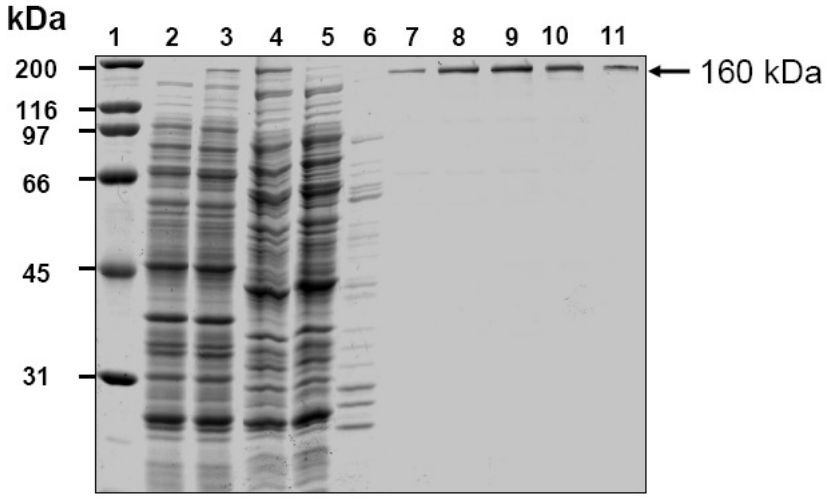
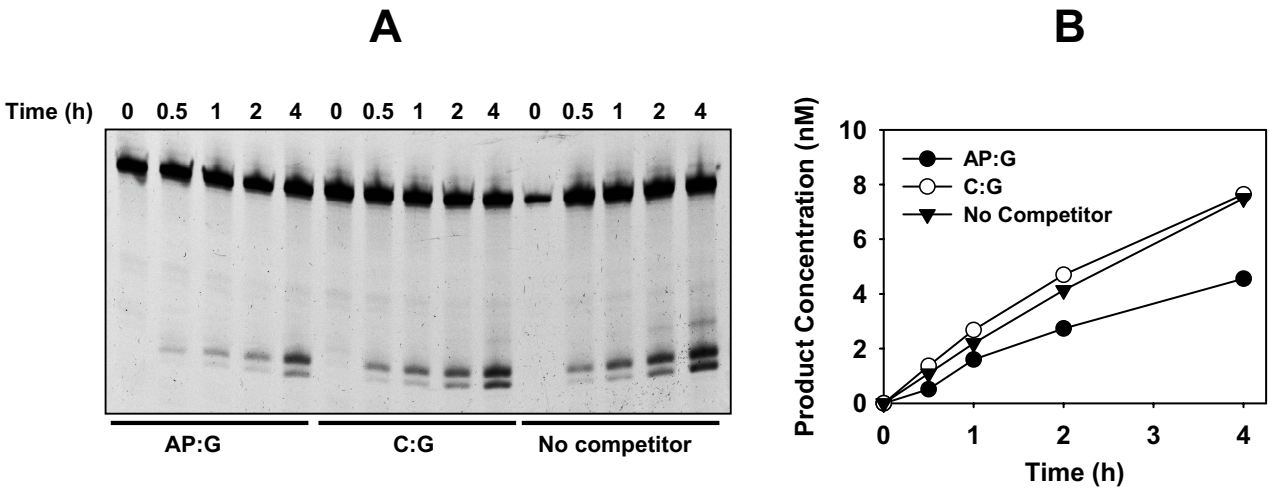
SUPPLEMENTARY FIGURE 1



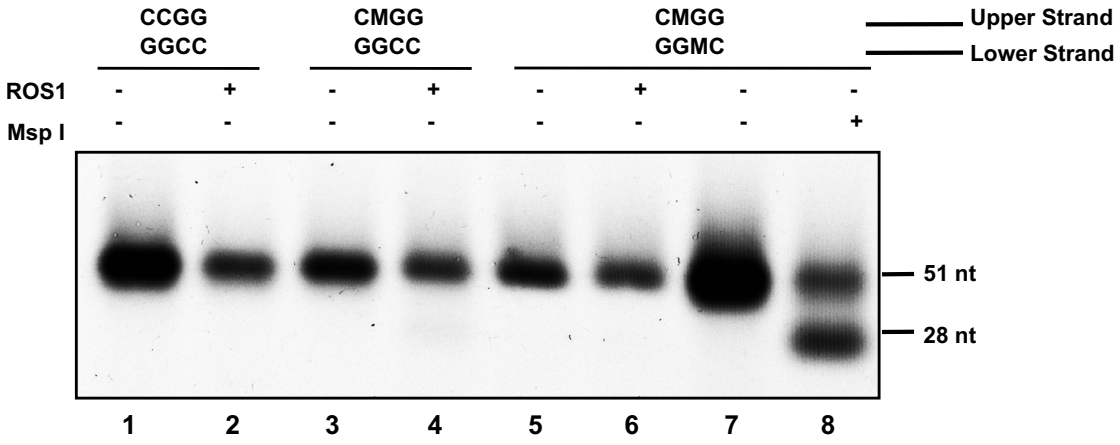
Supplementary Figure 1. Expression and purification of His-tagged ROS1 protein. Protein samples obtained during the purification process (see Materials and Methods) were analyzed by SDS-10% PAGE and Coomassie Blue staining. Protein markers (lane 1); cleared lysate before (lane 2) and after IPTG induction (lane 3); soluble material loaded onto the Ni^{2+}-sepharose column (lane 4); column flow through (lane 5); column wash with 100 mM imidazole SB (lane 6); imidazole-gradient elution fractions corresponding to the His-ROS1 protein peak (lanes 7-9); pooled fractions containing a single band (lane 10); dialyzed His-ROS1 protein used in enzymatic assays (lane 11).

