Supporting Information

Gold-Nanostar-Chitosan Mediated Delivery of SARS-CoV-2 DNA Vaccine for Respiratory Mucosal Immunization: Development and Proof-of-Principle

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Supplementary Figures:

Figure S1

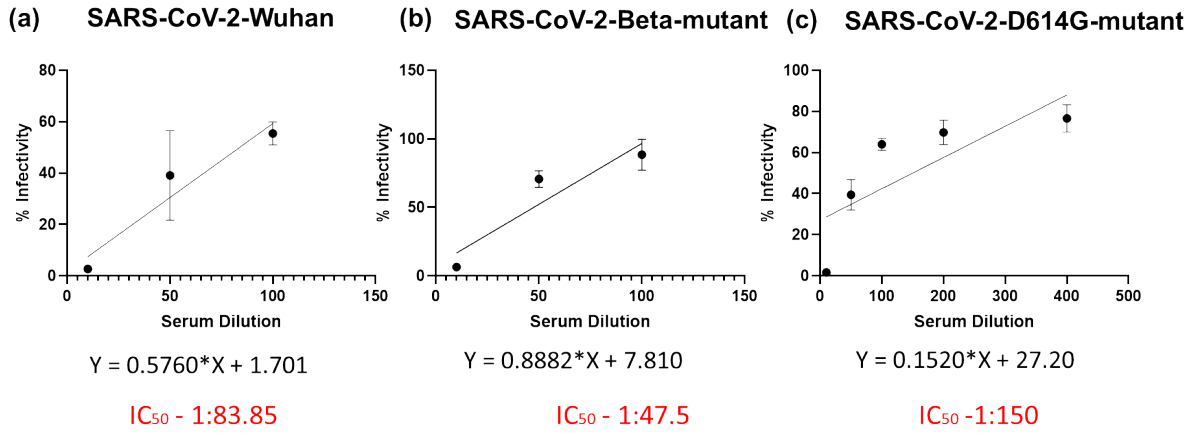
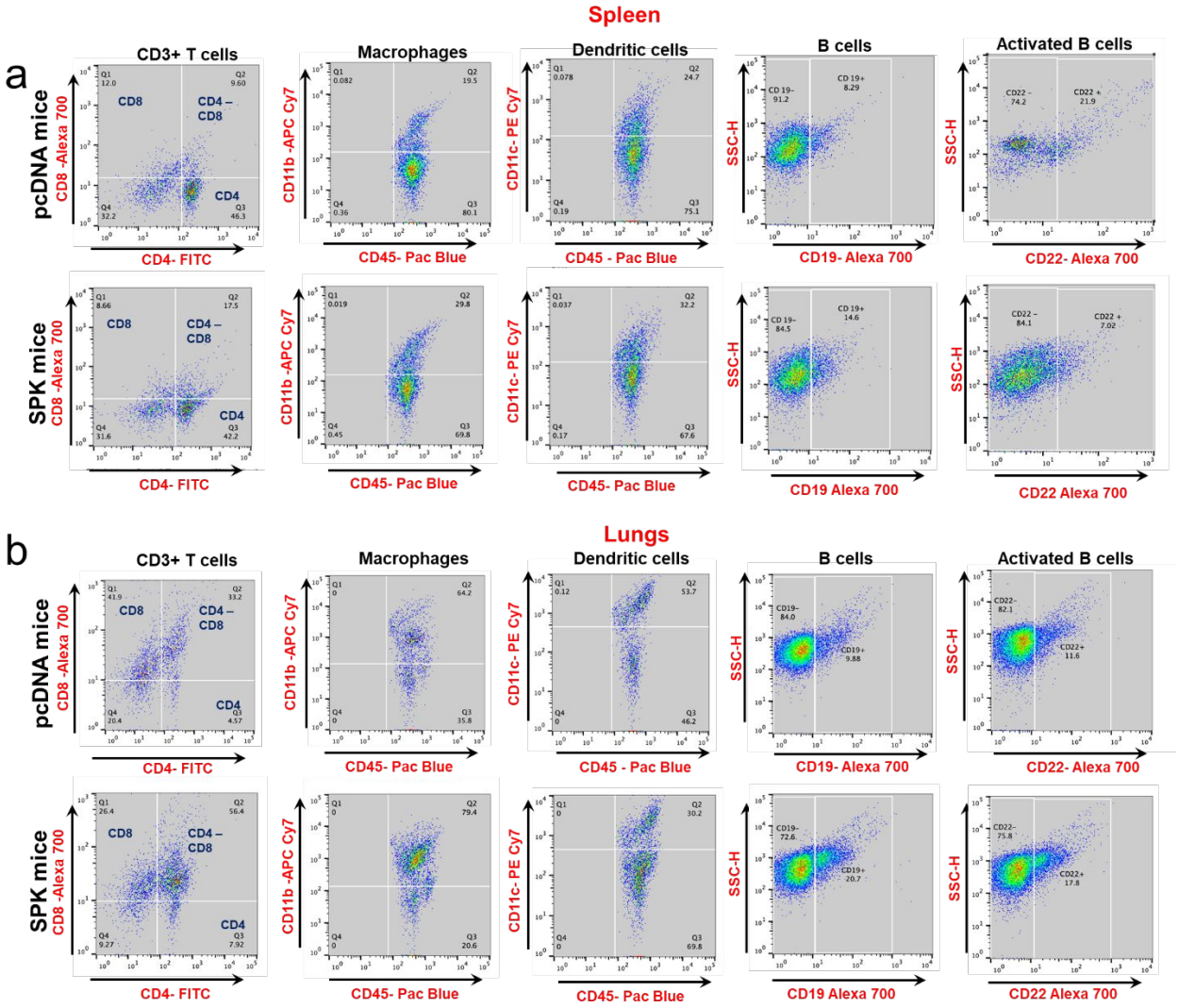


