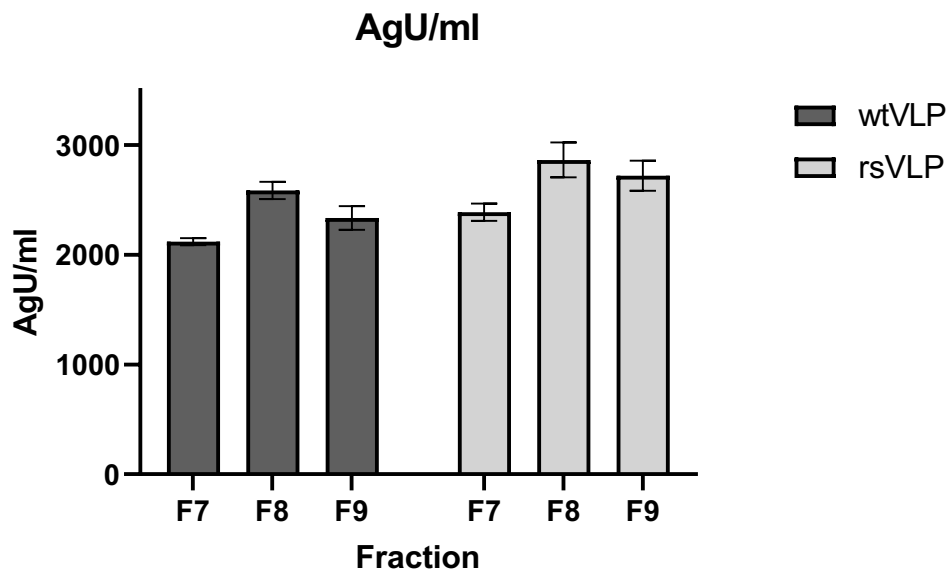


618 **Supplementary material:**

619 **Figure S1:**

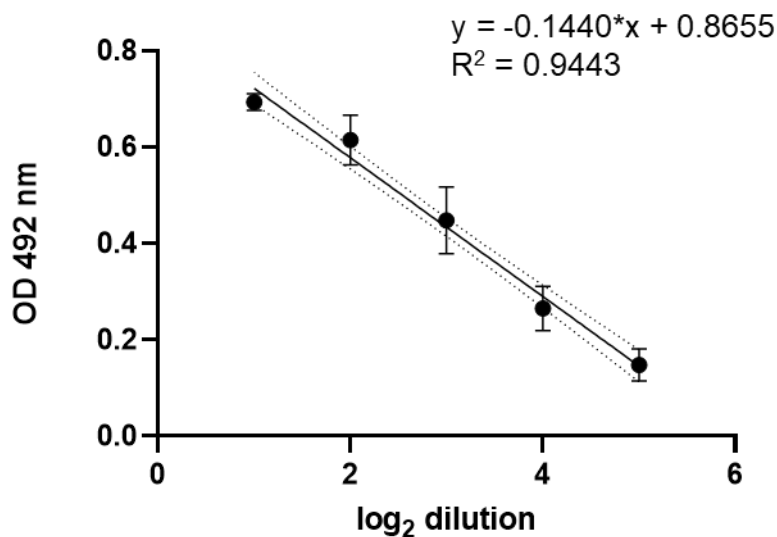


620

621 **Figure S1:** Antigen concentration (AgU/ml) of peak VLP fractions determined by the CT11F9
622 antibody relative to the 18/116 antigen standard.

623

624 **Figure S2:**



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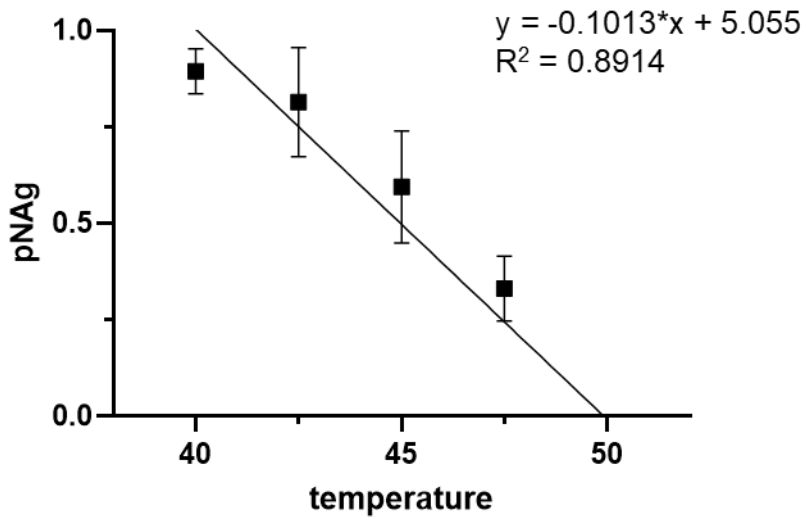
626 **Figure S2:** Relationship between OD and log₂ dilution of antigen, used to calculate the
627 proportion of HA_g in samples.

