

Supplementary Data - The mosaic sequences

>H1m

MKQGKTKATKMKAILVLLYTFATANADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLL
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VLVSLGAISFWMCSNGSLQCRICI*

>H3m

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RGQAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWS
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NIRCNICI*

>N1m

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>N2m

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WLHVCITGDDKNATASFIYNGRLVDSVVSWSKDILRTQESECVCINGTCTVVMTDGNATG
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TEVLWTSNSIVVFCGTSPTYGTGWSWPDGADLNLMPI*

Supplementary Table 1. The HA and NA proteins of strains used in this study were analyzed for identity and similarity to mosaic HA and NA sequences.

Antigen	Identity	Similarity	Antigen	Identity	Similarity
HAm-H1/NA _m -N1	100/100	100/100	HAm-H3/NA _m -N2	100/100	100/100
A/Michigan/45/2015	98.06/98.93	98.41/99.36	A/Kansas/14/2017	98.06/98.29	98.23/99.15
A/Victoria/2570/2019	95.94/96.38	97.35/97.65	A/Hong Kong/4801/2014	98.06/98.72	98.06/99.36
A/Guangdong-Maonan/ SWL1536/2019	96.64/96.80	97.70/97.87	A/Hong Kong/2671/2019	97.17/98.72	97.88/99.15
A/California/7/2009	98.76/98.08	99.12/99.15	A/Cambodia/E0826360/2020	97.17/97.44	97.88/98.08
A/Brisbane/02/2018	96.82/98.08	97.70/98.72	A/Texas/50/2012	98.41/98.51	99.12/99.36
A/California/04/2009	98.94/98.08	99.29/99.15	A/Singapore/INFIMH-16- 0019/2016	98.76/99.36	98.94/99.79
A/Hunan/42443/2015	78.27/90.19	87.10/93.82	A/Switzerland/971529/2013	98.41/98.93	98.94/99.36
A/Puerto Rico/8/1934	43.82/48.40	51.41/53.73	A/Aichi/2/1968	86.04/84.65	92.05/90.41

Qubit™ 4 Assay Run Results



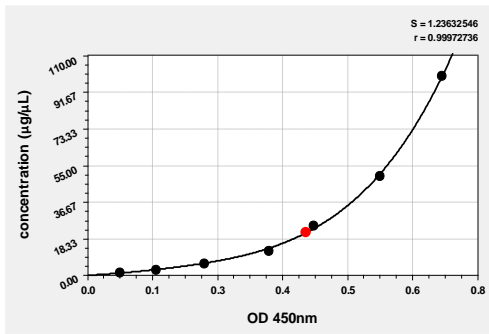
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Run ID	2023-06-26_103821
Assay Name	dsDNA High Sensitivity
Extended Low Range (ng/mL)	0.5-1.0
Core Range (ng/mL)	1.0-500.0
Extended High Range (ng/mL)	500.0-600.0
Excitation	Blue (430-495 nm)
Emission	Green (510-580 nm)
Std 1 RFU	38.47
Std 2 RFU	25366.31
Std 3 RFU	-

Test Name	Test Date	Sample Volume (µL)	Dilution Factor	Qubit Tube Conc.	Original Sample Con.	Green RFU
Sample_#230626-104532	26/06/2023 10:45:32 AM	2	100	*	*	38.54
Sample_#230626-104456	26/06/2023 10:44:56 AM	2	100	0.65 ng/mL	0.0001 µg/µL	69.70

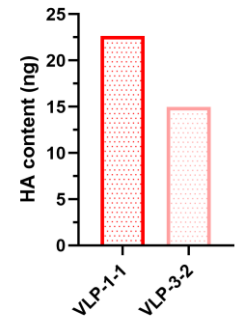
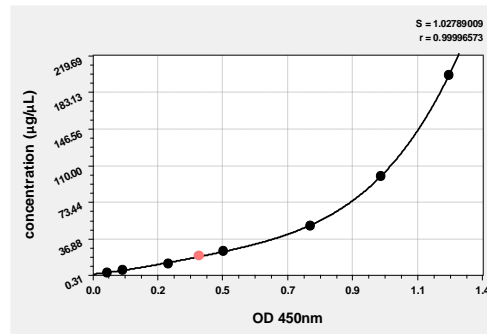
* Out of range

Supplementary Figure 1. The amount of residual baculovirus DNA in the mosaic VLPs by Qubit Fluorometric Quantification. The concentration of dsDNA in VLP-1-1 < 0.5 ng/mL, shown as “* Out of range”; The concentration of dsDNA in VLP-3-2 was 0.65 ng/mL. (Sample_#230626-104456 is VLP-1-1; Sample_#230626-104532 is VLP-3-2).

