Supporting Information

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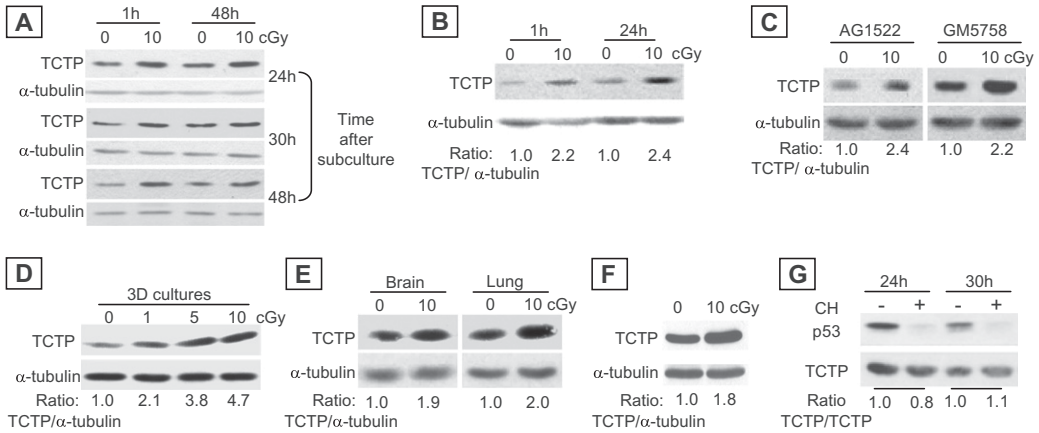

Fig. S1. Western blot analyses of translationally controlled tumor protein (TCTP) in normal human cells and in tissues of mice exposed to low-dose ^{137}Cs γ rays or 1 GeV protons. (*A*) Up-regulation of TCTP in asynchronous cells. Confluent normal human AG1522 cells were subcultured and exposed to acute γ rays at different time points: 24 h later, when the population was enriched in S-phase cells; at 30 h, when the population was enriched in G_2/M cells; and at 48 h, when dividing cells progressed in the following growth cycle. Cells were harvested 1 or 48 h after irradiation, and TCTP expression was quantified. (*B*) TCTP was induced up to 24 h after exposure of confluent AG1522 cells to acute 10 cGy of γ rays. (*C*) TCTP expression in confluent AG1522 or GM5758 normal human skin fibroblasts at 4 h after no exposure or acute exposure to 10 cGy of γ rays. (*D*) TCTP up-regulation in irradiated AG1522 cells maintained in a 3D architecture. Cells were exposed to low-dose γ rays and harvested 4 h later. (*E*) TCTP is up-regulated in tissues of C3H/HeJ mice exposed to whole-body irradiation by low-dose γ rays. Tissues were harvested for analysis 4 h after exposure. (*F*) TCTP is up-regulated in confluent AG1522 cells exposed to 1 GeV protons and harvested 24 h later. (*G*) Western blot analyses of TCTP in AG1522 confluent cell cultures exposed to 10 cGy irradiation at an acute dose rate (6 cGy/min) in the presence or absence of cycloheximide (2 μg/mL). Cells were incubated in cycloheximide for 30 min before irradiation and were harvested for analysis 24 or 30 h later.

