Statistics and Reproducibility

This file contains detailed information on the number of biological repeats, the counts of animal/cell lines used in each indicated figure panel as well as the statistical methods and the exact p value whenever possible. Note: when the $p < 1.00 \times 10^{-10}$, the GraphPad Prism software does not provide the exact p value.

Figure 1.

a, *DNA-PKcs*$^{KD/KD}$*;Tp53*$^{-/-}$ (n=20), *DNA-PKcs*$^{KD/KD}$*;Tp53*$^{+/-}$(n=38) , *DNA-PKcs*$^{-/-}$*;Tp53*$^{-/-}$ (n=31), *Xrcc4*$^{-/-}$*;Tp53*$^{-/-}$ (n=21) and *Tp53*$^{-/-}$ (n=56). Two-sided Mantel-Cox Log-rank test $p=1.00 \times 10^{-10}$ between *DNA-PKcs*$^{KD/KD}$*;Tp53*$^{-/-}$ and *DNA-PKcs*$^{KD/KD}$*;Tp53*$^{+/-}$; $p=5.58 \times 10^{-4}$ between *DNA-PKcs*$^{KD/KD}$*;Tp53*$^{-/-}$ and *DNA-PKcs*$^{-/-}$*;Tp53*$^{-/-}$; $p=1.01 \times 10^{-1}$ (n.s.) between *DNA-PKcs*$^{-/-}$*;Tp53*$^{-/-}$ and *Xrcc4*$^{-/-}$*;Tp53*$^{-/-}$, $p= 4.27 \times 10^{-5}$ between *DNA-PKcs*$^{KD/KD}$*;Tp53*$^{+/-}$ and *Tp53*$^{-/-}$ and finally the $p< 1.00 \times 10^{-10}$ when the survival curves of *DNA-PKcs*$^{-/-}$*;Tp53*$^{-/-}$, *DNA-PKcs*$^{KD/KD}$*;Tp53*$^{-/-}$ mice or *Xrcc4*$^{-/-}$*;Tp53*$^{-/-}$ groups were complared with the *Tp53*$^{-/-}$ group.

c. *DNA-PKcs*$^{KD/KD}$*;Tp53*$^{-/-}$ (n=8 myeloid, n=4 pro-B); *DNA-PKcs*$^{-/-}$*;Tp53*$^{-/-}$ (n=14 pro-B); *Xrcc4*$^{-/-}$*;Tp53*$^{-/-}$ (n=7 pro-B), *DNA-PKcs*$^{KD/KD}$*;Tp53*$^{+/-}$ (n=6 Thymic lymphoma, n=4 sarcoma). The p value was calculated using a two-sided unpaired Student's *t*-test. When the survival (days) were compared with the *DNA-PKcs*$^{KD/KD}$*;Tp53*$^{-/-}$ myeloid group, $p=4.15 \times 10^{-8}$ for *DNA-PKcs*$^{KD/KD}$*;Tp53*$^{-/-}$ pro-B cell lymphoma, $p=0.07 \times 10^{-8}$ for *DNA-PKcs*$^{-/-}$*;Tp53*$^{-/-}$ pro-B cell lymphoma, and $p=0.02 \times 10^{-8}$ for *Xrcc4*$^{-/-}$*;Tp53*$^{-/-}$ pro-B cell lymphoma. In comparison to the survival (days) of *DNA-PKcs*$^{KD/KD}$*;Tp53*$^{-/-}$ pro-B, the p-value for *DNA-PKcs*$^{-/-}$*;Tp53*$^{-/-}$ pro-B cell lymphoma is 9.85×10^{-2} (n.s.), for *Xrcc4*$^{-/-}$*;Tp53*$^{-/-}$ pro-B cell lymphoma is 0.51(n.s.). Finally the p value between the survival (days) of *DNA-PKcs*$^{-/-}$*;Tp53*$^{-/-}$ pro-B and *Xrcc4*$^{-/-}$*;Tp53*$^{-/-}$ pro-B cell lymphoma is 0.14 (n.s.).

