Table S1: RT-qPCR primers.

Gene	Primers [a]	Sequence (5′ → 3′)	Product location [b]	Product length (bp)	Annealing temperature (°C)	PCR efficiency [c]	
						Liquid media	Solid media
rpfA	rpfAF	GAGTCCGGCGGCAACTGGTC	+160 to +292	133	61	1.014 ± 0.022	1.020 ± 0.023
	rpfAR	GCTGGGACTTGCTCGCCTGG					
rpfB	SCO3150F	AGTACGGCGGTCTGGACTA	+1220 to +1330	111	60	N/A	N/A
	SCO3150R	CTTATCTGCTGGGAGCGACT					
rpfC	SCO3098F	GGCTCTACCAGTTCGACTCC	+236 to +296	61	57	1.045 ± 0.027	1.057 ± 0.029
	SCO3098R	TGCGCACGTAGAGCTTCTG					
rpfD	SCO0974F	GTTCGTACGGCTACCAGGTG	+1079 to +1168	90	60	1.041 ± 0.034	1.054 ± 0.038
	SCO0974R	GTCCGCTCTTCACGGAGAT					
rpfE	SCO7458F	ACCGGCAACGGCTACTAC	+199 to +345	147	66	N/A	N/A
	SCO7458R	CATCCCCTGCAGACGGG					

[a] Forward and reverse primers are denoted with letters F and R, respectively.

[b] Nucleotide sites are numbered relative to the start codon.

[c] Values are presented as mean ± standard deviation.

Table S2. Oligonucleotides used in this study

Primer name	Sequence (5' – 3')	Function
SCO0974 F	GGCACCGCCGGTATCAGCCGAACA	Disruption of *rpfD*
SCO0974 R2	GCTGCTGCTCGTCCTGCCGTCTTCC	Disruption of *rpfD*
SCO0974 up	AGTGCTCATTGGGTTGGCTC	*rpfD* complementation and disruption confirmation
SCO0974 down	CAGGTGATACCGGCGAGTG	*rpfD* complementation and disruption confirmation
SCO3097FWD	CAGCTCACCTCGCAGGCGTCGGTGAGGGGATCAACCATGATTCCGGGGATCCGTCGACC	*rpfA* knockout
SCO3097REV	CACGCACCGGGGCGGGAGTCACGGGACCGCCGCCGCTTATGTAGGCTGGAGCTGCTTC	*rpfA* and *rpfA/C* knockout
SCO3098FWD	CCGCGTGGTCCCCGTCGTCTTGTGAAAGGTCGTCGCATGATTCCGGGGATCCGTCGACC	*rpfC* and *rpfA/C* knockout
SCO3098REV	GGGACATACGGTCTCTAACCGTGACGCGAACTCCCTCTATGTAGGCTGGAGCTGCTTC	*rpfC* knockout
SCO3098 up	CGTGATCTGCGCGGCATGAAC	*rpfC* knockout confirmation
SCO3098 int	GCAGGCCGCCGTAGTACC	*rpfC* knockout confirmation
SCO3150FWD	CTGGGGGCCCGATCGGGACCCCTGGAGCGTGTGGGCGTGATTCCGGGGATCCGTCGACC	*rpfB* knockout
SCO3150REV	GCTCACCCCGCAAGAGTAGCCGGGGGCCGGGCGGCCTCATGTAGGCTGGAGCTGCTTC	*rpfB* knockout
3150 up	TTCAAGAACGCCCAGAACCTG	*rpfB* knockout confirmation
3150 down	ACCGTGTTGGCGTCGATGAC	*rpfB* knockout confirmation
