Supplementary Information

Figures

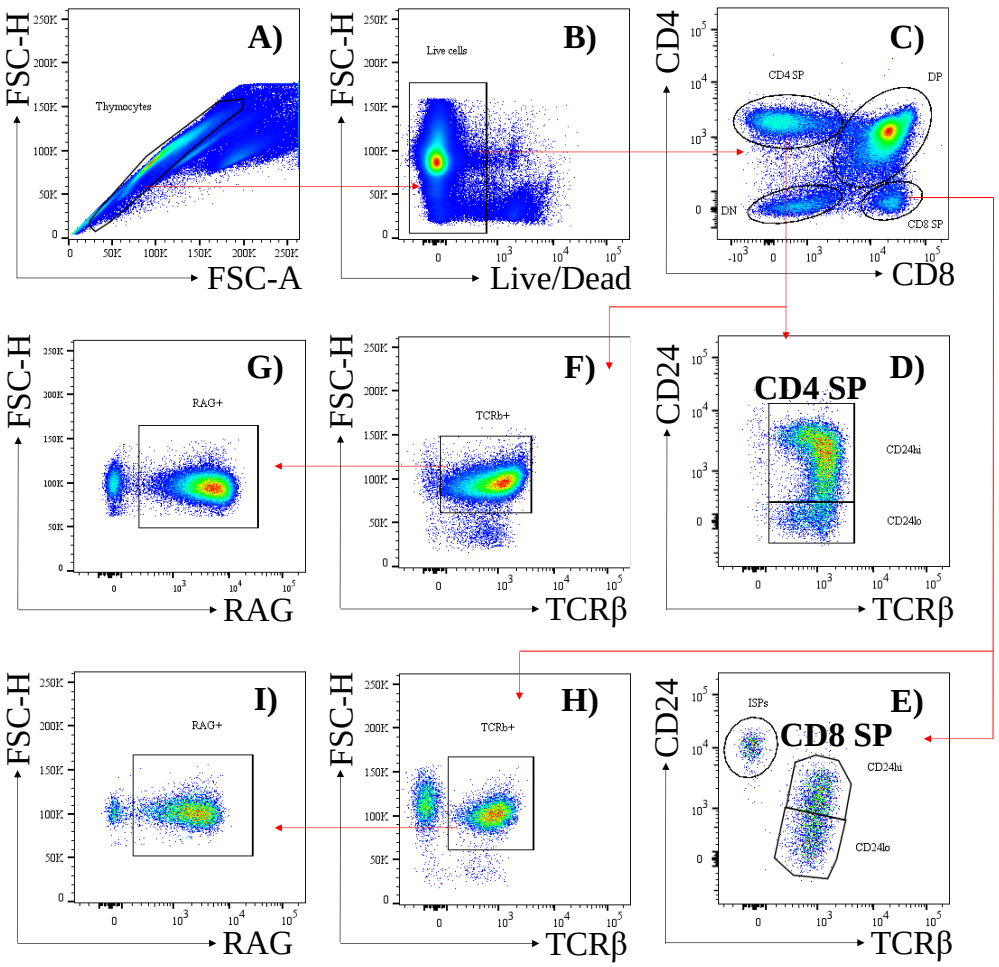

Figure S1: Gating strategy for quantification of thymocyte subsets, DN, DP and SPs subsets, CD24^{hi} and CD24^{lo} SP subsets, proportions of TCRβ^{+}RAG^{+} SPs and RAG MFI in TCRβ^{+}SPs. In naïve and 8-10 weeks post reconstituted mice, the thymocytes were harvested, and were analyzed by FACS. **A)** The singlets and **B)** live events were gated to generate CD4 versus CD8 plots, wherein the DN (CD4^{-}CD8^{-}), DP (CD4^{+}CD8^{+}), CD4 SP (CD4^{+}CD8^{-}) and CD8 SP (CD4^{-} CD8^{+}) cells were gated for quantification **(C)**. The CD4 and CD8 SPs were gated on the basis of CD24 and TCRβ expression to analyze the CD24^{hi} and CD24^{lo} SP subsets **(D and E)**. In the reconstituted mice, after gating on the CD45.1/.2 (+/+ BM-derived) and the CD45.2 (+/*w* BM-derived) thymocytes, the DN, DP and SP thymocytes were quantified along with the CD24^{hi} and CD24^{lo} SP subsets. In addition, **(F and G)** the TCRβ^{+} CD4 SPs and **(H and I)** the TCRβ^{+} CD8 SP cells were gated to quantify the proportions of RAG^{+} cells and the RAG-GFP geometric MFI in the respective gates.

