

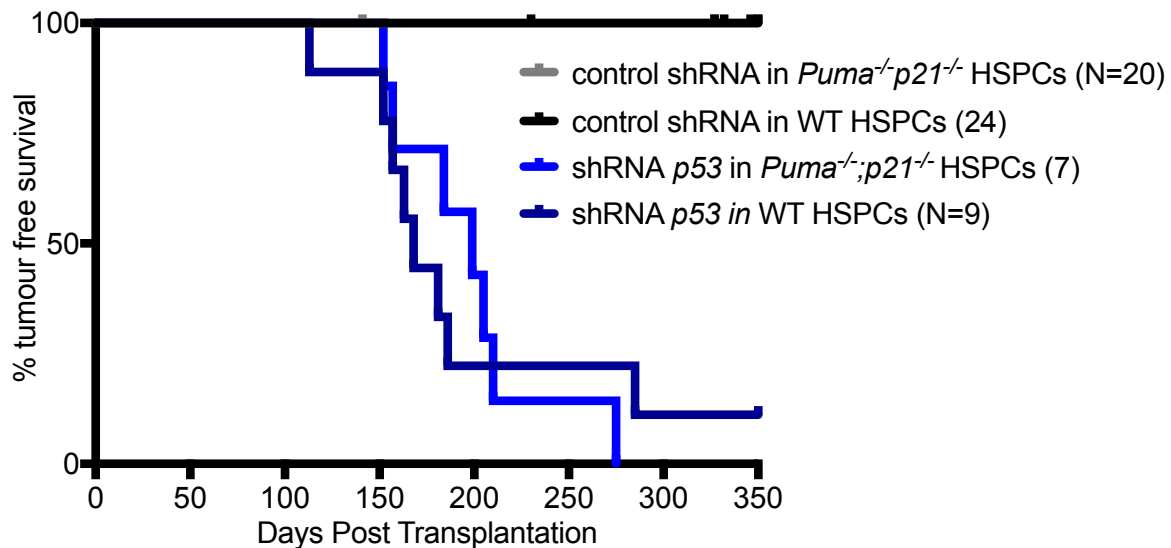
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DNA repair processes are critical mediators of p53-dependent tumor suppression

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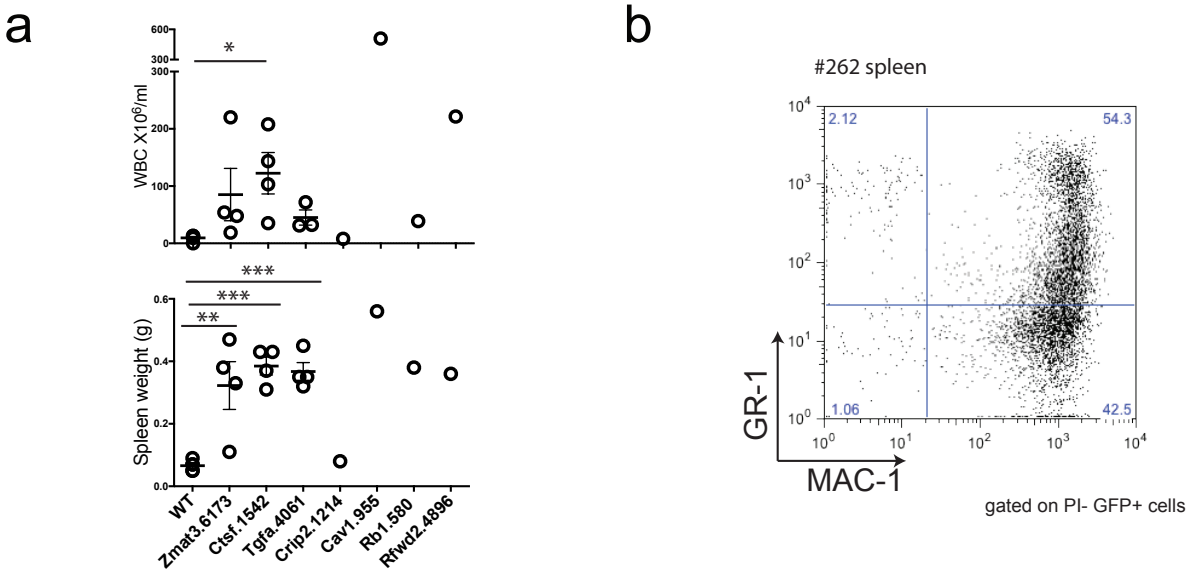
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Supplementary Figure 1



Supplementary Figure 1 Knockdown of p53 causes tumor development on a wt and a *Puma*^{-/-}*p21*^{-/-} background. Kaplan-Meier curve showing tumor-free survival of C57BL/6-Ly5.1 (wt) mice reconstituted with HSPCs of the indicated genotypes that had been transduced with the indicated shRNAs. Mice reconstituted with *p53.1124* shRNA transduced *Puma*^{-/-}*p21*^{-/-} HSPCs: median survival 168 days post-transplantation; mice reconstituted with *p53.1124* shRNA transduced wt HSPCs: median survival 199 days post-transplantation. There was no significant difference in tumor-free survival between these two cohorts. N represents the total number of reconstituted mice within a cohort. Note that the survival curves for mice reconstituted with wt and *Puma*^{-/-}*p21*^{-/-} HSPCs that had been transduced with a control shRNA vector (negative control) or with a *p53* shRNA (positive control) constitute the aggregate from several independent experiments and they are also shown as controls in Fig. 1b, 2a, 3a, and Supplementary Fig. 3a, b, c.

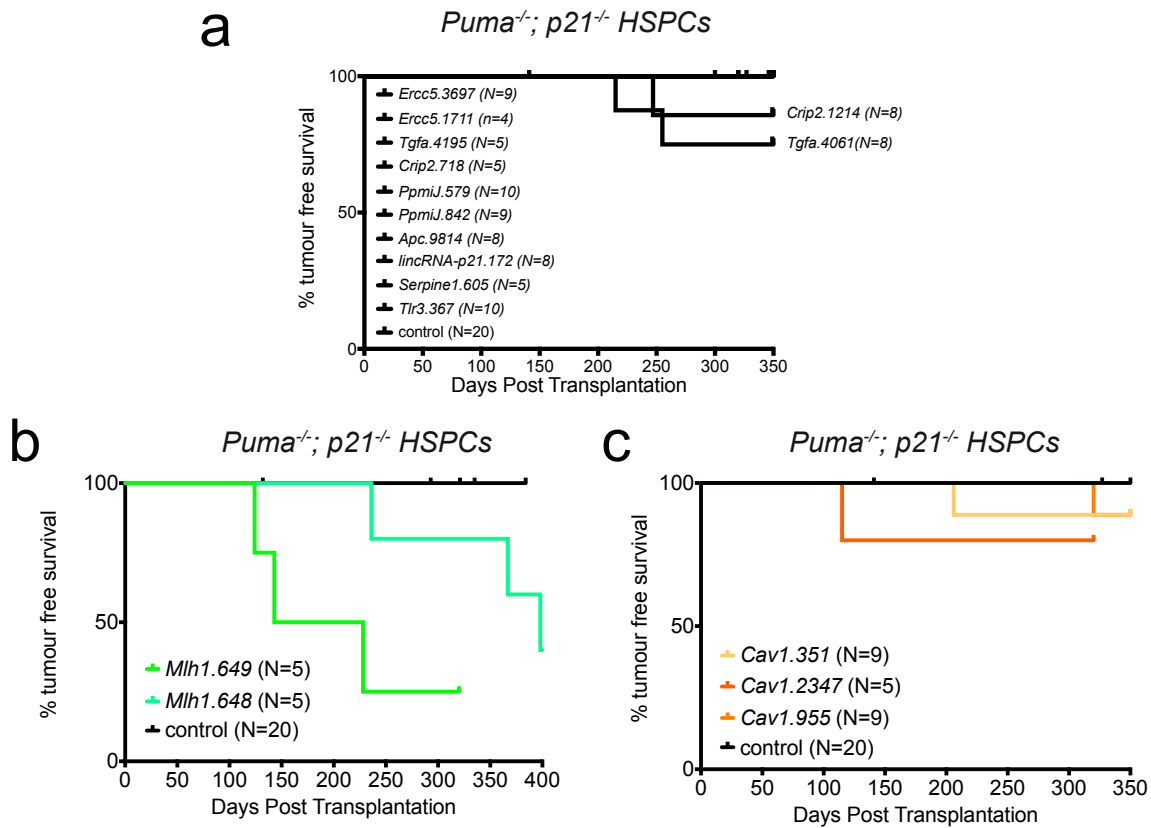
Supplementary Figure 2



Supplementary Figure 2 Characterisation of myeloid leukaemias arising in mice reconstituted with *Puma*^{-/-};*p21*^{-/-} HSPCs that had been transduced with different shRNAs

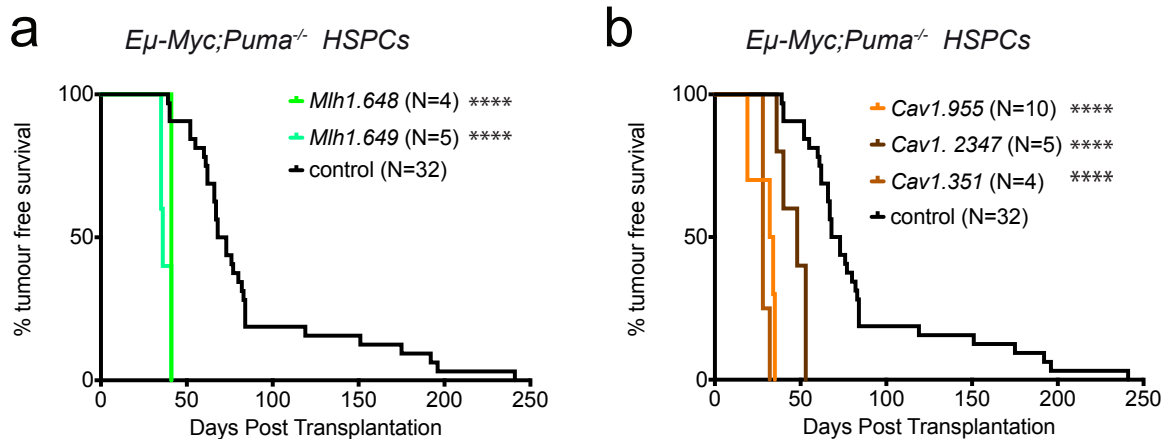
Numbers of leukocytes (WBC) in the blood and spleen weights of 1 year-old healthy control animals (N=4) and tumor-burdened C57BL/6-Ly5.1 (wt) recipient mice that had been reconstituted with *Puma*^{-/-};*p21*^{-/-} HSPCs that had been transduced with the indicated shRNAs, *Zmat3.6173* (N=4), *Ctsf.1542* (N=4), *Tgfa.4061* (N=3), *Crip2.1214* (N=1), *Cav1.955* (N=1), *Rb1.580* (N=1), *Rfwd2.4896* (N=1). Each circle represents a single reconstituted mouse. Data represent mean $\pm$ SEM. One-way ANOVA followed by Dunnett's multiple comparisons test. 95% confidence interval of difference WT vs *Ctsf.1542* is -219.4 to -6.705 in number of WBCs. 95% confidence interval of difference WT vs *Ctsf.1542* is -0.468 to -0.17, WT vs *Zmat3.6173* is -0.4055 to -0.1075, WT vs *Tgfa.4061* is -0.4505 to -0.1525 of spleen weights. **b** Flow cytometric analysis of a MAC-1⁺GR-1⁺ myeloid leukaemia found in a sick recipient mouse that had been reconstituted with *Puma*^{-/-};*p21*^{-/-} HSPCs that had been transduced with the *Cav1.955* shRNA. The flow cytometry profile shown was gated on live (PI⁻), donor derived (GFP⁺) cells. This is a representative example of 18 sick recipient mice that had been reconstituted with *Puma*^{-/-};*p21*^{-/-} HSPCs that had been transduced with different shRNA and developed myeloid leukemia.

