Systematic identification of novel regulatory interactions controlling biofilm formation in the bacterium *Escherichia coli*

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Supporting Information

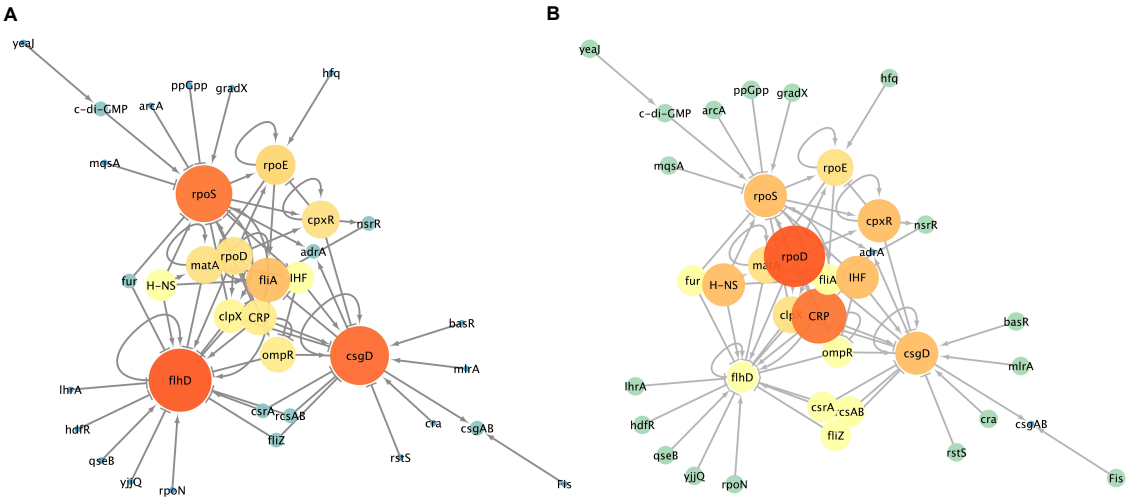

Figure S1. Flagella-biofilm transcriptional regulatory network. The principal nodes and paths, which drive the flagella-biofilm network were mapped using Cytoscape 3.4.1. The network was analyzed by using degree (A) and out-degree (B). Size of the nodes (circles) indicates number of interactions of the nodes. Color scale colors from light yellow to dark orange indicating the low to high values.

