SUPPLEMENTAL FIGURES

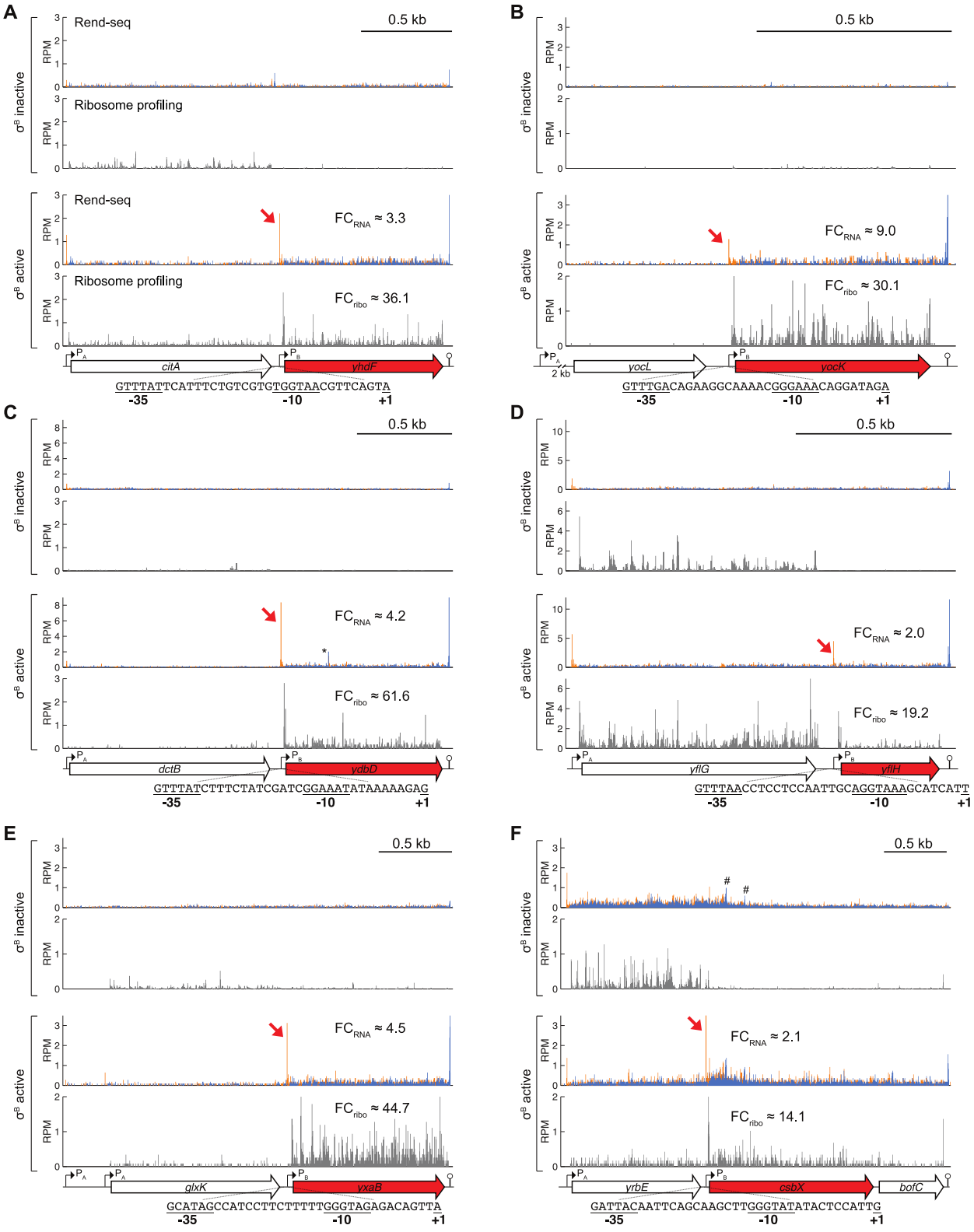

Figure S1. Additional translationally activated genes with σ^B transcript inside σ^A polycistron.

