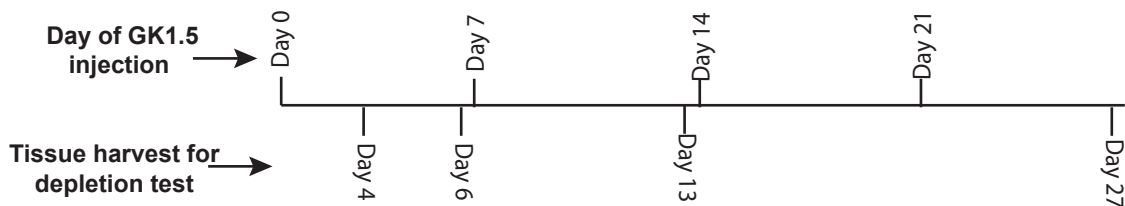
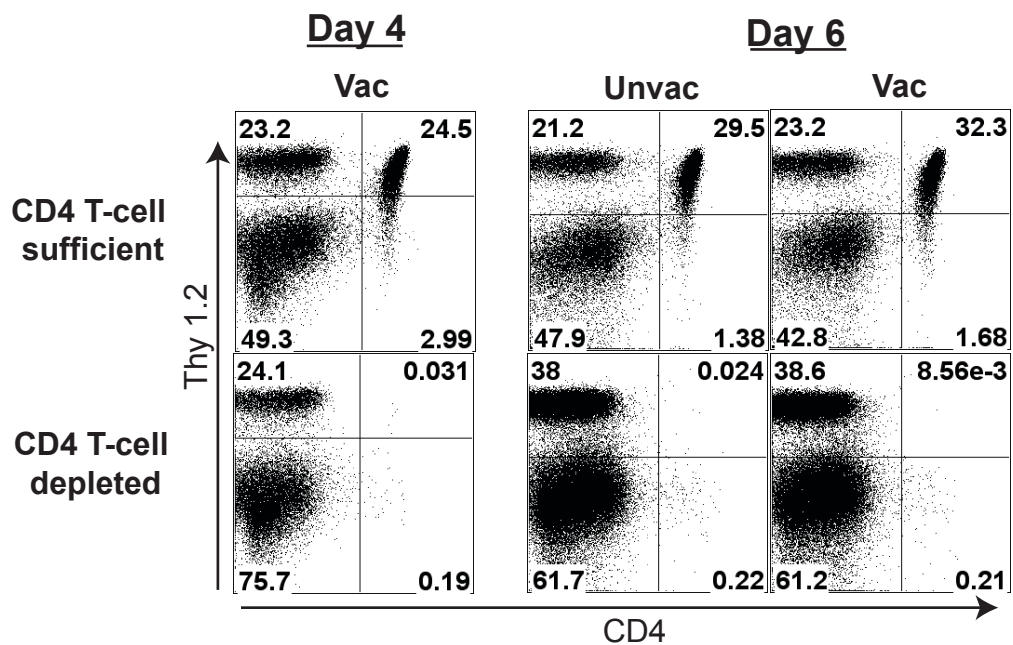


Supplementary Figure 1.

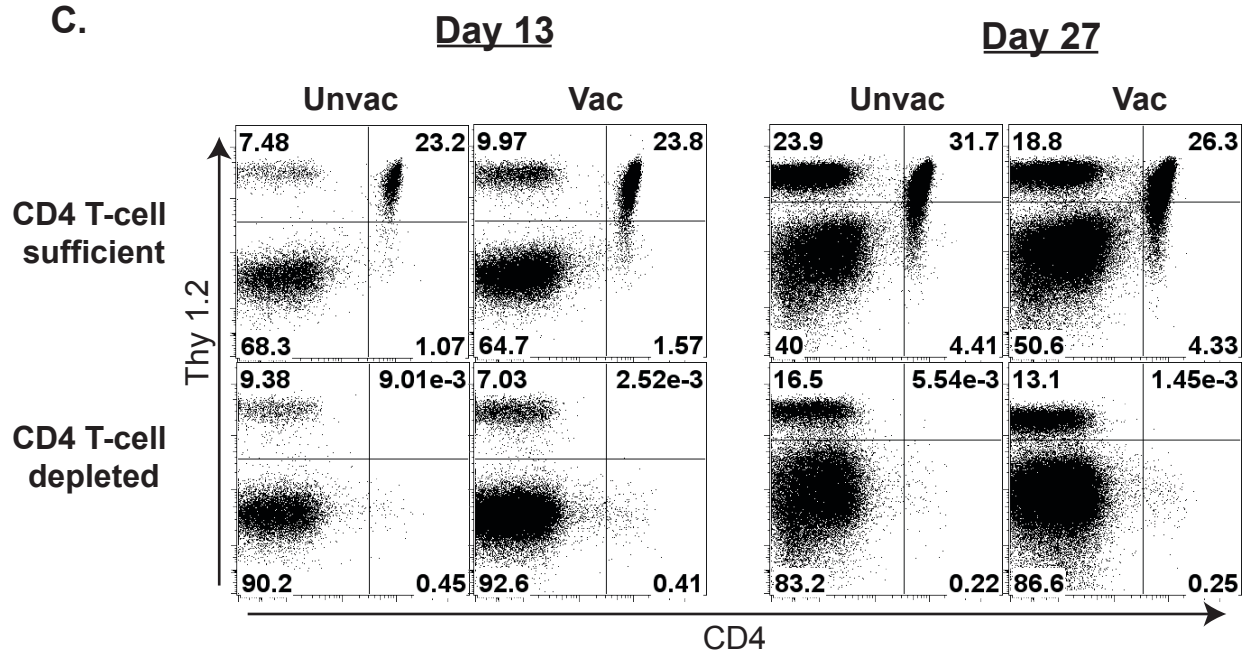
A.



B.

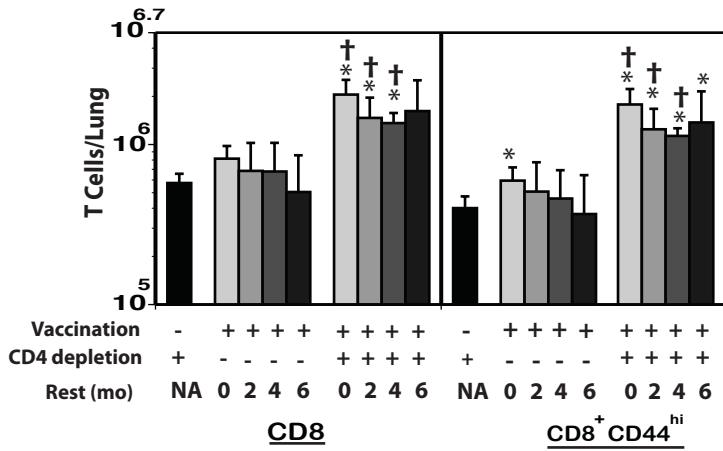


C.