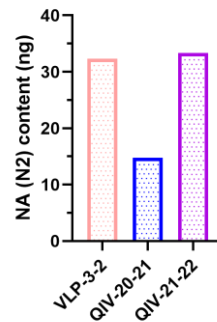
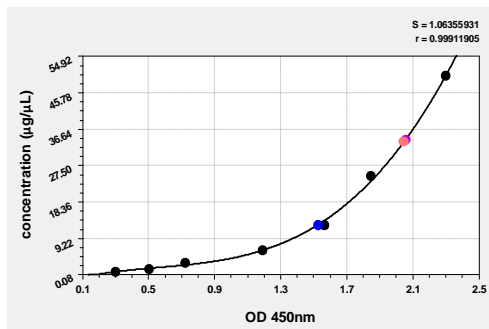
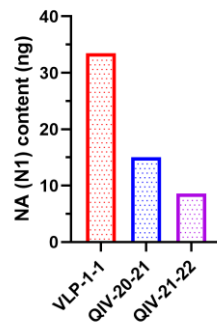
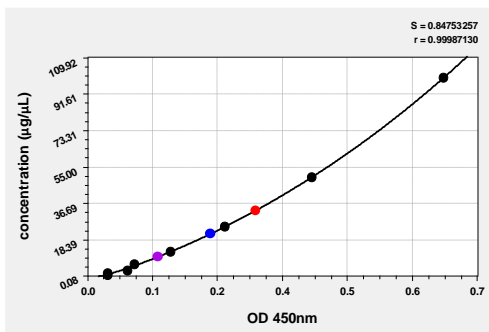
A



