Supporting Information

Grudzenski et al. 10.1073/pnas.1002213107

SI Materials and Methods

Measurement of Nuclear PAR Synthesis via Nicotinamide-Adenine Dinucleotide-^{32}P. [Adenylate-^{32}P] nicotinamide-adenine dinucleotide (NAD) (800 Ci/mM, 5 mCi/mL) was obtained from Perkin–Elmer. To measure PAR synthesis, HSF1 cells were treated with H_2O_2 or irradiated in a manner identical to that used in the experiments in Fig. 2. In detail, 10^6 cells were first incubated in PB buffer [100 mM KacO, 30 mM KCl, 10 nM Na_2HPO_4, 1 mM $MgCl_2$, 1 mM Na_2ATP, 1 μM DTT (pH 7.2)] containing 20 nM NAD-^{32}P and H_2O_2 at 4 °C for 30 min. Cells were then washed three times with cold PB buffer to remove residual H_2O_2. Irradiated samples were treated the same way with PB buffer not containing H_2O_2 and were irradiated at this step. All samples were then incubated in PB buffer with 20 nM fresh NAD-^{32}P for 15 min at 37 °C, washed twice in cold PB buffer, and fixed in cold PBS containing 1.5% (wt/vol) paraformaldehyde and 2% (wt/vol) sucrose for 5 min. After washing with PB buffer, cells were transferred in tubes and centrifugated at 400 × *g*. After washing with PBS, cells were resuspended in Nonidet P-40 buffer [10 mM Tris-HCl, 10 mM NaCl, 3 mM $MgCl_2$, 30 mM sucrose, 0.5% Nonidet P-40 (pH 7.0)], centrifugated at 1,500 × *g*, and resuspended again and centrifugated. Finally, cells were resuspended in $CaCl_2$ buffer [10 mM Tris-HCl, 10 mM NaCl, 3 mM $MgCl_2$, 30 mM sucrose, 10 mM $CaCl_2$ (pH 7.0)] and centrifugated at 1,500 × *g*. The resulting cell pellet was dissolved in 2 mL of scintillation liquid, and counts were measured in a scintillation counter.

Microarray Analysis. Highly confluent HSF1 cells were H_2O_2-treated or irradiated in a manner identical to that used in the experiments in Fig. 2. In short, H_2O_2 treatment was for 30 min at 4 °C in PBS, followed by incubation in cell culture medium at 37 °C for 5 h and cell harvest. Irradiated samples were also treated with PBS at 4 °C for 30 min (but without H_2O_2) and were then irradiated, incubated for 5 h, and lysed in RLT buffer (Qiagen). All the following steps were performed by Aros Applied Biotechnologies according to the methods detailed below.

Three hundred nanograms of total RNA was used as starting material for the cDNA preparation. The first- and second-strand cDNA synthesis was performed using the 3′ IVT express Kit (Affymetrix) according to the manufacturer's instructions. Labeled cRNA was prepared using the 3′ IVT express Kit according to the manufacturer's instructions.

Six micrograms of cRNA was fragmented at 94 °C for 35 min in fragmentation buffer containing 40 mM Tris-acetate (pH 8.1), 100 mM KOAc, and 30 mM MgOAc. Before hybridization, the fragmented cRNA in a 6× saline-sodium phosphate-EDTA-triton (SSPE-T) hybridization buffer [1 M NaCl, 10 mM Tris (pH 7.6), 0.005% Triton] was heated to 95 °C for 5 min and subsequently to 45 °C for 5 min before loading onto the Affymetrix GeneTitan fully automated hybridization, wash, and scan equipment. The fragmented cRNA was hybridized to the Affymetrix HT U133$^+$ PM array plate. The readings from the quantitative scanning were normalized with the Robust Multichip Averaging-method (RMA) using Affymetrix Gene Expression Analysis Software before loading into the MeV 4.1.1 for significance analysis of microarrays (SAM) using a false discovery rate threshold of ≥1 (1).

1. Tusher VG, Tibshirani R, Chu G (2001) Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci USA* 98:5116–5121.

Fig. S1. BrdU incorporation measured by flow cytometry analysis of highly confluent (*Upper*) or exponentially growing (*Lower*) primary human fibroblast line, HSF1. Cells were labeled with BrdU for 5 h before staining with anti-BrdU antibodies and propidium iodide. Cycling cells were observed in the confluent culture at a rate of 0.15% compared with 57.9% in growing cells.

