Supporting Information

del Blanco et al. 10.1073/pnas.1406551112

SI Materials and Methods

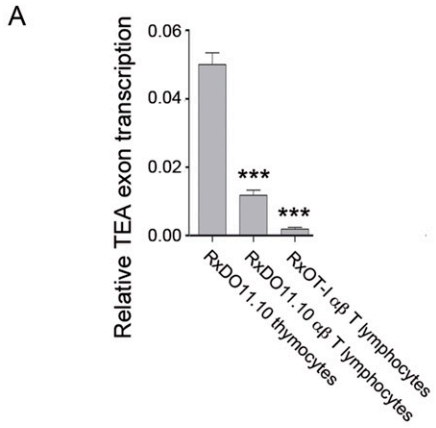
OT-I Mouse Strain. OT-I mice were described (1) and were obtained from Taconic on a *Rag2*$^{-/-}$ background. Animals were maintained in pathogen-free conditions within the Animal Houses at the IPBLN-CSIC. Five- to 8-wk-old mice were used in all experiments. Animal use adhered to CSIC Bioethical Guidelines.

Characterization of Jα61-Cα Transcripts. The PCR product from the RT-qPCR obtained using the Jα61 and Cα primers (Table S1) was extracted from the agarose gel by using the GeneClean Turbo kit (MP Biomedicals). The agarose-purified Jα61 amplified PCR product was ligated into the pGEM-T plasmid (Promega) as recommended by the manufacturer and cloned into DH5 *Escherichia coli* bacteria. Plasmid preparations from independent colonies were sequenced using a 3130XL Genetic Analyzer (Applied Biosystem). The sequences were aligned using the Pubmed Gene ID: 21473 database.

Western Blot. Thymocyte and T lymphocyte nuclear extracts were prepared as indicated above. Five micrograms of nuclear extract proteins were separated by 12% (wt/vol) SDS/PAGE, transferred to a nitrocellulose membrane (GE Healthcare Lifescience), and then incubated with E12 (sc-762), E47 (sc-763), or CDK9 (sc-484) Abs (Santa Cruz Biotechnology) at a 1:500 dilution. Diluted peroxidase-conjugated anti-rabbit IgG (1:500) (PerkinElmer Life Science) was used, and the bound Abs were detected using enhanced chemiluminescence (Perkin-Elmer Life Science).

Intracellular Staining for Flow Cytometry Analysis. Th cells were fixed and permeabilized using the BD Pharmingen Transcription Factor Buffer Set (BD Biosciences) as recommended by the manufacturer. Intracellular GATA-3 was detected by using GATA-3-PE Ab clone L50-823 (BD Biosciences).

1. Hogquist KA, et al. (1994) T cell receptor antagonist peptides induce positive selection. *Cell* 76(1):17–27.



B

Jα61	GTA	CAG	AAT	TAA	TAG	GAA	ATT	GGC	ATT	TTT	TTT	TTT	GGA	GCC	ATG	ACT	GGA	AGA	CTC	ATG	AAA	CTC	AGC
	V	Q	N	*	*	E	I	G	I	F	F	F	G	A	M	T	G	R	L	M	K	L	S

C

TGGGCATGCATGCTCCGGCCGCCATGGCGGCCGCGGGAATTCGATTGCCATGACTGGAAGACTCATGAAACTCAGCAAGTAACATCTGACAGAATTACTTTTTTTCTACCTGAAAA
TTGCTGGAGGGAGATGGAAGTGTTCCTTCAGGAATAGTAACAAAATATGGATATTTAATACAATTCAACTTAACAAATACTTATTAACAACCTACAATTTTCCCACGAATCATCCA
GGCCGATTTCTAAAAAGACATTGTCAATGTCCCAAGAGACGCAAAGGCTTTTGTCAATGGACCAGGGATATGAACGAACAGTAGTGGACTTTGTCGTGAGCCTAGGACATAGTTAA
CAGTGGCCCTGAGTGATGTGGCTTCAGAAAGAAAAAATGTGCATCACATGGGTAACCTTCAACACCAGACATCCAGAACCCAGAACCTGCTGTGTACCAGTTAAAAGATCCTCGGT
CTCAGGACAGCACCCTCTGCCTGTTCACCGACTTAATCACTAGTGAATTCGCGGCCGCCTGCAGGTCGACCATAT

Fig. S1. Analysis of TEA exon transcription in RxDO11.10 and Rag2$^{-/-}$ × OT-I (RxOT-I) αβ T lymphocytes and nucleotide/amino acid sequences of the Jα gene segments and the Jα61 transcripts analyzed in this study. (*A*) Analysis of TEA exon transcription in RxDO11.10 and RxOT-I thymocytes and peripheral T lymphocytes by RT-qPCR. The results were normalized to those for *Actb* and represent the mean ± SEM of duplicate RT-qPCR from three independent experiments. Two-tailed Student's *t* tests were used to determine the statistical significance between the values of the RT-qPCRs from RxDO11.10 thymocytes versus RxDO11.10 and RxOT-I T lymphocytes (***$P < 0.0005$). (*B*) Sequence of the Jα61 transcripts. (*C*) The Jα61 PCR product obtained by RT-PCR was subcloned into the pGEM-T plasmid to be sequenced using the M13 forward and reverse primers (Promega). Six of six independent clones were sequenced, and the same sequence was obtained in the analysis of all clones. The plasmid pGEM backbone sequence is represented in green; the Jα61 gene segment sequence is represented in black; the genomic region downstream of the Jα61 gene segment that is spliced onto the first exon of the Cα region is represented in red; the first Cα exon sequence is represented in blue. The sequences of the primers used to amplify the Jα61 transcripts are underlined.

