Transient genomic instability drives tumorigenesis
through accelerated clonal evolution

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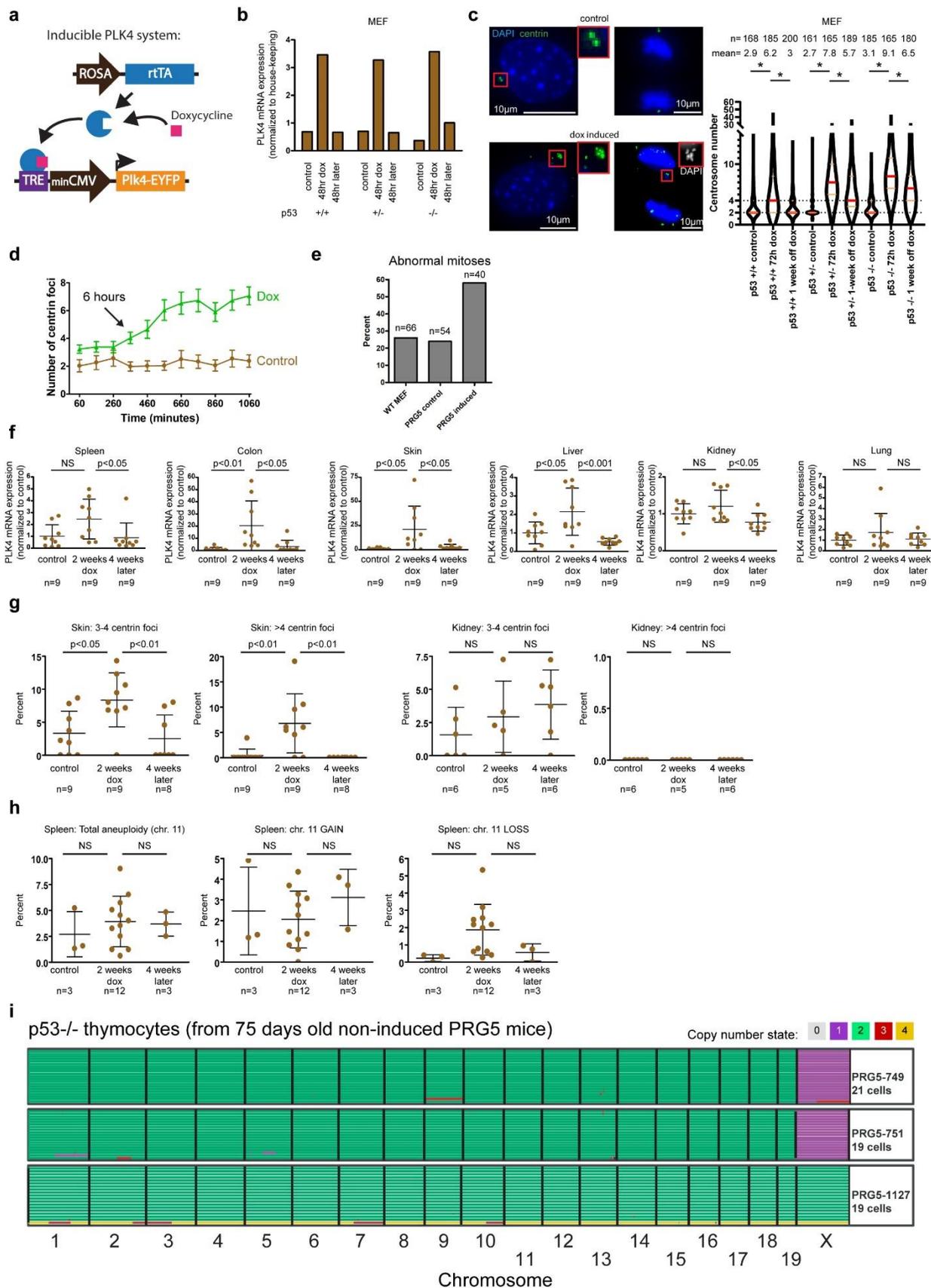
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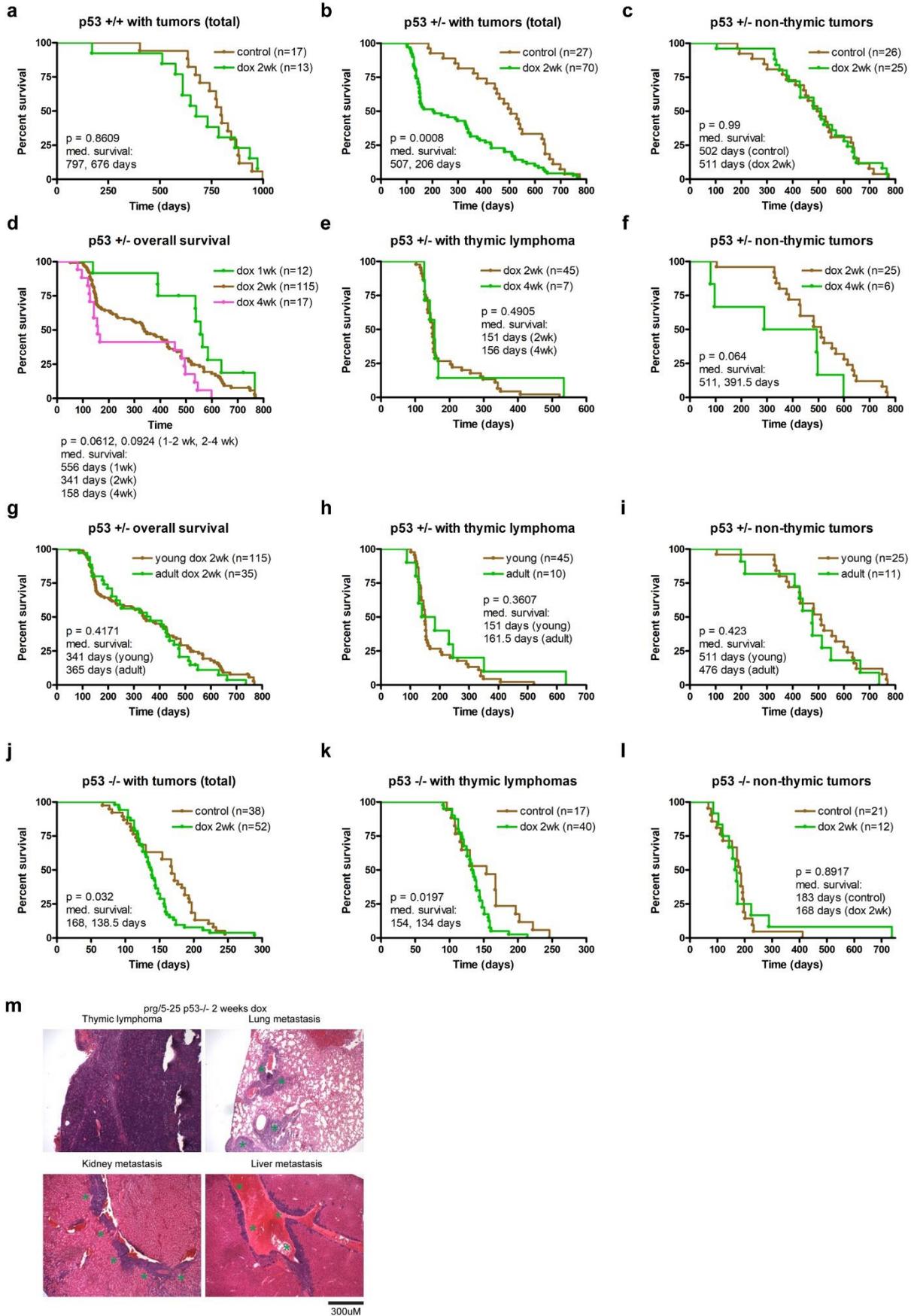
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a Inducible PLK4 system:

b MEF

c DAPI centrin

MEF

d

e Abnormal mitoses

f Spleen | Colon | Skin | Liver | Kidney | Lung

g Skin: 3-4 centrin foci | Skin: >4 centrin foci | Kidney: 3-4 centrin foci | Kidney: >4 centrin foci

h Spleen: Total aneuploidy (chr. 11) | Spleen: chr. 11 GAIN | Spleen: chr. 11 LOSS

i p53-/- thymocytes (from 75 days old non-induced PRG5 mice)

