Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supplementary Information

Mechanistic insight into the repair of C8-linked pyrrolobenzodiazepine monomer-mediated DNA damage

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Supplementary Figure Legends:

Figure S1: (A) General synthetic scheme for the synthesis of PBD core (10) and the conjugation of C8-side chain to the PBD core to obtain KMR-28-33 and KMR-28-35. (B) Synthesis of MPB building block 15 which is present in KMR-28-35.

Figure S2: (A) Molecular docking of KMR-28-33 and KMR-28-35 with 15 bp DNA sequence taken from the LexA box within the promoter of *recA* gene (5'-GTTCGCAAGATGTTC-3'; GC content – 46%). (B) Molecular docking of KMR-28-33 and KMR-28-35 with 15 bp DNA sequence taken from the sRNA gene *CCNA_R0074* (5'-CCCCTTCGCCCTCCT-3'; GC content – 73%) (C) Table of all the repair components deleted in this study and their ascribed functions; SSGR – single-strand gap repair, DSBR – double-strand break repair. (D) Representative images of wild type *Caulobacter crescentus* growth on increasing concentrations of DNA damaging agents UV, MMS and norfloxacin. Grey triangle at the bottom of each image panel depicts increasing dilution of the bacterial culture from left to right. Minimum of three independent experiments were performed for each condition.

Figure S3: Melt curves for AT-rich (Sequence 1) and GC-rich (Sequence 2) hairpin DNA after 3h or 24h incubation with (A) KMR-28-33, (B) KMR-28-35, (C) MMC and (D) GWL-78. For comparison, melt curves of 'only DNA' control (for Sequence 1 or Sequence 2) are included in these graphs. Each reaction was carried out in triplicates – black, red and green lines correspond to individual melt curves for only DNA control, and blue, purple and pink lines represent melt curves for DNA stabilized with drug.

Figure S4: (A) Fold change in rifampicin resistant colonies (Rif mutants) in KMR-28-33 (0.5 µg/ml), KMR-28-35 (2 µg/ml) or MMC (0.5 µg/ml) treated cells compared to control (no DNA damage). Mean and SD for data from three independent experiments is plotted. (B) [left] Representative images for cells with Gam-GFP foci upon treatment with norfloxacin in wild type *Caulobacter*. [right] Percentage cells with Gam-GFP foci upon treatment with norfloxacin in wild type *Caulobacter*. Mean and SD for data from three independent experiments is plotted (n ≤ 300 cells). (C) [left] Representative images for cells with Gam-GFP foci upon treatment with KMR-28-33, KMR-28-35 and MMC in wild type and *ΔrecN* strains. Scale bar – 4 µm. [right] Percentage cells with Gam-GFP foci upon treatment with KMR-28-33, KMR-28-35 and MMC in wild type and *ΔrecN* strains. Mean and SD for data from three independent experiments is plotted (n ≤ 330 cells). (D) Survival of wild type, *ΔmutL* and *ΔalkB* strains under increasing doses of KMR-28-33, KMR-28-35 and MMC. Minimum of three independent experiments were performed for each strain. Mean and SEM from all repeats for each strain is plotted (wild type data from Figure 3A for comparison).

