Supporting Information

Walker *et al.* 10.1073/pnas.0710146105

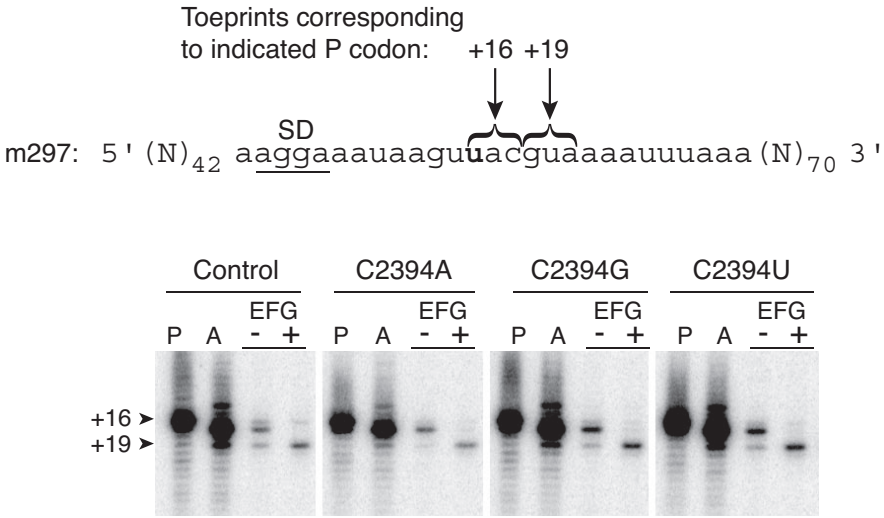

Fig. S1. Mutations at position 2394 of 23S rRNA have no appreciable effect on the efficiency of tRNA binding to the P and A sites or the extent of translocation as judged by toeprinting analysis. PRE complexes were formed by incubating control or mutant ribosomes (as indicated) with m297 and $tRNA^{Tyr2}$ to fill the P site (P lanes). *N*-Acetyl-Val-$tRNA^{Val}$ was then added to bind the A site (A lanes), after which complexes were diluted 5-fold with buffer 1. Portions of the diluted complexes were then added to equal volumes of buffer 2 containing GTP (− lanes) or EF-G plus GTP (+ lanes). These conditions mimic those used in the stopped-flow experiments. At each stage of the experiment, the position of mRNA was mapped by toeprinting.

