

Supplemental Information

Inventory of Supplementary Materials

Supplementary figures and legends:

Figure S1, related to Figure 1. Centrosome amplification does not promote DNA damage or cytokinesis failure.

Figure S2, related to Figure 2. There is no Plk4 overexpression or centrosome amplification in the brain of doxycycline-treated Plk4^{Dox} mice.

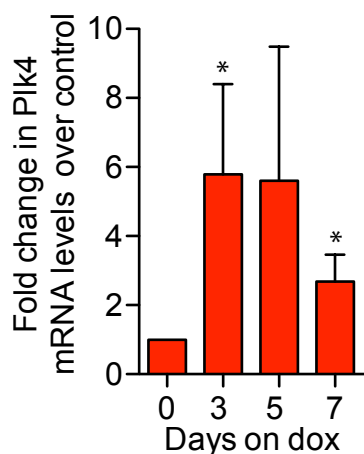
Figure S3, related to Figure 2. Centrosome amplification leads to progressive hair loss.

Figure S4, related to Figure 3 and Figure 4. Centrosome amplification leads to aneuploidy in the spleen of aged mice.

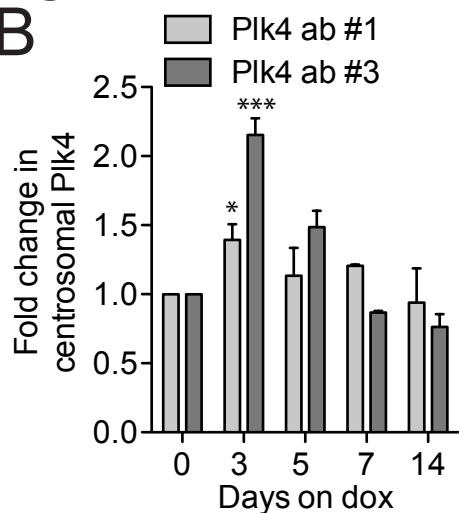
Figure S5, related to Figure 5. Transient Plk4 overexpression triggers spontaneous tumor development.