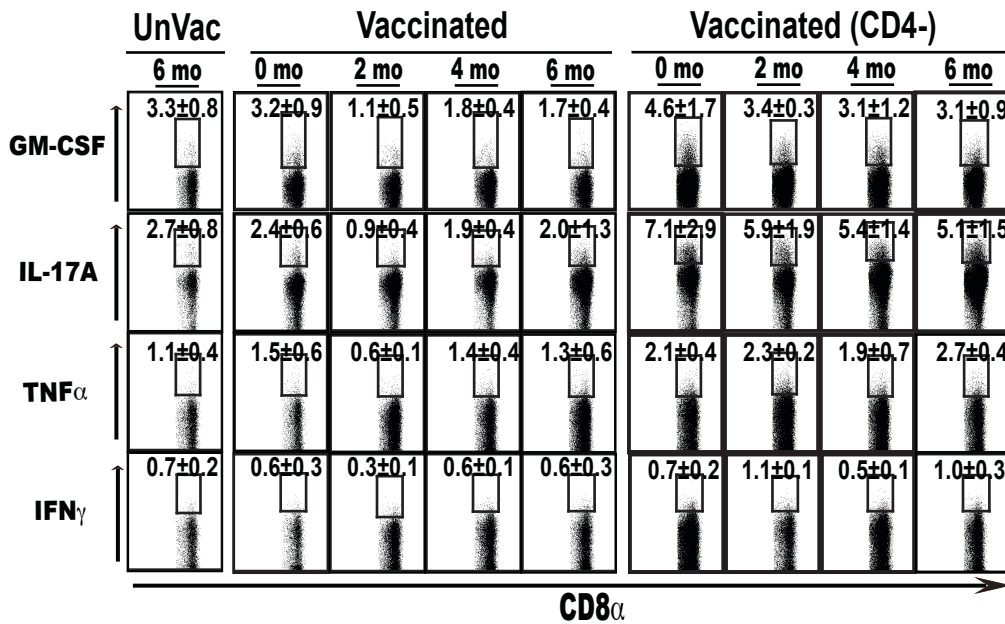


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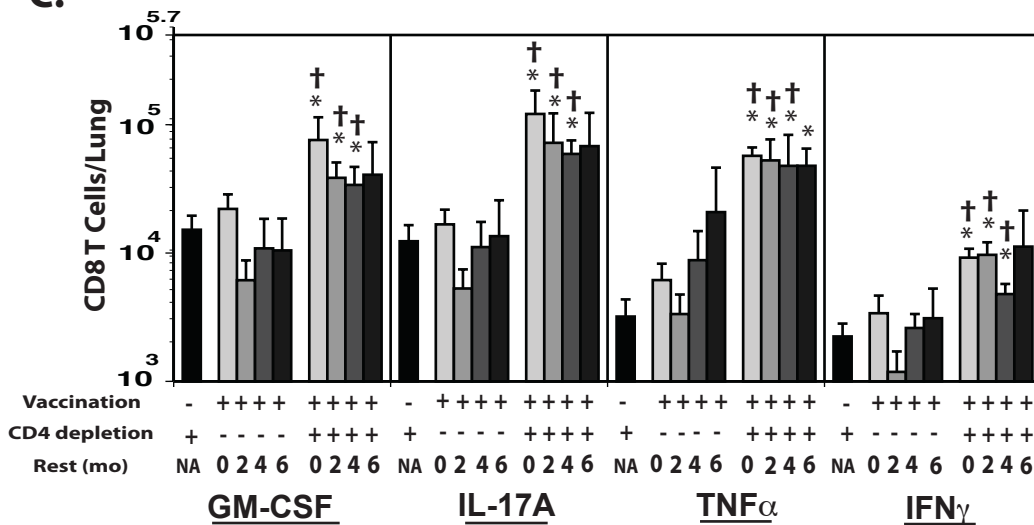
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B.

