

SUPPLEMENTAL INFORMATION

FIGURE LEGENDS

Supplemental Figure 1. Characterization of the memory subsets prior to co-transfer. The phenotype of the memory subsets was examined by flow cytometry prior to co-transfer. **(A)** Comparison of the indicated cell surface markers for the HP- and true-memory cells from pooled spleen and lymph nodes. Representative of 5 or more experiments (n=2-6).

Supplemental Figure 2. Competition between HP- and true-memory cells is observed in alternate infection systems and is not TRAIL dependent. Co-transferred populations were identified by congenic markers. The percentage of donor cells among total CD8⁺ cells in the PBL were measured. **(A)** HP- and true-memory cells generated as described in Fig. 1 were co-transferred into B6 hosts followed by infection with VSV.OVA. **(B)** HP- and true-memory P14 cells were co-transferred into a CD45.1 host followed by LCMV infection. **(C)** TRAIL-KO HP- (CD45.2) and true-memory (CD45.1.2) OT-I were co-transferred into CD45.1 hosts followed by infection with Lm.OVA. Representative of 2 or more experiments (n=3). Error bars indicate SD.

Supplemental Figure 3. Comparison of the HP- and true-memory cell death and proliferation rates following co-transfer and infection. BrdU incorporation, Annexin-V staining, and TUNEL staining were measured for the memory subsets in the co-transfer experiment described in Fig. 1. The percentage of donor cells that were: **(A)** BrdU⁺ in the spleen, **(B)** BrdU⁺ in the lymph nodes, **(C)** TUNEL⁺ in the spleen, **(D)** TUNEL⁺ in the lymph nodes, **(E)** Annexin-V⁺ in the spleen, **(F)** Annexin-V⁺ in the lymph nodes. Representative of 5 or more experiments. Error bars indicate SD.

Supplemental Figure 4. Phenotype of the co-transferred memory subsets on day 6 after infection.

Phenotype of memory subsets from co-transfer recipients on day 6 of Lm.OVA infection. Comparison of indicated cell surface markers in the **(A)** spleen **(B)** lymph nodes. Representative of 5 or more experiments (n=3-5).

Supplemental Figure 5. Timecourse of HP- and true-memory cell localization following co-transfer.

HP- and true-memory were co-transferred into B6 hosts and infected with Lm.OVA. Localization of donor cells in the spleen on days 1-10 after infection was evaluated by staining for CD45.1, Thy1.1, and B220. Representative of 5 or more experiments (n=3).

Supplemental Figure 6. Localization of the HP- or true-memory subsets following single transfers.

