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	MluI	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	A225T	A396C	A453G	C647G/C	A1401G	C2027T	A2275	TG2380/1CC	G2579A	A3674G	A3773A/G	C4018T	A5270T	C5391T	T5547C	C6437T	G6599C	T7026C/T	C8571T**	A9750C
Protein-AA position	NCR	C-silent	C-R100S	prM-silent	prM-T79R/T	E-silent	E-A364V	E-M447L	E-V482A	NS1-G53D	NS2A-D66G	NS2A-K99K/R	NS2A-L181F	NS3-E250V	NS3-silent	NS3-silent	NS4A-A21V	NS4A-G75A	NS4B-silent	NS5-silent	NS5-silent
D2-16681	C	A	A (R)	A					TG (V)	G (G0	A (D)	A (K)	C (L)	A (E)	C	T	C (A)	G (G)	T	C	A
D2-PDK-53	T	-	-	|						A (D)	-	-	**T (F)**	T (V)	-	c	-	C (A)	-	t	-
D4-1036					G (T)	A	C (A)	A (M)													
Cloned D2/4-V1 (pD2/4-VP1)	T	-	C (S)	g	-	g	T (V)	C (L)	CC (A)	A (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 nt 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 NgoMV: D2/4 junction engineered RE sites;

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

Fig. 2

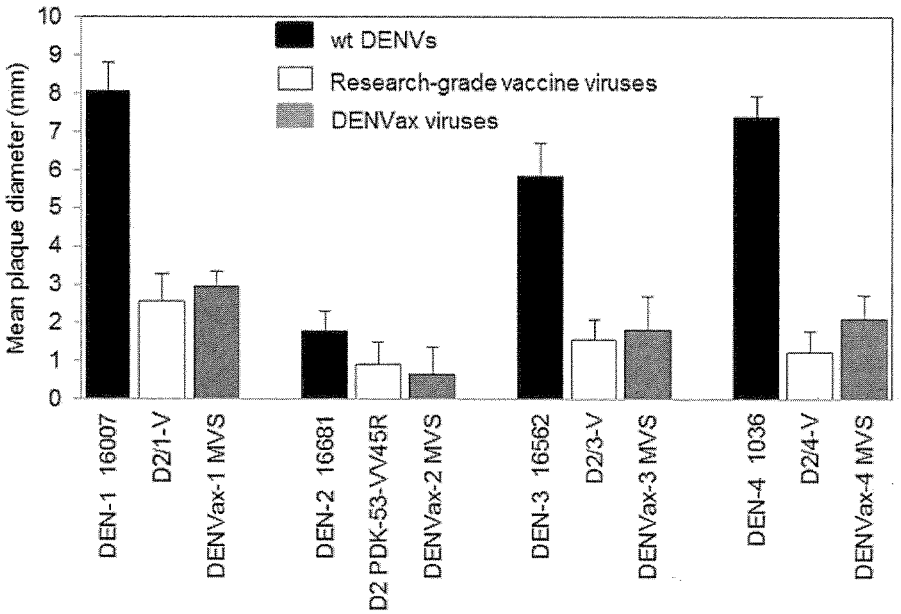

Mean plaque diameter (mm)
10
9
8
7
6
5
4
3
2
1
0
wt DENVs
Research-grade vaccine viruses
DENVax viruses
DEN-1 16007
D2/1-V
DENVax-1 MVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DEN-4 1036
D2/4-V
DENVax-4 MVS

Fig. 3

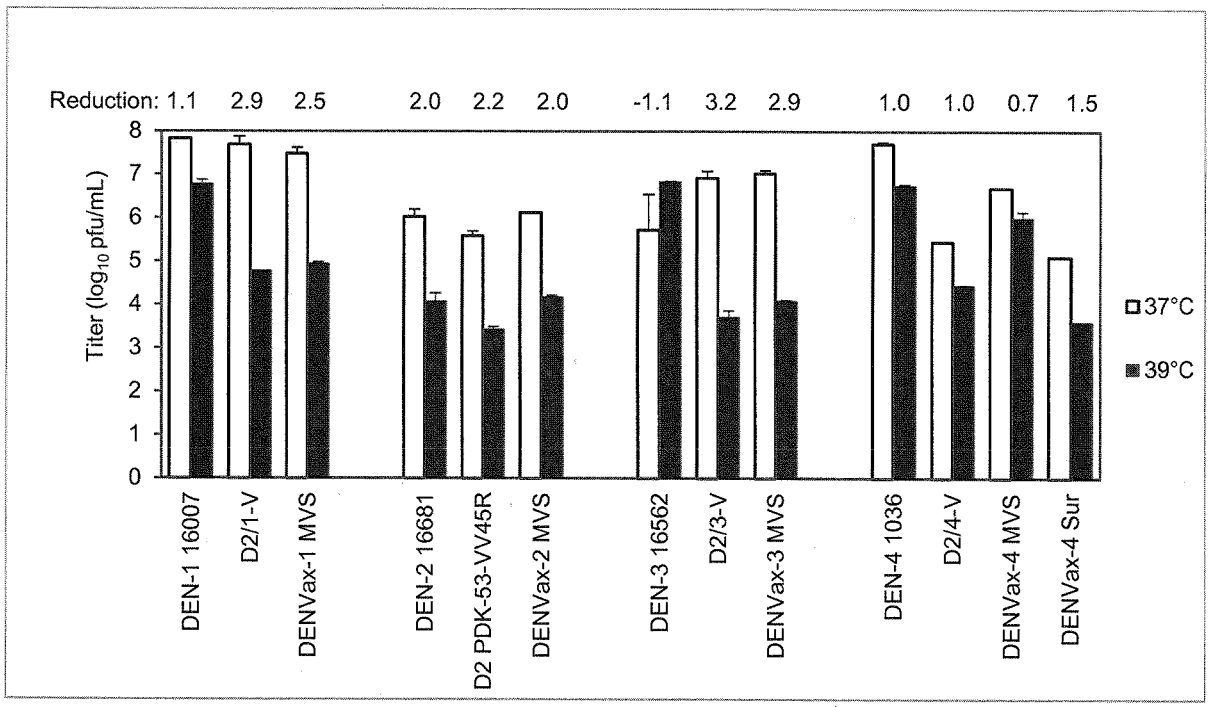

Reduction: 1.1 2.9 2.5 2.0 2.2 2.0 -1.1 3.2 2.9 1.0 1.0 0.7 1.5
Titer (log_{10} pfu/mL)
0
1
2
3
4
5
6
7
8
37°C
39°C
DEN-1 16007
D2/1-V
DENVax-1 MVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DEN-4 1036
D2/4-V
DENVax-4 MVS
DENVax-4 Sur

