SUPPLEMENTAL MATERIAL

Figure S1. **Phenotypic characterization of immune cell subsets.** Analysis in spleen (A), thymus (B), blood (C), lungs (D), mesenteric lymph nodes (MLN; E), and peripheral lymph nodes (PLN; F) from Sec22b−/− and Sec22b+/+ mice. Data are means of four independent biological replicates. CD4, CD8, CD25, CD44, CD62L, TCRb, and CD3 were used as T cell markers. B220 and CD19 as B cell markers. TCR-γδ was used as the γδ T cell marker. NK1.1 and NKp46 were used as natural killer markers. Gr-1 and Ly6C were used as granulocyte/neutrophil markers. CD11c was generally used as the DC marker, with the exception of lungs; alveolar macrophages (CD11c^high) were discriminated from DCs by analyzing Siglec F expression. CD103, I-Ab, H2-Kb, CD11b, CD8, CD40, and CD86 were used to analyze DC phenotypes. F4/80 was used as a macrophage marker. Shown are the pooled data of four independent experiments. Results were analyzed by two-way ANOVA with Bonferroni's multiple comparisons test for statistical significance. The means and SEM are shown. (G) Expression of the costimulatory molecule CD86 and of MHC class II in splenic CD11c+ DCs upon treatment with IFN-γ or TNF. (H) Expression of the costimulatory molecule CD86 and of MHC class II in BMDCs upon treatment with IFN-γ or TNF. For all the DC results, the pooled data of at least three independent experiments are shown. All results were analyzed by two-way ANOVA with Bonferroni's multiple comparisons test. *, 0.01 < P < 0.05; **, P < 0.01.

Figure S2. **Immunization of Sec22b^{+/+} and Sec22b^{−/−} mice to analyze endogenous responses.** (A) Total cells from popliteal (pLN; left) and inguinal (iLN; right) lymph nodes from Sec22b^{+/+} and Sec22b^{−/−} mice immunized with IFA-CpG-SIINFEKL were restimulated ex vivo with SIINFEKL or OVA. The percentage of OVA-specific T cells producing IFN-γ per 5×10^5 blood cells was determined by ELISPOT analysis. The means and SEM are shown. *, 0.01 < P < 0.05. (B) Phenotypic analysis of resident (res) and migratory (mig) DCs in lymph nodes. Gating strategy to identify the putative cross-presenting subsets CD8^{+} and