Supplementary Information for

Monitoring global protein thiol-oxidation and *S*-mycothiolation in *Mycobacterium smegmatis* under hypochlorite stress

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Running title: Protein thiol-oxidation and protein *S-mycothiolation* in *Mycobacterium smegmatis*

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Supplementary Figure legends

Figure S1. **ClustalΩ sequence alignments of NaOCl-sensitive thiol-switches with increased thiol-oxidation and/or *S*-mycothiolations in NaOCl-treated *M. smegmatis* cells.** The alignments include redox-sensitive transcriptional regulators (RseA, RshA, IdeR, MSMEG_4471, WhiB1/2), metabolic enzymes and ribosomal proteins, such as the myoinositol-phosphate synthase (Ino1), the IMP dehydrogenases (GuaB and GuaB2), the ribosomal protein (RpsM) and Zn-containing alcohol dehydrogenases (AdhB1/B2/E1/E2/C2/MSMEG_1138, MSMEG_3464, MSMEG_4400, MSMEG_1977). The alignments were performed using ClustalΩ (http://www.ebi.ac.uk/Tools/msa/clustalo/) and presented using Jalview (ver.2.9.0.b2). The purple color gradient indicates percentage of amino acid identity. Cysteine residues are marked in red and the oxidized Cys are bold-faced and marked with an asterisk (*). The *S*-mycothiolated Cys is marked with "^{M}".

(A) The iron-dependent repressor IdeR of *M. smegmatis* was aligned with *M. tuberculosis* IdeR (Rv2711) and DtxR from *C. glutamicum* (Cg2103) and *C. diphtheriae* (DIP1414). The conserved single Cys102 is *S*-mycothiolated in *M. smegmatis* after NaOCl stress.

(B) The inositol-3-phosphate synthase (Ino1) of *M. smegmatis* was aligned with its homologs of *M. tuberculosis* (Rv0046c), *C. glutamicum* (Cgl2996) and *C. diphtheriae* (DIP0115). The conserved Cys18 was *S*-mycothiolated in *M. smegmatis* with a 6.3% oxidation increase under NaOCl stress. Ino1 of *C. glutamicum* was *S*-mycothiolated at Cys79.

(C) The inosine-5'-monophosphate dehydrogenases GuaB and GuaB2 of *M. smegmatis* were aligned with the GuaB homologs of *M. tuberculosis* and *C. glutamicum*. The active site Cys325 of GuaB and Cys302 of GuaB2 were identified as *S*-mycothiolated and with 19% and 33% increased oxidations, respectively, under NaOCl stress. This Cys form a thioimidate intermediate during the catalysis and is one of the most conserved NaOCl-sensitive targets for *S*-thiolation across different bacteria.

(D) The Zn-containing alcohol dehydrogenases of *M. smegmatis* AdhB1/B2/E1/E2/C2, MSMEG_3464, MSMEG_4400, MSMEG_1977 were aligned. Two catalytic Cys residues are shown in red and the four Zn-binding Cys residues are labelled in blue. Interestingly, AdhE1 and MSMEG_1977 were oxidized at their catalytic N-terminal Cys58 and Cys39, while AdhB1/B2/E2 and MSMEG_1138 were all oxidized at one conserved Zn-binding Cys105, 106, 107 and 113.

(E) The ribosomal protein RpsM of *M. smegmatis* (MSMEG_1521) was aligned with RpsM homologs of *M. tuberculosis* (Rv3460c), *C. glutamicum* (Cg0673) and *C. diphtheriae* (DIP0546). The single Cys86 is conserved and *S*-mycothiolated in both *M. smegmatis* and *C. glutamicum*, as well as *S*-cysteinylated in *M. smegmatis* and *C. glutamicum mshC* mutants. Cys86 showed a 21.8% oxidation increase in the *M. smegmatis* wild type under NaOCl stress.

(F) The anti-sigma factors RshA (MSMEG_1915) and RseA (MSMEG_5071) of *M. smegmatis* were aligned with their homologs of *M. tuberculosis* (Rv3221A and Rv1222 respectively) and *C. glutamicum* (Cg0877 and Cg1272). RseA showed 37.5% increased oxidation in the Zn-binding (ZAS) motif at Cys67 and Cys70 and RshA was 38.2% higher oxidized at the conserved Cys76 not present in the ZAS motif.

(G) The MarR-type transcriptional regulator MSMEG_4471 of *M. smegmatis* was aligned with homologs of *M. tuberculosis* (Rv2327), *Rhodococcus equi* (REQ_39840) and *Nocardia cyriacigeorgica* (NOCYR_0508). Cys58 showed an 42.3 % oxidation increase in *M. smegmatis* wild-type strain under NaOCl stress.

(H) WhiB1 and WhiB2 of *M. smegmatis* (MSMEG_1919 and MSMEG_1831) were aligned with their homologs of *M. tuberculosis* (Rv3219 and Rv3260c) and *C. glutamicum* (Cg0878 and Cg0850). Conserved Cys residues of WhiB1 and WhiB2 coordinate an [4Fe-4S] cluster. The conserved Cys9 of WhiB1 and Cys67 of WhiB2 showed increased oxidation of 7.4% and 12.6%, respectively, under NaOCl stress.

Fig. S1A

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M.smegmatis_IdeR/1-230      1 MNDLVDTTEMYLRTIYDLEEEGVVPLRARIAERLDQSGPTVSQTVSRMERDGLLH  55
M.tuberculosis_IdeR/1-230   1 MNELVDTTEMYLRTIYDLEEEGVTPLRARIAERLDQSGPTVSQTVSRMERDGLLR  55
C.diphtheriae_DtxR/1-226    1 MKDLVDTTEMYLRTIYELEEEGVTPLRARIAERLEQSGPTVSQTVARMERDGLVV  55
C.glutamicum_DtxR/1-228     1 MKDLVDTTEMYLRTIYELEEEGIVPLRARIAERLEQSGPTVSQTVARMERDGLVH  55

M.smegmatis_IdeR/1-230     56 VAGDRHLELTDKGRALAVAVMRKHRLAERLLVDVIGLPWEDVHAEACRWEHVMSE  110
M.tuberculosis_IdeR/1-230  56 VAGDRHLELTEKGRALAIAVMRKHRLAERLLVDVIGLPWEEVHAEACRWEHVMSE  110
C.diphtheriae_DtxR/1-226   56 VASDRSLQMTPTGRTLATAVMRKHRLAERLLTDIIGLDINKVHDEACRWEHVMSD  110
C.glutamicum_DtxR/1-228    56 VSPDRSLEMTPEGRSLAIAVMRKHRLAERLLTDIIGLDIHKVHDEACRWEHVMSD  110

M.smegmatis_IdeR/1-230    111 EVERRLVQVLDNPTTSPFGNPIPGLTELGVTPGVNTEDVSLVRLTELPVGMPVAV  165
M.tuberculosis_IdeR/1-230 111 DVERRLVKVLNNPTTSPFGNPIPGLVELGVGPEPGADDANLVRLTELPAGSPVAV  165
C.diphtheriae_DtxR/1-226  111 EVERRLVKVLKDVSRSPFGNPIPGLDELGVGNSDA--AVPGTRVIDAATSMPRKV  163
C.glutamicum_DtxR/1-228   111 EVERRLVEVLDDVHRSPFGNPIPGLGEIGLDQADE--PDSGVRAIDLPLGENLKA  163

M.smegmatis_IdeR/1-230    166 VVRQLTEHVQGDTDLIGRLKEAGVVPNARVTVEANNNGGVMIVIPGHEQVELPHH  220
M.tuberculosis_IdeR/1-230 166 VVRQLTEHVQGDIDLITRLKDAGVVPNARVTVETTPGGGVTIVIPGHENVTLPHE  220
C.diphtheriae_DtxR/1-226  164 RIVQINEIFQVETDQFTQLLDADIRVGSEVEIV--DRDGHITLSHNGKDVELIDD  216
C.glutamicum_DtxR/1-228   164 RIVQLNEILQVDLEQFQALTDAGVEIGTEVDII--NEQGRVVITHNGSSVELIDD  216

