Supplementary Figures

Figure S1

Gel image of BsmAI restricted plasmid containing either the single damage or a 8-oxoG/Tg cluster following processing by *fpg mutY* strain of *E. coli*. The specific damage within the plasmid constructs is indicated above each lane. If the plasmid carries a mutation an additional band representing 1755 bp is present, following digestion with Bsma1.

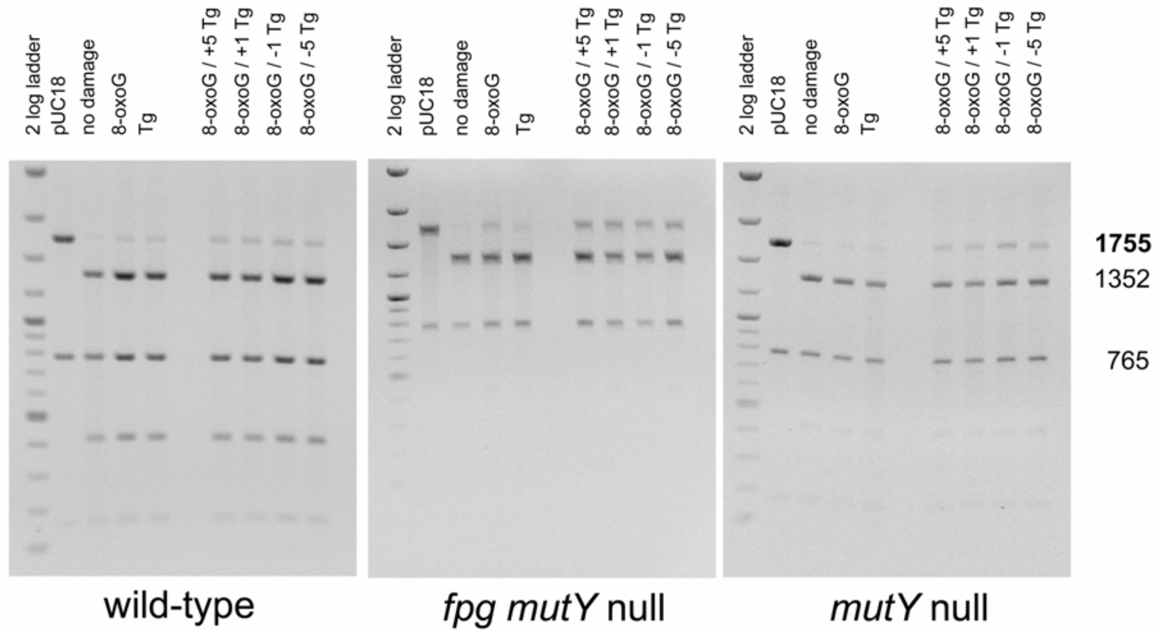

Figure S2

Mutation frequency of the 8-oxoG (Go)/Tg clustered damage site transformed into wild type, *nth nei* strain, *mutY* strain and *nth nei mutY* strains of *E. coli*. The types of clusters are shown along the x-axis. Error bars display the standard errors of the mean from three experiments.

