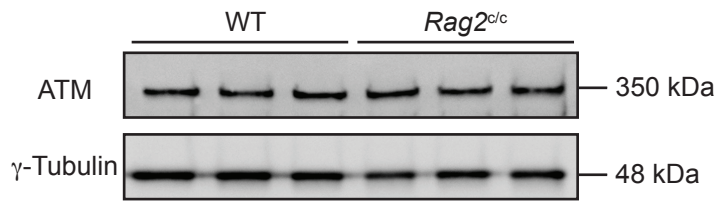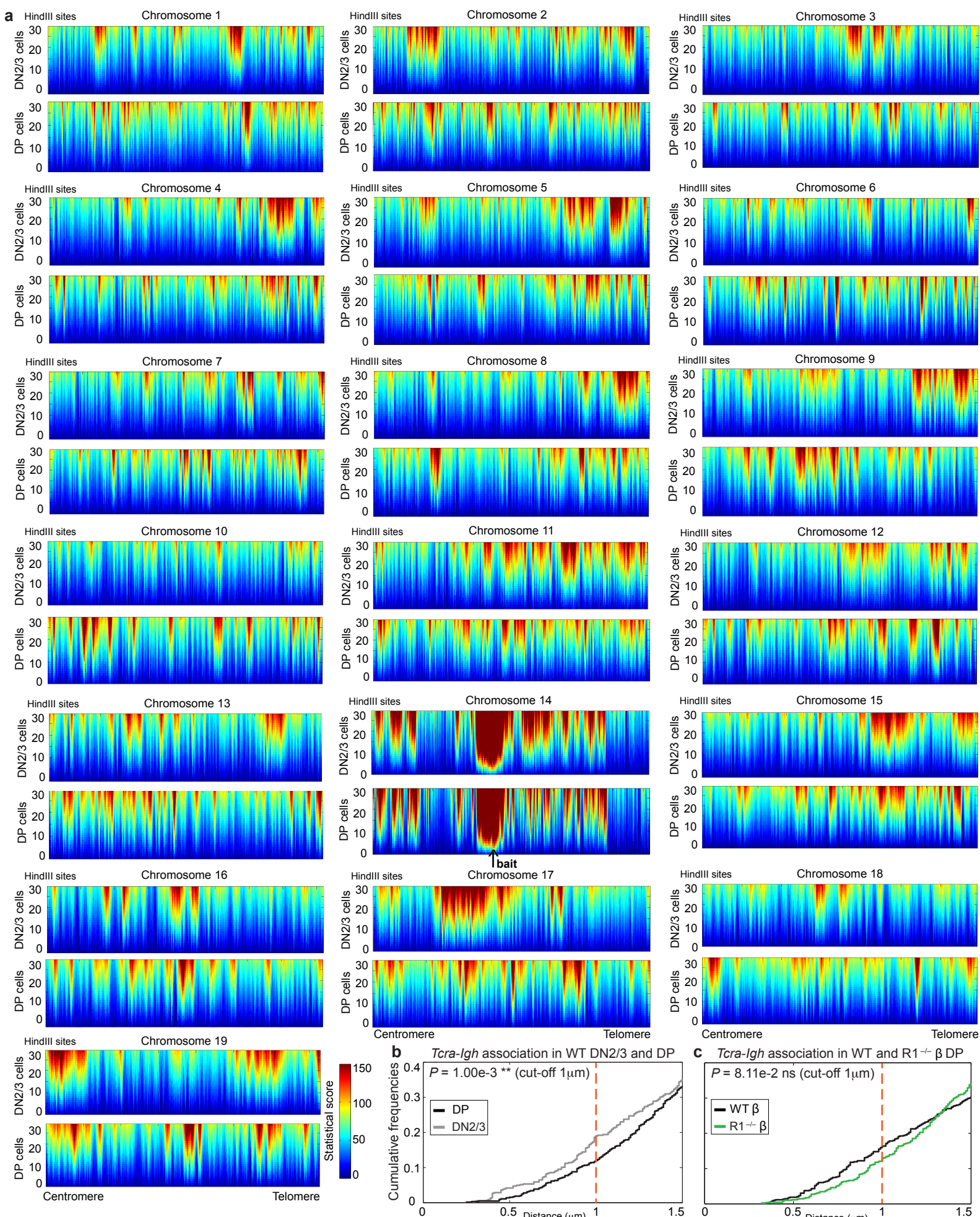**Supplementary Figures (S1-S3) and Supplementary Tables (S1-S10)**

The RAG2 C-terminus and ATM protect genome integrity by controlling antigen receptor gene cleavage

Julie Chaumeil, Mariann Micsinai, Panagiotis Ntziachristos, David B. Roth, Iannis Aifantis, Yuval Kluger, Ludovic Deriano, & Jane A. Skok.

