Supplementary Figures

for

STRUCTURE AND FUNCTIONAL ANALYSIS OF RifR, THE TYPE II THIOESTERASE FROM THE RIFAMYCIN BIOSYNTHETIC PATHWAY

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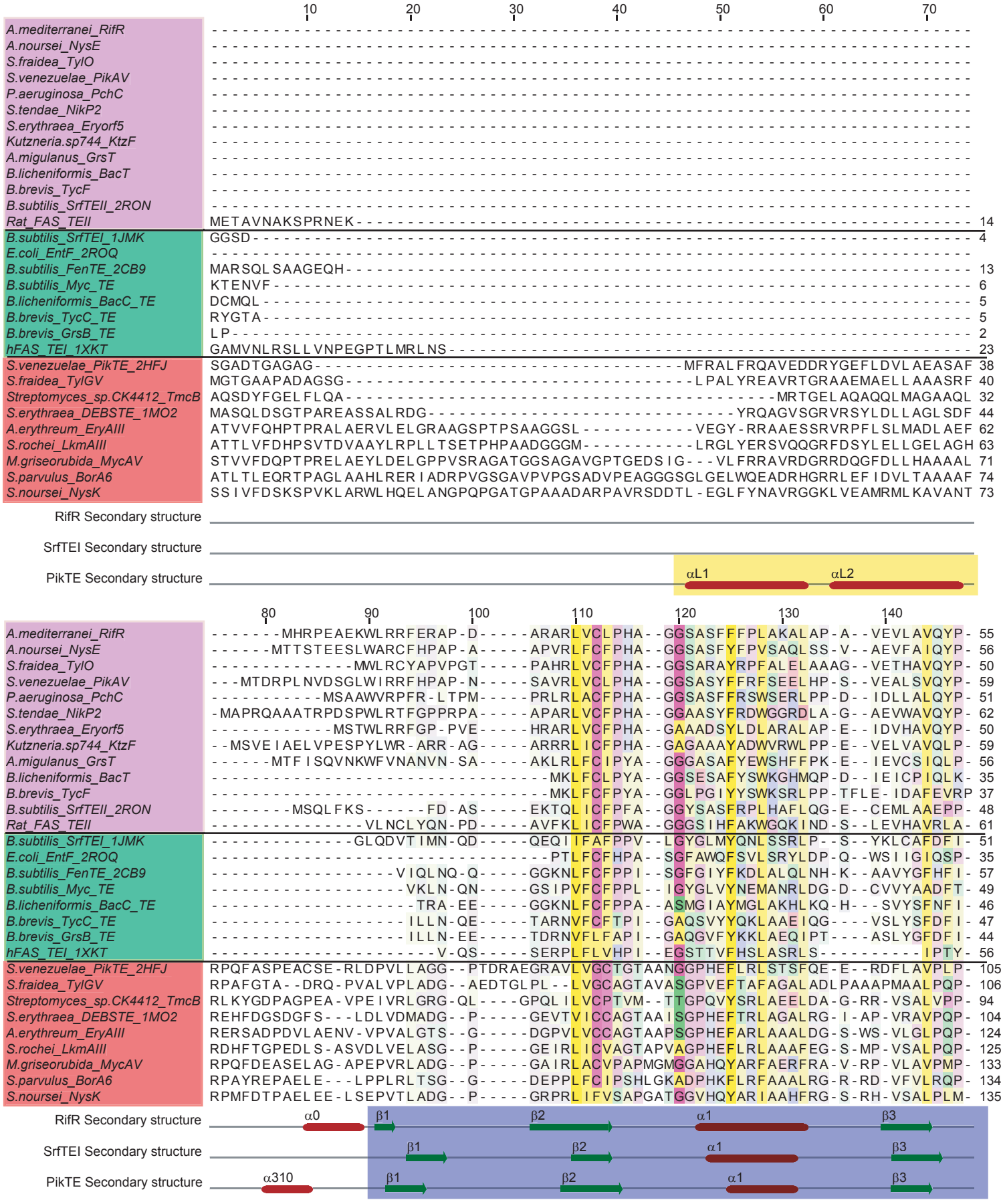
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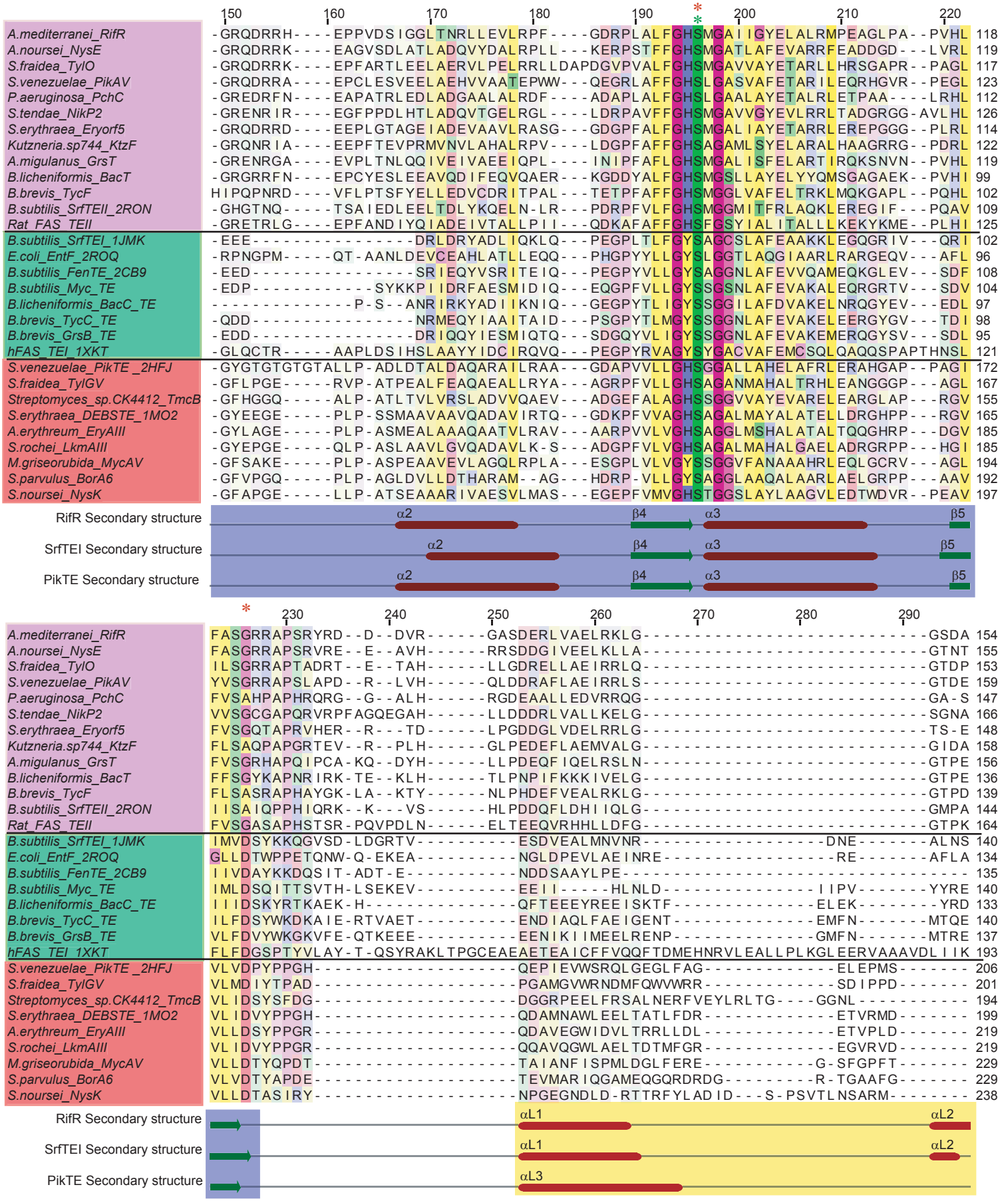
20
30
40
50
60
70
A.mediterranei_RifR
A.noursei_NysE
S.fraidea_TylO
S.venezuelae_PikAV
P.aeruginosa_PchC
S.tendae_NikP2
S.erythraea_Eryorf5
Kutzneria.sp744_KtzF
A.migulanus_GrsT
B.licheniformis_BacT
B.brevis_TycF
B.subtilis_SrfTEII_2RON
Rat_FAS_TEII
METAVNAKSPRNEK 14
B.subtilis_SrfTEI_1JMK
GGSD 4
E.coli_EntF_2ROQ
B.subtilis_FenTE_2CB9
MARSQLSAAGEQH 13
B.subtilis_Myc_TE
KTENVF 6
B.licheniformis_BacC_TE
DCMQL 5
B.brevis_TycC_TE
RYGTA 5
B.brevis_GrsB_TE
LP 2
hFAS_TEI_1XKT
GAMVNLRSLLVNPEGPTLMRLNS 23
S.venezuelae_PikTE_2HFJ
SGADTGAGAG 38
S.fraidea_TylGV
MGTGAAPADAGSG 40
Streptomyces_sp.CK4412_TmcB
AQSDYFGELFLQA 32
S.erythraea_DEBSTE_1MO2
MASQLDSGTPAREASSALRDG 44
A.erythreum_EryAIII
