKTNLNEIPAILALAESLGAAAHHIFLLV	201
CSA25_02885	RGVDGAFDRAINGIKIA-KAAGLDFQINTVITKTNLDEISAILTLAESLGAAAHHIFLLV	201
DVU_0855	RGVPGAFEQSMRGIGYL-RDAGIEFQINTTVTRDNLHSFKDIFKLCERIGAVAWHIFLLV	197
Ddes_0287	RGVPGAFEASMRGIEYL-KAAGVPFQINTTVTRNNLTSFKKIFELCERIGAAWHIFLLV	235
Mbur_1233	RGVPGAFESSMRGIEYL-KAGGLGFQINTTITKRNIDEIPAILEIATNIGAEAHHIFLLV	182
<b>Mbar_A1458</b>	<b>RGMPGAFERTLAGIEVL-RKADFPFQINTTVSKRNLEEITKTFELAKELGAVAYHVFFLV</b>	<b>185</b>
MA_0573	RGVPGAFDRTLAGEIEVL-RKDGFPFQINTTISRNLLEEIPKTFELAKDLGAVAYHVFFLV	185
MM_1737	RGVPGAFERTLAGIEIL-RKADFPFQINTTISRNLLEEIPKTFELAKELGAVAYHVFFLV	185
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Igni_0397	LTGRASKDLD--LTKDEYWDVSNFLYDASKY-GKTTIRTTEGPFRRVYRLRTVLDDEMGK	237
APE_1655	PTGRAARILD--LTPSEWEDVSNLYDASRY-GV-LVRTVEGPMFRRIALTRRLLENMGL	247
Hbut_0035	PVGRAQLELN--LTPEEWEDVSHFLYEASKY-GL-VVRTSEGPMFRRVAITRMLLELAGK	258
ST0127	PVGRGTLELD--IPREKYKDVIDFLVEVSRY-NI-VVRTVEAPFFRRAKLEYKEV-----	234
M1425_1048	PVGRGTTELD--IPRDKYKDVIDFLVEATRY-DL-VVRTVEAPFFRRAKLEYTPATT---	236
SSO1631	PVGRGTTELD--IPRDKYKDVIDFLVEATRY-DL-IVRTVEAPFFRRAKLEYTPATT---	236
SSO1840	PVGRGVIELD--IPKEKYKDVIDFLVETTRY-DL-VVRTVEAPFFRRAKLEYTPSTI---	236
Cmaq_1900	HVGRGIELEA--LTPEETEDVNVVLYDVSKY-GF-TVRTVEAPFYRRVVLHRYAFENNEI	244
Pisl_0113	VAGRAKEELD--ITPEEYEAQVFLVDVSTY-GL-QVRTVEAPFYRRAKLERLEG-----	246
Tneu_1901	VTGRAREELD--ISPEEYEAQVFLVDVSTY-GI-QVRTVEAPFYRRAKLERLEG-----	246
Pcal_1716	VTGRAREELD--ISPAEYESAVQVFLVDVSTY-GF-QVRTVEAPFYRRAKLERLRG-----	246
PAE0596	VTGRAREELD--ITPEEYEAQVFLVDVSTY-GF-QVRTVEAPFYRRAKLERLRG-----	246
Pars_2255	VTGRAREELD--ITPEEYEAQVFLVDVSTY-GF-QVRTVEAPFYRRAKLERLEG-----	246
Msed_0512	PTGRATKEMM--ITSEQAEEVMRTITKW-RM-EGLNVRMTCAPYLVRVMNEMGVVR----	231
AF_2413	PTGRAKAEMM--PTPQQFEDVLCWLYDLSKK-TGLNVKSSAATHLRRIELMRDRGE----	236
NP_1546A	PVGRGQILD--IQPERAESVLEWLQDVSEA-APFGVKTTEAPHYRRVALQRNGSA----	250
VNG_1185G	PVGRGRALDP--VSPARAEVMAWLDGVAR-S-EAFGVKTTEAPMYRRVRAQRGGD-----	242
Hlac_1215	AVGRGRILEP--IAPERAEVMEWLHGVAES-EPFGVKTTEAPFYRRVGLQSDGD-----	241
rrnAC3489	PVGRGRVLT--IDPERAERVVKWLHEVSDE-ASFGLKTTEAPHYRRVAMEQQDEG----	243
Hmuk_1679	PVGRGRVLTQ--ISPDRAERVMEWLHEVSES-EPFGLKTTEAPHYRRVTIERERD-----	242
LRZ99_07755	PTGRAVNIEQETLKAEQYKLLHRIMTKQQQ-VAIELKPTCAPQFMRIAKQLGVK-----	230
Memar_0879	PTGRGKEVVD--ISPEMYESLIRRLRLRAD-RGLSIRPTCAPQYVRIAAGMGLP-----	242
Mboo_0958	PTGRAGGTGP--ENPREYEDVIRRVLLKYCD-SNVNLRPTCAPQFRRIAADLGVT-----	237
Mpal_2626	PTGRARQIEP--RSPEEYEMIRRLIRYRD-SRINIRPTCAPQFRRIADCEGIA-----	260
Mthe_1135	PTGRGRAV--ECISPEMQRSLE---RIASEERSIEIRPTCAPQYGRVLMKKGS-----	225
C0624_00890	PTGRGEEIMNELITKEDYEEILSWHYEQEKNEDDILMRPTCAPHYRIVPQMAKAEGVDF	247
Dace_3005	PTGRGEEIMNELVSKEDYEEILSWHYEQEKNEDDILMRPTCAPHYRIVPQMAKAEGVDF	247
CSA32_02305	PTGRGEDIMAELIPEEEYEDILNWHYDMETESDLLVRPTCAPHYRIVLQRSKEEGSRF	243
DSY57_04415	PTGRGEEIMEELIPESEYEDMLNWHYDMKKESDMLVRPTCAPQYRIVLQRAKKEGEKF	243
B5M56_04105	PTGRGREMAEQAISAQDYENTLNWFYDQQKI-SSLQLKATCAPQYRIVLRQARARMENISV	253
DSCW_66730	PTGRGKYIVDQAIDAESYESTLNWFYDQREK-TSLQLKATCAPHYRILRQARARDEGKTI	260
DEB50_09370	PTGRGKYIVDTAIDAKEYEETLNWFYDQRDK-TSLQLKATCAPHYRILRQARAKADGKKV	260
CSA25_02885	PTGRGKYIVDTAIDAKEYEETLNWFYDQRDK-TSLQLKATCAPHYRILRQARAKAEGRKV	260
DVU_0855	PTGRAAGLSQDVISAAEYEEVLNWFYDQFRKT-TSMHLKATCAPHYRIMRQRAKEEGVSV	256
Ddes_0287	PMGRAAGLADQVITAQYEDVLHWLYDQFRKT-TKMHLKATCAPHYRIMRQRAKEEGVSV	294
Mbur_1233	PTGRGKELENEEIIPAEYERVNLNWFYDQQKH-VKIQLKATCAPHYFRIMRQRAKREGTEV	241
<b>Mbar_A1458</b>	<b>PTGRGDE--SDEVSPADYERILHWFYEMQKE-SKIQLKATCAPHYFRIMRQRAKKEGIEI</b>	<b>242</b>
MA_0573	PTGRGEE--SDEVSPADYERVNLHWFYDMQKE-SEIQLKATCAPHYFRIMRQRAKKEGIEI	242
MM_1737	PTGRGEE--SDEVSPADYERVNLHWFYDMQKE-SEIQLKATCAPHYFRIMRQRAKKEGIEI	242

