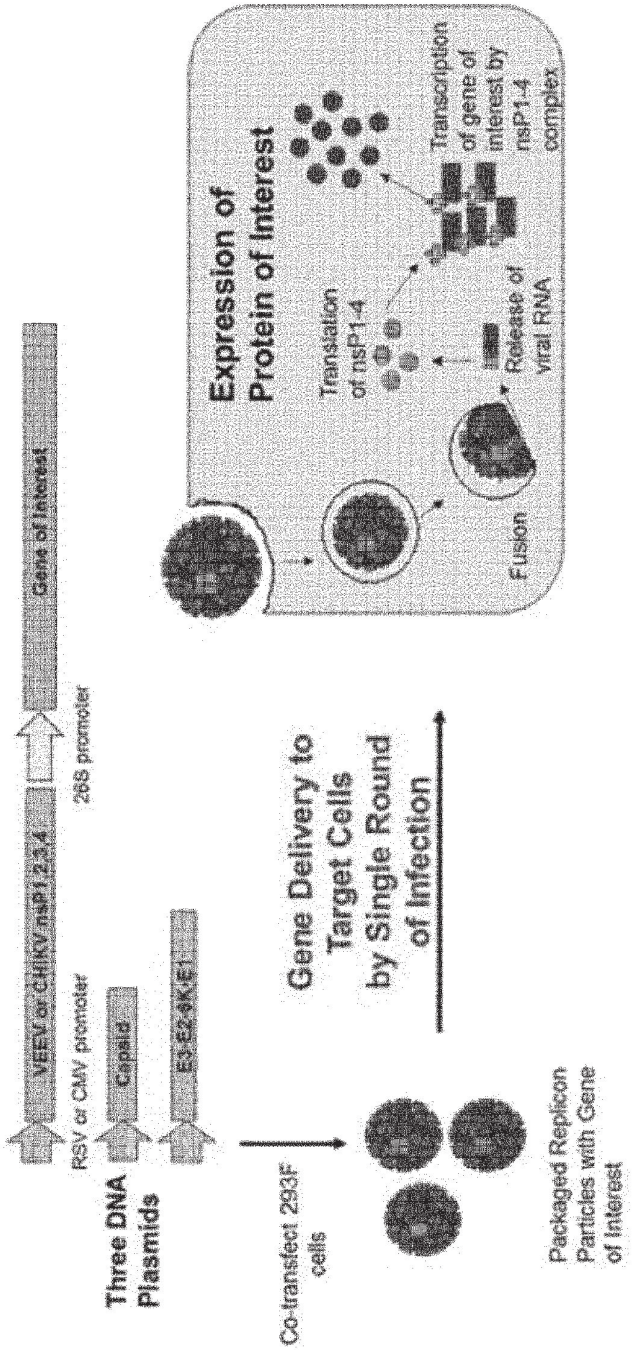
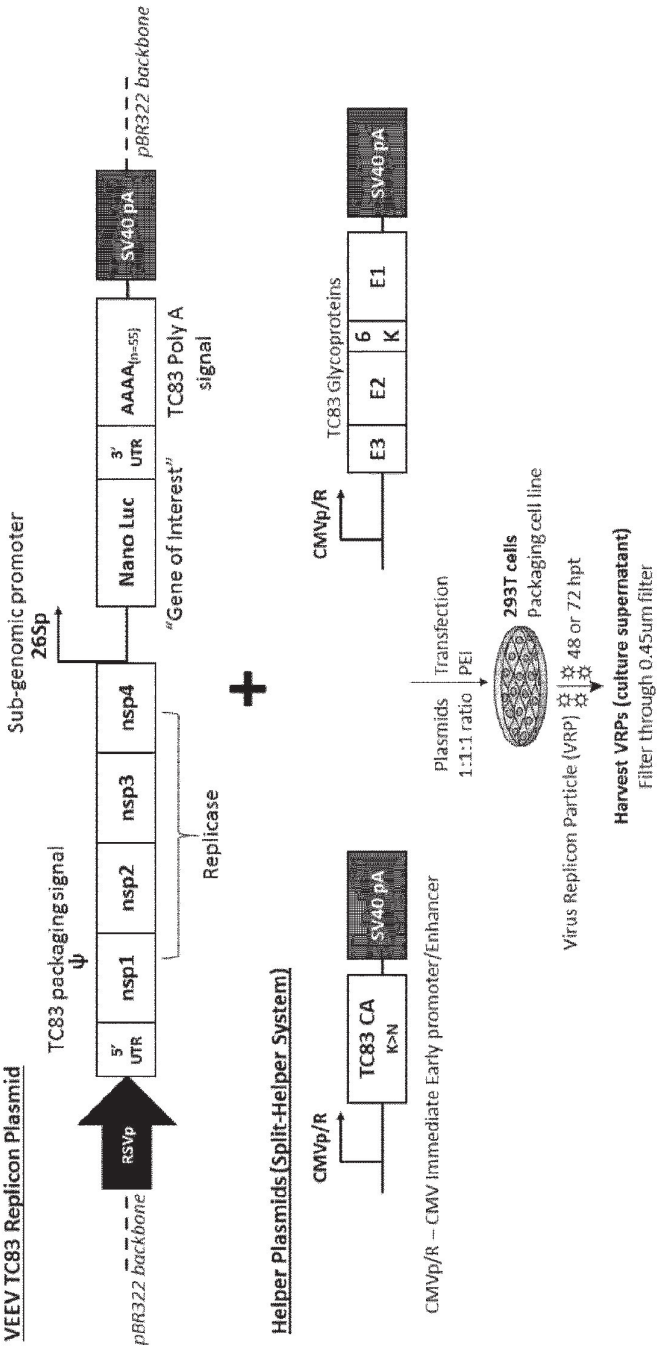
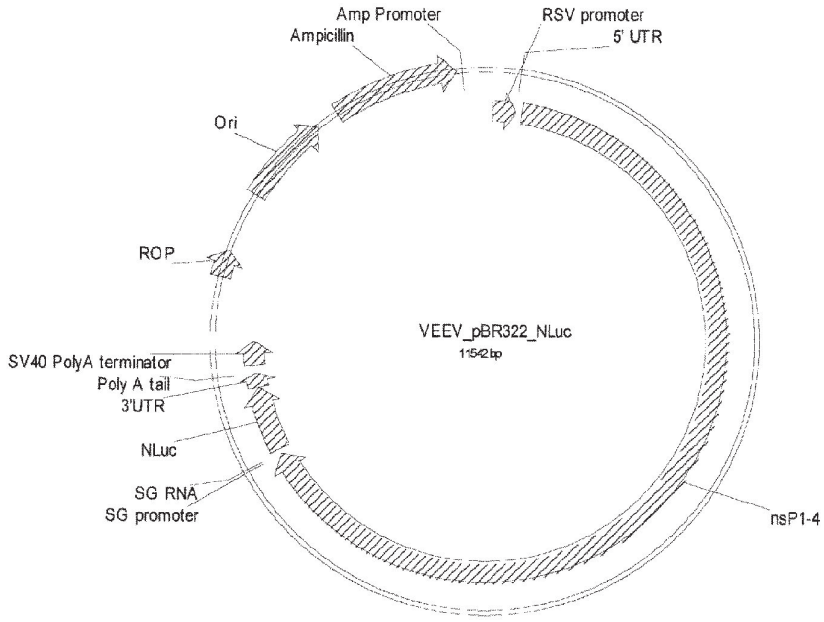
[Fig. 1]


