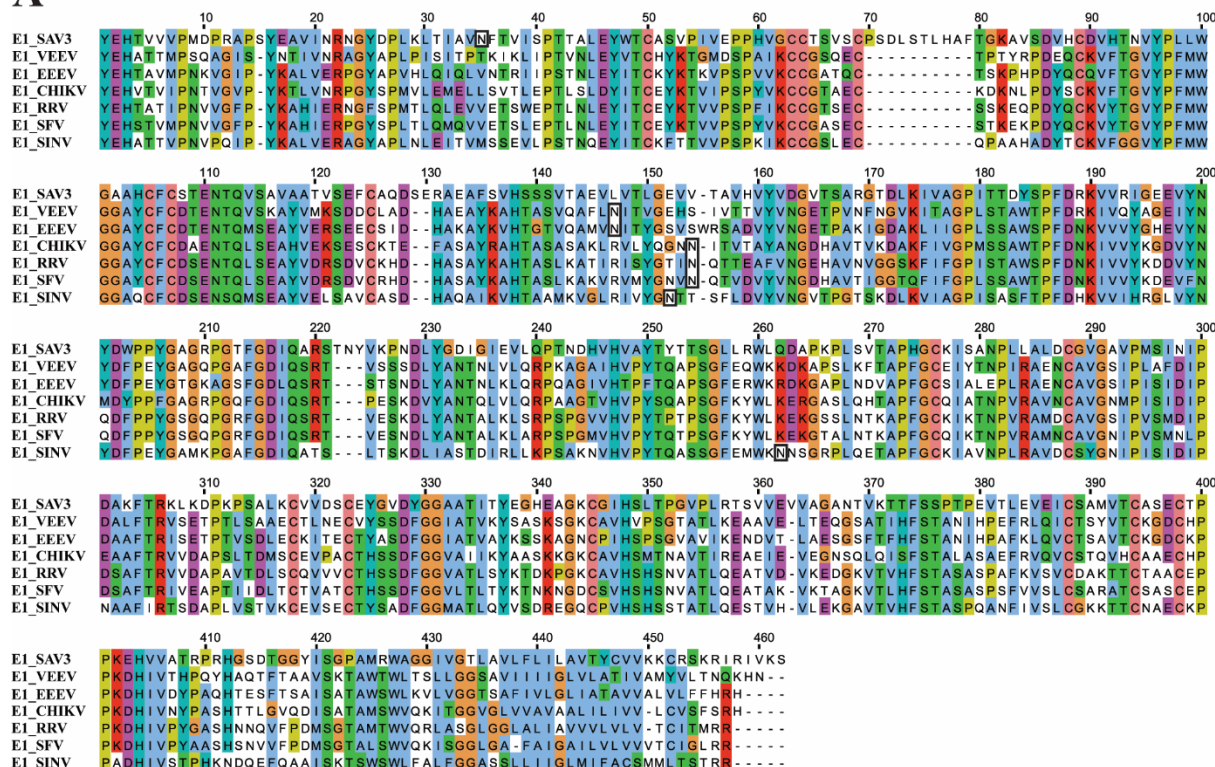
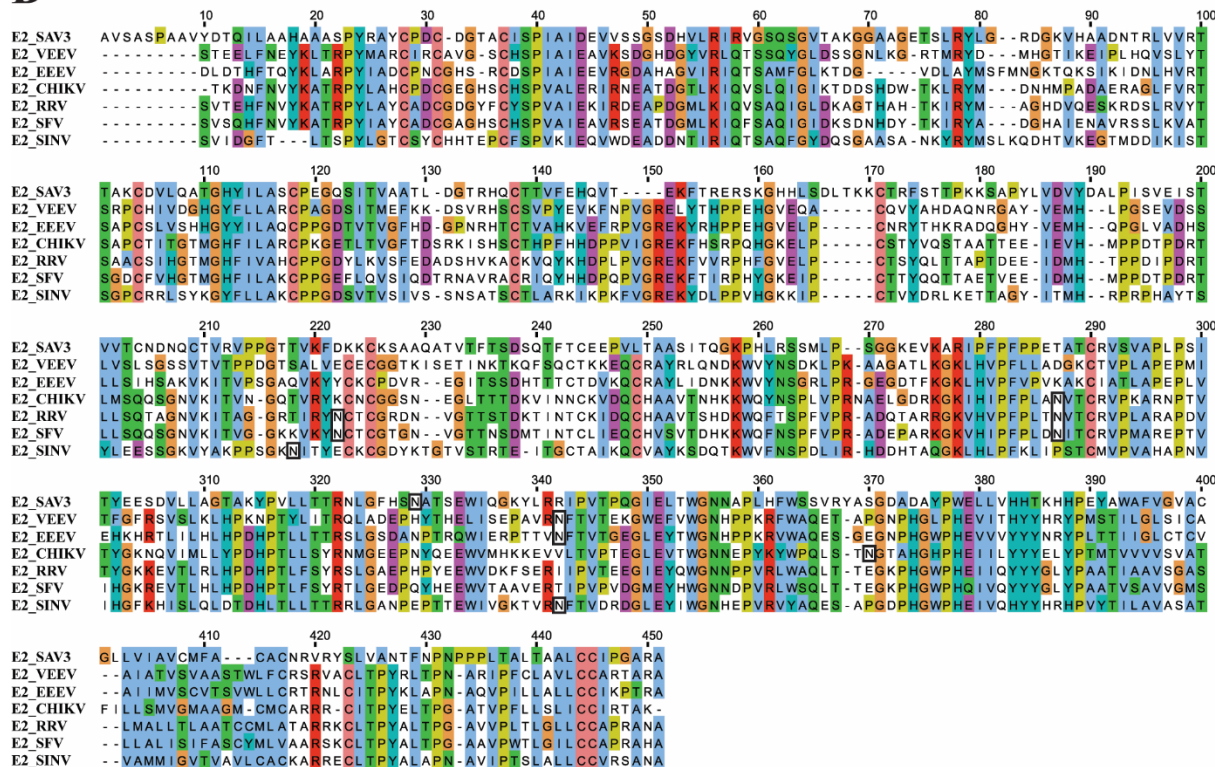




