SUPPLEMENTAL MATERIAL

Pseudomonas aeruginosa defense systems against microbicidal oxidants

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Running title: Defense systems against microbicidal oxidants

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Supplemental Figure / Table Legends

Fig. S1. Survival of PA14 after treatment with HOCl, HOBr or HOSCN in PBS buffer. PA14 wild-type cells were grown in MOPS-glucose media to mid-log phase. Then, cells were spun down, resuspended in PBS and incubated with the indicated concentrations of either HOCl, HOBr, or HOSCN for 30 min. Excess HOX was quenched by adding 10 mM sodium thiosulfate. The bacteria were serially diluted with 0.85% NaCl, spot-titered onto LB agar plates, and incubated overnight at 37°C. The experiments were repeated at least three independent times and a representative result is shown.

Fig. S2. Defining sublethal HOCl, HOBr or HOSCN stress conditions and gene enrichment analysis using Fisher's Exact Test. (**A**) PA14 wild-type cells were grown in MOPS-glucose media until mid-log phase was reached. Then, the cells were incubated with 0.5 mM HOCl, 0.15 mM HOBr or 0.25 mM HOSCN. Growth was recorded every 30 min for 6 h post treatment. Inset: Cells were incubated for 30 min with HOCl, HOBr or HOSCN before excess HOX was quenched by the addition of 10 mM sodium thiosulfate. Cells were serially diluted with 0.85% NaCl, spot-titered onto LB agar plates, and incubated overnight at 37°C. The experiments were repeated at least three independent times. **(B)** Enrichment of differentially expressed genes in each of 78 regulons was tested using one-sided Fisher's Exact Tests. *P*-values were adjusted for false discovery rate as described in *Experimental Procedures*. Regulons determined to be significantly enriched for differentially expressed genes (FDR < 0.05) are presented as heatmaps, using the heatmap function from the R package NMF. Log_2 - fold change values of each treatment group compared to the untreated control group were used for

clustering genes by their Euclidean distance with complete linkage. No significant enrichment was determined for the OxyR regulon.

Fig. S3. Effects of HOCl, HOBr or HOSCN on the aggregation propensity of the PA14 wild-type proteome. Exponentially growing PA14 wild-type cells in MOPS-glucose medium (A_{600}= 0.4-0.5) were either left untreated or exposed to HOCl (0.5 mM), HOBr (0.15 mM), or HOSCN (0.25 mM, 0.5 mM) for 30 min. Incubation was stopped by the addition of 10 mM sodium thiosulfate to quench excess of HOX. Cells were lysed, and the aggregated proteins were separated from the soluble fractions by centrifugation. The insoluble proteins were analyzed by SDS-PAGE.

Fig. S4. Identification of HOCl- and HOSCN-sensitive proteins *in vivo*. A SILAC experiment was performed to determine the identity of proteins aggregating in response to either 0.5 mM HOCl or 0.25 mM HOSCN treatment compared to untreated wild-type PA14. Heavy-labeled protein aggregates prepared from stress-treated cells were mixed 1:1 (vol) with light-labeled proteins from untreated cells, and identified by MS/MS analysis. The H/L ratio for each protein identified in the aggregates of (**A**) HOCl- or (**B**) HOSCN-treated PA14 was determined. Previously identified HOCl-targeted proteins are shown in blue while ribosomal proteins are highlighted in orange.

Fig. S5. HOCl, HOBr or HOSCN stress survival of wild-type PA14 and select mutant strains. Mid-log PA14 wild-type, *ppk*$^-$, *hslO*$^-$, and *ppk*$^-$*hslO*$^-$ cells were incubated with the indicated concentrations of HOCl, HOBr, or HOSCN for 30 min. After the remaining HOX was quenched by the addition of 10 mM sodium thiosulfate, cells were serially diluted with 0.85% NaCl, spot-titered onto LB agar plates, and incubated

overnight at 37°C. The experiments were repeated at least three independent times and a representative data set is shown.

