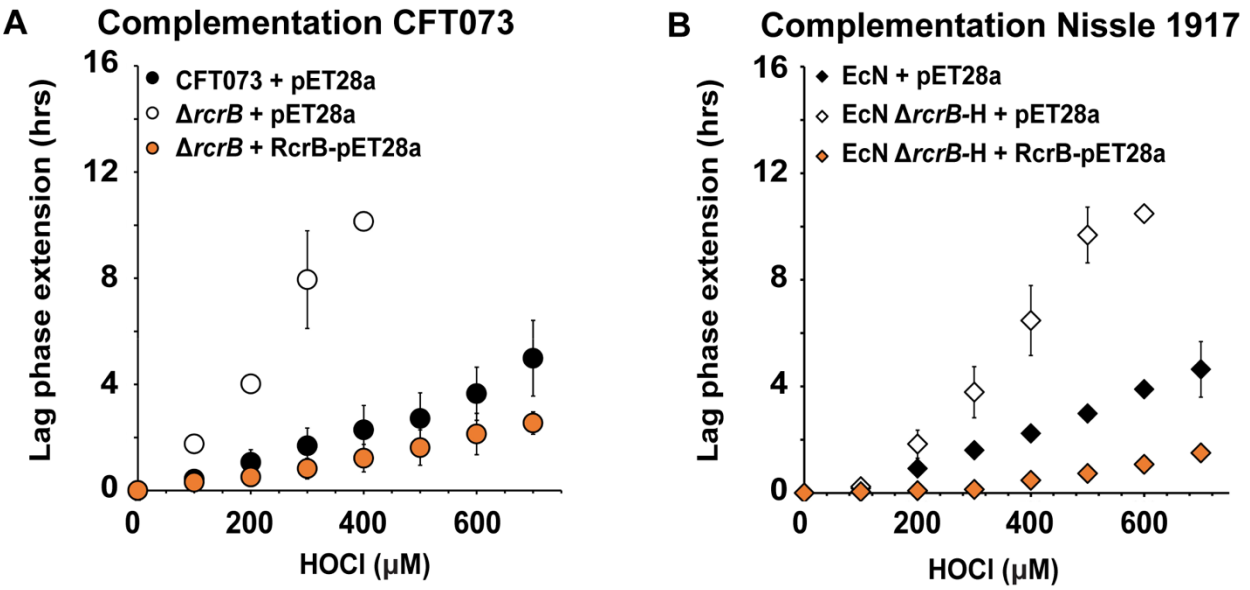
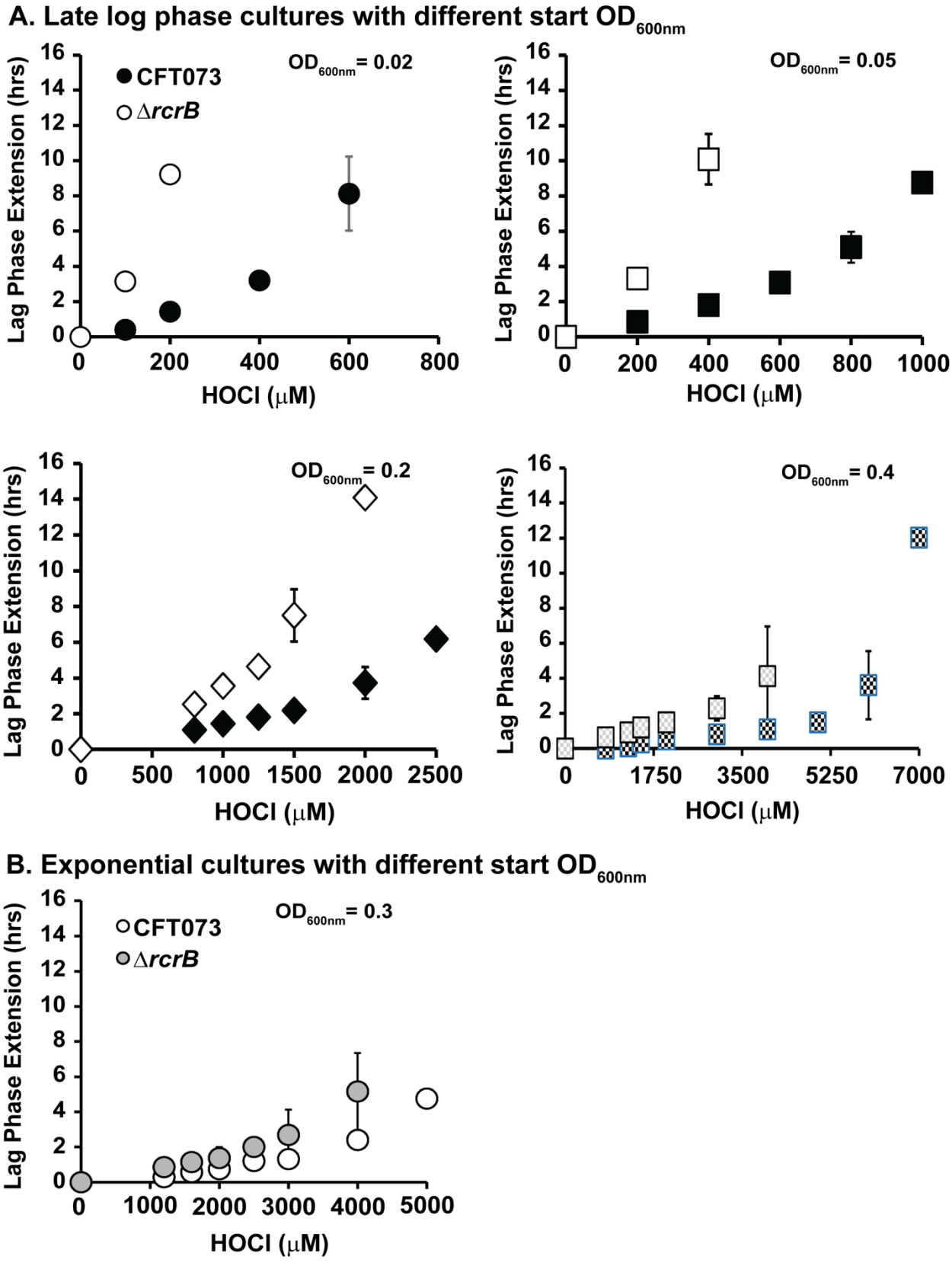
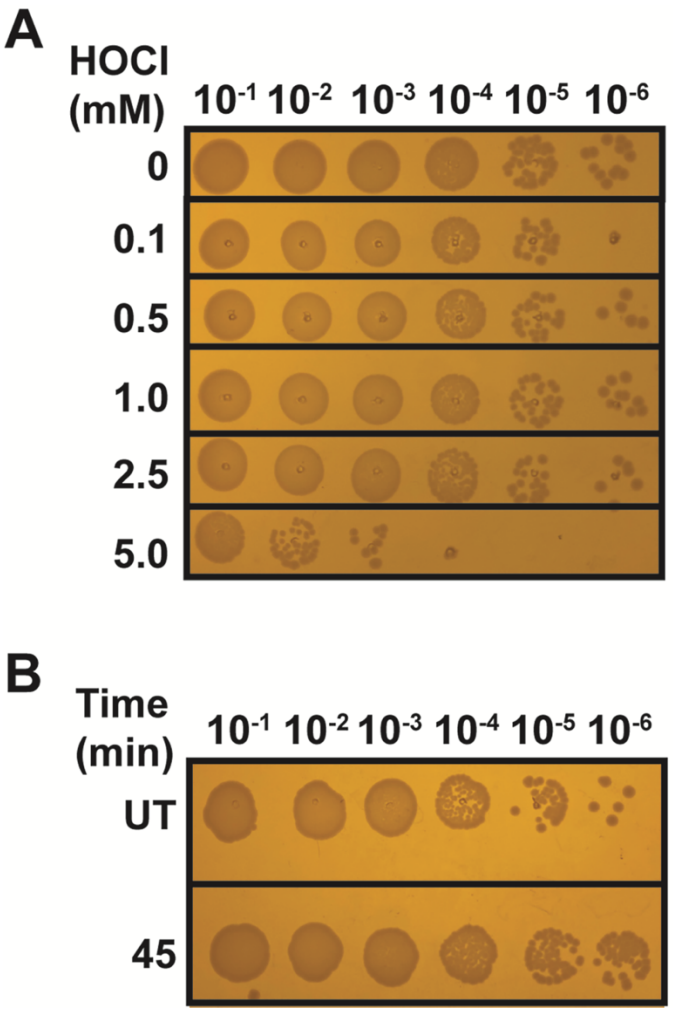
Supplemental Figures



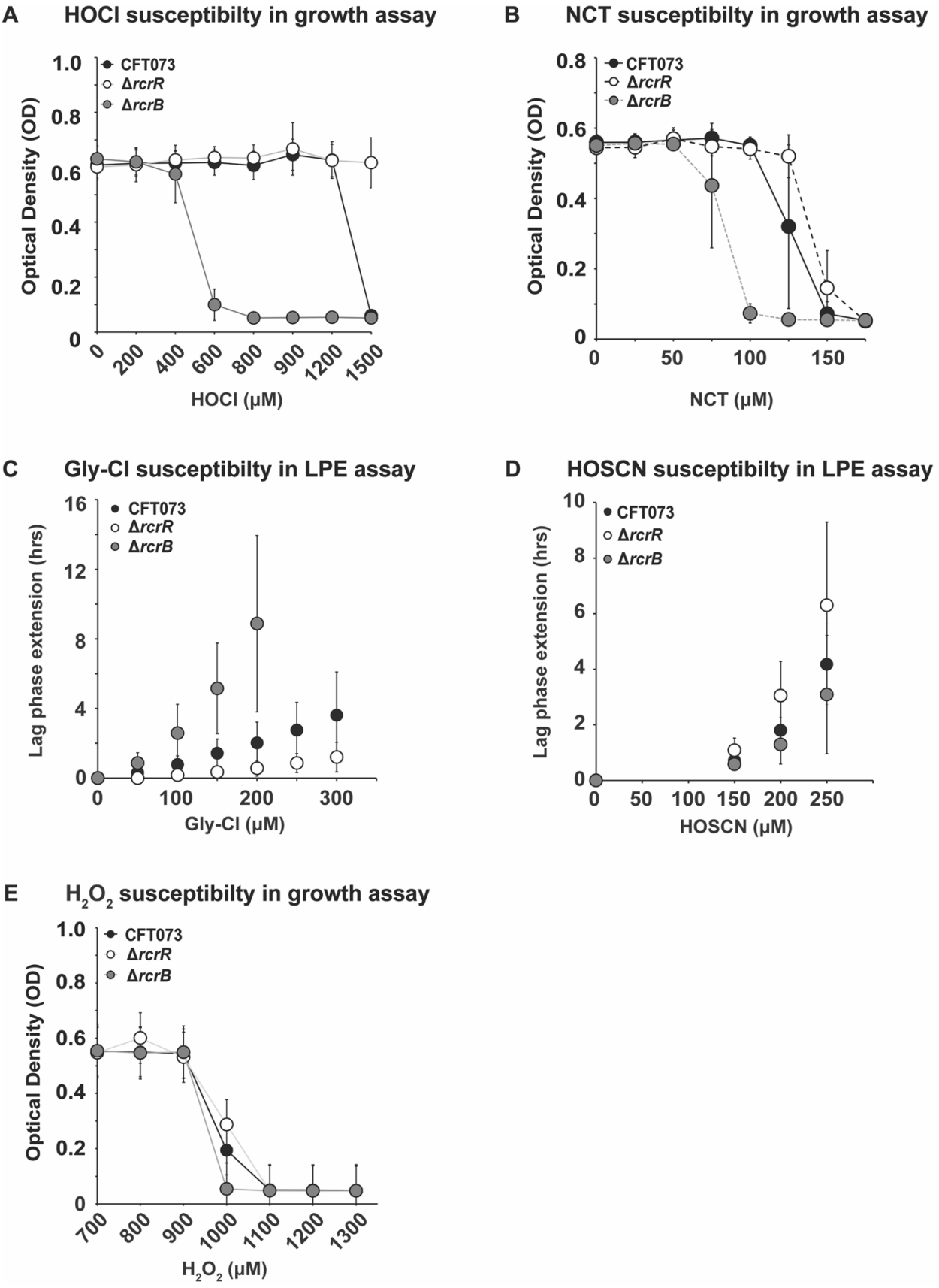
Supplementary FIG S1. Recombinant expression of CFT073 RcrB complements the HOCl sensitivity phenotypes of *E. coli* strains deficient in *rcrB* or its homolog. Growth phenotype analyses of the UPEC strain CFT073 and Nissle 1 917 carrying pET28a or *rcrB*-pET28a in the presence and absence of *rcrB* were performed in MOPSg media in the presence of the indicated HOCl concentrations. HOCl-mediated LPE was calculated for each strain (see Materials and Methods for a detailed protocol). Recombinant expression of RcrB in **(A)** CFT073 and Δ*rcrB*, and **(B)** *E. coli* Nissle 1917 and Δ*rcrB*-homolog (Δ*rcrB*-H) resulted in full complementation restoring HOCl resistance comparable to the corresponding wildtype strains CFT073 and EcN, respectively *(n = 4, ± S.D.)*.


