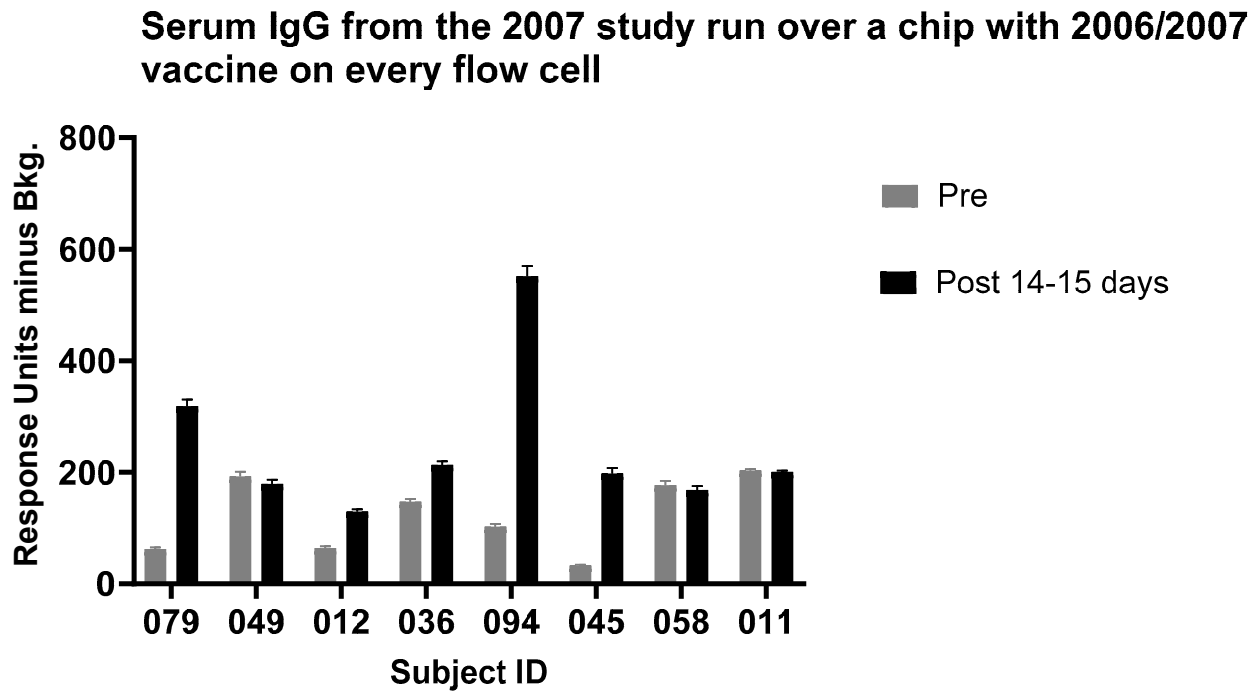
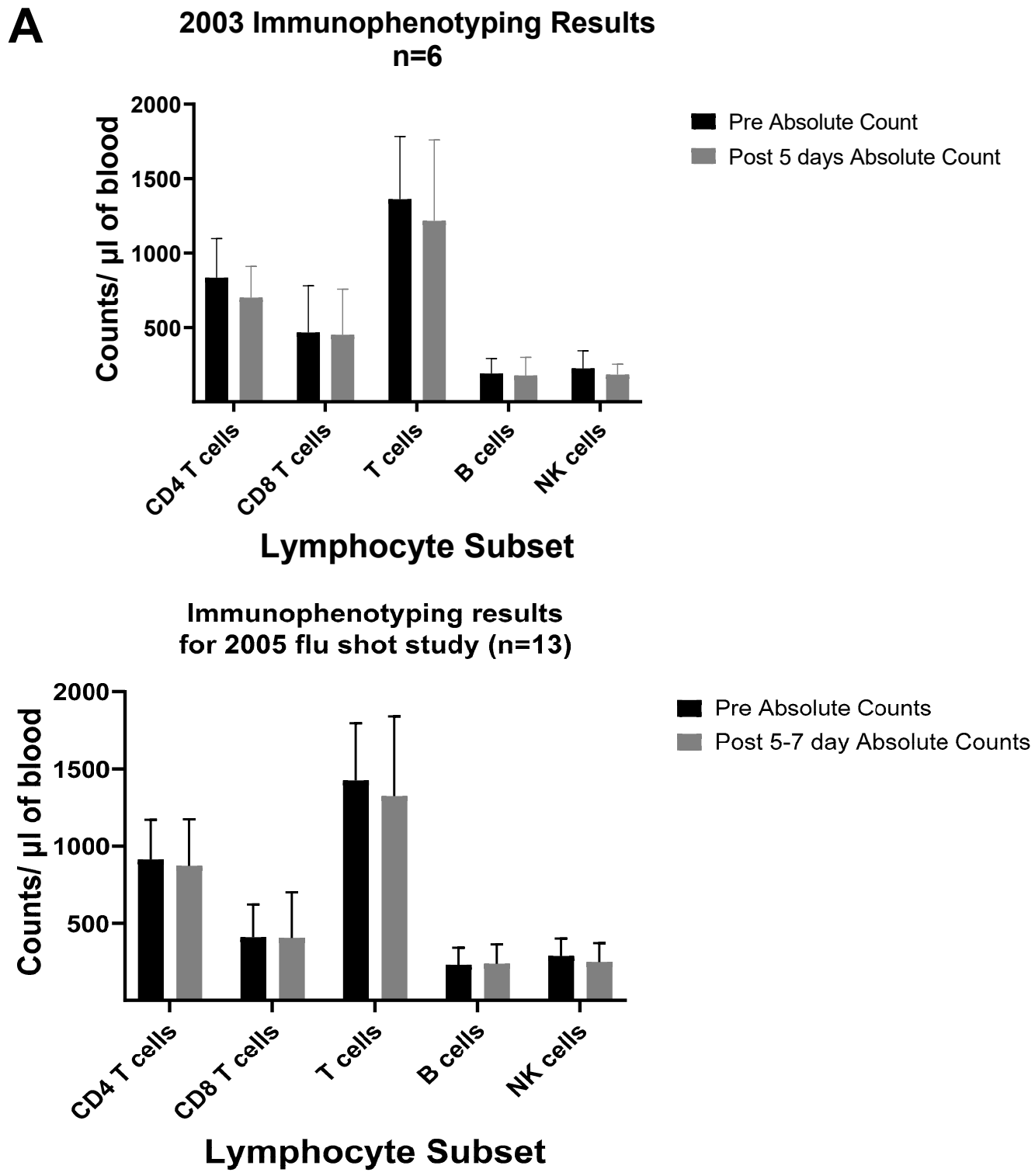
Figure S1



