Figure 1

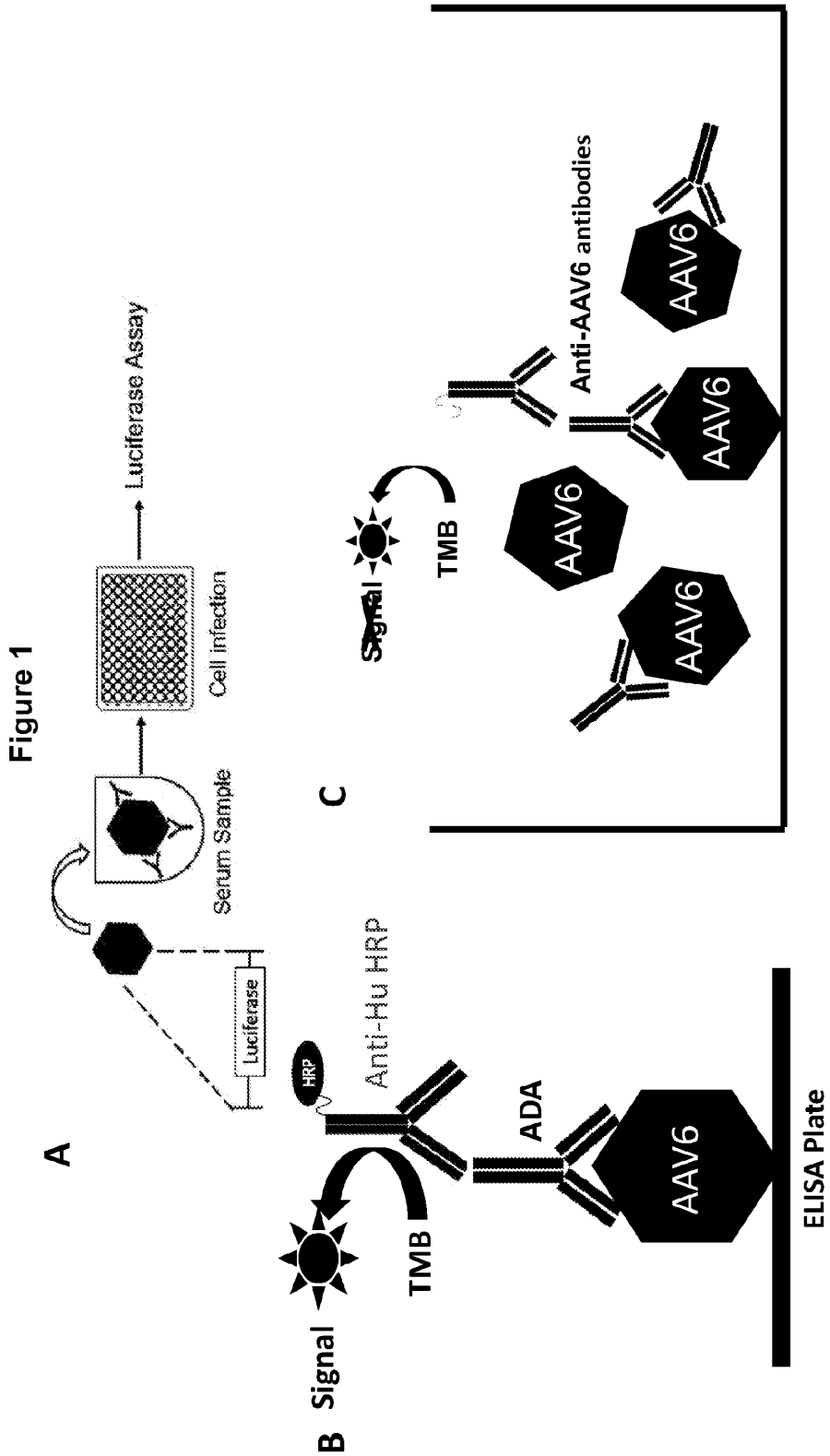

A
Luciferase Assay
Cell infection
Serum Sample
Luciferase
B
Signal
HRP
Anti-Hu HRP
TMB
ADA
AAV6
ELISA Plate
C
Signal
TMB
Anti-AAV6 antibodies
AAV6
AAV6
AAV6
AAV6

