Supplement to

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Cyanide enhances hydrogen peroxide toxicity by recruiting endogenous iron to trigger catastrophic chromosomal fragmentation

E. coli strains and plasmids (all strains except CP909 are in the BW25113 background).

Strain name	Relevant Genotype	Source/ CGSC# or Construction
CP909	*nuoG*::Tn*10 purF*	(Prüss *et al.*, 1994)
BW25113	F-, Δ*(araD-araB)567*, Δ*lacZ4787*(::rrnB-3), λ^-, *rph-1*, Δ*(rhaD-rhaB)568*, *hsdR514*	7636, (Baba *et al.*, 2006)
JW1721-1	Δ*katE731*::*kan*	9453
JW3914-1	Δ*katG729*::*kan*	10827
TM10	Δ*katE732*	JW1721-1 –> pCP20, 42°C, screen for Kn^S
TM12	Δ*katG729*::*kan* Δ*katE732*	TM10 x P1 JW3914-1
JW3820-1	Δ*fre-784*::*kan*	10763
JW0598-2	Δ*ahpC744*::*kan*	8713
JW1095-1	Δ*ndh-771*::*kan*	11791
TM17	*nuoG*::Tn*10 purF*	BW2113 x P1 CP909
JW0960-1	Δ*appC721*::*kan*	8956
JW0421-1	Δ*cyoB788*::*kan*	8585
JW0723-2	Δ*cydB782*::*kan*	8790
JW0669-2	Δ*fur-731*::*kan*	8758
JW3879-1	Δ*sodA768*::*kan*	10798
JW1648-1	Δ*sodB734*::*kan*	9402
TM11	Δ*sodB735*	JW1648-1–> pCP20, 42°C, screen for Kn^S
TM13	Δ*sodA768*::*kan* Δ*sodB735*	TM11 x P1 JW3879-1
JW0797-1	Δ*dps-784*::*kan*	8844
JW1893-1	Δ*ftnA-755*::*kan*	9575
JW3298-1	Δ*bfr-746*::*kan*	10467
TM12	Δ*ftnA-756*	JW1893-1–> pCP20, 42°C, screen for Kn^S
TM14	Δ*ftnA-756* Δ*bfr-746*::*kan*	TM12 x P1 JW3298-1
TM15	Δ*ftnA-756* Δ*fre-784*::*kan*	TM12 x P1 JW3820-1
TM16	Δ*ftnA-756* Δ*dps-784*::*kan*	TM12 x P1 JW0797-1

Plasmids		
pCP20	Flp recombinase gene on a temperature-sensitive replicon	(Datsenko & Wanner, 2000)
pMTL20	Cloning vector	(Chambers *et al.*, 1988)
pES1	*fre* overproducer	(Woodmansee & Imlay, 2002)

References

Baba, T., T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K. A. Datsenko, M. Tomita, B. L. Wanner & H. Mori, (2006) Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol. Syst. Biol.* **2**: 2006.0008.

Chambers, S. P., S. E. Prior, D. A. Barstow & N. P. Minton, (1988) The pMTL nic$^-$ cloning vectors. I. Improved pUC polylinker regions to facilitate the use of sonicated DNA for nucleotide sequencing. *Gene* **68**: 139-149.

Datsenko, K. A. & B. L. Wanner, (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. USA* **97**: 6640-6645.

Prüss, B. M., J. M. Nelms, C. Park & A. J. Wolfe, (1994) Mutations in NADH:ubiquinone oxidoreductase of *Escherichia coli* affect growth on mixed amino acids. *J. Bacteriol.* **176**: 2143-2150.

Woodmansee, A. N. & J. A. Imlay, (2002) Reduced flavins promote oxidative DNA damage in non-respiring *Escherichia coli* by delivering electrons to intracellular free iron. *J. Biol. Chem.* **277**: 34055-34066.

