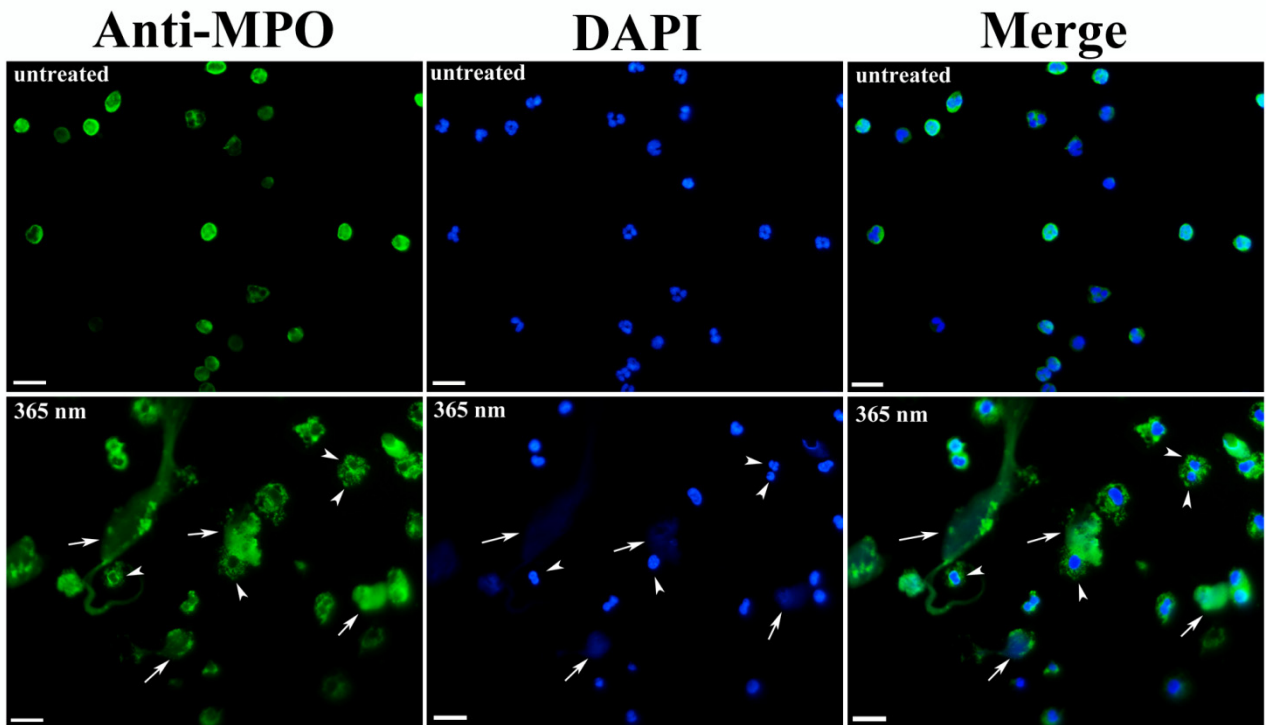
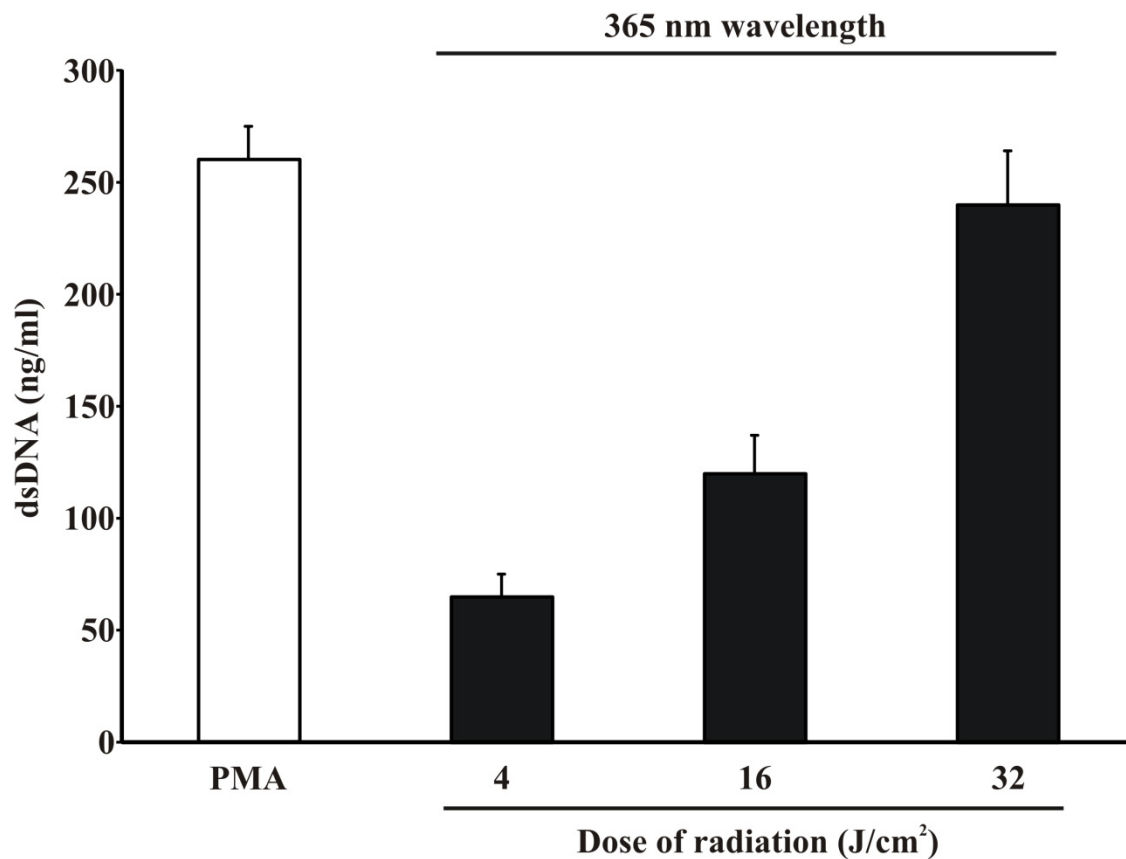


