

Figure S1. Gating strategy for identification and phenotypic analysis of antigen-specific CD8<sup>+</sup> T cells. B cells and monocytes were excluded prior to analysis. CD8<sup>+</sup> T cells were identified from the total lymphocytes based on CD3 and CD8 expression (top left). Antigen-specific CD8<sup>+</sup> T cells were detected using peptide-MHC dextramers (e.g., PSA<sub>153-161</sub>), shown in the top right panel, with colored dots representing peptide-binding cells. The phenotypic characterization was performed using CCR7 vs CD28 (bottom left) and CCR7 vs CD45RA (bottom right) plots. CD8<sup>+</sup> T cell subsets were defined as: naïve (CD45RA<sup>+</sup> CCR7<sup>+</sup> CD28<sup>+</sup>), central memory (CM; CD45RA<sup>-</sup> CCR7<sup>+</sup> CD28<sup>+</sup>), effector memory (EM; CD45RA<sup>-</sup> CCR7<sup>-</sup> CD28<sup>+</sup>), and terminal effector memory (TEMRA; CD45RA<sup>+</sup> CCR7<sup>-</sup> CD28<sup>-</sup>). These subsets were assessed both within the total CD8<sup>+</sup> T cell population and among antigen-specific cells. This strategy enables the comprehensive profiling of T-cell differentiation and antigen specificity. MHC, major histocompatibility complex; PSA, prostate-specific antigen; CCR7, C-C chemokine receptor type 7.

