

Supplementary Materials

Supplementary Tables

Table S1. Primers used in this study

Name	Sequences (5'-3')
Characterization of the transposon insertional sites	
MY-002	GAGTCAGTGAGCGAGGAA
For <i>rifZ</i> knock-out and complementation:	
0655U-F	TCCCTCGGCCACGGGCTCCC
0655U-R	CAAATCGTCCCCGAATCCGG
0655D-F	GCCGGTGACGCGGCGGCGGG
0655D-R	ACATGTAGCCGACCGCGACC
0655C-F	ACCGTGAACATCGGCAGACC
0655C-R	CTACGAGAACACCTTCCACG
0655CH-UF	GGTTGGTCGCCACCACGGAG
0655CH-UR	TGCCCTGGCACAGTGCAACC
0655CH-DF	GTGGGCCGGCAGCTGTTCAT
0655CH-DR	GTCCTGCGTGGTCATGCGCT
RT-PCR analysis:	
rt-rifS-F	GATCGGTGCGGGGCTCATCG
rt-rifS-R	CGTCCTTCGCGCCGATCCTC
rt-rifT-F	ATCCTGTCGTCCACGCCCA
rt-rifT-R	CGCCGATTCGTAGGCCGCTT
rt-orf35-F	TGGAGGCCGCGATGAGTGTT
rt-orf35-R	TTATTCGGCGATCTTCGTGC
rt-orf0-F	AGCTACGAGCTGGTCCGCGA
rt-orf0-R	CCAGGCACTGGTGGATGCCG
rt-rifA-F	CTCGCAGCAGCGGGTCATCC
rt-rifA-R	CGGACAGCGACTCCGTGCTC
rt-rifB-F	CAGGGATGCCGGGCAAGACG
rt-rifB-R	CAGCACCGGATGCGCACTGA
rt-rifC-F	GATGGCGTCGGTCGCGTTGA
rt-rifC-R	GACGTTCACTCCGCCCTGCC
rt-rifD-F	CGCGGTATCGCGGTGAACCA
rt-rifD-R	TCCTCCTCGGGCGCTTCCTC
rt-rifE-F	TTCGCAGCGGCTCGGGATG
rt-rifE-R	CTTGGTCTTGTGCCCGCGCT
rt-rifF-F	GGCGTGGACCTCGAAACGCT
rt-rifF-R	GGTGATCGTGCGCACCTGCT
rt-rif1-F	ATGACGGAAAAAGCGGGCCT
rt-rif1-R	TCACGGCGCCTTCTTCCGCG
rt-rifG-F	GCGTCGAGACCCTGCTGCTC

rt-rifG-R	CGAGCCCGGTGATCGCCTTC
rt-rifH-F	GGCCGAGGTGATCGAGCTGC
rt-rifH-R	TCCACGATCGGCGGGAGGAG
rt-rifI-F	CCAACCTCGACGGCATCTTC
rt-rifI-R	CATGTGGCTGCCGTAATGGA
rt-rifK-F	ATGGGCGGGGACGAGGTGAA
rt-rifK-R	GGCGTCGGTGCGGTAGATCG
rt-rifL-F	GTTACGAAGGCGACCGGCA
rt-rifL-R	CGCTCACCTGGCGCGTGTAT
rt-rifM-F	GTCCGGCACGCGGTCATCTT
rt-rifM-R	CAGCTCGCCGATGTCTGCCG
rt-rifN-F	GTCGCCTCCGCGTCGAGTC
rt-rifN-R	ATCCGCCAGGAACCCCGACA
rt-rifO-F	GACGACACCACCCTGCGG
rt-rifO-R	GGTGCGGAACGTGTCCTCCG
rt-rif2-F	GCGTCCGCGAGCTCAGAGAG
rt-rif2-R	ACACCCGGTCCGAGGTAGGC
rt-rifP-F	CTGTGGAGCGCGGTCAGCTC
rt-rifP-R	TCGTTCATGGCGATGCCAGC
rt-rifQ-F	GGCGACGCGAAGTTCTCGAT
rt-rifQ-R	CATGCCGGTGAACAGCCAGT
rt-orf3-F	ACGTTTCGACGTCTTGCCGTA
rt-orf3-R	GGACCTGAGCATGACCGAGA
rt-orf4-F	TTGCGGATGGTGGTGTCCGC
rt-orf4-R	CTCTACGACGGCCGCAAGGC
rt-orf5-F	GGCCATGATCAGCGGCACCA
rt-orf5-R	CCGCTACGAAGACGTCCGCC
rt-orf6-F	GAAGCCGGCGTCGGGTTTCA
rt-orf6-R	GGCGGTGCTGACGAACTCCG
rt-orf7-F	ATCGCCGAAAGCGGCCTGAC
rt-orf7-R	GCTTCCTCGGGCGAGAGCAC
rt-orf8-F	ACGAGCGCGGCGACTTCTTC
rt-orf8-R	AGCGCGAGGCACTCCTCGTA
rt-orf9-F	GACGTCCGTCACCACCTGCC
rt-orf9-R	GCCCGACCTGATGGAGCAGC
rt-orf10-F	GTTCGCCACCTGGTTCGTCGG
rt-orf10-R	TCGCCCCGCACGGAAATCAC
rt-orf11-F	ACGACGTCATCGGGGTGGGT
rt-orf11-R	CGCTCCCGGTGGGTTTCGAG
rt-orf17-F	GGCCGAGCACCCTCCACG
rt-orf17-R	CCGGATCTGCCCGATGACGC
rt-orf18-F	CTTCTCGGTTCGAGGGCCTGC
rt-orf18-R	GCGGTGAGCTCGGCGATGTT
rt-orf19-F	CCGGGCTTTCGTGGCGCAG

