Supplementary Information

Solid-state nanopore analysis of diverse DNA base modifications using a modular enzymatic labeling process

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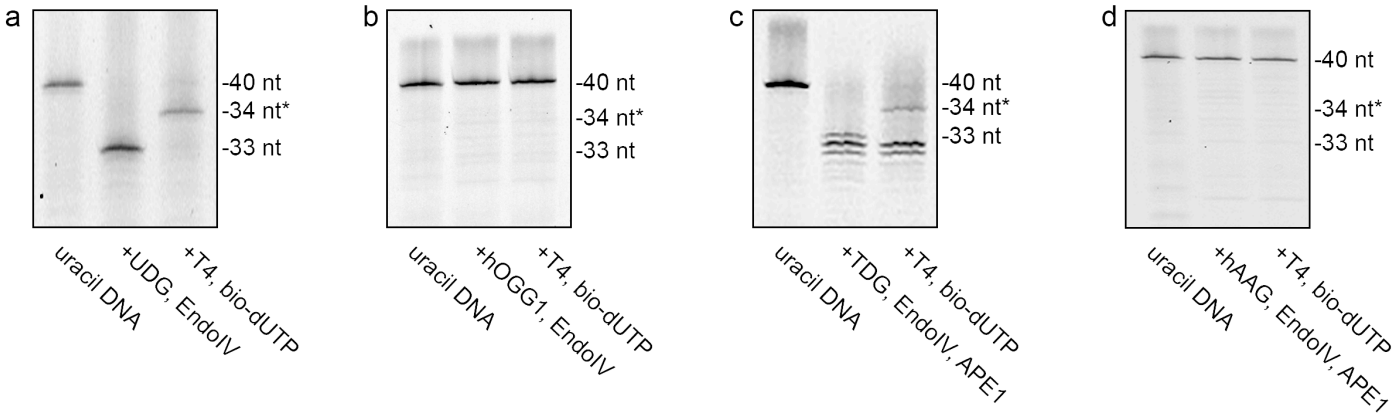

Figure S1. Cross-recognition for uracil modified bases Denaturing gel analyses for an oligonucleotide containing a single uracil treated with (a) UDG, (b) hOGG1, (c) TDG, and (d) hAAG. Lanes are labeled with treatment steps and * indicates 34 nt length plus the incorporated biotin. Aside from UDG, only TDG shows recognition, though yield of biotin-labeled product is very low due to additional exonuclease activity (lower bands) under our conditions.

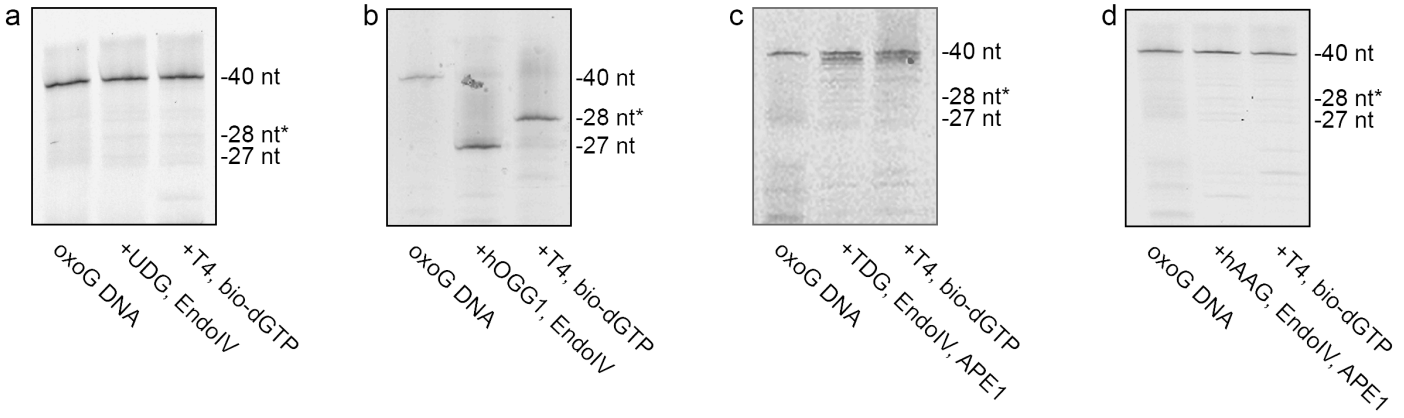

Figure S2. Cross-recognition for oxoG modified bases Denaturing gel analyses for an oligonucleotide containing a single oxoG treated with (a) UDG, (b) hOGG1, (c) TDG, and (d) hAAG. Lanes are labeled with treatment steps and * indicates 28 nt length plus the incorporated biotin. Only hOGG1 shows any significant yield of biotin-labeled product.

