Supporting Information

SI Text

Mice. Previously described $Hus1^{Flox}$ (1) and $Hus1^{\Delta 1}$ (2) mice were maintained on a 129S6 inbred genetic background. $p53^{+/-}$ mice with the Trp-53^{tm1Tyj} allele were maintained on a C57BL/6J background (3). Blg-Cre mice were maintained on a mixed background (4). $Hus1^{Flox/Flox}$ mice were bred to Blg-Cre transgenic (Cre^{+}) mice that also were heterozygous for the null $Hus1^{\Delta 1}$ allele to produce $Hus1^{Flox/\Delta 1}$ Cre^{+} conditional Hus1 knockout mice, as well as $Hus1^{+/Flox}$ Cre^{+} and $Hus1^{Flox/\Delta 1}$ Cre^{-} control animals. All animals were genotyped by PCR analysis of DNA extracted from tail tip biopsies. Mice were housed in accordance with institutional animal care and use guidelines.

Southern Blot. Genomic DNA for Southern blotting was isolated from fourth mammary gland or spleen tissue using Proteinase K digestion and ethanol precipitation. DNA was digested with *Nhe*I, run through a 0.8% agarose gel, transferred to a nylon membrane, and hybridized with a ^{32}P-labeled 190-bp *Eag*I fragment from plasmid pCR2.1–5′UTR-Δ2,3, as previously described (1). The extent of deletion of the conditional Hus1 allele was quantified using a PhosphorImager (GE Healthcare). Statistical analysis was by one-way ANOVA.

Whole Mounts and Histology. Conditional Hus1 knockout mice ($Hus1^{Flox/\Delta 1}$ Cre^{+}) or control mice ($Hus1^{+/Flox}$ Cre^{+} or $Hus1^{Flox/\Delta 1}$ Cre^{-}), either on $p53^{+/+}$ or $p53^{-/-}$ backgrounds, were mated with wild-type males overnight, and checked for copulatory plugs the following morning. The fourth mammary glands were harvested on the 18th day of pregnancy (P18), or the second (L2) or fourth (L4) day of lactation. For whole mount analysis, the fourth mammary gland was harvested, fixed in Carnoy's solution for 6 h, stained overnight in Carmine Alum solution at 4 °C, dehydrated, and stored in xylene. For histological analyses, the fourth mammary gland was fixed in 10% neutral-buffered formalin overnight, dehydrated, embedded in paraffin, and sectioned at 5–8 μm. Sections were deparaffinized in xylenes, rehydrated, and stained with Hematoxylin and Eosin or subjected to immunohistochemical analysis. The relative area of each mammary gland occupied by epithelial or adipose tissue was measured using ImageJ (National Institutes of Health) and Canvas 8 (ACD Systems) software applications.

Ki67 and γ-H2AX Immunohistochemistry. Mammary gland sections from at least three females of each genotype were stained for the presence of Ki67 antigen or γ-H2AX. Heat-mediated antigen retrieval was performed in 0.01 M citrate buffer (for Ki67) or 25 mM EDTA (for γ-H2AX) for 50 min. Sections were incubated with anti-Ki67 Clone MM1 (Vector Laboratories) or anti-γ-H2AX (Upstate Biotechnology) antibodies followed by biotinylated polyclonal rabbit anti-mouse (DAKO). Approximately 500 cells in at least two fields of vision (magnification, ×40) were counted per slide. Statistical analysis was by two-tailed Student's *t*-test.

TUNEL Staining. Mammary gland sections from three different female mice for each genotype were prepared and analyzed using the ApopTag peroxidase In Situ Apoptosis Detection Kit (Millipore) according to the manufacturer's directions. Approximately 500 cells in at least two fields of vision (magnification, ×40) were counted per slide. Statistical analysis was by two-tailed Student's *t*-test.

