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Gene silencing induced by oxidative DNA base damage: association with local decrease of histone H4 acetylation in the promoter region

Supplementary Material

Legends to Supplementary Figures

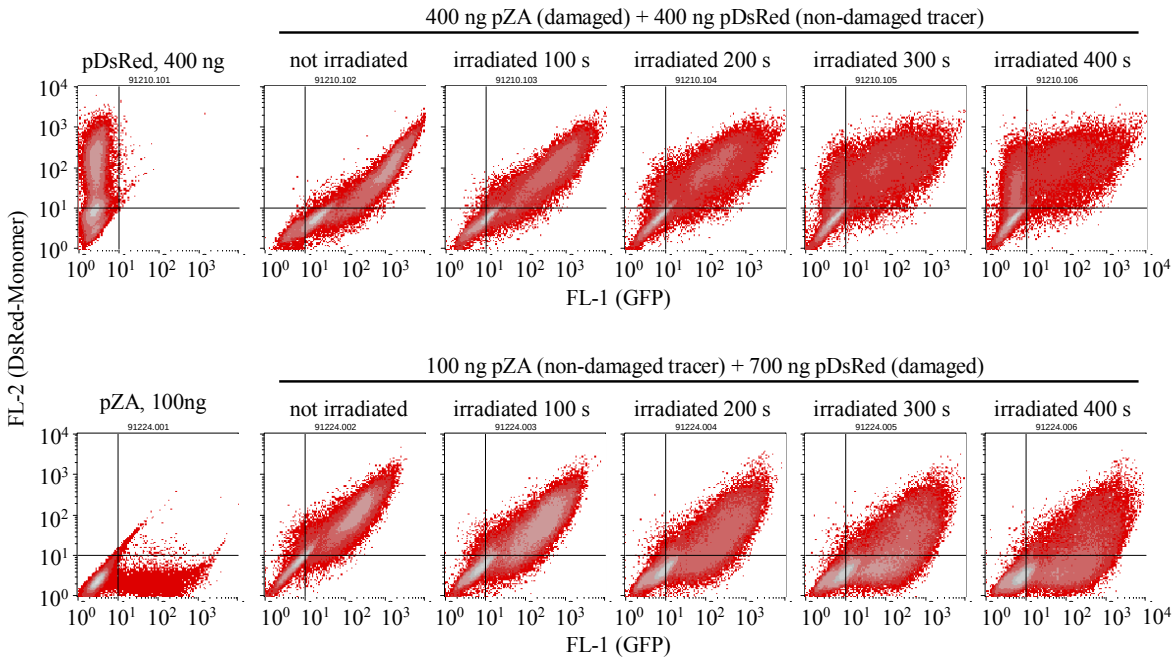
Supplementary Figure S1. Effects of various doses of oxidative DNA base damage on the expression of plasmid DNA in HeLa cells. Light exposure of 100 s in presence of methylene blue induced approximately one Fpg-sensitive base modification per plasmid molecule. Cells were transfected with the indicated amounts of either pZA (encoding for GFP), pDsRed (encoding for DsRed-Monomer), or co-transfected with both plasmids, one of which was damaged as indicated. (A) Dot density plots obtained by flow-cytometry of the transfected cells fixed 24 hours after transfections. (B) Expression of the damaged genes. Median fluorescence per cell calculated from the data shown in A in the cells with high expression levels of the reference undamaged plasmids, as described in Materials and Methods. Dotted lines show the background fluorescence levels in the cells transfected with reference plasmids only.

Supplementary Figure S2. Time course of expression of DsRed-Monomer in HeLa cells co-transfected with 100 ng of pZA (undamaged tracer plasmid) and 700 ng pDsRed dark-incubated in presence of methylene blue (oG=0) or exposed to a dose of visible light generating approximately 3 Fpg-sensitive sites per plasmid molecule (oG=3). (A) Dot density plots obtained by flow-cytometry of the transfected cells fixed at the indicated times after transfections. (B) Quantification of expression of the damaged pDsRed plasmid relative to the non-damaged pDsRed (red data series, mean and data range of two parallel transfection experiments). The expression of the pZA plasmid exposed to the same damaging conditions relative to the non-damaged pZA is plotted for comparison (green data series).