Fig. 4

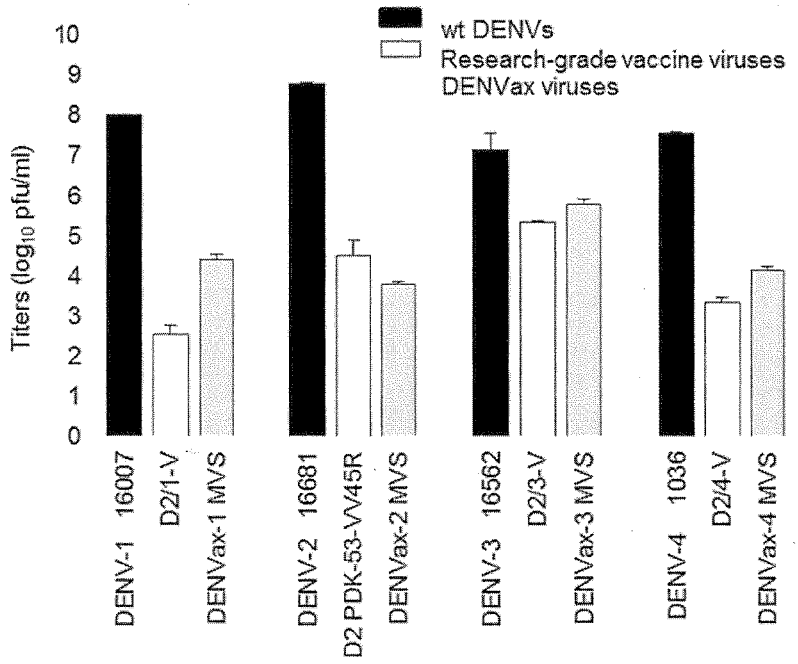

wt DENVs
Research-grade vaccine viruses
DENVax viruses
Titers (log10 pfu/ml)
10
9
8
7
6
5
4
3
2
1
0
DENV-1 16007
D2/1-V
DENVax-1 MVS
DENV-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DENV-3 16562
D2/3-V
DENVax-3 MVS
DENV-4 1036
D2/4-V
DENVax-4 MVS

Fig. 5

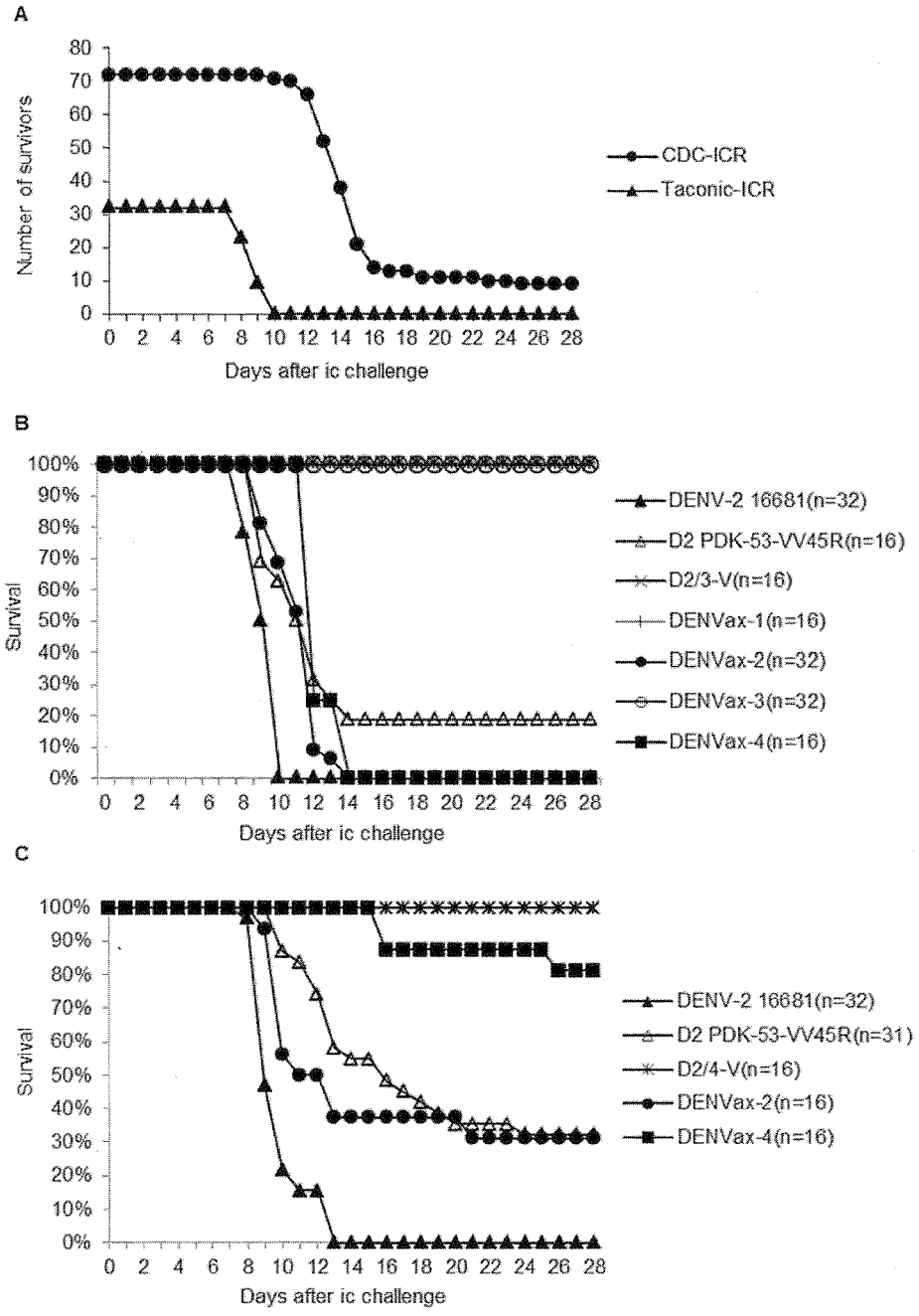

A
Number of survivors
Days after ic challenge
CDC-ICR
Taconic-ICR
B
Survival
Days after ic challenge
DENV-2 16681(n=32)
D2 PDK-53-VV45R(n=16)
D2/3-V(n=16)
DENVax-1(n=16)
DENVax-2(n=32)
DENVax-3(n=32)
DENVax-4(n=16)
C
Survival
Days after ic challenge
DENV-2 16681(n=32)
D2 PDK-53-VV45R(n=31)
D2/4-V(n=16)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

