Supplemental figures

Figure S1. Impact of pMHCII stability on the accumulation of $CD44^{hi}CD62L^{hi}$ antigen-specific CD4 T cells

(**A**) As displayed in Figure 2, antigen-specific CD4 T cells ($V\alpha11^{+}V\beta3^{+}CD44^{hi}CD62L^{hi}$) at day 7 in LN from B10.BR mice immunized with the indicated peptide. Profiles are pre-gated on DAPI negative cells that are $B220^{-}CD8^{-}CD11b^{-}$ and $V\alpha11^{+}V\beta3^{+}$. Numbers above boxed areas indicate percent cells in profile. (**B**) Total number of $CD44^{hi}CD62L^{hi}$ antigen-specific CD4 T cells 7 days after immunization with the indicated peptide. Data shown are derived from three independent experiments (mean ± SEM; n≥3 mice per group).

Figure S2. Impact of pMHCII stability on TCRα chain selection

Antigen-driven selection for preferred CDR3 features in α-chain of the TCR. Single antigen-specific CD4 T cells ($V\alpha11^{+}V\beta3^{+}CD44^{hi}CD62L^{lo}$) were sorted from draining lymph nodes of B10.BR mice immunized with the indicated peptide. Single-cell repertoire analysis was done as described in Materials and Methods. Each dot represents the sequence information from a single-cell. The y-axis represents (**A**) Jα usage (**B**) CDR3α length in number of amino acids (aa) (**C**) aa present at position α93, and **(D)** α95. The numbers of sequences used in the analysis are displayed (across three separate mice for each condition). Data shown are derived from three independent experiments (3 mice per group).

Figure S3. Impact of pMHCII stability on TCRβ chain selection

Antigen-driven selection for preferred CDR3 features in β-chain of the TCR. Single antigen-specific CD4 T cells ($V\alpha11^{+}V\beta3^{+}CD44^{hi}CD62L^{lo}$) were sorted from draining LN of B10.BR mice immunized with the indicated peptide. Single-cell repertoire analysis was done as described in Materials and Methods. Each dot represents the sequence information from a single-cell. The y-axis represents (**A**) Jβ usage, (**B**) aa present at position β102, (**C**) the CDR3β length in number of aa, and (**D**) aa present at position β100. The numbers of sequences used in the analysis are displayed (across three separate mice for each condition). Data shown are derived from three independent experiments (n≥3 mice per group).

Figure S4. Impact of pMHCII stability on TCRβ chain selection

All TCRβ amino acid sequences of antigen-specific CD4 T cells ($V\alpha11^{+}V\beta3^{+}CD44^{hi}CD62L^{lo}$) analyzed in this study. Frequency of TCRβ public, recurrent and private amino acid sequences in antigen-specific CD4 T cells sorted from draining LN of B10.BR mice immunized with the indicated peptide are depicted. Data shown are derived from three independent experiments (means ± SEM; n≥3 mice per group).

Figure S5. TCR binding properties of PCC-specific TCRαβ transgenic cells

Peptide-MHCII tetramer staining and TCR Vα11 expression levels of CD4 T cells isolated from LN of naïve ANDαβ (CDR3β: SLNNANSDY, Jβ1.2) and low affinity

2B4αβ (CDR3β: SLNWSQDTQ, Jβ2.5) (14) PCC-specific TCR transgenic mice. Mean fluorescence intensity of **(A)** pMHCII tetramer staining and **(B)** Vα11 expression of indicated TCRαβ cells. Representative histograms of two independent experiments are shown.

Figure S6. Antigen dose impacts clonotypic diversity

Single-cell repertoire analysis of individual antigen-specific CD4 T cells ($V\alpha11^{+}V\beta3^{+}CD44^{hi}CD62L^{lo}$) sorted from mice immunized with the indicated dose of PCC_{103K}. (**A-C**) Average number ± SEM of preferred CDR3 features per cell for the (A) whole TCR, (**B**) TCRα chain only or (**C**) TCRβ chain only. *, $P \leq 0.05$, between indicated peptides (unpaired Student's t-test). Data shown are derived from three independent experiments (n≥3 mice per group).

