Fig. S1

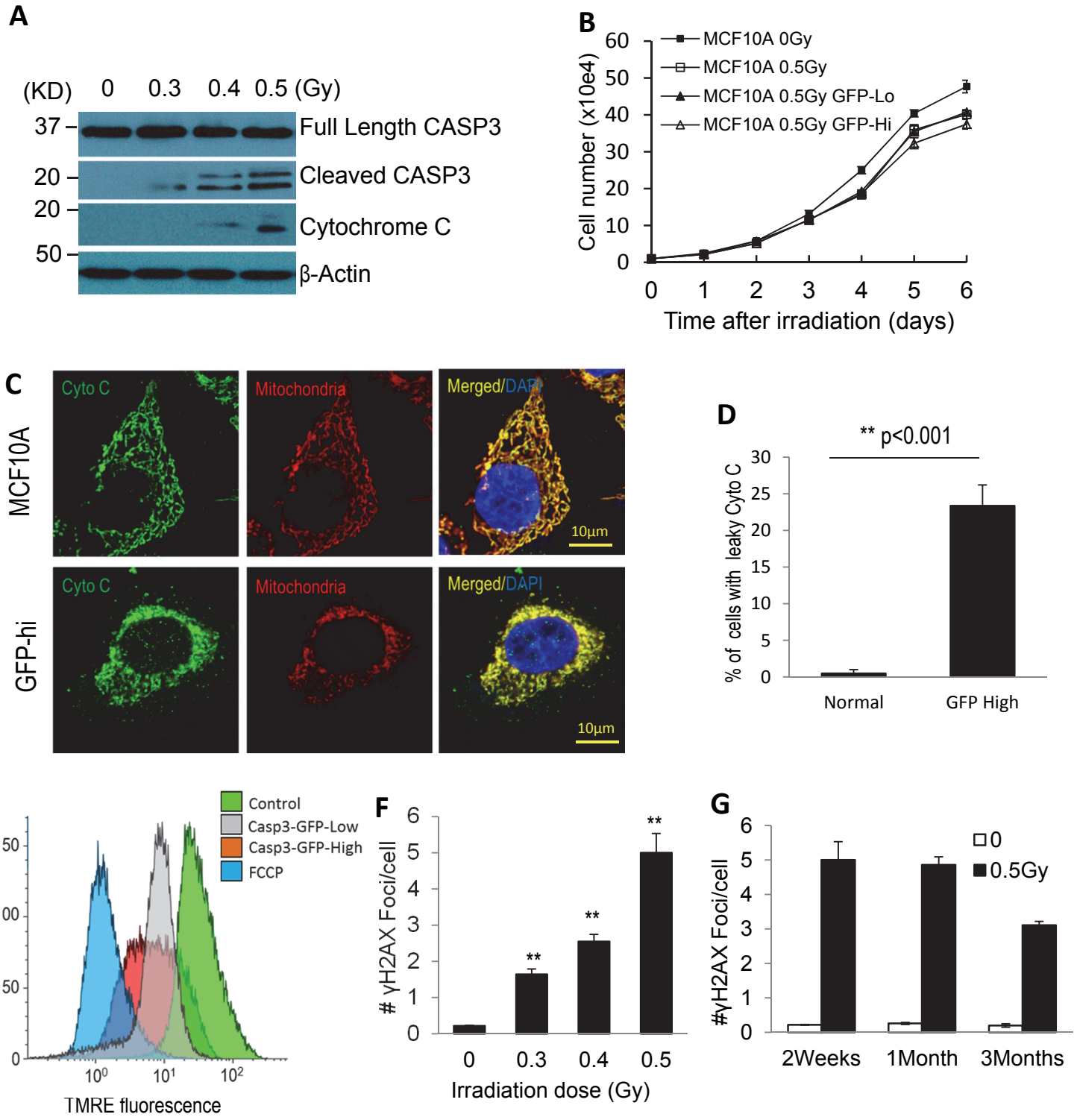
A
(KD) 0 0.3 0.4 0.5 (Gy)
37
Full Length CASP3
20
Cleaved CASP3
20
Cytochrome C
50
β-Actin
B
MCF10A 0Gy
MCF10A 0.5Gy
MCF10A 0.5Gy GFP-Lo
MCF10A 0.5Gy GFP-Hi
Cell number (x10e4)
Time after irradiation (days)
C
MCF10A
GFP-hi
Cyto C
Mitochondria
Merged/DAPI
10µm
D
** p<0.001
% of cells with leaky Cyto C
Normal
GFP High
E
Control
Casp3-GFP-Low
Casp3-GFP-High
FCCP
Cell Counts
TMRE fluorescence
F
γH2AX Foci/cell
Irradiation dose (Gy)
G
0
0.5Gy
#γH2AX Foci/cell
2Weeks
1Month
3Months

Figure S1 (related to Figure 1&2)**. Caspase 3 activation, cell proliferation, and γH2AX foci formation after exposure to radiation. A.** Western blot analysis of caspase 3 activation and cytochrome c release into the cytoplasm in MCF10A cells exposed to different doses of ^{56}Fe ions. **B.** Growth characteristics of irradiated (0.5 Gy) and sham-irradiated MCF10A cells. Irradiated, FACS sorted cells with high or low reporter activities were also evaluated together with non-sorted cells. **C.** Confocal microscopic imaging of Immunofluorescence staining of cytochrome c and mitochondria in parental and irradiated GFP-hi MCF10CA cells. There is clear cytochrome c staining in cytoplasmic areas outside the mitochondria. **D**. Percentage of parental and GFP-hi MCF10A cells with 'leaky" extra-mitochondrial cytochrome c staining pattern (similar to those shown in the lower panels of F**ig.S1C**). Error bars represent standard error of the mean. **E**. Flow cytometry of analysis of mitochondrial membrane potential in control MCF10A, FCCP-treated, Casp3-GFP-hi and Casp3-GFP-low MCF10A cells. Higher levels of fluorescence correlated with higher mitochondrial membrane potential. **F.** Dose-dependent γH2AX foci induction in MCF10A cells 14 days after exposure to ^{56}Fe ions (**p<0.01). **G.** Persistent γH2AX foci induction in MCF10A cells after exposure to ^{56}Fe ions.

