CHO cell production of a single, enveloped VLP vaccine targeting SARS-CoV-2, Influenza A and RSV.

Supplementary figures

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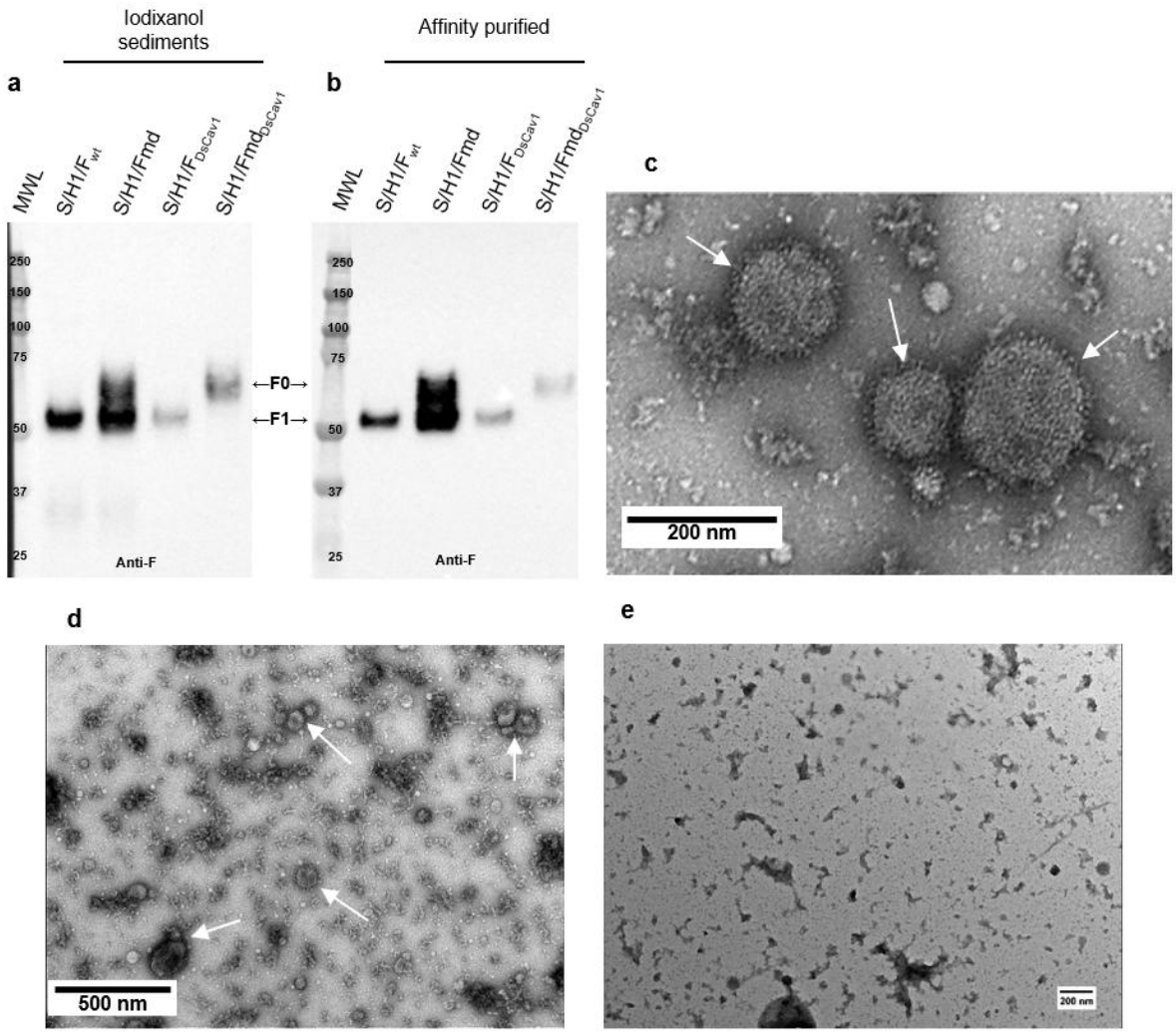
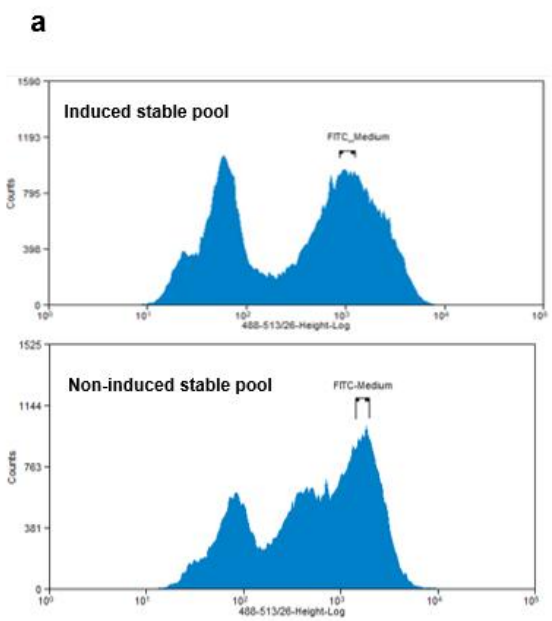
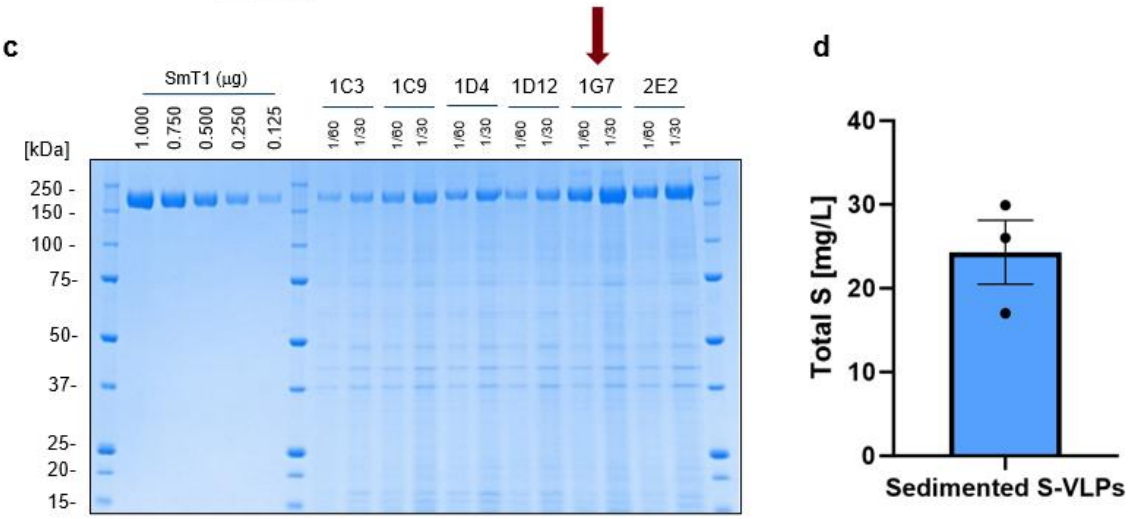