B

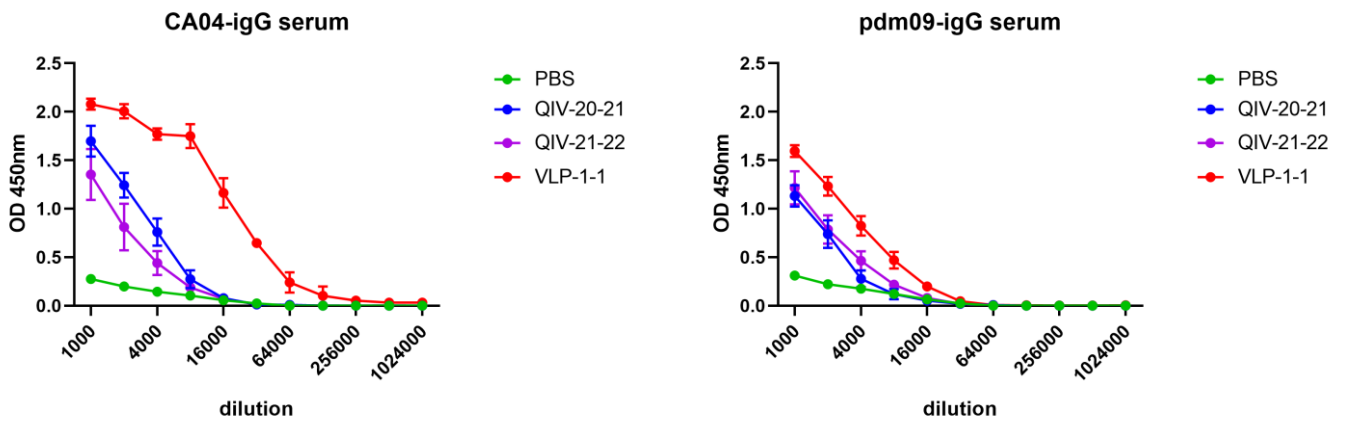


B

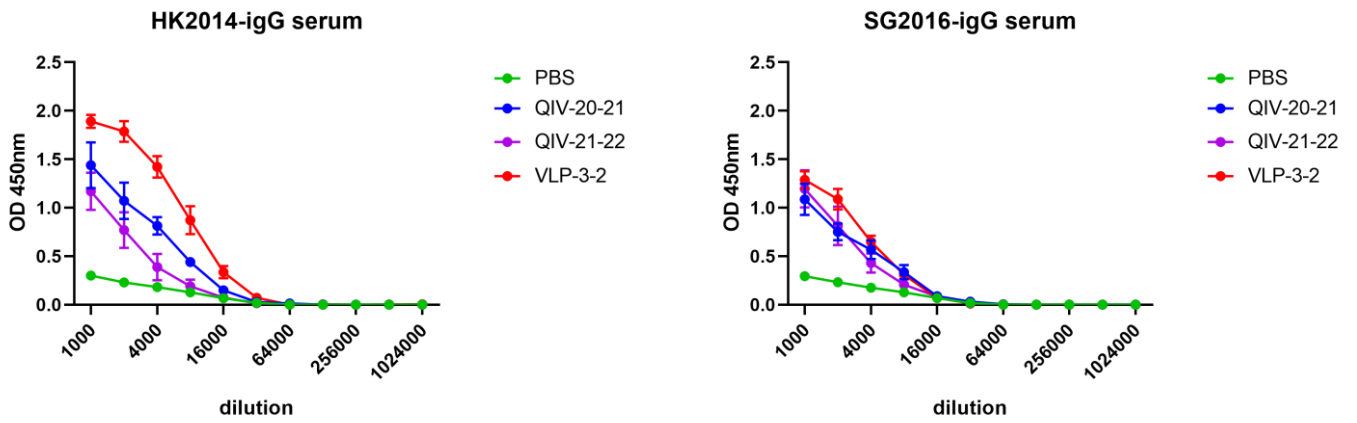


Supplementary Figure 2. Quantification of HA and NA in mosaic VLPs and QIV by ELISA. (A) Using decreasing concentrations of recombinant HA protein as standard protein, measured HA content in 1 μ g VLPs. A standard curve was generated for HA concentration based on absorbance values from standard protein (black dotted lines), the goodness of fit statistic for the standard curve is also displayed (polynomial fit). The concentration of NA in the mosaic VLPs was extrapolated from the curve (red and pink dot). (B) Using decreasing concentrations of recombinant NA protein as standard protein, measured NA content in 0.15 μ g HA of mosaic VLPs and QIV. A standard curve was generated for NA concentration based on absorbance values from standard protein (black dotted lines), the goodness of fit statistic for the standard curve is also displayed (polynomial fit). The concentration of NA in the mosaic VLPs was extrapolated from the curve (red, pink, blue and purple dot).

A

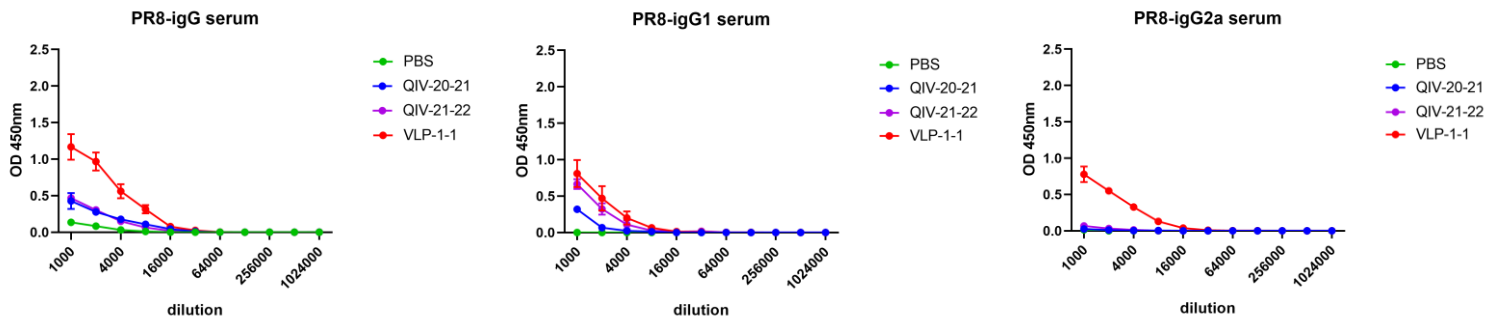


B

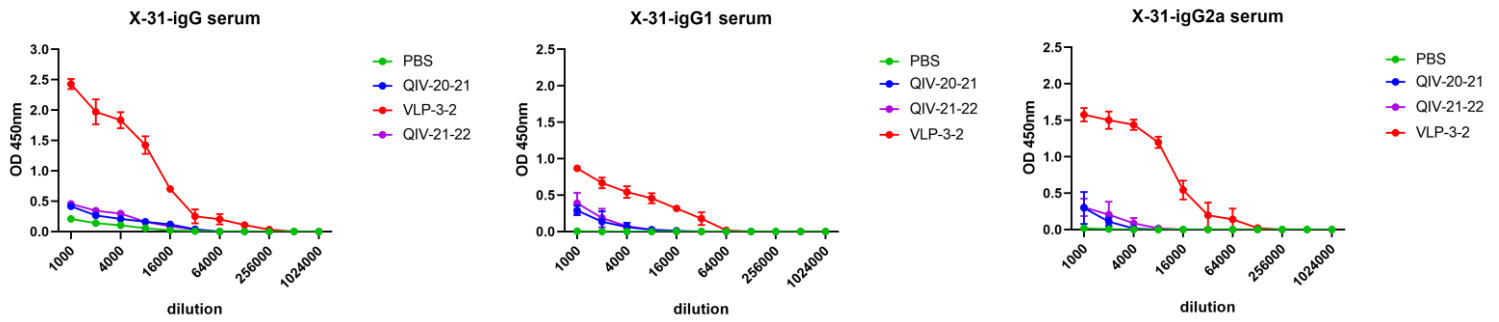


Supplementary Figure 3. Mosaic VLPs immunization elicits higher antibody titers in mice against H1 and H3 representative viruses. Antibody binding was determined by ELISA assay. (A) ELISA against H1 representative strains A/California/04/2009 (CA04) and A/California/7/2009 (pdm09). (B) ELISA against H3 representative strains A/Hong_Kong/4801/2014 (HK2014) and A/Singapore/INFIMH-16-0019/2016 (SG2016).

