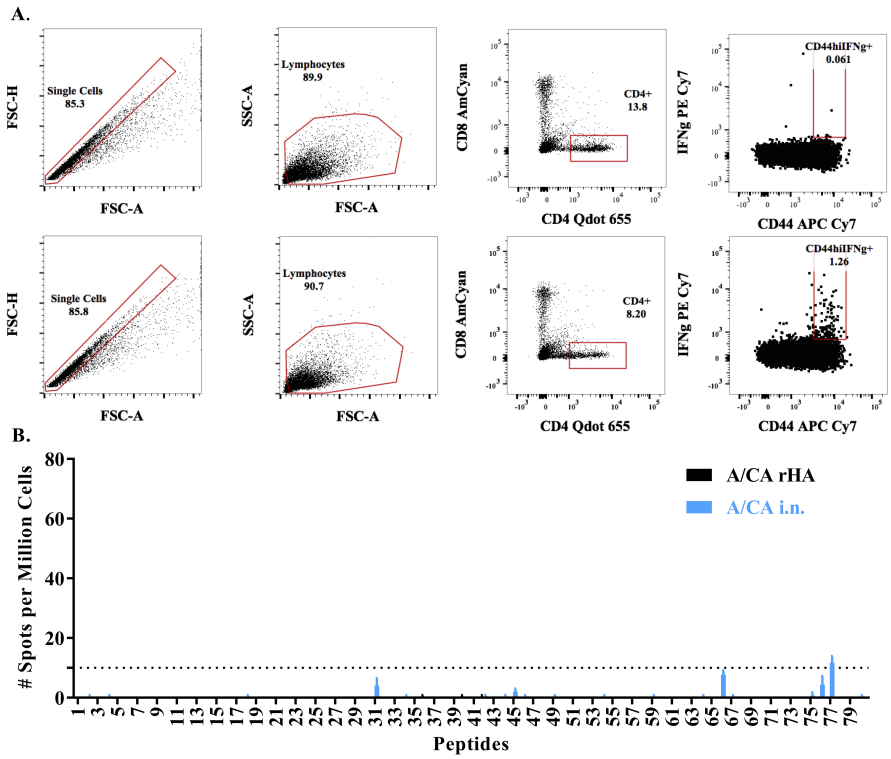
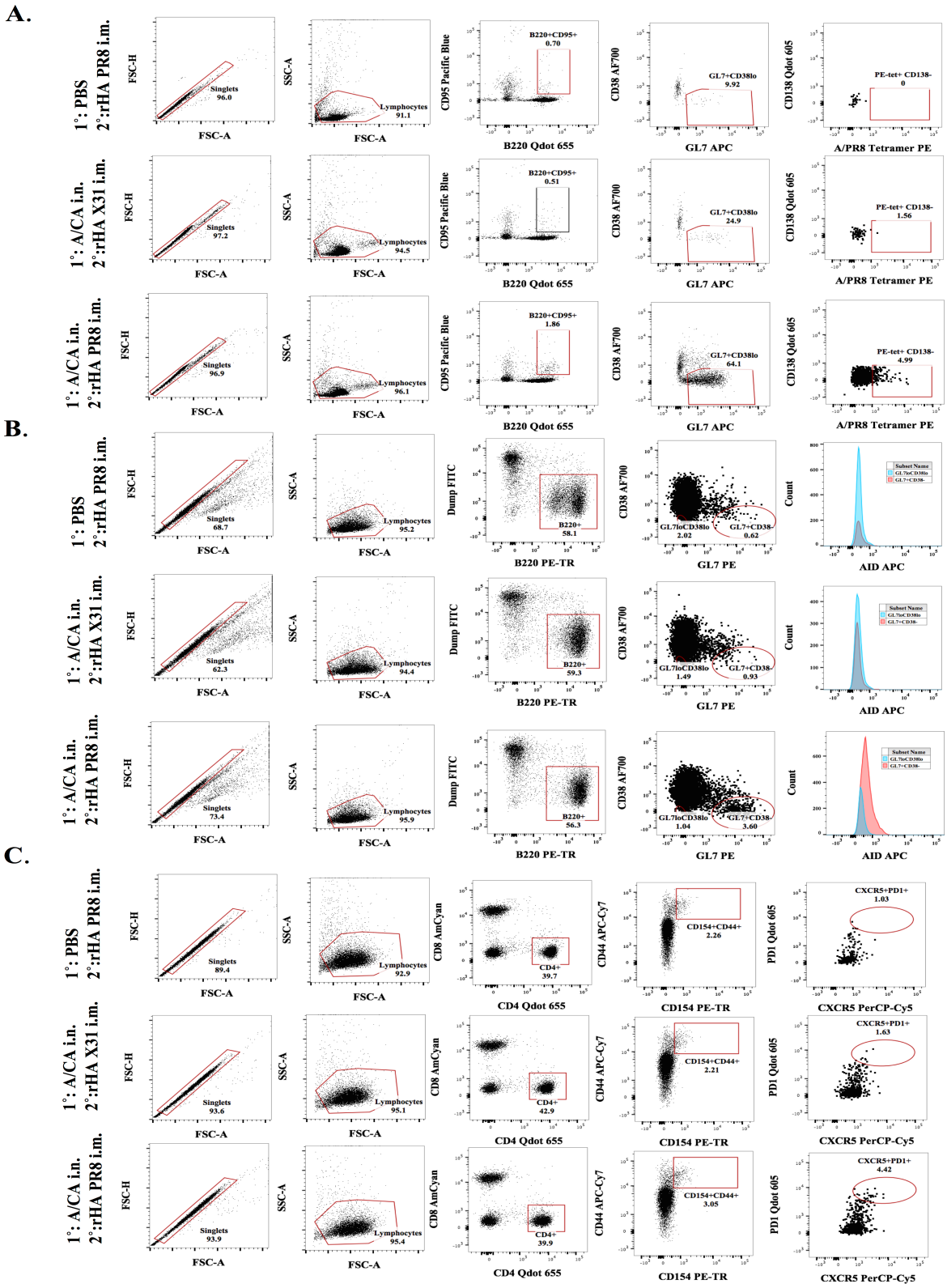
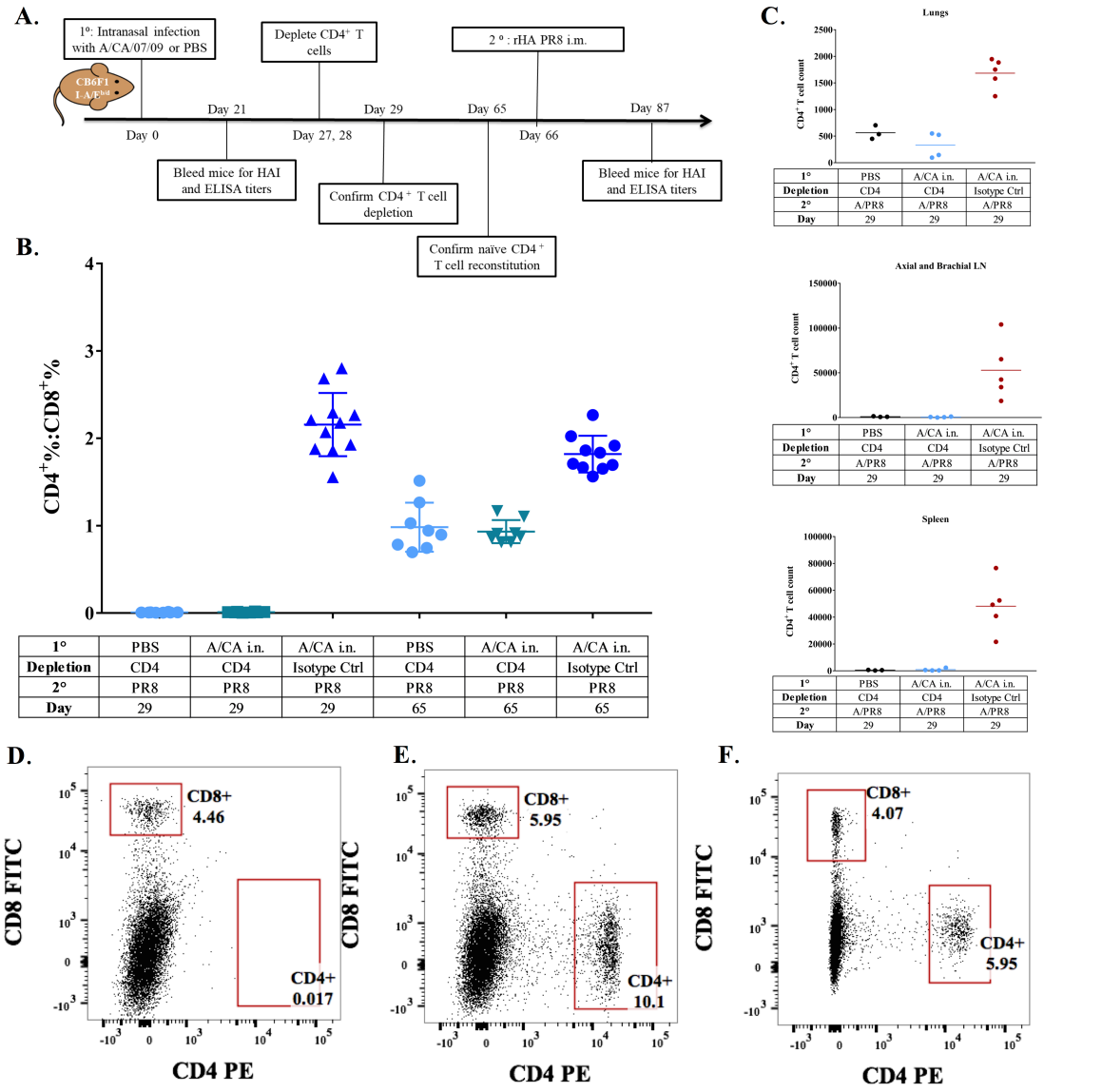


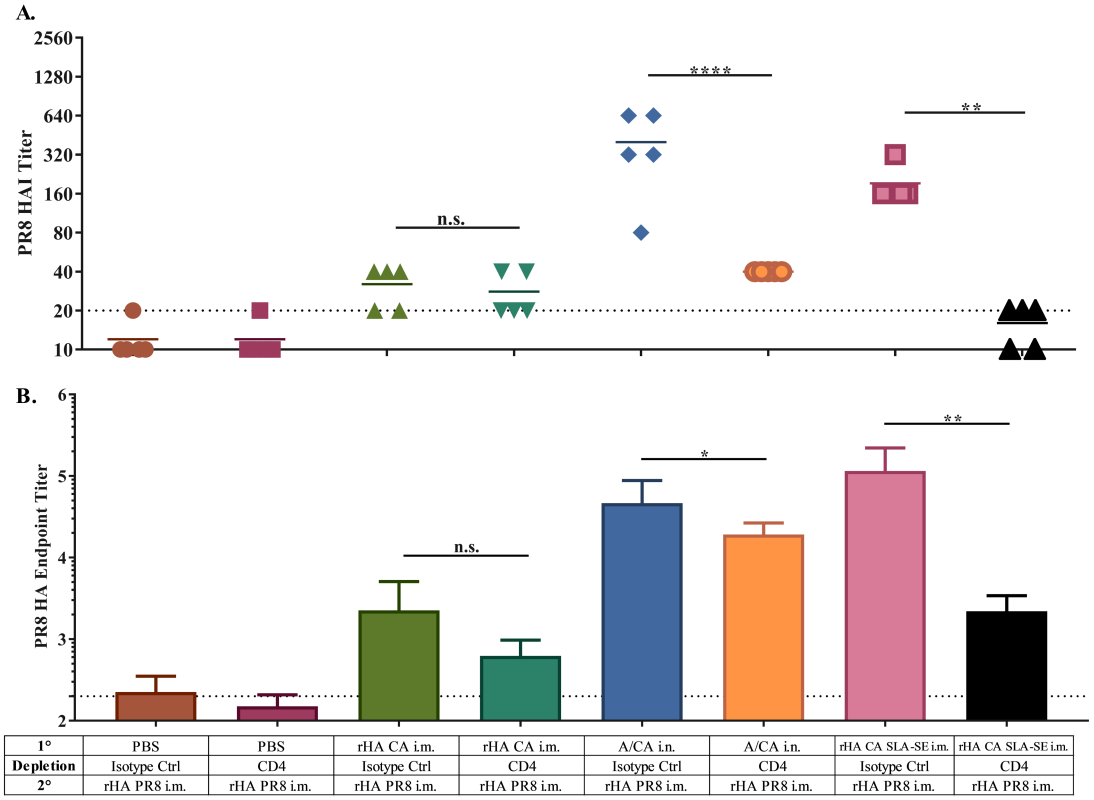
Supplementary Figure 1: Flow Cytometry gating strategy and representative flow plots of CD4^{+} T cell reactivity and IFN-γ ELISPOT. C57BL/6 (n=5 per group) or CB6F1 mice were intranasally infected with either A/PR8 or A/CA. Day 14 post infection spleens were harvested and stimulated with pooled peptides (1-10) of A/CA HA. Cells were then stained for analysis by flow cytometry and