

Supplementary Figures

for

**STRUCTURE AND FUNCTIONAL ANALYSIS OF RifR, THE TYPE II  
THIOESTERASE FROM THE RIFAMYCIN BIOSYNTHETIC PATHWAY**  
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Smith<sup>1,2</sup>**

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authors contributed equally to this work.

		10	20	30	40	50	60	70
<i>A.mediterranei_RifR</i>		-	-	-	-	-	-	-
<i>A.noursei_NysE</i>		-	-	-	-	-	-	-
<i>S.fraidea_TyIO</i>		-	-	-	-	-	-	-
<i>S.venezuelae_PikAV</i>		-	-	-	-	-	-	-
<i>P.aeruginosa_PchC</i>		-	-	-	-	-	-	-
<i>S.tendae_NikP2</i>		-	-	-	-	-	-	-
<i>S.erythraea_Eryorf5</i>		-	-	-	-	-	-	-
<i>Kutzneria.sp744_KtzF</i>		-	-	-	-	-	-	-
<i>A.migulanus_GrsT</i>		-	-	-	-	-	-	-
<i>B/licheniformis_BacT</i>		-	-	-	-	-	-	-
<i>B.brevis_TycF</i>		-	-	-	-	-	-	-
<i>B.subtilis_SrfTEII_2RON</i>		-	-	-	-	-	-	-
<i>Rat_FAS_TEII</i>	METAVNAKSPRNEK	-	-	-	-	-	-	14
<i>B.subtilis_SrfTEI_1JMK</i>	GGSD	-	-	-	-	-	-	4
<i>E.coli_EnvF_2ROQ</i>		-	-	-	-	-	-	-
<i>B.subtilis_FenTE_2CB9</i>	MARSQLSAAGEQH	-	-	-	-	-	-	13
<i>B.subtilis_Myc_TE</i>	KTENVF	-	-	-	-	-	-	6
<i>B/licheniformis_BacC_TE</i>	DCMQL	-	-	-	-	-	-	5
<i>B.brevis_TycC_TE</i>	RYGTA	-	-	-	-	-	-	5
<i>B.brevis_GrsB_TE</i>	LP	-	-	-	-	-	-	2
<i>hFAS_TEI_1XKT</i>	GAMVNRLRSLLVNPEGPTLMRLNS	-	-	-	-	-	-	23

<i>S.venezuelae_PikTE_2HFJ</i>	SGADTGAGAG	-	-	-	-	-	-	38
<i>S.fraidea_TyIGV</i>	MGTGAAPADAGSG	-	-	-	-	-	-	40
<i>Streptomyces_sp.CK4412_TmcB</i>	AQSDYFGELFLQA	-	-	-	-	-	-	32
<i>S.erythraea_DEBSTE_1MO2</i>	MASQLDSGT PAREASSALRDG	-	-	-	-	-	-	44
<i>A.eurythrum_EryAIII</i>	ATVVFQHPTPRAL AERVLELGRAAGSPTPSAAGGSL	-	-	-	-	-	-	62
<i>S.rochei_LkmAllI</i>	ATTLFVDHPSVTDAAYLRPLL TSETPHPAADGGGM	-	-	-	-	-	-	63
<i>M.griseorubida_MycAV</i>	STVVFDQPTPRELAEYLDELGPVSRAGATGGSAGAVGPTGEDS	I G	-	-	-	-	-	71
<i>S.parvulus_BorA6</i>	ATLTLEQRTPAGLA AHLRER IADRPVGSGAVPVPGSADVPEAGGGSGL GELWQEADR HGRRL EF IDVL TAAA AF	74	-	-	-	-	-	73
<i>S.noursei_NysK</i>	SSIVFDSKSPVKL ARWL HQEL ANGPQPGATGPAAADARPAVRSDDTL	-	-	-	-	-	-	-

RifR Secondary structure



SrfTEI Secondary structure



PikTE Secondary structure



	80	90	100	110	120	130	140
<i>A.mediterranei_RifR</i>	-	-	-	-	-	-	-
<i>A.noursei_NysE</i>	-	-	-	-	-	-	-
<i>S.fraidea_TyIO</i>	-	-	-	-	-	-	-
<i>S.venezuelae_PikAV</i>	-	-	-	-	-	-	-
<i>P.aeruginosa_PchC</i>	-	-	-	-	-	-	-
<i>S.tendae_NikP2</i>	-	-	-	-	-	-	-
<i>S.erythraea_Eryorf5</i>	-	-	-	-	-	-	-
<i>Kutzneria.sp744_KtzF</i>	-	-	-	-	-	-	-
<i>A.migulanus_GrsT</i>	-	-	-	-	-	-	-
<i>B/licheniformis_BacT</i>	-	-	-	-	-	-	-
<i>B.brevis_TycF</i>	-	-	-	-	-	-	-
<i>B.subtilis_SrfTEII_2RON</i>	-	-	-	-	-	-	-
<i>Rat_FAS_TEII</i>	-	-	-	-	-	-	-
<i>B.subtilis_SrfTEI_1JMK</i>	-	-	-	-	-	-	-
<i>E.coli_EnvF_2ROQ</i>	-	-	-	-	-	-	-
<i>B.subtilis_FenTE_2CB9</i>	-	-	-	-	-	-	-
<i>B.subtilis_Myc_TE</i>	-	-	-	-	-	-	-
<i>B/licheniformis_BacC_TE</i>	-	-	-	-	-	-	-
<i>B.brevis_TycC_TE</i>	-	-	-	-	-	-	-
<i>B.brevis_GrsB_TE</i>	-	-	-	-	-	-	-
<i>hFAS_TEI_1XKT</i>	-	-	-	-	-	-	-