A. Late log phase cultures with different start OD600nm
OD600nm= 0.02
CFT073
ΔrcrB
OD600nm= 0.05
OD600nm= 0.2
OD600nm= 0.4
Lag Phase Extension (hrs)
HOCl (μM)
B. Exponential cultures with different start OD600nm
OD600nm= 0.3
CFT073
ΔrcrB

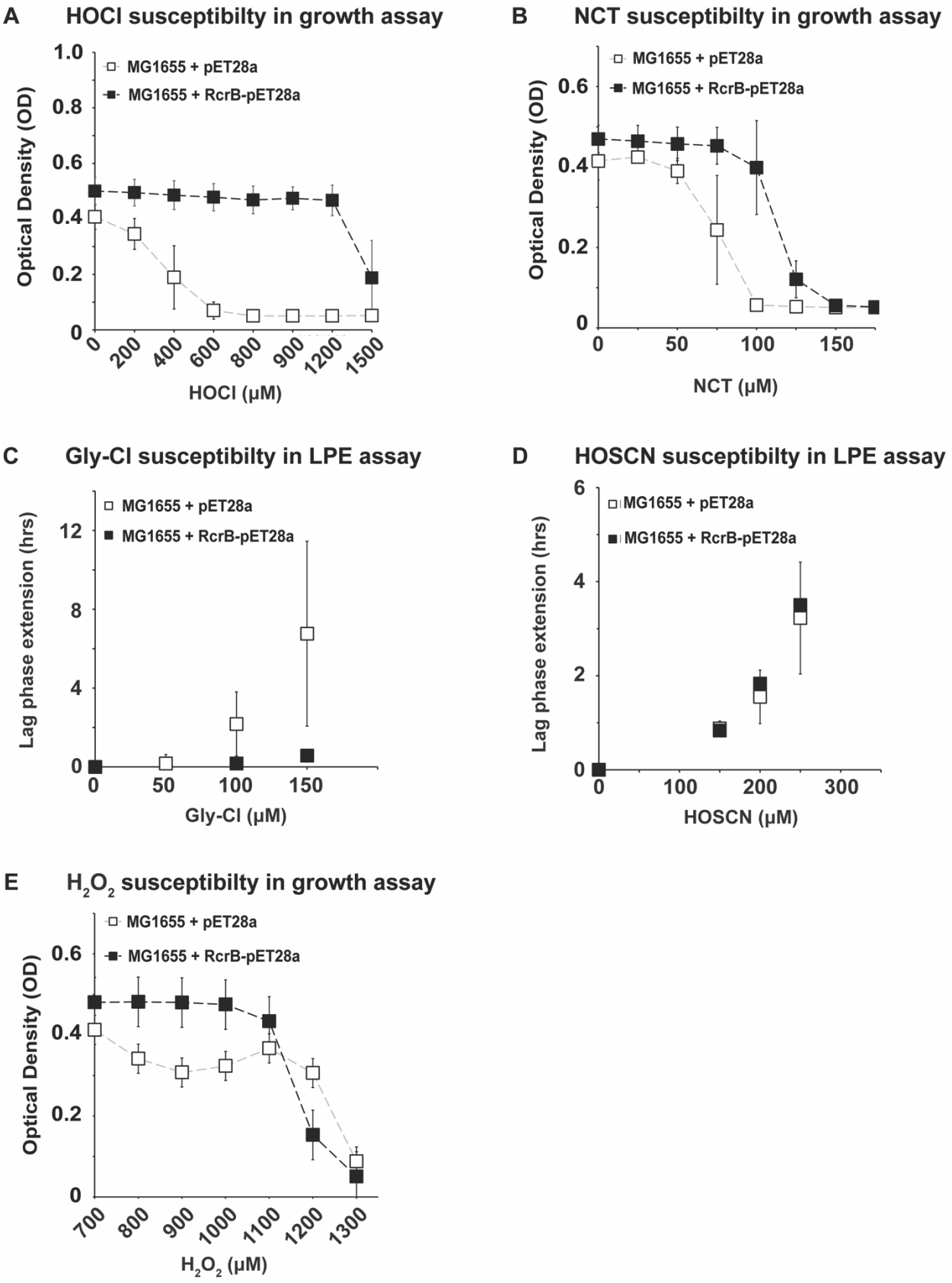
Supplementary FIG S2. RcrB plays an important role for UPEC's HOCl resistance under various growth conditions. Growth phenotype analyses of UPEC strains CFT073 and Δ*rcrB* were performed in MOPSg media in the presence of the indicated HOCl concentrations. HOCl-mediated LPE was calculated for each strain (see Materials and Methods for a detailed protocol). **(A)** *Late log cultures:* overnight cultures of CFT073 (black fillings) and Δ*rcrB* (white fillings) were diluted 25-fold into fresh MOPSg and grown until they reached late log / early stationary phase (OD_{600} ~2) before they were diluted again to the indicated start OD_{600} and cultivated in the presence of the indicated HOCl concentrations; *(n = 3-4, ± S.D.)*. **(B)** *Exponential cultures:* overnight cultures of CFT073 (white circles) and Δ*rcrB* (grey circles) were diluted into fresh MOPSg to an OD_{600} = 0.01 and grown until they reached an OD_{600} = 0.3 before they were split up and cultivated in the presence of the indicated HOCl concentrations; *(n = 3-4, ± S.D.)*.



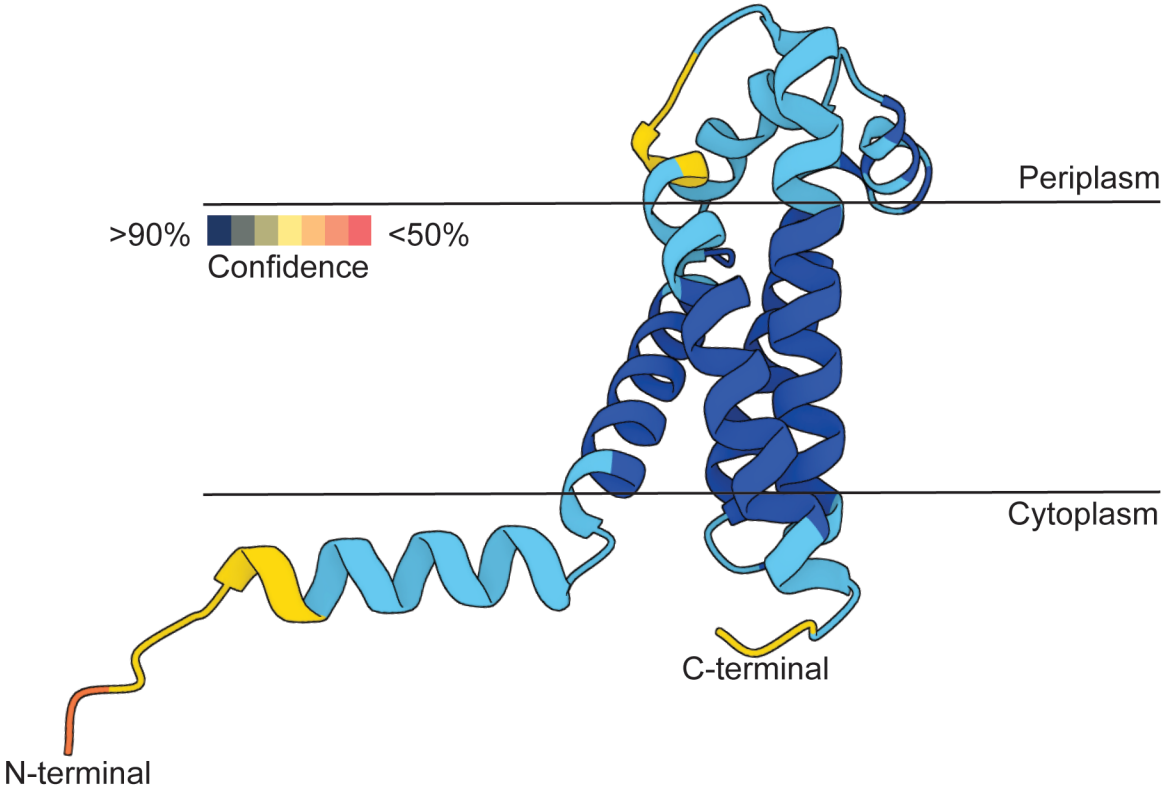