Fig. 6

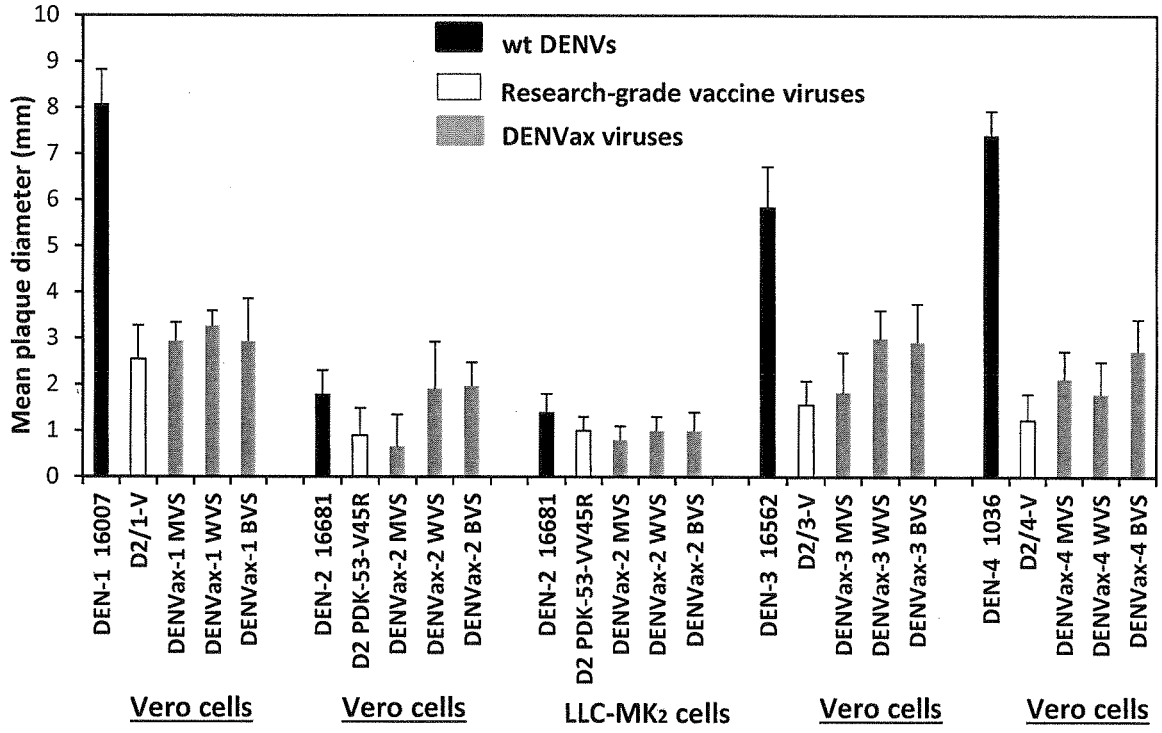

Mean plaque diameter (mm)
10
9
8
7
6
5
4
3
2
1
0
wt DENVs
Research-grade vaccine viruses
DENVax viruses
DEN-1 16007
D2/1-V
DENVax-1 MVS
DENVax-1 WVS
DENVax-1 BVS
DEN-2 16681
D2 PDK-53-V45R
DENVax-2 MVS
DENVax-2 WVS
DENVax-2 BVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DENVax-2 WVS
DENVax-2 BVS
DEN-3 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

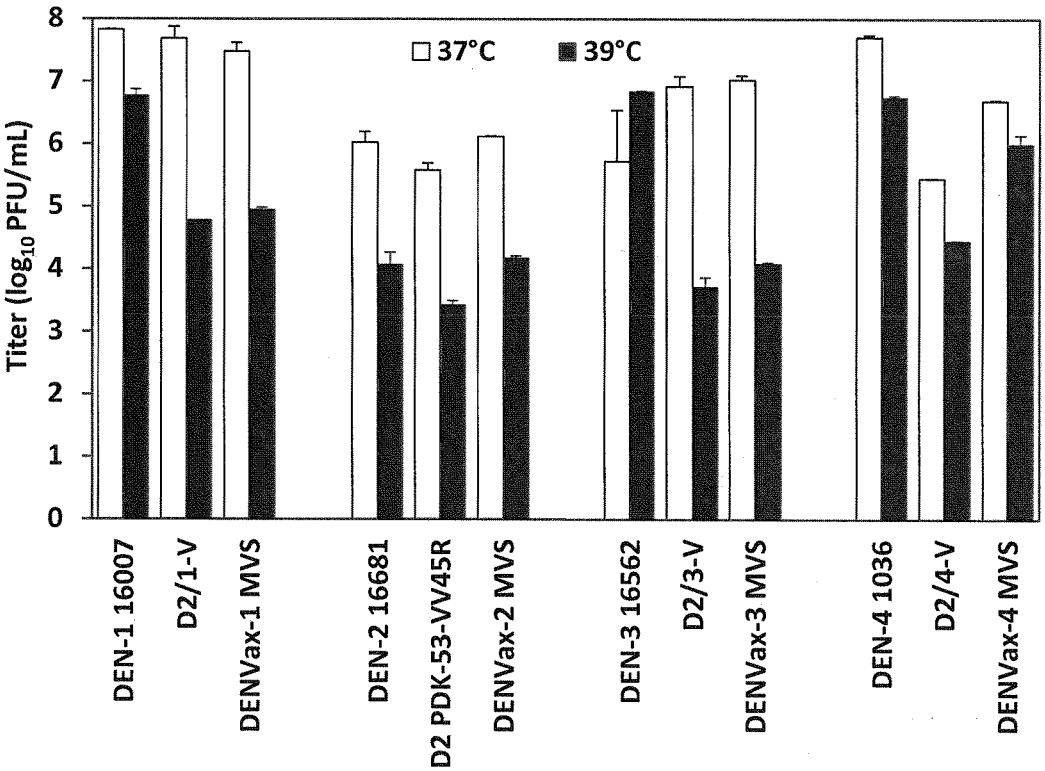

37°C
39°C
Titer (log_{10} PFU/mL)
0
1
2
3
4
5
6
7
8
DEN-1 16007
D2/1-V
DENVax-1 MVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DEN-4 1036
D2/4-V
DENVax-4 MVS

