Figure 1

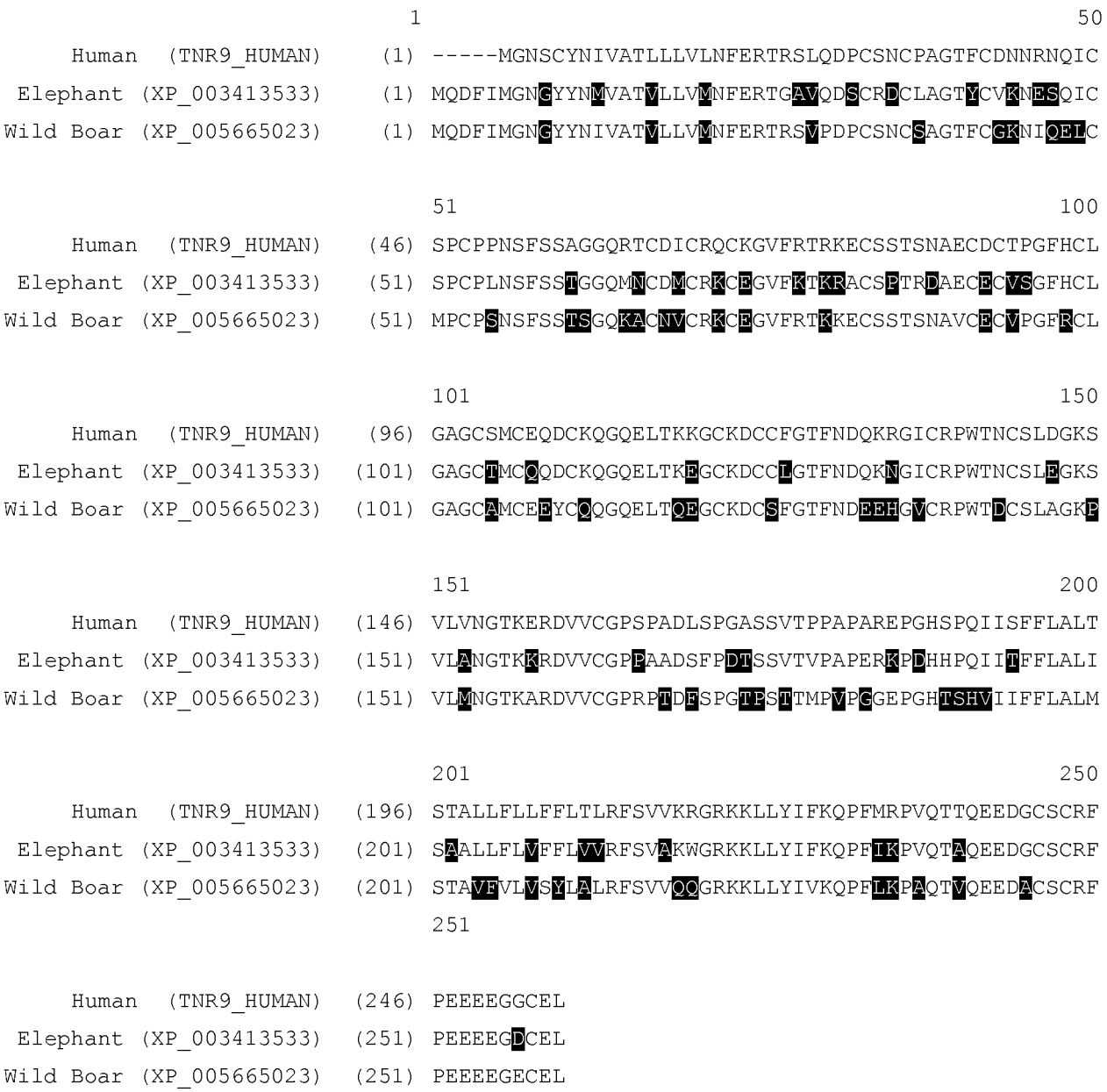

1 50
Human (TNR9_HUMAN) (1) -----MGNSCYNIVATLLLVLNFERTRSLQDPCSNCPAGTFCDNNRNQIC
Elephant (XP_003413533) (1) MQDFIMGNGYYNMVATVLLVMNFERTGAVQDSCRDCLAGTYCVKNESQIC
Wild Boar (XP_005665023) (1) MQDFIMGNGYYNIVATVLLVMNFERTRSVPDPCSNCSAGTFCGKNIQELC
51 100
Human (TNR9_HUMAN) (46) SPCPPNSFSSAGGQRTCDICRQCKGVFRTRKECSSTSNAECDCTPGFHCL
Elephant (XP_003413533) (51) SPCPLNSFSSTGGQMNCDMCRKCEGVFKTKRACSPTRDAECECVSGFHCL
Wild Boar (XP_005665023) (51) MPCPSNSFSSTSGQKACNVCRKCEGVFRTKKECSSTSNAVCECVPGFRCL
101 150
Human (TNR9_HUMAN) (96) GAGCSMCEQDCKQGQELTKKGCKDCCFGTFNDQKRGICRPWTNCSLDGKS
Elephant (XP_003413533) (101) GAGCTMCQQDCKQGQELTKEGCKDCCLGTFNDQKNGICRPWTNCSLEGKS
Wild Boar (XP_005665023) (101) GAGCAMCEEYCQQGQELTQEGCKDCSFGTFNDEEHGVCRPWTDCSLAGKP
151 200
Human (TNR9_HUMAN) (146) VLVNGTKERDVVCGPSPADLSPGASSVTPPAPAREPGHSPQIISFFLALT
Elephant (XP_003413533) (151) VLANGTKKRDVVCGPPAADSFPDTSSVTVPAPERKPDHHPQIITFFLALI
Wild Boar (XP_005665023) (151) VLMNGTKARDVVCGPRPTDFSPGTPSTTMPVPGGEPGHTSHVIIFFLALM
201 250
Human (TNR9_HUMAN) (196) STALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF
Elephant (XP_003413533) (201) SAALLFLVFFLVVRFSVAKWGRKKLLYIFKQPFIKPVQTAQEEDGCSCRF
Wild Boar (XP_005665023) (201) STAVFVLVSYLALRFSVVQQGRKKLLYIVKQPFLKPAQTVQEEDACSCRF
251
Human (TNR9_HUMAN) (246) PEEEEGGCEL
Elephant (XP_003413533) (251) PEEEEGDCEL
Wild Boar (XP_005665023) (251) PEEEEGECEL

Figure 2

1. TNR9_HUMAN
Shuffle
2. TNR9_HUMAN shuffle 1
Shuffle
3. TNR9_HUMAN shuffle 2
Shuffle
4. TNR9_HUMAN shuffle 3
Shuffle
5. TNR9_HUMAN shuffle 4
Shuffle
6. TNR9_HUMAN shuffle 5
Shuffle
7. TNR9_HUMAN shuffle 6
Shuffle
8. XP_003413533
9. XP_005665023
Shuffle 1
Shuffle 2
Shuffle 3
Shuffle 4
Shuffle 5
Shuffle 6

Figure 3

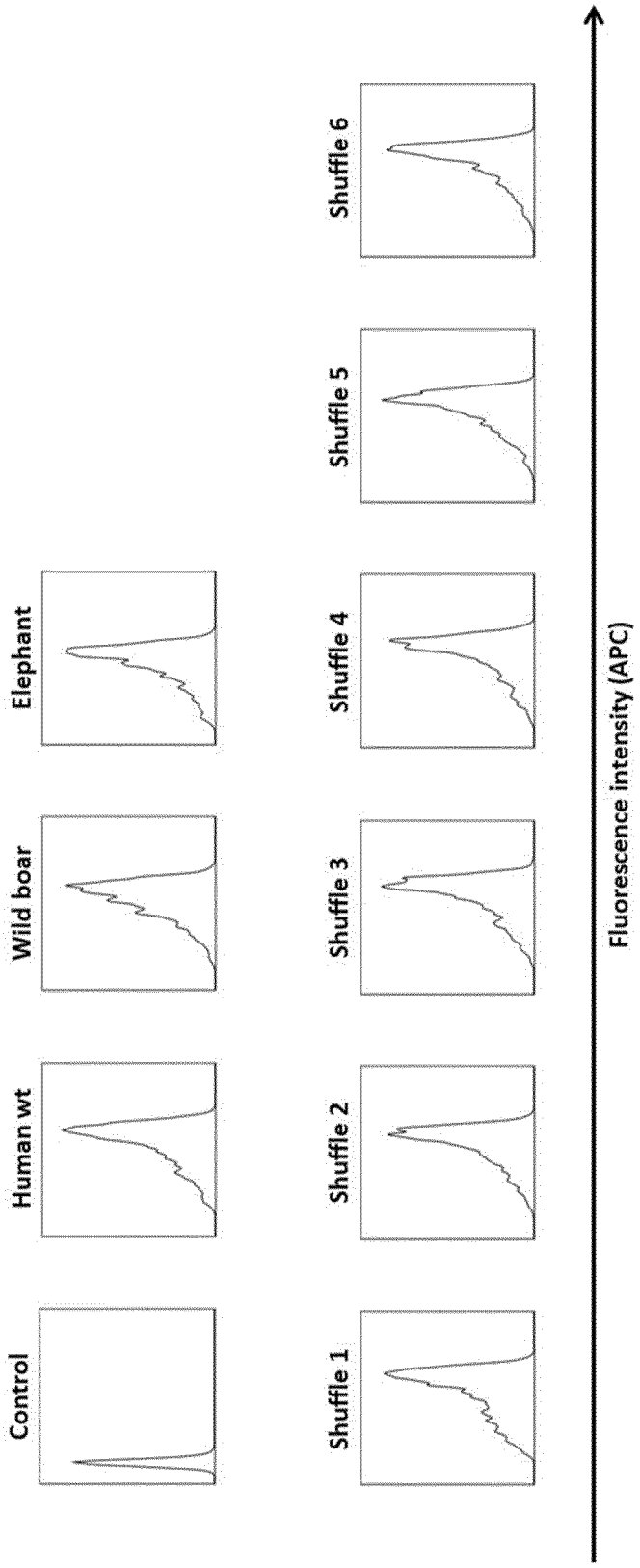

Control
Human wt
Wild boar
Elephant
Shuffle 1
Shuffle 2
Shuffle 3
Shuffle 4
Shuffle 5
Shuffle 6
Fluorescence intensity (APC)

Figure 4

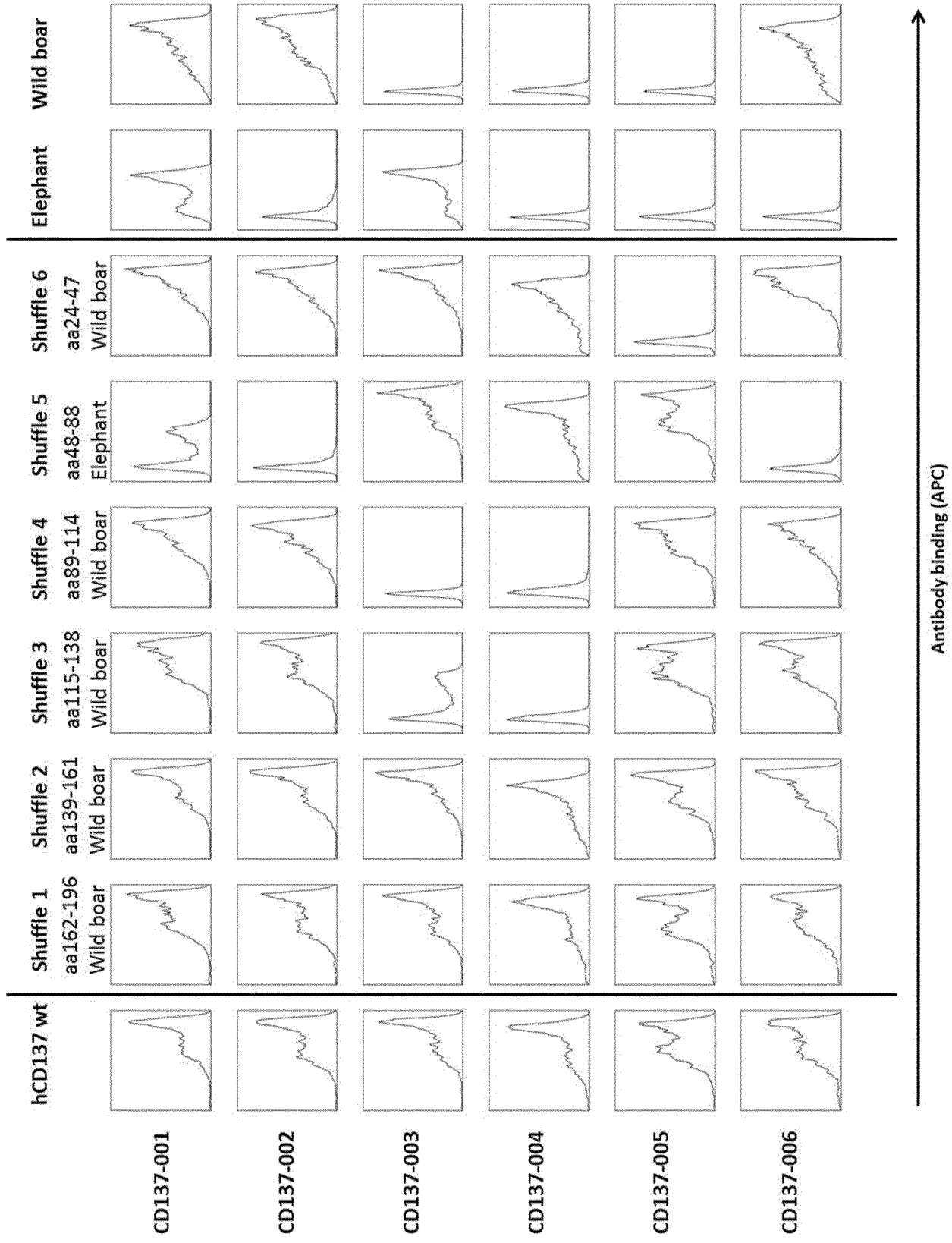

hCD137 wt
Shuffle 1 aa162-196 Wild boar
Shuffle 2 aa139-161 Wild boar
Shuffle 3 aa115-138 Wild boar
Shuffle 4 aa89-114 Wild boar
Shuffle 5 aa48-88 Elephant
Shuffle 6 aa24-47 Wild boar
Elephant
Wild boar
CD137-001
CD137-002
CD137-003
CD137-004
CD137-005
CD137-006
Antibody binding (APC)

Figure 4 (continued)