ATVVFQHPTPRALAERVLELGRAAGSPTPSAAGGSL 62
S.rochei_LkmAIII
ATTLVFDHPSVTDVAAYLRPLLTSETPHPAADGGGM 63
M.griseorubida_MycAV
STVVFDQPTPRELAEYLDELGPPVSRAGATGGSAGAVGPTGEDSIG 71
S.parvulus_BorA6
ATLTLEQRTPAGLAAHLRERIADRPVGSGAVPVPGSADVPEAGGGSGLGELWQEADRHGRRLEFIDVLTAAAAF 74
S.noursei_NysK
SSIVFDSKSPVKLARWLHQELANGPQPGATGPAAADARPAVRSDDTL 73
RifR Secondary structure
SrfTEI Secondary structure
PikTE Secondary structure
αL1
αL2
80
90
100
110
120
130
140
A.mediterranei_RifR
MHRPEAEKWLRRFERAP 55
A.noursei_NysE
MTTSTEESLWARCFHPAP 56
S.fraidea_TylO
MWLRCYAPVPGT 50
S.venezuelae_PikAV
MTDRPLNVDSGLWIRRFHPAP 59
P.aeruginosa_PchC
MSAAWVRPFRLTPM 51
S.tendae_NikP2
MAPRQAAATRPDSPWLRTFGPPRPA 62
S.erythraea_Eryorf5
MSTWLRRFGP 50
Kutzneria.sp744_KtzF
MSVEIAELVPESPYLWR 59
A.migulanus_GrsT
MTFISQVNKWFVNANVN 56
B.licheniformis_BacT
MKLFCLPYA 35
B.brevis_TycF
MKLFCFPYA 37
B.subtilis_SrfTEII_2RON
MSQLFKS 48
Rat_FAS_TEII
VLNCLYQN 61
B.subtilis_SrfTEI_1JMK
GLQDVTIMN 51
E.coli_EntF_2ROQ
PTLFCFHPA 35
B.subtilis_FenTE_2CB9
