Supplement

for the paper by Pooja Agashe and Andrei Kuzminov

Catalase inhibition by nitric oxide potentiates hydrogen peroxide to trigger catastrophic chromosome fragmentation in *Escherichia coli*

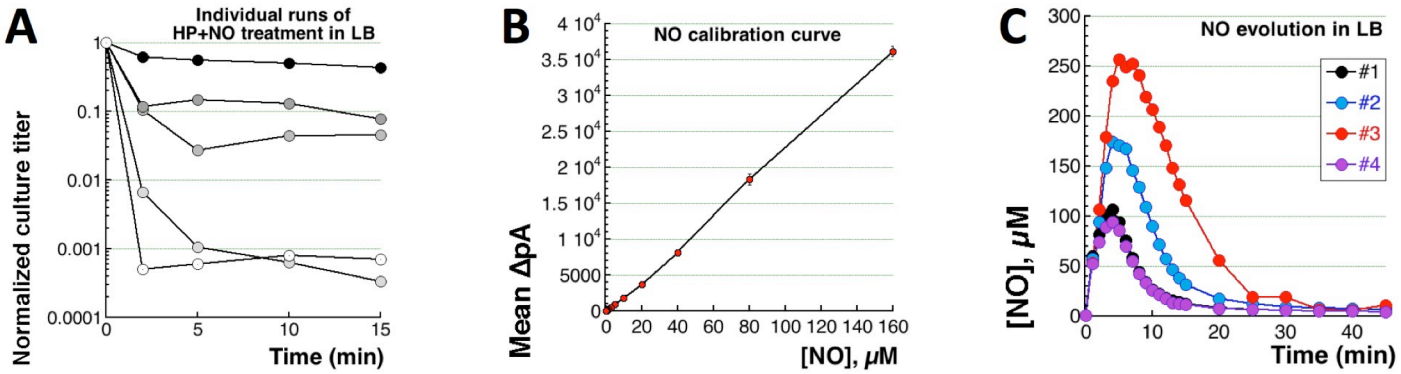

Fig. S1. Variation in the effects of HP+NO treatments in LB is due to variation of NO release in LB by 0.6 mM DEA NONOate.

A. HP+NO survival of WT cells. Individual runs were done on different days. Even though in all cases most of the killing happens in the first two minutes, the depth of the killing varies widely.

B. A calibration curve of the NO sensor (see Materials and Methods for the procedure). N=4, error bars are standard deviations in this case.

C. NO release from 0.6 mM DEA NONOate in non-buffered LB. Four individual runs are shown. Since the linear range of the NO sensor ends above 160 μM (hence the top [NO] in panel B), the real [NO] above 160 μM were approximated from the trendline.