Fig. 8

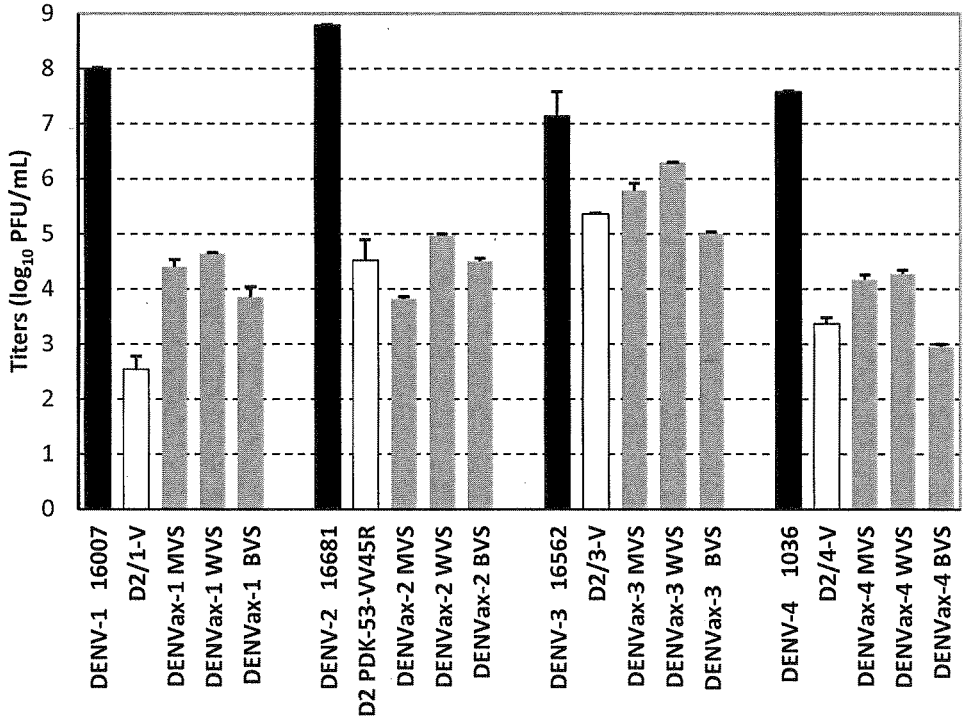

Titers (log_{10} PFU/mL)
0
1
2
3
4
5
6
7
8
9
DENV-1 16007
D2/1-V
DENVax-1 MVS
DENVax-1 WVS
DENVax-1 BVS
DENV-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DENVax-2 WVS
DENVax-2 BVS
DENV-3 16562
D2/3-V
DENVax-3 MVS
DENVax-3 WVS
DENVax-3 BVS
DENV-4 1036
D2/4-V
DENVax-4 MVS
DENVax-4 WVS
DENVax-4 BVS

Fig. 9

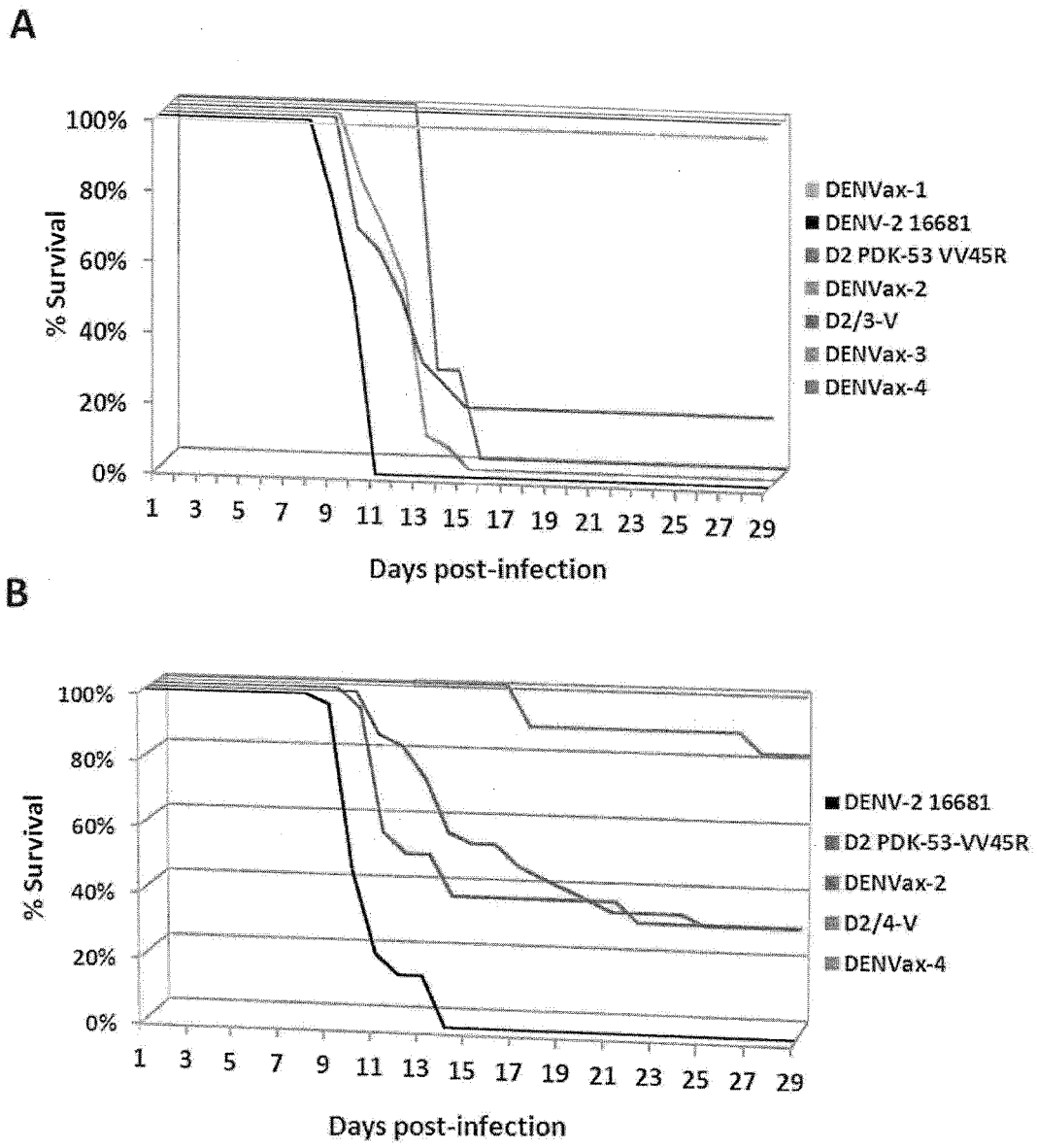

A
100%
80%
60%
40%
20%
0%
% Survival
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
Days post-infection
DENVax-1
DENV-2 16681
D2 PDK-53 VV45R
DENVax-2
D2/3-V
DENVax-3
DENVax-4
B
100%
80%
60%
40%
20%
0%
% Survival
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
Days post-infection
DENV-2 16681
D2 PDK-53-VV45R
DENVax-2
D2/4-V
DENVax-4

Fig. 10

Serotype	Strain	virus origin	C57T 5'NCR	A524T prM-D29V	T900C* M (silent)	C2055T E (silent)	G2579A NS3-G53D	C4018T NS2A-L181	A52703 NS3-E350V	T5547C NS3 (silent)	G6599C 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	..	t	A	T	T/A mix	c	C	t
	PDK53-V(VV45R)	Recombinant PDK-53-V	T	T	c	t	A	T	T	c	C	C
	PDK53-E(VE48R)	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
* Engineered silent clone marker to differentiate original PDK-53 and recombinant (clone-derived) viruses