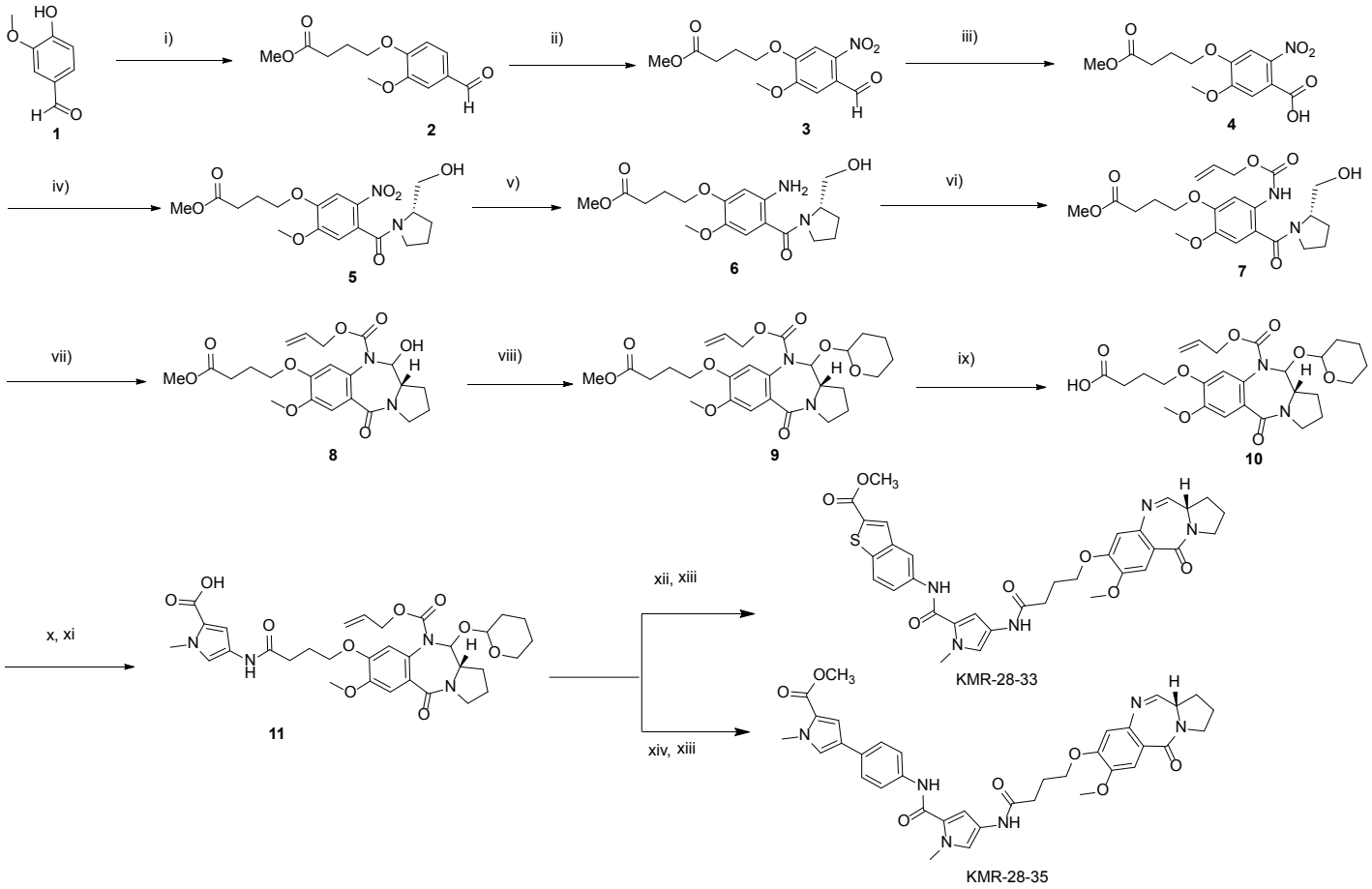
Figure S5: (A) Survival of wild type and *ΔuvrA* strains under increasing doses of UV, MMS and norfloxacin (NF) damage. Minimum of three independent experiments were performed for each strain. Mean and SEM from all repeats for each strain is plotted. (B) Survival of wild type, *ΔuvrA* and *Δmfd* strains under increasing doses of KMR-28-33, KMR-28-35 and MMC.

Minimum of three independent experiments were performed for each strain. Mean and SEM from all repeats for each strain is plotted (wild type and *ΔuvrA* data from Figures 3A and 5A for comparison).

Figure S6: Table summarizing findings from this study on DNA repair mechanisms essential for repair/tolerance of lesions induced by KMR-28-33, KMR-28-35 and MMC.

Figure S1

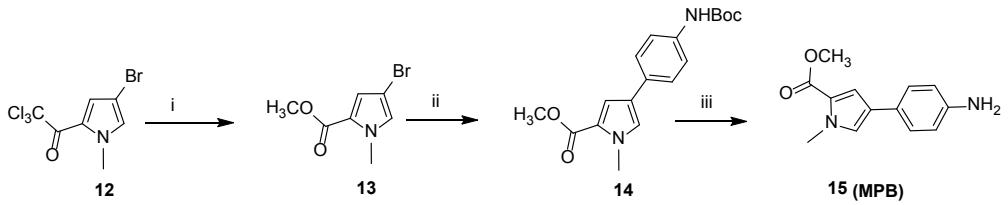
A.



Reagents and Conditions

i) K2CO3, DMF, Methyl 4-bromobutyrate, RT ii) KNO3, TFA, 0°C iii) KMnO4, Acetone, H2O 70°C, iv) Oxalyl Chloride, S pyrrolidine methanol, RT v) Ammonium formate, Pd/C, 60°C vi) Allylchloroformate, Pyridine, DCM, RT vii) TEMPO, BAIB, RT viii) pTSA, DHP, ix) NaOH, Dioxane, H2O, RT. X) EDCI, DMAP, methyl 4-amino-1-methyl-1H-pyrrole-2-carboxylate, RT xi) NaOH, Dioxane, H2O, RT. xii) EDCI, DMAP, methyl 5-aminobenzo[b]thiophene-2-carboxylate, RT. xiii) Pyrrolidine, PPh3, Pd(PPh3)4, DCM, RT xiv) EDCI, DMAP, MPB, RT

B.

