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Supplemental Information

Bacteria-enabled oral delivery of a replicon-based mRNA vaccine candidate protects against ancestral and delta variant SARS-CoV-2

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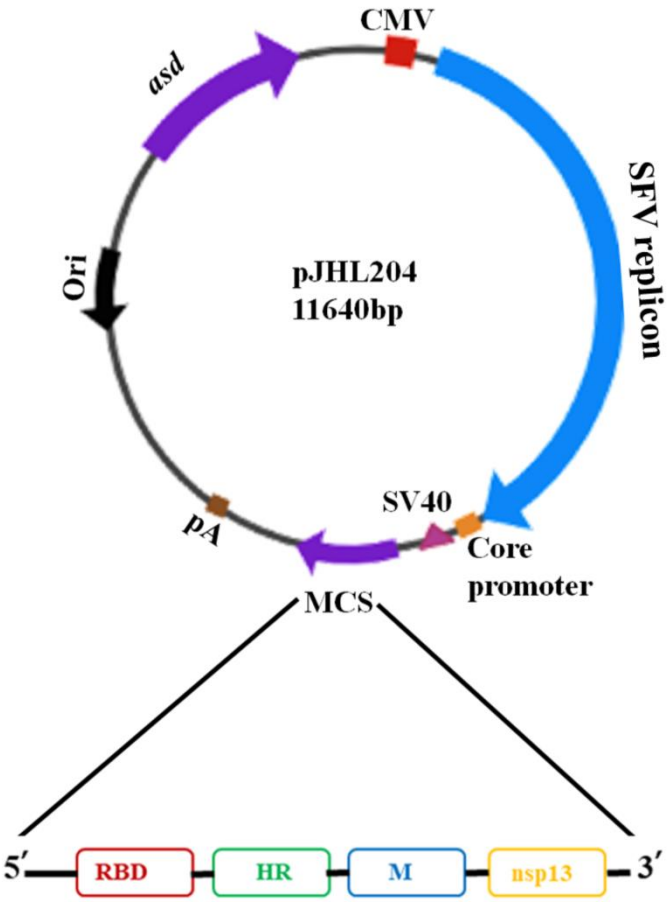
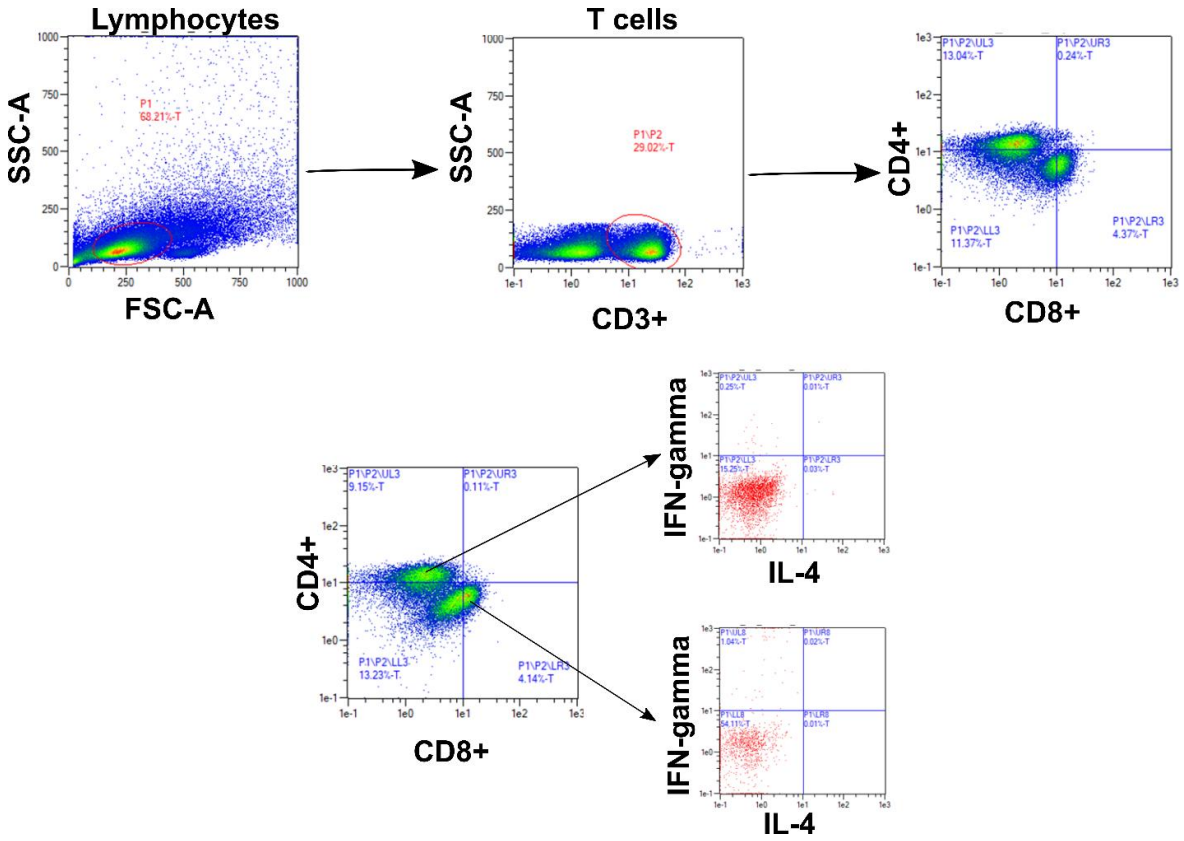


Supplementary Figure 1. pJHL204, a SFV replicon-based vector design

The CMV promoter, SFV replicon (nsp1–4), SFV sub-genomic core promoter, SV40 promoter, MCS region, polyadenylation signal (pA), asd marker and pBR origin are described.The SFV non-structural proteins (nsp1–4) form a replicase complex to drive transgene expression as a self-replicating and self-transcribing mRNA. MCS comprises the ApaI, AscI, PacI, AsiSI and PsiI restriction sites for gene cloning. The *asd* serves as an auxotrophic selection marker for antibiotic-free gene delivery. SFV MCS region has been enlarged to show the multicistronic vaccine construct containing RBD, HR, M and nsp13 epitopes of SARS-CoV-2, each target sequence is separated by viral self-cleaving peptide, P2A. SFV- Semliki Forest Virus.