rt-orf19-R	AACACCATGCACCAGCCCCG	
rt-orf20-F	TGCAGGTTGGTCAGCCGCAG	
rt-orf20-R	CAGCTTTCGGCCTGCGTGGA	
rt-rifR-F	GCCCCGGTGGTGTGCTCTCC	
rt-rifR-R	CGTTTCGAACGGGCACCGGA	
rt-orf13-F	TGCATCTCGTTGGCGACGCT	
rt-orf13-R	GCTTCCTCACCAACCCGCC	
rt-orf14-F	GTCCGCGACGCTCGAAGAGG	
rt-orf14-R	GTCCTTCGAACCGCTCCGCC	
rt-orf15A-F	GGCCTGTTCGGCCGGATGAC	
rt-orf15A-R	GGTCAGCGCCTTCTCCAGCG	
rt-orf15B-F	CCGCCGACATCTCGTCGTGG	
rt-orf15B-R	GCCTCGGTACCTCGAACGC	
rt-orf16-F	CTGCTGGCCGAGGCACTGG	
rt-orf16-R	ACCCGCGCTTTTCCTCCGAC	
rt-rifJ-F	CCAACCTGGGTGTGCTGGGC	
rt-rifJ-R	CAGGTGCAGCAACGCCTGTG	
rt-rifZ-F	ATGCAGCGGTCCTGTGACAC	
rt-rifZ-R	AGCGCCAGCACGACGTAACT	
rt-rpoB-F	TTCAGCGTCCGCGTCATCCC	
rt-rpoB-R	TGGATCAGCTCGCCGACGGT	
Co-transcription analysis:		Amplicon size (bp)
rifT-orf35-F	GATGCTCAAGGACTTCCTGG	
rifT-orf35-R	CACCTGCGTAACACTCATCG	333
orf35-0-F	GGAGAACACGCTCAAGATCC	
orf35-0-R	GACCTCGTCGAAGAACTTCC	756
orf0-rifA-F	GAGGACGTGAAGCTGGAGGG	
orf0-rifA-R	ATGTGCGAAAGGCTGTGGAA	1011
rifA-B-F	TACCTGCGCGTCGAACCTCT	
rifA-B-R	GCGCTGTTCTCCTGCTGAAG	383
rifB-C-F	TGGGCACAGCGAAGACGACC	
rifB-C-R	GCCCCGGAAGCTCTGCATCT	670
rifC-D-F	TCACCGTGGCCGAACAGGAA	
rifC-D-R	CCTCGTCAGCCACTGAACTC	561
rifD-E-F	TGCAACGGCGTGAACGACCA	
rifD-E-R	CACGAGCTGCCACAGGTCTT	326
rifF-G-F	CTACCTGACGGTCGAGAACG	
rifF-G-R	TCACCGCACAGTTCCTCCAC	543
rifH-I-F	GACGACGTGACGGAATGCCT	
rifH-I-R	TGCGGCACGGTACGAAGAT	375
rifI-K-F	GTGGTCGCCGACATCATCAT	
rifI-K-R	TCGCGCTCGAAGGAGTTCAC	417
rifK-L-F	ATCTACCGCACCGACGCCTT	
rifK-L-R	CTCGGTATGCAGGTAGTTGG	653

rifL-M-F	GAGCGCATCGGCGTCGAATA	
rifL-M-R	CTCATCACCGCGAAGCTGTC	307
rifM-N-F	CGTTCTTCGAGCACGTCATC	
rifM-N-R	TGTACAGCAGGTTCTCGCAG	682
rifN-O-F	ACGTCACCTACTACCGGCTG	
rifN-O-R	TGTAGCCGAGGAACTCGAGC	630
rifO-orf2-F	ATGTTGCGCACCGAGTGGTT	
rifO-orf2-R	CTGATGCCGTACGTGACGAT	609
orf2-rifP-F	GAACGTCGAGCTGCATCTGT	
orf2-rifP-R	CGAGATCTTCGGTGAGCTTG	562
rifP-Q-F	CGTTGACCTCCGGTGAGAAG	
rifP-Q-R	TCAGGTA CTGCCGTAGCAG	416
orf3-4-F	CGGTGTGTTCTTCCTGTTCTC	
orf3-4-R	GTCCCTTCGCGGTCGGTG	816
orf4-5-F	TGCTGGACGTCGGTGA ACTC	
orf4-5-R	GGAGAAGCTGCAGATCGACC	528
orf5-6-F	ACCTGGCGGACGTCTTCGTA	
orf5-6-R	AGTTCGTCGCCACGCACTGA	269
orf7-8-F	AACCTCTCCGCGCCAACG	
orf7-8-R	AGGCCGATTTCCGGGTCCA	725
orf9-10-F	TGGCCGATGTTACGTTGAC	
orf9-10-R	ACCGCTCCGGATACGAACTT	472
orf11-17-F	GTTCAGCTACTTCACCGTGC	
orf11-17-R	CTCGAACTCGATGCTCTGGT	462
orf17-18-F	GCAGATCCGGTCCATT CAGG	
orf17-18-R	ATCTCCGGCTGCACGATGAT	515
orf19-20-F	TCTGCCACTGGTAGCCGTTG	
orf19-20-R	CTCGCCGTGGTCAGCATCAT	493
rifR-orf13-F	GGAAGAAGAAGCTGGCCGAG	
rifR-orf13-R	CTGAGCGTCGCCAACGAGAT	344
orf14-15-F	AGGACGTCAGCCAGTACACC	
orf14-15-R	CATCGTCTCGTGGTTGCTGC	652
rifJ-Z-F	GGTCCACCTGAGCAACGTGT	
rifJ-Z-R	GACGTACCAGAACCGAGCCA	948
RifZ heterologous expression:		
RifZ-1EX-F	AAATCCATATGCAGCGGTCCTGTGACAC	
RifZ-2EX-F	AAATCCATATGTACGTCGGGTTGCTGATCAAG	
RifZ-3EX-F	AAATCCATATGGCGATGGCGGAAATCAGCAC	
RifZ-4EX-F	AAATCCATATGCACGACCTGGCGAAGGCTTC	
RifZ-5EX-F	AAATCCATATGGTGAACAAGCTCCGCGAGTAC	
RifZ-EX-R	AATTCCAAGCTTGCCTACGAGAACACCTTCCAC	
Cloning of promoters for preparation of FAM-probes:		Probe size (bp)
rifSP-F	ACTCGAACGGCACCTCATCGTC	
rifSP-R	CGCCGGTGGGAGCCATTACCAA	400

