Supplementary tables

Table S1. Primers for mutagenesis.

Primers	Sequence
EndoIII1 R61Q	Fw ATCCTCTCGCAG**CAG**ACCACCCACGCCGAC Rv GTCGGCGTGGGTGG**TCT**GCTGCGAGAGGAT
EndoIII1 Y100L	Fw CGCCGCAGCAAT**CTG**CCTGAGAGCAAGGCC Rv GGCCTTGCTCTCAGG**CAG**ATTGCTGCGGCG
EndoIII2 C199A	Fw GCCGCCGCGT**CGC**CCACGCCCGCA Rv TGCGGGCGTGG**GCG**ACGCGGCGGC
EndoIII2 C199S	Fw CCGCCGCGT**CAG**CCACGCCCG Rv CGGGCGTGG**CTG**ACGCGGCGG
EndoIII2 C206S	Fw GCAAGCCCC**AGA**GCCCGTCGTGC Rv GCACGACGGG**CTC**TGGGGCTTGC
EndoIII2 C209S	Fw CCA GTG CCC GTC **GAG** CGA ATT GGC GAG Rv CTC GCC AAT TCG **CTC** GAC GGG CAC TGG
EndoIII2 C215S	Fw GAATTGGCGAGCTTC**AGT**CCGAAAGTAGGGG Rv CCCCTACTTTCGGAC**TGA**AGCTCGCCAATTC
EndoIII3 G250TN251H	Fw ATGCCGGTGGAT**ACCCAC**ATGGAACGGGCG Rv CGCCCGTTCCAT**GTGGGT**ATCCACCGGCAT
EndoIII3 R139D	Fw GCAGCAAAACAC**CGA**CCGGGTCGCGCAG Rv CTGCGCGACCCG**GTC**GGTGTTTTGCTGC

Table S2. X-ray data collection and refinement statistics. Values in parentheses belong to the highest resolution shell.

	EIII1 R61Q	**EIII1 Y100L**	**EIII3 G250T/N251H**
Data collection			
Beamline	ALBA XALOC	ALBA XALOC	ALBA XALOC
Wavelength (Å)	0.97926	0.97926	0.97926
Space group	*C 1 2 1*	*C 1 2 1*	*C 1 2 1*
Unit cell parameters (Å, °)	*a* = 179.3, *b* = 38.0, *c* = 36.4, β= 91.0	*a* = 178.5, *b* = 37.8, *c* = 36.6, β= 90.6	*a* = 90.3, *b* = 38.8, *c* = 72.0, β= 99.7
Resolution (Å)	44.82 – 1.38 (1.47 – 1.38)	44.63 - 1.95 (2.05 – 1.95)	44.53 – 1.89 (1.99 – 1.89)
Nr. observations	146093 (22818)	56386 (9040)	65506 (9163)
Nr. unique reflections	50163 (8090)	17563 (2766)	19937 (3111)
Completeness (%)	98.9 (99.1)	97.1 (96.2)	99.1 (97.6)
Multiplicity	2.9 (2.8)	3.2 (3.3)	3.3 (2.9)
Mosaicity (°)	0.16	0.14	0.15
$CC_{1/2}$ (%)[a]	99.4 (46.2)	99.7 (49.7)	99.9 (49.8)
R_{sym} (%)[b]	5.8 (89.6)	4.9 (74.9)	4.2 (54.4)
R_{meas} (%)[c]	7.2 (83.9)	6.2 (91.4)	5.9 (71.2)
$R_{p.i.m.}$ (%)[d]	4.0 (31.3)	3.4 (31.1)	3.0 (28.3)
<I/σ(I)>	8.85 (0.99)	9.88 (1.07)	13.71 (1.61)

Wilson B ($Å^2$)	31.6	56.7	39.1
V_M ($Å^3$ Da^{-1})	2.08	2.08	1.98
Estimated solvent content (%)	41.0	40.8	37.8
Refinement			
R_{work} (%)[e]	15.1	19.4	23.8
R_{free} (%)[e]	18.0	24.7	28.9
rmsd for bond lengths (Å)	0.005	0.008	0.008
rmsd for bond angles (°)	0.696	0.824	0.870
Structure <*a.d.p.*> ($Å^2$)	36.1	75.8	50.6
Ramachandran plot			
Residues in favored regions (%)	97.9	94.5	97.0
Residues in allowed regions (%)	2.1	5.5	3.0
Residues in disallowed regions (%)	0	0	0
PDB code	8A5F	8A5C	8A5G

[a] $CC_{1/2}$ = Percentage of correlation between intensities from random half-datasets (Karplus & Diederichs, 2012).

