APPENDIX

Selective base excision repair of DNA damage by the non-base-flipping DNA glycosylase AlkC

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Appendix Table S1. Crystallographic data collection and refinement statistics

	SeMet-PfAlkC/THF-DNA	PfAlkC/1aR-DNA
Data collection		
Space group	$P2_12_12_1$	$P6_1$
Cell dimensions		
a, *b*, *c* (Å)	80.6, 94.9, 134.0	198.4, 198.4, 60.2
α, *β*, *γ* (°)	90.0, 90.0, 90.0	90.0, 90.0, 120.0
Resolution (Å)	50.00–2.40 (2.49–2.40) [a]	100.00–1.80 (1.86–1.80)
R_{sym}	0.109 (0.429)	0.092 (0.490)
Avg. $I/\sigma I$	23.0 (5.8)	24.8 (5.3)
Completeness (%)	99.7 (99.8)	99.1 (97.1)
Redundancy	9.8 (9.7)	9.7 (9.1)
Wilson *B*-factor (Å^2)	25.2	19.8
Refinement		
Resolution (Å)	40.42–2.40 (2.46–2.40)	49.29–1.80 (1.82–1.80)
No. reflections	40,519 (2,602)	124,478 (3,819)
R_{work}	0.168 (0.219)	0.141 (0.177)
R_{free} [b]	0.225 (0.333)	0.164 (0.202)
No. atoms [c]		
Protein	5,685	5,718
DNA	870	870
Solvent [d]	573	1,383
Avg. *B*-factors [c,e] (Å^2)		
Protein	25.6	23.0
DNA	25.6	25.1
Solvent [d]	29.2	37.5
R.m.s. deviations		
Bond lengths (Å)	0.008	0.008
Bond angles (°)	0.967	1.059
Ramachandran distribution (%)		
Favored	97.6	97.6
Allowed	2.4	2.2
Disallowed	0.0	0.1

[a] Statistics for the highest resolution shell are shown in parentheses.

[b] R_{free} was determined from the 5% of reflections excluded from refinement.

[c] Riding hydrogen atoms were not included in no. atoms or avg. *B*-factors.