d, Histological analyses were performed independently on 3 mice for each genotype with similar results to the reached conclusion.

e, Histological analyses were independently repeated on n=9 *DNA-PKcs*$^{+/+}$, n=9 *DNA-PKcs*$^{5A/5A}$, n=4 DNA-*PKcs*$^{5A/5A}$*; Tp53*$^{+/-}$, and n=3 *PKcs*$^{5A/5A}$*;Tp53*$^{-/-}$ mice with similar results.

Figure 2.

a. *DNA-PKcs*$^{5A/5A}$ (n=21), *DNA-PKcs*$^{5A/5A}$*;Tp53*$^{+/-}$(n=34), *DNA-PKcs*$^{5A/5A}$*;Tp53*$^{-/-}$(n=11), *DNA-PKcs*$^{5A/5A}$*;Ku70*$^{-/-}$ (n=9) and *Ku70*$^{-/-}$ (n=7). The p-value was obtained via a two-sided Mantel-Cox log-rank test. In comparison to the *DNA-PKcs*$^{5A/5A}$ group, p-value is $<0.01 \times 10^{-8}$ for *DNA-PKcs*$^{5A/5A}$*;Tp53*$^{+/-}$, 9.08×10^{-7} for *DNA-PKcs*$^{5A/5A}$*;Tp53*$^{-/-}$, 9.46×10^{-6} for *DNA-PKcs*$^{5A/5A}$*;Ku70*$^{-/-}$, and 1.33×10^{-5} for *Ku70*$^{-/-}$.

b and e. *DNA-PKcs*$^{+/+}$ (n=22), *DNA-PKcs*$^{5A/5A}$ (n=11), *DNA-PKcs*$^{5A/5A}$*; Ku70*$^{-/-}$ (n=3), *Ku70*$^{-/-}$ (n=3), and *DNA-PKcs*$^{-/-}$ (n=5). The p-value was obtained using a two-sided unpaired Student's *t*-test. For red blood cell counts (**panel b**) - in comparison to the *DNA-PKcs*$^{+/+}$ group, $p<<0.01 \times 10^{-8}$ for the *DNA-PKcs*$^{5A/5A}$, p=0.18 (n.s.) for *DNA-PKcs*$^{5A/5A}$*; Ku70*$^{-/-}$, p=0.16 (n.s.) for *Ku70*$^{-/-}$, and p=0.32 (n.s.) for *DNA-PKcs*$^{-/-}$ groups The p-value between the *DNA-PKcs*$^{5A/5A}$ group and the *DNA-PKcs*$^{5A/5A}$*;Ku70*$^{-/-}$ group is 3.04×10^{-5}. For MCV (panel e)- in comparison to the *DNA-PKcs*$^{+/+}$ group, $p=1.32 \times 10^{-9}$ for the *DNA-PKcs*$^{5A/5A}$ group, p=0.27(n.s.) for *DNA-PKcs*$^{5A/5A}$*; Ku70*$^{-/-}$ group, p=0.42 (n.s.) for *Ku70*$^{-/-}$, and $p=5.68 \times 10^{-2}$ for *DNA-PKcs*$^{-/-}$.

c and d. The FACS plot and the dot plot represent the collective results from *DNA-PKcs*$^{+/+}$ (n=9), *DNA-PKcs*$^{5A/5A}$ (n=9), *PKcs*$^{5A/5A}$*; Ku70*$^{-/-}$(n=4), and *Ku70*$^{-/-}$ (n=5) mice. The p-value was calculated using a two-sided unpaired Student's *t*-test. In comparison to the *DNA-PKcs*$^{5A/5A}$ group, the p= 4.53×10^{-5} for *DNA-PKcs*$^{+/+}$ and p=0.03 for *DNA-PKcs*$^{5A/5A}$*;Ku70*$^{-/-}$. In comparison to the *Ku70*$^{-/-}$, the p= 0.36 (n.s.) for *DNA-PKcs*$^{5A/5A}$*;Ku70*$^{-/-}$ and p= 0.10 (n.s.) for *DNA-PKcs*$^{+/+}$.