Figure S1: Neutralization IC 50 (serum dilution factor) for relative inhibition in infectivity of lentiviral particles engineered with S protein SC2-Wuhan **(a)**, SC2-Beta-mutant **(b)** and SC2-D614G-mutant **(c)** variants.

Figure S2


Spleen
a
CD3+ T cells
Macrophages
Dendritic cells
B cells
Activated B cells
pcDNA mice
SPK mice
CD8 -Alexa 700
CD4- FITC
CD11b -APC Cy7
CD45- Pac Blue
CD11c- PE Cy7
CD45 - Pac Blue
SSC-H
CD19- Alexa 700
CD19 Alexa 700
CD22- Alexa 700
CD22 Alexa 700
Lungs
b

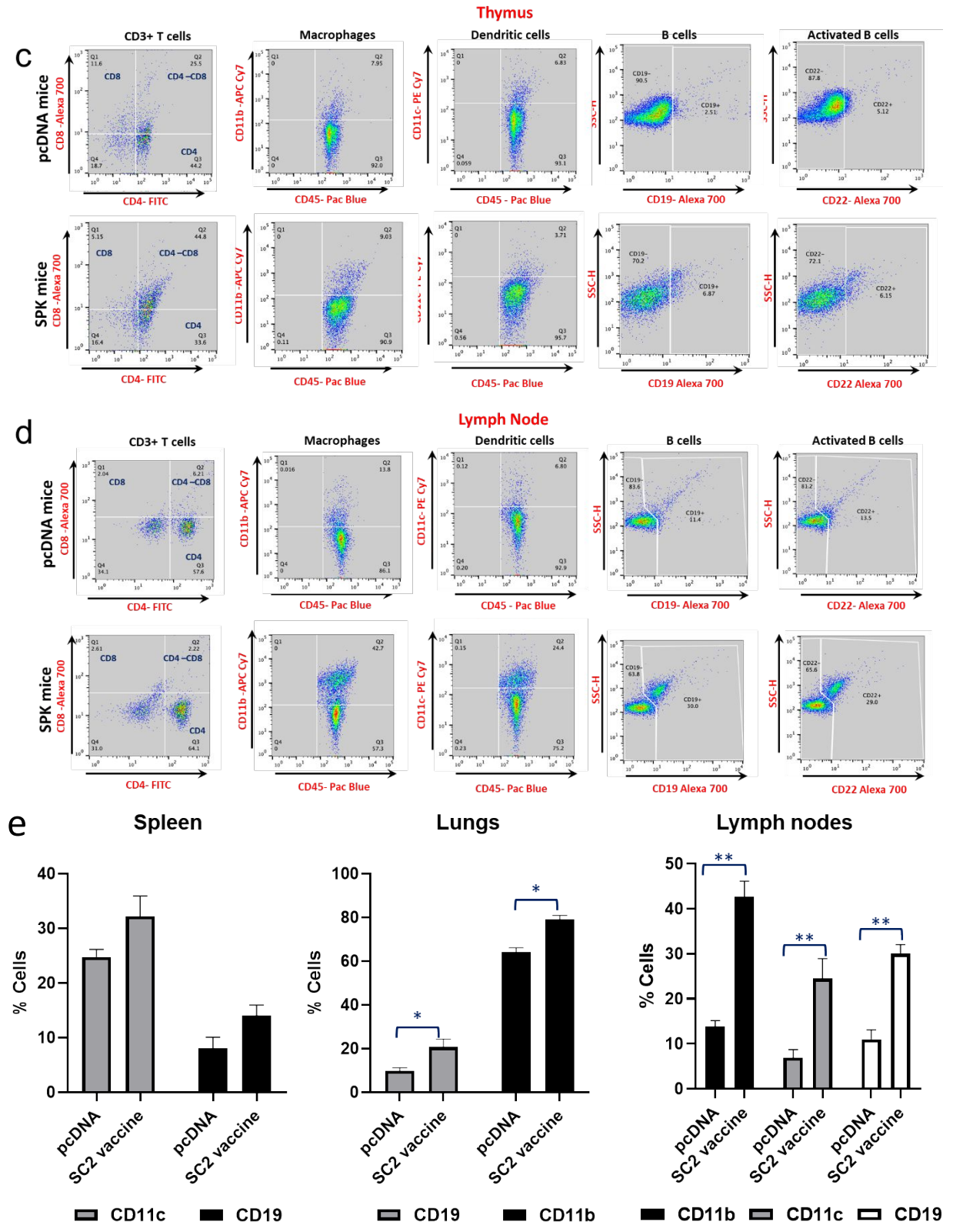


Figure S2. Resident T cell distribution in the spleen **(a)**, lungs **(b)**, thymus **(c)**, and lymph nodes **(d)** of mice IN treated with DNA vaccine expressing S protein of SC2. The DNA vaccine induced

CD4+ T cells expressing memory and tissue-resident markers were used for assessment. The number of lung resident CD4+ T cells increased in the lungs of the immunized mice compared to control DNA treated mice. The lungs, spleen, thymus and lymph nodes were dissected 14 weeks after treatment to evaluate for T cell subtypes. (T cell subtypes: CD3/CD4, CD3/CD8; macrophages: CD45/CD11b; dendritic cells: CD45/CD11c; B cells: CD19 and CD22) (n=3). (e) Quantitative representation of CD11c, CD19, and CD11b populations in spleen, lungs and lymph nodes. The data are presented as mean ± SD; One-way ANOVA with Bonferroni post hoc test was used to draw significance of comparisons as indicated. Adjusted *p*-values were considered statistically significant if *p*-values <0.05 and the symbols indicating statistical significance were as follows – * represents p<0.05, ** represents p<0.01 (n=3).

