Fig. 1

Genetic variations among D2/4 chimeras (compared to wt D2 16681 and D4-1036)

Genome	D2			junction prM	D4				junction	D2									
Genes	NCR	C			E					NS1	NS2A			NS3			NS4A		NS4B	NS5	
Mutation types*	PDK-53	seed	Eng	Mut	seed	Marker	Eng	Eng	NgoMIV	PDK-53	seed	seed	PDK-53	PDK-53	seed	PDK-53	seed	PDK-53	seed	PDK53	seed
Genome NT position	C57T	A125T	A398C	AA536	C947G/C	A1301G	C2027T	A2275	TG2380/CC	G2579A	A3674G	A5773A/G	C4018T	A5270T	C5391T	T5547C	C6437T	C6699C	T7036C/T	C8573T**	A9750C
Protein-AA position	NCR	C-silent	C-R106S	prM-silent	prM-T79I/T	E-silent	E-A364V	E-M-57L	E-V482A	NS1-G53D	NS2A-D66G	NS2A-K99K/R	NS2A-L181F	NS3-E250K	NS3-silent	NS3-silent	NS4A-A21V	NS4A-E75A	NS4B-silent	NS5-silent	NS5-silent
D2-16681	C	A	A (R)	A					TG (V)	G (G)	A (D)	A (K)	C (L)	A (E)	C	T	C (A)	G (G)	T	C	A
D2-PDK-53	T	-	-							G (D)	-	-	T (F)	T (V)	-	C	-	C (A)	-	T	-
D4-1036					G (T)	A	C (A)	A (M)													
Cloned D2/4-V1 (pD2/4-VP3)	T	-	C (S)	g	-	g	T (V)	C (L)	CC (A)	G (D)	-	-	T (F)	T (V)	-	C	-	C (A)	-	-	-
DENVax-4 (MVS)	T	t	C (S)	g	-	g	T (V)	C (L)	CC (A)	A (D)	G (G)	A/G (K/R)	T (F)	T (V)	t	c	T (V)	C (A)	c/t	-	c

*: same as wt D2 16681 or D4 1036; small or letter: silent mutation in open reading region

*: PDK-53: D2-PDK-53 specific genotype (vs 16681); Red: major attenuation PDK-53 loci; Seed: mutations found only in specified virus seed and not in the original clone; Eng: Engineered mutations for the D2/4 clones; Mlu and NgoMIV: D2/4 junction engineered RE sites;

**: C8571T (PDK-53 silent mutation) was not included in most D2/4 chimeric clones

Fig. 2

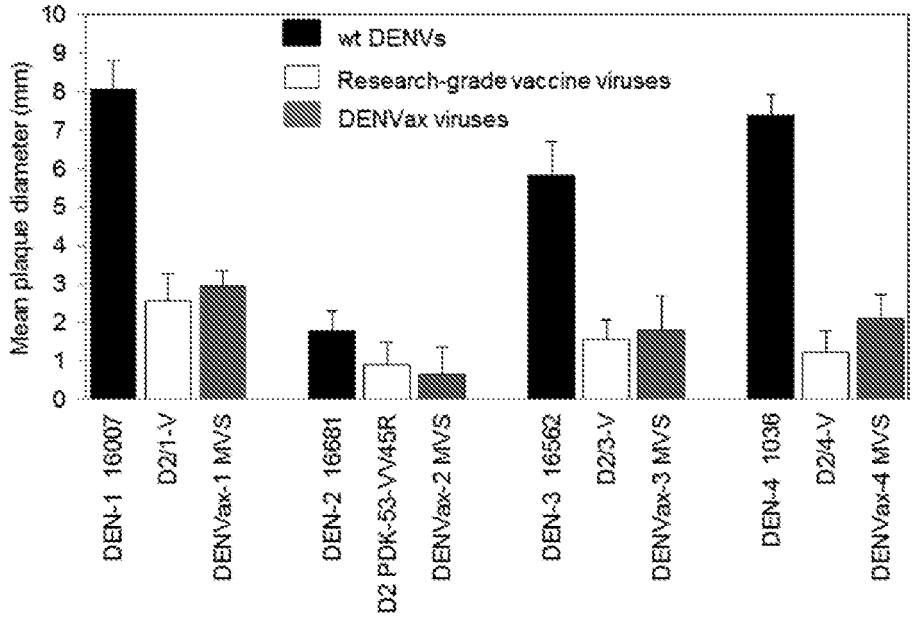

Mean plaque diameter (mm)
0
1
2
3
4
5
6
7
8
9
10
wt DENVs
Research-grade vaccine viruses
DENVax viruses
DEN-1 16007
D2/1-V
DENVax-1 MVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DEN-4 1036
D2/4-V
DENVax-4 MVS

