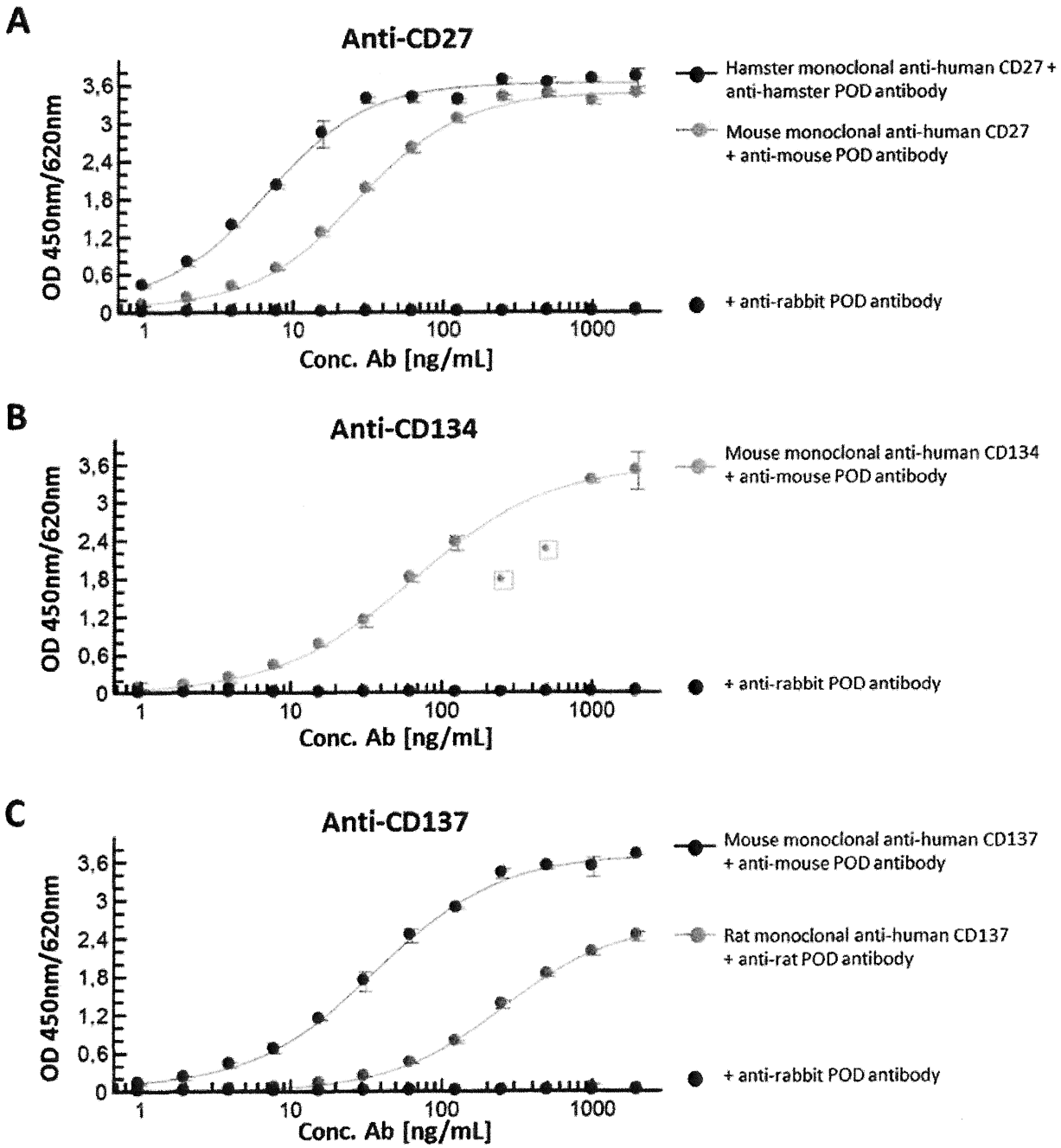

A
Anti-CD27
OD 450nm/620nm
3,6
3
2,4
1,8
1,2
0,6
0
1
10
100
1000
Conc. Ab [ng/mL]
Hamster monoclonal anti-human CD27 + anti-hamster POD antibody
Mouse monoclonal anti-human CD27 + anti-mouse POD antibody
+ anti-rabbit POD antibody
B
Anti-CD134
OD 450nm/620nm
Conc. Ab [ng/mL]
Mouse monoclonal anti-human CD134 + anti-mouse POD antibody
+ anti-rabbit POD antibody
C
Anti-CD137
OD 450nm/620nm
Conc. Ab [ng/mL]
Mouse monoclonal anti-human CD137 + anti-mouse POD antibody
Rat monoclonal anti-human CD137 + anti-rat POD antibody
+ anti-rabbit POD antibody

FIG. 1

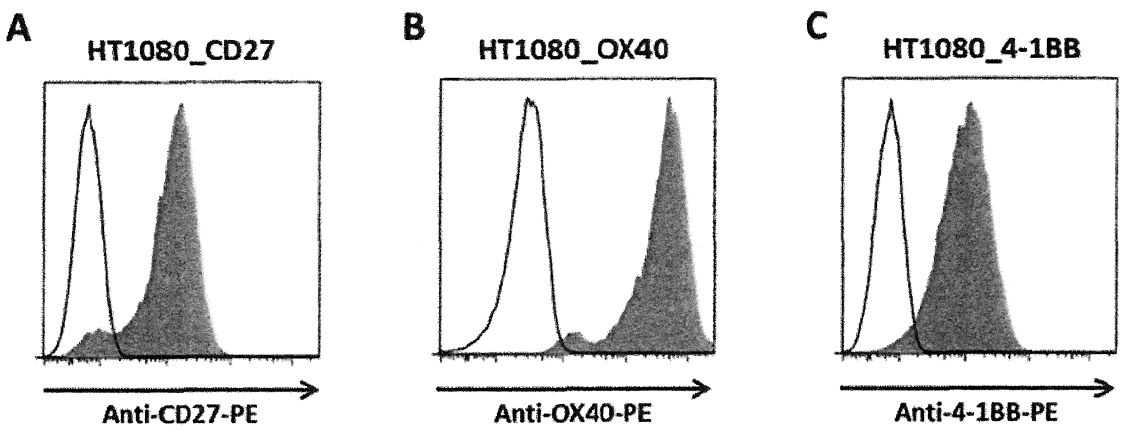

A
HT1080_CD27
Anti-CD27-PE
B
HT1080_OX40
Anti-OX40-PE
C
HT1080_4-1BB
Anti-4-1BB-PE

FIG. 2

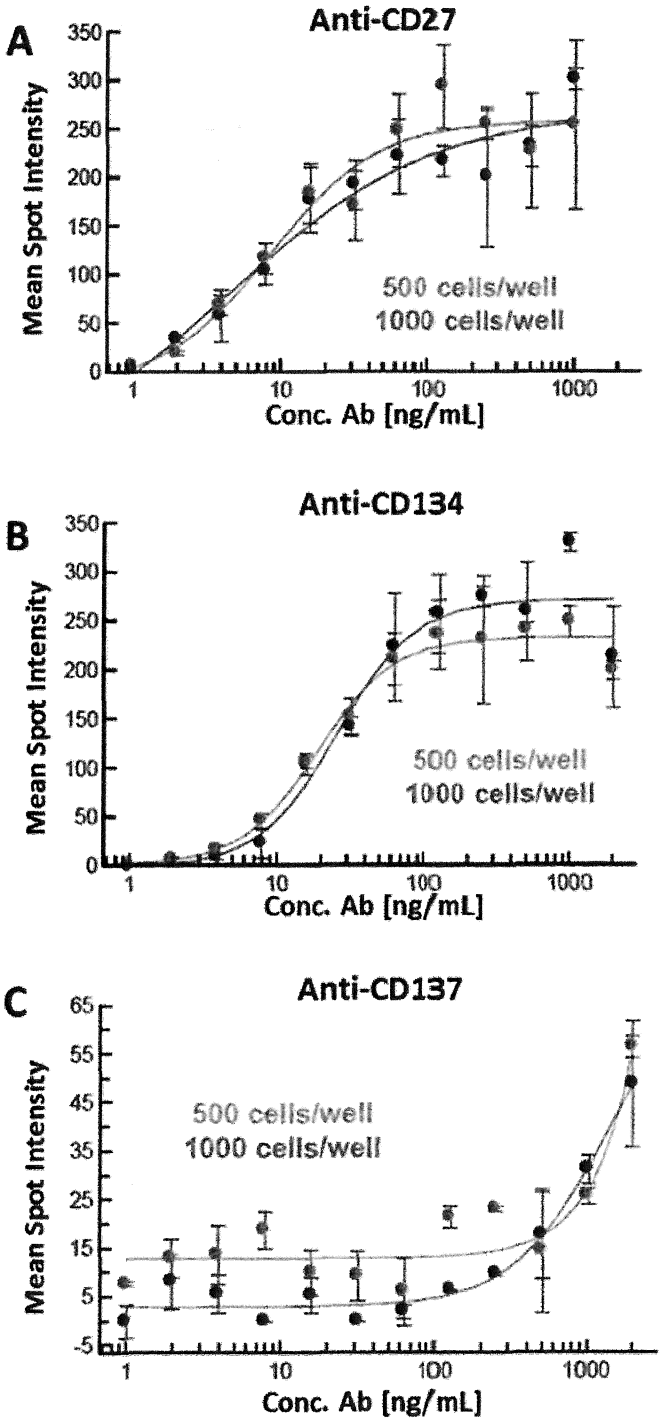

A
Anti-CD27
Mean Spot Intensity
350
300
250
200
150
100
50
0
500 cells/well
1000 cells/well
1
10
100
1000
Conc. Ab [ng/mL]
B
Anti-CD134
Mean Spot Intensity
350
300
250
200
150
100
50
0
500 cells/well
1000 cells/well
1
10
100
1000
Conc. Ab [ng/mL]
C
Anti-CD137
Mean Spot Intensity
65
55
45
35
25
15
5
-5
500 cells/well
1000 cells/well
1
10
100
1000
Conc. Ab [ng/mL]

