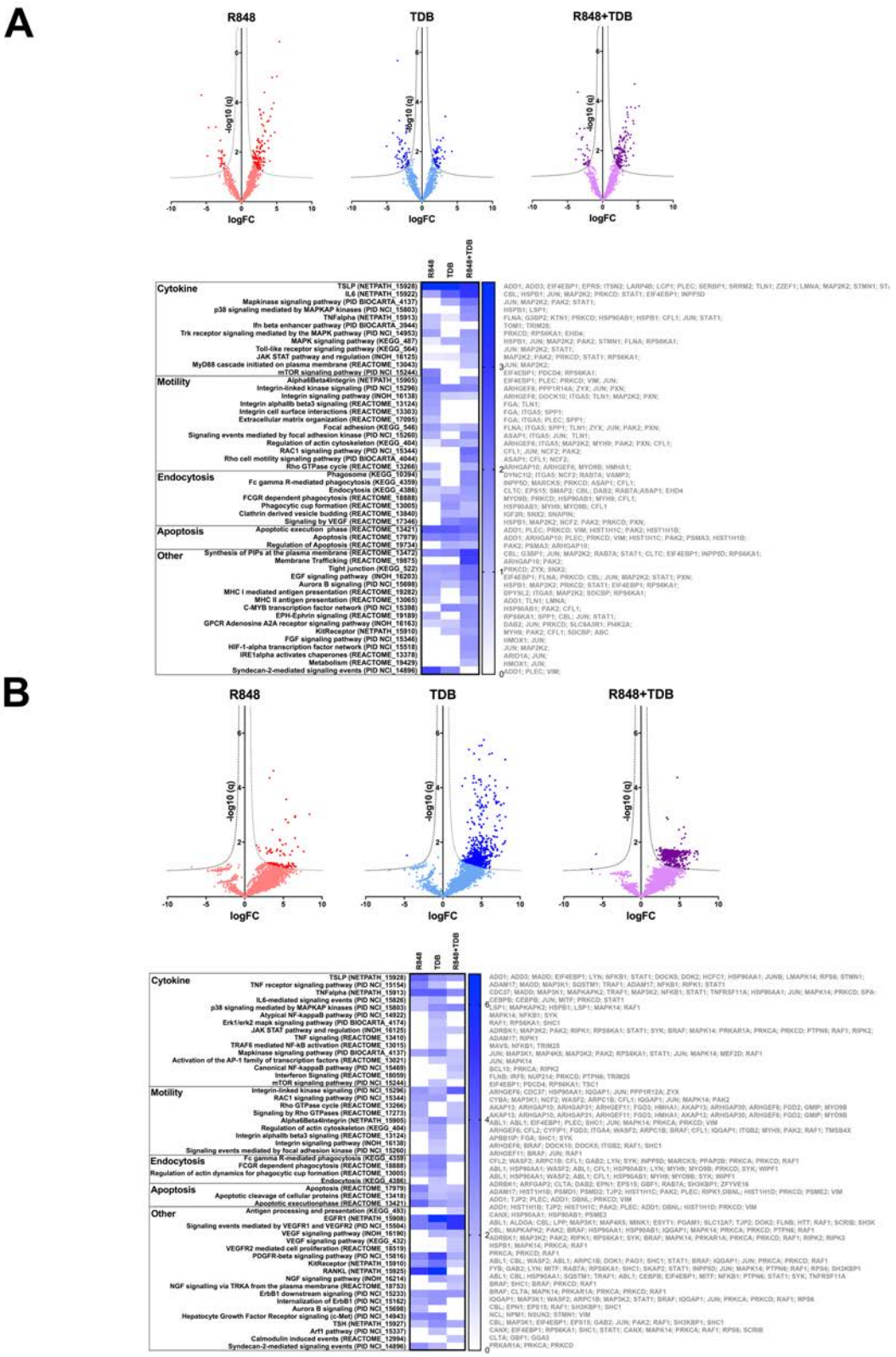