Three DNA Plasmids
VEEV or CHIKV nsP1,2,3,4
RSV or CMV promoter
Capsid
E3-E2-6K-E1
26S promoter
Gene of Interest
Co-transfect 293F cells
Packaged Replicon Particles with Gene of Interest
Gene Delivery to Target Cells by Single Round of Infection
Expression of Protein of Interest
Translation of nsP1-4
Release of viral RNA
Fusion
Transcription of gene of interest by nsP1-4 complex

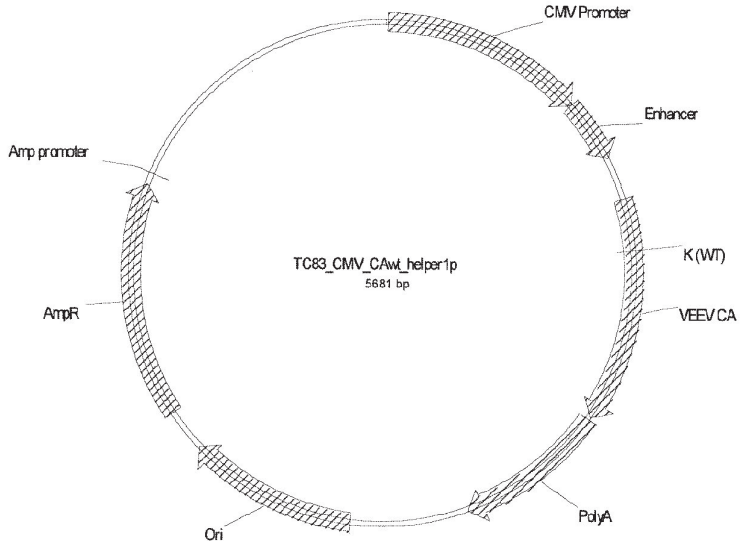
[Fig. 2]


VEEV TC83 Replicon Plasmid
pBR322 backbone
RSVp
TC83 packaging signal
Ψ
5' UTR
nsp1
nsp2
nsp3
nsp4
Sub-genomic promoter
26Sp
Nano Luc
3' UTR
AAAA(n=55)
SV40 pA
pBR322 backbone
Replicase
"Gene of interest"
TC83 Poly A signal
Helper Plasmids (Split-Helper System)
CMVp/R
TC83 CA K>N
SV40 pA
CMVp/R – CMV immediate Early promoter/Enhancer
CMVp/R
TC83 Glycoproteins
E3
E2
6K
E1
SV40 pA
Plasmids 1:1:1 ratio
Transfection PEI
293T cells
Packaging cell line
Virus Replicon Particle (VRP)
48 or 72 hpt
Harvest VRPs (culture supernatant)
Filter through 0.45um filter

