The influence of cdG on 8-oxodG excision by OGG1 and FPG glycosylases.

Supplementary Materials

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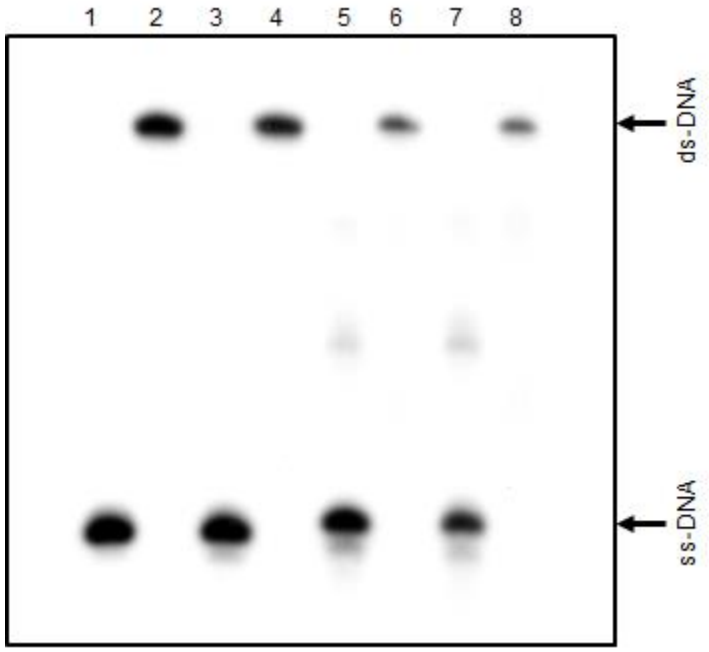

Figure S1. Visualization of efficient labeling (ssDNA) and hybridization (dsDNA) of oligonucleotides. Upper row of bands show 40-mer ds-DNA, lower row of bands show 40-mer ss-DNA. The order of tested oligonucleotides was as follows: -6/+6(H/dA) (lanes 3, 4), -6/+6(H/ScdG (lanes 5, 6), -6/+6(H/RcdG) (lanes 7, 8). Lanes 1 and 2 are not a part of this study.

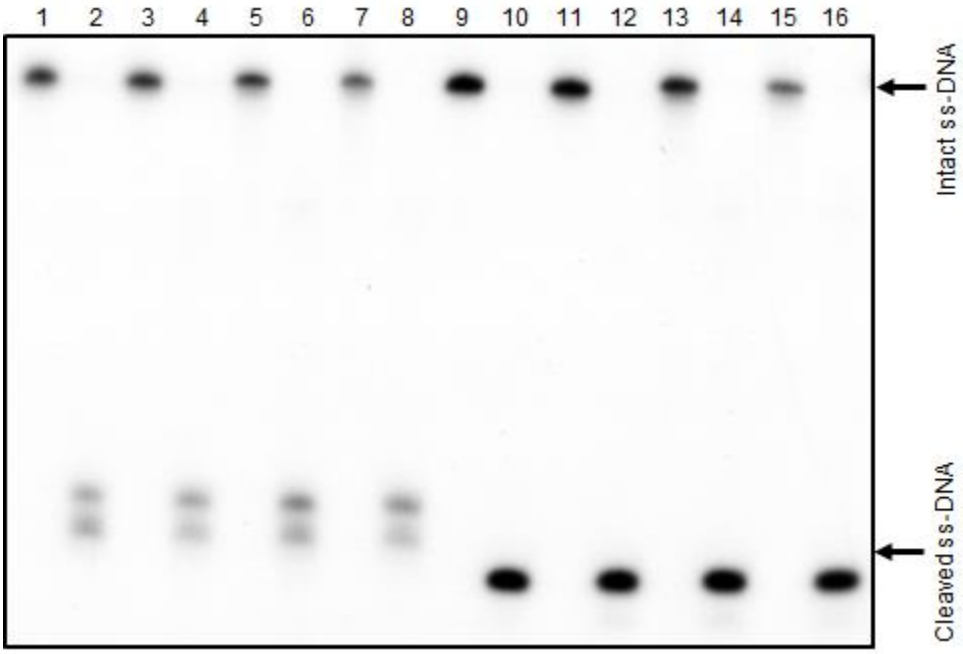

Figure S2. Visualization of ds-DNA oligonucleotides cleavage by 0.5U OGG1 (lanes 3-8) or 0.5U FPG (lanes 11-16). Upper row of bands shows intact 40-mer oligonucleotides, while lower row of bands shows cleavage after maximum reaction time (300 min for OGG1 and 120 min for FPG). The order of tested oligonucleotides was as follows: -6/+6(H/dA) (lanes 3-4, 11-12), -6/+6(H/ScdG (lanes 5-6, 13-14), -6/+6(H/RcdG) (lanes 7-8, 15-16). Lanes 1, 2, 9 and 10 are not a part of this study.