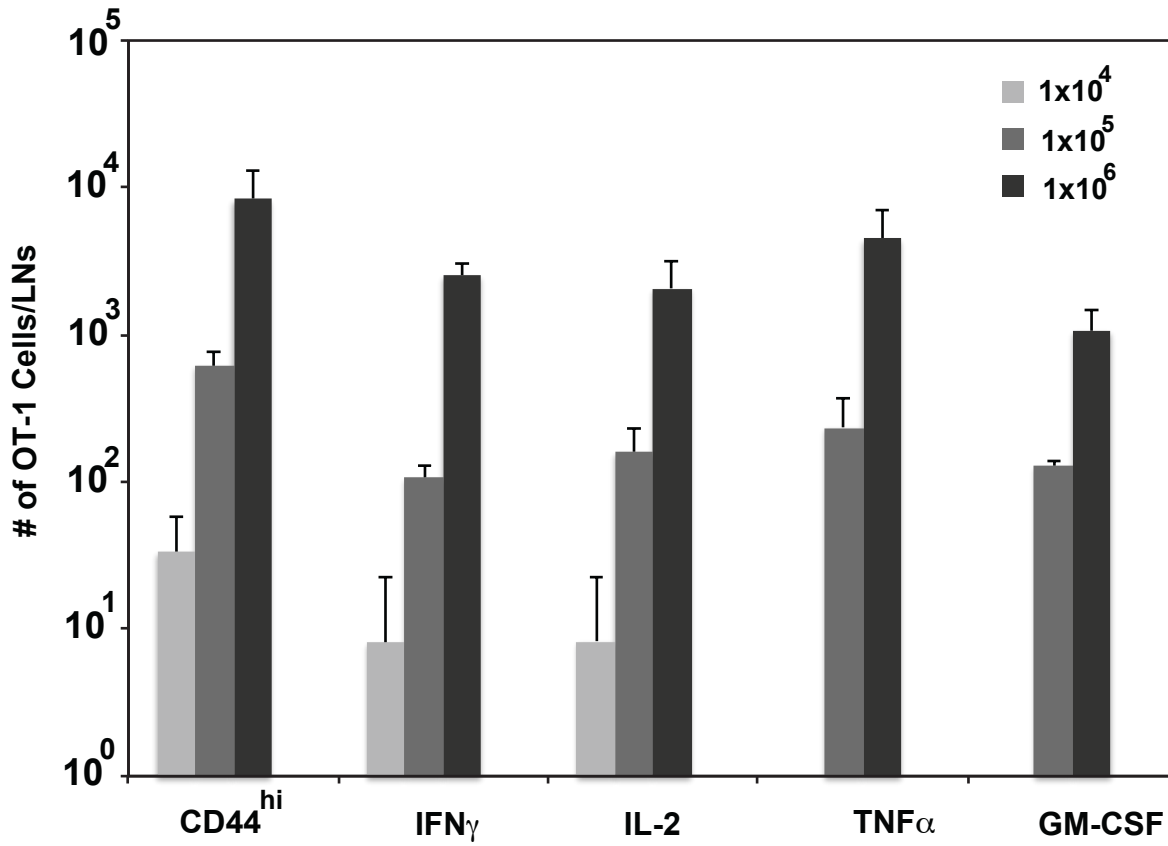


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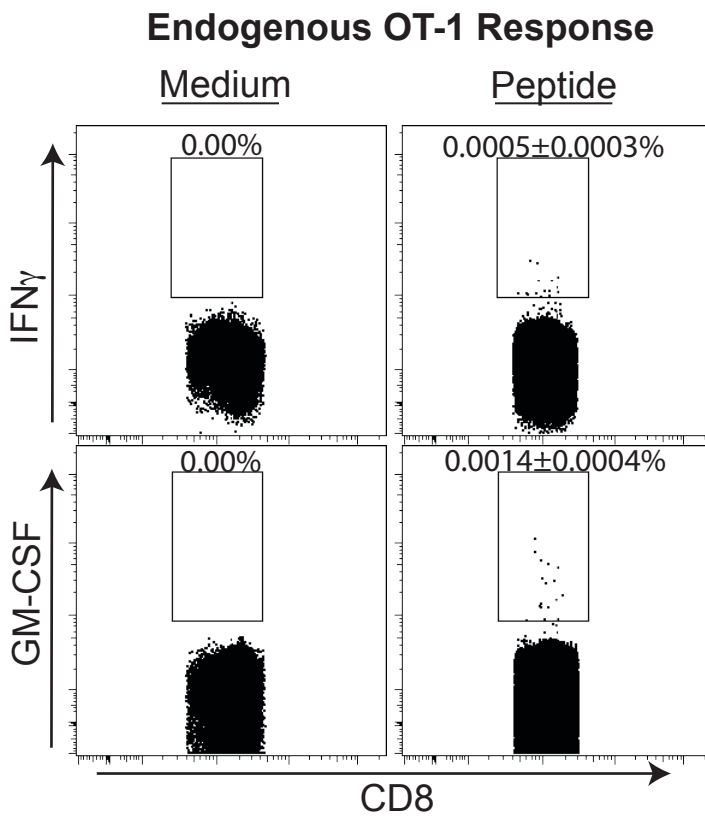


Supplemental Figure 3.

A.



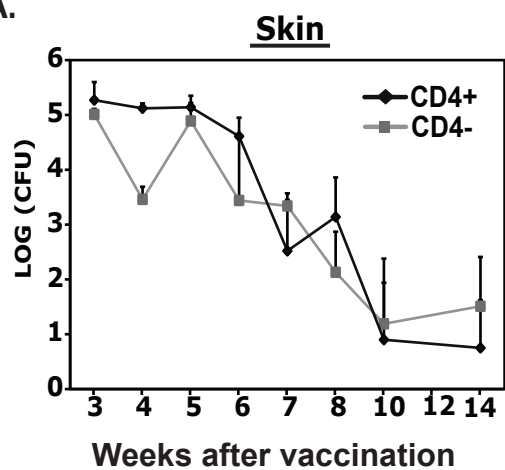
B.



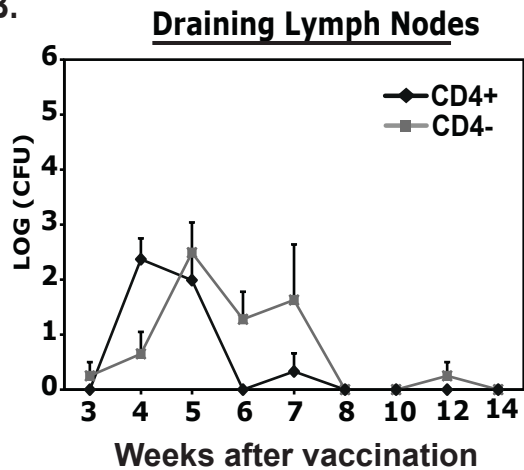
Supplementary Figure 4.