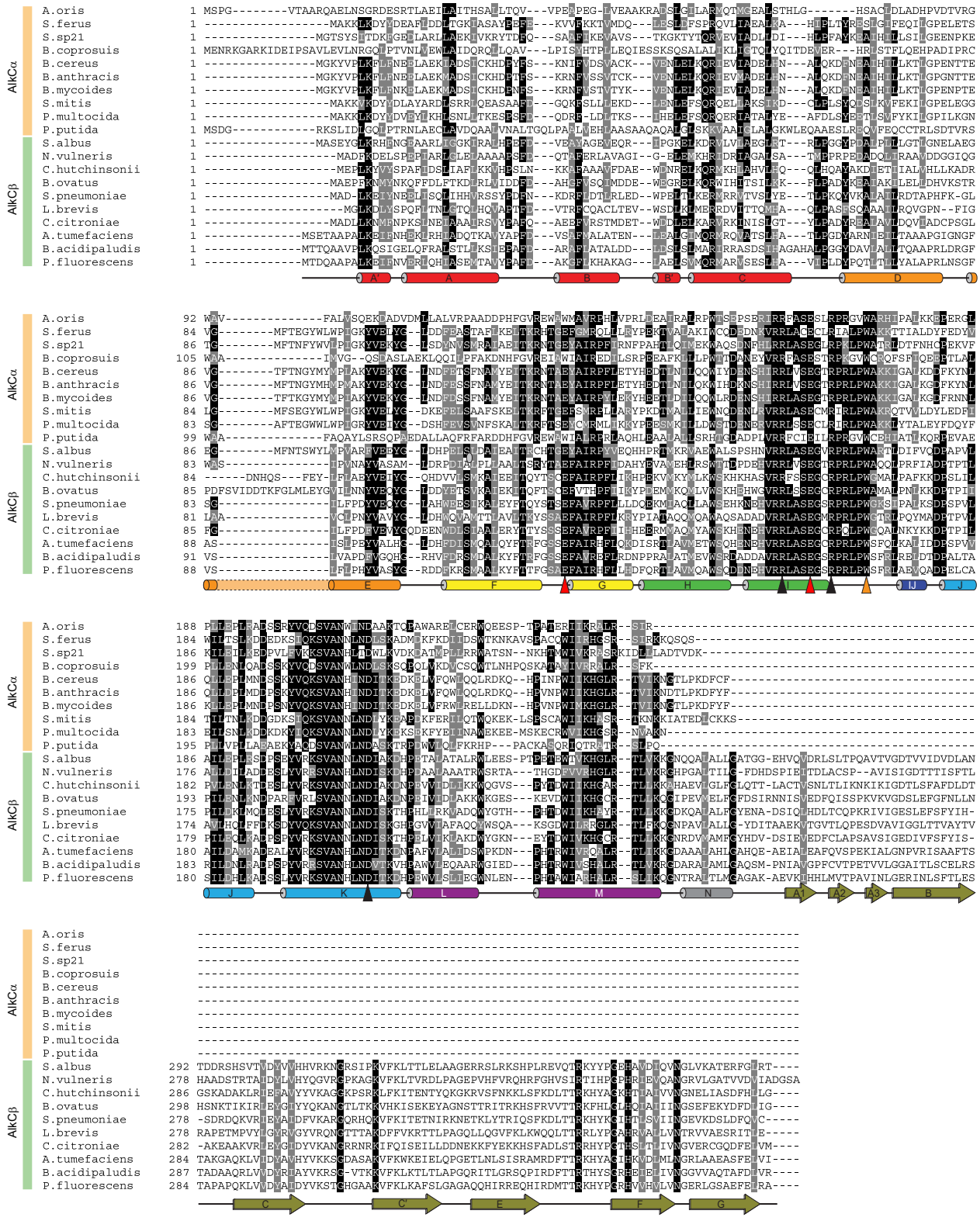
[d] In addition to water molecules, values for solvent include one PEG 4000 molecule in the THF structure and two Na+ ions, two pentaerythritol propoxylate, two glycerol, and three MES molecules in the 1aR structure.

[e] Equivalent isotropic *B*-factors were calculated in conjunction with TLS-derived anisotropic *B*-factors.

