Annex 1. Proteins present in conventionally purified RecN protein

The proteins present in the conventionally purified RecN sample, the M_R = 520,000 peak, were separated by preparative two-dimensional (2D) polyacrylamide gel electrophoresis (PAGE) using a linear pH gradient in the 3-10 pH range. The six protein spots were excised manually and the gel plugs were washed with 50 mM ammonium bicarbonate and treated with acetonitrile, before reduction with 10 mM DTT in 25 mM ammonium bicarbonate and alkylation with 55 mM iodoacetamide in 50 mM ammonium bicarbonate, as described (1). Proteins were digested with modified porcine trypsin (sequencing grade; Promega, Madison WI) at a final concentration of 15 ng/ml in 25 mM ammonium bicarbonate for 4 hrs at 37º C. Peptides were eluted from gel pieces with 0.5% trifluoroacetic acid in water for 30 min at 25º C, and the resulting peptides were analyzed using a Bruker Reflex IV MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) using FlexControl 1.1 software and then processed by Xtof 5.1.1 software, which analyzes raw data. Each spectrum was calibrated internally with two trypsin autolysis reference ions, specifically 842.510 and 2211.105 Da peptides, to reach a typical mass measurement accuracy of ±30 ppm in the 800-3000 m/z range. For protein identification, tryptic peptide masses were transferred to the BioTools 2.0 interface, provided by Bruker Daltonics, and searched against the non-redundant NCBI database using Mascot software (www.matrixscience.com; Matrix Science, London, UK). In all protein identifications, the probability Mowse scores were greater than the minimum score fixed as significant with a *p*-value less than 0.05.

The conventionally purified RecN sample, after FPLC purification, contained *circa* 1.2 % GroEL, 0.3 % AhpC , 0.3% PncB, 0.2 % PNPase and 0.1 % YqfO. Except for *pncB*, which is essential only in the presence of the *yrxA* gene product (see 2), the genes coding for the other polypeptides are not essential (data not shown). AhpC, which is the small subunit of the alkyl hydroperoxide reductase and has an estimated molecular mass of 20.4 kDa, migrated as a 19 kDa protein with a pI of 4.1 (Table 1). GroEL, a multisubunit chaperonin with M_R = ~ 840,000 and a subunit estimated molecular mass of 57.2 kDa, migrated as a 60 kDa protein with a pI of 4.6 (Table 1). PNPase, a multisubunit polynucleotide phosphorylase complex with M_R = ~ 235,000 and an estimated subunit molecular mass of 77.2 kDa, migrated as a 75 kDa protein with a pI of 4.9, similar to RecN. PncB (previously termed YueK), which is nicotinic acid phosphoribosyltransferase having an estimated molecular mass of 56.0 kDa, migrated as a 44 kDa protein with a pI of 5.1 (Table 1). A peptide fingerprint revealed that a truncated form of PncB was present in the RecN sample. YqfO, a multisubunit protein of unknown function with M_R = ~ 120,000 and an estimated molecular mass of 40.7 kDa, migrated as a 40 kDa protein with a pI of 5.3 (Table 1). Previously it was reported that the GroEL AhpC, PncB, PNPase, YqfO and RecN proteins were involved in nucleic acid metabolism or played a certain role with enzymes involved in nucleic acid metabolism (2-8).

Annex 2. Are PNPase, GroEL, AhpC, PncB and YqfO simply "contaminants" in the RecN sample?

B. subtilis GroEL genuinely interacts with AphC (9), and $GroEL_{Eco}$ interacts directly with PNPase in multiprotein complexes that are involved in mRNA processing and degradation (see 6,10). AhpC, is the founding member of the large AhpC/TSA family of peroxidases (11), and participates actively in DNA repair by reducing peroxides to their corresponding alcohols (5). PncB, the nicotinic acid phosphoribosyltransferase, is involved in the niacin salvage pathway and the metabolism of NAD (see 2), with NAD playing important roles in cellular metabolism. YqfO is a member of the widespread Nif3 family of proteins, although the precise function of this family still remains elusive.