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Igni_0397	GPE-ELGVGELYKLRERLEELMGVPV-----HGPPKQKRAPSAYTRDGYGIIFVAY	288
APE_1655	DWRNRLRPGSLYHKLARKTLELLG--D-----PPGEARAQTTGTRDGKGVIFVSN	295
Hbut_0035	NPDEALNTGPLYRQLVSRRLRQLLG--E-----PQGKPLASTTGTDRDGKGVIFVSY	306
ST0127	-----KNELIRKLKELLG--E-----SKSPVDKSILPTRDGAGVIFISY	271
M1425_1048	-----NDNELVSTLRELLG--E-----PVKEADKSILPTRDGAGVIFIGY	274
SSO1631	-----NDNELVSTLRELLG--E-----PVKEADKSILPTRDGAGVIFIGY	274
SSO1840	-----KSNELVSKLRELLG--E-----PVKDVDKSVLPTRDGSGVIFIGY	274
Cmaq_1900	N--IKPNLGPLYRQLYEGLIKVMGNEP-----PKLTKRPMVARTRDGDGIIFVAY	292
Pisl_0113	----KTYDSPLYRKLVGRLRELMG--P-----PRRGIDPTIVPTRDGFGIIFVAY	290
Tneu_1901	----KTYDSPLYRKLVRLGELMG--P-----PRRGVDPTVVPTRDGFGIIFVAY	290
Pcal_1716	----KAYSDDLKLVARLRELLG--P-----PQRGVDPTIVPTRDGFGIIFVGH	290
PAE0596	----REFGHPLYNQLVERLRNLMG--P-----PVRVDPTIVPTRDGFGIIFIAY	290
Pars_2255	----RIYDHPLYLQLVDKLRKLLG--P-----PTRGVDPTIVPTRDGFGIIFVAY	290
Msed_0512	----P-----LP-----PDKNYGRRSVNGARGCMAGNGYAFVAY	261
AF_2413	----MPAVGELYRLLERIEDFPEGEGIVVAGGHGKSLSTDGIRRAIGITDGRGMFFISH	292
NP_1546A	----T-----DAPDADAIGRRGGIIAGDGFVSH	276
VNG_1185G	-----APQRRAGVTAGDGFVSH	261
Hlac_1215	-----EEATRRRGGITAGRGFVSH	262
rrnAC3489	----A-----SGLKRRMGIRAGKGFVSH	264
Hmuk_1679	----E-----AGENDGLQRRTGIRAGQGFAVSH	267
LRZ99_07755	-----M-RFSKGCLAGTGyciisp	248
Memar_0879	-----VAEGERGCIAGIRYCRIDP	261
Mboo_0958	-----KPDWGRGCIAGISYCRIFA	256
Mpal_2626	-----NPAWGRGCLAGITYCRIFA	279
Mthe_1135	-----QARTAGGCIAGIRFVFISR	244
C0624_00890	ERRSL-----TFSTGGGKGCIAAQTICLIDC	273
Dace_3005	KRRSL-----TFSTGGGKGCIAAQTICLIDC	273
CSA32_02305	RRRSL-----KFSTGGSKGCLAGQLICLIDV	269
DSY57_04415	KRRNL-----KFSTGGSKGCLAGQLICLIDV	269
B5M56_04105	SFKTH-----GLD-AVTRGCLGGVSFCFISH	278
DSCW_66730	SFESH-----GLD-AVTRGCLAGTGFCFISH	285
DEB50_09370	SFETH-----GLD-AVTRGCLAGTGFCFISH	285
CSA25_02885	SFKTH-----GLD-AVTRGCLAGTGFCFISH	285
DVU_0855	TPDNF-----GMD-AMTRGCLGGTGFCFISH	281
Ddes_0287	TPENF-----GMD-ALTRGCLGGTGFCFISH	319
Mbur_1233	TVKTH-----GYE-AMTRGCLGGISFCFVSS	266
<b>Mbar_A1458</b>	<b>SVKTH-----GYE-AMTKGCLGGTGFCFVSS</b>	<b>267</b>
MA_0573	SVKTH-----GYE-AMTKGCLGGTGFCFVSS	267
MM_1737	SVKTH-----GYE-AMTKGCLGGTGFCFVSS	267

