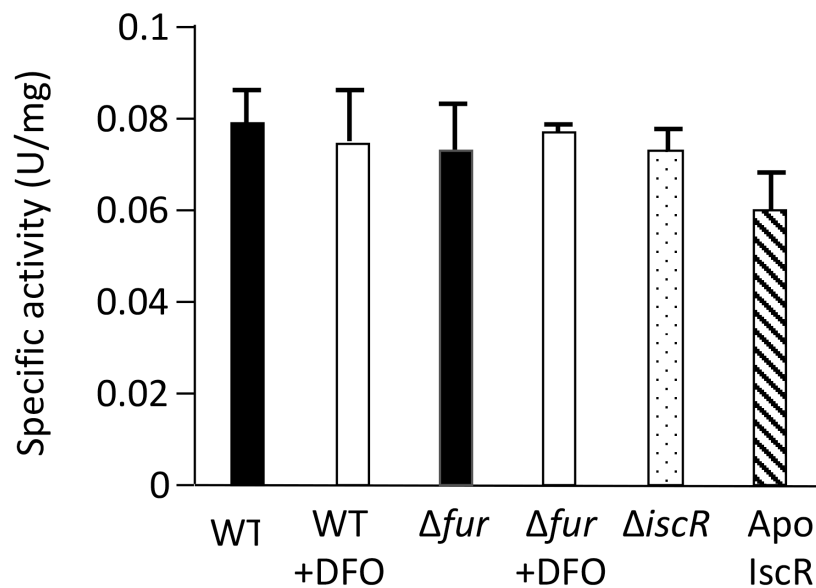


**Supplemental Material.**

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- Figure S1. The *clpSA-lacZ* fusion is not regulated by Fur or IscR.
- Figure S2. The transient phenotype of the  $Hpx^-$  *clpSA* mutant is statistically robust.
- Figure S3. ClpX and ClpP exhibit independent effects upon cell growth.
- Figure S4. The  $\Delta clpSA \Delta clpX$  mutant remains viable even when 8  $\mu M$   $H_2O_2$  blocks its growth.
- Figure S5.  $Hpx^- \Delta clpSA \Delta clpPX$  cells do not exhibit a significant growth defect in aerobic LB media.
- Figure S6. ClpX and ClpSA are not necessary to recover from an abrupt nutritional downshift.
- Figure S7. The Clp proteins work independently of the Suf cluster-assembly system.
- Figure S8. The protective effect of a *clpX* mutation depends upon Dps in a *recA* strain.
- Figure S9. Unlike Dps, ferritin and bacterioferritin did not affect the level of free iron available to participate in Fenton chemistry.
- Figure S10. Mn does not poison  $Hpx^-$  cells or  $Hpx^+ Clp^-$  cells.
- Table S1. Strains.
- Table S2. Primers.

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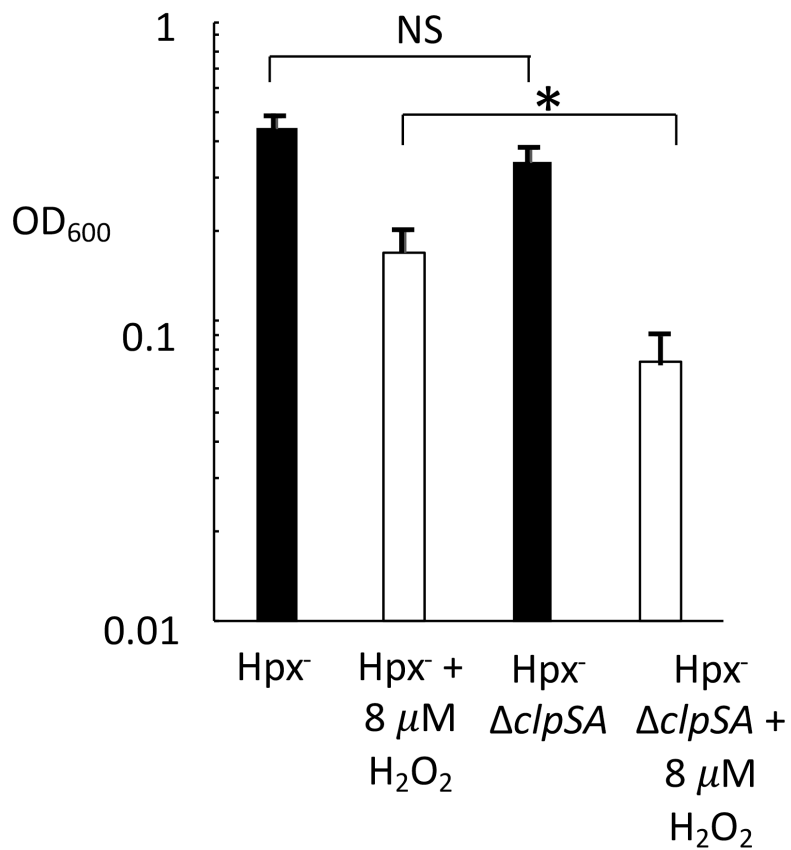
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23 **Supplementary Figure 1. The *clpSA-lacZ* fusion is not regulated by Fur or IscR.** The cells were  
24 grown aerobically in medium that included histidine and aromatic and branched-chain amino  
25 acids. Where indicated, 100  $\mu$ M of the cell-permeable iron chelator desferrioxamine (DFO) was  
26 supplied to deactivate the Fur:Fe(II) repressor. Apo-IscR strains lack the Fe-S cluster in IscR.  
27 Strains: ASE149, ASE227, ASE242, ASE248.