Supplementary Figure 3



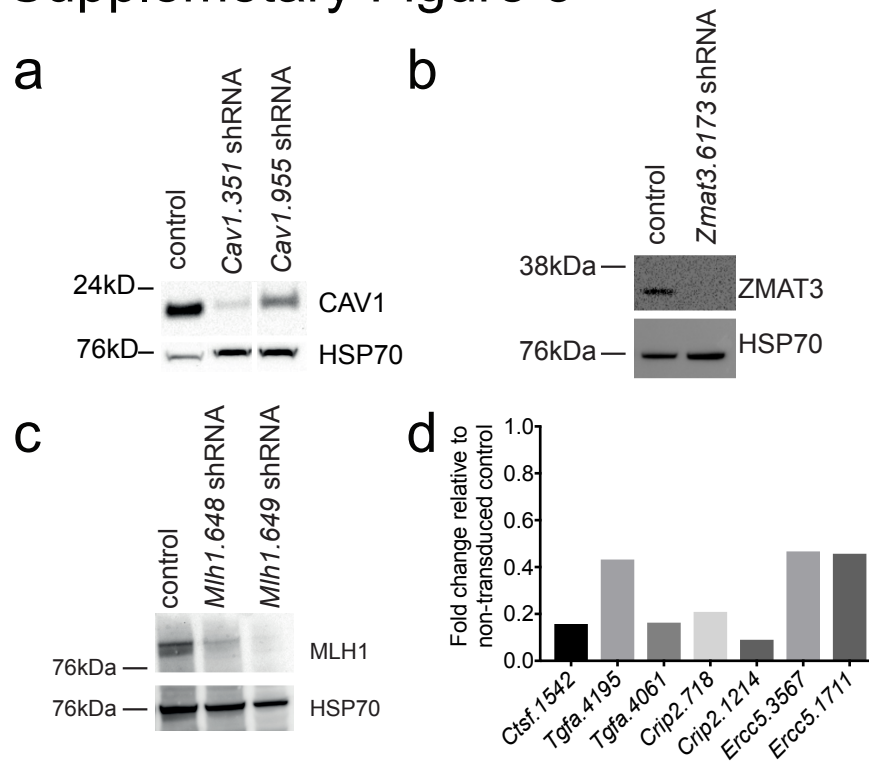
Supplementary Figure 3 Validation of candidate tumor suppressors using transduction of *Puma*^{-/-}; *p21*^{-/-} HSPCs with individual shRNAs. (a), (b) and (c) Kaplan-Meier curve showing tumor-free survival of C57BL/6-Ly5.1 (wt) mice reconstituted with *Puma*^{-/-}; *p21*^{-/-} HSPCs that had been transduced individually with the indicated shRNAs. Experiments with different shRNAs are presented in different panels for ease of viewing. N represents total number of reconstituted mice within a cohort. Note that the survival curves for mice reconstituted with *Puma*^{-/-}; *p21*^{-/-} HSPCs that had been transduced with a control shRNA vector (negative control) constitute the aggregate from several independent experiments and they are also shown as controls in Fig. 1b, 2a, and Supplementary Fig. 1.

Supplementary Figure 4



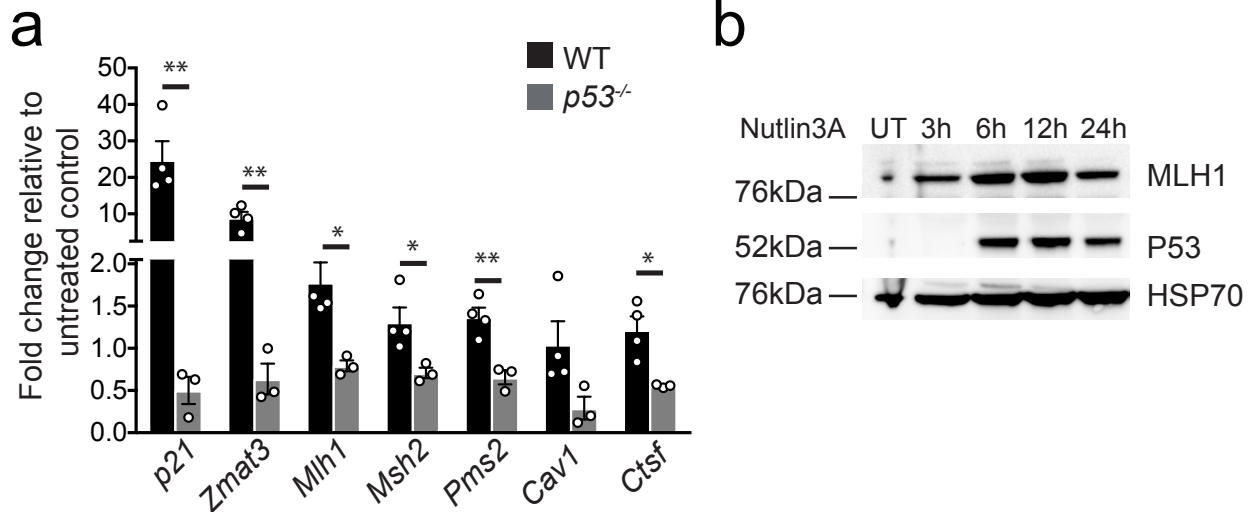
Supplementary Figure 4 Independent shRNAs targeting *Mlh1* or *Cav1* can accelerate c-MYC-driven lymphoma development even in the absence of PUMA. **a,b** Kaplan-Meier curve showing tumor-free survival of C57BL/6-Ly5.1 (wt) mice reconstituted with *Eμ-Myc;Puma*^{-/-} HSPCs that had been transduced individually with the indicated shRNAs. N represents total number of reconstituted mice within a cohort. **a** Median survival for control = 71 days post-transplantation, for *Mlh1.648* = 41 and *Mlh1.649* = 36 days post-transplantation **b** Median survival for control = 71 days post-transplantation, for *Cav1.955* = 33, *Cav1.2347* = 48 and *Cav1.351* = 28 days post-transplantation. Compared to control, ****p<0.0001. Log-rank (Mantel-Cox) test was used for comparison of mouse survival curves. Note that the survival curves for mice reconstituted with *Eμ-Myc;Puma*^{-/-} HSPCs that had been transduced with a control shRNA vector (negative control) constitute the aggregate from several independent experiments and they are also shown as controls in Fig. 1e and 2b.

Supplementary Figure 5



Supplementary Figure 5 Demonstration of the efficacy of the shRNAs targeting the different p53 target genes. **a** Western blot analysis of extracts from spleen cells from a pre-leukaemic C57BL/6-Ly5.1 (wt) mouse reconstituted with *Eμ-Myc;Puma*^{-/-} HSPCs that had been transduced with the *Cav1.351* or *Cav1.955* shRNAs and extracts from pre-leukaemic cells of control animals reconstituted with non-transduced *Eμ-Myc;Puma*^{-/-} HSPCs (control) probed for CAV1 protein or HSP70 (loading control). All extracts were loaded onto the same gel. **b** Western blot analysis of extracts from an *Eμ-Myc* lymphoma derived cell line that had been transduced with the *Zmat3.6173* shRNA or empty vector (negative control) probed for ZMAT3 or HSP70 (loading control). **c** Western blot analysis of extracts from primary (low passage) mouse embryonic fibroblasts (MEFs) that had been transduced with the *Mlh1.648* or *Mlh1.649* shRNA vectors or empty vector (negative control) probed for MLH1 protein or HSP70 (loading control). **d** QRT-PCR analysis of mRNA from *E1A+H-Ras* transformed wt MEFs that had been transduced with vectors encoding the indicated shRNAs. The mRNA levels were standardised to the control gene *Hmbs*. Data are expressed relative to non - transduced *E1A+H-Ras* transformed wt MEFs. The experiments shown in Supplementary Figures 5a-d have been performed once.

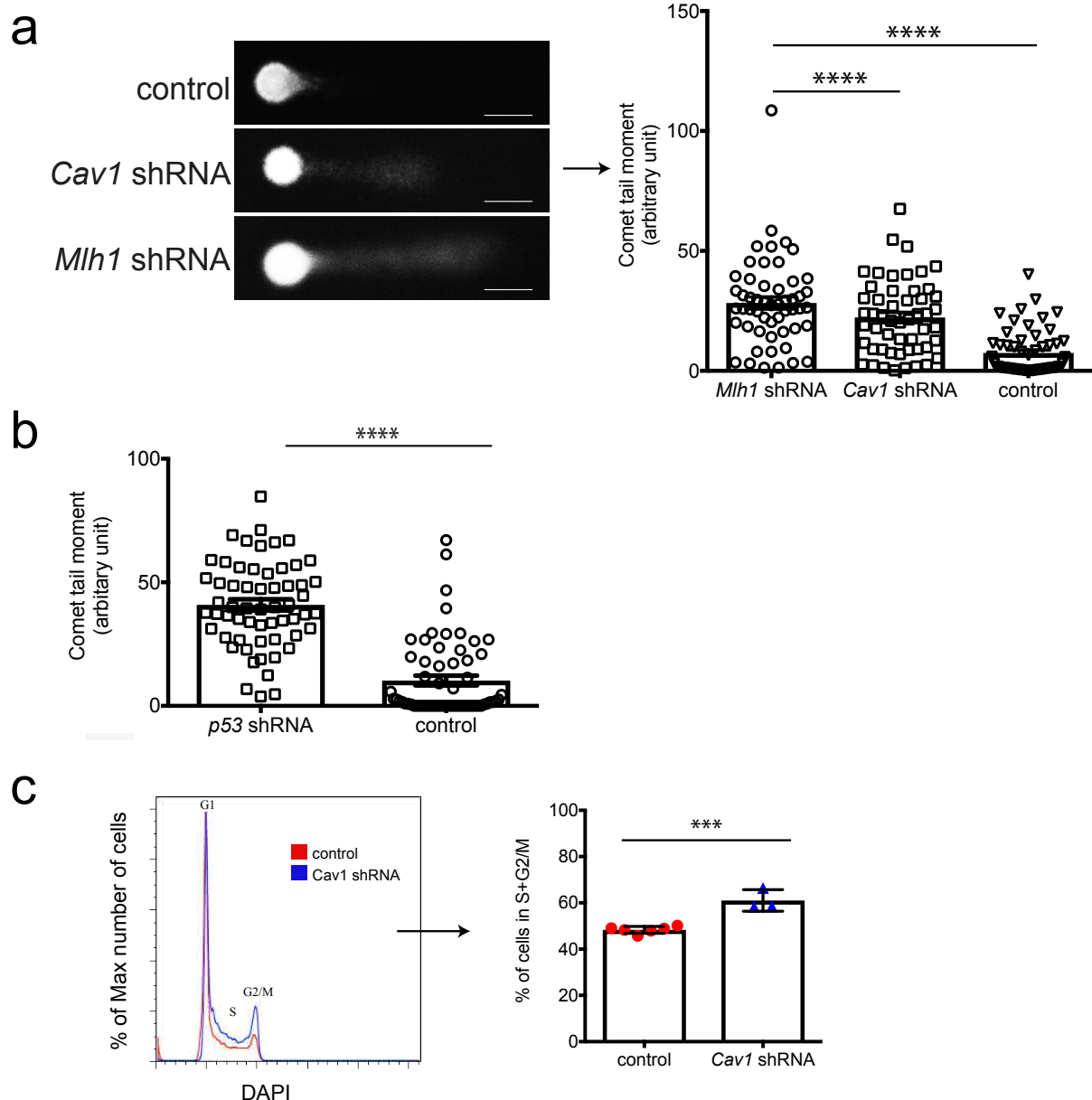
Supplementary Figure 6



Supplementary Figure 6 p53-dependent induction of the validated tumor suppressor genes.