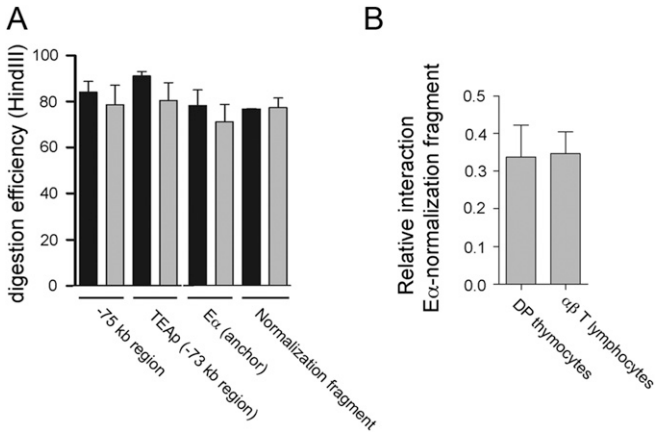


Fig. S2. Determination of HindIII digestion efficiency and relative interaction analysis of Eα with the normalization fragment from RxDO11.10 cells. (*A*) Digestion efficiencies at the indicated genomic fragments obtained from DP thymocyte-derived DNA HindIII digestions are represented as black bars, and those obtained from the HindIII digestion of DNA derived from T lymphocytes are represented as gray bars. The digestion efficiency was calculated according to a published protocol (1). Primers used for the determination of restriction efficiency are listed in Table S1. (*B*) Determination of relative interaction of Eα with the control −75 kb region measured by qPCR in DP thymocyte and T lymphocyte HindIII-digested and ligated DNA. Primers used are listed in Table S1. The results represent the mean ± SEM of duplicate qPCRs of three independent experiments.

1. Hagège H, et al. (2007) Quantitative analysis of chromosome conformation capture assays (3C-qPCR). *Nat Protoc* 2(7):1722–1733.

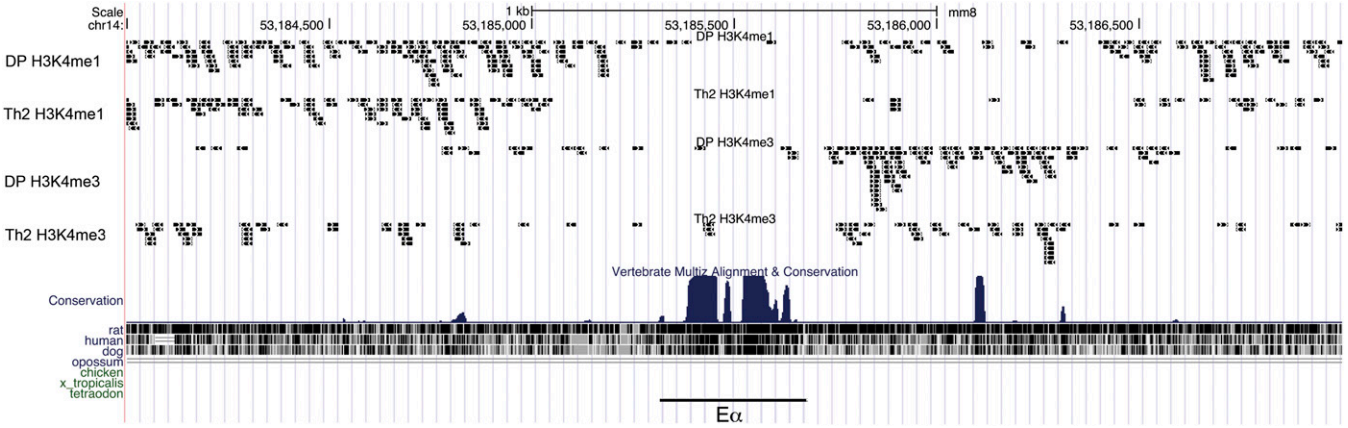


Fig. S3. Comparative H3K4me1 and H3K4me3 analyses in DP thymocytes and Th2 lymphocytes from WT mice by ChIP-sequencing. A public database of H3K4me1 and H3K4me3 results obtained from ChIP-sequencing experiments performed in WT DP thymocytes and Th2 cells (data from ref. 1) was analyzed. The figure shows a screenshot of the UCSC Mouse mm8 Genome Browser (data from ref. 2) with the alignments of the reads obtained from the different samples downloaded as BED files. The genomic location of Eα (Tα1–Tα4 core region) is also indicated.

