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## Supplemental Information

# DNA ADP-Ribosylation Stalls Replication and Is Reversed by RecF-Mediated Homologous Recombination and Nucleotide Excision Repair

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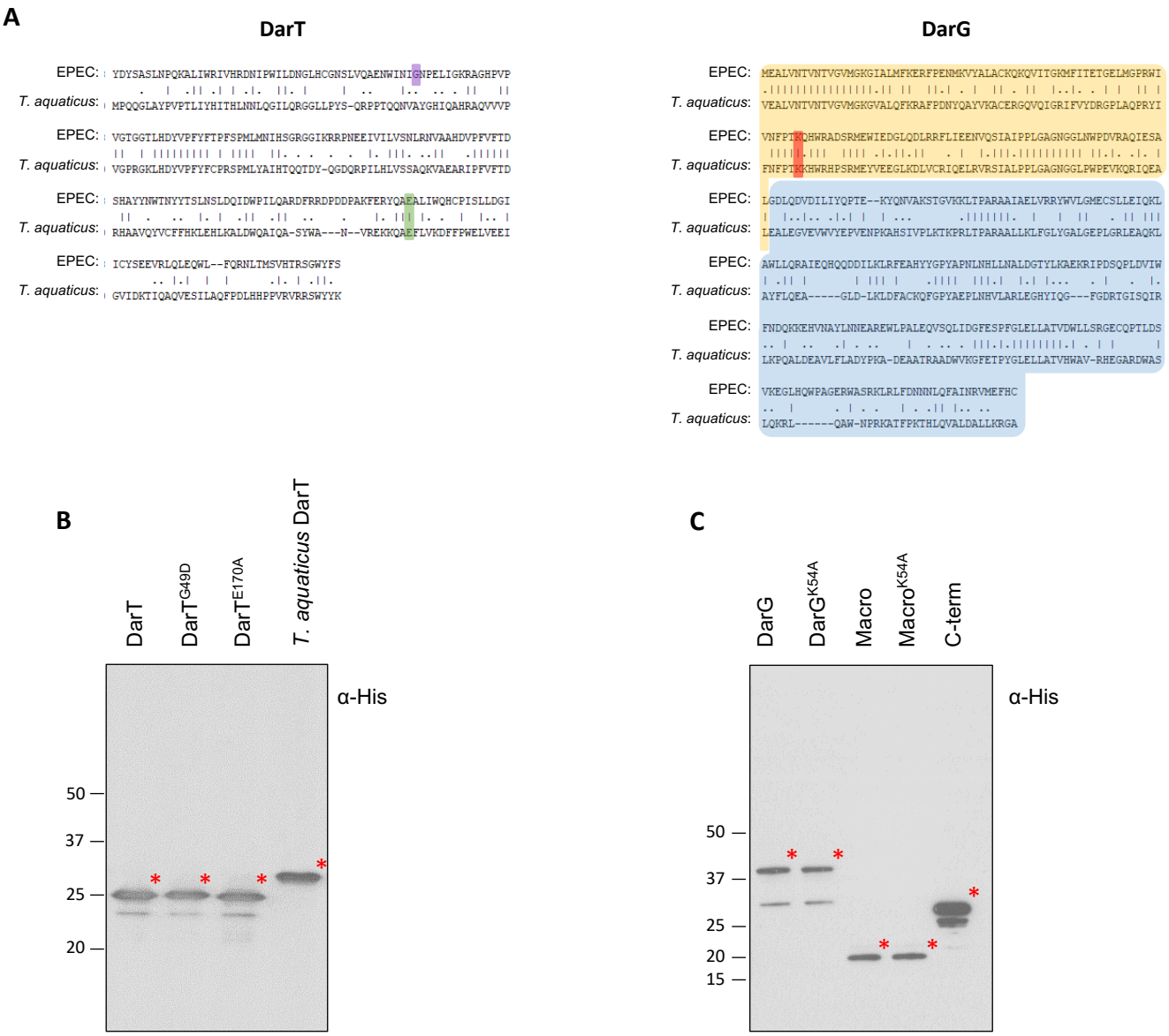



**Figure S1: Alignment of DarTG from *T. aquaticus* and EPEC, and the corresponding purified proteins after *in vitro* transcription/translation**, related to Figures 1 and 2. **(A)** The amino acid similarity between *T. aquaticus* and EPEC DarT or DarG is 47% and 59%, respectively. Identical residues are shown with a line, similar residues are shown with a dot. The mutated glycine in EPEC DarT (G49D) is shown in purple while the mutated glutamic acid residues (DarT$^{E160A}$ in *T. aquaticus* and DarT$^{E170A}$ in EPEC) are marked in green. The macrodomain of DarG is highlighted in yellow while the C-terminal domain of DarG is showed in blue. The catalytic lysine residues of DarG (K80A in *T. aquaticus* and K54A in EPEC) are marked in red. Alignment was performed by Clustal O with JalView software. Levels of His-tagged **(B)** DarT and **(C)** DarG variants (indicated with red asterisks) obtained after *in vitro* transcription/translation determined by Western blot analysis using an anti-His antibody.