Supplemental Figures

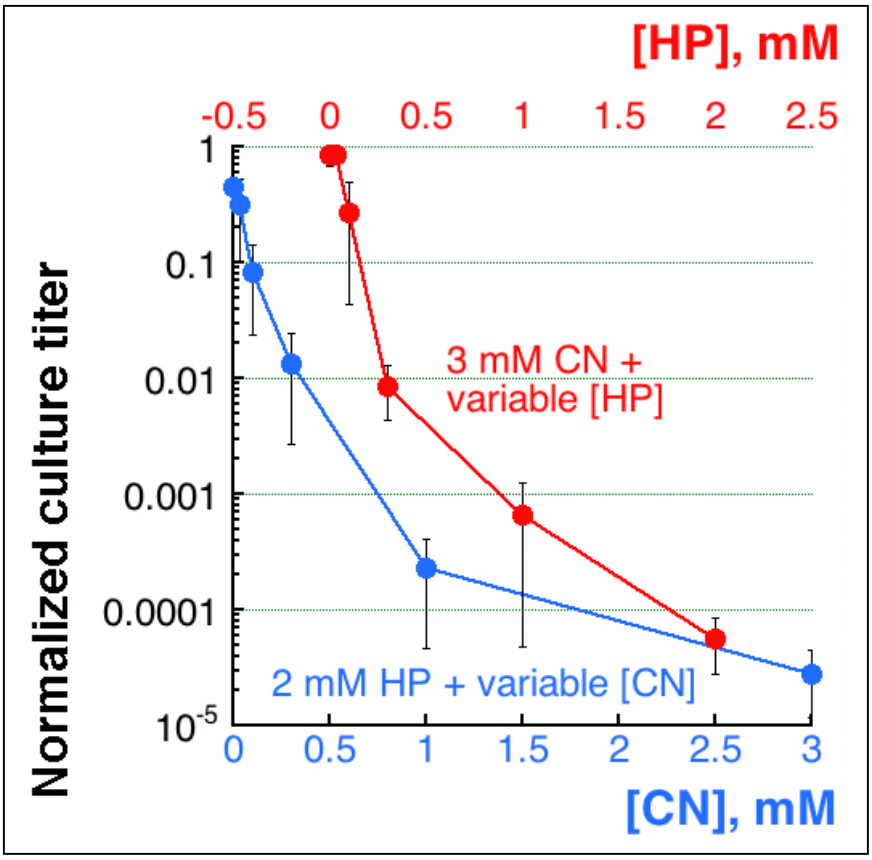

Fig. S1. Concentration-dependence of the CN+HP toxicity.

Concentration of one of the ingredient was kept constant (either 2 mM HP or 3 mM CN), while concentration of the other ingredient was decreased step-wise, and the survival was measured after 45 minutes of shaking at 30°C.

