Figure 1

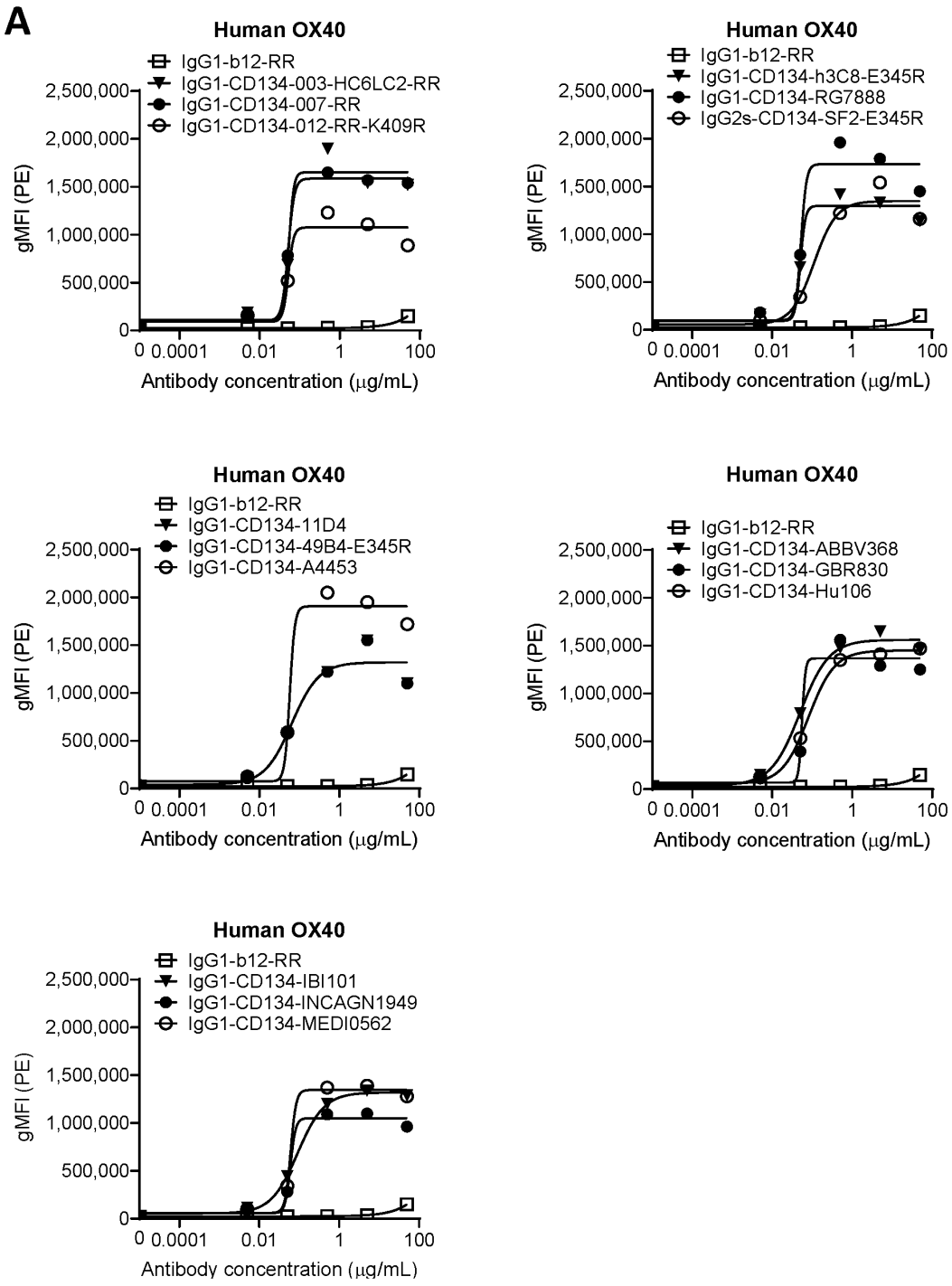

A
Human OX40
IgG1-b12-RR
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-007-RR
IgG1-CD134-012-RR-K409R
gMFI (PE)
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
0 0.0001 0.01 1 100
Antibody concentration (μg/mL)
Human OX40
IgG1-b12-RR
IgG1-CD134-h3C8-E345R
IgG1-CD134-RG7888
IgG2s-CD134-SF2-E345R
gMFI (PE)
Antibody concentration (μg/mL)
Human OX40
IgG1-b12-RR
IgG1-CD134-11D4
IgG1-CD134-49B4-E345R
IgG1-CD134-A4453
gMFI (PE)
Antibody concentration (μg/mL)
Human OX40
IgG1-b12-RR
IgG1-CD134-ABBV368
IgG1-CD134-GBR830
IgG1-CD134-Hu106
gMFI (PE)
Antibody concentration (μg/mL)
Human OX40
IgG1-b12-RR
IgG1-CD134-IBI101
IgG1-CD134-INCAGN1949
IgG1-CD134-MEDI0562
gMFI (PE)
Antibody concentration (μg/mL)