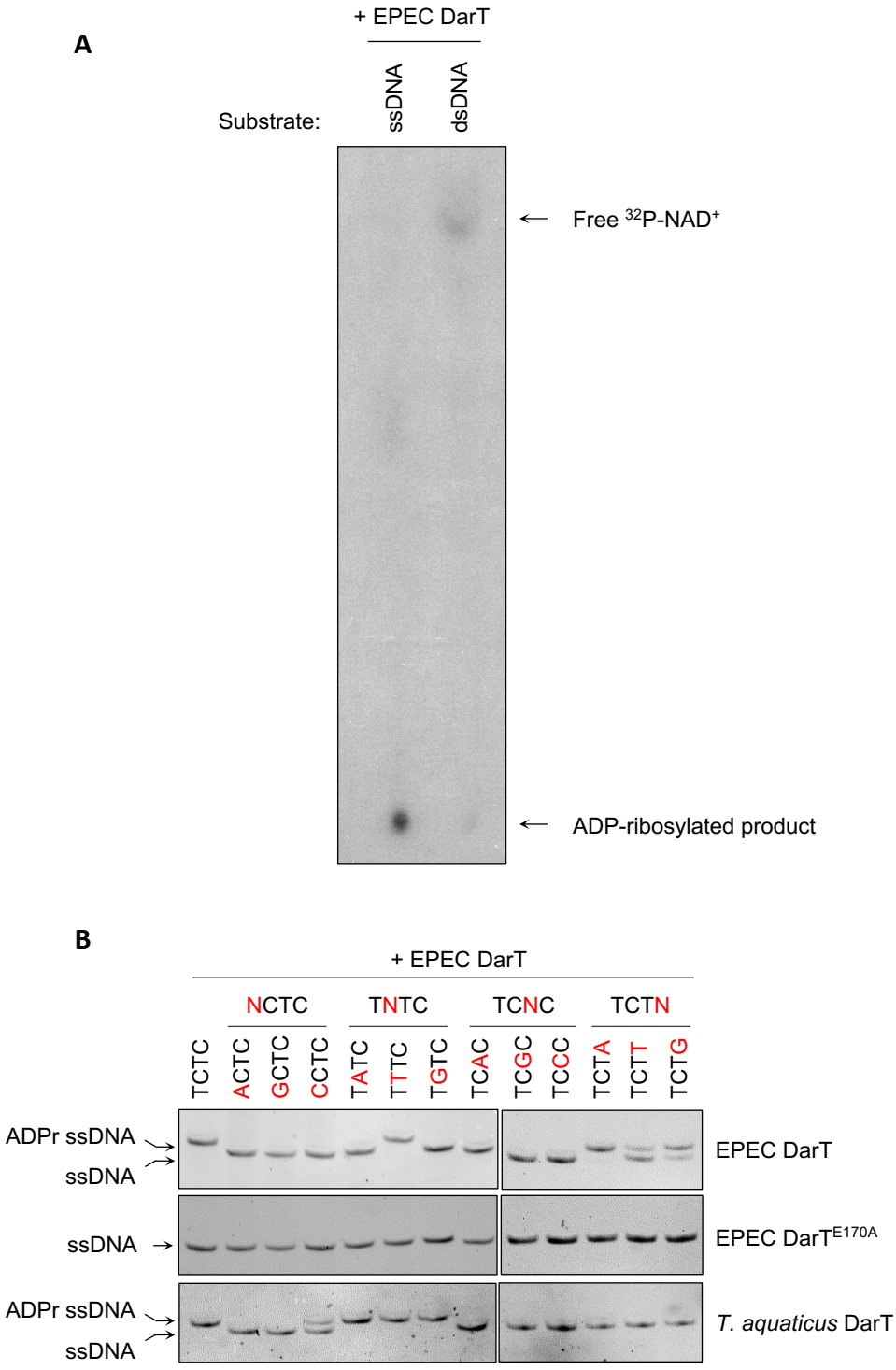



**Figure S2: EPEC DarT only ADP-ribosylates ssDNA, on a different DNA sequence than *T. aquaticus* DarT**, related to Figure 1. **(A)** ADP-ribosylation of 13-mer ssDNA or dsDNA oligonucleotides incubated with EPEC DarT with $^{32P}$NAD$^{+}$ and separated by thin-layer chromatography. Sequences of the oligonucleotides are provided in the Star methods. **(B)** ADP-ribosylation of 27-mer ssDNA oligonucleotides incubated with purified EPEC DarT or DarT$^{E170A}$ or *T. aquaticus* DarT. The region with different sequence is shown above each lane. ADPr ssDNA: ADP-ribosylated ssDNA. All oligonucleotides are listed in Table S1. n = 3, representative data from one experiment shown.

