Supporting Information

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SI Materials and Methods

RNAi Methods. For transient Mps1 transfections, fluorescein-labeled oligonucleotides specific for Mps1 (GGU UGA GUU UGU UGC UCA A) and a scrambled sequence (GGU UUG AUU GUU CGG UCA A) (Invitrogen) were transfected into ≈70% confluent cultures by using lipofectamine (Invitrogen). Cells were incubated at 37 °C 5% CO_2 for 48–72 h. For stable transfections, shRNA oligos (5′-CACCGGTCGTTACAGTCAAGCAATTCGAAAATTGC-TTGACTGTAACGACC-3′; 3′-CCAGCAATGTCAGTTCGT-TAAGCTTTTAACGAACTGACATTGCTGGAAAA-5′) were cloned into pENTR/H1/TO (Invitrogen) vector for tetracycline induction. These oligonucleotides were then transfected into cells with lipofectamine (Invitrogen) or by electroporation (Amaxa Biosystems). Stable transfectants were selected by using Blasticidin and Zeocin (Invitrogen). Cells were sustained in media supplemented with Tet-approved FBS (Invitrogen). Mps1 shRNA was then induced by adding 100 μg/mL tetracycline to media.

Time-Lapse Microscopy. For visualization of mitoses, cells were stably transfected with pBOS-H2B-GFP construct (BD Pharmingen) and selected for Blasticidin resistance. Cells were then selected for high fluorescence by using FACS and used in subsequent experiments. Cultures at 70% confluency were either mock transfected or transiently transfected with Mps1 siRNA or a scrambled sequence, recultured for 24 h, and photographed with either 40× or 20× magnification at 5 min intervals over 24 h by using a Nikon 2000E Fluorescence microscope. Images were analyzed by using NIS Elements AR (Nikon).

Analysis of Metaphase Spreads for Chromosome Numbers. Cells in log growth were incubated in the presence of 1 μg/mL colcemid at 37 °C for 1–3 h, washed with PBS, harvested by trypsinization, and pelleted by centrifugation. The pellet was then incubated with 5 mL of 0.5% KCl for 5 min, centrifuged, and fixed by drop-wise addition of methanol:glacial acetic acid (3:1). After another centrifugation, this step was repeated twice and the final pellet was suspended in 200 μL of fixative. Metaphase spreads were then prepared by releasing drops from a height of ≈10 cm onto slides, and after air drying, slides were stained with 0.5% Enzar-t (Armour Pharmaceuticals). Chromosomes were counted in satisfactory spreads in a blinded manner.

Fluorescent in Situ Hybridization (FISH). Cells stably transfected with a pENTR-^{1}H-TO Mps1 shRNA construct (or vector) were plated on chamber slides at low density in selective media and treated with 0 or 100 μg/mL tetracycline. Cells were grown for an additional 5–7 d and then fixed and incubated with chromagen-labeled FISH probes to Centromere 17 and Centromere 8 (green), and Centromere 11 and Centromere 7(red), by using manufacturer's protocols (Abbott Molecular). Cells were visualized at 40× magnification with an Olympus DX60 fluorescence microscope and assessed by using Image-Pro Plus (Media Cybernetics).

Immunohistochemistry. Explanted tumors of killed mice were fixed in 10% buffered formalin for at least 48 h, embedded in paraffin, and sectioned at 5 microns for immunohistochemical staining. The K_i-67 (MIB-1; Dako) and active caspase 3 (Abcam) primary antibodies were used at 1:500 and 1:100 dilutions, respectively, for 4 h according to manufacturers' instructions. After washings, a secondary antibody conjugated with peroxidase was applied to detect and visualize the specific antigen–antibody complexes by using LASB System-HRP assay kit (Dako). Scoring was performed by counting 100 cells in a representative field for each of eight tissue samples in each of the treatment groups. Data are reported as means ± SDs for those counts.

Annexin V Staining. Annexin V assays (PharMingen) were conducted according to the manufacturer's protocol. Live cells were harvested by using 0.25% trypsin-EDTA, washed with binding buffer, and incubated in 100 μL of Annexin V binding buffer and 5 μL of FITC-conjugated Annexin V in the dark at room temperature for 15 min. Dead cell discrimination was accomplished by using a propidium iodide counterstain, and at least 10,000 events were analyzed. Four hundred microliters of binding buffer was then added, and the cells were analyzed on a FACSCalibur flow cytometer by using CellQuest software (BD Biosciences).