Fig. 3

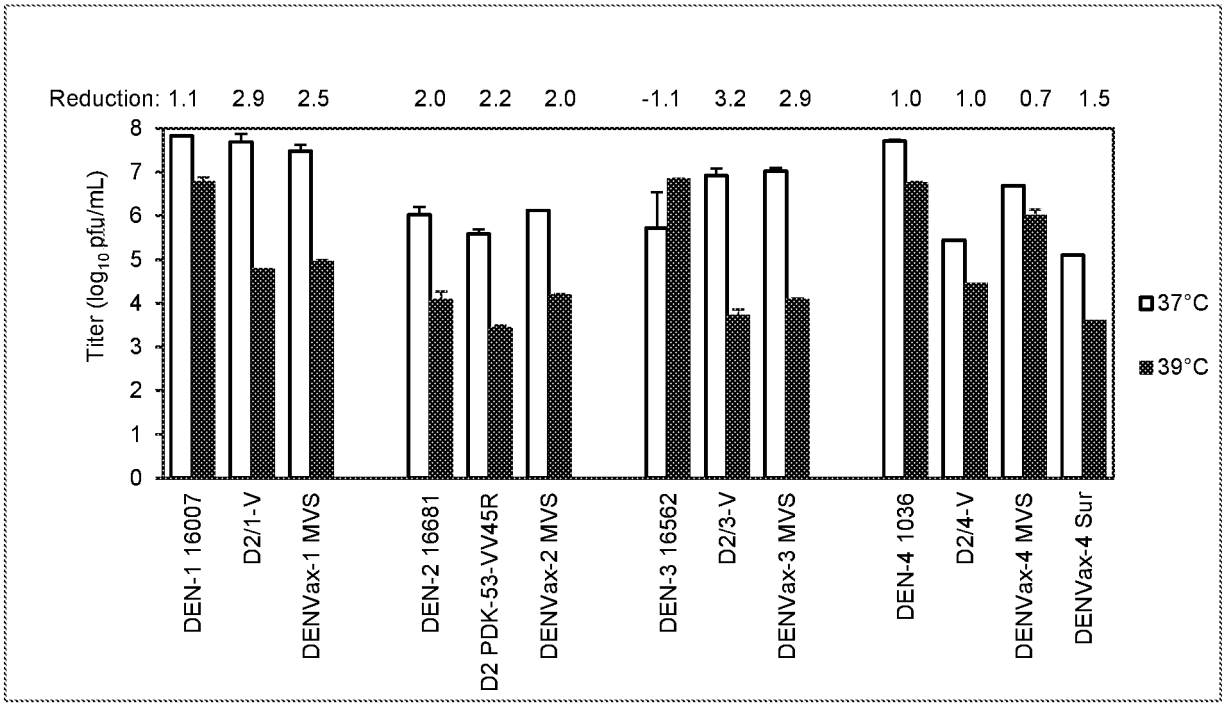
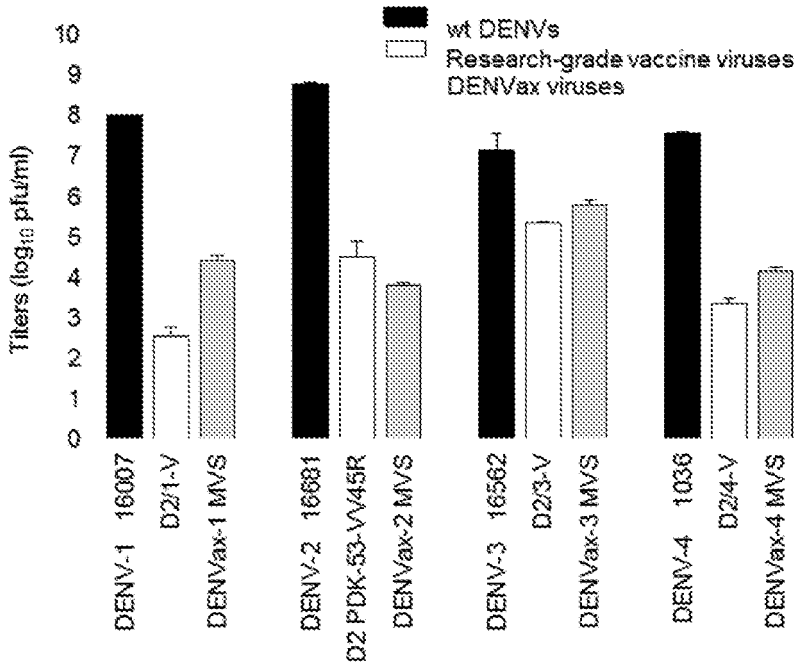

Reduction: 1.1 2.9 2.5 2.0 2.2 2.0 -1.1 3.2 2.9 1.0 1.0 0.7 1.5
Titer (log_{10} pfu/mL)
0
1
2
3
4
5
6
7
8
37°C
39°C
DEN-1 16007
D2/1-V
DENVax-1 MVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DEN-4 1036
D2/4-V
DENVax-4 MVS
DENVax-4 Sur

Fig. 4


wt DENVs
Research-grade vaccine viruses
DENVax viruses
Titers (log10 pfu/ml)
0
1
2
3
4
5
6
7
8
9
10
DENV-1 16007
D2/1-V
DENVax-1 MVS
DENV-2 16681
D2 PDK-53-V45R
DENVax-2 MVS
DENV-3 16562
D2/3-V
DENVax-3 MVS
DENV-4 1036
D2/4-V
DENVax-4 MVS

