Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Figure S1 (1) Sequence alignment of L31 (70 amino acids) and its paralog YkgM (87 amino acids). **(2)** Sequence alignment of L36 (38 amino acids) and its paralog YkgO (46 amino acids). Shaded boxes indicate conserved amino acids. Arrow for (1) and dots in L31 and L36 show the site cleaved by protease 7 and the zinc-binding site, respectively.

Figure S2 Depletion of L31 inhibits bacterial growth in both liquid EP and solid LB media, but the *zur* mutant rescues growth inhibition. **(A)** BW25113 (white circles), BW25113 Δ*rpmE::Km* (white triangles), and BW25113 Δ*rpmE::Km zur* (T200A) (black triangle) cells were cultured in EP medium at 37°C. Cell growth was monitored

by measuring turbidity (Klett units). The time scales are 0–8 h (1) and 1–8 days (2). The vertical axis shows normal logarithmic values for 0–7 h and a linear scale for 1–6 days. **(B)** Colony-forming unit (CFU) values of the three strains listed in (A). Cultured cells were harvested every day over 1–6 days of cultivation, and the CFU value was measured. **(C)** Bacterial colonies growth on LB solid medium. BW25113, BW25113 Δ*rpmE::Km*, and BW25113 Δ*rpmE::Km zur* (T200A) were incubated overnight (16–18 h) at 37°C on solid LB medium, and the sizes of the resultant colonies are shown.

Figure S3 Ribosomes prepared from BW25113 Δ*rpmE::Km zur* (T200A) cells contain one copy of YkgM. **(A)** BW25113 Δ*rpmE::Km* and Δ*rpmE::Km zur* (T200A) cells were grown at 37°C in EP medium and collected in logarithmic growth phase (Klett units: 50), and high-salt–washed ribosomes (HSRs) were prepared. HSR proteins were analyzed by RFHR 2D-PAGE. Gels were stained with CBB, and the resultant patterns are shown. Spots corresponding to r-proteins S10, L21, L25, L29, L30, and YkgM are indicated. **(B)** YkgM is contained in the 50S subunit but not in the 30S subunit. BW25113 Δ*rpmE::Km zur* (T200A) cells were grown at 37°C in EP medium and

collected in logarithmic growth phase (Klett units: 50). HSRs were suspended in dissociation buffer I and dialyzed against the same buffer overnight, as described in Experimental Procedures. The sample was fractionated after 10–40% SDG centrifugation in dissociation buffer I, and 50S and 30S fractions were collected. The r-proteins in each fraction were analyzed by RFHR 2D-PAGE, and the resultant gel patterns are shown.

Figure S4 (A) L31-lacking HSRs dissociate into 30S and 50S subunits during SDG centrifugation in low Mg^{2+} conditions, but YkgM-containing HSRs form 70S. **(A)** BW25113 Δ*rpmE::Km* and Δ*rpmE::Km & zur* (T200A) cells were grown at 37°C in EP medium and collected in logarithmic growth phase (Klett units: 50). HSRs were prepared and analyzed by 5–20% SDG centrifugation with the indicated Mg^{2+} concentrations. The resultant ribosome profiles are shown. **(B)** Dissociated ribosomes (50S+30S) lacking L31 form unstable 70S *in vitro*, but ribosomes containing YkgM form stable 70S. HSRs were prepared from BW25113 Δ*rpmE::Km* and Δ*rpmE::Km zur* (T200A) cells containing one copy of YkgM (1). HSRs of both strains were dissociated

to 30S and 50S subunits (2). Dissociated HSRs were incubated at 37°C for 30 min to allow re-association. Samples (60 pmol) were analyzed by 5–20% SDG centrifugation in 6 or 15 mM Mg^{2+}. The resultant ribosome profiles are shown in the re-association panels (3).

Figure S5 Ribosomes lacking L31 are defective in 100S formation in stationary phase, but YkgM restores the function. BW25113 Δ*rpmE::Km zur* (T200A) cells were grown at 37°C for 1, 3, or 6 days in EP medium, and then harvested. Crude ribosomes (CRs) from each cell were prepared and analyzed by RFHR 2D-PAGE and 5–20% SDG centrifugation in 5 or 15 mM Mg^{2+}. Copy numbers per ribosome of YkgM (dark gray) and RMF (black) in each CR sample are shown in the bar graph. Ribosome profiles after 5–20% SDG centrifugation in 5 or 15 mM Mg^{2+} are shown in the lower panels. For each CR sample, 150 pmol was used.

