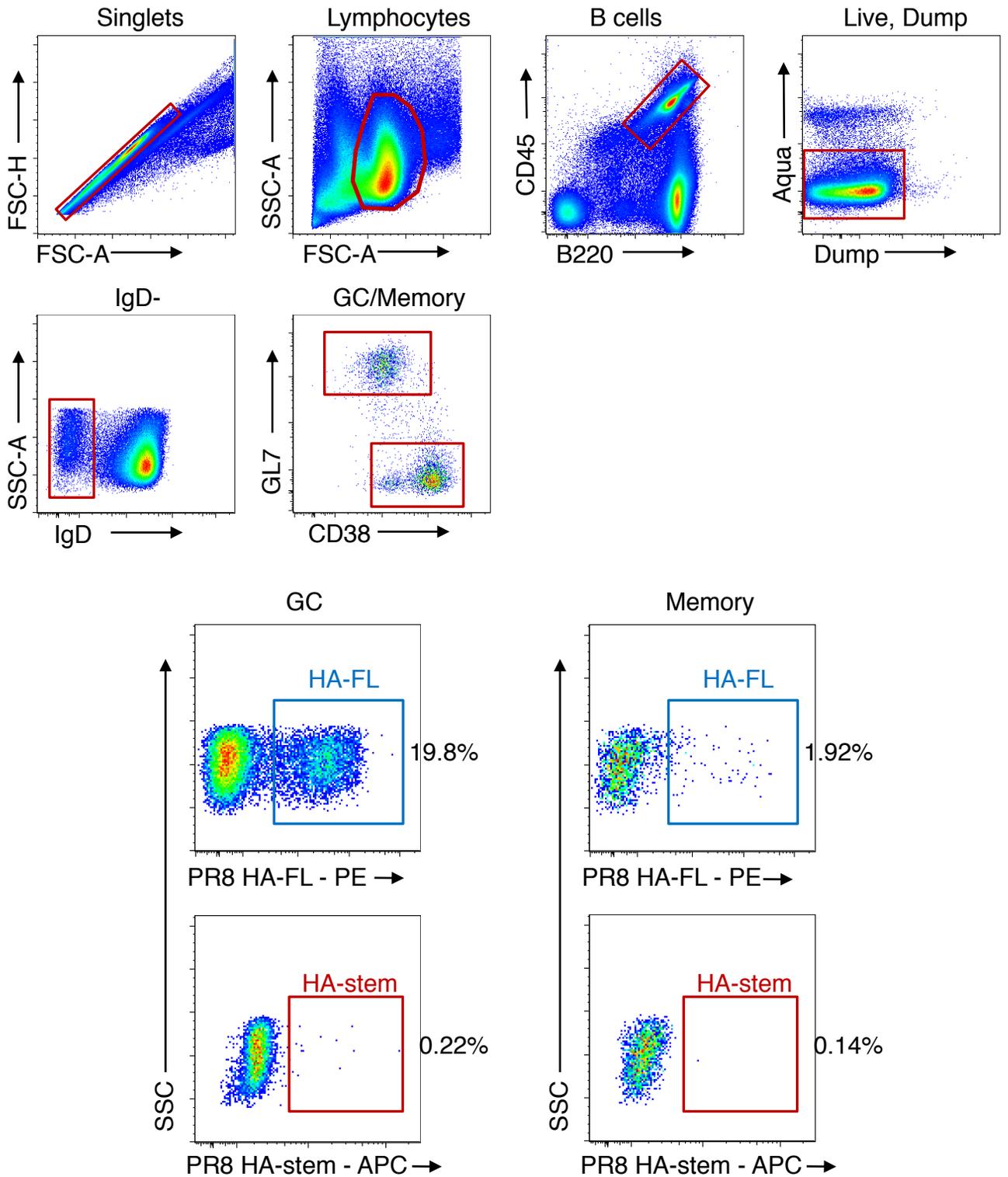


### Figure S1. Antigenicity of HA-FL and HA-stem proteins

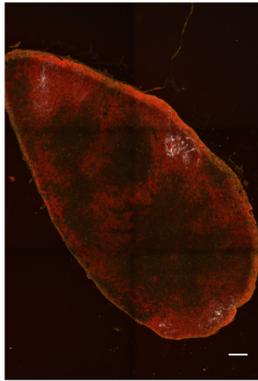
The antigenicity of full length HA0 ectodomains and stabilised HA-stem proteins was examined by ELISA using a panel of monoclonal antibodies: 445D1 (HA-head specific), TN1G09 (IGHV6-1 class, HA-stem/fusion peptide), C179 (HA-stem), CR9114 (HA-stem) and VRC01 (non-influenza control).



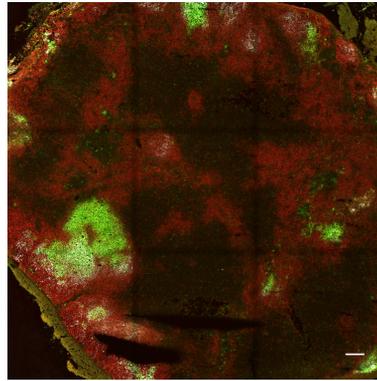
**Figure S2. Representative gating for B cell analyses of infected mice**

After doublet removal, CD45+B220+IgD- lymphocytes were gated to remove dead and CD3, F4/80 and streptavidin-specific cells before assessing binding to HA-FL or HA-stem probes within germinal centre (GL7+ CD38mid) and memory populations (GL7- CD38hi). Pictured is mediastinal lymph node (MLN) from an infected mouse 28 days after intranasal infection with A/Puerto Rico/08/1934.

