# Supplementary Information file

**Single-molecule live-cell imaging visualizes parallel pathways of prokaryotic nucleotide excision repair**

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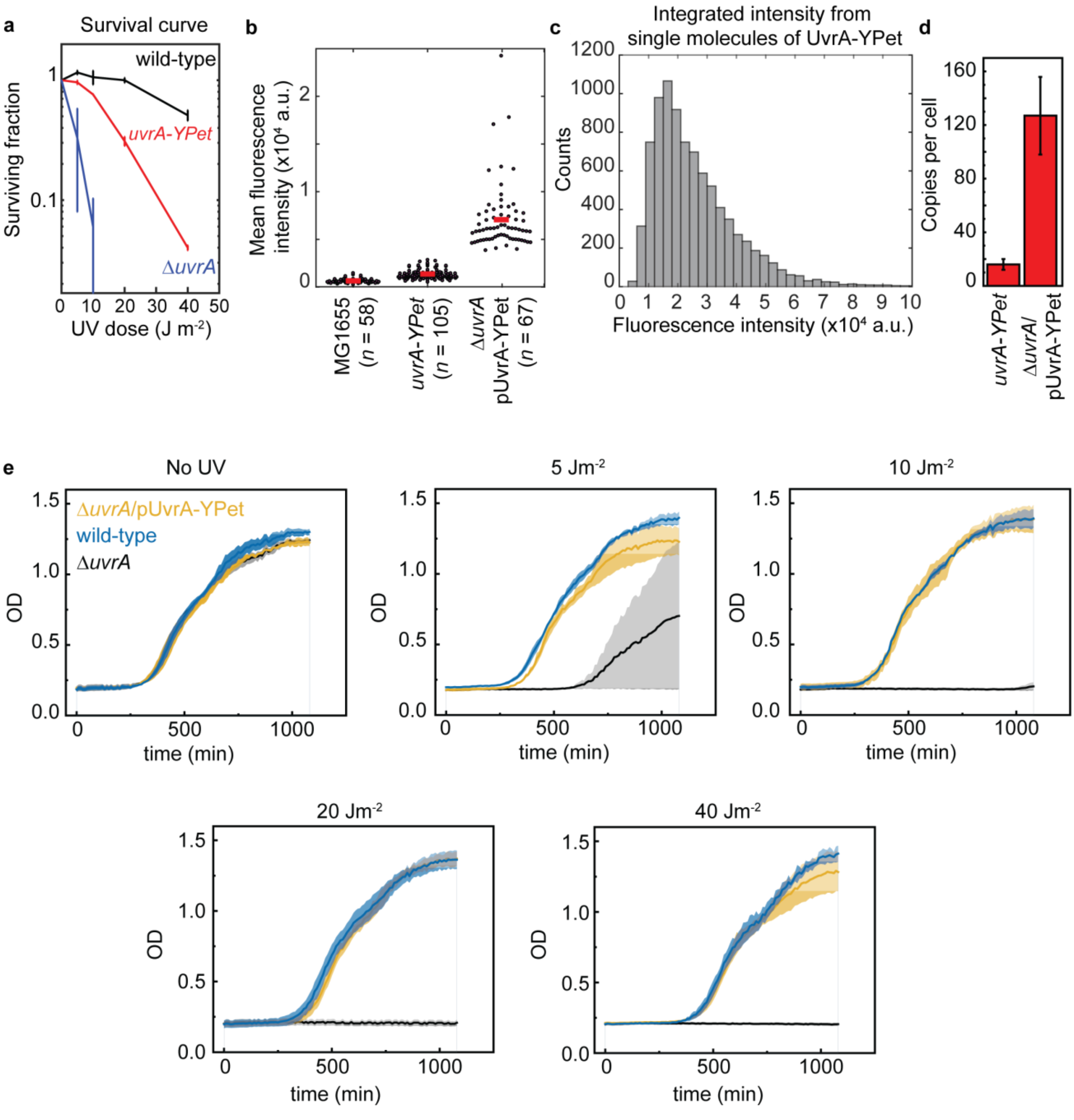
Supplementary References

## Supplementary Figures

### Supplementary Figure 1:



**Supplementary Figure 1:**
a. Survival assay showing survival of wild-type (black), *uvrA-YPet* (red) and Δ*uvrA* (blue) cells upon exposure to 20 $Jm^{-2}$ of 254-nm UV light. Error bars represent standard error of the mean from two independent experiments, each experiment involved three technical replicates.

b. Mean fluorescence intensity of wild-type MG1655, *uvrA-YPet* cells and cells carrying a low-copy plasmid expressing UvrA-YPet upon excitation with 514-nm light. Red line indicates mean of the distribution. *n*, number of cells.

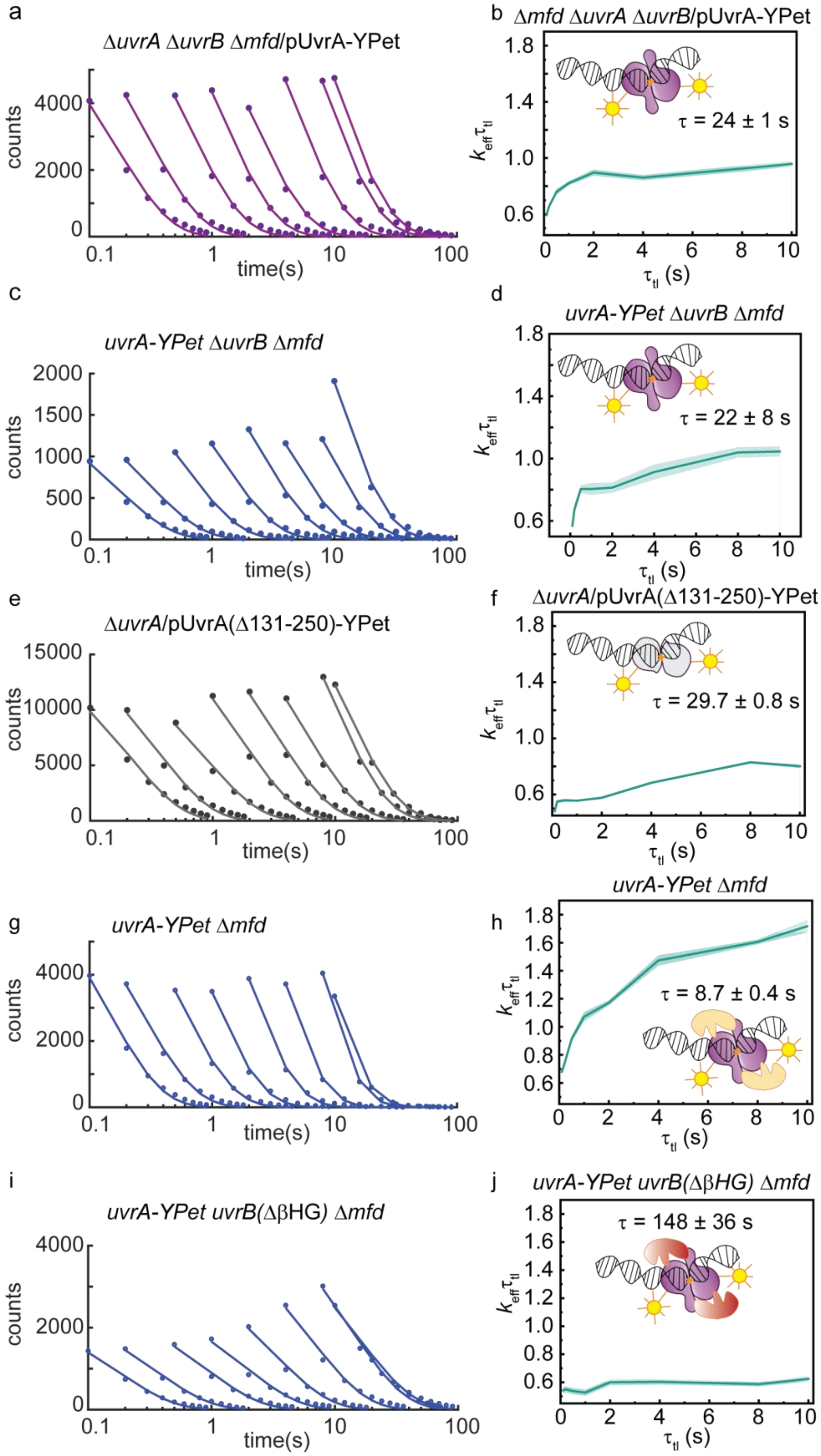
c. Histogram of integrated fluorescence intensities of single UvrA-YPet foci in *uvrA-YPet* cells upon excitation with 514-nm light.

