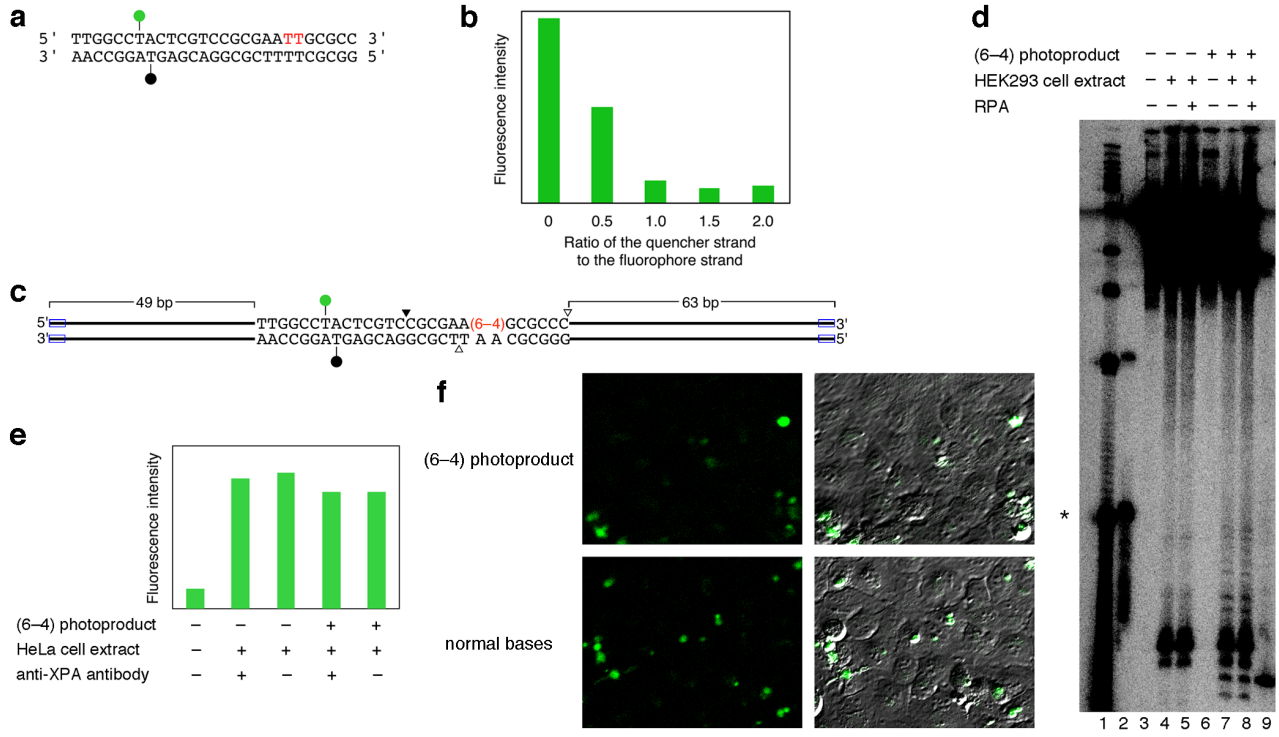
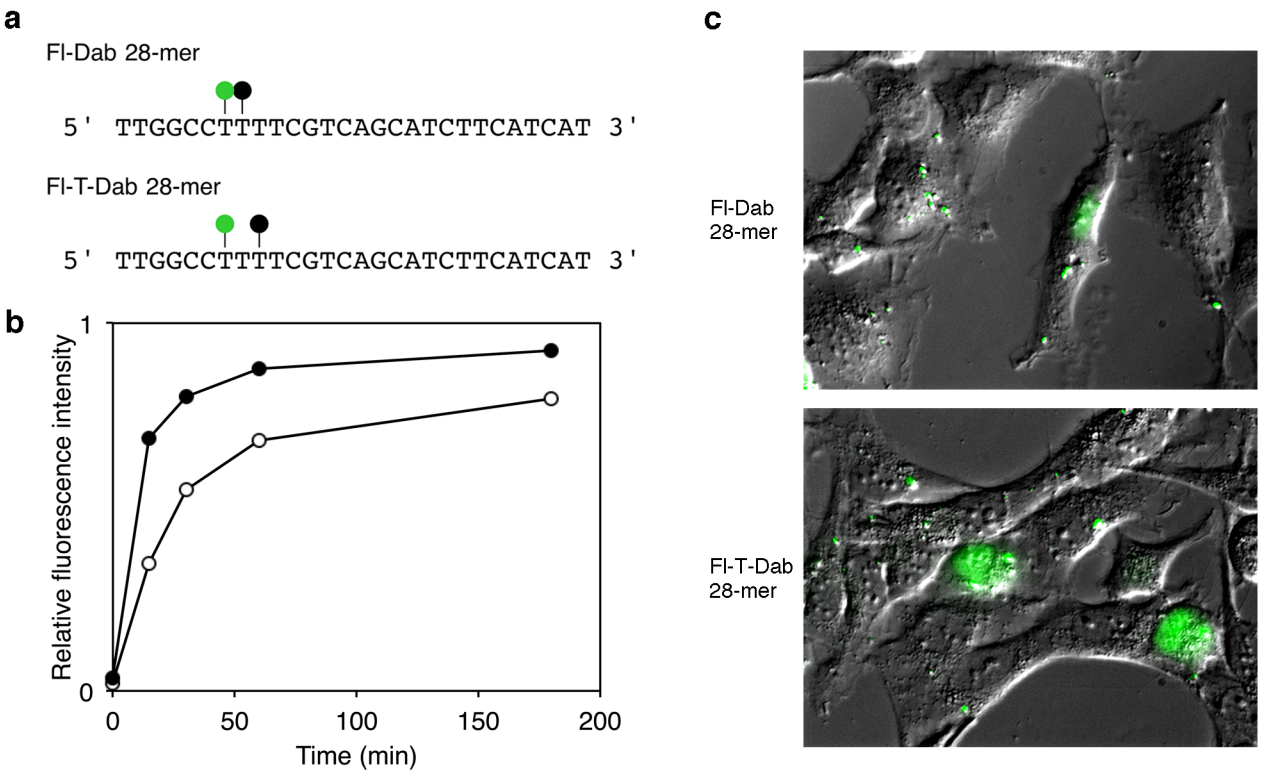
Supplementary Information

