

Supporting Information

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SI Materials and Methods

CD4-FITC (561835), CD8-FITC (553031), CD4-biotin (553044), CD8a-biotin (553028), CD44-PE (553134), and CD25-APC (557192) antibodies were purchased from BD Biosciences and used for cell

staining and sorting. H3K4me2 (07-030, Millipore), H3K27ac (ab4729), and P300 (C-20) (SC-585x) for H3ac (06-599; Millipore), H3K4me3 (39159; Active Motif), and CTCF (07-729; Millipore) antibodies were purchased and used for ChIP experiments.

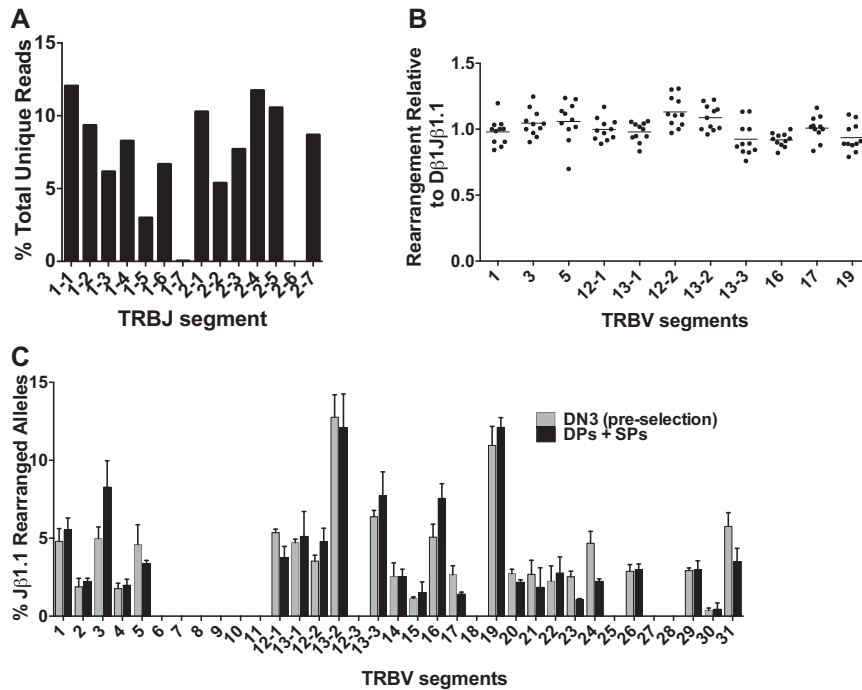


Fig. S1. Variable (V) β repertoire comparisons. (A) Joining (J) β use profile from high-throughput sequencing [mean ($n = 3$), 15,000–20,000 unique reads per sample]. (B) Distribution of rearrangements from high-throughput sequencing involving V β segments and each of the 11 functional J β segments. Shown are distributions for rearrangements of V β segments yielding at least 1,000 unique reads. Data are represented relative to the distribution of V β -J β 1.1, where percent total V β -J β 1.1 is set to a value of 1 (Fig. 1). Each circle represents a data point for a given J β segment. (C) Comparison of V β use in preselection and postselection thymocytes measured by the genomic DNA (gdNA) assay described in Fig. 1C (mean \pm SEM, $n = 3$).

Table S2. Primers and probes for V β utilization assay

Taqman probes (5'FAM and 3' TAMRA from Sigma Life Sciences)

J β 1.1 probe

J β 2.1 Probe

Primers for cloning V β J β template plasmids

J β 1.1 F

J β 1.1 R

J β 2.1 F

J β 2.1 R

V1-F

V1-R

V2-F

V2-R

V3-F

V3-R

V4-F

V4-R

V5-F

V5-R

V6-F

V6-R

V7-F

V7-R

V8-F

V8-R

V9-F

V9-R

V10-F

V10-R

V11-F

V11-R

V12-1-F

V12-1-R

V13-1-F

V13-1-R

V12-2-F

V12-2-R

V13-2-F

V13-2-R

V12-3-F

V12-3-R

V13-3-F

V13-3-R

V14-F

V14-R

V15-F

V15-R

V16-F

V16-R

V17-F

V17-R

V18-F

V18-R

V19-F

V19-R

V20-F

V20-R

V21-F

V21-R

V22-F

V22-R

V23-F

V23-R

Sequences

5'FAM-TGTGAGTCTGGTTCCTTACCAA-3'TAMRA

5'HEX-TAGGACGGTGAGTCGTGTCC-3'TAMRA

Sequences

5'-GACAGACGGATCCTGGCACTGTGCAAACACAGAAGTC-3'

