Supplementary Information

Oxidation of phosphorothioate DNA modifications leads to lethal genomic instability

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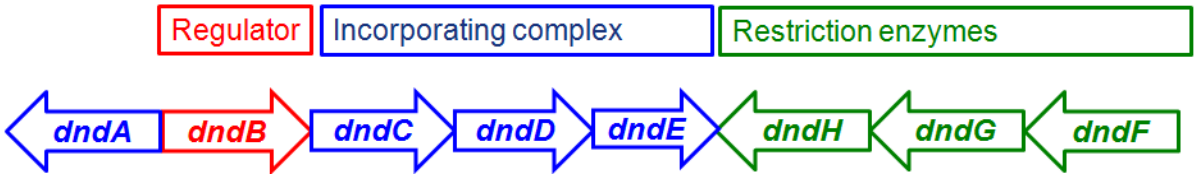
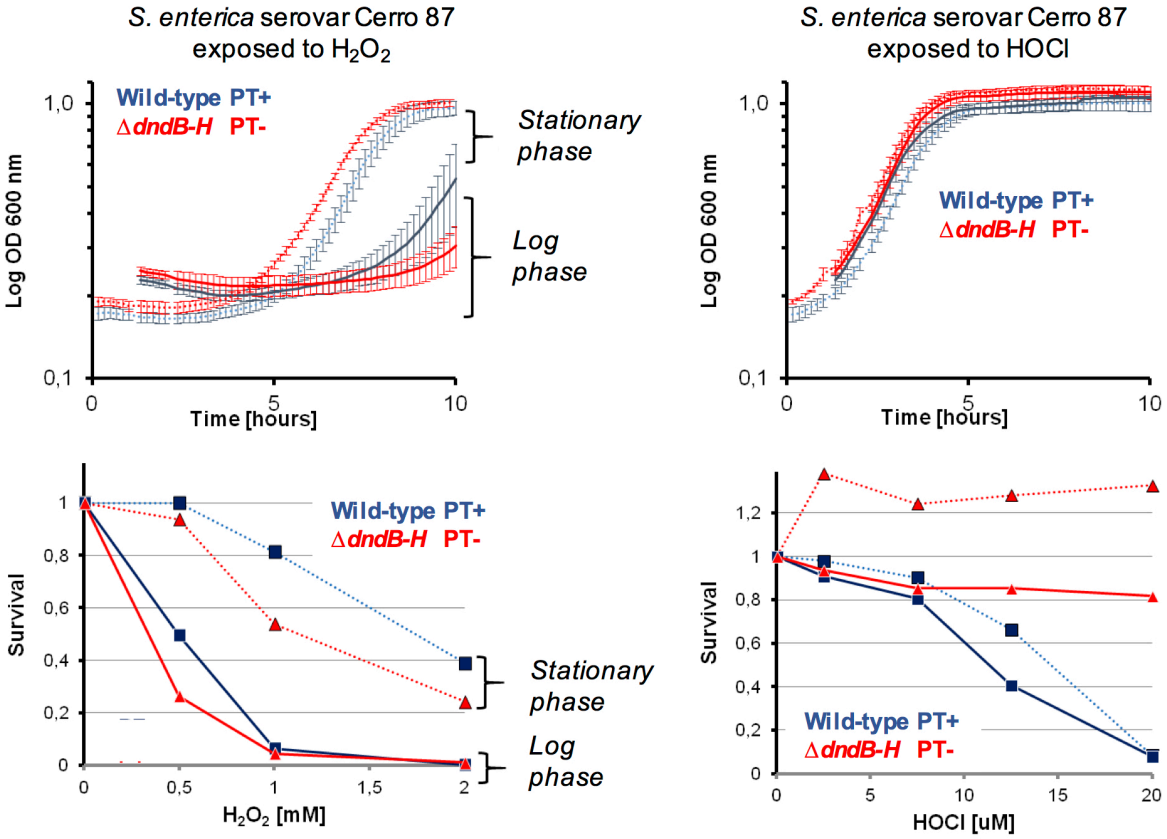
Content:

Supplementary Results

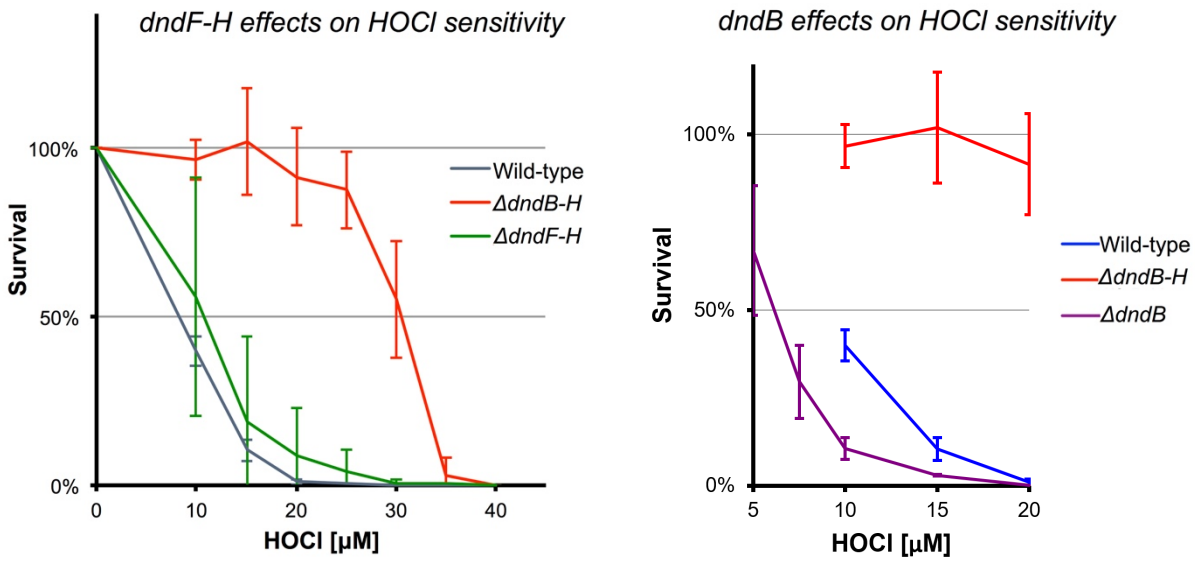
Supplementary Figure 1: *dnd* genes and their corresponding functions.