We consider it unlikely that RecN co-purifies fortuitously with PNPase. Previously it was shown that PNPase co-purifies with GroEL (10). Although GroEL constituted >1% of the

RecN preparation, other proteins that are involved in nucleic acid metabolism with an acid pI and that physically interact with the GroEL protein (e.g., Enolase [pI 4.5], DnaK [pI 4.8], AtpD [pI 4.8] or RecA [pI 4.9]) (9) were not present in the RecN sample (Supplemental Fig. 1). We cannot rule out the possibility that GroEL interacts with RecN. We have found that, by fully removing GroEL from the RecN sample, a decrease (~ 2-fold) in the ssDNA-dependent ATPase activity of the RecN protein stored at 4°C for one week was observed (data not shown). At present only 21 % of total proteins that interact with GroEL have been identified (9). We consider it unlikely that RecN was able to co-purify fortuitously with any protein(s) induced under stress conditions, because the *B. subtilis* strain (BG214) used for *recN* over-expression is deficient in σ^B (*sigB*37), and under this condition low levels of AhpC expression were expected during exponential growth (see 4). Other than PNPase and GroEL, proteins found in the degradosome-like complex in other organisms, such as enolase and DnaK (see 6), were not present in the RecN sample.

Table S1. Bacterial strains used in this study

Strain[a]	Doubling Time (min)[b]	Relevant genotype	Source or reference
BG214	29.4	*trpC*2 *metB*5 *amyE sigB*37 *xre*1 attSPβ *att*ICE*Bs*1	(12)
BG993	38.2	+ Δ*pnpA*	This work
BG190	32.3	+ Δ*recA*	(13)
BG439 (α)	32.1	+ Δ*recO*	(14)
BG125 (β)	34.9	+ *addA5*	(12)
BG281 (δ)	35.0	+ Δ*recN*	(15)
BG623 (ε)	31.0	+ Δ*recU*	This work
BG705 (ζ)	30.1	+ Δ*recJ*	(16)
BG713 (η)	34.5	+ Δ*recG*	(17)
BG995	48.3	+ Δ*recA* Δ*pnpA*	This work
BG997	46.6	+ Δ*recO* Δ*pnpA*	This work
BG1007	48.1	+ *addA*5 Δ*pnpA*	This work
BG1013	50.0	+ Δ*recN* Δ*pnpA*	This work
BG1009	44.4	+ Δ*recU* Δ*pnpA*	This work
BG1003	47.9	+ Δ*recJ* Δ*pnpA*	This work
BG1005	47.0	+ Δ*recG* Δ*pnpA*	This work
BG809	31.0	+ Δ*ku* (*ykoV*)	(18)
BG849	49.7	+ Δ*ku* Δ*recA*	(18)
BG1011	48.7	+ Δ*ku* Δ*pnpA*	This work
BG1047	ND	+ Δ*lexA*	This work
BG1048	ND	+ Δ*lexA* Δ*pnpA*	This work

[a] Parentheses denote the epistatic group in which the gene is classified, All strains were isogenic with the wt control (BG214), and the relevant genotype of the strains is indicated.
[b]Values represent the average from three independent experiments in which doubling times were measured for strains grown in LB broth at 37° C. ND, not done

Table S2. Expression of RecA protein in Δ*pnpA* cells

Strain	Level of induced RecA[a] - MMC	+ MMC
wt	~ 4,100 (5.6 μM)	~ 26,200 (35 μM)
Δ*pnpA*	~ 4,000	~ 25,900
Δ*recA*	ND	ND
Δ*lexA*	~ 26,100	~ 25,900
Δ*lexA* Δ*pnpA*	~ 25,800	~ 25,400

[a]Number of protein protomers based on the number of wt, Δ*pnpA*, Δ*rec*A and Δ*lex*A exponentially growing cells or those cells after exposure to MMC (3 μM or 1 μg/ml) for 30 min was estimated as described (19). In parentheses is indicated the estimated *in vivo* concentration of RecA, assuming a cell volume of 1.2 femtoliters. ND, not detected.

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Figure Legend

Figure S1. Analysis of proteins present in the RecN sample prior to purification by gel filtration chromatography. The six proteins (1 to 6) were identified as: 1, RecN; 2, AhpC; 3, GroEL; 4, PNPase; 5, PncB; 6, YqfO.

