Supplementary Information

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Figure S12. *Atm*$^{-/-}$ thymic lymphomas display *Tcrα/δ* and *Igh*-associated genomic instability.

Figure S13. Defective handling of V(D)J recombination intermediates in *Rag2*$^{c/c}$ lymphocytes.

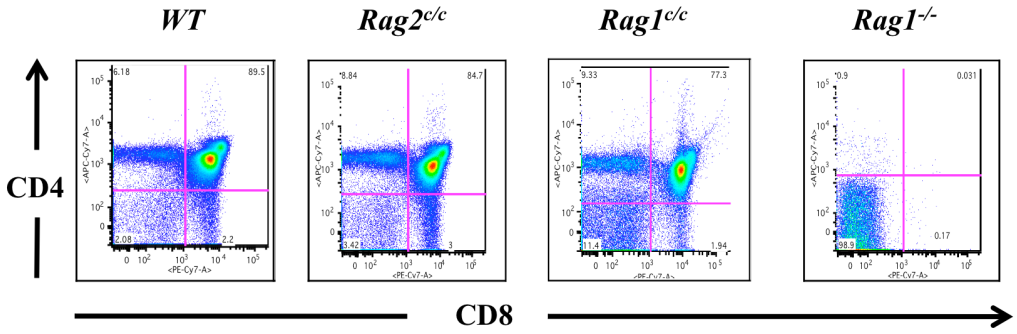
Table S1. Genomic instability in *Rag2*$^{-/-}$ *p53*$^{-/-}$ thymic lymphomas.

Supplementary Reference

Supplementary Figure S1.

a

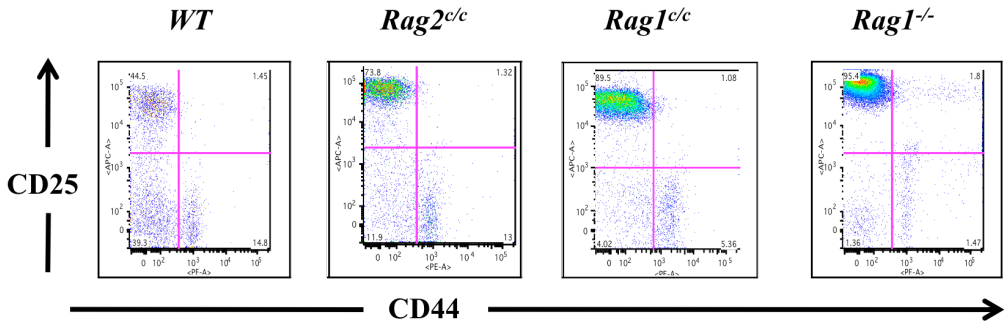
Thymus



	% CD4⁺CD8⁺	% CD4⁺	%CD8⁺	%CD4⁻CD8⁻
WT	88.37±1.59	6.42±1.37	2.31±0.62	1.85±0.46
RAG2$^{c/c}$	86.92±1.87	6.94±1.41	2.26±0.39	3.30±0.53
RAG1$^{c/c}$	77.70±1.40	10.29±0.58	2.72±0.41	9.27±1.59
RAG$^{-/-}$	0.06±0.00	0.39±0.01	0.17±0.14	99.10±0.28

b

CD4⁻CD8⁻ gated



	% CD44⁻CD25⁺ (DN3)	% CD44⁻CD25⁻ (DN4)
WT	38.92±11.81	46.10±11.73
RAG2$^{c/c}$	74.27±9.08	15.25±4.58
RAG1$^{c/c}$	90.70±2.39	5.25±0.89
RAG$^{-/-}$	95.5±1.13	1.71±1.06

Figure S1. T cell development in *Rag2$^{c/c}$* and *Rag1$^{c/c}$ knock-in* mice.
Thymocytes were stained with APC-Cy7-anti-CD4, PE-Cy7-anti-CD8, APC-anti-CD25 and PE-anti-CD44. **a.** Upper, representative FACScan profile of live thymic lymphocytes

from the indicated genotypes analyzed for the surface expression of CD4 and CD8. Lower, Percentages indicated are the mean and standard deviation of at least three repetitions of this experiment. **b.** Upper, representative FACScan profile of gated $CD4^-$ /$CD8^-$ thymocytes from the indicated genotypes analyzed for the surface expression of CD25 and CD44. Lower, Percentages indicated are the mean and standard deviation of at least three repetitions of this experiment. (Complete analysis of the *core-RAG2* and *core-RAG1 knock-in* mice have been previously published[15,24]).

Supplementary Figure S2.

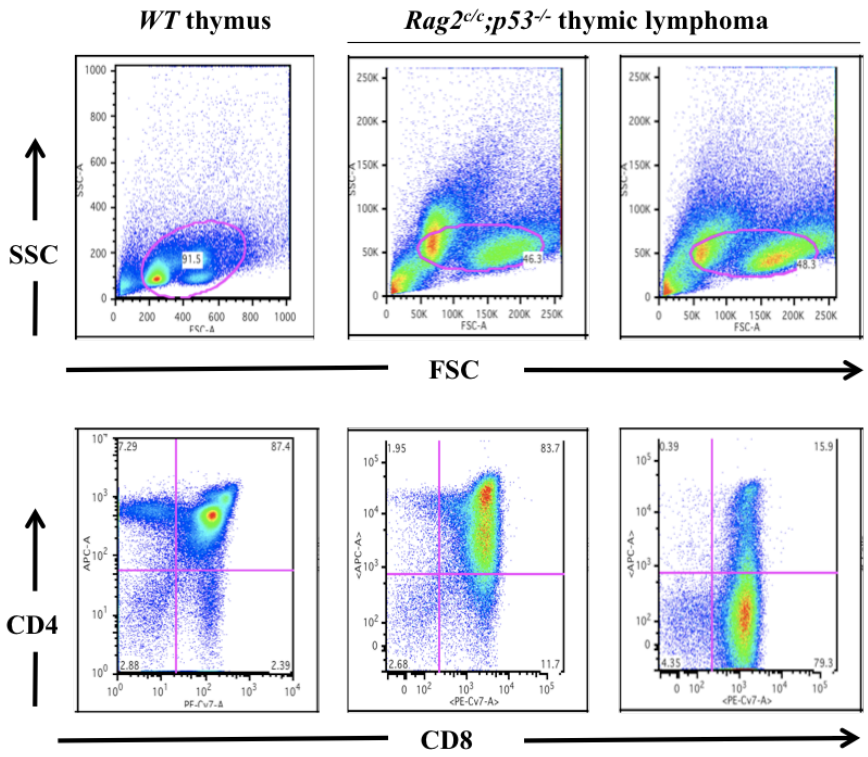
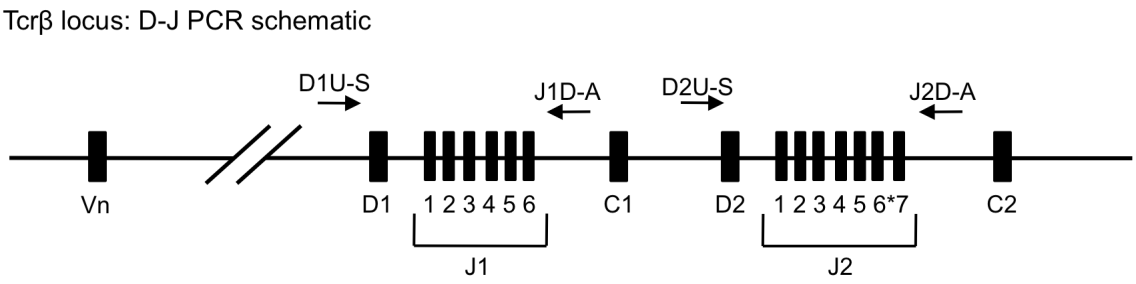
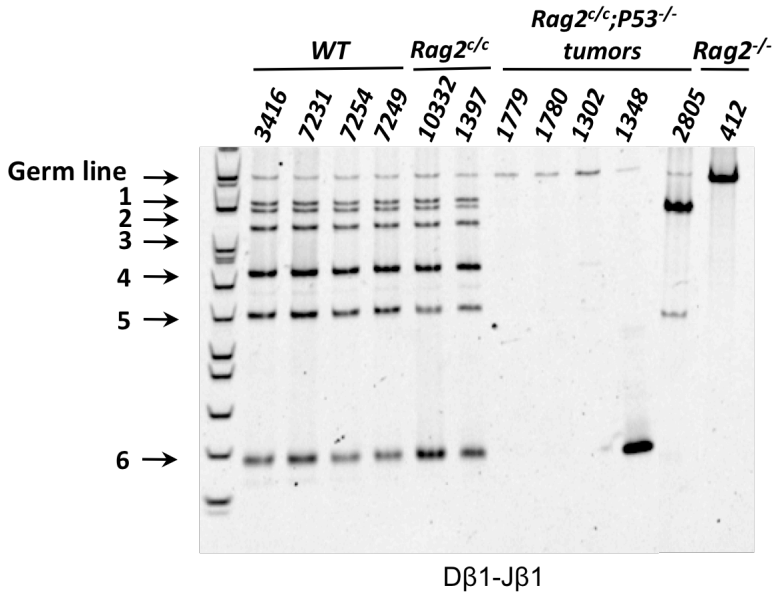


