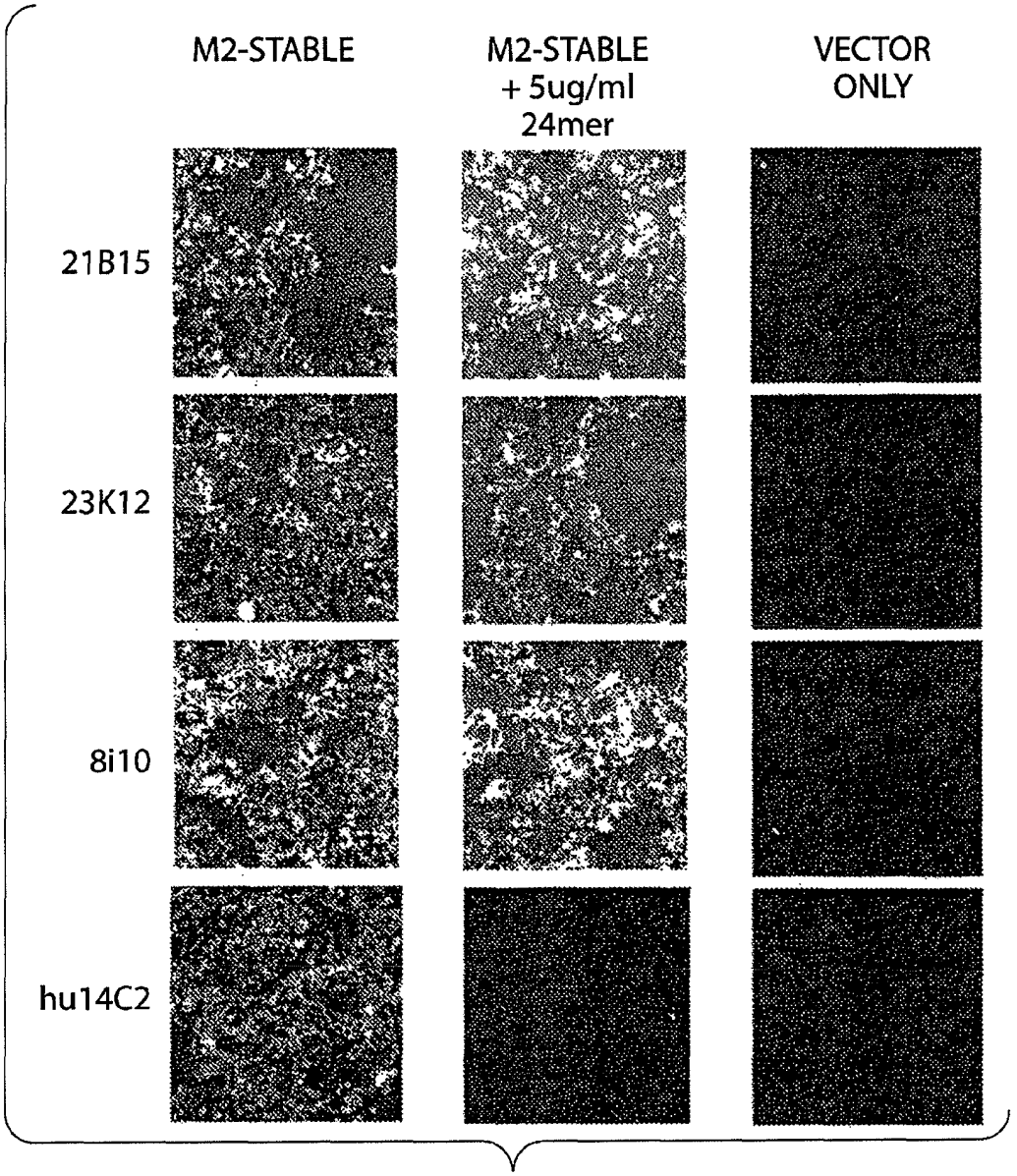

M2-STABLE
M2-STABLE + 5ug/ml 24mer
VECTOR ONLY
21B15
23K12
8i10
hu14C2

FIG. 1

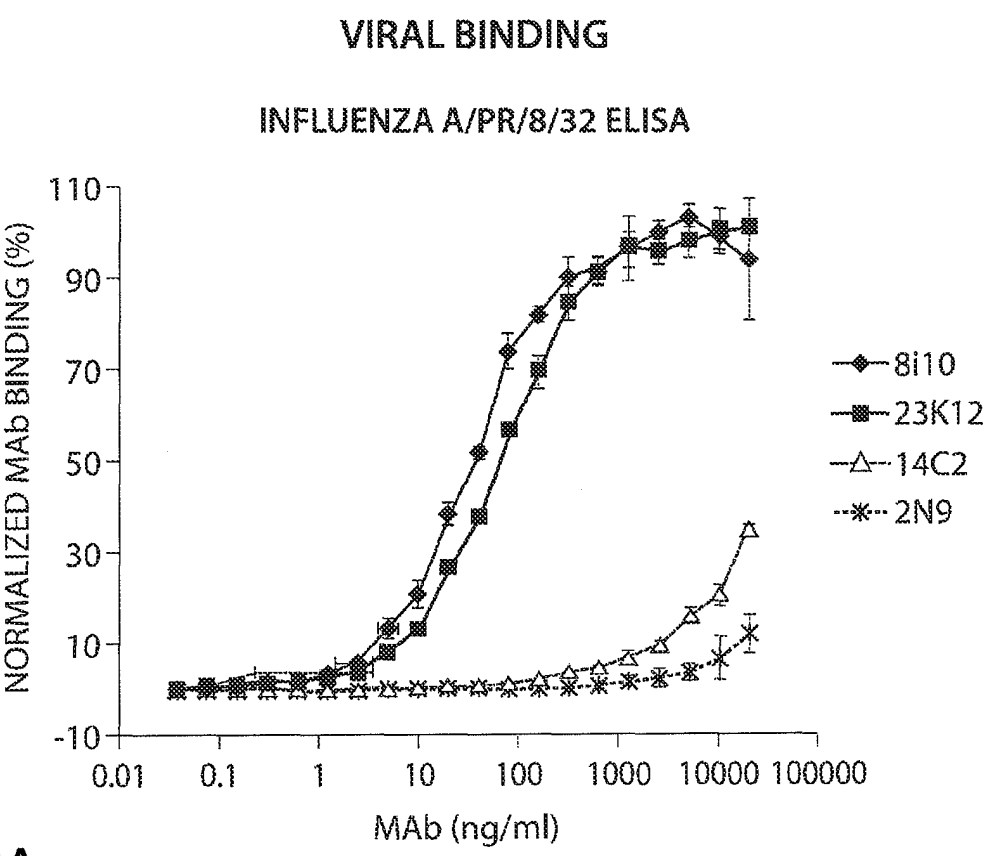

VIRAL BINDING
INFLUENZA A/PR/8/32 ELISA
NORMALIZED MAb BINDING (%)
110
90
70
50
30
10
-10
0.01
0.1
1
10
100
1000
10000
100000
MAb (ng/ml)
8i10
23K12
14C2
2N9

FIG. 2A

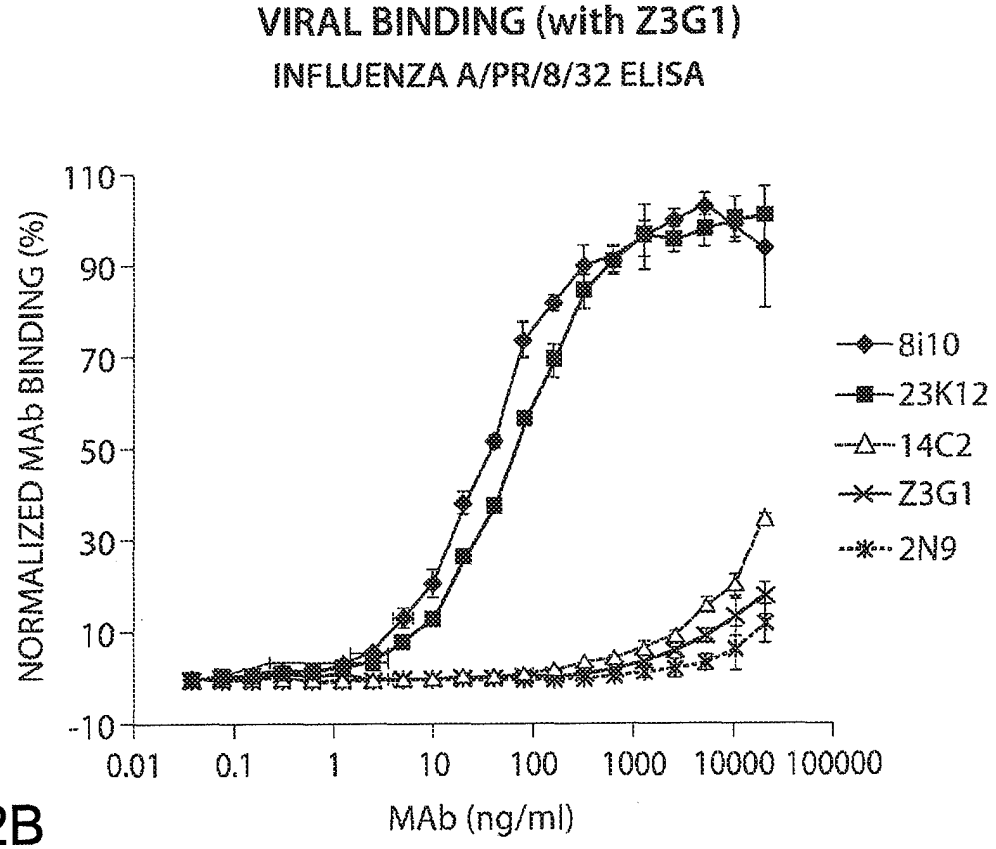

VIRAL BINDING (with Z3G1)
INFLUENZA A/PR/8/32 ELISA
NORMALIZED MAb BINDING (%)
110
90
70
50
30
10
-10
0.01
0.1
1
10
100
1000
10000
100000
MAb (ng/ml)
8i10
23K12
14C2
Z3G1
2N9

FIG. 2B

FULL-LENGTH M2 VARIANT BINDING

AMINO ACID SEQUENCES OF EXTRACELLULAR DOMAINS OF M2 VARIANTS.

										10								20							
1	A.Brevig Mission.1.1918.H1N1	M	S	L	L	T	E	V	E	T	P	T	R	N	E	W	G	C	R	C	N	D	S	S	D
2	A.Fort Monmouth.1.1947.H1N1	M	S	L	L	T	E	V	E	T	P	T	K	N	E	W	E	C	R	C	N	D	S	S	D
3	A.Singapore.02.2005.H3N2	M	S	L	L	T	E	V	E	T	P	I	R	N	E	W	E	C	R	C	N	D	S	S	D
272	A.Wisconsin.10.98.H1N1	M	S	L	L	T	E	V	E	T	P	I	K	N	G	W	E	C	K	C	N	D	S	S	D
5	A.Wisconsin.301.1976.H1N1	M	S	L	L	T	E	V	E	T	P	I	R	S	E	W	G	C	R	C	N	D	S	S	D
6	A.Panama.1.66.H2N2	M	S	F	L	P	E	V	E	T	P	I	R	N	E	W	G	C	R	C	N	D	S	S	D
7	A.New York.321.1999.H3N2	M	S	L	L	T	E	V	E	T	P	I	R	N	E	W	G	C	R	C	N	D	S	S	N
8	A.Caracas.1.71.H3N2	M	S	L	L	T	E	V	E	T	P	I	R	K	E	W	G	C	R	C	N	D	S	S	D
9	A.Taiwan.3.71.H3N2	M	S	F	L	T	E	V	E	T	P	I	R	N	E	W	G	C	R	C	N	D	S	S	D
10	A.Wuhan.359.95.H3N2	M	S	L	P	T	E	V	E	T	P	I	R	S	E	W	G	C	R	C	N	D	S	S	D
11	A.Hong Kong.1144.99.H3N2	M	S	L	L	P	E	V	E	T	P	I	R	N	E	W	G	C	R	C	N	D	S	S	D
12	A.Hong Kong.1180.99 H3N2	M	S	L	L	P	E	V	E	T	P	I	R	N	G	W	G	C	R	C	N	D	S	S	D
13	A.Hong Kong.1774.99 H3N2	M	S	L	L	T	E	V	E	T	P	T	R	N	G	W	E	C	R	C	S	G	S	S	D
14	A.New York.217.02 H1N2	M	S	L	L	T	E	V	E	T	P	I	R	N	E	W	E	Y	R	C	N	D	S	S	D
15	A.New York.300.2003.H1N2	M	S	L	L	T	E	V	E	T	P	I	R	N	E	W	E	Y	R	C	S	D	S	S	D
16	A.swine.Spain.54008.2004.H3N2	M	S	L	L	T	E	V	E	T	P	T	R	N	G	W	E	C	R	Y	S	D	S	S	D
17	A.Guangzhou.333.99.H9N2	M	S	F	L	T	E	V	E	T	L	T	R	N	G	W	E	C	R	C	S	D	S	S	D
18	A.Hong Kong.1073.99.H9N2	M	S	L	L	T	E	V	E	T	L	T	R	N	G	W	E	C	K	C	R	D	S	S	D
19	A.Hong Kong.1.68.H3N2	M	S	L	L	T	E	V	E	T	P	I	R	N	E	W	G	C	R	C	N	D	S	S	D
20	A.swine.Hong Kong.126.1982.H3N2	M	S	L	L	T	E	V	E	T	P	I	R	S	E	W	G	C	R	C	N	D	S	G	D
21	A.New York.703.1995.H3N2	M	S	L	L	T	E	V	E	T	P	I	R	N	E	W	E	C	R	C	N	G	S	S	D
22	A.swine.Quebec.192.81 H1N1	M	S	L	P	T	E	V	E	T	P	I	R	N	E	W	G	C	R	C	N	D	S	S	D
23	A.Puerto Rico.8.34.H1N1	M	S	L	L	T	E	V	E	T	P	I	R	N	E	W	G	C	R	C	N	G	S	S	D
24	A.Hong Kong.485.97.H5N1	M	S	L	L	T	E	V	D	T	L	T	R	N	G	W	G	C	R	C	S	D	S	S	D
25	A.Hong Kong.542.97.H5N1	M	S	L	L	T	E	V	E	T	L	T	K	N	G	W	G	C	R	C	S	D	S	S	D
26	A.silky chicken.Shantou.1826.2004.H	M	S	L	L	T	E	V	E	T	P	T	R	N	G	W	E	C	K	C	S	D	S	S	D
27	A.chicken.Taiwan.0305.04.H6N1	M	S	L	L	T	E	V	E	T	H	T	R	N	G	W	E	C	K	C	S	D	S	S	D
28	A.Quail.Arkansas.16309-7.94.H7N3	M	S	L	L	T	E	V	K	T	P	T	R	N	G	W	E	C	K	C	S	D	S	S	D
29	A.Hong Kong.486.97.H5N1	M	S	L	L	T	E	V	E	T	L	T	R	N	G	W	G	C	R	C	S	D	S	S	D
30	A.Chicken.Pennsylvania.13552-1.98	M	S	L	L	T	E	V	E	T	P	T	R	D	G	W	E	C	K	C	S	D	S	S	D
31	A.chicken.Heilongjiang.48.01.H9N2	M	S	L	L	T	E	V	E	T	P	T	R	N	G	W	G	C	R	C	S	D	S	S	D
32	A.swine.Korea.S5.2005.H1N2	M	S	L	L	T	E	V	E	T	P	T	R	N	G	W	E	C	K	C	N	D	S	S	D
33	A.Hong Kong.1073.99.H9N2	M	S	L	L	T	E	V	E	T	L	T	R	N	G	W	E	C	K	C	S	D	S	S	D
34	A.Wisconsin.3523.88.H1N1	M	S	L	L	T	E	V	E	T	P	I	R	N	E	W	G	C	K	C	N	D	S	S	D
35	A.X-31 Vaccine strain.H3N2	M	S	F	L	T	E	V	E	T	P	I	R	N	E	W	G	C	R	C	N	G	S	S	D
36	A.Chicken.Rostock.8.1934.H7N1	M	S	L	L	T	E	V	E	T	P	T	R	N	G	W	E	C	R	C	N	D	S	S	D
37	A.environment.New York. 16326-1.2	M	S	L	L	T	E	V	E	T	P	I	R	K	G	W	E	C	N	C	S	D	S	S	D
38	A.Indonesia.560H.2006.H5N1	M	S	L	L	T	E	V	E	T	P	T	R	N	E	W	E	C	R	C	S	D	S	S	D
39	A.Chicken.Hong Kong.SF1.03.H9N2	M	S	L	L	T	G	V	E	T	H	T	R	N	G	W	G	C	K	C	S	D	S	S	D
40	A.chicken.Hong Kong YU427.03.H9N	M	S	L	L	P	E	V	E	T	H	T	R	N	G	W	G	C	R	C	S	D	S	S	D

EXTRACELLULAR SEQUENCE OF D20 IS IDENTICAL
TO #19, HK483 TO #29, AND VN1203 TO #38.

Fig. 3A

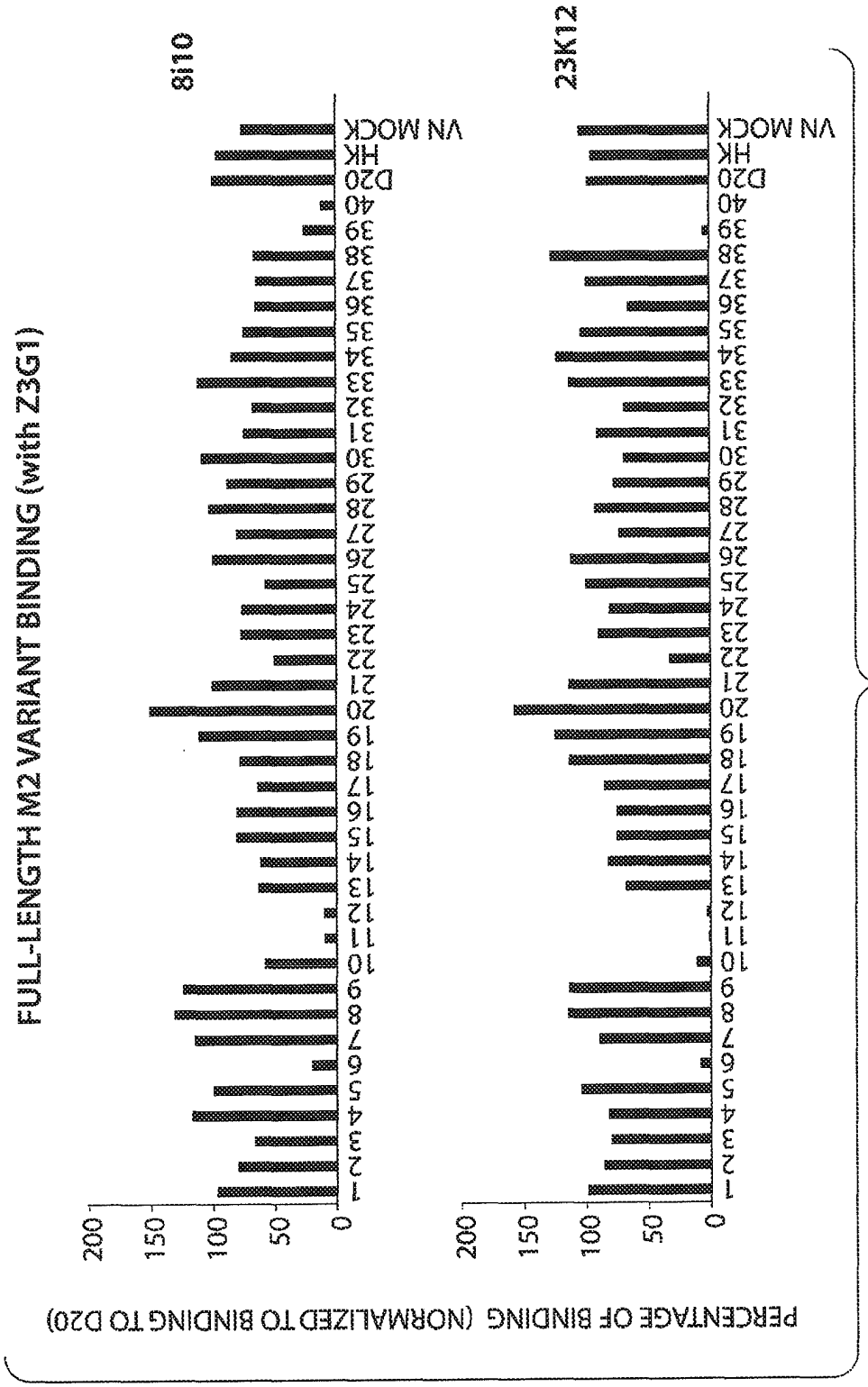

FULL-LENGTH M2 VARIANT BINDING (with Z3G1)
8i10
23K12
PERCENTAGE OF BINDING (NORMALIZED TO BINDING TO D20)
200
150
100
50
0
VN MOCK
HK
D20
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1

FIG. 3B-1

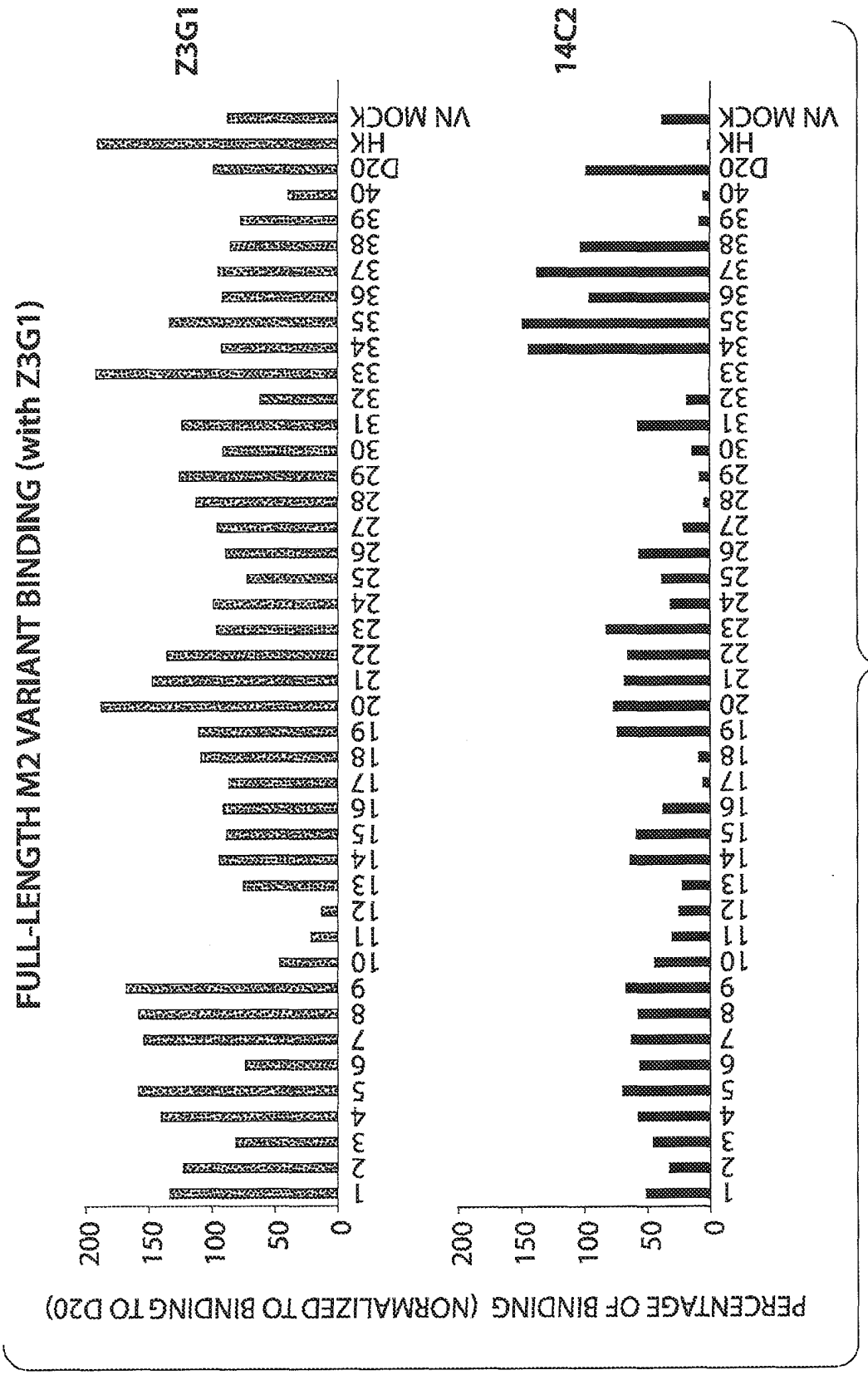

FULL-LENGTH M2 VARIANT BINDING (with Z3G1)
Z3G1
14C2
PERCENTAGE OF BINDING (NORMALIZED TO BINDING TO D20)
0
50
100
150
200
VN MOCK
HK
D20
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1

FIG. 3B-2

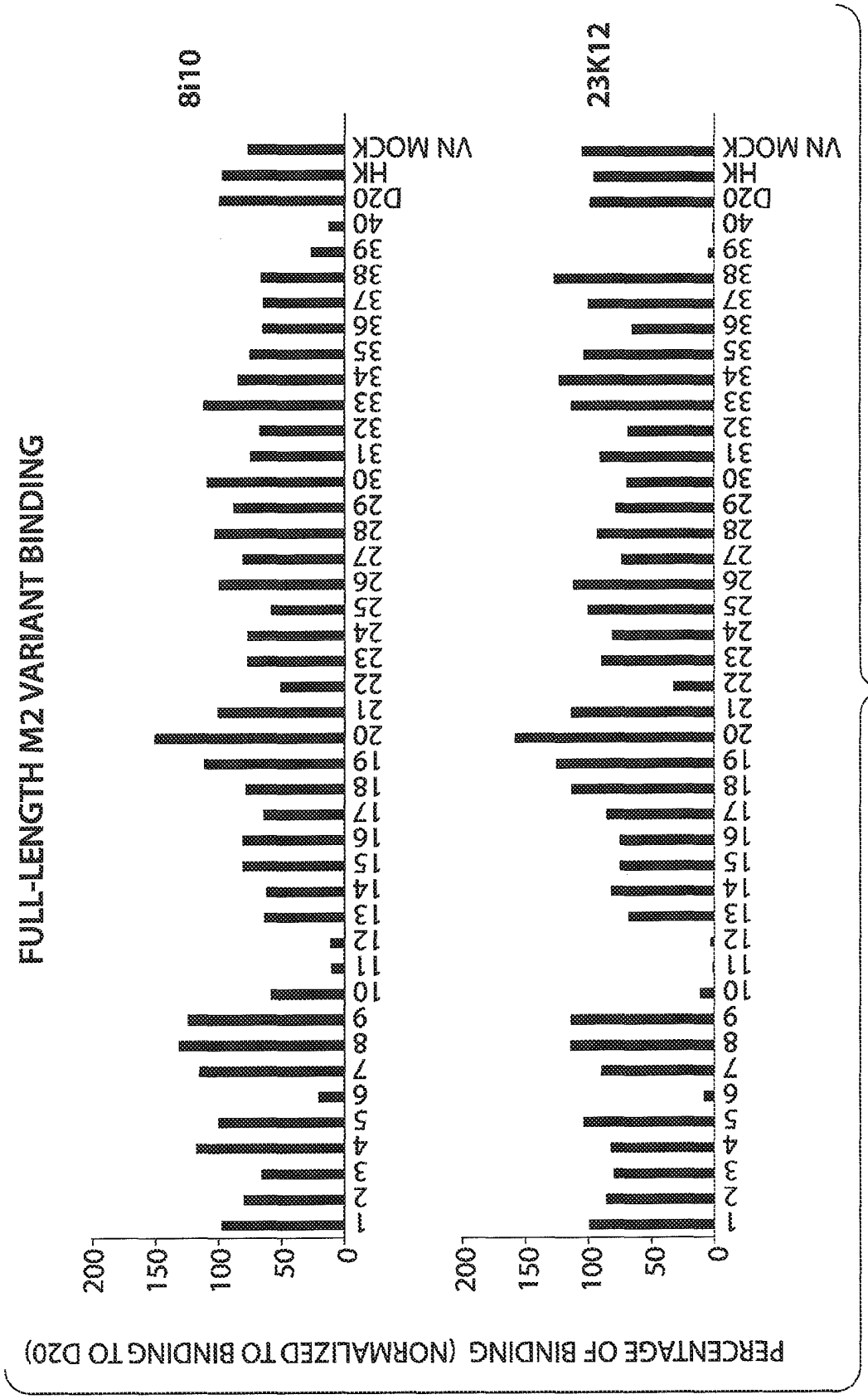

FULL-LENGTH M2 VARIANT BINDING
PERCENTAGE OF BINDING (NORMALIZED TO BINDING TO D20)
8i10
23K12
200
150
100
50
0
VN MOCK
HK
D20
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40

