Fig. 1

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

Genome Genes	D2				D4																									
	NCR		C		junction prM	E				junction	NS1		NS2A			NS3			NS4A			NS4B			NS5				
Mutation types*	PDK-53	seed	Eng	seed	MMul	Marmer	Eng	Eng		NgoMVr	PDK-53	seed	seed	PDK-53	PDK-53	seed	PDK-53	seed	seed	PDK-53	seed	PDK-53	seed	PDK53	seed					
Genome NT position	C57T	42357	43966	A4536	2647GAC	A46013	C28077	42275		T5238G/ICC	G25784	A36746	A37734/G	C46138T	A52787	C5931T	T5947C	C6437T	C6939C	T7025C/T	C8571***	A9756C								
Protein-44 position	NCR	C-silent	C-R1003K	prM-silent	prM-E-silent	E-A438V	E-M447K				NS1-G53D	NS2A-D66G	NS2A-K99K/R	NS2A-L181F	NS3-E250V	NS3-silent	NS3-silent	NS4A-A22V	NS4A-G75A	NS4B-silent			NS5-silent								
D2-16681	C	A	A							TG (V)	G (G)	A (D)	A (N)	C (L)	A (E)	C	T	C (A)	G (G)	T	C	A								
D2-PDK-53	T	-	-								A (D)	-	-	T (F)	T (V)	-	-	-	C (A)	-	T	-								
D4-1036				G (T)	A	C (A)	A (M)																							
Cloned D2/4-V1 (pD2/4-VP1) DENVs-4 (MMS)	T	-	C (R)	a	-	a	T (M)	C (R)		CC (A)	A (D)	-	-	T (F)	T (V)	-	-	-	C (A)	-	-	-								
	T	t	C (R)	a	-	a	T (M)	C (R)		CC (A)	A (D)	G (G)	A/G (K/R)	T (F)	T (V)	t	c	T (V)	C (A)	-	C/t	-								

*: 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;

***: 10857A1 (PDK-53 silent mutation) was not included in most D2/4 chimeric clones

Fig. 2

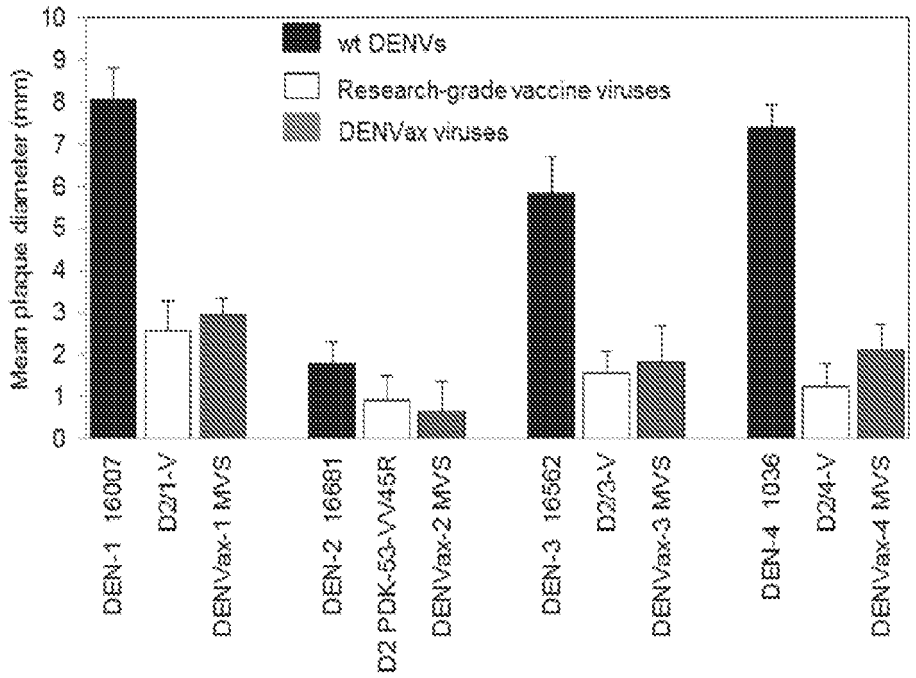

Mean plaque diameter (mm)
0
1
2
3
4
5
6
7
8
9
10
wt DENVs
Research-grade vaccine viruses
DENVax viruses
DEN-1 16007
D2/1-V
DENVax-1 MVS
DEN-2 16681
D2 PDK-53-V-V45R
DENVax-2 MVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DEN-4 1036
D2/4-V
DENVax-4 MVS