Mediastinal lymph node



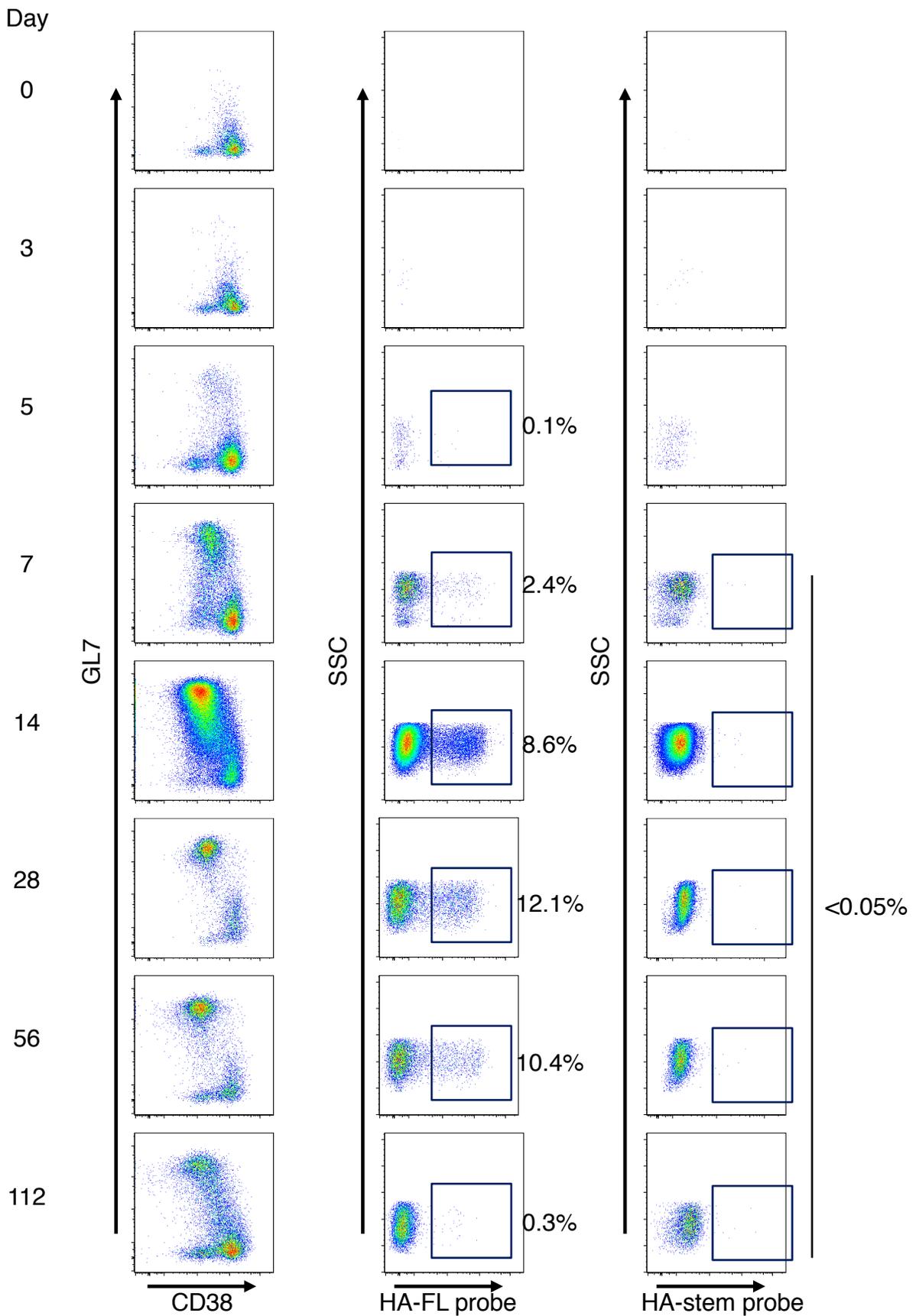
Uninfected



d28 post-infection

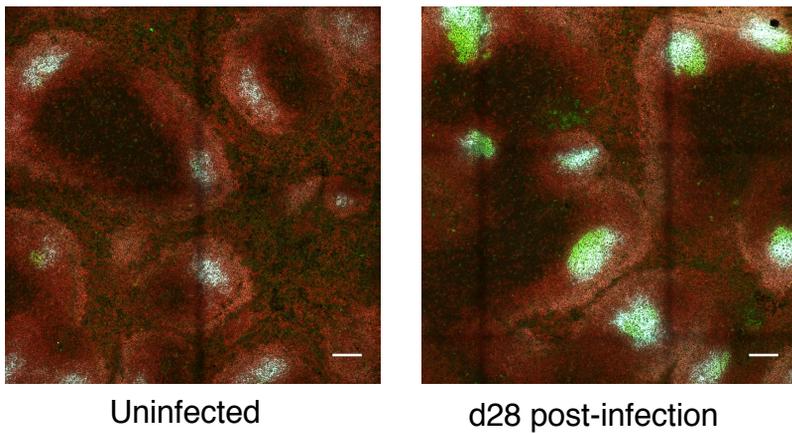
**Figure S3. Germinal centre expansion in the mediastinal lymph node following intranasal infection with A/Puerto Rico/08/1934**

Tissue sections were stained with CD35 (cyan), GL7 (green) and B220 (red) (scale bar – 100  $\mu$ m).



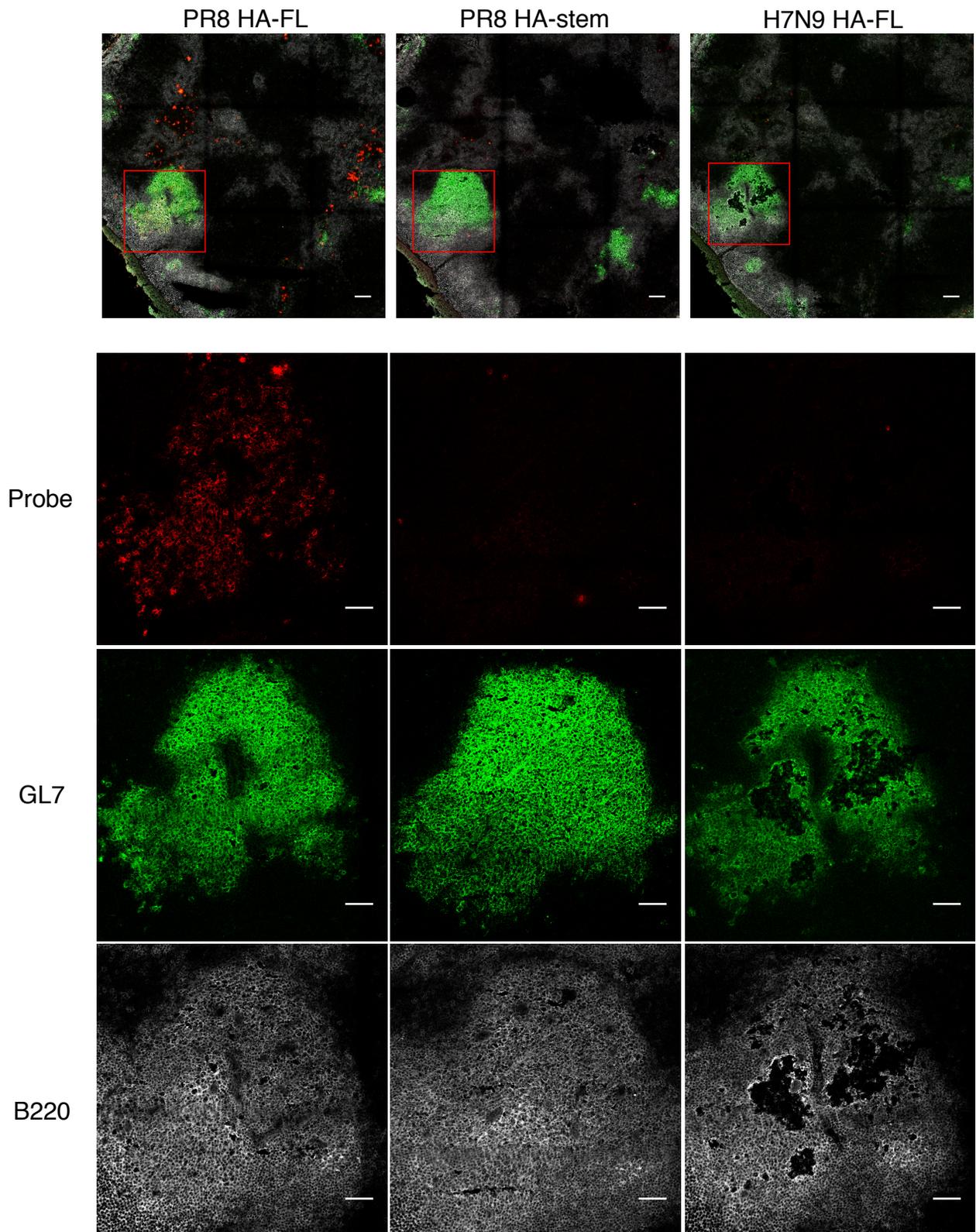
**Figure S4. Representative staining of B cell changes in the mediastinal lymph node following intranasal infection with A/Puerto Rico/08/1934. Within expanded GL7+ GC B cells, elevated proportions of HA-FL-specific but not HA-stem-specific B cells can be observed in infected mice.**

