

A**EXPERIMENTAL PLAN**

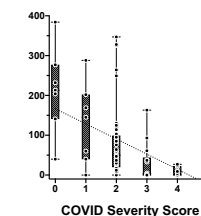
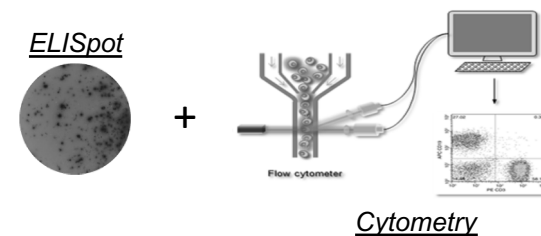
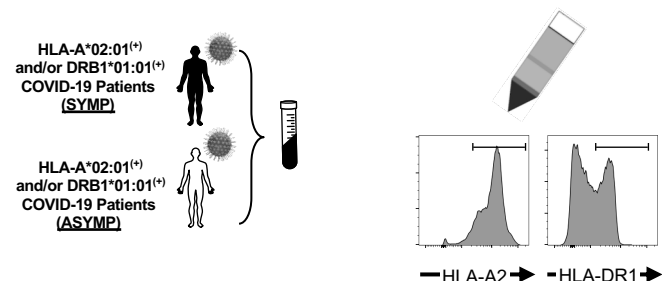
Collecting blood samples from ASYMP and SYMP individuals tested SARS-CoV-2 PCR+ (early after the onset of symptoms: 4.8-day average)

PBMCs extraction (Ficoll) and HLA screening and genotyping (PCR + FACS)

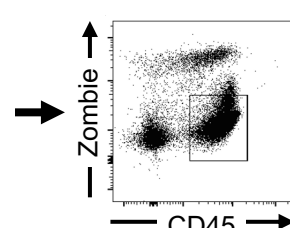
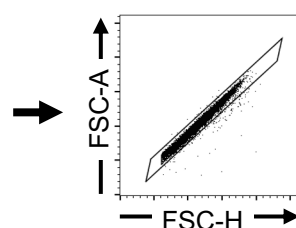
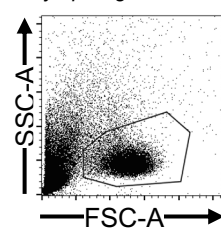
72h stimulation of 0.5×10^6 PBMCs with $10 \mu\text{g/ml}$ of SARS-CoV-2-derived CD4⁺ and CD8⁺ T cell epitopes-peptides previously identified (Class-II HLA-DRB1*01:01 and Class-I HLA-A*02:01 -restricted, respectively)

IFN- γ ELISpot and FACS staining of CD8⁺/CD4⁺ T cells. T-cells were gated on HLA-A*02:01 (for CD4⁺) and HLA-DRB1*01:01 (for CD8⁺)-restricted SARS-CoV-2 tetramers positive cells (Tet+)

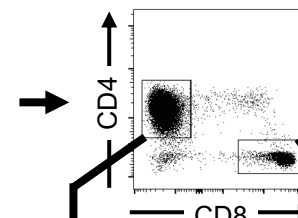
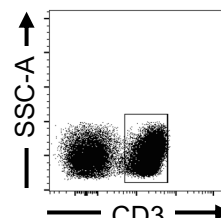
Analysis & correlation with the disease severity (disease severity score assessed by clinicians after patient discharge)