Fig. 3

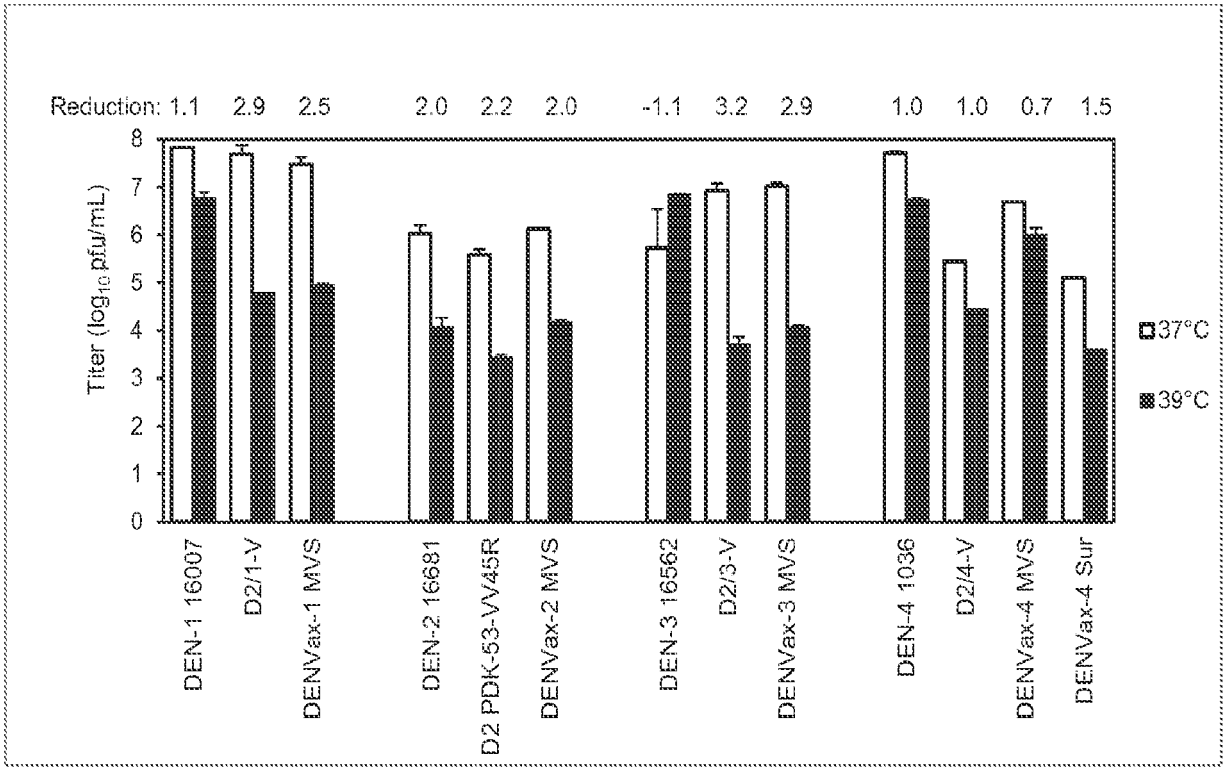

Reduction: 1.1 2.9 2.5 2.0 2.2 2.0 -1.1 3.2 2.9 1.0 1.0 0.7 1.5
Titer (log_{10} pfu/mL)
0
1
2
3
4
5
6
7
8
37°C
39°C
DEN-1 16007
D2/1-V
DENVax-1 MVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DEN-4 1036
D2/4-V
DENVax-4 MVS
DENVax-4 Sur

