FIGURE 1

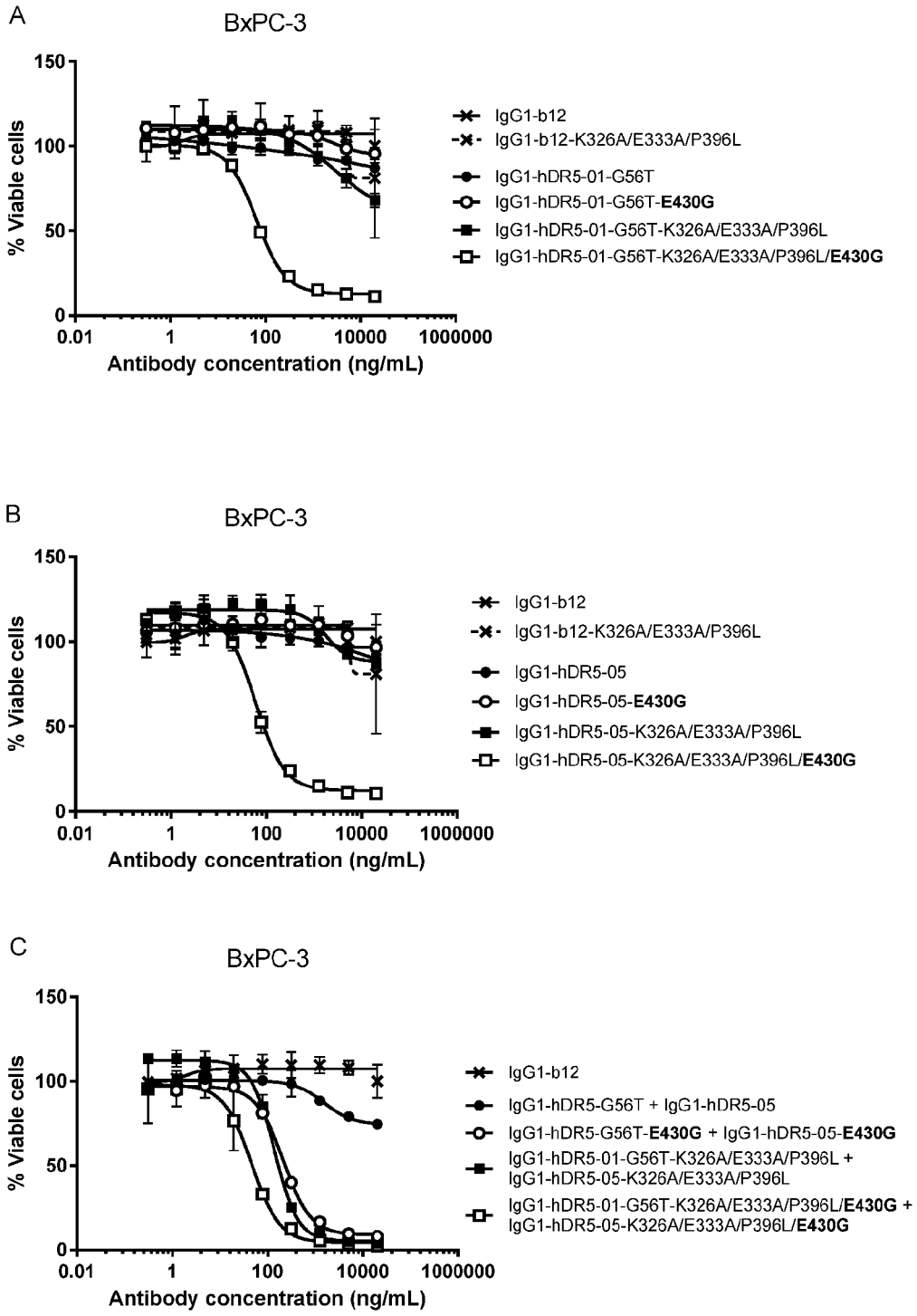

A
BxPC-3
% Viable cells
150
100
50
0
0.01
1
100
10000
1000000
Antibody concentration (ng/mL)
IgG1-b12
IgG1-b12-K326A/E333A/P396L
IgG1-hDR5-01-G56T
IgG1-hDR5-01-G56T-E430G
IgG1-hDR5-01-G56T-K326A/E333A/P396L
IgG1-hDR5-01-G56T-K326A/E333A/P396L/E430G
B
BxPC-3
% Viable cells
Antibody concentration (ng/mL)
IgG1-b12
IgG1-b12-K326A/E333A/P396L
IgG1-hDR5-05
IgG1-hDR5-05-E430G
IgG1-hDR5-05-K326A/E333A/P396L
IgG1-hDR5-05-K326A/E333A/P396L/E430G
C
BxPC-3
% Viable cells
Antibody concentration (ng/mL)
IgG1-b12
IgG1-hDR5-G56T + IgG1-hDR5-05
IgG1-hDR5-G56T-E430G + IgG1-hDR5-05-E430G
IgG1-hDR5-01-G56T-K326A/E333A/P396L +
IgG1-hDR5-05-K326A/E333A/P396L
IgG1-hDR5-01-G56T-K326A/E333A/P396L/E430G +
IgG1-hDR5-05-K326A/E333A/P396L/E430G

FIGURE 2

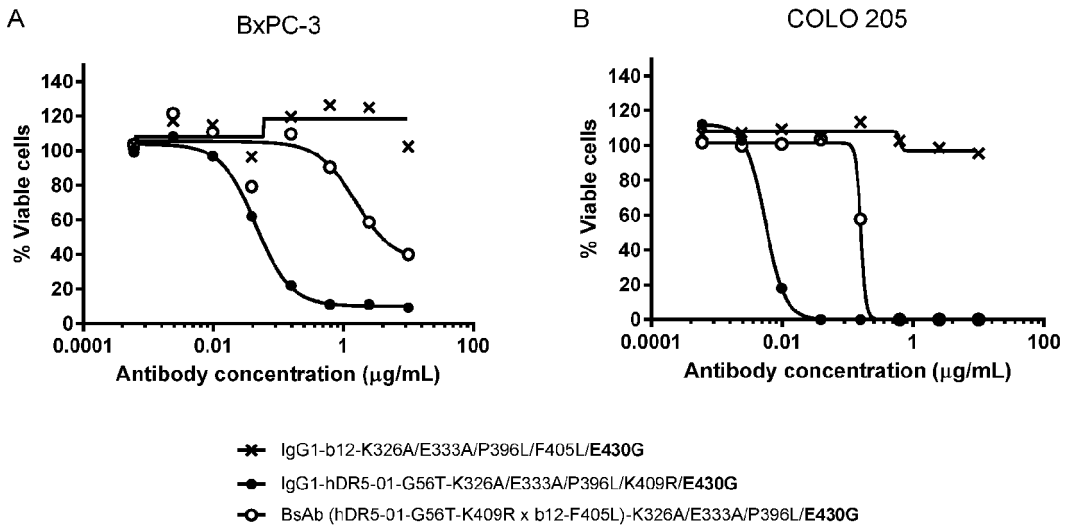

A
BxPC-3
% Viable cells
140
120
100
80
60
40
20
0
0.0001
0.01
1
100
Antibody concentration (µg/mL)
B
COLO 205
% Viable cells
140
120
100
80
60
40
20
0
0.0001
0.01
1
100
Antibody concentration (µg/mL)
IgG1-b12-K326A/E333A/P396L/F405L/E430G
IgG1-hDR5-01-G56T-K326A/E333A/P396L/K409R/E430G
BsAb (hDR5-01-G56T-K409R x b12-F405L)-K326A/E333A/P396L/E430G

FIGURE 3

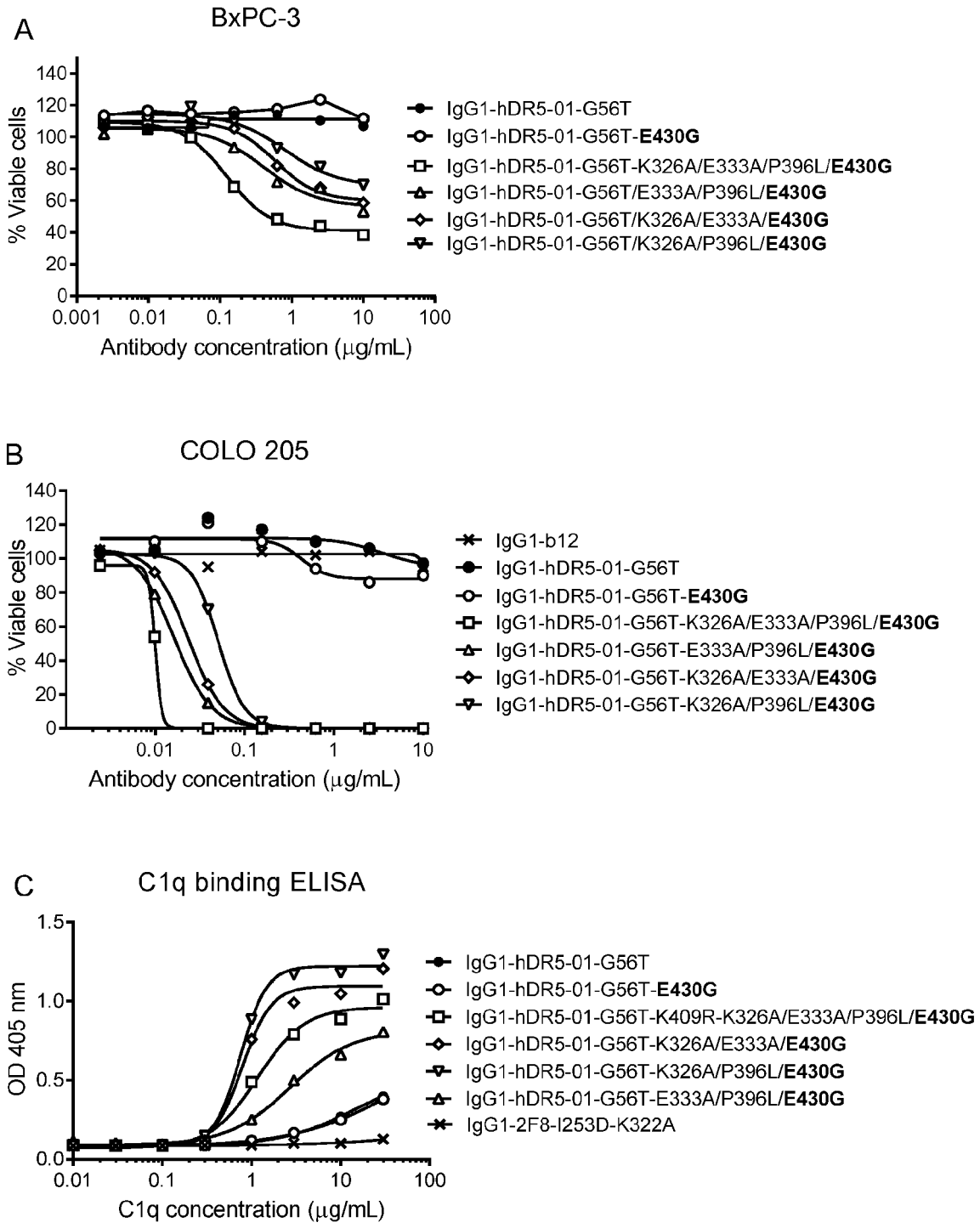

A
BxPC-3
% Viable cells
Antibody concentration (μg/mL)
IgG1-hDR5-01-G56T
IgG1-hDR5-01-G56T-E430G
IgG1-hDR5-01-G56T-K326A/E333A/P396L/E430G
IgG1-hDR5-01-G56T/E333A/P396L/E430G
IgG1-hDR5-01-G56T/K326A/E333A/E430G
IgG1-hDR5-01-G56T/K326A/P396L/E430G
B
COLO 205
% Viable cells
Antibody concentration (μg/mL)
IgG1-b12
IgG1-hDR5-01-G56T
IgG1-hDR5-01-G56T-E430G
IgG1-hDR5-01-G56T-K326A/E333A/P396L/E430G
IgG1-hDR5-01-G56T-E333A/P396L/E430G
IgG1-hDR5-01-G56T-K326A/E333A/E430G
IgG1-hDR5-01-G56T-K326A/P396L/E430G
C
C1q binding ELISA
OD 405 nm
C1q concentration (μg/mL)
IgG1-hDR5-01-G56T
IgG1-hDR5-01-G56T-E430G
IgG1-hDR5-01-G56T-K409R-K326A/E333A/P396L/E430G
IgG1-hDR5-01-G56T-K326A/E333A/E430G
IgG1-hDR5-01-G56T-K326A/P396L/E430G
IgG1-hDR5-01-G56T-E333A/P396L/E430G
IgG1-2F8-I253D-K322A

FIGURE 4

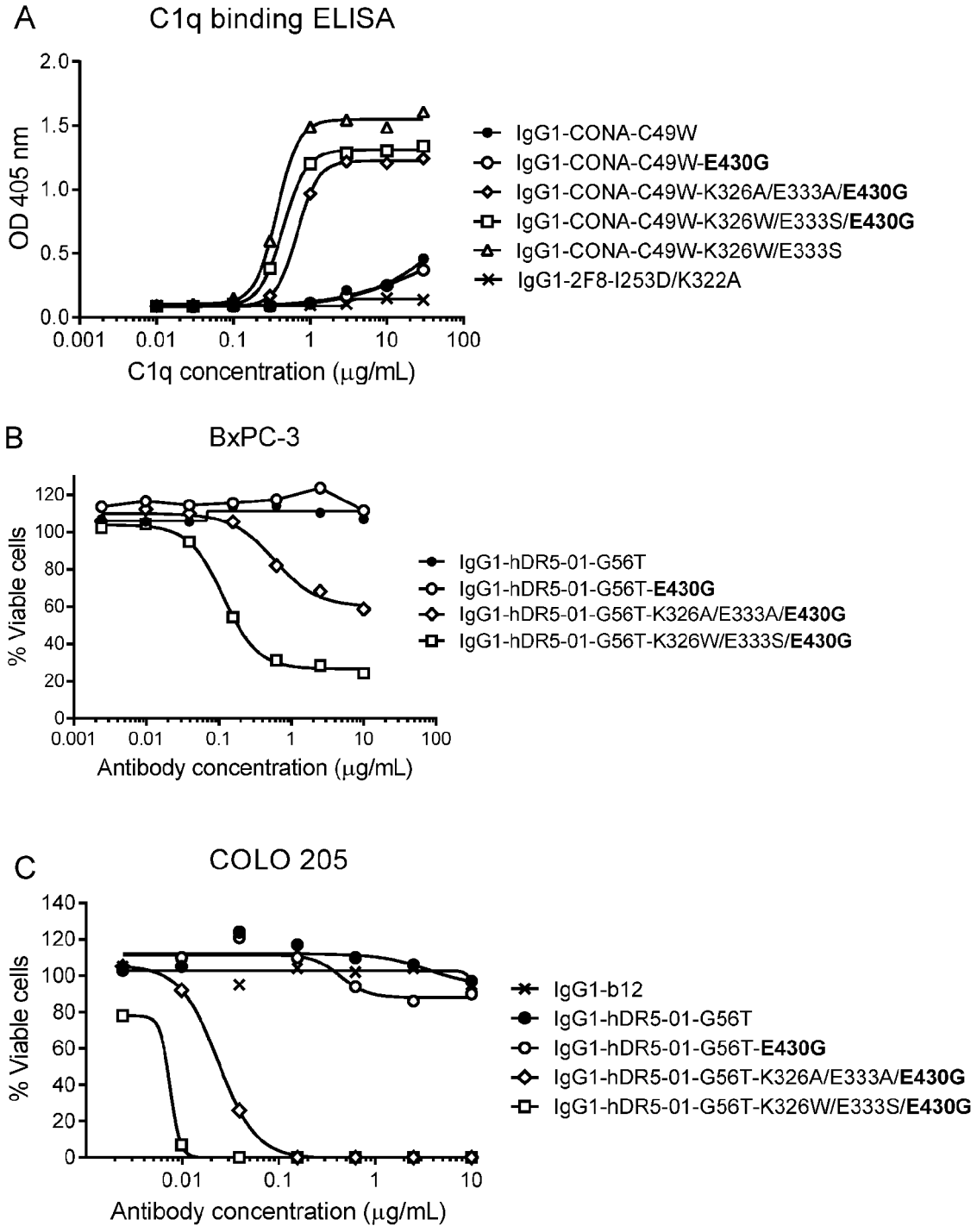

A
C1q binding ELISA
OD 405 nm
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
100
C1q concentration (µg/mL)
IgG1-CONA-C49W
IgG1-CONA-C49W-E430G
IgG1-CONA-C49W-K326A/E333A/E430G
IgG1-CONA-C49W-K326W/E333S/E430G
IgG1-CONA-C49W-K326W/E333S
IgG1-2F8-I253D/K322A
B
BxPC-3
% Viable cells
120
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Antibody concentration (µg/mL)
IgG1-hDR5-01-G56T
IgG1-hDR5-01-G56T-E430G
IgG1-hDR5-01-G56T-K326A/E333A/E430G
IgG1-hDR5-01-G56T-K326W/E333S/E430G
C
COLO 205
% Viable cells
140
120
100
80
60
40
20
0
0.01
0.1
1
10
Antibody concentration (µg/mL)
IgG1-b12
IgG1-hDR5-01-G56T
IgG1-hDR5-01-G56T-E430G
IgG1-hDR5-01-G56T-K326A/E333A/E430G
IgG1-hDR5-01-G56T-K326W/E333S/E430G