Figure 2

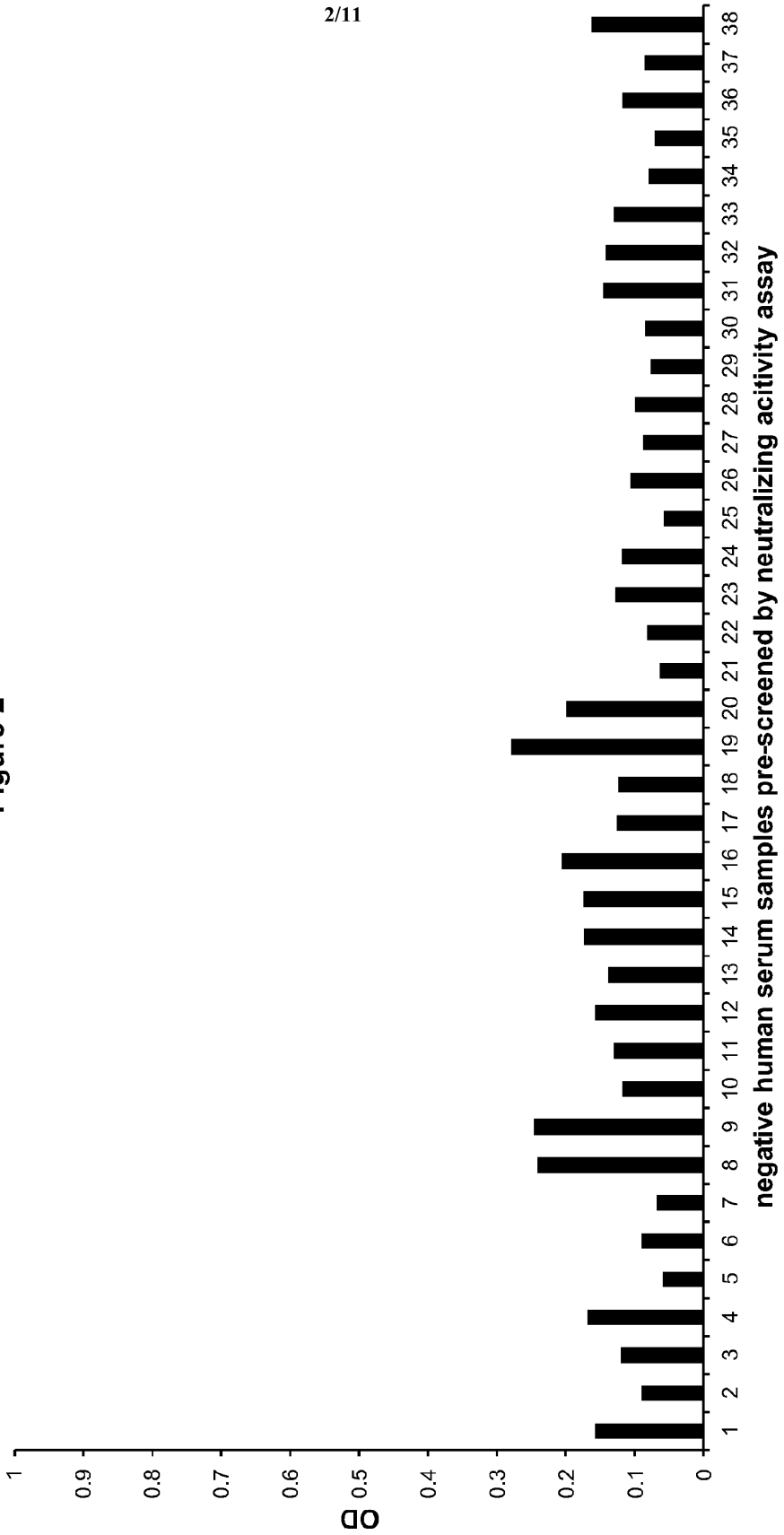

OD
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38
negative human serum samples pre-screened by neutralizing acitivity assay