Figure S1

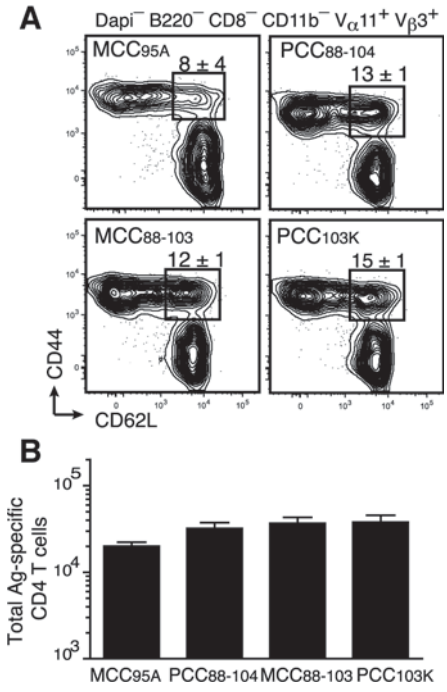

Figure S2

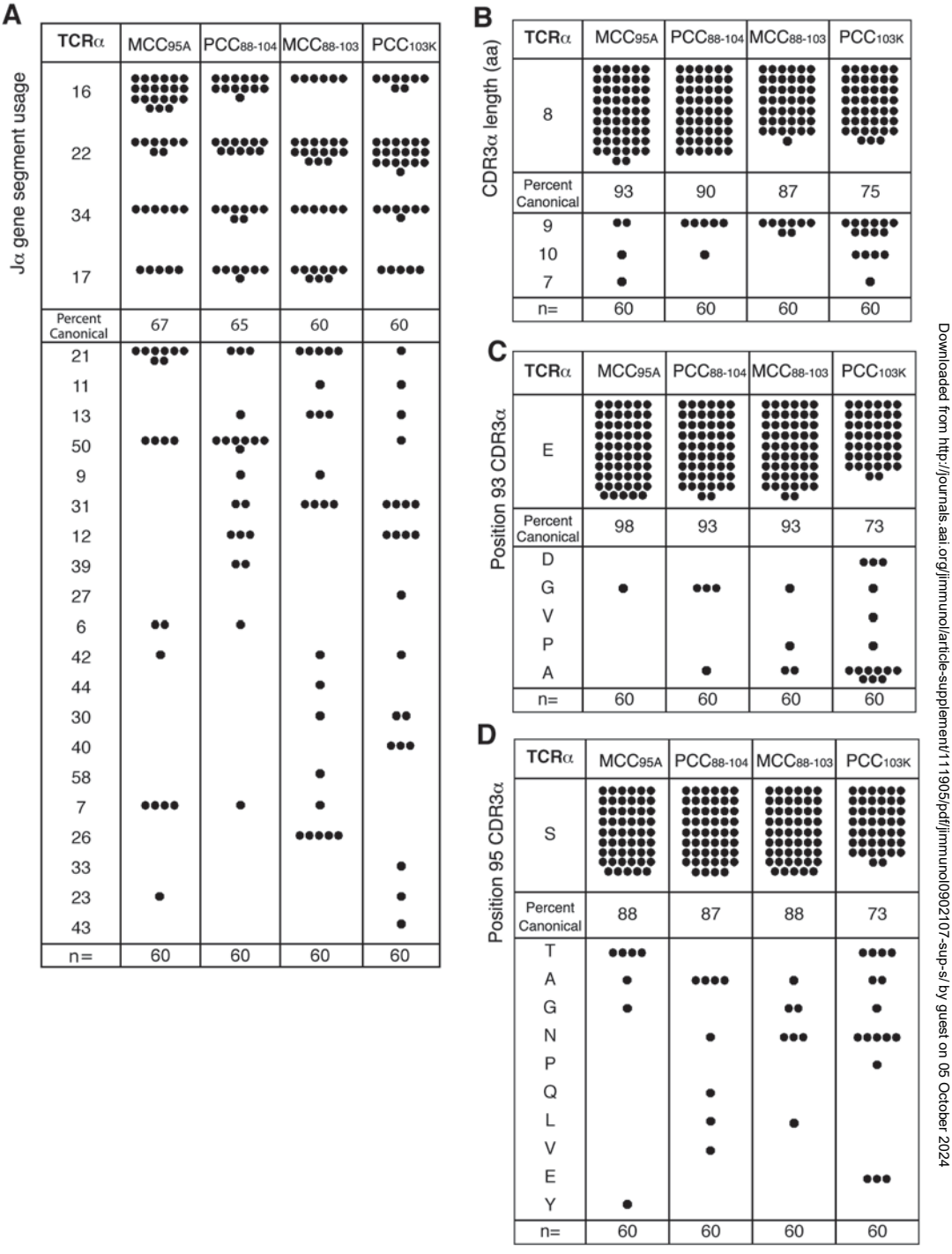

