Supporting Information

Peil et al. 10.1073/pnas.1310642110

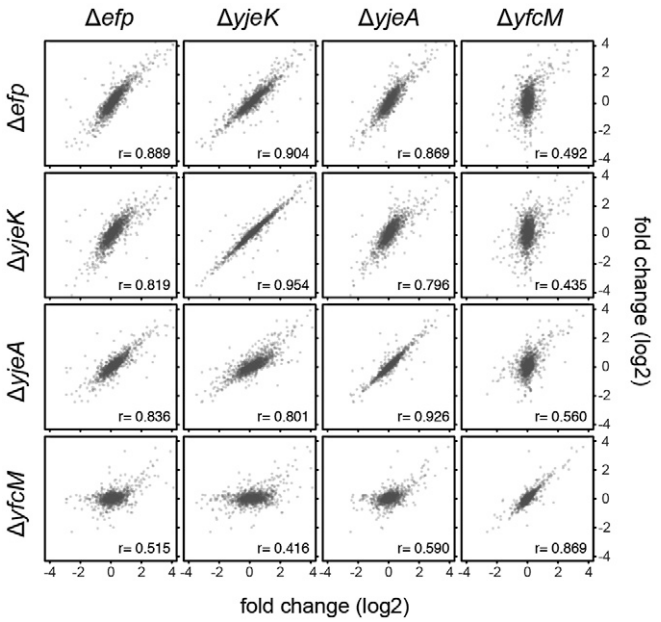

Fig. S1. Correlation for biological replicates of proteomics data from Δ*efp*, Δ*yjeK*, Δ*yjeA*, and Δ*yfcM* strains. Scatter plots showing correlation of inverted normalized heavy/light (H/L) ratios (log2-transformed) for biological replicates of SILAC (stable isotope labeling by amino acids in cell culture) data from the Δ*efp*, Δ*yjeK*, Δ*yjeA*, and Δ*yfcM* strains.

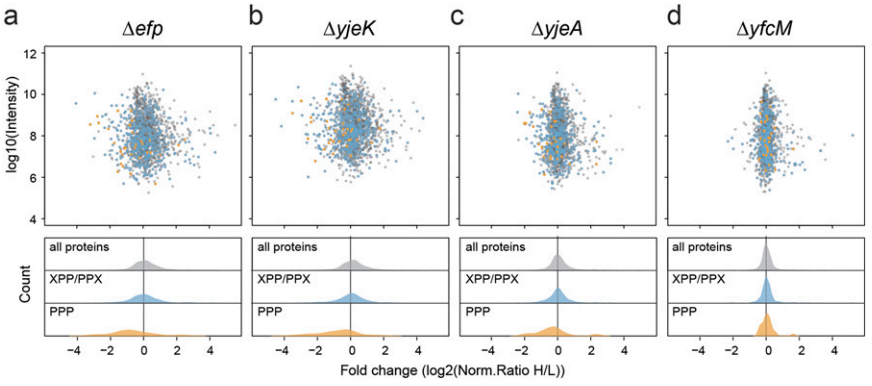

Fig. S2. Scatter plots of proteomics data from replicate 2 of Δ*efp*, Δ*yjeK*, Δ*yjeA*, and Δ*yfcM* strains. (*A*–*D*) Scatter plots of inverted normalized H/L ratios (log2-transformed) relative to the summed up protein intensity for a biological replicate of SILAC data from the Δ*efp* (*A*), Δ*yjeK* (*B*), Δ*yjeA* (*C*), and Δ*yfcM* (*D*) strains, including density plots showing distributions of PPP-containing (gold) and XPP/PPX-containing (blue) proteins relative to all proteins (gray).

Fig. S3. Gene Ontology (GO) analysis of complete proteomics data from Δ*efp*, Δ*yjeK*, Δ*yjeA*, and Δ*yfcM* strains. Heat map representation of hierarchical clustering of all GO terms that are up-regulated (top 25% quantile) or down-regulated (bottom 25% quantile), or remain unchanged (25–75% quantile). Color key as indicated: Gray indicates fewer than three genes; white indicates a *P* value of >0.05; different shades of red indicate increasing significance of *P* values, from salmon (<0.05) to red (<0.00005).

