Supporting Materials for the manuscript entitled "Repression of flagellar genes in exponential phase by CsgD and CpxR, two crucial modulators of *Escherichia coli* biofilm formation" by Dudin and co-authors.

Table S1. List of primers used in the present study. Primers with an asterisk were labeled with FITC for the gel retardation assay (Figure 5).

Primer name	Sequence (5' to 3')	Purpose
PrpoS1	GCTGGCTCGAGACGTGAGGAAATAC	Cloning of P*rpoS* into pZE GFP
PrpoS3	CGGAGAATTCAAGCAAAAGCCTG	Cloning of P*rpoS* into pZE GFP
rpoS atg KpnI	GGAGCGGTACCATGAGTCAGAATACGC	Cloning of *rpoS* CDS into pTOPO2
rpoS taa BamHI	AGATGGATCCTTACTCGCGGAACAGCG	Cloning of *rpoS* CDS into pTOPO2
rpoS ff1	ATAACGACACAATGCTGGTC	Verification of the *rpoS* deletion
rpoS fr2	GCTGGCAGCCTGCTTTGG	Verification of the *rpoS* deletion
B5bcsgD	GGATCCTGTACAACTTTTCTATCATTTC	Verification of the *csgD* deletion
csgDcdsRBamHI	ATAACGTTTCGGATCCTTATCGCCTGAGGTT	Verification of the *csgD* deletion
cpxR ff1	GGCCATGACGGCAGCGG	Verification of the *cpxR* deletion
cpxR fr2	TGCCCACTTATCAATCGCCC	Verification of the *cpxR* deletion
pAlon_Fw	TGGCAATTCCGACGTCTAAG	Verification of the promoters cloned into the pUA66 reporter plasmid (SC101 *ori*)
pAlon_Rv	CAACCAAAATTGGGACAACAC	Verification of the promoters cloned into the pUA66 reporter plasmid (SC101 *ori*)
flgM-EcoRI-F	GTGCTGGAATTCAATCAACTTGTTGAT	Construction of pRS*flgM*
flgM-BamHI-R	GGTTGTTTTTGGATCCCGGCTGTTCGTTA	Construction of pRS*flgM*
fliA- BamHI I-F	AAAAGCTAACCAGGATCCGCAGCAGGTTCT	Construction of pRS*fliA*
fliA-BamHI-R	CGAGTGTGGATCCATTACACCTTCA	Construction of pRS*fliA*
fliA-S1F	GCAACATAAAGCGCAATTT	Construction of FITC-labeled *fliA* DNA
flgM-S1F	TGGTATCGGGACAGGTAGT	Construction of FITC-labeled *flgM* DNA
lacZ-30R-FITC *	AGGGTTTTCCCAGTCACGACGTTGTAAAAC	FITC-labeled used in PCR with *fliA* / *flgM* S1F
CD6F	TTCTTGCCCGTCGCTGATTGCT	Construction of FITC-labeled *csgD* DNA
CD6R-FITC-R *	GCACTGCTGTGTGTAGTAAT	Construction of FITC-labeled *csgD* DNA
flgM_F	AACCGCGCGAAACCACTGACG	qRT-PCR on the *flgM* locus
flgM_R	CCGGTGTCCATTTTTAGTTCACC	qRT-PCR on the *flgM* locus
tar_F	GCGGTACGGATGATGATGG	qRT-PCR on the *tar* locus
tar_R	TTCCCTGGGTTGGCTGAGCG	qRT-PCR on the *tar* locus
16S_F	GGGGATAACTACTGGAAACG	qRT-PCR internal standard
16S_R	GCTAGGGATCGTCGCCTAGG	qRT-PCR internal standard
aer_F	AAGGGCAGCCGCACAACATGG	qRT-PCR on the *aer* locus
aer_R	CCGCGATCTCTTCATCCGTCG	qRT-PCR on the *aer* locus
fliC_F	TTCTGGCTTGCGTATTAACAGC	qRT-PCR on the *fliC* locus
fliC_R	ATCCAGACGGGATTTAATTTCG	qRT-PCR on the *fliC* locus
fliL_F	CCGATTCTGGTATTCATTACC	qRT-PCR on the *fliL* locus
fliL_R	GCGGGTAGCTTCATCTTTCAGG	qRT-PCR on the *fliL* locus
trg_F	CCGTTATTTGCCTGCATTCTAGG	qRT-PCR on the *trg* locus
trg_R	CTTGCTGCGACTGTTTAATCTCC	qRT-PCR on the *trg* locus
tsr_F	GATCAGAATAATATTGGTAGCGG	qRT-PCR on the *tsr* locus
tsr_R	TTTTGCCTGCACCTAACAGTTGG	qRT-PCR on the *tsr* locus
ves_F	TGAAACGCGCGAAATTTGCACG	qRT-PCR on the *ves* locus
ves_R	GTCCGCTGCAAAGGCAAAAGGC	qRT-PCR on the *ves* locus
ycgR_F	CAAAGCCGCAATTCCTTTGAGC	qRT-PCR on the *ycgR* locus
ycgR_R	AGGCGGTACGGTAATAAATGC	qRT-PCR on the *ycgR* locus

