Title: Surviving the Storm: Exploring the Role of Natural Transformation in Nutrition and DNA Repair of Stressed *Deinococcus radiodurans*

Supplementary Data file:

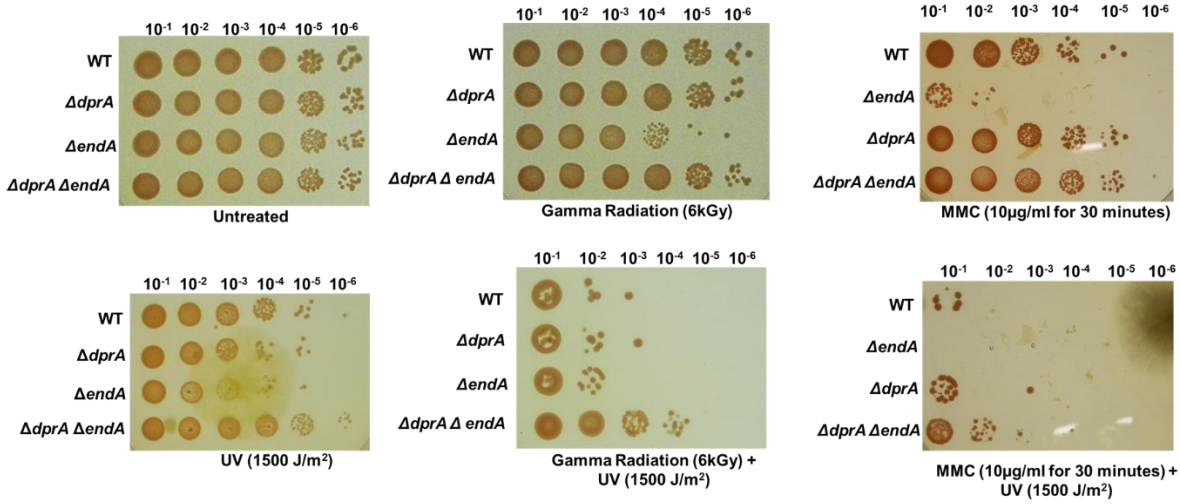


Figure S1: Survival of wild-type and natural transformation (NT) mutants of *D. radiodurans* following exposure to gamma radiation, MMC, and UV radiation, or combinations thereof. Exponentially growing cultures of the wild-type strain and NT mutants (*ΔendA*, *ΔdprA*, and *ΔendA ΔdprA*) were exposed to a 6 kGy dose of gamma radiation, or MMC (10 µg/ml) for 30 minutes, or 1500 J/m² of UV radiation, or a combination of both gamma radiation, and or a combination of MMC and UV radiation. After exposure, the cells were diluted, and aliquots were spotted onto TGY medium. Plates were then incubated at 32°C for 48 hours. The data presented here is representative of three independent experiments.