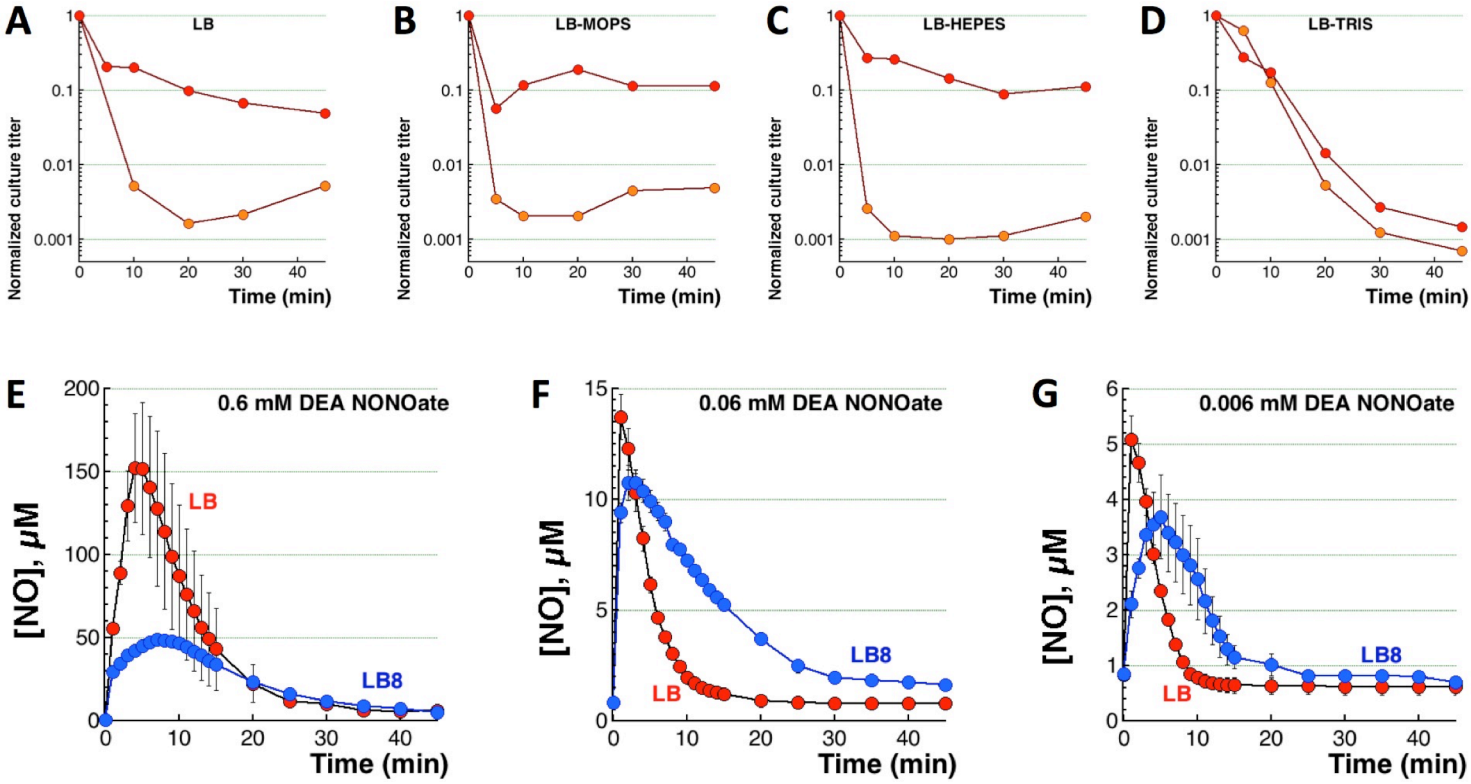

Fig. S2. Reproducibility of HP+NO treatment and NO release kinetics in LB with and without buffer.

A. HP+NO survival, LB without any buffer (pH ~7.2).

B. HP+NO survival, LB with 50 mM MOPS (pH ~7.2)

C. HP+NO survival, LB with 50 mM HEPES (pH ~7.6)

D. HP+NO survival, LB with 50 mM Tris-HCl (pH ~8.1) = "LB8"

E. NO release from 0.6 mM DEA NONOate in LB vs LB8.

F. NO release from 0.06 mM DEA NONOate in LB vs LB8.

G. NO release from 0.006 mM DEA NONOate in LB vs LB8.

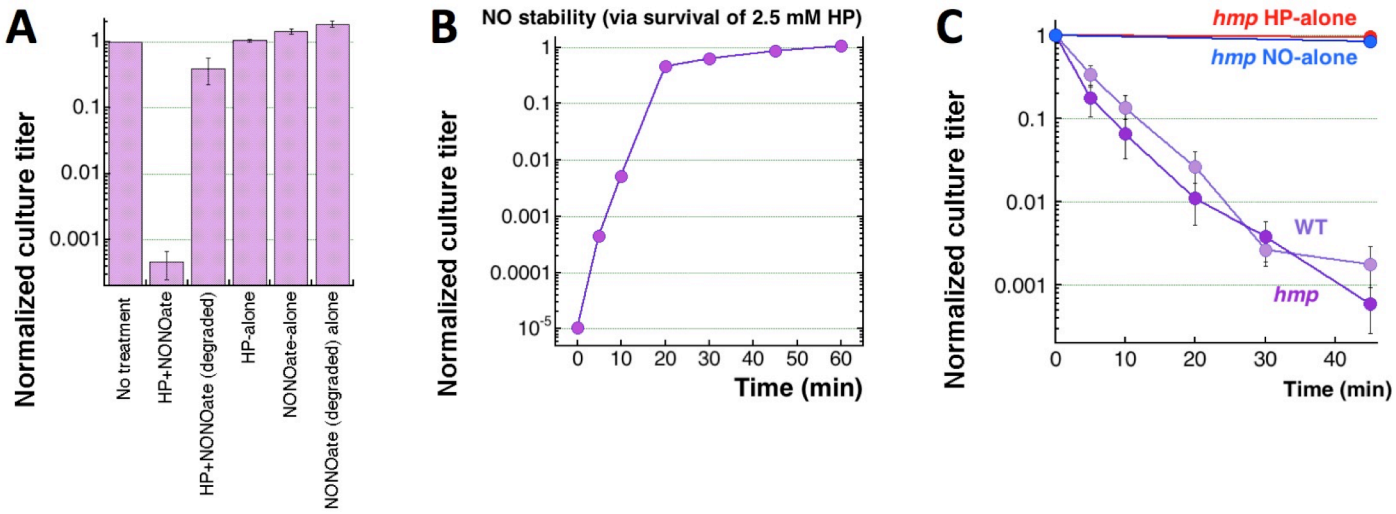

Fig. S3. NO stability in solutions and in cultures.

