Supplemental Data

Rsd family proteins make simultaneous interactions with regions 2 and 4 of the primary sigma factor

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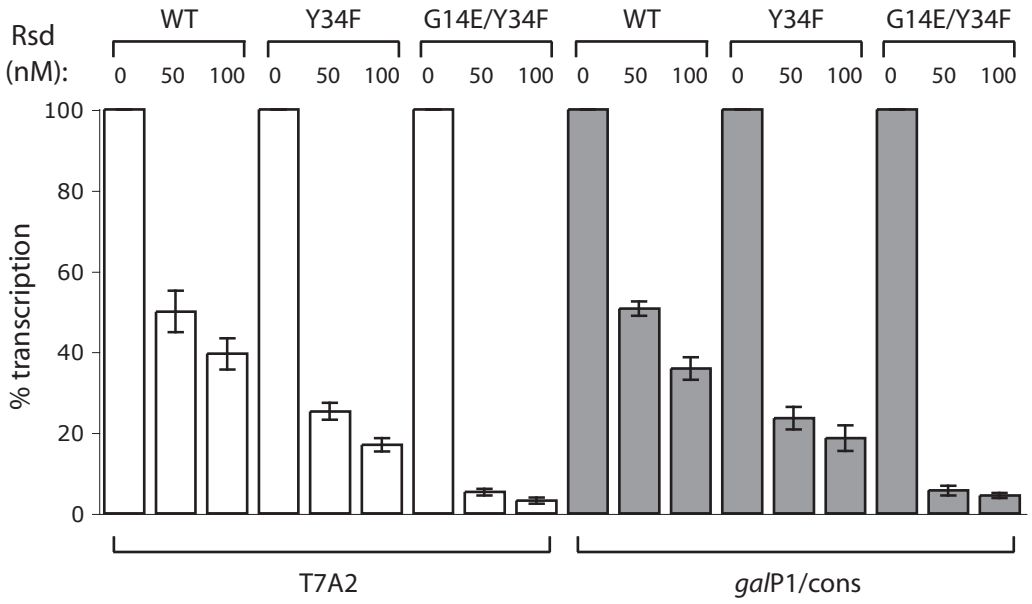

Figure S1. Enhanced-function Rsd mutants are better inhibitors of σ^{70}-dependent transcription than wild-type Rsd *in vitro*.

Results of single-round *in vitro* transcription assays performed with DNA templates bearing the T7A2 promoter or the *gal*P1/cons promoter in the absence or presence of increasing concentrations (50 or 100 nM) of the indicated Rsd protein. The graph shows the averages of three independent experiments and standard deviations.

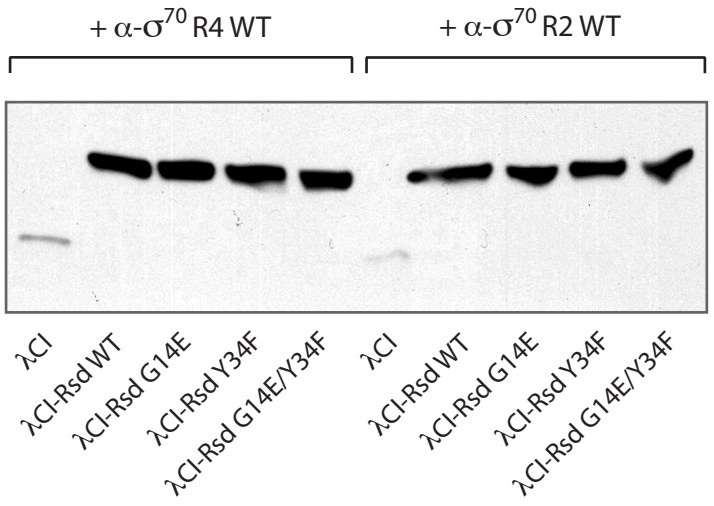

Figure S2. Rsd amino acid substitutions G14E and Y34F, tested individually or in combination, do not affect the intracellular levels of the λCI-Rsd fusion protein.

