Supplementary material

***Neutral comet assay in Xenopus egg extract*:** Nuclei assembled in Xenopus egg extract were challenged with 0.5 μg/ml phleomycin, which induced formation of DSBs. These nuclei were then isolated and transferred to a second extract, which lacked phleomycin and which was G6PD or mock depleted. We then monitored the amount of DSBs at 0 and 60 minutes from the incubation in the second extract using the neutral comet assay. The comet assay was performed as indicated in the main text.

Radiosensitivity assay: Human fibroblasts were transfected with the control or G6PD targeting siRNA 2 days in a row. 24h after the last transfection cells were trypsinized and seeded at $1.2*10^4$ in each 10cm plate, the day after the cells were exposed to 2 Gy. After 10 days the plates were placed on ice and rinsed in ice-cold 1x PBS. The cells were then fixed in ice-cold methanol for 10minutes and stained in 0.5% crystal violet in 25% methanol. The plates were rinsed in deionized water as long as the excess of crystal violet was removed. After air-dry the plates the colonies were counted.

ROS measurement: human fibroblasts were transfected with the indicated siRNA and at 24h from the last transfection the cells were divided in two plates. The day after the cells were rinsed in 1x PBS and incubated 10minutes at 37ºC in 1x PBS containing 10 μM dichlorofluorescein diacetate (DCF-DA, Molecular Probes). The cells were then exposed to 10 Gy, rinsed and collected in 1xPBS. The samples were finally filtered and DCF-DA intensity was determined by FACS analysis with a BD-Facscalibur flow cytometer.

Supplementary figure legends:

Figure S1: DHEA prevents IR-induced G6PD activation. Normal lymphoblasts were treated for 15 minutes with 100 μM DHEA or the equivalent amount of EtOH. The cells were then exposed to 10 Gy or left untreated, collected in PBS and processed as described in Material and Methods to determine G6PD activity. The graph represents G6PD activity over 10 minutes kinetic reading.

Figure S2: Quantification of Hsp27 in human cells. **A)** 5 and 10 μg of total protein lysates derived from human lymphoblasts and fibroblasts were loaded on an SDS-PAGE and analyzed by Western blot with anti-Hsp27 antibody. The amount of protein was compared with known amount of recombinant Hsp27, as indicated at the top of the table. **B)** Taking into account the total protein content, the relative Hsp27 content and the volume of the cell lysates the amount of Hsp27 was determined, as indicated in the table.

Figure S3: Radiosensitivity assay in G6PD silenced cells. Human fibroblasts were transfected with control and G6PD targeting siRNA. After 72h from the first round of transfection the cells were irradiated with 2 Gy or left untreated. After 14 days the Colony Forming Rate (CFR) was determined. The graph represents the average of three independent experiments.

Figure S4: DSB repair in Xenopus egg extract. **A)** Untreated or phleomycin treated nuclei were added to both mock and G6PD depleted extracts. The samples were incubated for 60 minutes and then analyzed by neutral comet assay. **B)** The histogram represents the phleomycin induced DSBs calculated as a ratio of average tail moment in the samples incubated with phleomycin treated nuclei and the correspondent control.

Figure S5: Human fibroblasts were irradiated with 10 Gy and incubated for 1h. The cells were then lysed and the total extract was analyzed by western blot with anti-Caspase-3 antibody.

Figure S6: Effect of DNA-PK inhibitor on DSB repair. Human fibroblasts were treated with 1 μM DNA-PKi or the vehicle for 1h. After that the cells were either left untreated or irradiated with 10 Gy. The amount of DSBs was determined by neutral comet assay at the indicated time points.

Figure S7: ROS levels upon IR in control and G6PD silenced cells. Human fibroblasts were transfected with the indicated siRNA. At 72h the cells were incubated with 20 μM DCF-DA and then irradiated with 10 Gy or left untreated. The amount of ROS was evaluated by FACS analysis. The graph represents the fold increase of ROS production in every cell line compared to the untreated control.