Supplementary Figure S1 | Western blot showing levels of ATM in WT and *Rag2*c/c thymocytes. γ-Tubulin was used as a control.

**Supplementary Figure S2 |** (**a**) 4C-seq - Domainograms for all the autosomes. See also Table S2. (**b,c**) Cumulative frequency curves (cut-off at 1.5 μm) for inter-locus distances of *Tcra* and *Igh* alleles in WT DN2/3 and DP cells (**b**), WT β and R1$^{-/-}$ β DP cells (**c**). *P*-values were calculated using a two-tail Fisher exact test for a cut-off at 1 μm. See Fig. 3 and Supplementary Table S3.

- Wild-type DP cells -
Mono-locus recombination of *Tcra* or *Igh*

- Mutant DP cells -
Bi-locus recombination of *Tcra* and *Igh*
in absence of ATM or RAG2 C-terminus
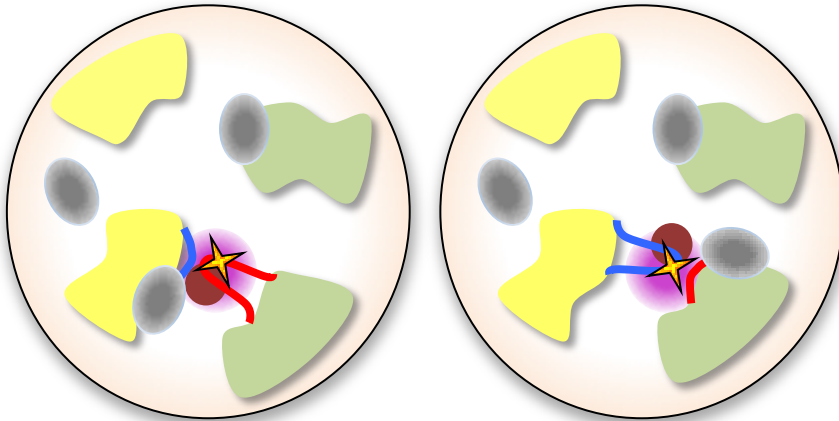
RAG-mediated *Tcra* / *Igh* pairing with mono-locus looping out of one locus. After mono-locus RAG-mediated cleavage, ATM and RAG2 C-terminus mediate the repositioning of the other locus to pericentromeric heterochromatin (PCH) and the modulation of looping, to pevent further cleavage.
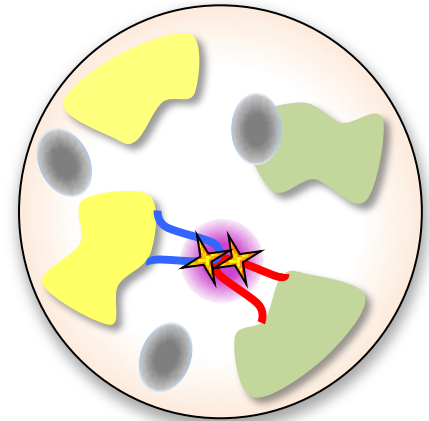
Pairing and looping out of both loci.
No repositioning to PCH or modulation of looping: Bi-locus cleavage and damage.

Tcra
Igh

14   12

Chromosome territories

RAG   PCH   ATM   Break
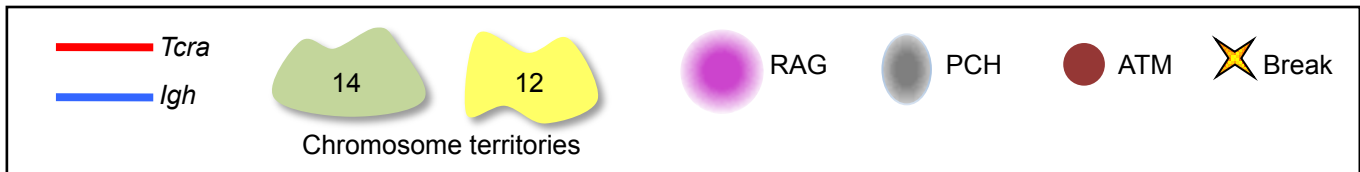
Supplementary Figure S3 | Model.