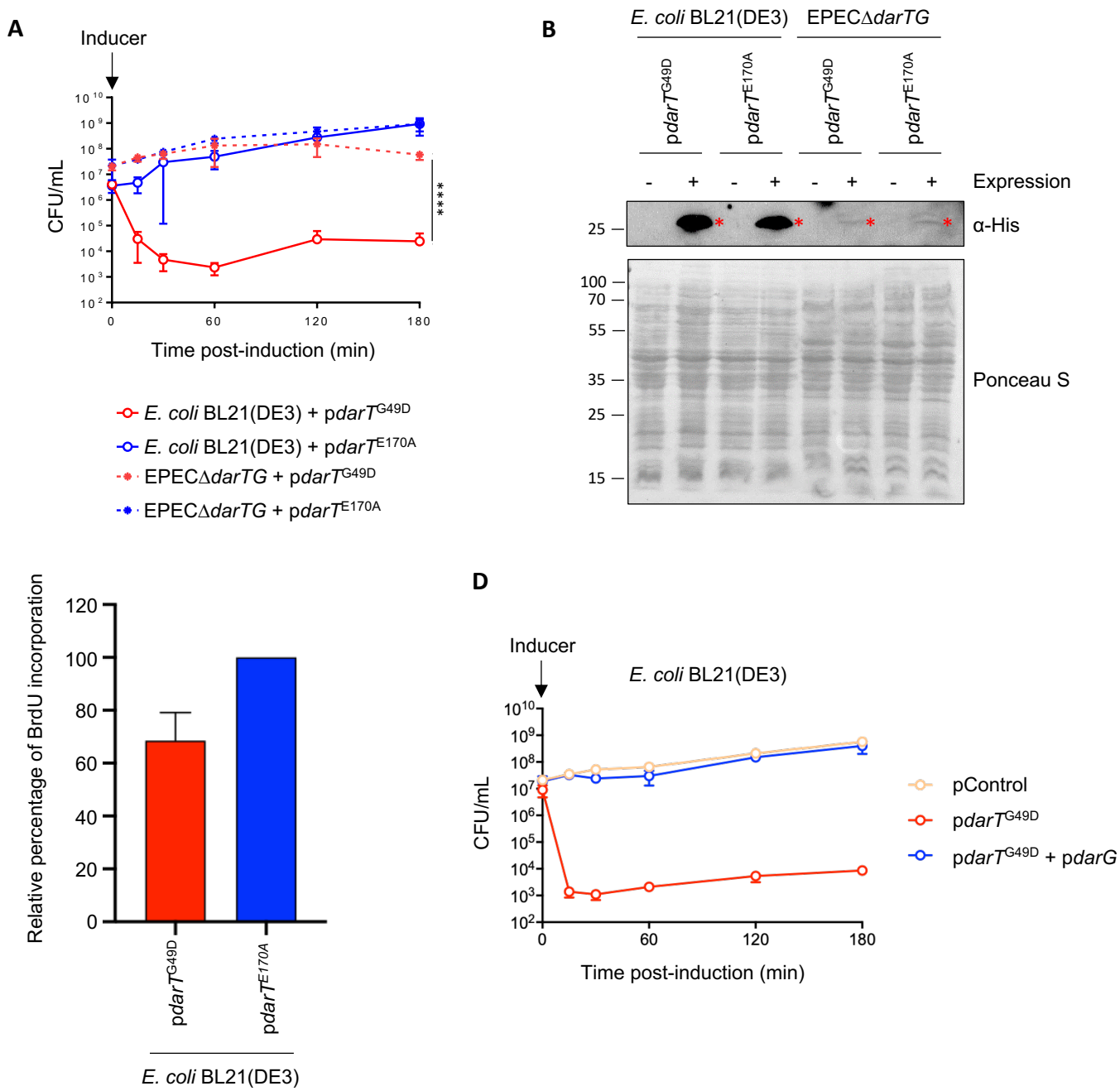



**Figure S3: Differential DarT$^{G49D}$/DarT$^{E170A}$ toxicity and expression levels in *E. coli* BL21(DE3) and EPEC, where DarT$^{G49D}$ inhibits DNA replication in *E. coli* BL21(DE3) within 5 min of expression**, related to Figures 1 and 5. **(A)** Viability of *E. coli* BL21(DE3) and EPECΔ*darTG* following expression of *darT*$^{G49D}$ or *darT*$^{E170A}$. n = 3 ± s. d., **** p < 0.0001 by two-way ANOVA between *E.coli* BL21(DE3) + p*darT*$^{G49D}$ and EPECΔ*darTG* + p*darT*$^{G49D}$. **(B)** Levels of His-tagged DarT$^{G49D}$ or DarT$^{E170A}$ (red asterisks) in cell lysates of *E. coli* BL21(DE3) and EPECΔ*darTG* using an anti-His antibody. Expression of His-DarT$^{G49D}$/His-DarT$^{E170A}$ is repressed by addition of 0.8% glucose or expressed by the addition 0.8% arabinose for 3 hrs. Ponceau S staining is shown as the loading control. n = 3, representative data from one experiment shown. **(C)** Relative values were obtained by calculating the ratio of the amount of BrdU incorporated following expression of *darT*$^{G49D}$ vs. *darT*$^{E170A}$. n = 2 ± s. d. **(D)** Viability of *E. coli* BL21(DE3) grown in M9 medium following expression of *darT*$^{G49D}$ with or without DarG. pControl, empty plasmid; n = 2 ± s. d.