Table S2. List of genes encoding transcriptional modulators slightly activated by RpoS in LB or M63 media for which insertion mutants are available in the Keio collection of strain BW25113. Regulatory genes (K-COG) differently expressed were selected from previous transcriptomic studies performed by the laboratory of H. Schellhorn (Hamilton, Canada) using Affymetrix gene chips (Geo data : GDS3123 and GSE12797, see corresponding references in the manuscript).

Gene name	b-number	Gene function
argP	b2916	DNA-binding transcriptional activator, replication initiation inhibitor
cpxR	b3912	DNA-binding response regulator in two-component regulatory system with CpxA
csgD	b1040	DNA-binding transcriptional activator in two-component regulatory system
csiE	b2535	stationary phase inducible protein
cueR	b0487	DNA-binding transcriptional activator of copper-responsive regulon genes
frvR	b3897	predicted regulator
gadW	b3515	DNA-binding transcriptional activator
gadX	b3516	DNA-binding transcriptional dual regulator
metR	b3828	DNA-binding transcriptional activator, homocysteine-binding
narL	b1221	Nitrite response regulator
nsrR	b4178	predicted DNA-binding transcriptional regulator
nusG	b3982	transcription termination factor
rhaR	b3906	DNA-binding transcriptional activator, L-rhamnose-binding
sfsB	b3188	DNA-binding transcriptional activator of maltose metabolism
yeaM	b1790	predicted DNA-binding transcriptional regulator
ygcP	b2768	predicted anti-terminator regulatory protein
ygfI	b2921	predicted DNA-binding transcriptional regulator
yhjB	b3520	predicted DNA-binding response regulator in two-component regulatory system
yiaG	b3555	predicted transcriptional regulator
yidL	b3680	predicted DNA-binding transcriptional regulator
yqgE	b2948	predicted protein

Figure S1. Expression of *fliA* in various growth conditions. (**A**) The time of entrance into stationary phase influences the timing of induction of FliA expression in exponential phase in an *rpoS* mutant strain. (**B**) Addition of casaminoacids favors fast growing and enhances the stimulatory effect of the *rpoS* deletion. The increase of *fliA* expression in presence of casamino acid is comparable to the induction observed in LB (as shown in Figure 5D of the manuscript). Promoter activity is expressed in RFU/min (relative fluorescence per time), derived from the kinetics of the absorbance and GFP reporter data.

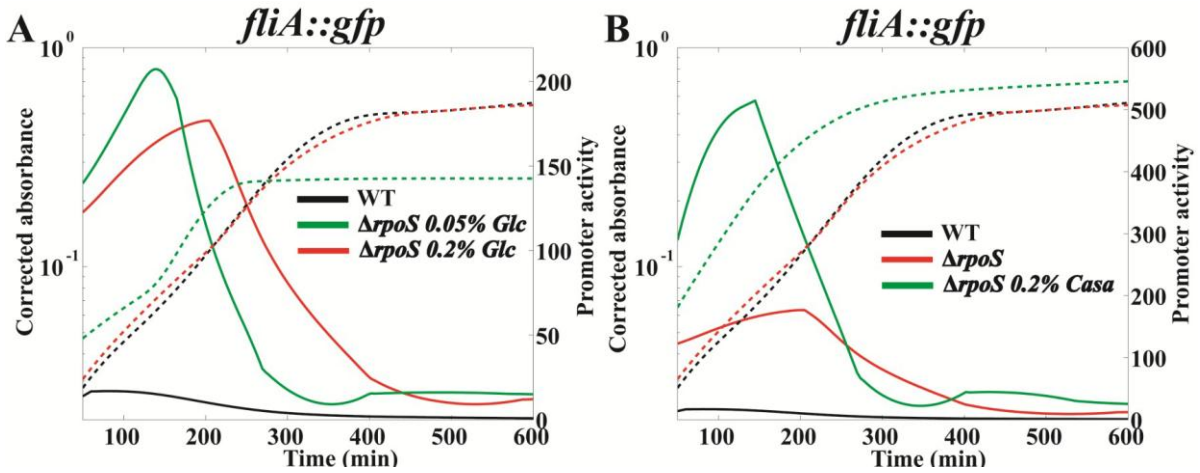


Figure S2. The *csgD* mutation affects swimming motility of *E. coli* in a temperature independent manner. Cell motility was assayed on LB soft agar plate (0.3%) incubated 15 hours (30°C, left panel) or 8 hours (37°C, right panel).