d. Bar plots show mean copy number of UvrA-YPet measured in *uvrA-YPet* cells ($n$ = 105 cells) and Δ*uvrA*/pUvrA-YPet cells ($n$ = 67 cells). Error bars are standard deviations.

e. Growth curves (OD 600 nm; LB medium 30°C in a 96-well plate) of UV irradiated (0, 5, 10, 20 and 40 $Jm^{-2}$ of UVC radiation) of the Δ*uvrA*/pUvrA-YPet strain (yellow line) compared to Δ*uvrA*/pJM1071 (empty vector; black line) and wild-type cells carrying the pJM1071 vector (blue line) reveal that UvrA-YPet expressed from the plasmid complements a Δ*uvrA* phenotype. Solid lines represent average of two biological replicates, each performed in triplicate. Shaded areas represent standard error of mean of two biological replicates.

Source data are provided as a Source Data file.

**Supplementary Figure 2:**



**Supplementary Figure 2:**
a. Cumulative residence time distributions (CRTDs, circles) obtained from interval imaging of UvrA-YPet in Δ*uvrA* Δ*uvrB* Δ*mfd* cells. Lines are mono-exponential fits to CRTDs.

b. The $k_{eff}\,\tau_{tl}$ plot obtained from fitting CRTDs of UvrA-YPet in Δ*uvrA* Δ*uvrB* Δ*mfd* cells. Shaded error bands are standard deviations from ten bootstrapped samples. Cartoon (inset) illustrates UvrA-YPet (purple) in complex with DNA.

c. Cumulative residence time distributions (CRTDs, circles) obtained from interval imaging of UvrA-YPet in *uvrA-YPet* Δ*uvrB* Δ*mfd* cells. Lines are mono-exponential fits to CRTDs.

d. The $k_{eff}\,\tau_{tl}$ plot obtained from fitting CRTDs of UvrA-YPet in *uvrA-YPet* Δ*uvrB* Δ*mfd* cells. Shaded error bands are standard deviations from ten bootstrapped samples. Cartoon (inset) illustrates UvrA-YPet (purple) in complex with DNA.

e. CRTDs (circles) obtained from interval imaging of UvrA(Δ131-250)-YPet in Δ*uvrA* cells. Lines are mono-exponential fits to CRTDs.

f. The $k_{eff}\,\tau_{tl}$ plot obtained from fitting CRTDs of UvrA(Δ131-250)-YPet in Δ*uvrA* cells. Shaded error bands are standard deviations from ten bootstrapped samples. Cartoon (inset) illustrates UvrA(Δ131-250)-YPet (grey) in complex with DNA.

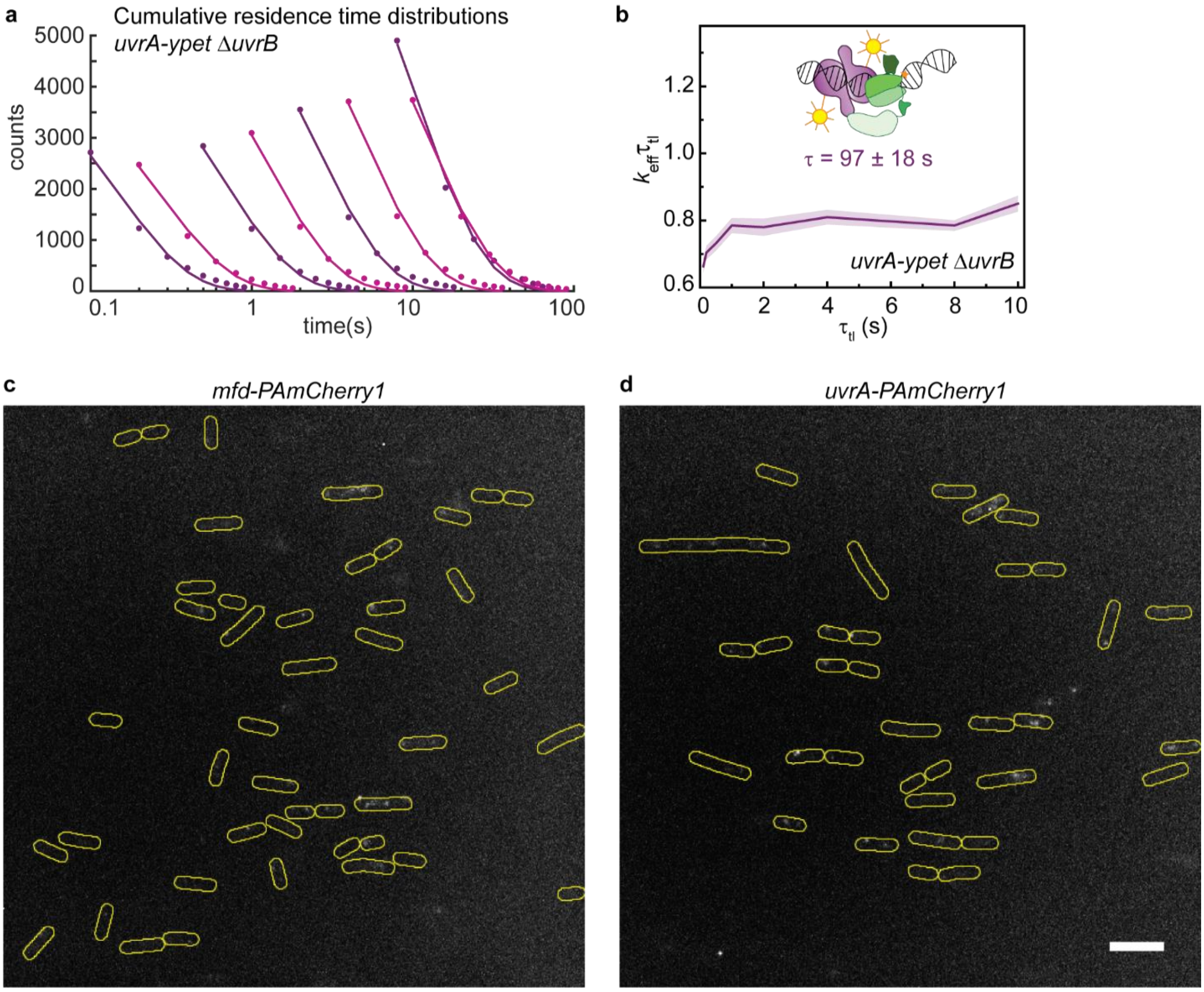
g. CRTDs (circles) obtained from interval imaging of UvrA-YPet in *uvrA-YPet* Δ*mfd* cells. Lines are mono-exponential fits to CRTDs.

h. The $k_{eff}\,\tau_{tl}$ plot obtained from fitting CRTDs of UvrA-YPet in *uvrA-YPet* Δ*mfd* cells. Shaded error bands are standard deviations from ten bootstrapped samples. Cartoon (inset) illustrates the complex formed by UvrA-YPet (purple) and UvrB (orange) with DNA.

i. CRTDs (circles) obtained from interval imaging of UvrA-YPet in *uvrA-YPet uvrB(ΔβHG)* Δ*mfd* cells. Lines are mono-exponential fits to CRTDs.

j. The $k_{eff}\,\tau_{tl}$ plot obtained from fitting CRTDs of UvrA-YPet in *uvrA-YPet uvrB(ΔβHG)* Δ*mfd* cells. Shaded error bands are standard deviations from ten bootstrapped samples. Cartoon (inset) illustrates the complex formed by UvrA-YPet (purple) and UvrB(ΔβHG) (red) with DNA.
Source data are provided as a Source Data file.