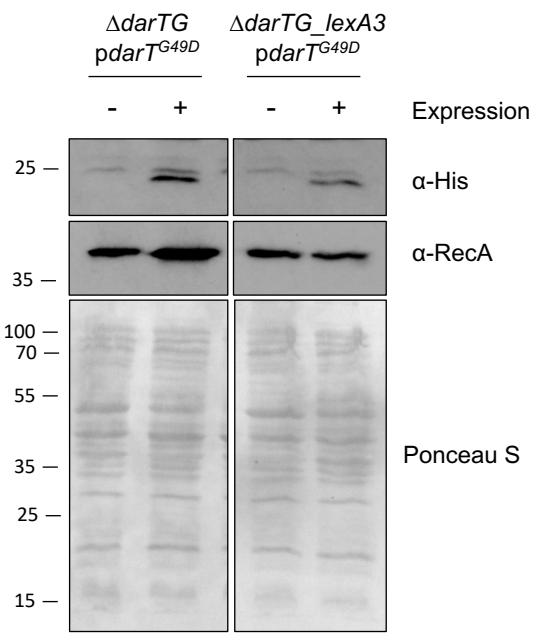
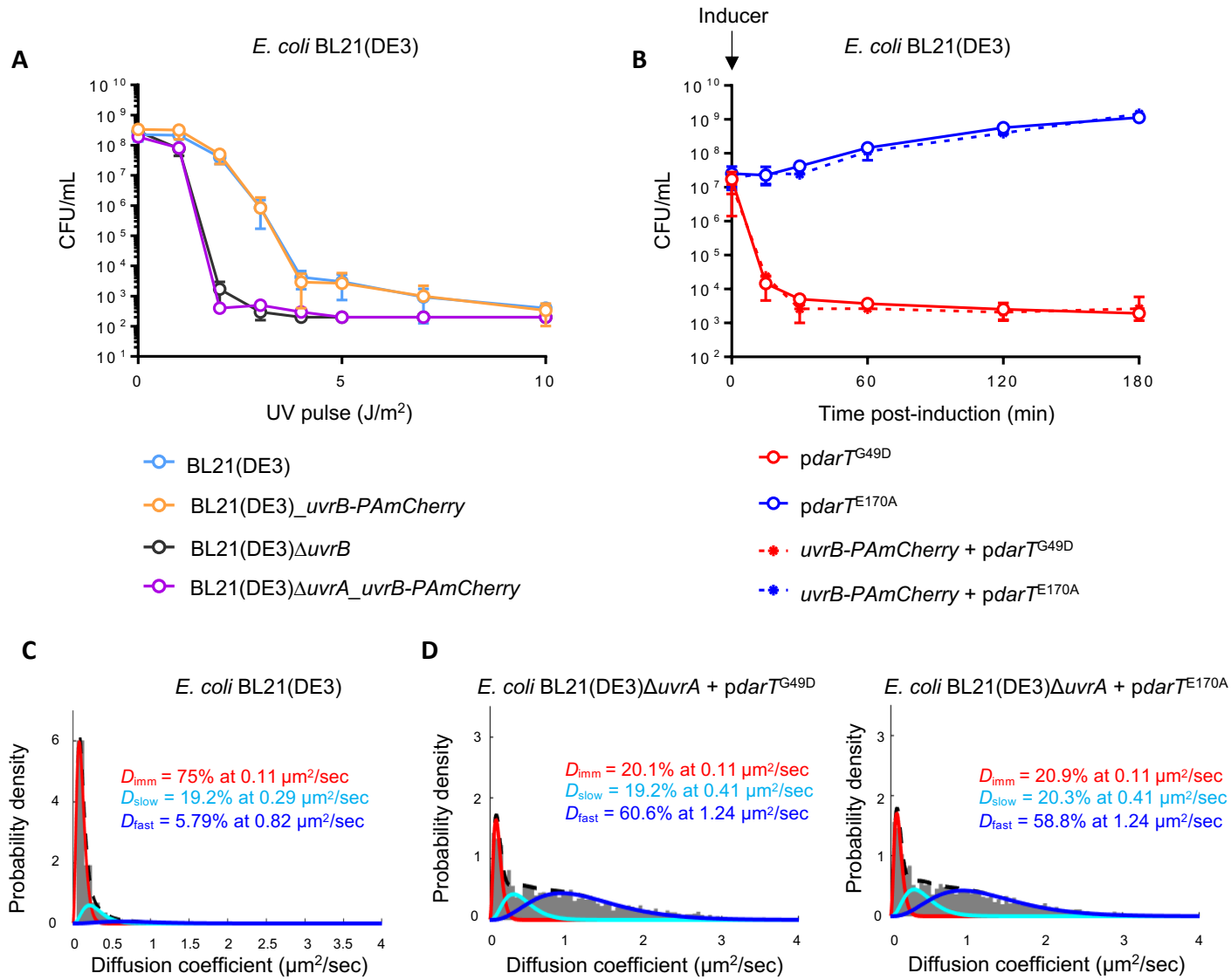



**Figure S4: Cellular RecA levels do not increase in the absence of an SOS response**, related to Figure 3. Western blot analysis of RecA expression levels in cell lysates of EPECΔ*darTG* and EPECΔ*darTG_lexA3* using an anti-His (to detect levels of His tagged DarT$^{G49D}$ or DarT$^{E170A}$) and anti-RecA antibody after 3 hours of *darT*$^{G49D}$ expression. Ponceau S staining is shown as the loading control. n = 3, representative data from one experiment shown.