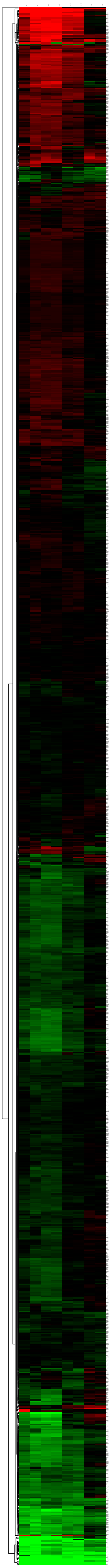

Fig. S4. Hierarchical clustering of complete proteomics data from Δ*efp*, Δ*yjeK*, Δ*yjeA*, and Δ*yfcM* strains. Heat map representation of hierarchical clustering of all proteins that are up-regulated (red) and down-regulated (blue) in the biological replicates of Δ*efp*, Δ*yjeK*, Δ*yjeA*, and Δ*yfcM* strains relative to wild-type strain.

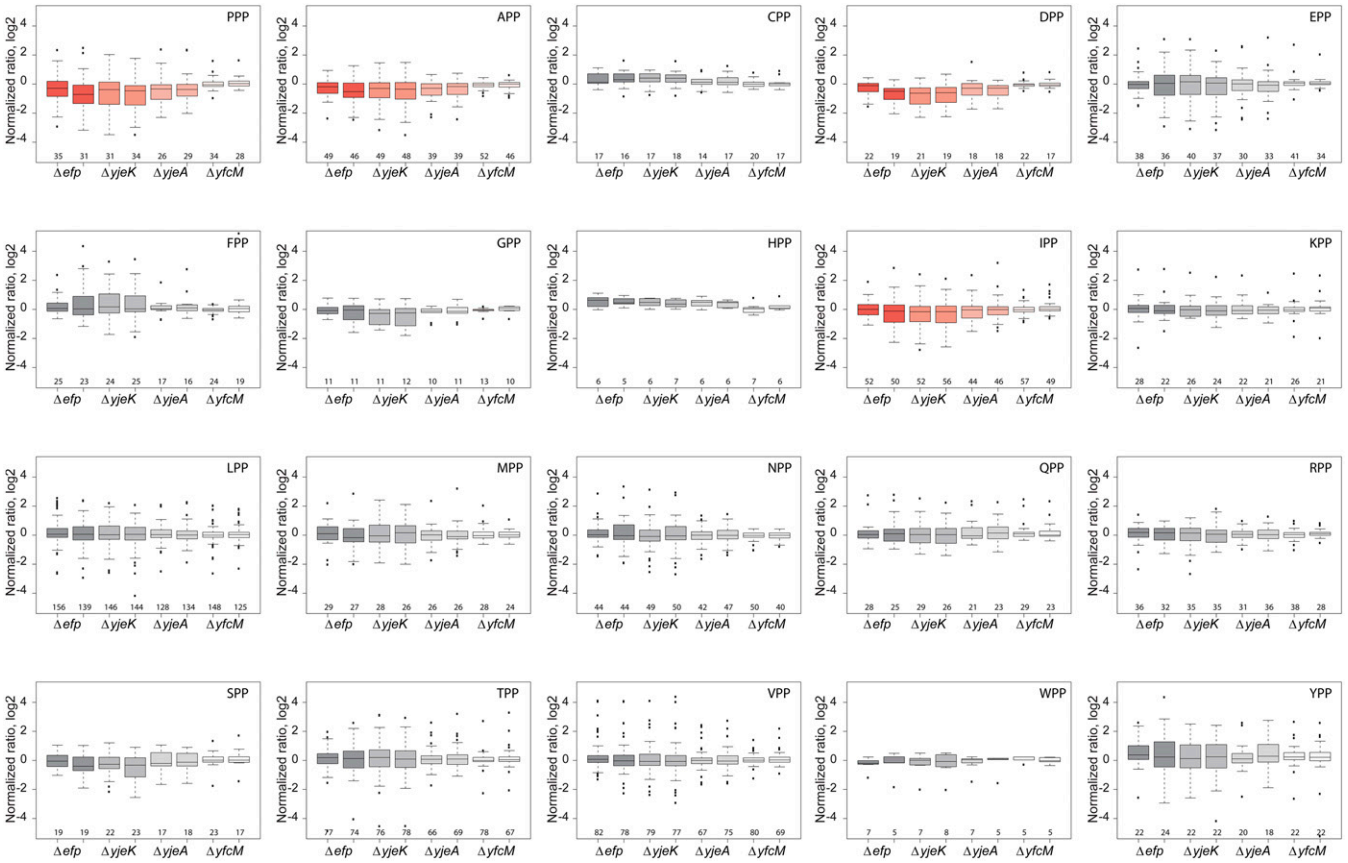

Fig. S5. Box plot representations of inverted normalized H/L ratios (log2-transformed) for proteins containing XPP motifs for Δ*efp*, Δ*yjeK*, Δ*yjeA*, and Δ*yfcM* strains.