Fig. S2

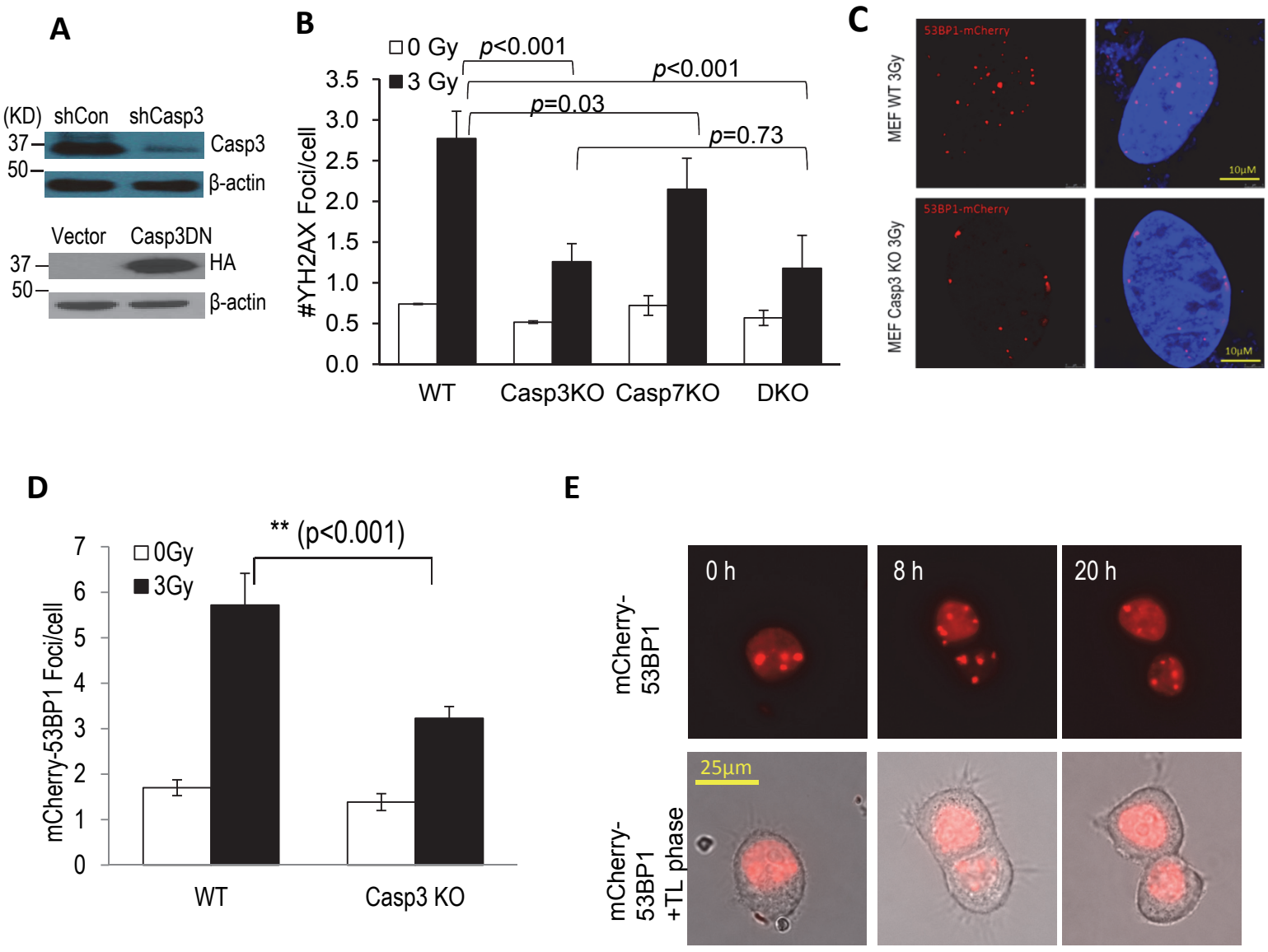
A
(KD) shCon shCasp3
37
50
Casp3
β-actin
Vector Casp3DN
37
50
HA
β-actin
B
0 Gy
3 Gy
p<0.001
p<0.001
p=0.03
p=0.73
#YH2AX Foci/cell
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
WT
Casp3KO
Casp7KO
DKO
C
MEF WT 3Gy
MEF Casp3 KO 3Gy
53BP1-mCherry
10μM
D
** (p<0.001)
0Gy
3Gy
mCherry-53BP1 Foci/cell
7
6
5
4
3
2
1
0
WT
Casp3 KO
E
mCherry-53BP1
mCherry-53BP1 +TL phase
0 h
8 h
20 h
25μm

Figure S2 (related to Figure 2). **Additional data on caspase 3 activation and DNA damage foci formation. A.** Western blot analysis of the effect of a small hairpin gene against the *CASP3* gene (*shCasp3*) in MCF10A cells (top panels), and a dominant-negative caspase 3 gene expression (Casp3DN) as detected by the presence of a hemaglutinin (HA) tag, which is part of *CASP3DN* gene. **B**. The number of γH2AX foci in irradiated MEFs with caspase 3 and/or 7 knockout 5 days after irradiation with 3 Gy of x-rays. Error bars represent standard deviation. **C.** Microscopic images of 53BP1 foci in sham and 3 Gy x-rays irradiated WT and casp3-KO MEF cells transduced with the fusion reporter *mCherry-53BP1* gene. **D.** The average number of 53BP1 foci in sham and 3 Gy x-rays irradiated MEF cells transduced with the fusion reporter *mCherry-53BP1* gene. Error bars represent standard deviation. All values are derived from the average of triplicate experiments. In each experiment, at least 200 cells were counted. Student's t-test was used to calculate the P values. **E.** Persistent, *de novo* generation of 53BP1 foci in irradiated MCF10A cells 14 days after cellular exposure to radiation. Shown are select images of the same cell with the mCherry-53BP1 reporter at different time points after the start of observation. Notice persistent foci presence in the progeny of the parent cell (middle and right panels).

Fig. S3

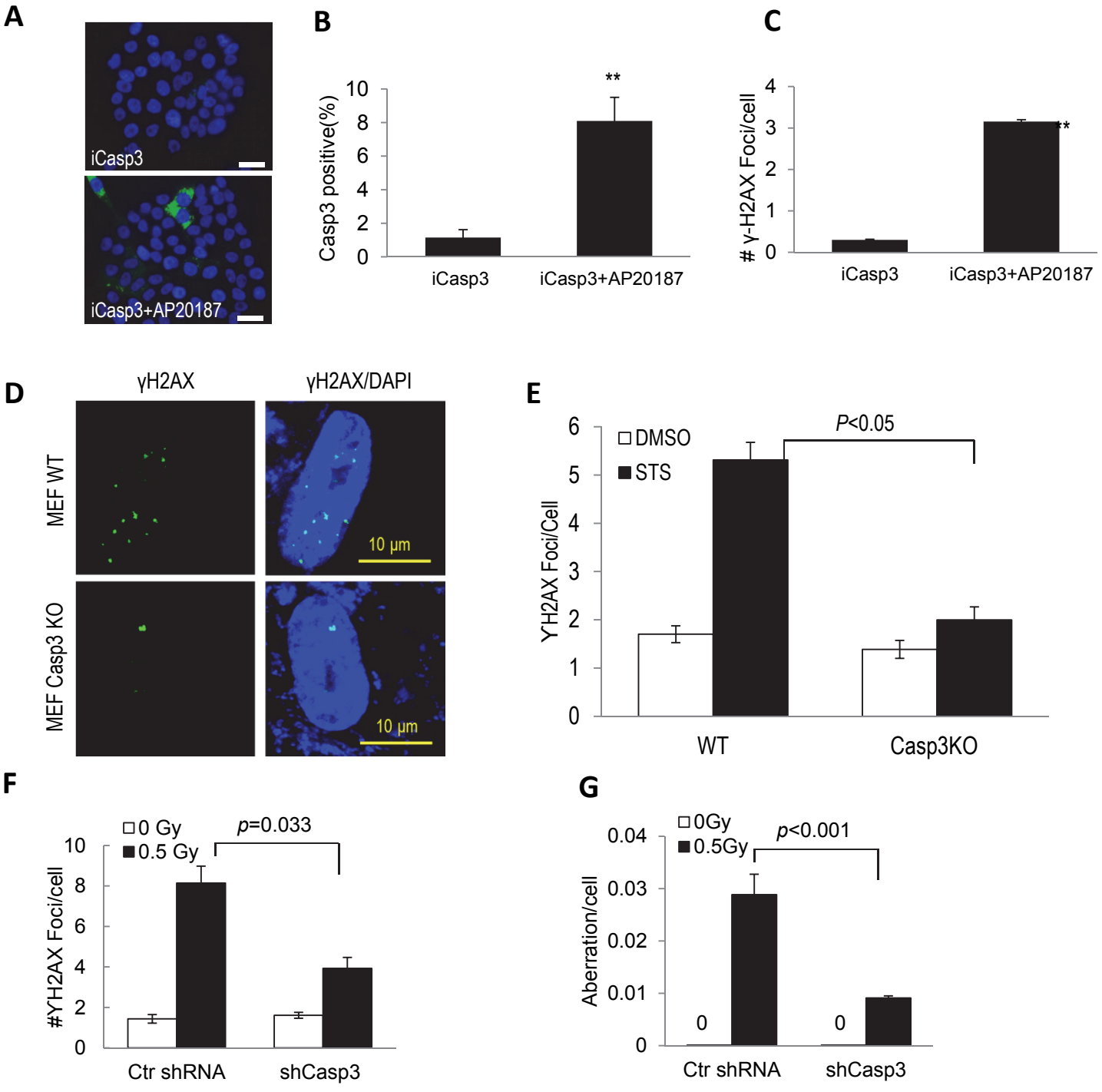
A
iCasp3
iCasp3+AP20187
B
Casp3 positive(%)
10
8
6
4
2
0
**
iCasp3
iCasp3+AP20187
C
γ-H2AX Foci/cell
4
3
2
1
0
**
iCasp3
iCasp3+AP20187
D
γH2AX
γH2AX/DAPI
MEF WT
MEF Casp3 KO
10 μm
10 μm
E
DMSO
STS
P<0.05
γH2AX Foci/Cell
6
5
4
3
2
1
0
WT
Casp3KO
F
0 Gy
0.5 Gy
p=0.033
#γH2AX Foci/cell
10
8
6
4
2
0
Ctr shRNA
shCasp3
G
0Gy
0.5Gy
p<0.001
Aberration/cell
0.04
0.03
0.02
0.01
0
0
0
Ctr shRNA
shCasp3