M.smegmatis_IdeR/1-230    221 MAHAVKVEKV--  230
M.tuberculosis_IdeR/1-230 221 MAHAVKVEKV--  230
C.diphtheriae_DtxR/1-226  217 LAHTIRIEEL--  226
C.glutamicum_DtxR/1-228   217 LAHAVRVEKVEG  228
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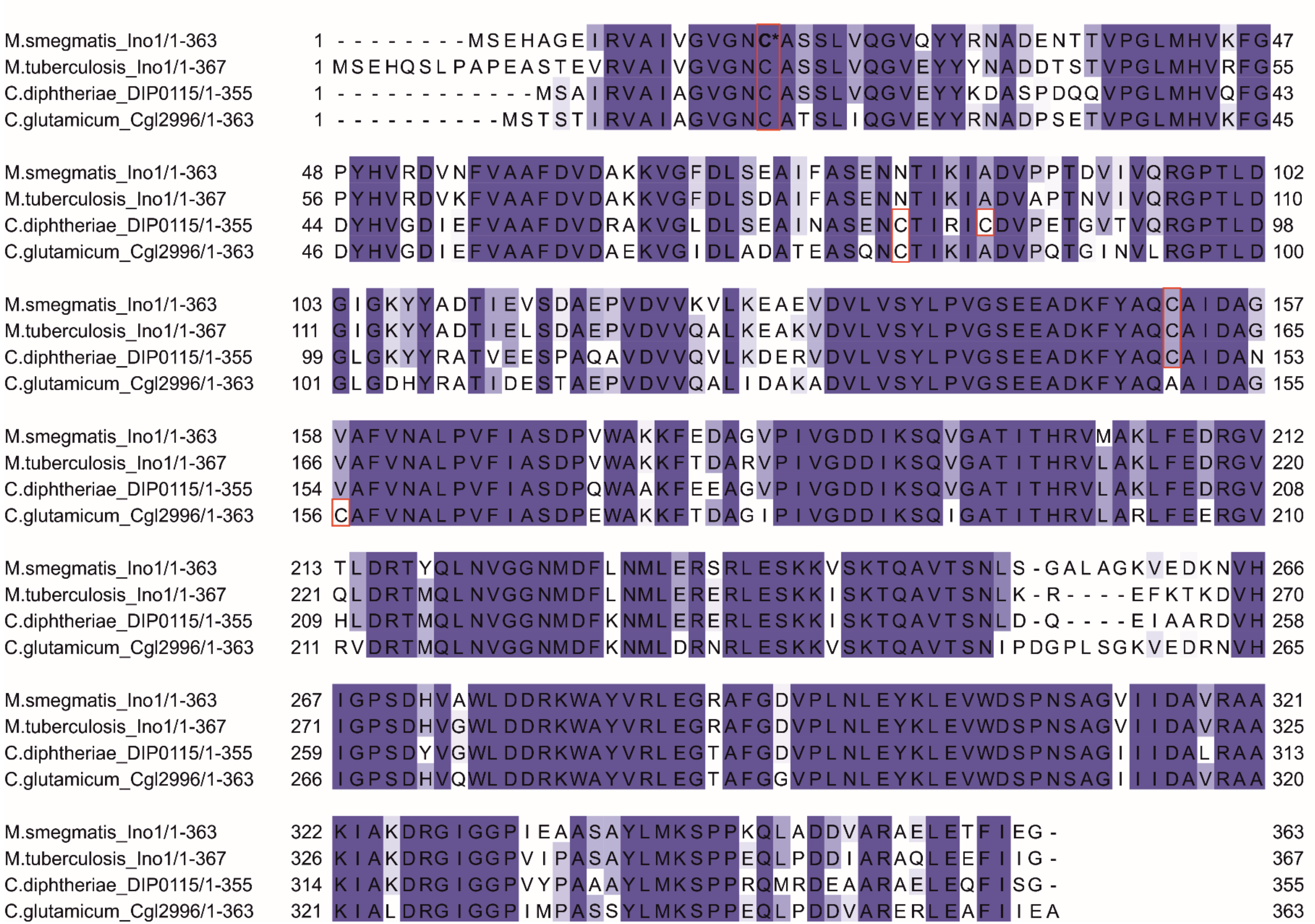
Fig. S1B



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M.tuberculosis_Ino1/1-367       1 MSEHQSLPAPEASTEVRVAIVGVGNCASSLVQGVEYYYNADDTSTVPGLMHVRFG 55
C.diphtheriae_DIP0115/1-355     1 ------------MSAIRVAIAGVGNCASSLVQGVEYYKDASPDQQVPGLMHVQFG 43
C.glutamicum_Cgl2996/1-363      1 ----------MSTSTIRVAIAGVGNCATSLIQGVEYYRNADPSETVPGLMHVKFG 45

M.smegmatis_Ino1/1-363         48 PYHVRDVNFVAAFDVDAKKVGFDLSEAIFASENNTIKIADVPPTDVIVQRGPTLD 102
M.tuberculosis_Ino1/1-367      56 PYHVRDVKFVAAFDVDAKKVGFDLSDAIFASENNTIKIADVAPTNVIVQRGPTLD 110
C.diphtheriae_DIP0115/1-355    44 DYHVGDIEFVAAFDVDRAKVGLDLSEAINASENCTIRICDVPETGVTVQRGPTLD 98
C.glutamicum_Cgl2996/1-363     46 DYHVGDIEFVAAFDVDAEKVGIDLADATEASQNCTIKIADVPQTGINVLRGPTLD 100

M.smegmatis_Ino1/1-363        103 GIGKYYADTIEVSDAEPVDVVKVLKEAEVDVLVSYLPVGSEEADKFYAQCAIDAG 157
M.tuberculosis_Ino1/1-367     111 GIGKYYADTIELSDAEPVDVVQALKEAKVDVLVSYLPVGSEEADKFYAQCAIDAG 165
C.diphtheriae_DIP0115/1-355    99 GLGKYYRATVEESPAQAVDVVQVLKDERVDVLVSYLPVGSEEADKFYAQCAIDAN 153
C.glutamicum_Cgl2996/1-363    101 GLGDHYRATIDESTAEPVDVVQALIDAKADVLVSYLPVGSEEADKFYAQAAIDAG 155

M.smegmatis_Ino1/1-363        158 VAFVNALPVFIASDPVWAKKFEDAGVPIVGDDIKSQVGATITHRVMAKLFEDRGV 212
M.tuberculosis_Ino1/1-367     166 VAFVNALPVFIASDPVWAKKFTDARVPIVGDDIKSQVGATITHRVLAKLFEDRGV 220
C.diphtheriae_DIP0115/1-355   154 VAFVNALPVFIASDPQWAAKFEEAGVPIVGDDIKSQVGATITHRVLAKLFEDRGV 208
C.glutamicum_Cgl2996/1-363    156 CAFVNALPVFIASDPEWAKKFTDAGIPIVGDDIKSQIGATITHRVLARLFEERGV 210

M.smegmatis_Ino1/1-363        213 TLDRTYQLNVGGNMDFLNMLERSRLESKKVSKTQAVTSNLS-GALAGKVEDKNVH 266
M.tuberculosis_Ino1/1-367     221 QLDRTMQLNVGGNMDFLNMLERERLESKKISKTQAVTSNLK-R----EFKTKDVH 270
C.diphtheriae_DIP0115/1-355   209 HLDRTMQLNVGGNMDFKNMLERERLESKKISKTQAVTSNLD-Q----EIAARDVH 258
C.glutamicum_Cgl2996/1-363    211 RVDRTMQLNVGGNMDFKNMLDRNRLESKKVSKTQAVTSNIPDGPLSGKVEDRNVH 265

M.smegmatis_Ino1/1-363        267 IGPSDHVAWLDDRKWAYVRLEGRAFGDVPLNLEYKLEVWDSPNSAGVIIDAVRAA 321
M.tuberculosis_Ino1/1-367     271 IGPSDHVGWLDDRKWAYVRLEGRAFGDVPLNLEYKLEVWDSPNSAGVIIDAVRAA 325
C.diphtheriae_DIP0115/1-355   259 IGPSDYVGWLDDRKWAYVRLEGTAFGDVPLNLEYKLEVWDSPNSAGIIIDALRAA 313
C.glutamicum_Cgl2996/1-363    266 IGPSDHVQWLDDRKWAYVRLEGTAFGGVPLNLEYKLEVWDSPNSAGIIIDAVRAA 320