Figure 3

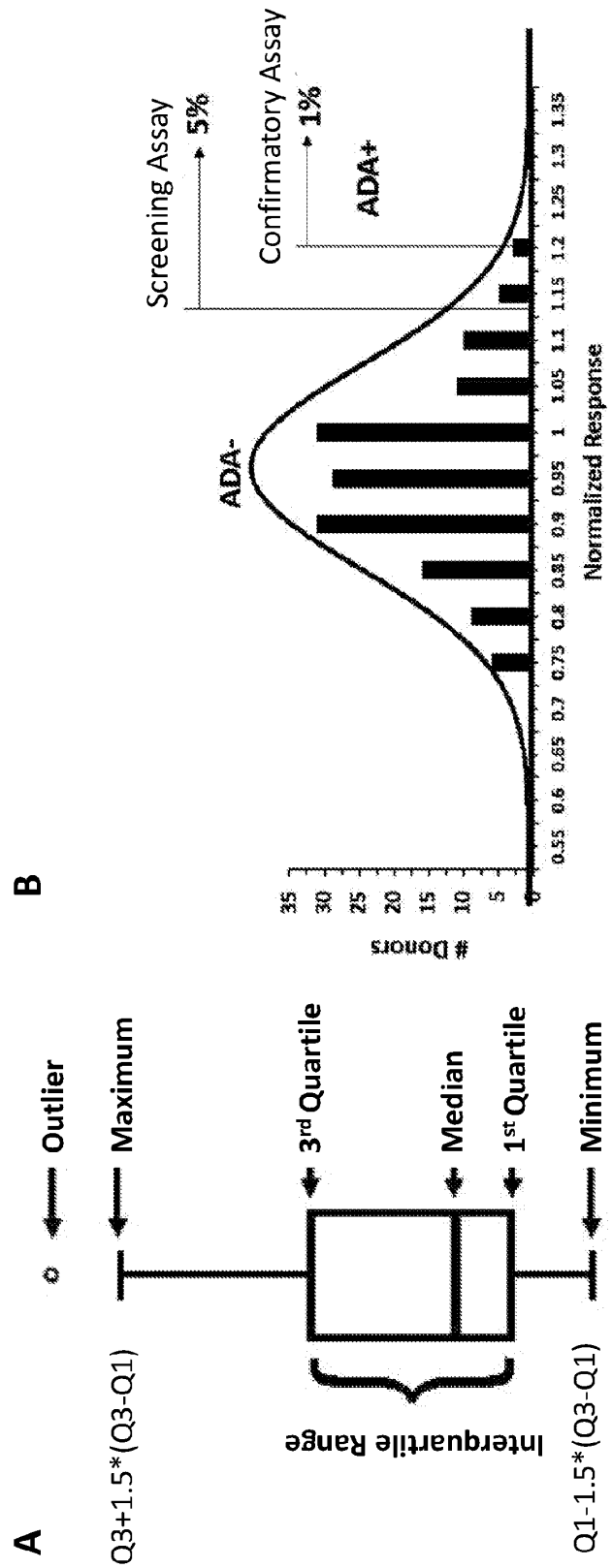

A
Outlier
Maximum
3rd Quartile
Median
1st Quartile
Minimum
Q3+1.5*(Q3-Q1)
Q1-1.5*(Q3-Q1)
Interquartile Range
B
Screening Assay
5%
Confirmatory Assay
1%
ADA+
ADA-
Donors
Normalized Response
0 5 10 15 20 25 30 35
0.55 0.6 0.65 0.7 0.75 0.8 0.85 0.9 0.95 1 1.05 1.1 1.15 1.2 1.25 1.3 1.35