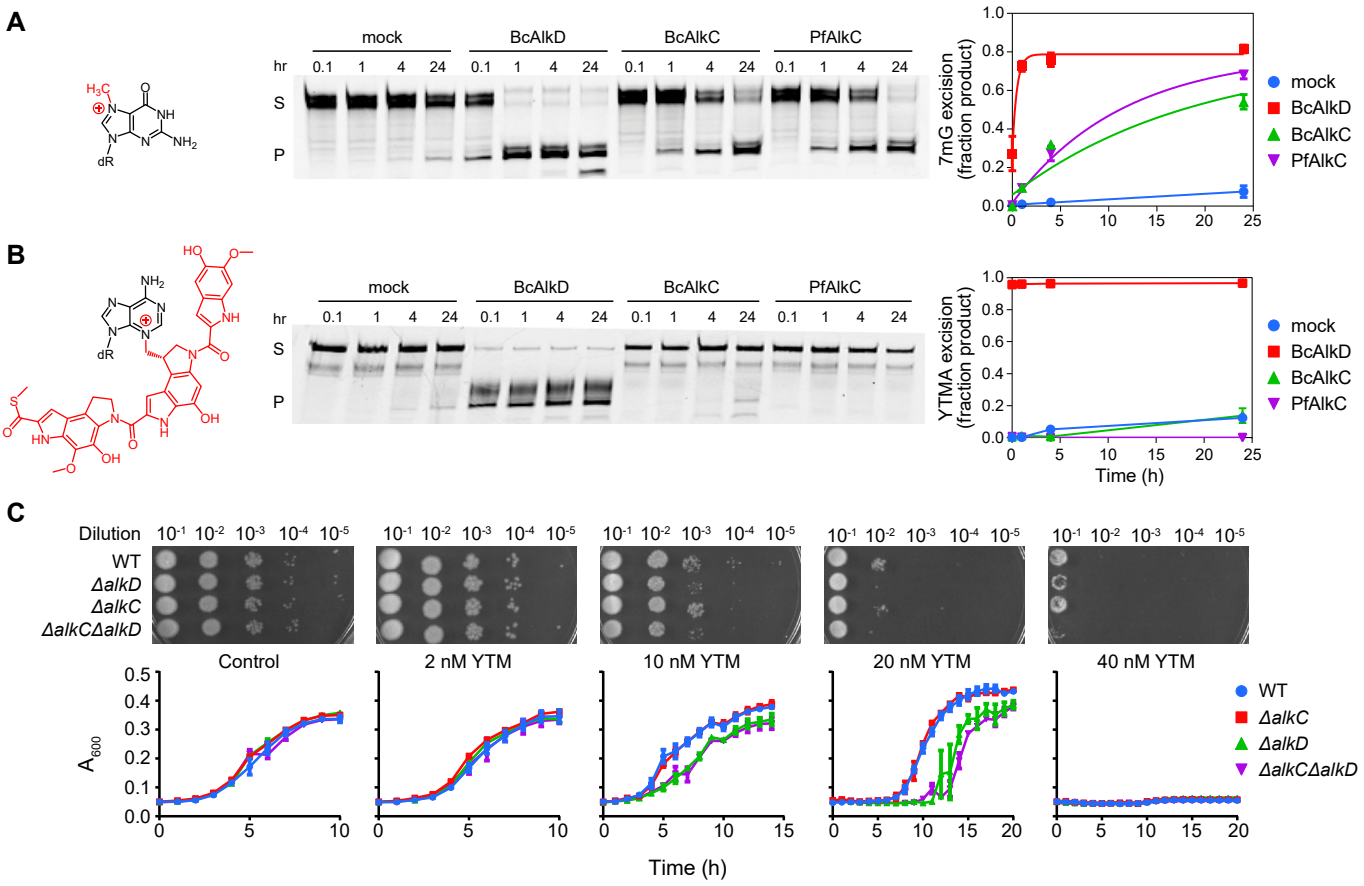
Appendix Table S2. Base excision activities [a]

	7mG	3mC	1mA
BcAlkD	1.3×10^{-2} [b]	6.1×10^{-7}	n.d.[c]
BcAlkC	1.5×10^{-5}	1.7×10^{-4}	6.9×10^{-6}
PfAlkC	2.4×10^{-5}	5.2×10^{-4}	2.4×10^{-5}
PfAlkC E121A		n.d.	n.d.
PfAlkC E156A		n.d.	n.d.
PfAlkC W164A		6.1×10^{-5}	5.6×10^{-6}

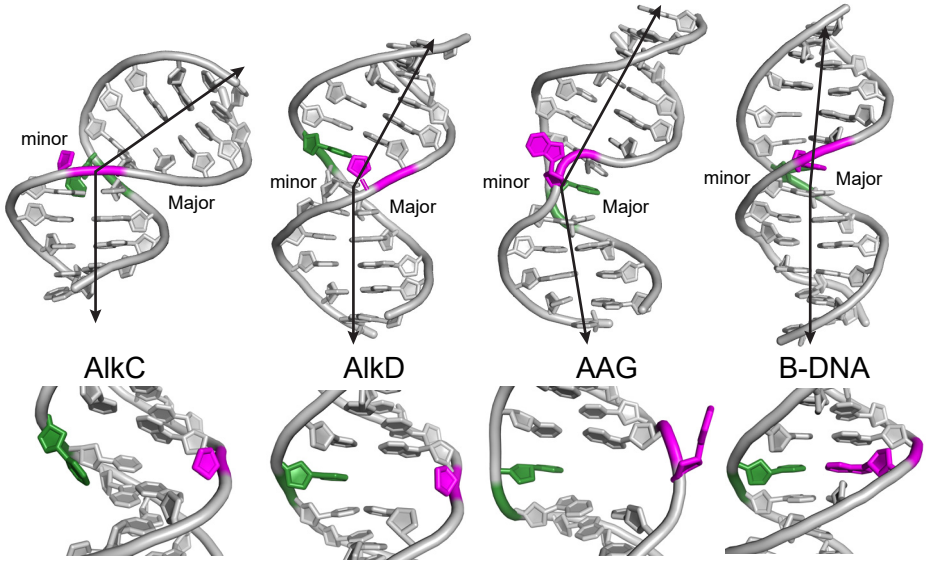
[a] Values are single-turnover rate constants (s^{-1}), averaged from three independent measurements. Errors (SD) were between 10-20% for each.
[b] From Parsons et al (2016) *J. Am. Chem. Soc.*, 138: 11485-8.
[c] n.d., none detected.



