Supplementary Material-

RedJ Variant	Decanoyl-RedQ	Acetyl-RedQ
Wild-Type	100	100
L150N	ND*	ND
V158T	39.8±1.6	49±5.3
L162N	ND	ND
L187N	ND	ND
I215N	ND	ND

Table S1. Activity of $RedJ_{FL}$ variants with substitutions in the hydrophobic pocket. The activities were determined as in Figure S5 using full-length RedJ. Errors represent the spread of results in three replicate experiments.
*No detectable activity.

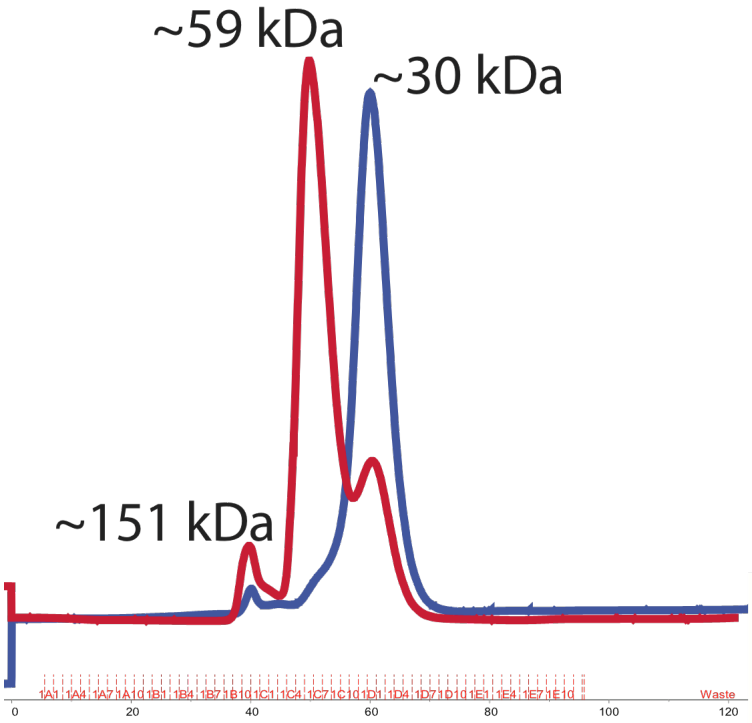

Figure S1. Elution chromatograms of $RedJ_{FL}$ (blue) and $RedJ_T$ (red) from an S100 gel filtration column. The apparent molecular weights indicate that $RedJ_{FL}$ is a monomer (apparent molecular weight 30 kDa) while the majority of $RedJ_T$ is a dimer (apparent molecular weight 59 kDa).

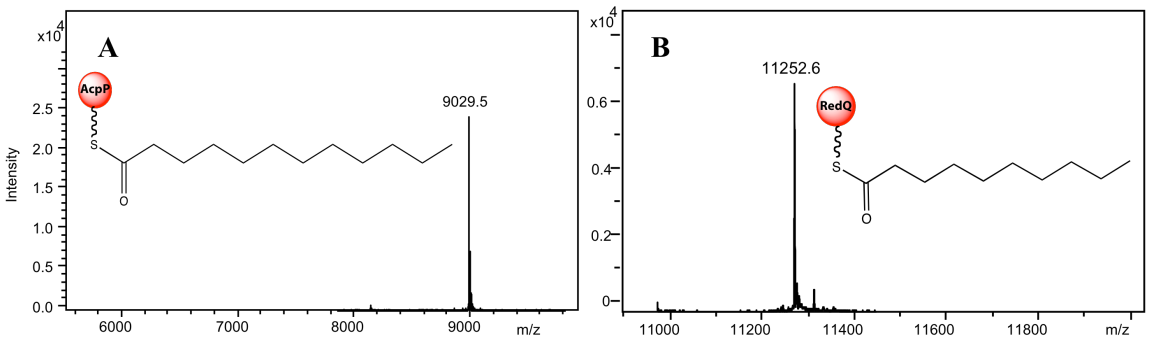

Figure S2. Deconvoluted ESI mass spectrum of products of Sfp reaction with (A): apo-AcpP and dodecanoyl-CoA, (B) apo-RedQ and decanoyl-CoA.