Rend-seq and ribosome profiling data from conditions with inactive/active σ^B for the operons containing (**A**) *yhdF*, (**B**) *yocK*, (**C**) *ydbD*, (**D**) *yflH*, (**E**) *yxaB*, and (**F**) *csbX*. Orange and blue bars represent 5'- and 3'-mapped read counts, respectively, and the black scale bars correspond to 0.5 kb. Rend-seq data indicating the σ^B-dependent transcription start sites are marked by red arrows. Fold changes (FC) for Rend-seq and ribosome profiling between σ^B active and σ^B inactive conditions are shown. Putative σ^B-dependent promoter sequences are indicated for each gene (+1 corresponds to the 5' end of the σ^B-dependent isoform mapped by Rend-seq). The *yocK* σ^A polycistron includes multiple upstream genes not shown for clarity. A likely post-lysis RNase A 5'/3' doublet in *ydbD* is marked by *. PnpA and Rho dependent 3' ends (Lalanne et al., 2018) in *csbX* are indicated with #. See also Fig. 2.

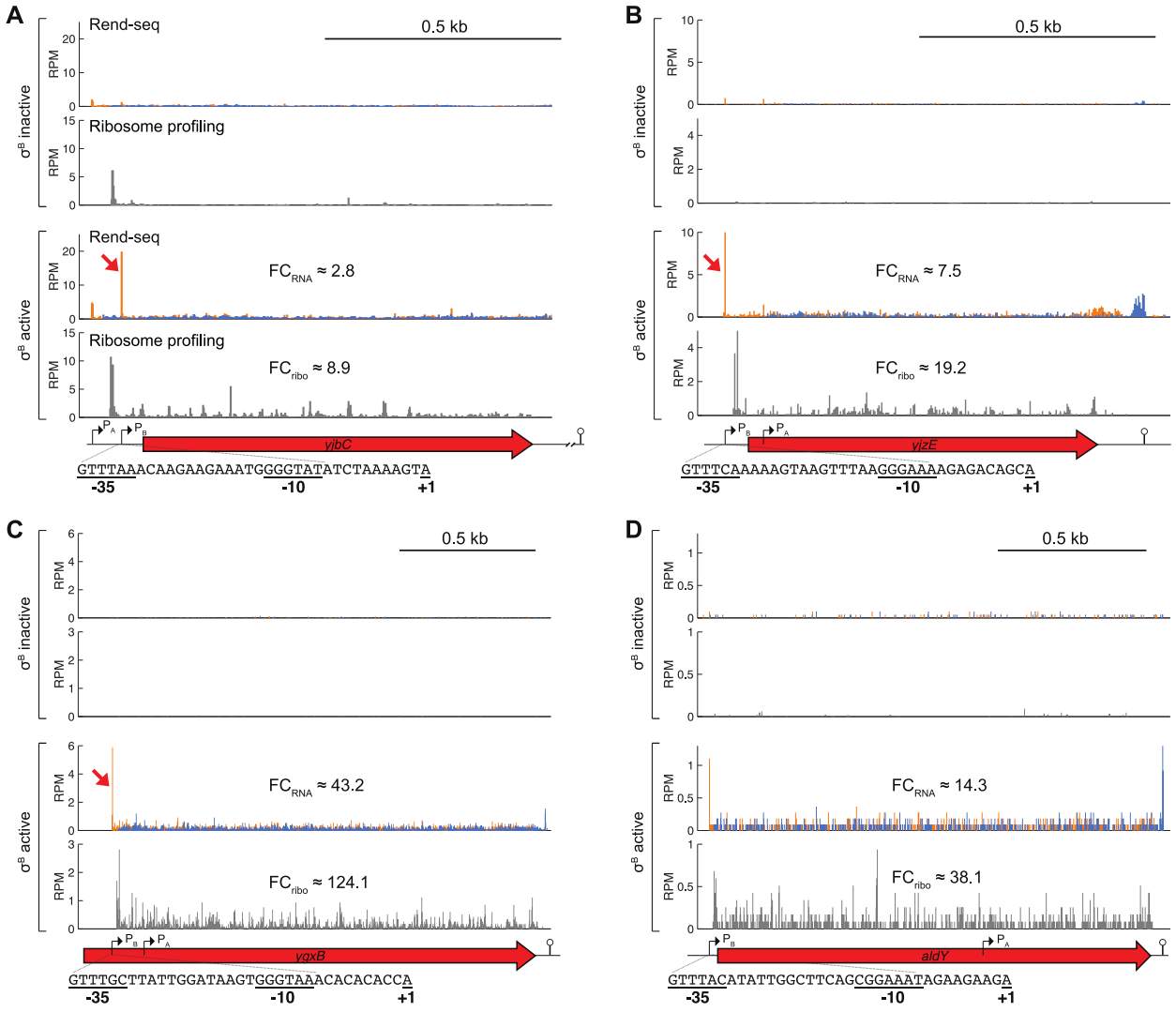

Figure S2. Additional translationally activated σ^B regulon genes with changes in mRNA isoform.

Rend-seq and ribosome profiling data from conditions with inactive/active σ^B for the operons containing (**A**) *yjbC*, (**B**) *yjzE*, (**C**) *ygxB*, and (**D**) *aldY*. Orange and blue bars represent 5'- and 3'-mapped read counts, respectively, and the black scale bars correspond to 0.5 kb. Rend-seq data indicating the σ^B-dependent transcription start sites are marked by red arrows. Fold changes (FC) for Rend-seq and ribosome profiling between σ^B active and σ^B inactive conditions are shown. Putative σ^B-dependent promoter sequences are indicated for each gene (+1 corresponds

to the 5' end of the σ^B-dependent isoform mapped by Rend-seq). *yjbC*'s mRNA is part of a polycistronic transcript including *spxA*, which is not shown for clarity. Due to low sequencing coverage, Rend-seq data from (Lalanne et al., 2018) was used to annotate some transcript isoforms in the σ^B inactive condition. We note that the annotation for *ygxB* is likely incorrect based on our ribosome profiling data.