Figure 4

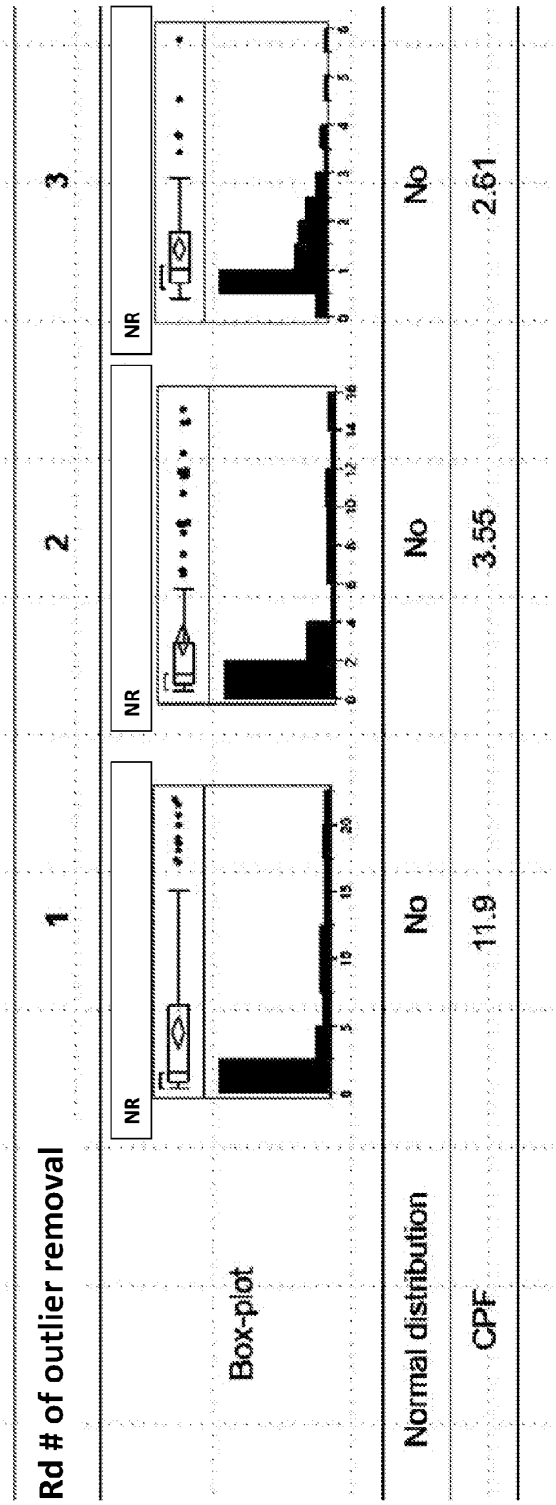

A
Screening assay
Rd # of outlier removal
1
2
3
NR
NR
NR
Box-plot
Normal distribution
No
No
No
CPF
11.9
3.55
2.61

Figure 4

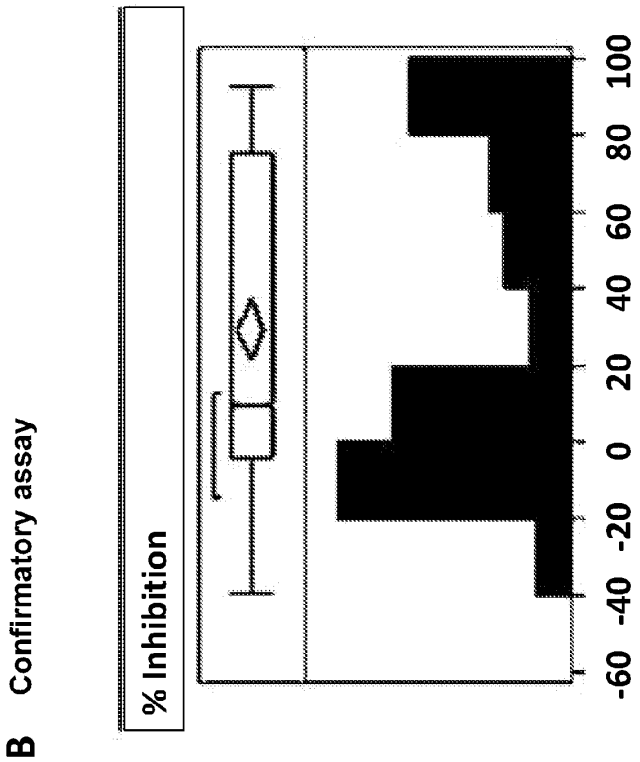
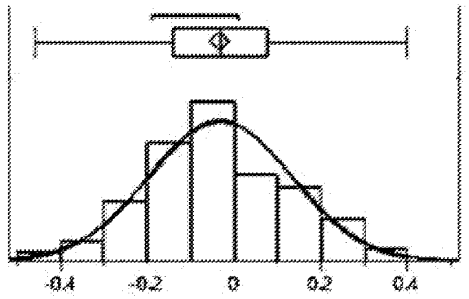


Figure 5A

Distributions

LogNR



Quantiles		
100.0%	maximum	0.3975160999
99.5%		0.3961949968
97.5%		0.2973620563
90.0%		0.1957929723
75.0%	quartile	0.0781425295
50.0%	median	-0.031389793
25.0%	quartile	-0.139694149
10.0%		-0.228019804
2.5%		-0.356265953
0.5%		-0.457897518
0.0%	minimum	-0.45813997

Summary Statistics	
Mean	-0.030174
Std Dev	0.1637422
Std Err Mean	0.0114085
Upper 95% Mean	-0.007681
Lower 95% Mean	-0.052667
N	206

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	-0.030174	-0.052667	-0.007681
Dispersion	σ	0.1637422	0.1493101	0.181287

Measure	
-2*LogLikelihood	-161.8956
AICc	-157.8365
BIC	-151.2398

Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.992401	0.3637

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

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Figure 5B

Distributions

% inhibition

Normal(1.77581,9.75368)