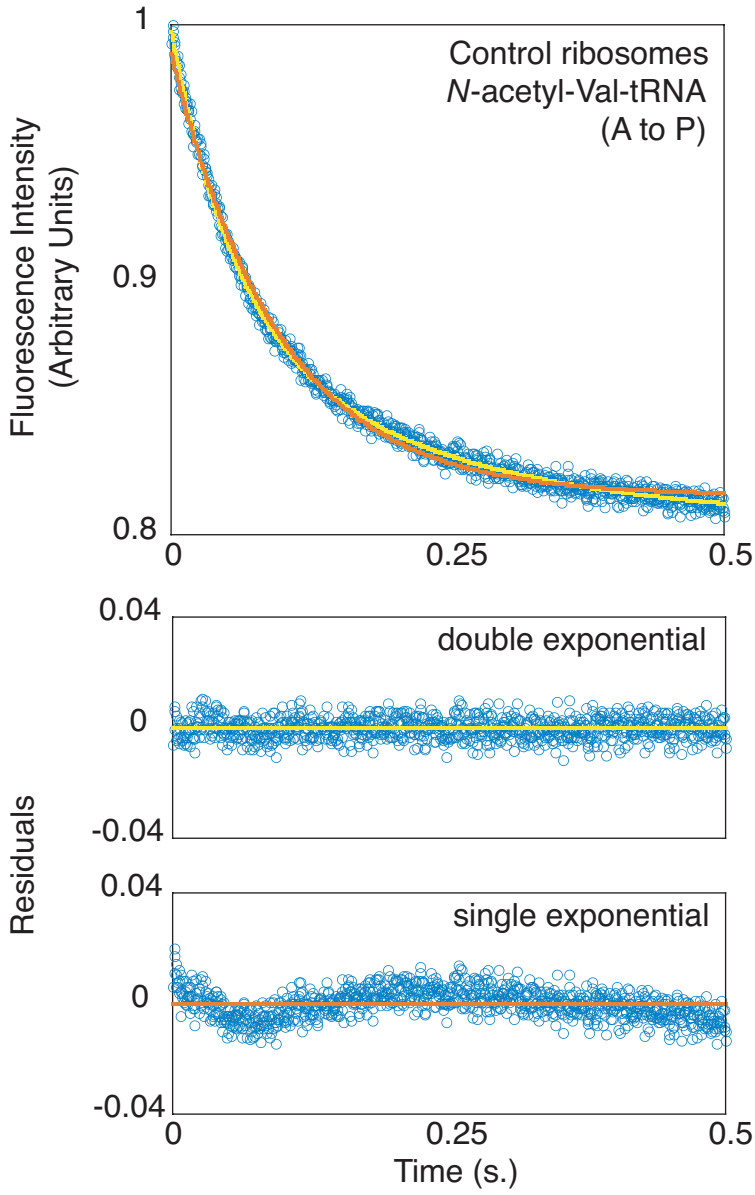

Fig. S2. Example of an experiment monitoring translocation of pyrene-labeled mRNA paired to *N*-acetyl-Val-tRNA^{Val} in control (wild-type) ribosomes. (*Top*) In this experiment, syringe 1 was loaded with PRE complexes programmed with m433 and containing tRNA^{Tyr} in the P site and *N*-acetyl-Val-tRNA^{Val} in the A site. Syringe 2 contained EF-G (8 μM) and GTP (1 mM). Upon rapid mixing, decreased fluorescence intensity (open blue circles) was observed as a function of time. (*Middle* and *Bottom*) Residual plots in which the data in *Top* were fit to either a single (*Bottom*, orange trace) or double (*Middle*, yellow trace) exponential function. Based on inspection of the residual plots, two exponential terms were necessary to provide a reasonable fit to the data. No fluorescence change was observed when EF-G was omitted from syringe 2 under otherwise identical conditions (data not shown).

