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Supplemental Information

Restricted Expression of the Thymoproteasome Is Required for Thymic Selection and Peripheral Homeostasis of $CD8^+$ T Cells

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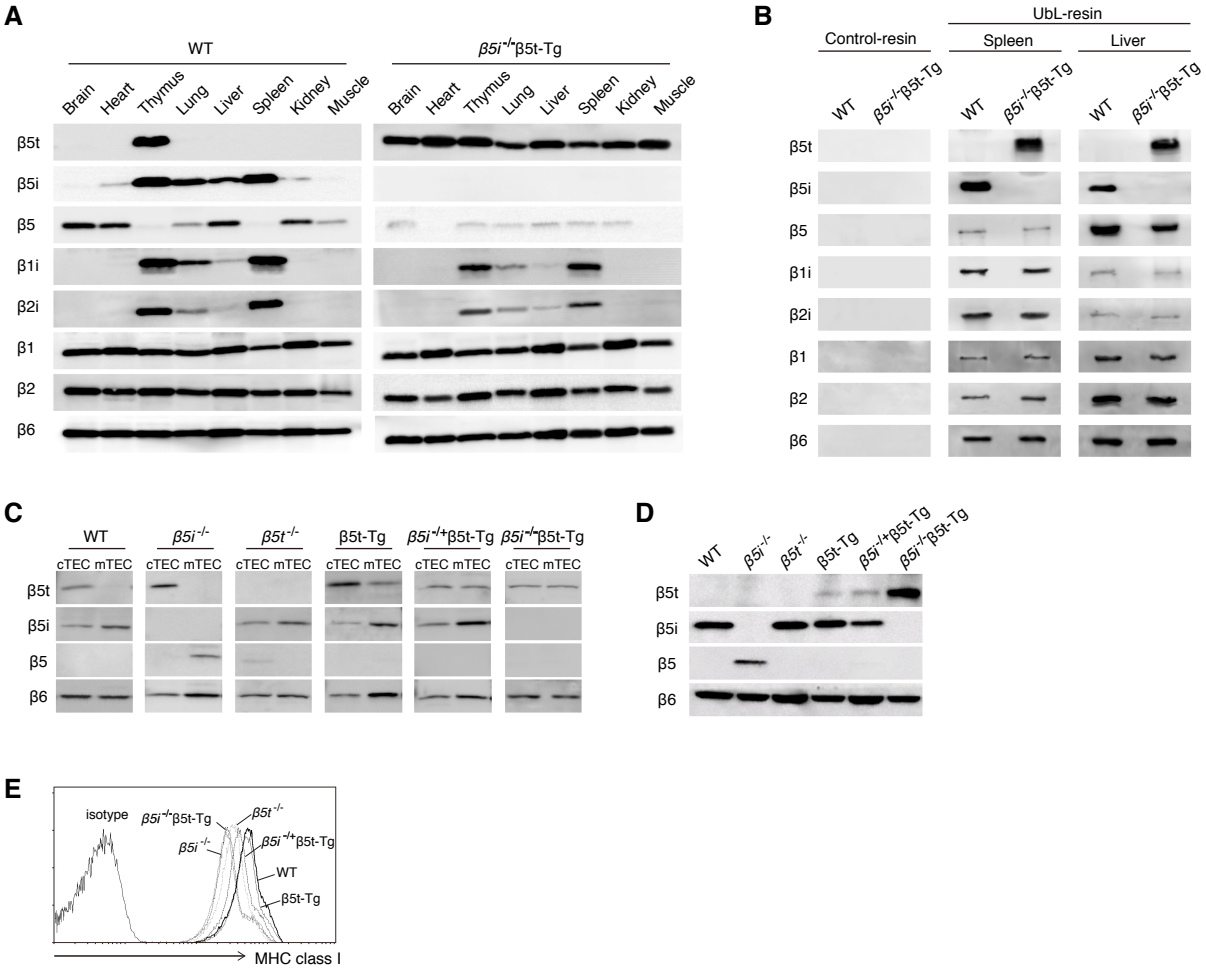