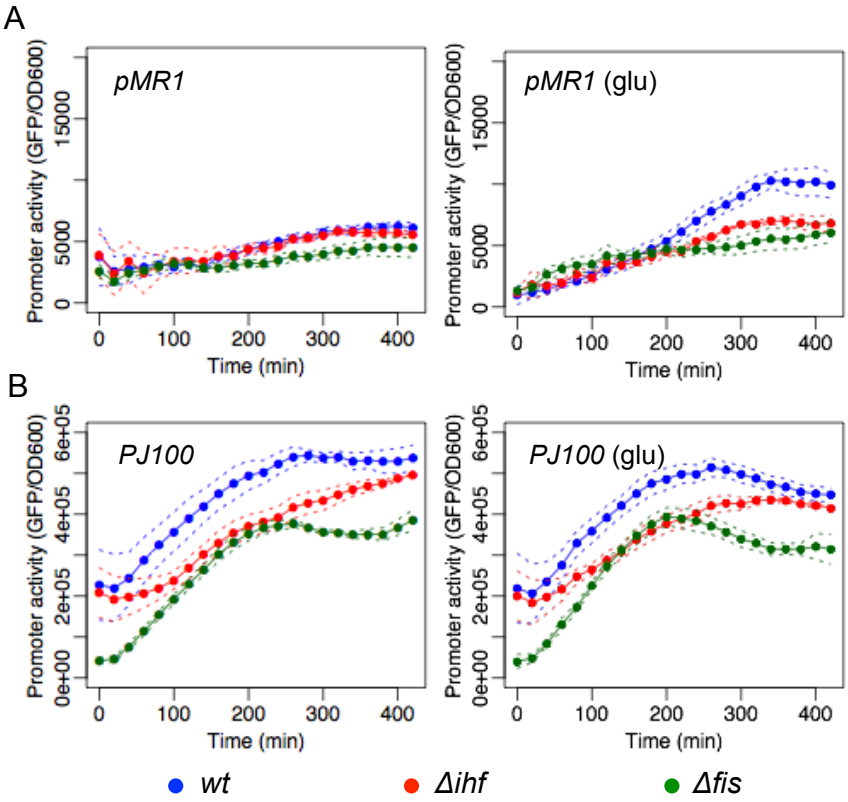

Figure S2. Effect of CRP, IHF and Fis GRs over the controls. Promoter activity assay of (A) pMR1 empty vector and (B) pMR1-*Pj100*, a synthetic constitutive strong promoter. The systems were evaluated in *E. coli* BW25113 *wild-type* (blue line), Δ*ihf* (red line) and Δ*fis* (green line) in 96-well plate as described in methods in the absence (left panel) or presence (right panel) of 0.4% of glucose. GFP fluorescence was measured every 20 minutes over 8 hours growth at 37 ºC in static conditions (normalized by OD600). Solid lines represent the mean of three independent experiments, while dashed lines are the upper and lower limits of standard error (SE) of the mean.

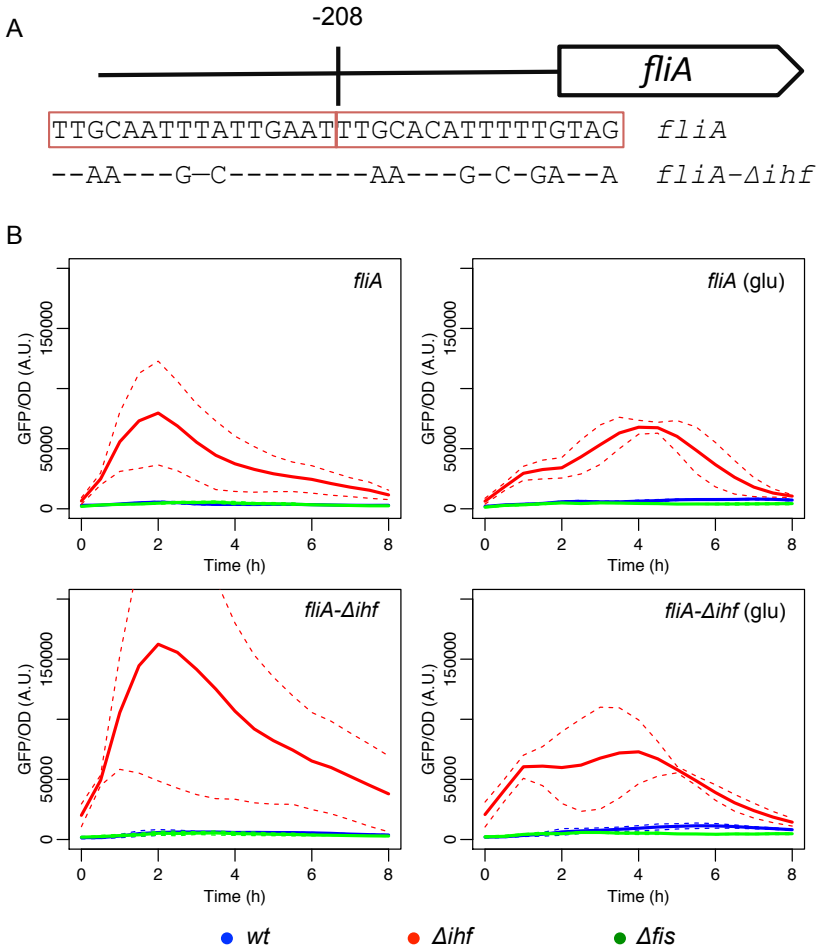

Figure S3. Effect of CRP, IHF and Fis on wild-type and mutant versions of *fliA* promoter. (A) Identification of two putative IHF binding sites at the *fliA* promoter using Virtual Footprint (http://prodoric.tu-bs.de/vfp/index2.php). The inserts represented the original and mutated versions constructed (the putative IHF sites are boxed). (B) Assays of the two promoters were evaluated in *E. coli* BW25113 *wild-type* (blue line), Δ*ihf* (red line) and Δ*fis* (green line) in 96-well plate as described in methods in the absence (left panels) or presence (right panels) of 0.4% of glucose. GFP fluorescence was measured every 20 minutes over 8 hours growth at 37 ºC in static conditions (normalized by OD600). Solid lines represent the mean of three independent experiments, while dashed lines are the upper and lower limits of SE.