orf35P-F	AGACCTGGAGGCGTTGATGCT	
orf35P-R	CTCATCGCGGCCTCCACCTGT	335
rifKP-F	CGTGGTCGCCGACATCATCA	
rifKP-R	AGGGAATTCCGGTGCCTTTTCG	311
orf6P-F	CGACGCCGTGGTCATCACCT	
orf6P -R	ACCATGTCGTATCGATCCCTGC	315
orf11P -F	CCAGCAGCGCCGAATAGCCCTC	
orf11P -R	CTCCCAGTGGCCATAACGGCAAA	345
orf18P -F	GGAGATGAAGAAGCTGCTG	
orf18P -R	CCGCCATGGACATGACCGC	223
orf14P -F	CGGCGACCACTTCCGGTGAGAAC	
orf14P -R	CGGCTTCGTCACCAGTACCCCT	341
orf16P -F	CGCCGACCAGCTCGCCCTC	
orf16P -R	GCGTTTTCGGTCACTTTGGTCGT	391

Table S2. All the possible RifZ binding sites in *A. mediterranei* U32

Motif Sequence*	Motif start-end site		Strand	Genes	Genes sharing the promoter	Verified?
CTATC <u>TAATTGT</u> AGGATG	346101	346118	+	<i>AMED_0332</i>		No
CTACCCACG <u>TGGT</u> AAATG	626166	626183	+	<i>AMED_0613 (rifS)</i>		Yes
CTACCCAGAG <u>TGAGG</u> GATG	628058	628075	+	<i>AMED_0615</i>		Yes
CTACCAAGCCG <u>AGG</u> GATG	684103	684120	+	<i>AMED_0627 (rifK)</i>		Yes
CTACCCAG <u>TGAGG</u> GATG	697352	697369	+	<i>AMED_0639</i>	<i>AMED_0638</i>	Yes
CTATCAGGACG <u>AGG</u> GATG	701716	701733	+	<i>AMED_0643</i>	<i>AMED_0642</i>	Yes
CTATCAGGTCGGG <u>G</u> GATG	703784	703801	+	<i>AMED_0645</i>		No
CTATCAGAACG <u>AGG</u> GATG	710319	710336	+	<i>AMED_0650</i>		Yes
CTATCAGACCG <u>AGG</u> GATG	714461	714478	+	<i>AMED_0654</i>	<i>AMED_0653</i>	Yes
CTATC <u>TAATTGT</u> AGGATG	3466909	3466926	+	<i>AMED_3213</i>		No
CTATC <u>TAATTGT</u> AGGATG	5217291	5217308	+	<i>AMED_4744</i>		No
CTACCTCGGGG <u>G</u> GATG	6088879	6088896	+	<i>AMED_5484</i>	<i>AMED_5483</i>	No

*, The internal DNA sequences were underlined.

Supplementary Figures

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      RIFZ-1EX-F
1  ATG CAG CGG TCC TGT GAC ACC GCT GGT TTC CTC CGC CGC TCG GCC TCG TCG TGG TTC AAC GGG CGG CTC GAC ACG GGG TTG CAC TGT GCC 90
1  Met Gln Arg Ser Cys Asp Thr Ala Gly Phe Leu Arg Arg Ser Ala Ser Ser Trp Phe Asn Gly Arg Leu Asp Thr Gly Leu His Cys Ala 30

      RIFZ-2EX-F
91 AGG GCA GCG GCC ACG GGC AAC CGC CGG GAA TCC GAC GAA CTC AGC CAG CTG GCT CGG TTC TGG TAC GTC GGG TTG CTG ATC AAG GCC CGG 180
31 Arg Ala Ala Ala Thr Gly Asn Arg Arg Glu Ser Asp Glu Leu Ser Gln Leu Ala Arg Phe Trp Tyr Val Gly Leu Leu Ile Lys Ala Arg 60

      RIFZ-3EX-F
181 GAC CTC GAC GCG GGC CGC CGG GTG CTC GAG TCG GCG GAG GCG GAT CGG GCG GGG GCA GCC TCC GAC CGC ATG ACC GCC GTC GAG CAC AAC 270
61 Asp Leu Asp Ala Gly Arg Arg Val Leu Glu Ser Ala Glu Ala Asp Arg Arg Gly Ala Ala Ser Asp Arg Met Thr Ala Val Glu His Asn 90

      RIFZ-4EX-F
271 GTG CGG GGC AGC CTG CTG TTC GCC ATG GGC AGC GTC GAC GAC GCG ATG GCG GAA ATC AGC ACC GGG CTG CGG ATC GCC GAG CGC TGT GGT 360
91 Val Arg Gly Ser Leu Leu Phe Ala Met Gly Ser Val Asp Asp Ala Met Ala Glu Ile Ser Thr Gly Leu Arg Ile Ala Glu Arg Cys Gly 120

361 GAC CGG TCG CTG CGG CGC CCC AGT TAC GTC GTG CTG GCG CTG GGT GCC CTT CGC CGC GCG GAC ATG CGG GCG TGC CGC CAC TTC GTC GAC 450
121 Asp Arg Ser Leu Arg Pro Pro Ser Tyr Val Val Leu Ala Leu Gly Ala Leu Arg Arg Ala Asp Met Arg Ala Cys Arg His Phe Val Asp 150

451 AAG CTG TCG GAC GAA GCG CTG CTC GGT TAC TTC GGG CAG GCG CCG GGA GCG TGG GTG GCC GCG CAG GCC GTG GAA GCC CGC AGC GGC GTG 540
151 Lys Leu Ser Asp Glu Ala Leu Leu Gly Tyr Phe Gly Gln Ala Pro Gly Ala Trp Val Ala Ala Gln Ala Val Glu Ala Arg Ser Gly Val 180