1. Wei G, et al. (2011) Genome-wide analyses of transcription factor GATA3-mediated gene regulation in distinct T cell types. *Immunity* 35(2):299–311.
2. Karolchik D, et al. (2004) The UCSC Table Browser data retrieval tool. *Nucleic Acids Res* 32(Database issue):D493–D496.

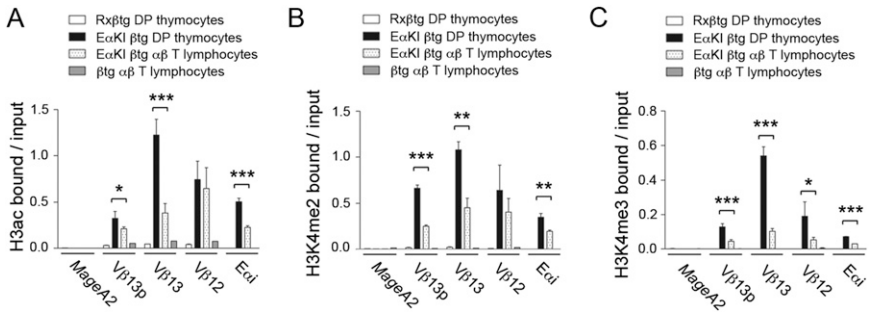


Fig. S4. Comparative H3ac, H3K4me2, and H3K4me3 analyses in DP thymocytes and T lymphocytes from EαKI alleles by qChIP. Analysis of indicated histone H3Ac (*A*), H3K4me2 (*B*), and H3K4me3 (*C*) at Vβ13p, Vβ13, and Vβ12 gene segments, and Eαi in DP thymocytes from Rxßtg and EαKI Rxßtg mice, and T lymphocytes from EαKI βtg and βtg mice. *MageA2*, negative control (1). Data represent the mean ± SEM of duplicate qPCR analysis of three independent experiments. Two-tailed Student's *t* test were used to determine the statistical significance between the values of the qChIPs from DP thymocytes versus T lymphocytes (*$P < 0.05$, **$P < 0.005$, and ***$P < 0.0005$).

1. Hao B, Krangel MS (2011) Long-distance regulation of fetal Vδ gene segment TRDV4 by the *Tcrd* enhancer. *J Immunol* 187(5):2484–2491.