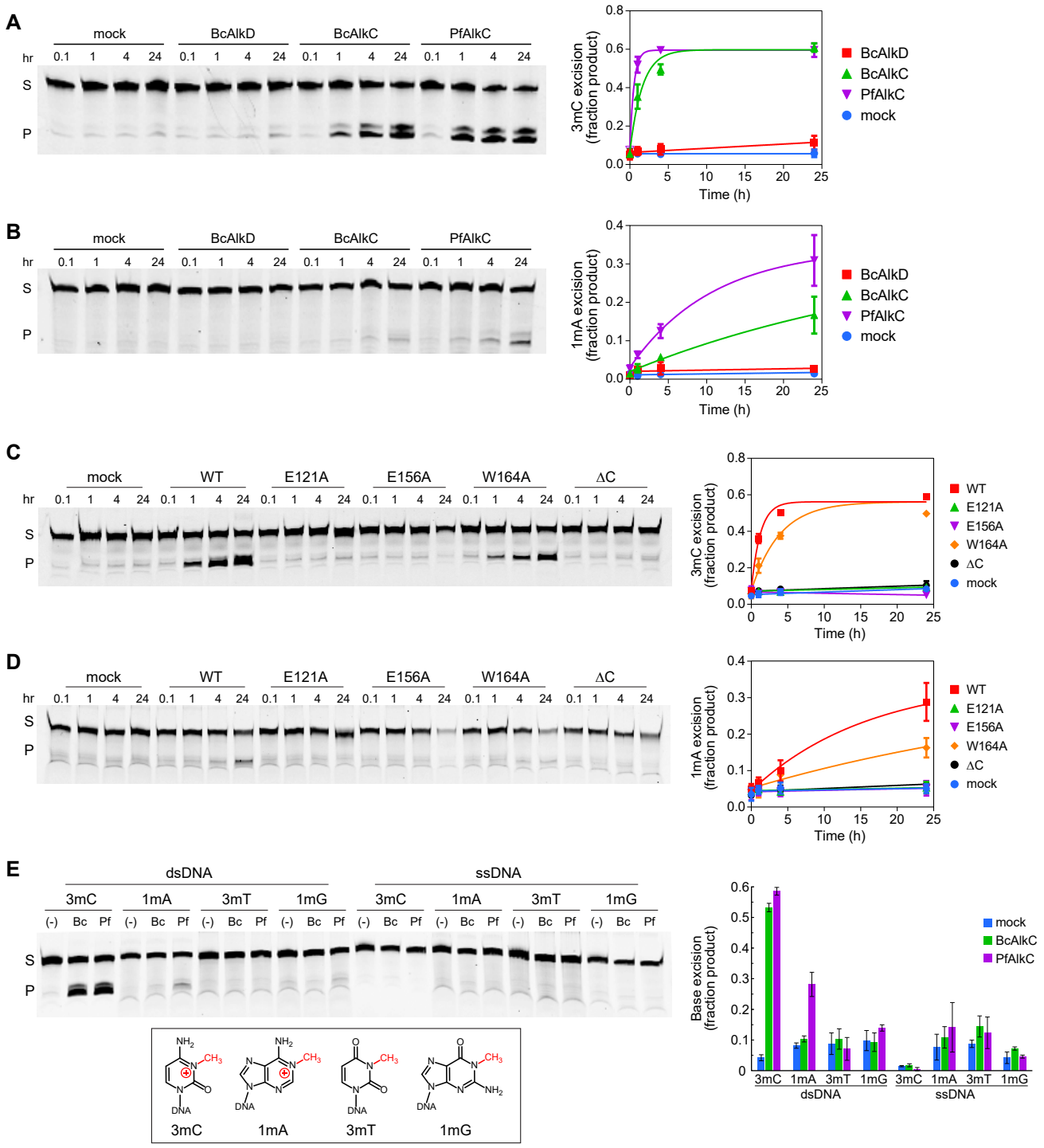
Appendix Figure S1. AlkCα and AlkCβ sequence alignment. Twenty selected sequences from four representative phyla (Actinobacteria, Bacteroideles, Firmicutes and Proteobacteria) were aligned using Clustal Omega and annotated using BoxShade (http://www.ch.embnet.org/software/BOX_form.html). Shaded residues have >50% sequence identity (black) and similarity (grey). Secondary structural elements identified from the PfAlkC crystal structure are shown below the sequences. Triangles designate PfAlkC residues important for base excision activity (red), nucleobase binding (orange) and for stabilizing the DNA backbone in the vicinity of the lesion (black).



