**Supplementary Data of Manuscript number- AEM01948-23R1:**

**Title-** Natural transformation specific DprA coordinate DNA double strand break repair pathways in heavily irradiated *D. radiodurans*

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**List of Supplementary data-**

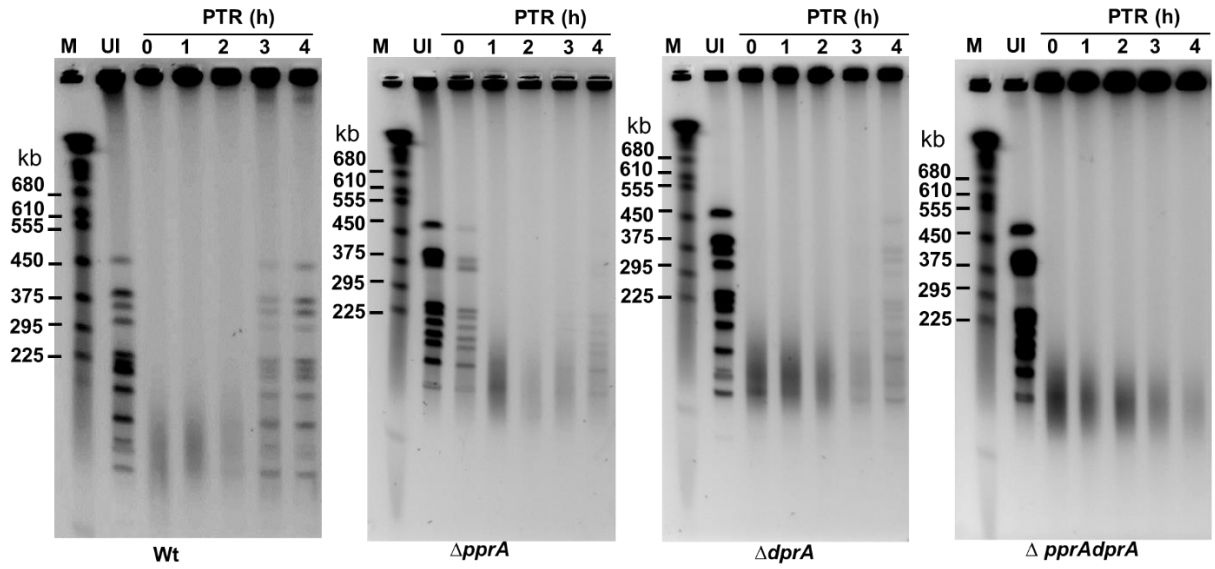


**Fig. S1: The repair kinetics of DNA double-strand breaks (DSBs) in *D. radiodurans* wild-type and mutant strains upon treatment with MMC.** The kinetics of DSB repair are shown for four strains, namely wild type (Wt), *ΔpprA*, *ΔdprA*, and *ΔpprA ΔdprA* mutants. The evaluation of the repair kinetics was performed using pulsed-field gel electrophoresis (PFGE). The NotI-digested DNA from untreated cells (UI) and cells treated with 20µg/ml mitomycin C (MMC) for 30 min were allowed to recover in TGY medium for 4 hours post-treatment (PTR). Cell samples collected at different time points were analyzed on PFGE to assess the kinetics of DSB repair. *S. cerevisiae* molecular mass standards (lane-M).