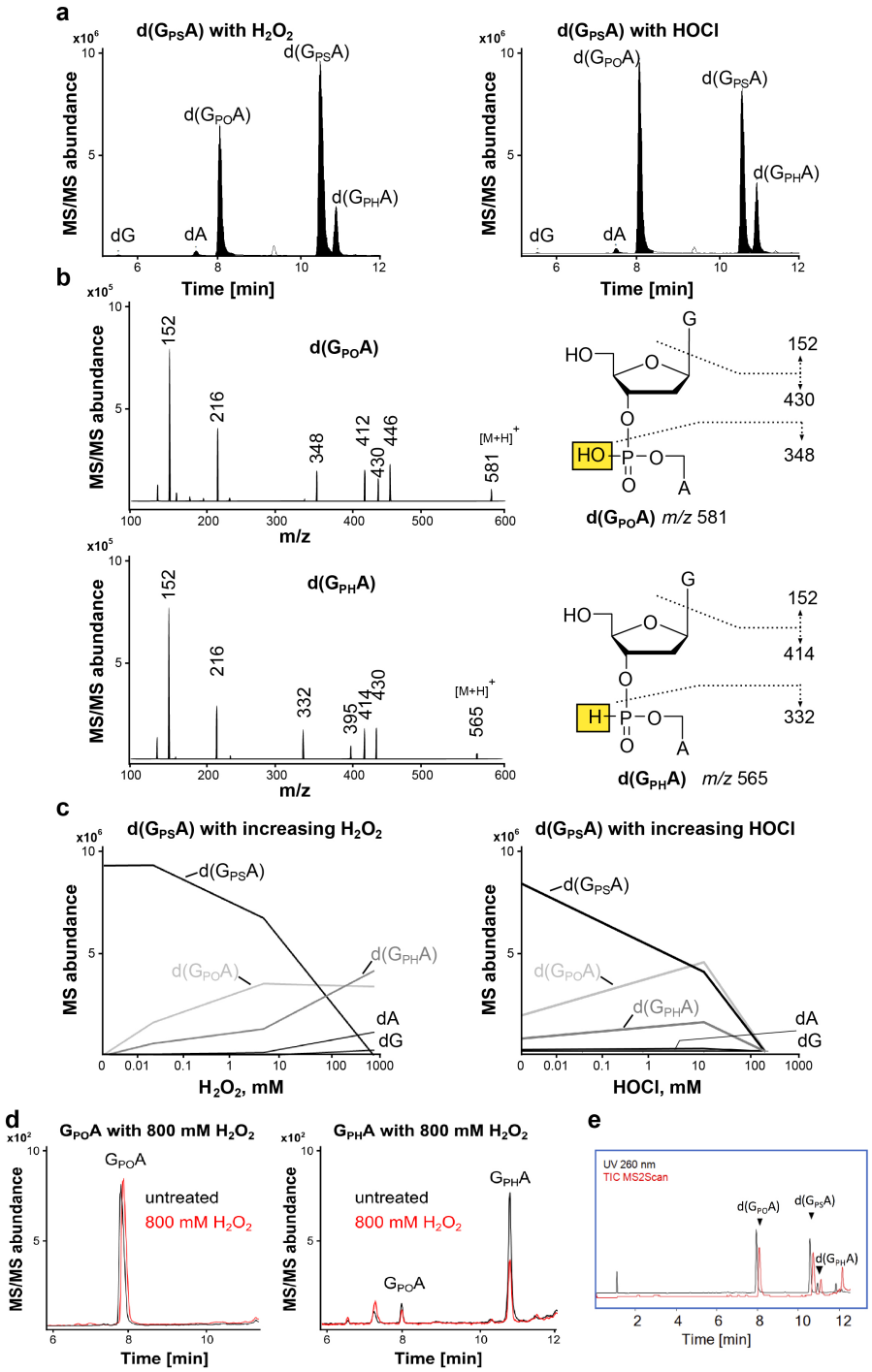
Supplementary Figure 2: Influence of growth state on the response to oxidant exposure. Wild-type (blue; PT+) and *ΔdndB-H* (red; PT-) *S. enterica* serovar Cerro 87 cultures were exposed to either H_2O_2 (left column) or HOCl (right column) in stationary phase (following from overnight growth; dashed lines) or log phase (after 1 h of growth in fresh medium; solid lines). While exposure to H_2O_2 leads to a difference in both growth and survival depending on the growth state and experimental conditions, no such effect was observed for HOCl. Data for growth rate represent mean ± SD for 3 technical replicates. Data for survival represent a single illustrative experiment.



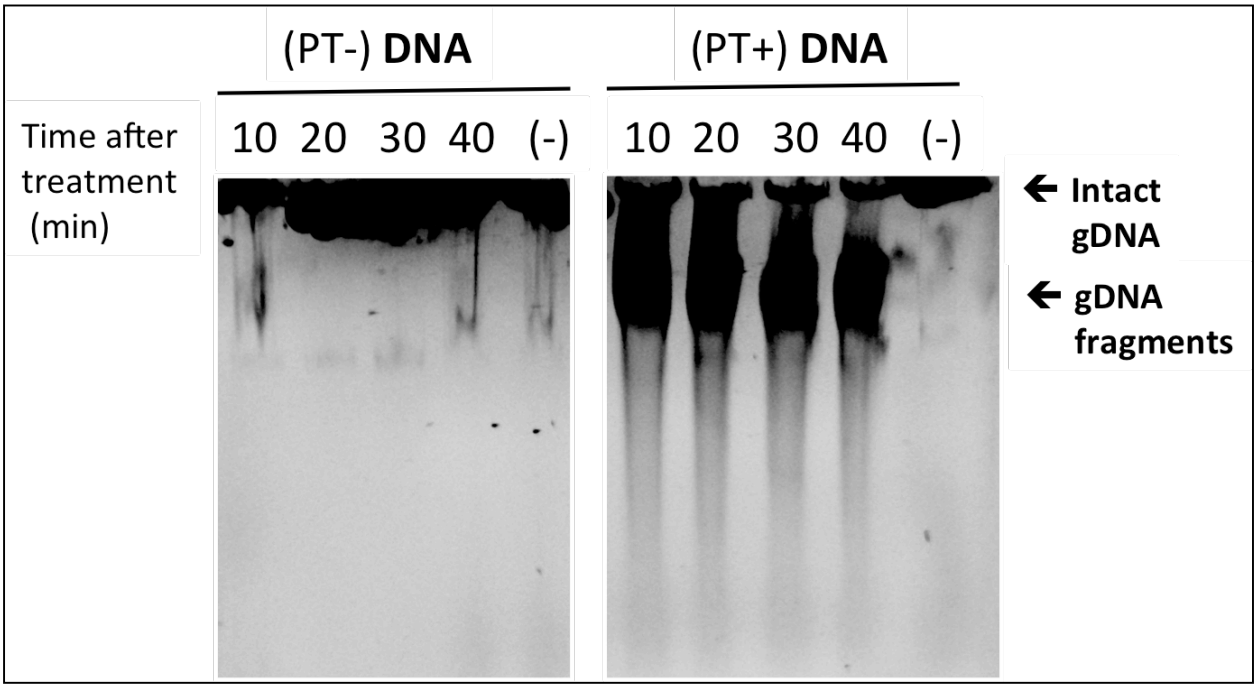
Supplementary Figure 3: HOCl sensitivity of *ΔdndF-H* and *ΔdndB S. enterica* strains compared with wild-type and *ΔdndB-H*. Data represent mean ± SD for 3 biological replicates.