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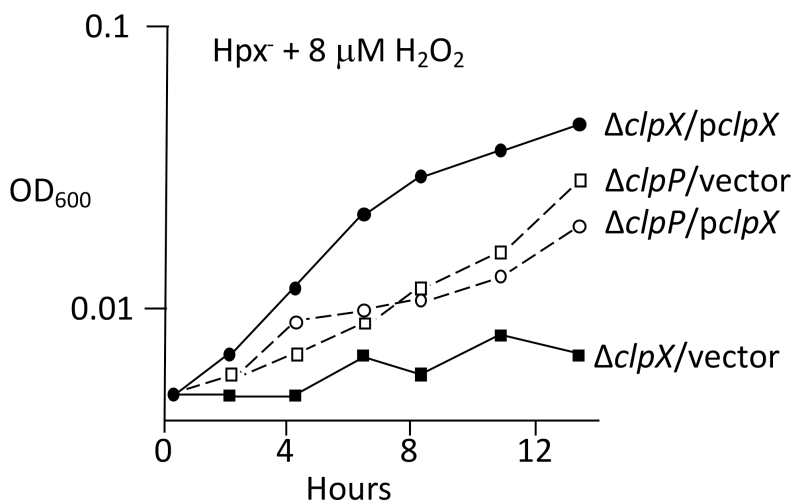
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31 **Supplementary Figure 2. The transient phenotype of the Hpx<sup>-</sup> *clpSA* mutant is statistically**32 **robust.** Anaerobic cultures were diluted into aerobic medium with or without 8 μM H<sub>2</sub>O<sub>2</sub> at

33 time zero. The time points were measured after 12 hours. Strains: LC106, ASE11.

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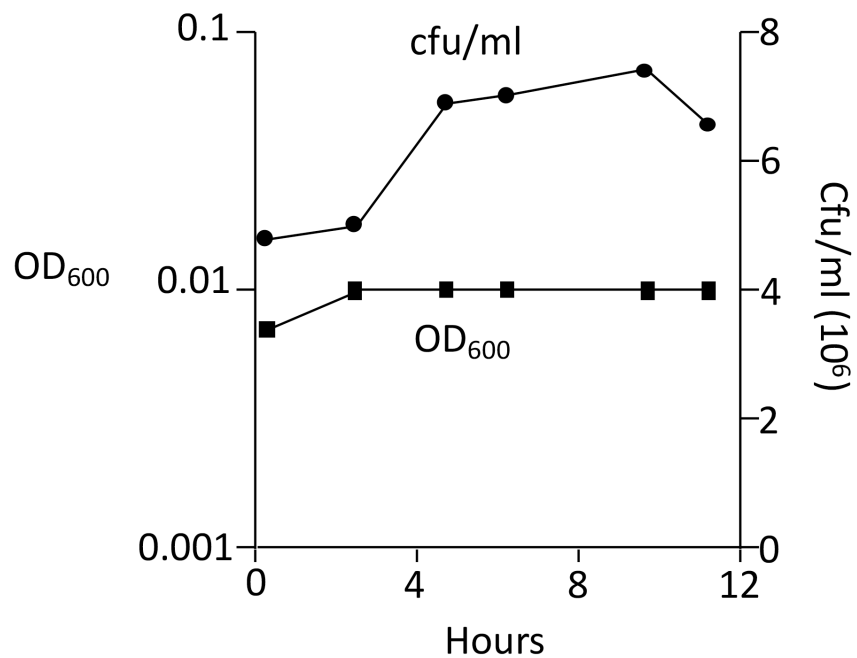
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### 38 **Supplementary Figure 3. ClpX and ClpP exhibit independent effects upon cell growth.**