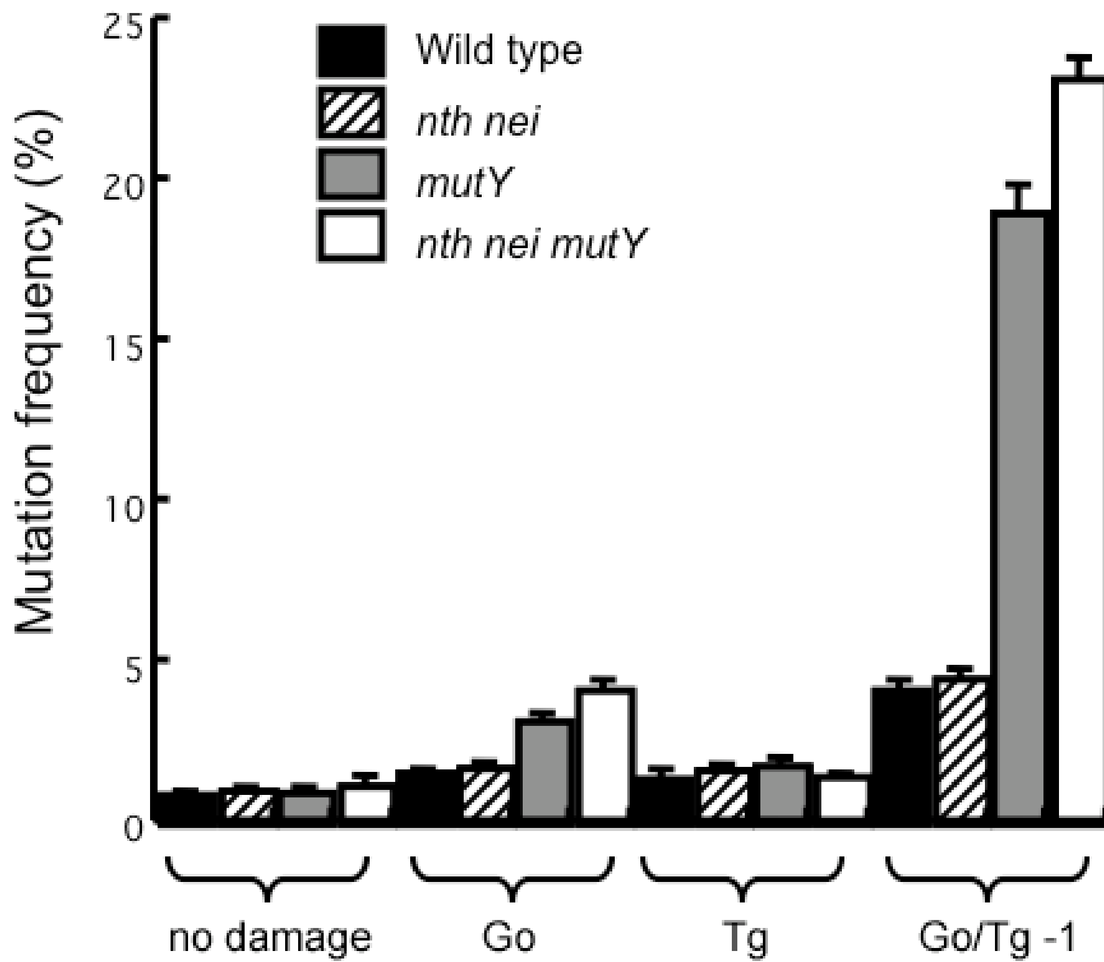

Figure S3

The proportion of individual clones that have a given level of completeness of mutation (i.e., the relative extent of both non-mutant and mutant plasmid in a clone), plotted in ascending order from 0% (non-mutant) to 100% (completely mutant) in 10% intervals. Note that the experimental determination of completeness is subject to measurement uncertainties, so that the 0-10% group is best regarded as non-mutant (so that approx. 45-55% of clones tested carry no mutant plasmid in all strains/damage types tested), and similarly the 90-100% group is best regarded as clones carrying only mutant plasmid. Mutation frequency of a clone was determined by restriction cutting and gel analysis of the retrieved plasmids. Frequencies of clones are shown for 8-oxoG/Tg -1 cluster in *fpg mutY* (light grey bar) and 8-oxoG/Tg +1 cluster in *fpg mutY* (striped). Numbers of clones analyzed for these strains/damage types were 100.