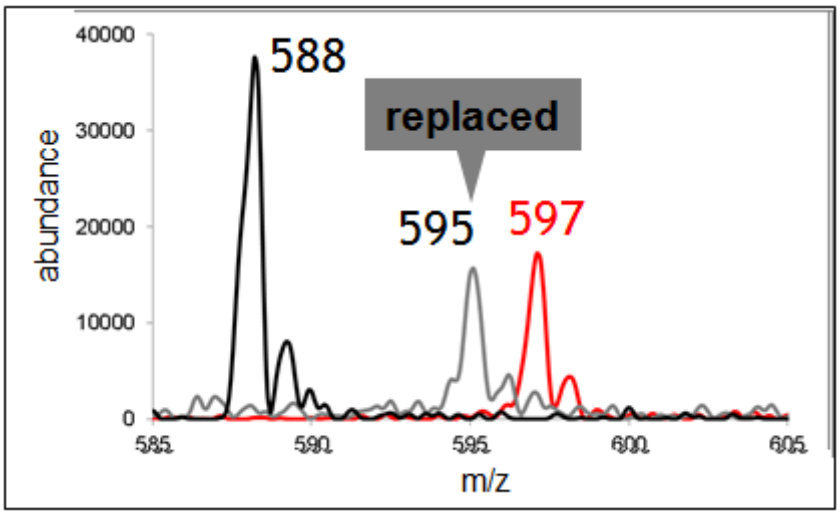
Supplementary Figure 4: Products arising in reactions of PT dinucleotides with H_2O_2 and HOCl. (**a**) LC-MS/MS analysis of the reaction of d(G_{ps}A) with 8 mM H_2O_2 and 1 mM HOCl reveals desulfuration to form phosphate in d(G_{PO}A) and phosphonate in d(G_{PH}A), as well as dG and dA indicative of strand-breaks. (**b**) Positive ion mode, collision-induced dissociation product ion mass spectra of d(G_{PO}A) and d(G_{PH}A), difference highlighted in yellow boxes. (**c**) Concentration dependence of the reactions of d(G_{ps}A) with H_2O_2 and HOCl. Products were quantified as MS signal intensity in LC-MS analysis. (**d**) Reaction of d(G_{PO}A) and d(G_{PH}A) with 800 mM H_2O_2. The decrease in d(G_{PH}A) is consistent with an oxidation-induced strand-break (**Fig. 3**, main text), but not d(G_{PO}A) formation. (**e**) Comparison of the total ion chromatogram (TIC) and UV absorption for products formed in the reaction of d(G_{PS}A) to 1 mM HOCl. Note that the elution time offset of the UV and MS signals is due to the fact that the in-line UV detector is positioned ahead of the MS system.