Figure 1 – continued

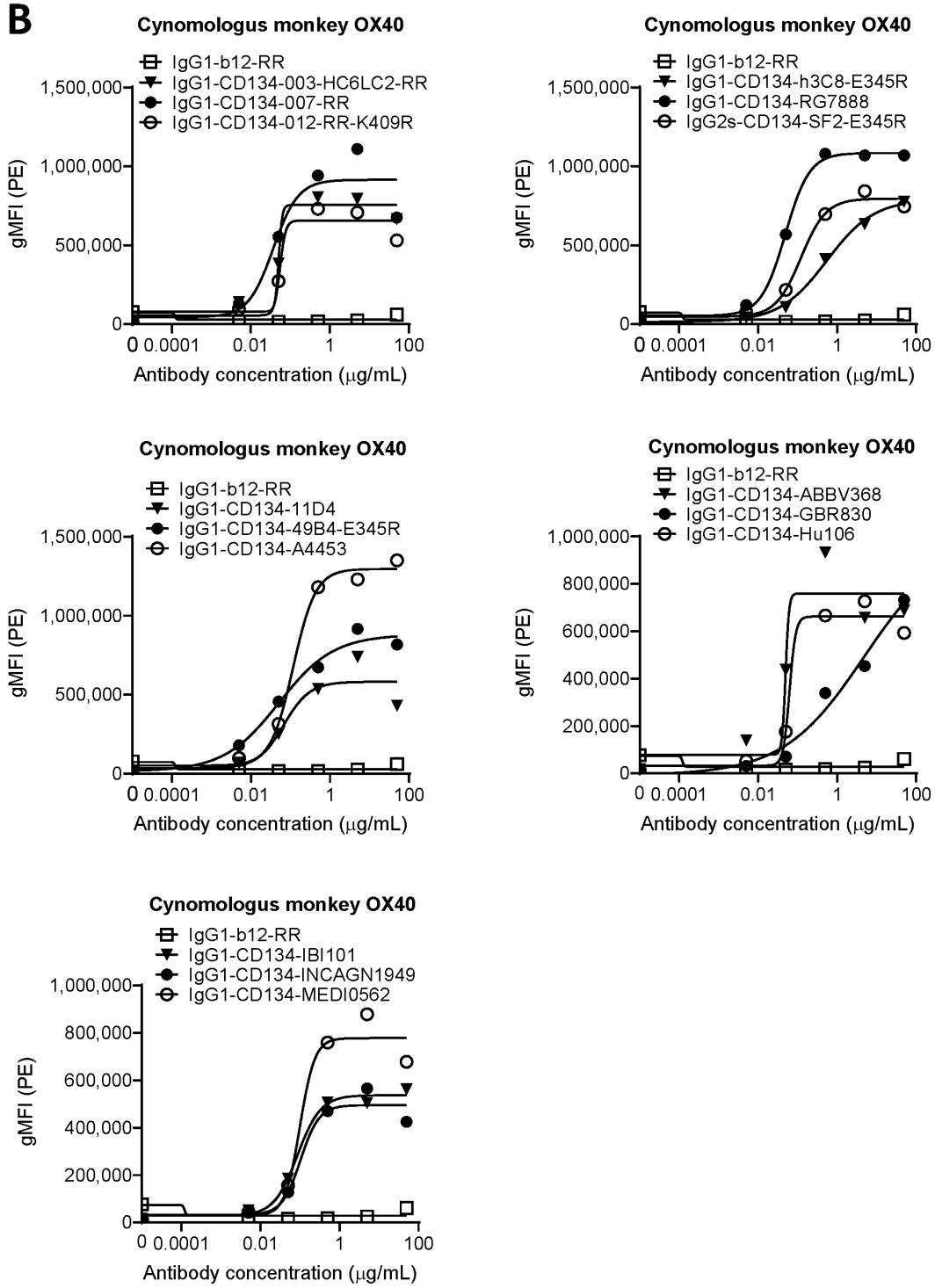


Figure 2

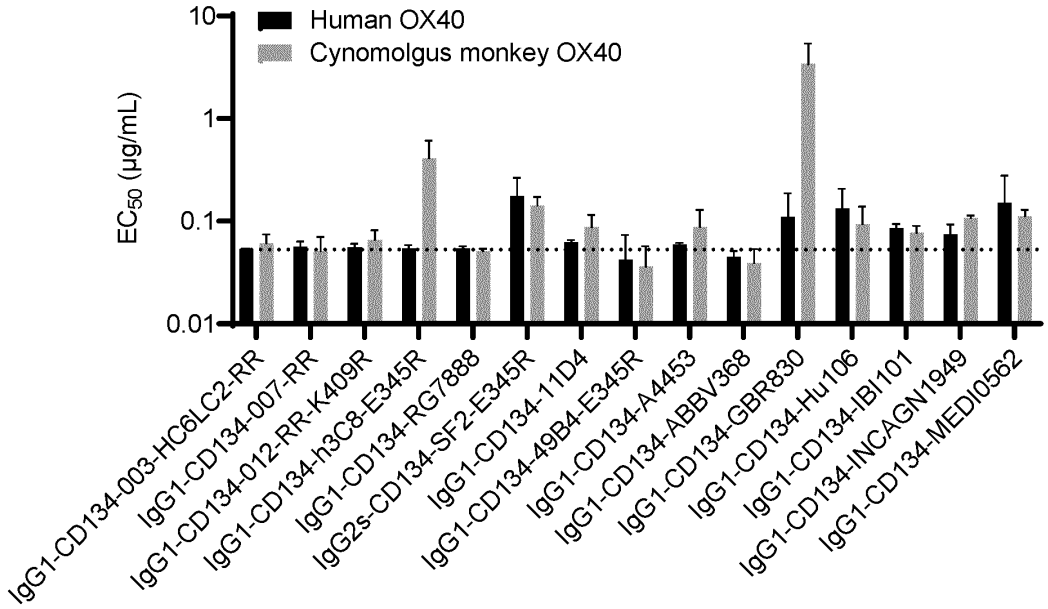

Human OX40
Cynomolgus monkey OX40
EC50 (µg/mL)
10
1
0.1
0.01
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-007-RR
IgG1-CD134-012-RR-K409R
IgG1-CD134-h3C8-E345R
IgG1-CD134-RG7888
IgG2s-CD134-SF2-E345R
IgG1-CD134-11D4
IgG1-CD134-49B4-E345R
IgG1-CD134-A4453
IgG1-CD134-ABBV368
IgG1-CD134-GBR830
IgG1-CD134-Hu106
IgG1-CD134-IBI101
IgG1-CD134-INCAGN1949
IgG1-CD134-MEDI0562