<i>A.mediterranei_RifR</i>	GLQDVTIMN-QD	-	QEQQIIFAFPPV	-	LGYGLMYQNLSSRLP	-	-	51
<i>A.noursei_NysE</i>	-	-	-	-	-	-	-	56
<i>S.fraidea_TyIO</i>	-	-	-	-	-	-	-	50
<i>S.venezuelae_PikAV</i>	-	-	-	-	-	-	-	59
<i>P.aeruginosa_PchC</i>	-	-	-	-	-	-	-	51
<i>S.tendae_NikP2</i>	-	-	-	-	-	-	-	62
<i>S.erythraea_Eryorf5</i>	-	-	-	-	-	-	-	50
<i>Kutzneria.sp744_KtzF</i>	-	-	-	-	-	-	-	59
<i>A.migulanus_GrsT</i>	-	-	-	-	-	-	-	56
<i>B/licheniformis_BacT</i>	-	-	-	-	-	-	-	35
<i>B.brevis_TycF</i>	-	-	-	-	-	-	-	35
<i>B.subtilis_SrfTEII_2RON</i>	-	-	-	-	-	-	-	48
<i>Rat_FAS_TEII</i>	-	-	-	-	-	-	-	61
<i>B.subtilis_SrfTEI_1JMK</i>	-	-	-	-	-	-	-	51
<i>E.coli_EnvF_2ROQ</i>	-	-	-	-	-	-	-	35
<i>B.subtilis_FenTE_2CB9</i>	-	-	-	-	-	-	-	57
<i>B.subtilis_Myc_TE</i>	-	-	-	-	-	-	-	49
<i>B/licheniformis_BacC_TE</i>	-	-	-	-	-	-	-	46
<i>B.brevis_TycC_TE</i>	-	-	-	-	-	-	-	47
<i>B.brevis_GrsB_TE</i>	-	-	-	-	-	-	-	44
<i>hFAS_TEI_1XKT</i>	-	-	-	-	-	-	-	56

<i>S.venezuelae_PikTE_2HFJ</i>	RPQFASPEACSE-RLDPVLLAGG	-	-	-	-	-	-	105
<i>S.fraidea_TyIGV</i>	-	-	-	-	-	-	-	106
<i>Streptomyces_sp.CK4412_TmcB</i>	-	-	-	-	-	-	-	94
<i>S.erythraea_DEBSTE_1MO2</i>	-	-	-	-	-	-	-	104
<i>A.eurythrum_EryAIII</i>	-	-	-	-	-	-	-	124
<i>S.rochei_LkmAllI</i>	-	-	-	-	-	-	-	125
<i>M.griseorubida_MycAV</i>	-	-	-	-	-	-	-	133
<i>S.parvulus_BorA6</i>	-	-	-	-	-	-	-	134
<i>S.noursei_NysK</i>	-	-	-	-	-	-	-	135