SUPPLEMENTARY TABLES

Statistical analyses were performed using a two-tail Fisher exact test or a two-tail χ^2 test (Significance at $\alpha = 0.05$).

ns =	$P > 5.00e\text{-}2$	Not significant
* =	$1.00e\text{-}2 < P \leq 5.00e\text{-}2$	Significant
** =	$1.00e\text{-}3 < P \leq 1.00e\text{-}2$	Very significant
*** =	$P \leq 1.00e\text{-}3$	Highly significant

Supplementary Table S1.

Promiscuous *Igh* rearrangement overlaps with *Tcrd* and *Tcra* recombination in DN2/3 and DP cells (Fig. 1c).

Igh			No association number	No association %	γ-H2AX association number	γ-H2AX association %	Sample size (alleles)
DN2/3	WT	Exp1	203	95.5	9	4.2	212
		Exp2	291	92.7	23	7.3	314
		TOTAL	**494**	**93.9**	**32**	**6.1**	**526**
DN2/3	R1$^{-/-}$	Exp1	298	97.4	8	2.6	306
		Exp2	190	96.9	6	3.1	196
		TOTAL	**488**	**97.2**	**14**	**2.8**	**502**
DP	WT	Exp1	212	96.4	8	3.6	220
		Exp2	413	96.5	15	3.5	428
		TOTAL	**625**	**96.5**	**23**	**3.5**	**648**
DP	R1$^{-/-}$ β	Exp1	225	98.7	3	1.3	228
		Exp2	459	98.1	9	1.9	468
		TOTAL	**684**	**98.3**	**12**	**1.7**	**696**
Statistical analysis (Fisher exact test).					*P*-value		Level
WT vs. R1$^{-/-}$			DN2/3		1.07e-2		*
			DP		3.58e-2		*

Tcra/d			No association number	No association %	γ-H2AX association number	γ-H2AX association %	Sample size (alleles)
DN2/3	WT	Exp1	192	90.6	20	9.4	212
		Exp2	290	92.4	24	7.6	314
		TOTAL	**482**	**91.6**	**44**	**8.4**	**526**
DN2/3	R1$^{-/-}$	Exp1	299	97.7	7	2.3	306
		Exp2	192	98	4	2	196
		TOTAL	**491**	**97.8**	**11**	**2.2**	**502**
DP	WT	Exp1	147	66.8	73	33.2	220
		Exp2	266	62.1	162	37.9	428
		TOTAL	**413**	**63.7**	**235**	**36.3**	**648**
DP	R1$^{-/-}$ β	Exp1	220	96.5	8	3.5	228
		Exp2	438	93.6	30	6.4	468
		TOTAL	**658**	**94.5**	**38**	**5.5**	**696**
Statistical analysis (Fisher exact test).					*P*-value		Level
WT vs. R1$^{-/-}$			DN2/3		1.10e-5		***
			DP		1.10e-44		***

Supplementary Table S2.

The C-terminus of RAG2 and ATM regulate mono-locus RAG-mediated cleavage in DN2/3 cells.