A



B



1

2

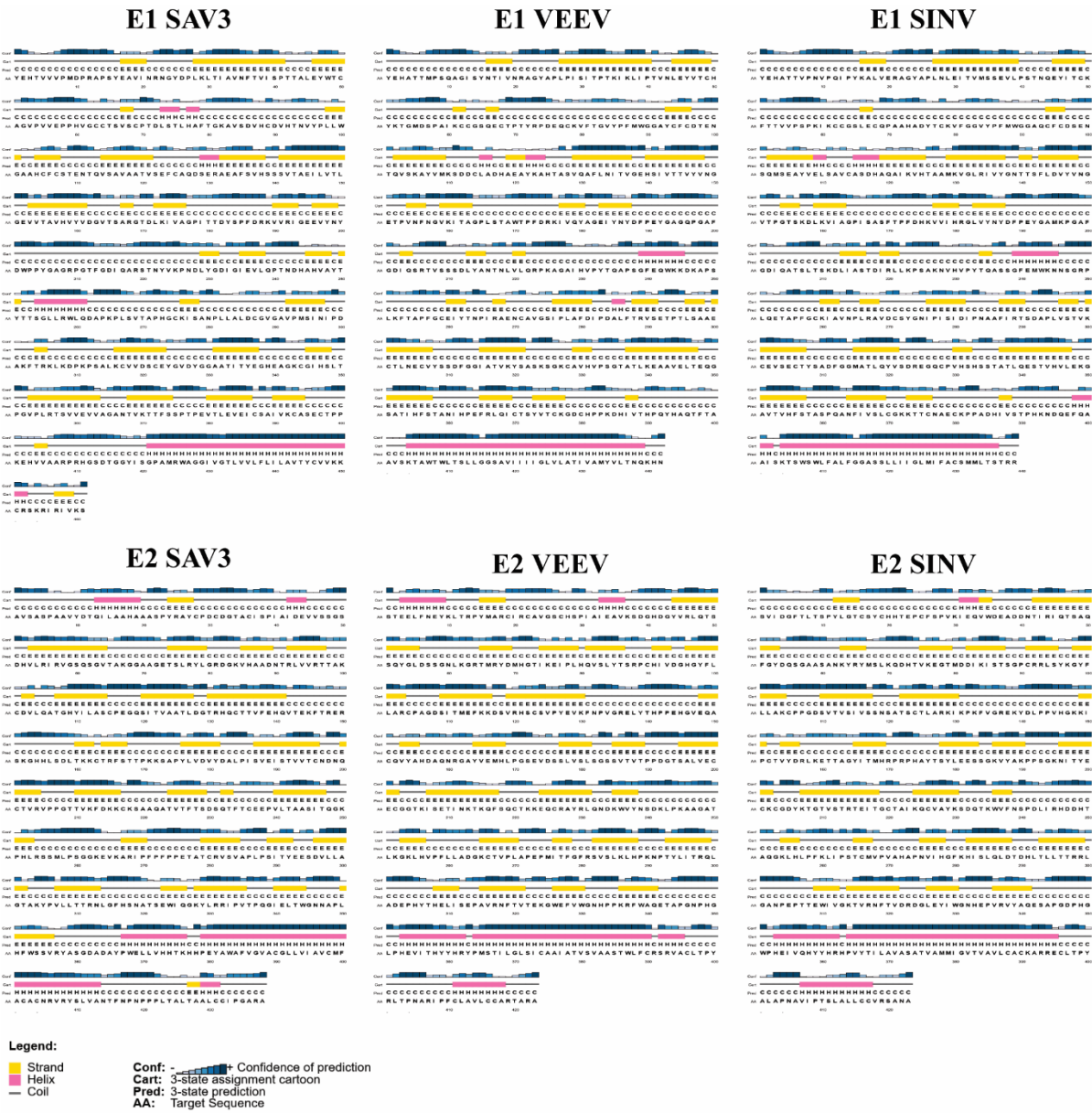
3

4

5

**Figure S1.** Multiple sequence alignments of the A1 and B) E2 protein sequences from SAV3 and the alphaviruses Venezuelan equine encephalitis virus (VEEV) (3J0C\_A/B, P09592 (like)), Eastern equine encephalitis virus (EEEV) (ANB41743), Chikungunya virus (CHIKV) (AEA10291), Ross river virus (RRV) (P08491), Semliki forest virus (SFV) (NP\_819008, NP\_819006) and Sinbis virus (SINV)

(CAA24684). The predicted N-glycosylation sites for SAV E1 and E2, and those from the other alphaviruses, are shown in black boxes. Dotted lines in bold indicate sequence sections removed for display purposes.



**Figure S2.** PSIPRED secondary structure predictions of **A** E1 and **B** E2 from SAV3 and the alphaviruses Venezuelan equine encephalitis virus (VEEV) (3J0C\_A/B, P09592 and Sinbid virus (SINV) (CAA24684).

**Table S1.** Pairwise amino acid sequence identities (above diagonal) and similarities (below diagonal) between E1 from SAV3 and selected alphaviruses (%).

	SAV3	VEEV	EEEV	CHIKV	RRV	SFV	SINV