39 Anaerobic cultures were diluted into aerobic medium containing 8 μM H<sub>2</sub>O<sub>2</sub> at time zero. The  
 40 *clpX* mutant consistently exhibits greater sensitivity to H<sub>2</sub>O<sub>2</sub> than does a *clpP* mutant. A *clpX*  
 41 plasmid complements the *clpX* but not the *clpP* mutation, indicating that the *clpP* phenotype  
 42 does not arise from a polar effect upon *clpX*. Strains: ASE398, ASE400, ASE413, ASE414.

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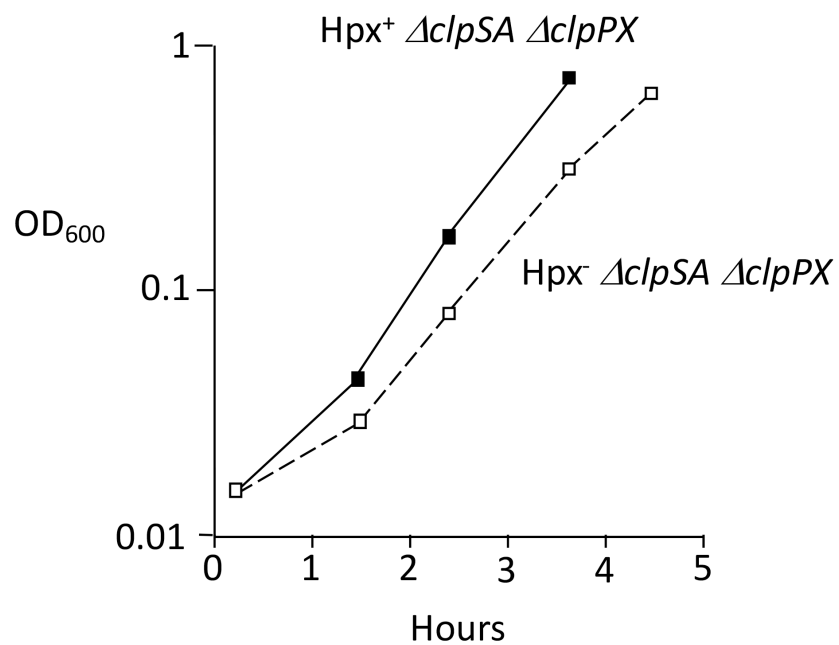
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47 **Supplementary Figure 4. The  $\Delta clpSA \Delta clpX$  mutant remains viable even when 8  $\mu M$  H<sub>2</sub>O<sub>2</sub>**

48 **blocks its growth.** Anaerobic cultures of Hpx<sup>-</sup>  $\Delta clpSA \Delta clpX$  cells (ASE21) were diluted at time  
49 zero into aerobic media containing 8  $\mu M$  H<sub>2</sub>O<sub>2</sub>. At intervals aliquots of the cells were removed  
50 the cells were plated on anoxic medium to determine viability.

