Supporting Information

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SI Materials and Methods

Maturation of dendritic cells (DCs) and macrophages from mononuclear cells. Briefly, peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll–Hypaque density gradient centrifugation from buffy coats of blood donors obtained from the blood bank of the University Medical Center Mainz. PBMCs were seeded in six-well plates (Corning/Costar) at a density of 1 to 1.5 × 10^7 cells/3 mL/well in RPMI 1640 (PAA) containing 1.5% autologous plasma and incubated for 60 min (37 °C, 5% CO_2). Nonadherent cells were removed, and the remaining cells were further cultured in X-VIVO-15 in the presence of 800 U/mL GM-CSF and 50 U/mL IL-4, to differentiate them into immature DCs. Cells were fed on the third and sixth day with X-VIVO-15, 1600 U/mL GM-CSF, and 50 U/mL IL-4. After 1 wk of cultivation, DCs were harvested. For the generation of macrophages, PBMCs were seeded in six-well plates at 0.5 × 10^7 cells/3 mL/well in RPMI 1640 containing 1.5% autologous plasma for 60 min (37 °C, 5% CO_2). Nonadherent cells were removed, and the remaining cells were further cultured in X-VIVO-15 with 800 U/mL GM-CSF. Macrophages were harvested after 6 d. All cell preparations were checked as to cell surface markers by flow cytometry, and preparations that were of low purity (<75%) were excluded. In some experiments, monocytes were isolated from PBMCs by Miltenyi columns ($CD14^+$) according to the manufacturer's protocol.

Quantification of Apoptosis. Apoptosis was measured by flow cytometry.

Sub-G_1 assay. After treatment with tert-butyl hydroperoxide (BOOH), H_2O_2, or ionizing radiation, monocytes, DCs, and macrophages were washed in PBS and fixed in 70% ethanol for a minimum of 30 min at −20 °C. DNA in the cells was stained with propidium iodide (16.5 μg/mL) in PBS after RNase (0.03 μg/mL) digestion. For each sample, 10^4 cells were analyzed on a FACSCalibur device (Becton Dickinson). The number of apoptotic cells per sample was calculated using the computer program WinMDI 2.9 (Joseph Trotter).

Annexin V/propidium iodide assay. The annexin V/propidium iodide (PI) assay distinguishes between early apoptotic cells and late apoptotic/necrotic cells by using annexin V/PI double staining of unfixed cells. Cells were suspended in 50 μL binding buffer (10 mM Hepes, pH 7.4, 140 mM NaCl, 2.5 mM CaCl, 0.1% BSA) and annexin V-FITC (2.5 μL; Miltenyi Biotec) was added to each sample. After 15 min incubation in the dark, 430 μL binding buffer and 1 μg/mL PI per sample were added. The flow cytometric analysis was carried out by using a FACSCalibur device (Becton Dickinson). For each sample, 10^4 cells were analyzed. Calculation of apoptotic/necrotic cell populations was performed by using the computer program WinMDI 2.9 (Joseph Trotter).

Reactive Oxygen Species Measurement. Living cells were incubated for dye uptake with 0.1 μM 5-(and 6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) for 1 h in the dark at 37 °C (Invitrogen). The acetates of CM-H_2DCFDA are cleaved by intracellular esterases releasing the reduced fluorescein (i.e., 2′,7′-dichlorofluorescein), which is converted to (dichloro)fluorescein in the presence of reactive oxygen species (ROS) and can be detected by FACS. In brief, dye-loaded cells were washed, returned to prewarmed growth media, and treated with BOOH. Oxidation of CM-H_2DCFDA was detected by monitoring the increase in fluorescence with FACSCalibur device (Becton Dickinson). The relative median values of the curves were calculated as (dichloro)fluorescein fluorescence.