FIG. 3

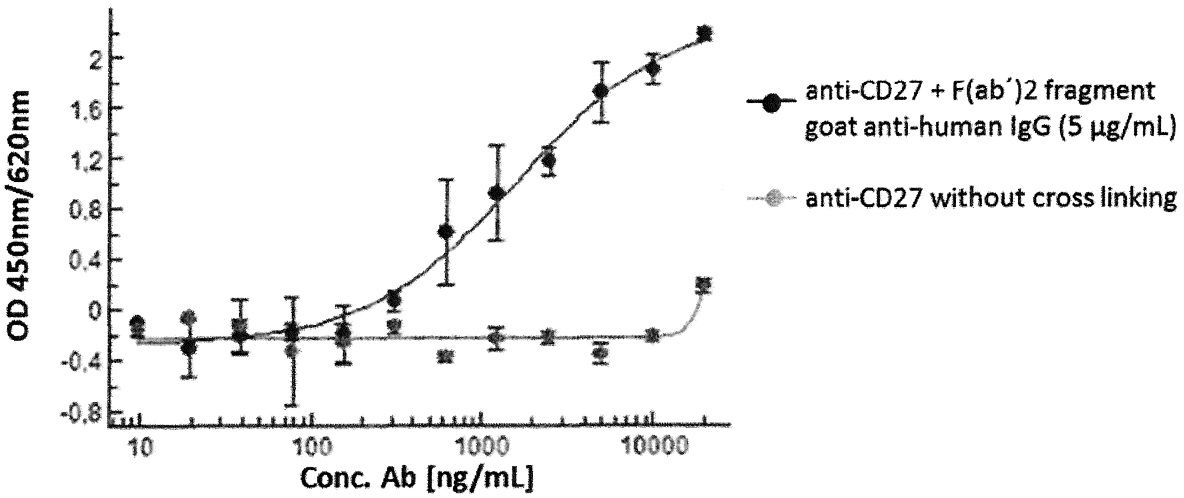

OD 450nm/620nm
2
1.6
1.2
0.8
0.4
0
-0.4
-0.8
10
100
1000
10000
Conc. Ab [ng/mL]
anti-CD27 + F(ab´)2 fragment goat anti-human IgG (5 µg/mL)
anti-CD27 without cross linking

FIG. 4

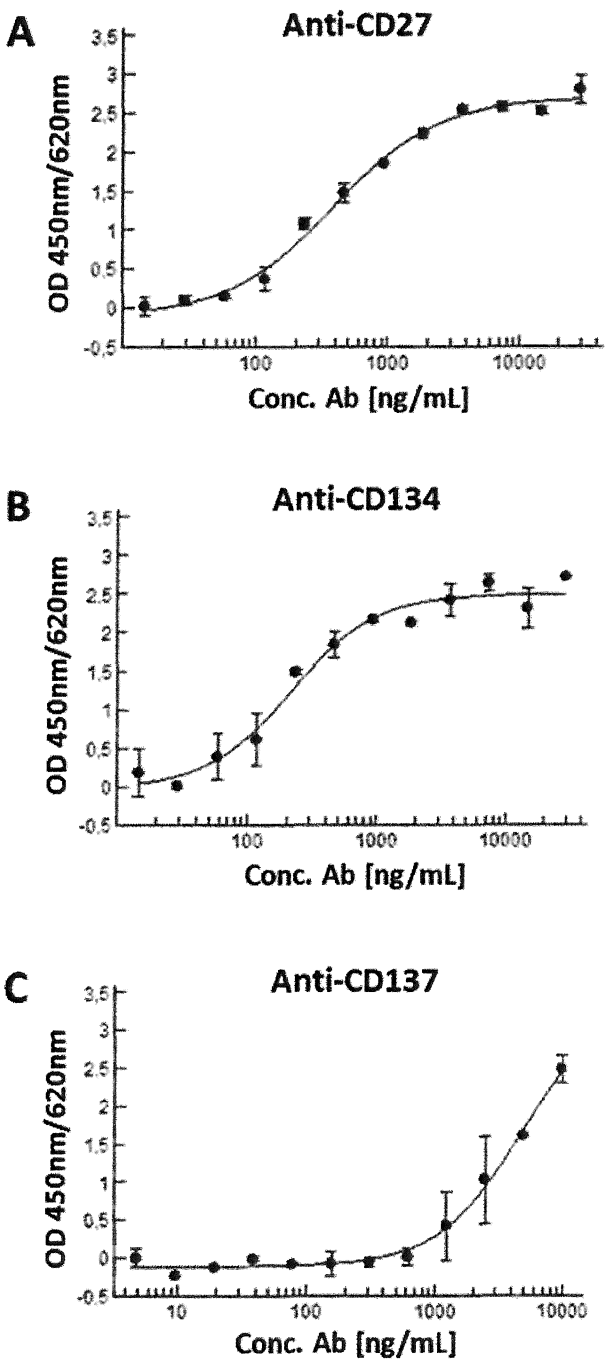
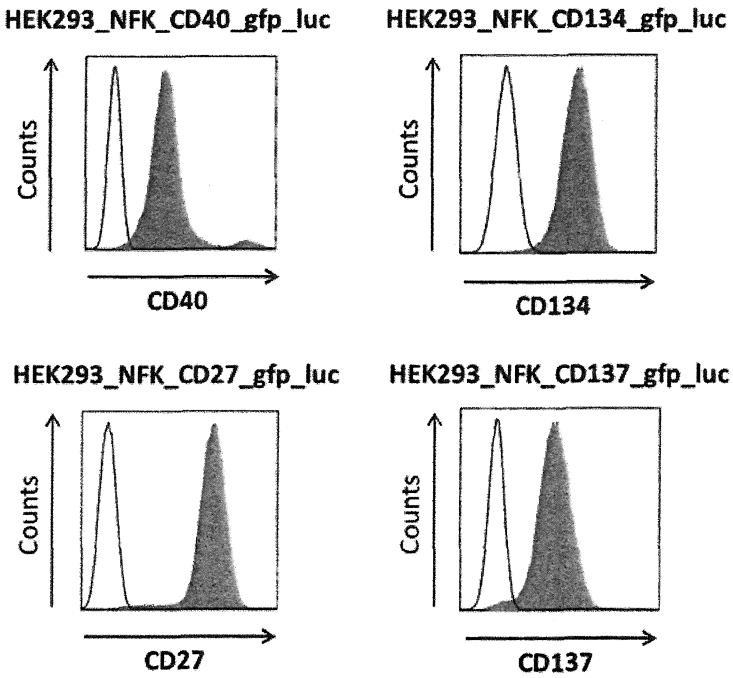

A
Anti-CD27
OD 450nm/620nm
3,5
3
2,5
2
1,5
1
0,5
0
-0,5
100
1000
10000
Conc. Ab [ng/mL]
B
Anti-CD134
OD 450nm/620nm
3,5
3
2,5
2
1,5
1
0,5
0
-0,5
100
1000
10000
Conc. Ab [ng/mL]
C
Anti-CD137
OD 450nm/620nm
3,5
3
2,5
2
1,5
1
0,5
0
-0,5
10
100
1000
10000
Conc. Ab [ng/mL]

FIG. 5

A


HEK293_NFK_CD40_gfp_luc
Counts
CD40
HEK293_NFK_CD134_gfp_luc
Counts
CD134
HEK293_NFK_CD27_gfp_luc
Counts
CD27
HEK293_NFK_CD137_gfp_luc
Counts
CD137

