

Antigenic refocusing of a SAT2 FMDV through epitope dampening

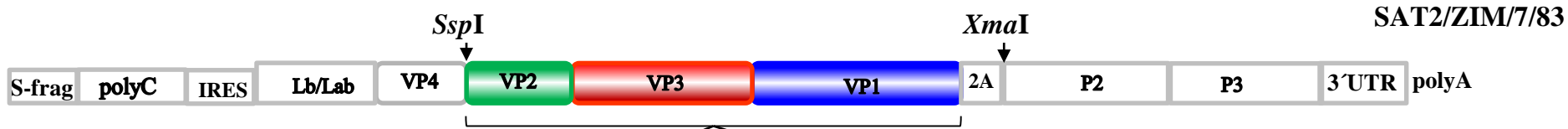
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Introduction

- Antigenic variation → multiple SAT2 subtypes
- Strain specific immunity → poor intra-serotype cross-protection
- Pathogens mechanism to evade immune system → deceptive imprinting
- Surface-exposed, hypervariable, immunogenic regions → decoy epitopes
- Masking or removing of decoy epitopes → **immune refocusing**
- **AIM**: To identify the antigenic determinants of a SAT2 virus utilising antigenic refocusing strategy
 - charge-dampening and replacement of hypervariable surface exposed residues.

Viable SAT2 mutant viruses



Epitope-replaced mutants (SAT2/EGY/9/2012)

vEGY^{VP3}SAT2 129IETDRLPK¹³⁶ → 129VAV QMPS¹³⁶ :: 187AVF¹⁸⁹ → 187VVL¹⁸⁹

vEGY^{site3}SAT2 43LTNRTAFA⁵⁰ → 43HTNKTSFV⁵⁰

vEGY^{EHHER}SAT2 81LGEHERVW⁸⁸ → 81VGDHTRVF⁸⁸

vEGY^{HNN}SAT2 108SHNN¹¹¹ → 108AKGG¹¹¹

vEGY^{site5}SAT2 135KYTQQS¹⁴⁰ → 135VYTKAA¹⁴⁰

vEGY^{GH}SAT2 135 GH 160

vEGY^{Ct}SAT2 196 Ct 216

vEGY^{GH&Ct}SAT2 135 GH Ct 216

vEGY/SAT2 VP2 VP3 VP1

vEGY^{ZIMGH}/SAT2 VP2 VP3 ZIMGH

Charge-dampened mutants (Alanine residues)