Figure S1. Expression of proteasome subunits and MHC class I in tested mice, Related to Figures 1 and 3. (A, C, and D) Western blot analysis of proteasome subunits in tissue lysates from WT and *β5i*$^{-/-}$β5t-Tg mice (A), cell lysates from sorted cTECs, mTECs (C), and DCs (D) in the indicated mice. (B) Immunoblot using enriched proteasomes from tissue lysates. For controls, spleen tissue lysates from WT and *β5i*$^{-/-}$β5t-Tg mice were incubated with control resin. Spleen and liver tissue lysates from WT and *β5i*$^{-/-}$β5t-Tg mice were incubated with UbL-resin to enrich proteasomes. (E) Flow cytometric analysis of MHC class I expression in splenic cells from the indicated mice.

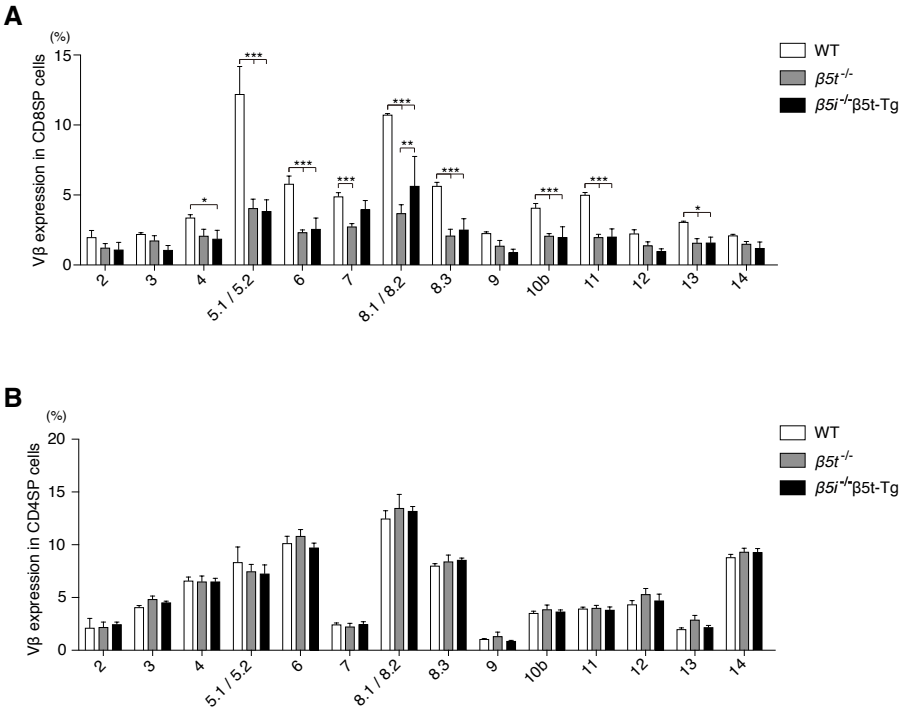

Figure S2. TCR Vβ repertoire in WT, *β5t*$^{-/-}$, and *β5i*$^{-/-}$ β5t-Tg mice, Related to Figure 1.