Figure S2. Chromosomal segregation in Δ*pnpA* cells. Exponentially growing cells were fixed, stained with DAPI, and analyzed by fluorescence microscopy to visualize the nucleoid. Two images are shown from each of the indicated strains. Grey arrows indicate anucleate cells and white arrows denote aberrant and misplaced nucleoids.

Figure S3. Survival of Δ*pnpA* strains exposed to a chronic dose of H_2O_2, MMS or MMC. The strains were grown to OD_{560} = 0.4 in LB medium and serially diluted (10^{-3} to 10^{-7}) and plated on LB agar containing the indicated concentration of the DNA damaging agent. The strains used are identified by their relevant genotype. – Drug, no drug added. Only the relevant drug concentration that highlight differences are shown. In *A*, cells were exposed to 1 mM H_2O_2, 2.7 mM MMS or 180 pM MMC. In *B*, cells were exposed to 0.5 (*addA*5 and *addA*5 Δ*pnpA*) or 0.6 (Δ*recN* and Δ*recN* Δ*pnpA*) mM H_2O_2, 1.2 mM MMS or 90 pM MMC. In *C*, cells were exposed to 0.2 mM H_2O_2, 70 μM MMS or 10 pM MMC.

Figure S4. Survival of Δ*pnpA* strains exposed to an acute dose of MMS or H_2O_2. The strains were grown to OD_{560} = 0.4 in LB medium and exposed to increasing concentrations of MMS (*A*, *B* and *C*) or H_2O_2 (*D*, *E* and *F*) for 15 min, and serial dilutions were plated onto LB plates to measure survival rates. A given recombination-deficient strain in the Δ*pnpA* context is denoted by its equivalent filled symbol. In *A* and *D* the Δ*recQ* Δ*pnpA* and Δ*recJ* Δ*pnpA* strains showed similar survival curves; hence, only the former strain is included. In *C* and *F*, the Δ*recO* Δ*pnpA* and Δ*recR* Δ*pnpA* strains showed similar survival curves; hence only the former strain is included.

Figure S5. Survival of the null *ku* mutation in the Δ*pnpA* context. *A*, survival of cells exposed acutely to increasing drug concentrations. *B* and *C,* survival of cells exposed chronically to DNA damaging agents. In *B*, cells were exposed to 1 mM H_2O_2, 2.7 mM MMS, 200 nM 4NQO or 180 pM MMC. In *C*, cells were exposed to 0.2 mM H_2O_2, 70 μM MMS, 7.5 nM 4NQO or 10 pM MMC.

