Supplemental Materials and Methods

Immunohistochemical Analysis

IHC conditions for BrdU (Calbiochem, #NA20), p53 (Novacastra, CM5), MCM7 (NeoMarkers, # ab47DC141), pRb (BD Pharmingen, #554136) and p16 (Santa Cruz, M156) were previously described (17). IHC analysis for Bmi-1 (Upstate, #05-637), ERα (Santa Cruz, MC20), pRb S807/S811 (Cell Signaling, #9308S) were as follows after deparaffinization. The slides were immersed in 2N HCl for 20 minutes to unmask further. For ERα analysis, slides were then blocked in a mixture of 5% powdered milk and horse serum diluted in PBS for 30min to 1 hour. For Bmi-1, the slides were blocked in 1.0% horse serum diluted in PBS for 30 min. For vimentin (Neomarkers, #MS129P), the slides were blocked in 1.5% horse serum and 0.1% BSA diluted in PBS for 30 min. For all other analysis, slides were blocked in 5% horse serum diluted in PBS for 30 min. Primary antibody (in blocking solution) was applied to the sections at 1:125-150 for Bmi-1, 1:100 for ERα, 1:150 pRb S807/S811, and 1:400 for vimentin (in 1.5% horse serum without BSA) overnight at 4°C. Sections were subjected to several PBS washes. A universal biotinylated secondary antibody was used at a concentration of 1:50-1:200 followed by streptavidin-peroxidase application per instructions from the Vectastain ABC kit (Vector Labs, PK-6200). The sections were developed with 3,3'-diaminobenzidine (DAB) solution (Vector Labs, SK-4100) or ImmPACT DAB (Vector Labs, SK-4105) and stopped with H_2O. The sections were then counterstained with hematoxylin, rehydrated in a sequence of graded alcohol/water mixtures and xylenes and then covered with a cover slip.

Immunofluorescence Analysis

For E-cadherin (BD Pharmingen, #610181) and N-cadherin (BD Pharmingen, #610920) double staining, the slides were unmasked by boiling for 20 minutes in 10mM citrate buffer, pH 6 after dehydration. The slides were then rinsed in PBS and then blocked in 10% horse serum diluted in PBS for 2 hours at room temperature. Primary antibody was applied to the sections at 1:100 diluted in blocking solution for E-cadherin overnight at 4°C. A universal secondary biotinylated antibody (Vector Labs, PK-6200) was used at 1:50 diluted in PBS for 30 min at RT. SA-Texas Red (Vector, TI-2000) was then applied to the sections at 1:150 for 30min at RT. N-cadherin was then applied at 1:100 diluted in blocking solution overnight at 4°C. Finally, a mouse-anti-fluorescein antibody (Vector, FI-2000) was applied at 1:100 for 30min at RT.

For K8 (DSHB, #Troma-I) and K14 double staining, the sections were blocked with 10% goat serum and 3% BSA diluted in PBS for 1 hour at RT after dehydration. K8 was used at 1:250 in 3% BSA/PBS overnight at 4°C. A goat anti-rat biotinylated secondary (BD Pharmingen, #554014) was applied to the sections at 1:50 in 3%BSA/PBS for 30min. Avidin-Rhodamine (Vector, A-1100) was applied to the sections at 1:100 in 3%BSA/PBS for 30min. Then K14-FITC (Covance, #PRB-155P) was used at 1:500 diluted in 3%BSA/PBS for at least 3 hours to overnight. A drop of mounting medium containing DAPI was applied to each section and covered with a cover slip.

Supplemental Figure Legends

Figure 1. Acute properties of p53-insufficiency in the mouse cervix.

A. Shown are sections of cervical epithelium from mice treated with exogenous estrogen for six weeks and stained for p53 by IHC. Mice were either unirradiated (*a,c*) or irradiated (*b,d*) to induce p53 (brown nuclei). p53 is induced in $p53^{f/f}$ mice upon irradiation (*b*) but is undetectable in the absence of DNA damage (*a*). p53 is not detected in either unirradiated or irradiated epithelia (*c,d*) of $K14Crep53^{f/f}$ mice.

B. p53 activity is lost upon conditional deletion in the cervical epithelium. Shown are sections of cervical epithelium from mice acutely treated with estrogen and either given ionizing radiation or untreated and injected with BrdU prior to sacrifice. The sections were stained for BrdU (brown nuclei).