SAV3	-	38.2	40.0	37.4	38.8	39.7	39.4
VEEV	49.3	-	60.1	50.8	52.3	51.4	50.3
EEEV	50.8	70.5	-	51.7	54.6	56.6	52.8
CHIKV	49.4	64.0	65.1	-	60.5	61.2	46.7
RRV	50.0	62.3	66.9	71.9	-	77.9	50.9
SFV	49.3	60.5	67.4	71.9	86.5	-	49.3

SINV	50.6	60.4	62.6	57.4	59.8	58.7	-
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**Table S2.** Pairwise amino acid sequence identities (above diagonal) and similarities (below diagonal) between E2 from SAV3 and selected alphaviruses (%).

	SAV3	VEEV	EEEV	CHIKV	RRV	SFV	SINV
SAV3	-	27.2	28.3	29.5	28.2	26.8	25.8
VEEV	38.3	-	48.1	36.1	38.9	36.0	40.7
EEEV	38.1	59.0	-	40.5	40.5	39.0	43.8
CHIKV	42.0	49.6	52.4	-	56.3	57.5	35.6
RRV	38.9	49.8	53.6	65.6	-	69.2	40.8
SFV	37.9	48.1	51.0	66.5	78.4	-	39.6
SINV	37.4	50.8	56.0	46.3	51.2	49.1	-

**Table S3.** Ct values following recovery of viral RNA in CHH-1 supernatant after infection with SAV3 with mutation in N-glycosylation sites in E1 and E2.

