Supplementary data

Title: Temporal expression of Bim limits the development of agonist selected thymocytes and skews their TCRβ repertoire.

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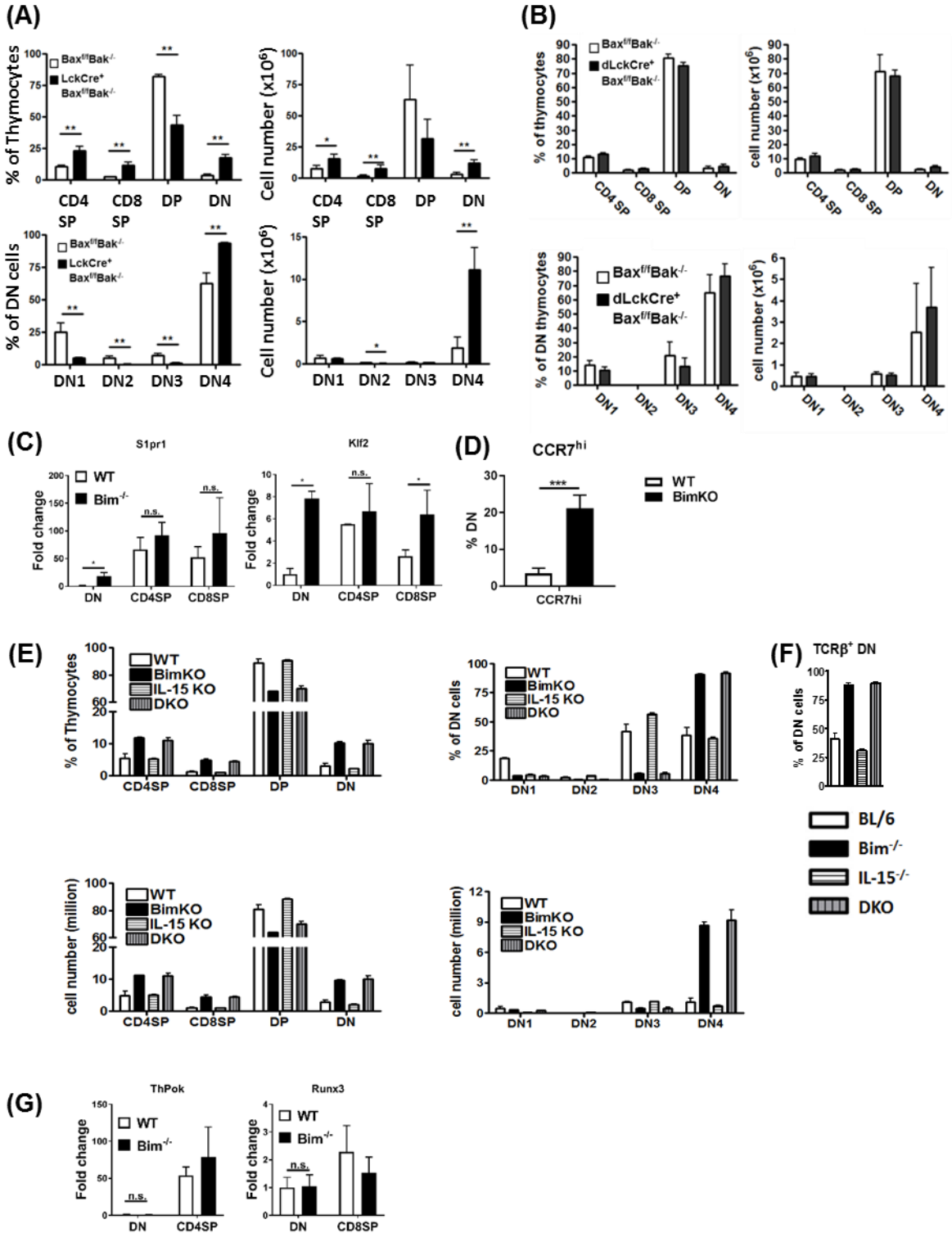
Running title: Bim temporally limits the development and TCR repertoire of CD8αα precursors.

