Supplementary Information for:

A CENTRAL INTERDOMAIN PROTEIN JOINT IN EF-G REGULATES ANTIBIOTIC SENSITIVITY, GTP HYDROLYSIS, AND RIBOSOME TRANSLOCATION

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EF-G binding and hydrolysis of mant-GTP

Binding affinities (Supplementary Table 1) of EF-G for mant-GDP, and for mant-GTP in the absence of hydrolysis, were derived by fitting data (e.g., Supplementary Fig. 2) to:

$$\Delta F = \Delta F_{max}[\text{mGNP}] / (K_d + [\text{mGNP}])$$

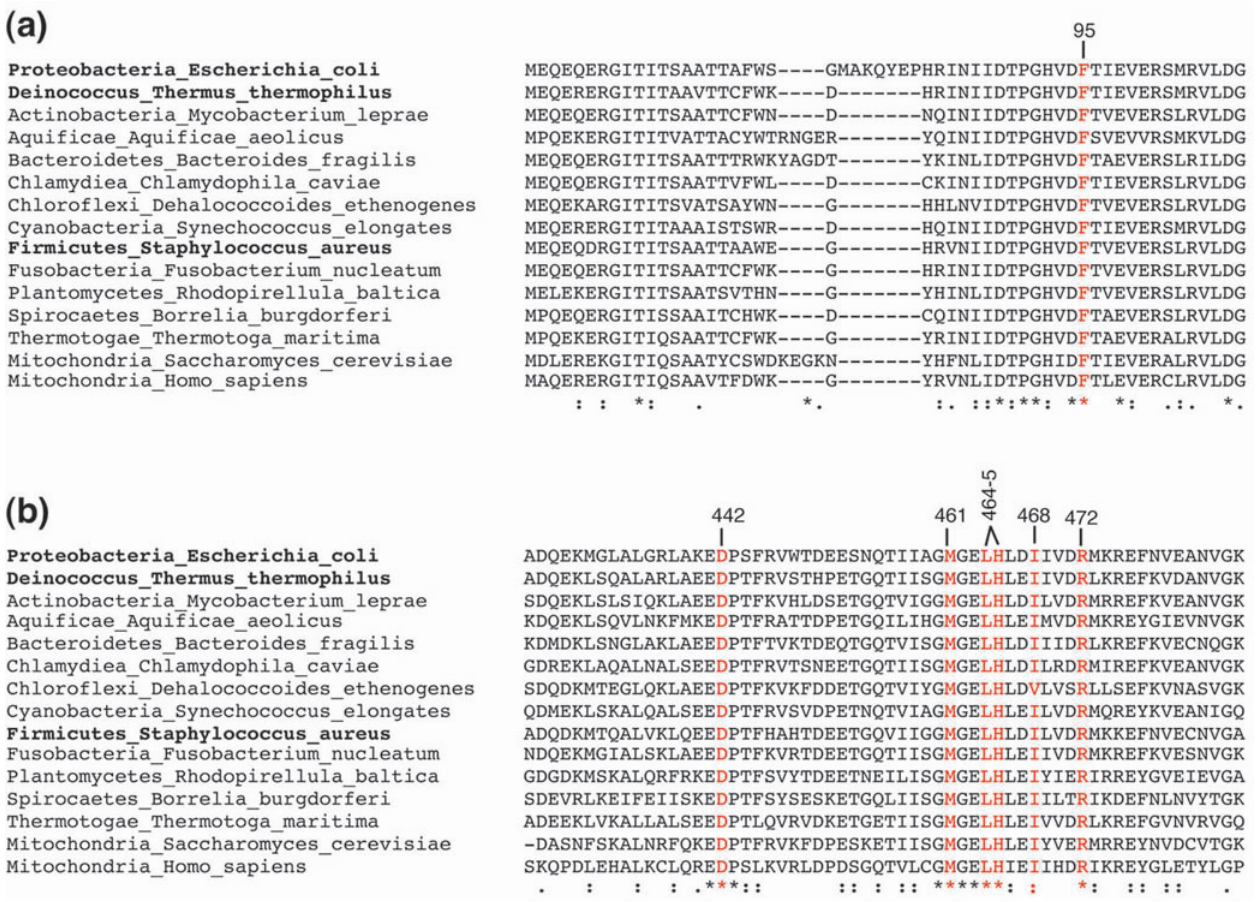
where: ΔF is the fluorescence change, [mGNP] is the concentration of mant-GDP or mant-GTP, K_d is the dissociation equilibrium constant for mant-nucleotide binding to EF-G, and ΔF_{max} is the maximal fluorescence change extrapolated to infinite [mGNP].