		No γ-H2AX association		γ-H2AX association		Sample size
		number	%	number	%	(cells)
Exp1	WT	213	96.8	7	3.2	220
	R1$^{-/-}$	220	100	0	0	220
Exp2	WT	115	95.8	5	4.2	120
	R1$^{-/-}$	112	100	0	0	112
Exp1	WT	202	98.5	3	1.5	205
	53bp1$^{-/-}$	186	98.9	2	1.1	188
Exp2	WT	98	98	2	2	100
	53bp1$^{-/-}$	115	98.3	2	1.7	117
Exp1	WT	152	96.8	5	3.2	157
	Atm$^{-/-}$	137	90.7	14	9.3	151
Exp2	WT	104	98.1	2	1.9	106
	Atm$^{-/-}$	98	94.2	6	5.8	104
Exp1	WT	104	98.1	2	1.9	106
	Rag2$^{c/c}$	102	91.1	10	8.9	112
	Rag2$^{c/c}$ *p53*$^{-/-}$	107	96.4	4	3.6	111
Exp2	WT	152	96.8	5	3.2	157
	Rag2$^{c/c}$	123	93.9	8	6.1	131
	Rag2$^{c/c}$ *p53*$^{-/-}$	140	92.7	11	7.3	151
TOTAL :	**WT**	**1140**	**97.4**	**31**	**2.6**	**1171**
	R1$^{-/-}$	**332**	**100**	**0**	**0**	**332**
	***53bp1*$^{-/-}$**	**301**	**98.7**	**4**	**1.3**	**305**
	***Atm*$^{-/-}$**	**235**	**92.2**	**20**	**7.8**	**255**
	***Rag2*$^{c/c}$**	**225**	**92.6**	**18**	**7.4**	**243**
	***Rag2*$^{c/c}$ *p53*$^{-/-}$**	**247**	**94.3**	**15**	**5.7**	**262**
Statistical analysis (Fisher exact test).				P-value		Level
WT vs. R1$^{-/-}$				2.74e-3		**
WT vs. *53bp1*$^{-/-}$				1.72e-1		ns
WT vs. *Atm*$^{-/-}$				5.10e-5		***
WT vs. *Rag2*$^{c/c}$				2.20e-4		***
WT vs. *Rag2*$^{c/c}$ *p53*$^{-/-}$				1.06e-2		*

Supplementary Table S3.

The C-terminus of RAG2 and ATM regulate mono-locus RAG-mediated cleavage in DP cells.

		No γ-H2AX association		γ-H2AX association		Sample size
		number	%	number	%	(cells)
Exp1	WT	229	95.8	10	4.2	239
	R1$^{-/-}$ β	229	97.9	5	2.1	234
Exp2	WT	106	96.4	4	3.6	110
	R1$^{-/-}$ β	113	99.1	1	0.9	114
Exp1	WT	199	96.1	8	3.9	207
	53bp1$^{-/-}$	210	95.9	9	4.1	219
Exp2	WT	107	100	0	0	107
	53bp1$^{-/-}$	100	96.2	4	3.8	104
Exp1	WT	204	95.3	10	4.7	214
	Atm$^{-/-}$	175	81.8	39	18.2	214
Exp2	WT	104	94.5	6	5.5	110
	Atm$^{-/-}$	88	83	18	17	106
Exp1	WT	95	95	5	5	100
	Rag2$^{c/c}$	82	81.2	19	18.8	101
	Rag2$^{c/c}$ p53$^{-/-}$	176	83.4	35	16.6	211
Exp2	WT	101	98.1	2	1.9	103
	Rag2$^{c/c}$	83	82.2	18	17.8	101
	Rag2$^{c/c}$ p53$^{-/-}$	92	83.6	18	16.4	110
TOTAL :	**WT**	**1145**	**96.2**	**45**	**3.8**	**1190**
	R1$^{-/-}$	**342**	**98.3**	**6**	**1.7**	**348**
	53bp1$^{-/-}$	**310**	**96**	**13**	**4**	**323**
	Atm$^{-/-}$	**263**	**82.2**	**57**	**17.8**	**320**
	Rag2$^{c/c}$	**164**	**81.7**	**37**	**18.3**	**202**
	Rag2$^{c/c}$ p53$^{-/-}$	**268**	**83.5**	**53**	**16.5**	**321**
	Statistical analysis (Fisher exact test).			*P*-value		Level
WT	vs. R1$^{-/-}$			5.84e-2		ns
WT	vs. 53bp1$^{-/-}$			8.40e-1		ns
WT	vs. Atm$^{-/-}$			6.80e-19		***
WT	vs. Rag2$^{c/c}$			3.80e-16		***
WT	vs. Rag2$^{c/c}$ p53$^{-/-}$			2.10e-16		***

Supplementary Table S4.
4C-seq data (Fig. 3a and Fig. S2a).

	Tcra DN	Tcra DP
Total number of reads sequenced	3,674,583	2,559,768
Number of mapped reads after pre-preprocessing	917,974	943,119
DP_mnase_Input	3,674,583	2,559,768
Reads per chromosome:		
1	20105	31200
2	23206	34885
3	15238	21522
4	17115	25664
5	19322	26764
6	15048	24880
7	15885	24321
8	12539	18287
9	12575	17492
10	11231	20253
11	22392	29869
12	12493	20067
13	11478	18034
14	658135	551444
15	11403	16634
16	8260	14012
17	13030	18414
18	6386	10979
19	8379	12851
X	3615	5356
Y	139	191

Supplementary Table S5.