Flow cytometric analysis using TCR Vβ Abs panel on thymic CD8SP cells (A) and CD4SP cells (B). Data are shown as percentages of indicated TCR Vβ-positive cells among CD8SP or CD4SP cells. Data were pooled from three independent experiments and are expressed as means ± s.d.. Statistical significance was analyzed by one-way ANOVA with multiple comparison post-test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

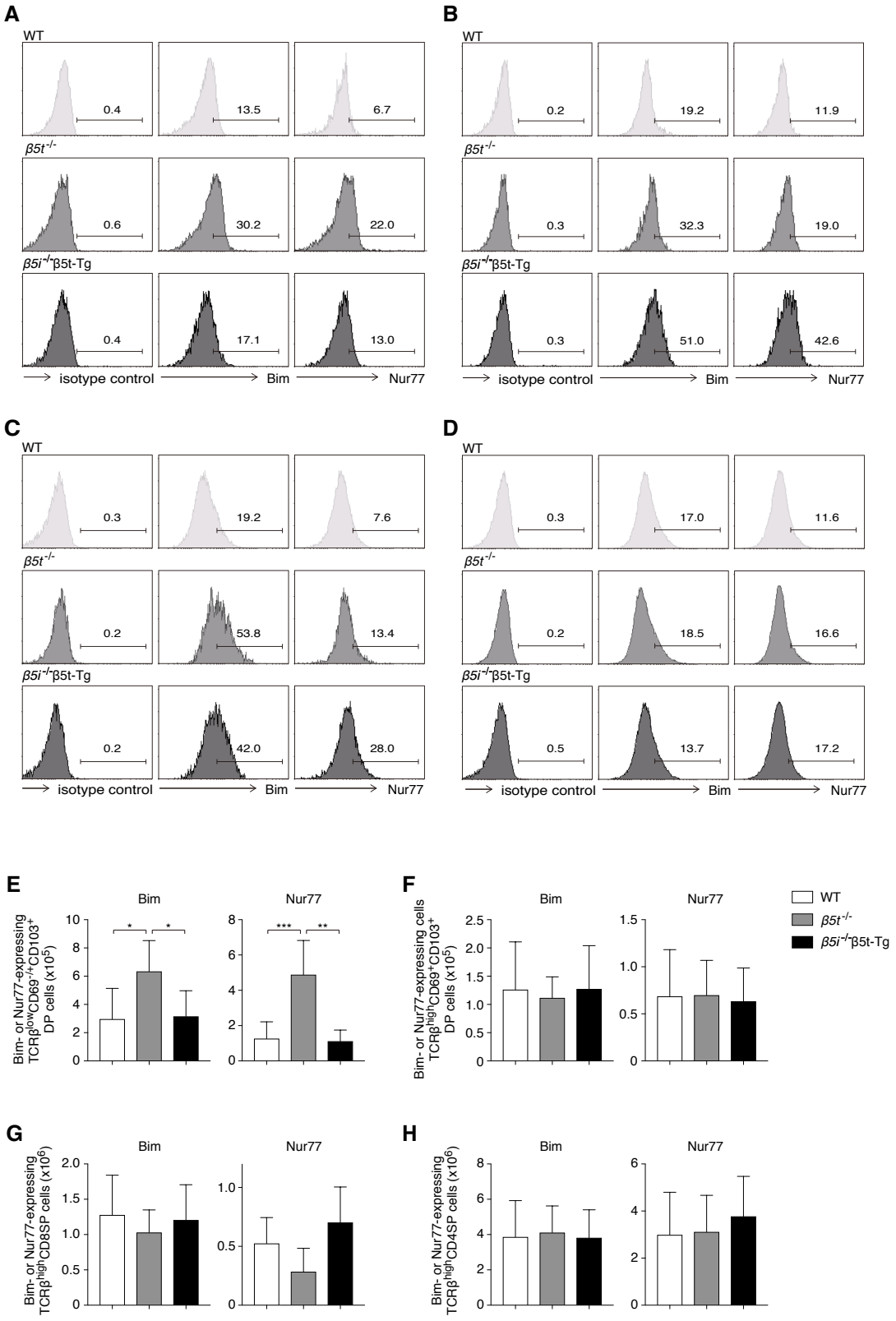

Figure S3. Bim and Nur77 expression in thymocytes, Related to Figure 2.

