Supplementary Materials for

Translation elongation factor 4 (LepA) contributes to tetracycline susceptibility by stalling elongating ribosomes

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Supplementary Table 1. Mechanism of the tested antibiotics

Antibiotic	Mechanism of action
Streptomycin	Interacts with 16S rRNA and ribosomal protein S12, which causes misreading of mRNA(1, 2).
Tetracycline	Prevents the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site(3).
Spectinomycin	Sterically blocks swiveling of the head domain of the small ribosomal subunit to disrupt translocation(4).
Puromycin	Causes premature termination during translation. Part of the molecule resembles the 3' end of the aminoacylated tRNA. It enters the A site and transfers to the growing chain, causing the formation of a puromycylated nascent chain and premature chain release(5).
Erythromycin	Binds to the nascent peptide exit tunnel close to the peptidyl transferase center and prevents synthesis of peptides longer than eight amino acid(6).
Hygromycin B	Prevents movement of the A site bound tRNA into the P site with an overall net effect of sequestering tRNA in the A site(7).
Gentamicin	Binds the 30S subunits of the bacterial ribosome and assists read-through of termination codon(8).

Supplementary Table 2. Primers used in sequencing library construction

Primer name	Sequence (5' to 3')
RTP	5′-(Phos)-AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGC-(SpC18)-CACTCA-(SpC18) TTCAGACGTGTGCTCTTCCGATCTATTGATGG TGCCTACAG-3′
5s-1	5′_/5Biosg/AGTTCGGCATGGGGTCAGGTGGGACCACCGCGCTAC_3'
5s-2	5′_/5Biosg/CGCTACGGCGTTTCACTTCTGA_3'
5s-3	5′_/5Biosg/CTCTCGCATGGGGAGACCCCACACTACCATCGGC_3'
16s-1	5′_/5Biosg/CCACTCGTCAGCAAAGAAGCAAGCTTCTTCCTGT_3'
16s-2	5′_/5Biosg/GTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCT_3'
16s-3	5′_/5Biosg/CCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGG_3'
16s-4	5′_/5Biosg/GCCTCAAGGGCACAACCTCCAAGTCGACAT_3'
23s-1	5′_/5Biosg/GGCATTTGTTGCTTCAGCACCGTAGTGCCTCGTCATCACGCCTCAG_3'
23s-2	5′_/5Biosg/TACCACGTGTCCCGCCCTACTCATCGAGCTCA_3'
23s-3	5′_/5Biosg/TGTCCCGCCCTACTCATCGAGCTCACAATATG_3'
23s-4	5′_/5Biosg/CATAAGCGTCGCTGCCGCAGCTTCGGTGCATGGTTTA_3'
Forward library PCR primer	5′-AATGATACGGCGACCACCGAGATCTACAC-3′
Reverse PCR primer WT	5'-CAAGCAGAAGACGGCATACGAGATACTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCG-3′
Reverse PCR primer ΔEF4	5'-CAAGCAGAAGACGGCATACGAGATATGCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCG-3′
Reverse PCR primer WT tetra	5'-CAAGCAGAAGACGGCATACGAGATACGTCGGTGACTGGAGTTCAGACGTGTGCTCTTCCG-3′
Reverse PCR primer ΔEF4 tetra	5'-CAAGCAGAAGACGGCATACGAGATAGCTGCGTGACTGGAGTTCAGACGTGTGCTCTTCCG-3′
linker	5'-rAppCTGTAGGCACCATCAAT–NH2-3′

References:

1. **Finken M, Kirschner P, Meier A, Wrede A, Bottger EC.** 1993. Molecular-Basis of Streptomycin Resistance in Mycobacterium-Tuberculosis - Alterations of the Ribosomal-Protein S12 Gene and Point Mutations within a Functional 16s Ribosomal-Rna Pseudoknot. Mol Microbiol **9:**1239-1246.
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3. **Chopra I, Roberts M.** 2001. Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol R **65:**232-260.
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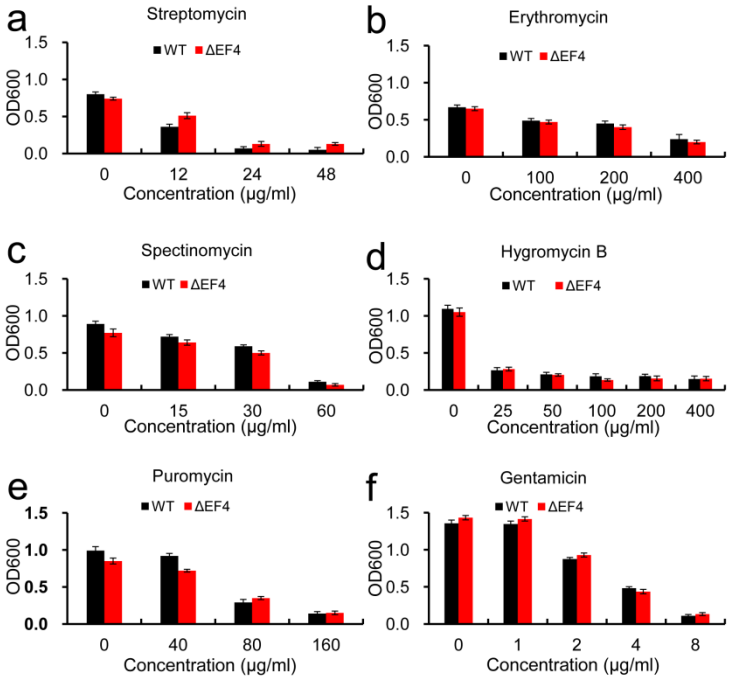
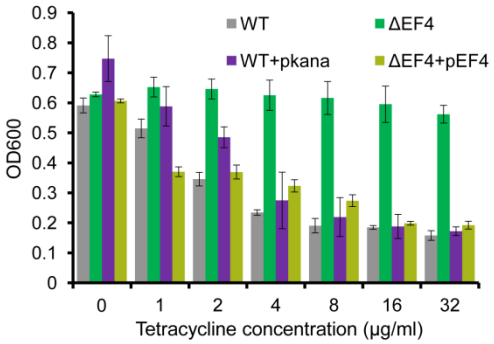
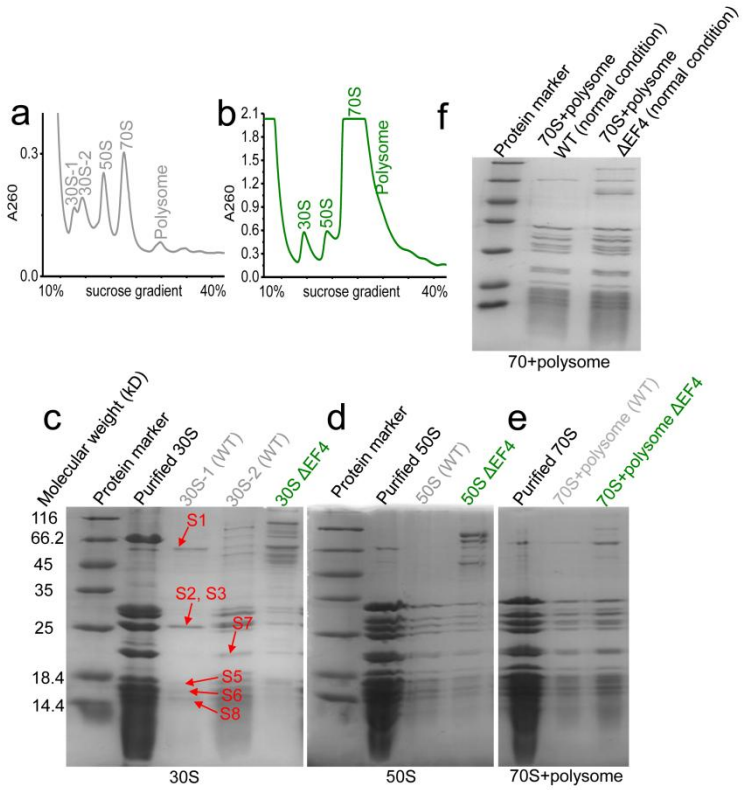
8. **Bellais S, Le Goff C, Dagoneau N, Munnich A, Cormier-Daire V.** 2010. In vitro readthrough of termination codons by gentamycin in the Stuve-Wiedemann Syndrome. Eur J Hum Genet **18:**130-132.



