Supplementary Material for

Functional analysis of BipA in *E. coli* reveals the natural plasticity of 50S subunit assembly

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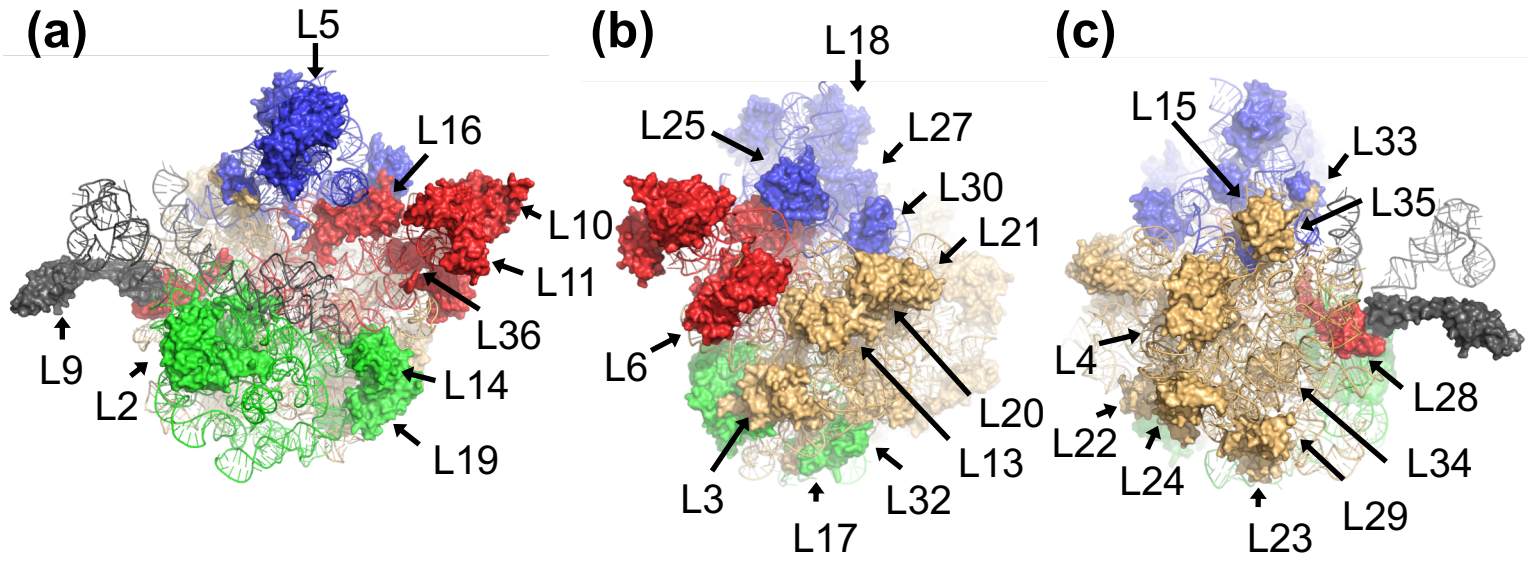

Figure S1. Assembly blocks of the large subunit (LSU). Interface (a), A-site side (b), and E-site side (c) views of the subunit (PDB 4YBB), with proteins in the foreground labeled. RNA, cartoon ladder; protein, surface representation. Color-coding is based on experimental evidence (Davis *et al.* 2016) or inferred from the structure of the mature LSU. Block 1, tan; block 2, blue; block 3, green; block 4, red; flexible/unassigned, gray.