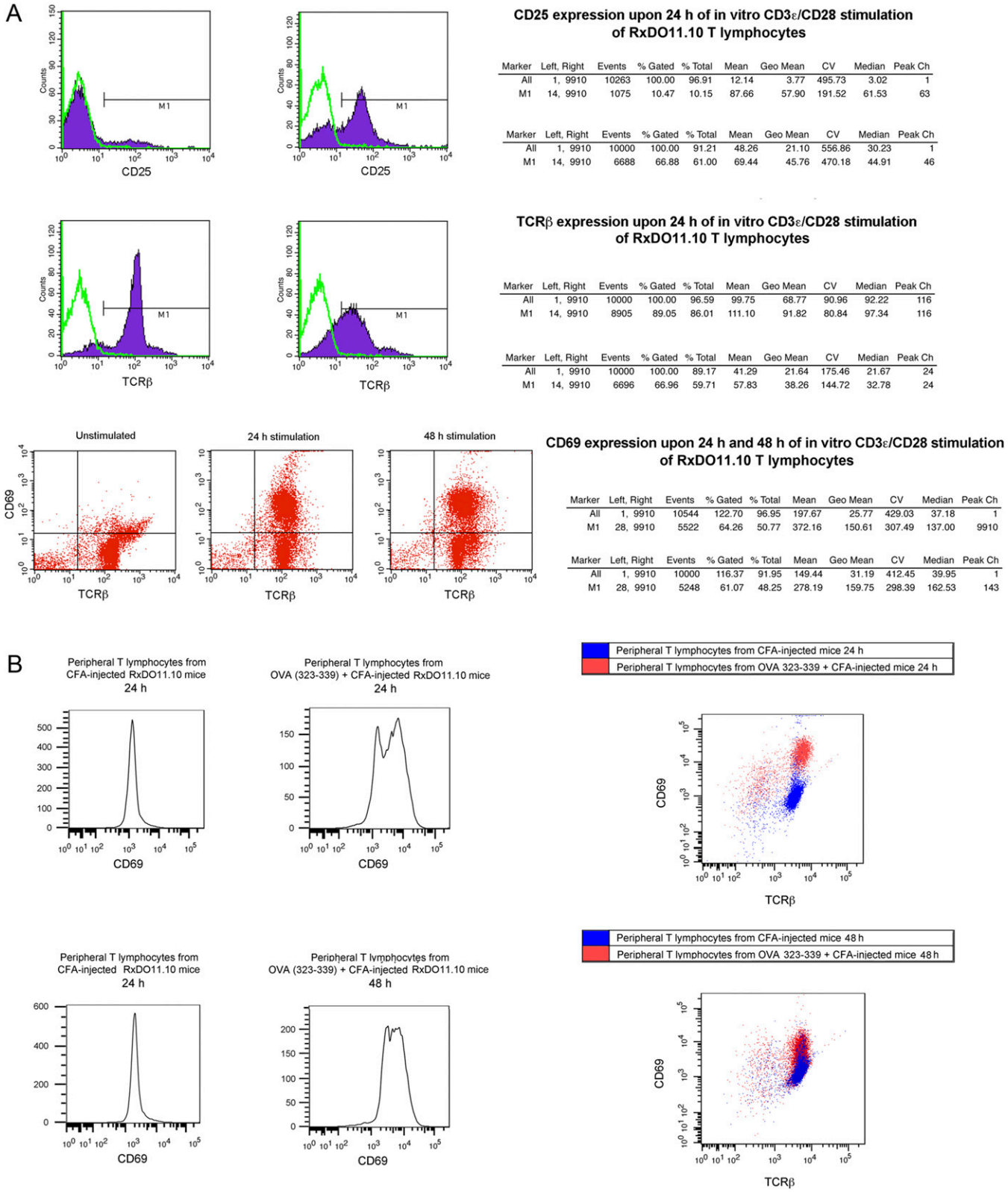


Fig. S5. Measure of CD25, TCRβ, and CD69 upon TCR–mediated stimulation of T lymphocytes by flow cytometry. (*A*) CD25 and CD69 expression is up-regulated, whereas TCRβ expression is down-regulated upon in vitro TCR-mediated stimulation of T lymphocytes. CD25, TCRβ, and CD69 expression was analyzed by flow cytometry in unstimulated and plate-bound CD3ε and CD28 Ab-stimulated RxDO11.10 T lymphocytes. The green line represents staining with the control Ab, and the purple graphic represents staining with specific CD25 or TCRβ Abs after 24 h of stimulation. Similar TCRβ/CD69 double-expression patterns were observed upon 24 h and 48 h of T lymphocyte stimulation. (*B*) CD69 expression is up-regulated upon in vivo TCR-mediated stimulation of T lymphocytes. CD69 up-regulation is maximal at 24 h after OVA 323–339 + CFA-immunization, whereas it is down-modulated 48 h after immunization.