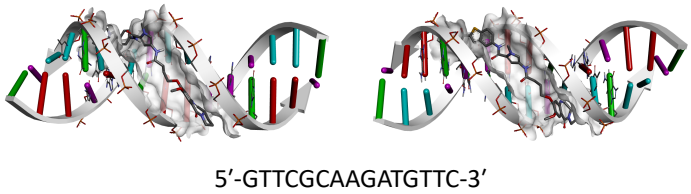


Reagents and Conditions

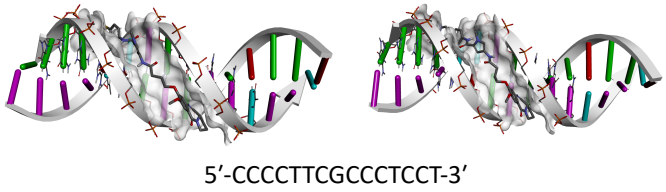
i) Na-Methoxide, MeOH (anhydrous), H2SO4, reflux; ii) 4-(tert-butoxycarbonylamino)phenylboronic acid, (PPh3)4Pd, K2CO3, Ethanol:Tolune:Water 9:3:1, MW iii) 4M HCl in Dioxane, RT.

Figure S2

A.



B.



C.

Repair mechanisms		Components deleted in this study	
		Gene name	**Implicated function**
SOS response		*lexA*	Prevents expression of SOS inducible genes in the absence of DNA damage
		recA	Expression of SOS inducible genes under DNA damage
Mismatch repair		*mutL*	Lesion recognition
Alkylation repair		*alkB*	1-methyladenine and 3-methylcytosine repair protein
Nucleotide excision repair		*uvrA*	Lesion recognition
		mfd	Transcription coupled repair
Homologous recombination	SSGR	*recA*	Recombinase; filaments on single stranded DNA
		recF	RecA loading
		recO	RecA loading
		recR	RecA loading
	DSBR	*recA*	Recombinase; filaments on single stranded DNA
		recN	Homology search
		addAB	Helicase-nuclease
Translesion synthesis		*dnaE2*	Low-fidelity polymerase

D.