CD4^{+}CD44^{hi}IFNγ^{+} cells frequency of parent percentage (FoP %) were quantified in Figure 2. Representative flow plots and gating strategy are shown in panel (**A**). C57BL/6 mice (n=10 per group) mice were infected with A/CA or immunized with A/CA rHA (1 μg) then 14 days later splenocytes were isolated, resuspended and stimulated with 10 μg/mL of individiual 15-mer peptides from A/CA HA, media alone or PMA to measure IFN-γ production by ELISPOT in panel (**B**). Data show results from one of two experiments (n = 10).


A.
1°: PBS
2°:rHA PR8 i.m.
FSC-H
FSC-A
Singlets
96.0
SSC-A
Lymphocytes
91.1
CD95 Pacific Blue
B220 Qdot 655
B220+CD95+
0.70
CD38 AF700
GL7 APC
GL7+CD38lo
9.92
CD138 Qdot 605
A/PR8 Tetramer PE
PE-tet+ CD138-
0
1°: A/CA i.n.
2°:rHA X31 i.m.
Singlets
97.2
Lymphocytes
94.5
B220+CD95+
0.51
GL7+CD38lo
24.9
PE-tet+ CD138-
1.56
1°: A/CA i.n.
2°:rHA PR8 i.m.
Singlets
96.9
Lymphocytes
96.1
B220+CD95+
1.86
GL7+CD38lo
64.1
PE-tet+ CD138-
4.99
B.
1°: PBS
2°:rHA PR8 i.m.
Singlets
68.7
Lymphocytes
95.2
Dump FITC
B220 PE-TR
B220+
58.1
CD38 AF700
GL7 PE
GL7loCD38lo
2.02
GL7+CD38-
0.62
Count
AID APC
Subset Name
GL7loCD38lo
GL7+CD38-
1°: A/CA i.n.
2°:rHA X31 i.m.
Singlets
62.3
Lymphocytes
94.4
B220+
59.3
GL7loCD38lo
1.49
GL7+CD38-
0.93
1°: A/CA i.n.
2°:rHA PR8 i.m.
Singlets
73.4
Lymphocytes
95.9
B220+
56.3
GL7loCD38lo
1.04
GL7+CD38-
3.60
C.
1°: PBS
2°:rHA PR8 i.m.
Singlets
89.4
Lymphocytes
92.9
CD8 AmCyan
CD4 Qdot 655
CD4+
39.7
CD44 APC-Cy7
CD154 PE-TR
CD154+CD44+
2.26
PD1 Qdot 605
CXCR5 PerCP-Cy5
CXCR5+PD1+
1.03
1°: A/CA i.n.
2°:rHA X31 i.m.
Singlets
93.6
Lymphocytes
95.1
CD4+
42.9
CD154+CD44+
2.21
CXCR5+PD1+
1.63
1°: A/CA i.n.
