Supporting Information

Chiba et al. 10.1073/pnas.1018867108

SI Methods

Immunofluorescence. Cells were grown on Lab-Tek chamber slides, fixed with 4% paraformaldehyde 0, 10, or 30 min after being exposed to 10 Gy of γ-irradiation, and permeabilized with 0.2% Triton X-100 for 5 min. After blocking with 10% goat serum and 0.2% Triton X-100 in PBS for 1 h at room temperature, cells were incubated at 4 °C overnight with the following primary antibodies: mouse anti-phosphorylated histone H2AX (anti–γ-H2AX) (Ser-139; Abcam); rabbit anti-p53 binding protein 1 (anti-53BP1) (Cell Signaling), rabbit anti-histone H4 dimethyl K20 (Abcam), mouse anti-ataxia telangiectasia mutated (anti-ATM) (Ser1981; Rockland), and anti–mediator of DNA-damage checkpoint 1 (anti-MDC1) (Bethyl). Proteins were visualized by incubation with secondary antibodies labeled with Alexa Fluor 488 or Alexa Fluor 585 (Invitrogen). Slides were mounted with ProLong Gold antifade reagent containing DAPI (Invitrogen). Images were obtained on a Nikon microscope with Delta Vision deconvolution software. Images used for comparisons of different treatments and/or cell lines were acquired with the same instrument settings and exposure times and were processed equally. The number of positive nuclei was counted in nine fields in each experiment.

Cell-Cycle Analysis. Cells were harvested, washed twice in PBS, resuspended in 0.1% saponin/PBS (Sigma) containing 10 μg/mL RNase (Sigma) and 50 μg/mL propidium iodide (Sigma), and incubated for 30 min at 37 °C. Samples were analyzed by flow cytometry using the FACScalibur and CellQuest software (Becton Dickinson).