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631 **Figure S3:**

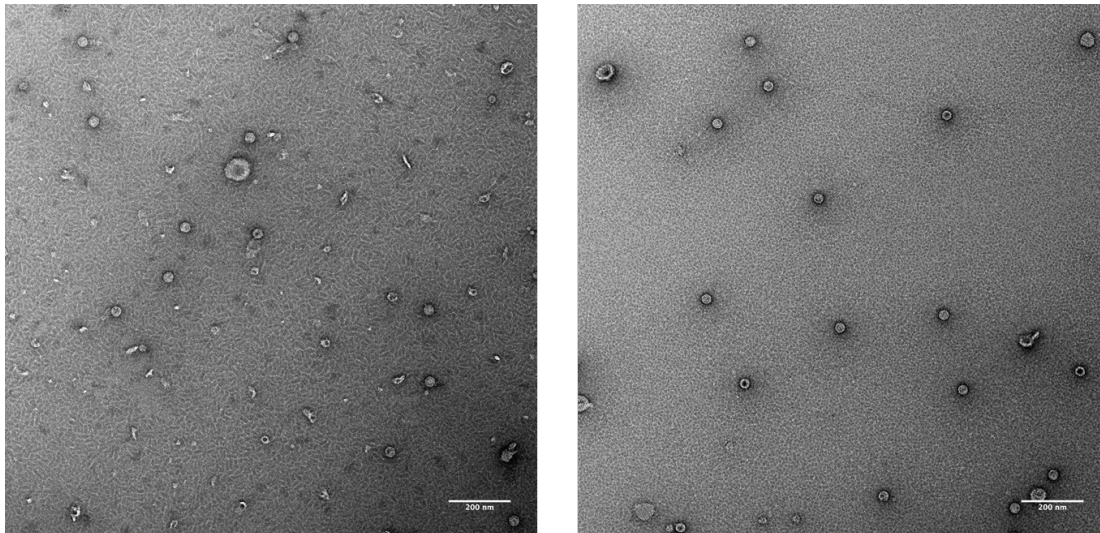


632

633 **Figure S3:** Linear regression to estimate antigenic conversion temperature of rsVLPs
634 between 40°C and 50°C.

635

636 **Figure S4:**



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638 **Figure S4:** Negative stain TEM (using 2% UA) of samples of wtVLPs (left) and rsVLPs (right),
639 scale bar = 200 nm.

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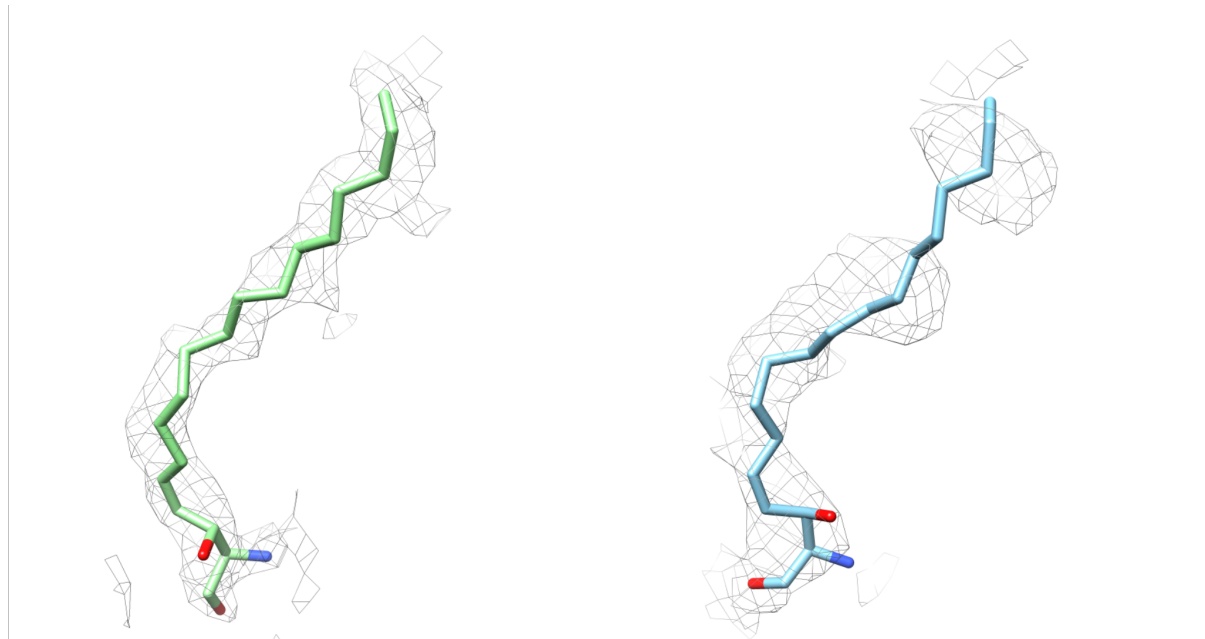
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646 **Figure S5:**