FIG. 3C-1

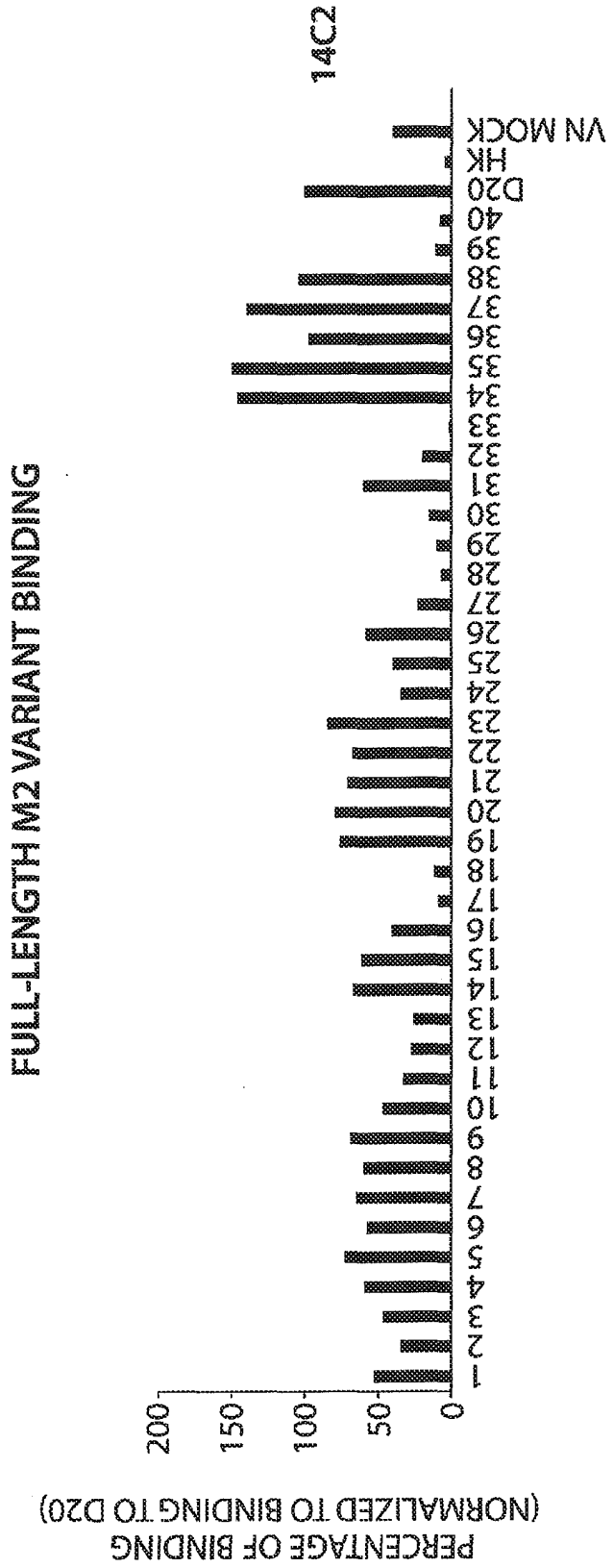

FULL-LENGTH M2 VARIANT BINDING
14C2
VN MOCK
HK
D20
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
50
100
150
200
PERCENTAGE OF BINDING
(NORMALIZED TO BINDING TO D20)

FIG. 3C-2

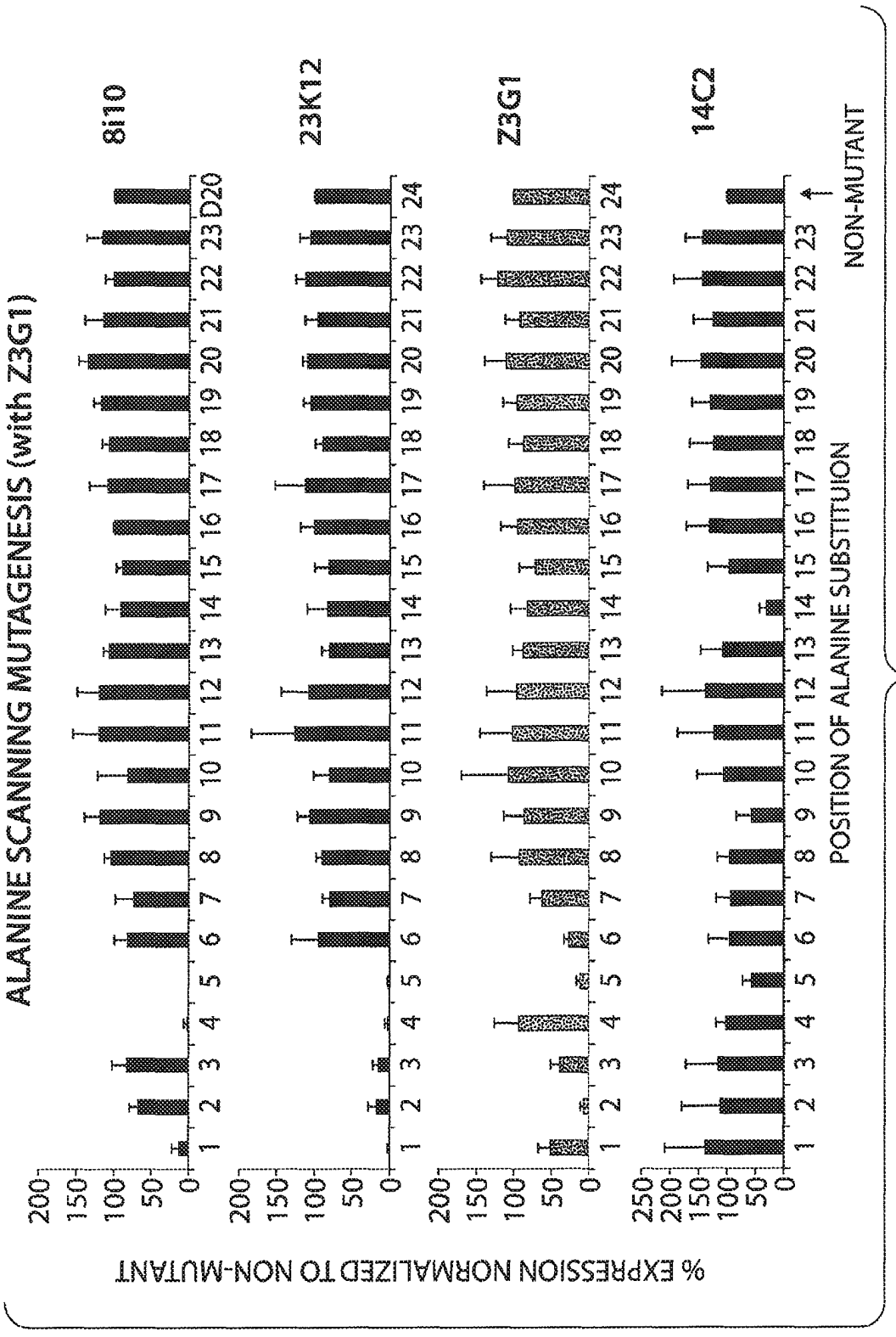

ALANINE SCANNING MUTAGENESIS (with Z3G1)
8i10
23K12
Z3G1
14C2
% EXPRESSION NORMALIZED TO NON-MUTANT
POSITION OF ALANINE SUBSTITUION
NON-MUTANT

FIG. 4A

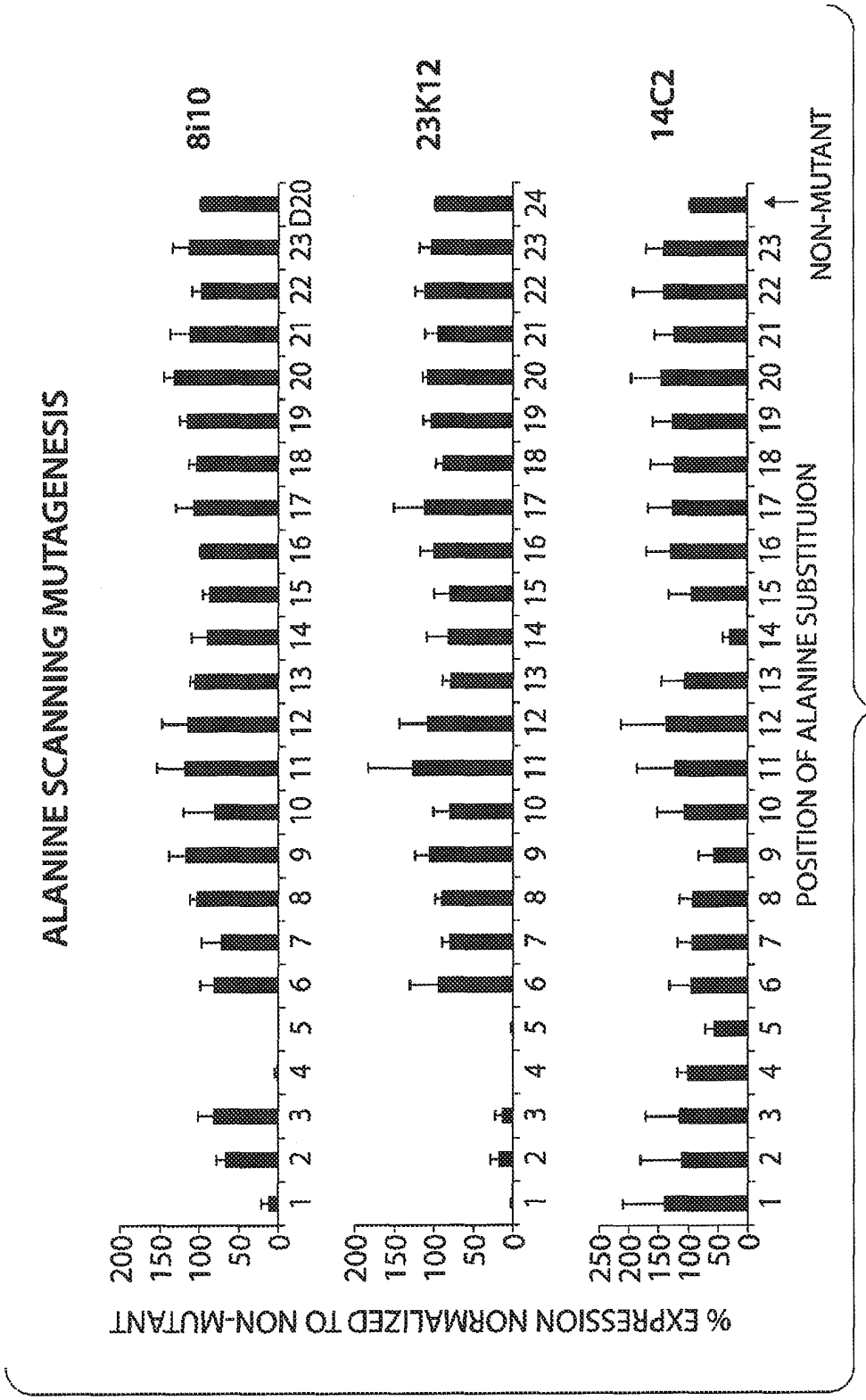

ALANINE SCANNING MUTAGENESIS
8i10
23K12
14C2
% EXPRESSION NORMALIZED TO NON-MUTANT
POSITION OF ALANINE SUBSTITUION
NON-MUTANT
D20

FIG. 4B

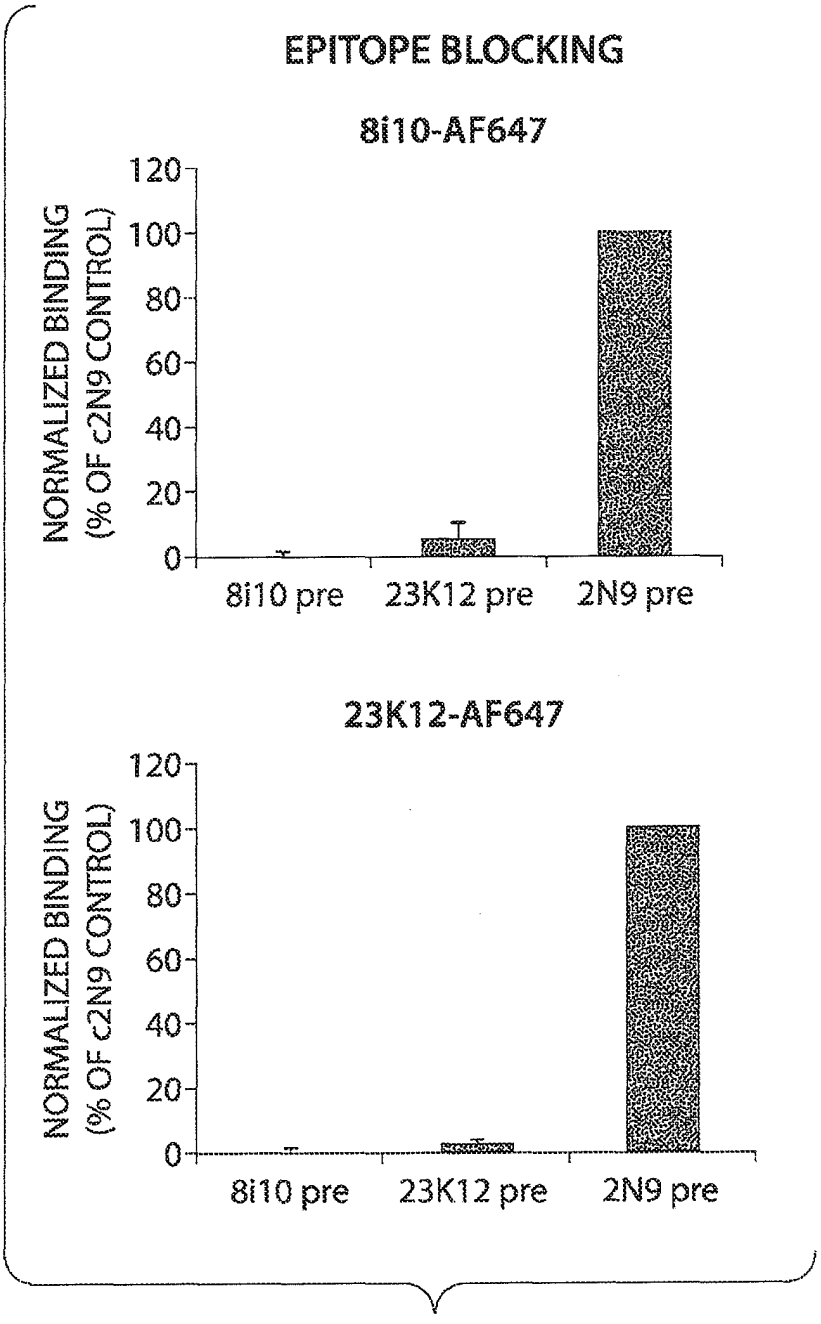

EPITOPE BLOCKING
8i10-AF647
NORMALIZED BINDING
(% OF c2N9 CONTROL)
120
100
80
60
40
20
0
8i10 pre
23K12 pre
2N9 pre
23K12-AF647
NORMALIZED BINDING
(% OF c2N9 CONTROL)
120
100
80
60
40
20
0
8i10 pre
23K12 pre
2N9 pre

FIG. 5

CROSS REACTIVITY BINDING OF ANTI-M2 ANTIBODIES TO VARIANT M2 PEPTIDES

				ELISA (OD 450)			
seqNo	Name	Size	Description	14C2	8i10	23K12	2N9
273	M2	23 aa	SLLTEVETPIRNEWGCRCNDSSD	+	-	-	-
274	M2SG	23 aa	SLLTEVETPIRSEWGCRCNDSGD	+	-	-	-
275	M2EG	23 aa	SLLTEVETPIRNEWECRCNGSSD	+	-	-	-
276	M2P	23 aa	SLPTEVETPIRNEWGCRCNDSSD	+	-	-	-
277	M2G	23 aa	SLLTEVETPIRNEWGCRCNGSSD	+	-	-	-
278	M2DLTGS	23 aa	SLLTEVDTLTRNGWGCRCSDSSD	-	-	+	-
279	M2KNS	23 aa	SLLTEVETPIRKEWGCNCSDSSD	+	-	-	-
280	M2LGS	23 aa	SLLTEVETLIRNGWGCRCSDSSD	-	-	-	-
281	M2LTKGS	23 aa	SLLTEVETLTKNGWGCRCSDSSD	-	-	-	-
282	M2SY	23 aa	SLLTEVETPIRSEWGCRYNDSSD	+	-	-	-
283	M2TGEKS	23 aa	SLLTEVETPTRNGWECKCSDSSD	+	-	-	-
284	M2HTGEKS	23 aa	SLLTEVETHTRNGWECKCSDSSD	-	-	-	-
285	M2KTGEKS	23 aa	SLLTEVKTPTRNGWECKCSDSSD	-	-	-	-
286	M2LTGS	23 aa	SLLTEVETLTRNGWGCRCSDSSD	-	-	+	-
287	M2TDGEKS	23 aa	SLLTEVETPTRDGWECKCSDSSD	+	-	-	-
288	M2TGS	23 aa	SLLTEVETPTRNGWGCRCSDSSD	+	-	W	-
289	M2TGEK	23 aa	SLLTEVETPTRNGWECKCNDSSD	+	-	-	-
290	M2LTGEKS	23 aa	SLLTEVETLTRNGWECKCSDSSD	-	-	W	-
291	M2K	23 aa	SLLTEVETPIRNEWGCKCNDSSD	+	W	+	-
292	M2FG	23 aa	SFLTEVETPIRNEWGCRCNGSSD	+	W	-	-
293	M2TGE	23 aa	SLLTEVETPTRNGWECRCNDSSD	+	-	-	-
294	M2KGENS	23 aa	SLLTEVETPIRKGWECNCSDSSD	+	-	-	-
295	M2TES	23 aa	SLLTEVETPTRNEWECRCSDSSD	+	-	-	-
296	M2GHTGKS	23 aa	SLLTGVETHTRNGWGCKCSDSSD	-	-	-	-
297	M2PHTGS	23 aa	SLLPEVETHTRNGWGCRCSDSSD	-	-	-	-

PERCENTAGE COMPARED RELATIVE TO BINDING TO WILD-TYPE PEPTIDE (Seq 1) NOTE: mAbs WERE TESTED AT 5 µg/mL

>25 %	-	NO BINDING
25 - 40 %	W	WEAK BINDING
> 40 %	+	POSITIVE BINDING

Fig. 6A

BINDING ACTIVITY OF M2 ANTIBODIES TO TRUNCATED M2 PEPTIDES

seqNo	Name	Size	Description	14C2	8i10	23K12	2N9
273	M2	23 aa	SLLTEVETPIRNEWGCRCNDSSD	3.85	0.11	0.22	0.06
298	M16	16 aa	LLTEVETPIRNEWGCR	3.94	0.09	0.21	0.09
299	M15	15 aa	LTEVETPIRNEWGCR	3.95	0.09	0.21	0.09
300	M12	12 aa	VETPIRNEWGCR	0.15	0.09	0.20	0.09
301	CM17	17 aa	ETPIRNEWGCRCNDSSD	0.19	0.11	0.34	0.11
302	CM16	16 aa	TPIRNEWGCRCNDSSD	0.23	0.13	0.35	0.12
303	CM15	15 aa	PIRNEWGCRCNDSSD	0.19	0.12	0.34	0.11
304	CM14	14 aa	IRNEWGCRCNDSSD	0.23	0.14	0.36	0.13
305	CM13	13 aa	RNEWGCRCNDSSD	0.22	0.14	0.34	0.13
306	CM12	12 aa	NEWGCRCNDSSD	0.27	0.14	0.39	0.14
307	NM17	17 aa	SLLTEVETPIRNEWGCR	3.99	0.26	0.58	0.10
308	NM16	16 aa	SLLTEVETPIRNEWGC	3.90	0.29	0.62	0.09
309	NM15	15 aa	SLLTEVETPIRNEWG	3.97	0.12	0.30	0.11
310	NM14	14 aa	SLLTEVETPIRNEW	3.97	0.11	0.24	0.09
311	NM13	13 aa	SLLTEVETPIRNE	0.18	0.11	0.25	0.10
312	NM12	12 aa	SLLTEVETPIRN	0.20	0.10	0.24	0.09
313	NM11	11 aa	SLLTEVETPIR	0.21	0.13	0.30	0.12
314	NM10	10 aa	SLLTEVETPI	0.17	0.10	0.24	0.10
315	NM8	8 aa	SLLTEVET	0.15	0.10	0.20	0.09
316	NM7	7 aa	SLLTEVE	0.14	0.10	0.20	0.08
271	NM9	9 aa	SLLTEVETP	0.21	0.12	0.30	0.19
1	M2e	24 aa	MSLLTEVETPIRNEWGCRCNDSSD	3.98	0.13	0.43	0.10
CMV	HVIR1			0.16	0.11	0.21	3.99

NOTE: mAbs WERE TESTED AT 5 µg/mL

Fig. 6B

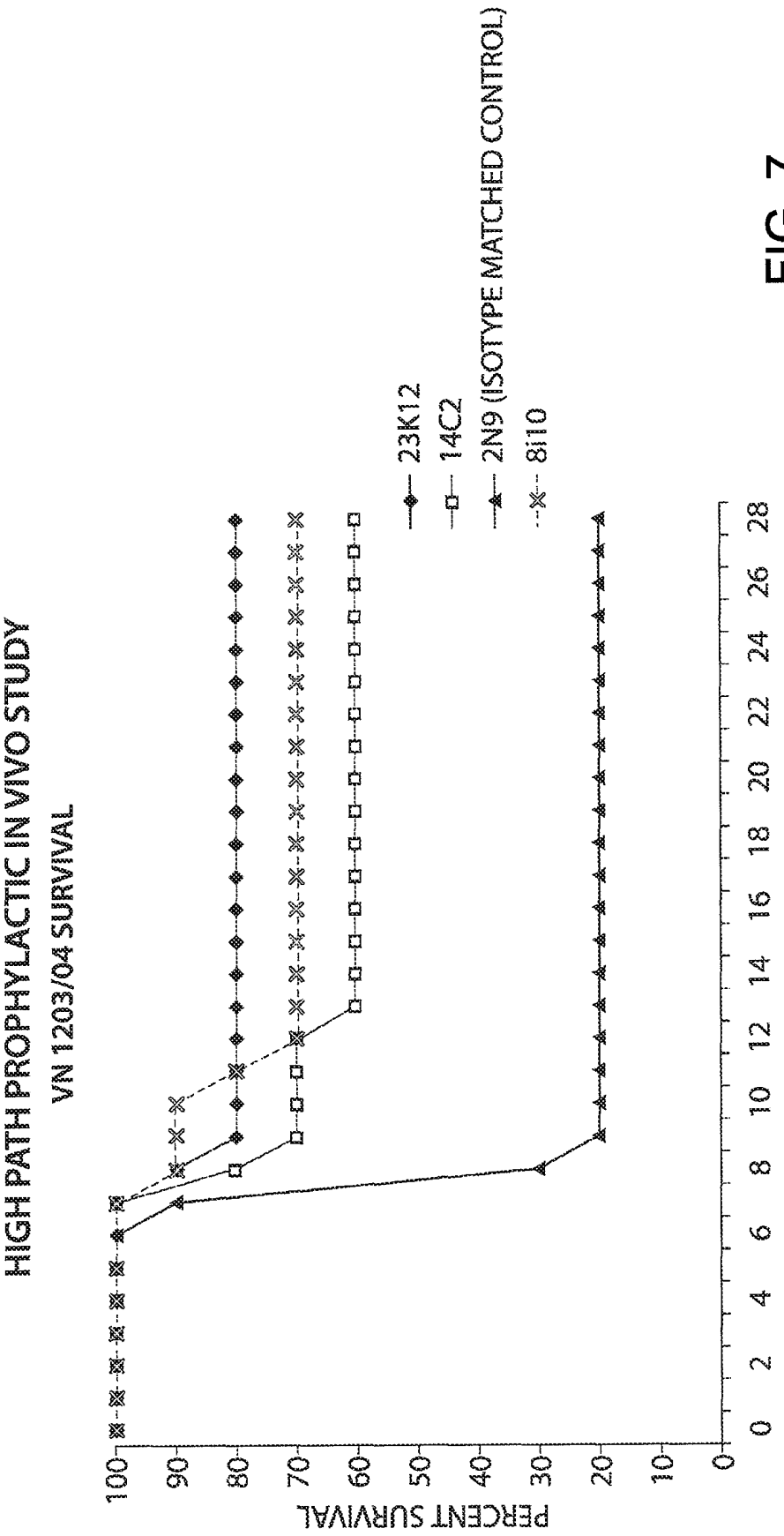

HIGH PATH PROPHYLACTIC IN VIVO STUDY
VN 1203/04 SURVIVAL
PERCENT SURVIVAL
DAYS POST INFECTION
23K12
14C2
2N9 (ISOTYPE MATCHED CONTROL)
8i10
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28

FIG. 7

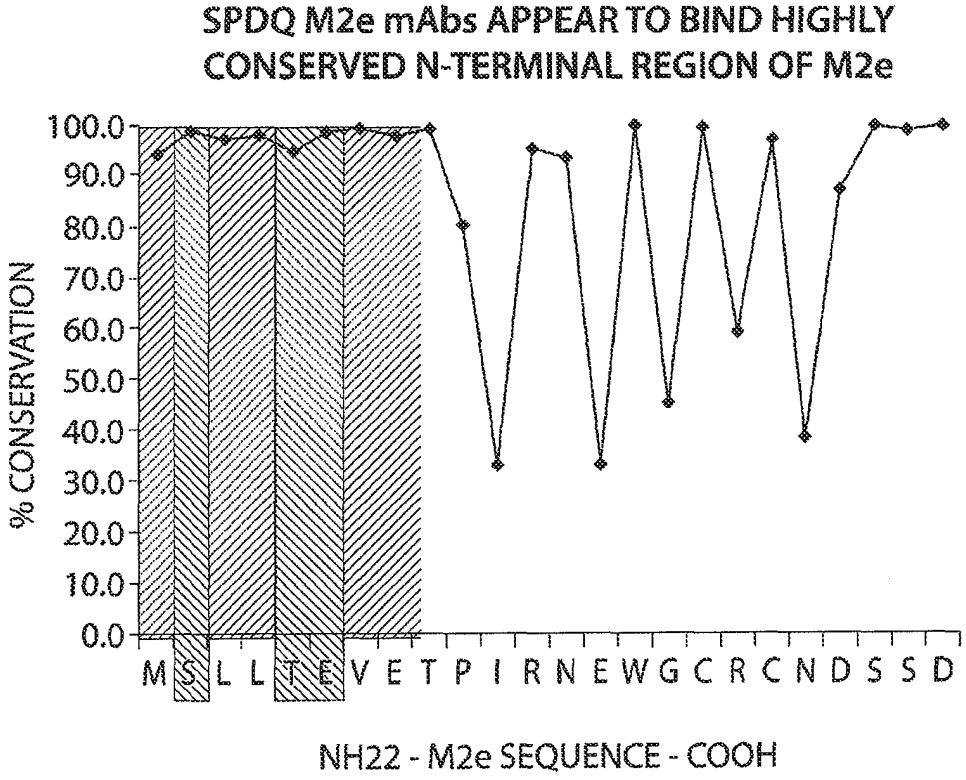

SPDQ M2e mAbs APPEAR TO BIND HIGHLY CONSERVED N-TERMINAL REGION OF M2e
% CONSERVATION
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
M S L L T E V E T P I R N E W G C R C N D S S D
NH22 - M2e SEQUENCE - COOH

FIG. 8

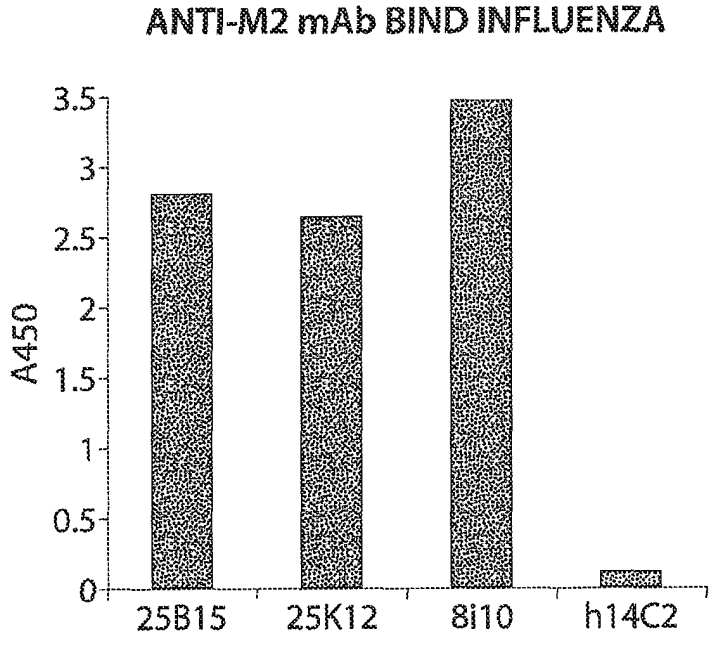

ANTI-M2 mAb BIND INFLUENZA
A450
3.5
3
2.5
2
1.5
1
0.5
0
25B15
25K12
8i10
h14C2

FIG. 9

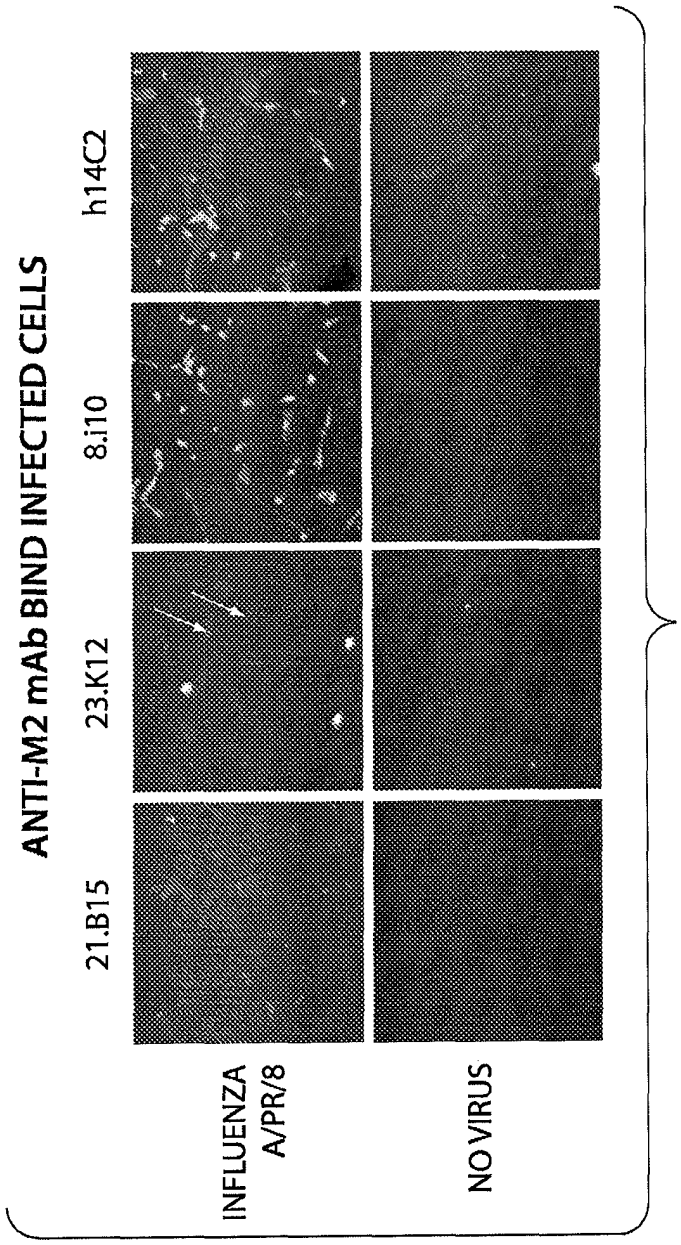

ANTI-M2 mAb BIND INFECTED CELLS
21.B15
23.K12
8.i10
h14C2
INFLUENZA A/PR/8
NO VIRUS

FIG. 10

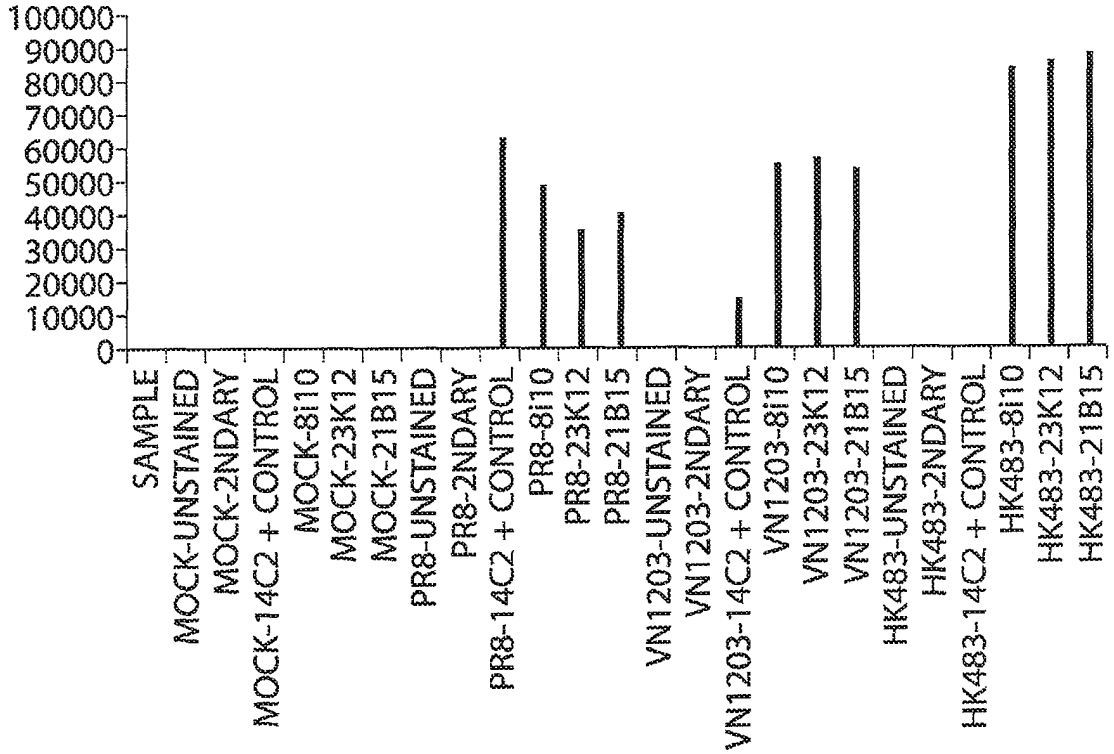

100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
SAMPLE
MOCK-UNSTAINED
MOCK-2NDARY
MOCK-14C2 + CONTROL
MOCK-8i10
MOCK-23K12
MOCK-21B15
PR8-UNSTAINED
PR8-2NDARY
PR8-14C2 + CONTROL
PR8-8i10
PR8-23K12
PR8-21B15
VN1203-UNSTAINED
VN1203-2NDARY
VN1203-14C2 + CONTROL
VN1203-8i10
VN1203-23K12
VN1203-21B15
HK483-UNSTAINED
HK483-2NDARY
HK483-14C2 + CONTROL
HK483-8i10
HK483-23K12
HK483-21B15

