Supplemental Information

Memory CD4$^+$T-cells protect against influenza through multiple synergizing mechanisms

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Supplemental Table I: Viral escape mutants emerge in the absence of host CD8+ T cell and B cells

Viral Isolate	Peptide Sequence [a]
Native PR8 $HA_{126-138}$	H N T N G V T A A C S H E
Control PR8	H N T N G V T A A C S H E
3.1	H N T N G V T A A C **x** H E
4.1	H N T N G V T A A C **T** H E
8.1	H N T N G V T A A C **P** H E
9.2	H N T N G V T A **T** C S H E
3.2	H N T **S** G V T A A C S H E
9.2	H N T N G V T A **S** C S H E
11.2	H N T **K** G V T A A C S H E
12.2	H N T N G V T A A **A** S H E
13.2	H N T N G **G** T A A C S H E
15.2	H N T N G V T **C** A C S H E
4.3[b]	H N T N G V T A A C **P** H E
Native $OVA_{323-339}$	I S Q A V H A A H A E I N E A G R
Control PR8-OVA_{II}	I S Q A V H A A H A E I N E A G R
1.1	I S Q A V H A **T** H A E I N E A G R
2.1	I S Q - - - - - - - - - - - - - - - - - - G R
3.1	I S Q A V H A **T** H A E I N E A G R
4.1	I S Q A V H A A H A E I N E **T** G R

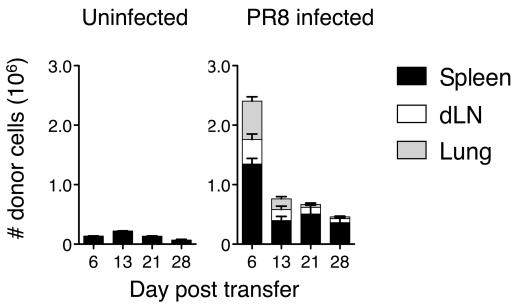
[a] Nucleotide mutations and their corresponding translated aa location are underlined. Aa mutations are in bold blue text, a deletion is demarked as a dash (-), one silent mutation is simply underlined, and one unresolved aa is demarked as x.

[b] Mutant viral isolate obtained from recipient of *Prf1*$^{-/-}$ HNT CD4 T cells.

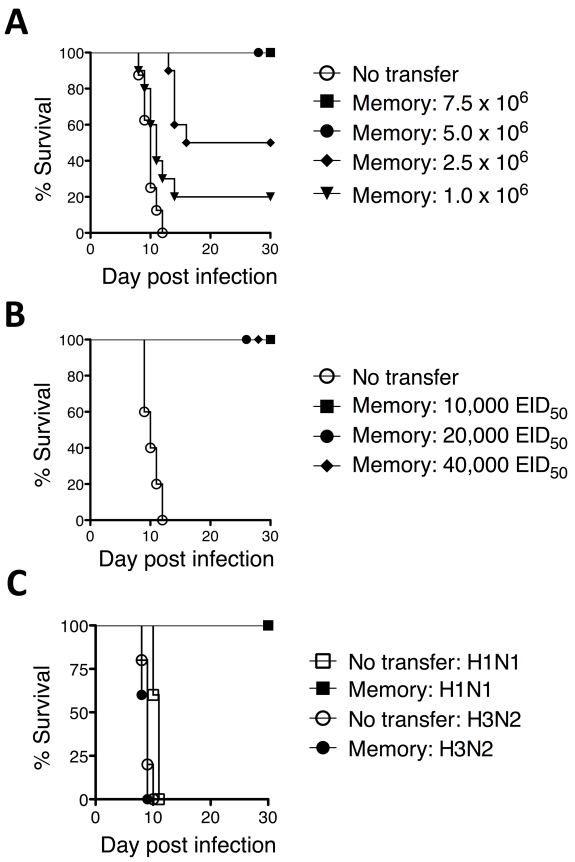
Supplemental Table II: Viral escape mutants emerge in *Rag*$^{-/-}$ recipients of T_H1-polarized memory OT-II CD4$^+$ TCR Tg cells challenged with PR8-OVA_{II} virus

Viral Isolate	Peptide Sequence [a]
Native $OVA_{323-339}$	I S Q A V H A A H A E I N E A G R
Control PR8-OVA_{II}	I S Q A V H A A H A E I N E A G R
1.1	I S Q A V H A **A** H **E** E I N E A G R
2.1	I S Q A V H A A **T** **T** E I N E A G R
3.1	I S Q A V H A A H A E I N E **T** G R
4.1	I S Q A V H A A H A E I N E **T** G R
5.1	I S Q A V H A A H A E I N E **T** G R
6.1	I **Y** Q A V H A **T** H - E I N E A G R
7.1	I S Q A V H A A H A E **T** N E A G R
8.1	I S Q A V H A A H A E I N E **T** G R
9.1	I S Q A V H A A H A E **F** N E A G R
10.1	I - Q - V H A A H A E - N E A G R