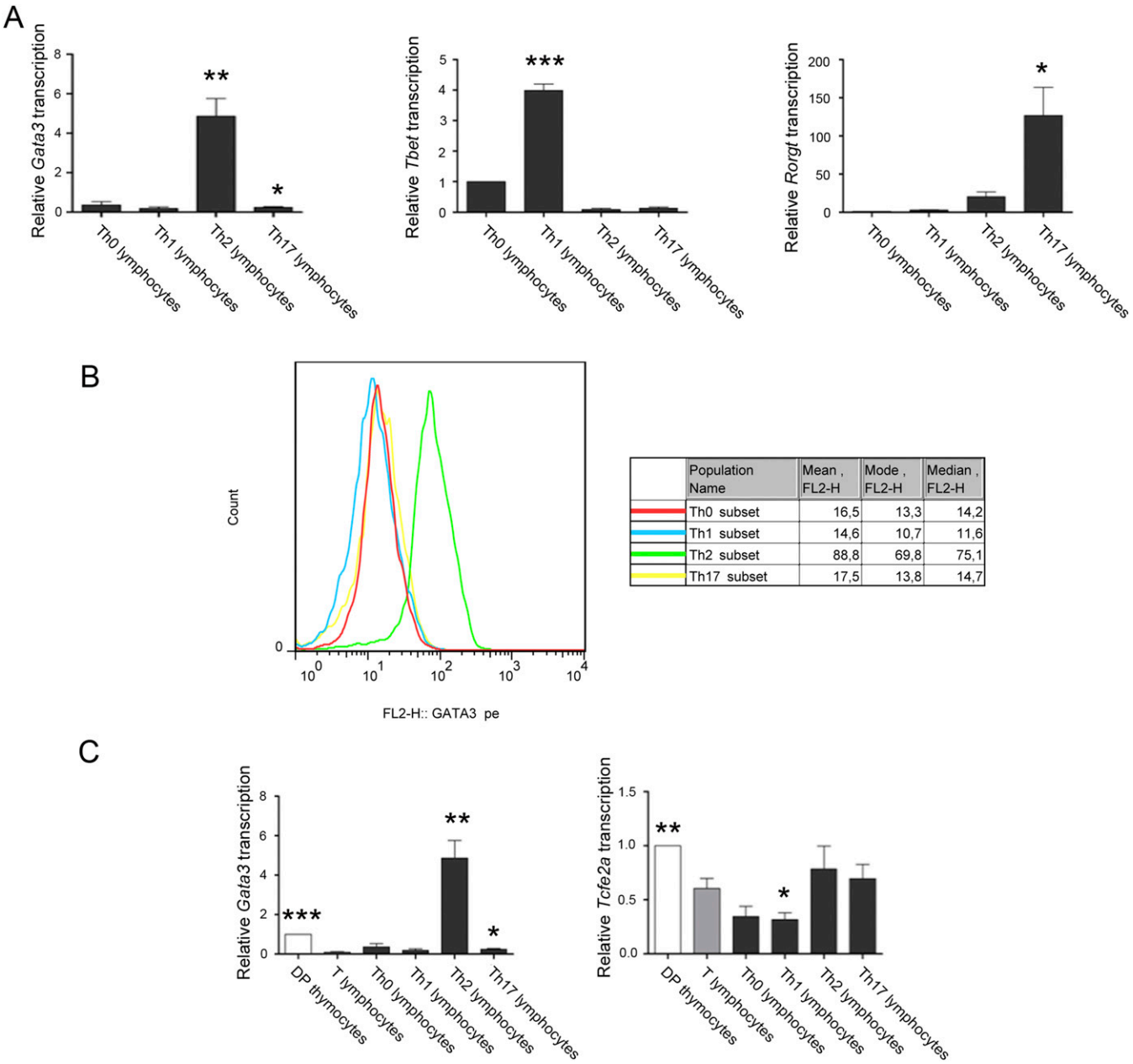


Fig. S6. Measure of *Gata3*, *Tbet*, *Rorgt*, and *Tcfe2a* transcription upon Th differentiation. (*A*) Analysis of *Gata3*, *Tbet*, and *Rorgt* transcripts in Th0, Th1, Th2, and Th17 cell populations from RxDO11.10 mice by RT-qPCR. (*B*) Comparative analysis by flow cytometry of intracellular GATA-3 expression in Th0 (red), Th1 (1), Th2 (green), and Th17 (yellow) cells. (*C*) Analysis of *Gata3* and *Tcfe2a* transcripts in sorted DP thymocytes, T lymphocytes, and Th0, Th1, Th2, and Th17 cell populations from RxDO11.10 mice by RT-qPCR. The results were normalized to those for *Actb* and represent the mean ± SEM of duplicate RT-qPCR from three independent experiments. Two-tailed Student's *t* tests were used to determine the statistical significance between the values of the RT-qPCRs from Th0 vs. Th populations in *A* and T lymphocytes vs. DP thymocytes or Th populations *C* (**P* < 0.05, ***P* < 0.005, and ****P* < 0.0005).

1. Takeda S, Rodewald H-R, Arakawa H, Bluethmann H, Shimizu T (1996) MHC class II molecules are not required for survival of newly generated CD4$^+$ T cells, but affect their long-term life span. *Immunity* 5(3):217–228.

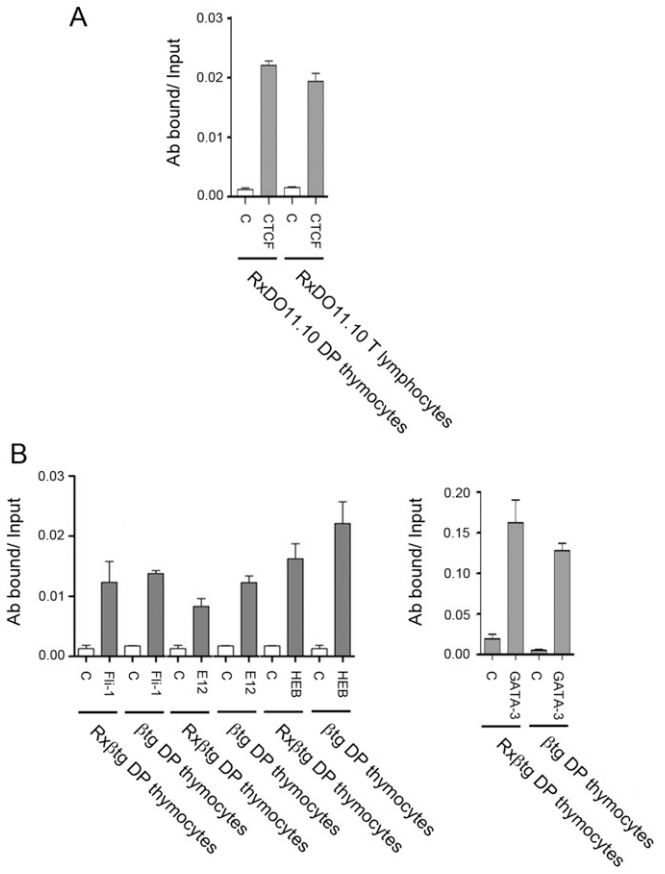


Fig. S7. Comparative CTCF binding analysis to TEAp in DP thymocytes and T lymphocytes, and Fli-1, E-12, HEB, and GATA-3 binding analyses in DP thymocytes from unrearranged and rearranged *Tcra* alleles by qChIP. (*A*) Analysis of CTCF binding to TEAp in DP thymocytes and T lymphocytes from RxDO11.10 mice. (*B*) Analysis of the indicated TF binding to Eα in DP thymocytes from Rxβtg mice (unrearranged *Tcra* alleles) and βtg mice (rearranged *Tcra* alleles) using the control (c) or indicated Abs. DNA purified from the input and Ab-bound fractions was used as a template for qPCR to evaluate the presence of TEAp and Eα. The results are representative of three independent experiments.