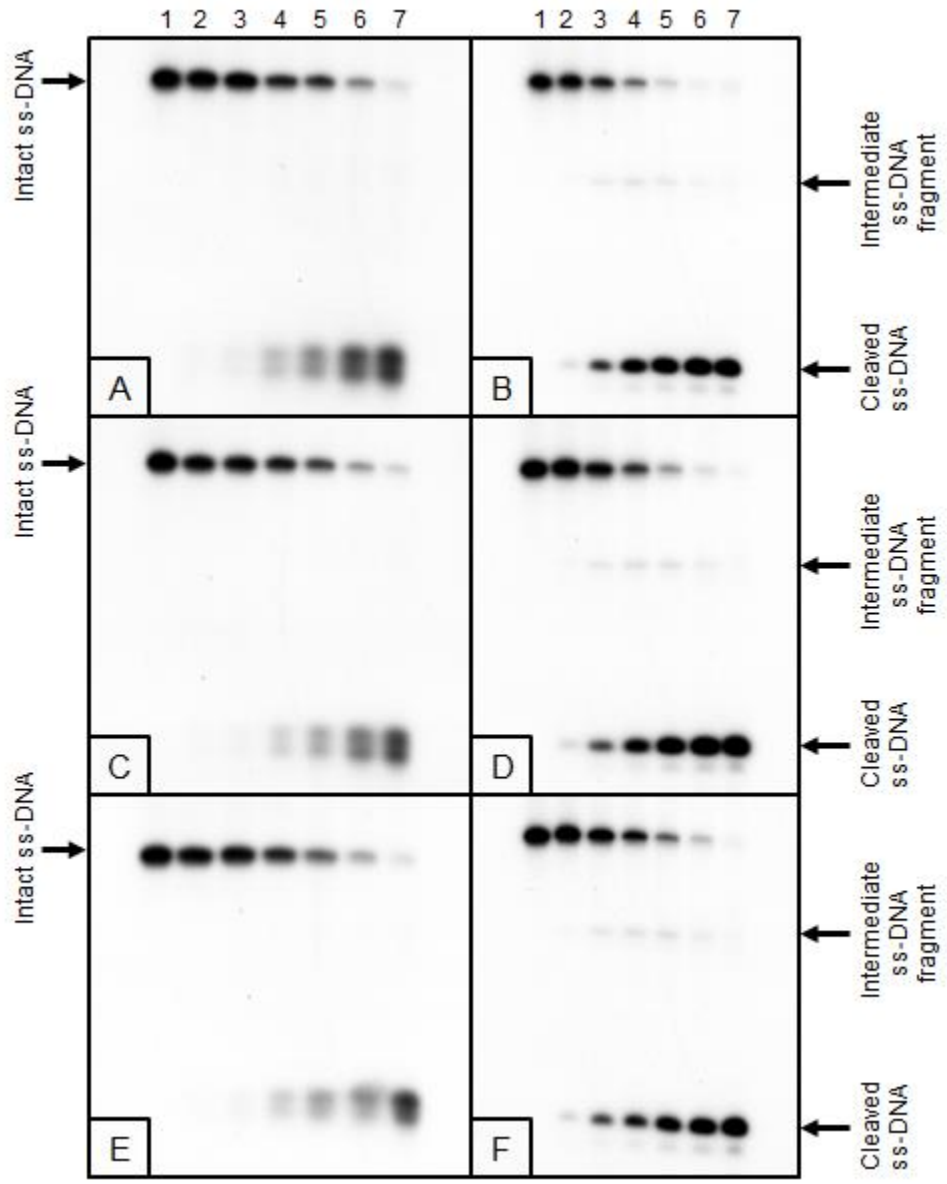

Figure S3. Cleavage assay of -6/+6(H/dA) by 0.5U OGG1 (A ,C, E) or 0.5U FPG (B, D, F). (A), (C) and (E) show 7 lanes each, which correspond to reaction times 0, 30, 60, 120, 180, 240, and 300 min starting from the left. (B), (D) and (F) show 7 lanes each, which correspond to reaction times 0, 1, 5, 15, 30, 60 and 120 min starting from the left.

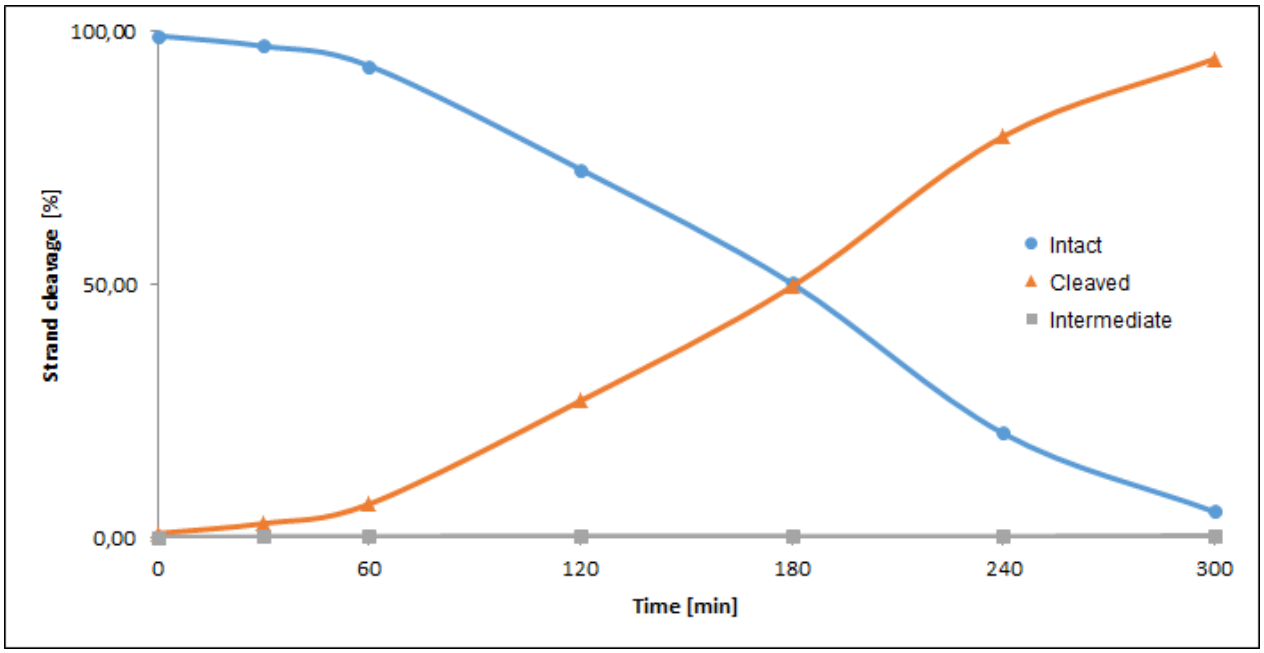

Figure S4. Cleavage assay of -6/+6(H/dA) by 0.5U OGG1. The quantity loss of intact ssDNA (blue), the quantity increase of SSB-DNA (orange) and an intermediate oligo fragment (grey).

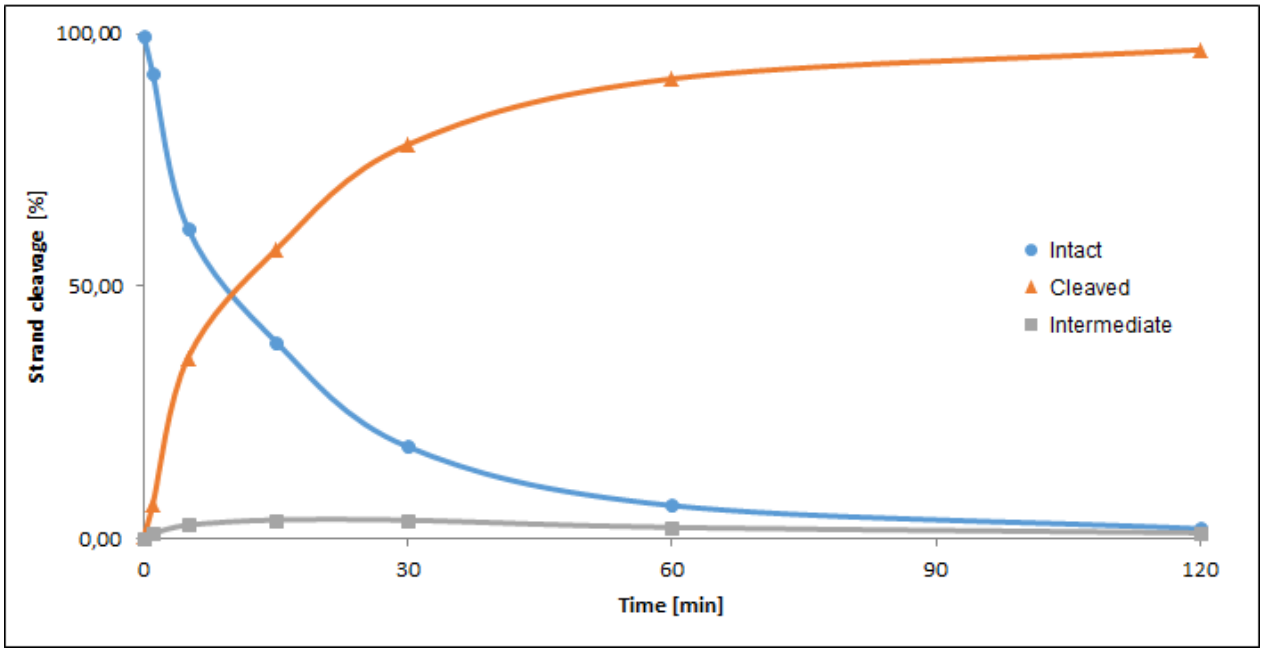

Figure S5. Cleavage assay of -6/+6(H/dA) by 0.5U FPG. The quantity loss of intact ssDNA (blue), the quantity increase of SSB-DNA (orange) and an intermediate oligo fragment (grey).