Appendix Figure S2. Substrate specificities of AlkC and AlkD. A,**B**. Excision activity of BcAlkD and PfAlkC against oligodeoxynucleotides containing 7mG (A) and YTMA (B). Representative denaturing polyacrylamide gels show substrates (S) and hydroxide-nicked products (P) as a function of time. Quantification of the data is shown on the right; values are mean ± SD (n=3). The extra band below the 12-mer product in panel a corresponds to a nuclease contaminant in the AlkD preparation. The smearing in the AlkD-YTMA product band is a result of incompletely denatured GC-rich duplex DNA after hydroxide nicking. **C**. Growth of *B. anthracis* wild-type (blue), *ΔalkC* (red), *Δalk D* (green), and *ΔalkCΔalkD* (purple) in the presence of varying concentrations of yatakemycin (YTM). *Bacillus anthracis ΔalkC* cells were generated and their resistance to YTM assayed as described for *ΔalkD* (Mike et al, 2014; Mullins et al, 2015; Stauff & Skaar, 2009). Briefly, growth curves were obtained by growing cell cultures in the presence or absence of YTM and recording cell density every hour for 20 hours. Spot assays were performed by serial dilution of early-mid-log phase cells on LB plates prepared with or without YTM. Growth curves and spot assays were performed in triplicate.



Appendix Figure S3. Glycosylase-induced DNA distortion. DNA models from glycosylase-DNA co-crystal structures of PfAlkC/1aR-DNA (this work), BcAlkD/1aR-DNA (PDB 5KUB), and base-flipping human AAG/1,N^{6}-ethenoadenine-DNA (PDB 1EWN) compared to unbound B-DNA (PDB 1BNA). The modified and partner nucleotides are colored magenta and green, respectively. Black arrows depict the helical axes



Appendix Figure S4. AlkC excision of 3mC and 1mA. Representative denaturing electrophoresis gels show substrate (S) and NaOH-nicked abasic-DNA product (P) over a 24-hour incubation. Values in the plots are mean ± SD (n=3). **A,B.** 3mC-DNA (A) and 1mA-DNA (B) excision by no enzyme (mock), BcAlkD, BcAlkC, or PfAlkC. **C,D.** 3mC (C) and 1mA (D) excision by no enzyme (mock), wild-type PfAlkC (WT), or PfAlkC mutants (E121A, E156A, W164A, ΔC). **E.** 24-hour incubation of no enzyme [(-), mock], BcAlkC, or PfAlkC with double-stranded (ds) or single-stranded (ss) oligodeoxynucleotides containing a 3mC, 1mA, 1mG, or 3mT modification.

Appendix References

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