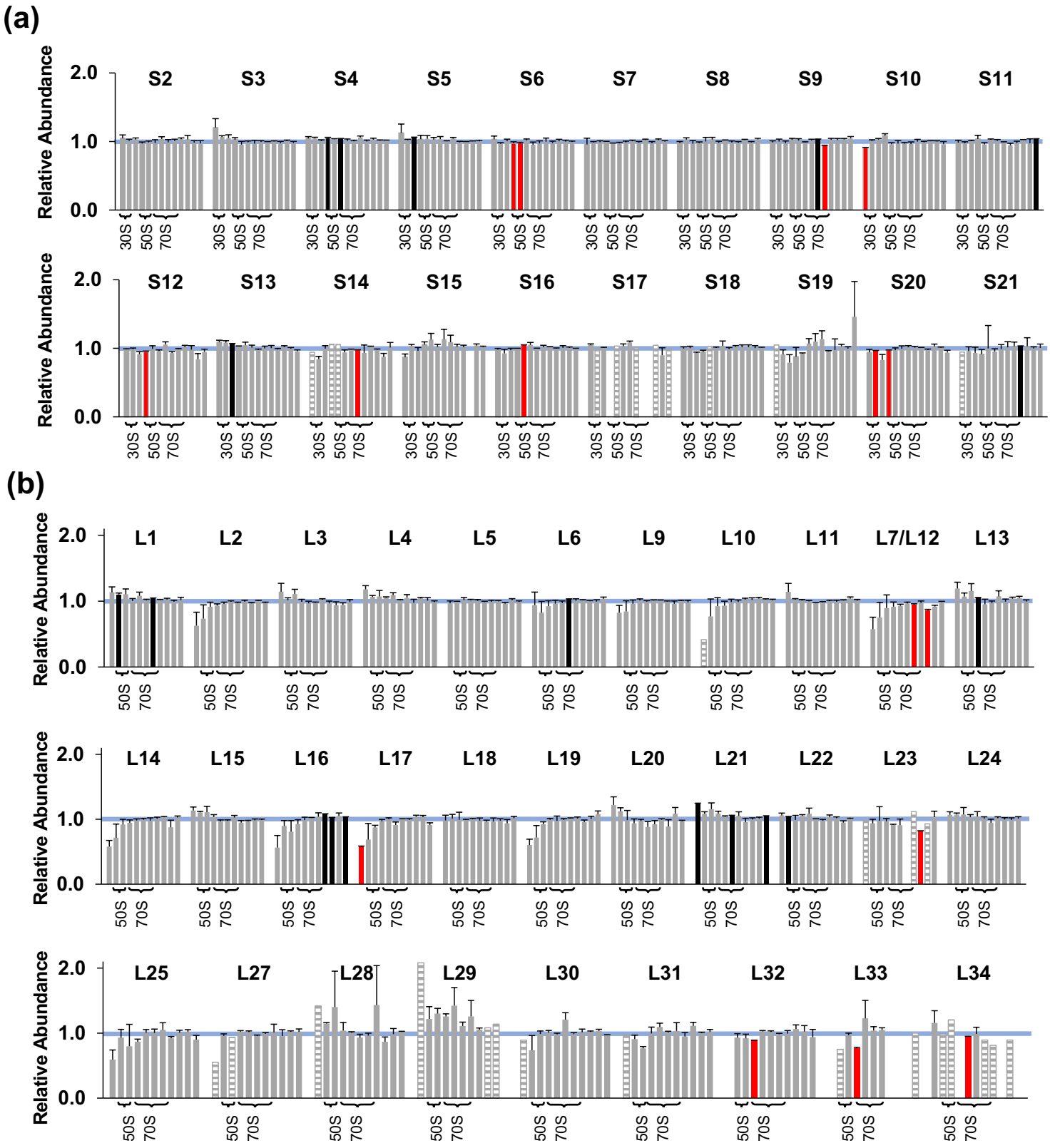

Figure S2. Ribosomal protein composition of SSU (a) and LSU (b) particles in the mutant *ΔbipA* strain grown at 37 °C. Shown are normalized isotope ratios (*ΔbipA*/WT) in fractions 6-18 (a) and fractions 8-18 (b) with fractions 6-7, 9-10, and 11-14 containing the 30S, 50S, and 70S respectively (as indicated). Data represents mean ± SEM from three independent experiments. Full datasets are included in Table S1. Red and black bars indicate values ≠1.0 based on Student's t test (uncorrected p value < 0.05). Striped bars indicate cases in which only one measurement was obtained. The blue line marks 1.0.