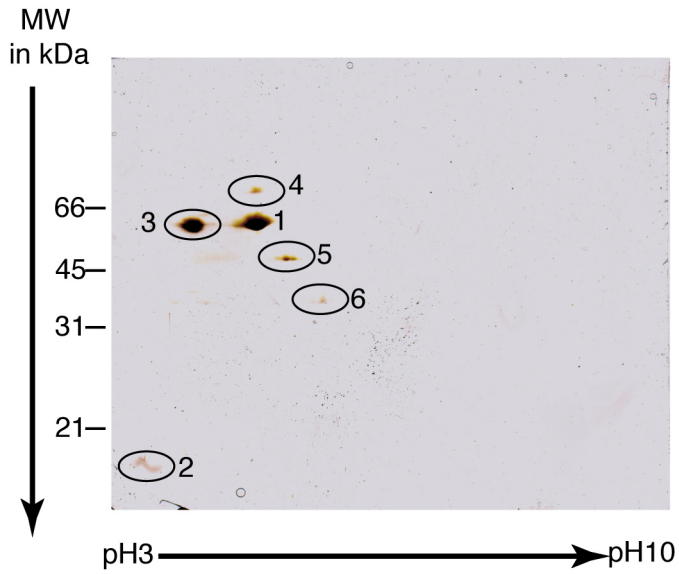


Figure S1