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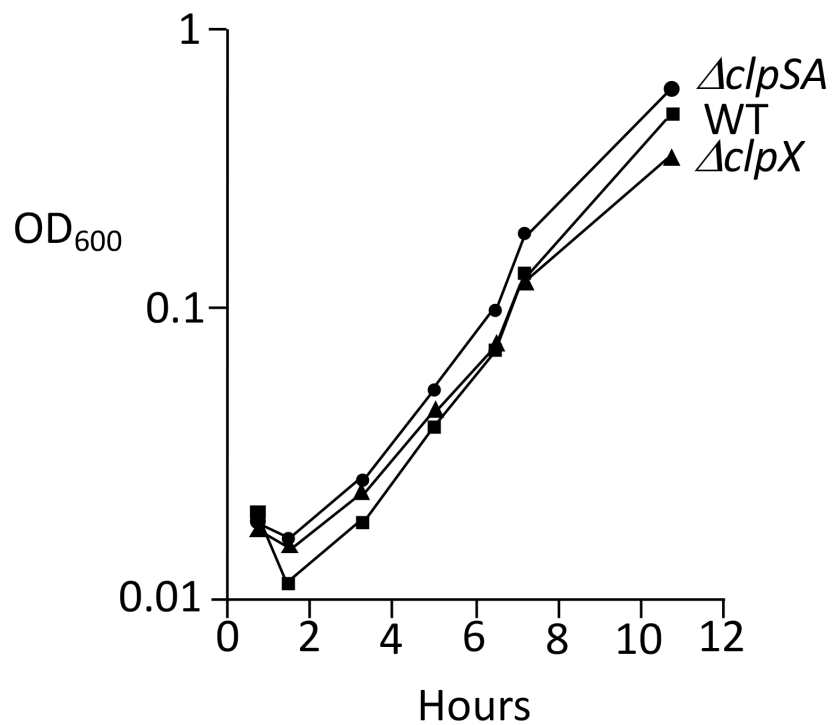
55 **Supplementary Figure 5. Hpx<sup>-</sup> ΔclpSA ΔclpPX cells do not exhibit a significant growth defect in**

56 **aerobic LB media.** Anaerobic cultures were diluted into aerobic LB at time zero. Strains:

57 ASE125, ASE217.

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62 **Supplementary Figure 6. ClpX and ClpSA are not necessary to recover from an abrupt**

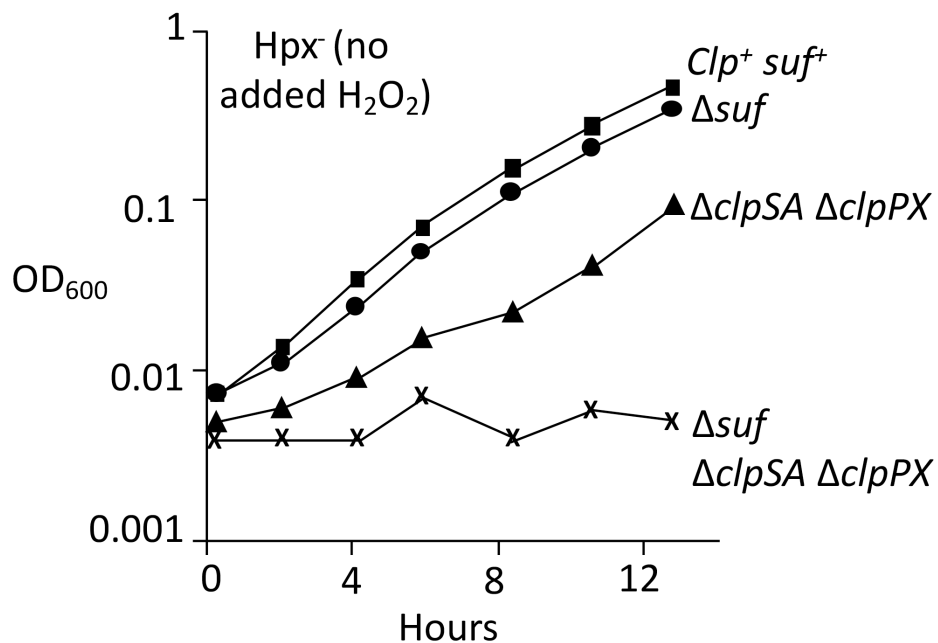
63 **nutritional downshift.** The cells were precultured aerobically in LB media to OD 0.1. They were

64 then centrifuged and resuspended at time zero in minimal glucose medium containing Phe, Tyr,

