Supplementary Information for

Prophage excision switches the primary ribosome rescue pathway and rescue-associated gene regulations in *Escherichia coli*

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Supplementary Figures (included in this file)
- Figure S1
- Figure S2
- Figure S3
- Figure S4
- Figure S5

Supplementary Tables (separated in the individual files)
- Table S1
- Table S2
- Table S3
- Table S4
- Table S5
- Table S6

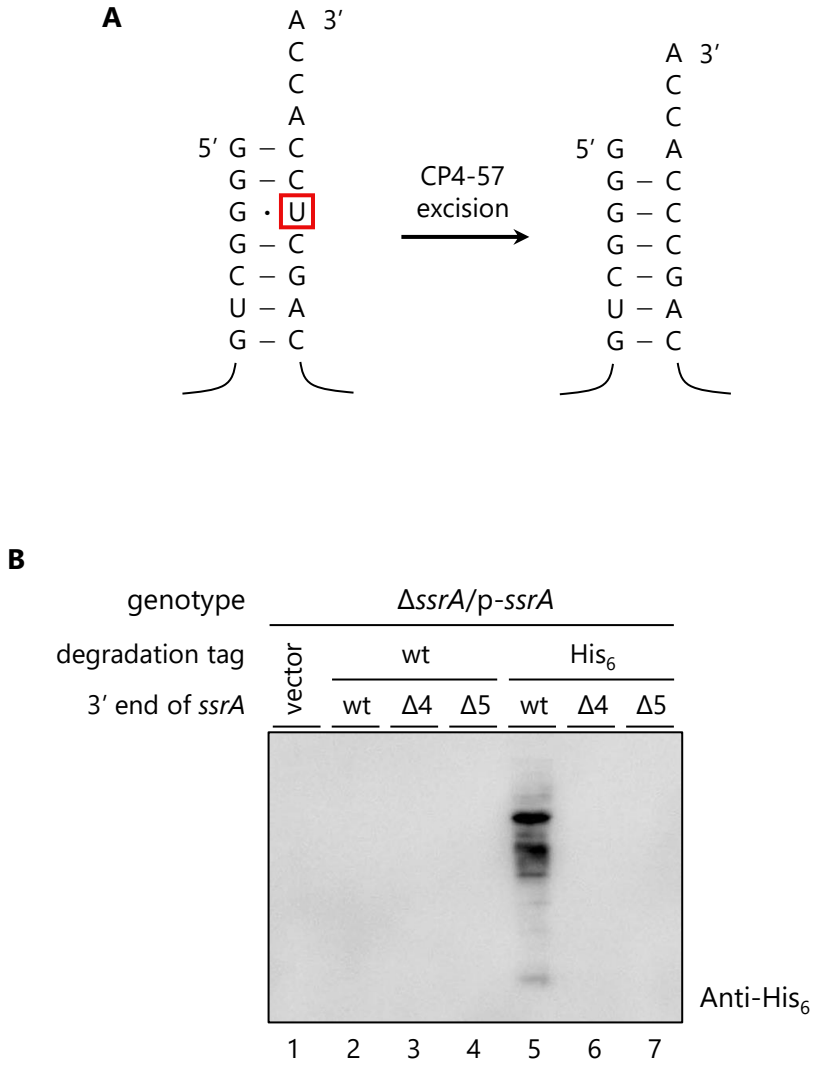

Fig. S1.

(A) The acceptor stem-loop structure at the 3' end of *E. coli* tmRNA and the tmRNAΔU357 mutant. CP4-57 excision deletes the T357 residue of the *ssrA* gene, which forms a G·U wobble base pair in the acceptor stem in the tRNA-like domain.

(B) Inactivation of tmRNA^{His} by prophage excision-introduced mutations in other bacterial species. The *E. coli* Δ*ssrA* strain harboring pMW118 (vector control, lane 1) and its derivatives, p-*ssrA* (lane 2), p-*ssrA*Δ4 (induced by the excision of Ype11X in *Yersinia pestis* CO92, which deletes four nucleotides at the 3' end, lane 3), p-*ssrA*Δ5 (induced by the excision of Stm27X in *Salmonella enterica* serovar Typhimurium LT2, Eco48X in *Escherichia coli* RS218 and Oi108 in *Escherichia coli* O157:H7 EDL933, which deletes five nucleotides at the 3' end, lane 4), p-*ssrA*^{His} (lane 5), p-*ssrA*^{His}Δ4 (lane 6) and p-*ssrA*^{His}Δ5 (lane 7) were grown in LB medium until mid-log phase. Cellular extracts were fractionated by SDS-PAGE and analyzed by western blotting using an anti-His$_6$ antibody.