1. Levitt PS, Liu H, Manning C, Weiss RS (2005) Conditional inactivation of the mouse Hus1 cell cycle checkpoint gene. *Genomics* 86:212–224.
2. Weiss RS, Enoch T, Leder P (2000) Inactivation of mouse Hus1 results in genomic instability and impaired responses to genotoxic stress. *Genes Dev* 14:1886–1898.
3. Jacks T, et al. (1994) Tumor spectrum analysis in p53-mutant mice. *Curr Biol* 4:1–7.
4. Selbert S, et al. (1998) Efficient BLG-Cre mediated gene deletion in the mammary gland. *Transgenic Res* 7:387–396.

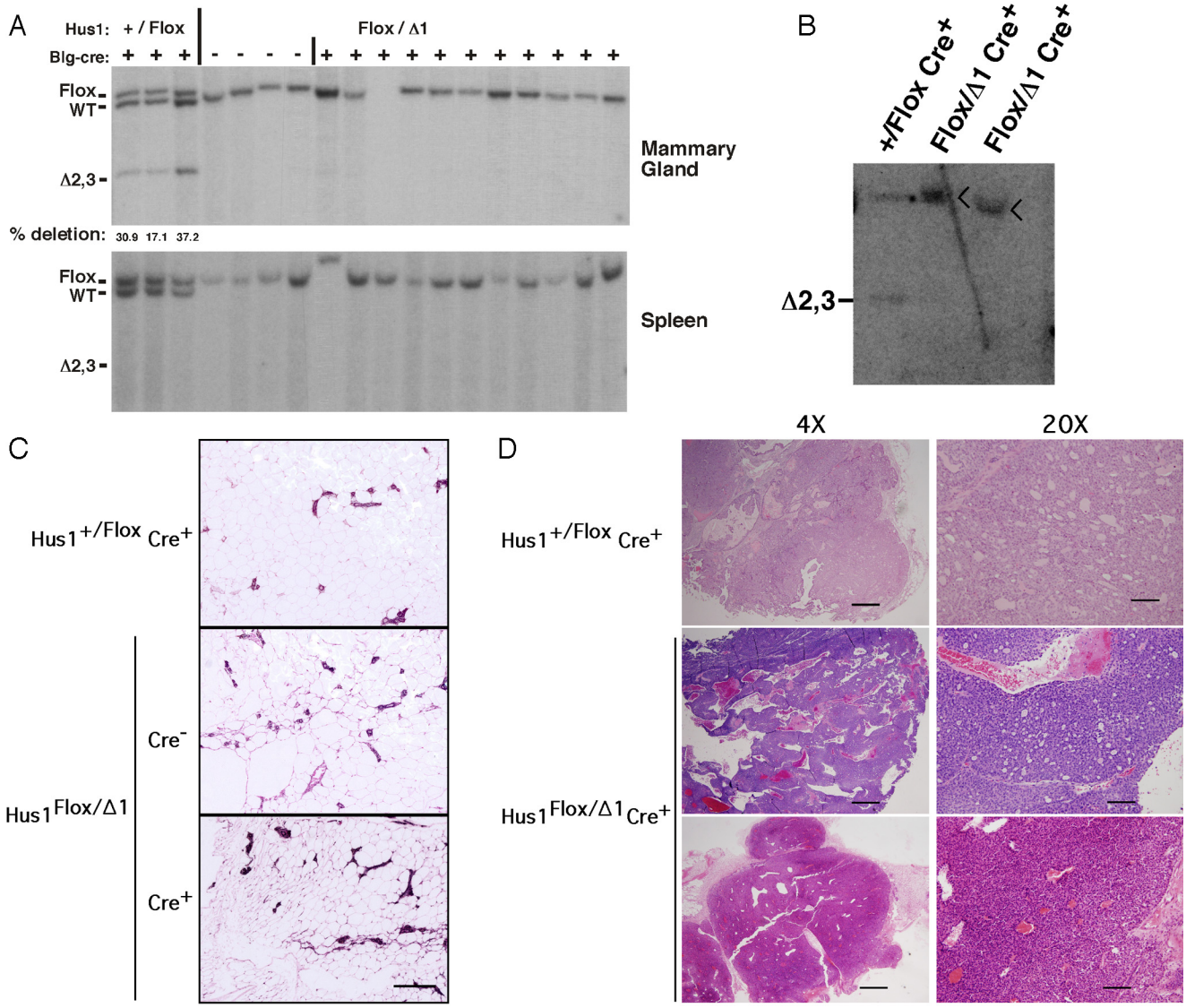

Fig. S1. Mammary glands from conditional Hus1 knockout mice following multiple rounds of pregnancy show depletion of Hus1-deficient cells and grossly normal morphology. (*A*) DNA was extracted from mammary glands of multiparous mice, and probed by Southern blot using a Hus1-specific probe. Southern blot analysis of spleen DNA was included to confirm mammary gland-specific Cre expression. (*B*) Hus1-deficient cells are not detectable in mammary tumors from multiparous conditional Hus1 knockout mice. DNA was isolated from mammary gland tumors from conditional Hus1 knockout and control multiparous females and probed by Southern blot using a Hus1-specific probe. The position of the bands for $Hus1^{Flox}$ (<), wild-type Hus1, and the recombined inactivated Hus1 allele, $Hus1^{\Delta 2,3}$, are indicated. (*C*) Representative images of histological