CHO cell production of a single, enveloped VLP vaccine co-displaying SARS-CoV-2, Influenza and RSV antigens

Supplementary figures

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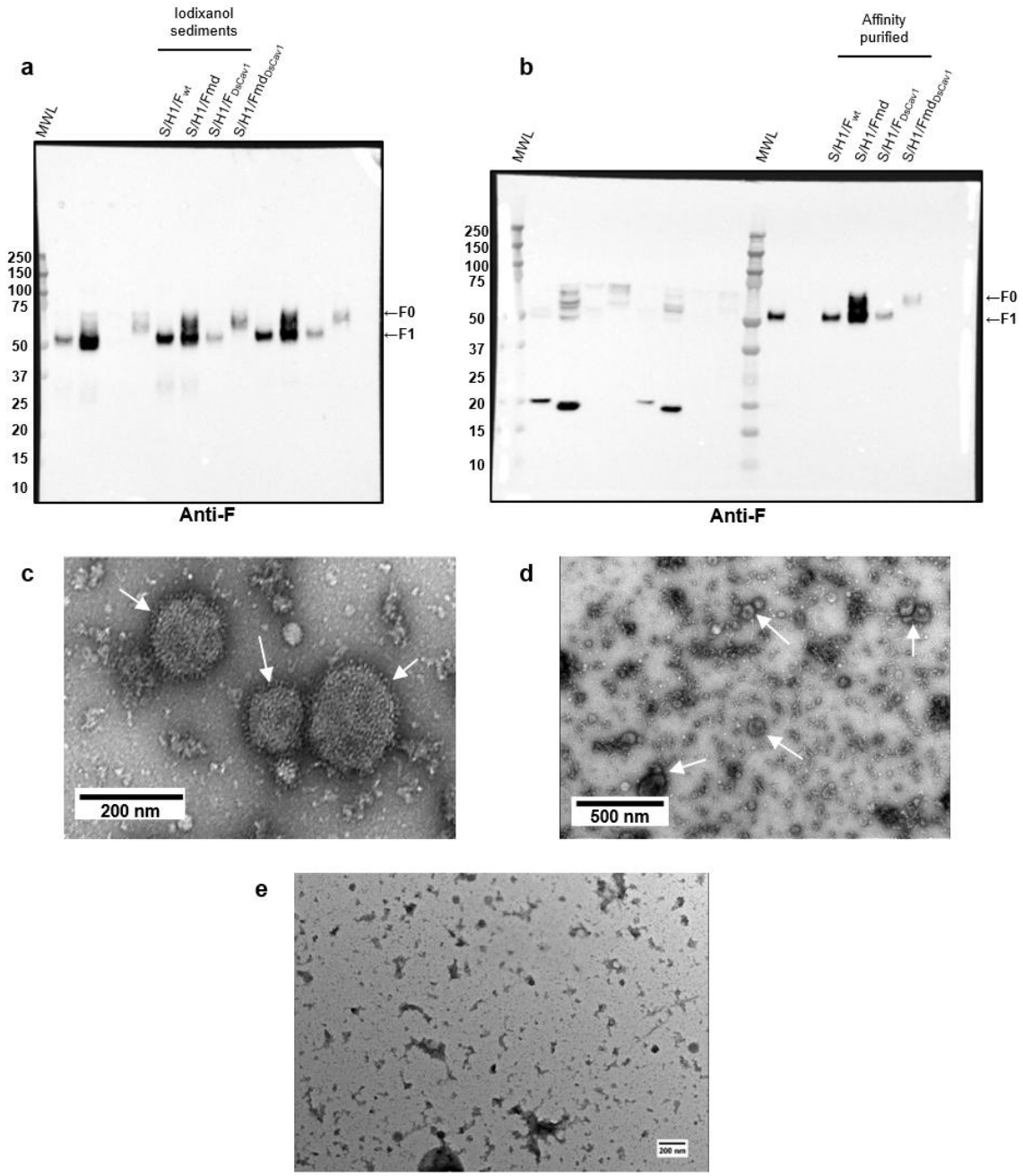
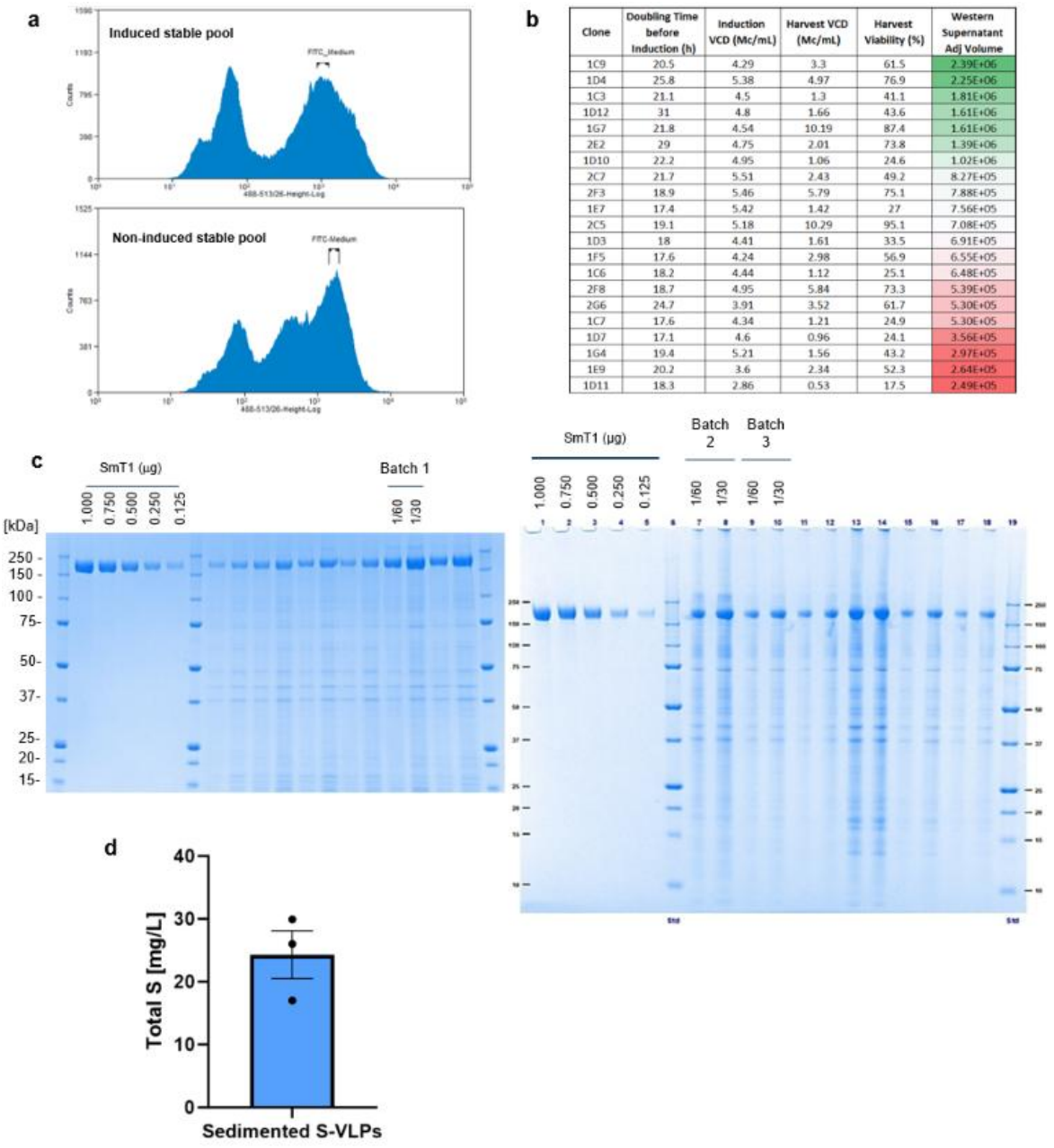
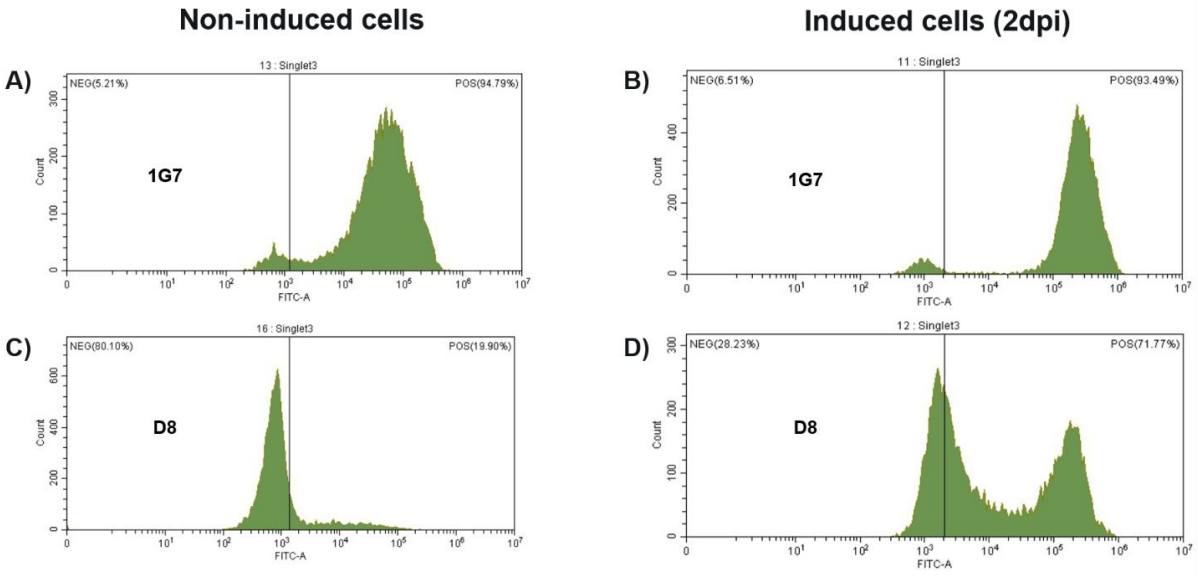


Supplementary Figure S1. **Comparison of S/H1/F VLP fractions with different F constructs and TEM of sedimented Fmd-VLPs**. Anti-F western blot of iodixanol-sedimented (**a**) or S-affinity purified (**b**) VLP samples produced by co-expression of S/H1/F with different F constructs. Representative TEM images of iodixanol cushion-sediments from CHO-C2 cells transfected with pTT5-RSV-Fmd (**c-d**) or with empty pTT5 vector as mock control (**e**). Fmd-VLPs are indicated with white arrows. Scale bars are shown at the bottom of each image.