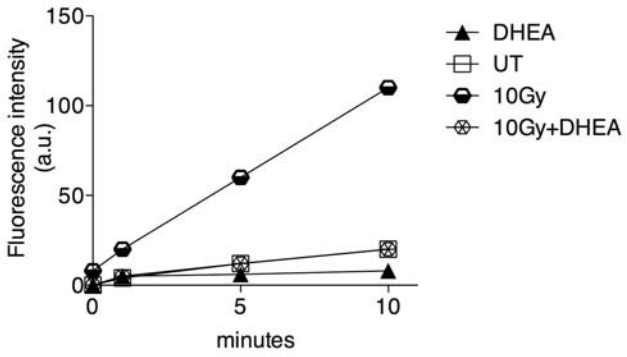
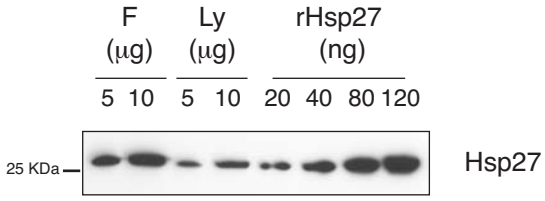

150
100
50
0
Fluorescence intensity (a.u.)
0
5
10
minutes
DHEA
UT
10Gy
10Gy+DHEA

Fig S1

A



B

	Total protein content				Hsp27content			
	μl	μg/μl	μg	ng/cell	ng/μl	μg/sample	pg/cell	%tot
F	100.00	1.84	184.00	0.35	14.81	1.48	2.85	0.80
Ly	100.00	0.78	78.00	0.15	3.13	0.31	0.60	0.40

Fig S2

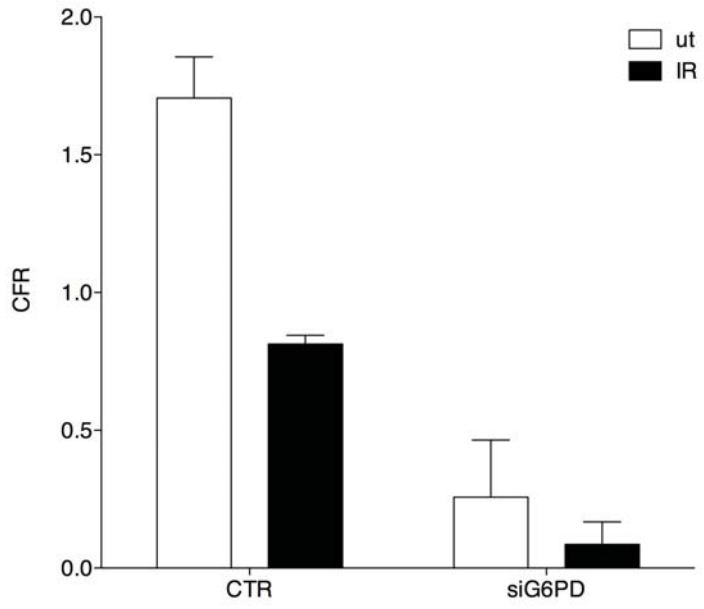
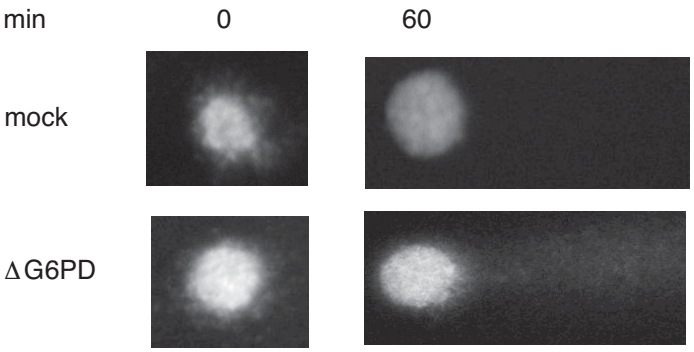