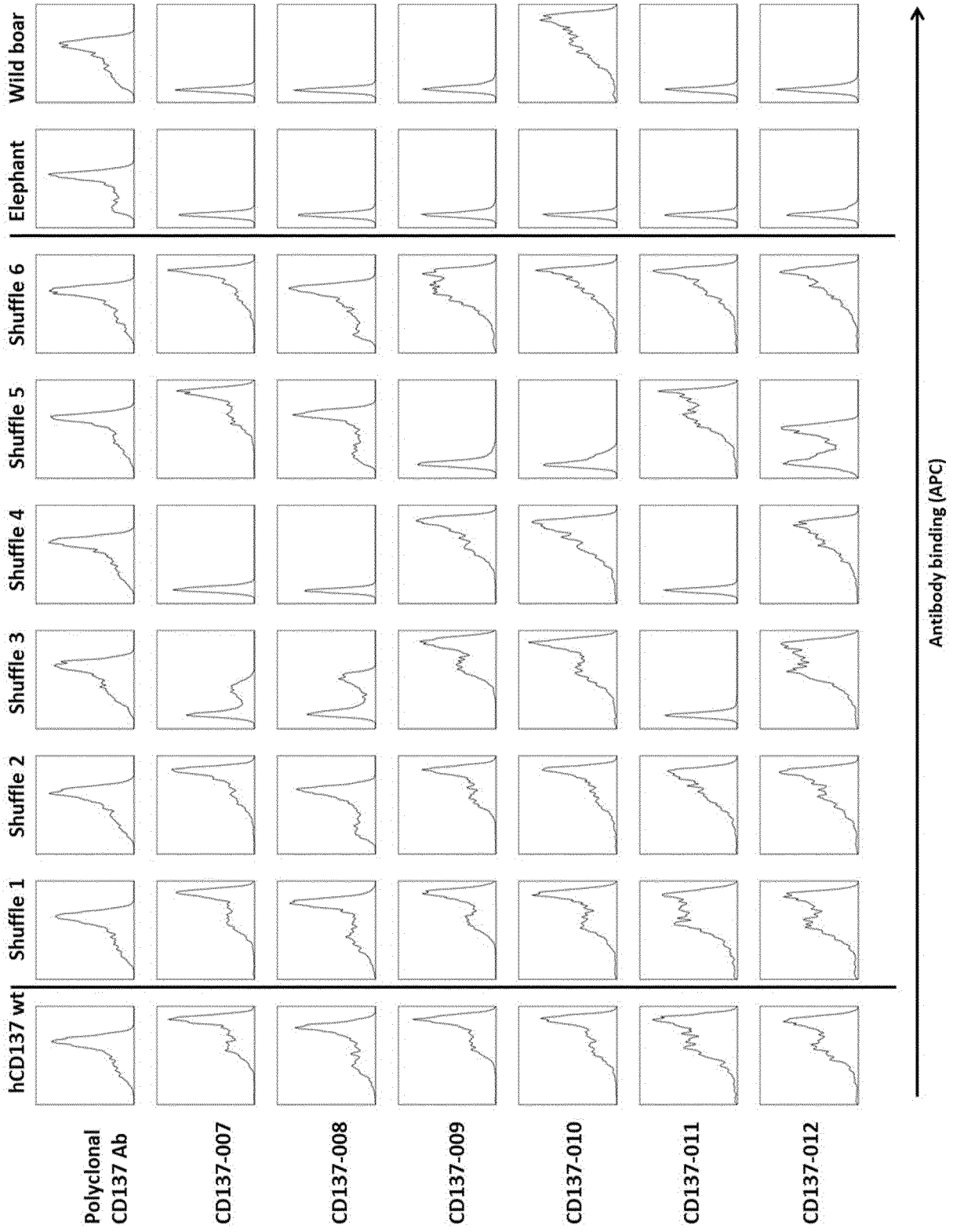


Figure 5

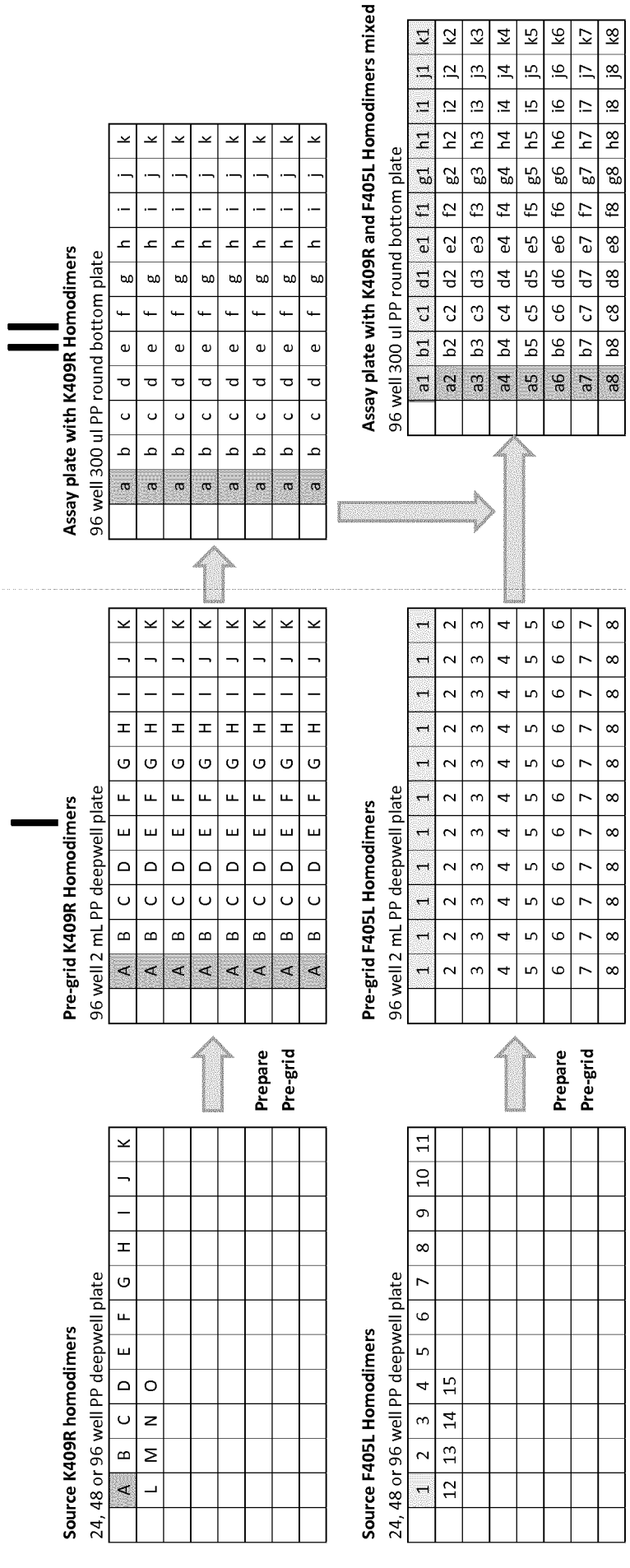

Source K409R homodimers
24, 48 or 96 well PP deepwell plate
Source F405L Homodimers
24, 48 or 96 well PP deepwell plate
Prepare Pre-grid
Prepare Pre-grid
Pre-grid K409R Homodimers
96 well 2 mL PP deepwell plate
Pre-grid F405L Homodimers
96 well 2 mL PP deepwell plate
Assay plate with K409R Homodimers
96 well 300 ul PP round bottom plate
Assay plate with K409R and F405L Homodimers mixed
96 well 300 ul PP round bottom plate

Figure 6

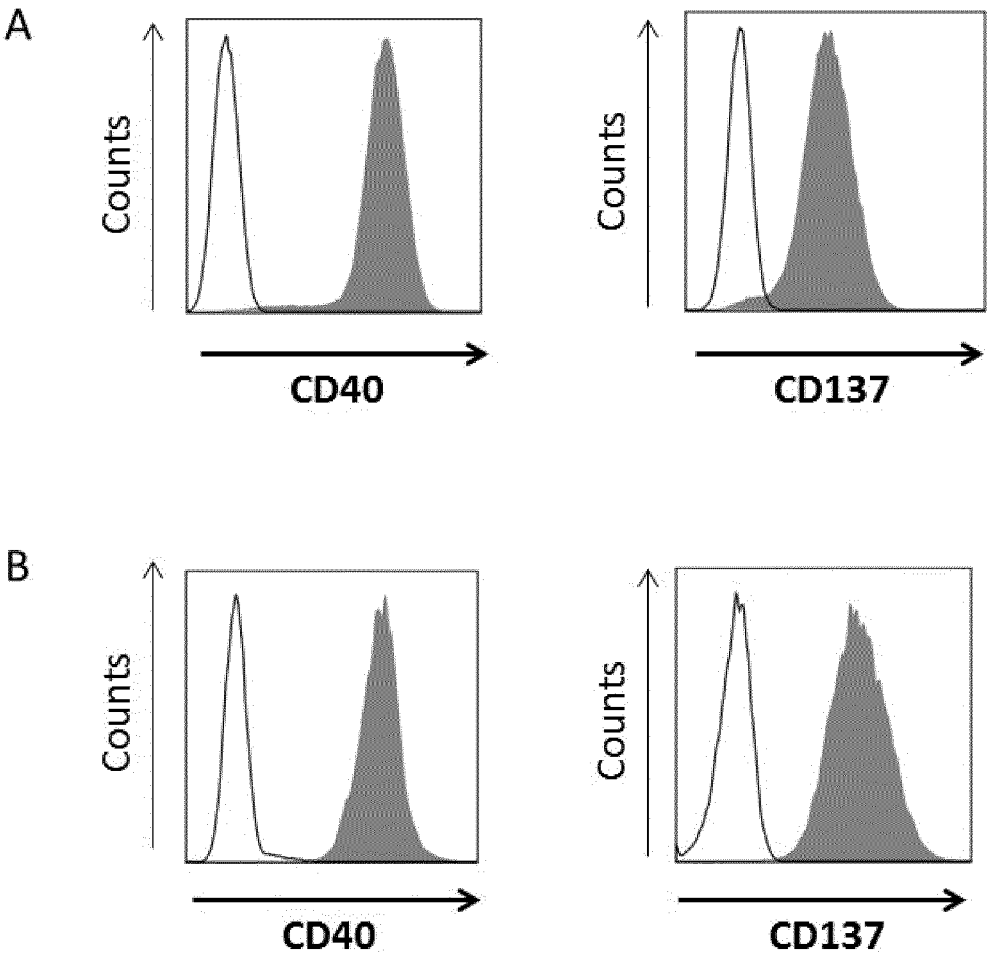


Figure 7

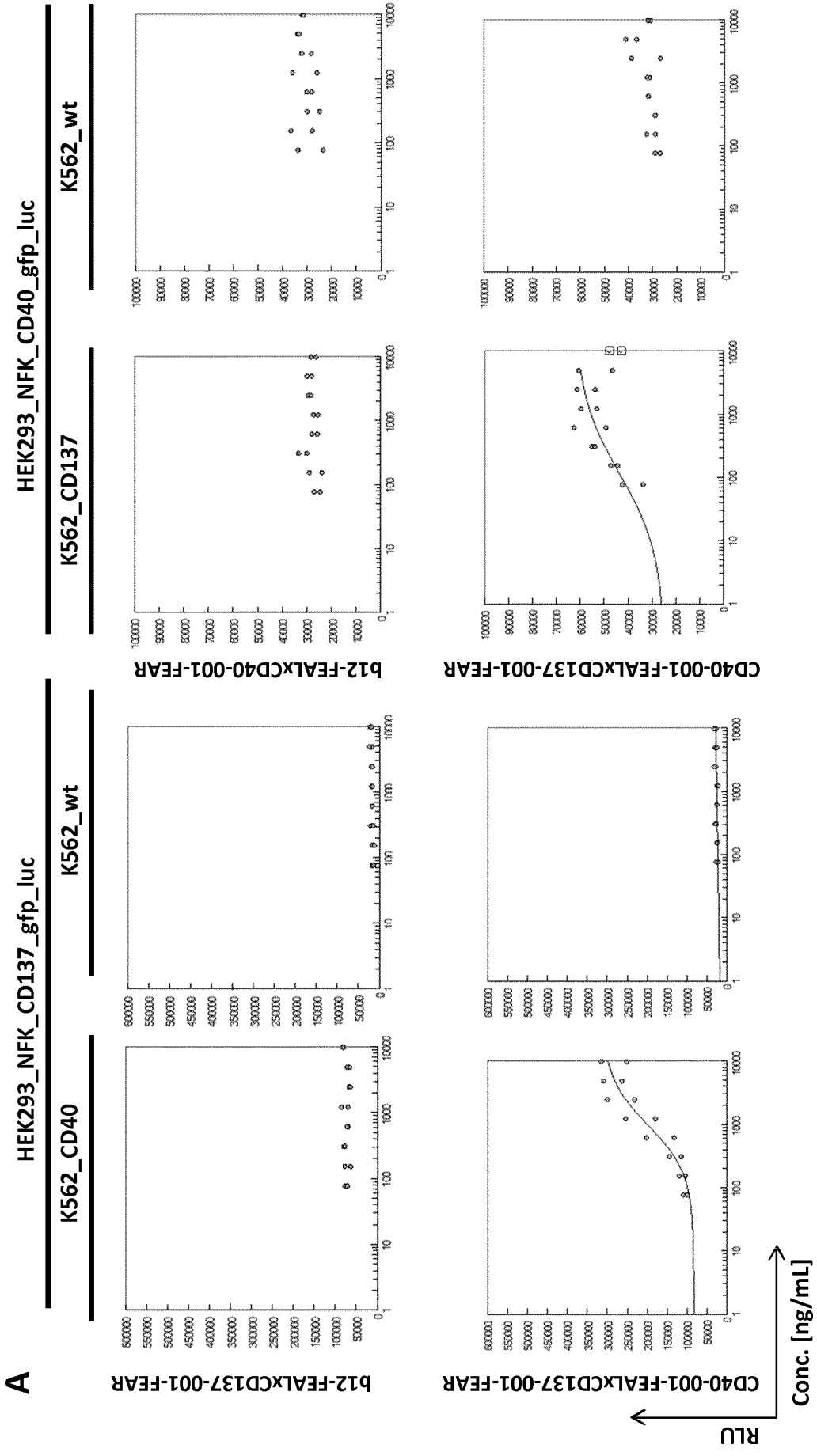

A
HEK293_NFK_CD137_gfp_luc
HEK293_NFK_CD40_gfp_luc
K562_CD40
K562_wt
K562_CD137
K562_wt
b12-FEALxCD137-001-FEAR
CD40-001-FEALxCD137-001-FEAR
b12-FEALxCD40-001-FEAR
CD40-001-FEALxCD40-001-FEAR
RLU
Conc. [ng/mL]

Figure 7 (continued)

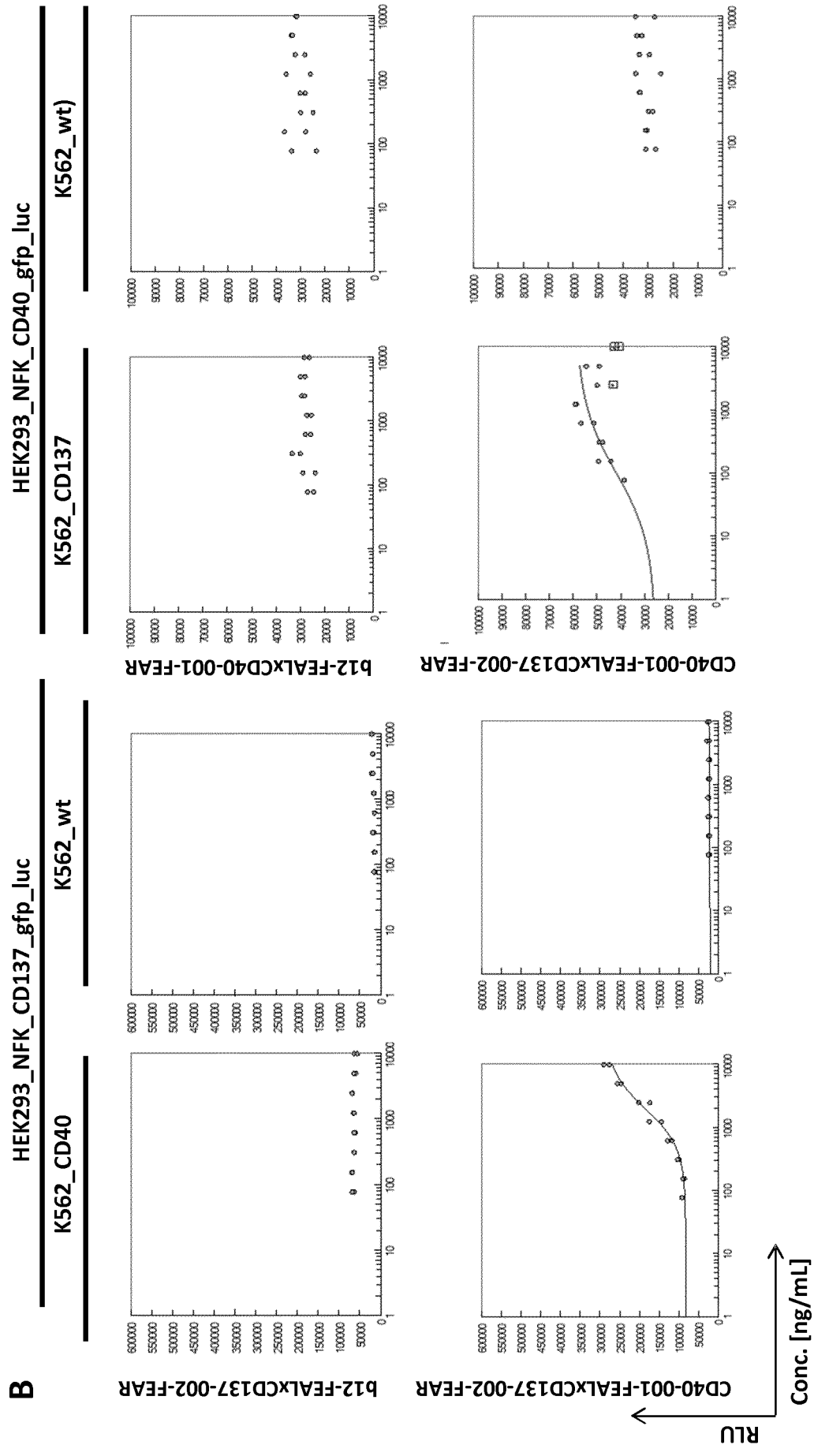


Figure 7 (continued)