FIGURE 5

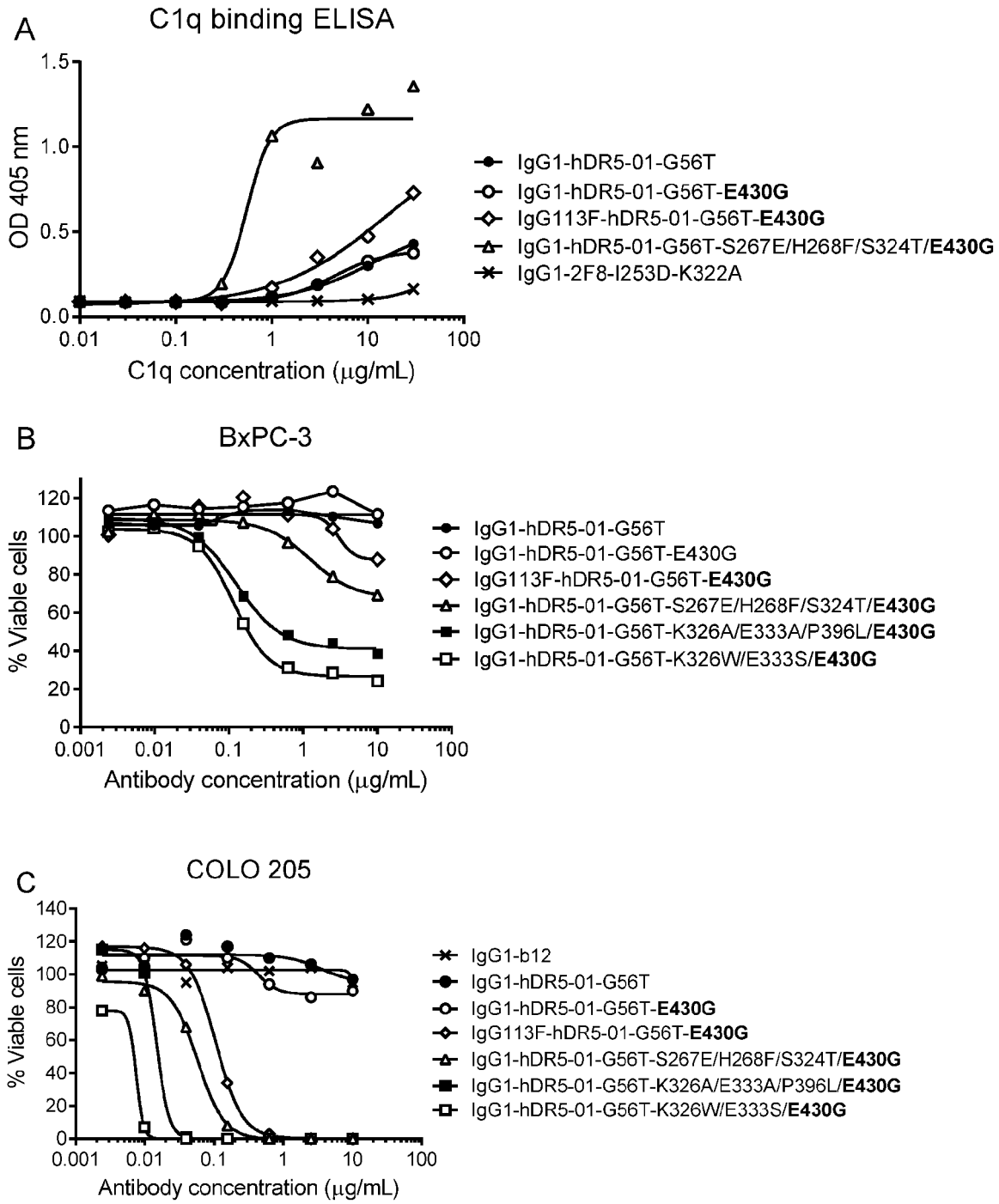

A
C1q binding ELISA
OD 405 nm
1.5
1.0
0.5
0.0
0.01
0.1
1
10
100
C1q concentration (µg/mL)
IgG1-hDR5-01-G56T
IgG1-hDR5-01-G56T-E430G
IgG113F-hDR5-01-G56T-E430G
IgG1-hDR5-01-G56T-S267E/H268F/S324T/E430G
IgG1-2F8-I253D-K322A
B
BxPC-3
% Viable cells
120
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Antibody concentration (µg/mL)
IgG1-hDR5-01-G56T
IgG1-hDR5-01-G56T-E430G
IgG113F-hDR5-01-G56T-E430G
IgG1-hDR5-01-G56T-S267E/H268F/S324T/E430G
IgG1-hDR5-01-G56T-K326A/E333A/P396L/E430G
IgG1-hDR5-01-G56T-K326W/E333S/E430G
C
COLO 205
% Viable cells
140
120
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Antibody concentration (µg/mL)
IgG1-b12
IgG1-hDR5-01-G56T
IgG1-hDR5-01-G56T-E430G
IgG113F-hDR5-01-G56T-E430G
IgG1-hDR5-01-G56T-S267E/H268F/S324T/E430G
IgG1-hDR5-01-G56T-K326A/E333A/P396L/E430G
IgG1-hDR5-01-G56T-K326W/E333S/E430G

FIGURE 6

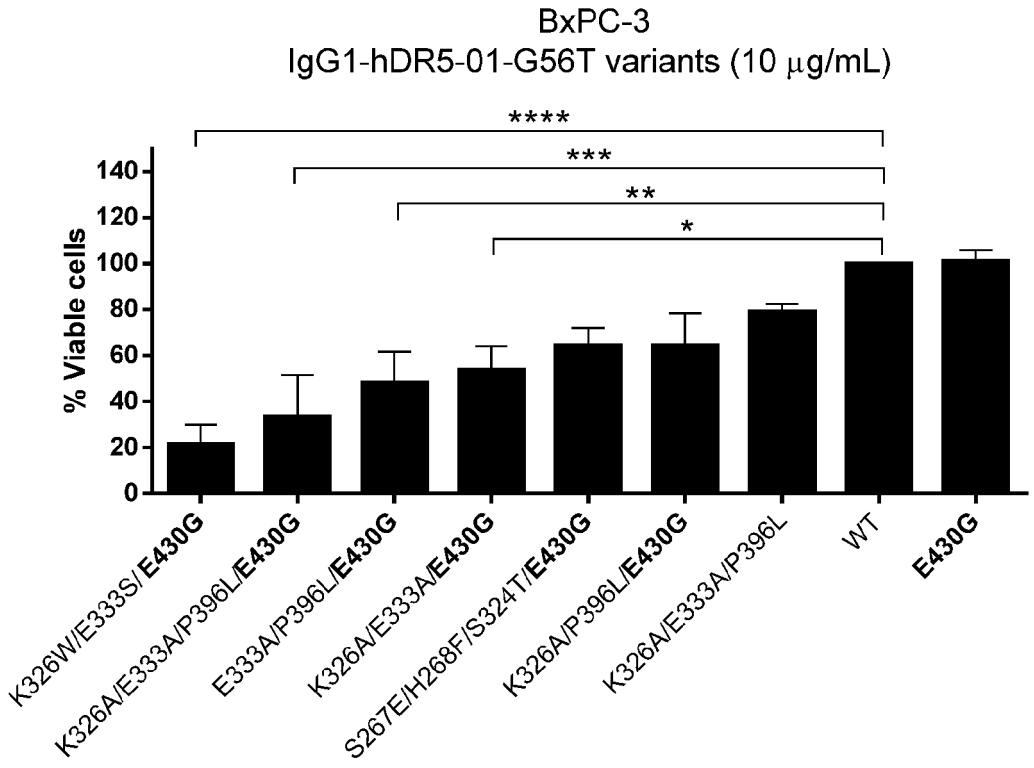

BxPC-3
IgG1-hDR5-01-G56T variants (10 μg/mL)
% Viable cells
0
20
40
60
80
100
120
140
****
***
**
*
K326W/E333S/E430G
K326A/E333A/P396L/E430G
E333A/P396L/E430G
K326A/E333A/E430G
S267E/H268F/S324T/E430G
K326A/P396L/E430G
K326A/E333A/P396L
WT
E430G

FIGURE 7

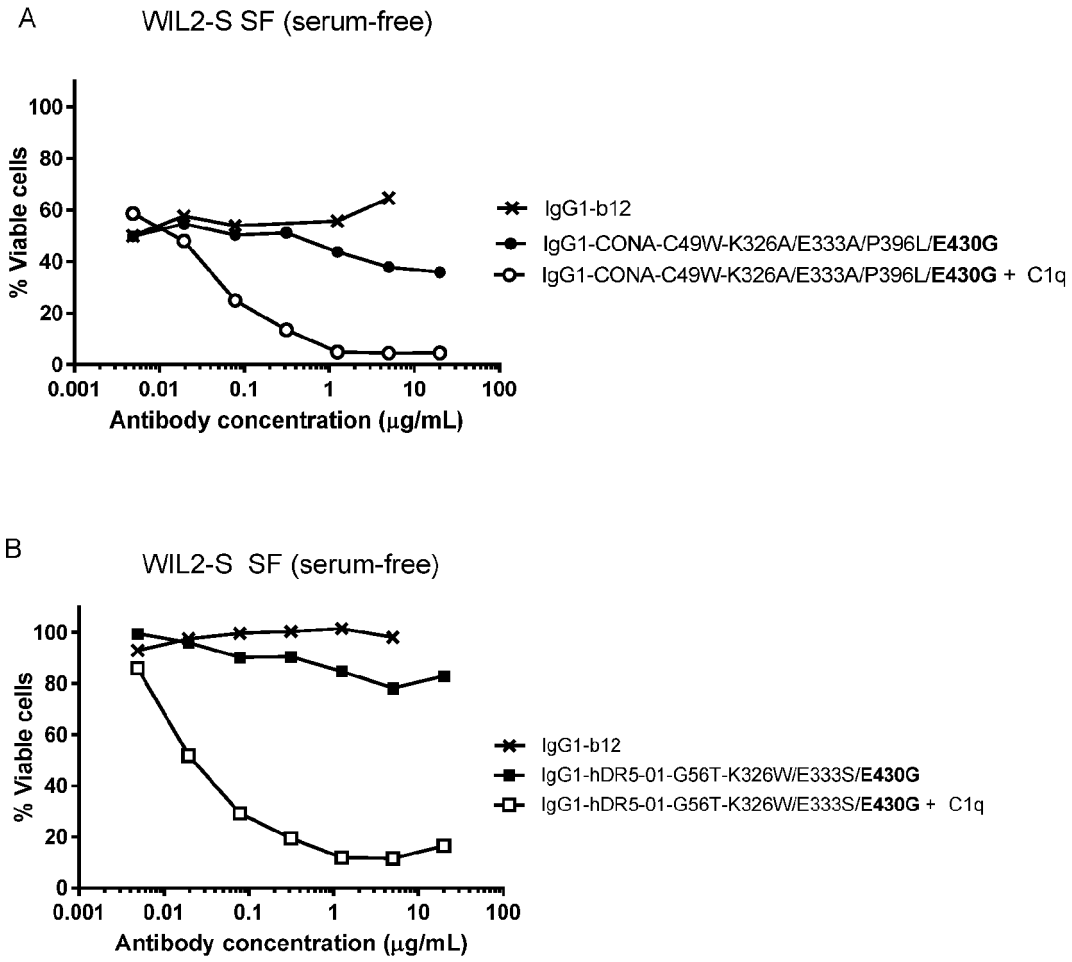

A
WIL2-S SF (serum-free)
% Viable cells
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Antibody concentration (µg/mL)
IgG1-b12
IgG1-CONA-C49W-K326A/E333A/P396L/E430G
IgG1-CONA-C49W-K326A/E333A/P396L/E430G + C1q
B
WIL2-S SF (serum-free)
% Viable cells
Antibody concentration (µg/mL)
IgG1-b12
IgG1-hDR5-01-G56T-K326W/E333S/E430G
IgG1-hDR5-01-G56T-K326W/E333S/E430G + C1q