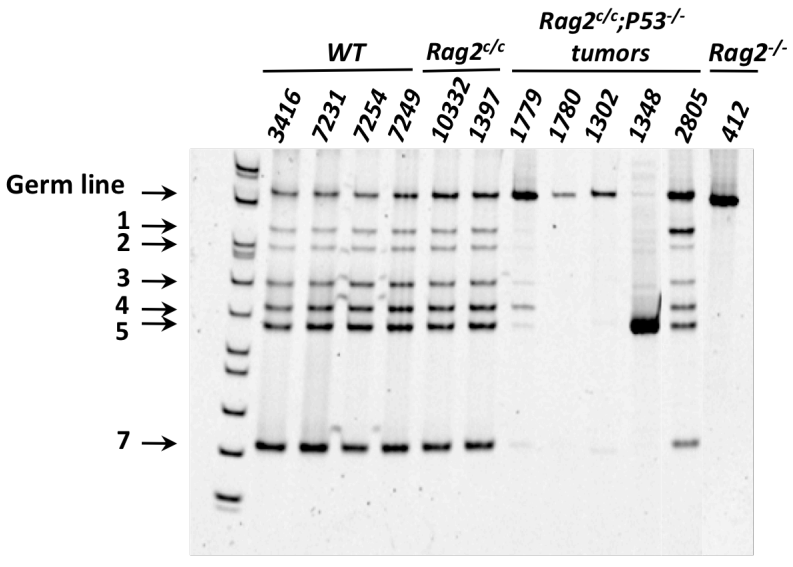
Figure S2. Flow cytometry analysis of *Rag2$^{c/c}$ p53$^{-/-}$* thymic lymphomas. Representative FACScan profile of thymic lymphoma in *Rag2$^{c/c}$ p53$^{-/-}$*. Thymocytes derived from two lymphomas in *Rag2$^{c/c}$ p53$^{-/-}$* mice and from a healthy wild type mouse were analyzed for the surface expression of CD4 and CD8. Plots with the FSC (x axis) versus SSC (y axis) indicate the large-sized lymphoma blasts in the tumors.

Supplementary Figure S3.





Dβ1-Jβ1



Dβ2-Jβ2

Figure S3. PCR analysis of rearrangements at the *Tcrβ* locus in thymocytes and thymic lymphomas.
PCR analysis of Dβ1 to Jβ1 and Dβ2 to Jβ2 rearrangements in *WT*, *Rag2*$^{c/c}$, *Rag2*$^{-/-}$ thymocytes and *Rag2*$^{c/c}$ *p53*$^{-/-}$ thymic lymphomas was performed using primers specific for Dβ to Jβ rearrangements (upper panel), as previously reported[39]. The bands marked by numbered arrows represent rearrangements of Dβ to one of the Jβ segments (except for pseudogene Jβ2.6*). Representative experiments are shown. The PCR primers, specific for the Dβ1 and Jβ1.6 and for the Dβ2 and Jβ2.7 segments, amplified respectively six Dβ1-Jβ1 and six Dβ2-Jβ2 rearrangements from *wild-type* and *core Rag2* animals, reflecting the polyclonal nature of thymocytes in normal and *Rag2*$^{c/c}$ mice. In contrast, tumor cells from the *Rag2*$^{c/c}$ *p53*$^{-/-}$ mice displayed generally one or two predominant rearrangements, indicating a clonal or oligoclonal origin.

Supplementary Figure S4.

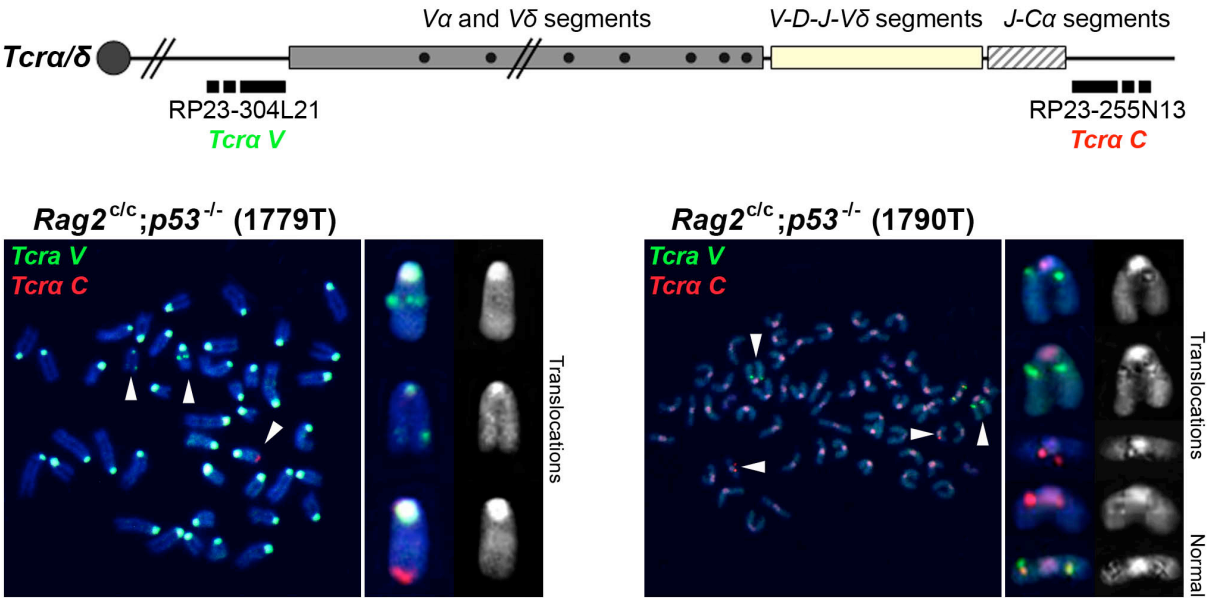
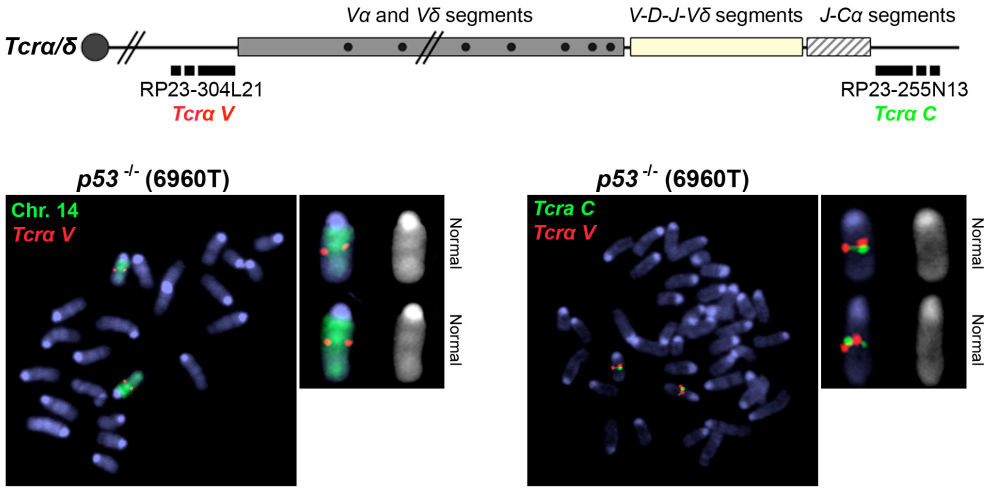


Figure S4. *Tcrα/δ*-associated genomic instability in *Rag2$^{c/c}$ p53$^{-/-}$* thymic lymphomas.
Top panel: schematic of the *Tcrα/δ* locus, with positions of the BACs used for generation of DNA FISH probes indicated. Bottom panels: representative metaphases from two *Rag2$^{c/c}$ p53$^{-/-}$* thymic lymphomas (1779T and 1790T) analyzed by DNA FISH using the *Tcrα/δ V* BAC probe (green signal) in combination with the *Tcrα/δ C* BAC probe (red signal). Arrow heads point translocation events.