A. Degraded DEA NONOate does not synergize with HP. DEA NONOate was either preincubated for 2 hours at 37°C in LB ("degraded") before adding to the HP-treated cultures or added right away.

B. NO stability in *E. coli* culture was assessed by its ability to synergize with subsequently added 2.5 mM HP. HP addition times are plotted in X-axes. Subsequent HP-treatment was 45 minutes at 37°C.

C. Kinetics of death of the *hmp* mutant treated with 2.5 mM HP-alone, 0.6 mM NO-alone, or with both agents together. Survival of WT cells treated with HP+NO in parallel is shown as a control.

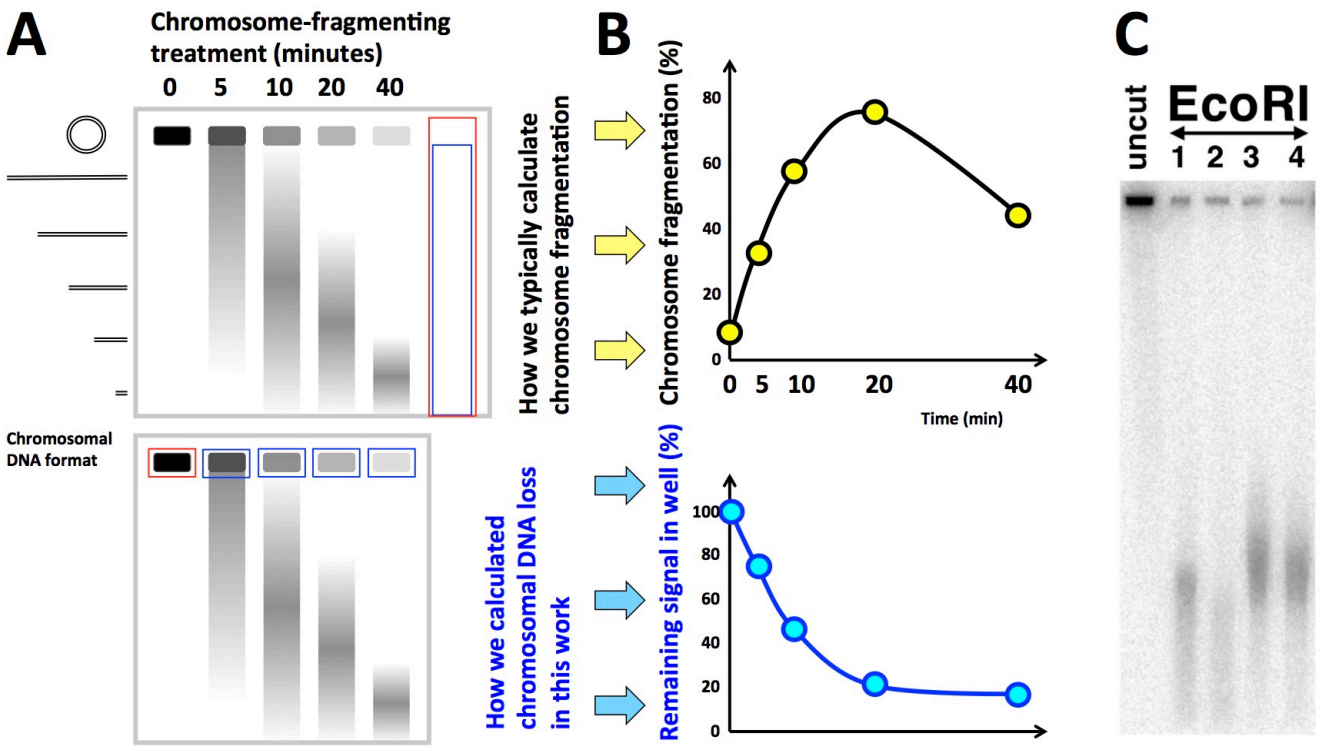

Fig. S4. Pulsed-field gel electrophoresis detection and quantification of chromosome fragmentation and chromosomal DNA loss.

