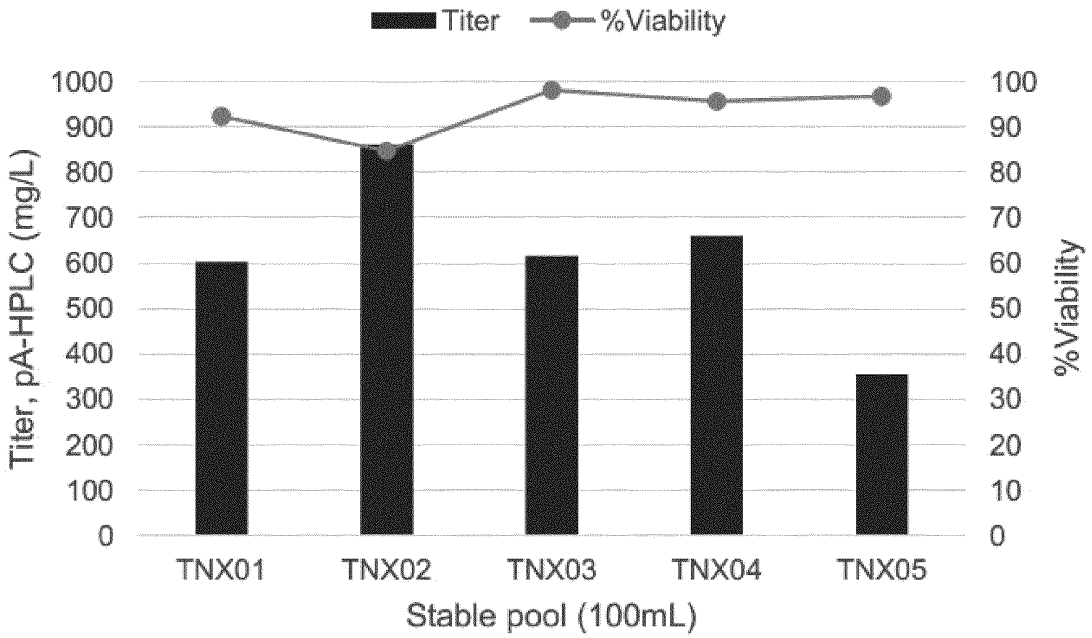
Figure 1



TNX01_HumIgG1_HC_N297Q
TNX02_HumIgG1_HC_wt
TNX03_HumIgG1_HC_N297G
TNX04_HumIgG1_HC_Fc silent (C220S, C226S, C229S, P238S)
TNX05_HumIgG4_HC_S228P, L235A

Figure 2

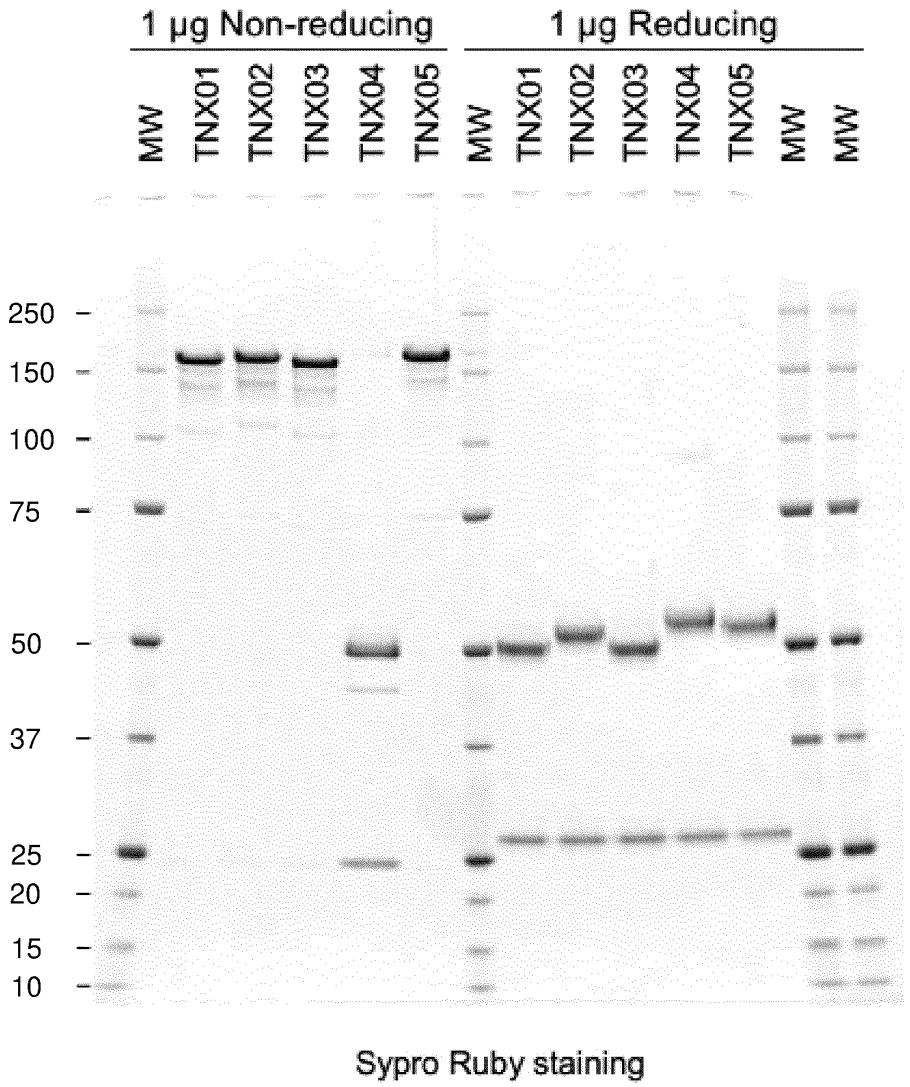

1 µg Non-reducing
1 µg Reducing
MW
TNX01
TNX02
TNX03
TNX04
TNX05
MW
TNX01
TNX02
TNX03
TNX04
TNX05
MW
MW
250
150
100
75
50
37
25
20
15
10
Sypro Ruby staining