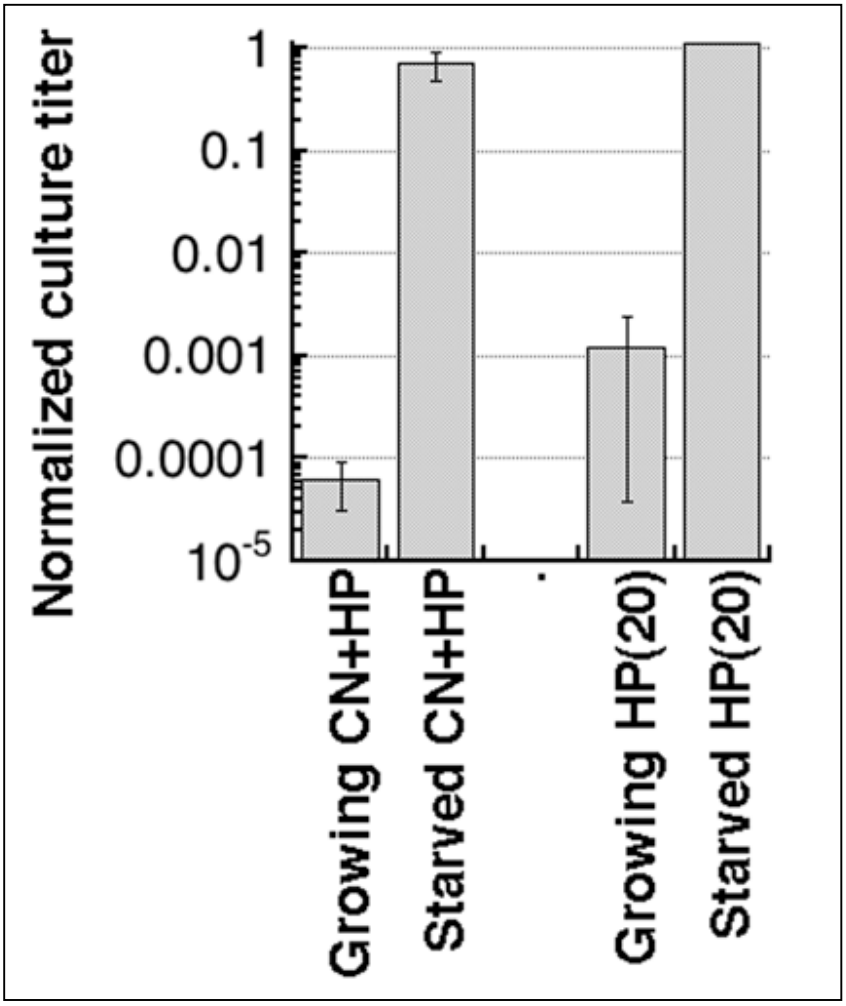

Fig. S2. Starved cell survival. Cultures growing in LB till OD_{600} ~0.3 were pelleted, resuspended in M9 salts and shaken at 37°C for 2 hours before the indicated treatments.

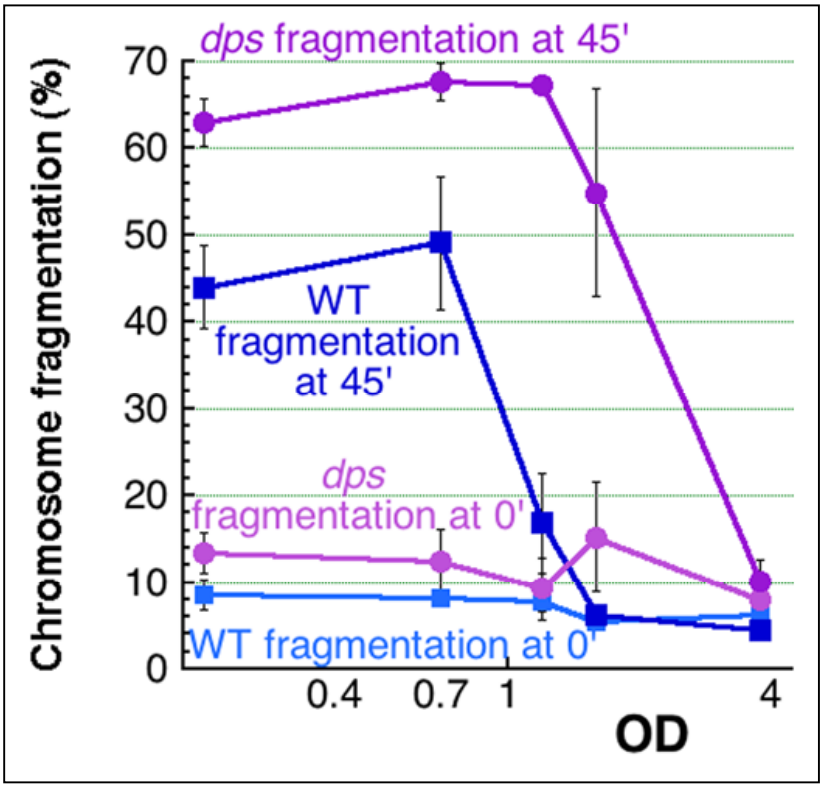

Fig. S3. OD_{600}-dependence of fragmentation in Δ*dps* mutants — quantification of several gels like in Fig. 5G. The cultures were *not* diluted before the treatment. The corresponding wild type curves from Fig. 5C are shown for comparison.