vSAT^{2a} 70SD⁷¹ → 70AA⁷¹

vSAT^{2b} 132KD¹³³ → 132AA¹³³

vSAT^{2c} 191QMQ¹⁹³ → 191AAA¹⁹³

vSAT^{VP3} 129IETDRL¹³⁴ → 129AAAAAA¹³⁴

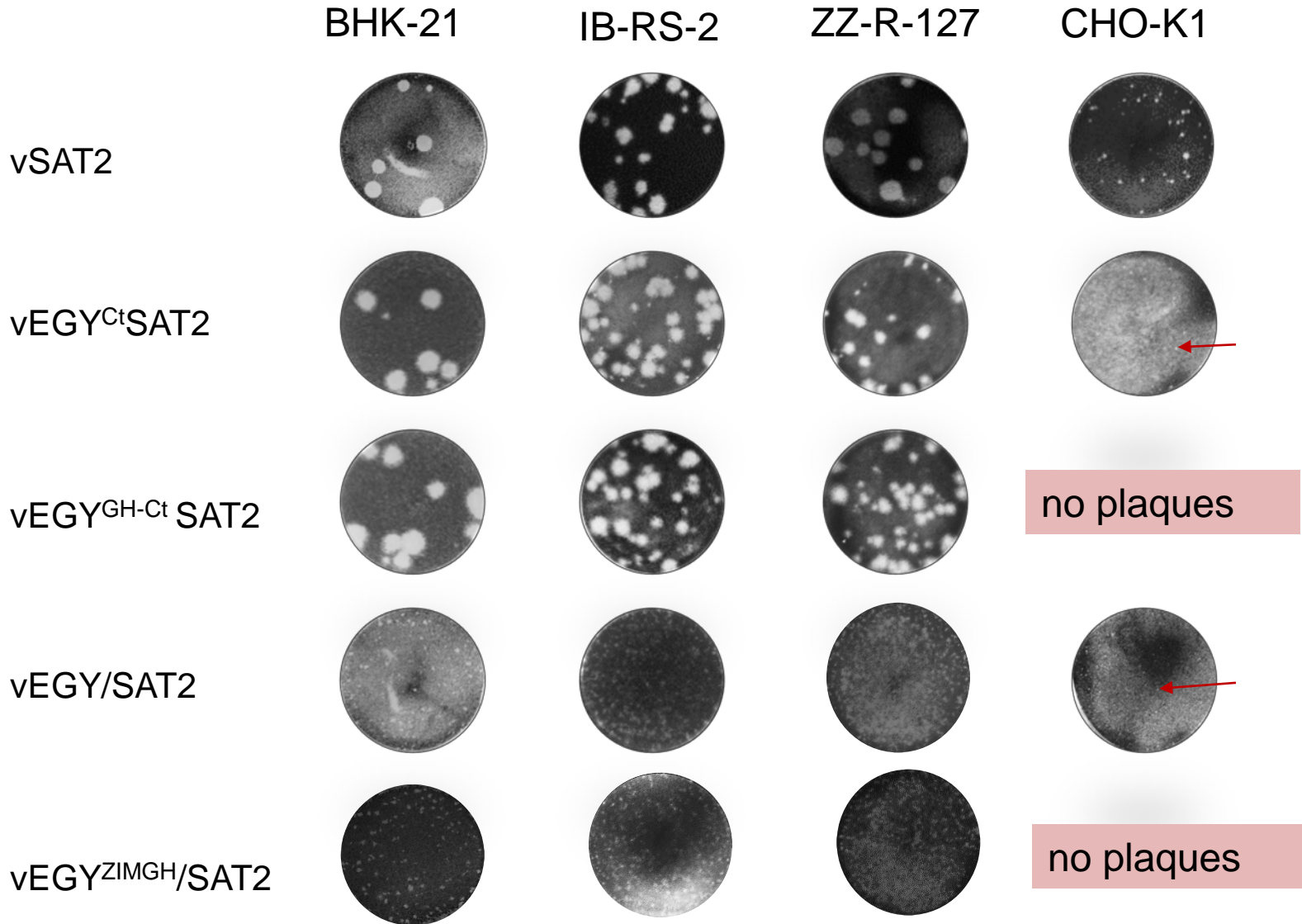
vSAT^{site3} 46RTAFA⁵⁰ → 46ATAAA⁵⁰

vSAT^{EHHER} 81IGEHER⁸⁷ → 81AGAAAA⁸⁷

vSAT^{HNN} 109HNN¹¹¹ → 109AAA¹¹¹

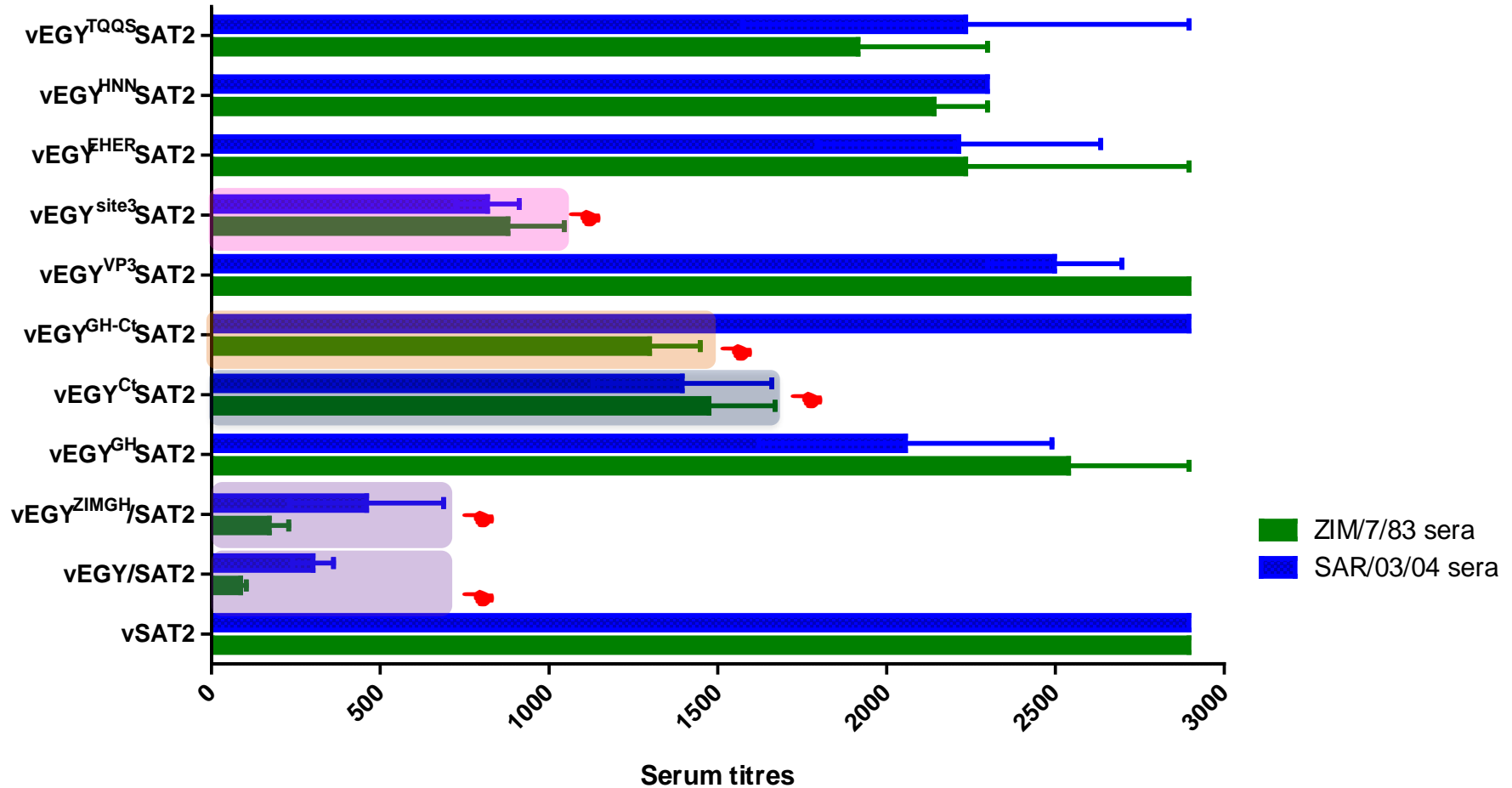
vSAT^{site5} 137TQQS¹⁴⁰ → 137AAAA¹⁴⁰

Mutants phenotype



Micro : <1 mm
S : 1-2 mm
M : 3-5 mm
L : 7-8 mm

Antigenicity of epitope-replaced viruses

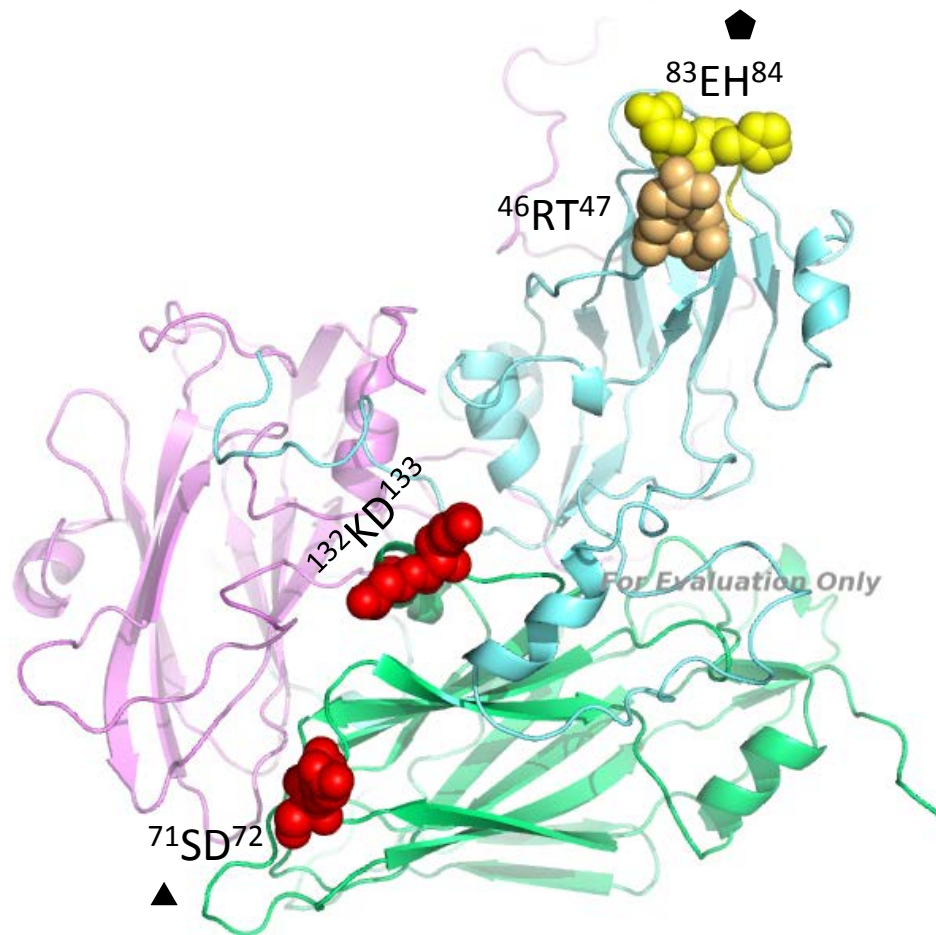


Antigenic profiling with SAT2-specific mAbs

	Mutants	ID5	GG1	GE11	GD12	DA10	Capsid position