M.smegmatis_Ino1/1-363        322 KIAKDRGIGGPIEAASAYLMKSPPKQLADDVARAELETFIEG-             363
M.tuberculosis_Ino1/1-367     326 KIAKDRGIGGPVIPASAYLMKSPPEQLPDDIARAQLEEFIIG-             367
C.diphtheriae_DIP0115/1-355   314 KIAKDRGIGGPVYPAAAYLMKSPPRQMRDEAARAELEQFISG-             355
C.glutamicum_Cgl2996/1-363    321 KIALDRGIGGPIMPASSYLMKSPPEQLPDDVARERLEAFIIEA             363
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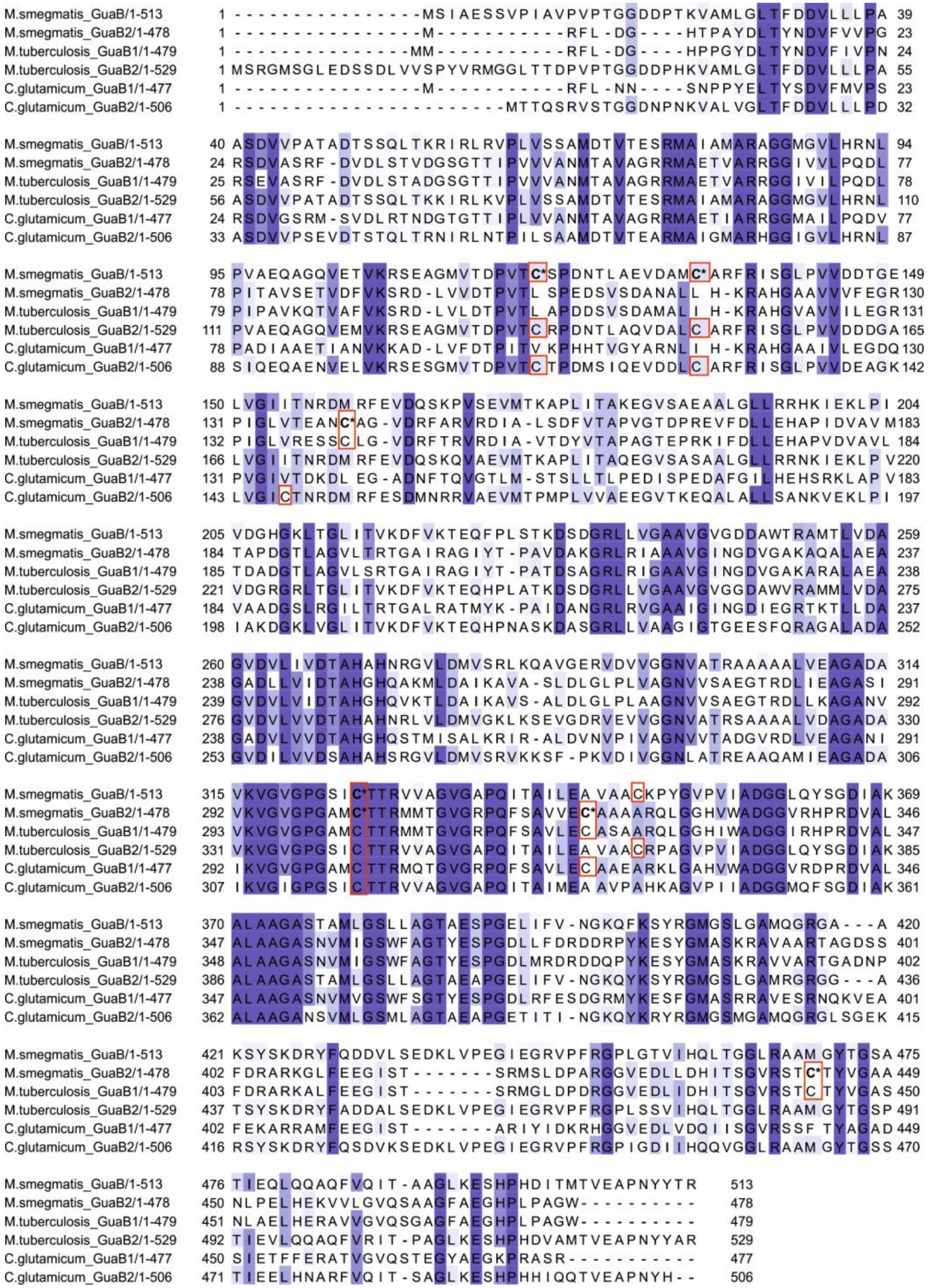
Fig. S1C



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M.smegmatis_GuaB2/1-478          1 ----------------M-----------RFL-DG----HTPAYDLTYNDVFVVPG 23
M.tuberculosis_GuaB1/1-479       1 ---------------MM-----------RFL-DG----HPPGYDLTYNDVFIVPN 24
M.tuberculosis_GuaB2/1-529       1 MSRGMSGLEDSSDLVVSPYVRMGGLTTDPVPTGGDDPHKVAMLGLTFDDVLLLPA 55
C.glutamicum_GuaB1/1-477         1 ----------------M-----------RFL-NN----SNPPYELTYSDVFMVPS 23
C.glutamicum_GuaB2/1-506         1 -----------------------MTTQSRVSTGGDNPNKVALVGLTFDDVLLLPD 32

M.smegmatis_GuaB/1-513          40 ASDVVPATADTSSQLTKRIRLRVPLVSSAMDTVTESRMAIAMARAGGMGVLHRNL 94
M.smegmatis_GuaB2/1-478         24 RSDVASRF-DVDLSTVDGSGTTIPVVVANMTAVAGRRMAETVARRGGIVVLPQDL 77
M.tuberculosis_GuaB1/1-479      25 RSEVASRF-DVDLSTADGSGTTIPVVVANMTAVAGRRMAETVARRGGIVILPQDL 78
M.tuberculosis_GuaB2/1-529      56 ASDVVPATADTSSQLTKKIRLKVPLVSSAMDTVTESRMAIAMARAGGMGVLHRNL 110
C.glutamicum_GuaB1/1-477        24 RSDVGSRM-SVDLRTNDGTGTTIPLVVANMTAVAGRRMAETIARRGGMAILPQDV 77
C.glutamicum_GuaB2/1-506        33 ASDVVPSEVDTSTQLTRNIRLNTPILSAAMDTVTEARMAIGMARHGGIGVLHRNL 87

M.smegmatis_GuaB/1-513          95 PVAEQAGQVETVKRSEAGMVTDPVTC*SPDNTLAEVDAMC*ARFRISGLPVVDDTGE 149
M.smegmatis_GuaB2/1-478         78 PITAVSETVDFVKSRD-LVVDTPVTL SPEDSVSDANALL H-KRAHGAAVVVFEGR 130
M.tuberculosis_GuaB1/1-479      79 PIPAVKQTVAFVKSRD-LVLDTPVTL APDDSVSDAMALI H-KRAHGVAVVILEGR 131
M.tuberculosis_GuaB2/1-529     111 PVAEQAGQVEMVKRSEAGMVTDPVTC RPDNTLAQVDALC ARFRISGLPVVDDDGA 165
C.glutamicum_GuaB1/1-477        78 PADIAAETIANVKKAD-LVFDTPITV KPHHTVGYARNLI H-KRAHGAAIVLEGDQ 130
C.glutamicum_GuaB2/1-506        88 SIQEQAENVELVKRSESGMVTDPVTC TPDMSIQEVDDLC ARFRISGLPVVDEAGK 142

M.smegmatis_GuaB/1-513         150 LVGIITNRDM RFEVDQSKPVSEVMTKAPLITAKEGVSAEAALGLLRRHKIEKLPI 204
M.smegmatis_GuaB2/1-478        131 PIGLVTEANC*AG-VDRFARVRDIA-LSDFVTAPVGTDPREVFDLLEHAPIDVAVM 183
M.tuberculosis_GuaB1/1-479     132 PIGLVRESSC LG-VDRFTRVRDIA-VTDYVTAPAGTEPRKIFDLLEHAPVDVAVL 184
M.tuberculosis_GuaB2/1-529     166 LVGIITNRDM RFEVDQSKQVAEVMTKAPLITAQEGVSASAALGLLRRNKIEKLPV 220
C.glutamicum_GuaB1/1-477       131 PVGIVTDKDL EG-ADNFTQVGTLM-STSLLTLPEDISPEDAFGILHEHSRKLAPV 183
C.glutamicum_GuaB2/1-506       143 LVGICTNRDM RFESDMNRRVAEVMTPMPLVVAEEGVTKEQALALLSANKVEKLPI 197

M.smegmatis_GuaB/1-513         205 VDGHGKLTGLITVKDFVKTEQFPLSTKDSDGRLLVGAAVGVGDDAWTRAMTLVDA 259
M.smegmatis_GuaB2/1-478        184 TAPDGTLAGVLTRTGAIRAGIYT-PAVDAKGRLRIAAAVGINGDVGAKAQALAEA 237
M.tuberculosis_GuaB1/1-479     185 TDADGTLAGVLSRTGAIRAGIYT-PATDSAGRLRIGAAVGINGDVGAKARALAEA 238
M.tuberculosis_GuaB2/1-529     221 VDGRGRLTGLITVKDFVKTEQHPLATKDSDGRLLVGAAVGVGGDAWVRAMMLVDA 275
C.glutamicum_GuaB1/1-477       184 VAADGSLRGILTRTGALRATMYK-PAIDANGRLRVGAAIGINGDIEGRTKTLLDA 237
C.glutamicum_GuaB2/1-506       198 IAKDGKLVGLITVKDFVKTEQHPNASKDASGRLLVAAGIGTGEESFQRAGALADA 252