Fig. 4

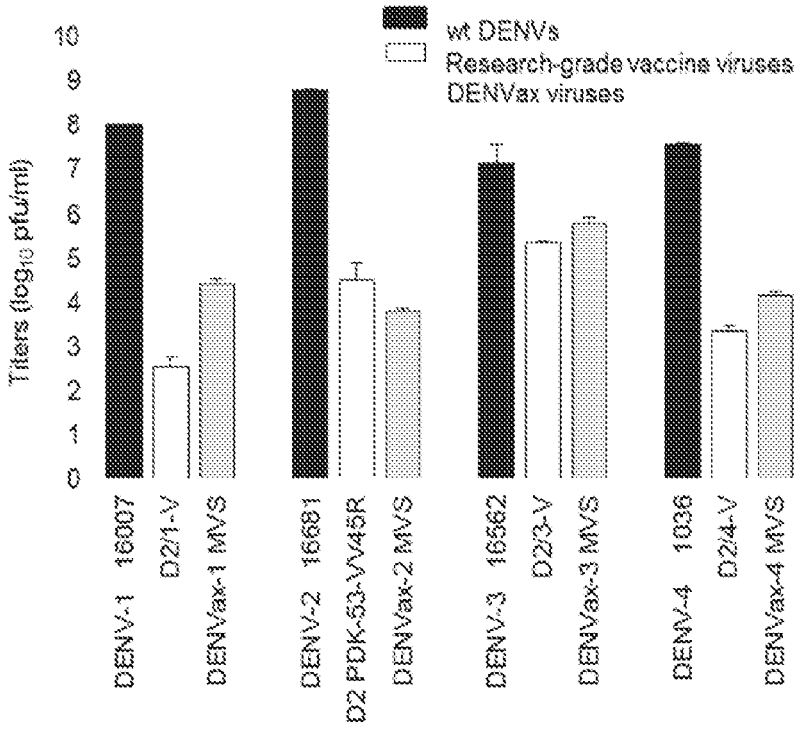

wt DENVs
Research-grade vaccine viruses
DENVax viruses
Titers (log_{10} pfu/ml)
0
1
2
3
4
5
6
7
8
9
10
DENV-1 16007
D2/1-V
DENVax-1 MVS
DENV-2 16681
D2 PDK-53-V45R
DENVax-2 MVS
DENV-3 16562
D2/3-V
DENVax-3 MVS
DENV-4 1036
D2/4-V
DENVax-4 MVS