Live vaccine persistence

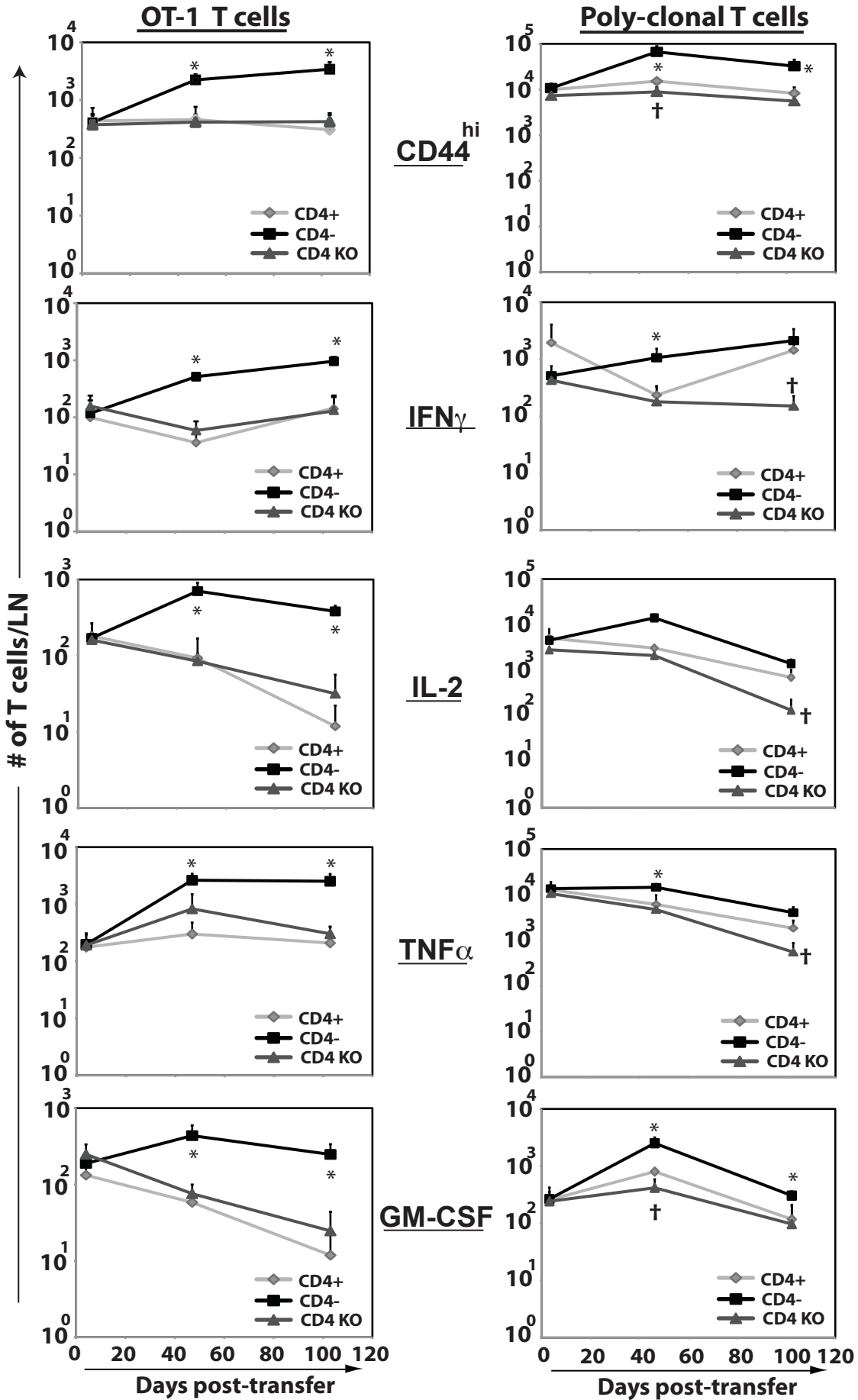
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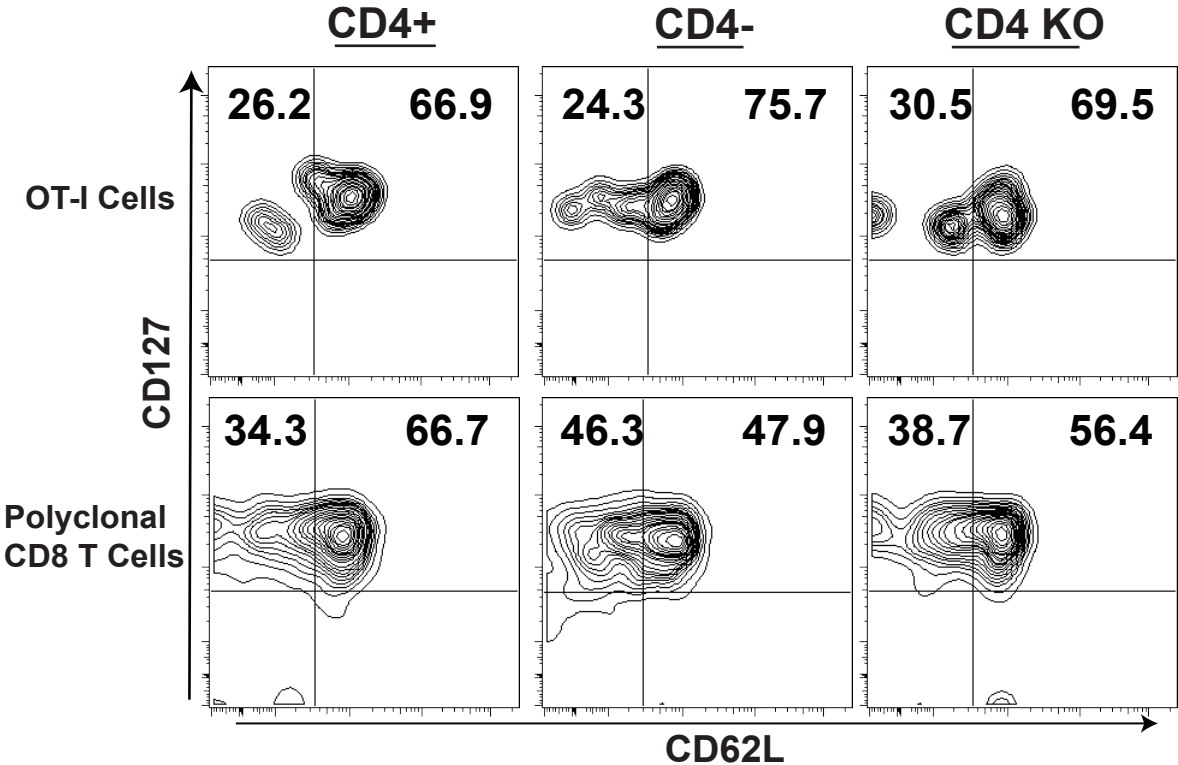
Supplementary Figure 5.



Supplementary Figure 6.

