Supplemental Information

for

Direct IL-6 signals maximize protective secondary CD4 T cell responses against influenza

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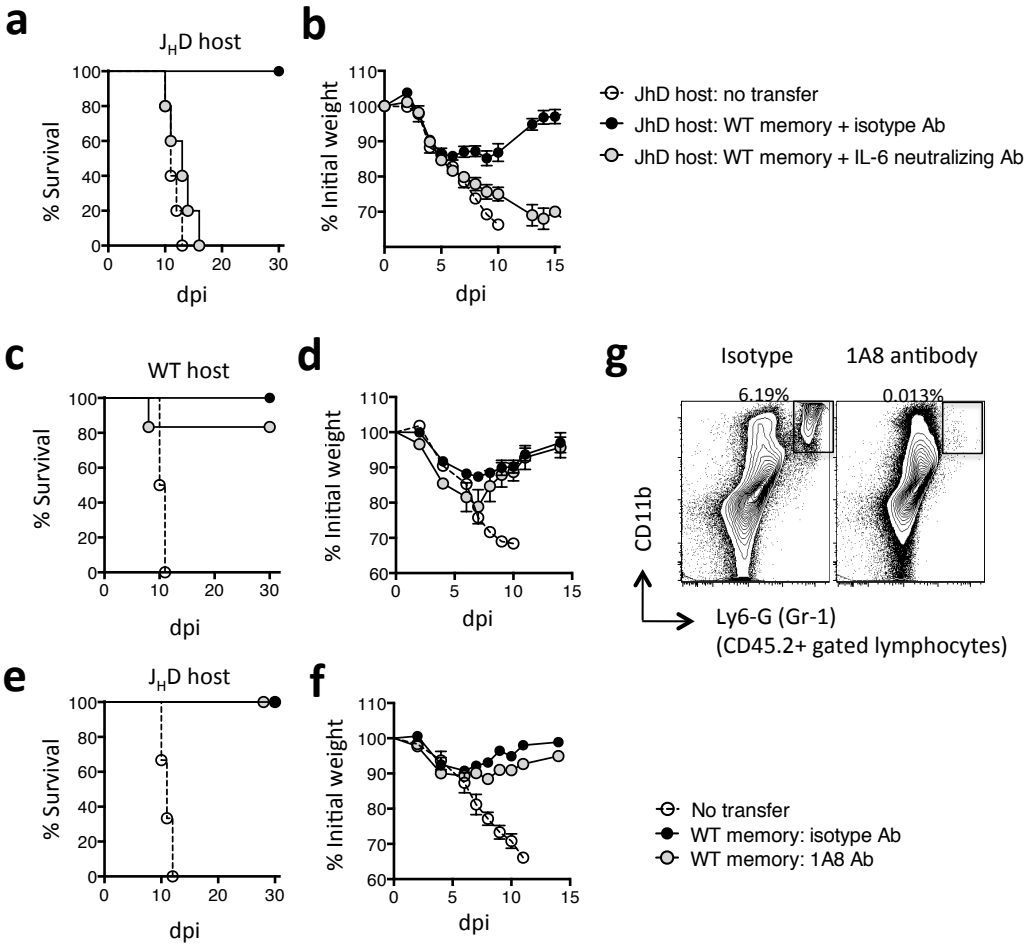
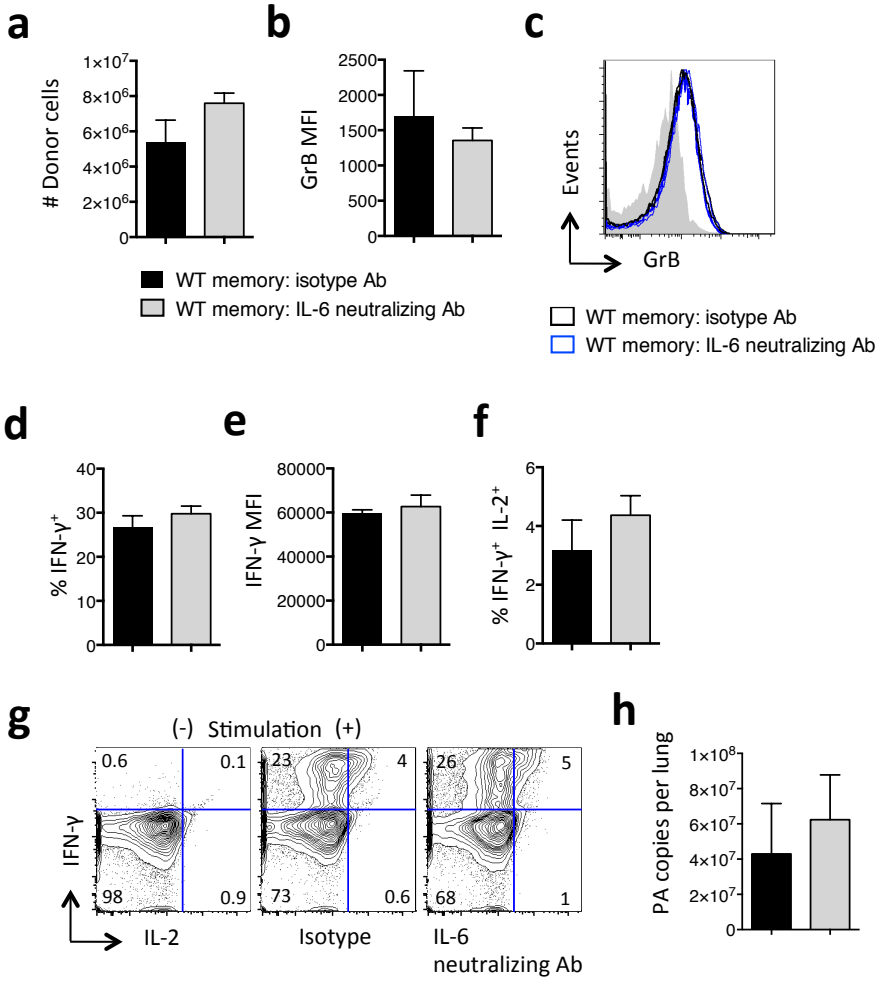
Supplemental Figure 1