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Igni_0397	NGDVYPSGFLPYKVGNVREASLKEIYQNSKALKMIRDPSNFRPP	CGTCKFNFMCGGSRAR	348								
APE_1655	KGLVYPSGFLPYVGDVRKSSLKEIYQSSPELEGLRK-AVFKGR	CGRCEFSQLCGGSRAR	354								
Hbut_0035	NGTVYPSGFMPYPLGNIRVKSLEIYRENILKRLRG-ARFEGRC	CGRCEFREICGGSRAR	365								
ST0127	NGDIYPSGFLPLKLGNVREDRLIDVYRNSELLKMIKA-GKLKGK	CGICAFSNICGGSRAR	330								
M1425_1048	NGDVYPSGFLPLYLGNVKKESLVDIYRKSEVLKKIKD-SRFEGK	CGICKYNNICGGSRAR	333								
SSO1631	NGDVYPSGFLPLYLGNVKKESLVDIYRKSEVLKKIKD-SRFEGK	CGICKYNNICGGSRAR	333								
SSO1840	NGDVYPSGFLPLYLGNVRKESIVDIYRKSEVLKKIKD-GRFDGK	CGVCKFNNICGGSRAR	333								
Cmaq_1900	NGDVSPSGFLPIKLGNVKEESLVKIYRENPVLLRIRR-GEYGGRC	GLCEFRFICGGSRAR	351								
Pisl_0113	DGTVYPSGFLPYPLGNVRRRSLVEIYREHPLLQKMRR-GEFGGRC	GVCKYKDICGGSRAR	349								
Tneu_1901	DGTVYPSGFLPYPLGNVRRRSLVEIYRDHPLLQKMRR-GEFGGRC	GVCKYKDICGGSRAR	349								
Pcal_1716	DGTVTPSGFLPYPLGNVRKRLVDIYRNHPLLVKMRR-GEFEGRC	GVCEYKDICGGSRAR	349								
PAE0596	DGTVHPSGFLPYPLGNVRKQSLVKIYREHPLLQKMRR-GEFGGRC	GVCEYKDICGGSRAR	349								
Pars_2255	DGTVHPSGFLPYPLGNVRRQSLVEIYRNHPLLQKMRR-GEFGGRC	GVCRYKDICGGSRAR	349								
Msed_0512	DGTVYP	CGFLPIPAGNVRRFRFSEIYEQSPVFKSLREPSKLGKCG	GLCEYRSVCGGCRAR	321							
AF_2413	IGEYVPSGFLPIVAGNVNRTSLKEIYYSSEIFVNLRDPDR	LKGKCGRCEYRKICGGSRAR	352								
NP_1546A	TGELFPSGFLPKSAGNVTDSDLVSLYQDSTLFESLRDR	DLKKGKCGACEFRHVCGGSRAR	336								
VNG_1185G	TGAVYPSGFLPSSAGNVRRERSVVDSYRNSELFQRLRDTEAL	TGKCGACSFRTVCGGSRAR	321								
Hlac_1215	TGEAYPSGFLPESAGNVHDSIVDIYRNGDLFESLREPDR	LKGKCGACEFRQVCGGSRAR	322								
rrnAC3489	TGEVFPSPGFLPKSAGSVREESVVDIYRDSPLFQQLRDD	DALTGKCGACRYRTVCGGSRAR	324								
Hmuk_1679	TGEMYPSGFLPESAGNVRRSEGVVDLYRESALFQRLRDD	SALEGKCGACPYRGVCGGSRAR	327								
LRZ99_07755	KGIVQPCAYLNI	PAGNVRET	PFSEIWRD	NAVFNR	LRNQ-PLQGGCGTCDYQKICGGCRAR	307					
Memar_0879	TGEVTPCPYLP	PLGLGNIRRT	PF	FAEIWNGSEV	FAALRS	GEGLRGKCGACEYRSACGGCRAR	321				
Mboo_0958	NGDVT	PCPYLPVS	AGNV	RDIPF	DIWNE	SHLFAL	RD	PNRLTGKCGRCEYKTTCCGGCRAR	316		
Mpal_2626	NGDVT	PCPYLPVS	AGNV	RTT	PFSEI	WNN	SPLFA	AL	RDPSRLTGKCGRCSFKTSCGGCRAR	339	
Mthe_1135	TGDV	FPCGYF	PLS	AGSIR	DRSFSEI	WSS	PLL	N	DLRER-RLKGR	CGSCNYVRICGGCRAR	303
C0624_00890	FGNLKPCSYFHSS	VGNVKQIPFKEL	WFNNKVFNDLR	DFSKYKKGKCGECE	FLNVC	GGCRAR	333				
Dace_3005	FGNLKPCSYFHSS	VGNVKQIPFKDL	WFNSKVFNDLR	DFSKYKKGKCGECE	FINVC	GGCRAR	333				
CSA32_02305	DGNILPCSYFPLSD	SNIRDKSFKDI	WENSPL	LLTDMRNFSQYKGS	CGRCEYIQV	CGGCRAR	329				
DSY57_04415	DGNVLP	CSYF	PKAAGNIRS	QSFQDI	WENSTLFHEL	RD	FKSYKGS	CGHCEYVNV	CGGCRAR	329	
B5M56_04105	VGIVQPC	G	FLELNC	GDVTSQH	FGEI	WRDSVIFNN	LRNYDML	GGKCGICEYKRV	CGGCRAR	338	
DSCW_66730	TGIVQPC	GYTD	VKCGDIT	TRDTF	GHVWRNSP	VFLKLR	DFKQLEGK	CGRCEYRAV	CGGCRAR	345	
DEB50_09370	VGRVQTC	G	FLDVT	CGDIK	TS	HF	KDVWENSEVFN	KLRDFNNLEPK	CGICEYKQV	CGGCRAR	345
CSA25_02885	VGRVQTC	G	FLDVT	CGDIK	TH	HF	KDVWENSEVFN	KLRNFNNLDPK	CGLCEYKQV	CGGCRAR	345
DVU_0855	TGQVQPC	G	YLELDC	GNVRNT	PFPEI	WRKSEH	FRQFRTQEE	YTGKCGPCEYHKV	CGGCRAR	341	
Ddes_0287	VGQVQPC	G	YLELDC	GNVRQT	PF	PKI	WRESKH	FLQFRDQSCYSGK	CGECEYHKV	CGGCRAR	379
Mbur_1233	TGDVQPC	G	YLP	VIAGNI	KEKSFKEI	WEDSVL	FN	DLRDYDKLGGK	CGRCSYKNV	CGGCRAR	326
<b>Mbar_A1458</b>	<b>VGKVFPCGYLPV</b>	<b>LAGNIREQPF</b>	<b>REI</b>	<b>WENAEVFR</b>	<b>KLRDPEEL</b>	<b>KGKCGICEYK</b>	<b>KVCAGCRAR</b>	<b>327</b>			
MA_0573	VGEVYPC	G	YLPV	LAGNIREQ	PFKEI	WENSEVFR	KLRDPEEL	KGKCGICEYK	KVCAGCRAR	327	
MM_1737	VGEVYPC	G	YLPV	LAGDIRA	QPF	KDVWENSEVFR	KLRDPEEL	KGKCGICEYK	KVCAGCRAR	327	