Supplementary FIG S3. Survival assays performed in conjunction with gene expression analyses. UPEC strain CFT073 was grown in MOPSg to mid-log phase (OD_{600} ~0.5-0.55) before it was incubated with the indicated HOCl concentrations for the indicated time. Remaining HOCl was quenched with 5-fold excess of thiosulfate after **(A)** 15 min and **(B)** 45 min of exposure to 1 mM HOCl, cells serially diluted and spot-titered onto LB agar plates for overnight incubation at 37 °C. The experiments were repeated at least three independent times.


A
HOCl susceptibilty in growth assay
CFT073
ΔrcrR
ΔrcrB
Optical Density (OD)
1.0
0.8
0.6
0.4
0.2
0
0
200
400
600
800
900
1200
1500
HOCl (μM)
B
NCT susceptibilty in growth assay
CFT073
ΔrcrR
ΔrcrB
Optical Density (OD)
0.8
0.6
0.4
0.2
0
0
50
100
150
NCT (μM)
C
Gly-Cl susceptibilty in LPE assay
CFT073
ΔrcrR
ΔrcrB
Lag phase extension (hrs)
16
12
8
4
0
0
100
200
300
Gly-Cl (μM)
D
HOSCN susceptibilty in LPE assay
CFT073
ΔrcrR
ΔrcrB
Lag phase extension (hrs)
10
8
6
4
2
0
0
50
100
150
200
250
HOSCN (μM)
E
H2O2 susceptibilty in growth assay
CFT073
ΔrcrR
ΔrcrB
Optical Density (OD)
1.0
0.8
0.6
0.4
0.2
0
700
800
900
1000
1100
1200
1300
H2O2 (μM)