**Supplementary Figure 3:**



**Supplementary Figure 3:**
a. CRTDs (circles) obtained from interval imaging of UvrA-YPet in *uvrA-YPet* Δ*uvrB* cells. Lines are mono-exponential fits to CRTDs.

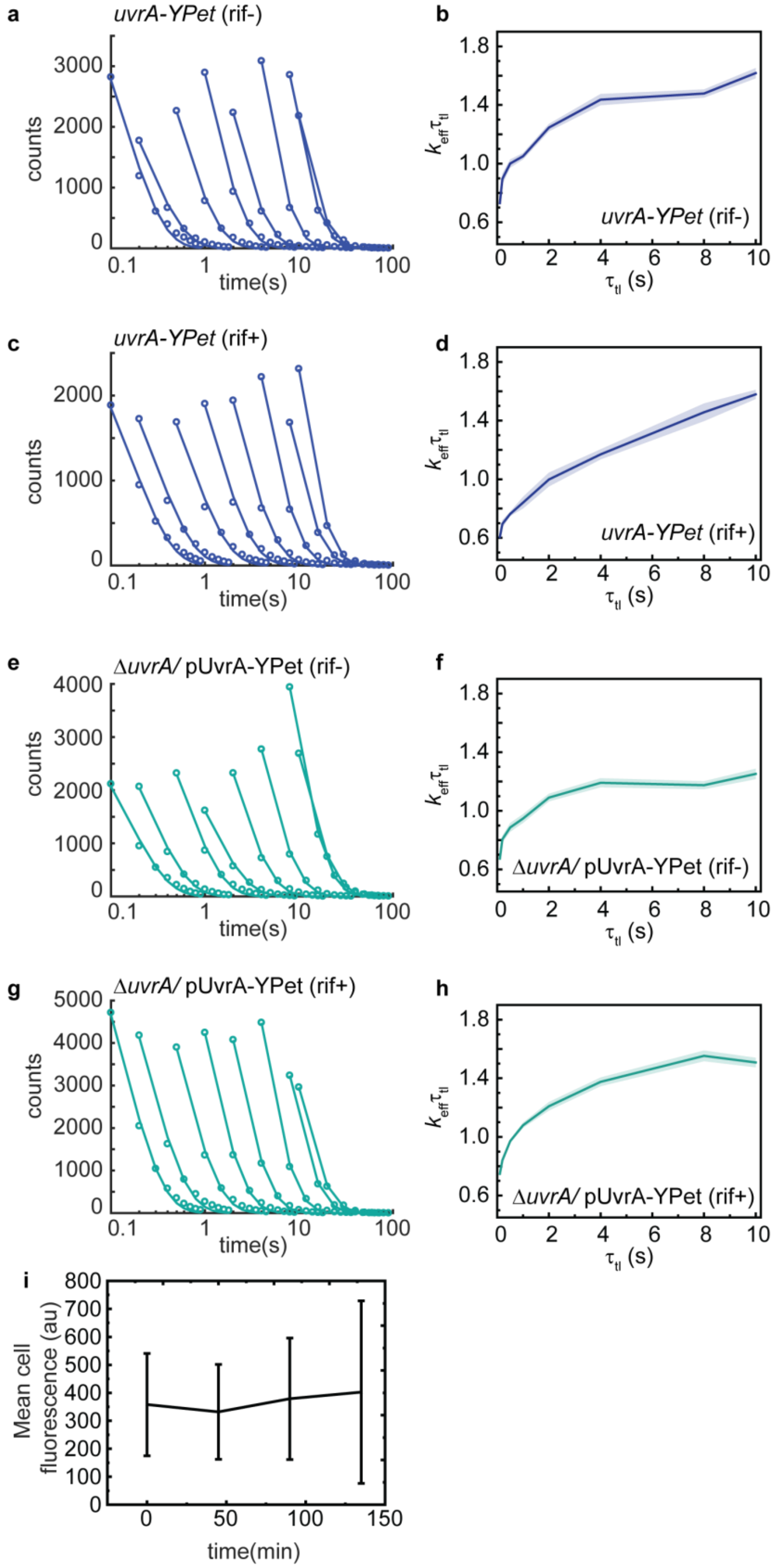
b. The $k_{eff}\,\tau_{tl}$ plot obtained from fitting CRTDs of UvrA-YPet in *uvrA-YPet* Δ*uvrB* cells. Shaded error bands are standard deviations from ten bootstrapped samples. Cartoon (inset) illustrates the arrested complex formed by UvrA-YPet (purple) and Mfd (green) in *uvrA-YPet* Δ*uvrB* cells.

c. Maximum intensity projection of *mfd-PAmCherry1* cells obtained upon exposure to 405-nm and 568-nm light.

d. Maximum intensity projection of *uvrA-PAmCherry1* cells obtained upon exposure to 405-nm and 568-nm light.

Note the significantly fewer localizations obtained in these strains compared to the YPet tagged constructs (Figure 2) and reference 1. Cell outlines (yellow) are provided as guide to the eye. Scale bar represents 5 μm. Source data are provided as a Source Data file.

**Supplementary Figure 4:**



**Supplementary Figure 4:**
a. CRTDs (circles) obtained from interval imaging of UvrA-YPet in *uvrA-YPet* cells. Lines are mono-exponential fits to CRTDs.

b. The $k_{eff}\tau_{tl}$ plot obtained from fitting CRTDs of UvrA-YPet in *uvrA-YPet* cells. Shaded error bands are standard deviations from ten bootstrapped samples.

c. CRTDs (circles) obtained from interval imaging of UvrA-YPet in rif-treated *uvrA-YPet* cells. Lines are mono-exponential fits to CRTDs.

d. The $k_{eff}\tau_{tl}$ plot obtained from fitting CRTDs of UvrA-YPet in rif-treated *uvrA-YPet* cells. Shaded error bands are standard deviations from ten bootstrapped samples.

e. CRTDs (circles) obtained from interval imaging of UvrA-YPet in Δ*uvrA*/pUvrA-YPet cells. Lines are mono-exponential fits to CRTDs.

f. The $k_{eff}\tau_{tl}$ plot obtained from fitting CRTDs of UvrA-YPet in Δ*uvrA*/pUvrA-YPet cells. Shaded error bands are standard deviations from ten bootstrapped samples.