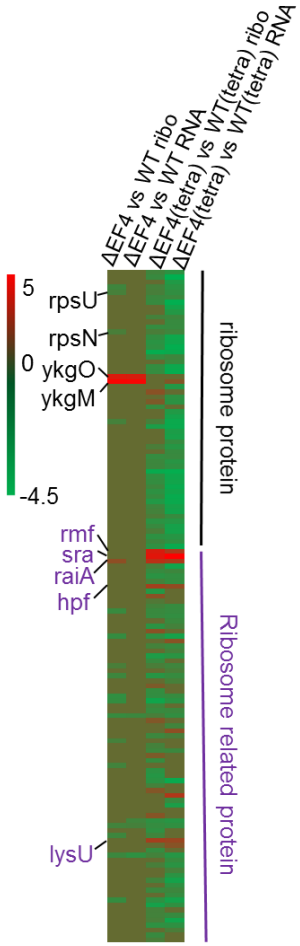
Supplementary Fig. 1 Growth of the WT and ΔEF4 strains under different concentrations of antibiotics, including streptomycin (a), erythromycin (b), spectinomycin (c), hygromycin B (d), puromycin (e), gentamicin (f).



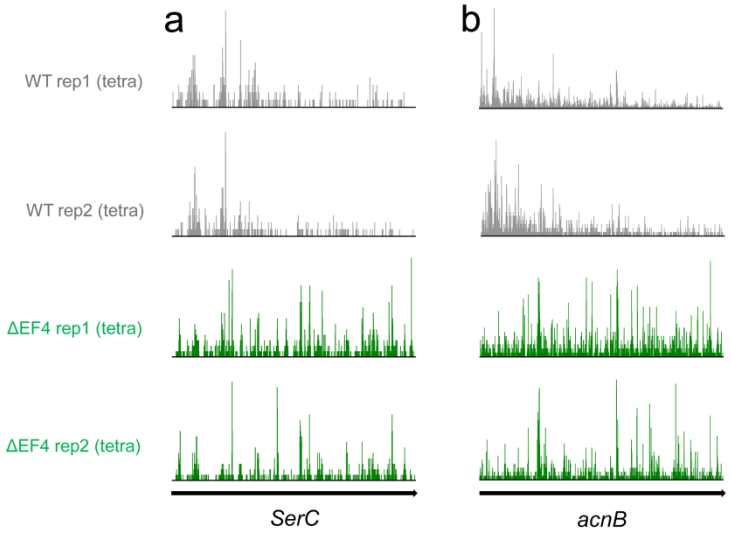
Supplementary Fig. 2 Growth of the WT strain, the ΔEF4 strain, the WT strain with an empty vector containing a kanamycin-resistant gene (pkana), and the ΔEF4 strain with a plasmid expressing EF4 (pEF4) under different concentrations of tetracycline.