## Spleen

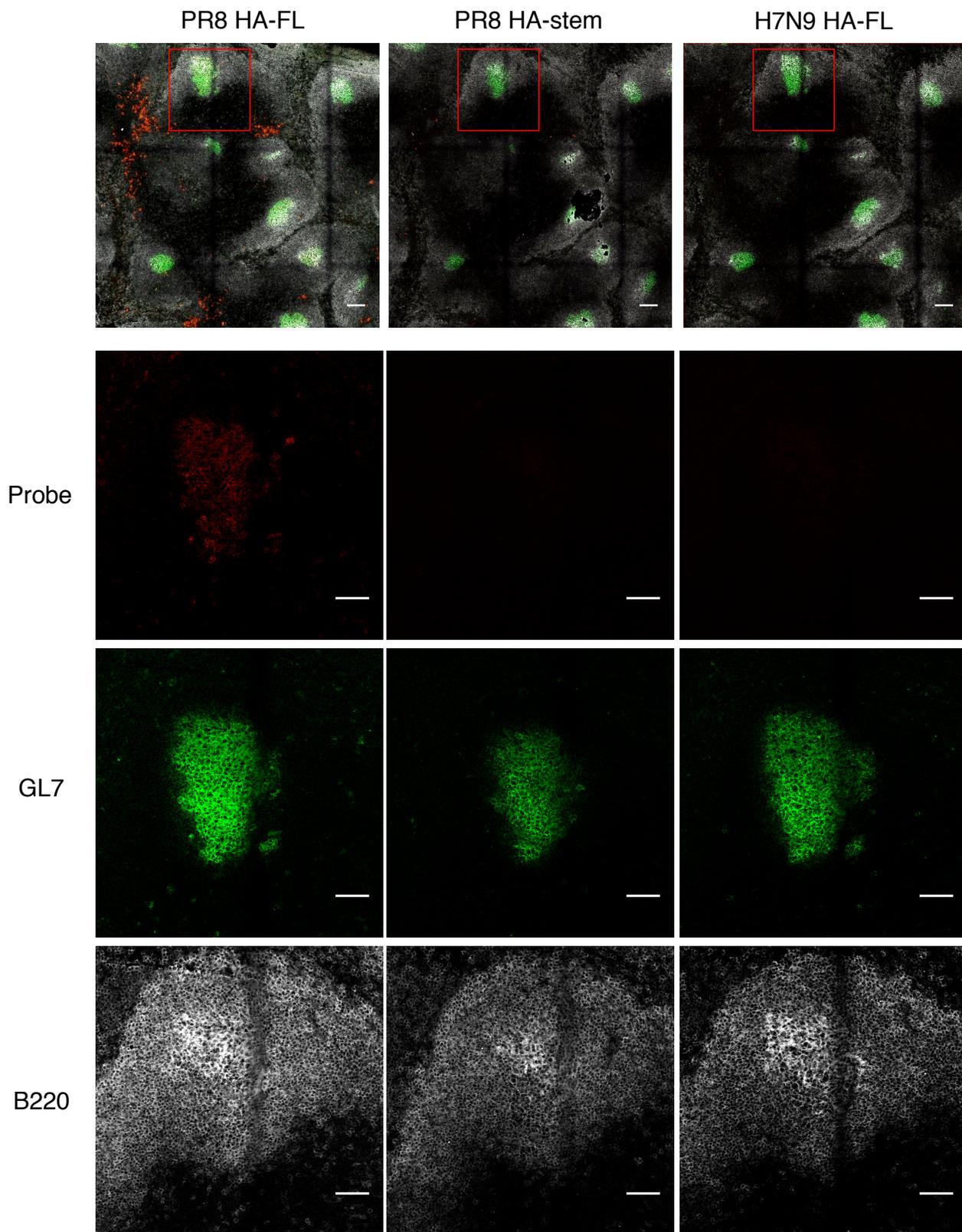


**Figure S5. Germinal centre expansion in the spleen following intranasal infection with A/Puerto Rico/08/1934**

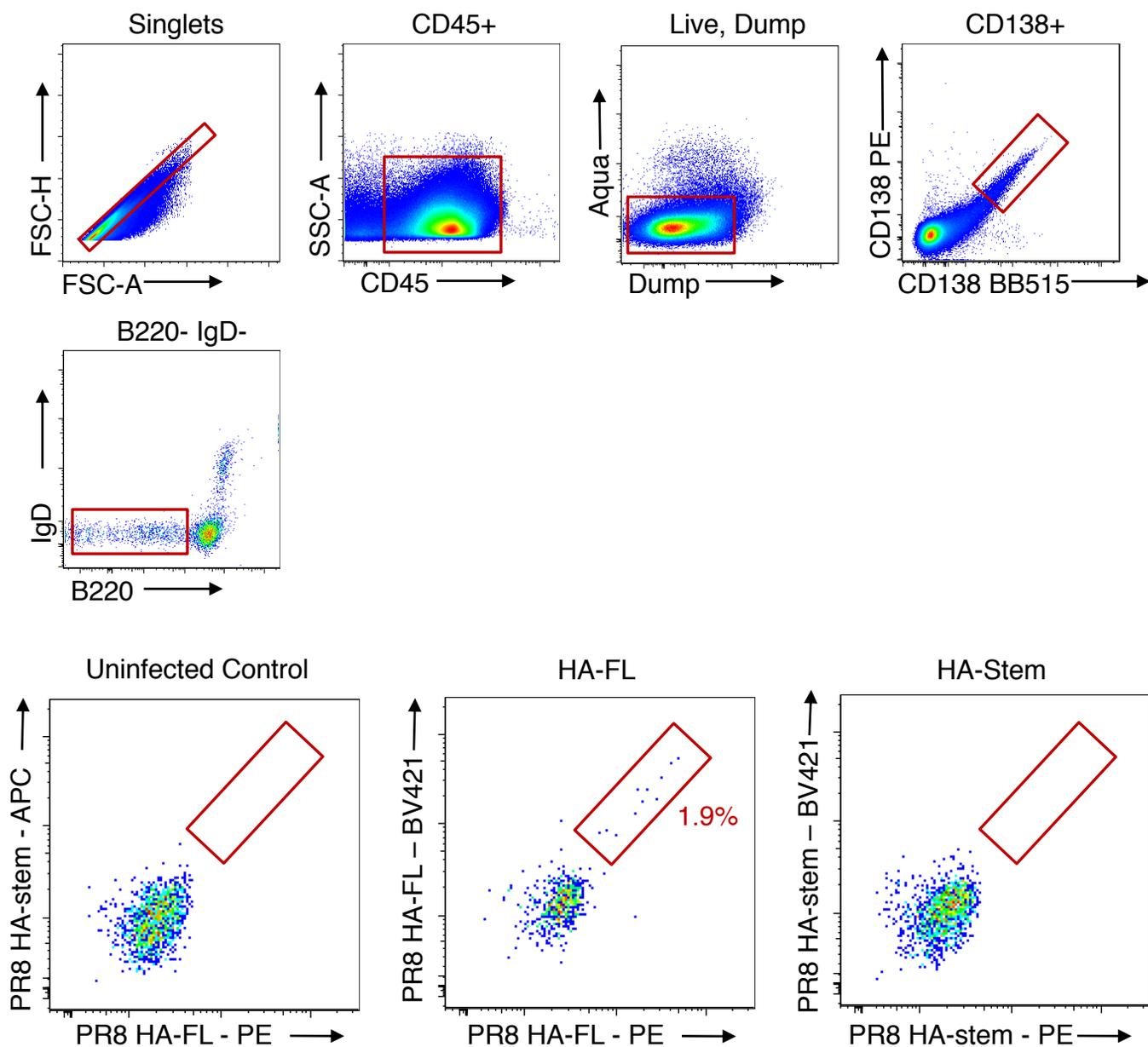
Tissue sections were stained with CD35 (cyan), GL7 (green) and B220 (red) (scale bar – 100  $\mu\text{m}$ ).



**Figure S6. In situ staining for antigen specific B cells in the MLN of infected mice**  
Tissue sections were stained with HA probe (red), GL7 (green) and B220 (grey) (Gross tissue scale bar – 100  $\mu$ m; inset GC scale bar – 50  $\mu$ m).

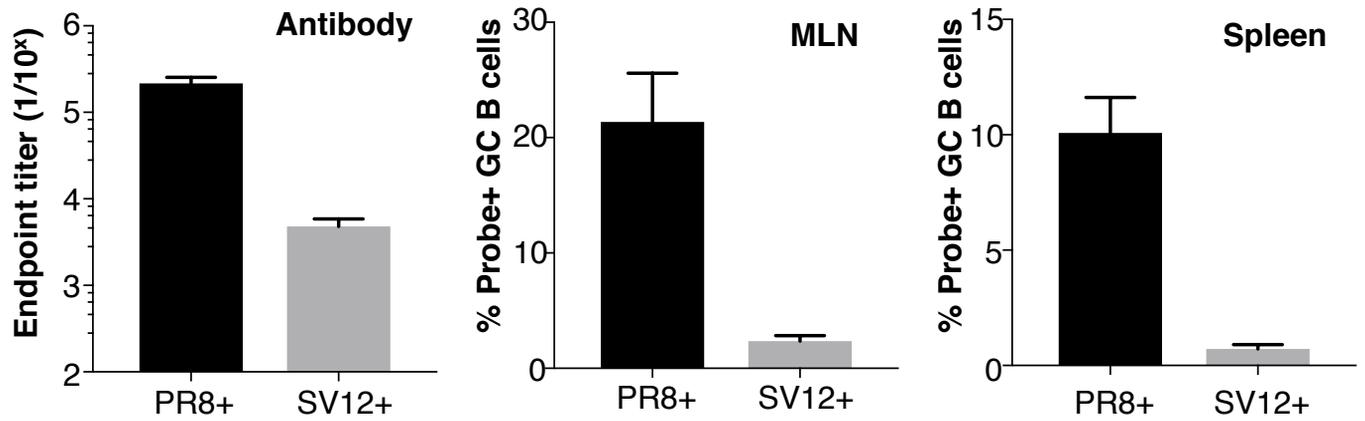


**Figure S7. In situ staining for antigen specific B cells in the spleen of infected mice**  
Tissue sections were stained with HA probe (red), GL7 (green) and B220 (grey) (Gross tissue scale bar – 100  $\mu$ m; inset GC scale bar – 50  $\mu$ m).