3150 internal	AGCAGTTCACTCACGTCGTC	*rpfB* knockout confirmation
SCO7458FWD	GGGCGACCGGTGGTCACCTGGTGTAACAGTCTTCTTATGATTCCGGGGATCCGTCGACC	*rpfE* knockout
SCO7458REV	GTCAACCCGGCGCGGCCGGGGCCCGGTTTGGCACCGTCATGTAGGCTGGAGCTGCTTC	*rpfE* knockout
7458 up	ACAAGGAGGCGGTCCACG	*rpfE* complementation and knockout confirmation
7458 down	GTTGGTCGGCCTACTCGG	*rpfE* complementation and knockout confirmation
7458 internal	CGACATCCCCTGCAGACG	*rpfE* knockout confirmation
SCO0974PP 5'	CAGTACCATATGGCCGACGCGCGACCTGGGAC	Overexpression of RpfD
SCO0974PP 3'	CAGTACGGATCCTCAGATCCTGACGCCGCCGGC	Overexpression of RpfD
SCO3097PP 5'	CAGTACCATATGGCCACCGCGTCCGAGTGGGAC	Overexpression of RpfA
SCO3097PP 3'	CGAAGTGGATCCTTACTTCAGGTGCAGCTGCTG	Overexpression of RpfA
SCO3098PP 5'	CAGTACCATATGGCCGACTCCACGAACTGGGAC	Overexpression of RpfC
SCO3098PP 3'	CAGTACGGATCCCTACTTTTCGCCCGATTCGCC	Overexpression of RpfC
SCO3150ΔDUFPP5'	CAGTACCATATGGCGACCGGCTTCCCGC	Overexpression of RpfBΔDUF348
SCO3150PP 5'	CAGTACCATATGGCCAAGGACAAGGCGGTCGAG	Overexpression of RpfB
SCO3150PP 3'	CAGTACGGATCCTCATTCCCGCAGCCGGGCGCC	Overexpression of RpfB
SCO7458PP 5'	CGAAGTCATATGGCGCCCTCGGCGCC	Overexpression of RpfE
SCO7458PP 3'	CGAAGTGGATCCTCAGGCGCAGCCCC	Overexpression of RpfE
PrpfA-F	GCACTGGATCC**A**AAAGTAGAGGGAGTTCGCG	Cloning of *rpfA* promoter
PrpfA-R	GCACTGGTACCGAGCGGTCATGACCGTAGAC	Cloning of *rpfA* promoter
SCO3152 pFlux Fwd	TGATGAGGATCCTCTGAGGGGATCCATAGGGC	Cloning of *SCO3152* promoter
SCO3152 pFlux Rev	TGATGAGGTACCCACGGCGCTCTCACTCAT	Cloning of *SCO3152* promoter
SCO3098 pFlux Fwd	TGATGAGGATCCGCATCCACTACTGCCTCGG	Cloning of *rpfC* promoter
SCO3098 pFlux Rev	AAGAAGGGTACCGACGACCGTTCCCGGAGAG	Cloning of *rpfC* promoter
rpfD pFlux Fwd	TGATGAGGATCCCCTAGTGACCTTGGTGTCCG	Cloning of *rpfD* promoter
rpfD pFlux Rev	AAGAAGGGTACCCGGTTGATCCTGTTCGGCTG	Cloning of *rpfD* promoter
rpfE pFlux Fwd	TGATGAGGATCCCCGTGTCCATCGTCTCCAC	Cloning of *rpfE* promoter
rpfE pFlux Rev	TGATGAGGTACCAAGAAGACTGTTACAGGTGAC	Cloning of *rpfE* promoter
ermEF-B	GCACTGGATCCAGCCCGACCCGAGCACGCGC	Cloning of *ermE** promoter
ermER-K	GCACTGGTACCGATCCTACCAACCGGCACGA	Cloning of *ermE** promoter