FIGURE 8

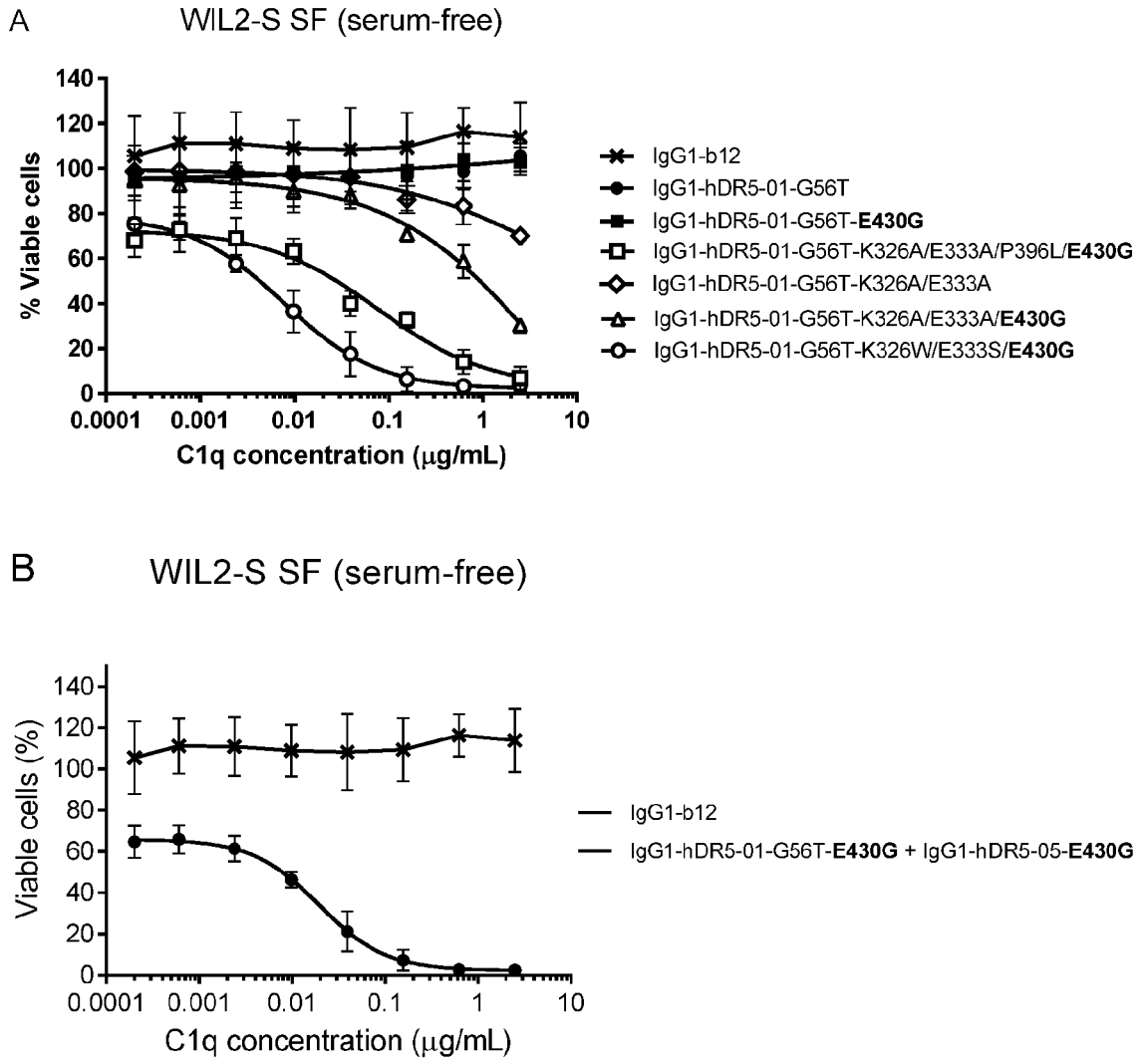
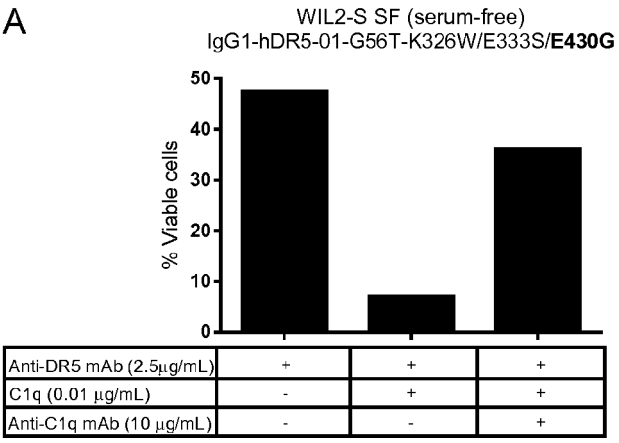

A
WIL2-S SF (serum-free)
% Viable cells
140
120
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
C1q concentration (µg/mL)
IgG1-b12
IgG1-hDR5-01-G56T
IgG1-hDR5-01-G56T-E430G
IgG1-hDR5-01-G56T-K326A/E333A/P396L/E430G
IgG1-hDR5-01-G56T-K326A/E333A
IgG1-hDR5-01-G56T-K326A/E333A/E430G
IgG1-hDR5-01-G56T-K326W/E333S/E430G
B
WIL2-S SF (serum-free)
Viable cells (%)
140
120
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
C1q concentration (µg/mL)
IgG1-b12
IgG1-hDR5-01-G56T-E430G + IgG1-hDR5-05-E430G

FIGURE 9

A


WIL2-S SF (serum-free)
IgG1-hDR5-01-G56T-K326W/E333S/E430G
% Viable cells
50
40
30
20
10
0
Anti-DR5 mAb (2.5µg/mL) + + +
C1q (0.01 µg/mL) - + +
Anti-C1q mAb (10 µg/mL) - - +

B

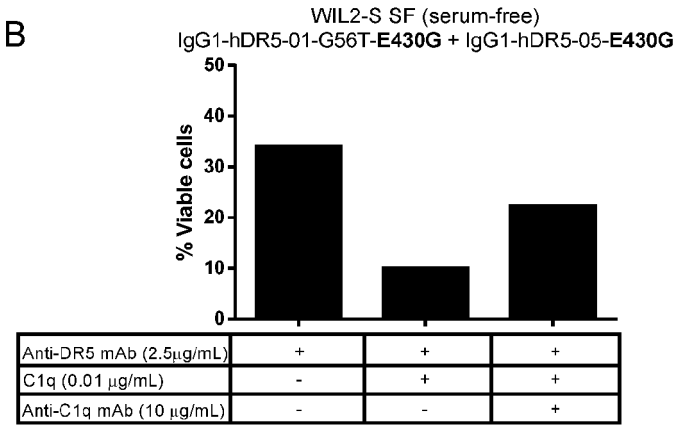

WIL2-S SF (serum-free)
IgG1-hDR5-01-G56T-E430G + IgG1-hDR5-05-E430G
% Viable cells
50
40
30
20
10
0
Anti-DR5 mAb (2.5µg/mL) + + +
C1q (0.01 µg/mL) - + +
Anti-C1q mAb (10 µg/mL) - - +

FIGURE 10

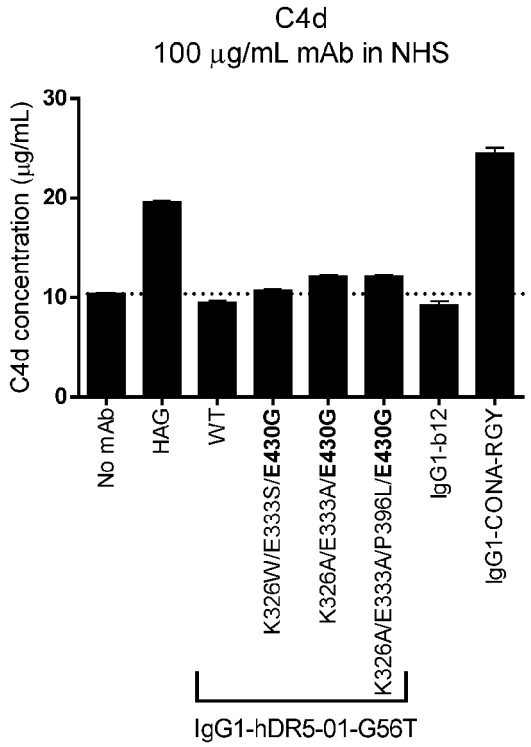

C4d
100 μg/mL mAb in NHS
C4d concentration (μg/mL)
30
20
10
0
No mAb
HAG
WT
K326W/E333S/E430G
K326A/E333A/E430G
K326A/E333A/P396L/E430G
IgG1-b12
IgG1-CONA-RGY
IgG1-hDR5-01-G56T

FIGURE 11

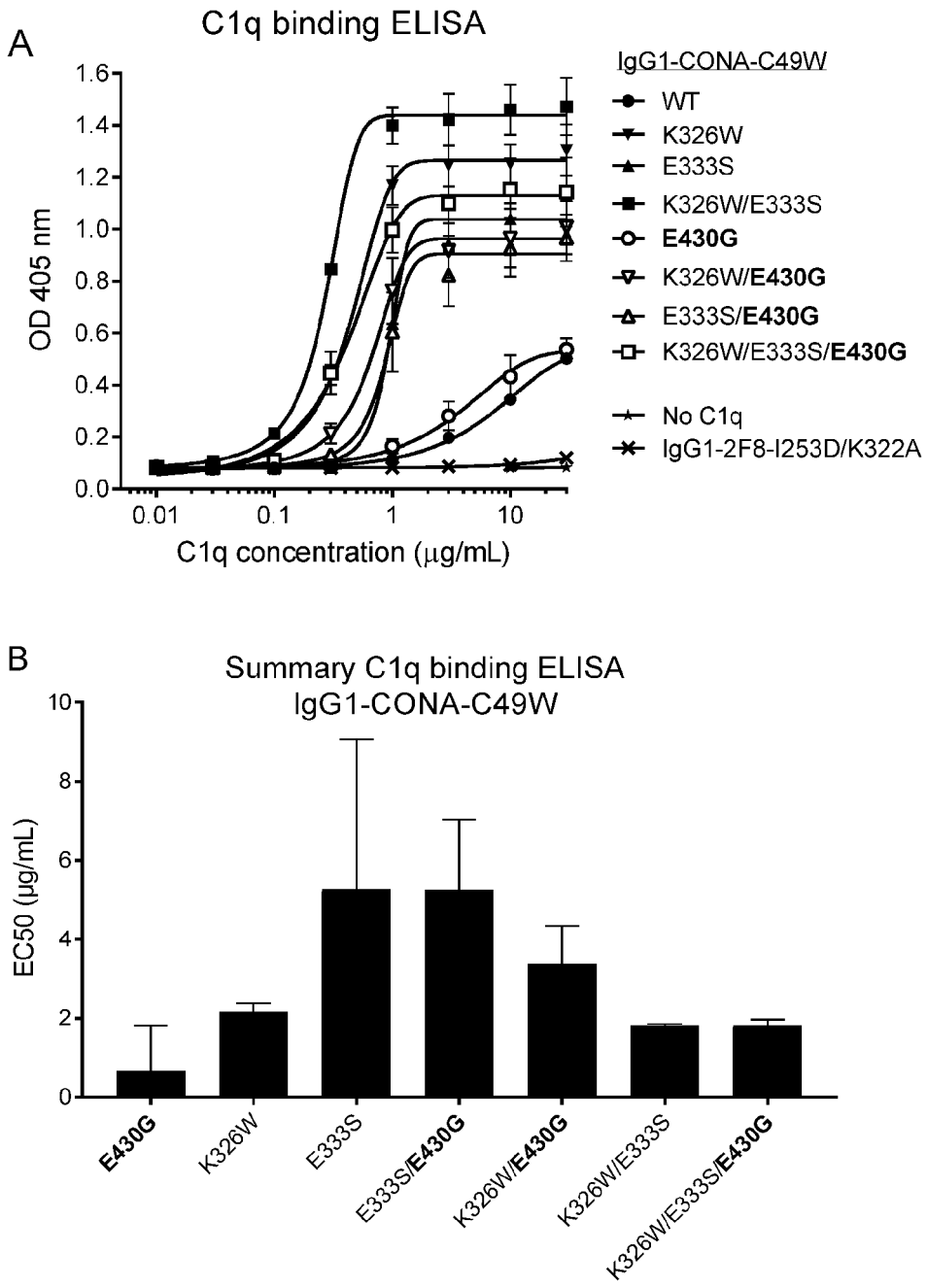

A
C1q binding ELISA
OD 405 nm
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.01
0.1
1
10
C1q concentration (µg/mL)
IgG1-CONA-C49W
WT
K326W
E333S
K326W/E333S
E430G
K326W/E430G
E333S/E430G
K326W/E333S/E430G
No C1q
IgG1-2F8-I253D/K322A
B
Summary C1q binding ELISA
IgG1-CONA-C49W
EC50 (µg/mL)
10
8
6
4
2
0
E430G
K326W
E333S
E333S/E430G
K326W/E430G
K326W/E333S
K326W/E333S/E430G

FIGURE 11 continued

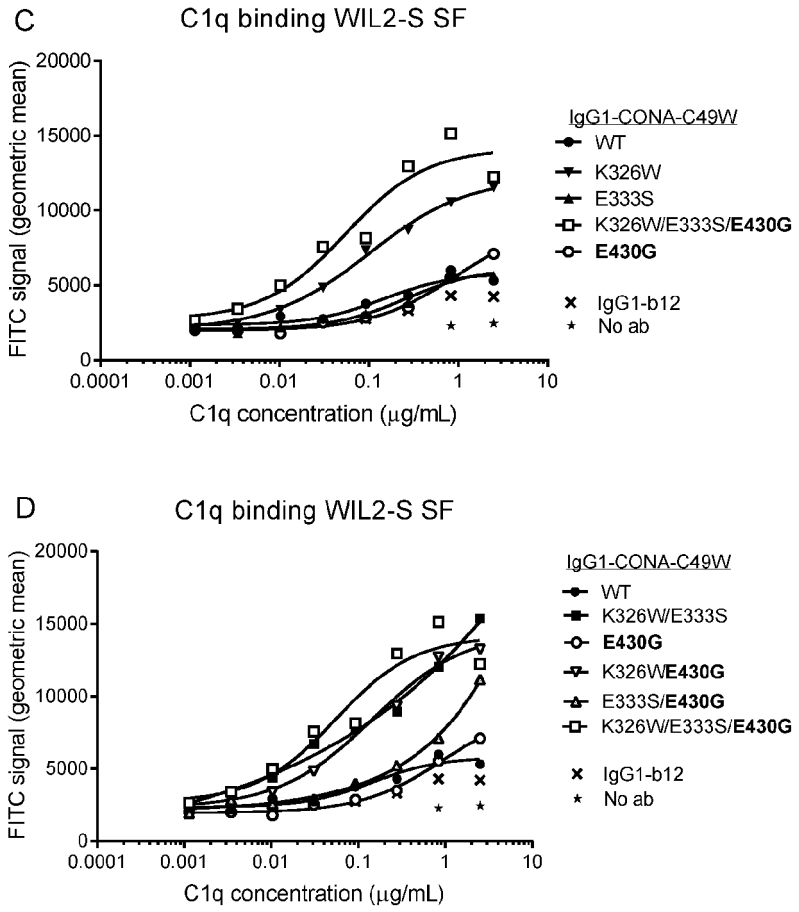
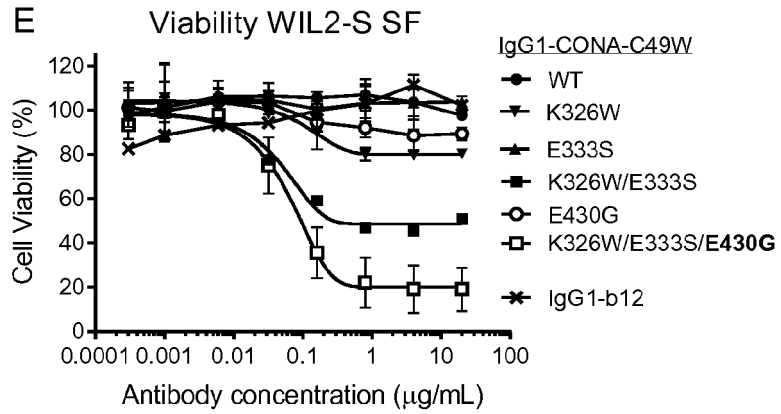
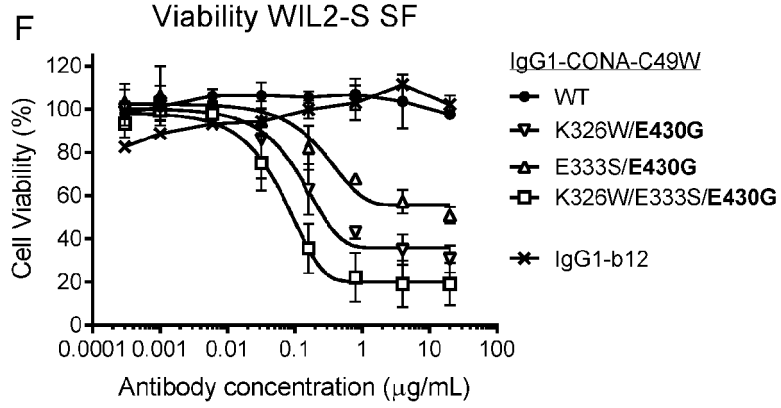