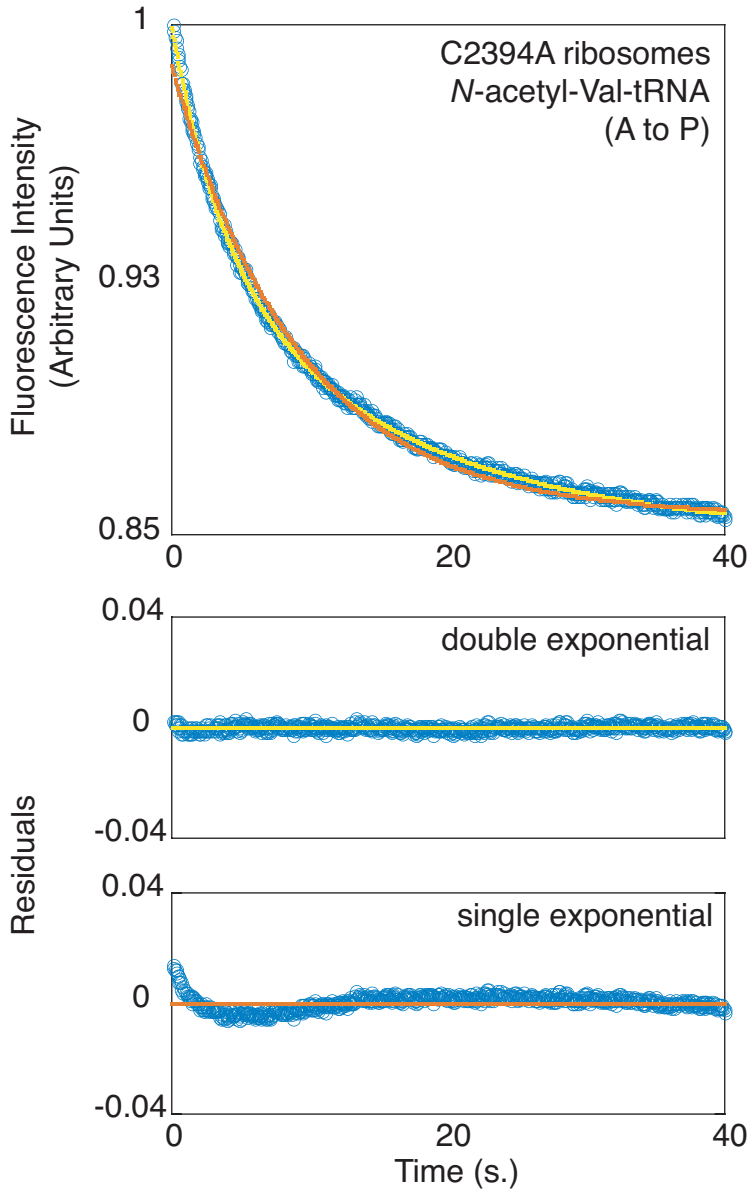

Fig. S3. Example of an experiment monitoring translocation of pyrene-labeled mRNA paired to *N*-acetyl-Val-tRNA^{Val} in ribosomes harboring C2394A. Conditions and annotations were as described for Fig. S2.

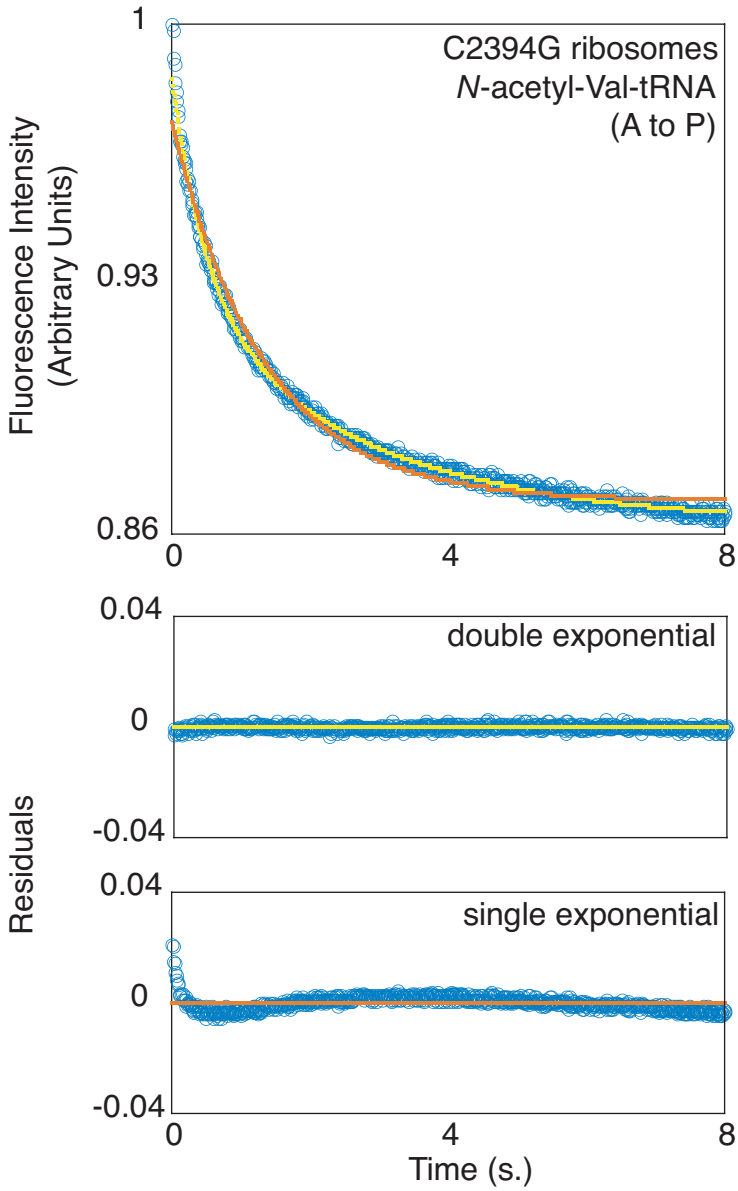

Fig. S4. Example of an experiment monitoring translocation of pyrene-labeled mRNA paired to *N*-acetyl-Val-tRNA^{Val} in ribosomes harboring C2394G. Conditions and annotations were as described for Fig. S2.