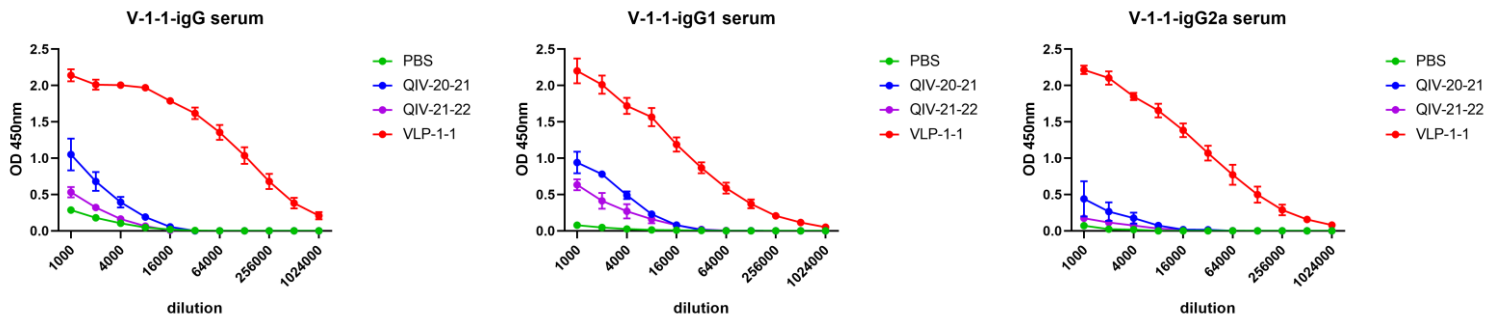
A



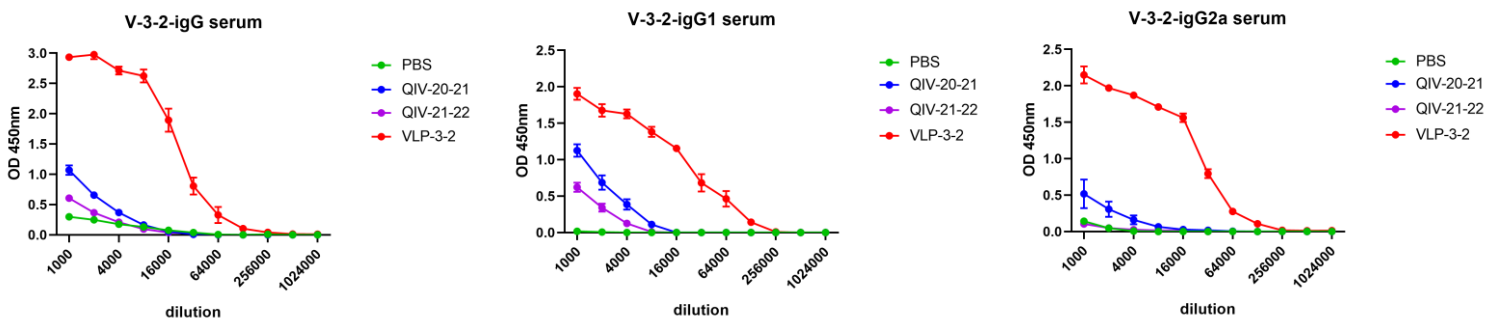
B



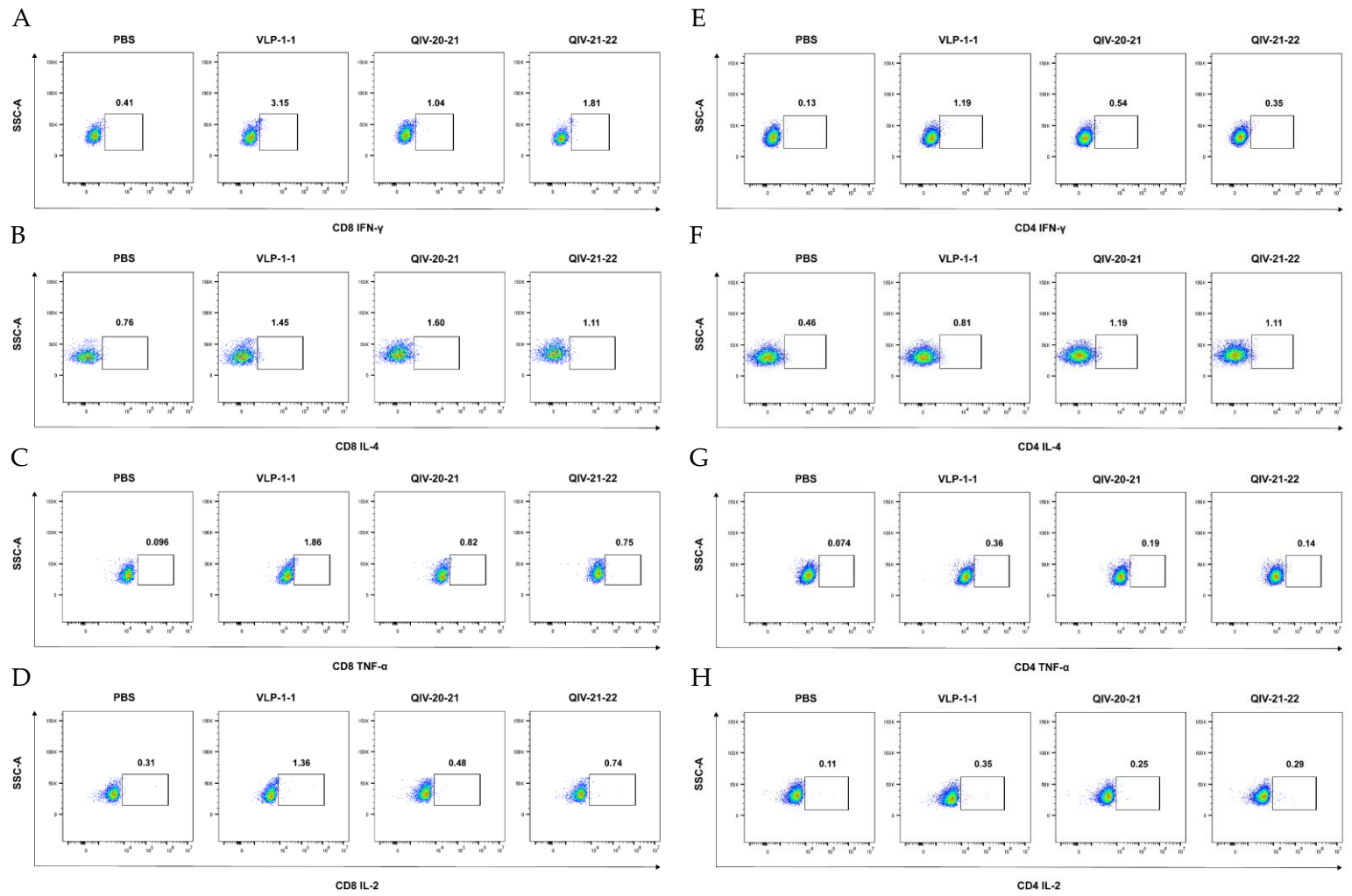
C



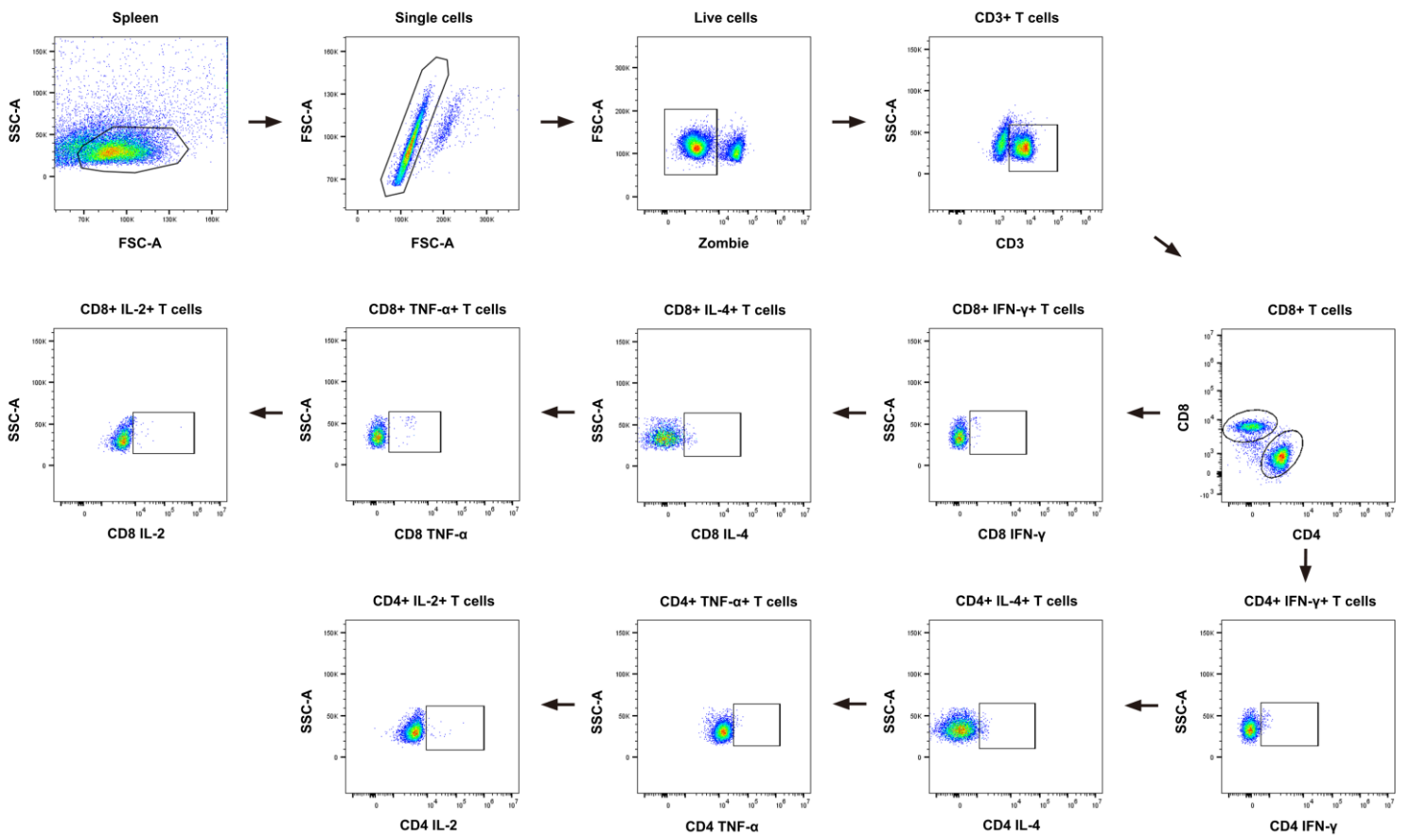