Supplementary Figure S1. **Comparison of S/H1/F VLP fractions with different F constructs and TEM of sedimented Fmd-VLPs**. Anti-F western blot of iodixanol-sedimented (**a**) or S-affinity purified (**c**) VLP samples produced by co-expression of S/H1/F with different F constructs. Representative TEM 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.

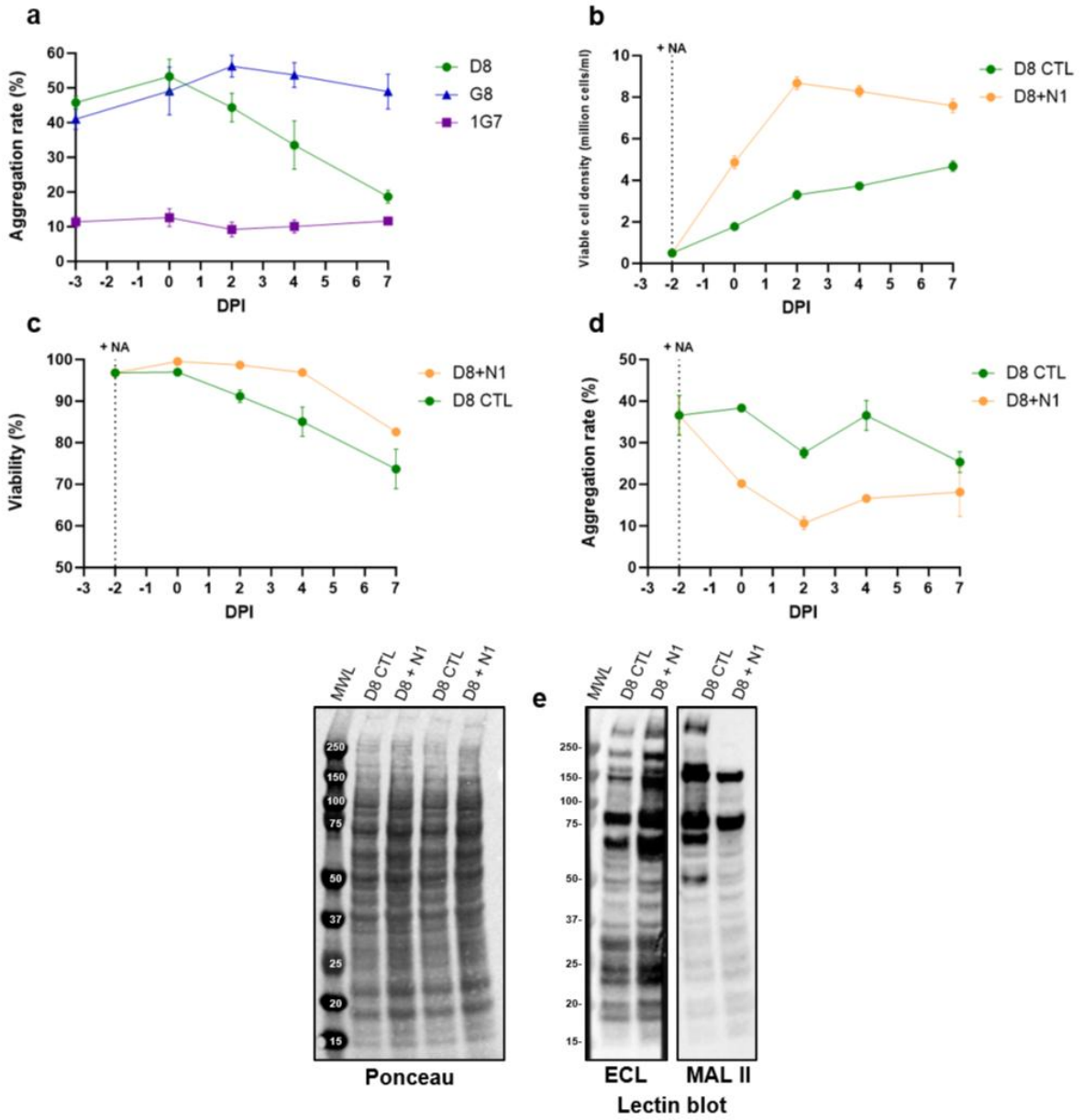


b

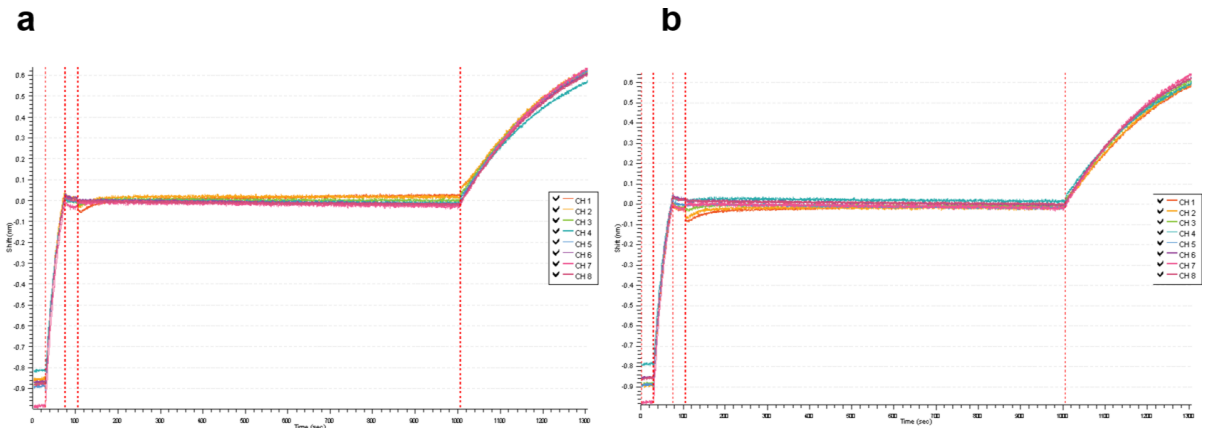
Clone	Doubling Time before Induction (h)	Induction VCD (Mc/mL)	Harvest VCD (Mc/mL)	Harvest Viability (%)	Western Supernatant Adj Volume
1C9	20.5	4.29	3.3	61.5	2.39E+06
1D4	25.8	5.38	4.97	76.9	2.25E+06
1C3	21.1	4.5	1.3	41.1	1.81E+06
1D12	31	4.8	1.66	43.6	1.61E+06
1G7	21.8	4.54	10.19	87.4	1.61E+06
2E2	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



