PNAS

Supporting Information for

Type III interferon drives thymic B cell activation and regulatory T cell generation

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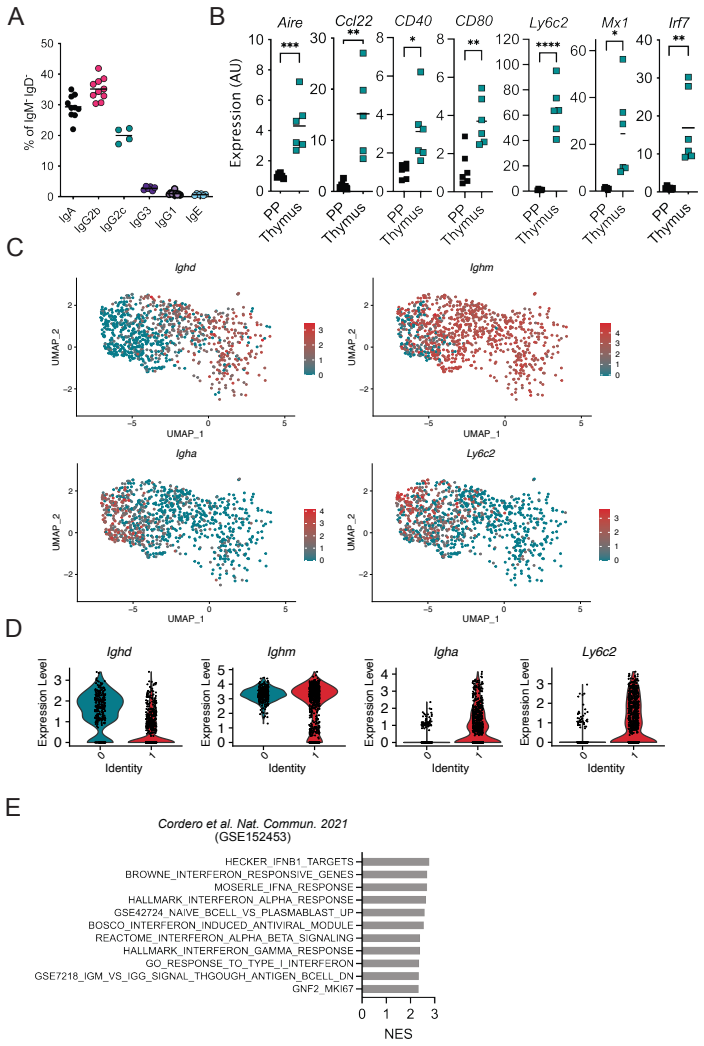

Figure S1 Phenotype of activated and licensed thymic B cells. (**A**) Quantification of the proportion of individual isotypes in the isotype class-switched IgM$^-$IgD$^-$ thymic B cell population. (**B**) RT-qPCR of *Aire*, *Ccl22*, *CD40, CD80*, *Ly6c2*, *Mx1* and *Irf7* in sorted IgM$^-$IgD$^-$ B cells from thymus (blue) and PP (black) normalized to *Gapdh*. (**C**) Feature plots of *Ighd*, *Ighm*, *Igha* and *Ly6c2* for thymic B cells from scRNA-seq data. (**D**) Violin plots of thymic B cell scRNA-Seq for *Ighd*, *Ighm*, *Igha* and *Ly6c2* separated by cluster. (**E**) Gene set enrichment analysis NES for ranked RNA-Seq data from GSE152453. Positive NES are pathways over-represented by thymic B cells (CD21$^+$CD35$^+$) compared to cord blood B cells.

