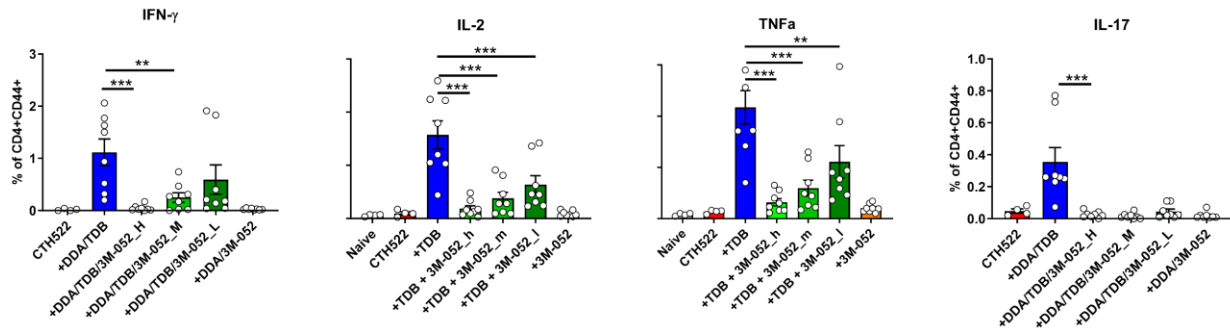
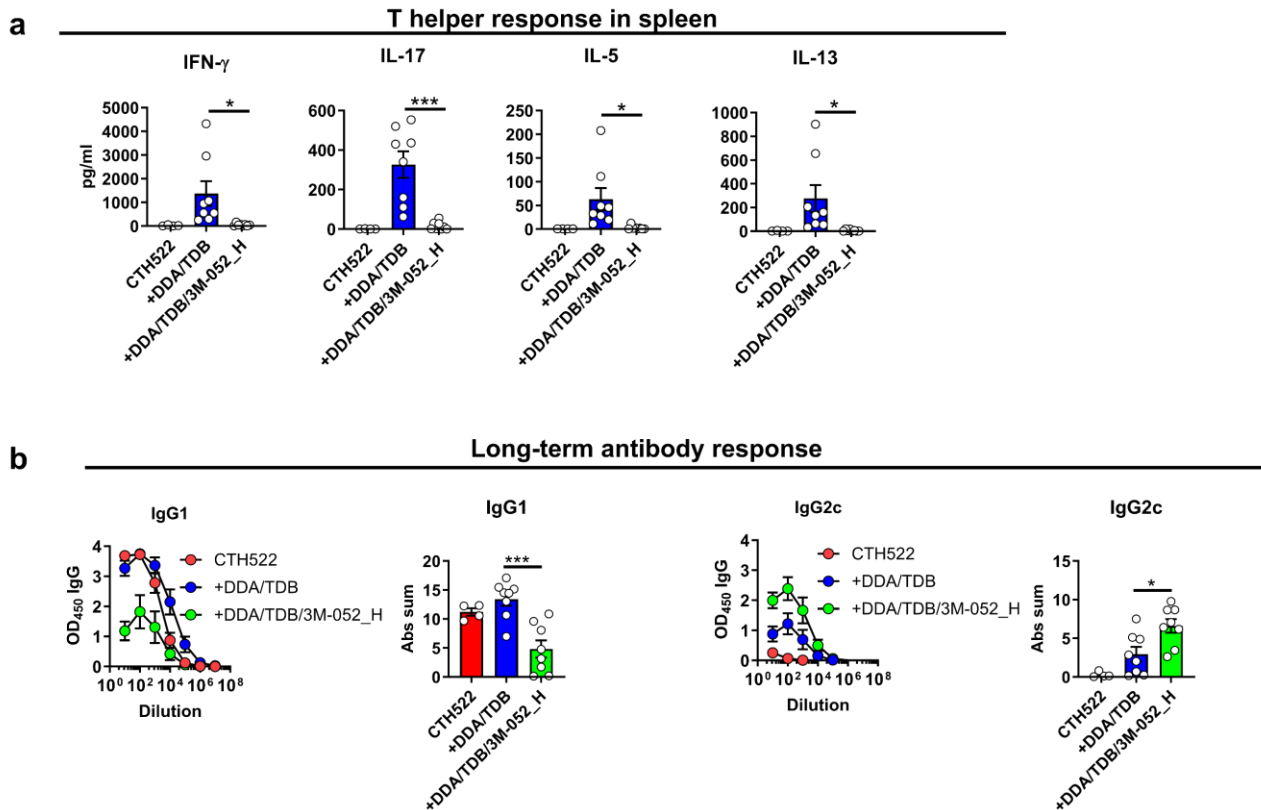