541 GAG AGC GCG GCG AGC CTG ATC GCC GGG ATC GTG ACG AAC CCG GTG GTG CTC CGG CAG CTG CTG GTG TCG GAA CCG GCG GCG GCG TCC TGG 630
181 Glu Ser Ala Ala Ser Leu Ile Ala Gly Ile Val Thr Asn Pro Val Val Leu Arg Gln Leu Leu Val Ser Glu Pro Ala Ala Ala Ser Trp 210

      RIFZ-5EX-F
631 CTG GTC CGC GCG TGC GTC AAG CTC GGC GCG CAC GAC CTG GCG AAG GGT TCG GCG GCG GAG TCC GCC GCG CTG GCC GCC GAG CAG CCG GAG 720
211 Leu Val Arg Ala Cys Val Lys Leu Gly Ala His Asp Leu Ala Lys Ala Ser Ala Ala Glu Ser Ala Ala Leu Ala Ala Glu Gln Pro Glu 240

721 TTC AGC GTG ATC CGC GGC TCC GCC CTG CAC GCG GCG GGC CTG CTG GAG CAG GAC GCG GCC AAG CTC CAC GAG GCG GCC GAC ATC CAC CCC 810
241 Phe Ser Val Ile Arg Gly Ser Ala Leu His Ala Ala Gly Leu Leu Glu Gln Asp Ala Ala Lys Leu His Glu Ala Ala Asp Ile His Pro 270

811 GAC CGC TGG TGC GCG GCC TCG GCG CGC GAG GAC CTG GCG AGC CTG CTG ACC GTC CGG TGT TCC GAA CGC GAC CGG ACG ATC AGG ATC CTC 900
271 Asp Arg Trp Cys Ala Ala Ser Ala Arg Glu Asp Leu Ala Ser Leu Leu Thr Val Arg Cys Ser Glu Arg Asp Arg Thr Ile Arg Ile Leu 300

      RIFZ-6EX-F
901 GAG TCG GTC CTG GAC ACG TAC ACG TCG GTG AGC GCC ACC CGC GAC TCG GCC CGG GTG GTG AAC AAG CTC CGC GCG TAC GAG TAC GGC GTC CGC CGC 990
301 Glu Ser Val Leu Asp Thr Tyr Thr Ser Val Ser Ala Thr Arg Asp Ser Ala Arg Val Val Asn Lys Leu Arg Glu Tyr Gly Val Arg Arg 330

991 GGC ACG ACC CGG ACG GTC GAG TGC GAA GGC ACC GTC CCG CAC GGG CTG ACG AAC ACG GAG TTC GCG GTG GCG GAG CTG GTG AGC CAG GGC 1080
331 Gly Thr Thr Arg Thr Val Glu Cys Glu Gly Thr Val Pro His Gly Leu Thr Asn Thr Glu Phe Ala Val Ala Glu Leu Val Ser Gln Gly 360

1081 CAC ACC AAC AAC GAG GTG GGC CGG CAG CTG TTC ATC TCC CCG CAC ACG GTG GCG TTC CAC CTG AAG AAG GTG TTC CAG AAG ATG AAC CTC 1170
361 His Thr Asn Asn Glu Val Gly Arg Gln Leu Phe Ile Ser Arg His Thr Val Ala Phe His Leu Lys Lys Val Phe Gln Lys Met Asn Leu 390

      RIFZ-EX-R
1171 ACC TCC AGG GTC GAG CTG GCG GCG GCG TGG AAG GTG TTC TCG TAG 1215
391 Thr Ser Arg Val Glu Leu Ala Ala Ala Trp Lys Val Phe Ser End
  
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Figure S1. The schematic depiction of heterologous expression of truncated RifZ. The primers used for the expression of different truncated RifZ were labeled upon the nucleic acids sequence. And the predicted DNA binding domain of RifZ indicated by shaded boxes.

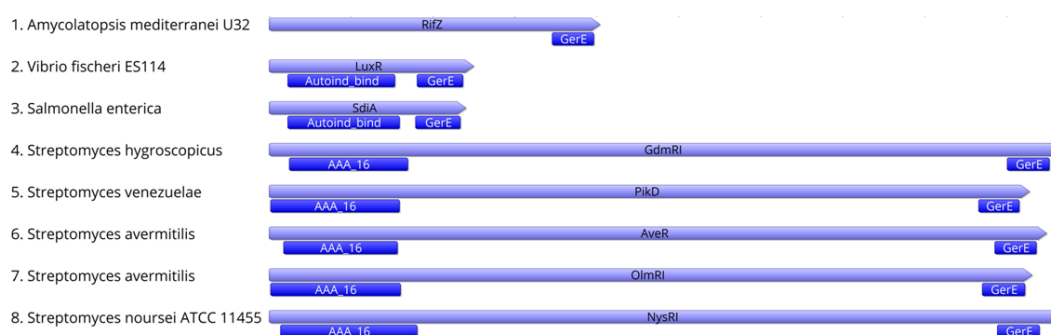


Figure S2. Conserved domains in RifZ and representatives of LuxR-family proteins. NCBI GI numbers for proteins used: RifZ (GI: 300782586), LuxR (GI: 59714108), SdiA (GI: 2992525), GdmRI (GI: 115299630), PikD (GI: 3800841), AveR (GI: 5921165), OlmRI (GI: 29606552) and NysRI (GI: 8050852). Analysis was performed using the Pfam v28.0 database. GerE(pfam00196): bacterial regulatory proteins, LuxR family; Autoind_bind(pfam03472): autoinducer binding domain; AAA_16(pfam13191): AAA ATPase domain.

