Supplemental Fig. 1

Strategy to enhance anti-tumor/malaria immunity by combination of 7DW8-5 and a TLR agonist (MPLA) as adjuvants. A glycolipid, 7DW8-5, is known to be presented by CD1d molecules expressed by APCs to iNKT cells through invariant TCR and activate iNKT cells. Activated iNKT cells, in turn, induce activation/maturation of APCs. MPLA binds TLR4 and also activate APCs. Thus 7DW8-5 and MPLA together may synergistically enhance the peptide presentation capability by APCs to specific CD8 + T cells, thereby increasing the level of anti-tumor/malaria CD8 + T cell responses elicited by the peptide vaccine.

Supplemental Fig. 2

Generation of a C1498 cell line co-expressing WT-1 and HLA-A2. A C1498 cell line co-expressing WT-1 and HLA-A2 was constructed as described in material and methods and its expression determined by a flow cytometric analysis (A) and RT-PCR (B).

Supplemental Fig. 3

Lack of IFN-γ response in mice administered 7DW8-5 alone. A group of BALB/c mice (n = 4) received two doses of i.m. immunization with a PyCSP-derived peptide (SYVPSAEQI; 20 μg), 7DW8-5 (10 μg), or both with 3-week interval. Two weeks after the last immunization, splenocytes were collected from immunized mouse groups as well as a naïve mouse group, and the level of PyCSP-specific CD8 + T-cell response was determined by ELISpot assay. Results of IFN-γ-secreting cells/million splenocytes were expressed as mean bars and error for each group.