FIG. 11

FIG. 12A

Heavy Chain

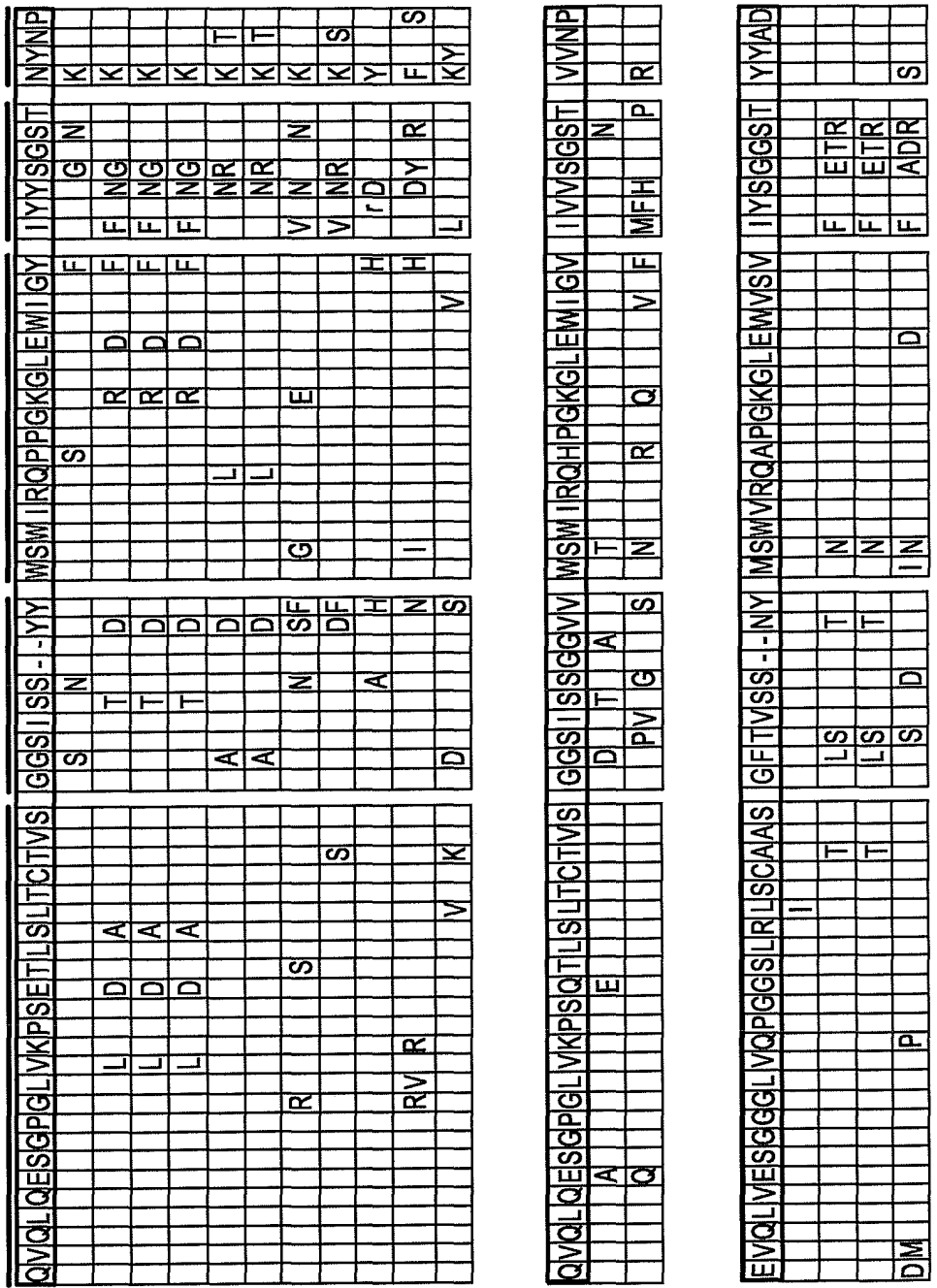


FIG. 12A (continued)

Heavy Chain

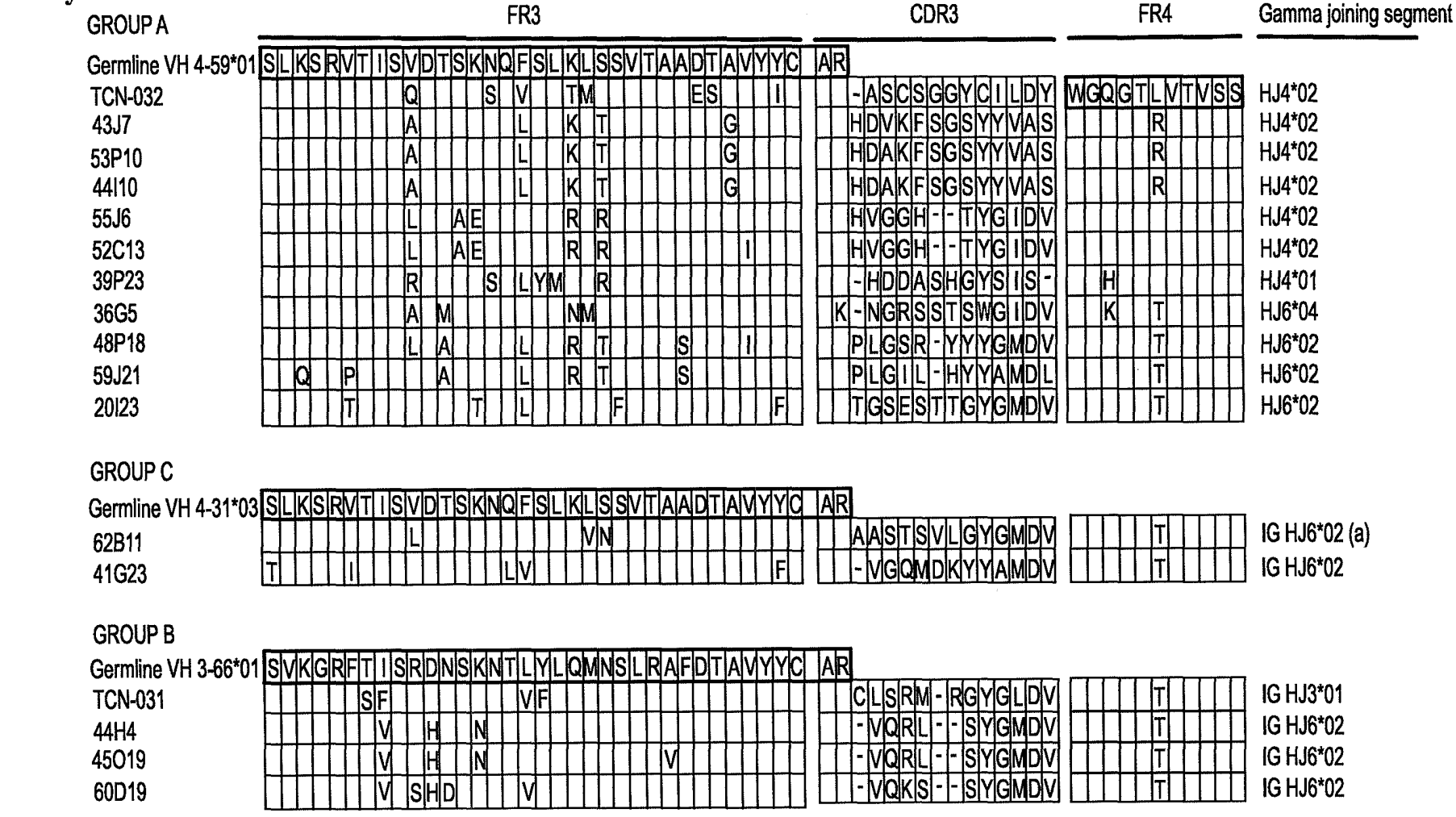


FIG. 12B
Kappa Chain

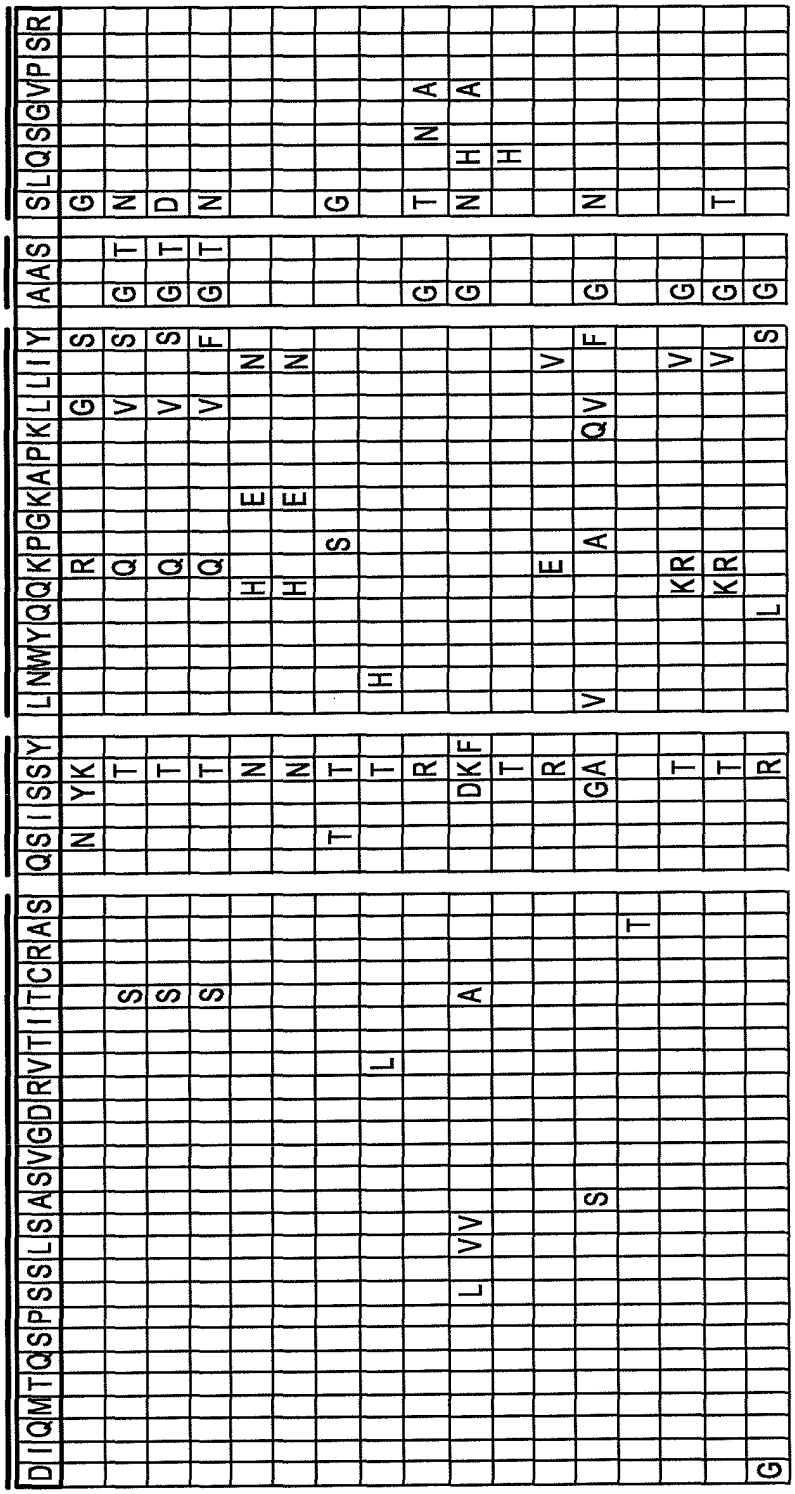


FIG. 12B (continued)

	FR3																							CDR3							FR4									Kappa joining segment
Germline Vkappa 1-39*01	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	Q	S	Y	S	T	P			F	G	G	G	T	R	V	E	I	K	
TCN-032															T																	P		L	T											KJ4*01
43J7																															N			I			Q		K				KJ2*01 or KJ2*02 or KJ2*03 or KJ2*04
53P10																															N			I			Q		K	L			KJ2*01 or KJ2*02 or KJ2*03 or KJ2*04
44I10																															N			I			Q		K	L			KJ2*01 or KJ2*02 or KJ2*03 or KJ2*04
55J6		A								S			G																		N			I			P			L			KJ5*01
52C13		A																													N			I			Q			L			KJ5*01
39P23																											F				N						Q		K				KJ1*01
36G5				R																D											P						P		K		D	M	KJ3*01
48P18																						S				L					V		A	-				K			V	KJ4*01
59J21		A											T	N	I		T									L				F	V		A	-				K				KJ4*01
20I23									A																						P			I			Q			L			KJ5*01
62B11																																					Q			L			KJ5*01
41G23																											F			T				I			Q			L			KJ5*01
23K12															G																	M		A	-			Q		K	L			KJ2*01 or KJ*02
44H4														A								S		V					T		I			-			Q			L			KJ5*01
45O19														A								S							T		I			-			Q			L			KJ5*01
60D19				T					E																				T	F		I			-			Q		K	L			KJ2*01 or KJ2*02 or KJ2*03 or KJ2*04 (a)

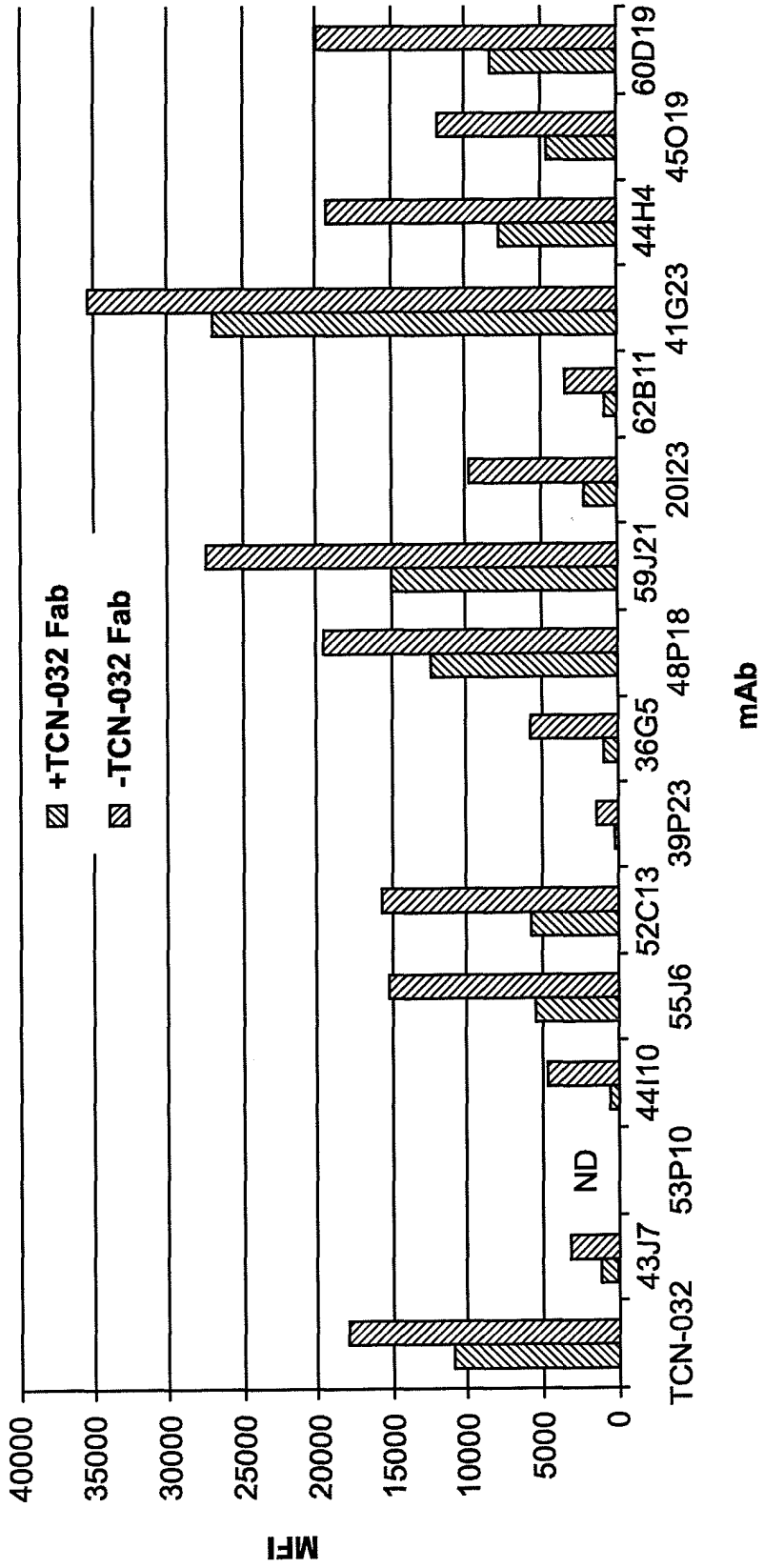

+TCN-032 Fab
-TCN-032 Fab
MFI
40000
35000
30000
25000
20000
15000
10000
5000
0
TCN-032
43J7
53P10
ND
44I10
55J6
52C13
39P23
36G5
48P18
59J21
20I23
62B11
41G23
44H4
45O19
60D19
mAb

FIG. 13

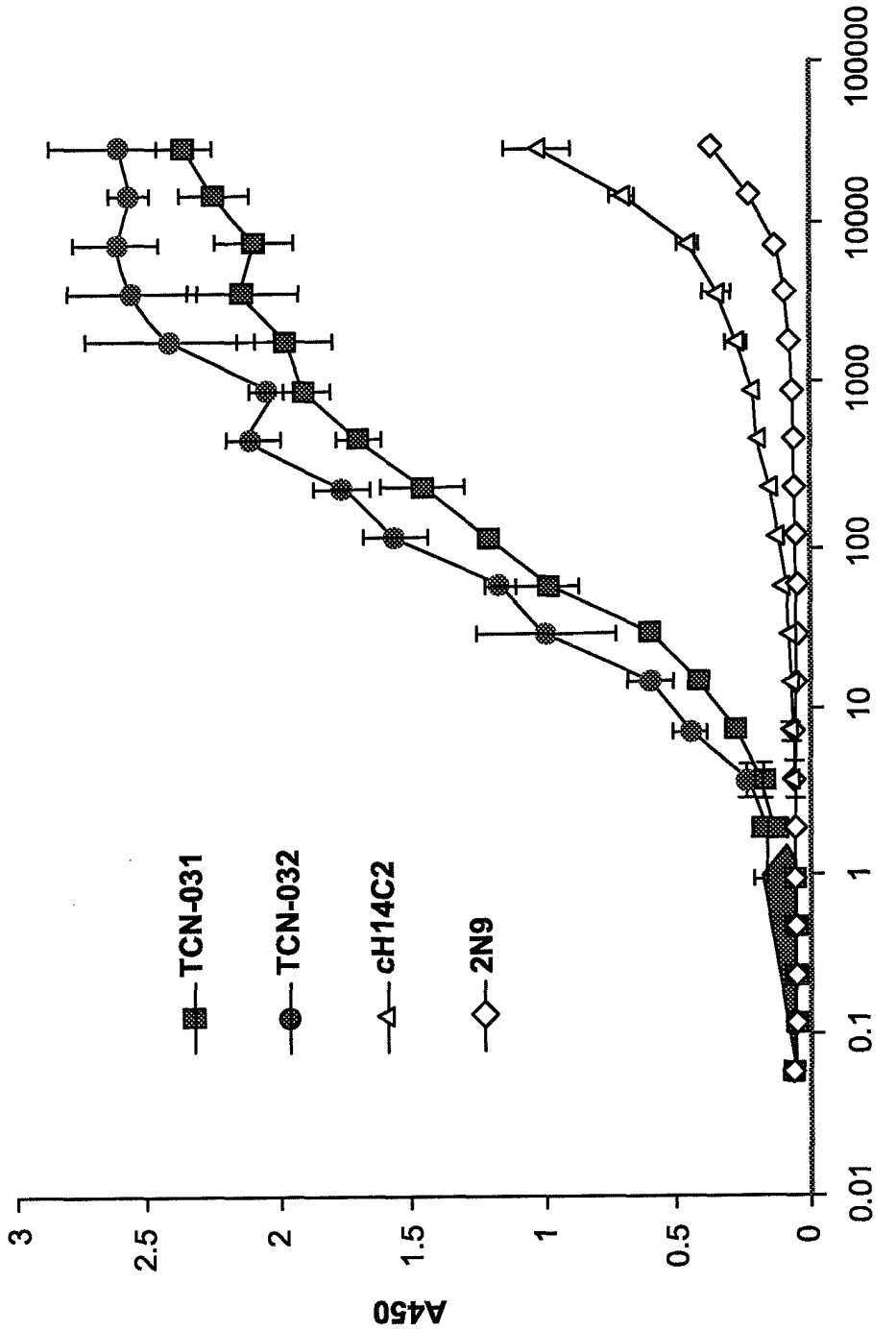

A450
3
2.5
2
1.5
1
0.5
0
0.01
0.1
1
10
100
1000
10000
100000
mAb(ng/ml)
TCN-031
TCN-032
cH14C2
2N9

FIG. 14A

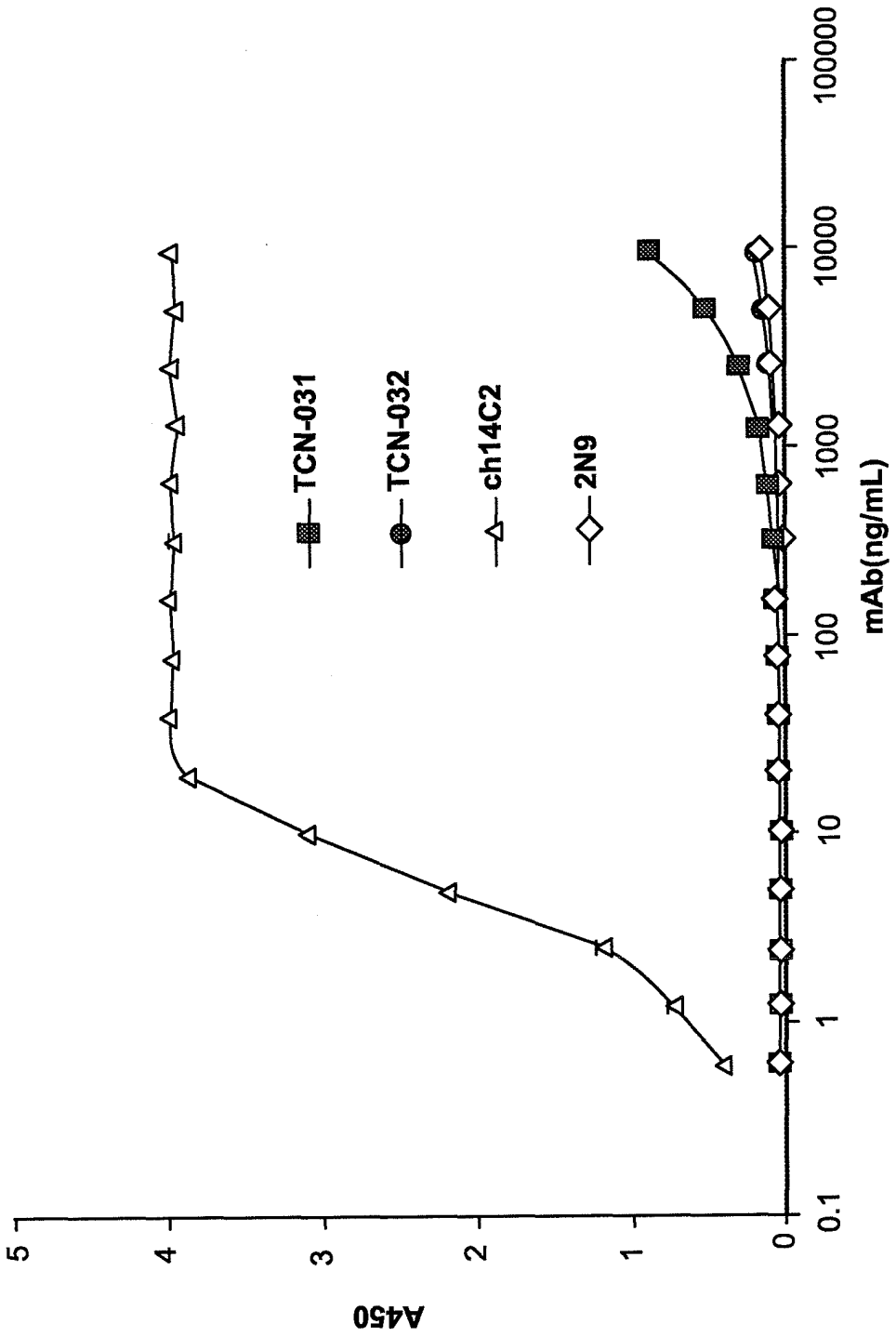

A450
0
1
2
3
4
5
0.1
1
10
100
1000
10000
100000
mAb(ng/mL)
TCN-031
TCN-032
ch14C2
2N9

FIG. 14B

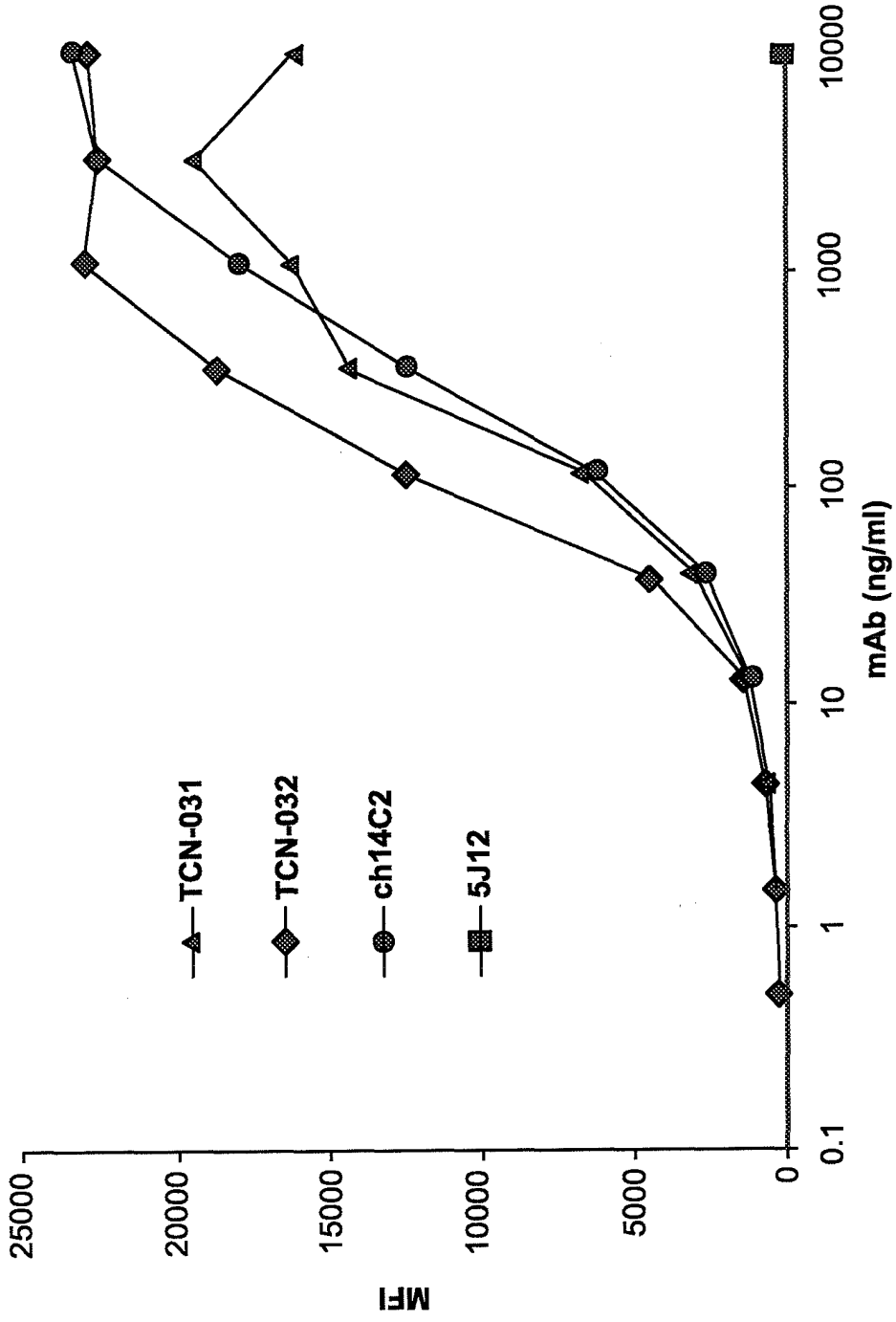

MFI
25000
20000
15000
10000
5000
0
0.1
1
10
100
1000
10000
mAb (ng/ml)
TCN-031
TCN-032
ch14C2
5J12