Copy number state: 0 1 2 3 4

Chromosome

2

Supplemental Figure S1 | Effect of PLK4 overexpression in vitro and in vivo. (**a**) Inducible Plk4 expression using the tetracycline-on system. Doxycycline provided in mouse chow binds the reverse tetracycline-controlled trans-activator (rtTA, inserted in the Rosa locus) that together bind a Tet Response Element (TRE) upstream of Plk4 (inserted into the Col1A locus). (**b**) Plk4 mRNA levels in mouse embryonic fibroblasts derived from p53+/+, p53+/-, and p53-/- PRG5 mice, before (control) immediately after (48hr dox) and 48 hou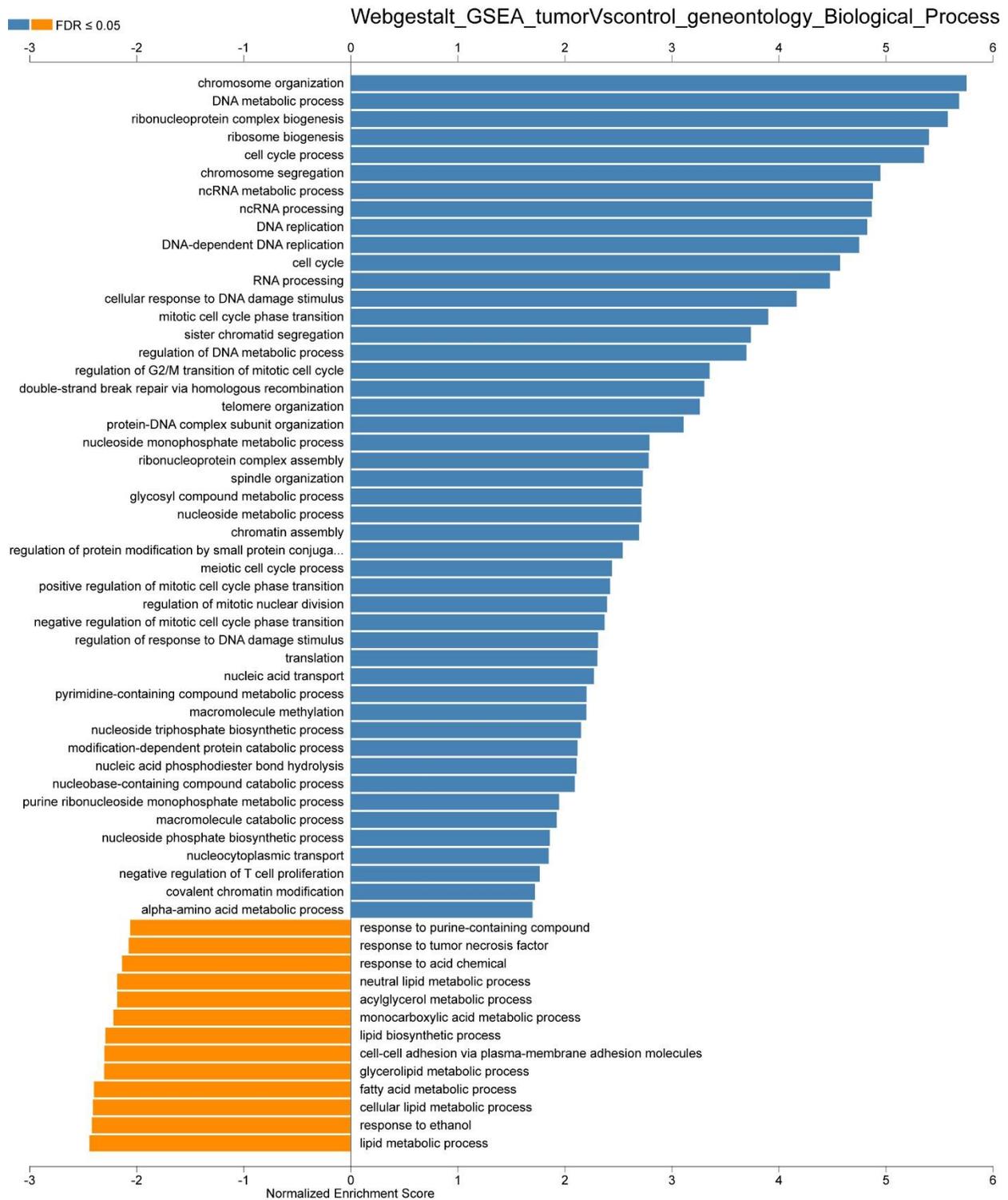rs after (48hr later) doxycycline administration. (**c**) Frequency of centrosome amplification as determined using centring-GFP in mouse embryonic fibroblasts derived from p53+/+, p53+/-, and p53-/- PRG5 mice, before (control) immediately after (72hr dox) and 1 week after (1 week off dox) doxycycline administration. Violin plots show median (red) and quartiles (orange). *p<0.0001 determined using one-way ANOVA with Tukey's Multiple Comparison Test. Data from two independent experiments are presented. Representative images of control and doxycycline induced MEFs are shown. (**d**) Measurements of centrin-GFP foci (starting one hour following Plk4 induction, mean ± SD, n=37-54 and n=43-77 per time point for control and induced cells, respectively). (**e**) Mitotic abnormalities (anaphase bridges and lagging chromosomes) in p53+/- control PRG5 MEFs, or starting 8 hours after Plk4 induction, and in wild-type MEFs (C57BL/6 mice). Similar levels of basal CIN were observed in both wild-type MEFs and in control PRG5 MEFs indicating there is minimal or no leakiness in the Plk4 doxycycline system. (**f-h**) Plk4 mRNA levels (**f**), measurement of centrin-GFP foci (**g**), and percent aneuploidy for chromosome 11 (using interphase DNA-FISH, **h**) in indicted tissues from PRG5 mice before (control) immediately after (2 weeks dox) and one month after (4 weeks later) doxycycline administration. Mean ± SD of indicated mice per group are presented. *p-values determined using one-way ANOVA with Tukey's Multiple Comparison Test. (**i**) Heatmaps showing DNA copy number using single cell whole-genome sequencing of cells collected from normal thymic tissues of 75-days old p53-/- PRG5 mice.

a

p53 +/+ with tumors (total)

control (n=17)
dox 2wk (n=13)

p = 0.8609
med. survival:
797, 676 days

x-axis: Time (days); y-axis: Percent survival

b

p53 +/- with tumors (total)

control (n=27)
dox 2wk (n=70)

p = 0.0008
med. survival:
507, 206 days

x-axis: Time (days); y-axis: Percent survival

c

p53 +/- non-thymic tumors

control (n=26)
dox 2wk (n=25)

p = 0.99
med. survival:
502 days (control)
511 days (dox 2wk)

x-axis: Time (days); y-axis: Percent survival

d

p53 +/- overall survival

dox 1wk (n=12)
dox 2wk (n=115)
dox 4wk (n=17)

x-axis: Time; y-axis: Percent survival

p = 0.0612, 0.0924 (1-2 wk, 2-4 wk)
med. survival:
556 days (1wk)
341 days (2wk)
158 days (4wk)

e

p53 +/- with thymic lymphoma

dox 2wk (n=45)
dox 4wk (n=7)

p = 0.4905
med. survival:
151 days (2wk)
156 days (4wk)

x-axis: Time (days); y-axis: Percent survival

f

p53 +/- non-thymic tumors

dox 2wk (n=25)
dox 4wk (n=6)

p = 0.064
med. survival:
511, 391.5 days

x-axis: Time (days); y-axis: Percent survival

g

p53 +/- overall survival

young dox 2wk (n=115)
adult dox 2wk (n=35)

p = 0.4171
med. survival:
341 days (young)
365 days (adult)

x-axis: Time (days); y-axis: Percent survival

h

p53 +/- with thymic lymphoma

young (n=45)
adult (n=10)

p = 0.3607
med. survival:
151 days (young)
161.5 days (adult)

x-axis: Time (days); y-axis: Percent survival

i

p53 +/- non-thymic tumors

young (n=25)
adult (n=11)

p = 0.423
med. survival:
511 days (young)
476 days (adult)

x-axis: Time (days); y-axis: Percent survival

j

p53 -/- with tumors (total)

control (n=38)
dox 2wk (n=52)

p = 0.032
med. survival:
168, 138.5 days

x-axis: Time (days); y-axis: Percent survival

k

p53 -/- with thymic lymphomas

control (n=17)
dox 2wk (n=40)

p = 0.0197
med. survival:
154, 134 days

x-axis: Time (days); y-axis: Percent survival

l

p53 -/- non-thymic tumors

control (n=21)
dox 2wk (n=12)

p = 0.8917
med. survival:
183 days (control)
168 days (dox 2wk)