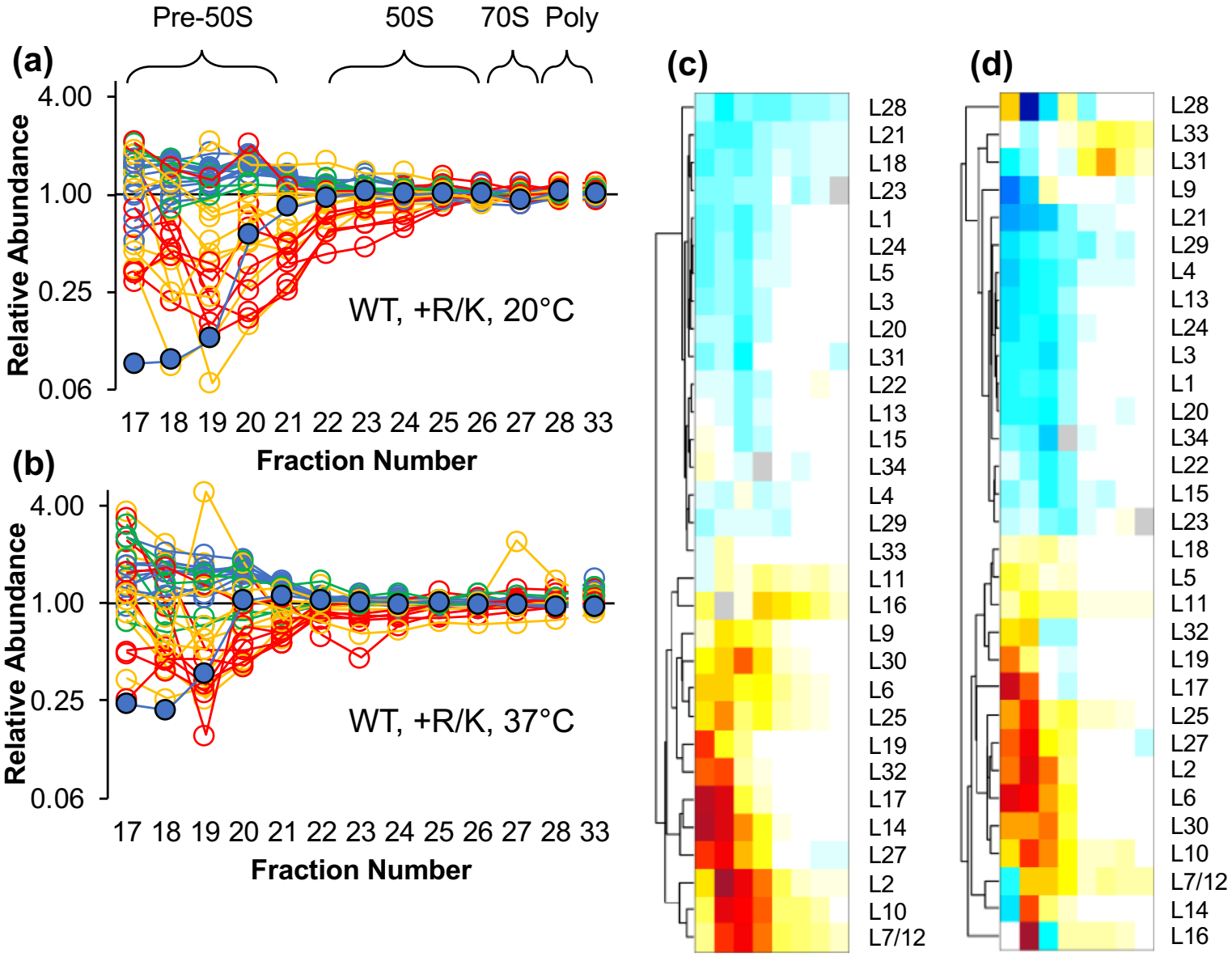

Figure S3. Effect of temperature on WT cells grown with arginine and lysine supplementation. Plotted are the normalized isotope ratios indicating the relative abundance of each LSU protein in each fraction (17-28, 33) from WT cells grown at 20 °C (a) or 37 °C (b) across the sucrose gradient. Color coding corresponds to temporal stages of assembly as defined by Chen and Williamson, 2013, (blue, early; green, middle; yellow, middle-late; red, late). L17 is highlighted with a black outline. Data represent the mean of 3 independent experiments. Full datasets are included in Table S1. Hierarchical clustering of the data in (a) and (b) is shown in (c) and (d), respectively. Color scale as in Figure 1(d).