VIQLNQ 57
B.subtilis_Myc_TE
VKLN 49
B.licheniformis_BacC_TE
TRA 46
B.brevis_TycC_TE
ILLN 47
B.brevis_GrsB_TE
ILLN 44
hFAS_TEI_1XKT
V 56
S.venezuelae_PikTE_2HFJ
RPQFASPEACSE 105
S.fraidea_TylGV
RPAFGTA 106
Streptomyces_sp.CK4412_TmcB
RLKYGDPAGPEA 94
S.erythraea_DEBSTE_1MO2
REHFDGSDGFS 104
A.erythreum_EryAIII
RERSADPDVLAENV 124
S.rochei_LkmAIII
RDHFTGPEDLS 125
M.griseorubida_MycAV
RPQFDEASELAG 133
S.parvulus_BorA6
RPAYREPAELE 134
S.noursei_NysK
RPMFDTPAELEE 135
RifR Secondary structure
α0
β1
β2
α1
β3
SrfTEI Secondary structure
β1
β2
α1
β3
PikTE Secondary structure
α310
β1
β2
α1
β3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A.mediterranei_RifR
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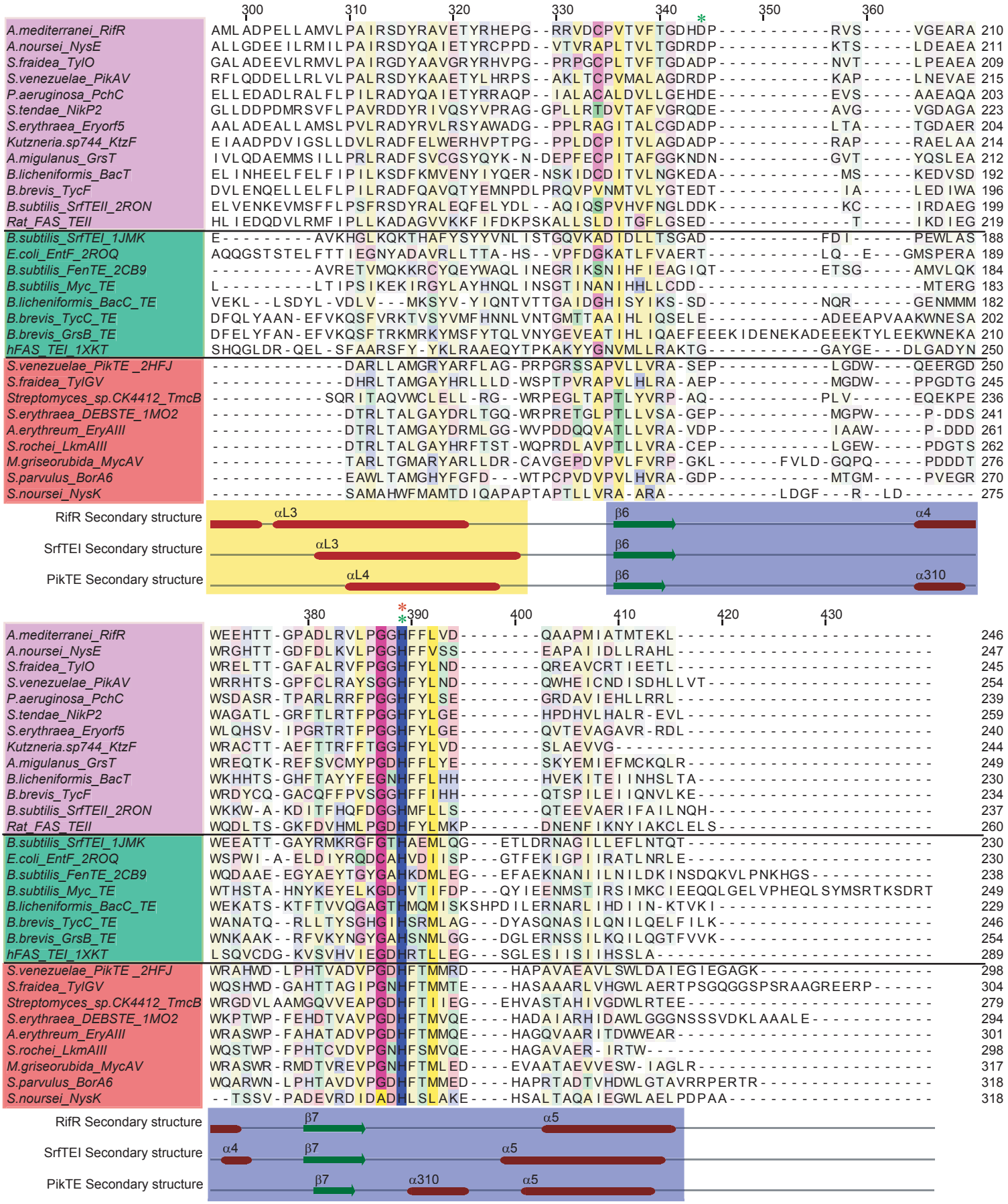
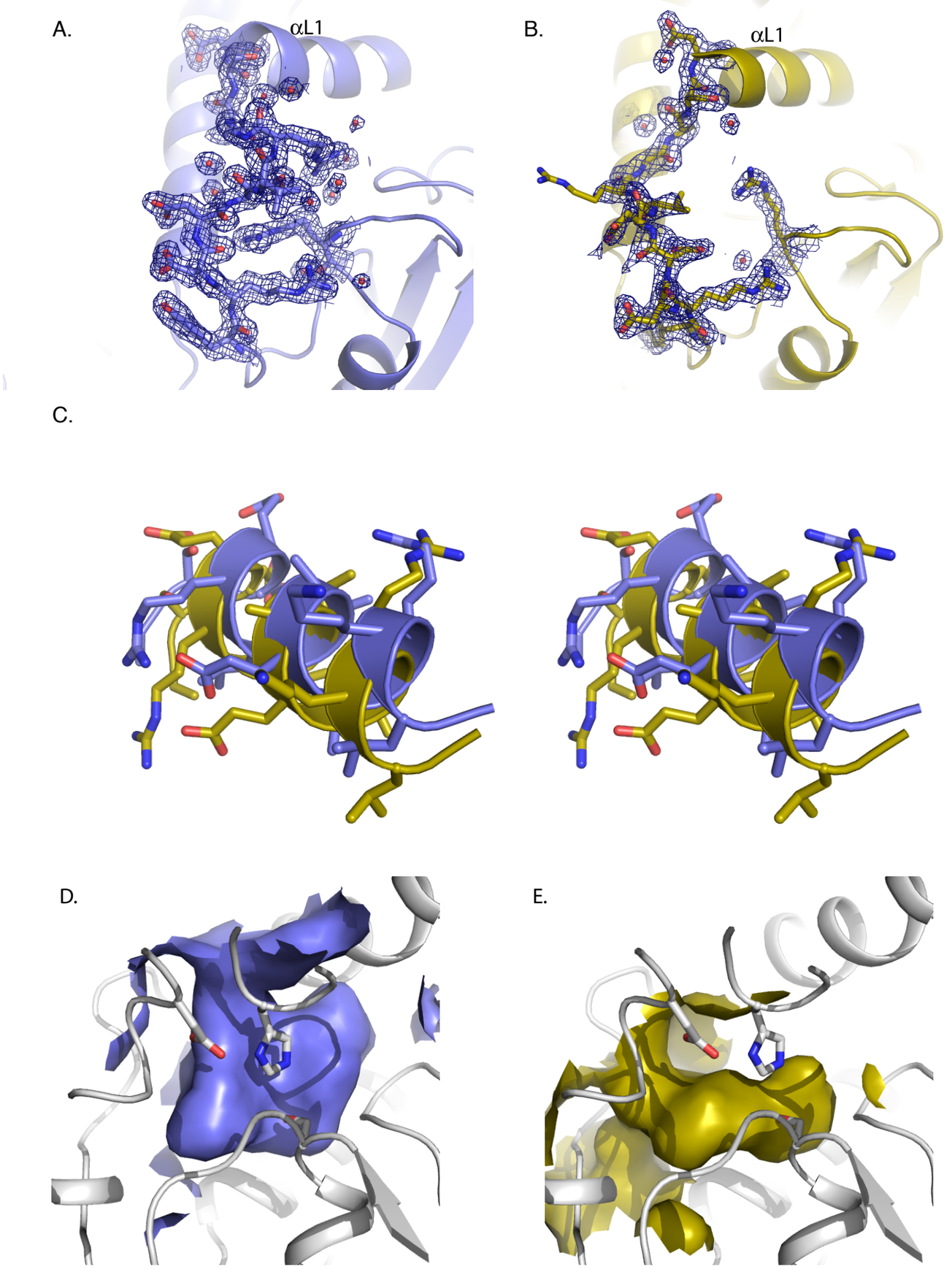
B.subtilis_FenTE_2CB9
B.subtilis_Myc_TE
B.licheniformis_BacC_TE
B.brevis_TycC_TE
B.brevis_GrsB_TE
hFAS_TEI_1XKT
S.venezuelae_PikTE _2HFJ
S.fraidea_TylGV
Streptomyces_sp.CK4412_TmcB
S.erythraea_DEBSTE_1MO2
