

# DNA VACCINE candidate encoding SARS-CoV-2 spike proteins elicited potent humoral and Th1 cell-mediated immune responses in mice

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## Abstract

More than 426 million people have been confirmed infection with SARS-CoV-2 and more than 5 million have died from COVID-19 and this pandemic remains critical worldwide. Effective vaccines are one of the most important strategies to limit the pandemic. Here, we report a construction strategy of DNA vaccine candidates expressing full length wild type SARS-CoV-2 spike (S) protein, S1 or S2 region and their immunogenicity in mice. All DNA vaccine constructs of pCMVkan-S, -S1 and -S2 induced high levels of specific binding IgG that showed a balance of IgG1/IgG2a response. However, only the sera from mice vaccinated with pCMKkan-S or -S1 DNA vaccines could inhibit viral RBD and ACE2 interaction. The highest neutralizing antibody (NAb) titer was found in pCMVkan-S group, followed by -S1, while -S2 showed the lowest PRNT50 titers. The geometric mean titers (GMTs) were 2,551, 1,005 and 291 for pCMVkan-S, -S1 and -S2, respectively. pCMVkan-S construct vaccine also induced the highest magnitude and breadth of T cells response. Analysis of IFN- $\gamma$  positive cells after stimulation with SARS-CoV-2 spike peptide pools were 2,991, 1,376 and 1,885 SFC/106 splenocytes for pCMVkan-S, -S1 and -S2, respectively. Our findings highlighted that full-length S antigen is more potent than the truncated spike (S1 or S2) in inducing of neutralizing antibody and robust T cell responses.

## Background

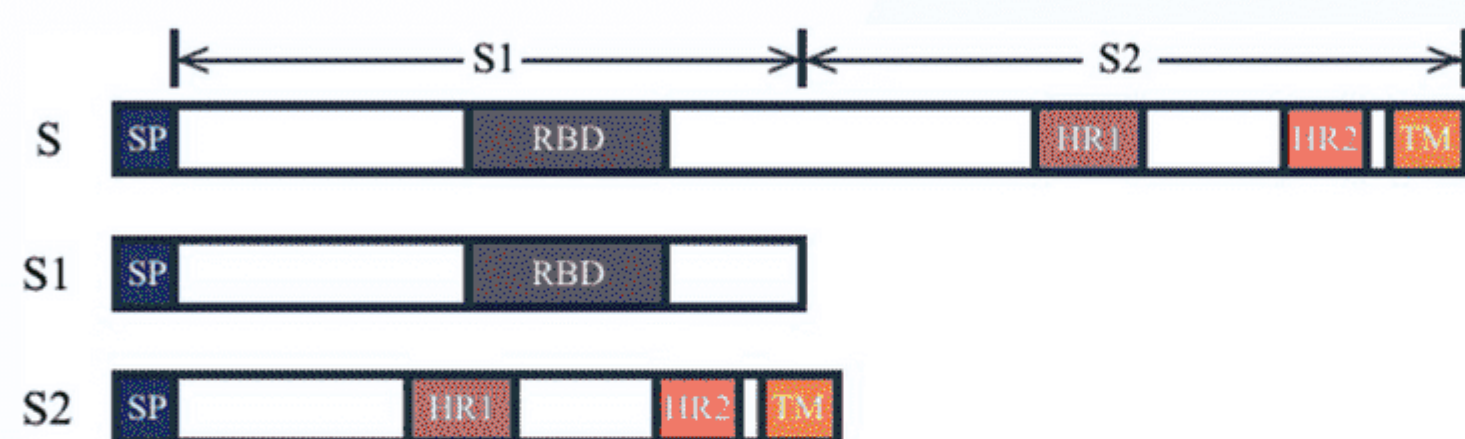
The vaccine for the current outbreak must be produced at a high speed and be easily scalable. Recombinant protein, viral-vectored or nucleic acid-based (recombinant plasmid DNA and mRNA) vaccines should be considered to shorten the vaccine production timeframe. The lessons learnt and information from previous related coronaviruses outbreak, such as SARS in 2003 and MERS in 2012, revealed that the immune responses against viral spike (S) protein played an important role in viral infection inhibition.

## Objectives

The objective was to investigate the immunogenicity of SARS-CoV-2 spike either as a full-length wild type or truncated as S1 or S2 in this DNA vaccine design platform in murine model.