The rate of hydrolysis of mant-GTP was determined by rapidly mixing EF-G with mant-GTP in a stopped-flow device.(1) The rate (v) of increase in the fluorescence signal was measured during the initial (linear) phase of the reaction. Data were fitted to:

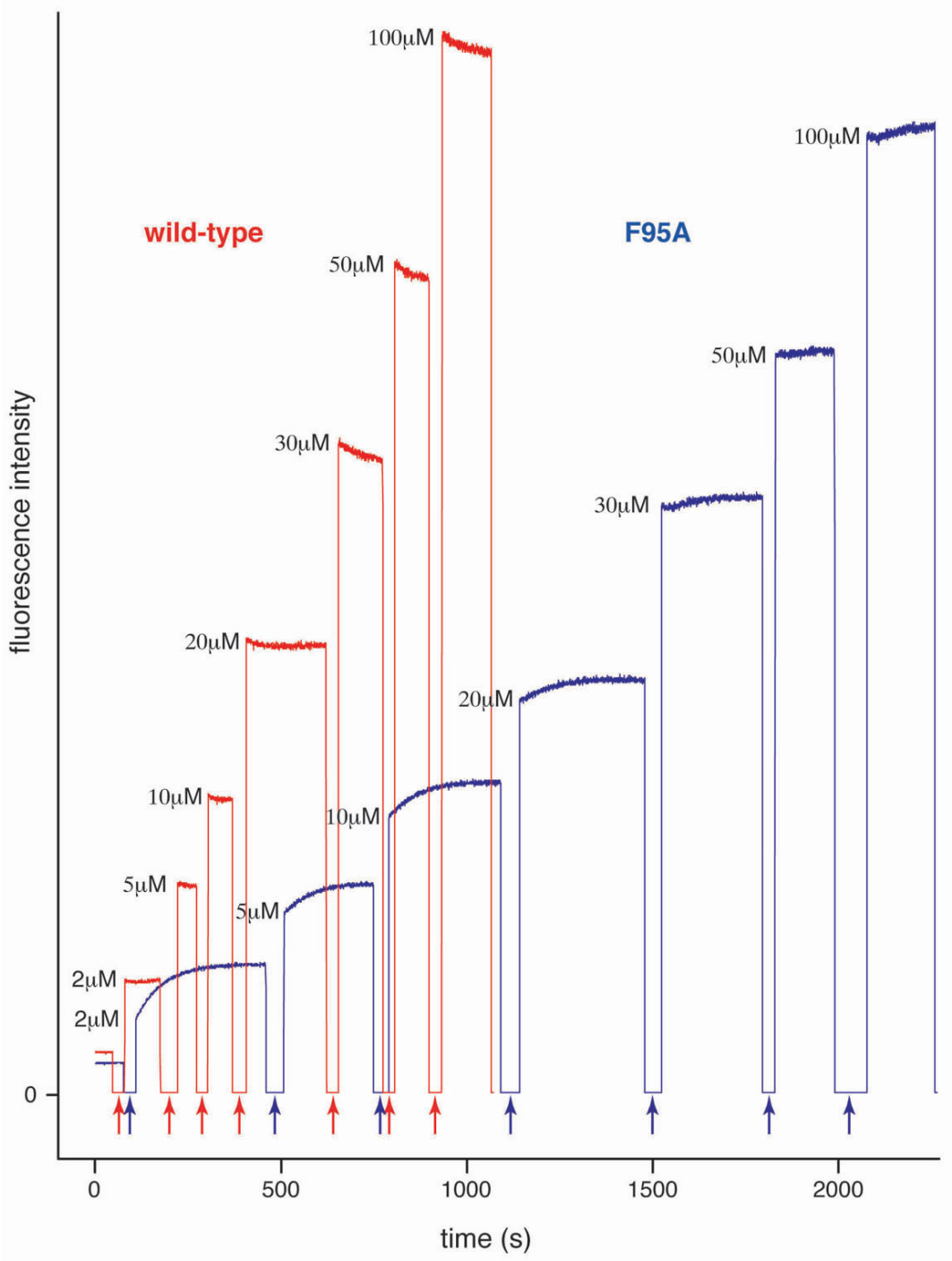
$$v = v_0 + V_{max} / (K_m + [\text{mGTP}])$$