Fig. S3 (related to Fig. 2&3). **Additional data on the effect of caspase 3 attenuation and the role of p53 in radiation-induced foci formation. A.** MCF10A transduced with iCasp3 incubated with or without AP20187 for 48 h, then stained with anti-active casp3 antibody (Green). DAPI staining for cell count. Scale bar: 50μm. **B.** Caspase3 activation cells in MCF10A transduced with iCasp3 incubated with or without AP20187 (**p<0.01). **C**. The number of γH2AX foci in MCF10A transduced with iCasp3 incubated with or without AP20187 (**p<0.01). **D.** Immunofluorescence staining images of phosphorylated H2AX foci formation in wild type and Casp3KO MEF cells exposed to staurosporine (STS). Cells were processed 3 days after exposure with 0.2 uM of staurosporine for 2 hrs. **E**. Quantitative estimate of average number of γH2AX foci per cell in WT and Casp3KO MEF cells after STS treatment. The error bars represent standard error of the mean. Two-tailed student's t-test was used for calculation of the p-value. **F.** Knockdown caspase3 in IMR90 cells decreased radiation (0.5 Gy ^{56}Fe ions) induced γH2AX foci. **G.** Radiation-induced chromosome aberrations in IMR90 cells.

Fig. S4

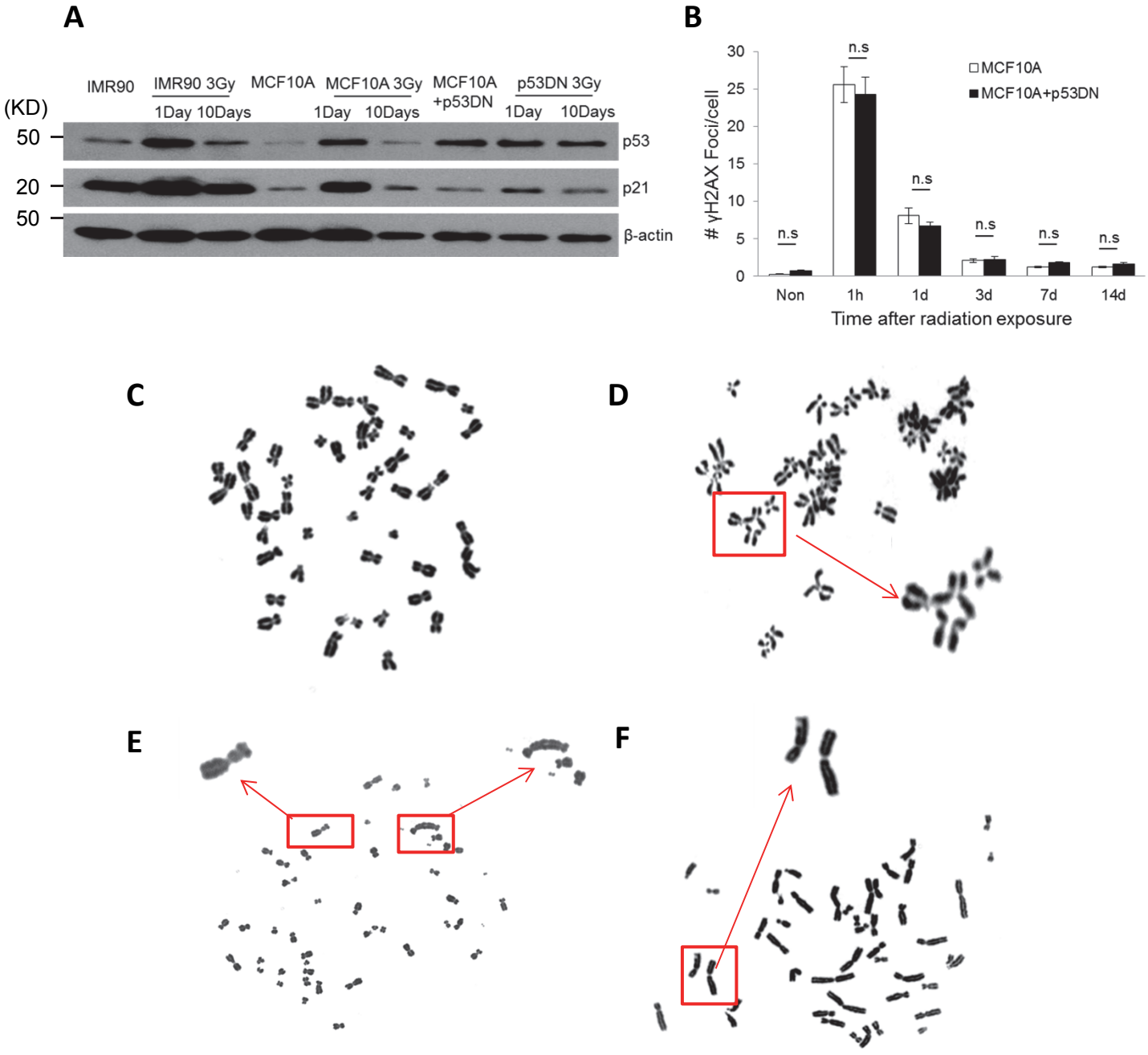
A
IMR90
IMR90 3Gy
1Day 10Days
MCF10A
MCF10A 3Gy
1Day 10Days
MCF10A +p53DN
p53DN 3Gy
1Day 10Days
(KD)
50
20
50
p53
p21
β-actin
B
γH2AX Foci/cell
30
25
20
15
10
5
0
n.s
MCF10A
MCF10A+p53DN
Non
1h
1d
3d
7d
14d
Time after radiation exposure
C
D
E
F

Figure S4 (related to Figure 3 & 4). **A** Western blot analysis of p53 and p21 protein expression levels in MCF10A cells irradiated with 3 Gy of x-rays. IMR90 cells were used as a normal cell control. **B.** Average number of γH2AX foci in irradiated MCF10A cells transduced with dominant negative p53 (p53DN) (n.s., not significant) .**C.** A normal chromosome spread of MCF10A. **D.** A chromosome spread with a chromosome exchange in irradiated MCF10A cells. The exchange is shown as an enlarged image at lower left corner. **E**. A chromosome spread with a dicentric (left) and a dicentric and interstitial deletion (right) in MCF10A. **F.** A chromosome spread with a chromatid break in a MCF10A cell.