Fig. 5

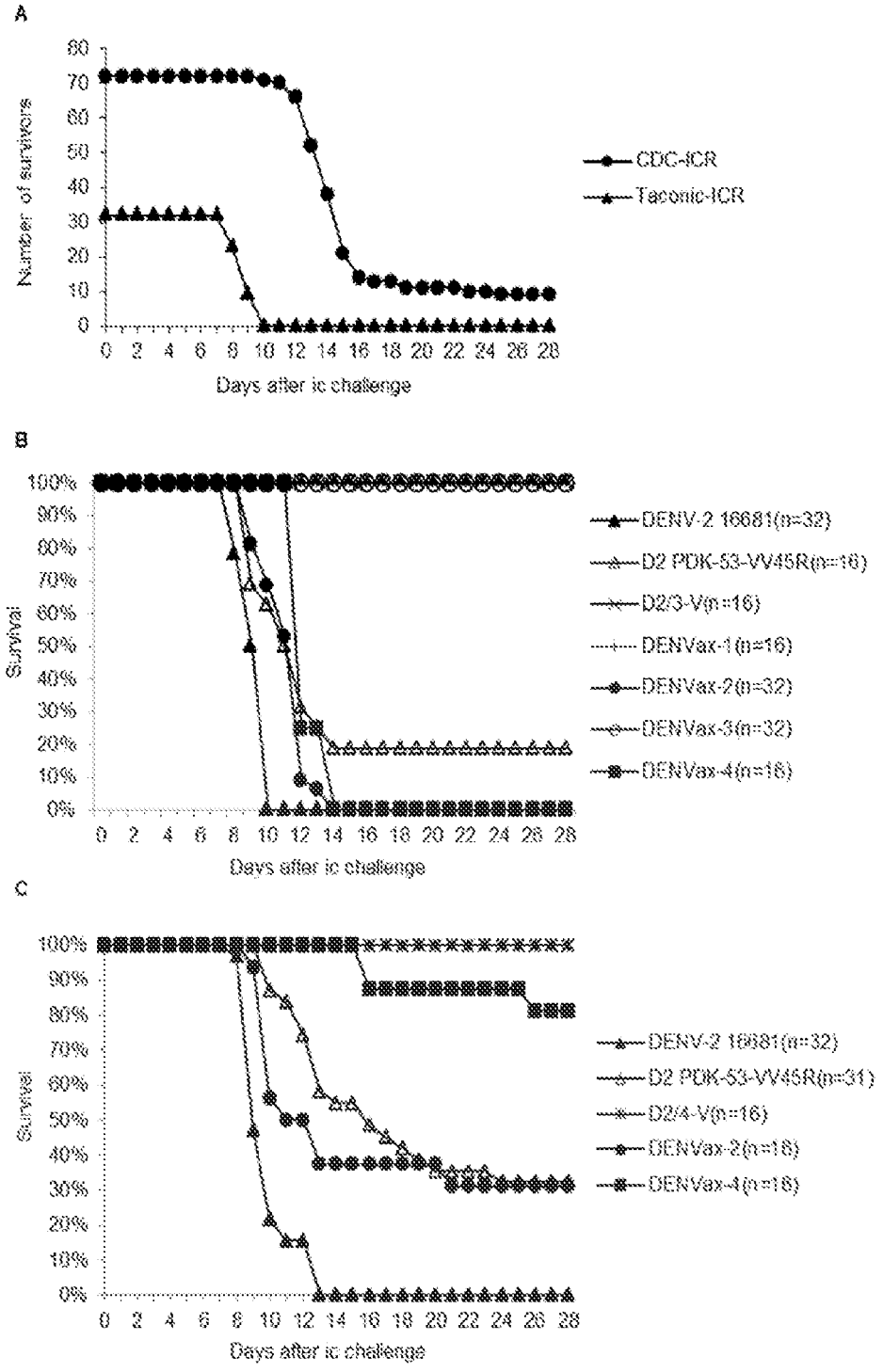

A
Number of survivors
Days after ic challenge
CDC-ICR
Taconic-ICR
B
Survival
Days after ic challenge
DENV-2 16681(n=32)
D2 PDK-53-VV45R(n=16)
D2/3-V(n=16)
DENVax-1(n=16)
DENVax-2(n=32)
DENVax-3(n=32)
DENVax-4(n=16)
C
Survival
Days after ic challenge
DENV-2 16681(n=32)
D2 PDK-53-VV45R(n=31)
D2/4-V(n=16)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