Supplemental Table 1. Summary of lower reproductive tract histopathology in mice treated with estrogen

Genotype	NH	VAIN1	VAIN2	VAIN3	MIC	LIC
$p53^{f/f}$	5	1	1			
$K14Crep53^{f/f}$	6	11	2	1	5	
$K14E6^{WT}p53^{f/f}$	12	8	2			
$K14E6^{\Delta}p53^{f/f}$	3	7				
$K14E6^{WT}Crep53^{f/f}$	6		3		5	2
$K14E6^{\Delta}Crep53^{f/f}$	3	2		5	6	
$K14E7^{WT}p53^{f/f}$	2	2	6	1	5	
$K14E7^{WT}Crep53^{f/f}$			2	2	2	8
$K14E6^{WT}E7^{WT}p53^{f/f}$	1	1	1		5	1
$K14E6^{WT}E7^{WT}Crep53^{f/f}$					1	4

Supplemental Table 2. Comparison of tumor development in the lower reproductive tract in mice treated with estrogen

Genotype	Cancer Incidence (%)	Incidence of LIC (%)	Tumor Multiplicity
$p53^{f/f}$ (n=7)	0	0	0.00
$K14Crep53^{f/f}$ (n=25)	20	0	0.20
$K14E6^{WT}p53^{f/f}$ (n=22)	0	0	0.00
$K14E6^{WT}Crep53^{f/f}$ (n=16)	43.7	12.5[a]	0.94[c]
$K14E6^{\Delta}Crep53^{f/f}$ (n=16)	37.5	0	0.50
$K14E7^{WT}p53^{f/f}$ (n=16)	31.3	0	0.44
$K14E7^{WT}Crep53^{f/f}$ (n=14)	71.4	57.1[b]	1.90[d]
$K14E6^{WT}E7^{WT}p53^{f/f}$ (n=9)	66.7	11.1	1.22
$K14E6^{WT}E7^{WT}Crep53^{f/f}$ (n=5)	100	100	ND

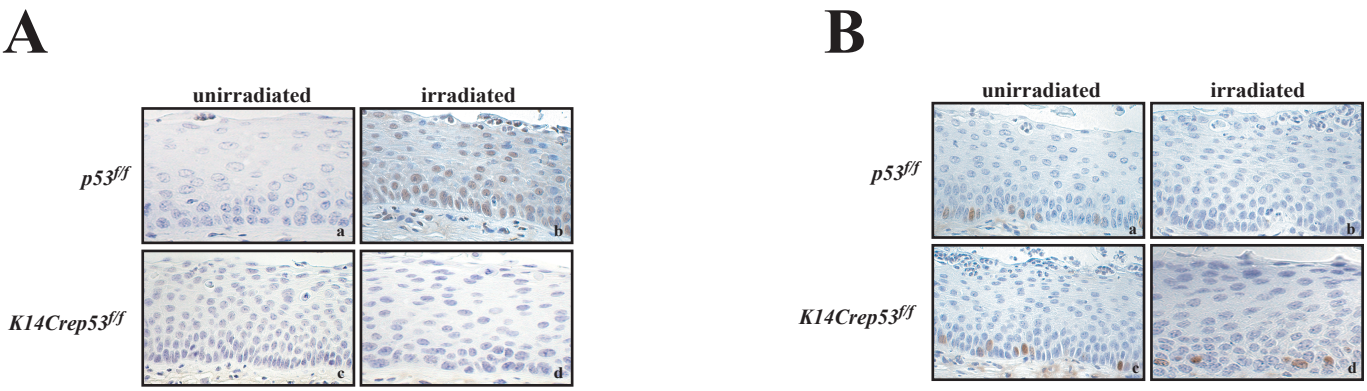
NOTE: Tumor multiplicity was not determined in $K14E6^{WT}E7^{WT}Crep53^{f/f}$ mice due to difficulty in delineating individual tumors. All statistical analyses are two-sided.

[a] Although $K14E6^{WT}Crep53^{f/f}$ mice have an increase in the incidence of LIC, this was not significant when compared to $K14Crep53^{f/f}$ mice, p=0.15, Fisher's Exact Test.
[b] The incidence of LIC in $K14E7^{WT}Crep53^{f/f}$ mice is significant when compared to $K14E6^{WT}E7^{WT}p53^{f/f}$ mice, p=0.04, Fisher's Exact Test.
[c] Tumor multiplicity in $K14E6^{WT}Crep53^{f/f}$ mice is significant when compared to $K14Crep53^{f/f}$ mice, p=0.05, Wilcoxon-Rank Sum.
[d] Tumor multiplicity in $K14E7^{WT}Crep53^{f/f}$ mice is significant when compared to $K14E7^{WT}p53^{f/f}$ mice, p=0.005, Wilcoxon-Rank Sum.

Supplemental Table 3. Comparison of biomarker expression in cervical tumors

Genotype	Bmi-1	p16	pRb	pRb-ppp	MCM7
$p53^{ff}$	±	±	+	+	+
$crep53^{ff}$	± (- to +)	++ (± to +++)	± (- to +)	± (- to +)	++ (± to +++)
$E6^{WT}crep53^{ff}$	+ (- to ++)	++ (± to +++)	± (- to +)	± (- to +)	++ (+ to +++)
$E7^{WT}crep53^{ff}$	++	+++	±	±	+++
$E6^{WT}E7^{WT}crep53^{ff}$	++	+++	±	±	+++

Note: –, negative; ±, ≤ 5%; + ≤ 20%; ++ ≤ 50%; +++, > 50%. Average biomarker expression as shown in Figure 4. Some genotypes have variable expression of biomarkers and thus include a range below the average.



Shai *et al*., Supplementary Figure 1