Supplemental Figure 1: *IL-6 enhances memory CD4 T cell-mediated protection independently of B cell and neutrophils*. J_HD host receiving $5x10^6$ memory HNT CD4 T cells were treated with 0.5 mg of isotype or IL-6 neutralizing Ab on days 0, 2, 4, 6, and 8-post challenge with 2,500 EID_{50} A/PR8. Survival (**a**) and weight loss (**b**) was monitored (n=5 mice per group). Separate WT and J_HD mice receiving $5x10^6$ memory HNT CD4 T cells were challenged with a lethal dose of A/PR8 (10,000 EID_{50} for WT and 2,500 EID50 J_HD), and were treated with 0.5 mg of neutrophil-depleting Ab (clone 1A8) or isotype control (2A3) on 0, 2, 4, and 6 dpi. Survival **(c** and **e)** and weight loss **(d** and **f)** of each host was monitored (n = 2 for WT no transfer controls, n=6 for memory WT recipients, and n=4 for all J_HD host groups). Results summarize 2 replicate

experiments for each host. Representative staining **(g)** verifying the efficient depletion of neutrophils in the lungs of WT mice at 6 dpi. The depletion of neutrophils was as efficient in J_HD hosts (not shown).

Supplemental Figure 2



Supplemental Figure 2. *Memory CD8 T cell recall is largely unaffected by IL-6.* Memory CD8 T cells, 1x10^6, generated *in vitro* from naïve HA TcR Tg cells were transferred to unprimed WT BALB/c subsequently infected with 2,500 EID_{50} A/PR8. Groups of mice either received 0.5mg of isotype or IL-6 neutralizing Ab on days 0, 2, 4, and 6 dpi. At 7 dpi, **(a)** the number of donor cells in the lung**, (b)** donor granzyme B (GrB) expression as MFI, with representative staining shown in **(c)**, the frequency of donor IFN-γ^+ cells **(d)** and IFN-γ MFI **(e)**, and dual IFN-γ^+IL-2^+ cytokine

production (**f**) was determined from 4 mice/group**.** Representative ICCS staining with and without stimulation is shown in **(g)**. In separate experiments, viral titer **(h)** was determined at 7 dpi in mice that received $5x10^6$ memory CD8 T cells and were subsequently challenged with 2,500 EID_{50} A/PR8, and treated with anti-IL-6 or isotype control Ab as above (n = 4 mice/group).