Supplementary Figure 1

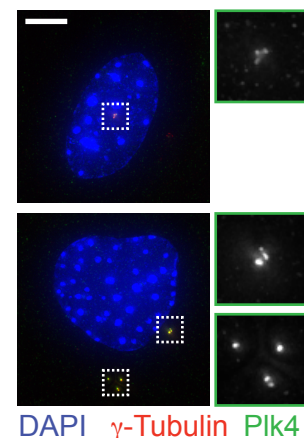
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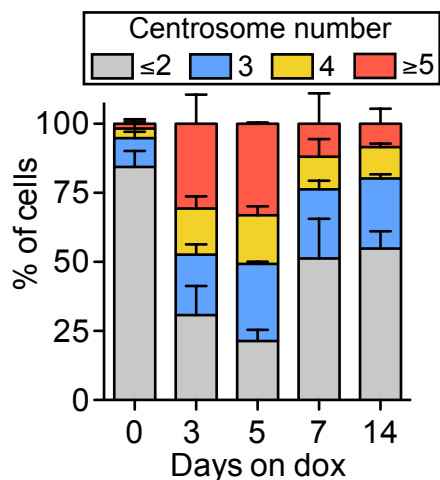
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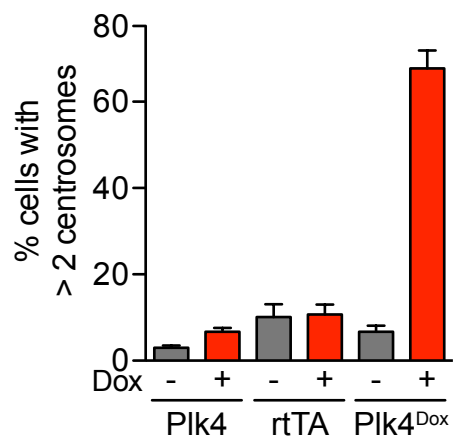
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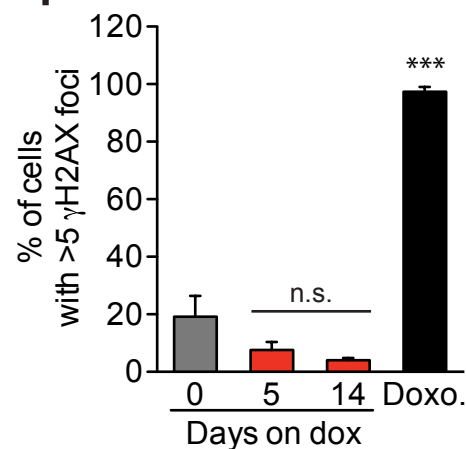
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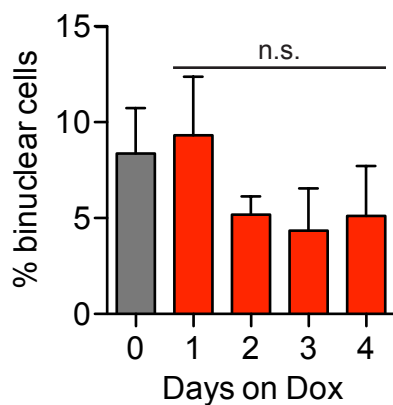