Fig. 6

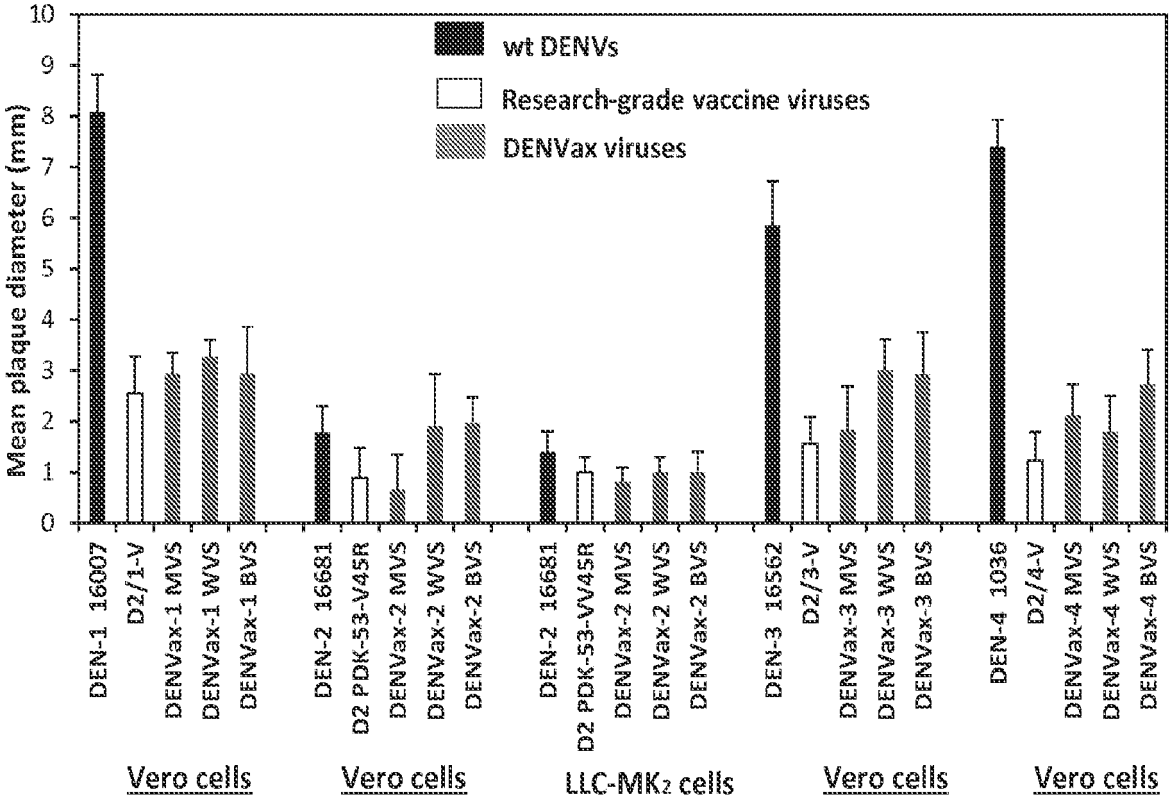

Mean plaque diameter (mm)
wt DENVs
Research-grade vaccine viruses
DENVax viruses
DEN-1 16007
D2/1-V
DENVax-1 MVS
DENVax-1 WVS
DENVax-1 BVS
DEN-2 16681
D2 PDK-53-V45R
DENVax-2 MVS
DENVax-2 WVS
DENVax-2 BVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DENVax-2 WVS
DENVax-2 BVS
DEN-3 16562
D2/3-V
DENVax-3 MVS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

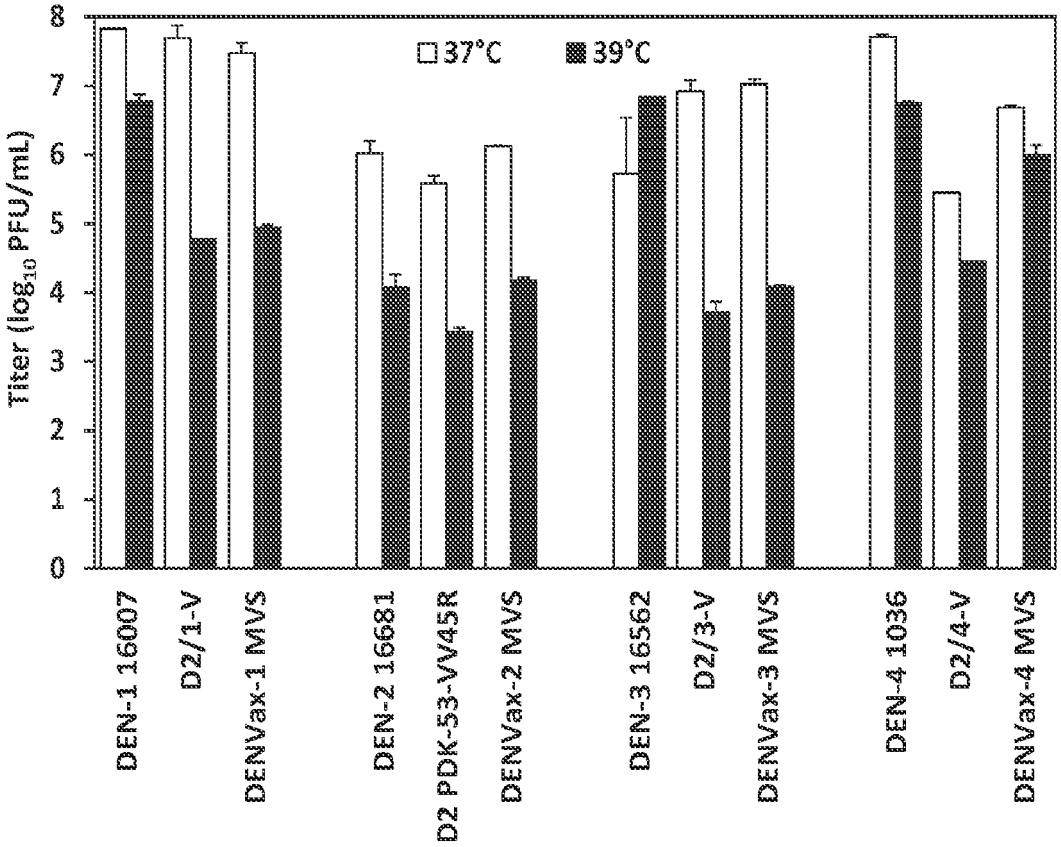

37°C
39°C
Titer (log_{10} PFU/mL)
0
1
2
3
4
5
6
7
8
DEN-1 16007
D2/1-V
DENVax-1 MVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DEN-4 1036
D2/4-V
DENVax-4 MVS