Fig. S1. (PDF)

Fig. S2. Enlarged versions of the representative images of γ-H2AX staining shown in Fig. 1*A*. Each white circle indicates a γ-H2AX focus (green). Several foci are induced at 15 min after 200 mGy in each cell, but the foci are only marked in one cell (inside the rectangle). Note that the images were generated by maximum intensity projection of many image stacks (*z* = 40), which significantly reduces foci intensity compared with the situation of online foci scoring under the microscope. This effect is particularly relevant at early times when the foci are still small. Similarly, it is difficult to discriminate between foci inside nuclei and background signals above or below, which appear to localize to the nucleus in the 2D representations.

Fig. S2. (PDF)

Fig. S3. Spontaneous and IR-induced pATM focus levels in the primary human fibroblast line, HSF1. (*A*) Representative images of pATM staining. Each white circle marks a pATM focus (red). Several foci are induced at 15 min after 200 mGy in each cell, but the foci are only marked in one cell (inside the rectangle). (*B*) Background numbers of foci were assessed in 14 different samples from seven independent experiments. (*C*) Focus numbers at 5 min after irradiation with 2.5–200 mGy. The line represents a linear fit to the data points (~20 foci per 1 Gy). (*D*) Kinetics for the loss of foci in HSF1 cells after different radiation doses. Mean values from three different experiments are displayed. The black part of the columns represents the background values of the samples analyzed in parallel with the respective irradiated samples. Error bars represent the SEM.

Fig. S3. (PDF)

Fig. S4. γ-H2AX focus levels at 24 h after 10 mGy. Cells were either untreated, mock-treated, or treated with different concentrations of H_2O_2 (0.01–10 μM) before irradiation. Higher but not lower concentrations of H_2O_2 are able to "activate" repair of γ-H2AX foci. Background foci numbers (~0.1 focus per cell) were subtracted. Error bars represent the SEM.

Fig. S4. (PDF)

Fig. S5. Immunofluorescence staining of 53BP1 (green) in heart, small intestine, and kidney sections of C57BL/6 mice at 10 min and 72 h after in vivo irradiation with 10 mGy. Unirradiated mice served as controls. Each white circle marks a 53BP1 focus. The images are similar to the images in Fig. 3*A* but represent considerably larger tissue sections. In irradiated tissue samples, several discrete nuclear foci can be observed at 10 min after irradiation. The IR-induced focus levels decline by about 50% within 24 h but are still substantially higher than the focus levels of unirradiated tissue samples.

Fig. S5. (PDF)

Fig. S6. 53BP1 staining (green) in the heart, small intestine, and kidney of unirradiated or irradiated C57BL/6 mice analyzed at 10 min or 5 h after in vivo irradiation with 1 Gy. Unirradiated tissues are predominantly negative for 53BP1 foci, whereas several discrete nuclear foci can be observed at 10 min and, to a lesser extent, 5 h after irradiation.

Fig.S6. (PDF)

Fig. S7. Spontaneous and IR-induced γ-H2AX foci in various mouse tissues. (*A*) Focus levels were assessed in the heart, small intestine, and kidney of eight unirradiated mice and three mice analyzed at 10 min after 10 mGy. (*B*) Focus levels were assessed in the heart, small intestine, and kidney of three mice analyzed at 10 min after 100 mGy and two to three mice analyzed at 10 min after 1 Gy.

Fig. S7. (PDF)

Fig. S8. Kinetics for IR-induced γ-H2AX foci in various mouse tissues. Focus levels in the heart, small intestine, and kidney of C57BL/6 mice analyzed at 10 min, 5 h, or 24 h after in vivo irradiation with 10 mGy, 100 mGy, or 1 Gy are shown. Mean values from three mice are displayed. Background foci numbers were subtracted. Error bars represent the SEM.