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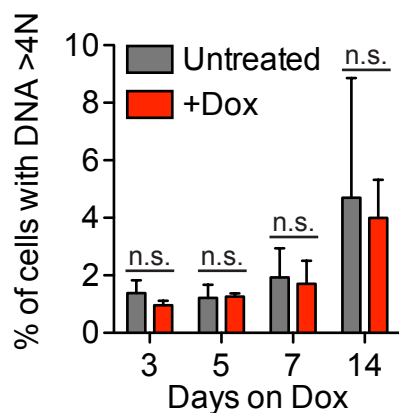
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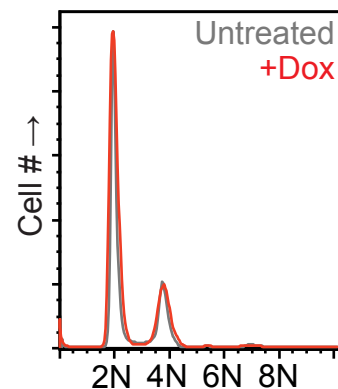
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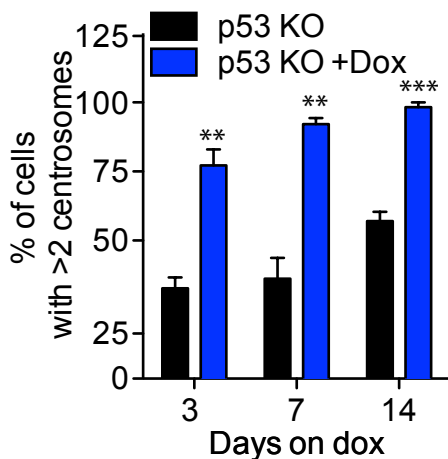
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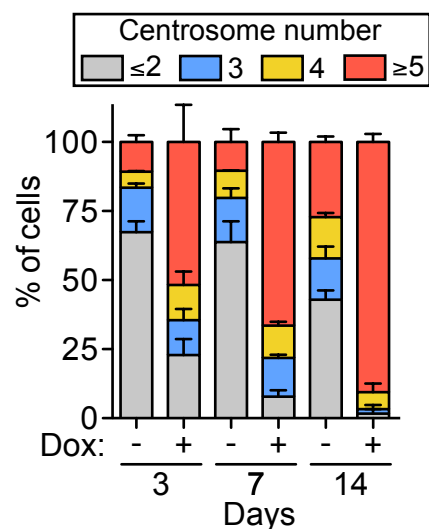