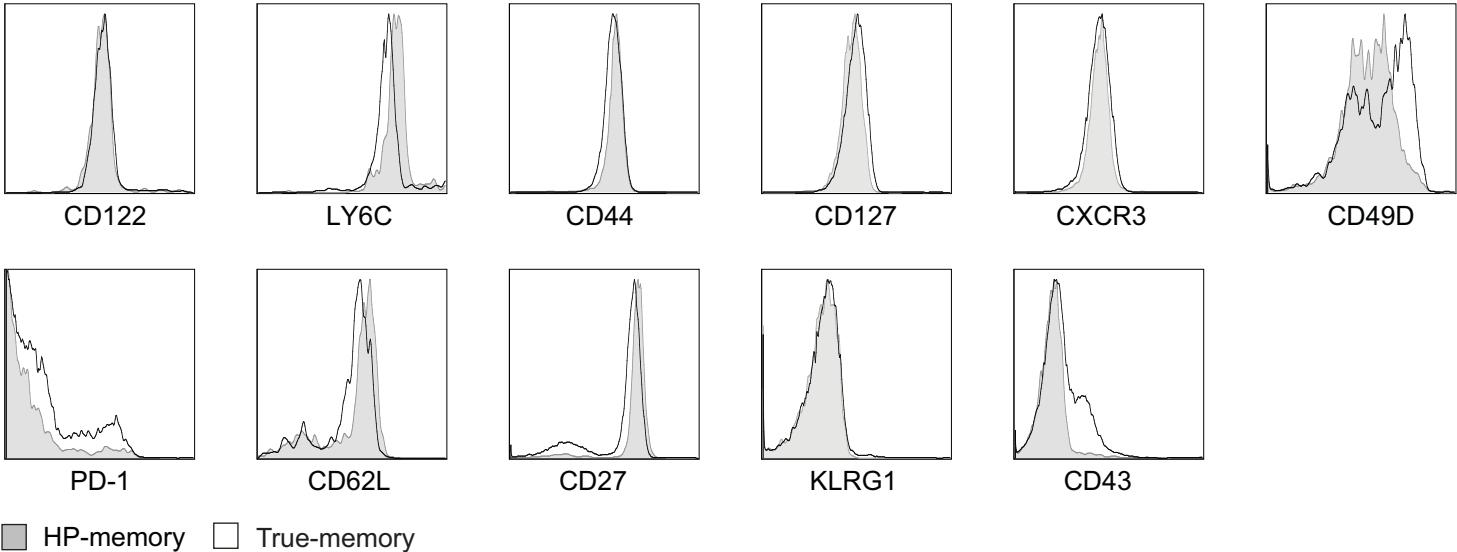
HP- or-true memory cells were transferred separately into B6 hosts as shown in Fig 1A. Localization of donor cells in the spleen was evaluated by staining for CD45.1, CD4 and B220. **(A)** Single transfer of HP-memory cells on day 6 of infection. **(B)** Single transfer of true-memory cells on day 5 of infection. Images are taken at 20X and are representative of 2 or more experiments (n = 2-3).

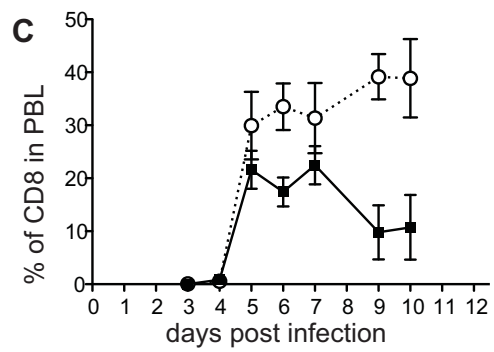
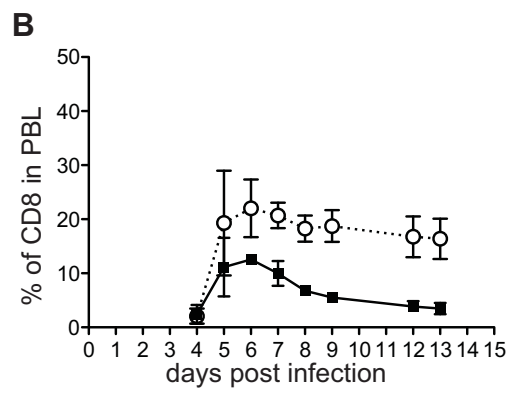
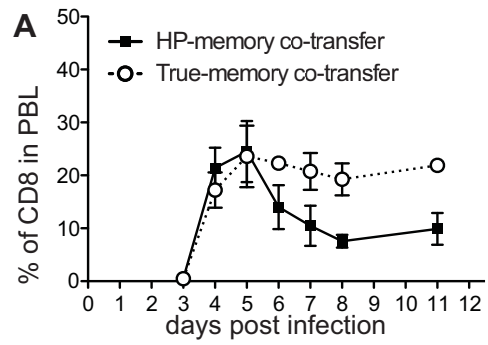
Supplemental Figure 7. Chemokine receptor mRNA expression and chemotaxis for memory subsets.