SUPPLEMENTARY FIGURE 2



Supplementary Figure 2. Effect of preincubation with DNA containing a reduced AP site on ROS1 activity. Purified ROS1 (22.5 nM) was preincubated for 30 min with reaction buffer (filled triangles) or with 40 nM unlabelled duplex oligonucleotide containing either an AP:G (filled circles) or a C:G pair (open circles). Then, the fluorescein-labelled 5-meC:G substrate (40 nM) was added and the reactions were monitored for 4 h. Products were separated in a 12% denaturing polyacrylamide gel (A), and the relative amount of incised oligonucleotide was quantified by fluorescence scanning (B).

SUPPLEMENTARY FIGURE 3



Supplementary Figure 3. Analysis of double-strand break formation during ROS1 processing of hemimethylated and bimethylated DNA. Purified ROS1 (22.5 nM) was incubated with 40 nM duplex DNA labelled on the upper strand that contained a single non-methylated (lanes 1 and 2), hemimethylated (lanes 2 and 3) or bimethylated (lanes 5, and 6) CG site. Reactions were stopped at the indicated times and products were separated in a 3% agarose gel. A control reaction was performed by digesting a bimethylated DNA substrate with MspI at 37ºC for 20 min (lanes 7 and 8).

Supplementary Table 1. DNA sequence of oligonucleotides used as substrates.

Name	DNA sequence[a]	Strand	**X =**
FL-CGF	5´-TCACGGGATCAATGTGTTCTTTCAGCTC**X**GGTCACGCTGACCAGGAATACC-3´	Upper	C
FL-meCGF	5´-TCACGGGATCAATGTGTTCTTTCAGCTC**X**GGTCACGCTGACCAGGAATACC-3´	Upper	5-meC
FL-TGF	5´-TCACGGGATCAATGTGTTCTTTCAGCTC**X**GGTCACGCTGACCAGGAATACC-3´	Upper	T
FL-HUGF	5´-TCACGGGATCAATGTGTTCTTTCAGCTC**X**GGTCACGCTGACCAGGAATACC-3´	Upper	5-HU
FL-HmeUGF	5´-TCACGGGATCAATGTGTTCTTTCAGCTC**X**GGTCACGCTGACCAGGAATACC-3´	Upper	5-HmeU
FL-FUGF	5´-TCACGGGATCAATGTGTTCTTTCAGCTC**X**GGTCACGCTGACCAGGAATACC-3´	Upper	5-FU
FL-BrUGF	5´-TCACGGGATCAATGTGTTCTTTCAGCTC**X**GGTCACGCTGACCAGGAATACC-3´	Upper	5-BrU
FL-BrCGF	5´-TCACGGGATCAATGTGTTCTTTCAGCTC**X**GGTCACGCTGACCAGGAATACC-3´	Upper	5-BrC
FL-APGF	5´-TCACGGGATCAATGTGTTCTTTCAGCTC**X**GGTCACGCTGACCAGGAATACC-3´	Upper	THF[b]
CGR	3´-AGTGCCCTAGTTACACAAGAAAGTCGAGGCCAGTGCGACTGGTCCTTATGG-5´	Lower	-
meCGR	3´-AGTGCCCTAGTTACACAAGAAAGTCGAGG**X**CAGTGCGACTGGTCCTTATGG-5´	Lower	5-meC
FL-CGR	3´-AGTGCCCTAGTTACACAAGAAAGTCGAGGCCAGTGCGACTGGTCCTTATGG-5´	Lower	-
FL-3GmeCGAF	5´-AAGCTGCGATAAGCTG**X**GATAAGCTG**X**GATAAGCTG**X**GATAAGCTGCGATAACT-3´	Upper	5-meC
FL-3GUGAF	5´-AAGCTGCGATAAGCTG**X**GATAAGCTG**X**GATAAGCTG**X**GATAAGCTGCGATAACT-3´	Upper	U
3GCGAR	3´-AGTTATCGCAGCTTATCGCAGCTTATCGCAGCTTATCGCAGCTTATCGCAGCTT-5´	Lower	-

[a] Relevant regions are boxed.

[b] THF = tetrahydrofuran AP site analogue