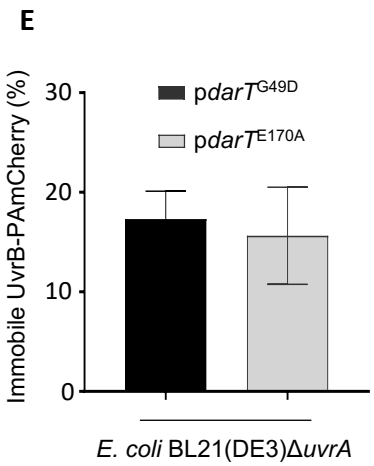



**Figure S5: UvrB fused to PAmCherry is functional, does not affect bacterial viability following UV exposure and UvrB-PAmCherry recruitment does not occur in a Δ*uvrA* strain**, related to Figure 5. **(A)** Viability of *E. coli* BL21(DE3), *E. coli* BL21(DE3)*_uvrB-PAmCherry*, *E. coli* BL21(DE3)Δ*uvrB* and *E. coli* BL21(DE3)Δ*uvrA_uvrB-PAmCherry* after UV exposure. n = 3 ± s. d. **(B)** Viability of *E. coli* BL21(DE3) and *E. coli* BL21(DE3)*_uvrB-PAmCherry* following expression of $darT^{G49D}$ or $darT^{E170A}$. n = 3 ± s. d. **(C)** To determine the immobile D*, cells were fixed with 3% paraformaldehyde to crosslink UvrB-PAmCherry molecules to DNA (and so render them immobile). Using unconstrained fitting, the calculated immobile D* of UvrBPAmCherry is 0.11 μm2/sec. This value was used as a constraint in the fit to measure the percentage of immobile UvrBPAmCherrymolecules in live cells with all three species (immobile, slow, fast) present. **(D)** Determination of D* values of UvrB-PAmCherry in *E. coli* BL21(DE3)Δ*uvrA*, fitted with a three constrained species model following 15 min expression of $darT^{G49D}$ or $darT^{E170A}$. **(E)** Percentage of immobile UvrB-PAmCherry in *E. coli* BL21(DE3)Δ*uvrA* after 15 min expression of $darT^{G49D}$ or $darT^{E170A}$. n = 2 biological replicates ± s. d.; total number of cells = 3,698; total number of tracks = 38,918.