M.smegmatis_GuaB/1-513         260 GVDVLIVDTAHAHNRGVLDMVSRLKQAVGERVDVVGGNVATRAAAAALVEAGADA 314
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M.tuberculosis_GuaB1/1-479     239 GVDVLVIDTAHGHQVKTLDAIKAVS-ALDLGLPLAAGNVVSAEGTRDLLKAGANV 292
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C.glutamicum_GuaB1/1-477       238 GADVLVVDTAHGHQSTMISALKRIR-ALDVNVPIVAGNVVTADGVRDLVEAGANI 291
C.glutamicum_GuaB2/1-506       253 GVDILVVDSAHAHSRGVLDMVSRVKKSF-PKVDIVGGNLATREAAQAMIEAGADA 306

M.smegmatis_GuaB/1-513         315 VKVGVGPGSIC*TTRVVAGVGAPQITAILEA VAACKPYGVPVIADGGLQYSGDIAK 369
M.smegmatis_GuaB2/1-478        292 VKVGVGPGAMC*TTRMMTGVGRPQFSAVVEC*AAAARQLGGHVWADGGVRHPRDVAL 346
M.tuberculosis_GuaB1/1-479     293 VKVGVGPGAMC TTRMMTGVGRPQFSAVLEC ASAARQLGGHIWADGGIRHPRDVAL 347
M.tuberculosis_GuaB2/1-529     331 VKVGVGPGSIC TTRVVAGVGAPQITAILEA VAACRPAGVPVIADGGLQYSGDIAK 385
C.glutamicum_GuaB1/1-477       292 IKVGVGPGAMC TTRMQTGVGRPQFSAVLEC AAEARKLGAHVWADGGVRDPRDVAL 346
C.glutamicum_GuaB2/1-506       307 IKVGIGPGSIC TTRVVAGVGAPQITAIMEA AVPAHKAGVPIIADGGMQFSGDIAK 361

M.smegmatis_GuaB/1-513         370 ALAAGASTAMLGSLLAGTAESPGELIFV-NGKQFKSYRGMGSLGAMQGRGA---A 420
M.smegmatis_GuaB2/1-478        347 ALAAGASNVMIGSWFAGTYESPGDLLFDRDDRPYKESYGMASKRAVAARTAGDSS 401
M.tuberculosis_GuaB1/1-479     348 ALAAGASNVMIGSWFAGTYESPGDLMRDRDDQPYKESYGMASKRAVVARTGADNP 402
M.tuberculosis_GuaB2/1-529     386 ALAAGASTAMLGSLLAGTAEAPGELIFV-NGKQYKSYRGMGSLGAMRGRGG---A 436
C.glutamicum_GuaB1/1-477       347 ALAAGASNVMVGSWFSGTYESPGDLRFESDGRMYKESFGMASRRAVESRNQKVEA 401
C.glutamicum_GuaB2/1-506       362 ALAAGANSVMLGSMLAGTAEAPGETITI-NGKQYKRYRGMGSMGAMQGRGLSGEK 415

M.smegmatis_GuaB/1-513         421 KSYSKDRYFQDDVLSEDKLVPEGIEGRVPFRGPLGTVIHQLTGGLRAAM GYTGSA 475
M.smegmatis_GuaB2/1-478        402 FDRARKGLFEEGIST-------SRMSLDPARGGVEDLLDHITSGVRSTC*TYVGAA 449
M.tuberculosis_GuaB1/1-479     403 FDRARKALFEEGIST-------SRMGLDPDRGGVEDLIDHITSGVRSTC TYVGAS 450
M.tuberculosis_GuaB2/1-529     437 TSYSKDRYFADDALSEDKLVPEGIEGRVPFRGPLSSVIHQLTGGLRAAM GYTGSP 491
C.glutamicum_GuaB1/1-477       402 FEKARRAMFEEGIST-------ARIYIDKRHGGVEDLVDQIISGVRSSF TYAGAD 449
C.glutamicum_GuaB2/1-506       416 RSYSKDRYFQSDVKSEDKLVPEGIEGRVPFRGPIGDIIHQQVGGLRAAM GYTGSS 470

M.smegmatis_GuaB/1-513         476 TIEQLQQAQFVQIT-AAGLKESHPHDITMTVEAPNYYTR    513
M.smegmatis_GuaB2/1-478        450 NLPELHEKVVLGVQSAAGFAEGHPLPAGW----------    478
M.tuberculosis_GuaB1/1-479     451 NLAELHERAVVGVQSGAGFAEGHPLPAGW----------    479
M.tuberculosis_GuaB2/1-529     492 TIEVLQQAQFVRIT-PAGLKESHPHDVAMTVEAPNYYAR    529
C.glutamicum_GuaB1/1-477       450 SIETFFERATVGVQSTEGYAEGKPRASR-----------    477
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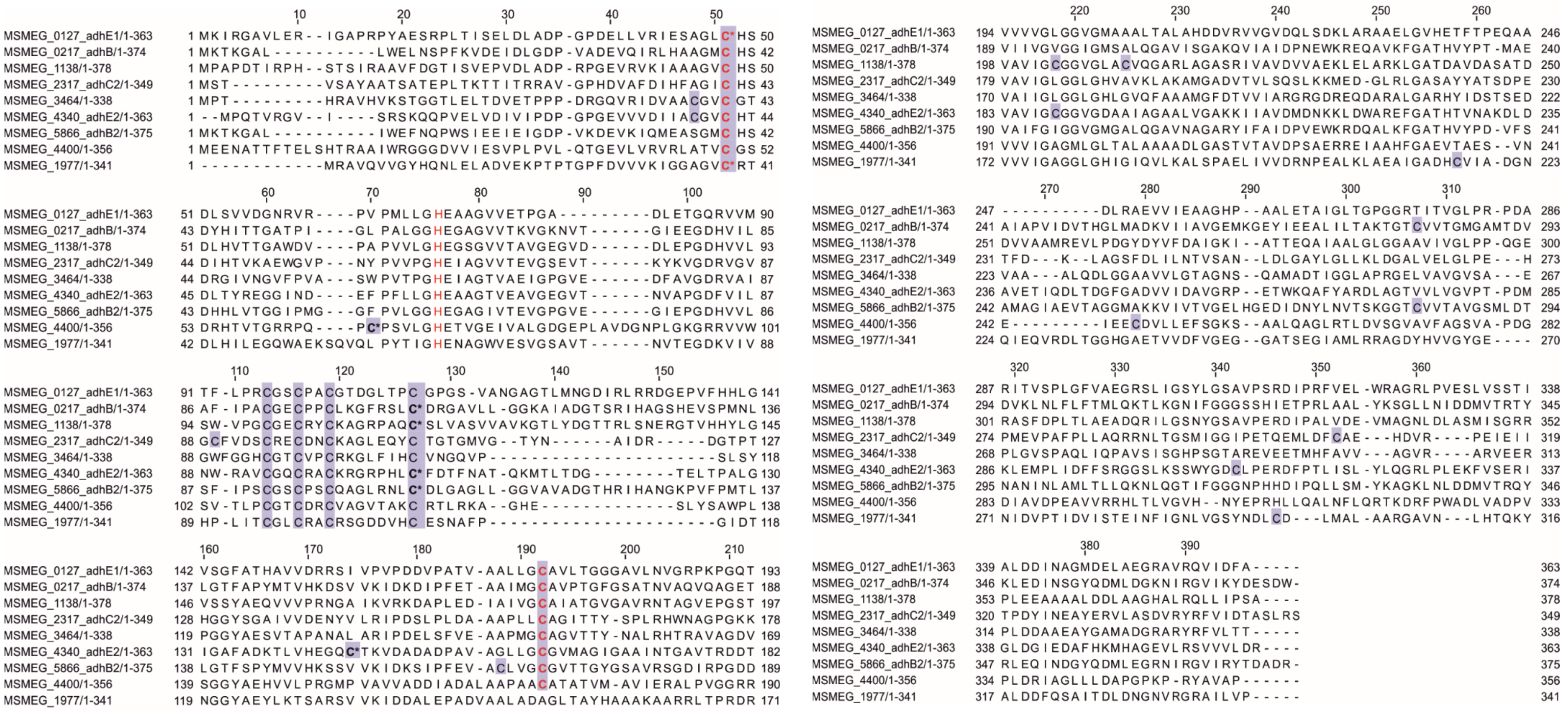
Fig. S1D