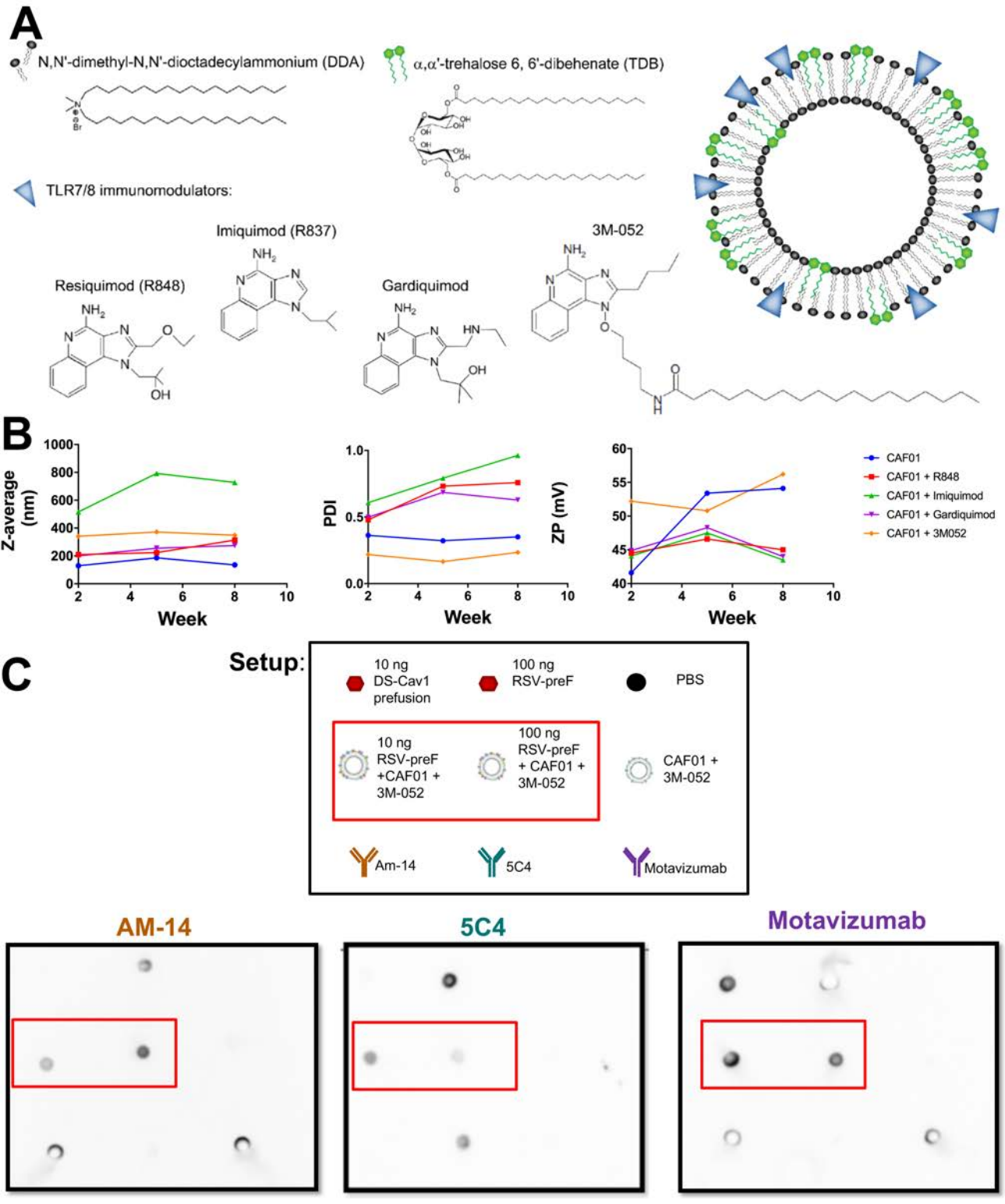
Supplementary Figure 1.