ChIP-seq data (Fig. 3c and 5a).

Histone marks	Mapped reads	Unique mapped reads
DN_H3K4me3	9,368,703	8,783,451
DN_H3K9Ac	15,428,075	13,345,701
DN_mnase_Input	10,644,827	10,191,160
DP_H3K4me3	12,466,417	11,412,239
DP_H3K9Ac	12,482,225	10,466,783
DP_mnase_Input	13,332,872	12,858,986

Frequency of γ-H2AX association on *Tcra* and/or *Igh* alleles in *Tcra-Igh* pairs in DP cells (Fig. 4a).

Frequency of γ-H2AX association:	No γ-H2AX association		γ-H2AX association						Sample size
			On *Tcra*		On both *Tcra / Igh*		On *Igh*		
	number	%	number	%	number	%	number	%	(pairs)
Exp1 WT	76	58.5	48	36.9	6	4.6	0	0	130
53bp1$^{-/-}$	26	53.1	20	40.8	3	6.1	0	0	49
Atm$^{-/-}$	36	47.4	17	22.4	18	23.7	5	6.6	76
Rag2$^{c/c}$	24	51.1	14	29.8	8	17	1	2.1	47
Rag2$^{c/c}$ p53$^{-/-}$	47	54	22	25.3	12	13.8	6	6.9	87
Exp2 WT	61	61	38	38	1	1	0	0	100
53bp1$^{-/-}$	24	63.2	13	34.2	1	2.6	0	0	38
Atm$^{-/-}$	20	33.3	31	51.7	9	15	0	0	60
Rag2$^{c/c}$	19	45.2	14	33.3	5	11.9	4	9.5	42
Rag2$^{c/c}$ p53$^{-/-}$	35	66	11	20.8	6	11.3	1	1.9	53
TOTAL WT	**137**	**59.6**	**86**	**37.4**	**7**	**3**	**0**	**0**	**230**
53bp1$^{-/-}$	**50**	**57.5**	**33**	**37.9**	**4**	**4.6**	**0**	**0**	**87**
Atm$^{-/-}$	**56**	**41.2**	**48**	**35.3**	**27**	**19.9**	**5**	**3.7**	**136**
Rag2$^{c/c}$	**43**	**48.3**	**28**	**31.5**	**13**	**14.6**	**5**	**5.6**	**89**
Rag2$^{c/c}$ p53$^{-/-}$	**82**	**58.6**	**33**	**23.6**	**18**	**12.9**	**7**	**5**	**140**

Statistical analysis (Fisher exact test).						*P*-value	Level
Entire distribution	WT	vs.	53bp1$^{-/-}$			7.82e-1	ns
	WT	vs.	Atm$^{-/-}$			1.00e-8	***
	WT	vs.	Rag2$^{c/c}$			2.60e-6	***
	WT	vs.	Rag2$^{c/c}$ p53$^{-/-}$			2.10e-6	***
γ-H2AX on both *Tcra / Igh*	WT	vs.	53bp1$^{-/-}$			5.00e-1	ns
	WT	vs.	Atm$^{-/-}$			8.60e-8	***
	WT	vs.	Rag2$^{c/c}$			1.30e-4	***
	WT	vs.	Rag2$^{c/c}$ p53$^{-/-}$			2.60e-4	***

Frequency of *Tcra-Igh* pairing in DP cells:	Paired *Tcra-Igh*		Total *Tcra-Igh* distances	*Statistical analysis (Fisher exact test).WT vs. Mutant:*	
	number	%		*P*-value	Level
TOTAL WT	**230**	**13.7**	**1682**	-	-
53bp1$^{-/-}$	**87**	**13.5**	**646**	9.10e-1	ns
Atm$^{-/-}$	**136**	**21.3**	**640**	1.70e-4	***
Rag2$^{c/c}$	**89**	**22**	**404**	4.40e-4	***
Rag2$^{c/c}$ p53$^{-/-}$	**140**	**21.8**	**642**	5.80e-5	***

Supplementary Table S7. RAG mediated heterologous pairing is not a general phenomenon that occurs between RAG2 enriched genes. Pairing between loci in individual DN2/3 and DP cells (Fig. 5b,c).