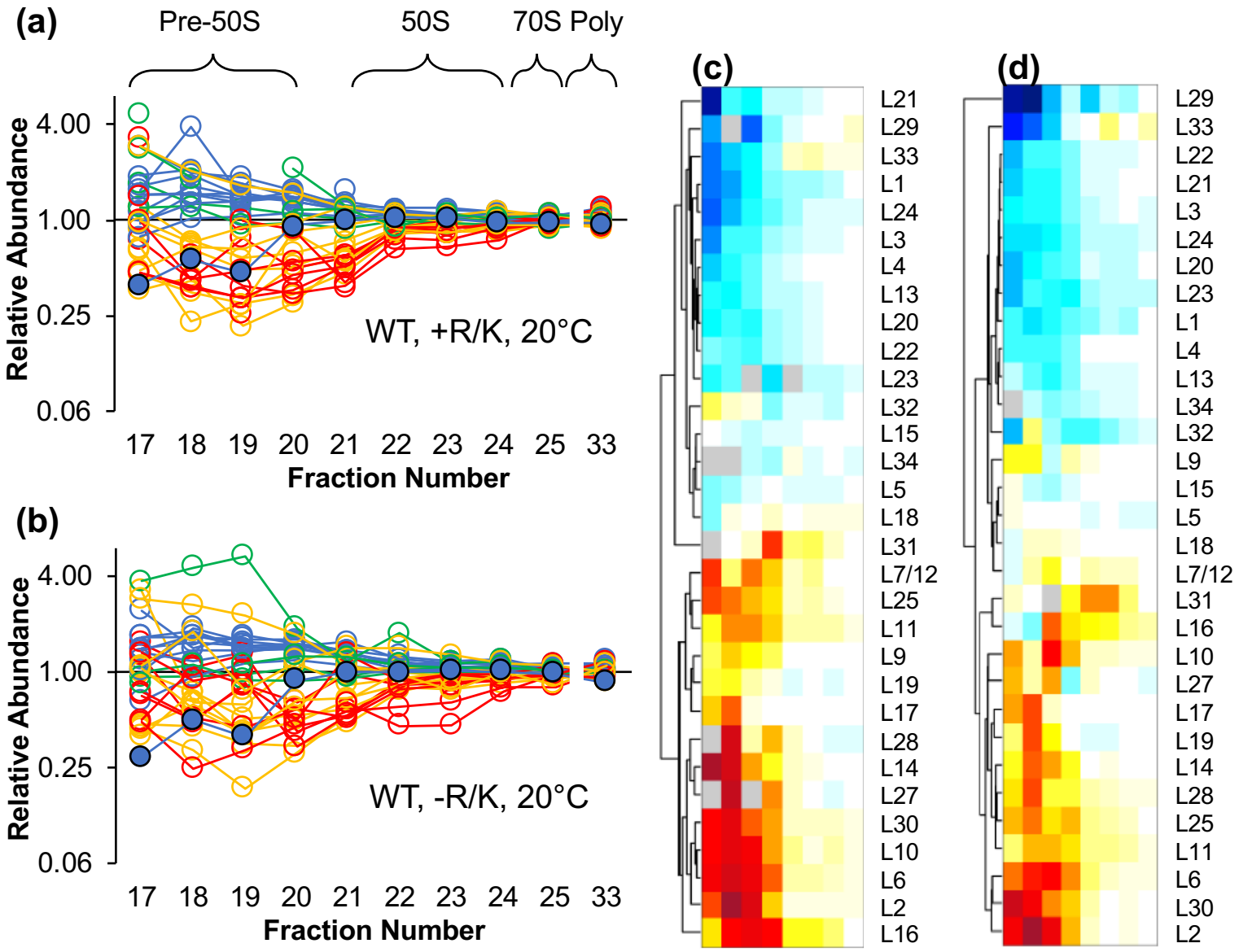

Figure S4. Evaluation of LSU assembly in WT cells grown at suboptimal temperature. Shown are the relative abundance of LSU proteins in various ribosomal particles from the WT prototroph grown at 20 °C with (a) or without (b) arginine and lysine. Color coding is based on temporal stages defined by Chen and Williamson, 2013 (blue, early; green, middle; yellow, middle-late; red, late). L17 is highlighted with a black outline. Data represent the mean of 3 independent experiments. Full datasets are included in Table S1. Hierarchical clustering of the data in (a) and (b) is shown in (c) and (d), respectively. Color scale as in Figure 1(d).