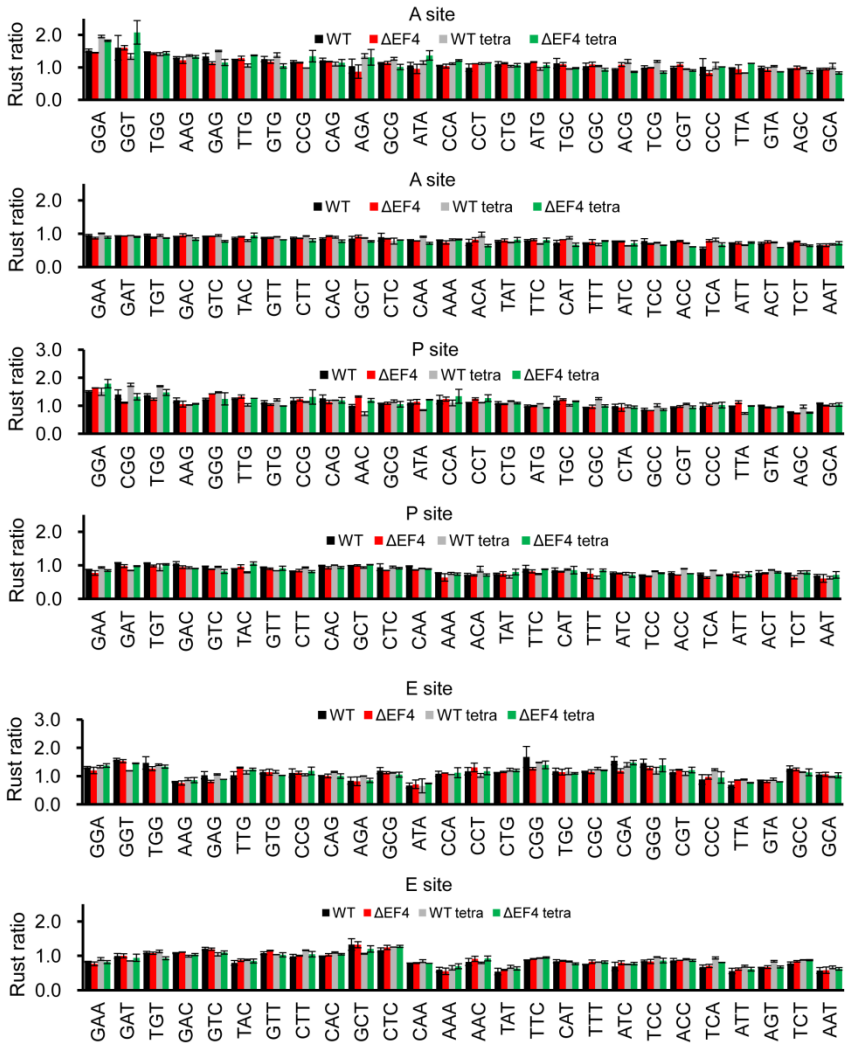
Supplementary Fig. 3 Polysome profiles and SDS-PAGE of ribosome fractions. A-B, polysome profiles for the WT (a) and ΔEF4 (b) strains that were treated with tetracycline. The polysome profiles were obtained by centrifuging at 40000 rpm for 3 hours in 10%-40% sucrose. C-F, SDS-PAGE of ribosome fractions prepared from strains treated with (c-e) or without (f) tetracycline.



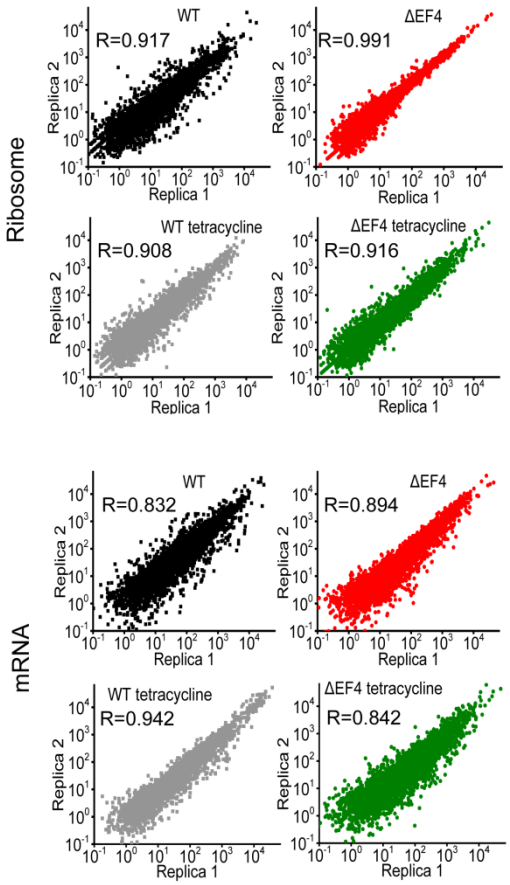
Supplementary Fig. 4 Expression analysis of ribosomal proteins and ribosome related proteins between samples. The green and red colors strand for down- and up-regulation in the ΔEF4 strain, respectively. Ribo and RNA represent expression analysis at the translational and transcriptional levels, respectively.



Supplementary Fig. 5 Profiles of ribosomal footprints on mRNA for gene *SerC* (a) and *acnB* (b) in the WT and ΔEF4 strains with tetracycline treatment.



Supplementary Fig. 6 The rust ratio values for the codons at A, P and E sites, which were not showed in Figure 4.



Supplementary Fig. 7 Correlation between replicates in all samples for both ribosomal footprints and mRNA data sets.