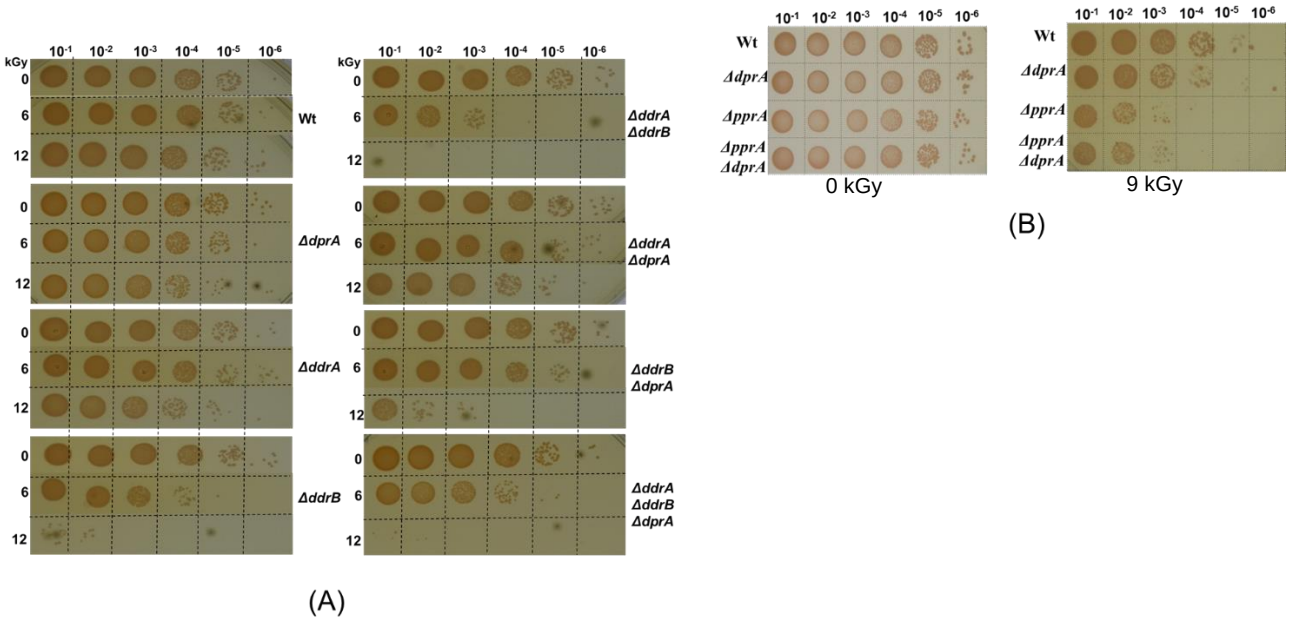


**Fig. S2: Gamma radiation survival of various mutants of *D. radiodurans*.**

(A) Exponential growth phase cells of wild type and mutants including *Δdp*rA, *ΔddrA*, *Δddr*B, *ΔddrA ΔddrB*, *ΔddrA ΔdprA*, *ΔddrB ΔdprA*, and *ΔddrA ΔddrB ΔdprA* were exposed to gamma radiation doses ranging from 0 to12kGy. After dilution, aliquots were spotted on TGY medium and incubated at 32°C for 48 hours.

(B) Wild type and mutants including *ΔdprA*, *ΔpprA* and *ΔpprA ΔdprA* were exposed to 9kGy gamma radiation doses. After dilution, aliquots were spotted on TGY medium and incubated at 32°C for 48 hours.