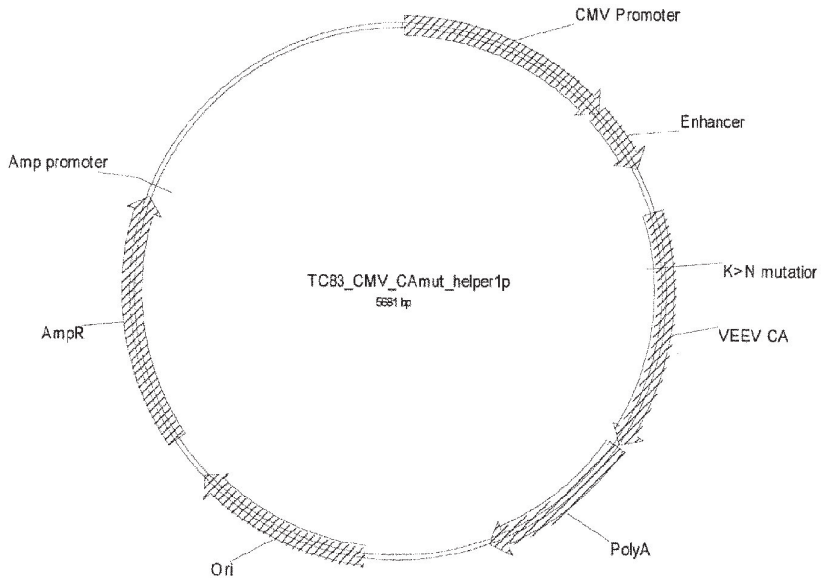
[Fig. 3]


Amp Promoter
Ampicillin
RSV promoter
5' UTR
Ori
ROP
VEEV_pBR322_NLuc
11542bp
SV40 PolyA terminator
Poly A tail
3'UTR
NLuc
SG RNA
SG promoter
nsP1-4

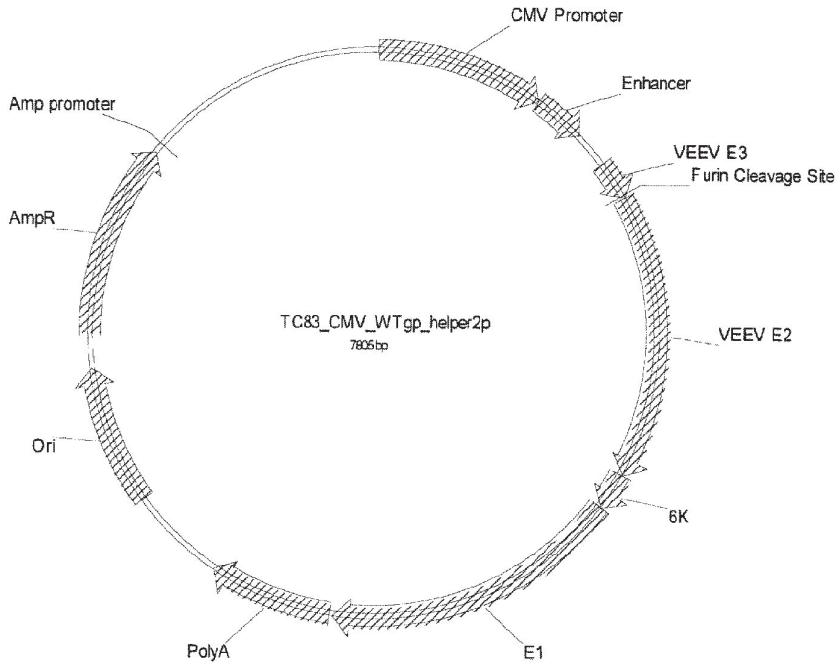
[Fig. 4]


CMV Promoter
Enhancer
Amp promoter
K (WT)
TC83_CMV_CAwt_helper1p
5681 bp
AmpR
VEEV CA
PolyA
Ori

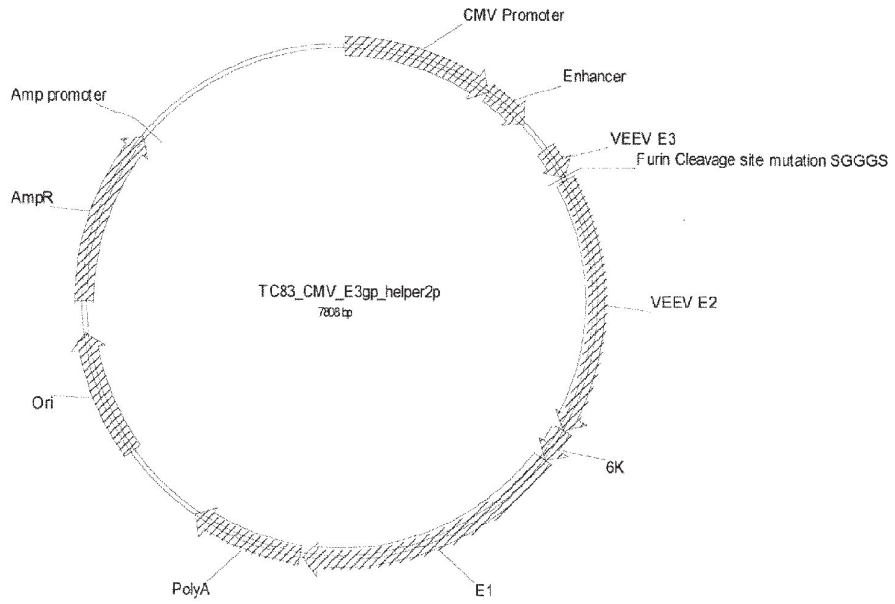
[Fig. 5]


