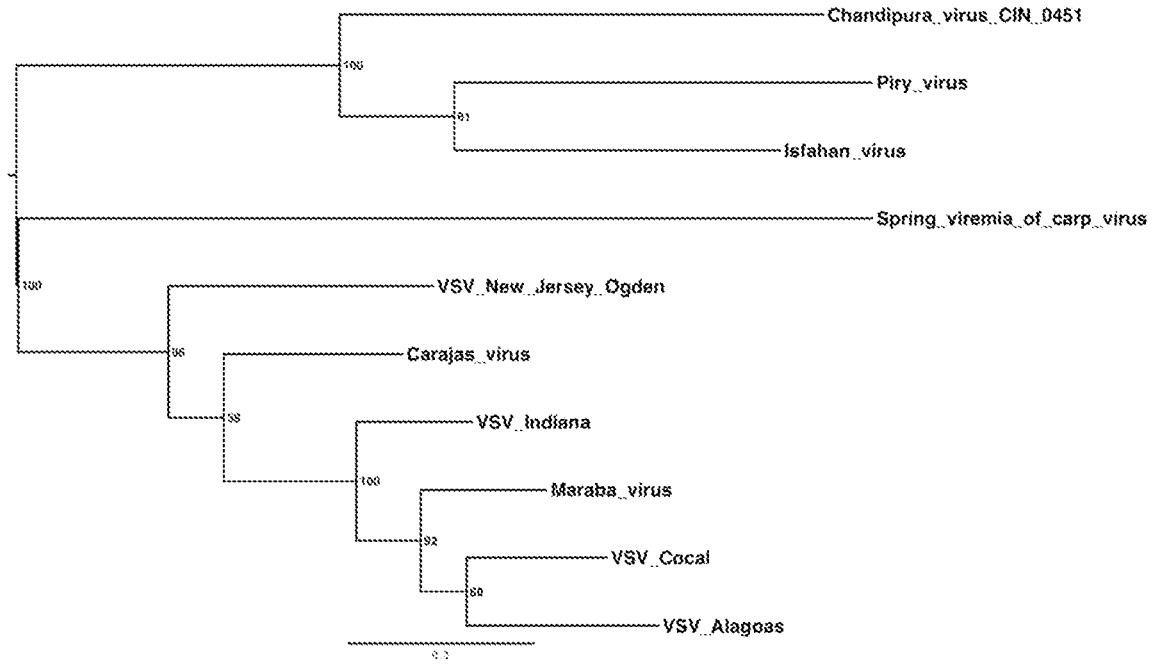


FIG. 1

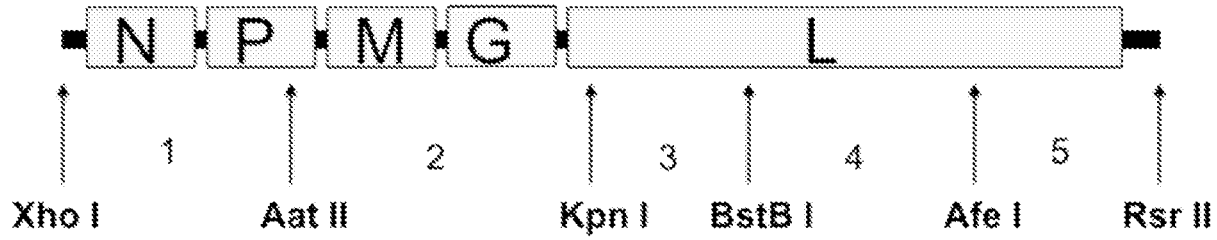


FIG. 2

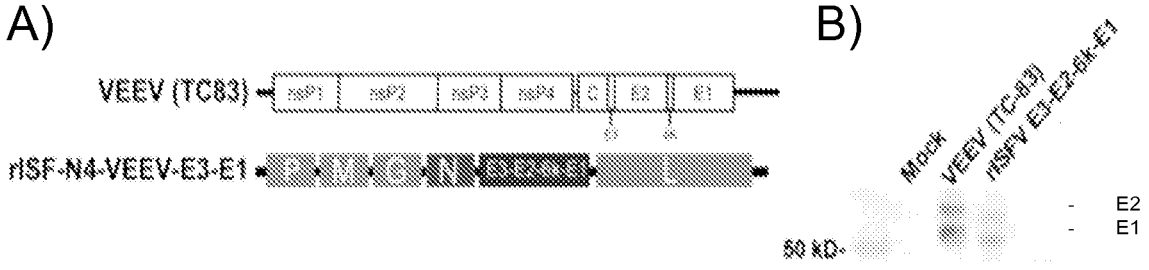


FIG. 3A-3B

```
ISF N          1 -mrvvkkitdtgsetlevlpaeedpstpdqfklqppgkirvciur---klkp---arlrqyvsqlkngdrnvchinaplyprlkktrcdeeqsdvisfgtslekqivsrfdt-imsdytrgeapdgcked
VSVi N         1 -mrvvsrlidntvvrpklpaeedpvkekk-kapiyrnr---tkal---adirqyvqqlkkqdrnvsiinvsyiidgvsalkvrtkdgysdm
VSV NJ_N       1 -mspvkkiindsiiqpklpaeedpvspaqyf-knntslyvar---kval---mdlrayvsqlkeqnpeaiihnayiyaalkgvegtldtdsrvsifdlsvraalktvpdgksdp
Chandipura_N   1 -mesqrtcastggtvsvslpaeedpvrtpgatfppnerkrvyikk---esdl---alirshvtdqikdqavrvsqcinsrlymwlkdirekydnntafyvelgkkeapdgsilnvedvkpkwlekkcgd
Piry_N         1 -mesvkkiisrgtgtvsalpaeedpvrtpaayfkkmpgrikvfiar---rldl---dkirqyvkdmirtvgrnvchinaryiyrlquiedeerdnnvafirvtiradnlrtpdfkeve
Cocal_N        1 -mesvkkiidsvsipklpaeedpvrtpgadyf-kktsrdpyiat---tksl---aelrqfvyqqlkegrvsiihvnaplyvalkdvtsgkiidtdnrvcfingtcdaanvgiidlvsrkqleqvpdgvsda
VSV_Alagoas_N  1 -maavkkiisdtlivpklpaeedpvtepadyf-kktaeipllfiat---tksl---aelrqfvyqqlkegrvsiihvnaplyvalkdvtsgkaldrdntsfgrnigtcadaanvgiidlvsrkqleqvpdgvsda
Spring viraemia 1 --mavi-rikcnatvasvlpaeedqadypatff-eggneairlyvnrgekidy------ikqyvsqlqekhkvgtpqdkigpfalvrvedtpdqigdklna
vesiculo_Pike_Fr 1 ---mavi-rikcnaavasvlpaeedqadypatff-eggneairlyvnrgekidy------ikqyvsqlqekhkvgtpqdkigpfalvrvedtpdqigdklna
rhabdo_Sea Trout 1 mdnalvvvrtkaadfpvpipledekvqpsatfva---gkipafdhy---yakkrqaellgmifgalaeertpaevifqvlrnvaaplkaqwvekldaaeesygtrdigdlmervinaaklmsrgvaacqipdykvga

ISF N          376 grtidddwklpmliiglgrvvsrsatqedvtkellqklgvqapkdirspgaeelvedaeafyvrnsdnfldkivaaidmdfhrdkfnhdad-aglrvgtiverrhkdaaalsclehvpkvtgksirhevtlsvdlkes