Supplementary Figure S5.

a



b

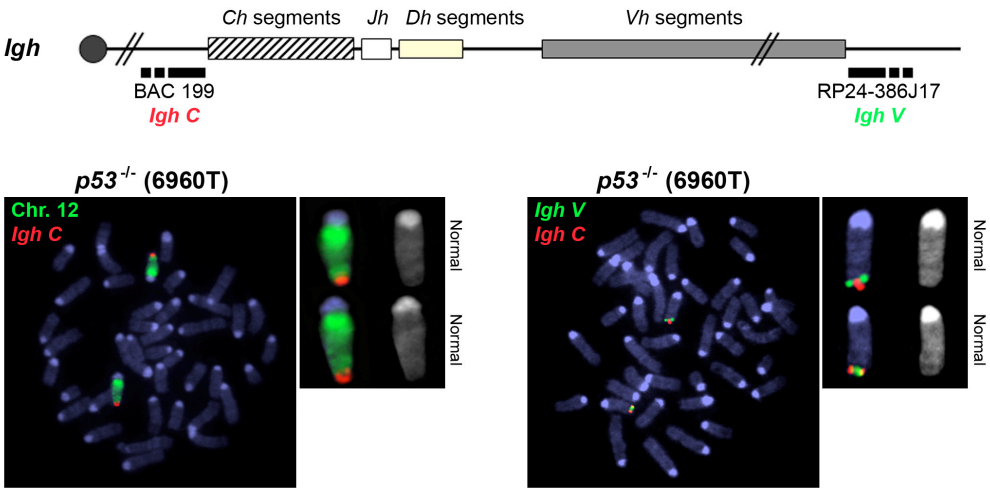
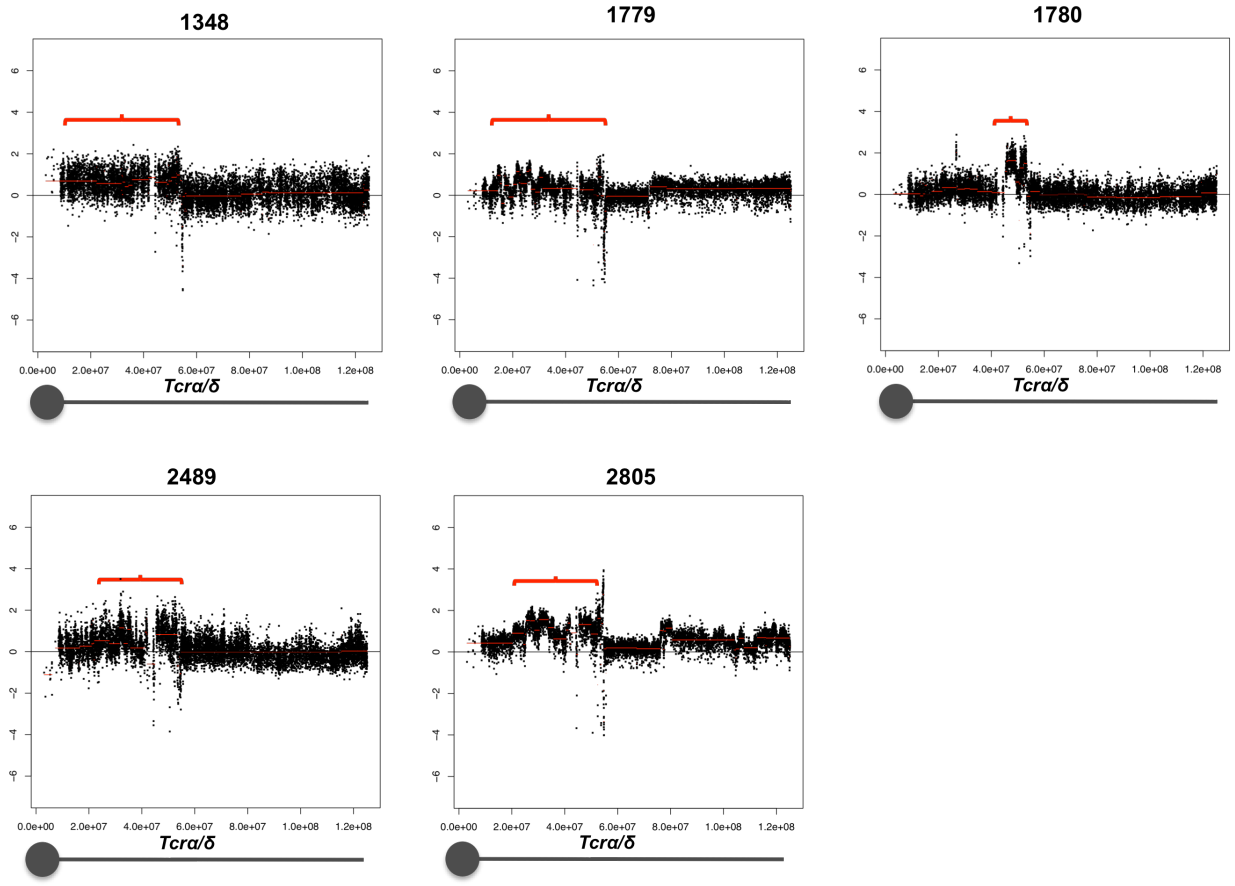


Figure S5. Absence of *Tcrα/δ* and *Igh*-associated genomic aberrations in *p53*$^{-/-}$ thymic lymphomas.

a. Top panel: schematic of the *Tcrα/δ* locus, with positions of the BACs used for generation of DNA FISH probes indicated. Bottom panel representative metaphases from one *p53*$^{-/-}$ thymic lymphomas (6960T) analyzed by DNA FISH using the *Tcrα/δ V* BAC probe (red signal) combined with a chromosome 14 paint (green signal; left panel) or with the *Tcrα/δ C* BAC probe (green signal; right panel). **b.** Top panel: schematic of the *Igh* locus, with positions of the BACs used for generation of DNA FISH probes indicated. Bottom panels: representative metaphases from one *p53*$^{-/-}$ thymic lymphoma (6960T) analyzed by DNA FISH using the *Igh C* BAC probe (red signal) combined with a chromosome 12 paint (green signal; left panel), or with the *Igh V* BAC probe (green signal; right panel).

Supplementary Figure S6.

a Chromosome 14



Supplementary Figure S6.