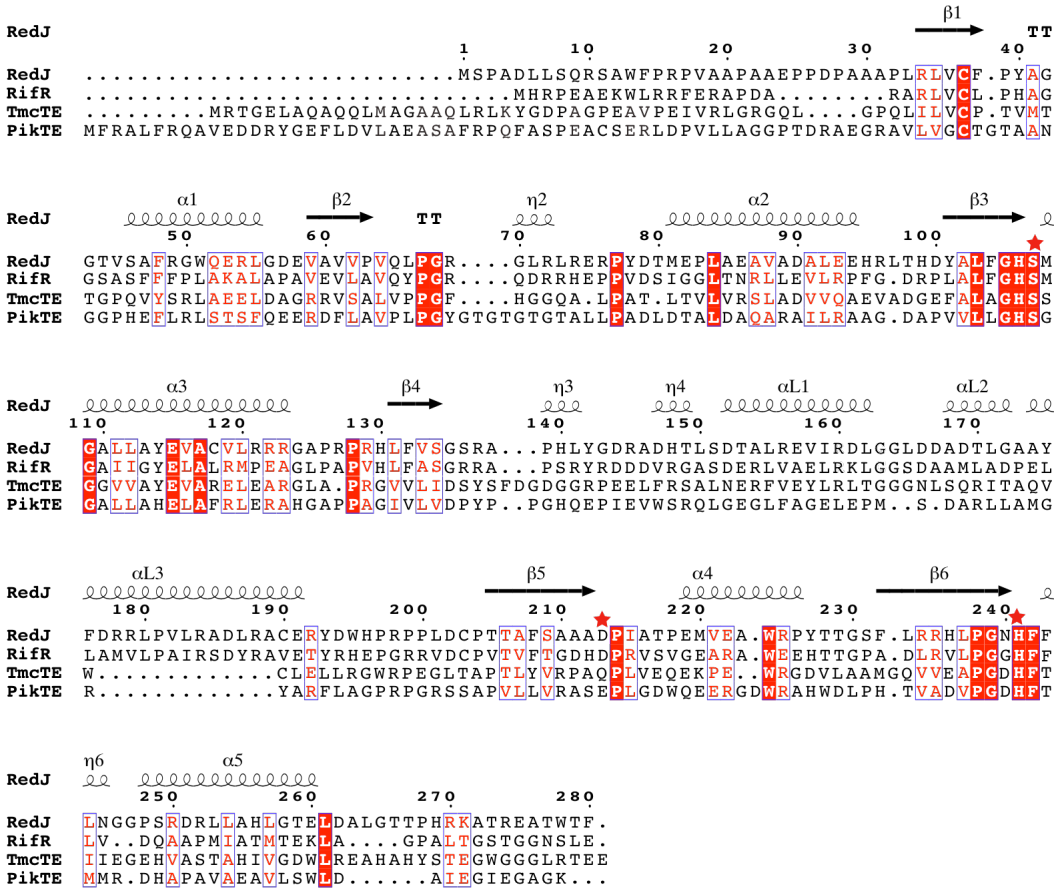

Figure S3. Structure-based multiple sequence alignment of RedJ (accession NP_630015) with the TE II from the rifamycin pathway in *Amycolatopsis mediterranei* (RifR, 38% sequence identity to RedJ, accession AAG52991), the TE I from the tautomycetin pathway of *Streptomyces griseochromogenes* (TmcTE, 14% identity to RedJ, accession ABI94380), and the TE I from the pikromycin pathway of *Streptomyces venezuelae* (PikTE, 15% identity to RedJ, accession AAC69332). RedJ and RifR catalytic triad residues are designated with red stars. The Asp residue of the catalytic triad in TmcTE and PikTE is analogous to Gly135 in RedJ. Sequence alignment was generated with MUSCLE (Edgar, R.C., Nucleic Acids Res, 2004. 32: p. 1792-7). Invariant residues are in white with a red background, other conserved sites are in red. No conservation is highlighted in regions that lack structure similarity: the N-termini and the lid (RedJ residues 144-192). ESPript (Gouet, P. *et al*., Bioinformatics, 1999. 15: 305-8) was used to generate secondary structure annotations for RedJ.