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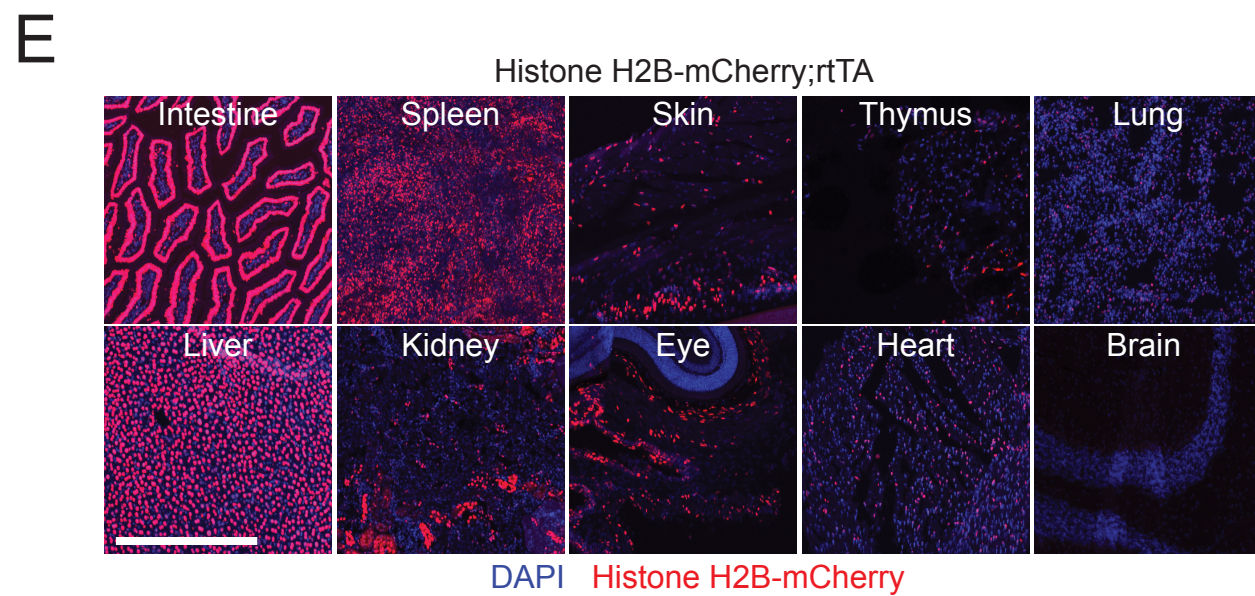
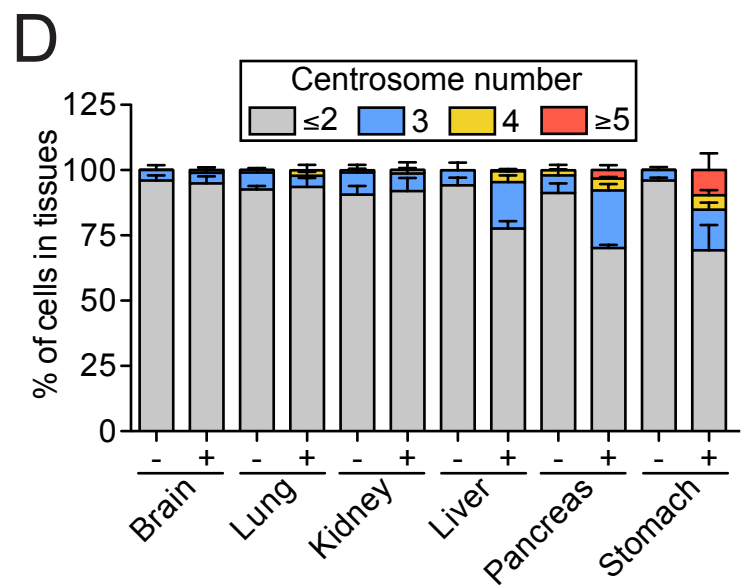
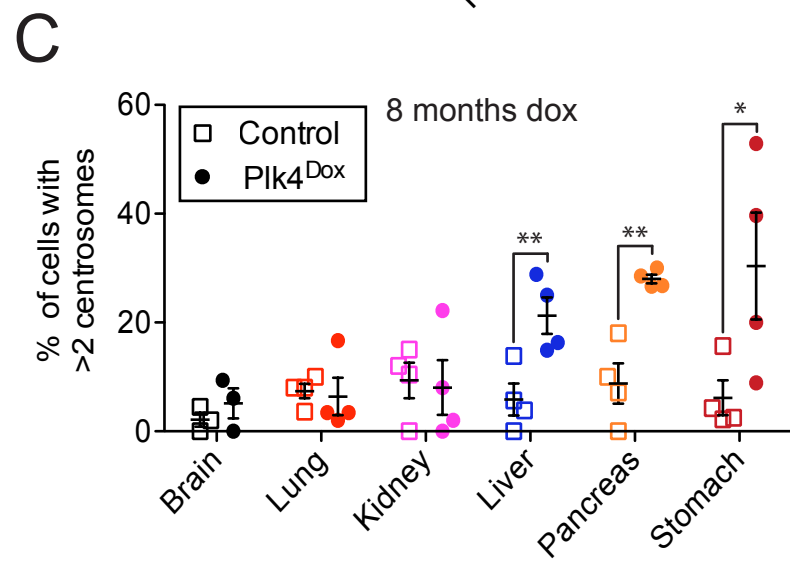
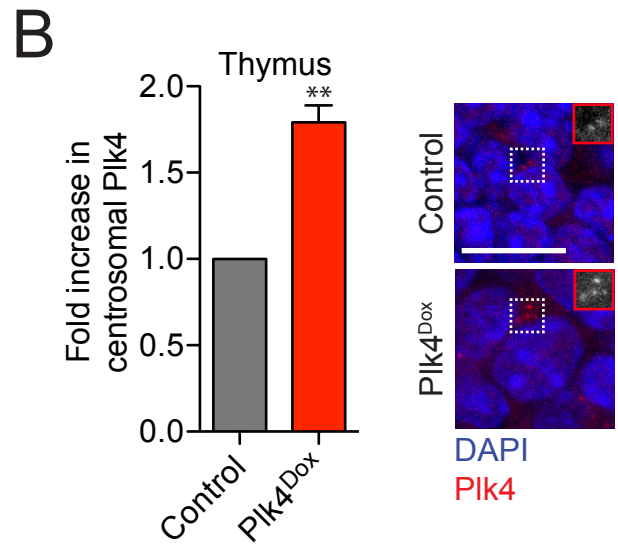
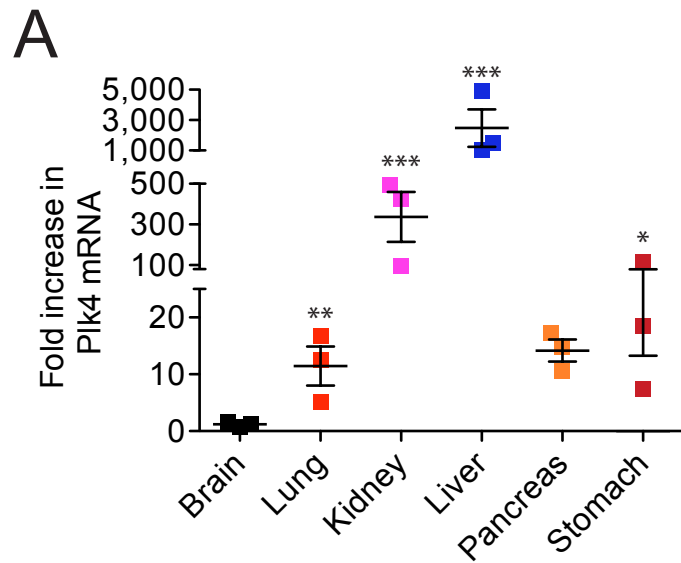
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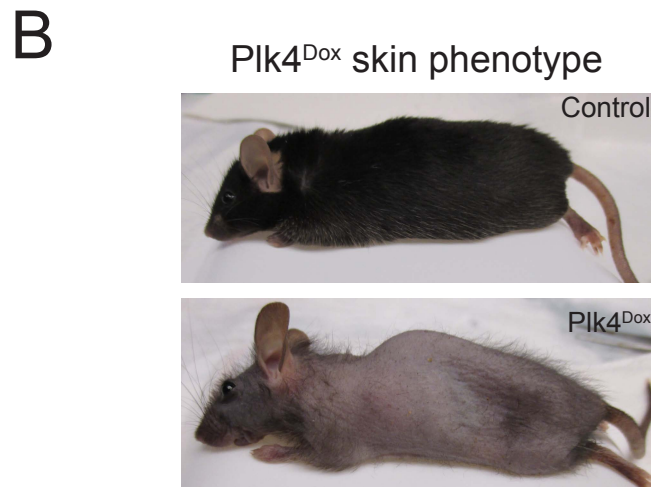
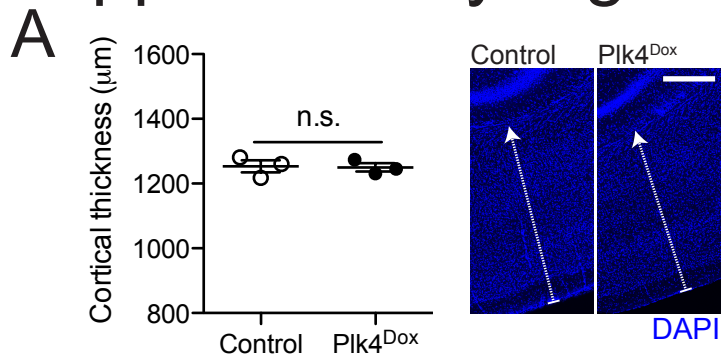
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Supplementary Figure 2

