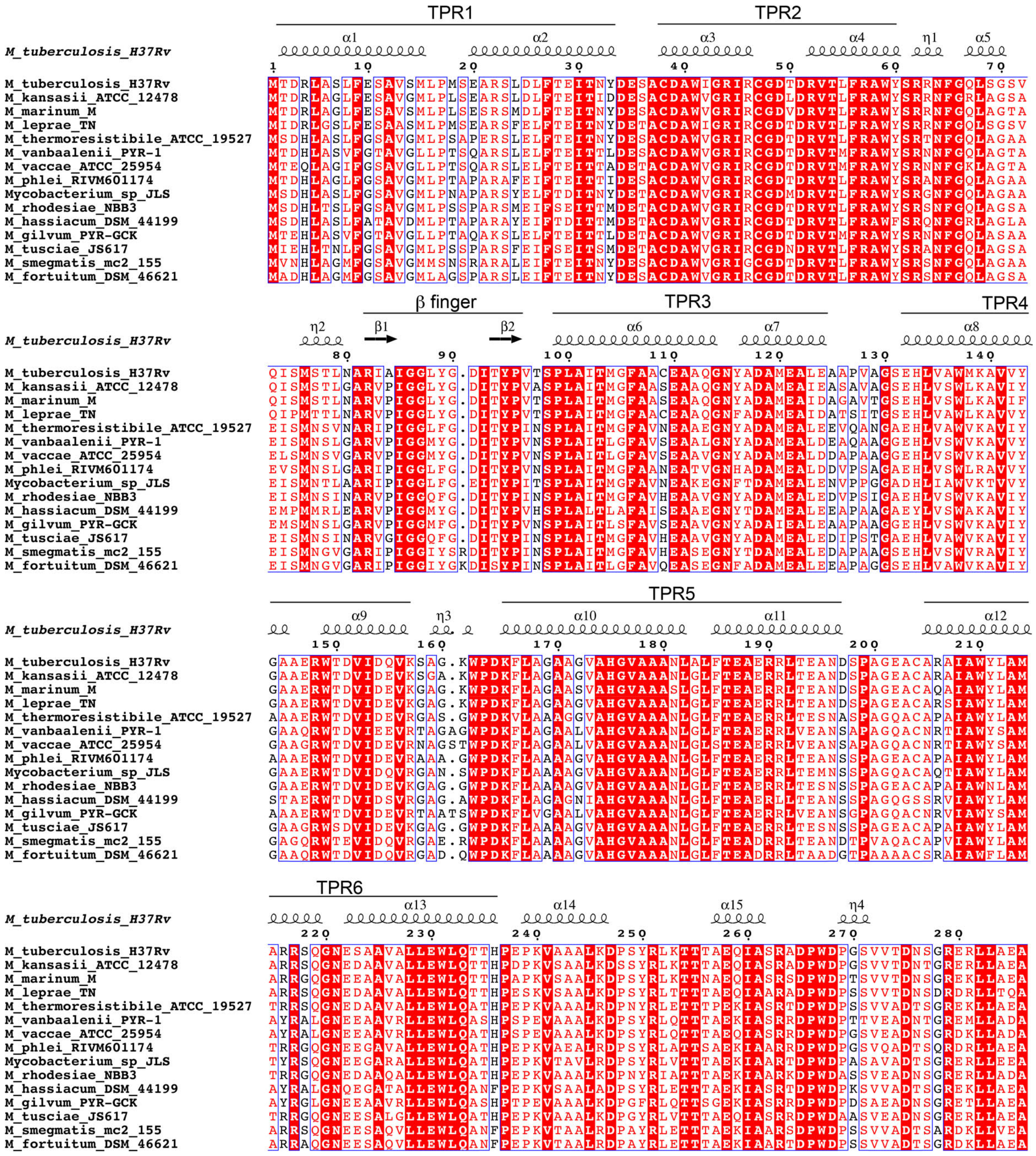
# Supporting information

## Crystal structure of the N-terminal domain of EccA$_1$ ATPase from the ESX-1 secretion system of *Mycobacterium tuberculosis*