Fig. 8

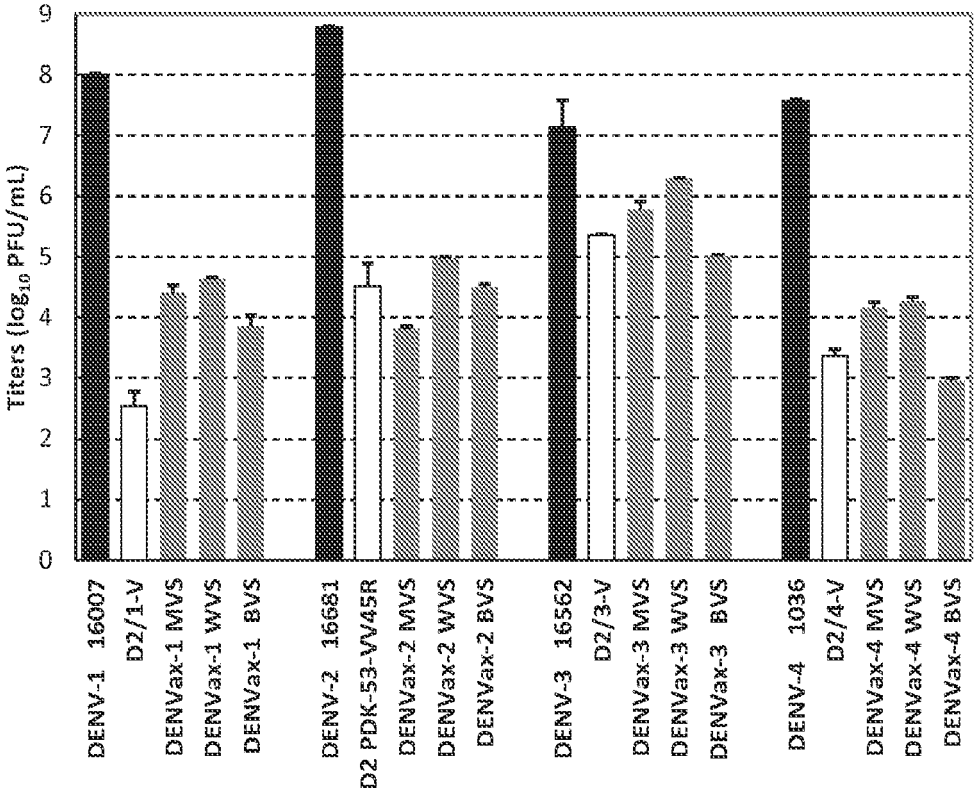

Titers (log_{10} PFU/mL)
0
1
2
3
4
5
6
7
8
9
DENV-1 16007
D2/1-V
DENVax-1 MVS
DENVax-1 WVS
DENVax-1 BVS
DENV-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DENVax-2 WVS
DENVax-2 BVS
DENV-3 16562
D2/3-V
DENVax-3 MVS
DENVax-3 WVS
DENVax-3 BVS
DENV-4 1036
D2/4-V
DENVax-4 MVS
DENVax-4 WVS
DENVax-4 BVS