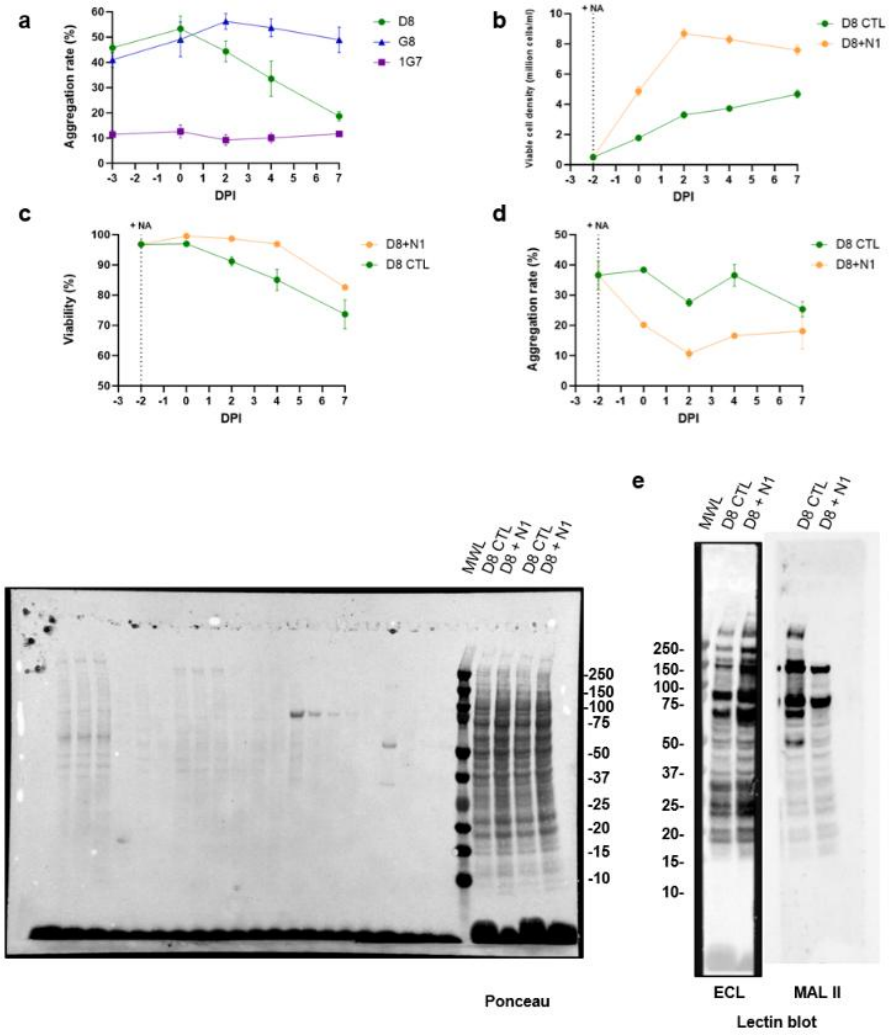


Clone	Doubling Time before Induction (h)	Induction VCD (Mc/mL)	Harvest VCD (Mc/mL)	Harvest Viability (%)	Western Supernatant Adj Volume
1C9	20.5	4.29	3.3	61.5	2.39E+06
1D4	25.8	5.38	4.97	76.9	2.25E+06
1C3	21.1	4.5	1.3	41.1	1.81E+06
1D12	31	4.8	1.66	43.6	1.61E+06
1G7	21.8	4.54	10.19	87.4	1.61E+06
2E2	29	4.75	2.01	73.8	1.39E+06
1D10	22.2	4.95	1.06	24.6	1.02E+06
2C7	21.7	5.51	2.43	49.2	8.27E+05
2F3	18.9	5.46	5.79	75.1	7.88E+05
1E7	17.4	5.42	1.42	27	7.56E+05
2C5	19.1	5.18	10.29	95.1	7.08E+05
1D3	18	4.41	1.61	33.5	6.91E+05
1F5	17.6	4.24	2.98	56.9	6.55E+05
1C6	18.2	4.44	1.12	25.1	6.48E+05
2F8	18.7	4.95	5.84	73.3	5.39E+05
2G6	24.7	3.91	3.52	61.7	5.30E+05
1C7	17.6	4.34	1.21	24.9	5.30E+05
1D7	17.1	4.6	0.96	24.1	3.56E+05
1G4	19.4	5.21	1.56	43.2	2.97E+05
1E9	20.2	3.6	2.34	52.3	2.64E+05
1D11	18.3	2.86	0.53	17.5	2.49E+05

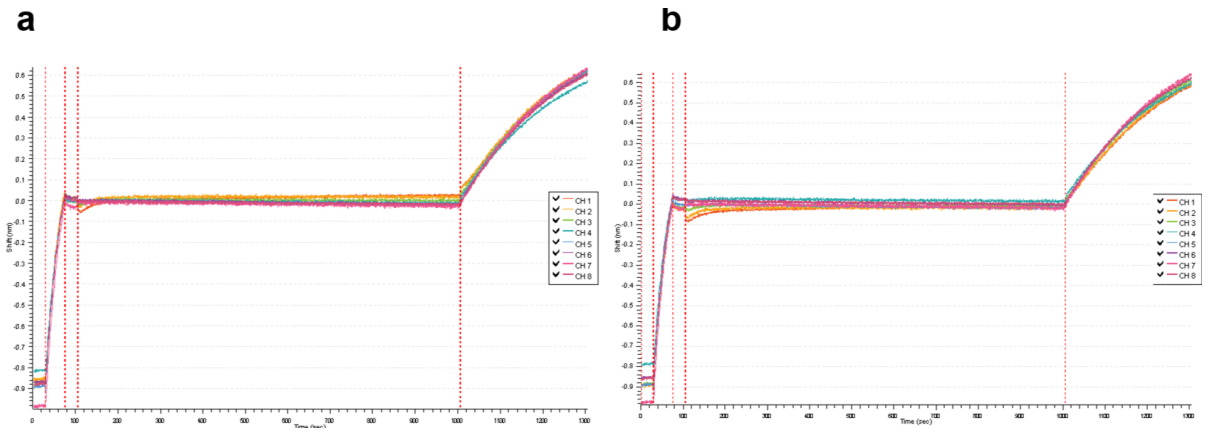
Supplementary Figure S2. Generation of stable CHO clones for S-VLP production (**a**) FACS-based single-cell sorting based on medium surface expression of S, using induced