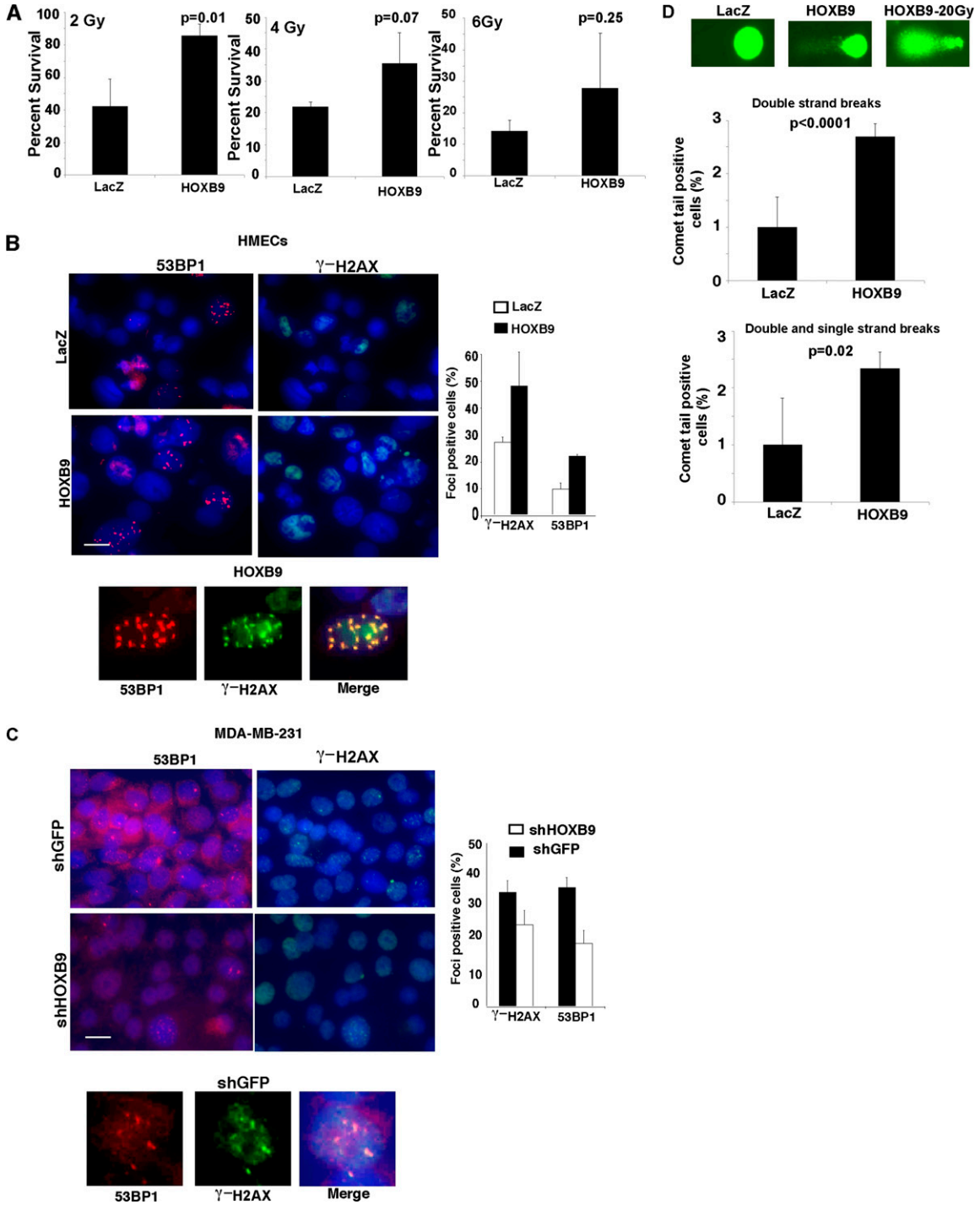

Fig. S1. Homeobox B9 (HOXB9) expression promotes clonogenic survival and DNA damage responses in nonirradiated cells. (*A*) The effect of HOXB9 expression on radiosensitivity. Cultures of LacZ- and HOXB9-expressing MCF10A cells were exposed to graded doses of ionizing radiation, and colony-forming efficiency was determined. Percent survival was calculated as the percent of colonies formed per number of cells plated ×100 ($n = 3$). (*B*) (*Upper*) The expression of γ-H2AX and 53BP1 was evaluated in human mammary epithelial cells (HMECs) expressing either LacZ or HOXB9. (*Lower*) High-magnification individual and merged images of γ-H2AX and 53BP1 foci in HOXB9-expressing cells demonstrate colocalization of the two proteins. (*C*) (*Upper*) The expression of γ-H2AX and 53BP1 was evaluated in MDA-MB-231 cells in which HOXB9 expression was knocked down with shHOXB9. Cells infected with shGFP were used as control. Bar graphs beside each panel show the percentage of nuclei positive for γ-H2AX and 53BP1 foci. The mean was derived from the percentage of cells that scored positive for γ-H2AX and 53BP1 across nine fields. (*Lower*) High-magnification individual and merged images of γ-H2AX and 53BP1 foci in HOXB9-expressing cells demonstrate colocalization of the two proteins. (*D*) The presence of DNA damage in LacZ and HOXB9-MCF10A cells was assessed by single-cell gel electrophoresis assay under neutral (double-strand breaks) and alkaline (double- and single-strand breaks) conditions. More than 70 cells were evaluated for each condition, and the comet tail-positive cells in the control sample was set at 1. (Scale bars, 10 μM.)