Jonathan M. Wagner, Timothy J. Evans, Konstantin V. Korotkov



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                                 1        10        20        30        40        50        60        70
M_tuberculosis_H37Rv             MTDRLASLFESAVSMLPMSEARSLDLFTEITNYDESACDAWIGRIRCGDTDRVTLFRAWYSRRNFGQLSGSV
M_kansasii_ATCC_12478            MTDRLAGLFESAVGMLPLSEARSLDLFTEITIDDESACDAWVGRIRCGDLDRVTLFRAWYSRRNFGRLAGSA
M_marinum_M                      MTDRLAGLFESAVSMLPLSESRSMDLFTEITNYDESACDAWVGRIRCGDVDRVTLFRAWYSRRNFGQLAGTA
M_leprae_TN                      MIDRLGSLFESAASMLPMSEARSFELFTEITNYDETACDAWIGRIRCGDTDRVTLFRAWYSRRNFGQLSGSV
M_thermoresistibile_ATCC_19527   MSDHLASLFGSAVGMLPSAPERSLELFTEITNYDESACDAWVGRIRCGDTDRVTLFRAWYSRTNFGQLAGAA
M_vanbaalenii_PYR-1              MTDHLASVFGTAVGLLPTSQARSLELFTEITTLDESACDAWVGRIRCGDTDRVTMFRAWYSRNNFGQLAGTA
M_vaccae_ATCC_25954              MTEQLAGIFGSAVGLLPTSQARSLELFTEITTADETACDAWVGRIRCGDTDRVTMFRAWYSRNNFGKLAGTA
M_phlei_RIVM601174               MTDHLAGLFGSAVGMLPTAPARAFEIFTEITTIDESACDAWVGRIRCGDTDRVTLFRAWYSRANFGQLAGAA
Mycobacterium_sp_JLS             MSDHLASLFGSAVGMLPNAPARSYELFTDITNYDETACDAWVGRIRCGDMDRVTLFRAWYSRGNFGQLAGAA
M_rhodesiae_NBB3                 MSDHLTSLFGSAVGMLPSSPARSMEIFSEITTMDETACDAWVGRIRCGDTDRVTLFRAWYSRSNFGQLAGSA
M_hassiacum_DSM_44199            MSDHLASLFATAVDMLPTAPARAYEIFTDITTMDETACDAWVGRIRCGDTDRVTLFRAWFSRQNFGRLAGLA
M_gilvum_PYR-GCK                 MTEHLASVFGTAVGLLPTAQAKSLELFTEITTLDETACDAWVGRIRCGDTDRVTMFRAWYSRNNFGQLASAA
M_tusciae_JS617                  MIEHLTNLFGSAVGMLPSSPARSFEIFSEITSMDETACDAWVGRIRCGDNDRVTLFRAWYSRANFGQLAGSA
M_smegmatis_mc2_155              MVNHLAGMFGSAVGMMSNSRARALEIFTEITNYDESACDAWVGRIGCGDTDRVTLFRAWYSRSNFGQLAGAA
M_fortuitum_DSM_46621            MADHLAGMFGSAVGMLAGSPARSLEIFTEITNYDESACDAWVGRIRCGDTDRVTLFRAWYSRSNFGQLAGSA

