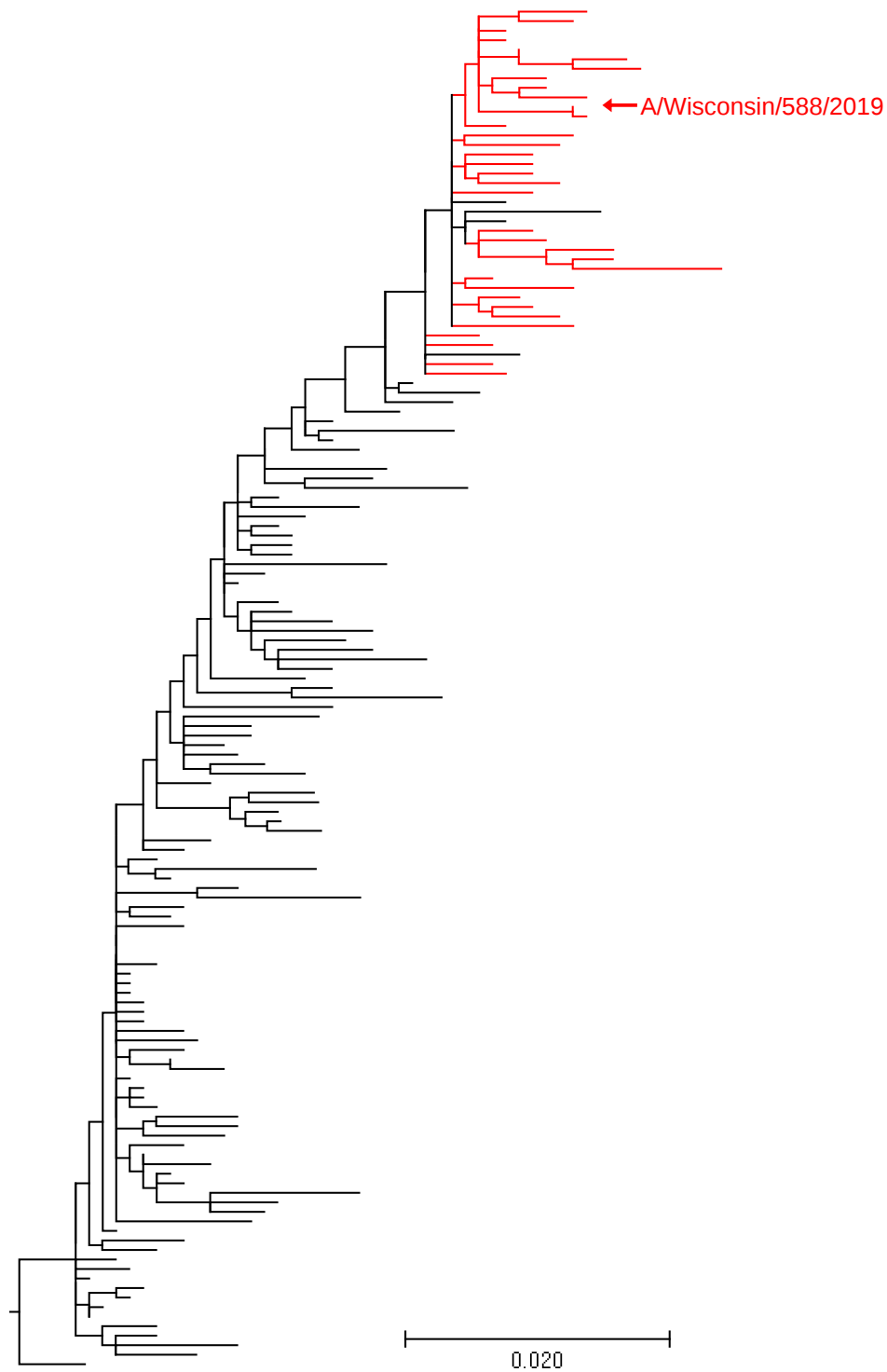


Supplementary Materials for

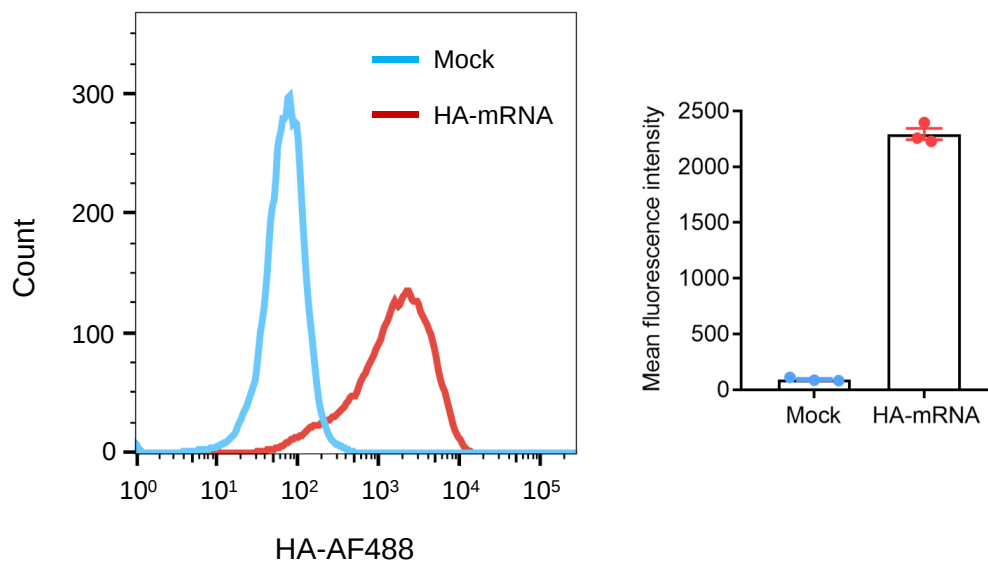
Rational development of a combined mRNA vaccine against COVID-19 and influenza

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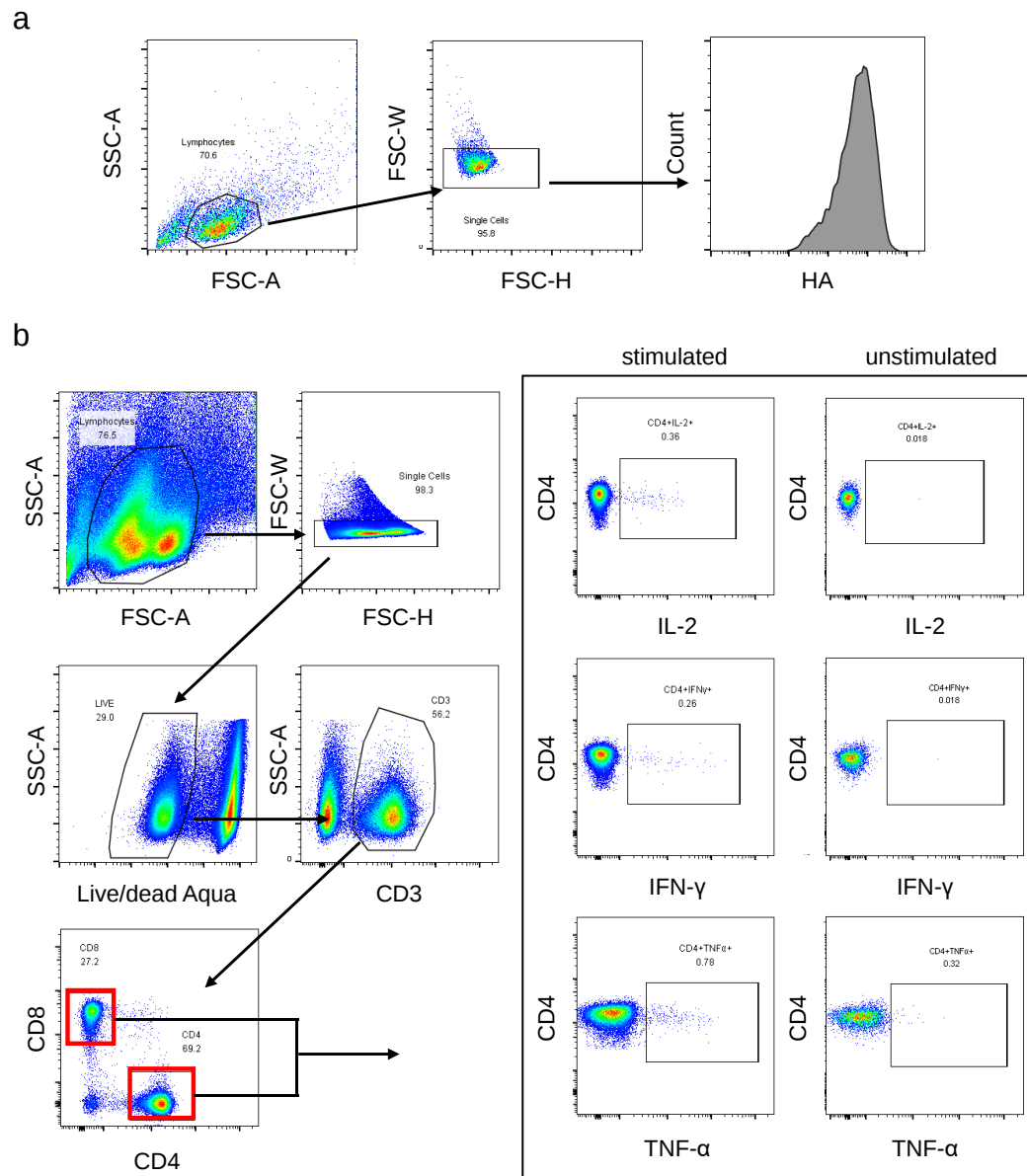