B

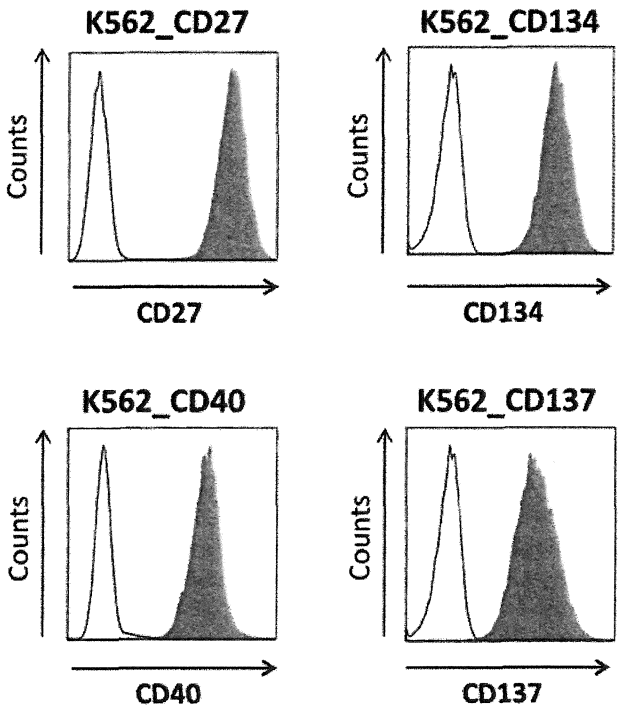

K562_CD27
Counts
CD27
K562_CD134
Counts
CD134
K562_CD40
Counts
CD40
K562_CD137
Counts
CD137

FIG. 6

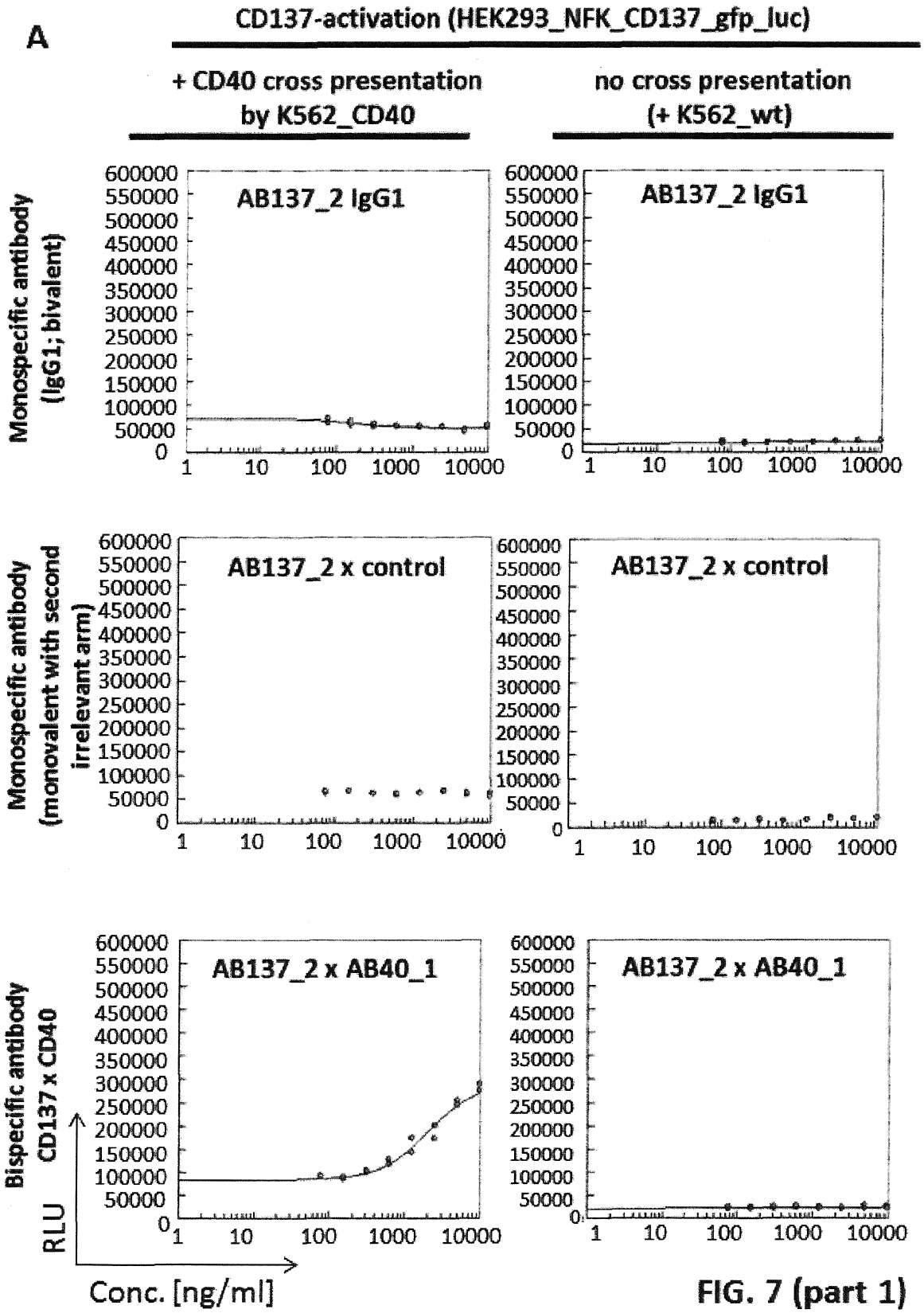

A
CD137-activation (HEK293_NFK_CD137_gfp_luc)
+ CD40 cross presentation by K562_CD40
no cross presentation (+ K562_wt)
Monospecific antibody (IgG1; bivalent)
AB137_2 IgG1
AB137_2 IgG1
Monospecific antibody (monovalent with second irrelevant arm)
AB137_2 x control
AB137_2 x control
Bispecific antibody CD137 x CD40
AB137_2 x AB40_1
AB137_2 x AB40_1
RLU
Conc. [ng/ml]
600000
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
1
10
100
1000
10000

FIG. 7 (part 1)

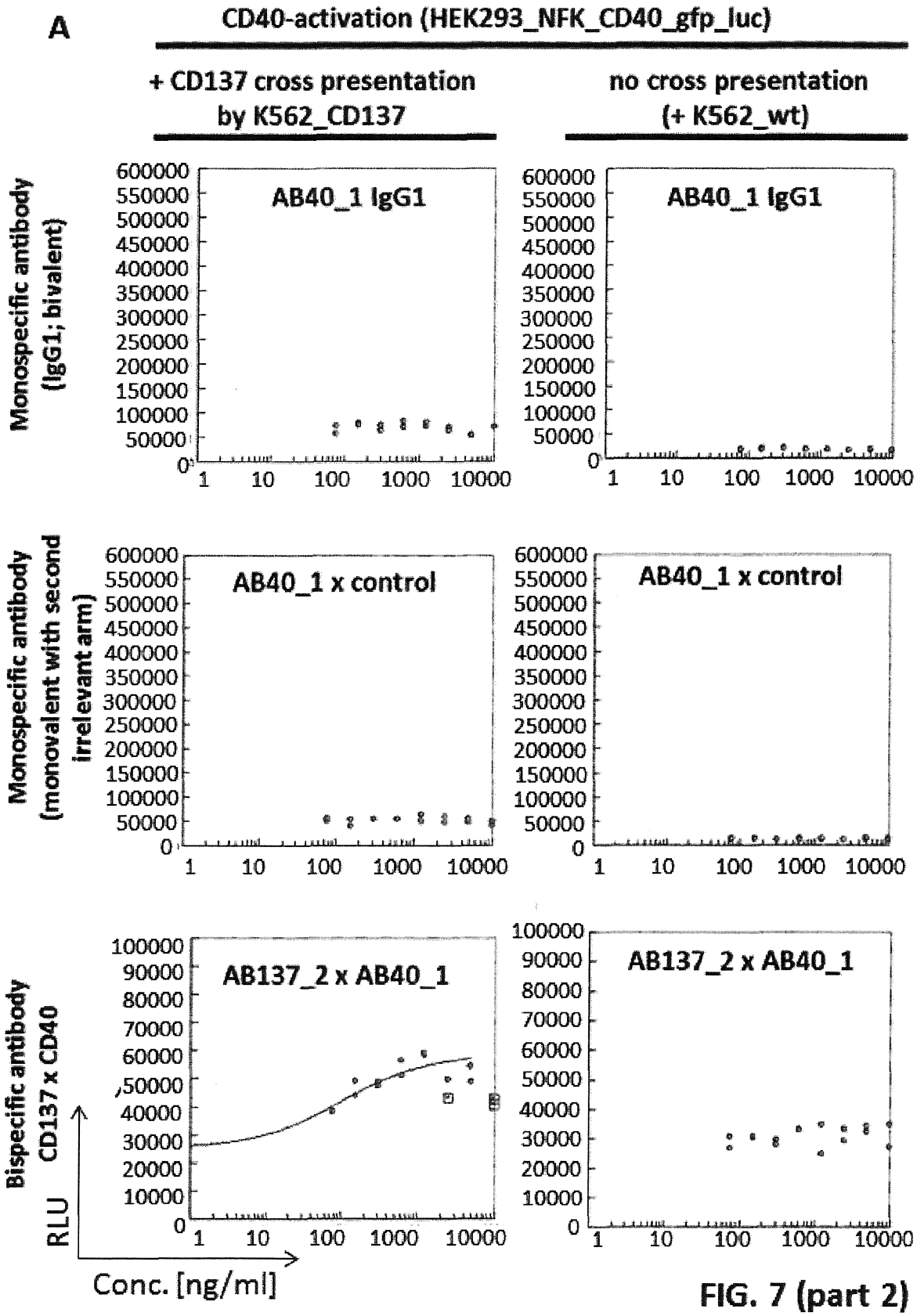

A
CD40-activation (HEK293_NFK_CD40_gfp_luc)
+ CD137 cross presentation by K562_CD137
no cross presentation (+ K562_wt)
Monospecific antibody (IgG1; bivalent)
AB40_1 IgG1
Monospecific antibody (monovalent with second irrelevant arm)
AB40_1 x control
Bispecific antibody CD137 x CD40
AB137_2 x AB40_1
RLU
Conc. [ng/ml]