Table S3. Conditions for Rpf protein over expression and purification.

Protein	Size (kDa)*	[IPTG] (mM)	Induction time (h)	Induction temperature (˚C)	[Imidazole] (mM) for elution
RpfA	21.7	1	2.5	30	250
RpfB	36.6	0.25	14	16	250
RpfBΔDUF348	21.5	0.25	14	16	250
RpfC	40.0	1	14	16	150
RpfD	42.6	1	14	16	70
RpfE	10.2	1	2.5	30	250
sIHF	13.3	1	14	16	250

*Calculated excluding the signal peptide but including the 6 × His tag from pET15b.

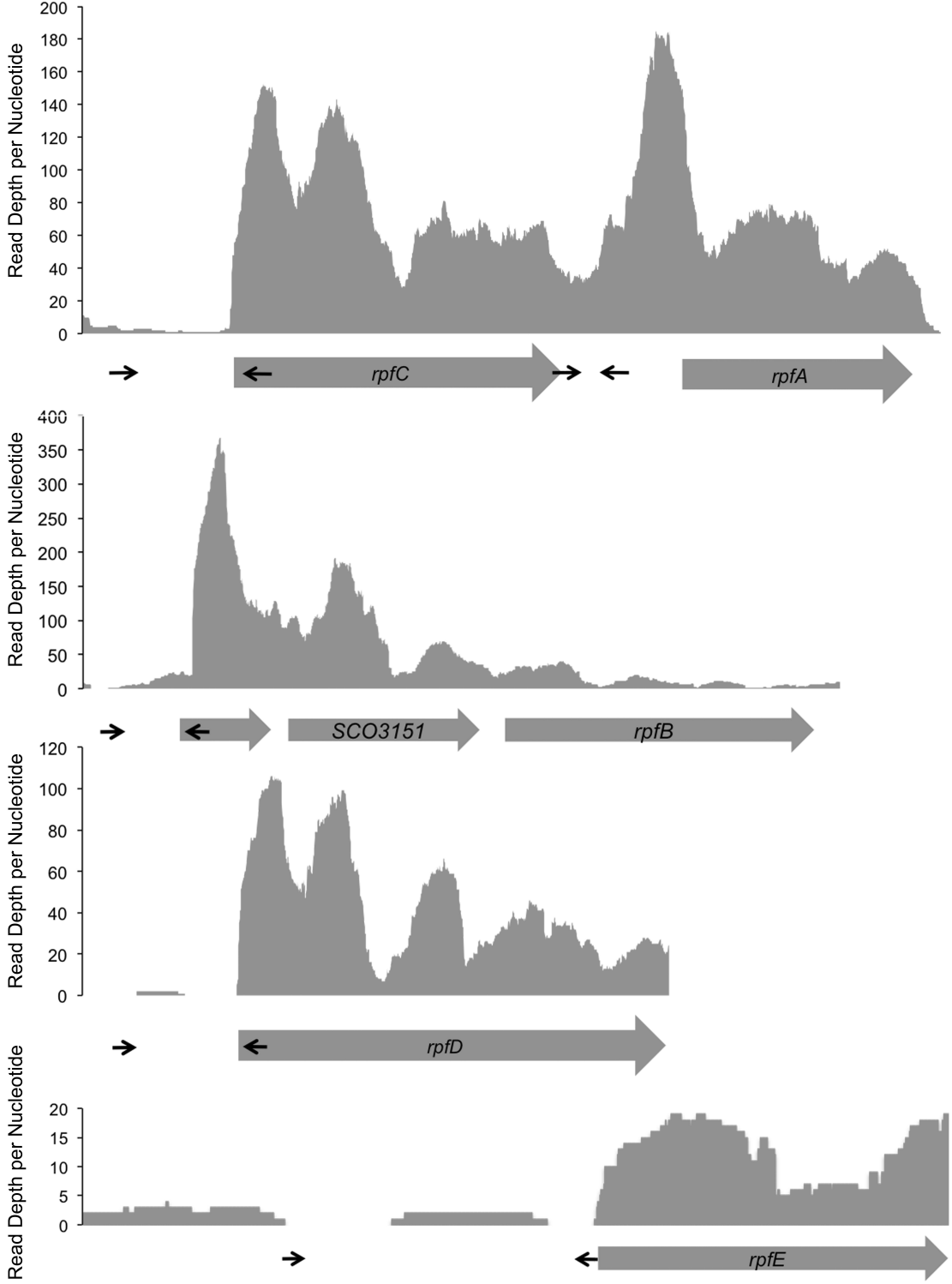


Figure S1. Primer location for luciferase assays. *Primers for luciferase assays were positioned to amplify the promoter region for each rpf gene, based on previously published RNA sequencing data (Moody et al., 2013). Graphs show the number of reads at each nucleotide for both the protein-coding sequence and region upstream of each rpf. Protein-coding sequences are indicated with grey arrows. Primer locations are indicated with black arrows.*