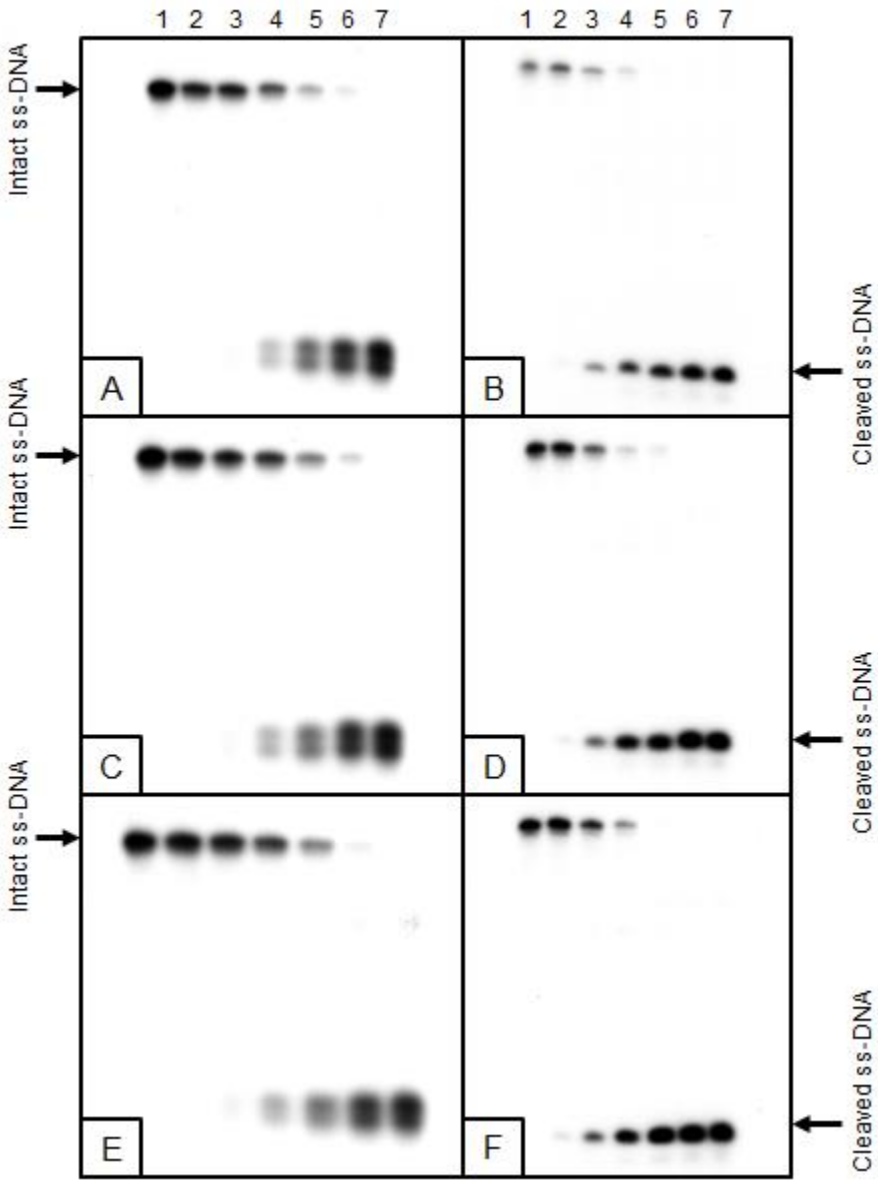

Figure S6. Cleavage assay of -6/+6(H/ScdG) by 0.5U OGG1 (A,C,E) or 0.5U FPG (B,D,F). (A), (C) and (E) show 7 lanes each, which correspond to reaction times 0, 30, 60, 120, 180, 240, and 300 min starting from the left. (B), (D) and (F) show 7 lanes each, which correspond to reaction times 0, 1, 5, 15, 30, 60 and 120 min starting from the left.

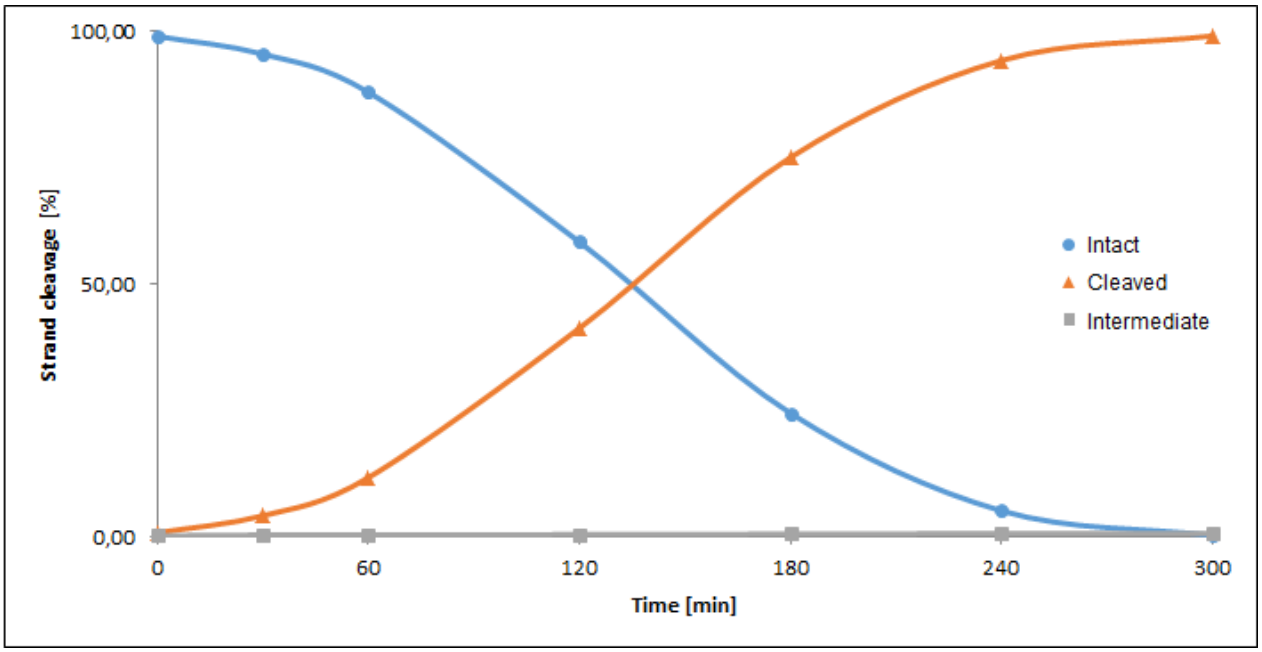

Figure S7. Cleavage assay of -6/+6(H/ScdG) by 0.5U OGG1. The quantity loss of intact ssDNA (blue), the quantity increase of SSB-DNA (orange) and an intermediate oligo fragment (grey).