Figure S3

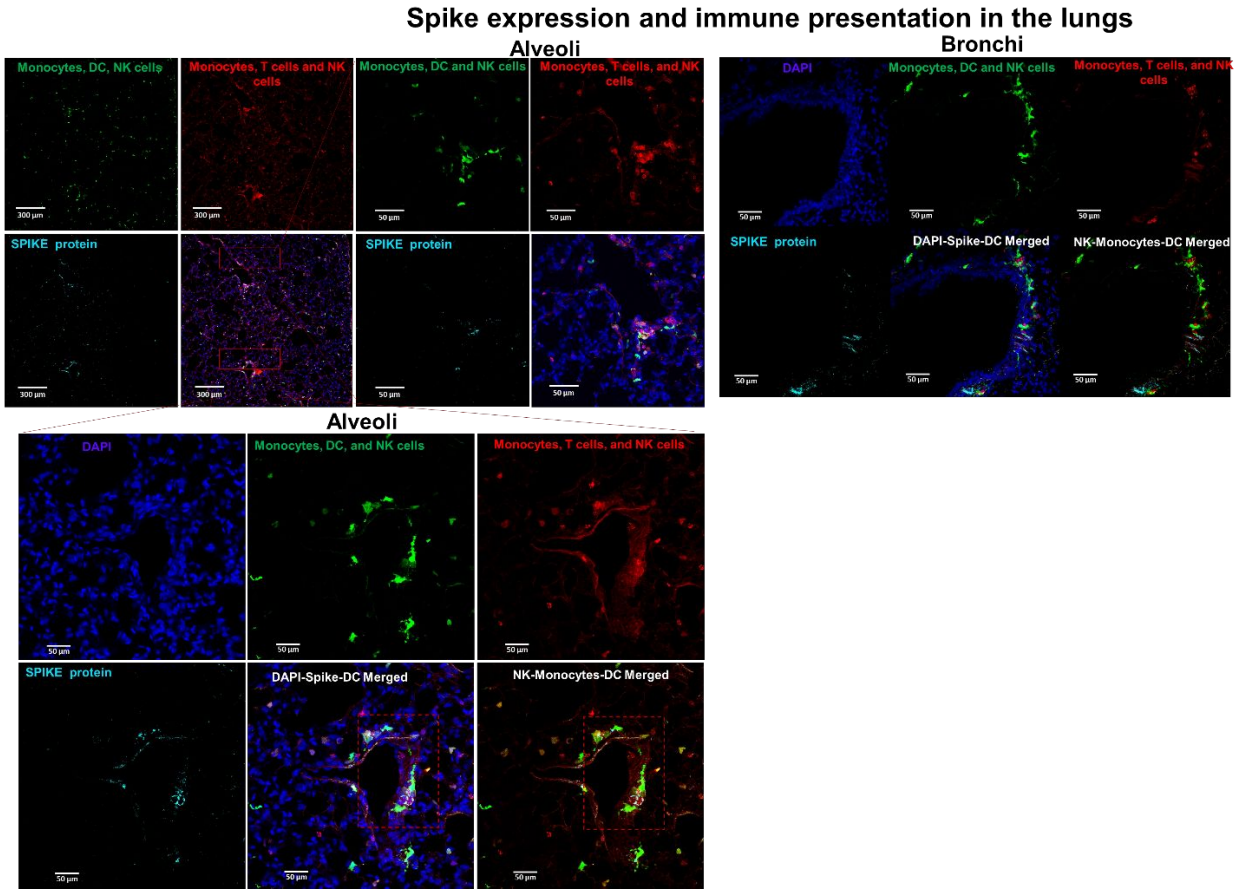


Figure S3. Interaction of the immune-surveillant DCs present in the airway epithelium with cells expressing SPK antigen enacts their role as professional antigen-presenting cells to NK cells and stimulates T cell response. The phagocytic alveolar macrophages also complement DCs in antigen presentation but at relatively low levels as they lack costimulatory molecules. The color codes represent the respective markers of cells.