RifR Secondary structure



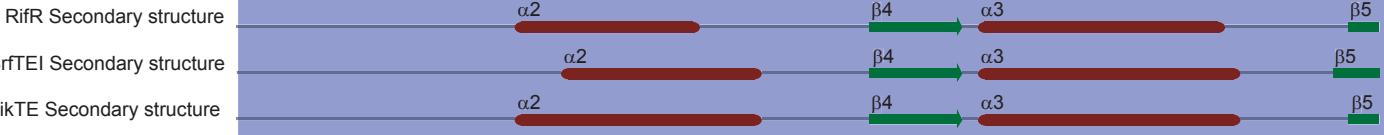
SrfTEI Secondary structure



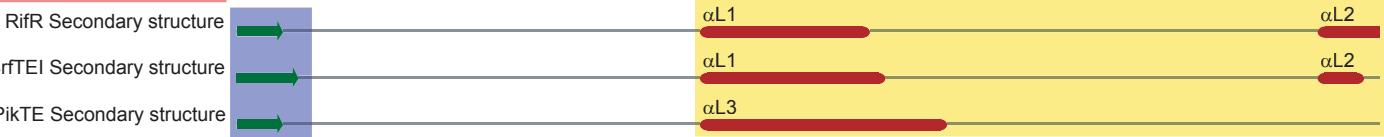
PikTE Secondary structure



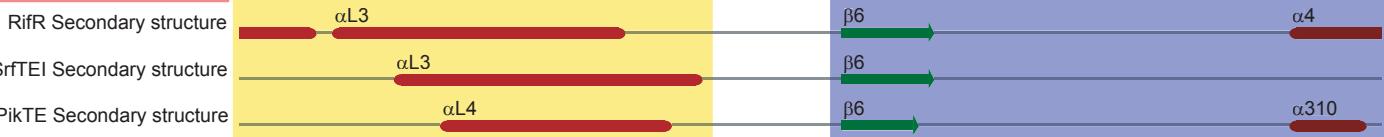
	150	160	170	180	190	*	200	210	220									
<i>A.mediterranei_RifR</i>	-GRQDRRH	-EPPVDSIGGL	TNRLLLEVLRPF	-GDRPLALF	GHSMGAI	I	GYELALRMP	EAGLPA	-PVHL	118								
<i>A.noursei_NysE</i>	-GRQDRRK	-EAVSDSLATLADQVYDAL	RPLL	-KERPSTFF	GHSMGAT	I	AFEVARRF	EADDGD	-LVRL	119								
<i>S.fraidea_TyI</i> O	-GRQDRRK	-EPFARTLEELAERVLP	ELRRLLADPDGV	PVALFGHSMGAVVAYETARLL	HRSGAPR	-	PAGL			117								
<i>S.venezuelae_PikAV</i>	-GRQDRRA	-EPCLESVEELAEHVVAATE	EPWV	-QEGRALAFFGHSLGASVAFETAR	I	LEQRHGVR	-PEGL			123								
<i>P.aeruginosa_PchC</i>	-GREDRFN	-EAPATRLEDLADGAALAL	RDF	-ADAPLALFGHSLGAALAYETALRL	ETPAA	-	LRHL			112								
<i>S.tendae_NikP2</i>	-GRENRIR	-EGFPFDLHTLADQVTGEL	RGL	-LDRPAVFFGHS	MGAVVGYEV	LRRLLADGRGG	-AVLHL			126								
<i>S.erythraea_Eryorf5</i>	-GRQDRRD	-EEPLGTAGEI	ADEVAAVLRA	SGRASG	-LDPGP	FALFGHS	MGA	I	AYETARRLEREPGGG	-PLRL	114							
<i>Kutzneria.sp744_KtzF</i>	-GRQNR	IA	-EEPFTEVPRMV	NVLAHALRPV	-LDGPFA	LGFS	AGAMISY	YELARAL	HAACRRG	-PDRL	122							
<i>A.migulanus_GrsT</i>	-GRENRGA	-EVPLTNLQQ	I	VAEEIQPL	-INIPFAFL	GHS	MGA	I	SELART	IRQKSNVN	-PVHL	119						
<i>B/licheniformis_BacT</i>	-GRGRRFN	-EPCYESLEEAVQD	I	FEQVQAER	-KGDDYALFGHS	MGSLLAYEL	YYQMMSGAGAEK	-		PVHI	99							
<i>B.brevis_TycF</i>	HIPQPNRD	-VFLPTSFYELL	EDVC	CDITPAL	TETPF	FAFFGHS	MGGLVA	FE	TRKL	MKGAPL	-PQHL	102						
<i>B.subtilis_SrfTEII_2RON</i>	-GHGTNQ	-TSAI	EDLEEL	TDLYKQELN-LR	-PDRPF	VLF	GHS	MGGM	I	TFRLAQKLEREGIF	-PQAV	109						
<i>Rat FAS TEII</i>	-GRETRLG	-EPFANDI	YQIADE	IVTALLP	I	QDKAFA	FFGHS	FSYI	ALITALL	KEKYKME	-PLHI	125						
<i>B.subtilis_SrfTEI_1JMK</i>	-EEE	-	DRLDRYADL	I	QKQLQ	-PEGPL	TLFGYS	AGCOSL	AFEEAKKL	EGQGRIV	-QR	102						