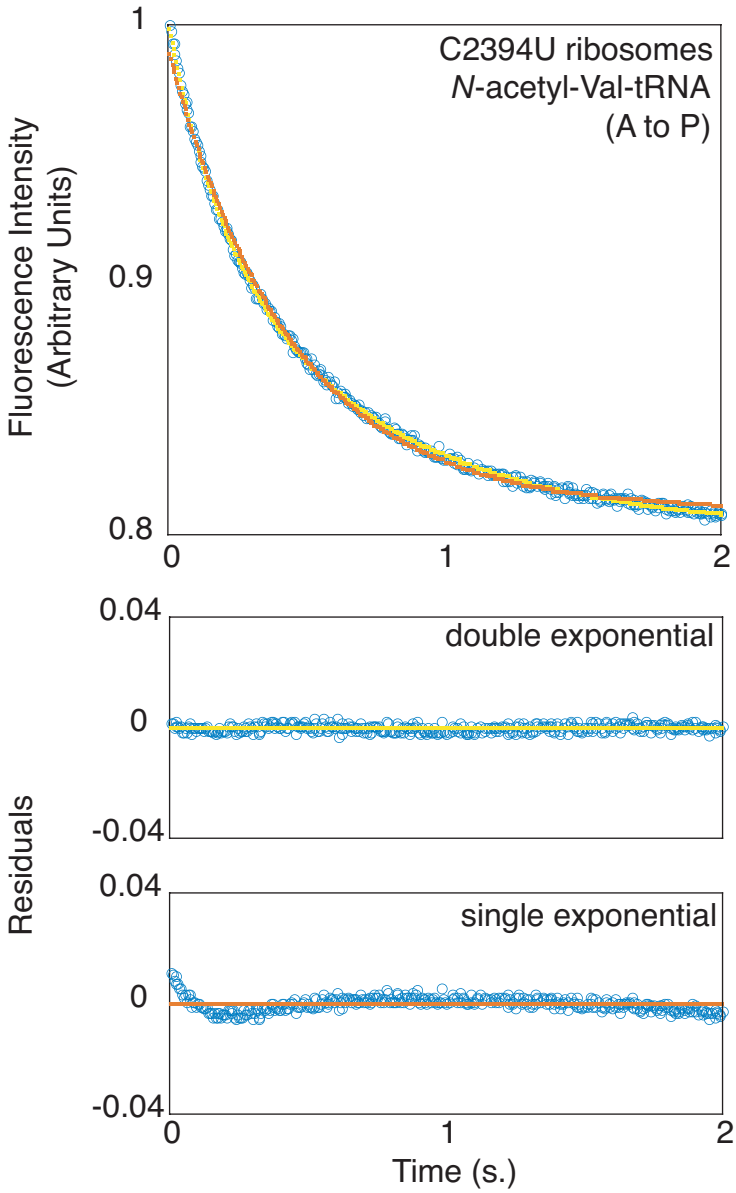

Fig. S5. Example of an experiment monitoring translocation of pyrene-labeled mRNA paired to *N*-acetyl-Val-tRNA^{Val} in ribosomes harboring C2394U. Conditions and annotations were as described for Fig. S2.