Fig. 1

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

Genome	D2			junction	D4				junction	D2											
Genes	NCR	C		prM	E					NS1	NS2A			NS3			NS4A		NS4B	NS5	
Mutation types*	PDK-53	seed	Eng	MluI	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	A225T	A396C	A453G	C647G/C	A1401G	C2027T	A2275	TG2580/1CC	G2579A	A3574G	A3773A/G	C4018T	A5270T	C5391T	T5547C	C6437T	G6599C	T7026C/T	C8571T**	A8750C
Protein-AA position	NCR	C-silent	C-R100S	prM-silent	prM-T198/T	E-silent	E-A364V	E-M447L	E-V482A	NS1-G53D	NS2A-D66G	NS2A-K99K/R	NS2A-L181F	NS3-E250V	NS3-silent	NS3-silent	NS4A-A21V	NS4A-G75A	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)	C (G)	T	C	A
D2-PDK-53	T	-	-							A (D)	-	-	T (F)	T (V)	-	c	-	C (A)	-	T	-
D4-1036					G (T)	A	C (A)	A (M)													
Cloned D2/4-V1 (pD2/4-VP1)	T	-	C (S)	g	-	g	T (V)	C (L)	CC (A)	A (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 nt 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

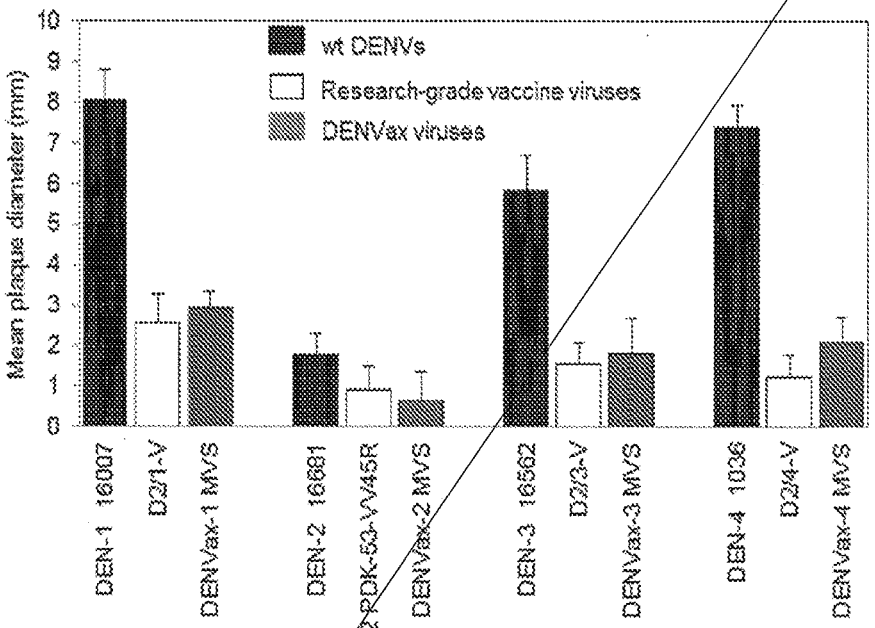

Mean plaque diameter (mm)
0
1
2
3
4
5
6
7
8
9
10
wt DENVs
Research-grade vaccine viruses
DENVax viruses
DEN-1 16007
D2/1-V
DENVax-1 MVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DEN-4 1036
D2/4-V
DENVax-4 MVS

Fig. 3

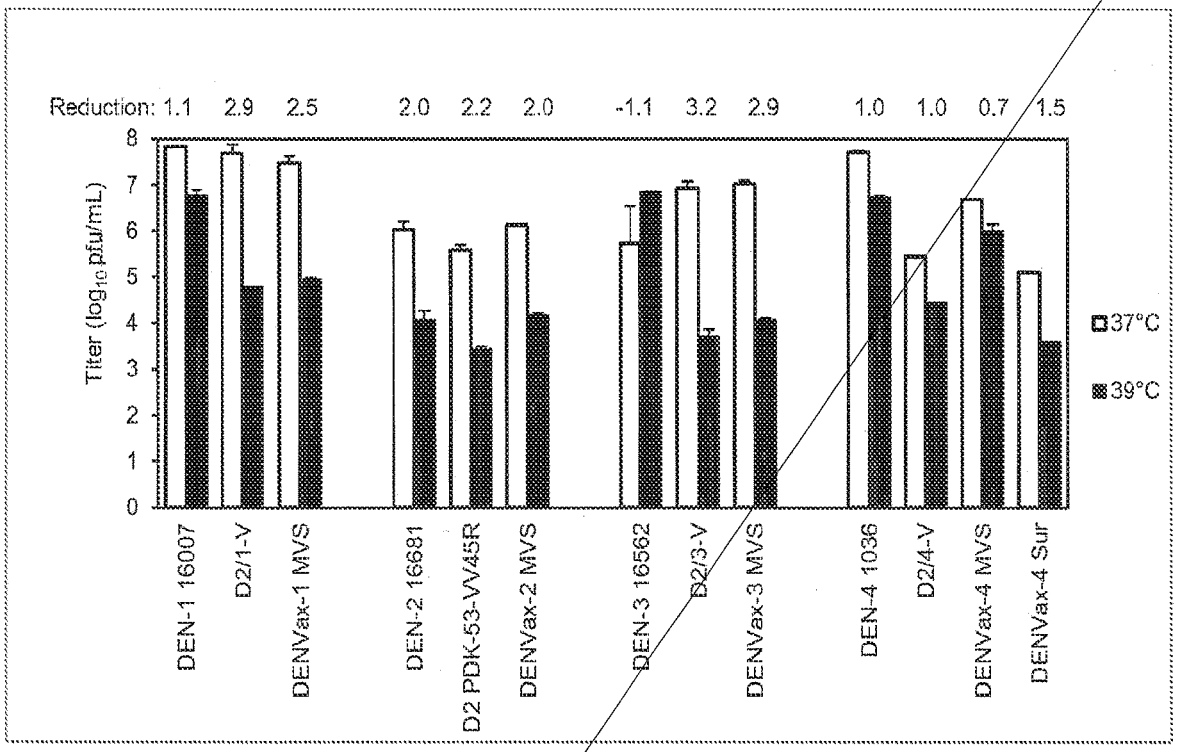

Reduction: 1.1 2.9 2.5 2.0 2.2 2.0 -1.1 3.2 2.9 1.0 1.0 0.7 1.5
Titer (log_{10} pfu/mL)
0
1
2
3
4
5
6
7
8
37°C
39°C
DEN-1 16007
D2/1-V
DENVax-1 MVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DEN-4 1036
D2/4-V
DENVax-4 MVS
DENVax-4 Sur