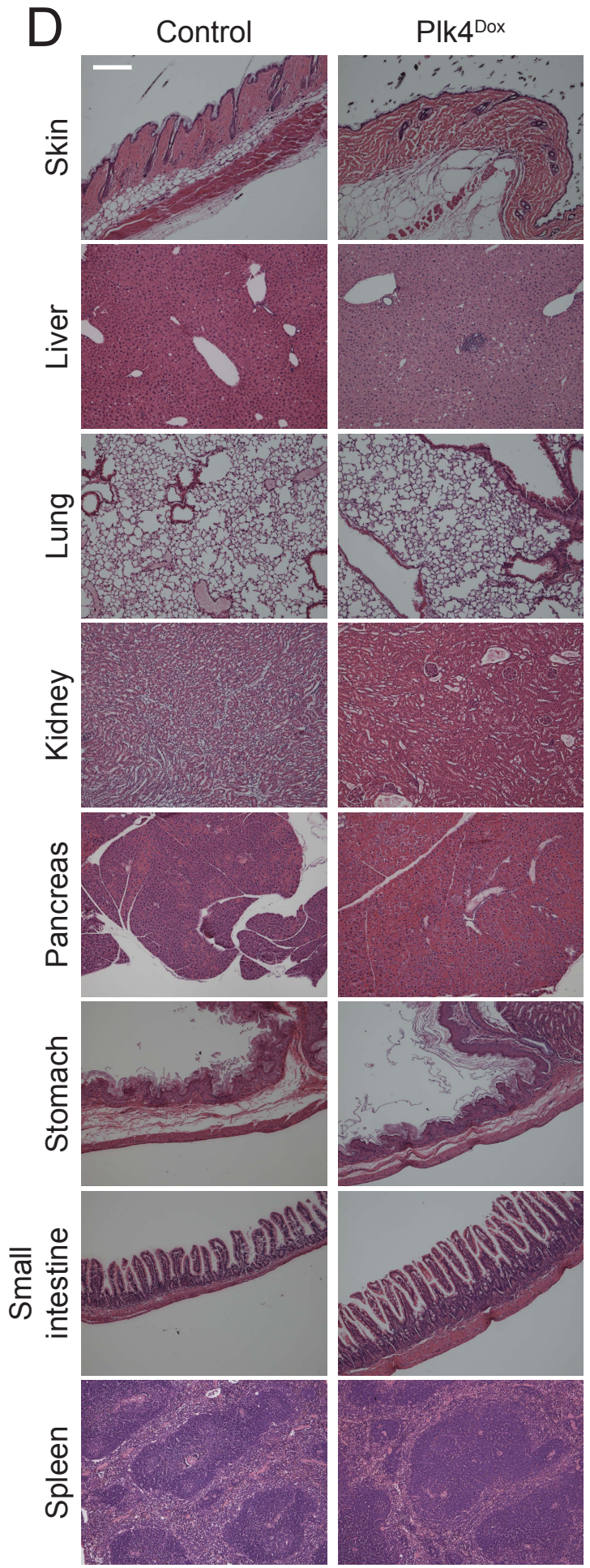


Supplementary Figure 3

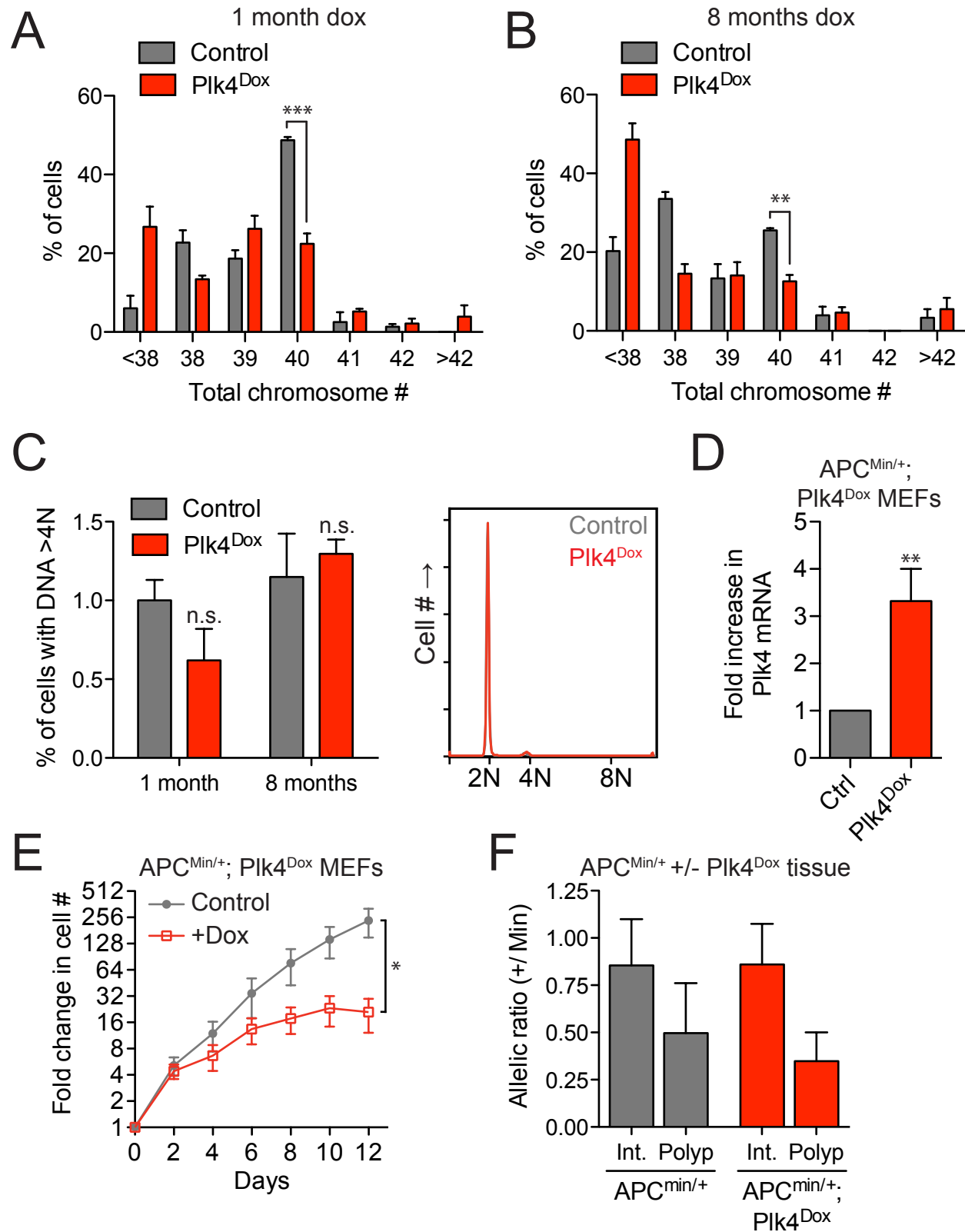


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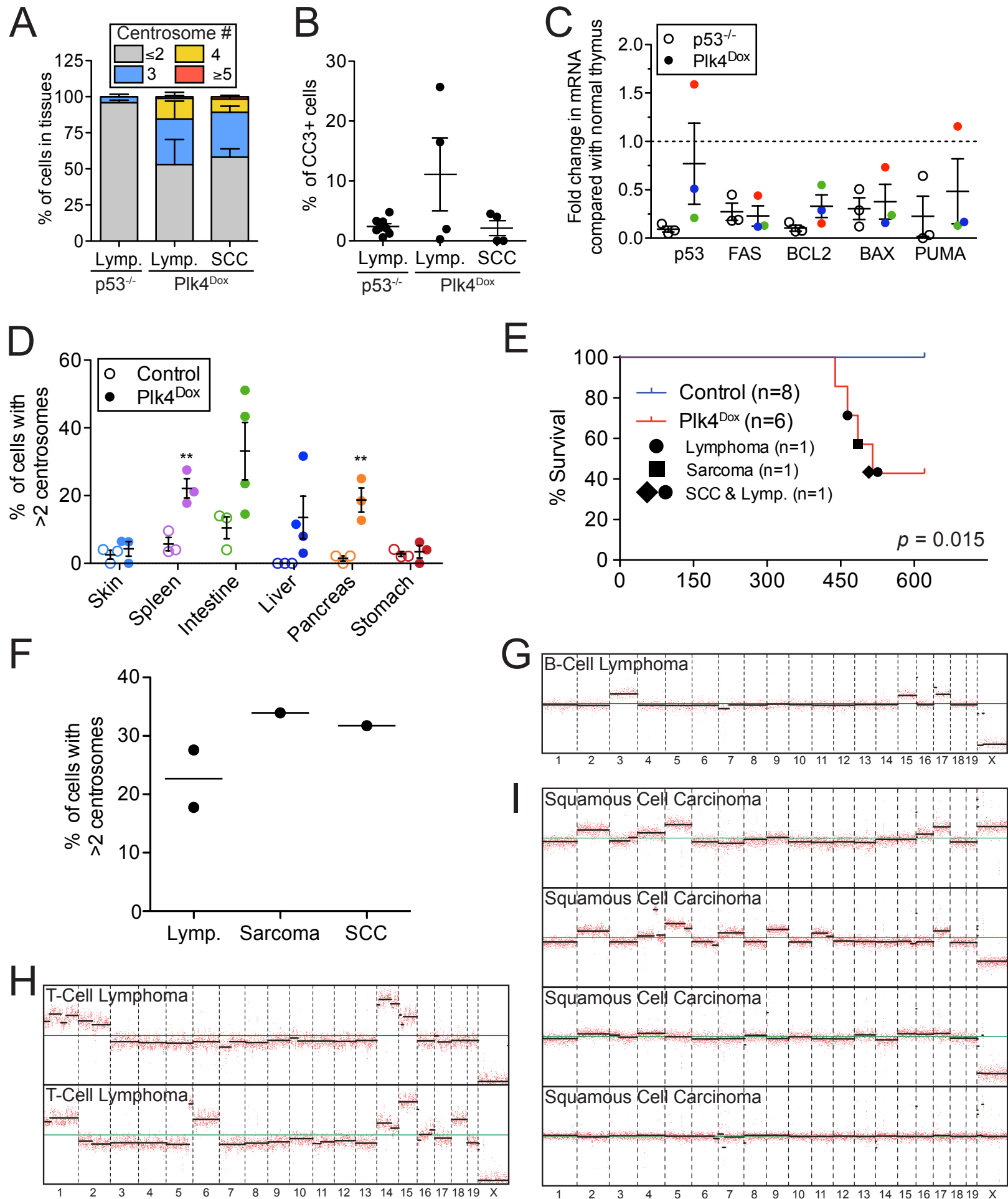
Tissues	Pathology
Skin	Small, widely-spaced follicles; sparse hair; hyperkeratosis
Liver	Normal
Lungs	Normal
Kidney	Normal
Pancreas	Normal
Stomach	Normal
Intestine	Normal
Spleen	Modestly elevated white pulp
Bone Marrow	Normal
Thymus	Normal
Lymph Nodes	Modestly reactive
Heart	Normal
Reproductive System	Normal



