

Supplemental Figure 1. hCD2 expression in bone marrow proB and preB/B cells (A) and splenic NK cells (B). Total Bone marrow cells were stained with FITC conjugated anti-CD43, APC conjugated anti-B220, PE conjugated anti-hCD2 and 7AAD. Total spleen cells were stained with PE conjugated anti-DX5, FITC conjugated anti-CD3, FITC conjugated anti-hCD2, PE-Cy5 conjugated B220 and 7AAD. Events that are shown in the contour plot are pre-gated on viable cells. hCD2 staining of the gated population from wild-type, V $\beta$ 8.2<sup>CD2/+</sup> and V $\beta$ 8.2<sup>CD2/CD2</sup> mice is presented as overlayed histogram.



**Supplemental Figure 2. hCD2 expression in gdT cells.** hCD2 expression in thymic gdT cells (A), splenic gdT cells (B) and gdT cells in IELs (C) from 4~6-week old V $\beta$ 8.2<sup>CD2/CD2</sup>, V $\beta$ 8.2<sup>CD2/+</sup> and wild type mice. The gating of gdT cells is indicated in the contour plots. hCD2 expression in gated T cells from V $\beta$ 8.2<sup>CD2/CD2</sup> (black line), V $\beta$ 8.2<sup>CD2/+</sup> (dotted line), and wild type (shade) mice is presented by overlayed histograms. Percentages of hCD2 positive cells are also shown in the histograms. Results are representative of at least 3 separate experiments.



## Supplemental Figure 3. Gating and Purity of DN2/3 thymocyte sorting for

**quantitative PCR analysis.** Total thymocytes from wild-type,  $V\beta 8.2^{CD2/+}$  and  $V\beta 8.2^{CD2/CD2}$  mice were stained as described in *Method*. Gating and frequency of gated population in CD4/CD8 depleted thymocytes prior to sorting are shown in the pre-sort dot plot. A small fraction of sorted cells were subjected to four-color FACS analysis directly after cell sorting. Purity of sorted DN2/3 thymocytes is shown in the post sort dot plot. Events are pre-gated on viable cells. Graphs of wild-type sample are shown as a representative of all three genotypes.



Supplemental Figure 3. Gating and purity of DN2 and DN3 thymocyte sorting for quantitative PCR analysis. Total thymocytes from B6 and SJL/B6 F1 mice were stained as described in *Method*. Cells were enriched for CD25 positive staining on AUTO MACS before sorting. Gating and frequency of DN2 and DN3 cells were shown in pre-sort plot. A small fraction of sorted cells were subjected to four-color FACS analysis directly after cell sorting. Events that are shown in the plots are pre-gated on viable cells. c-kit expression in sorted DN2 thymocytes is also shown as histogram. Graphs of B6 sample are shown as a representative of both strains.

GAPDHfor	5'- CCT GGA GAA ACC TGC	Forward primer for GAPDH
	CAA GTA TG -3'	
GAPDHrev	5'-AGA GTG GGA GTT GCT	Reverse primer for GAPDH
	GTT GAA GTC 3'	
TCRVβ8.2for	5'-ATG GTG CTG GCA GCA	Specific for TCR V $\beta$ 8.2 exon;
	CTG AG-3'	Forward primer for V $\beta 8.2^{CD2}$
		knock-in allele germline
		transcription
LoxPrev	5'-CTT CGA ATA GCA TAC	Specific for loxP sequece;
	ATT ATA CGA AGT TAT CC-3'	Reverse primer for V $\beta 8.2^{CD2}$
		knock-in allele germline
		transcription
TCRVβ8.2L/efor	5'-CTC CTG TGT TCA AAA	Specific for 3' of TCRVβ8.2
-	CAC ATG GAG G -3'	leading exon and 5'of
		TCRV $\beta$ 8.2 exon; Forward
		primer for V $\beta$ 8.2 germline
		transcription
TCRVβ8.2R4GL	5'-GGA ACT TTC TTA GGT	Reverse primer for Vβ8.2
•	ACA CAG GCA C -3'	germline transcription
TCRV <sub>β4L</sub> /efor	5'-TCC TGG TGG CAG GTC	Specific for 3' of TCRVβ4
•	CAG TCG ACC -3'	leading exon and 5' of TCRV64
		exon; Forward primer for $V\beta4$
		germline transcription
TCRVβ4 R4GL	5'- GAC CAG TTA CAT CAA	Reverse primer for Vβ4
	CAG TTT CCT GG-3'	germline transcription
TCRV <sub>β5.1L</sub> /efor	5'-CCT GGG AAC AAG TTC	Specific for 3' of TCRV <sub>β</sub> 5.1
•	AGC AAA TTC -3'	leading exon and 5'of
		TCRV $\beta$ 5.1 exon; Forward
		primer for VB5.1 germline
		transcription
TCRV65 1R4GL	5'-CCT CTC CAG TAC ATC	Reverse primer for VB5 1
	ACC AGC TGG -3'	germline transcription
TCRVB12L/efor	5'-CCT TGA CAC AGT ACT	Specific for 3' of TCRVB12
	GTC TGA AGC -3'	leading exon and 5'of
		TCRVB12 exon: Forward
		primer for VB12 germline
		transcription
TCRVR12 RACI		Reverse primer for VR12
	AAT AGT CCC AGG -3'	germline transcription
1		

Supplemental Table 1. Primer se	quence used for q	uantitative PCR analysis
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