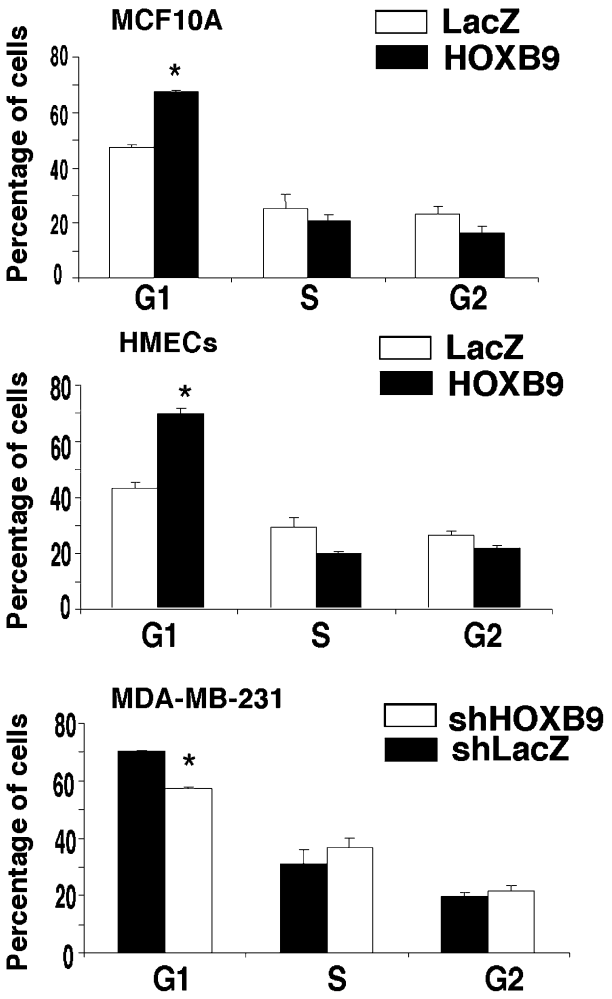

Fig. S2. Cell-cycle analysis of MCF10A cells (*Top*) and HMECs (*Middle*) expressing LacZ and HOXB9 demonstrates that HOXB9 increases the fraction of cells in the G1 phase. (*Bottom*) Knockdown of HOXB9 in MDA-MB-231 cells decreases the fraction of cells in the G1 phase of the cell cycle. *$P < 0.001$.