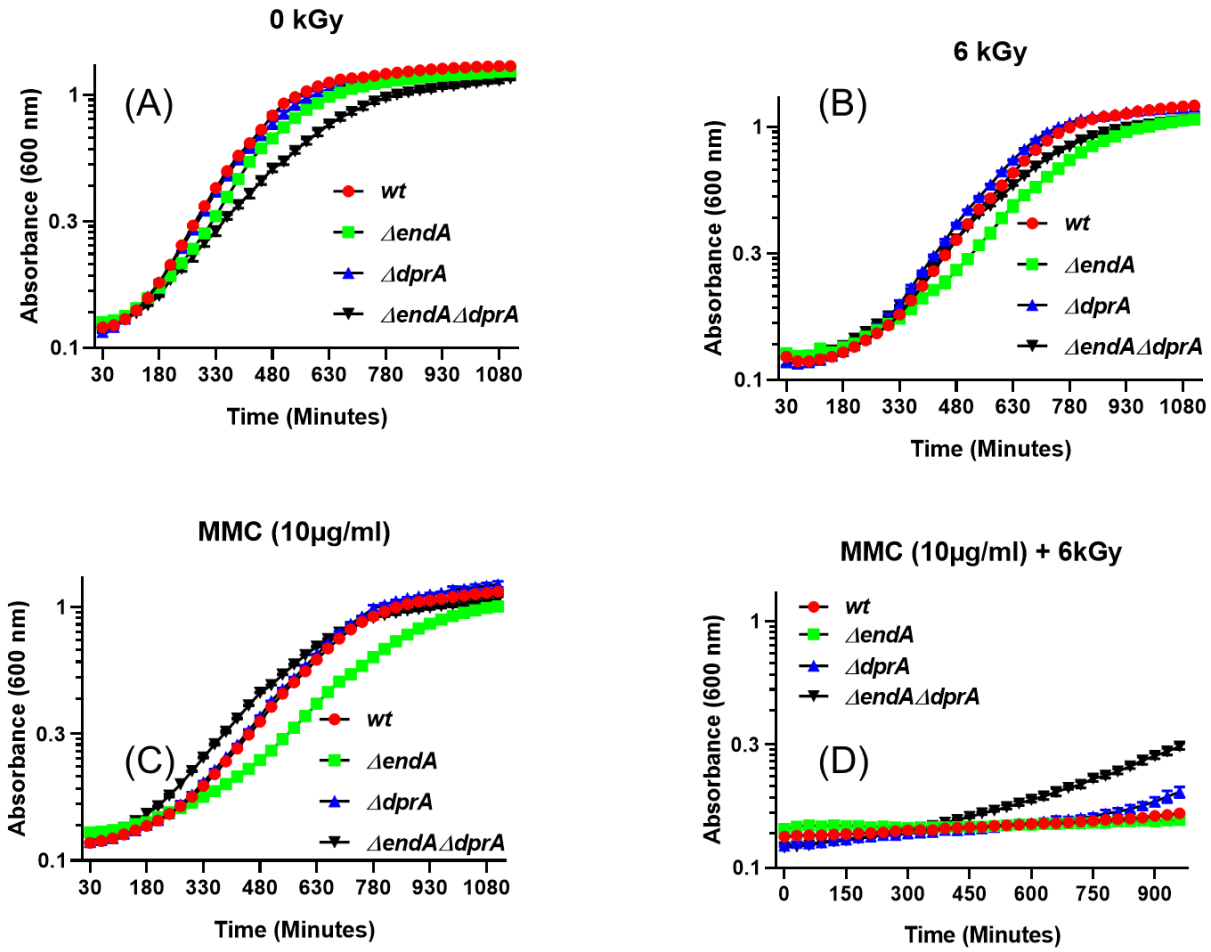


Figure S2: Cell viability and growth curve of wild type and NT mutants after exposure to gamma radiation. Optical density at 600 nm was continuously monitored using a microtiter-based density reader. The growth medium (TGY) was used as a blank throughout the incubation period, and data was normalized to this blank optical density. Panel (A) shows the growth of normal, untreated cells; panel (B) depicts cells exposed to 6 kGy of gamma radiation; panel (C) illustrates cells treated with MMC; and panel (D) presents cells treated with both MMC and 6 kGy of gamma radiation. The data presented here are the mean ± SEM from three independent experiments.