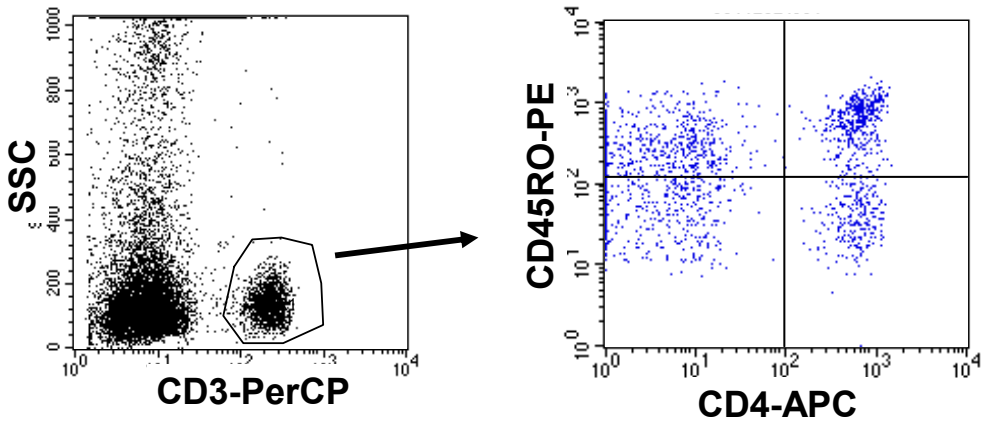
The vaccine from the 2006/2007 season was attached by amine coupling to a CM5 chip at ~5000 units on flow cells, F2, F3, and F4. Flow cell F1 was used as a background binding negative control. Purified IgG (both pre- and post-14-15 days) at 500 µg/ml from each subject was injected over the chip as described in the methods section. Bar graphs represent average RU minus background RU ± Standard Error (SE) for each subject.

