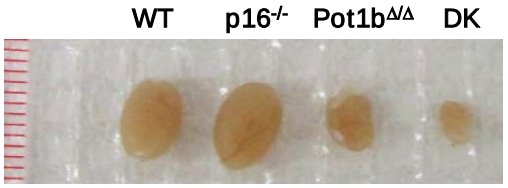
A



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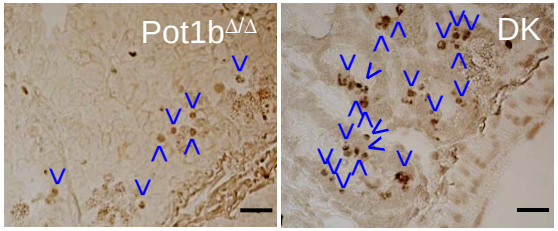

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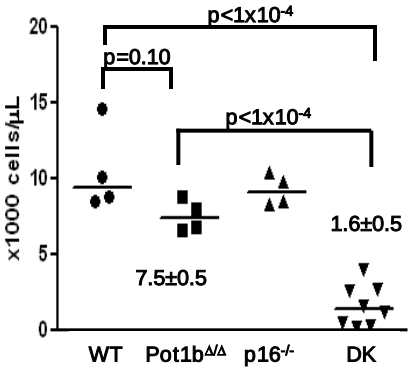
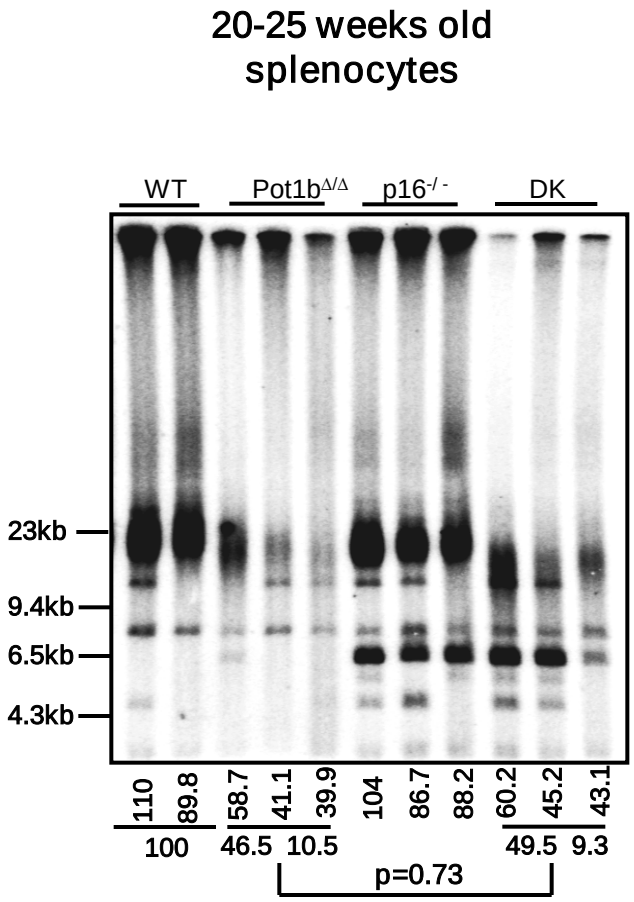