Fig. 9

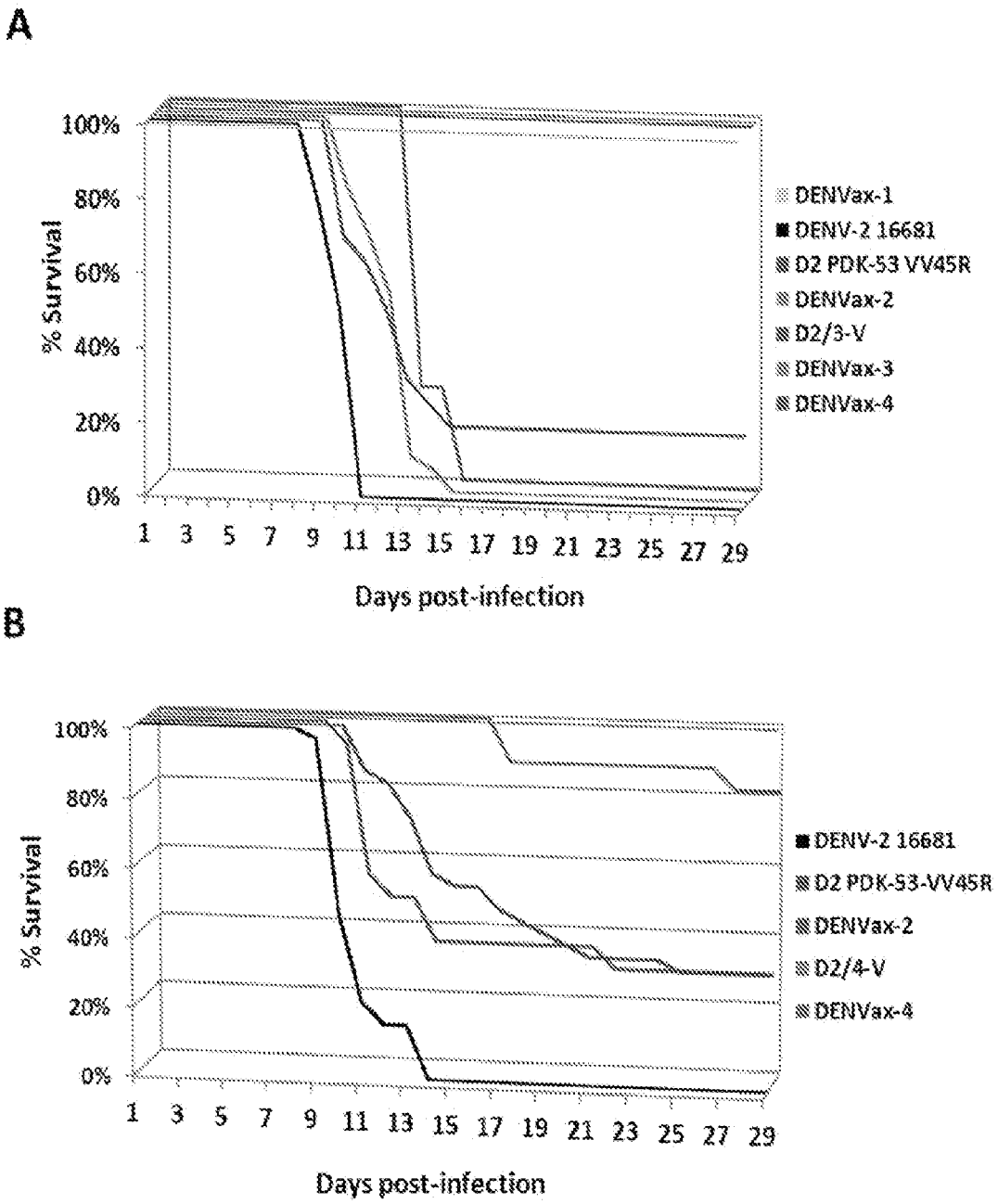

A
100%
80%
60%
40%
20%
0%
% Survival
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
Days post-infection
DENVax-1
DENV-2 16681
D2 PDK-53 VV45R
DENVax-2
D2/3-V
DENVax-3
DENVax-4
B
100%
80%
60%
40%
20%
0%
% Survival
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
Days post-infection
DENV-2 16681
D2 PDK-53-VV45R
DENVax-2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

Fig. 10

Serotype	Strain	virus origin	C671 E/NS3	A2247 prM-V29V	T3004C M (silent)	C3035T E (silent)	G4572A NS1-G20D	C4818T NS2A-L101	A5203 NS2B-E68K	T5347C NS3 (silent)	G6896C NS4A-G75A	C8871T NS5 (silent)
DENV-3	16681	isolate from human	C	A	T	C	G	C	A	T	G	C
	PDK-53	PDK cell pass of 16681	T	T	.	.	A	T	EA mix	.	C	.
	PDK53-V(V345R)	Recombinant PDK-53-V	T	T	.	.	A	T	T	.	C	C
	PDK53-E(T464R)	Recombinant PDK-53-E	T	T	.	.	A	T	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: Different nt sequence between PDK-53 and clone derived V or E virus
* Engineered silent clone number to differentiate original PDK-53 and recombinant (clone-derived) viruses