Chimeric O1K foot-and-mouth disease virus with SAT2 outer capsid as an FMD vaccine candidate

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Supplementary figure 1

Cryo-EM density maps, (a) SAT2 wt (b) SAT2/O (c) SAT2/O mut 2 (d) ts-SAT2/O. Depth-cueing is used such that colour indicates radius (<110 Å: cyan; 120–140 Å: yellow; >150 Å red). (e) A snapshot of atomic model fitted into density map of ts-SAT2.

(a)

(b)

(c)

(d)

(e)
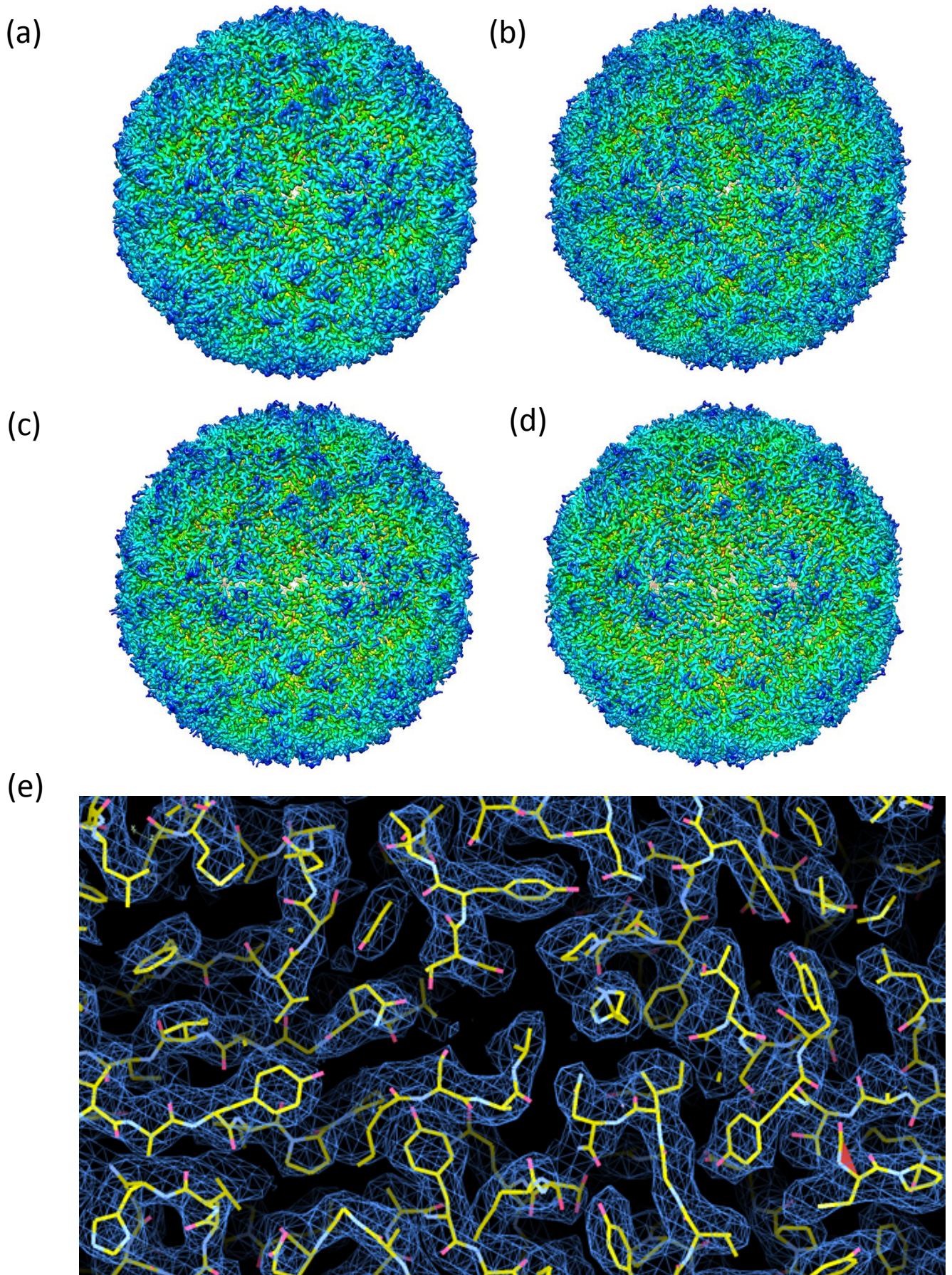
Supplementary figure 2

Disorder prediction of amino acids on VP4. The disorder predication of amino acid residues on VP4 was carried out using MoreRONN (unpublished). The residues with disorder probabilities higher than 0.5 are considered as disordered.
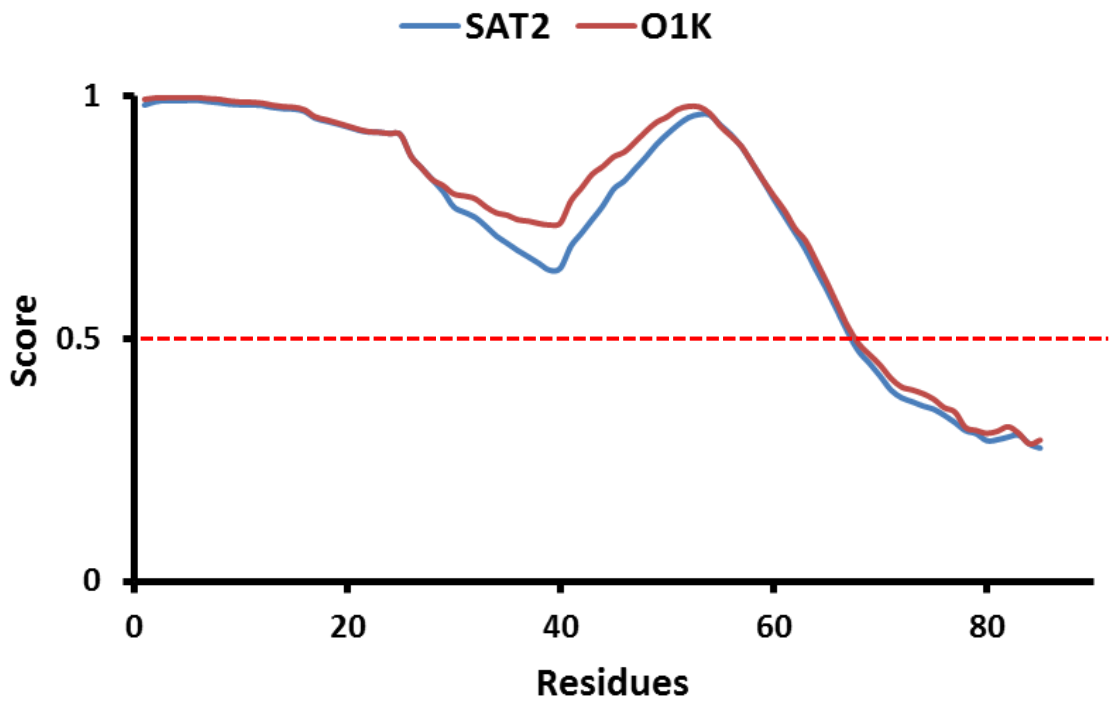
Supplementary figure 3