Figure S4

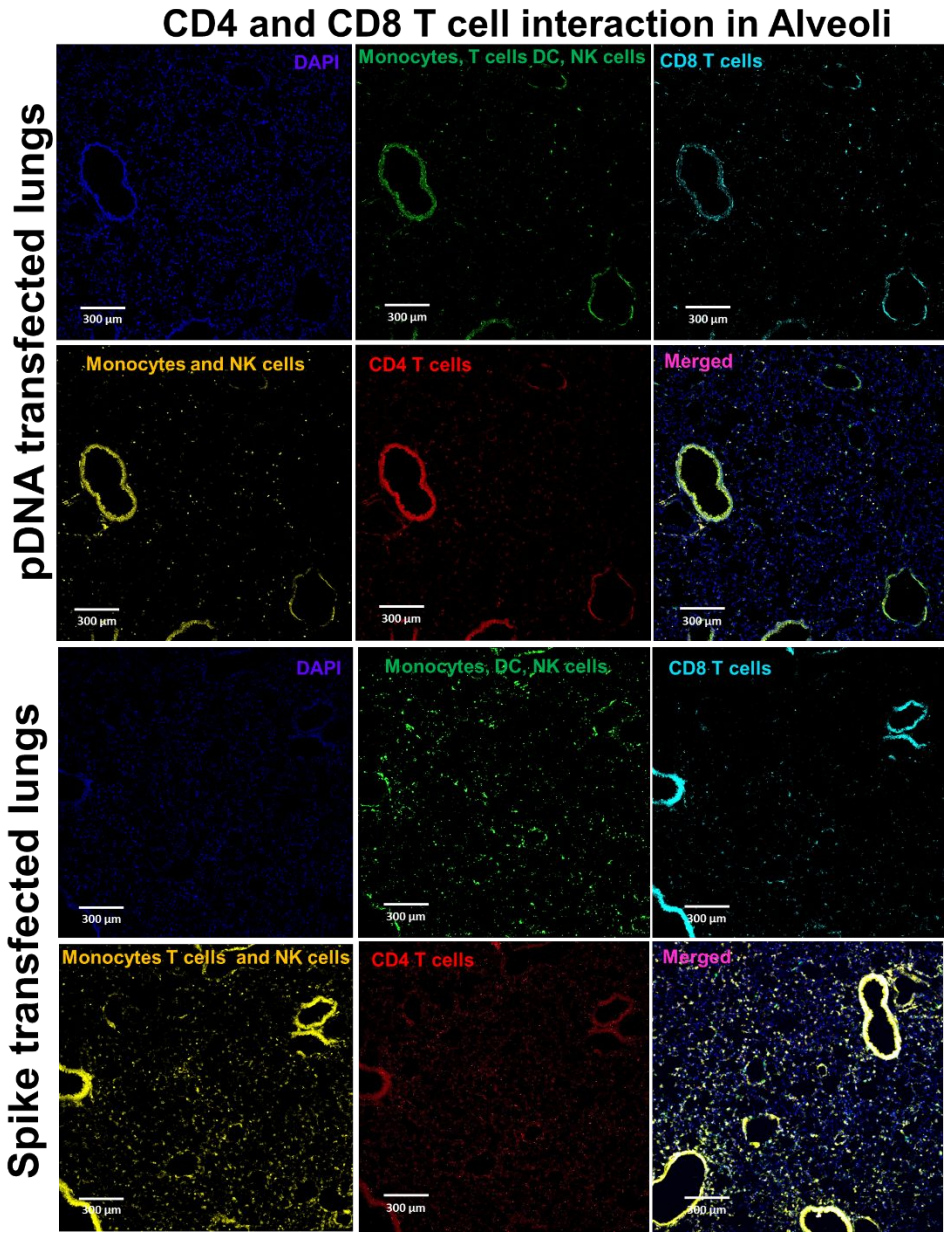


Figure S4. Evaluation of role of NK cells on DC and T cell responses in lungs upon IN administration of SPK vaccine. The increase in peripheral, immature local DCs in the SPK vaccinated lungs and their maturation and migration to draining lymph nodes drives the T cell response (CD4+ and CD8+ T cells) from the lymph nodes. As result of this effector T response CD4+ and CD8+ T cells increase in lungs. On the other hand, increase in classical effector cells of the innate immune system *i.e.* NK cell complements in mounting T cell mediated SPK immunogenicity and also play a protective role in mitigating inflammation and tissue damage by modulating DC function to impact T cell responses.