2°:rHA PR8 i.m.
Singlets
93.9
Lymphocytes
95.4
CD4+
39.9
CD154+CD44+
3.05
CXCR5+PD1+
4.42

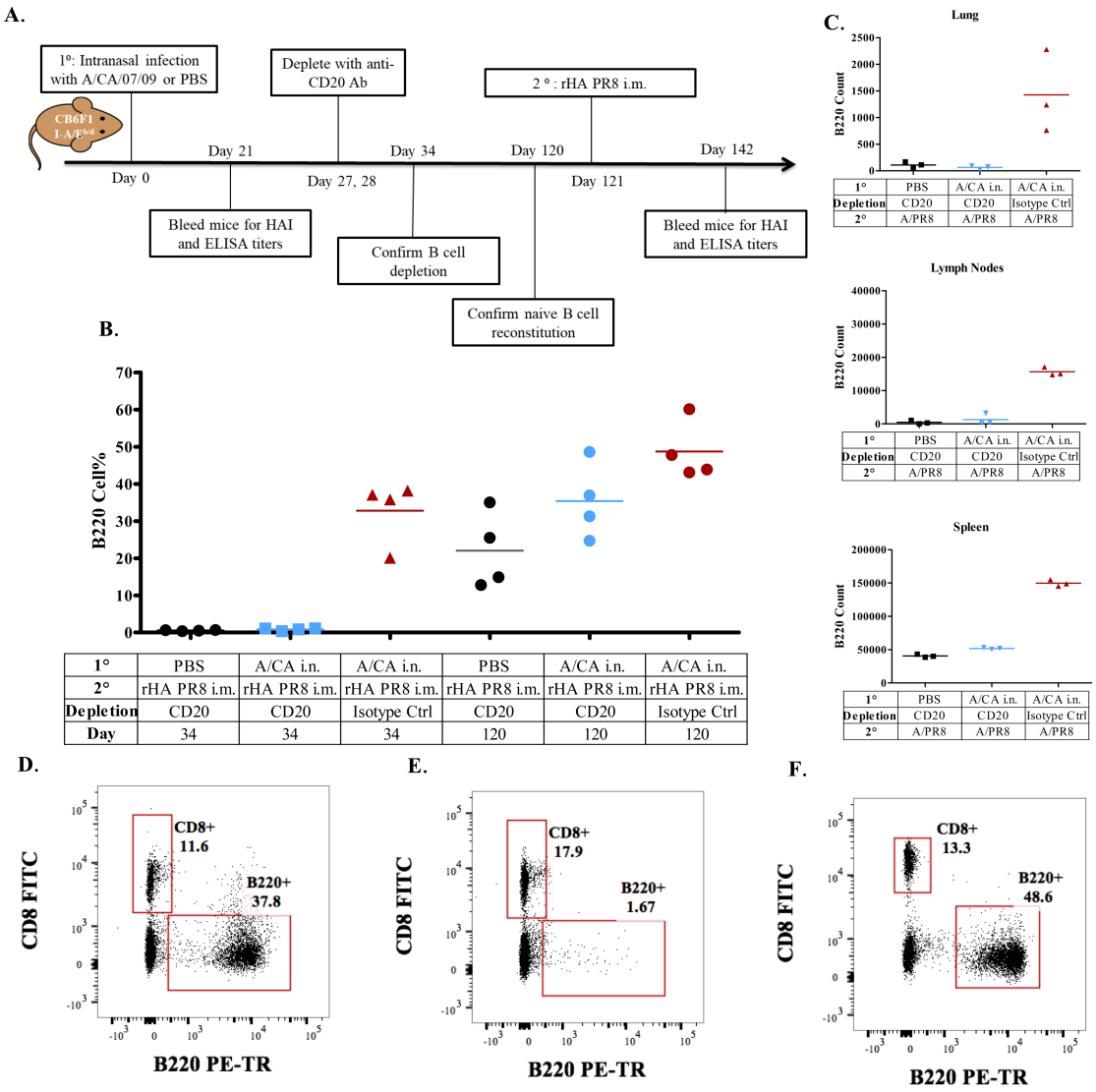
Supplementary Figure 2: Increased Germinal Center A/PR8-specific B and T follicular helper cells in response to drifted immunization in previously exposed mice but not unexposed mice. Representative flow plots show increased A/PR8-specific GC cells ($B220^{+}$ $CD95^{+}$ $GL7^{+}$ $CD38^{lo}$ PR8-Tet^{+} $CD138^{lo}$) in previously exposed CB6F1 mice immunized with rHA A/PR8 or rHA X-31, as a negative control, in panel (**A**). Representative flow plots show increased GC cells ($B220^{+}$ $GL7^{+}$ $CD38^{lo}$) in previously exposed in panel (**B**) and these GC B cells express higher levels of activation-induced cytidine deaminase (AID) in panel (**B**). Representative flow plots show increased Tfh cells ($CD4^{+}$ $CD154^{+}$ $CD44^{+}$ $CXCR5^{hi}$ $PD1^{hi}$) in previously exposed CB6F1 mice immunized with PR8 rHA (1 μg) in panel (**C**).