x-axis: Time (days); y-axis: Percent survival

m

prg/5-25 p53-/- 2 weeks dox

Thymic lymphoma   Lung metastasis

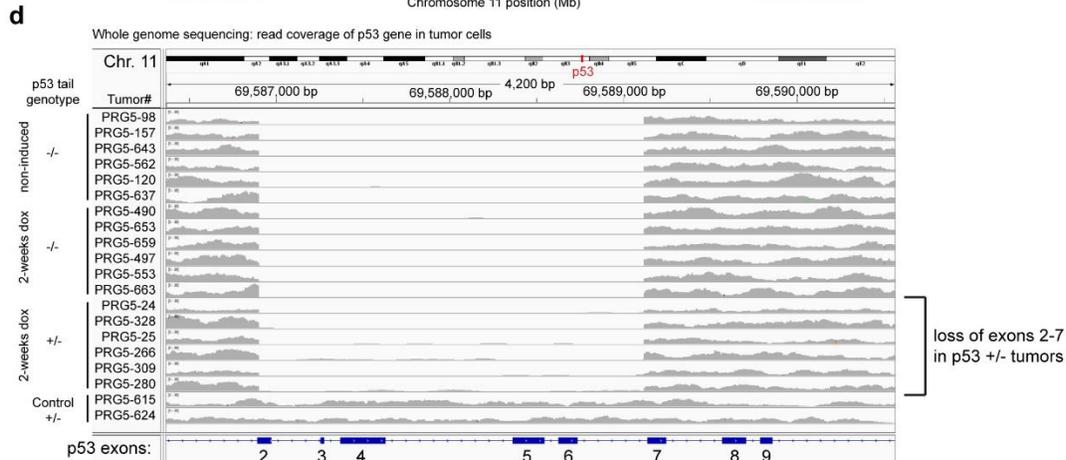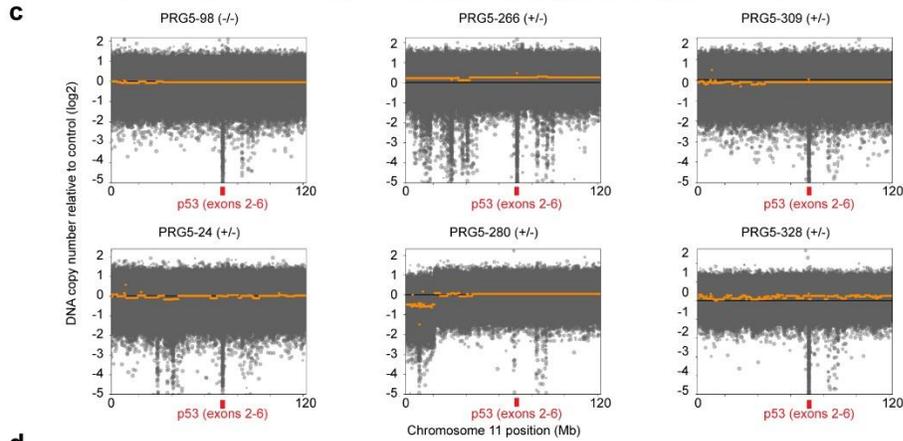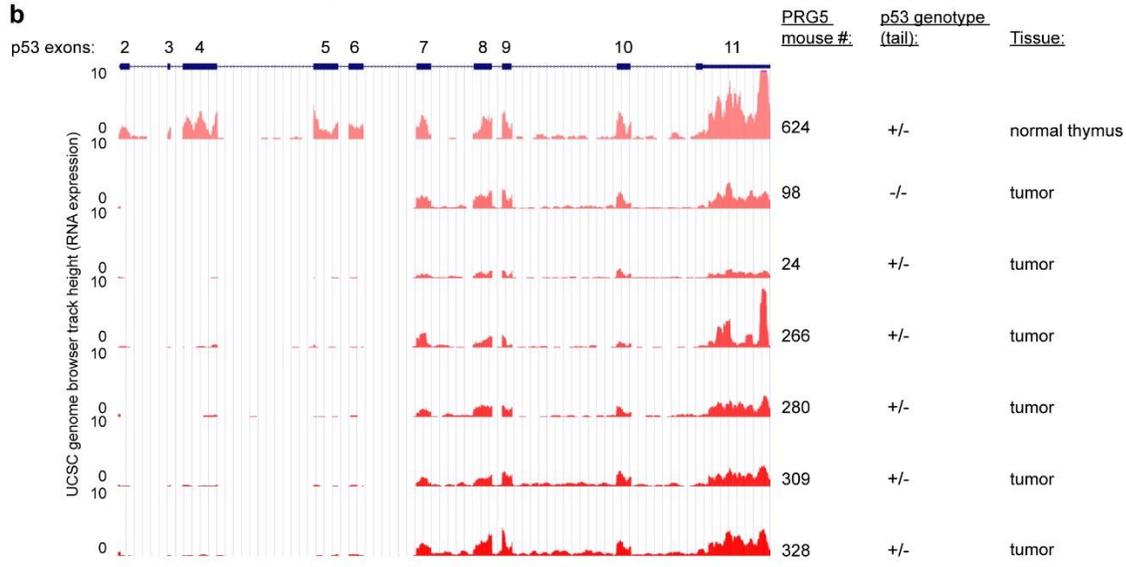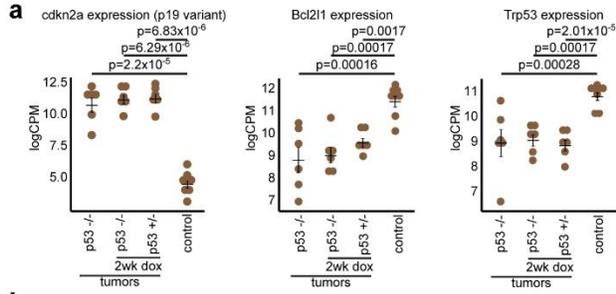Kidney metastasis   Liver metastasis

300uM

4

Supplemental Figure S2 | Effects of timing and duration of chromosome instability on tumor formation. (**a-l**) Survival (Kaplan-Meier) plots of PRG5 mice not treated (control) or treated with the indicated doxycycline regimens (dox 1wk/2wk/4wk – treated with doxycycline for 1/2/4 weeks at the age of 30 days; young/adult dox 2wk – 2 weeks treatment of doxycycline starting at 30/100 days of age) and with the indicated p53 backgrounds (p53+/+, p53+/-, p53-/-). Overall survival and survival of mice bearing either thymic or non-thymic tumors are presented. Comparison of indicated number of mice done using log rank test. (**m**) Representative images of thymic lymphoma metastases from a 2-weeks Plk4 induced p53-/- PRG5 mouse. See Supplemental Table 1 for a histology analysis of tissues from a total of 104 mice.

a

1325 differentially expressed genes with p<0.05

Z_Score
4
2
0
-2
-4

Cohort

PRG5 p53−/−
non-induced

PRG5 p53−/−
2-weeks doxycycline

PRG5 p53+/−
2-weeks doxycycline

} Thymic lymphomas

PRG5 Control
normal thymus

387 978 819 961 980 615 787 562 266 497 490 653 24 818 120 157 328 309 637 98 25 659 553 280 663 643

b

FDR ≤ 0.05

Webgestalt_GSEA_tumorVscontrol_pathway_KEGG

Cell cycle
RNA transport
Pyrimidine metabolism
DNA replication
Ribosome biogenesis in eukaryotes
Purine metabolism
Biosynthesis of amino acids
Arachidonic acid metabolism
PPAR signaling pathway

-2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5
Normalized Enrichment Score

6

Supplemental Figure S3 | Differential gene expression analysis comparing thymic lymphomas and normal thymus. (**a**) Differentially expressed genes as determined using RNA sequencing between control normal thymuses and PRG5 derived thymic lymphomas. See Supplemental Table 2 for a complete list of the differentially expressed genes (**b**) Significantly enriched KEGG pathways in control thymuses and in PRG5 derived thymic lymphomas determined using Webgestalt GSEA analysis.

Webgestalt_GSEA_tumorVscontrol_geneontology_Biological_Process

chromosome organization
DNA metabolic process
ribonucleoprotein complex biogenesis
ribosome biogenesis
cell cycle process
chromosome segregation
ncRNA metabolic process
ncRNA processing
DNA replication
DNA-dependent DNA replication
cell cycle
RNA processing
cellular response to DNA damage stimulus
mitotic cell cycle phase transition
sister chromatid segregation
regulation of DNA metabolic process
regulation of G2/M transition of mitotic cell cycle
double-strand break repair via homologous recombination
telomere organization
protein-DNA complex subunit organization
nucleoside monophosphate metabolic process
ribonucleoprotein complex assembly
spindle organization
glycosyl compound metabolic process
nucleoside metabolic process
chromatin assembly
regulation of protein modification by small protein conjuga...
meiotic cell cycle process
positive regulation of mitotic cell cycle phase transition
regulation of mitotic nuclear division
negative regulation of mitotic cell cycle phase transition
regulation of response to DNA damage stimulus
translation
nucleic acid transport
pyrimidine-containing compound metabolic process
macromolecule methylation
nucleoside triphosphate biosynthetic process
modification-dependent protein catabolic process
nucleic acid phosphodiester bond hydrolysis
nucleobase-containing compound catabolic process
purine ribonucleoside monophosphate metabolic process
macromolecule catabolic process
nucleoside phosphate biosynthetic process
nucleocytoplasmic transport
negative regulation of T cell proliferation
covalent chromatin modification
alpha-amino acid metabolic process

response to purine-containing compound
response to tumor necrosis factor
response to acid chemical
neutral lipid metabolic process
acylglycerol metabolic process
monocarboxylic acid metabolic process
lipid biosynthetic process
cell-cell adhesion via plasma-membrane adhesion molecules
glycerolipid metabolic process
fatty acid metabolic process
cellular lipid metabolic process
response to ethanol
lipid metabolic process

Normalized Enrichment Score

FDR ≤ 0.05

8

Supplemental Figure S4 | Pathway analysis reveals differences between thymic lymphomas and normal thymus. Significantly enriched Gene Ontology (Biological Processes) pathways in control thymuses and in PRG5 derived thymic lymphomas determined using Webgestalt GSEA analysis.

a

cdkn2a expression (p19 variant)
p=6.83x10^{-6}
p=6.29x10^{-8}
p=2.2x10^{-5}

Bcl2l1 expression
p=0.0017
p=0.00017
p=0.00016

Trp53 expression
p=2.01x10^{-5}
p=0.00017
p=0.00028

b

PRG5 mouse #:	p53 genotype (tail):	Tissue:
624	+/-	normal thymus
98	-/-	tumor
24	+/-	tumor
266	+/-	tumor
280	+/-	tumor
309	+/-	tumor
328	+/-	tumor

p53 exons: 2 3 4 5 6 7 8 9 10 11

UCSC genome browser track height (RNA expression)

c

PRG5-98 (-/-)   PRG5-266 (+/-)   PRG5-309 (+/-)

PRG5-24 (+/-)   PRG5-280 (+/-)   PRG5-328 (+/-)

DNA copy number relative to control (log2)

p53 (exons 2-6)