FIG. 14C

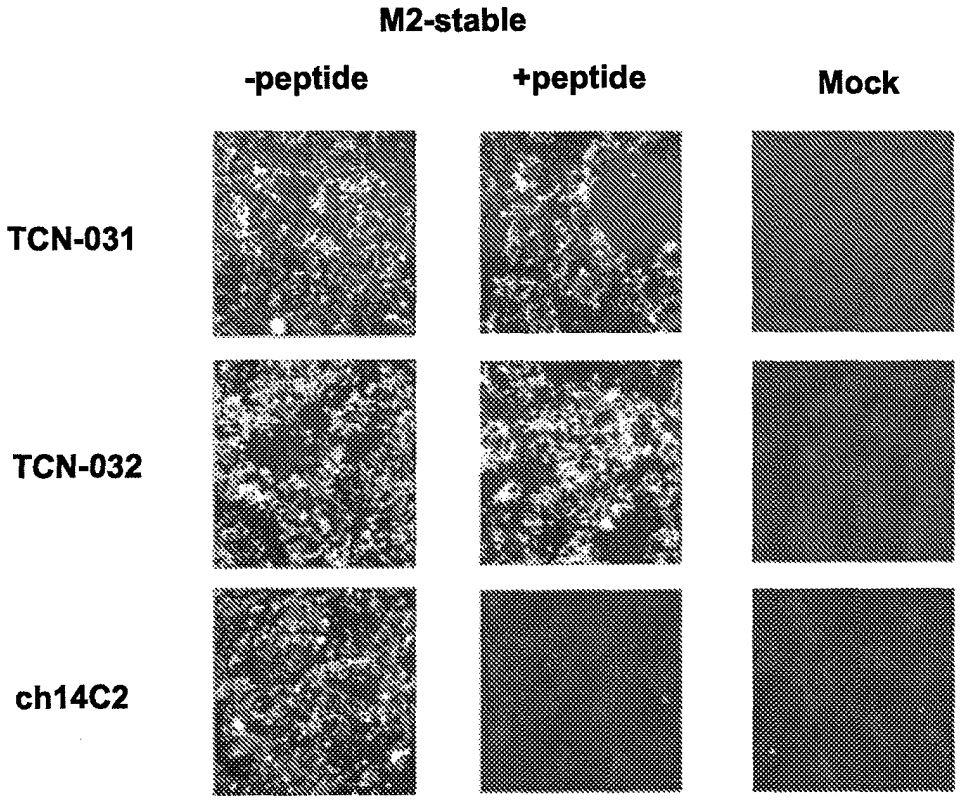


FIG. 14D

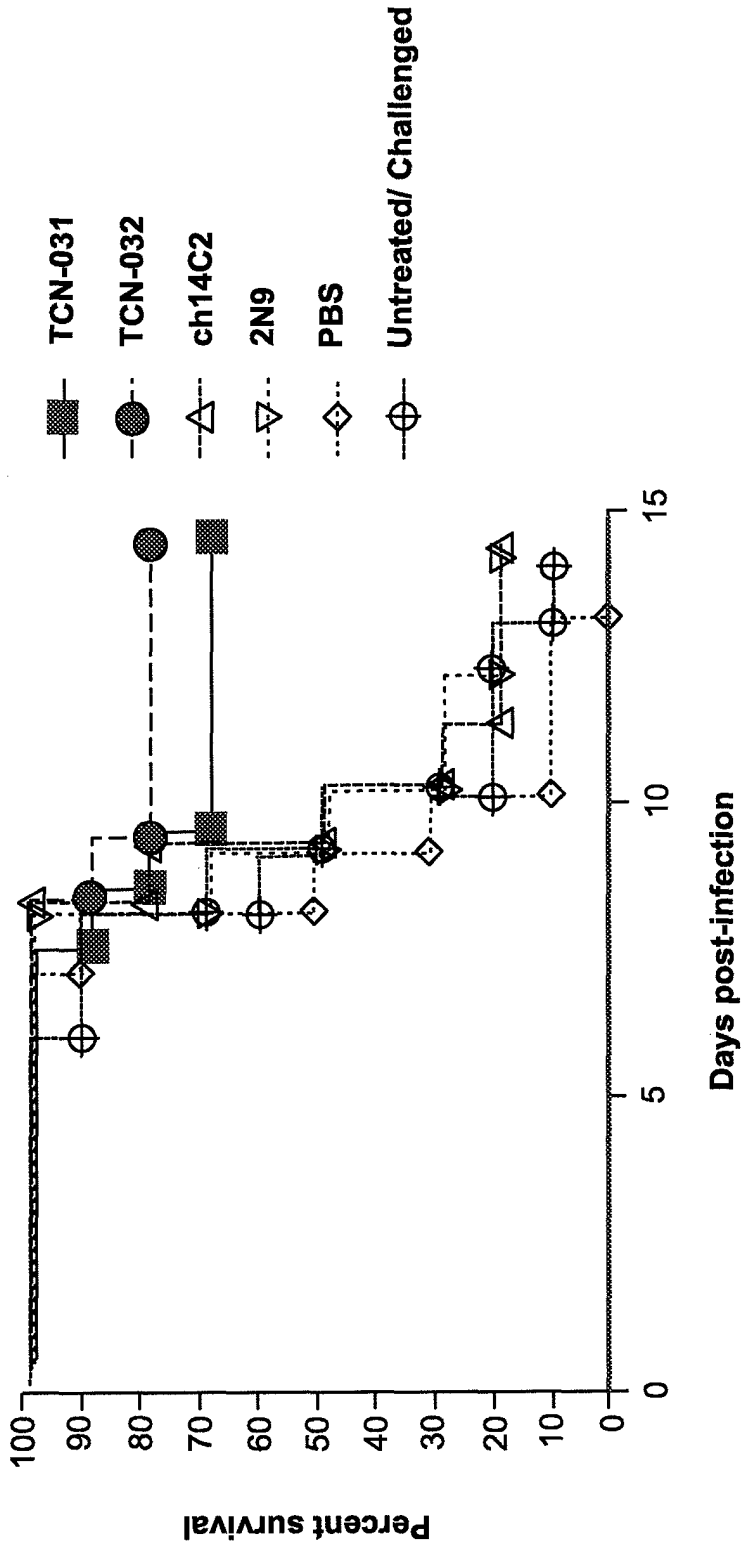

TCN-031
TCN-032
ch14C2
2N9
PBS
Untreated/ Challenged
Percent survival
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
Days post-infection

FIG. 15A

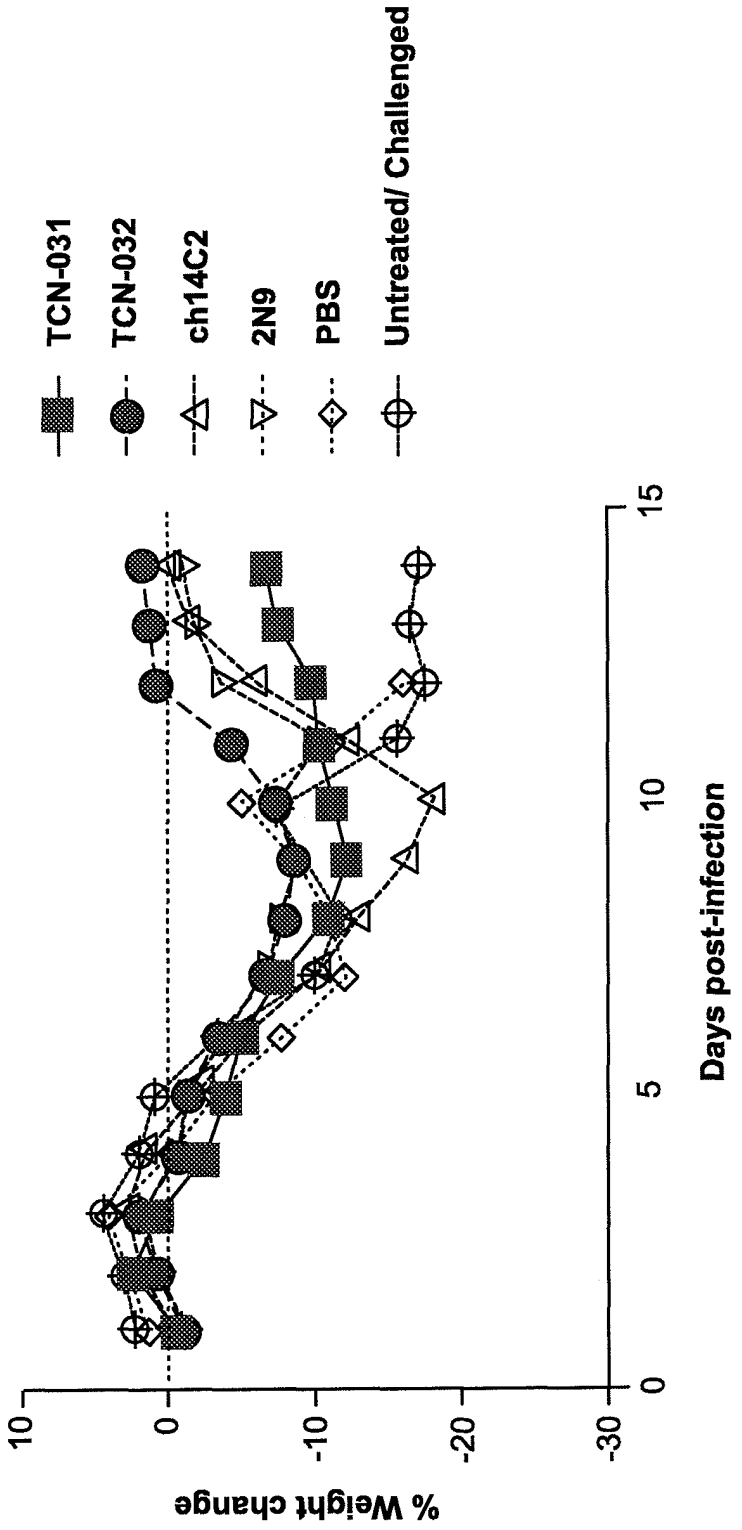

TCN-031
TCN-032
ch14C2
2N9
PBS
Untreated/ Challenged
% Weight change
10
0
-10
-20
-30
0
5
10
15
Days post-infection

FIG. 15B

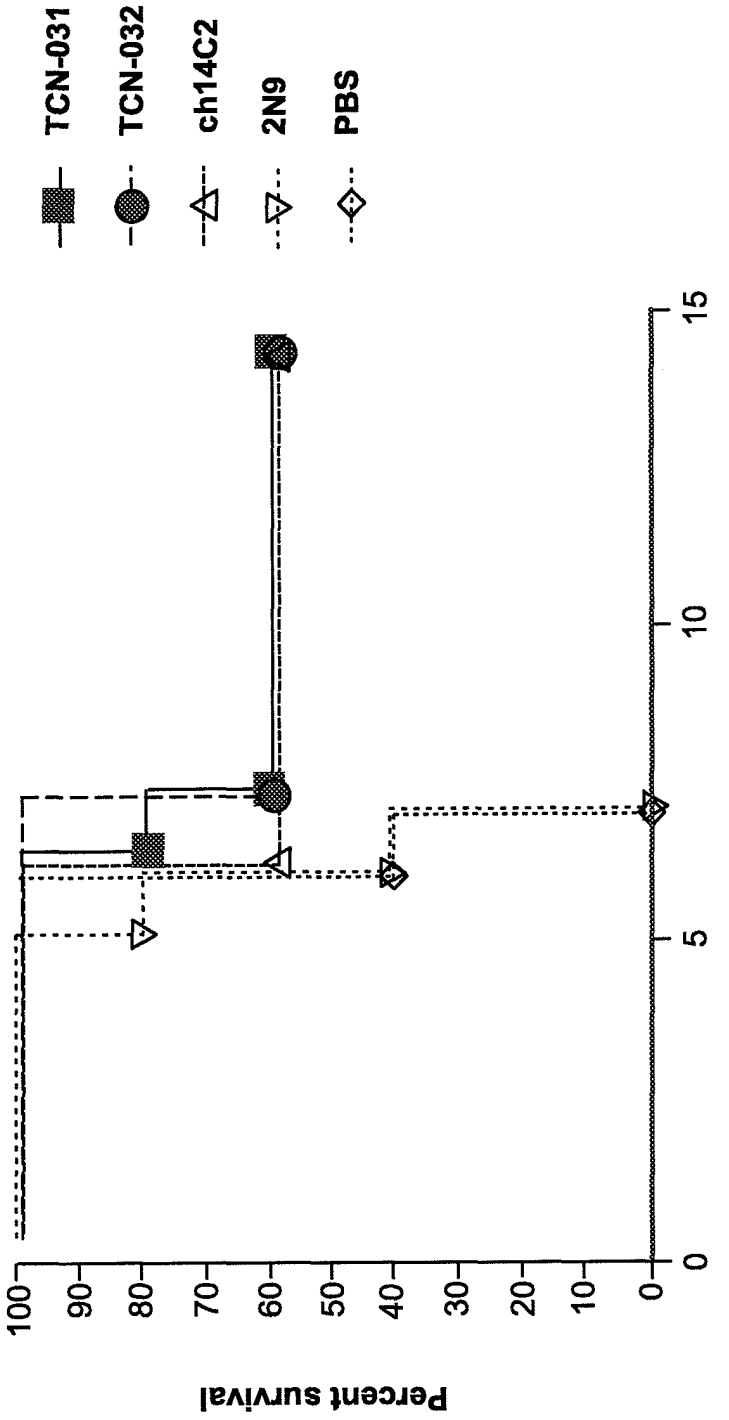

TCN-031
TCN-032
ch14C2
2N9
PBS
Percent survival
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
Days post-infection

FIG. 15C

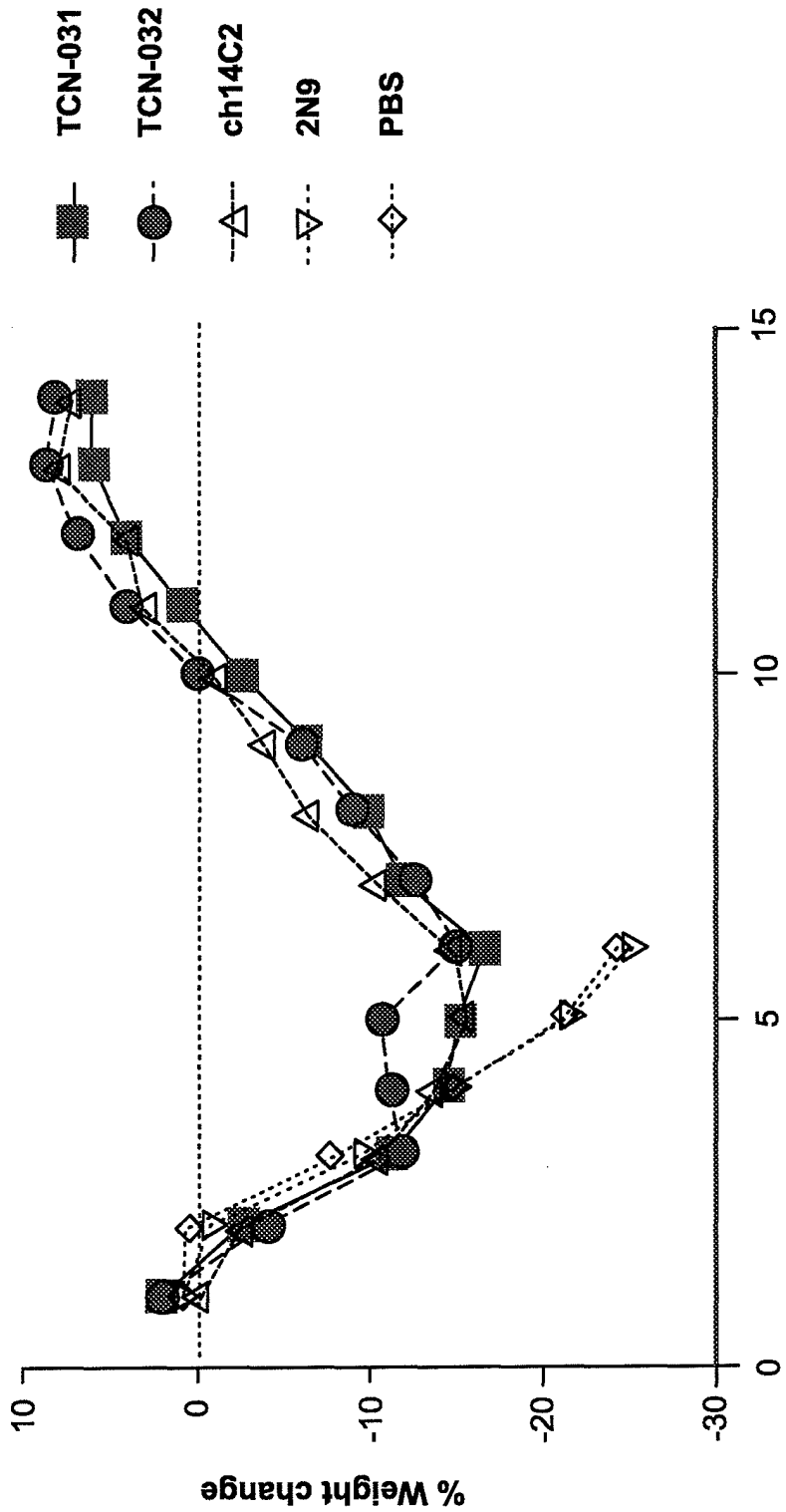

TCN-031
TCN-032
ch14C2
2N9
PBS
% Weight change
10
0
-10
-20
-30
0
5
10
15
Days post-infection

FIG. 15D

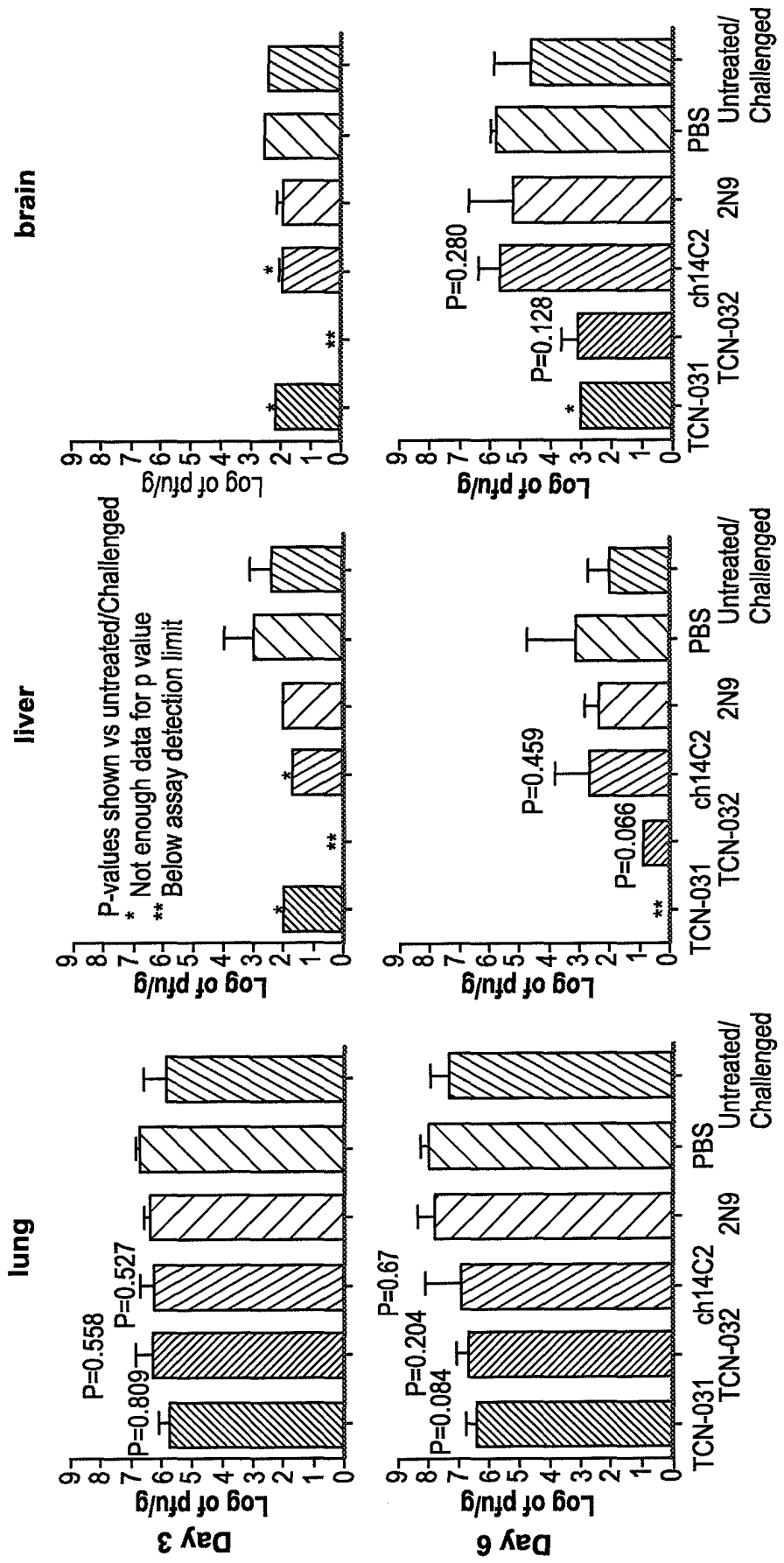

lung
liver
brain
Day 3
Day 6
Log of pfu/g
P=0.558
P=0.809
P=0.527
P=0.084
P=0.204
P=0.67
P=0.066
P=0.459
P=0.128
P=0.280
P-values shown vs untreated/Challenged
* Not enough data for p value
** Below assay detection limit
TCN-031
TCN-032
ch14C2
2N9
PBS
Untreated/
Challenged

FIG. 16

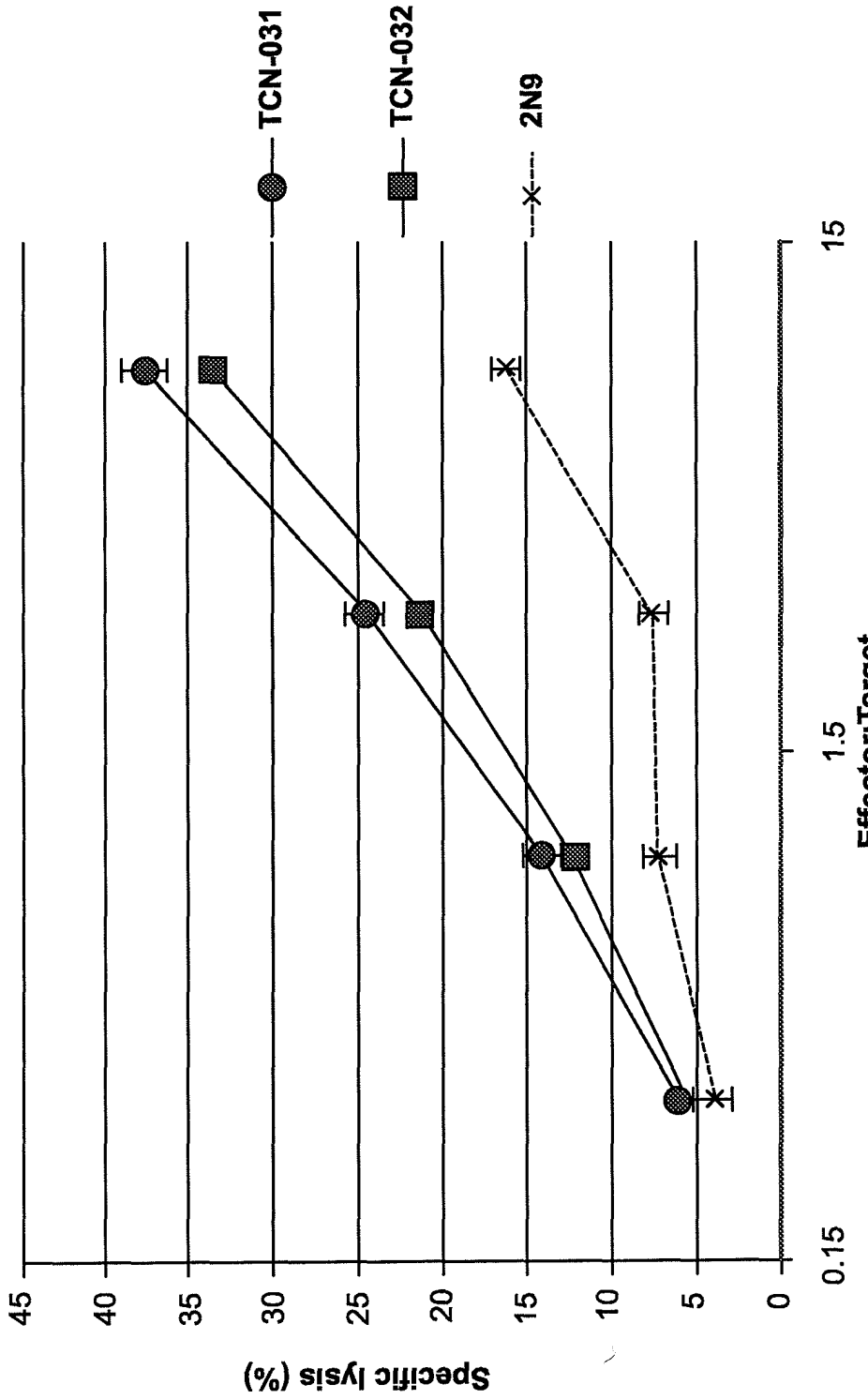

TCN-031
TCN-032
2N9
Specific lysis (%)
45
40
35
30
25
20
15
10
5
0
0.15
1.5
15
Effector:Target

FIG. 17

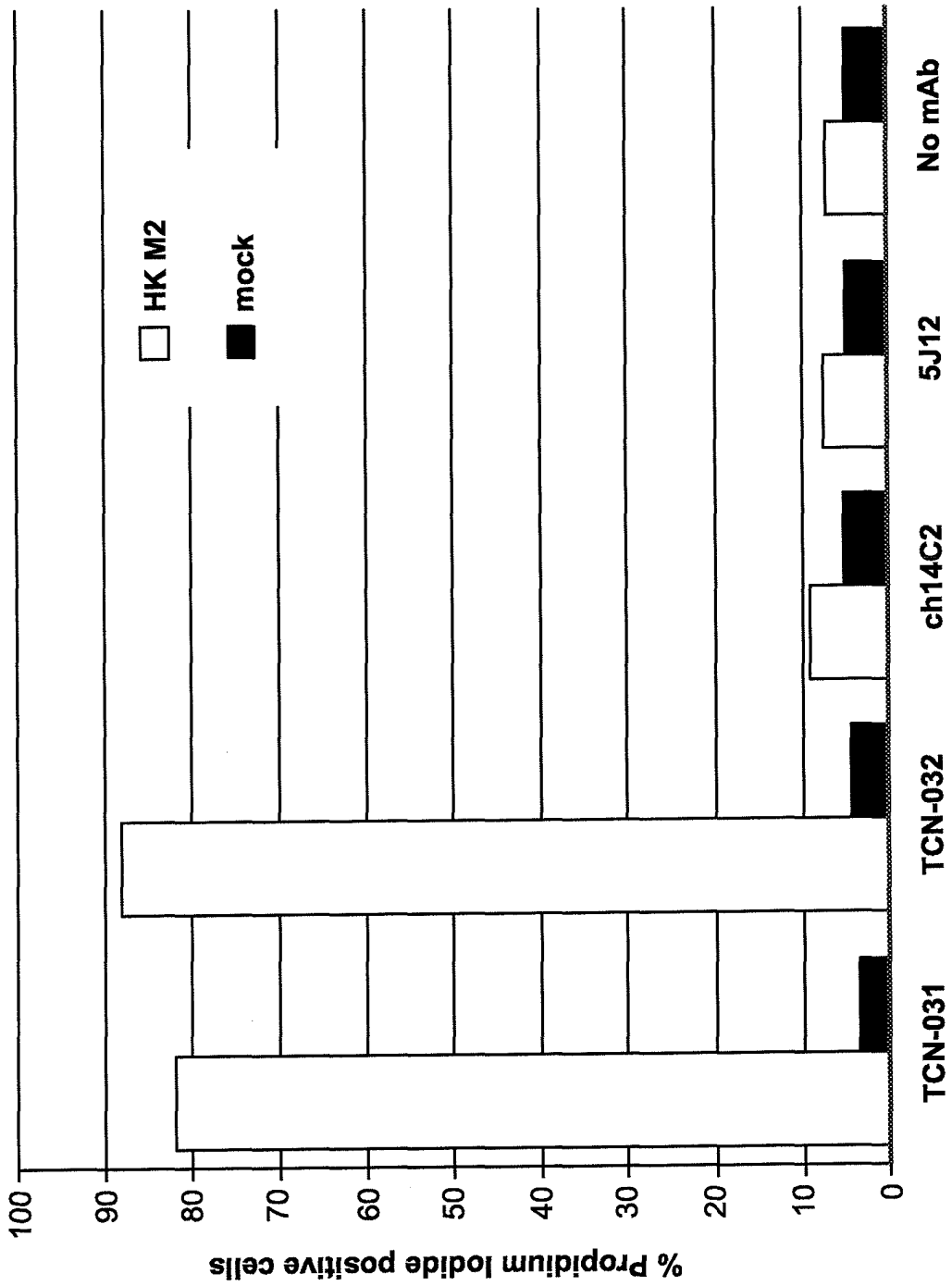

% Propidium Iodide positive cells
100
90
80
70
60
50
40
30
20
10
0
HK M2
mock
TCN-031
TCN-032
ch14C2
5J12
No mAb