Human Breast Cancer Xenografts. To establish xenografts, 2×10^6 cells were implanted directly into the mammary fat pad of irradiated (500 cGy), anesthetized (xylazine/ketamine) female, athymic nude mice (Harlan). At 1 d or 12 d after inoculation, randomly selected mice were switched to feed containing doxycycline hyclate (Harlan), a diet designed to deliver a daily dose of 2–3 mg of doxycycline based on consumption of 4–5 grams of food daily per mouse. Tumors were measured weekly by using calipers in two dimensions, and tumor volume was estimated as the product of the longitudinal measurement, the transverse measurement, and the average of these two measurements. Mice were killed 28 d after inoculation, and tumors were removed for histological examination. Statistical significance between groups was determined by using a two-tailed, nonparametric Student's *t* test.

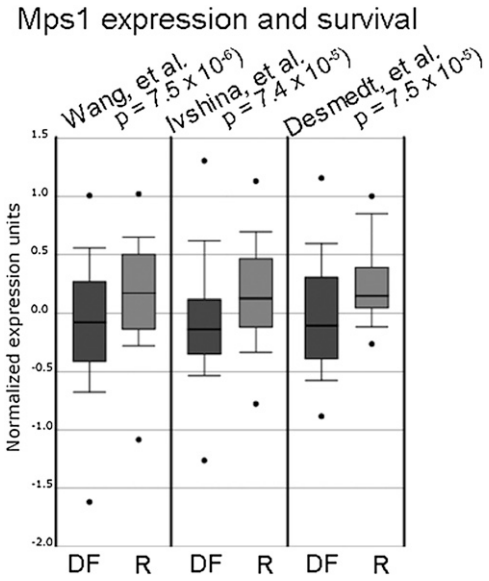


Fig. S1. Correlations between levels of Mps1 and disease-free survival of breast cancer patients. Microarray datasets of breast cancer gene expression were probed for expression of Mps1, and correlations between Mps1 levels and disease-free survival at 5 y were determined by using Oncomine version 3. DF, levels of Mps1 in tumors of patients who were disease-free at 5 y; R, levels of Mps1 in tumors of patient who suffered recurrence/metastases within 5 y of initial treatment. The data comes from Wang and coworkers (1), Ivshina et al. (2), and Desmedt et al. (3). A Student's two-class *t* test was used to determine significance of differences in Mps1 levels between R and DF groups.

1. Wang Y, et al. (2005) Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. *Lancet* 365:671–679.
2. Ivshina AV, et al. (2006) Genetic reclassification of histologic grade delineates new clinical subtypes of breast cancer. *Cancer Res* 66:10292–10301.
3. Desmedt C, et al. (2008) Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes. *Clin Cancer Res* 14:5158–5165.

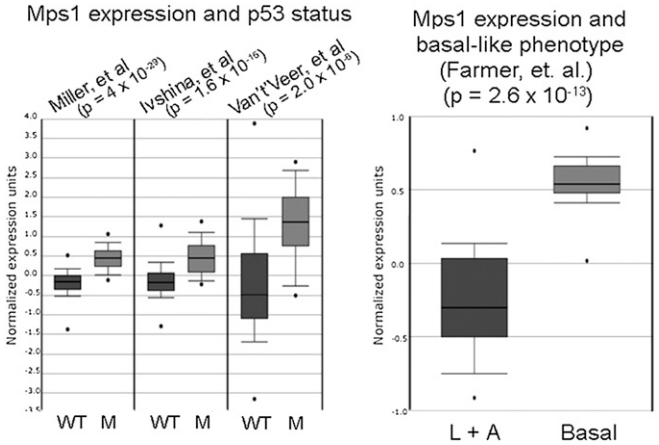


Fig. S2. Correlation between Mps1 levels and p53 mutations or basal-like phenotype. Microarray datasets of breast cancer gene expression were probed for expression of Mps1 and correlated with p53 status and basal-like phenotype by using Oncomine version 3. WT, designates levels of Mps1 in tumors with wild-type p53; M, levels of Mps1 in tumors with p53 mutations; L + A, levels of Mps1 in tumors with luminal or apocrine phenotype; Basal, levels of Mps1 in tumors with basal-like phenotype. The designated studies include Miller et al. (1), Ivshina et al. (2), Van't Veer et al. (3), and Farmer et al. (4). A Student's two-class *t* test was used to determine significance of differences between two groups.