Immunostaining. Before immunostaining, cells were fixed with 4% paraformaldehyde (1× PBS, 4% paraformaldehyde) for 20 min. Fixed cells were permeabilized with 0.5% Triton X-100/PBS for 10 min. For detection of 8-oxo-guanine (8oxoG), cells were incubated in 2 M HCl for 30 min to denaturize the DNA and make the damage accessible to antibodies. Blocking was done in PBST (1× PBS, 0.1% Tween 20) containing 4% BSA for 10 min at room temperature. Antibodies were diluted in PBST containing 2% BSA. Between application of primary and secondary antibodies, washing steps were performed in PBST for 15 min. Incubations with all antibodies were performed for 1 h at room temperature. 8oxoG was detected with polyclonal rabbit anti-8oxoG antibody (Squarix) and then with goat anti-rabbit antibody $F(ab')_2$ fragment conjugated with Cy3 (Jackson ImmunoResearch). Phosphorylated H2AX (ser139, γH2AX) was detected with monoclonal mouse anti-phoshpo-γH2AX (Ser-139) antibody (Millipore) and then with goat anti-mouse antibody $F(ab')_2$ fragment conjugated with Alexa Fluor 488 (Invitrogen). Nuclei were counterstained by using 1 μM ToPro3 (Invitrogen) for 15 min in the dark; preparations were mounted in antifade medium and sealed with nail polish. The cells were analyzed with an LSM710 laser scanning microscope (Carl Zeiss).

Single-Cell Gel Electrophoresis (Comet Assay). DNA single- and double-strand breaks were determined and quantified by the highly sensitive alkaline and neutral comet assay (1). Analysis of DNA migration was performed by using the image analysis system of Kinetic Imaging (Komet 4.0.2; Optilas). The mean tail moment (defined as percentage of DNA in the tail multiplied by the tail length) of 50 cells per sample was determined.

In Vitro Base Excision Repair Assay. ***Preparation of whole-cell extracts.*** Cells were harvested, washed once with ice-cold PBS solution, frozen at −80 °C for at least 1 h, and suspended for lysis in one packed cell volume of buffer I [10 mM Tris (pH 7.8), 200 mM KCl, 1 μg/mL protease inhibitors (leupeptin, chymostatin, pepstatin, aprotinin), 1 mM DTT, 1 μM phenylmethylsulfonyl fluoride]. After adding two packed cell volumes of buffer II [10 mM Tris (pH 7.8), 600 mM KCl, 2 mM EDTA, 40% glycerol, 0.2% Nonidet P-40], cell suspension was mixed and rocked at 4 °C for 2 h. Cell debris was removed by centrifugation at 90,000 × *g* for 20 min at 4 °C; supernatants were collected and dialyzed overnight in 25 mM Hepes, 10 mM KCl, 12 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, and 17% glycerol in dialysis bags (Zelu-Trans 6.0, Carl Roth, molecular-weight cutoff 8,000–10,000) at 4 °C under constant stirring. Remaining cell debris was removed by centrifugation at 16,000 × *g* for 10 min at 4 °C.

Preparation of oligonucleotide. Synthetic oligonucleotides were purchased from Eurogentec. The uracil-containing oligonucleotide 5′-ATCTACCGAGTCCGTCCGA**U**CACGCTTATTGGCTA-CCGA-3′ was [γ-^{32}P]ATP-5′ end-labeled by using T4-polynucleotide kinase (Roche Diagnostics). After labeling, the oligonucleotide was purified by Micro Bio-Spin 6 Chromatography columns (Bio-Rad) and annealed to a twofold excess of the complementary oligonucleotide at 90 °C for 5 min before slow cooling to room temperature. After annealing, an apurinic (AP) site was created by removing the uracil residue by uracil-DNA-glycosylase (Roche Diagnostics), and the AP site containing duplex DNA molecule was purified by Micro Bio-Spin 30 chro-

matography columns (Bio-Rad). Hybridization efficiency and freezing stability were checked by native PAGE.

Repair reaction. Reaction mixtures contained 600 fmol of [γ-^{32}P] ATP-5′ end-labeled oligonucleotide containing a single AP site, 52.5 mM Hepes-KOH (pH 7.8), 5 mM KCl, 11 mM $MgCl_2$, 0.6 mM EDTA, 0.55 mM DTT, 8.5% glycerol, 2 mM ATP, 20 μM of each dNTP, 250 μM NAD, 25 mM phosphocreatine, 50 ng/μL of creatine phosphokinase, 20 ng/μL Carrier DNA (a single-stranded 30mer oligonucleotide), and 8 μg of whole-cell extracts. After 0, 5, 10, 30, 60, and 90 min at 37 °C, the reactions were terminated and oligonucleotides denaturated by adding stop solution containing formamide and heating at 95 °C for 5 min. Products were separated by denaturing 20% PAGE. The base excision repair (BER) products were detected and quantified on a Storm 860 molecular imager (GMI).