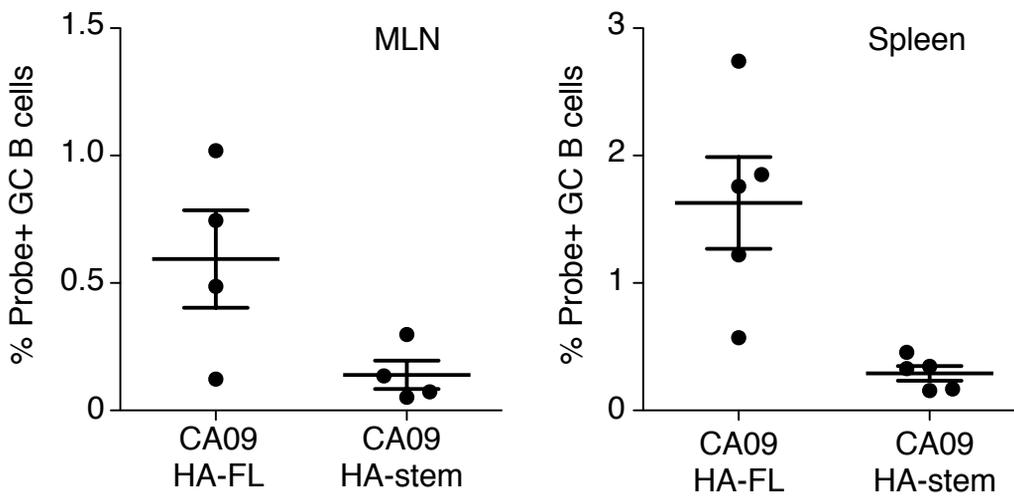
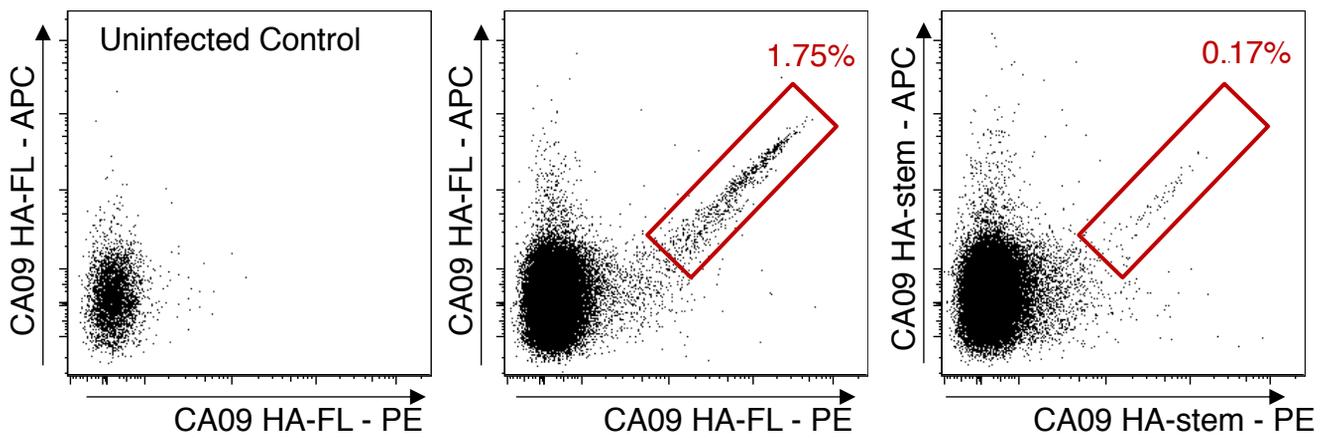
**Figure S8. Representative gating for plasma cell analyses of infected mice**

CD45+ bone marrow cells were gated to remove doublets, dead, CD3+, F4/80+ and streptavidin-binding cells. Plasma cells were identified based upon double staining for CD138 and negative for B220 and IgD. Intracellular binding to HA-FL or HA-stem probes was assessed. Pictured is an uninfected control and an infected mouse 56 days after intranasal infection with A/Puerto Rico/08/1934.



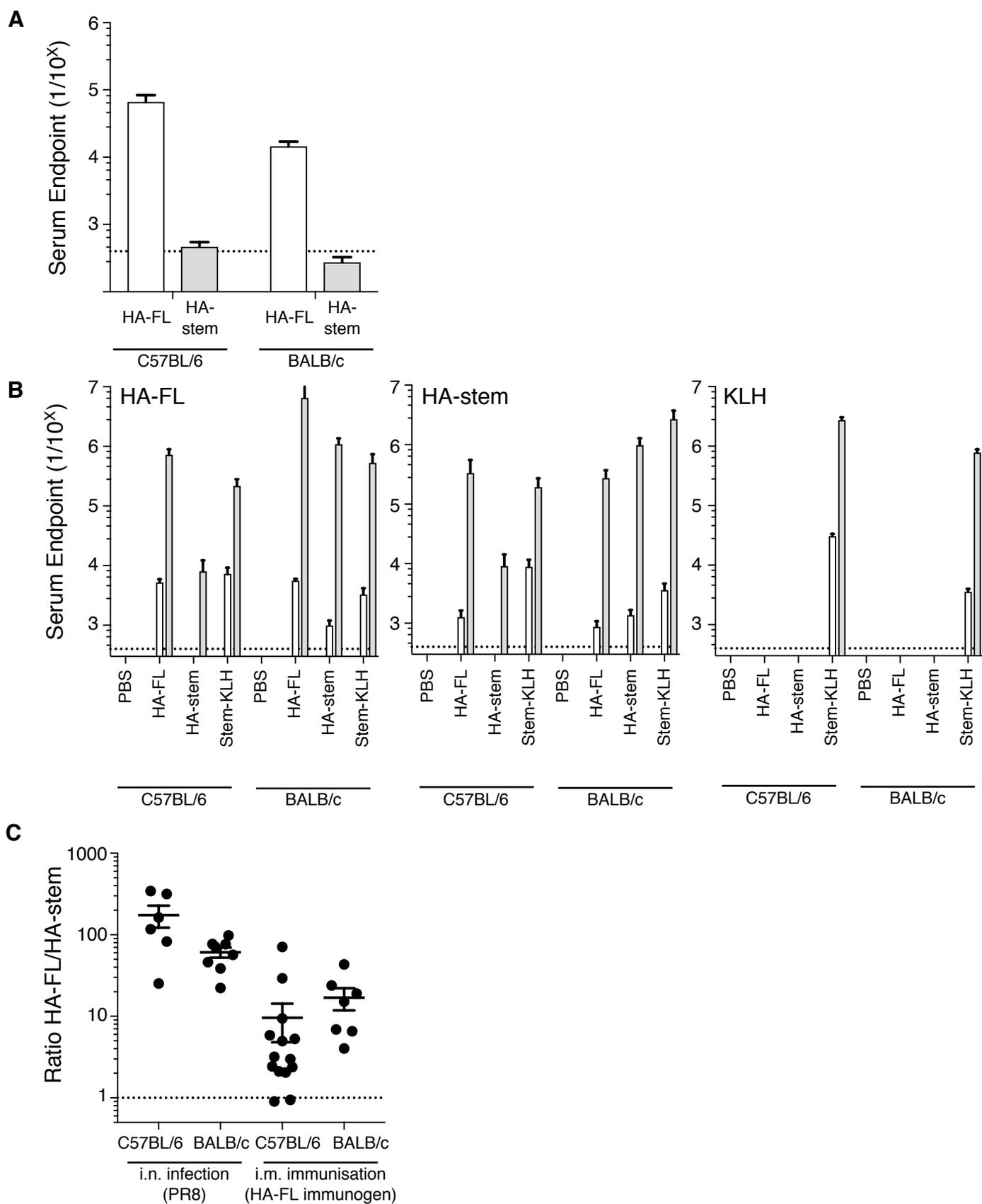
**Figure S9. Antibody and B cell responses in PR8 infected mice are focused upon canonical epitopes within the HA head domain**

Antibody and B cells specific for PR8 HA or SV12 HA were measured 14 days post-infection in mice infected intranasally with A/Puerto Rico/08/1934 (n = 5). Serum endpoint IgG titres were determined by ELISA using recombinant PR8 HA or SV12 HA. The proportion of GC B cells (B220+IgD-GL7+CD38<sup>lo</sup>) binding PR8 or SV12 HA probes within MLNs and spleens of infected mice was assessed by flow cytometry.



