Figure 1

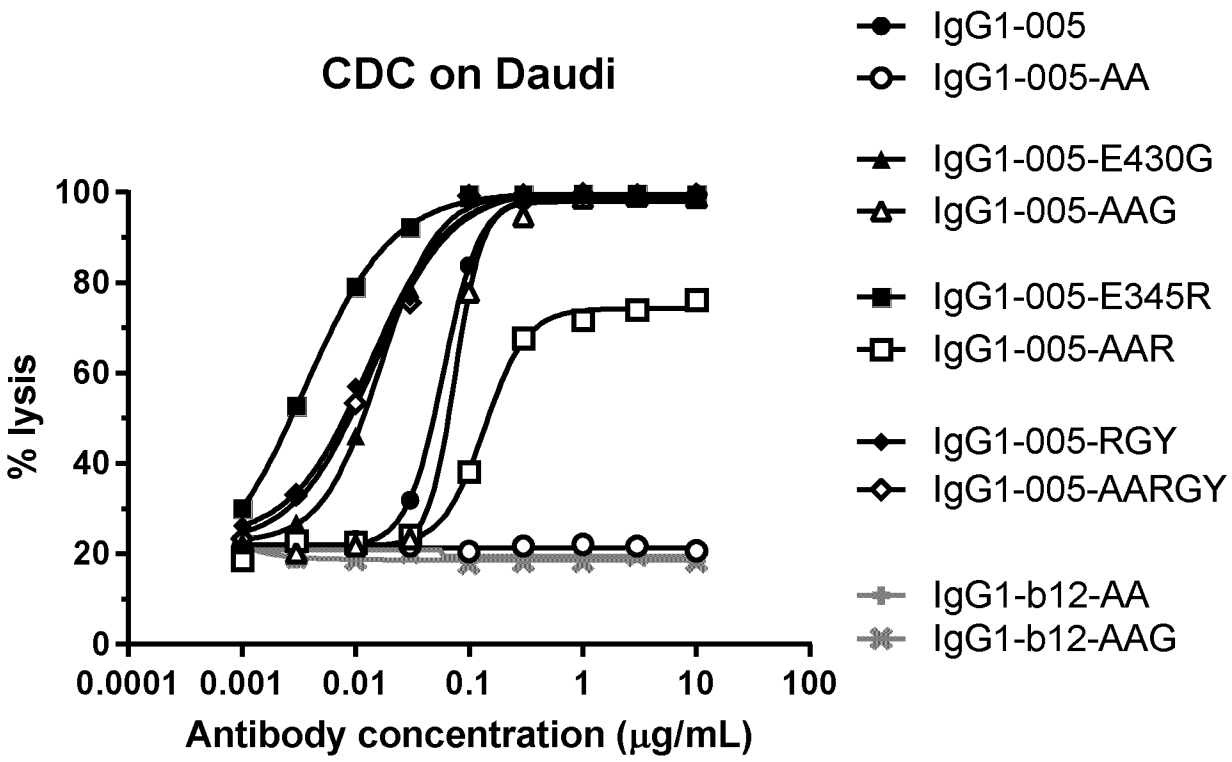

CDC on Daudi
% lysis
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration (µg/mL)
IgG1-005
IgG1-005-AA
IgG1-005-E430G
IgG1-005-AAG
IgG1-005-E345R
IgG1-005-AAR
IgG1-005-RGY
IgG1-005-AARGY
IgG1-b12-AA
IgG1-b12-AAG

Figure 2

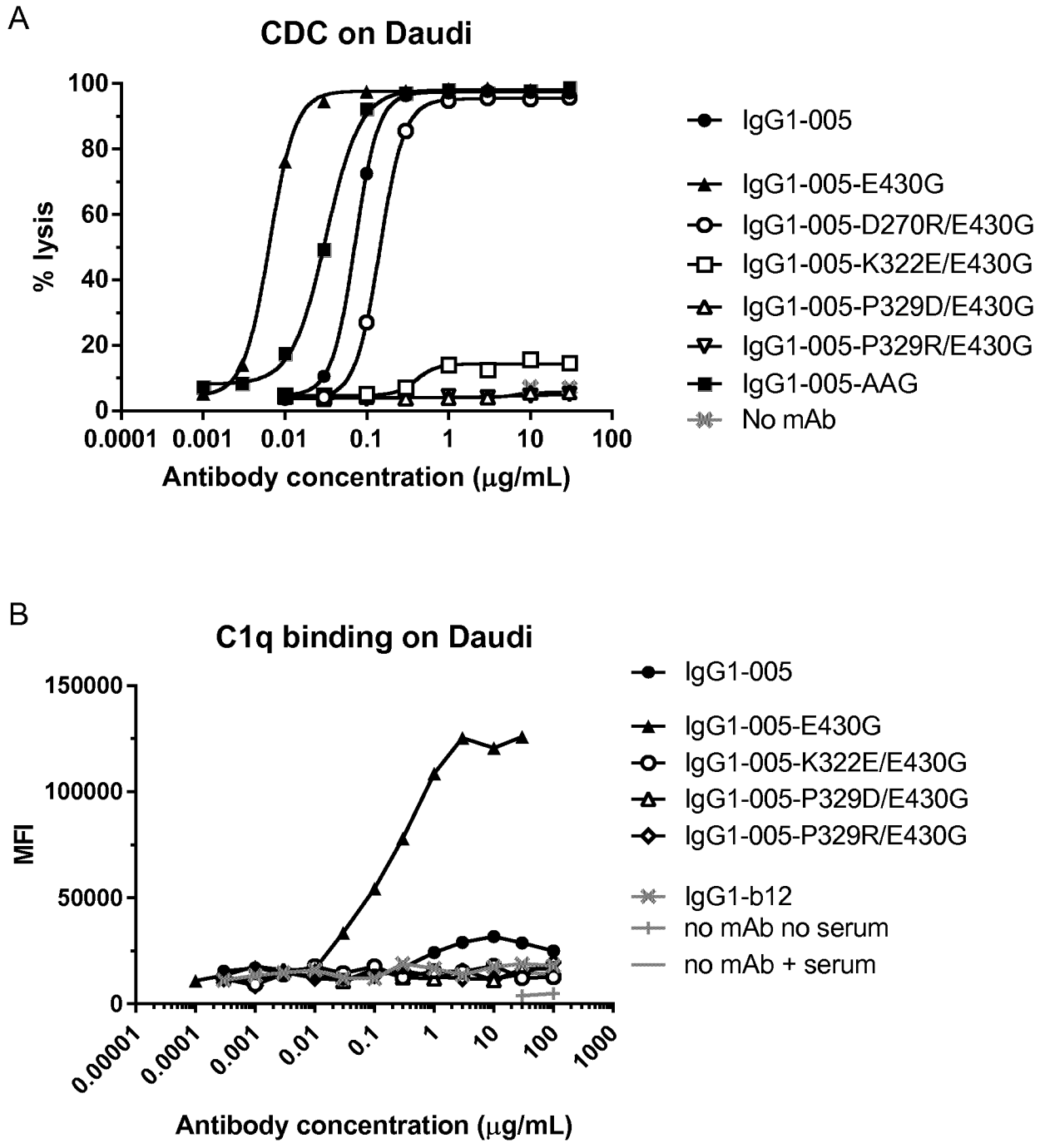

A
CDC on Daudi
% lysis
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration (µg/mL)
IgG1-005
IgG1-005-E430G
IgG1-005-D270R/E430G
IgG1-005-K322E/E430G
IgG1-005-P329D/E430G
IgG1-005-P329R/E430G
IgG1-005-AAG
No mAb
B
C1q binding on Daudi
MFI
150000
100000
50000
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000
Antibody concentration (µg/mL)
IgG1-005
IgG1-005-E430G
IgG1-005-K322E/E430G
IgG1-005-P329D/E430G
IgG1-005-P329R/E430G
IgG1-b12
no mAb no serum
no mAb + serum

Figure 3

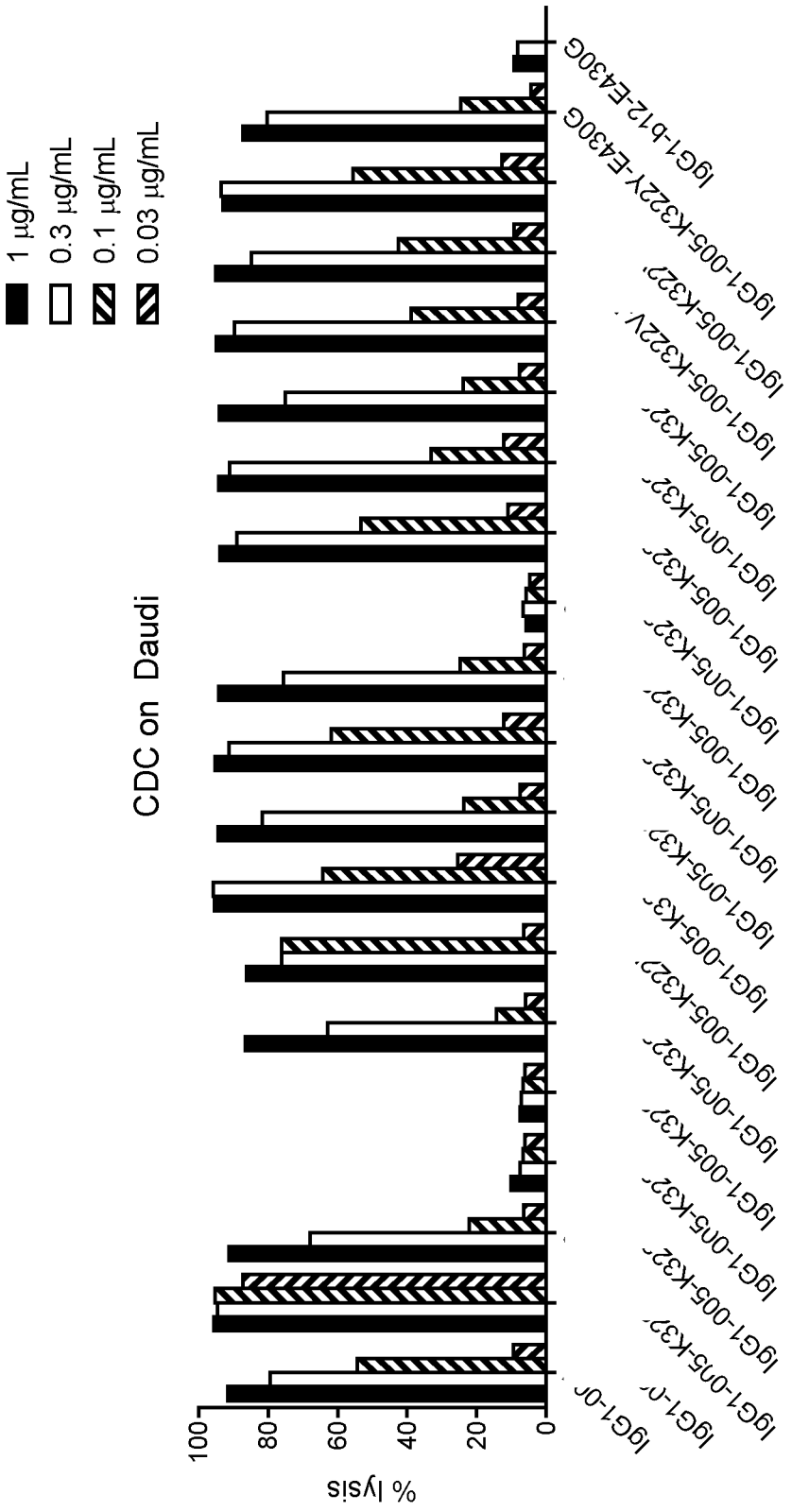

1 µg/mL
0.3 µg/mL
0.1 µg/mL
0.03 µg/mL
CDC on Daudi
% lysis
100
80
60
40
20
0
IgG1-0
IgG1-005-K32
IgG1-005-K32
IgG1-005-K32

Figure 4

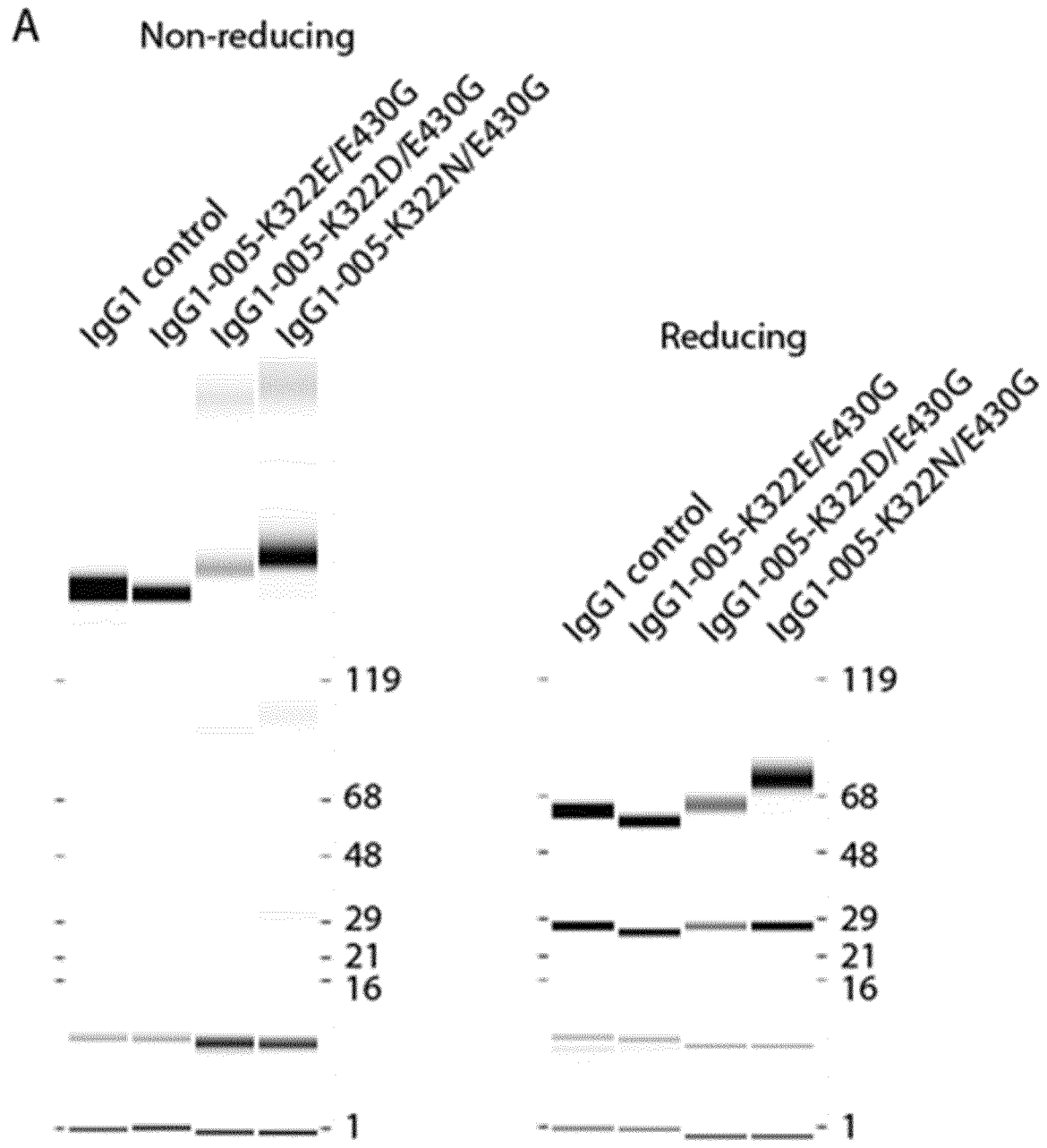

A
Non-reducing
IgG1 control
IgG1-005-K322E/E430G
IgG1-005-K322D/E430G
IgG1-005-K322N/E430G
Reducing
IgG1 control
IgG1-005-K322E/E430G
IgG1-005-K322D/E430G
IgG1-005-K322N/E430G
119
68
48
29
21
16
1
119
68
48
29
21
16
1