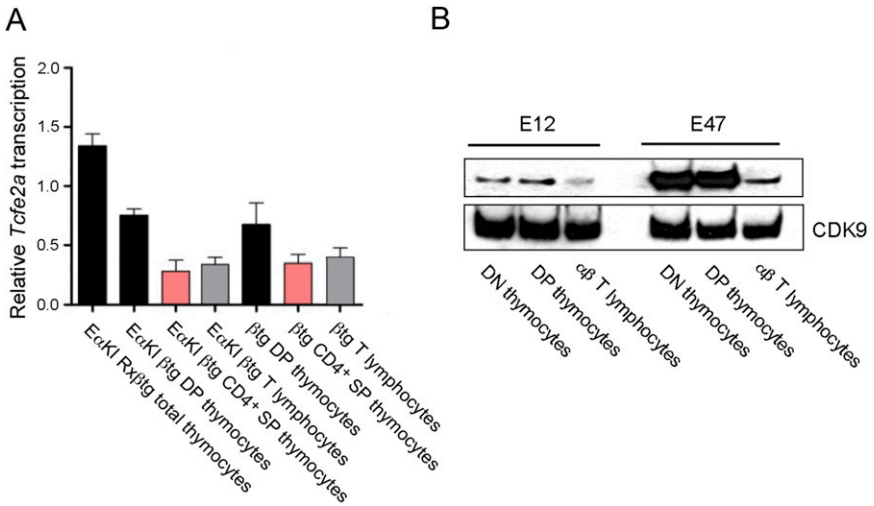


Fig. S8. E2A expression is down-regulated in peripheral T lymphocytes compared with DP thymocytes. (*A*) Analysis of *Tcfe2a* (E2A) transcription in DP thymocytes from EαKI Rxβtg mice, sorted DP and $CD4^+$ SP thymocyte populations and peripheral T lymphocytes from EαKI βtg and βtg mice by RT-qPCR. Transcript level was normalized to that of *Actb* in each sample. (*B*) Western blot of the E2A isoforms E12 and E47 present in DN3 thymocytes from $Rag2^{-/-}$ mice, DP thymocytes from Rxβtg mice, and peripheral T lymphocytes from βtg mice. The presence of cyclin-dependent kinase 9 (CDK9) was analyzed as loading control.