<i>E.coli_EntF_2ROQ</i>	-RPNPGM	-QT	AANLDEVCEAHL	ATLLEQQ	-PHGPPYLL	GYS	LGTTLA	AGQIAARL	RARGEQV	-AFL	96							
<i>B.subtilis_FenTE_2CB9</i>	-EED	-	-SR	I	EQYVSR	ITEI	Q	-PEGPYVLL	GYSAGGNL	AEEVQAMEQKGL	EV	-SDF	108					
<i>B.subtilis_Myc_TE</i>	-EDP	-	SYKKP	I	DRDFAESMID	IQ	EQGP	FVLL	GYSSGSNL	AEEVAKALE	ERGRRTV	-SDV	104					
<i>B/licheniformis_BacC_TE</i>	-	-P-S-	ANR	I	RKYAD	I	IKNIQ	-GEGPYTL	I	GYSSGGI	LAFFDVAKEL	NRQGYEV	-EDV	97				
<i>B.brevis_TycC_TE</i>	-QDD	-	-NR	MEQY	IAA	ITA	ID	-PSGPYTL	MGYSSGGNL	AF	EVAKEL	EERGYGV	-TD	98				
<i>B.brevis_GrsB_TE</i>	-EDD	-	-DR	I	QQY	I	ESMIQTQ	-SDGQYVL	I	GYSSGGNL	AF	EVAKEMERQGYSV	-SDL	95				
<i>hFAS TEI 1XKT</i>	-GLQC	TR	-AAPLDS	I	HSLAA	YYI	DC	-IRQVQ	-PEGPYRVAG	GYACVAFEM	CSQL	QAQQSPAPTHNSL		121				
<i>S.venezuelae_PikTE_2HFJ</i>	-GYGTG	TGTGTALLP	-ADLD	TALDAQARA	I	LRAA	-GDA	PVVLL	GHS	GCALLA	HEL	AFRLERAH	GAP	-PAG	172			
<i>S.fraidea_TyIGV</i>	-GFLP	GPE	-	-RVP	-ATPEAL	FEAQAE	ALLRYA	-AGRPF	VLL	GHS	AGANMA	HAL	TRHL	EANGGP	-AGL	167		
<i>Streptomyces_sp.CK4412_TmcB</i>	-GFHGGQ	-	-ALP	-ATL	TVL	VRSL	ADVQ	-ADGE	FALAGH	SSG	VVA	YEVAREL	EARGL	AP	-RGV	155		
<i>S.erythraea_DEBSTE_1MO2</i>	-GYEEGE	-	-PLP	-SSMAA	VAAVQAD	ADAV	I	IRTQ	-GDKPF	VVAGHS	AGAL	MAYAL	ATELL	DRGHPP	-RGV	165		
<i>A.erythreum_EryAIII</i>	-GYLAGE	-	-PLP	-ASMEAL	AAAQ	QAT	VLRAV	-AARP	PVVL	VGH	SAGGLMS	HAL	ATALT	TQQGHRP	-DGV	185		
<i>S.rochei_LkmAIII</i>	-GYEPGE	-	-QLP	-ASLAAVL	GQADAV	KLK	S	-	-ADGPF	VLL	VGH	SAGAL	MAHAL	GAEL	ADRGRPP	-HG	185	
<i>M.griseorubida_MycAV</i>	-GFSAKE	-	-PLP	-ASPEAAVE	VL	AGQLR	PLA	-	-ESGPL	VLL	VGH	YSSGGV	FANAAA	HRL	EQLGCRV	-AGL	194	
<i>S.parvulus_BorA6</i>	-GFVPGQ	-	-PLP	-AGLDV	L	DTHAR	AM	-	-HDRP	VLL	GYS	AGGL	AAQAL	AARL	AEL	GRPP	-AAV	192
<i>S.noursei_NysK</i>	-GFAPGE	-	-LLP	-ATSEAAAR	I	VAESV	LMAS	-	-EGEPF	VMV	GHS	TGGSL	AYLA	AGV	LED	TWDVR	-PEAV	197