and non-induced stable pools. (**b**) Doubling times, viable cell densities (VCD) and viabilities of expanded clones screened for S-VLP production via western blot. (**c-d**) S-VLP yields from three independent productions with the CHO-1G7 clone. (**c**) Representative Coomassie-stained SDS-PAGE gels used for densitometric analysis. (**d**) Total S protein yield (mg/L), presented as mean ± SEM.



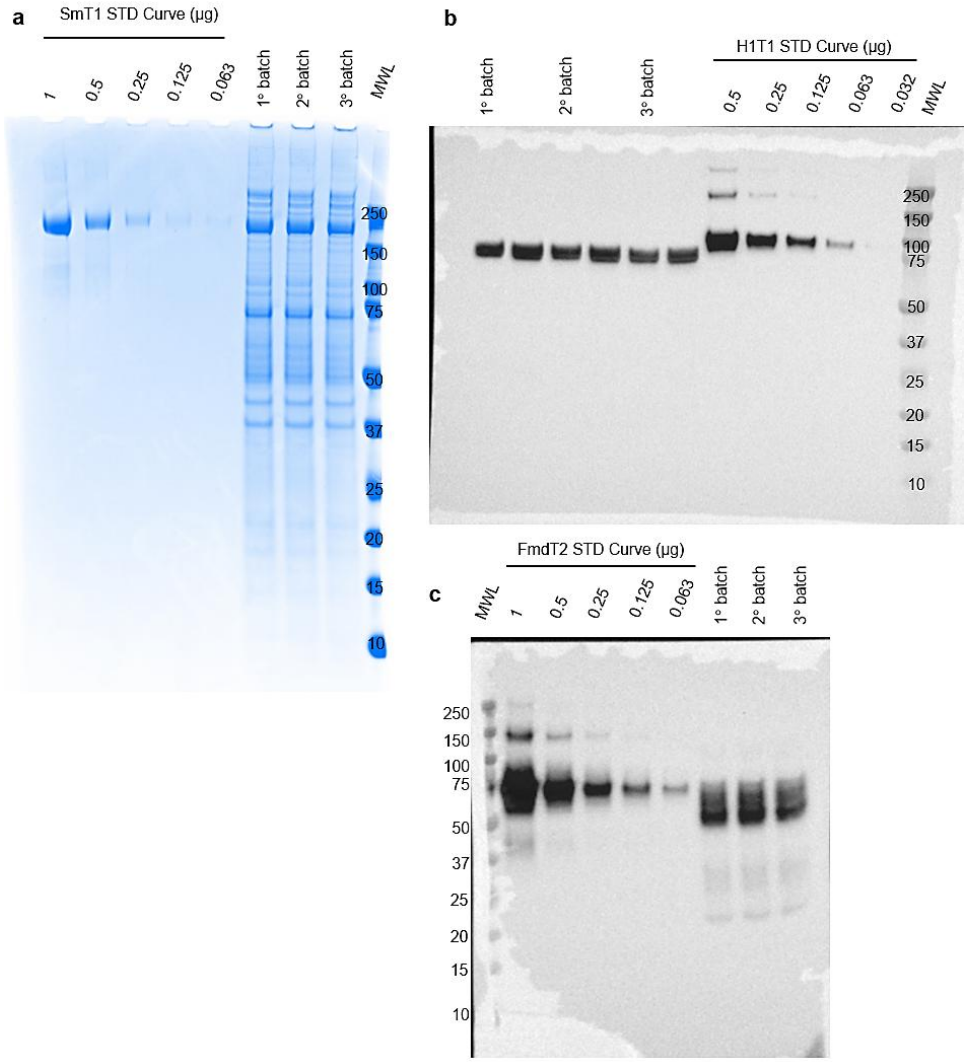
Supplementary Figure S3. Surface Spike expression in parental 1G7 and trivalent-derived D8 CHO clones under non-induced and induced conditions. Flow-cytometry histograms of anti-Spike staining in non-induced (**a,c**) and induced cells at 2 dpi (**b,d**). Compared with 1G7, D8 showed lower basal Spike expression and a more heterogeneous induction profile, with enrichment of lower-S-expressing subpopulations despite retention of a high-S fraction. Cells were stained with human anti-S VHH-72Fc followed by anti-human IgG-AF488 (Jackson 109-545-088). Gates were defined using induced 1G7 cells stained only with the secondary antibody.