Figure S3

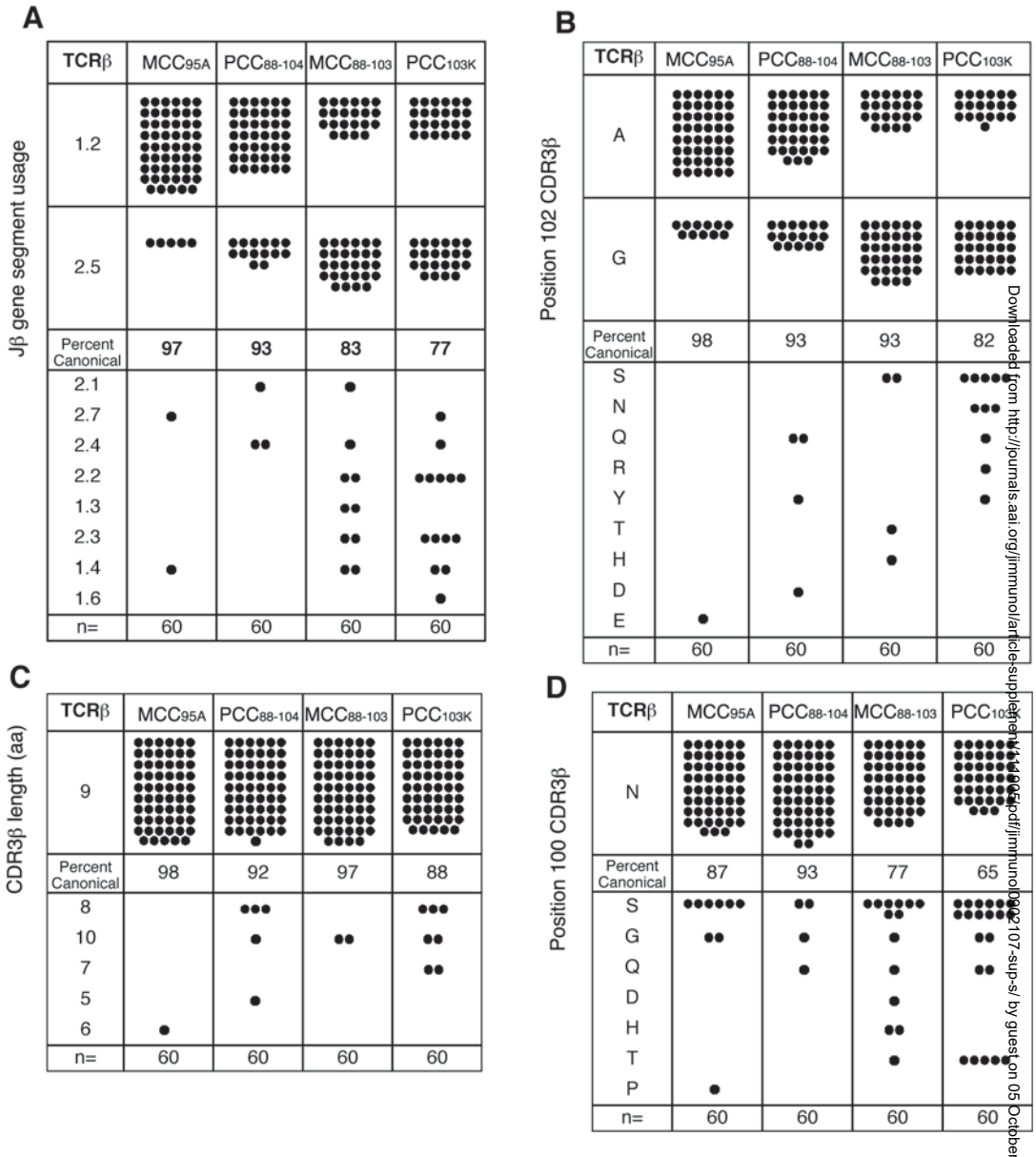

Figure S4