Supplementary FIG S4. The RcrR regulon protects from HOCl stress and its byproducts but not from H_2O_2 and HOSCN. Growth phenotype analyses of UPEC strains CFT073, Δ*rcrB*, and Δ*rcrR* were performed in MOPSg media in the presence of the indicated stressor concentrations. LPE were calculated for each strain (see Materials and Methods for a detailed protocol). The absence of *rcrB* in CFT073 caused a reduction in HOCl resistance, while constitutive RcrB expression (i.e. Δ*rcrR*) caused increased resistance to all stressors except HOSCN and H_2O_2, *(n = 4-6, ± S.D.)*.


A
HOCl susceptibilty in growth assay
MG1655 + pET28a
MG1655 + RcrB-pET28a
Optical Density (OD)
HOCl (μM)
B
NCT susceptibilty in growth assay
NCT (μM)
C
Gly-Cl susceptibilty in LPE assay
Lag phase extension (hrs)
Gly-Cl (μM)
D
HOSCN susceptibilty in LPE assay
HOSCN (μM)
E
H2O2 susceptibilty in growth assay
H2O2 (μM)

Supplementary FIG S5. The RcrR regulon protects from HOCl stress and its byproducts but not from H_2O_2 and HOSCN. Growth phenotype analyses of the K-12 *E. coli* strain MG1655 carrying pET28a or *rcrB*-pET28a, respectively, were performed in MOPSg media in the presence of the indicated stressor concentrations. LPE were calculated for each strain (see Materials and Methods for a detailed protocol). Recombinant expression of RcrB in MG1655 resulted in an increased resistance to all stressors except for HOSCN and H_2O_2 compared to the empty vector control, *(n = 4-6, ± S.D.)*.



Supplementary FIG S6. The predicted alphafold structure of RcrB.