b Chromosome 12

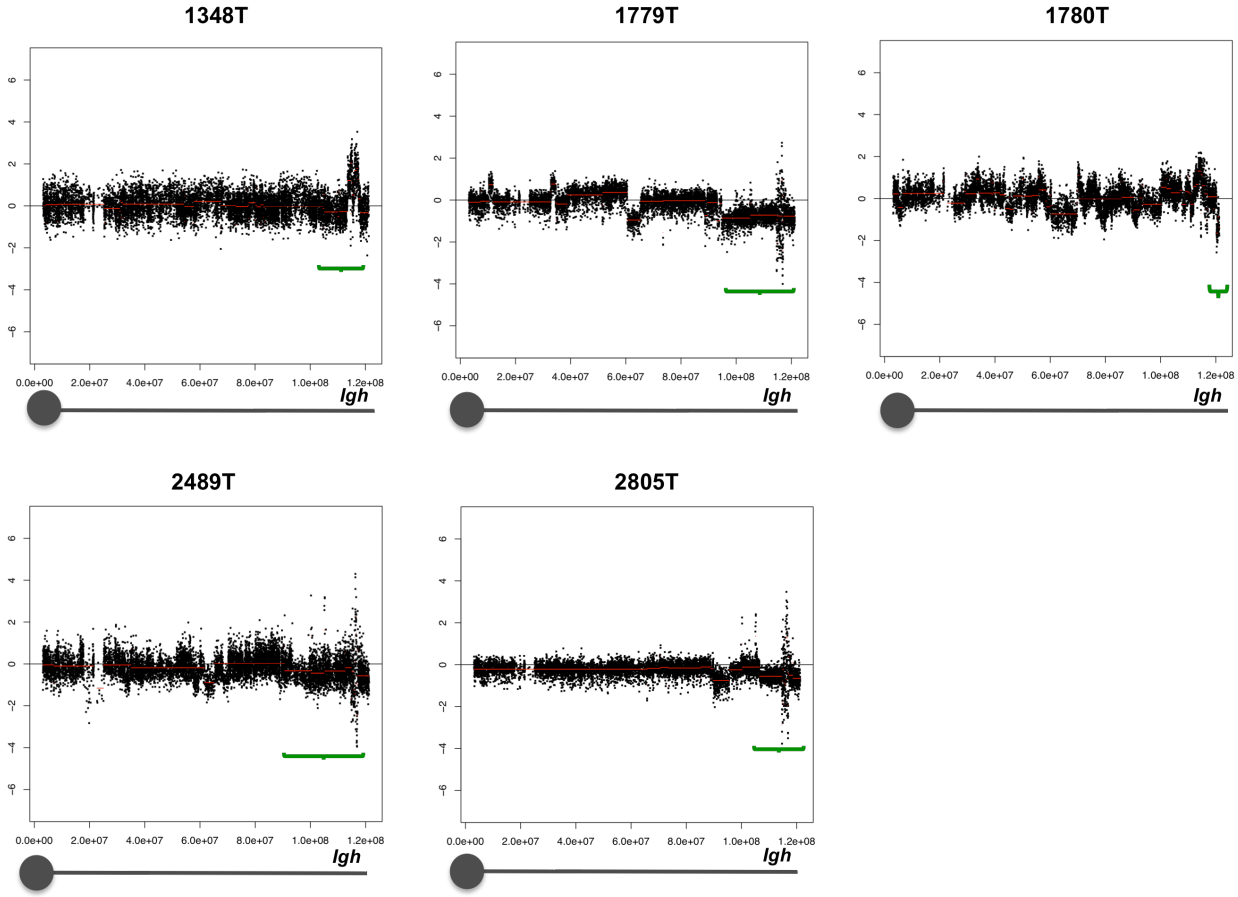


Figure S6. array-CGH profile of chromosomes 14 and 12 from *Rag2*$^{c/c}$ *p53*$^{-/-}$ thymic lymphomas .

Typical a-CGH profiles of chromosomes 14 (**a**) and 12 (**b**) from five *Rag2*$^{c/c}$ *p53*$^{-/-}$ thymic lymphomas (1348T, 1779T,1780T, 2489T and 2805T). Red braces indicate gain of DNA material and green braces show loss of DNA material. The normalized hybridization signal (region mean in red) is plotted against the genomic location of the probes. Relative genomic positions of the *Tcra/δ* locus (on chromosome 14) and Igh locus (on chromosome 12) are indicated.

Supplementary Figure S7.

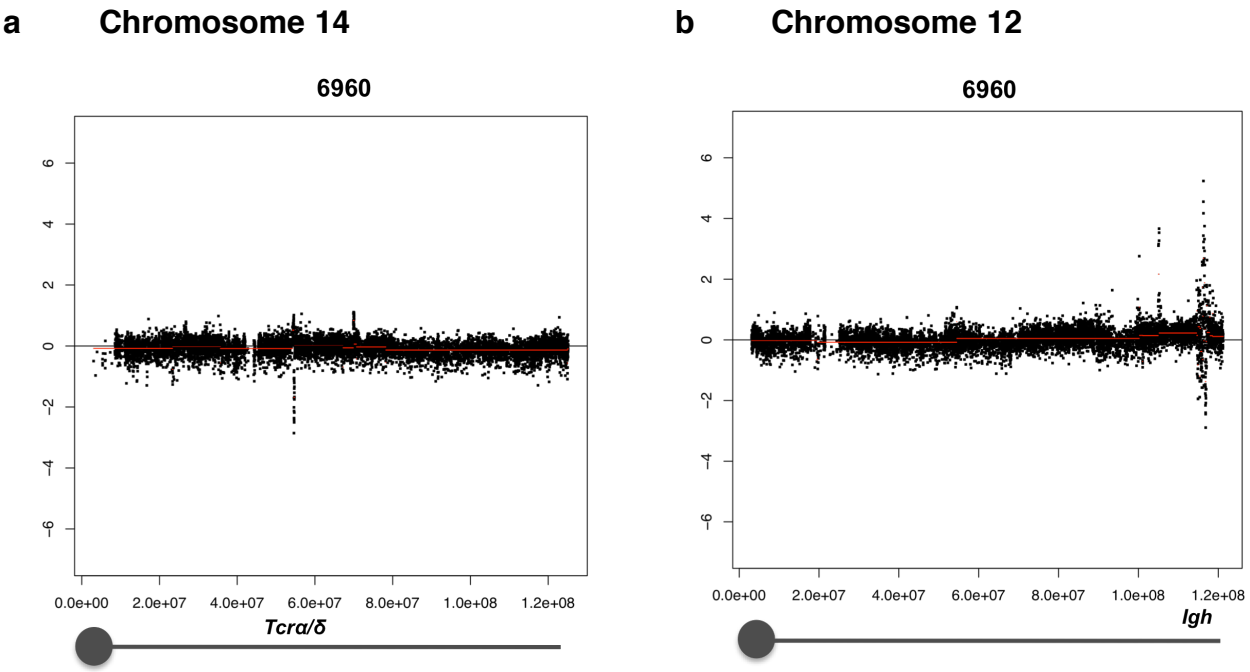
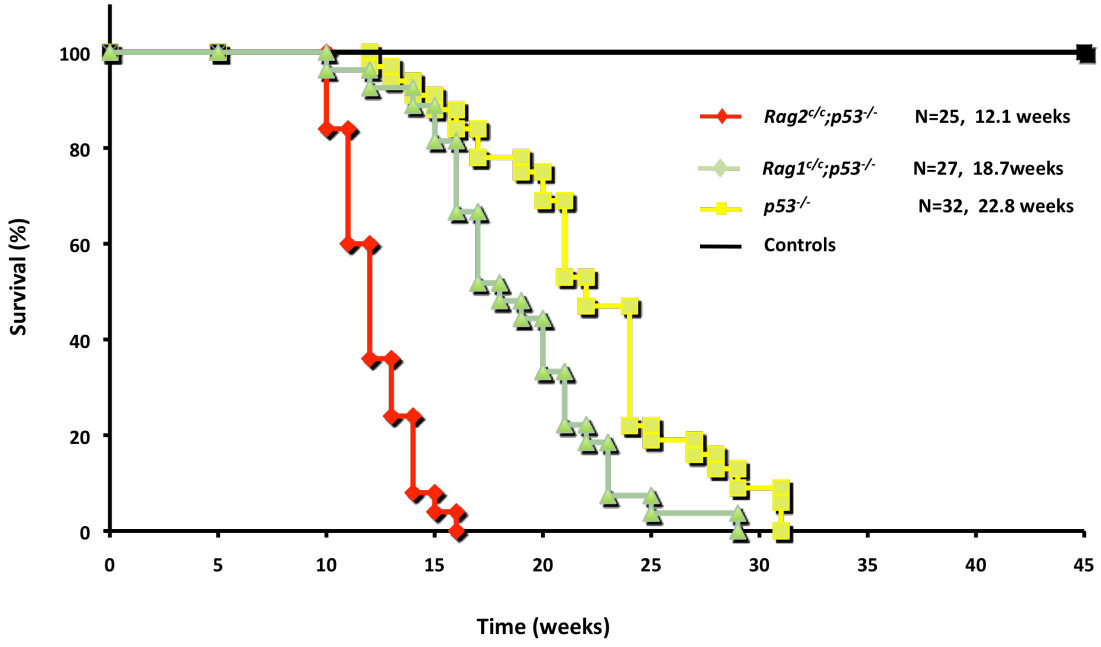


Figure S7. array-CGH profile of chromosomes 14 and 12 from *p53*$^{-/-}$ thymic lymphoma.
Typical a-CGH profiles of chromosomes 14 (**a**) and 12 (**b**) from one *p53*$^{-/-}$ thymic lymphomas (6960). The normalized hybridization signal (region mean in red) is plotted against the genomic location of the probes. Relative genomic positions of the *Tcrα/δ* locus (on chromosome 14) and Igh locus (on chromosome 12) are indicated.

