Supporting Information

Kannan et al. 10.1073/pnas.1417334111

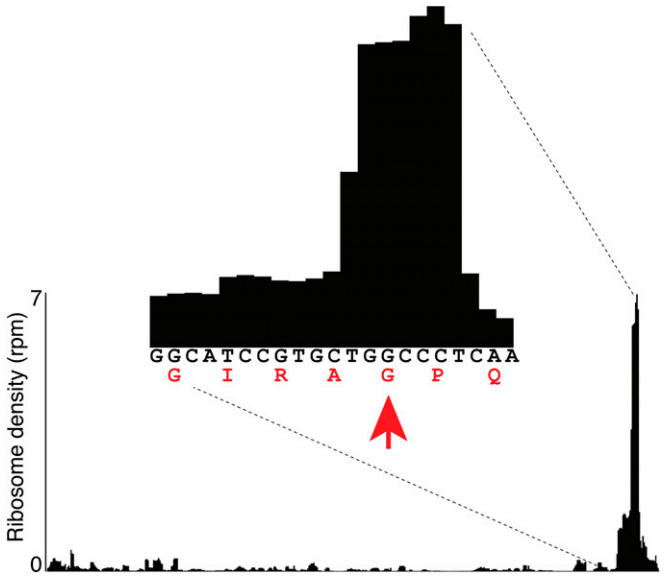

Fig. S1. Translation of *secM* in the absence of antibiotics is arrested when the Gly_{165} codon enters the ribosomal P site (1). Because of the ambiguity of assigning the ribosome placement from the analysis of sequenced protected mRNA fragments, the peak of the computed ribosome density spans the P- and A-site codons of mRNA in the stalled ribosome.

1. Muto H, Nakatogawa H, Ito K (2006) Genetically encoded but nonpolypeptide prolyl-tRNA functions in the A site for SecM-mediated ribosomal stall. *Molec Cell* 22(4):545–552.

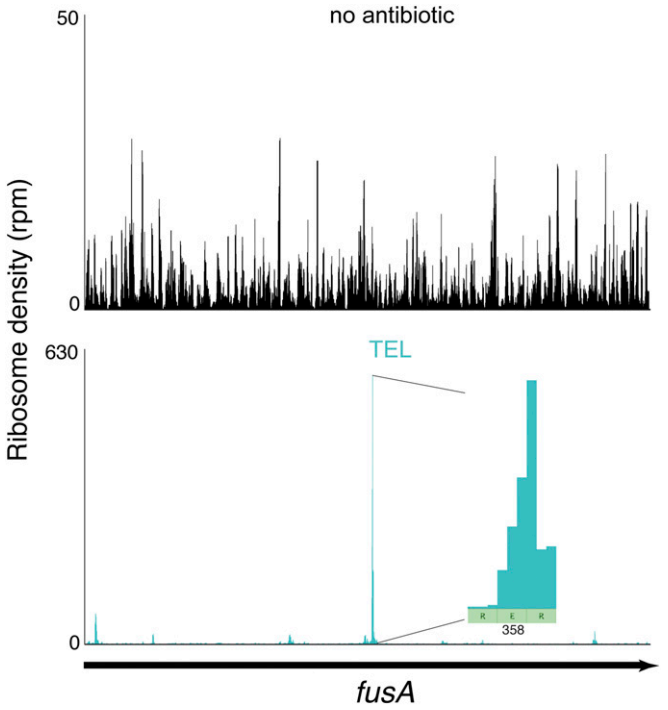

Fig. S2. Ribosome profiling shows TEL-induced ribosome stalling during translation of the *fusA* gene in vivo at the site that agrees with the previous observation of TEL-dependent arrest at the middle codon of the $RE_{358}R$ sequence in vitro (1).

1. Kannan K, Vazquez-Laslop N, Mankin AS (2012) Selective protein synthesis by ribosomes with a drug-obstructed exit tunnel. *Cell* 151(3):508–520.