sections of involuted mammary glands from multiparous conditional Hus1 knockout and control females are shown. At approximately 5 months following the weaning of the final litter, the fourth mammary glands from females of the indicated genotypes were harvested, fixed, embedded, sectioned, and stained with H&E. (Scale bar, 400 μm.) (*D*) Representative images of mammary tumors from multiparous females of the indicated genotypes were taken at low (left) or high (right) magnification. (Scale bars, 500 μm and 100 μm respectively.) The tumor incidence in multiparous conditional Hus1 knockout (2 of 22) and control (1 of 16) females was not significantly different ($P = 1.000$; χ^2 test). Tumor incidence values also were not significantly different when including a third multiparous $Hus1^{Flox/\Delta 1}$ Cre^+ female with a palpable mammary mass that subsequently regressed ($P = 0.624$; χ^2 test).

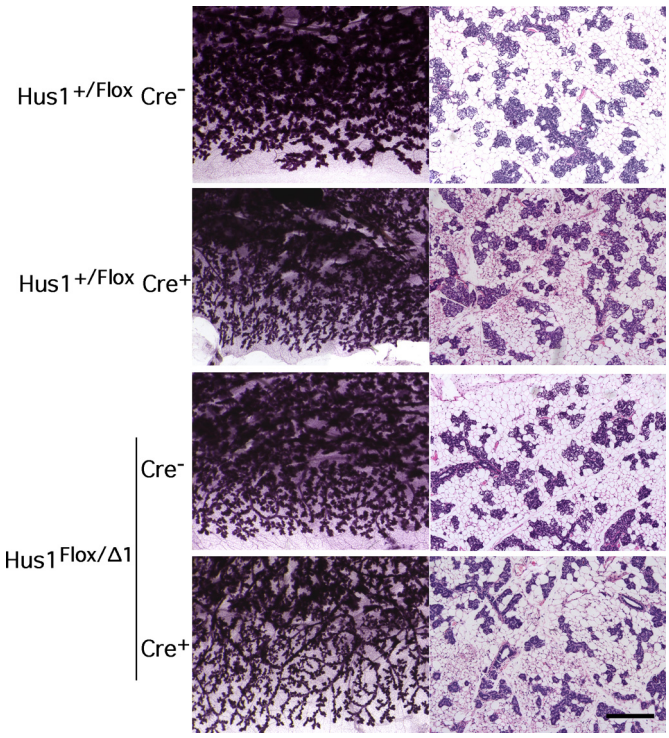

Fig. S2. Grossly normal mammary gland morphology in conditional Hus1 knockout mice. Representative images of whole mounts and histological sections of mammary glands from conditional Hus1 knockout and control females are shown. The fourth mammary glands of mice of the indicated genotype at P18 were harvested, fixed, and stained with Carmine Alum. The contralateral mammary glands were harvested, fixed, embedded, sectioned, and stained with H&E. (Scale bar, 400 μm.)