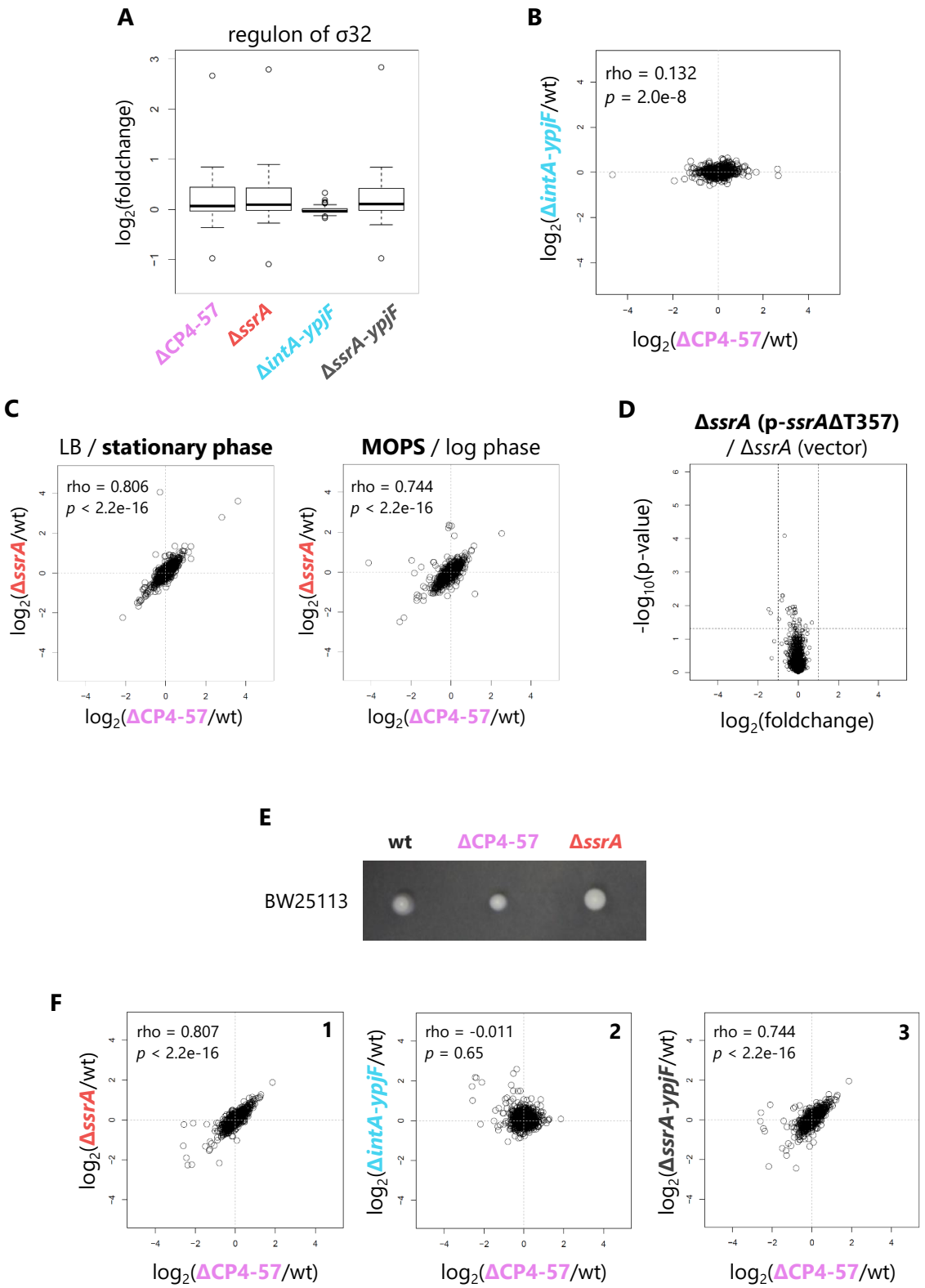
A
regulon of σ32
log2(foldchange)
ΔCP4-57
ΔssrA
ΔintA-ypjF
ΔssrA-ypjF
B
rho = 0.132
p = 2.0e-8
log2(ΔintA-ypjF/wt)
log2(ΔCP4-57/wt)
C
LB / stationary phase
rho = 0.806
p < 2.2e-16
log2(ΔssrA/wt)
log2(ΔCP4-57/wt)
MOPS / log phase
rho = 0.744
p < 2.2e-16
log2(ΔssrA/wt)
log2(ΔCP4-57/wt)
D
ΔssrA (p-ssrAΔT357)
/ ΔssrA (vector)
-log10(p-value)
log2(foldchange)
E
wt
ΔCP4-57
ΔssrA
BW25113
F
rho = 0.807
p < 2.2e-16
1
log2(ΔssrA/wt)
log2(ΔCP4-57/wt)
rho = -0.011
p = 0.65
2
log2(ΔintA-ypjF/wt)
log2(ΔCP4-57/wt)
rho = 0.744
p < 2.2e-16
3
log2(ΔssrA-ypjF/wt)
log2(ΔCP4-57/wt)