**B****GATING STRATEGY**

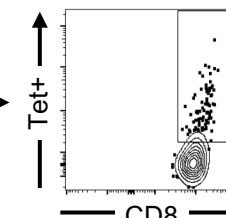
~ 200,000 lympho-gated cells



140,000 alive
CD45+ cells



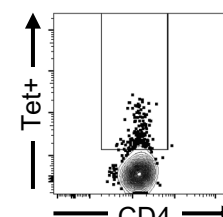
CD8⁺ T cells



HLA-A*02 Tetramers specificity

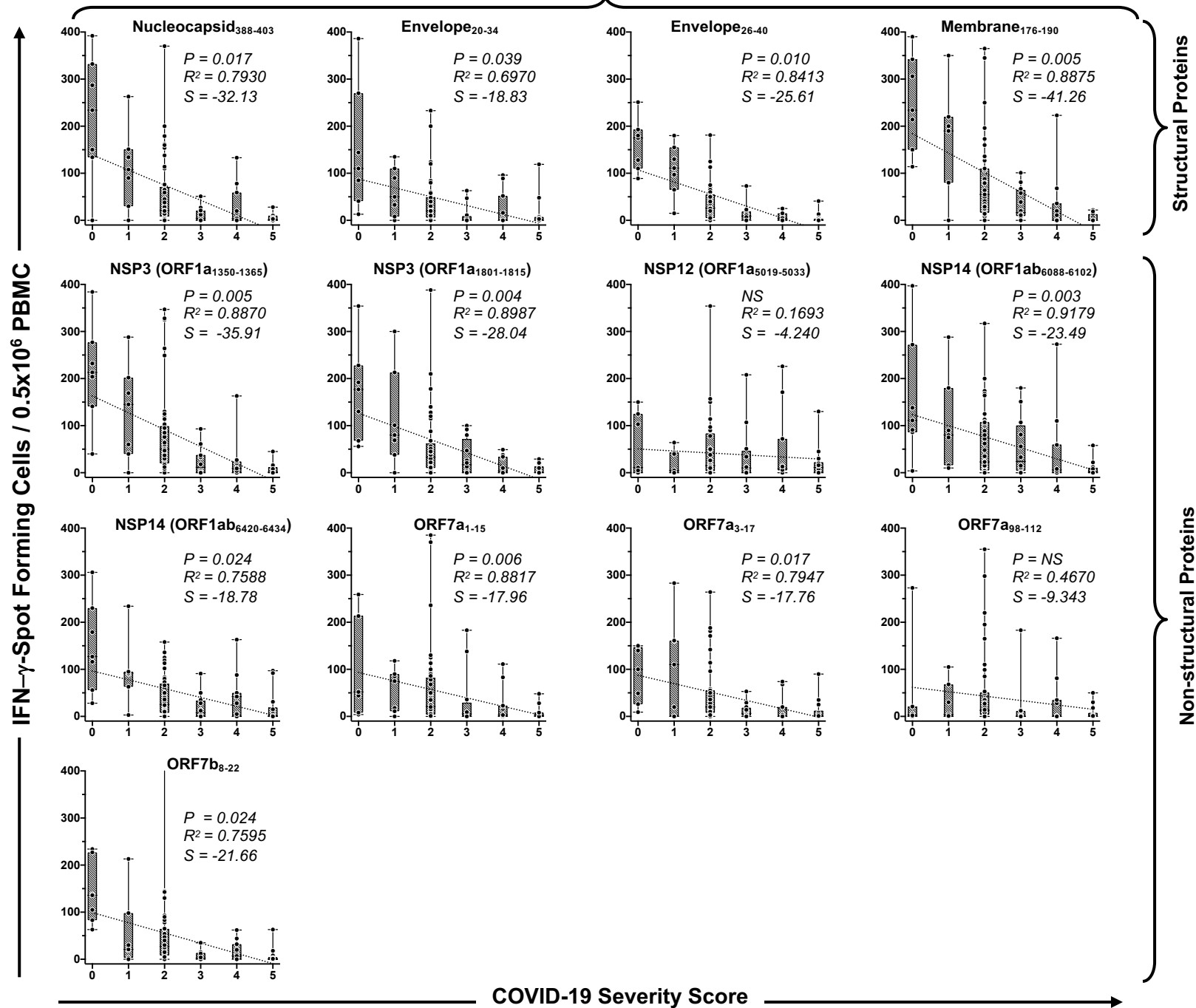
- Orf1ab₂₂₁₀₋₂₂₁₆
- Orf1ab₄₂₈₃₋₄₂₉₁
- S₉₇₆₋₉₈₄
- S₁₂₂₀₋₁₂₂₈
- ORF10₃₋₁₁

