**Supplementary material**

# Compromised DNA repair and signaling in human granulocytes

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## Material and methods

*Cell death detection by AnnexinV/PI staining*

Cells were pelleted and resuspended in 50 µl annexin V binding buffer (10 mM HEPES; pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$, 0.1 % BSA) and 2.5 µl annexin V-FITC. Samples incubated on ice in the dark for 20 min before samples were topped up with 200 µl annexin V binding buffer + 1 µl of 50 µg/ml PI. Cell death was measured in a FACS Canto II (Becton±Dickinson, Heidelberg, Germany) and data was analysed using the FACS Canto II Diva software.

*RNA isolation, cDNA synthesis and quantitative PCR (qPCR)*

Total RNA was isolated using the NucleoSpin RNA II kit (Machery and Nagel, Düren, Germany) according to the manufacturer's instructions. 200 ng RNA were transcribed into cDNA using random hexamer primers (Verso cDNA kit, Thermo Scientific, Darmstadt, Germany). qPCR was performed using the SensiMix™ SYBR Green & Fluorescein Kit (Bioline, Luckenwalde, Germany). The primers were purchased from Primerdesign Ltd (Cambridge, UK); *ACTB* (for 5'-TGGCATCCACGAAACTACC-3', rev 5'-GTGTTGGCGTACAGGTCTT-3'), *ATM* (for 5'-TATCTGCTGCCGTCAACTA-3' and rev 5'-GGAATCTGAATGCCGATCTAG-3'), *ATR* (for 5'-AAGGCAGTTGTATTGAAT-3' and rev 5'-CAGATGACTTCACAGATT-3') and *MGMT* (For 5'-CTCTTCACCATCCCGTTT-3' and Rev 5'-AATCACTTCTCCGAATTTCAC-3').

*Alternative cell lysis protocol for Western blot analysis*

Based on Kurosawa *et al.*, cell pellets were lysed in a buffer containing 6 M urea, 2 % SDS, 0.5 % glycerol, 2 % β-mercaptoethanol, 50 mM Tris-HCl pH 6.8, and 0.1 % bromophenol blue (1). Lysates were heated at 95°C for 2 min and then sonicated 2 x 10 s. SDS-PAGE and Western blotting was performed as described in the main text.

Reference:

Kurosawa, A., Shinohara, K.-i., Watanabe, F., Shimizu-Saito, K., Koiwai, O., Yamamoto, K. and Teraoka, H. (2003) Human neutrophils isolated from peripheral blood contain Ku protein but not DNA-dependent protein kinase, *The International Journal Biochemistry & Cell Biology,* **35,** 86–94.