Figure 4 continued

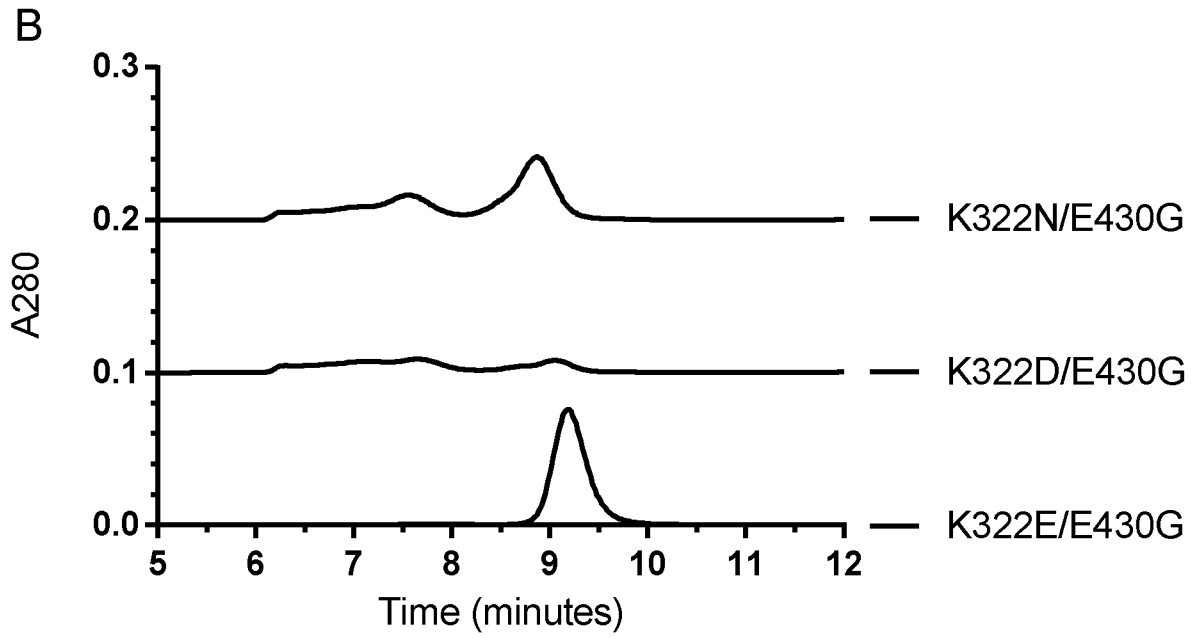


Figure 5

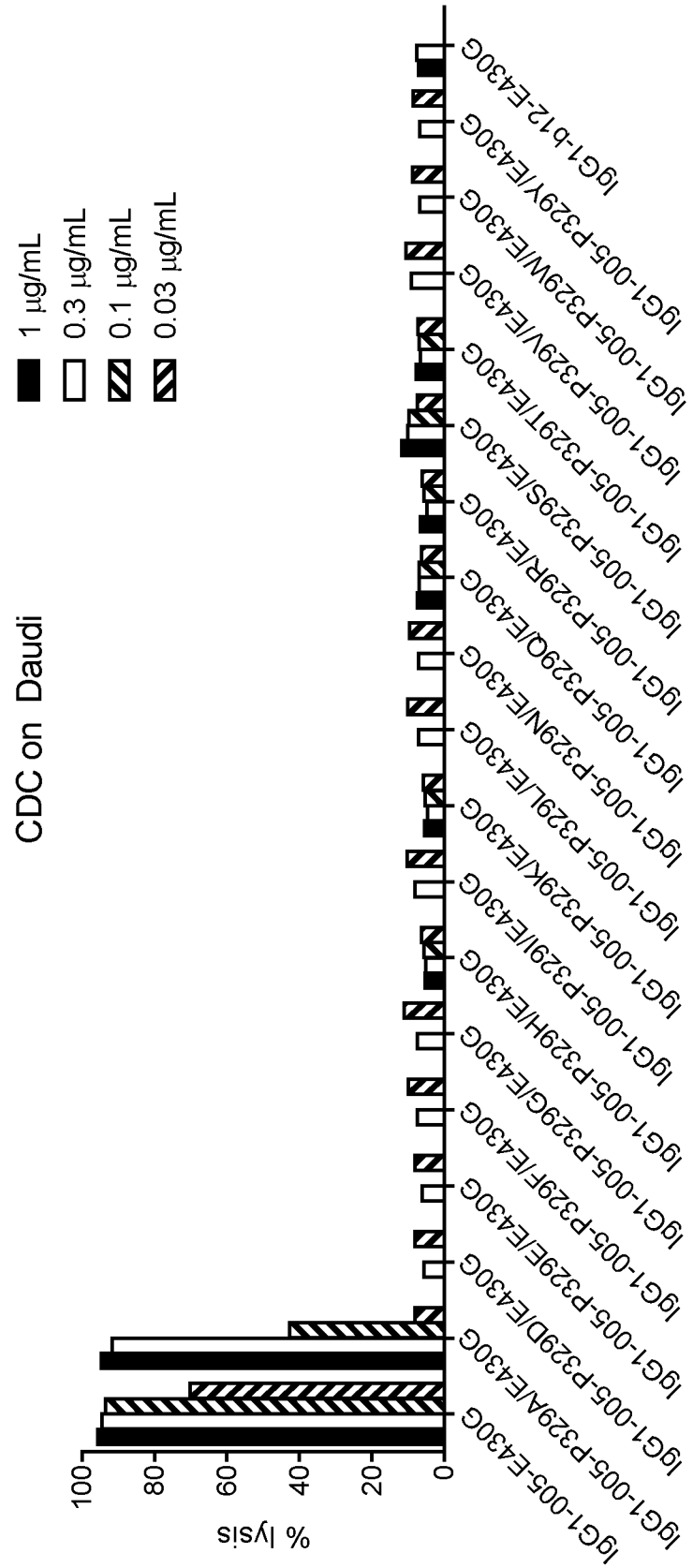

CDC on Daudi
1 µg/mL
0.3 µg/mL
0.1 µg/mL
0.03 µg/mL
% lysis
0
20
40
60
80
100
IgG1-005-E430G
IgG1-005-P329A/E430G
IgG1-005-P329D/E430G
IgG1-005-P329E/E430G
IgG1-005-P329F/E430G
IgG1-005-P329G/E430G
IgG1-005-P329H/E430G
IgG1-005-P329I/E430G
IgG1-005-P329K/E430G
IgG1-005-P329L/E430G
IgG1-005-P329N/E430G
IgG1-005-P329Q/E430G
IgG1-005-P329R/E430G
IgG1-005-P329S/E430G
IgG1-005-P329T/E430G
IgG1-005-P329V/E430G
IgG1-005-P329W/E430G
IgG1-005-P329Y/E430G
IgG1-b12-E430G

Figure 6

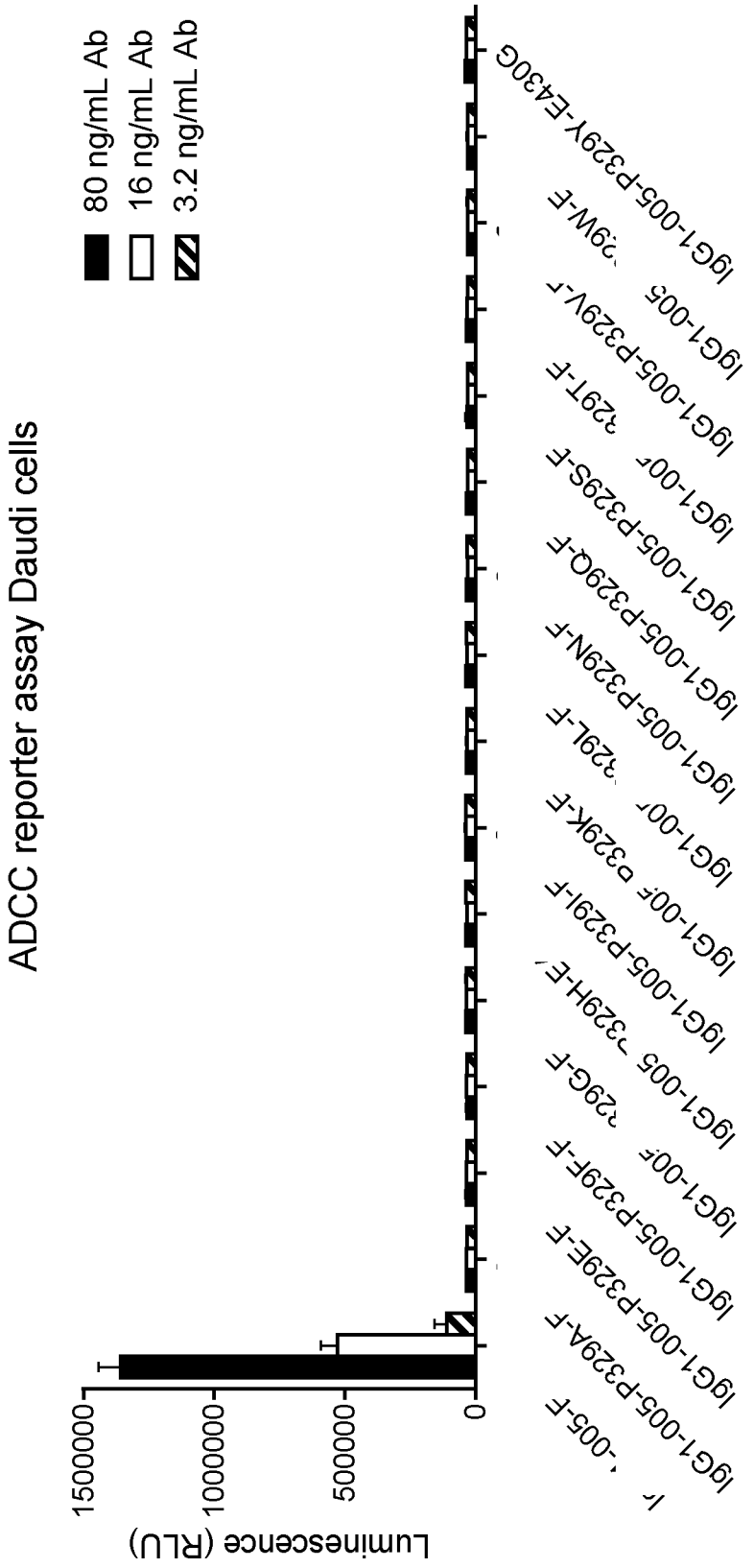

ADCC reporter assay Daudi cells
80 ng/mL Ab
16 ng/mL Ab
3.2 ng/mL Ab
Luminescence (RLU)
1500000
1000000
500000
0
IgG1-005-F
IgG1-005-P329A-F
IgG1-005-P329E-F
IgG1-005-P329K-F
IgG1-005-P329L-F
IgG1-005-P329N-F
IgG1-005-P329Q-F
IgG1-005-P329S-F
IgG1-005-P329T-F
IgG1-005-P329V-F
IgG1-005-P329W-F
IgG1-005-P329Y-E430G

Figure 7

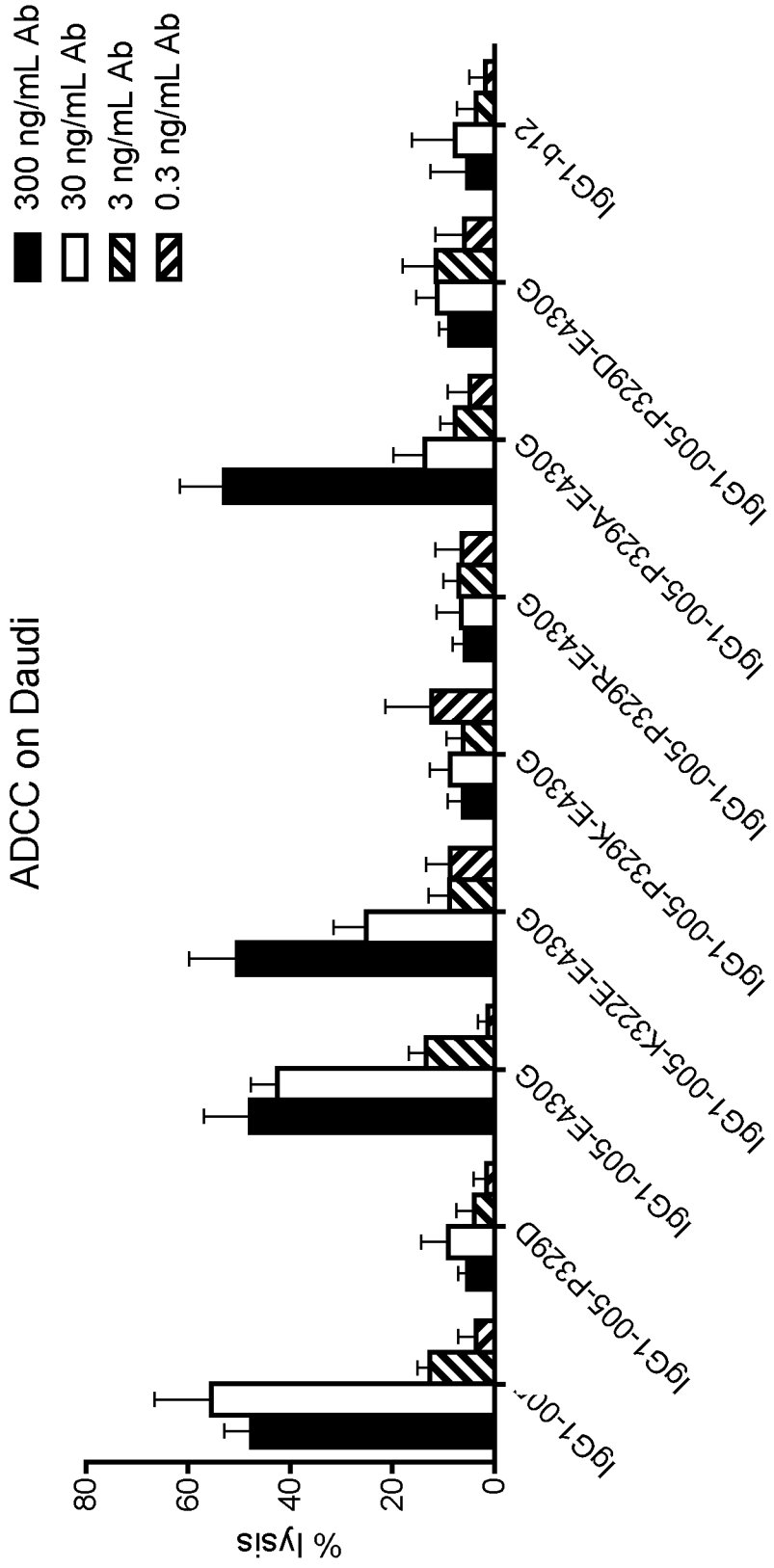

ADCC on Daudi
% lysis
0
20
40
60
80
300 ng/mL Ab
30 ng/mL Ab
3 ng/mL Ab
0.3 ng/mL Ab
IgG1-b12
IgG1-005-P329D-E430G
IgG1-005-P329A-E430G
IgG1-005-P329R-E430G
IgG1-005-P329K-E430G
IgG1-005-K322E-E430G
IgG1-005-E430G
IgG1-005-P329D
IgG1-0