FIG. 7 (part 2)

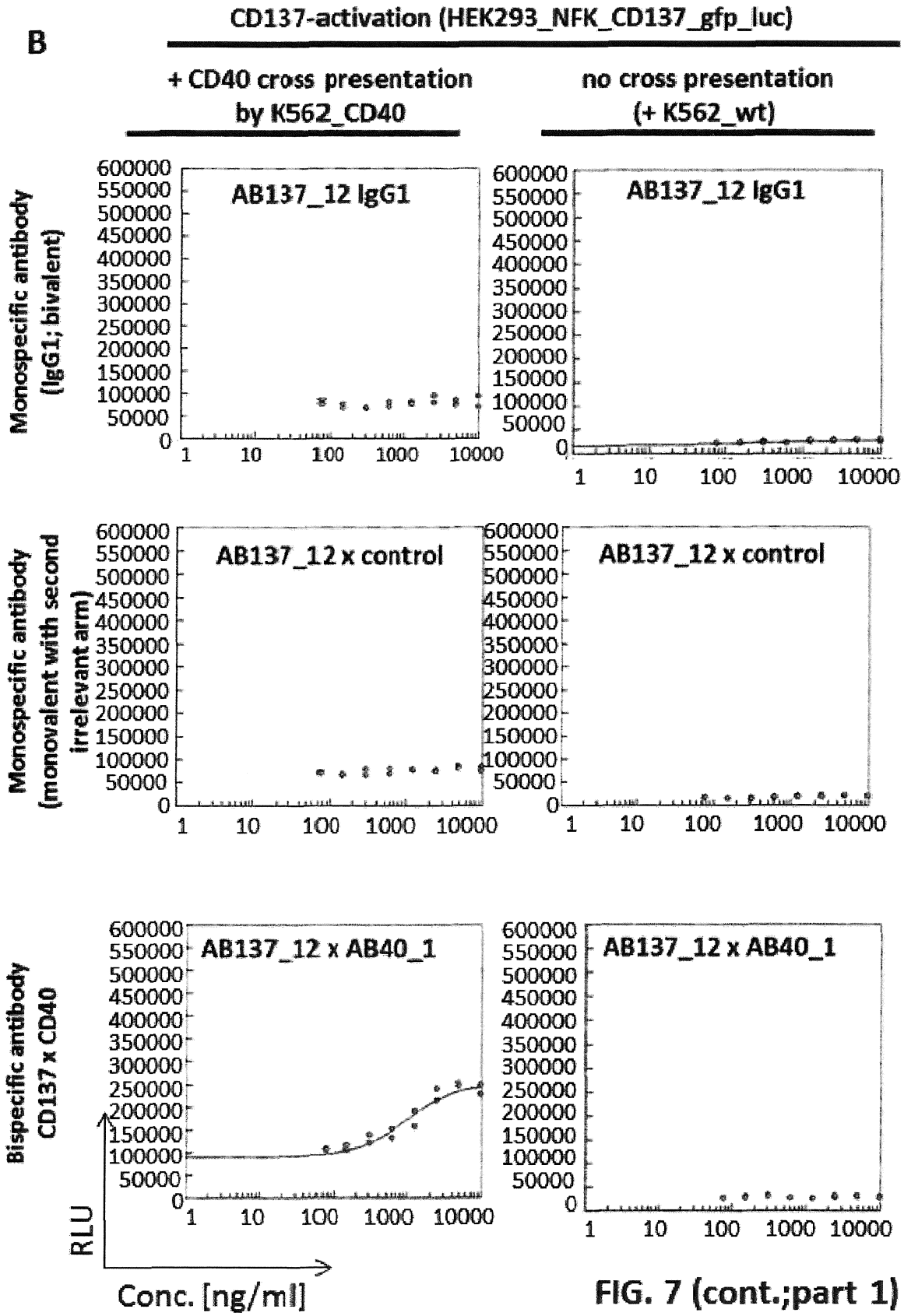



FIG. 7 (cont.;part 1)

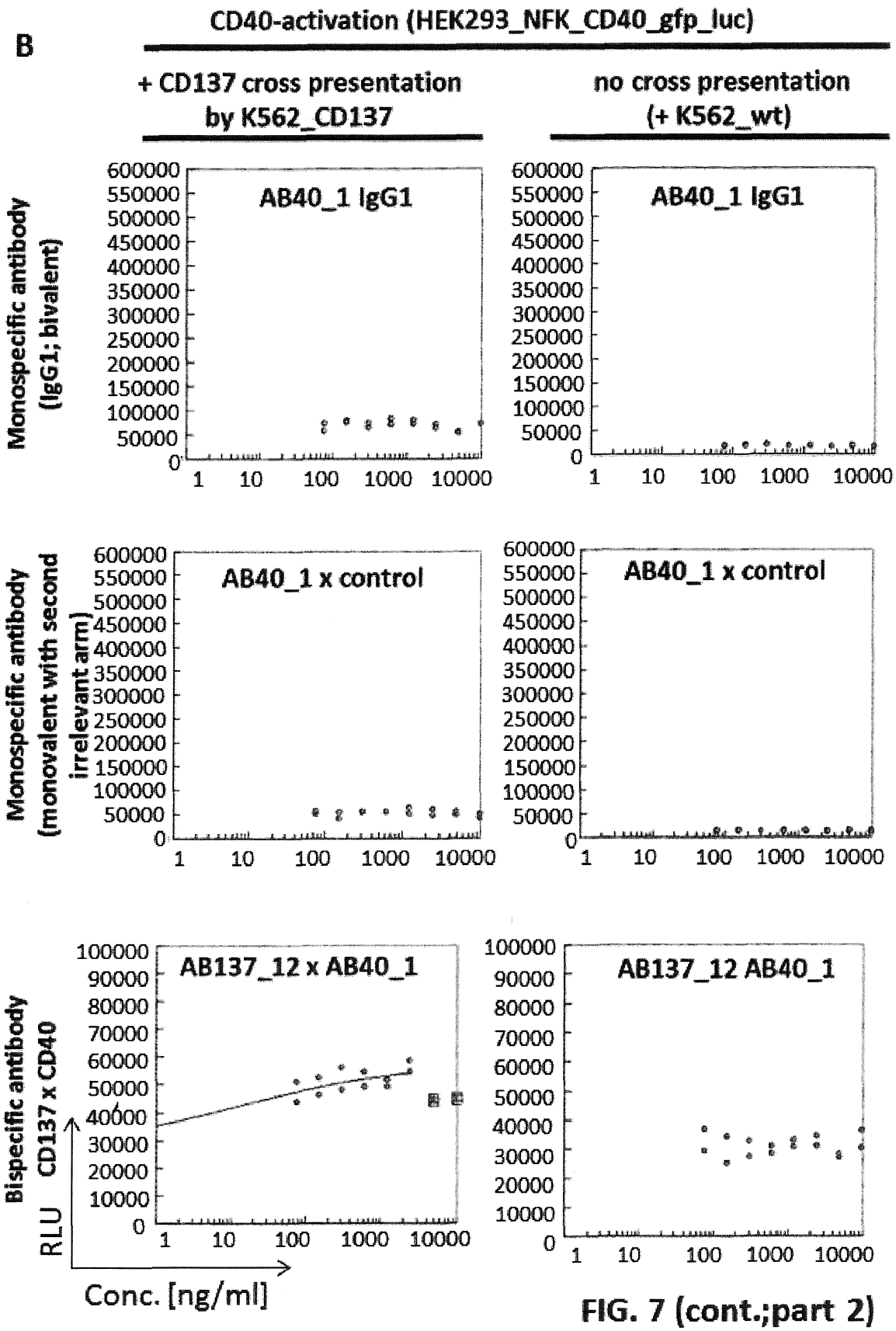



FIG. 7 (cont.;part 2)