Figure 3

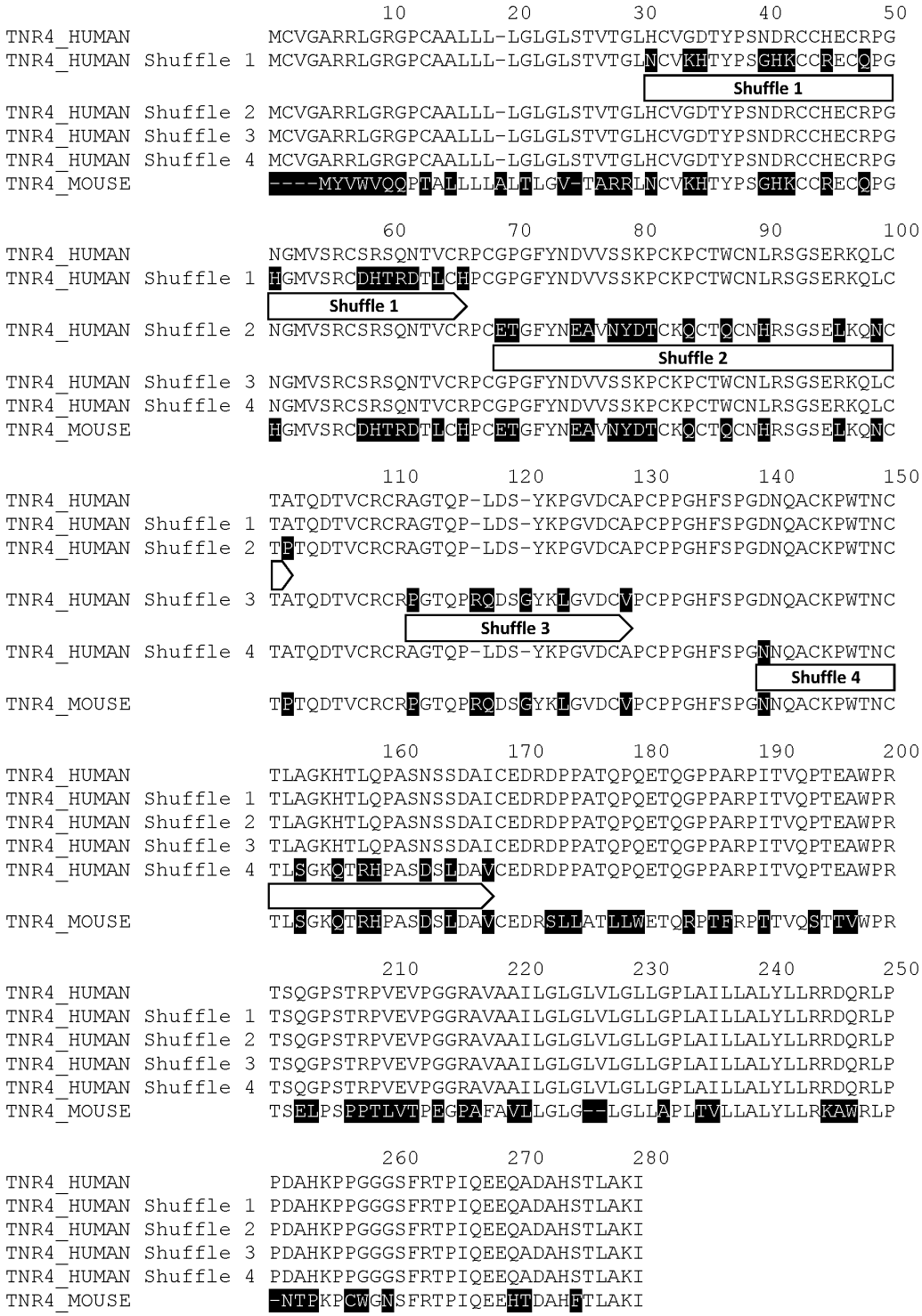

10 20 30 40 50
TNR4_HUMAN MCVGARRLGRGPCAALLL-LGLGLSTVTGLHCVGDTYPSNDRCCHECRPG
TNR4_HUMAN Shuffle 1 MCVGARRLGRGPCAALLL-LGLGLSTVTGLNCVKHTYPSGHKCCRECQPG
Shuffle 1
TNR4_HUMAN Shuffle 2 MCVGARRLGRGPCAALLL-LGLGLSTVTGLHCVGDTYPSNDRCCHECRPG
TNR4_HUMAN Shuffle 3 MCVGARRLGRGPCAALLL-LGLGLSTVTGLHCVGDTYPSNDRCCHECRPG
TNR4_HUMAN Shuffle 4 MCVGARRLGRGPCAALLL-LGLGLSTVTGLHCVGDTYPSNDRCCHECRPG
TNR4_MOUSE ----MYVWVQQPTALLLLALTLGV-TARRLNCVKHTYPSGHKCCRECQPG
60 70 80 90 100
TNR4_HUMAN NGMVSRCSRSQNTVCRPCGPGFYNDVVSSKPCKPCTWCNLRSGSERKQLC
TNR4_HUMAN Shuffle 1 HGMVSRCDHTRDTLCHPCGPGFYNDVVSSKPCKPCTWCNLRSGSERKQLC
Shuffle 1
TNR4_HUMAN Shuffle 2 NGMVSRCSRSQNTVCRPCETGFYNEAVNYDTCKQCTQCNHRSGSELKQNC
Shuffle 2
TNR4_HUMAN Shuffle 3 NGMVSRCSRSQNTVCRPCGPGFYNDVVSSKPCKPCTWCNLRSGSERKQLC
TNR4_HUMAN Shuffle 4 NGMVSRCSRSQNTVCRPCGPGFYNDVVSSKPCKPCTWCNLRSGSERKQLC
TNR4_MOUSE HGMVSRCDHTRDTLCHPCETGFYNEAVNYDTCKQCTQCNHRSGSELKQNC
110 120 130 140 150
TNR4_HUMAN TATQDTVCRCRAGTQP-LDS-YKPGVDCAPCPPGHFSPGDNQACKPWTNC
TNR4_HUMAN Shuffle 1 TATQDTVCRCRAGTQP-LDS-YKPGVDCAPCPPGHFSPGDNQACKPWTNC
TNR4_HUMAN Shuffle 2 TPTQDTVCRCRAGTQP-LDS-YKPGVDCAPCPPGHFSPGDNQACKPWTNC
TNR4_HUMAN Shuffle 3 TATQDTVCRCRPGTQPRQDSGYKLGVDCVPCPPGHFSPGDNQACKPWTNC
Shuffle 3
TNR4_HUMAN Shuffle 4 TATQDTVCRCRAGTQP-LDS-YKPGVDCAPCPPGHFSPGNNQACKPWTNC
Shuffle 4
TNR4_MOUSE TPTQDTVCRCRPGTQPRQDSGYKLGVDCVPCPPGHFSPGNNQACKPWTNC
160 170 180 190 200
TNR4_HUMAN TLAGKHTLQPASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPR
TNR4_HUMAN Shuffle 1 TLAGKHTLQPASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPR
TNR4_HUMAN Shuffle 2 TLAGKHTLQPASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPR
TNR4_HUMAN Shuffle 3 TLAGKHTLQPASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPR
TNR4_HUMAN Shuffle 4 TLSGKQTRHPASDSLDAVCEDRDPPATQPQETQGPPARPITVQPTEAWPR
TNR4_MOUSE TLSGKQTRHPASDSLDAVCEDRSLLATLLWETQRPTFRPTTVQSTTVWPR
210 220 230 240 250
TNR4_HUMAN TSQGPSTRPVEVPGGRAVAAILGLGLVLGLLGPLAILLALYLLRRDQRLP
TNR4_HUMAN Shuffle 1 TSQGPSTRPVEVPGGRAVAAILGLGLVLGLLGPLAILLALYLLRRDQRLP
TNR4_HUMAN Shuffle 2 TSQGPSTRPVEVPGGRAVAAILGLGLVLGLLGPLAILLALYLLRRDQRLP
TNR4_HUMAN Shuffle 3 TSQGPSTRPVEVPGGRAVAAILGLGLVLGLLGPLAILLALYLLRRDQRLP
TNR4_HUMAN Shuffle 4 TSQGPSTRPVEVPGGRAVAAILGLGLVLGLLGPLAILLALYLLRRDQRLP
TNR4_MOUSE TSELPSPPTLVTPEGPAFAVLLGLG--LGLLAPLTVLLALYLLRKAWRLP
260 270 280
TNR4_HUMAN PDAHKPPGGGSFRTPIQEEQADAHSTLAKI
TNR4_HUMAN Shuffle 1 PDAHKPPGGGSFRTPIQEEQADAHSTLAKI
TNR4_HUMAN Shuffle 2 PDAHKPPGGGSFRTPIQEEQADAHSTLAKI
TNR4_HUMAN Shuffle 3 PDAHKPPGGGSFRTPIQEEQADAHSTLAKI
TNR4_HUMAN Shuffle 4 PDAHKPPGGGSFRTPIQEEQADAHSTLAKI
TNR4_MOUSE -NTPKPCWGNSFRTPIQEEHTDAHETLAKI