                                        80        90        100       110       120       130       140
M_tuberculosis_H37Rv             QISMSTLNARIAIGGLYG.DITYPVTSPLAITMGFAACEAAQGNYADAMEALEAAPVAGSEHLVAWMKAVVY
M_kansasii_ATCC_12478            QISMSTLGARVPIGGLYG.DITYPVASPLAITMGFAASEAAQGNYADAMEAIEASAVAGSEHLVSWLKAVIY
M_marinum_M                      QISMSTLNARVPIGGLYG.DITYPVTSPLAITMGFAASEAAQGNYADAMEAIDAGAVTGSEHLVSWLKAVIF
M_leprae_TN                      QIPMTTLNARVPIGGLYG.DITYPVTSPLAITMGFAACEAAQGNFADAMEAIDATSITGSEHLVAWLKAVVY
M_thermoresistibile_ATCC_19527   EISMNSVNARIPIGGLFG.DITYPINSPLAITMGFAVNEAAEGNYADAMEALEEVQANGAEHLVAWVKAVIY
M_vanbaalenii_PYR-1              EISMNSLGARVPIGGMYG.DITYPVNSPLAITLGFAVSEAALGNYADAMEALDEAQAAGGEHLVSWVKAVVY
M_vaccae_ATCC_25954              ELSMNSVGARVPIGGMYG.DITYPVNSPLAITLGFAVSEAAVGNYADAMEALDDAPAAGGEHLVSWVKAVVY
M_phlei_RIVM601174               EVSMNSLGARIPIGGLFG.DITYPVNSPLAITMGFAANEATVGNHADAMEALDDVPSAGAEHLVSWLRAVVY
Mycobacterium_sp_JLS             EISMNTLAARIPIGGLFG.EITYPITSPLAITMGFAVNEAKEGNFTDAMEALENVPPGGADHLIAWVKTVVY
M_rhodesiae_NBB3                 EISMNSINARVPIGGQFG.DITYPINSPLAITMGFAVHEAAVGNYADAMEALEDVPSIGAEHLVSWVKAVIY
M_hassiacum_DSM_44199            EMPMMRLEARVPIGGMYG.DITYPVHSPLALTLAFAISEAAEGNYTDAMEALEEAPAAGAEYLVSWAKAVIY
M_gilvum_PYR-GCK                 EMSMNSLGARVPIGGMFG.DITYPVNSPLAITLSFAVSEAAVGNYADAIEALEAAPAAGGEHLVSWVKAVIY
M_tusciae_JS617                  EISMNSINARVGIGGQFG.DITYPINSPLAITMGFAVHEAAVGNYADAMEALEDIPSTGAEHLVAWVKAVIY
M_smegmatis_mc2_155              EISMNGVGARIPIGGIYSRDITYPINSPLAITMGFAVHEASEGNYTDAMEALEDAPAAGSEHLVSWVRAVIY
M_fortuitum_DSM_46621            EISMNGVGARIPIGGIYGKDISYPINSPLAITLGFAVQEASEGNFADAMEALEEAPAGGSEHLVAWVKAVIY

                                     150       160       170       180       190       200       210
M_tuberculosis_H37Rv             GAAERWTDVIDQVKSAG.KWPDKFLAGAAGVAHGVAAANLALFTEAERRLTEANDSPAGEACARAIAWYLAM
M_kansasii_ATCC_12478            GAAERWTDVIDEVKSGA.KWPDKFLAGAAGVAHGVAAANLGLFTEAERRLTEANDSPAGEACARAIAWYLAM
M_marinum_M                      GAAERWTDVIDEVKGAG.KWPDKFLAGAASVAHGVAAASLGLFTEAERRLTEANDSPAGEACAQAIAWYLAM
M_leprae_TN                      GAAERWTDVIDEVKGAG.KWPDKFLAGAAGVAHGVAAANLGLFTEAERRLTEANDSPAGEACARSIAWYLAM
M_thermoresistibile_ATCC_19527   AAAERWTDVIDEVRGAS.GWPDKVLAAAGGVAHGVAAANLGLFTEAERRLTESNASPAGQACAPAIAWYLAM
M_vanbaalenii_PYR-1              GAAQRWTDVIEEVRTAGAGWPDKFLAGAALVAHGVAAANLGLFTEAERRLVEANSAPAGQACNRTIAWYSAM
M_vaccae_ATCC_25954              GAAGRWTDVIDEVRNAGSTWPDKFLAGAALVAHGVAAANLGLSTEAERRLVEANSAPAGQACNRTIAWYSAM
M_phlei_RIVM601174               AAAERWTDVIDEVRAAA.GWPDKFLAAAAGVAHGVAAANLGLFTEAERRLTEANSSPAGEACAPAIAWYLAM
Mycobacterium_sp_JLS             GAAERWTDVIDQVRGAN.SWPDKFLAAAAGVAHGVAAANLGLFTEAERRLTEMNSSPAGQACAQTIAWYLAM
M_rhodesiae_NBB3                 GAAERWTDVIDEVKGAG.GWPDKFLAAAAGVAHGVAAANLGLFTEAERRLTESNSSPAGEACAPAIAWNLAM
M_hassiacum_DSM_44199            STAERWTDVIDSVRGAG.AWPDKFLAGAGNIAHGVAAANLGLFTEAERLLTEANSSPAGQGSSRVIAWYLAM
M_gilvum_PYR-GCK                 AAAERWTDVIDEVRTAATSWPDKFLVGAALVAHGVAAANLGLFTEAERRLVEANSGPAGQACNRVIAWYSAM
M_tusciae_JS617                  GAAGRWSDVIDEVKGAG.GWPDKFLAAAAGVAHGVAAANLGLFTEAERRLTESNSSPAGEACAPAIAWYLAM
M_smegmatis_mc2_155              GAGQRWTEVIDQVRGAE.RWPDKFLAAAAGVAHGVAAANLGLFTEADRRLTEANDTPVAQACAPVIAWYLAM
M_fortuitum_DSM_46621            GAAQRWTDVIDQVRGAD.QWPDKFLAAAAGVAHGVAAANLGLFTEADRRLTAADGTPAAAACSRAIAWFLAM