Figure S5

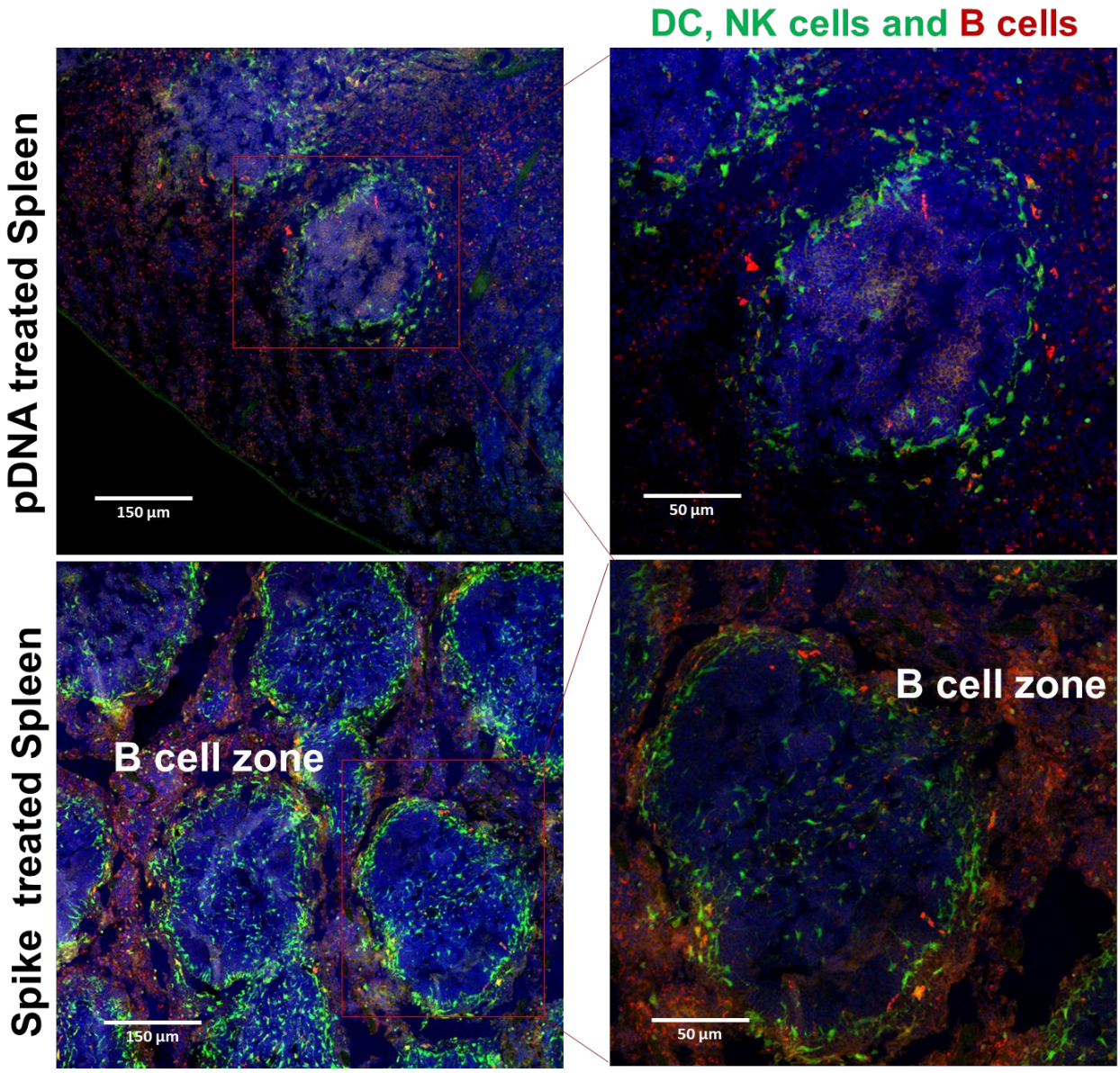


Figure S5. Marginal zone (MZ) B cells are the major constituent of the marginal zone, together with myeloid, dendritic, and stromal cells. These MZ B cells are the main producers of IgM antibodies against S antigen. The enrichment and mobilization of MZ B cells indicate the characteristics of splenic immune response.