	WT DN2/3		R1^{−/−} DN2/3		WT DP	
	Paired / Total	%	Paired / Total	%	Paired / Total	%
Tcra-Igh	184 / 892	20.6	90 / 664	13.6	76 / 648	11.7
Tcra-Hmgb1	39 / 252	15.5	32 / 212	15.1	66 / 346	19.1
Tcra- Gapdh	26 / 200	13	31 / 256	12.1	68 / 332	20.5
Tcra-Lat	21 / 252	8.3	35 / 212	16.5	81 / 346	23.4
Tcra- Cd3g	28 / 200	14	60 / 256	23.4	87 / 332	26.2
Tcra- Ly6d	21 / 252	8.3	24 / 212	11.3	58 / 346	16.8
Tcra- Satb1	14 / 200	7	36 / 256	14.1	57 / 332	17.2
Tcra-Igh	184 / 892	20.6	90 / 664	13.6	76 / 648	11.7
Hmgb1-Ly6d	19 / 252	7.5	25 / 212	11.8	62 / 346	17.9
Hmgb1-Lat	20 / 252	7.9	28 / 212	13.2	74 / 346	21.4
Ly6d-Lat	22 / 252	8.3	30 / 212	14.2	81 / 346	23.4
Gapdh-Satb1	14 / 200	7	28 / 256	10.9	43 / 332	13
Gapdh-Cd3g	11 / 200	5.5	36 / 256	14.1	66 / 332	19.9
Satb1-Cd3g	10 / 200	5	24 / 256	9.4	51 / 332	15.4

Supplementary Table S8.

Looping out of *Tcra* and/or *Igh* from their chromosome territories in *Tcra-Igh* pairs in DP cells (Fig. 6a).

Paired loci	No loop		*Tcra* loops		*Tcra* and *Igh* loops		*Igh* loops		Sample size
	number	%	number	%	number	%	number	%	(pairs)
WT	68	75.6	16	17.8	1	1.1	5	5.6	90
Atm$^{-/-}$	24	43.6	24	43.6	4	7.3	3	5.5	55
Rag2$^{c/c}$ *p53*$^{-/-}$	27	48.2	17	30.4	8	14.3	4	7.1	56
Statistical analysis (two-tail χ^2 or Fisher exact test):							*P*-value		Level
WT vs. *Atm*$^{-/-}$			Entire distribution				5.50e-4		***
			Tcra and *Igh* loops				4.55e-2		*
WT vs. *Rag2*$^{c/c}$ *p53*$^{-/-}$			Entire distribution				1.01e-3		**
			Tcra and *Igh* loops				1.29e-3		**

Supplementary Table S9.

Frequency of PCH association of *Tcra* and/or *Igh* alleles in *Tcra-Igh* pairs in DP cells (Fig. 5c).

Paired loci	No PCH association		*Tcra*		Both *Tcra* and *Igh*		*Igh*		Sample size
	number	%	number	%	number	%	number	%	(pairs)
WT	132	60.6	9	4.1	16	7.3	61	28	218
Atm$^{-/-}$	148	81.3	10	5.5	2	1.1	22	12.1	182
Rag2$^{c/c}$ *p53*$^{-/-}$	160	87	6	3.3	1	0.5	17	9.2	184
Statistical analysis (two-tail χ^2 or Fisher exact test):							*P*-value		Level
WT vs. *Atm*$^{-/-}$							5.40e-6		***
WT vs. *Rag2*$^{c/c}$ *p53*$^{-/-}$							2.00e-8		***

Supplementary Table S10.

Frequency of damage on both *Tcra* and *Igh* in invidual DP cells (Fig. 5e).

Tcra and *Igh*	No damage on both loci		Damage on both loci		Sample size
	number	%	number	%	(cells)
WT	329	99.7	1	0.3	330
53bp1$^{-/-}$	171	100	0	0	171
Atm$^{-/-}$	177	91.2	17	8.8	194
Rag2$^{c/c}$ *p53*$^{-/-}$	116	92.8	9	7.2	125
Statistical analysis (Fisher exact test):	*P*-value		Level		
WT vs. *53bp1*$^{-/-}$	4.71e-1		ns		
WT vs. *Atm*$^{-/-}$	2.80e-7		***		
WT vs. *Rag2*$^{c/c}$ *p53*$^{-/-}$	7.50e-6		***		