D



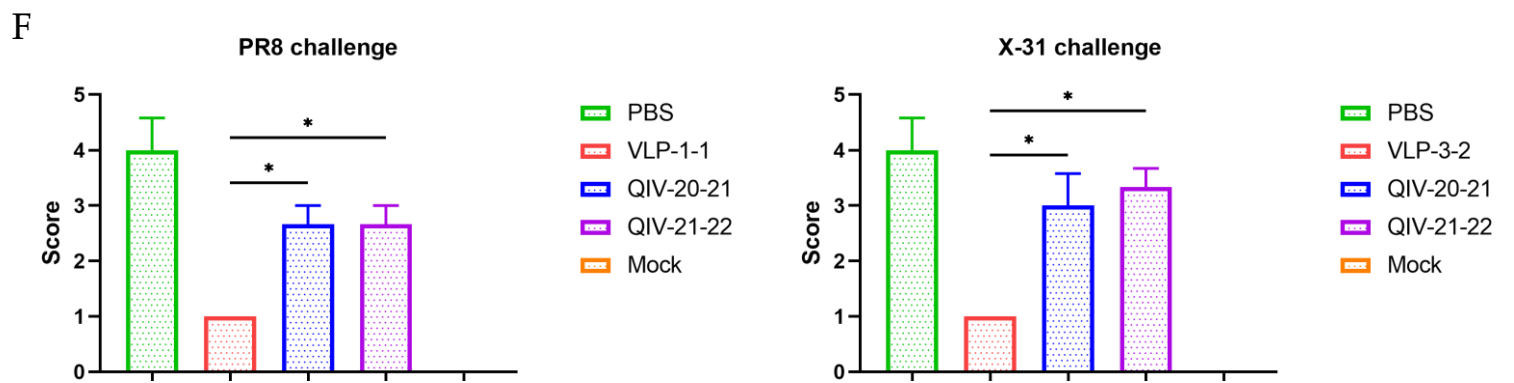
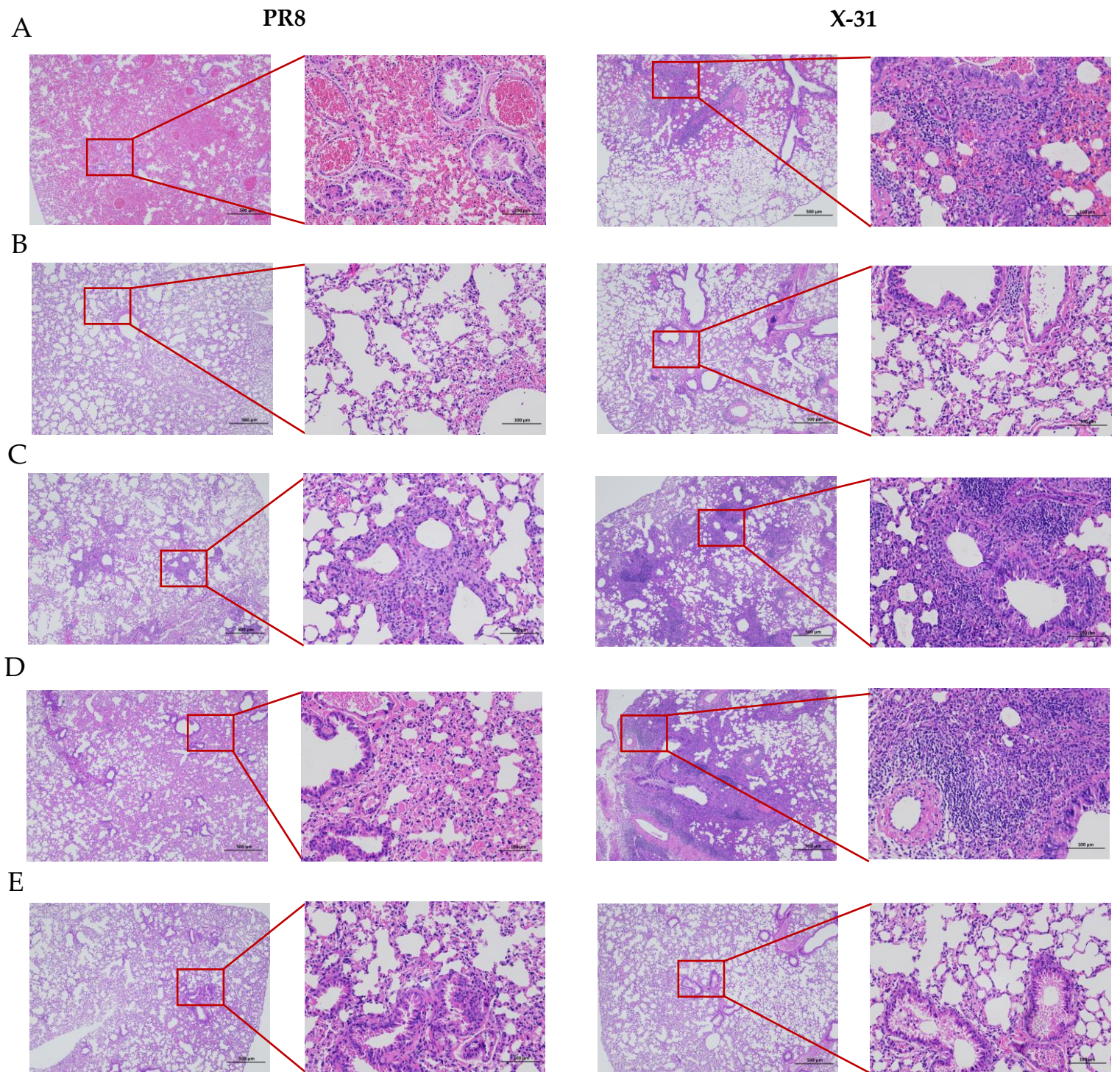
Supplementary Figure 4. Antigenicity of mosaic VLPs and QIVs. Antibody binding was determined by ELISA assay. ELISA against (A) A/Puerto Rico/8/1934 (PR8), (B) A/Aichi/2/1968 (X-31), (C) mosaic H1N1 VLP and (D) mosaic H3N2 VLP.