FIG. 18

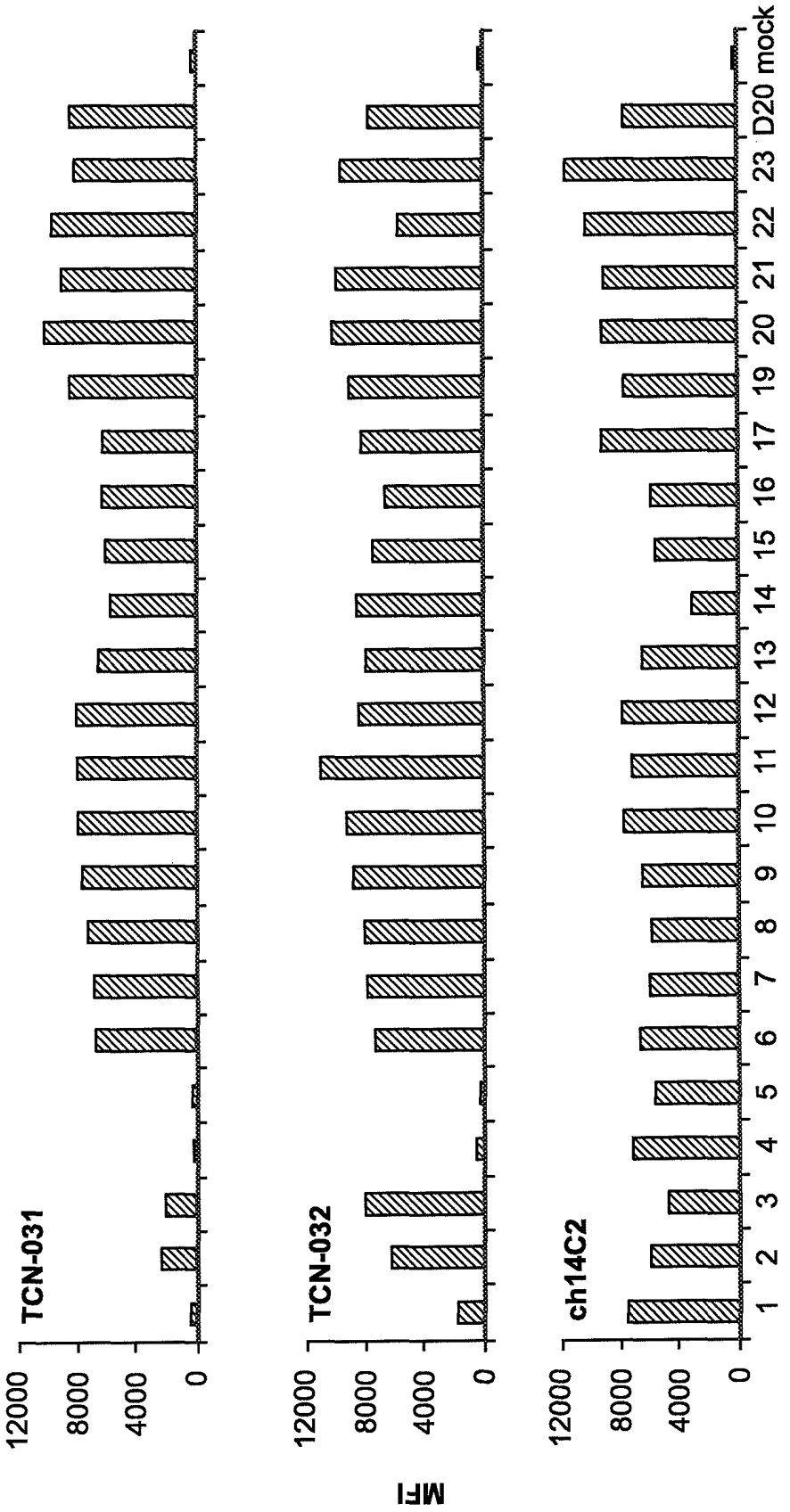

TCN-031
TCN-032
ch14C2
MFI
12000
8000
4000
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 19 20 21 22 23 D20 mock
Position of Alanine substitution in M2e

FIG. 19A

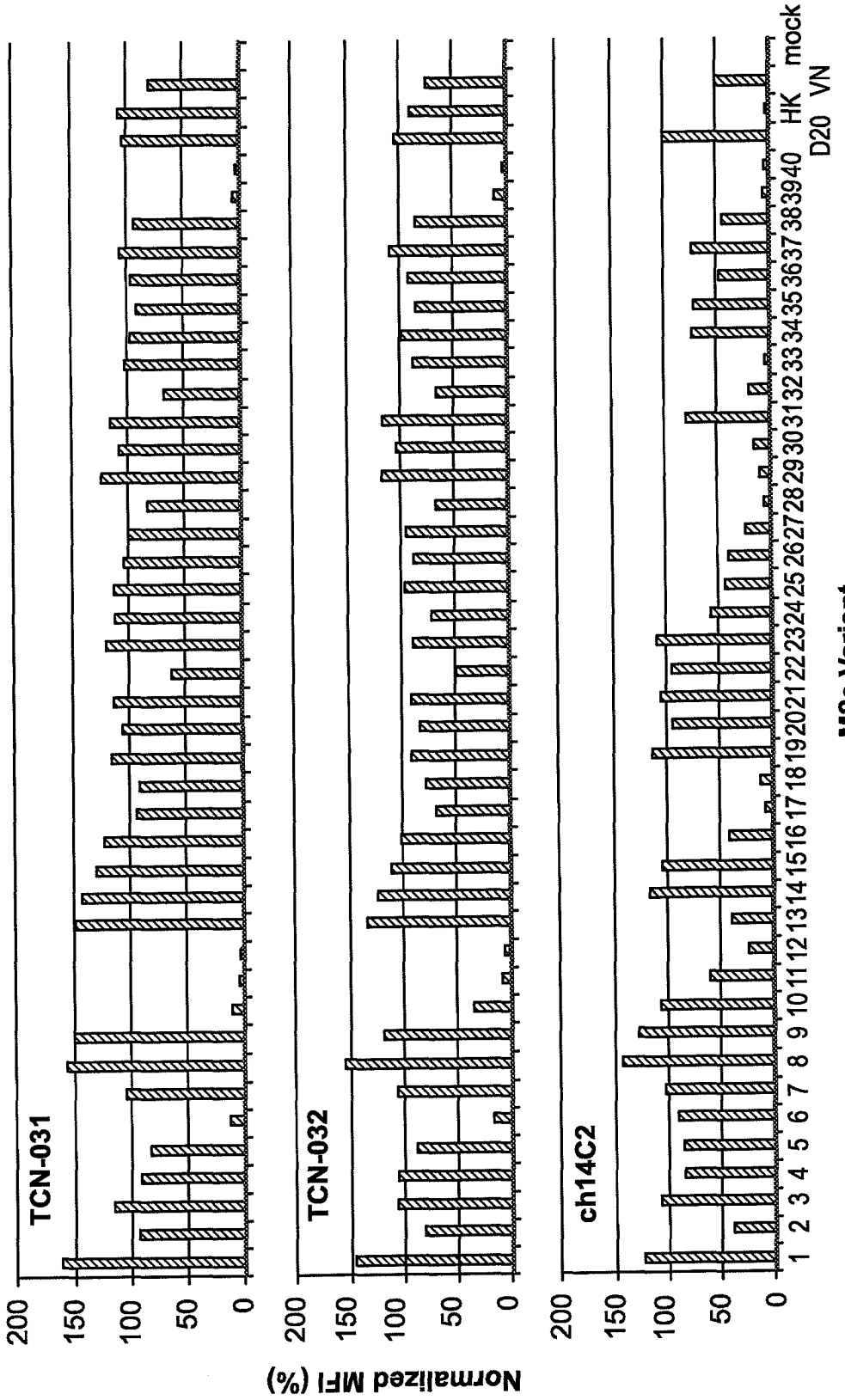

TCN-031
TCN-032
ch14C2
Normalized MFI (%)
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 HK mock
D20 VN
M2e Variant

FIG. 19B

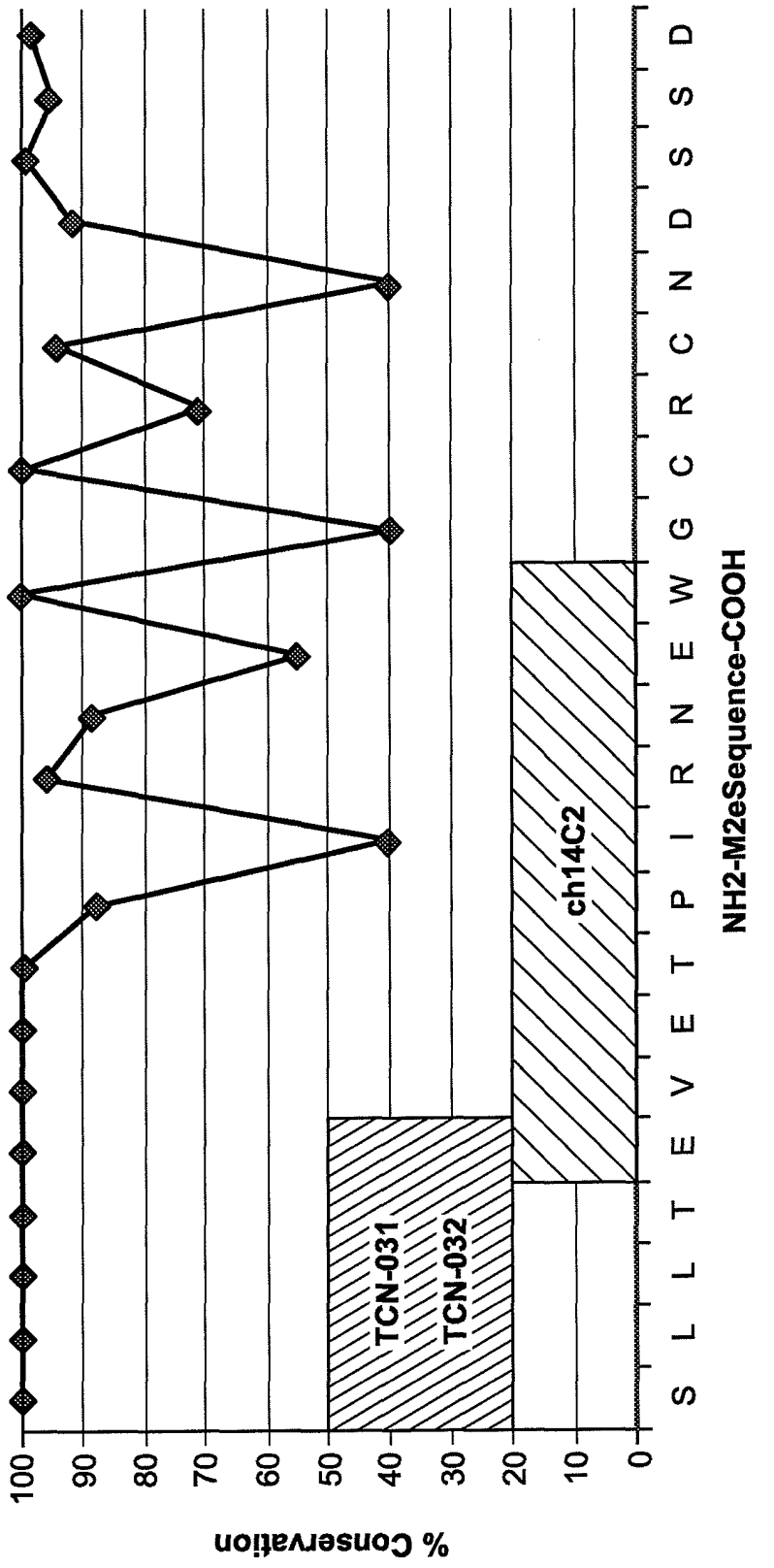

% Conservation
100
90
80
70
60
50
40
30
20
10
0
TCN-031
TCN-032
ch14C2
S L L T E V E T P I R N E W G C R C N D S S D
NH2-M2eSequence-COOH

FIG. 19C

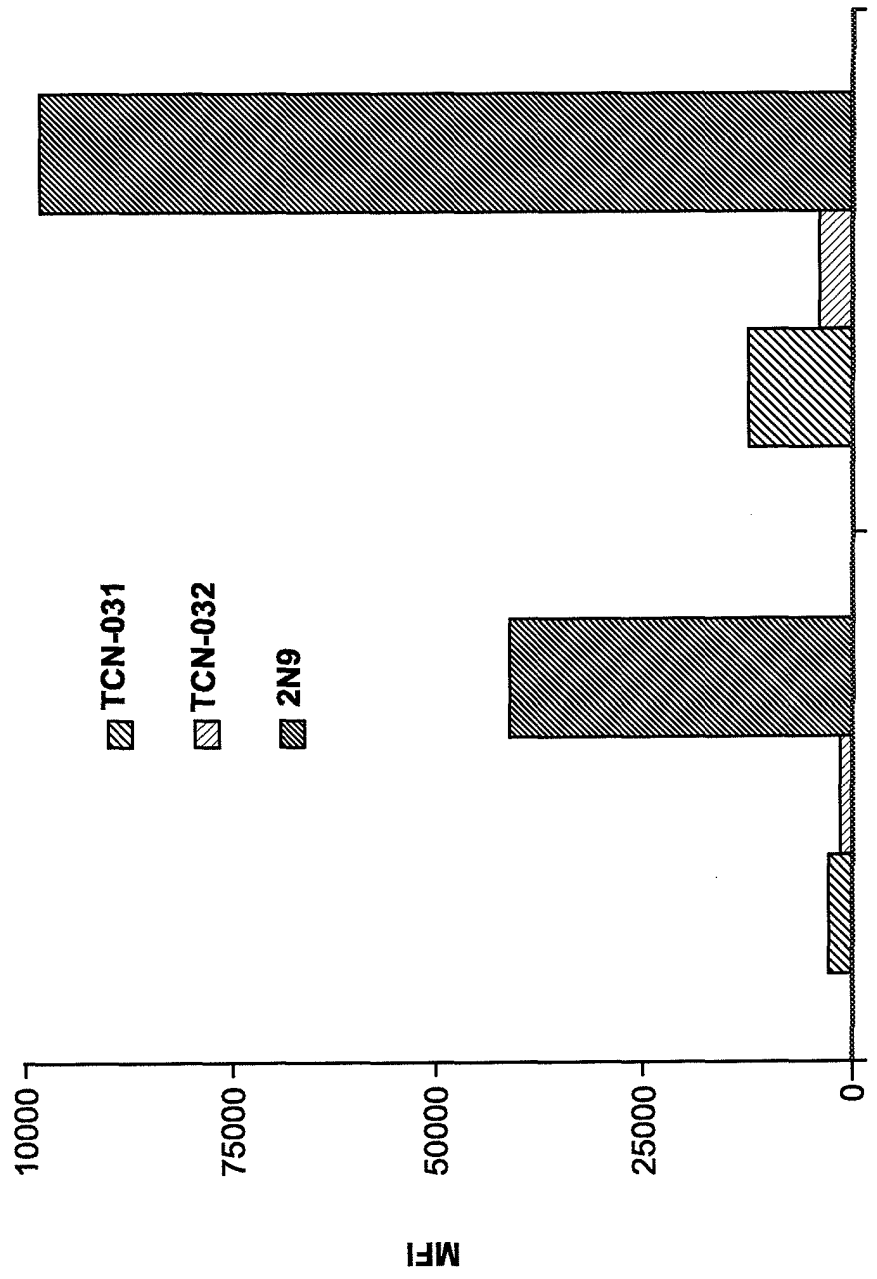

MFI
10000
75000
50000
25000
0
TCN-031
TCN-032
2N9
TCN-031 AF647
TCN-032 AF647

FIG. 20

FIG. 21

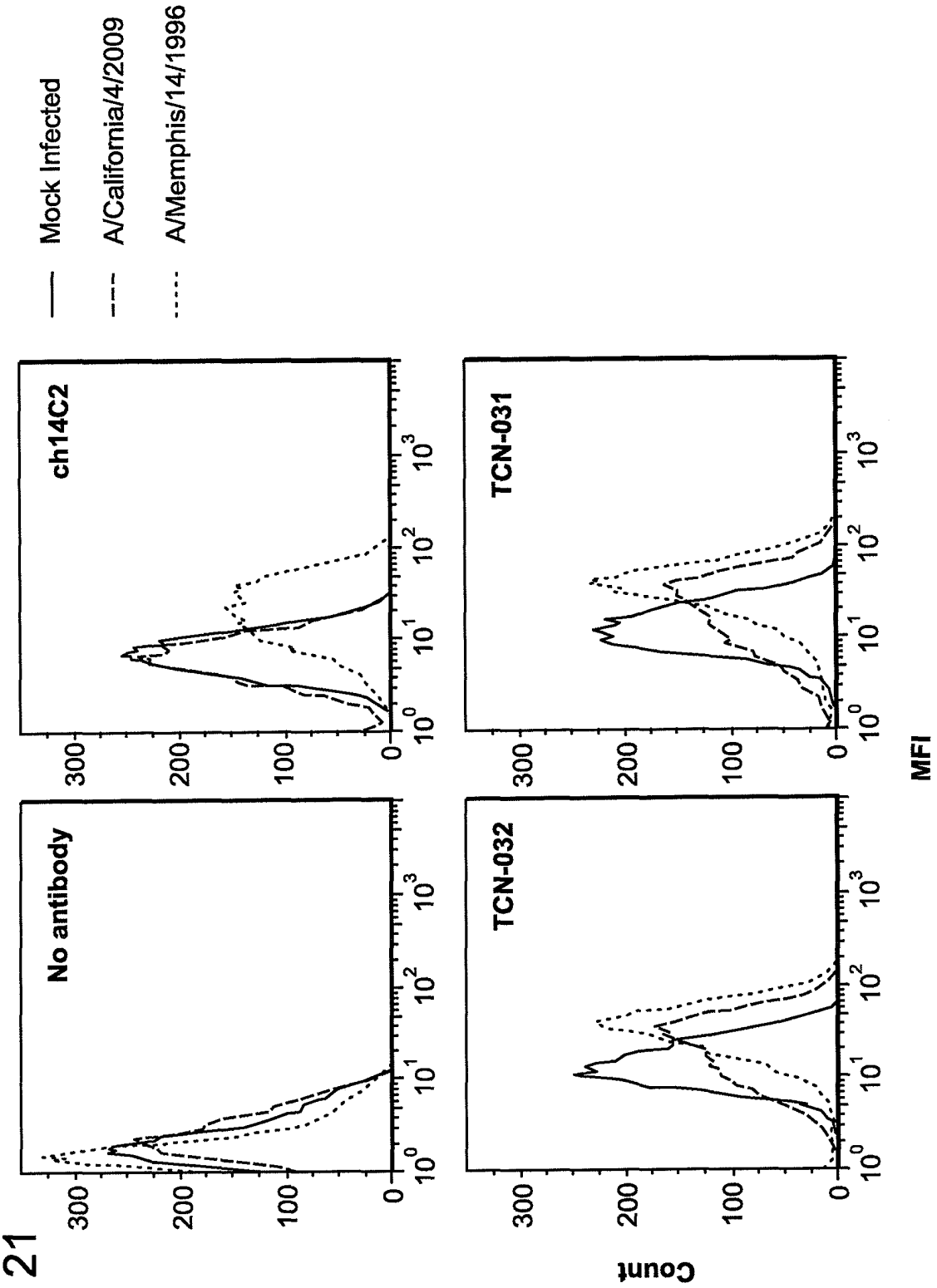
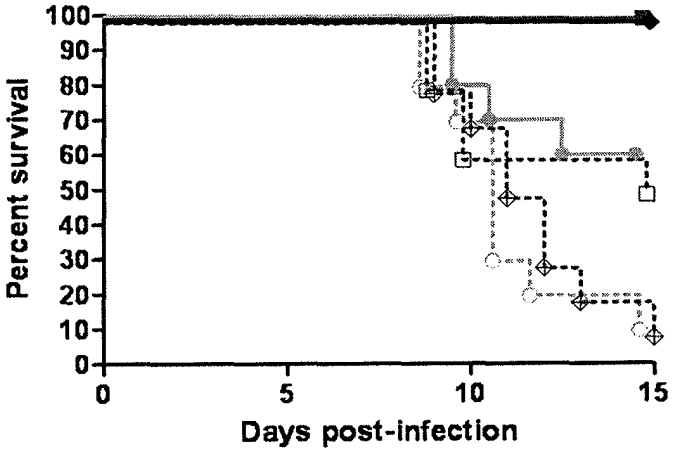
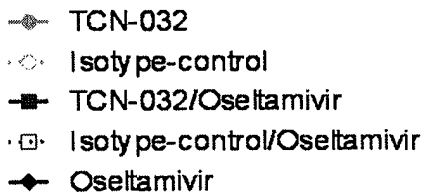

Mock Infected
A/California/4/2009
A/Memphis/14/1996
No antibody
ch14C2
TCN-031
TCN-032
Count
MFI

FIGURE 22

H5N1(A/VN/1203/04) Challenge at 5LD50

Group size = 10 mice
mAb at 20 mg/kg on day 1, 3, 5
Oseltamivir at 10 mg/kg bid on day 1-5



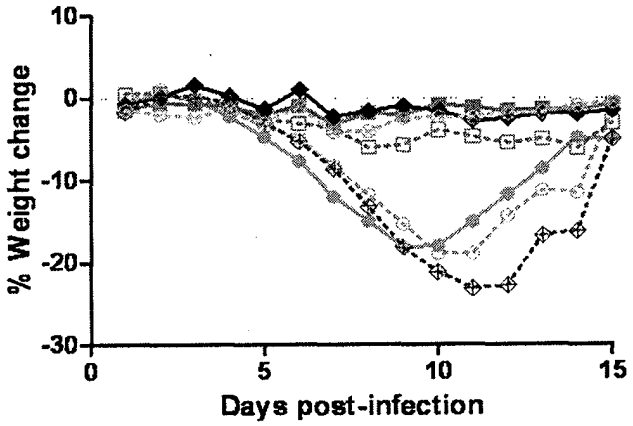


Comparison	P-value
TCN-032 vs Isotype	0.027
TCN-032/Oseltamivir vs Isotype/Oseltamivir	0.012
TCN-032/Oseltamivir vs Oseltamivir	1.000
TCN-032 vs untreated	0.031
TCN-032/Oseltamivir vs untreated	<0.0001
Oseltamivir vs untreated	<0.0001

FIGURE 23

H5N1(A/VN/1203/04) Challenge at 5LD50

Group size = 10 mice
mAb at 20 mg/kg on day 1, 3, 5
Oseltamivir at 10 mg/kg bid on day 1-5



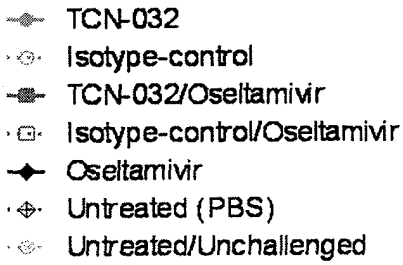
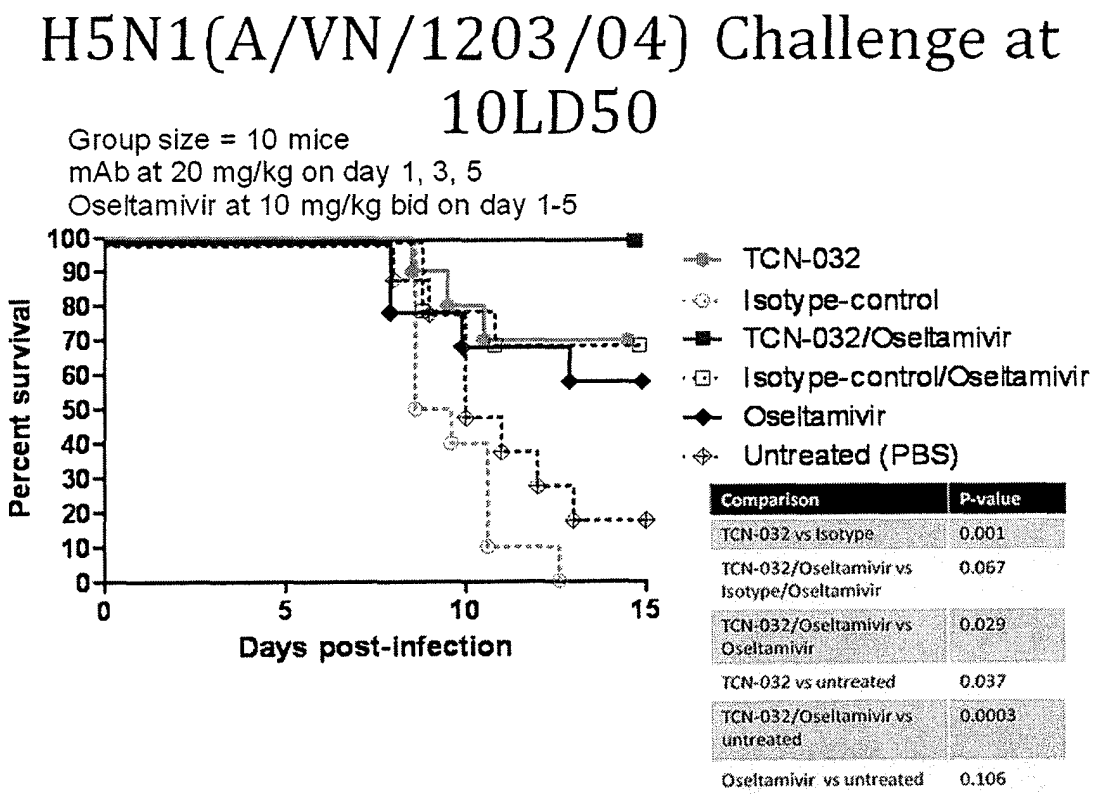


FIGURE 24



Comparison	P-value
TCN-032 vs Isotype	0.001
TCN-032/Oseltamivir vs Isotype/Oseltamivir	0.067
TCN-032/Oseltamivir vs Oseltamivir	0.029
TCN-032 vs untreated	0.037
TCN-032/Oseltamivir vs untreated	0.0003
Oseltamivir vs untreated	0.106

FIGURE 25

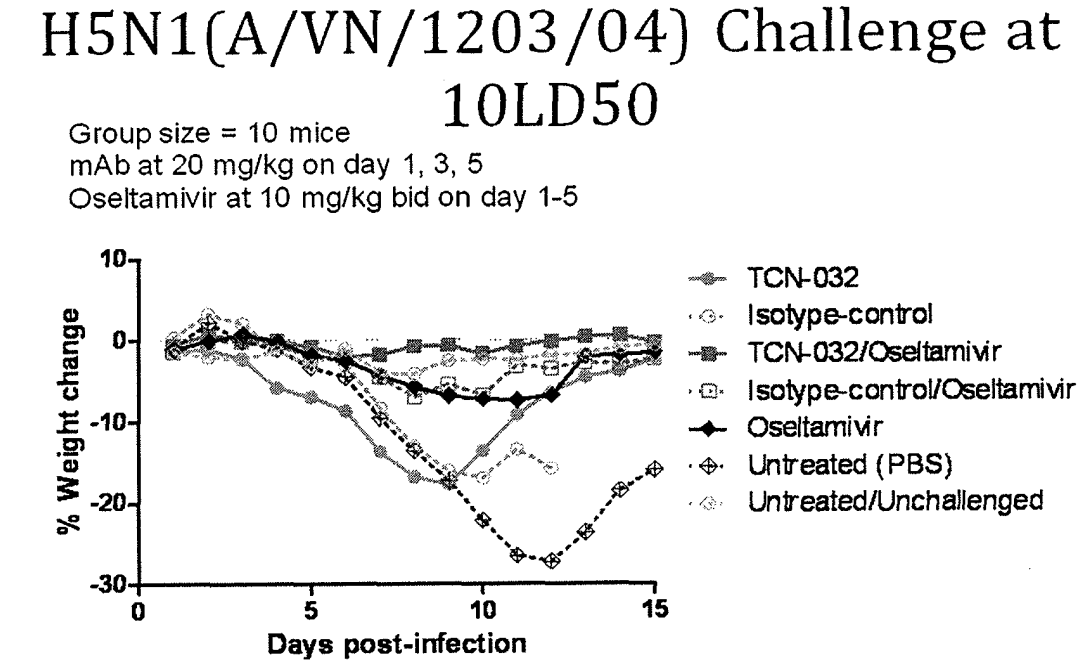
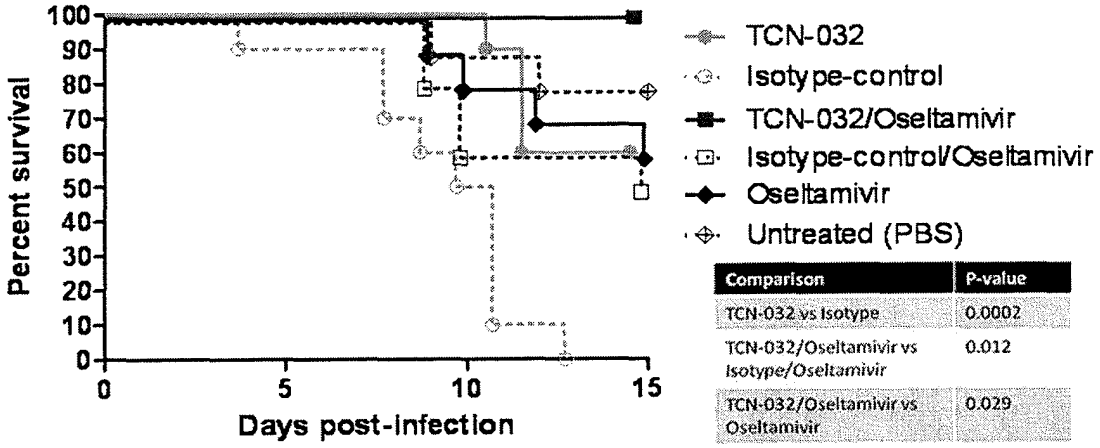