5'-TACATCGCGGCCGCACTCGAATATGGACACGGAGGACA-3'

5'-GACAGACGGATCCGTAACATATGCTGAGCAGTTCTTCGGACC-3'

5'-TACATCGCGGCCGAGTCCTGGAAATGCTGGCACAACAAC-3'

5'-TATCTCGAGCTGGAGCAAAACCCAAGGTG-3'

5'-CGAGAAGCTTTGCAGTACAAGTTCTGCCCT-3'

5'-TATCTCGAGCGAAAATTATCCAGAAACCAA-3'

5'-CGAGAAGCTTGACAGAAAGTATGTGGCCGAG-3'

5'-TATCTCGAGCAGATGGTGACCCCTCAATTGT-3'

5'-TAGCGAAGCTTTAAGCTGCTGGCACAGAAG-3'

5'-TATCTCGAGGACGGCTGTTTTCCAGACT-3'

5'-CGAGAAGCTTTGGCACAGAGATACACAGCAG-3'

5'-TATCTCGAGGATATCTAATCTGGGAAGAGC-3'

5'-CGAGAAGCTTCTGCCGTGGATCCAGAAGACT-3'

5'-TATCTCGAGGTTACAGACATGGGACAGAAATGTCA-3'

5'-CGAGAAGCTTAGCTGCTGGCATAACATAGTGGAGT-3'

5'-TATCTCGAGAGCAGGCTCTGCTTCTGACTTGT-3'

5'-CGAGAAGCTTAGAACAGTGCAGAGTCTTTGGCT-3'

5'-TAGCCTCGAGCATTGACTCCCAAATCAT-3'

5'-TAGCGAAGCTTCTGTGCATGATCTGGAGAC-3'

5'-TATCTCGAGGTGACACAATTTCTGGTCTACTGG-3'

5'-CGAGAAGCTTCTTCTGGCACAGAGATAGATGCCT-3'

5'-TATCTCGAGGTTGGAATCACCCAGACACCTAGATA-3'

5'-CGAGAAGCTTAGTACATGGAGTCTGGTTGGAACG-3'

5'-TATCTCGAGAGGCACTTCTGATATGTGGCCTCT-3'

5'-CGAGAAGCTTAGTTAGAAAACCATGGCTTTGCC-3'

5'-TATCTCGAGCTGACGTGATTTCCCATCTCT-3'

5'-TAGCGAAGCTTCCAGTCCAAAGGCACTCATG-3'

5'-TATCTCGAGTGGTTAGCCCAAGTGTGCTTCTCT-3'

5'-CGAGAAGCTTAAGCCAATTCCAGCAGGAGGAAGA-3'

5'-TATCTCGAGCATTGCTGCTGCTGCTGCTGC-3'

5'-TAGCGAAGCTTACACGGCAGAGTCTCTAG-3'

5'-TATCTCGAGTCTGTGTTCAAGTGAAGTGGT-3'

5'-CGAGAAGCTTTGGTCTGGAGGCCCTTGATCCAT-3'

5'-TAGCCTCGAGCCTTCTCCCAAGGTCAGC-3'

5'-TAGCGAAGCTTACACAGTAAAGTCTCTAGGTCC-3'

5'-TATCTCGAGGACGATATGATCAGGCTTTG-3'

5'-TAGCGAAGCTTAGAAAATACAGTGTCTGAG-3'

5'-TATCTCGAGTATGCAGTCTACAGGAAGGGCAA-3'

5'-CGAGAAGCTTAAACTGCTGGCACAGAGATAGGTG-3'

5'-TATCTCGAGCAGACCCAGACATGAGGT-3'

5'-CGAGAAGCTTACAGCTGAGTCTTGGGTTCTG-3'

5'-TATCTCGAGCACCTAGGCACAAGGTGACA-3'

5'-TAGCGAAGCTTACAGACTCAGCGGTGATCT-3'

5'-TATCTCGAGGATACTACGGTTAAGCAGAAC-3'

5'-TAGCGAAGCTTAGCACAGAGGTACATGGCAG-3'

5'-TAGCCTCGAGGCTGGTGTACCACGAACCT-3'

5'-TAGCGAAGCTTCTGTCATCTCCAGATCTGC-3'

5'-TATCTCGAGCTCAGACACCCAAATCCTGA-3'