Fig. S2.

(A) Fold change values of the heat shock regulons (σ32) were extracted from the results in **Fig. 2**, and represented as a boxplot.

(B) Two-dimensional plots of the fold change values in the *E. coli* BW25113 ΔCP4-57 (horizontal axis) and Δ*intA-ypjF* strains (vertical axis). The plot is represented with Spearman's rho and *P*-values calculated by Spearman's rank correlation tests.

(C) Two-dimensional plots comparing the proteomic rearrangements in the BW25113 ΔCP4-57 and BW25113 Δ*ssrA* strains in LB medium at the stationary growth phase (left) or in MOPS minimal medium at the mid-log growth phase (right). Cells were grown at 37 °C in the indicated medium and then subjected to the SWATH-MS analysis, as shown in **Fig. 2**. Each plot is represented with Spearman's rho and *P*-values calculated by Spearman's rank correlation tests.

(D) A volcano plot showing the poor proteomic change in the Δ*ssrA* strain expressing tmRNAΔU357, compared to the Δ*ssrA* strain harboring an empty vector. Fold change and *P*-values of each protein are represented by dots and plotted according to its $\log_2$ fold change on the horizontal axis and *P*-value on the vertical axis. The lines indicate a *P*-value of 0.05 and 2-fold change.

(E) Swimming motility of BW25113 wild-type strain and its derivatives, ΔCP4-57 and Δ*ssrA* mutant. Colonies were inoculated onto semisolid agar plates and incubated at 30 °C for 20 hrs.

(F) Two-dimensional plots of the fold change values in the *E. coli* MG1655 mutant indicated below and on the side of the graph. Each plot is represented with Spearman's rho and *P*-values calculated by Spearman's rank correlation tests.