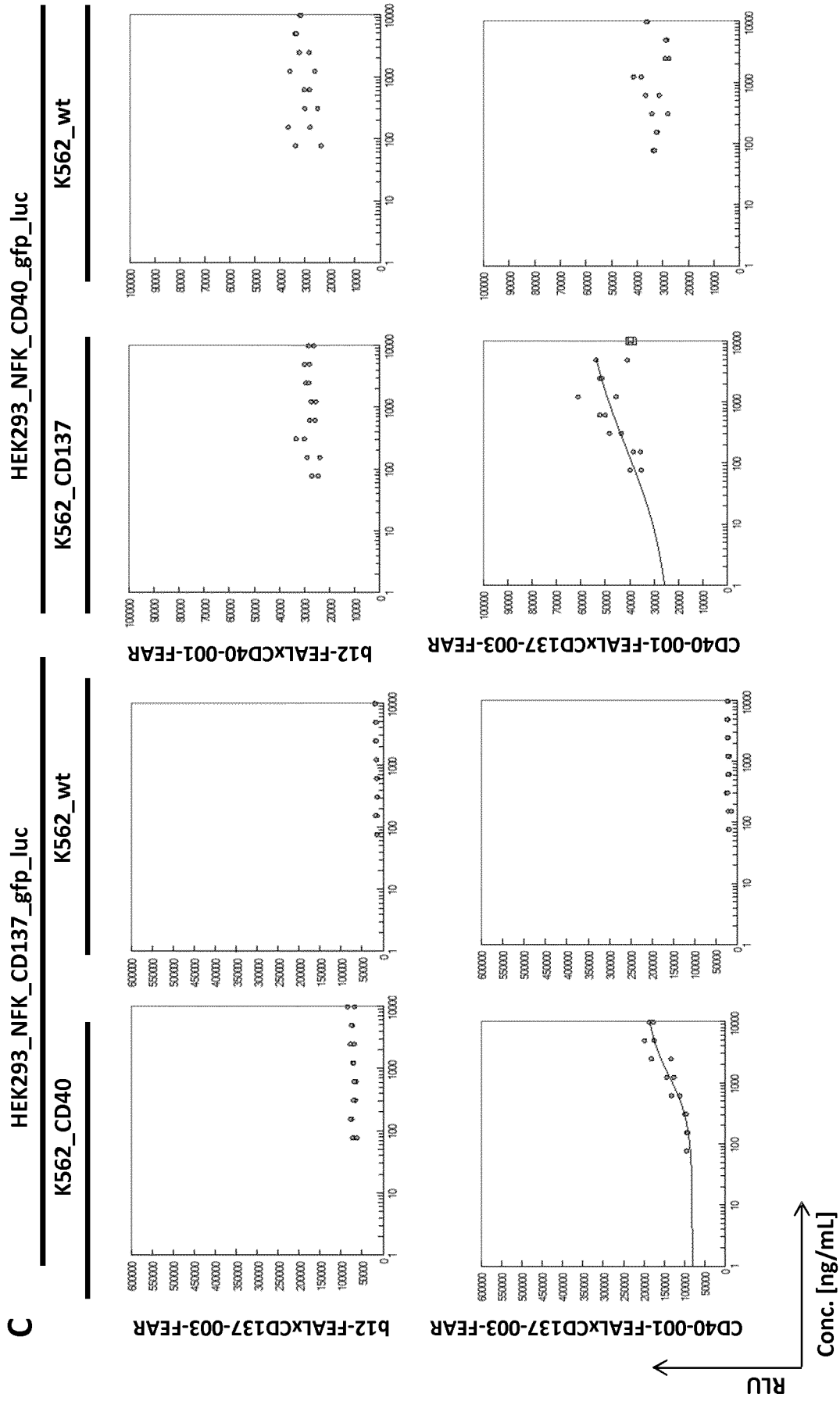


Figure 7 (continued)

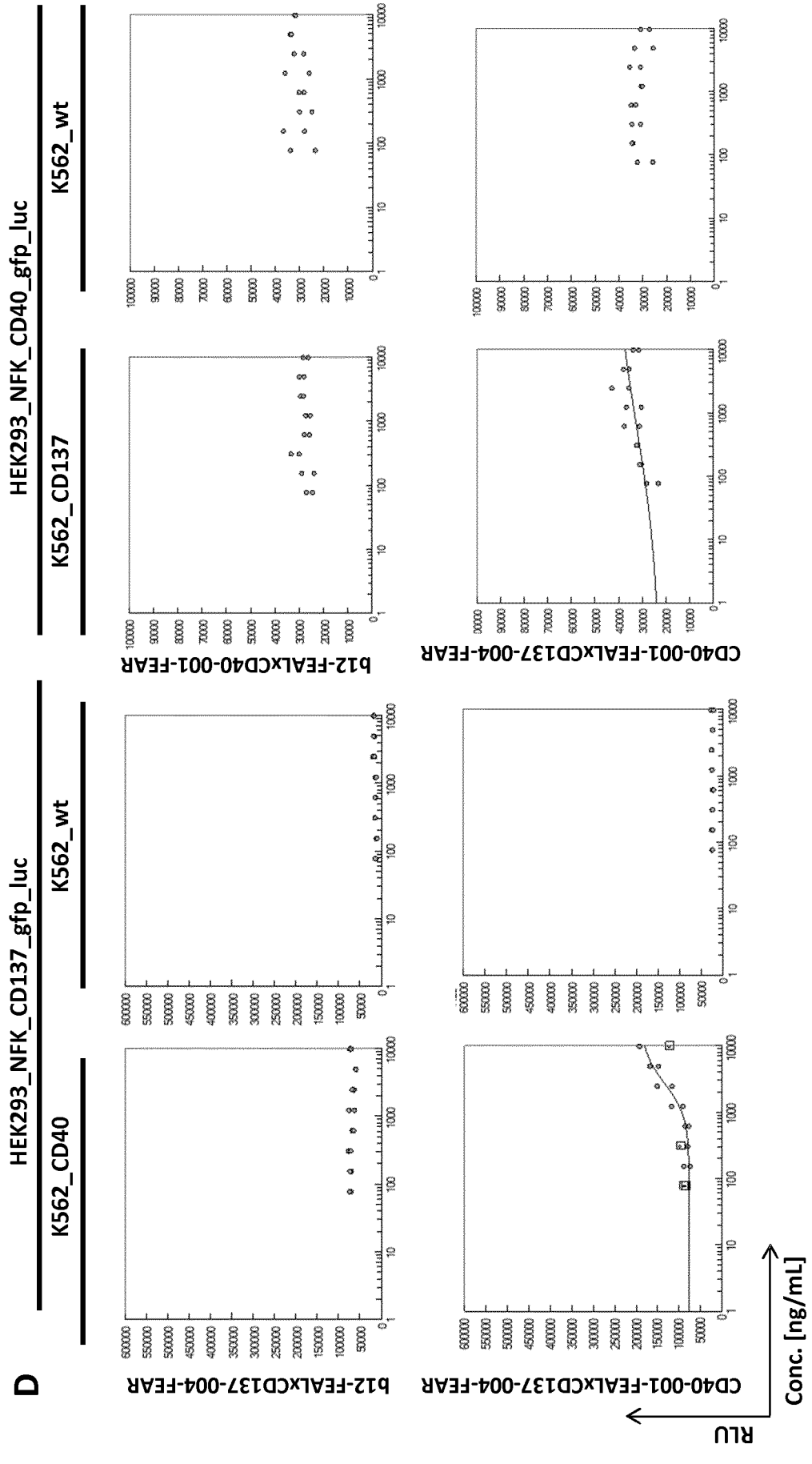


Figure 7 (continued)

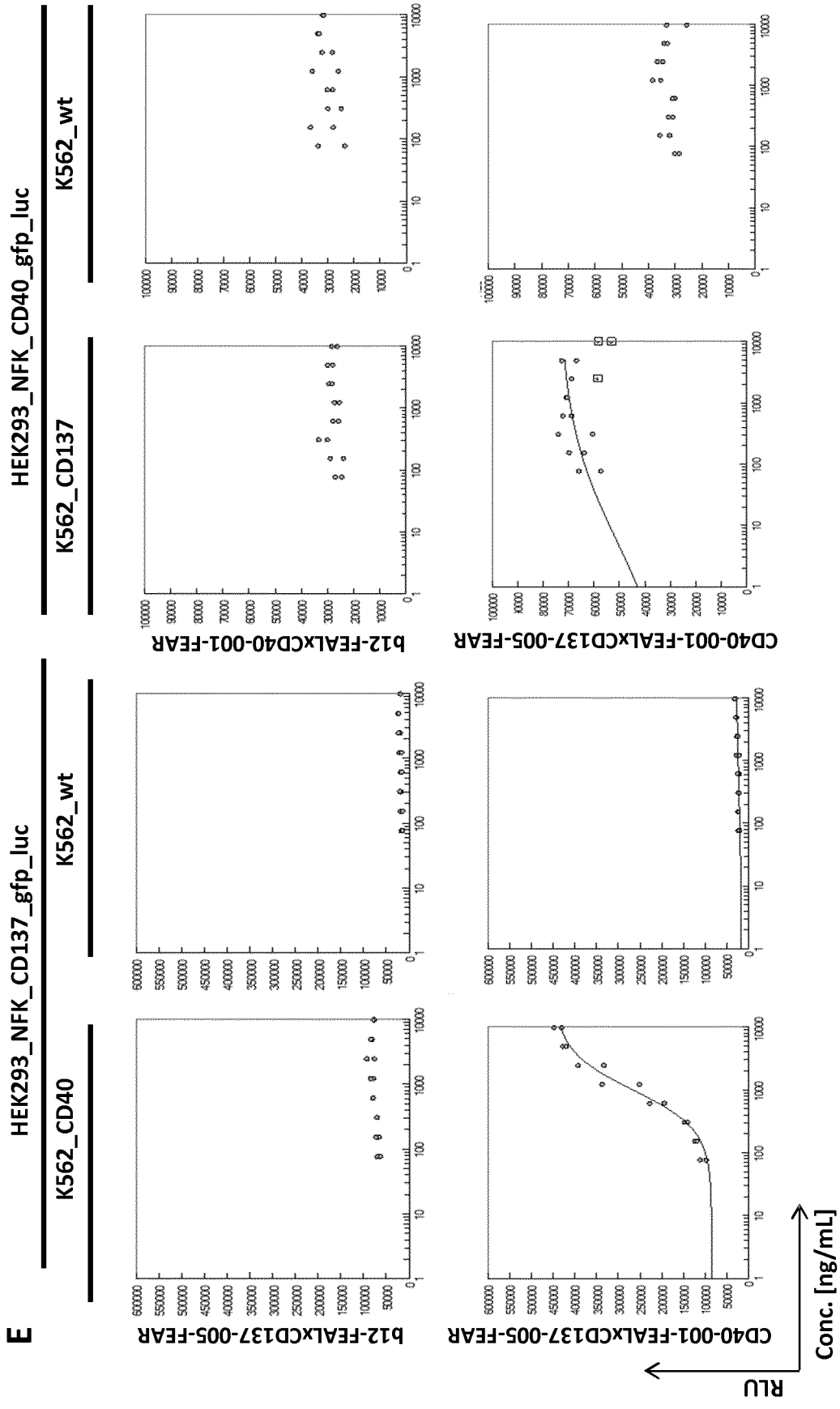


Figure 7 (continued)

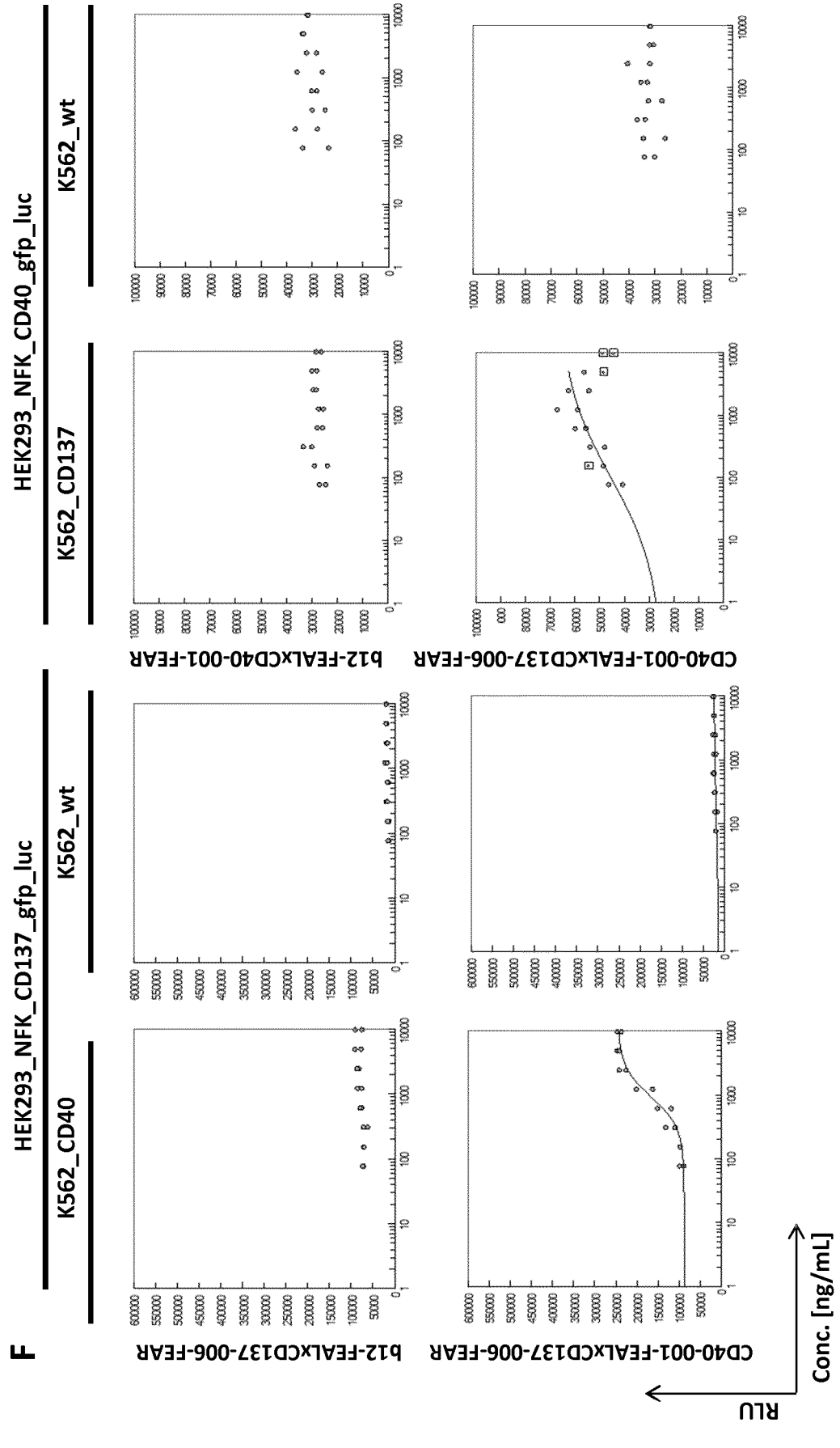


Figure 7 (continued)