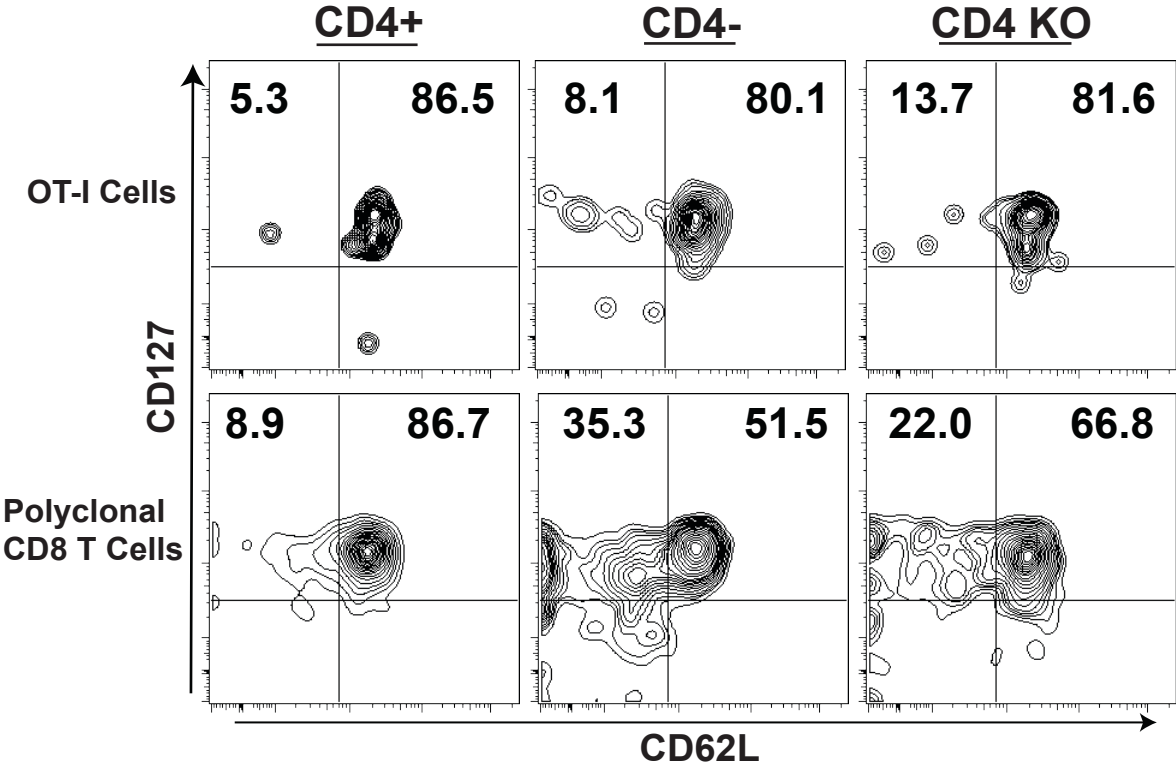
A.

T cells in LNs (Day 1 post-transfer)



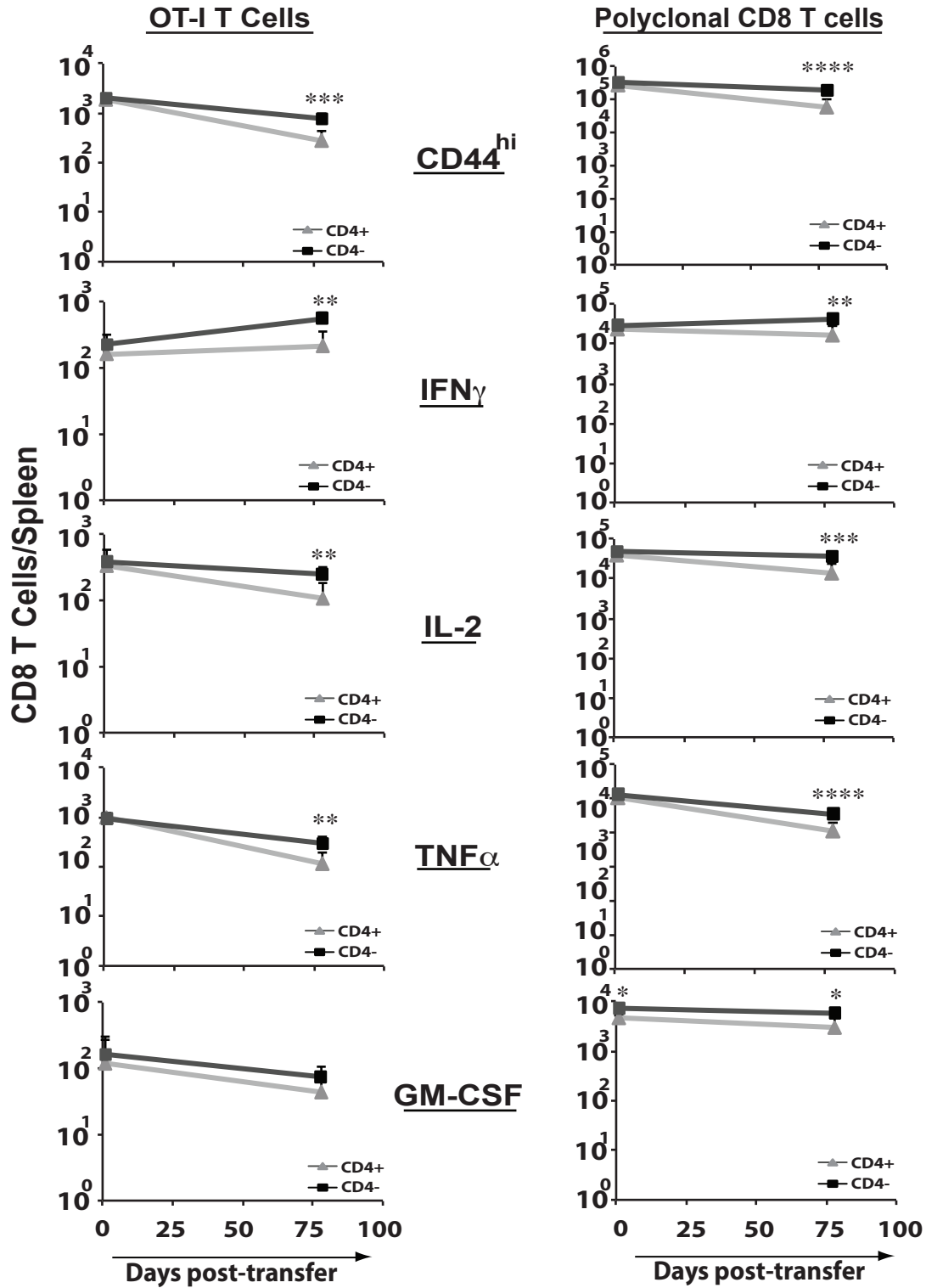
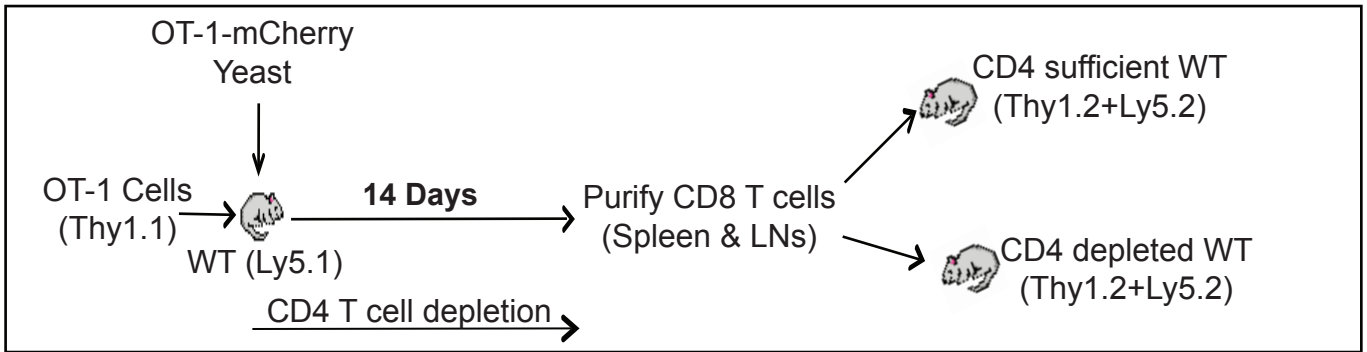
B.