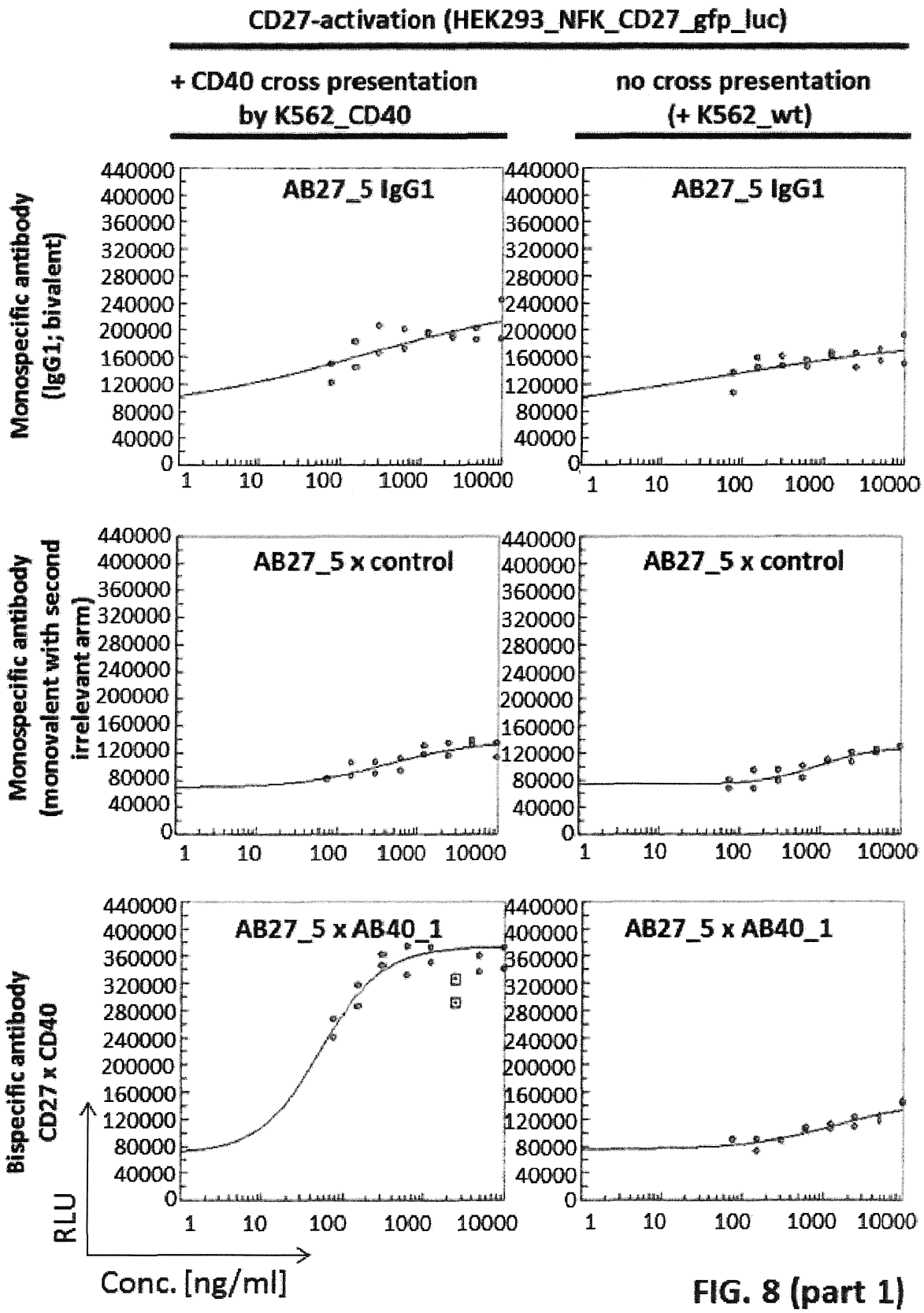

CD27-activation (HEK293_NFK_CD27_gfp_luc)
+ CD40 cross presentation by K562_CD40
no cross presentation (+ K562_wt)
Monospecific antibody (IgG1; bivalent)
AB27_5 IgG1
AB27_5 IgG1
Monospecific antibody (monovalent with second irrelevant arm)
AB27_5 x control
AB27_5 x control
Bispecific antibody CD27 x CD40
AB27_5 x AB40_1
AB27_5 x AB40_1
RLU
Conc. [ng/ml]
440000
400000
360000
320000
280000
240000
200000
160000
120000
80000
40000
0
1
10
100
1000
10000

FIG. 8 (part 1)

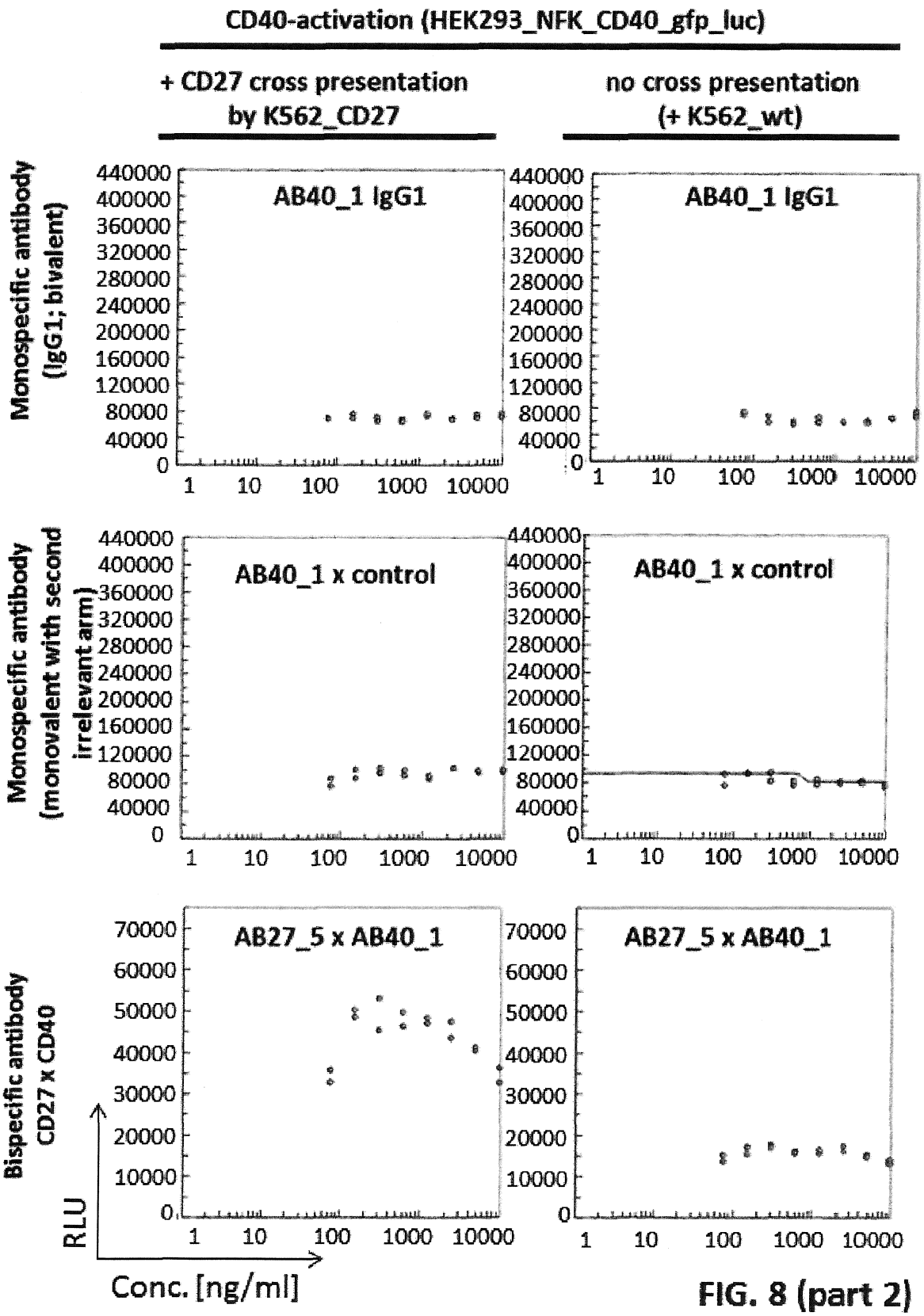

CD40-activation (HEK293_NFK_CD40_gfp_luc)
+ CD27 cross presentation by K562_CD27
no cross presentation (+ K562_wt)
Monospecific antibody (IgG1; bivalent)
AB40_1 IgG1
AB40_1 IgG1
Monospecific antibody (monovalent with second irrelevant arm)
AB40_1 x control
AB40_1 x control
Bispecific antibody CD27 x CD40
AB27_5 x AB40_1
AB27_5 x AB40_1
RLU
Conc. [ng/ml]
1
10
100
1000
10000

FIG. 8 (part 2)