65 Trp, and His. Strains: MG1655, ASE3, ASE9.

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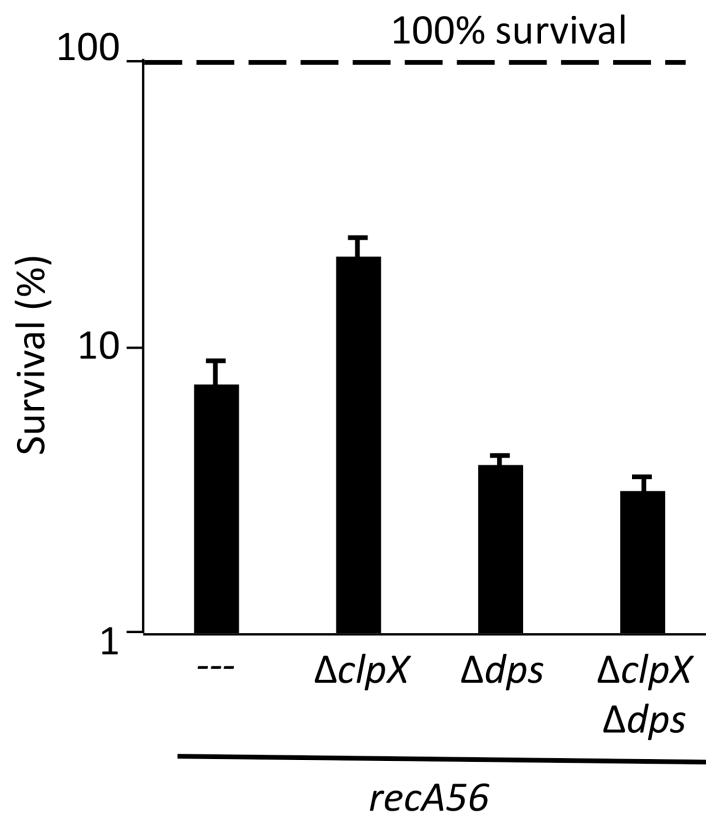
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70 **Supplementary Figure 7. The Clp proteins work independently of the Suf cluster-assembly**71 **system.** Anaerobic cultures of Hpx<sup>-</sup> mutants were diluted at time zero into aerobic medium.72 No additional H<sub>2</sub>O<sub>2</sub> was added, indicating that the phenotypes arise from only the ~ 1 μM H<sub>2</sub>O<sub>2</sub>73 that arises in Hpx<sup>-</sup> cultures. Strains: LC106, SJ1017, ASE213, and ASE300.

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78 **Supplementary Figure 8. The protective effect of a *clpX* mutation depends upon Dps in a *recA***79 **strain.** Anaerobic cultures were aerated, and 2.5 mM H<sub>2</sub>O<sub>2</sub> was added for 2 minutes. Catalase

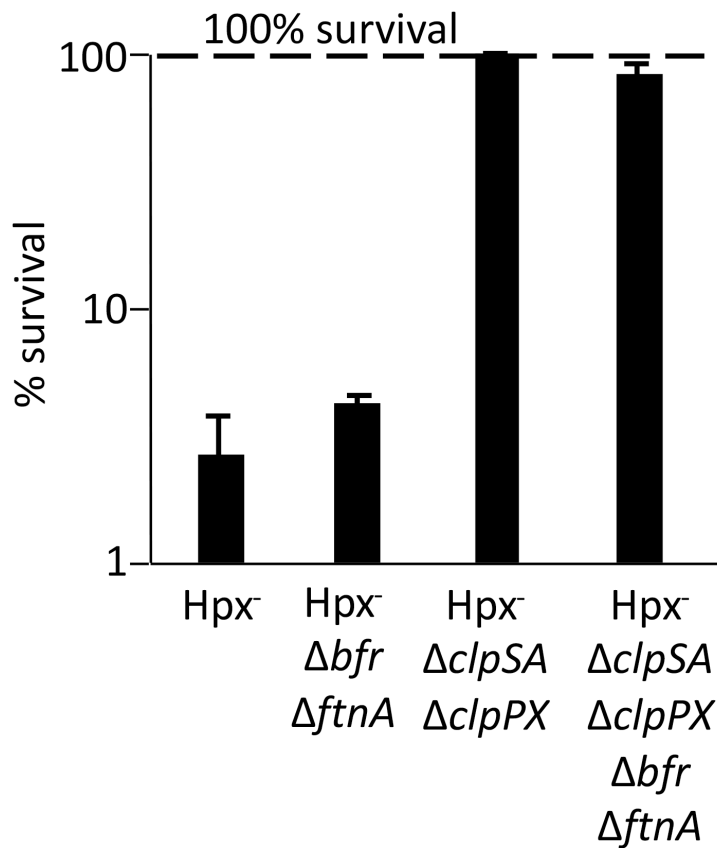
80 was then added, and viability was determined by plating on anaerobic LB plates. The use of

81 *recA* strains allowed DNA damage to be perceived without the amplification of Fenton

82 chemistry by cystine addition, as in Fig. 10. Strains: ASE386, ASE388, ASE390.