T cells in LNs (Day 100 post-transfer)



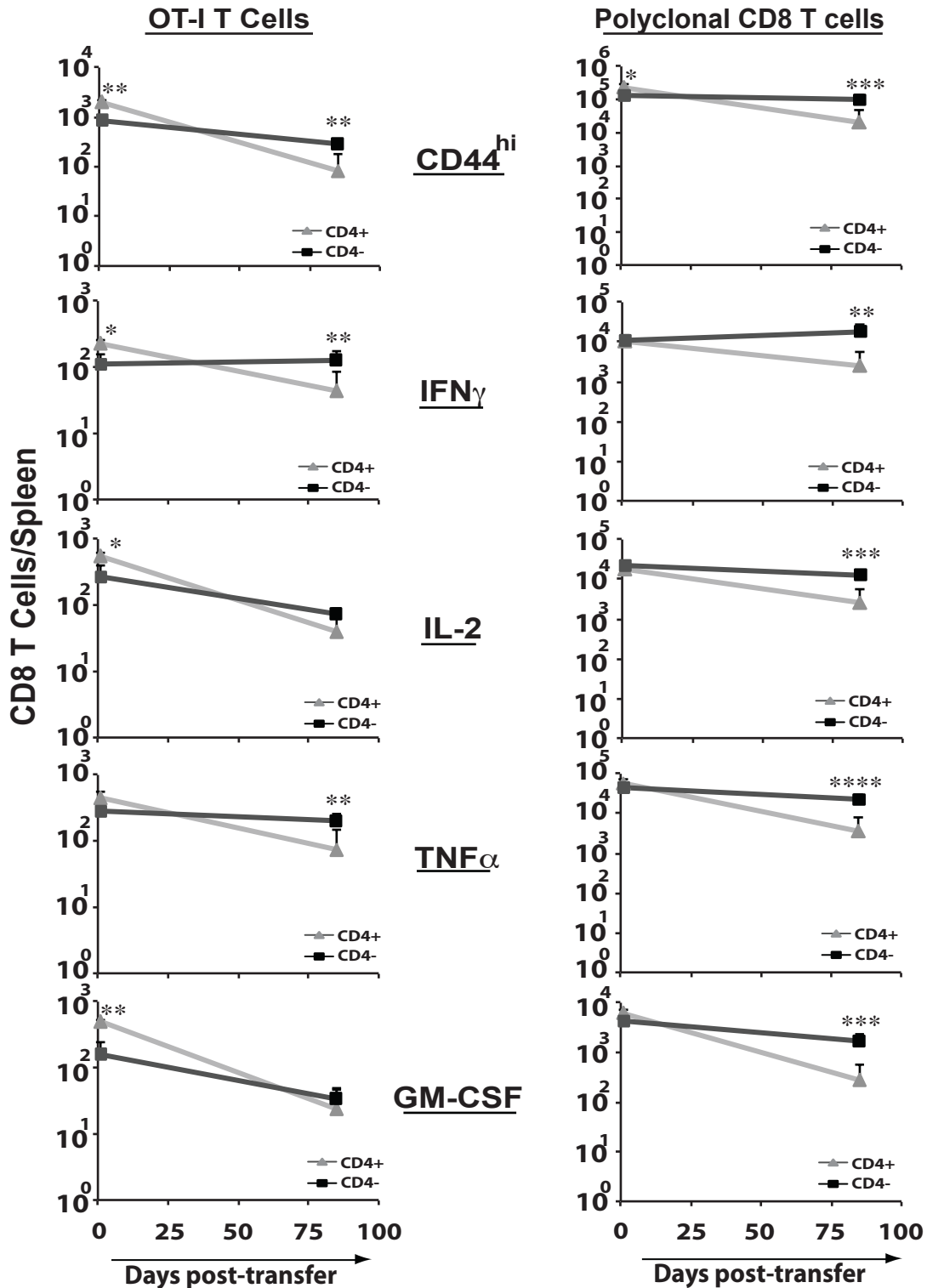
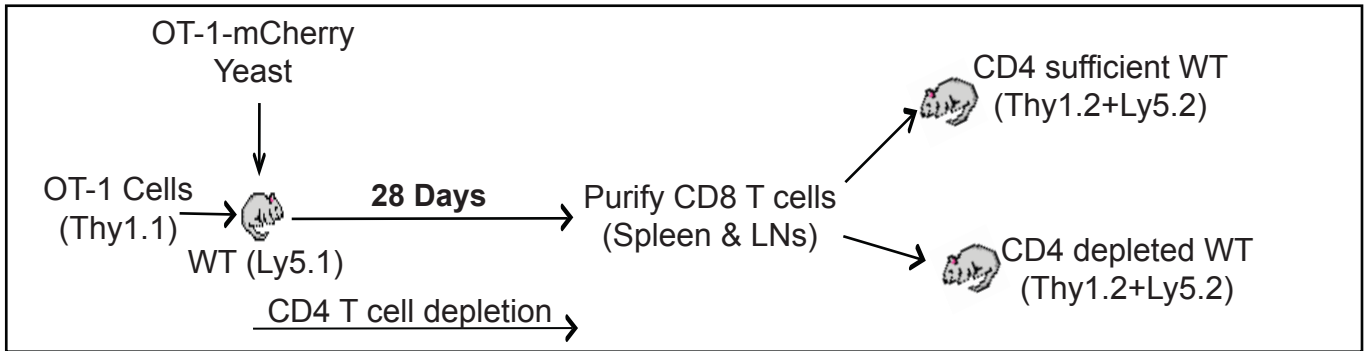
Supplementary Figure 7A.