Figure 4

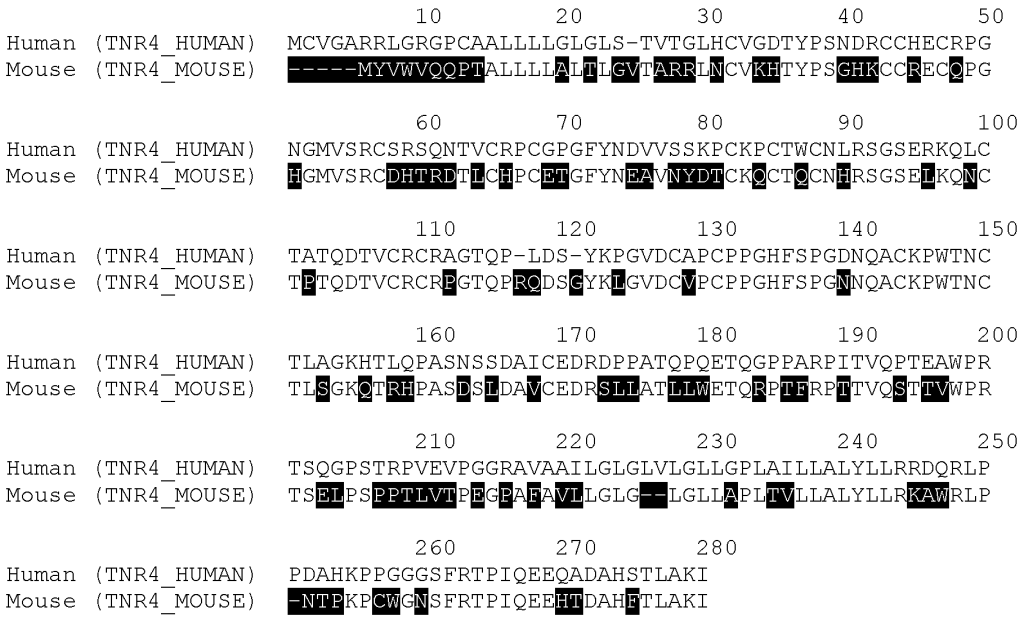

10 20 30 40 50
Human (TNR4_HUMAN) MCVGARRLGRGPCAALLLLGLGLS-TVTGLHCVGDTYPSNDRCCHECRPG
Mouse (TNR4_MOUSE) -----MYVWVQQPTALLLLALTLGVTARRLNCVKHTYPSGHKCCRECQPG
60 70 80 90 100
Human (TNR4_HUMAN) NGMVSRCSRSQNTVCRPCGPGFYNDVVSSKPCKPCTWCNLRSGSERKQLC
Mouse (TNR4_MOUSE) HGMVSRCDHTRDTLCHPCETGFYNEAVNYDTCKQCTQCNHRSGSELKQNC
110 120 130 140 150
Human (TNR4_HUMAN) TATQDTVCRCRAGTQP-LDS-YKPGVDCAPCPPGHFSPGDNQACKPWTNC
Mouse (TNR4_MOUSE) TPTQDTVCRCRPGTQPRQDSGYKLGVDCVPCPPGHFSPGNNQACKPWTNC
160 170 180 190 200
Human (TNR4_HUMAN) TLAGKHTLQPASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPR
Mouse (TNR4_MOUSE) TLSGKQTRHPASDSLDAVCEDRSLLATLLWETQRPTFRPTTVQSTTVWPR
210 220 230 240 250
Human (TNR4_HUMAN) TSQGPSTRPVEVPGGRAVAAILGLGLVLGLLGPLAILLALYLLRRDQRLP
Mouse (TNR4_MOUSE) TSELPSPPTLVTPEGPAFAVLLGLG--LGLLAPLTVLLALYLLRKAWRLP
260 270 280
Human (TNR4_HUMAN) PDAHKPPGGGSFRTPIQEEQADAHSTLAKI
Mouse (TNR4_MOUSE) -NTPKPCWGNSFRTPIQEEHTDAHFTLAKI

Figure 5

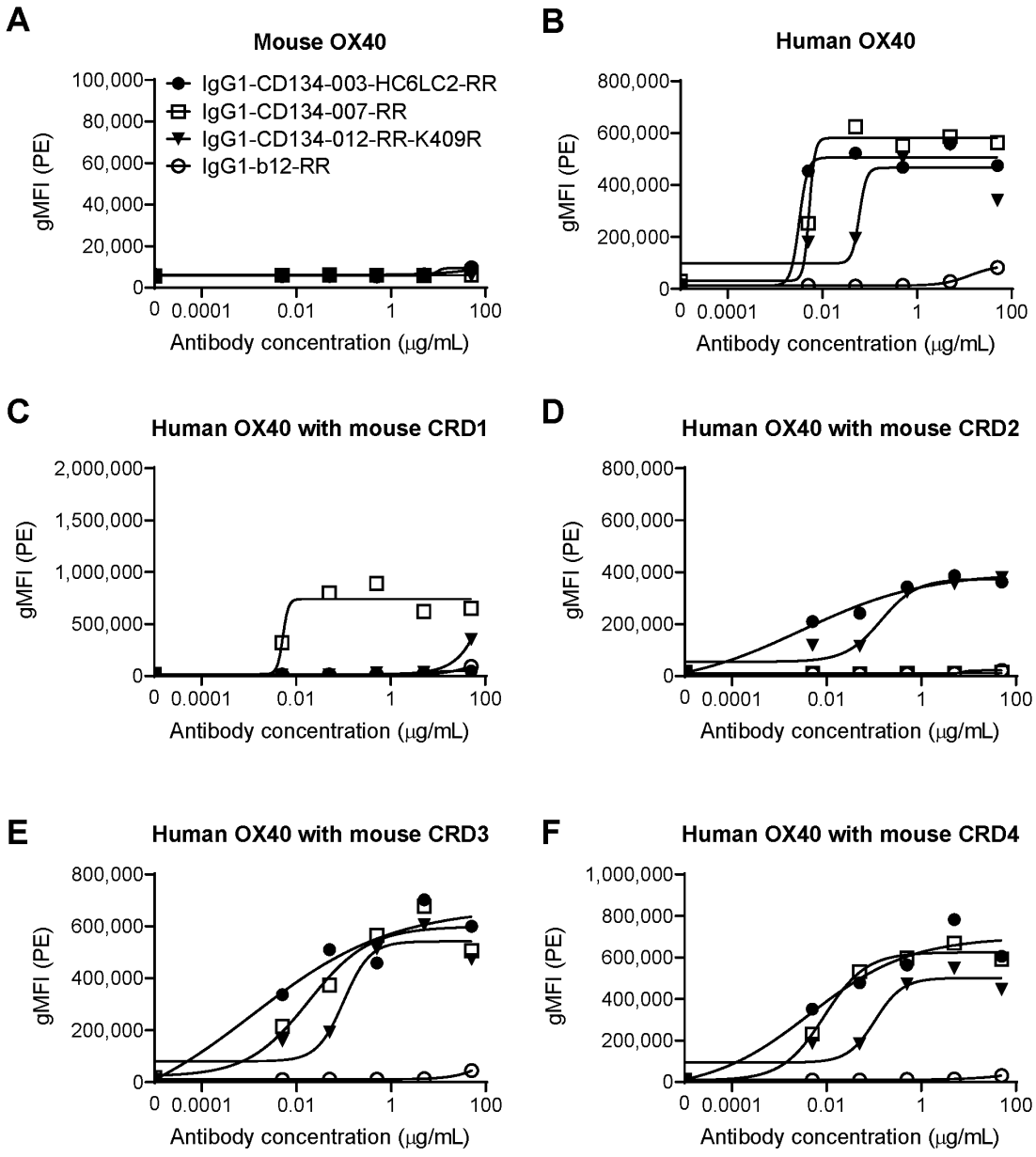

A
Mouse OX40
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-007-RR
IgG1-CD134-012-RR-K409R
IgG1-b12-RR
gMFI (PE)
Antibody concentration (µg/mL)
B
Human OX40
C
Human OX40 with mouse CRD1
D
Human OX40 with mouse CRD2
E
Human OX40 with mouse CRD3
F
Human OX40 with mouse CRD4