(A–D) Representative data for histogram of Bim- or Nur77-expressing cells in TCRβ^{low}CD69$^{-/+}$CD103^{+} DP cells (A), in TCR^{high}CD69^{+}CD103^{+} DP cells (B), in TCR^{high} CD8SP cells (C), and in TCR^{high} CD4SP cells (D) from the indicated mice. Numbers in histograms indicate percentages. (E–H) Numbers of Bim- or Nur77-expressing cells in TCRβ^{low}CD69$^{-/+}$CD103^{+} DP cells (E), in TCR^{high}CD69^{+}CD103^{+} DP cells (F), in TCR^{high} CD8SP cells (G), and in TCR^{high} CD4SP cells (H) from the indicated mice. Data are expressed as means ± s.d. (E–H). Data were pooled from at least three independent experiments. Statistical significance was analyzed by one-way ANOVA with multiple comparison post-test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

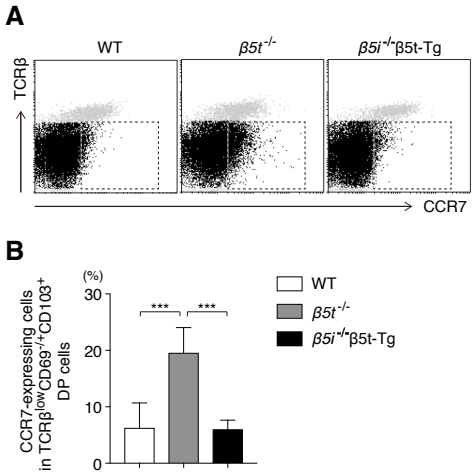

Figure S4. Aberrant expression of CCR7 in TCRβ^{low}CD69$^{-/+}$CD103^{+} DP cells of *β5t*$^{-/-}$ mice, Related to Figure 2. Representative data of dot-plot profiles (A) and percentages of CCR7-expressing cells in TCRβ^{low}CD69$^{-/+}$CD103^{+} DP cells (B). Data were pooled from three independent experiments and are expressed as means ± s.d.. Statistical significance was analyzed by one-way ANOVA with multiple comparison post-test: *** $P < 0.001$.

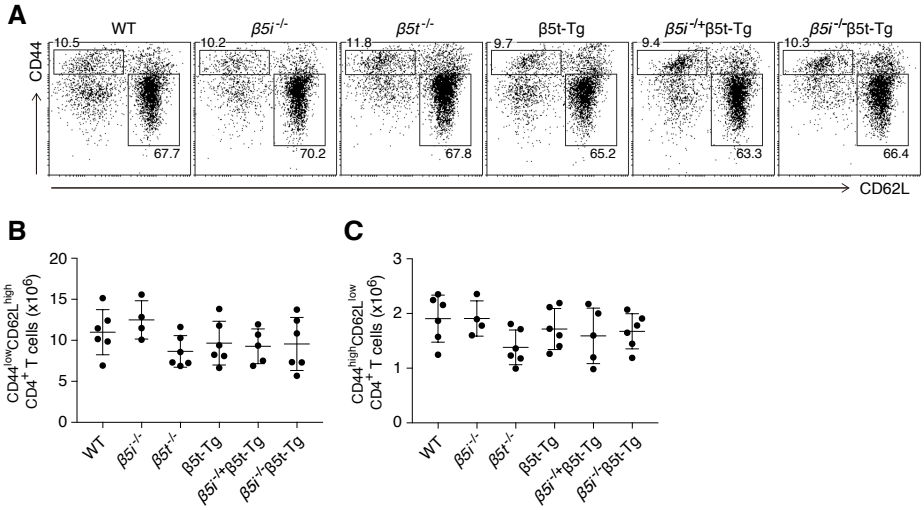

Figure S5. Expression of CD44 and CD62L in peripheral CD4$^+$ T cells, Related to Figure 3.