Figure 3

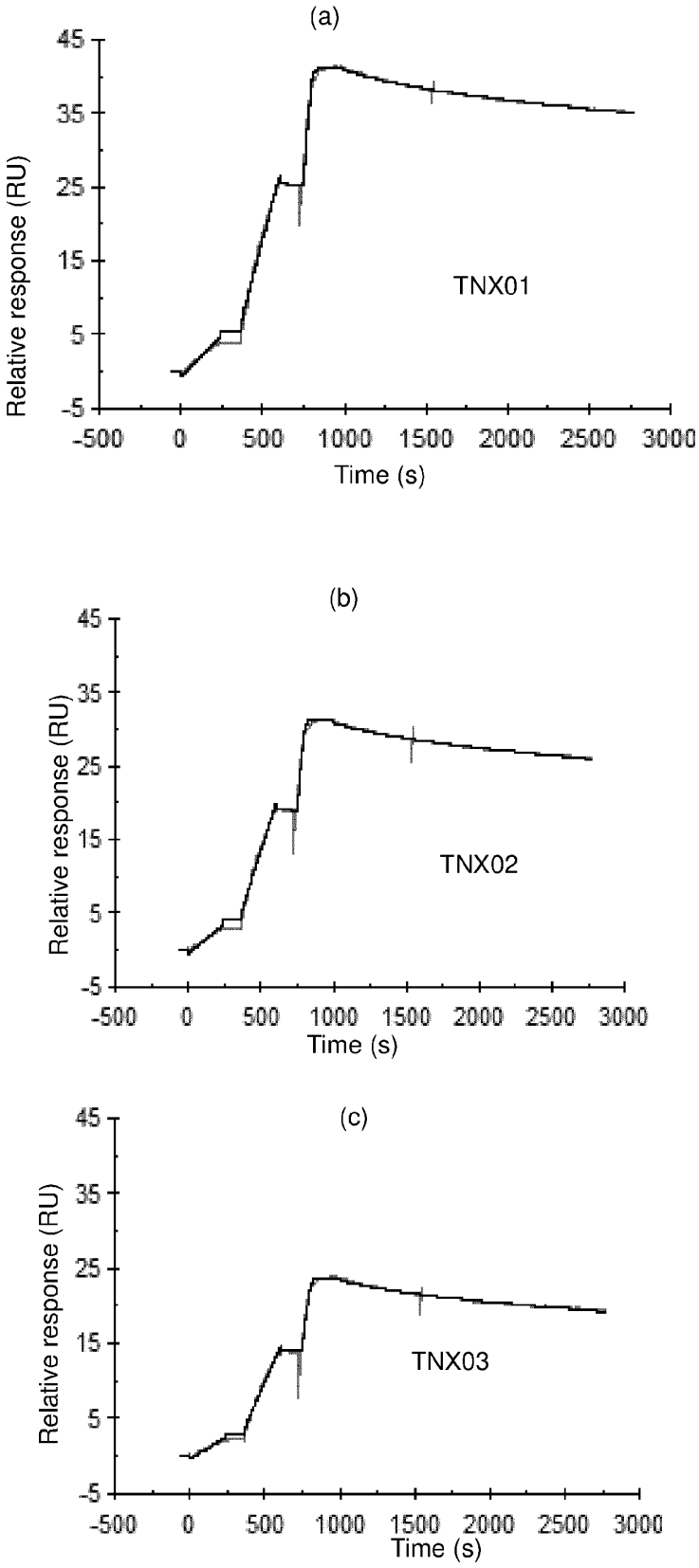
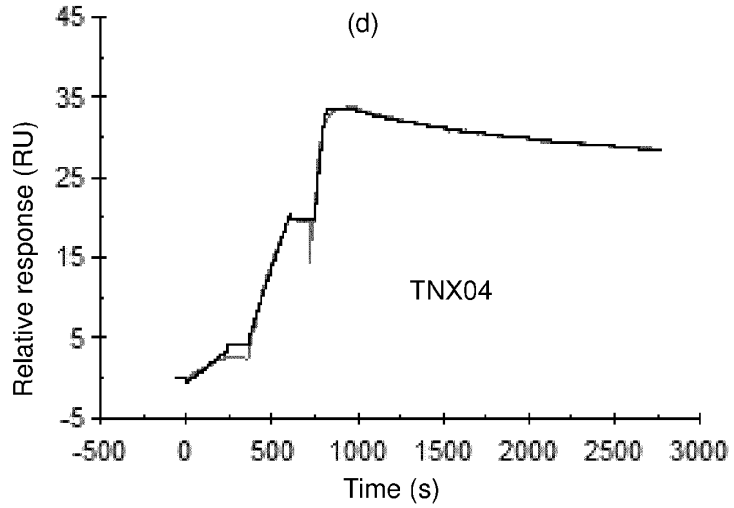
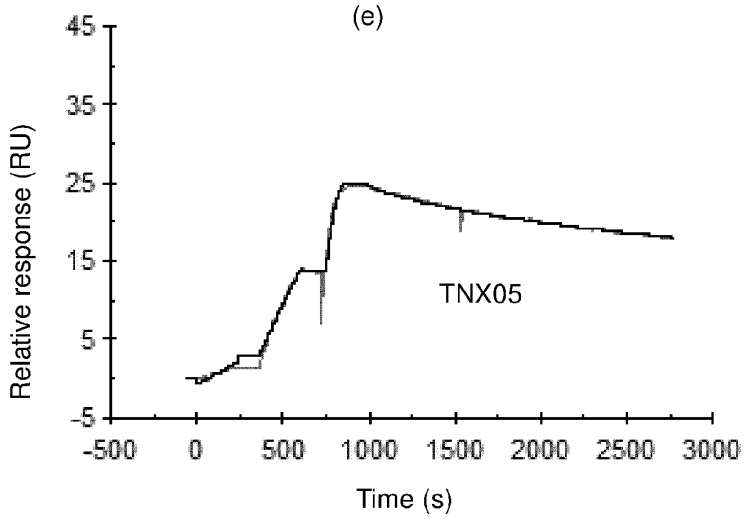

(a)
Relative response (RU)
45
35
25
15
5
-5
-500
0
500
1000
1500
2000
2500
3000
Time (s)
TNX01
(b)
Relative response (RU)
Time (s)
TNX02
(c)
Relative response (RU)
Time (s)
TNX03

Figure 3





(f)

	Ka (1/Ms)	Kd (1/s)	KD (M)
TNX01	2.683E+7	2.040E-4	7.603E-12
TNX02	2.379E+7	2.322E-4	9.759E-12
TNX03	2.147E+7	2.666E-4	1.242E-11
TNX04	2.597E+7	2.245E-4	8.643E-12
TNX05	1.377E+7	4.189E-4	3.043E-11

Figure 4 : Anti-CD154 CD40L Binding assessment by SPR

(a)

mAb	KD Ave (M)	SD	Fold weaker TX02
TNX01	8.00E-12	5.E-13	0.80
TNX02	1.00E-11	1.E-12	1.00
TNX03	1.3E-11	1.E-12	1.30
TNX04	9.5E-12	1.E-12	0.95
TNX05	2.3E-11	6.E-12	2.30