Virus	Passage number				
	P0	P1	P2	P3	P4
rSAV3 E1 <sub>35Q</sub>	21.4	29.5	35.5	no Ct	no Ct
rSAV3 E1 <sub>35A</sub>	22.9	26.5	31.5	no Ct	no Ct
rSAV3 E1 <sub>35Q</sub> E2 <sub>319Q</sub>	22.2	27.1	32.3	no Ct	no Ct
rSAV3 E1 <sub>35A</sub> E2 <sub>319A</sub>	21.8	26.1	30.7	30.2	no Ct
rSAV3	24.9	23.3	20.1	22.1	23.1
rSAV E2 <sub>319Q</sub>	22.8	21.7	18.2	18.3	24.7
rSAV E2 <sub>319A</sub>	18.7	23.9	19.1	19.4	21.5

Assays were repeated for constructs containing mutations in E1 to verify pattern of decreasing Ct-values with no Cts at P4. Sanger sequencing on RNA isolated from P4 confirmed that the mutations in rSAV E2<sub>319Q</sub> and rSAV E2<sub>319A</sub> were intact.

**Table S4.** Cytopathic effect in CHH-1 cells infected rSAV3, rSAV3 E2<sub>319Q</sub> and rSAV3 E2<sub>319A</sub> as measured by total RNA concentration (ng/μL) in adherent cells.

Virus	4 hpi		24 hpi		3 dpi		7 dpi		9 dpi		14 dpi	
	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2
rSAV3	175	157	202	193	287	285	181	211	134	142	47	65
rSAV E2 <sub>319Q</sub>	169	165	155	196	331	284	326	210	322	248	256	305
rSAV E2 <sub>319A</sub>	153	184	143	186	278	263	326	212	232	308	267	309

Biological parallels (well #) for each virus at each timepoint. hpi=hours post infection, dpi= days post infection. Total RNA concentration in ng/μL.

**Table S5.** Viral Ct values of CHH-1 cells infected with rSAV3, rSAV3 E2<sub>319Q</sub> and rSAV3 E2<sub>319A</sub>

Virus	4 hpi		12 hpi		24 hpi		3 dpi		7 dpi		9 dpi		14 dpi	
	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2
rSAV3	32.7	33.0	31.7	33.6	<i>no Ct</i>	<i>no Ct</i>	19.3	20.5	19.8	17.8	29.4	13.4	18.8	20.2
	32.2	32.8	31.8	33.7	<i>no Ct</i>	27.6	19.2	20.3	19.8	18.0	29.8	13.5	18.2	19.8
	-	-	-	-	<i>no Ct</i>	27.4	-	-	-	-	-	-	-	-
	-	-	-	-	<i>no Ct</i>	27.7	-	-	-	-	-	-	-	-
	-	-	-	-	<i>no Ct</i>	25.9	-	-	-	-	-	-	-	-
	-	-	-	-	<i>no Ct</i>	25.8	-	-	-	-	-	-	-	-
rSAV E2 <sub>319Q</sub>	35.0	33.4	33.0	34.0	27.9	27.4	21.7	20.5	18.4	19.5	15.2	14.5	22.7	24.2
	35.9	33.7	33.0	33.6	27.9	27.8	21.5	20.6	18.4	19.3	15.0	14.7	22.7	24.2
rSAV E2 <sub>319A</sub>	33.0	32.8	31.9	32.8	27.4	26.9	21.5	20.4	20.6	18.7	13.3	16.1	24.7	24.9
	33.0	32.7	31.7	33.0	27.1	27.0	21.4	20.1	20.5	18.7	12.9	16.2	24.8	25.0

Biological (well #) and PCR parallels for each virus at each timepoint. hpi=hours post infection, dpi= days post infection. Data in italics have not been included in Figure. 7. See main text for more details.