Supplementary Figure 1. Phosphopeptide quantification and pathway analysis.

Newborn (A) and adult **(B)** MoDCs were stimulated with 50 µM R848, 100 µg/ml TDB, combination, or vehicle control for 30 minutes. Heat map intensity indicates –log p Value obtained with pathway overrepresentation analysis of phosphopeptides quantified through mass-spectrometry. (Hypergemoetric test, InnateDB.com)

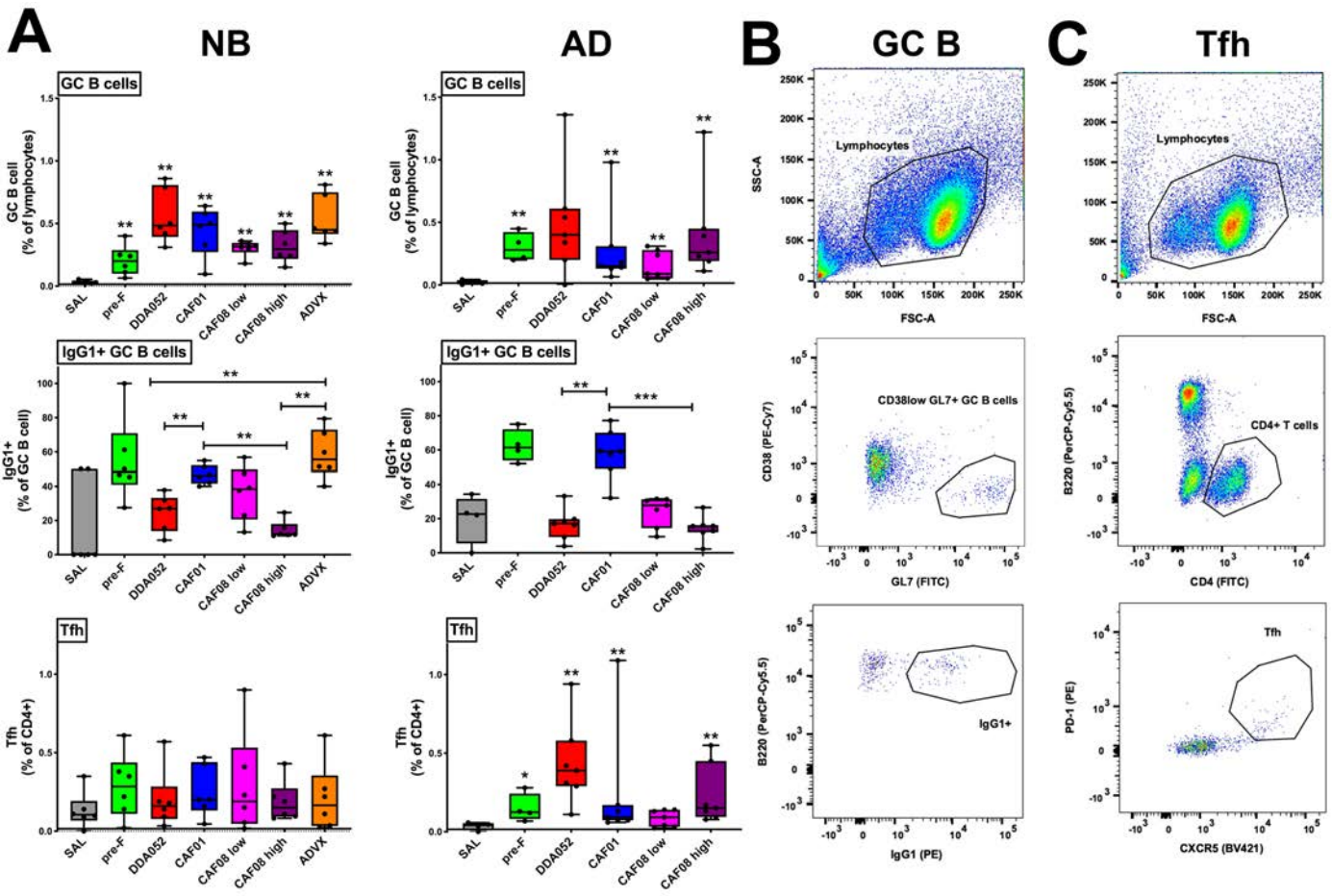
Supplementary Figure 2.



Supplementary Figure 2. Formulation optimization.

Different R848 congeners **(A)** were tested for icorporation into DDA and CAF01 liposomes. Liposomal stability was assessed by multiple dynamic light scattering measurements over 8 weeks **(B)**. Dot Blot of spotted formulations show the availability of three different antigenic epitopes on the pre-F protein after formulation, using monoclonal antibodies against pre-F as indicated **(C)**.