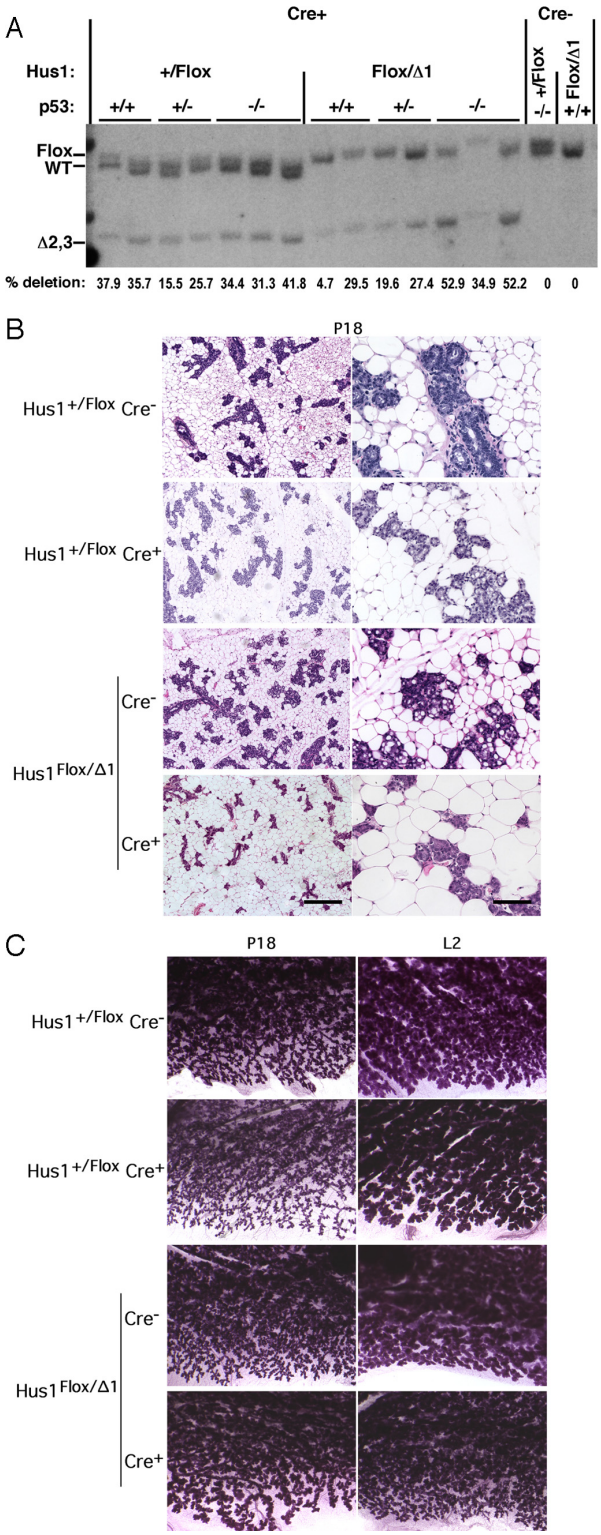

Fig. S3. Increased retention of Hus1-null cells in mammary glands from p53-deficient conditional Hus1 knockout mice at P18. (*A*) Southern blot analysis was performed to measure Hus1 deletion in the mammary glands of conditional Hus1 knockout mice that differ in p53 status. DNA was extracted from mammary glands of mice of the indicated genotypes at P18 and probed by Southern blot as described in the legend of Fig. 1. (*B*) Representative histological sections of mammary glands from p53-deficient conditional Hus1 knockout and control mice are shown. The fourth mammary glands of mice of the indicated genotype at P18 were harvested, fixed, embedded, sectioned, and stained with H&E. [Scale bars, 400 μm for low-magnification images (*Left*); 100 μm for high-magnification images (*Right*).] (*C*) Representative images of whole mounts from p53-deficient conditional Hus1 knockout and control mice are shown. The fourth mammary glands of mice of the indicated genotype at P18 or L2 were harvested, fixed, and stained with Carmine Alum.