VSVi N         373 srvsaddkwipifvlglfrvvgtrqmgvgvrdkmldgltncgkmeinedfeplpngegrdifdvsgmdanytkfvaevdmffhmdkfkhet-asfrvgtivsrfdcaaiatfghlckitghsmevdtvnlmre
VSV NJ_N       125 drsaeddkwipifvlglfrvvgtrqvsvgvrdkmldgleqgirvasrfreivedgidtrdiwrnpdfrfvaevdmffhmdkfkher-apfrvgtivsrfkdcaaiatfghlskvgdsieidtlswlmre
Chandipura_N   126 tdipvddlwiptlifglfrvvsesqvgvgktlmsalvpgvkdirtrpdaeifhsvandnpfkhkfkvaevdmffhmdkfkdvh-apfrfgtivsrfkdcaaiatlehlgkvtqdpieevftwfmns
Piry_N         126 altqvvdevdvpmvflglfrvvgtrqtvgvgtdervrgdlydhicagpklkspefeslgpapadnpvsavvkdsfihkfkhee-avrrfgtivsrfkdcaaiatmshlvkvtqmpveeamlevflnkp
Cocal_N        125 trfeaddqwipifvlglfrvvgtrqmtdpvrtkmldglnqcgkmsinedfepipegrdifdvsgmdanytkfvaevdmffhmdkfkdeh-asfrvgtivsrfkdcaaiatfghvckitgmsteevrtwllnre
VSV_Alagoas_N  125 srtaaddkwipifvlglfrvvgtrqmtdpvrtrlmdglnqcgkmdinedfeplpegrdifdvsgmdanytkfiesvdmffhmdkfkher-aylrvgtivsrfkdcaaiatfghvckitgmsteevrtwmlnre
Spring viraemia 123 evsaeddawipifvlglfrvvgtrqaaetayrtllmeslkpqkaikadnvspprtatkyfdvsgpdgnylkavacvdmfymdkfkaik-atfrwgtivsrfkdcaaiatlghvvkitgltieevttwvlqra
vesiculo_Pike_Fr 123 evsaeddawipifvlglfrvvgtraeetayrtllmeslkpqkaikadnvspprtatkyfdvsgpdgnylkvacvdmfymdkfkaik-atfrwgtivsrfkdcaaiatlghlvkitgltieevttwvlqra
rhabdo_Sea Trout 127 vpaeddpvsmvrflleqvrtivqtmeesqvqrtvmqtl--qnqidslgakkiesvqlkmtcliktsppsyllkvtipgaevdmdfytkfkkmneksvvvslgstlmdceaiaclnmhvpfdqlillevidnttdq

ISF N          763 vedeirnmkerqevdkedsqpqyllldgistkppsevkqpcfnkvqqiallvhsnhrakmavvpedqypnsitvaaldsfayawqsrsgleqtfcs-dd-syy-------qeseditkqlgvvkqqcs-rdv