TNX01_HumIgG1_HC_N297Q
TNX02_HumIgG1_HC_wt
TNX03_HumIgG1_HC_N297G
TNX04_HumIgG1_HC_Fc silent (C220S, C226S, C229S, P238S)
TNX05_HumIgG4_HC_S228P, L235A

(b)

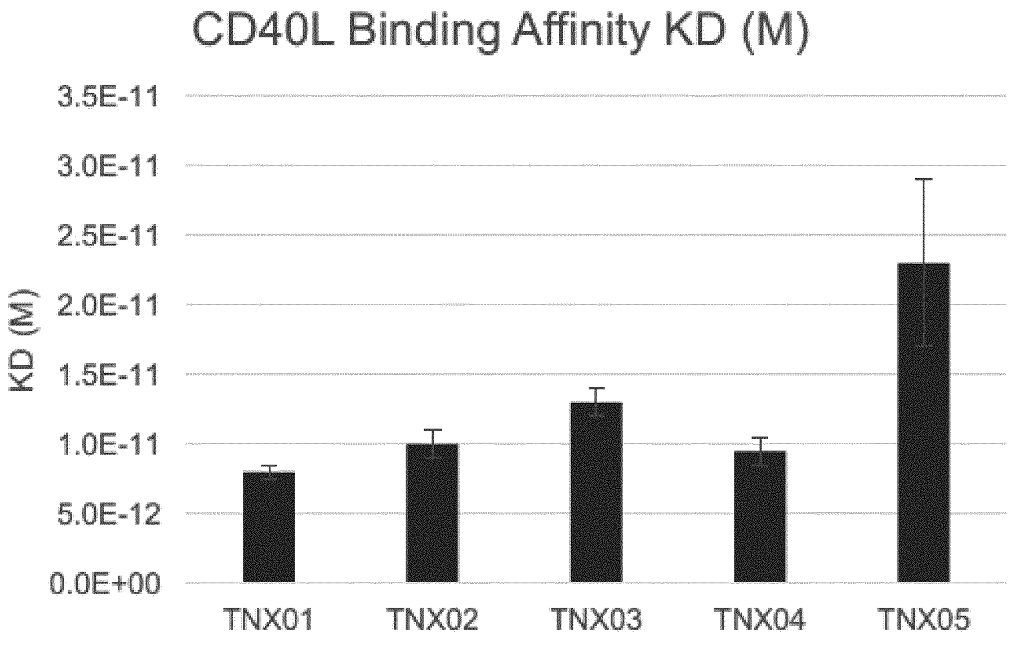


Figure 5

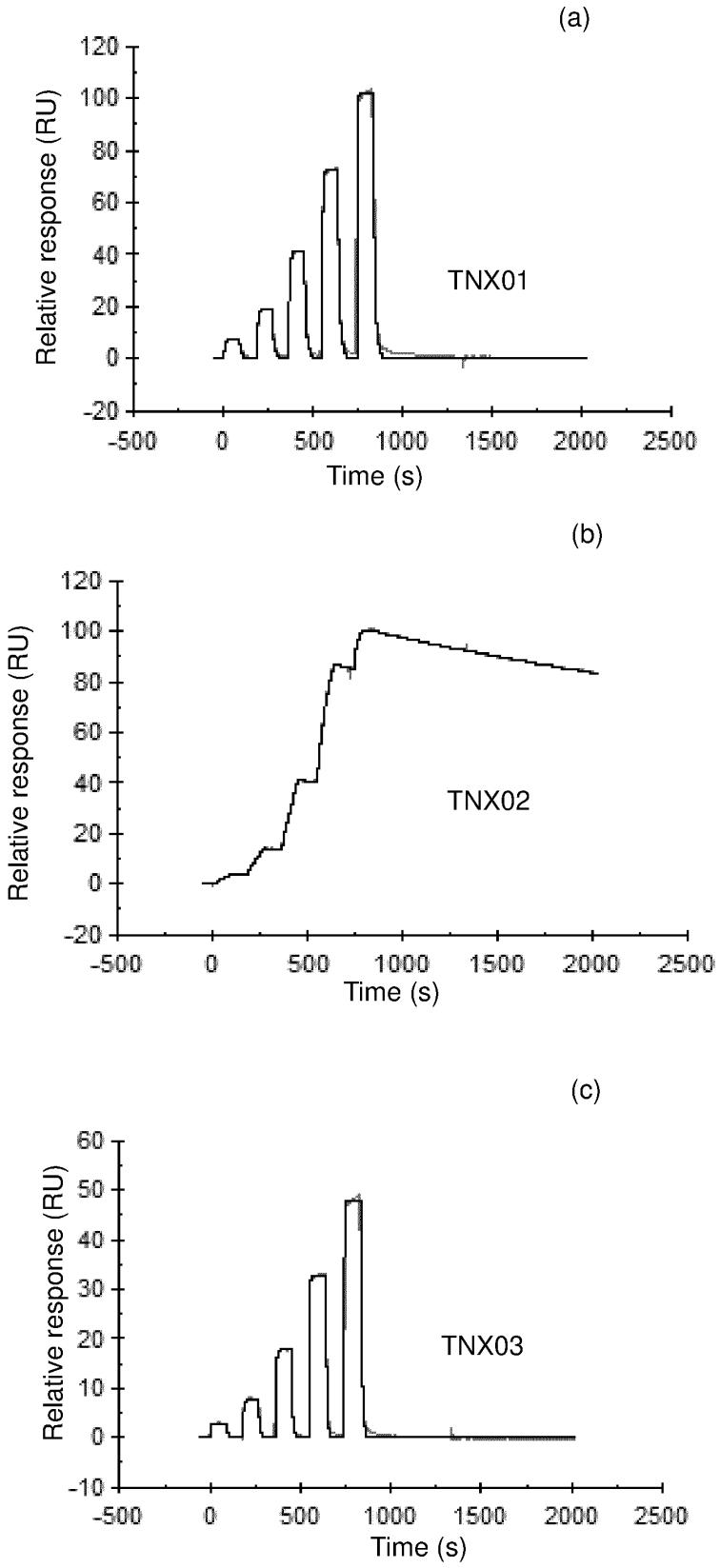
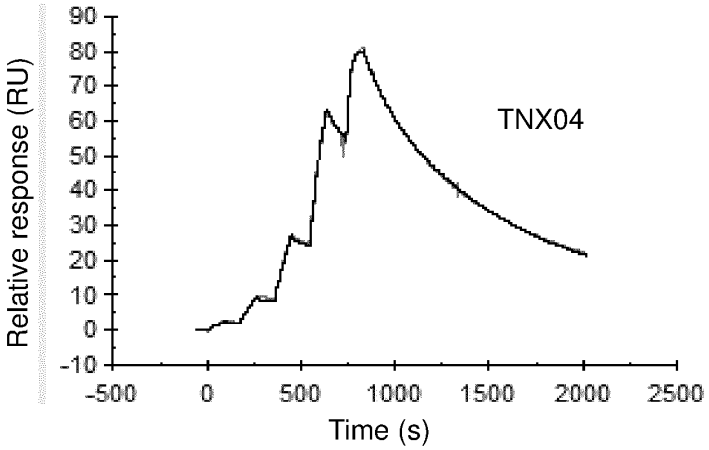
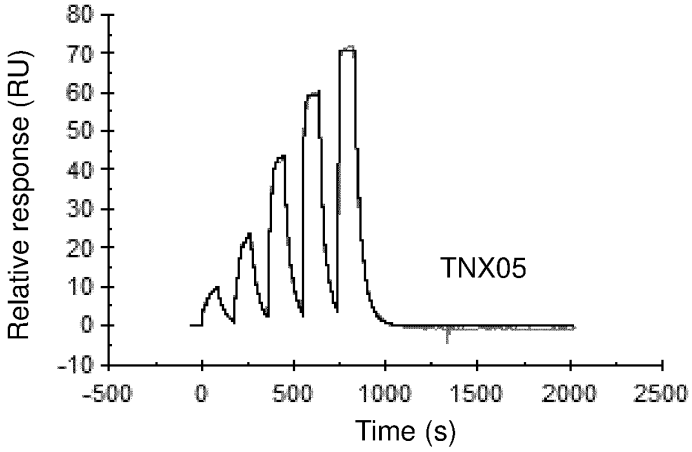