[b] R_{sym} = Σhkl Σi |Ii(hkl) - <I(hkl)>|/ Σhkl Σi Ii (hkl), where Ii(hkl) is the observed intensity and <I(hkl)> is the average intensity of multiple observations from symmetry-related reflection (Arndt et al., 1968).

[c] R_{meas} = Σhkl [N/(N(hkl) -1)]1/2 Σi |Ii(hkl) - <I(hkl) >|/Σhkl Σi Ii (hkl), where N(hkl) is the data multiplicity, Ii(hkl) is the observed intensity and <I(hkl)> is the average intensity of multiple observations from symmetry-related reflections. It is an indicator of the agreement between symmetry related observation (Diederichs & Karplus, 1997).

[d] $R_{p.i.m.}$ = Σhkl [1/(N(hkl) -1)]1/2 Σi |Ii(hkl) - <I(hkl) >|/Σhkl Σi Ii (hkl), where N(hkl) is the data multiplicity, Ii(hkl) is the observed intensity and <I(hkl)> is the average intensity of multiple observations from symmetry-related reflections. It is an indicator of the precision of the final merged and averaged data set (Weiss, 2001).

[e] R_{work} refers to the actual working data set used in refinement, while R_{free} refers to a cross validation set that is not directly used in refinement and is therefore free from refinement bias.

Table S3. Oligonucleotides used for probing DrEndoIII activity.

SUBSTRATE	SEQUENCE
TG SUSBTRATE (6-FAM)	TGTCAATAGCAAG(**ThyGly**)GGAGAAGTCAATCGTGAGTCT
URACIL SUSBTRATE (6-FAM)	TGTCAATAGCAAG**U**GGAGAAGTCAATCGTGAGTCT
REVERSE G	AGACTCACGATTGACTTCTCC**G**CTTGCTATTGACA
REVERSE A	AGACTCACGATTGACTTCTCC**A**CTTGCTATTGACA
THF SUBSTRATE (6-FAM)	AGCTACCATGCCTGCACGAA**X**TAAGCAATTCGTAATCATGGTCAT
REVERSE G	ATGACCATGATTACGAATTGCTTA**G**TTCGTGCAGGCATGGTAGCT
FORWARD C	AGCTACCATGCCTGCACGAA**C**TAAGCAATTCGTAATCATGGTCAT

Table S4. DrEndoIII native and mutated enzymes temperature melting point.

Protein	TM (°C)
EndoIII1	63
EndoIII2	62
EndoIII3	61
EndoIII1 R61Q	62.4
EndoIII1 Y100L	62.13
EndoIII3 G250T/N251H	60

Table S5. Determination in percentage through RR, of the amount of [3Fe-4S] and [4Fe-4S] present in solution in DrEndoIII2 C199S.