Figure 8

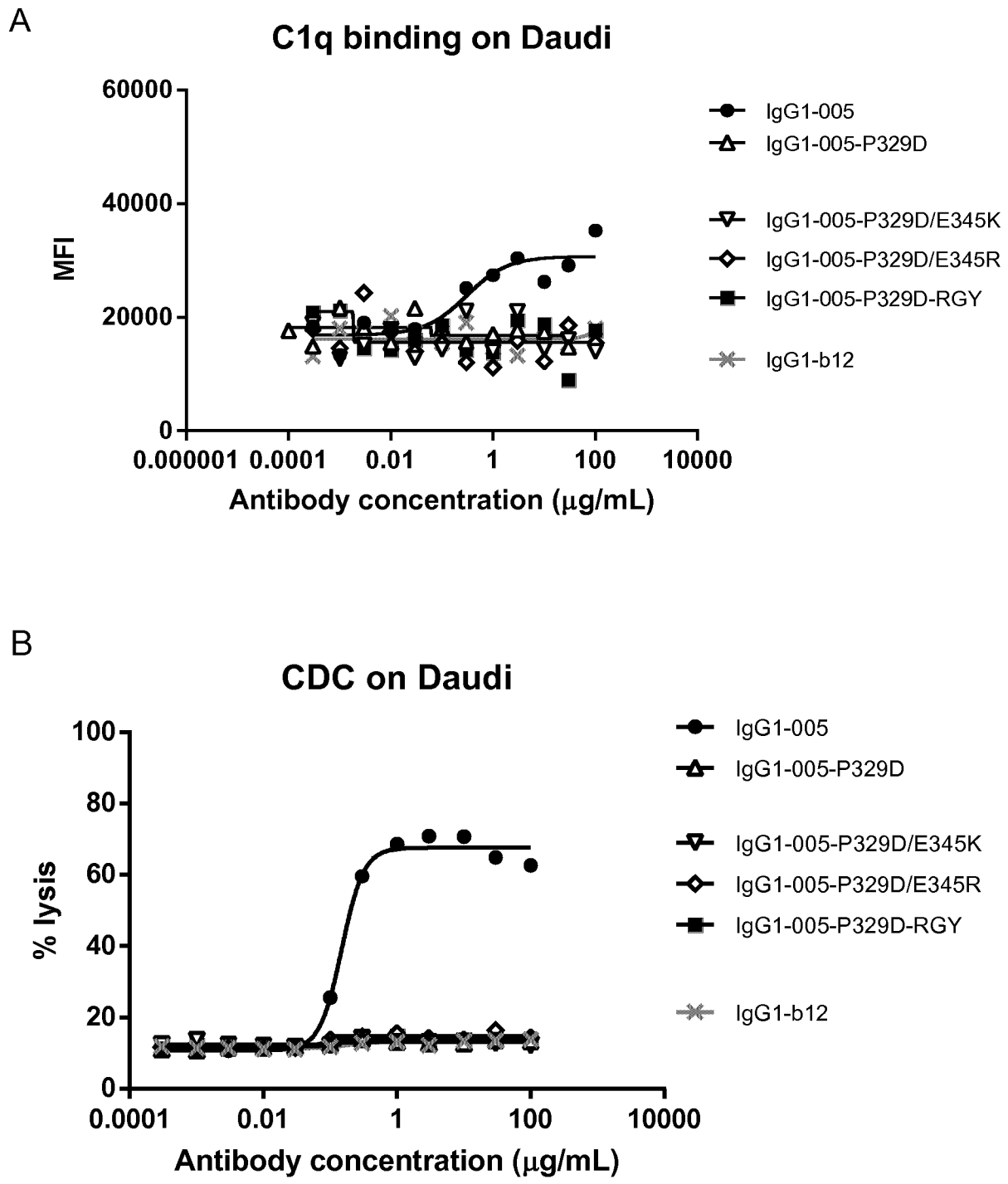
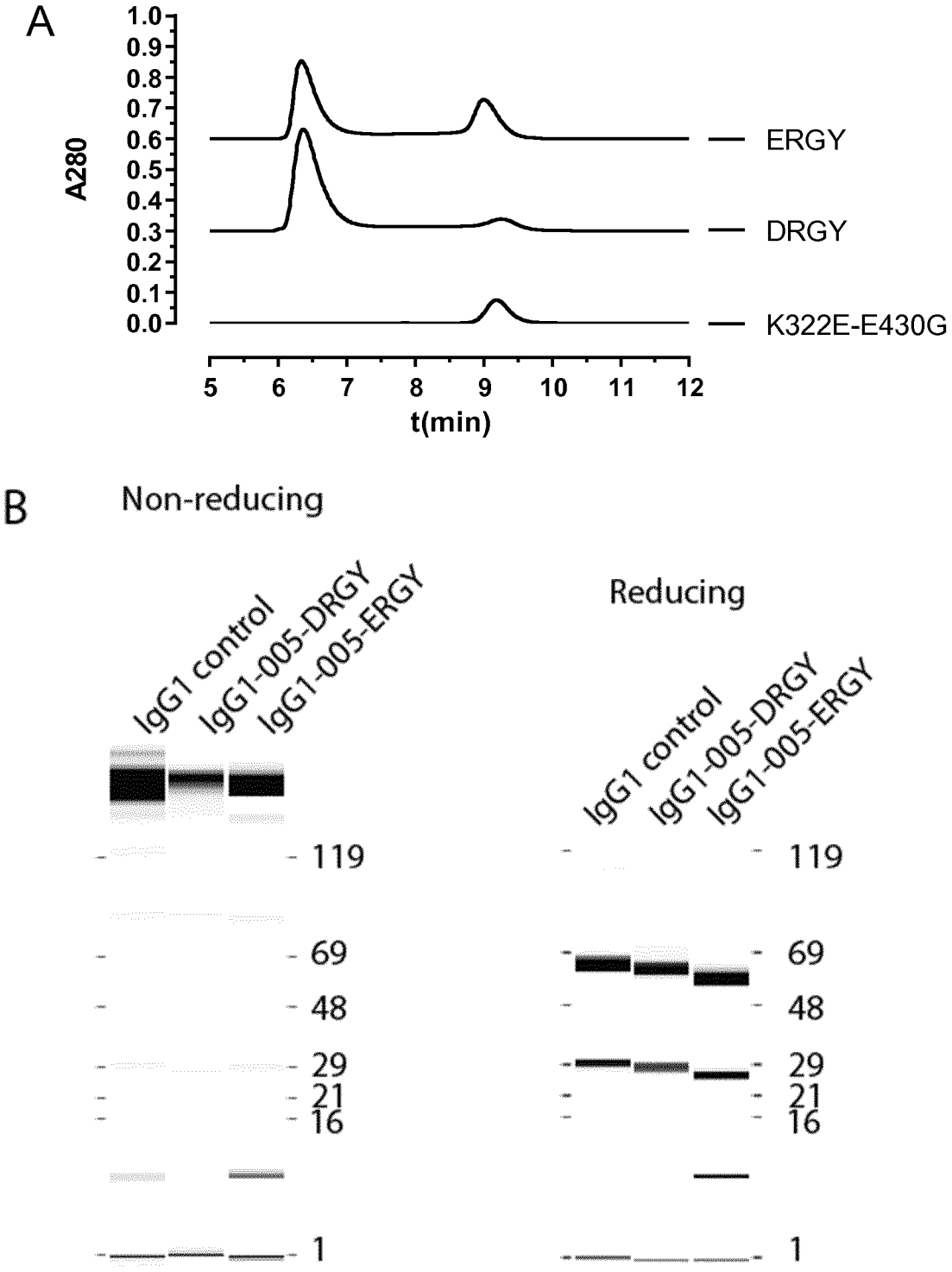

A
C1q binding on Daudi
MFI
60000
40000
20000
0
0.000001 0.0001 0.01 1 100 10000
Antibody concentration (µg/mL)
IgG1-005
IgG1-005-P329D
IgG1-005-P329D/E345K
IgG1-005-P329D/E345R
IgG1-005-P329D-RGY
IgG1-b12
B
CDC on Daudi
% lysis
100
80
60
40
20
0
0.0001 0.01 1 100 10000
Antibody concentration (µg/mL)
IgG1-005
IgG1-005-P329D
IgG1-005-P329D/E345K
IgG1-005-P329D/E345R
IgG1-005-P329D-RGY
IgG1-b12

Figure 9


A
A280
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
5
6
7
8
9
10
11
12
t(min)
ERGY
DRGY
K322E-E430G
B
Non-reducing
IgG1 control
IgG1-005-DRGY
IgG1-005-ERGY
Reducing
IgG1 control
IgG1-005-DRGY
IgG1-005-ERGY
119
69
48
29
21
16
1
119
69
48
29
21
16
1

C

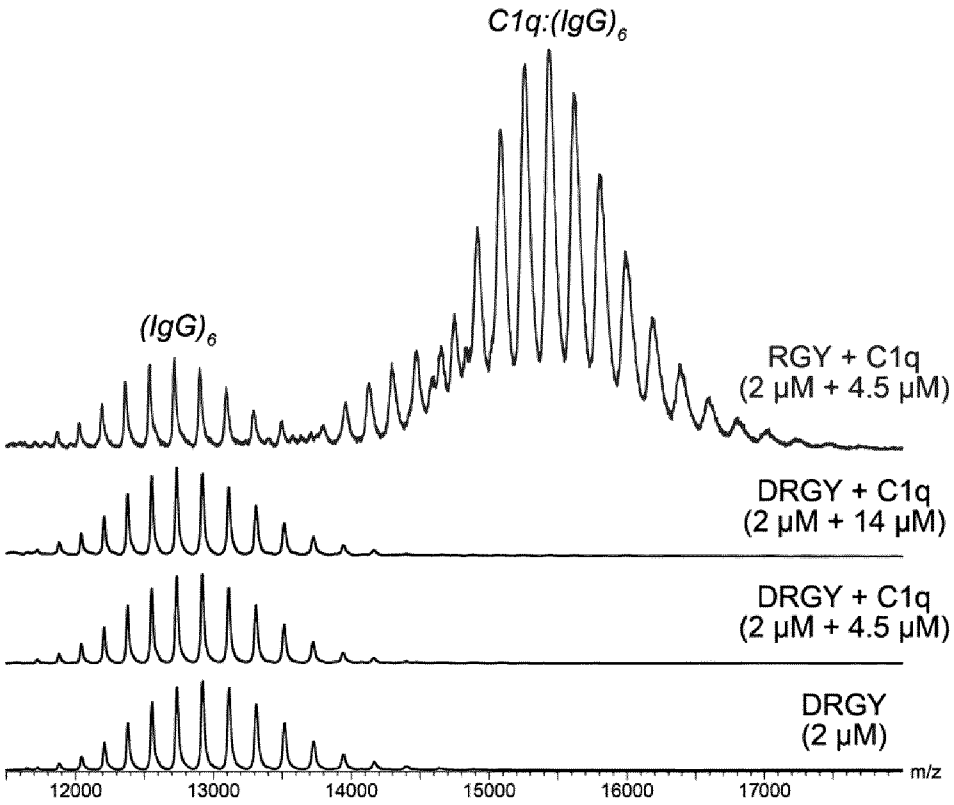

C1q:(IgG)6
(IgG)6
RGY + C1q
(2 µM + 4.5 µM)
DRGY + C1q
(2 µM + 14 µM)
DRGY + C1q
(2 µM + 4.5 µM)
DRGY
(2 µM)
m/z
12000
13000
14000
15000
16000
17000

Figure 10

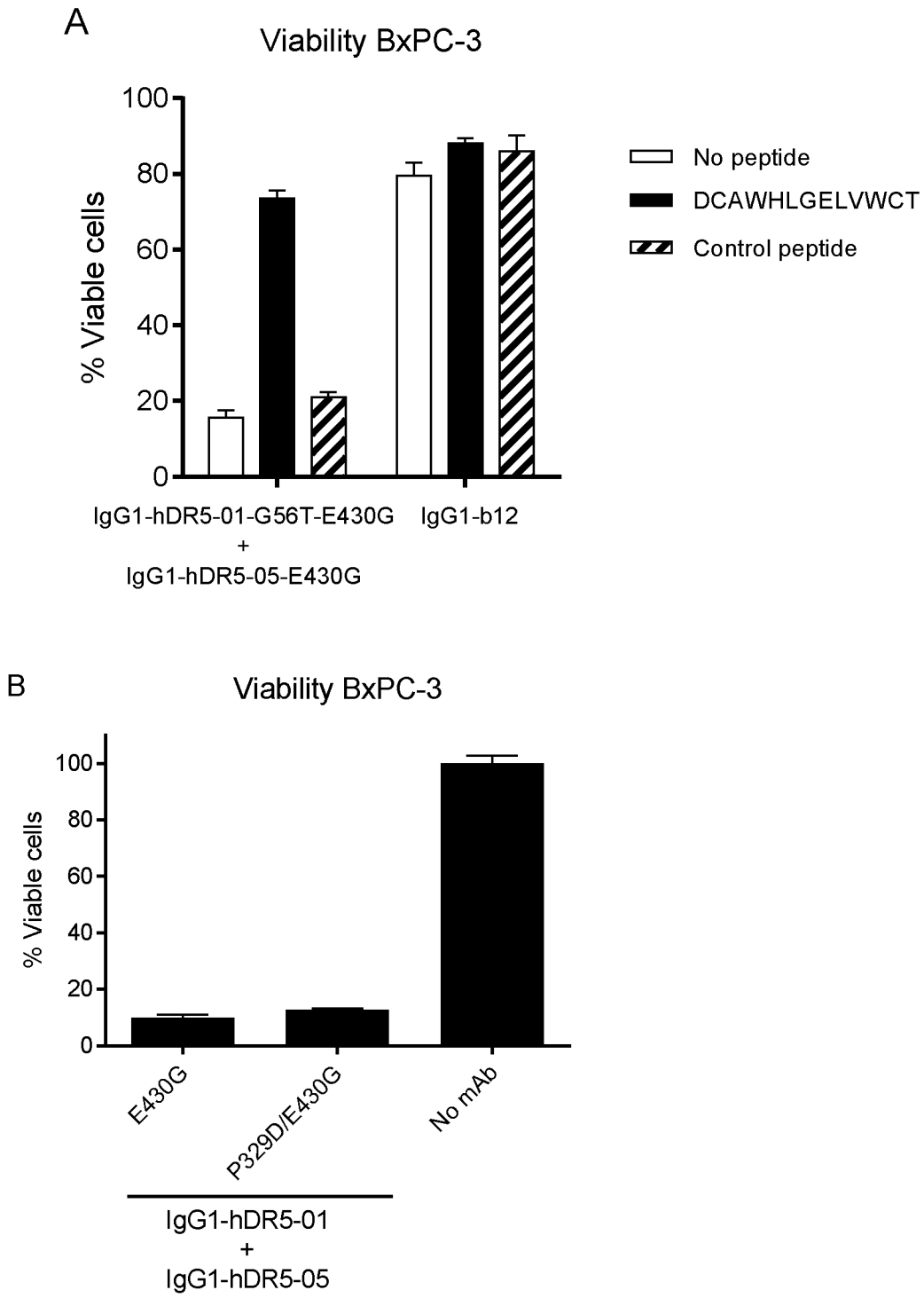

A
Viability BxPC-3
% Viable cells
100
80
60
40
20
0
No peptide
DCAWHLGELVWCT
Control peptide
IgG1-hDR5-01-G56T-E430G
+
IgG1-hDR5-05-E430G
IgG1-b12
B
Viability BxPC-3
% Viable cells
100
80
60
40
20
0
E430G
P329D/E430G
No mAb
IgG1-hDR5-01
+
IgG1-hDR5-05

Figure 10 continued

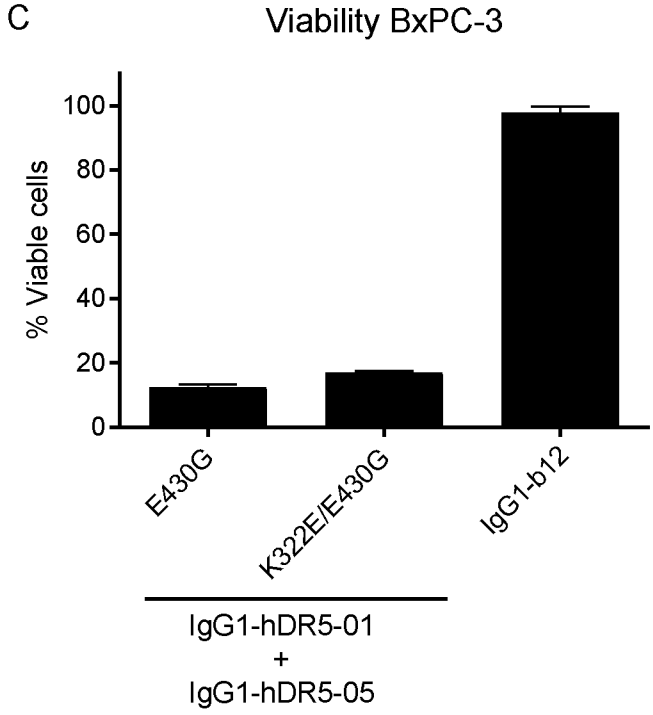


Figure 11

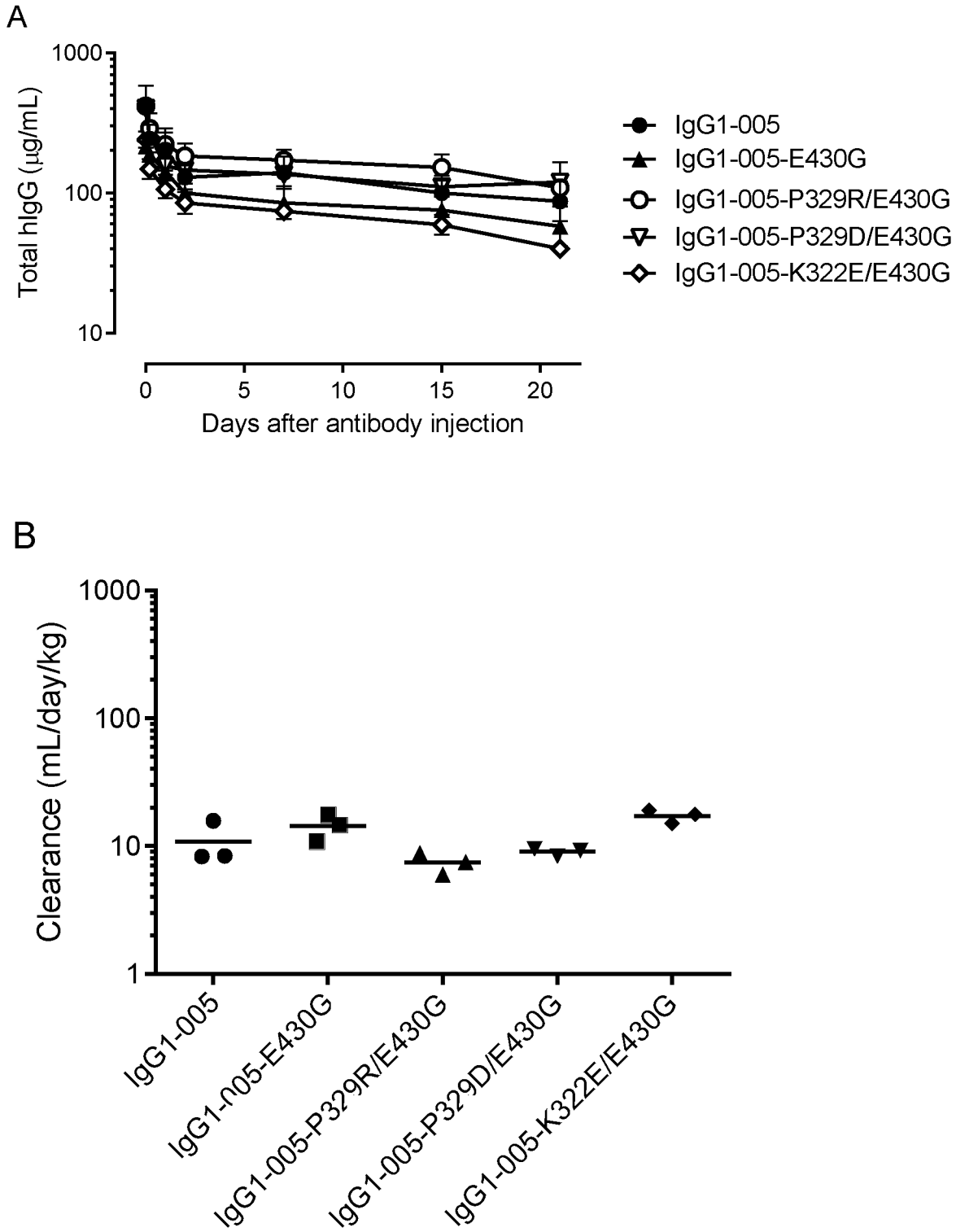

A
1000
100
10
Total hIgG (µg/mL)
0
5
10
15
20
Days after antibody injection
IgG1-005
IgG1-005-E430G
IgG1-005-P329R/E430G
IgG1-005-P329D/E430G
IgG1-005-K322E/E430G
B
1000
100
10
1
Clearance (mL/day/kg)
IgG1-005
IgG1-005-E430G
IgG1-005-P329R/E430G
IgG1-005-P329D/E430G
IgG1-005-K322E/E430G