Figs. 5A-5C

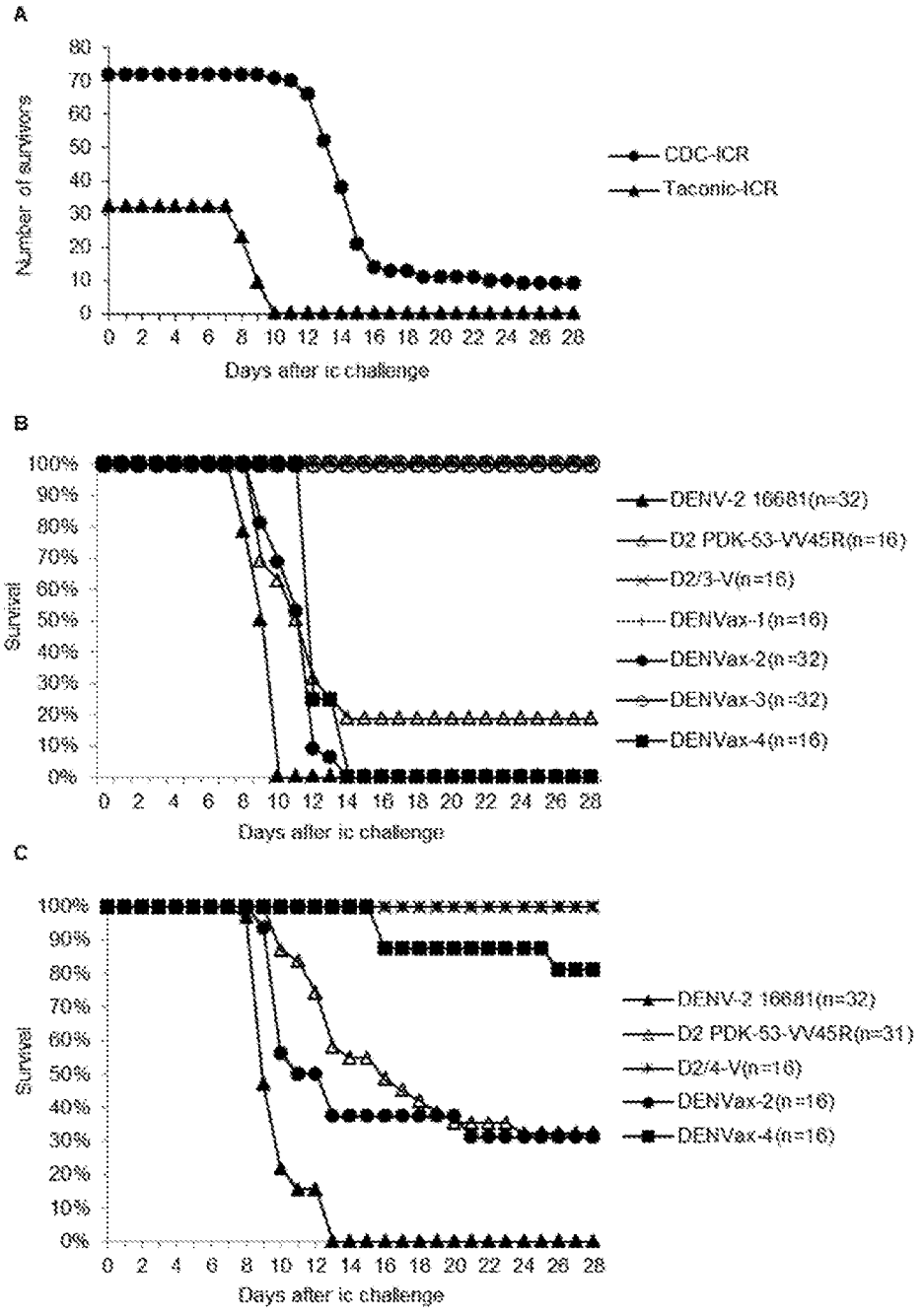

A
Number of survivors
Days after ic challenge
CDC-ICR
Taconic-ICR
B
Survival
Days after ic challenge
DENV-2 16681(n=32)
D2 PDK-53-VV45R(n=16)
D2/3-V(n=16)
DENVax-1(n=16)
DENVax-2(n=32)
DENVax-3(n=32)
DENVax-4(n=16)
C
Survival
Days after ic challenge
DENV-2 16681(n=32)
D2 PDK-53-VV45R(n=31)
D2/4-V(n=16)
DENVax-2(n=16)
DENVax-4(n=16)

Fig. 6

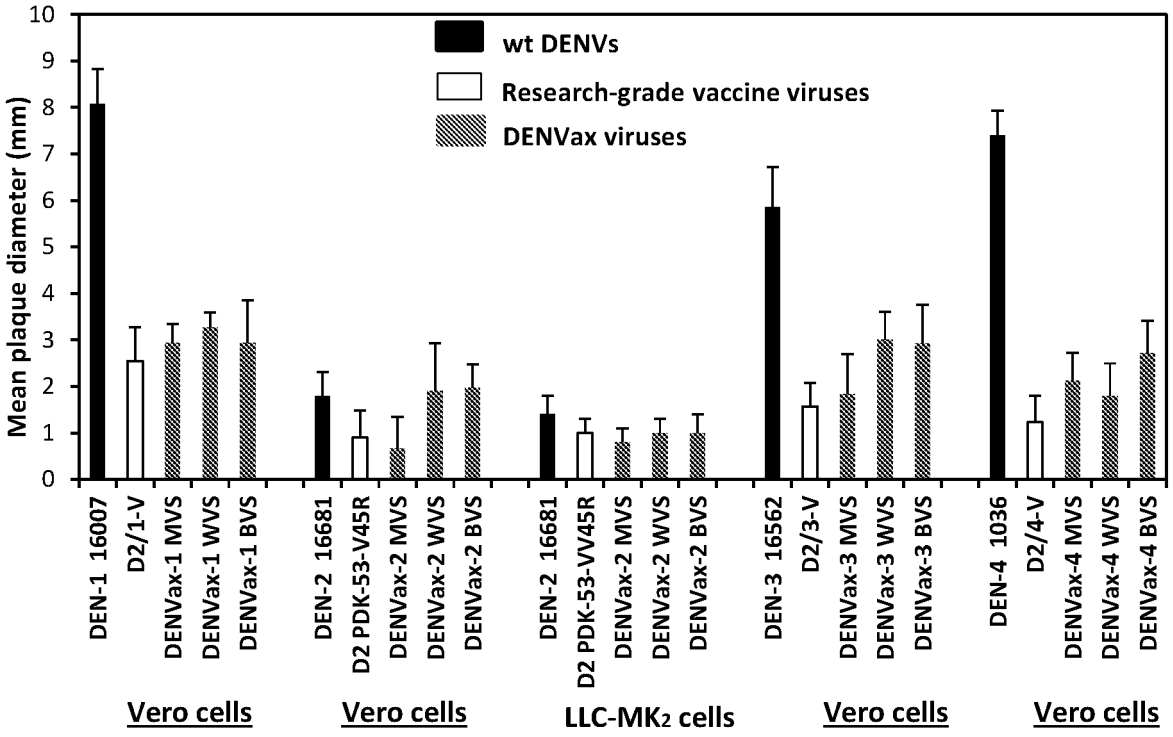

Mean plaque diameter (mm)
wt DENVs
Research-grade vaccine viruses
DENVax viruses
DEN-1 16007
D2/1-V
DENVax-1 MVS
DENVax-1 WVS
DENVax-1 BVS
DEN-2 16681
D2 PDK-53-V45R
DENVax-2 MVS
DENVax-2 WVS
DENVax-2 BVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DENVax-2 WVS
DENVax-2 BVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DENVax-3 WVS
DENVax-3 BVS
DEN-4 1036
D2/4-V
DENVax-4 MVS
DENVax-4 WVS
DENVax-4 BVS
Vero cells
Vero cells
LLC-MK2 cells
Vero cells
Vero cells