Fig. S5

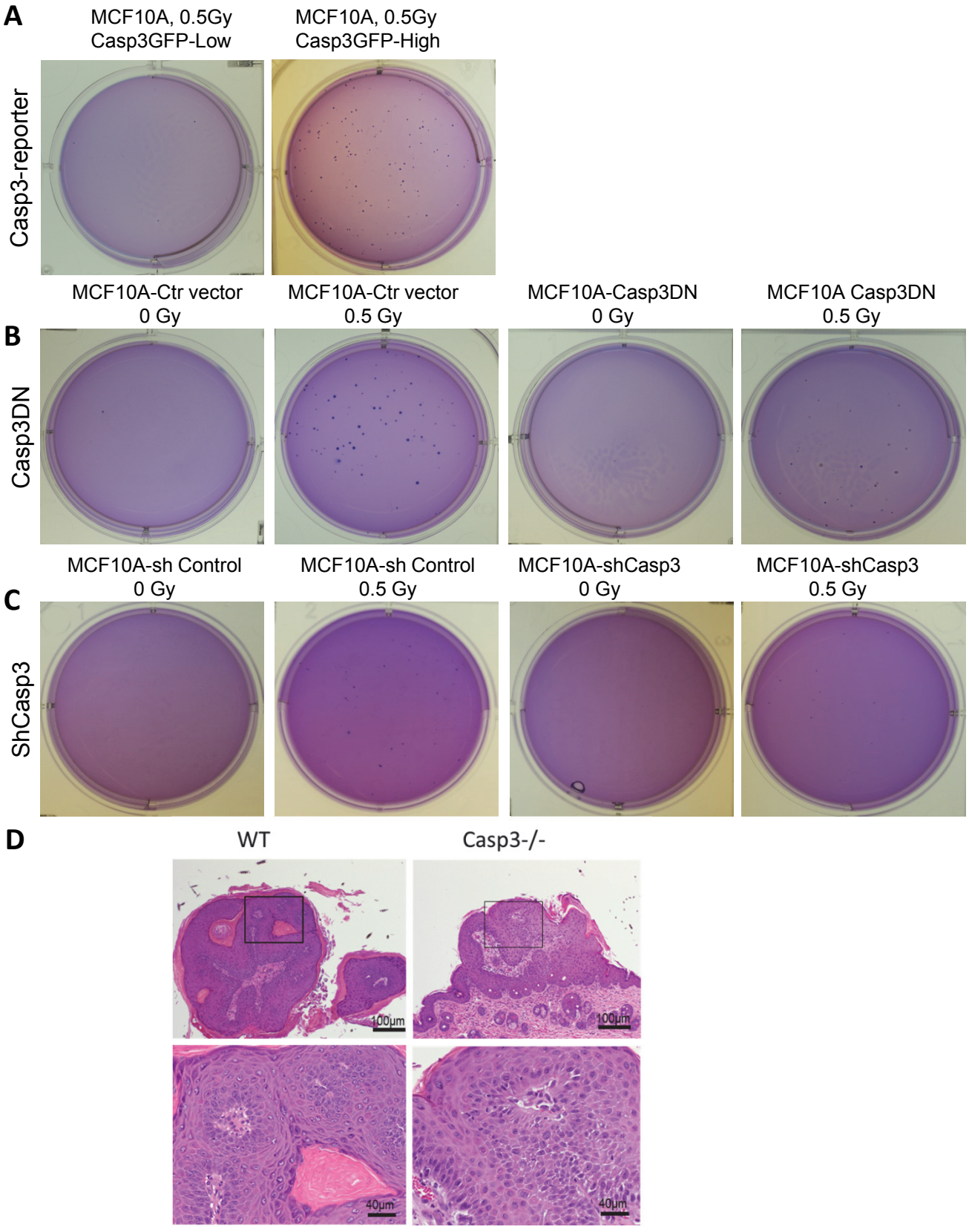
A
MCF10A, 0.5Gy
Casp3GFP-Low
MCF10A, 0.5Gy
Casp3GFP-High
Casp3-reporter
B
MCF10A-Ctr vector
0 Gy
MCF10A-Ctr vector
0.5 Gy
MCF10A-Casp3DN
0 Gy
MCF10A Casp3DN
0.5 Gy
Casp3DN
C
MCF10A-sh Control
0 Gy
MCF10A-sh Control
0.5 Gy
MCF10A-shCasp3
0 Gy
MCF10A-shCasp3
0.5 Gy
ShCasp3
D
WT
Casp3-/-
100μm
100μm
40μm
40μm

Figure S5 (related to Figure 5&6). **A.** Soft agar growth from cells with high and low Casp3GFP reporter expression after ^{56}Fe ions irradiation. **B**. Soft agar formation from cells transduced with vector control or *Casp3DN* with or without ^{56}Fe ions irradiation. **C.** Soft agar growth cells transduced with vector control or *shCASP3DN* expression. **D**. Representative H&E staining of a paraffin-embedded tumor section derived from the two-stage carcinogenesis.

Fig. S6

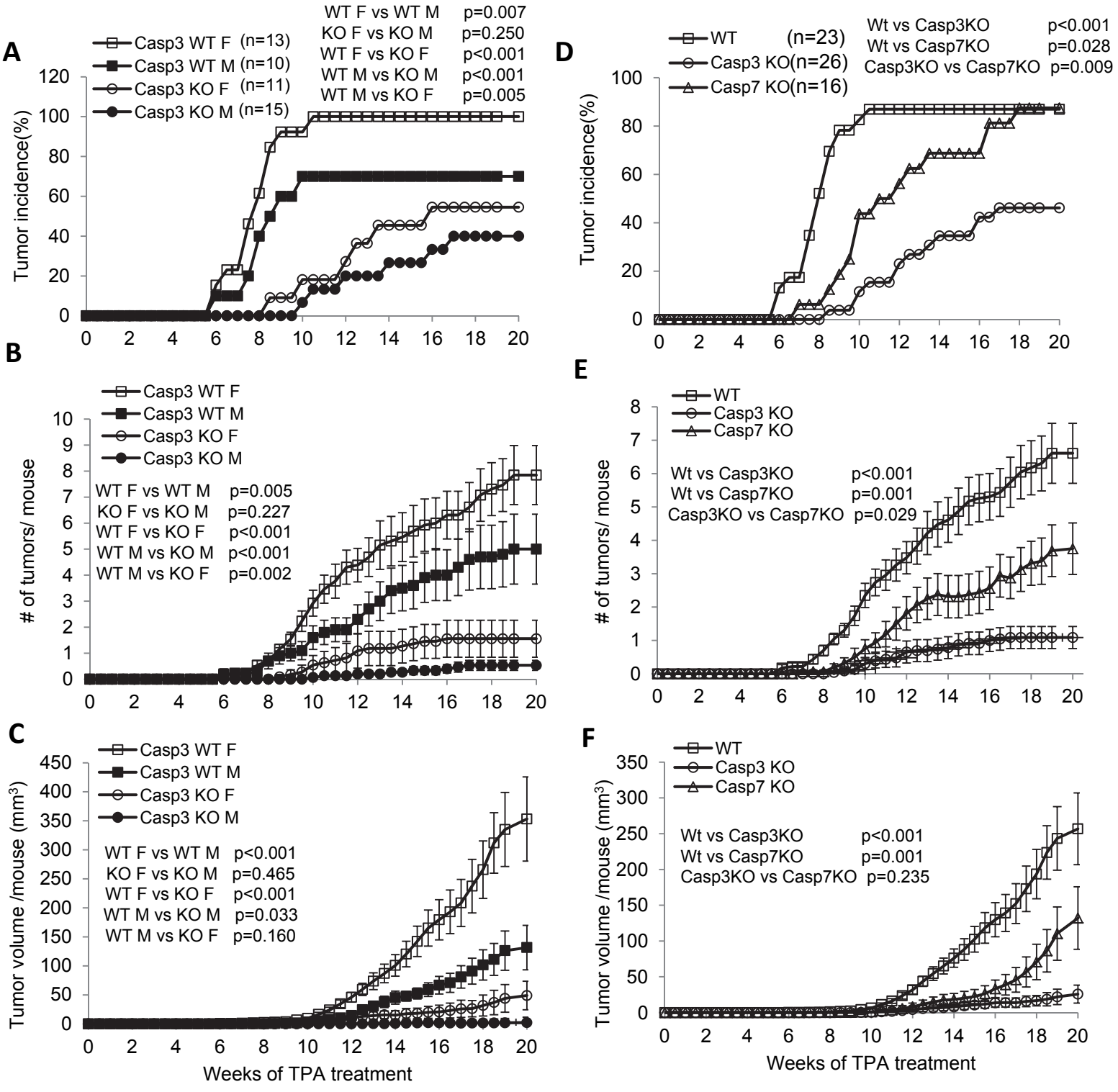
A
Casp3 WT F (n=13)
Casp3 WT M (n=10)
Casp3 KO F (n=11)
Casp3 KO M (n=15)
WT F vs WT M p=0.007
KO F vs KO M p=0.250
WT F vs KO F p<0.001
WT M vs KO M p<0.001
WT M vs KO F p=0.005
Tumor incidence(%)
B
Casp3 WT F
Casp3 WT M
Casp3 KO F
Casp3 KO M
WT F vs WT M p=0.005
KO F vs KO M p=0.227
WT F vs KO F p<0.001
WT M vs KO M p<0.001
WT M vs KO F p=0.002
# of tumors/ mouse
C
Casp3 WT F
Casp3 WT M
Casp3 KO F
Casp3 KO M
WT F vs WT M p<0.001
KO F vs KO M p=0.465
WT F vs KO F p<0.001
WT M vs KO M p=0.033
WT M vs KO F p=0.160
Tumor volume /mouse (mm3)
Weeks of TPA treatment
D
WT (n=23)
Casp3 KO(n=26)
Casp7 KO(n=16)
Wt vs Casp3KO p<0.001
Wt vs Casp7KO p=0.028
Casp3KO vs Casp7KO p=0.009
Tumor incidence(%)
E
WT
Casp3 KO
Casp7 KO
Wt vs Casp3KO p<0.001
Wt vs Casp7KO p=0.001
Casp3KO vs Casp7KO p=0.029
# of tumors/ mouse
F
WT
Casp3 KO
Casp7 KO
Wt vs Casp3KO p<0.001
Wt vs Casp7KO p=0.001
Casp3KO vs Casp7KO p=0.235
Tumor volume /mouse (mm3)
Weeks of TPA treatment

