# Supporting Information

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### SI Materials and Methods

**Lymphoma Reconstitution Assays.** Once visible lymphoma nodules were identified from the *Eμ-Myc; Rosa26-CreER; p53*$^{LSL\text{-}25,26/+}$ and *Eμ-Myc; Rosa26-CreER; p53*$^{LSL\text{-}25,26,53,54/+}$ mice, lymphoma tissue was dissected out from cervical, inguinal, mesenteric, and mediastinal lymph nodes. Tumor chunks were minced with a razor blade in cold PBS, and the cell suspension was passed through a 40-μm nylon cell strainer and then treated with red blood cell lysis buffer (0.15 M $NH_4Cl$, 10 mM $KHCO_3$, 0.1 mM EDTA). To establish in vitro cultures, lymphoma cells were grown in B-cell medium (DMEM + IMDM 1:1, 10% FCS, 4 mM L-glutamine, 48.3 μM 2-Mercaptoethanol) on feeder cells (30 Gy IR-irradiated 3T3 cells). To induce recombination of the *Lox-Stop-Lox* element, lymphoma cells in culture were treated with 1 μM 4-OHT for 4 d. To reconstitute lymphoma in vivo, 1–1.5 × $10^6$ lymphoma cells per recipient were retroorbitally injected into syngeneic 2- to 6-mo-old recipient mice. A total of 13 recipient mice received lymphoma cells from two *Eμ-Myc; Rosa26-CreER; p53*$^{LSL\text{-}25,26/+}$ donor mice (donor I to 7 recipients and donor II to 6 recipients), and 11 recipient mice received lymphoma cells from 2 *Eμ-Myc; Rosa26-CreER; p53*$^{LSL\text{-}25,26,53,54/+}$ donor mice (donor I to 7 recipients and donor II to 4 recipients). Tumors forming from major lymph nodes were monitored and dissected out once clearly visible for lymphoma cell isolation and tissue fixation.

**Spontaneous Tumor Analysis.** *p53* mutant strains were crossed to *Rosa26-CreER*$^{T2}$ mice (1). A total of 5 mg tamoxifen (Sigma) dissolved in 2% ethanol in corn oil (vol/vol) was administered for 3 consecutive days by oral gavage, a protocol that results in relatively efficient deletion of the *Lox-Stop-Lox* element and expression of p53 mutants in the thymus (~50% of the cells), among other tissues (2). Mice were aged until moribund and then subjected to necropsy to analyze tumor incidence.


1. Ventura A, et al. (2007) Restoration of p53 function leads to tumour regression in vivo. *Nature* 445:661–665.
2. Brady CA, et al. (2011) Distinct p53 transcriptional programs dictate acute DNA-damage responses and tumor suppression. *Cell* 145:571–583.


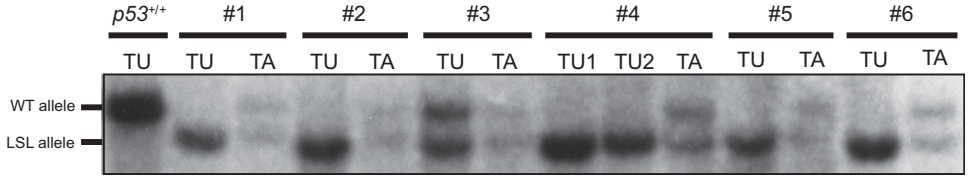


**Fig. S1.** The majority of lymphomas arising from *Eμ-Myc; p53*$^{LSL\text{-}25,26/+}$ mice display Loss of Heterozygosity (LOH) at the *p53* locus. Southern blot analysis of lymphoma DNA (TU, tumor) from five out of six *Eμ-Myc; p53*$^{LSL\text{-}25,26/+}$ mice (#1–6, except #3) showed loss of the wild-type *p53* allele in contrast to tail DNA from the same mouse (TA), which retained both the *p53*$^{LSL\text{-}25,26}$ and the wild-type *p53* alleles. Southern blotting was performed using the EcoRI digestion and probe shown in Fig. 1*A*. Tumor DNA from two different sites (TU1 from mediastinal lymph node and TU2 from inguinal lymph node) were included for mouse #4. DNA from an *Eμ-Myc; p53*$^{+/+}$ tumor was used as a wild-type *p53* allele control in the first lane.