FIGURE 11 continued





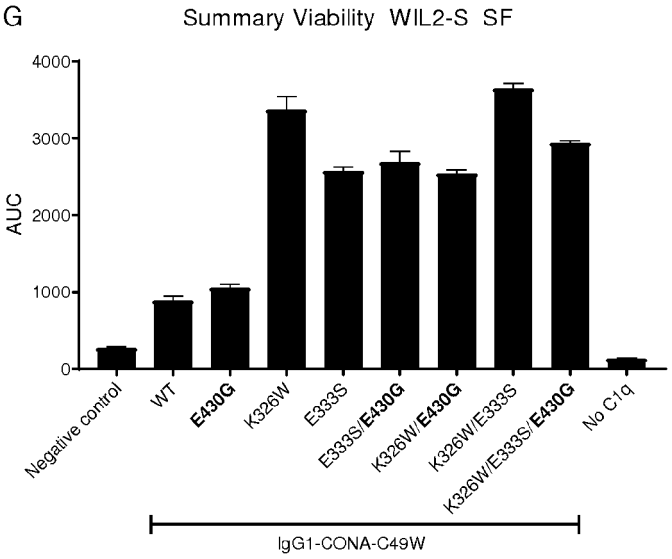
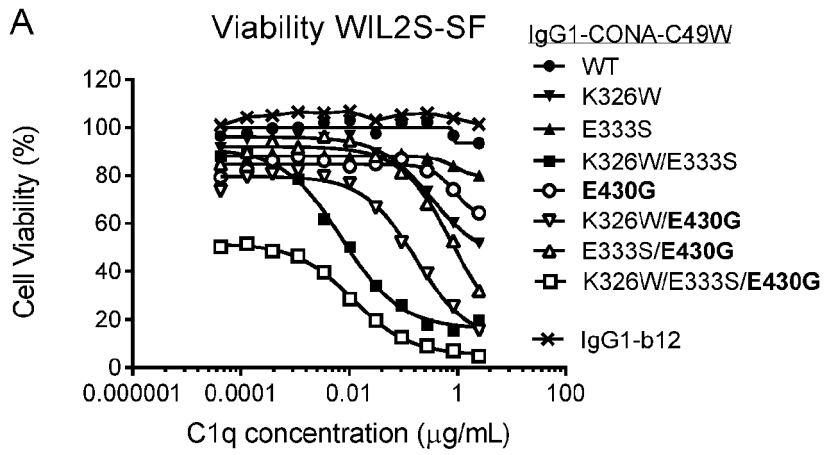


FIGURE 12


A
Viability WIL2S-SF
IgG1-CONA-C49W
WT
K326W
E333S
K326W/E333S
E430G
K326W/E430G
E333S/E430G
K326W/E333S/E430G
IgG1-b12
Cell Viability (%)
120
100
80
60
40
20
0
0.000001
0.0001
0.01
1
100
C1q concentration (µg/mL)

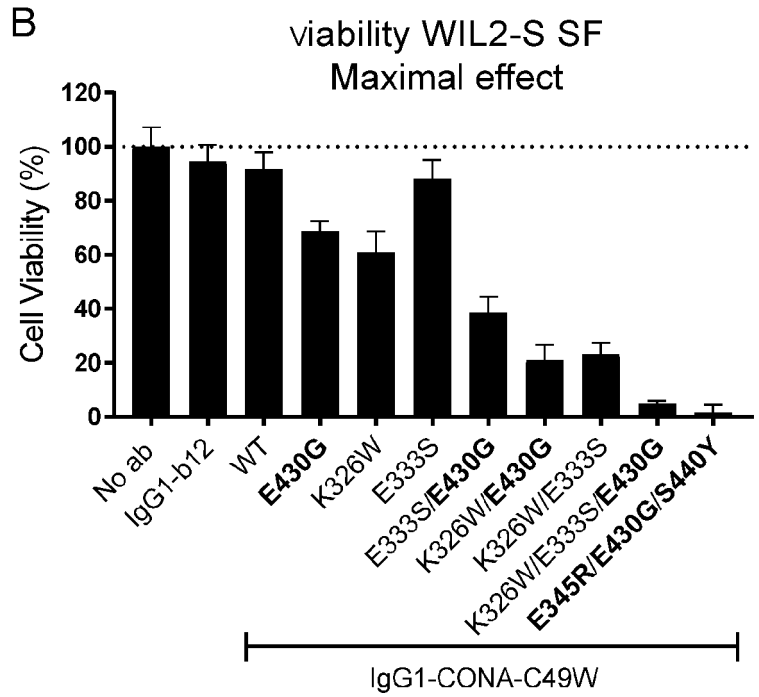

B
viability WIL2-S SF
Maximal effect
Cell Viability (%)
120
100
80
60
40
20
0
No ab
IgG1-b12
WT
E430G
K326W
E333S
E333S/E430G
K326W/E430G
K326W/E333S
K326W/E333S/E430G
E345R/E430G/S440Y
IgG1-CONA-C49W

FIGURE 12 continued

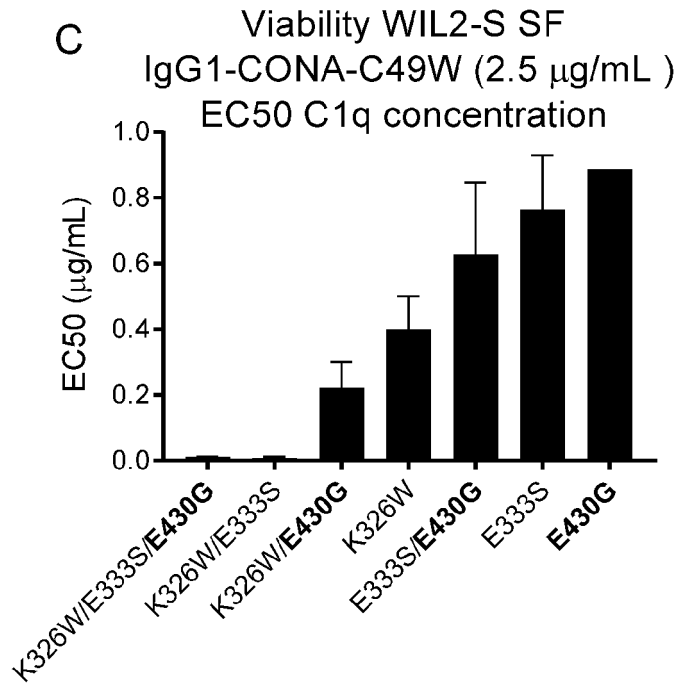


FIGURE 13

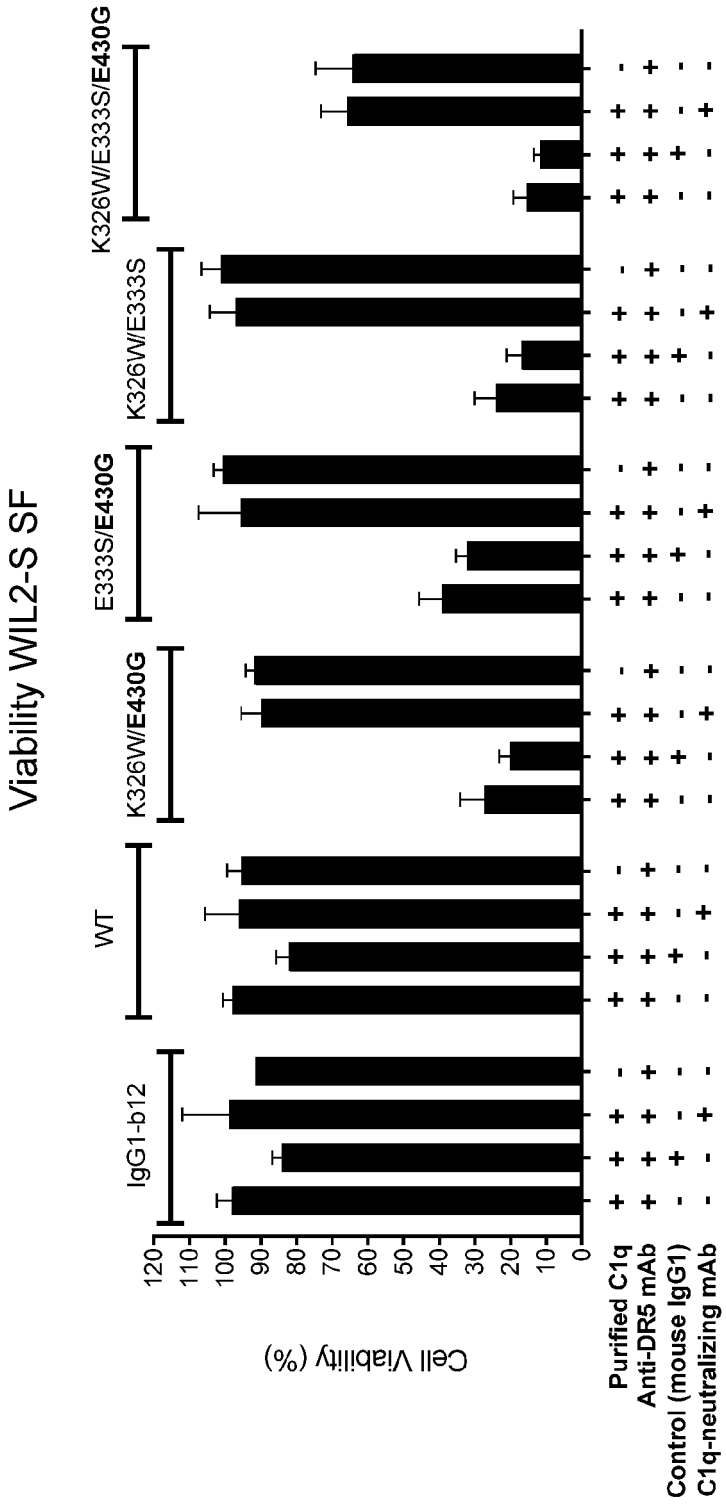

Viability WIL2-S SF
IgG1-b12
WT
K326W/E430G
E333S/E430G
K326W/E333S
K326W/E333S/E430G
Cell Viability (%)
120
110
100
90
80
70
60
50
40
30
20
10
0
Purified C1q
Anti-DR5 mAb
Control (mouse IgG1)
C1q-neutralizing mAb

FIGURE 14

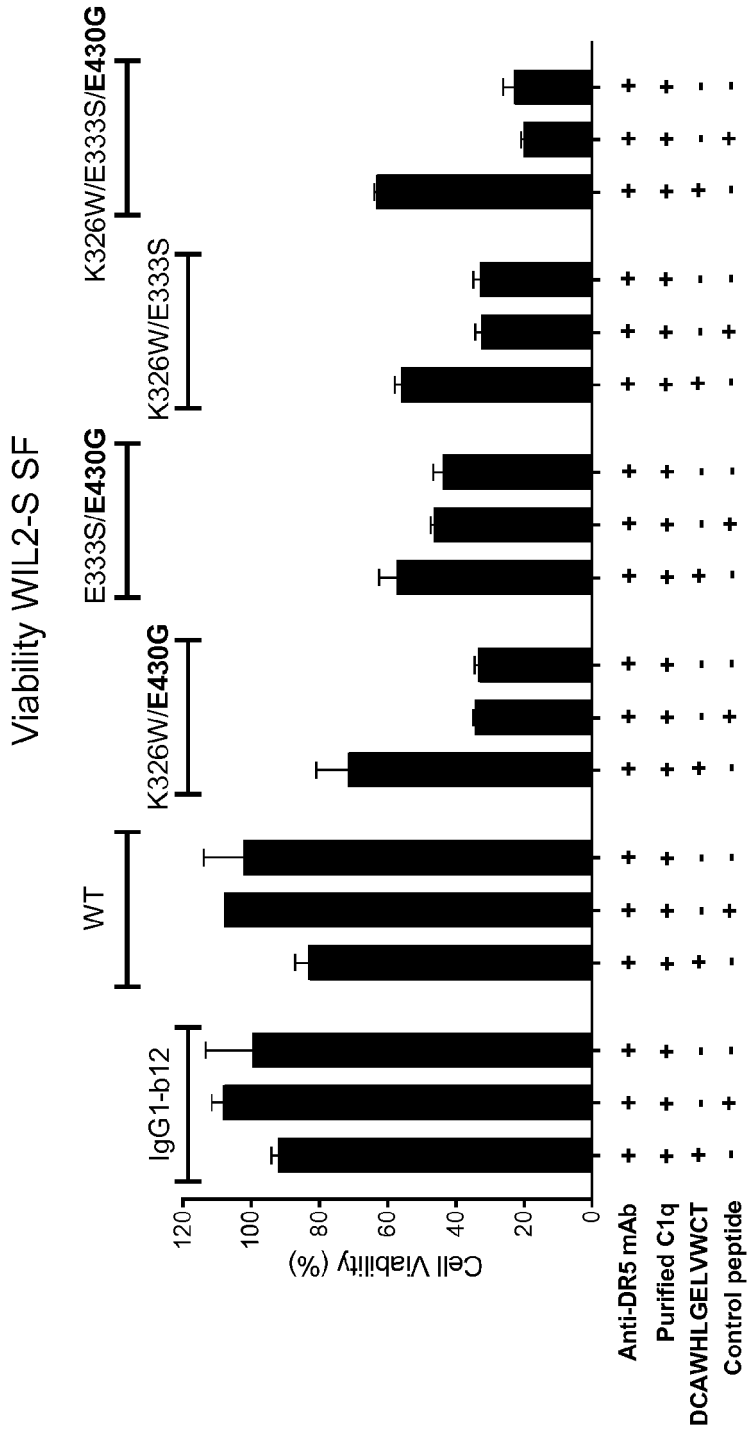

Viability WIL2-S SF
Cell Viability (%)
120
100
80
60
40
20
0
IgG1-b12
WT
K326W/E430G
E333S/E430G
K326W/E333S
K326W/E333S/E430G
Anti-DR5 mAb
Purified C1q
DCAWHLGELVWCT
Control peptide