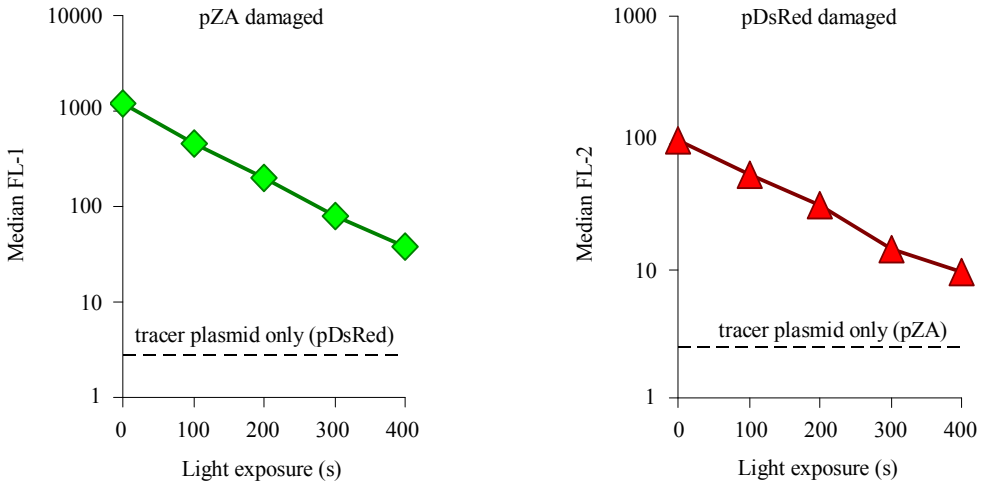
Supplementary Figure S3. Quantitative analyses of the association of acetylated histone H4 (AcH4) with promoter regions of the plasmid genes (DsRed-Monomer and GFP). Data of five ChIP experiments independently performed with chromatin preparations obtained from three independent transfections. In each ChIP experiment, recoveries of the promoter fragment of pZA plasmids (each containing no (oG=0) or three (oG=3) Fpg-sensitive oxidative base modifications) are directly compared to the recoveries of the corresponding fragment of pDsRed (undamaged reference) measured in the same sample. Filled bars: immunoprecipitations with antibody specific to AcH4; open bars: immunoprecipitations with non-immune IgG. Error bars show standard deviation of the mean calculated from n real-time PCR determinations.