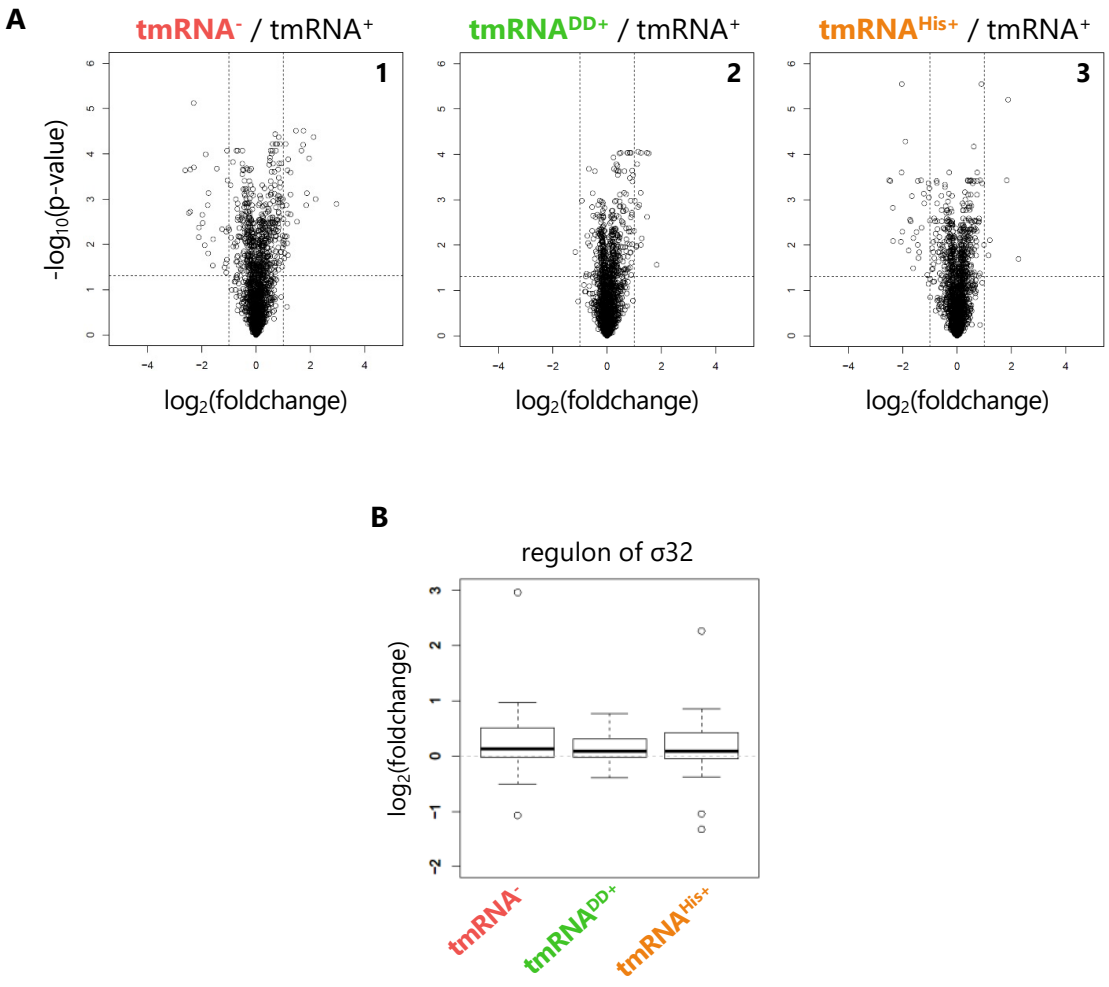

Fig. S3.

(A) Proteomic rearrangements in proteolysis-deficient tmRNA-expressing strains. The fold change relative to the wild-type tmRNA expressing strain and the *P*-value of each protein in the Δ*ssrA* strain harboring pMW118 (panel 1) and its derivatives, p-*ssrA*^{DD} (panel 2) and p-*ssrA*^{His} (panel 3), are represented by volcano plots, as shown in Fig. 2. (B) Fold change values of the heat shock regulons (σ32) were extracted from the results in Fig. 3, and are represented by boxplots.