In Vitro Ligation Assay. The preparation of whole-cell extracts, the repair reaction, and separation by electrophoresis was performed as described for the BER assay.

Preparation of oligonucleotide: The oligonucleotide 5′-CCACGCTTATTGGCTACCGA-3′ was 5′-end labeled by using T4-polynucleotide kinase (Roche Diagnostics) and [γ-^{32}P]ATP. After labeling, the oligonucleotide was purified by Micro Bio-Spin 6 Chromatography columns (Bio-Rad) and annealed to oligonucleotides 5′-ATCTACCGAGTCCGTCCGA-3′ and 5′-TCGGTAGCCAATAAGCGTGGTCGGACGGACTCGGTAGAT-3′ at 90 °C for 5 min before slow cooling to room temperature.

Preparation of Whole-Cell Extracts and Western Blot Analysis. Cells were harvested, washed once with ice-cold PBS solution, and lysed on ice in an appropriate amount of lysis buffer containing 50 mM Tris-HCl (pH 7.5), 250 mM NaCl, 1 mM EDTA, 0.1% Triton X-100, 2 μg/mL aprotinin, 2 μg/mL leupeptin, 1 μg/mL pepstatin, and 97 μg/mL PMSF. After 30 min incubation, lysates were centrifuged at 13.000 × *g* at 4 °C for 20 min, and the supernatant was recovered. Cell extract (30 μg) was separated on a 10% or 7.5% SDS polyacrylamide gel at 100 V and was blotted onto a nitrocellulose membrane for 1 h at 300 mA by using buffer composed of 25 mmol/L Tris-HCl, 86 mmol/L glycine, and 20% methanol. The antibodies used were $pATM_{Ser1981}$ (Millipore), $pChk1_{Ser317}$ (Bethyl), $pChk2_{Thr68}$ (Epitomics), p53 (Dianova), $pATR_{Ser428}$, Chk1, Chk2, cleaved caspase-8, cleaved caspase-3, cleaved caspase-7 (Cell Signaling), *N*-methylpurine-DNA glycosylase (Abcam), 8oxoG DNA glycosylase (OGG1/2), AP endonuclease (Novus), polymerase β (Neomarkers), proliferating cell nuclear antigen, flap endonuclease 1 (BD Biosciences), XRCC1, Lig I (Abcam), Lig IIIα, poly(ADP-ribose) polymerase-1 (BD Biosciences), p53 (Dianova), catalytic subunit of DNA protein kinase (DNA-PKcs; Calbiochem), Ku70, Ku80, XRCC4, Lig IV (Biozol Diagnostica), and ERK2 (Santa Cruz Biotechnology) as protein loading control.

Preparation of RNA, RT-PCR, and Real-Time RT-PCR. Total RNA was isolated from cells by using the RNA II Isolation Kit (Macherey–Nagel). One microgram of RNA was transcribed into cDNA by using the Reverse-iT first-strand synthesis kit (ABgene) in a volume of 40 μL, and 3 μL was subjected to RT-PCR performed by the use of specific primers (MWG) and Red-Taq Ready Mix (Sigma-Aldrich). Primer sequences used for RT-PCR were as follows: FasR (up, 5′-AAGGGATTGGAATTGAGGAAGACTG-3′; low, 5′-GTGGAATTGGCAAAAGAAGAAGACA-3′), XRCC1 (up, 5′-ACGAATGCCAGGGAGGGGTTGTC-3′; low, 5′-AGCGGTGGCAGCGGAGATGAAG-3′), Lig IIIα (up, 5′-GCTATATGTCTTTGGCTTTC-3′; low, 5′-GGAATAGGCACAGTTCTT-3′), Parp-1 (up, 5′-GGCCGCATACTCCATCCTCA-3′; low, 5′-CATTCGCCTTCACGCTCTATCTTA-3′), DNA PKc (up, 5′-CTTTGTCGTGTGGAGGGAT-3′; low, 5′-CACAACGGGGTTCAGAAGTT-3′), and β-actin (up, 5′-TCCGCTGCCCTGAGGCACTC-3′; low, 5′-GAGCCGCCGATCCACACGGA-3′), which was used as loading control. Real-time PCR was performed using the SensiMix Plus SYBR and Fluorescein Kit (Bioline) and the MyIQ real-time PCR cycler (BioRad). Primer sequences used for real-time RT-PCR were as follows: XRCC1 (up, 5′-GAGGAAGTTGGATTTGAA-3′; low, 5′-ATTTAGGTCTCTTGGGAA-3′), Lig IIIα (up, 5′-GCTATATGTCTTTGGCTTTC-3′; low, 5′-GGAATAGGCACAGTTCTT-3′), Parp-1 (up, 5′-GAACGACCTGATCTGGAA-3′; low, 5′-CACTTGCTGCTTGTTGAA-3′), DNA PKc (up, 5′-CTTTGTCGTGTGGAGGGAT-3′; low, 5′-CACAACGGGGTTCAGAAGTT-3′), DNA PKc II (up, 5′-TGCAGCTGATTCACTGGTTC-3′; low, 5′-CTCCTGCTGCTGTGGTGTTA-3′), and β-actin (up, 5′-TGGCATCCACGAAACTACC-3′; low, 5′-GTGTTGGCGTACAGGTCTT-3′).