Fig. S6. Effect of HOSCN treatment on the expression of select heat-shock genes in wild-type and a *ppk* deficient PA14 mutant strain as determined by RT-PCR. Mid-log phase PA14 wild-type and *ppk*$^{-}$ cells were incubated with 0.25 mM HOSCN for 20 min. Incubation was stopped by the addition of ice-cold methanol. Total RNA was extracted, reverse-transcribed into cDNA, and RT-qPCR was performed using primers for detection of heat-shock genes *rpoH*, *dnaK*, and *ibpA*. Gene expression was normalized to the expression of *rrsD* (encoding 16S rRNA), which did not change under the conditions tested, and fold-changes were calculated relative to the expression of each gene in untreated PA14 wild-type using the ΔΔCT method. Data represent the mean of three independent experiments ± s.d. Two-way ANOVA analysis was conducted with a = n.s.; b = $0.01<p<0.05$; c: $0.001<p<0.01$.

Tables S1A-C. RNAseq gene expression analysis of wild-type PA14 before and after 20 min treatment with sublethal concentrations of HOCl (A), HOBr (B) or HOSCN (C).

Table S2. SILAC analysis of wild-type PA14 before and after 30 min treatment with sublethal concentrations of HOCl or HOSCN.

Table S3. Strains, plasmids and primers used in this study.