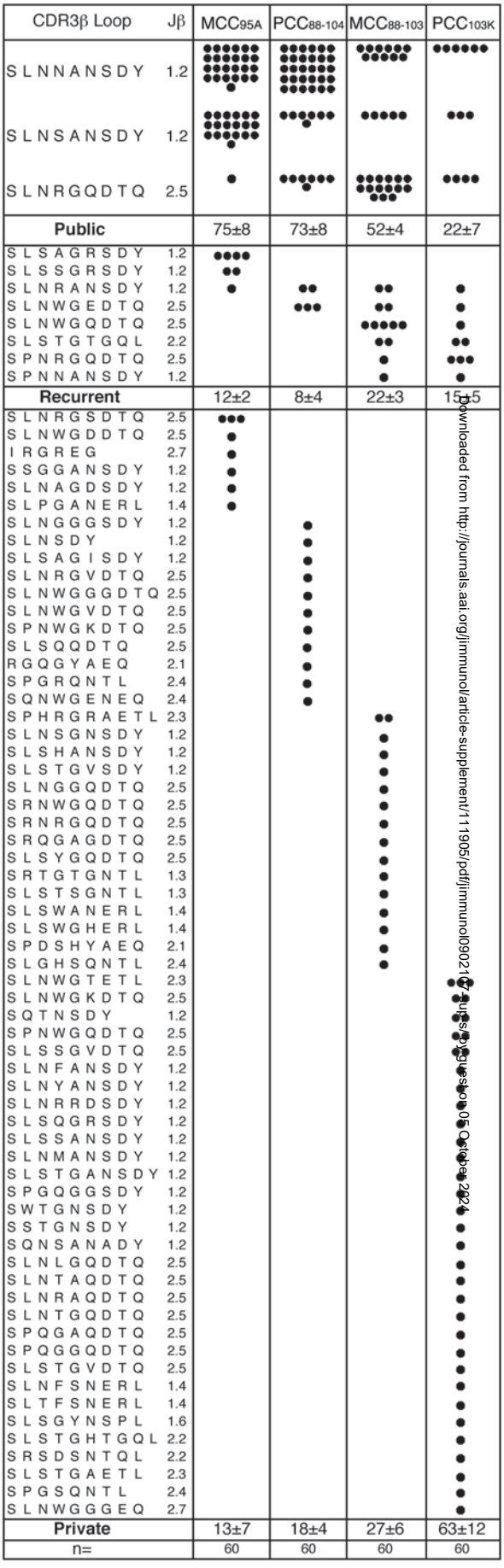

Figure S5

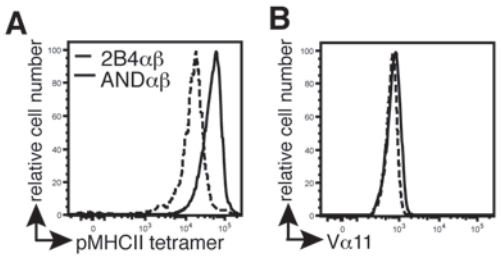

Figure S6