Fig. 4

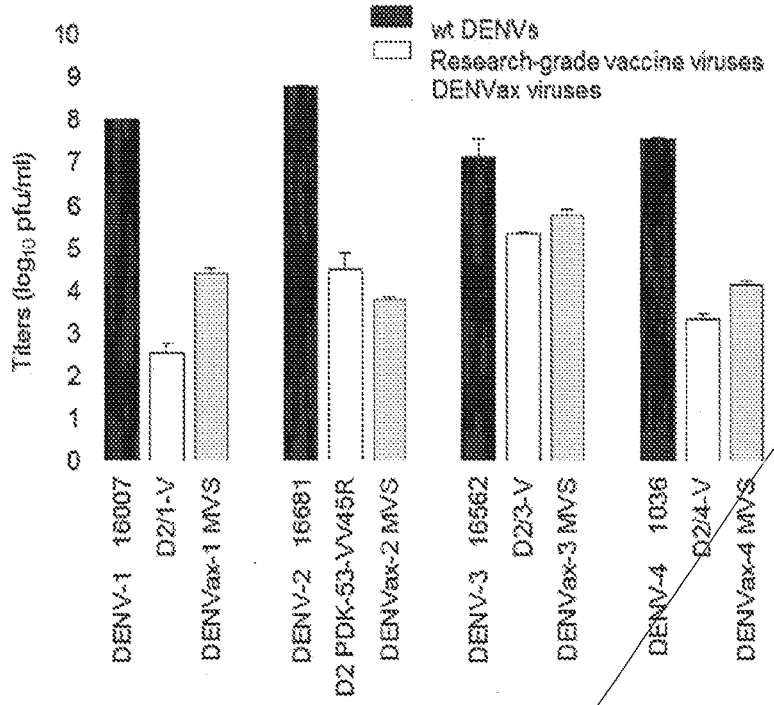

wt DENVs
Research-grade vaccine viruses
DENVax viruses
Titers (log10 pfu/ml)
0
1
2
3
4
5
6
7
8
9
10
DENV-1 16007
D2/1-V
DENVax-1 MVS
DENV-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DENV-3 16562
D2/3-V
DENVax-3 MVS
DENV-4 1036
D2/4-V
DENVax-4 MVS

Fig. 5

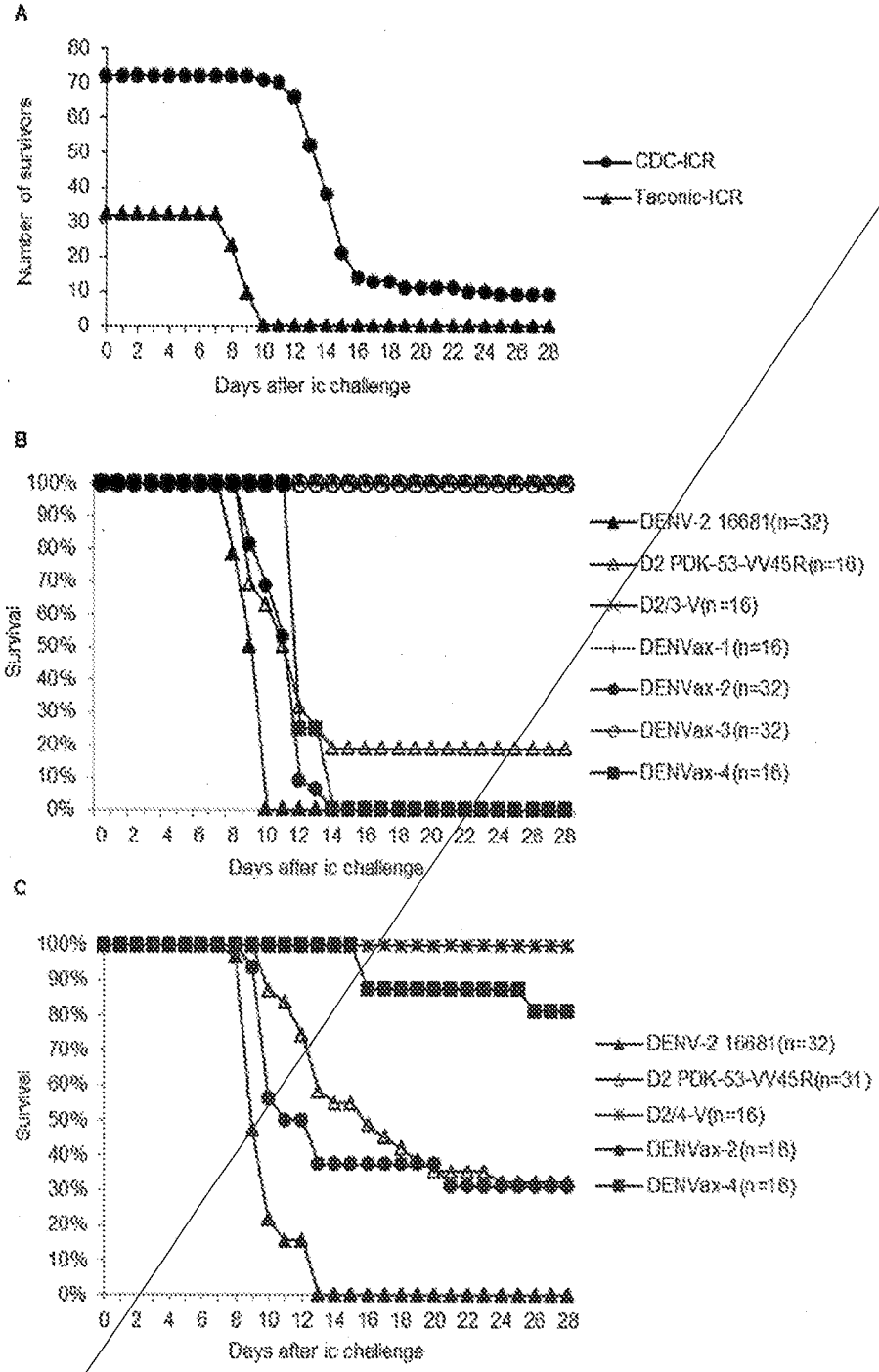

A
Number of survivors
Days after ic challenge
CDC-ICR
Taconic-ICR
B
Survival
Days after ic challenge
DENV-2 16681(n=32)
D2 PDK-53-VV45R(n=16)
D2/3-V(n=16)
DENVax-1(n=16)
DENVax-2(n=32)
DENVax-3(n=32)
DENVax-4(n=16)
C
Survival
Days after ic challenge
DENV-2 16681(n=32)
D2 PDK-53-VV45R(n=31)
D2/4-V(n=16)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