where: v_0 is the rate without EF-G, V_{max} is the maximal rate extrapolated to infinite [mGTP], and K_m is the Michaelis enzyme constant.

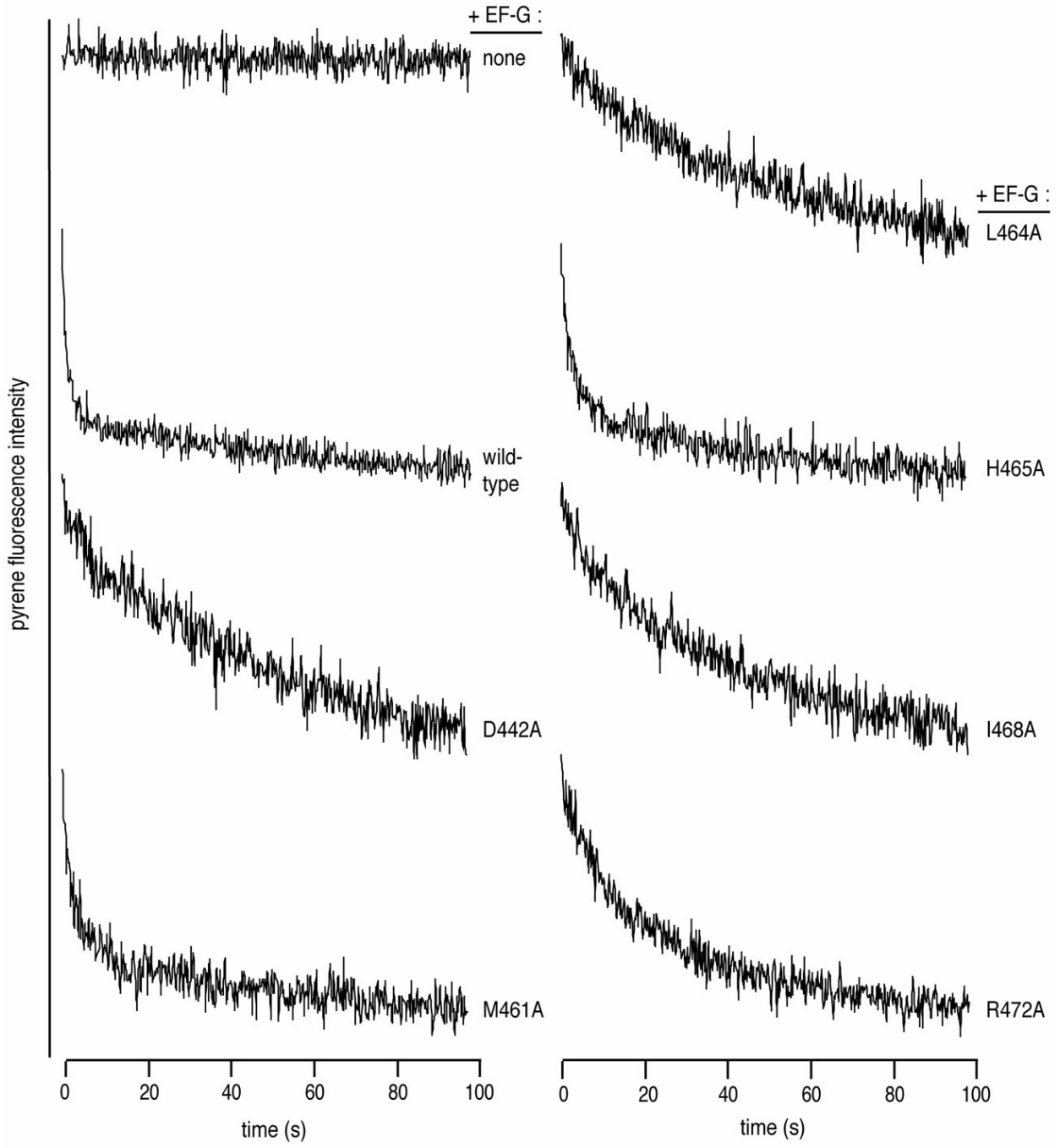
SUPPLEMENTARY FIGURES



Supplementary Fig. 1. Conservation of Fus^R mutations, isolated from *S. aureus* and other bacteria (2-6) EF-G amino acid sequences are aligned from species from different bacterial phyla and eukaryotic mitochondria. Portions of this sequence alignment include: *A*. the switch 2 region in the G domain; *B*. domain III. Secondary structural elements are based on free and ribosome-bound *T. thermophilus* EF-G (6,7).