The western blot shows protein levels of either λCI or the indicated λCI-Rsd fusion protein in FW102 O_L2–62 cells containing two compatible plasmids, one encoding either λCI or one of the four λCI-Rsd variants, and the other encoding either an α-σ^{70} region 4 or an α-σ^{70} region 2 fusion protein. The cells were grown in the presence of 10 μM IPTG, and normalized samples of cell lysates were processed for western blotting as described in (Deighan and Hochschild, 2007). The anti-λCI antibody used to detect λCI and the λCI-Rsd fusion proteins was a generous gift from J. Beckwith.

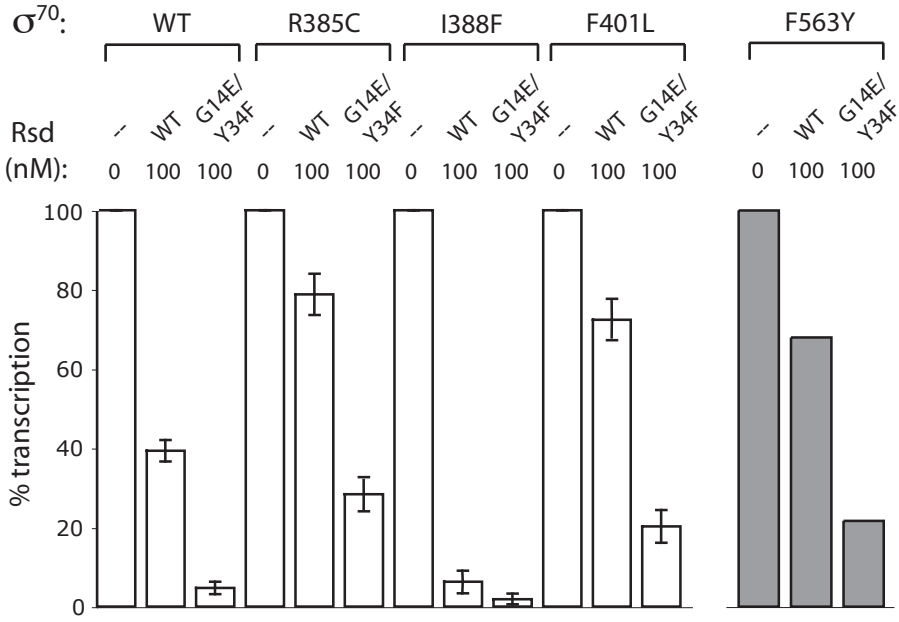

Figure S3. Amino acid substitutions in σ^{70} affect Rsd's ability to inhibit σ^{70}-dependent transcription *in vitro*.

Results of single-round *in vitro* transcription assays performed with a DNA template bearing the T7A2 promoter using RNAP holoenzyme reconstituted with the indicated σ^{70} protein in the absence or presence of either wild-type Rsd or Rsd G14E/Y34F (100 nM). The graph shows the averages of three independent experiments and standard deviations (white bars) or the averages of two independent experiments (gray bars).