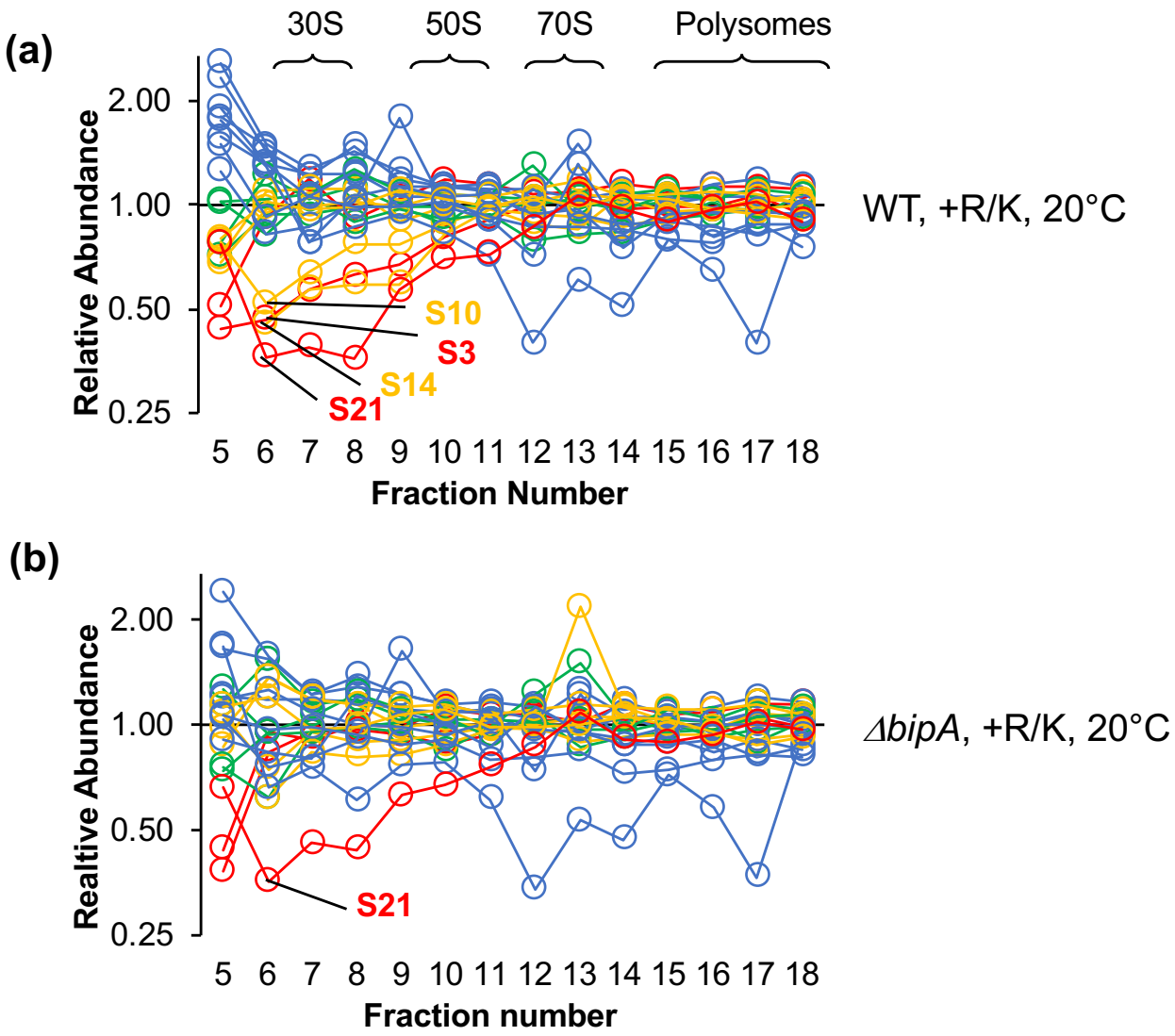

Figure S5. Abundance of small subunit proteins in cells grown at 20 °C. Plotted are the normalized isotope ratios indicating the relative abundance of each SSU protein in each fraction (5-18) from WT (a) and *ΔbipA* (b) cells. Color coding corresponds to temporal stages of assembly as defined by Chen and Williamson, 2013, (blue, early; green, middle; yellow, middle-late; red, late). Data represent the mean of 3 independent experiments. Full datasets are included in Table S1.