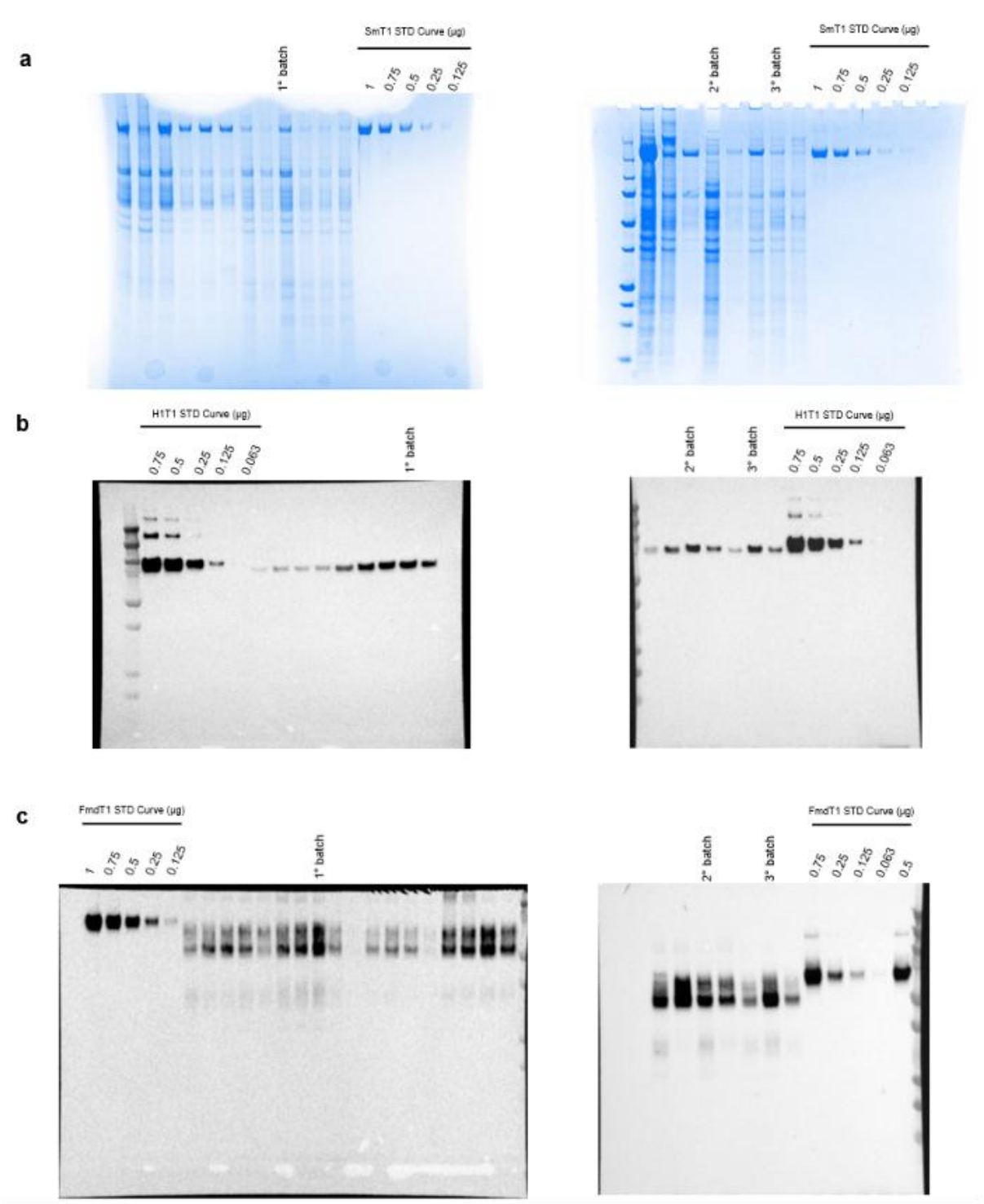
Supplementary Figure S4. CHO cell aggregation induced by stable PR8/IAV H1 expression is reduced through N1 disruption of α2,3-sialoglycans. Aggregation rate comparison of D8 and G8 clones stably expressing S/H1/Fmd VLPs and parental 1G7 clone stably expressing S-VLPs (**a**). CHO-D8 viable cell density (**b**), viability (**c**), and aggregation rates (**d**) with or without recombinant N1 supplementation. Errors bars are +/- one SEM for three independent experiments. (**e**) MALII and ECL lectin blots of D8 cell extracts at day 1 post-induction from fed-batch cultures ± N1 supplementation. Ponceau S staining was used as a protein loading control (20 µg total protein per lane). Following transfer and Ponceau S staining, the membrane was sectioned prior to lectin incubation, and separate sections were probed with MALII and ECL, respectively; therefore, the images shown represent the complete membrane sections available.