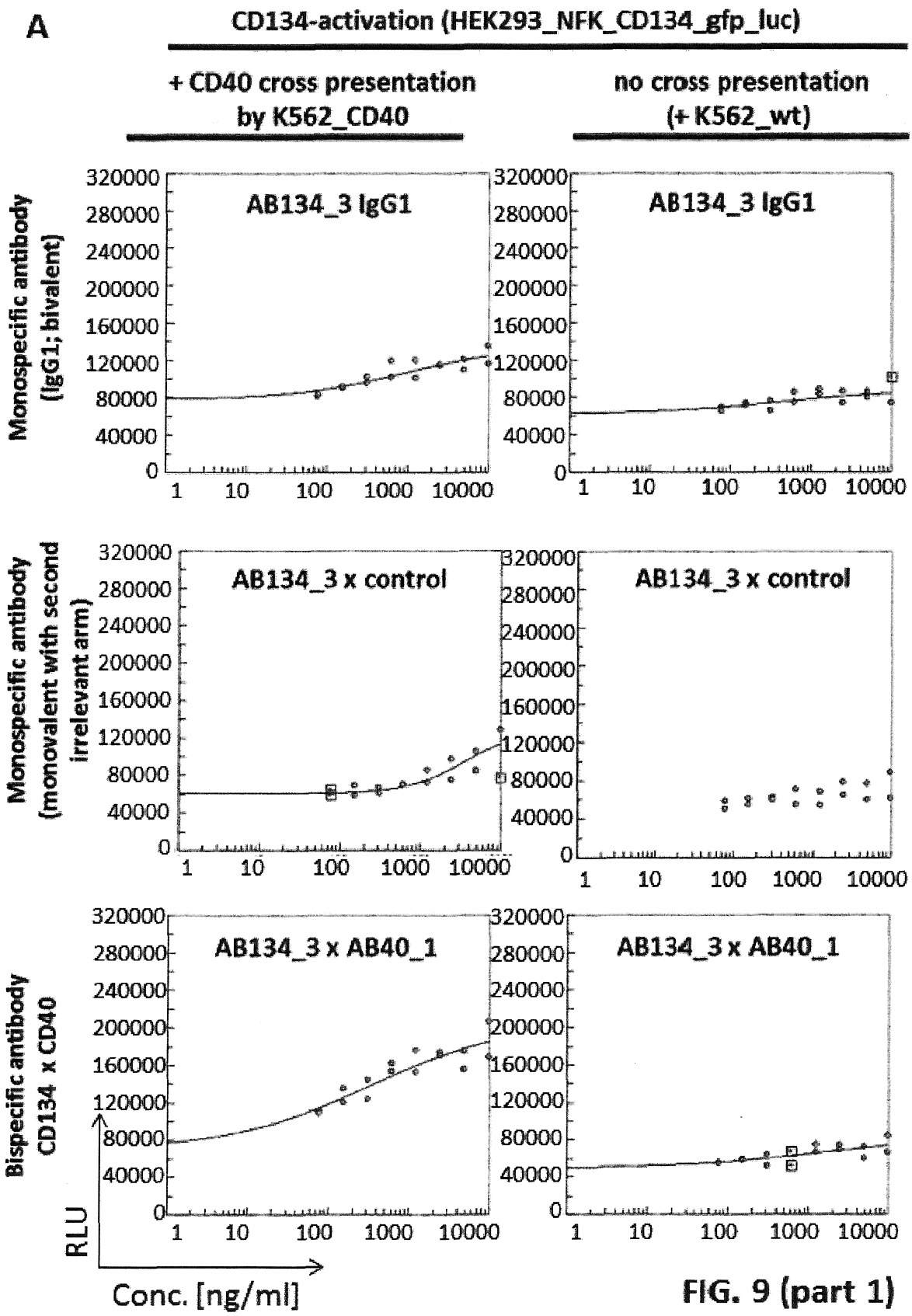

A
CD134-activation (HEK293_NFK_CD134_gfp_luc)
+ CD40 cross presentation by K562_CD40
no cross presentation (+ K562_wt)
Monospecific antibody (IgG1; bivalent)
AB134_3 IgG1
AB134_3 IgG1
Monospecific antibody (monovalent with second irrelevant arm)
AB134_3 x control
AB134_3 x control
Bispecific antibody CD134 x CD40
AB134_3 x AB40_1
AB134_3 x AB40_1
RLU
Conc. [ng/ml]
320000
280000
240000
200000
160000
120000
80000
40000
0
1
10
100
1000
10000

FIG. 9 (part 1)

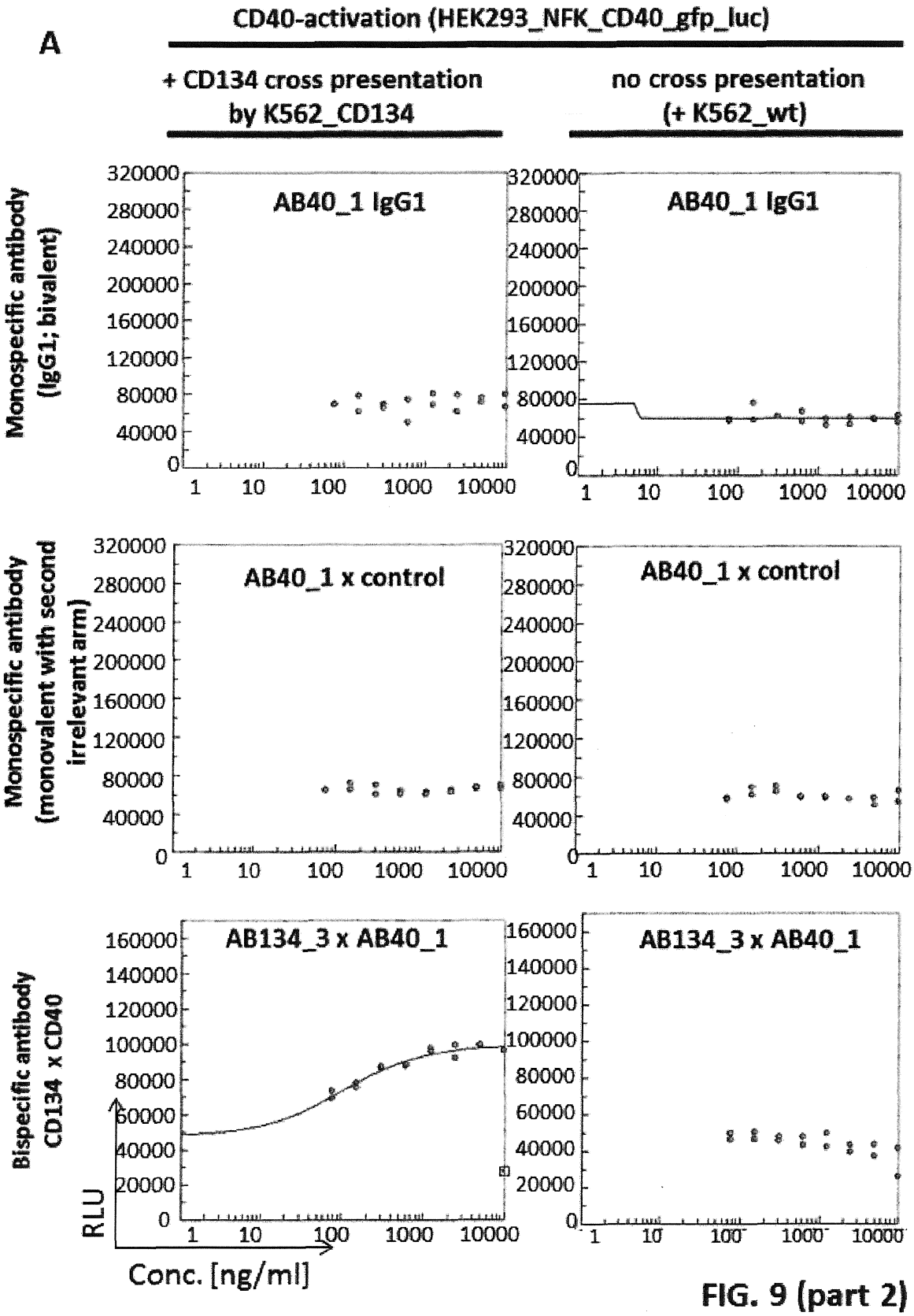

A
CD40-activation (HEK293_NFK_CD40_gfp_luc)
+ CD134 cross presentation by K562_CD134
no cross presentation (+ K562_wt)
Monospecific antibody (IgG1; bivalent)
AB40_1 IgG1
AB40_1 IgG1
Monospecific antibody (monovalent with second irrelevant arm)
AB40_1 x control
AB40_1 x control
Bispecific antibody CD134 x CD40
AB134_3 x AB40_1
AB134_3 x AB40_1
RLU
Conc. [ng/ml]

FIG. 9 (part 2)