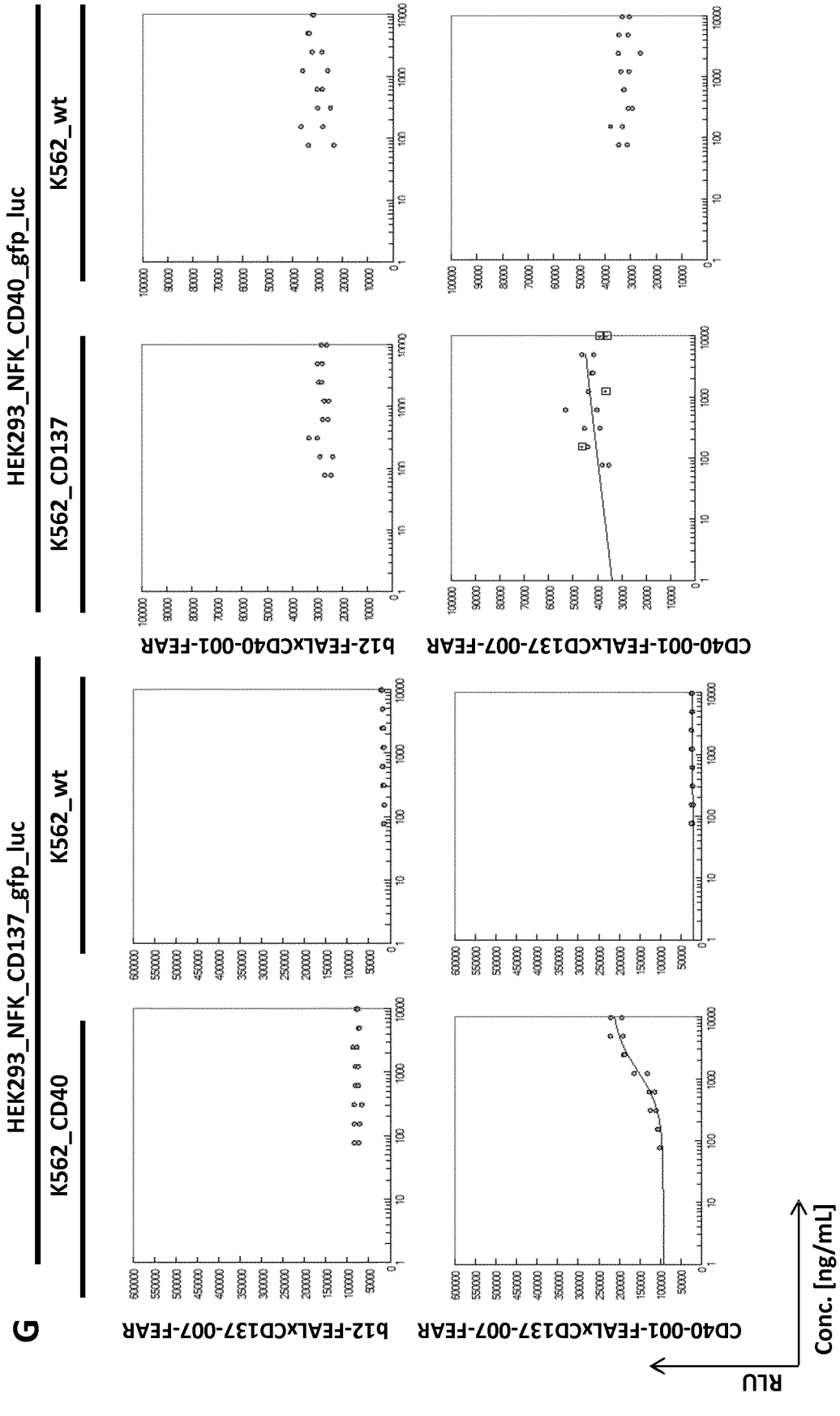


Figure 7 (continued)

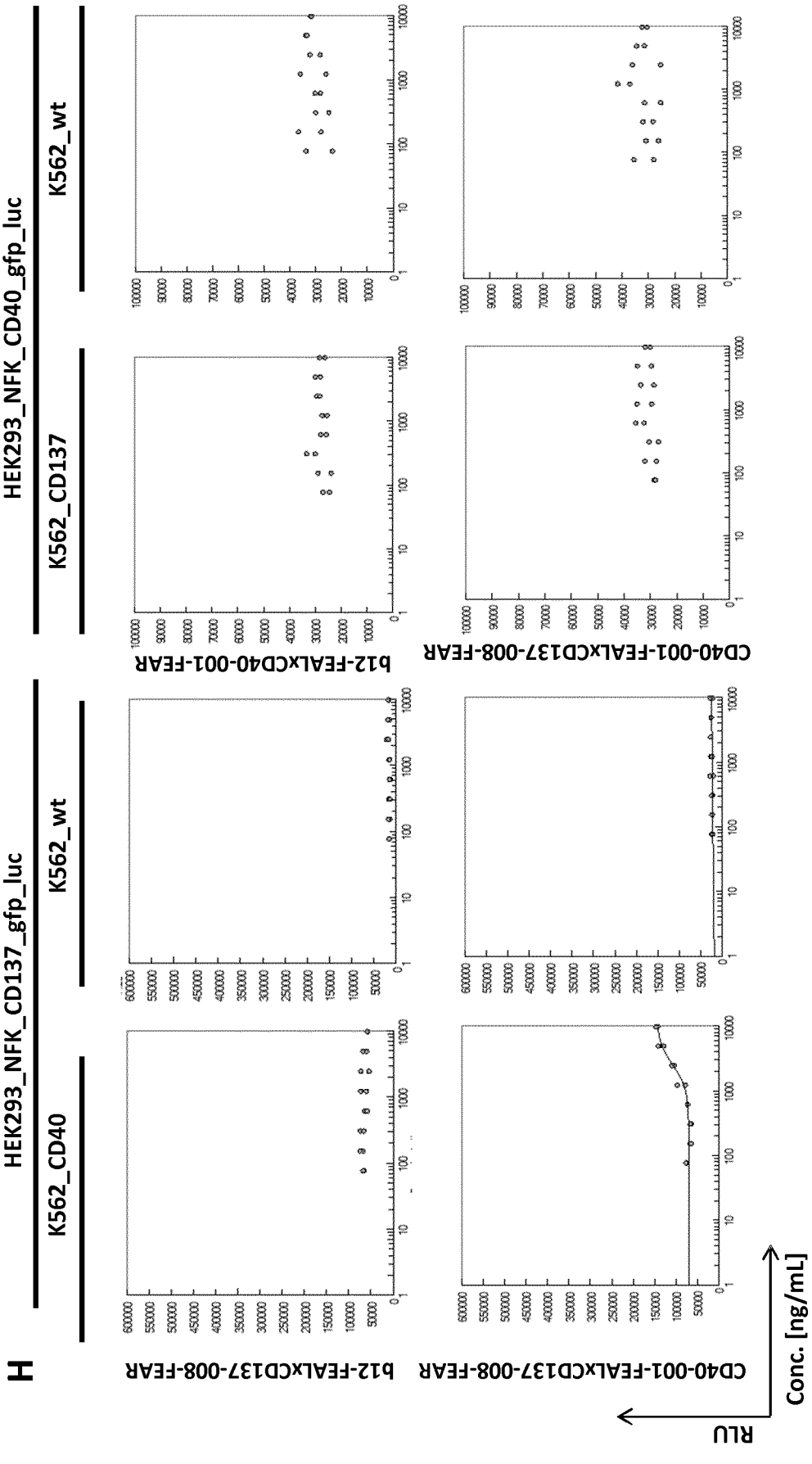


Figure 7 (continued)

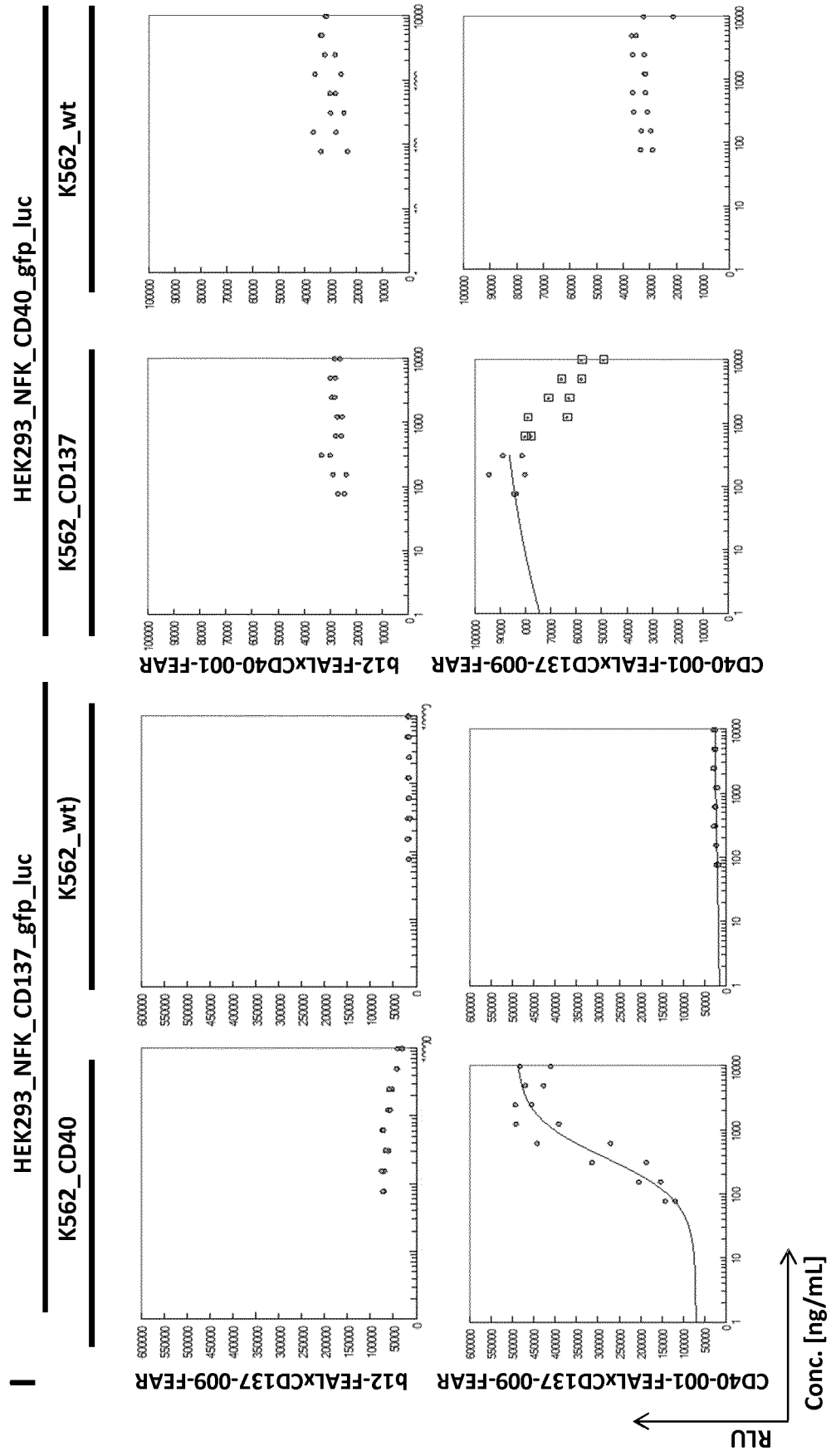


Figure 7 (continued)

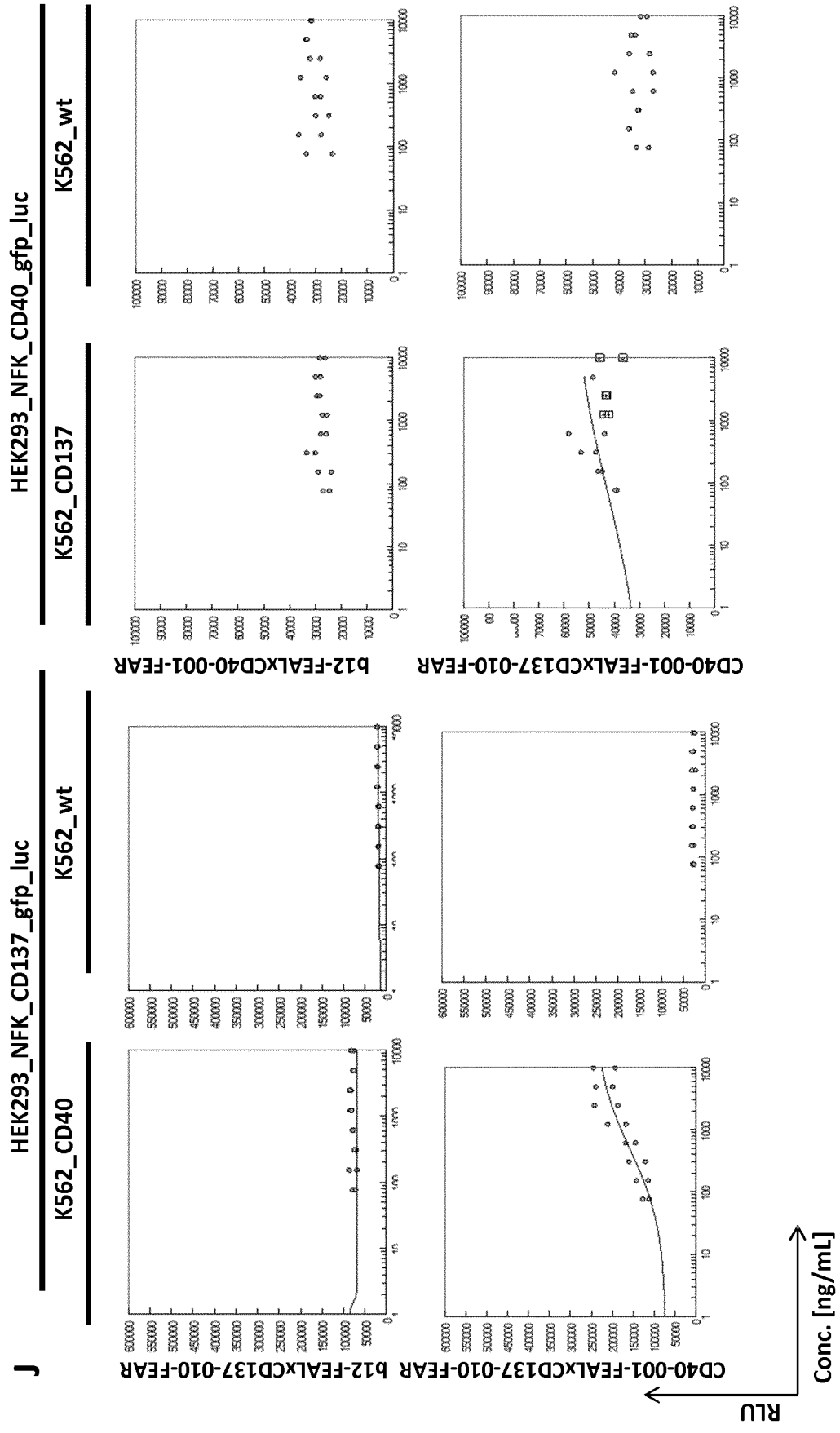


Figure 7 (continued)