VSVi N         760 vedenvqmalpqpeidkeasmvrlidkglesksqpserkqaefksvqqiallrsrsrakmavqpddeyrsltsaglyavavgsseadlaqdfcv-pd-nky-------tpd-dscgqiltnaqpqgadv
VSV NJ_N       254 vaeicqmavpqpeidkeasmvpmidkgiaqkppserkqaefksvqqiallirtrsrakmavqpddeytsaltsaglyavavgsseadlaqdfyiged-kys-------teddsilkeavv-pkkg-rav
Chandipura_N   255 vqdillnmrppqeidkeasmvpmidkgiaskppserkqaefksvqqiafivesarsknalvpvddeyphelttsaglyavavgsseiaqdfvl-ng-kcf-------tpekssckhnqtpsee-gnv
Piry_N         255 vedevrnmrppqeidkeasmvpmidkgiakvpvserkqaefksvqqiafivnsarsknalvpddpypnsltvaaldsfayawqsrsgsadlaqdfvl-hn-kcy-------tpekssckhnqtpsee-gnv
Cocal_N        254 vqdemvqmalpqpeidkeasmvpmidkgiaqkppsevkqpcfnkvqqiallirsrsrakmavqpddeyrsltsaglyavavgsseadlaqdfyv-qd-dky-------tpdss-cgqiltnaqpqgadv
VSV_Alagoas_N  254 vqdemvqmalpqpeidkeasmvpmidkgiaqkppsevkqpcfnkvqqiallirsrsrakmavqpddeyrsltsaglyavavgsseadlaqdfym-qd-nky-------tpdss-dgqiltnaqpqgadv
Spring viraemia 252 vadelvkmakpqpeidkeasmvpmidkgiaqkppsetkqpcfnkvqqiallrtrsrakmavqpddeyrsltsaglyavavgsseadlnaqdfqdet-pd-tyr-------kpskeasy-ivaeqpkn-rsv
vesiculo_Pike_Fr 252 vadelvrmakpqpeidkeasmvpmidkgiaqrppsetkqpcfnkvqqiallrtrsrakmavqpddeyrsltsaglyavavgsseadlnaqdfqdet-pd-tyr-------kppke--gsvlvteapkt--rav
rhabdo_Sea Trout 255 vamniarnmkpqpeidkeasmvpmidkgiaqkppsetkqpcfnkvqqiallgtkrsrakmavqpddeyrsltsaglyavavgsseiaqdfvdet-pd-tyr-------kpdkeasmvpmidkgiaqkppsetkqpcfnkvqqiallgtkrsrakmavqpddeyrsltsaglyavavgsseiaqdfvdet-pd-tyr-------ep
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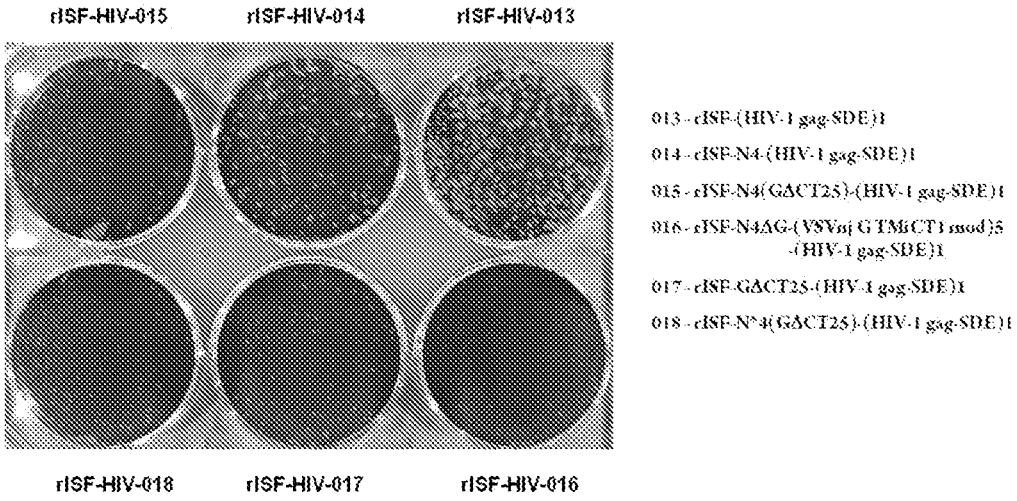

rISF-HIV-015
rISF-HIV-014
rISF-HIV-013
rISF-HIV-018
rISF-HIV-017
rISF-HIV-016
013 - rISF-(HIV-1 gag-SDE)1
014 - rISF-N4-(HIV-1 gag-SDE)1
015 - rISF-N4(GΔCT25)-(HIV-1 gag-SDE)1