Quantiles		
100.0%	maximum	25.386040766
99.5%		25.377495908
97.5%		21.472473445
90.0%		13.356135468
75.0%	quartile	8.9434001729
50.0%	median	2.7354020891
25.0%	quartile	-5.039460585
10.0%		-12.21646571
2.5%		-18.65174216
0.5%		-24.66458332
0.0%	minimum	24.68152866

Summary Statistics	
Mean	1.7758085
Std Dev	9.7536841
Std Err Mean	0.6896896
Upper 95% Mean	3.1358464
Lower 95% Mean	0.4157705
N	200

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	1.7758085	0.4157705	3.1358464
Dispersion	σ	9.7536841	8.8823133	10.816103

Measure	
-2*LogLikelihood	1477.6334
AICc	1481.6944
BIC	1488.2301

Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.990307	0.1981

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Figure 6

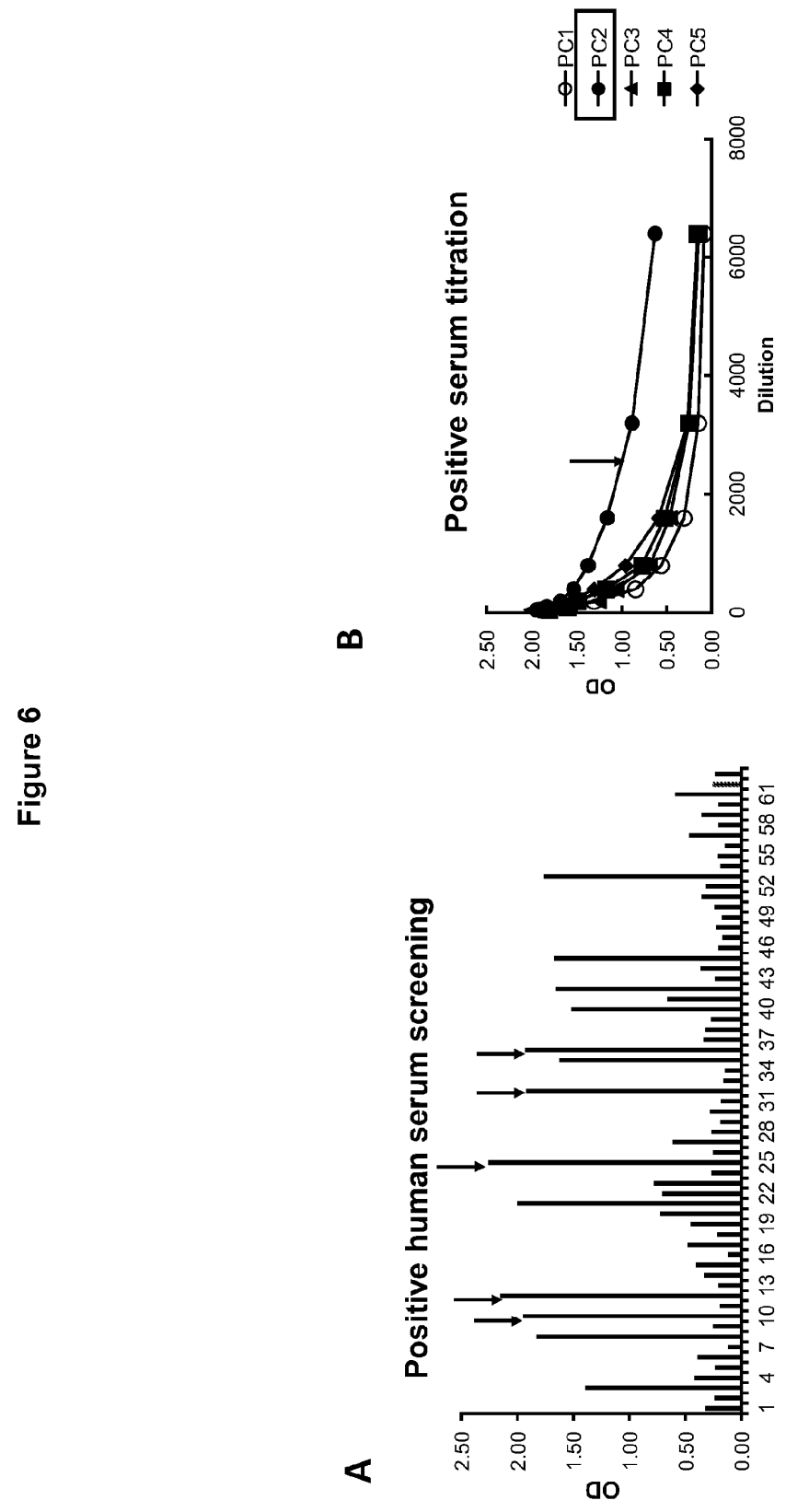

A
Positive human serum screening
OD
2.50
2.00
1.50
1.00
0.50
0.00
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61
B
Positive serum titration
OD
2.50
2.00
1.50
1.00
0.50
0.00
0 2000 4000 6000 8000
Dilution
PC1
PC2
PC3
PC4
PC5