Supplementary Figure 5. Representative flow images of CD8⁺ IFN- γ ⁺ T cells (A), CD8⁺ IL-4⁺ T cells (B), CD8⁺ TNF- α ⁺ T cells (C), CD8⁺ IL-2⁺ T cells (D), CD4⁺ IFN- γ ⁺ T cells (E), CD4⁺ IL-4⁺ T cells (F), CD4⁺ TNF- α ⁺ T cells (G), and CD4⁺ IL-2⁺ T cells (H) generated by virus A/PR/8/1934 (PR8) stimulation.

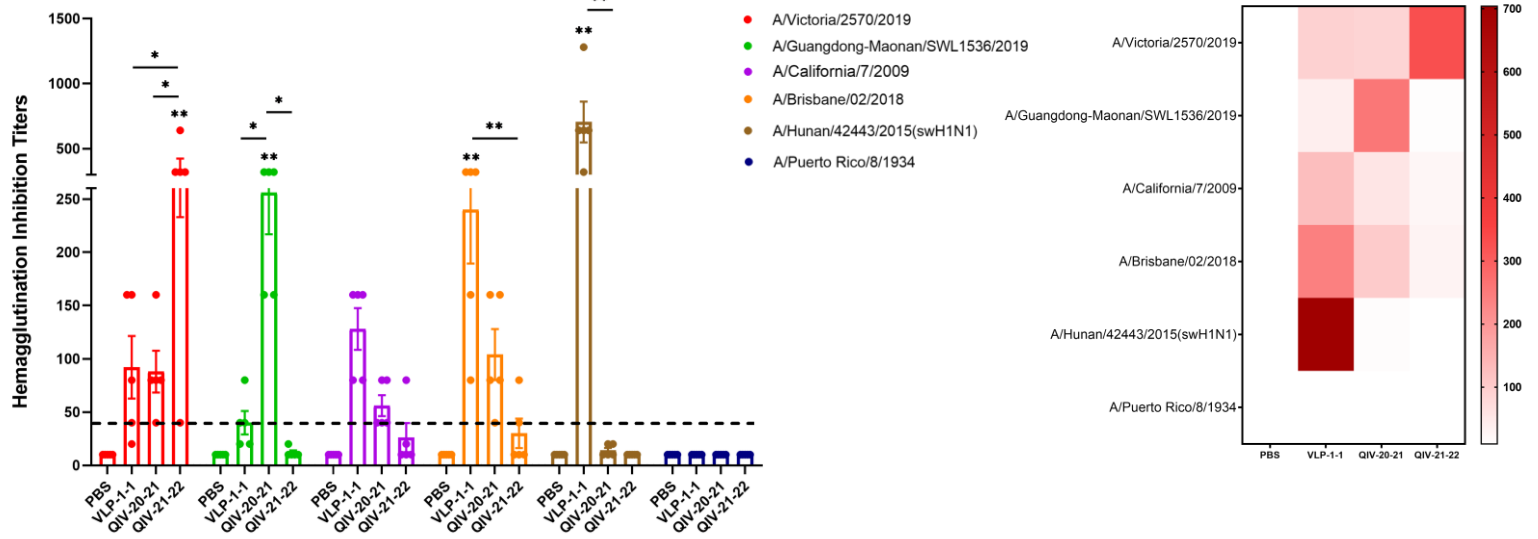


Supplementary Figure 6. Flow cytometric gating strategies of CD8⁺ IFN- γ ⁺, CD8⁺ IL-4⁺, CD8⁺ TNF- α ⁺, CD8⁺ IL-2⁺, and CD4⁺ IFN- γ ⁺, CD4⁺ IL-4⁺, CD4⁺ TNF- α ⁺, CD4⁺ IL-2⁺ T cells.