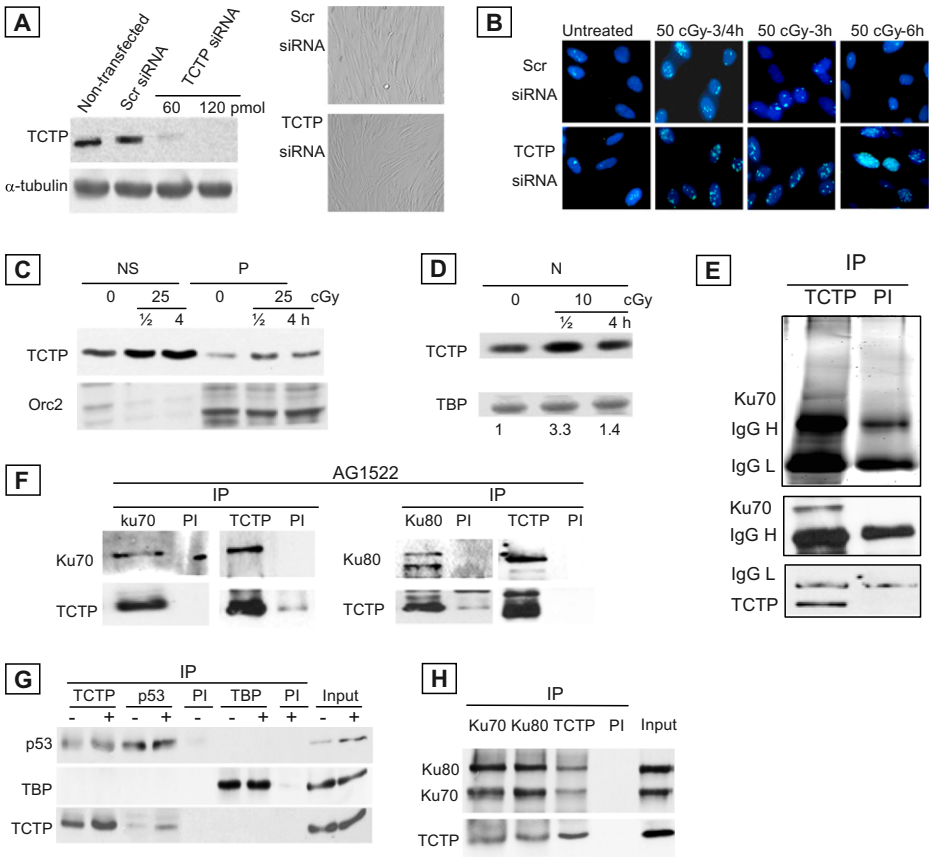

Fig. S2. Involvement of TCTP in sensing and repair of ^{137}Cs γ ray-induced DNA damage. (*A*) (*Left*) Western blot analysis of TCTP in untreated AG1522 cells or in AG1522 cells transfected with scrambled (Scr) siRNA or TCTP siRNA. (*Right*) Representative images of AG1522 confluent cells 72 h after transfection with Scr siRNA or TCTP siRNA. (*B*) Immunostaining for H2A.X phosphorylation (Ser139; green) in Scr siRNA- or TCTP siRNA-transfected AG1522 cells at different times after 50-cGy irradiation. (*C*) The γ ray-induced up-regulation of TCTP in soluble nuclear fraction (NS) and chromatin-enriched fraction (P). Extracts of AG1522 confluent cells exposed to no or 25-cGy irradiation were fractionated as described in *Materials and Methods*. Orc2, a chromatin-associated protein, was blotted, showing relative loading. (*D*) Immunoblot analyses show that TCTP levels are up-regulated in nuclei of U2OS confluent cells 0.5 and 4 h after exposure to 10 cGy. (*E*) (*Top*) Ku70 detection in mock/preimmune (PI) and TCTP-immunoprecipitated proteins from nuclei of U2OS confluent cells resolved by SDS/PAGE and stained with SYBR Gold. Heavy (H) and light (L) IgG bands are indicated. Western blots for TCTP (*Bottom*) and Ku70 (*Middle*) from the same immunoprecipitated samples. IP, immunoprecipitation. (*F*) Western blot analyses of TCTP, Ku70, and Ku80 following immunoprecipitations with respective antibodies against TCTP, Ku70, and Ku80 in isolated nuclear fractions of AG1522 confluent cells. Rabbit preimmune serum (PI) was used as a control. (*G*) Ethidium bromide-treated nuclear extracts isolated 30 min after exposure of U2OS confluent cells to no or acute-dose-rate 50-cGy radiation were immunoprecipitated with anti-TCTP, anti-p53, or control anti-TBP antibodies. Mouse or rabbit preimmune serum (PI) was used as a control. Immunoblotting was performed using antibodies against p53, TCTP, or TATA-binding protein (TBP). (*H*) Immunoblotting of TCTP, Ku70, and Ku80 in ethidium bromide-treated nuclear extracts of control U2OS confluent cells after immunoprecipitation with normal serum (PI) or with antibodies against TCTP, Ku70, or Ku80.