Supplementary Figure 4



Supplementary Figure 5



Supplemental Figure Legends

Figure S1, related to Figure 1. Centrosome amplification does not promote DNA damage or cytokinesis failure

(A) Quantification of the level of Plk4 mRNA in Plk4^{Dox} MEFs at different times after doxycycline treatment. Data are means \pm SEM (N = 3, performed in triplicate).

(B) Quantification of the level of centrosomal Plk4 at different times after doxycycline treatment. Data are means \pm SEM from two different Plk4 antibodies (Plk4 ab #1 and #3) (N = 3, >150 centrosomes per experiment).

(C) Representative immunofluorescent images of centrosomal Plk4 in Plk4^{Dox} MEFs.

(D) Quantification of centrosome number in Plk4^{Dox} MEFs at different times after doxycycline treatment. Data are means \pm SEM (N = 3, >150 cells per experiment).

(E) Quantification of the level of centrosome amplification in Plk4-EYFP, rtTA and Plk4^{Dox} MEFs at two days after doxycycline addition. Data are means \pm SEM (N = 3, >150 cells per experiment).

(F) Quantification of DNA damage foci in Plk4^{Dox} MEFs at different times after doxycycline treatment. Doxorubicin treatment (Doxo.) is shown as a control. Data are means \pm SEM (N = 3, >150 cells per experiment).

(G) Quantification of the fraction of binuclear Plk4^{Dox} MEFs at different times after doxycycline treatment. Data are means \pm SEM (N = 3, >135 cells per experiment).

(H) Quantification of the fraction of Plk4^{Dox} MEFs with >4N DNA content at different times after doxycycline treatment. Data are means \pm SEM (N = 3, 10,000 cells per experiment).

(I) Representative flow cytometry profiles show cell cycle analysis in Plk4^{Dox} MEFs.

(J) Quantification of the level of centrosome amplification in Plk4^{Dox}; p53^{-/-} MEFs at different times after doxycycline addition. Data are means \pm SEM (N = 3, >150 cells per experiment).

(K) Quantification of centrosome number in Plk4^{Dox}; p53^{-/-} MEFs at different times after doxycycline treatment. Data are means \pm SEM (N = 3, >150 cells per experiment).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ and NS (not significant) indicates $P > 0.05$; two-tailed Student's *t*-test.

Figure S2, related to Figure 2. There is no Plk4 overexpression or centrosome amplification in the brain of doxycycline-treated Plk4^{Dox} mice

(A) Graph showing the fold increase in Plk4 mRNA in tissues from Plk4^{Dox} mice treated with doxycycline for 1 month. Data are means \pm SEM (N = 3, performed in triplicate).

(B) Quantification of the level of centrosomal Plk4 in tissues from Plk4^{Dox} mice treated with doxycycline for 1 month. Representative immunofluorescent images show centrosomal Plk4 in tissue sections. Data are means \pm SEM (N = 3). Scale bar represents 10 μ m.

(C) Quantification of the level of centrosome amplification in tissues from Plk4^{Dox} mice treated with doxycycline for 8 months. Data are means \pm SEM (N = 4).

(D) Quantification of centrosome number in tissues from Plk4^{Dox} mice treated with doxycycline for 1 month. Data are means \pm SEM (N = \geq 4).