CD103^{+} DCs in resDCs and migDCs, respectively. (C) Percentages (left) and cell count (right) of CD8^{+} resDCs (top) and CD103^{+} migDCs (bottom). Shown are the pooled data of four independent experiments. Results were analyzed by two-way ANOVA with Bonferroni's multiple comparisons test for statistical significance. The means are shown.
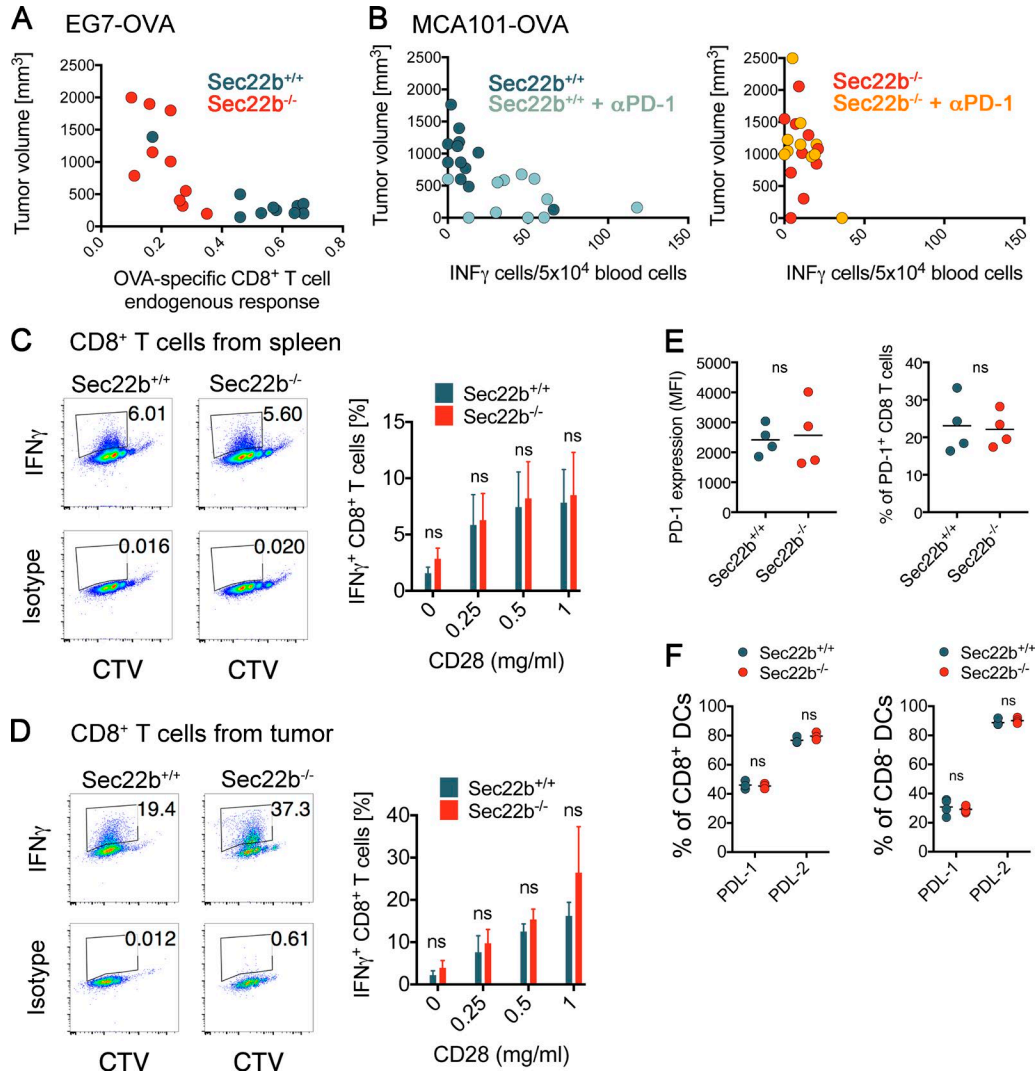
Figure S3. **Correlative analysis of tumor growth and T cell responses.** (A) CD8^{+} T cell response (measured by OVA-specific tetramers) versus EG7 tumor growth (the day of sacrifice). (B, left) CD8^{+} T cell response (quantified by IFN-γ–producing cells after restimulation with MHC class I–restricted peptide) versus MCA101-OVA tumor growth in Sec22b^{+/+} mice, without or with anti–PD-1 treatment. (Right) CD8^{+} T cell response (quantified by IFN-γ–producing cells after restimulation with MHC class I–restricted peptide) versus MCA101-OVA tumor growth in Sec22b^{−/−} mice, without or with anti–PD-1 treatment. (C) Intracellular staining for IFN-γ production in CD8^{+} T cells from spleens of mice injected with MCA-OVA tumor and treated with anti–PD-1, after 72 h of proliferation in a CD3-coated plate with increasing concentrations of CD28. Example of gating strategy (left) and pooled data (right) of four mice. Shown are the means and SEM. Results were analyzed by two-way ANOVA with Bonferroni's multiple comparisons test for statistical significance. (D) Intracellular staining for IFN-γ production in CD8^{+} T cells from tumors of mice injected with MCA-OVA tumor and treated with anti–PD-1, after 72 h of proliferation in a CD3-coated plate with increasing concentrations of CD28. Example of gating strategy (left) and pooled data (right) of four mice. Shown are the means and SEM. Results were analyzed by two-way ANOVA with Bonferroni's multiple comparisons test for statistical significance. (E) PD-1 expression in CD8^{+} T cells from tumors of Sec22b^{+/+} and Sec22b^{−/−} mice injected with MCA-OVA tumor and treated with anti–PD-1. (F) PD-L1 and PD-L2 expression in splenic DCs from Sec22b^{+/+} and Sec22b^{−/−} mice.