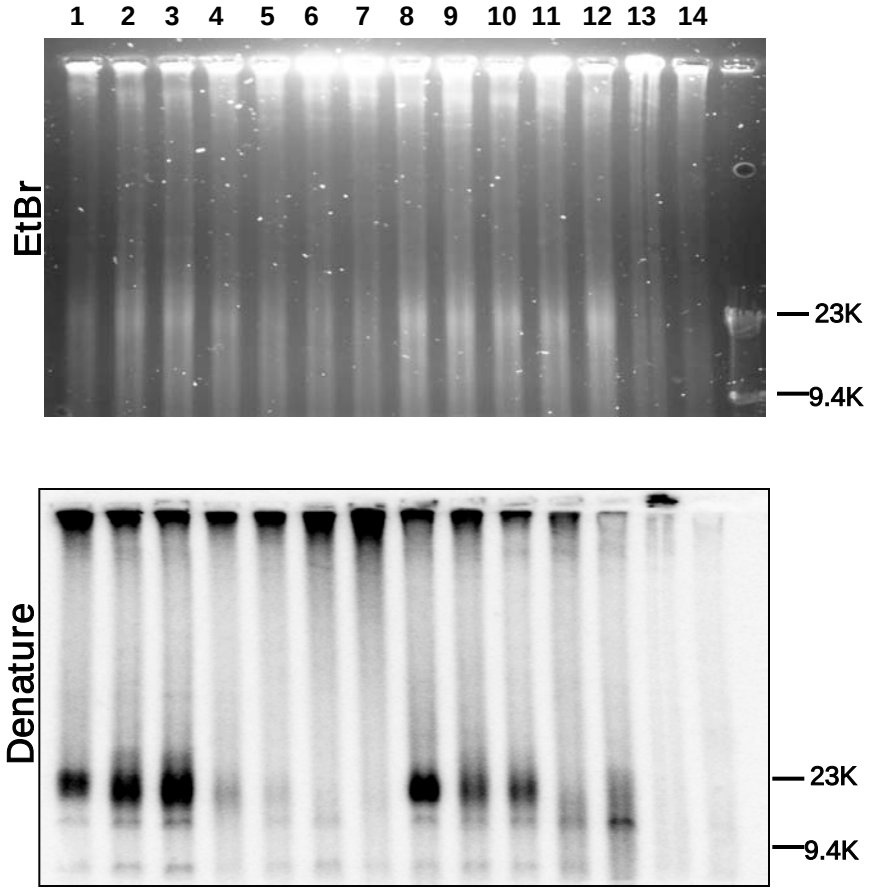
Wang et al., Supplementary Figure 1

Supplementary Figure 1. Defects in DK proliferative tissues. (A) Representative testes from mice of the indicated genotypes are shown. (B) Representative images of TUNEL stained small intestines isolated from age-matched *Pot1b*$^{\Delta/\Delta}$ and DK mice. Arrowheads point to apoptotic nuclei (magnification x40). (C) Peripheral white blood cell (WBC) counts from 40-45-weeks-old mice of the indicated genotypes. Each experiment was repeated in triplicate. Two-tailed t-test was used to calculate statistical significance.



Wang et al., Supplementary Figure 2

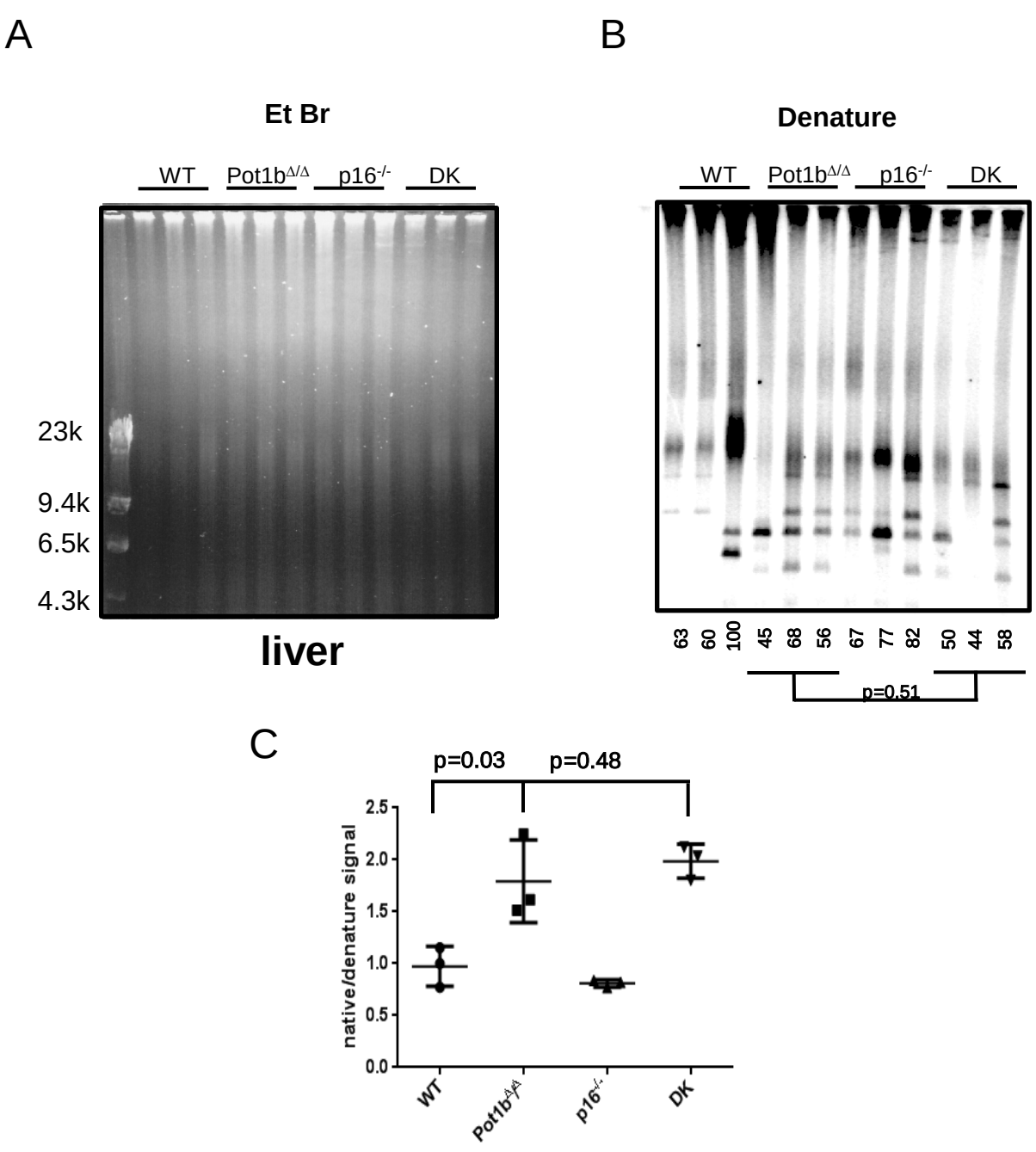
Supplementary Figure 2. Telomere Restriction Fragment (TRF) length analysis of 20-25 weeks old splenocytes. TRF length analysis of total telomeres from splenocytes isolated from 20-25 weeks old mice of the indicated genotypes. HinfI/RsaI digested genomic DNA were hybridized under denatured conditions with a ^{32}P-labeled $[CCCTAA]_4$-oligo to detect total telomere DNA. Telomere signal intensity (%) was quantified by setting WT total telomere DNA as 100%.