Chromosome 11 position (Mb)

d

Whole genome sequencing: read coverage of p53 gene in tumor cells

Chr. 11   p53

69,587,000 bp   69,588,000 bp   4,200 bp   69,589,000 bp   69,590,000 bp

p53 tail genotype	Tumor#
non-induced -/-	PRG5-98
	PRG5-157
	PRG5-643
	PRG5-562
	PRG5-120
	PRG5-637
2-weeks dox -/-	PRG5-490
	PRG5-653
	PRG5-659
	PRG5-497
	PRG5-553
	PRG5-663
2-weeks dox +/-	PRG5-24
	PRG5-328
	PRG5-25
	PRG5-266
	PRG5-309
	PRG5-280
Control +/-	PRG5-615
	PRG5-624

loss of exons 2-7 in p53 +/- tumors

p53 exons: 2 3 4 5 6 7 8 9

10

Supplemental Figure S5 | p53 LOH occurs through whole-chromosome mis-segregation without structural changes. (**a**) Comparison of mRNA expression levels (log[counts per million]) of indicated PRG5 tumors (n=6 tumors from each group) and control thymuses (n=8). Mean ± SD, and p-values calculated using one-way ANOVA are presented. (**b**) UCSC genome browser tracks showing RNA expression of exons 2-11 of p53 in indicated mice. (**c**) CNVkit derived DNA copy number plots of chromosome 11 in tumors from the indicated PRG5 mice. Black line represents the diploid control and the orange line represents the mean copy number of the indicated sample. The location of the *p53* gene is indicated in red. (**d**) Zoom on exons 2-6 of the p53 gene using Integrated Genome Viewer (IGV 2.3.97) showing number of reads in the indicated samples.
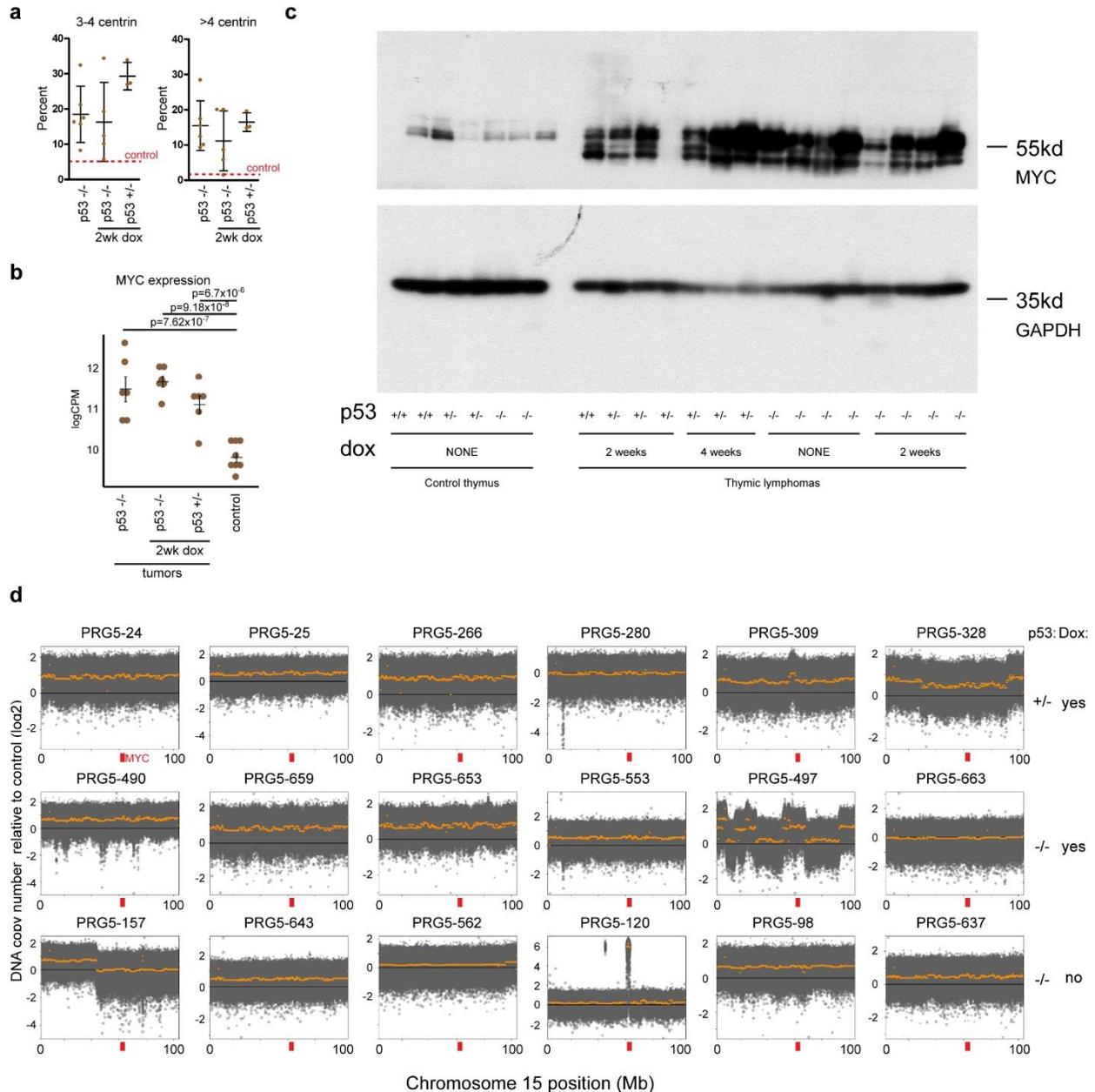
Supplemental Figure S6 | Common molecular events in spontaneous and induced lymphomas. (**a**) Measurement of centrin-GFP foci in thymic lymphomas from indicated PRG5 mice. Mean ± SD of n=6 (p53-/-), n=5 (p53-/- 2wk dox), and n=3 (p53+/- 2wk dox) are presented. Dashed red line represents the control shown in Figure 2f. (**b**) Comparison of mRNA expression levels (log[counts per million]) of indicated PRG5 tumors (n=6 tumors from each group) and control thymuses (n=8). Mean ± SD, and p-values calculated using one-way ANOVA are presented. (**c**) Myc protein levels as seen using