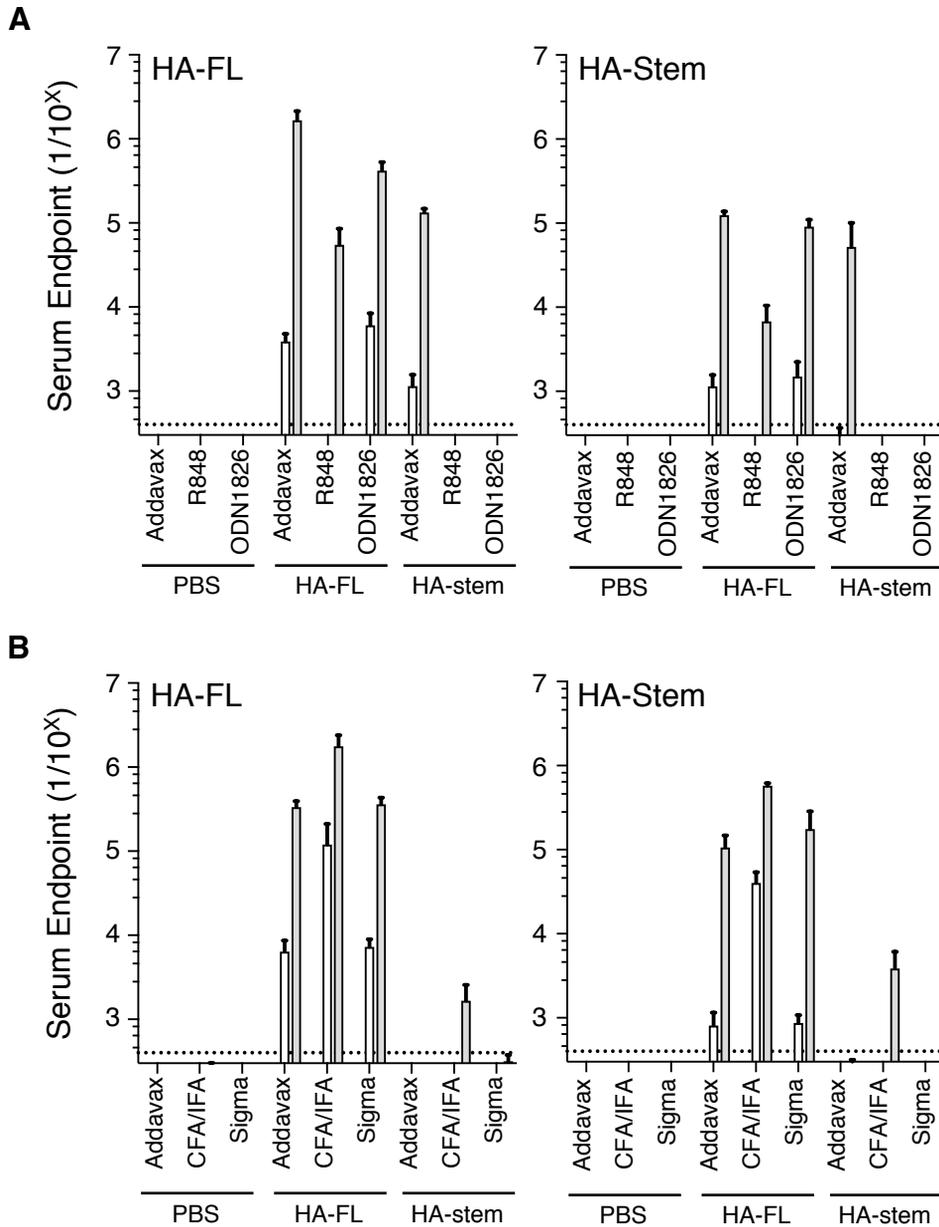
**Figure S10. Head- and stem-specific B cells in CA09 infected mice**

The proportion of B cells binding HA-FL or the HA-stem probes was assessed in mice infected intranasally with A/California/04/2009 (500 TCID<sub>50</sub>) (n = 4-5). Spleen and MLNs were harvested 14 days post-infection and the specificity of GC B cells (B220+IgD-GL7+CD38<sup>lo</sup>) binding HA probes assessed by flow cytometry.



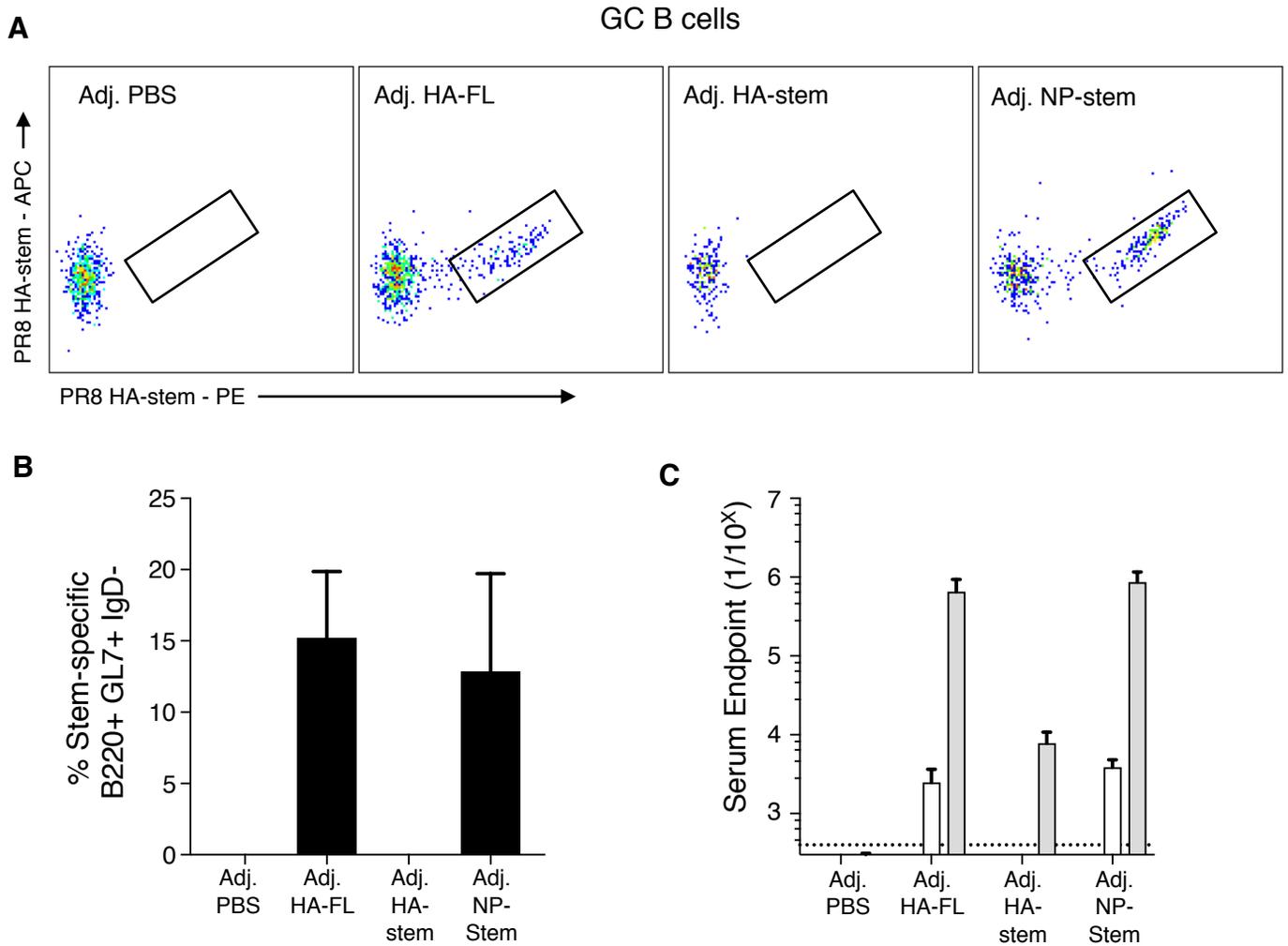
**Figure S11. Comparison of serological responses in C57BL/6 and BALB/c mice after vaccination or infection**

Serum endpoint total IgG titres were measured by ELISA using HA-FL, HA-stem or KLH proteins in mice that were (A) infected intranasally with A/Puerto Rico/08/1934 ( $n = 6$  C57BL/6,  $n = 8$  BALB/c) or (B) immunised twice at 3-week intervals with adjuvanted (Addavax) immunogens ( $n = 5$  per group). Serum was analysed 2 weeks post-immunisation or infection. (C) HA-FL immunodominance over HA-stem by measurement of serum endpoint IgG titre ratios compiled from infected ( $n = 6$  C57BL/6,  $n = 8$  BALB/c) or HA-FL immunised mice ( $n = 15$  C57BL/6,  $n = 7$  BALB/c). Dotted lines denote detection cut off (dilution 1:400).