a Thymocytes from wt and *p53*^{-/-} mice were either left untreated or exposed to 5 Gy γ -irradiation and harvested after 6 h in culture. The mRNA levels of the genes indicated were determined by qRT-PCR and standardised to the control gene *Hmbs*. Data are expressed relative to untreated thymocytes of the same genotype (cultured for 6 h). Data represent mean $\pm$ SEM, and for *p21* ***p*=0.0098, for *Zmat3* ***p*=0.0071, for *Mlh1* **p*=0.0183, for *Msh2* **p*=0.0353, for *Pms2* **p*=0.0049, for *Ctsf* **p*=0.0169, is calculated by two-tailed t-test. Each circle represents a single mouse, N= 3 for *p53*^{-/-} mice and N=4 for wt mice. **b** Western blot analysis of extracts from wt thymocytes that had been left untreated (UT) or had been treated with 10 μ M Nutlin-3a and harvested after 3, 6, 12 or 24 h, probed for MLH1, p53 and HSP70 (loading control). This experiment has been performed once.

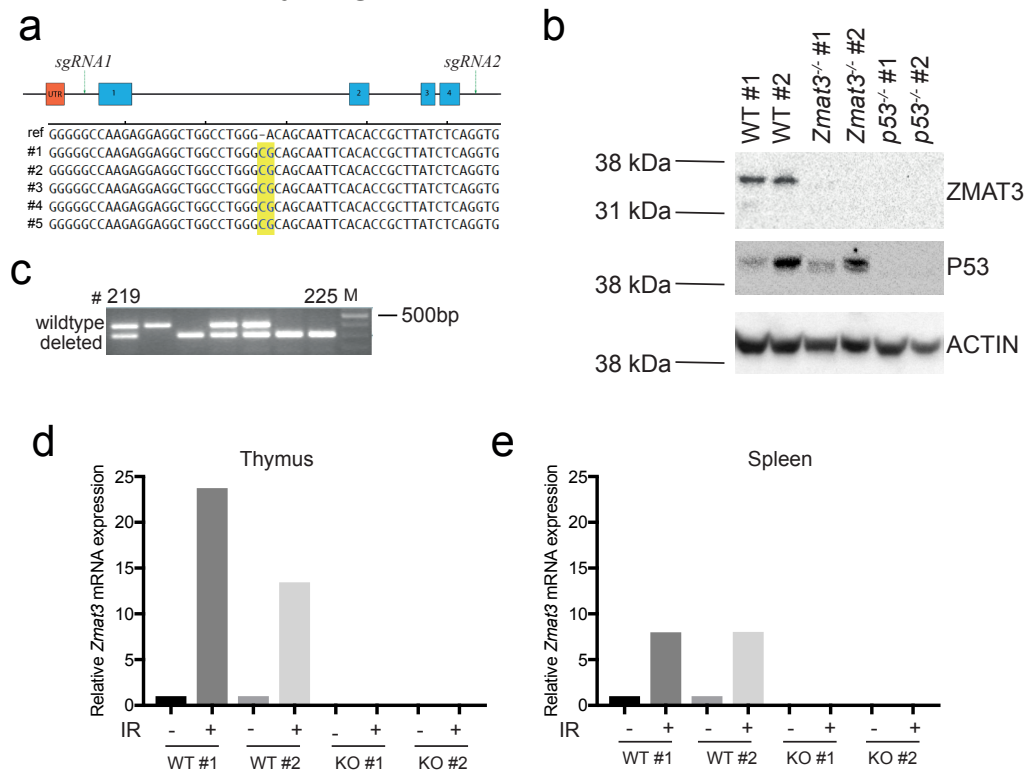
Supplementary Figure 7



Supplementary Figure 7 Knockdown of MLH1 or CAV1 increases DNA damage in pre-leukaemic cells and knockdown of CAV1 also increases cell cycling. **a** DNA damage in pre-leukaemic *Eμ-Myc;Puma^{-/-}* bone marrow derived B lymphoid cells was assessed by the comet assay; (left) representative comet images and (right) quantification of comet tail moment (indicator of level of DNA damage). For each sample, a total of ~50 nuclei in two fields were inspected and scored. Data represent mean $\pm$ SEM of N=3 C57BL/6-Ly5.1 recipient mice reconstituted with *Eμ*-

Myc;Puma^{-/-} HSPCs that had been transduced with vectors encoding the following shRNAs: *Mlh1.649* shRNA, *Cav1.351* or empty vector control. Each circle represents a single cell inspected, ****p<0.0001 (two-tailed *t*-test). **b** DNA damage in pre-leukaemic *Eμ-Myc;Puma*^{-/-} bone marrow derived B lymphoid cells (B220⁺GFP⁺) was assessed by using the comet assay. For each sample, a total of ~50 nuclei in two fields were inspected and scored. Data represent mean ±SEM of N=3 recipient mice reconstituted with *Eμ-Myc;Puma*^{-/-} HSPCs that had been transduced with the *p53.1124* shRNA vector or an empty control vector. Each circle represents a single cell inspected, ****p<0.0001 (two-tailed *t*-test). Scale bar = 50 μm. **c** Representative cell cycle distribution histogram (DAPI staining) of pre-leukaemic bone marrow derived B lymphoid cells (B220⁺GFP⁺). Percentages of cells within the given cell cycle stages were calculated by using FlowJo software. Data represent mean ±SEM from N=3 for *Cav1.351* shRNA and N= 6 for empty vector (control) C57BL/6-Ly5.1 recipient mice that had been reconstituted with *Eμ-Myc;Puma*^{-/-} HSPCs. Each circle represents a single reconstituted mouse inspected, *** p=0.0004 (two-tailed *t*-test).

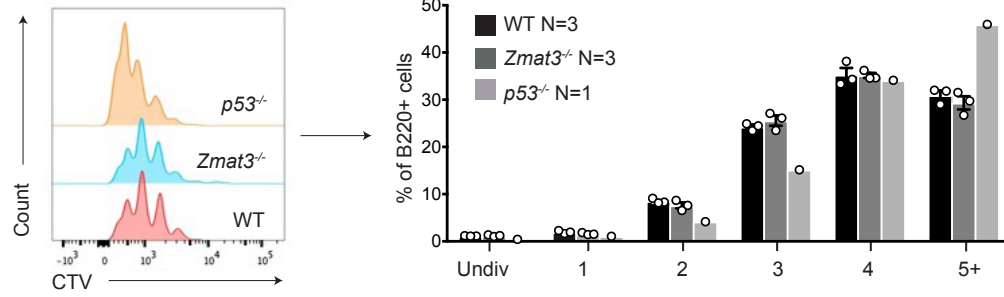
Supplementary Figure 8



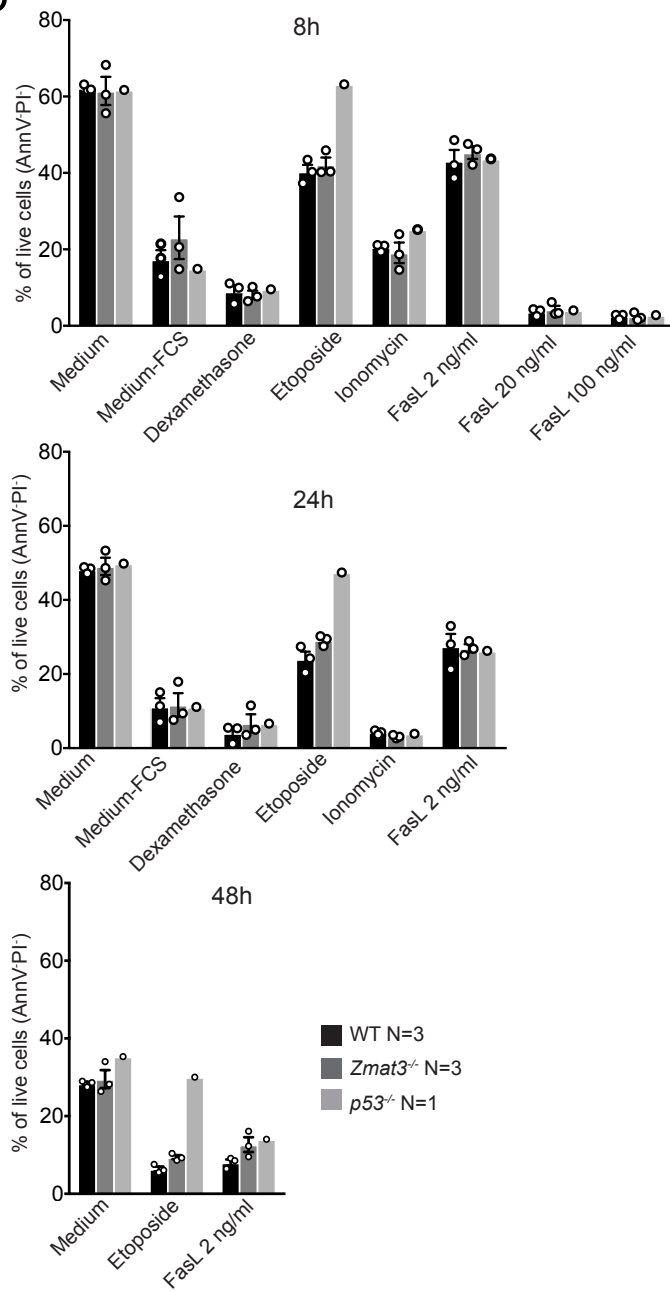
Supplementary Figure 8 Generation of ZMAT3-deficient mice using CRISPR/CAS9 technology. **a** Targeting strategy for the *Zmat3* deletion mouse strain by CRISPR/CAS9. Next Generation DNA Sequencing data of five F1 *Zmat3* gene targeted mice (#1, #2, #3, #4 & #5) from one founder were aligned to a predicted deleted allele sequence (ref). Highlighted (yellow) bases indicate InDels on top of the predicted deleted allele. **b** The absence of ZMAT3 protein but presence of p53 in the *Zmat3* mutant mice were confirmed by Western blot analysis of extracts from low passaged MEFs that had been exposed to 10 Gy γ -irradiation and harvested after a further 6 h in culture. MEFs from wt and *p53*^{-/-} mice were used as controls. Probing for actin was used as a loading control. This experiment was performed once. **c** All mice were genotyped before examination. A successful targeted deletion of *Zmat3* yielded a 308-bp PCR product, whereas the wt allele produced a 416-bp band when subjected to gel electrophoresis. This experiment was performed once. **d** and **e** Thymocytes (**d**) and spleen cells (**e**) from wt and *Zmat3* mutant mice were either left untreated or exposed to 5 Gy γ -irradiation and harvested after 6 h in culture. The *Zmat3* mRNA levels were determined by qRT-PCR and standardised to the control gene *Hmbs*. N= 2 mice/genotype.