CMV Promoter
Enhancer
Amp promoter
K>N mutatior
TC83_CMV_CAmut_helper1p
5681 bp
AmpR
VEEV CA
PolyA
Ori

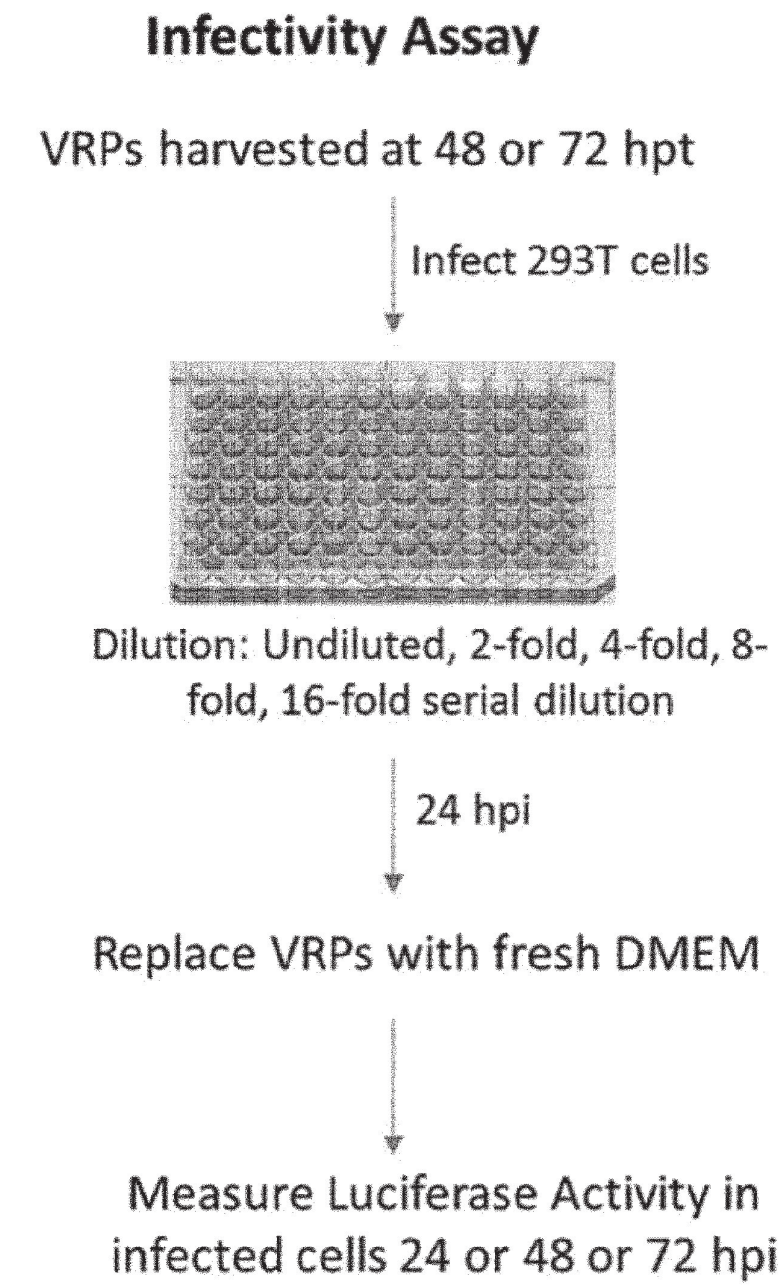
[Fig. 6]


CMV Promoter
Enhancer
Amp promoter
VEEV E3
Furin Cleavage Site
AmpR
TC83_CMV_WTgp_helper2p
7805bp
VEEV E2
Ori
6K
PolyA
E1

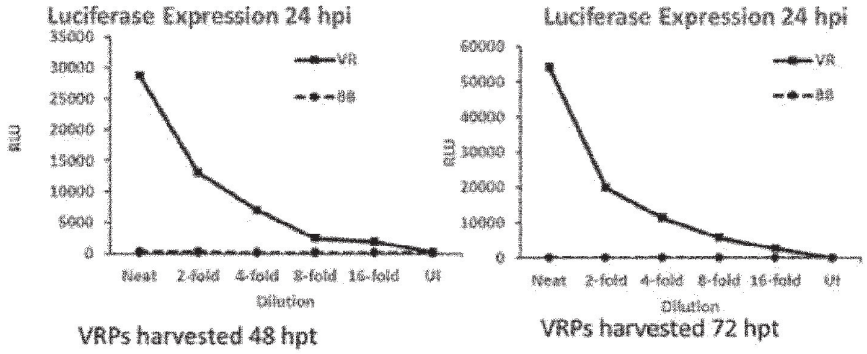
[Fig. 7]