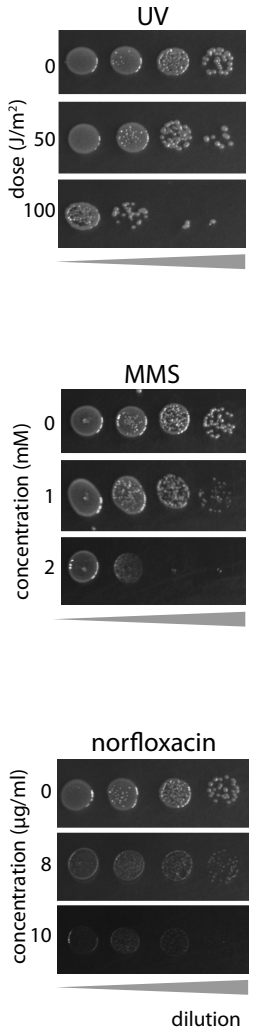


Figure S3

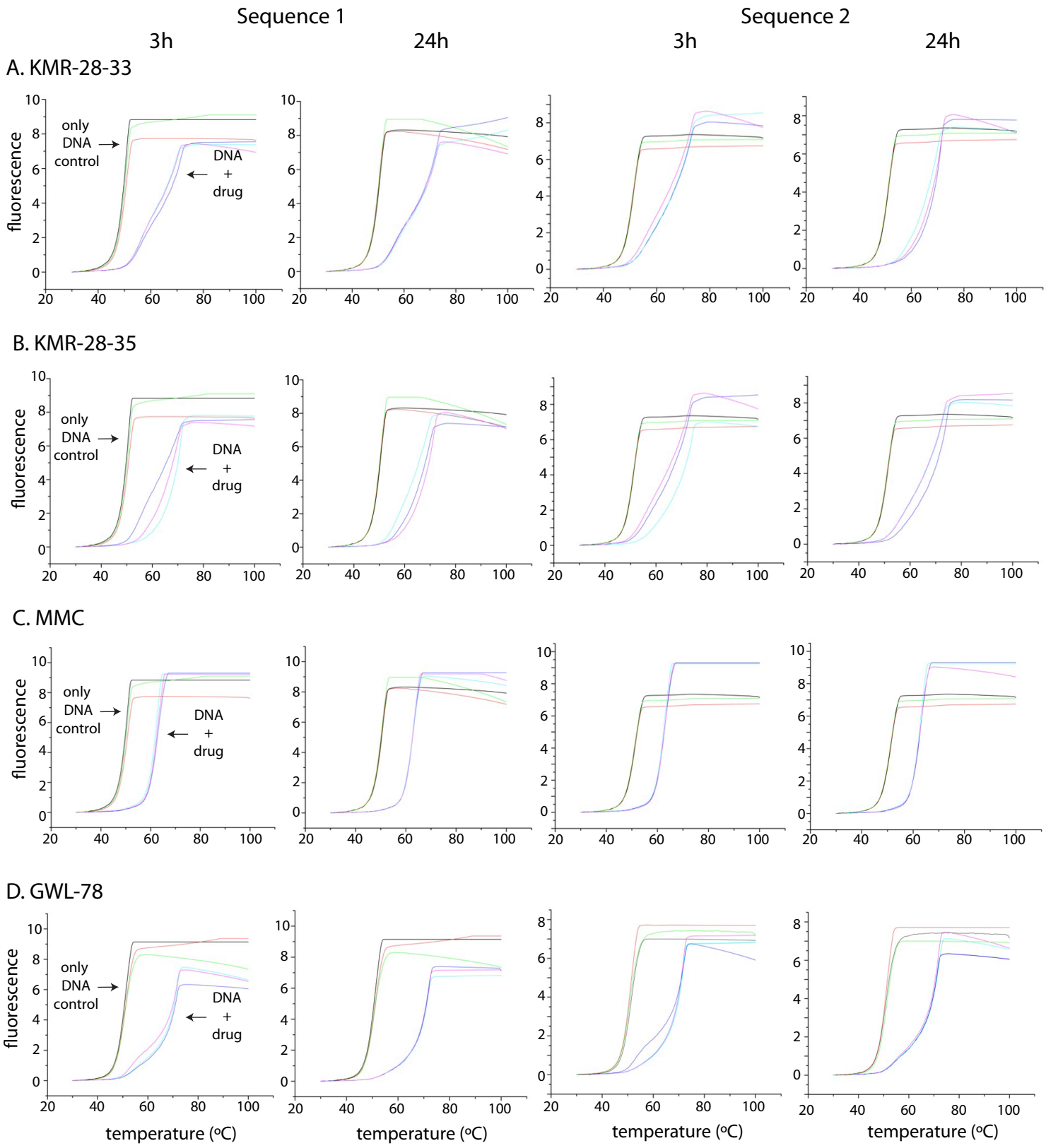

Sequence 1
Sequence 2
3h
24h
3h
24h
A. KMR-28-33
B. KMR-28-35
C. MMC
D. GWL-78
fluorescence
only DNA control
DNA + drug
temperature (°C)