Figure S2


A
2003 Immunophenotyping Results
n=6
Counts/ µl of blood
2000
1500
1000
500
CD4 T cells
CD8 T cells
T cells
B cells
NK cells
Lymphocyte Subset
Pre Absolute Count
Post 5 days Absolute Count
B
Immunophenotyping results
for 2005 flu shot study (n=13)
Counts/ µl of blood
2000
1500
1000
500
CD4 T cells
CD8 T cells
T cells
B cells
NK cells
Lymphocyte Subset
Pre Absolute Counts
Post 5-7 day Absolute Counts

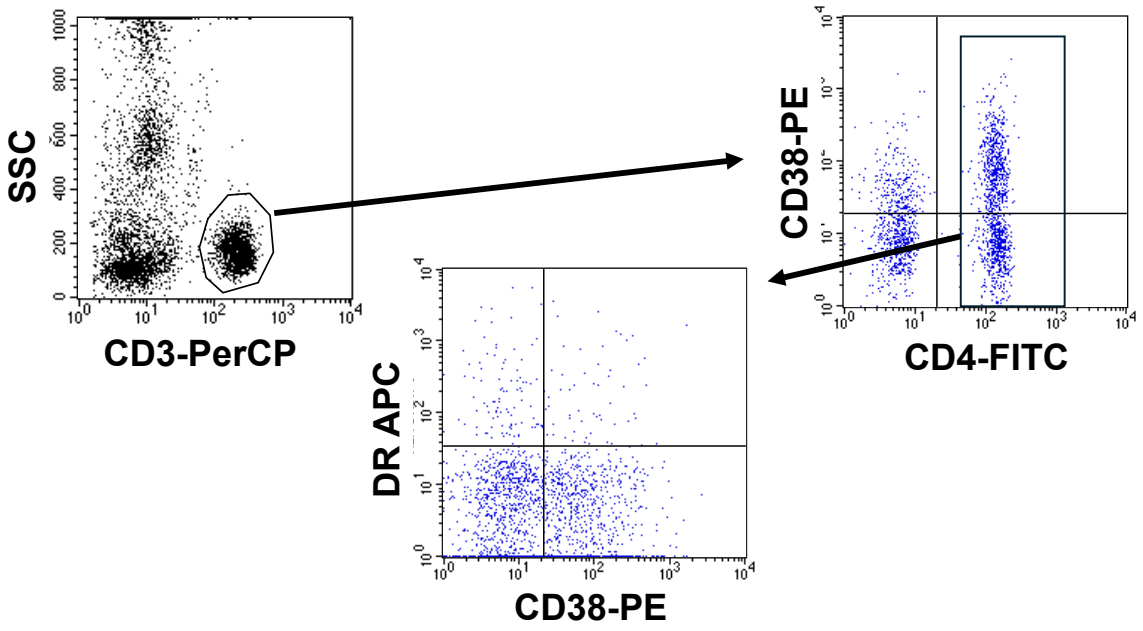
Immunophenotyping results from the 2003/2004 (A) and 2005/2006 (B) flu seasons. Study subjects were immunophenotyped with the 4-color MultiTest reagents with Trucount tubes from BD Biosciences according to the manufacturer's protocol. Stained samples were run and analyzed on a FACSCalibur flow cytometer with MultiSet software v1.1.1 (BD Biosciences, San Jose, CA, USA). Graph bars indicate average counts ± SE.