CMV Promoter
Enhancer
VEEV E3
Furin Cleavage site mutation SGGGS
VEEV E2
6K
E1
PolyA
Ori
AmpR
Amp promoter
TC83_CMV_E3gp_helper2p
7808bp

[Fig. 8]

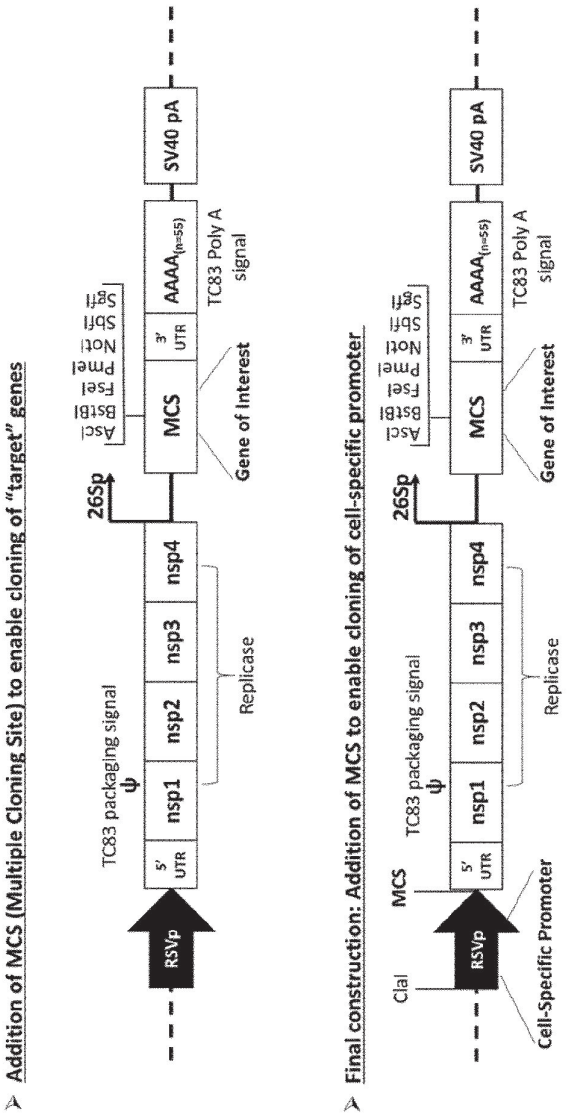

Infectivity Assay
VRPs harvested at 48 or 72 hpt
Infect 293T cells
Dilution: Undiluted, 2-fold, 4-fold, 8-fold, 16-fold serial dilution
24 hpi
Replace VRPs with fresh DMEM
Measure Luciferase Activity in infected cells 24 or 48 or 72 hpi

[Fig. 9]

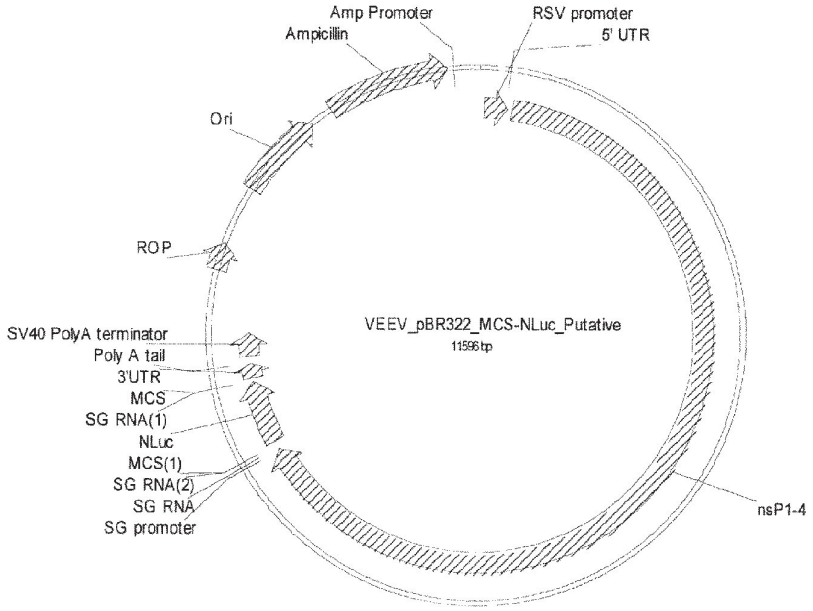


BB – Backbone without nsp 1-4 but has RSVp, 26S promoter, Nluc ORF and SV40 pA (Negative Control)