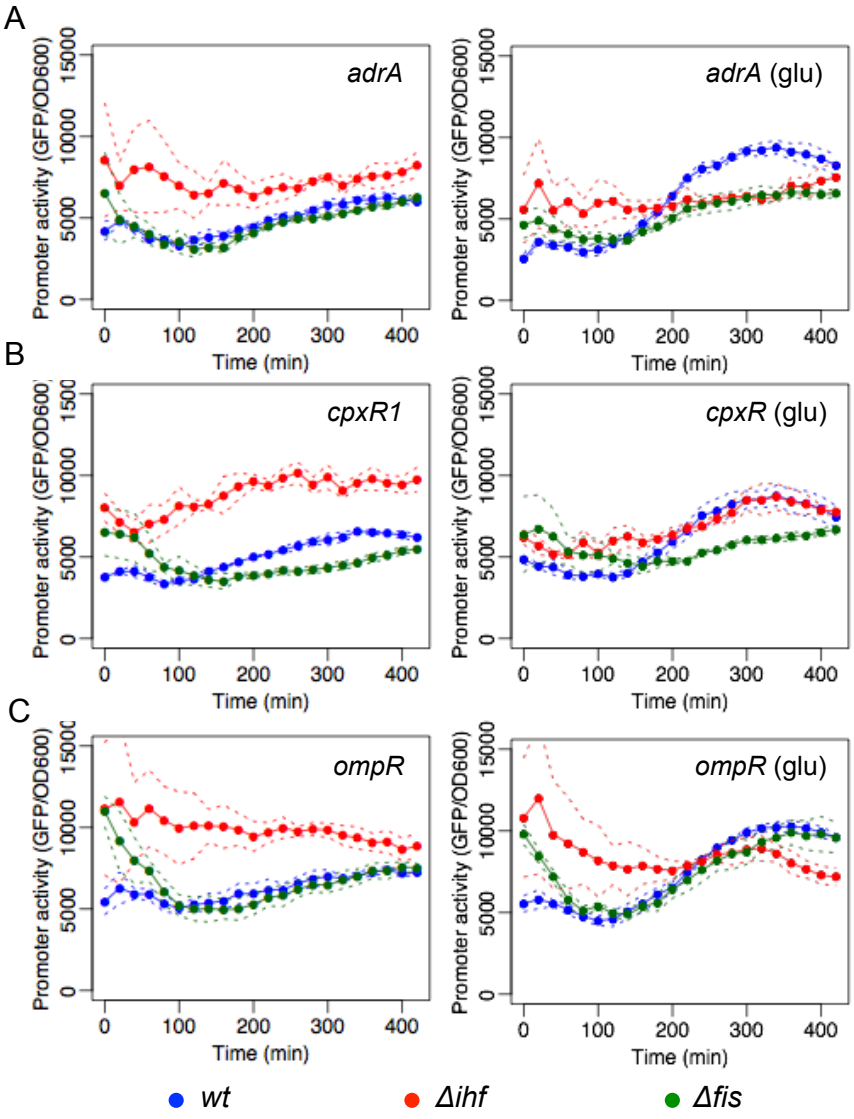

Figure S4. Effect of CRP, IHF and Fis GRs over the promoter activity of *adrA*, *cpxR* and *ompR*. Promoter activity assay of (A) pMR1-*PadrA,* (B) pMR1-*PcpxR,* and (C) pMR1-*PompR* were evaluated in *E. coli* BW25113 *wild-type* (blue line), Δ*ihf* (red line) and Δ*fis* (green line) in 96well plate as described in methods in the absence (left panel) or presence (right panel) of 0.4% of glucose. GFP fluorescence was measured every 20 minutes for 8 hours growth at 37 ºC in static conditions (normalized by OD600). Solid lines represent the mean of three independent experiments, while dashed lines are the upper and lower limits of SE.