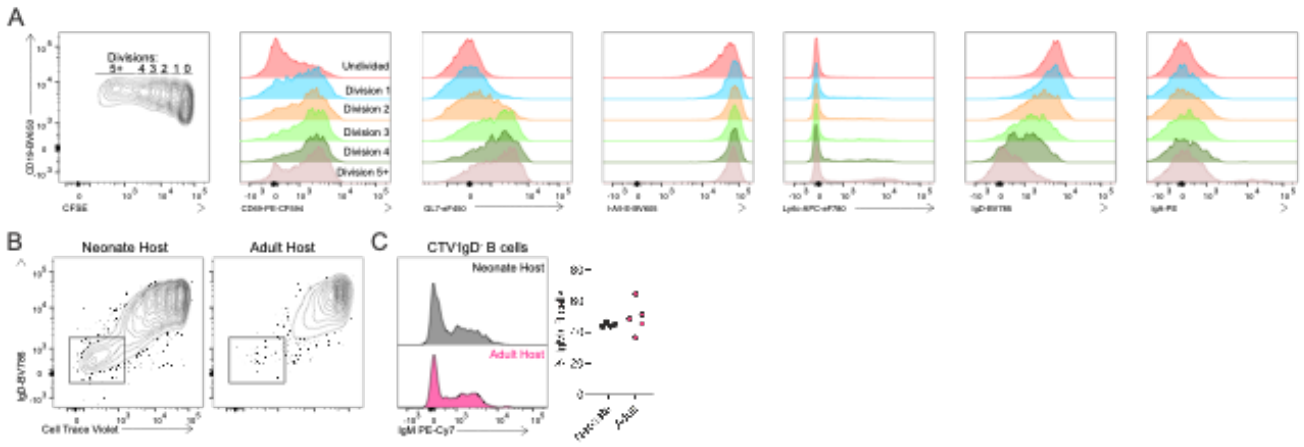

Figure S2 Phenotype of transferred splenic B cells in the thymic microenvironment. (**A**) Flow cytometry plots of transferred splenic B cells separated by division. Flow cytometric histograms demonstrate expression of activation and isotype class-switching markers CD69, GL7, I-A/I-E, Ly6c, IgD, IgA as a function of division. (**B**) Flow cytometry of splenic B cells following intrathymic transfer showing IgD and Cell Trace Violet (CTV) expression in neonatal (left) and adult (middle) host mice with number adjacent to outlined areas identifying proportion of cells within the area. (**C**) Flow cytometry histograms of $CTV^{-}IgD^{-}$ B cells identified in (**B**) showing IgM expression in neonatal (black) or adult (pink) hosts. Graphs on right show quantification of IgM^{+} $CTV^{-}IgD^{-}$ B cells following intrathymic transfer.

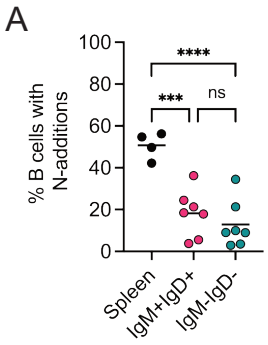

Figure S3 Reduced N-nucleotide additions in thymic B cells. (**A**) Quantification of N-additions in the B cell repertoire of FACS sorted splenic or thymic switched and unswitched B cells from 3H9 mice (data from GSE85366). Each symbol represents an individual mouse; small horizontal lines indicate the group mean. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 and ****P ≤ 0.0001 (**A** One way ANOVA).