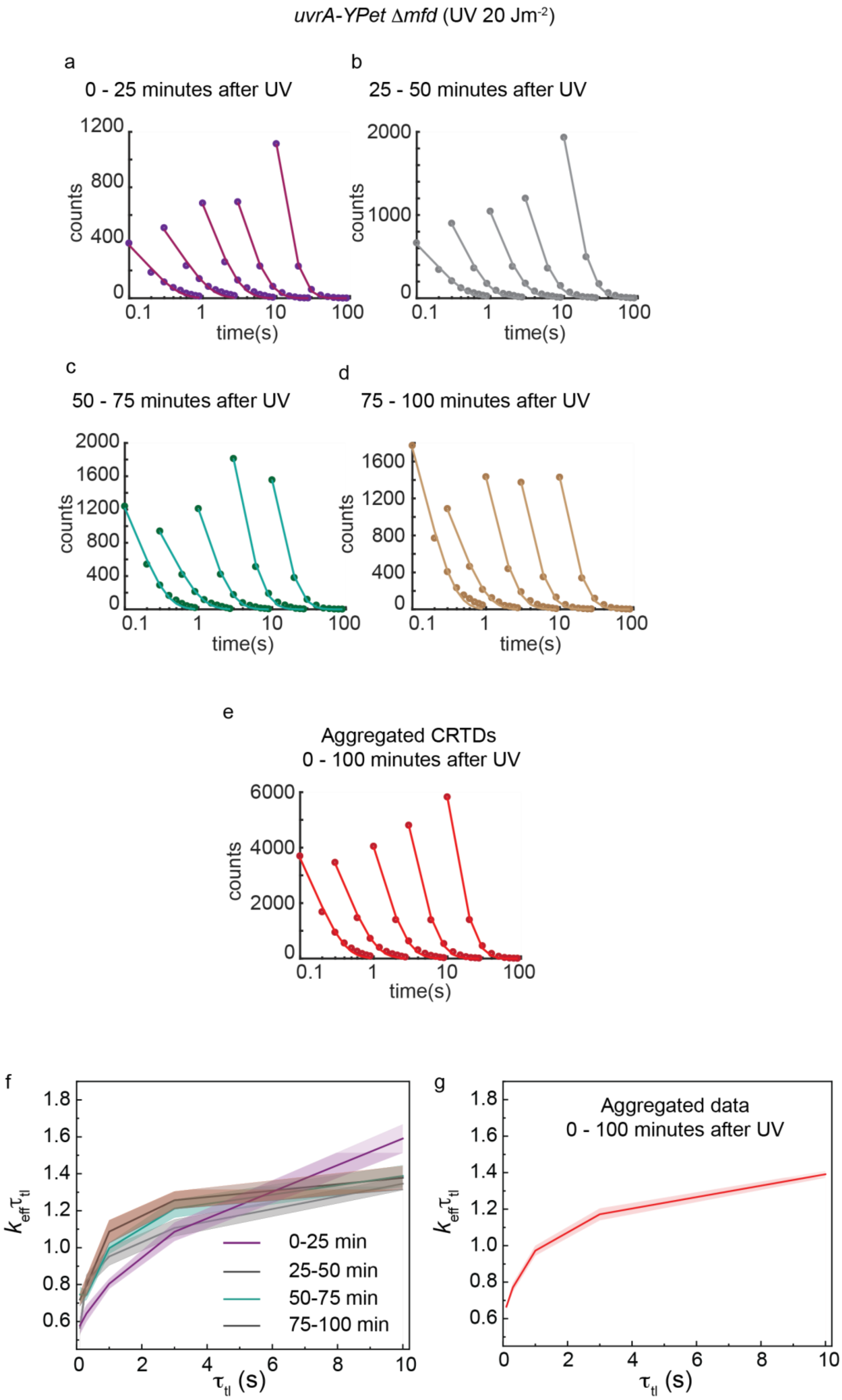
g. CRTDs (circles) obtained from interval imaging of UvrA-YPet in rif-treated Δ*uvrA*/pUvrA-YPet cells. Lines are mono-exponential fits to CRTDs.

h. The $k_{eff}\tau_{tl}$ plot obtained from fitting CRTDs of UvrA-YPet in rif-treated Δ*uvrA*/pUvrA-YPet cells. Shaded error bands are standard deviations from ten bootstrapped samples.

i. Mean pixel intensity (corrected for background) and standard deviation of all pixel intensities in cells (between 50 – 150 cells were analyzed at the indicated time points t = 0, 45, 90 and 135 min) following 30 min rif treatment. Cell fluorescence remains constant within error of measurement.

Source data are provided as a Source Data file.

**Supplementary Figure 5:**



**Supplementary Figure 5:**

a. CRTDs (circles) obtained from interval imaging of UvrA-YPet in *uvrA-YPet* $\Delta$*mfd* cells within the first 25 minutes following exposure to a 20 $Jm^{-2}$ dose of 254-nm light. Lines are mono-exponential fits to CRTDs.

b. CRTDs (circles) obtained from interval imaging of UvrA-YPet in *uvrA-YPet* $\Delta$*mfd* cells 25-50 minutes following exposure to a 20 $Jm^{-2}$ dose of 254-nm light. Lines are mono-exponential fits to CRTDs.

c. CRTDs (circles) obtained from interval imaging of UvrA-YPet in *uvrA-YPet* $\Delta$*mfd* cells 50-75 minutes following exposure to a 20 $Jm^{-2}$ dose of 254-nm light. Lines are mono-exponential fits to CRTDs.

d. CRTDs (circles) obtained from interval imaging of UvrA-YPet in *uvrA-YPet* $\Delta$*mfd* cells 75-100 minutes following exposure to a 20 $Jm^{-2}$ dose of 254-nm light. Lines are mono-exponential fits to CRTDs.

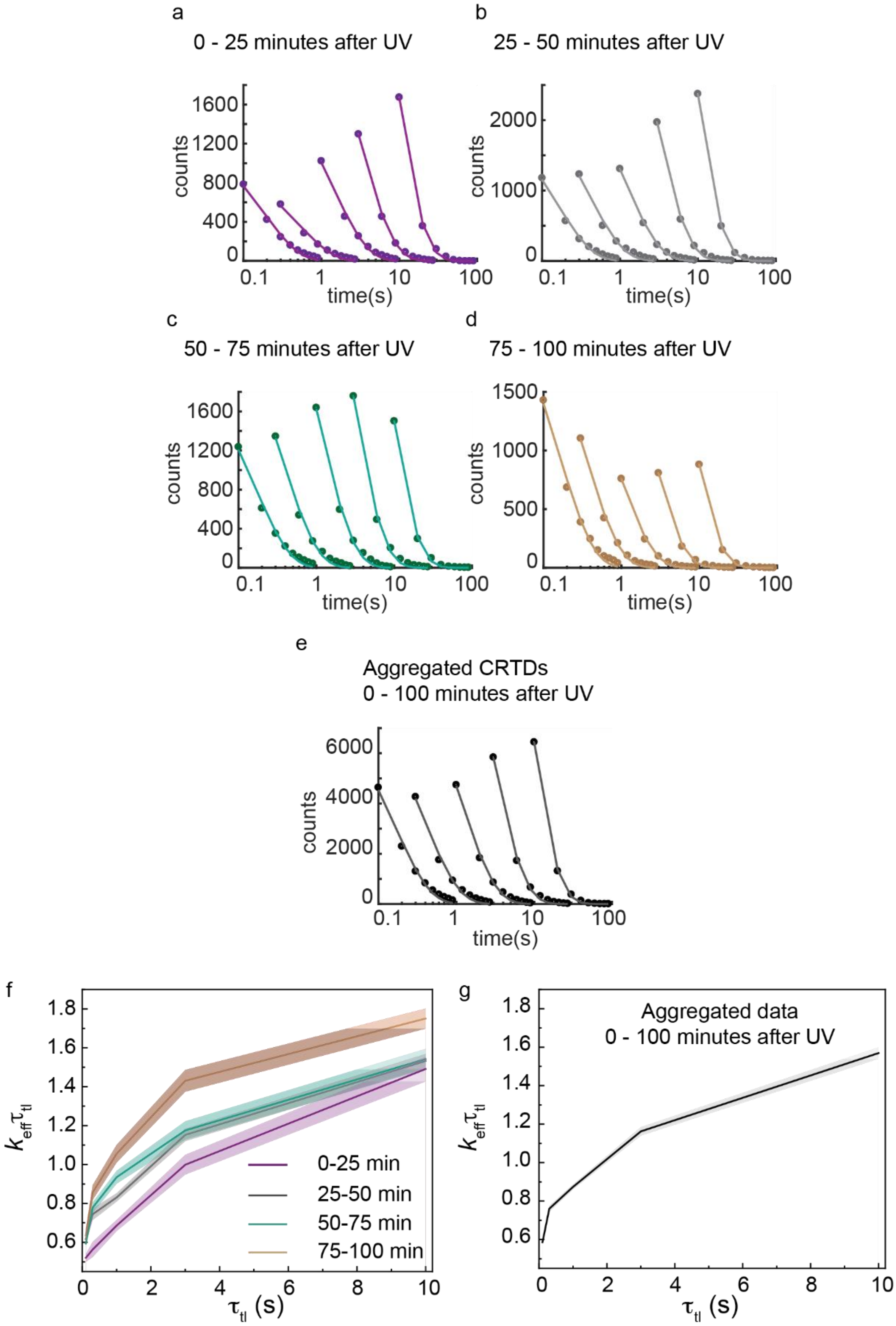
e. CRTDs (circles) obtained from interval imaging of UvrA-YPet in *uvrA-YPet* $\Delta$*mfd* cells within the first 100 minutes following exposure to a 20 $Jm^{-2}$ dose of 254-nm light. Lines are mono-exponential fits to CRTDs.