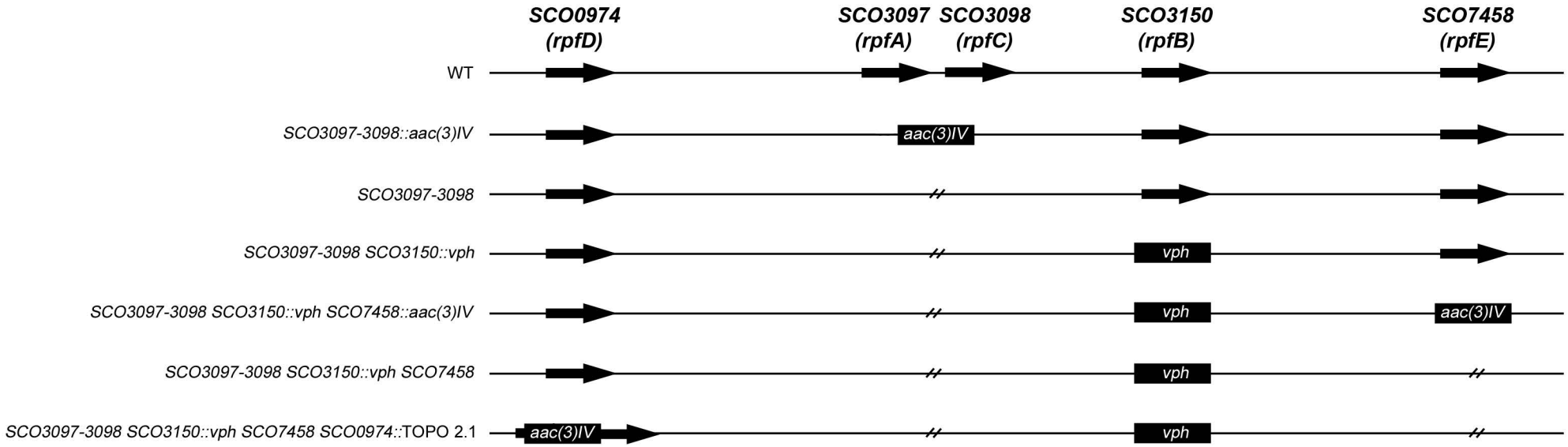
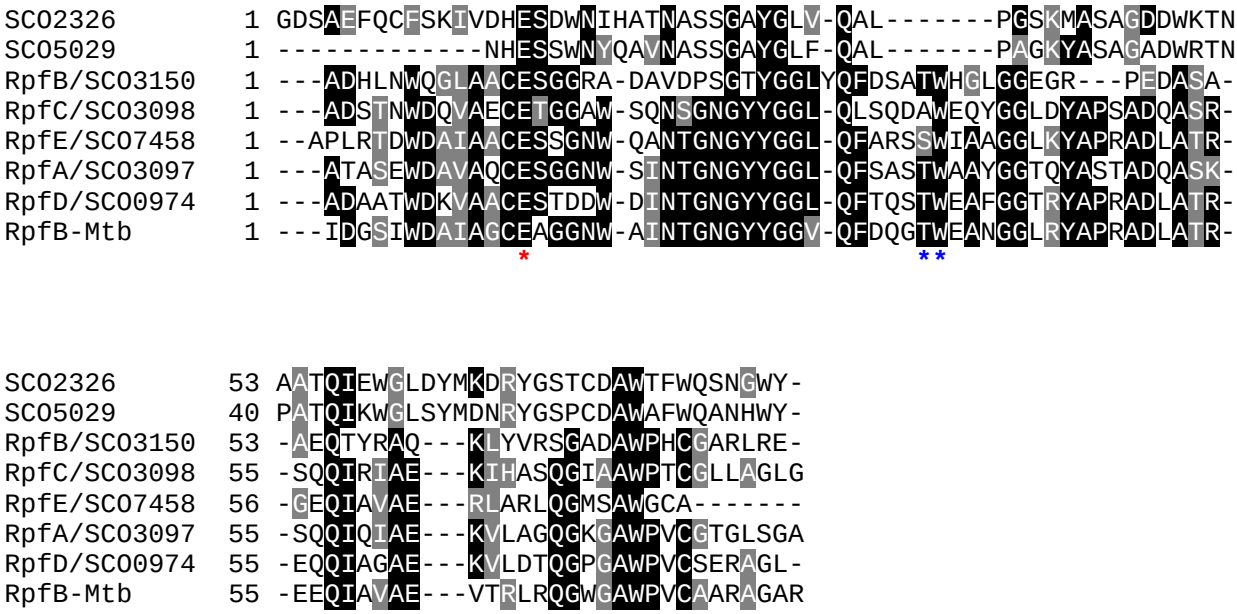


Figure S2 . Construction of multiple *rpf* mutants. *rpf* genes were replaced with antibiotic resistance cassettes in the order shown. Double diagonal lines represent 'scar' sequences left after removal of the antibiotic resistance cassettes. Abbreviations: *aac(3)IV,* apramycin resistance cassette; *vph*, viomycin resistance cassette.



* Active site residues
* Key cleft residues

Figure S3. Alignment of distantly related Rpf-like domains from SCO2326 and SCO5029 with RpfB from *Mycobacterium tuberculosis* and the more closely related Rpfs from *S. coelicolor*. Identical amino acid residues are highlighted in black, while similar residues indicated in grey. The red asterisk marks the key catalytic residue, while the blue asterisks mark important substrate-binding residues.

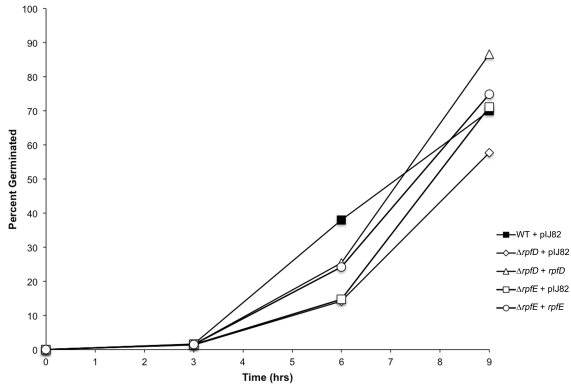


Figure S4. Partial complementation of Δ*rpfD* and Δ*rpfE* confirmed by examining germination profiles. Spores of complemented and vector alone-containing *rpf* mutants were plated on MS agar overlaid with cellophane discs. Spores were monitored for the presence or absence of germ tubes at the indicated time points. Data are representative of three independent trials (N > 100 per timepoint per strain). WT, wild type.

Figure S5. Alignment of the Rpf domain of RpfC orthologues from diverse *Streptomyces* species. The key catalytic residue is indicated with a red asterisk, while important substrate-binding residues are marked with blue asterisks.