016 - rISF-N4ΔG-(VSVnj GTMiCT1 mod)5 -(HIV-1 gag-SDE)1
017 - rISF-GΔCT25-(HIV-1 gag-SDE)1
018 - rISF-N*4(GΔCT25)-(HIV-1 gag-SDE)1

FIG. 5

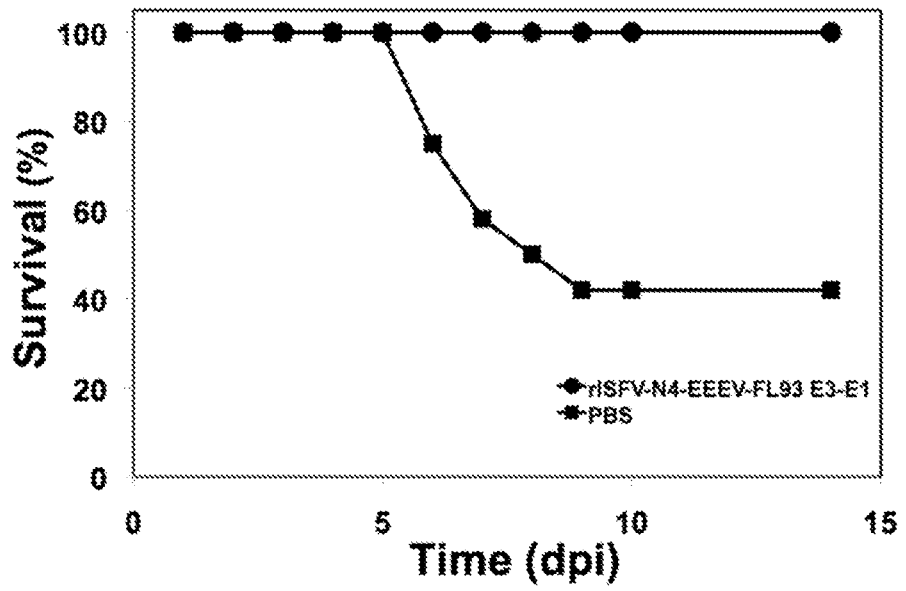

Survival (%)
100
80
60
40
20
0
0
5
10
15
Time (dpi)
rISFV-N4-EEEV-FL93 E3-E1
PBS

FIG. 6

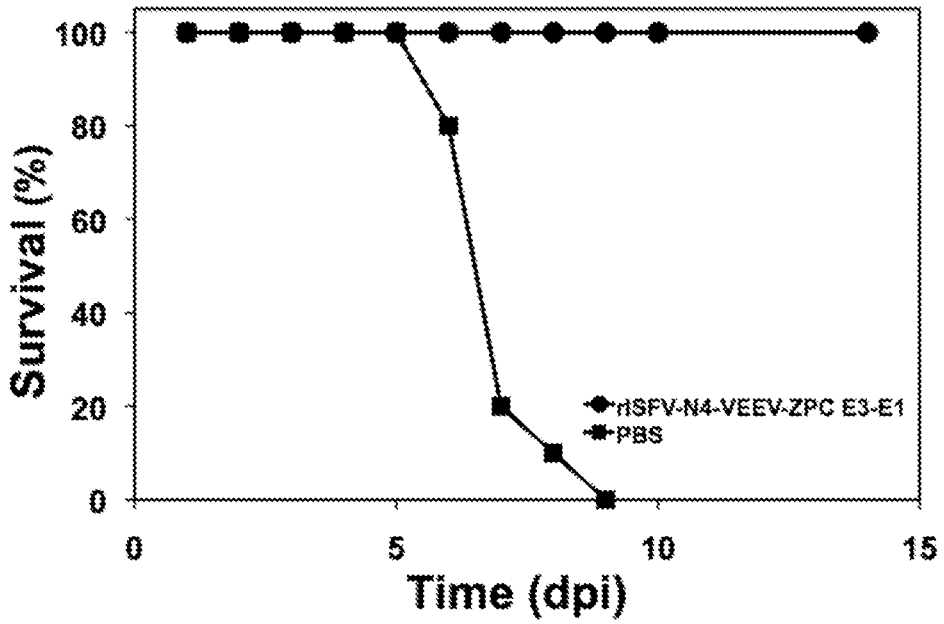

100
80
60
40
20
0
Survival (%)
0
5
10
15
Time (dpi)
rSFV-N4-VEEV-ZPC E3-E1
PBS

FIG. 7

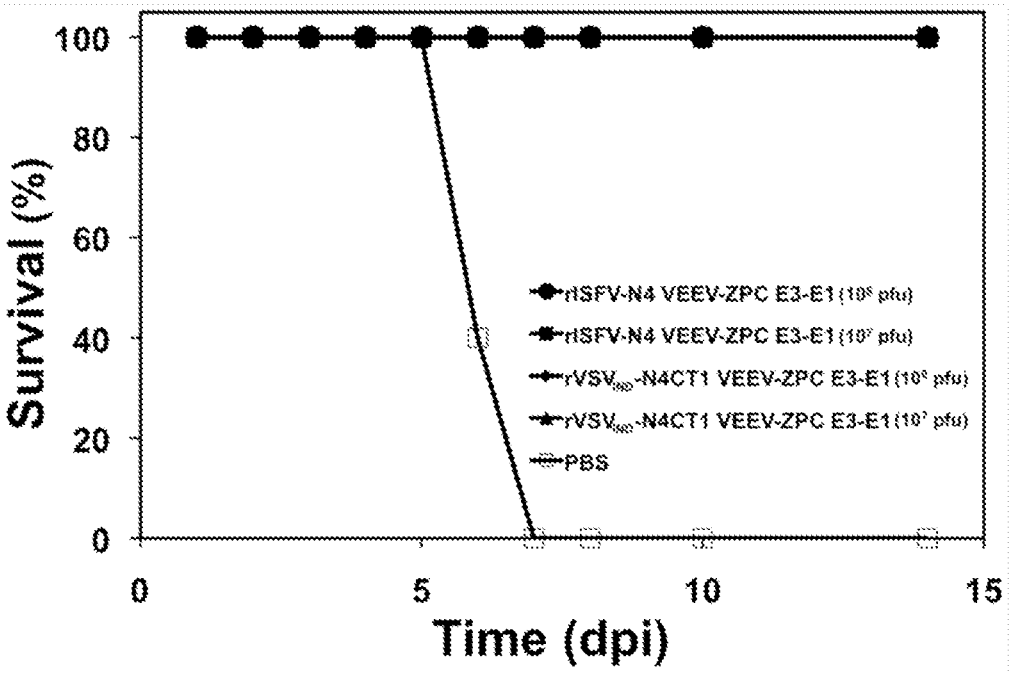

100
80
60
40
20
0
Survival (%)
0
5
10
15
Time (dpi)
rSFV-N4 VEEV-ZPC E3-E1 (10⁶ pfu)
rSFV-N4 VEEV-ZPC E3-E1 (10⁷ pfu)
rVSV-N4CT1 VEEV-ZPC E3-E1 (10⁶ pfu)
rVSV-N4CT1 VEEV-ZPC E3-E1 (10⁷ pfu)
PBS

FIG. 8

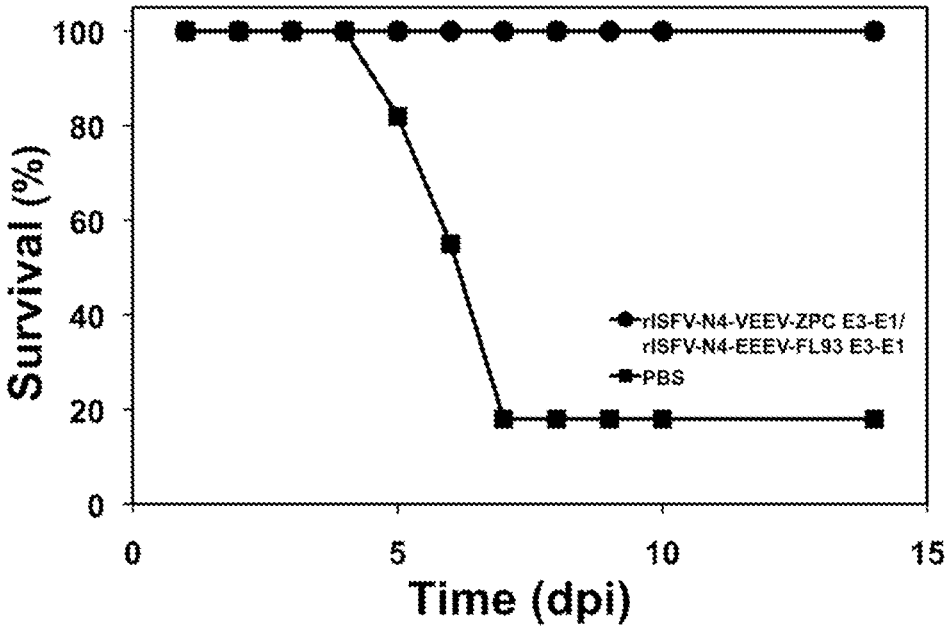

100
80
60
40
20
0
Survival (%)
0
5
10
15
Time (dpi)
rISFV-N4-VEEV-ZPC E3-E1/
rISFV-N4-EEEV-FL93 E3-E1
PBS

FIG. 9

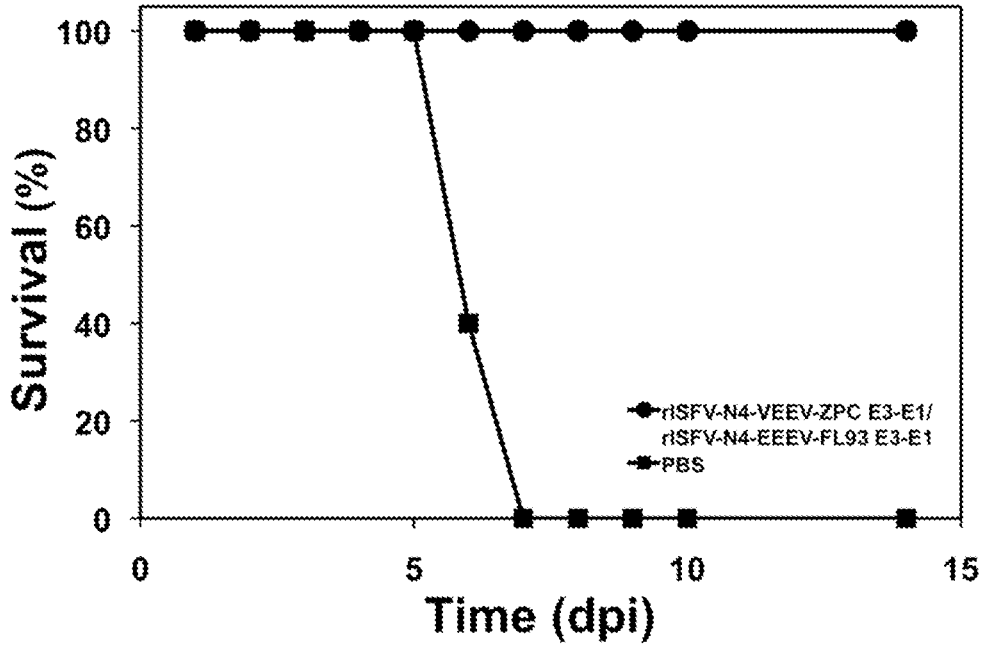

100
80
60
40
20
0
Survival (%)
0
5
10
15
Time (dpi)
rISFV-N4-VEEV-ZPC E3-E1/
rISFV-N4-EEEV-FL93 E3-E1
PBS

FIG. 10

```
Chandipura N          ...mmtpgqeidqadsympylidmglstkspysstknp...
Piry N                ...mmtpgqeidkadsympylidfglstkspyssvknp...
Cocal N               ...mmypgqeidksdsympylidlglsqkspystvknp...
VSV Alagoas N         ...mmkpgqeidkadsympylidfglsqkspystvknp...
Spring Viraemia       ...mmkpgqeidkstsympylidmgisakspystiknp...
Vesiculo Pike Fr      ...mmkpgqeidngasympylidmgisakspystiknp...
VSVnj N               ...mmypgqeidkadsympymidfglsqkspyssvknp...
VSVi N                ...mmlpgqeidkadsympylidfglsskspyssvknp...
ISF N                 ...mmkerqevdkadsympylidfgistkspyssvknp...
                                  ↓↓      ↓  ↓
ISF N*                ...mmkerqevdqsdsympymidmgistkspyssvknp...