f and g. *DNA-PKcs*$^{+/+}$ (n=10), *DNA-PKcs*$^{5A/5A}$ (n=9), *DNA-PKcs*$^{5A/5A}$*; Ku70*$^{-/-}$ (n=3), *Ku70*$^{-/-}$ (n=3) and *DNA-PKcs*$^{-/-}$ (n=5). The p-value was calculated using a two-sided unpaired Student's *t*-test. **For S2%**: in comparison to *DNA-PKcs*$^{+/+}$ group, p= 6.05×10^{-4} for *DNA-PKcs*$^{5A/5A}$, p= 0.69×10^{-2} for *DNA-PKcs*$^{5A/5A}$*;Ku70*$^{-/-}$, p=0.49 (n.s.) for *Ku70*$^{-/-}$, and p= 0.60(n.s.) for *DNA-PKcs*$^{-/-}$. **For S3%**: in comparison to *DNA-PKcs*$^{+/+}$ group, p= 6.87×10^{-8} for *DNA-PKcs*$^{5A/5A}$, p= 0.21(n.s.) for *DNA-PKcs*$^{5A/5A}$*;Ku70*$^{-/-}$, p=0.93(n.s.) for *Ku70*$^{-/-}$, and p= 0.08 (n.s.) for *DNA-PKcs*$^{-/-}$. **For S4%**: in comparison to *DNA-PKcs*$^{+/+}$ group, p= 9.08×10^{-6} for *DNA-PKcs*$^{5A/5A}$, p= 0.43 (n.s.) for *DNA-PKcs*$^{5A/5A}$*;Ku70*$^{-/-}$, p=0.13 (n.s.) for *Ku70*$^{-/-}$, and p= 0.77 (n.s.) for *DNA-PKcs*$^{-/-}$. There were no adjustments for multiple comparisons. Each group was analyzed independently, without assuming a consistent standard deviation (SD).

h and i, *DNA-PKcs*$^{+/+}$ (n=6) or *DNA-PKcs*$^{+/-}$ (n=5), *DNA-PKcs*$^{5A/5A}$(n=8), *Ku70*$^{-/-}$(n=3), and *DNA-PKcs*$^{-/-}$ (n=5). The p-values were calculated using a two-sided unpaired Student's *t*-test. **For S1-OP-Puro low%**: in comparison to *DNA-PKcs*$^{+/+}$ or *DNA-PKcs*$^{+/-}$ Ctrl group, p= 0.43×10^{-2} for *DNA-PKcs*$^{5A/5A}$, p= 0.06 for *Ku70*$^{-/-}$, and p= 0.73 for *DNA-PKcs*$^{-/-}$. **For S2-OP-Puro low%**: in comparison to *DNA-PKcs*$^{+/+}$ or *DNA-PKcs*$^{+/-}$ Ctrl group, p= 0.60×10^{-3} for *DNA-PKcs*$^{5A/5A}$, p= 0.42 for *Ku70*$^{-/-}$, and p= 0.79 for *DNA-PKcs*$^{-/-}$. **For S3-OP-Puro low%:** in comparison to *DNA-PKcs*$^{+/+}$ or *DNA-PKcs*$^{+/-}$ Ctrl group, p= 0.02 for *DNA-PKcs*$^{5A/5A}$, p= 0.56 for *Ku70*$^{-/-}$, p= 0.53 for *DNA-PKcs*$^{-/-}$. There were no adjustments for multiple comparisons. Each group was analyzed independently, without assuming a consistent SD.