Fig. S1

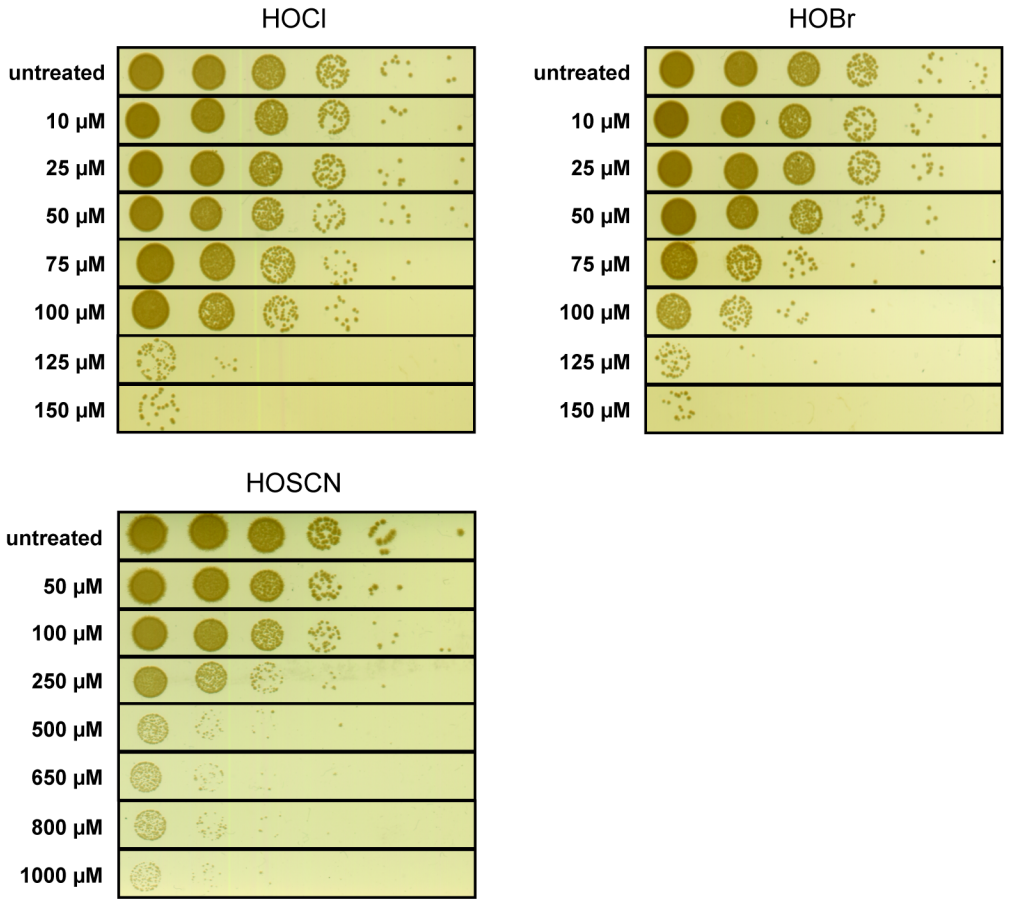