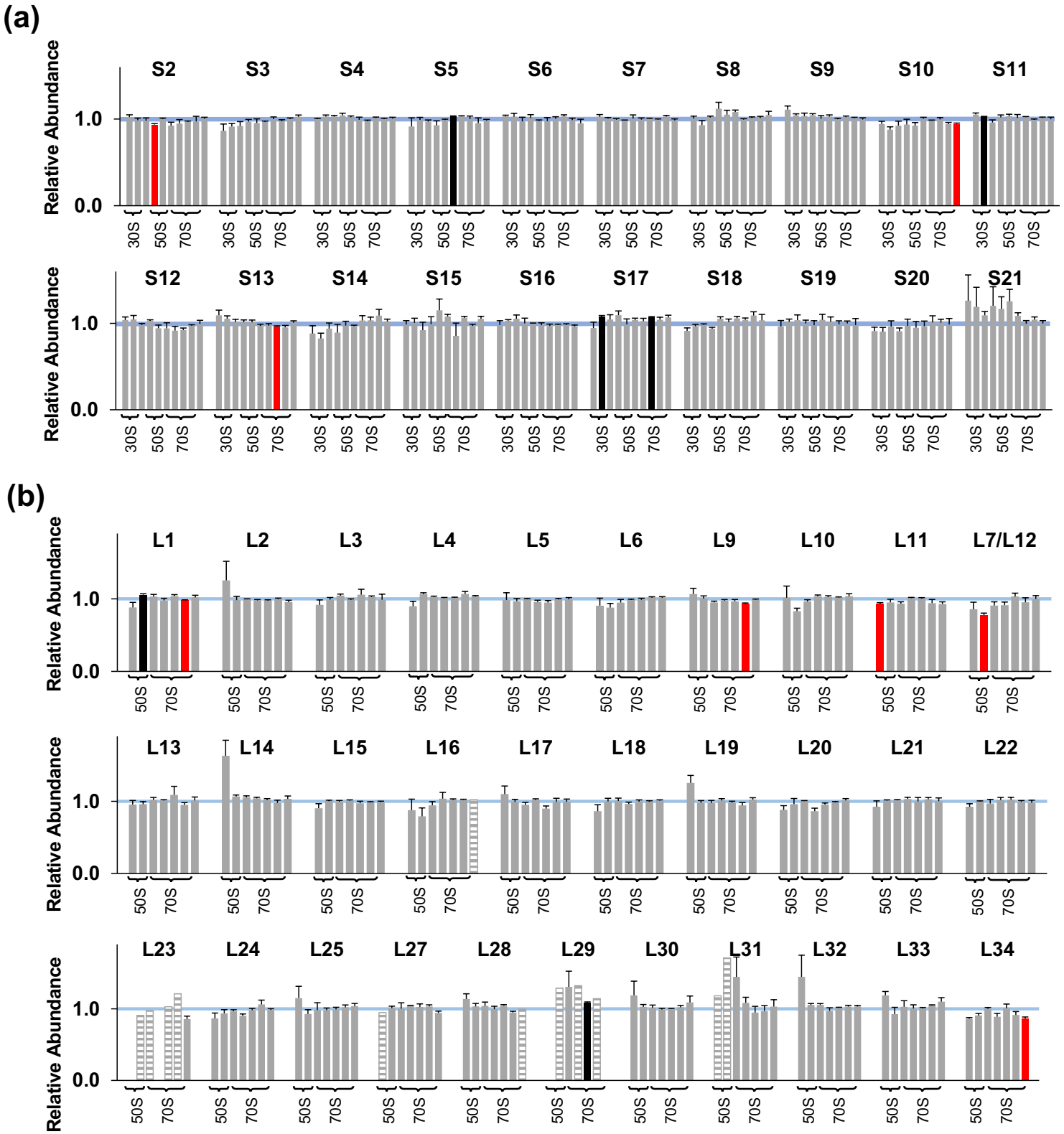

Figure S6. Mutation *ΔychF* has no obvious effect on ribosome assembly. Shown are normalized isotope ratios (*ΔychF*/WT) in fractions 6-15 (a) and fractions 9-15 (b) with fractions 6-7, 9-10, and 11-14 containing the 30S, 50S, and 70S respectively (as indicated). Data represents mean ± SEM from three independent experiments. Full datasets are included in Table S1. Red and black bars indicate values ≠1.0 based on Student's t test (uncorrected p value < 0.05). Striped bars denote cases where only one measurement was obtained. The blue line marks 1.0.