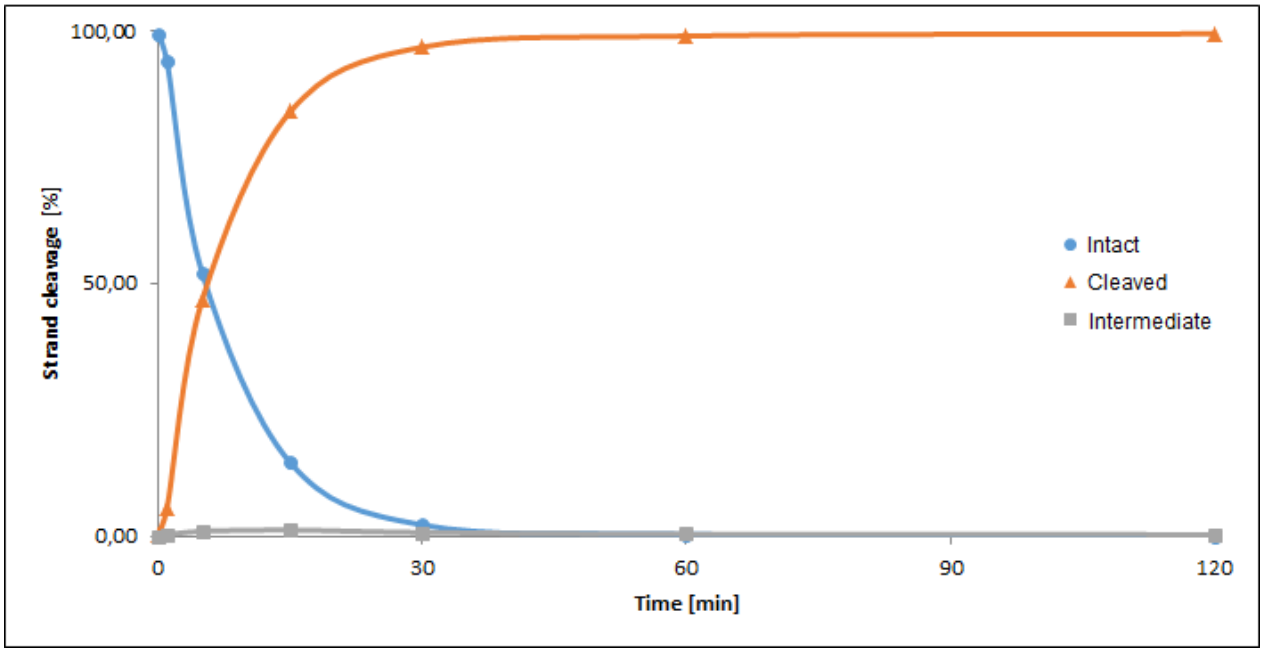

Figure S8. Cleavage assay of -6/+6(H/ScdG) by 0.5U FPG. The quantity loss of intact ssDNA (blue), the quantity increase of SSB-DNA (orange) and an intermediate oligo fragment (grey).

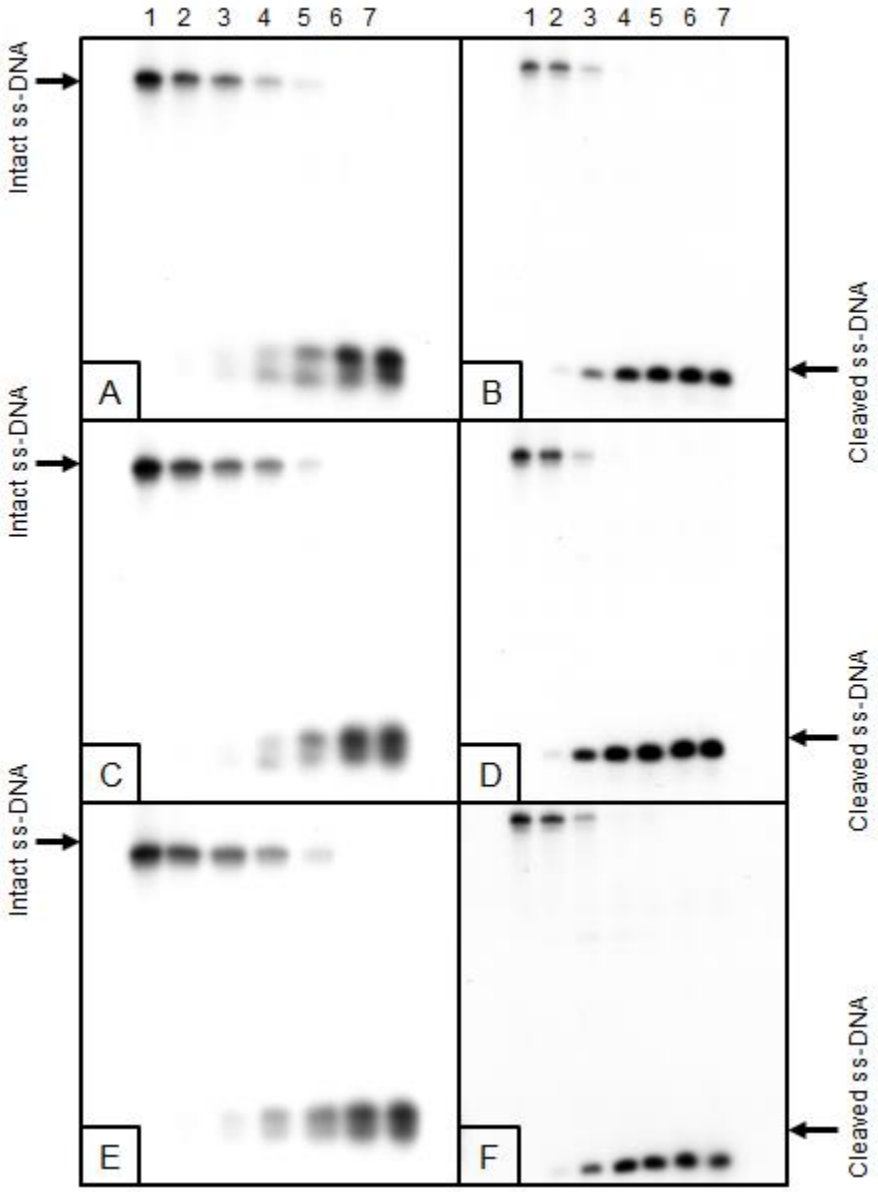

Figure S9. Cleavage assay of -6/+6(H/RcdG) by 0.5U OGG1 (A,C,E) or 0.5U FPG (B,D,F). (A), (C) and (E) show 7 lanes each, which correspond to reaction times 0, 30, 60, 120, 180, 240, and 300 min starting from the left. (B), (D) and (F) show 7 lanes each, which correspond to reaction times 0, 1, 5, 15, 30, 60 and 120 min starting from the left.

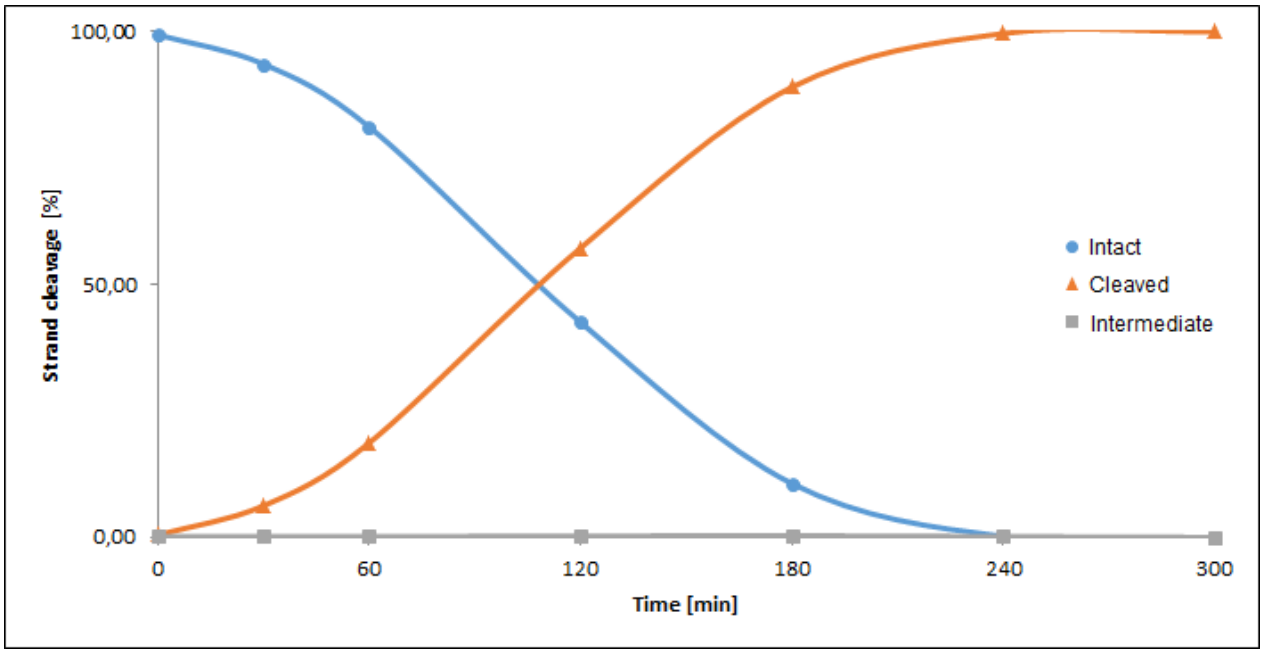

Figure S10. Cleavage assay of -6/+6(H/RcdG) by 0.5U OGG1. The quantity loss of intact ssDNA (blue), the quantity increase of SSB-DNA (orange) and an intermediate oligo fragment (grey).