Figure 12

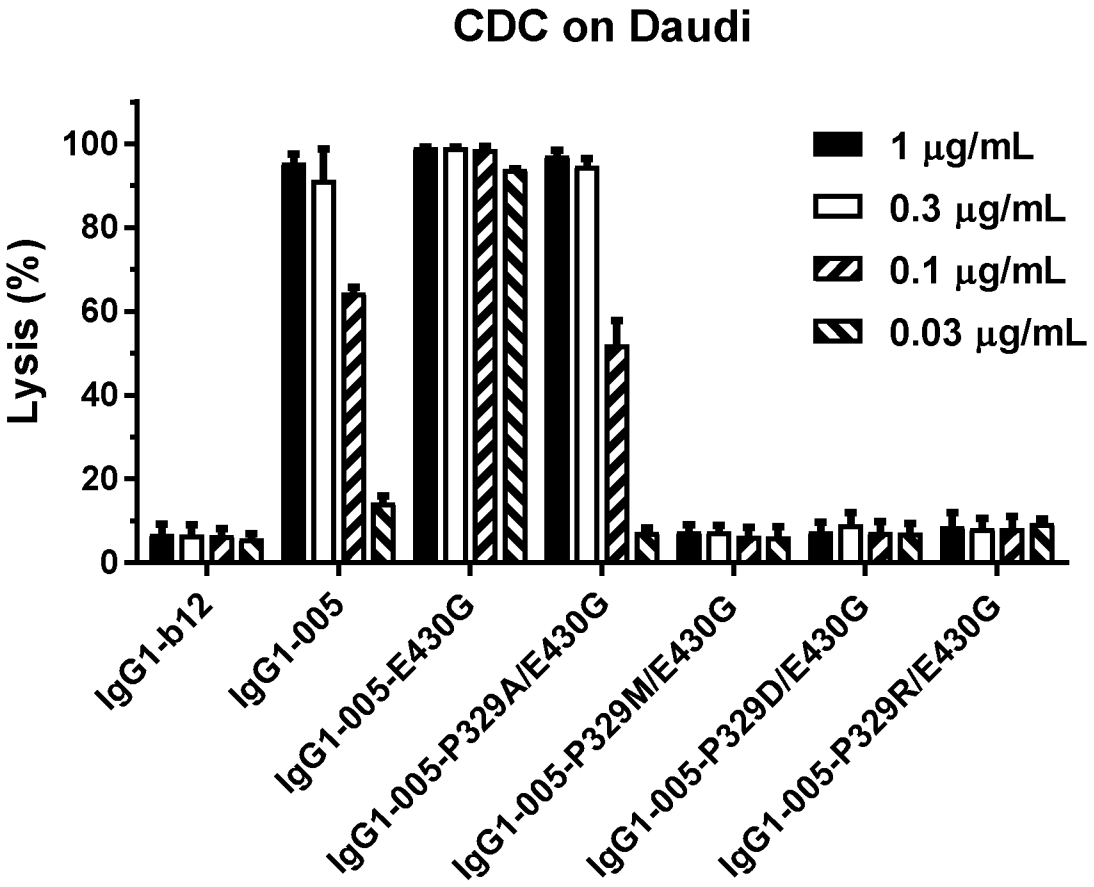
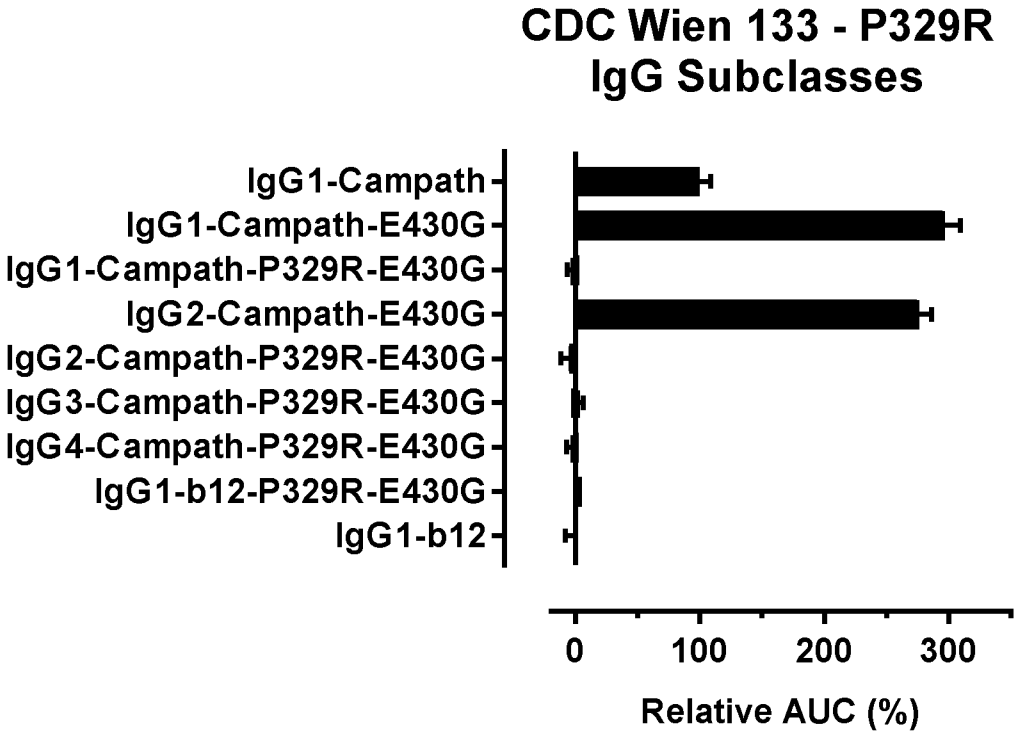

CDC on Daudi
Lysis (%)
100
80
60
40
20
0
1 µg/mL
0.3 µg/mL
0.1 µg/mL
0.03 µg/mL
IgG1-b12
IgG1-005
IgG1-005-E430G
IgG1-005-P329A/E430G
IgG1-005-P329M/E430G
IgG1-005-P329D/E430G
IgG1-005-P329R/E430G

Figure 13


CDC Wien 133 - P329R
IgG Subclasses
IgG1-Campath
IgG1-Campath-E430G
IgG1-Campath-P329R-E430G
IgG2-Campath-E430G
IgG2-Campath-P329R-E430G
IgG3-Campath-P329R-E430G
IgG4-Campath-P329R-E430G
IgG1-b12-P329R-E430G
IgG1-b12
0
100
200
300
Relative AUC (%)

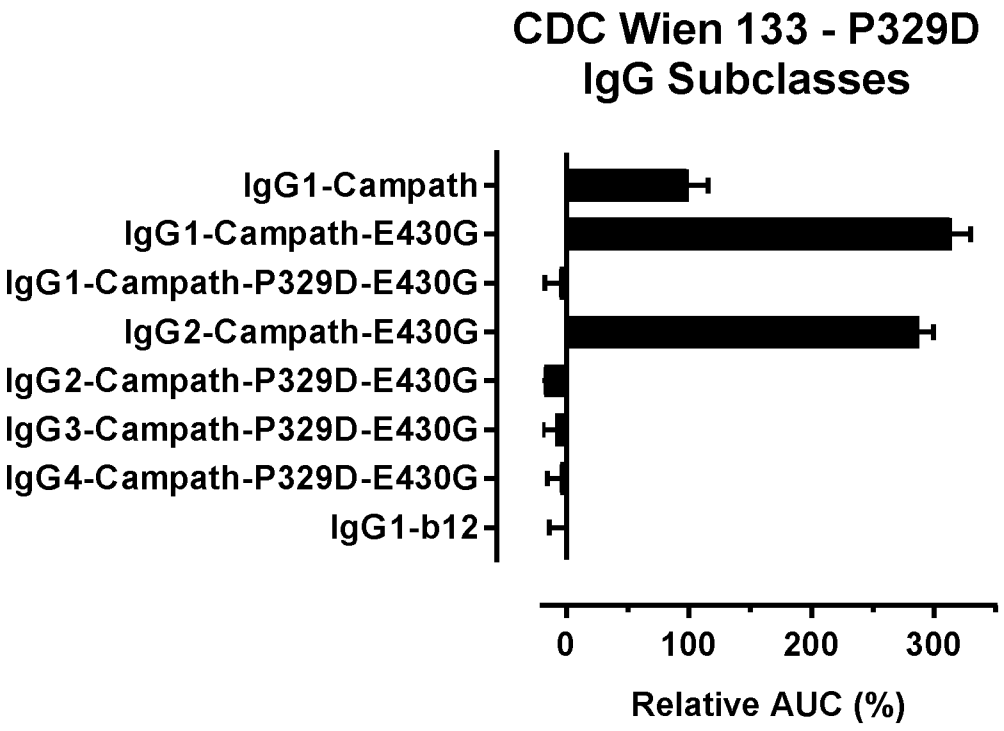

CDC Wien 133 - P329D
IgG Subclasses
IgG1-Campath
IgG1-Campath-E430G
IgG1-Campath-P329D-E430G
IgG2-Campath-E430G
IgG2-Campath-P329D-E430G
IgG3-Campath-P329D-E430G
IgG4-Campath-P329D-E430G
IgG1-b12
0
100
200
300
Relative AUC (%)

Figure 14

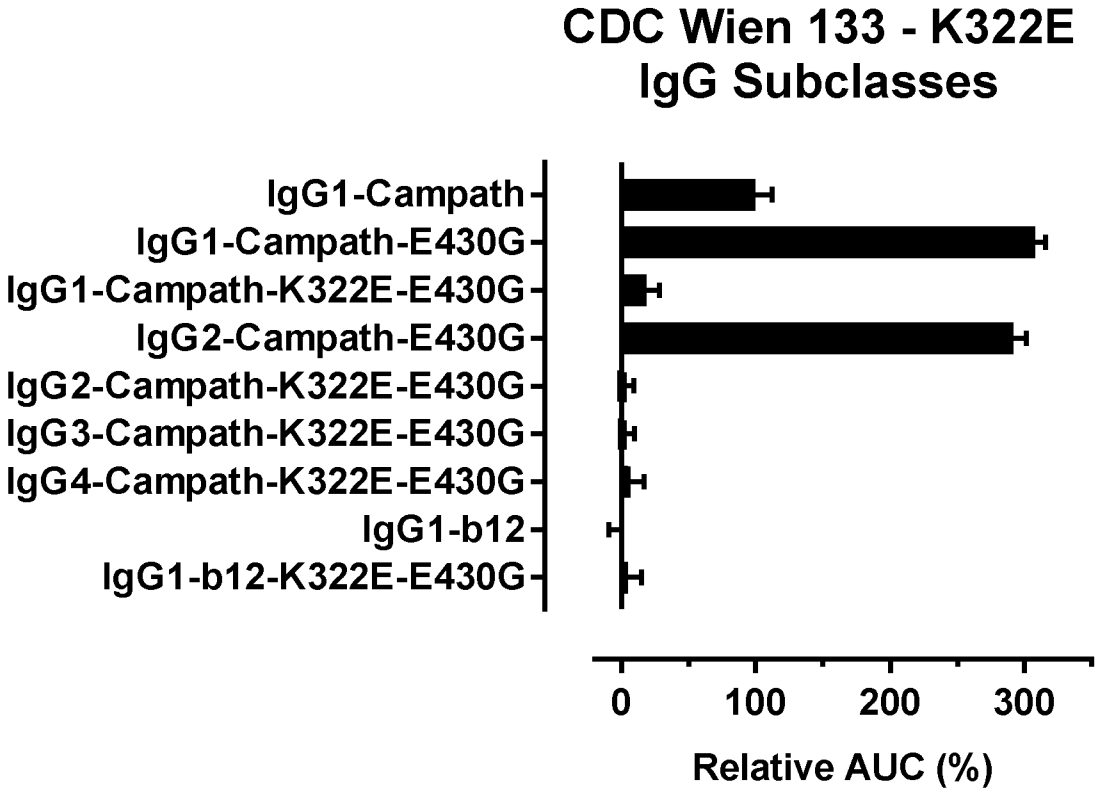

CDC Wien 133 - K322E
IgG Subclasses
IgG1-Campath
IgG1-Campath-E430G
IgG1-Campath-K322E-E430G
IgG2-Campath-E430G
IgG2-Campath-K322E-E430G
IgG3-Campath-K322E-E430G
IgG4-Campath-K322E-E430G
IgG1-b12
IgG1-b12-K322E-E430G
0
100
200
300
Relative AUC (%)

Figure 15

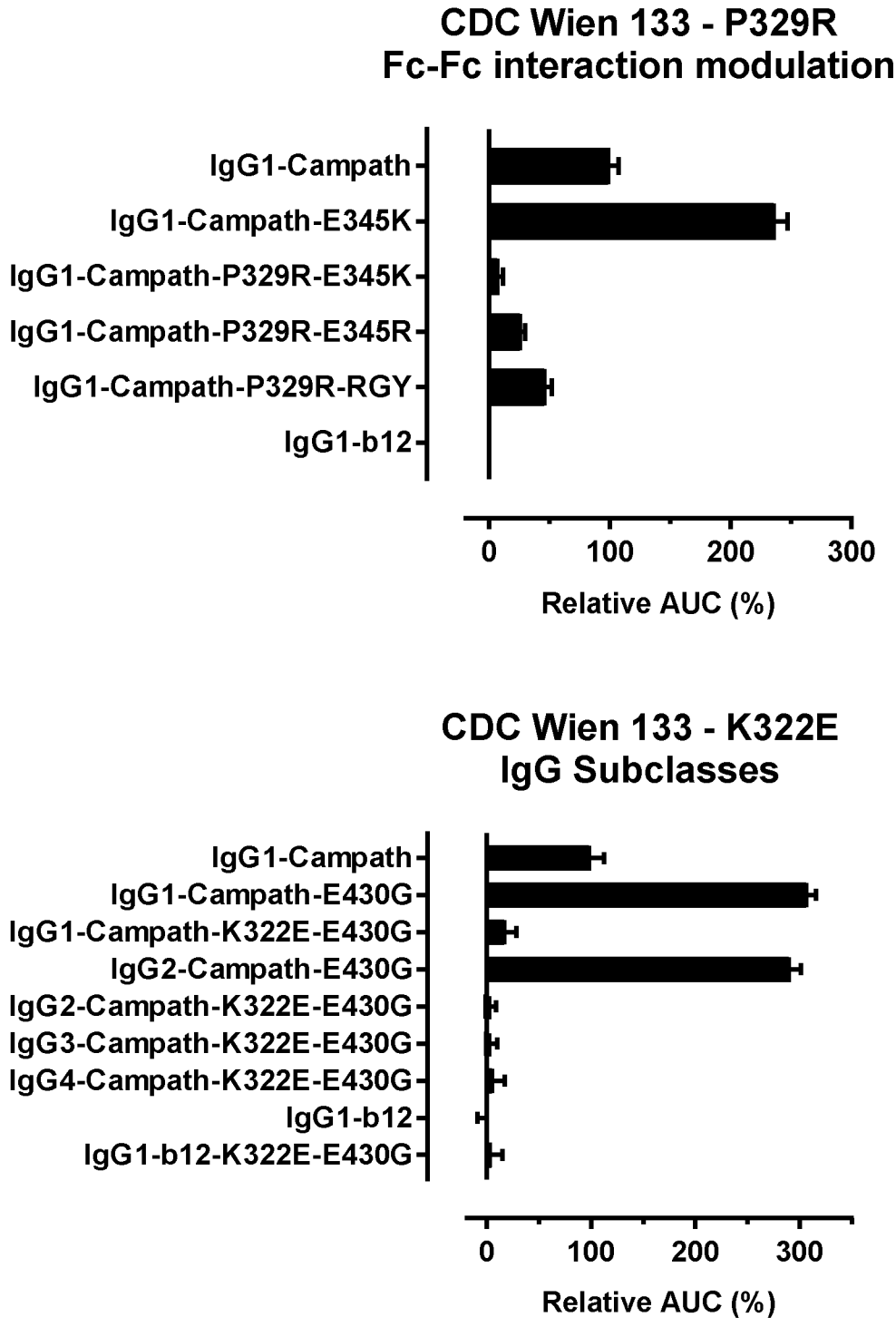

CDC Wien 133 - P329R
Fc-Fc interaction modulation
IgG1-Campath
IgG1-Campath-E345K
IgG1-Campath-P329R-E345K
IgG1-Campath-P329R-E345R
IgG1-Campath-P329R-RGY
IgG1-b12
0
100
200
300
Relative AUC (%)
CDC Wien 133 - K322E
IgG Subclasses
IgG1-Campath
IgG1-Campath-E430G
IgG1-Campath-K322E-E430G
IgG2-Campath-E430G
IgG2-Campath-K322E-E430G
IgG3-Campath-K322E-E430G
IgG4-Campath-K322E-E430G
IgG1-b12
IgG1-b12-K322E-E430G
0
100
200
300
Relative AUC (%)