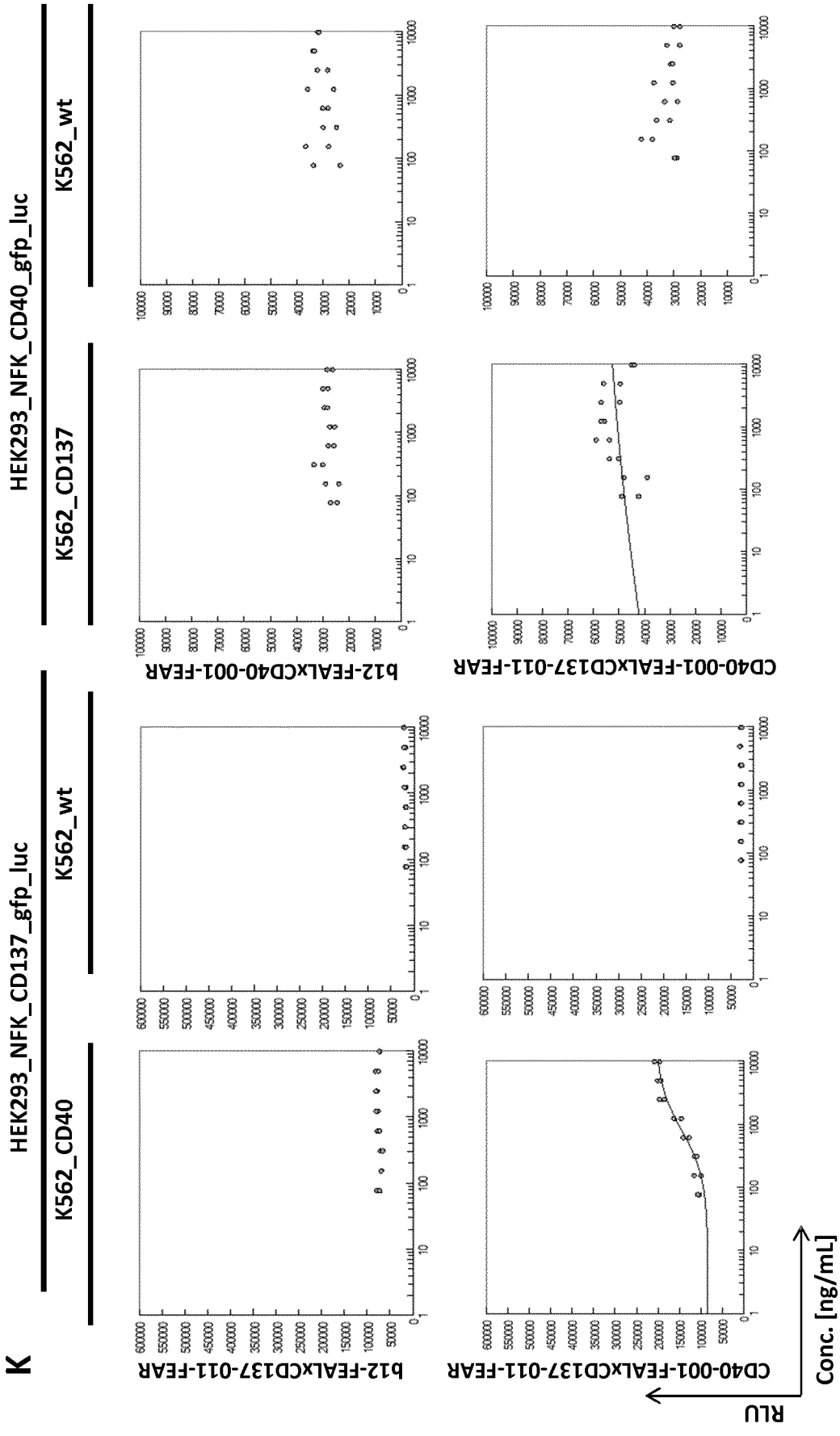


Figure 7 (continued)

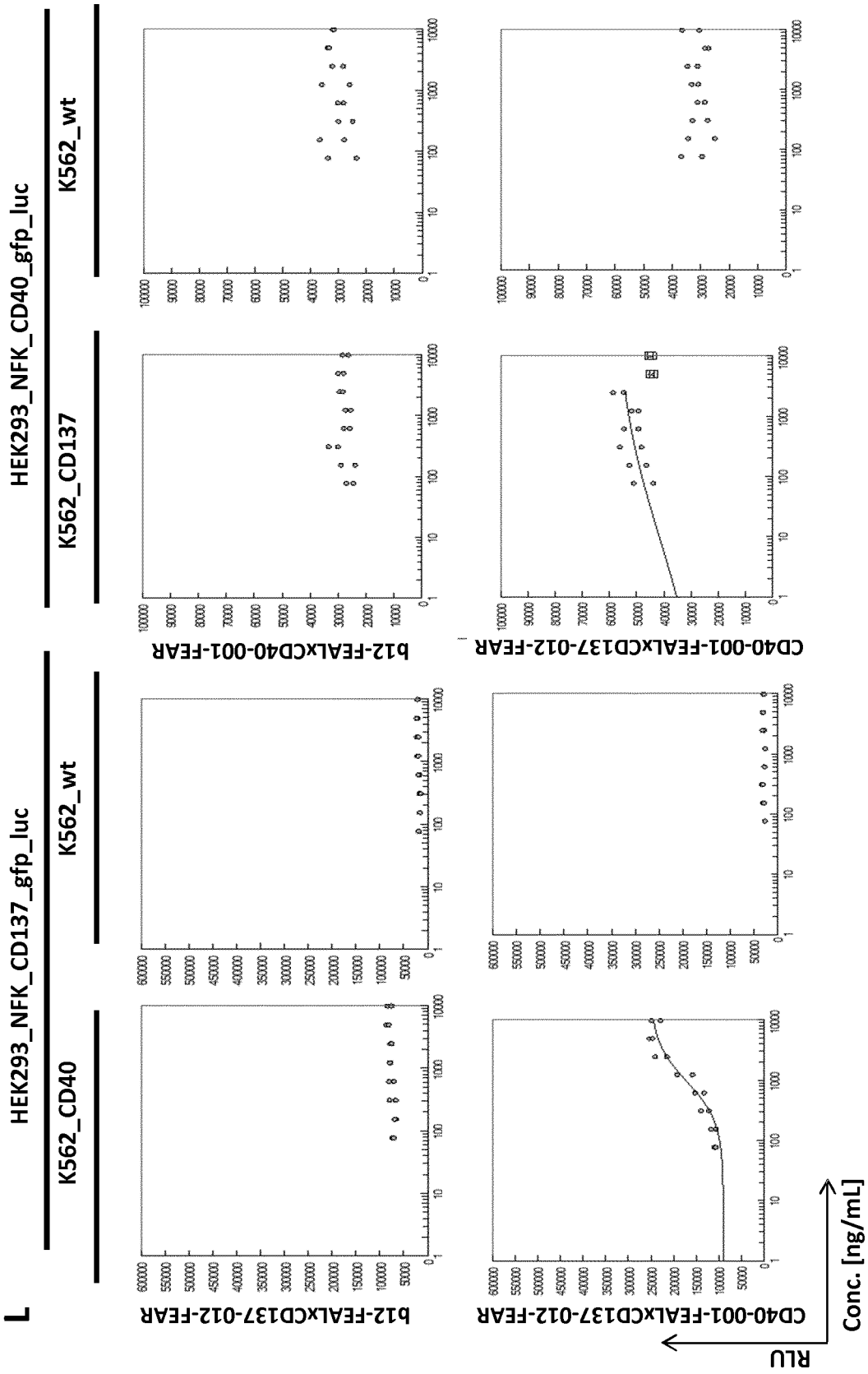


Figure 8
A

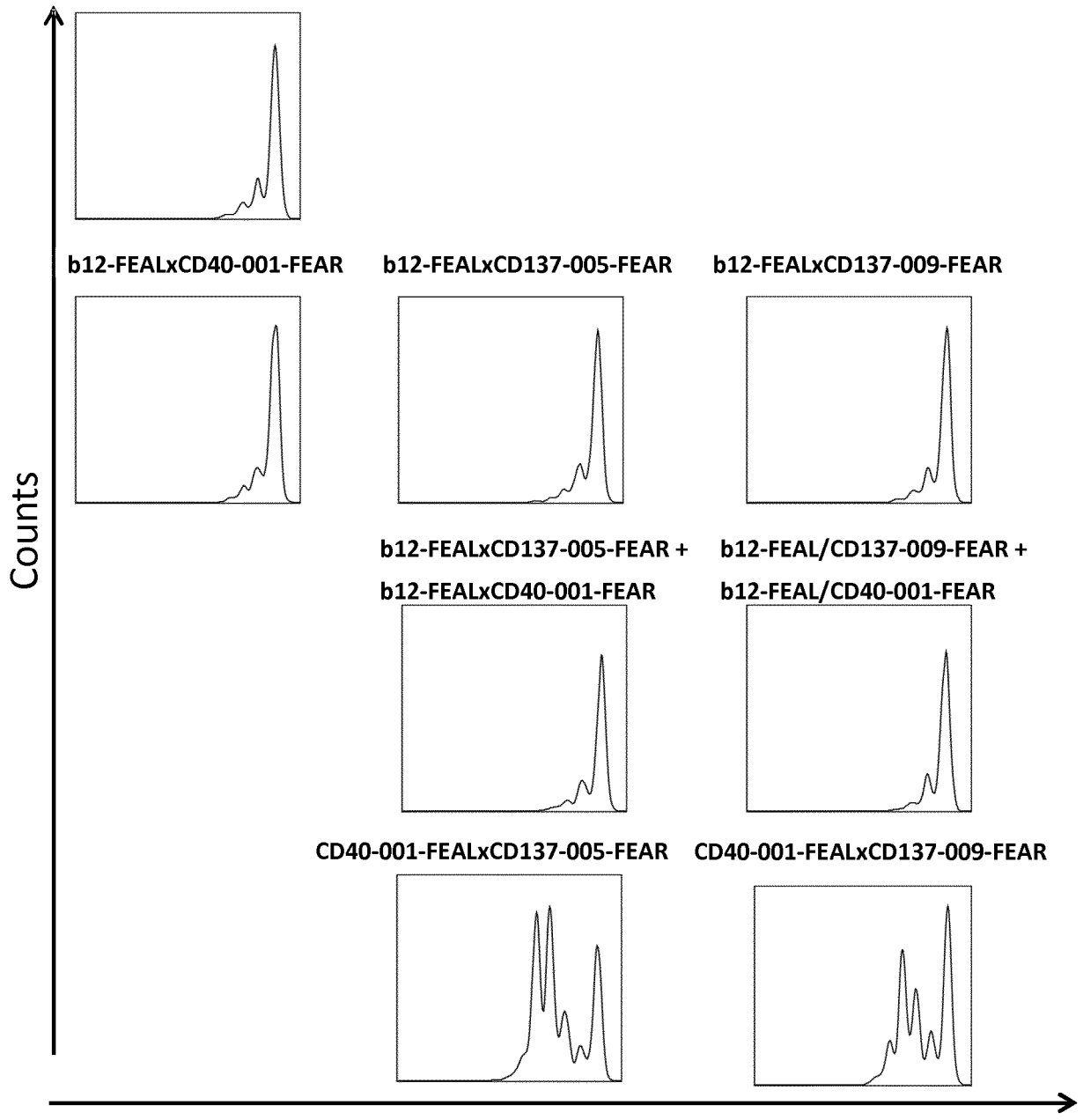
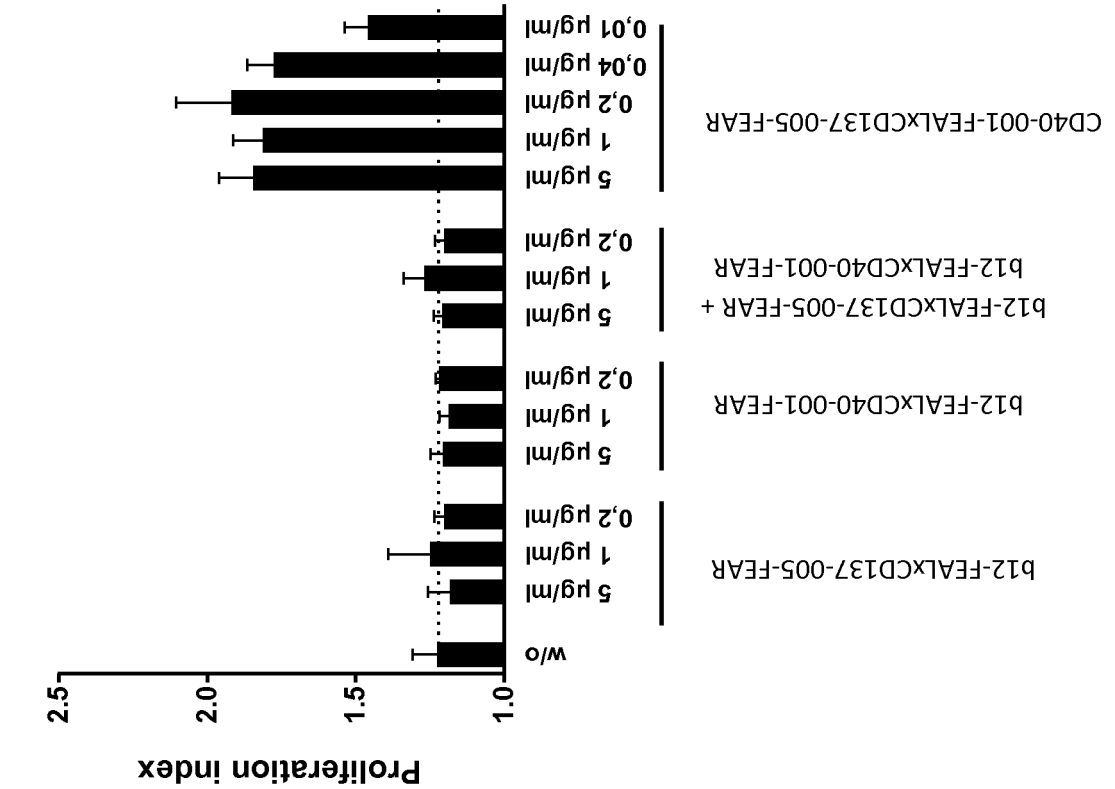

Counts
b12-FEALxCD40-001-FEAR
b12-FEALxCD137-005-FEAR
b12-FEALxCD137-009-FEAR
b12-FEALxCD137-005-FEAR +
b12-FEALxCD40-001-FEAR
b12-FEAL/CD137-009-FEAR +
b12-FEAL/CD40-001-FEAR
CD40-001-FEALxCD137-005-FEAR
CD40-001-FEALxCD137-009-FEAR

Figure 8 (continued)