\* . : .. : : : : \*\* \* : \*.\*\*.\*

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Igni_0397	AYS	FKDPFGHDPACKLPEVLES-LDPQVVEEALRPFGRAX <sub>5</sub>	393
APE_1655	AYS	TGDPLGEDPACAYRPGEFRSLLQELGVGEADVYGLVEX <sub>8</sub>	403
Hbut_0035	AYAV	TGKPFGEDPACPYPRTGTFSSVMVSKLGVRVENNVREIE <sub>9</sub>	415
ST0127	AYAV	YGDPLAEDPACPY	347
M1425_1048	AFAV	YNNPFAEDPMCPY	350
SSO1631	AFAV	YNNPFAEDPMCPY	350
SSO1840	AFAV	YNNPFAEDPMCPY	350
Cmaq_1900	AYAE	YGDPLAEDPACVYSPGTIRLPESLSP	382
Pisl_0113	AFAY	FKDPLAEDPACIYSPPL	370
Tneu_1901	AFAY	FKDPLAEDPACVYKP	368
Pcal_1716	AFAV	FKNPLAEDPACVYIPSSWKSVMTR	378
PAE0596	AFAY	FKDPLAEDPACVYKPHRNL	372
Pars_2255	AFAY	YKDPLAEDPACIYKPT	369
Msed_0512	AFSL	TENFMDEDPFCTYVPRTLRVRA	347
AF_2413	AYAV	HGDYLAEEPCCIYIPQSSR	375
NP_1546A	AYAV	TGDPLASDPLCAYRPSGYDGPLPDRQAAADR	371
VNG_1185G	AYAT	TGEPMASDPLCPHVPDGYDGPLPDRQATADD	356
Hlac_1215	AFAT	TGDPLESDPLCPYVPDGYDGELPPTLKDGFEGSTSAPX <sub>4</sub>	367
rrnAC3489	AYAT	TGDPLAADPLCDYRPDGFEGSVDPQHPAD	357
Hmuk_1679	AYAA	TGDPMGSDPLCSFVPDEYDGPLPATHGERVPTD	364
LRZ99_07755	AYYY	HGDYMAEEPWCILYHGRKGY	330
Memar_0879	AYGVTQE	RSGTGDCLAEDPWCLYEPGVR	349
Mboo_0958	AYRGAEAFSSRWCDGLLKPSAIGELCAEDPWCPYEPGGS		356
Mpal_2626	AYRQEDAASPLWCDGLATPDIVNGEICGEDPWCPYQPPDVIP		381
Mthe_1135	AYAL	TGDYLGEDPTCAWRGSIG	325
C0624_00890	ADAV	YGDYMQQEPFCNYVPERMRKKMHKEAEEMAPK	369
Dace_3005	ADAV	YGDYMAEEPFCNYIPRKTRLRMEKEAAENRGE	369
CSA32_02305	AYAM	TGDYMAEEPFCITYQPSRKMRTQL	356
DSY57_04415	AYAV	TGDYLAPEPFCSYQPK	349
B5M56_04105	AYEA	TGDYLAEEPLCVYQPAKQSQPL	364
DSCW_66730	AYEA	TGNYMAEEPCLCAYQPEKAAPC	370
DEB50_09370	AYEA	TGNYLAQEPLCTYQPPRH	367
CSA25_02885	AYEA	TGNYLAQEPLCTYQPARHKSAV	371
DVU_0855	AYNM	SGDHMAEEPCLCSYKPRRMTPCR	367
Ddes_0287	AHSM	DGDHMGEEPLCTYIPAKMRKKGRDGKS GEEK	414
Mbur_1233	AYAA	TGDYMAEEPYCIYNPR	346
<b>Mbar_A1458</b>	<b>AYAA</b>	<b>TGDYLEEEPYCIYRPGKK</b>	<b>349</b>
MA_0573	AYAA	TGDYLEEEPYCIYRPGKK	349
MM_1737	AYAA	TGDYLEEEPYCIYRPGKK	349
	*	* * *	

**Figure S1.** Amino acid sequence alignment of AhbD sequences from different archaea and sulfate-reducing bacteria. Conserved N-terminal cysteine residues coordinating the RS cluster are highlighted in violet purple, C-terminal cysteine residues in raspberry, and partially conserved cysteine residues of the full SPASM motif in salmon. Sequences are specified by gene numbers.