Figure S4

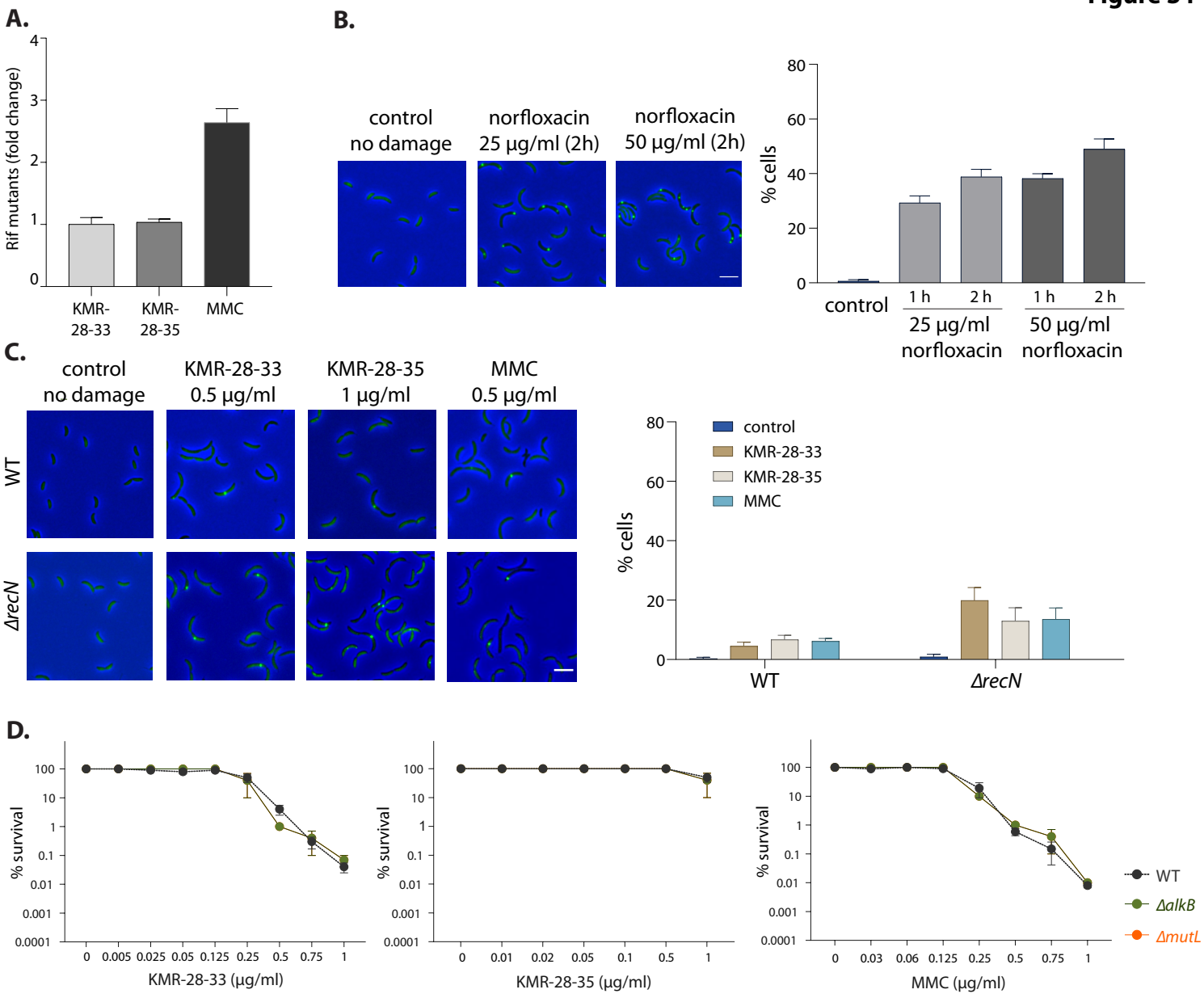
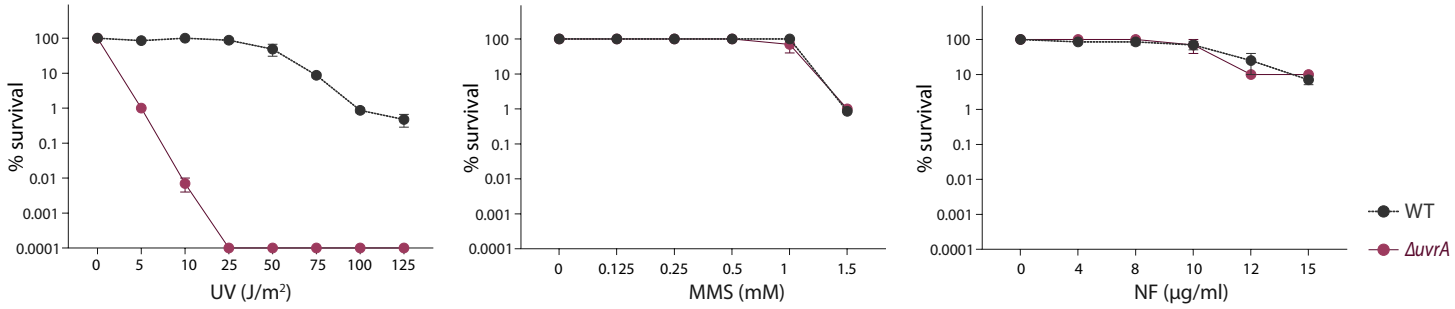

A.
Rif mutants (fold change)
KMR-28-33
KMR-28-35
MMC
B.
control no damage
norfloxacin 25 µg/ml (2h)
norfloxacin 50 µg/ml (2h)
% cells
control
1 h
2 h
1 h
2 h
25 µg/ml norfloxacin
50 µg/ml norfloxacin
C.
control no damage
KMR-28-33 0.5 µg/ml
KMR-28-35 1 µg/ml
MMC 0.5 µg/ml
WT
ΔrecN
control
KMR-28-33
KMR-28-35
MMC
% cells
WT
ΔrecN
D.
% survival
KMR-28-33 (µg/ml)
KMR-28-35 (µg/ml)
MMC (µg/ml)
WT
ΔalkB
ΔmutL

Figure S5

A.


% survival
100
10
1
0.1
0.01
0.001
0.0001
0 5 10 25 50 75 100 125
UV (J/m²)
0 0.125 0.25 0.5 1 1.5
MMS (mM)
0 4 8 10 12 15
NF (µg/ml)
WT
ΔuvrA

B.