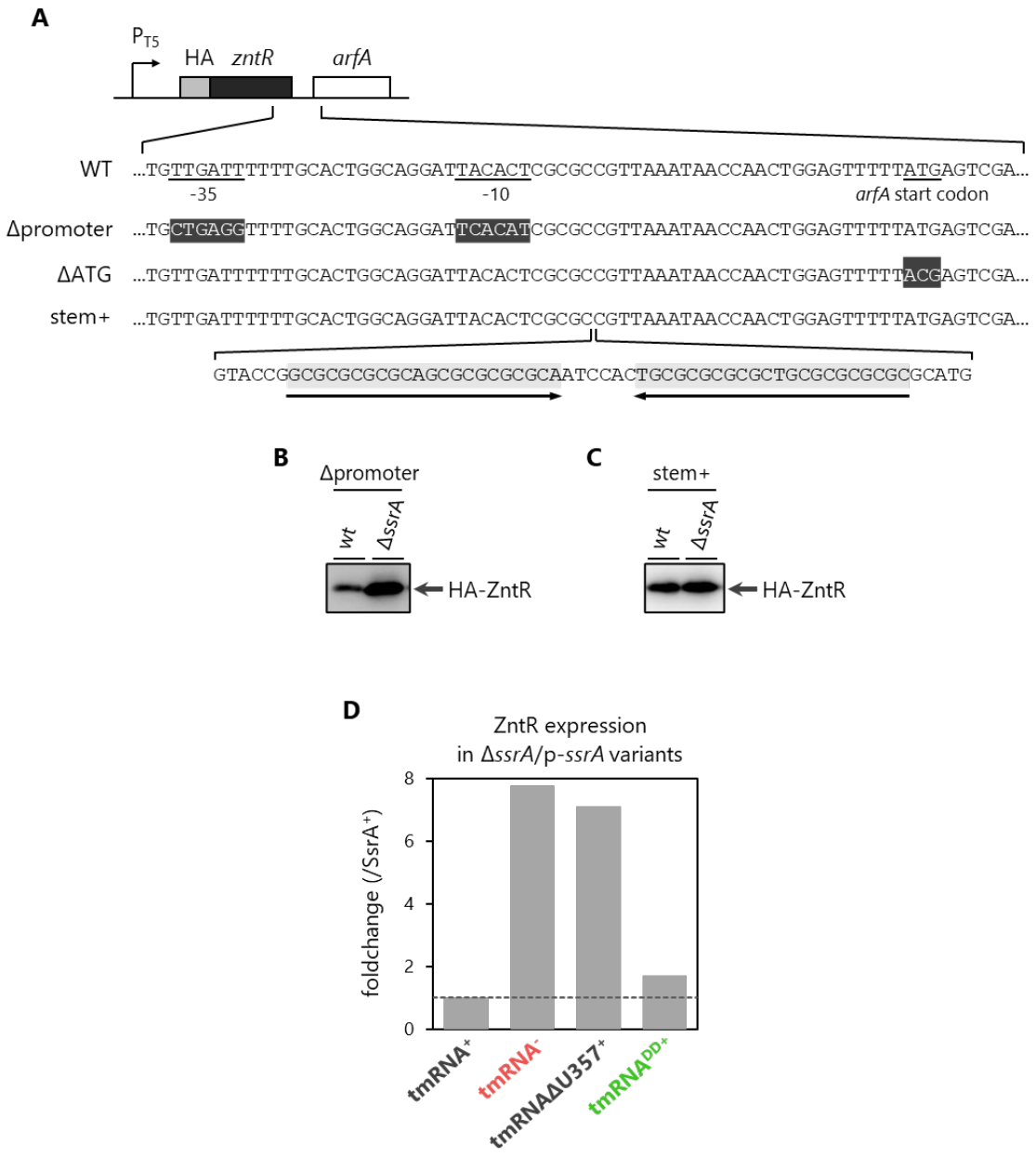

Fig. S4.

(A) Schematic representation of the mutations introduced into the *zntR-arfA* operon: WT (wild-type), ΔATG (disruption of the initiation codon of *arfA*), Δpromoter (disruption of *arfA* promoter) and stem+ (insertion of an artificial stem-loop between *zntR* and *arfA,* indicated by arrows).

(B) Expression of HA-tagged ZntR from *zntR-arfA* lacking the *arfA* promoter. BW25113 and the Δ*ssrA* strain harboring pOH020 (*zntR-arfA* Δpromoter) were grown in LB until the OD_{660} reached 0.2-0.3. IPTG (100 μM) was then added to induce the expression of

zntR, and cells were grown until the OD_{660} doubled. Cellular extracts were prepared and analyzed as in **Fig. 4D**.
(C) Expression of HA-tagged ZntR from *zntR-arfA* with the insertion of an artificial stem-loop. BW25113 and the Δ*ssrA* strain harboring pOH021 (*zntR-arfA* stem+) were grown and analyzed as in **Fig. 4D**.
(D) Expression levels of ZntR in tmRNA variant-expressing cells, extracted from the SWATH-MS analyses in **Fig. 3**. The dashed line indicates a fold change value of 1.