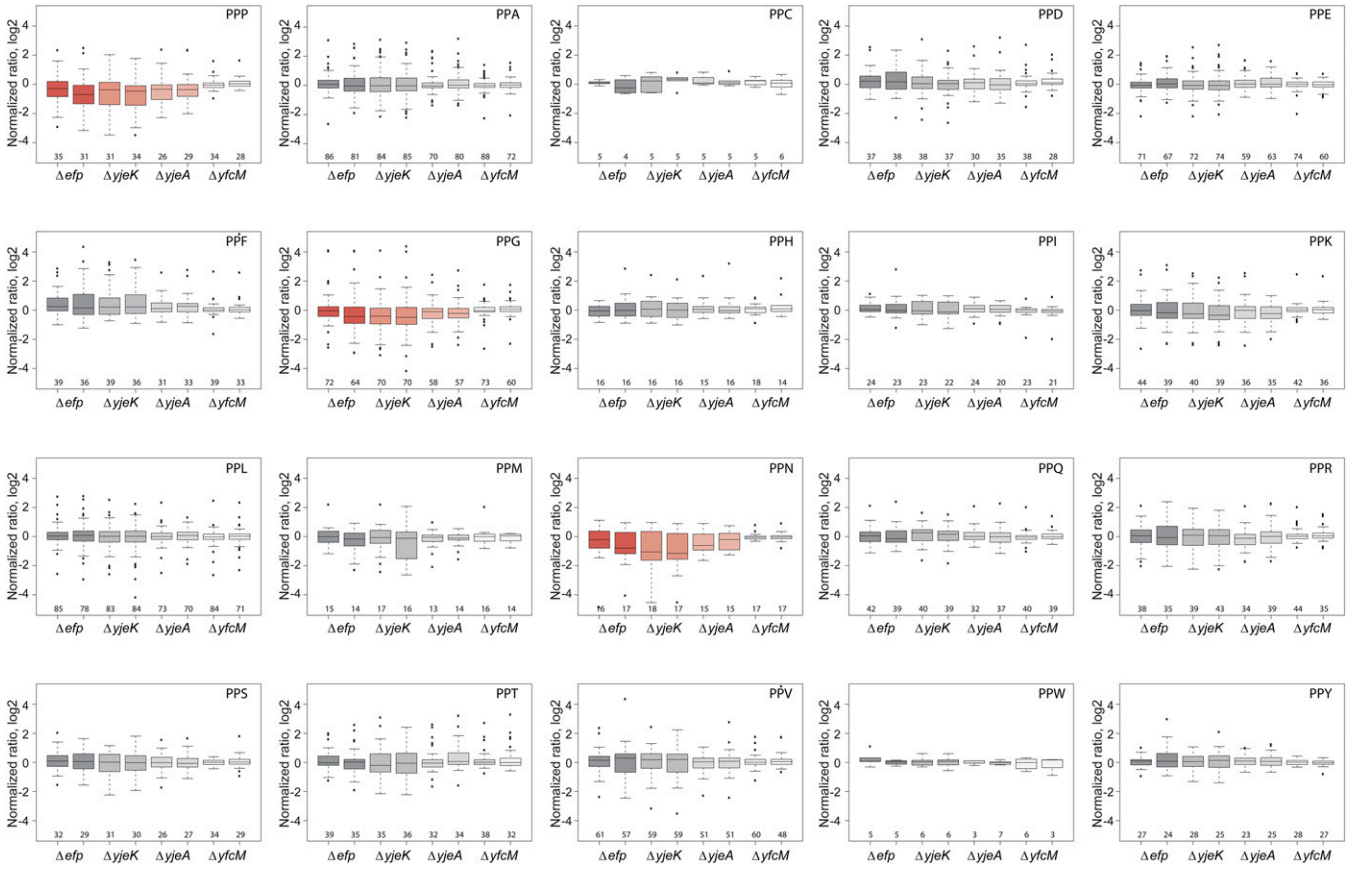

Fig. S6. Box plot representations of inverted normalized H/L ratios (log2-transformed) for proteins containing PPX motifs for Δ*efp*, Δ*yjeK*, Δ*yjeA*, and Δ*yfcM* strains.