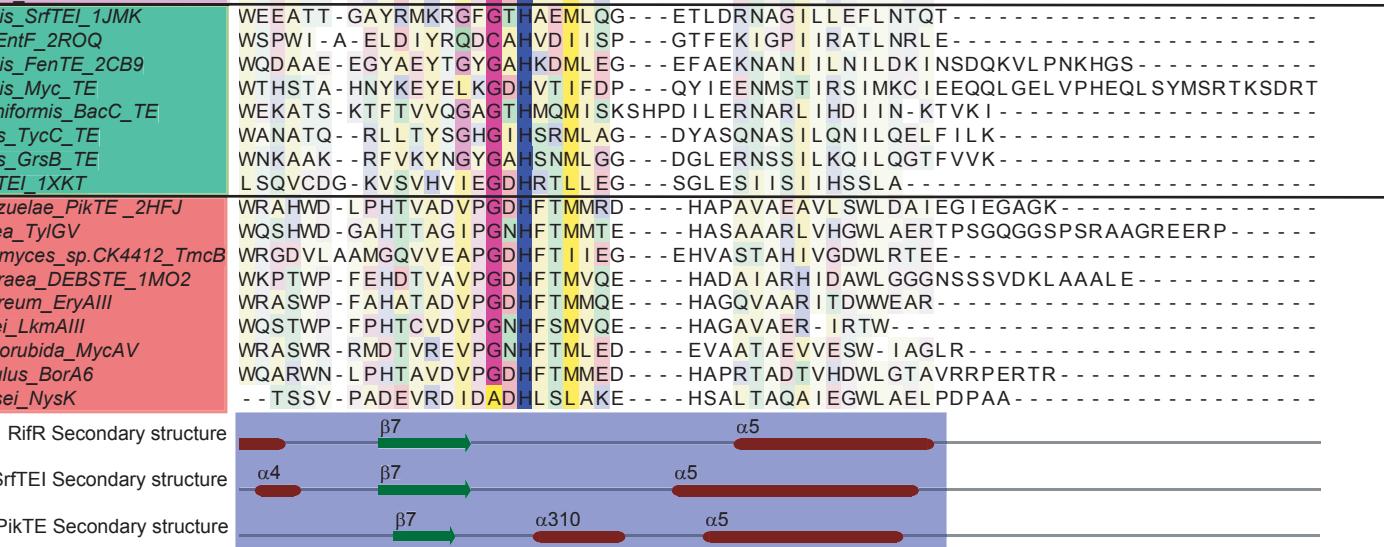
	*	230	240	250	260	270	280	290															
<i>A.mediterranei_RifR</i>	FASGRAP	SRYRD	-D	-DVR	-	-GASDER	LVAELRKLG	-	-GSDA	154													
<i>A.noursei_NysE</i>	FASGRAP	SRSVRE	-E	-AVH	-	-RRSDDG	I	VEELKLLA	-GTNT	155													
<i>S.fraidea_TyI</i> O	I	LSGRAP	TADRT	-E	-TAH	-	-LLGDRELL	AEIRRLLQ	-GTDP	153													
<i>S.venezuelae_PikAV</i>	YVSGRAP	SLAPD	-R	-LVH	-	-QLDDRAFL	AEIRRLLQ	-	-GTDE	159													
<i>P.aeruginosa_PchC</i>	FVSAHPA	HQRQG	-G	-ALH	-	-RGDEA	ALL	EDVRRQG	-GA-S	147													
<i>S.tendae_NikP2</i>	VVSGCGA	PQRV	RPFAQGEAH	-	-LLDDDR	LVAL	LKELG	-	-SGNA	166													
<i>S.erythraea_Eryorf5</i>	FVSGQT	APRVER	-R	-TD	-	-LPGDDGL	VDEL	RRRLG	-TS-E	148													
<i>Kutzneria.sp744_KtzF</i>	FLSAQ	PAPGRTE	-R	-PLH	-	-GLPEDEF	LAEMVALG	-	-GIDA	158													
<i>A.migulanus_GrsT</i>	FVSGR	HAPQI	PC	-KQ	-DYH	-LLP	DEQF	I	QELRSLN	-GTPE	156												
<i>B/licheniformis_BacC_TE</i>	FFSGY	KAPNR	I	RK	-TE	-KLH	-	-TLPNP	I	FKKK	IVE	LG	EL	-GTPE	136								
<i>B.brevis_TycF</i>	FLSAS	RAPHAY	KG	-LA	-KTY	-	-NL	PHDEF	VEALR	KLG	-	GTPD	139										
<i>B.subtilis_SrfTEII_2RON</i>	I	ISA	IQPPH	I	QRK	-K	-VS	-	-HL	PDDQFL	DH	I	QLG	-GMPA	144								
<i>Rat FAS TEII</i>	FVSGAS	A	PH	TSR	-PQVPDLN	-	-EL	TEEQVRHH	LLDFG	-	-GTPK	164											
<i>B.subtilis_SrfTEI_1JMK</i>	IMVD	SYKKQGVSD	-LDGRTV	-	-ESD	VAL	MNVNR	-	-DNE	-	-ALNS	140											
<i>E.coli_EntF_2ROQ</i>	GLLD	TWP	PETQNW	Q-EKEA	-	-NGLD	PEVLA	EINRE	-RE	-	-AFLA	134											
<i>B.subtilis_FenTE_2CB9</i>	I	IVD	YKKDQ	SIT	-ADT	-E	-	-NDD	SAAYL	PE	-	135											
<i>B.subtilis_Myc_TE</i>	IMLD	DSQ	I	TTSVTH	-L	SEKEV	-	EE	I	I	-	140											
<i>B/licheniformis_BacC_TE</i>	III	IDS	KYRT	KA	E	-H	-	-QF	TEEEY	YREE	-ELEK	-YRD	133										
<i>B.brevis_TycC_TE</i>	I	LF	D	SYW	KDKA	I	E	-RTVAET	-END	I	AQL	FAE	I	GENT	-EMFN	-MTQE	140						
<i>B.brevis_GrsB_TE</i>	VL	LD	TYW	KGV	FE	-	-QTKEEE	-	-EEN	I	KI	I	MEEL	REN	-GMFN	-MTRE	137						
<i>hFAS TEI 1XKT</i>	FL	FDG	SPT	YV	L	A	-T	-QSYRAKLT	PGCEAE	TEA	I	CF	VQQFT	DMEHNRV	LE	ALL	PL	KGL	EERVAA	AVDL	I	IK	193
<i>S.venezuelae_PikTE_2HFJ</i>	VLVDP	YPPG	-	-	-	-QEP	I	EVWSRQL	GEGL	FAG	-	-ELE	EPMS	-	-	-	-	-	206				
<i>S.fraidea_TyIGV</i>	VLMD	I	YTP	AD	-	-	-	-PGAM	GVWR	NDMF	QWW	WRR	-	-SD	I	IPP	-	-	-	201			
<i>Streptomyces_sp.CK4412_TmcB</i>	VL	IDS	Y	SFDG	-	-	-DGG	RP	EEL	FRS	AL	NER	F	VEYL	R	LG	-	-	-	194			
<i>S.erythraea_DEBSTE_1MO2</i>	VL	1	DVY	PPG	H	-	-QDAM	NAWL	EEL	TATL	FDR	-	-	-ETVR	MD	-	-	-	-	199			
<i>A.erythreum_EryAIII</i>	VLL	D	SY	PPG	GR	-	-QDA	VEGW	I	DVL	TRR	LL	-	-ETV	P	LD	-	-	-	219			
<i>S.rochei_LkmAIII</i>	VL	I	DVY	PPG	GR	-	-QQAVQ	QWL	AEL	TDT	MFGR	-	-	-EGVR	V	RD	-	-	-	219			
<i>M.griseorubida_MycAV</i>	VLL	D	DT	Y	QP	D	-	-TA	I	ANF	I	SPML	DGL	FER	-	-G	-SFGPFT	-	-	229			
<i>S.parvulus_BorA6</i>	VL	V	D	TY	AP	D	-	-TE	VMAR	I	QGAME	QGQR	DRDG	-	-R	-TGA	AFG	-	-	229			
<i>S.noursei_NysK</i>	VLL	D	TAS	I	RY	-	-NPGE	GN	DLD	-RT	TR	FY	LAD	ID	-S	-PSV	T	NSARM	-	238			