SDS gel electrophoresis immunoblotting in the indicated control or tumor samples. GAPDH is shown as a loading control. (**d**) CNVkit derived DNA copy number plots of chromosome 15 in tumors from the indicated PRG5 mice. Black line represents the diploid control and the orange line represents the mean copy number of the indicated sample. The location of the MYC gene is indicated in red.
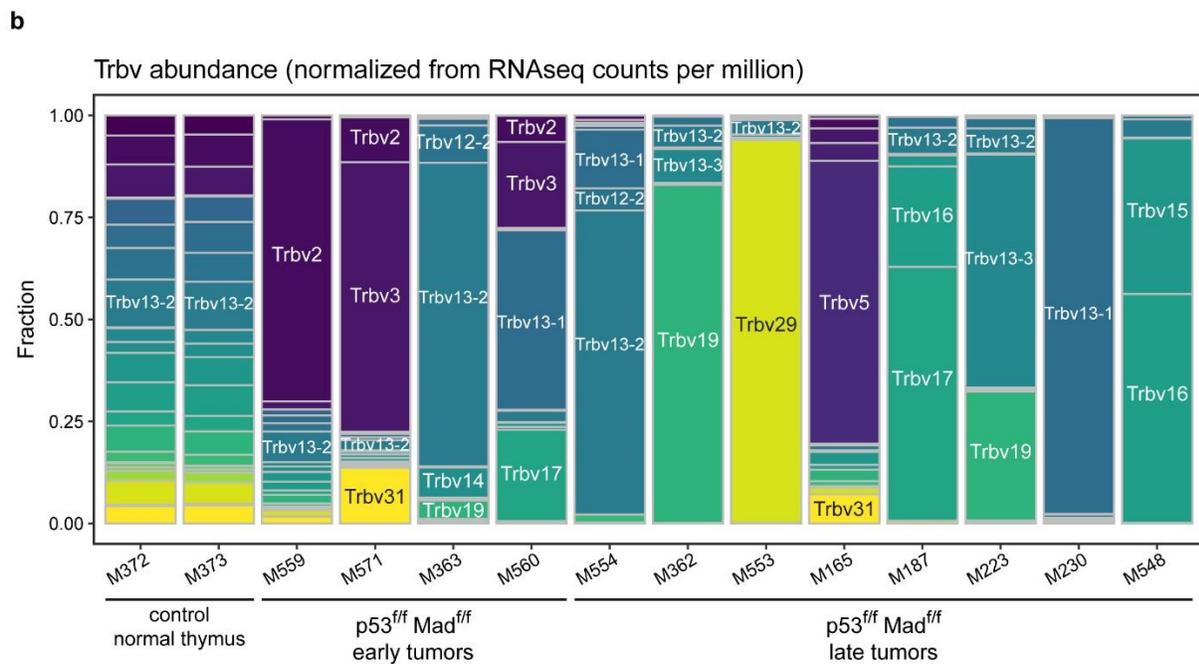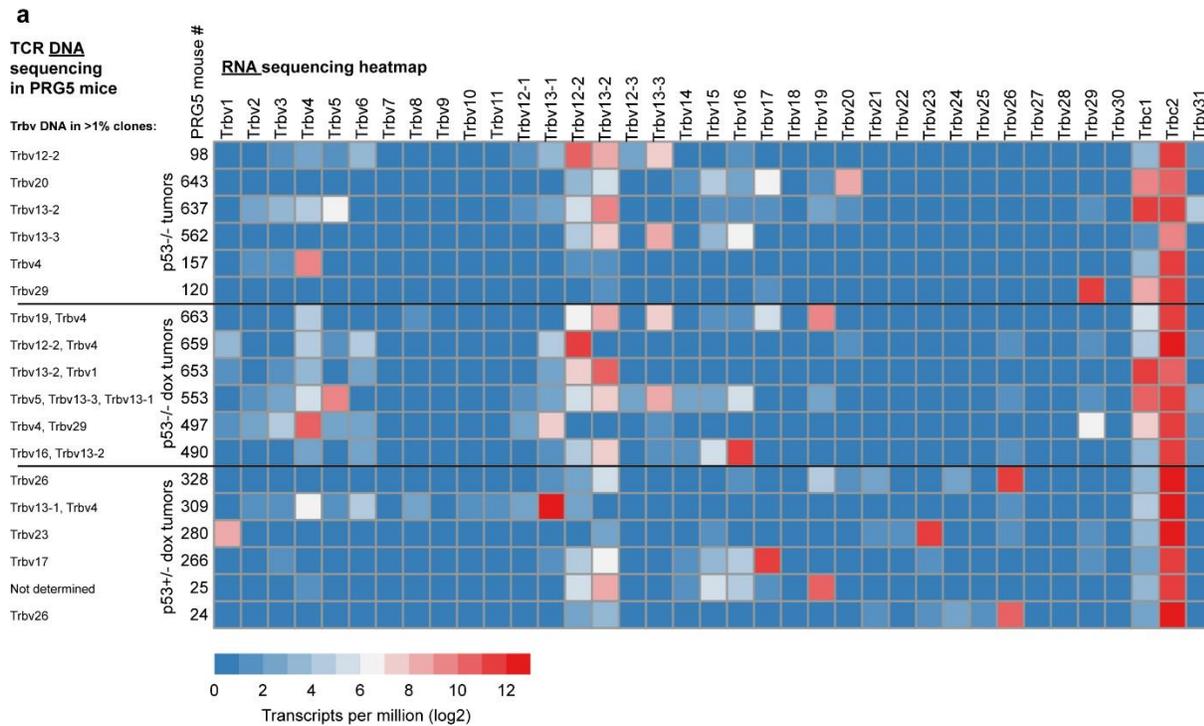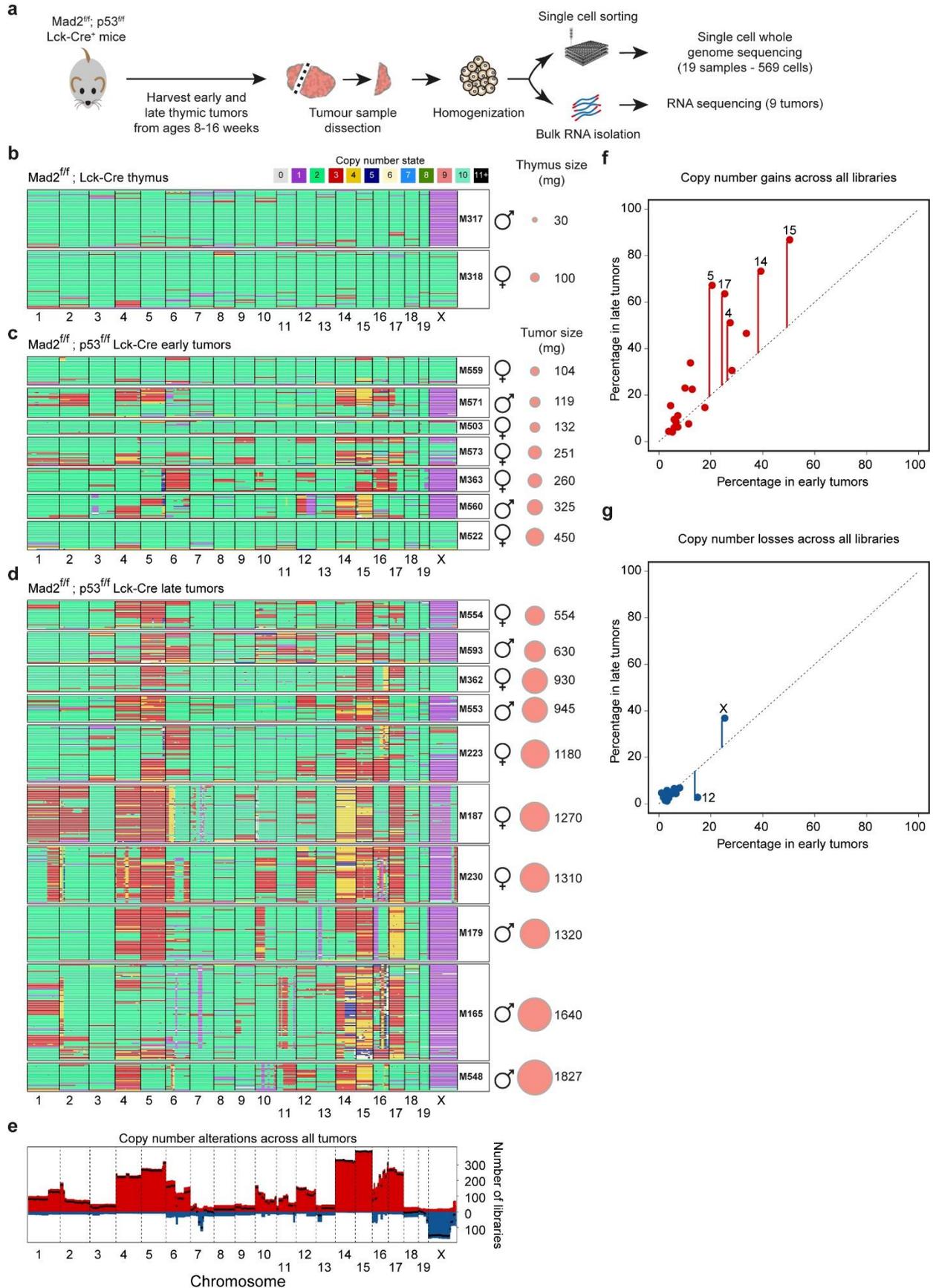
Supplemental Figure S7 | Tumor clonality derived from RNA sequencing data. (a-b) Expression of T cell receptor variable region genes (Trbv genes) as determined using RNA sequencing in tumors from (**a**) PRG5 mice and (**b**) Mad2 mice. Trbv genes with frequency > 1% in PRG5 tumors as detected using T cell receptor DNA sequencing (Figure 3a) are shown in **a**.
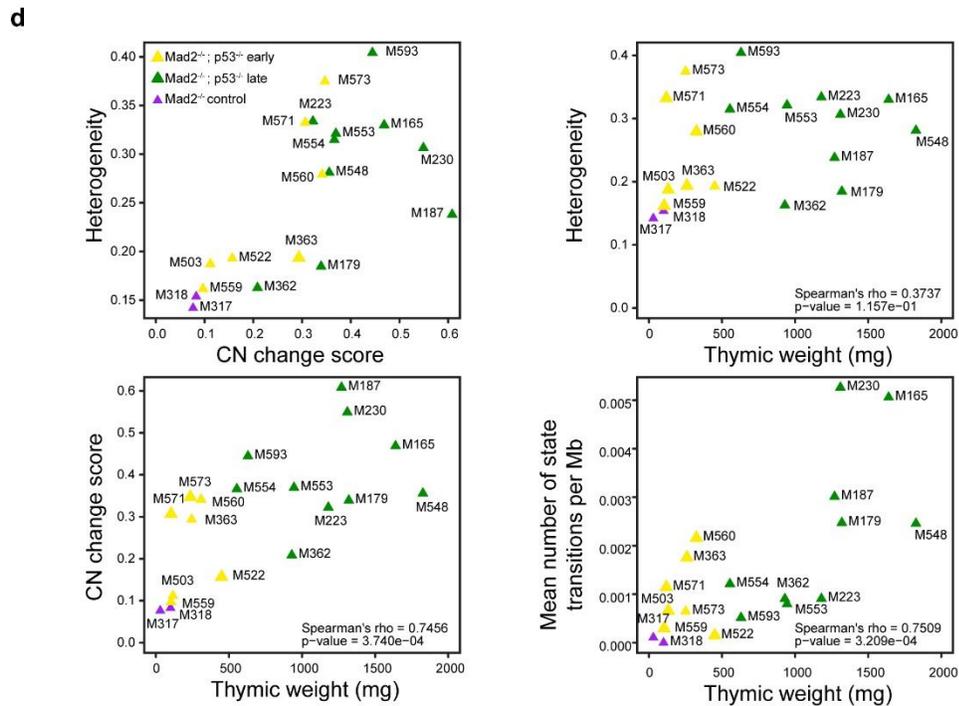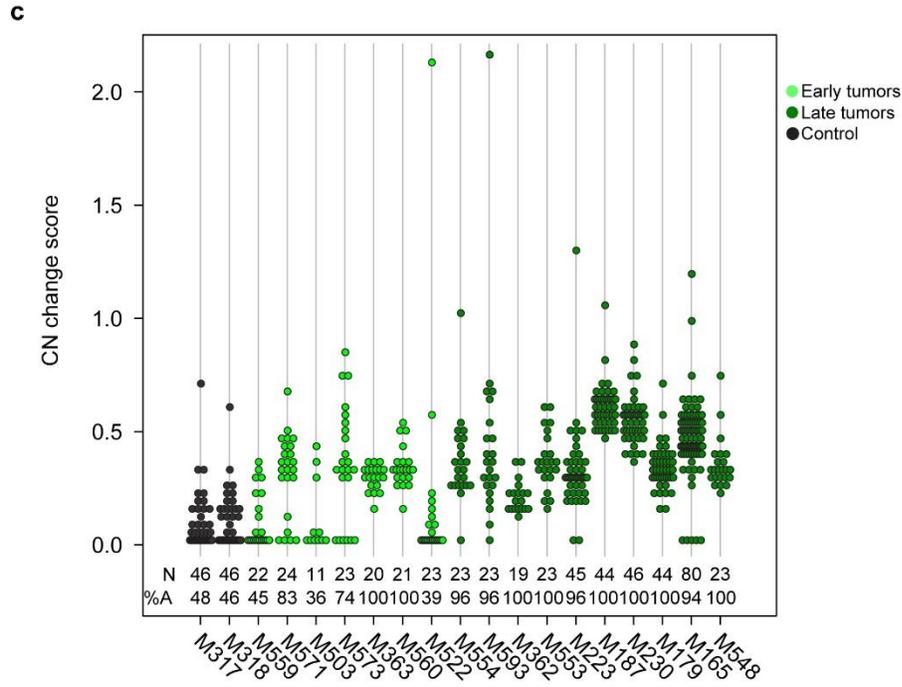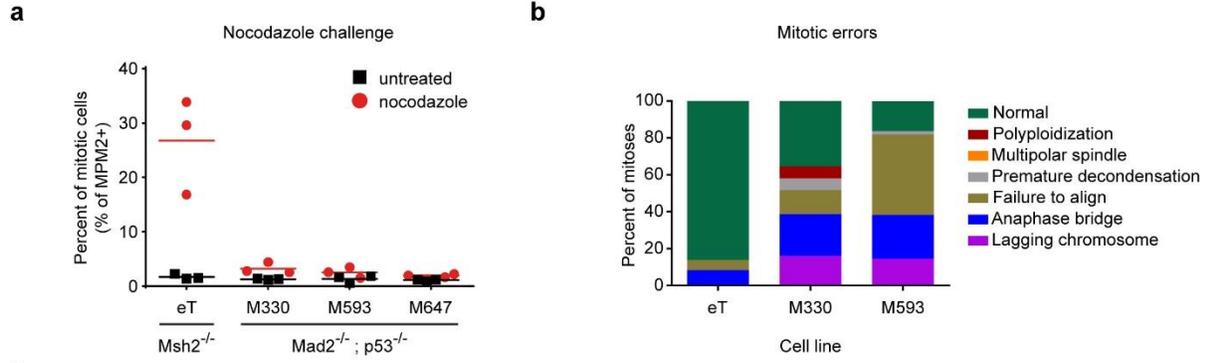
14