                                  qs      m  m
```

FIG. 11

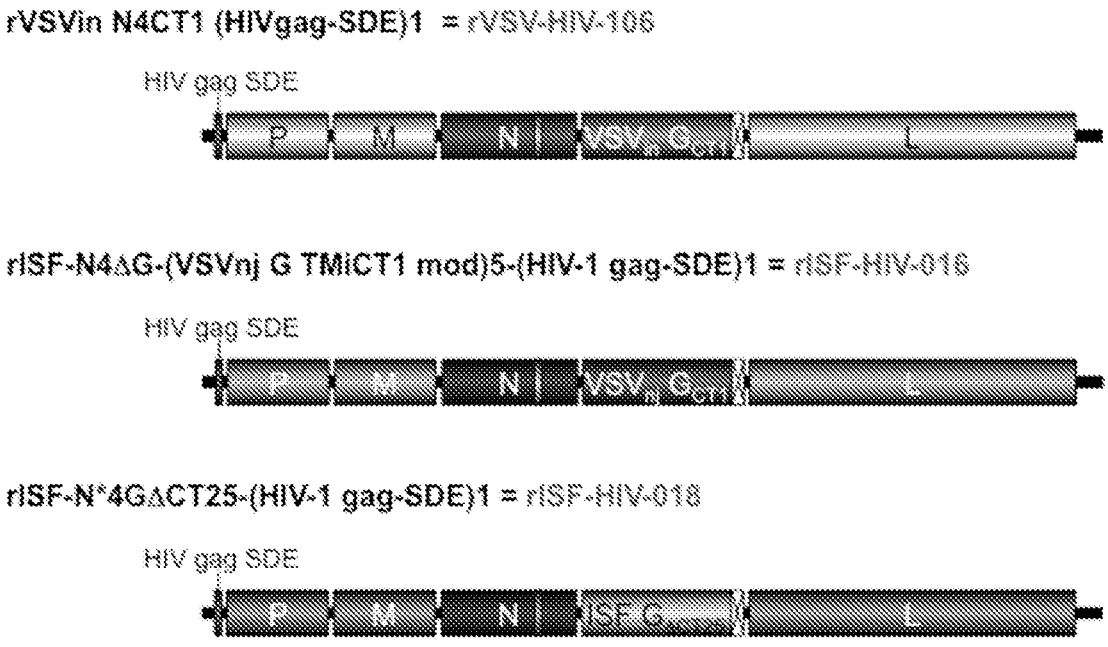


FIG. 12

Group #	# mice	Vaccine	Vaccine dose	Route
1	5	rVSV$_{IN}$ N4CT1 (HIVgag-SDE)1 (rVSV-HIV-106)	10^7 pfu	I.M
2	5	rISF-N4ΔG-(VSV$_{IN}$ G TMCT1 mod)5-(HIV-1 gag-SDE)1 (rISF-HIV-016)	10^7 pfu	I.M
3	5	rISF- N*4GΔCT2S-(HIVgag-SDE)1 (rISF-HIV-018)	10^7 pfu	I.M

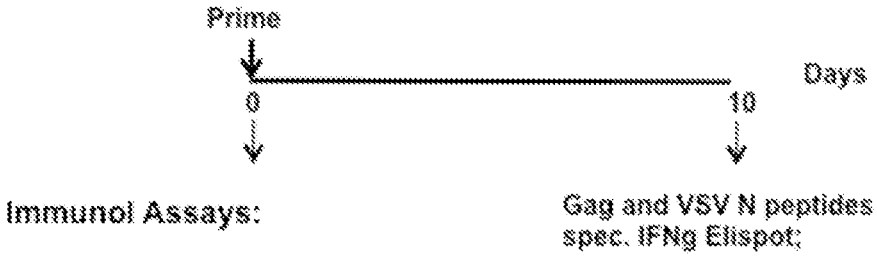
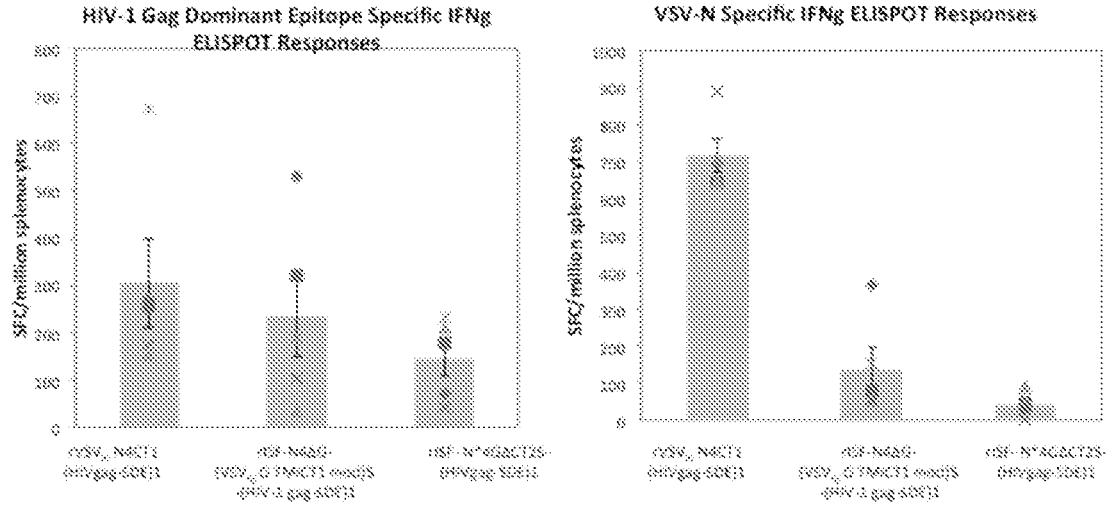


FIG. 13



Conclusion: 1) the two rISF candidates elicited similar Ag specific CMI, slightly lower than those from rVSV.
2) rISF N* mutation reduces the cross responses to rVSV N. It may benefit the prime/boost. Further test it in Mu062b.

FIG. 14

rISF-N4GΔCT25-(HIV-1 gag-SDE)1 = rISF-HIV-018



rISF-N*4GΔCT25-(HIV-1 gag-SDE)1 = rISF-HIV-018



FIG. 15

Group #	# mice	Prime Vaccine	Vaccine dose	Boost Vaccine	Vaccine dose	Route
1	5	NA	NA	rVSVin ΔG/CT1 (HIVgag-SDE)1 rVSV-HIV-018	10^7 pfu	I.M.
2	5	rVSVng ΔG/CT1 (HIVgag-SDE)1 rVSV-HIV-018	10^7 pfu	rVSVin ΔG/CT1 (HIVgag-SDE)1 rVSV-HIV-018	10^7 pfu	I.M.
3	5	rISF-N*4GΔCT25-(HIVgag-SDE)1 rISF-HIV-018	10^7 pfu	rVSVin ΔG/CT1 (HIVgag-SDE)1 rVSV-HIV-018	10^7 pfu	I.M.
4	5	rVSVin ΔG/CT1 (HIVgag-SDE)1 rVSV-HIV-018	10^7 pfu	rISF-N*4GΔCT25-(HIVgag-SDE)1 rISF-HIV-018	10^7 pfu	I.M.
5	5	rISF-N4GΔCT25-(HIVgag-SDE)1 rISF-HIV-018	10^7 pfu	rVSVin ΔG/CT1 (HIVgag-SDE)1 rVSV-HIV-018	10^7 pfu	I.M.