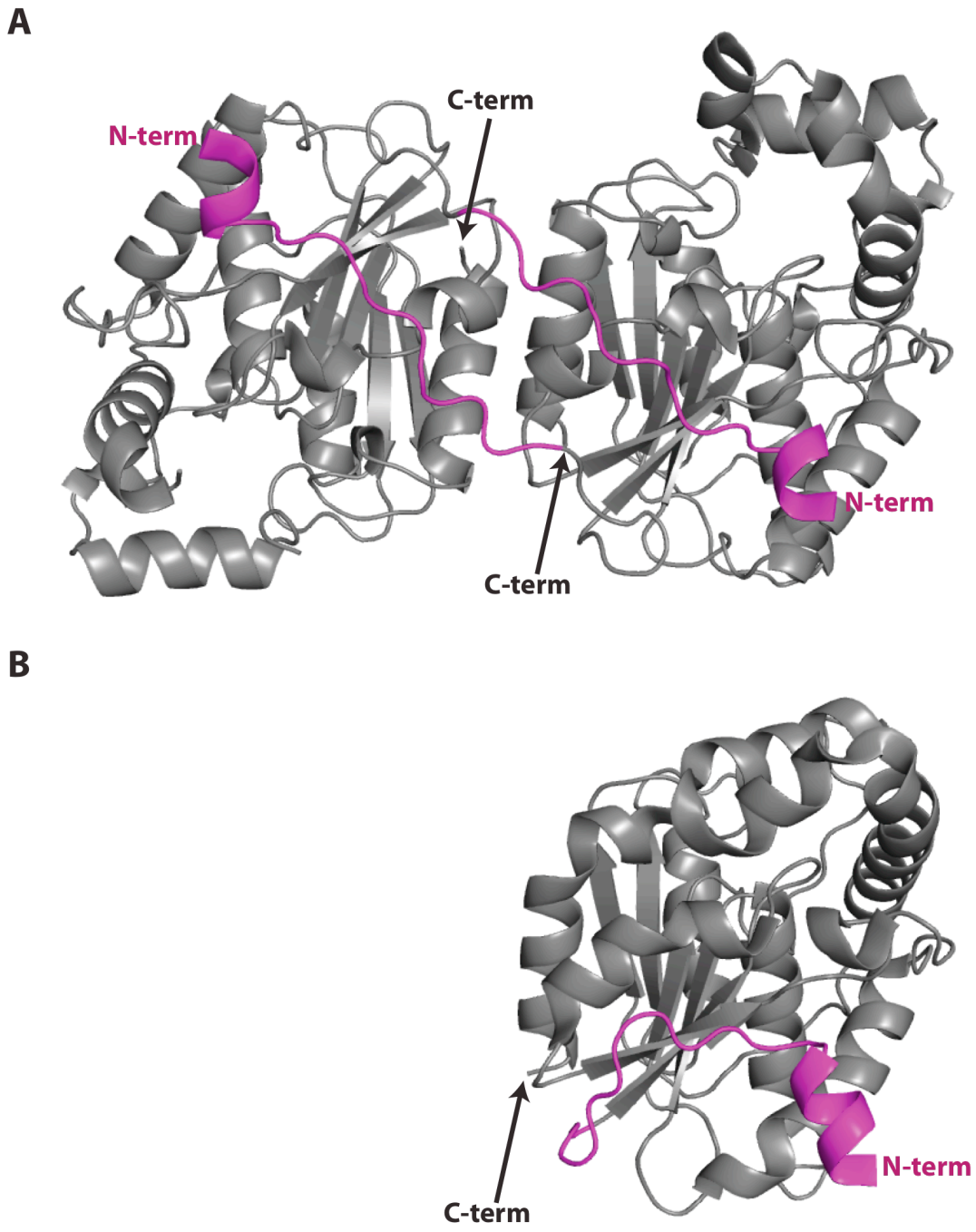

Figure S4. A. Domain-swapped RedJ$_T$ dimer. The domain-swapped N-terminus (residues 9-24) is shown in magenta. B. RifR structure (Claxton et al., J Biol Chem, 2009. 284: p. 5021-5029). RifR residues 2-20, homologous to residues 6-24 of RedJ, are shown in magenta.