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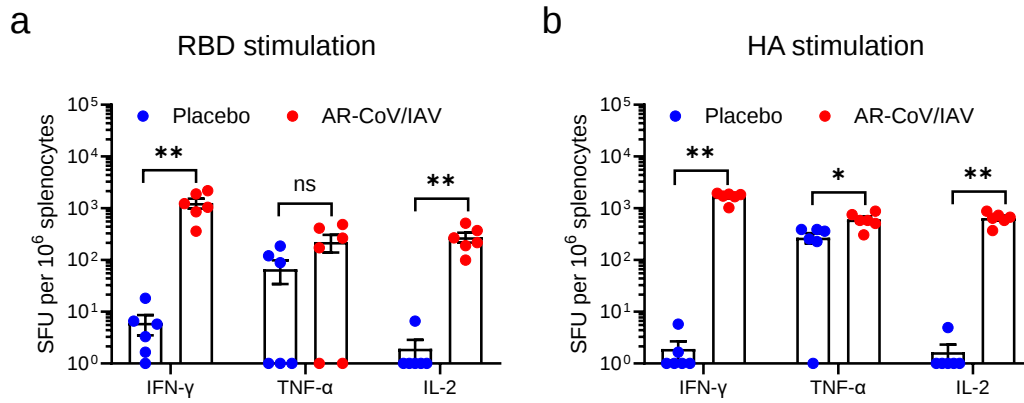
Supplementary Fig. 1 A phylogenetic tree was constructed by a maximum likelihood method based on the nucleotide sequence of the HA gene of influenza A (H1N1) virus. The circulating strains isolated from 2018 to 2020 in the Northern Hemisphere are colored red.