j. The data represents 3 biologically independent samples for each genotype. The p-values were calculated using a two-sided paired Student's *t*-test. The p-value is 1.54×10^{-7} between *DNA-PKcs*$^{+/+}$ with or without OP-Puro. The p-value is 0.90×10^{-2} between *DNA-PKcs*$^{+/+}$ +/- NU7441 (DNA-PK inhibitor), is 0.89 (n.s.) between *DNA-PKcs*$^{-/-}$ +/- NU7441, and p= 0.02 between *Ku70*$^{-/-}$ +/- NU7441. Finally, the p-value is 0.03 between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{-/-}$, and is 0.02 between *DNA-PKcs*$^{+/+}$ and *Ku70*$^{-/-}$.

Figure 3.

c, The experiments were independently repeated 4 times. One additional repeat was shown in Extended Data Figure 8f.

d, The experiments were independently repeated 3 times.

Ext. Data Figure 1.

a, All the *DNA-PKcs*$^{KD/KD}$*;Tp53*$^{-/-}$ mice (n=4 at the time of the first submission) that succumbed to pro-B cell lymphomas show similar accumulation of B220^{+}IgM^{-} proB cells in both bone marrow and spleen. One representative flow cytometry plot is shown.

b, Experiments were repeated on 3 mice for each genotype. The p-value for two-sided unpaired Student's *t*-test is 0.17 x10^{-2} for the number of megakaryocytes per field, and 0.74 x10^{-3} for the number of megakaryocytes with hyperchromatic/pyknotic nuclei per field in spleen between *DNA-PKcs*$^{KD/KD}$*;Tp53*$^{-/-}$ and *DNA-PKcs*$^{+/+}$*;Tp53*$^{-/-}$ mice.

c, A subset of *DNA-PKcs*$^{KD/KD}$*;Tp53*$^{-/-}$ mice (3 out of the 8 at the time of the first submission) succumbed to myeloid disease have an accumulation of cKit^{+}CD11b^{+}GR1^{+} cells in the bone marrow. The frequency of cKit^{+} fraction among CD11b^{+}GR1^{+} cells varies from 10-20%. One representative plot was shown.

Ext. Data Figure 2.

d, Over 5 correctly targeted clones were identified by Southern Blotting wit ah 5' probe, and subsequently confirmed with a 3' probe and Sanger sequencing. One representative Southern blot of a positive clone is shown.

e and f, *DNA-PKcs*$^{+/+}$ (n=5) and *DNA-PKcs*$^{PQR/PQR}$ (n=4). A two-sided unpaired Student's *t*-test was used to calculate the p-value. The p=0.27 for RBC counts (**e**) and p= 0.99 for PLT counts (**f**) between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{PQR/PQR}$ mice.

g, *DNA-PKcs*$^{+/+}$ (n=22), *DNA-PKcs*$^{5A/5A}$(n=11) and *DNA-PKcs*$^{-/-}$ (n=5). A two-sided unpaired Student's *t*-test was used to calculate the p-value. In comparison to the *DNA-PKcs*$^{+/+}$ group, the p-value is 2.72x10^{-7} for *DNA-PKcs*$^{5A/5A}$, p=0.06 for *DNA-PKcs*$^{-/-}$.

h and i, The pale kidney and liver were observed in every *DNA-PKcs*$^{5A/5A}$ mice (n=11 or more) analyzed. One representative picture is shown.

Ext. Data Figure 3.

a and b, Representative flow cytometry panels and statistic summary from independent analyses performed on n=9 DNA-*PKcs*$^{+/+}$, n=9 *DNA-PKcs*$^{5A/5A}$, n=4 *DNA-PKcs*$^{5A/5A}$;*Ku70*$^{-/-}$, and n=5 *Ku70*$^{-/-}$ mice. A two-sided unpaired Student's *t*-test was used to calculate the p-value. When compared to the Lin^{-}Scal^{-}cKit^{+} cells frequency in the *DNA-PKcs*$^{+/+}$ group, the p-value is 1.67x10^{-5} for *DNA-PKcs*$^{5A/5A}$, 0.03 for *DNA-PKcs*$^{5A/5A}$; *Ku70*$^{-/-}$, and 0.04 for *Ku70*$^{-/-}$.