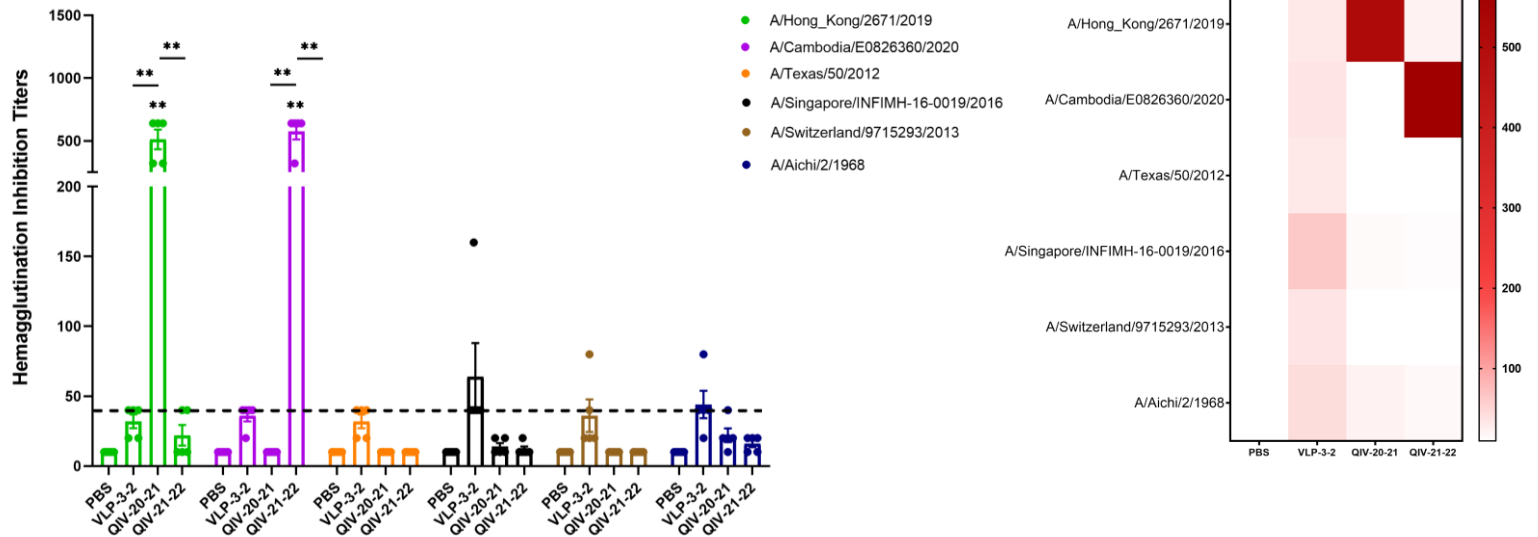


Supplementary Figure 7. Lung histology of challenged mice. Mice were challenged with A/Puerto Rico/8/34 (PR8) and A/Aichi/2/1968 (X-31) viruses at week 5 following immunization with vaccines PBS (A), mosaic VLPs (B), QIV20-21 (C), and QIV21-22 (D). Non-challenged control mice (E). At days 4 post-infection, the mice were euthanized and lungs were harvested. Lungs were inflated with 10% formalin, fixed and processed for paraffin embedding, and 5-mm sections stained for H&E. Observations were made at 40 \times and 200 \times magnification. (F) Lungs were scored according to severity of overall lesions, where 0 indicates no abnormalities detected, 1 indicates minimal abnormalities (5 to 20% lung area), 2 indicates mild abnormalities (20 to 40% lung area), 3 indicates moderate abnormalities (40 to 60% lung area), 4 indicates marked abnormalities (60 to 80% lung area), and 5 indicates severe abnormalities (80 to 100% lung area). (n = 3; one-way ANOVA with Tukey multiple comparison), * p < 0.05, ** p < 0.01.

A



B



Supplementary Figure 8. Mosaic VLPs immunization elicits robust cross-reactive antibody responses in mice against H1 and H3 representative viruses. BALB/c mice were vaccinated with VLPs and compared to QIV-vaccinated mice. The HI antibody response to H1 (A) or H3 (B) representative strains was determined by HI assay in mice immunized with prime immunization. A heat map of HI titers was further constructed to better visualize cross-reactive antibody response for each vaccine. Data are presented as the mean with standard error (SEM) ($n = 5$; two-way ANOVA with Tukey multiple comparison), $*p < 0.05$, $**p < 0.01$.