A.erythreum_EryAIII
S.rochei_LkmAIII
M.griseorubida_MycAV
S.parvulus_BorA6
S.noursei_NysK
RifR Secondary structure
SrfTEI Secondary structure
PikTE Secondary structure
α2
β4
α3
β5
230
240
250
260
270
280
290
αL1
αL2
αL3


300
310
320
330
340
350
360
A.mediterranei_RifR
A.noursei_NysE
S.fraidea_TylO
S.venezuelae_PikAV
P.aeruginosa_PchC
S.tendae_NikP2
S.erythraea_Eryorf5
Kutzneria.sp744_KtzF
A.migulanus_GrsT
B.licheniformis_BacT
B.brevis_TycF
B.subtilis_SrfTEII_2RON
Rat_FAS_TEII
B.subtilis_SrfTEI_1JMK
E.coli_EntF_2ROQ
B.subtilis_FenTE_2CB9
B.subtilis_Myc_TE
B.licheniformis_BacC_TE
B.brevis_TycC_TE
B.brevis_GrsB_TE
hFAS_TEI_1XKT
S.venezuelae_PikTE _2HFJ
S.fraidea_TylGV
Streptomyces_sp.CK4412_TmcB
S.erythraea_DEBSTE_1MO2
A.erythreum_EryAIII
S.rochei_LkmAIII
M.griseorubida_MycAV
S.parvulus_BorA6
S.noursei_NysK
RifR Secondary structure
αL3
β6
α4
SrfTEI Secondary structure
αL3
β6
PikTE Secondary structure
αL4
β6
α310
380
390
400
410
420
430
RifR Secondary structure
β7
α5
SrfTEI Secondary structure