(a)
Relative response (RU)
Time (s)
TNX01
(b)
Relative response (RU)
Time (s)
TNX02
(c)
Relative response (RU)
Time (s)
TNX03

Figure 5

(d)



(e)



(f)

	Ka (1/Ms)	Kd (1/s)	KD (M)
TNX01	1.539E+6	0.08620	5.601E-8
TNX02	5.934E+6	1.710E-4	2.882E-11
TNX03	1.583E+6	0.1422	8.980E-8
TNX04	7.028E+6	0.002172	3.090E-10
TNX05	5.803E+6	0.05108	8.803E-9

Figure 6 : Anti-CD154 FcγRIA (CD64) binding assessment by SPR

(a)

mAb	KD Ave (M)	SD	Fold weaker TNX02
TNX01	7.65E-08	1.E-09	2602
TNX02	2.94E-11	7.E-13	1
TNX03	9.2E-08	1.E-09	3121
TNX04	3.3E-10	1.E-11	11
TNX05	8.7E-09	3.E-10	297

TNX01_HumIgG1_HC_N297Q
TNX02_HumIgG1_HC_wt
TNX03_HumIgG1_HC_N297G
TNX04_HumIgG1_HC_Fc silent (C220S, C226S, C229S, P238S)
TNX05_HumIgG4_HC_S228P, L235A

(b)

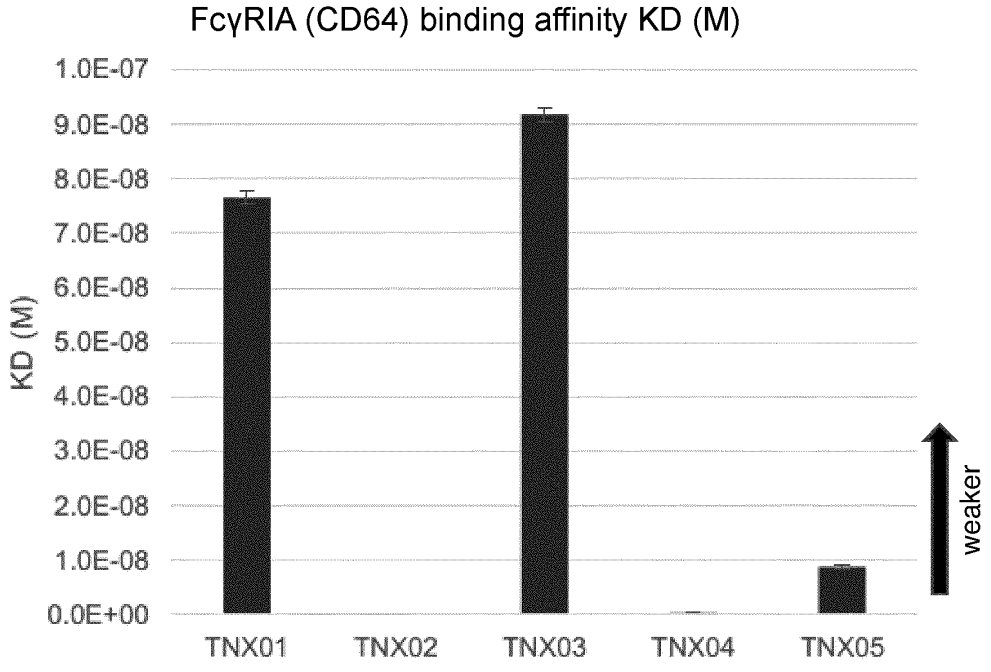


Figure 7 : Anti-CD154 Low-Affinity FcγR binding assessment by SPR

(a)

Mean KD (M)	TNX01	TNX02	TNX03	TNX04	TNX05
CD16aF	>> 5E-06	1.7E-06	>> 5E-06	>> 5E-06	8.115E-06
CD16aV	>> 5E-06	3.2E-07	>> 5E-06	1.87E-05	6.04E-06
CD32aH	>> 12E-06	5.0E-07	>> 12E-06	3.66E-05	7.17E-06
CD32bF	>> 12E-06	9.1E-07	>> 12E-06	1.8E-05	4.9E-06

TNX01_HumIgG1_HC_N297Q
TNX02_HumIgG1_HC_wt
TNX03_HumIgG1_HC_N297G
TNX04_HumIgG1_HC_Fc silent (C220S, C226S, C229S, P238S)
TNX05_HumIgG4_HC_S228P, L235A

(b)