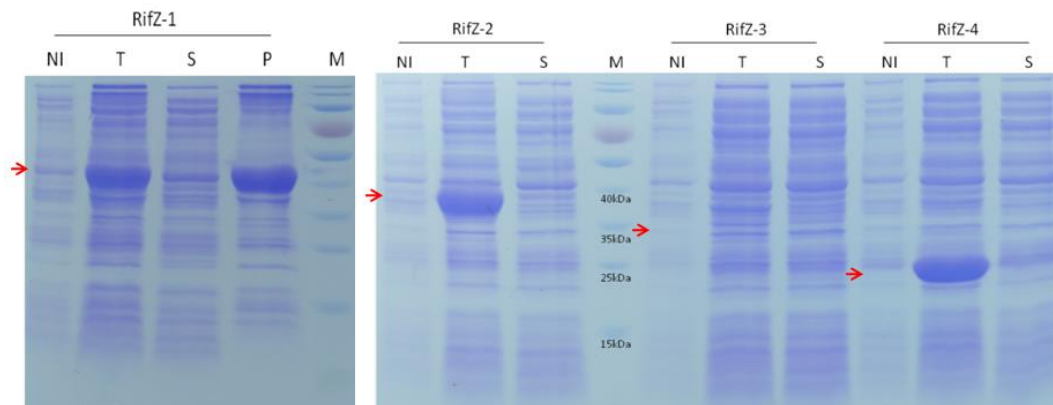


Figure S3. Heterologous expression of recombinant His-tagged RifZ-1, RifZ-2, RifZ-3 and RifZ-4. Protein samples were analyzed by 12.5% SDS-PAGE followed by coomassie blue staining. Symbols used: M, PageRuler™ Prestained Protein Ladder (Thermo Fisher Scientific); NI, total lysates of cells without IPTG induction; T, total lysates of cells after IPTG induction; S, supernatant of T; P, precipitate of T.

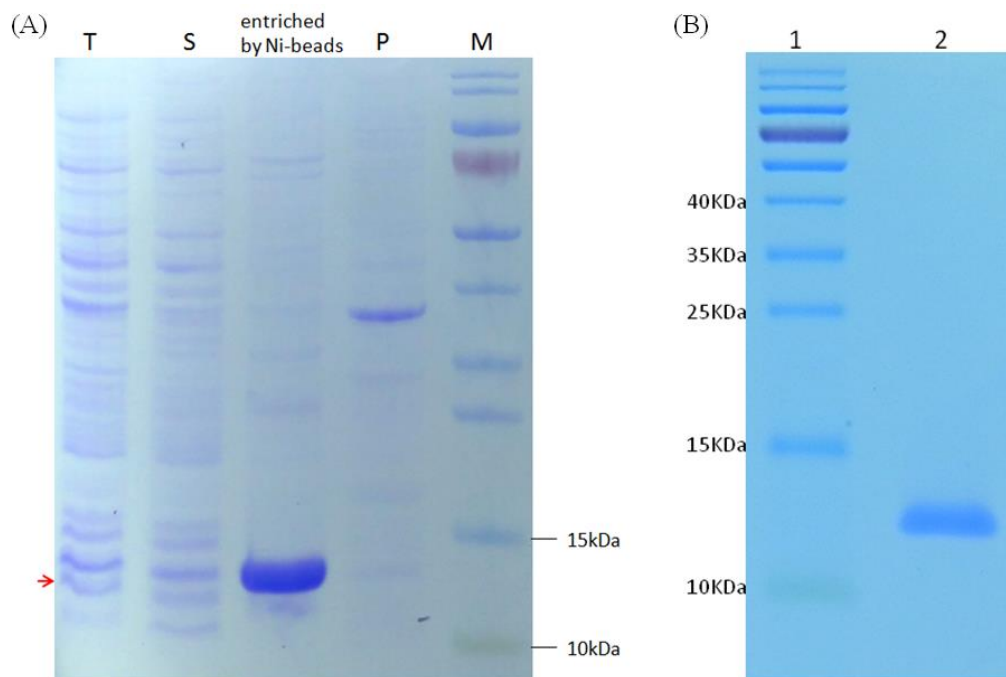


Figure S4. The expression and purification of recombinant His-tagged RifZ-5. (A) Protein samples were analyzed by 12.5% SDS-PAGE. M, PageRuler™ Prestained Protein Ladder (Thermo Fisher Scientific); T, total lysates of cells after IPTG induction; S, supernatant of T; P, precipitate of T. (B) Analysis of the purified recombinant His-tagged RifZ-5 by 15% SDS-PAGE followed by coomassie blue staining. Lane 1: PageRuler™ Prestained Protein Ladder (Thermo Fisher Scientific); Lane 2: 2 µg of the purified RifZ-5.