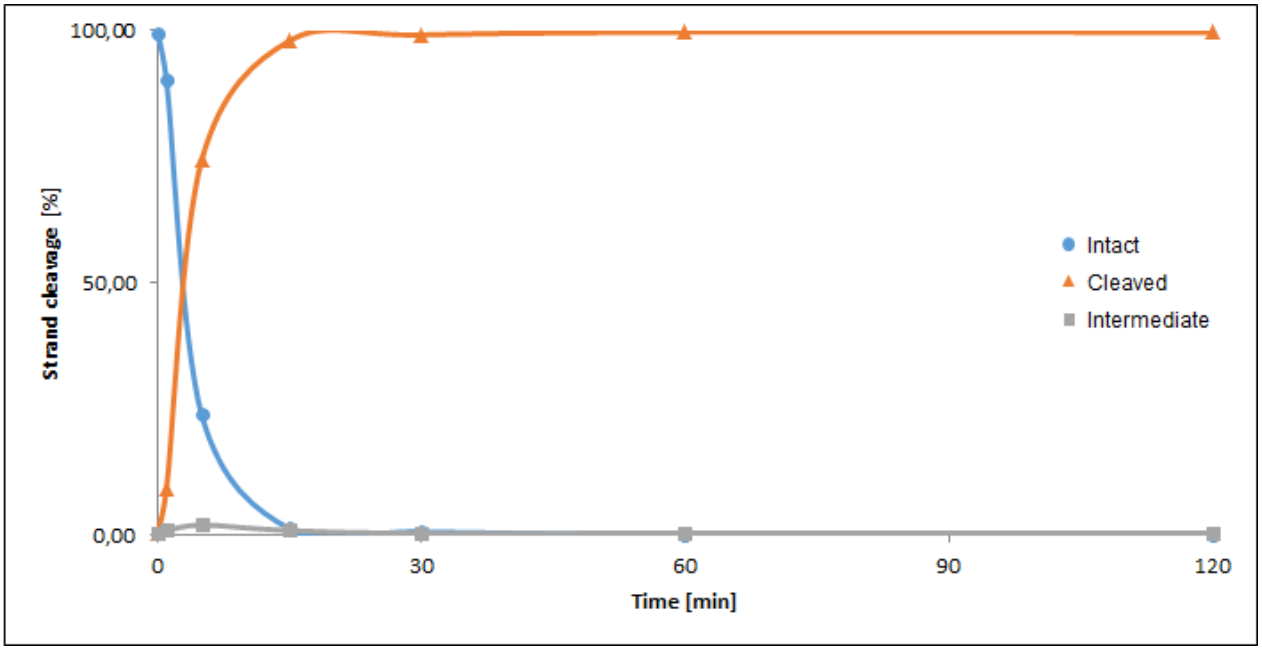

Figure S11. Cleavage assay of -6/+6(H/RcdG) by 0.5U FPG. The quantity loss of intact ssDNA (blue), the quantity increase of SSB-DNA (orange) and an intermediate oligo fragment (grey).

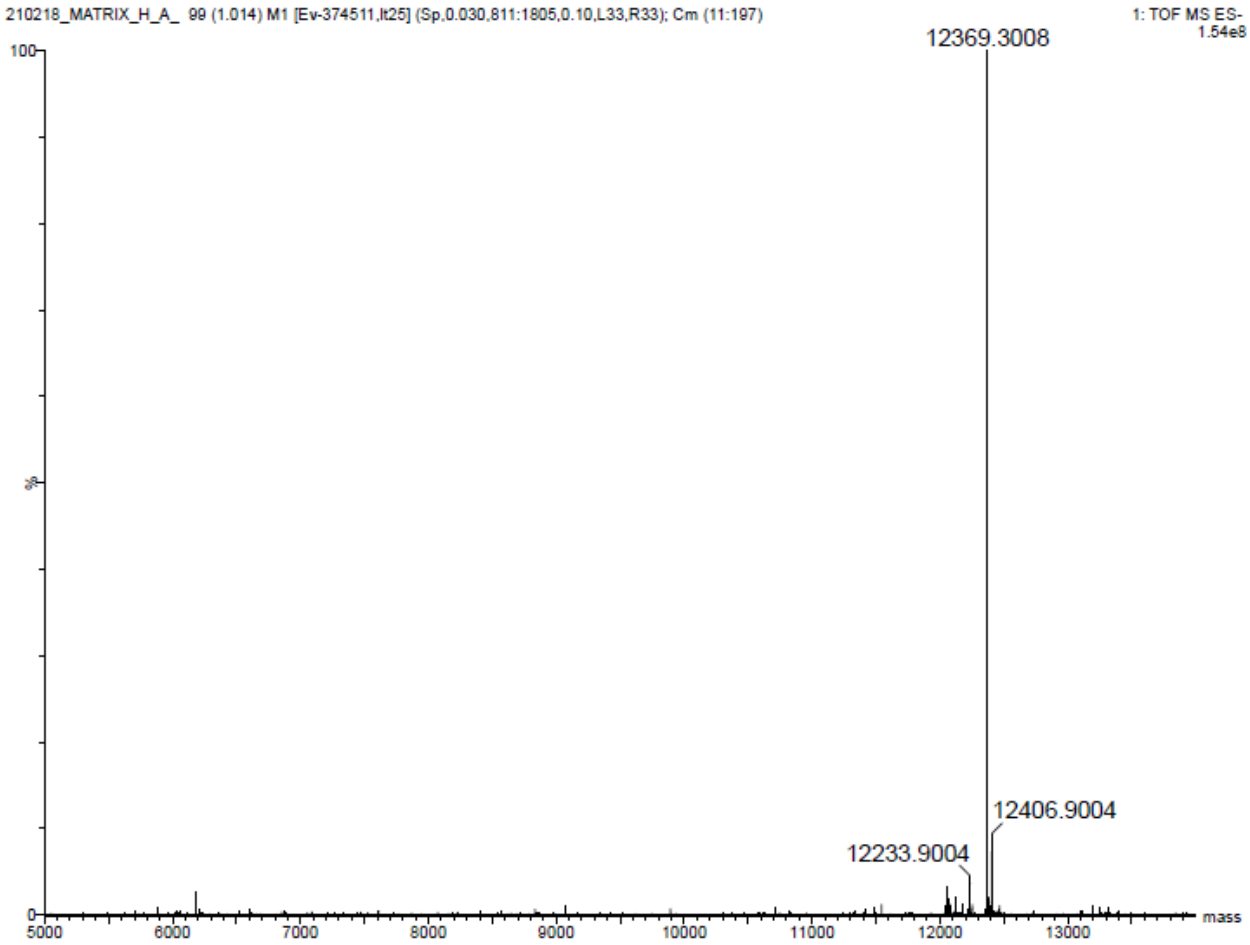

Figure S12. Mass spectrum of Matrix H/A.