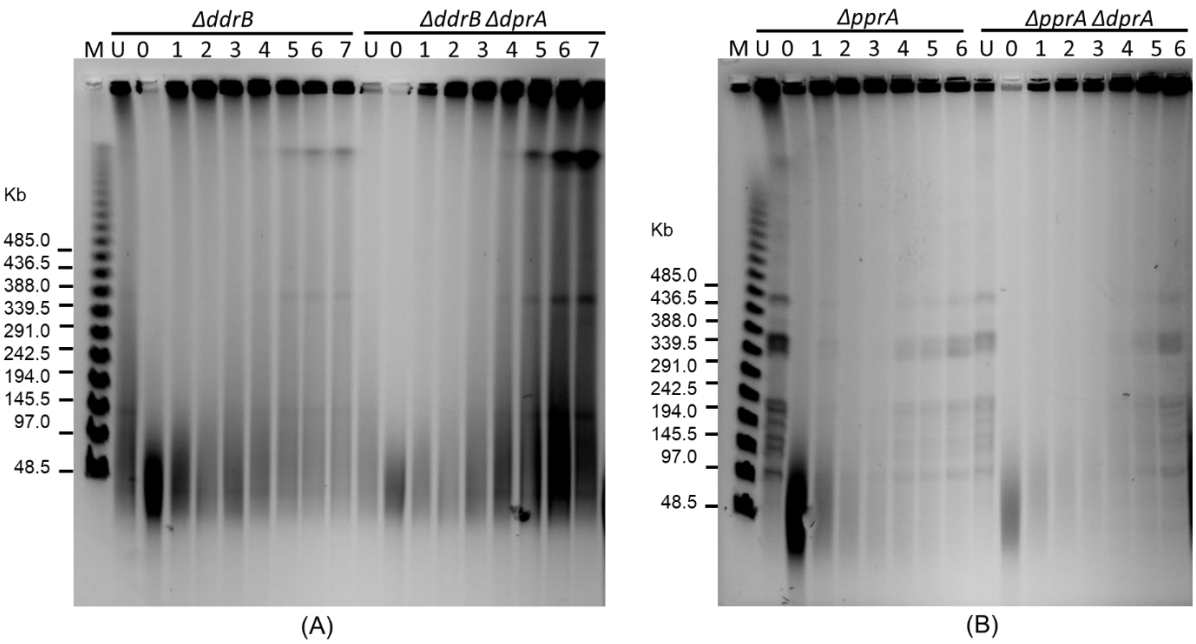


**Fig. S3: The DNA double-strand breaks (DSBs) repair kinetics of *D. radiodurans* wild-type and its mutants.**

PFGE was employed to assess the repair kinetics of DSBs. Panel (A) presents the repair kinetics of DSBs in *ΔddrB* and *ΔddrB ΔdprA* mutants, while panel (B) displays the repair kinetics of DSBs in *ΔpprA* and *ΔpprA ΔdprA* mutants. The NotI-digested DNA samples obtained from unirradiated cells (U) and cells irradiated with 6 kGy at different post-irradiation time points (PIR) were visualized on the gel immediately after irradiation (0) and at specific incubation times (in hours). Lambda PFG molecular mass standards were loaded in lane-M for size reference. The data suggests that the *ΔddrB ΔdprA* mutant exhibits relatively improved DSB repair compared to the *ΔddrB* mutant, while the *ΔpprA ΔdprA* mutant shows weaker DSB repair compared to the *ΔpprA* mutant.

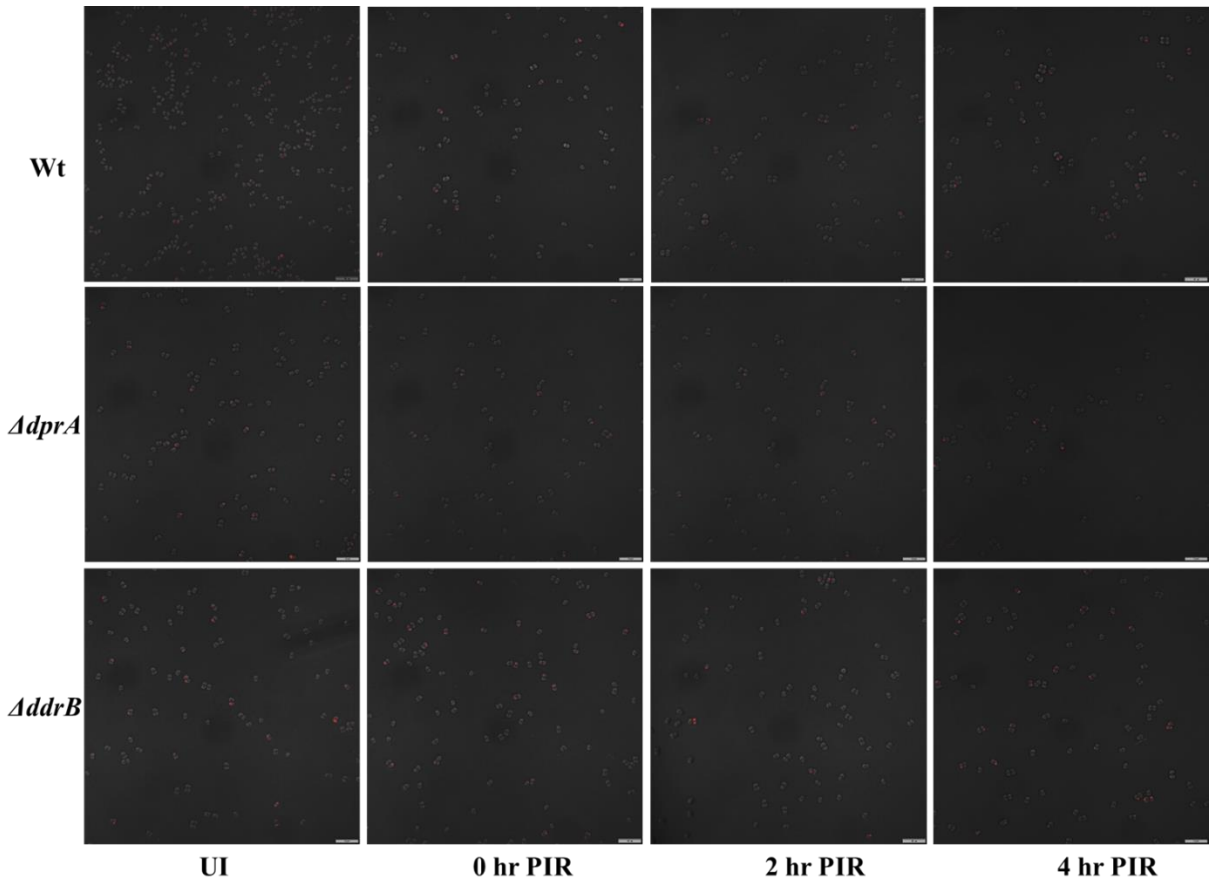


**Fig. S4 RecA$^{RFP}$ foci formation in wild type, *ΔdprA* and *ΔddrB* mutants.**

For fixed-cell imaging, *D. radiodurans* cells expressing pRADrecA-RFP were fixed and observed using Olympus FV3000 confocal microscope, specifically. These cells were examined in the RFP channel (561 nm) and under bright field (DIC) to determine the localization of RecA$^{RFP}$. Image analysis was performed using automated cellSens software. The scale bar indicates a length of 10 µm.