Supplementary Figure 9

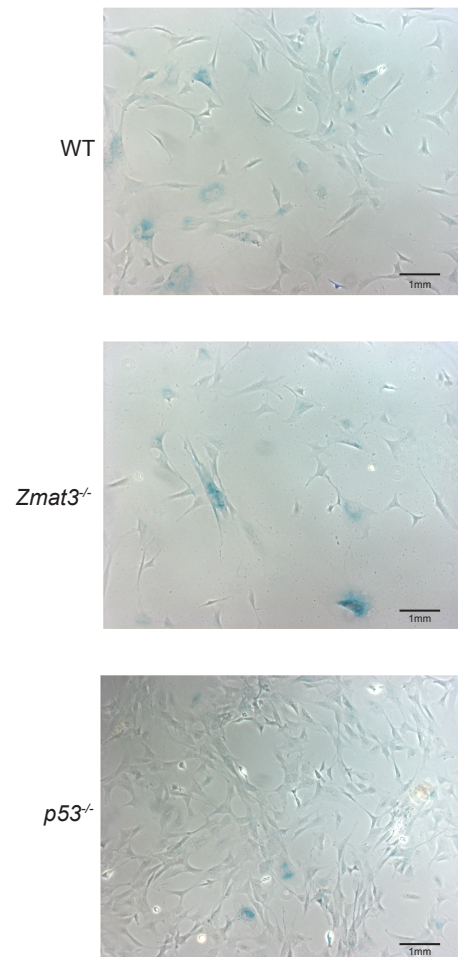
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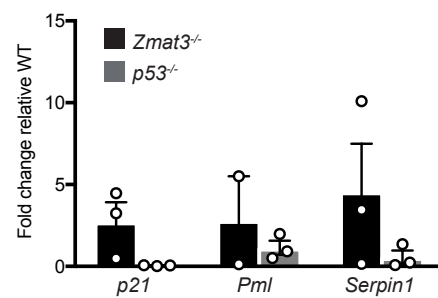
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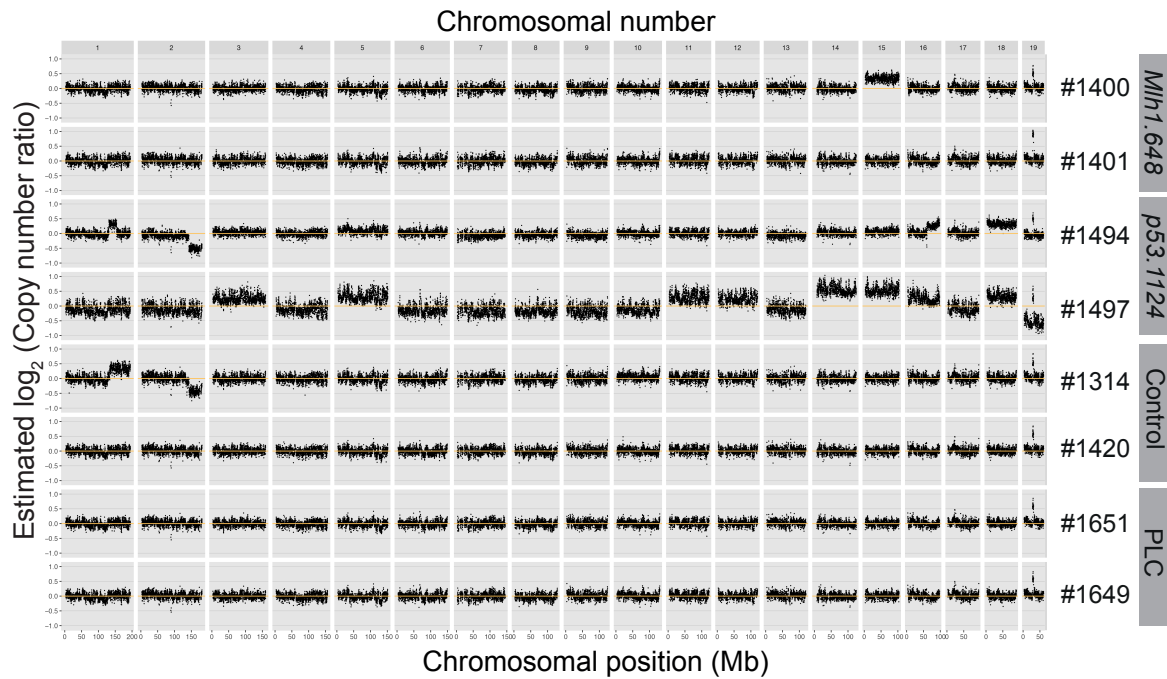


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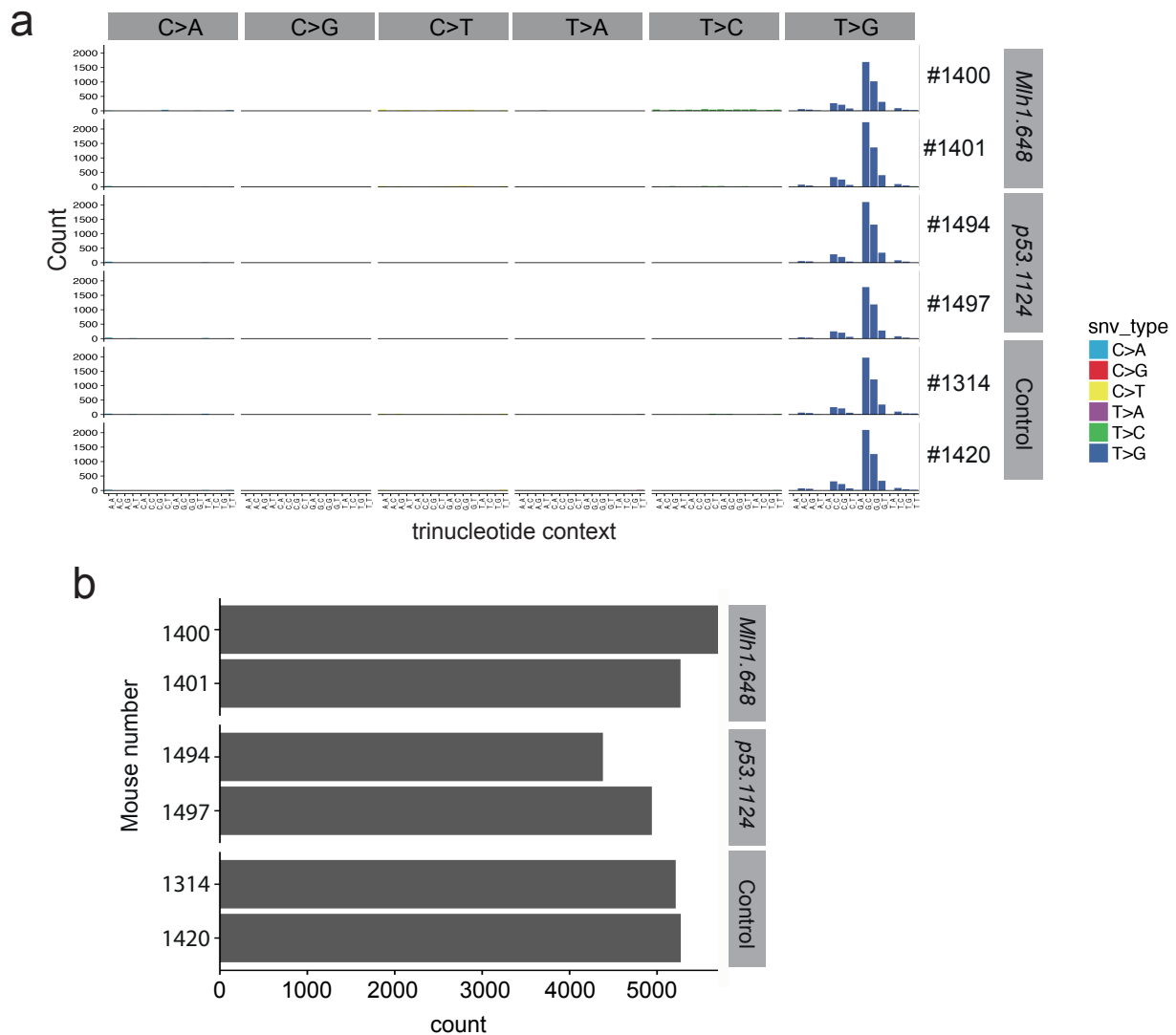
Supplementary Figure 9 Loss of *Zmat3* does not affect cell proliferation, cell death or cellular senescence. **a** Flow cytometric analysis of the dilution of CTV in B cells from *Zmat3* mutant, wt and *p53*^{-/-} mice stimulated for 96 h with anti-CD40 antibodies plus anti-IgM Fab₂ antibody fragments. Data represent mean ±SEM of the percentage of cells that have undergone multiple divisions. N represents number of mice. **b** Thymocytes from *Zmat3* mutant, wt and *p53*^{-/-} mice were exposed to a range of cell death stimuli *in vitro* (growth factor deprivation = medium without addition of cytokines and/or without addition of FCS, dexamethasone (1 μM), etoposide (1 μM), ionomycin (500 ng/mL) and Fc-FasL (2, 20 or 100 ng/mL). Cell survival was analysed by flow cytometry at 8, 24 and 48 h, using staining with PI plus FITC conjugated Annexin-V (Life Technologies). PI⁺ Annexin-V⁻ cells were regarded as viable thymocytes. Data represent mean ±SEM. N represents number of mice. **c** Phase contrast images of SA-β-galactosidase staining and morphology of MEFs of the indicated genotypes during the final time points of the senescence assays (passage 6-7). This experiment has been performed once using N=3 wt, N=3 *p53*^{-/-} and N=2 *Zmat3*^{-/-} MEFs that were generated from E13.5 embryos. Scale bar = 1 mm. **d** qRT-PCR analysis of the expression of the senescence-related p53 target genes *p21*, *Serpin-1* and *Pml* in MEFs of the indicated genotypes during their final time points (passage 6-7) of the senescence assays. Data are presented as averages ± SEM of 3 samples/genotype.