Fig. 7

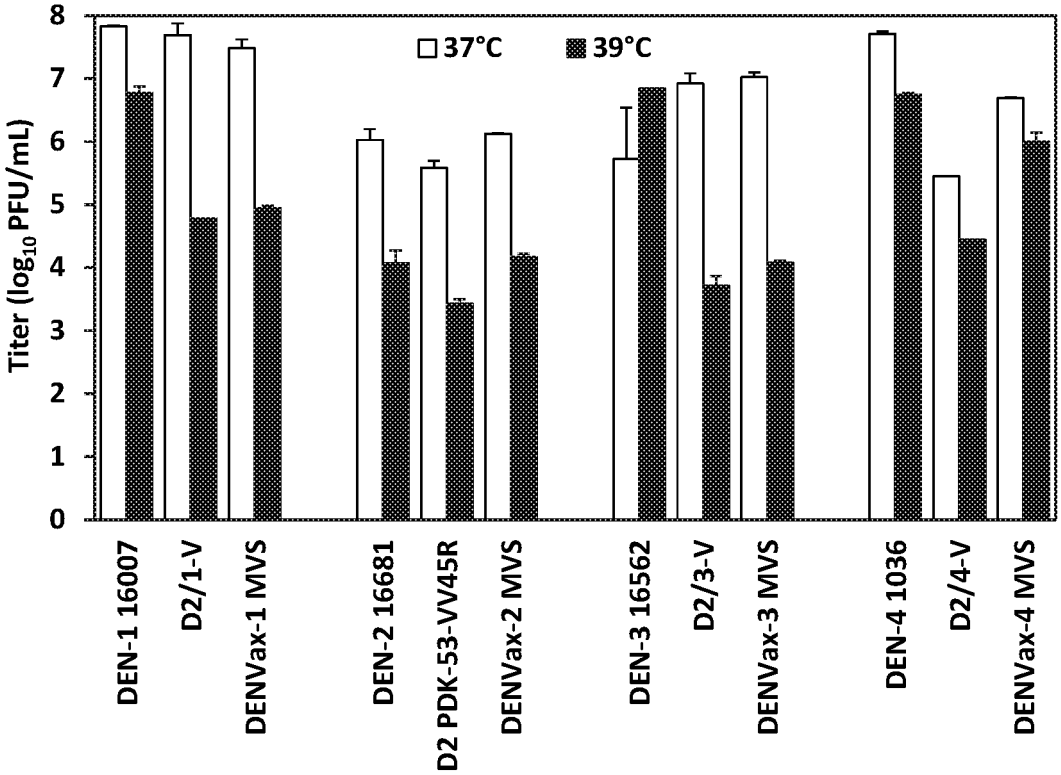
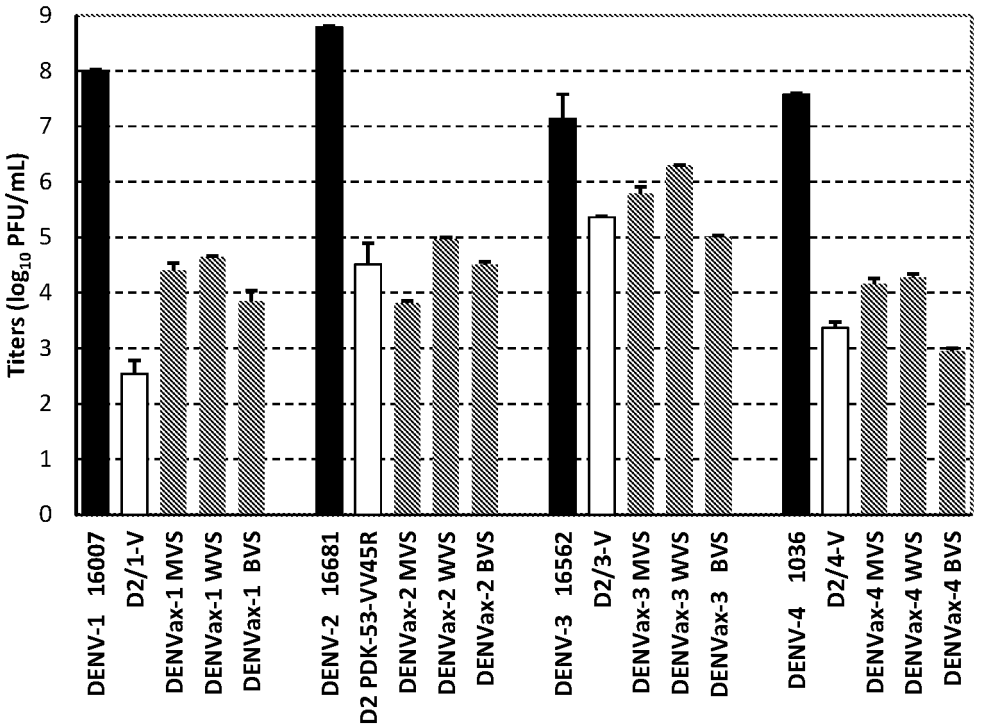

37°C
39°C
Titer (log_{10} PFU/mL)
0
1
2
3
4
5
6
7
8
DEN-1 16007
D2/1-V
DENVax-1 MVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DEN-4 1036
D2/4-V
DENVax-4 MVS

Fig. 8


Titers (log_{10} PFU/mL)
0
1
2
3
4
5
6
7
8
9
DENV-1 16007
D2/1-V
DENVax-1 MVS
DENVax-1 WVS
DENVax-1 BVS
DENV-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DENVax-2 WVS
DENVax-2 BVS
DENV-3 16562
D2/3-V
DENVax-3 MVS
DENVax-3 WVS
DENVax-3 BVS
DENV-4 1036
D2/4-V
DENVax-4 MVS
DENVax-4 WVS
DENVax-4 BVS