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MSMEG_0217_adhB/1-374       1 MKTKGAL----------LWELNSPFKVDEIDLGDP-VADEVQIRLHAAGMCHS 42
MSMEG_1138/1-378            1 MPAPDTIRPH--STSIRAAVFDGTISVEPVDLADP-RPGEVRVKIAAAGVCHS 50
MSMEG_2317_adhC2/1-349      1 MST---------VSAYAATSATEPLTKTTITRRAV-GPHDVAFDIHFAGICHS 43
MSMEG_3464/1-338            1 MPT---------HRAVHVKSTGGTLELTDVETPPP-DRGQVRIDVAACGVCGT 43
MSMEG_4340_adhE2/1-363      1 --MPQTVRGV--I----SRSKQQPVELVDIVIPDP-GPGEVVVDIIACGVCHT 44
MSMEG_5866_adhB2/1-375      1 MKTKGAL----------IWEFNQPWSIEEIEIGDP-VKDEVKIQMEASGMCHS 42
MSMEG_4400/1-356            1 MEENATTFTELSHTRAAIWRGGGDVVIESVPLPVL-QTGEVLVRVRLATVCGS 52
MSMEG_1977/1-341            1 ------------MRAVQVVGYHQNLELADVEKPTPTGPFDVVVKIGGAGVC*RT 41

                                  60        70        80        90       100
MSMEG_0127_adhE1/1-363     51 DLSVVDGNRVR----PVPMLLGHEAAGVVETPGA---------DLETGQRVVM 90
MSMEG_0217_adhB/1-374      43 DYHITTGATPI----GLPALGGHEGAGVVTKVGKNVT------GIEEGDHVIL 85
MSMEG_1138/1-378           51 DLHVTTGAWDV----PAPVVLGHEGSGVVTAVGEGVD------DLEPGDHVVL 93
MSMEG_2317_adhC2/1-349     44 DIHTVKAEWGVP---NYPVVPGHEIAGVVTEVGSEVT------KYKVGDRVGV 87
MSMEG_3464/1-338           44 DRGIVNGVFPVA---SWPVTPGHEIAGTVAEIGPGVE------DFAVGDRVAI 87
MSMEG_4340_adhE2/1-363     45 DLTYREGGIND----EFPFLLGHEAAGTVEAVGEGVT------NVAPGDFVIL 87
MSMEG_5866_adhB2/1-375     43 DHHLVTGGIPMG---GFPVLGGHEGAGIVTEVGPGVE------GIEPGDHVVL 86
MSMEG_4400/1-356           53 DRHTVTGRRPQ----PC*PSVLGHETVGEIVALGDGEPLAVDGNPLGKGRRVVW 101
MSMEG_1977/1-341           42 DLHILEGQWAEKSQVQLPYTIGHENAGWVESVGSAVT------NVTEGDKVIV 88

                               110       120       130       140       150
MSMEG_0127_adhE1/1-363     91 TF-LPRCGSCPACGTDGLTPCGPGS-VANGAGTLMNGDIRLRRDGEPVFHHLG 141
MSMEG_0217_adhB/1-374      86 AF-IPACGECPPCLKGFRSLC*DRGAVLL-GGKAIADGTSRIHAGSHEVSPMNL 136
MSMEG_1138/1-378           94 SW-VPGCGECRYCKAGRPAQC*SLVASVVAVKGTLYDGTTRLSNERGTVHHYLG 145
MSMEG_2317_adhC2/1-349     88 GCFVDSCRECDNCKAGLEQYCTGTGMVG--TYN------AIDR-----DGTPT 127
MSMEG_3464/1-338           88 GWFGGHCGTCVPCRKGLFIHCVNGQVP----------------------SLSY 118
MSMEG_4340_adhE2/1-363     88 NW-RAVCGQCRACKRGRPHLC*FDTFNAT-QKMTLTDG--------TELTPALG 130
MSMEG_5866_adhB2/1-375     87 SF-IPSCGSCPSCQAGLRNLC*DLGAGLL-GGVAVADGTHRIHANGKPVFPMTL 137
MSMEG_4400/1-356          102 SV-TLPCGTCDRCVAGVTAKCRTLRKA--GHE-------------SLYSAWPL 138
MSMEG_1977/1-341           89 HP-LITCGLCRACRSGDDVHCESNAFP----------------------GIDT 118

                              160       170       180       190       200       210
MSMEG_0127_adhE1/1-363    142 VSGFATHAVVDRRSIVPVPDDVPATV-AALLGCAVLTGGGAVLNVGRPKPGQT 193
MSMEG_0217_adhB/1-374     137 LGTFAPYMTVHKDSVVKIDKDIPFET-AAIMGCAVPTGFGSATNVAQVQAGET 188
MSMEG_1138/1-378          146 VSSYAEQVVVPRNGAIKVRKDAPLED-IAIVGCAIATGVGAVRNTAGVEPGST 197
MSMEG_2317_adhC2/1-349    128 HGGYSGAIVVDENYVLRIPDSLPLDA-AAPLLCAGITTY-SPLRHWNAGPGKK 178
MSMEG_3464/1-338          119 PGGYAESVTAPANALARIPDELSFVE-AAPMGCAGVTTY-NALRHTRAVAGDV 169
MSMEG_4340_adhE2/1-363    131 IGAFADKTLVHEGQC*TKVDADADPAV-AGLLGCGVMAGIGAAINTGAVTRDDT 182
MSMEG_5866_adhB2/1-375    138 LGTFSPYMVVHKSSVVKIDKSIPFEV-ACLVGCGVTTGYGSAVRSGDIRPGDD 189
MSMEG_4400/1-356          139 SGGYAEHVVLPRGMPVAVVADDIADALAAPAACATATVM-AVIERALPVGGRR 190
MSMEG_1977/1-341          119 NGGYAEYLKTSARSVVKIDDALEPADVAALADAGLTAYHAAAKAARRLTPRDR 171

                                   220       230       240       250       260
MSMEG_0127_adhE1/1-363    194 VVVVGLGGVGMAAALTALAHDDVRVVGVDQLSDKLARAAELGVHETFTPEQAA 246
MSMEG_0217_adhB/1-374     189 VIIVGVGGIGMSALQGAVISGAKQVIAIDPNEWKREQAVKFGATHVYPT-MAE 240
MSMEG_1138/1-378          198 VAVIGCGGVGLACVQGARLAGASRIVAVDVVAEKLELARKLGATDAVDASATD 250
MSMEG_2317_adhC2/1-349    179 VAVIGLGGLGHVAVKLAKAMGADVTVLSQSLKKMED-GLRLGASAYYATSDPE 230
MSMEG_3464/1-338          170 VAIIGLGGLGHLGVQFAAAMGFDTVVIARGRGDREQDARALGARHYIDSTSED 222
MSMEG_4340_adhE2/1-363    183 VAVIGCGGVGDAAIAGAALVGAKKIIAVDMDNKKLDWAREFGATHTVNAKDLD 235
MSMEG_5866_adhB2/1-375    190 VAIFGIGGVGMGALQGAVNAGARYIFAIDPVEWKRDQALKFGATHVYPD-VFS 241
MSMEG_4400/1-356          191 VVVIGAGMLGLTALAAAADLGASTVTAVDPSAERREIAAHFGAEVTAES--VN 241
MSMEG_1977/1-341          172 VVVIGAGGLGHIGIQVLKALSPAELIVVDRNPEALKLAEAIGADHCVIA-DGN 223

                                270       280       290       300       310
MSMEG_0127_adhE1/1-363    247 ----------DLRAEVVIEAAGHP--AALETAIGLTGPGGRTITVGLPR-PDA 286
MSMEG_0217_adhB/1-374     241 AIAPVIDVTHGLMADKVIIAVGEMKGEYIEEALILTAKTGTCVVTGMGAMTDV 293
MSMEG_1138/1-378          251 DVVAAMREVLPDGYDYVFDAIGKI--ATTEQAIAALGLGGAAVIVGLPP-QGE 300
MSMEG_2317_adhC2/1-349    231 TFD---K--LAGSFDLILNTVSAN--LDLGAYLGLLKLDGALVELGLPE---H 273
MSMEG_3464/1-338          223 VAA---ALQDLGGAAVVLGTAGNS--QAMADTIGGLAPRGELVAVGVSA---E 267
MSMEG_4340_adhE2/1-363    236 AVETIQDLTDGFGADVVIDAVGRP--ETWKQAFYARDLAGTVVLVGVPT-PDM 285
MSMEG_5866_adhB2/1-375    242 AMAGIAEVTAGGMAKKVIVTVGELHGEDIDNYLNVTSKGGTCVVTAVGSMLDT 294
MSMEG_4400/1-356          242 E---------IEECDVLLEFSGKS--AALQAGLRTLDVSGVAVFAGSVA-PDG 282
MSMEG_1977/1-341          224 QIEQVRDLTGGHGAETVVDFVGEG--GATSEGIAMLRRAGDYHVVGYGE---- 270