Fig. S8. (PDF)

Table S1. List of the genes that are significantly up-regulated in confluent primary human fibroblasts treated with 200 mGy IR, 10 μM H_2O_2, or 10 mGy IR (relative to untreated control cells)

Public ID	Gene Title	Gene Symbol	Fold change 200 mGy vs. control
AI251399	protein kinase D2	PRKD2	-1.7283
NM_002594	proprotein convertase subtilisin/kexin type 2	PCSK2	1.3162
AI927479	hypothetical protein LOC552889	LOC552889	1.3705
NM_005823	Mesothelin	MSLN	1.3851
BU619319	KIAA1731	KIAA1731	1.3891
BC019100	YTH domain containing 2	YTHDC2	1.4042
AI739378	euchromatic histone-lysine N-methyltransferase 1	EHMT1	1.4092
AI290971	myotubularin related protein 6	MTMR6	1.4463
AW235355	translocated promoter region (to activated MET oncogene)	TPR	1.4503
AI768512	WNK lysine deficient protein kinase 1	WNK1	1.4536
W52819	SEC22 vesicle trafficking protein homolog C (S. cerevisiae)	SEC22C	1.4561
BF673049	CAP-GLY domain containing linker protein 1	CLIP1	1.4563
NM_017571	coiled-coil domain containing 88A	CCDC88A	1.4569
T68150	pleckstrin homology-like domain, family B, member 2	PHLDB2	1.4655
AW771935	TEA domain family member 1 (SV40 transcriptional enhancer factor)	TEAD1	1.4744
NM_025090	ubiquitin specific peptidase 36	USP36	1.4863
NM_018353	chromosome 14 open reading frame 106	C14orf106	1.4877
AI808395	NADH dehydrogenase (ubiquinone) Fe-S protein 1	NDUFS1	1.4980
AL045717	ERO1-like beta (S. cerevisiae)	ERO1LB	1.5076
AI803727	---	---	1.5257
Z25431	NIMA (never in mitosis gene a)-related kinase 1	NEK1	1.5308
AI049791	biorientation of chromosomes in cell division 1-like	BOD1L	1.5456
NM_007211	Ras association (RalGDS/AF-6) domain family (N-terminal) member 8	RASSF8	1.6221
AI916242	early endosome antigen 1	EEA1	1.6340
AW968823	dedicator of cytokinesis 11	DOCK11	1.6666

Public ID	Gene Title	Gene Symbol	Fold change 10 μM H_2O_2 vs. control
U75667	arginase, type II	ARG2	2.1922
AI561354	myosin X	MYO10	1.9791
AI803727	---	---	1.9509
AI049791	biorientation of chromosomes in cell division 1-like	BOD1L	1.9034
Z25431	NIMA (never in mitosis gene a)-related kinase 1	NEK1	1.7835
AF250311	tribbles homolog 3 (Drosophila)	TRIB3	1.7457
AF199015	ezrin	EZR	1.7327
AW131754	SWI/SNF related, matrix associated, actin dependent regulator of chromatin	SMARCA2	1.7161
AU149225	MAX gene associated	MGA	1.7067
AI927479	hypothetical protein LOC552889	LOC552889	1.6857
NM_007211	Ras association (RalGDS/AF-6) domain family (N-terminal) member 8	RASSF8	1.6589
AI768512	WNK lysine deficient protein kinase 1	WNK1	1.5935
AF283773	DDB1 and CUL4 associated factor 11	DCAF11	1.5251
AA864758	---	---	1.5018
BF056459	coiled-coil domain containing 18	CCDC18	1.4678
AI739378	euchromatic histone-lysine N-methyltransferase 1	EHMT1	1.4621
BF663141	ezrin	EZR	1.4496
AA211909	TOX high mobility group box family member 2	TOX2	1.4192

Public ID	Gene Title	Gene Symbol	Fold change 10 mGy vs. control
AI803727	---	---	1.5397
AA336502	structural maintenance of chromosomes flexible hinge domain containing 1	SMCHD1	1.7977
AI640348	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 7	PSMD7	1.4038
BF313832	DEAH (Asp-Glu-Ala-His) box polypeptide 9	DHX9	1.5936
AA825721	---	---	1.5012
AI290971	myotubularin related protein 6	MTMR6	1.4635
AF011499	guanine nucleotide binding protein (G protein), alpha 11 (Gq class)	GNA11	1.4124