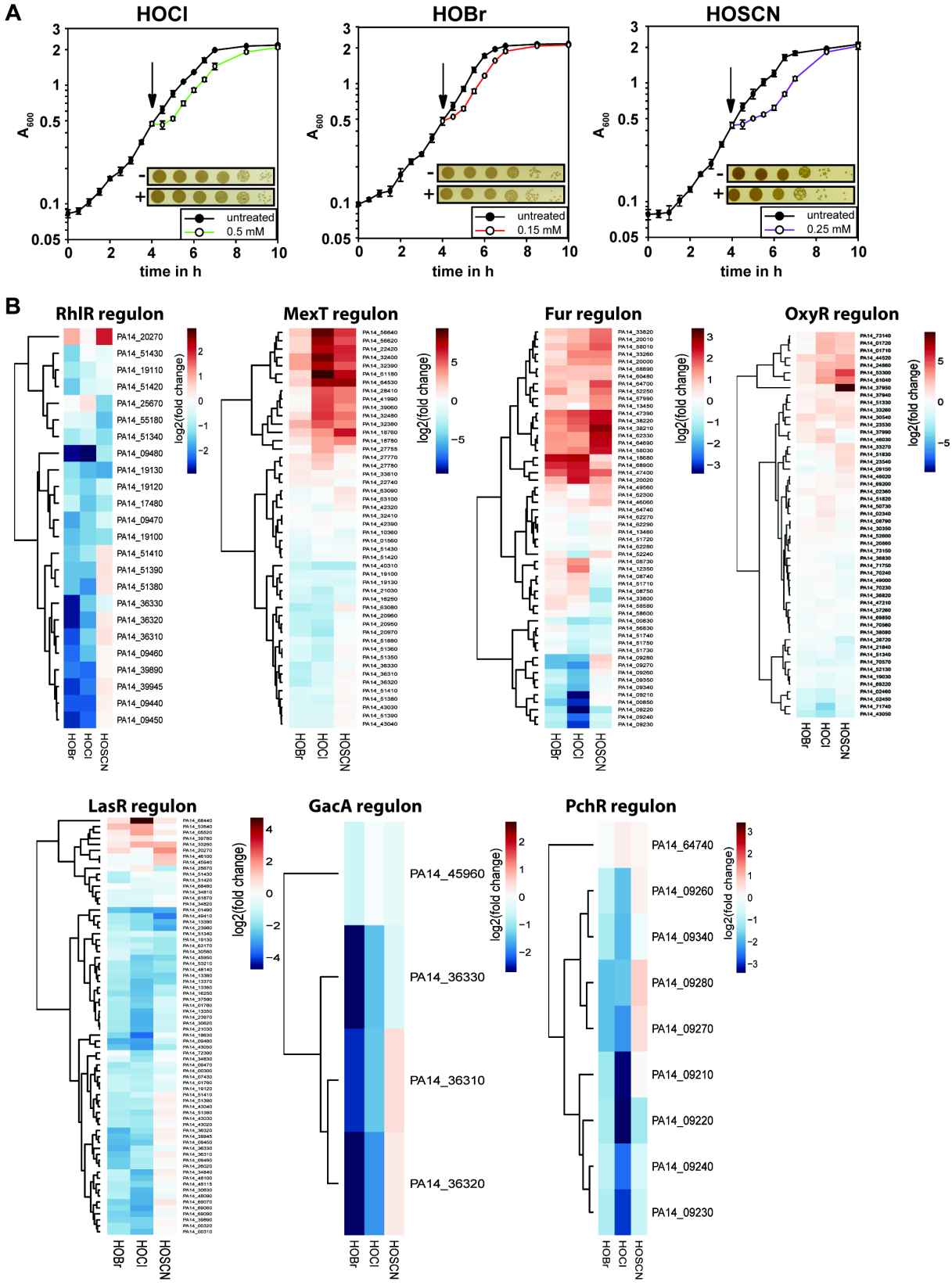