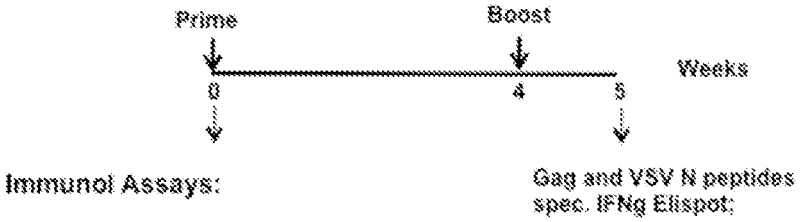


FIG. 16

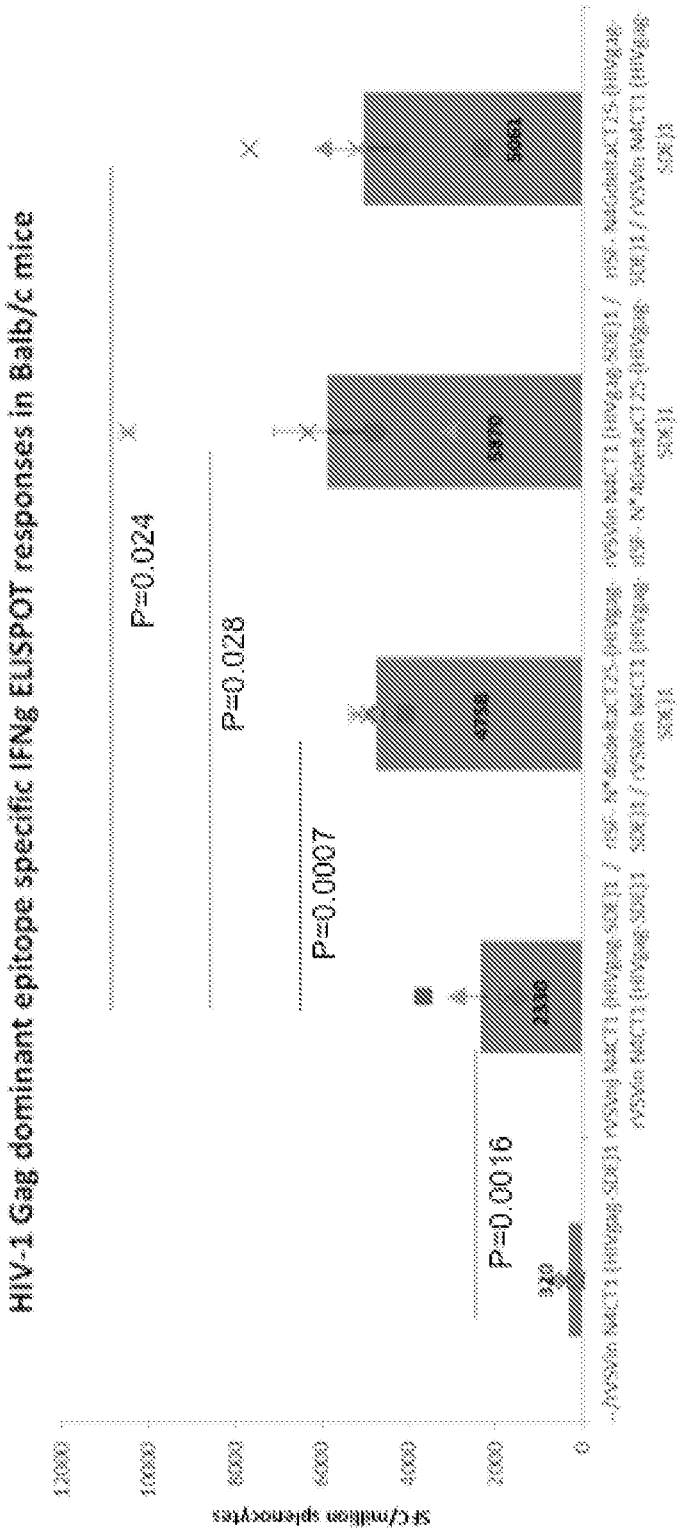

HIV-1 Gag dominant epitope specific IFNg ELISPOT responses in Balb/c mice
SF-C/million splenocytes
12000
10000
8000
6000
4000
2000
0
P=0.024
P=0.028
P=0.0007
P=0.0016
3366
4738
5814
5961
SC#11
SC#13

FIG. 17

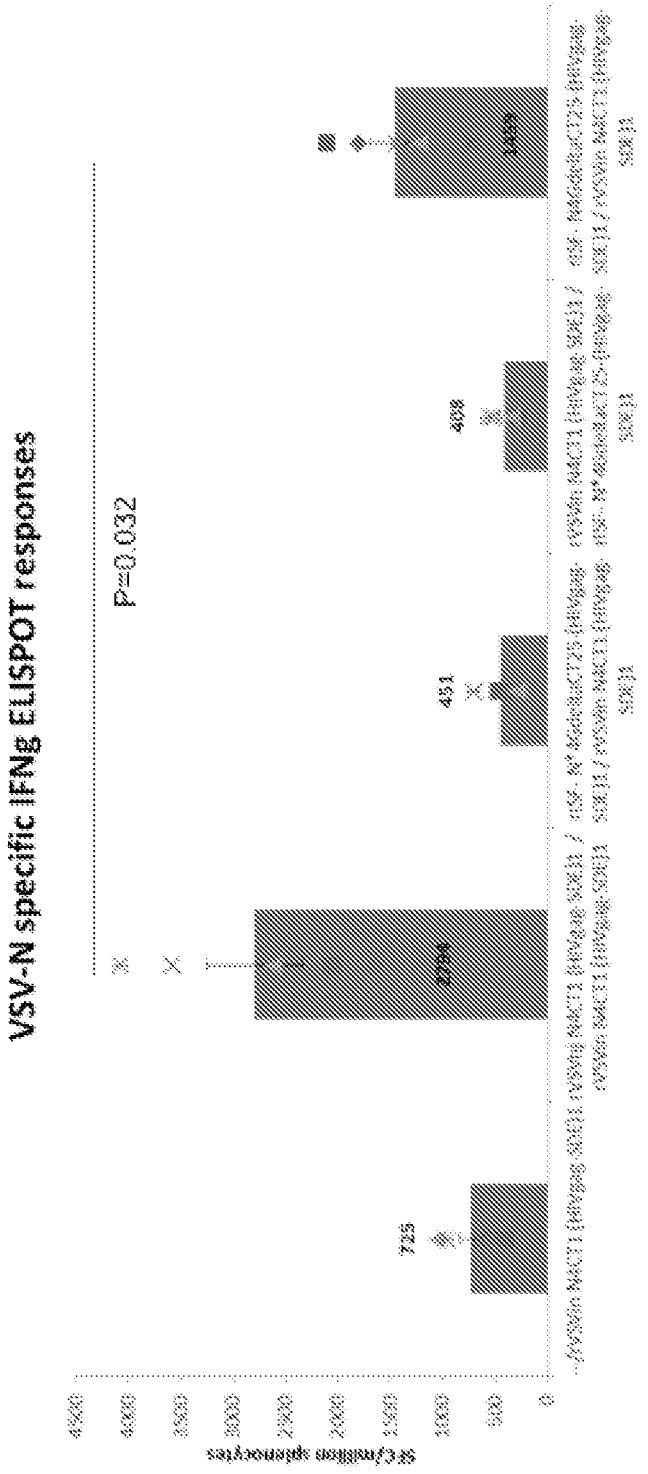

VSV-N specific IFNg ELISPOT responses
P=0.032
725
2294
451
408
1453