a

Single biopsies:

b

Multi-focal biopsies:

15
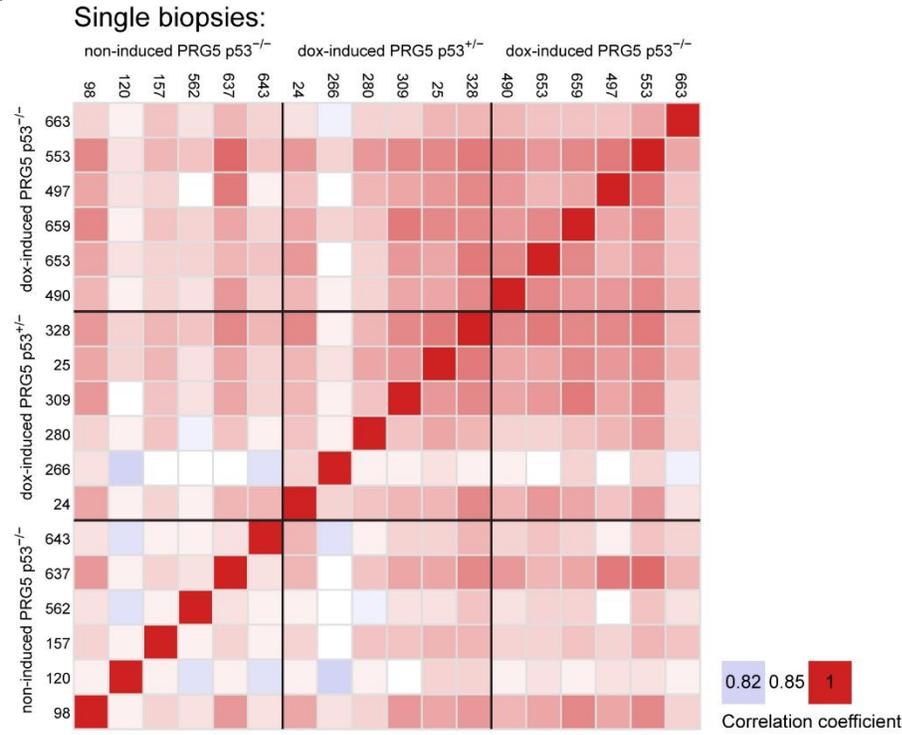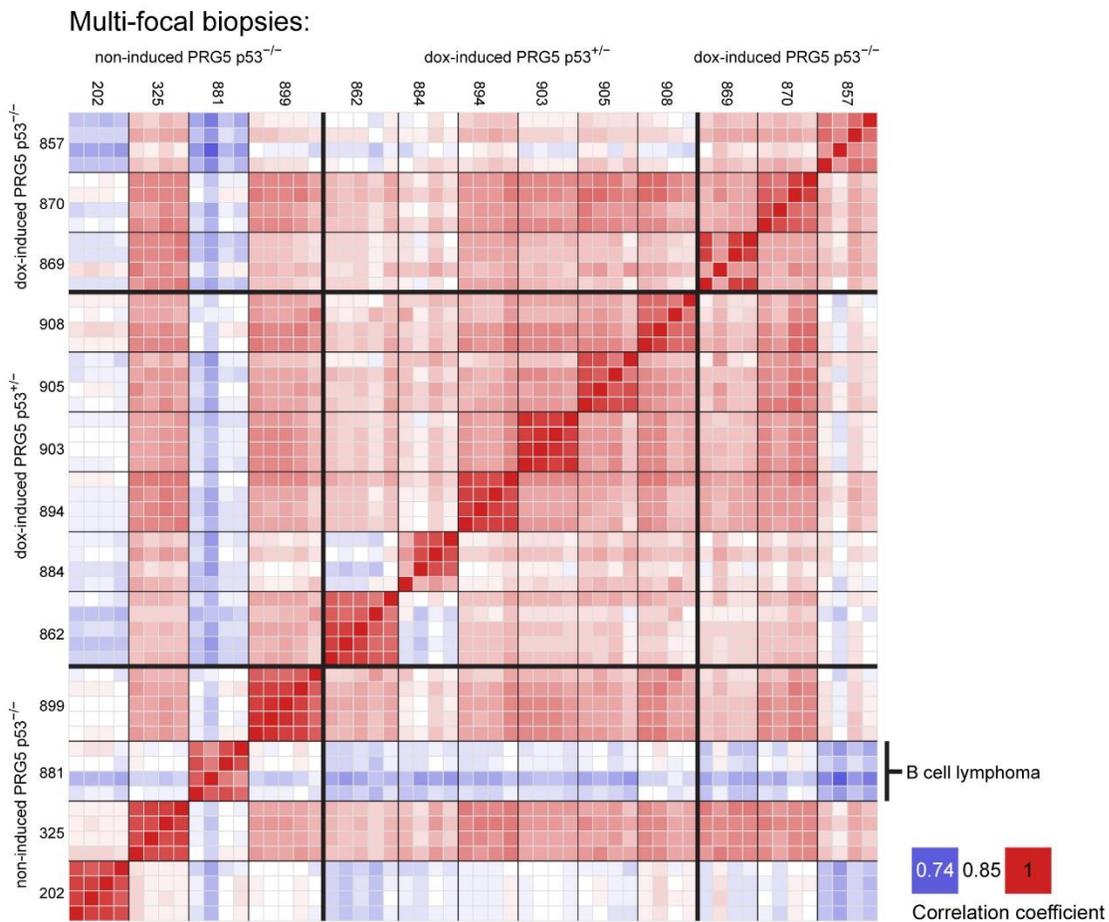
Supplemental Figure S8 | Comparison of CIN-induced and non-induced thymic lymphoma transcriptomes. (**a-b**) Heatmaps showing pair-wised Pearson correlation coefficients among samples (**a** - tumor single biopsies, **b** – multi-focal tumor biopsies) using transcript-per-million (TPM) values as determined using RNA sequencing of the PRG5 mice.
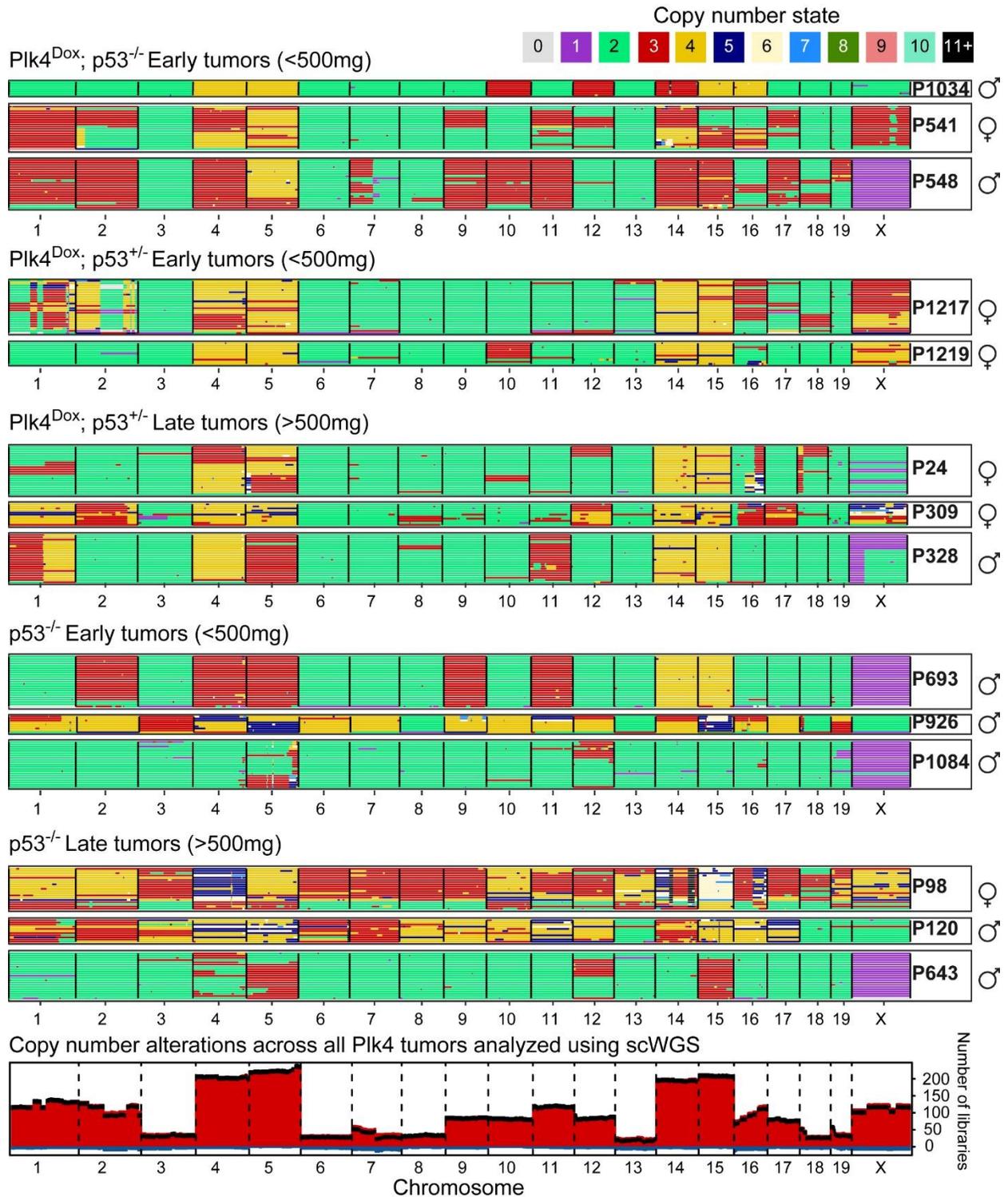
a Single biopsies
Whole genome DNA sequencing: 100kb binning