Figure 6

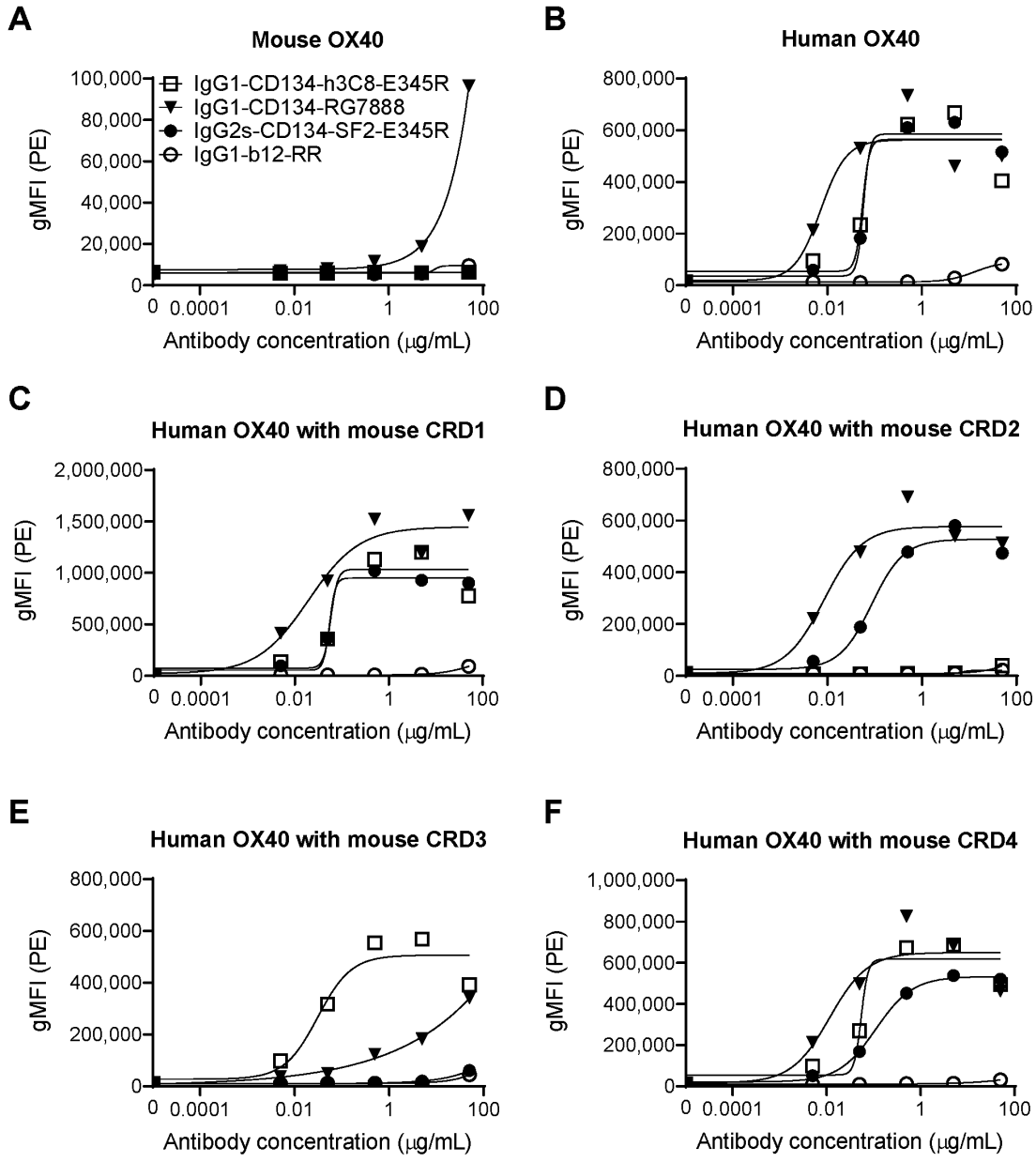

A
Mouse OX40
IgG1-CD134-h3C8-E345R
IgG1-CD134-RG7888
IgG2s-CD134-SF2-E345R
IgG1-b12-RR
gMFI (PE)
Antibody concentration (µg/mL)
B
Human OX40
C
Human OX40 with mouse CRD1
D
Human OX40 with mouse CRD2
E
Human OX40 with mouse CRD3
F
Human OX40 with mouse CRD4

Figure 7

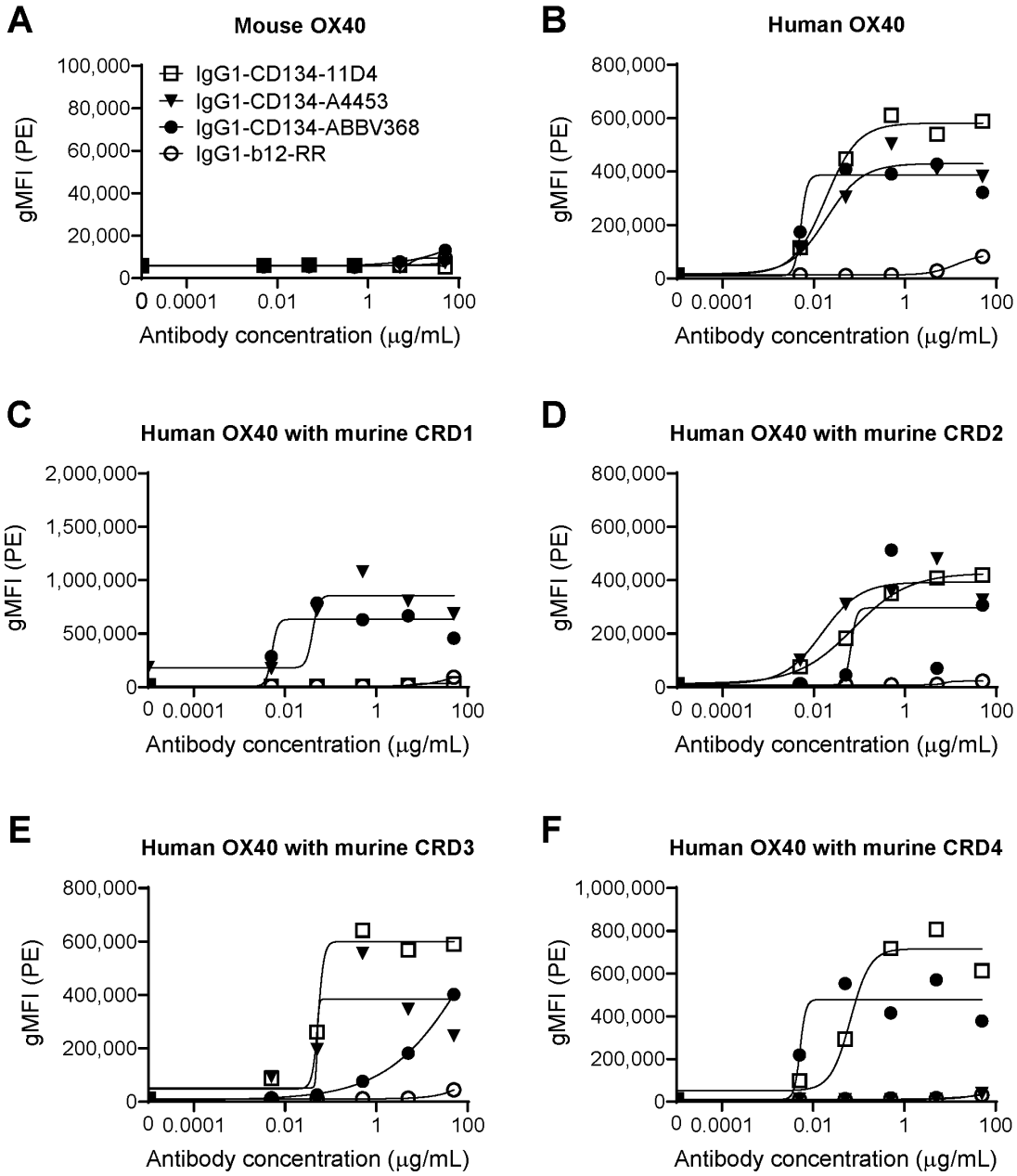

A
Mouse OX40
IgG1-CD134-11D4
IgG1-CD134-A4453
IgG1-CD134-ABBV368
IgG1-b12-RR
gMFI (PE)
Antibody concentration (μg/mL)
B
Human OX40
C
Human OX40 with murine CRD1
D
Human OX40 with murine CRD2
E
Human OX40 with murine CRD3
F
Human OX40 with murine CRD4