Figure 16

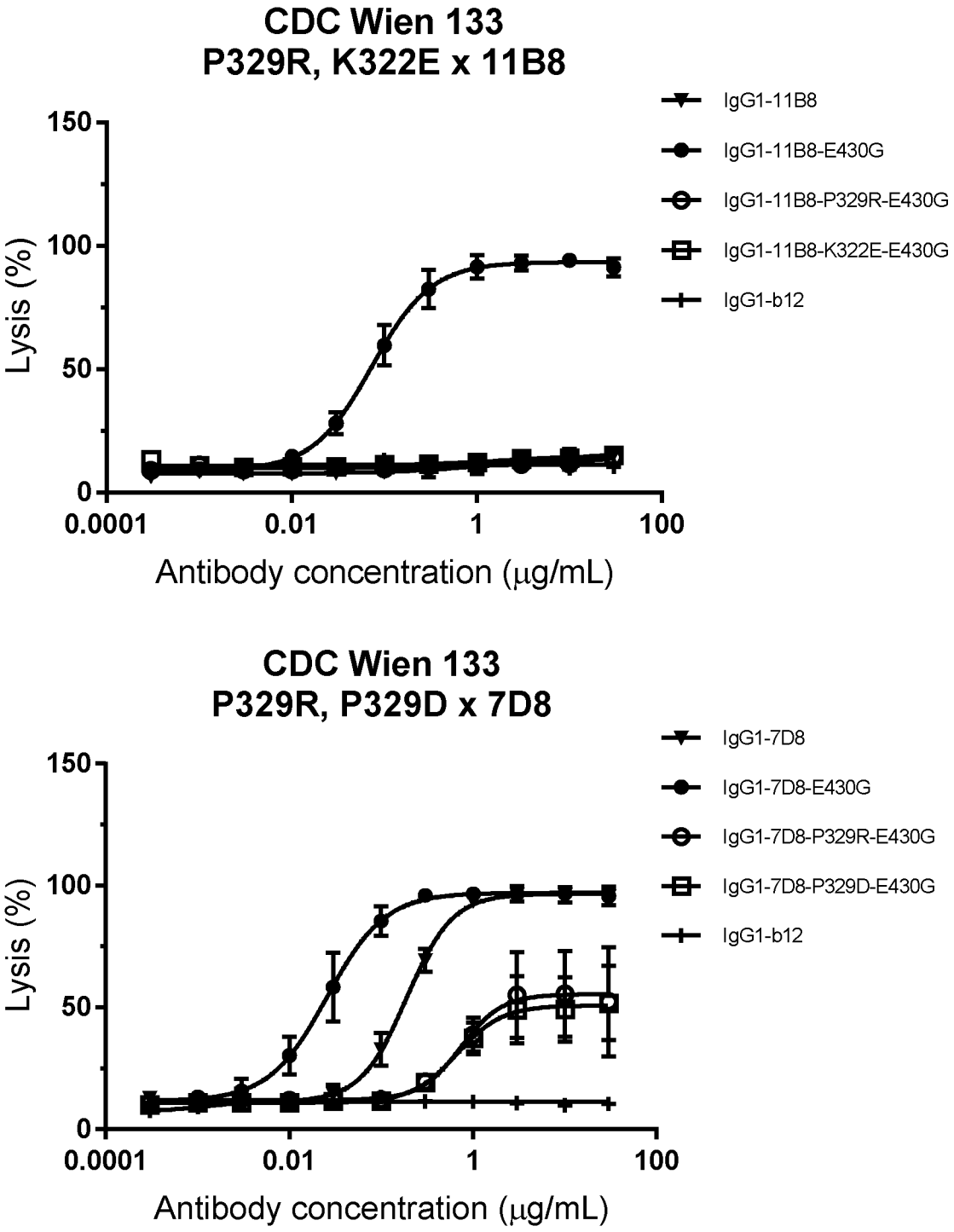

CDC Wien 133
P329R, K322E x 11B8
IgG1-11B8
IgG1-11B8-E430G
IgG1-11B8-P329R-E430G
IgG1-11B8-K322E-E430G
IgG1-b12
Lysis (%)
150
100
50
0
0.0001
0.01
1
100
Antibody concentration (μg/mL)
CDC Wien 133
P329R, P329D x 7D8
IgG1-7D8
IgG1-7D8-E430G
IgG1-7D8-P329R-E430G
IgG1-7D8-P329D-E430G
IgG1-b12
Lysis (%)
150
100
50
0
0.0001
0.01
1
100
Antibody concentration (μg/mL)

Figure 17

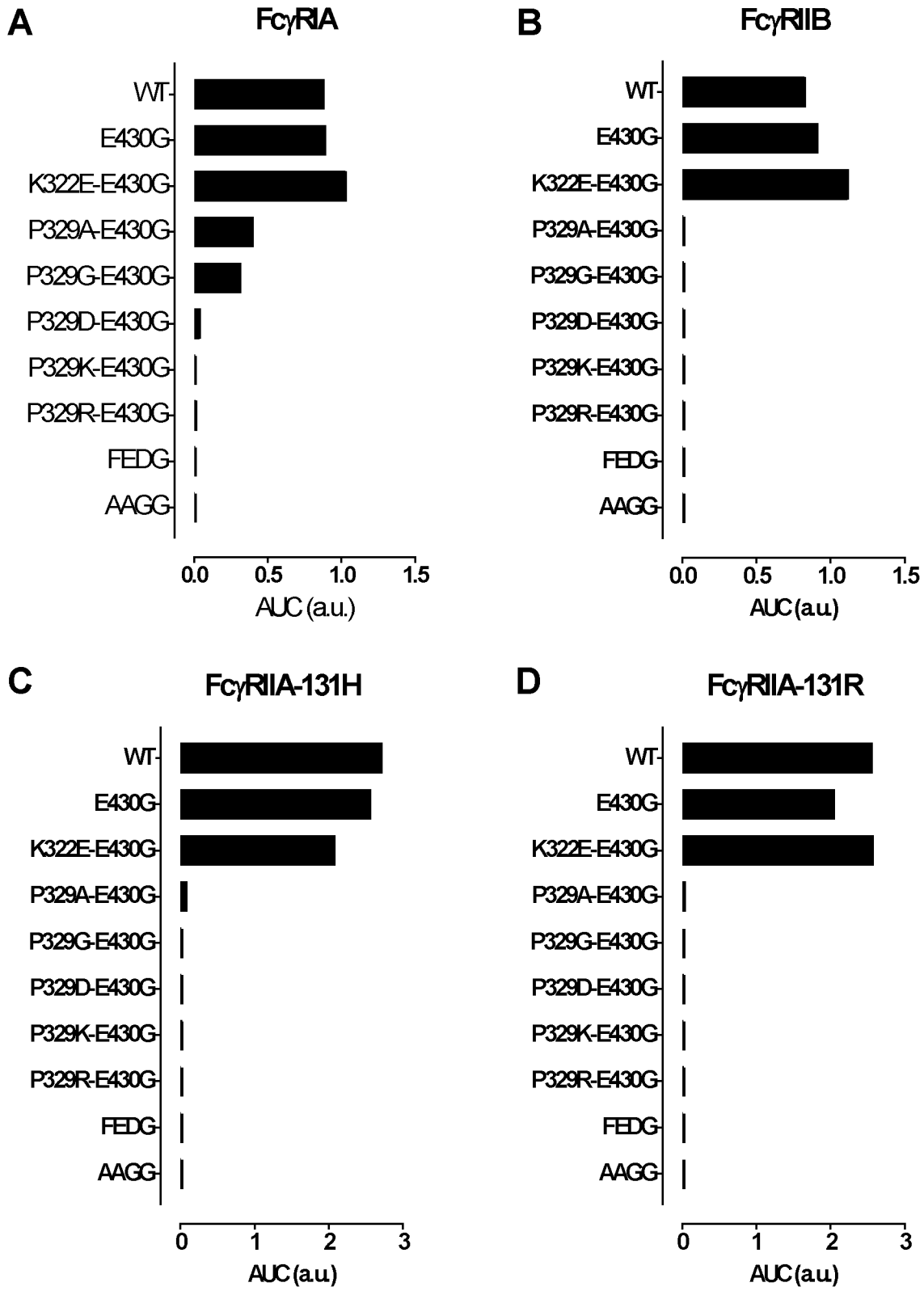

A
FcγRIA
WT
E430G
K322E-E430G
P329A-E430G
P329G-E430G
P329D-E430G
P329K-E430G
P329R-E430G
FEDG
AAGG
0.0
0.5
1.0
1.5
AUC (a.u.)
B
FcγRIIB
WT
E430G
K322E-E430G
P329A-E430G
P329G-E430G
P329D-E430G
P329K-E430G
P329R-E430G
FEDG
AAGG
0.0
0.5
1.0
1.5
AUC (a.u)
C
FcγRIIA-131H
WT
E430G
K322E-E430G
P329A-E430G
P329G-E430G
P329D-E430G
P329K-E430G
P329R-E430G
FEDG
AAGG
0
1
2
3
AUC (a.u)
D
FcγRIIA-131R
WT
E430G
K322E-E430G
P329A-E430G
P329G-E430G
P329D-E430G
P329K-E430G
P329R-E430G
FEDG
AAGG
0
1
2
3
AUC (a.u)

Figure 17 Continued

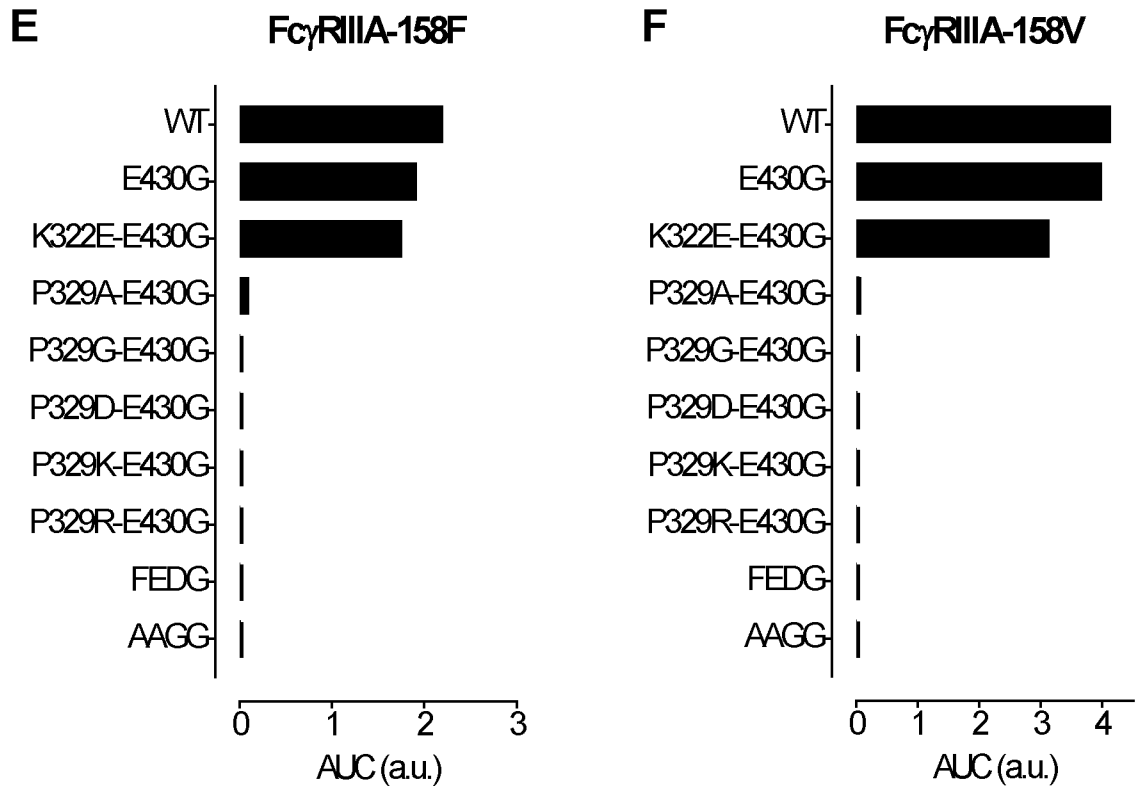
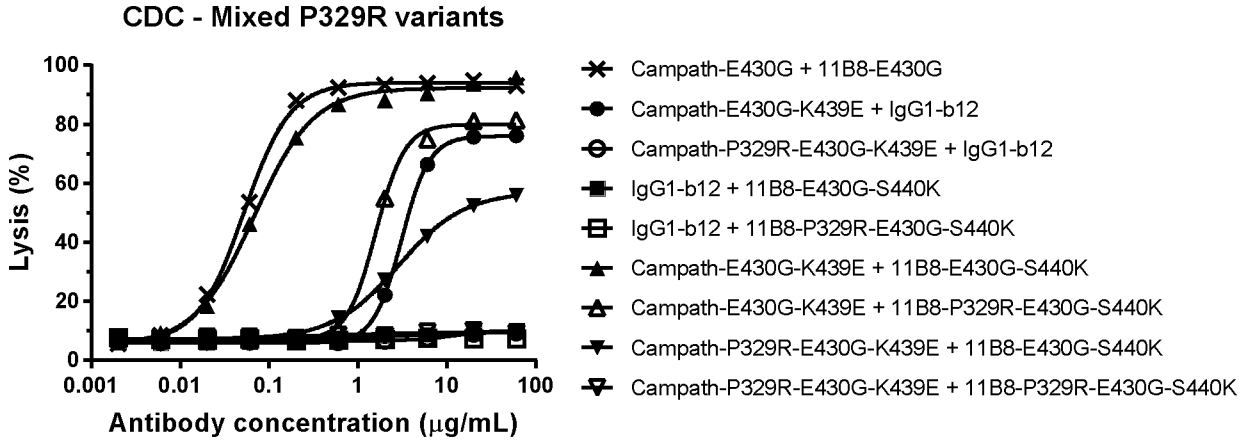


Figure 18


CDC - Mixed P329R variants
Lysis (%)
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Antibody concentration (µg/mL)
Campath-E430G + 11B8-E430G
Campath-E430G-K439E + IgG1-b12
Campath-P329R-E430G-K439E + IgG1-b12
IgG1-b12 + 11B8-E430G-S440K
IgG1-b12 + 11B8-P329R-E430G-S440K
Campath-E430G-K439E + 11B8-E430G-S440K
Campath-E430G-K439E + 11B8-P329R-E430G-S440K
Campath-P329R-E430G-K439E + 11B8-E430G-S440K
Campath-P329R-E430G-K439E + 11B8-P329R-E430G-S440K

