Supplementary Methods

L11 cloning, overproduction and purification

The *rplK* ORF coding for ribosomal protein L11 was amplified from genomic *E. coli* DNA by polymerase chain reaction (PCR) using forward primer 5'-TCGAGGCATATGGCTAAGAAAGTACAAGCCTAT-3' and reverse primer 5'-CTTATACTCGAGTTAGTCCTCCACTACCAGG-3'. Primers were designed to introduce restriction sites for NdeI and XhoI endonucleases for the purpose of cloning the PCR fragment into the high copy number vector pET-28a (Novagen).

The pET-28a vector containing the *rplK* gene was used to transform *E. coli* strain BL21(DE3) for further L11-His6 protein overproduction. Cell cultures were grown overnight at 37°C at 210 rpm shaking, diluted 100 times to inoculate LB medium supplemented with 25 μg/mL kanamycin and grown at 37°C. Upon reaching optical density A_{600} of 0.4-0.6 cell cultures were induced with 1 mM IPTG, grown for an additional 3-4 hours and then cells were harvested by centrifugation.

For purification, the cell pellet was resuspended in cell opening buffer (25 mM Tris pH 7.5, 150 mM NaCl, 2 mM $MgCl_2$, 1 mM β-ME) supplemented with 1 μg/ml DNAase I (Sigma) and 0.2 mM PMSF (Sigma). Cells were disrupted using a High Pressure Homogenizer (EmulsiFlex-C3, Avestin), and the lysate was clarified by centrifugation for 30 min at 15 000 rpm. The supernatant was loaded onto a Ni-NTA agarose prepacked column (HisTrap FF, GE Healthcare) equilibrated in

washing buffer (25 mM Tris pH 7.5, 150 mM NaCl, 2 mM $MgCl_2$, 5 mM imidazole, 1 mM β-ME). The column was washed with 5-10 volumes of washing buffer, followed by 3-5 volumes of washing buffer supplemented with 1M NaCl in order to remove contaminating high Mw nucleic acids. Protein was eluted by a linear imidazole gradient up to the final concentration of 300 mM in washing buffer. Fractions containing L11-His6 were pooled together, dialyzed against the Polymix buffer [1] and concentrated with centrifugal filters (Amicon Ultra, Ultra-15, MWCO 10 kDa, Sigma).

Western blot analysis

RelA-His6 protein-containing samples were transferred to a nitrocellulose membrane (Invitrogen) using the Dot Blot system (The Convertible, Biometra). The membrane was blocked for 1 hour at room temperature by shaking with PBST buffer (50 mM TrisHCl pH 7.5, 150 mM NaCl, 0.1% Tween-20) containing 5% non-fat dry milk powder. Blots were then incubated with primary antibodies recognizing His6 tag (Quattromed) diluted 1:1000 in PBST buffer containing 2% NFDM powder for 1 hour at room temperature with shaking, followed by three washes (5 min each) with PBST. Next, the membrane was incubated with goat anti-mouse HRP-conjugated antibody (Icosagen) diluted 1:10000 in PBST buffer with 2% NFDM for 1 hour at room temperature. After washing three times with PBST (5 min each), blots were developed with ECL Plus reagent (Amersham). Specificity of the Anti-His6 antibodies and absence of the cross-reactivity with other components of the *in vitro* system was verified by the SDS PAGE followed by Western blotting (data not shown).

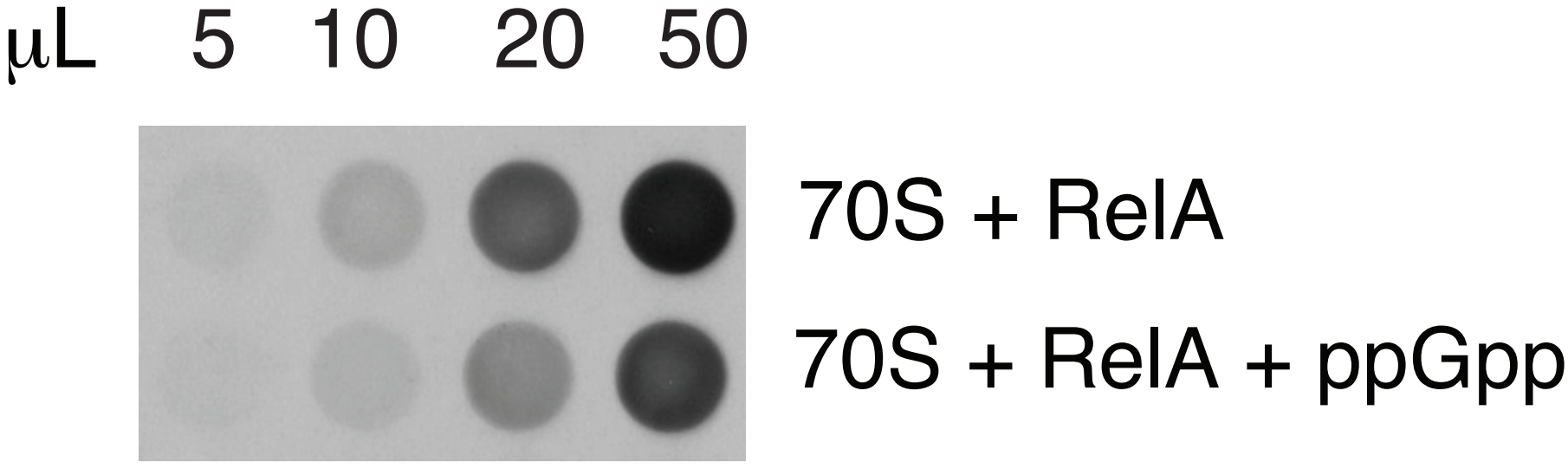
Supplementary Figure legends

Fig S1 | Analysis of RelA binding to 70S in the presence and absence of 100 μM ppGpp. 70S ribosomes were separated from RelA using an S-300 analytical Gel-filtration column. Increasing volumes (5, 10, 20 and 50 μL) were transferred to the membrane and subjected to quantification by anti-6His Western blotting.