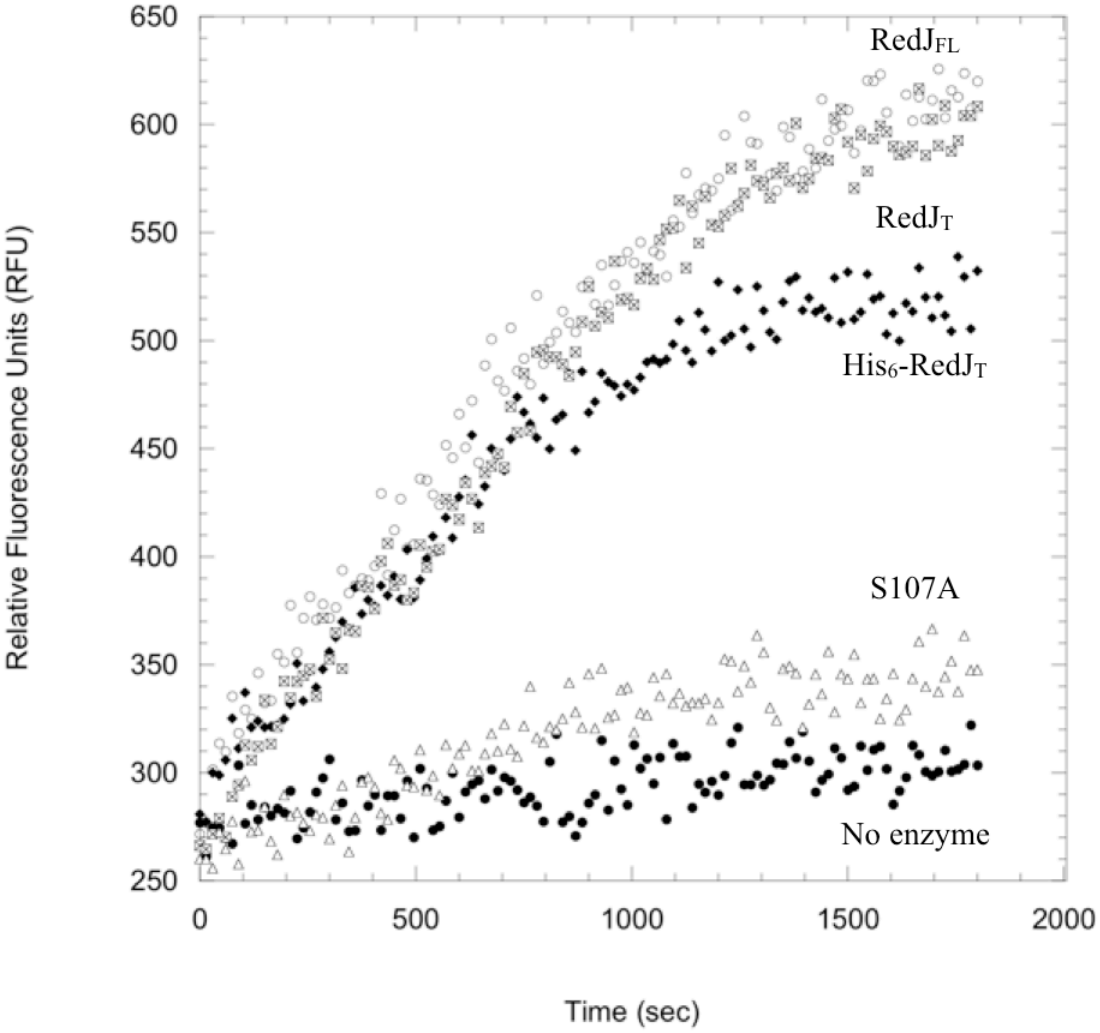

Figure S5. Catalytic activity of full-length and truncated RedJ. Example traces are shown for $RedJ_{FL}$ (○), His_6-$RedJ_T$ (◆), TEV-cleaved $RedJ_T$ (☒), no RedJ (●), and TEV-cleaved $RedJ_T$ S107A (△), in which the catalytic serine was substituted with an alanine. The activities were determined using ThioGlo 1 (Calbiochem). Reducing agents were removed from all recombinant RedJ solutions by exchange into buffer A. The 110 µL reaction mixture (10µM decanoyl-RedQ, 10 µM ThioGlo reagent, and 0.1 M potassium phosphate buffer pH 7.4) was pre-incubated for 5 min at room temperature in a black 96-well Greiner plate. Recombinant RedJ (1 µM) was added to initiate the reaction and fluorescence measurements (λ_{ex}=379 nm and λ_{em}=513 nm) were taken every 15 sec for 30 min at 37°C. Initial slopes of each line were determined to compare levels of activity. $RedJ_{FL}$ and TEV-cleaved $RedJ_T$ have similar activity. His_6-RedJT has approximately 70% of the activity of $RedJ_{FL}$.