## Methods

### • Vaccine construction



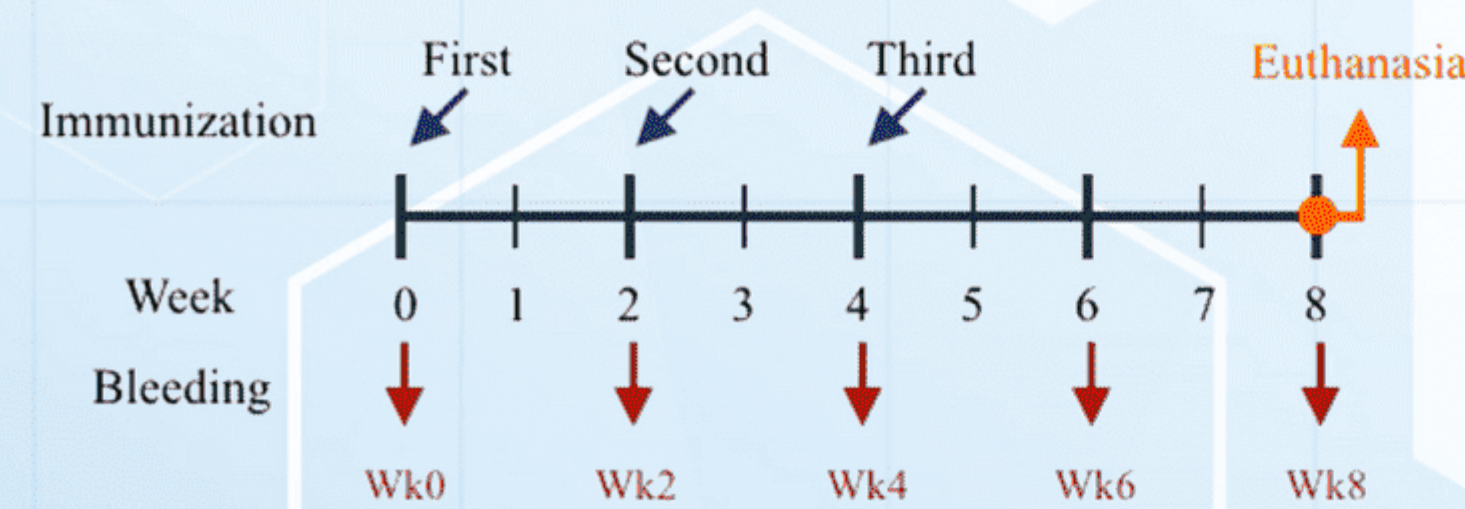
**Fig 1:** Schematic diagram of three DNA vaccine constructs; SP: signal peptide, RBD: receptor binding domain, HR: heptad repeat, TM: transmembrane domain.

### • Protein expression analysis

HEK293T cells were transfected with pCMVkan-S, pCMVkan-S1, pCMVkan-S2 or empty vector. At 24 h post-transfection, Transfected cell were analyzed for in vitro target protein expression under fluorescence microscope (Olympus, Japan).