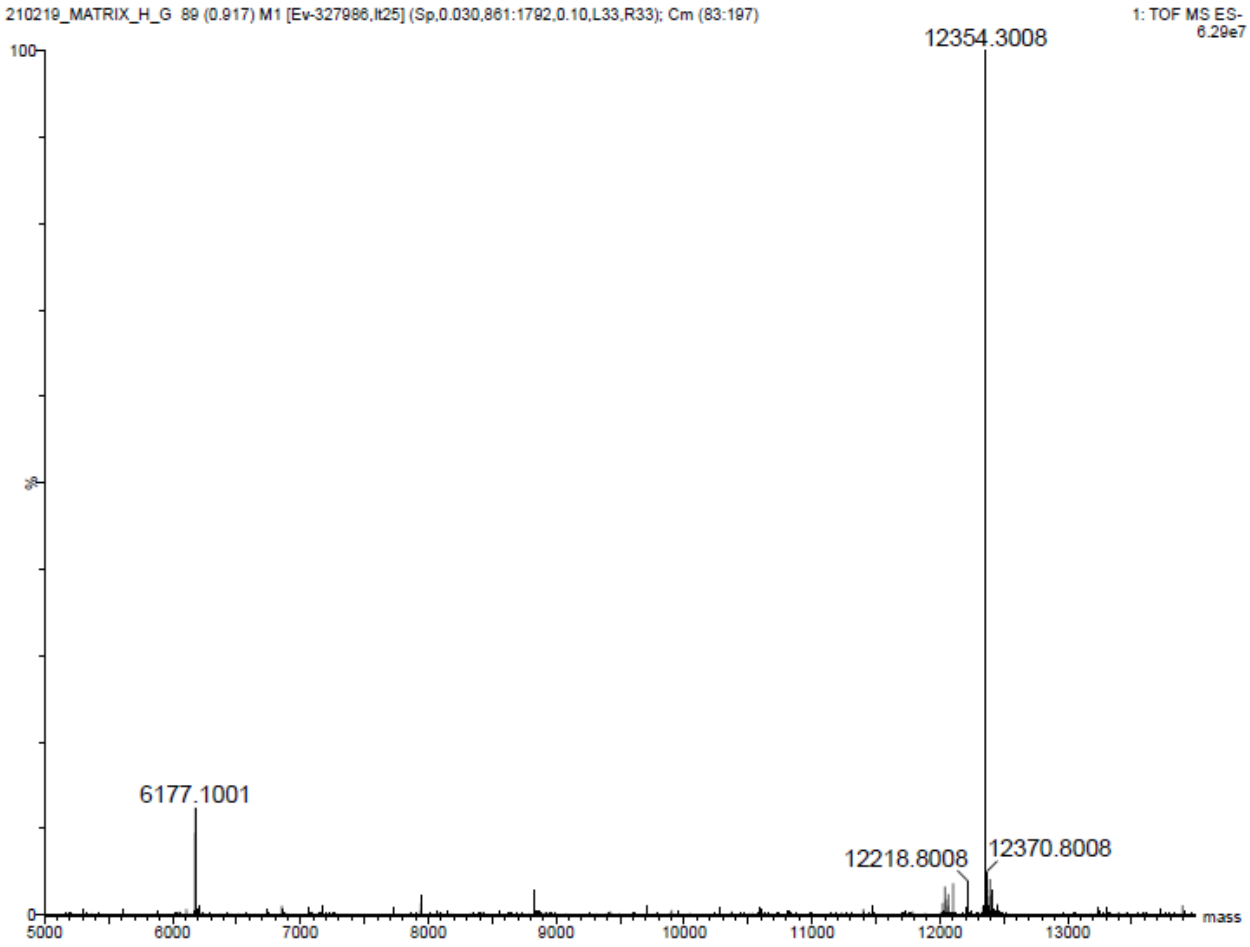

Figure S13. Mass spectrum of Matrix H/G.

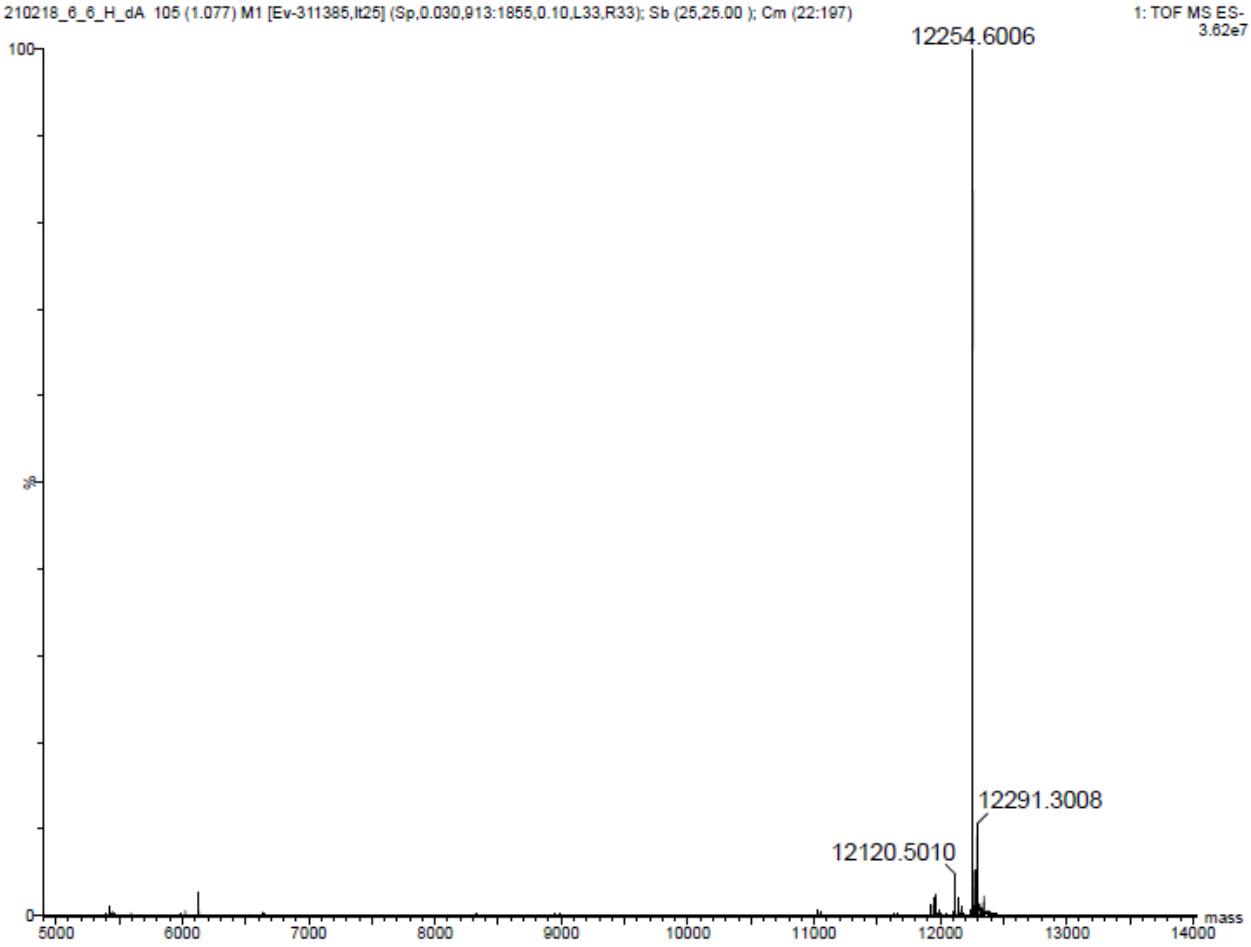

Figure S14. Mass spectrum of -6/+6(H/dA).