5'-CGAGAAGCTTGCTATACTGCTGGCACAGAGA-3'

5'-TATCTCGAGCTCTATCAATATCCAGAAG-3'

5'-CGAGAAGCTTAGCACACAGAGATATAAGCC-3'

5'-TAGCCTCGAGGTTGTCCAGAATCCTAGACAT-3'

5'-TAGCGAAGCTTGTACACAGCTGAATCTGTTAG-3'

5'-TATCTCGAGCCAAGTTATCCAGACTCCAT-3'

5'-TAGCGAAGCTTATAACTGAGTCTCCAGCCTC-3'

5'-TATCTCGAGGAAAGGCCAGGAAGCAGAGAT-3'

5'-CGAGAAGCTTGCTGGAGCAAGTACAGTGC-3'

Table S3. Primers and probes for 3C assay

3C anchor primers and Taqman probes (5' FAM and 3' TAMRA from Sigma Life Sciences)	Sequences
D β _1 HindIII probe	5'-AAGGCATTGTTGCATGATCCT-3'
D β _2 HindIII probe	5'-AAATGCTGGGCCTCTGTAGA-3'
E β _ HindIII probe	5'-CATAAGCATTGTGATGTTTGTGACA-3'
ERCC3 HindIII probe	5'-AAAGCTTGACCCCTGCTTTAGTGGCC-3'
D β _1 HindIII primer	5'-TGAAATTTTTCTGCCGAAAGGAC-3'
D β _2 HindIII primer	5'-GCGGGATCCAAGAGAACTCA-3'
E β _ HindIII primer	5'-GAAAATTGGCATCGTTTTGC-3'
HindIII primers	Sequences
V1	5'-TATCTCTGTGGGCATGCAG-3'
V2	5'-TTTCATTCACAGCCGACCAG-3'
V3	5'-TTTCATTCACAGCCGACCAG-3'
V4	5'-AGCTCGACACAGAAAGCAAGTT-3'
V5	5'-AGCTCGACACAGAAAGCAAGTT-3'
V6	5'-GGTCCCTTCACTTCCCACA-3'
V7	5'-GTCCGCTAGCAGCCAGAGTT-3'
V8	5'-GTCCGCTAGCAGCCAGAGTT-3'
V9	5'-ACCAGAGGGCAGCTGAAAAT-3'
V10	5'-GTGCCTGTACCATGCTGTGG-3'
V11	5'-TTCAGCAAGTAGGTGCGAAGA-3'
V12-1	5'-TGGTGGGATCCTGACAGCTTATA-3'
V13-1	5'-CCATCTGCATGAACACCTTCTT-3'
V12-2	5'-CCATCTGCATGAACACCTTCTT-3'
V13-2	5'-CCATCTGCATGAACACCTTCTT-3'
V12-3	5'-GGATCTTGGTCTCGGGAGGT-3'
V13-3	5'-CTCAGCTGCACCCTCACAAAC-3'
V14	5'-CAGGCTTTTGAGTGCCATGT-3'
V15	5'-AGGCAGGAGGTGAGTCTTGG-3'
V16	5'-TATCATGCCCAGCTGCATTC-3'
V17	5'-GTTAGGCCGACTGGATTGGA-3'
V18	5'-GGCAGTGTTACAGAACCCAGTG-3'
V19	5'-GGCAGTGTTACAGAACCCAGTG-3'
V20	5'-TGTGATGGGTTGTCATCTGGA-3'
V22	5'-CCAAGGGATGATGTCACAGG-3'
V23	5'-TACACCGGCCAGGAGAGACT-3'
V24	5'-ACTAGGCCAGCAGAGGATGC-3'
V25	5'-ACTAGGCCAGCAGAGGATGC-3'
V26	5'-AGCATAGGATTGGGCCTCAG-3'
V27	5'-CATCACTGCGCCTAGCAATC-3'
V28	5'-GCGTGTGCCACGTTTTTGTA-3'
V29	5'-CTCTAGCAATCCCCCTGTGC-3'
V31	5'-AAGGAGAGAGCAGGCCACAG-3'
D β _1	5'-AAGGCATTGTTGCATGATCC-3'
D β _2	5'-TGGGGCCCTCACTTTTCTTA-3'
E β	5'-TCCTAAGGAGAGGCAGAGTGG-3'
ERCC3	5'-GACTTCTCACCTGGGCCTACA-3'