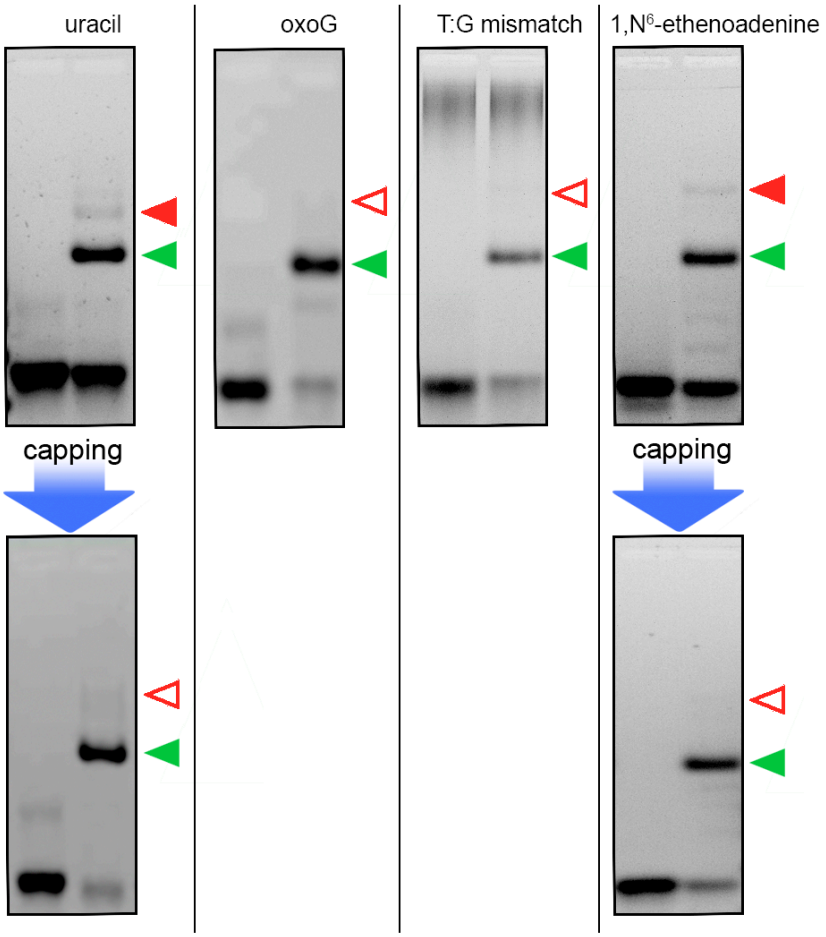

Figure S3. Limiting off-target T4 polymerase end-labeling with terminal transferase Electromobility shift assay (EMSA) gels for all specifically-labeled DNA constructs. In each image, labeled DNA is visualized without (lane 1) and with (lane 2) MS. Green arrows indicate target DNA-MS construct. For uracil (left) and 1,N^6-ethenoadenine (right), an additional band was observed (red arrow), indicating superfluous end-labeling of the construct by the T4 polymerase, possibly driven by incorporation kinetics of dUTP and dATP, respectively. By capping the 3' ends of these oligonucleotides with terminal transferase and ddATP (see Materials and Methods) prior to labeling (blue arrows), we find that multiple biotin-labeling is inhibited, with results similar to those of oxoG and T:G mismatch (open red arrows).