Lane 1: WT 45 wks
2: Pot1b$^{\Delta/\Delta}$ 8wks
3: Pot1b$^{\Delta/\Delta}$ 8wks
4: Pot1b$^{\Delta/\Delta}$ 25 wks
5: Pot1b$^{\Delta/\Delta}$ 25 wks
6: Pot1b$^{\Delta/\Delta}$ 45 wks
7: Pot1b$^{\Delta/\Delta}$ 45 wks
8: p16$^{-/-}$ 45 wks
9: DK 8 wks
10: DK 8 wks
11: DK 25 wks
12: DK 25 wks
13: DK 45 wks
14: DK 45 wks

Wang et al., Supplementary Figure 3

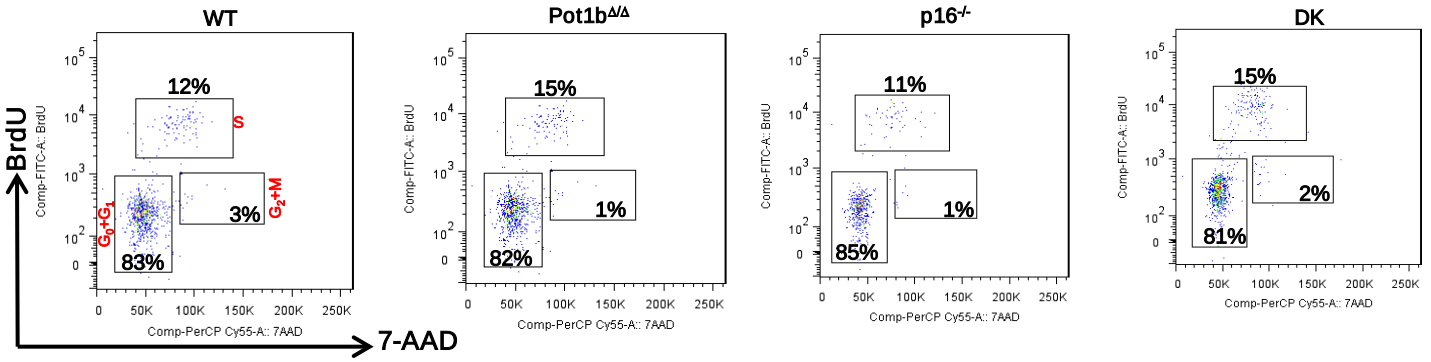
Supplementary Figure 3. TRF length analysis of total telomeres in DK spleenocytes isolated from 8, 25 and 45 weeks old mice. The upper panel shows ethidium bromide staining indicating equal loading of genomic DNA per lane. In-gel hybridization was performed under native conditions to detect single-stranded telomereic DNA (middle panel) and subsequently under denaturing conditions to detect total telomeric DNA (lower panel).