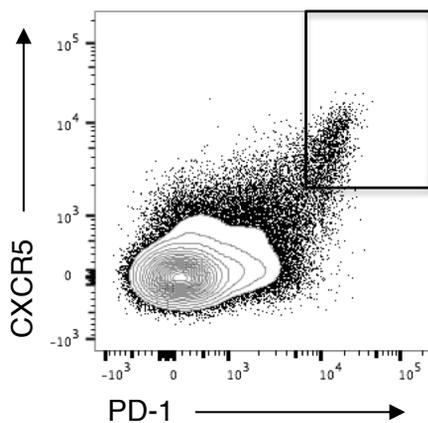
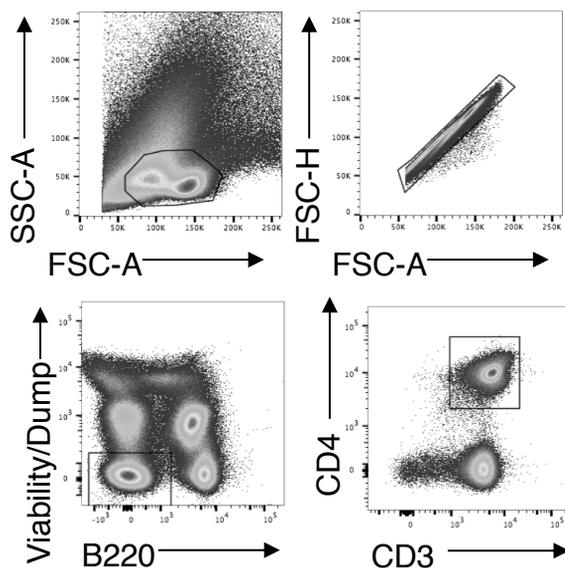
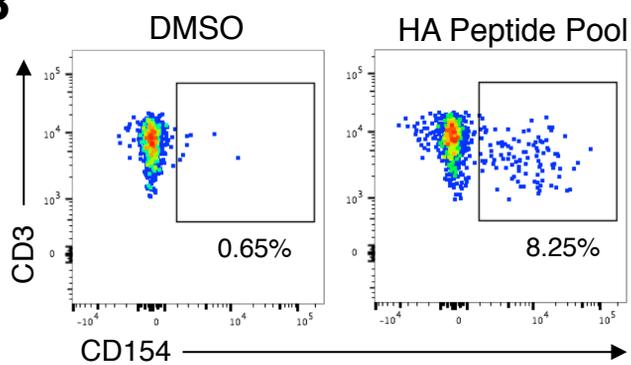
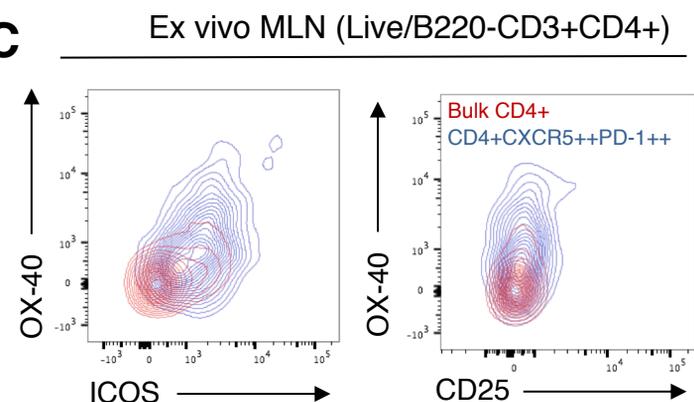
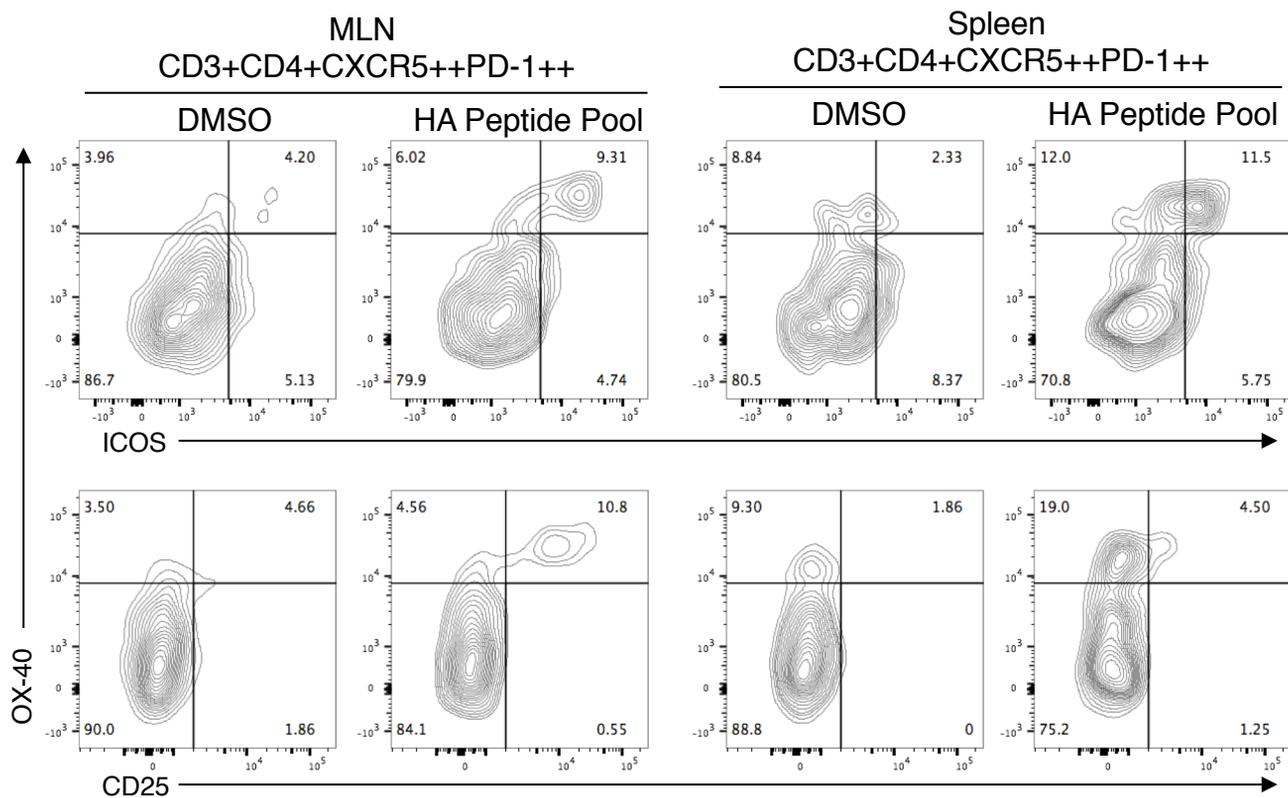
**Figure S12. Adjuvants fail to rescue HA-stem antibody responses in C57BL/6 mice**

Serum endpoint total IgG titres were measured by ELISA using HA-FL or HA-stem proteins in mice that were immunised (A) intramuscularly or (B) subcutaneously twice at 3-week intervals with adjuvanted immunogens (n = 5). Serum was analysed 2 weeks post-immunisation. Dotted lines denote detection cut off (dilution 1:400).



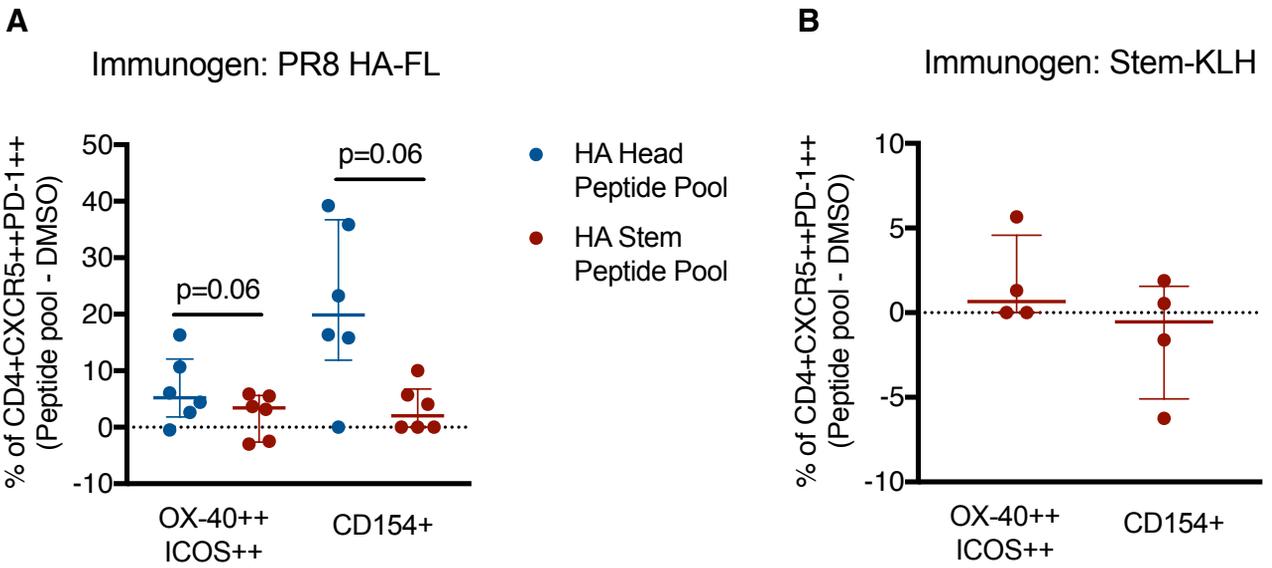
**Figure S13. Nanoparticle (NP) carrier rescues B cell and antibody responses against HA-stem**

(A) Representative flow cytometry plots and (B) frequency of MLN GC B cells (B220+/IgD-/CD38lo/GL7+) double stained with HA-stem probes (A/Puerto Rico/08/1934). (C) Serum endpoint total IgG titres were measured by ELISA using HA-stem proteins. Mice were vaccinated twice intramuscularly at 3-week intervals with adjuvanted (Addavax) immunogens (n = 5). B cell probe staining was performed 2 weeks after the last immunisation and serum was analysed every 2 weeks post-immunisation. Dotted lines denote detection cut off (dilution 1:400). Data represent mean  $\pm$  SEM.