Figs. 9A and 9B

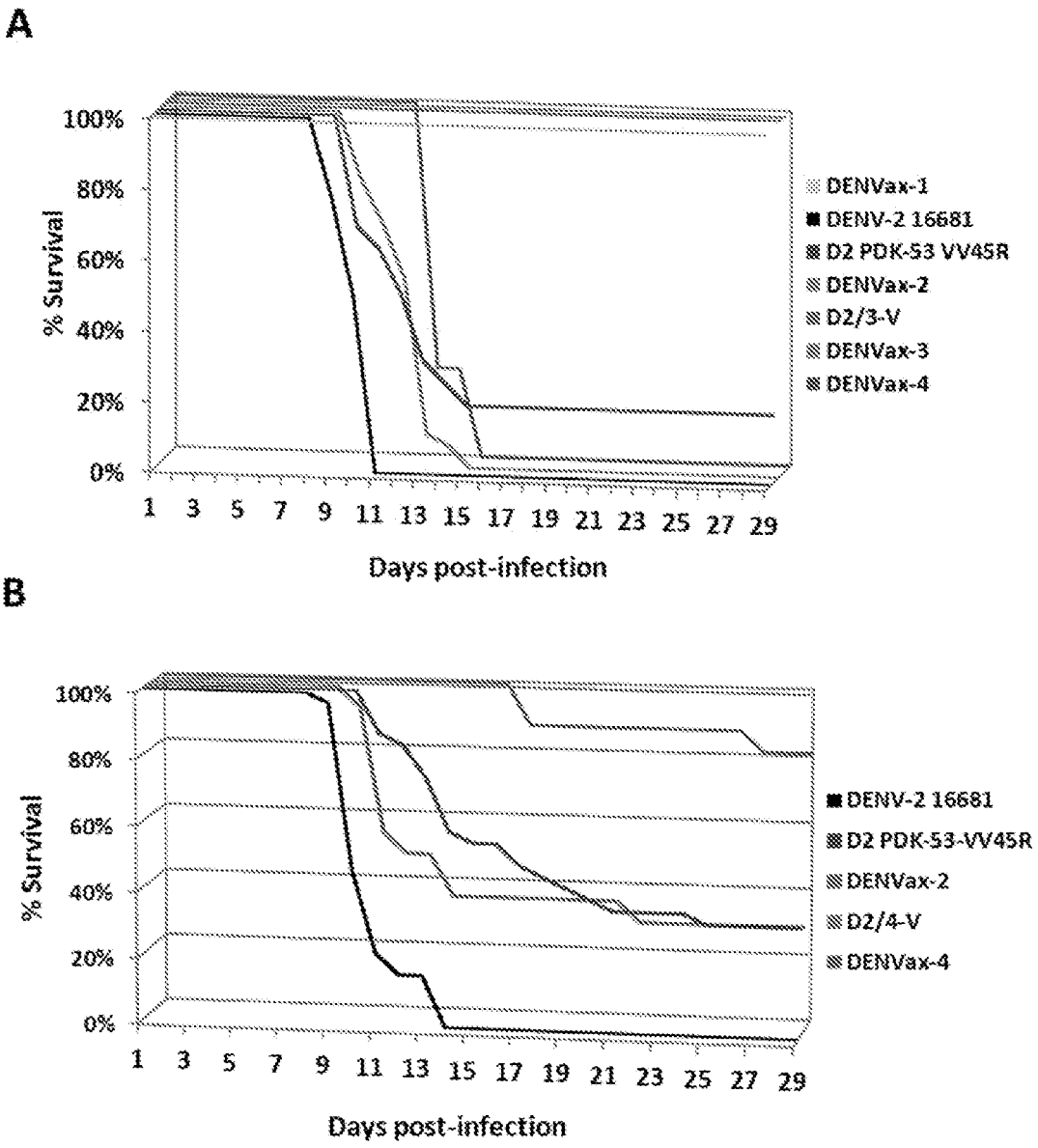


Fig. 10

Serotype	Strain	virus origin	C5'UTR 5'NCR	A52T prM-D39V	T90C M (silent)	C205T E (silent)	G2729A NS1-G53D	C4018T NS2A-L181	A5270G NS3-E350V	T5547C NS3 (silent)	G6699C NS4A-G75A	C8571T NS5 (silent)
DENV-2	16681	isolate from human	C	A	T	C	G	C	A	T	G	C
	PDK-53	PDK cell pass of 16681	T	T	Y	t	A	T	T/A mix	c	C	t
	PDK53-V(V345R)	Recombinant PDK-53-V	T	T	c	t	A	T	T	c	C	C
	PDK53-E(V345R)	Recombinant PDK-53-E	T	T	c	t	A	T	A	c	C	C

Underlined Mutations: the 3 most important attenuation loci of PDK-53
Red font: PDK-53 specific sequence (change from 16681)
Bold font: Different nt sequence between PDK-53 and clone-derived V or E virus
[a] Engineered silent clone marker to differentiate original PDK-53 and recombinant (clone-derived) viruses

FIG. 11A

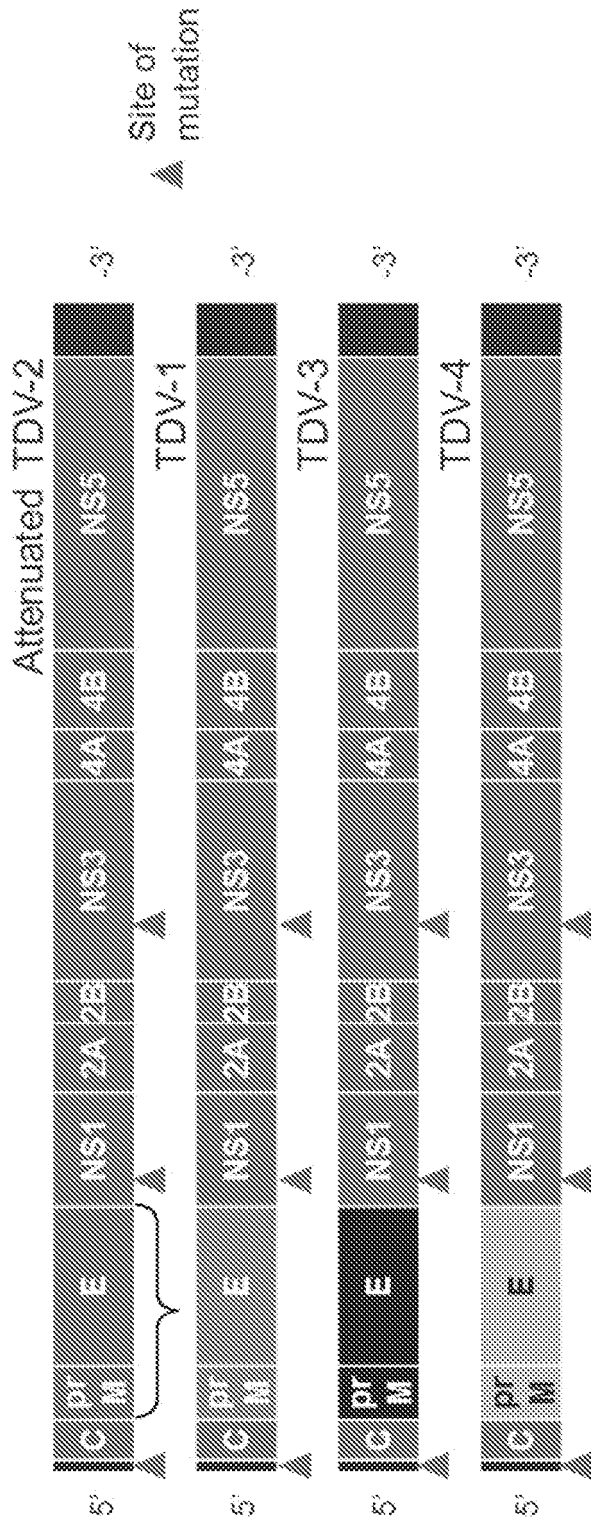

Site of mutation
Attenuated TDV-2
TDV-1
TDV-3
TDV-4
5'
3'
C
prM
E
NS1
2A
2B
NS3
4A
4B
NS5