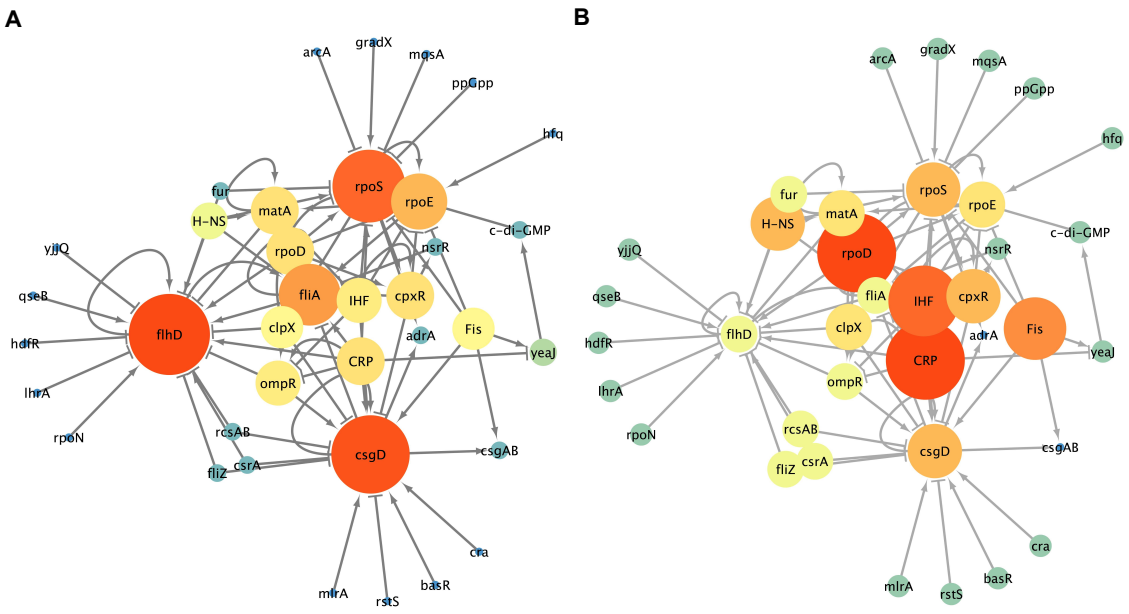

Figure S5. Flagella-biofilm transcriptional regulatory network with new interactions added. The principal nodes and paths, which drive the flagella-biofilm network upon the introduction of seven new interactions were analyzed using Cytoscape 3.4.1. The network was analyzed by using degree (A) and out-degree (B). Size of the nodes (circles) indicates number of interactions of the nodes. Scale colors from red to bright to dark indicate the high to low values.

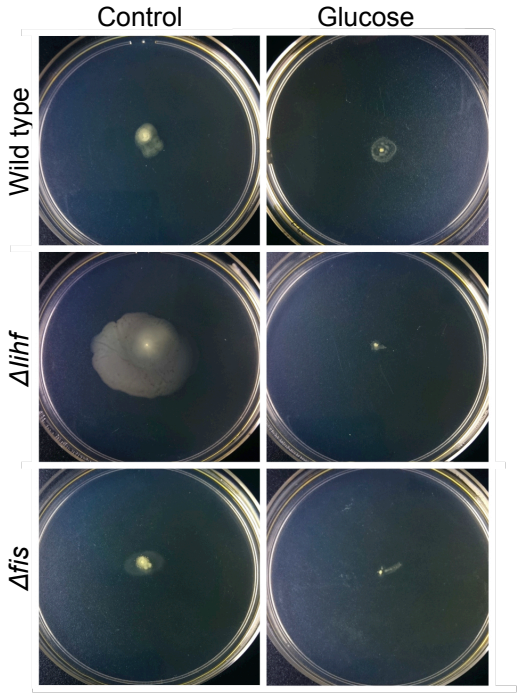

Figure S6. Effect of GRs in the motility program at 18h. Motility phenotype of *E. coli* BW25113 wild-type and mutant strains were evaluated by cell motility assay at 18h. in the presence or absence of glucose as depicted. Divergent motility capability is observed between the different conditions, proving the effect of the GRs CRP, IHF and Fis to modulate the motility program. The results are representative of 3 independent experiments.