FIGURE 15

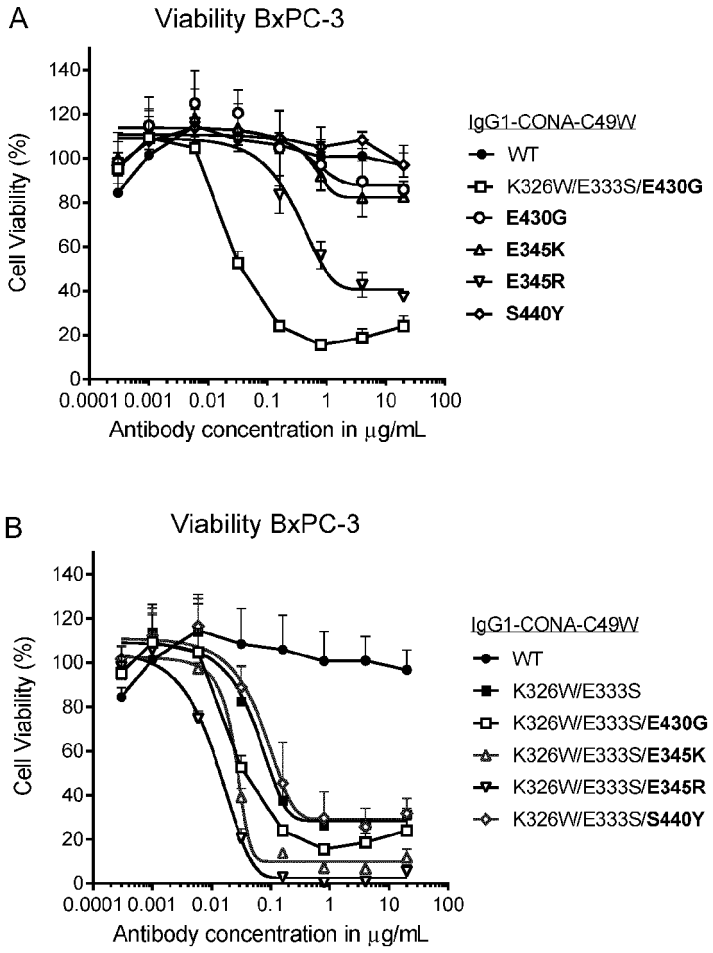

A
Viability BxPC-3
Cell Viability (%)
140
120
100
80
60
40
20
0
0.0001 0.001 0.01 0.1 1 10 100
Antibody concentration in µg/mL
IgG1-CONA-C49W
WT
K326W/E333S/E430G
E430G
E345K
E345R
S440Y
B
Viability BxPC-3
Cell Viability (%)
140
120
100
80
60
40
20
0
0.0001 0.001 0.01 0.1 1 10 100
Antibody concentration in µg/mL
IgG1-CONA-C49W
WT
K326W/E333S
K326W/E333S/E430G
K326W/E333S/E345K
K326W/E333S/E345R
K326W/E333S/S440Y

FIGURE 16

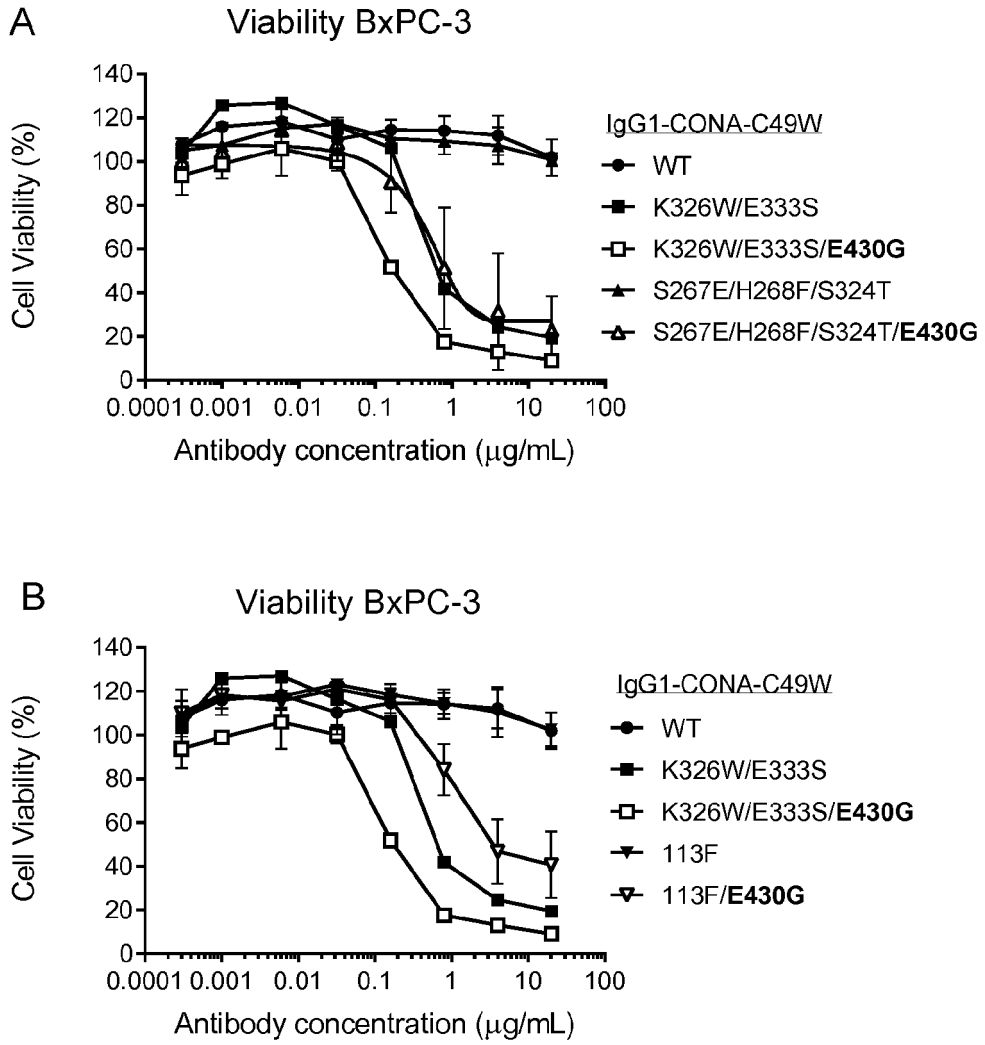

A
Viability BxPC-3
Cell Viability (%)
140
120
100
80
60
40
20
0
0.0001 0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
IgG1-CONA-C49W
WT
K326W/E333S
K326W/E333S/E430G
S267E/H268F/S324T
S267E/H268F/S324T/E430G
B
Viability BxPC-3
Cell Viability (%)
140
120
100
80
60
40
20
0
0.0001 0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
IgG1-CONA-C49W
WT
K326W/E333S
K326W/E333S/E430G
113F
113F/E430G

FIGURE 17

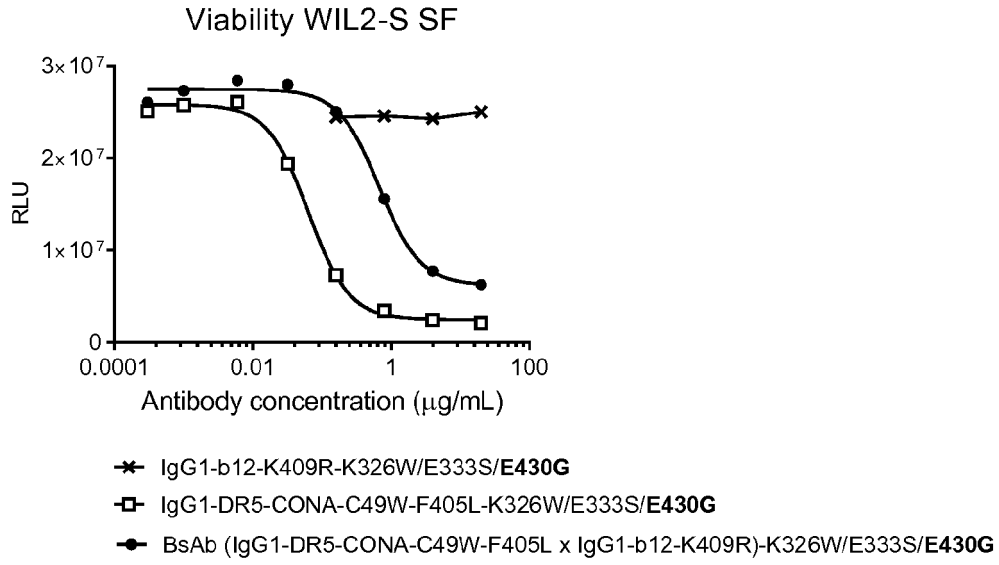

Viability WIL2-S SF
3×10^7
2×10^7
1×10^7
0
RLU
0.0001
0.01
1
100
Antibody concentration (μg/mL)
IgG1-b12-K409R-K326W/E333S/E430G
IgG1-DR5-CONA-C49W-F405L-K326W/E333S/E430G
BsAb (IgG1-DR5-CONA-C49W-F405L x IgG1-b12-K409R)-K326W/E333S/E430G

FIGURE 18

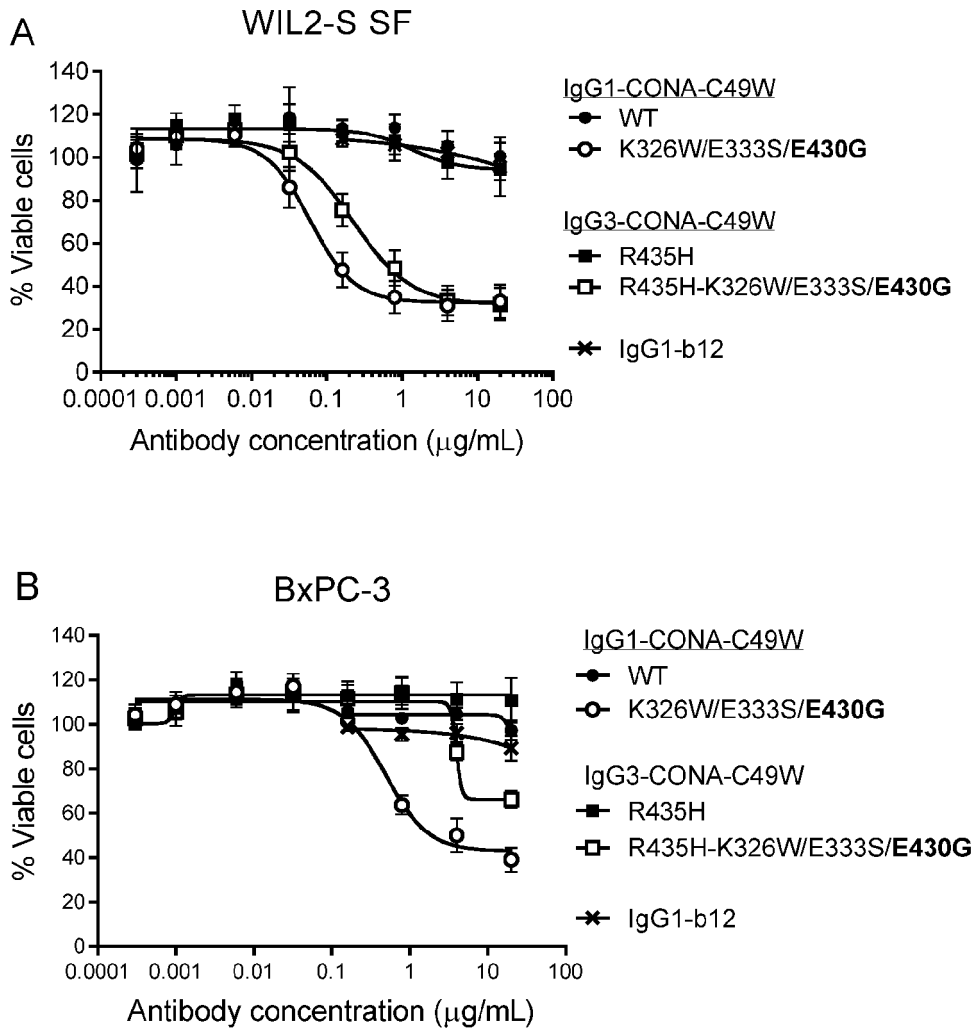

A
WIL2-S SF
% Viable cells
140
120
100
80
60
40
20
0
0.0001 0.001 0.01 0.1 1 10 100
Antibody concentration (μg/mL)
IgG1-CONA-C49W
WT
K326W/E333S/E430G
IgG3-CONA-C49W
R435H
R435H-K326W/E333S/E430G
IgG1-b12
B
BxPC-3
% Viable cells
140
120
100
80
60
40
20
0
0.0001 0.001 0.01 0.1 1 10 100
Antibody concentration (μg/mL)
IgG1-CONA-C49W
WT
K326W/E333S/E430G
IgG3-CONA-C49W
R435H
R435H-K326W/E333S/E430G
IgG1-b12

FIGURE 18 continued

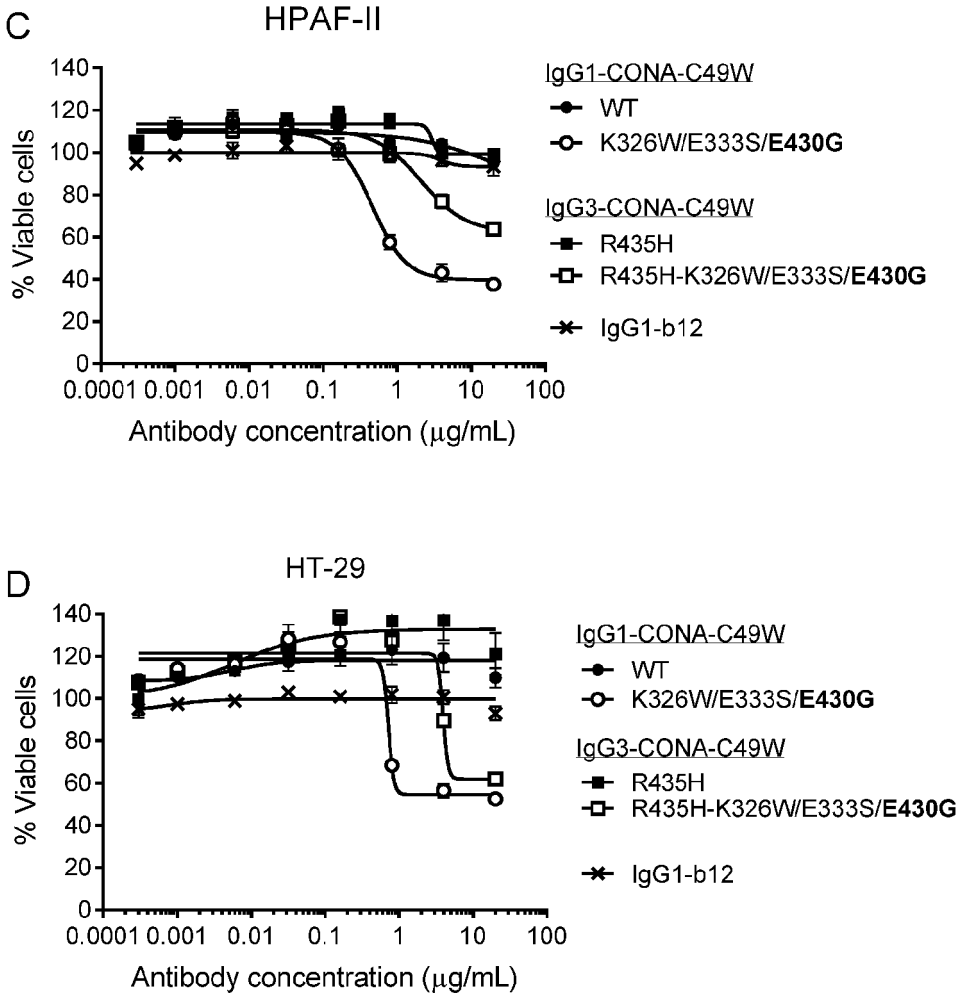


FIGURE 19

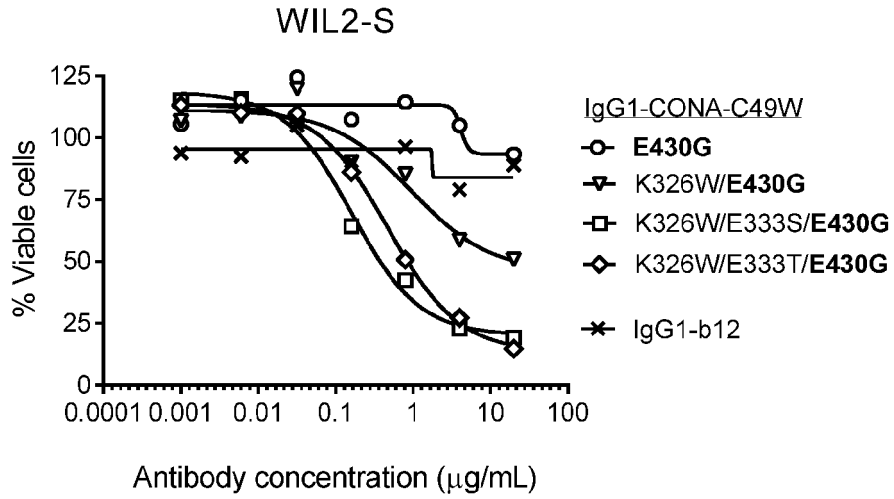

WIL2-S
% Viable cells
125
100
75
50
25
0
0.0001 0.001 0.01 0.1 1 10 100
Antibody concentration (μg/mL)
IgG1-CONA-C49W
E430G
K326W/E430G
K326W/E333S/E430G
K326W/E333T/E430G
IgG1-b12