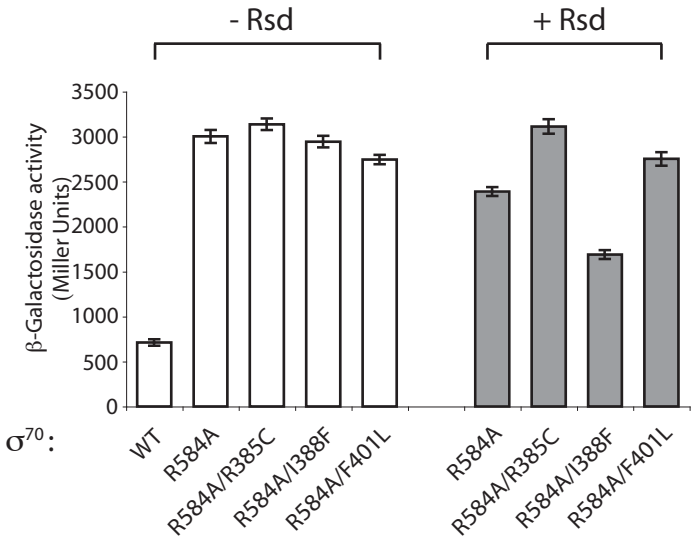

Figure S4. Amino acid substitutions in σ^{70} region 2 affect Rsd's ability to inhibit σ^{70}-dependent transcription *in vivo*.

Results of β-galactosidase assays performed with BG77 cells containing two compatible plasmids: one encoding the indicated σ^{70} protein (pLXσ^{70}) and the other encoding either wild-type Rsd (pACRsd) or no Rsd (pACΔCI). The plasmids directed the synthesis of σ^{70} and Rsd under the control of a weak-constitutive promoter and an IPTG-inducible promoter, respectively, and the cells were grown in the presence of 10 μM IPTG. Strain BG77 also contains chromosomally encoded wild-type Rsd. The graph shows the averages of three independent experiments and standard deviations.