                                     220       230       240       250       260       270       280
M_tuberculosis_H37Rv             ARRSQGNESAAVALLEWLQTTHPEPKVAAALKDPSYRLKTTTAEQIASRADPWDPGSVVTDNSGRERLLAEA
M_kansasii_ATCC_12478            ARRSQGNEDAAVALLEWLQTTHPDPKVSAALKDSSYRLKTTTAEQIASRADPWDPGSVVTDNTGRERLLAEA
M_marinum_M                      ARRGQGNEEAAVALLEWLQTTHPAPKVSAALKDPSYRLKTTNAEQIASRSDPWDPTSVVTDNSGREKLLAEA
M_leprae_TN                      ARRSQGNEDAAVALLEWLQTTHPESKVSAALKDPSYRLTTTTAEQIAARADPWDPSSVVTDNSDRDRLLTQA
M_thermoresistibile_ATCC_19527   TRRSQGNEDAAVALLEWLQATHPEPKVAAALRDPNYRLETTTPEKIASRTDPWDPSSVVADTSGREKLLAEA
M_vanbaalenii_PYR-1              AYRALGNEEAAVRLLEWLQASHPSPEVAAALKDPSYRLQTTTAEKIASRRDPWDPTTVEADNTGREMLLADA
M_vaccae_ATCC_25954              AYRALGNEEAAVRLLEWLQATHPSPEVAAALKDPSYRLQTTTAEQIASRRDPWDPGSVEADNSGRDKLLADA
M_phlei_RIVM601174               TRRGQGNEEGAVALLEWLQATHPEPKVAEALRDPSYRLATTSAEKIAARRDPWDPGSVQADTSQRDRLLAEA
Mycobacterium_sp_JLS             TYRSQGNEEGARALLEWLQATHPEPKVTAVLRDPSYRLVTTTAEKIAARTDPWDPASAVADTSGRERLLEEA
M_rhodesiae_NBB3                 TRRGQGNEDAAQALLEWLQATHPEPKVAAALKDPNYRIATTTAEKIAARKDPWDASSVEADNSGRERLLADA
M_hassiacum_DSM_44199            AYRALGNQEGATALLEWLQANFPEPKVSAALADPSYRLETTTAEKIASRTDPWDPKSVVADTSGREKLLAEA
M_gilvum_PYR-GCK                 AYRGLGNEEAAVRLLEWLQASHPTPEVAAALKDPGFRLQTTSGEKIASRRDPWDPDSAEADNSGRETLLAEA
M_tusciae_JS617                  TRRGQGNEESALGLLEWLQATHPEPKVAAALKDPGYRLVTTTAEQIASRRDPWDAASVEADNSGRERLLAEA
M_smegmatis_mc2_155              ARRSQGNEESAQVLLEWLQANFPEPKVTAALRDPAYRLETTTAEKIAARSDPWDPSSVVADTSARDKLLVEA
M_fortuitum_DSM_46621            ARRAQGNEESAQVLLEWLQANFPEPKVTAALRDPAYRLETTTAEKIAARTDPWDPSSVVADTSGRDKLLAEA
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**Figure S1** (continues on the next page)