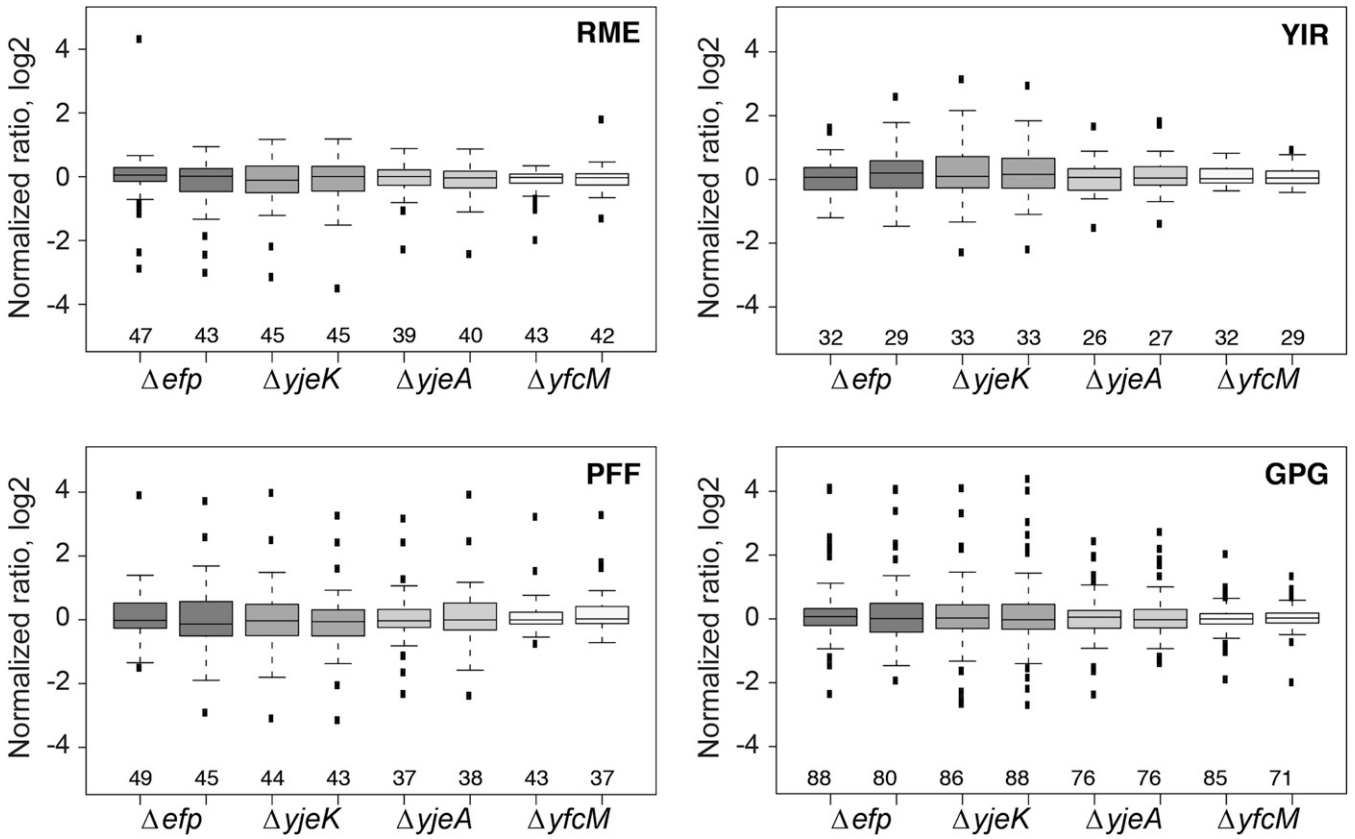

Fig. S7. Box plot representations of inverted normalized H/L ratios (log2-transformed) for proteins containing RME, YIR, PFF, and GPG motifs for Δ*efp*, Δ*yjeK*, Δ*yjeA*, and Δ*yfcM* strains.