c and d, The representative flow cytometry analyses and the summary were collected from n=3 DNA-*PKcs*$^{+/+}$, n=6 *DNA-PKcs*$^{+/5A}$. n=4 *DNA-PKcs*$^{5A/5A}$, and n=4 *DNA-PKcs* $^{KD/KD}$ embryos. A two-sided unpaired Student's *t*-test was used to compare the frequency of Lin^{-}Scal^{+}cKit^{+} cells in the fetal liver. In comparison to the *DNA-PKcs*$^{+/+}$ controls, the p-value is 0.19 for *DNA-PKcs*$^{+/5A}$, 6.72x10^{-5} for *DNA-PKcs*$^{5A/5A}$, and 6.43x10^{-6} for *DNA-PKcs* $^{KD/KD}$.

e, This gating strategy was used in all experiments, including the OP-Puro data shown in Extended Data Figure 7d.

Ext. Data Figure 4.

a and b, The flow cytometry plots (a) and the summary (b) were collected from n=4 mice for each genotype. A two-sided unpaired Student's *t*-test was used to calculate the p-value. In comparison to *DNA-PKcs*$^{+/+}$, the p= 3.80x10^{-6} for *DNA-PKcs*$^{5A/5A}$, p= 0.33 for *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{-/-}$, and p= 1.28x10^{-6} for *DNA-PKcs*$^{-/-}$;*Tp53*$^{-/-}$. In addition, p= 7.85x10^{-6} between *DNA-PKcs*$^{5A/5A}$ and *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{-/-}$ groups

c, *DNA-PKcs*$^{+/+}$(n=22), *DNA-PKcs*$^{5A/5A}$(n=11), *DNA-PKcs*$^{5A/5A}$; *Tp53*$^{+/-}$ (n= 4), *DNA-PKcs*$^{5A/5A}$; *Tp53*$^{-/-}$ (n= 3), *Tp53*$^{-/-}$(n= 3), and *DNA-PKcs*$^{-/-}$(n=5). A two-sided unpaired Student's *t*-test was used to calculate the p-value. The p<0.01x10^{-8} between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{5A/5A}$, p= 0.01 between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{+/-}$, p= 0.65 between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{-/-}$, p= 0.93 between *DNA-PKcs*$^{+/+}$ and *Tp53*$^{+/-}$, and p= 0.32 between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{-/-}$.

d, e, *DNA-PKcs*$^{+/+}$(n=22), *DNA-PKcs*$^{5A/5A}$(n=6), *DNA-PKcs*$^{5A/5A}$;*Ku70*$^{-/-}$ (n= 3), *DNA-PKcs*$^{-/-}$ (n= 5), *Ku70*$^{-/-}$ (n= 3), *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{+/-}$ (n= 4), *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{-/-}$ (n= 3), and *Tp53*$^{+/-}$(n=3). A two-sided unpaired Student's *t*-test was used to calculate the p-value. **For lymphocyte counts (d)**, in comparison to the *DNA-PKcs*$^{+/+}$ group, the p-values are 2.15x10^{-5} for *DNA-PKcs*$^{5A/5A}$, 0.08x10^{-2} for *DNA-PKcs*$^{5A/5A}$;*Ku70*$^{-/-}$, 0.12x10^{-2} for *Ku70*$^{-/-}$, 0.14x10^{-2} for *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{+/-}$, 0.096 for *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{-/-}$, 0.88 for *Tp53*$^{+/-}$, and finally 2.05x10^{-4} for *DNA-PKcs*$^{-/-}$. **For neutrophil counts (e)**, in comparison to the *DNA-PKcs*$^{+/+}$ group, the p-values are 1.20x10^{-3} for *DNA-PKcs*$^{5A/5A}$, 0.10 for *DNA-PKcs*$^{5A/5A}$; *Ku70*$^{-/-}$, 0.85 for *Ku70*$^{-/-}$, p= 0.055 for *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{+/-}$, p= 0.73 for *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{-/-}$, p= 0.82 for *Tp53*$^{+/-}$, and p= 0.55 for *DNA-PKcs*$^{-/-}$.