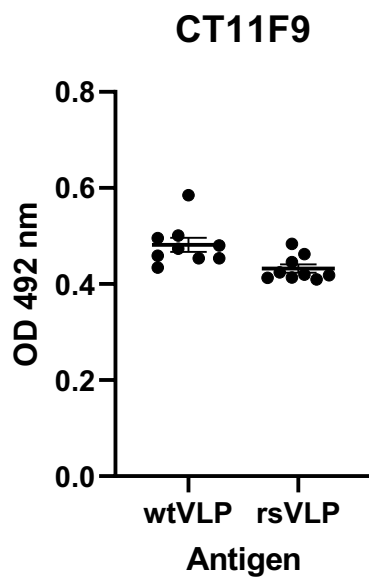


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648 **Figure S5:** Density indicating occupancy of the pocket, present in the major population (left)
649 and the minor population (right) shown at 2.5 rmsd.

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651 **Figure S6:**



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653 **Figure S6:** Reactivity of VLP (captured on ELISA plates) with CT11F9 antibody.

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658 **Table S1:** Antigen composition of samples at a range of temperatures

$$\text{proportion HAg} = 2^{(OD\ 4^\circ\text{C} - OD\ 55^\circ\text{C})/\text{incline}}$$

Temperature (°C)	pHAg rsVLP	pNAg rsVLP	pHAg wtVLP	pNAg wtVLP
4	0.13	0.87	1.11	-0.11
30	0.11	0.89	0.80	0.20
35	0.10	0.90	0.69	0.31
37.5	0.11	0.89	0.84	0.16
40	0.14	0.86	0.97	0.03
42.5	0.31	0.69	1.16	-0.16
45	0.46	0.54	1.18	-0.18
47.5	0.86	0.14	1.32	-0.32
50	1.10	-0.10	1.54	-0.54
52.5	1.07	-0.07	1.17	-0.17
55	1.00	0.00	1.00	0.00
60	0.80	0.20	0.73	0.27

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663 **Table S2:**

	rsVLP
Microscope	FEI Titan Krios
Detector mode	Counting
Camera	Falcon IV
Voltage (kV)	300
Pixel size (Å)	0.91
Nominal magnification	130,000×
Exposure time (s)	5
Total dose (e ⁻ /Å ²)	31
Number of fractions	42
Defocus range (μm)	-0.8 to -2.9
Number of micrographs	20,419
Acquisition software	Thermo Scientific EPU

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669 **Table S3:**

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Model	<i>rsVLP</i>
EMDB ID	EMD-16450
PDB ID	PDB-8C6D
CryoEM map processing	
<i>Symmetry imposed</i>	11
<i>Number of particles contributing to map</i>	11,789
<i>Map resolution (FSC = 0.143) (Å)</i>	2.4
<i>Map resolution range at atomic coordinates (Å)</i>	2.4-2.8
<i>Map sharpening B factor (Å²)</i>	-133.6
Residues modelled	
	A 58-297 B 13-45, 83-323 C 1-242
RMSD	
<i>Bond lengths (Å)</i>	0.0096
<i>Bond angles (°)</i>	1.33
Validation	
<i>All-atom clashscore</i>	8.17
<i>MolProbity score</i>	2.21
<i>Rotamer outliers (%)</i>	3.89
Ramachandran plot	
<i>Favoured (%)</i>	95.45
<i>Allowed (%)</i>	4.55
<i>Outliers (%)</i>	0.00

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