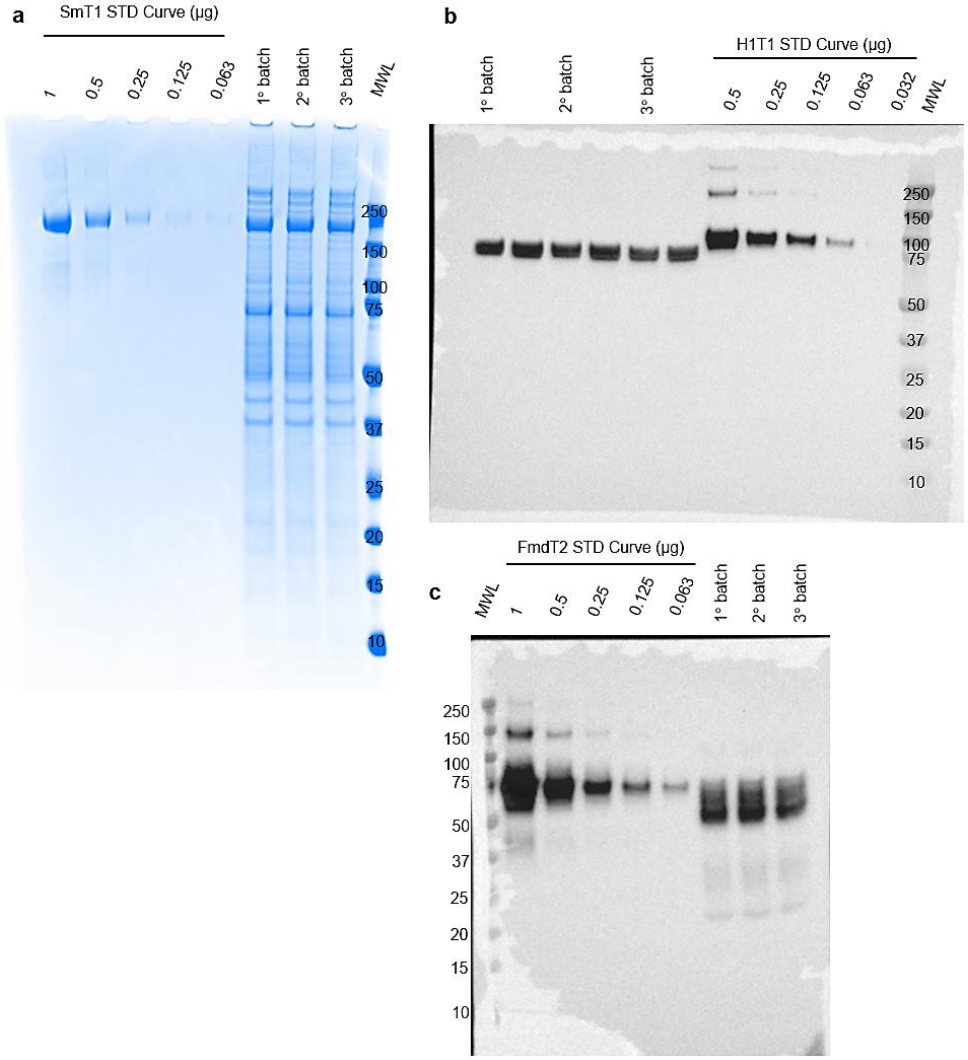
Supplementary Figure S2. Generation of stable CHO clones for S-VLP production (**a**) FACS-based single-cell sorting based on medium surface expression of S, using induced and non-induced stable pools. (**b**) Doubling times, viable cell densities (VCD) and viabilities of expanded clones screened for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



Supplementary Figure S3. 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 blot of D8 cell extracts at day 1 post-induction from fed-batch cultures ± N1 supplementation. Ponceau S-stained membrane is used as a protein loading control (20 µg total protein per lane).