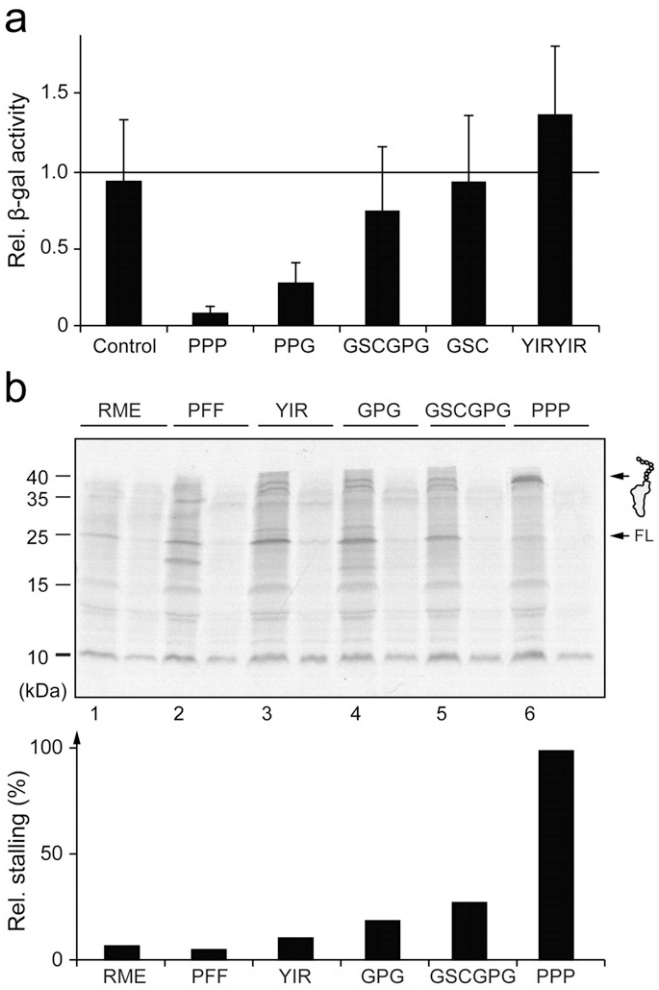

Fig. S8. Translation of non-XPPX motifs in the absence of elongation factor P (EF-P). (*A*) β-galatosidase activities (normalized in wild-type *Escherichia coli* strains relative to Δ*efp* strain) of LacZ constructs containing control (no stalling motif), PPP, or PPG, compared with LacZ constructs containing YIRYIR or PoxB motifs GSCGPG or GSC. (*B*) Autoradiographs of SDS–polyacrylamide gels indicating [^{35}S]Met-labeled in vitro translation of reporters containing (lanes 1–6) RME, PFF, YIR, GPG, GSCGPG compared with PPP. Reactions were performed in the absence (–) of active EF-P. Expected peptidyl-tRNA and full-length (FL) product sizes are indicated. The bar graph presents the relative stalling, which is calculated as the amount of stalled peptidyl-tRNA product/(stall + FL product). All relative stalling values are presented relative to that determined for PPP, which was assigned as 100%.

Table S1. Kruskal–Wallis significance for protein ratio distribution differences between the motif subsets and complete dataset