Supplementary information



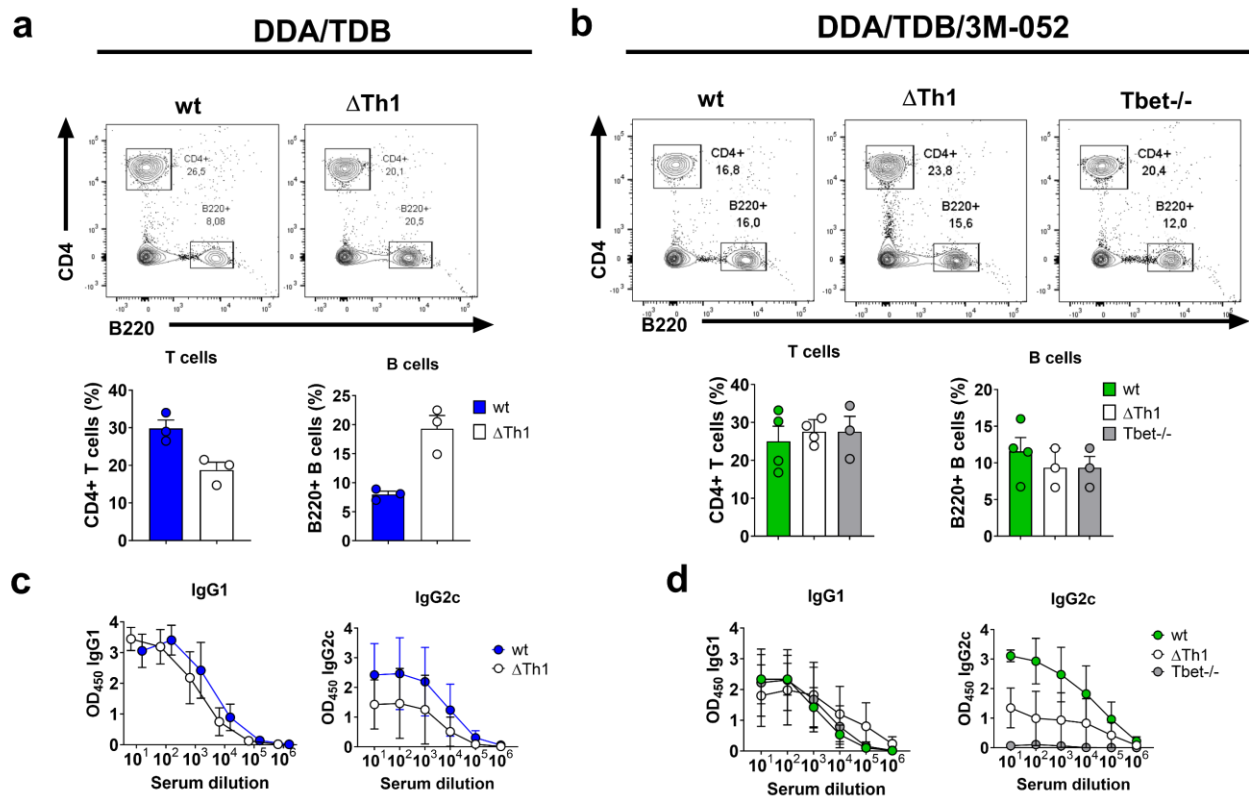
Supplementary Figure 1. Splenic Th1 responses are impaired when 3M-052 is incorporated in DDA/TDB

CB6F1 mice were vaccinated subcutaneously with 2 μ g of the recombinant protein antigen CTH522 either alone in the presence of the indicated cationic liposomal adjuvant. Adjuvants tested were cationic DDA liposomes containing either TDB (DDA/TDB), 3M-052 (DDA/3M-052) or both (DDA/TDB/3M-052). DDA/TDB/3M-052 was tested with three different doses of 3M-052 (H, M, L -corresponding to a dose of 10 μ g, 2 μ g or 0.4 μ g, respectively). Mice were immunized twice, with three weeks apart, and sacrificed three weeks after the second immunization. Splenic CD4 T cell responses were measured by intracellular staining of the indicated cytokines by flow cytometry. The cells were gated as CD4+CD44+. Groups consisted of 4 (antigen alone) or 8 (antigen +adjuvants) mice. The experiment was performed once. Each point represents individual mice (bars indicate mean $\pm$ SEM). Statistically significant differences between groups are indicated by ** and *** (One-way ANOVA with Tukey's correction for multiple group comparison, using the DDA/TDB group as reference and significance levels of p<0.01 and p<0.001 respectively).



Supplementary Figure 2. Incorporating 3M-052 into DDA/TDB liposomes abrogates Th1/Th17 responses

Mice were vaccinated subcutaneously with 2 μ g of the recombinant protein antigen CTH522 either alone in the presence of the indicated cationic liposomal adjuvant. Mice were immunized twice and sacrificed three months after the second immunization. **A)** T helper cell responses in the spleen were measured by re-stimulation with antigen and measuring secreted IFN- γ , IL-17, IL-5 and IL-13. **B)** Antibody responses were measured in serum by ELISA and displayed as the ratio between IgG2c and IgG1 responses. The experiment was performed once. Data are depicted as mean+SEM. Statistically significant differences between groups are indicated by * and *** (Two-tailed unpaired t-test, with significance levels of $p < 0.05$ and $p < 0.001$ respectively).



Supplementary Figure 3. IgG2c switching requires T-bet but not Th1 cells

Bone marrow chimeras lacking Th1 cells, or lacking T-bet in both T and B cells, were generated by cell transfer to RAG1^{-/-} mice. The mice were immunized with the CTH522 antigen formulated in cationic DDA liposomes containing either TDB (DDA/TDB) or TDB and 3M-052 (DDA/TDB/3M-052), using the medium dose of 3M-052 (2 μ g). The mice received three doses each three weeks apart. **A)** Frequencies of CD4⁺ T cells and B220⁺ B cells in blood measured one week after the second immunization. **B)** Antigen-specific IgG1 and IgG2c responses measured in serum three weeks after the third immunization with TDB (DDA/TDB) (left panel) or TDB (DDA/TDB/3M-052) (right panel). Groups consisted of 3 - 4 mice and data are depicted as mean+SEM. The experiment was performed once.