                              320       330       340       350       360
MSMEG_0127_adhE1/1-363    287 RITVSPLGFVAEGRSLIGSYLGSAVPSRDIPRFVEL-WRAGRLPVESLVSSTI 338
MSMEG_0217_adhB/1-374     294 DVKLNLFLFTMLQKTLKGNIFGGGSSHIETPRLAAL-YKSGLLNIDDMVTRTY 345
MSMEG_1138/1-378          301 RASFDPLTLAEADQRILGSNYGSAVPERDIPALVDE-VMAGNLDLASMISGRR 352
MSMEG_2317_adhC2/1-349    274 PMEVPAFPLLAQRRNLTGSMIGGIPETQEMLDFCAE---HDVR----PEIEII 319
MSMEG_3464/1-338          268 PLGVSPAQLIQPAVSISGHPSGTAREVEETMHFAVV---AGVR----ARVEER 313
MSMEG_4340_adhE2/1-363    286 KLEMPLIDFFSRGGSLKSSWYGDCLPERDFPTLISL-YLQGRLPLEKFVSERI 337
MSMEG_5866_adhB2/1-375    295 NANINLAMLTLLQKNLQGTIFGGGNPHHDIPQLLSM-YKAGKLNLDDMVTRQY 346
MSMEG_4400/1-356          283 DIAVDPEAVVRRHLTLVGVH--NYEPRHLLQALNFLQRTKDRFPWADLVADPV 333
MSMEG_1977/1-341          271 NIDVPTIDVISTEINFIGNLVGSYNDLCD---LMAL-AARGAVN---LHTQKY 316

                                    380       390
MSMEG_0127_adhE1/1-363    339 ALDDINAGMDELAEGRAVRQVIDFA----- 363
MSMEG_0217_adhB/1-374     346 KLEDINSGYQDMLDGKNIRGVIKYDESDW- 374
MSMEG_1138/1-378          353 PLEEAAAALDDLAAGHALRQLLIPSA---- 378
MSMEG_2317_adhC2/1-349    320 TPDYINEAYERVLASDVRYRFVIDTASLRS 349
MSMEG_3464/1-338          314 PLDDAAEAYGAMADGRARYRFVLTT----- 338
MSMEG_4340_adhE2/1-363    338 GLDGIEDAFHKMHAGEVLRSVVVLDR---- 363
MSMEG_5866_adhB2/1-375    347 RLEQINDGYQDMLEGRNIRGVIRYTDADR- 375
MSMEG_4400/1-356          334 PLDRIAGLLLDAPGPKP-RYAVAP------ 356
MSMEG_1977/1-341          317 ALDDFQSAITDLDNGNVRGRAILVP----- 341
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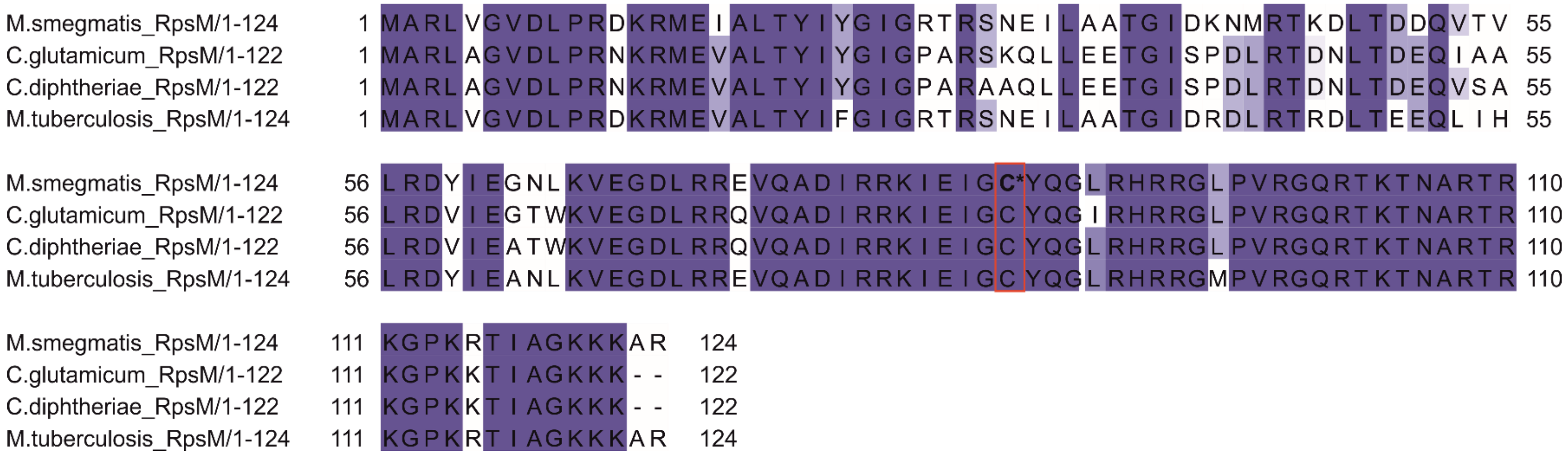
Fig. S1E



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M.smegmatis_RpsM/1-124        1 MARLVGVDLPRDKRMEIALTYIYGIGRTRSNEILAATGIDKNMRTKDLTDDQVTV  55
C.glutamicum_RpsM/1-122       1 MARLAGVDLPRNKRMEVALTYIYGIGPARSKQLLEETGISPDLRTDNLTDEQIAA  55
C.diphtheriae_RpsM/1-122      1 MARLAGVDLPRNKRMEVALTYIYGIGPARAAQLLEETGISPDLRTDNLTDEQVSA  55
M.tuberculosis_RpsM/1-124     1 MARLVGVDLPRDKRMEVALTYIFGIGRTRSNEILAATGIDRDLRTRDLTEEQLIH  55

M.smegmatis_RpsM/1-124       56 LRDYIEGNLKVEGDLRREVQADIRRKIEIGC*YQGLRHRRGLPVRGQRTKTNARTR 110
C.glutamicum_RpsM/1-122      56 LRDVIEGTWKVEGDLRRQVQADIRRKIEIGC YQGIRHRRGLPVRGQRTKTNARTR 110
C.diphtheriae_RpsM/1-122     56 LRDVIEATWKVEGDLRRQVQADIRRKIEIGC YQGLRHRRGLPVRGQRTKTNARTR 110
M.tuberculosis_RpsM/1-124    56 LRDYIEANLKVEGDLRREVQADIRRKIEIGC YQGLRHRRGMPVRGQRTKTNARTR 110

M.smegmatis_RpsM/1-124      111 KGPKRTIAGKKKAR 124
C.glutamicum_RpsM/1-122     111 KGPKKTIAGKKK-- 122
C.diphtheriae_RpsM/1-122    111 KGPKKTIAGKKK-- 122
M.tuberculosis_RpsM/1-124   111 KGPKRTIAGKKKAR 124
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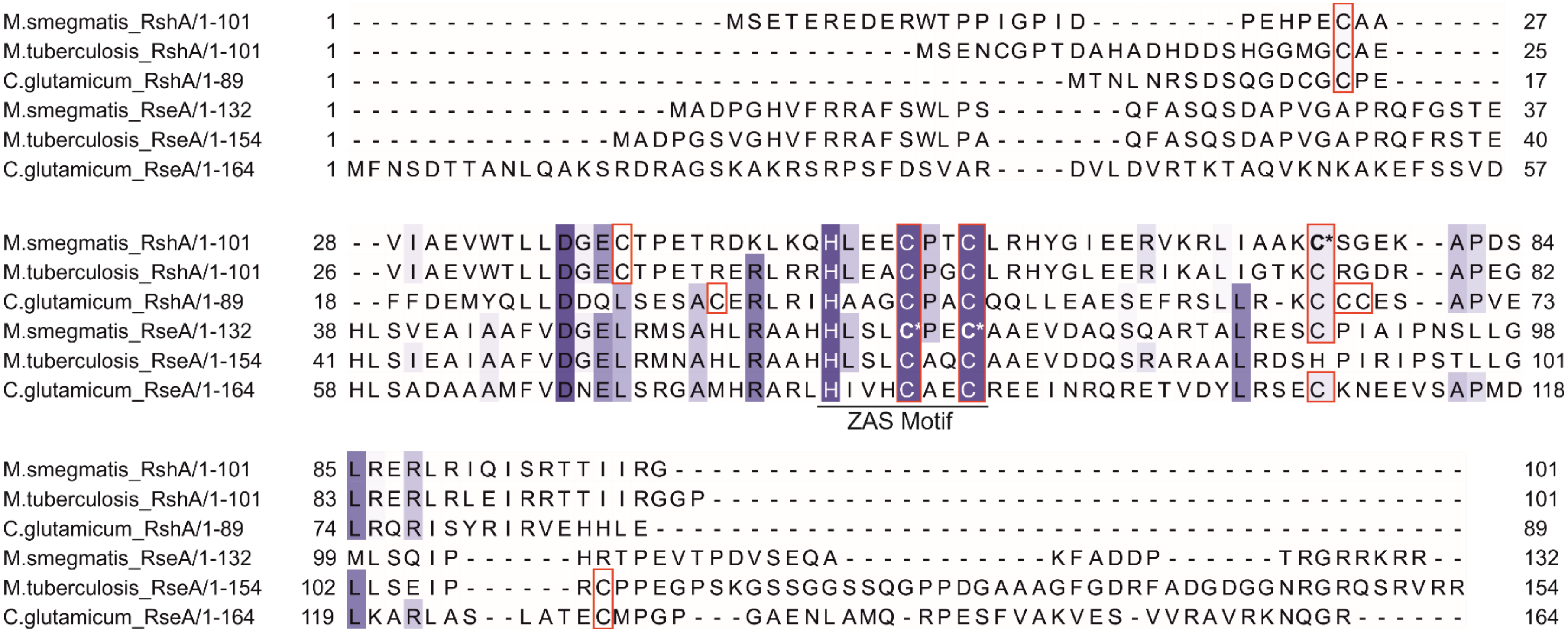
Fig. S1F