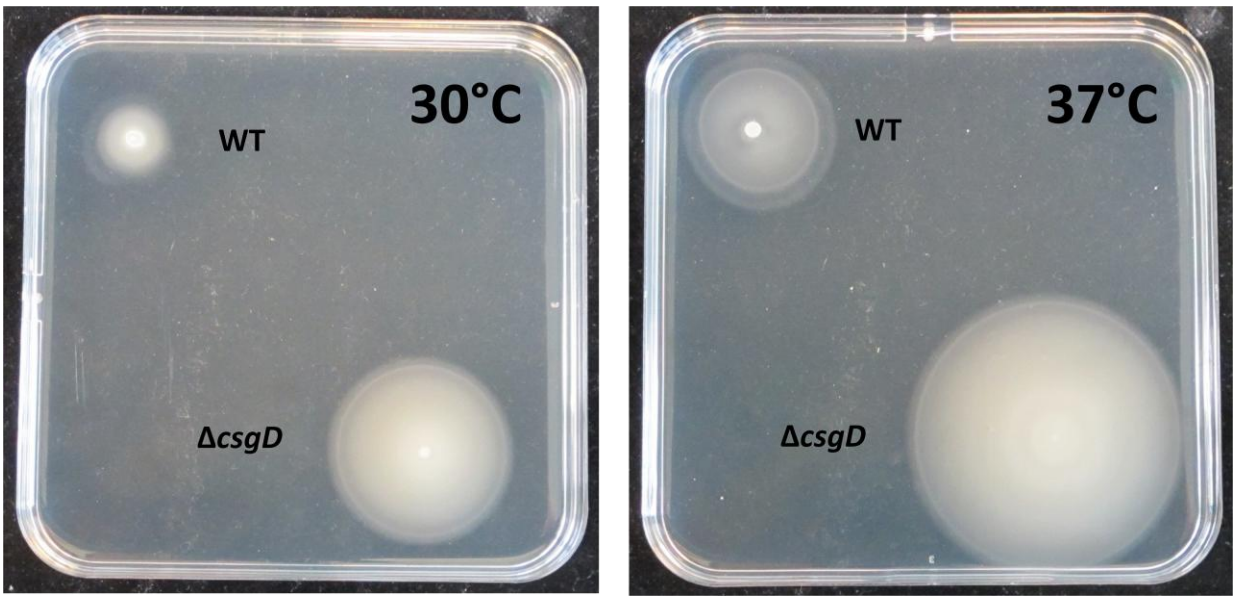
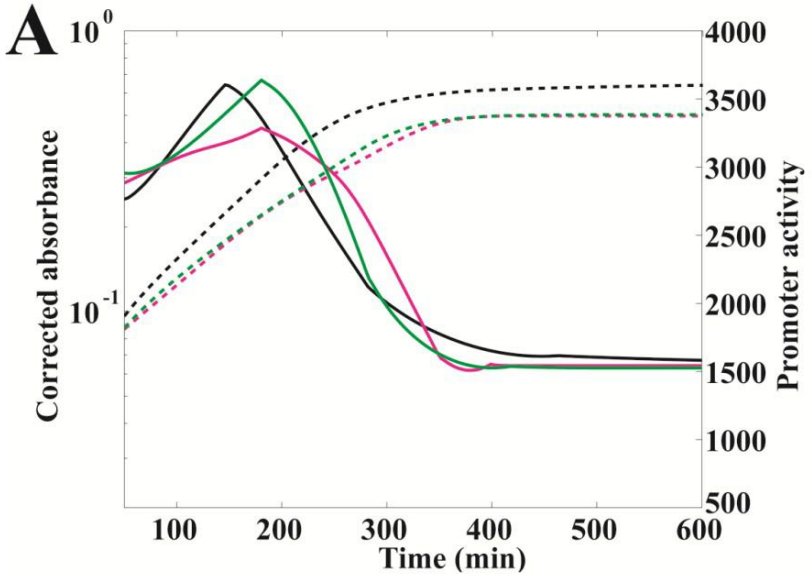
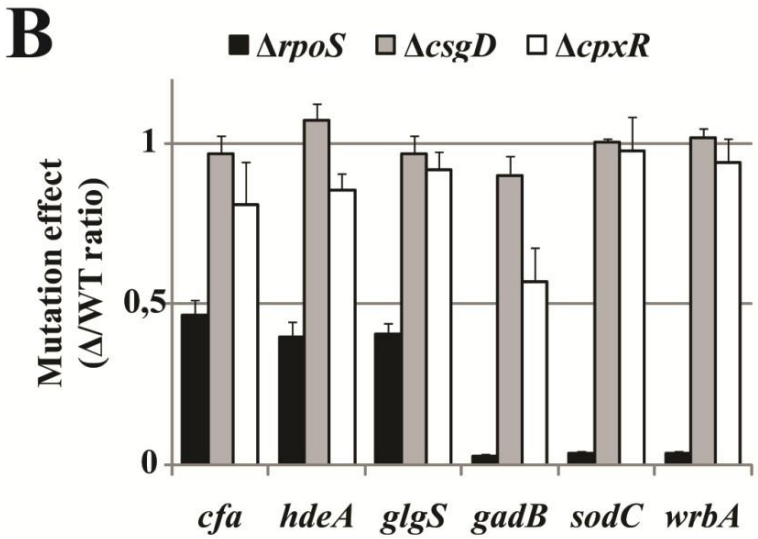