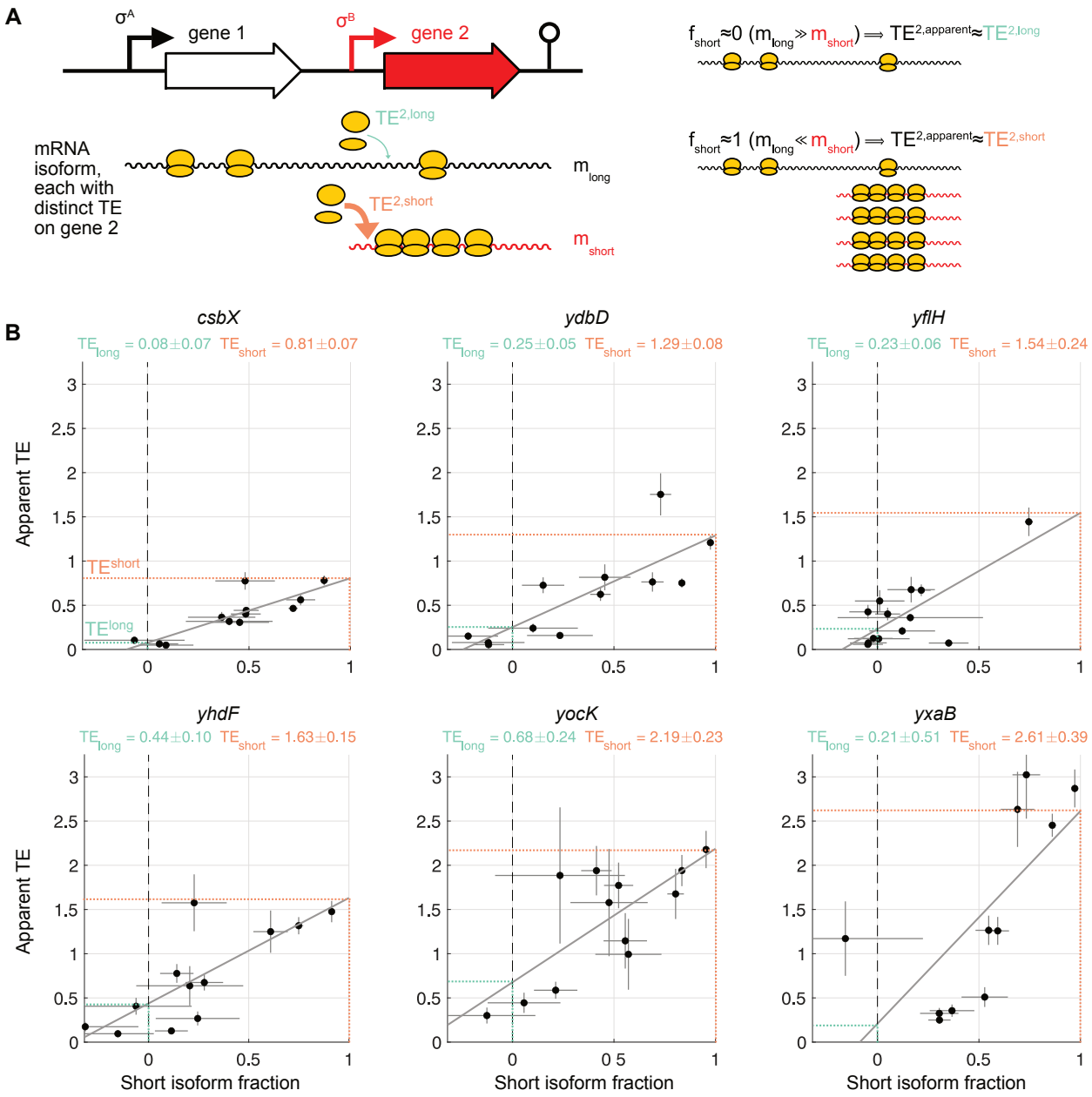

Figure S3. Estimations of isoform-specific TE for additional translationally activated σ^B regulon genes.

(**A**) Schematic illustration of the mathematical analysis used to identify the isoform-specific TE. (**B**) Estimation of the isoform-specific TE for the short, σ^A-dependent and long, σ^B-dependent isoforms of *csbX*, *ydbD*, *yflH*, *yhdF*, *yocK*, and *yxaB*. Each point is an experimental condition which has a different short isoform fraction and correspondingly different apparent TE. Error

bars correspond to standard deviations from subsampling bootstraps. The gray lines are linear regressions, whereas the dashed lines indicate estimates of isoform-specific TE calculated from the fits (Methods). Estimated isoform-specific TEs and errors (standard deviations) from a bootstrapped linear fit (Methods) are shown. See also Fig. 3.