time (h)	[3Fe-4S]	[4Fe-4S]
0	74	26
5	79	21
16	85	15

Supplementary Figures

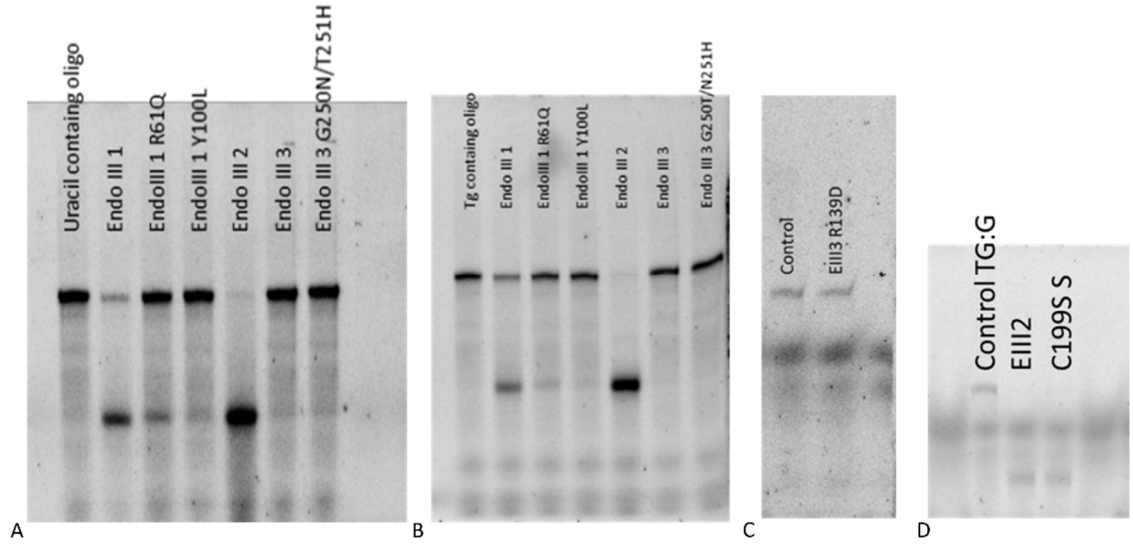

Figure S1. Denaturing gel electrophoresis analysis of the processing of Tg containing DNA by DrEndoIII1 and DrEndoIII2 and DrEndoIII3 (wild-type and mutants). AP-lyase activity (A), DNA glycosylase activity (B), activity of DrEndoIII3 R139D (C) and activity of DrEndoIII2 C199S (D).

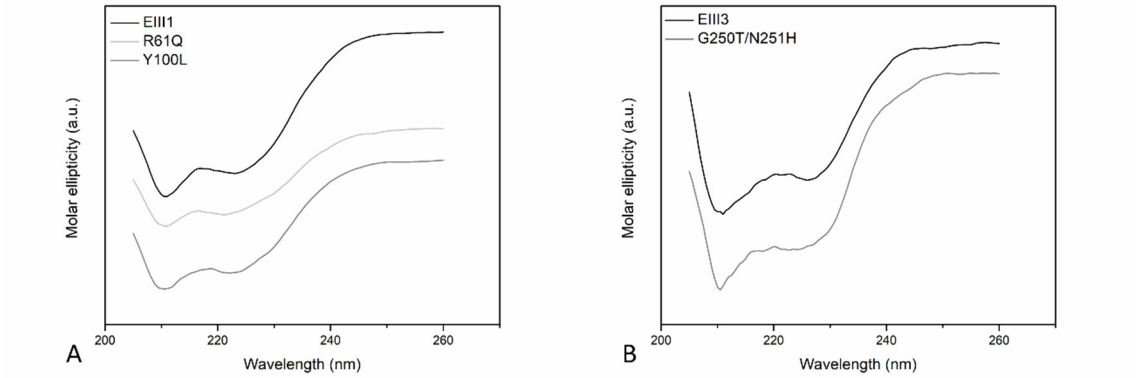

Figure S2. CD spectra of DrEndoIII1, DrEndoIII1 R61Q and Y100L (A), and DrEndoIII3 and DrEndoIII3 G250T/N251H (B).