Figure S6 (related to Figure 6). Additional data on DMBA+TPA induced tumor formation in C57BL/6 with various genetic backgrounds. Sex difference in terms of DMBA+TPA induced tumor incidence (**A**), tumor numbers (**B**), and aggregate tumor size (**C**) in wild type vs casp3KO mice. The comparative roles of caspase 3 vs caspase 7 are shown in terms tumor incidence (**D**), tumor number per mouse (**E**), and aggregate tumor volume per mouse (**F**). P-values were obtained from log rank test.

Fig. S7

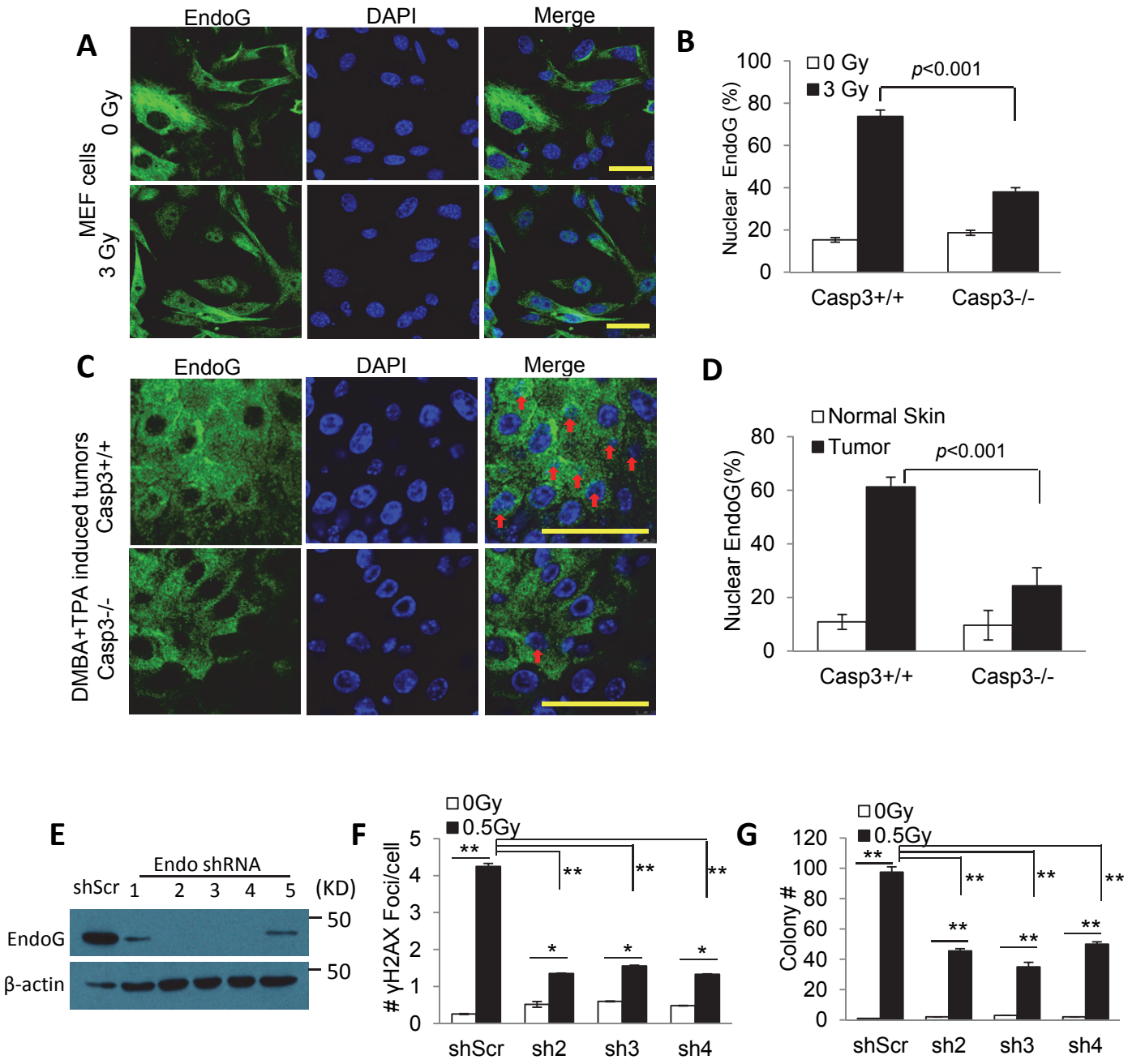
A
EndoG
DAPI
Merge
MEF cells
0 Gy
3 Gy
B
0 Gy
3 Gy
p<0.001
Nuclear EndoG (%)
100
80
60
40
20
0
Casp3+/+
Casp3-/-
C
EndoG
DAPI
Merge
DMBA+TPA induced tumors
Casp3+/+
Casp3-/-
D
Normal Skin
Tumor
p<0.001
Nuclear EndoG(%)
80
60
40
20
0
Casp3+/+
Casp3-/-
E
Endo shRNA
shScr 1 2 3 4 5 (KD)
EndoG
β-actin
50
50
F
0Gy
0.5Gy
γH2AX Foci/cell
5
4
3
2
1
0
**
*
shScr
sh2
sh3
sh4
G
0Gy
0.5Gy
Colony #
120
100
80
60
40
20
0
**
shScr
sh2
sh3
sh4

Figure S7 (related to Figure 7). **Additional data on the relationship among** caspase 3 activation, endoG migration, γH2AX foci formation, and soft agar growth. **A&B**. Immunofluorescence analysis endoG location in irradiated wild type and casp3-/- MEF cells. **C&D.** Immunofluorescence analysis of endoG staining in DMBA+TPA induced skin tumors as well as adjacent normal skins. Red arrows show endoG in cellular nuclei. Scale bar, 40μm. **E.** Western blot analysis of the efficacy of 5 different shRNA on endoG expression levels in MCF10A cells. The attenuation of of effective shRNAs (shEndoG2-4) on radiation induced γH2AX foci formation and soft agar formation are shown in **F** and **G,** respectively. *$p<0.05$; **$p<0.001$.