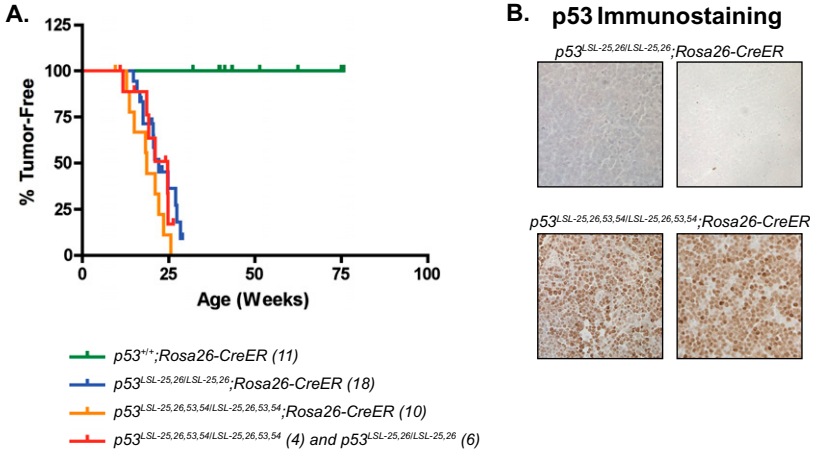


**Fig. S2.** p53$^{25,26}$, but not p53$^{25,26,53,54}$, suppresses spontaneous tumor development. (*A*) Kaplan–Meier analysis showing tumor-free survival of tamoxifen-treated *p53*$^{+/+}$*; Rosa26-CreER*, *p53*$^{LSL\text{-}25,26/LSL\text{-}25,26}$*; Rosa26-CreER*, *p53*$^{LSL\text{-}25,26,53,54/LSL\text{-}25,26,53,54}$*; Rosa26-CreER*, and *p53* null controls (comprising *p53*$^{LSL\text{-}25,26/LSL\text{-}25,26}$ and *p53*$^{LSL\text{-}25,26,53,54/LSL\text{-}25,26,53,54}$ mice). The number of mice analyzed is indicated. The tumor-free survival of *p53*$^{+/+}$*; Rosa26-CreER* mice is significantly different from that of mice of the other genotypes ($P \leq 0.0002$, by log rank test), which are not significantly different from each other ($P \geq 0.0607$, by log rank test). (*B*) p53 immunohistochemistry in thymic lymphomas from *p53*$^{LSL\text{-}25,26/LSL\text{-}25,26}$*; Rosa26Cre-ER* or *p53*$^{LSL\text{-}25,26,53,54/LSL\text{-}25,26,53,54}$*; Rosa26-CreER* mice. Selection against p53$^{25,26}$ expression is always observed in tumors arising in *p53*$^{LSL\text{-}25,26/LSL\text{-}25,26}$*; Rosa26Cre-ER* mice. Tumors in *p53*$^{LSL\text{-}25,26,53,54/LSL\text{-}25,26,53,54}$*; Rosa26-CreER* mice can arise from cells expressing p53$^{25,26,53,54}$ or *p53* null cells (*p53*$^{LSL\text{-}25,26,53,54/LSL\text{-}25,26,53,54}$ cells failing to delete the stop element), consistent with the initial mixed population of p53$^{25,26,53,54}$-expressing and *p53* null cells in the normal thymus and with the *p53*$^{25,26,53,54}$ allele being functionally equivalent to a *p53* null allele. The selection against p53$^{25,26}$ but not p53$^{25,26,53,54}$ expression in tumors provides further support for the idea that p53$^{25,26}$ displays tumor suppressor activity.