Supplementary Figure 1.



Supplementary Figure 1. Thymic profiles of different genetic backgrounds. (A) Thymocytes from LckCre^{+}Bax$^{f/f}$Bak$^{-/-}$ mice or Bax$^{f/f}$Bak$^{-/-}$ control littermates (n=3) were stained with Abs and analyzed by flow cytometry. The bar graphs show the frequency and cell numbers of each population from either Bax$^{f/f}$Bak$^{-/-}$ (white bars) or LckCre^{+}Bax$^{f/f}$Bak$^{-/-}$ (black bars) mice. Results are representative of at least two independent experiments. (B) Thymocytes from dLckCre^{+}Bax$^{f/f}$Bak$^{-/-}$ mice or Bax$^{f/f}$Bak$^{-/-}$ control littermates (n=3) were analyzed by flow cytometry. The bar graphs show the frequency and cell numbers of each population from either Bax$^{f/f}$Bak$^{-/-}$ (white bars) or dLckCre^{+}Bax$^{f/f}$Bak$^{-/-}$ (black bars) mice. Results are representative of at least two independent experiments. (C) The expression levels of S1pr1 and Klf2 were examined in sorted DN (CD4^{-}CD8^{-}), CD4SP (CD4^{+}CD8^{-}), or CD8SP(CD4^{-}CD8^{+}) thymocytes from either C57BL/6 WT mice (white bars) or Bim$^{-/-}$ mice (black bars). (D) The CCR7^{hi} frequency among DN (CD4^{-}CD8^{-}) thymocytes from either C57BL/6 WT mice (white bars) or Bim$^{-/-}$ mice (black bars) were tested by flow cytometry. (E) Thymocytes from C57BL/6 (BL/6), Bim$^{-/-}$, IL-15$^{-/-}$, or Bim$^{-/-}$ IL-15$^{-/-}$ (DKO) mice (n=3) were analyzed by flow cytometry. The bar graphs show the frequency and cell numbers of each population from either BL/6 (white bars), Bim$^{-/-}$ (black bars), IL15 KO (horizontal striped bars), or IL-15$^{-/-}$Bim$^{-/-}$ (DKO, vertical striped bars). (F) TCRβ^{+} frequency amount CD25^{-}CD44^{-} DN thymocytes is shown in the bottom panel. Results are representative of at least two independent experiments. (G) The ThPok or Runx3 mRNA expression levels of purified DN (CD4^{-}CD8^{-}), CD4SP (CD4^{+}CD8^{-}), or CD8SP(CD4^{-}CD8^{+}) thymocytes from either C57BL/6 or Bim$^{-/-}$ mice were analyzed by real-time PCR.

(Mean ± SD; * $p<0.05$; ** $p<0.01$; *** $p<0.001$).

The primers used are listed below:

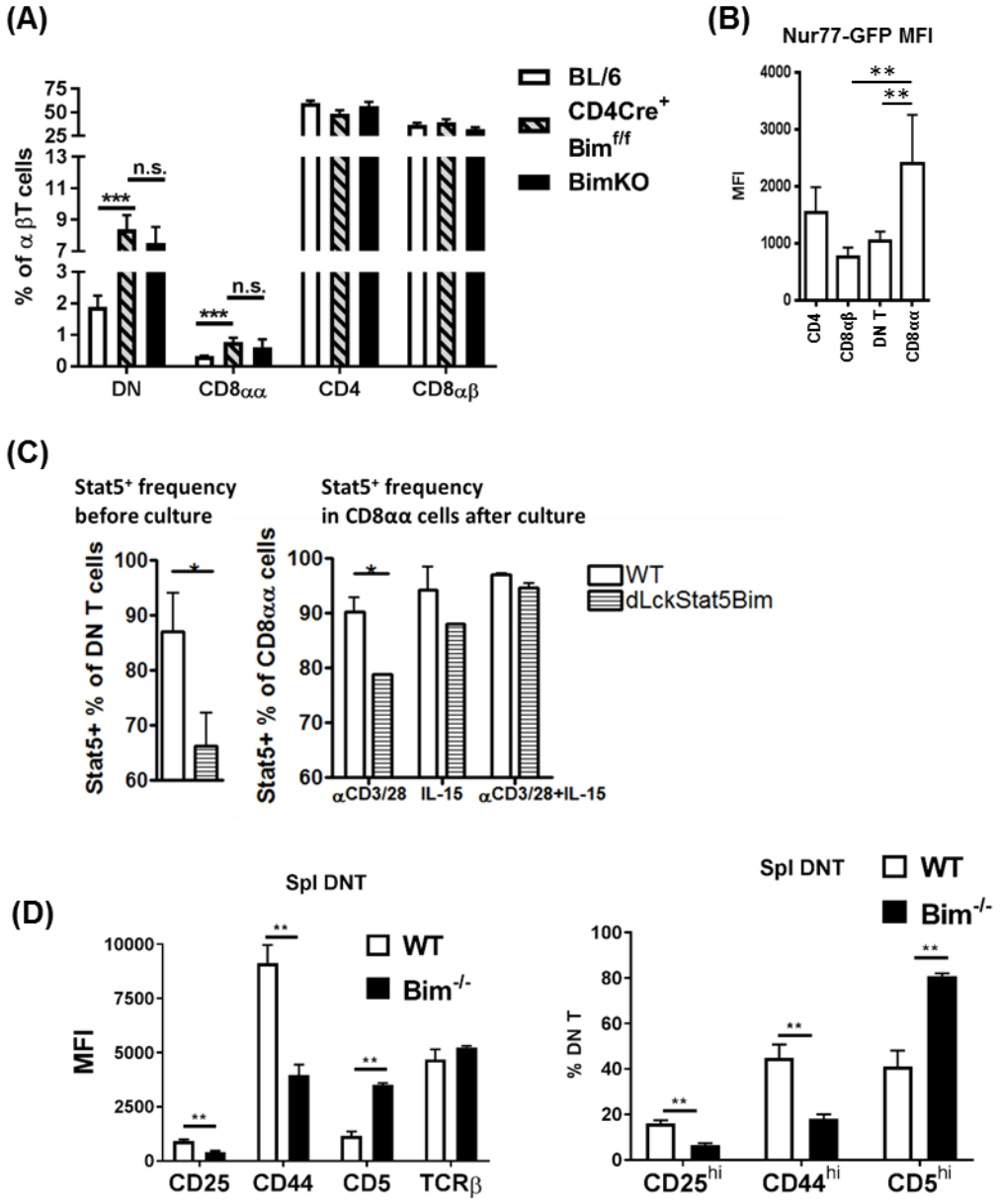
S1pr1: Fw: TATGGAGCTTTTCCTTGGCT; Rv: CGGTGTAGACCCAGAGTCCT.

Klf2: Fw: GTGGCAGGTGGAGCCAAG; Rv: GTTGCACTACGGGCCTCC.

ThPok: Fw: ATGGGATTCCAATCAGGTCA; Rv: TTCTTCCTACACCCTGTGCC.

Runx3: Fw: GGTCACCACCGTTCCATC; Rv: ACTTCCTCTGCTCCGTGCT.