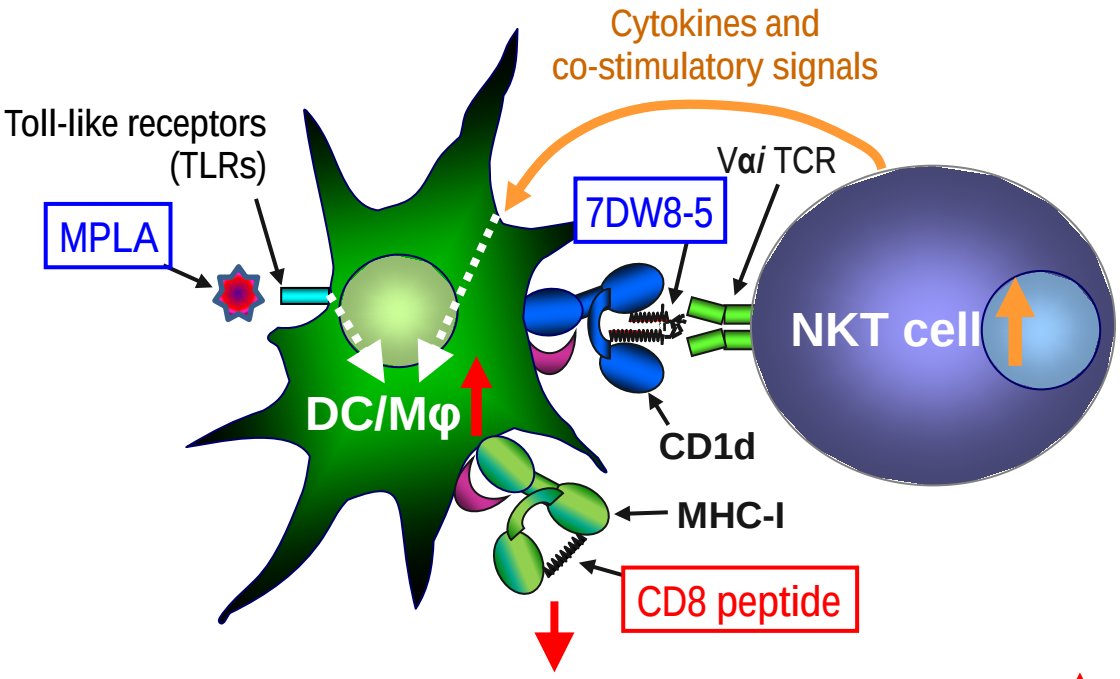
Supplemental Fig. 4

Gating strategy for flow cytometry results for NKT cells in HLA-A2 Tg (B6 background) mice, shown in Fig. 3. The data analyses were performed using FlowJo Software version 10.0.6 (Tree Star Inc., Ashland, OR, USA). First, a gate on Time versus FSC was built to exclude events acquired initially in order to avoid carry over from the previous sample. After that, a singlet gate on FSC-Area versus FSC-height was set to remove doublets. From singlets, NKT-cell subset was defined as SSClowCD3+/lowCD4-CD8-NK1.1+. NKT-cell memory subsets were evaluated for the expression of memory markers (CD44 and CD62L). The NKT-cell subsets CD44-CD62L-/+, CD44+ CD62L+ and CD44+ CD62L− were defined as naïve, central, and effector NKT cells, respectively. CD11a expression 677 was also evaluated in each subset and histograms were set for the selection of the CD11ahigh population (equal or above 104 fluorescence intensity). Contour plots showing CD3 versus Granzyme B or IFN-γ were set for all the three subsets. The capacity of NKT subsets on producing IFN-γ and granzyme B were also defined by gating on the positive population (taking the naive control as reference). Results were expressed in percentage of parent subset.

Supplemental Fig. 5

Gating strategy for flow cytometry results for granzyme B and IFN-γ-secreting CD8 + T-cells after SYVPSAEQI peptide stimulation in BALB/c mice, shown in Fig. 5. The data analyses were performed using FlowJo Software version 10.0.6 (Tree Star Inc., Ashland, OR, USA). Primary analyses on the lymphocytic subsets were performed as demonstrated in Fig. 4. Mock peptide-stimulated and SYVPSAEQI-stimulated tubes are displayed as well as Isotype control for SYVPSAEQI-stimulated sample. After selection of Naïve (CD44− CD62L−/+), Central (CD44+ CD62L+) and effector (CD44+ CD62L−) memory CD8+ T-cells, these subsets were analyzed for intracellular Granzyme B and IFN-γ and CD11a expression. Contour plots showing CD11a versus Granzyme B or IFN-γ were set for all the three subsets. Gating of CD11aHigh was performed. Gates for Granzyme B or IFN-γ were set based on Isotype controls. Results were expressed in percentage of parent subset.

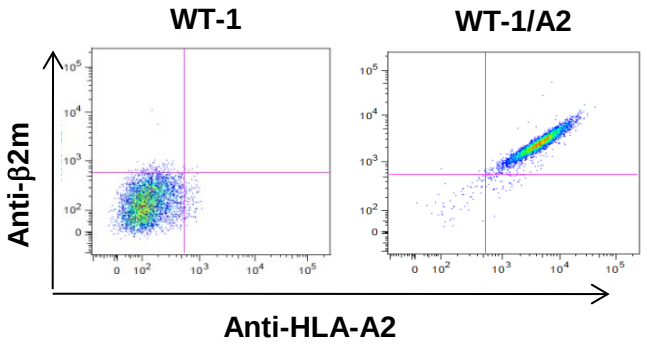
Supplemental Figure 1



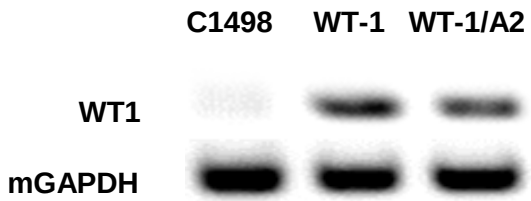
Anti-tumor/malaria CD8+ T-cell responses ↑