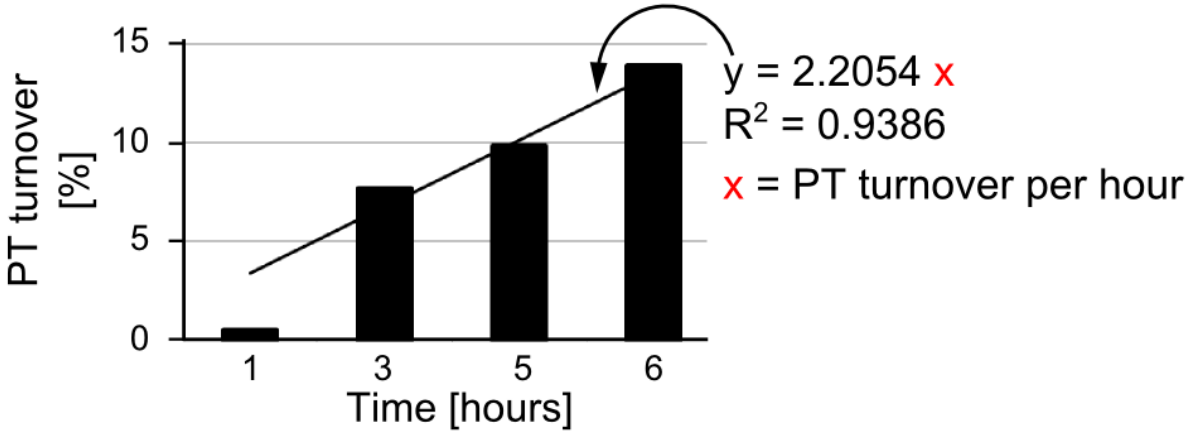
Supplementary Figure 5: Agarose gel analysis of DNA isolated from oxidant-treated bacteria after 10 to 40 min of incubation. Wild-type (right panel; PT+) and *ΔdndB-H* (left panel; PT-) *S. enterica* strains were exposed to 150 µM HOCl and genomic DNA (gDNA) was isolated at various times. The gDNA was then resolved on a 0.7% agarose gel. Lanes marked "(-)" contain DNA from unexposed bacteria.



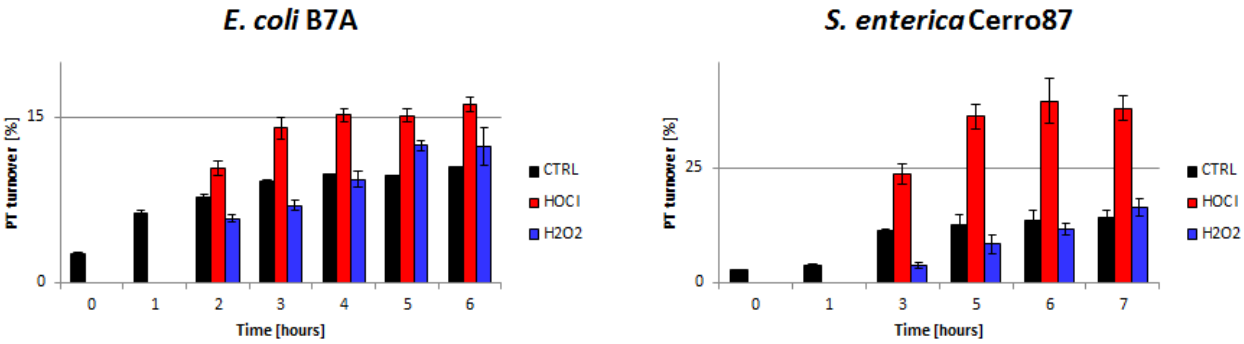
Supplementary Figure 6: Mass spectra showing the different d(G_{PS}T) isotopomers. The red mass spectrum with molecular ion $[M+H]^+$ *m/z* 597 represents the [^{34}S]/[^{15}N]-labeled d(G_{PS}T) in original DNA strands. The black mass spectrum with molecular ion $[M+H]^+$ *m/z* 588 represents [^{32}S]/[^{14}N]-labeled d(G_{PS}T) in newly replicated DNA strands. The grey mass spectrum with molecular ion $[M+H]^+$ *m/z* 595 represents [^{32}S]/[^{15}N]-labeled d(G_{PS}T) in which original PT [^{34}S] has been replaced with [^{32}S].