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
SIK31

FIG. 18

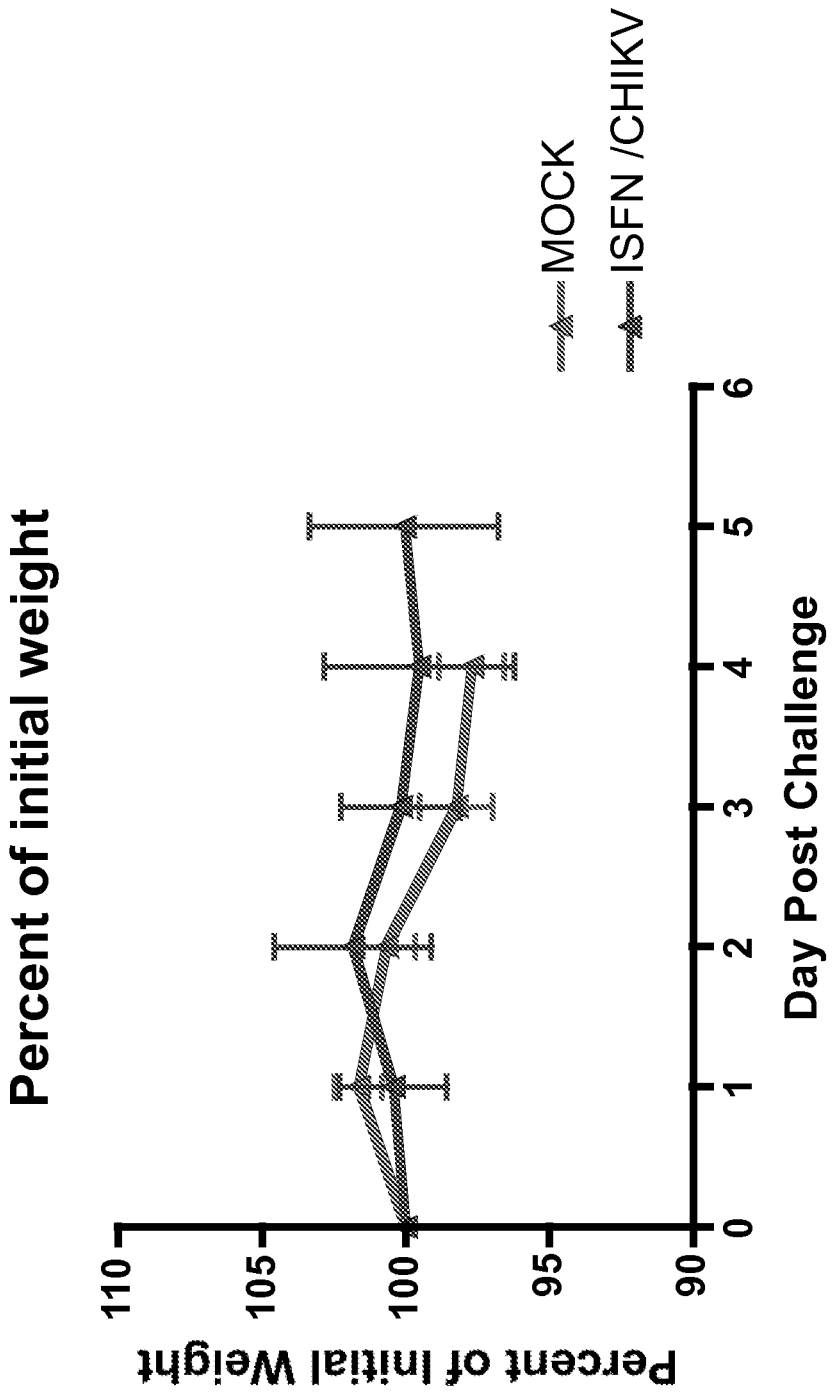

Percent of initial weight
Percent of Initial Weight
110
105
100
95
90
0
1
2
3
4
5
6
Day Post Challenge
MOCK
ISFN /CHIKV

FIG. 19

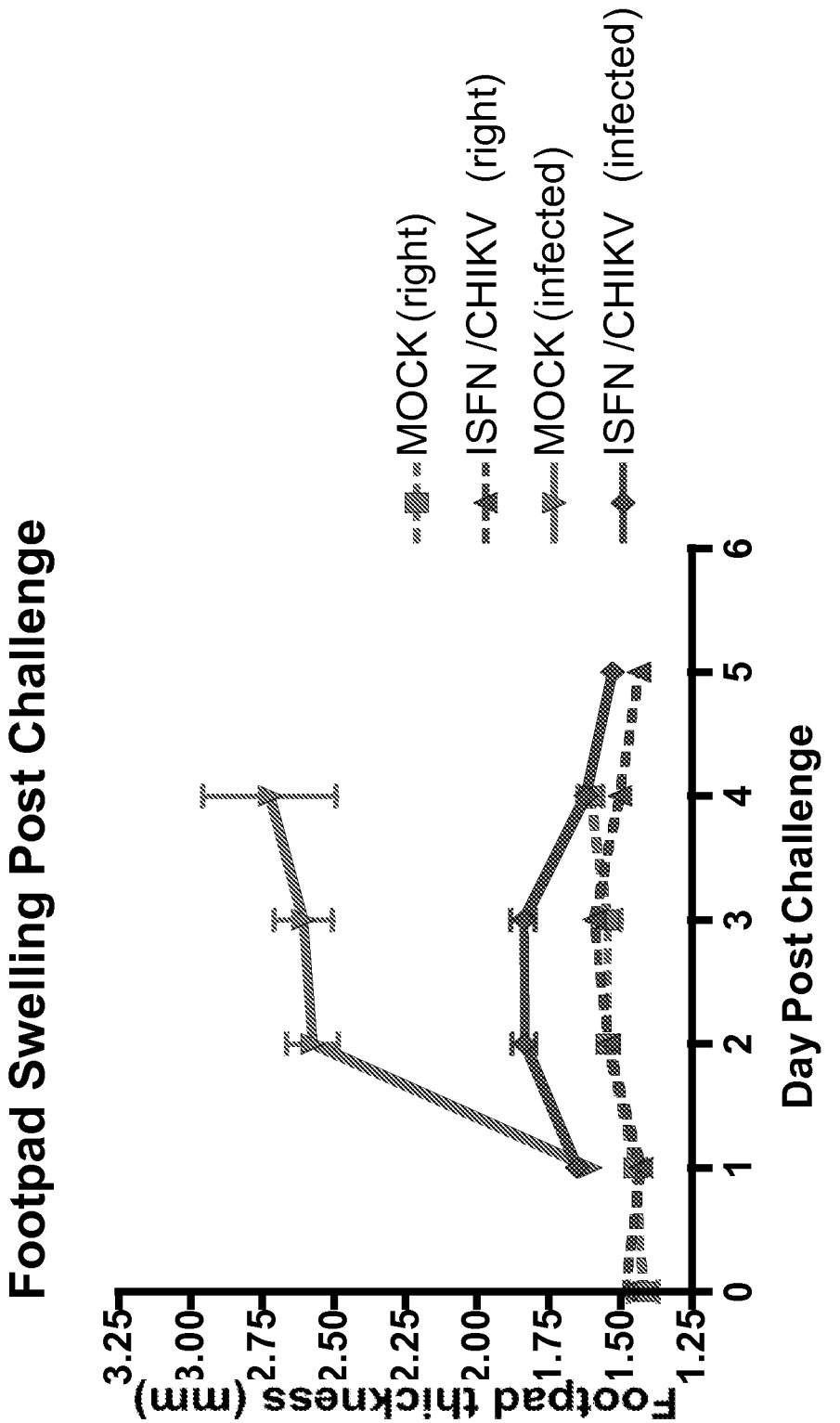

Footpad Swelling Post Challenge
Footpad thickness (mm)
3.25
3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
0
1
2
3
4
5
6
Day Post Challenge
MOCK (right)
ISFN /CHIKV (right)