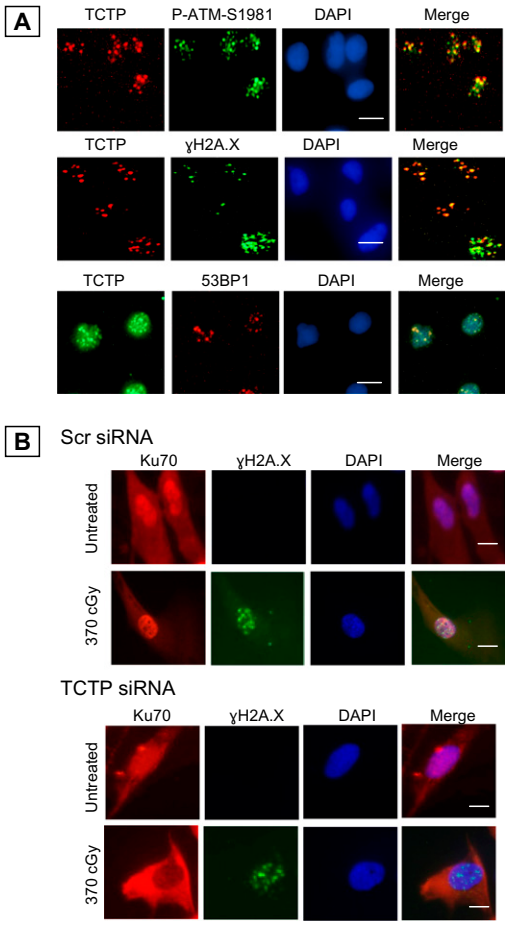

Fig. S3. (*A*) TCTP colocalizes with P-ATM (S1981), γH2A.X, and 53BP1 following ^{137}Cs γ irradiation. U2OS cells exposed to 100-cGy radiation were preextracted and were fixed 1 h later; they were immunostained with anti-TCTP, anti-P-ATM (S1981), anti-γH2A.X, or anti-53BP1 antibodies. (Scale bars, 10 μm.) (*B*) U2OS cells transfected with Scr or TCTP siRNA were left untreated or were exposed to 370-cGy radiation. The cells were fixed 0.5 h later and were immunostained in situ with anti-Ku70 (red) and anti-γH2A.X (green) antibodies. (Scale bars, 10 μm.)

Table S1. Top-ranked proteins modulated in AG1522 confluent cells exposed to 10 cGy of ^{137}CS γ rays (0.2 cGy/h) as revealed by the isobaric tags for relative and absolute quantitation shotgun proteomics approach

Change	UniProt accession no.	Protein name	Ratio*	SD
Increase	P13693	Translationally controlled tumor protein (TCTP) (p23) (histamine-releasing factor) (HRF)	1.811	0.072
Increase	P04083	Annexin A1	1.618	0.292
Increase	Q16740	Putative ATP-dependent Clp protease proteolytic subunit, mitochondrial precursor	1.566	0.293
Increase	P11413	Glucose-6-phosphate 1-dehydrogenase	1.544	0.252
Increase	P04264	Keratin, type II cytoskeletal 1 (Cytokeratin 1)	1.499	0.495
Increase	P17317	Histone H2A.Z (H2A/Z)	1.495	0.16
Increase	Q9P2J5	Leucyl-tRNA synthetase, cytoplasmic	1.466	0.091
Increase	P08253	72-kDa type IV collagenase precursor	1.456	0.223
Increase	Q15366	Poly(rC)-binding protein 2	1.445	0.499
Increase	Q10567	Adapter-related protein complex 1 beta 1 subunit	1.435	0.114
Increase	P51991	Heterogeneous nuclear ribonucleoprotein A3	1.433	0.081
Increase	O60664	Mannose-6-phosphate receptor binding protein 1	1.421	0.195
Increase	Q13596	Sorting nexin 1	1.407	0.093
Increase	O43399	Tumor protein D54 (hD54)	1.4	0.089
Increase	P82675	Mitochondrial 28S ribosomal protein S5 (S5mt)	1.395	0.094
Increase	Q13151	Heterogeneous nuclear ribonucleoprotein A0 (hnRNP A0)	1.391	0.047
Increase	O95340	Bifunctional 3′′-phosphoadenosine 5′-phosphosulfate synthethase 2 (PAPS synthethase 2)	1.362	0.333
Increase	P48681	Nestin	1.357	0.318
Increase	Q15046	Lysyl-tRNA synthetase	1.35	0.078
Increase	P09651	Heterogeneous nuclear ribonucleoprotein A1	1.325	0.028
Increase	Q8WWX9	Selenoprotein M precursor	1.319	0.08
Increase	P23381	Tryptophanyl-tRNA synthetase	1.317	0.329
Decrease	P62873	Guanine nucleotide-binding protein G(I)/G(S)/G(T) beta subunit 1 (Transducin beta chain 1)	0.613	0.38
Decrease	P99999	Cytochrome *c*	0.620	0.403
Decrease	P10620	Microsomal GST 1	0.714	0.235
Decrease	Q15629	Translocation associated membrane protein 1	0.717	0.194
Decrease	P35556	Fibrillin 2 precursor	0.732	0.01
Decrease	P61619	Protein transport protein Sec61 alpha subunit isoform 1	0.735	0.134
Decrease	P30049	ATP synthase delta chain, mitochondrial precursor	0.736	0.099
Decrease	P53680	Clathrin coat assembly protein AP17 (Clathrin coat associated protein AP17)	0.746	0.155
Decrease	Q9Y3D6	Tetratricopeptide repeat protein 11	0.748	0.286

*Ratio: protein level in irradiated vs. nonirradiated cells.