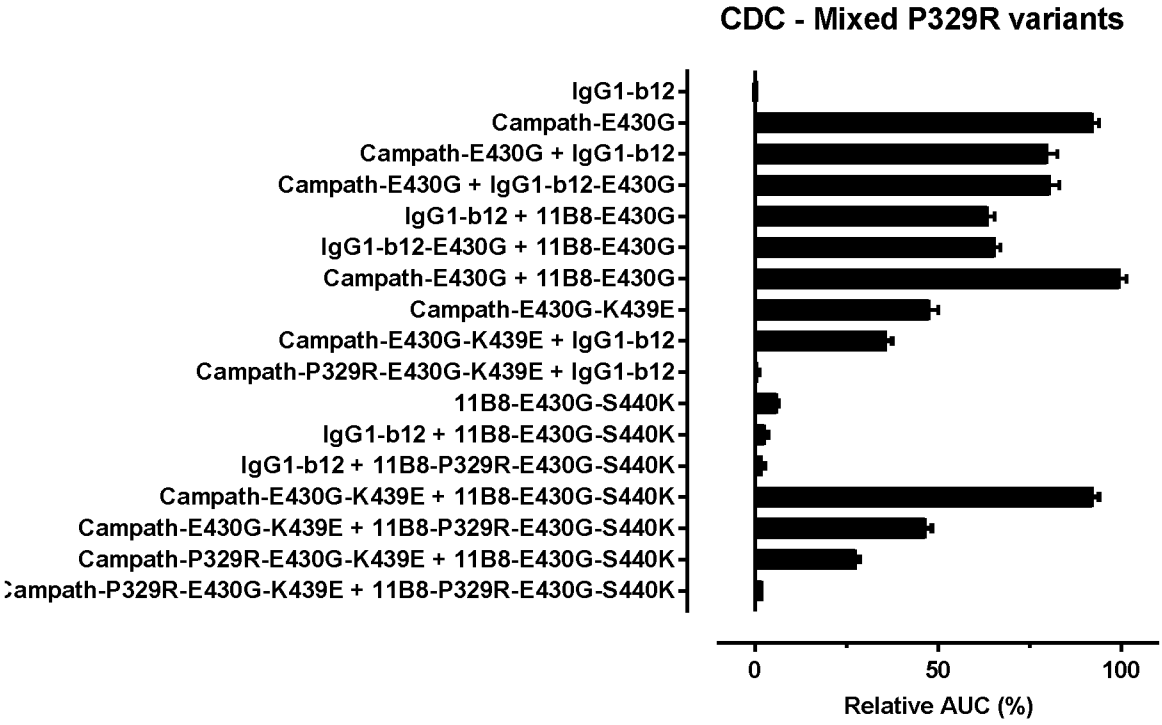
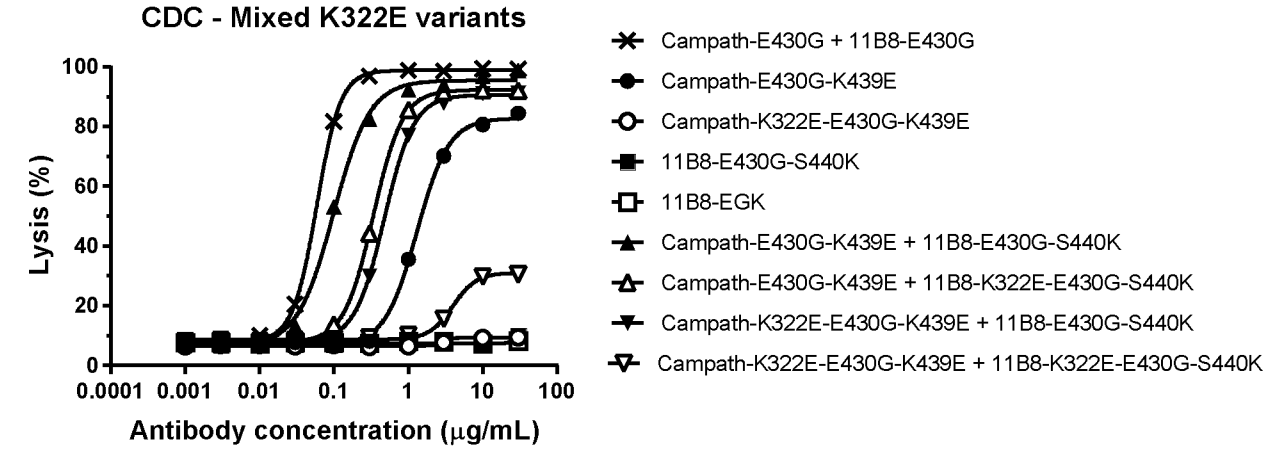

CDC - Mixed P329R variants
IgG1-b12
Campath-E430G
Campath-E430G + IgG1-b12
Campath-E430G + IgG1-b12-E430G
IgG1-b12 + 11B8-E430G
IgG1-b12-E430G + 11B8-E430G
Campath-E430G + 11B8-E430G
Campath-E430G-K439E
Campath-E430G-K439E + IgG1-b12
Campath-P329R-E430G-K439E + IgG1-b12
11B8-E430G-S440K
IgG1-b12 + 11B8-E430G-S440K
IgG1-b12 + 11B8-P329R-E430G-S440K
Campath-E430G-K439E + 11B8-E430G-S440K
Campath-E430G-K439E + 11B8-P329R-E430G-S440K
Campath-P329R-E430G-K439E + 11B8-E430G-S440K
Campath-P329R-E430G-K439E + 11B8-P329R-E430G-S440K
0
50
100
Relative AUC (%)

Figure 19


CDC - Mixed K322E variants
Lysis (%)
100
80
60
40
20
0
0.0001 0.001 0.01 0.1 1 10 100
Antibody concentration (μg/mL)
Campath-E430G + 11B8-E430G
Campath-E430G-K439E
Campath-K322E-E430G-K439E
11B8-E430G-S440K
11B8-EGK
Campath-E430G-K439E + 11B8-E430G-S440K
Campath-E430G-K439E + 11B8-K322E-E430G-S440K
Campath-K322E-E430G-K439E + 11B8-E430G-S440K
Campath-K322E-E430G-K439E + 11B8-K322E-E430G-S440K

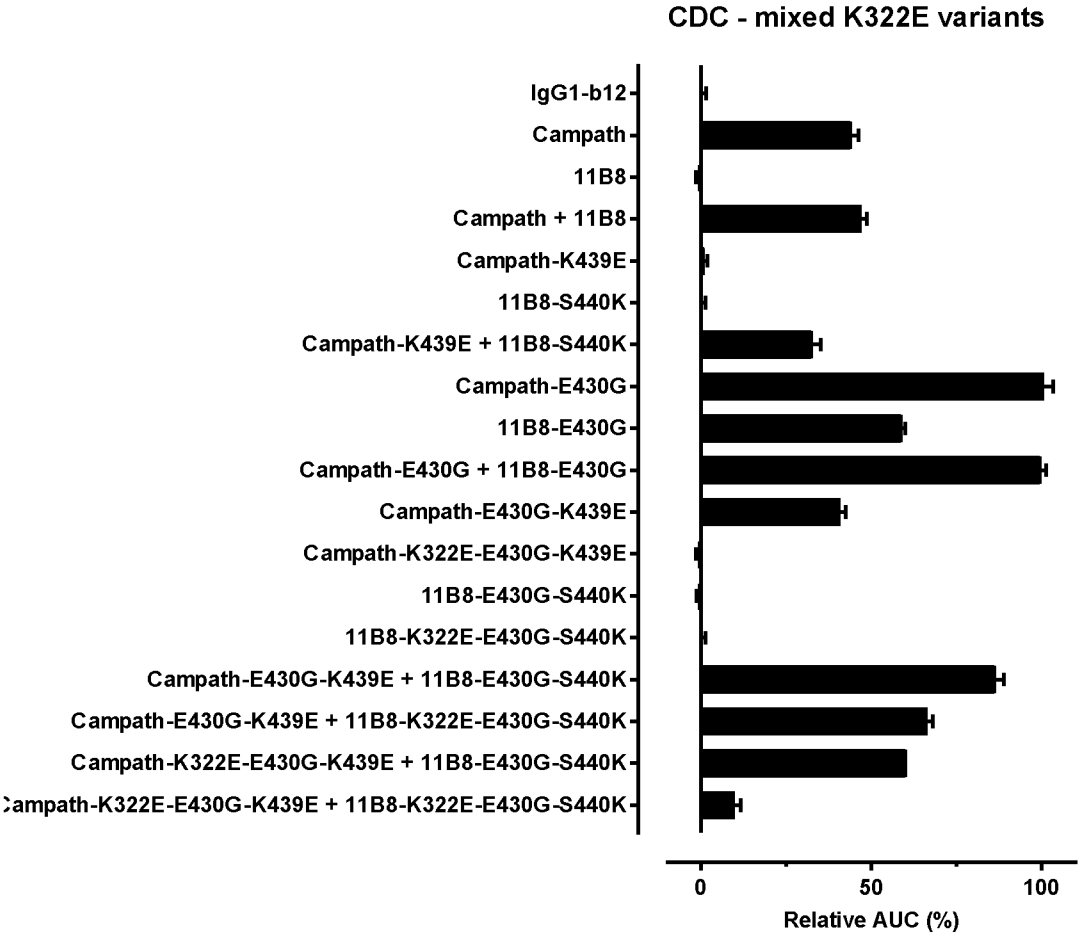

CDC - mixed K322E variants
IgG1-b12
Campath
11B8
Campath + 11B8
Campath-K439E
11B8-S440K
Campath-K439E + 11B8-S440K
Campath-E430G
11B8-E430G
Campath-E430G + 11B8-E430G
Campath-E430G-K439E
Campath-K322E-E430G-K439E
11B8-E430G-S440K
11B8-K322E-E430G-S440K
Campath-E430G-K439E + 11B8-E430G-S440K
Campath-E430G-K439E + 11B8-K322E-E430G-S440K
Campath-K322E-E430G-K439E + 11B8-E430G-S440K
Campath-K322E-E430G-K439E + 11B8-K322E-E430G-S440K
0
50
100
Relative AUC (%)

Figure 20

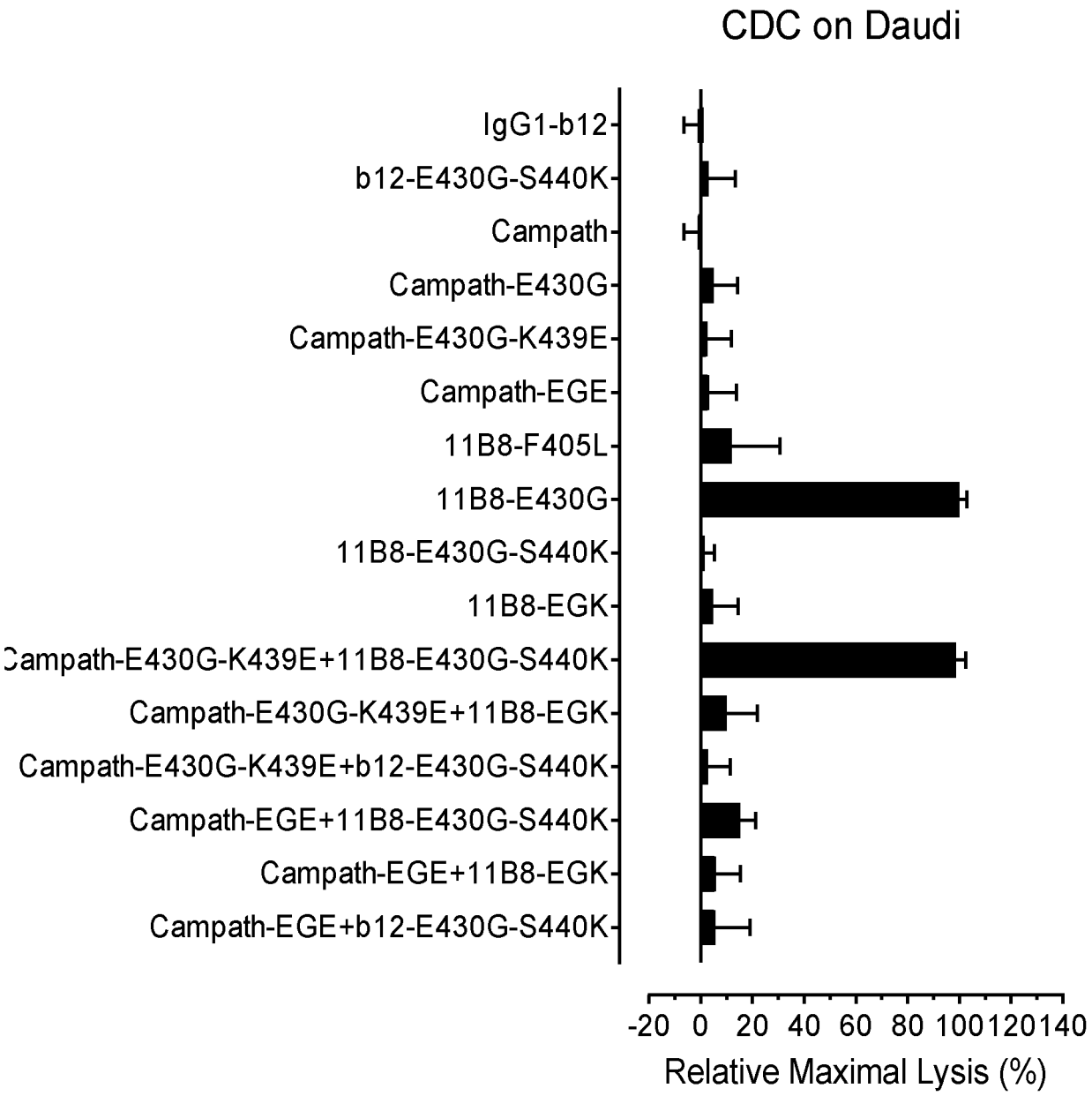

CDC on Daudi
IgG1-b12
b12-E430G-S440K
Campath
Campath-E430G
Campath-E430G-K439E
Campath-EGE
11B8-F405L
11B8-E430G
11B8-E430G-S440K
11B8-EGK
Campath-E430G-K439E+11B8-E430G-S440K
Campath-E430G-K439E+11B8-EGK
Campath-E430G-K439E+b12-E430G-S440K
Campath-EGE+11B8-E430G-S440K
Campath-EGE+11B8-EGK
Campath-EGE+b12-E430G-S440K
-20 0 20 40 60 80 100 120 140
Relative Maximal Lysis (%)