FIGURE 20

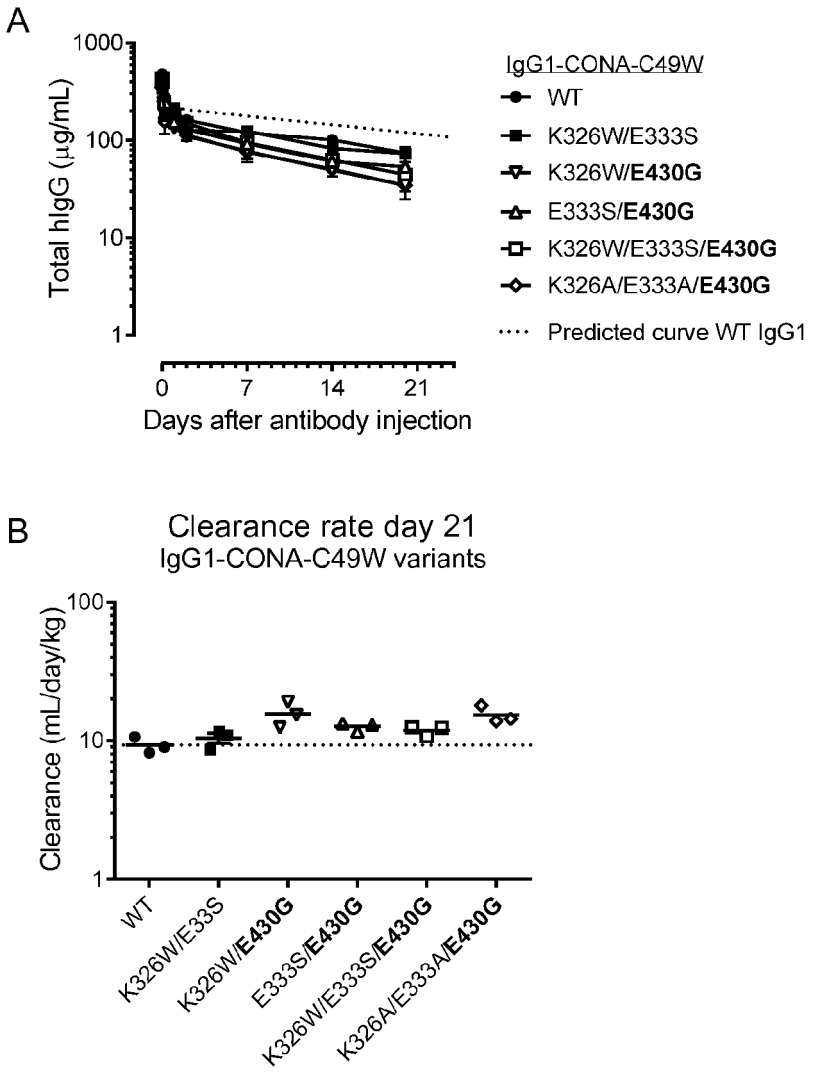

A
1000
100
10
1
Total hIgG (µg/mL)
0
7
14
21
Days after antibody injection
IgG1-CONA-C49W
WT
K326W/E333S
K326W/E430G
E333S/E430G
K326W/E333S/E430G
K326A/E333A/E430G
Predicted curve WT IgG1
B
Clearance rate day 21
IgG1-CONA-C49W variants
100
10
1
Clearance (mL/day/kg)
WT
K326W/E33S
K326W/E430G
E333S/E430G
K326W/E333S/E430G
K326A/E333A/E430G

FIGURE 21

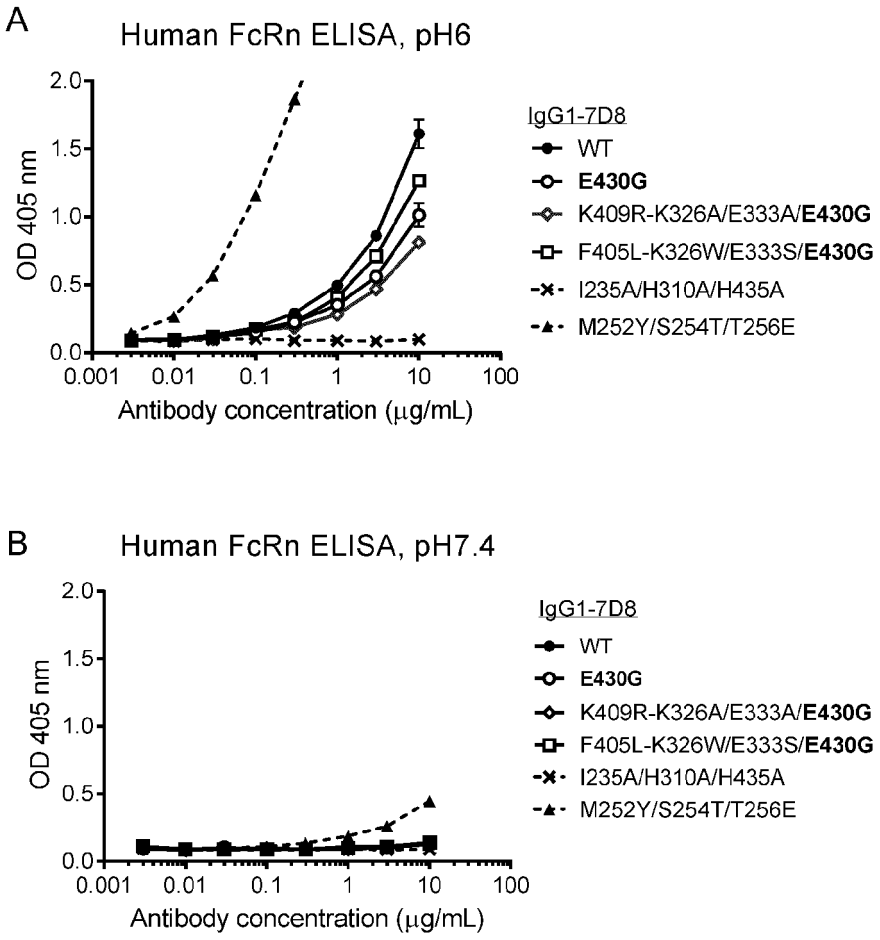

A
Human FcRn ELISA, pH6
OD 405 nm
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
100
Antibody concentration (μg/mL)
IgG1-7D8
WT
E430G
K409R-K326A/E333A/E430G
F405L-K326W/E333S/E430G
I235A/H310A/H435A
M252Y/S254T/T256E
B
Human FcRn ELISA, pH7.4
OD 405 nm
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
100
Antibody concentration (μg/mL)
IgG1-7D8
WT
E430G
K409R-K326A/E333A/E430G
F405L-K326W/E333S/E430G
I235A/H310A/H435A
M252Y/S254T/T256E

FIGURE 22

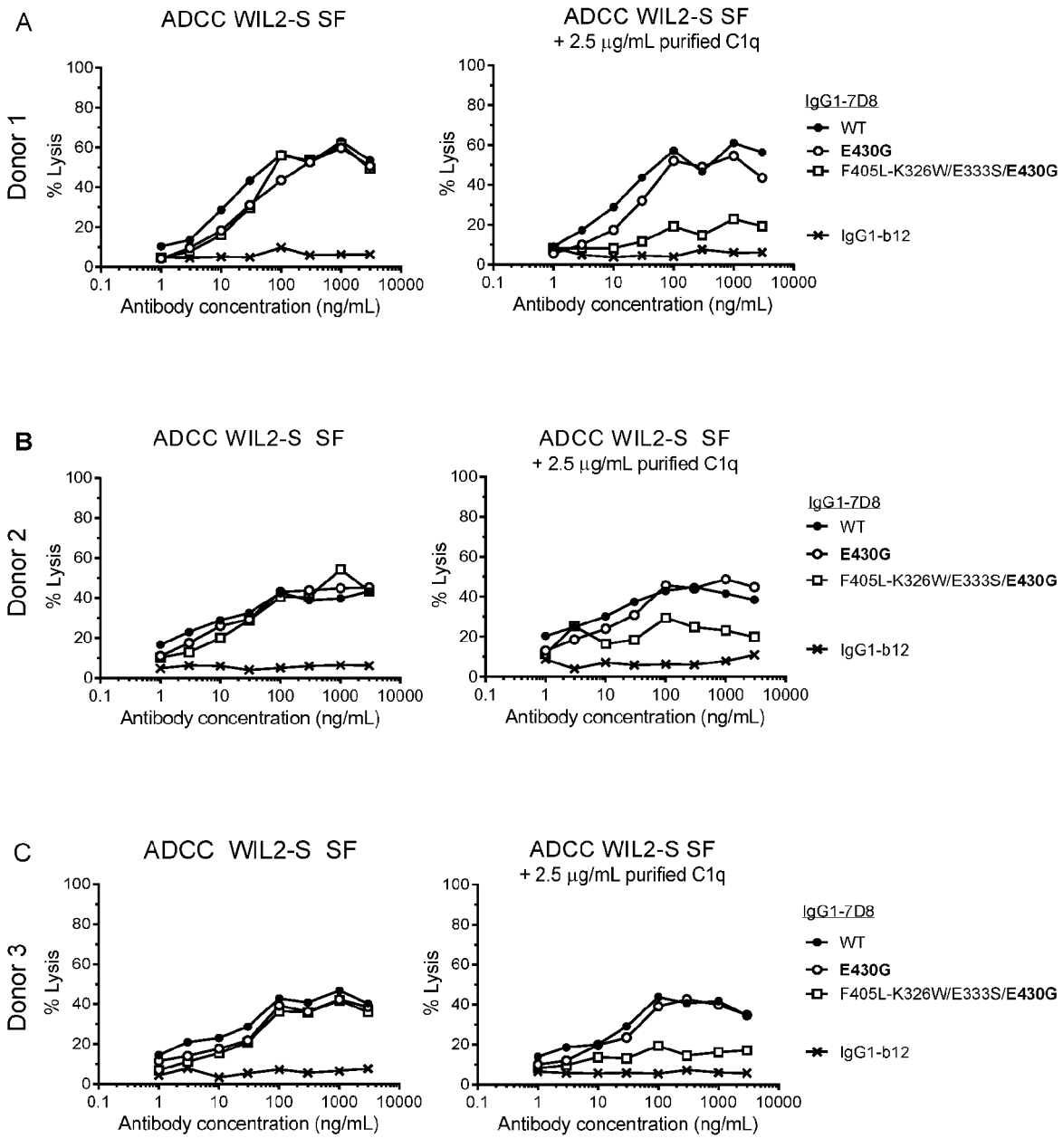

A
ADCC WIL2-S SF
ADCC WIL2-S SF
+ 2.5 µg/mL purified C1q
Donor 1
% Lysis
Antibody concentration (ng/mL)
IgG1-7D8
WT
E430G
F405L-K326W/E333S/E430G
IgG1-b12
B
ADCC WIL2-S SF
ADCC WIL2-S SF
+ 2.5 µg/mL purified C1q
Donor 2
C
ADCC WIL2-S SF
ADCC WIL2-S SF
+ 2.5 µg/mL purified C1q
Donor 3

FIGURE 23

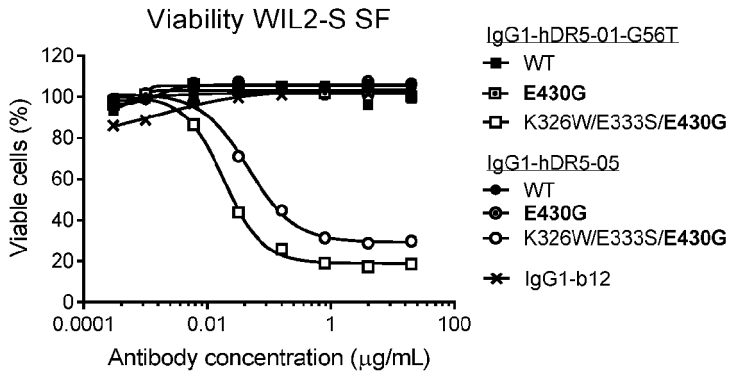

Viability WIL2-S SF
Viable cells (%)
120
100
80
60
40
20
0
0.0001
0.01
1
100
Antibody concentration (μg/mL)
IgG1-hDR5-01-G56T
WT
E430G
K326W/E333S/E430G
IgG1-hDR5-05
WT
E430G
K326W/E333S/E430G
IgG1-b12

FIGURE 24

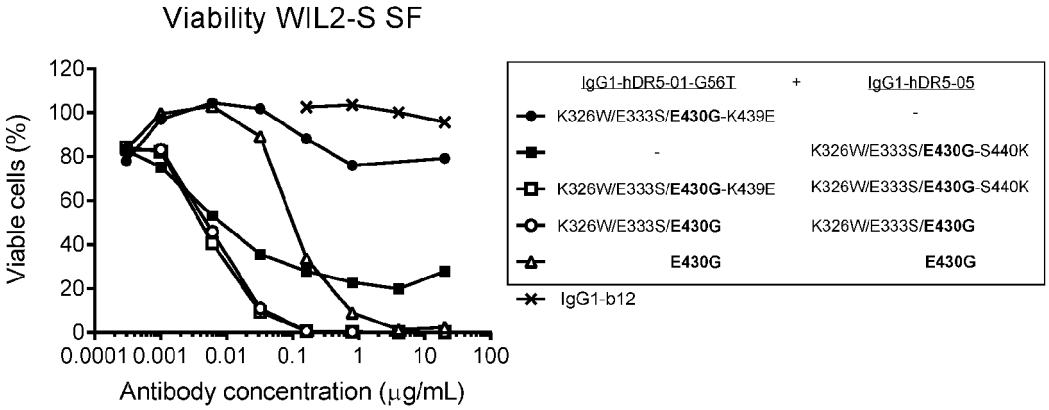

Viability WIL2-S SF
Viable cells (%)
120
100
80
60
40
20
0
0.0001 0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
IgG1-hDR5-01-G56T + IgG1-hDR5-05
K326W/E333S/E430G-K439E -
- K326W/E333S/E430G-S440K
K326W/E333S/E430G-K439E K326W/E333S/E430G-S440K
K326W/E333S/E430G K326W/E333S/E430G
E430G E430G
IgG1-b12