Alanine replacements	vSAT2 ^{site3}						VP1; 46-50
	vSAT2 ^{EHER}	57	60	58	72	67	VP1; 81-87
	vSAT2 ^{HNN}				80	80	VP1; 109-111
	vSAT2 ^{site5}						VP1; 137-140
	vSAT2 ^{2A}	22	9	12		8	VP2; 70-71
	vSAT2 ^{2B}	64	55	70		64	VP2; 132-133
	vSAT2 ^{2C}						VP2; 191-193
	vSAT2 ^{VP3}	65	66	59	60	59	VP3; 129-134
Epitope replacements	vEGY ^{VP3} SAT2						VP3; 129-136 :: 187-189
	vEGY ^{site3} SAT2					30	VP1; 43-50
	vEGY ^{EHER} SAT2						VP1; 81-88
	vEGY ^{HNN} SAT2						VP1; 108-111
	vEGY ^{TQQS} SAT2						VP1; 135-140
	vEGY ^{GH} SAT2						VP1; 135-160
	vEGY ^{Ct} SAT2						VP1; 135-216
	vEGY ^{GH-Ct} SAT2				71		VP1; 196-216
	vEGY/SAT2		41	57	33		VP1, VP2 and VP3
	vSAT2						-

0-25
 25-65
 65-80
 80-100

mAb binding footprint



Conclusions

- Replacement of VP1 C-terminus or in combination with GH-loop resulted in reduction of neutralising titre
- Region 43-50 in VP1 (**site3**) seems to play a role of a decoy epitope
- Confirms residues 70-71 (**site 2A**) and 132-133 (**site 2B**) in VP2 and residues 82-85 (**EHER**) of VP1 as antigenic sites of SAT2

Collaborators

Agricultural Research Council



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