**A****B****C****D**

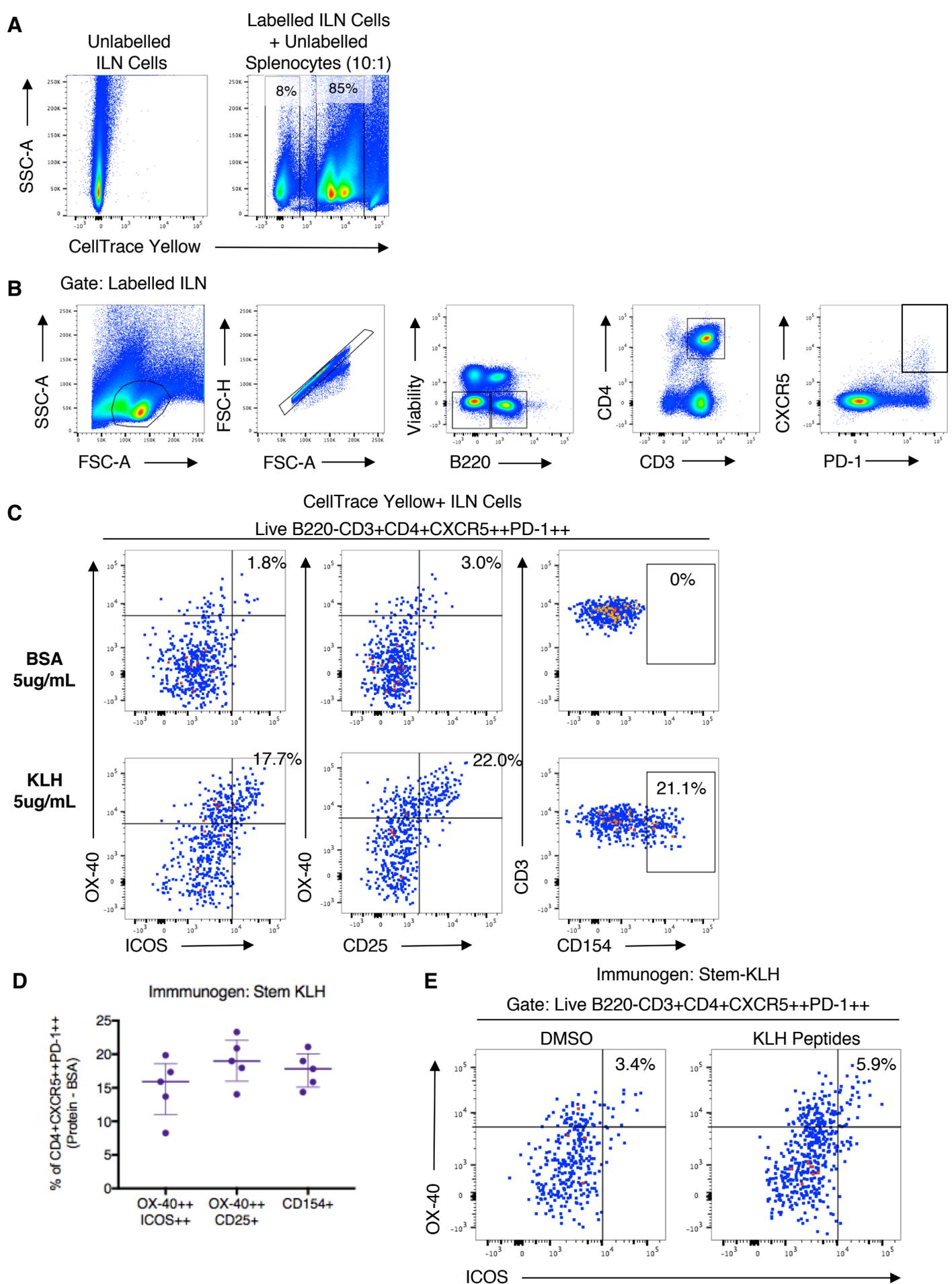
**Figure S14. Identification of bulk and antigen-specific Tfh cells in the lymph node and spleen**

**(A)** Bulk Tfh cells were identified first by gating on lymphocytes (FSC-A vs SSC-A) and single cells (FSC-A vs FSC-H). After removal of dead/F4-80+/B220+ cells, CD3+CD4+ T cells were identified and the Tfh population was defined as CXCR5++PD-1++ cells. **(B)** At day 14 post-infection with A/Puerto Rico/08/1934 virus, MLN-derived cells or splenocytes were stimulated with an HA peptide pool in the presence of anti-CD154 antibody to identify antigen-specific upregulation of CD154 compared to a DMSO control. **(C)** Representative ex vivo staining of OX-40, ICOS and CD25 expression on MLN CD4+ T cells and Tfh cells. **(D)** Identification of antigen-specific Tfh cells following 18 hours of peptide stimulation by gating on OX-40++ICOS++ and OX-40++CD25+ cells. Representative staining is shown for MLN and splenocyte-derived Tfh cells.



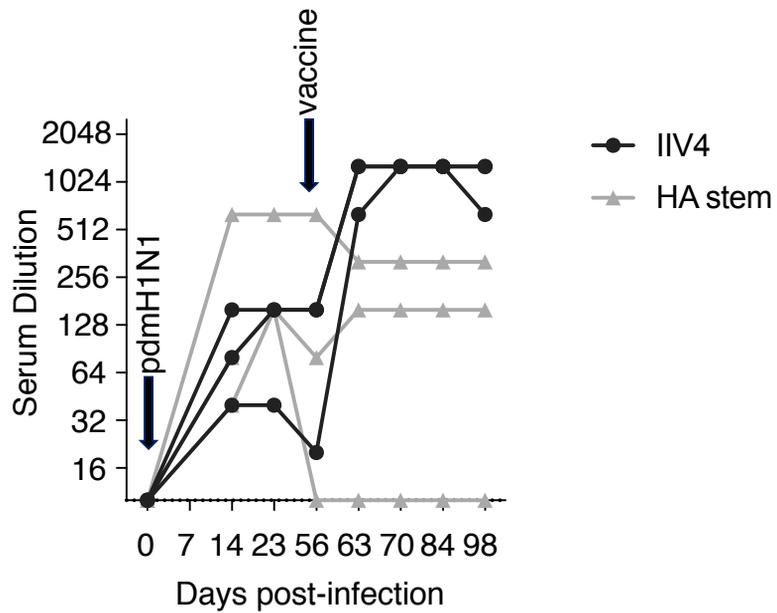
**Figure S15. Identification of antigen-specific Tfh cells in BALB/c mice**

ILN cells were collected at day 14 post-vaccination with (A) HA-FL protein (n = 6) or (B) stem-KLH protein (n = 4) and stimulated with HA Head (blue dots) or HA Stem (red dots) peptide pools. Antigen-specific Tfh cells were quantified according to surface marker upregulation or CD154 expression as in Figure 4. Bars indicate median and interquartile range.