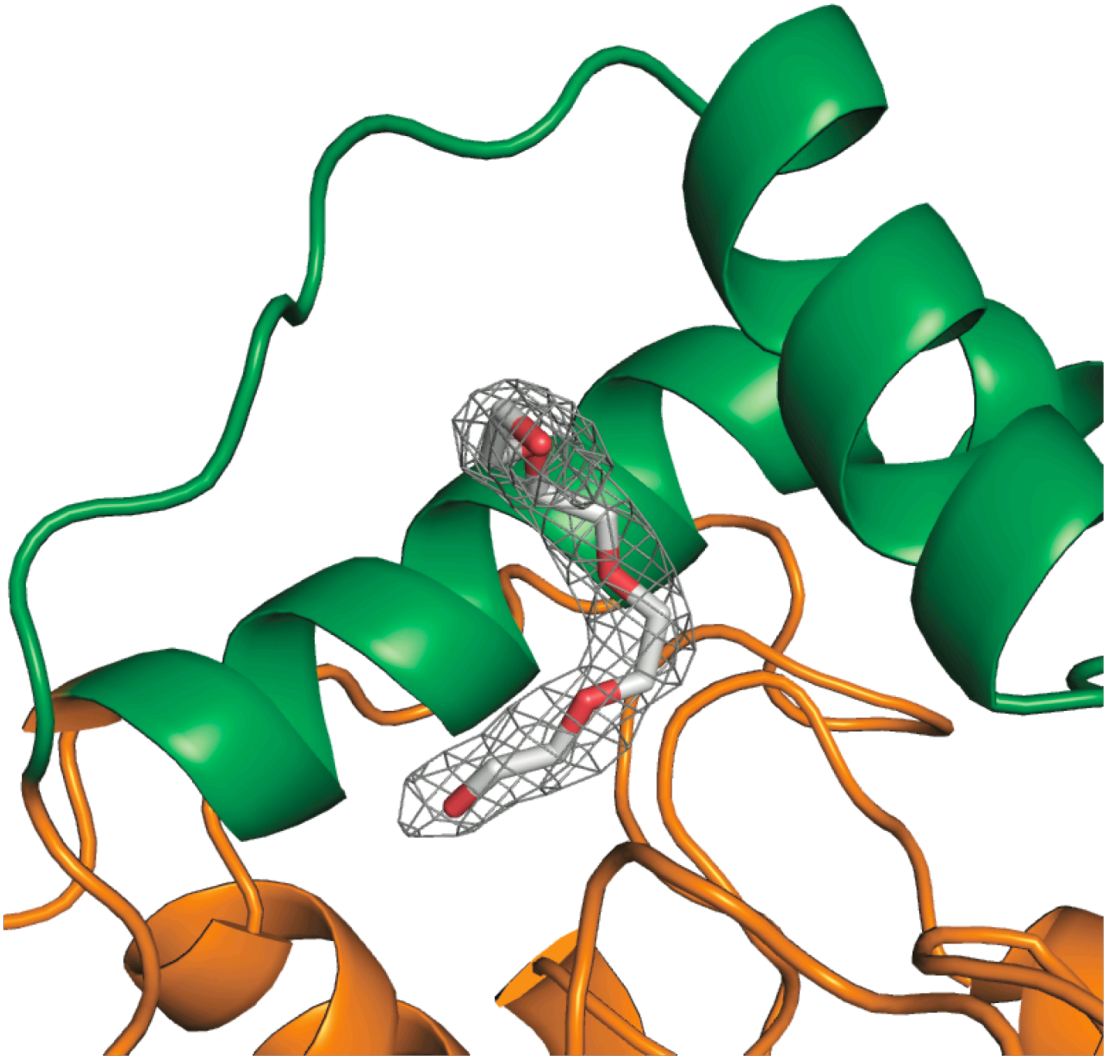

Figure S6. *Fo-Fc* omit map contoured at 1 σ for the polyethylene glycol molecule bound in the hydrophobic pocket of RedJ.