Supplementary material



Supplementary Figure S1. Immunofluorescence images of intact and irradiated human neutrophils.

Irradiation of human neutrophils at UV-A (365 nm wavelength) with energy dose of 32 J/cm² caused NETs formation. For immunofluorescence staining, neutrophils were fixed with 4% glutaraldehyde for 10 min, washed with PBS, and permeabilized using 0.1 % Triton X-100 in PBS for 2 min at room temperature. Nonspecific binding was reduced by pre-incubation of cells with blocking buffer (including human immunoglobulins) for 20 min. Immunofluorescence staining was performed with FITC-conjugated monoclonal mouse anti-human MPO priming antibodies (Invitrogen, USA). Blue color: staining of chromatin with DAPI. Green color: staining of MPO with FITC-conjugated anti-MPO monoclonal antibodies. NETs-forming cells are indicated with arrows, intact cells are indicated with arrow heads. Scale bars, 25 μ m.



Supplementary Figure S2. Quantification of released dsDNA in culture supernatants determined by PicoGreen reagent.

Human neutrophils were seeded on the 96-well plate at 1×10^5 cells per well (100 μ l). NETs formation was induced by irradiation of neutrophils at 365 nm wavelength (UV-A) with different energy doses. After irradiation, 1.5 U of micrococcal nuclease (Worthington) was added to the wells and plate was incubated for another 15 min at 37°C. The culture supernatant of each well (100 μ l) was collected, adjusted to 2.5 mM EDTA, centrifuged at 300 g for 5 min to remove cellular debris, and saved for analysis of DNA. DNA content of each supernatant was measured with Quant-iT™ PicoGreen dsDNA Assay Kit (Molecular Probes, Invitrogen) using ELx808 Absorbance Microplate Reader (Bio-Tec Instruments Inc., USA). The excitation wavelength and emission wavelength were 485 nm and 535 nm, respectively. The DNA concentration was calculated using a standard curve generated from a series of Lambda DNA standard provided by the manufacturer. Data represent mean \pm SD from five independent experiments (n=5).