1. Miller LD, et al. (2005) An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. *Proc Natl Acad Sci USA* 102: 13550–13555.
2. Ivshina AV, et al. (2006) Genetic reclassification of histologic grade delineates new clinical subtypes of breast cancer. *Cancer Res* 66:10292–10301.
3. van 't Veer LJ, et al. (2002) Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 415:530–536.
4. Farmer P, et al. (2005) Identification of molecular apocrine breast tumours by microarray analysis. *Oncogene* 24:4660–4671.

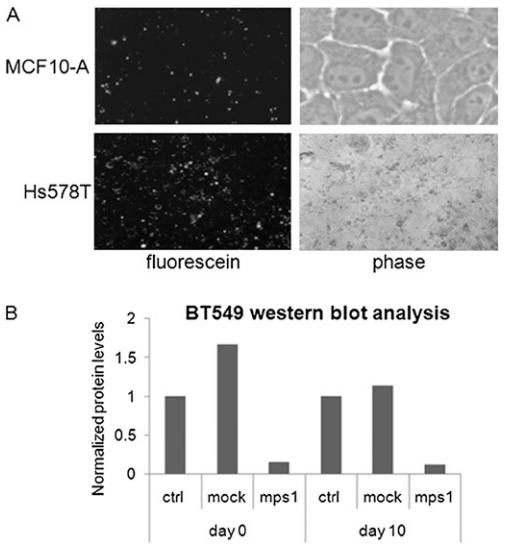


Fig. S3. Measurements of Mps1 siRNA transfection efficiency and effects on Mps1 protein levels. (*A*) Successful transfection of Mps1 siRNA was measured by immunofluorescence of fluorescein-labeled Mps1 siRNA oligos in both Hs578T and MCF10A. Fluorescent and phase contrast micrographs are shown. (*B*) Quantitation of Mps1 protein in BT549 immunoblots both at the time of transfection and after 10 d growth in fresh media. Proteins are normalized to controls.

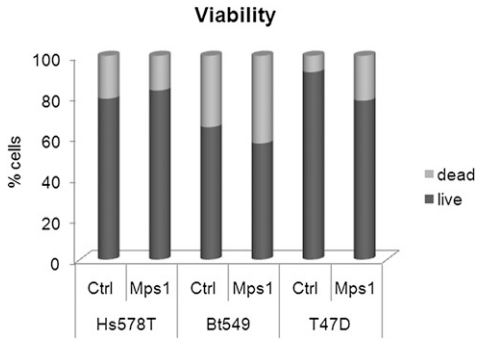


Fig. S4. Viability of cells immediately after Mps1 siRNA transfection. Viability of cells was determined immediately after transfection and incubation for 72 h (day 0) for Hs578T, BT549, and T47D. Viability is measured by Trypan blue exclusion, using a hemocytometer.