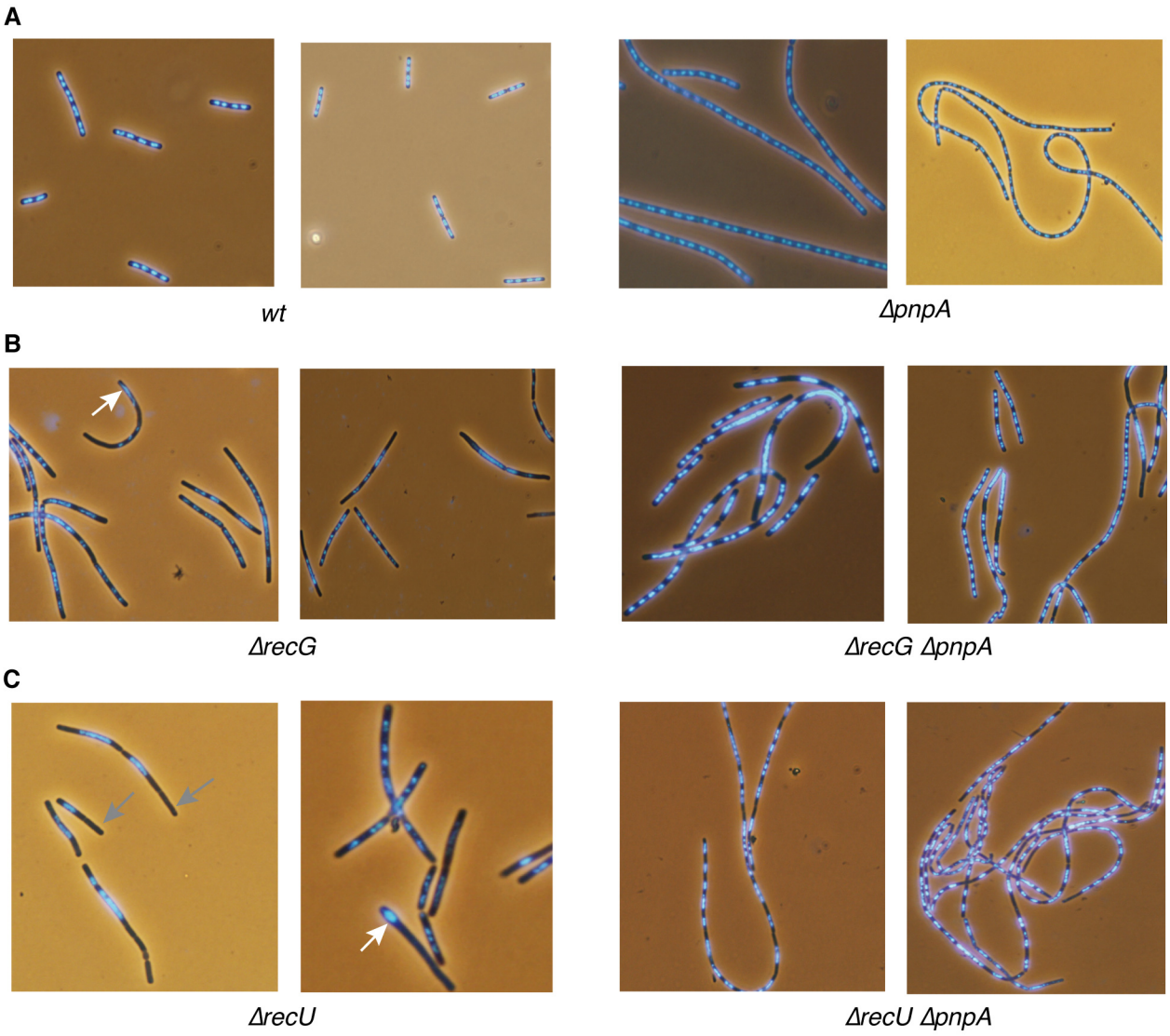
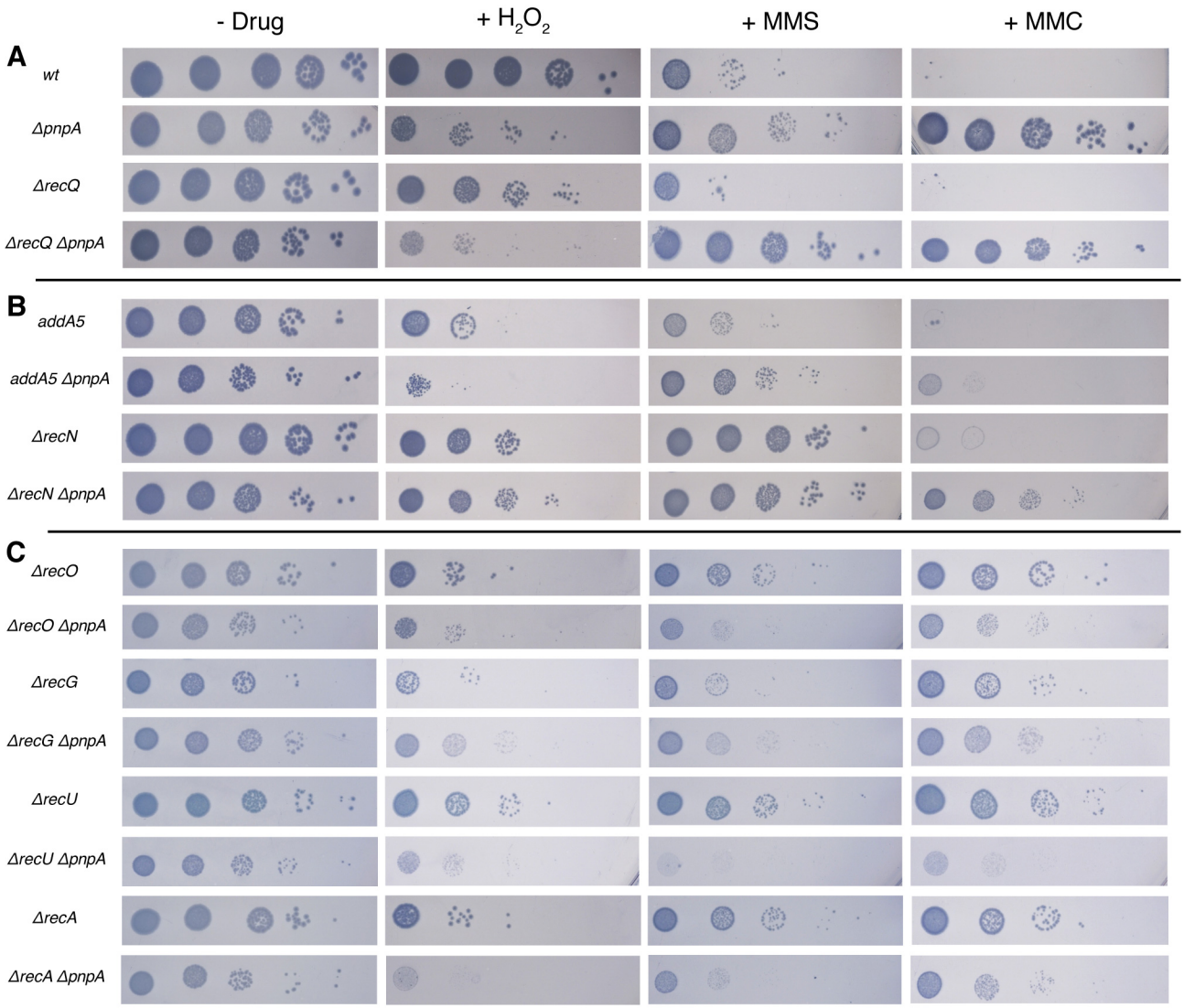