### • Mice immunization

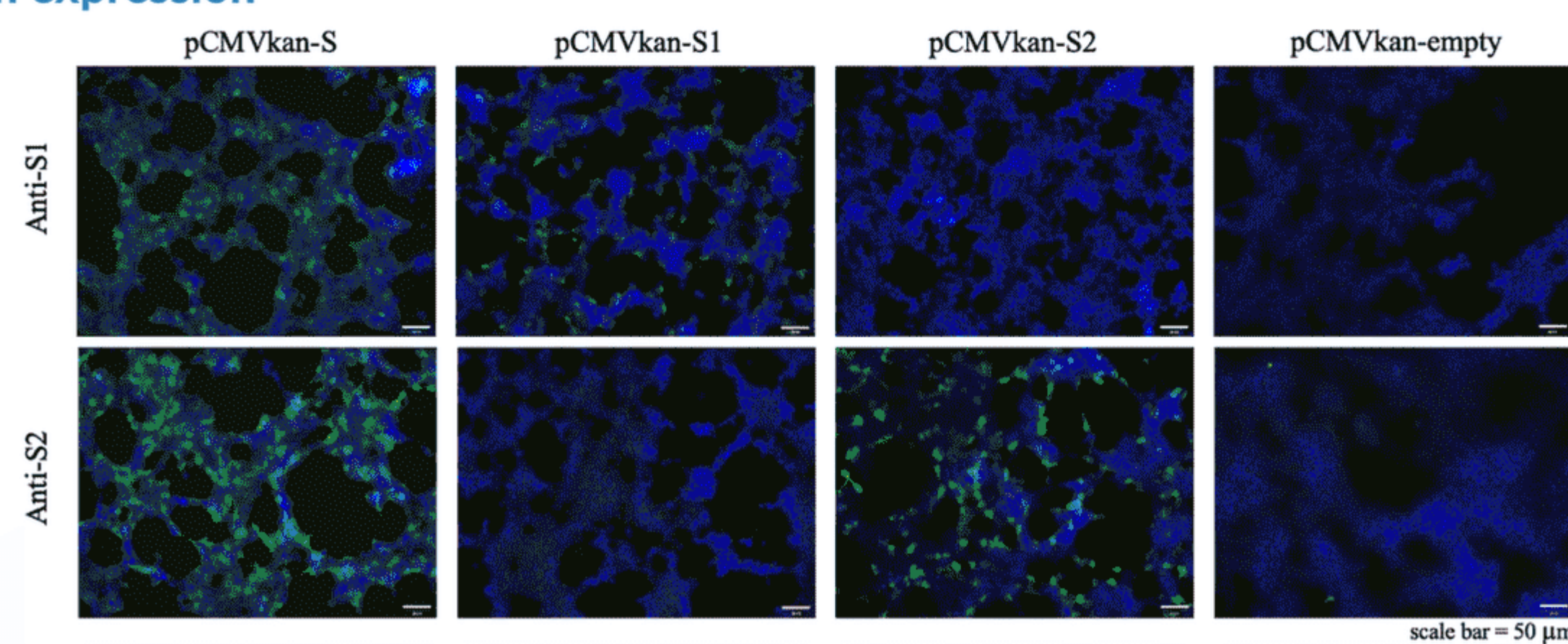
Six-week old of female ICR mice (20–25 grams) were randomly allocated into 4 groups with 5 mice/group. Mice in each group were immunized with 100  $\mu$ g of recombinant plasmids via intramuscular electroporation using TriGrid delivery system (Ichor Medical System, CA, USA). The plasmids were administered 3 times, 2-week interval. Blood samples were collected at weeks 0, 2, 4, 6 and 8. At week 8, the mice were euthanized by 30% CO<sub>2</sub> inhalation and splenocytes collected for T cells response analysis.