Fig S3

A


min
0
60
mock
ΔG6PD

B

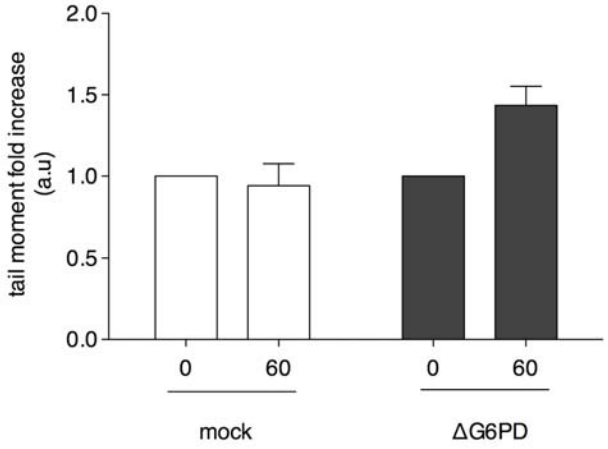

tail moment fold increase (a.u)
2.0
1.5
1.0
0.5
0.0
0
60
0
60
mock
ΔG6PD

Fig S4

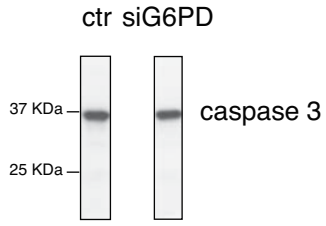


Fig S5

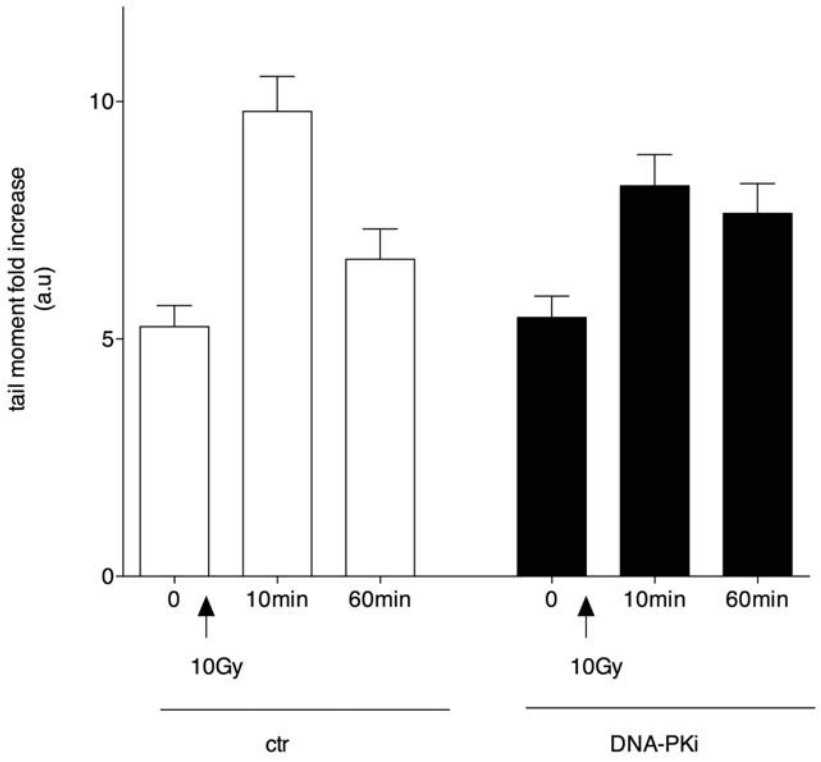

tail moment fold increase
(a.u)
10
5
0
0
10min
60min
10Gy
0
10min
60min
10Gy
ctr
DNA-PKi

Fig S6

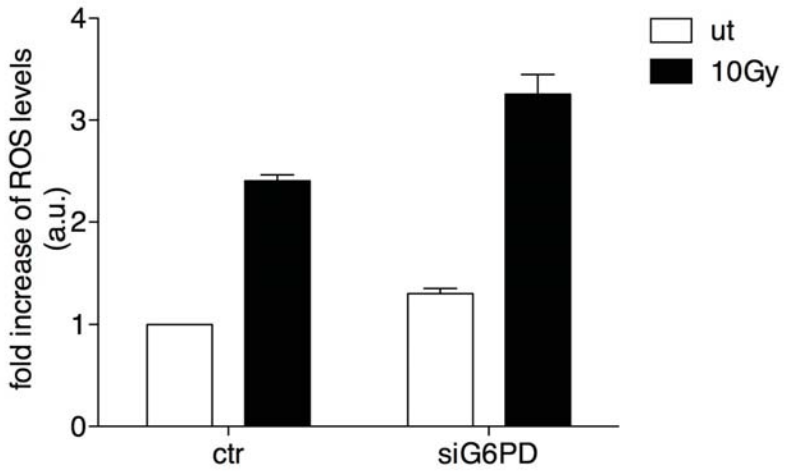


Fig S7