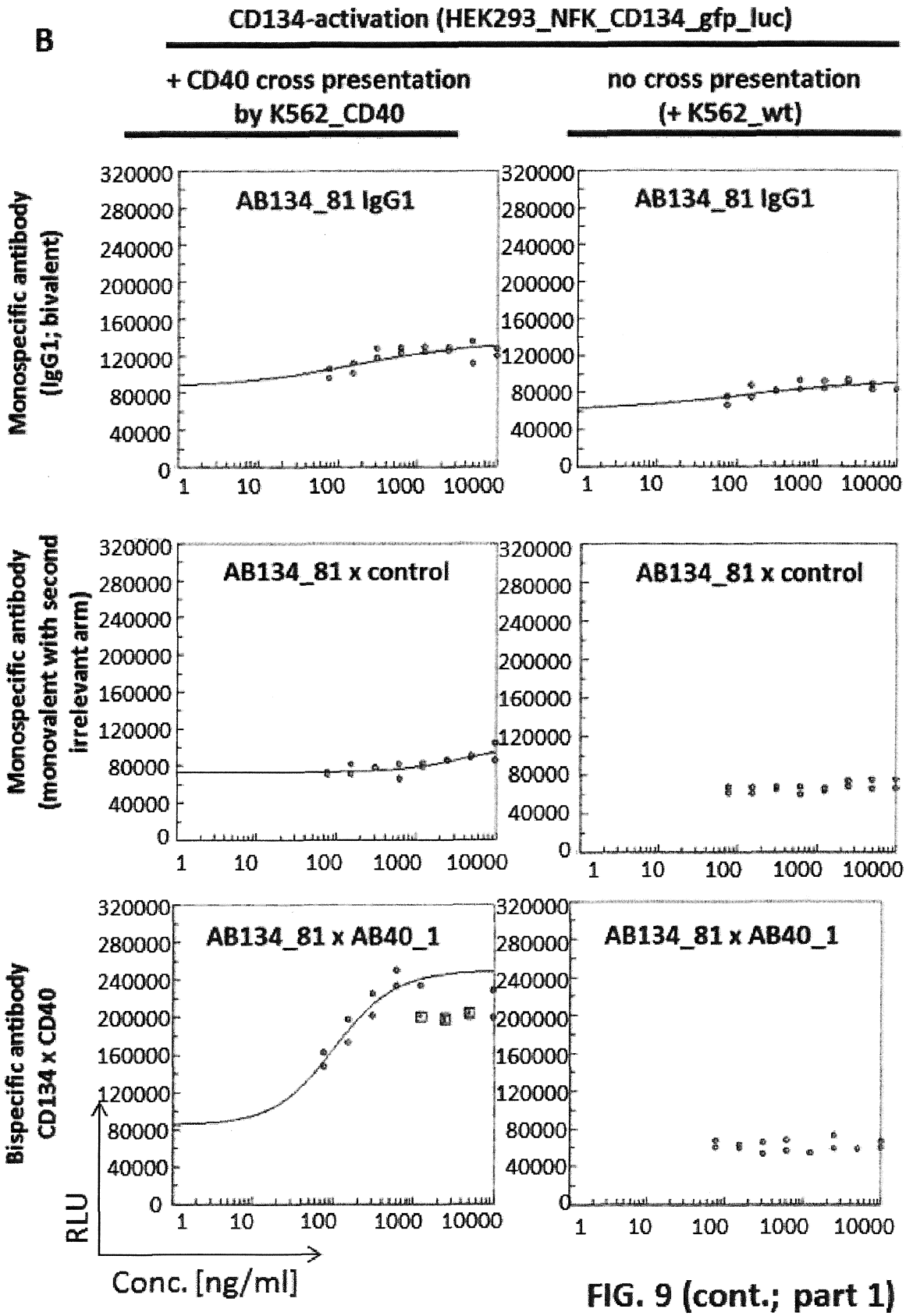



FIG. 9 (cont.; part 1)

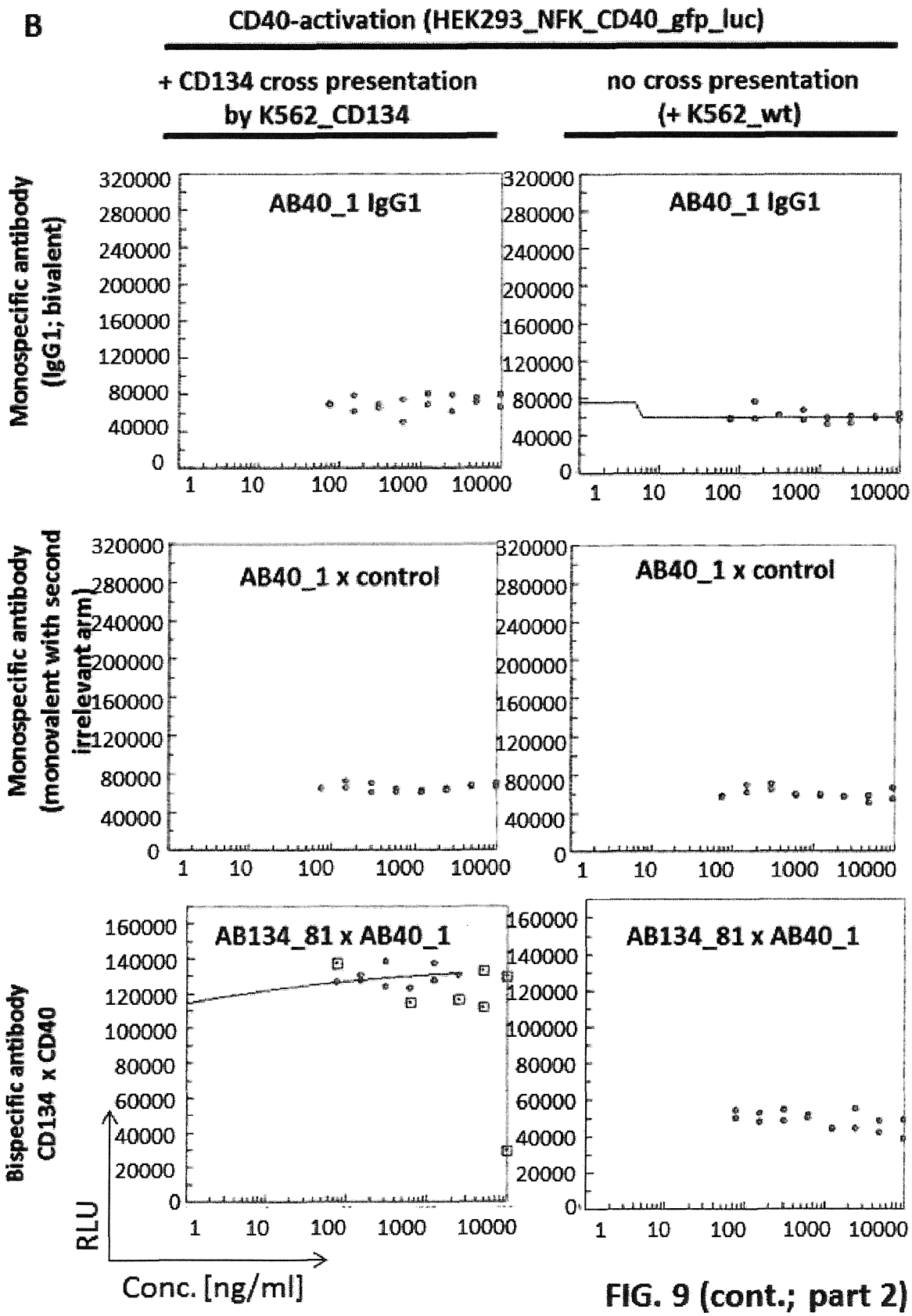



FIG. 9 (cont.; part 2)

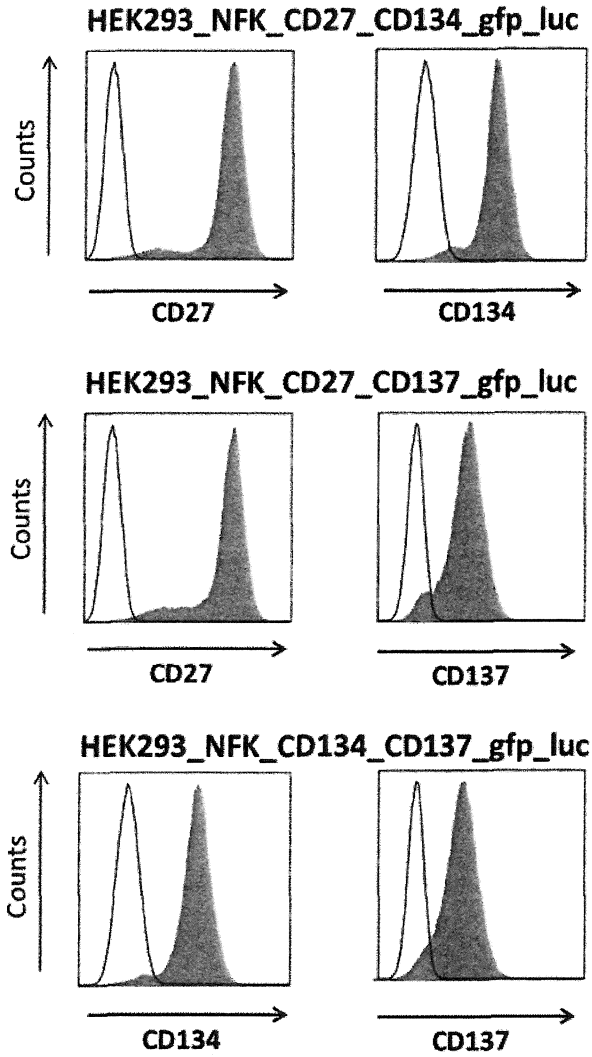

HEK293_NFK_CD27_CD134_gfp_luc
Counts
CD27
CD134
HEK293_NFK_CD27_CD137_gfp_luc
Counts
CD27
CD137
HEK293_NFK_CD134_CD137_gfp_luc
Counts
CD134
CD137