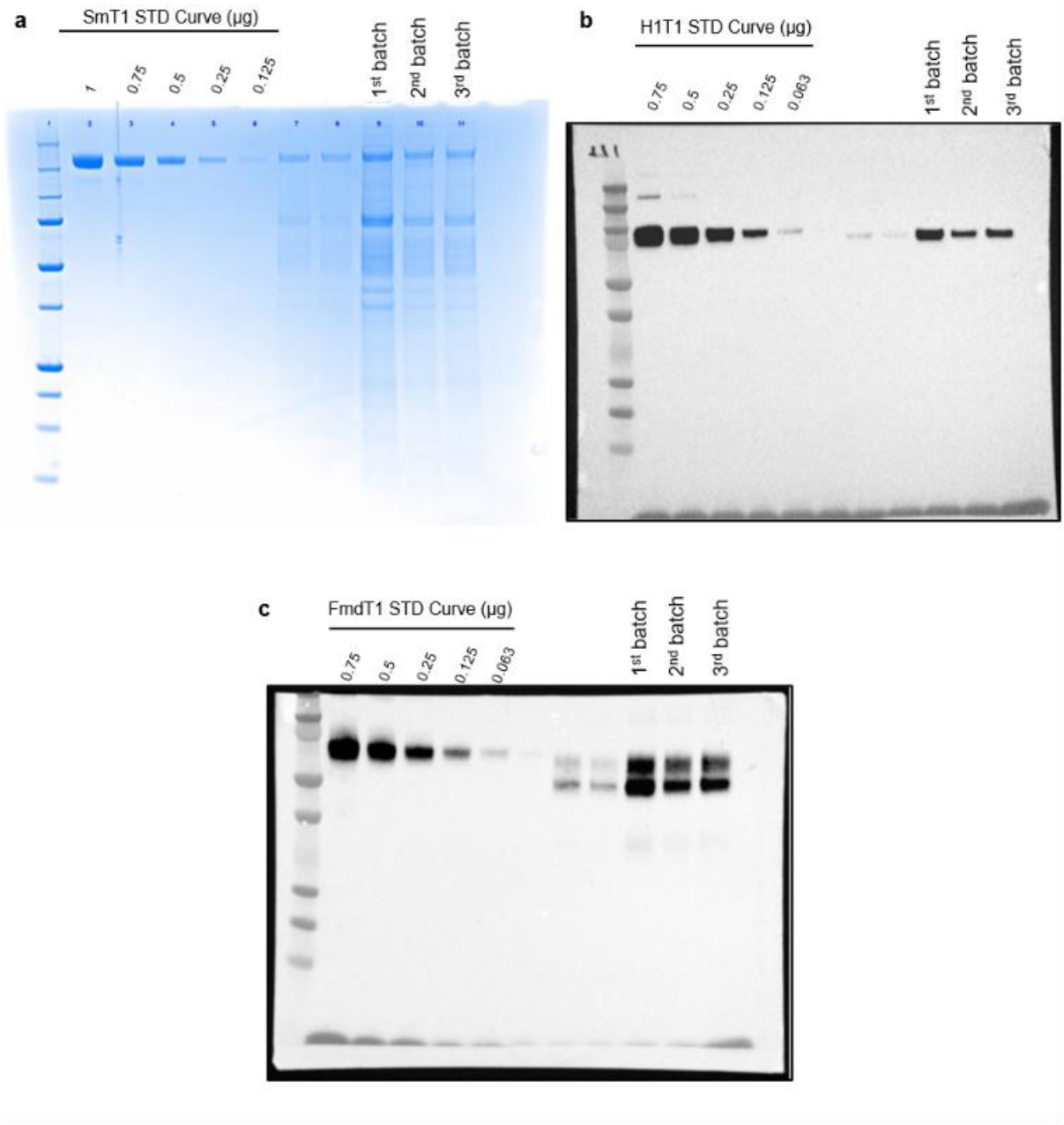
Supplementary Figure S5. PCA BLI sensograms of negative controls. Biosensor tips were loaded with FmdT1 antigen, then dipped into mouse serum (2-fold serially diluted), and then into a fixed concentration of palivizumab. Representative BLI sensograms showing lack of competitive inhibition of palivizumab binding to immobilized FmdT1 trimer by (**a**) naïve and (**b**) adjuvant-only immunized mouse serum.