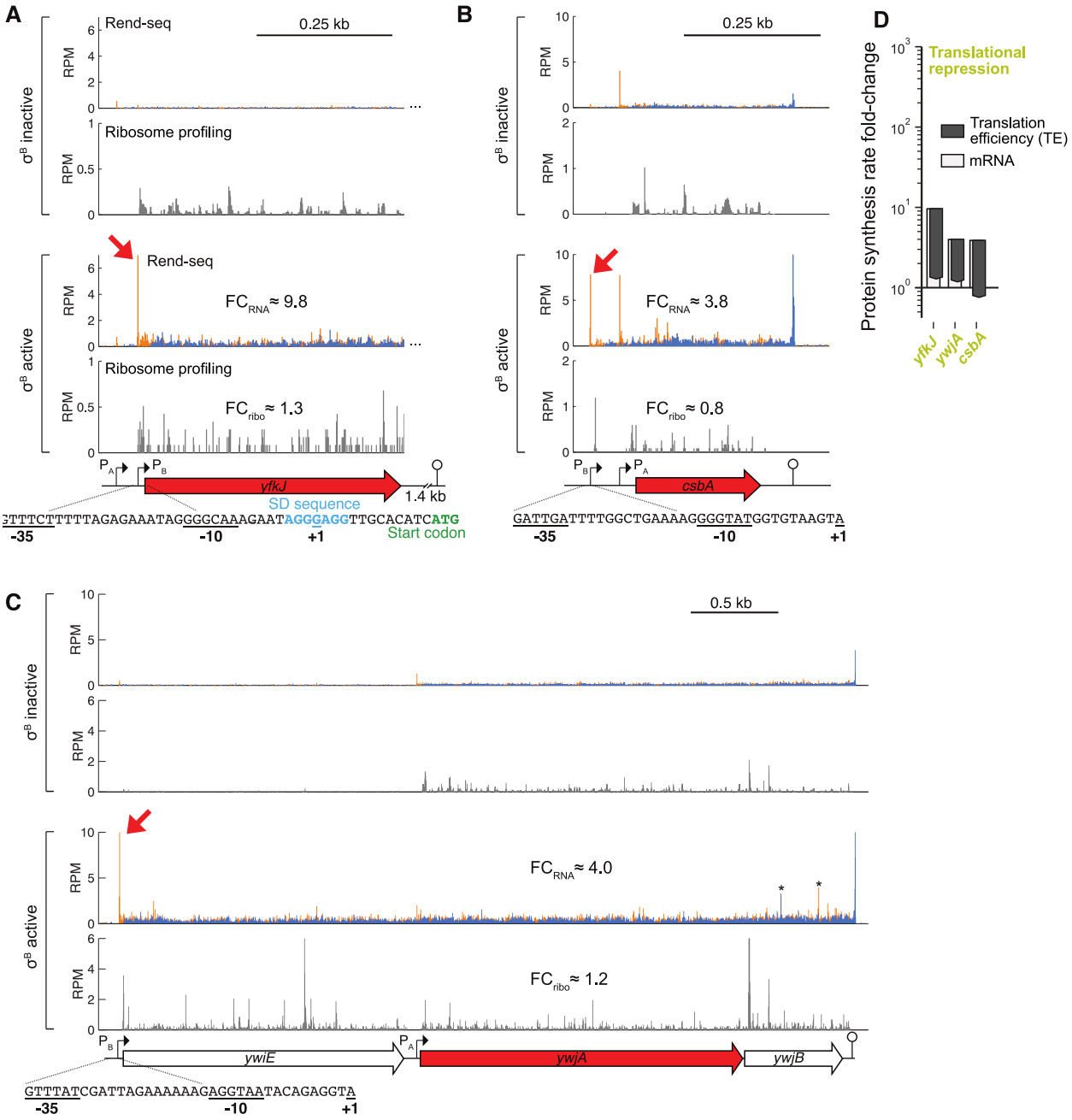

Figure S4. Translationally repressed σ^B regulon genes with changes in mRNA isoform.

Rend-seq and ribosome profiling data from conditions with inactive/active σ^B for the operons containing (**A**) *yfkJ*, (**B**) *csbA*, and (**C**) *ywjA*. Orange and blue bars represent 5'- and 3'-mapped read counts, respectively, and the black scale bars correspond to 0.5 kb. Rend-seq data indicating the σ^B-dependent transcription start sites are marked by red arrows. Fold changes (FC) for Rend-

seq and ribosome profiling between σ^B active and σ^B inactive conditions are shown. Putative σ^B-dependent promoter sequences are indicated for each gene (+1 corresponds to the 5' end of the σ^B-dependent isoform mapped by Rend-seq). * in (C) marks a spurious RNase A cleavage site that likely occurred post-lysis. (**D**) Analogous to Fig. 1E and F, for translationally repressed genes. Fold-changes in mRNA and translation efficiency are represented in light and dark grey, respectively.