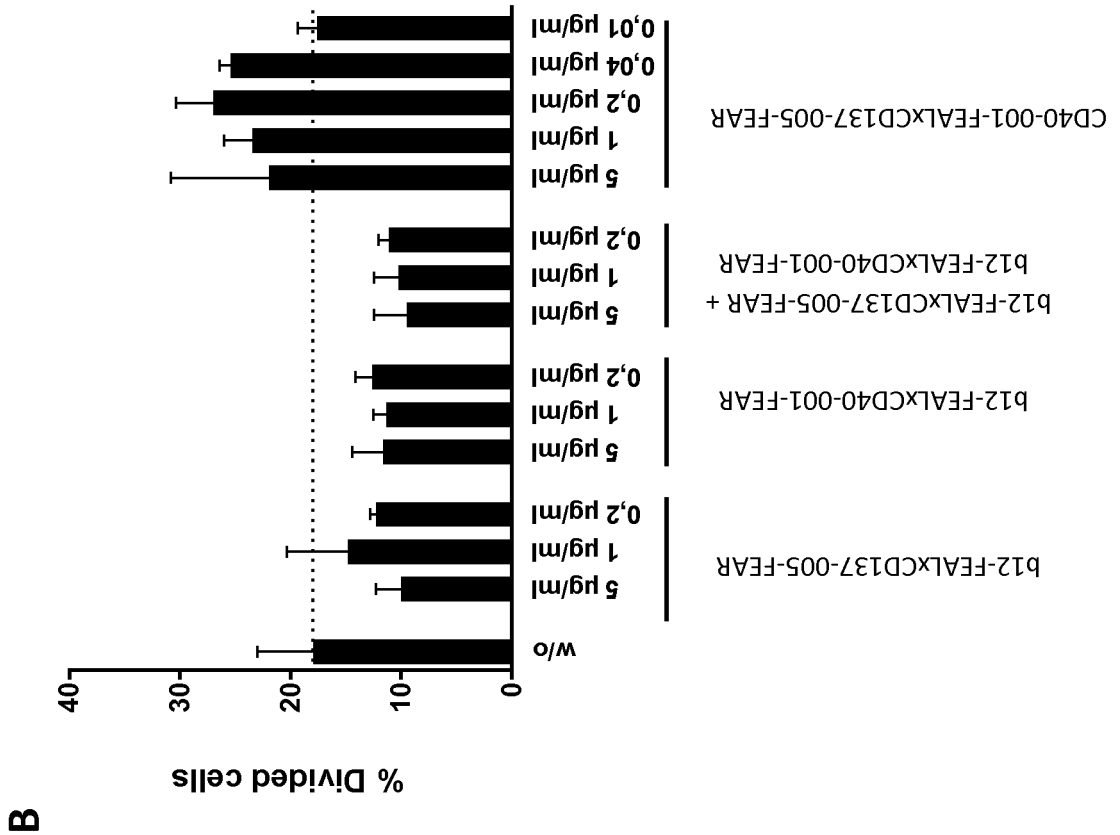
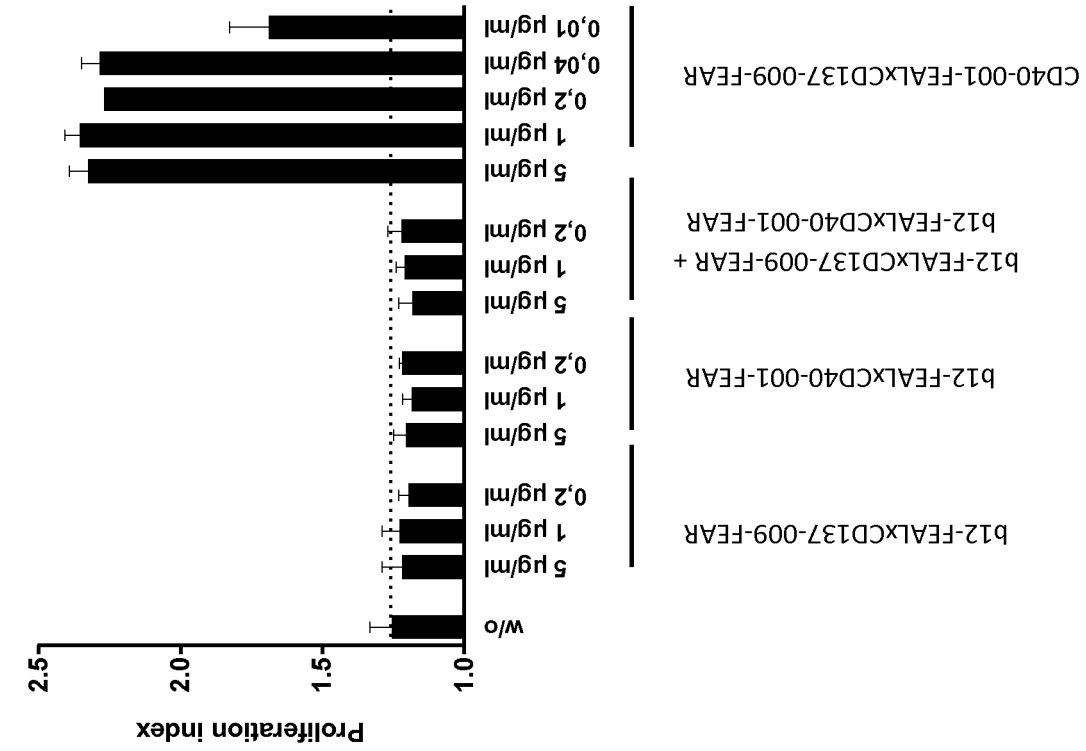
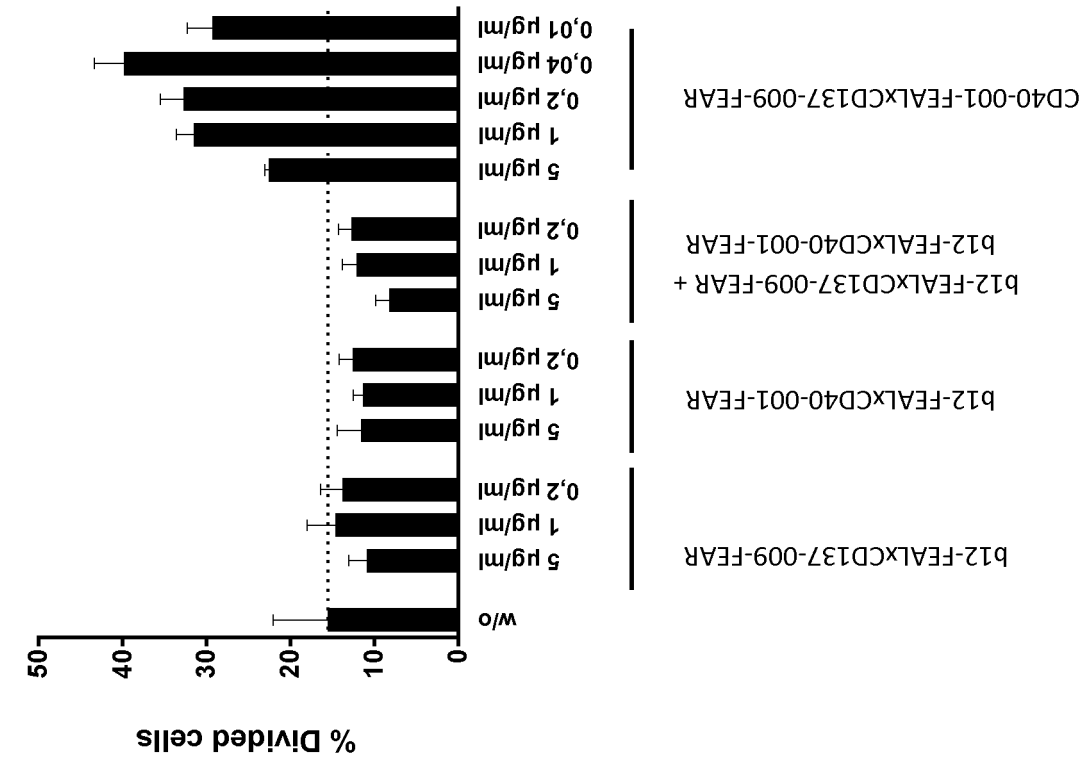


Figure 8 (continued)



C



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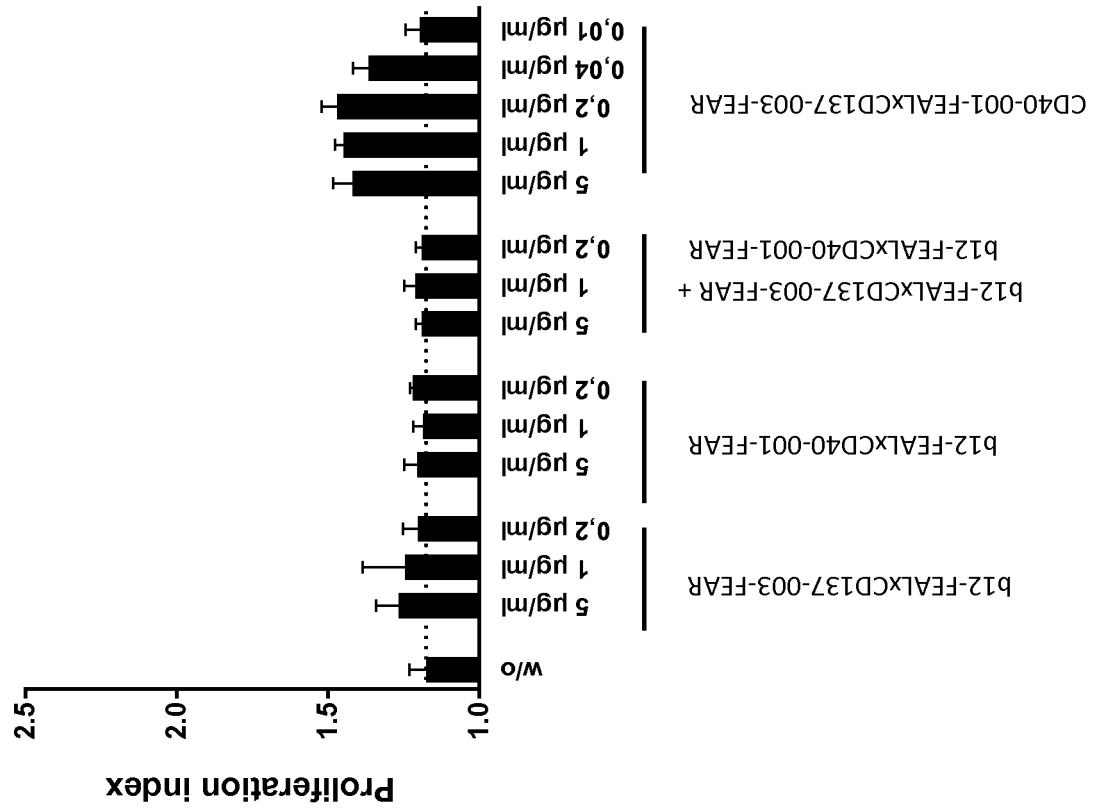


D

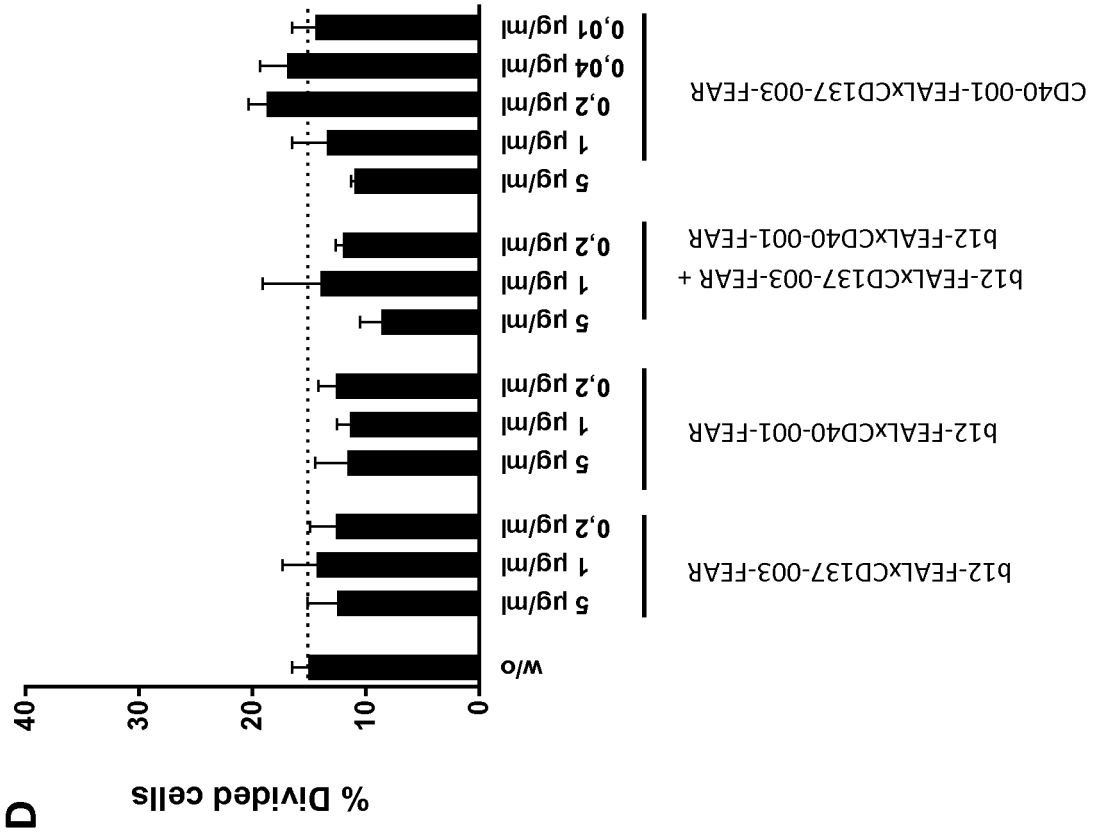
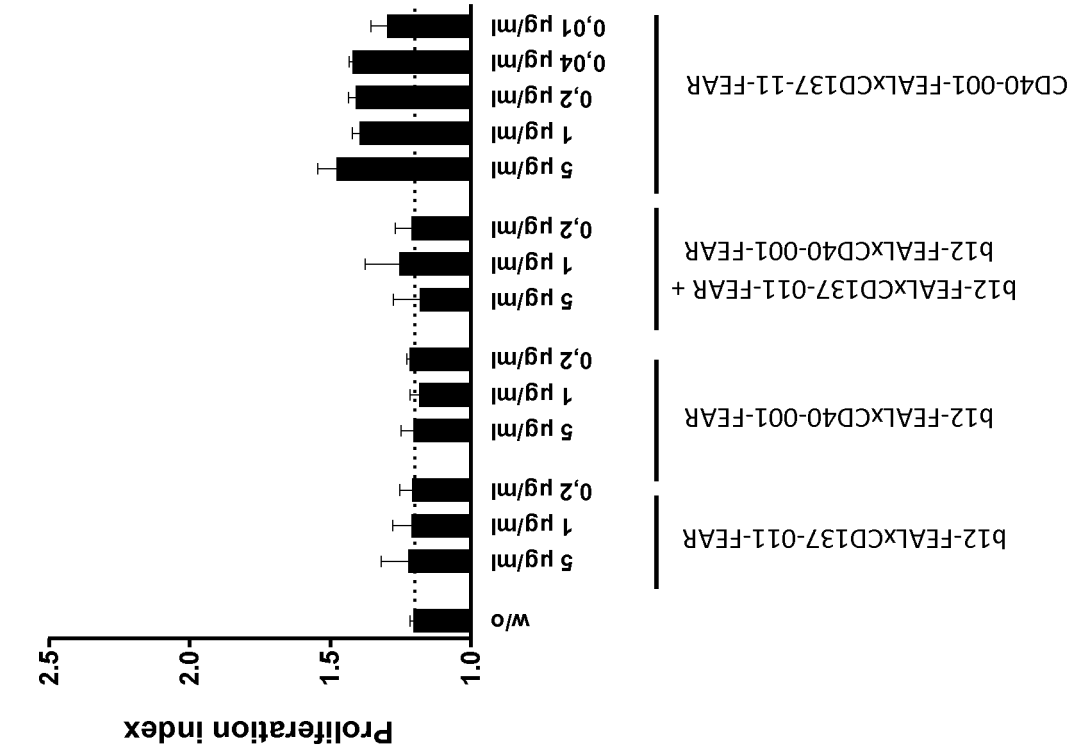


Figure 8 (continued)