FIGURE 26

H5N1(A/VN/1203/04) Challenge at 20LD50

Group size = 10 mice
mAb at 20 mg/kg on day 1, 3, 5
Oseltamivir at 10 mg/kg bid on day 1-5



Comparison	P-value
TCN-032 vs Isotype	0.0002
TCN-032/Oseltamivir vs Isotype/Oseltamivir	0.012
TCN-032/Oseltamivir vs Oseltamivir	0.029
TCN-032 vs untreated	0.397
TCN-032/Oseltamivir vs untreated	0.146
Oseltamivir vs untreated	0.363

FIGURE 27

H5N1(A/VN/1203/04) Challenge at 20LD50

Group size = 10 mice
mAb at 20 mg/kg on day 1, 3, 5
Oseltamivir at 10 mg/kg bid on day 1-5

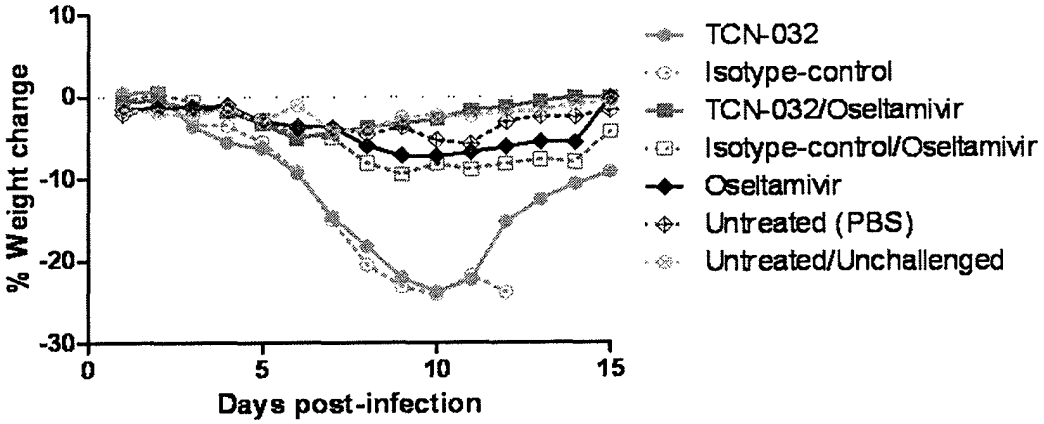


FIGURE 28

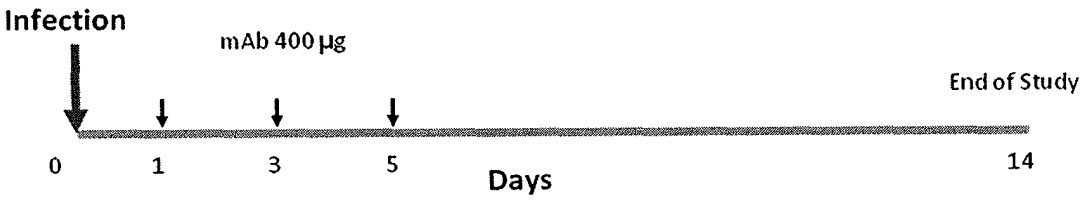


FIGURE 29

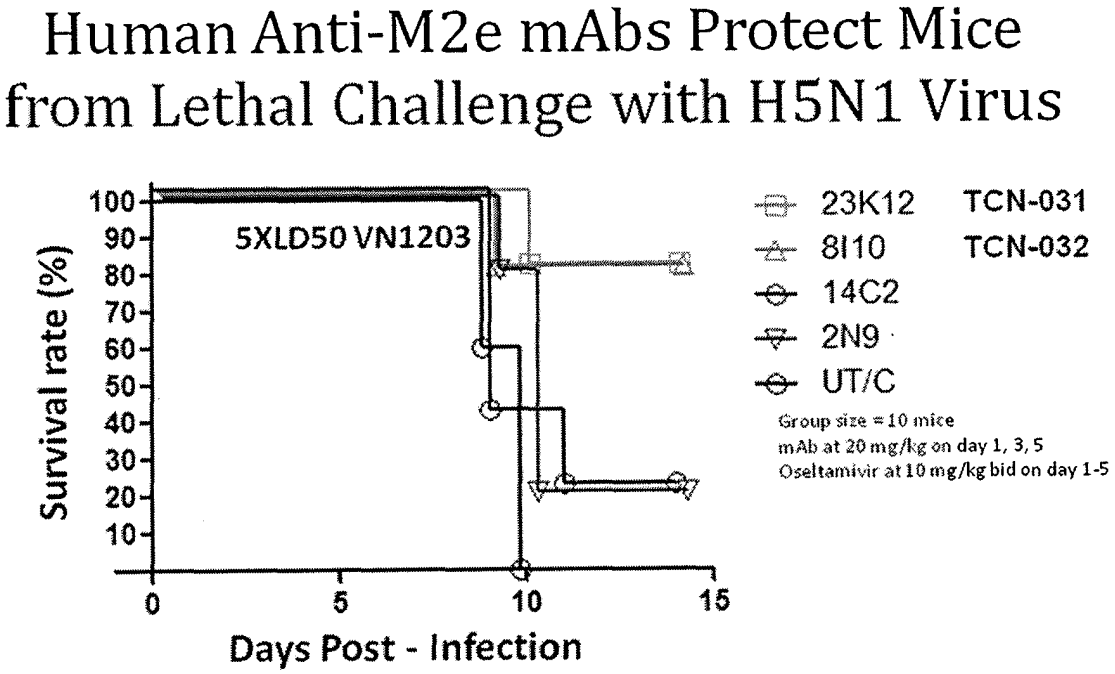


FIGURE 30

Failure of Oseltamivir (10mg/kg) to Protect Mice in VN1203 Lethal Challenge Model

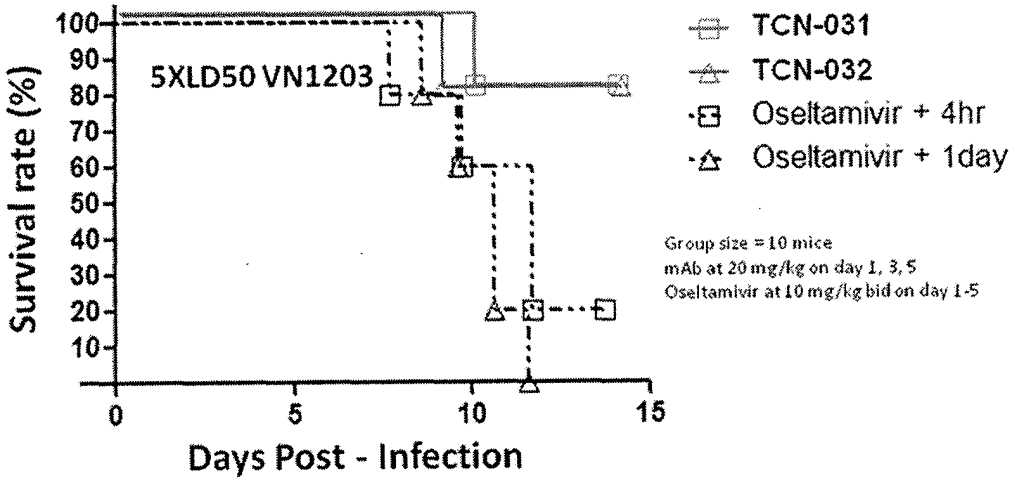
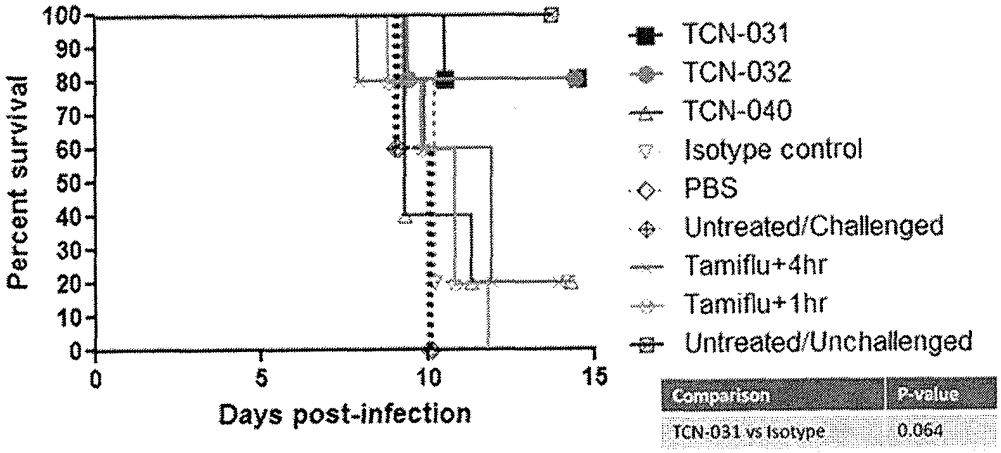


FIGURE 31

Mice are protected from lethal avian H5N1 flu infection (5 MLD_{50} VN1203/04) after treatment with anti-M2 mAbs



Comparison	P-value
TCN-031 vs Isotype	0.064
TCN-032 vs Isotype	0.130
TCN-040 vs Isotype	0.769
Tamiflu+4hr vs PBS	0.122
Tamiflu+1hr vs PBS	0.085

FIGURE 32

Oseltamivir does not protect, even when given within 4 hours of infection, against Vn1203/04

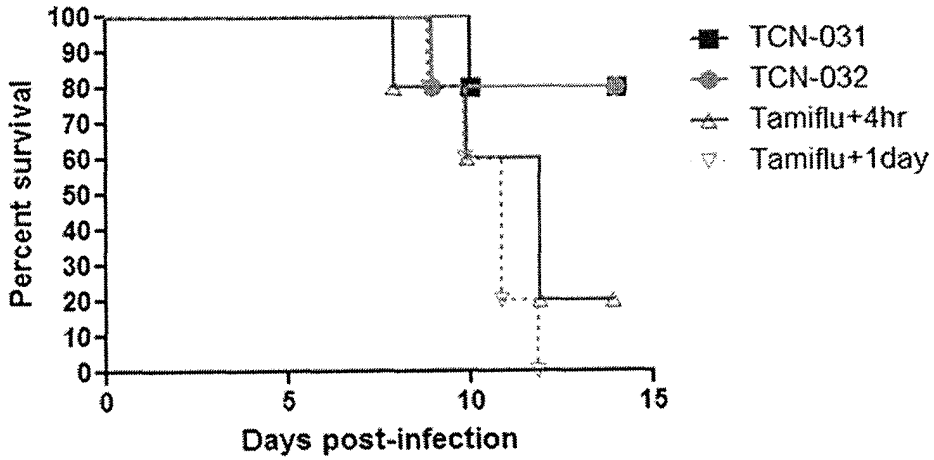


FIGURE 33

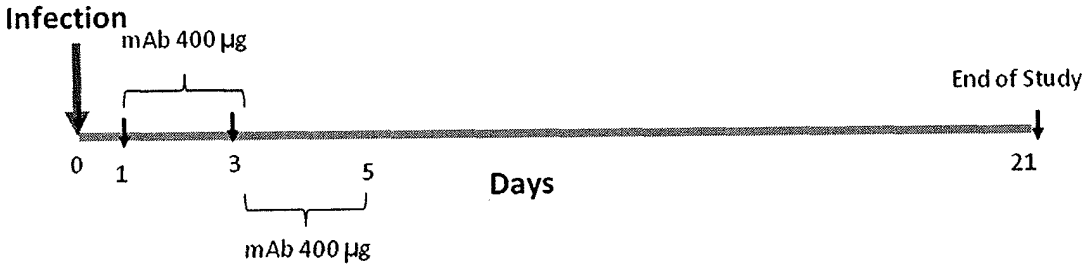


FIGURE 34

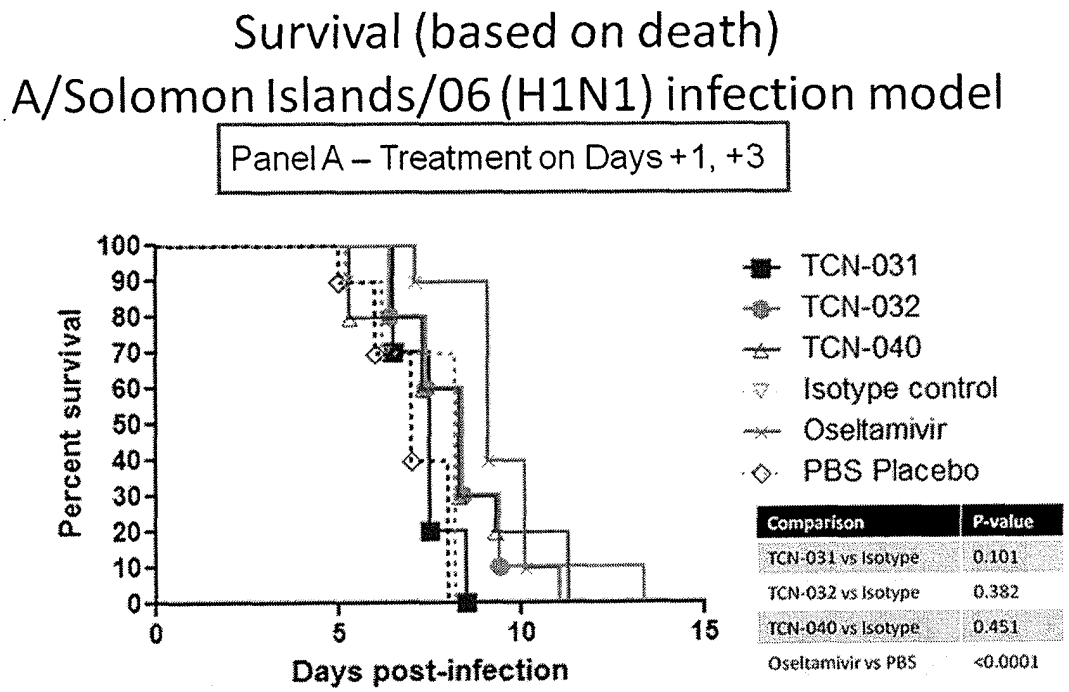


FIGURE 35

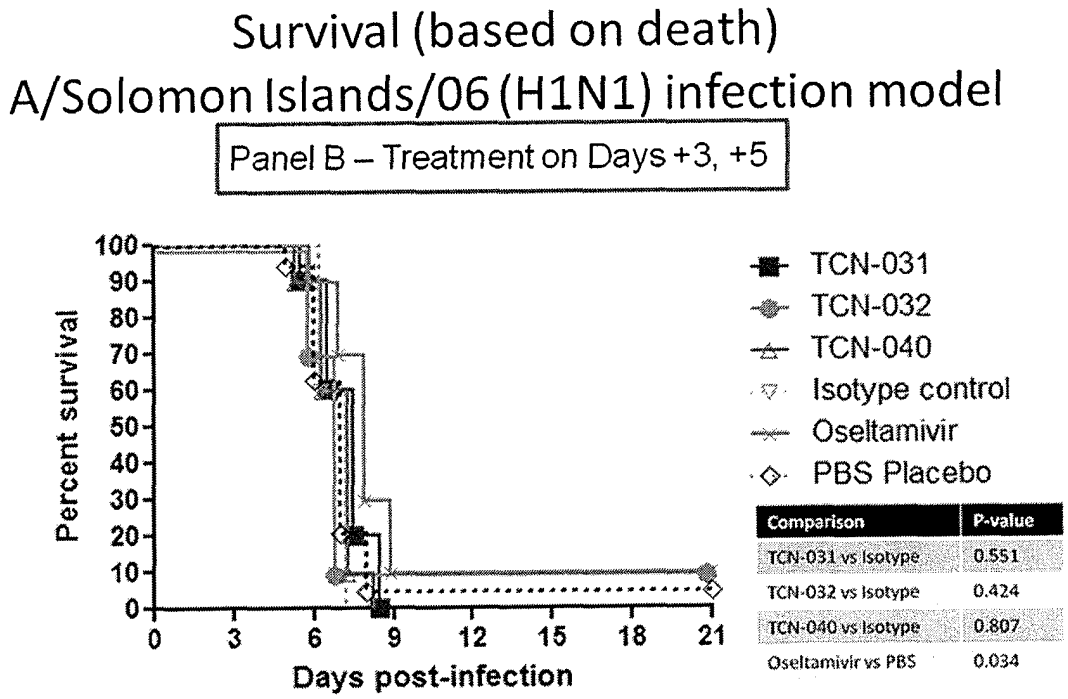


FIGURE 36

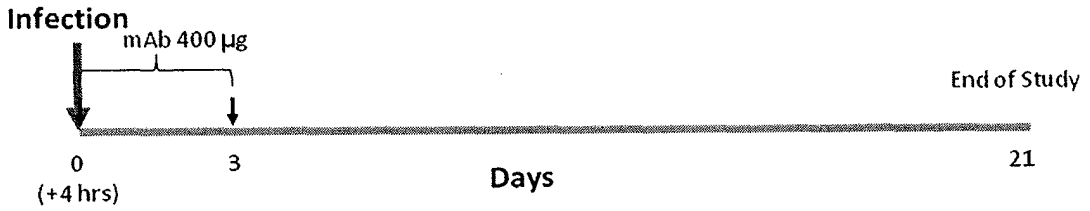


FIGURE 37

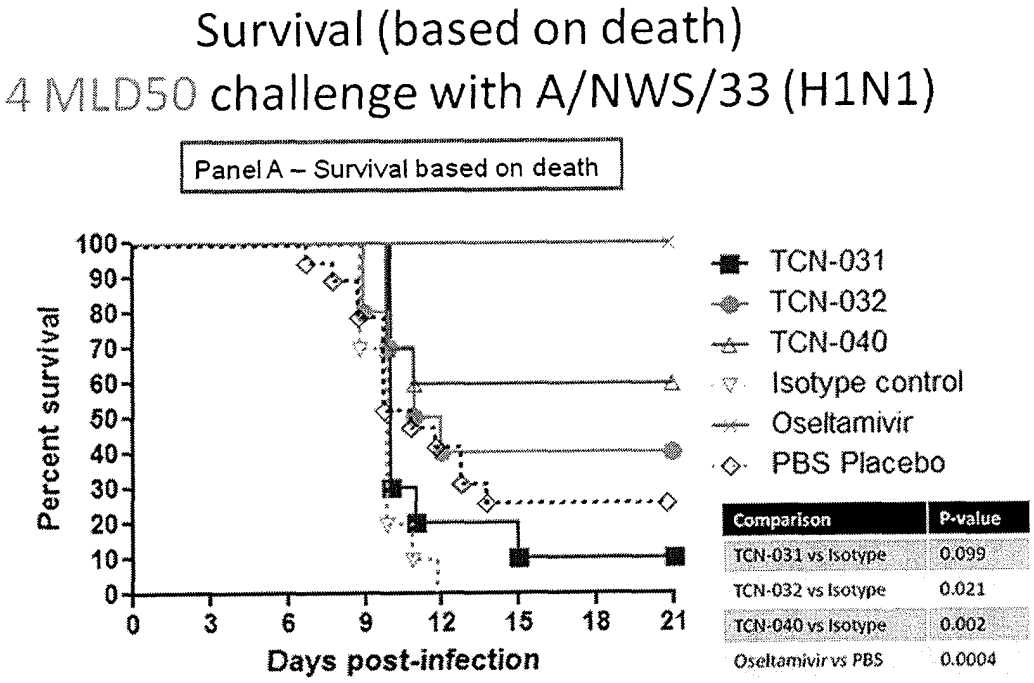
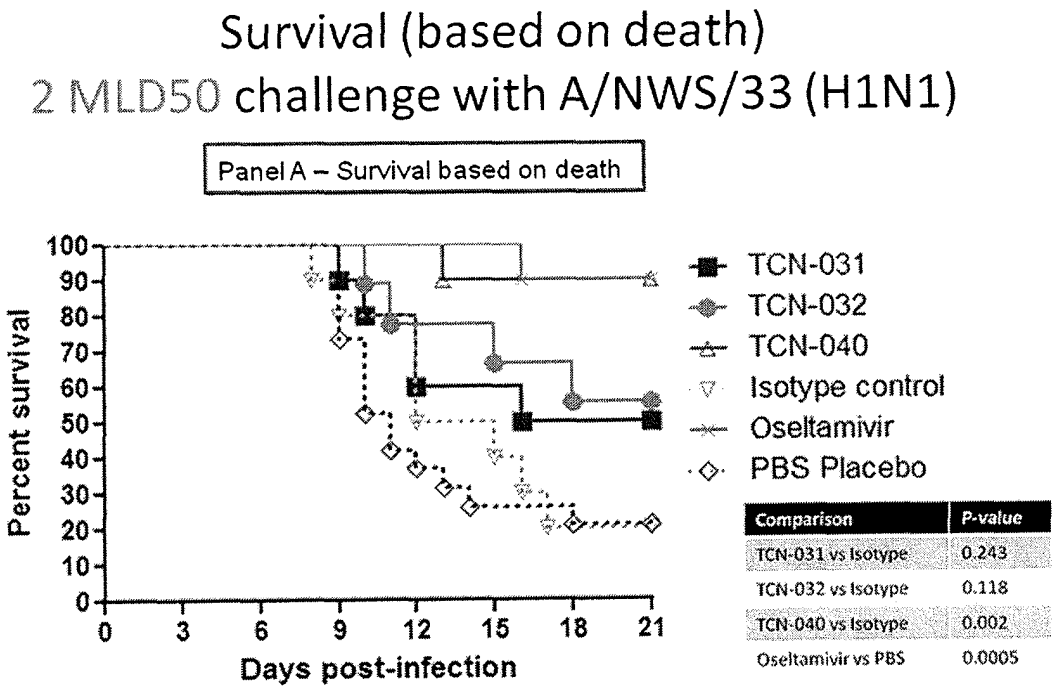
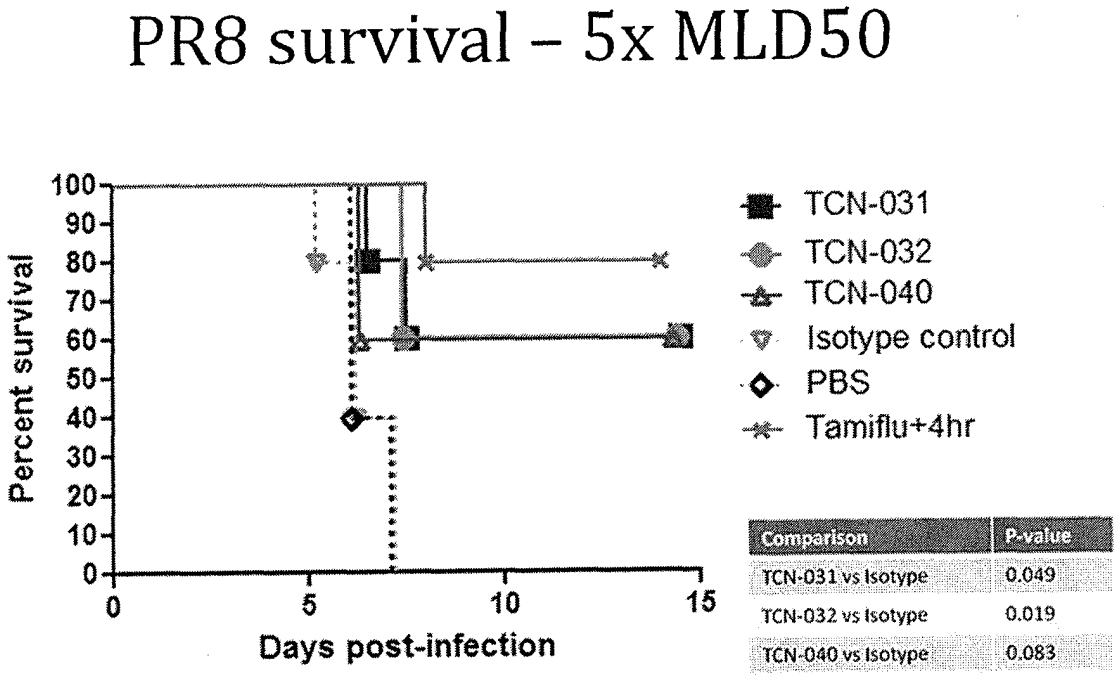


FIGURE 38



Comparison	P-value
TCN-031 vs Isotype	0.243
TCN-032 vs Isotype	0.118
TCN-040 vs Isotype	0.002
Oseltamivir vs PBS	0.0005

FIGURE 39



Comparison	P-value
TCN-031 vs Isotype	0.049
TCN-032 vs Isotype	0.019
TCN-040 vs Isotype	0.083
Tamiflu+4hr vs PBS	0.002

FIGURE 40

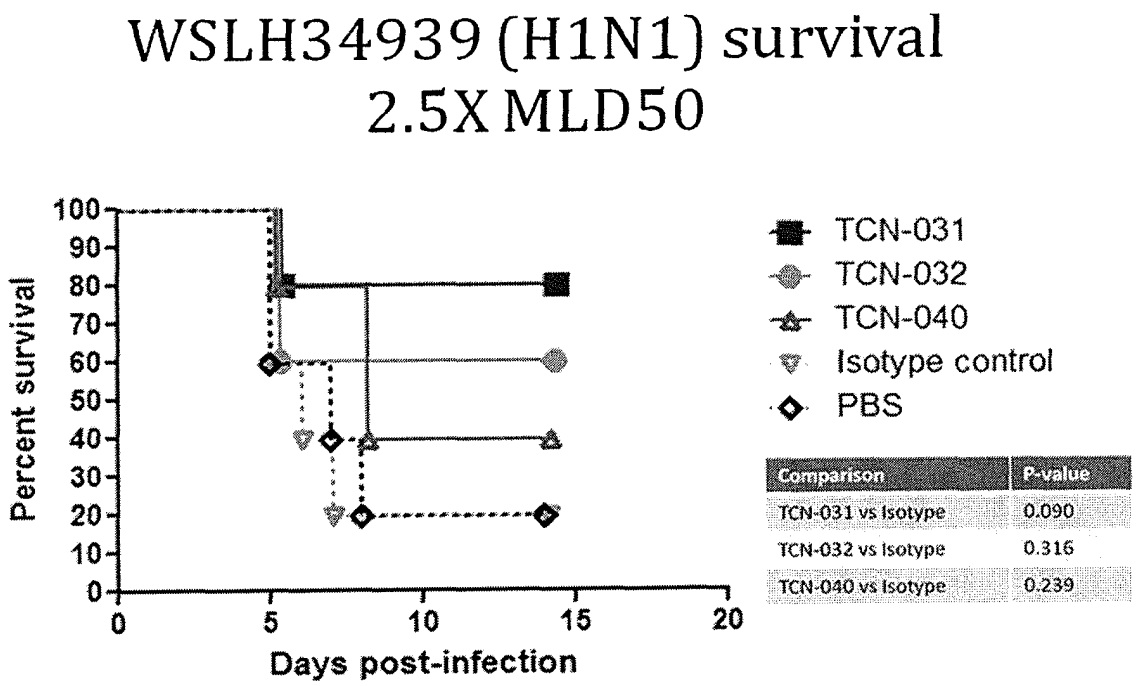


FIGURE 41

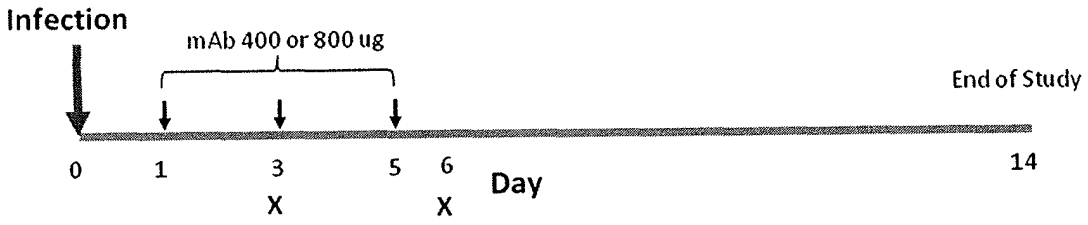
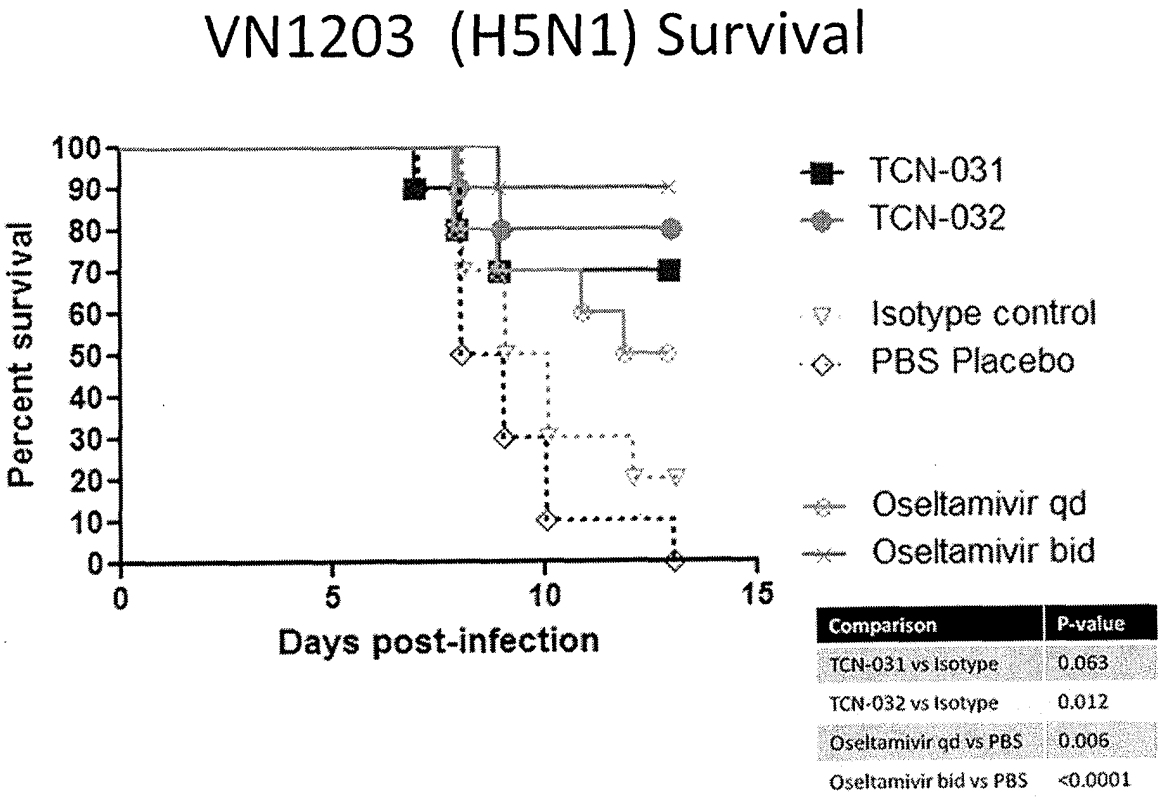
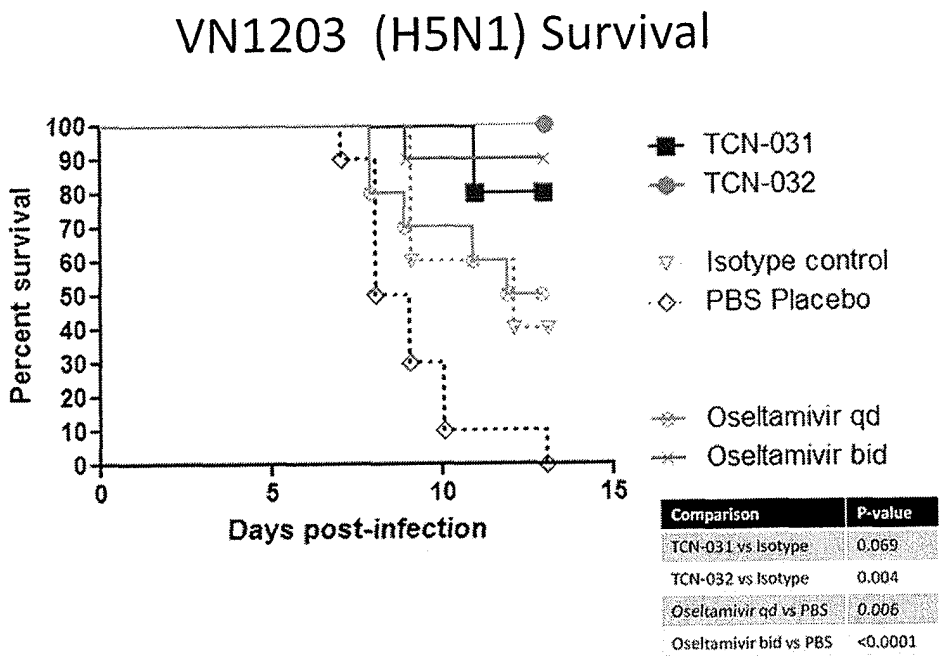