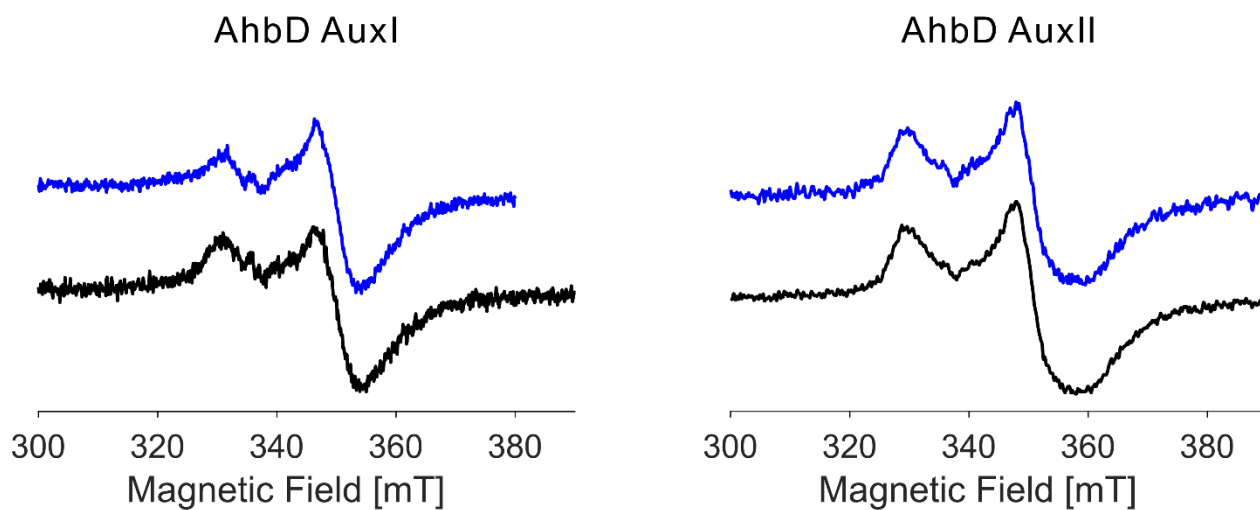


**Figure S2**

anSME_CPE0635	6	LLIKPASSGCNLKITYCFYHSLSDNRNVKSYGIMRDEVLES MVKRVLNE--ADGHCSFAF	63
SuiB_STRSU	108	ELVIYPSMYCDLKQGFCLANREDRN-----AKPAKDWERILRQAKDNGVLSVSI	157
PQQE_KLEPN	13	WLLAELTYRCPLQCPYCSNPLDFARQDK-----ELTTEQWIEVFRQARAMGSVQLGF	64
MFTC_MYCTU	21	CLTWELTYACNLACVHCLSSSG-KRDPG-----ELSTRQCKDIIDELERMQGFYVNI	71
DVU_0855	22	LIWEVTRSCNLACKHCRAEAHMEPYPG-----EFSTDEAKALIDTFPDVGNPIIIF	73
Ddes_0287	60	LIWEVTRSCNLACKHCRAEAHPEPYPG-----ELSTAEAKALIDTFTEVGKPIIIF	111
Mbur_1233	7	LIAWELTWGCNLACVHCRGSSTSEIPEG-----ELSTSEAKHFVDEIVEMGDPILIL	58
<b>Mbar_A1458</b>	<b>10</b>	<b>LIAWELTAGCNLCVHCRGASTSSVPAG-----ELTTDEAKHFIDEVASIGKPIILIL</b>	<b>61</b>
		: : * * * . *	. . . :
anSME_CPE0635		QGGEPILAGLEFFERLMELQRKHNYKNLKIYNSLQTNGTLIDESWAKFLSENKF-LVGLS	122
SuiB_STRSU		LGGEPTRYFD-IDN----LLI--ACEELKIKTTITNAQLIKKSTVEILAKSKYITPVLS	210
PQQE_KLEPN		SGGEPLTRKD-LPE----LIR--AARDLGFYTNLITSGIGLTSKLDASFSEAGLDHIQIS	117
MFTC_MYCTU		GGGEPTVRPD-FWE----LVD--YATAHHVGKFKSTNGVRITPEVATRLAATDYVDVQIS	124
DVU_0855		TGGDPMMRGD-VYE----LIA--YATDKGLRCVMSPNGTLITPEHAQRMKASGVQRC SIS	126
Ddes_0287		TGGDPMIRPD-VYE----LVA--YAHSGKLPCAFSPNGTLITPETAQKIKNAGVNRCSIS	164
Mbur_1233		TGGEPLVRKD-VYE----IAR--YATDKGLRVALATNGTLNDGVVKKLKDAGVQRV SIS	111
<b>Mbar_A1458</b>		<b>SGGEPLTRPD-VFE----IAR--YGTDAGLRVVLATNGTLTPEIVEKLRAGVQRLSVS</b>	<b>114</b>
		**:* . : : . : . . : : *	
anSME_CPE0635		MDGPKEIHNLNRKDCGLDTFSKVERAAELFKKYKV-EFNILCVVTSNTARHVNKIYRYF	181
SuiB_STRSU		LQTLDSKLNFEMLMGR--PD--RQIKLAKYFNEVGK-KCRINAVYTKQSYEQIIELVDFC	265
PQQE_KLEPN		FQASDEVNLNAALAGNK--KAFQOKLAMAKAVKARDY-PMVLNFVLHRRHNDQLDKIIELC	174
MFTC_MYCTU		LDGATAEVDNDAIRGT---GSFDMAVRALQNLAAAGFAGVKISVITRRNVAQLDEFATLA	181
DVU_0855		IDGPDAAASHDAFRGVP--GAFEQSMRGIYLRDAGI-EFQINTTVTRDNLHSEFKDIFKLC	183
Ddes_0287		IDGADAASHDSFRGVP--GAFEASMRGIEYLKAAGV-PFQINTTVTRNNLTSFKKIFELC	221
Mbur_1233		IDGSAQTHDDFRGVP--GAFESSMRGIEYLKAGGL-GFQINTTITKRNIIDEIPALIEIA	168
<b>Mbar_A1458</b>		<b>IDGANAETHDNFRGMP--GAFERTLAGIEVLRKADF-PFQINTTVSKRNLEETIKTFELA</b>	<b>171</b>
		:: : . . . . . : . . . . .	
anSME_CPE0635		KEKDFKFLQFINCLDPLYEEKGYNYSLKPQDY-TKFLKNLFDLW-----YEDFLNGNR	234
SuiB_STRSU		IENKIDRFVS-ANYSEVTGYTKIKKK-YDLADL-RRLNEYVTD----YITQREANLNFAT	318