Supplemental Figure 2

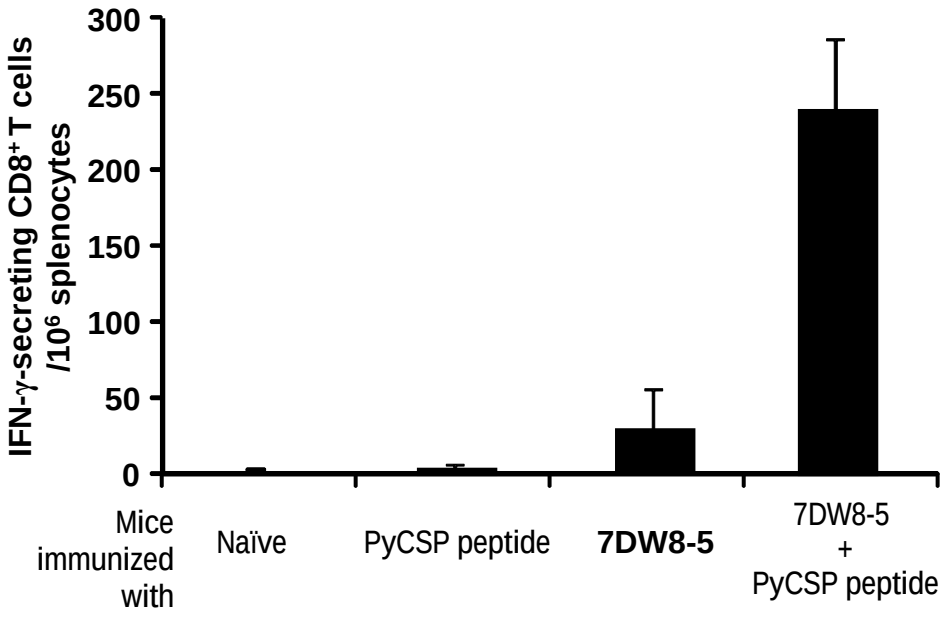
A



B

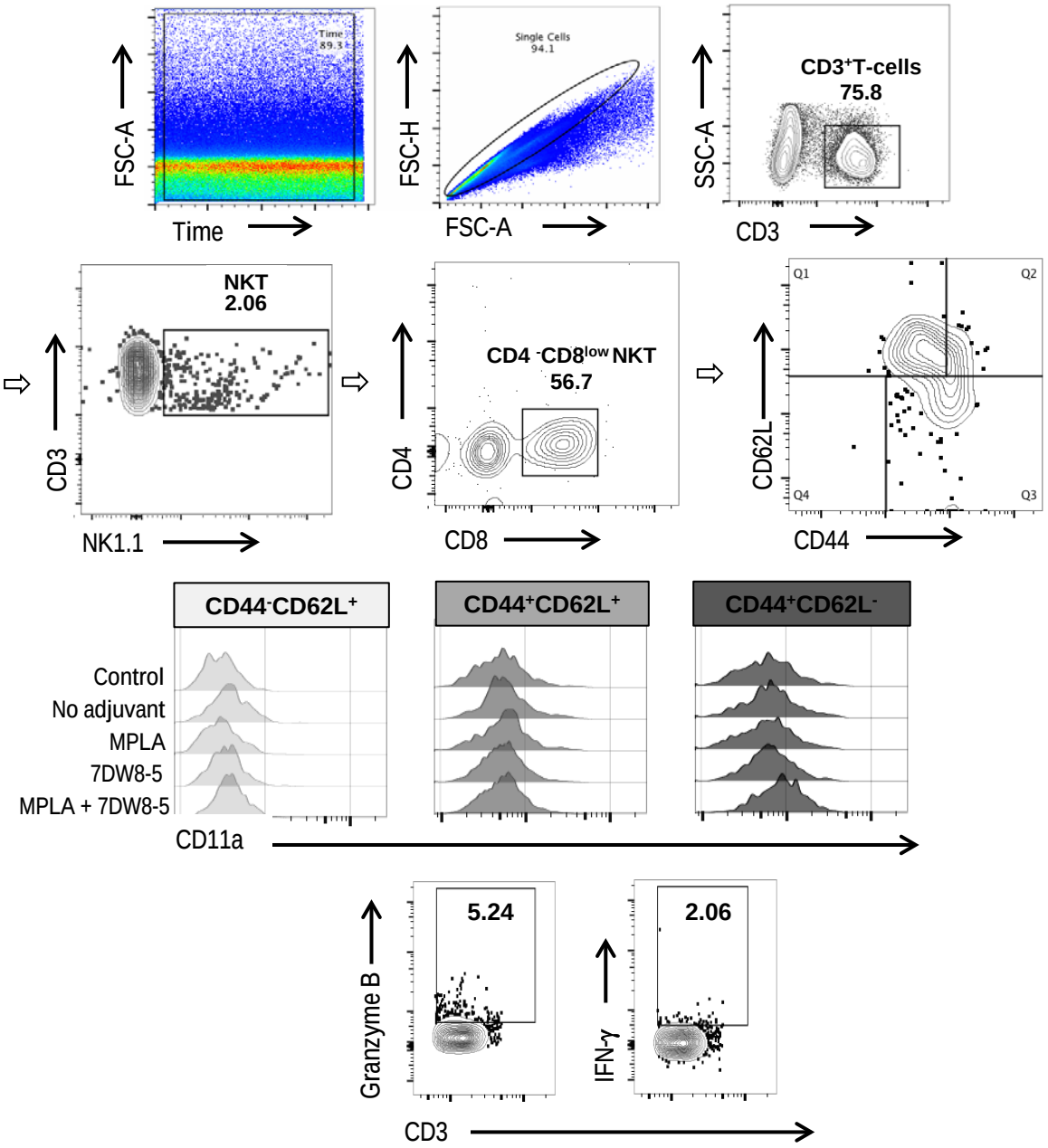


Supplemental Figure 3



Supplemental Figure 4

NKT cell gating strategy (for data in Fig. 3)



Supplemental Figure 5