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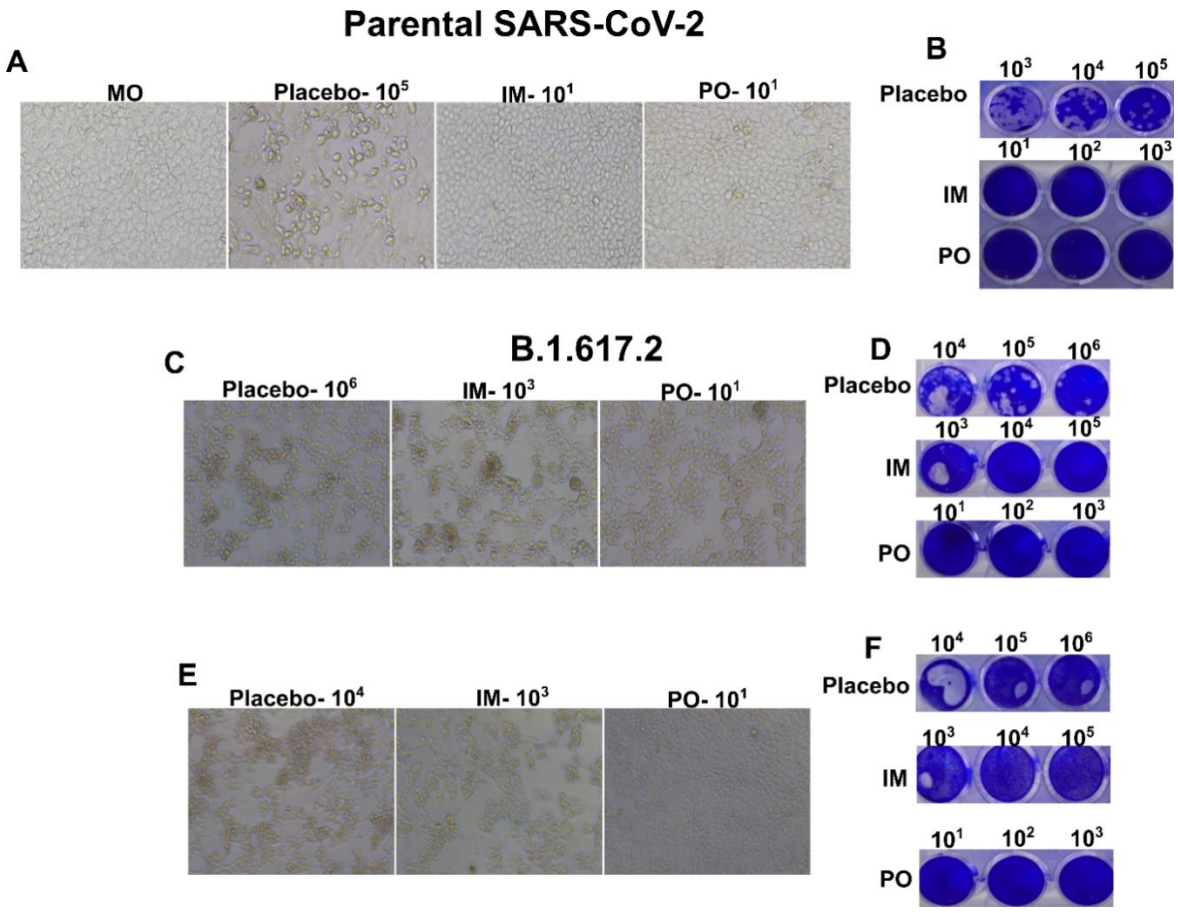
13 **Supplementary Figure 2. Gating strategy and analysis of CD4 and CD8 T cells**

14 Firstly, lymphocytes were gated by side and forward scatter properties. CD3+ T cells were
15 stratified into CD4 and CD8 T cells. Finally, population of cells expressing IFN-γ and IL-4
16 intracellular markers were profiled for CD4 and CD8 T cells.



Supplementary Figure 3 (Related to Figure 5). Assessment of live viral load in vaccinated and placebo hamsters.

Hamsters were immunized via intramuscular and oral routes. The intramuscular injection consisted of a single dose of 2×10^7 colony-forming units (CFU). Whereas, the oral route of administration consisted of two doses of 2×10^8 CFU at a 2-week interval. Hamsters were challenged three weeks after the final immunization with 1×10^4 PFU of either parental strain or delta variant. Representative CPE (A, C and E) and plaque (B, D and F) images are shown. (A-B) and (C-D), respectively, lung sample data from hamsters challenged with parental and delta variant SARS-CoV-2. (E-F) Nasal wash data from hamsters challenged with delta variant SARS-CoV-2. IM- Intramuscular; PO- Per os; B.1.617.2- Delta variant SARS-CoV-2.