PQQE_KLEPN		IELEADDVEL-AT-----CQFYGWAFLNREGLLPTREQIARAEQ	212
MFTC_MYCTU		SRYGAT-LRI-TRLRPSGRGTDVWADLHPTADQQVQLYDWLVSKGERV-----L	228
DVU_0855		ERIGAVAWHI-FLLVPTGRAAGLSQVISAEEY-EEVLNWFYDFRKTTSMHL-----KAT	236
Ddes_0287		ERIGAAAWHI-FLLVPMGRAAGLADQVITAQEY-EDVLHWLYDFRKTTKMHL-----KAT	274
Mbur_1233		TNIGAEAAHI-FLLVPTGRGKELENEEIPPAEY-ERVNLWFYDQQKHVKIQL-----KAT	221
<b>Mbar_A1458</b>		<b>KELGAVAYHV-FFLVPTGRGDE--SDEVSPADY-ERILHWFYEMQKESKIQL-----KAT</b>	<b>222</b>
		. . . . .	
anSME_CPE0635		VSIRYFDGLL-----ETIL--LG-----KSSSGMNGTCTCQFVVESDGSVYPCDFYVLD	282
SuiB_STRSU		EGCHLFTAYP-----ELINNSIEFSEFDEMYYGCRAKYTKMEIM--SNGDILPCIAFLGV	371
PQQE_KLEPN		VVADYRQKMAASGN-LTNLLFVTDPDYEEERPCKGMGWGSIFLSVTPEGTALPCHSARQL	271
MFTC_MYCTU		TGDSFFHLAP-----LGQSGALAGLNMCGAGRVVCLID--PVGDVYACPFIAHD	275
DVU_0855		CAPHYYRIMRQRAKEEGVSVPDNFGMDAMTRGCLGGTGFCFIS--HTGQVQPCGYLEL-	293
Ddes_0287		CAPHYYRIMRQRAKEEGVSVPENFGMDALTRGCLGGTGFCFIS--HVGQVQPCGYLEL-	331
Mbur_1233		CAPHYFRIMRQRAKREGTEVTVKTHGYEAMTRGCLGGISFCFVS--STGDVQPCGYLPV-	278
<b>Mbar_A1458</b>		<b>CAPHYFRIMRQQAQKKEGIEISVKTHGYEAMTKGCLGGTGFCFVS--SVGKVFPCGYLPV-</b>	<b>279</b>
		. . . . . * *	
anSME_CPE0635		KWRLGN-IQDMTMKELFETNKNHFEIKSSFVHEEKKCKWFKLCKGGCRRCRDSKEDSD	341
SuiB_STRSU		NQTKQN-AFEKDLLDVWYDDPLYGGIRSFRTKNSKQLSCGLLKIIEGGCYVNLIKEKSPE	430
PQQE_KLEPN		PVAFPS-VLEQSLESIWYDSFGFNRYRGYDWMPEPCRS CDEKEKDFGGCRCAQAFMLTGSA	330
MFTC_MYCTU		HFLAGNVLSGGGFQNVWKNSSLFRELREPQS-AGACGSCGHYDSRGGCMAAKFFTGLPL	334
DVU_0855		--DCGN-VRNTPFPEIWRKSEHFQRTQEEYTGKGPCEYHKVC-GGCRARAYNMSGDH	349
Ddes_0287		--DCGN-VRQTPFPKIWRKSEHFLQFRDQSCYSGKCGCEYHKVC-GGCRARAHSMGDH	387
Mbur_1233		--IAGN-IKEKSFKIEWEDSVLFNDLRDYDKLGKCGRC SYKNVC-GGCRARAYAATGDY	334
<b>Mbar_A1458</b>		<b>--LAGN-IREQPFREIWENAEVFRKLDPPEELKKGCGICEYKKVC-GGCRARAYAATGDY</b>	<b>335</b>
		. : : .:: . : * * . .**	
anSME_CPE0635		LELNYYCQSYKEFFEYAFPRLI-----NVANNIK-----	370
SuiB_STRSU		YFRDSVCQL-----	439
PQQE_KLEPN		DNADPVCCKSPHHHKILEARRE-----AACSDIKVSQ---LQFRNRTRSQLIYQTRDL	380
MFTC_MYCTU		DGPDPECVQGHSEPALARERHLPRPRADHSRGRRVSKPVPLTLSMRPPKRPC--N-ESPV	391
DVU_0855		MAEEPSCSYKPRRMTPC-----R-----	367
Ddes_0287		MGEELPLCTYIPAKMRKK-----GRDGKSGEEK-----	414
Mbur_1233		MAEEPYCINPR-----	346
<b>Mbar_A1458</b>		<b>LEEEPYCIRPGKK-----</b>	<b>349</b>
		: *	