f. The $k_{\mathrm{eff}}\,\tau_{\mathrm{tl}}$ plots obtained from fitting CRTDs of UvrA-YPet in UV-treated *uvrA-YPet* $\Delta$*mfd* cells as a function of time following UV exposure. Shaded error bands are standard deviations from ten bootstrapped samples. Purple, 0-25 minutes; grey, 25-50 minutes; cyan, 50-75 minutes; brown, 75-100 minutes.

g. The $k_{\mathrm{eff}}\,\tau_{\mathrm{tl}}$ plot obtained from fitting CRTDs of UvrA-YPet in UV-treated *uvrA-YPet* $\Delta$*mfd* cells within the first 100 minutes following UV exposure.
Source data are provided as a Source Data file.

**Supplementary Figure 6:**

***uvrA-YPet* (UV 20 $Jm^{-2}$)**



**Supplementary Figure 6:**
a. CRTDs (circles) obtained from interval imaging of UvrA-YPet in *uvrA-YPet* cells within the first 25 minutes following exposure to a 20 $Jm^{-2}$ dose of 254-nm light. Lines are mono-exponential fits to CRTDs.

b. CRTDs (circles) obtained from interval imaging of UvrA-YPet in *uvrA-YPet* cells 25-50 minutes following exposure to a 20 $Jm^{-2}$ dose of 254-nm light. Lines are mono-exponential fits to CRTDs.

c. CRTDs (circles) obtained from interval imaging of UvrA-YPet in *uvrA-YPet* cells 50-75 minutes following exposure to a 20 $Jm^{-2}$ dose of 254-nm light. Lines are mono-exponential fits to CRTDs.

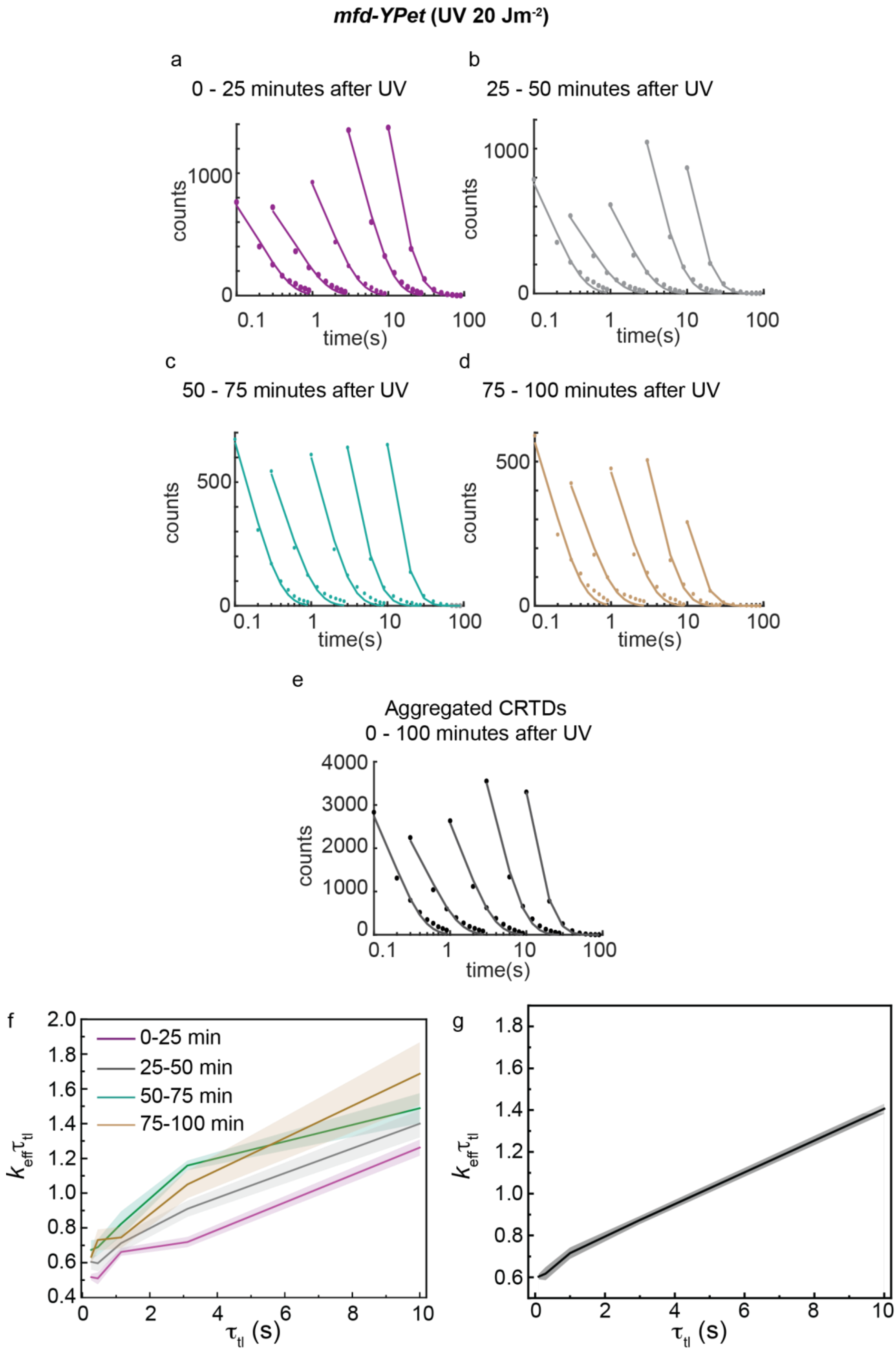
d. CRTDs (circles) obtained from interval imaging of UvrA-YPet in *uvrA-YPet* cells 75-100 minutes following exposure to a 20 $Jm^{-2}$ dose of 254-nm light. Lines are mono-exponential fits to CRTDs.

e. CRTDs (circles) obtained from interval imaging of UvrA-YPet in *uvrA-YPet* cells within the first 100 minutes following exposure to a 20 $Jm^{-2}$ dose of 254-nm light. Lines are mono-exponential fits to CRTDs.

f. The $k_{eff}\tau_{tl}$ plots obtained from fitting CRTDs of UvrA-YPet in UV-treated *uvrA-YPet* cells as a function of time following UV exposure. Shaded error bands are standard deviations from ten bootstrapped samples. Purple, 0-25 minutes; grey, 25-50 minutes; cyan, 50-75 minutes; brown, 75-100 minutes.

g. The $k_{eff}\tau_{tl}$ plot obtained from fitting CRTDs of UvrA-YPet in UV-treated *uvrA-YPet* cells within the first 100 minutes following UV exposure.
Source data are provided as a Source Data file.