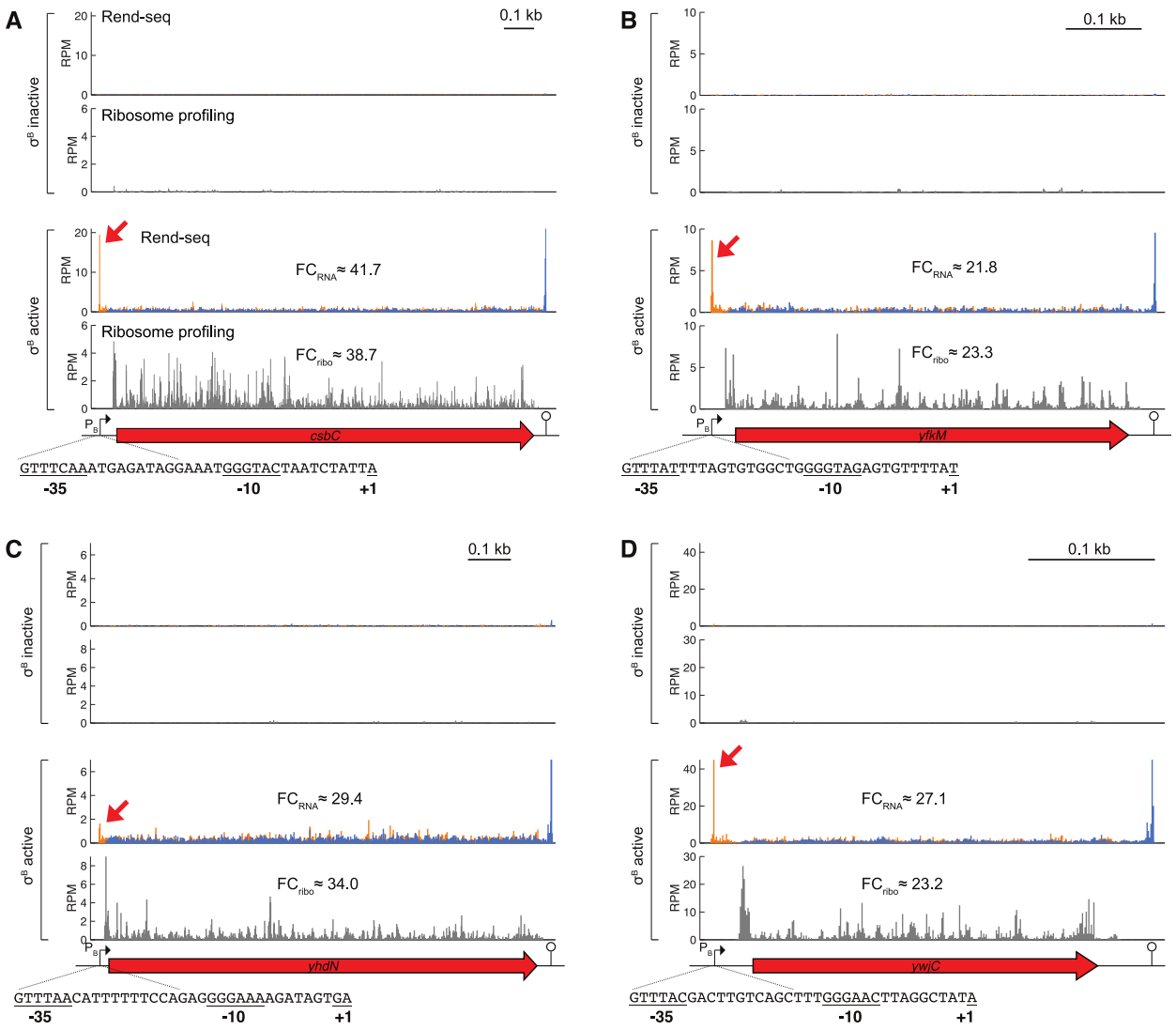

Figure S5. Examples of induced σ^B regulon genes without complex isoform architecture.

Rend-seq and ribosome profiling data from conditions with inactive/active σ^B for the operons containing (**A**) *csbC*, (**B**) *yfkM*, (**C**) *yhdN*, and (**D**) *ywjC*. Orange and blue bars represent 5'- and 3'-mapped read counts, respectively, and the black scale bars correspond to 0.5 kb. Rend-seq data indicating the σ^B-dependent transcription start sites are marked by red arrows. Fold changes (FC) for Rend-seq and ribosome profiling between σ^B active and σ^B inactive conditions are shown. Putative σ^B-dependent promoter sequences are indicated for each gene (+1 corresponds to the 5' end of the σ^B-dependent isoform mapped by Rend-seq).

REFERENCES

Lalanne JB, Taggart JC, Guo MS, Herzel L, Schieler A, Li GW. 2018. Evolutionary Convergence of Pathway-Specific Enzyme Expression Stoichiometry. *Cell* **173**: 749-761.e38.