E

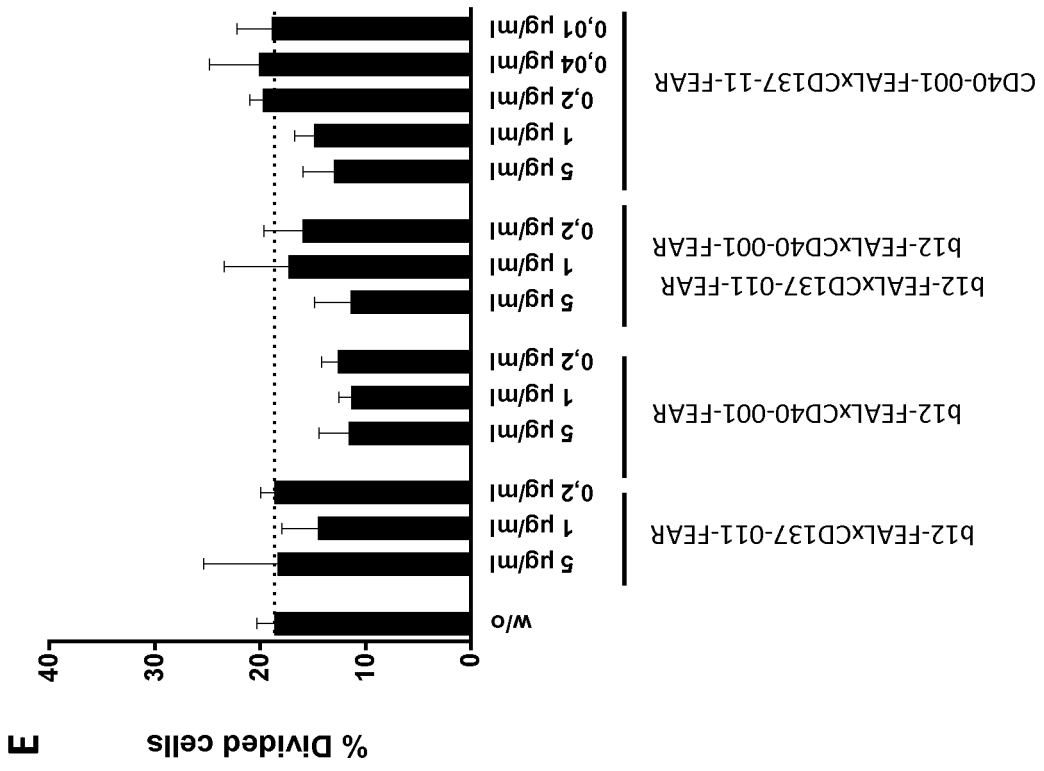


Figure 9
A

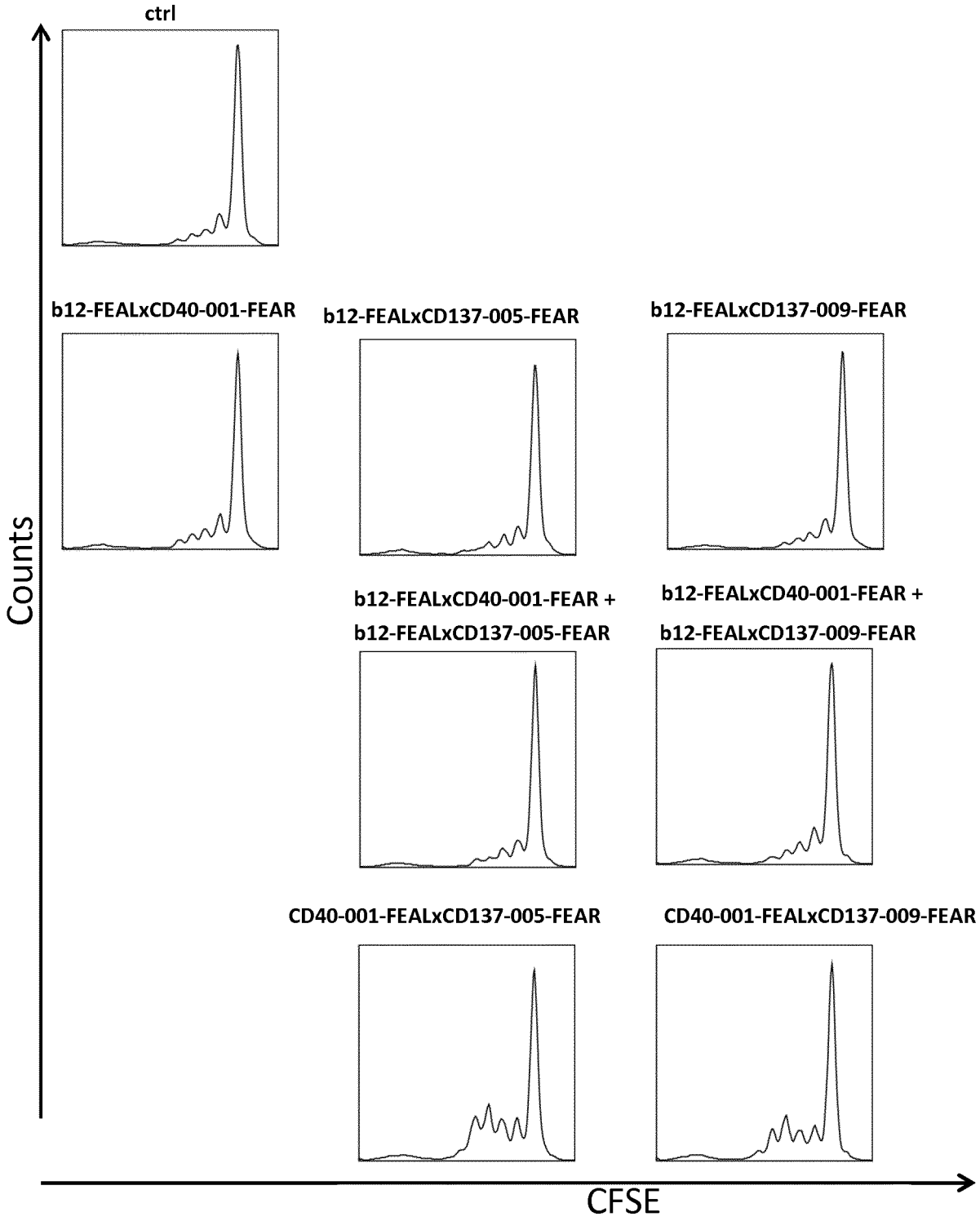
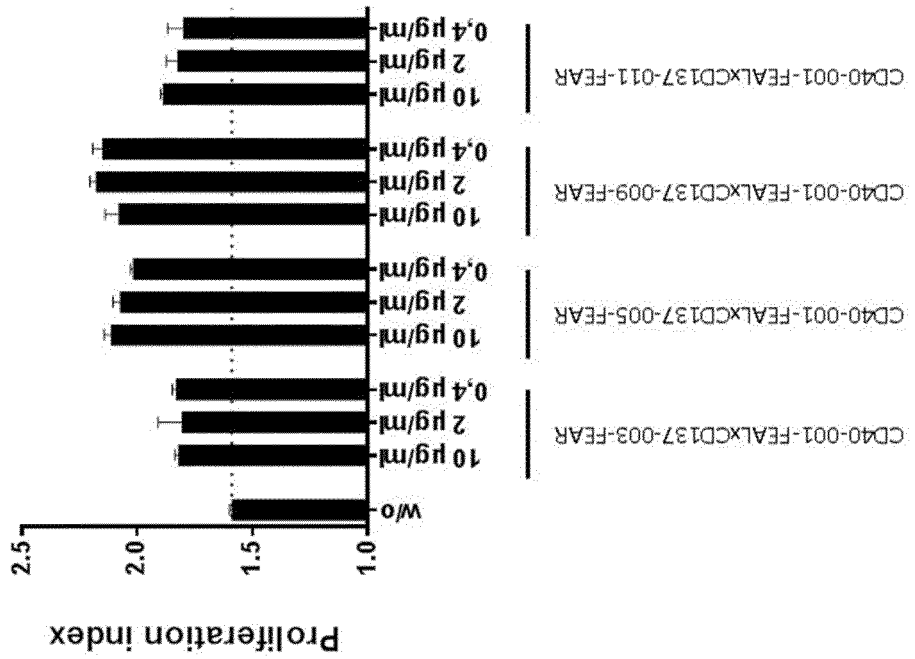

ctrl
b12-FEALxCD40-001-FEAR
b12-FEALxCD137-005-FEAR
b12-FEALxCD137-009-FEAR
b12-FEALxCD40-001-FEAR +
b12-FEALxCD137-005-FEAR
b12-FEALxCD40-001-FEAR +
b12-FEALxCD137-009-FEAR
CD40-001-FEALxCD137-005-FEAR
CD40-001-FEALxCD137-009-FEAR
Counts
CFSE

Figure 9 (continued)



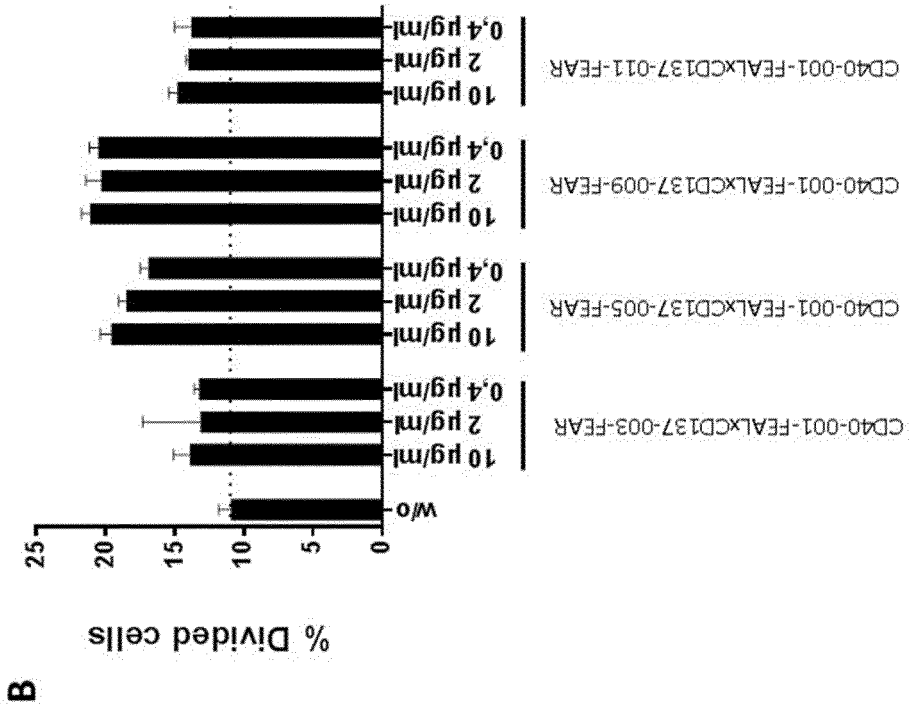
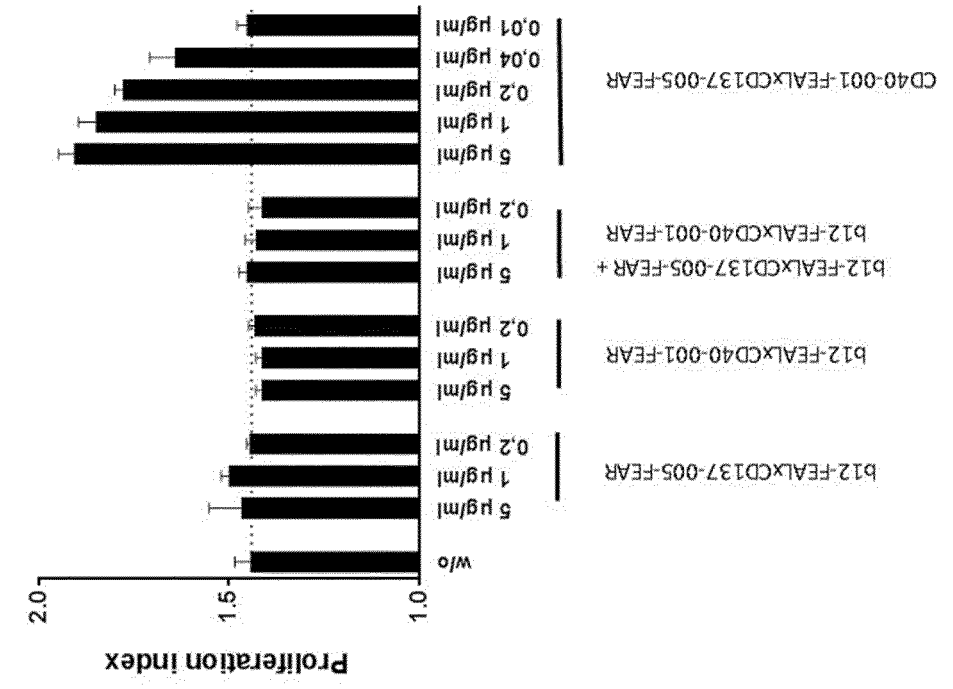


Figure 9 (continued)



C

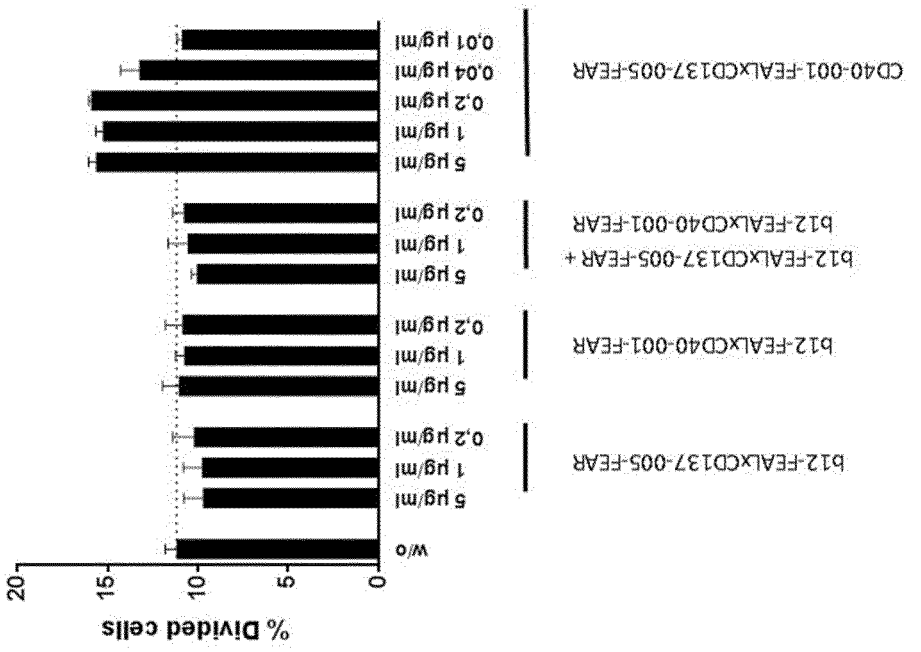


Figure 9 (continued)

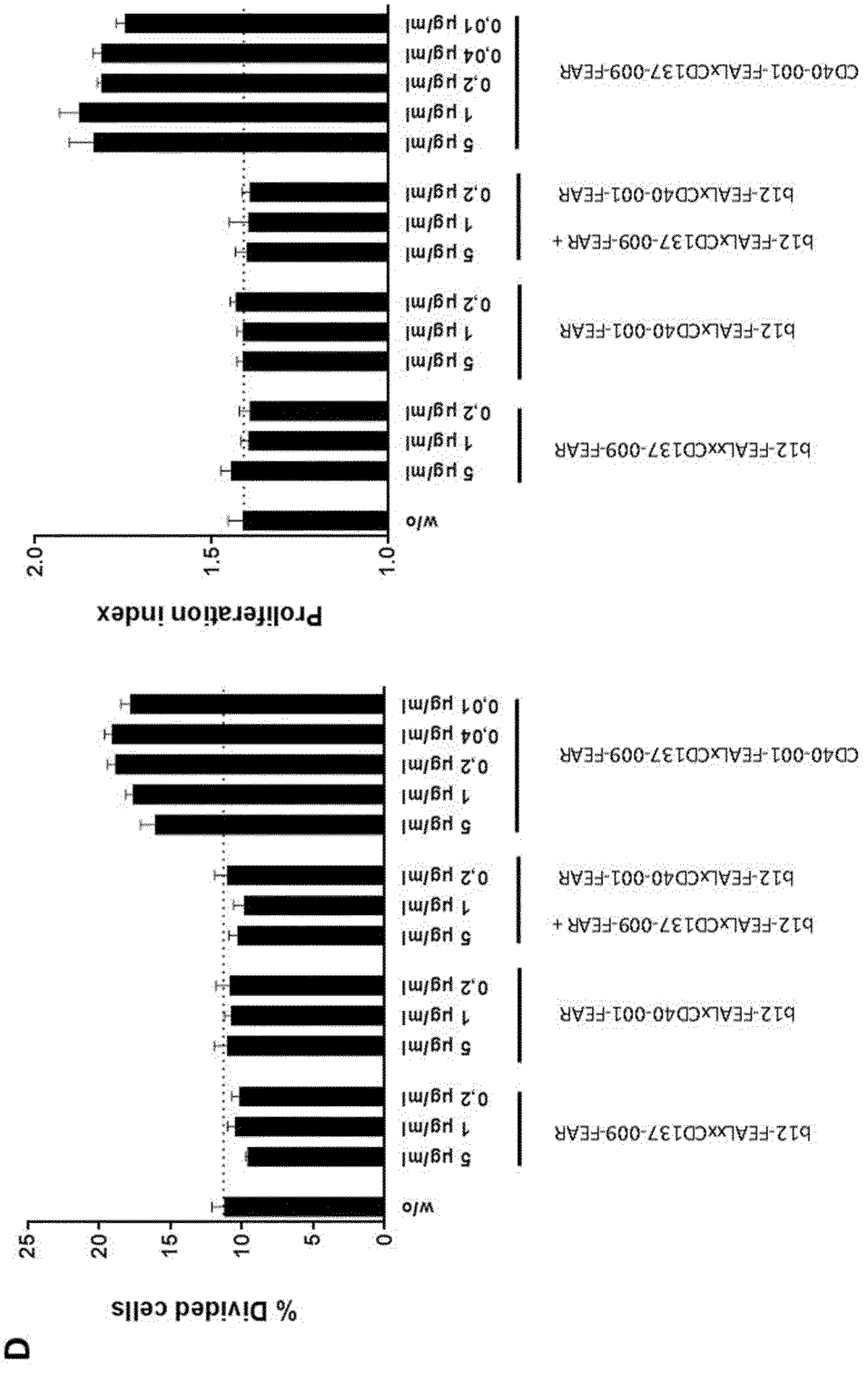


Figure 9 (continued)