FIG. 11B

Per dose	TDV-1	TDV-2	TDV-3	TDV-4	Total
TDV formulation (Plaque Forming Units)	2×10^4 PFU	5×10^4 PFU	1×10^5 PFU	3×10^5 PFU	4.7×10^5 PFU

FIG. 12A

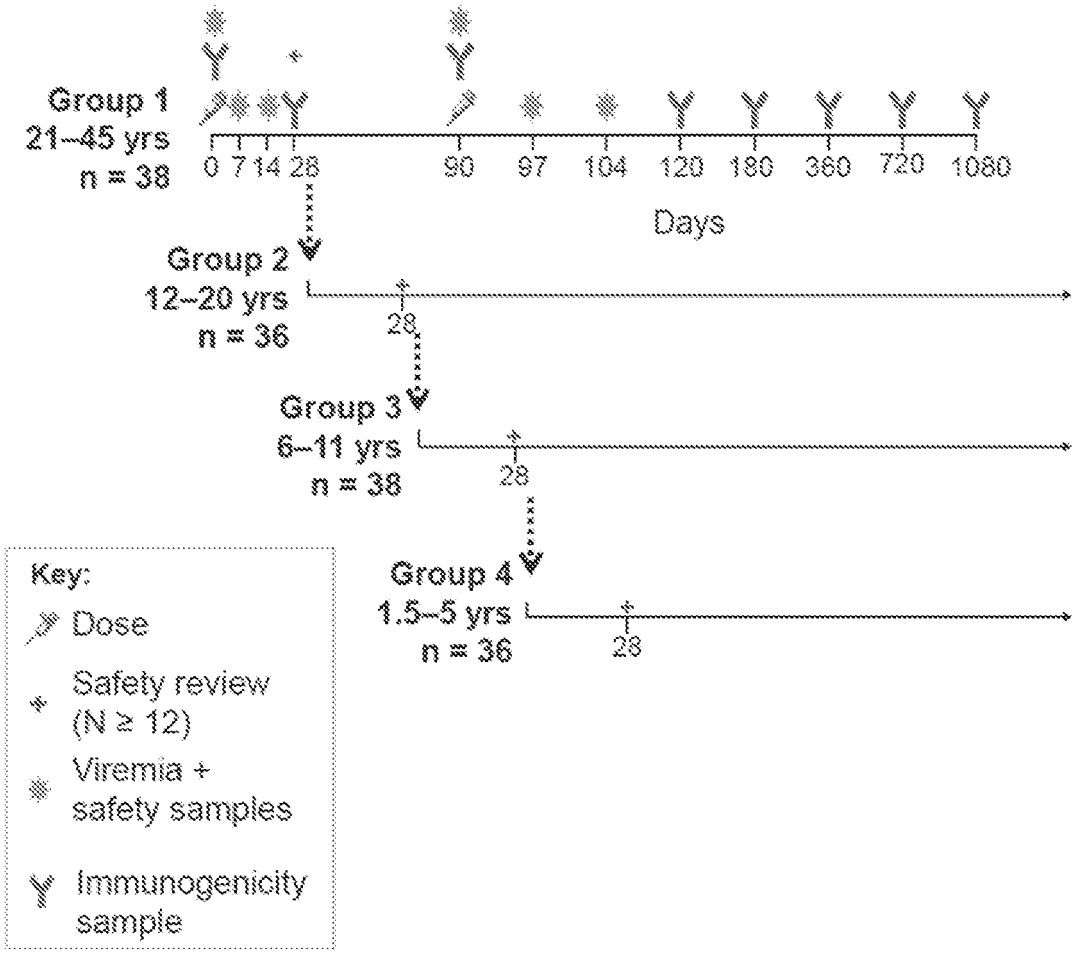

Group 1
21–45 yrs
n = 38
0 7 14 28 90 97 104 120 180 360 720 1080
Days
Group 2
12–20 yrs
n = 36
28
Group 3
6–11 yrs
n = 38
28
Group 4
1.5–5 yrs
n = 36
28
Key:
Dose
Safety review
(N ≥ 12)
Viremia +
safety samples
Immunogenicity
sample