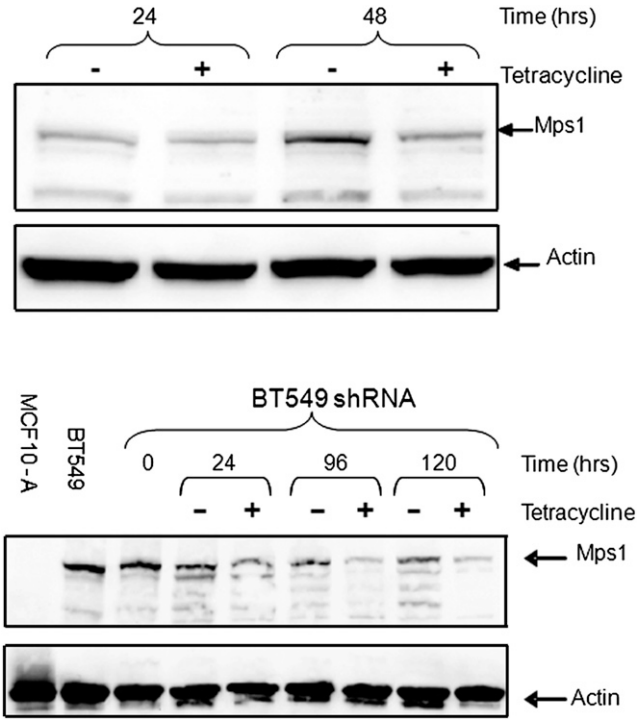


Fig. S5. Measurements of effects of Mps1 shRNA transfection on Mps1 protein levels. Western blot analysis of Hs578T and Bt549 cells stably transfected with tetracycline-inducible Mps1 shRNA after tetracycline induction. Note Mps1 remains reduced as long as tetracycline is present.

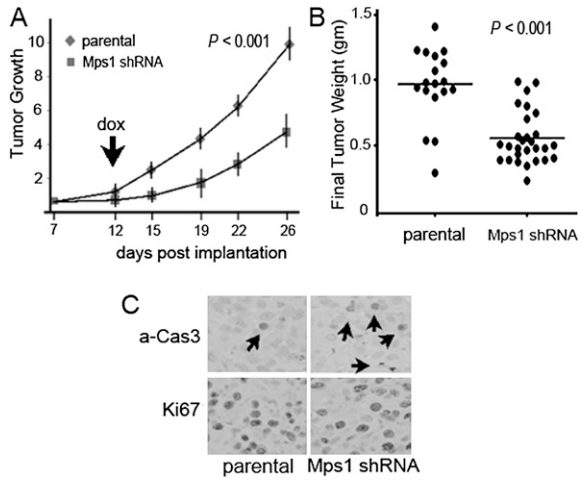
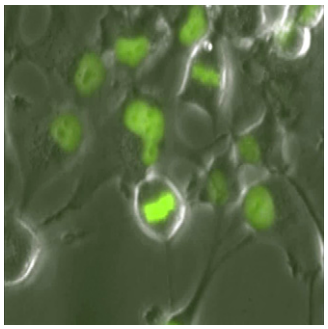
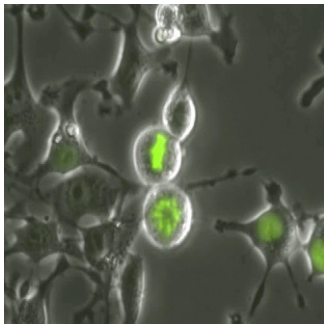


Fig. S6. Reduced Mps1 levels result in decreased in vivo growth of breast cancer xenografts. (*A*) Growth of mouse xenografts tumors of Hs578T cells with doxycycline-inducible Mps1 shRNA compared with xenografts with cells from parental controls. In this experiment, doxycylcine treatment began 12 d after inoculation of tumor cells. Error bars indicate SEM. (*B*) Weights of tumors explanted from animal killed 20 d after inoculation. *P* values were calculated by using Student's *t* test. (*C*) Representative staining of tissues for active caspase 3 and K_i-67.



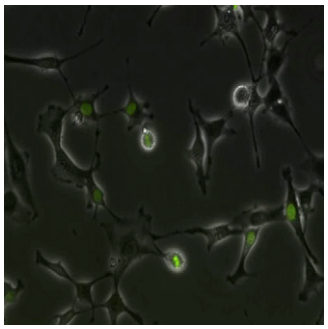
Movie S1. Control Hs578T cells progressing through mitosis. Two control cells in metaphase complete mitosis in ≈25 min. Selected individual frames are shown in Fig. 4*A*.

Movie S1



Movie S2. Hs578T cells with reduced Mps1 progressing through mitosis. Two Mps1 siRNA-treated cells, one in prometaphase and one in metaphase, unsuccessfully attempt cell division. The bottom cell remains in prometaphase before finally undergoing apoptosis after ≈90 min. Note the top metaphase cell makes several attempts to divide before retrogressing to a prometaphase-like state with condensed but unaligned chromosomes. The cell undergoes apoptosis after 170 min.

Movie S2



Movie S3. Hs578T cells with reduced Mps1 progressing through mitosis. Mps1 siRNA-treated Hs578T cells divide but fail to undergo cytokinesis and eventually become multinucleated.

Movie S3