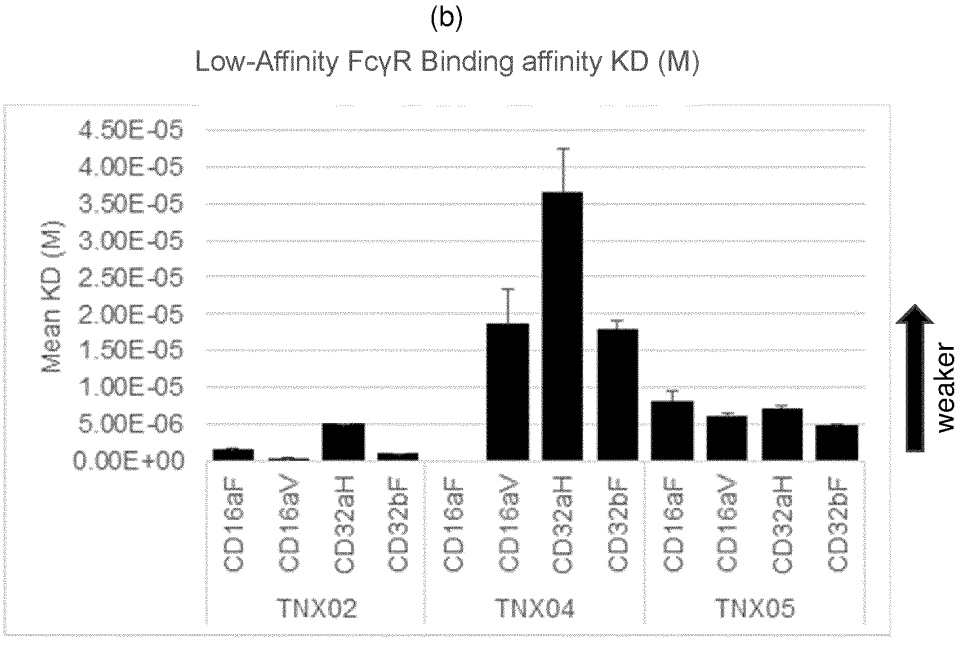
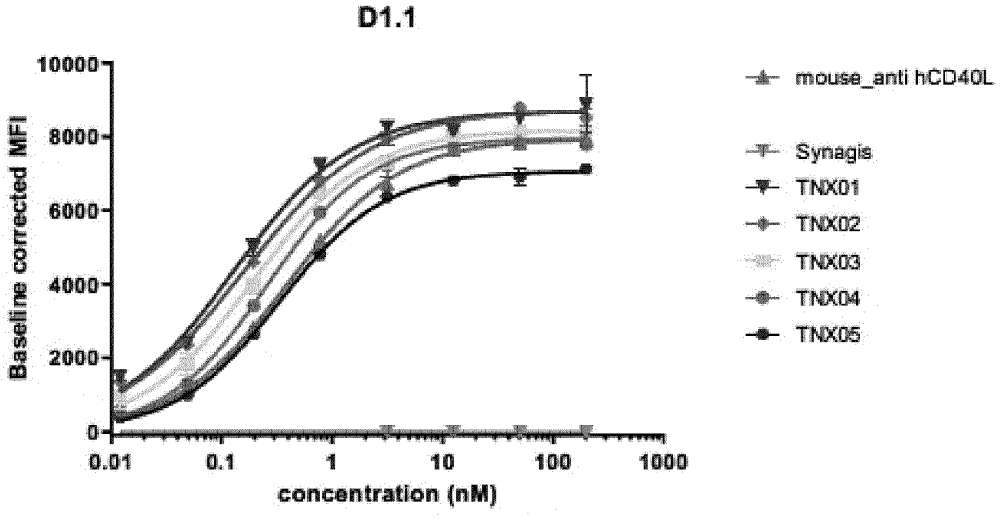
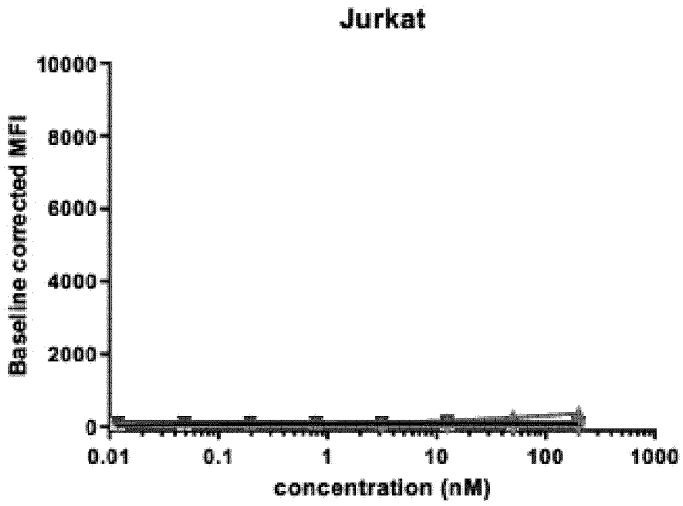


Figure 8

(a)



(b)



(c)

One site – Specific binding with Hill slope Best fit values	Mouse anti hCD40L	TNX01	TNX02	TNX03	TNX04	TNX05	Synagis
Bmax	7953	8693	8730	8191	7967	7075	No detectable binding
H	0.855	0.811	0.755	0.846	0.941	0.927	
Apparent Kd	0.390	0.128	0.162	0.195	0.261	0.338	