Determination of O^6-Methylguanine-DNA Methyltransferase Activity. O^6-Methylguanine-DNA methyltransferase activity was determined as previously described (2).

DNA-PK Activity Assay. The assay was performed as reported (3). In brief, cells were resuspended in two volumes of extraction buffer [50 mM NaF, 20 mM Hepes (pH 7.8), 450 mM NaCl, 25% glycerol, 0.2 mM EDTA, and 0.5 mM DTT in the presence of protease inhibitors (complete, Mini, EDTA-free; Roche)]. Cells were frozen in liquid nitrogen and thawed at 30 °C three times. After centrifugation (12,000 rpm for 30 min at 4 °C), the supernatant was shock-frozen and stored at −80 °C. Endogenous DNA was removed by DEAE Sepharose Fast Flow (GE Healthcare). DNA-PK activity was determined in 10 μg protein extract by using a Sigma TECT DNA-Dependent Protein Kinase Assay System (Promega). The amount of ^{32}P incorporated into DNA-PK biotinylated peptide substrates was determined by liquid scintillation counting and expressed as counts per minute per microgram of protein. For background control, which was subtracted from the assays, the reaction was performed in the absence of the specific DNA-PK biotinylated peptide.

1. Olive PL, Wlodek D, Banáth JP (1991) DNA double-strand breaks measured in individual cells subjected to gel electrophoresis. *Cancer Res* 51:4671–4676.
2. Briegert M, Enk AH, Kaina B (2007) Change in expression of MGMT during maturation of human monocytes into dendritic cells. *DNA Repair (Amst)* 6:1255–1263.
3. Shao CJ, Fu J, Shi HL, Mu YG, Chen ZP (2008) Activities of DNA-PK and Ku86, but not Ku70, may predict sensitivity to cisplatin in human gliomas. *J Neurooncol* 89:27–35.

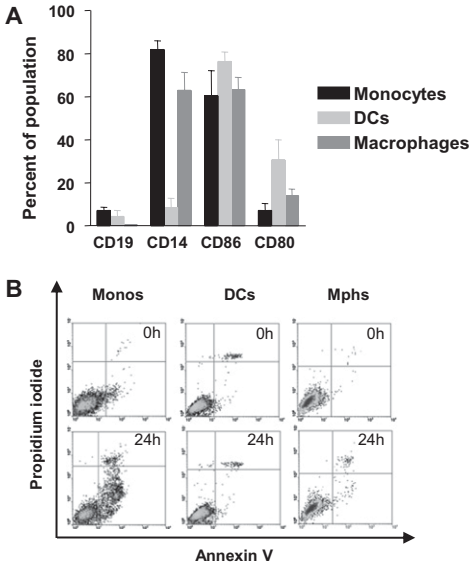

Fig. S1. Characterization of monocytes, DCs, and macrophages (Mphs), which occurred for each sample under study. (*A*) Surface markers studied by FACS. In each experiment, DCs and monocytes were derived from the same population of monocytes isolated from peripheral blood. (*B*) Representative density plots of untreated and 400 μM BOOH-treated monocytes, DCs, and macrophages. Cells were harvested 24 h after treatment and subjected to annexin V/PI double staining.