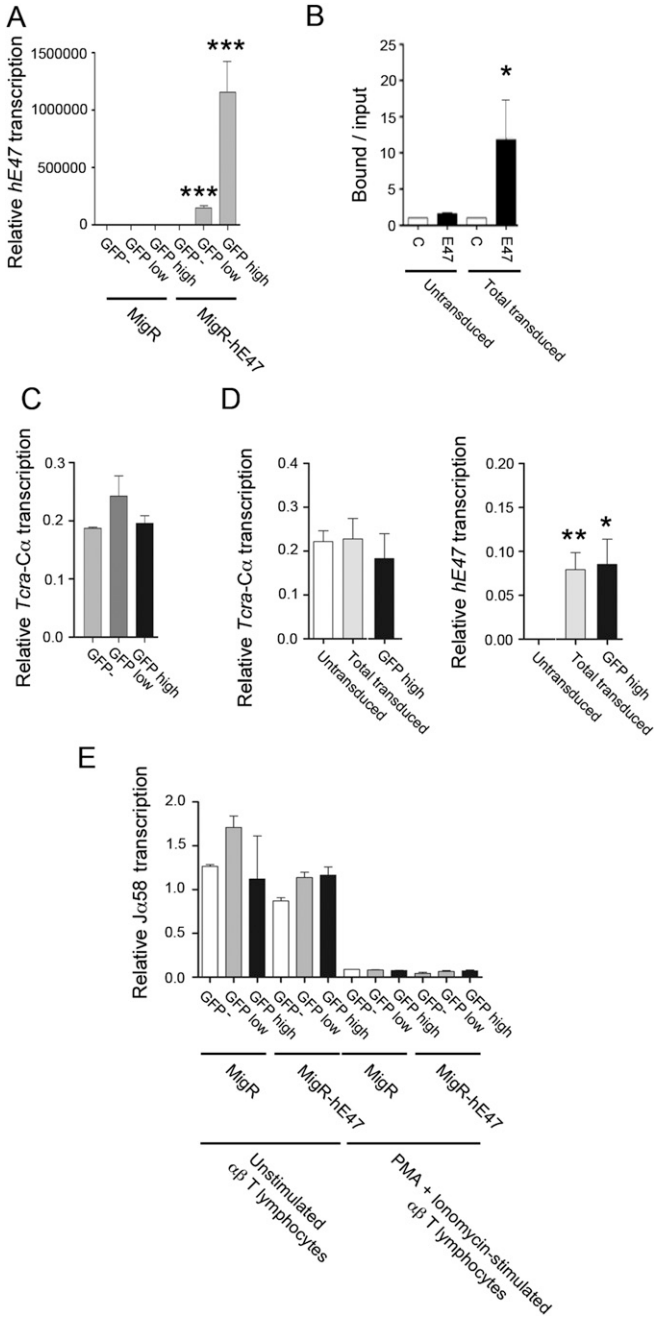


Fig. S9. Analysis of expression of hE47 and *Tcra*-Cα transcription, binding of E2A to Eα, and Jα58 transcription upon TCR-mediated cell stimulation after forced hE47 expression in transduced T lymphocytes. (*A*) Forced expression of hE47 by retroviral transduction of T lymphocytes from RxDO11.10 and EαKI βtg mice. GFP$^-$, GFP-low–, and GFP-high–expressing transduced populations expressing MigR or MigR-hE47 were sorted, and the levels of hE47 transcription were quantified by RT-qPCR. (*B*) Analysis of E2A binding to Eα by qChIP upon retroviral transduction of T lymphocytes from βtg mice. Chromatin preparations from untransduced or transduced T cells with MigR-hE47 were immunoprecipitated with control (c) or E47 Abs. DNA purified from the input and Ab-bound fractions was used as a template for qPCR to evaluate the presence of Eα. Data represent the mean ± SEM of duplicate qPCR from two independent experiments. These ChIPs correspond to the samples presented in *D*. (*C*) Analysis of *Tcra*-Cα transcription in sorted GFP$^-$, GFPlow and GFPhi hE47 transduced RxDO11.10 T lymphocytes by RT-qPCR. (*D*) Analysis of *Tcra*-Cα and hE47 transcription in untransduced, and hE47-transduced and -sorted GFPhi βtg T lymphocytes by RT-qPCR. (*E*) Analysis of Jα58 transcription after forced expression of hE47 by retroviral transduction in unstimulated and PMA + ionomycin-stimulated T lymphocytes from RxDO11.10 mice. GFP$^-$, GFP-low, and GFP-high–transduced populations expressing MigR (control) or MigR-hE47 (hE47) were sorted, and Jα58 transcription was analyzed by RT-qPCR. The results represent the mean ± SEM of duplicate RT-qPCR from three independent experiments. Two-tailed Student's *t* tests were used to determine the statistical significance between the values of the RT-qPCR from MigR- and MigR-hE47-transduced T lymphocytes by comparing MigR GFP$^-$ cells with MigR-hE47 GFP$^-$ cells, MigR GFP-low cells with MigR-hE47 GFP-low cells, and MigR GFP-high cells with MigR-hE47 GFP-high cells (*$P < 0.05$, **$P < 0.005$, and ***$P < 0.0005$).