Figure 9

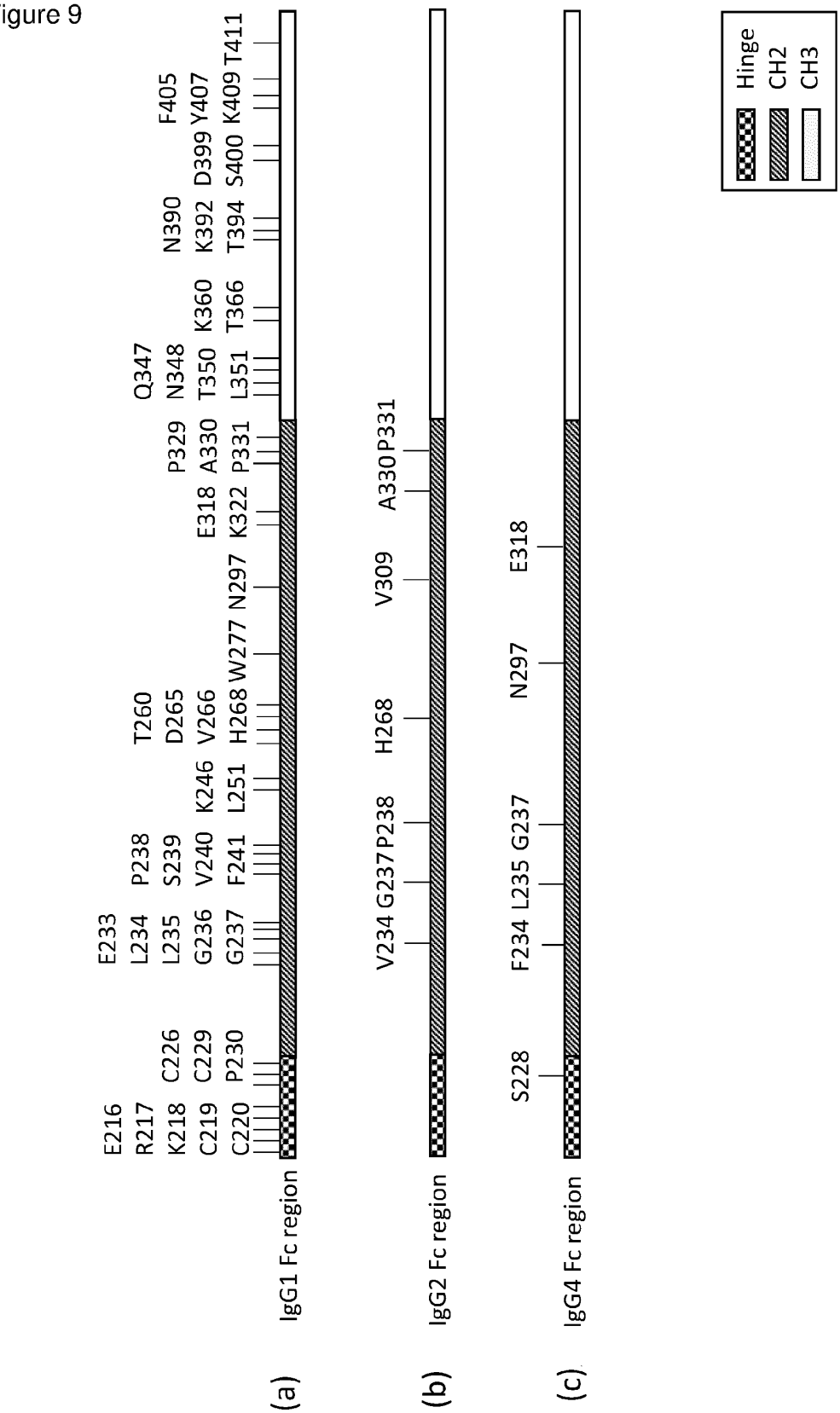

(a)
IgG1 Fc region
E216 R217 K218 C219 C220 C226 C229 P230 E233 L234 L235 G236 G237 P238 S239 V240 F241 K246 L251 T260 D265 V266 H268 W277 N297 E318 K322 P329 A330 P331 Q347 N348 T350 L351 K360 T366 N390 K392 T394 D399 S400 F405 Y407 K409 T411
(b)
IgG2 Fc region
V234 G237 P238 H268 V309 A330 P331
(c)
IgG4 Fc region
S228 F234 L235 G237 N297 E318
Hinge
CH2
CH3

Figure 10

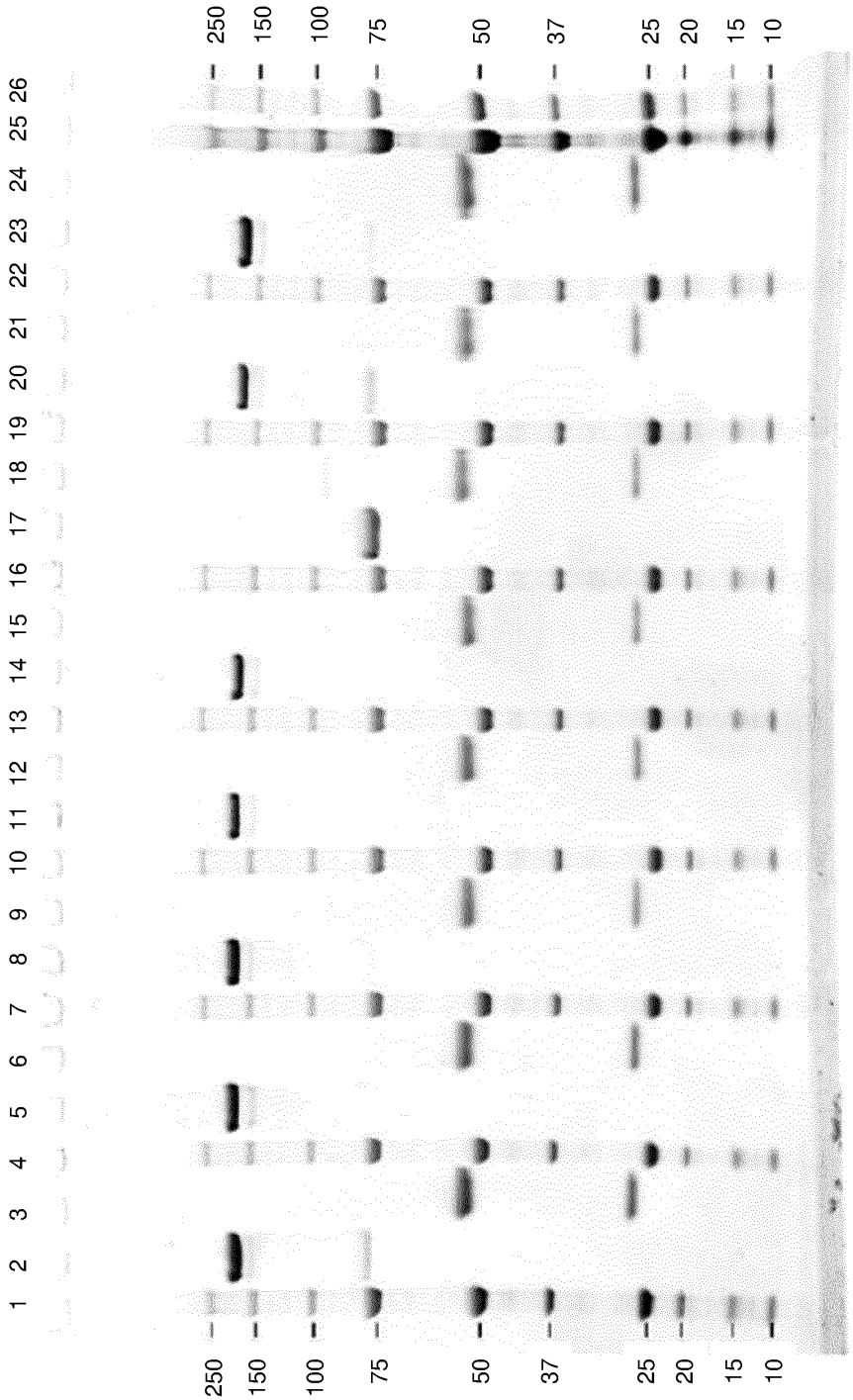

250
150
100
75
50
37
25
20
15
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
250
150
100
75
50
37
25
20
15
10