g and h, Four independent v-abl transformed cell lines with single integration of pMX-INV V(D)J recombination substrates were generated and analyzed. The results were all consistent. A representative result from a *DNA-PKcs*$^{5A/5A}$ and a *DNA-PKcs*$^{+/+}$ lines are shown.

i, *DNA-PKcs*$^{+/+}$(n=5), *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{+/-}$ (n=3) and *DNA-PKcs*$^{-/-}$;*Tp53*$^{+/-}$(n=3) Left: A two-sided unpaired Student's *t*-test was used to calculate the p-value. The p= 0.56 (n.s.) between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{+/-}$, and p= 4.29x10^{-3} between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{-/-}$;*Tp53*$^{+/-}$, right: p= 0.09 between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{+/-}$, and p= 2.43x10^{-3} between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{-/-}$;*Tp53*$^{+/-}$.

Ext. Data Figure 5.

a,b. The experiments were repeated 4 times with independently derived MEFs (2 *DNA-PKcs*$^{5A/5A}$ MEFs line for each repeat). Each experiment was plated in triplicates or quadruplicates for each treatment.

c, **d**. The experiments were independently repeated 2 times in triplicates for each line and two independent *DNA-PKcs*$^{5A/5A}$ lines per experiment were used. The representative results and quantification for one experiment (n=3 for *DNA-PKcs*$^{+/+}$ and n=6 for *DNA-PKcs*$^{5A/5A}$) are shown. A two-sided unpaired Student's *t*-test was used to calculate p-value. p= 0.17 (n.s., 250nM), p= 0.27 (n.s., 500nM) and p= 0.69 (n.s., 1000nM) between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{5A/5A}$.

e, The experiments were independently repeated 2 times with duplication for each line and two independently derived *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{5A/5A}$ ESCs per experiment, which made n=4

biologically independent experiments. A two-sided unpaired Student's *t*-test was used to calculate the p-value at each IR dose independently from each other. No technical replicates were included.

f, The experiments were independently repeated 2 times in triplicates for each line. The specific experiments shown include independently derived primary MEFs from *DNA-PKcs*$^{+/+}$ (n=2), *DNA-PKcs*$^{-/-}$ (n=1), and *DNA-PKcs*$^{5A/5A}$ (n=3).

g, The experiments were independently repeated 3 times using independently derived primary MEFs. One representative FACS from each type is shown. Experiments were repeated n=3 times for *DNA-PKcs*$^{+/+}$, n=4 times for *DNA-PKcs*$^{5A/5A}$, n=2 times for *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{-/-}$ and n=2 times for *DNA-PKcs*$^{-/-}$ yielding similar results.

Ext. Data Figure 6.

a, b The experiments were independently repeated 3 times with two independently derived ESC for each genotype and plated in triplicates. Similar results were obtained. Randomly selected colonies were measured *DNA-PKcs*$^{+/+}$ (n=9), *DNA-PKcs*$^{5A/5A}$ (n=11) and *DNA-PKcs*$^{5A/5A}$;*Ku80*$^{-/-}$ (n=15) A two-sided unpaired Student's *t*-test was used to calculate p-value. The p= 0.02 between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{5A/5A}$ and p= 0.01 between *DNA-PKcs*$^{5A/5A}$ and *DNA-PKcs*$^{5A/5A}$*;Ku80*$^{-/-}$.

c, A total of n=4 independently derive ESCs of each genotype were analyzed. The representative panel shows two independently derived ESCs for each genotype.