Supplementary Figure S4. 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.



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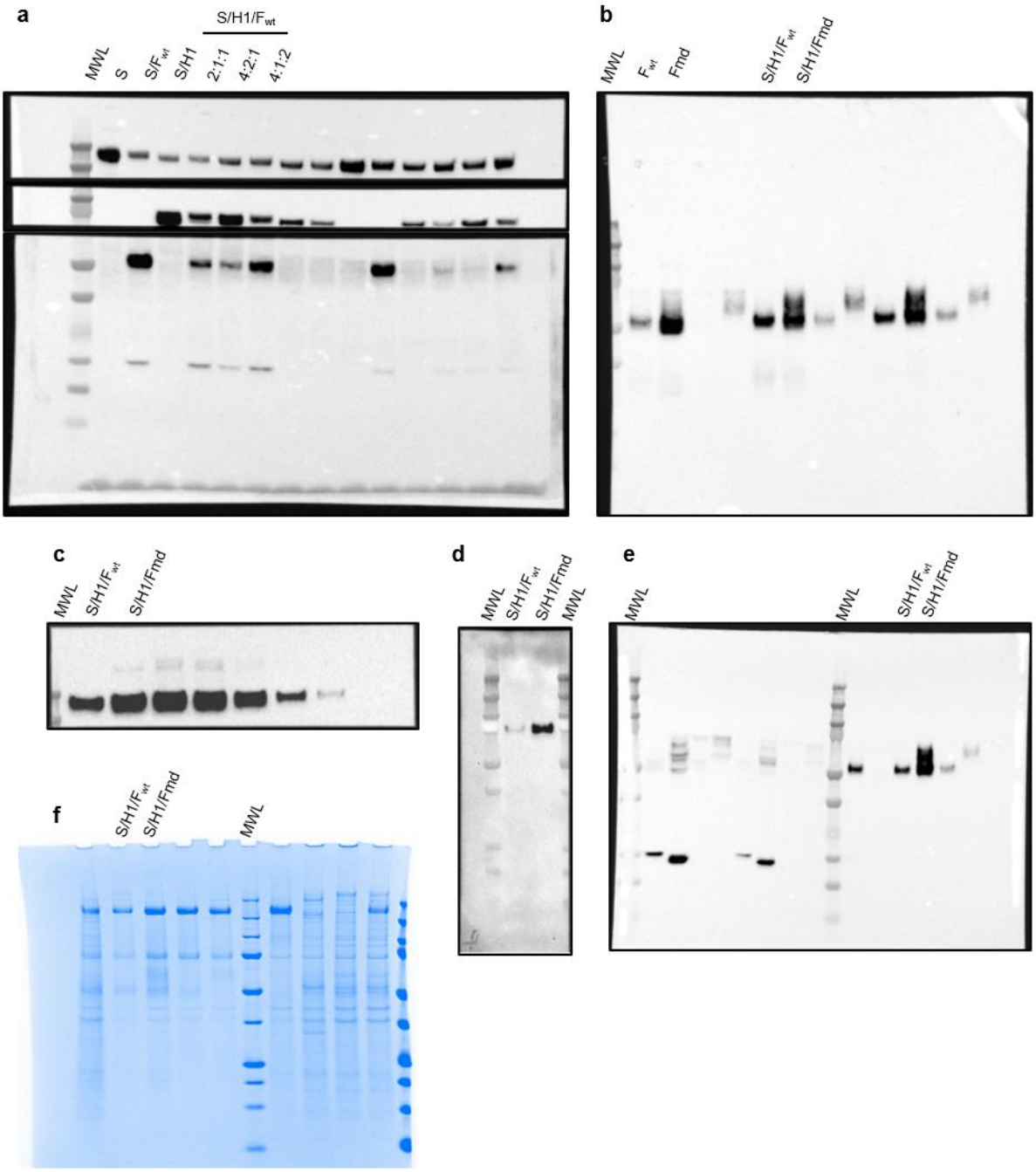
48 **Supplementary Figure S5**. **Representative gel and blot images used for antigen**
49 **quantification in sedimented S/H1/Fmd VLPs.** (**a**) Coomassie-stained SDS-PAGE for S
50 densitometry. (**b**) Western blot for H1 densitometry. (**c**) Western blot for F densitometry.