Figure 11 : SPR comparability score

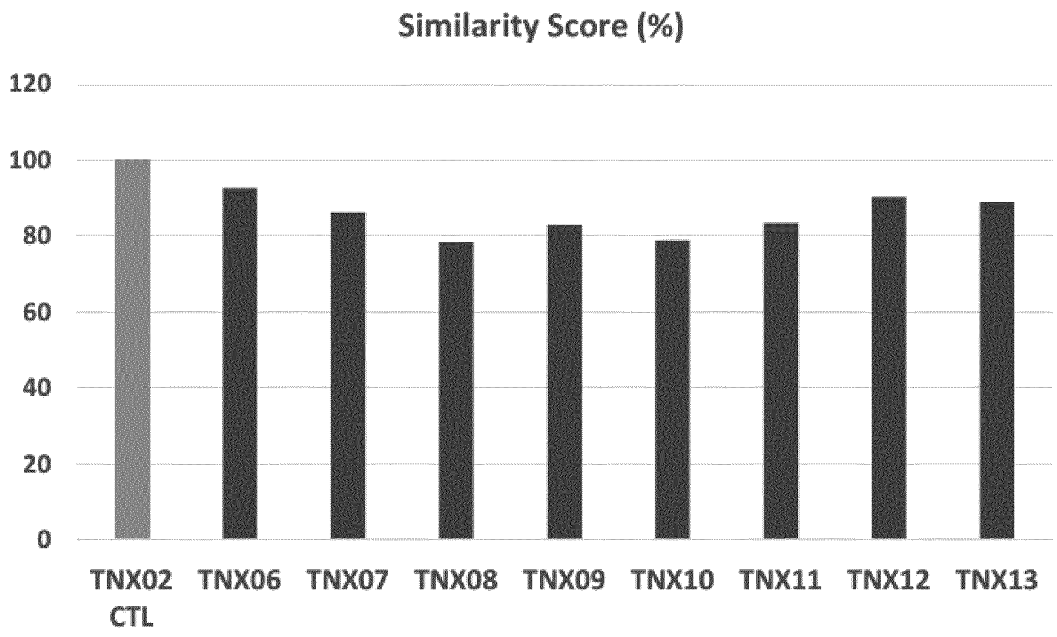


Figure 12 : Anti-CD154 FcγRIA (CD64) binding assessment by SPR

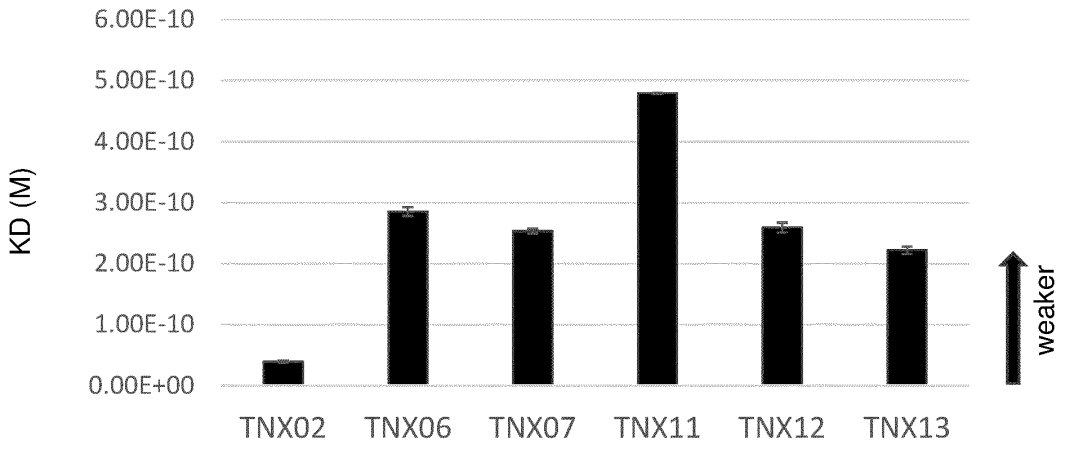
(a)

mAb	KD Ave (M)	SD	Fold weaker TNX02
TNX02	3.91E-11	1.9E-12	1
TNX06	2.85E-10	7E-12	7
TNX07	2.53E-10	4E-12	6
TNX08	1.03E-07	1.1E-11	2638
TNX09	1.47E-07	1E-09	3764
TNX10	6.87E-08	4.5E-10	1758
TNX11	4.79E-10	1E-12	12
TNX12	2.59E-10	8E-12	7
TNX13	2.22E-10	6E-12	6

TNX02_HumIgG1_HC_wt
TNX06_HumIgG4_HC_wt
TNX07_HumIgG4_HC_S228P
TNX08_HumIgG1_HC_S228P,L235E
TNX09_HumIgG1_HC_S228P,F234A,L235A
TNX10_HumIgG1_HC_L234A,L235A
TNX12_HumIgG1_HC_C229S,P238S
TNX13_HumIgG1_HC_C226S,P238S

Figure 12 : Anti-CD154 FcγRIA (CD64) binding assessment by SPR

(b)



(c)