(A) Expression of CCR7 and CXCR5 mRNA by memory subsets from single or co-transfers. Memory subsets were sorted from indicated transfers on day 6 of infection. Relative expression of CCR7 and CXCR5 mRNA are shown compared to the true-memory single transfer population. **(B)** Percent of absolute cell numbers for each memory subset that migrated in response

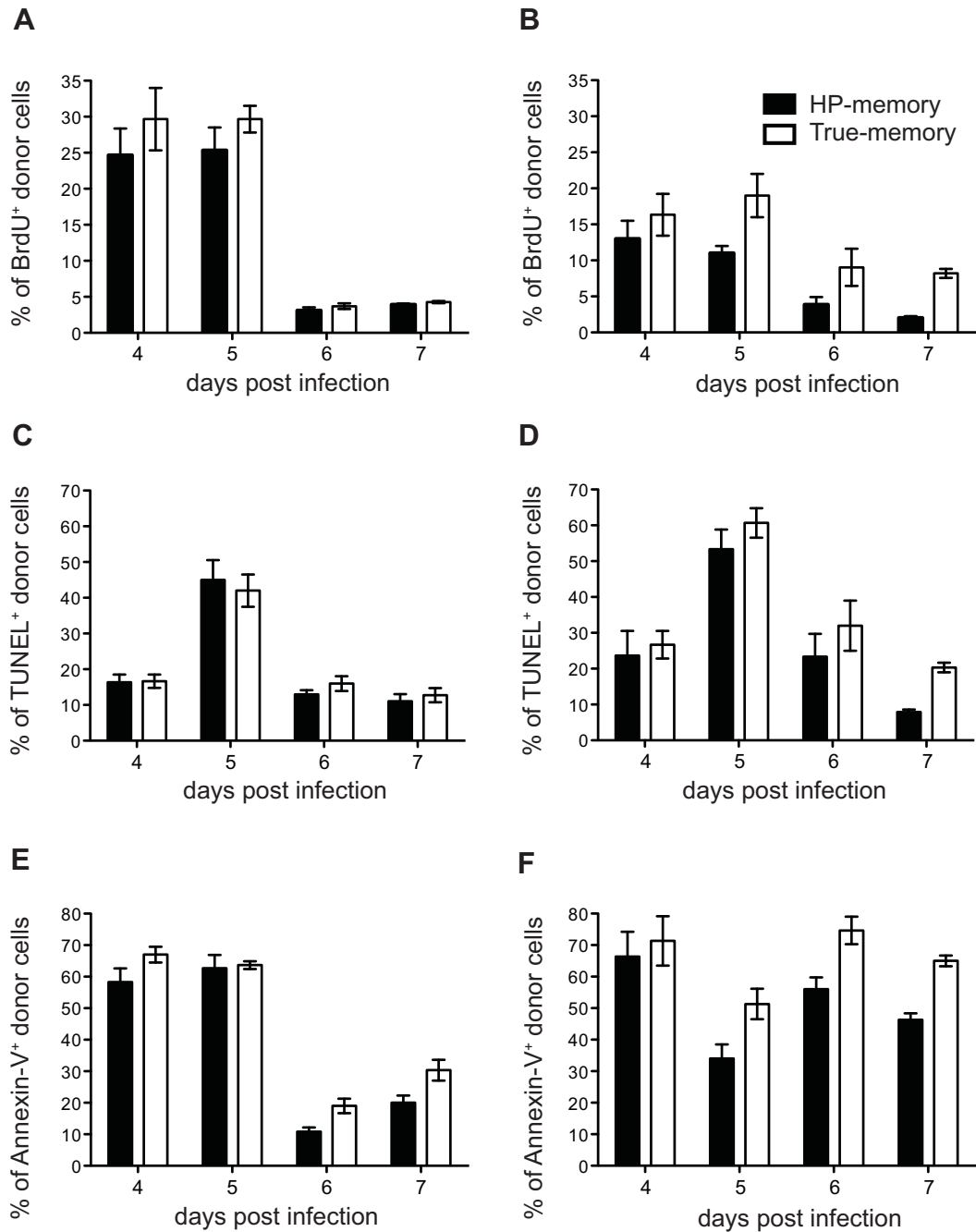
to CCL19 (1 $\mu\text{g/mL}$), CCL21 (3 $\mu\text{g/mL}$) in 3 hr at 37°C: HP-memory single transfer, true-memory single transfer, HP-memory co-transfer and true-memory co-transfer.