A. A scheme of pulsed-field gel separation of intact and broken bacterial chromosome. Chromosomal DNA is prepared by cell lysis in agarose plugs, which are then inserted into the wells of a gel. During PFGE, intact (circular) chromosomal DNA cannot enter the gel and stays at the origin. Fragmented chromosomal DNA enters the gel and runs according to its molecular weight, from ~10 kbp at the bottom of the gel all the way to 2 Mbp close to the origin (in our conditions). Top gel: typically, to derive percentage of low-to-modest fragmentation, we divide the signal in the lane (the blue rectangle) by the sum of the signal in the well+lane (the red rectangle) and multiply the fraction by 100. Bottom gel: since fragmentation in this case is so complete, we concentrated on the surviving intact chromosomal DNA, normalizing the remaining fraction of it to the original signal before the treatment.

B. The model plots of the values, calculated according to the schemes in "A". Top, our standard calculation of the chromosome fragmentation. The mismatch between these values and the true extent of the fragmentation is evident. Bottom, our new calculation of the signal remaining in well.

C. EcoRI digestion of genomic DNA plugs defines the background of the non-DNA signal remaining in the well, calculated to be 7.2±1.0% (mean ±SEM, n=5).

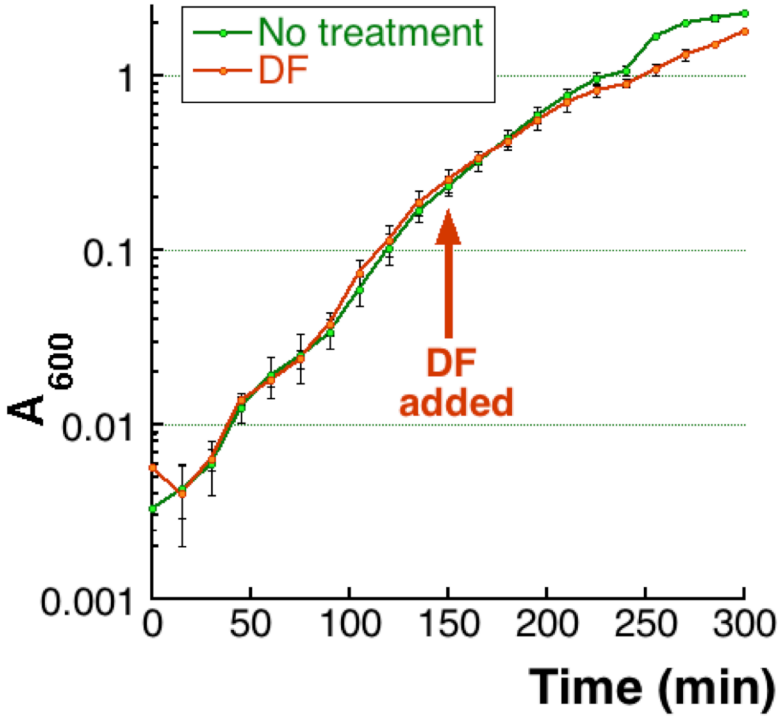

Fig. S5. 20 mM deferoxamine inhibits growth of *E. coli* only slightly.

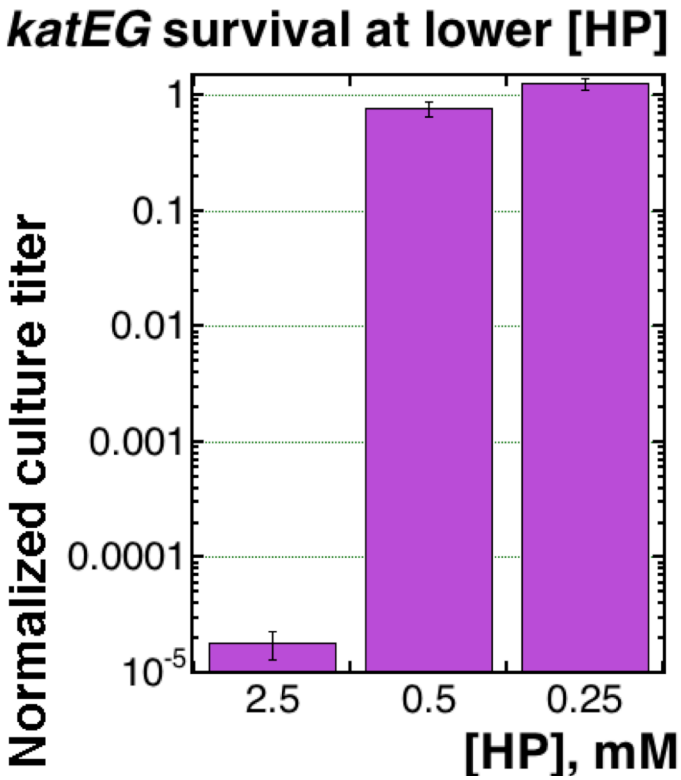

Fig. S6. The *katEG* mutant survives at lower [HP], 45 min treatments.