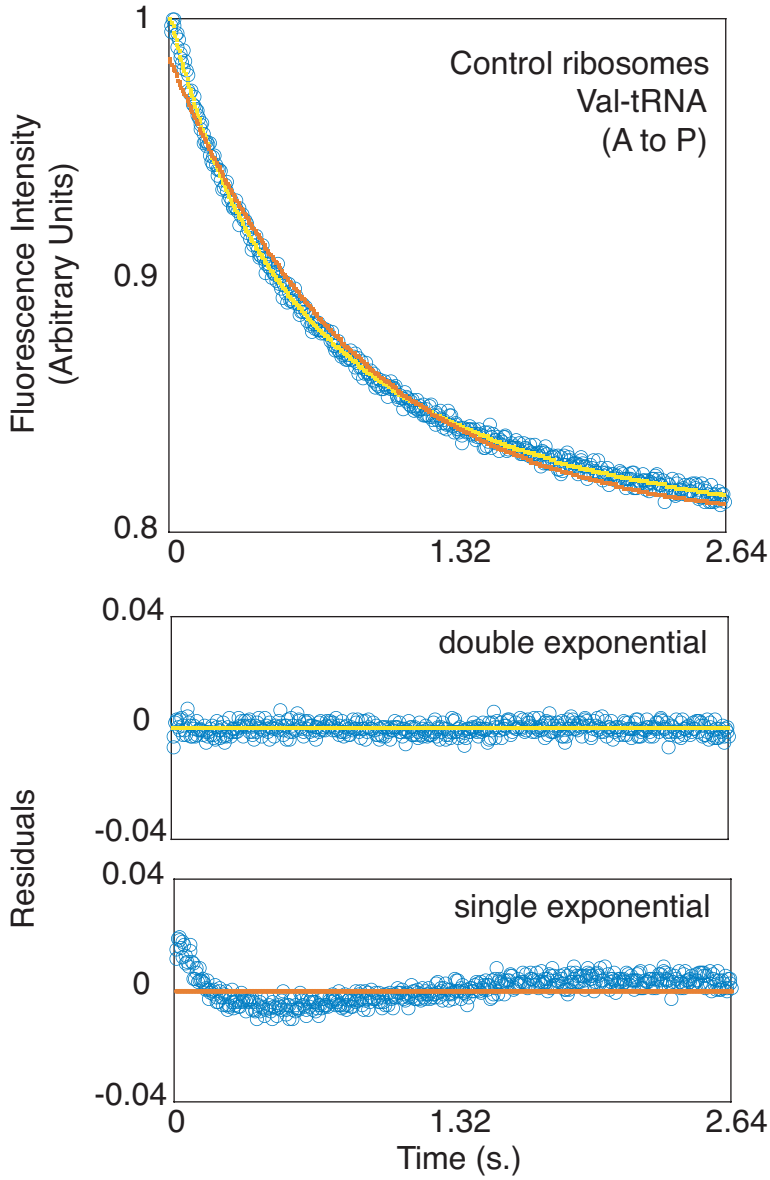

Fig. S6. Example of an experiment monitoring translocation of pyrene-labeled mRNA paired to Val-tRNA^{Val} in control (wild-type) ribosomes. In this experiment, syringe 1 was loaded with PRE complexes programmed with m433 and containing tRNA^{Tyr} in the P site and Val-tRNA^{Val} in the A site. Conditions were otherwise the same as those described for Fig. S2.

