Supplementary Information for:

Title: GTPases IF2 and EF-G bind GDP and the SRL RNA in a mutually exclusive manner

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Supporting information for Mitkevich et al.

SI Methods:

Cloning, overexpression and purification of E. coli L7/L12

The *E. coli rplL* gene encoding L7/L12 protein was amplified from *E. coli* genomic DNA using forward primer 5' TCGAGGCATATGTCTATCACTAAAGATCAAATCA 3' and reverse primer 5' CTTATCCTCGAGTTATTTAACTTCAACTTCAGCG 3'. Primers were designed to introduce restriction sites for NdeI and XhoI endonucleases for cloning the PCR fragment into high copy number vector pET-28a (Novagen). A pET-28a containing *rplL* gene was used to transform *E. coli* strain BL21(DE3) for L7/L12-His_6 protein overproduction. Fresh selective LB medium was inoculated with an overnight culture, and incubated at 37°C 2-3 h with 200 rpm shaking, and expression was induced at optical density A_{600} 0.4-0.6 by addition of 1mM IPTG. Cells were grown for an additional 3-4 h, harvested by centrifugation, 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 ug/ml DNAase I (Sigma) and 0.2 mM PMSF and disrupted by a microfluidizer. Cell lysate was clarified by centrifugation at 15 000 rpm for 30 min. The supernatant was loaded on a prepacked Ni-NTA agarose column (HisTrap FF Column, GE Healthcare) equilibrated in the washing buffer A (25 mM Tris pH 7.5, 150 mM NaCl, 2 mM $MgCl_2$, 5 mM imidazole, 1 mM β-ME), followed by first, a washing step of 5-10 volumes of washing buffer A, and, second, a washing step of 3-5 volumes of the washing buffer B (25 mM Tris pH 7.5, 1M NaCl, 2 mM $MgCl_2$, 5 mM imidazole, 1 mM β-ME). Protein was eluted with a linear imidazole gradient up to 100% final

concentration of elution buffer (25 mM Tris pH 7.5, 150 mM NaCl, 2 mM $MgCl_2$, 300 mM imidazole 1 mM β-ME). Fractions containing L7/L12-His_6 protein were collected and dialyzed against Polymix buffer [1] and concentrated by centrifugal filters (Amicon Ultra, Ultra-15, MWCO 10 kDa, Sigma). Homogeneity and purity of the final protein sample were assessed by SDS-PAAG electrophoresis and spectroscopy. Protein concentration was estimated with the Bradford assay. From the absence of L7/L12 monomers in our gel filtration analysis we concluded that addition of N-terminal 6His did not inhibit L7/L12 dimerization.

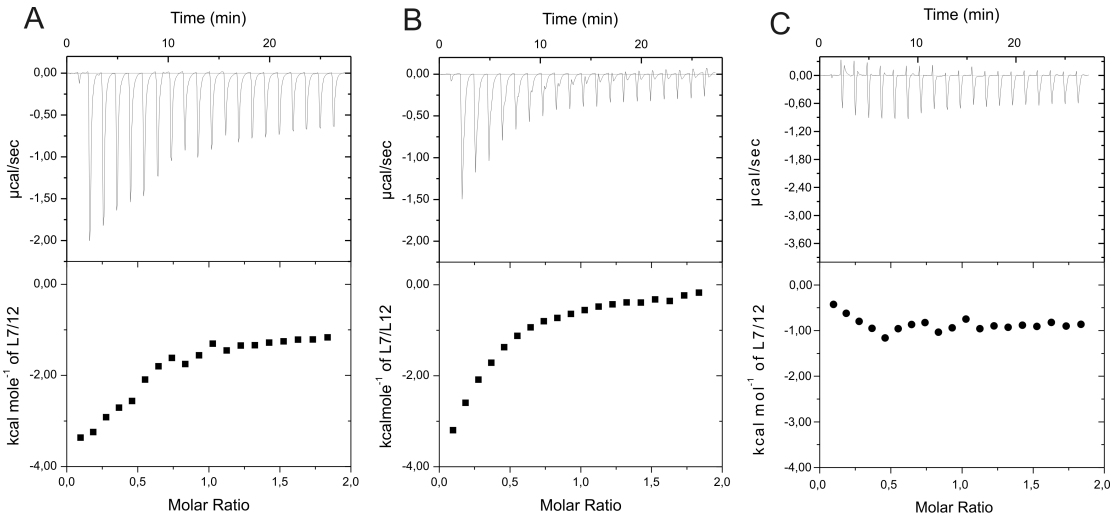

Figure S1: EF-G and L7/12 interact weakly. ITC titration curves (upper panel) and binding isotherms (lower panel) for L7/12 titration into EF-G (A), L7/12 dilution in to the buffer (B) and L7/12 titration into to EF-G after subtraction of L7/12 dilution (C).

SI Reference:

1 Antoun, A., Pavlov, M. Y., Tenson, T. & Ehrenberg, M. M. Ribosome formation from subunits studied by stopped-flow and Rayleigh light scattering. *Biol Proced Online* 6, 35-54, (2004).