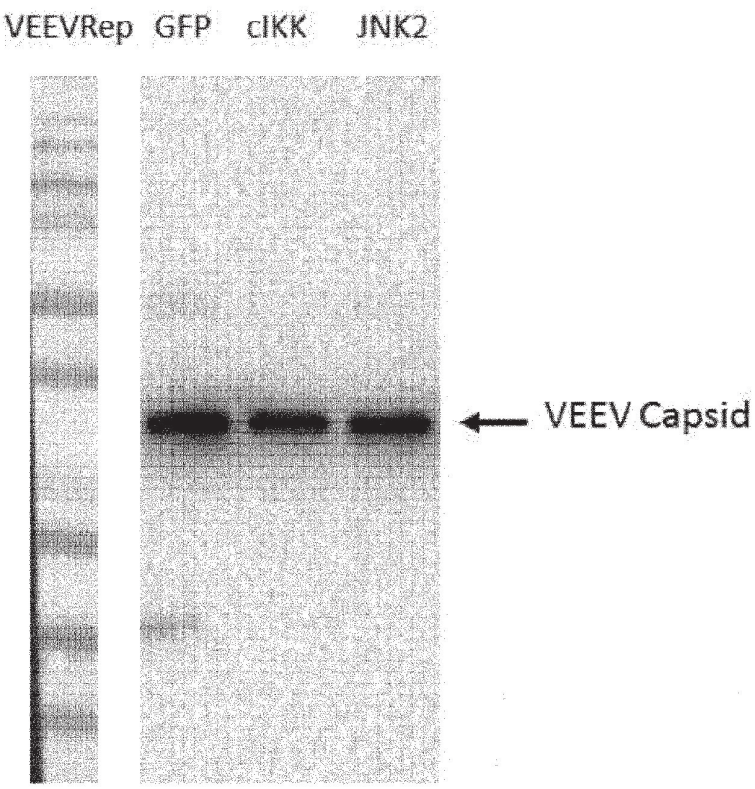
[Fig. 10]


Addition of MCS (Multiple Cloning Site) to enable cloning of "target" genes
RSVp
5' UTR
TC83 packaging signal
Ψ
nsp1
nsp2
nsp3
nsp4
Replicase
26Sp
AscI
BstBI
FseI
PmeI
NotI
SbfI
SgfI
MCS
3' UTR
AAAA(n=55)
TC83 Poly A signal
Gene of Interest
SV40 pA
Final construction: Addition of MCS to enable cloning of cell-specific promoter
ClaI
MCS
RSVp
Cell-Specific Promoter
5' UTR
TC83 packaging signal
Ψ
nsp1
nsp2
nsp3
nsp4
Replicase
26Sp
AscI
BstBI
FseI
PmeI
NotI
SbfI
SgfI
MCS
3' UTR
AAAA(n=55)
TC83 Poly A signal
Gene of Interest
SV40 pA

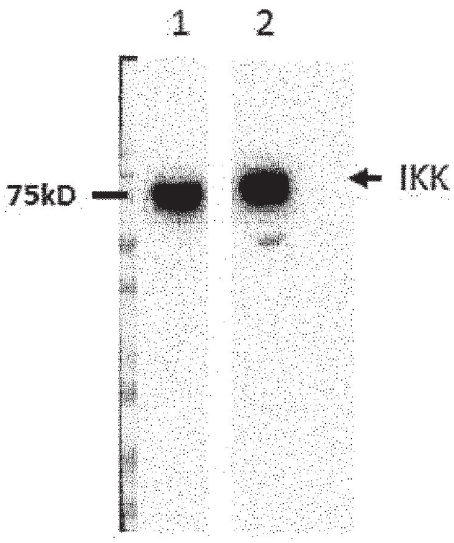
[Fig. 11]


Amp Promoter
Ampicillin
RSV promoter
5' UTR
Ori
ROP
VEEV_pBR322_MCS-NLuc_Putative
11596bp
SV40 PolyA terminator
Poly A tail
3'UTR
MCS
SG RNA(1)
NLuc
MCS(1)
SG RNA(2)
SG RNA
SG promoter
nsP1-4

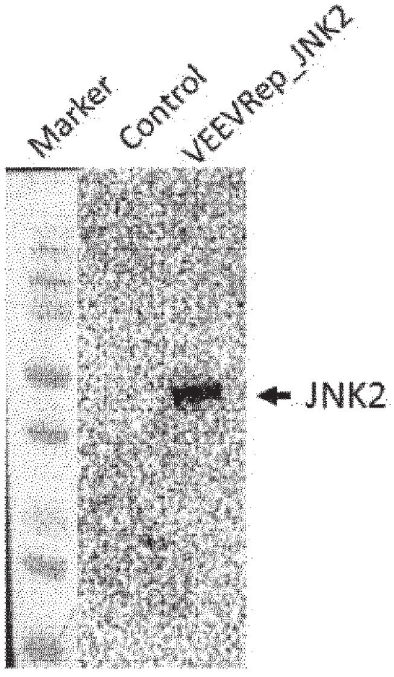
[Fig. 12]


