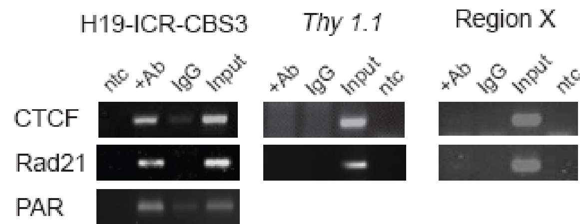
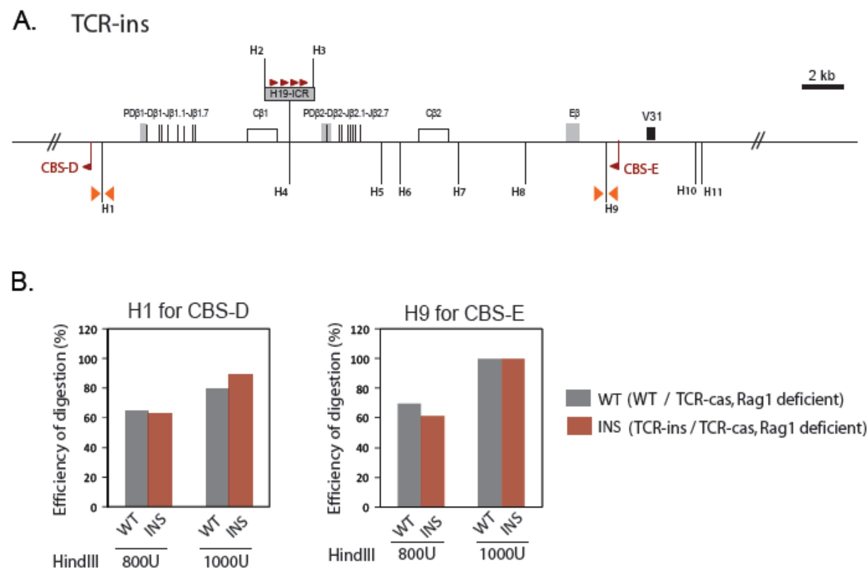
Supplementary Information



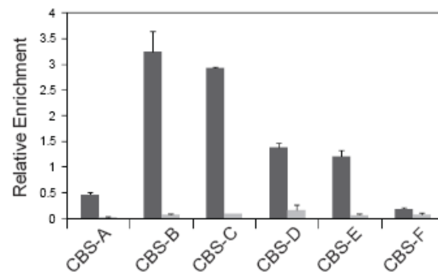
Supplementary Figure 1 : **ChIP-PCR analysis to estimate binding of CTCF, Rad-21 and presence of PAR moieties at control regions**. Data was collated from ChIPs on DN thymocytes. H19-ICR-CBS3 (mm10:chr7: 142580733-142580840) was used as a positive control. *Thy 1.1* exonic region (mm10: chr 9: 44047850-44047960) and TCRb locus non-genic Region X (mm10:chr6: 41562424-41562527; located between CBS-E and CBS-F) served as negative control regions for anti-CTCF and anti-Rad21 ChIPs. A suitable negative region for anti-PAR ChIP is difficult to predict as Poly-ADP-ribosylation is involved in many cellular and nuclear processes including DNA repair, replication and transcriptional regulation and several DNA binding proteins exhibit dynamically regulated Parylation.