## Figures

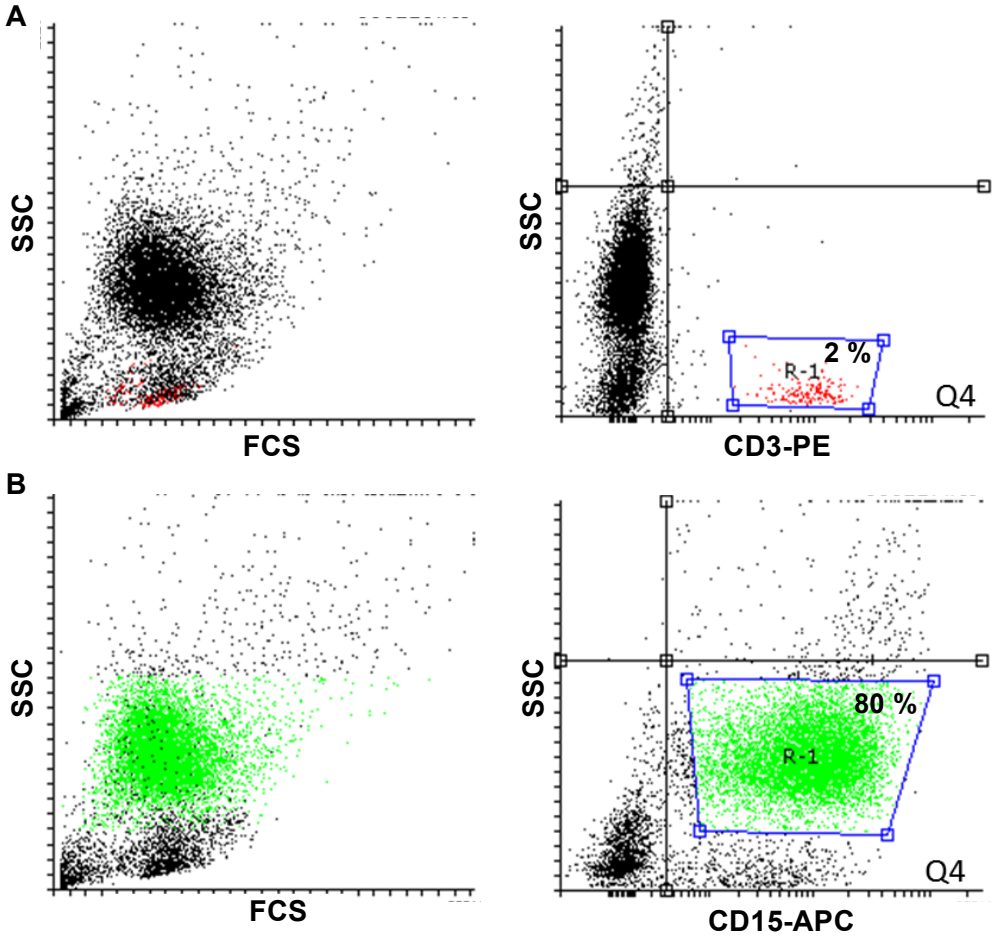


**Fig. S1** Granulocytes were stained with CD3-PE (A, red) or CD15-APC (B, green) after Optiprep density centrifugation and analyzed by flow cytometry.

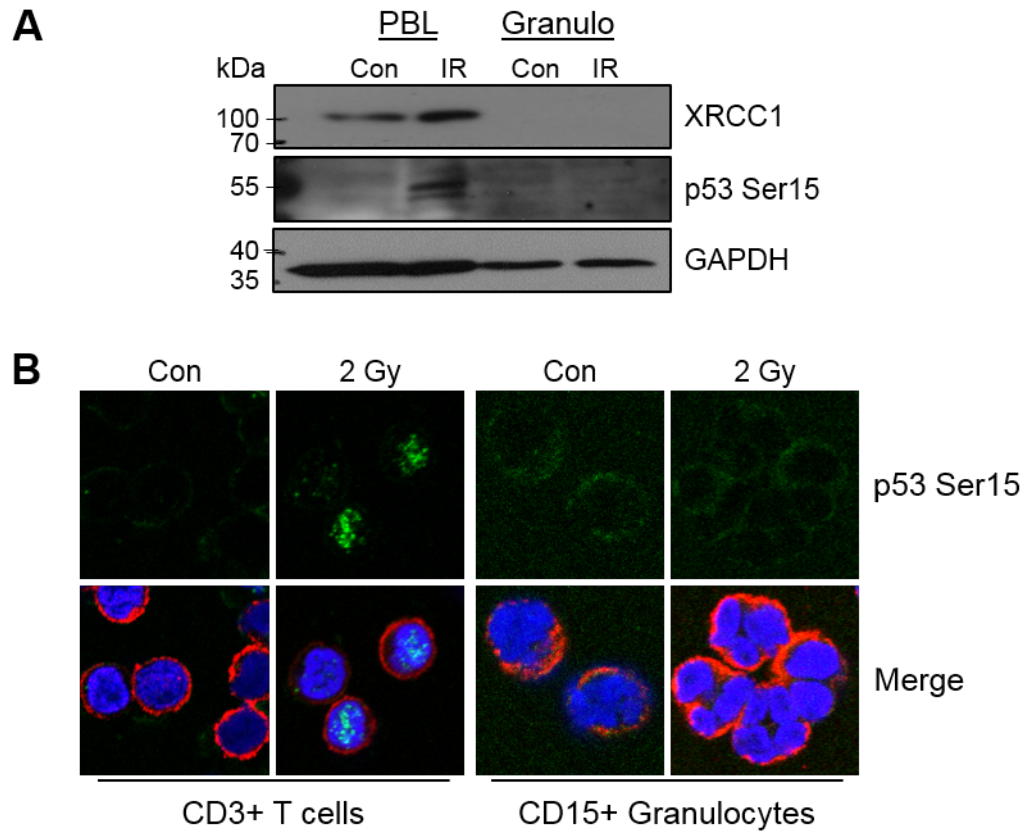


**Fig. S2** A) Determination of XRCC1 and p53Ser15 in granulocytes and PBL from buffy coat (6 h after taking blood). Cells were isolated and irradiated with 2 Gy. After 1 h cells were harvested for Western Blot analysis. B) Immunofluorescence staining of p53Ser15 in CD15+ granulocytes and CD3+ T cells after 2 Gy irradiation and 1 h post-incubation time. Red, surface marker CD3+ for T cells and CD15+ for granulocytes; green, p53Ser15; blue, TO-PRO-3. Representative images are shown.