VEEVRep
GFP
cIKK
JNK2
VEEV Capsid

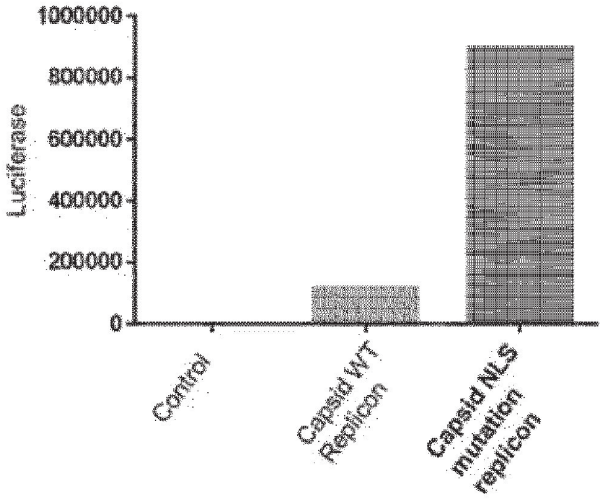
[Fig. 13]



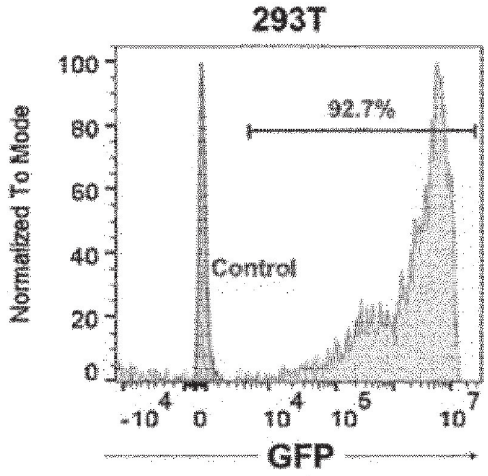
[Fig. 14]



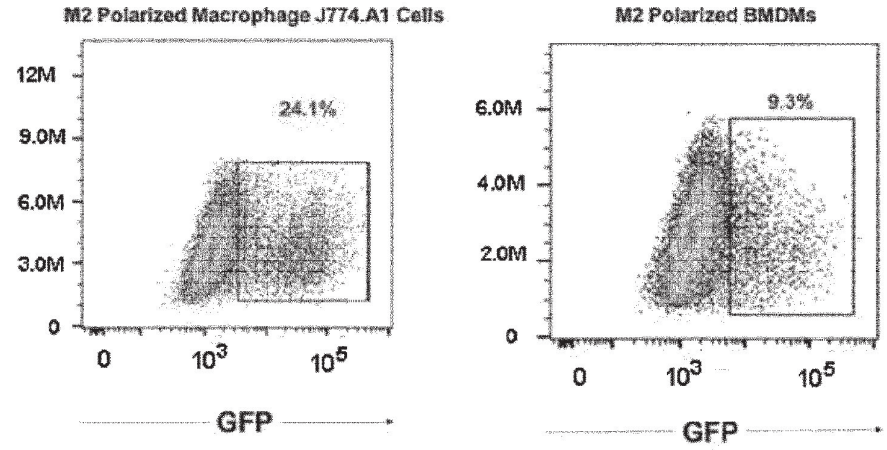
[Fig. 15]



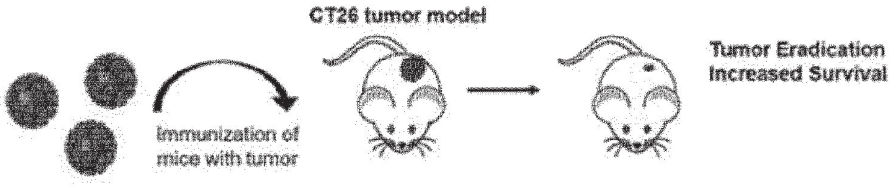
[Fig. 16]