Supplementary Figure 10



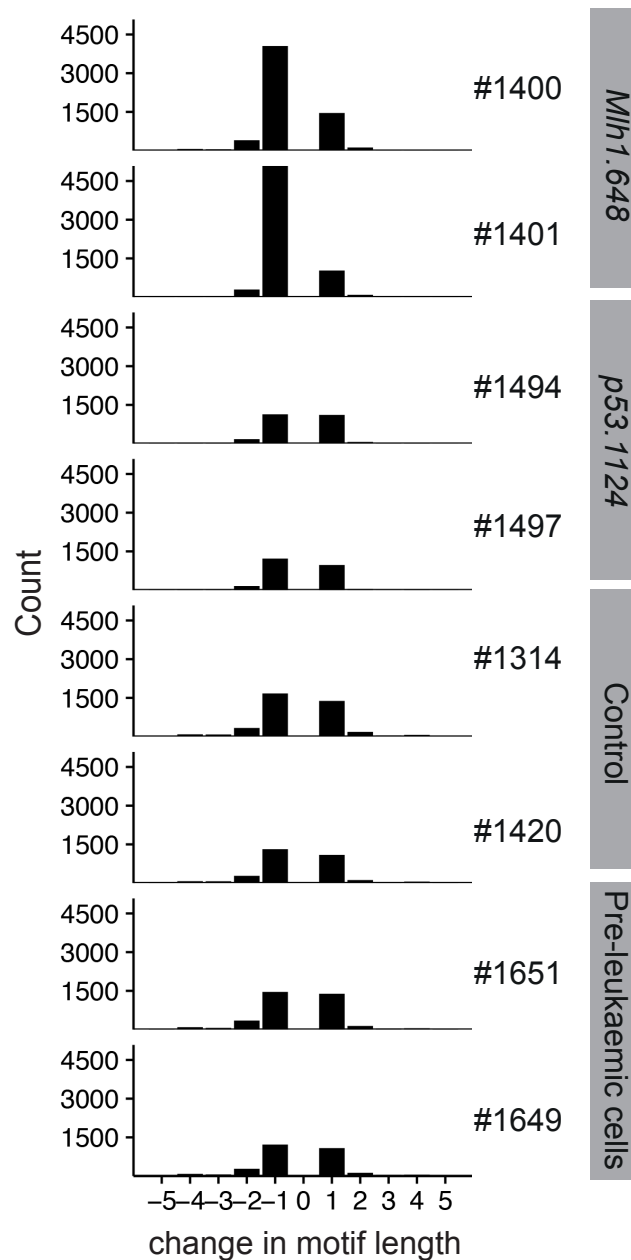
Supplementary Figure 10 Increased copy number variations (CNVs) in *Eμ-Myc* lymphomas accelerated by downregulation of *p53*. CNVs in *Eμ-Myc* lymphomas accelerated by shRNAs against *p53* or shRNAs against *Mlh1* and in *Eμ-Myc* control lymphomas and in pre-leukemic *Eμ-Myc* B lymphoid cells (PLC) (same samples as in Fig. 3c,d).

Supplementary Figure 11



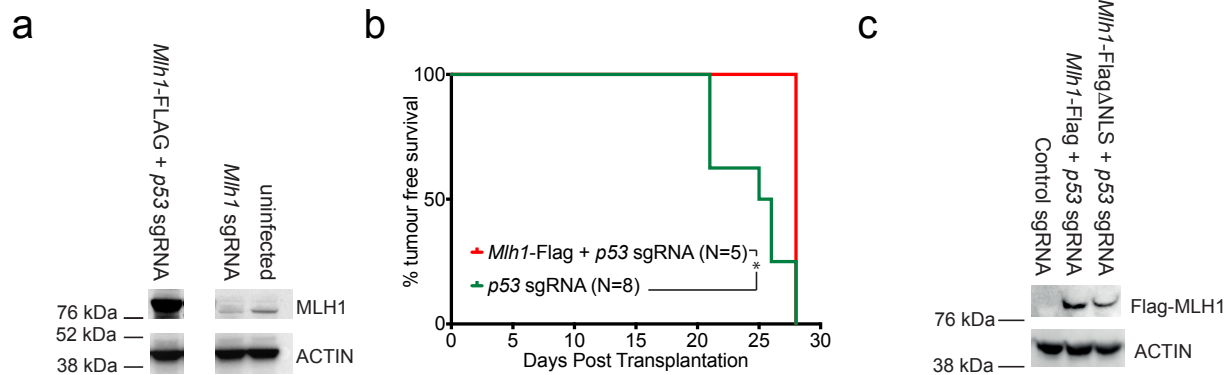
Supplementary Figure 11 Single nucleotide variant (SNVs) analysis of lymphomas. a Counts of unique single-nucleotide substitutions per sample in *Eμ-Myc* lymphomas accelerated by shRNAs against *p53* or shRNAs against *Mlh1* and in *Eμ-Myc* control lymphomas and in pre-leukemic *Eμ-Myc* B lymphoid cells (PLC) (same samples as in Fig. 3c,d), arranged by trinucleotide context. **b** Overall substitution counts by sample.

Supplementary Figure 12



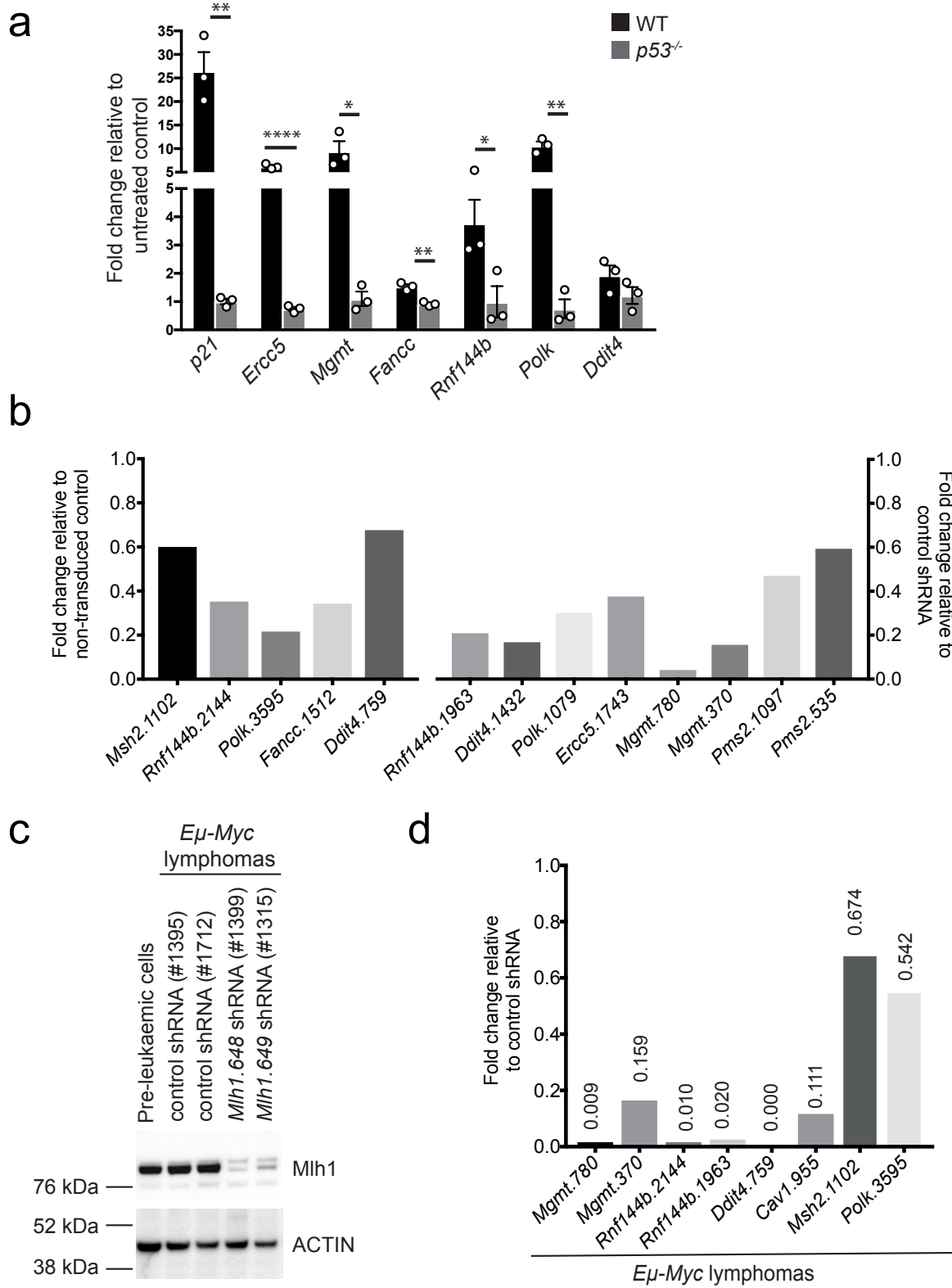
Supplementary Figure 12 Defects in MLH1-mediated DNA repair contribute to microsatellite instability. Changes in the lengths of short tandem repeat motifs across the *Eμ-Myc* lymphomas accelerated by shRNAs against *p53* or shRNAs against *Mlh1* and in *Eμ-Myc* control lymphomas and in pre-leukaemic *Eμ-Myc* B lymphoid cells (PLC) (same samples as in Fig. 3c,d). Changes more than 5nt in length have been excluded.

Supplementary Figure 13



Supplementary Figure 13 Impact of restored MLH1 expression on the acceleration of *Eμ-Myc* lymphoma development driven by loss of p53. **a** Western blot analysis of extracts from *Cas9* transgenic primary (low passage) mouse embryonic fibroblasts (MEFs) that had not been transduced or transduced with the *Mlh1* sgRNA or the *Mlh1*-FLAG expression plus the *p53* sgRNA. The blots were probed for MLH1 or Actin (loading control). All extracts were loaded onto the same gel. This experiment has been performed once. **b** Kaplan-Meier curves showing tumor-free survival of mice reconstituted with *Eμ-Myc;Cas9* HSPCs that had been transduced with the indicated vectors (see above). Median survival for *p53* sgRNA = 26 days, for *Mlh1*-FLAG + *p53* sgRNA = 28 days (compared to *p53* sgRNA, **p*=0.0143). Log-rank (Mantel-Cox) test was used for comparison of mouse survival curves. **c** Western blot analysis of extracts from *E1A+H-Ras* transformed wt MEFs that had not been manipulated or transduced with vectors encoding *Mlh1*-FLAGΔNLS plus a *p53* sgRNA or *Mlh1*-FLAG plus a *p53* sgRNA, probed for MLH1 or Actin (loading control). This experiment has been performed once.