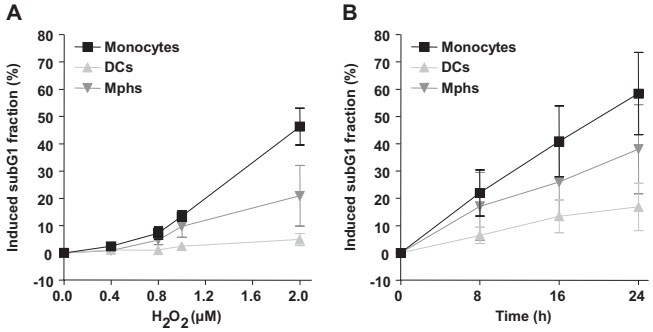

Fig. S2. Apoptotic response of monocytes, DCs, and macrophages (Mphs) following treatment with H_2O_2. (*A*) Dose response of cells treated with H_2O_2 after 24 h. (*B*) Time dependence of the apoptotic response of cells after treatment with 2 mM H_2O_2. Apoptosis was determined by quantifying the sub-G1 fraction by flow cytometry. Data of at least three independent experiments are pooled.

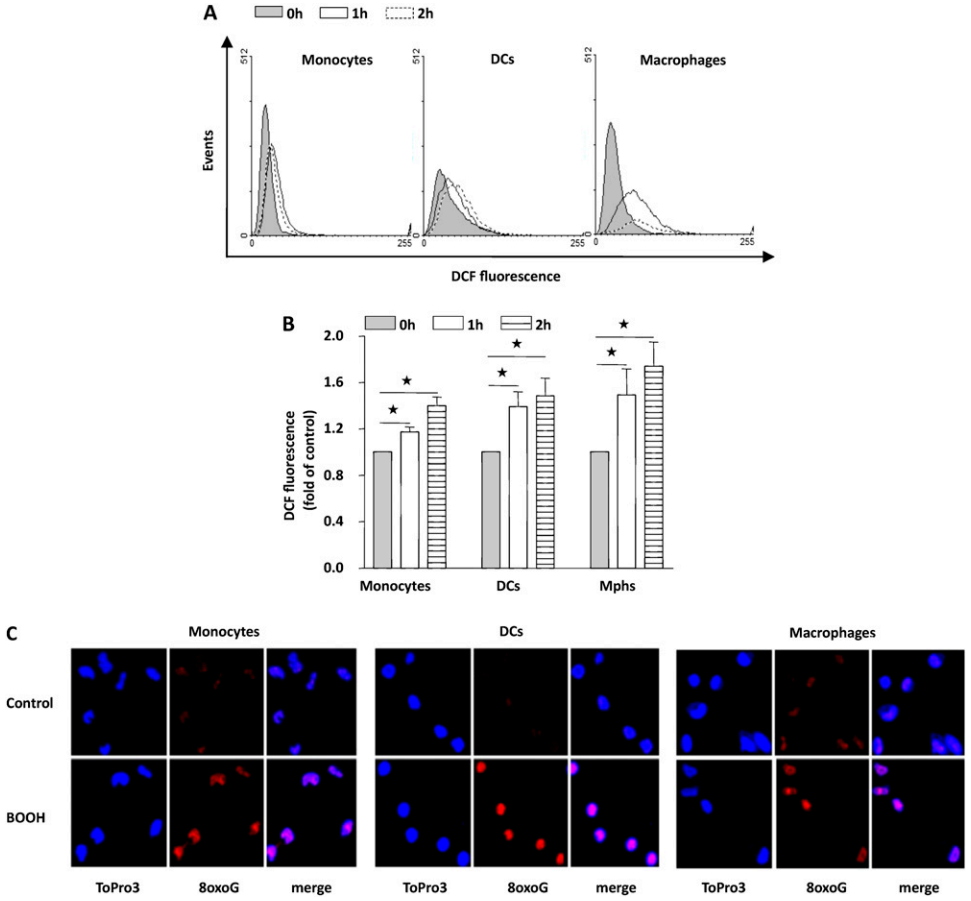

Fig. S3. Induction of ROS in monocytes, DCs, and macrophages (Mphs) following BOOH treatment. (*A*) Representative histograms of untreated cells (gray) and cells treated with 400 μM BOOH for 1 h (black line) and 2 h (dotted line). (*B*) Quantification of intracellular ROS following BOOH treatment. Data are the mean of at least three independent experiments (*$P < 0.05$, *t* test, untreated vs. BOOH-treated cells). (*C*) Formation of 8oxoG in monocytes, DCs, and macrophages 20 min after treatment with 400 μM BOOH, determined by immunostaining. ToPro3, nuclear staining; merge, overlay nuclear and 8oxoG staining.

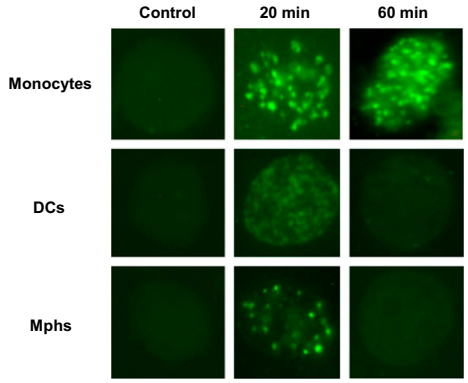