Supplementary Figure 3: Depletion of memory CD4$^+$ T cells and reconstitution of naïve CD4$^+$ T cells in blood and tissues of CB6F1 mice. Experimental outline in panel **(A) :** CB6F1 mice were i.n. exposed to A/CA (500 pfu/mouse) or PBS and then depleted of CD4$^+$ T cells in between primary and secondary immunization using 500 µg Ab GK 1.5 given i.p., then allowed to reconstitute their naïve CD4$^+$ T cell population prior to secondary drifted immunization to A/PR8 rHA (1 µg). CD4$^+$ T cells (n=10 per group) were quantified in blood in panel (**B**) post depletion with anti-CD4$^+$ Ab and isotype control and upon reconstitution using Flow Cytometry. Penetration of tissue CD4$^+$ T cell depletion (n=5 per group) shown in lungs, axial and brachial lymph nodes and spleen in panel (**C**). Flow Cytometry plots to confirm CD4$^+$ T cell depletion with anti-CD4 Ab in panel (**D**) and isotype control in panel (**E**) and reconstitution in blood in panel (**F**)**.**



Supplementary Figure 4: Preexposure with adjuvanted A/CA HA protein enhanced hAb responses to A/PR8 HA immunization and enhancement is CD4$^+$ T cell dependent. CB6F1 mice (n=5 per group) are intranasally exposed to A/CA (500 pfu/mouse) or PBS or rHA CA (1 µg/mouse) with and without adjuvant (SLA-SE) and then depleted of CD4$^+$ T cells in between primary and secondary exposure using 500 µg Ab GK 1.5 given i.p., then allowed to reconstitute their naïve CD4$^+$ T cell population prior to secondary intramuscular immunization to rHA PR8 (1 µg /mouse)**.** A/PR8 HAI titer in panel (**A**) and endpoint titer ± SD (Log_{10}) in panel (**B**) at day 21 post-secondary rHA PR8 immunization in CB6F1 mice. (**p<0.01, ****p<0.0001, One-way ANOVA). Data show results from one of two experiments with similar results (n = 5 mice).



Supplementary Figure 5: Experimental determination of memory B cell necessity in CB6F1 mouse model. Experimental model timeline: CB6F1 mice are intranasally exposed to A/CA (500 pfu/mouse) or PBS and then depleted of B cells in between primary and secondary immunization using 500 µg anti-CD20 antibody given i.p., then allowed to reconstitute their naïve B cell population prior to secondary intranasal or intramuscular exposure to A/PR8 rHA (1 µg/mouse) as shown in panel (**A**). B cells were quantified in blood post depletion and upon reconstitution using Flow Cytometry in panel (**B**). Penetration of tissue B cell depletion (n=3 per group) shown in lungs, axial and brachial lymph nodes and spleen in panel (**C**). Flow Cytometry plots to confirm B cell depletion in panel (**D**), isotype control in panel (**E**) and reconstitution in blood in panel (**F**). Data show results from one of two experiments with similar results (n = 4 mice).