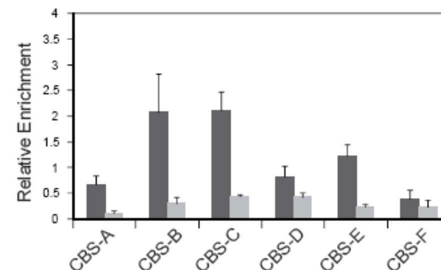
Supplementary Figure 2 : Estimation of efficiency of chromatin digestion by HindIII between maternally inherited TCR-ins and wild type TCRb alleles during 3C analysis. **A.** Schematic map of TCR-ins allele showing the location of HindIII sites (H1-H11) and CBS sites CBS-D and CBS-E in the vicinity of recombination center encompassing PDb1-DJCb1, PDb2-DJCb2 and Eb. **B**. Efficiency of HindIII based digestion of chromatin. Crosslinked Chromatin was digested with 800U or 1000U of HindIII. DNA was isolated from digested and undigested chromatin fractions and used to determine efficiency of digestion by qPCR as described (Hagege et al, 2007). Digestion efficiencies at HindIII sites H1 and H9, in the vicinity of CBS-D and CBS-E, were analyzed. Primers for qPCR (orange triangles) across H1 and H9 were allele specific and amplified DNA specifically from maternally inherited (*domesticus*) wild type TCRb allele and TCR-ins allele. Data shown is from two HindIII digested libraries of each genotype. Each library was created by pooling thymocytes form 8-10 mice of specified genotypes.

A. Wild typeTCRb locus

B. CTCF



Rad 21



Sample key

anti-CTCF or anti-Rad21 IgG (control for the adjacent left sample)

Supplementary Figure 3 : Presence of CTCF and Rad-21 at TCRb locus in Double Positive (DP) thymocytes. **A.** Schematic map showing the analyzed CTCF binding sites (CBS), Trbv segments (V1-V31) and recombination center encompassing PDb1-DJCb1, PDb2-DJCb2 and Eb. **B.** ChIP-qPCR analysis to estimate binding of CTCF and Rad-21. The ChIP-pPCR signals were normalized to the values observed for CBS-3 of H19-ICR at the *Igf2/H19* locus. Values represent mean enrichments (+SEM) from three biological replicates. For isolating DP cells, total thymocytes of OT2-Tg mice, were stained with anti-CD4 PE (clone RM4-5) and anti-CD8a-FITC (clone 53-6.7) antibodies and sorted using BD FACS AriaIII.

Supplementary Table 1 : Genomic Coordinates of the CBS analyzed

CTCF Binding Sites			Closest Transcriptional Segments		
Name	Motif Coordinates	Orientation	Name	Coordinates	Orientation
CBS-A	41091302-41091320	plus	V10	41091935-41092228	plus
CBS-B	41113049-41113069	plus	V12.1	41113567-41114067	plus
CBS-C	41137170-41137198	plus	V14	41135167-41135616	plus
CBS-5'PC	41505556-41505537	minus	Prss2	41521776 - 41525079	plus
CBS-D	41530175-41530194	minus	D1	41533201-41533212	plus
CBS-E	41556570-41556588	minus	V31	41557693-41558371	minus
CBS-F	41563427-41563447	minus	Gm38582	41558555 - 41603451	plus

The coordinates are according to GRCm38/mm10 (accession number NC_000072.6) and the orientations of CBS are based on comparison with PWM defined earlier (Kim et al, 2007).

Supplementary Table 2 :

Specificity of amplification during allele specific 3C-qPCR analysis

	Amplicon	Ct of Dom/Dom (3C library)	Ct of Cas/Cas (3C library)
CBS-B as anchor	CBS-B/CBS-A	33.04	36.61
	CBS-B/CBS-C	35.5	39.4
	CBS-B/CBS-5'PC	39.06	Undetected
	CBS-B/CBS-D	36.43	39.25
	CBS-B/CBS-E	37.76	42.14
CBS-E as anchor	CBS-E/CBS-A	39.65	Undetected
	CBS-E/CBS-B	40.20	Undetected
	CBS-E/CBS-C	38.58	Undetected
	CBS-E/CBS-5'PC	36.19	Undetected
	CBS-E/CBS-D	37.48	Undetected
Eb as anchor	Eb/V4	36.5	Undetected
	Eb/V12.1	36.3	Undetected
	Eb/V19	35.4	Undetected
	Eb/V26	36	Undetected
	Eb/V31	32.6	42
PDb2-DJb2 as anchor	PDb2-DJb2/V4	35	Undetected
	PDb2-DJb2/V12.1	34.5	Undetected
	PDb2-DJb2/V19	33	41
	PDb2-DJb2/V26	34	Undetected
	PDb2-DJb2/V31	33.2	Undetected