d, e, and f, Telomere instability was measured in n=4 independent derived MEF lines for each genotype. A two-sided unpaired Student's *t*-test was used: p= 0.04 between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{5A/5A}$, p= 2.20x10^{-3} between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{-/-}$, and p= 0.28 (n.s.) between *DNA-PKcs*$^{5A/5A}$ and *DNA-PKcs*$^{-/-}$. The raw data were included in panel f.

g, The RBC size were independently measured in n=10 *DNA-PKcs*$^{+/+}$ and n=5 *DNA-PKcs*$^{-/-}$ mice. Similar results were obptained.

h, The OP-Puro levels in erythroblasts were measured in n=6 *DNA-PKcs*$^{+/+}$, n=3 *Ku70*$^{-/-}$ and n=5 *DNA-PKcs*$^{-/-}$ mice with similar results obtained in each experiment. One representative set is shown.

Ext. Data Figure 7.

a, *DNA-PKcs*$^{+/+}$ (n=22), *DNA-PKcs*$^{5A/5A}$ (n=11), *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{+/-}$ (n= 4), *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{-/-}$ (n= 3) and *Tp53*$^{-/-}$(n= 3). A two-sided unpaired Student's *t*-test was used to calculate the p value. The p= 1.32x10^{-8} between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{5A/5A}$, p= 0.10 between *DNA-PKcs*$^{5A/5A}$ and *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{+/-}$, and p= 0.003 between *DNA-PKcs*$^{5A/5A}$ and *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{-/-}$.

b, *DNA-PKcs*$^{+/+}$ (n=5) and *DNA-PKcs*$^{5A/5A}$ (n=4) fetal livers. A two-sided unpaired Student's *t*-test calculated p-value between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{5A/5A}$ is 0.71x10^{-2} for S1, 0.02 for S2 and 0.01 for S3.

c, n=8 mice for each genotype. A two-sided unpaired multi Student's *t*-test calculated p= 7.02x10^{-5} between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{5A/5A}$.

d, Data collected from n=4 independent *DNA-PKcs*$^{+/+}$ mice were used to generate the plot. The high OP-puro levels in the erythroblast lineage were consistent in all animal tested. The means and standard errors were marked on the plot.

e, f, *DNA-PKcs*$^{+/+}$ (n=6) and *DNA-PKcs*$^{5A/5A}$ (n=8) fetal livers. A two-sided unpaired Student's *t*-test was used to calculate the p-values between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{5A/5A}$ is 2.68×10^{-2} for S1, 5.63×10^{-3} for S2 and 6.20×10^{-3} for S3.

g, Control (*DNA-PKcs*$^{+/+}$ or *DNA-PKcs*$^{+/5A}$, n=5) and *DNA-PKcs*$^{5A/5A}$;*Tp53*$^{+/-}$ (n=3). A two-sided unpaired Student's *t*-test was used to calculate the p-values between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{5A/5A}$ are 7.26×10^{-3} for S1 and 2.18×10^{-4} for S2.

h, The experiments were performed n=4 times independently. The relative OP-Puro levels of each line were normalized to the OP-Puro levels of the WT (*DNA-PKcs*$^{+/+}$) controls measured at the same time. The results from all four experiments were plotted together. A two-sided unpaired Student's *t*-test was used to calculate the p-values: $p=6.76 \times 10^{-7}$ between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{3A/3A}$ and $p= 2.84 \times 10^{-2}$ between *DNA-PKcs*$^{+/+}$ and *DNA-PKcs*$^{KD/KD}$.

i, The experiments were performed 3 times independently and plotted together. The relative OP-Puro levels of ATM inhibitor treated lines were presented as a fraction of the OP-Puro levels in the DMSO treated sample measured at the same time. The results from the n=3 independent experiments were plotted together. A two-sided unpaired Student's *t*-test was used to calculate the p-value: p=0.38 (n.s.) between DMSO and ATMi.