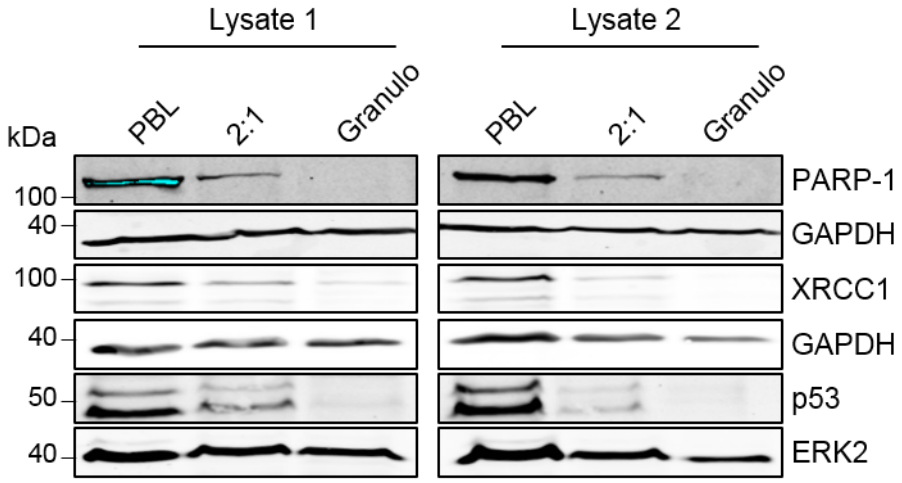


**Fig. S3** BER protein expression of granulocytes and PBL was analysed using an alternative lysis protocol (see supplemental material and methods). Cells were isolated from two different donors (lysate 1 and lysate 2, respectively). Granulocytes, PBL and a mixture of

PBL and granulocytes in a 2:1 ratio were subjected to SDS-PAGE followed by Western blot analysis. The BER proteins PARP-1 and XRCC1 as well as p53 were detected in PBL, but not in granulocytes. GAPDH and ERK2 were used as loading controls.

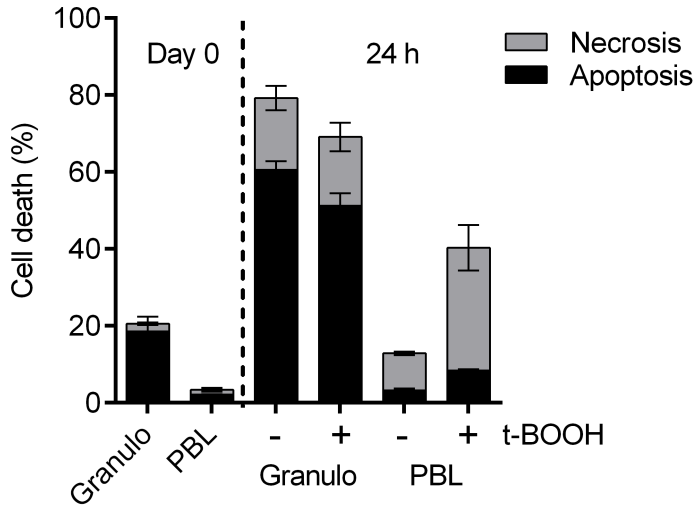


**Fig S4** Cell death trough apoptosis and necrosis was measured in granulocytes and PBL on the day of isolation in cells from buffy coat (Day 0) and 24 h later after *in vitro* culture and *t*-BOOH (100 µM) treatment. Cell death was quantified by flow cytometry using annexin V/PI staining. Data are the mean of three independent experiment ± SEM.

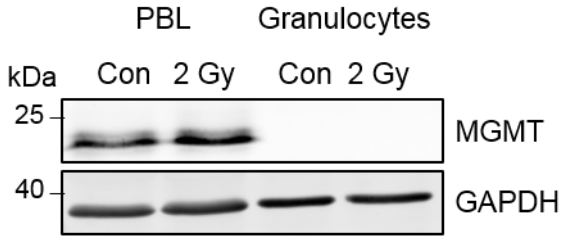


**Fig. S5** PBL and granulocytes were isolated from buffy coats and subjected to Western blot analysis using the standard lysis protocol described in material and methods. The alkyltransferase MGMT was clearly expressed in PBL, but not in granulocytes.