FIGURE 42



Comparison	P-value
TCN-031 vs Isotype	0.063
TCN-032 vs Isotype	0.012
Oseltamivir qd vs PBS	0.006
Oseltamivir bid vs PBS	<0.0001

FIGURE 43



Comparison	P-value
TCN-031 vs Isotype	0.069
TCN-032 vs Isotype	0.004
Oseltamivir qd vs PBS	0.006
Oseltamivir bid vs PBS	<0.0001

FIGURE 44

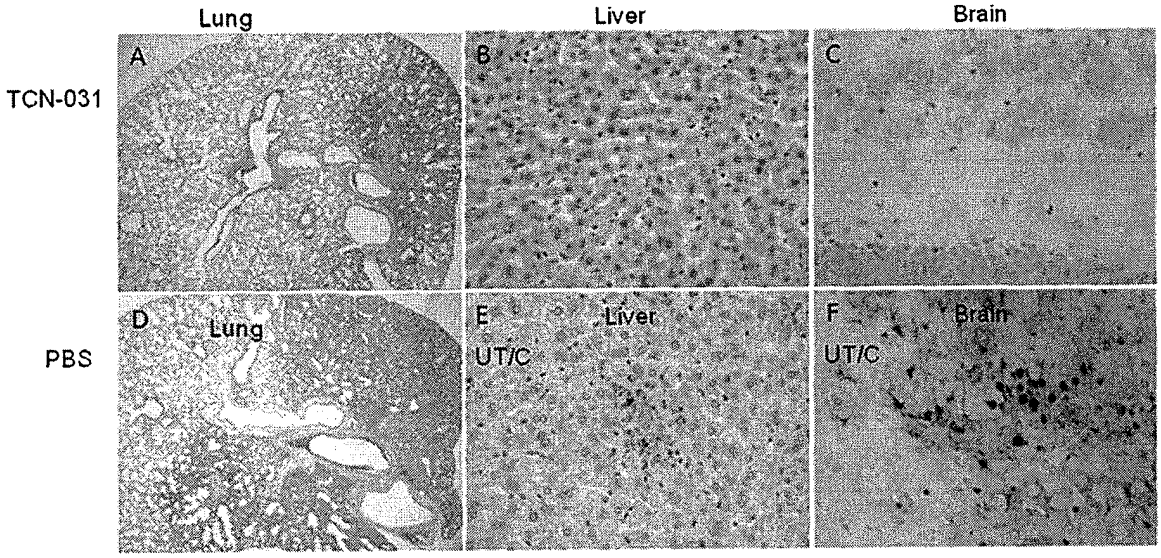
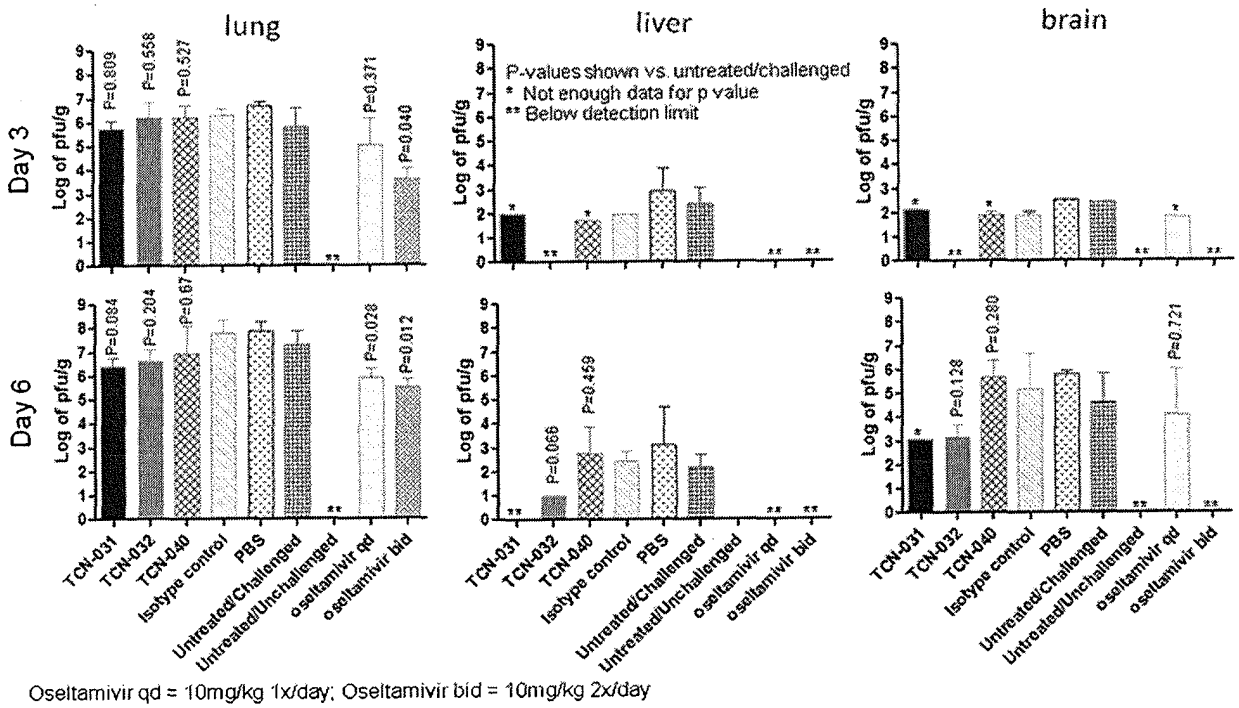


FIGURE 45



Oseltamivir qd = 10mg/kg 1x/day; Oseltamivir bid = 10mg/kg 2x/day

FIGURE 46

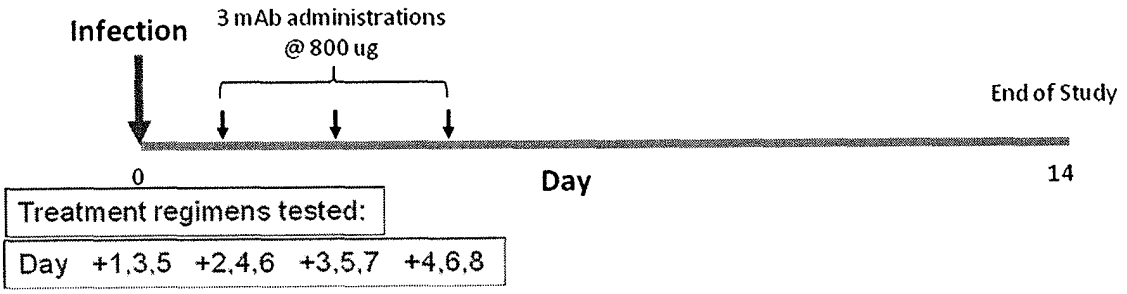
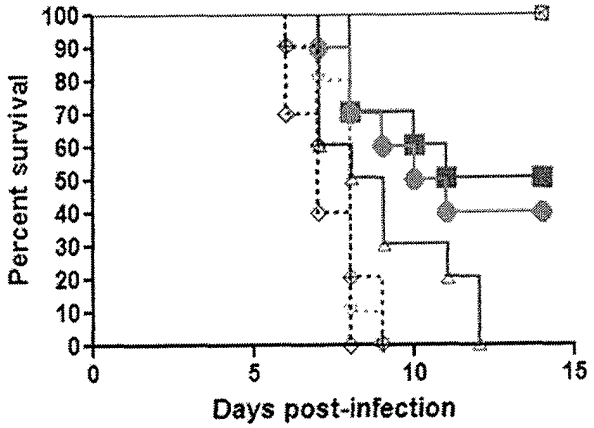
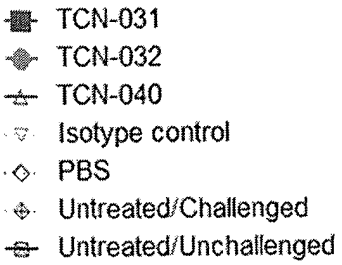


FIGURE 47

Mice are protected from lethal avian H5N1 flu infection (5 MLD_{50} VN1203/04) after 800 ug Day 1,3,5 treatment with anti-M2e mAbs



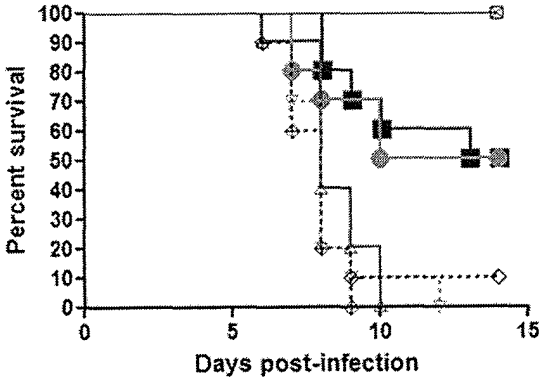


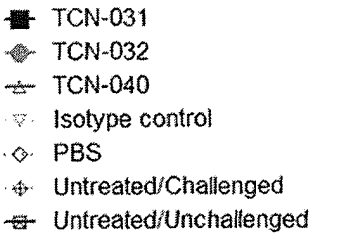
Based on death independent of weight loss

Comparison	P-value
TCN-031 vs Isotype	0.0008
TCN-032 vs Isotype	0.004
TCN-040 vs Isotype	0.181
TCN-031 vs Untreated/Challenged	0.0007
TCN-032 vs Untreated/Challenged	0.003
TCN-040 vs Untreated/Challenged	0.116

FIGURE 48

Mice are protected from lethal avian H5N1 flu infection (5 MLD_{50} VN1203/04) after 800 ug Day 2,4,6 treatment with anti-M2e mAbs



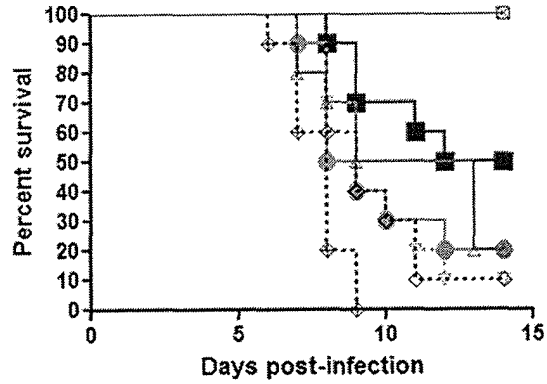


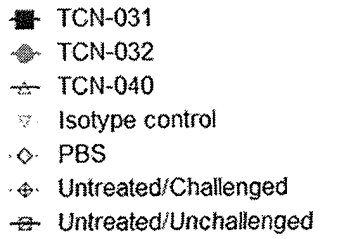
Comparison	P-value
TCN-031 vs Isotype	0.001
TCN-032 vs Isotype	0.009
TCN-040 vs Isotype	0.580
TCN-031 vs Untreated/Challenged	0.0005
TCN-032 vs Untreated/Challenged	0.003
TCN-040 vs Untreated/Challenged	0.099

Based on death independent of weight loss

FIGURE 49

Mice are protected from lethal avian H5N1 flu infection (5 MLD_{50} VN1203/04) after 800 ug Day 3,5,7 treatment with anti-M2e mAbs



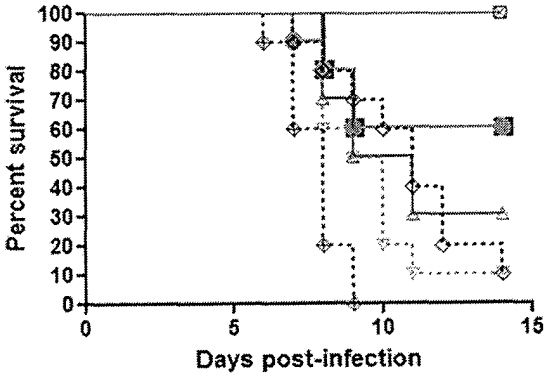


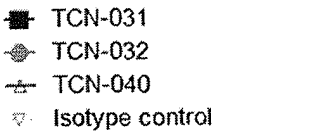
Comparison	P-value
TCN-031 vs Isotype	0.039
TCN-032 vs Isotype	0.840
TCN-040 vs Isotype	0.307
TCN-031 vs Untreated/Challenged	0.0002
TCN-032 vs Untreated/Challenged	0.023
TCN-040 vs Untreated/Challenged	0.010

Based on death independent of weight loss

FIGURE 50

Mice are protected from lethal avian H5N1 flu infection (5 MLD_{50} VN1203/04) after 800 ug Day 4,6,8 treatment with anti-M2e mAbs



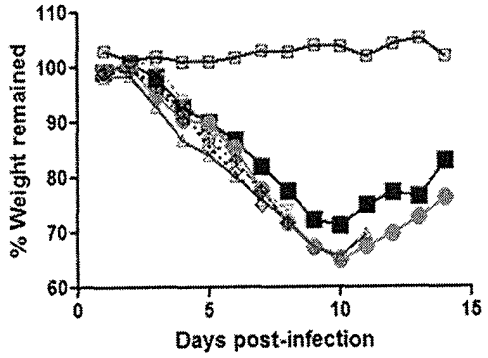


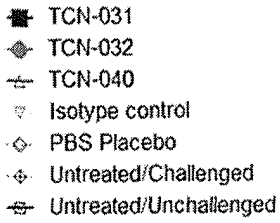
Comparison	P-value
TCN-031 vs Isotype	0.046
TCN-032 vs Isotype	0.059
TCN-040 vs Isotype	0.264
TCN-031 vs Untreated/Challenged	0.0009
TCN-032 vs Untreated/Challenged	0.002
TCN-040 vs Untreated/Challenged	0.003

Based on death independent of weight loss

FIGURE 51

Weight loss in H5N1 flu infection (5 MLD_{50} VN1203/04) after 800 ug Day 1,3,5 treatment with anti-M2e mAbs

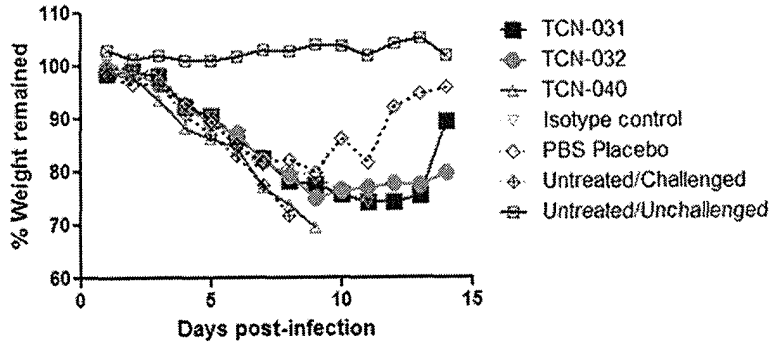




Based on death independent of weight loss

FIGURE 52

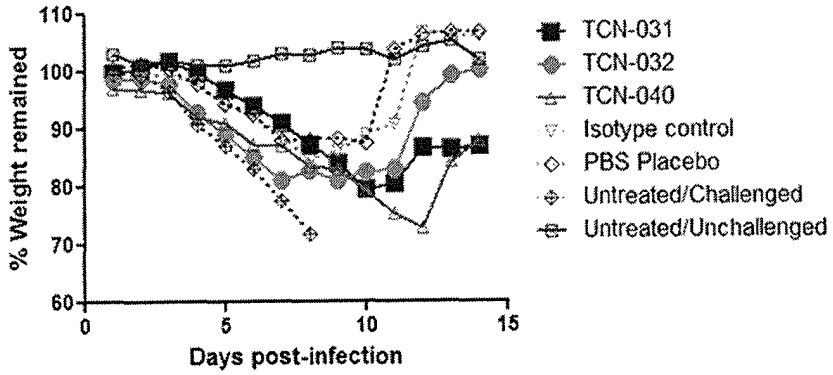
Weight loss in H5N1 flu infection (5 MLD_{50} VN1203/04) after 800 ug Day 2,4,6 treatment with anti-M2e mAbs



Based on death independent of weight loss

FIGURE 53

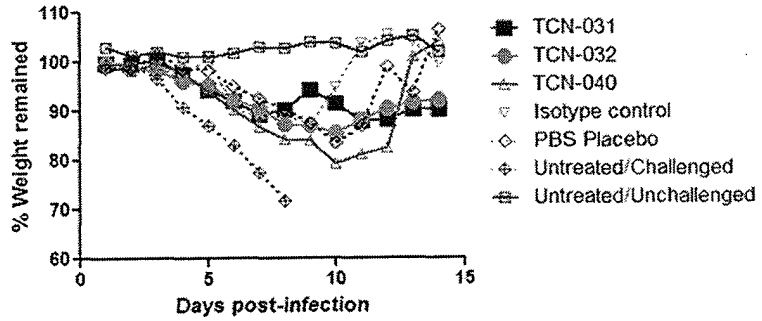
Weight loss in H5N1 flu infection (5 MLD_{50} VN1203/04) after 800 ug Day 3,5,7 treatment with anti-M2e mAbs



Based on death independent of weight loss

FIGURE 54

Weight loss in H5N1 flu infection (5 MLD_{50} VN1203/04) after 800 ug Day 4,6,8 treatment with anti-M2e mAbs



Based on death independent of weight loss

FIGURE 55

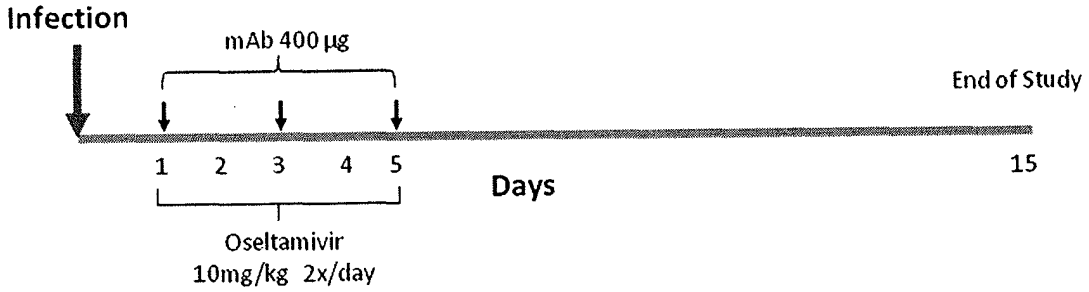
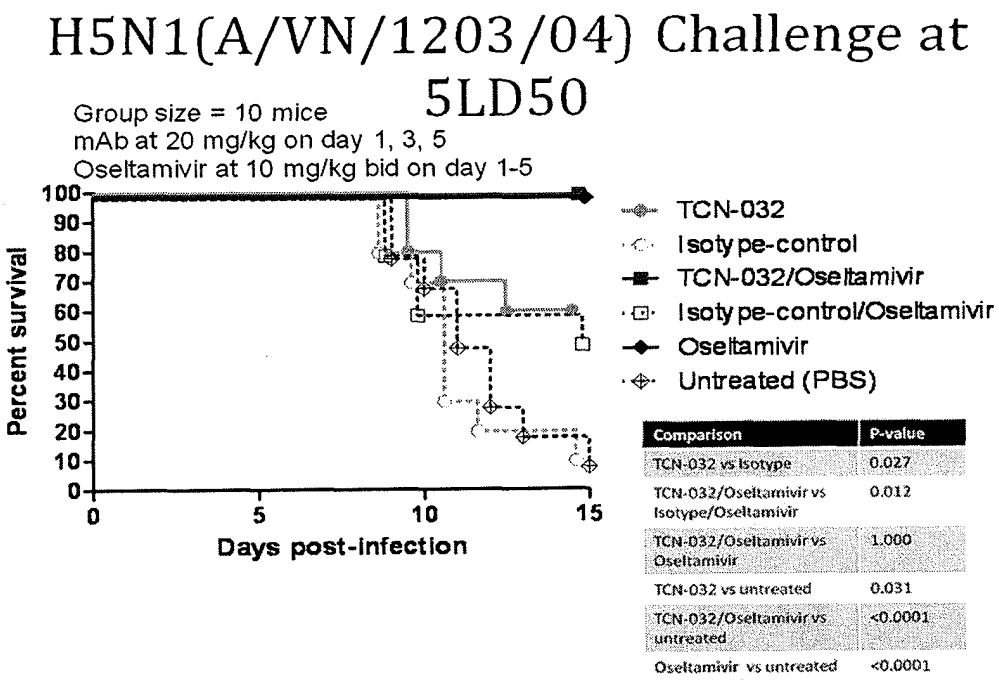


FIGURE 56



Comparison	P-value
TCN-032 vs Isotype	0.027
TCN-032/Oseltamivir vs Isotype/Oseltamivir	0.012
TCN-032/Oseltamivir vs Oseltamivir	1.000
TCN-032 vs untreated	0.031
TCN-032/Oseltamivir vs untreated	<0.0001
Oseltamivir vs untreated	<0.0001

FIGURE 57

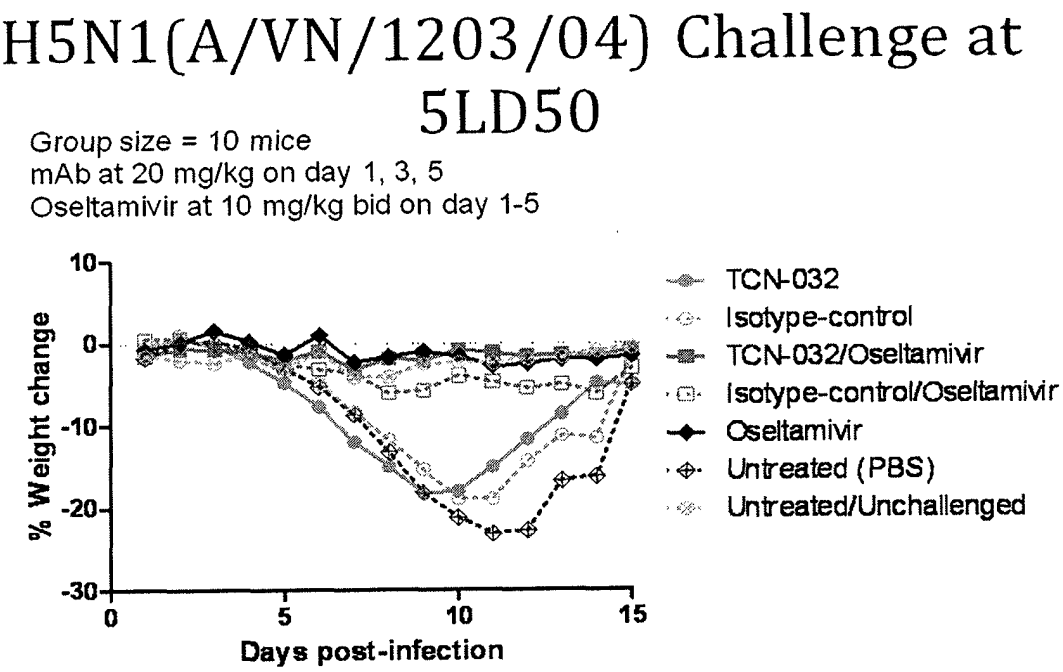
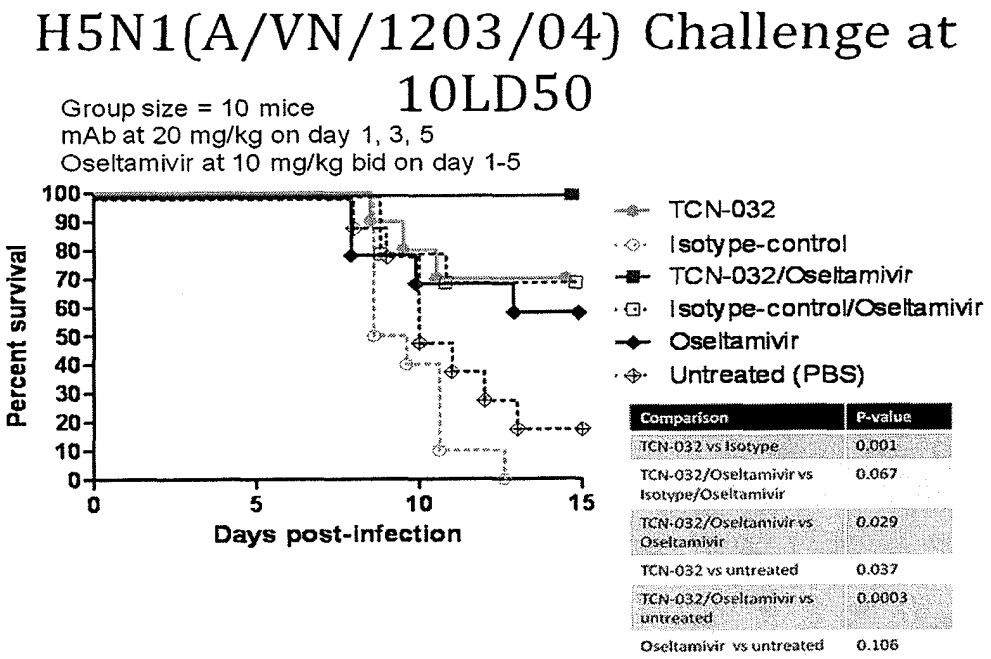


FIGURE 58



Comparison	P-value
TCN-032 vs Isotype	0.001
TCN-032/Oseltamivir vs Isotype/Oseltamivir	0.067
TCN-032/Oseltamivir vs Oseltamivir	0.029
TCN-032 vs untreated	0.037
TCN-032/Oseltamivir vs untreated	0.0003
Oseltamivir vs untreated	0.106