Figure 8

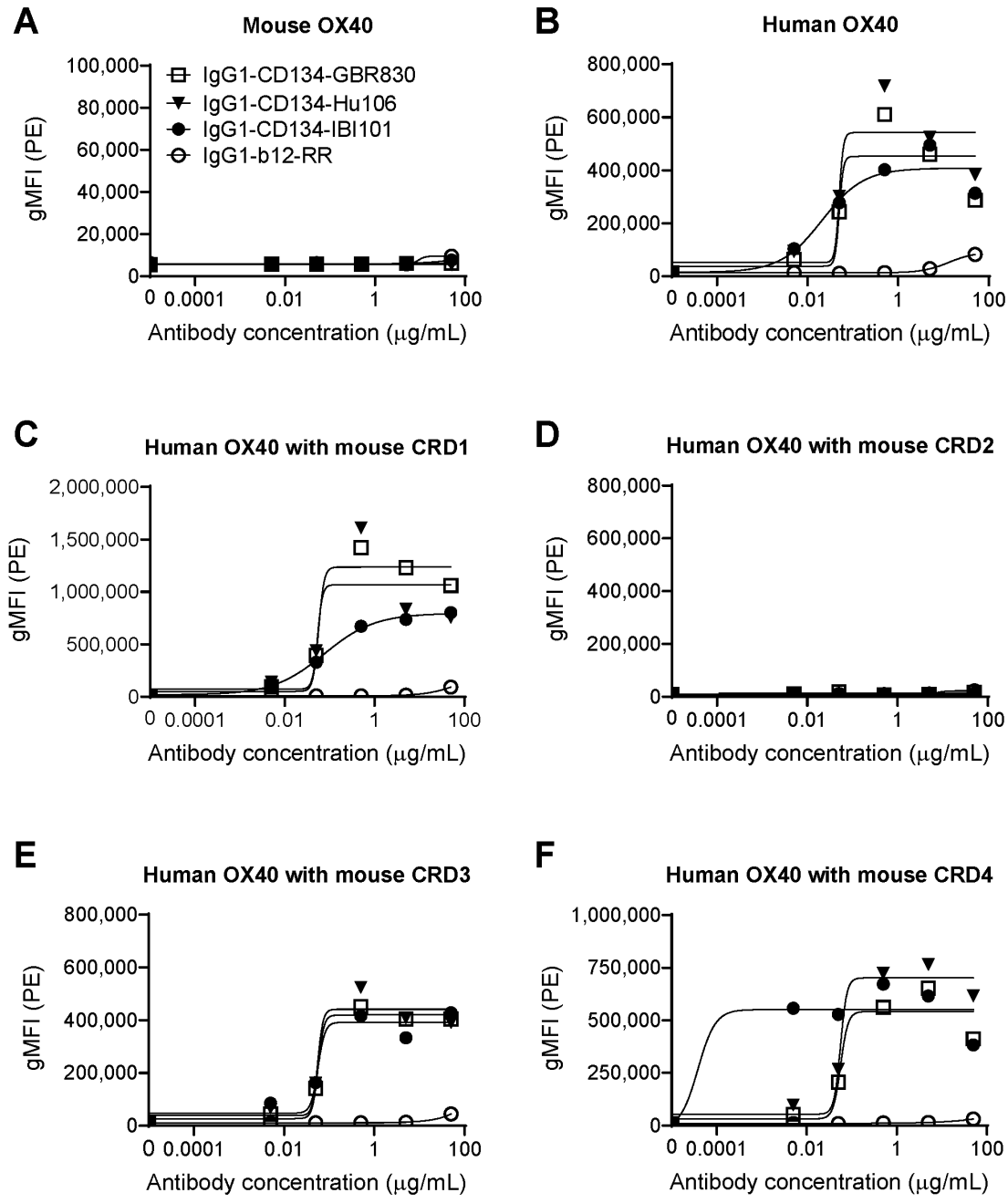

A
Mouse OX40
gMFI (PE)
100,000
80,000
60,000
40,000
20,000
0
IgG1-CD134-GBR830
IgG1-CD134-Hu106
IgG1-CD134-IBI101
IgG1-b12-RR
0 0.0001 0.01 1 100
Antibody concentration (μg/mL)
B
Human OX40
gMFI (PE)
800,000
600,000
400,000
200,000
0
0 0.0001 0.01 1 100
Antibody concentration (μg/mL)
C
Human OX40 with mouse CRD1
gMFI (PE)
2,000,000
1,500,000
1,000,000
500,000
0
0 0.0001 0.01 1 100
Antibody concentration (μg/mL)
D
Human OX40 with mouse CRD2
gMFI (PE)
800,000
600,000
400,000
200,000
0
0 0.0001 0.01 1 100
Antibody concentration (μg/mL)
E
Human OX40 with mouse CRD3
gMFI (PE)
800,000
600,000
400,000
200,000
0
0 0.0001 0.01 1 100
Antibody concentration (μg/mL)
F
Human OX40 with mouse CRD4
gMFI (PE)
1,000,000
750,000
500,000
250,000
0
0 0.0001 0.01 1 100
Antibody concentration (μg/mL)