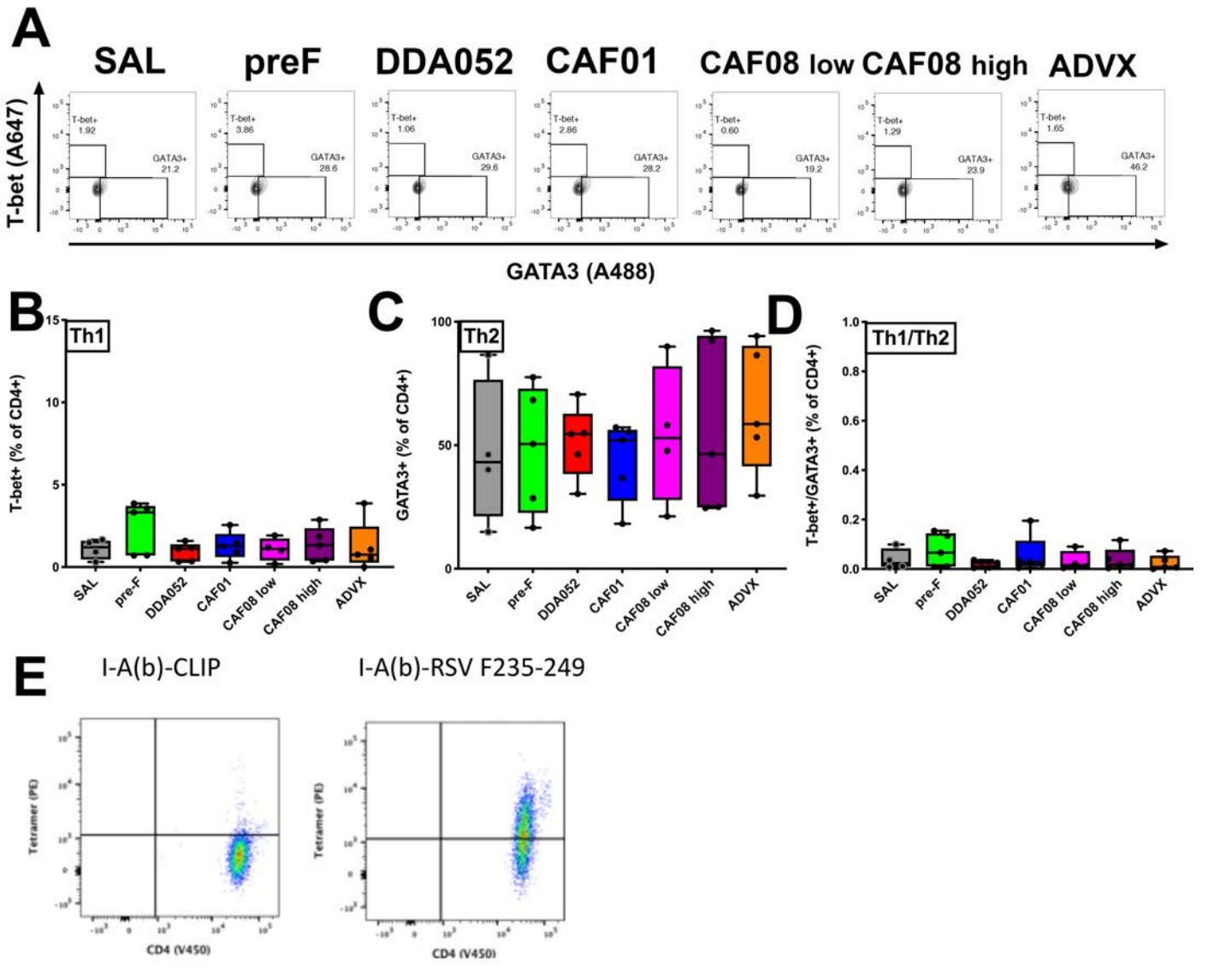
Supplementary Figure 3.



Supplementary Figure 3. Combination adjuvant-containing liposomes promote formation of germinal center IgG1- B cells and generation of Tfh cells.

Brachial or inguinal lymph node cell suspensions from immunized animals were analyzed phenotypically by flow cytometry. Immunization with formulations containing pre-F increased B220+GL7+CD38lo germinal center (GC) B cells in newborn and adult mice. A reduction in IgG1+ GC B cells was noted after treatment with 3M-052-containing formulations, consistent with serology results in Fig. 3 indicating isotype switching to IgG2a/c in those groups. An increase in B220-CD4+PD-1hiCXCR5+ T follicular helper (Tfh) cells occurred in adult mice immunized with DDA052, CAF01 or CAF08b_h formulations, but not in newborn mice. All statistical comparisons are Mann-Whitney U-test comparisons to saline groups.