Figure S6

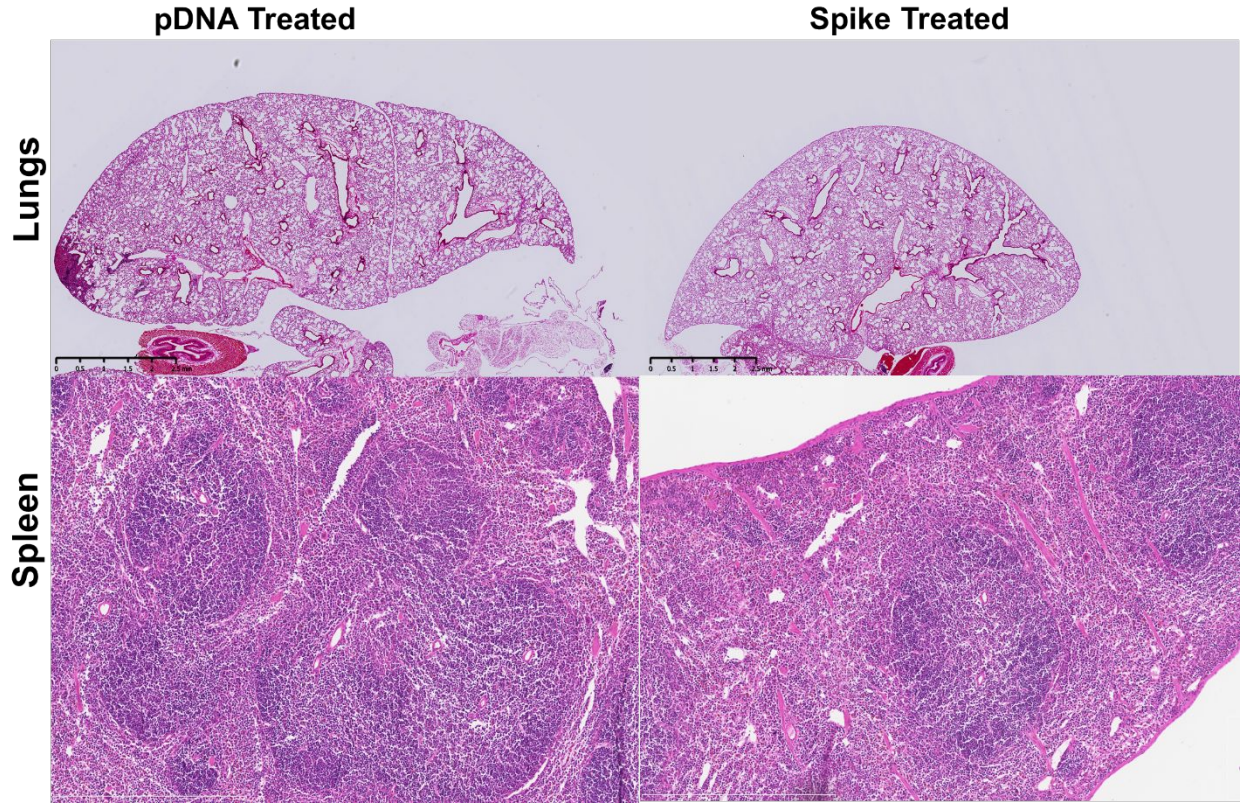


Figure S6. Hematoxylin and Eosin staining of histological slices of lung and spleen tissues collected from mice treated with pcDNA or pcDNA-SC2-spike delivered using AuNS-chitosan for toxicological observations.