Experimental outline:



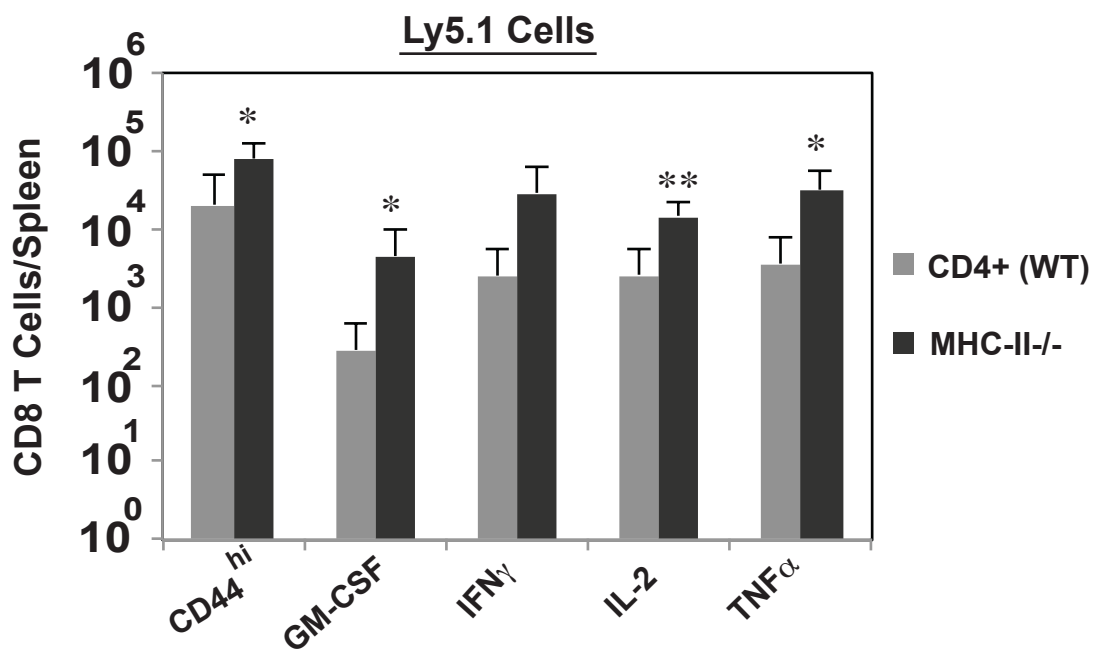
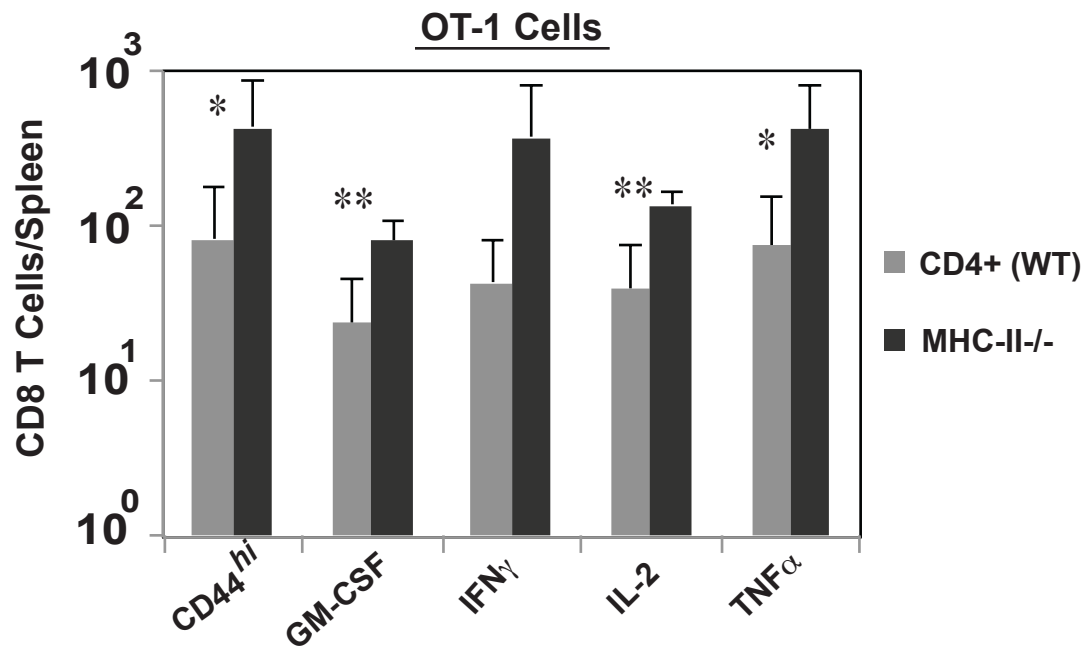
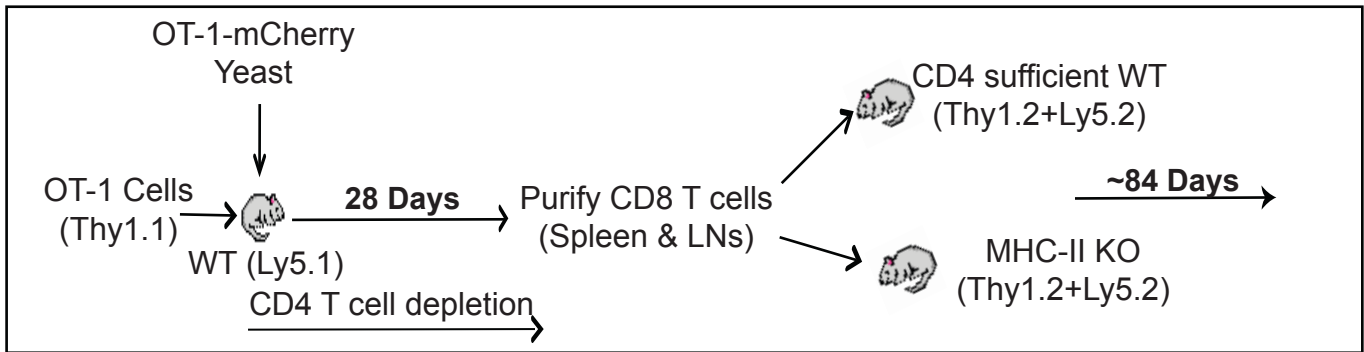
Supplementary Figure 7B.