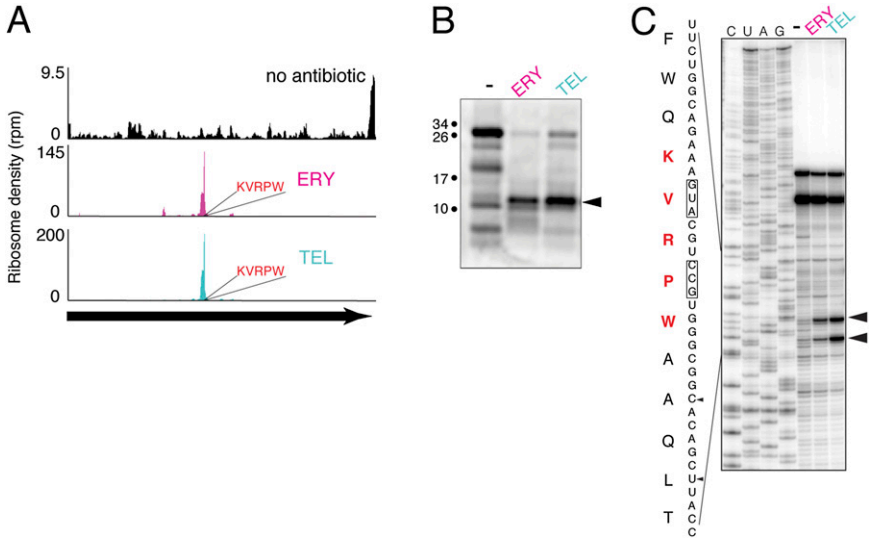

Fig. S3. Defining the exact sites of macrolide-induced ribosome stalling in the *rseA* (ORF) containing the XP (RPW) motif. *A* represents the ribosome density within the *rseA* ORF in the ribosome profiling experiments, the gel in *B* shows fractionation of the [^{35}S]-labeled protein products of translation of the ORF in the S30 cell-free system, and the sequencing gel in *C* shows the results of toe-printing analysis of ribosome stalling during translation of the 5′-terminally truncated ORF in the in vitro system composed of purified components. Arrowheads show the main translation products and the main toe-printing bands corresponding to the peaks of the ribosome density in the profiling experiments. Note that in the *rseA* ORF macrolides induce ribosome stalling at two sites: Val codon within the KVR sequence representing the [+]X[+] motif and Pro codon of the XP motif.