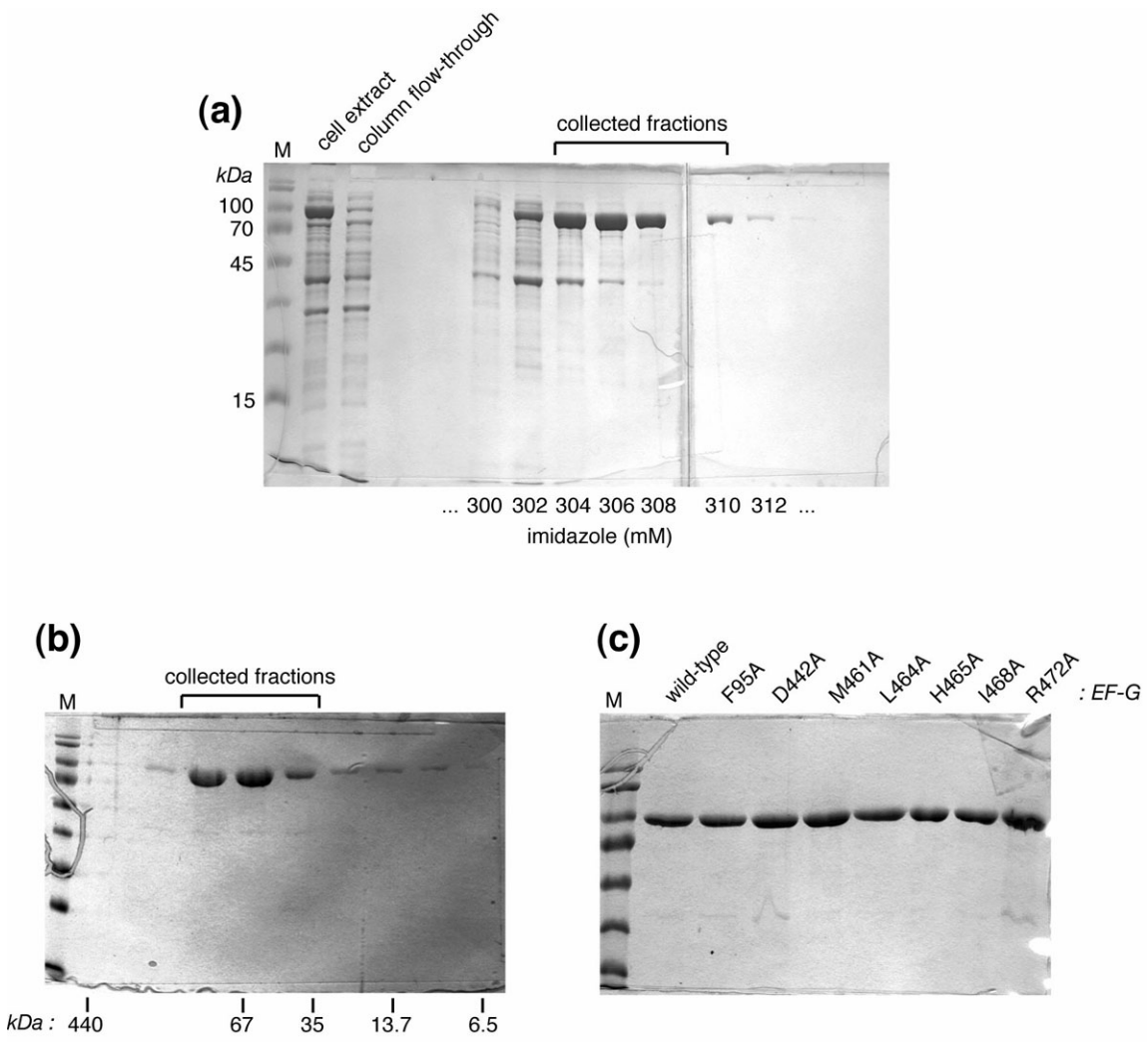


Supplementary Fig. 2. Interactions of mant-GTP with EF-G (*E. coli* wild-type and mutant F95A). EF-G (1 μM) was titrated with mant-GTP (from 2 μM to 100 μM, as indicated in the graph) in polymix buffer at 20°C. A cuvette containing EF-G was initially mixed with a concentrated stock of mant-GTP (2 μM, final concentration) and placed in our fluorimeter's sample chamber. The sample was excited at 290 nm (tryptophan residues in EF-G) and the fluorescence emission at 445 nm (excited mant-GTP bound to EF-G) via fluorescence resonance energy transfer from neighboring tryptophan residues (8). The fluorescence signal was allowed to stabilize over time. Measurements were continued by mixing subsequent aliquots of concentrated mant-GTP (indicated by the lower arrows in the graph).



Supplementary Fig. 3. Ribosome translocation catalyzed by EF-G Fus^R mutants, assayed by using pyrene-mRNA.(1) Reactions at 20^{o}C were monitored by a stopped-flow device attached to our fluorimeter. In this device, EF-G (2.5 μM) + GTP (1 mM) were loaded into syringe A. Pretranslocational ribosome complexes containing pyrene-labeled mRNA (at 0.25 μM; see Methods) were loaded into syringe B. The reactants in the two syringes were rapidly mixed together, and shot into our fluorimeter's sample chamber. The pyrene fluorescence was monitored over time. The exponential decay represents ribosome translocation by one codon along the mRNA; i.e., movement of the pyrene into the ribosome.