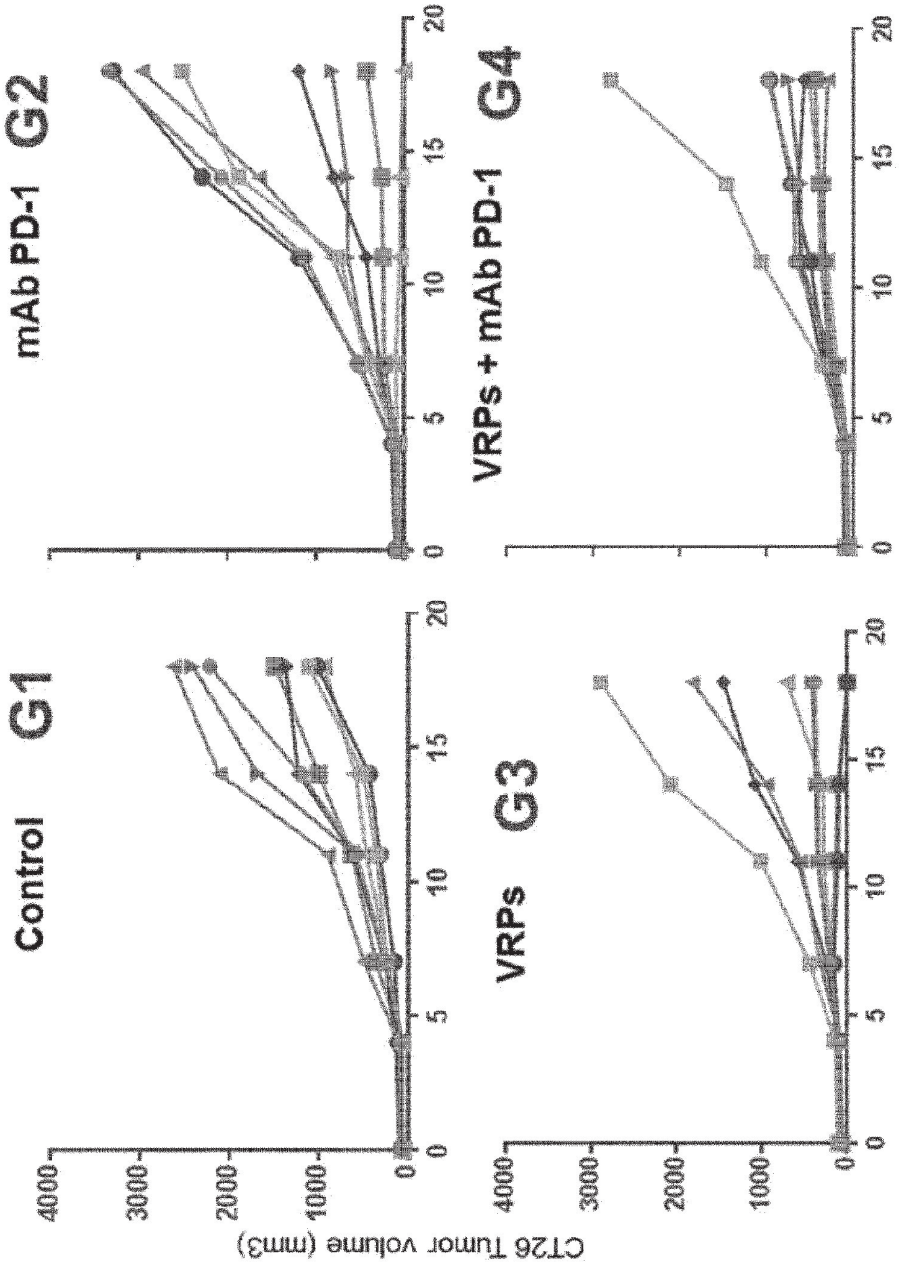
[Fig. 17]



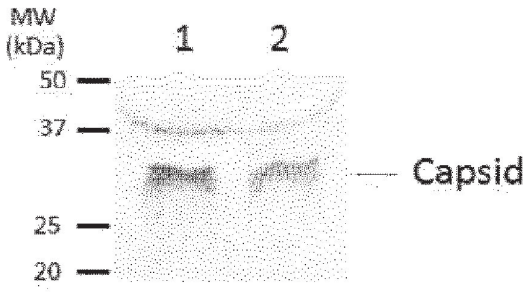
[Fig. 18]


CT26 tumor model
Immunization of mice with tumor
Tumor Eradication
Increased Survival

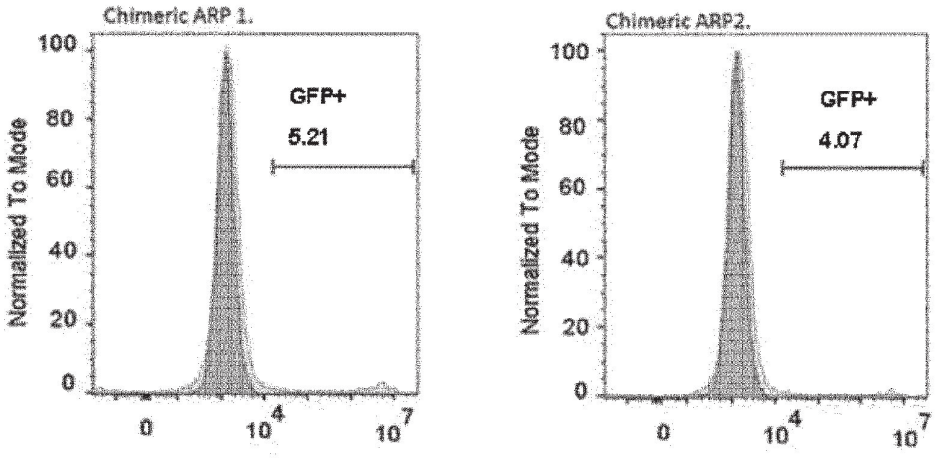
[Fig. 19]


mAb PD-1 G2
VRPs + mAb PD-1 G4
Control G1
VRPs G3
CT26 Tumor volume (mm3)
0
1000
2000
3000
4000
5
10
15
20

[Fig. 20]


MW
(kDa)
1
2
50
37
25
20
Capsid

[Fig. 21]


Chimeric ARP 1.
Normalized To Mode
GFP+
5.21
Chimeric ARP2.
GFP+
4.07