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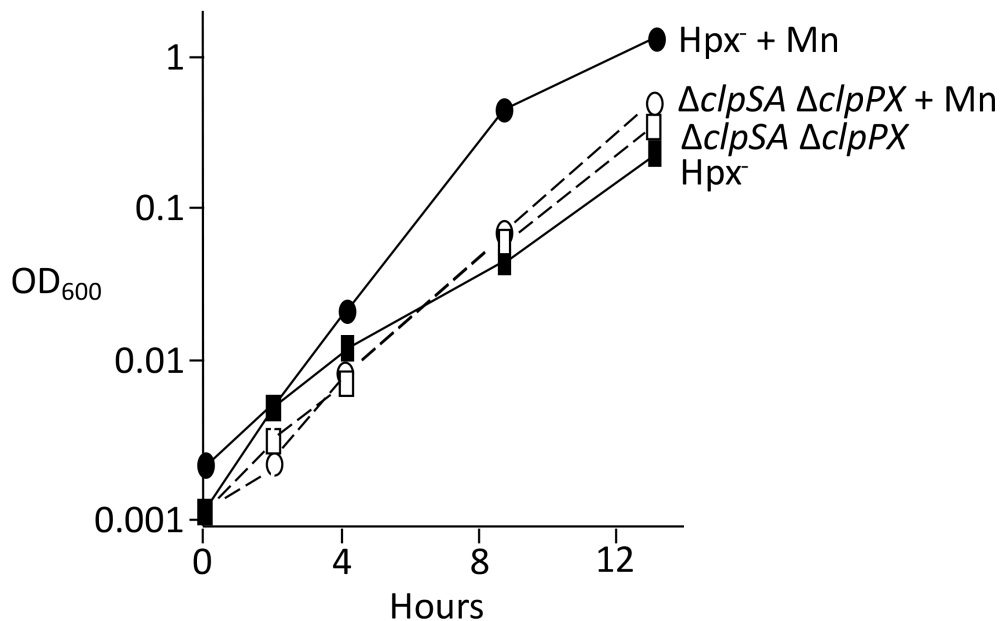
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87 **Supplementary Figure 9. Unlike Dps, ferritin and bacterioferritin did not affect the level of**  
 88 **free iron available to participate in Fenton chemistry.** Anaerobic cells were then aerated for  
 89 two hours. They were diluted in the same medium, incubated for 3 min with 0.5 mM cystine,  
 90 and exposed to 2.5 mM H<sub>2</sub>O<sub>2</sub> for 2 minutes. Catalase was added, and viability was determined  
 91 by plating. Strains: LC106, ASE125, ASE308, ASE310.

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96 **Supplementary Figure 10. Mn does not poison  $Hpx^-$  cells or  $Hpx^+ Clp^-$  cells.** Anaerobic cultures  
 97 of  $Hpx^-$  cells (LC106) were diluted into aerobic medium at time zero, with or without the  
 98 addition of 4  $\mu M$  manganese. The  $Hpx^+ \Delta clpSA \Delta clpX$  cells (ASE217) were precultured  
 99 aerobically and then diluted in media with or without 4  $\mu M$  manganese.