Supplementary Figure S8.

a



b

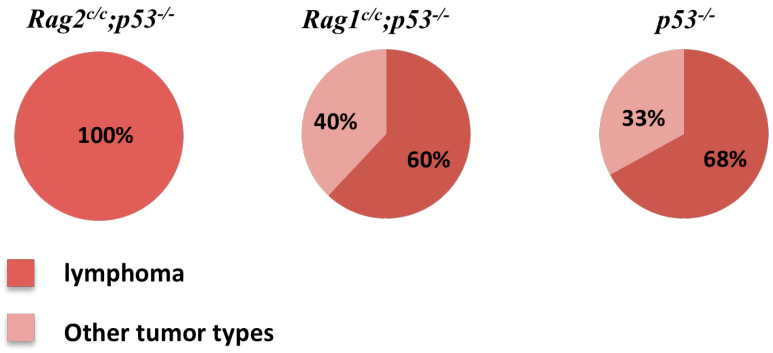


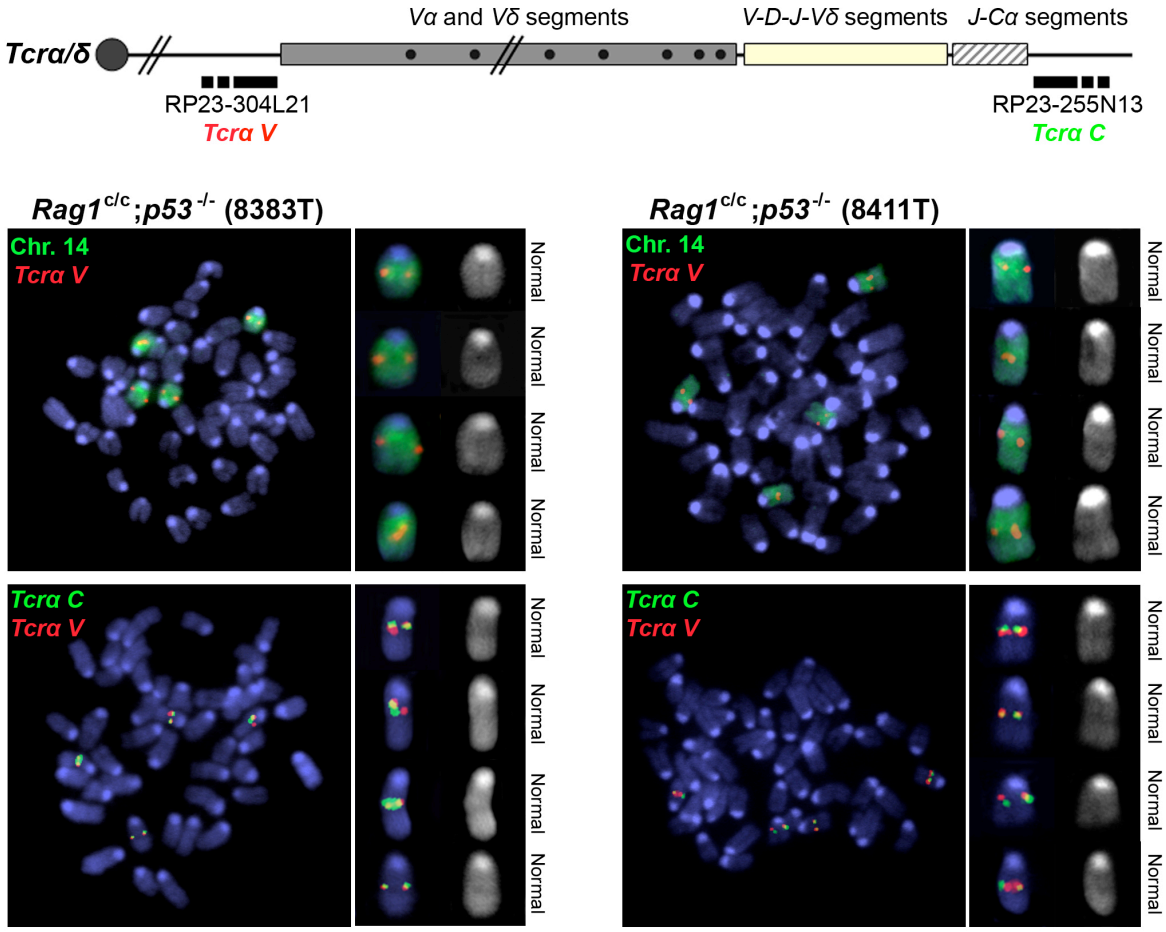
Figure S8. The "non core" region of RAG2, but not RAG1, is a tumor suppressor in developing thymocytes.

a. Kaplan-Meier tumor-free survival analysis for cohorts of control (*WT*, n=12 and *Rag2*$^{c/c}$, n=19), *p53*$^{-/-}$ (n=32), *Rag2*$^{c/c}$ *p53*$^{-/-}$ (n=25) mice (as shown in fig. 1) and *Rag1*$^{c/c}$ *p53*$^{-/-}$ (n=27) mice. Animals were monitored for 50 weeks. The average age of death in weeks is shown for *p53*$^{-/-}$ (22.8 weeks), *Rag2*$^{c/c}$ *p53*$^{-/-}$ (12.1 weeks) and *Rag1*$^{c/c}$ *p53*$^{-/-}$ (18.7 weeks) genotypes. the *P*-value were determined by the Wilcoxon rank sum test; *Rag1*$^{c/c}$ *p53*$^{-/-}$ significantly different than *p53*$^{-/-}$ (P(two-sided) = 0.006) and *Rag1*$^{c/c}$ *p53*$^{-/-}$

highly significantly different than *Rag2*$^{c/c}$ *p53*$^{-/-}$ (P(two-sided) < 0.0001) **b.** Pie chart showing the tumor spectrum observed for *Rag2*$^{c/c}$ *p53*$^{-/-}$ (n=25), *p53*$^{-/-}$ (n=27) mice (as shown in Fig 1) and *Rag1*$^{c/c}$ *p53*$^{-/-}$ (n=27) mice. All *Rag2*$^{c/c}$ *p53*$^{-/-}$ animals (n=25) showed enlarged thymus. Both *p53*$^{-/-}$ and *Rag1*$^{c/c}$ *p53*$^{-/-}$ animals showed either enlarged thymus and/or spleen or other non lymphoid tumor mass with no statistical difference (Fisher's Exact Test, 2-Tail : $p > 0.5$).

Supplementary Figure S9.

a



Supplementary Figure S9.