FIG. 10

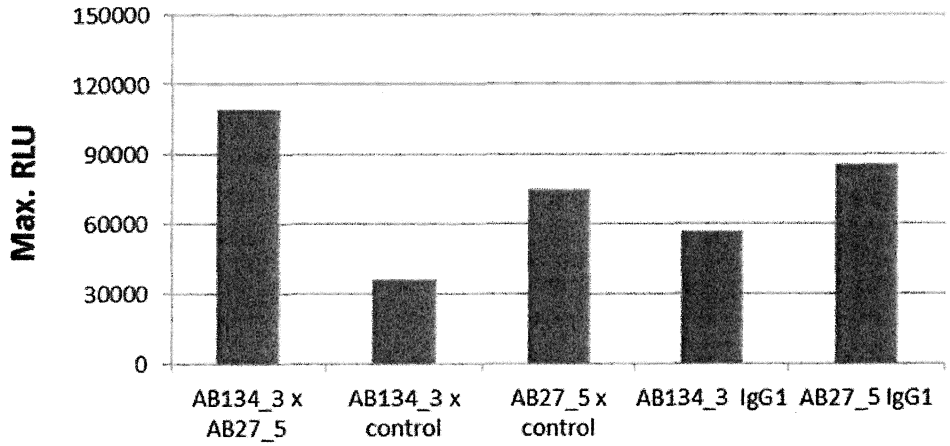
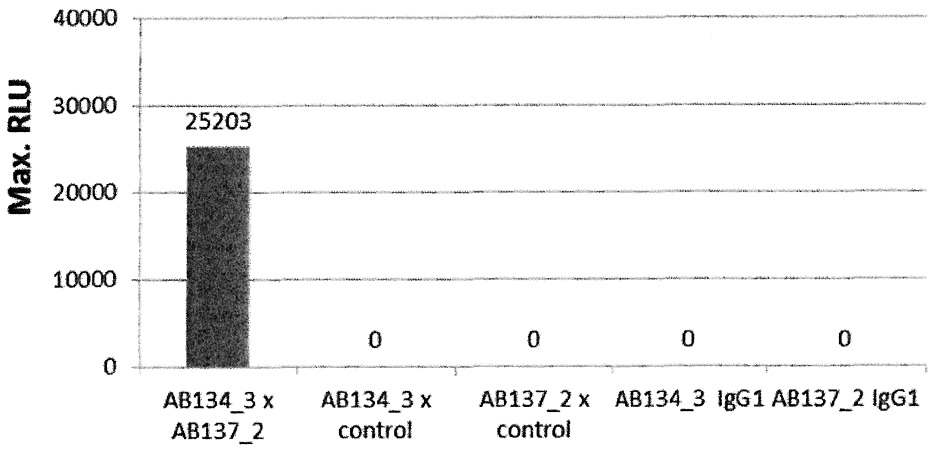

Max. RLU
150000
120000
90000
60000
30000
0
AB134_3 x AB27_5
AB134_3 x control
AB27_5 x control
AB134_3 IgG1
AB27_5 IgG1

FIG. 11

A


40000
30000
20000
10000
0
Max. RLU
25203
0
0
0
0
AB134_3 x AB137_2
AB134_3 x control
AB137_2 x control
AB134_3 IgG1
AB137_2 IgG1

B

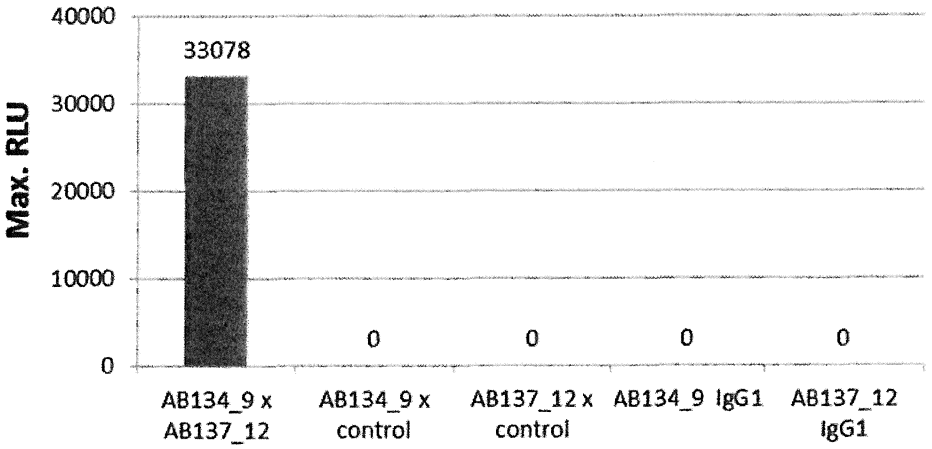
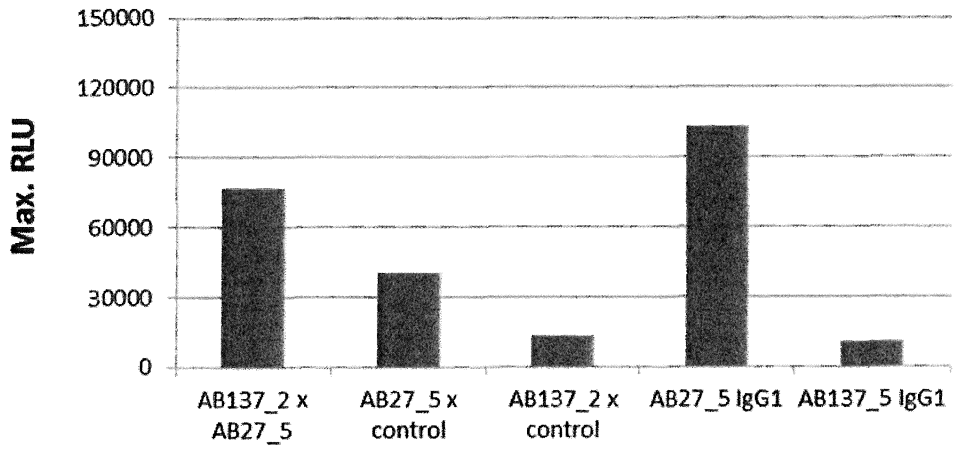

40000
30000
20000
10000
0
Max. RLU
33078
0
0
0
0
AB134_9 x AB137_12
AB134_9 x control
AB137_12 x control
AB134_9 IgG1
AB137_12 IgG1

FIG. 12

A


150000
120000
90000
60000
30000
0
Max. RLU
AB137_2 x AB27_5
AB27_5 x control
AB137_2 x control
AB27_5 IgG1
AB137_5 IgG1

B

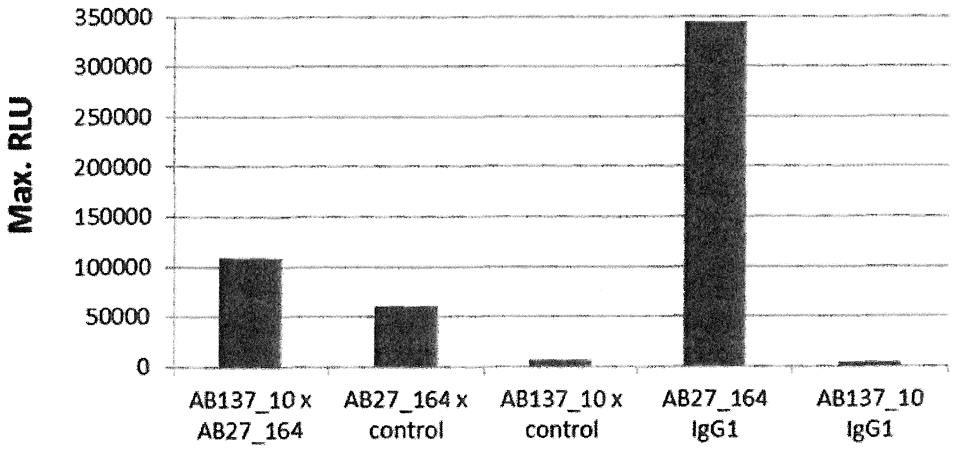

350000
300000
250000
200000
150000
100000
50000
0
Max. RLU
AB137_10 x AB27_164
AB27_164 x control
AB137_10 x control
AB27_164 IgG1
AB137_10 IgG1

FIG. 13