Table S4. Luciferase assay cloning primers

Primer name	Sequences
Eβ-F	5'-ATTGGATCCGTTAACCAGGCACAGTAGGACC-3'
Eβ-R	5'-ATTGGATCCCATGGTGCATACTGAAGGCTTC-3'
Pro-V1F	5'-TAGCCTCGAGGAGTACTAGTTACTTCTGC-3'
Pro-V1R	5'-TAGCGAAGCTTCTCTGAGACCTCAGTTTCTC-3'
Pro-V3-F	5'-TATCTCGAGGGGACTCAGTTCAGTAGTC-3'
Pro-V3-R	5'-CGAGAAGCTTAGTAGGGTCACGGCAGGAA-3'
Pro-V4F	5'-TAGCCTCGAGTGTGCTAAGGGCACCAATGAAT-3'
Pro-V4R	5'-TAGCGAAGCTTGTGGGTCAAGGCAGGGCAAAT-3'
Pro V5-FX	5'-TAGCCTCGAGTATCCATTGTATGCTCTGTTTG-3'
Pro V5-RH	5'-TAGCGAAGCTTGGTGAATCAGGCTCCAGACG-3'
Pro V6-FX	5'-TAGCCTCGAGCTACAAGCTCCAAGAGAGAG-3'
Pro V6-RH	5'-TAGCGAAGCTTCTCTGGAGAAGACAGAGGAC-3'
Pro-V7F	5'-TAGCCTCGAGGCTGCTGAATAGCAAGTTTCCAG-3'
Pro-V7R	5'-TAGCGAAGCTTTGGAGGTTTGGATCTGTAGTCT-3'
Pro V9-FX	5'-TAGCCTCGAGGGAACCTTTCATGTGAGGAGA-3'
Pro V9-RH	5'-TAGCGAAGCTTCTGCAAAAATAAAGTTGTGAACAG-3'
Pro V10-FX	5'-TAGCCTCGAGGGGATATCTCTATGCTTTAATG-3'
Pro V10-RH	5'-TAGCGAAGCTTCTGGAGAAGGAGGCATAAGGA-3'
Pro-V11F	5'-TAGCCTCGAGTTCCTACAGTGTCAAGGGCTG-3'
Pro-V11R	5'-TAGCGAAGCTTGTACCCACAGGGTTGTTCTCA-3'
Pro-V12-2-F	5'-TAGCCTCGAGCAACTGACTCAGAGAAAAAC-3'
Pro-V12-2-F	5'-TAGCGAAGCTTTCCTCTCAGGATACTGGTCTCT-3'
Pro-V14F	5'-TACATCGCTAGCCATTTATGTGTACCATAATAAT-3'
Pro-V14R	5'-TAGCCTCGAGGGCAGATTGAGGGCAGAGGAG-3'
Pro-V16F	5'-TAGCCTCGAGTTGCAATCTACCTCTGCTGCTC-3'
Pro-V16R	5'-TAGCGAAGCTTTGTGATGACACCACTGTCTCCG-3'
Pro V17-FX	5'-TAGCCTCGAGGCAGGTGTGACCTACGATAAC-3'
Pro V17-RH	5'-TAGCGAAGCTTGGATGTCCAGAACAGGAAA-3'
Pro-V19-F	5'-TATCTCGAGCATTGAGAAAAGACAACAA-3'
Pro-V19-R	5'-CGAGAAGCTTAGTTTGGAGGGACTTCTT-3'
Pro-V20F	5'-TAGCCTCGAGGATAAGGTAAGTGAAGCGGGA-3'
Pro-V20R	5'-TAGCGAAGCTTCTCAGTGTGACTTACACCC-3'
Pro-V22F	5'-TAGCCTCGAGGATGAAATATGGTAACAAGG-3'
Pro-V22R	5'-TAGCGAAGCTTAGGAGATAAAGGGCTACATA-3'
Pro-V24F	5'-TACATCGCTAGCCCAATGATATGTGACAGAGATGA-3'
Pro-V24R	5'-TAGCCTCGAGGATCACACTAGGCCAGCAGAG-3'
Pro-V25F	5'-TAGCCTCGAGCAATTGGGCCATCTTCTGCCAC-3'
Pro-V25R	5'-TAGCGAAGCTTCAGGTGGATACTTCAATCC-3'
Pro-V28F	5'-TAGCCTCGAGAGTTGTCTTGTGGGCAACTCTG-3'
Pro-V28R	5'-TAGCGAGATCTGCTAGATAGCCTCAAGGCTGCAAA-3'