Table S1. Primary antibodies used in this study for western blot and immunofluorescence analyses (related to Figures 1-4, 6-7**)**

Target protein	**Antibody source**	**Clone information**
Caspase 3 (full length)	Cell Signaling Technology	8G10 , Rabbit mAb
Caspase 3 (cleaved, activated)	Cell Signaling Technology	5A1E, Rabbit mAb
Caspase 3 (active form)	Millipore	3D9.3, Mouse mAb
Cytochrome C	Cell Signaling Technology	Goat polyclonal
p53	Cell Signaling Technology	7F5, Rabbit mAb
p21	Cell Signaling Technology	12D1, Rabbit mAb
HA epitope	Novus Biologicals	Goat polyclonal
Endo G	Chemicon	Rabbit polyclonal
TATA binding protein(TBP)	Abcam	1TBP18, Mouse mAb
β-Actin	Novus Biologicals	Mouse mAb
γH2AX	Upstate Biotechnology	JBW301, Mouse mAb
Mitochondria maker	Abcam	MTC02, Mouse mAb
GFP	Abcam	Goat polyclonal

Table S2. Radiation-induced chromosome aberrations in MCF10A cells (related to Figures 4&7**).**

		Ctr Vector		Casp3DN		shScramble		shEndoG2	
		0 Gy	0.5Gy	0 Gy	0.5Gy	0 Gy	0.5Gy	0 Gy	0.5Gy
Exp1	Total score	150	150	150	150	150	150	150	150
	Chromatid break	0	8	1	2	1	5	1	3
	Chromatid exchange	0	2	1	1	0	3	1	3
	Chromosome dicentric	0	6	1	1	0	5	0	3
	Interstitial deletion	0	9	2	5	1	8	3	5
Exp2	Total score	150	150	150	150	150	150	150	150
	Chromatid break	0	4	0	0	0	4	0	0
	Chromatid exchange	0	3	0	3	0	3	0	1
	Chromosome dicentric	0	3	0	0	0	3	0	5
	Interstitial deletion	0	12	2	3	0	12	0	3
Exp3	Total score	150	150	150	150	150	150	150	150
	Chromatid break	0	5	0	1	0	6	0	1
	Chromatid exchange	0	4	0	4	0	3	0	1
	Chromosome dicentric	0	3	0	1	0	2	0	4
	Interstitial deletion	0	11	3	3	0	10	0	5
M±SD	Aberrations/cell	0	0.16±0.01	0.02±0.01	0.05±0.01	0	0.14±0.01	0.01±0.01	0.07±0.01

Table S3. Radiation-induced chromosome aberration in bone marrow cells of wild type and Casp3-/- C57BL/6 mice 3 days after irradiation with x-rays (3 Gy) (related to Figure 4)**.**

		Non-irradiated		3 Gy	
		Casp3+/+	Casp3-/-	Casp3+/+	Casp3-/-
Exp1	Total score	150	150	150	150
	Chromatid break	0	0	1	0
	Chromatid exchange	0	1	3	2
	Chromosome dicentric	0	1	6	4
	Chromosome interstitial deletion	0	0	9	4
Exp2	Total score	150	150	150	150
	Chromatid break	0	0	1	0
	Chromatid exchange	0	0	1	1
	Chromosome dicentric	0	0	0	0
	Chromosome interstitial deletion	0	0	10	3
Exp3	Total score	150	150	150	150
	Chromatid break	0	0	2	1
	Chromatid exchange	0	0	2	0
	Chromosome dicentric	0	0	2	1
	Chromosome interstitial deletion	0	0	8	4
M±SD	Aberrations /cell	0	0.004±0.007	0.10±0.03	0.04±0.02

Supplemental Experimental Procedures

Cell lines and tissue culture

Early passage, immortalized, non-transformed human breast epithelial cell line, MCF10A, was a kind gift from Dr. Hatsumi Nagasawa of Colorado State University (Fort Collins, CO). The cells were cultured following a published protocol. Briefly, MCF10A growth medium was composed of DMEM/F12 (Sigma, St. Louis, MO) supplemented with 5% donor horse serum (Sigma), 20 ng/ml epidermal growth factor (EGF; R&D, Minneapolis, MN), 0.5 μg/ml hydrocortisone (Sigma), 100 ng/ml cholera toxin (Sigma), 10 μg/ml insulin (Invitrogen, Grand Island, NY), and 100 unites/ml penicillin and 100 μg/ml streptomycin. For γH2AX foci assays, the cells were cultured in 2% horse serum without EGF. Human lung fetal fibroblasts, IMR90, were obtained from the Coriell Institute (Camden, NJ) and cultured in Eagle's minimum essential medium (MEM) supplemented with 10% FBS and non-essential amino acid.

Primary mouse embryonic fibroblasts (MEFs) were generated from caspase 3 or deficiency mice (Jackson Laboratory, Bar Harbor, ME). To generate caspase 3&7 double knockout mice, caspase3 knockout (casp3-/-) male mice were cross with caspase7 knockout (casp7-/-) female mice to generate caspase3 and 7 heterozygous mice (casp3+/-, casp7+/-). These mice were them mated with each other to obtain MEFs with various genotypes: casp3-/-, casp7-/- (DKO); casp3-/-, casp7+/+ (Casp3KO); casp3+/+, casp7-/- (Casp7KO); casp3+/+, casp7+/+ (WT). Primary MEFs were isolated from E13.5 embryos and propagated in MEM supplemented with 10% FBS, 100 U/ml penicillin/100 μg/ml streptomycin. For genotype analysis, DNA were prepared from MEFs and subjected to standard PCR analysis following the Jackson Lab`s procedure. All

experimental protocols involving animals were approved by the Animal Care and Use Committee of the Duke University Medical Center (Durham, NC).

Plasmids, molecular cloning, and lentivirus production

A caspase 3 recognition site (DEVD) was engineered into the 5' end of the luciferase-GFP fusion gene. In addition, a flexible linker $(Gly3Ser)_3$ sequence was incorporated in between the luciferase-GFP fusion gene. The stop codon for the luciferase gene was removed to allow a full read-through. Subsequently, a 9-unit polyubiquitin domain was then ligated into the 5' end of the luciferase-GFP gene. The fully assembled caspase 3 reporter genes were transferred into the lentiviral vector pLEX (Open Biosystems, Huntsville, AL).

The human dominant-negative caspase3 gene (*CASP3DN*) was obtained from Addgene (deposited by Dr. Guy Salvesen from Burnham Institute, La Jolla, CA), which contains a single mutation (C163A) that disables its proteolytic function. The dominant-negative caspase3 gene (*CASP3DN*) was cloned into pLEX vector. A hemaglutinin tag (HA) was inserted at the 3` end of dominant-negative caspase3 gene to enable detection and distinction from the endogenous caspase 3 protein.