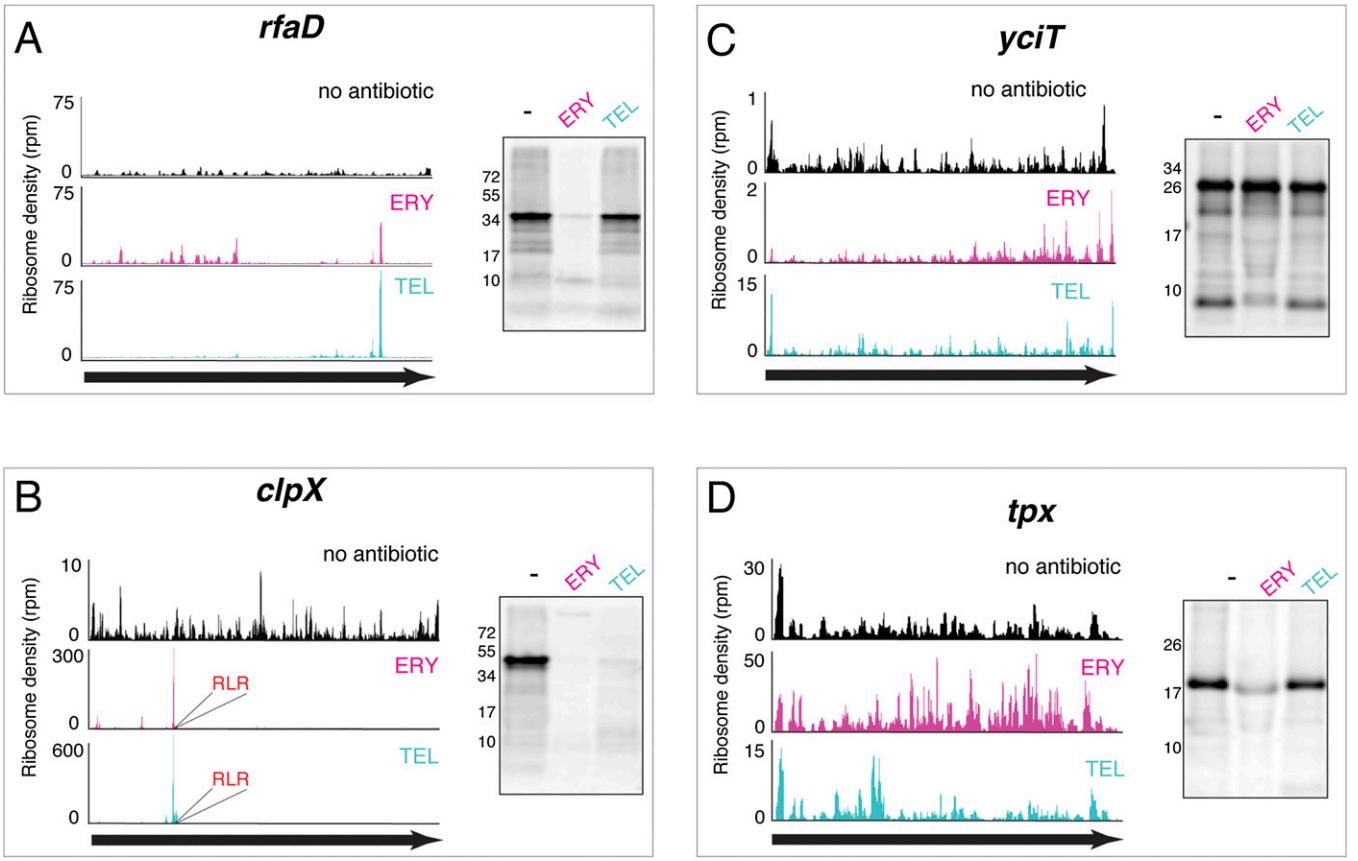

Fig. S4. Some of the in vivo effects of macrolide antibiotics are not readily reproduced in vitro. (*A*) Late arrest induced by TEL during translation of the *rfaD* ORF in the bacterial cell (*Left*) is not observed during in vitro translation (*Right*, SDS gel). (*B*) ERY and TEL cause strong ribosome stalling at the $RL_{99}R$ motif of *clpX* in vivo (Fig. S4), but the corresponding ~10-kDa truncated polypeptide was not observed during cell-free translation, possibly due to inhibition of the *clpX* in vitro synthesis at the earlier codons. Although translation of the *yciT* (*C*) and *tpx* (*D*) ORFs is resistant to macrolides in vivo, only *yciT* maintains resistance to both macrolides in a cell-free system, whereas *tpx* is susceptible to ERY inhibition in vitro.

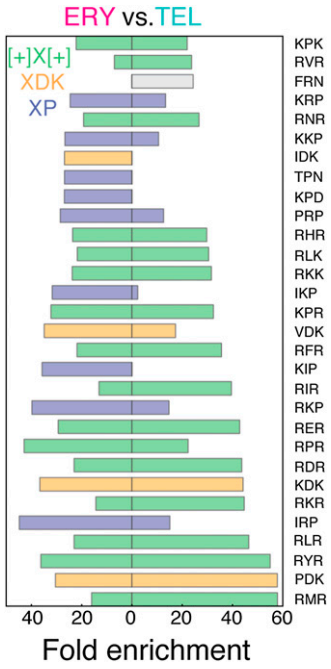

Fig. S5. Comparison of the top TEL and ERY arrest motifs shows that the chemical structure of the antibiotic significantly affects sequence specificity of the drug action.

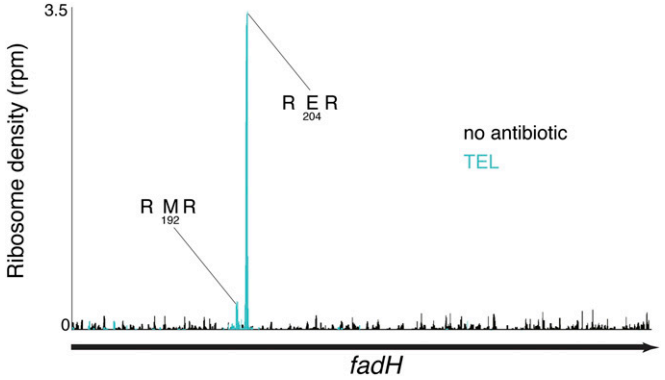

Fig. S6. Translation of *fadH* in the presence of TEL is poorly arrested at the $RM_{192}R$ site, which carries the triamino acid sequence with the highest enrichment in the sites of TEL-induced arrest (Fig. 3*B*), but is stalled at the downstream $RE_{204}R$ site.