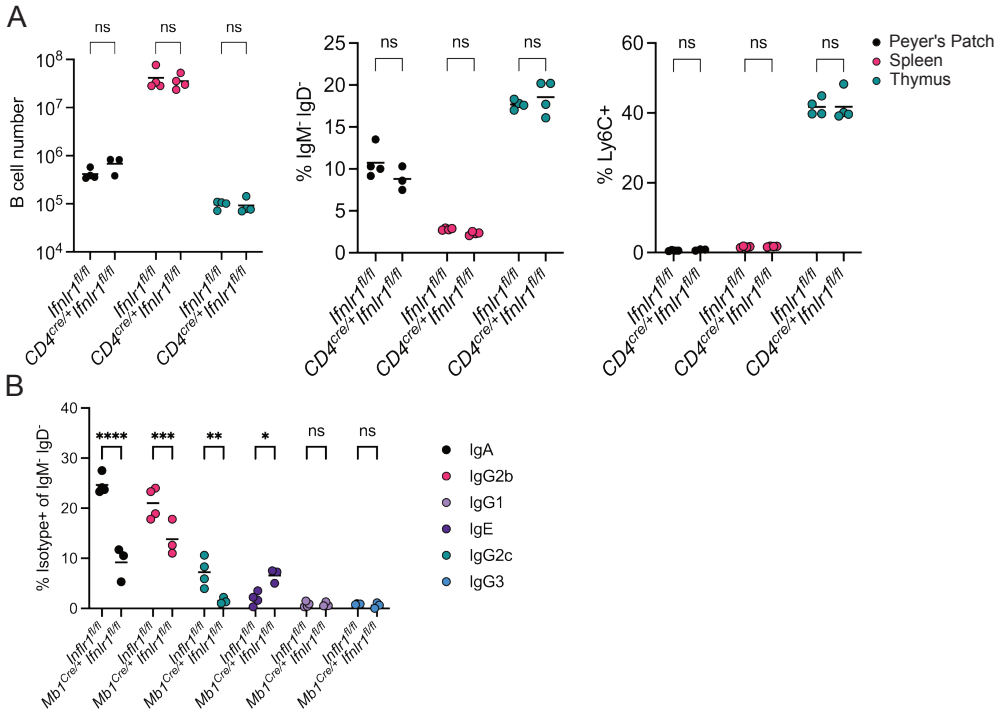

Figure S4 Contribution of type III IFN signaling on T and B cells. (**A**) Quantification of the B cell number and proportion of isotype class-switched (IgM^-IgD^-) and Ly6c expressing B cells in *Ifnlr1*$^{fl/fl}$ and *Cd4*$^{Cre/+}$*Ifnrl1*$^{fl/fl}$ mice from Peyer's Patch (black), spleen (pink) and thymus (aqua).(**B**) Quantification of the proportion of individual isotypes in the isotype class-switched IgM^-IgD^- thymic B cell population in *Ifnlr1*$^{fl/fl}$ and *Mb1*$^{Cre/+}$*Ifnrl1*$^{fl/fl}$ mice. Each symbol (**A-B**) represents an individual mouse; small horizontal lines indicate the group mean. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 and ****P ≤ 0.0001 (**A,B** Two-way ANOVA with Sidak's multiple comparisons test).

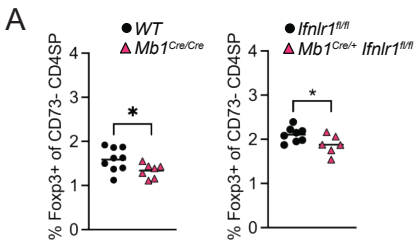

Figure S5 Nascent CD73^{-} Treg development in *Mb1$^{Cre/Cre}$* and *Mb1$^{Cre/+}$Ifnlr1$^{fl/fl}$* mice. (**A**) Quantification of the proportion of Foxp3+ Tregs of CD73^{-} CD4SP thymocytes in Mb1$^{Cre/Cre}$ (left) and Mb1$^{Cre/+}$Ifnlr1$^{fl/fl}$ (right) mice. Each symbol represents an individual mouse; small horizontal lines indicate the group mean. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 and ****P ≤ 0.0001 (Two-tailed unpaired Student's t-test).

	SOURCE	IDENTIFIER
Antibodies		
BUV395 Rat Anti-mouse CD45R/B220 (Clone: RA3 6B2)	BD Biosciences	(BD Biosciences Cat# 563793, RRID:AB_2738427)
FITC anti-mouse H-2K^{b} Antibody (Clone: AF6-88.5)	BioLegend	(BioLegend Cat# 116506, RRID:AB_313733)
PerCP/Cyanine5.5 anti-mouse TCR β chain Antibody (Clone: H57-597)	BioLegend	(BioLegend Cat# 109228, RRID:AB_1575173)
PE anti-mouse IgA Antibody (Clone: mA-6E1)	Thermo Fisher Scientific	(Thermo Fisher Scientific Cat# 12-4204-82, RRID:AB_465917)
PE-CF594 Hamster Anti-Mouse CD69 (Clone: H1.2F3)	BD Biosciences	(BD Biosciences Cat# 562455, RRID:AB_11154217)
PE/Cyanine7 anti-mouse IgM Antibody (Clone: RMM-1)	BioLegend	(BioLegend Cat# 406514, RRID:AB_10642031)
BV786 Rat Anti-Mouse IgD (Clone: 11-26c.1)	BD Biosciences	(BD Biosciences Cat# 563618, RRID:AB_2738322)
APC anti-mouse CD183 (CXCR3) Antibody (Clone: CXCR3-173)	BioLegend	(BioLegend Cat# 126512, RRID:AB_1088993)
APC-eFluor 780 Ly-6C Antibody (Clone: HK1.4)	Thermo Fisher Scientific	(Thermo Fisher Scientific Cat# 47-5932-82, RRID:AB_2573992)
Alexa Fluor 700 CD38 Antibody (Clone: 90)	Thermo Fisher Scientific	(Thermo Fisher Scientific Cat# 56-0381-82, RRID:AB_657740)
LIVE/DEAD Fixable Aqua Dead Cell Stain Kit	Invitrogen	L34966 A
BV605 Rat Anti-Mouse I-A/I-E (Clone: M5/114.15.2)	BD Biosciences	(BD Biosciences Cat# 563413, RRID:AB_2738190)
Brilliant Violet 650 anti-mouse CD19 Antibody (Clone: 6D5)	BioLegend	(BioLegend Cat# 115541, RRID:AB_11204087)
Alexa Fluor 350 Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody	Thermo Fisher Scientific	(Thermo Fisher Scientific Cat# A-11045, RRID:AB_2534100)
BV786 Rat Anti-Mouse CD4 (Clone: GK1.5)	BD Biosciences	(BD Biosciences Cat# 563331, RRID:AB_2738140)