Fluorescence detection of cellular nucleotide excision repair of damaged DNA

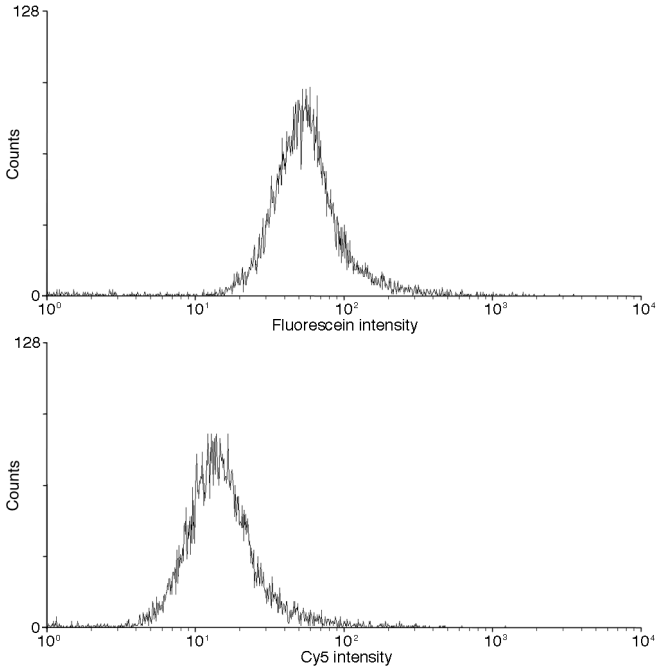
Tatsuya Toga, Isao Kuraoka, Shun Watanabe, Eiji Nakano, Seiji Takeuchi, Chikako Nishigori, Kaoru Sugasawa & Shigenori Iwai



Supplementary Figure S1 | Linear substrate for NER. (**a**) The duplex used to test the fluorophore–quencher system. The green and black circles represent fluorescein and Dabcyl, respectively, and their chemical structures are shown in Fig. 2b. Mismatches were incorporated on the assumption of the existence of a photoproduct at the TT site shown in red. (**b**) Quenching of the fluorescence upon hybridization. The two strands were mixed at various ratios, and after annealing and dilution, the fluorescence intensity was measured at 20 °C in a buffer containing 20 mM sodium phosphate (pH 8.0) and 50 mM NaCl. (**c**) Structure of the 140 bp duplex containing the (6–4) photoproduct. Each strand was prepared by ligation of oligonucleotides at the sites indicated by triangles. For the incision assay, ^{32}P-phosphate was incorporated at the site indicated by the filled triangle. Blue boxes represent the regions of phosphorothioate modification. A 140 bp duplex containing normal thymines in place of the (6-4) photoproduct was also prepared. (**d**) Dual-incision assay using a HEK293 cell extract. The 140 bp duplexes containing TT (lanes 3–5) and the (6–4) photoproduct (lanes 6–8) were incubated without the cell extract (lanes 3 and 6), with the cell extract (lanes 4 and 7), or with the cell extract plus RPA (lanes 5 and 8), at 37 °C for 1 h, and after separation by denaturing PAGE, the products were detected by radioactivity measurement. Lanes 1 and 2, markers (the 25-mer is indicated by an asterisk); lane 9, a 14-mer, d(CGCGAATTGCGCCC). (**e**) Measurement of the fluorescence intensity. After incubation with a HeLa cell extract, the mixtures were diluted with 1 M Tris-HCl (pH 8.0), and the fluorescence intensity was measured at 37 °C. (**f**) Fluorescence detection in XPA cells. The cells were transfected with the duplex containing the (6–4) photoproduct (upper panels) or TT (lower panels), and the fluorescence images (left panels) were obtained at 6 h after transfection. The right panels are overlays with cell images.



Supplementary Figure S2 | Fluorophore–quencher system for detecting the degradation of the dual-incision product. (**a**) Oligonucleotides mimicking the dual-incision product. The green and black circles represent fluorescein and Dabcyl, respectively, and their chemical structures are shown in Fig. 2b. (**b**) Changes in fluorescence intensity by incubations of Fl-Dab 28-mer (open circles) and Fl-T-Dab 28-mer (filled circles) with a HeLa cell extract. An oligonucleotide without Dabcyl was treated in the same manner, and the values obtained for this control were used to normalize the data. The fluorescence intensities at time 0 show the efficient quenching in the single-stranded oligonucleotides. (**c**) Fluorescence detection in HeLa cells transfected with Fl-Dab 28-mer (upper panel) and Fl-T-Dab 28-mer (lower panel). The fluorescence images were obtained at 6 h after transfection and overlaid on the cell images.



Supplementary Figure S3 | Flow cytometry analysis of HeLa cells without transfection with the probe or the transfection reporter.