Fig. 6

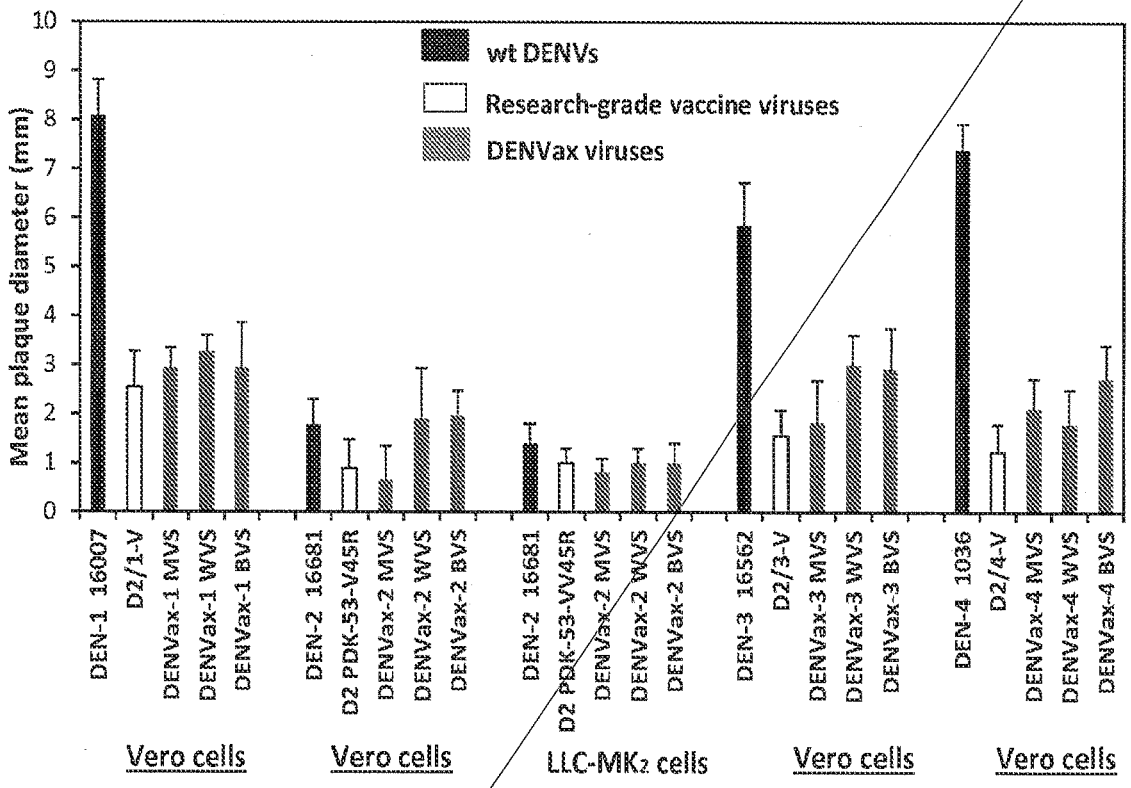

Mean plaque diameter (mm)
wt DENVs
Research-grade vaccine viruses
DENVax viruses
DEN-1 16007
D2/1-V
DENVax-1 MVS
DENVax-1 WVS
DENVax-1 BVS
DEN-2 16681
D2 PDK-53-V45R
DENVax-2 MVS
DENVax-2 WVS
DENVax-2 BVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DENVax-2 WVS
DENVax-2 BVS
DEN-3 16562
D2/3-V
DENVax-3 MVS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

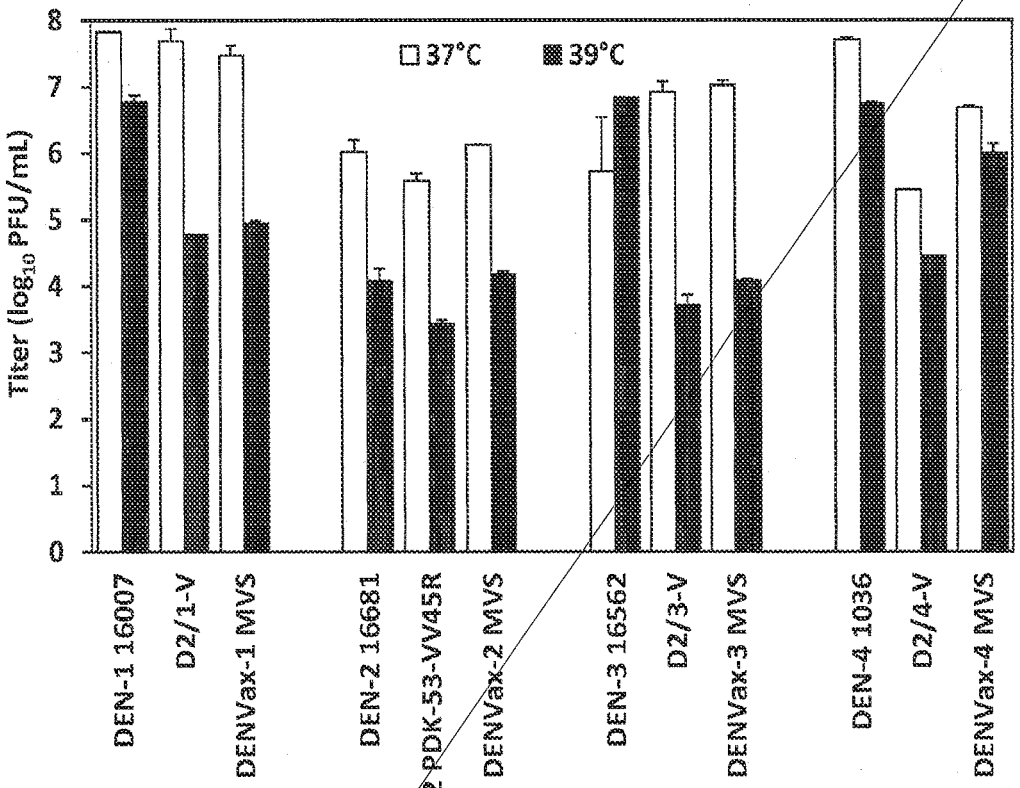

37°C
39°C
Titer (log_{10} PFU/mL)
0
1
2
3
4
5
6
7
8
DEN-1 16007
D2/1-V
DENVax-1 MVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DEN-4 1036
D2/4-V
DENVax-4 MVS