BUV395 Rat Anti-Mouse CD8a (Clone: 53-6.7)	BD Biosciences	(BD Biosciences Cat# 563786, RRID:AB_2732919)
APC-eFluor 780 CD11c Antibody (Clone: N418)	Thermo Fisher Scientific	(Thermo Fisher Scientific Cat# 47-0114-82, RRID:AB_1548652)
APC-eFluor 780 CD11b Antibody (Clone: M1/70)	Thermo Fisher Scientific	(Thermo Fisher Scientific Cat# 47-0112-82, RRID:AB_1603193)
APC-eFluor 780 F4/80 Antibody (Clone: BM8)	Thermo Fisher Scientific	(Thermo Fisher Scientific Cat# 47-4801-82, RRID:AB_2735036)
APC-eFluor 780 CD45R (B220) Antibody (Clone: RA3-6B2)	Thermo Fisher Scientific	(Thermo Fisher Scientific Cat# 47-0452-82, RRID:AB_1518810)
Brilliant Violet 421 anti-mouse CD25 Antibody (Clone: PC61)	BioLegend	(BioLegend Cat# 102034, RRID:AB_11203373)
Alexa Fluor 647 anti-mouse/rat/human FOXP3 Antibody (Clone: 150D)	BioLegend	(BioLegend Cat# 320014, RRID:AB_439750)
PE/Cyanine7 anti-mouse CD357 (GITR) Antibody (Clone: YGITR765)	BioLegend	(BioLegend Cat# 120222, RRID:AB_528907)
PE Hamster Anti-Mouse CD154 (Clone: MR1)	BD Biosciences	(BD Biosciences Cat# 553658, RRID:AB_394978)
APC Anti-BrdU (Clone BU20A)	Thermo Fisher Scientific	(Thermo Fisher Scientific Cat# 17-5071-42, RRID:AB_11040534)
eFluor 450 GL7 Antibody (Clone: GL7)	Thermo Fisher Scientific	(Thermo Fisher Scientific Cat# 48-5902-82, RRID:AB_10870775)
UAE-1	Vector Laboratories	(Vector Laboratories Cat# FL-1061, RRID:AB_2336767)
Anti-β5t (Mouse) pAb	MBL International	(MBL International Cat# PD021, RRID:AB_2171885)
Alexa Fluor 647 Rat Anti-mouse CD19 (Clone: 1D3)	BD Biosciences	(BD Biosciences Cat# 557684, RRID:AB_396794)
PE-Cy7-SA PBS-57 tetramer	NIH tetramer core	-