α4
β7
α5
PikTE Secondary structure
β7
α310
α5

Supplementary Figure 1. Structure-based sequence alignment of TEIs and TEIIs. PKS/NRPS/FAS TEIIs and TEIs were first aligned using the EXPRESSO(3DCoffee) server (1), then manually edited based on 3-D structures. Conserved residues were highlighted with Jalview 2.4 (2). Asterisks mark the active site residues of both TEIs and TEIIs. TEII sequences are highlighted in violet, FAS/NRPS TEIs in green, and PKS TEIs in red. The following TEs were aligned: *A. mediterranei*_RifR (AAG52991), *A. noursei*_NysE (AAF71777), *S.fraidea*_TylO (AAA21345), *S.venezuelae*_PikAV (AAC69333), *P.aeruginosa*_PchC (CAA57967), *S.tendae*_NikP2 (CAC11138), *S.erythraea*_Eryorf5 (AAA26497), *Kutzneria.sp744* (ABV56586), *A.migulanus*_GrsT (P14686), *B.licheniformis*_BacT (BAA36683), *B.brevis*_TycF (AAC45933), *B.subtilis*_SrfTEII_2RON (AAF87217), Rat_FAS_TEII (CAA68411), *B.subtilis*_SrfTEI_1JMK (1JMK), *E.Coli*_EntF_2ROQ (2ROQ), *B.subtilis*_FenTE_2CB9 (2CB9), *B.subtilis*_Myc_TE (AAF08797), *B.licheniformis*_BacC_TE (AAC06348), *B.brevis*_TycC_TE (AAC45930), *B.brevis*_GrsB_TE (AAC06348), hFAS_TEI_1XKT (1XKT), *S.venezuelae*_PikTE_2HFJ (2HFJ), *S.fraidea*_TylGV (AAB66508.1), *Streptomyces_sp.CK4412*_tmcB (AB194380), *S.erythraea*_DEBSTE_1MO2 (1MO2), *A.erythreum*_EryAIII (AAU93805.2), *S.rochei*_LkmAIII (BAC76491), *M.griseorubida*_MycAV (BAC57032.1), *S.parvulus*_BorA6 (CAE45672.1), *S.noursei*_NysK (AAF71768).



Supplementary Figure 2. Electron density and model for flexible linker region for form 1 (A. – experimental density contoured at 1σ) and for form 2 (B. – 2Fo-Fc refined density contoured at 1σ). C. Stereodiagram of αL1 helices from form 1 (blue) and form 2 (gold). Form 1 (D.) and form 2 (E.) substrate chambers have unique shapes, and different access to the exterior of RifR.

References

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2. Clamp, M., Cuff, J., Searle, S. M., and Barton, G. J. (2004) *Bioinformatics* **20**, 426-427