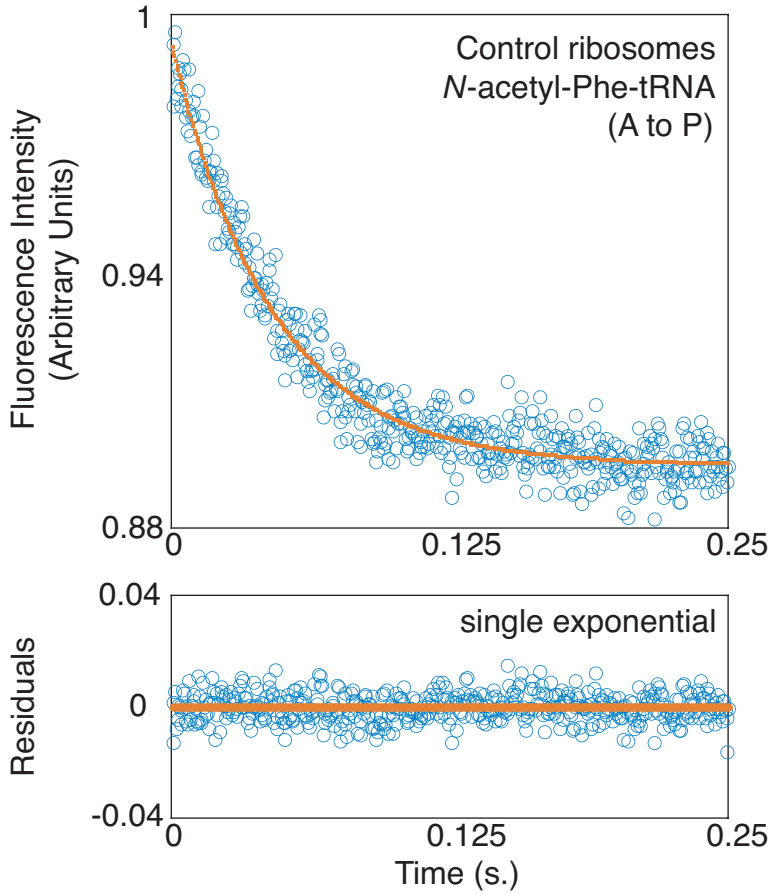

Fig. S7. Example of an experiment monitoring translocation of pyrene-labeled mRNA paired to *N*-acetyl-Phe-tRNA^{Phe} in control (wild-type) ribosomes. (*Upper*) In this experiment, syringe 1 was loaded with PRE complexes programmed with m432 and containing tRNA^{Tyr} in the P site and *N*-acetyl-Phe-tRNA^{Phe} in the A site. Syringe 2 contained EF-G (6 μM) and GTP (1 mM). Upon rapid mixing, decreased fluorescence intensity (open blue circles) was observed as a function of time. (*Lower*) These data were fit to a single (orange trace) exponential function. In this context only (at each concentration of EF-G), the data fit well to a single exponential function.

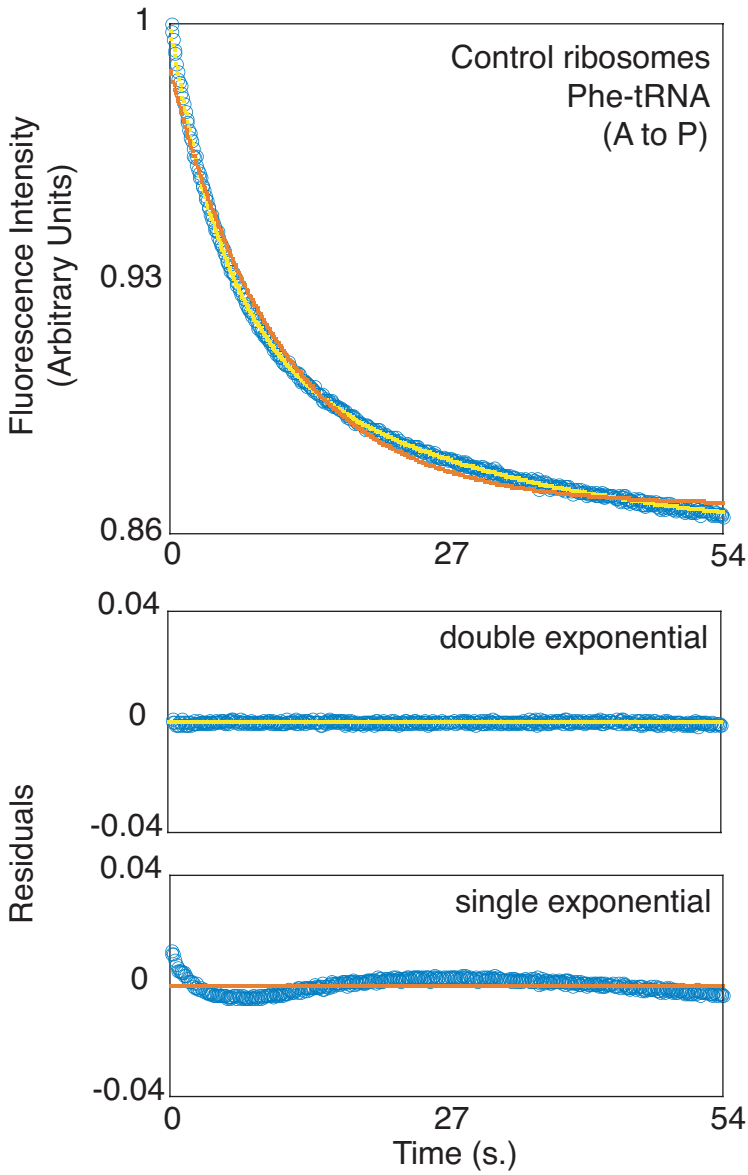

Fig. S8. Example of an experiment monitoring translocation of pyrene-labeled mRNA paired to Phe-tRNA^{Phe} in control (wild-type) ribosomes. In this experiment, syringe 1 was loaded with PRE complexes programmed with m432 and containing tRNA^{Tyr} in the P site and Phe-tRNA^{Phe} in the A site. Syringe 2 contained EF-G (6 μM) and GTP (1 mM). This figure was annotated as described for Fig. S2.

Other Supporting Information Files

SI Appendix (PDF)