To knock down the *CASP3* gene, we obtained the GIPZ lentiviral vectors from Open Biosystems (Huntsville, AL) encoding a mir30-based shRNA minigene against the *CASP3* gene. The sequence of Caspase 3 shRNA minigene is:

5`- TGCTGTTGACAGTGAGCGAGGAAACATTCAGAAACTTGAATAGTGAAGCCACAG

ATGTATTCAAGTTTCTGAATGTTTCCCTGCCTACTGCCTCGGA-3'. Red sequences indicate sense and anti-sense targeting sequences while blue sequences indicate the loop region in shRNA.

To knock down genes encoding endonuclease G (Endo G), we obtained pLKO.1puro lentiviral vector from Sigma (St. Louis, MO) encoding shRNA minigene against the endoG gene. The sequences for the EndoG shRNA minigenes are:

shEndoG1, 5`- CCGGGCTGATGGGAAATCCTACGTACTCGAGTACGTAGGATTTCCCATCAGCTTTTTG -3`;

shEndoG2, 5`- CCGGCCTGGAACAACCTGGAGAAATCTCGAGATTTCTCCAGGTTGTTCCAGGTTTTTG -3`;

shEndoG3, 5`- CCGGGACACGTTCTACCTGAGCAAACTCGAGTTTGCTCAGGTAGAACGTGTCTTTTTG -3`;

shEndoG4, 5`- CCGGCCGTGCCACCAACGCCGACTACTCGAGTAGTCGGCGTTGGTGGCACGGTTTTTG -3`;

shEndoG5, 5`- CCGGCCGCAGCTTGACCCGCAGCTACTCGAGTAGCTGCGGGTCAAGCTGCGGTTTTTG -3`;

Red sequences indicate sense and anti-sense targeting sequences while the blue sequence indicate the loop region.

To determine if Casp3 activation alone can cause DNA damage, we obtained an inducible human caspase3 from Addgene (deposited by Dr. Spencer DM from Baylor college of medicine, Houston, TX), which contains two mutated FKBP (FK506-binding protein)-12 domains (FKBP12) and procaspase3. The resulting molecule, FKBP12-caspase3, namedd inducible caspase3 (iCasp3), can be dimerized with the administration of AP20187, a nontoxic lipid-permeable dimeric FK506 analogue that specifically binds to the attached F506-binding protein, FKBP12. The iCasp3 gene was cloned into pLEX vector.

To produce lentiviral vectors, lentiviral plasmids with the target genes were transduced into 293T cells together with second generation packaging plasmids (psPAX2, pMD2.G) following previously published procedures: http://tronolab.epfl.ch/lentivectors.

Exposure to higher energy ^{56}Fe ions and x rays

To conduct irradiation with ^{56}Fe ions, cells to be irradiated or sham-irradiated were shipped by FedEx to the National Aeronautics and Space Administration (NASA)-sponsored Space Radiation Laboratory at Brookhaven National Laboratory (BNL; Brookhaven, Long Island, NY) in sealed T-25 flasks. The iron beam energy used was 600 MeV/μ. The dose rate for exposure was 0.5 Gy/min. After irradiation, the cells were immediately shipped back to our laboratory in Durham, NC for further analysis. In some instances, cells were irradiated with x-rays in an XRad320 irradiator (Precision X-rays, North Branford, CT) located in the vivarium of Duke University Medical Center. The dose rate was 3 Gy/min.

Monitoring caspase 3 reporter activation and sorting of cells with high or low reporter activities

After being irradiated, the caspase3 reporter lentiviral vector infected cells were plated at 1×10^4 cells/well in 6-well plates, the reporter activities in the cells were subsequently imaged with Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek, Winooski, VT) by use of luciferin at 150 μg/ml at different time points after plating. Quantification of luciferase activities was carried out by using software supplied by the manufacturer. In other instances, caspase 3 reporter activities were also monitored in individual cells by use of a fluorescence-activated cell sorting

analyzer (FACS; BD FACSVantage SE). FACS sorting was also used successfully to separate high and low GFP expression cell populations from irradiated MCF10A cells (0.5Gy) with the caspase 3 reporter.

To sort out cells with different levels of caspase 3 activation, MCF10A cells were stably transduced with Casp3-GFP reporter and exposed to different radiation. After 72-96 hrs of radiation, MCF10A cells were gated into 8 groups (M1-M8) in according to their Casp3EGFP reporter activities. Cells from different gates were then sorted one cell per well into 96-well by use of a FACS apparatus. Three weeks later, the number of colonies in different 96-well plates were counted and plotted.

Western blot analysis

Cells were lysed in Laemmli buffer. Samples were separated by SDS-PAGE and transferred to PVDF) membranes (Bio-Rad, Hercules, CA). The membranes were then blotted with an assortment of antibodies. Detailed information for all antibodies is listed in supplementary **Table S1**. Appropriate secondary antibodies and enhanced chemiluminescence substrate (Thermo scientific, Rockford, IL) were used to visualize protein bands.

Subcellular fractionation and western blot analyses

To analyze release of different apoptotic proteins (endoG, AIF, Smac/Diablo, and cytochromeC) from the mitochondria, it necessary to obtained fractionate cellular proteins into mitochondria, cytoplasm, and nuclear fractions. To obtain the mitochondria protein, MCF10A cells were harvested and isolated using a mitochondrial isolation kit for cultured cells (Abcam, Cambridge,

MA) according to the manufacturer`s protocol. Briefly, MCF10A cells were detached by trypsinization and centrifuged. Cells were re-suspended in reagent A and homogenized. The homogenate was centrifuged to remove the supernatant. The pellet was re-suspended in reagent B, homogenized and centrifuged at 1,000 g for 10 min. The combined supernatants were further centrifuged at 12,000 g for 15 min. The resulting supernatant was collected as the cytosolic fraction and the mitochondria pellet re-suspended in reagent C.

To obtain cellular nuclei, MCF10A cells were harvested in cytoplasmic lysis buffer (Millipore, Billerica) containing 0.5 mM DTT and protease inhibitor cocktail. Samples were homogenized and centrifuged at 8,000 g for 20 min at 4°C. The supernatant contained the cytosolic portion, and the remaining pellet contained the nuclear portion. The nuclear pellet was re-suspended in nuclear extraction buffer, homogenized and centrifuged at 16,000 g for 5 min at 4°C, the supernatant was used as the nuclear extract. Manufacturer's procedure was followed throughout the extraction procedure.

γH2AX foci assay

To detect the radiation-induced DNA double strand breaks, γH2AX foci in the irradiated cells was examined through immunofluorescence by use of an established protocol (Paull et al., 2000; Rogakou et al., 1998). Briefly, irradiated or sham-irradiated MCF10A, IMR90, or MEF cells were plated on the glass-bottom 35mm petri dishes (MatTek, Ashland, MA) and cultured with growth medium (see beginning of Methods section for the makeup of growth mediam) for overnight, then changed to assay medium (see beginning of Methods section) for another 72 h. Cells were fixed with 4% paraformaldehyde (PFA), permeabilized with 0.2% Triton X-100/PBS

and blocked with 10% goat serum. Cells were then incubated with a primary antibody against γH2AX (Upstate Biotechnology, Lake Placid, NY), washed with PBS and incubated with a secondary antibody conjugated with Alexa Fluor® 488 (Invitrogen, Carlbad, CA). Cells were mounted with mounting medium (Vector Laboratories, CA) containing DAPI. Fluorescent images of γH2AX were acquired with a Zeiss fluorescence microscope with 63x oil objective (Axio Observer Z1).