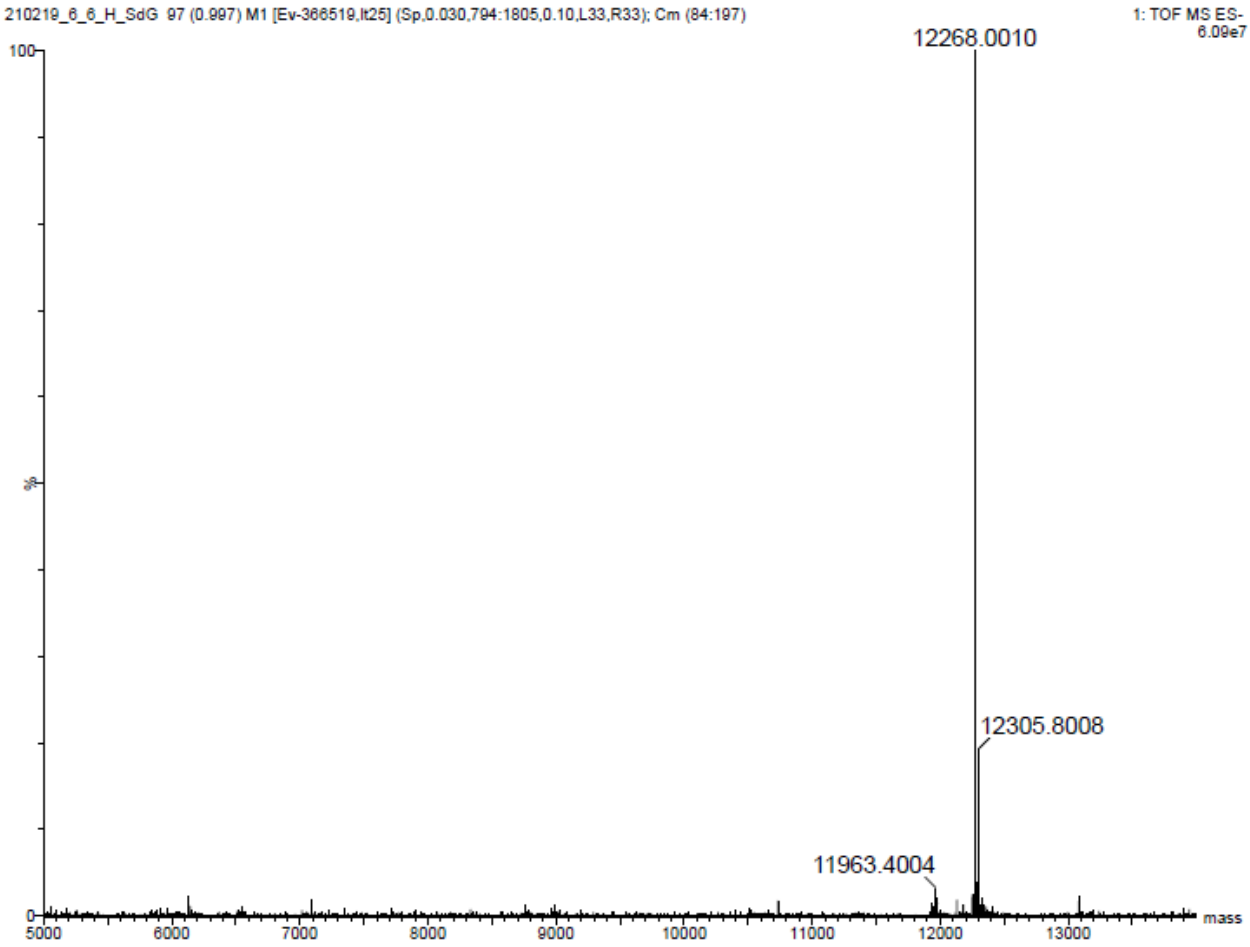

Figure S15. Mass spectrum of -6/+6(H/ScdG).

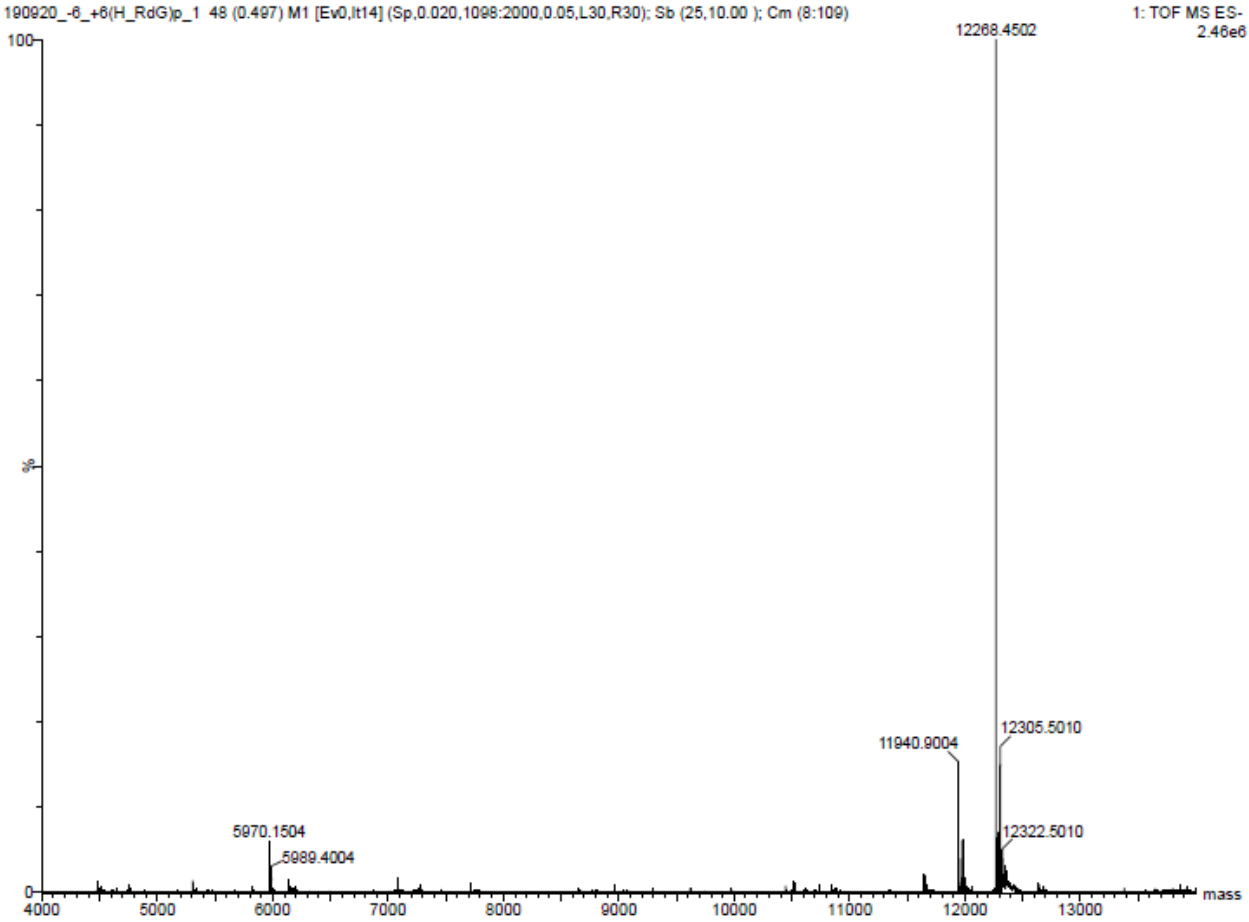

Figure S16. Mass spectrum of -6/+6(H/RcdG).

Table S1. Raw numerical data of OGG1 cleavage assays obtained from Quantity One software.