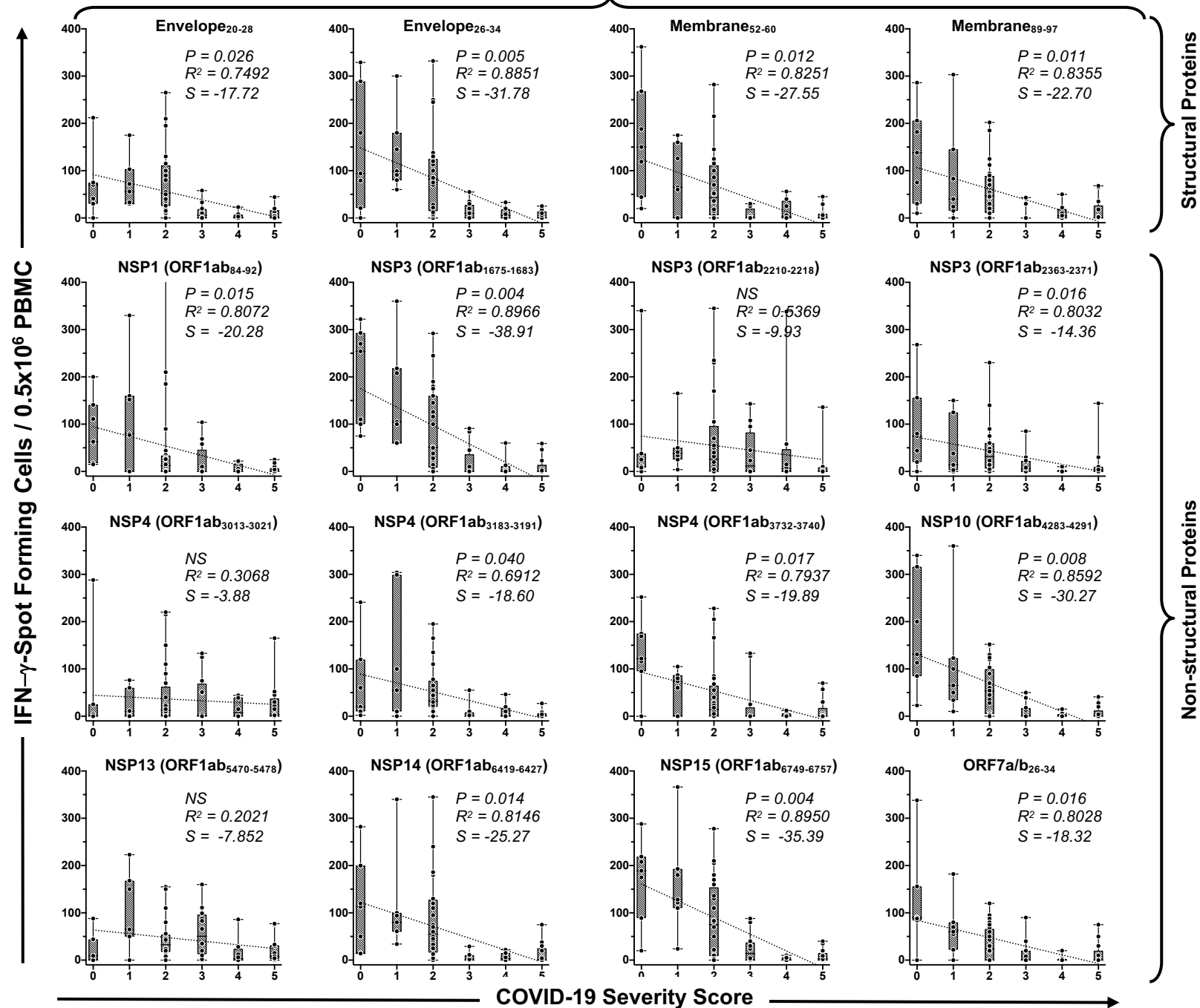
CD4⁺ T cells



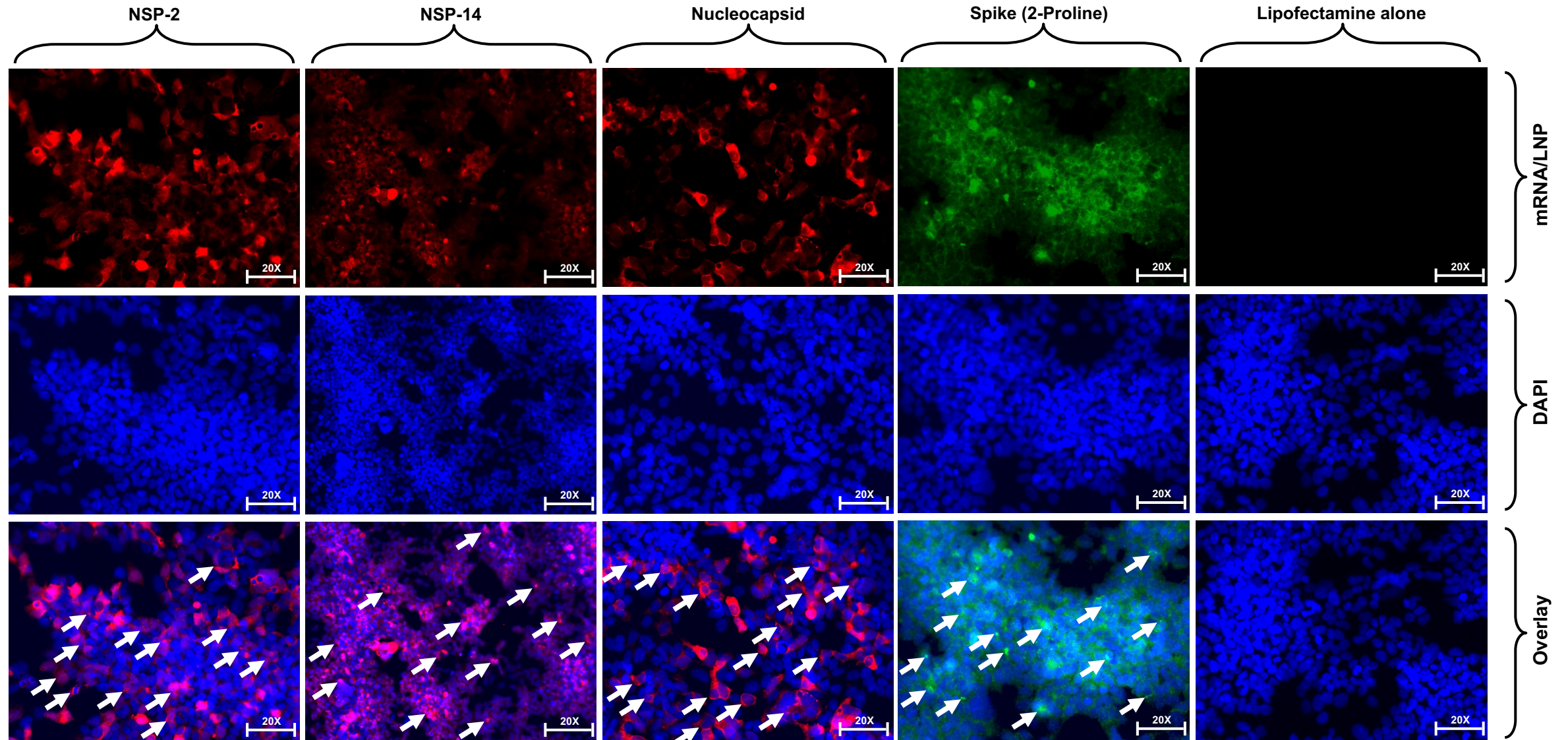
HLA-DRB*01 Tetramers specificity

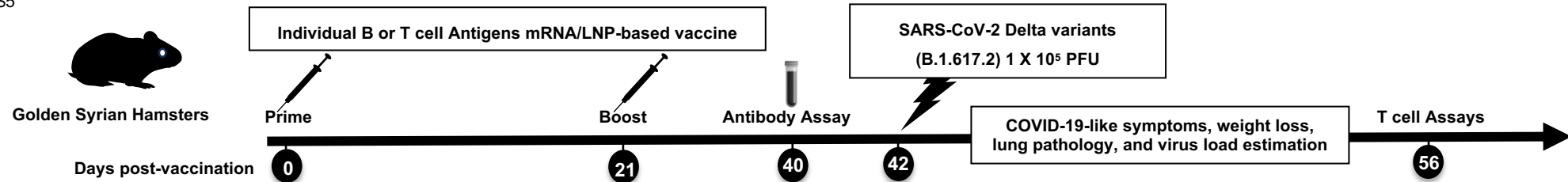
- ORF1a₁₃₅₀₋₁₃₆₅
- S₁₋₁₃
- E₂₆₋₄₀
- M₁₇₆₋₁₉₀
- ORF6₁₂₋₂₆

CD4⁺ T cells

CD8⁺ T cells

Efficient expression of individual Large Sequence Antigen mRNA/LNP vaccines *in vitro* in HEK293 cells



A**B**