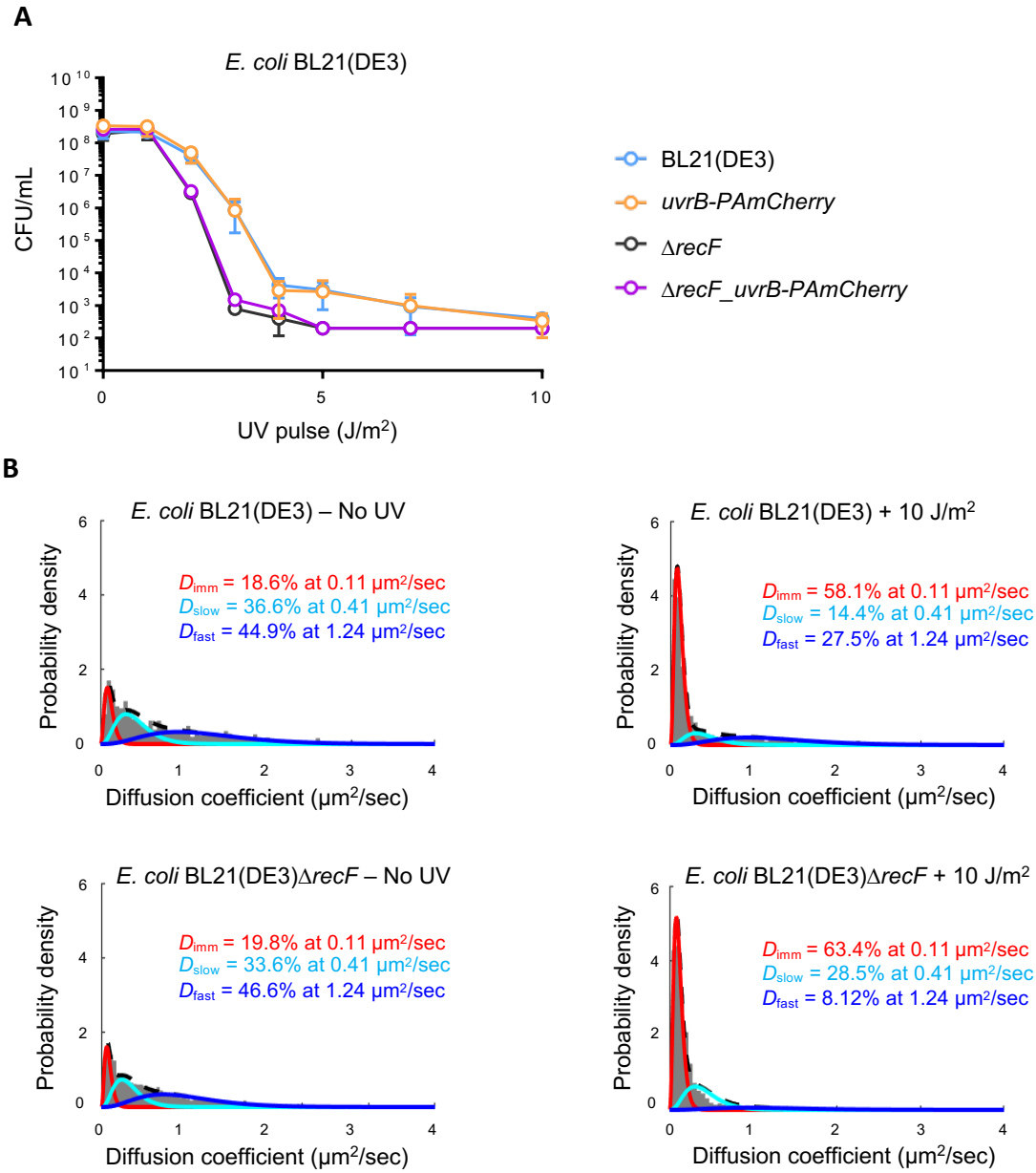



**Figure S6: NER is functional in a Δ*recF* strain,** related to Figure 6. **(A)** Viability of BL21(DE3), BL21(DE3)_*uvrB-PAmCherry*, BL21(DE3)Δ*recF* and BL21(DE3)Δ*recF*_*uvrB-PAmCherry* after exposure to different UV doses. n = 3 ± s. d. **(B)** Determination of D* values of UvrB-PAmCherry in *E. coli* BL21(DE3) and BL21(DE3)Δ*recF*, fitted with a three species model (three constraints fit) with or without UV exposure. **(C)** Percentage of immobile UvrB-PAmCherry in *E. coli* BL21(DE3) and *E. coli* BL21(DE3)Δ*recF* after UV exposure. n = 3 biological replicates ± s. d.; total number of cells = 12,976; total number of tracks = 126,225.