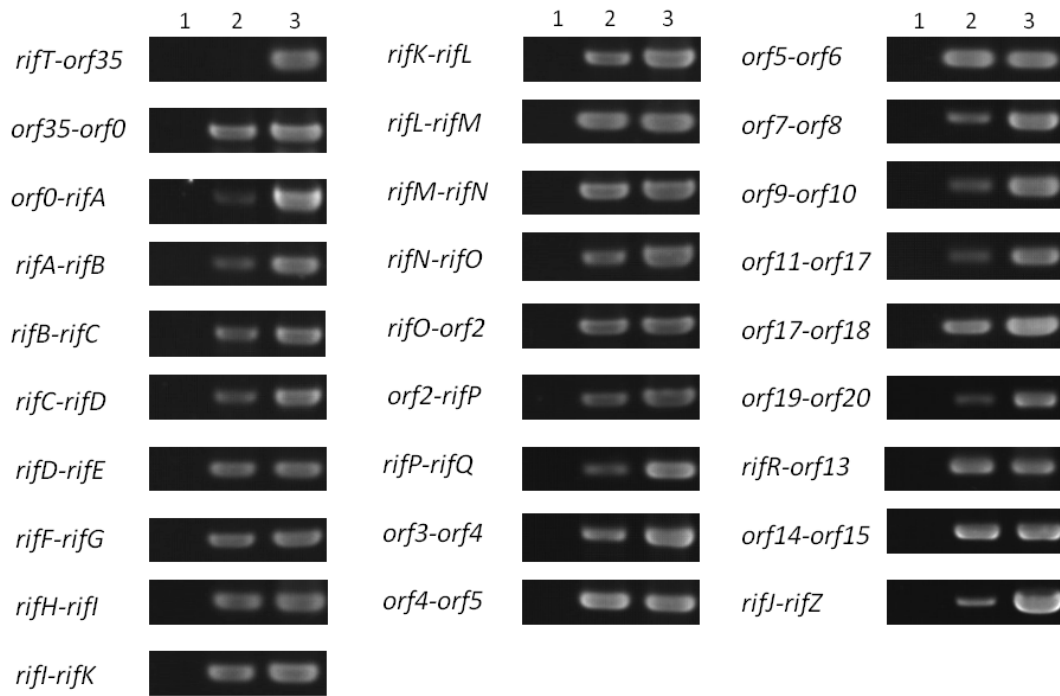
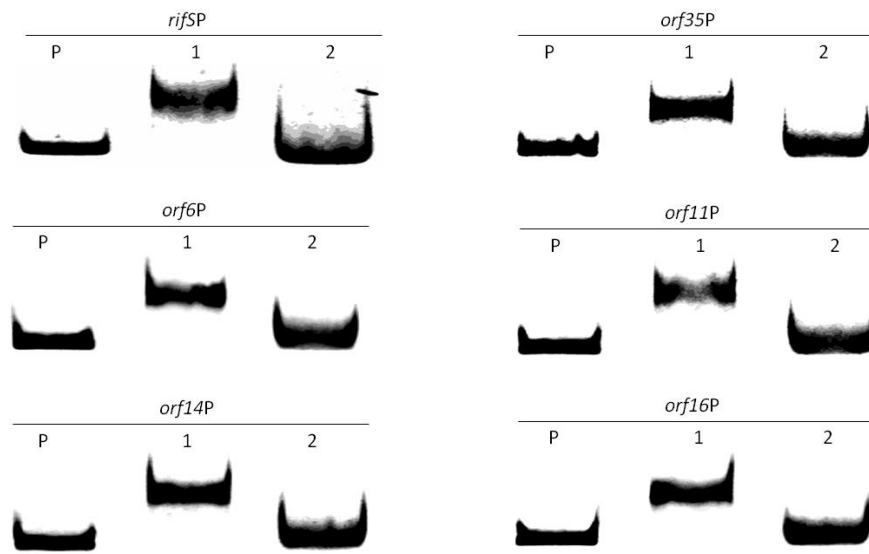


Figure S5. Co-transcription analysis of the *rif* genes with RT-PCR. For all reactions, 30 cycles of PCR amplification were applied, and details of the RT-PCR assay were described in the “Materials and Methods”. Lane 1: 20 ng total RNA of U32 as the template for PCR amplification (negative control); lane 2: 20 ng cDNA mixture as the template; lane 3: 20 ng gDNA of U32 as the template (positive control). Some genes that overlapped with their previous one were not analyzed here.

(A)

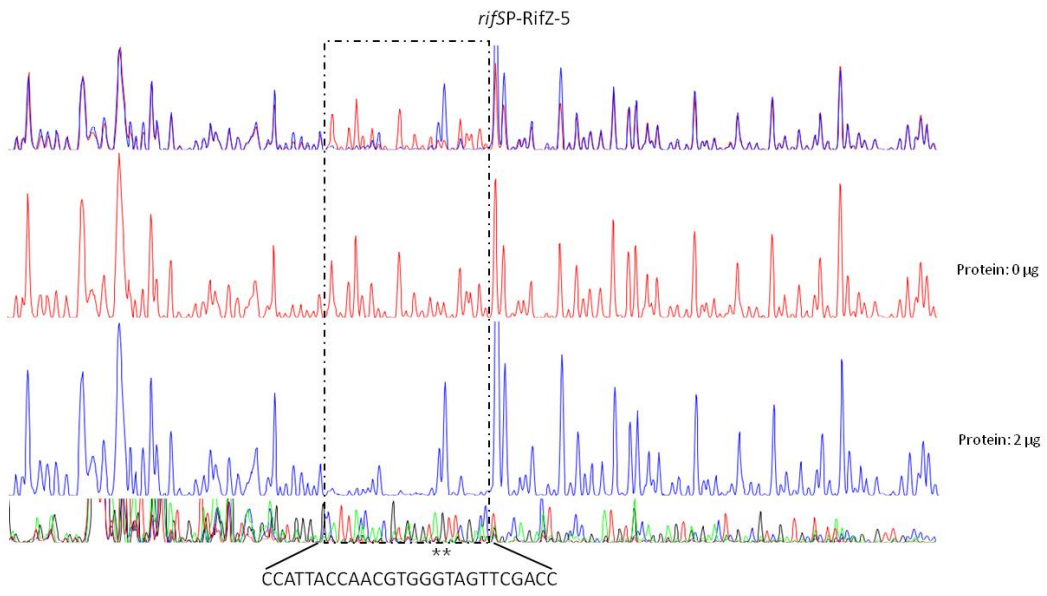


(B)