Figure S3. CsgD and CpxR neither affect *rpoS* transcription nor RpoS level and activity. (A) Expression profile of an *rpoS::gfp* transcriptional fusion in the wild-type strain (black), *csgD* mutant (pink) and *cpxR* mutant (green) in M9 medium at 37°C. Absorbance measurements are represented with dashed curves. Promoter activity is expressed in RFU /min. (B) Six RpoS-dependent fusions were measured in *rpoS, csgD,* or *cpxR* mutant strains. The GFP concentrations (RFU) were measured in the wild-type and mutant strains in stationary phase (Abs_{600} = 0.6) when the target genes of RpoS are fully expressed (Dudin *et al.,* 2013). The histograms show the expression ratios relative to the WT strain as determined from at least three independent experiments. (C) Detection of RpoS by Western blot analysis in exponential phase (OD_{600nm} = 0.6). Crude cell extracts (cell-pellet from about 0.8 mL of M9 culture at 37°C resuspended in a loading blue containing β-mercapto-ethanol and C-complement (Roche) protease inhibitor) were loaded onto a denaturing 4-12% acrylamide gel (Invitrogene) and separated on a glycine / Tris-base running buffer. An anti-RpoS mouse monoclonal serum (Santa-Cruz Biotechnology) was used together with a peroxydase conjugate for revelation (ECL Amersham). The genotype of the strains is indicated above the lanes.





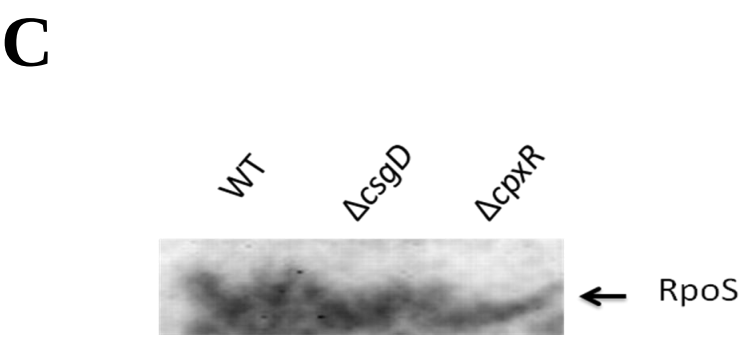


Figure S4. Detection of FliA in the *csgD* and *cpxR* mutant strains in exponential phase. The Western blot was performed as described in Figure S3 using anti-FliA mouse monoclonal serum (Santa-Cruz Biotechnology). The genotype of the strain is indicated above the lanes. FliA was undetectable in the WT strain in our growth conditions.