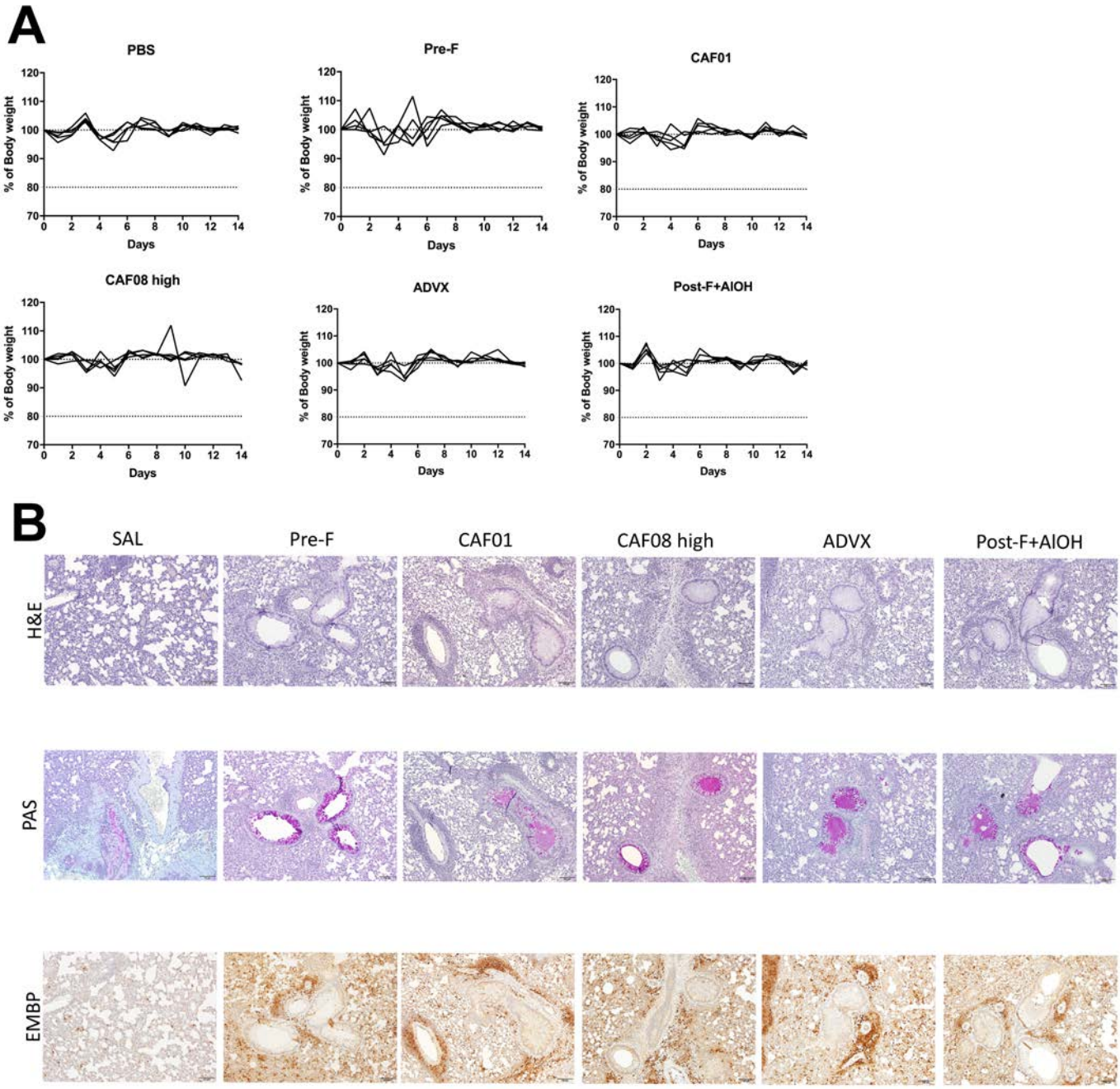
Supplementary Figure 4.



Supplementary Figure 4. Tetramer-negative T cell phenotype and teramer-positive clone development.

Spleen and lymph node cell suspensions were stained for flow cytometry with a pre-F -specific MHC II tetramer (see figure 4) and stained for intracellular presence of Th1 transcription factor T-bet or Th2 transcription factor GATA3. Representative histograms of teramer-negative cells are shown in panel **(A).** The phenotype of tetramer-negative cells was predominantly Th2-skewed in all treatment groups, in accordance with the neonatal phenotype. This indicated that Th1 induction by CAF08 was antigen-specific and did not result in systemic changes in Th1/Th2 balance. **(B).** LN cells from a CAF08-immunized mouse were stained with the tetramer and tetramer-positive cells were single-cell sorted and expanded using anti-CD3/28 beads. This resulted in the generation of a Tetramer-positive T cell clone **(C)**.

Supplementary Figure 5.



Supplementary Figure 5. Pathological evaluation of RSV-infected CB6F1 mice

Immunized mice as described in Figures 4+5 were challenged with live RSV A2 strain to assess protective potential of the vaccine formulations. Weight of each animal was measured over a period of 14 days (A). **(A).** Lungs from 3 animals in each immunization group were stained with H&E. PAS and EMBP for histopathological assessment of damage and immune cell infiltration **(B).**