A

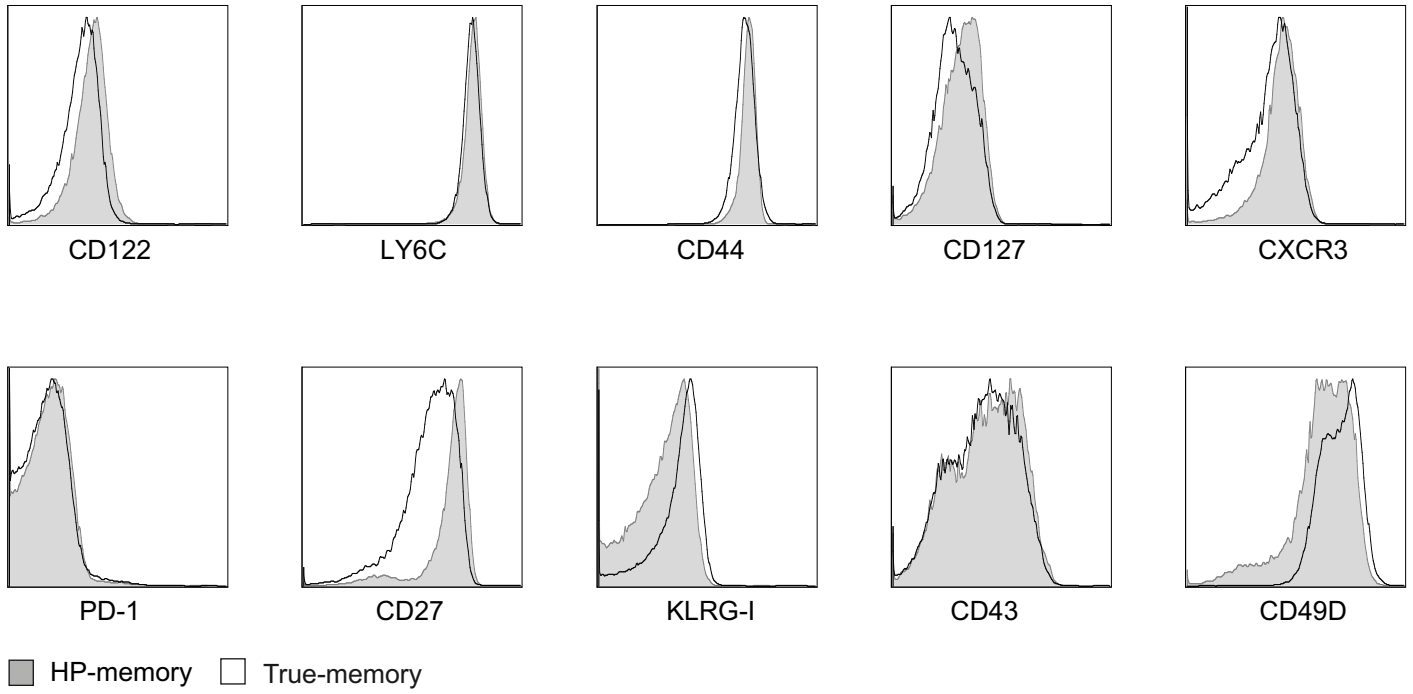




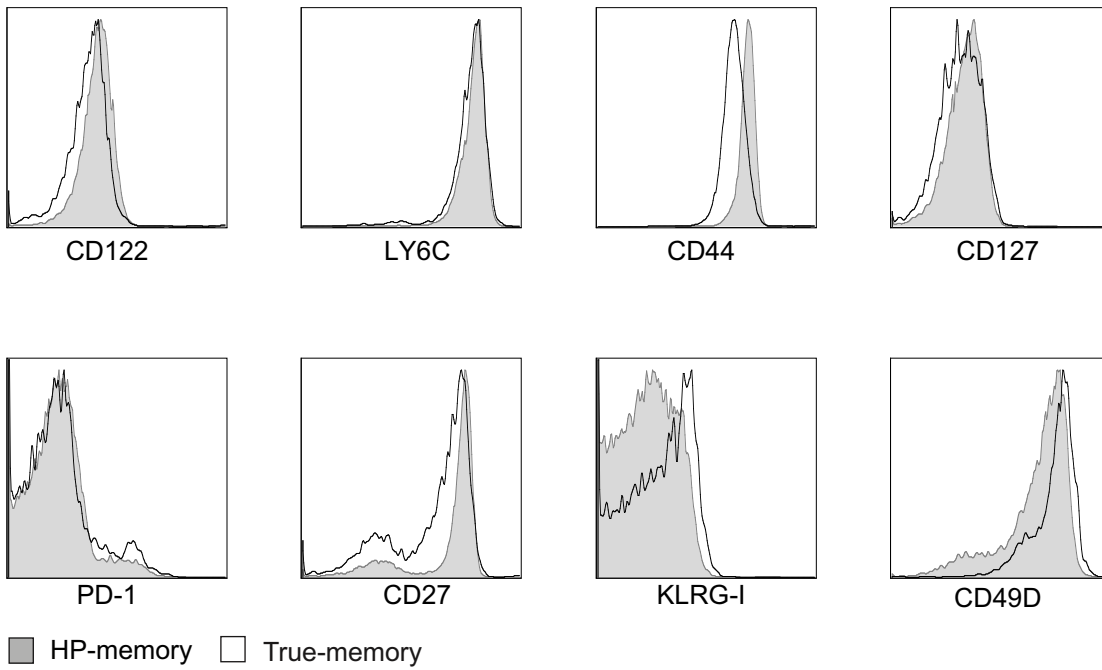
Supplemental Figure 3, Cheung et al.



A

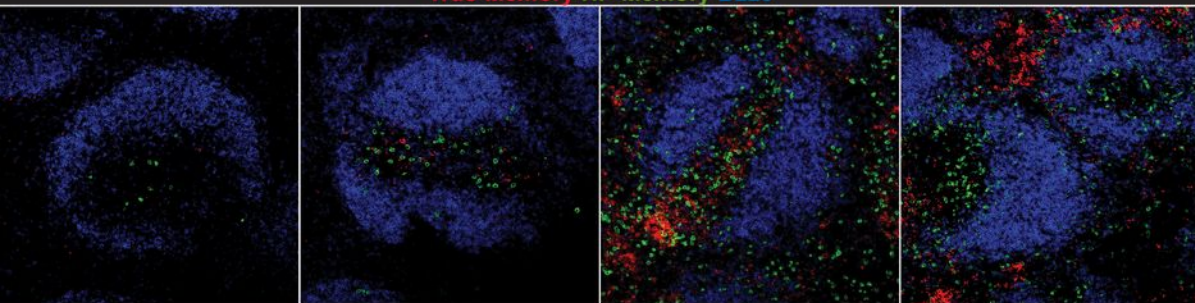


B