Figure 7

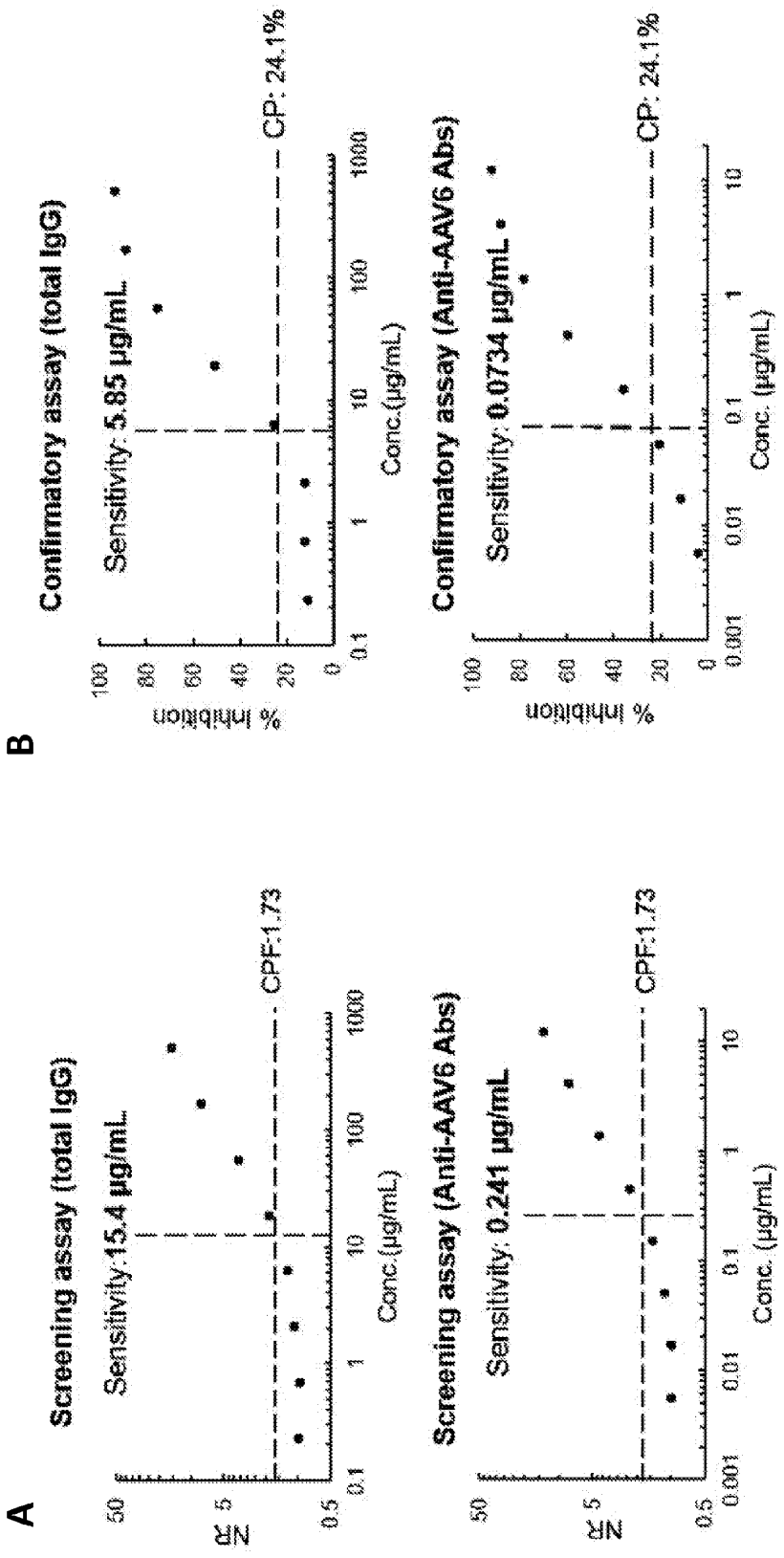

A
Screening assay (total IgG)
Sensitivity: 15.4 µg/mL
CPF: 1.73
NR
Conc. (µg/mL)
Screening assay (Anti-AAV6 Abs)
Sensitivity: 0.241 µg/mL
CPF: 1.73
NR
Conc. (µg/mL)
B
Confirmatory assay (total IgG)
Sensitivity: 5.85 µg/mL
CP: 24.1%
% Inhibition
Conc. (µg/mL)
Confirmatory assay (Anti-AAV6 Abs)
Sensitivity: 0.0734 µg/mL
CP: 24.1%
% Inhibition
Conc. (µg/mL)