Figure S3: Gating Strategy for $CD4^+$ and $CD8^+$ T cells, Naive and Memory Cells



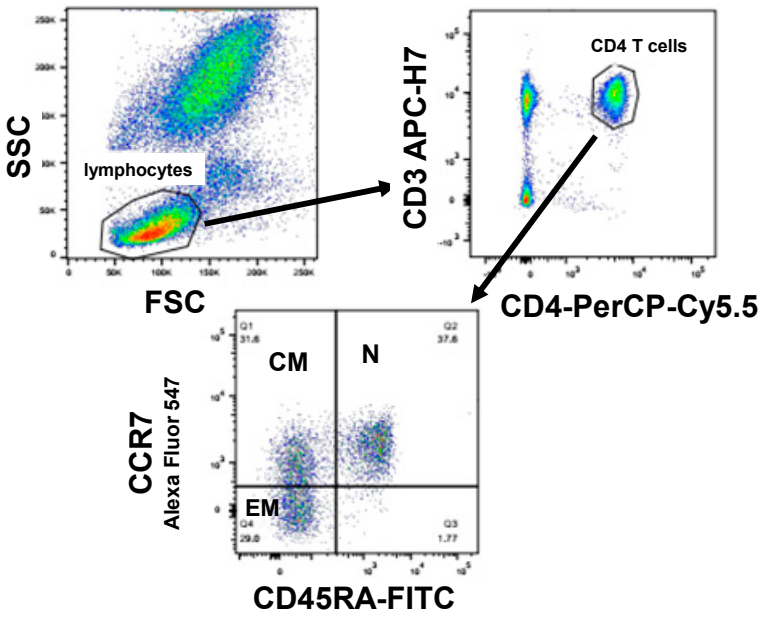
The method for determining the percentage of memory $CD4^+$ and $CD8^+$ T cells. In a plot of CD3 versus side scatter the $CD3^+$ cells are gated upon, and this gate is applied to a plot of $CD4^+$ versus $CD45RO^+$ (memory) cells. In this plot the quadrant statistics give the percentages of memory and naïve cells for $CD4^+$ and $CD8^+$ T cells. Analysis by CellQuest software v3.3 (BD Biosciences, San Jose, CA, USA).

Figure S4: Gating Strategy for HLA-DR$^+$/CD38$^+$/CD4$^+$ T cells



The method for determining the percentage of CD38$^+$ and HLA-DR$^+$ CD4$^+$ T cells. In a plot of CD3$^+$ versus side scatter the CD3$^+$ cells are gated upon, and this gate is applied to a plot of CD4$^+$ versus CD38$^+$ cells. In this plot the CD4$^+$ T cells are gated upon, and this gate is applied to a plot of CD38$^+$ versus HLA-DR$^+$ cells. Quadrant statistics will give the percentage of CD38$^+$/HLA-DR$^+$/CD4$^+$ T cells. Analysis by CellQuest software v3.3.

Figure S5: Gating Strategy for CD4⁺ T cell Naïve, Effector Memory, and Central Memory Cells



The method for determining the percentage of naïve (N), effector memory (EM), and central memory (CM) CD4⁺ T cells. In a plot of forward scatter versus side scatter the lymphocytes are gated upon and this gate was applied to a plot of CD4⁺ versus CD3⁺ cells. In this plot the CD4⁺ T cells were gated. This gate was applied to a plot of CD45RA versus CCR7 to identify naïve (N), effector memory (EM) and central memory cells (CM). The quadrant statistics gave the percentages of each CD4⁺ T cell subset. Analysis by FlowJo software v10.7.1_CL (Tree Star. Inc. Oregon, USA).