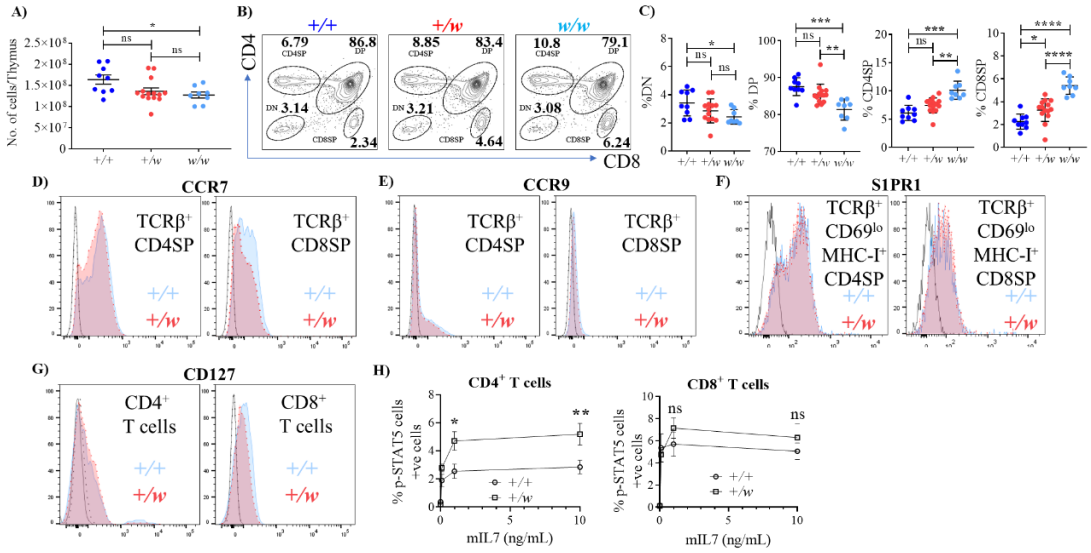

Figure S2: The WHIM allele skews the distribution of thymocyte subpopulations with increased proportions of CD8 SP cells. Thymi from 5-8 week old mice were harvested. **A)** Total thymic cellularity. **(B and C)** Thymocyte subset analysis by CD4 and CD8 expression. **B)** Representative example using thymocytes from *+/+*, *+/w* and *w/w* mice as indicated above each contour-plot. Subset names and frequencies are adjacent to the gates. +, wild type allele; *w*, WHIM allele; DN; DP and CD4SP and CD8SP. **C)** Summary data for thymocyte subset distribution. In **A** and **C**, mouse *Cxcr4* genotypes (+, wild type allele; *w*, WHIM allele) are indicated on the x-axes. Summary data are the mean ± SEM of at least 7 mice per genotype from 4 independent experiments. ns, not significant; *, p< 0.05, **, p< 0.01, ***, p< 0.001 and ****, p< 0.0001 as determined by the one-way ANOVA for **A** and **C**. **(D-F)** CCR7, CCR9 and S1PR1 expression on thymocytes. Representative histograms of **D)** CCR7 and **E)** CCR9 cell-surface expression on $TCR\beta^+CD4SP$ and $TCR\beta^+CD8SP$ WT (blue) and WHIM (red) thymocytes are overlayed on WT (solid line) and WHIM (dashed line) isotype controls. **(F)** S1PR1 expression on mature ($TCR\beta^+CD69^{lo}MHC\text{-}I^+$) WT (blue) and WHIM (red) thymocytes are shown as histograms (fluorescence minus one as control). **(G)** Representative histograms of CD127 (IL7Rα) expression on bone marrow T cells. The $CD4^+$ T cells and $CD8^+$ T cells were gated to analyze the cell-surface expression of CD127. Representative histograms are shown with fluorescence minus one as control, WT (blue) and WHIM (red). **(H)** p-STAT5 expression in recombinant mouse IL7 activated T cells. WT and WHIM bone marrow cells were stimulated with the indicated doses of IL7 (Catalog #: 407-ML-005/CF, R&D Systems®) for 20 mins at 37ºC and percentage T cells expressing intracellular p-STAT5 were quantified. The data is depicted as mean ± SEM. The results in panels **D-G** are representative of 4-5 mice per genotype from a single experiment. The results in panels **H** are from a single experiment with each condition tested in replicates from 4-5 mice per genotype. *, p<0.05 and **, p< 0.01, as determined by two-way ANOVA for the +/+ vs *+/w* comparison in panel **H**.