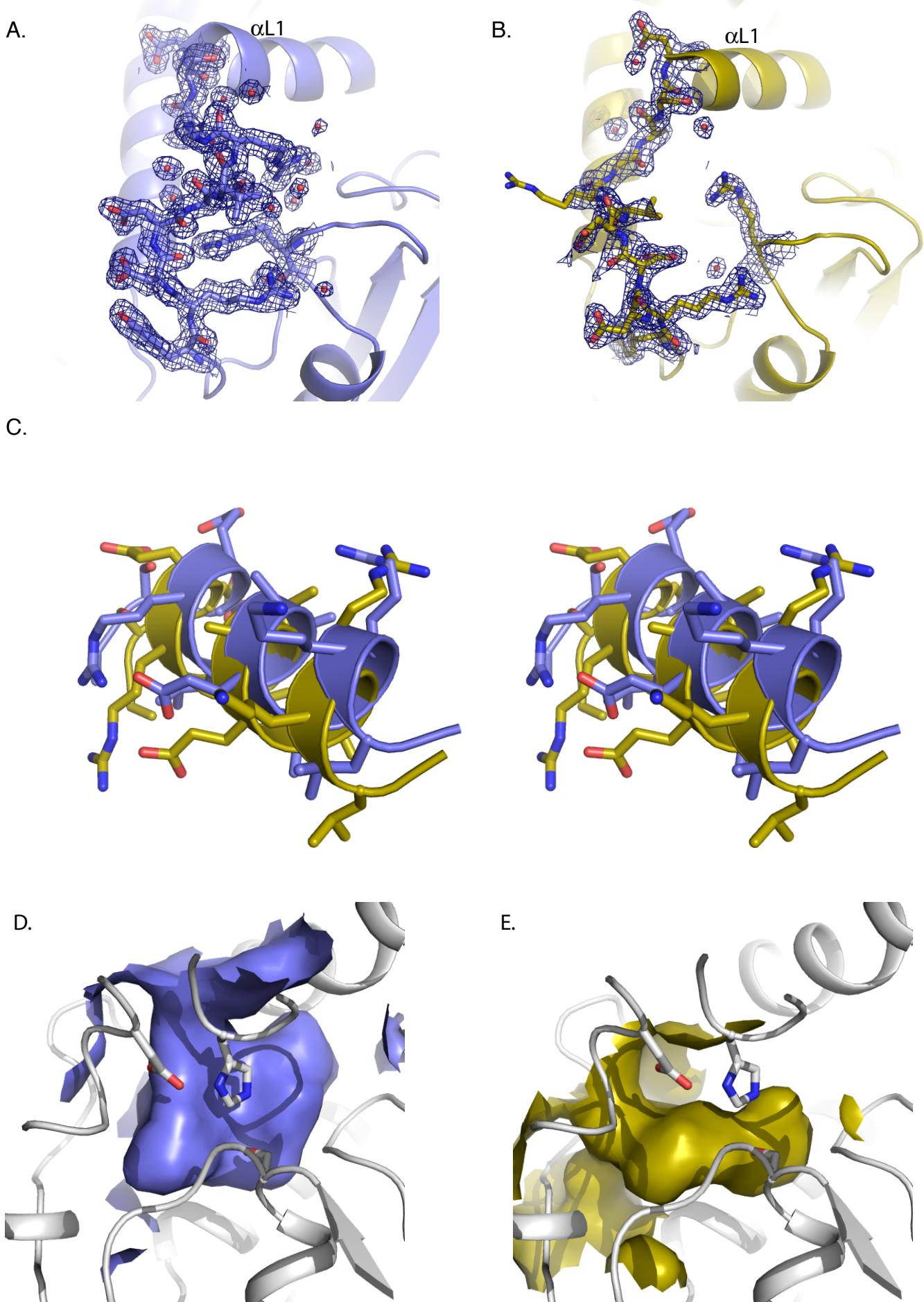
	300	310	320	330	340	*	350	360	
<i>A.mediterranei_RifR</i>	AML ADPELL AMVL PA IRS DYRAVET YRHEPG -	RRV DCP VTV FTD GDHDP -	-	-	RVS -	-	VGEARA	210	
<i>A.noursei_NysE</i>	ALL GDEE I LRM I L PA IRS DYQA I ETYRCPPD -	VT VRAP L TVL T QGD RDP -	-	-	KTS -	-	LDEAE A	211	
<i>S.fraidea_TyI0</i>	GAL ADEEV L RML V PA IRS GDYAA VGRYR HVPG -	PR PG CPL TV FTD GDADP -	-	-	NVT -	-	LPEAEA	209	
<i>S.venezuelae_PikAV</i>	RFL QDD DEL L RLV L PAL RSDY KAA E TYL HRPS -	AKL T CP VMAL AGDR DP -	-	-	KAP -	-	LNEVAE	215	
<i>P.aeruginosa_PchC</i>	ELL EDAD L RAL FLP I LRAD Y QA I ETY RR AQP -	I AL ACAL DV L GEH DE -	-	-	EVS -	-	AAEAQA	203	
<i>S.tendae_NikP2</i>	GL L DDP DMRS VFL PAV R D DYR I VQSY V PRAG -	GPL L RT D VT AF V GRQ DE -	-	-	AVG -	-	VGDAGA	223	
<i>S.erythraea_Eryorf5</i>	AAL ADE ALL A MSL PVL RAD Y RV L R S Y AWADG -	PPL RAG I T AL CGD ADP -	-	-	LTA -	-	TGDAER	204	
<i>Kutzneria.sp744_KtzF</i>	E I AA DP DV I GS L LD VL R AD FEL WER H VP T PG -	PPL DCP I TV L AGD ADP -	-	-	RAP -	-	RAEL AA	214	
<i>A.migulanus_GrsT</i>	I VL QDA EMMS I LL P RL RAD F SV CGS Y QYK -	N - DEPF ECPI T AF GG KND N -	-	-	GVT -	-	YQS LEA	212	
<i>B.licheniformis_BacT</i>	EL I NHEEL FEL F I P I L KSD F KM VEN Y I YQER -	NSK I DC D I TV L NG KEDA -	-	-	MS -	-	KED VSD	192	
<i>B.brevis_TycF</i>	DVL EN QEL L E L FLP I LRAD F QAV QT YEMN PDL PR QVP VN M T VL Y GT E DT -	-	-	-	IA -	-	L ED I WA	196	
<i>B.subtilis_SrTEII_2RON</i>	EL VEN KEV MS FFL PS FR SDY RAL EQ FEL Y DL -	AQ I QSP V HF NGL DDK -	-	-	KC -	-	IRDAEG	199	
<i>Rat_FAS_TEII</i>	HL I ED QD V L R MF I PL L KAD AGV VVK F I FD KPS K ALL SLD I TG F GL GS ED -	-	-	-	T -	-	I KD I EG	219	
<i>B.subtilis_SrTEII_1JMK</i>	- - - - - AV KH GL KQ K THAF Y S Y V NL I ST QGV KAD I DLL T SGAD -	-	-	-	FD I -	-	PEWL AS	188	
<i>E.coli_EntF_2ROQ</i>	AQQ G S T STE L FTT I EG NYA DA VRL L T TA - HS -	- VPF D Q KAT L F V A E RT -	-	-	LQ - E -	-	GMSPERA	189	
<i>B.subtilis_FenTE_2CB9</i>	- - - - - AV RET V M QK K RC Y Q E Y WA QL I NE GR I K S N I HF I EA G I QT -	-	-	-	ETSG -	-	AMVL QK	184	