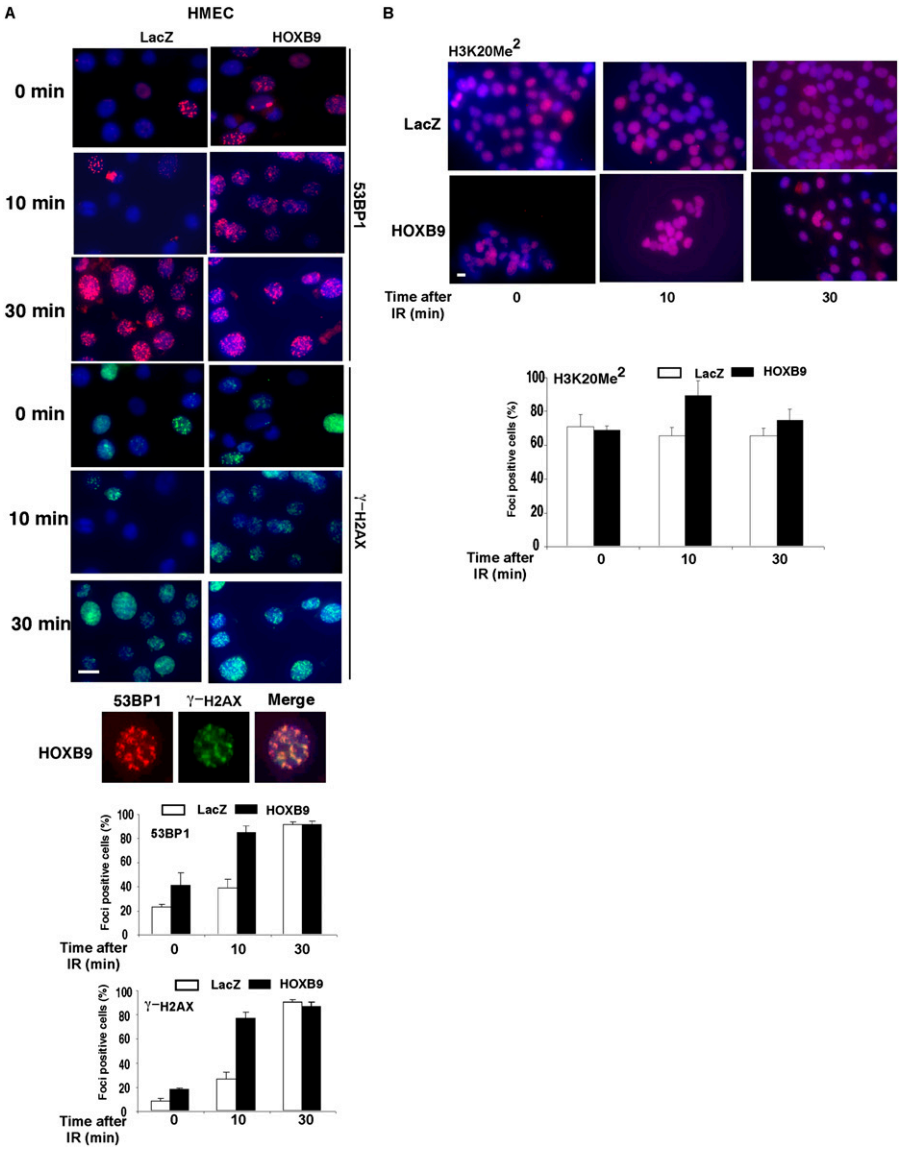

Fig. S3. HOXB9 expression leads to a rapid radiation-induced DNA damage response. (*A*) (*Upper*) HMECs expressing LacZ or HOXB9 were irradiated (10 Gy), and γ-H2AX and 53BP1 expression was visualized and quantified 0, 10, and 30 min after γ-irradiation (IR). HOXB9-expressing HMECs demonstrate colocalization of 53BP1 and γ-H2AX proteins 10 min after irradiation. (*Lower*) Bar graphs show the percentage of nuclei positive for γ-H2AX or 53BP1 foci. The mean was derived from the percentage of cells that scored positive for γ-H2AX or 53BP1 foci across nine fields. (Scale bar, 10 μM.) (*B*) Dimethylation of histone 4, lysine 20 (H4K20me2) expression is not altered by HOXB9 expression. (*Upper*) MCF10A cells expressing LacZ or HOXB9 were irradiated (10 Gy), and H4K20me2 expression was visualized 0, 10, and 30 min after γ-irradiation. (*Lower*) Bar graph shows the percentage of nuclei positive for H4K20me2. The mean was derived from the percentage of cells that scored positive for H4K20me2 across nine fields.

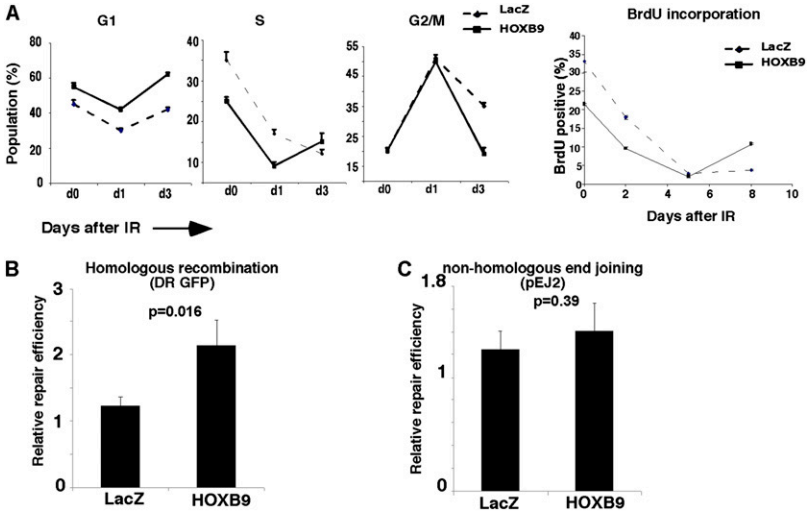

Fig. S4. **(***A*) (*Left*) Cell-cycle analysis of LacZ- or HOXB9-expressing MCF10A cells before and 1 and 3 d after exposure to ionizing radiation (IR). (*Right*) The population of LacZ- or HOXB9-expressing MCF10A cells in the S phase was monitored using BrdU incorporation. (*B*) The homologous recombination repair substrate DR-GFP with or without SceI endonuclease was expressed in vector and HOXB9-expressing MCF10A cells. The frequency of double-stranded DNA break repair was determined 4 d after transfection by flow cytometric analysis on a BD Biosciences FACScan. Relative repair efficiency was calculated as the ratio of GFP^+ cells in cultures transfected with DR-GFP plus Sce1 and those in cultures transfected with DR-GFP alone (n = 3). (*C*) Nonhomologous end-joining repair was evaluated by transfecting the reporter constructs pEJ2 with or without SceI endonuclease in MCF10A cells expressing vector or HOXB9. Relative repair efficiency was determined 4 d after transfection by flow cytometric analysis on a BD Biosciences FACScan. Repair efficiency was calculated as the ratio of GFP^+ cells in cultures transfected with pEJ2 plus Sce1 and those in cultures transfected with pEJ2 alone (n = 3).