Alexa Fluor 647 goat anti-mouse IgG2b	Thermo Fisher Scientific	(Thermo Fisher Scientific Cat# A-21242, RRID:AB_2535811)
BV605 Rat Anti-Mouse IgG1 (Clone: A85-1)	BD Biosciences	(BD Biosciences Cat# 563285, RRID:AB_2738116)
BV510 Rat Anti-Mouse IgE (Clone: R35-72)	BD Biosciences	(BD Biosciences Cat# 563097, RRID:AB_2738003)
Goat pAb to Ms IgG2c (FITC)	Abcam	(Abcam Cat# ab97254, RRID:AB_10679820)
Alexa Fluor 594 Goat anti-Mouse IgG3 Cross-Adsorbed Secondary Antibody	Thermo Fisher Scientific	(Thermo Fisher Scientific Cat# A-21155, RRID:AB_2535785)
Anti-Mouse CD16/CD32 (Fc Shield) (Clone: 2.4G2)	Tonbo Biosciences	(Tonbo Biosciences Cat# 70-0161, RRID:AB_2621487)
redFlour 710 Anti-Human/Mouse CD44 (Clone: IM7)	Tonbo Biosciences	(Tonbo Biosciences Cat# 80-0441, RRID:AB_2621985)
CountBright Absolute Counting Beads	Thermo Fisher Scientific	C36950
Biotin anti-Mouse CD4 (Clone: GK1-5)	BioLegend	(BioLegend Cat# 100404, RRID:AB_312689)
Biotin anti-Mouse CD8α (Clone: 53-6.7)	BioLegend	(BioLegend Cat# 100704, RRID:AB_312743)
Chemicals, peptides, and recombinant proteins		
Collagenase D	Roche	11088882001
Dnase I	Qiagen	79254
DAPI	Invitrogen	D1306
BrdU	Sigma-Aldrich	B5002
Polymyxin B sulfate salt	Sigma-Aldrich	P4932
Neomycin trisulfate salt hydrate	Sigma-Aldrich	N6386
ProLong Diamond Antifade Moutant	Thermo Fisher Scientific	P36961
HBSS with Ca^{2+},Mg^{2+}	Hyclone	SH30030.02
HBSS without Ca^{2+},Mg^{2+}	Hyclone	SH30031.02
EDTA (0.5M Solution/pH 8.0)	Thermo Fisher Scientific	BP2482100
HEPES (1M)	Thermo Fisher Scientific	15630080
BSA	Sigma-Aldrich	A7906-500G
O.C.T Compound	Sakura Finetek	4583
Acetone	Sigma-Aldrich	320110
TWEEN 20	Sigma-Aldrich	P9416
PBS without Ca^{2+},Mg^{2+}	Corning	21-040-CV
Fetal Bovine Serum	Atlas Biologicals	FS-0500-AD

Bovine Serum Albumin	Sigma-Aldrich	A7906
Complete Freund's Adjuvant	Sigma-Aldrich	F5881
Cre_{61-71} peptide (RKWFPAEPEDV)	GenScript	(1)
L-Glutamine, 100x, Liquid	Corning	25-005-CI
RNAprotect Cell Reagent	Qiagen	76526
D-glucose	WVR	4912
HEPES	Thermo Fisher Scientific	BP310-1
Critical commercial assays		
RNEasy Plus Mini Kit	Qiagen	74134
SuperScript III First-Strand Synthesis SuperMix for qRT-PCR	Thermo Fisher Scientific	11752050
Fast SYBR Green Master Mix	Thermo Fisher Scientific	4385614
CellTrace Violet Cell Proliferation Kit	Thermo Fisher Scientific	C34557
CellTrace CFSE Cell Proliferation Kit	Thermo Fisher Scientific	C34554
BD Cytofix/Cytoperm	BD	554714
Foxp3 / Transcription Factor Staining Buffer Kit	Tonbo Biosciences	NB-0607-KIT
Anti-PE MicroBeads	Miltenyi Biotec	130-105-639
Anti-APC MicroBeads	Miltenyi Biotec	130-090-855
Anti-Biotin MicroBeads	Miltenyi Biotec	130-090-485
Mouse B Cell Isolation Kit	StemCell Technologies	19854
Deposited data		
Experimental models: Organisms/strains		
B6.129S7-*Ifngr1*^{tm1Agt}/J (Ifngr1$^{-/-}$)	The Jackson Laboratory	RRID:IMSR_JAX:003288
B6.Cg-*Mx1*$^{tm1.1Agsa}$/J (*Mx1*^{gfp})	A. García-Sastre	RRID:IMSR_JAX:033219
Ifnlr1$^{tm1.1Svko}$	Sergei V Kotenko	(2)
Ifnlr1$^{tm1.2Svko}$	Sergei V Kotenko	(2)
B6.SJL-Ptprc^{a}Pepc^{b}/BoyCrl (B6.SJL),	Charles River Laboratories	RRID:IMSR_CRL:494
C57BL/6NCrl (B6)	Charles River Laboratories	RRID:IMSR_CRL:027
B6.C(Cg)-Cd79a$^{tm1(cre)Reth}$*/EhobJ* (*Cd79a*^{cre})	The Jackson Laboratory	RRID:IMSR_JAX:020505
B6.Cg-Tg(Cd4-cre)1Cwi/BfluJ (*Cd4*^{cre})	The Jackson Laboratory	RRID:IMSR_JAX:022071
B6.129S2-Ifnar1^{tm1Agt}*/Mmjax* (*Ifnar1*$^{-/-}$)	The Jackson Laboratory	RRID:MMRRC_032045-JAX
B6.129S(Cg)-*Stat1*^{tm1Dlv}/J (*Stat1*$^{-/-}$)	The Jackson Laboratory	RRID:IMSR_JAX:012606
B6.129S2-*Tcra*^{tm1Mom}/J (*Tcra*$^{-/-}$)	The Jackson Laboratory	RRID:IMSR_JAX:002116
B6.Cg-Foxp3tm2Tch/J (*Foxp3*^{eGFP})	The Jackson Laboratory	RRID:IMSR_JAX:006772