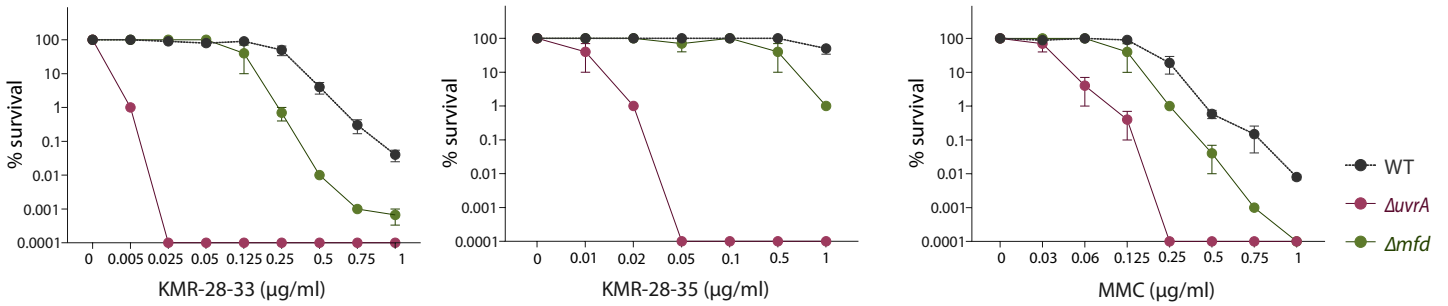

% survival
100
10
1
0.1
0.01
0.001
0.0001
0 0.005 0.025 0.05 0.125 0.25 0.5 0.75 1
KMR-28-33 (µg/ml)
0 0.01 0.02 0.05 0.1 0.5 1
KMR-28-35 (µg/ml)
0 0.03 0.06 0.125 0.25 0.5 0.75 1
MMC (µg/ml)
WT
ΔuvrA
Δmfd

Figure S6

DNA damage	Lesion type	Repaired by
KMR-28-33	Mono-adducts	Nucleotide excision repair Recombination repair
KMR-28-35	Mono-adducts	Nucleotide excision repair Recombination repair
Mitomycin C (MMC)	Mono-adducts intra-strand crosslinks inter-strand crosslinks	Nucleotide excision repair Translesion synthesis Recombination repair

Table S1: Strains used in present study

Strain name	Genotype	Strain construction
CB15N		
NABC2	*CB15N; ΔrecA*	[1]
NABC29	*CB15N; ΔdnaE2*	CB15N was transformed with pNABC148 plasmid to generate deletion of *dnaE2* through two-step recombination procedure.
NABC238	*CB15N; ΔuvrA*	CB15N transformed with pNABC417 plasmid to generate deletion of *uvrA* through two-step recombination procedure.
NABC239	*CB15N; ΔaddAB::gent*	CB15N transduced with lysate of strain harboring deletion of *addAB* linked to $gent^R$
NABC265	*CB15N; ΔrecA::*kan^R*, ΔlexA::*tet^R*, ΔsidA*	*CB15N; ΔlexA::*tet^R*, ΔsidA strain* transduced with lysate of strain harboring *recA* deletion linked to kan^R
NABC268	*CB15N;* P_{xyl}*-Gam-GFP::*$spec^R$	CB15N transformed with pNABC592 plasmid
NABC439	*CB15N; ΔrecN*	[2]
NABC499	*CB15N; ΔrecF*	CB15N transformed with pYY193 plasmid to generate deletion of *recF* (3rd-100th codon) through two-step recombination procedure.
NABC502	*CB15N; ΔrecR*	CB15N transformed with pYY115 plasmid to generate deletion of *recR* through two-step recombination procedure.
NABC505	*CB15N; ΔrecO*	CB15N transformed with pYY119 plasmid to generate deletion of *recO* through two-step recombination procedure.
NABC544	*CB15N; Δmfd*	CB15N transformed with pNABC590 plasmid to generate deletion of *mfd* through two-step recombination procedure.
NABC579	*CB15N; ΔalkB*	CB15N transformed with pNABC591 plasmid to generate deletion of *alkB* through two-step recombination procedure.
NABC580	*CB15N; ΔmutL*	CB15N transformed with pNABC416 plasmid to generate deletion of *mutL* through two-step recombination procedure.
NABC581	*CB15N;* P_{sidA}*-YFP::*kan^R	CB15N transformed with pNABC420 [3]
NABC582	*CB15N; ΔuvrA;* P_{xyl}*-Gam-GFP::*$spec^R$	NABC238 transformed with pNABC592 plasmid
NABC583	*CB15N; ΔrecN::*$spec^R$*;* P_{xyl}*-Gam-GFP::*$spec^R$	NABC439 transformed with pNABC592 plasmid