Fig. S4. H2AX phosphorylation in monocytes, DCs, and macrophages following treatment with 400 μM BOOH. Cells were fixed 20 and 60 min after the addition of BOOH to the medium.

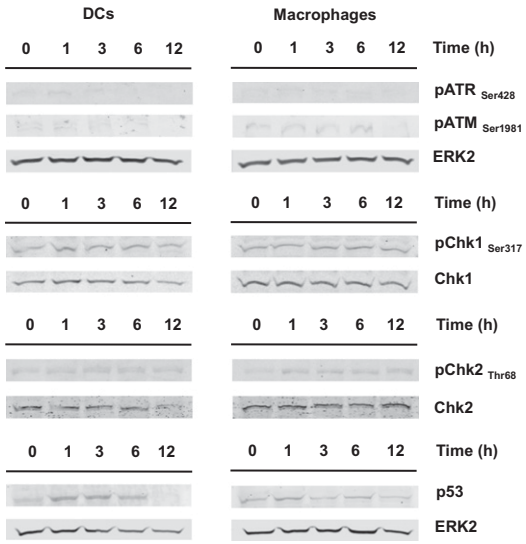

Fig. S5. DNA damage signaling in DCs and macrophages induced by BOOH. Western blot analysis of ATM, ATR, Chk1, and Chk2 phosphorylation and p53 stabilization in extracts of DCs and macrophages after treatment with 400 μM BOOH. The experiments were done in parallel and blots were exposed in the same way as those shown in Fig. 2*D* for monocytes. Compared with monocytes, nearly no ATM/ATR activation was observed.

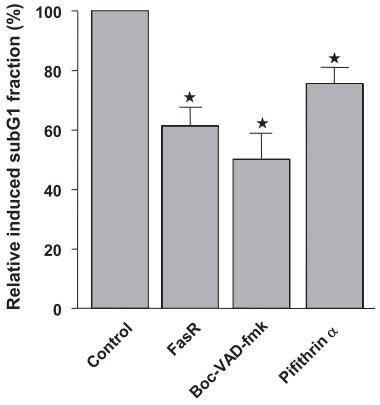

Fig. S6. Induced apoptosis in monocytes following treatment with 400 μM BOOH and posttreatment with Fas receptor inhibiting antibody (FasR), the caspase inhibitor Boc-VAD-fmk, and pifithrin-α. Cells were pretreated with 30 μM pifithrin-α, or 50 μM Boc-VAD-fmk or 1 μg/ml FasR for 1 h and, thereafter, treated with 400 μM BOOH together with 15 μM pifithrin-α or 25 μM Boc-VAD-fmk or 0.5 μg/ml FasR for 24 h, respectively. DMSO was added to the medium in appropriate amount as control (**$P < 0.01$, DMSO control vs. pifithrin-α–treated monocytes, *t* test). Data of three independent experiments ± SD are shown.