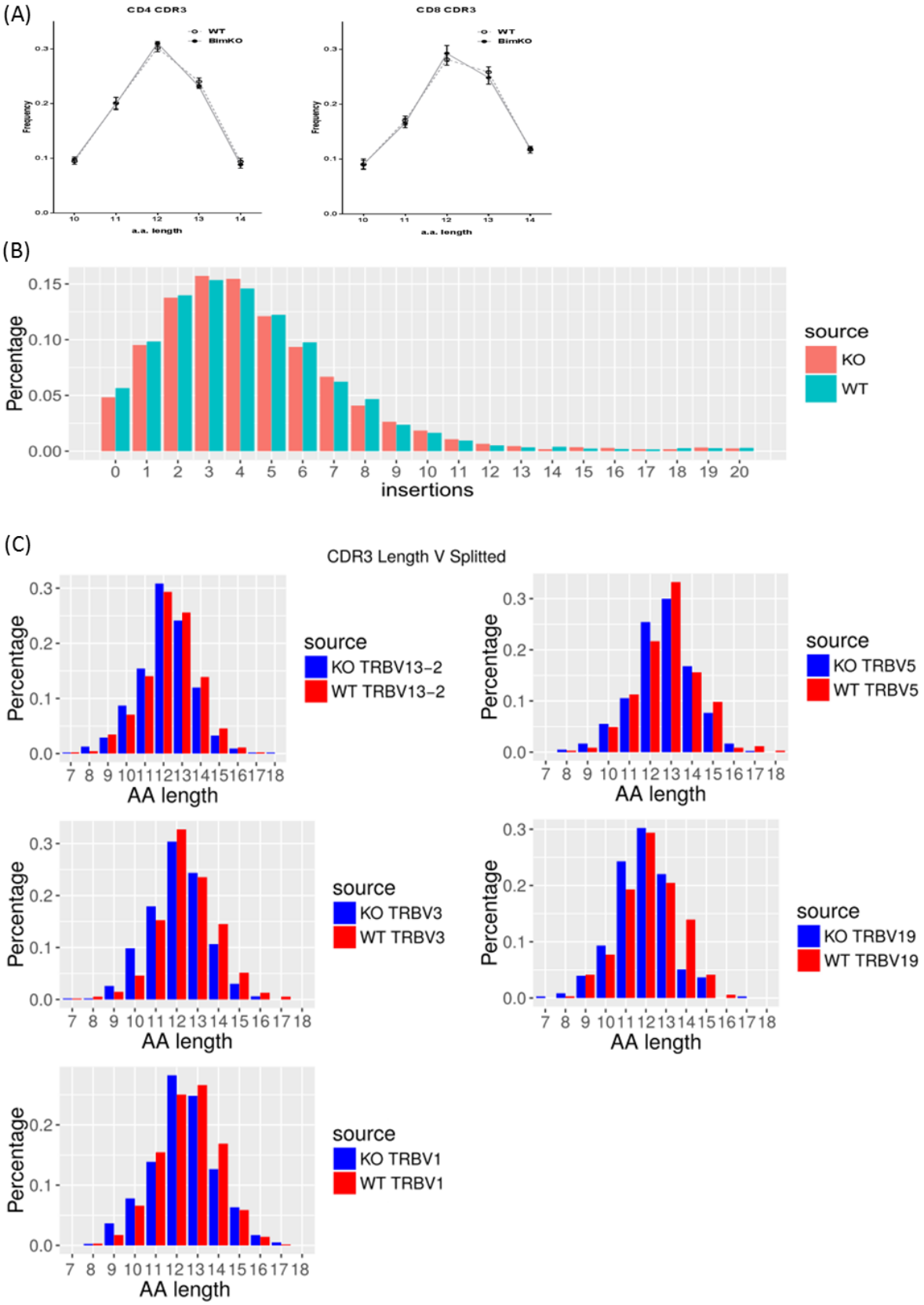
Supplementary Figure 2.