Living cell imaging analysis of 53BP1 foci

To carry out live, non-invasive monitoring of DNA double strand breaks, we obtained mCherry-53BP1 plasmid from Addgene (Cambridge, MA, deposited by Dr. Titia de Lange from The Rockefeller University, New York, NY) (Dimitrova et al., 2008). The 53BP1-mCherry reporter consists of the fusion between mCherry and a polypeptide domain representing amino acids 1220-1711 of 53BP1. The 53BP1 subdomain lacks most of the functions of 53BP1 but can form foci similar to wild type proteins (Dimitrova et al., 2008). Irradiated or sham-irradiated MCF10A cells were transfected with mCherry-53BP1 plasmid with lipofectamine 2000 (Invitrogen). Puromycin was added to select the stably expressing mCherry-53BP1 fusion protein cells. Twenty-four hours prior to microscopy, the stably transduced cells were plated on glass-bottom 35mm petri dishes (MatTek, Ashland, MA) and cultured with growth medium. Cells were imaged by use of a Leica SP5 confocal scanning microscope. For time-lapse video imaging, cells were imaged by use of a Zeiss Axio Z1 inverted microscope with 40x/1.3 oil objective and a QuantEM back thinned EM-CCD camera. The microscope was surrounded by a custom enclosure to maintain constant 37°C temperature and CO_2 concentration at 5%. Images were acquired every 10 min in phase-contrast and mCherry channels using MetaMorph software

(Molecular Devices, Sunnyvale, CA). Images were combined to movie sequences using MetaMorph software.

Alkaline Comet Assay

The alkaline comet assay to measure DNA strand breaks is well-established(Olive et al., 1990; Ostling and Johanson, 1984; Singh et al., 1988). Radiation-induced DNA damage was also assessed by single cell gel electrophoresis (alkaline comet assay) following manufacturer's procedures (Trevigen, Gaithersburg, MD). Cells were harvested and counted and then mixed into liquid low melting agarose and layered on the slides and allowed to solidify on ice. Slides embedded with cells were then placed into cold lysis buffer to solubilize cellular proteins so as to free nuclear DNA. After cell lysis, the slides were drained off lysis buffer and kept in DNA unwinding buffer containing 200 mM NaOH, 1 mM EDTA, pH>13 for 20 min at room temperature. The slides were then placed into an electrophoresis tray filled with fresh, chilled alkaline electrophoresis buffer, and electrophoresis was carried out for 30 min at 21 Volts in cold conditions. The slides were drained and immersed with neutralization buffer and were subsequently stained with SYBR Gold. All the steps described above were conducted under reduced light conditions to prevent additional DNA damage. SYBR Gold-stained DNA on each slide was then visualized at 20x magnification using fluorescence microscopy as comets with fluorescence head and tail. The "comet" images were captured with a Zeiss fluorescence microscope (Axio Observer Z1). Commercially available software for comet analysis (Comet Assay IV, Perceptive Instruments, UK) was used to quantitate the amount of DNA in the comet tails. A total of 50 cells per sample were analyzed. The percentage of DNA in the tail is a

normalized measure of percent of total DNA found in the tail that reflects DNA damage of host cells. Three independent experiments were performed for each treatment condition.

Chromosome aberration analysis

To observe chromosomal aberrations in irradiated MCF10A, IMR90 or MEF cells, 0.1μg/ml of colcemid was added to the cells for 4 h to arrest cells in metaphase with condensed chromosomes. Cells were treated with hypotonic solution (0.075 M KCl) at 37°C for 15 min, then fixed by Carnoy's fixative (methanol/acetic acid, 3:1), and dropped onto slides for analysis.

To analyze *in vivo* chromosomal aberrations, wild type (*CASP3*+/+) and caspase3-deficient (*CASP3*-/-) C57BL/6 mice (Kuida et al., 1996) (Jackson Laboratory, Bar Harbor, ME) were irradiated at 3 Gy whole body with x-rays in an XRad320 irradiator (Precision X-rays, North Branford, CT) located in the vivarium of Duke University Medical Center. The dose rate was 3 Gy/min. Three days later, mice were sacrificed. One hour before their sacrifice, 0.1 ml of 0.5% colchicine was intraperitoneally injected to arrest proliferating cells at the metaphase stage. After the mice were sacrificed, bone marrow of the femurs was flushed out by using of hypotonic solution (0.075 M KCl) and treated at 37°C for 15 min. The bone marrow cells were then fixed by Carnoy's fixative and dropped onto slides for analysis. The scored structural chromosomal aberrations included dicentric, interstitial deletions, chromatid breaks, chromatid exchange. At least three independent metaphase spreads were used for each experiment.

To examine chromosome translocations in irradiated mice, metaphase chromosome spreads of mice bone marrow cells was prepared. Subsequently, chromosome painting was carried out with

whole-chromosome probes for mouse chromosome 1 labeled with FITC, and chromosome 2 labeled with Rhodamine, following standard manufacturer's procedures (Applied Spectral Imaging, Carlsbad, CA). The painting probes are visible using a standard triple DAPI/FITC/Rhodamine filter. Fluorescent images were acquired with a Zeiss fluorescence microscope with 63x oil objective (Axio Observer Z1).

Soft-Agar Assay

About 5×10^3 irradiated MCF10A cells in growth medium were plated into 6-well plates with 1.5 ml 0.3% (w/v) low-melting agar (BD, Sparks, MD), which was overlaid onto 1.5 ml 0.6% (w/v) bottom agar layer. Soft-agar cultures were maintained at 37°C and fed twice a week by drop-wise addition of Growth medium for colony formation. After three to four weeks in cultures, the colonies were counted after staining with 0.005% crystal violet.

Tumorigenic assay for MCF10A cells

To confirm the tumorigenicity of irradiated MCF10A cells, about 2×10^6 cells were injected subcutaneously into the flanks of 6-8 weeks-old, female athymic nude mice (Jackson Laboratories) in 50 µl of PBS. After inoculation, the growth of tumors was evaluated once a week for 12 weeks. Animals were sacrificed at the end of study, and tumors were removed, fixed and sectioned. Tumor sections were stained with Hematoxylin and Eosin (H&E).

Two-stage carcinogenesis

We crossed male and female heterozygous *Casp3* (*Casp3*+/-) mice to generate littermate with different caspase 3 status. Virgin littermates with different genotypes were used for the 7,12-Dimethylbenz(a)anthracene + 12-O-tetradecanoyphobol-13-acetate (DMBA/TPA, Sigma) two-stage chemical carcinogenesis experiments according published procedures(Abel et al., 2009). Briefly, the dorsal hair of 6- to 8-week-old mice, both males and females, were shaved with electric hair clippers 2 days before the beginning of the protocol. The DMBA treatment consisted of a single application of 100 μg of DMBA in 200 μl of acetone (400 nmol) on the shaved dorsal skin; TPA treatment was initiated 1 week after DMBA initiation by topical application of 6 μg of TPA in 200 μl of acetone (10 nmol) twice a week. Animals were monitored at the time of TPA applications, and tumors were counted and measured with a caliper twice a week. At the end of experiments, the mice were euthanized and tumor samples were collected and fixed for histology.

Immunofluorescence analysis

Cells were cultured on glass-bottom 35mm petri dishes. Cells were fixed with 4% paraformaldehyde (PFA) in PBS for 15min, permeabilized and blocked with PBS containing 5% donkey serum, 0.1% Triton X-100 and 1% BSA for 45 min. Fixed cells were incubated with primary antibodies in 1% BSA overnight at 4°C, followed by incubated with appropriate Alexa Fluor 488, 555 or 633-conjugated secondary antibodies (Invitrogen, Carlsbad, CA) for 1 h and mounted with mounting medium (Vector Laboratories, CA) containing DAPI. See supplementary Table S1 for information on the antibodies. Fluorescent images were acquired using Leica SP5 confocal scanning microscope.

Supplementary References

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