Figure S6 (A) *rpmJ* deletion mutant formed small colonies, but a strain carrying a *rpmJ*+ plasmid formed normal colonies at 25, 37, and 42°C. W3110

Δ*rpmJ::Km/*pBAD22 and Δ*rpmJ::Km/*pBAD22-*rpmJ*$^{+}$ cells were grown overnight at 37°C. The cultures were diluted 1:10^{6} in saline and spread 0.1ml on L-broth plates; the plates were incubated for 1–4 days at 25, 37, and 42°C. Colony size on each plate was measured in photographs. **(B)** Cells depleted L36 inhibits bacterial growth in EP liquid medium at 37°C, but the *zur* mutant and pBAD22-*rpmJ*$^{+}$ cells recover the growth inhibition. W3110 Δ*ompT* (rhombus), Δ*ompT* Δ*rpmJ::Km* (square), Δ*ompT* Δ*rpmJ* Δ*zur::Km* (triangle) and Δ*ompT* Δ*rpmJ::Km* /pBAD22-*rpmJ*$^{+}$cells (cross) were cultured in EP or EP medium containing 0.02% Arabinose for Δ*ompT* Δ*rpmJ::Km* /pBAD22-*rpmJ*$^{+}$cells at 37°C. Cell growth was monitored by measuring turbidity (OD600 or Klett units). The time scales are 0–6 h (1) and 1–9 days (2). The vertical axis shows normal logarithmic values for 0–6 h and a linear scale for 1–9 days.

Figure S7 HSRs lacking L36, like those containing YkgO, do not dissociate into 30S and 50S subunits even during SDG centrifugation under low Mg^{2+} conditions. HSRs of W3110 Δ*ompT::Km*, Δ*rpmJ* Δ*ompT::Km*, and Δ*zur* Δ*rpmJ* Δ*ompT::Km* cells were grown at 37°C in EP medium and collected in logarithmic growth phase (Klett units:

50). Three HSRs were prepared from each strain and analyzed by 5–20% SDG centrifugation at the indicated Mg^{2+} concentrations. The resultant ribosome profiles are shown.

Figure S8 Ribosomes lacking L36 form 100S ribosomes in the stationary phase. W3110 Δ*ompT::Km*, Δ*ompT* Δ*rpmJ::Km*, and Δ*zur* Δ*rpmJ* Δ*ompT::Km* cells were grown at 37°C for 1 or 3 days in EP medium, and then harvested. Crude ribosomes (CRs) from each strain were prepared and analyzed by RFHR 2D-PAGE and 5–20% SDG centrifugation in 5, 8, or 15 mM Mg^{2+}. Copy numbers per ribosome of intact L31, L36, YkgM, YkgO, or RMF (black) in each CR sample are shown in the bar graph. Ribosome profiles after 5–20% SDG centrifugation in 5, 8, or 15 mM Mg^{2+} are shown in the lower panels. For each CR sample, 150 pmol was used.

Figure S9 r-protein patterns of 50S and 40S particles prepared from the *rpmJ* deletion mutant cells. 50S and 40S particles fractionated from W3110 Δ*ompT* Δ*rpmJ::Km* cells were the same as those described in legends of Fig. 9A,B. The r-proteins of the

collected 40S or 50S fractions were analyzed by RFHR 2D-PAGE. The upper panels show the RFHR 2D-PAGE gel patterns of the 50S fraction (left) and the 40S fraction (right). The middle panels show expanded gels of the 50S fraction (left) and the 40S fraction (right), respectively. The lower panel shows the expanded gel of wild-type 50S (Ueta *et al*, 2017).

Table S1

Bacterial strains used in this study.

Table S2

Primers used to determine mutation sites in *ykgM* or *zur*.

Table S3

Distribution of genes encoding zinc-binding motifs in r-proteins (L31, L36, L33, S14, S4, S18, L28, and L32) in bacterial and organellar genomes.

C^+ and C^- indicate zinc binding and zinc-free, respectively.

References for supplemental materials

Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., ….Mori, H. (2006) Construction of *Escherichia coli* K-12 in-frame, single-gene knock-out mutants the Keio collection. *Mol. Systems Biol.,* 2*,* 2006. 0008. https://doi:10.1038/msb4100050.

Ueta, M., Wada, C., Bessho, Y., Maeda, M., & Wada, A. (2017) Ribosomal protein L31 in *Escherichia coli* contributes to ribosome subunit association and translation, whereas short L31 cleaved by protease 7 reduces both activities. *Genes Cells*, 22(5), 452-471. https://doi: 10.1111/gtc.12488.