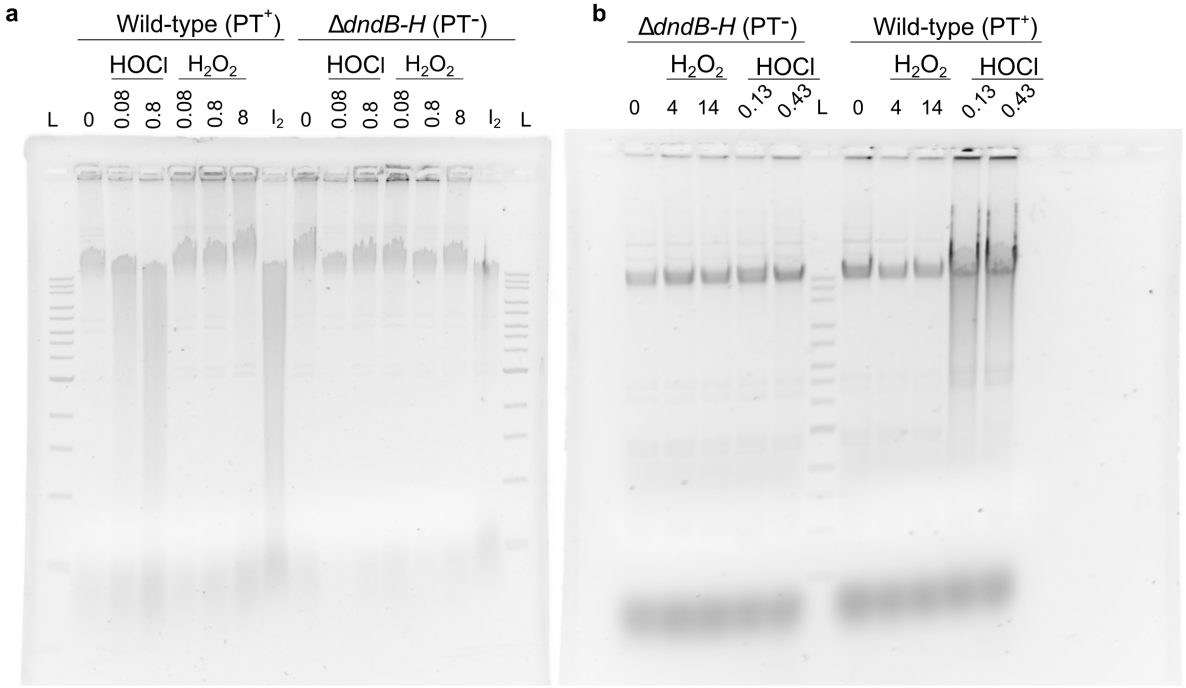
Supplementary Figure 7: Estimation of the rate of PT turnover in untreated wild-type *S. enterica serovar Cerro 87.* To calculate PT turnover events per hour ("rate") as in **Fig. 5c** for *E. coli* B7A, PT turnover events quantified as the percentage of original PT levels are plotted versus sampling time. Regression analysis reveals a reasonable linear fit of these data, with the slope defining the percentage of PTs turning over per hour. The data shown in the graph are derived from untreated wild-type *S. enterica serovar Cerro 87* and illustrate the regression analysis performed to estimate PT turnover rates for *B7A* and *Cerro 87* in **Fig. 5d**. Data in the graph below represent mean values for 2 technical replicates from a single time course experiment, with the slopes from three different experiments averaged to yield the mean ± SD data plotted in **Figure 5d**.



Supplementary Figure 8: Percentage of all PT replaced, repaired or moved over time. On the left, PT turnover in *E. coli* B7A wild-type is shown. On the right, PT turnover for *S. enterica* Cerro 87 wild-type is shown. Used doses: *E. coli* B7A 0.8 mM H_2O_2 (52% ±1.1% survival), 17.5 µM HOCl (50% ±1% survival) and *S. enterica* 1 mM H_2O_2 (60% ±8% survival), 7.5 µM HOCl (36% ±8% survival). The error bars represent the standard deviation of the mean for 3 biological replicates.