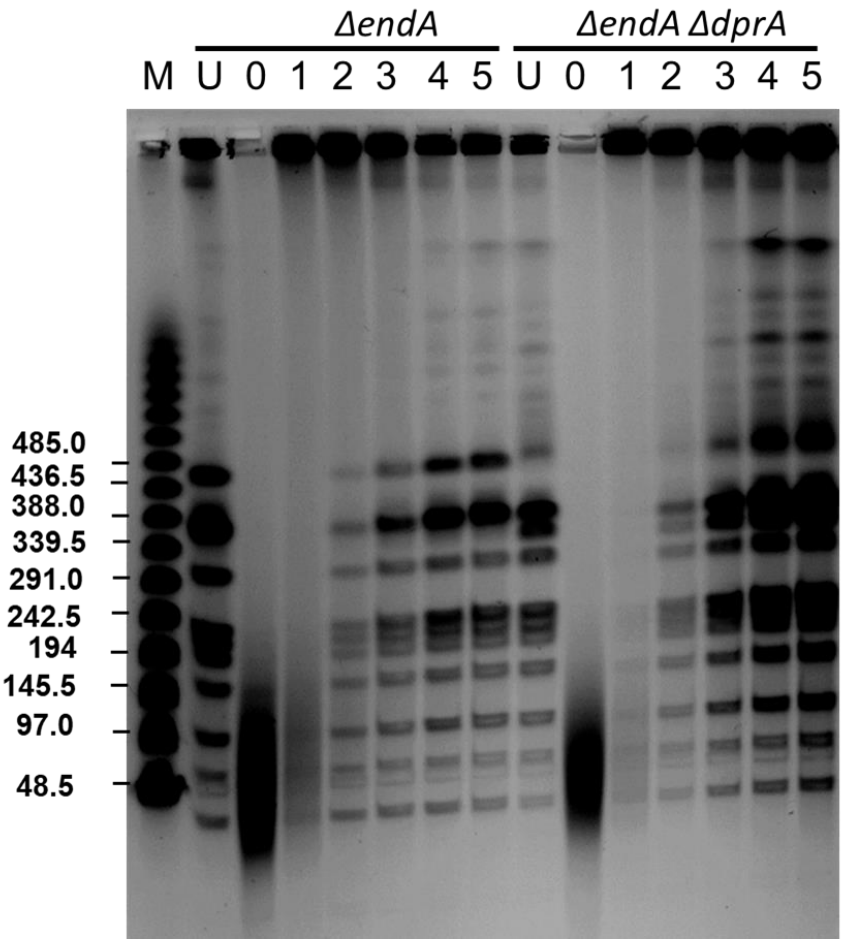


Figure S3: The DNA double-strand breaks (DSBs) repair kinetics in *D. radiodurans* wild-type and its NT-specific gene mutants.

PFGE was utilized to evaluate the repair kinetics of DSBs. The repair processes in *ΔendA* and *ΔendAΔdprA* mutants were specifically monitored. NotI-digested DNA samples were prepared from both unirradiated cells (U) and cells exposed to 6 kGy of radiation, with samples collected at various post-irradiation recovery times (PIR). The DNA was analyzed immediately after irradiation (0 hours) and at designated recovery intervals (in hours). Lambda PFG molecular mass standards were included in lane M for size calibration. The results indicate that the *ΔendA ΔdprA* double mutant demonstrates a relatively faster and more efficient DSB repair capability compared to the *ΔendA* mutant. The data presented here is representative of two independent experiments.