FIGURE 59

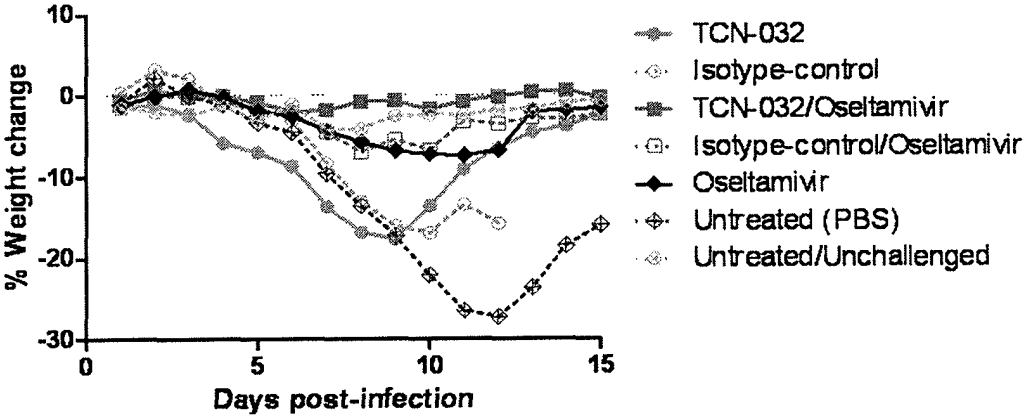


FIGURE 60

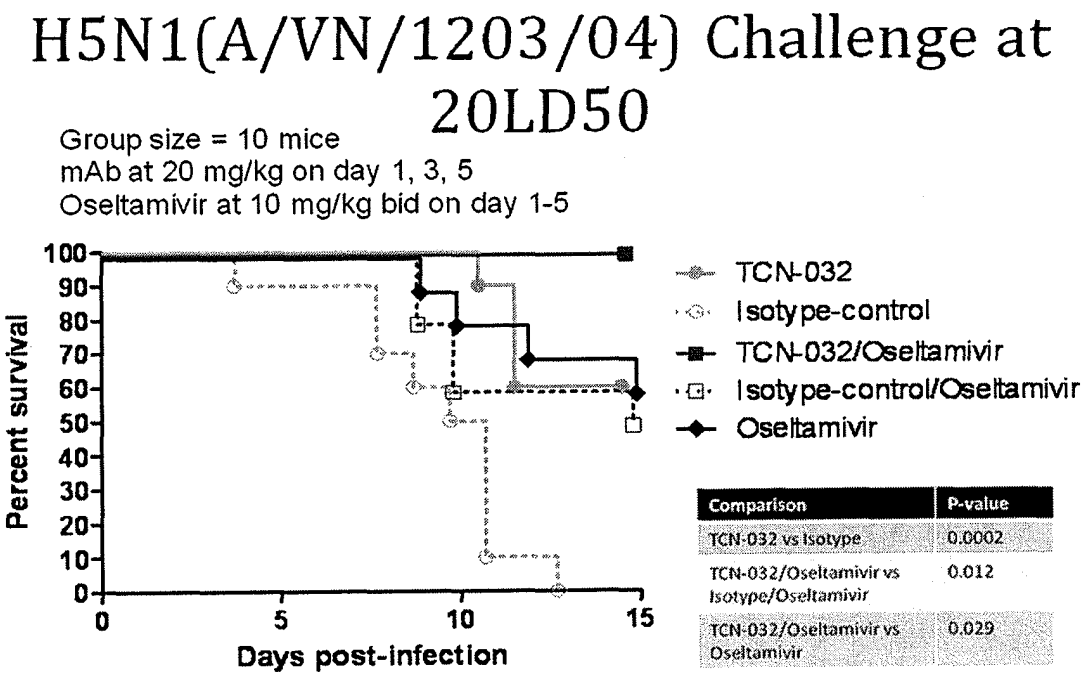


FIGURE 61

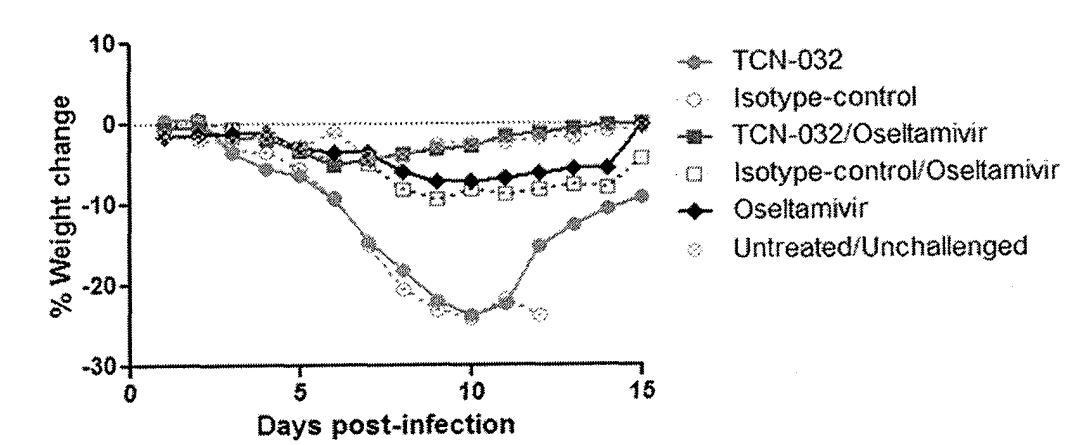


FIGURE 62

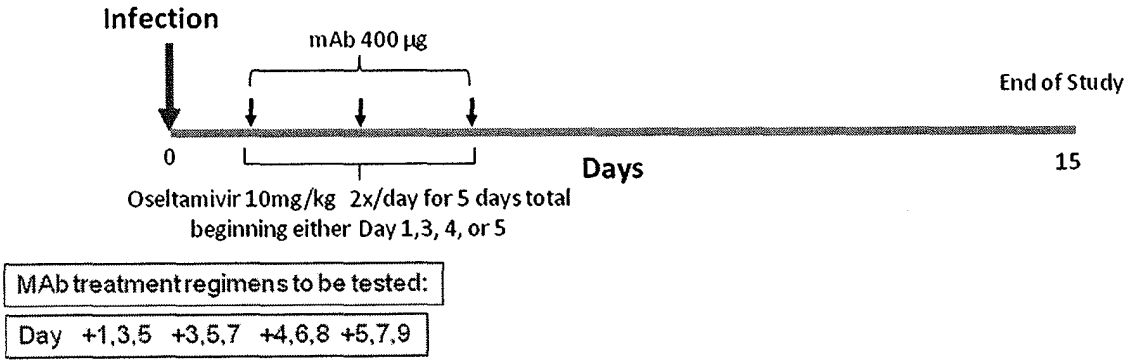

Infection
mAb 400 µg
End of Study
0
Days
15
Oseltamivir 10mg/kg 2x/day for 5 days total
beginning either Day 1,3, 4, or 5
MAb treatment regimens to be tested:
Day +1,3,5 +3,5,7 +4,6,8 +5,7,9

FIGURE 63

H5N1(A/VN/1203/04) Challenge at 20LD50

Group size = 10 mice; mAb at 20 mg/kg; Oseltamivir at 10 mg/kg bid

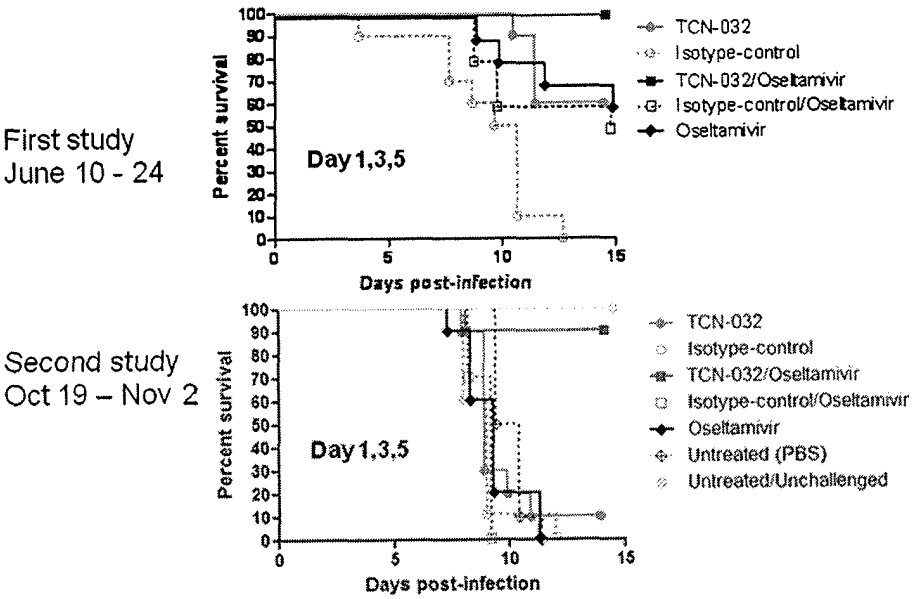


FIGURE 64

H5N1(A/VN/1203/04) Challenge at 20LD50

Group size = 10 mice; mAb at 20 mg/kg; Oseltamivir at 10 mg/kg bid

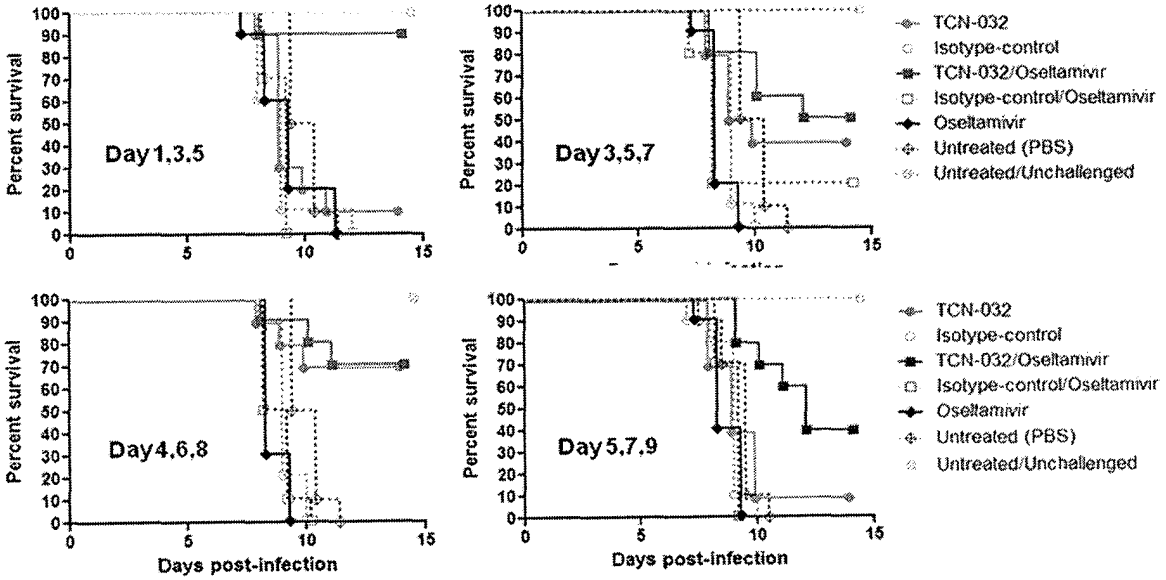


FIGURE 65

H5N1(A/VN/1203/04) Challenge at 20LD50

Group size = 10 mice; mAb at 20 mg/kg; Oseltamivir at 10 mg/kg bid

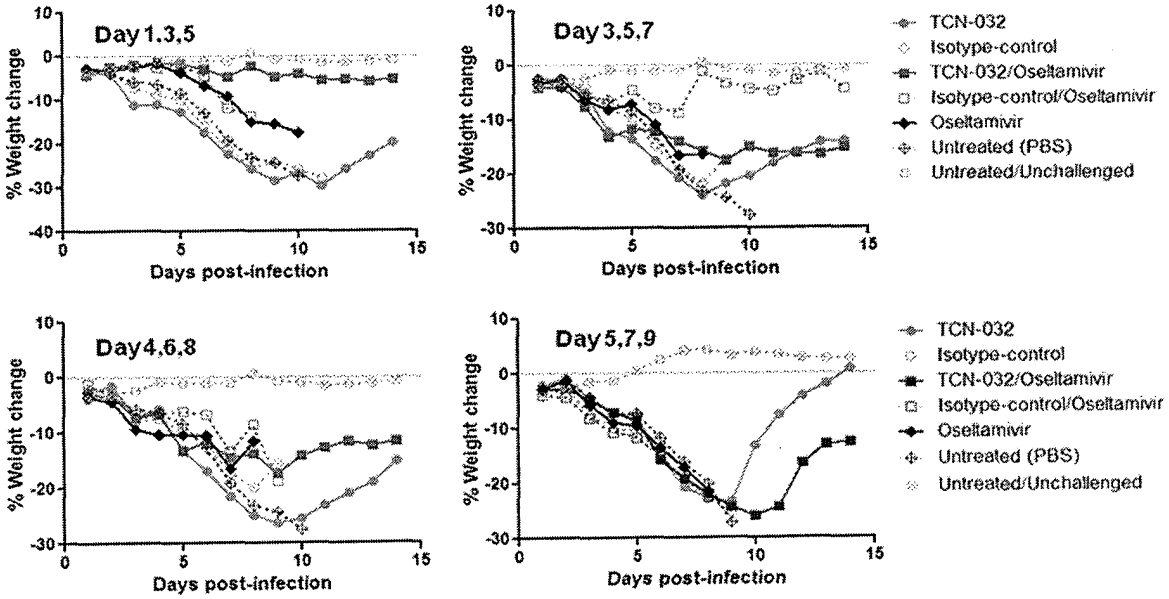


FIGURE 66

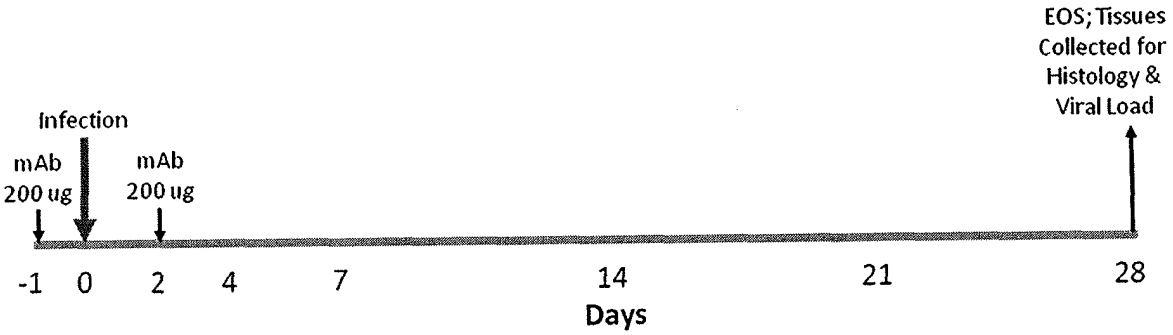


FIGURE 67

TCN Human Anti-M2e mAbs are Protective In Rodent Lethal Challenge Model (H5N1)

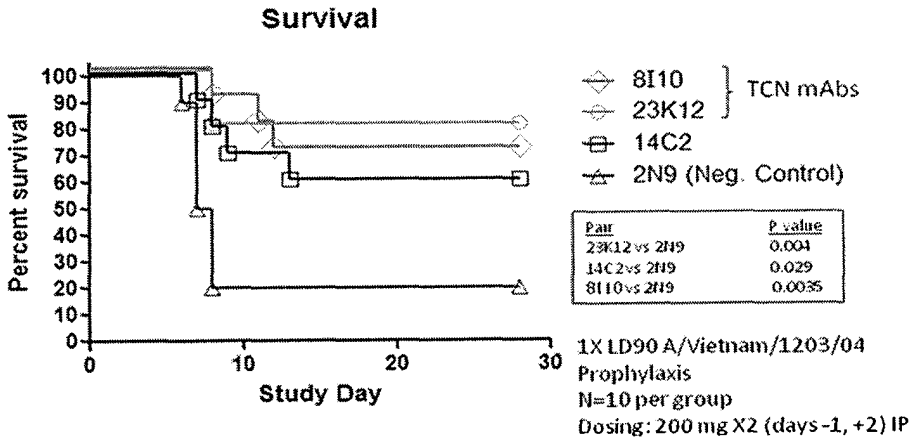


FIGURE 68

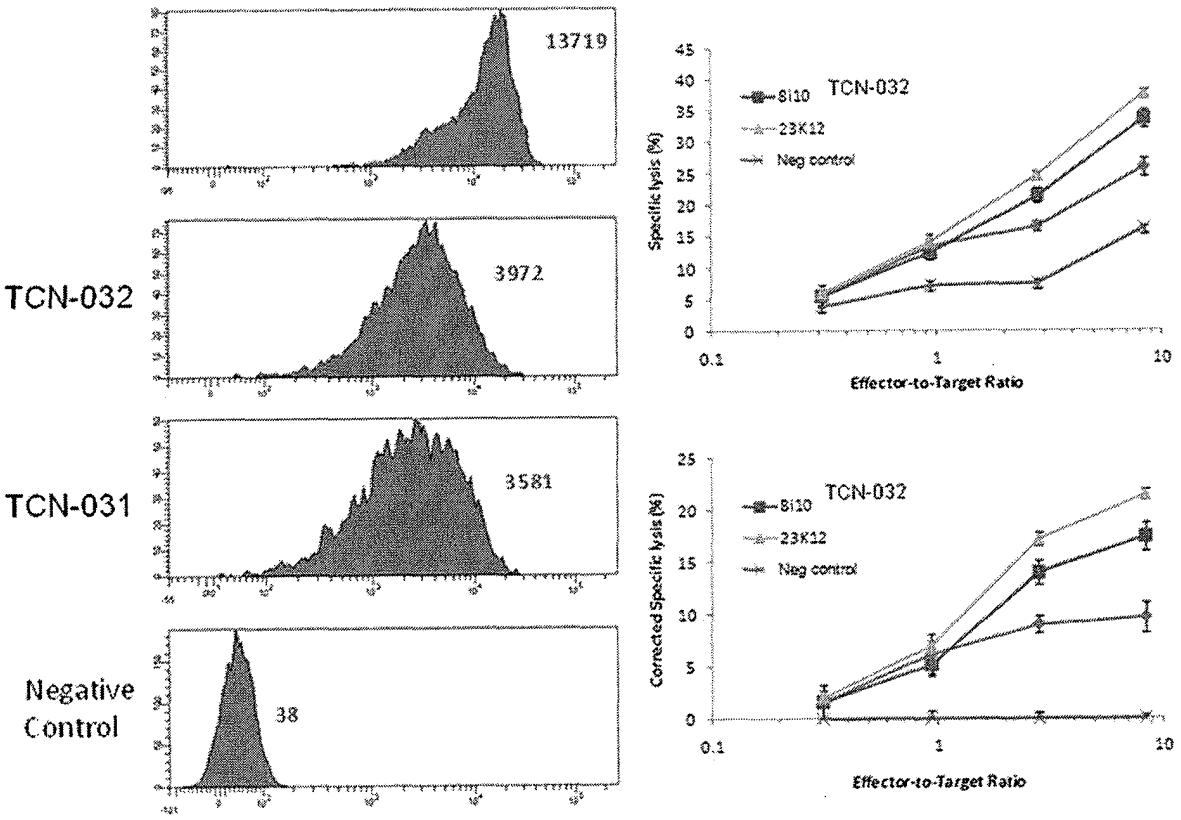


FIGURE 69

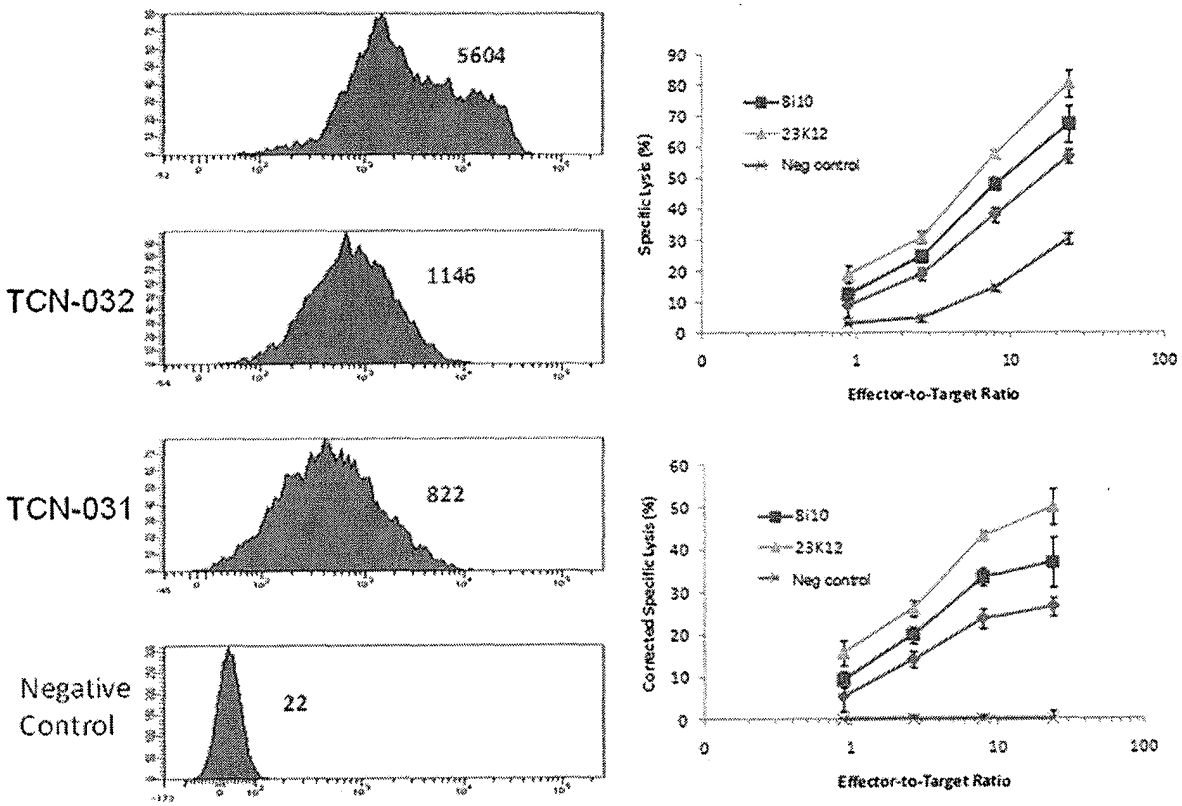

TCN-032
5604
1146
TCN-031
822
Negative Control
22
Specific Lysis (%)
Effector-to-Target Ratio
8i10
23K12
Neg control
Corrected Specific Lysis (%)
Effector-to-Target Ratio
8i10
23K12
Neg control

FIGURE 70

M2e mAbs stain M2 transfectants at 1.25 ug/ml

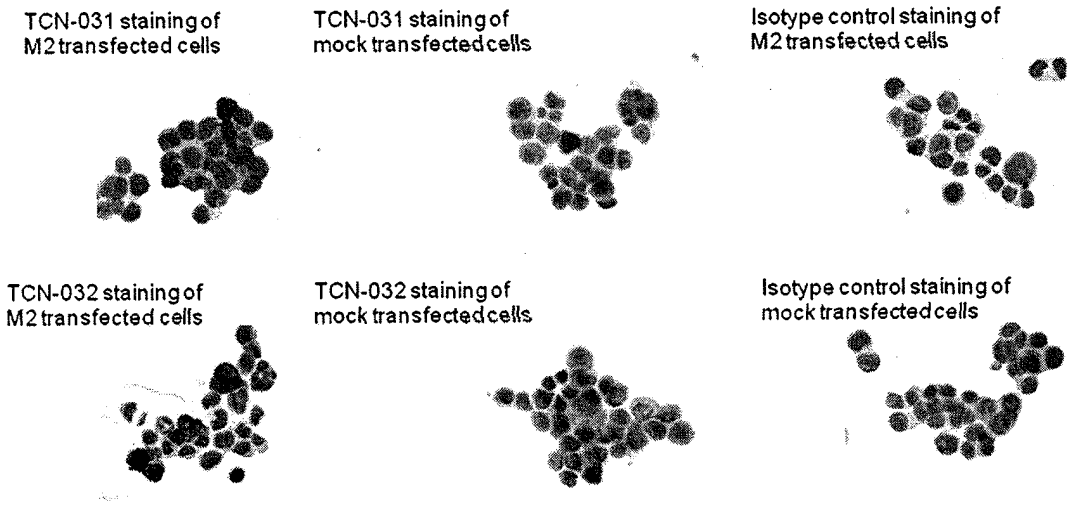


FIGURE 71

M2e mAbs are negative for normal lung tissue staining

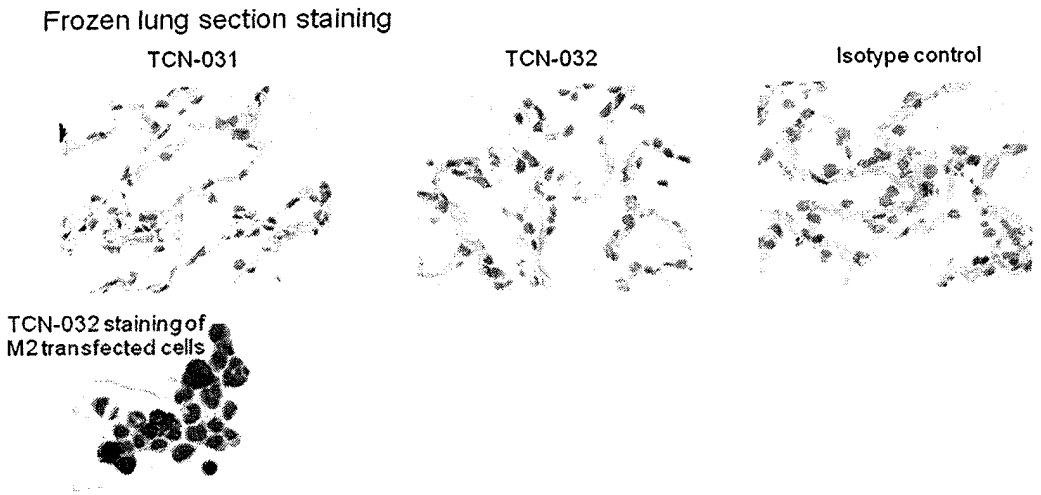


FIGURE 72

96-well CDC Assay Protocol via CellTiter-Glo Luminescence

Pre-incubate DG44.VNM2 with TCN-032

4°C for 1 hr

Wash cells

Add human complement

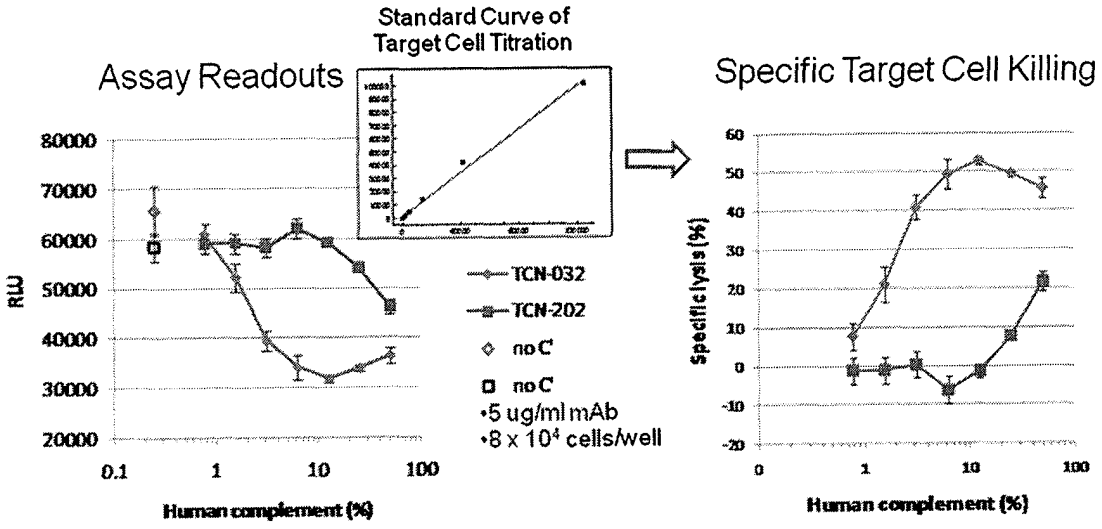
37°C for 2 hr

Add CellTiter-Glo reagent

Quantify RLU on plate reader

FIGURE 73

TCN-032 in 96-well CDC Assay



Maximal target cell lysis was obtained with 5-10% complement (v/v)

FIGURE 74

96-well Homogeneous CDC Assay Protocol via CellTiter-Glo Luminescence

Mix TCN-032, human complement and DG44.VNM2 cells

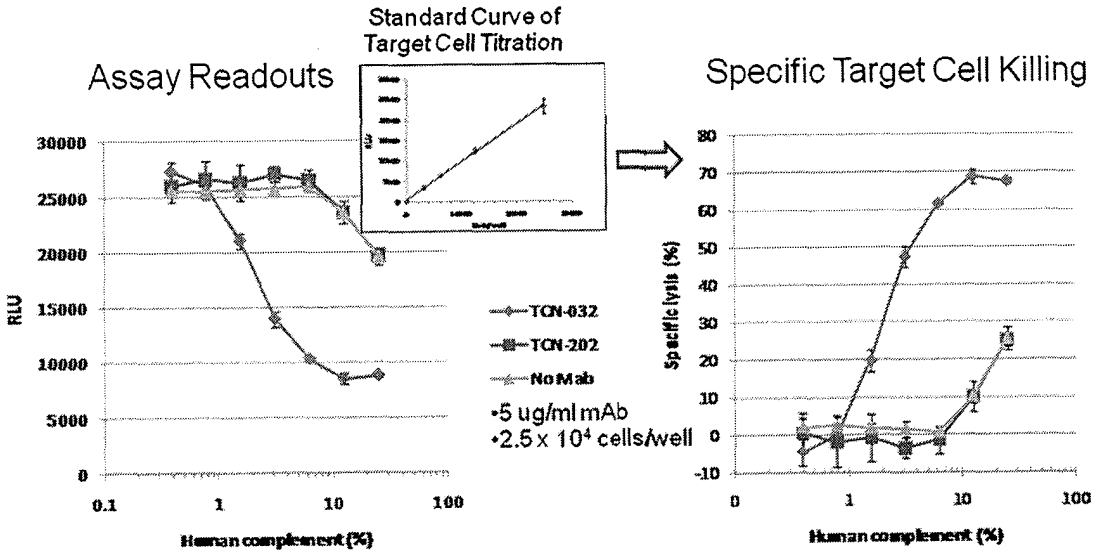
37°C for 2 hr

Add CellTiter-Glo reagent

Quantify RLU on plate reader

FIGURE 75

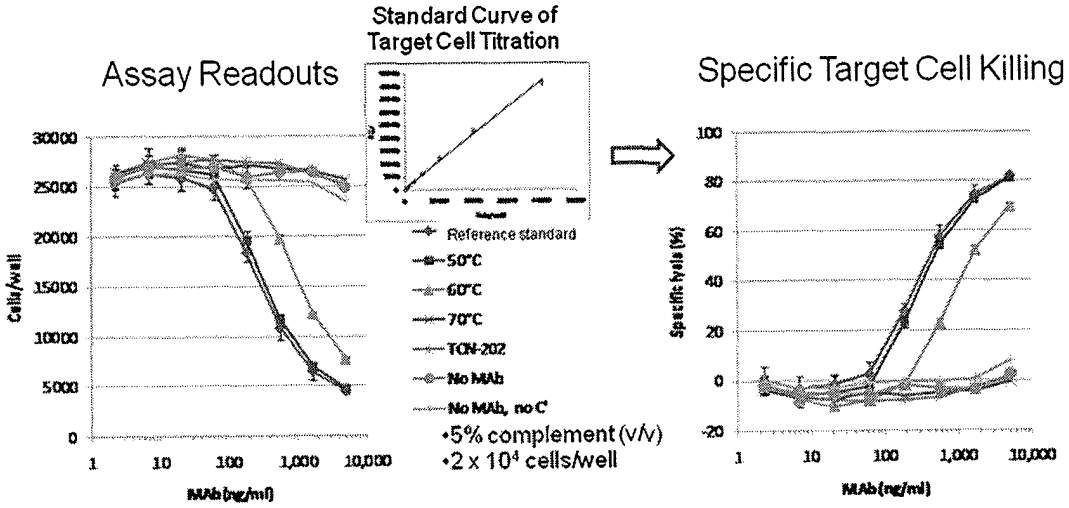
TCN-032 in Homogeneous CDC Assay



Maximal target cell lysis with minimal negligible background lysis was obtained with 6.25% complement (v/v)

FIGURE 76

Analysis of Temperature-stressed TCN-032 in Homogeneous CDC Assay



TCN-032 stressed at >60°C showed diminished CDC activity