FIGURE 25

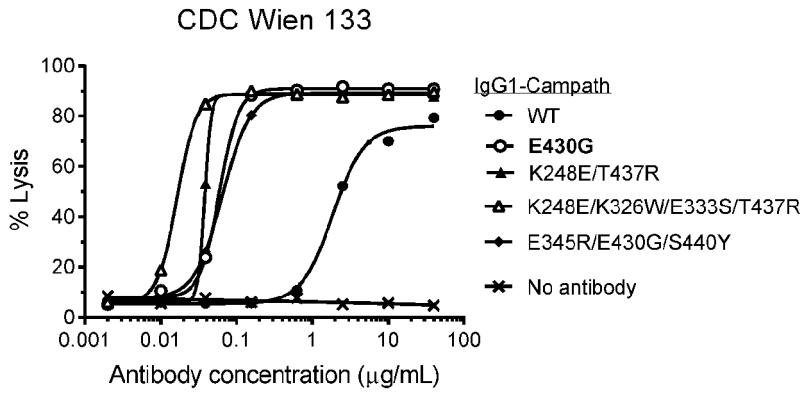

CDC Wien 133
% Lysis
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Antibody concentration (µg/mL)
IgG1-Campath
WT
E430G
K248E/T437R
K248E/K326W/E333S/T437R
E345R/E430G/S440Y
No antibody

FIGURE 26

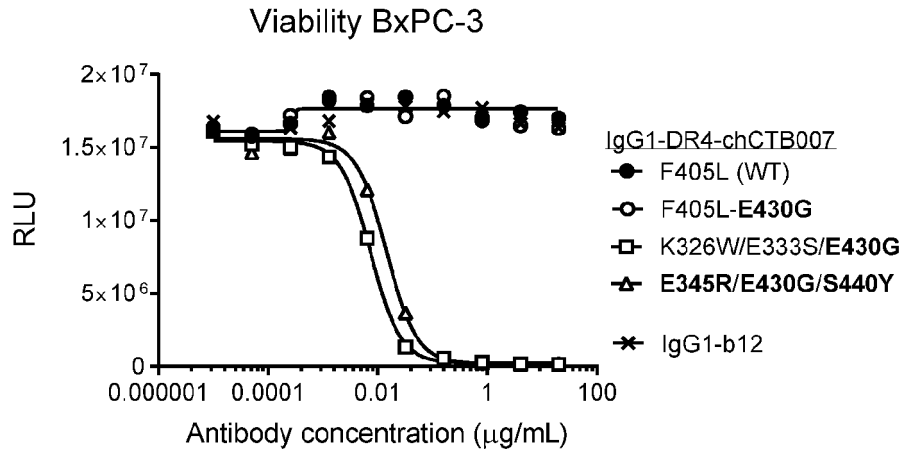

Viability BxPC-3
RLU
2×10^7
1.5×10^7
1×10^7
5×10^6
0
0.000001
0.0001
0.01
1
100
Antibody concentration (μg/mL)
IgG1-DR4-chCTB007
F405L (WT)
F405L-E430G
K326W/E333S/E430G
E345R/E430G/S440Y
IgG1-b12

Figure 27

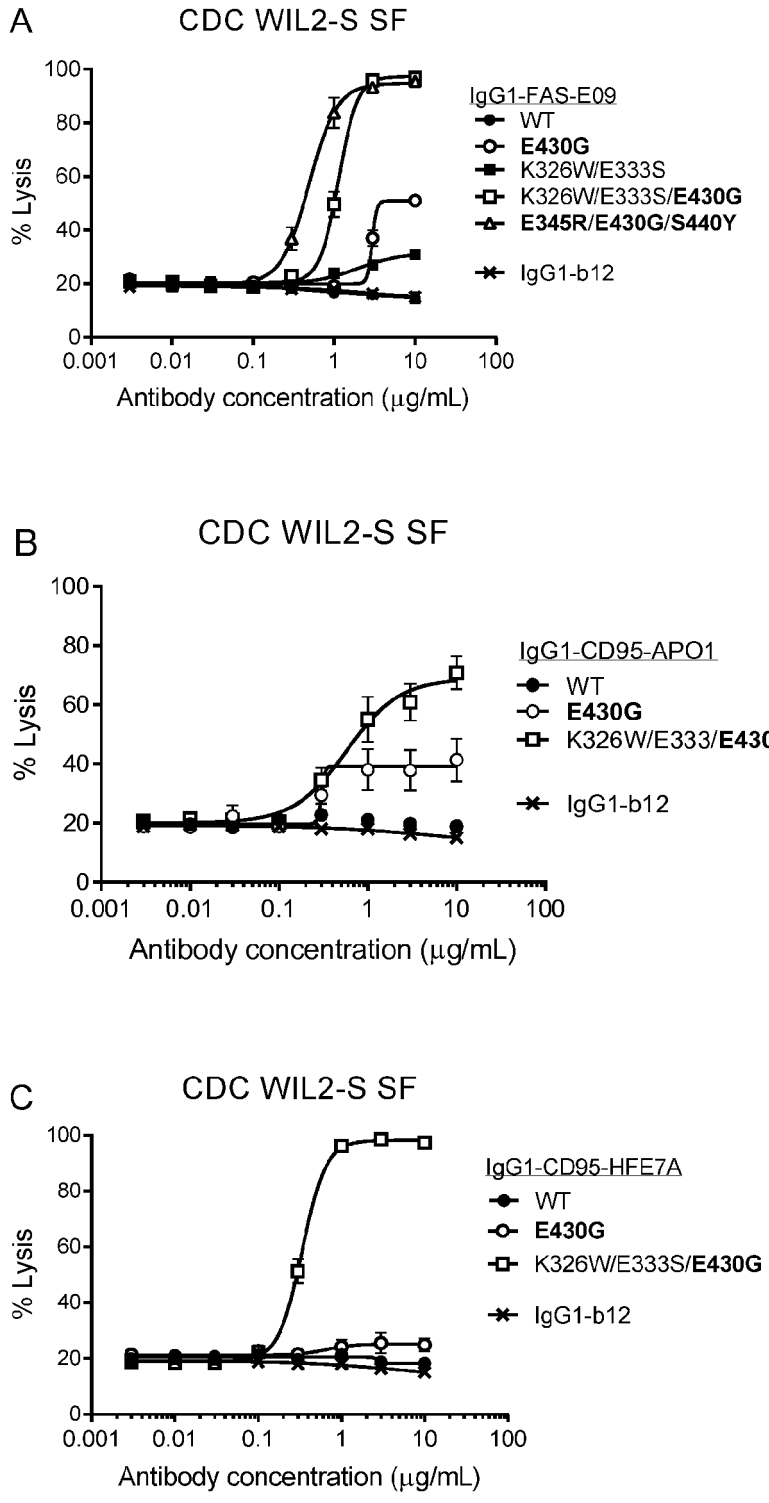

A
CDC WIL2-S SF
% Lysis
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Antibody concentration (µg/mL)
IgG1-FAS-E09
WT
E430G
K326W/E333S
K326W/E333S/E430G
E345R/E430G/S440Y
IgG1-b12
B
CDC WIL2-S SF
% Lysis
Antibody concentration (µg/mL)
IgG1-CD95-APO1
WT
E430G
K326W/E333/E430G
IgG1-b12
C
CDC WIL2-S SF
% Lysis
Antibody concentration (µg/mL)
IgG1-CD95-HFE7A
WT
E430G
K326W/E333S/E430G
IgG1-b12

FIGURE 28

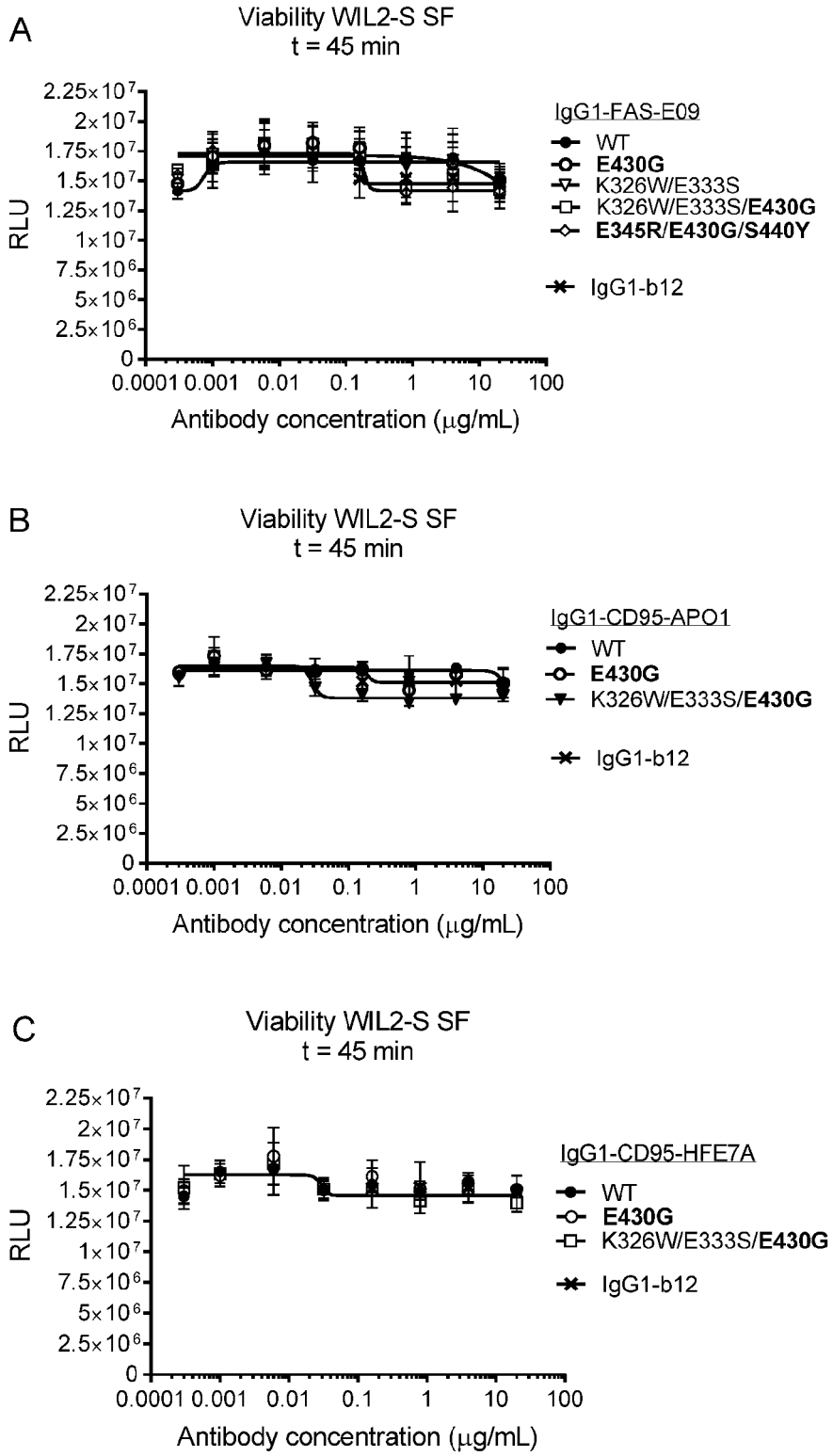

A
Viability WIL2-S SF
t = 45 min
RLU
2.25×10^7
2×10^7
1.75×10^7
1.5×10^7
1.25×10^7
1×10^7
7.5×10^6
5×10^6
2.5×10^6
0
0.0001 0.001 0.01 0.1 1 10 100
Antibody concentration (μg/mL)
IgG1-FAS-E09
WT
E430G
K326W/E333S
K326W/E333S/E430G
E345R/E430G/S440Y
IgG1-b12
B
Viability WIL2-S SF
t = 45 min
RLU
Antibody concentration (μg/mL)
IgG1-CD95-APO1
WT
E430G
K326W/E333S/E430G
IgG1-b12
C
Viability WIL2-S SF
t = 45 min
RLU
Antibody concentration (μg/mL)
IgG1-CD95-HFE7A
WT
E430G
K326W/E333S/E430G
IgG1-b12

FIGURE 29

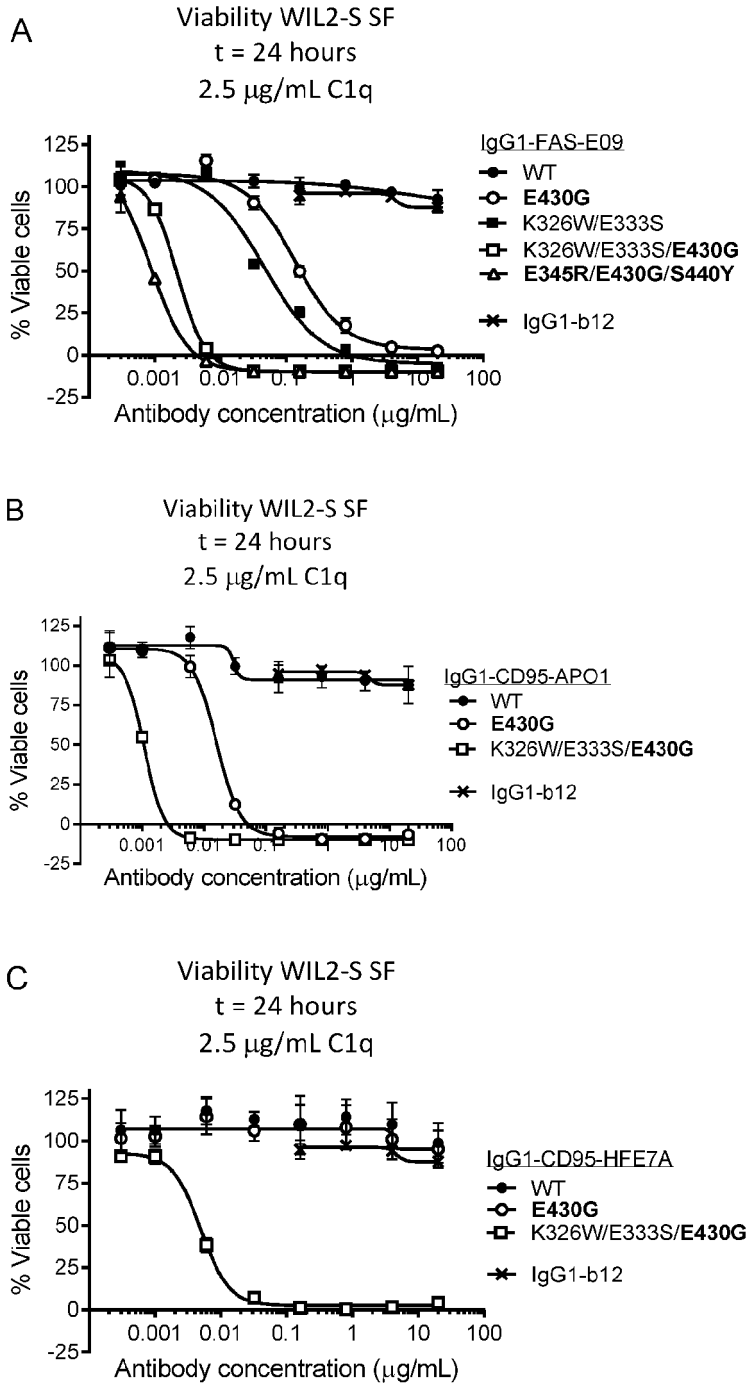

A
Viability WIL2-S SF
t = 24 hours
2.5 µg/mL C1q
% Viable cells
125
100
75
50
25
0
-25
0.001
0.01
0.1
1
10
100
Antibody concentration (µg/mL)
IgG1-FAS-E09
WT
E430G
K326W/E333S
K326W/E333S/E430G
E345R/E430G/S440Y
IgG1-b12
B
Viability WIL2-S SF
t = 24 hours
2.5 µg/mL C1q
% Viable cells
Antibody concentration (µg/mL)
IgG1-CD95-APO1
WT
E430G
K326W/E333S/E430G
IgG1-b12
C
Viability WIL2-S SF
t = 24 hours
2.5 µg/mL C1q
% Viable cells
Antibody concentration (µg/mL)
IgG1-CD95-HFE7A
WT
E430G
K326W/E333S/E430G
IgG1-b12