Supplementary Figure 14

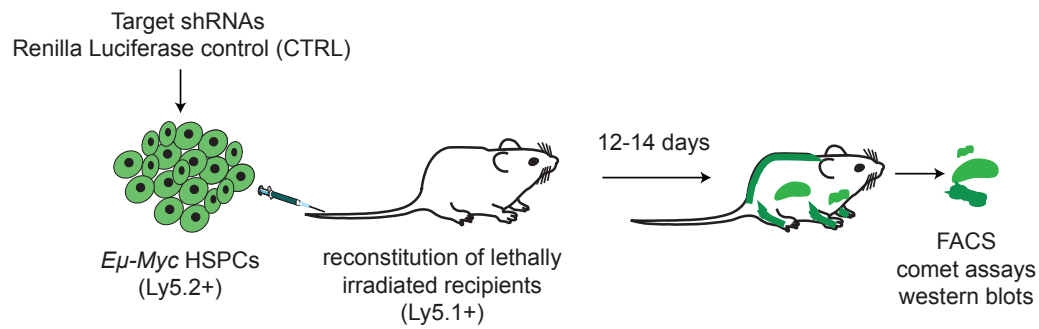


Supplementary Figure 14 Analysis of p53-regulated genes implicated in DNA repair a

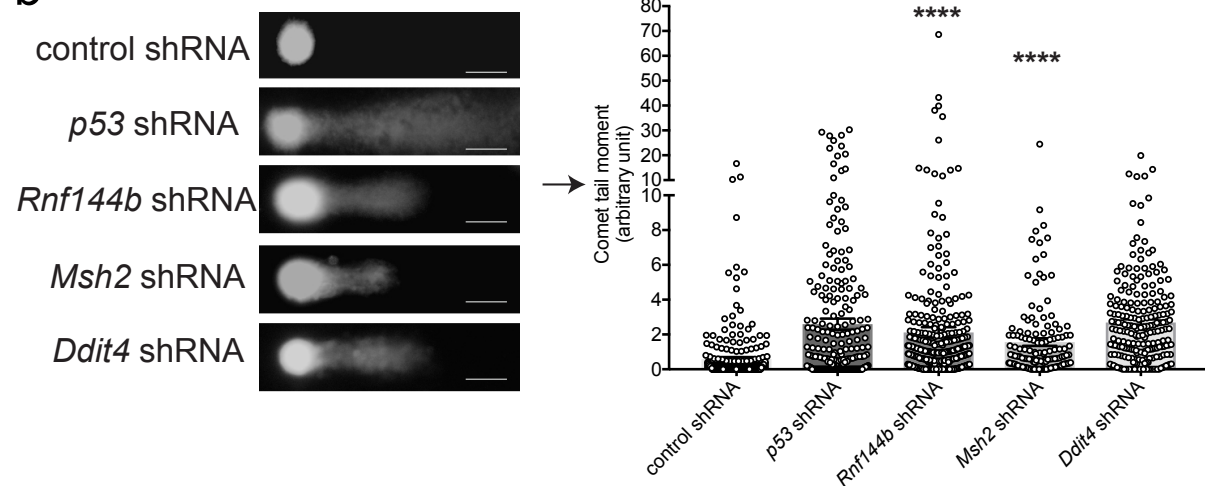
Thymocytes from wt or *p53*^{-/-} mice were either left untreated or exposed to 5 Gy γ -irradiation and harvested after 6 h in culture. The mRNA levels of the genes indicated were determined by qRT-PCR analysis with the mRNA levels standardised to the control gene *Hmbs*. Data are expressed relative to untreated thymocytes of the same genotype (cultured for 6 h). N= 3 mice/genotype and data represent mean $\pm$ SEM, and for *p21* **p=0.0032, for *Mgmt* *p=0.0171, for *Rnfl44b* *p=0.0498, for *Polk* **p=0.0013, for *Ercc5* ****p<0.0001, for *Fancc* **p=0.002, calculated by two-tailed t-test. **b** QRT-PCR analysis of mRNA from GFP⁺PI⁻ FACS sorted *E1A+H-Ras* transformed wt MEFs that had been transduced with the indicated shRNAs. The mRNA levels were standardised to the control gene *Hmbs*. Data are expressed relative to non-transduced *E1A+H-Ras* transformed wt MEFs or *E1A+H-Ras* transformed wt MEFs transduced with a control shRNA targeting Renilla luciferase. **c** Western blot analysis of extracts from FACS sorted B220⁺PI⁻ pre-leukaemic *E μ -Myc* B lymphoid cells (lane 1) and FACS sorted GFP⁺PI⁻ lymphoma cells that arose in mice that had been reconstituted with *E μ -Myc* HSPCs transduced with the *Mlh1.648* or *Mlh1.649* shRNA vectors (lanes 4 and 5) and extracts from FACS sorted GFP⁺PI⁻ control lymphoma cells that arose in animals that had been reconstituted with *E μ -Myc* HSPCs transduced with a vector encoding an shRNA targeting Renilla Luciferase (lanes 2 and 3), probed for MLH1 protein or Actin (loading control). This experiment has been performed once. **d** QRT-PCR analysis of mRNA from FACS sorted GFP⁺PI⁻ lymphoma cells that arose in mice that had been reconstituted with *E μ -Myc* HSPCs transduced with vectors encoding the indicated shRNAs. The mRNA levels were standardised to the control gene *Hmbs*. Data are expressed relative to mRNA levels in FACS sorted control GFP⁺PI⁻ lymphoma cells that arose in animals that had been reconstituted with *E μ -Myc* HSPCs transduced with an shRNA targeting Renilla luciferase.

Supplementary Figure 15

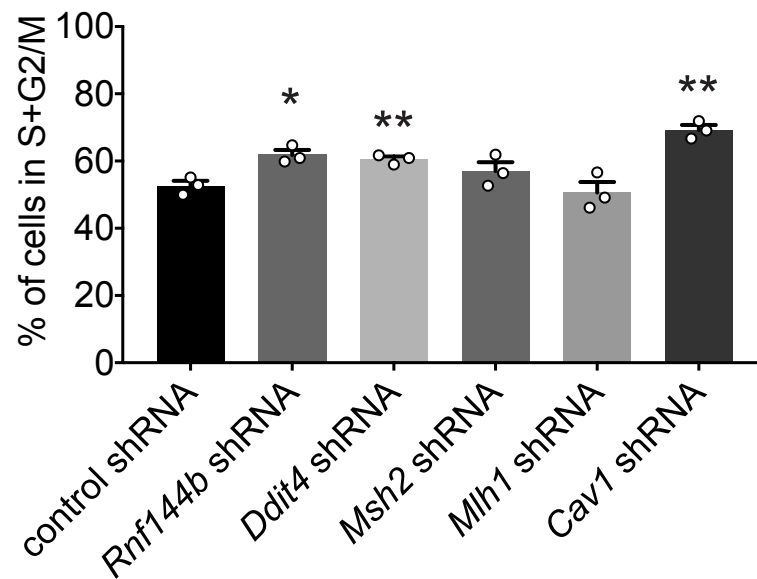
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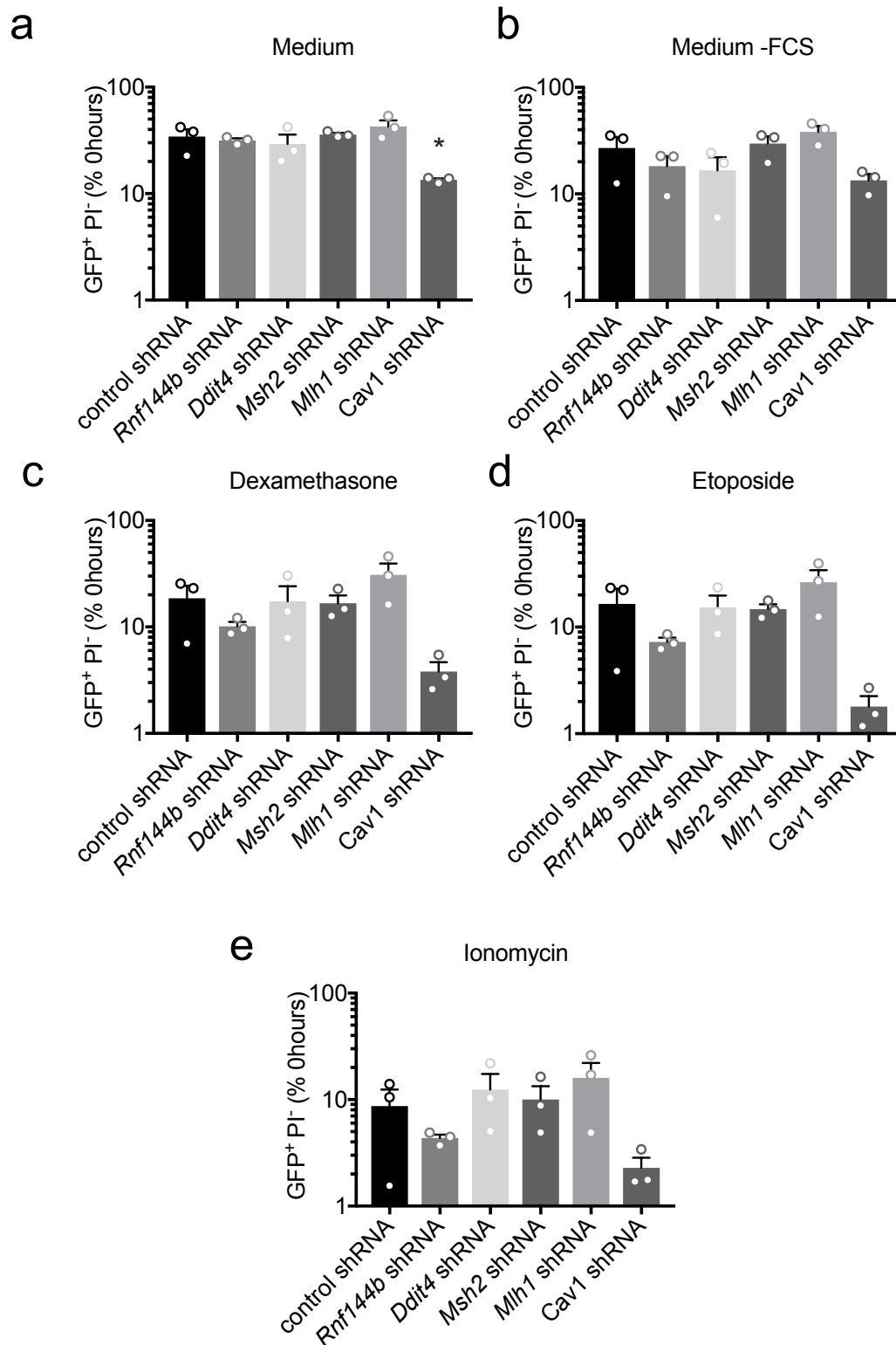
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Supplementary Figure 15 Functional analysis of p53-regulated genes implicated in DNA repair