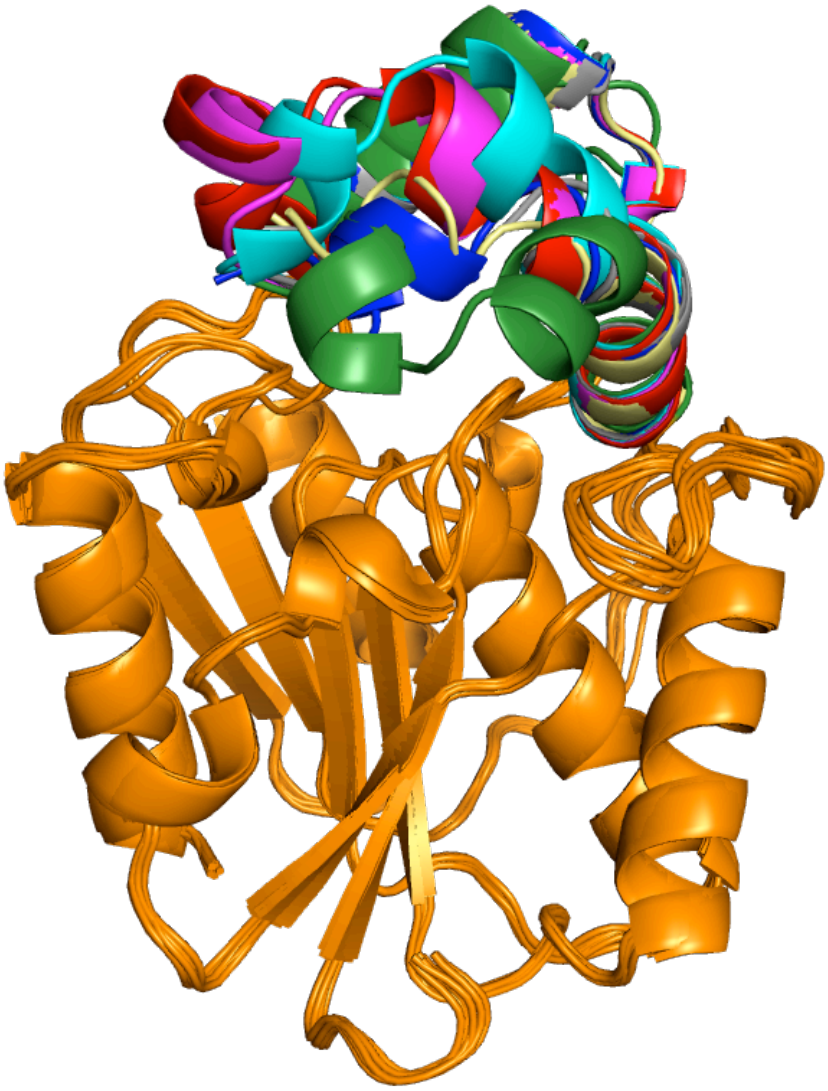

Figure S7. Superposition of the eight RedJ molecules from the $RedJ_T$ and SeMet His_6-$RedJ_T$ crystal structures. The core domain is orange, the lid domain in the open-entrance conformation is cyan, the lid domain in the closed entrance conformation is green, and the lid domains in the intermediate conformation are red, magenta, yellow, and blue. The positions of αL2 and residues 173-179 of αL3 are not shown for two of the RedJ molecules due to disorder.

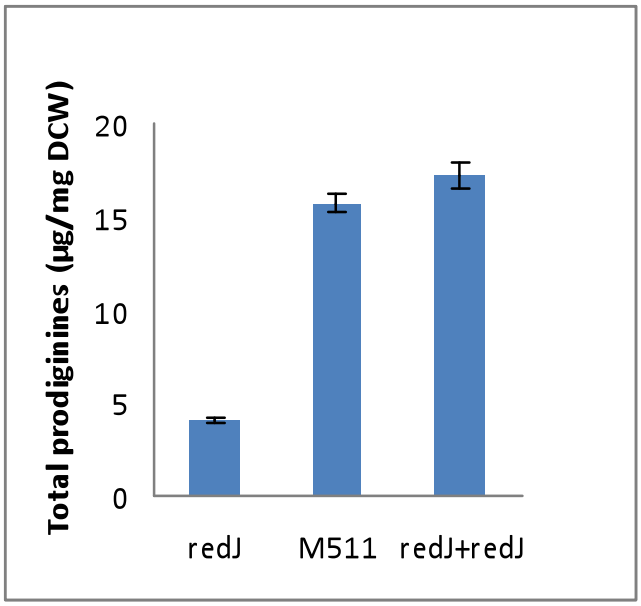

Figure S8. Levels of total prodiginine production in wild type *S. coelicolor* (M511), a *redJ* deletion mutant of the M511 strain (redJ) and the genetically complemented mutant (redJ+redJ).

Figure S9. Sequence alignment of FabC (accession NP_626635), AcpP (accession AAB27925), and RedQ (accession NP_630008) generated by clustalw (Chena *et al*., Nucleic Acids Res, 2003. 13: p. 3497-500). The blue box indicates the residues of FabC in the additional helical turn at the C-terminus of helix 1. The red boxes indicate residues on α-helix 3 that are proposed to either prevent (FabC) or promote (RedQ and AcpP) interaction with RedJ.