Thymocytes derived from Rag1 deficient TCRb-wt/TCRb-wt (Dom/Dom) and Rag1 deficient TCR-cas/TCR-cas (Cas/Cas) mice were used to construct 3C libraries. Equal amount of DNA was used as template for 3C-qPCR using Taqman probes. Relative Ct values were compared for the 3C libraries for various test amplicons. While Dom/Dom gave amplification, Cas/Cas based amplification was undetected or considerably lower in each case. Hence, these amplicons were suitable for *domesticus* allele specific detection of 3C products in the 3C-qPCR assay. Primer "Ins" was located on the H19-ICR. Therefore, all amplicons that used it (Ins-CBS-B, Ins-CBS-E etc.) were *domesticus* allele specific. Amplicons detecting negative regions (N1-N4) were not verified for allele specificity. Their signals were very low in the 3C libraries. Dom, *domesticus*; Cas, *castaneus.*

Supplementary Table 3 :

Extent of change in long range interactions as influenced by the allele and the point of interaction

	Interaction	Relative crosslinking frequency (RCF) normalized to TCRb wild type allele			Inference based on Two Way ANOVA
		Wild type	**TCR-ins**	**TCR-mut**	
Anchor CBS-B or CBS-E	CBS-B/CBS-A	1	0.61	0.95	source of variation Alleles : Significant (p <0.0001) Point of interaction : not significant (p=0.0956)
	CBS-B/CBS-C	1	0.57	0.94	
	CBS-B/CBS-5'PC	1	0.62	1.19	
	CBS-B/CBS-D	1	0.45	0.83	
	CBS-B/CBS-E	1	0.22	0.67	
	CBS-E/CBS-A	1	0.43	1.2	
	CBS-E/CBS-B	1	0.59	0.96	
	CBS-E/CBS-C	1	0.52	1.16	
	CBS-E/CBS-5'PC	1	0.24	0.88	
	CBS-E/CBS-D	1	0.42	1.14	
	Average	**1**	**0.47**	**0.99**	
	SEM	**0**	**0.047**	**0.056**	
Anchor Eb	Eb/V4	1	0.47	0.81	source of variation Alleles :Significant (p <0.0002) Point of interaction: not significant (p=0.2683)
	Eb/V12.1	1	0.51	1.04	
	Eb/V19	1	0.45	0.70	
	Eb/V26	1	0.53	0.92	
	Average	**1**	**0.49**	**0.87**	
	SEM	**0**	**0.017**	**0.073**	
Anchor PDb2-DJb2	PDb2-DJb2/V4	1	0.64	1.39	source of variation Alleles : Significant (p <0.0033) Point of interaction : not significant (p=0.5250)
	PDb2-DJb2/V12.1	1	0.48	0.86	
	PDb2-DJb2/V19	1	0.42	1.59	
	PDb2-DJb2/V26	1	0.29	1.40	
	Average	**1**	**0.46**	**1.31**	
	SEM	**0**	**0.075**	**0.157**	

Relative crosslinking frequencies (RCF) were estimated using allele specific 3C-qPCR analysis. For each allele, at each point of interaction, the RCF was normalized to that observed in the wild type allele. Two Way ANOVA was used to determine the influence of the two factors (allele type and point of interaction) on RCF for each set of long range interactions. In each case, it was evident that RCF was influence by ALLELE and it was not influenced by POINT OF INTERACTION (p-value based on F distribution calculated by ANOVA). Average of normalized interactions is also shown in Figure 3B and Figure 4B-4C.