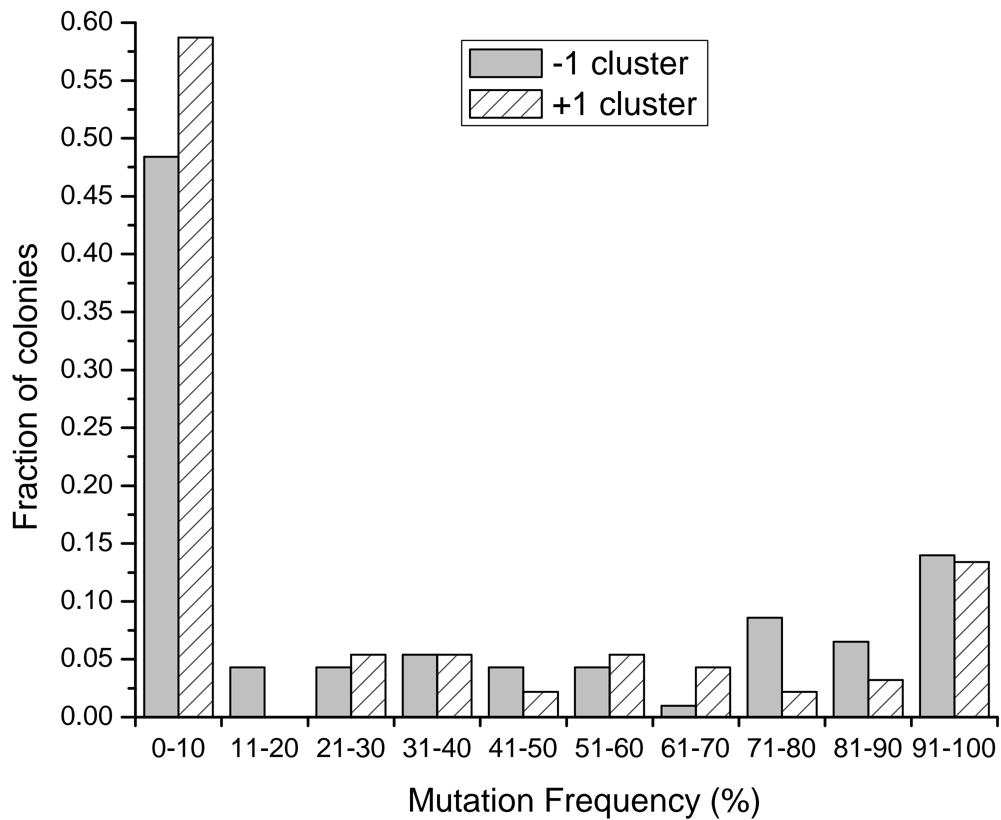

Figure S4

Representative denaturing polyacrylamide gel showing the rejoining of an AP-site **(A)** or HAP1-SSB **(B)** in the absence and presence of Tg at positions +1, +5, -1 and -5 following incubation with xrs5 nuclear extracts for the times shown. ssDNA band represents single-stranded DNA band before rejoining of the SSB. ssDNA +1 or +5 bases bands represent single-stranded DNA following addition of the respective number of bases but before the SSB is sealed, seen as restoration of the 40mer band (rejoined band).