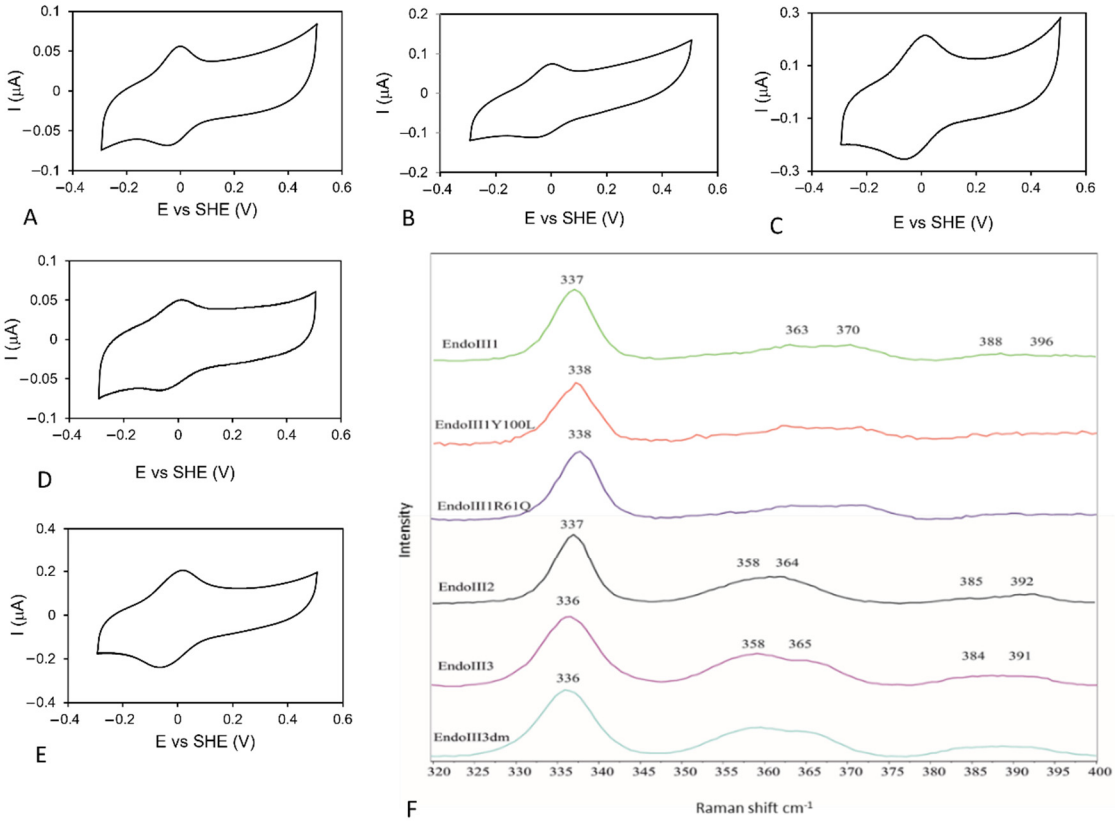

Figure S3. CV of DrEndoIII1 R61Q (A) and Y100L (B); DrEndoIII3 (C) and DrEndoIII3G250T/N251H (D); DrEndoIII2 (E), on Au electrodes coated with 11-mercaptoundecanoic acid (MUA) in 50 mM Tris-Hcl pH 8.5, 50 mM NaCl buffer. Scan rate: 50 mV/s. F) RR spectra DrEndoIII1, DrEndoIII1R61Q and DrEndoIII1Y100L; DrEndoIII2; DrEndoIII3 and DrEndoIII3G250T/N251H in the resting state.

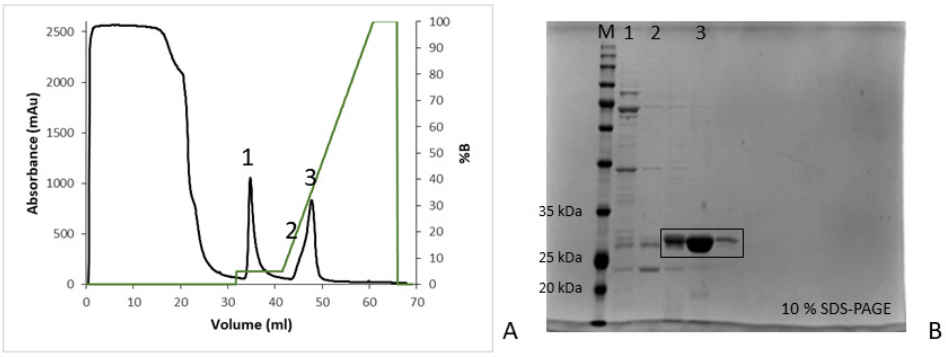

Figure S4. Chromatogram of HisTrap purification of DrEndoIII2 C199S (A) and SDSPAGE analysis of DrEndoIII2 C199S purification from different peaks (1, 2 and 3) highlighted in the chromatogram (B) (M- NZYColour protein marker II from NZYTech).

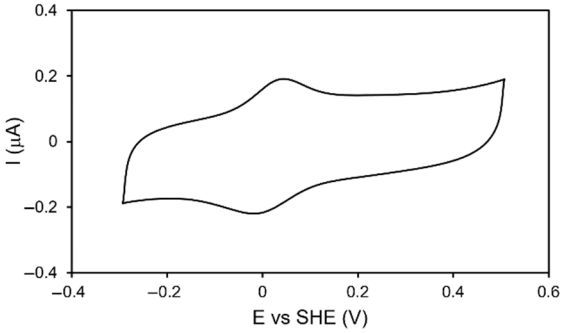

Figure S5. CV DrEndoIII2 C199S immobilized on Au electrodes coated with 11-mercaptoundecanoic acid (MUA) in 50 mM Tris-Hcl pH 7.5 (B), 50 mM NaCl buffer terminated SAM. V_{scan} 50 mV/s.