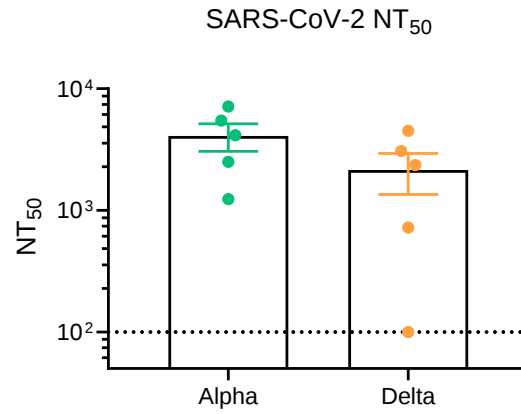
Supplementary Fig. 2 In vitro expression of membrane-bound HA was determined by flow cytometry. HEK293T cells were transfected with 4 μ g of HA-encoded mRNA for 48 h. Cells were collected, stained with antibody for H1N1-HA, and analyzed by flow cytometry. The data are representative from three independent replicates.



Supplementary Fig. 3. Gating strategies for H1N1-HA staining and intracellular cytokine staining. **a** Gating strategy for HA expression in HEK293T cells presented on Supplementary Fig. 2. **b** Flow cytometric gating strategy for the investigation of T cell responses in AR-CoV/IAV immunized mice presented on Fig. 3a-d.



Supplementary Fig. 4 AR-CoV/IAV immunization elicits an antigen-specific T cell immune response in BALB/c mice. Production of IFN- γ , TNF- α or IL-2 in splenocytes stimulated with the RBD (a) or HA (b) peptide pool was determined by ELISPOT assay. Statistical differences were analyzed by using two-tailed unpaired t tests. * $P < 0.05$, ** $P < 0.01$.



Supplementary Fig. 5 AR-CoV/IAV immunization elicits neutralizing antibodies against SARS-CoV-2 variants. NT₅₀ titers against SARS-CoV-2 Alpha and Delta variant were determined 73 days post-initial immunization by using VSV-based pseudovirus.