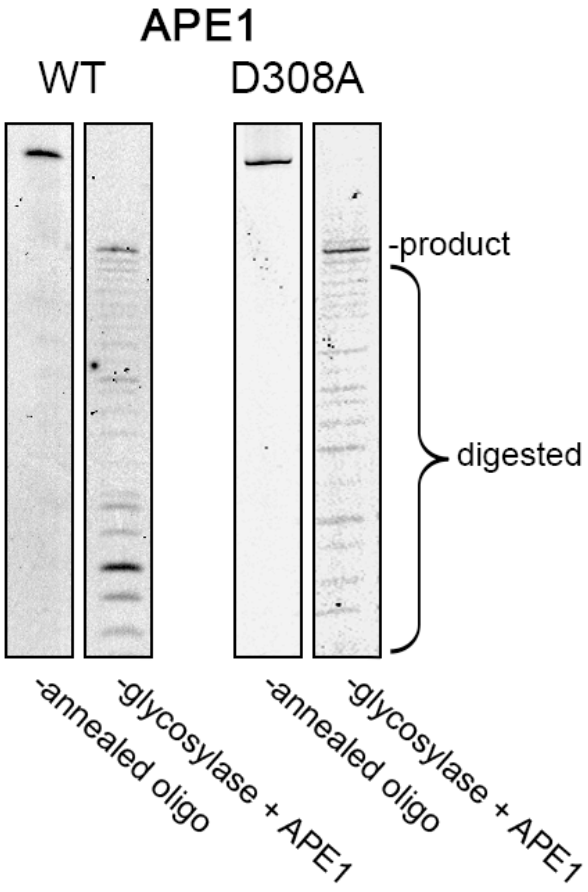

Figure S4. WT APE1 and D308A APE1 exonuclease activity Denaturing gel analysis of a FAM-labeled 40 nt T:G mismatch DNA oligonucleotide before and after treatment with TDG and APE1 in buffer conditions described in Materials and Methods section of main document. Left gels show WT APE1 behavior, resulting in massive loss of product due to 3'-5' exonuclease activity. Right gels show same treatment with the APE1 D308A mutant. While some exonuclease activity is observed in our buffer conditions, it is significantly reduced compared to WT.

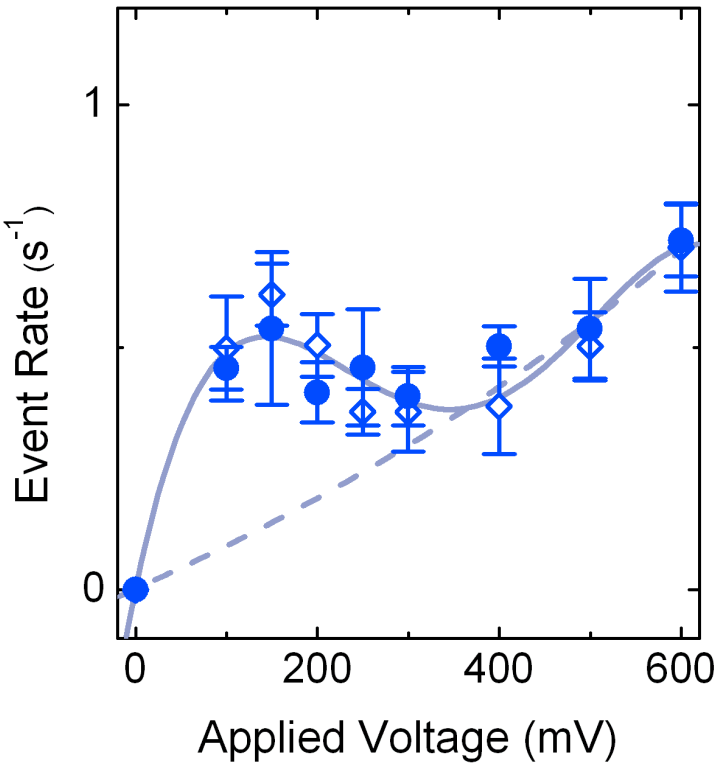

Figure S5. Additional SS-nanopore data for labeled 1,N^6-ethenoadenine DNA Filled circles and open diamonds represent two additional independent measurements on different SS-nanopore devices of 250 nM 1,N^6-ethenoadenine DNA that was biotin labeled with hAAG and mixed with MS. The solid line is a guide to the eye. In some preparations, we observed a reproducible artifact for this material wherein an anomalously high event rate was measured at low voltages (<400 mV) that returned to the expected exponential relationship (dashed line) at higher voltages. One possible explanation for this behavior may be in the structure of 1,N^6-ethenoadenine, which is known to cause kinking in DNA [Guliaev et al. (2000) Carcinogenesis, (9):1727-36] and potentially alter interactions with the nanopore during translocation. While capping DNA reduced the apparent emergence of end labeling (see Fig. S3), gel analysis could not confirm the location of the single biotin tag. It is possible that some population of monobiotinylated DNA could be end labeled while maintaining the 1,N^6-ethenoadenine. As a result, a kinked structure may be maintained. We hypothesize that the high event rate at low voltages could be due to orientation-specific interactions of the kinked DNA-MS construct threading through the pore, which are not present at high voltage due to a greater electrophoretic force. Future measurements will focus on elucidating the basis of this effect and optimizing experimental variables that include nanopore diameter, event thresholding, and efficiency of the labeling protocol.