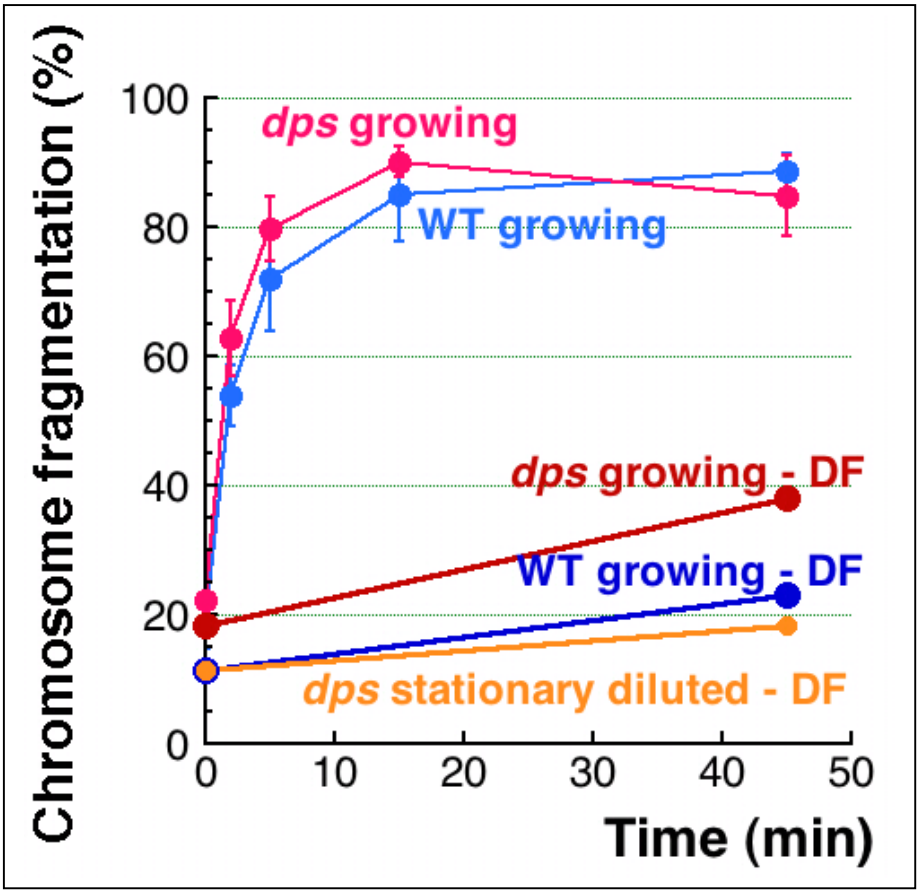

Fig. S4. Deferoxamine blocks chromosomal fragmentation in *dps* mutants. WT or *dps* mutant cultures were treated with CN+HP in the presence of iron chelator deferoxamine (DF) for the indicated amount of time. DF, deferoxamine added to 20 mM five minutes before the CN+HP treatment. The values for the corresponding growing cultures without DF-pretreatment from Fig. 5I are also shown for comparison.