Fig S2 | Restoration of ΔL11 70S functionality by addition of L11 protein. Turnover rates of ppGpp production in the presence of 100 μM ppGpp were measured in the presence of 0.5 μM wild type 70S (red fill), 0.5 μM ΔL11 70S (gray fill), 15 μM L11 protein (dashed bar stroke) and in the absence of 70S (no fill). Error bars represent standard deviations of the turnover estimates by linear regression, each experiment was performed at least three times.

Supplementary references:

1. Jelenc PC, Kurland CG (1979) Nucleoside triphosphate regeneration decreases the frequency of translation errors. *Proc Natl Acad Sci U S A* **76:** 3174-3178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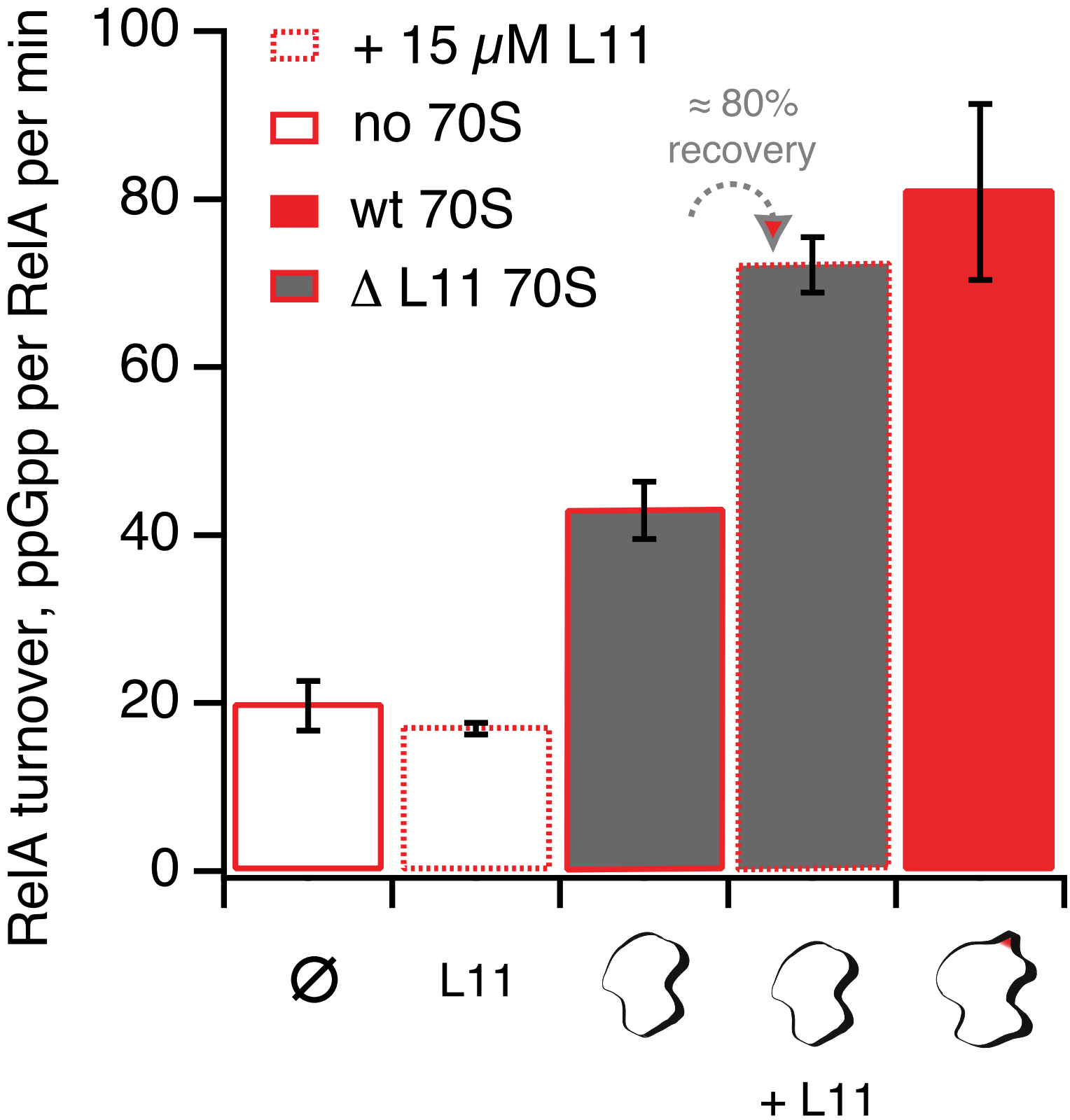
70S + RelA
70S + RelA + ppGpp


RelA turnover, ppGpp per RelA per min
100
80
60
40
20
0
+ 15 μM L11
no 70S
wt 70S
Δ L11 70S
≈ 80%
recovery
Ø
L11
+ L11