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105**Table S1: Strains**

MG1655	<i>F</i> wild-type <i>E. coli</i>
LC106	$\Delta$ ahpF::kan $\Delta$ (katG17::Tn10)1 $\Delta$ (katE12::Tn10)1
AS015	LC106 with pclpSA
ASE3	MG1655 $\Delta$ clpX::cat
ASE7	LC106 $\Delta$ clpX::cat
ASE9	MG1655 $\Delta$ clpSA::cat~zjb-1230::Tn10
ASE11	LC106 $\Delta$ clpSA::cat~zjb-1230::Tn10
ASE15	ASE11 with pWKS30
ASE17	ASE11 with pclpSA
ASE21	LC106 $\Delta$ clpX::cat $\Delta$ clpSA::cat~zjb-1230::Tn10
ASE36	LC106 with pclpPX
ASE38	ASE7 with pWKS30
ASE40	ASE7 with pclpPX
ASE67	LC106 $\Delta$ clpP::cat
ASE117	LC106 $\Delta$ dps::cat $\Delta$ (clpX::cat)
ASE119	LC106 $\Delta$ clpPX::cat
ASE125	LC106 $\Delta$ clpPX::cat $\Delta$ (clpSA::cat)
ASE149	MG1655 att $\lambda$ ::[pSJ501::clpS'-lacZ']~cat
ASE151	ASE125 with pWKS30
ASE153	ASE125 with pclpSA
ASE155	LC106 att $\lambda$ ::[pSJ501::clpS'-lacZ']~cat
ASE157	LC106 $\Delta$ oxyR::spec att $\lambda$ ::[pSJ501::clpS'-lacZ']~cat
ASE173	MG1655 att $\lambda$ ::[pSJ501::clpS'-lacZ'] with pACYC184
ASE175	MG1655 att $\lambda$ ::[pSJ501::clpS'-lacZ'] with pGS058
ASE197	MG1655 $\Delta$ clpPX::cat
ASE201	MG1655 clpX::cat $\Delta$ (clpSA::cat)
ASE203	MG1655 $\Delta$ clpP::cat
ASE207	LC106 $\Delta$ (clpP::cat) $\Delta$ (clpSA::cat)
ASE213	LC106 $\Delta$ (clpPX::cat) $\Delta$ (clpSA::cat)
ASE217	MG1655 $\Delta$ (clpPX::cat) $\Delta$ (clpSA::cat)
ASE225	MG1655 $\Delta$ oxyR::spec att $\lambda$ ::[pSJ501::clpS'-lacZ']
ASE227	MG1655 $\Delta$ fur::kan att $\lambda$ ::[pSJ501::clpS'-lacZ']
ASE242	MG1655 $\Delta$ iscR1::cat att $\lambda$ ::[pSJ501::clpS'-lacZ']
ASE248	MG1655 iscR-(C92A/C92A/C104A)~zfh-3600::kan att $\lambda$ ::[pSJ501::clpS'-lacZ']
ASE252	MG1655 att $\lambda$ ::[pSJ501::clpPX'-lacZ']~cat
ASE254	LC106 att $\lambda$ ::[pSJ501::clpPX'-lacZ']~cat
ASE270	MG1655 $\Delta$ dps::cat
ASE272	LC106 $\Delta$ dps::cat $\Delta$ (clpPX::cat) $\Delta$ (clpSA::cat)
ASE282	LC106 $\Delta$ dps::cat

ASE296	LC106 $\Delta bfr::cat$
ASE298	LC106 $\Delta bfr::cat \Delta(clpPX::cat) \Delta(clpSA::cat)$
SJ1017	LC106 $\Delta sufBCDSE1::cat$
ASE300	LC106 $\Delta sufBCDSE1::cat \Delta(clpPX::cat) \Delta(clpSA::cat)$
ASE308	LC106 $\Delta ftnA::cat \Delta(bfr::cat)$
ASE310	LC106 $\Delta ftnA::cat \Delta(bfr::cat) \Delta(clpPX::cat) \Delta(clpSA::cat)$
ASE386	MG1655 $\Delta clpX::cat recA56-srlC300::Tn10$
ASE388	MG1655 $\Delta dps::cat recA56-srlC300::Tn10$
ASE390	MG1655 $\Delta clpX::cat \Delta(dps::cat) recA56-srlC300::Tn10$
ASE394	LC106 with pCKR101
ASE396	LC106 with p <i>clpX</i>
ASE398	ASE7 with pCKR101
ASE400	ASE7 with p <i>clpX</i>
ASE402	ASE119 with pCKR101
ASE404	ASE119 with p <i>clpX</i>
ASE406	ASE207 with pCKR101
ASE408	ASE207 with p <i>clpX</i>
ASE412	LC106 $iucC::lacZ \Delta clpX::cat$
ASE413	ASE67 with pCKR101
ASE414	ASE67 with p <i>clpX</i>
ASE425	MG1655 pColV( <i>iucC::lacZ</i> ) $\Delta clpX::cat$