MOCK (infected)
ISFN /CHIKV (infected)

FIG. 20

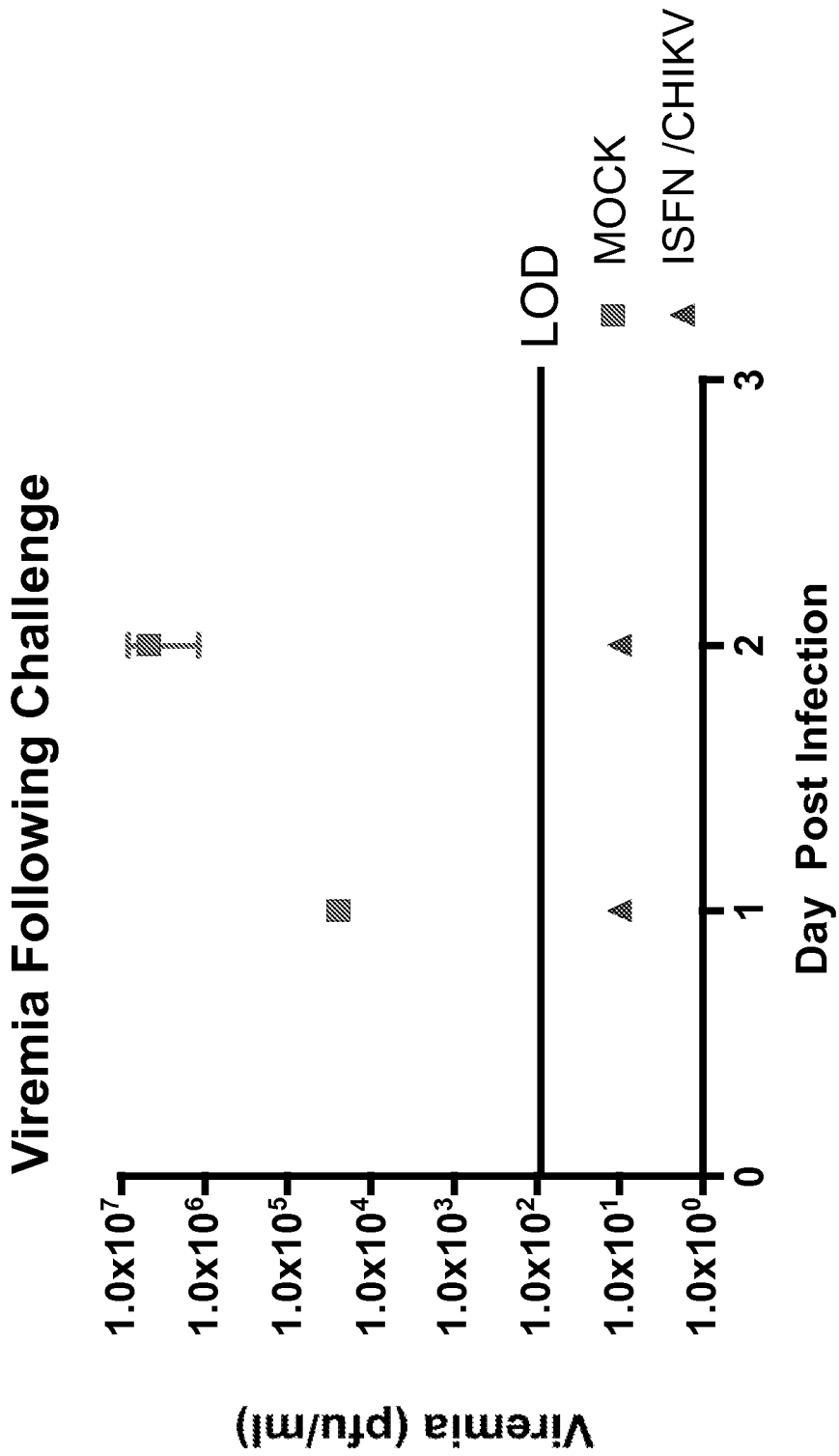

Viremia Following Challenge
Viremia (pfu/ml)
1.0x10^7
1.0x10^6
1.0x10^5
1.0x10^4
1.0x10^3
1.0x10^2
1.0x10^1
1.0x10^0
LOD
MOCK
ISFN /CHIKV
0
1
2
3
Day Post Infection

FIG. 21

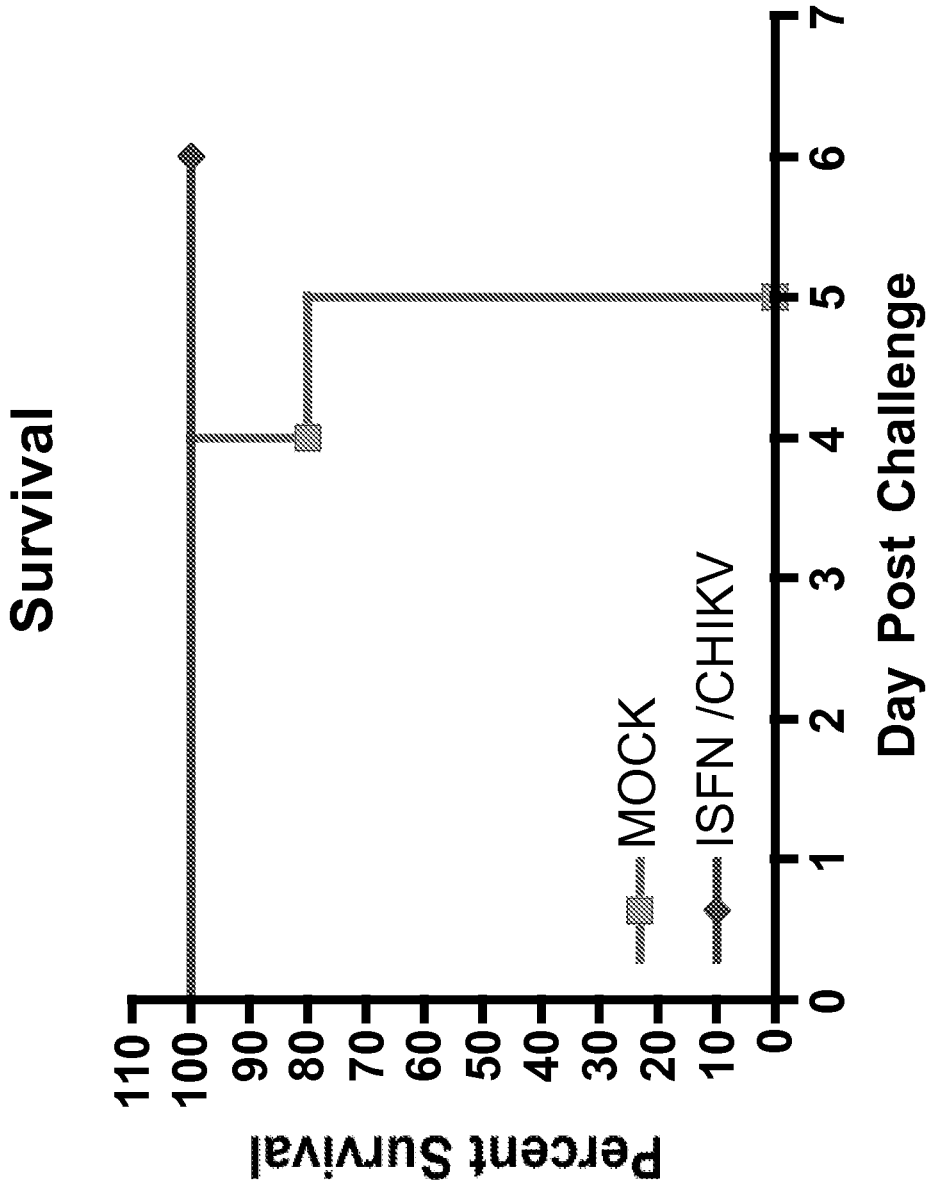

Survival
Percent Survival
110
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
Day Post Challenge
MOCK
ISFN /CHIKV

FIG. 22