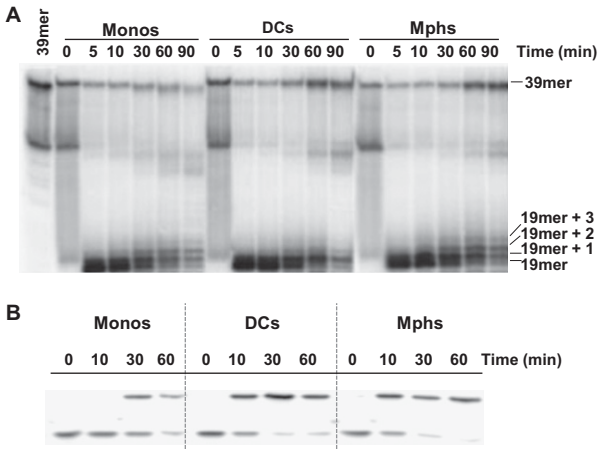

Fig. S7. (*A*) Formation and repair of BER intermediates in cell extracts of monocytes, DCs, and macrophages (Mphs). Repair reactions were analyzed on a 20% urea-denaturating polyacrylamide gel and show the AP oligonucleotide after incubation with cell extracts for increasing periods of time (0, 5, 10, 30, 60, and 90 min). The 39-mer fragment (full length) represents molecules that contain the lesion or are fully repaired. The 19-mer fragment indicates that incision occurred; fragment of 19-mer + 1(2,3)-mer indicates resynthesis by short/long patch BER. The midsize oligo represents very likely nonannealed ssDNA that becomes degraded by nucleases in the extracts. (*B*) DNA ligation assay. In vitro ligation reactions were performed using 20 μg whole-cell extracts and duplex oligonucleotides containing a single nick. The oligonucleotide containing the single-strand break was labeled with ^{32}P. The representative blot shows the kinetics of the ligation reaction in cell extracts of monocytes, DCs, and macrophages. Repair reactions were analyzed on a 20% urea-denaturating polyacrylamide gel after incubation of the oligonucleotide with cell extracts for increasing periods of time (0, 10, 30, 60 min).

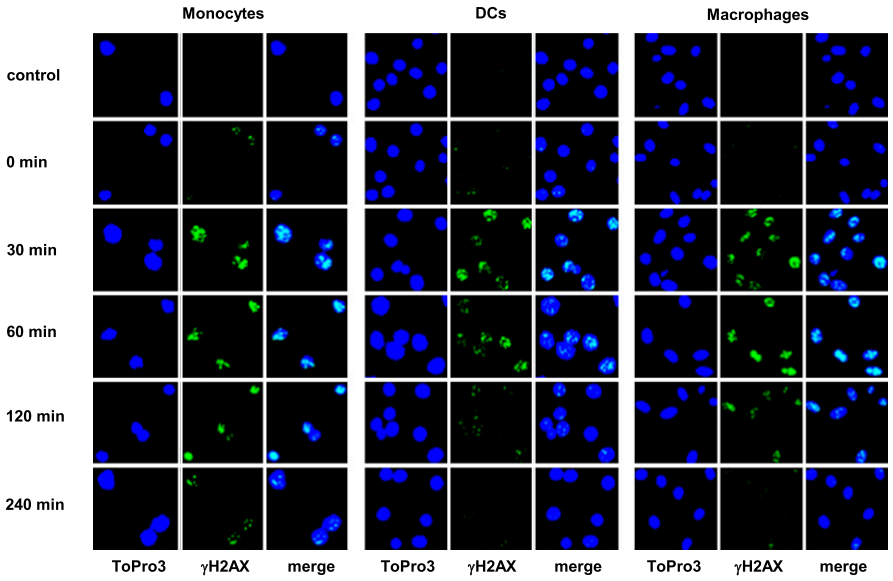

Fig. S8. Formation and removal of γH2AX foci in monocytes, DCs, and macrophages as a function of time after irradiation with 5 Gy. ToPro3, nuclear staining; γH2AX, staining with γH2AX antibody.