HOCl
untreated
10 µM
25 µM
50 µM
75 µM
100 µM
125 µM
150 µM
HOBr
untreated
10 µM
25 µM
50 µM
75 µM
100 µM
125 µM
150 µM
HOSCN
untreated
50 µM
100 µM
250 µM
500 µM
650 µM
800 µM
1000 µM

Fig. S2


A
HOCl
HOBr
HOSCN
A600
time in h
untreated
0.5 mM
0.15 mM
0.25 mM
B
RhlR regulon
MexT regulon
Fur regulon
OxyR regulon
LasR regulon
GacA regulon
PchR regulon
log2(fold change)
HOBr
HOCl
HOSCN
PA14_45960
PA14_36330
PA14_36310
PA14_36320
PA14_64740
PA14_09260
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PA14_09220
PA14_09240
PA14_09230

Fig. S3

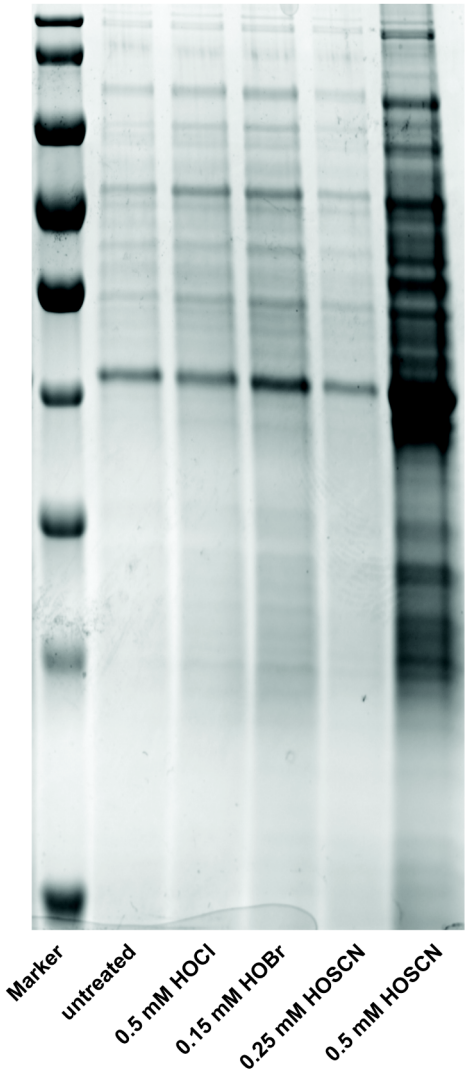

Marker
untreated
0.5 mM HOCl
0.15 mM HOBr
0.25 mM HOSCN
0.5 mM HOSCN

Fig. S4

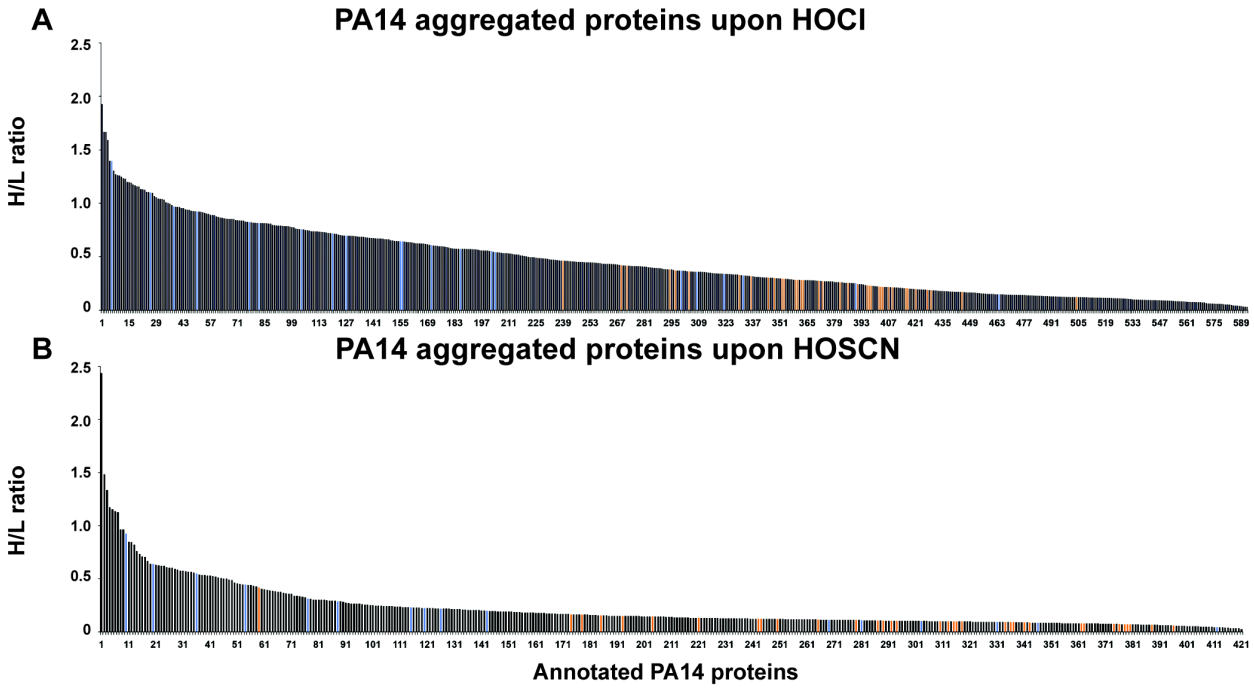

A
PA14 aggregated proteins upon HOCl
H/L ratio
2.5
2.0
1.5
1.0
0.5
0
1 15 29 43 57 71 85 99 113 127 141 155 169 183 197 211 225 239 253 267 281 295 309 323 337 351 365 379 393 407 421 435 449 463 477 491 505 519 533 547 561 575 589
B
PA14 aggregated proteins upon HOSCN
H/L ratio
2.5
2.0
1.5
1.0
0.5
0
1 11 21 31 41 51 61 71 81 91 101 111 121 131 141 151 161 171 181 191 201 211 221 231 241 251 261 271 281 291 301 311 321 331 341 351 361 371 381 391 401 411 421
Annotated PA14 proteins