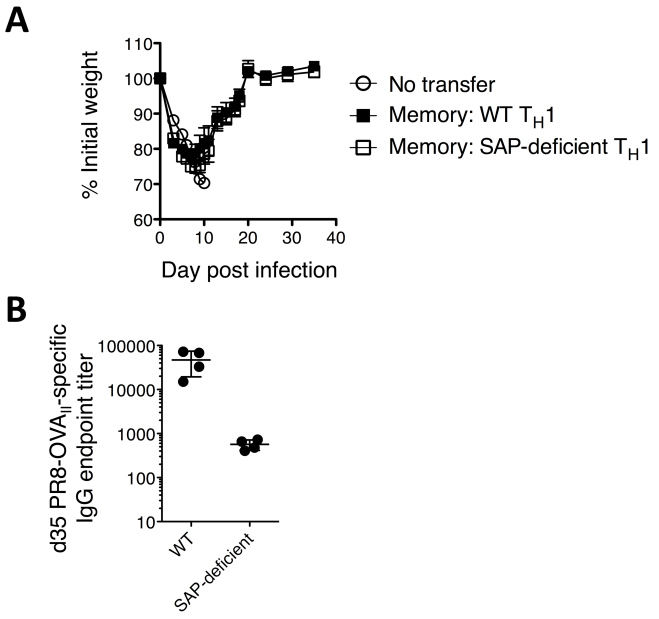
[a] Nucleotide mutations and their corresponding translated aa location are underlined. Aa mutations are in bold blue text, a deletion is demarked as a dash (-). No mutations were detection in the HNT peptide aa sequence ($HA_{126-139}$) in any isolates.



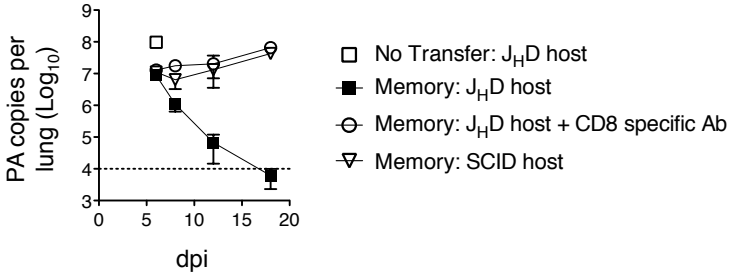
Supplemental Figure 1: Number of HNT cells present post PR8 priming. $1x10^6$ naive HNT cells were transferred to Thy-disparate WT hosts. Mice were either left uninfected (left panel) or challenged with 1,000 EID_{50} PR8 (right panel). On stated days post-infection, the absolute number of donor cells present in the spleen, draining mediastinal lymph nodes (dLN), and lung were determined from groups of 3 mice/day.



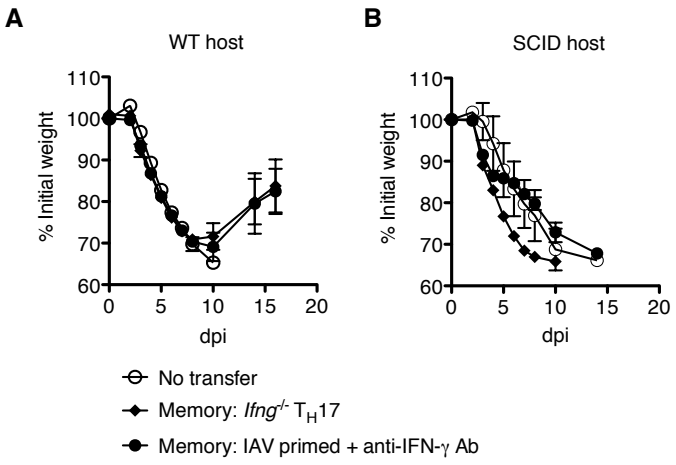
Supplemental Figure 2: Protective impact of memory $CD4^+$ T cells against lethal viral doses is dependent on cell number and is antigen-specific. **(A)** Stated numbers of T_H1-polarized *in vitro*-generated memory HNT cells were transferred to WT hosts then infected with 10,000 EID_{50} PR8. Survival from groups of at least 5 mice is shown. **(B)** WT mice received $5x10^6$ memory HNT cells and were challenged with stated doses of PR8 or **(C)** infected with a lethal dose of either PR8 (H1N1) or A/Philippines/2/82/x-79 (H3N2). Survival compared to mice not receiving memory HNT cells is shown (n=5/group). These results are representative of 3 separate independent experiments.



Supplemental Figure 3: Memory $CD4^+$ T cell synergy with B cells is independent of SAP-dependent helper functions. C57BL/6 nude mice received $5x10^6$ WT or SAP-deficient OT-II memory cells and were challenged with a lethal dose of either PR8-OVA_{II}. **(a)** Weight loss, n=5/group and **(b)** mean PR8-OVA_{II}-specific total IgG endpoint titers (horizontal bars) are shown (n=4/group (dots)). These results are representative of 2 separate independent experiments.



Supplemental Figure 4: Viral titers in different host mice receiving memory $CD4^+$ T cells. $5x10^6$ WT HNT memory cells were transferred to J_HD mice, J_HD mice depleted of CD8 T cells by Ab treatment as in Figure 5A and B (J_HD host + CD8 specific Ab) or SCID mice. All mice were challenged with 2,500 EID_{50} PR8 and viral titers determined on indicated days as described in Methods (n=3/group/day).



Supplemental Figure 5: T_H17-polarized and IAV-primed memory $CD4^+$ T cells do not protect SCID mice in the absence of IFN-γ. $5x10^6$ T_H17-polarized memory HNT cells generated from *Ifng*$^{-/-}$ precursors or $5x10^6$ IAV-primed WT memory HNT cells were transferred to **(A)** WT or **(B)** SCID hosts (4 mice/group). Control mice (No transfer) received no cells. Recipients of IAV primed memory cells also received anti-IFN-γ Ab throughout infection. WT hosts challenged with 10,000 EID_{50} PR8 were fully protected by both memory populations until the experiment was terminated while both populations failed to protect SCID hosts challenged with 2,500 EID_{50} PR8 (all mice succumbed by 15 dpi).