Oligo	Time [min]	1	2	3	Avg	SD	1	2	3	Avg	SD	1	2	3	Avg	SD
		Intact oligonucleotide [%]					Cleaved strand [%]					Intermediate fragment [%]				
Native control oligonucleotides																
-6/+6 (H/dA)	0	98,58	98,95	99,99	99,17	0,73	1,36	1,03	0,01	0,80	0,70	0,06	0,02	0,00	0,03	0,03
	30	95,73	97,22	98,40	97,12	1,34	4,27	2,47	1,54	2,76	1,39	0,00	0,31	0,06	0,12	0,16
	60	90,13	94,70	94,84	93,23	2,68	9,44	5,30	5,04	6,59	2,47	0,43	0,00	0,12	0,18	0,22
	120	64,17	77,51	76,60	72,76	7,45	35,28	22,49	23,22	26,99	7,18	0,55	0,00	0,18	0,24	0,28
	180	45,33	53,14	52,15	50,21	4,25	54,42	46,70	47,60	49,58	4,22	0,24	0,16	0,25	0,22	0,05
	240	18,42	19,48	23,87	20,59	2,89	81,25	80,49	75,99	79,24	2,84	0,33	0,03	0,15	0,17	0,15
	300	4,40	5,96	4,90	5,09	0,80	95,26	93,77	94,70	94,58	0,75	0,34	0,27	0,40	0,34	0,06
Oligonucleotides containing cdG																
-6/+6 (H/ScdG)	0	98,11	99,14	99,91	99,06	0,90	1,63	0,59	0,09	0,77	0,78	0,26	0,26	0,00	0,18	0,15
	30	92,99	96,30	97,30	95,53	2,26	6,67	3,49	2,27	4,14	2,27	0,34	0,21	0,43	0,33	0,11
	60	86,47	88,14	89,31	87,97	1,43	13,24	11,68	10,17	11,70	1,53	0,29	0,19	0,52	0,33	0,17
	120	54,11	59,45	61,41	58,32	3,78	45,89	40,05	37,90	41,28	4,13	0,00	0,50	0,69	0,39	0,35
	180	16,64	26,91	29,76	24,43	6,90	83,07	72,73	69,39	75,06	7,13	0,29	0,36	0,86	0,50	0,31

	240	4,38	6,52	4,41	5,11	1,23	94,99	92,92	94,93	94,28	1,18	0,62	0,56	0,65	0,61	0,05
	300	0,00	0,16	0,56	0,24	0,29	99,38	99,36	98,71	99,15	0,38	0,62	0,48	0,73	0,61	0,13
-6/+6 (H/RcdG)	0	98,96	99,32	99,75	99,34	0,39	1,04	0,24	0,21	0,50	0,47	0,00	0,44	0,04	0,16	0,24
	30	90,23	94,78	95,70	93,57	2,93	9,77	5,07	3,96	6,27	3,08	0,00	0,15	0,33	0,16	0,17
	60	73,92	86,71	82,76	81,13	6,55	26,08	12,80	17,24	18,71	6,76	0,00	0,49	0,00	0,16	0,28
	120	26,26	55,45	45,88	42,53	14,88	73,74	43,93	53,96	57,21	15,17	0,00	0,62	0,16	0,26	0,32
	180	6,59	12,83	12,54	10,66	3,52	93,07	86,51	87,43	89,00	3,55	0,34	0,66	0,03	0,34	0,31
	240	0,00	0,31	0,30	0,20	0,18	99,61	99,69	99,59	99,63	0,05	0,39	0,00	0,11	0,16	0,20
	300	0,00	0,00	0,01	0,00	0,01	100,00	100,00	99,95	99,98	0,03	0,00	0,00	0,04	0,01	0,03

Table S2. Raw numerical data of FPG cleavage assays obtained from Quantity One software.

Oligo	Time [min]	1	2	3	Avg	SD	1	2	3	Avg	SD	1	2	3	Avg	SD
		Intact oligonucleotide [%]					Cleaved strand [%]					Intermediate fragment [%]				
Native control oligonucleotides																
-6/+6 (H/dA)	0	99,10	99,89	99,41	99,47	0,39	0,28	0,11	0,54	0,31	0,22	0,62	0,00	0,05	0,22	0,34
	1	91,77	92,68	92,23	92,23	0,46	7,04	6,48	6,64	6,72	0,29	1,19	0,85	1,13	1,05	0,18
	5	54,66	63,77	65,86	61,43	5,95	42,37	33,68	31,47	35,84	5,76	2,97	2,55	2,66	2,73	0,22
	15	26,28	40,14	50,61	39,01	12,20	69,64	56,43	45,65	57,24	12,01	4,08	3,43	3,73	3,75	0,32
	30	9,00	17,09	28,60	18,23	9,85	87,43	79,42	67,35	78,07	10,11	3,57	3,50	4,04	3,70	0,30
	60	2,98	4,88	11,99	6,62	4,75	94,82	92,86	85,57	91,08	4,87	2,21	2,26	2,44	2,30	0,12
	120	2,45	1,48	2,09	2,00	0,49	95,84	97,75	96,84	96,81	0,95	1,70	0,77	1,07	1,18	0,48
Oligonucleotides containing cdG																
-6/+6 (H/ScdG)	0	99,75	99,58	99,34	99,56	0,21	0,25	0,27	0,58	0,37	0,19	0,00	0,15	0,08	0,08	0,07
	1	95,93	94,09	92,40	94,14	1,76	4,07	5,49	6,88	5,48	1,40	0,00	0,41	0,72	0,38	0,36
	5	45,91	52,21	58,36	52,16	6,22	53,70	46,63	40,11	46,81	6,79	0,39	1,16	1,53	1,03	0,58
	15	10,00	10,62	23,36	14,66	7,54	89,60	87,94	74,70	84,08	8,16	0,40	1,45	1,94	1,26	0,79
	30	0,45	4,80	1,65	2,30	2,24	99,49	94,09	97,08	96,88	2,70	0,06	1,11	1,27	0,82	0,66
	60	0,04	0,45	0,86	0,45	0,41	99,96	98,98	98,29	99,08	0,84	0,01	0,57	0,84	0,47	0,43
	120	0,01	0,08	0,21	0,10	0,10	99,97	99,53	99,07	99,52	0,45	0,02	0,39	0,72	0,38	0,35
-6/+6 (H/RcdG)	0	99,51	99,63	99,55	99,56	0,06	0,42	0,33	0,00	0,25	0,22	0,07	0,04	0,45	0,19	0,23
	1	89,11	90,27	90,85	90,07	0,89	10,23	9,33	7,70	9,09	1,29	0,66	0,40	1,46	0,84	0,55
	5	24,20	15,33	31,86	23,80	8,27	74,24	83,57	64,99	74,27	9,29	1,57	1,10	3,15	1,94	1,07
	15	1,04	0,62	1,92	1,19	0,66	98,32	99,02	96,62	97,98	1,24	0,64	0,36	1,46	0,82	0,57
	30	0,00	0,00	1,77	0,59	1,02	99,92	99,87	97,62	99,13	1,31	0,08	0,13	0,61	0,28	0,29
	60	0,00	0,00	0,00	0,00	0,00	99,81	99,80	99,43	99,68	0,21	0,19	0,20	0,57	0,32	0,21
	120	0,00	0,00	0,08	0,03	0,04	99,82	99,82	99,35	99,66	0,27	0,18	0,18	0,58	0,31	0,23

Table S3. Calculated and found molecular masses [Da] of applied oligonucleotides.

Oligonucleotide	Calculated mass	Found mass
Matrix H/A	12369.10	12369.30
Matrix H/G	12354.10	12354.30
-6/+6(H/dA)	12254.00	12254.60
-6/+6(H/ScdG)	12269.00	12268.00
-6/+6(H/RcdG)	12269.00	12268.45