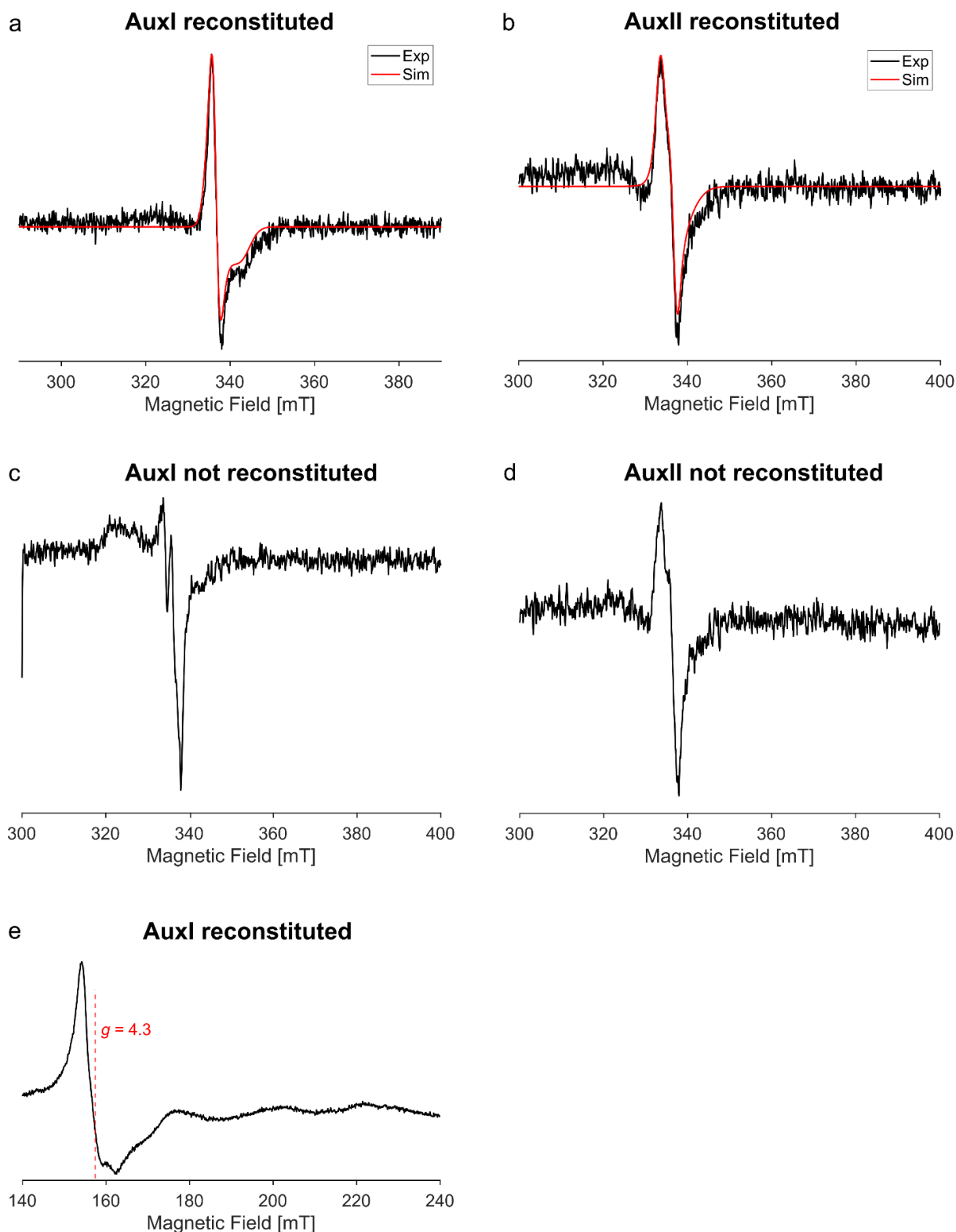
**Figure S2.** Amino acid sequence alignment of AhbD with the SPASM domain-containing Radical SAM enzymes anSME, SuiB, PqqE and MftC. Cluster-coordinating cysteine residues are highlighted in color as in Figure S1. Sequences are specified by UniProt entry names or gene numbers.