Table S1. Sequence of oligucleotides used as PCR primers

Gene or transcript and application	Primer sequence
RT-qPCR	
Actb	5′- ACACCCGCCACCAGTTC
	5′- TACAGCCCGGGGAGCAT
Tcfe2a	5′- CAGATGGTGGCCTGGATACT
	5′- CATCCCTGCTGTAGCTGTCA
Gata3	5′- GAGGTGGTGTCTGCATTCCAA
	5′- TTTCACAGCACTAGAGACCCTGTTA
hE47	5′- CAGTTCGGAGGTTCAGGTCTT
	5′- AGAGGCTGCTGTGCGACT
Tbet	5′- AGCAAGGACGGCGAATGTT
	5′- GGGTGGACATATAAGCGGTTC
Rorgt	5′- CCGCTGAGAGGGCTTCAC
	5′- TGCAGGAGTAGGCCACATTACA
Vα transcripts	
TRAV21	5′- CAATAGCCAGGAAAGCCATAC
	5′- TAGCCACGCCACAATCAGT
TRAV17	5′- GCCTCCAGTTTCTCCTCAAA
	5′- GCTGGGGATTTCTTCAGGTT
TEA transcripts	
TEA exon	5′- TCCAAGATTCCTGGGGACAAC
	5′- AAGCAGGAGCCTTTGCATTA
Spliced Jα transcripts	
Jα61	5′- GCCATGACTGGAAGACTCAT
Jα58	5′- TGGGTCTAAGCTGTCATTTGG
Jα47	5′- GCTTGGGAACCATTTTGAGA
Cα	5′- AAGTCGGTGAACAGGCAGAG
Vβ transcripts	
Vβ11	5′- AAGGAACGATTCTCAGCTC
	5′- CTCTGTGTCTAAGCTGCTT
Vβ12	5′- TCAGCTGTGTATCTGTGTG
	5′- AAAGTTCAGAACTGGTGGG
Vβ13	5′- ACAGCCACCTATCTCTGT
	5′- GGAGTATAAGAAATAGTCCC
qChIP	
Jα61	5′- GCCATGACTGGAAGACTCAT
	5′- TCCATATTTTGTTACTATTCCTGAAG
Jα58	5′- TGGGTCTAAGCTGTCATTTGG
	5′- TTGACTGGACTTGAATTTTGG
Jα47	5′- GCTTGGGAACCATTTTGAGA
	5′- CACCTGGAGCTTTGTTTGTTT
Eα	5′- AGCCCTGCTTTGGGTAAAA
	5′- CCATTTCCTGTTCTACTTCTGG
Eα CTCF	5′- AGGAAGTCGCAGAACCTGAA
	5′- CGTAGGATGCAGGGATTTTC
TEAp	5′- ATGGGAAAGGGACCGATAAG
	GCTCAAAGGACACTGGAAGG
Vβ13p	5′- CTTACCATTCCCTATGGAAGTTCAG
	5′- CATATGCACAGACTGCATGTGAG
Vβ13	5′- ACAGCCACCTATCTCTGT
	5′- GGAGTATAAGAAATAGTCCC
Vβ12	5′- TCAGCTGTGTATCTGTGTG
	5′- AAAGTTCAGAACTGGTGGG
EαKI - 1	5′- ACCACACATATGTGTAGAGTC
	5′- ATCTGACCAGCTTACCCATTTC
EαKI - 2	5′- TGTCAATACCAATCTCTTGATGTC
	5′- CCATTTCCTGTTCTACTTCTGG
MageA2	5′-AACGTTTTGTGAACGTCCTGAG
	5′-GACGCTCCAGAACAAAATGGC
Actb promoter	5′-CGCCATGGATGACGATATCG
	5′-CGAAGCCGGCTTTGCACATG
2-Oct	5′-CGGGTGTGAGAGGTGTGG
	5′-CGAGTCTGAAGCAAGCCAGT
HindIII digestion efficiency and 3C	
TEAp	5′- GCGGATTTGGGCAAGAATAA (3C)

Table S1. Cont.

Gene or transcript and application	Primer sequence
	5′-CACAGGCTGCTCCAGAGATG
Eα	5′- AGCATGGGAGACAGACGTAATG (3C)
	5′-ATTCCGGTCTCCGTGTGAAT
Normalization	5′- GGCAAAATCAGAAGACAAGATG (3C)
	5′- GCAGGAGTCGGTTCTTTCC
−75 region	5′- CTGCCCAAAAGAGAGCTTGG (3C)
	5′- GCATGTGAATGAGAGAGGATGG

The primer for Jα49 transcripts was previously described (1). The primers for Vβ11, Vβ13, and Vβ12 germ-line transcription were described as primers for ChIP experiments of the gene segments (2). The primers for *Actb*, *Gata3*, and *Tcfe2a* were described (3–5). The primers for qChIP of Eαi-1, Vβ13p, Vβ13, and Vβ12 were described (2). The Eαi-1 primers were used in the qChIP experiments performed with mononucleosomes (Fig. S4), whereas the Eαi-2 primers were used in the qChIP experiments performed with sonicated chromatin (Figs. 4 and 6). The *MageA2* and *Oct2* primers were described for qChIP (6, 7). The HindIII digestion efficiency and 3C primers for TEAp, Eα, and −75 region were described (8). Each pair of primers was used for HindIII digestion efficiency determinations, whereas the primers labeled 3C were used for 3C experiments.

1. Abarrategui I, Krangel MS (2006) Regulation of T cell receptor-α gene recombination by transcription. *Nat Immunol* 7(10):1109–1115.
2. Jackson A, Kondilis HD, Khor B, Sleckman BP, Krangel MS (2005) Regulation of T cell receptor β allelic exclusion at a level beyond accessibility. *Nat Immunol* 6(2):189–197.
3. Dionne CJ, et al. (2005) Subversion of T lineage commitment by PU.1 in a clonal cell line system. *Dev Biol* 280(2):448–466.
4. David-Fung ES, et al. (2009) Transcription factor expression dynamics of early T-lymphocyte specification and commitment. *Dev Biol* 325(2):444–467.
5. Yui MA, Rothenberg EV (2004) Deranged early T cell development in immunodeficient strains of nonobese diabetic mice. *J Immunol* 173(9):5381–5391.
6. Hao B, Krangel MS (2011) Long-distance regulation of fetal Vδ gene segment TRDV4 by the *Tcrd* enhancer. *J Immunol* 187(5):2484–2491.
7. del Blanco B, García-Mariscal A, Wiest DL, Hernández-Munain C (2012) *Tcra* enhancer activation by inducible transcription factors downstream of pre-TCR signaling. *J Immunol* 188(7): 3278–3293.
8. Seitan VC, et al. (2011) A role for cohesin in T-cell-receptor rearrangement and thymocyte differentiation. *Nature* 476(7361):467–471.