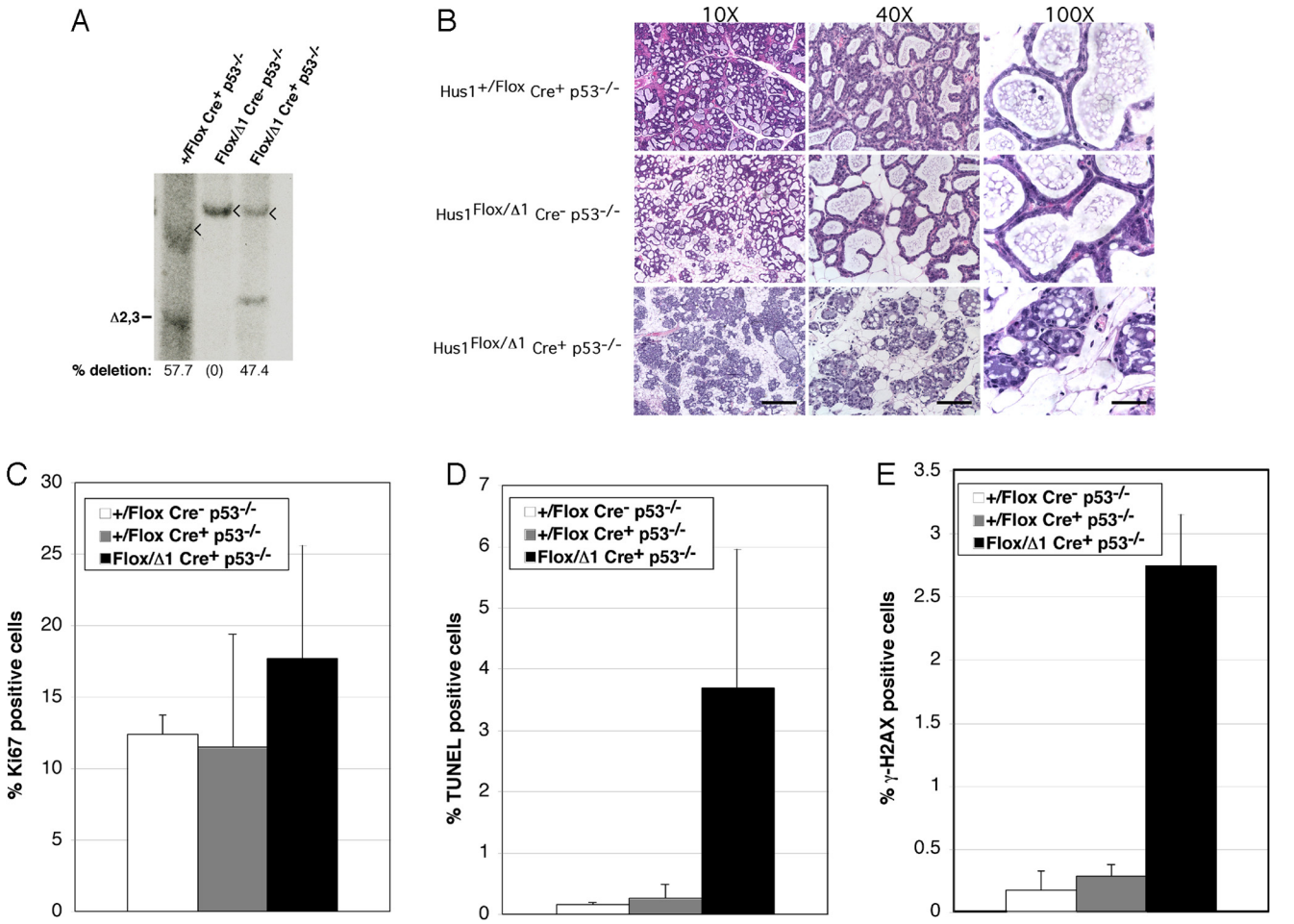

Fig. S4. Aberrant morphology and increased frequency of damaged and dying cells in L4 mammary glands from p53-null conditional Hus1 knockout mice. (*A*) Hus1 deletion in the mammary glands of conditional Hus1 knockout mice at L4 was measured by extracting DNA from mammary tissue from mice of the indicated genotypes and probing by Southern blot as described in the legend of Fig. 1. The position of the band for $Hus1^{Flox}$ is marked with a ''<'' symbol. (*B*) Representative histological sections of mammary glands from p53-deficient conditional Hus1 knockout and control mice are shown. The fourth mammary glands of mice of the indicated genotype at L4 were harvested, fixed, embedded, sectioned, and stained with H&E. [Scale bars, 400 μm for magnification $\times$10 images (*Left*); 100 μm for magnification, $\times$40 images (*Center*); 40 μm for magnification $\times$100 images (*Right*).] (*C–E*) Sections from the fourth mammary gland of p53-deficient conditional Hus1 knockout mice at L4 were stained for Ki67 to assess proliferation, by TUNEL assay to detect apoptosis, or for γ-H2AX to detect DNA damage. Bar graphs show the average percentage of cells positive for (*C*) Ki67, (*D*) TUNEL, or (*E*) γ-H2AX staining. Values are the mean for three independent mammary gland regions from a single mouse of each genotype; error bars, standard deviation.