Copy number relative to 2N
2 1 0 -1 -2

Cohort
PRG5 p53-/- non-induced
PRG5 p53-/- dox-induced
PRG5 p53+/- dox-induced

PRG5 mouse#:
98, 157, 643, 562, 120, 637, 490, 653, 659, 497, 553, 663, 24, 328, 25, 266, 309, 280

Chromosome: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X

b Multi-focal biopsies
Whole genome DNA sequencing: 100kb binning

Copy number relative to 2N
2 1 0 -1 -2

Cohort
PRG5 p53-/- non-induced
PRG5 p53-/- dox-induced
PRG5 p53+/- dox-induced

PRG5 mouse#:
325.3, 325.1, 325.5, 325.7, 870.5, 870.7, 870.1, 870.3, 884.2, 884.6, 884.5, 884.8

Chromosome: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X

c RNA sequencing: correlation coefficients

PRG5-325: 325.1 325.3 325.5 325.7
0.97, 0.97, 0.98, 0.97, 0.97, 0.98, 1, 1, 1, 1

PRG5-870: 870.1 870.3 870.5 870.7
0.97, 0.96, 0.95, 0.95, 0.94, 0.98, 1, 1, 1, 1

PRG5-884: 884.2 884.5 884.6 884.8
0.9, 0.92, 0.9, 0.97, 0.96, 0.97, 1, 1, 1, 1

d TCR sequencing

PRG5-325
1		7
	5	
3		

PRG5-870
1		7
	5	
3		

PRG5-884
| 2 | 5 | 8 |
| | 6 | |

biopsy # indicated for each biopsy

e RNA sequencing: Expression across chromosomes relative to control

chrX **, **
chr19 ***, ***, ***
chr18
chr17
chr16 *, ***
chr15 ***, ***
chr14 ***
chr13 **, ***
chr12 ***, ***
chr11 ***, ***
chr10 ***
chr9 *
chr8
chr7 ***, ***
chr6 ***, ***
chr5 ***, ***
chr4 ***, ***
chr3 ***, **
chr2 **, **
chr1

Legend:
p53+/- Plk4 Dox
p53-/- Plk4 Dox
p53-/-

Fold Change (relative to normal thymus)
1/4 1/2 1 2 4

17

Supplemental Figure S9 | DNA copy number analysis in CIN-induced and non-induced thymic lymphomas. (**a**) Heatmap showing 100-kb DNA copy number changes in late tumors (>500mg) from non-induced p53-/- PRG5 mice (black), two-weeks doxycycline treated (at the age of 30 days) p53-/- PRG5 mice (dark grey) and p53+/- PRG5 mice (light grey) as determined using whole-genome sequencing. (**b**) Heatmap showing 100-kb DNA copy number changes in multiple regions taken from late tumors (>500mg) from non-induced p53-/- PRG5 mice (black), two-weeks doxycycline treated (at the age of 30 days) p53-/- PRG5 mice (dark grey) and p53+/- PRG5 mice (light grey) as determined using whole-genome sequencing. (**c**) Heatmaps showing pair-wised Pearson correlation coefficients among samples using TPM values as determined by RNA sequencing of the indicated PRG5 tumor biopsies. (**d**) Top ten T cell receptor frequencies (indicative of T cell clones) in thymic T cell lymphomas from PRG5 mice in multi-regional biopsies as determined using T cell receptor sequencing (these samples are also shown in Figure 3b and presented here for convenience). (**e**) Gene expression levels from each chromosome. The fold change of each gene between each tumor group and the control (n=6 for each group) was calculated, and boxplots of grouping all genes by chromosome for each comparison are presented. p-values (comparing expression levels from individual chromosomes between of each tumor group and the control) were determined using wilcoxon rank sum test, *: $p < 0.05$; **:$p < 0.01$ and ***:$p < 0.001$.

a Mad2^f/f; p53^f/f Lck-Cre^+ mice → Harvest early and late thymic tumors from ages 8-16 weeks → Tumour sample dissection → Homogenization → Single cell sorting → Single cell whole genome sequencing (19 samples - 569 cells); Bulk RNA isolation → RNA sequencing (9 tumors)

b Mad2^f/f; Lck-Cre thymus

Copy number state: 0 1 2 3 4 5 6 7 8 9 10 11+

M317 ♂ Thymus size (mg): 30
M318 ♀ 100

Chromosomes: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X

c Mad2^f/f; p53^f/f Lck-Cre early tumors

Tumor size (mg):
M559 ♀ 104
M571 ♂ 119
M503 ♀ 132
M573 ♀ 251
M363 ♀ 260
M560 ♂ 325
M522 ♀ 450

Chromosomes: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X

d Mad2^f/f; p53^f/f Lck-Cre late tumors

M554 ♀ 554
M593 ♂ 630
M362 ♀ 930
M553 ♂ 945
M223 ♀ 1180
M187 ♀ 1270
M230 ♀ 1310
M179 ♂ 1320
M165 ♂ 1640
M548 ♂ 1827

Chromosomes: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X

e Copy number alterations across all tumors

Number of libraries

Chromosome: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X

f Copy number gains across all libraries

Percentage in late tumors vs Percentage in early tumors
Labeled points: 5, 17, 4, 14, 15

g Copy number losses across all libraries

Percentage in late tumors vs Percentage in early tumors
Labeled points: X, 12

19

Supplemental Figure S10 | Aneuploidy evolution in thymic lymphomas under chronic CIN driven by deletion of Mad2. (**a**) Overview of thymic tumors collection from Lck-Cre+; Mad2f/f; p53f/f mice sampled at 8-16 weeks of age. (**b-d**) Heatmaps showing DNA copy number using single cell whole-genome sequencing of cells collected from (**b**) Lck-Cre+; Mad2f/f mice (**c**) Lck-Cre+; Mad2f/f; p53f/f mice with developing (early) tumors and (**d**) Lck-Cre+; Mad2f/f; p53f/f mice with terminal (late) tumors. Genomic position in order from chromosome 1 to X are in the x-axis and individual cells are in the y-axis. Colors indicate the copy number state as determined by AneuFinder. Data of samples M187 and M179 was previously reported in Foijer et al. elife, 2017. Sample M165 is a new sequencing experiment of a sample also shown in Foijer et al. elife, 2017. (e) Genome-wide overview of cumulative copy number (1 Mb bins) gains (red) and losses (blue) across all thymic lymphomas presented in panels **c-d**. Black line presents the net change; difference between number of libraries with a copy number gain and the number of libraries with a copy number loss. (**f-g**) Comparison of copy number gains (**f**) and losses (**g**) between early and late tumors. Each dot represents one chromosome.