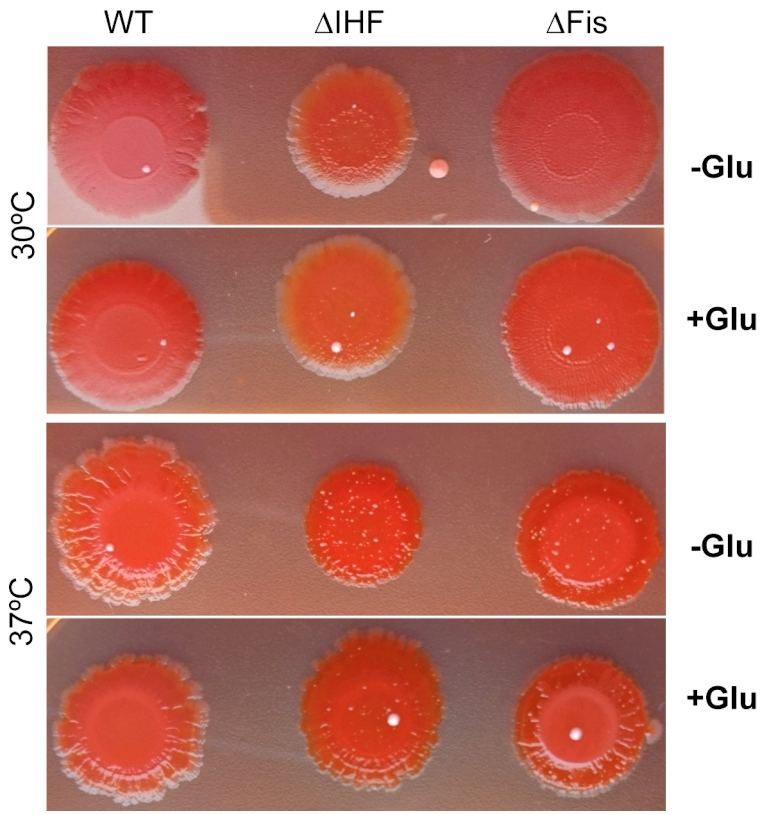

Figure S7. Capability of *E. coli* and mutant strains to develop mature biofilm. Mature biofilm formation of *E. coli* BW25113 *wt*, Δ*ihf* and Δ*fis* strains were perform using Congo red plate assay. Comparisons of the mature biofilm morphological characteristics of wild-type and mutant strains at 30 ºC and 37˚C in the presence or absence of glucose is shown. The results are representative of 3 independent experiments.

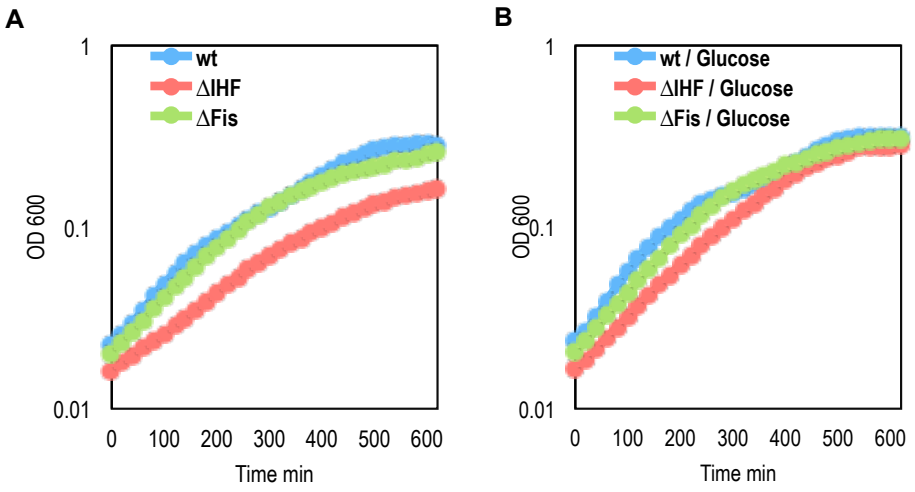

Figure S8. Growth curve of *E. coli* wild-type and mutant strains under the conditions used for promoter analysis. Experiments were performed in the absence (A) or presence (B) of 0.4% of glucose. Optical density at 600nm (OD600) was measured every 20 minutes over 8 hours growth at 37 ºC in static conditions. The curves represent the mean from three independent experiments.

Global Regulator	Connection	Node	Contribution
CRP	activates	*rpoS*	confirmed
IHF	inhibits	*rpoS*	confirmed
Fis	inhibits	*rpoS*	New
IHF	inhibits	*rpoE*	New
Fis	inhibits	*rpoE*	New
CRP	activates	*csgD*	confirmed
IHF	activates	*csgD*	confirmed
Fis	activates	*csgD*	New
CRP	activates	*flhD*	confirmed
CRP	activates	*fliA*	New
IHF	inhibits	*fliA*	New
CRP	inhibits	*yeaJ*	New
Fis	activates	*yeaJ*	New

Table S1. New regulatory interactions in the flagella-biofilm regulatory network.