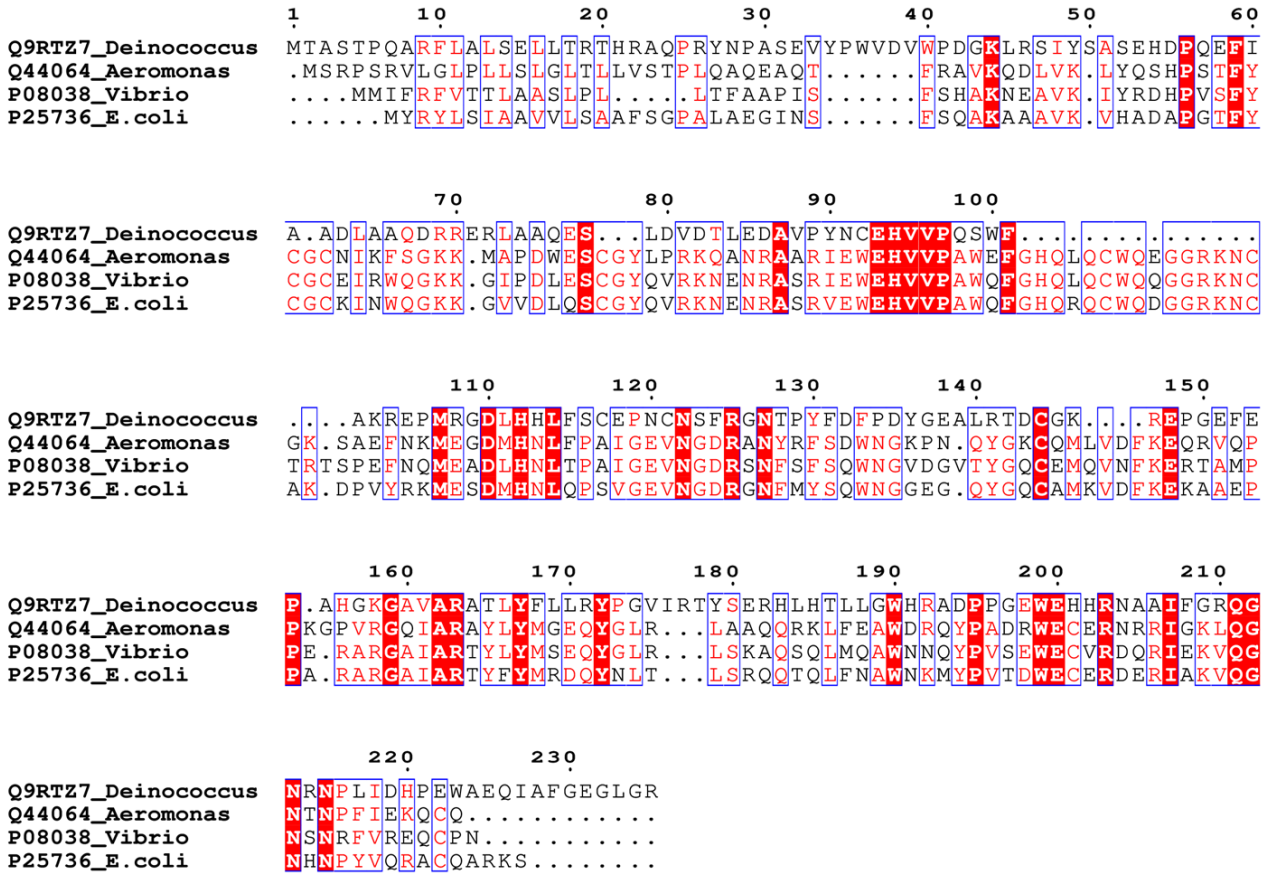
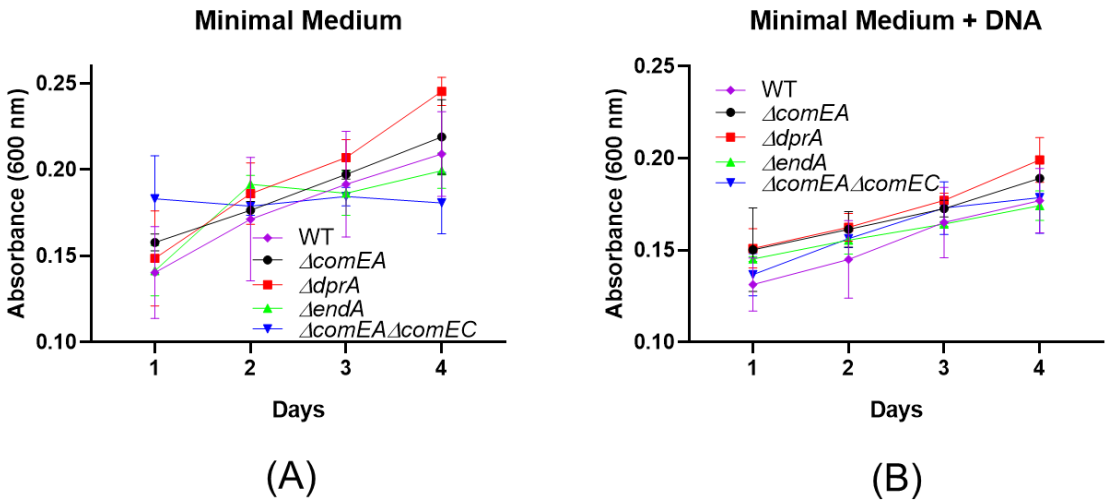