Polyclonal serum that detects both 146S (intact virus) and 12S (capsid subunits) was used for ELISA to confirm the presence of comparable levels of antigen before and after incubation at different temperatures. Wild type SAT2/O and SAT2/O viruses containing one (SAT2/O mut 1, SAT2/O mut 2, SAT2/O mut 3) or three targeted (SAT2/O mut 1/2/3) VP4 mutations, were incubated at 4°C, 40°C or 47°C for 10 minutes.
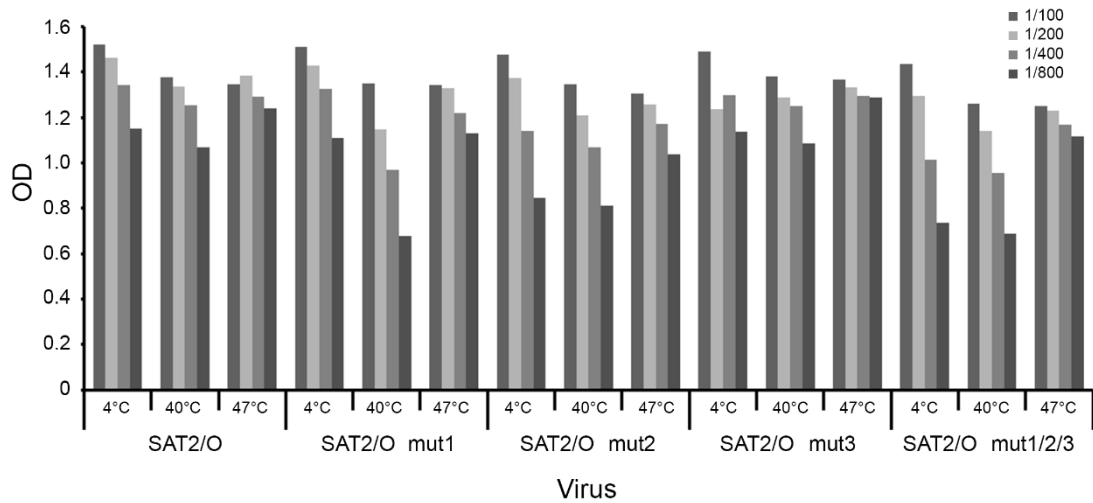
Supplementary Table 1

Wild type SAT2/O and SAT2/O viruses containing one (SAT2/O mut 1, SAT2/O mut 2, SAT2/O mut 3) or three targeted (SAT2/O mut 1/2/3) VP4 mutations, were incubated at 4°C, 40°C or 47°C for 10 minutes. Virus samples were then analysed by ELISA using a single-domain llama antibody (M377F VHH) that specifically recognises intact FMDV capsid (146S).