Supplementary Figure 9: Full, uncut agarose gel images for Figures 4a and 4b. a) Uncropped and unedited agarose gel for Figure 4a. Isolated DNA from wild-type (PT^+) and *ΔdndB–H* (PT^-) *E. coli* were exposed to 0.08–0.8 mM HOCl or 0.08–8 mM H_2O_2; iodine (I_2) exposure serves as a positive control for PT-dependent strand breaks. HOCl-induced strand-breaks are apparent as smearing in the lane for DNA containing PT (PT^+), but not for DNA lacking PT (PT^-); strand-breaks are not detectable for H_2O_2 exposure in any case. **b)** Uncropped and unedited agarose gel for Figure 4b. WT and *ΔdndB–H E. coli* cells were exposed to 7.5- and 25-times the WT LD_{50} concentration of H_2O_2 or HOCl for 10 min (4 and 14 mM for H_2O_2; 0.13 and 0.43 for HOCl). Again, DNA isolated from HOCl-exposed WT bacteria, but not PT^- bacteria, shows strand-breaks, while no strand breaks are apparent after H_2O_2 treatment in either strain.



Supplementary Tables

Supplementary Table 1: Overview of bacterial strains and their PT levels

Strain	*PT per 10^6 nts*
E. coli B7A WT[6]	875 ± 68
E. coli B7A *ΔdndB-H*[6]	Not detectable
E. coli B7A *ΔdndF-H*[6]	858 ± 36
S. enterica serovar Cerro 87 WT[13]	620±41
S. enterica serovar Cerro 87 *ΔdndB*[13]	1236 ± 53
S. enterica serovar Cerro 87 *ΔdndB-H*[13]	Not detectable
S. enterica serovar Cerro 87 *ΔdndF-H*[13]	658 ± 38

Supplementary Table 2: Parameters for LC-MS/MS detection of PT isotopomers

Compound	Precursor ion *m/z*	Product ion *m/z*	Fragmentor (V)	Collision energy (eV)	Ret Time (min)	ΔRet Time (min)
dA	252.2	136.1	170	20	5	2
[^{15}N]-dA	257.2	141.1	170	20	5	2
[^{15}N]/[^{34}S]-d(G_{ps}A)	609.4	141.1	120	40	8	2
[^{15}N]/[^{32}S]-d(G_{ps}A)	607.4	141.1	120	40	8	2
[^{14}N]/[^{32}S]-d(G_{ps}A)	597.4	136.1	120	40	8	2
[^{14}N]/[^{34}S]-d(G_{ps}A)	599.4	136.1	120	40	8	2
[^{13}C]-d(G_{ps}A)	617.4	141.1	120	40	8	2
[^{15}N]/[^{34}S]-d(G_{ps}T)	597.4	157.1	110	17	9.5	2
[^{15}N]/[^{32}S]-d(G_{ps}T)	595.4	157.1	110	17	9.5	2
[^{14}N]/[^{32}S]-d(G_{ps}T)	588.4	152.1	110	17	9.5	2
[^{14}N]/[^{34}S]-d(G_{ps}T)	590.4	152.1	110	17	9.5	2
[^{13}C]-d(G_{ps}T)	608.4	157.1	110	17	9.5	2

Supplementary Table 3: Doses of H_2O_2 and HOCl used in analysis of DNA damage in bacteria. Data represent mean ±SD for 3 biological replicates.

		H_2O_2, μM				HOCl, μM			
	Strain	**LD_{50}**	**LD_{80}**	**7.5xLD_{50}**	**25x LD_{50}**	**LD_{50}**	**LD_{80}**	**7.5x LD_{50}**	**25x LD_{50}**
E. coli	Wild-type	555±163	2100	4162	13875	17±0.9	27	128	425
	ΔdndB								
	ΔdndB-H	383±59				21±2.8			
S. enterica	Wild-type	524±81	1350	3930	13100	6.0±0.3	15.6	45	150
	ΔdndB					3.7±1.8			
	ΔdndB-H	381±128				29±0.2			
	ΔdndF-H					9.5±4.8			