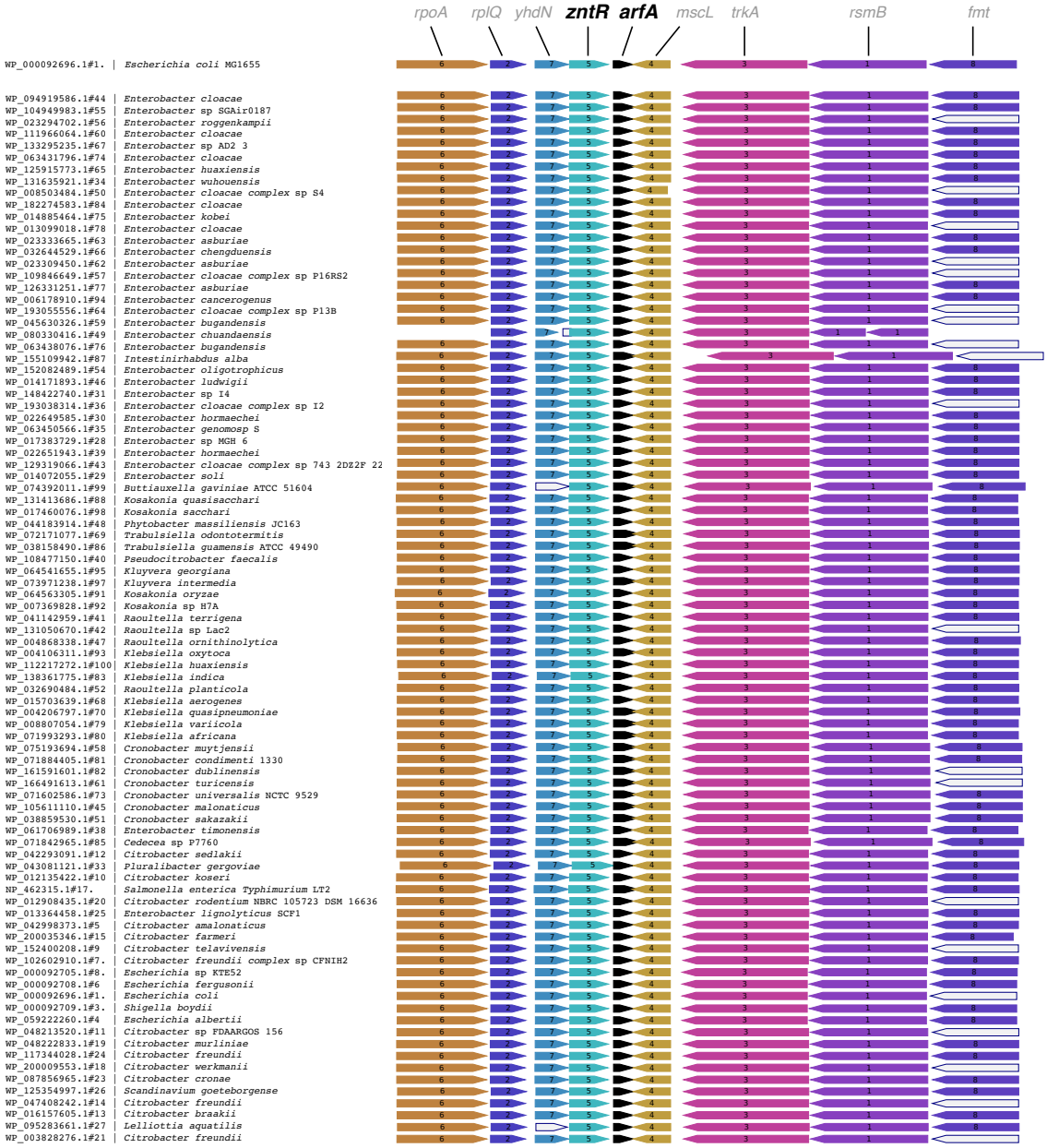

Fig. S5.

The genetic structure of *arfA*-surrounding genes among enterobacteria described by webFlaGs software (Saha *et al*., 2020). Arrow boxes from 1 to 8 indicate the ORFs homologous to *rpoA* (no.6), *rplQ* (no. 2), *ydhN* (no.7), *zntR* (no.5), *mscL* (no. 4), *trkA* (no. 3), *rsmB* (no. 1) and *fmt* (no.8), respectively. Colorless arrowed boxes without the number indicates the ORFs that do not exist in *E. coli* MG1655 strain.