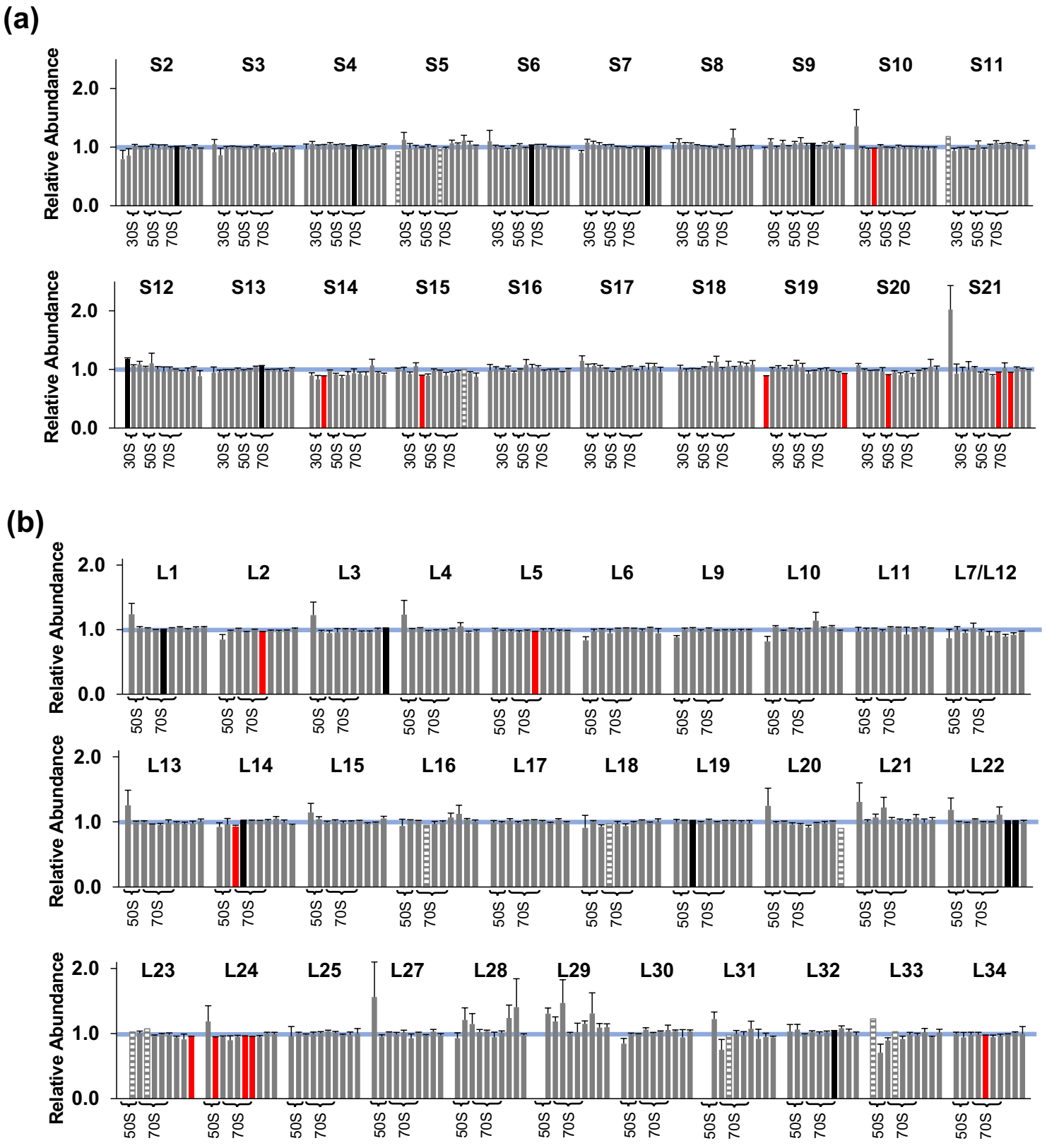

Figure S7. Mutation *ΔhflX* has no obvious effect on ribosome assembly. Shown are normalized isotope ratios (*ΔhflX*/WT) in fractions 5-18 (a) and fractions 9-18 (b) with fractions 6-7, 9-10, and 11-14 containing the 30S, 50S, and 70S respectively (as indicated). Data represents mean ± SEM from three independent experiments. Full datasets are included in Table S1. Red and black bars indicate values ≠1.0 based on Student's t test (uncorrected p value < 0.05). Striped bars denote cases where only one measurement was obtained. The blue line marks 1.0.