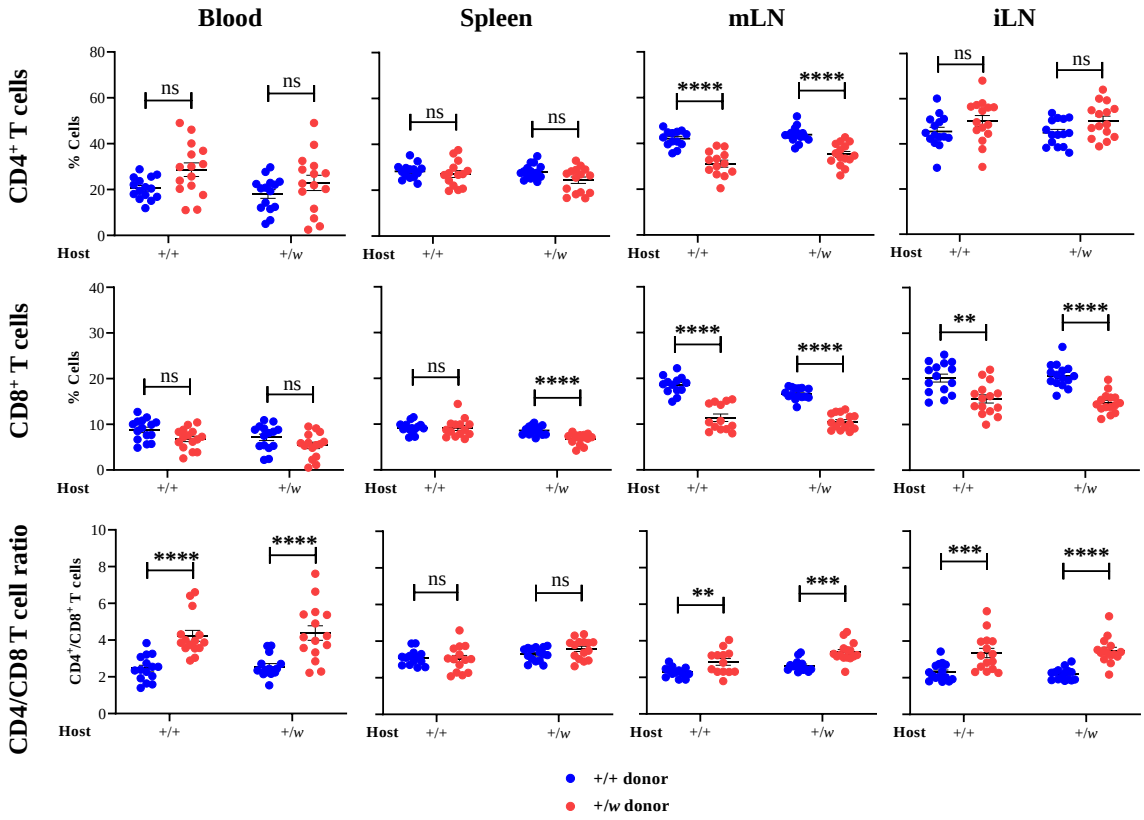

Fig. S3. The WHIM mutation does not promote CD8$^+$ T cell accumulation in the blood, spleen and lymph nodes. At approximately 6 weeks of age, male and female CD45.1/.2 +/+ and CD45.2 +/*w* mice expressing RAG-GFP were sacrificed, and their BM cells were harvested. After T cell depletion, the +/+ and +/*w* BM cells were mixed in a ratio of 1:1 and i.v. injected into lethally irradiated CD45.1/.2 +/+ and +/*w* mice (not expressing RAG-GFP). After 8-10 weeks of BM reconstitution, the samples indicated on the top of the first row of panels were harvested and stained for cell-surface CD45.1, CD45.2, CD4 and CD8 to quantify CD4$^+$ and CD8$^+$ T cells and CD4/CD8 ratios in each tissue, as designated on the left of each graph row. The +/*w* BM-derived CD45$^+$ leukocyte chimerism was approximately 8.5%, 21%, 23% and 17% in blood, spleen, mLN and iLN, respectively. The *Cxcr4* genotypes (+, wild type allele; *w*, WHIM allele) of the recipient mice are given on the x-axes of the panels and the *Cxcr4* genotype color code for the donor-derived leukocytes is given at the bottom of the figure. Data are summarized as the mean ± SEM of 13-16 mice per genotype from 7 independent experiments. ns: not significant, **, p< 0.01, ***, p< 0.001 and ****, p< 0.0001, as determined by the multiple t-tests.

Table S1: List of antibodies used for flow cytometry.

Antigen Specificity	Clone	Vendor	Catalog no.	Dilution	Conjugate
CD3ε	145-2C11	BioLegend®	100312	1:25	APC
CD3ε	145-2C11	BioLegend®	100351	1:25	BV605™
CD3	SK7	BD Biosciences	341091	1:100	PE/Cy7
CD3	7D6	Invitrogen	MHCD0301-4	1:20	FITC