Experimental outline:



Supplemental Figure 7C.

Experimental outline:



Supplemental figure legends

Fig. S1: Efficiency of CD4 T-cell depletion.

(A) Schematic diagram of the experimental design. (B & C) Unvaccinated and vaccinated mice were injected intravenously with a weekly dose of 100µg of anti-CD4 (clone GK1.5) antibody. On indicated days, spleens and LNs were harvested to assess the efficiency of CD4 T cell depletion by flow cytometry. Dot plots show Thy1.2⁺ CD4 T cells in the spleens.

Fig. S2: Maintenance of protective anti-fungal memory CD8 T cells in the absence of CD4 T cell help.

Mice were vaccinated and rested as described in figure 1, except that they were vaccinated at different ages so that they were the same age at the time of experimental infection. Lungs were harvested 4 days after challenge and cells were stained using anti-CD8, anti-CD44, anti-CD62L, anti-CD127, anti-CD122 and anti-CD27 antibodies. (A) Total number of lung CD8 T cells and CD44^{hi} CD8 T cells. (B&C) Cytokine expression. Lung CD8 T cells were re-stimulated *ex vivo* with anti-CD3 and anti-CD28 antibodies for 5 hours in the presence of Golgi stop. Intracellular IFN- γ , IL-17A, GM-CSF and TNF- α was measured as described in Methods. Percentage (B) and total number (C) of cytokine-producing CD8 T cells in different groups of mice were enumerated by flow cytometry. Results are expressed as mean \pm SD of 4-5 mice/group. $p \leq 0.05$ is denoted by (*) for vaccinated vs. unvaccinated groups; and by (†) for vaccinated CD4-depleted (CD4⁻) vs. CD4-sufficient mice.

Fig. S3: Impact of precursor frequency on OT-I T-cell responses following vaccination.

(A) Indicated numbers of Thy1.1^{+ve} OT-I cells were adoptively transferred into naïve Thy1.2^{+ve} mice. GK1.5 antibody was used weekly to deplete CD4 T cells. Mice were vaccinated with 1x10⁶ cfu SIINFEKL-expressing yeast. On day 15, draining LNs were harvested for analysis. Data show the numbers of activated (CD44^{hi}) and cytokine producing OT-I T cells. Data are mean values ± SD of 4 mice per group. (B) Endogenous OVA-specific CD8 T cell responses to recombinant yeast. Naïve 7-8 wk old mice were treated weekly with GK1.5 and vaccinated with SIINFEKL-expressing yeast. At day 15, draining LNs were harvested and re-stimulated *ex-vivo* with OVA (SIINFEKL) peptide to assess antigen-specific CD8 T cell responses. Dot plots show frequency of cytokine-producing cells among CD8 T cells. N=4 mice.

Fig. S4: Persistence of live yeast after vaccine administration.

Following vaccination, the skin sites of vaccine administration (A) and the draining LNs (B) were collected at serial time points to assess residual live vaccine yeast by CFU enumeration.

Fig. S5: Long-term maintenance of antigen-specific anti-fungal memory CD8 T cells in the absence of vaccine antigen and CD4 T-cell help.

Persistence of antigen-specific anti-fungal memory CD8 T cells in draining LNs. A flow diagram of the experimental design is shown in figure 5A. Thy1.1^{+ve} OT-I Tg T cells (~1x10⁶) were adoptively transferred into naïve Ly5.1^{+ve} congenic mice. A day later mice were vaccinated s.c. with SIINFEKL expressing *B. dermatitidis* yeast (10⁶ cfu), as shown. CD4 T cells were depleted using GK1.5 antibody throughout vaccination. On day 28 post-vaccination, spleens and draining LNs were collected and CD8 T cells were purified using autoMACS by negative

enrichment. 0.88×10^5 OT-I T cells along with endogenous $\text{Ly5.1}^+ \text{CD44}^{\text{hi}}$ (1.42×10^6) T cells were transferred into groups of naïve $\text{Ly5.2}^+ \text{Thy1.2}^+$ congenic mice: CD4-sufficient (CD4^+), CD4-depleted (CD4^-) and $\text{CD4}^{-/-}$ (CD4 KO). At serial times, (1, 44 and 100 days post-transfer), draining LNs were collected to enumerate activated (CD44^{hi}) and cytokine producing- OT-I and polyclonal CD8 T cells by flow cytometry. Data are mean values \pm SD from 4 mice per group. $p \leq 0.05$ is denoted by (*) for CD4^- vs. CD4^+ groups; and by (†) for CD4 KO vs. CD4^+ groups.

Fig. S6: Phenotypic attributes of antigen specific anti-fungal memory CD8 T cells maintained in the absence of antigen and CD4 T-cell help.

At day 1 (A) and day 100 (B) following cell transfers described in figure S3, lymph node cells were directly surface stained with anti-Thy1.1, anti-CD8, anti-Ly5.1, anti-CD127 and anti-CD62L antibodies and analyzed by flow cytometry. Plots are gated on $\text{CD44}^{\text{hi}} \text{Thy1.1}^+ / \text{Ly5.1}^+$ CD8 T cells. Numbers in the plots are percentages. N=3-4 mice/group.

Fig. S7: Long-term maintenance of antigen-specific anti-fungal CD8 T cells.

$\text{Thy1.1}^{+\text{ve}}$ OT-I cells ($\sim 1 \times 10^6$) were adoptively transferred into naïve $\text{Ly5.1}^{+\text{ve}}$ mice. Mice were treated with GK1.5 antibody to deplete CD4 T cells and vaccinated with SIINFEKL-expressing yeast ($\sim 1 \times 10^6$). At day 14 (A) or day 28 (B & C), spleens and LNs were harvested, and CD8 T cells were purified by autoMACS, and adoptively transferred into groups of naïve $\text{Thy1.2}^{+\text{ve}}$ $\text{Ly5.2}^{+\text{ve}}$ congenic mice: CD4 T cell sufficient (CD4^+), CD4 T cell depleted (CD4^-) or Class II deficient ($\text{MHC-II}^{-/-}$). At days 1 and 78 (A) or 85 (B & C), spleens were collected to enumerate activated (CD44^{hi}) and cytokine-producing OT-I and polyclonal CD8 T cells by flow cytometry.

Data are mean values \pm SD from 4-7 mice per group. Statistical significance was defined at $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***) and $p \leq 0.0001$ (****) vs. the respective control group.