Supplemental Figure 5, Cheung et al.

True-Memory HP-Memory B220

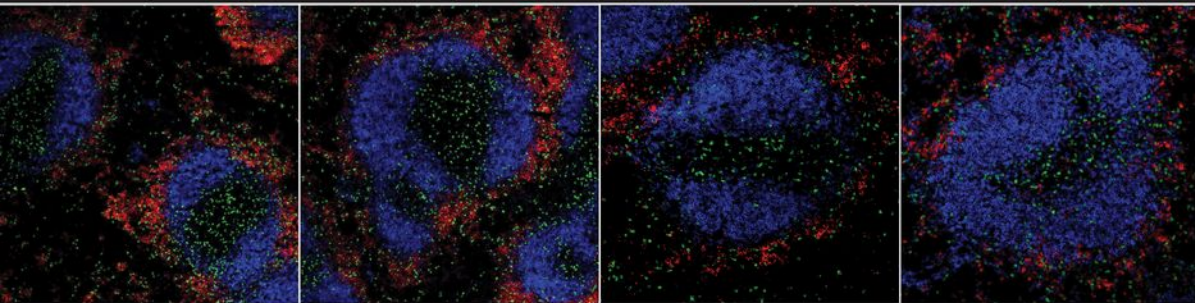


Day 1

Day 2

Day 3

Day 4



Day 5

Day 6

Day 7

Day 10

Supplemental Figure 6, Cheung et al.