Figure S2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+ H2O2
+ MMS
+ MMC
A
wt
ΔpnpA
ΔrecQ
ΔrecQ ΔpnpA
B
addA5
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ΔrecN
ΔrecN ΔpnpA
C
ΔrecO
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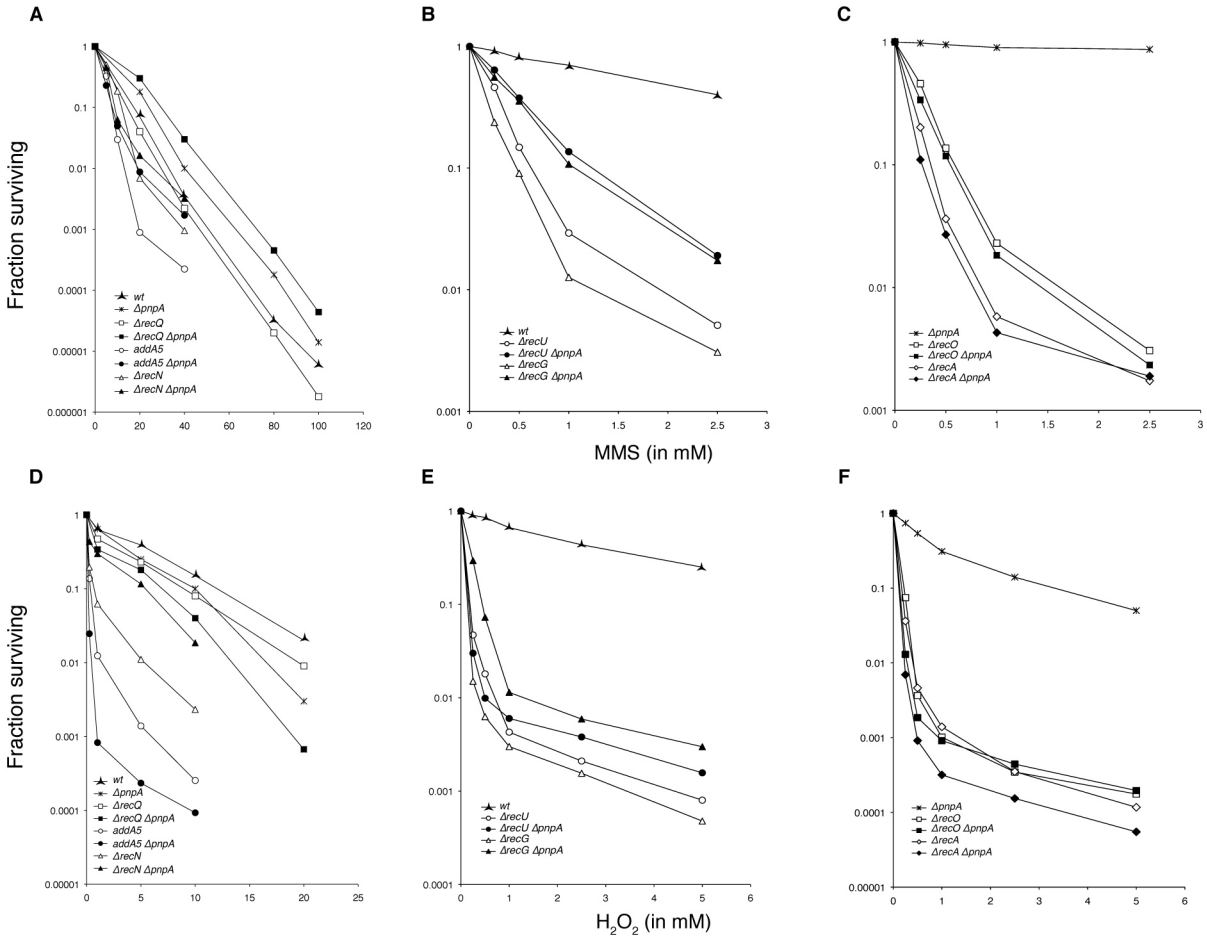
Figure S3