**Supplementary Figure 7:**



**Supplementary Figure 7:**
a. CRTDs (circles) obtained from interval imaging of Mfd-YPet in *mfd-YPet* cells within the first 25 minutes following exposure to a 20 $Jm^{-2}$ dose of 254-nm light. Lines are mono-exponential fits to CRTDs.

b. CRTDs (circles) obtained from interval imaging of Mfd-YPet in *mfd-YPet* cells 25-50 minutes following exposure to a 20 $Jm^{-2}$ dose of 254-nm light. Lines are mono-exponential fits to CRTDs.

c. CRTDs (circles) obtained from interval imaging of Mfd-YPet in *mfd-YPet* cells 50-75 minutes following exposure to a 20 $Jm^{-2}$ dose of 254-nm light. Lines are mono-exponential fits to CRTDs.

d. CRTDs (circles) obtained from interval imaging of Mfd-YPet in *mfd-YPet* cells 75-100 minutes following exposure to a 20 $Jm^{-2}$ dose of 254-nm light. Lines are mono-exponential fits to CRTDs.

e. CRTDs (circles) obtained from interval imaging of Mfd-YPet in *mfd-YPet* cells within the first 100 minutes following exposure to a 20 $Jm^{-2}$ dose of 254-nm light. Lines are mono-exponential fits to CRTDs.

f. The $k_{eff}\tau_{tl}$ plots obtained from fitting CRTDs of Mfd-YPet in UV-treated *mfd-YPet* cells as a function of time following UV exposure. Shaded error bands are standard deviations from ten bootstrapped samples. Purple, 0-25 minutes; grey, 25-50 minutes; cyan, 50-75 minutes; brown, 75-100 minutes.

g. The $k_{eff}\tau_{tl}$ plot obtained from fitting CRTDs of Mfd-YPet in UV-treated *mfd-YPet* cells within the first 100 minutes following UV exposure.
Source data are provided as a Source Data file.

## Supplementary Tables

**Supplementary Table 1.** Bacterial strains. All strains are in *E. coli* K-12 MG1655 background.

| **Strain/genotypes** | **Source/Technique** |
|---|---|
| *uvrA-YPet* | This study/ λ Red recombination |
| *uvrA-PAmCherry1* | This study/ λ Red recombination |
| *uvrA::kanR* | This study |
| *uvrB::kanR* | This study/ λ Red recombination |
| *mfd::kanR* | This laboratory |
| *ΔuvrA Δmfd ΔuvrB* | This study |
| *ΔuvrA/*pUvrA-YPet | This study |
| *ΔuvrA Δmfd ΔuvrB/*pUvrA-YPet | This study |
| *ΔuvrA Δmfd ΔuvrB/* pUvrA(Δ131-250)-YPet | This study |
| *uvrA-YPet Δmfd* | This study/ P1 transduction |
| *uvrA-YPet uvrB(ΔβHG)* | This study/ CRISPR-Cas9 assisted λ Red recombination |
| *uvrA-YPet uvrB(ΔβHG) Δmfd* | This study/ P1 transduction |
| *uvrA-YPet ΔuvrB* | This study/ P1 transduction |
| *mfd-YPet* | This laboratory |
| *mfd-PAmCherry1* | This study |

**Supplementary Table 2.** Oligonucleotides used for colony PCR and CRISPR-Cas9 assisted λ Red recombination.

| Oligo names | Sequence |
|---|---|
| UvrA_YPet_fw | TCGCG GAGTG CGAAG CATCA CACAC GGCAC GCTTC CTTAA GCCGA TGCTG TCGGCTGGCTCCGCTGCTGGTTCTGGCGAATTC ATG TCT AAA GGT GAA GAA TTA TTC ACT GGT G |
| UvrA_rev | GCTGG TGCAA CTCTG AAAGG AAAAG GCCGC TCAGA AAGCG GCCTT AACGA GAA GTT CCT ATT CTC TAG AAA GTA TAG G |
| UvrA_del_507_fw | CGGTA GCACC ATGCC ACCGG GCAAA AAAGC GTTTA ATCCG GGAAA GGTGA ccctttcgtcttcaagaattc |
| UvrA_del_507_rev | GCTGG TGCAA CTCTG AAAGG AAAAG GCCGC TCAGA AAGCG GCCTT AACGA ggccacgatgcgtccggcgta |
| uvrA_del_seq_fw1 | AACCTGGCCAGACATTGTTAC |
| uvrA_del_seq_rev1 | CTGTTTGCGATGGTGAAAGA |
| UvrB_del_seq_fw | CCC AAC TCC TTC AGG TAG CG |
| UvrB_del_seq_rev | TAG GCC TGA TAA GCG TAG CG |
| UvrA_FWc | TGGGTACCGGGCCCGCGATTGTACCATTACCAATAG |
| UvrAYPet_revc | AGCCAGCCGAagcgatcgcCAGCATCGGCTTAAGGA |
| RecASZ2 | GTGGCGGCCGCTCTA |
| *Colony PCR for Cas9 verification* | |
| dCas9dL5_303_F | CAGACCGCCACAGTATCAAA |
| pCas9_6700_R | GGAAGGTATCCGACTGCTG |
| *Cloning of pCRISPR variants* | |
| pCRISPR_UvrB_Y96A_S | AAA CCC TAC TAC GAC TAC TAT CAG CG |
| pCRISPR_UvrB_Y96A_AS | AAA ACG CTG ATA GTA GTC GTA GTA GG |
| *Recombineric ssDNA* | |
| UvrB_ΔβHG_ ssDNA | CAA TAT GTT CGT TAA CCG AGG CAT CTT TCT CAA TGA AAG TGC CAT AAT AGT CGT AGT AGG AAA CGA AAT ATT CCA CCG CGT TTT CCG |

**Supplementary Table 3.** Plasmids.

| Plasmid | Source/Technique |
|---|---|
| pHH001 (pSC101-based) | Plasmid backbone carrying a pSC101 origin, spectinomycin marker and expresses Mfd-YPet under the native *mfd* promoter[1]. pJM1071 used as a backbone was a generous gift from the Woodgate laboratory.[2] |
| pHH002 | Plasmid backbone carrying a pSC101 origin, spectinomycin marker and expresses Mfd-YPet under the native *mfd* promoter. This lab[1]. |
| pYPet | This lab (synthetic gene obtained from GeneArt AG) |
| pUvrA-YPet | This study/Sub-cloning into pHH001 |
| pUvrA(Δ131-250)-YPet | This study/Sub-cloning into pHH002 |
| pKD46 | Cox lab[3] |
| pCas9 | Addgene # 42876, Marraffini lab[4] |
| pCRISPR | Addgene # 42875, Marraffini lab[4] |
| pCRISPR-UvrB-Y96A | This study/Sub-cloning into pCRISPR |