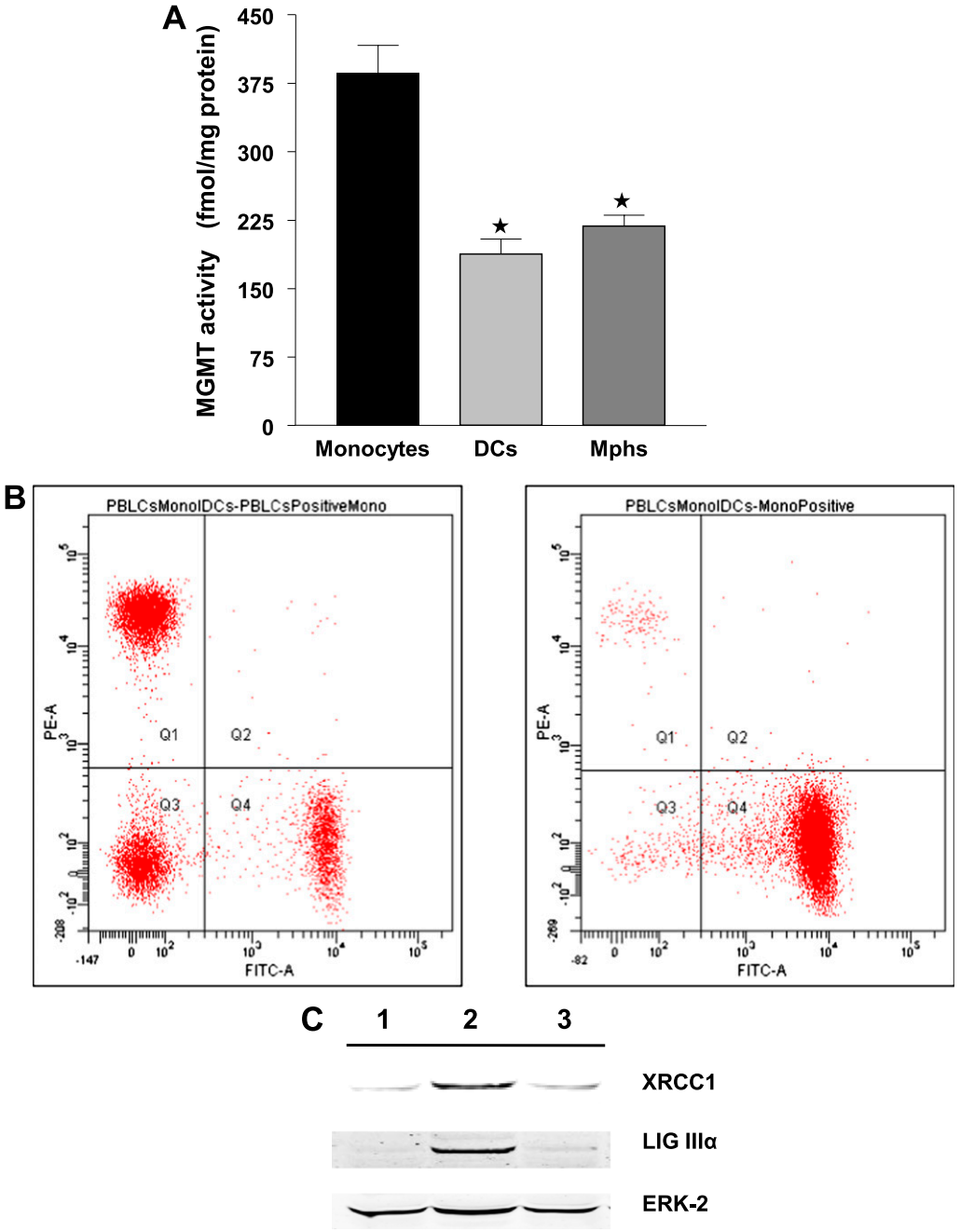

Fig. S9. (*A*) O^6-methylguanine-DNA methyltransferase (MGMT) activity in monocytes, DCs, and macrophages (Mphs) obtained from three different healthy donors (**$P < 0.01$, monocytes vs. DCs and macrophages, *t* test). (*B*) Blood cell populations isolated by Miltenyi columns and analyzed by flow cytometry. Q1, T cells; Q4, monocytes. *Left*: PBMCs following gradient centrifugation. *Right*: Cells after bead separation. (*C*) Lane 1, Western blot of extract of monocytes isolated by the method of adherence; lane 2, extract of DCs derived by GM-CSF and IL-4 from monocytes; lane 3, extract of monocytes isolated by CD14 Miltenyi beads.