A
B
C
D
E
F
Fraction surviving
MMS (in mM)
H2O2 (in mM)
wt
ΔpnpA
ΔrecQ
ΔrecQ ΔpnpA
addA5
addA5 ΔpnpA
ΔrecN
ΔrecN ΔpnpA
wt
ΔrecU
ΔrecU ΔpnpA
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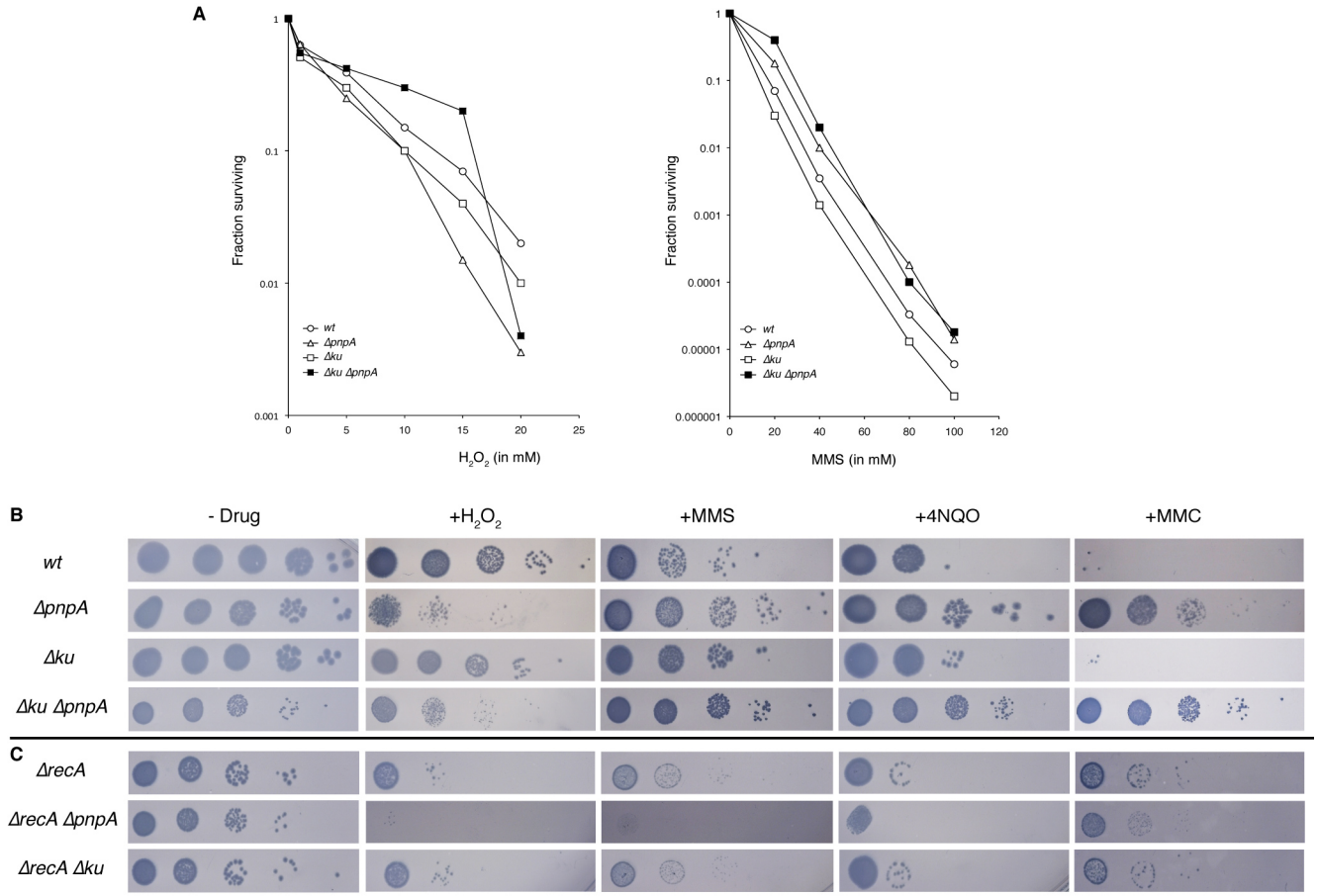
ΔpnpA
ΔrecO
ΔrecO ΔpnpA
ΔrecA
ΔrecA ΔpnpA

Figure S4


A
Fraction surviving
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0.1
0.01
0.001
0
5
10
15
20
25
H2O2 (in mM)
wt
ΔpnpA
Δku
Δku ΔpnpA
Fraction surviving
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
0
20
40
60
80
100
120
MMS (in mM)
wt
ΔpnpA
Δku
Δku ΔpnpA
B
- Drug
+H2O2
+MMS
+4NQO
+MMC
wt
ΔpnpA
Δku
Δku ΔpnpA
C
ΔrecA
ΔrecA ΔpnpA
ΔrecA Δku

Figure S5