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107 MG1655 (1) is a wild-type strain that was obtained from the *E. coli* Genetic Stock Center. LC106  
108 (2) is an Hpx<sup>-</sup> derivative of it. The plasmids pWKS30 (3) and pACYC184 and pGS058 are  
109 described (4). Derivative strains listed above were constructed using alleles that were provided  
110 by colleagues. The  $\Delta dps::cat$  and  $\Delta oxyR::spec$  (5) alleles were obtained in strains from Stephen  
111 Finkel and Gigi Storz, respectively. The  $\Delta fur::kan$  allele was transduced from EM1256 (6). The  
112  $\Delta iscR1::cat$  allele was generated in (7). The *iscR*-(C92A/C92A/C104A)~*zfh-3600::kan* allele was  
113 obtained in PK7887 (8); the  $\Delta sufBCDSE1::cat$  allele (7) was made in our lab. The pCKR101  
114 plasmid (7) was provided by Jeff Gardner. The *recA56* allele was reported (9). The  
115 pColV(*iucC::lacZ*) plasmid (10) was obtained from Joe Niellands.

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Table S2: Primers

<i>clpSA</i> deletion	5'-GGCGTTCTGCCGATAACCGTAACCGAAGATGATAACTGACATGTAGGCTGGAGCTGCTTCG-3' 5'-TTCGAGATTACGGACTTGACCAACCTACCTAACAATCAGACATATGAATATCCTCCTTAG-3'
<i>clpX</i> deletion	5'-GTGTGCGGCACAAAGAACAAGAAGAGGTTTTGACCCATGTGTAGGCTGGAGCTGCTTCG-3' 5'-CCCTTTTTGGTTAACTAATTGTATGGGAATGGTTAATTACATATGAATATCCTCCTTAG-3'
<i>clpP</i> deletion	5'-CGGTACAGCAGGTTTTTTCAATTTTATCCAGGAGACGGAATGTAGGCTGGAGCTGCTTCG-3' 5'-ATGAAGCGCCATAAGTTCATTCATGCGCCCTTTAACTTTCCATATGAATATCCTCCTTAG-3'
<i>suf</i> deletion	5'-CTGTTATACGCTGAAAGCGATGAAGTGAGGTAATCGATGTGTAGGCTGGAGCTGCTTCG-3' 5'-CAATGTGAGCCAACCGGATGAAAGCTGTCCTTTTAGTTTACATATGAATATCCTCCTTAG-3'
<i>dps</i> deletion	5'-TAATTACTGGGACATAACATCAAGAGGATATGAAATTATGTGTAGGCTGGAGCTGCTTCG-3' 5'-TATCGGGTACTAAAGTTCTGCACCATCAGCGATGGATTTACATATGAATATCCTCCTTAG-3'
<i>bfr</i> deletion	5'-TCTACTCTTCAAAGAGTGGAAGCGAAGGAGTCAAAAATGTGTAGGCTGGAGCTGCTTCG-3' 5'-TCTTATTAACCGGGAGGGTTCTCCCTCCCGACACGGCTCACATATGAATATCCTCCTTAG-3'
<i>ftnA</i> deletion	5'-GCCACAGCAACAAATATAACCTTTGTGGAGCACTATCATGTGTAGGCTGGAGCTGCTTCG-3' 5'-TTTGAACGGCGGCAGTAAACCTGCCGCCGAGAGCATTACATATGAATATCCTCCTTAG-3'
<i>clpSA::lacZ</i>	5'-AAGATCGTCGGTACCCTGGCATTTCCTTTAGGA-3' 5'-GCGTAGCGGAATTCCATTGTCAGTTATCATCTTC-3'
<i>clpPX::lacZ</i>	5'-ACGTAAGTGTGACGCGGTGTTAGCGTAACAACAA-3' 5'-ATGCTAGCTGGTACCCATGGGTCAAACCTCTTC-3'
Cloning <i>clpPX</i>	5'-TGACAGGGATCCAAAGCCTCTTTCGGTGTAGCGT-3' 5'-ATACGTATCTGCAGGATGTTTCCCCACATTCAA-3'
Cloning <i>clpSA</i>	5'-TAATATAATGGATCCATAAACCGGGTTCAGAGAGG-3' 5'-ATAATATAACTGCAGACCTCCTCCACCCCATAAAC-3'
Cloning <i>clpX</i>	5'-ACGTACGTGGTACCCAAAGAAGAGGTTTTGACCCATGACAGATAAACGC-3' 5'-AGTTCACTTGTCGACGGTTAATTATCACCAGATGCCTGTTGC-3'

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**References for supplementary material.**

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