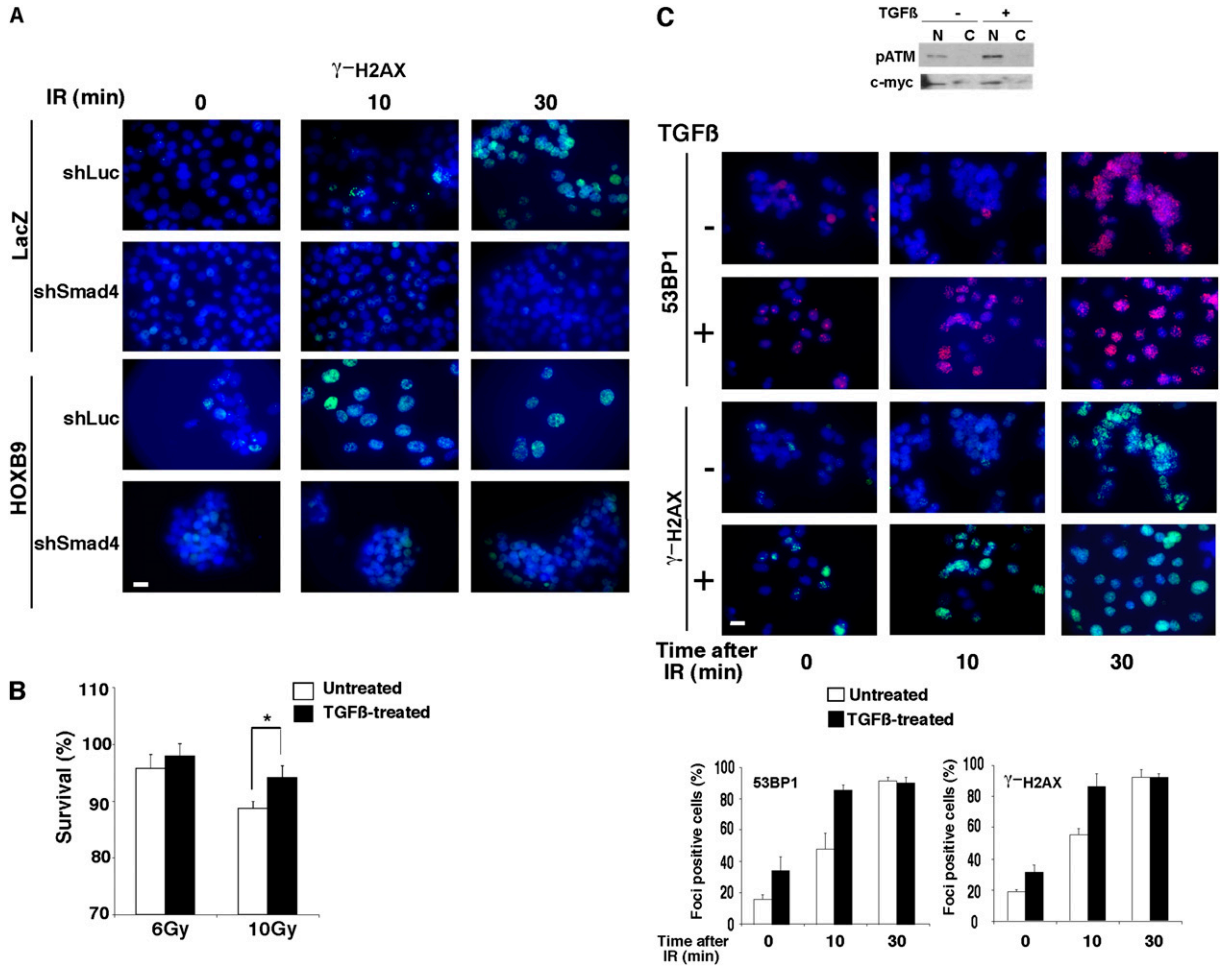

Fig. S5. (*A*) MCF10A cells expressing LacZ-shLuc, LacZ-shSmad4, HOXB9-shLuc, or HOXB9-shSmad4 were irradiated (10 Gy), and γ-H2AX expression was visualized 0, 10, and 30 min after irradiation (IR). Representative images of cells are shown. (*B*) MCF10A cells untreated or treated with 200 pM TGF-β for 3 d were irradiated with 6 Gy or 10 Gy of γ-radiation. Cell survival was measured 24 h after exposure to irradiation. (*C*) (*Top*) Phosphorylated ATM (pATM) is elevated in TGF-β–treated cells. Nuclear (N) and cytoplasmic (C) fractions of untreated and TGF-β–treated cells were analyzed for pATM and myelocytomatosis viral oncogene (c-myc) expression. (*Middle*) MCF10A cells untreated or treated with 200 pM TGF-β for 3 d were irradiated with 10 Gy γ-radiation, and γ-H2AX or 53BP1 expression was visualized 0, 10, and 30 min after irradiation. (*Bottom*) Bar graph demonstrates the percentage of nuclei positive for γ-H2AX or 53BP1 foci. The mean was derived from the percentage of cells that scored positive for γ-H2AX or 53BP1 foci across nine fields. (Scale bars, 10 μM.)