For clarity, the fluorescent traces have been offset along the y-axis. By fitting the fluorescent traces to an exponential first-order rate equation, $F_t = F_\infty + \Delta F \times exp(-k \times t)$,(1) we obtained the rate constants (k) that are listed in Table 1.



Supplementary Fig. 4. Purification of EF-G proteins.(8) Shown in the first two panels is an example purification of wild-type E. coli EF-G tagged with six histidines (His_6) at its C-terminal end. In all three panels, samples were analyzed by SDS-polyacrylamide gels (stain: Coomassie blue).

A. Initial purification of EF-G by His_6-affinity chromatography. Soluble cell extract from E. coli BL21(DE3) cells expressing EF-G from a plasmid (9) was loaded onto a Ni-NTA agarose column, and eluted with a concentration gradient of imidazole.

B. Further purification by gel filtration chromatograpy. The collected fractions from panel A were loaded onto a Superdex 200 (10/300) FPLC column, and eluted with 5 % v/v glycerol, 10 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 0.2 M KCl, 1 mM DTT. Globular protein molecular mass standards are marked below the gels.

C. All purified EF-G proteins, wild-type and Fus^R mutants. Collected fractions from panel B were dialyzed against 50% v/v glycerol, 10 mM Tris-HCl (pH 8.0), 0.1 M KCl, 3 mM DTT, and stored at -20°C.

SUPPLEMENTARY REFERENCES

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