Figure S4: Multiple sequence alignment (MSA) for EndA homologs. MSA of EndA homologs was performed using clustal omega (https://www.ebi.ac.uk/jdispatcher/msa/clustalo). The EndA sequence of *D. radiodurans* (uniprot ID Q9RTZ7), *E. coli* (P25736), *V. cholerae* (P08038), and *A. hydrophila* (Q44064) were retrieved from uniprot database. The identical residues have been highlighted with red background while similar residues have been boxed and marked with red font.



(A) (B)

Figure S5: *D. radiodurans* wild type and NT mutants growth measurement in minimal medium. Optical density at 600 nm monitored continuously in a microtiter-based density reader. The minmal medium was used as a blank throughout the incubation period, and data was normalized to this blank optical density. Panel (A) shows the growth of cells in minimal medium; panel (B) cells in minimal medium supplemented with 0.5mg/ml calf thymus DNA (cfDNA). The data presented here are the mean ± SEM from three independent experiments.

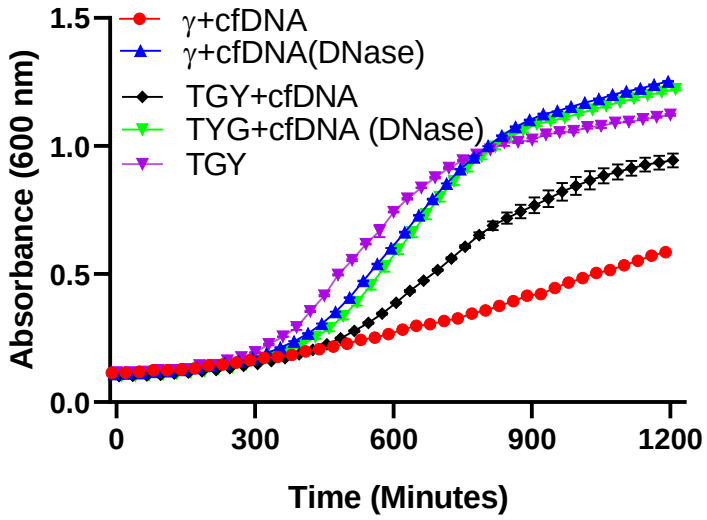


Figure S6: Growth curve of wild-type *D. radiodurans* cells in the presence of cfDNA or DNase-treated cfDNA in the TGY medium with and without gamma radiation exposure. Wild-type *D. radiodurans* cells were cultured in TGY, TGY + cfDNA, and TGY+cfDNA (DNAase-digested) with and without gamma irradiation (6kGy). Equal numbers of cells (0.1 OD_{600}) inoculated in 24 wells microtiter plate in triplicate and the optical density at 600 nm (OD_{600}) was continuously monitored using a microtiter-based density reader. TGY medium served as the blank throughout the incubation period, and the data were normalized to this blank optical density. The data shown represent the mean ± SEM from three independent experiments.

Table S1: Bacterial strains, plasmids, and primers Used in this study

A. Bacterial Strains

Bacterial strains	Organism	Genotype	Source
D. radiodurans R1	*D. radiodurans*	Wild type strain ATCC13939	Lab stock
E. coli Novablue	*E. coli*	*end*A1 *hsd*R17(*r* $_{K12}$ $^{-}$ *m* $_{K12}$ $^{+}$) *sup*E44 *thi-1* *rec*A1 *gyr*A96 *rel*A1 *lac*F''[*pro*A^{+}B^{+} *lac*I^{q} *Z*ΔM15::T*n*10] (Tet^{R})	Lab stock