**Fig 2:** Mice immunization and sample collection schedule.

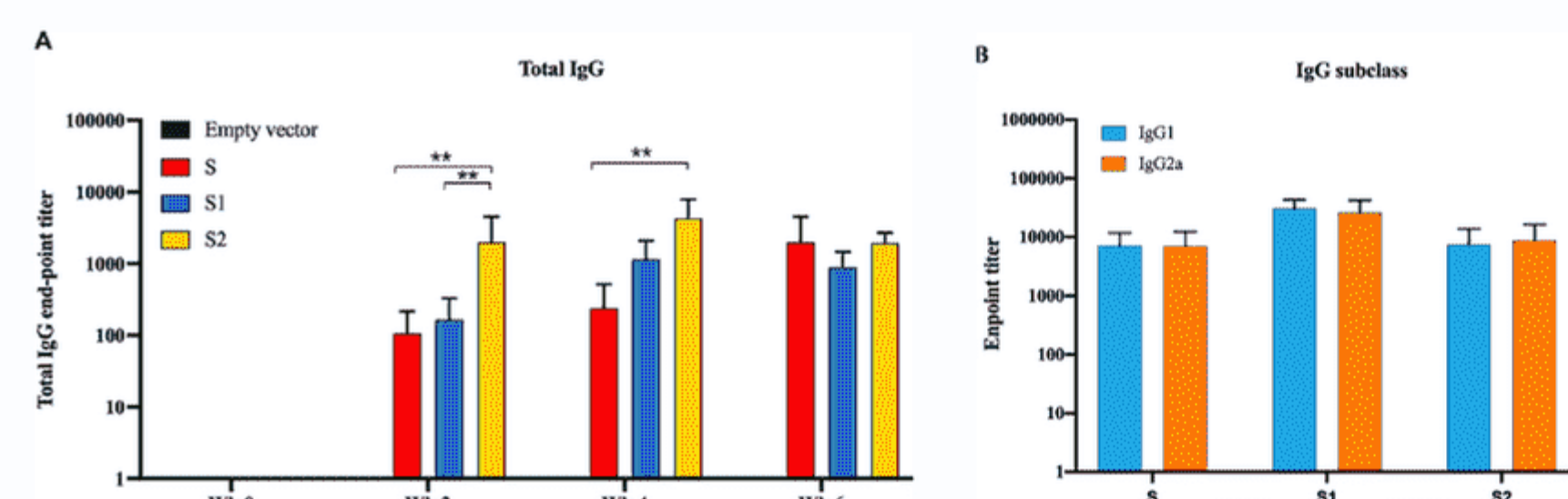
## Results

### • Protein expression

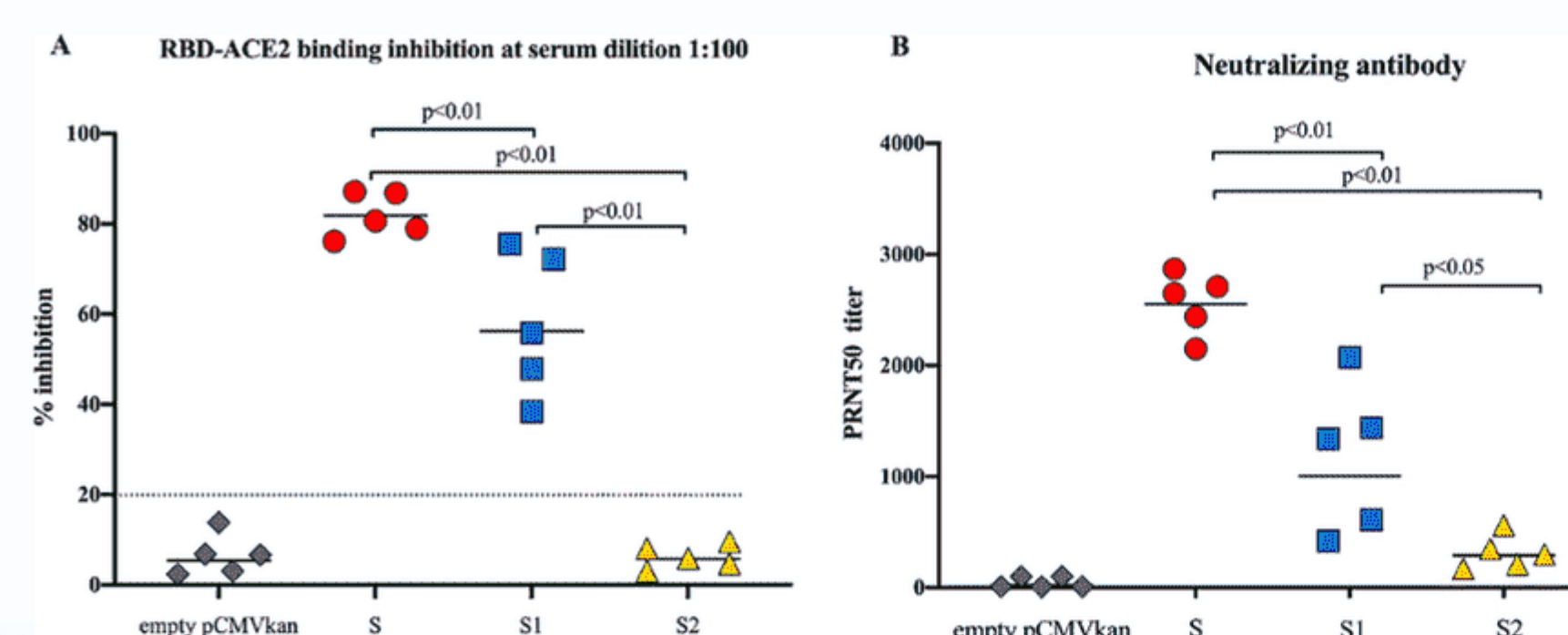


**Fig 3:** Intracellular protein expression analysis. Target proteins expression were detected employing anti-S1 (top) and anti-S2 (bottom) antibodies. Pictures were analyzed under fluorescent microscope with 10X objective lens.