Motif	efp-1	efp-2	yjeK-1	yjeK-2	yjeA-1	yjeA-2	yfcM-1	yfcM-2
PPP	1.31E-04	1.80E-06	7.37E-05	5.70E-08	2.81E-05	1.08E-04	3.98E-01	9.78E-01
APP	3.31E-06	1.94E-08	9.03E-08	2.99E-07	5.71E-06	8.24E-05	2.02E-02	5.76E-02
CPP	1.64E-01	1.26E-01	1.98E-01	9.46E-02	3.32E-01	3.12E-01	8.68E-01	5.63E-01
DPP	1.03E-03	1.03E-06	1.76E-07	2.37E-06	8.33E-04	2.96E-04	1.03E-01	3.01E-01
EPP	3.04E-02	1.93E-01	2.81E-01	1.24E-01	3.91E-01	5.81E-02	7.00E-01	5.91E-01
FPP	8.91E-01	9.71E-01	5.59E-01	6.53E-01	6.58E-01	4.52E-01	1.53E-01	9.89E-01
GPP	1.77E-01	1.64E-01	7.87E-03	7.55E-03	9.49E-02	1.63E-01	2.55E-01	8.24E-01
HPP	3.65E-02	1.16E-01	1.88E-01	2.08E-01	2.33E-02	3.83E-02	7.05E-01	2.23E-01
IPP	3.85E-02	8.82E-04	4.19E-05	3.92E-05	1.64E-02	4.01E-02	1.32E-01	6.38E-01
KPP	2.31E-01	2.05E-01	3.49E-02	6.41E-02	3.71E-01	1.69E-01	6.86E-01	6.75E-01
LPP	9.09E-01	5.05E-01	1.88E-01	1.04E-01	7.22E-01	2.85E-01	5.83E-01	6.77E-01
MPP	8.03E-01	1.53E-01	4.62E-01	7.37E-01	4.98E-01	2.54E-01	6.00E-01	9.15E-01
NPP	5.43E-01	5.86E-01	3.19E-02	2.31E-01	3.52E-01	5.09E-01	2.37E-01	1.80E-01
QPP	5.30E-01	4.84E-01	3.04E-01	3.57E-01	9.53E-01	4.29E-01	4.44E-01	5.68E-01
RPP	6.73E-01	9.20E-01	3.23E-01	2.37E-01	8.33E-01	6.17E-01	7.97E-01	1.48E-01
SPP	2.35E-01	7.09E-03	1.22E-02	1.69E-03	2.15E-01	3.54E-01	7.86E-01	7.16E-01
TPP	8.06E-01	6.78E-01	8.13E-01	4.88E-01	9.96E-01	6.61E-01	7.30E-01	1.95E-01
VPP	6.27E-01	7.82E-02	1.75E-02	1.09E-02	1.82E-01	2.65E-01	8.77E-01	7.57E-01
WPP	5.30E-02	4.22E-01	1.99E-01	2.87E-01	4.95E-01	7.41E-01	6.48E-01	7.70E-01
YPP	2.54E-02	4.68E-01	8.08E-01	7.24E-01	5.61E-01	1.46E-01	5.54E-03	2.65E-02
PPA	6.12E-01	1.84E-01	5.72E-02	6.37E-02	3.54E-01	7.63E-01	3.24E-01	9.70E-01
PPC	9.98E-01	3.42E-01	7.57E-01	5.93E-01	2.87E-01	4.64E-01	3.06E-01	8.77E-01
PPD	5.83E-01	6.67E-01	2.95E-01	2.09E-01	7.05E-01	4.10E-01	1.64E-01	4.41E-02
PPE	3.36E-01	2.22E-02	7.82E-02	1.51E-01	1.32E-01	4.84E-02	2.96E-01	8.68E-01