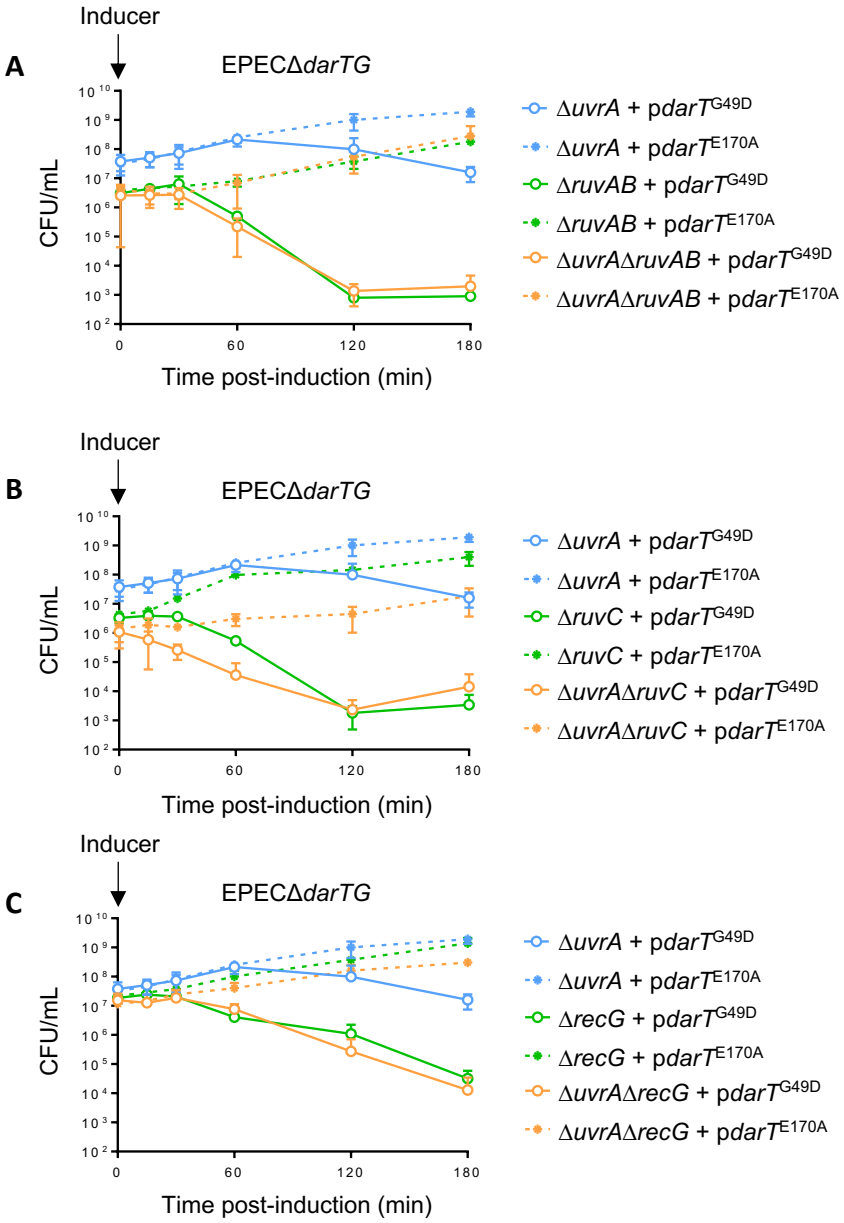



**Figure S7: Investigation of the sequentiality between RecF-mediated HR and NER pathways**, related to Figure 6. Viability of strains following expression of *darT*$^{G49D}$ or *darT*$^{E170A}$ in EPECΔ*darTG* lacking RecF-mediated HR and/or NER **(A)** EPECΔ*darTG*Δ*ruvAB* and EPECΔ*darTG*Δ*uvrA*Δ*ruvAB*, **(B)** EPECΔ*darTG*Δ*ruvC* and EPECΔ*darTG*Δ*uvrA*Δ*ruvC*, **(C)** EPECΔ*darTG*Δ*recG* and EPECΔ*darTG*Δ*uvrA*Δ*recG*. n = 3 ± s. d. NS = p > 0.9999 by two-way ANOVA between EPECΔ*darTG*Δ*ruvAB*/Δ*ruvC*/Δ*recG* + p*darT*$^{G49D}$ and EPECΔ*darTG*Δ*uvrA*Δ*ruvAB*/Δ*ruvC*/Δ*recG* + p*darT*$^{G49D}$.

| Name | Sequence |
|---|---|
| ADPr-EL1 | GAGCTGTACAAGTCAGATCTCGAGCTC |
| ADPr-EL2 | GAGCTGTACAAGTCAGAACTCGAGCTC |
| ADPr-EL3 | GAGCTGTACAAGTCAGAGCTCGAGCTC |
| ADPr-EL4 | GAGCTGTACAAGTCAGACCTCGAGCTC |
| ADPr-EL5 | GAGCTGTACAAGTCAGATATCGAGCTC |
| ADPr-EL6 | GAGCTGTACAAGTCAGATTTCGAGCTC |
| ADPr-EL7 | GAGCTGTACAAGTCAGATGTCGAGCTC |
| ADPr-EL8 | GAGCTGTACAAGTCAGATCACGAGCTC |
| ADPr-EL9 | GAGCTGTACAAGTCAGATCGCGAGCTC |
| ADPr-EL10 | GAGCTGTACAAGTCAGATCCCGAGCTC |
| ADPr-EL11 | GAGCTGTACAAGTCAGATCTAGAGCTC |
| ADPr-EL12 | GAGCTGTACAAGTCAGATCTTGAGCTC |
| ADPr-EL13 | GAGCTGTACAAGTCAGATCTGGAGCTC |
| ADPr-EL14 | GAGCTGTACAAGTCAGTTCTCGAGCTC |
| ADPr-EL15 | GAGCTGTACAAGTCAGGTCTCGAGCTC |
| ADPr-EL16 | GAGCTGTACAAGTCAGCTCTCGAGCTC |
| ADPr-EL17 | GAGCTGTACAAGTCAGATCTCTAGCTC |
| ADPr-EL18 | GAGCTGTACAAGTCAGATCTCGAGCTC |
| ADPr-EL19 | GAGCTGTACAAGTCAGATCTCCAGCTC |
| ADPr-EL20 | CTCGACATGATCAGTCGTCTCCTCGAG |
| ADPr-EL21 | CTCGACATGATCAGTCGTTTCCTCGAG |