Wang et al., Supplementary Figure 4

Supplementary Figure 4. TRF length analysis of total telomeres from liver of 40-45 weeks old mice of the indicated genotypes. HinfI/RsaI digested, EtBr stained (A) genomic DNA was hybridized under denaturing (B) conditions with a ^{32}P-labeled $[CCCTAA]_4$-oligo to detect single-strand overhang and total telomere DNA. Total telomere signal intensity (%) was quantified (C) by setting WT telomere DNA as 100%. The two-tailed t-test was used to calculate statistical significance.

A



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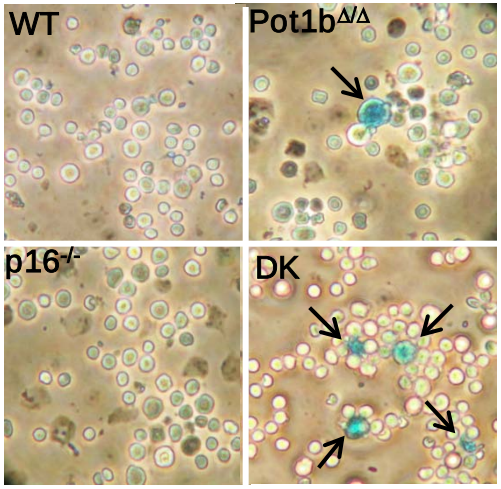

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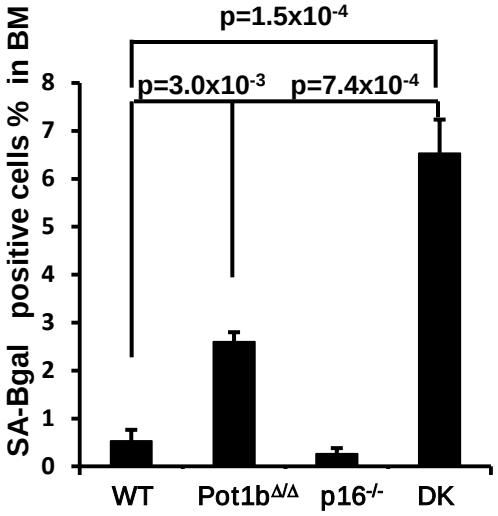


Wang et al., Supplementary Figure 5

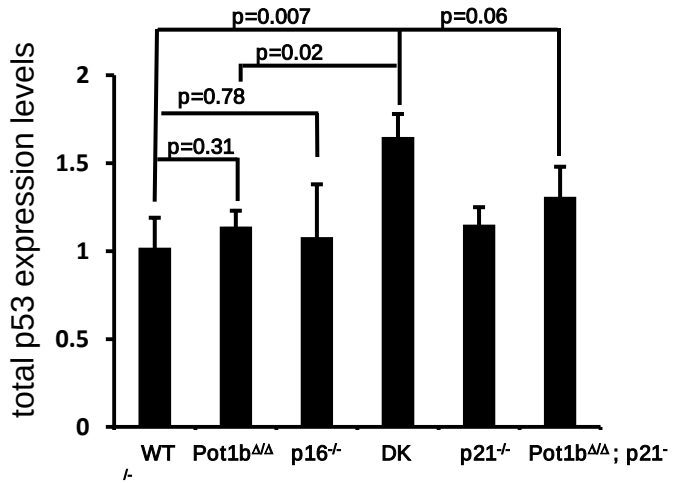
Supplementary Figure 5. Cell cycle profiles and cellular senescence in WT and mutant mice. (A) No significant cell cycle differences were observed in BrdU labeled LSK cells isolated from 4-5 weeks old mice. Representative FACs analysis of multi-lineage negative cell populations derived from the BM of mice of the indicated genotypes. FACS plots represent experiments with at least three mice per genotype. Cell cycle stages are labeled in red. (B) SA-β-galactosidase staining and (C) quantification of SA-β-galactosidase positive cells in the BM of mice of the indicated genotypes. Mean values were derived from at least three experiments. The two-tailed t-test was used to calculate statistical significance. Error bars: standard error of the mean (s.e.m.).