b

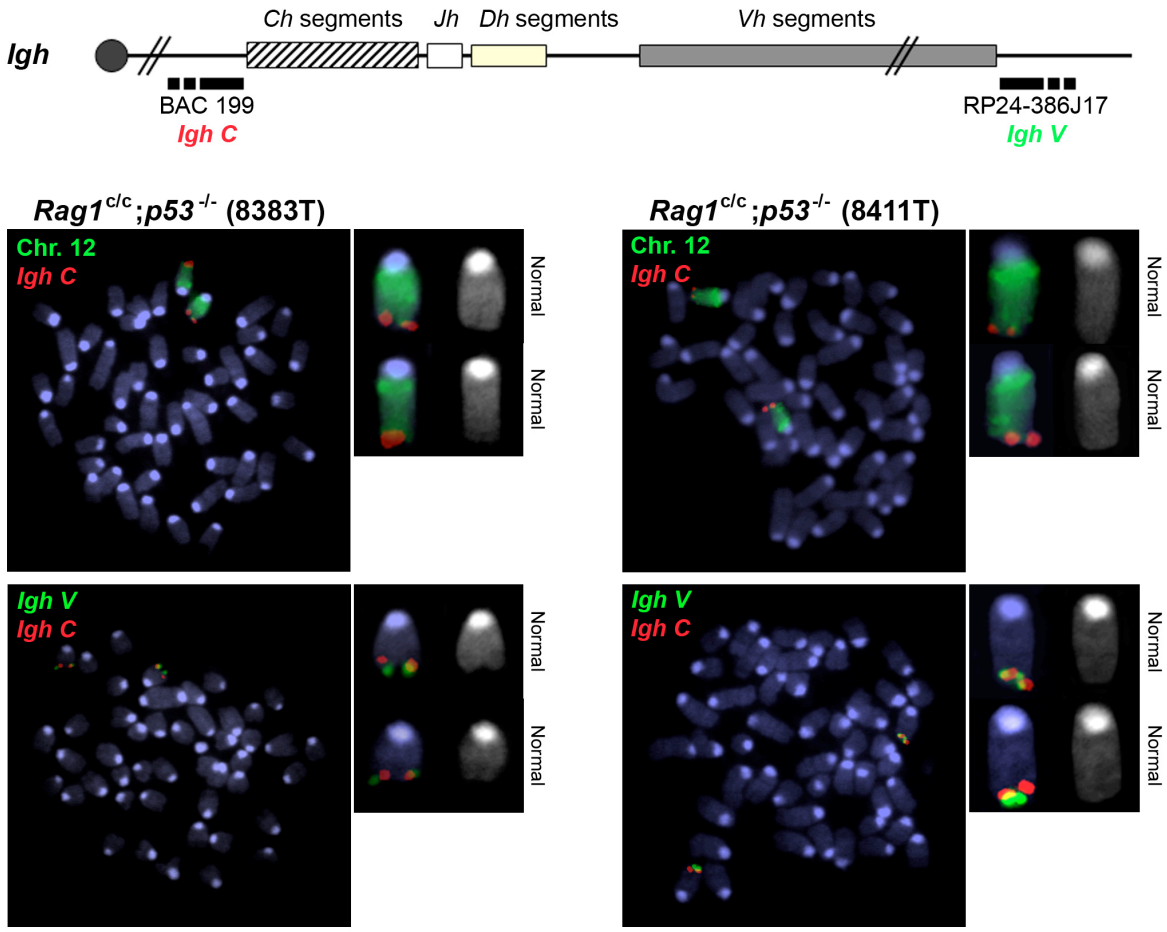
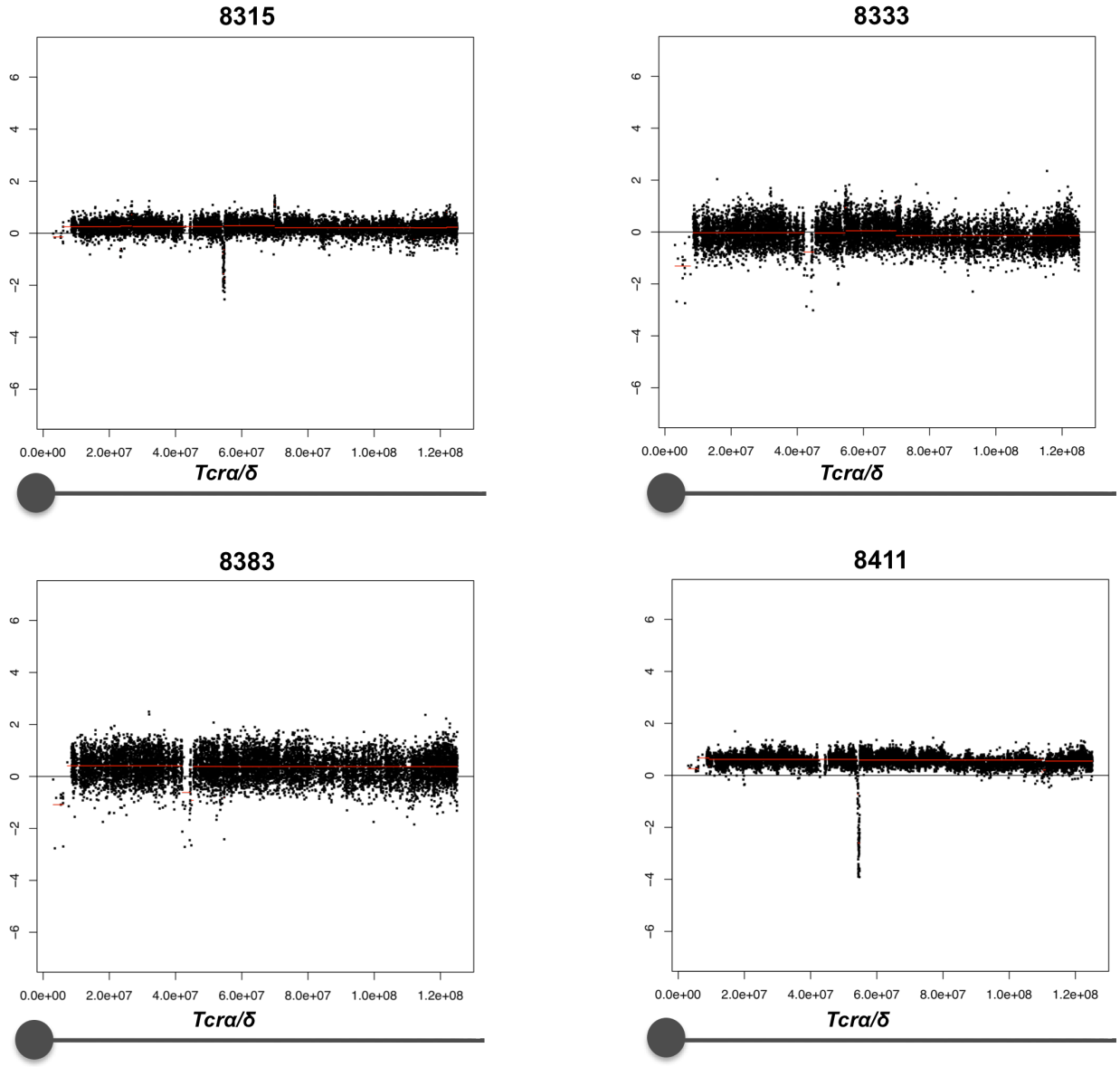


Figure S9. Absence of *Tcra/δ* and *Igh*-associated genomic aberrations in *Rag1$^{c/c}$ p53$^{-/-}$* thymic lymphomas.
a. Top panel: schematic of the *Tcra/δ* locus, with positions of the BACs used for generation of DNA FISH probes indicated. Bottom panels: representative metaphases from two *Rag1$^{c/c}$ p53$^{-/-}$* thymic lymphomas (8383T and 8411T) analyzed by DNA FISH using the *Tcra/δ V* BAC probe (red signal) combined with a chromosome 14 paint (green signal; top panels) or with the *Tcra/δ C* BAC probe (green signal; lower panels).
b. Top panel: schematic of the *Igh* locus, with positions of the BACs used for generation of DNA FISH probes indicated. Bottom panels: representative metaphases from two *Rag1$^{c/c}$ p53$^{-/-}$* thymic lymphomas (8383T and 8411T) analyzed by DNA FISH using the *Igh C* BAC probe (red signal) combined with a chromosome 12 paint (green signal; top panels), or with the *Igh V* BAC probe (green signal; bottom panels).

Supplementary Figure S10.

a Chromosome 14



Supplementary Figure S10.