Figure 9

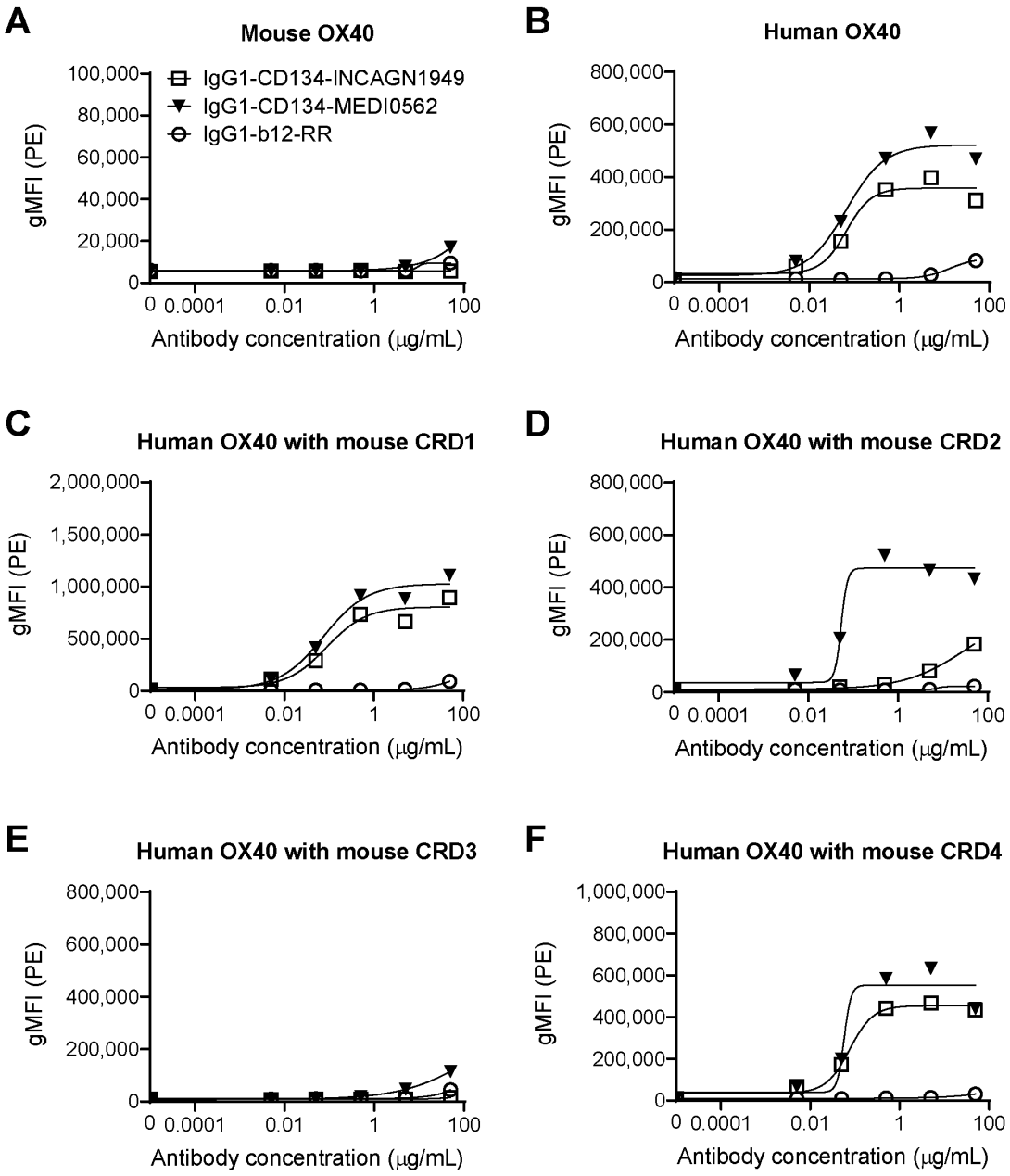

A
Mouse OX40
IgG1-CD134-INCAGN1949
IgG1-CD134-MEDI0562
IgG1-b12-RR
gMFI (PE)
100,000
80,000
60,000
40,000
20,000
0
0 0.0001 0.01 1 100
Antibody concentration (μg/mL)
B
Human OX40
gMFI (PE)
800,000
600,000
400,000
200,000
0
0 0.0001 0.01 1 100
Antibody concentration (μg/mL)
C
Human OX40 with mouse CRD1
gMFI (PE)
2,000,000
1,500,000
1,000,000
500,000
0
0 0.0001 0.01 1 100
Antibody concentration (μg/mL)
D
Human OX40 with mouse CRD2
gMFI (PE)
800,000
600,000
400,000
200,000
0
0 0.0001 0.01 1 100
Antibody concentration (μg/mL)
E
Human OX40 with mouse CRD3
gMFI (PE)
800,000
600,000
400,000
200,000
0
0 0.0001 0.01 1 100
Antibody concentration (μg/mL)
F
Human OX40 with mouse CRD4
gMFI (PE)
1,000,000
800,000
600,000
400,000
200,000
0
0 0.0001 0.01 1 100
Antibody concentration (μg/mL)