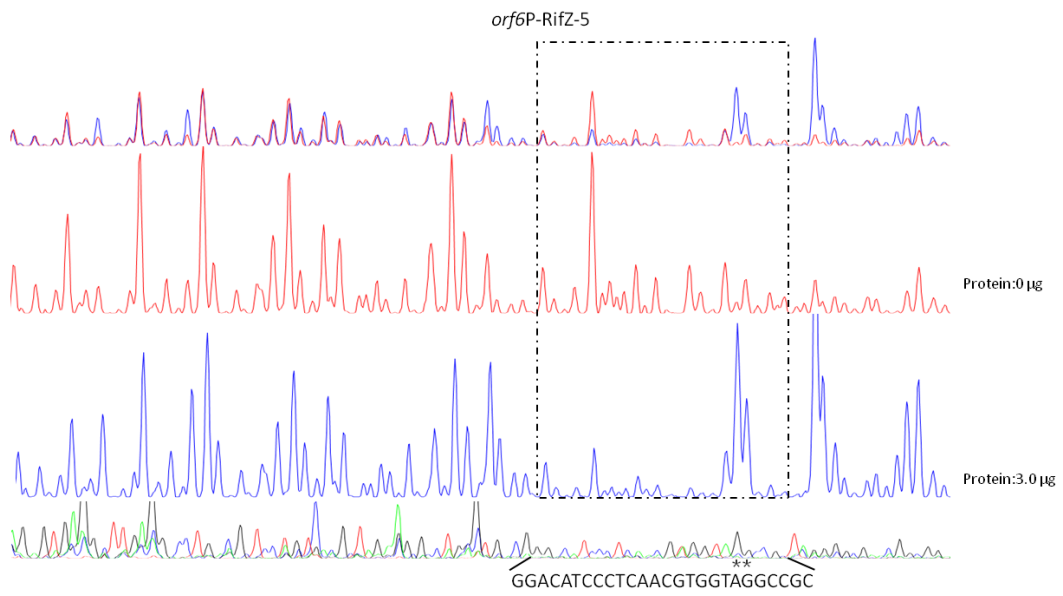


Figure S6. EMSA experiment employing purified His-tagged RifZ-5 with different probes. In each panel: lane P, 40ng FAM labeled probe without protein; lane 1, 40ng FAM labeled probe with 2 μg protein; lane 2, 40ng FAM labeled probe and excess (100 fold) unlabelled probe with 2 μg protein. And an excess of sheared salmon sperm DNA is included in every lane as internal control to avoid an unspecific binding. The assays were repeated three times. (A) Five promoter regions were specifically bound by RifZ; (B) EMSA result confirmed the specific binding of RifZ to the promoter regions of *rifK* and *orf18*.

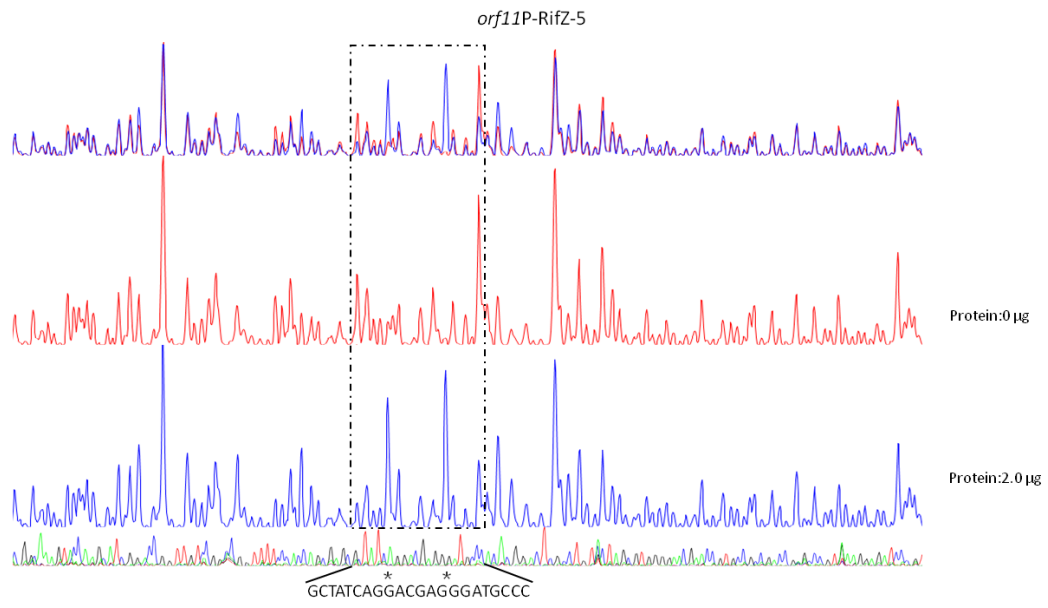
(A)



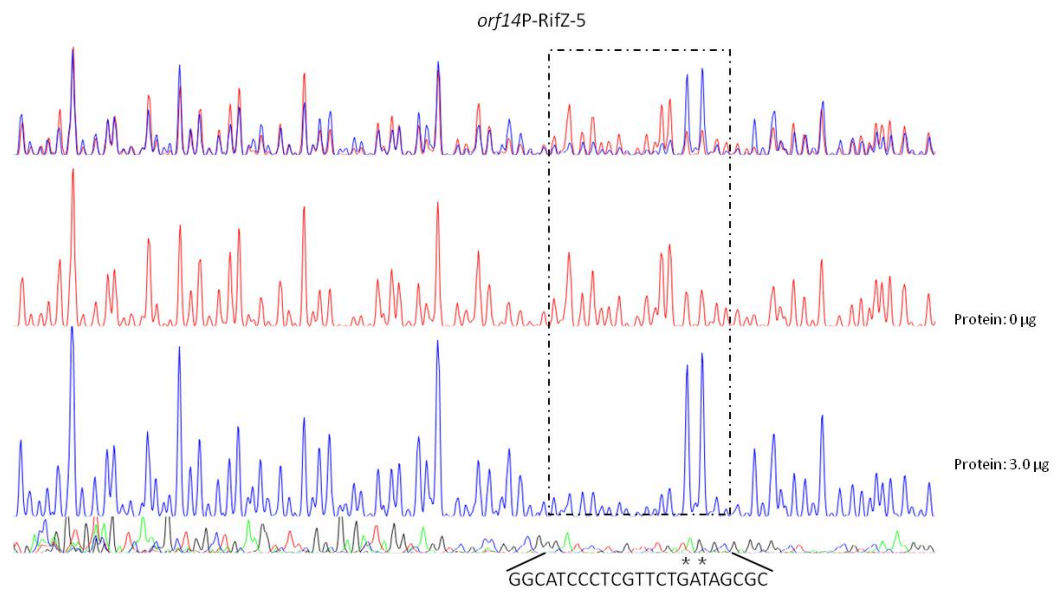
(B)



(C)



(D)



(E)