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M.smegmatis_RshA/1-101        1 -------------------MSETEREDERWTPPIGPID--------PEHPECAA------ 27
M.tuberculosis_RshA/1-101     1 -----------------------------MSENCGPTDAHADHDDSHGGMGCAE------ 25
C.glutamicum_RshA/1-89        1 -------------------------------------MTNLNRSDSQGDCGCPE------ 17
M.smegmatis_RseA/1-132        1 ----------------MADPGHVFRRAFSWLPS-------QFASQSDAPVGAPRQFGSTE 37
M.tuberculosis_RseA/1-154     1 -------------MADPGSVGHVFRRAFSWLPA-------QFASQSDAPVGAPRQFRSTE 40
C.glutamicum_RseA/1-164       1 MFNSDTTANLQAKSRDRAGSKAKRSRPSFDSVAR---DVLDVRTKTAQVKNKAKEFSSVD 57

M.smegmatis_RshA/1-101       28 --VIAEVWTLLDGECTPETRDKLKQHLEECPTCLRHYGIEERVKRLIAAKC*SGEK--APDS 84
M.tuberculosis_RshA/1-101    26 --VIAEVWTLLDGECTPETRERLRRHLEACPGCLRHYGLEERIKALIGTKCRGDR--APEG 82
C.glutamicum_RshA/1-89       18 --FFDEMYQLLDDQLSESACERLRIHAAGCPACQQLLEAESEFRSLLR-KCCCES--APVE 73
M.smegmatis_RseA/1-132       38 HLSVEAIAAFVDGELRMSAHLRAAHHLSLC*PEC*AAEVDAQSQARTALRESCPIAIPNSLLG 98
M.tuberculosis_RseA/1-154    41 HLSIEAIAAFVDGELRMNAHLRAAHHLSLCAQCAAEVDDQSRARAALRDSHPIRIPSTLLG 101
C.glutamicum_RseA/1-164      58 HLSADAAAMFVDNELSRGAMHRARLHIVHCAECREEINRQRETVDYLRSECKNEEVSAPMD 118
                                                         ZAS Motif

M.smegmatis_RshA/1-101       85 LRERLRIQISRTTIIRG------------------------------------------ 101
M.tuberculosis_RshA/1-101    83 LRERLRLEIRRTTIIRGGP---------------------------------------- 101
C.glutamicum_RshA/1-89       74 LRQRISYRIRVEHHLE------------------------------------------- 89
M.smegmatis_RseA/1-132       99 MLSQIP------HRTPEVTPDVSEQA----------KFADDP-------TRGRRKRR-- 132
M.tuberculosis_RseA/1-154   102 LLSEIP------RCPPEGPSKGSSGGSSQGPPDGAAAGFGDRFADGDGGNRGRQSRVRR 154
C.glutamicum_RseA/1-164     119 LKARLAS--LATECMPGP---GAENLAMQ-RPESFVAKVES-VVRAVRKNQGR------ 164
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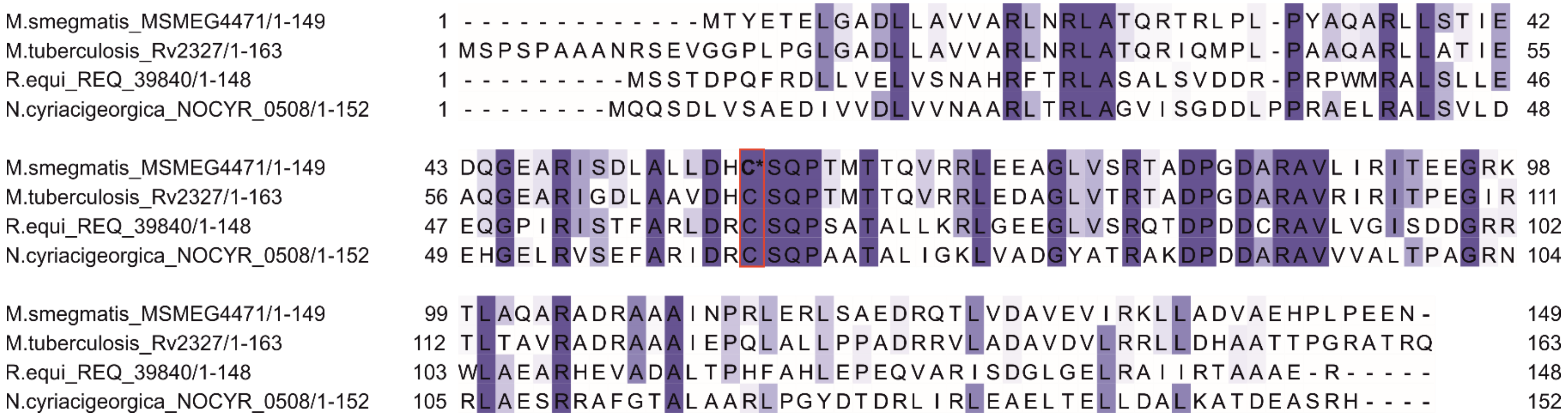
Fig. S1G



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M.smegmatis_MSMEG4471/1-149            1 -------------MTYETELGADLLAVVARLNRLATQRTRLPL-PYAQARLLSTIE 42
M.tuberculosis_Rv2327/1-163            1 MSPSPAAANRSEVGGPLPGLGADLLAVVARLNRLATQRIQMPL-PAAQARLLATIE 55
R.equi_REQ_39840/1-148                 1 ---------MSSTDPQFRDLLVELVSNAHRFTRLASALSVDDR-PRPWMRALSLLE 46
N.cyriacigeorgica_NOCYR_0508/1-152     1 --------MQQSDLVSAEDIVVDLVVNAARLTRLAGVISGDDLPPRAELRALSVLD 48

M.smegmatis_MSMEG4471/1-149           43 DQGEARISDLALLDHC*SQPTMTTQVRRLEEAGLVSRTADPGDARAVLIRITEEGRK 98
M.tuberculosis_Rv2327/1-163           56 AQGEARIGDLAAVDHCSQPTMTTQVRRLEDAGLVTRTADPGDARAVRIRITPEGIR 111
R.equi_REQ_39840/1-148                47 EQGPIRISTFARLDRCSQPSATALLKRLGEEGLVSRQTDPDDCRAVLVGISDDGRR 102
N.cyriacigeorgica_NOCYR_0508/1-152    49 EHGELRVSEFARIDRCSQPAATALIGKLVADGYATRAKDPDDARAVVVALTPAGRN 104