PPF	3.56E-02	3.41E-01	3.89E-01	2.22E-01	1.42E-01	1.45E-01	8.43E-01	6.54E-01
PPG	3.71E-03	8.62E-07	1.80E-08	1.03E-08	7.12E-04	1.60E-04	6.33E-01	2.11E-01
PPH	1.10E-01	4.75E-01	6.22E-01	3.60E-01	8.10E-01	7.15E-01	4.51E-01	2.53E-01
PPI	9.70E-01	7.50E-01	4.09E-01	3.81E-01	5.24E-01	9.99E-01	3.89E-01	1.33E-01
PPK	1.28E-01	1.14E-01	1.02E-02	3.98E-03	1.60E-01	7.25E-03	9.92E-01	7.19E-01
PPL	2.84E-01	2.40E-01	2.37E-02	5.17E-02	4.27E-01	6.96E-01	9.91E-02	3.29E-01
PPM	3.25E-01	4.99E-02	9.07E-02	3.46E-02	1.72E-01	1.18E-01	4.56E-01	4.39E-01
PPN	8.86E-02	7.93E-04	2.67E-03	7.14E-04	1.57E-02	4.18E-02	1.83E-01	3.05E-01
PPQ	4.44E-01	1.89E-01	6.72E-01	4.23E-01	8.71E-01	2.86E-01	3.76E-02	4.53E-01
PPR	2.64E-01	3.89E-01	6.44E-02	1.56E-01	8.99E-03	2.53E-01	6.75E-01	4.25E-01
PPS	8.38E-01	5.08E-01	7.27E-02	6.62E-02	4.53E-01	3.58E-01	8.93E-01	8.38E-01
PPT	4.58E-01	1.18E-01	4.26E-02	1.46E-01	2.22E-01	4.17E-01	9.31E-01	8.04E-01
PPV	8.00E-01	5.49E-01	7.15E-01	2.74E-01	4.81E-01	5.73E-01	9.60E-01	6.29E-01
PPW	7.00E-01	5.57E-01	5.75E-01	5.68E-01	8.69E-01	5.27E-01	6.09E-01	9.54E-01
PPY	5.02E-01	8.76E-01	3.20E-01	5.14E-01	8.09E-01	1.00E+00	9.69E-01	3.14E-01
(A/D/I)PP(G/N)	5.24E-05	5.20E-09	2.70E-09	1.30E-09	7.01E-07	2.65E-06	1.05E-01	5.81E-01
RME	3.44E-01	1.69E-02	1.04E-02	3.49E-02	2.28E-01	1.01E-01	4.00E-02	1.04E-01
PFF	1.64E-01	5.23E-02	4.53E-02	1.15E-02	3.53E-01	5.26E-01	7.81E-01	2.75E-01
GPG	4.16E-01	2.39E-01	1.06E-01	1.02E-01	8.36E-01	3.87E-01	5.81E-01	8.56E-01
YIR	4.49E-01	8.39E-01	9.21E-01	9.66E-01	8.64E-01	8.54E-01	3.19E-01	7.63E-01

Nonparametric Kruskal–Wallis test was applied to test for protein ratio distribution differences between the PPP (orange), XPP (pink), PPX (blue), and non-diprolyl (green) motif subsets and the complete dataset (without the corresponding motif subset) for each strain; corresponding *P* values are listed, with $P < 0.05$ highlighted in red.

Other Supporting Information Files

Dataset S1 (XLSX)