**Table S1**, related to STAR method**:** Oligonucleotides used for ADP-ribosylation assays

| Name | Strain | Relevant genotype or description | Reference |
|---|---|---|---|
| EL131 | Dh5α | *fhuA*2 Δ(*argF-lacZ*) *U169 phoA glnV44* Φ*80* Δ(*lacZ*)*M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17* | (Hanahan, 1983) |
| EL183 | SM10 | *thi thr leu tonA lacy supE recA::RP4-2-Tc::Mu* λpir; *kan*$^R$ | (Simon, 1983) |
| EL615 | *E. coli* λpir | λpir Δ*dapA* Δ*recA; apra*$^R$ *erm*$^R$ *zeo*$^R$ | (Herrero et al., 1990) |
| EL270 | BTH101 | *F- cya-99 araD139 galE15 galK16 rpsL1 (Strr) hsdR2 mcrA1 mcrB1* | (Karimova et al., 2000) |
| EL67 | BL21(DE3) | *E. coli* expression strain: F$^-$ *omp*T *hsd*S($r_B^-$ $m_B^-$) *dcm*$^+$ Tet$^r$ *gal* λ(DE3) *end*A [*arg*U *pro*L] | Stratagene |
| EL572 | BL21(DE3)Δ*recF* | BL21(DE3)Δ*recF* | This study |
| EL646 | BL21(DE3)Δ*uvrA_uvrB-PAmCherry* | BL21(DE3)Δ*uvrA* with chromosomal *uvrB* fused to PAmCherry; Kan$^R$ | This study |
| EL653 | BL21(DE3)Δ*uvrB* | BL21(DE3)Δ*uvrB* | This study |
| EL325 | BL21(DE3)*_uvrB-PAmCherry* | BL21(DE3) with chromosomal *uvrB* fused to PAmCherry; Kan$^R$ | This study |
| EL614 | BL21(DE3)Δ*recF_uvrB-PAmCherry* | BL21(DE3)Δ*recF* with chromosomal *uvrB* fused to PAmCherry; *kan*$^R$ | This study |
| EL1 | EPEC | Enteropathogenic *E. coli* isolate O127:H6 str. E2348/69; Str$^R$ | (Knutton et al., 1987) |
| EL2 | EPECΔ*darTG* | EPEC E2348/69Δ*darTG*; Str$^R$ | This study |
| EL268 | EPECΔ*darTG*Δ*dinB* | EPEC E2348/69Δ*darTG*Δ*dinB*; Str$^R$ | This study |
| EL326 | EPECΔ*darTG*Δ*dinB*Δ*polB*Δ*umuD* | EPEC E2348/69Δ*darTG*Δ*dinB*Δ*polB*Δ*umuD*; Str$^R$ | This study |
| EL377 | EPECΔ*darTG*Δ*fpg* | EPEC E2348/69Δ*darTG*Δ*fpg*; Str$^R$ | This study |
| EL415 | EPECΔ*darTG_lexA3* | EPEC E2348/69 containing a point mutation (G85D) in chromosomal *lexA*; Str$^R$ | This study |
| EL226 | EPECΔ*darTG*Δ*mutH* | EPEC E2348/69Δ*darTG*Δ*mutH*; Str$^R$ | This study |
| EL228 | EPECΔ*darTG*Δ*mutS* | EPEC E2348/69Δ*darTG*Δ*mutS*; Str$^R$ | This study |
| EL378 | EPECΔ*darTG*Δ*nei* | EPEC E2348/69Δ*darTG*Δ*nei*; Str$^R$ | This study |
| EL379 | EPECΔ*darTG*Δ*nfo* | EPEC E2348/69Δ*darTG*Δ*nfo*; Str$^R$ | This study |
| EL269 | EPECΔ*darTG*Δ*polB* | EPEC E2348/69Δ*darTG*Δ*polB*; Str$^R$ | This study |
| EL203 | EPECΔ*darTG*Δ*recA* | EPEC E2348/69Δ*darTG*Δ*recA*; Str$^R$ | This study |
| EL588 | EPECΔ*darTG*Δ*recD* | EPEC E2348/69Δ*darTG*Δ*recD*; Str$^R$ | This study |
| EL526 | EPECΔ*darTG*Δ*recF* | EPEC E2348/69Δ*darTG*Δ*recF*; Str$^R$ | This study |
| EL527 | EPECΔ*darTG*Δ*recG* | EPEC E2348/69Δ*darTG*Δ*recG*; Str$^R$ | This study |
| EL468 | EPECΔ*darTG*Δ*ruvAB* | EPEC E2348/69Δ*darTG*Δ*ruvAB*; Str$^R$ | This study |
| EL528 | EPECΔ*darTG*Δ*ruvC* | EPEC E2348/69Δ*darTG*Δ*ruvC*; Str$^R$ | This study |
| EL307 | EPECΔ*darTG*Δ*umuD* | EPEC E2348/69Δ*darTG*Δ*umuD*; Str$^R$ | This study |
| EL224 | EPECΔ*darTG*Δ*uvrA* | EPEC E2348/69Δ*darTG*Δ*uvrA*; Str$^R$ | This study |
| EL530 | EPECΔ*darTG*Δ*uvrA*Δ*recF* | EPEC E2348/69Δ*darTG*Δ*uvrA*Δ*recF*; Str$^R$ | This study |
| EL531 | EPECΔ*darTG*Δ*uvrA*Δ*recG* | EPEC E2348/69Δ*darTG*Δ*uvrA*Δ*recG*; Str$^R$ | This study |
| EL533 | EPECΔ*darTG*Δ*uvrA*Δ*ruvAB* | EPEC E2348/69Δ*darTG*Δ*uvrA*Δ*ruvAB*; Str$^R$ | This study |
| EL532 | EPECΔ*darTG*Δ*uvrA*Δ*ruvC* | EPEC E2348/69Δ*darTG*Δ*uvrA*Δ*ruvC*; Str$^R$ | This study |
| EL412 | EPECΔ*darTG*Δ*xth* | EPEC E2348/69Δ*darTG*Δ*xth*; Str$^R$ | This study |

**Table S2**, related to STAR method **:** Strains used in this study