Supplementary Figure S1

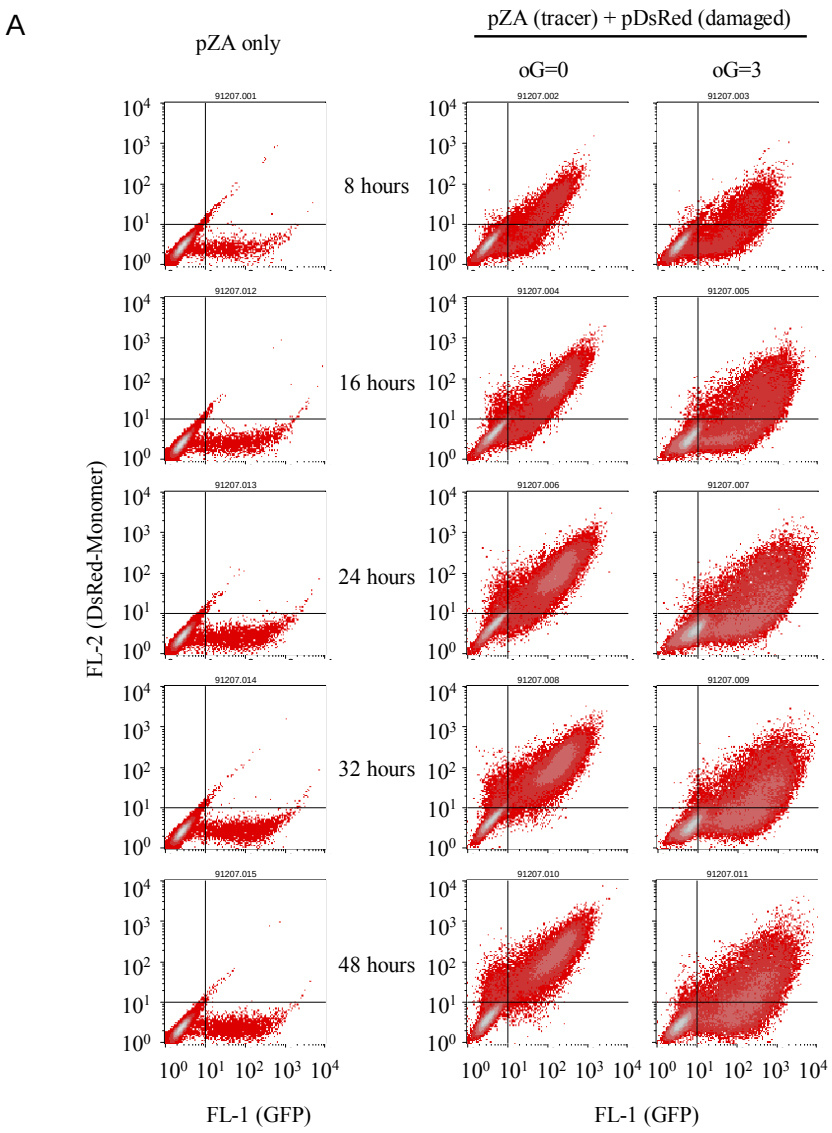
A

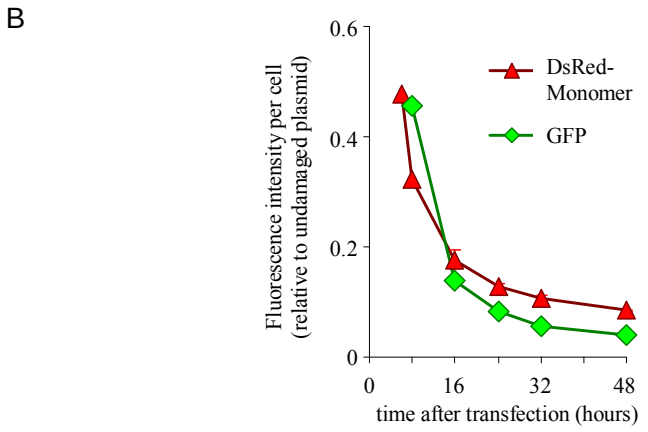


B

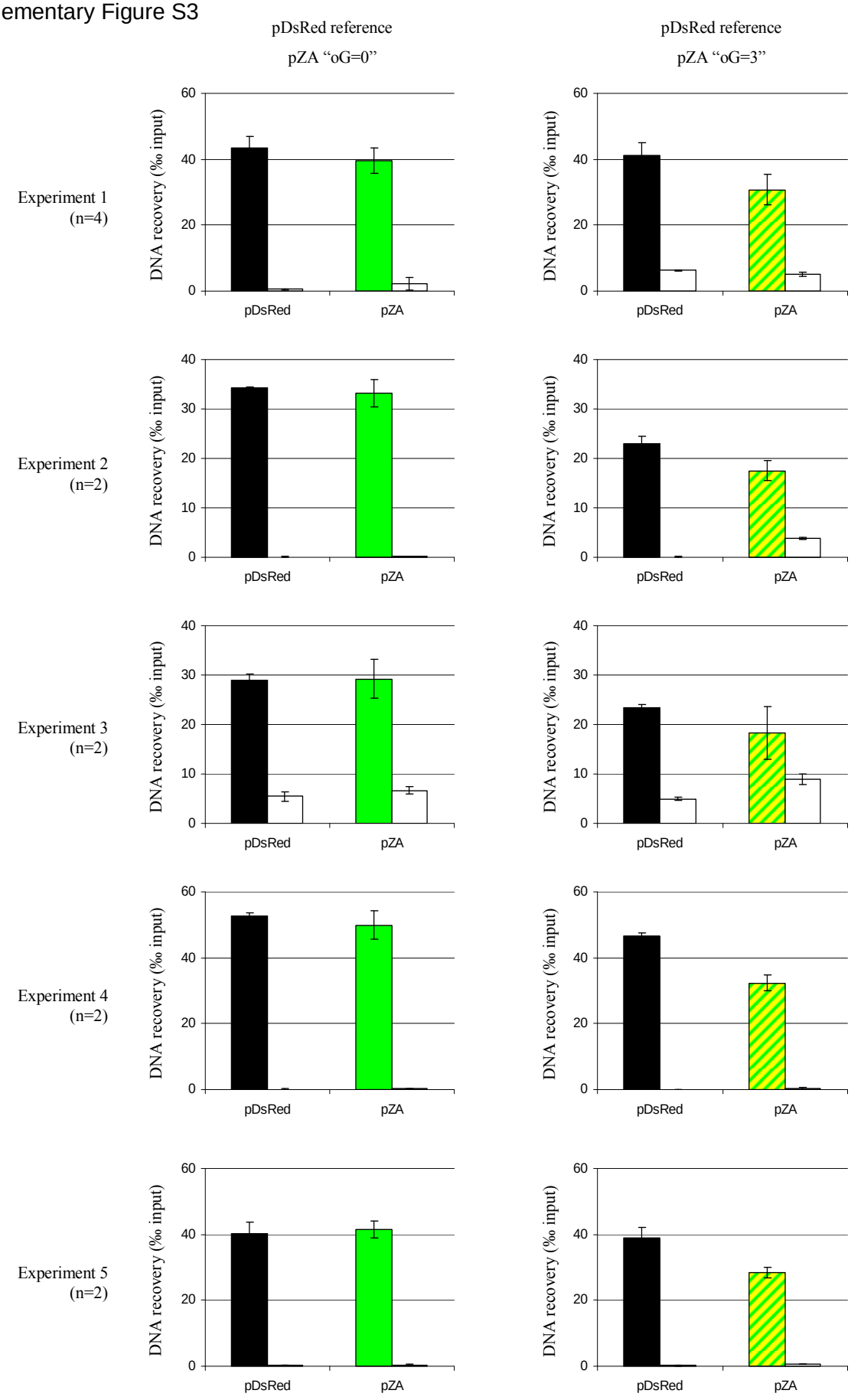


Supplementary Figure S2





Supplementary Figure S3



Supplementary Tables

Supplementary Table S1. Antibodies used in chromatin immunoprecipitation (ChIP).

Specificity	Designation	Manufacturer	Cat. No.	Origin, type	Amount used in ChIP
Non-immune	N-Imm	Chemicon	PP64	Rabbit IgG	8 µg
RNA Polymerase II	Pol2	Upstate	05-952	Mouse ascites	8 µl
Histone H1	H1	Santa Cruz Biotechnology	sc-8030	Mouse monoclonal IgG	8 µg
Acetyl-histone H3 (K9, 14)	AcH3	Upstate	06-599	Rabbit polyclonal IgG	8 µl
Acetyl-histone H4 (K5, 8, 12, 16)	AcH4	Upstate	06-866	Rabit antiserum	8 µl
di-methyl-histone H3 (K9)	H3K9Me2	Cell Signaling Technology	9753	Rabbit	8 µl
tri-methyl –histone H3 (K27)	H3K27Me3	Cell Signaling Technology	9733	Rabbit mAb	8 µl

Supplementary Table S2. Oligonucleotides used for ChIP analyses.
PCR-amplified DNA fragments are listed as the regions of the plasmid DNA (pZA or pDsRed) or as the chromosome regions according to the human gene nomenclature.

Target DNA fragment	Template	position relative to the transcription start	Sequence (5′ → 3′)	T_{ann} (°C)[a]	T_{acq} (°C)[b]	PCR product length (bp)
promoter[c]	pZA, pDsRed	-46	TGTACGGTGGGAGGTCTAT			
	pZA	+182	GGTGGTGCAGATGAACTTC	65	88	228