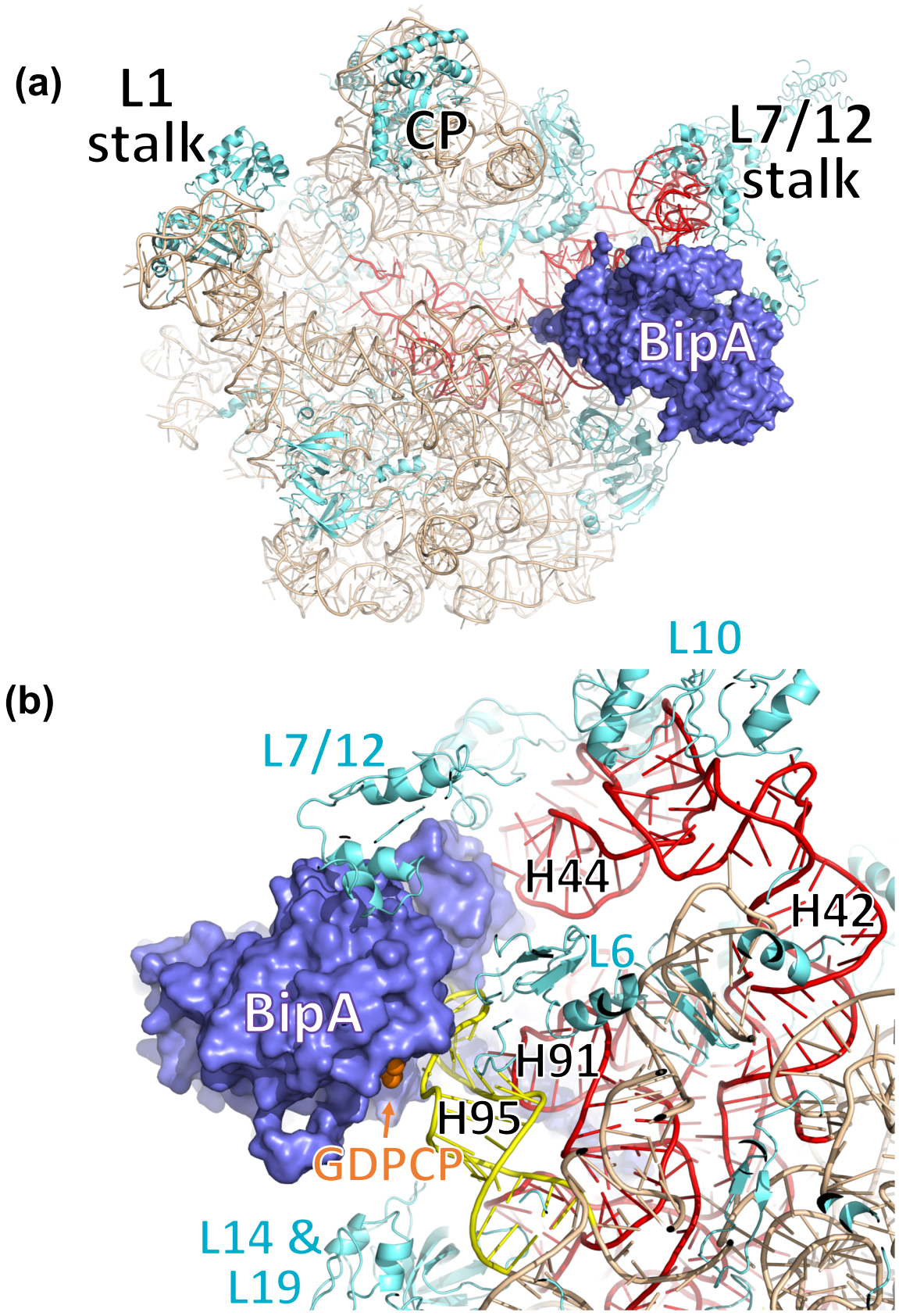

Figure S8. Interactions between BipA and the large subunit (LSU). Structure of BipA·GDPCP bound to the ribosome (PDB 5A9Z), with the small subunit computationally removed to reveal the BipA-LSU contacts. (a) Overview from the subunit interface perspective, with the central protuberance (CP), L1 stalk, and L7/12 stalk indicated. Block 4 rRNA is highlighted in red. (b) Close-up view, orthogonal to that above, showing BipA contacts to the sarcin-ricin loop (SRL) of H95 (yellow, part of block 1) and rRNA helices of block 4. Various helices and proteins in the foreground are indicated.