FIG. 12B

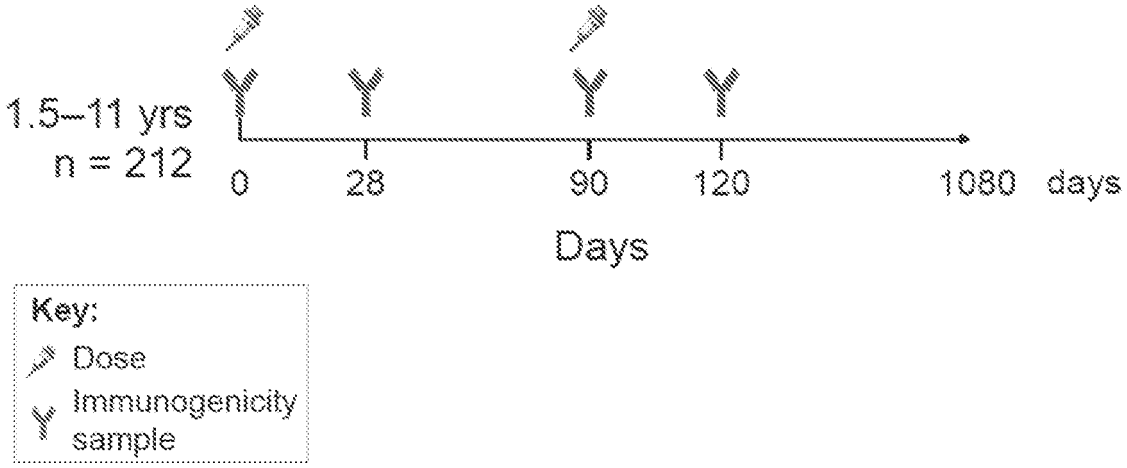

1.5–11 yrs
n = 212
0
28
90
120
1080
days
Days
Key:
Dose
Immunogenicity sample

FIG. 13A

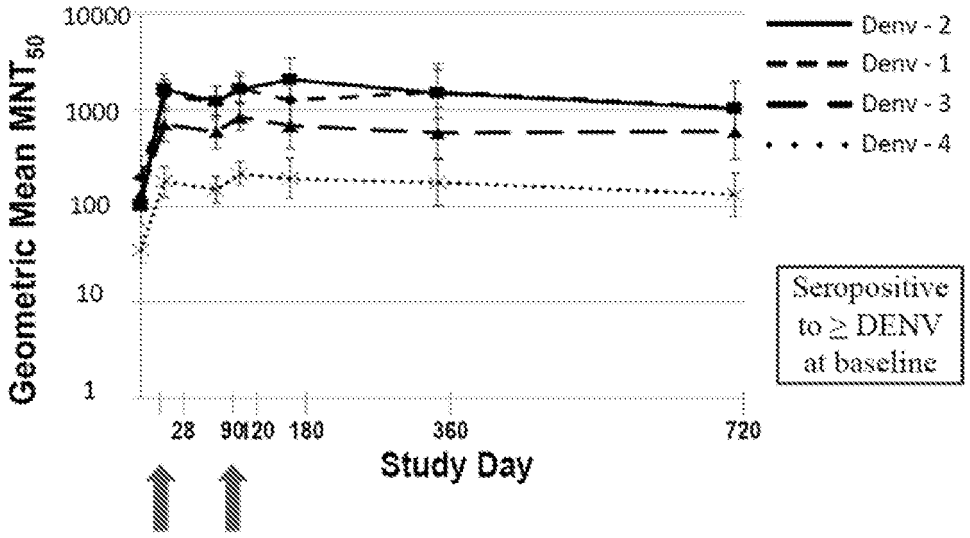

Geometric Mean MNT50
10000
1000
100
10
1
28
90
120
180
360
720
Study Day
Denv - 2
Denv - 1
Denv - 3
Denv - 4
Seropositive to ≥ DENV at baseline

FIG. 13B

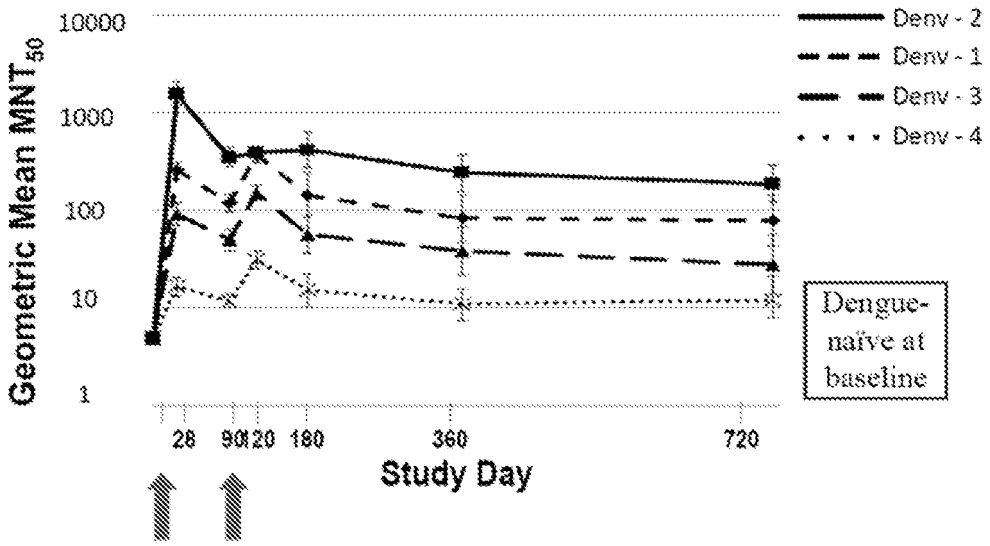

Geometric Mean MNT50
10000
1000
100
10
1
28
90
120
180
360
720
Study Day
Denv - 2
Denv - 1
Denv - 3
Denv - 4
Dengue-naïve at baseline

FIG. 14

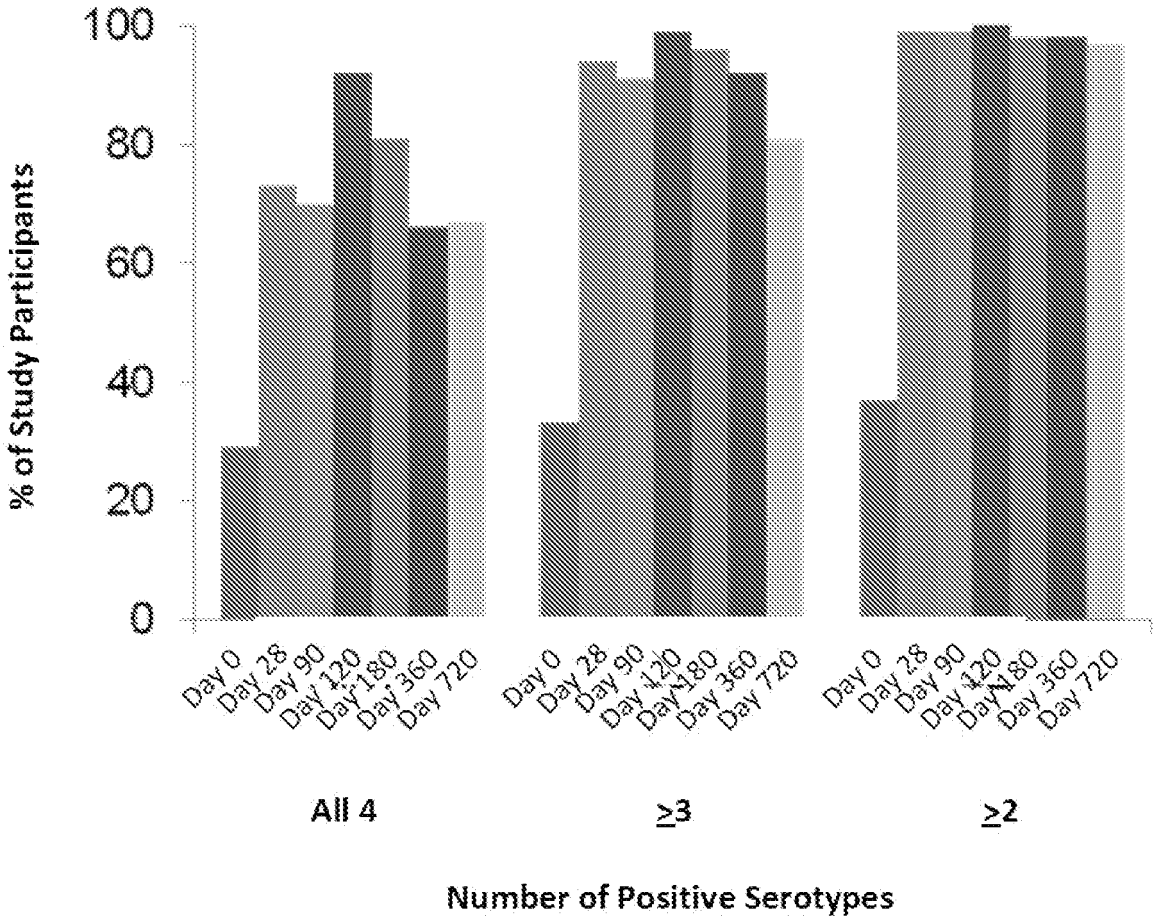

100
80
60
40
20
0
% of Study Participants
Day 0
Day 28
Day 90
Day 120
Day 180
Day 360
Day 720
All 4
≥3
≥2
Number of Positive Serotypes