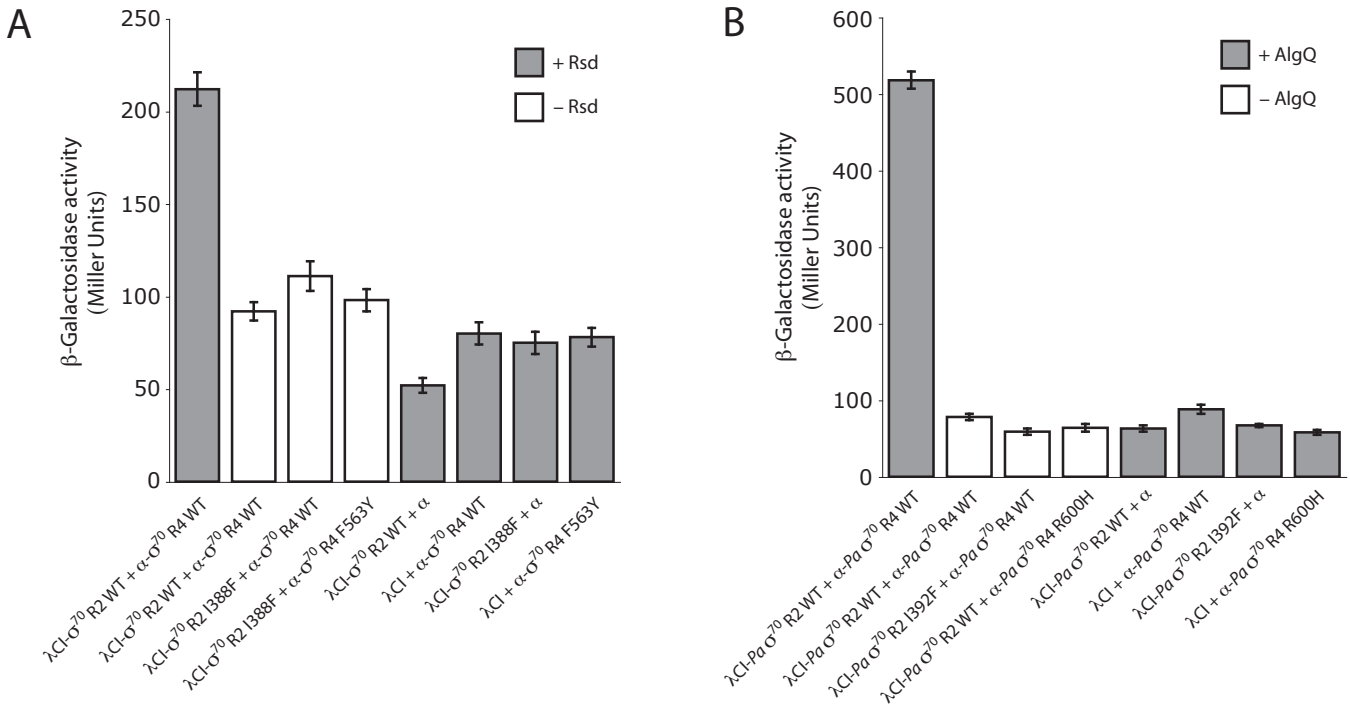

Figure S5. The ability of Rsd or AlgQ to activate transcription in a bacterial two-hybrid assay adapted to detect bridging interactions depends on the presence of both the λCI-σ^{70} region 2 and the α-σ^{70} region 4 fusion proteins.

(A) Results of β-galactosidase assays performed with FW102 O_L2–62 cells containing three compatible plasmids, one encoding either λCI or the indicated λCI-σ^{70} region 2 fusion protein, a second encoding either α or the indicated α-σ^{70} region 4 fusion protein, and a third encoding either unfused wild-type Rsd (gray bars) or no Rsd (white bars). The plasmids directed the synthesis of the fusion proteins (or Rsd) under the control of IPTG-inducible promoters, and the cells were grown in the presence of 50 μM IPTG. Strain FW102 O_L2–62 also contains chromosomally encoded wild-type Rsd. The graph shows the averages of three independent experiments and standard deviations.

(B) Results of β-galactosidase assays performed with FW102 O_L2–62 cells containing three compatible plasmids, one encoding either λCI or the indicated λCI-*Pa* σ^{70} region 2 fusion protein, a second encoding either α or the indicated α-*Pa* σ^{70} region 4 fusion protein, and a third encoding either unfused wild-type AlgQ (gray bars) or no AlgQ (white bars). The plasmids directed the synthesis of the fusion proteins (or AlgQ) under the control of IPTG-inducible promoters, and the cells were grown in the presence of 50 μM IPTG. The graph

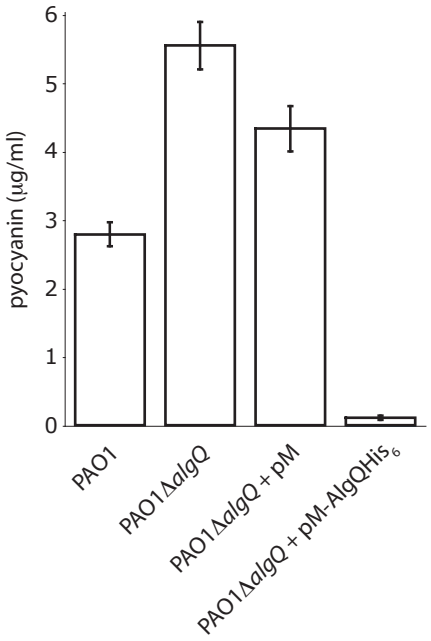

Figure S6. AlgQ inhibits pyocyanin production in *P. aeruginosa*.

Pyocyanin was assayed in supernatants of the indicated cultures following 24 hours of growth. Pyocyanin quantification is based on the absorbance of pyocyanin at 520 nm in acidic solution (Essar *et al*., 1990). Assays were performed essentially as described (Essar *et al*., 1990) with the following modifications: 5 ml cultures where grown for 24 hours in LB, and 2.5 ml of culture supernatants were extracted 3 times with 0.5 ml chloroform. The concentration of pyocyanin in a particular culture (in µg/ml) was calculated according to (Essar *et al*., 1990).