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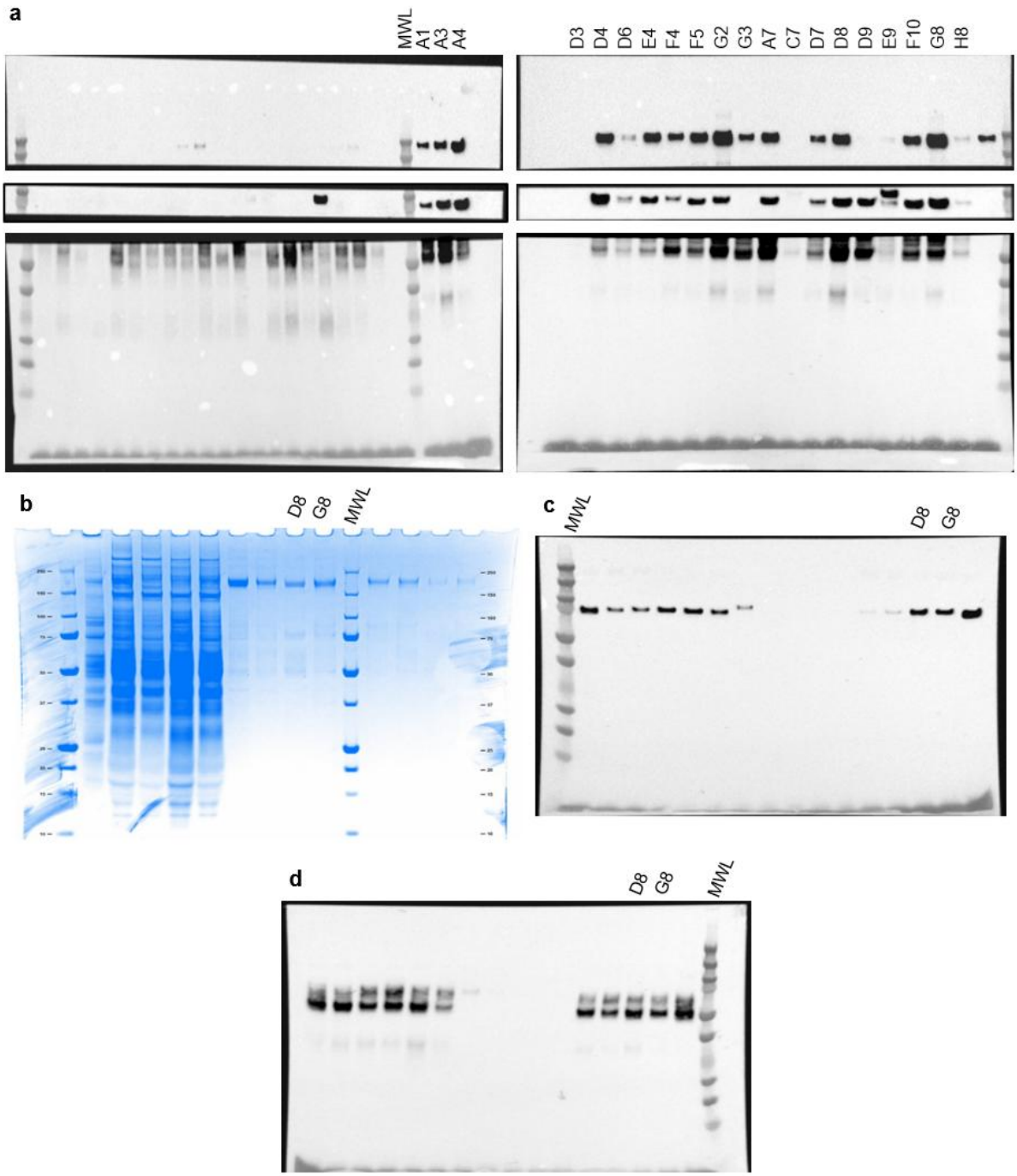
52 **Supplementary Figure S6**. **Digital uncropped images of gels and blots.** (**a**) Western blot
53 corresponding to Fig. 1b. (**b**) Western blot corresponding to Fig. 1c. (**c-e**) Western blots
54 corresponding to Fig. 1d. (**f**) Coomassie-stained SDS-PAGE gel corresponding to Fig. 1e.



Supplementary Figure S7. **Digital uncropped images of gels and blots.** (**a**) Western blots corresponding to Fig. 2d. (**b**) Coomassie-stained SDS-PAGE gel corresponding to Fig. 2e. (**c-d**) Western blots corresponding to Fig. 2f.