ΔcomEAΔcomEC	*D. radiodurans*	*D. radiodurans* R1 *comEA comEC Ω kan*	Prof. Pascale Servant lab, France
ΔcomEA	*D. radiodurans*	*D. radiodurans* R1 *comEA Ω Kan*	This work
∆*dprA*	*D. radiodurans*	*D. radiodurans* R1 *dprA (dr_0120) Ω spec*	[24]
ΔendA	*D. radiodurans*	*D. radiodurans* R1 *endA (dr_1600) Ω kan*	This work
ΔendA ΔdprA	*D. radiodurans*	*D. radiodurans* R1 *endA Ω kan , dprA Ω spec*	This work
ΔpilT	*D. radiodurans*	*D. radiodurans* R1 *pilT (dr_1963) Ω spec*	This work

Plasmids:

Names	**Characteristics and Source**	lab stock
pVHS559	A shuttle vector between *D. radiodurans* and *E. coli* ($Spec^R$)	lab stock
pRADgro	pRAD1 carrying 261bp *Bgl*II-*Xba*I fragment of promoter (Pgro) from *D. radiodurans*	lab stock
pNOK*comEA*	pNOK suicidal vector carrying upstream and downstream DNA fragment of *comEA* gene (*dr_1855*)	This work
pNOK*endA*	pNOK suicidal vector carrying upstream and downstream DNA fragment of *endA* gene (*dr_1600*)	This work
pNOS*dprA*	pNOS suicidal vector carrying upstream and downstream DNA fragment of *dprA* gene (*dr_0120*)	This work
pNOS*pilT*	pNOS suicidal vector carrying upstream and downstream DNA fragment of *pilT* gene (*dr_1963*)	This work
pRAD*dprA*	*dprA* gene cloned in pRADgro plasmid at	This work

	ApaI and XbaI restriction sites	
pRAD*endA*	*endA* gene cloned in pRADgro plasmid at ApaI and XbaI restriction sites	This work

Primers used:

Sl. No.	**Name of primer and**	**Sequence (5' to 3')**	**Purpose**
1	*comEA*-UF	AAA GGG CCC GGC AAT CAC GTT CAT CAG	*ΔcomEA* mutant generation
2	*comEA*-UR	ATA GGA TCC GCG TGA CGC TCG CAG TGG	
3	*comEA*-DF	ATA GGA TCC GAA GCC CTT CCC AAA GTC	
4	*comEA*-DR	TAG TCT AGA AGA AGC CCT CAT CAA ACG	
7	*endA*-UF	ATA GGT ACC TGC CCG ACT TCC TGC ATT	*ΔendA* mutant generation
8	*endA*-UR	AAA GGG CCC AGG AAA AGA GGT GGT GCA	
9	*endA*-DF	ATA GGA TCC TTG CCC CTT AAT GCA GCA	
10	*endA*-DR	TAG TCT AGA TAA CGG CGG GGT GAA ACG	
11	*dprA*-UF	ATA GGT ACC TAA GCG CCT ATC AAG CCC TC	*ΔdprA* mutant generation
12	*dprA*-UR	AAA GGG ATG CCG CCG CAG GTT TTC GAT	
13	*dprA*-DF	ATA GGA TCC GAA CTG AAC AAG GCG GCA GA	
14	*dprA*-DR	TAG TCT AGA ACG CGG CAA CAG AGA GAA GTC	
15	*pilT*-UF	AAA GGG CCC CAT CGA GAA CTT CAA CAT	*ΔpilT* mutant generation
16	*pilT*-UR	ATA GGA TCC ATG AAC TCG ATG GGG TCT T	
17	*pilT*-DF	ATA GGA TCC AGC TCG CCA ACA ACC TCG T	
18	*pilT*-DR	ATG TCT AGA AAC GAT TCC ACC GCA ATC GG	
19	*dprA*-pRAD-F	ATGGGCCCGTGACCCTTCCCTCC	

20	*dprA*-pRAD-R	GCTCTAGATCAGCGACTCCAACG	Cloning of *dprA* in pRADgro
21	*endA*-pRAD-F	ATGGGCCCATGACGGCGAGCACCCCG	Cloning of *endA* in pRADgro
22	*endA*-pRAD-R	GCTCTAGACTAACGCCCCAGCCCTTC	