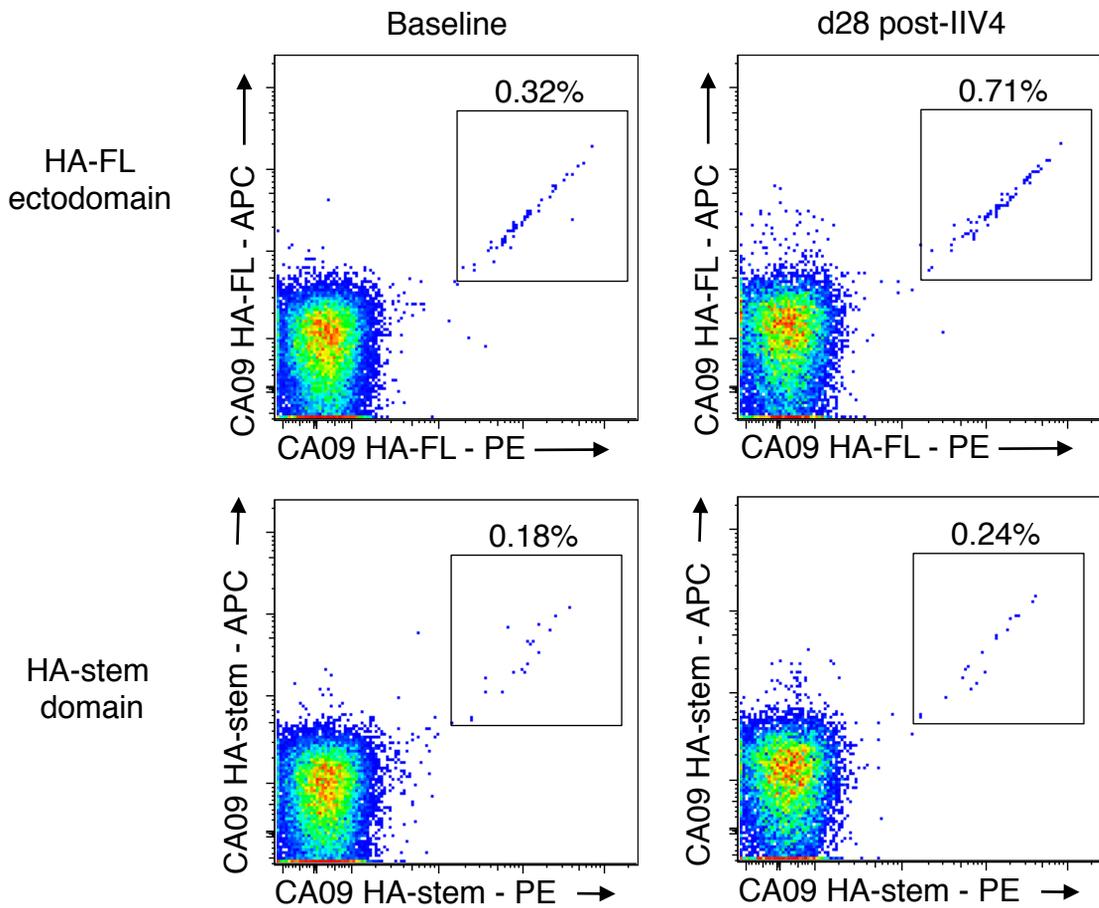
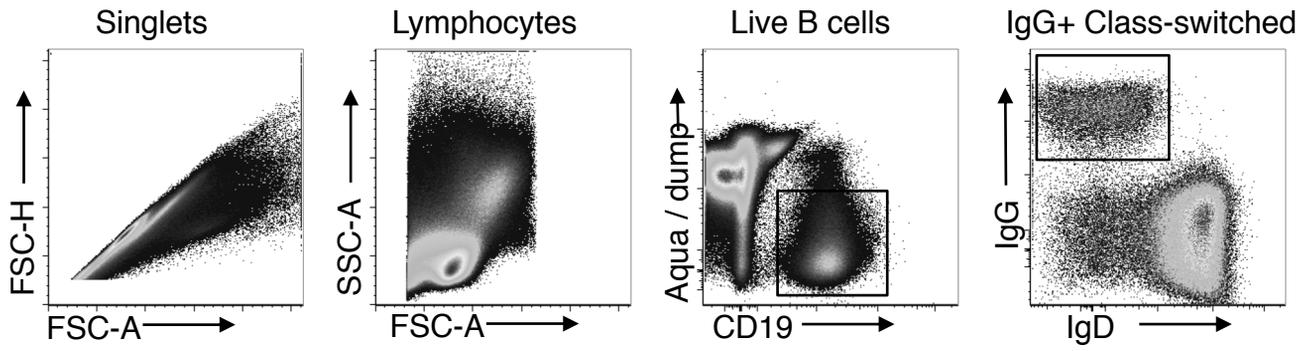


**Figure S16. Identification of KLH-specific Tfh cells using a whole protein assay**

(A) ILN-derived cells were labelled with CellTrace yellow dye and co-cultured for 18 hours with unlabelled splenocytes at a 10:1 ratio. Plots demonstrate identification of CellTrace yellow+ ILN cells after 18 hours of culture, with unlabelled, cultured ILN cells used as a gating control. (B) Tfh cells were identified as previously described, by gating on lymphocytes (FSC-A vs SSC-A), single cells (FSC-A vs FSC-H) and B220- viable cells. Tfh cells were defined as CD3+CD4+CXCR5++PD-1++ cells. (C) ILN/splenocyte co-cultures from stem-KLH vaccinated animals were stimulated with 5 µg/mL of either BSA or KLH protein in the presence of anti-CD154 antibody, and the expression of OX-40, ICOS and CD154 was assessed. Plots show a representative KLH-specific response in ILN cells from a single animal. (D) KLH-specific Tfh cells were quantified in n = 5 animals at day 14 post-vaccination with stem-KLH protein. (E) A pool of 20 15-mer KLH peptides, predicted by Tepitool to be immunogenic in C57BL/6 mice, was used to identify KLH-specific Tfh responses in ILN cells.

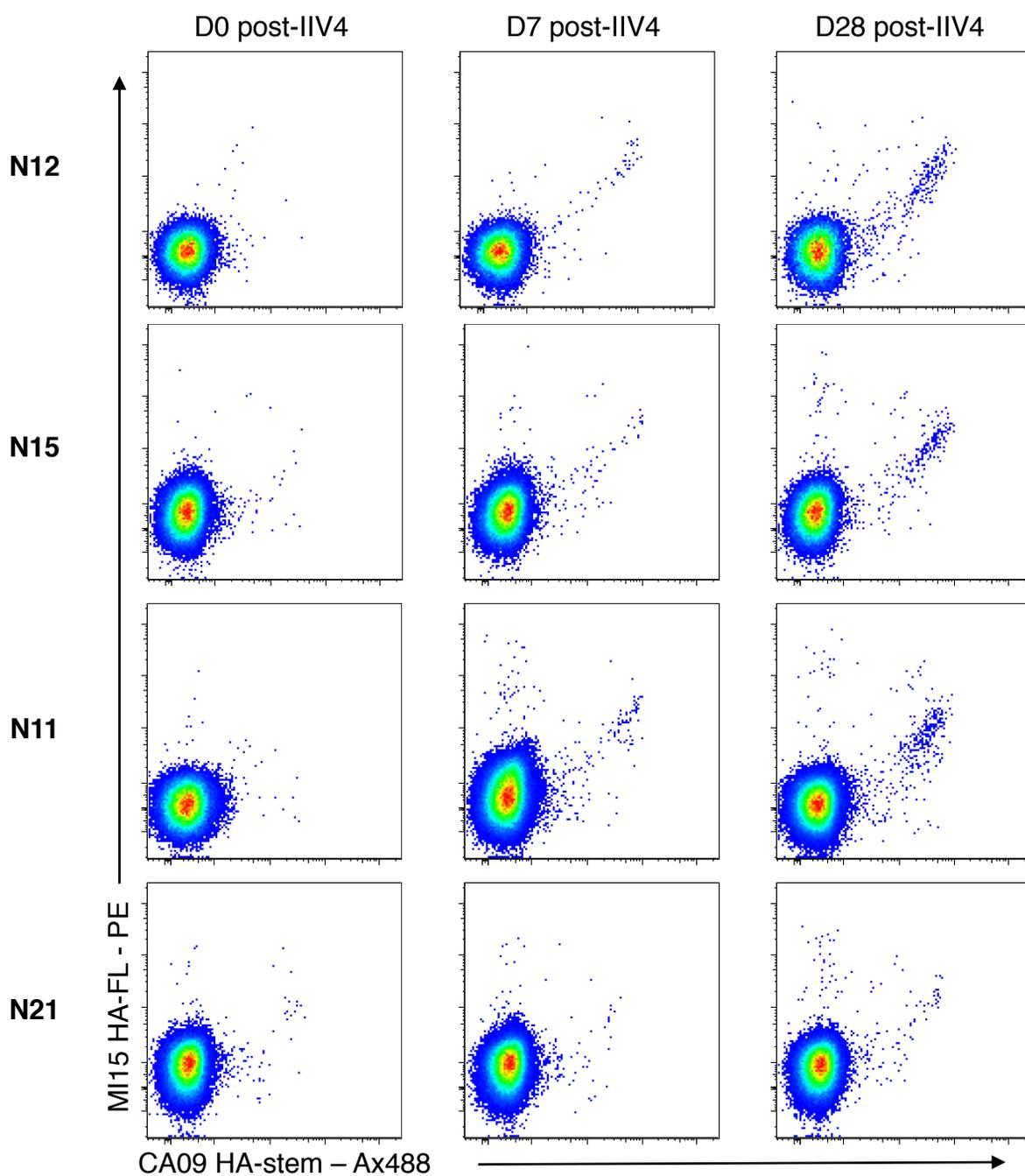


**Figure S17. Neutralisation activity against CA09 (focus reduction assay) in the sera of macaques infected with pdmH1N1 (D0) and immunised with IIV4 (n = 3) or HA-stem (n = 3) on day 56.**



**Figure S18. Representative gating scheme of human cryopreserved PBMC stained with HA-FL and HA-stem probes**

After doublet exclusion, and dumping of dead cells (Aqua Live Dead) and CD3/8/10/16/14 positive lymphocytes, IgG+ memory B cells (CD19+IgD-IgG+) enumerated based upon binding to recombinant HA probes.



**Figure S19. Marked expansion of stem-specific IgG+ memory B cells in selected individuals (4 out of 21 donors shown; N11/12/15/21 denotes donor codes) following immunisation with IIV4 (2017).**