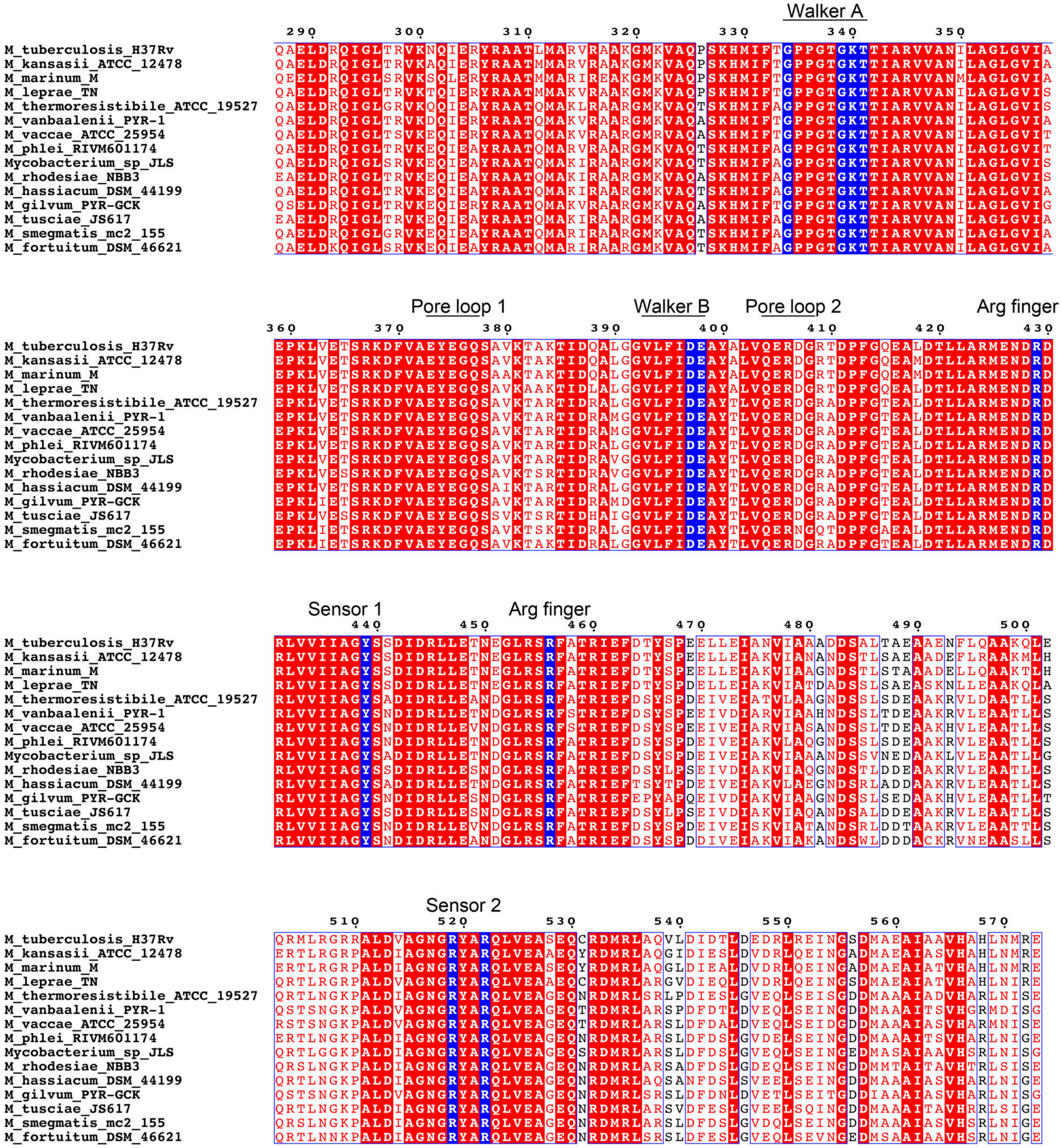

**Figure S1**

Family sequence alignment of EccA$_1$ homologs. Representative members of EccA$_1$ family from mycobacteria are included in the alignment. Sequences with high sequence identity (>98%) are excluded. The secondary structure elements and TPR motifs of *M. tuberculosis* EccA$_1$ are indicated above the sequences. The conserved residues from key elements of AAA+ domain are highlighted in blue and are indicated above the sequences.