FIGURE 30

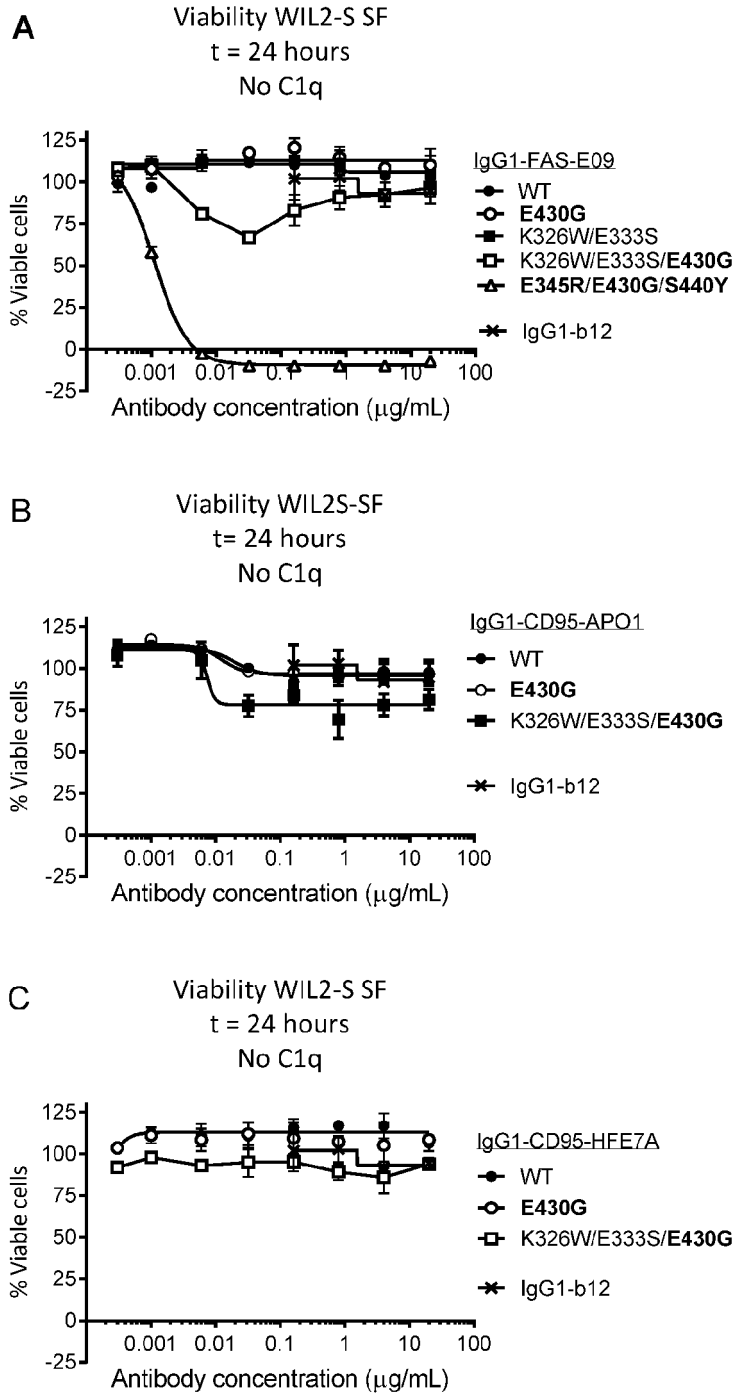

A
Viability WIL2-S SF
t = 24 hours
No C1q
% Viable cells
125
100
75
50
25
0
-25
0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
IgG1-FAS-E09
WT
E430G
K326W/E333S
K326W/E333S/E430G
E345R/E430G/S440Y
IgG1-b12
B
Viability WIL2S-SF
t= 24 hours
No C1q
% Viable cells
125
100
75
50
25
0
-25
0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
IgG1-CD95-APO1
WT
E430G
K326W/E333S/E430G
IgG1-b12
C
Viability WIL2-S SF
t = 24 hours
No C1q
% Viable cells
125
100
75
50
25
0
-25
0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
IgG1-CD95-HFE7A
WT
E430G
K326W/E333S/E430G
IgG1-b12

FIGURE 31

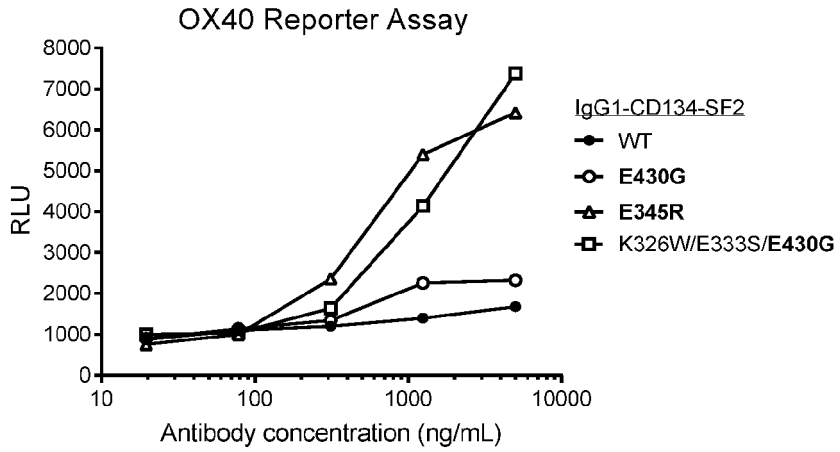

OX40 Reporter Assay
RLU
8000
7000
6000
5000
4000
3000
2000
1000
0
10
100
1000
10000
Antibody concentration (ng/mL)
IgG1-CD134-SF2
WT
E430G
E345R
K326W/E333S/E430G

FIGURE 32

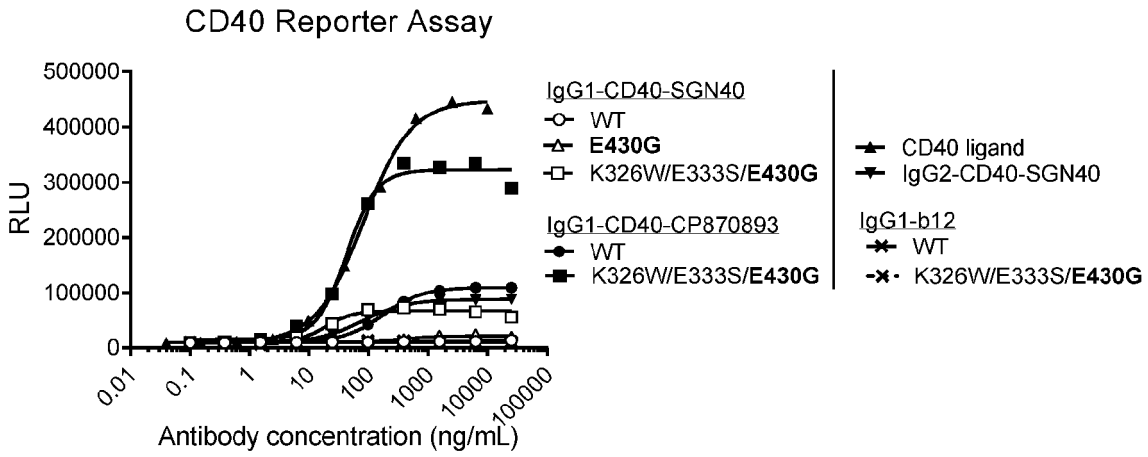

CD40 Reporter Assay
RLU
500000
400000
300000
200000
100000
0
0.01
0.1
1
10
100
1000
10000
100000
Antibody concentration (ng/mL)
IgG1-CD40-SGN40
WT
E430G
K326W/E333S/E430G
IgG1-CD40-CP870893
WT
K326W/E333S/E430G
CD40 ligand
IgG2-CD40-SGN40
IgG1-b12
WT
K326W/E333S/E430G

FIGURE 33

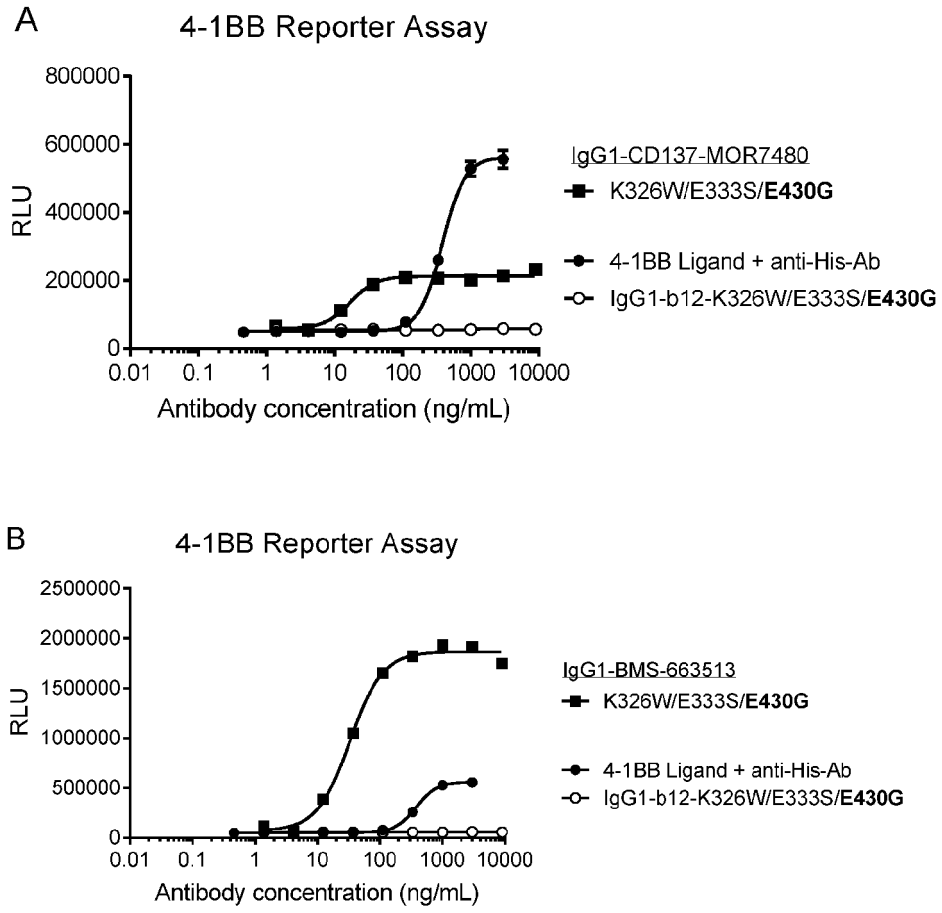

A
4-1BB Reporter Assay
800000
600000
400000
200000
0
RLU
0.01 0.1 1 10 100 1000 10000
Antibody concentration (ng/mL)
IgG1-CD137-MOR7480
K326W/E333S/E430G
4-1BB Ligand + anti-His-Ab
IgG1-b12-K326W/E333S/E430G
B
4-1BB Reporter Assay
2500000
2000000
1500000
1000000
500000
0
RLU
0.01 0.1 1 10 100 1000 10000
Antibody concentration (ng/mL)
IgG1-BMS-663513
K326W/E333S/E430G
4-1BB Ligand + anti-His-Ab
IgG1-b12-K326W/E333S/E430G

FIGURE 34

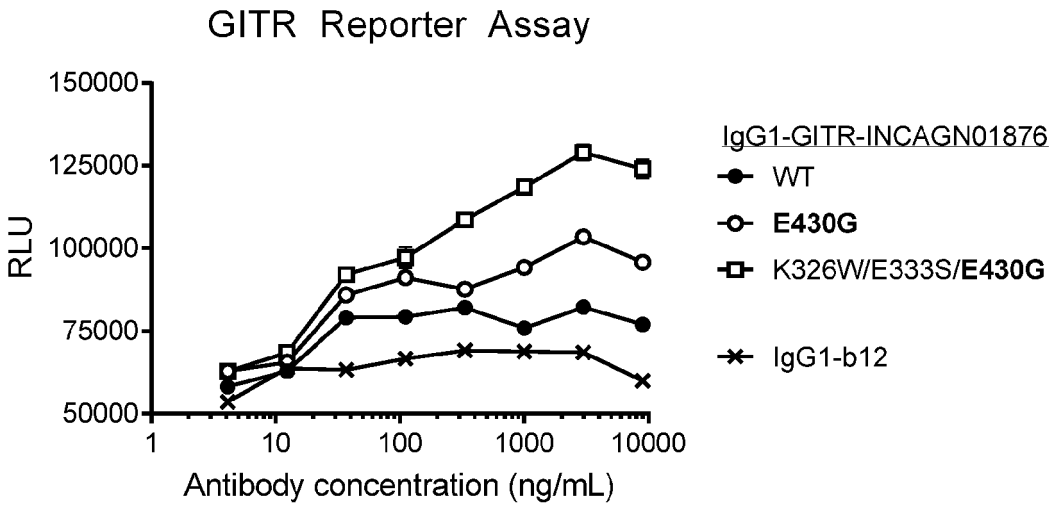
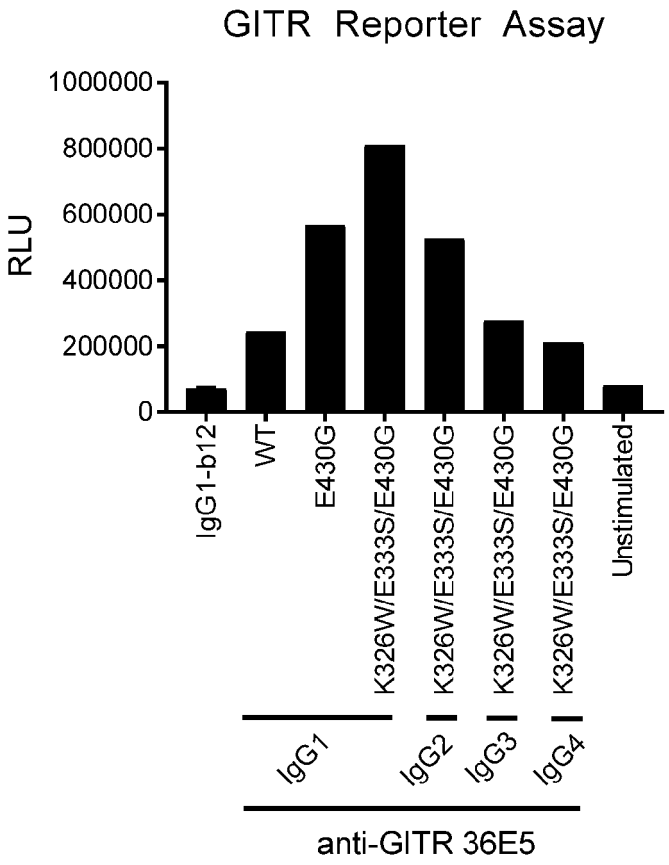

GITR Reporter Assay
150000
125000
100000
75000
50000
RLU
1
10
100
1000
10000
Antibody concentration (ng/mL)
IgG1-GITR-INCAGN01876
WT
E430G
K326W/E333S/E430G
IgG1-b12

FIGURE 35


GITR Reporter Assay
RLU
1000000
800000
600000
400000
200000
0
IgG1-b12
WT
E430G
K326W/E333S/E430G
K326W/E333S/E430G
K326W/E333S/E430G
K326W/E333S/E430G
Unstimulated
IgG1
IgG2
IgG3
IgG4
anti-GITR 36E5