a Nocodazole challenge

■ untreated
● nocodazole

b Mitotic errors

Normal
Polyploidization
Multipolar spindle
Premature decondensation
Failure to align
Anaphase bridge
Lagging chromosome

c

● Early tumors
● Late tumors
● Control

| N | 46 | 46 | 22 | 24 | 11 | 23 | 20 | 21 | 23 | 23 | 23 | 19 | 23 | 45 | 44 | 46 | 44 | 80 | 23 |
| %A | 48 | 46 | 45 | 83 | 36 | 74 | 100 | 100 | 39 | 96 | 96 | 100 | 100 | 96 | 100 | 100 | 100 | 94 | 100 |

d

▲ Mad2⁻/⁻ ; p53⁻/⁻ early
▲ Mad2⁻/⁻ ; p53⁻/⁻ late
▲ Mad2⁻/⁻ control

Spearman's rho = 0.3737
p-value = 1.157e-01

Spearman's rho = 0.7456
p-value = 3.740e-04

Spearman's rho = 0.7509
p-value = 3.209e-04

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Supplemental Figure S11 | Mad2/p53-deficient thymic lymphomas lack a functional SAC and display ongoing CIN. (**a**) Percentage of mitotic (MPM2+) cells in primary Mad2-/-; p53-/- (M330, M593, M647), or Msh2-/- (euploid Thymic, eT) thymic lymphoma cultures with or without a nocodazole challenge for six hours. (**b**) Frequency of abnormal mitoses observed in Mad2-/-; p53-/-; (M330, M593), or Msh2-/- (eT) primary thymic lymphoma cultures as determined using live-cell time-lapse imaging after transduction with H2B-mCherry. (**c**) Analysis of copy number changes of the samples pf Figure 1b-d. The numbers below the dot plots indicate the total number of cells and the percentage of cells that has at least one whole chromosome gain or loss. Controls (M317 and M318) are cells sequenced from normal (non-tumor) Mad2-/- (p53 wild-type) mice. (**d**) Genome-wide karyotype measures (heterogeneity score and CN [copy number] change score) for all samples shown in Figure 1b-d. Structural aberration scores (Mean number of state transitions per Mb) were defined by the number of copy number state transitions per Mb plotted against the weight of the thymus at time of harvest. Correlations were determined using Spearman's rank-order test. See methods for details about the karyotype measures displayed.

Plk4^Dox; p53^-/- Early tumors (<500mg)

Plk4^Dox; p53^+/- Early tumors (<500mg)

Plk4^Dox; p53^+/- Late tumors (>500mg)

p53^-/- Early tumors (<500mg)

p53^-/- Late tumors (>500mg)

Copy number alterations across all Plk4 tumors analyzed using scWGS

Supplemental Figure S12 | Single cell DNA sequencing of early and late PRG5 tumors. (**a**) Heatmaps showing DNA copy number using single cell whole-genome sequencing of Plk4 induced or control early and late PRG5 tumors with the indicated p53 backgrounds. Genomic position in order from chromosome 1 to X are in the x-axis and individual cells are in the y-axis. Colors indicate the copy number state as determined by AneuFinder. (**b**) Genome-wide overview of cumulative copy number (1 Mb bins) gains (red) and losses (blue) across all thymic lymphomas presented in panel **a**. Black line presents the net change; difference between number of libraries with a copy number gain and the number of libraries with a copy number loss.
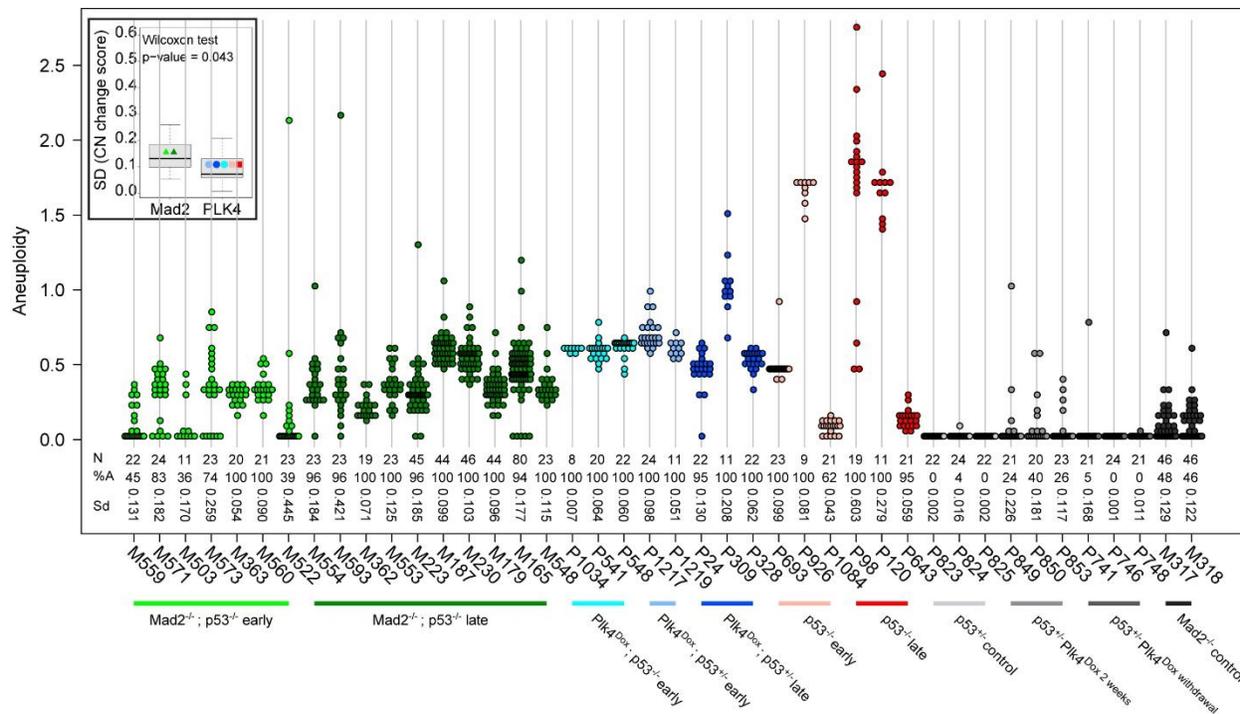
Supplemental Figure S13 | Differences between chronic and transient CIN in driving thymic lymphomas. Analysis of copy number changes of the entire Mad2 and PRG5 cohorts. The numbers below the dot plots indicate the total number of cells (N), the percentage of cells that has at least one whole chromosome gain or loss (%A) and the standard deviation of the copy number change scores of each sample (Sd). The insert boxplot shows the distribution of standard deviation values of the Mad2 and PLK4 samples. A Wilcoxon rank-sum test was used to determine if there is a significant difference between the SD values of the Mad2 and PLK4 samples.

ID	qvalue
REACTOME_M_PHASE	0.00003
REACTOME_MITOTIC_G1_G1_S_PHASES	0.00003
REACTOME_DNA_REPLICATION	0.00003
REACTOME_S_PHASE	0.00007
KEGG_CELL_CYCLE	0.00011
REACTOME_REGULATION_OF_CHOLESTEROL_BIOSYNTHESIS_BY_SREBP_SREBF	0.00034
REACTOME_CELL_CYCLE_CHECKPOINTS	0.00053
REACTOME_ACTIVATION_OF_ATR_IN_RESPONSE_TO_REPLICATION_STRESS	0.00241
REACTOME_ACTIVATION_OF_GENE_EXPRESSION_BY_SREBF_SREBP	0.00451
REACTOME_DNA_REPLICATION_PRE_INITIATION	0.00481
REACTOME_MITOTIC_PROMETAPHASE	0.00543
REACTOME_SWITCHING_OF_ORIGINS_TO_A_POST_REPLICATIVE_STATE	0.00655
REACTOME_ACTIVATION_OF_THE_PRE_REPLICATIVE_COMPLEX	0.00850
REACTOME_ONCOGENE_INDUCED_SENESCENCE	0.01062
REACTOME_MITOTIC_METAPHASE_AND_ANAPHASE	0.01988
REACTOME_G1_PHASE	0.03175
REACTOME_METABOLISM_OF_STEROIDS	0.03213

Supplemental Figure S14 | Acquisition of the aneuploidy specific profile reshapes gene expression of cell cycle, replication, and stress networks. Significantly enriched pathways (identified using msigdb and R/Bioconductor package clusterProfiler) in PRG5 and Mad2 derived thymic lymphomas with high similarity to the GISTIC analysis using all tumor cohorts as shown in Figure 4 (see gene list in Supplemental Table 5 and heatmap in Figure 6a).

Supplemental Tables (see attached files):

Supplemental Table 1 | Pathological analysis of tissue sections from PRG5 mice.

Supplemental Table 2 | Differentially expressed genes between thymic tumors and control thymuses as determined using RNA sequencing.

Supplemental Table 3 | Top 10 T cell receptor sequences sequences per sample as determined by T cell receptor sequencing

Supplemental Table 4 | Similarity to the GISTIC profile of PRG5 and Mad2 tumors (score ranges between 0-lowest and 1-highest).

Supplemental Table 5 | Gene expression analysis in correlation with the similarity index of each tumor in PRG5 mice (values represent Z scores)