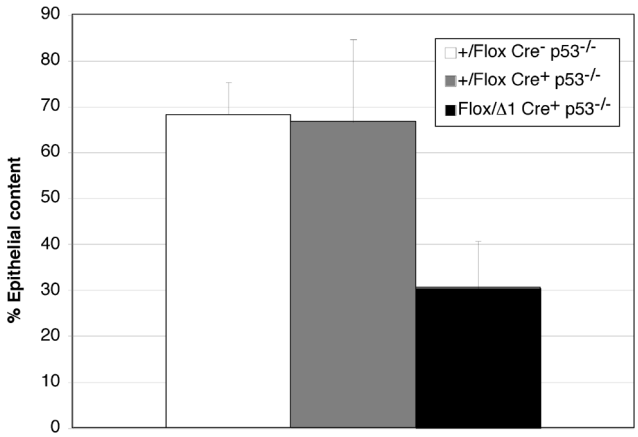

Fig. S5. Significantly reduced epithelial content in mammary glands from p53-null conditional Hus1 knockout mice. Digital images of H&E-stained L2 mammary gland tissue were analyzed using ImageJ and Canvas 8 software applications to determine the total area occupied by epithelial or adipose tissue. At least three independent 40× fields were analyzed from each animal, including $Hus1^{+/Flox}$ Cre^{-} $p53^{-/-}$ (two mice), $Hus1^{+/Flox}$ Cre^{+} $p53^{-/-}$ (three mice), and $Hus1^{Flox/\Delta1}$ Cre^{+} $p53^{-/-}$ (two mice). The bar graph shows the mean value for each genotype; error bars, standard deviation. The difference in epithelial content between $Hus1^{Flox/\Delta1}$ Cre^{+} $p53^{-/-}$ and control $Hus1^{+/Flox}$ Cre^{+} $p53^{-/-}$ mammary glands was significantly different ($P < 0.001$) as determined by Student's *t* test.

Table S1. Genotyping results for weanlings from $Hus1^{Flox/Flox}$ X $Hus1^{+/\Delta 1}$ Cre^{+} breedings

Genotype	Expected, *n* (%)	Observed, *n* (%)
$Hus1^{+/Flox}$ Cre^{-}	104 (25)	98 (26.2)
$Hus1^{+/Flox}$ Cre^{+}	104 (25)	115 (22.6)
$Hus1^{Flox/\Delta 1}$ Cre^{-}	104 (25)	94 (27.6)
$Hus1^{Flox/\Delta 1}$ Cre^{+}	104 (25)	109 (23.6)

Genomic DNA was isolated from tail biopsies from weanlings and then genotyped by PCR as described in *Materials and Methods*.