(E) Images of tissue sections taken from Histone H2B-mCherry;rtTA mice treated with doxycycline for 1 month. The Histone H2B-mCherry expression construct was integrated at the same location as the Plk4-EYFP transgene. This reporter showed

widespread doxycycline-inducible expression, but was undetectable in the brain. Scale bar represents 200 μm .

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ and NS (not significant) indicates $P > 0.05$; two-tailed Student's t -test.

Figure S3, related to Figure 2. Centrosome amplification leads to progressive hair loss.

(A) Quantification of cortex thickness measured in four-month old control or Plk4^{Dox} animals treated with doxycycline for 3 months (N = 3). Images show the cerebral cortex in control and Plk4^{Dox} mice. Scale bar represents 200 μm .

(B) Images show hair loss in eight-month old Plk4^{Dox} animals fed doxycycline chronically from one week of age.

(C) Pathology report from eight-month old Plk4^{Dox} animals fed doxycycline chronically from one week of age. Tissues analyzed by a veterinary pathologist (N = 4 for control and Plk4^{Dox} mice).

(D) Images show hematoxylin and eosin stained tissue sections from eight-month old control and Plk4^{Dox} mice fed doxycycline chronically from one week of age. Scale bars represent 200 μm .

Figure S4, related to Figure 3 and Figure 4. Centrosome amplification leads to aneuploidy in the spleen of aged mice

(A and B) Fraction of splenocytes with the indicated number of chromosomes. Cells were derived from control and $Plk4^{Dox}$ mice treated with doxycycline for either 1 or 8 months. Data are means \pm SEM (N = 3, 50 cells per experiment).

(C) Quantification of the fraction of $Plk4^{Dox}$ or control splenocytes with $>4N$ DNA content at different times after doxycycline treatment. Data are means \pm SEM (N = 3, 10,000 cells per experiment). Representative flow cytometry profiles show cell cycle analysis in $Plk4^{Dox}$ and control splenocytes.

(D) Quantification of the level of $Plk4$ mRNA in $APC^{Min/+}$; $Plk4^{Dox}$ MEFs at different times after doxycycline treatment. Data are means \pm SEM (N = 3, performed in triplicate).

(E) Graph showing the fold increase in cell number for $APC^{Min/+}$; $Plk4^{Dox}$ MEFs grown in the presence and absence of doxycycline. Data are means \pm SEM (N = 5, performed in triplicate).

(F) Graph showing the APC^+ to APC^{Min} PCR ratios generated from intestinal adenomas (Polyp) and normal intestine (Int.) from $APC^{Min/+}$ and $APC^{Min/+}$; $Plk4^{Dox}$ mice. The mean APC^+/APC^{Min} value for adenomas from APC^{Min} and $APC^{Min};Plk4^{Dox}$ mice is reduced compared to normal tissue from these animals, indicating partial loss of the APC^+ allele. Data are means \pm SEM (N = ≥ 2 , performed in duplicate).

** $P < 0.01$, *** $P < 0.001$ and n.s. (not significant) indicates $P > 0.05$; two-tailed Student's t -test.

Figure S5, related to Figure 5. Transient $Plk4$ overexpression triggers spontaneous tumor development

(A) Quantification of centrosome number in tumors that arise in $Plk4^{Dox}$ mice chronically

fed doxycycline. Data are means \pm SEM. (N = \geq 4).

(B) Quantification of the fraction of cleaved caspase 3 positive cells in tumors from Plk4^{Dox} and p53^{-/-} mice. Data are means \pm SEM. (N = \leq 4, with each data point representing a single tumor).

(C) Graph showing the expression level of p53 target genes in tumors formed in Plk4^{Dox} and p53^{-/-} mice.

(D) Quantification of the level of centrosome amplification in tissues from 16-18 month old Plk4^{Dox} mice treated with doxycycline for 1 month. Data are means \pm SEM (N = \geq 3).

(E) Kaplan-Meier survival analysis of Plk4^{Dox} and control (C57BL/6J) mice fed doxycycline at 1 month of age for one month. *P* value was calculated using the Log-rank test.

(F) Quantification of the level of centrosome amplification in tumors from Plk4^{Dox} mice fed doxycycline for one month. Each point represents a single tumor and horizontal lines represent the mean.

(G) Low-coverage whole-genome sequencing (WGS) plots for a B-cell Lymphoma derived from Plk4^{Dox} mice fed doxycycline for one month.

(H-I) Low-coverage whole-genome sequencing plots of two T-Cell Lymphomas, and four Squamous Cell Carcinomas (SCC) derived from Plk4^{Dox} mice chronically treated with doxycycline.