A

Quantification of Western Blot results in Figure 5E

	+ control	WT	Pot1b$^{\Delta/\Delta}$	Pot1b$^{\Delta/\Delta}$	p16$^{-/-}$	p16$^{-/-}$	DK	DK	DK
p-RPA	7.6	0.09	1	0.64	0.15	0.09	3.2	3.5	4.5
p-ATR	2.4	0.16	1	0.68	0.14	0.09	2	2.7	3.1
p-CHk1	3.8	0.1	1	0.77	0.14	0.14	3.1	3.2	3.8
p-p53	>5.5	0.12	1	0.7	0.16	0.09	1.9	1.6	1.5
p21	0.41	0.04	1	0.48	0.07	0.05	2.7	3.3	3

B



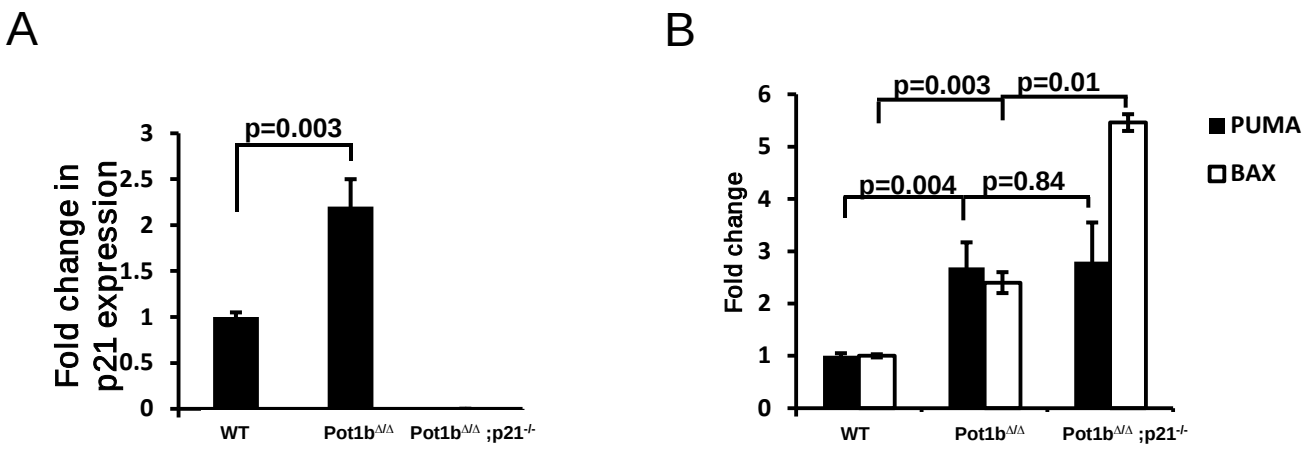
C

Quantification of Western Blot results in Figure 5G

	+ control	10 weeks	10 weeks	10 weeks	25 weeks	25 weeks	25 weeks	45 weeks	45 weeks
p-RPA	2.5	0.12	0.05	0.13	1	0.69	0.65	3.1	2.1
p-ATR	3.3	0.08	0.14	0.07	1	1.1	1.4	3.9	2.7
p-CHk1	3.7	0.39	0.21	0.18	1	0.87	0.82	2.7	1.9
p21	0.36	0.11	0.06	0.1	1	0.85	1.5	5.2	4

