Supplemental Figure 1 Cosme-Blanco et al.
Supplemental Figure 1. A. Quantitation of γ-H2AX foci in p53<sup>+/+</sup>, p53<sup>−/−</sup> and p53<sup>P/P</sup> MEFs after infection of TRF2<sup>ΔBΔM</sup> or vector control. Error bars represent SEM. B. Intergenerational mating scheme used to generate mouse cohorts. C. Tissues derived from mTerc<sup>+/−</sup>, p53<sup>P/P</sup> and iG1 p53<sup>−/−</sup> mice are not positive for SA-β-galactosidase. Organs from mice of the indicated genotypes were harvested and immediately processed for frozen sections and stained for SA-β-galactosidase activity. Compared to iG1 p53<sup>P/P</sup> tissues (Fig. 4C), only low-level background staining was detected in these tissues.

Supplemental Materials and Methods

**Immunohistochemistry.** Tissues were fixed in 10% formalin and paraffin embedded. Antigens were retrieved using 10mM Citrate Buffer pH 6.0 for 15 minutes. Primary antibodies used: p21 (Santa Cruz, sc-6246, 1:50), p53 (Novocastra, NCL-p53-CM5p, 1:500), and Ki-67 (Dako, M7249, 1:200). Sections were incubated with biotinylated rabbit-anti-mouse F(ab) at 1:250 dilution (for p21), envision plus labeled polymer, anti-rabbit-HRP (for p53), and SA-HPR (for Ki-67). Immunoreactivity was revealed using diaminobenzidine (DAB).

**Cell cultures, Western blot analysis and senescence-associated β-gal assay.** Mouse embryonic fibroblasts (MEFs) were prepared from individual day 13.5 p53<sup>P/P</sup> and p53<sup>+/−</sup> embryos. A minimum of 3 independently derived MEF cell lines of each genotype was used in all analyses, and harvested 4 days after retroviral infections. Murine myc-TRF2<sup>ΔBΔM</sup> was a kind gift from Jan Karlseder (Smogorzewska and de Lange, 2002). Western analysis was performed as described (Wu et al., 2006). The antibodies used for Western analyses were anti-mouse p21 (Santa Cruz, 1:500), anti-mouse p53 (Cell Signaling, 1:250), phosphor-γH2AX (JBW301, Upstate, 1:500) and anti-Myc (Sigma, 1:1000). Anti-mouse γ-tubulin (Sigma, 1:10,000) was used as a loading control. SA β-gal assay was performed as described (Dimri et al., 1995).

**Cytogenetics analysis.** Metaphase chromosomes from MEFs and bone marrows (BM) were prepared 1-4 hours after colcemid treatment as previously described (Wu et al., 2006) and subjected to Giemsa staining and/or fluorescence in-situ hybridization (FISH).
with Cy-3-labeled TcAG peptide-nucleic acid (PNA) probe as described (Wu et al., 2006). Images were captured on a Nikon Eclipse 800 microscope. Depending on the quality of metaphase spreads, 10-20 metaphases from each sample were analyzed in detail.

Supplemental Bibliography