Fig. 8

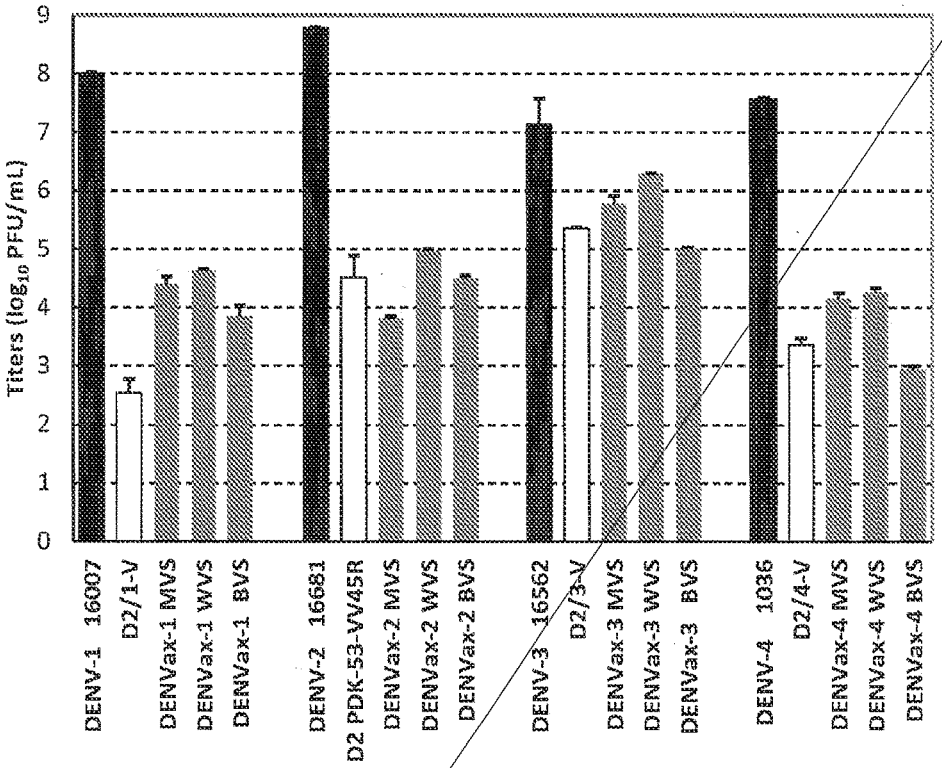

Titers (log_{10} PFU/mL)
0
1
2
3
4
5
6
7
8
9
DENV-1 16007
D2/1-V
DENVax-1 MVS
DENVax-1 WVS
DENVax-1 BVS
DENV-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DENVax-2 WVS
DENVax-2 BVS
DENV-3 16562
D2/3-V
DENVax-3 MVS
DENVax-3 WVS
DENVax-3 BVS
DENV-4 1036
D2/4-V
DENVax-4 MVS
DENVax-4 WVS
DENVax-4 BVS

Fig. 9

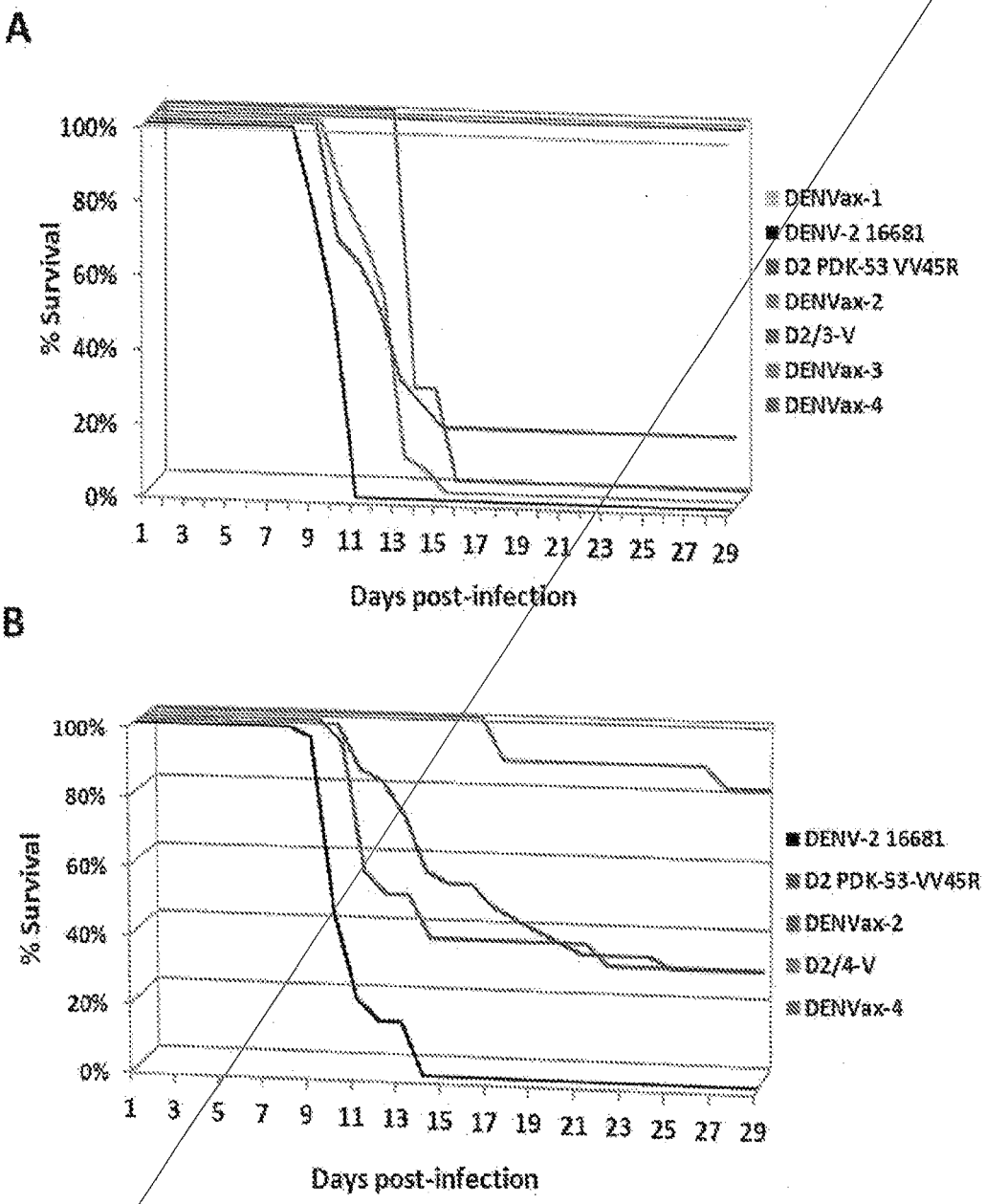

A
100%
80%
60%
40%
20%
0%
% Survival
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
Days post-infection
DENVax-1
DENV-2 16681
D2 PDK-53 VV45R
DENVax-2
D2/3-V
DENVax-3
DENVax-4
B
100%
80%
60%
40%
20%
0%
% Survival
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
Days post-infection
DENV-2 16681
D2 PDK-53-VV45R
DENVax-2
D2/4-V
DENVax-4

Fig. 10

Serotype	Strain	virus origin	C511T NS2A-T20I	A2247T prM-D29V	T9006C M (silent)	C20355T E (silent)	G22220A NS1-G53D	C24018T NS2A-L181	A25230T NS3-E249R	T5547C NS3 (silent)	G6996C NS4A-A75A	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	i	i	A	T	T/A mix	a	c	t
	PDK53-V(V165R)	Recombinant PDK-53-V	T	T	t	t	A	T	T	c	C	C
	PDK53-E(V165E)	Recombinant PDK-53-E	T	T	t	t	A	T	A	a	C	C

Underlined Mutations: the 3 most important attenuation loci of PDK-53
Red font: PDK-53 specific sequence (change from 16681)
Bold font: Differences in sequence between PDK-53 and closer-derived V or E virus
* Engineered silent clone markers to differentiate original PDK-53 and recombinant (clone-derived) viruses