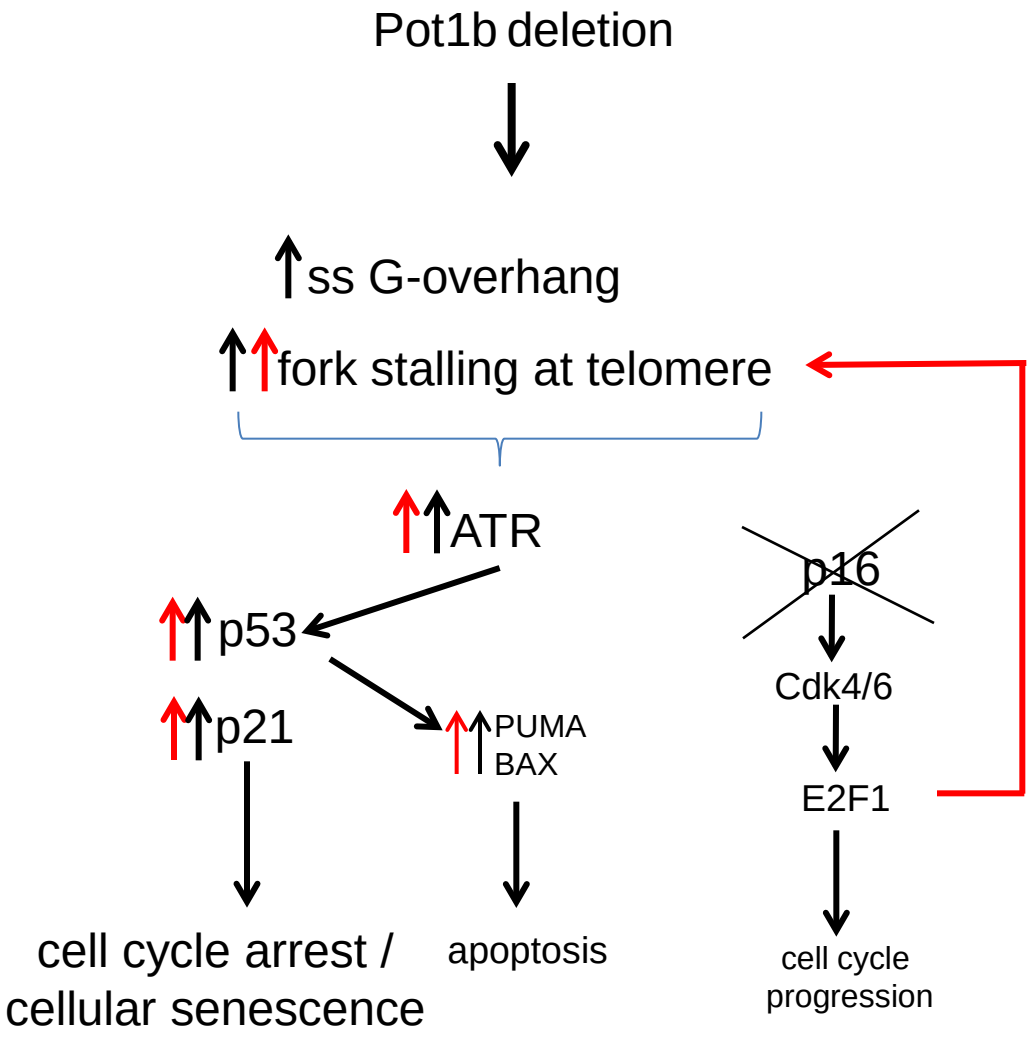
Wang et al., Supplementary Figure 6

Supplementary Figure 6. Quantification of Western blots and RT-PCR analysis of p53 expression in splenocytes of WT and mutant mice. (A) Quantification of the expression of indicated checkpoint proteins from Figure 5E, using IMAGE analysis software. Expression of checkpoint proteins in one *Pot1b* null sample was arbitrarily set to1 (100%) and the expression levels of all other samples were compared to this sample. (B) Real time PCR analysis of p53 expression in splenocytes of the indicated genotypes. Error bars represent s.e.m. and the two-tailed t-test was used to calculate statistical significance. The WT, $Pot1b^{\Delta/\Delta}$, $p16^{-/-}$ and DK mice were 40-45 weeks old at time of sacrifice while the $p21^{-/-}$ and $Pot1b^{\Delta/\Delta}; p21^{-/-}$ mice were 70-80 weeks old. (C) Quantification of the expression of indicated checkpoint proteins from Figure 5G. Expression of checkpoint proteins in 25 weeks old DK cells were arbitrarily set at 1 (100%).



Wang et al., Supplementary Figure 7

Supplementary Figure 7. RT-PCR analysis of p21, Bax and PUMA expression in WT and mutant LSK cells. Representative RT-PCR quantification of p21 (A), PUMA and Bax (B) mRNA expression levels in sorted LSK cells from 40-45 weeks old mice of the indicated genotypes. Each experiment was done in triplicate. The two-tailed t-test was used to calculate statistical significance. Error bars represent s.e.m.



Wang et al., Supplementary Figure 8

Supplementary Figure 8. Speculative schematic of DNA damage checkpoint activations in DK cells. Please see text for details.