List of supplementary files for the following manuscript:

Regulation of global transcription in E. coli by Rsd and 6S RNA

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Supplementary Figures

Figure S1: Sample growth curves of wild-type *E. coli* and the mutant strains used for RNA-Seq. Overnight cultures were diluted 1:100 in fresh M9 Glucose medium and growth was estimated by measuring optical density at 600 nm.

Figure S2: (A) Boxplots of log2 fold change in gene expression (Δ*rsd*/wild-type) for genes whose expression is significantly reduced in Δ*rpoS* in the indicated growth phase, compared to all other genes. (B) Boxplots of log2 fold change in gene expression (Δ*rsd*/ wild-type) for reported σ^{38} targets in the indicated growth phase, compared to all other genes. (C) Boxplots of log2 fold change in gene expression (Δ*rsd*/wild-type) for genes whose expression is significantly increased in Δ*rpoS* in the indicated growth phase, compared to all other genes. p-values are for Wilcoxon Test.

Figure S3: Boxplots of log2 fold change in gene expression (Δ*rsd*/wild-type) for 270 genes whose expression is controlled by constitutive σ^{70}-dependent promoters, compared to all other genes, in the indicated growth phase. p-values are for Wilcoxon Test.

Figure S4: Western blot showing expression of σ^{38} (RpoS) during stationary phase, in the five strains used for RNA-Seq. GroEL was used as a loading control.

Figure S5: A) Schematic of plasmid pTopo-*ssrS*, used for overexpressing 6S RNA. This plasmid was produced by amplifying a DNA segment containing the *ssrS* gene, both its promoters and its terminator, from genomic DNA by PCR (primers in Table S4), and cloning it into the pCR2.1-TOPO vector using the TOPO TA Cloning Kit (Invitrogen, K4500). B) Fold change in expression (qRT-PCR) of *ssrS* (dark gray) and *fau* (light gray) RNA in wild-type (both set to 1), Δ*ssrS*, wild-type transformed with empty vector, and wild-type transformed with pTopo-*ssrS*, during stationary phase. Data represent mean +/- SEM for 3 biological replicates. Empty vector was produced by digesting pTOPO-ssrS with EcoRI to remove the insert, and eluting and self-ligating the digested vector.

Figure S6: (A) Boxplots of log2 fold change in gene expression (Δ*ssrS*/wild-type) for genes whose expression is significantly reduced in Δ*rpoS* in the indicated growth phase, compared to all other genes. (B) Boxplots of log2 fold change in gene expression

(Δ*ssrS*/wild-type) for reported σ^{38} targets in the indicated growth phase, compared to all other genes. (C) Boxplots of log2 fold change in gene expression (Δ*ssrS*/wild-type) for genes whose expression is significantly increased in Δ*rpoS* in the indicated growth phase, compared to all other genes. p-values are for Wilcoxon Test.

Figure S7: Boxplots of log2 fold change in gene expression (Δ*ssrS*/wild-type) for 270 genes whose expression is controlled by constitutive σ^{70}-dependent promoters, compared to all other genes, in the indicated growth phase. p-values are for Wilcoxon Test.

Figure S8: Boxplots of log2 fold change in gene expression (Δ*ssrS*/wild-type) during the transition to stationary phase, for 710 genes repressed by ppGpp, 3159 genes unaffected by ppGpp, and 704 genes activated by ppGpp. p-values are for Wilcoxon Test.

Figure S9: Heatmaps showing the log2 fold change in expression, in successive growth phases, of (A) 16 genes whose expression was increased in Δ*ssrS*/wild-type, with the magnitude of this increase increasing in successive growth phases, and (B) 20 genes whose expression was reduced in Δ*ssrS*/wild-type, with the magnitude of this reduction increasing in successive growth phases.

Figure S10: Western blot showing expression of RNA polymerase β subunit (RpoB) during stationary phase, in the five strains used for RNA-Seq. GroEL was used as a loading control.

Figure S11: (A) Boxplots of log2 fold change in gene expression (Δ*ssrS*/wild-type) for genes as a function of the number of nucleotides in their promoter -35 sequence that match to consensus, for a set of 312 mapped σ^{70} promoters, based on the data of Cavanagh *et al*. (2008) (B) Boxplots showing log2 fold change in expression (Δ*ssrS*/ wild-type) of the same genes in our dataset. (C) Boxplots showing log2 fold change in gene expression (Δ*ssrS*/wild-type) for 77 genes whose promoters have a weak -35 element and extended -10 element, compared to 72 genes with a weak -35 element only, based on the data of Cavanagh *et al*. (2008) (D) Boxplots showing log2 fold change in gene expression (Δ*ssrS*/wild-type) for the same genes in our dataset. p-values are for Wilcoxon Test.

Figure S12: A schematic showing various pathways by which Rsd and 6S RNA have been proposed to regulate RNA polymerase. Bold lines represent sequestration into inactive complexes. Blue lines represent pathways which are affected only in the Rsd-6S RNA double knockout. The three arrows connecting Rsd and 6S RNA show that Rsd inhibits expression of 6S RNA (in stationary phase) and activates it (in ME phase), while 6S RNA increases expression of Rsd. The question mark indicates the possibility that 6S RNA is autoregulatory.

Supplementary Tables

Table S1: Strains and plasmids used in this study

Table S2: Summary of RNA-Seq

Table S3: Results of qRT-PCR validation

Table S4: Primers used in this study

Additional Files

File S1: Selection of model parameters

File S2: Differential Equations for Model

File S3: Fold changes and FDR-adjusted p-values for all strains and conditions.