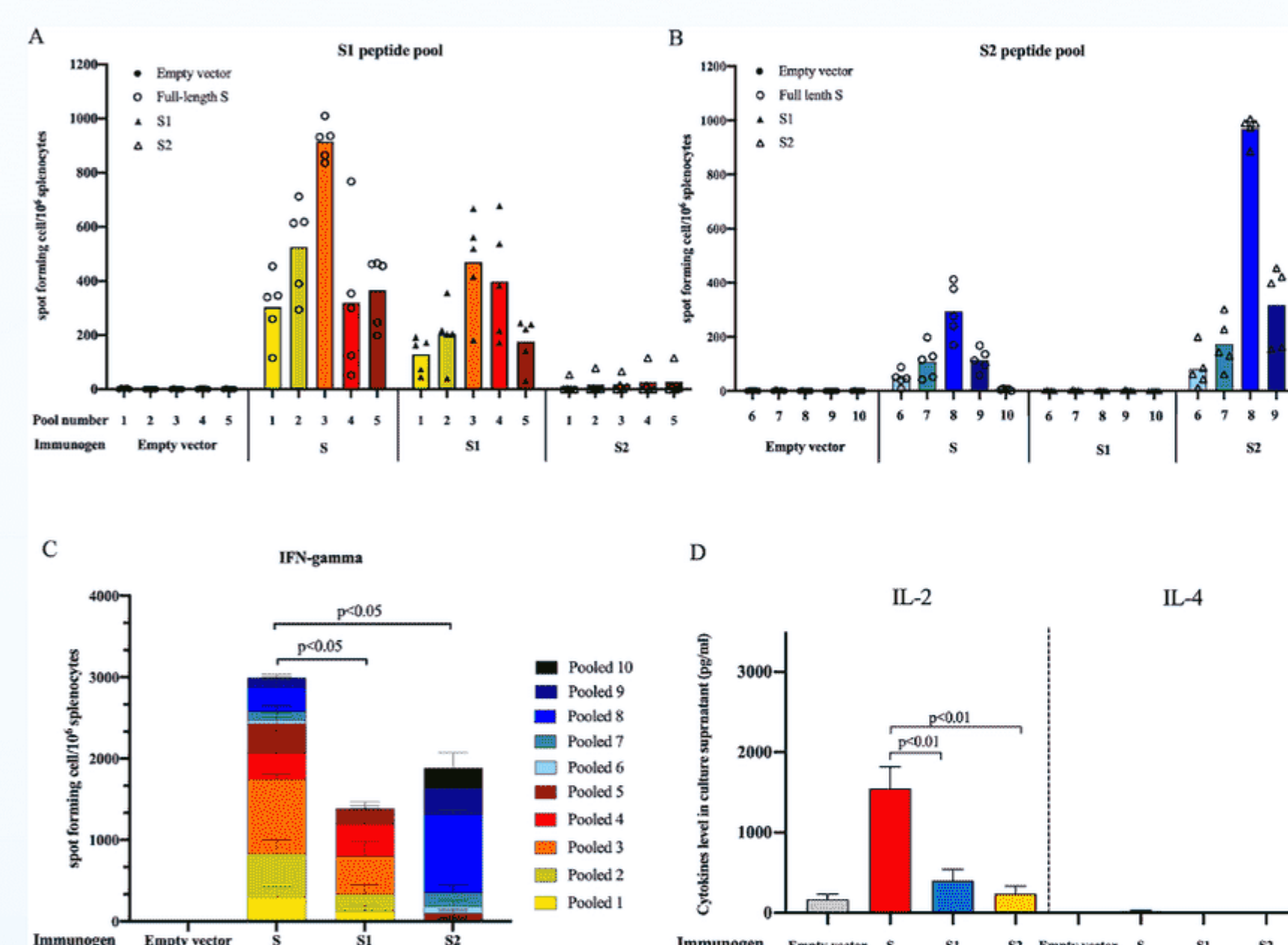
### • Vaccine immunogenicity



**Fig 4:** Titers of SARS-CoV-2 spike-specific total IgG analyzed at baseline and weeks 2, 4 and 6 (A), IgG subclass; IgG1 and IgG2a in the immunized mice sera collected on week 8 (B). Data presented as mean  $\pm$  SD of the endpoint titers in each mice vaccination group (n = 5).



**Fig 5:** Functional antibody analysis. (A) Inhibition of RBD-ACE2 interaction; immunized mice sera collected at week 6 were diluted 100-fold and analyzed for their activity on the inhibition of RBD-ACE2 binding. Percent inhibition below the dash line is considered negative. (B) NAb responses measured by standard plaque assay. Data presented the PRNT50 titer of individual mice (n = 5), horizontal lines indicated the geometric mean.



**Fig 6:** SARS-CoV-2 spike-specific T cells responses analyzed by ELISpot. Mice splenocytes were stimulated with pooled peptides from S1 (A) or S2 (B) regions. Each bar represents mean of IFN- $\gamma$  spot forming unit (SPF) per million splenocytes responses to each peptide pool. (C) Sum of IFN- $\gamma$  responses in mice (N = 5) immunized with different DNA vaccine constructs. Mann-Whitney test was used to compare the different in the total IFN- $\gamma$  responses from each group. (D) Ex vivo cytokines expression collected from the culture supernatant after splenocytes from S-immunized group were stimulated with spike pooled peptides for 24 h.

## Conclusion

This study provides crucial information regarding selection of antigens for SARS-CoV-2 vaccine development. SARS-CoV-2 full length S (S1+S2) is more potent in induction of both NAb and T cells responses than the truncated S1 or S2 immunogen. This finding could be further applied for development of other vaccine modalities.

## Acknowledgments

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