<i>B.subtilis_Myc_TE</i>	L - - - - - LT I P S I KE K I RG Y L AY H N Q L I NS GT I N A N I H H L C DD -	-	-	-	M TERG -	-		183	
<i>B.licheniformis_BacC_TE</i>	VEKL - L SDYL - VDL V - - MK SYV - Y I QNT VTT GA I D G H I S Y I KS - SD -	-	-	-	NQR -	-	GEN NMM	182	
<i>B.brevis_TycC_TE</i>	DF QL YA AN - EF VK QSF VR KTV S Y V M F H N N L V NT G M T T A A I HL I Q SELE -	-	-	-	-	-	ADEE AP V AAK W N E SA	202	
<i>B.brevis_GrsB_TE</i>	DF E L Y FAN - EF VK QSF TR KMR K YM S F Y T Q L V NY G E V E AT I HL I Q AE FEE E K I D E N E K A D E E K T Y L E E K W N E KA	-	-	-	-	-		210	
<i>hFAS_TEI_1XKT</i>	SH QGL DR - Q EL - SFA AR SFY - Y K L RAA E QY T P K A Y K G N V M L L R A K T G -	-	-	-	-	-	-	-	
<i>S.venezuelae_PikTE_2HFJ</i>	- - - - - D A R L A M G R Y A R F L A G - P R P G R S S A P V L L V R A - S E P -	-	-	-	L GDW -	-	QE ERG D	250	
<i>S.fraidea_TyIGV</i>	- - - - - D H R L T A M G A Y H R L L D - W S P T P V R A P V L H R L A - A E P -	-	-	-	M GDW -	-	PPG DT G	245	
<i>Streptomyces_sp.CK4412_TmcB</i>	- - - - - S Q R I T A Q V W C L E L - R G - - W R P E G L T A P T L Y V R P - A Q -	-	-	-	P L V -	-	EQ E K P E	236	
<i>S.erythraea_DEBSTE_1MO2</i>	- - - - - D T R L T A L G A Y D R L T G Q - W R P R E T G L P T L L V S A - G E P -	-	-	-	M GPW -	-	P - DDS	241	
<i>A.erythreum_EryAll</i>	- - - - - D T R L T A M G A Y D R M L G G - W V P D D Q Q V A T L L V R A - V D P -	-	-	-	I A A W -	-	P - D D D	261	
<i>S.rochei_LkmAIII</i>	- - - - - D T R L T A L G A Y H R F T S T - W Q P R D L A V P T L L V R A - C E P -	-	-	-	L GEW -	-	PDG TS	262	
<i>M.griseorubida_MycAV</i>	- - - - - T A R L T G M A Y A R L L D R - C A V G E P D V P V L F V R P - G K L -	-	-	-	F V LD -	-	G Q P Q	276	
<i>S.parvulus_BorA6</i>	- - - - - E A W L T A M G H Y F G F D - - W T P C P V D V P V L H V R A - G D P -	-	-	-	M T G M -	-	P V E G R	270	
<i>S.noursei_NysK</i>	- - - - - S A M A H W F M A M T D I Q A P A P T A P T L L V R A - A R A -	-	-	-	L D G F -	-	R - L D	275	