**Table S1: Statistical analysis of RecA$^{RFP}$ foci formation in wild-type, *ΔddrB*, and *ΔdprA* mutants**

| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant? | Summary | Adjusted P Value |
|---|---|---|---|---|---|
| Wt vs. *ΔddrB* | 51.72 | 27.12 to 76.32 | Yes | ** | 0.0064 |
| Wt vs. *ΔdprA* | 52.17 | 27.58 to 76.77 | Yes | ** | 0.0062 |
| *ΔddrB* vs. *ΔdprA* | 0.4550 | -24.14 to 25.05 | No | ns | 0.9967 |

**Table S2: Bacterial strains, plasmids, and primers used in this study**

| **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*$I^{q}$ ZΔM15::T*n*10] ($Tet^{R}$) | Lab stock |
| Δ*pprA* | *D. radiodurans* | *D. radiodurans* R1 *pprA Ω chl* | Prof. Issay Narumi lab |
| *ΔddrA* | *D. radiodurans* | *D. radiodurans* R1 *ddrA Ω chl* | Prof. Pascale Servant lab |
| *ΔddrB* | *D. radiodurans* | *D. radiodurans* R1 *ddrB Ω kan* | Prof. Pascale Servant lab |
| *ΔddrA ΔddrB* | *D. radiodurans* | *D. radiodurans* R1 *ddrA Ω chl , ddrB Ω kan* | Prof. Pascale Servant lab |
| *ΔpprA ΔdprA* | *D. radiodurans* | *D. radiodurans* R1 *pprA Ω chl, dprA Ω spec* | This work |
| Δ*dprA* | *D. radiodurans* | *D. radiodurans* R1 *dprA Ω spec* | This work |
| *ΔddrA ΔdprA* | *D. radiodurans* | *D. radiodurans* R1 *ddrA Ω chl, dprA Ω spec* | This work |
| *ΔddrB ΔdprA* | *D. radiodurans* | *D. radiodurans* R1 *ddrB Ω kan, dprA Ω spec* | This work |
| *ΔddrA ΔddrB ΔdprA* | *D. radiodurans* | *D. radiodurans* R1 *ddrA Ω chl, ddrB Ω kan, dprA Ω spec* | This work |
| *D. radiodurans (thy-)* | *D. radiodurans* | *D. radiodurans* R1 *thy-* | This work |
| *ΔdprA (thy-)* | *D. radiodurans* | *D. radiodurans* R1 *dprA Ω spec, thy-* | This work |
| *ΔpprA (thy-)* | *D. radiodurans* | *D. radiodurans* R1 *pprA Ω chl , thy-* | This work |
| *ΔpprA ΔdprA (thy-)* | *D. radiodurans* | *D. radiodurans* R1 *pprA Ω chl, dprA Ω spec, thy-* | This work |
| *ΔddrB (thy-)* | *D. radiodurans* | *D. radiodurans* R1 *ddrB Ω kan, thy-* | This work |
| *ΔddrB ΔdprA (thy-)* | *D. radiodurans* | *D. radiodurans* R1 *ddrB Ω kan, dprA Ω spec, thy-* | This work |
| **Plasmids:** | | | |
| **Names** | **Characteristics and Source** | | lab stock |
| | | | |
| pRADgro | pRAD1 carrying 261bp *Bgl*II-*Xba*I fragment of promoter (Pgro) from *D. radiodurans* | | lab stock |
| pDSRED-*recA* | pDSRED carrying dr*recA* at *BamHI* and *KpnI* | | [64] |
| pRAD*recA*-RFP | pRAD carrying dr*recA* at *ApaI* and *EcoRV* | | [64] |
| pNOS*dprA* | pNOS suicidal vector carrying upstream and downstream DNA fragment of *dprA* gene (*dr_0120*) | | This work |
| **Primer details** | | | |
| **Sl. No.** | **Name of primer and Sequence (5' to 3')** | | **Purpose** |
| 1. | *dprA*-UF (ATA GGT ACC TAA GCG CCT ATC AAG CCC TC) | | *ΔdprA* mutant generation |
| 2. | *dprA*-UR (AAA GGG ATG CCG CCG CAG GTT TTC GAT) | | |
| 3. | *dprA*-DF (ATA GGA TCC GAA CTG AAC AAG GCG GCA GA) | | |
| 4. | *dprA*-DR (TAG TCT AGA ACG CGG CAA CAG AGA GAA GTC) | | |