Supplementary Figure 2. Characterization of splenic DN T cells. (A) Splenocytes from $Bim^{f/f}$ (WT) or $CD4^{+}Bim^{f/f}$ mice (n=3) were analyzed by flow cytometry. The bar graphs show the frequency of each population among $TCR\beta^{+}$ T cells. Results are representative of at least three independent experiments. (B) C57BL/6-Tg (Nr4a1-EGFP/cre)820Khog/J ($Nur77^{GFP}$) mice were

obtained from Jackson Labs (Bar Harbor, ME, USA). Splenocytes from Nur77^{GFP} mice (n=3) were stained with Abs and analyzed by flow cytometry. The bar graphs show the MFI of GFP fluorescence among each population of TCRβ^{+} T cells. Results are representative of at least two independent experiments. (Mean ± SD). (C) The sorted splenic DN T cells (CD4^{-}CD8α^{-}TCRβ^{+} CD19^{-}MHC II^{-}NK1.1^{-}) from either BL/6 or dLckCre^{+}Bim$^{f/f}$Stat5$^{f/f}$ mice were cultured for CD8αα T cell induction. The cells were stimulated with plate-bound anti-CD3/CD28 Ab and/or 20 ng/ml of recombinant IL-15 for 24 hr. The left bar graphs show the frequency of Stat5^{hi} cells within *ex vivo* splenic DN T cells from either BL/6 (white bars) or dLckCre^{+}Bim$^{f/f}$Stat5$^{f/f}$ (striped bars) mice before culture. The right bar graphs show the frequency of Stat5^{hi} cells within CD8αα T cells induced by different treatments. (D) Splenic DN T cells from C57BL/6 (WT) or Bim$^{-/-}$ mice (n=3) were analyzed by flow cytometry. The MFI or frequencies of indicated markers or populations were shown. (Mean ± SD; * $p<0.05$; ** $p<0.01$).

Supplementary Figure 3.



Supplementary Figure 3. TCRβ CDR3 analysis. Splenic $CD4^+$ ($CD4^+TCR\beta^+$), $CD8^+$ ($CD8^+TCR\beta^+$) conventional T cells, or DN T cells were isolated from C57BL/6 (WT) or $Bim^{-/-}$ mice, and the TCRβ CDR3 repertoire of were sequenced by Illumina MiSeq system. (A) Amino acid lengths of CD4 or CD8 T cells are shown in histogram. (WT: n=5; KO: n=3; Mean ± SD). (B) Among DN T cell repertoire, the number of N-insertion nucleotides among the germline V/D and D/J junctions were counted for each sample and plotted against their relative frequency. (C) Among DN T cell repertoire, the subsampled clonotypes for DN T cells from either $Bim^{-/-}$ or WT mice were sorted according to their Vβ chains. Subsequently, the clonotypes were sorted regarding their CDR3 lengths. The CDR3 lengths of the five most frequent Vβ were plotted in the lower panel.

Supplementary Table 1.

Number of cells, raw reads and unique TCRβ sequences of T cells from naïve mice.

Mouse	Cell numbers ($x10^6$)	Nb. of raw reads ($x10^6$)	Nb. of CDR3 containing reads ($x10^6$)	Unique CDR3 sequences
WT1 CD4	2	1.3	1.24	99387
WT1 CD8	2	1.3	1.22	127095
WT2 CD4	2	1.6	1.53	119260
WT2 CD8	2	1.4	1.32	146567
WT2 DN	0.023	1.7	1.02	4902
WT3 CD4	2	1.62	1.55	129990
WT3 CD8	2	1.73	1.67	177702
WT3 DN	0.023	2.08	0.35	2029
WT4 CD4	0.5	2.55	2.4	159387
WT4 CD8	0.5	2.48	2.30	156463
WT4 DN	0.04	2.7	1.5	15566
WT5 CD4	0.5	2.69	2.5	157957
WT5 CD8	0.5	1.97	1,7	117955
WT5 DN	0.04	2	1.67	16121
KO1 CD4	2	0.99	0.9	70606
KO1 CD8	2	0.97	0.9	113156
KO1 DN	0.16	1.47	1.3	41058
KO2 CD4	2	1.32	1.2	83484
KO2 CD8	2	1.65	1.5	148902
KO2 DN	0.108	1.54	1.1	14557
KO3 CD4	2	1.91	1.8	85209
KO3 CD8	2	1.86	1.8	170501
KO3 DN	0.14	1.78	1.6	56916