Tg(Tcrb51–11.5)AR1251Ayr (Tclib)	N/A	(3)
B6.129P2-*Aicda*$^{tm1(cre)Mnz}$/J	The Jackson Laboratory	RRID:IMSR_JAX:007770
Oligonucleotides		
Ccl22	IDT	Mm.PT.58.15758573
Cd40 Fwd- 5'-GTT TAA AGT CCC GGA TGC GA-3' Rev-5'-CTC AAG GCT ATG CTG TCT GT-3'	IDT	N/A
Cd80 Fwd- 5'-GGC AAG GCA GCA ATA CCT TA-3' Rev- 5'-CTC TTT GTG CTG CTG ATT CG-3'	IDT	N/A
Ly6c2 Fwd- 5'-GGA CAG TAC TCA CGC TAC AAA G-3' Rev- 5'-ATT GGC ACT CCA TAG CAC TC-3'	IDT	N/A
Mx1 Fwd- 5'-CTC ACC TCC CAC ATC TGT AAA TC-3' Rev- 5'-GTA TGT CTG CAC CGT ACT TCT G-3'	IDT	N/A
Irf7	IDT	MM.PT.58.32394021.G
Gapdh Fwd- 5'-TGG CCT ACA TGG CCT CCA-3' Rev- 5'-TCC CTA GGC CCC TCC TGT TAT-3'	IDT	N/A
Ifnlr1 Fwd- 5'-GCT CTG AGG CTG GCA TAA A-3' Rev- 5'-CTG GTA GTG TTA GGA AGG TTG AG-3'	IDT	N/A
Aire Fwd- 5'- TGC ATA GCA TCC TGG ACG GCT TCC-3' Rev- 5'-CCT GGG CTG GAG ACG CTC TTT GAG-3'	IDT	N/A
Software and algorithms		
R	R version 4.1.0	R Project for Statistical Computing (RRID:SCR_001905)
CHURP	CHURP v0.2.1	https://github.com/msi-ris/CHURP
Prism	Version 9.3.1	PRISM (RRID:SCR_005375)
Other		

Supplemental Table 1. Materials

SI References

1. F. P. Legoux, *et al.*, CD4 + T Cell Tolerance to Tissue-Restricted Self Antigens Is Mediated by Antigen-Specific Regulatory T Cells Rather Than Deletion. *Immunity* **43**, 896–908 (2015).

2. J.-D. Lin, *et al.*, Distinct Roles of Type I and Type III Interferons in Intestinal Immunity to Homologous and Heterologous Rotavirus Infections. *PLoS Pathog* **12**, e1005600 (2016).

3. P. Wong, A. W. Goldrath, A. Y. Rudensky, Competition for Specific Intrathymic Ligands Limits Positive Selection in a TCR Transgenic Model of CD4 $^{+}$ T Cell Development. *J Immunol* **164**, 6252–6259 (2000).