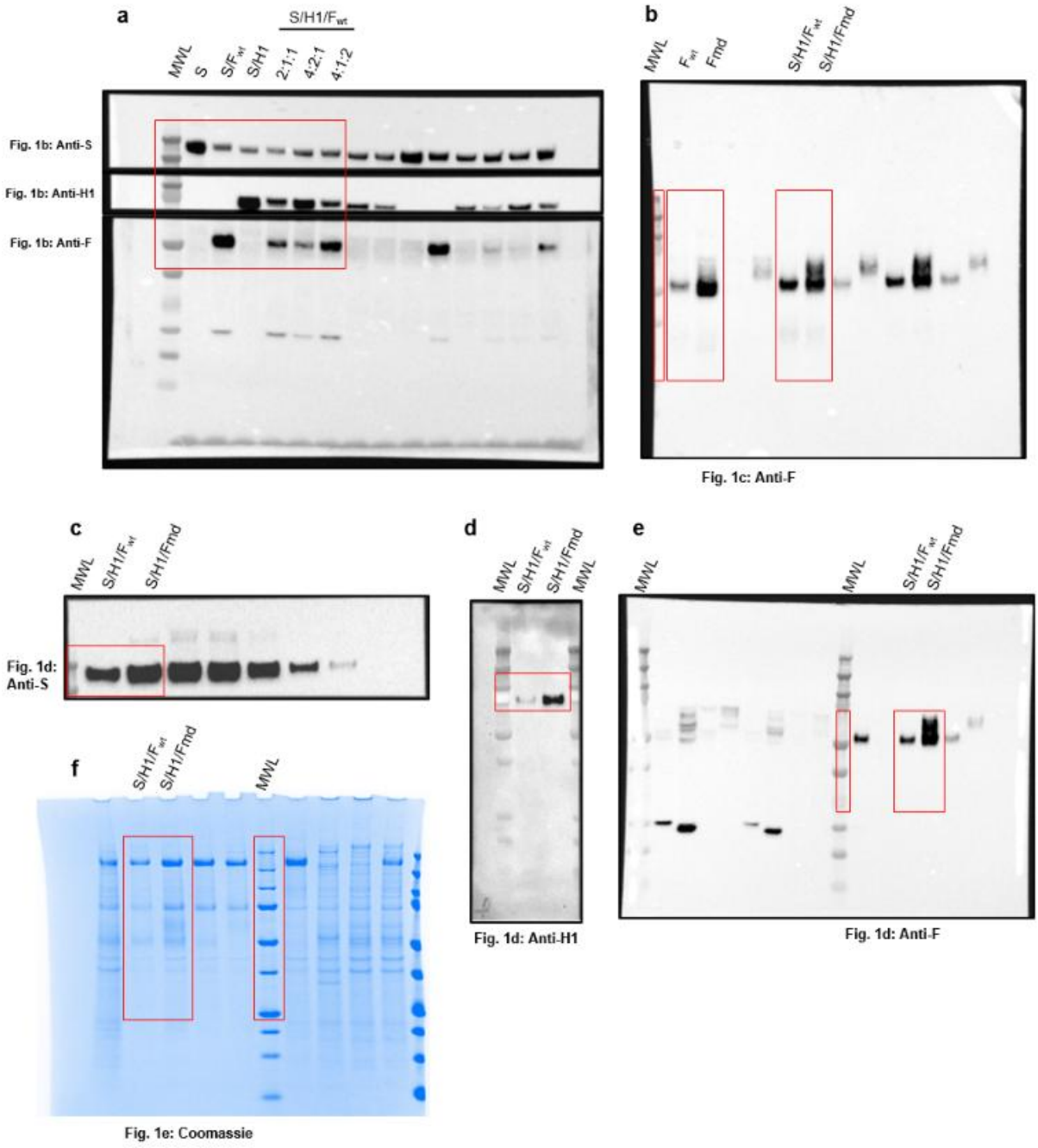
Supplementary Figure S6. Representative gel and blot images used for antigen quantification in sedimented S/H1/Fmd VLPs produced by TGE. (**a**) Coomassie-stained SDS-PAGE for S densitometry. (**b**) Western blot for H1 densitometry. (**c**) Western blot for F densitometry.