Figure 20 Continued

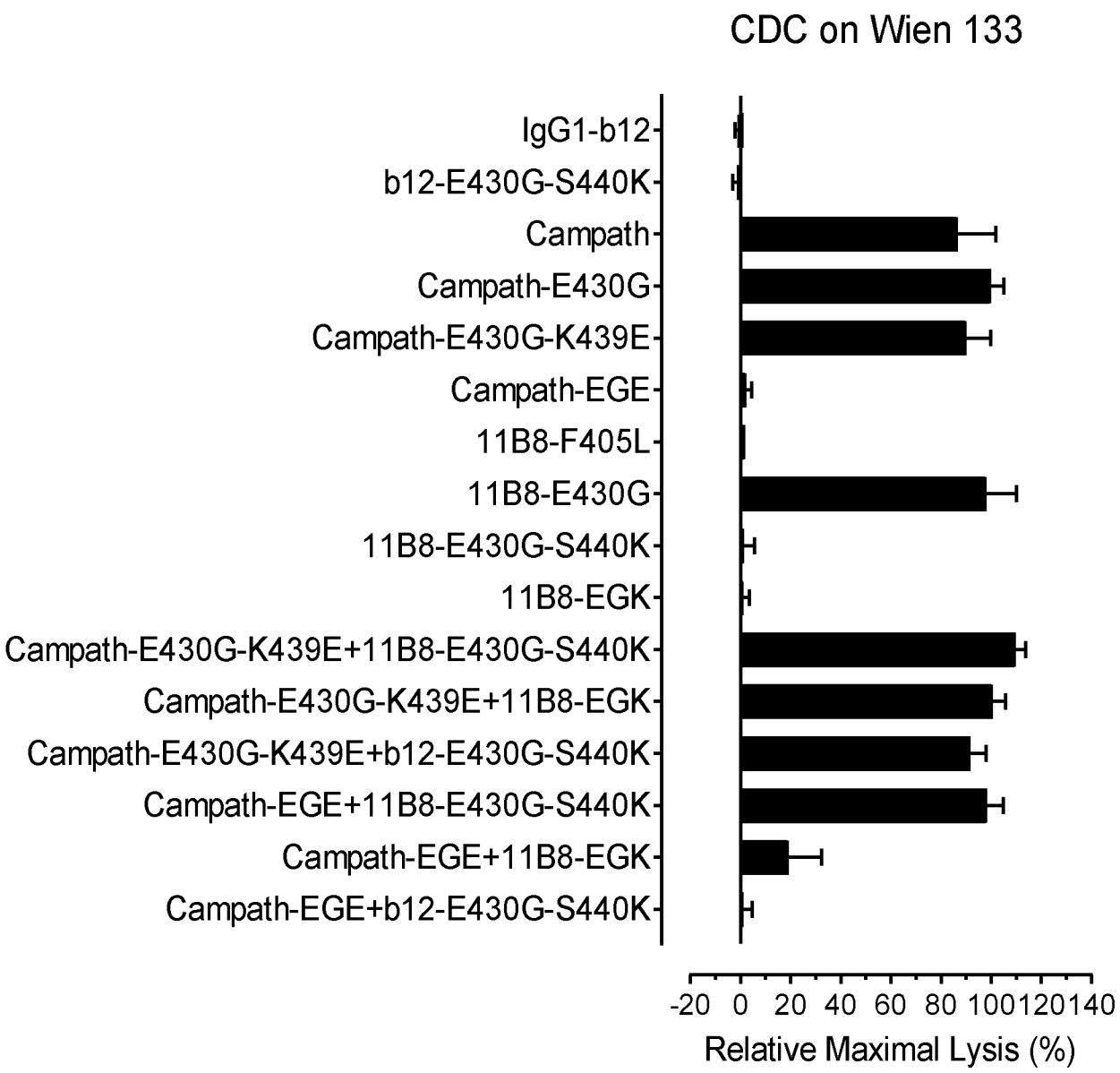


Figure 20 Continued

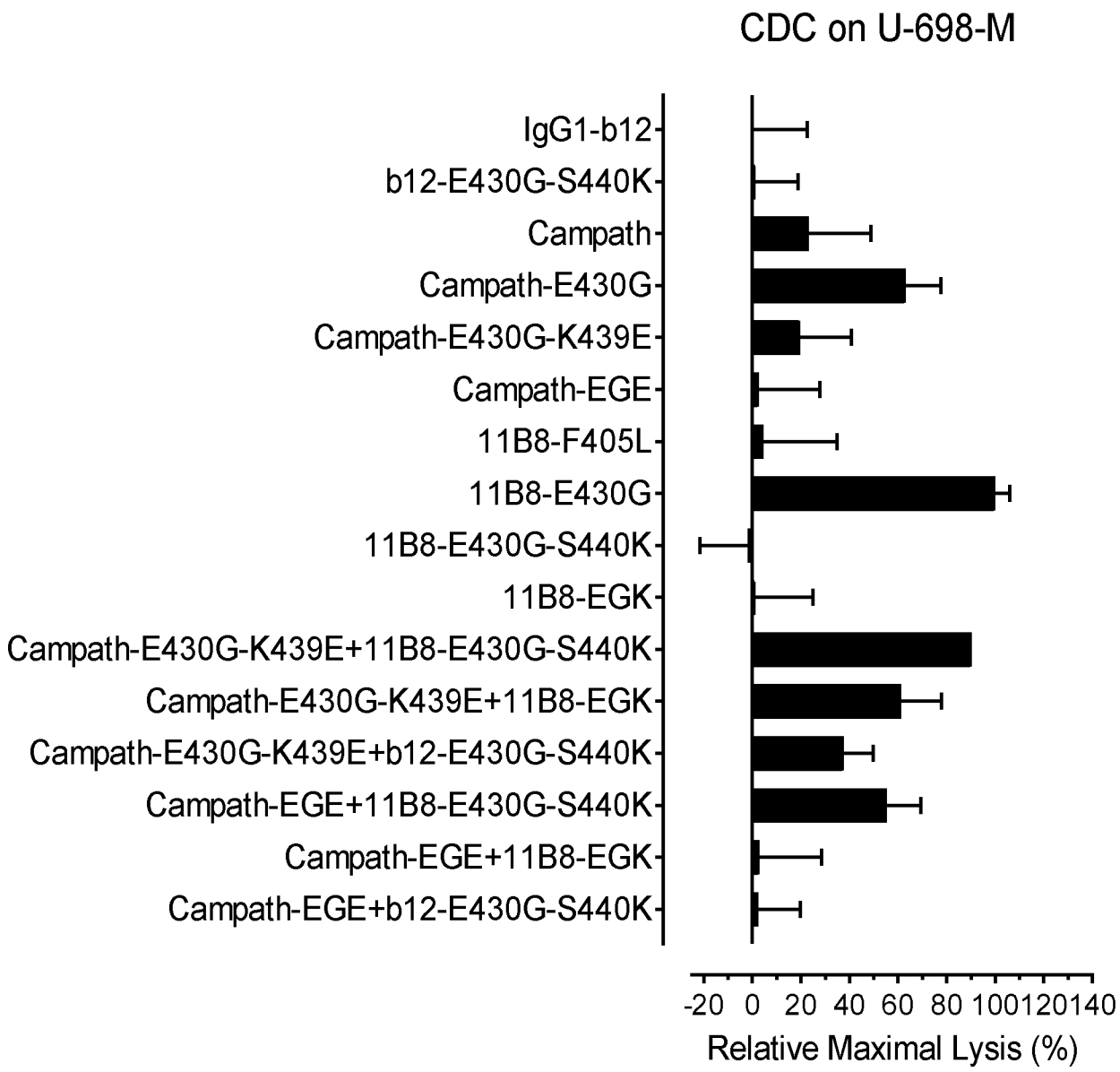


Figure 20 Continued

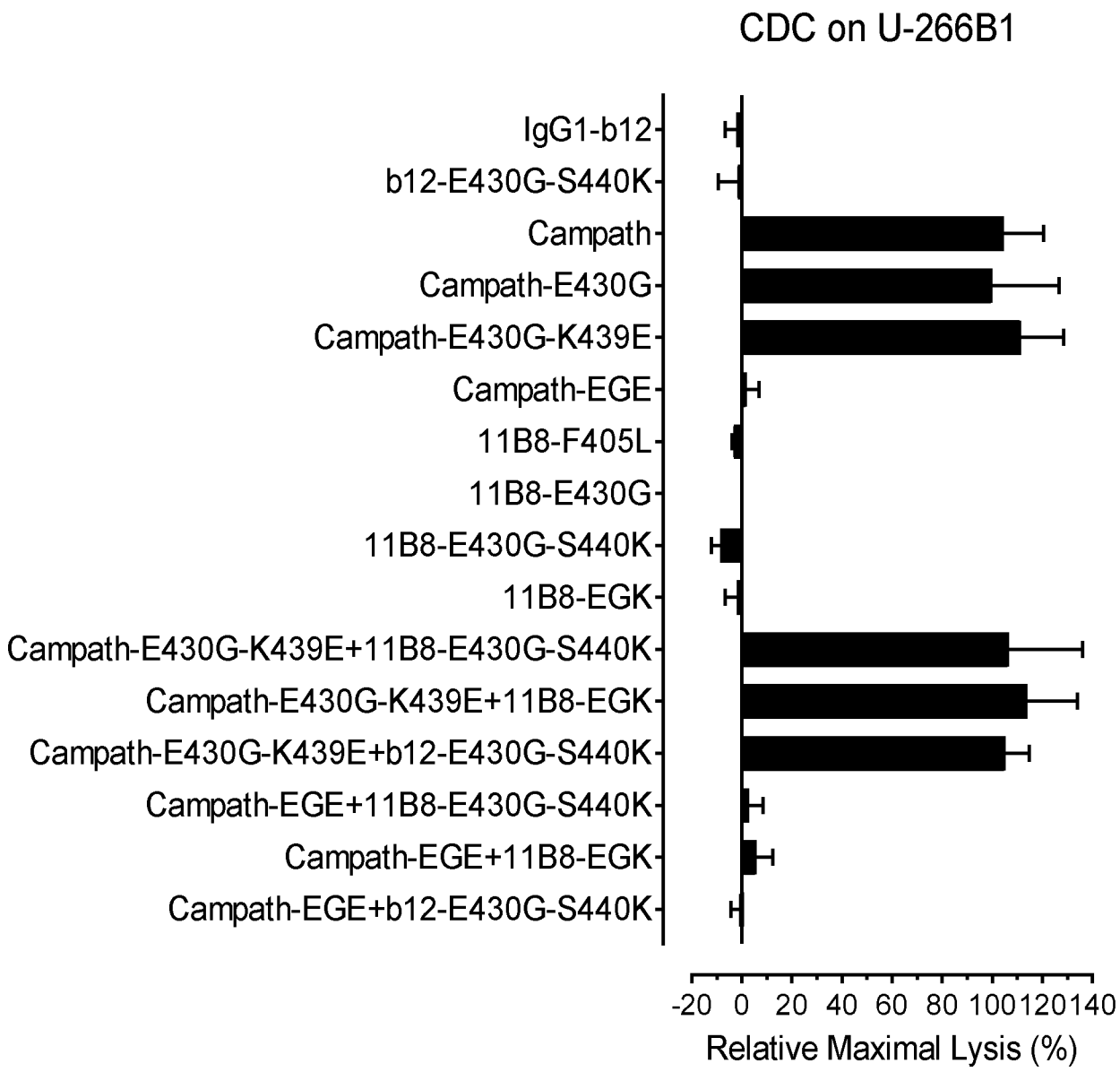


Figure 20 Continued

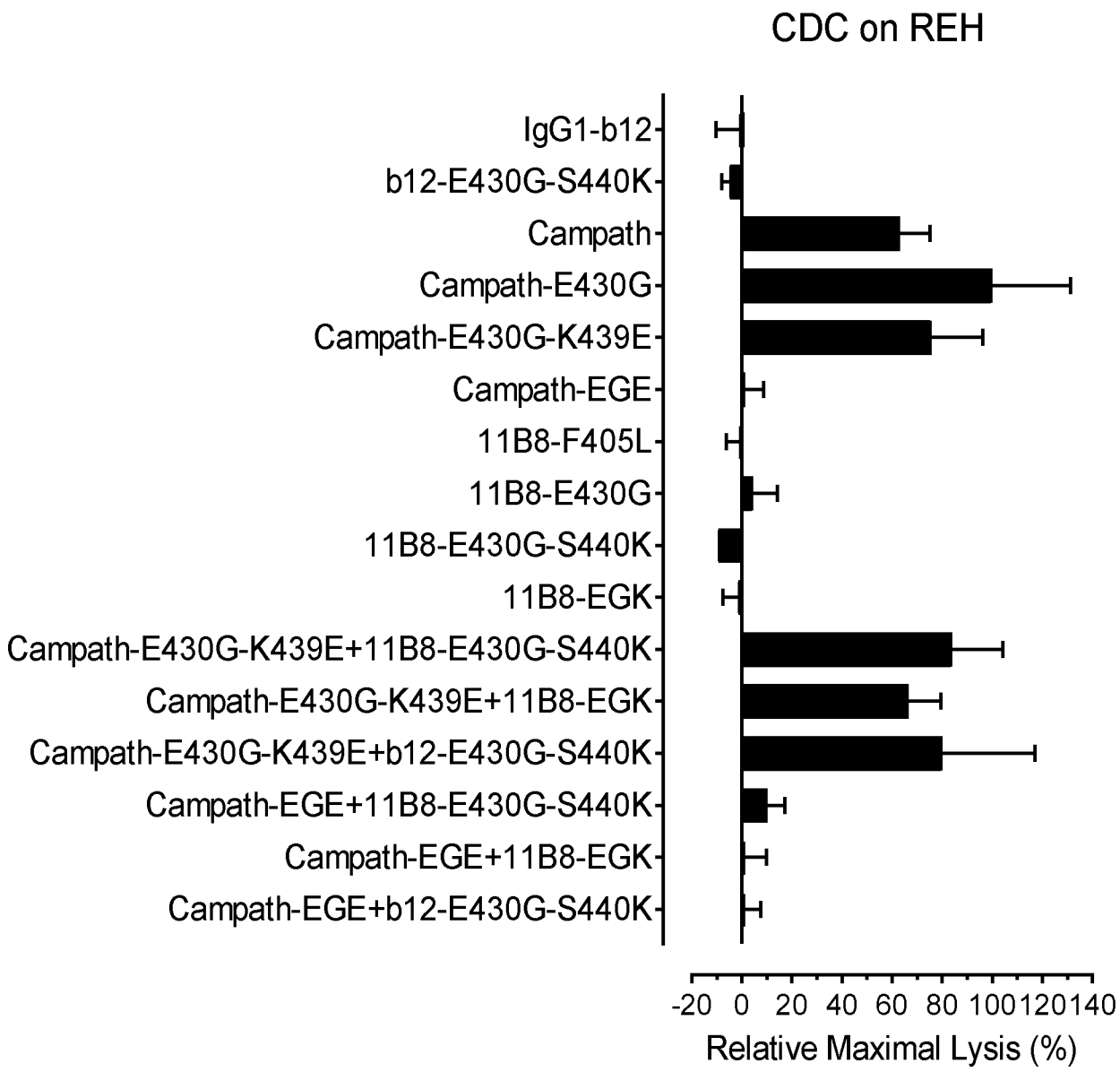


Figure 20 Continued

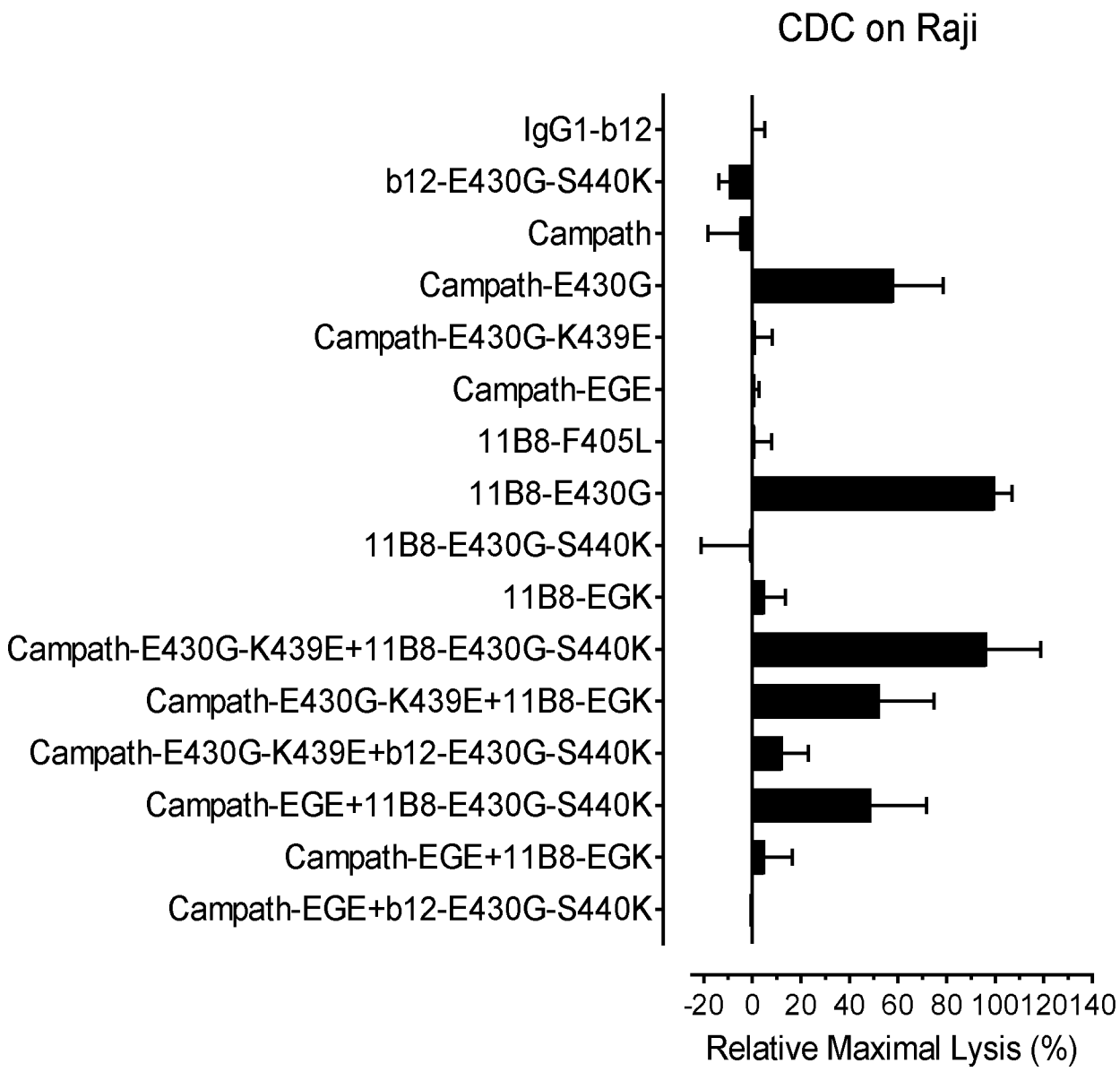


Figure 20 Continued

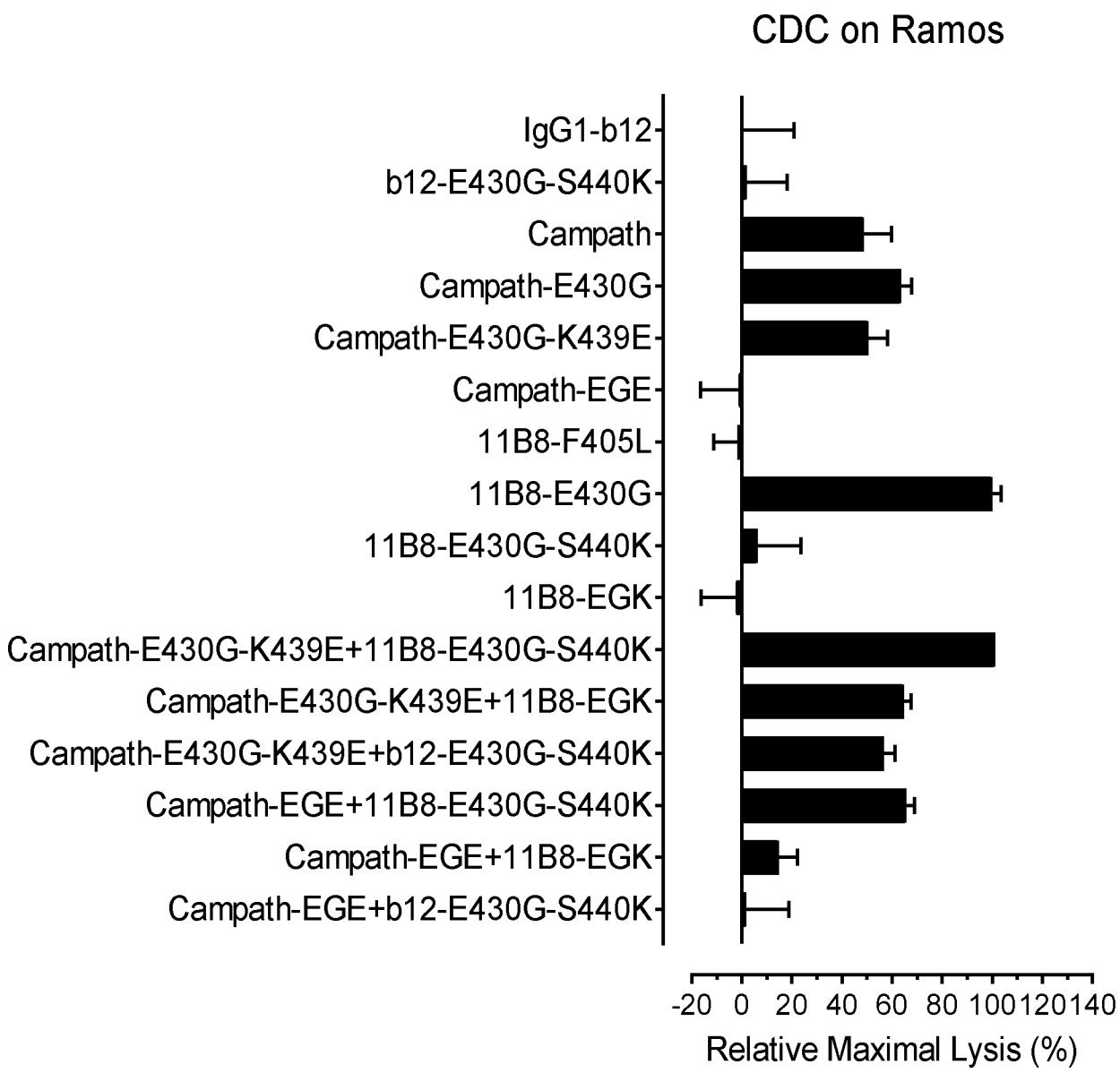


Figure 21

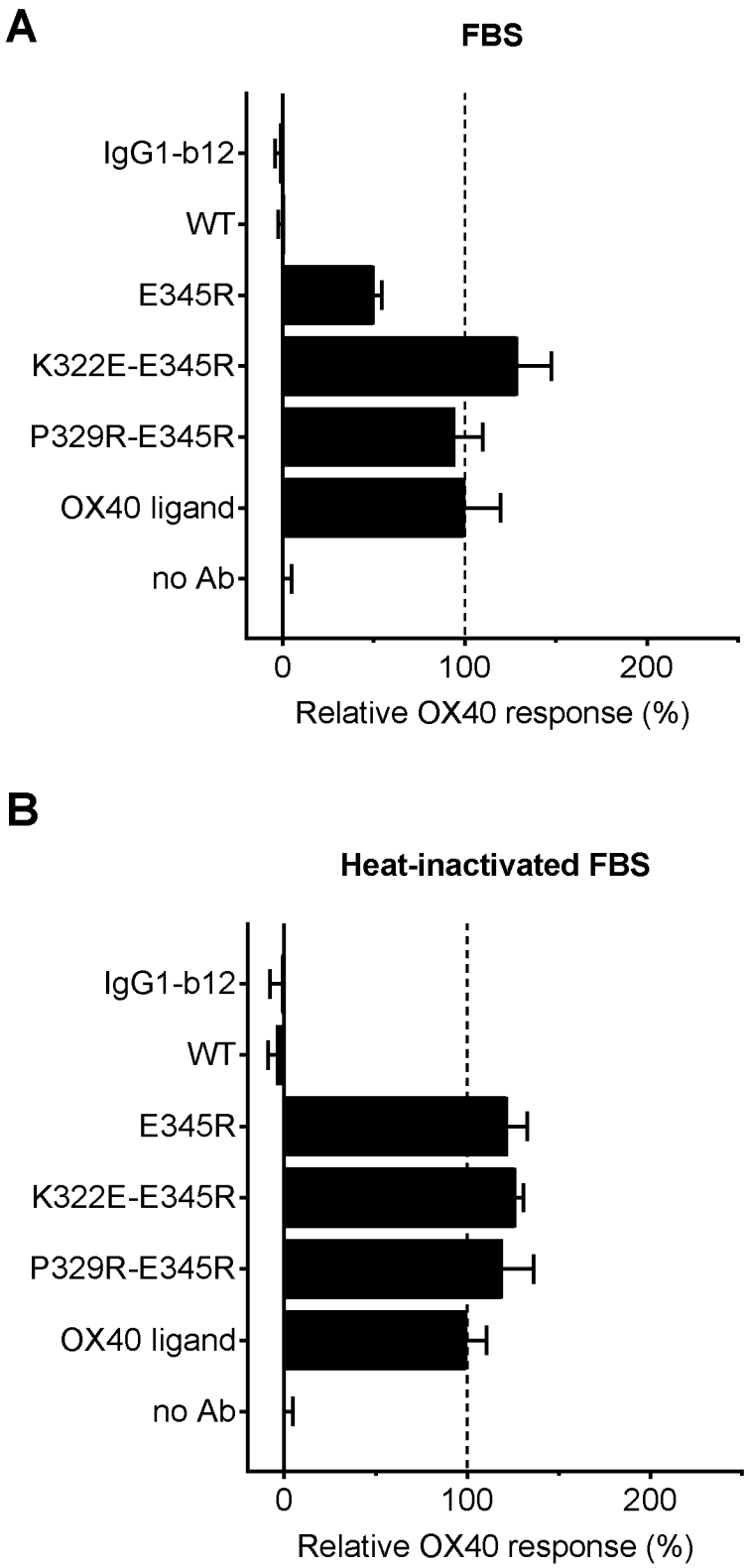
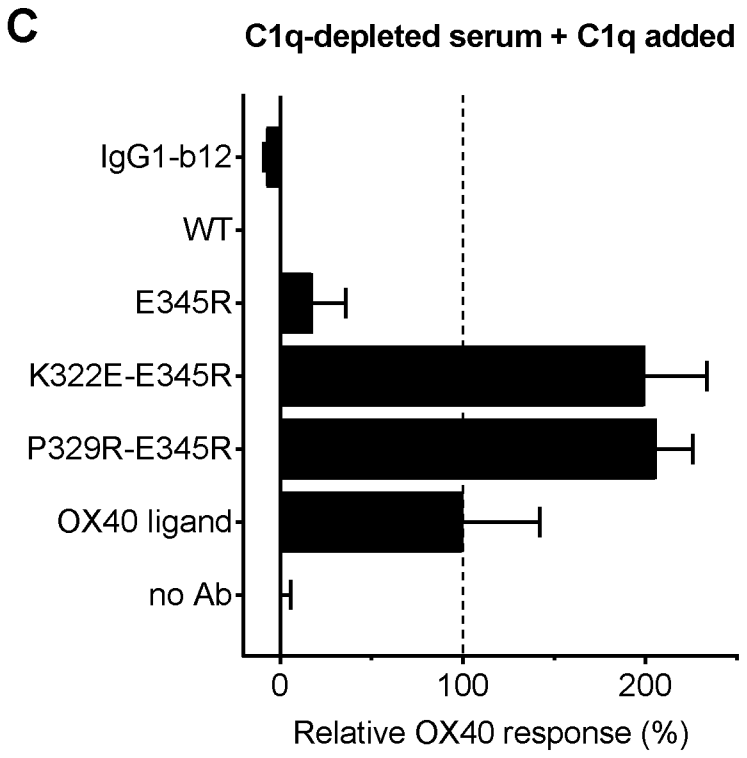

A
FBS
IgG1-b12
WT
E345R
K322E-E345R
P329R-E345R
OX40 ligand
no Ab
0
100
200
Relative OX40 response (%)
B
Heat-inactivated FBS
IgG1-b12
WT
E345R
K322E-E345R
P329R-E345R
OX40 ligand
no Ab
0
100
200
Relative OX40 response (%)

Figure 21 Continued



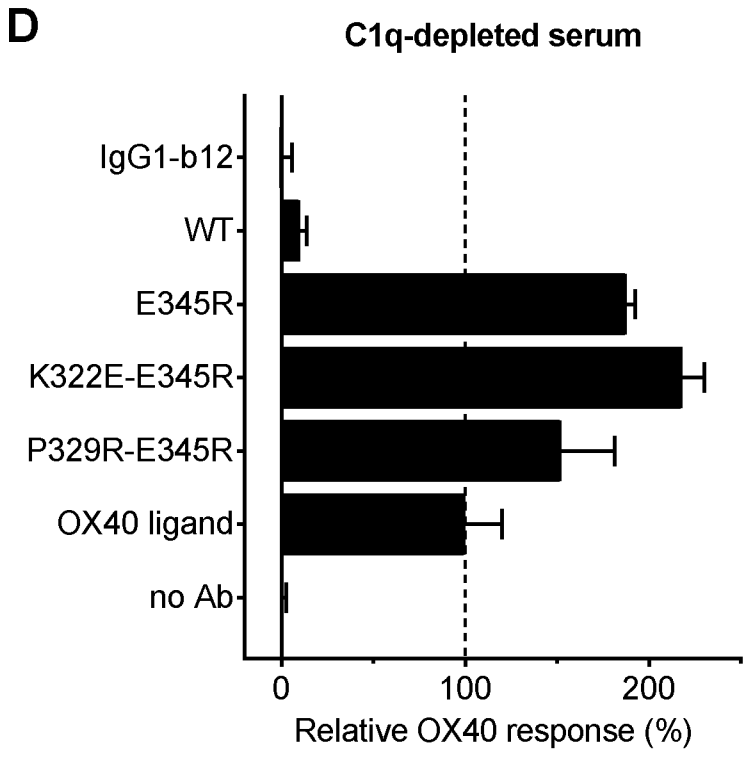



Figure 21 Continued

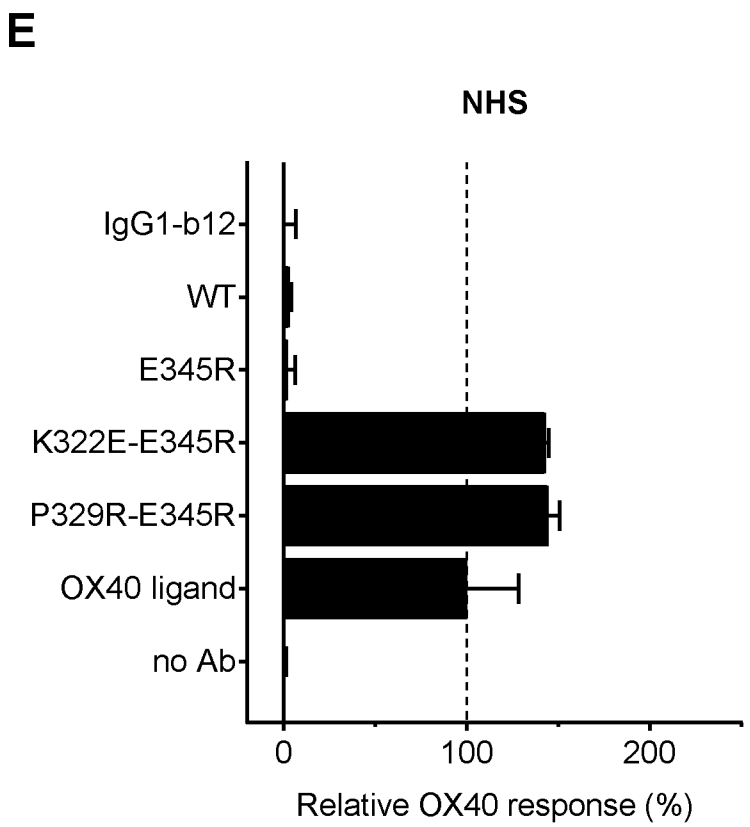


Figure 22

```
IgG1  247 PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL 306
IgG1f     PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
IgG2      PKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVL
IgG3      PKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVL
IgG4      PKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL
IgE       SPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQRNGTLTVTSTL
IgA1      ALEDLLLGSEANLTCTLTGLRDASG-VTFTWTPSSGKSAVQGP--PERDLCGCYSVSSVL
IgA2      ALEDLLLGSEANLTCTLTGLRDASG-ATFTWTPSSGKSAVQGP--PERDLCGCYSVSSVL
IgM       SFASIFLTKSTKLTCLVTDLTTYDS-VTISWTRQNGEAVKTHTNISESHPNATFSAVGEA
IgD       AVQDLWLRDKATFTCFVVGSDLKDAHLTWEVAG-KVPTGGVEEGLLERHSNGSQSQHSRL
                    : :   . .** :..    .                      * .  .     .

IgG1  307 TVLHQDWLNGKEYKCKVSNKALPAPIEKTIS-KAKGQPREPQVYTLPPSRDELTK-NQVS 364
IgG1f     TVLHQDWLNGKEYKCKVSNKALPAPIEKTIS-KAKGQPREPQVYTLPPSREEMTK-NQVS
IgG2      TVVHQDWLNGKEYKCKVSNKGLPAPIEKTIS-KTKGQPREPQVYTLPPSREEMTK-NQVS
IgG3      TVLHQDWLNGKEYKCKVSNKALPAPIEKTIS-KTKGQPREPQVYTLPPSREEMTK-NQVS
IgG4      TVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS-KAKGQPREPQVYTLPPSQEEMTK-NQVS