CD4	RPA-T4	Invitrogen	17-0049-42	1:100	APC
CD4	S3.5	Invitrogen	MHCD0431	1:100	PerCP
CD4	RM4-5	BioLegend®	100516	1:100	APC
CD8	RPA-T8	BD Biosciences	565165	1:100	APC-R700
CD8	3B5	Invitrogen	MHCD0831	1:20	PerCP
CD8a	53-6.7	eBioscience™	48-0081-82	1:200	eFluor™ 450
CD8a	53-6.7	BioLegend®	100708	1:100	PE
CD8a	53-6.7	BD Biosciences	563332	1:50	BUV786
CD24	M1/69	BioLegend®	101827	1:200	BV605™
CD44	IM7	BioLegend®	103032	1:100	PerCP/Cyanine5.5
CD45	30-F11	BioLegend®	103126	1:25	Pacific Blue™
CD45	30-F11	BioLegend®	103106	1:100	PE
CD45	HI30	Invitrogen	47-0459-42	1:100	APC-eFluor™ 780
CD45.1	A20	eBioscience™	25-0453-82	1:50	PE/Cy7
CD45.2	104	eBioscience™	48-0454-82	1:25	eFluor® 450
CD127	15-1271-82	eBioscience™	A7R34	1:200	PE/Cy5
IFN-γ	505813	BioLegend®	XMG1.2	1:200	AlexaFluor® 488
IL-2	503808	BioLegend®	JES6-5H4	1:200	PE
Granzyme B	GB11	BioLegend®	515403	1:100	FITC
KLRG1	2F1/KLRG1	BioLegend®	138416	1:200	PE/Cy7
TCRβ	H57-597	BioLegend®	109220	1:50	APC/Cy7
TNF-α	MP6-XT22	BioLegend®	506324	1:100	PE/Cyanine7
CCR7	4B12	BioLegend®	120105	1:10	PE
CCR9	9B1	BioLegend®	129707	1:20	PE
CXCR4	2B11	eBioscience™	61-9991-82	1:50	PE-eFluor®610
CXCR4	L276F12	BioLegend®	146505	1:50	PE
S1PR1 (S1P1)	713412	R&D Systems	MAB7089	1:12.5	Unconjugated
Rat IgG	Polyclonal	Jackson ImmunoResearch	712-136-153	1:200	APC
Phospho-STAT5	SRBCZX	Invitrogen	12-9010-42	1:20	PE
Isotype control	MOPC-21	BioLegend®	400114	1:80	PE
Isotype control	R35-95	BD Biosciences	553930	1:10-1:20	PE
Isotype control	eB149/10H5	eBioscience™	61-4031-80	1:50	PE-eFluor®610
Isotype control	RTK4530	BioLegend®	400607	1:50	PE
TruStain FcX™	93	BioLegend®	101320	1:50	