Fig. S5

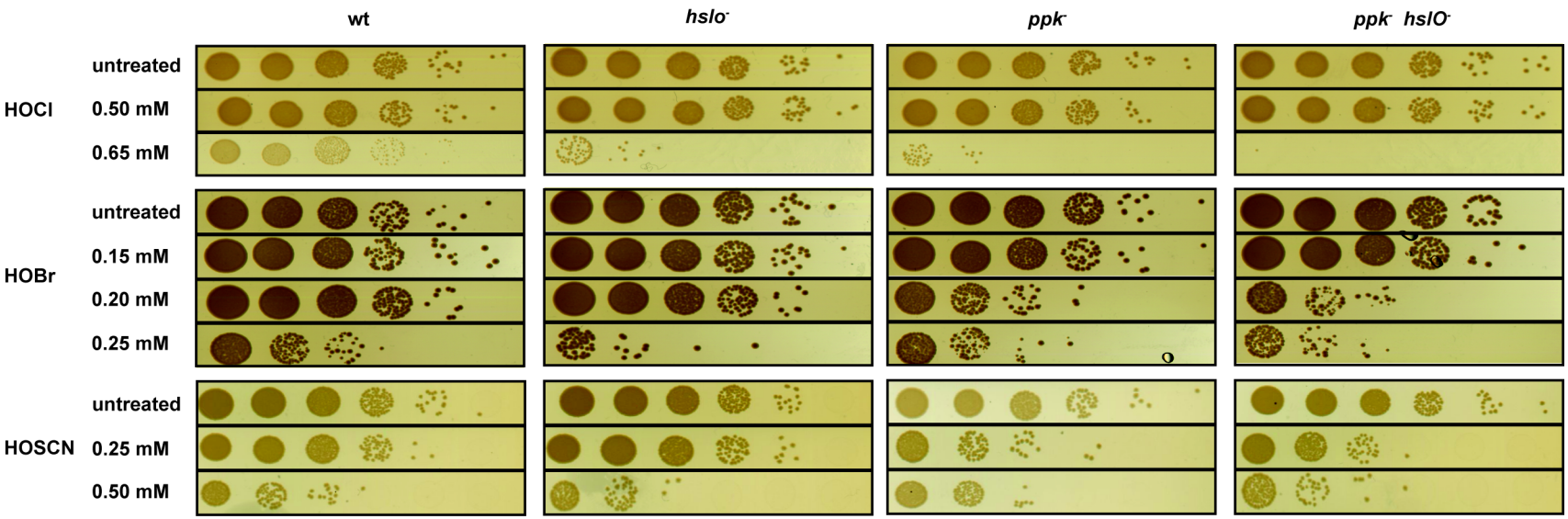
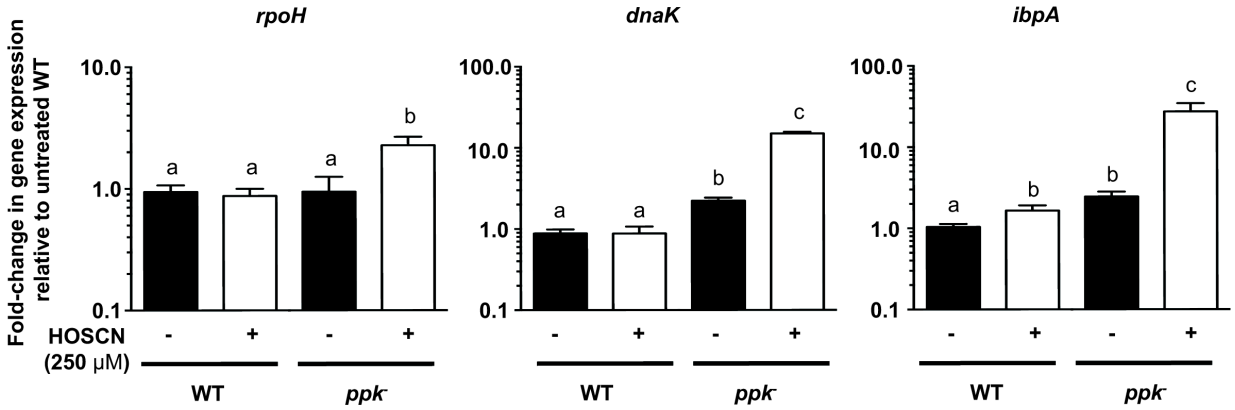

wt
hslo-
ppk-
ppk- hslO-
HOCl
untreated
0.50 mM
0.65 mM
HOBr
untreated
0.15 mM
0.20 mM
0.25 mM
HOSCN
untreated
0.25 mM
0.50 mM

Fig. S6


rpoH
dnaK
ibpA
Fold-change in gene expression relative to untreated WT
10.0
1.0
0.1
100.0
a
b
c
HOSCN (250 μM)
-
+
WT
ppk-