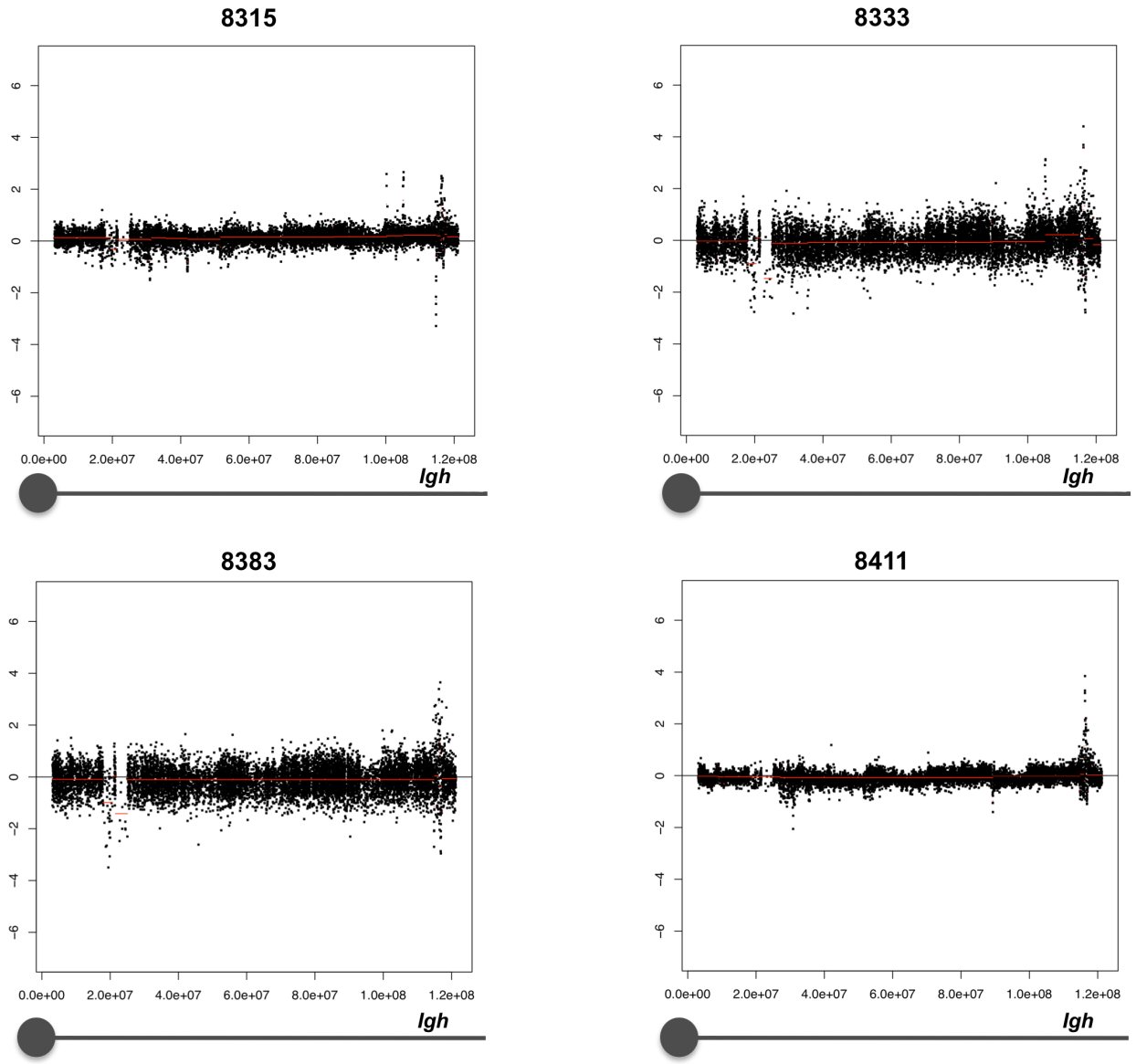


Figure S10. array-CGH profile of chromosomes 14 and 12 from *Rag1*$^{c/c}$ *p53*$^{-/-}$ thymic lymphomas .

Typical a-CGH profiles of chromosomes 14 (**a**) and 12 (**b**) from four *Rag1*$^{c/c}$ *p53*$^{-/-}$ thymic lymphomas (8315, 8333, 8383 and 8411). The normalized hybridization signal (region mean in red) is plotted against the genomic location of the probes. Relative genomic positions of the *Tcra/δ* locus (on chromosome 14) and Igh locus (on chromosome 12) are indicated.

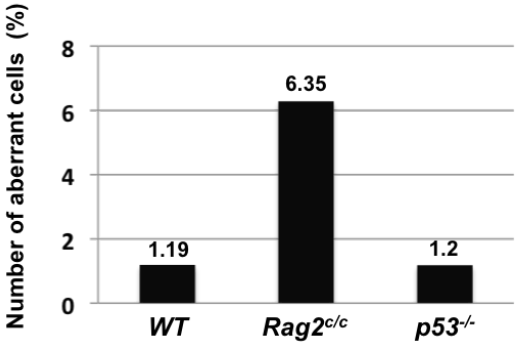
Supplementary Figure S11.

Experiment 1 (shown in Fig. 3d)	normal (2C+2V)	missing end (C and/or V)	number of cells analyzed
Wild type	98.81%	1.19%	335
Rag2$^{c/c}$	93.65%	6.35%	378
p53$^{-/-}$	98.80%	1.20%	417

Statistics analysis (Fisher's two-tailed Exact Test)

sample A	Sample B	probability	significance
WT	*Rag2$^{c/c}$*	3.30E-04	***
WT	*p53$^{-/-}$*	1.00E+00	ns
Rag2$^{c/c}$	*p53$^{-/-}$*	9.00E-05	***

ns not significant	* significant	** very significant	*** highly significant
p>0.05	0.01<p<0.05	0.001<p<0.01	p<0.001

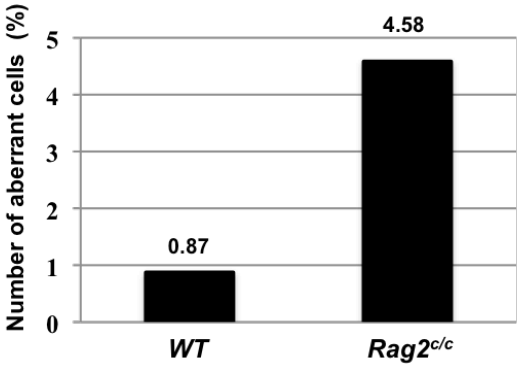


Experiment 2	normal (2C+2V)	missing end (C and/or V)	number of cells analyzed
Wild type	99.13%	0.87%	343
Rag2$^{c/c}$	95.42%	4.58%	371

Statistics analysis (Fisher's two-tailed Exact Test)

sample A	Sample B	probability	significance
WT	*Rag2$^{c/c}$*	2.60E-03	**

ns not significant	* significant	** very significant	*** highly significant
p>0.05	0.01<p<0.05	0.001<p<0.01	p<0.001



Experiment 3	normal (2C+2V)	missing end (C and/or V)	number of cells analyzed
Wild type	95.17%	4.83%	290
Rag2$^{c/c}$	85.32%	14.68%	218
p53$^{-/-}$	98.56%	1.44%	208

Statistics analysis (Fisher's two-tailed Exact Test)

sample A	Sample B	probability	significance
WT	*Rag2$^{c/c}$*	1.50E-04	***
WT	*p53$^{-/-}$*	4.60E-02	*
Rag2$^{c/c}$	*p53$^{-/-}$*	1.88E-07	***

ns not significant	* significant	** very significant	*** highly significant
p>0.05	0.01<p<0.05	0.001<p<0.01	p<0.001

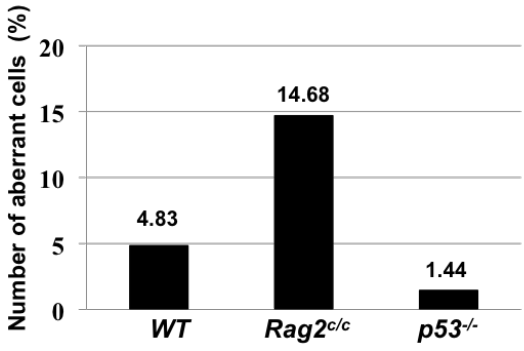


Figure S11. *Tcrα/δ* locus integrity in *wild type*, *Rag2$^{c/c}$* and *p53$^{-/-}$* double positive thymocytes.
Three experiments showing the frequency at which the *Tcrα/δ V* and/or the *Tcrα/δ C* signals are lost in *wild-type*, *p53$^{-/-}$* and *Rag2$^{c/c}$* thymocytes.

Supplementary Figure S12.