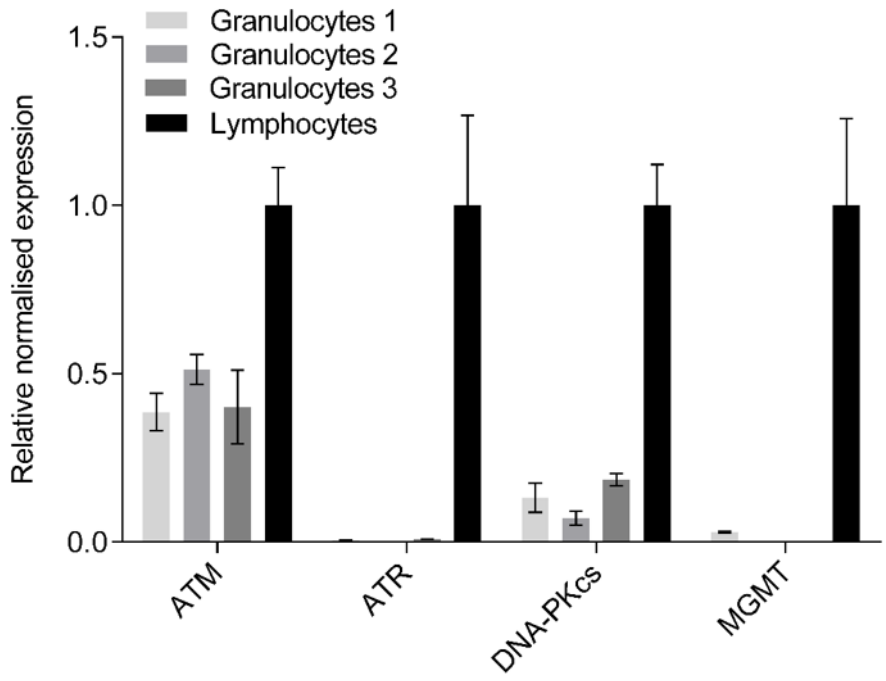


**Fig. S6** Expression of mRNA encoding ATM, ATR, DNA-PKcs and MGMT in PBL and three granulocyte samples, analysed by qPCR.

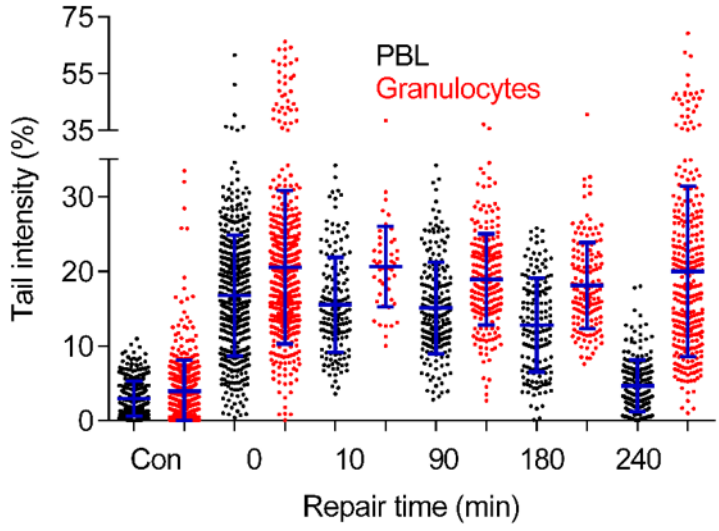


**Fig. S7** DSB repair kinetics after 2 Gy treatment was analysed in PBL (containing mostly T lymphocytes) and granulocytes on single cell level by the neutral comet assay. Data from three independent experiments are pooled. The mean value ± SD from three experiments is given for each treatment by the blue bar.

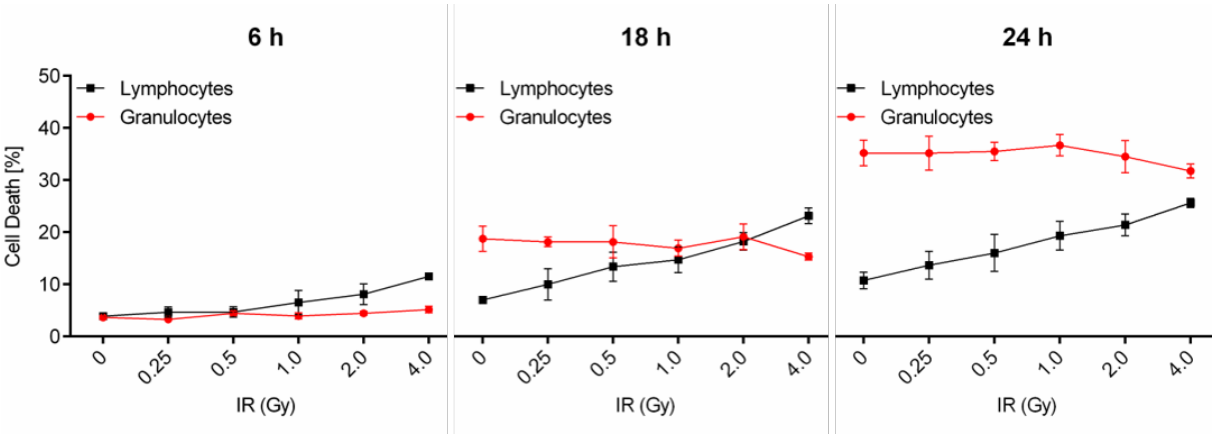


**Fig. S8 Cell death in PBL (containing mostly T cells) and granulocytes after ionising irradiation.** This is a corresponding graph to Fig. 5C. Cell death was measured 6, 18 and 24 h after treating the cells with IR in a dose range from 0.25 to 4 Gy. Data are the mean of three independent experiments ± SEM.