Dilution	SAT2/O			SAT2 Rd1			SAT2 Rd2			SAT2 Rd3			SAT2 Rd1/2/3		
	Intact 4°C	40°C	47°C	Intact 4°C	40°C	47°C	Intact 4°C	40°C	47°C	Intact 4°C	40°C	47°C	Intact 4°C	40°C	47°C
1/100	1.728	1.633	1.454	1.684	1.57	1.395	1.592	1.235	0.305	1.673	1.595	1.449	1.551	1.444	0.449
1/200	1.619	1.493	1.029	1.525	1.312	1.086	1.236	0.784	0.133	1.582	1.528	1.237	1.155	1.086	0.267
1/400	1.352	1.182	0.678	1.227	0.948	0.695	0.844	0.445	0.1	1.347	1.294	0.905	0.732	0.644	0.225
1/800	1.009	0.757	0.412	0.824	0.56	0.438	0.452	0.205	0.062	0.976	0.864	0.623	0.401	0.338	0.139

Supplementary Table 2

Thermofluor analysis to determine capsid stability of parental and chimeric SAT FMDVs at different pH values (pH 6, 6.3, 6.5, 6.8, 7.0, 8.0, 8.5 and 9.0). The respective temperatures of genome release, and hence capsid disassembly, are shown.

Virus	pH 6	pH 6.3	pH 6.5	pH 6.8	pH 7	pH 8	pH 8.5	pH 9
SAT2	28.7 ± 0.5	36.5 ± 0.3	39.3 ± 0.3	43.9 ± 0.1	46 ± 0.1	51.9 ± 0.1	51.1 ± 0.3	49.5 ± 0.0
SAT2/O	28.6 ± 0.3	36.6 ± 0.1	40.4 ± 0.3	45.5 ± 0.1	47 ± 0.1	53 ± 0.0	52 ± 0.2	50.5 ± 0.0
SAT2/O mut 1/2/3	ND	ND	41.3 ± 0.1	46.5 ± 0.0	47 ± 0.0	50.9 ± 0.1	50.9 ± 0.1	48.5 ± 0.0
SAT2/O mut 1	34.4 ± 0.1	42.7 ± 0.2	45.6 ± 0.0	48.5 ± 0.1	50 ± 0.1	53.9 ± 0.0	52.5 ± 0.0	50.9 ± 0.1
SAT2/O mut 2	26.5 ± 0.3	31.4 ± 0.3	34.8 ± 0.3	41.2 ± 0.1	42.9 ± 0.0	49 ± 0.1	48.1 ± 0.1	46.4 ± 0.4
SAT2/O mut 3	31.8 ± 0.4	40.1 ± 0.1	42.9 ± 0.0	46.5 ± 0.1	49.1 ± 0.1	52.9 ± 0.1	51.5 ± 0.0	50 ± 0.1

Supplementary Table 3

Cryo-EM data collection and refinement statistics.

Samples	SAT2	SAT2/O	SAT2/O mut 2	ts-SAT2/O
Data Collection				
Microscope	FEI Tecnai Polara	FEI Titan Krios	FEI Titan Krios	FEI Titan Krios
Detector	Gatan K2 Summit	Gatan K2 Summit	Gatan K2 Summit	Gatan K2 Summit
Filter	Gatan GIF Quantum	Gatan GIF Quantum	Gatan GIF Quantum	Gatan GIF Quantum
Pixel size (Å)	1.35	1.06	1.06	1.3
Defocus range (µm)	-2.8 to -1.0	-2.8 to -1.0	-2.8 to -0.8	-2.8 to -0.8
Voltage (kV)	300	300	300	300
Electron dose (e$^-$ Å^{-2})	30	30	30	30
Reconstruction				
Number of particles	8935	6606	3554	8776
Software	RELION 1.3	RELION 1.4	RELION 1.4	RELION 1.4
Symmetry	I1	I1	I1	I1
Final resolution (Å)	3.5	2.8	3.7	3.6
Map sharpening B-factor (Å^2)	-121.7	-57.4	-122.0	-141.1
Atomic model refinement				
Software	Phenix	Phenix	Phenix	Phenix
Resolution limit (Å)	3.5	2.8	3.7	3.6
Number of atoms				
Protein atoms	5400	5400	5400	5207
Other atoms	-	-	-	-
Residues				
Map CC (around atoms)	80%	79%	77%	75%
RMS bond lengths (Å)	0.01	0.01	0.01	0.01
RMS bond angles (°)	0.94	0.96	0.83	0.97
Validation				
Clashscore, all atoms (percentile)	3.13	3.75	3.89	2.56
Rotamer outliers (%)	0.1	1.1	0.2	0.7
C-beta deviations >0.25 Å	0	0	0	0
Ramachandran plot (%)				
Favored	89.5	93.8	90.5	88.7
Allowed	10.4	6.1	9.3	11.0
Outliers	0.1	0.1	0.2	0.3