Table S2: Plasmids used in present study

Plasmid name	Construct details	Antibiotic marker
pNPTS138	[4]	Kanamycin
pXGFPC1	[5]	Spectinomycin
pXYFPC2	[5]	Kanamycin
pNABC148	600 bp fragments upstream and downstream of *dnaE2* genomic locus were amplified from *C. crescentus* gDNA using RR_oligo_021/RR_oligo_022 (upstream fragment) and RR_oligo_023/RR_oligo_024 (downstream fragment) primer pairs. These fragments were assembled with BamH1/Nhe1 linearized pNPTS138 vector using Gibson assembly.	Kanamycin
pNABC416	600 bp fragments upstream and downstream of *mutL* genomic locus were amplified from *C. crescentus* gDNA using PS_oligo_049/AMJ_oligo_061 (upstream fragment) and AMJ_oligo_062/PS_oligo_054 (downstream fragment) primer pairs. These fragments were assembled with BamH1/Nhe1 linearized pNPTS138 vector using Gibson assembly.	Kanamycin
pNABC417	600 bp fragments upstream and downstream of *uvrA* genomic locus were amplified from *C. crescentus* gDNA using PS_oligo_037/AMJ_oligo_057 (upstream fragment) and AMJ_oligo_058/PS_oligo_042 (downstream fragment) primer pairs. These fragments were assembled with BamH1/Nhe1 linearized pNPTS138 vector using Gibson assembly.	Kanamycin
pYY193	500 bp fragments upstream and downstream of *recF* (from the 3rd to the 100th codon) were amplified from *C. crescentus* gDNA using UPdel00158_F/UPdel00158_R (upstream fragment) and DWNdel00158_F/DWNdel00158_R (downstream fragment) primer pairs. These fragments were assembled with EcoRI/BamHI linearized pNPTS138 vector using Gibson assembly.	Kanamycin
pYY115	500 bp fragments upstream and downstream of *recR* were amplified from *C. crescentus* gDNA using UPdel00270_F/UPdel00270_R (upstream fragment) and DWNdel00270_F/DWNdel00270_R (downstream fragment) primer pairs. These fragments were assembled with EcoRI/BamHI linearized pNPTS138 vector using Gibson assembly.	Kanamycin

pYY119	500 bp fragments upstream and downstream of *recO* were amplified from *C. crescentus* gDNA using UPdel01635_F/UPdel01635_R (upstream fragment) and DWNdel01635_F/DWNdel01635_R (downstream fragment) primer pairs. These fragments were assembled with EcoRI/BamHI linearized pNPTS138 vector using Gibson assembly.	Kanamycin
pNABC420	pXYFPC2 vector was amplified using AB_oligo_651 and AB_oligo_652 and P_{sidA}-*YFP* fragment was amplified from a replicating plasmid habouring YFP under P_{sidA} promoter using AC_oligo_322 and AC_oligo_321. The vector and insert fragments were assembled with Gibson assembly.	Kanamycin
pNABC590	600 bp fragments upstream and downstream of *mfd* genomic locus were amplified from *C. crescentus* gDNA using SD_oligo_088/SD_oligo_089 (upstream fragment) and SD_oligo_090/SD_oligo_0091 (downstream fragment) primer pairs. These fragments were assembled with BamH1/Nhe1 linearized pNPTS138 vector using Gibson assembly.	Kanamycin
pNABC591	600 bp fragments upstream and downstream of *alkB* genomic locus were amplified from *C. crescentus* gDNA using AMJ_oligo_041/AMJ_oligo_053 (upstream fragment) and AMJ_oligo_054/PS_oligo_044 (downstream fragment) primer pairs. These fragments were assembled with BamH1/Nhe1 linearized pNPTS138 vector using Gibson assembly.	Kanamycin
pNABC592	*gam_GFP* was amplified using SD_oligo_019 and SD_oligo_020 from an *E. coli* strain harboring *gam-gfp* (Shee *et al*., 2013) and assembled with Nde1/Nhe1 digested pXYFPC-1 using Gibson assembly.	Spectinomycin

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Table S3: Oligos used in present study