Figure S7

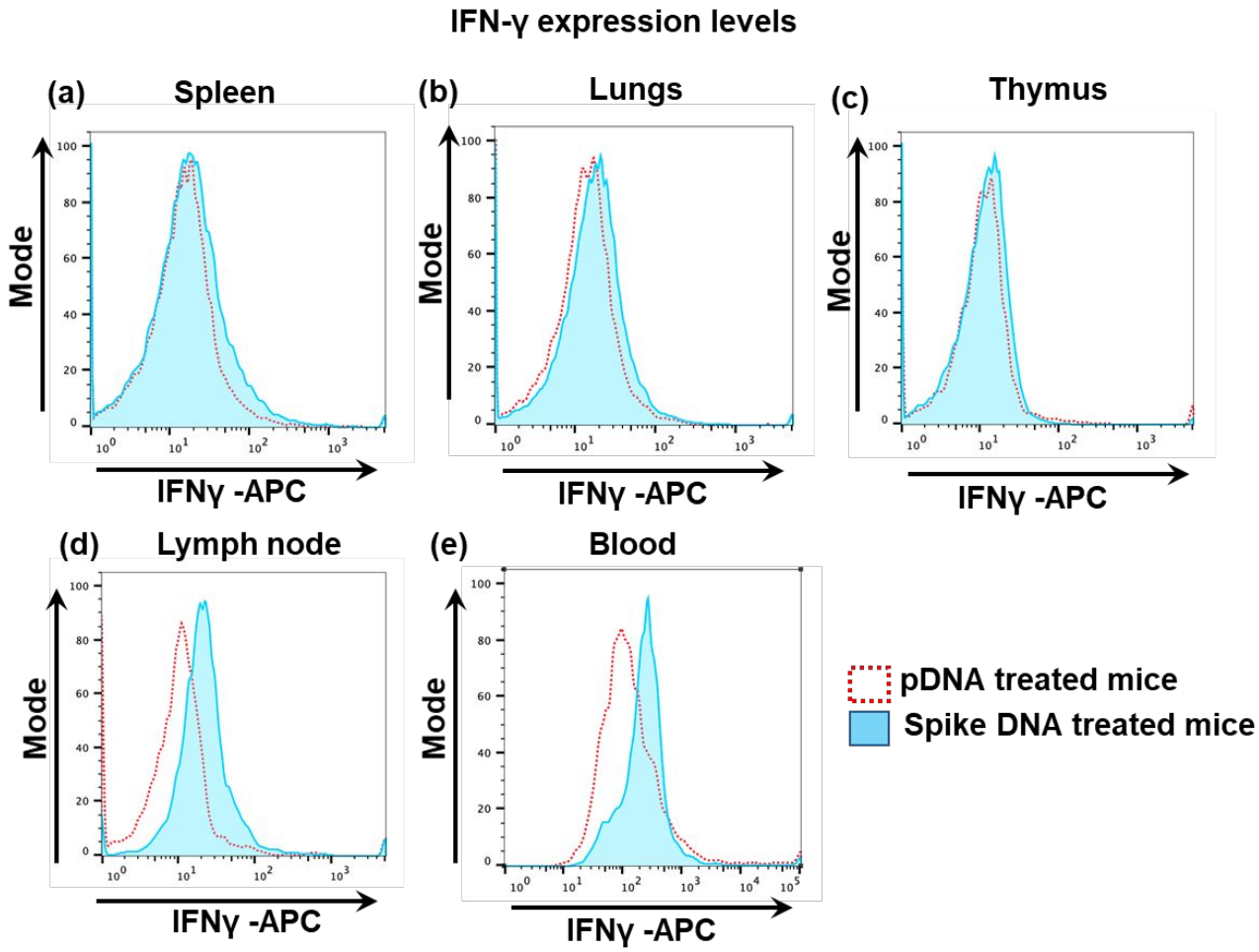


Figure S7. Flow cytometry analysis of IFNγ expression in CD45+ T cells isolated from spleen, lungs, thymus, lymph nodes, and blood of BALB/c mice treated with pcDNA and SC2-spike DNA(n=3).