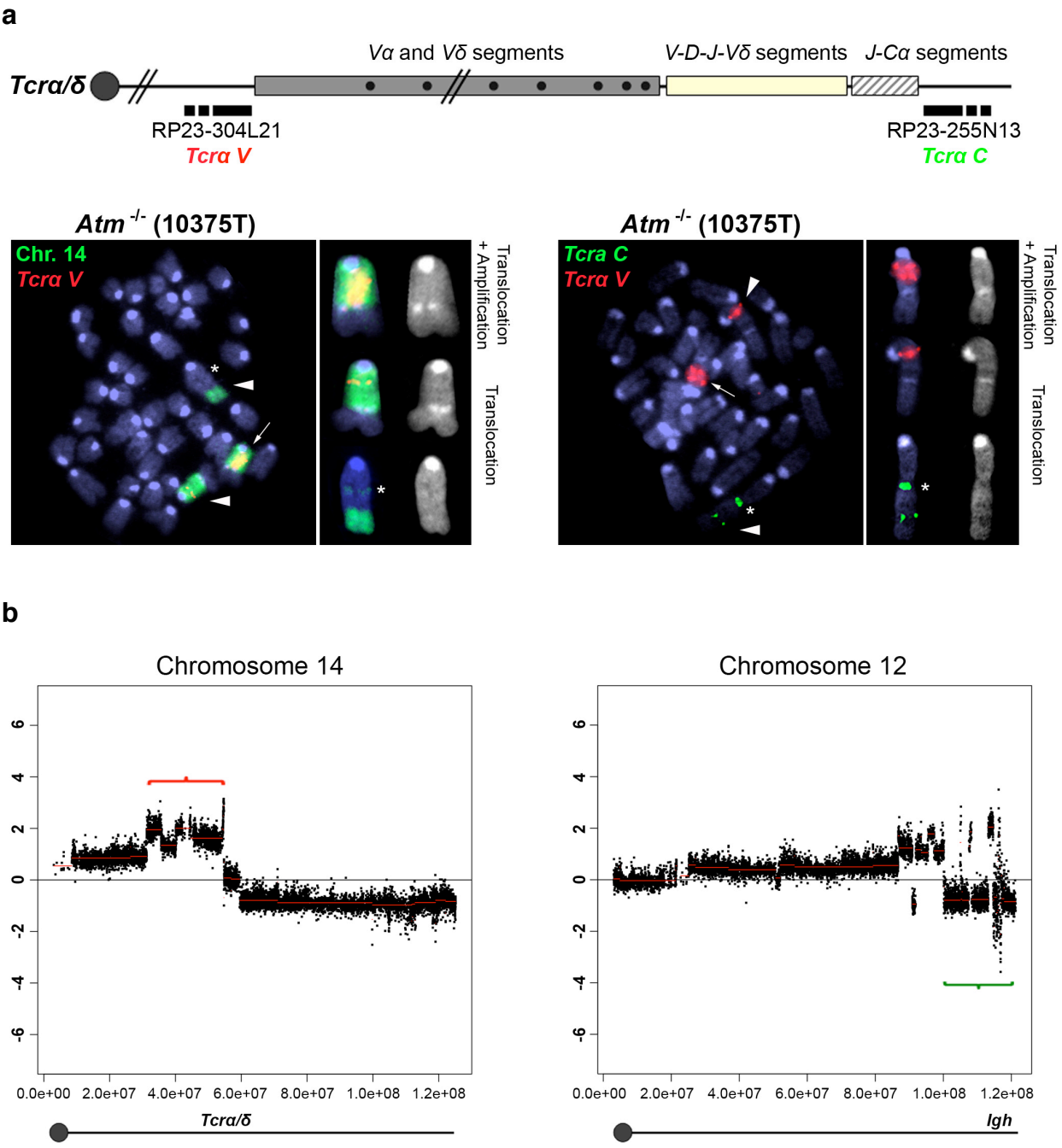


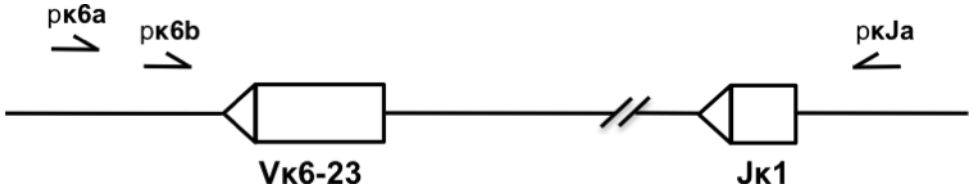
Figure S12. *Atm*$^{-/-}$ thymic lymphomas display *Tcrα/δ* and *Igh*-associated genomic instability.

a. Top panel: schematic of the *Tcrα/δ* locus, with positions of the BACs used for generation of DNA FISH probes indicated. Bottom panels: Representative metaphases from one *Atm*$^{-/-}$ thymic lymphoma analyzed by DNA FISH using the *Tcrα/δ V* BAC probe (red signal) combined with a chromosome 14 paint (green signal; left panel) or with the

Tcra/δ C BAC probe (green signal; right panel). Arrows point to the amplification of the *Tcra/δ V* region, arrow heads point to the translocated chromosome 14 and asterisks show a second translocation event of the *Tcra/δ* C region on the chromosome that carries also the translocated distal end of chromosome 14. **b.** A typical array-CGH profile of chromosomes 14 and 12 from the same $Atm^{-/-}$ tumor as in **a**. Red braces indicate gain of DNA material and green braces show loss of DNA material. The normalized hybridization signal (region mean in red) is plotted against the genomic location of the probes. Relative genomic positions of the *Tcra/δ* locus (on chromosome 14) and Igh locus (on chromosome 12) are indicated.

Supplementary Figure S13.

a



b

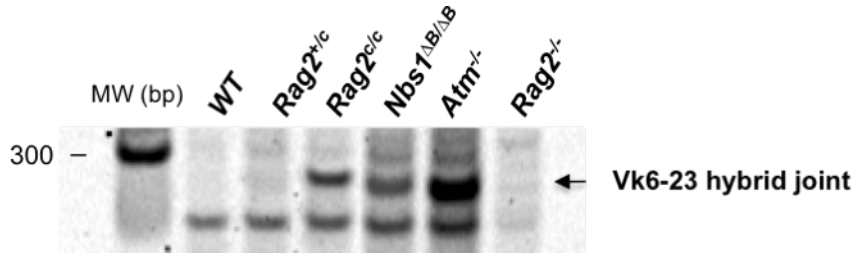


Figure S13. Defective handling of V(D)J recombination intermediates in *Rag2*$^{c/c}$ lymphocytes.
a. Schematic showing the relative orientation of the *Vκ6-23* to *Jκ1* gene segments. RSS are shown as open triangle; arrows denote PCR primers. **b.** PCR analysis of *Vκ6-23* to *Jκ1* hybrid joints in splenocytes of indicated mouse genotypes using 300 ng of genomic DNA. PCR experiments were performed as previously described[3,28].

Supplementary Table S1.

Sample #	Genotype	Number of metaphase analyzed	% of normal diploid metaphase	% of aberrant metaphase	% of cells with breaks	% of cells with fusions	Fusion/ metaphase	% of tetraploid cells
2595	*WT*	34	97	3	3	0	0	0
2598	*WT*	35	100	0	0	0	0	0
6902	$p53^{-/-}$	34	82.3	17.6	0	14.7	0.2	0
4156	$p53^{-/-}$	35	65.7	28.6	2.9	28.6	0.3	5.7
1758	$Rag2^{c/c}$ $p53^{-/-}$	34	79.4	17.6	5.9	11.8	0.1	2.9
1780	$Rag2^{c/c}$ $p53^{-/-}$	36	75	19.4	2.8	16.7	0.2	5.6
1779	$Rag2^{c/c}$ $p53^{-/-}$	36	83.3	8.3	0	8.3	0.08	11.1
1774	$Rag2^{c/c}$ $p53^{-/-}$	36	61.1	16.7	2.8	11.1	0.2	30.6
1735	$Rag2^{c/c}$ $p53^{-/-}$	17	17.7	82.3	0	82.3	0.9	5.9
1790	$Rag2^{c/c}$ $p53^{-/-}$	35	34.3	25.7	0	22.9	0.4	60
1799	$Rag2^{c/c}$ $p53^{-/-}$	35	31.4	17.1	5.7	11.4	0.1	62.9
1795	$Rag2^{c/c}$ $p53^{-/-}$	36	61.1	11.1	2.8	8.3	0.1	30.5
1800	$Rag2^{c/c}$ $p53^{-/-}$	37	75.7	18.9	0	18.9	0.2	0
1736	$Rag2^{c/c}$ $p53^{-/-}$	32	31.2	68.8	9.4	68.8	0.8	0
1743	$Rag2^{c/c}$ $p53^{-/-}$	37	40.5	54.1	5.4	45.9	0.5	5.4
1745	$Rag2^{c/c}$ $p53^{-/-}$	34	5.9	94.1	2.9	94.1	1.1	2.9

the three highlighted $Rag2^{c/c}$ $p53^{-/-}$ thymic lymphomas have been further analyzed in Figure 2.

Table S1. Genomic instability in *$Rag2^{-/-}$ $p53^{-/-}$* thymic lymphomas.
Analysis of Giemsa stained metaphase spreads prepared from 12 $Rag2^{c/c}$ $p53^{-/-}$ thymic lymphomas, two $p53^{-/-}$ thymic lymphomas and two *wild type* thymi.

Supplementary Reference

39 Gartner, F. *et al.* Immature thymocytes employ distinct signaling pathways for allelic exclusion versus differentiation and expansion. *Immunity* **10**, 537-546, doi:S1074-7613(00)80053-9 [pii] (1999).