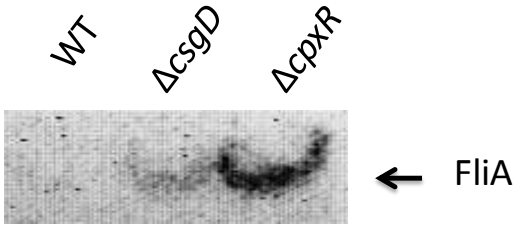


Figure S5. Negative regulation by csgD and cpxR of class 3 motility genes in exponential phase. RNA was prepared by hot-phenol extraction of cultures grown in M9 glucose medium at exponential phase (OD_{600nm}: 0.6). RT-qPCR was performed after random priming reverse transcription (SuperScript II, Invitrogene) and PCR (Mesagreen Eurogentec) using the 16S rRNA as internal control (ΔΔCT method) on an Applied Biosystems 7500 real time PCR system. The primer sequences are given in Table S1. The Mutant/WT expression ratios (in log10 scale) correspond to the average of technical triplicates giving very similar results.

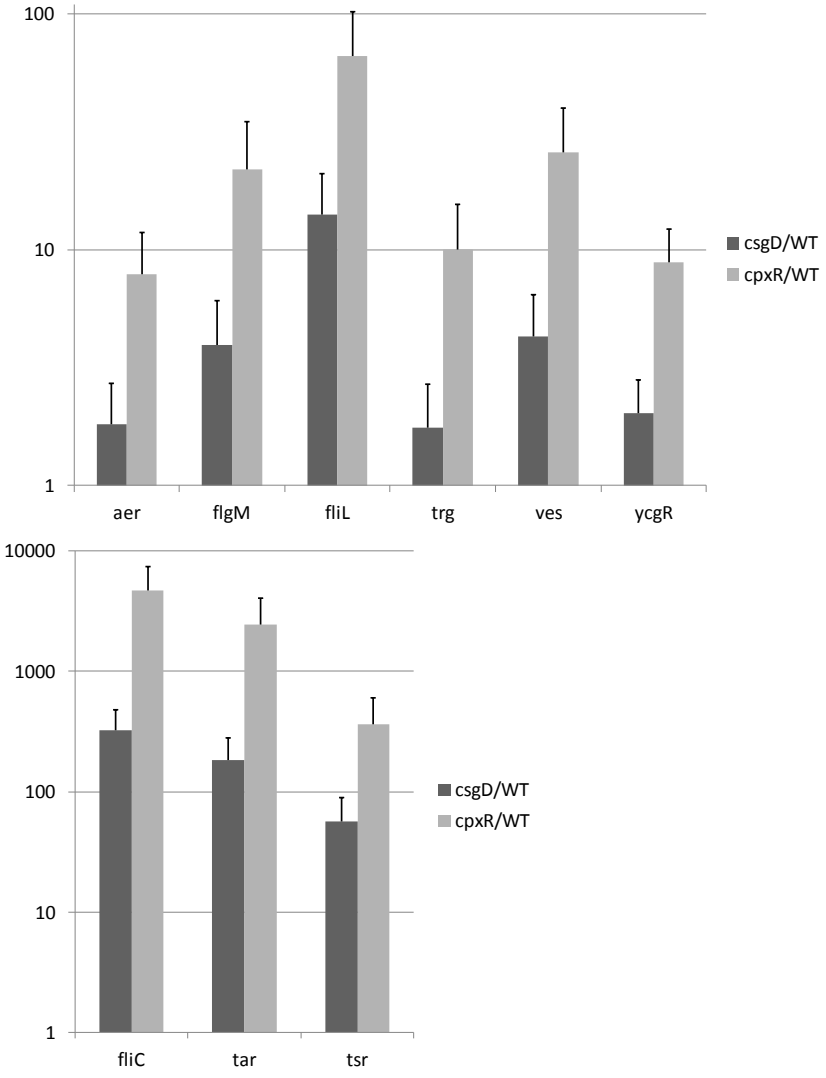


Figure S6. CRP-cAMP activates *fliA*. (**A**) *fliA::gfp* gene expression profiles in the wild-type (black), *rpoS* (red) and *cya* (pink) mutant strains. The *cya* mutant strain was supplemented with exogenous cyclic-AMP at 500 µM (orange) or 1 mM (blue) added at time 0. The black dashed curve shows a typical growth curve (Abs_{600}). (**B**) *fliA::gfp* expression was measured in a *cya* mutant strain after addition of exogenous cAMP (1 mM) at time 0 min (blue), 200 min (grey), 300 min (orange), 400 min (green) or in the absence of cAMP (pink). The complementation with cAMP is observed immediately in all growth phases. The experiments were performed in M9 minimal medium at 37°. Promoter activity is expressed in RFU/min (relative fluorescence per time), derived from the kinetics of the absorbance and GFP reporter data.