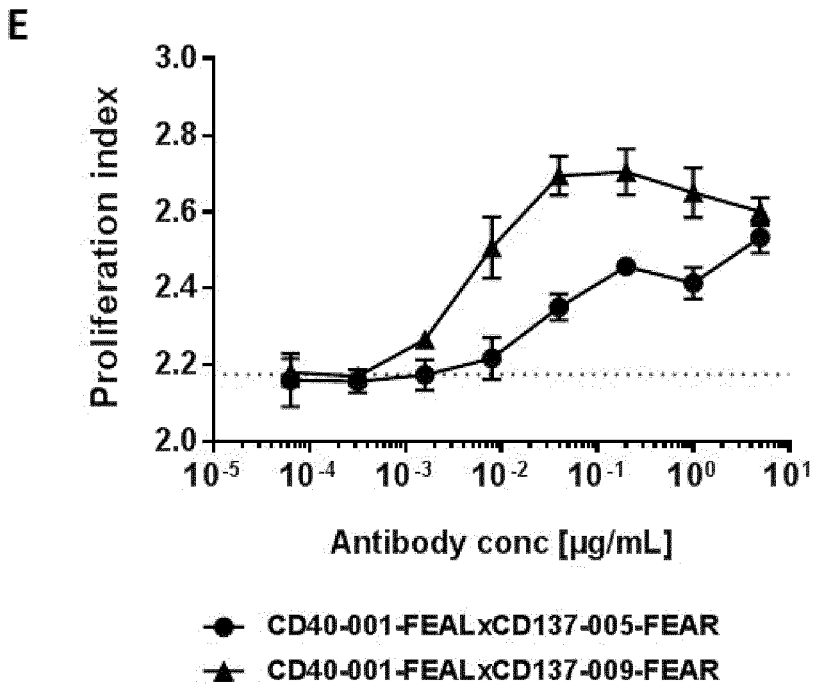
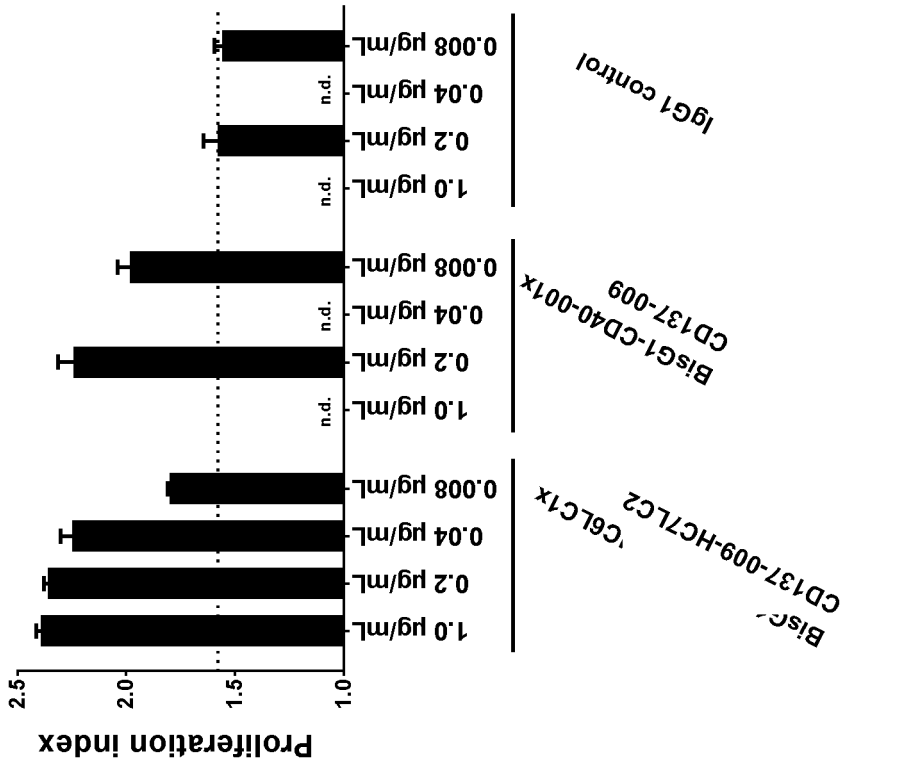


Figure 10


Proliferation index
2.5
2.0
1.5
1.0
1.0 µg/mL
0.2 µg/mL
0.04 µg/mL
0.008 µg/mL
n.d.
BisG1-CD40-001x CD137-009-HC7LC2
BisG1-CD40-001x CD137-009
IgG1 control

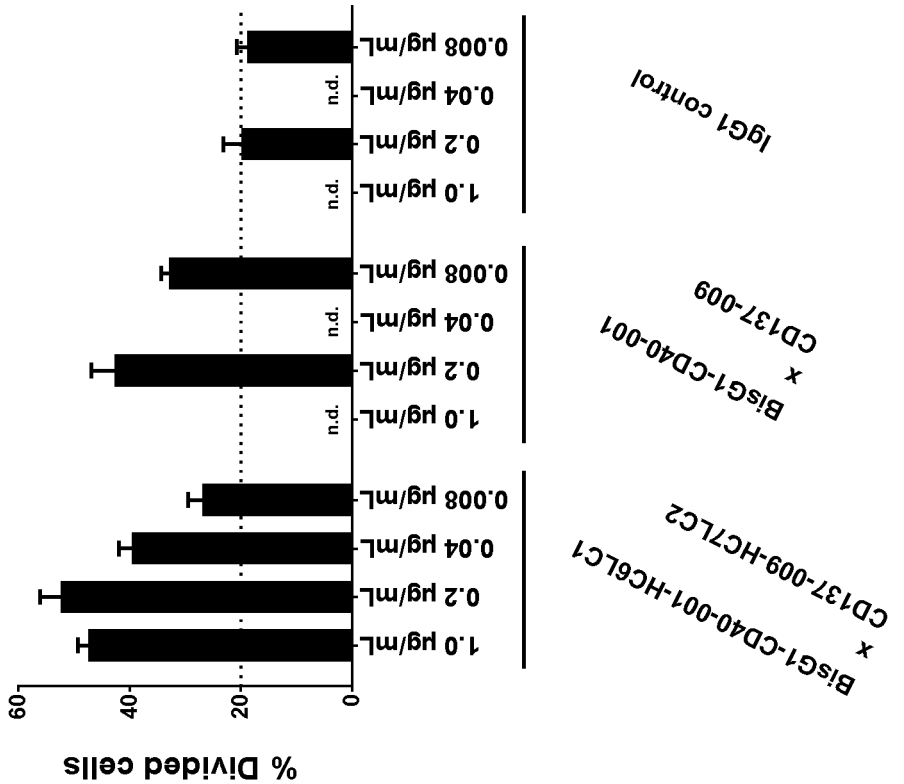
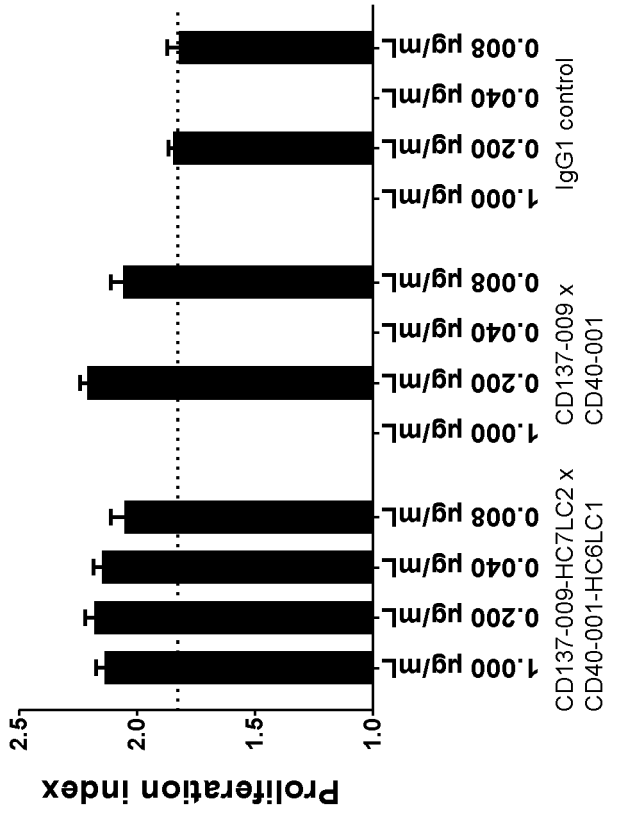

% Divided cells
60
40
20
0
1.0 µg/mL
0.2 µg/mL
0.04 µg/mL
0.008 µg/mL
n.d.
BisG1-CD40-001-HC6LC1 x CD137-009-HC7LC2
BisG1-CD40-001 x CD137-009
IgG1 control

Figure 11


Proliferation index
2.5
2.0
1.5
1.0
CD137-009-HC7LC2 x CD40-001-HC6LC1
CD137-009 x CD40-001
IgG1 control
1.000 µg/mL
0.200 µg/mL
0.040 µg/mL
0.008 µg/mL

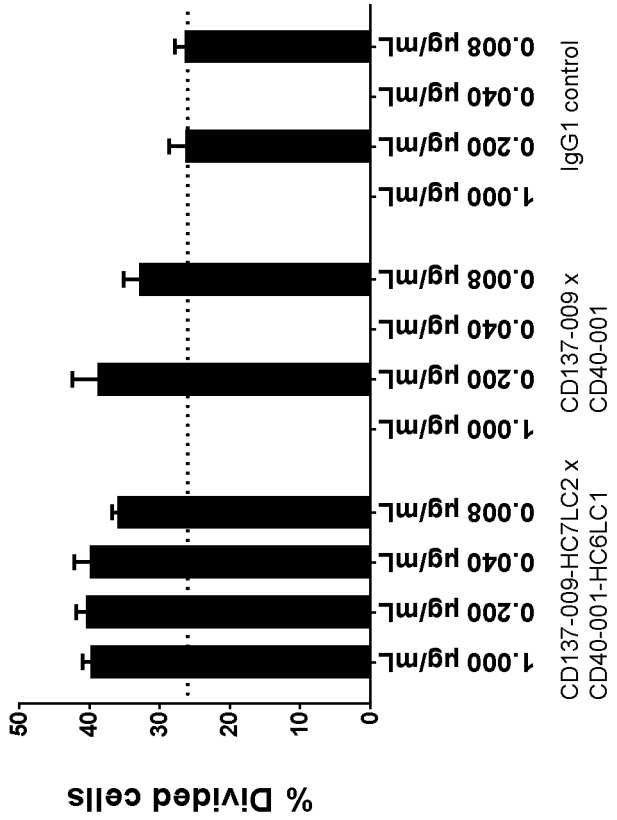
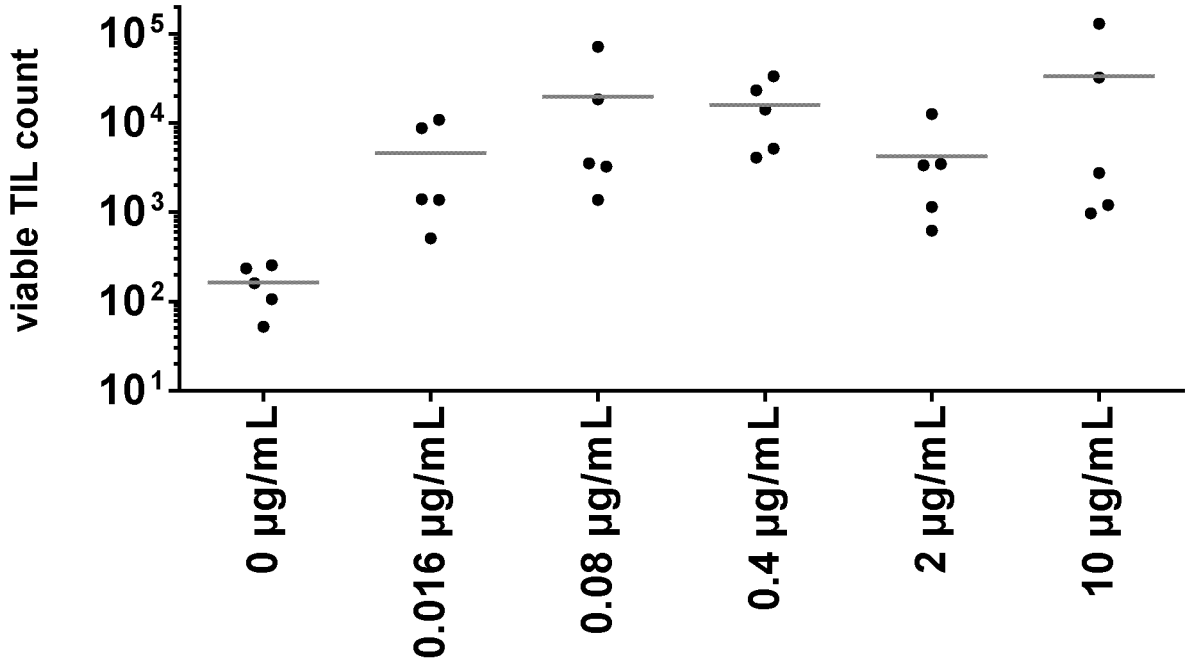

% Divided cells
50
40
30
20
10
0
CD137-009-HC7LC2 x CD40-001-HC6LC1
CD137-009 x CD40-001
IgG1 control
1.000 µg/mL
0.200 µg/mL
0.040 µg/mL
0.008 µg/mL

Figure 12


viable TIL count
10^5
10^4
10^3
10^2
10^1
0 µg/mL
0.016 µg/mL
0.08 µg/mL
0.4 µg/mL
2 µg/mL
10 µg/mL

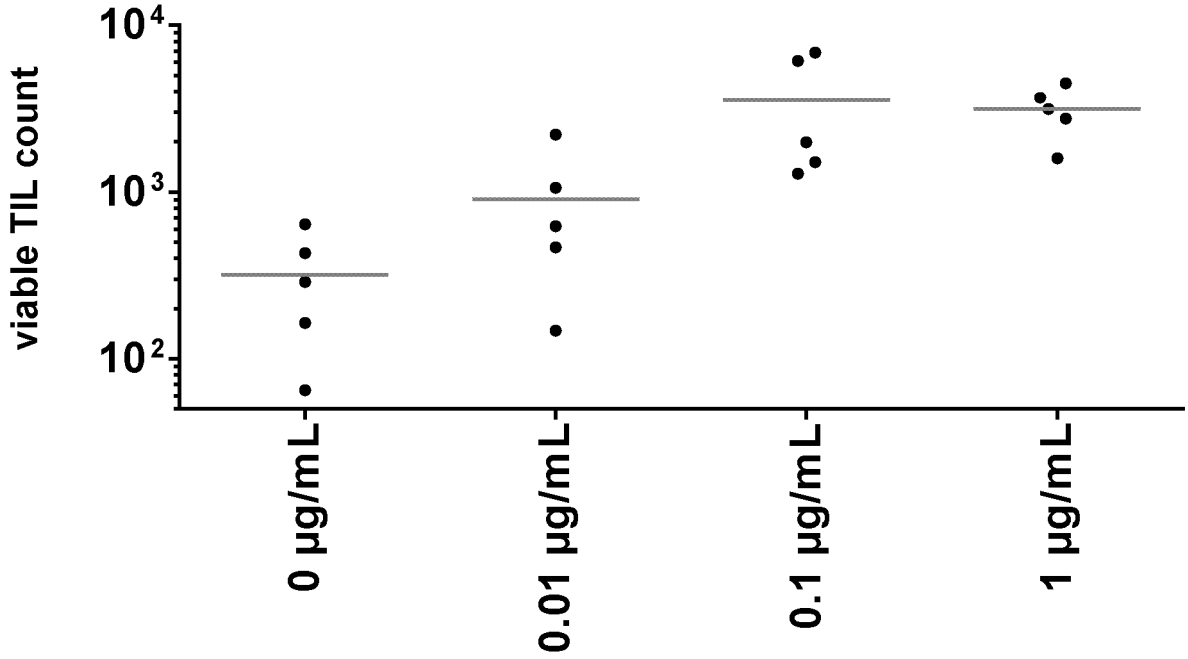



Figure 13