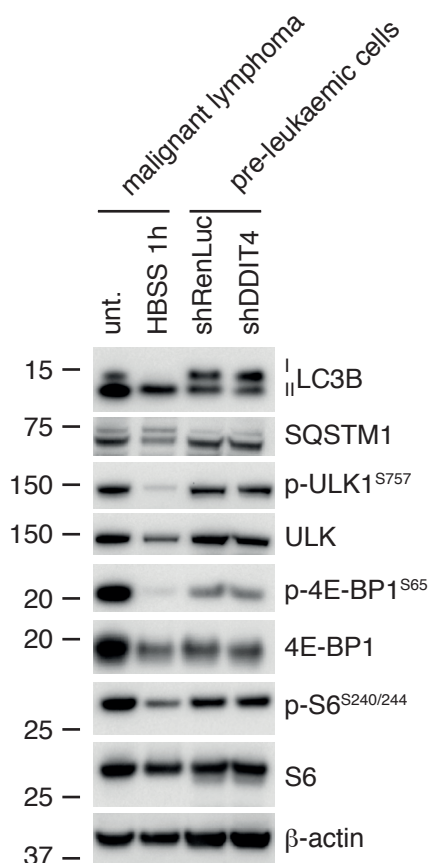
a Experimental strategy of the pre-leukemic analysis. **b** DNA damage in pre-leukemic bone marrow derived *Eμ-Myc* B lymphoid cells was assessed by the comet assay; (left) representative comet images and (right) quantification of comet tail moment. For total of (control shRNA N=257, *Ddit4* shRNA N=222, *Rnf114b* shRNA N=377, *Msh2* shRNA N=150, *p53* shRNA N=260) nuclei in two fields were inspected and scored in a blinded manner. Data represent mean $\pm$ SEM recipient mice reconstituted with *Eμ-Myc* HSPCs that had been transduced with vectors encoding shRNAs targeting Renilla luciferase (N=3), *p53.1124* (N=1), *Rnf144b.2144* (N=3), *Msh2.1102* (N=3) or *Ddit4.759* (N=2). **** $p < 0.0001$ (two-tailed *t*-test). Scale bar = 50 μ m. **c** Cell proliferation was monitored by DAPI staining of pre-leukemic bone marrow derived B lymphoid cells followed by flow cytometric analysis. GFP⁺B220⁺ cells were regarded as donor-derived and vector transduced B cells. Percentages of cells within the given cell cycle stages were calculated by using FlowJo software. Data represent mean $\pm$ SEM from N=3 C57BL/6-Ly5.1 recipient mice reconstituted with *Eμ-Myc* HSPCs transduced with vectors encoding the indicated shRNAs, using an shRNA targeting Renilla luciferase as a control, for *Rnf114b* shRNA * $p = 0.0117$, for *Ddit4* shRNA ** $p = 0.0095$, for *Cav1* shRNA ** $p = 0.0014$ (two-tailed *t*-test).

Supplementary Figure 16



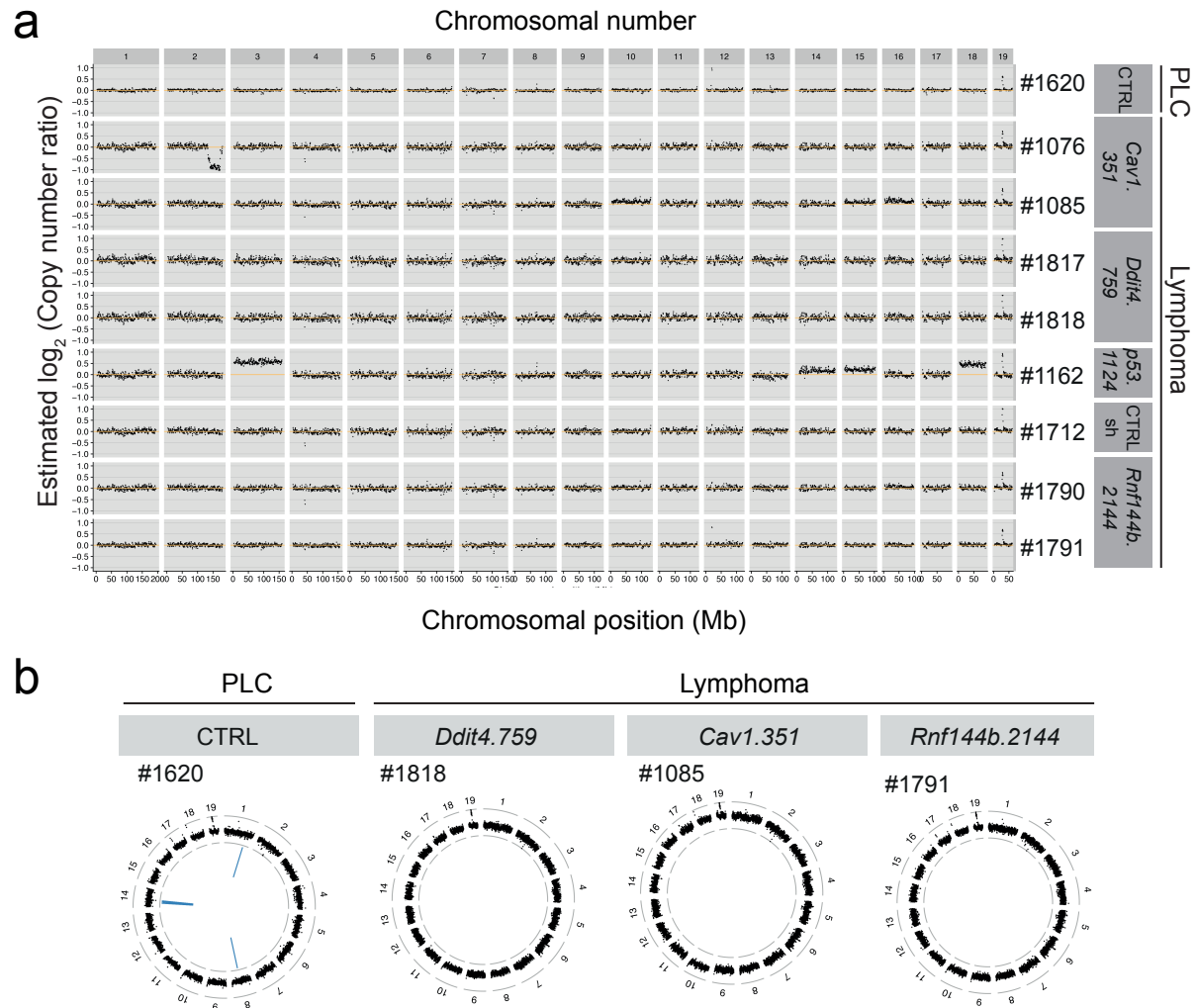
Supplementary Figure 16 Bone marrow derived pre-leukemic *Eμ-Myc* B lymphoid cells expressing shRNAs against *Rnf144b*, *Ddit4*, *Msh2*, *Mlh1* or *Cav1* exhibit normal sensitivity to a broad range of cell death stimuli. **a-e** Bone marrow derived pre-leukaemic *Eμ-Myc* B lymphoid cells transduced with vectors encoding the indicated shRNAs were exposed in tissue culture to a range of cell death stimuli, including **a** growth factor deprivation (medium without addition of cytokines), **b** growth factor deprivation (medium without addition of FCS and cytokines), **c** dexamethasone (1 μ M), **d** etoposide (1 μ M) and **e** ionomycin (500 ng/mL). Cell survival was monitored at 0 and 8 h by staining with PI followed by flow cytometric analysis. PI⁺ GFP⁺ cells were regarded as viable cells. Data represent mean $\pm$ SEM from N=3 C57BL/6-Ly5.1 recipient mice reconstituted with *Eμ-Myc* HSPCs that had been transduced with vectors encoding the indicated shRNAs using an shRNA targeting Renilla luciferase as a control. *p=0.0236, 95% confidence interval on the difference is 3.79 to 37.91, as determined by one way ANOVA followed by Dunnett's multiple comparisons test.

Supplementary Figure 17



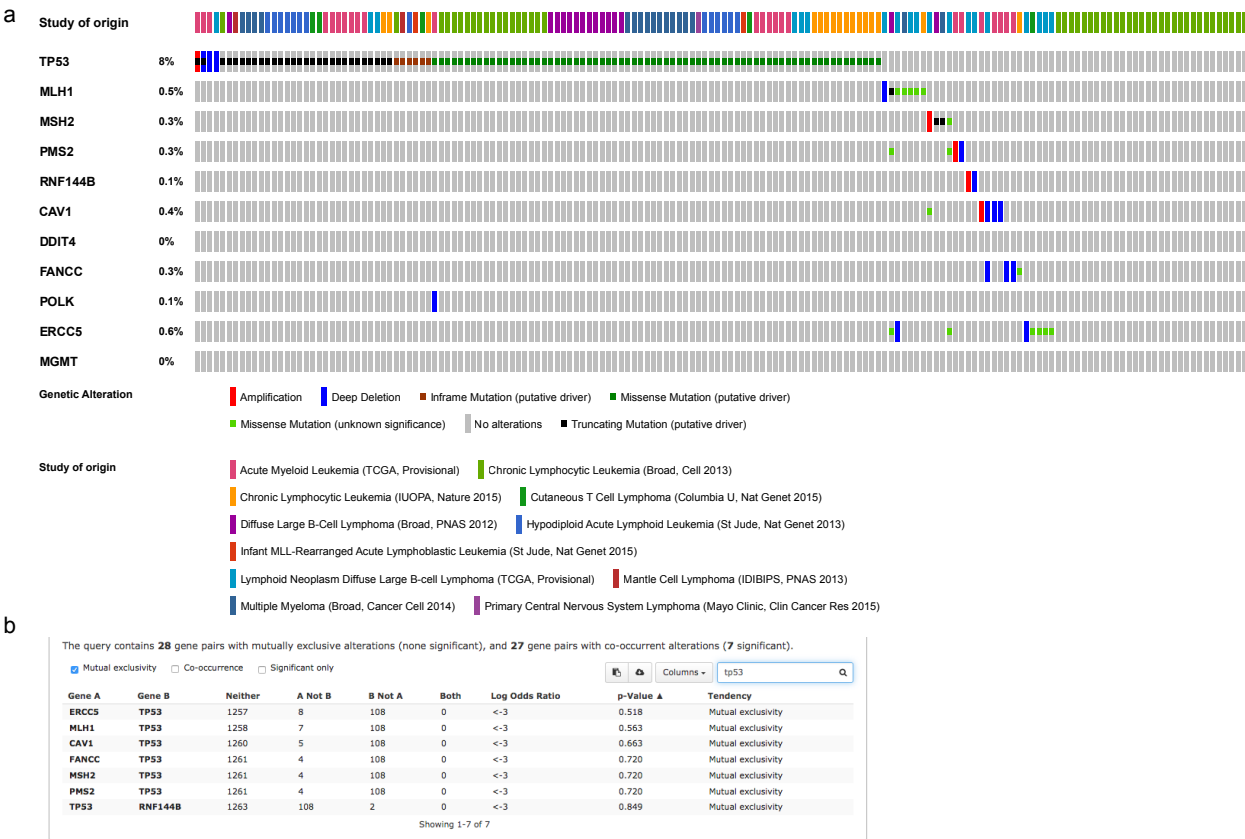
Supplementary Figure 17 Knockdown of DDIT4 does not affect mTORC1 signaling in pre-leukaemic *Eμ-Myc* B lymphoid cells. Western blot analysis of extracts from FACS sorted B220⁺GFP⁺PI⁻ pre-leukaemic B lymphoid cells from mice reconstituted with *Eμ-Myc* HSPCs that had been transduced with vectors encoding an shRNA targeting Renilla luciferase (control: lane 3) or *Ddit4* shRNA (lane 4). Three mice were pooled per genotype. Malignant *Eμ-Myc* lymphoma cells were starved in Hank's Balanced Salt Solution (HBSS) for 1 h as a positive control for mTORC1 inhibition and autophagy induction prior to harvesting (lanes 1 and 2). This experiment has been performed once.