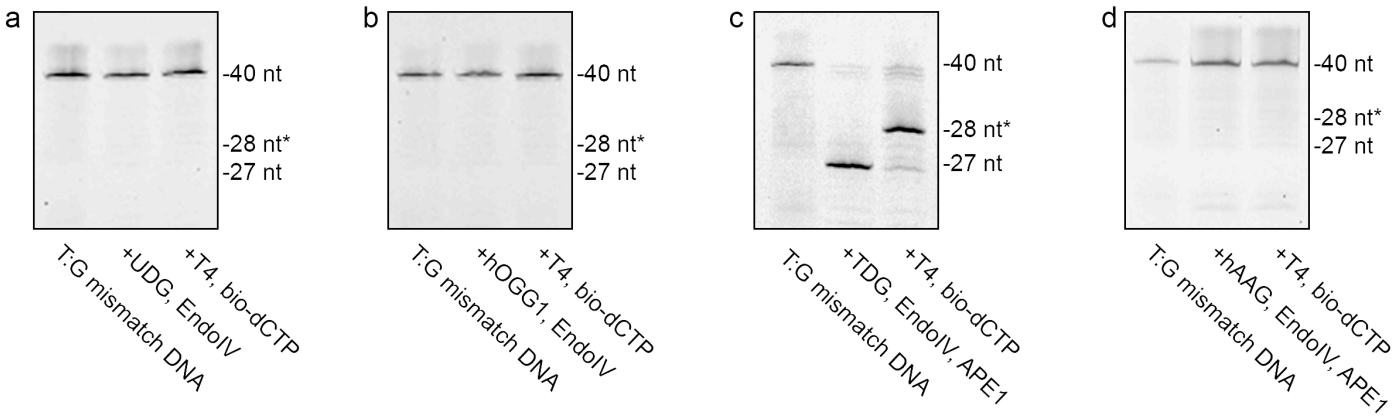

Figure S6. Cross-recognition for T:G mismatch Denaturing gel analyses for an oligonucleotide containing a single T:G mismatch treated with (a) UDG, (b) hOGG1, (c) TDG, and (d) hAAG. Lanes are labeled with treatment steps and * indicates 28 nt length plus the incorporated biotin. Only TDG shows any significant yield of biotin-labeled product.

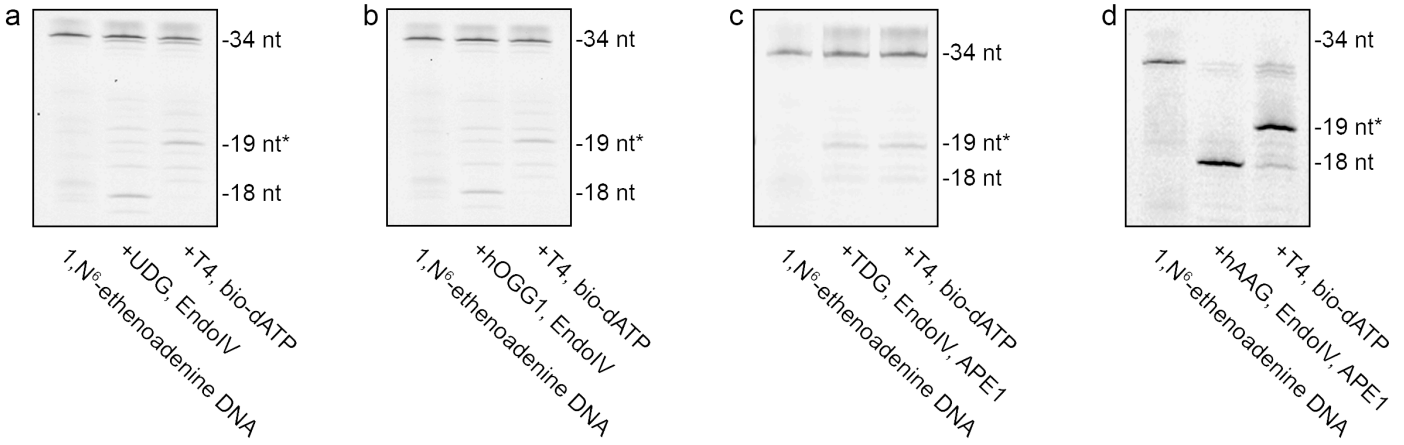

Figure S7. Cross-recognition for 1,N^6-ethenoadenine modified bases Denaturing gel analyses for an oligonucleotide containing a single 1,N^6-ethenoadenine treated with (a) UDG, (b) hOGG1, (c) TDG, and (d) hAAG. Lanes are labeled with treatment steps and * indicates 19 nt length plus the incorporated biotin. Some minor recognition is observed for off-target glycosylases, possibly due to DNA kinks induced by 1,N^6-ethenoadenine, but only hAAG shows a high yield of biotin-labeled product.