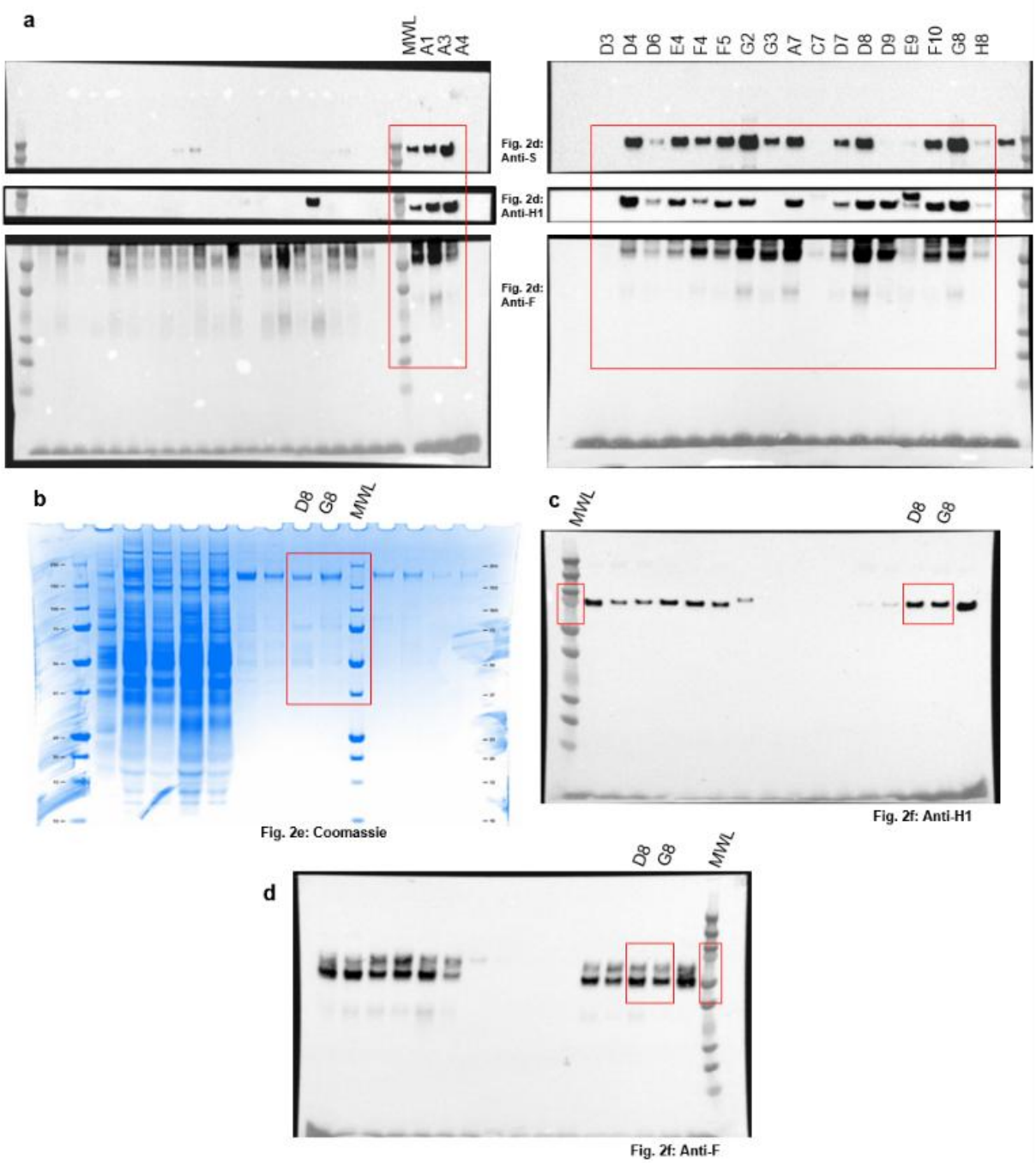
Supplementary Figure S7. **Representative gel and blot images used for antigen quantification in sedimented S/H1/Fmd VLPs produced by SGE.** (**a**) Coomassie-stained SDS-PAGE for S densitometry. (**b**) Western blot for H1 densitometry. (**c**) Western blot for F densitometry.



Supplementary Figure S8. **Representative gel and blot images used for antigen quantification in purified S/H1/Fmd VLPs produced by SGE.** (**a**) Coomassie-stained SDS-PAGE for S densitometry. (**b**) Western blot for H1 densitometry. (**c**) Western blot for F densitometry.



Supplementary Figure S9. **Original uncropped gel and blot images corresponding to Figure 1.** (a) From top to bottom: Anti-S, anti-H1, and anti-F western blots corresponding to Fig. 1b. (b) Anti-F western blot corresponding to Fig. 1c. (c-e) Anti-S, anti-H1, and anti-F western blots, respectively, corresponding to Fig. 1d. (f) Coomassie-stained SDS-PAGE gel corresponding to Fig. 1e. Red boxes indicate the regions displayed in the corresponding manuscript panels.



Supplementary Figure S10. Original uncropped gel and blot images corresponding to Figure 2. (a) From top to bottom: Anti-S, anti-H1, and anti-F western blots corresponding to Fig. 2d. (b) Coomassie-stained SDS-PAGE gel corresponding to Fig. 2e. (c) Anti-H1 and (d) anti-F western blots corresponding to Fig. 2f. Red boxes indicate the regions displayed in the corresponding manuscript panels.