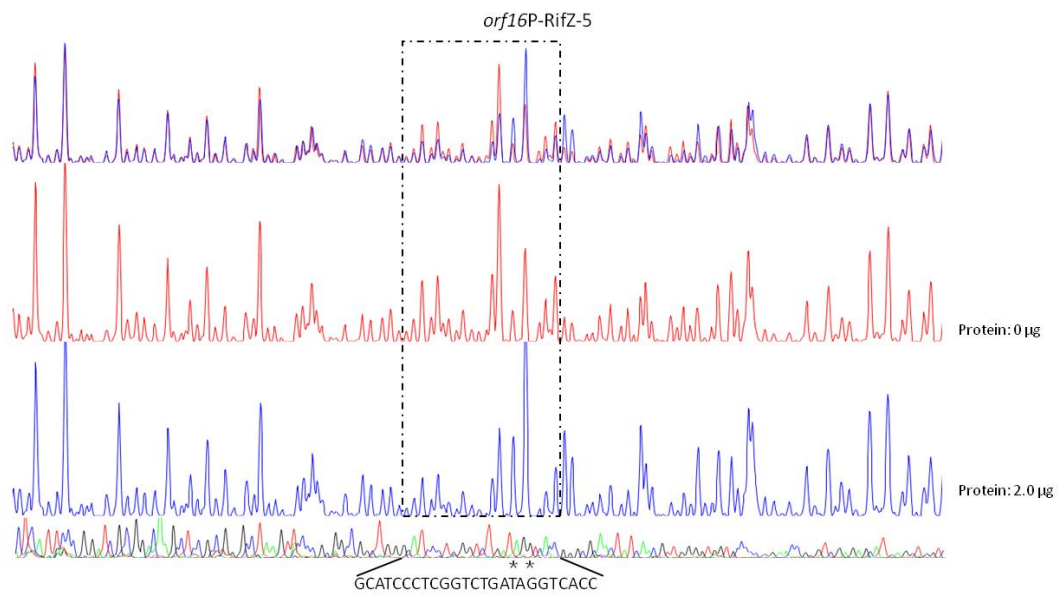


Figure S7. Characterization of the RifZ-protected DNA sequences in different promoter regions. The electropherogram of the control reaction (without RifZ-5, red line) and experimental reaction (with RifZ-5, blue line) were shown in the upper panel, and the lower panel showed the DNA sequencing results. Precise DNA sequences protected by RifZ-5 were indicated at the bottom, and hypersensitive sites were also indicated with asterisks.

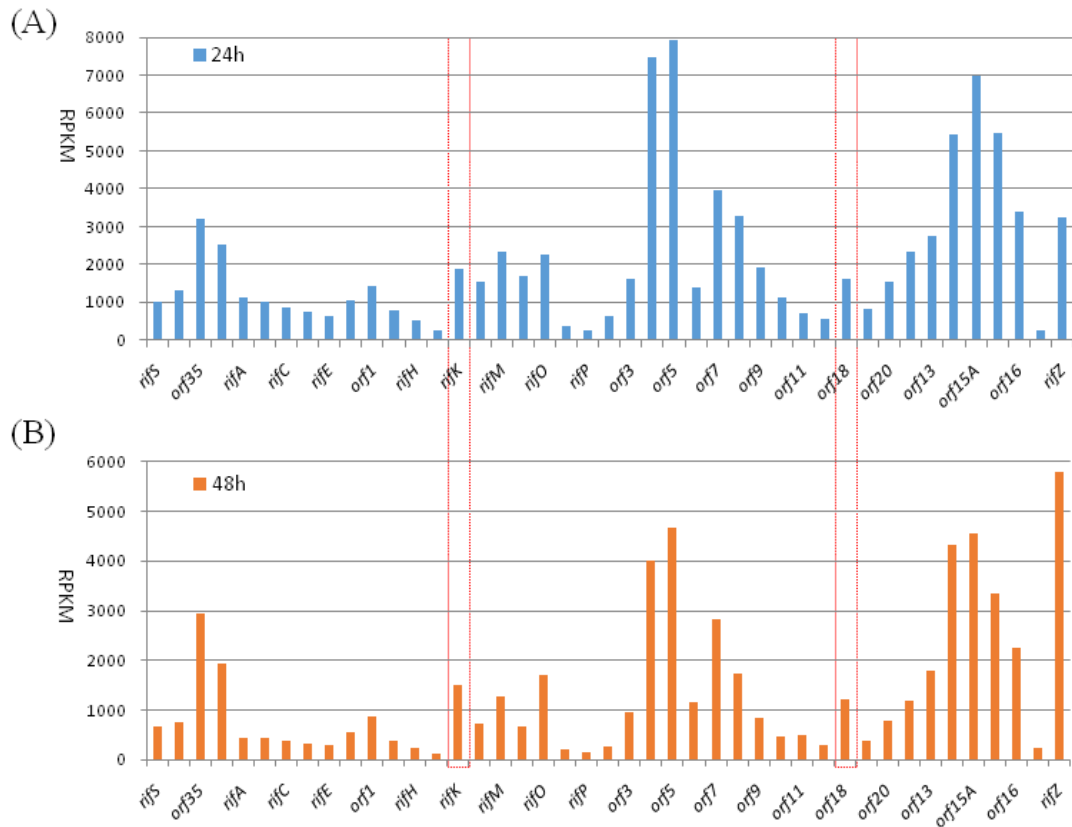


Figure S8. The transcriptional profiles of genes in *rif* cluster in *A. mediterranei* U32. The transcriptomic data were from Shao *et al.* (1). The transcriptional level was represented with the value of RPKM (Reads Per Kilo bases per Million reads). Through employing the RifZ binding motif as the query sequence in search of the whole U32 genome, six new RifZ targets were identified (Table S2), among which *rifK* and *orf18* (*AMED_0645*) within the *rif* cluster were boxed by dashed red line.

References

1. Shao ZH, Ren SX, Liu XQ, Xu J, Yan H, Zhao GP, Wang J. 2015. A preliminary study of the mechanism of nitrate-stimulated remarkable increase of rifamycin production in *Amycolatopsis mediterranei* U32 by RNA-seq. *Microb Cell Fact* **14**:75.