Table S5. Recombination substrate oligoes

Primer name	Sequences
RS V1 F	TAGCCTCGAGATACGGAGCTGAGGCTGCAAG
RS V1 R	TACATCGCGCCGCGAGTACCTTATAACTCATGCA
RS V15F	TAGCCTCGAGCCTTCTCCACTCTGAAGATTCTC
RS V15R	TACATCGCGCCGCTTCCACCCAAAGATTCTTAA
RS V16F	TAGCCTCGAGACTCAACTCTGAAGATCCAGA
RS V16R	TACATCGCGCCGCTAATGTAATACTCGTTACCAT
RS V18F	TAGCCTCGAGCCCAACATCCTAAAGTG
RS V18R	TACATCGCGCCGCTTCTCCGTAAGCATGGTG
RS V20F	TAGCCTCGAGCAGTATCCCAACTTATCCT
RS V20R	TACATCGCGCCGCTCCTGGGTACCCTCCCATTTC
RS V23F	TAGCCTCGAGCACTCTGCAGCCTGGGAATC
RS V23R	TACATCGCGCCGCTGACTTGGTCTGGGTGTGCTG
RS V24F	TAGCCTCGAGAGTGCATCCTGAAATCCTAT
RS V24R	TACATCGCGCCGCGAGACCTGGCCTGTTTCTCATG
RS V26F	TAGCCTCGAGCAAGAAGTTCTCAGCAAATA
RS V26R	TACATCGCGCCGCGATACAGGTTTCTCAGTTAGTT

Table S6. Computational analysis coefficients for determinants of V β frequencies (all *Tcrb* V gene segments): Classifier step, three features

Features	Estimate	SE	<i>t</i>	<i>Pr</i> (> <i>t</i>)
Intercept	1.09059	1.52205	0.717	0.47903
Recombination signal information content (RIC) score	0.08803	0.02619	3.362	0.00207
Formaldehyde-assisted isolation of regulatory elements (FAIRE)	0.03185	0.01639	1.944	0.06105
RNA Pol II	0.65913	0.26654	2.473	0.01909

Table S7. Computational analysis coefficients for determinants of V β frequencies (all *Tcrb* V gene segments): Combinatorial analysis of 13 features and their correlation to recombination frequency

Number of features	Pearson correlation coefficient	<i>P</i> value
13	0.77954	0.4707
8	0.74191	0.1015
7	0.72604	0.07434
6	0.71277	0.04925
5	0.68818	0.03779
4	0.66405	0.0265
3	0.64982	0.01359
2	0.60304	0.01089
1	0.53998	0.00782

Table S8. Coefficients for determinants of V β frequencies (rearranging V β segments)

Features	Estimate	SE	<i>t</i>	<i>Pr</i> (> <i>t</i>)
All <i>Tcrb</i> V gene segments (regressor step, 13 features)				
Intercept	0.08707	5.81E+00	0.015	0.9882
RIC score	0.08817	3.72E-02	2.373	0.0273
3C cross-linking	-2.17745	3.14E+00	-0.693	0.4961
Transcription	-0.1299	1.56E-01	-0.83	0.4156
CCCTC-binding factor (CTCF)	0.9394	1.24E+00	0.756	0.4579
FAIRE	0.01538	2.46E-02	0.625	0.5384
H3ac	-0.31847	5.05E-01	-0.63	0.5353
H3K27ac	0.03124	3.86E-02	0.81	0.4271
H3K4me1	0.16488	6.88E-01	0.24	0.813
H3K4me2	0.0194	1.67E-02	1.159	0.2595
H3K4me3	-0.08483	3.68E-01	-0.231	0.8197
H3K9me2	0.74873	1.27E+00	0.59	0.5618
P300	-0.03168	3.03E-02	-1.047	0.3069
RNA Pol II	1.10351	5.21E-01	2.119	0.0462
All <i>Tcrb</i> V gene segments (Regressor step, 5 features)				
Intercept	0.62866	0.35047	1.794	0.0907
Transcription	-0.0613	0.0467	-1.313	0.2066
CTCF	0.31418	0.25634	1.226	0.2371
H3K4me2	0.00779	0.00365	2.137	0.0475
H3K4me3	0.16139	0.0666	2.423	0.0268
P300	-0.0066	0.00646	-1.027	0.319

Other Supporting Information Files

[Dataset S1 \(XLSX\)](#)