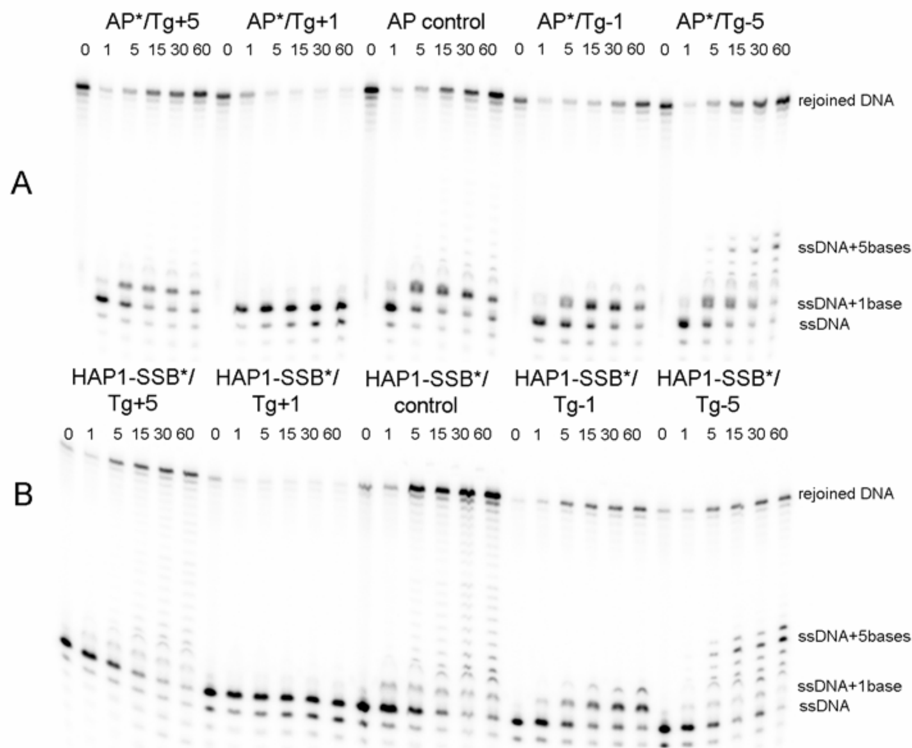

Figure S5

Relative amount of DNA rejoined in 60 min for **(A)** HAP1-SSB control and **(B)** HAP1-SSB/Tg+5 when the repair assay was undertaken using the full complement of dNTP (dCTP, dGTP, dTTP and dATP) or when either ddGTP, ddATP or ddTTP was substituted for its dNTP counterpart. The histograms show the total level of repair of the HAP1-SSB in 60 min of the repair assay for the HAP1-SSB control and HAP1-SSB/Tg+5, normalized to the amount of DNA rejoined in 60 min when a full complement of dNTPs was used in the repair assays. dGTP is the first base to be inserted into the repair gap and the substitution with ddGTP completely halts rejoining of the HAP1-SSB. dATP is the second base to be inserted into the repair gap and substitution with ddATP prevents LP-BER. The amount of DNA rejoined in 60 min is reduced by about 20% as this is the proportion of DNA rejoined by LP-BER. dTTP is the fifth base to be inserted into the repair gap. Repair of HAP1-SSB control and HAP1-SSB/Tg+5 does not involve LP-BER with a repair patch greater than 3-4 bases, thus the substitution with ddTTP should not affect the repair efficiency of these substrates if repair is via the BER and not a result of chain elongation. As can be seen in the histogram the relative amount of rejoining in 60 min is slightly higher when ddTTP is substituted for dTTP, demonstrating that repair is indeed via the BER pathway.

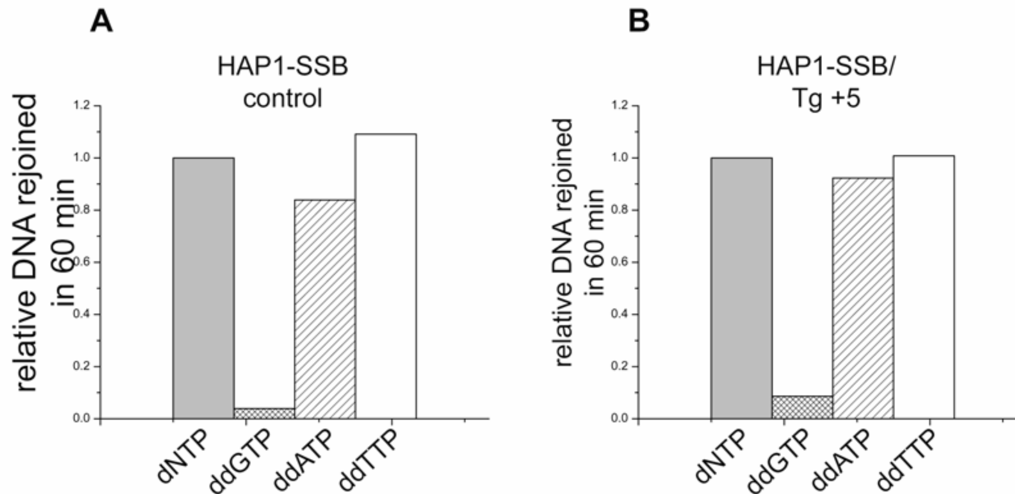

Figure S6

The efficiency of removal of either an AP site, Tg or 8-oxoG when present as single lesions in ds oligonucleotides by Nei.