Figure 8

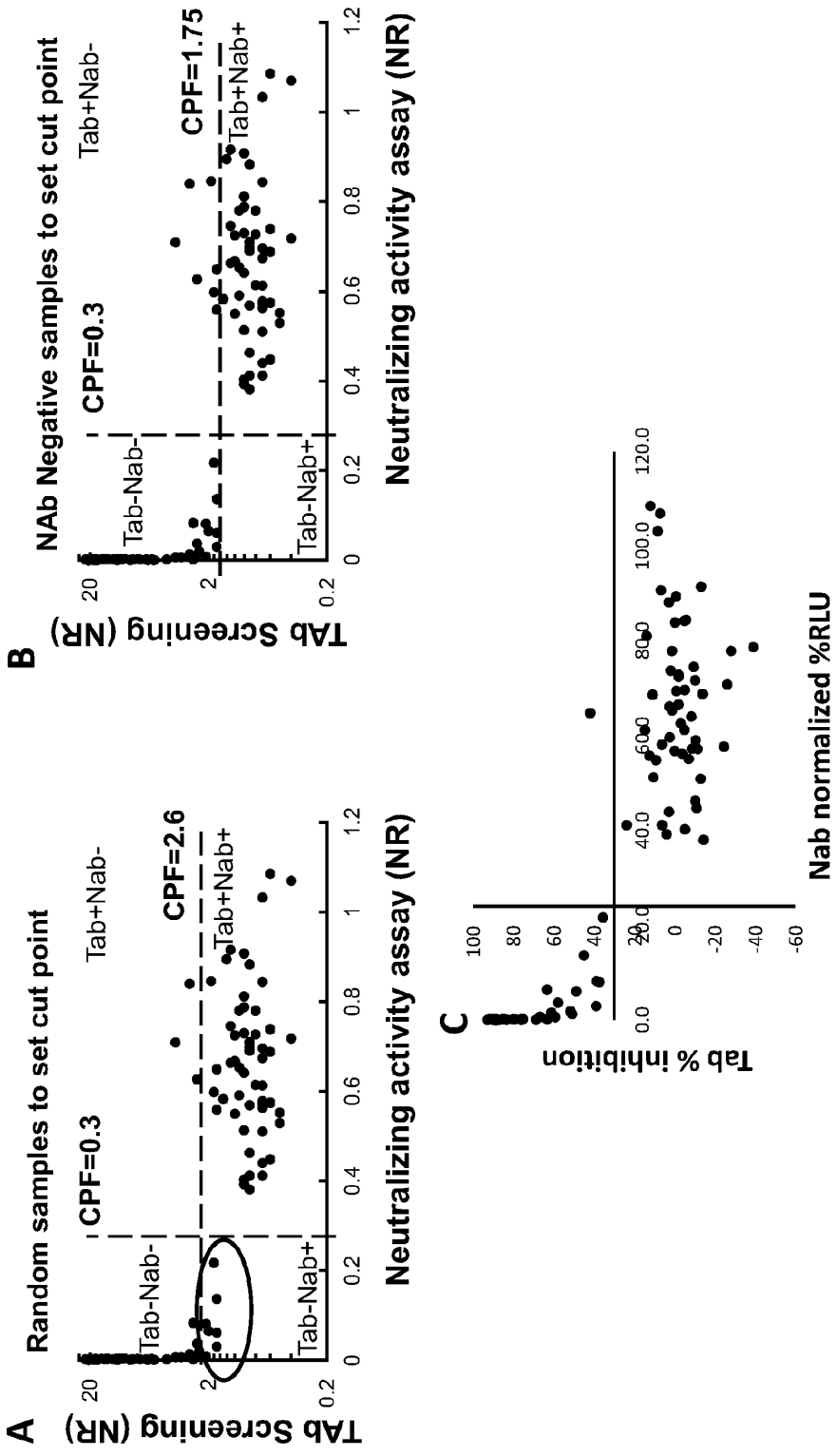
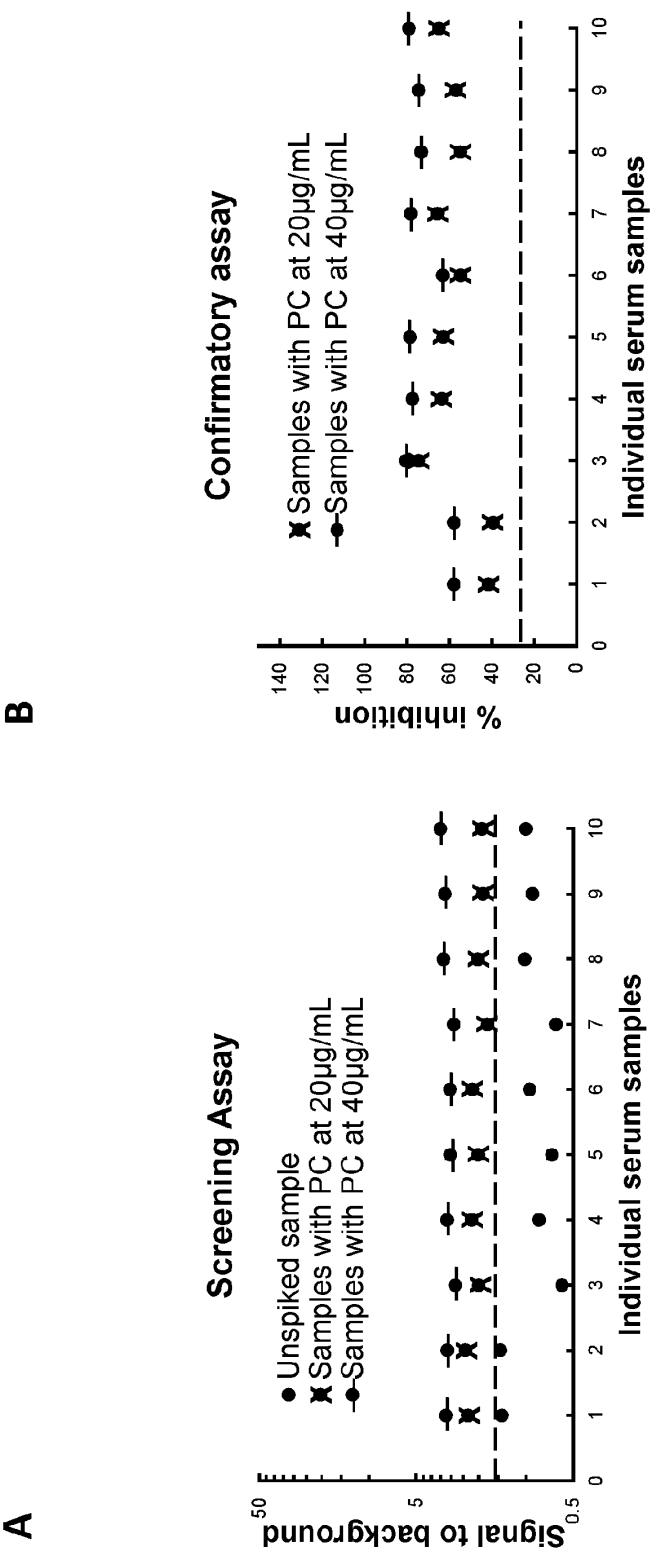

A
Random samples to set cut point
CPF=0.3
CPF=2.6
Tab+Nab-
Tab+Nab+
Tab-Nab-
Tab-Nab+
TAb Screening (NR)
Neutralizing activity assay (NR)
B
NAb Negative samples to set cut point
CPF=0.3
CPF=1.75
Tab+Nab-
Tab+Nab+
Tab-Nab-
Tab-Nab+
TAb Screening (NR)
Neutralizing activity assay (NR)
C
Tab % inhibition
Nab normalized %RLU

Figure 9


A
Screening Assay
Unspiked sample
Samples with PC at 20µg/mL
Samples with PC at 40µg/mL
Signal to background
50
5
0.5
0 1 2 3 4 5 6 7 8 9 10
Individual serum samples
B
Confirmatory assay
Samples with PC at 20µg/mL
Samples with PC at 40µg/mL
% inhibition
140 120 100 80 60 40 20 0
0 1 2 3 4 5 6 7 8 9 10
Individual serum samples