	pDsRed	+187	CCCTCGATCTCGAAGTAGTG	65	86	233
CDS	pZA	+156	CTGACCCTGAAGTTCATCTG			
		+355	GTCTTGTAGTTGCCGTCGTC	65	88	200
3′UTR	pZA	+570	GACCACTACCAGCAGAACAC			
		+785	GCCTGTGCTTCTGCTAGGAT	65	88	216
GAPDH	chromosome 12	-121	TAGCTCAGGCCTCAAGACCTT			
		+40	AAGAAGATGCGGCTGACTGTC	65	88	161
MYOD1	chromosome 11	-93	TTCCTATTGGCCTCGGACTC			
		+120	AAGTGCTGGCAGTCTGAATG	65	84	213
AFM	chromosome 4	+438	GGTAACAGTGGAACAATAGAT			
		+674	ACACCTCCTCTAGACATATGA	60	78	237

[a] Annealing temperature during the PCR reactions
[b] Fluorescence acquisition temperature during the PCR reactions.
[c] The amplified fragment corresponds to the proximal promoter region including the transcription start site

Supplementary Table S3. Oligonucleotides used for cDNA analyses.

Target transcript	Amplified templates	Sequence (5′ → 3′)	T_{ann} (°C)[d]	T_{acq} (°C)[e]	PCR product length (bp)
GFP	pZA, cDNA	CTGACCCTGAAGTTCATCTG GTCTTGTAGTTGCCGTCGTC	65	88	200
DsRed-Monomer	pDsRed, cDNA	TCAAGGAGTTCATGCAGTTC GAAGGACAGCTTCATGTAGT	65	88	239
GAPDH	exon 9, cDNA	AAGAGCACAAGAGGAAGAGA TTATTGATGGTACATGACAAG	60	84	158
MYOD1	exon 3, cDNA	CTAGACTGCCTGTCCAGCAT CAGAGCACCTGGTATATCGG	65	84	218
AFM	exon 8, cDNA	GCAAGGTTATGAACCATATT GGTCAGCATCTCGTTCTT	60	78	195

[d] Annealing temperature during the PCR reactions
[e] Fluorescence acquisition temperature during the PCR reactions.