Representative data of dot-plot quadrants (A) and cell numbers of naïve CD44^{low}CD62L^{high} (B) and memory CD44^{high}CD62L^{low}CD4$^+$ T cells (C). Numbers adjacent to outlined areas indicate percentages (A). Each symbol represents an individual mouse; the long horizontal line represents the mean, and short horizontal lines represent s.d.. Data were pooled from three independent experiments.

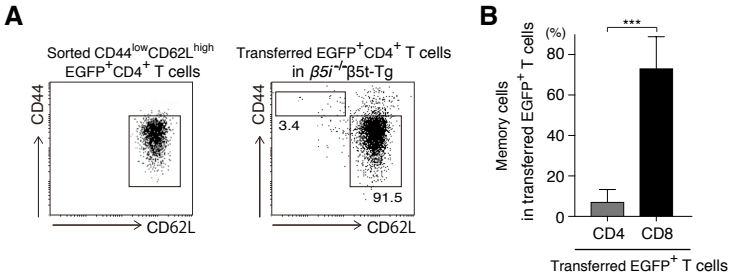

Figure S6. Naïve CD44^{low}CD62L^{high} CD4^{+} T cells transferred into *β5i*$^{-/-}$β5t-Tg mice, Related to Figure 4.
Flow cytometric analysis of CD44 and CD62L on transferred EGFP^{+}CD4^{+} T cells. CD44^{low}CD62L^{high} CD4^{+} T cells from EGFP mice were transferred into *β5i*$^{-/-}$β5t-Tg. Representative data of dot-plot profiles 12 days after the transfer are shown (A). No change to the memory phenotype was observed in transferred naïve CD4^{+} T cells (B). Numbers adjacent to outlined areas indicate percentages (A). Data were pooled from three independent experiments and are expressed as means ± s.d.. Statistical significance was analyzed by Student' s t-test: *** $P < 0.001$.

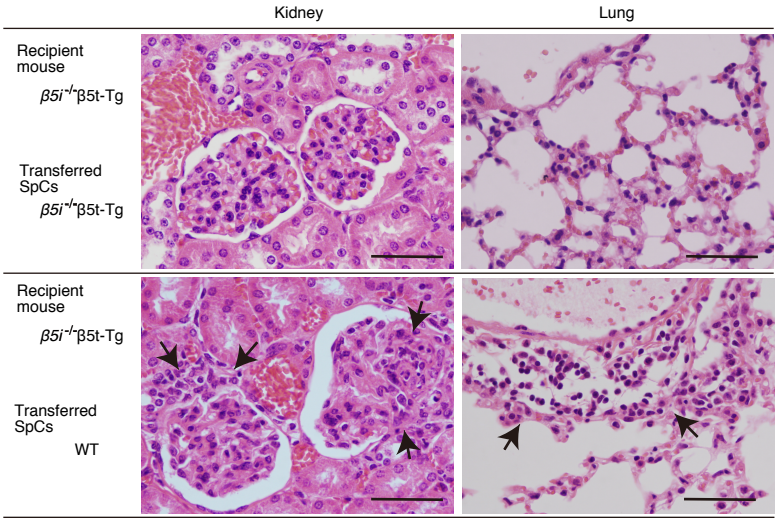

Figure S7. Representative histological images of kidney and lung tissues of recipient mice, Related to Figure 5. Arrows indicate inflammatory foci. The scale bar represents 50 μm.

Table S1. Expression of proteasomes in antigen presenting cells of the thymus and peripheral lymphoid tissues, Related to Figures 1 and 3.

	WT	*β5i*$^{-/-}$	*β5t*$^{-/-}$	β5t-Tg	*β5i*$^{-/+}$β5t-Tg	*β5i*$^{-/-}$β5t-Tg
cTECs	β5t	β5t	β5i	β5t	β5t	β5t
mTECs	β5i	β5	β5i	β5i/β5t	β5i/β5t	β5t
DCs	β5i	β5	β5i	β5i/β5t	β5i/β5t	β5t