**Figure S3**



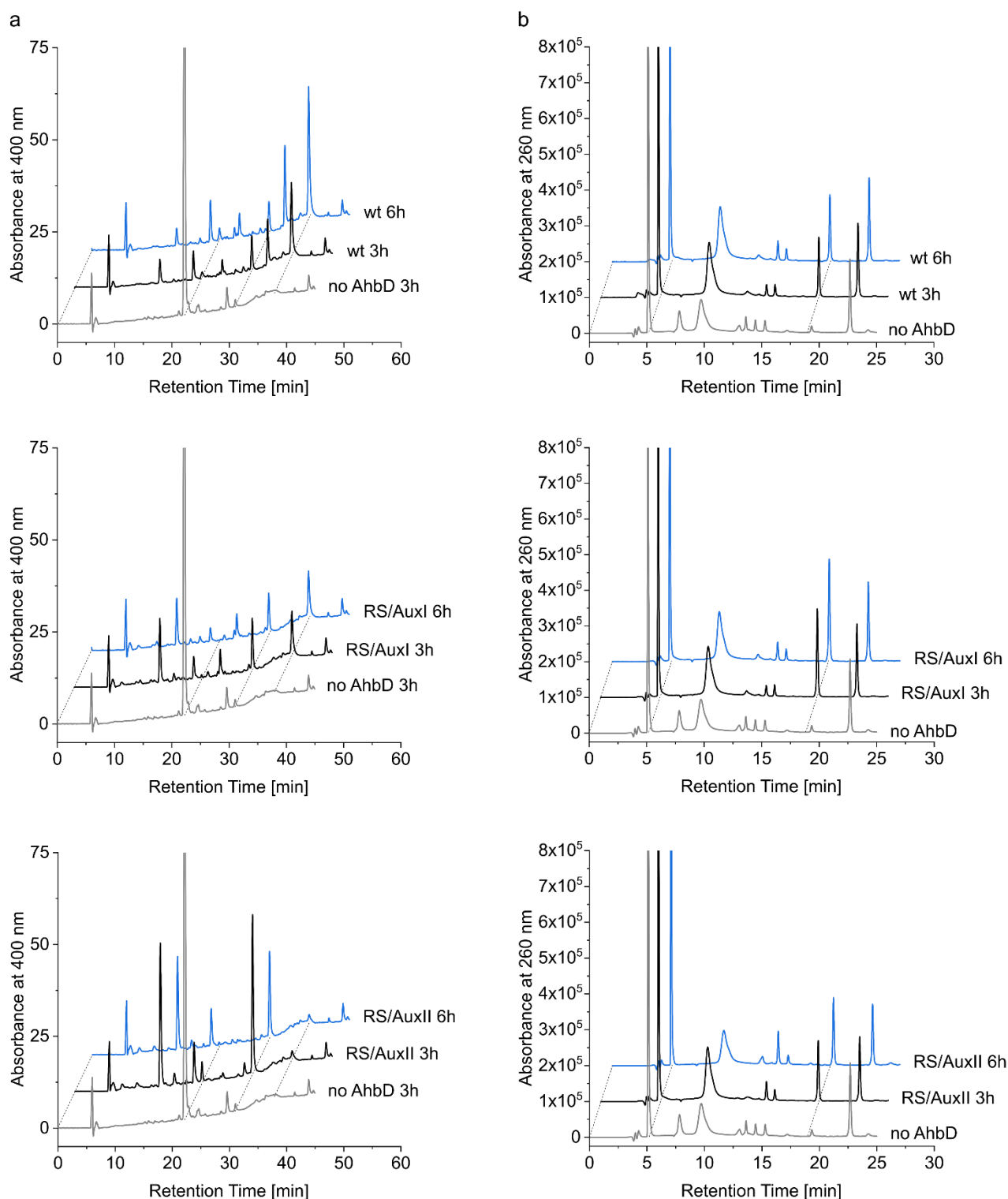
**Figure S3.** EPR Cw X-band spectra of the dithionite reduced AhbD variants AuxI (left) and AuxII (right) recorded before (blue) or after (black) iron-sulfur cluster reconstitution. Spectra were recorded at  $T = 13$  K and  $P_{\text{MW}} = 1$  mW. All other experimental parameters are given in the main text.

**Figure S4**



**Figure S4.** EPR Cw X-band spectra of the oxidized AhbD variants AuxI (a, c) and AuxII (b, d) recorded before (c, d) or after (a, b) iron-sulfur cluster reconstitution. Spectra a and b were simulated (red line) with  $g_x$ ;  $g_y$ ;  $g_z = 2.016$ ; 2.010; 1.973 and  $g_x$ ;  $g_y$ ;  $g_z = 2.026$ ; 2.007; 1.998, respectively. e) shows the signal of 'free' Fe(III) at  $g = 4.3$  contained within the reconstituted AuxI variant. Similar spectra with the same intensity of the peak at  $g = 4.3$  were obtained from the other samples. Spectra were recorded at  $T = 13$  K and  $P_{MW} = 1$  mW. All other experimental parameters are given in the main text.

**Figure S5**



**Figure S5.** HPLC analysis of enzyme activity assay mixtures containing AhbD wt (top), variant RS/AuxI (middle) or RS/AuxII (bottom). a, HPLC separation of tetrapyrroles extracted from enzyme activity assay mixtures after 3 and 6 hours of incubation. FeCopro elutes at a retention time of 22.1 min, the monovinyl-intermediate at 31 min and heme at 37.8 min. b, HPLC separation of SAM cleavage products formed after 3 and 6 hours of incubation. SAM and 5'-dA elute at retention times of about 5 and 18.9 min, respectively.

**Table S1****Table S1.** Oligonucleotide primers for site-directed mutagenesis of the *ahbD* gene.

Primer designation	Sequence (5' → 3') <sup>1</sup>
Mbar_C23A_C26A_rv	CTTGCACCACG <u>AGC</u> ATGAAC <u>AGC</u> ATTCAGATTACAACC
Mbar_C23A_C26A_fw	GGTTGTAATCTGAAT <u>GCT</u> GTTTCAT <u>GCT</u> CGTGGTGCAAG
Mbar_C256A_rv	AACCGGTGCCACCCAG <u>GCG</u> CACCTTTGGTCATTGCTTC
Mbar_C256A_fw	GAAGCAATGACCAAAGGT <u>GCG</u> CTGGGTGGCACCGGTT
Mbar_C274A_rv	GAACCGGCAGATAACCC <u>GCG</u> CGGAAAACTTTACCAACGC
Mbar_C274A_fw	GCGTTGGTAAAGTTTTCC <u>GCG</u> GGTTATCTGCCGGTTC
Mbar_C312A_C315A_rv	CTTTTTTATACTC <u>GCG</u> GATGCC <u>AGC</u> TTTACCTTTTCAGTTCTTCC
Mbar_C312A_C315A_fw	GGAAGAACTGAAAGGTAAAGCTGGCATC <u>GCG</u> GAGTATAAAAAAG
Mbar_C321A_rv	CACGACAACCTGCC <u>GCA</u> ACTTTTTTATACTCGGCGATG
Mbar_C321A_fw	CATCGCCGAGTATAAAAAAGTT <u>GCG</u> GCAGGTTGTCGTG
Mbar_C324A_rv (for Q5 Kit)	ACTTTTTTATACTCGCAGATG
Mbar_C324A_fw (for Q5 Kit)	TTGTGCAGGT <u>GCT</u> CGTGCACGTG

<sup>1</sup> Mutated codons are underlined.