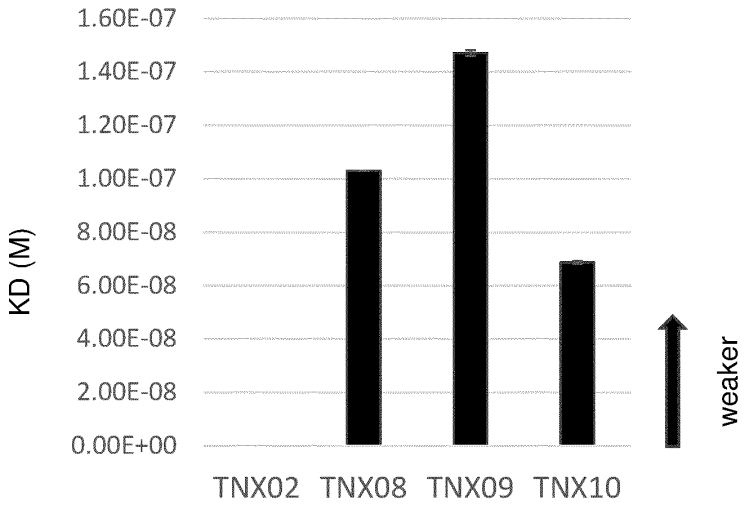
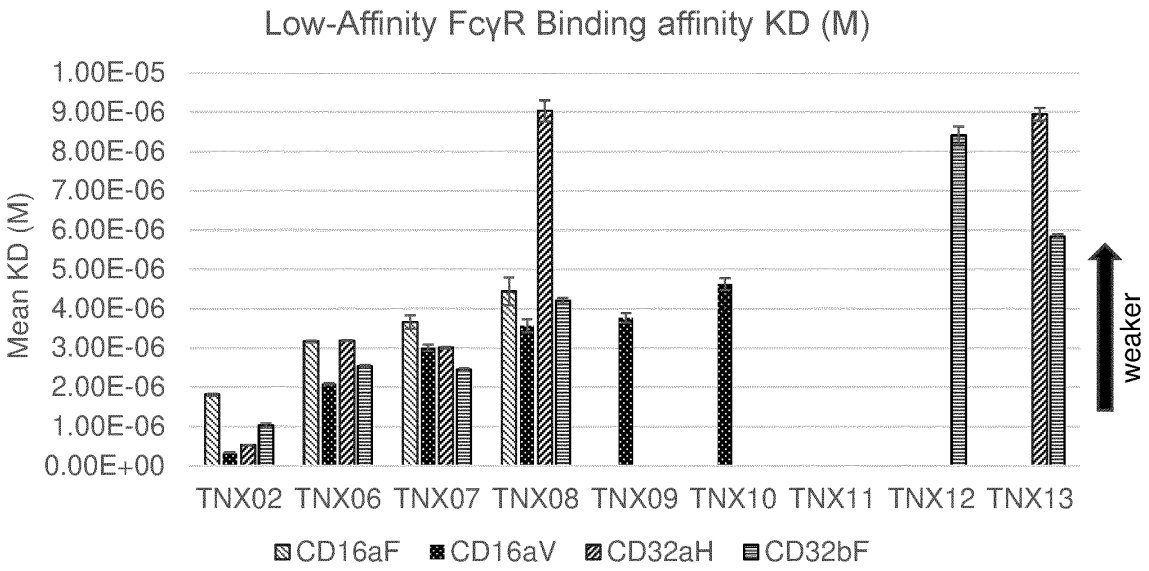


Figure 13(a) : Anti-CD154 Low Affinity FcγR binding assessment by SPR

Binding KD (M)	TNX02	TNX06	TNX07	TNX08	TNX09	TNX10	TNX11	TNX12	TNX13
CD16F	1.81E-06	3.17E-06	3.66E-06	4.44E-06	>>5 µM	>>5 µM	>>5 µM	>>5 µM	>>5 µM
CD16v	3.33E-07	2.09E-06	3.01E-06	3.56E-06	3.77E-06	4.63E-06	>>5 µM	>>5 µM	>>5 µM
CD32aH	5.25E-07	3.19E-06	3.01E-06	9.03E-06	>>12 µM	>>12 µM	>>12 µM	>>12 µM	8.95E-06
CD32bF	1.03E-06	2.53E-06	2.45E-06	4.20E-06	>>12 µM	>>12 µM	>>12 µM	8.41E-06	5.84E-06

TNX02_HumIgG1_HC_wt
TNX06_HumIgG4_HC_wt
TNX07_HumIgG4_HC_S228P
TNX08_HumIgG1_HC_S228P,L235E
TNX09_HumIgG1_HC_S228P,F234A,L235A
TNX10_HumIgG1_HC_L234A,L235A
TNX12_HumIgG1_HC_C229S,P238S
TNX13_HumIgG1_HC_C226S,P238S

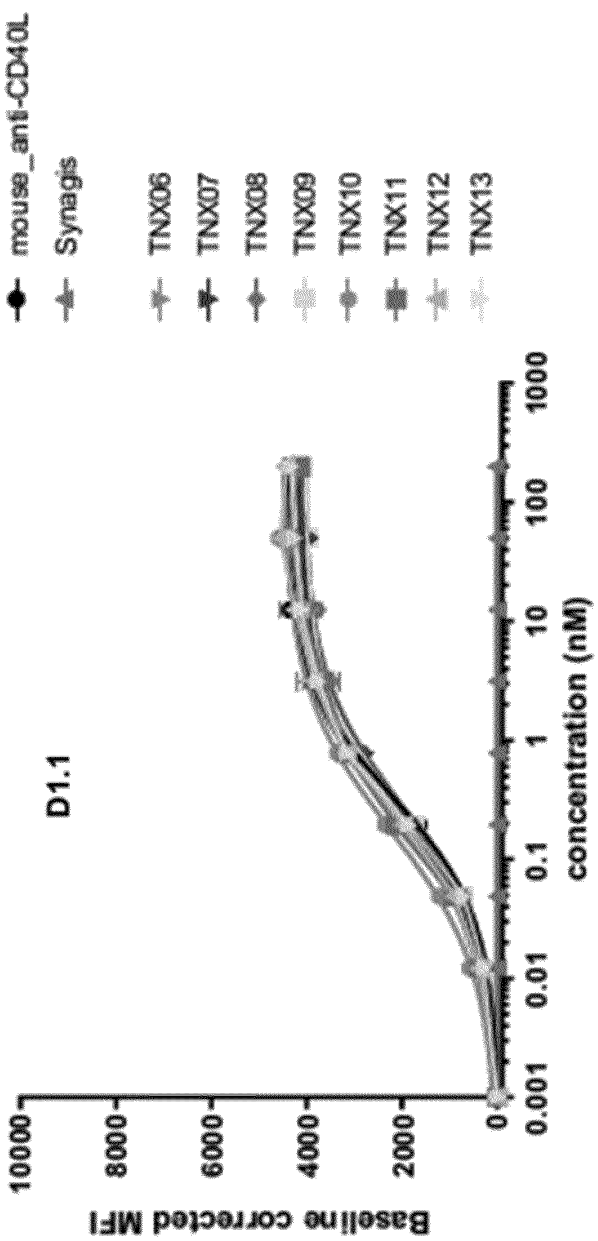
Figure 13(b)



* Note : No data (0M KD) indicates that no binding was detected at the FcγR concentrations tested

Figure 14

(a)



(b)

One site – Specific binding with Hill slope. Best fit values	Mouse_ anti-CD40L	TNX06	TNX07	TNX08	TNX09	TNX10	TNX11	TNX12	TNX13	Synagis
Bmax	4419	4207	4183	4069	4039	4483	4250	4503	4283	No detectable binding
h	0.869	0.774	0.772	0.801	0.868	0.737	0.802	0.786	0.897	
Kd	0.3394	0.285	0.296	0.293	0.278	0.177	0.213	0.277	0.268	