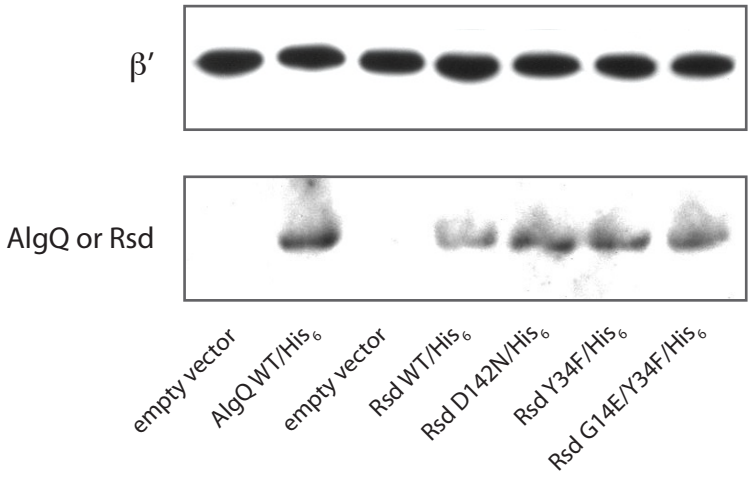

Figure S7. Plasmid-encoded wild-type and mutant Rsd proteins are produced at comparable levels in strain PAO1Δ*algQ*.

The western blot shows protein levels of the β' subunit of RNA polymerase (top panel, loading control), and wild-type AlgQ, wild-type Rsd, or the indicated Rsd mutant (bottom panel) from the same cells used in Figure 6C (lanes 3-7). All AlgQ and Rsd proteins bear a C-terminal hexahistidine tag. Normalized samples of cell lysates were processed for western blotting with either an anti-β' antibody (NeoClone) or His-Probe (Pierce) as described in (Deighan and Hochschild, 2007).

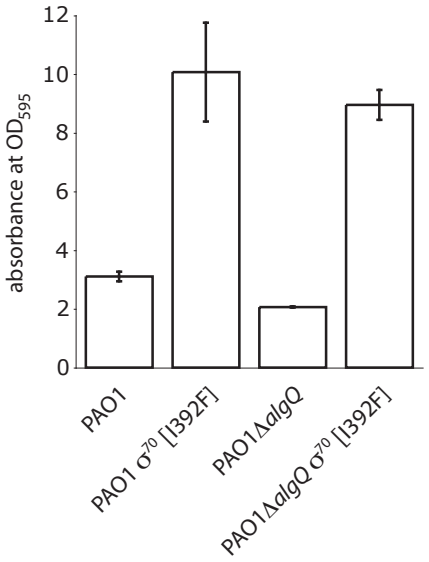

Figure S8. Amino acid substitution I392F in region 2 of *P. aeruginosa* σ^{70} promotes biofilm formation.

Biofilm formation by the indicated strains after 24 hours is shown. Duplicate cultures of PAO1, PAO1 σ^{70} [I392F], PAO1Δ*algQ*, and PAO1Δ*algQ* σ^{70} [I392F] were grown overnight at 37°C in LB with aeration. The overnight culture was used to inoculate 1mL TB media (contained in a 24-well tissue culture plate (Corning)) to a final OD_{600} of 0.0025. Plates were incubated at 37°C for 24 hours, after which media and unattached cells were removed, and 1.5mL 0.1% crystal violet was added to each well. Following incubation at room temperature for 30 minutes, the crystal violet solution was removed, and the plate was washed 4 times by immersion in water. The plate was allowed to dry for 1 hour at room temperature and the remaining crystal violet was dissolved in 33% acetic acid, and absorbance at OD_{595} was measured.

Supplemental References

Deighan, P. and Hochschild, A. (2007) The bacteriophage λQ anti-terminator protein regulates late gene expression as a stable component of the transcription elongation complex. *Mol Microbiol* **63:** 911-920.

Essar, D.W., Eberly, L., Hadero, A., and Crawford, I. P. (1990) Identification and characterization of genes for a second anthranilate synthase in *Pseudomonas aeruginosa*: interchangeability of the two anthranilate synthases and evolutionary implications. *J Bacteriol* **172:** 884-900.