**Supplementary Table 4: Sequences of plasmids and geneblocks used in this work**

| Plasmid | Sequence |
|---|---|
| pEAW507 | cgaggccctttcgtcttcaagaattcGAAGTTCCTATAGTTTCTAGAGAATAGGAACTTCgatcttt<br>agaaaaactcatcgagcatcaaatgaaactgcaatttattcatatcaggattatcaataccatatttttgaaaaag<br>ccgtttctgtaatgaaggagaaaactcaccgaggcagttccataggatggcaagatcctggtatcggtctgcgatt<br>ccgactcgtccaacatcaatacaacctattaatttcccctcgtcaaaaataaggttatcaagtgagaaatcaccat<br>gagtgacgactgaatccggtgagaatggcaaaagcttatgcatttctttccagacttgttcaacaggccagccatt<br>acgctcgtcatcaaaatcactcgcatcaaccaaaccgttattcattcgtgattgcgcctgagcgagacgaaatacg<br>cgatcgctgttaaaaggacaattacaaacaggaatcgaatgcaaccggcgcaggaacactgccagcgcatcaac<br>aatattttcacctgaatcaggatattcttctaatacctggaatgctgttttcccggggatcgcagtggtgagtaacca<br>tgcatcatcaggagtacggataaaatgcttgatggtcggaagaggcataaattccgtcagccagtttagtctgacc<br>atctcatctgtaacatcattggcaacgctacctttgccatgtttcagaaacaactctggcgcatcgggcttcccatac<br>aatcgatagattgtcgcacctgattgcccgacattatcgcgagcccatttatacccatataaatcagcatccatgtt<br>ggaatttaatcgcggcctcgagcaagacgtttcccgttgaatatggctcataacaccccttgtattactgtttatgta<br>agcagacagttttattgttcatgatgatatatttttatcttgtgcaatgtaacatcagagattttgagacacaacgtg<br>gctttcccccccccccgatccccgggtaccgagctcgaatttcgaccaattcGAAGTTCCTATACTTTCTA<br>GAGAATAGGAACTTCcgggatcctctacgccggacgcatcgtggccggcatcaccggcgccacaggtgcg<br>gttgctggcgcctatatcgccgacatccaccgatggggaagatcgggctcgccacttcgggctcatgagcgcttgt<br>ttcggcgtgggtatggtggcaggccccgtggccgggggactgttgggcgccatctccttgcatgcaccattccttgc<br>ggcggcggtgctcaacggcctcaacctactactggcgctgcttcctaatgcaggagtcgcataagggagagcgtc<br>gaccgatgcccttgagagccttcaacccagtcagctccttccggtgggcgcggggcatgactatcgtcgccgcact<br>tatgactgtcttctttatcatgcaactcgtaggacaggtgccggcagcgctctgggtcattttcggcgaggaccgctt<br>tcgctggagcgcgacgatgatcggcctgtcgcttgcggtattcggaatcttgcacgccctcgctcaagccttcgtca<br>ctggtcccgccaccaaacgtttcggcgagaagcaggccattatcgccggcatggcggccgacgcgctgggctacg<br>tcttgctggcgttcgcgacgcgaggctggatggccttccccattatgattcttctcgcttccggcggcatcgggatgc<br>ccgcgttgcaggccatgctgtccaggcaggtagatgacgaccatcagggacagcttcaaggatcgctcgcggctc<br>ttaccagcctaacttcgatcactggaccgctgatcgtcacggcgatttatgccgcctcggcgagcacatggaacgg<br>gttggcatggattgtaggcgccgccctataccttgtctgcctccccgcgttgcgtcgcggtgcatggagccgggcca<br>cctcgacctgaatggaagccggcggcacctcgctaacggattcaccactccaagaattggagccaatcaattcttg<br>cggagaactgtgaatgcgcaaaccaacccttggcagaacatatccatcgcgtccgccatctccagcagccgcacg |

| | |
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| pYPet | CTAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTC<br>ATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCG<br>AGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATTCGCCATTCAGGCTGCGCAACT<br>GTTGGGAAGGGCGTTTCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGG<br>GGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTT<br>GTAAAACGACGGCCAGTGAGCGCGACGTAATACGACTCACTATAGGGCGAATTGGCG<br>GAAGGCCGTCAAGGCCGCATATGTCTAAAGGTGAAGAATTATTCACTGGTGTTGTCCC |

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| | AATTTTGGTTGAATTAGATGGTGATGTTAATGGTCACAAATTTTCTGTCTCCGGTGAAG<br>GTGAAGGTGATGCTACGTACGGTAAATTGACCTTAAAATTACTCTGTACTACTGGTAA<br>ATTGCCAGTTCCATGGCCAACCTTAGTCACTACTTTAGGTTATGGTGTTCAATGTTTTGC<br>TAGATACCCAGATCATATGAAACAACATGACTTTTTCAAGTCTGCCATGCCAGAAGGTT<br>ATGTTCAAGAAAGAACTATTTTTTTCAAAGATGACGGTAACTACAAGACCAGAGCTGA<br>AGTCAAGTTTGAAGGTGATACCTTAGTTAATAGAATCGAATTAAAAGGTATTGATTTTA<br>AAGAAGATGGTAACATTTTAGGTCACAAATTGGAATACAACTATAACTCTCACAATGTT<br>TACATCACTGCTGACAAACAAAAGAATGGTATCAAAGCTAACTTCAAAATTAGACACA<br>ACATTGAAGATGGTGGTGTTCAATTAGCTGACCATTATCAACAAAATACTCCAATTGGT<br>GATGGTCCAGTCTTGTTACCAGACAACCATTACTTATCCTATCAATCTGCCTTATTCAAA<br>GATCCAAACGAAAAGAGAGACCACATGGTCTTGTTAGAATTTTTGACTGCTGCTGGTA<br>TTACCGAGGGTATGAATGAATTGTACAAAGAGCTCTAAGAAGTTCCTATAGTTTCTAG<br>AGAATAGGAACTTCGATCTTTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTT<br>ATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAG<br>AAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCC<br>GACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAA<br>GTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGCTTATGCA<br>TTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCAT<br>CAACCAAACCGTTATTCATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGCT<br>GTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAG<br>CGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTT<br>CCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTT<br>GATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTA<br>ACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTT<br>CCCATACAATCGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTAT<br>ACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGAGCAAGACGTTTCC<br>CGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATT<br>GTTCATGATGATATATTTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACACAAC<br>GTGGCTTTCCCCCCCCCCCCGATCCCCGGGTACCGAGCTCGAATTTCGACCAATTCGAA<br>GTTCCTATACTTTCTAGAGAATAGGAACTTCCTGGGCCTCATGGGCCTTCCGCTCACTG<br>CCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAACATGGTCATAGCTGTTT |

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| | CCTTGCGTATTGGGCGCTCTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTC<br>GGGTAAAGCCTGGGGTGCCTAATGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTA<br>AAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAA<br>AAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGC<br>GTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGAT<br>ACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGG<br>TATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGT<br>TCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGAC<br>ACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATG<br>TAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAAC<br>AGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGC<br>TCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCA<br>GATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCT<br>GACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAA<br>GGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATAT<br>ATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGC<br>GATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGAT<br>ACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGAACCACGCTCA<br>CCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGT<br>GGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGT<br>AAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTG<br>GTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCG<br>AGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCG<br>TTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAA<br>TTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAA<br>GTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGG<br>GATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTC<br>GGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACT<br>CGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAA<br>AACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAA<br>TACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAG |

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| | CGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTC CCCGAAAAGTGCCAC |
| UvrA d131_250 (gene block) | TGGGTACCGGGCCCGCGATTGTACCATTACCAATAGCGCTTTTACTATGTTGTGACCTC GGTTCCGGGAAACAAACCTGGCCAGACATTGTTACACAACACTCCGGGTAATGCATTC CAATACTGTATATTCATTCAGGTCAATTTGTGTCATAATTAACCGTTTGTGATCGCCGGT AGCACCATGCCACCGGGCAAAAAAGCGTTTAATCCGGGAAAGGTGAATGGATAAGAT CGAAGTTCGGGGCGCCCGCACCCATAATCTCAAAAACATCAACCTCGTTATCCCCCGCG ACAAGCTCATTGTCGTGACCGGGCTTTCGGGTTCTGGCAAATCCTCGCTCGCTTTCGAC ACCTTATATGCCGAAGGGCAGCGCCGTTACGTTGAATCCCTTTCCGCCTACGCGCGGC AGTTTCTGTCACTGATGGAAAAGCCGGACGTCGATCATATTGAGGGGCTTTCTCCTGC CATCTCAATTGAGCAGAAATCGACGTCTCATAACCCGCGTTCTACGGTGGGGACAATC ACCGAAATCCACGACTATTTGCGTTTGTTATTCGCCCGCGTTGGCGAGCCGCGCTGTCC GGACCACGACGTCCCGCTGGCGGCGCAAGGCTTCGCCTGCCCAATTTGCGGCTACAGT ATGCGTGAACTGGAGCCGCGACTGTTTTCGTTTAACAACCCGGCGGGGGCCTGCCCGA CCTGCGACGGCCTTGGCGTACAGCAATATTTCGATCCTGATCGAGTGATCCAGAATCC GGAACTGTCGCTGGCTGGTGGTGCGATCCGTGGCTGGGATCGCCGCAACTTCTATTAT TTCCAGATGCTGAAATCGCTGGCAGATCACTATAAGTTCGACGTCGAAGCGCCGTGGG GCAGCCTGAGCGCGAACGTGCATAAAGTGGTGTTGTACGGTTCTGGCAAAGAAAACA TTGAATTCAAATACATGAACGATCGTGGCGATACCTCCATTCGTCGTCATCCGTTCGAA GGCGTGCTGCATAATATGGAGCGCCGCTATAAAGAGACGGAATCCAGCGCGGTACGC GAAGAATTAGCCAAGTTTATCAGTAATCGTCCGTGCGCCAGCTGCGAAGGGACGCGTC TGCGTCGGGAAGCGCGCCACGTGTATGTCGAGAATACGCCGCTGCCTGCTATCTCCGA CATGAGCATTGGTCATGCGATGGAATTCTTCAACAATCTCAAACTCGCAGGTCAGCGG GCGAAGATTGCAGAAAAAATCCTTAAAGAGATCGGCGATCGTCTGAAATTCCTCGTTA ACGTCGGCCTGAATTACCTGACGCTTTCCCGCTCGGCAGAAACGCTTTCTGGCGGTGA AGCACAGCGTATCCGTCTGGCGAGCCAGATTGGTGCGGGCCTGGTTGGCGTTATGTAC GTGCTGGACGAGCCGTCTATCGGCCTGCACCAGCGTGATAACGAGCGCCTGTTGGGT ACGCTTATCCATCTGCGCGATCTCGGTAATACCGTGATTGTGGTGGAGCACGACGAAG ACGCAATTCGCGCCGCTGACCATGTGATCGACATTGGCCCGGGCGCAGGTGTTCACGG CGGTGAAGTGGTCGCAGAAGGTCCGCTGGAAGCGATTATGGCGGTGCCGGAGTCGTT GACCGGGCAGTACATGAGCGGCAAACGCAAGATTGAAGTGCCGAAGAAACGCGTTCC |

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| | GGCGAATCCGGAAAAAGTGCTGAAGCTGACAGGCGCACGCGGCAACAACCTGAAGG<br>ACGTGACGCTGACGCTGCCGGTGGGTCTGTTTACCTGCATCACCGGGGTTTCAGGTTC<br>CGGTAAATCGACGCTGATTAACGACACACTGTTCCCGATTGCCCAACGCCAGTTGAAT<br>GGGGCGACCATCGCCGAACCAGCACCGTATCGCGATATTCAGGGGCTGGAGCATTTC<br>GATAAAGTGATCGATATCGACCAAAGCCCAATTGGTCGTACTCCACGTTCTAACCCGG<br>CGACCTATACCGGCGTGTTTACGCCTGTGCGCGAACTGTTTGCGGGCGTACCGGAATC<br>CCGTGCGCGCGGCTATACGCCGGGACGTTTCAGCTTTAACGTTCGTGGCGGACGCTGC<br>GAGGCCTGTCAGGGCGATGGCGTGATCAAAGTGGAGATGCACTTCCTGCCGGATATC<br>TACGTGCCGTGCGACCAGTGCAAAGGTAAACGCTATAACCGTGAAACGCTGGAGATT<br>AAGTACAAAGGCAAAACCATCCACGAAGTGCTGGATATGACCATCGAAGAGGCGCGT<br>GAGTTCTTTGATGCCGTACCTGCACTGGCGCGTAAGCTGCAAACGTTGATGGACGTTG<br>GCCTGACGTACATTCGACTGGGGCAGTCCGCAACCACCCTTTCAGGCGGTGAAGCCCA<br>GCGCGTGAAGCTGGCGCGTGAACTGTCAAAACGCGGCACCGGGCAGACGCTGTATAT<br>TCTCGACGAGCCGACCACCGGTCTGCACTTCGCCGATATTCAGCAACTGCTCGACGTAC<br>TGCATAAACTGCGCGATCAGGGCAACACCATTGTGGTGATTGAGCACAATCTCGACGT<br>GATCAAAACCGCTGACTGGATTGTCGACCTGGGACCAGAAGGCGGCAGTGGTGGCGG<br>CGAGATCCTCGTCTCCGGTACGCCAGAAACCGTCGCGGAGTGCGAAGCATCACACACG<br>GCACGCTTCCTTAAGCCGATGCTGGCGATCGCTTCGGCTGGCTCCGCTGCAGGTTCTG<br>GCGAATTCGGGTAATGATTCAGTTCTAGAGCGGCCGCCACTAA |

## Supplementary Notes

### Supplementary Note 1

The chromosomal fusions of *uvrA-YPet* and *uvrA-PAmCherry1* and *mfd-PAmCherry1* were created using λ Red recombination as previously described and the primers indicated in supplementary table 2[3]. Sequence verified recombinant strains carrying Mfd-PAmCherry1 and UvrA-PAmCherry1 were then screened by imaging at the single-cell level. Unlike their YPet tagged counterparts, the PAmCherry1 labelled proteins were poorly expressed in MG1655 (Supplementary Fig. 3).

### Supplementary Note 2: Copy numbers of UvrA after SOS induction

UvrA copy numbers have been suggested to increase from 25 to 250 copies per cell after SOS induction[5]. We note that since the experimental conditions associated with these measurements are not available in the published literature, we are unable to effectively compare our measurements with these numbers. Nevertheless, the basal levels and the exact extent of fold-induction after SOS induction may depend on the nature and dosage of the genotoxin, as well as growth conditions such as medium and temperature. Consistent with this argument, the copy numbers of UvrA were found to rise six-fold within 40 minutes of UV exposure (40 $Jm^{-2}$) in cells growing at 37 °C in previous work [6].

## Supplementary References


1 Ho HN, van Oijen AM, Ghodke H. The transcription-repair coupling factor Mfd associates with RNA polymerase in the absence of exogenous damage. *Nat Commun* **9**, 1570 (2018).

2 Churchward G, Belin D, Nagamine Y. A pSC101-derived plasmid which shows no sequence homology to other commonly used cloning vectors. *Gene* **31**, 165-171 (1984).

3 Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci U S A* **97**, 6640-6645 (2000).

4 Jiang W, Bikard D, Cox D, Zhang F, Marraffini LA. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. *Nat Biotechnol* **31**, 233-239 (2013).

5 Selby, C. P. & Sancar, A. Structure and function of the (A)BC excinuclease of *Escherichia coli*. *Mutat Res* **236**, 203-211 (1990).

6 Crowley DJ, Hanawalt PC. Induction of the SOS Response Increases the Efficiency of Global Nucleotide Excision Repair of Cyclobutane Pyrimidine Dimers, but Not 6-4 Photoproducts, in UV-Irradiated *Escherichia coli*. *J Bacteriol* **180**, 3345-3352 (1998).