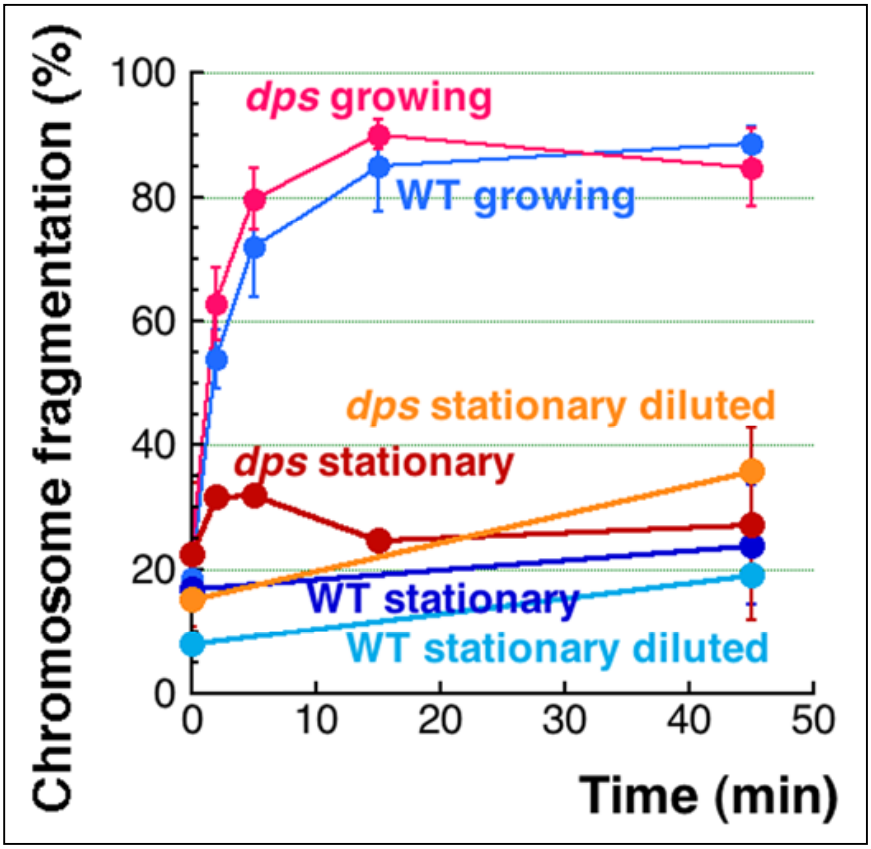

Fig. S5. Kinetics of chromosome fragmentation in stationary cultures. WT or Δ*dps* mutant stationary cultures were treated with CN+HP for the indicated time either undiluted or 10x diluted into "conditioned" medium from fully stationary cultures, and chromosome fragmentation was measured by PFGE. The values for the corresponding growing cultures without DF-pretreatment from Fig. 5I are also shown for comparison.

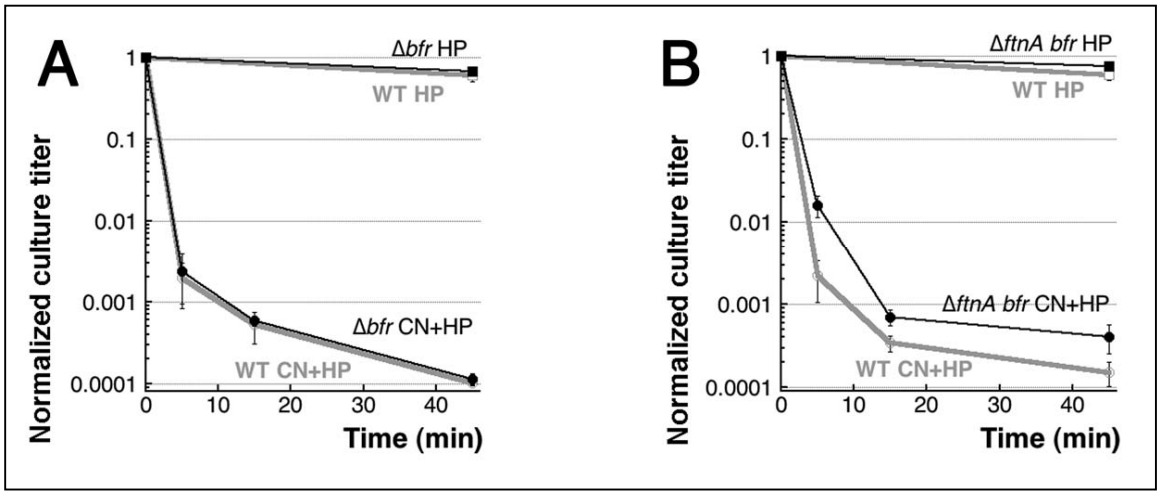

Fig. S6. Bacterioferritin mutants show WT or close to WT sensitivities to both HP-alone and CN+HP. A. The single *bfr* mutant. B. The double *bfr ftnA* mutant.