Primer name	Sequence
AB_oligo_651	CTGGACCTCTTGCCCATGACCGA
AB_oligo_652	GCTAGCTGCAGCCCGGGGG
AC_oligo_321	AACTAGTGGATCCCCCGGGCTGCAGCTAGCTTACTTGTACAGCTCGTCCATGCCGA
AC_oligo_322	GGTCAGGTCGGTCATGGGCAAGAGGTCCAGCACCCGCCCATCACCCACAGATGC
AMJ_oligo_041	CAAGCTTCTCTGCAGGATATCTGTGCGGCCAATCAGGCGCTTGATCG
AMJ_oligo_044	CGGAGACGCGTCACGGCCGAAGAGCCGGCGGATCGCAACCTCC
AMJ_oligo_053	AGAGTCAGATTGATCCGGCCTACGTCAAAGCCGGGGACAACGGT
AMJ_oligo_054	TTGTCCCCGGCTTTGACGTAGGCCGGATCAATCTGACTCTGCGACG
AMJ_oligo_057	GCCTGCTGAGCCGCCTTAGTTTTCCGGAACGTTGGAC
AMJ_oligo_058	GTCCAACGTTCCGGAAAACTAAGGCGGCTCAGCAGGC
AMJ_oligo_061	TAGGGGGCGCTCTGGCCTCACATCAAGCGGACTTTCACGGG
AMJ_oligo_062	CCCGTGAAAGTCCGCTTGATGTGAGGCCAGAGCGCCCCCTA
PS_oligo_037	CAAGCTTCTCTGCAGGATATCTGCTTGGCGATGGCGTCACCT
PS_oligo_042	CGGAGACGCGTCACGGCCGAAGTCTACGCAGACGTGGATCTTG
PS_oligo_049	CAAGCTTCTCTGCAGGATATCTGGTCAAATGCTTCTCCAGCCG
PS_oligo_054	CGGAGACGCGTCACGGCCGAAGGAAGGAGACGAGACGATGGA
SD_oligo_019	CAGACGCTCGAGTTTTGGGGAGACGACCATATGGCTAAACCAGCAAAACGTATCAAG
SD_oligo_020	GAACTAGTGGATCCCCCGGGCTGCAGCTAGCGCAGCCGGATCCCTTATTTGTATAGTTC
SD_oligo_088	CAAGCTTCTCTGCAGGATATCTGTTCGCTATCGACCACTATCT
SD_oligo_089	TGGGCCGGCGTGTCCCATTCATGACCAGGGCGTCGAAGCC
SD_oligo_090	GGCTTCGACGCCCTGGTCATGAATGGGACACGCCGGCCCA
SD_oligo_091	CGGAGACGCGTCACGGCCGAAGCAGAAGTTCAAGGACCCGGAGAAA
RR_oligo_021	CAAGCTTCTCTGCAGGATATCTGGACGCTGGCGCCGTTGATC
RR_oligo_022	ATCGCGCCCCGCTCACATGTTAGGTCCTCCCCCTCGC
RR_oligo_023	GGAGGACCTAACATGTGAGCGGGGCGCGATCCT
RR_oligo_024	CGGAGACGCGTCACGGCCGAAGGCGACATGCGGGTCAGCA
UPdel01635_F	TTCTCTGCAGGATATCTGGATCCACAATCGACGGCGAGACCTGGCTGGC
UPdel01635_R	TGACGGTTCTTAGAGCTCCAGGCTCAAGCGGGGGTTCCCCACGCT
DWNdel01635_F	CGCTTGAGCCTGGAGCTCTAAGAACCGTCACAAAGCGGCGCTATC
DWNdel01635_R	TCACGGCCGAAGCTAGCGAATTCCGCCGGCGTCGTGCGGGCCCGGCCCA
UPdel00270_F	TTCTCTGCAGGATATCTGGATCCATCGAGGGGAGGGGAACTTGAAGTTG
UPdel00270_R	GGGAATTTGTCAAGCGGCCATCAGGTCCTTCGGATAGGCGGTGCG
DWNdel00270_F	AAGGACCTGATGGCCGCTTGACAAATTCCCGAGCGTTCTTCGGGA
DWNdel00270_R	TCACGGCCGAAGCTAGCGAATTCGGGTGGCGCTCTGATCCTCCAGCAGG
UPdel00158_F	TTCTCTGCAGGATATCTGGATCCCATCCGTCGGGCGGTCGTCTCTATCG
UPdel00158_R	GGTGCGACGCGCCGCCGCCATGCGGGCGTTCTTAAGCGGTGTCGG
DWNdel00158_F	AACGCCCGCATGGCGGCGGCGCGTCGCACCGTGCGGCTCGAGGGCGAG
DWNdel00158_R	TCACGGCCGAAGCTAGCGAATTCCCGTCGCGGGCCCGCGCGGACGCCAGGG

References:

1 J. W. Modell, T. K. Kambara, B. S. Perchuk and M. T. Laub, A DNA damage-induced, SOS-independent checkpoint regulates cell division in Caulobacter crescentus, *PLoS Biol.*, 2014, **12**, e1001977.

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4 J. M. Skerker, M. S. Prasol, B. S. Perchuk, E. G. Biondi and M. T. Laub, Two-Component Signal Transduction Pathways Regulating Growth and Cell Cycle Progression in a Bacterium: A System-Level Analysis, *PLoS Biol.*, 2005, **3**, e334.

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