Supplementary Figure 18



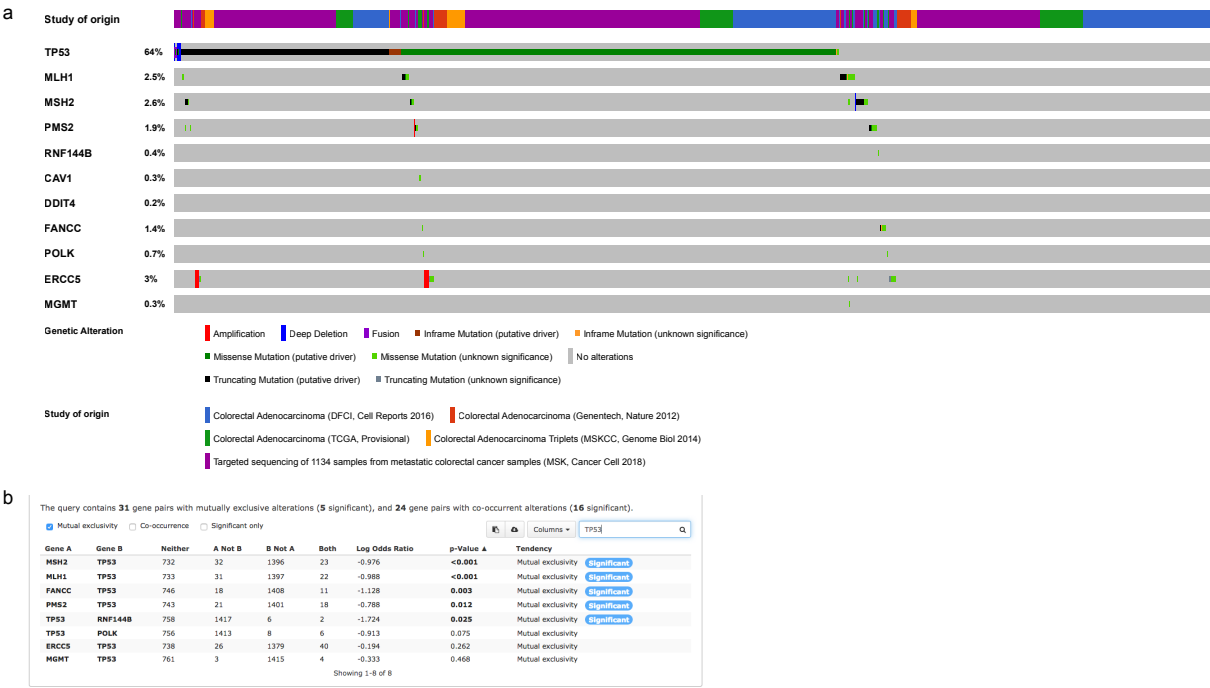
Supplementary Figure 18 Increased copy number variations (CNVs) in *Eμ-Myc* lymphomas accelerated by knockdown of *p53* compared to *Eμ-Myc* lymphomas accelerated by knockdown of *Rnf144b*, *Cav1*, *Ddit4* or *Mlh1*. **a CNVs in *Eμ-Myc* lymphomas accelerated by shRNAs against *p53* or shRNAs against *Rnf144b*, *Ddit4*, *Cav1* (same samples as in Fig. 4b) and in *Eμ-Myc* control lymphomas, and as a reference in pre-leukemic lymphoid cells (PLC) isolated from the blood of young (6 weeks-old) *Eμ-Myc* mice (sample #1620). **b** CIRCOS plots of log2 fold-change in copy number (black dots) and intra-chromosomal structural variants (blue lines) in *Eμ-Myc* lymphomas accelerated by shRNAs against *Cav1* (#1085), *Ddit4* (#1818), *Rnf144b* (#1791) and in control *Eμ-Myc* pre-leukaemic cells (PLC, sample #1620; see above).**

Supplementary Figure 19



Supplementary Figure 19 Mutual exclusivity between mutations in *p53* and mutations in *p53*-regulated DNA damage repair genes in human hematological cancers. Representative map illustrating mutations in *p53* and mutations in *p53* regulated-DNA damage repair genes. Mutual exclusivity relative to mutations in *p53* was evaluated using cBioPortal tools querying 1373 samples. P values were calculated using the Fisher exact test.

Supplementary Figure 20



Supplementary Figure 20 Mutual exclusivity between mutations in *p53* and mutations in *p53*-regulated DNA damage repair genes in human colorectal cancers. Representative map illustrating mutations in *p53* and mutations in *p53* regulated-DNA damage repair genes. Mutual exclusivity relative to mutations in *p53* was evaluated using cBioPortal tools querying 2183 samples. P values were calculated using the Fisher exact test.

Supplementary Table 1 *p53* gene targeted shRNA library. List of the 930 shRNAs used for the sensitized genetic screens.

Number of mice with enriched shRNA in tumours			
4	3	2	1
<i>Trp53</i> .848	<i>Rnf144b</i> .2037	<i>Anin</i> .5107 <i>Crip2</i> .1214 <i>Ercc5</i> .3697 <i>Ppm1j</i> .579 <i>Serpine</i> .605 <i>Tlr3</i> .367	<i>Abcb1a</i> .4209 <i>Abcb1b</i> .2171 <i>Bid</i> .658 <i>Cav1</i> .955 <i>Ctsd</i> .264 <i>Ctsf</i> .533 <i>Cx3cl1</i> .3044 <i>Dusp5</i> .412 <i>Ercc5</i> .1743 <i>Gdf15</i> .879 <i>Gml</i> .652 <i>lincRNA-p21</i> .172 <i>Lrdd</i> .2825 <i>Mad1l1</i> .867 <i>Mad1l1</i> .2591 <i>Mgmt</i> .412 <i>Mlh1</i> .204 <i>P2rx6</i> .215 <i>P2rx6</i> .1202 <i>Pcbp4</i> .945 <i>Pcna</i> .364 <i>Pcna</i> .480 <i>Phlda3</i> .969 <i>Pmaip1</i> .783 <i>Ppm1j</i> .842 <i>Prkab1</i> .1842 <i>Ptprv</i> .3260 <i>Rb1</i> .580 <i>Rb1</i> .3707 <i>Rfwd2</i> .1442 <i>Rfwd2</i> .4896 <i>Rnf144b</i> .1963 <i>Rps27l</i> .435 <i>S100a2</i> .207 <i>Scara3</i> .3049 <i>Scn3b</i> .1920 <i>Sh2d1a</i> .457 <i>Slc38a2</i> .3361 <i>Spata18</i> .1651 <i>Tap1</i> .2063 <i>Tgfa</i> .4061 <i>Trp53inp1</i> .5062 <i>Trpm2</i> .5599 <i>Ttc28</i> .2360 <i>Tyrp1</i> .2302 <i>Zmat3</i> .2279 <i>Zmat3</i> .2485 <i>Zmat3</i> .6173

Supplementary Table 2 Number of tumors with enriched shRNAs found in the sensitized genetic screens. List showing numbers of independent tumors in which a particular shRNA was found enriched in mice that were reconstituted with *Puma*^{-/-} *p21*^{-/-} HSPCs transduced with the p53

target gene directed shRNA library.

Number of independent shRNAs		
3	2	1
<i>Zmat3</i>	<i>Erc5</i>	<i>Abcb1a</i>
	<i>Mad1</i>	<i>Abcb1b</i>
	<i>P2rx6</i>	<i>Anln</i>
	<i>Pcna</i>	<i>Bid</i>
	<i>Ppm1j</i>	<i>Cav1</i>
	<i>Rb1</i>	<i>Crip2</i>
	<i>Rfwd2</i>	<i>Ctsd</i>
	<i>Rnf144b</i>	<i>Ctsf</i>
		<i>Cx3cl1</i>
		<i>Dusp5</i>
		<i>Gdf15</i>
		<i>Gml</i>
		<i>lincRNA-p21</i>
		<i>Lrdd</i>
		<i>Mgmt</i>
		<i>Mlh1</i>
		<i>Pcbp4</i>
		<i>Phlda3</i>
		<i>Pmaip1</i>
		<i>Prkab1</i>
		<i>Ptpv</i>
		<i>Rps27l</i>
		<i>S100a2</i>
		<i>Scara3</i>
		<i>Scn3b</i>
		<i>Serpine1</i>
		<i>Sh2d1a</i>
		<i>Slc38a2</i>
		<i>Spata18</i>
		<i>Tap1</i>
		<i>Tgfa</i>
		<i>Tlr3</i>
		<i>Trp53</i>
		<i>Trp53inp1</i>
		<i>Trpm2</i>
		<i>Ttc28</i>
		<i>Tyrrp1</i>
		<i>Xpc</i>
		<i>1190002H23Rik</i>
		<i>9630033F20Rik</i>

Supplementary Table 3 Number of independent shRNAs found enriched in the sensitized genetic screens. List showing numbers of shRNAs targeting particular genes that were found enriched in tumors arising in mice reconstituted with *Puma*^{-/-} *p21*^{-/-} HSPCs transduced with the p53

target gene directed shRNA library

Gene symbol	Reported function downstream of p53	References
<i>Mlh1</i>	DNA damage response	Chen and Sadowski, 2004;Sengupta and Harris, 2005, Nature Reviews;Kenzelmann Broz et al.,Genes Dev.2013;Tonelli et al., Oncotarget, 2013
<i>Ctsf</i>	unknown	Brady et al., Cell, 2011
<i>Zmat3</i>	Apoptosis, Cell proliferation	Vilborg et al., CDD, 2011;Vilborg et al., PNAS, 2009;Bersani et al., Oncogene,2014; Kenzelmann Broz et al.,Genes Dev.2013
<i>Cav1</i>	DNA damage response, Cell proliferation	Bist et al., Biochemistry 2000; Razani et al., J. Biol. Chem, 2001;Zhu et al., PLoS ONE, 2010
<i>Ddit4</i>	DNA damage response	Ellisen et al., Mol Cell 2002; Kenzelmann Broz et al.,Genes Dev.2013
<i>Pms2</i>	DNA damage response	Chen and Sadowski, 2004;Sengupta and Harris, 2005, Nature Reviews
<i>Msh2</i>	DNA damage response	Scherer et al., JBC 2000; Warnick et al., JBC, 2001;Kenzelmann Broz et al.,Genes Dev.2013;
<i>Fancc</i>	DNA damage response	Liebetrau et al., Hum Mol Genet. 1997
<i>Rnf144b</i>	DNA damage response, Apoptosis	Ng et al., 2003, Oncogene; Ho et al., 2014 PNAS;
<i>Polk</i>	DNA damage response	Brady et al., Cell, 2011; Kenzelmann Broz et al.,Genes Dev.2013;Tonelli et al., Oncotarget, 2013; Bugni et al., Oncogene. 2009
<i>Mgmt</i>	DNA damage response	Brady et al., Cell, 2011;Kenzelmann Broz et al.,Genes Dev.2013;Tonelli et al., Oncotarget, 2013
<i>Ercc5</i>	DNA damage response	Brady et al., Cell, 2011;Kenzelmann Broz et al.,Genes Dev.2013;

Supplementary Table 4 Direct p53 target genes found in tumors from the sensitized genetic screen. List of genes with their reported function and references.