IgE       PVGTRDWIEGETYQCRVTHPHLPRALMRSTT-KTSGPRAAPEVYAFATPEWPGSR-DKRT
IgA1      PGCAEPWNHGKTFTCTAAYPESKTPLTATLS--KSGNTFRPEVHLLPPPSEELALNELVT
IgA2      PGCAQPWNHGETFTCTAAHPELKTPLTANIT--KSGNTFRPEVHLLPPPSEELALNELVT
IgM       SICEDDWNSGERFTCTVTHTDLPSPLKQTISRPKGVALHRPDVYLLPPAREQLNLRESAT
IgD       TLPRSLWNAGTSVTCTLNHPSLPPQRLMALREPAAQAPVKLSLNLLASSDPPEAA---SW
                  .     *  *    *                              .:  :...

IgG1  365 LTCLVKGFYPSDIAVEWESNGQ--PENNYKTTPPVLDS---DGSFFLYSKLTVDKSRWQQ 419
IgG1f     LTCLVKGFYPSDIAVEWESNGQ--PENNYKTTPPVLDS---DGSFFLYSKLTVDKSRWQQ
IgG2      LTCLVKGFYPSDIAVEWESNGQ--PENNYKTTPPMLDS---DGSFFLYSKLTVDKSRWQQ
IgG3      LTCLVKGFYPSDIAVEWESSGQ--PENNYNTTPPMLDS---DGSFFLYSKLTVDKSRWQQ
IgG4      LTCLVKGFYPSDIAVEWESNGQ--PENNYKTTPPVLDS---DGSFFLYSRLTVDKSRWQE
IgE       LACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTK---GSGFFVFSRLEVTRAEWEQ
IgA1      LTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKK
IgA2      LTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKK
IgM       ITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEP-QAPGRYFAHSILTVSEEEWNT
IgD       LLCEVSGFSPPNILLMWLEDQREVNTSGFAPARPPPQP--GSTTFWAWSVLRVPAPPSPQ
                  : *   .* * :: : *           . .  .          :   * * *

IgG1  420 GNVFSCSVMHEALHN-HYTQKSLSLSPGK------------------ 447
IgG1f     GNVFSCSVMHEALHN-HYTQKSLSLSPGK------------------
IgG2      GNVFSCSVMHEALHN-HYTQKSLSLSPGK------------------
IgG3      GNIFSCSVMHEALHN-RFTQKSLSLSPGK------------------
IgG4      GNVFSCSVMHEALHN-HYTQKSLSLSLGK------------------
IgE       KDEFICRAVHEAASPSQTVQRAVSVNPGK------------------
IgA1      GDTFSCMVGHEALPL-AFTQKTIDRLAGKPTHVNVSVVMAEVDGTCY
IgA2      GDTFSCMVGHEALPL-AFTQKTIDRLAGKPTHVNVSVVMAEVDGTCY
IgM       GETYTCVA-HEALPN-RVTERTVDKSTGKPTLYNVSLVMSDTAGTCY
IgD       PATYTCVVSHEDSRTLLNASRSLEVSYVTDHGPMK------------
                    : * . **       ..:::.    .
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