M.smegmatis_MSMEG4471/1-149           99 TLAQARADRAAAINPRLERLSAEDRQTLVDAVEVIRKLLADVAEHPLPEEN-     149
M.tuberculosis_Rv2327/1-163          112 TLTAVRADRAAAIEPQLALLPPADRRVLADAVDVLRRLLDHAATTPGRATRQ     163
R.equi_REQ_39840/1-148               103 WLAEARHEVADALTPHFAHLEPEQVARISDGLGELRAIIRTAAAE-R-----     148
N.cyriacigeorgica_NOCYR_0508/1-152   105 RLAESRRAFGTALAARLPGYDTDRLIRLEAELTELLDALKATDEASRH----     152
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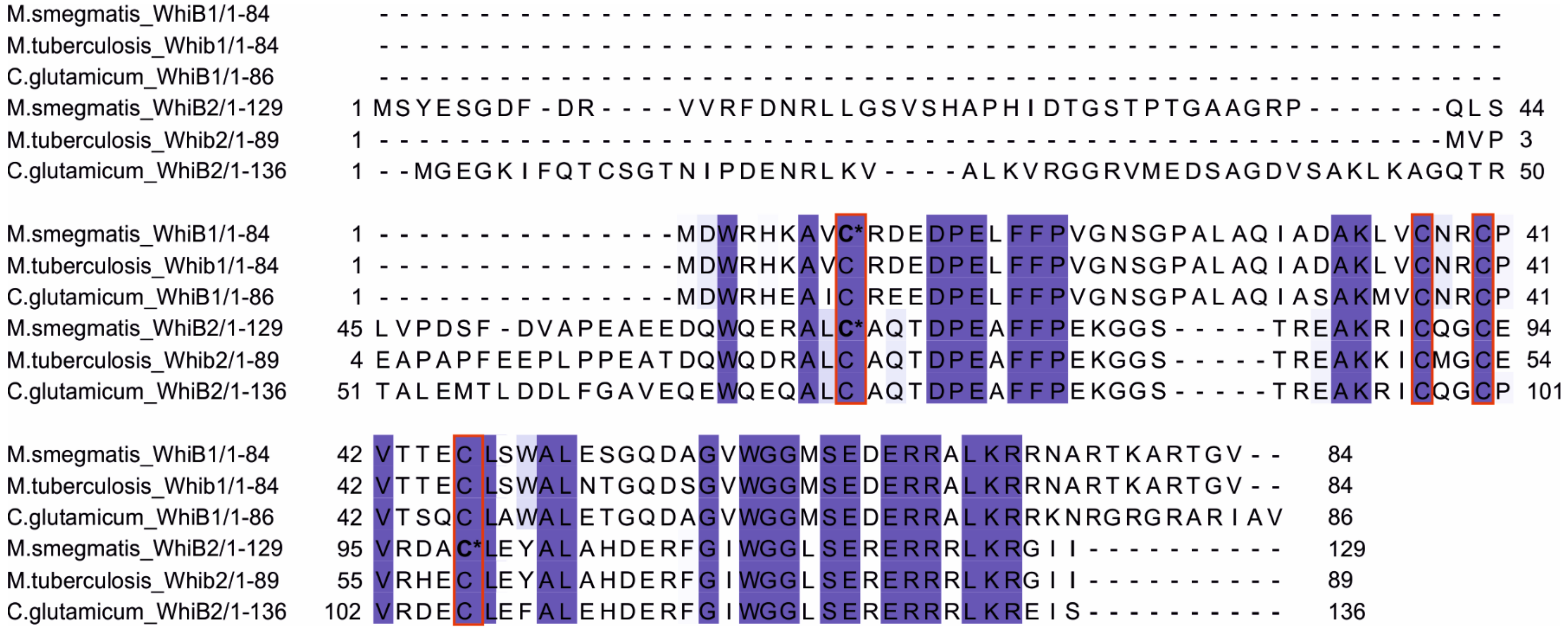
Fig. S1H



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M.smegmatis_WhiB1/1-84         --------------------------------------------------------
M.tuberculosis_Whib1/1-84      --------------------------------------------------------
C.glutamicum_WhiB1/1-86        --------------------------------------------------------
M.smegmatis_WhiB2/1-129      1 MSYESGDF-DR----VVRFDNRLLGSVSHAPHIDTGSTPTGAAGRP-------QLS 44
M.tuberculosis_Whib2/1-89    1 -----------------------------------------------------MVP 3
C.glutamicum_WhiB2/1-136     1 --MGEGKIFQTCSGTNIPDENRLKV----ALKVRGGRVMEDSAGDVSAKLKAGQTR 50

M.smegmatis_WhiB1/1-84       1 ---------------MDWRHKAVC*RDEDPELFFPVGNSGPALAQIADAKLVCNRCP 41
M.tuberculosis_Whib1/1-84    1 ---------------MDWRHKAVC RDEDPELFFPVGNSGPALAQIADAKLVCNRCP 41
C.glutamicum_WhiB1/1-86      1 ---------------MDWRHEAIC REEDPELFFPVGNSGPALAQIASAKMVCNRCP 41
M.smegmatis_WhiB2/1-129     45 LVPDSF-DVAPEAEEDQWQERALC*AQTDPEAFFPEKGGS-----TREAKRICQGCE 94
M.tuberculosis_Whib2/1-89    4 EAPAPFEEPLPPEATDQWQDRALC AQTDPEAFFPEKGGS-----TREAKKICMGCE 54
C.glutamicum_WhiB2/1-136    51 TALEMTLDDLFGAVEQEWQEQALC AQTDPEAFFPEKGGS-----TREAKRICQGCP 101

M.smegmatis_WhiB1/1-84      42 VTTEC LSWALESGQDAGVWGGMSEDERRALKRRNARTKARTGV--  84
M.tuberculosis_Whib1/1-84   42 VTTEC LSWALNTGQDSGVWGGMSEDERRALKRRNARTKARTGV--  84
C.glutamicum_WhiB1/1-86     42 VTSQC LAWALETGQDAGVWGGMSEDERRALKRRKNRGRGRARIAV  86
M.smegmatis_WhiB2/1-129     95 VRDAC*LEYALAHDERFGIWGGLSERERRRLKRGII----------  129
M.tuberculosis_Whib2/1-89   55 VRHEC LEYALAHDERFGIWGGLSERERRRLKRGII----------  89
C.glutamicum_WhiB2/1-136   102 VRDEC LEFALEHDERFGIWGGLSERERRRLKREIS----------  136
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Figure S2

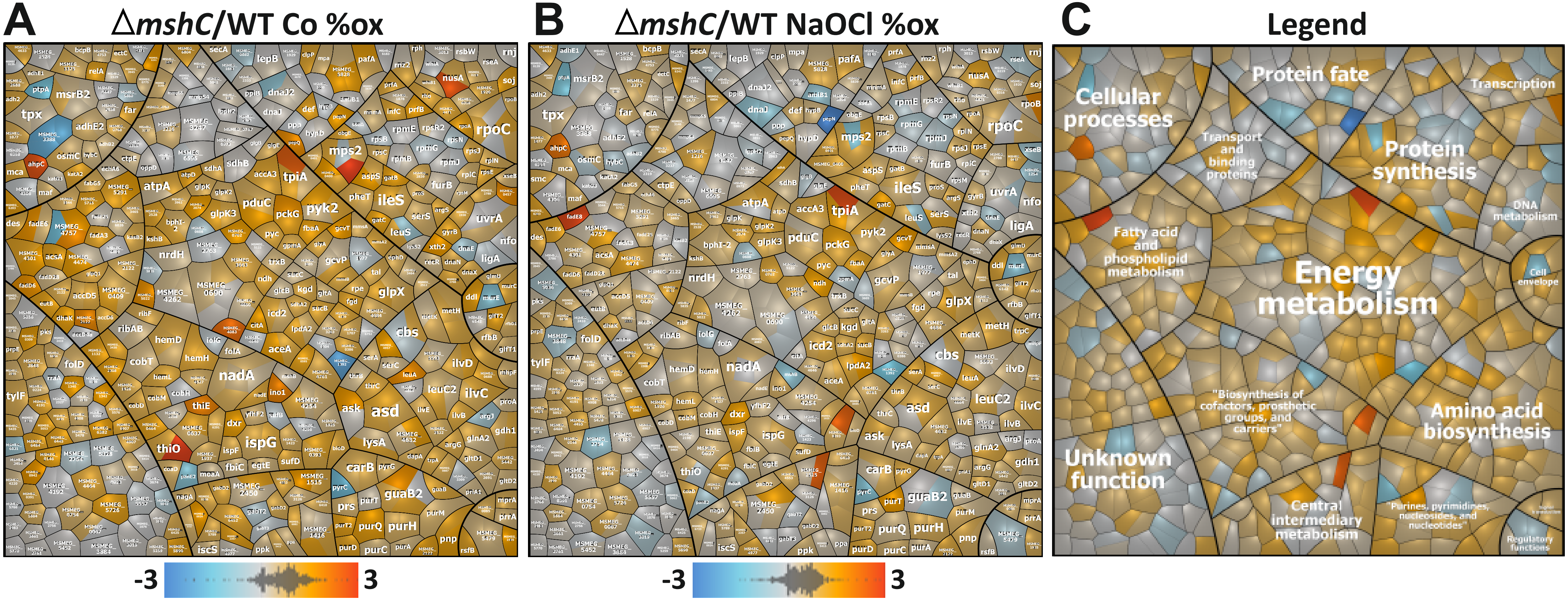

Fig. S2. Differential Voronoi redox treemaps for comparison of the thiol-oxidation level between the *M. smegmatis* wild type and the *mshC* mutant. The Voronoi redox treemaps visualize the log2 fold-change of the percentages of thiol-oxidations in the *mshC* mutant versus the wild type under control (A) and NaOCl stress conditions (B). The increased basal level oxidation in the mshC mutant control (A) is shown by the blue-yellow-red color gradient ranging from log2 fold-change oxidation of -3 to +3. The treemap in (C) is used as legend for the functional classification of the proteins displayed in (B). The treemaps were generated using the Paver software (Decodon) and proteins were classified according to the *M. smegmatis* TIGRfam annotation. The treemap results indicate that MSH is required to maintain the reduced state of the majority of protein thiols in *M. smegmatis*.