FIG. 15

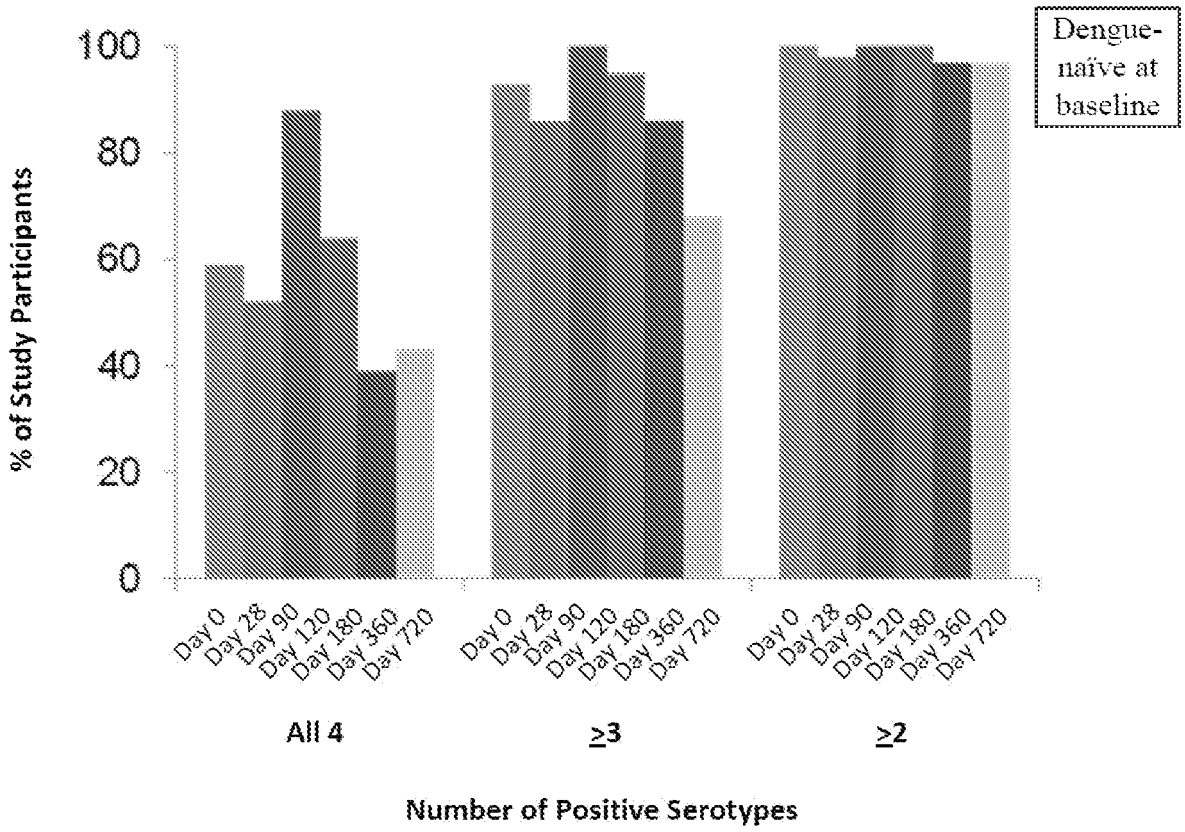
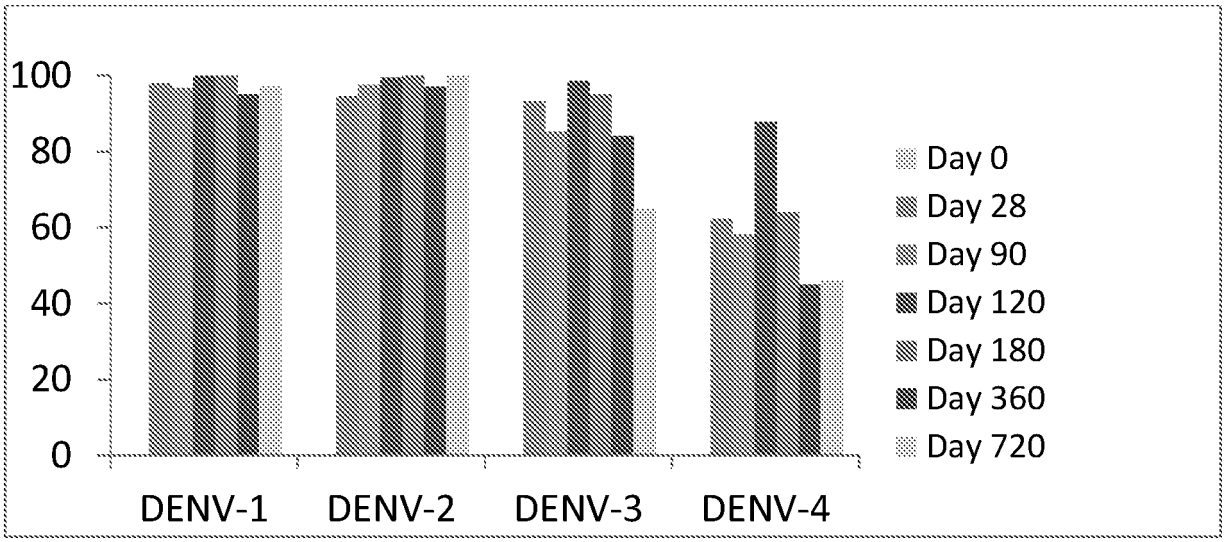

100
80
60
40
20
0
% of Study Participants
Dengue-naïve at baseline
Day 0
Day 28
Day 90
Day 120
Day 180
Day 360
Day 720
All 4
≥3
≥2
Number of Positive Serotypes

FIG. 16



*Dengue-naïve children/young adults at baseline and after receiving TDV (tetravalent)

FIG. 17



*Dengue-naïve children/young adults at baseline and after receiving TDV (tetravalent)