	380	*390	400	410	420	430	
<i>A.mediterranei_RifR</i>	WEEHTT - GPADL RVL PGGHFFL VD -		QAAPMI IATMTEKL -	- - - - -	- - - - -	- - - - -	246
<i>A.noursei_NysE</i>	WRGHTT - GDFDL KVL PGGHFFFVSS -		EAPA I IDLLRAHL -	- - - - -	- - - - -	- - - - -	247
<i>S.fraidea_TyIO</i>	WREL TT - GAFA LRV PGPGH FFYLN D -		QREAVCRT I EETL -	- - - - -	- - - - -	- - - - -	245
<i>S.venezuelae_PikAV</i>	WRRHTS - GPFCL RAYSGGH FYLN ND -		QWHE I CND I SDHLL VT	- - - - -	- - - - -	- - - - -	254
<i>P.aeruginosa_PchC</i>	WSDASR - TPARL RRF PGGH FYL SE -		GRDAV I EHLL RRL -	- - - - -	- - - - -	- - - - -	239
<i>S.tendae_NikP2</i>	WAGATL - GRFTL RTFPGGH FYL GE -		HPDHVL HALR - EVL -	- - - - -	- - - - -	- - - - -	259
<i>S.erythraea_Eryorf5</i>	WLQHSV - I PGRT RTFPGGH FYL GE -		QVTEVAGAVR - RDL -	- - - - -	- - - - -	- - - - -	240
<i>Kutzneria.sp744_KtzF</i>	WRACTT - AEFTTRFF TG GH FYL VD -		SLAEVVG - - - - -	- - - - -	- - - - -	- - - - -	244
<i>A.migulanus_GrsT</i>	WREQTK - REF S VCMY PGD HFFL YE -		SKYEMI I EFMC KQLR -	- - - - -	- - - - -	- - - - -	249
<i>B/licheniformis_BacT</i>	WKHHHTS - GHFT TAYY FEGN HFFL HH -		HVEK I TEI I INHSLTA -	- - - - -	- - - - -	- - - - -	230
<i>B.brevis_TycF</i>	WRDYCQ - GACQFFF PVSGGH FFH I HH -		QTSP I LEI I IQNVLK E -	- - - - -	- - - - -	- - - - -	234
<i>B.subtilis_SrTEII_2RON</i>	WKKW - A - KD I FT HQFDGGH MFML LS -		QTEEEVAER I FAI L NQH -	- - - - -	- - - - -	- - - - -	237
<i>Rat_FAS_TEII</i>	WQDL TS - GKFDVHML PGD H FYL MKP -		DNENF I KNY I AKCLELS -	- - - - -	- - - - -	- - - - -	260



**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.sp*744 (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  $1\sigma$ ) and for form 2 (B.–  $2\text{Fo}-\text{Fc}$  refined density contoured at  $1\sigma$ ). C. Stereodiagram of  $\alpha\text{L1}$  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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