Figure 10

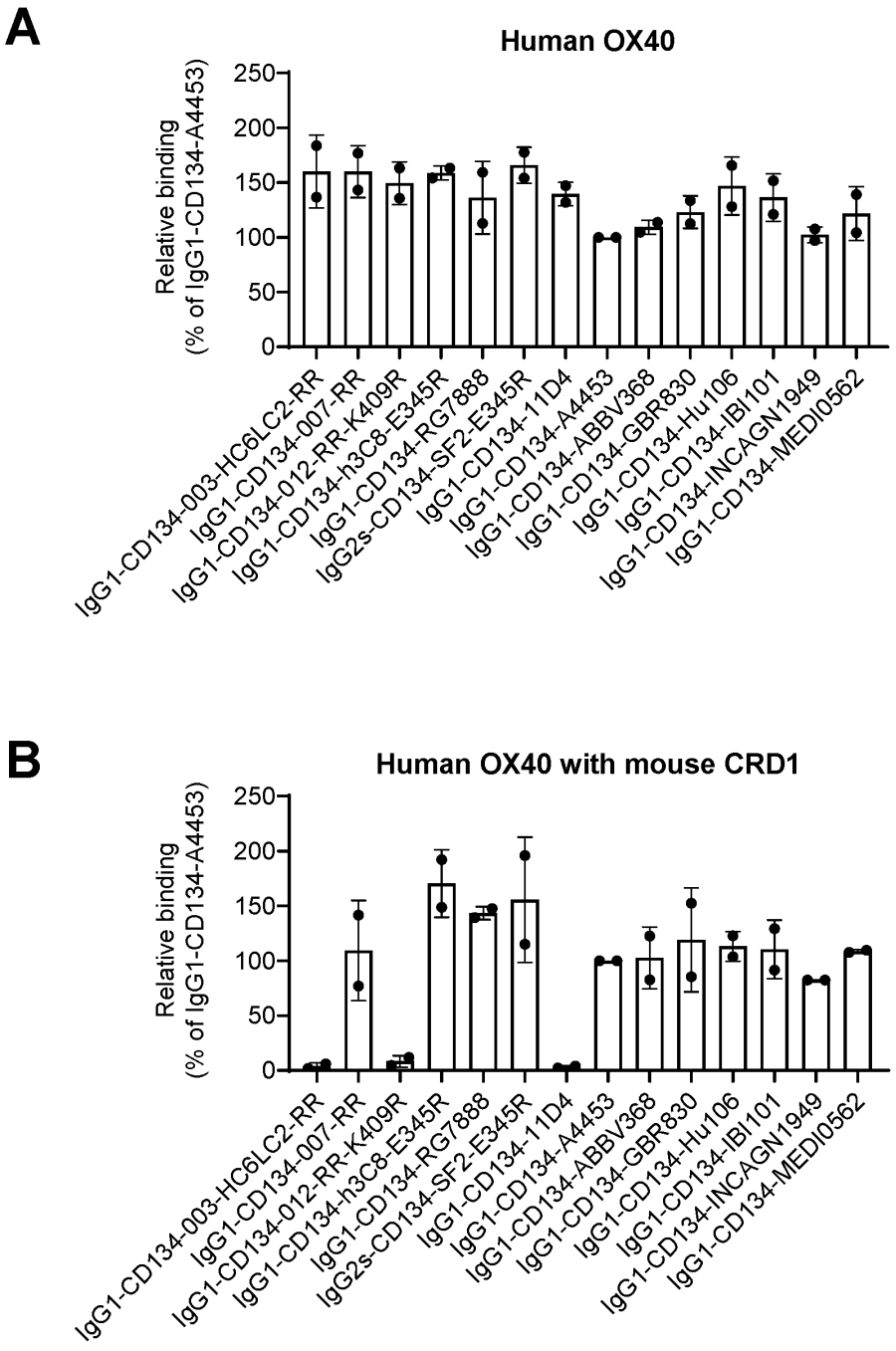

A
Human OX40
Relative binding
(% of IgG1-CD134-A4453)
250
200
150
100
50
0
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-007-RR
IgG1-CD134-012-RR-K409R
IgG1-CD134-h3C8-E345R
IgG1-CD134-RG7888
IgG2s-CD134-SF2-E345R
IgG1-CD134-11D4
IgG1-CD134-A4453
IgG1-CD134-ABBV368
IgG1-CD134-GBR830
IgG1-CD134-Hu106
IgG1-CD134-IBI101
IgG1-CD134-INCAGN1949
IgG1-CD134-MEDI0562
B
Human OX40 with mouse CRD1
Relative binding
(% of IgG1-CD134-A4453)
250
200
150
100
50
0
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-007-RR
IgG1-CD134-012-RR-K409R
IgG1-CD134-h3C8-E345R
IgG1-CD134-RG7888
IgG2s-CD134-SF2-E345R
IgG1-CD134-11D4
IgG1-CD134-A4453
IgG1-CD134-ABBV368
IgG1-CD134-GBR830
IgG1-CD134-Hu106
IgG1-CD134-IBI101
IgG1-CD134-INCAGN1949
IgG1-CD134-MEDI0562

Figure 10 – continued

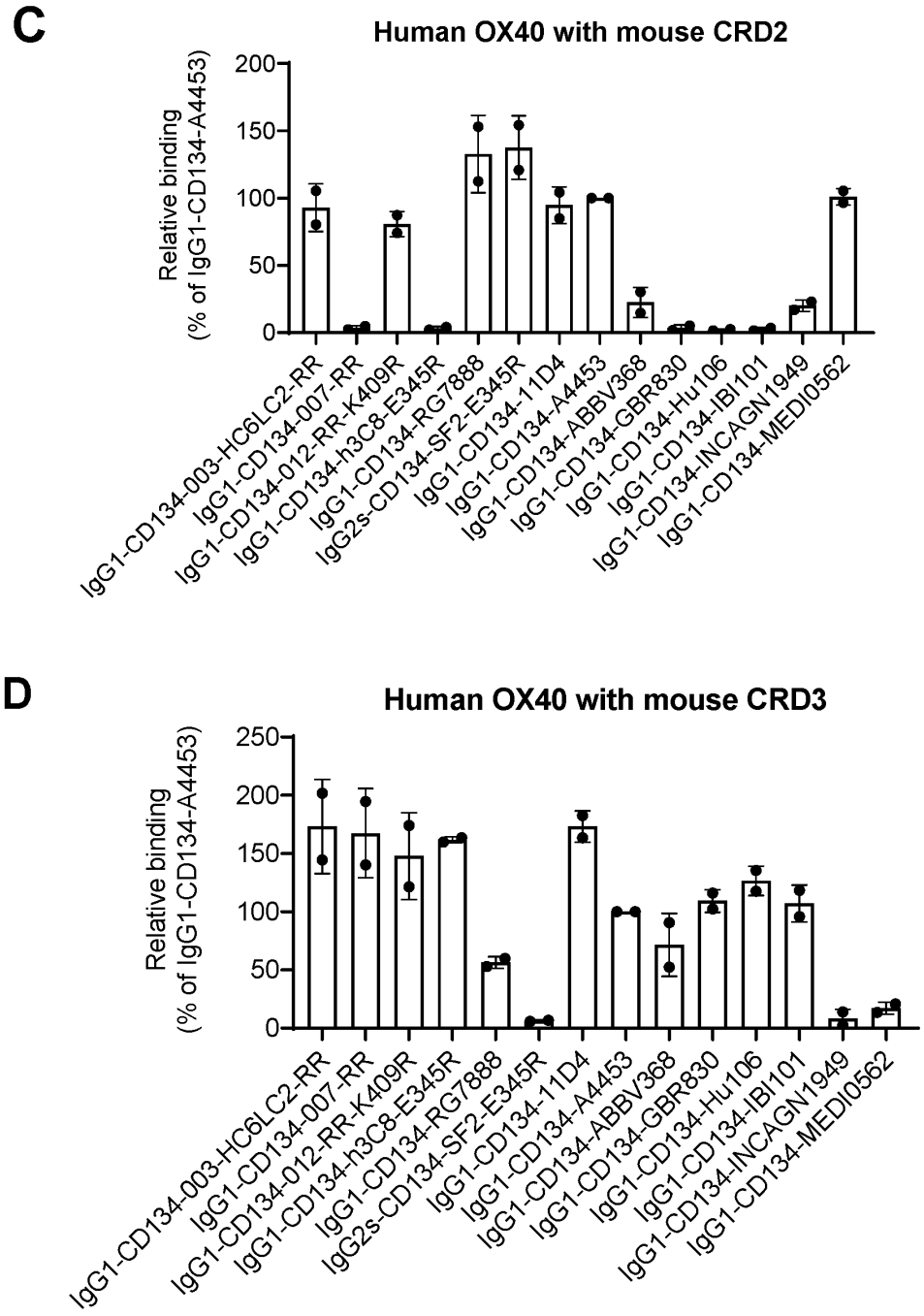


Figure 10 – continued

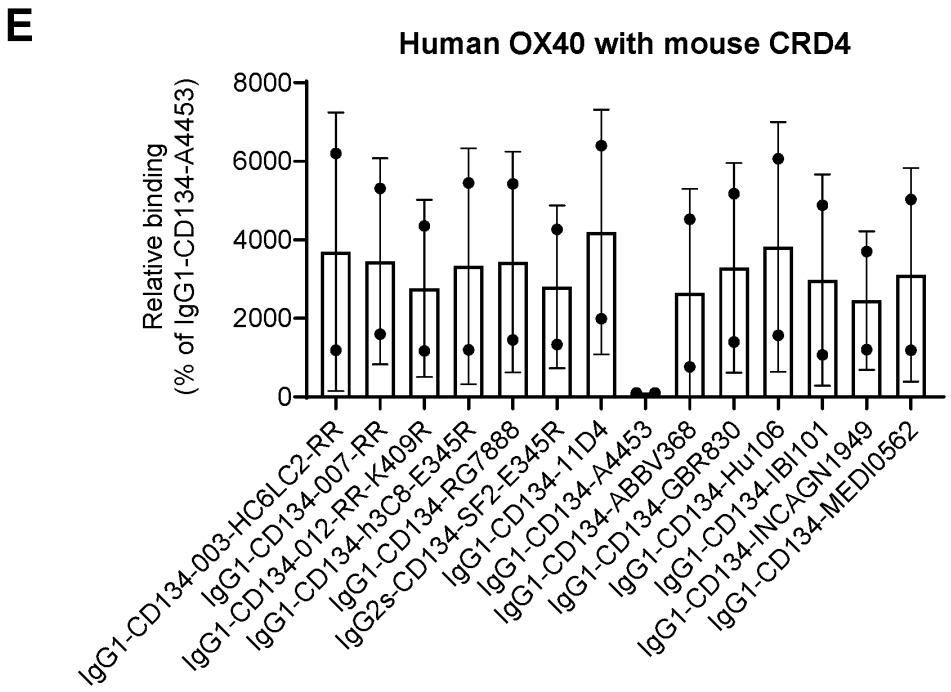


Figure 11

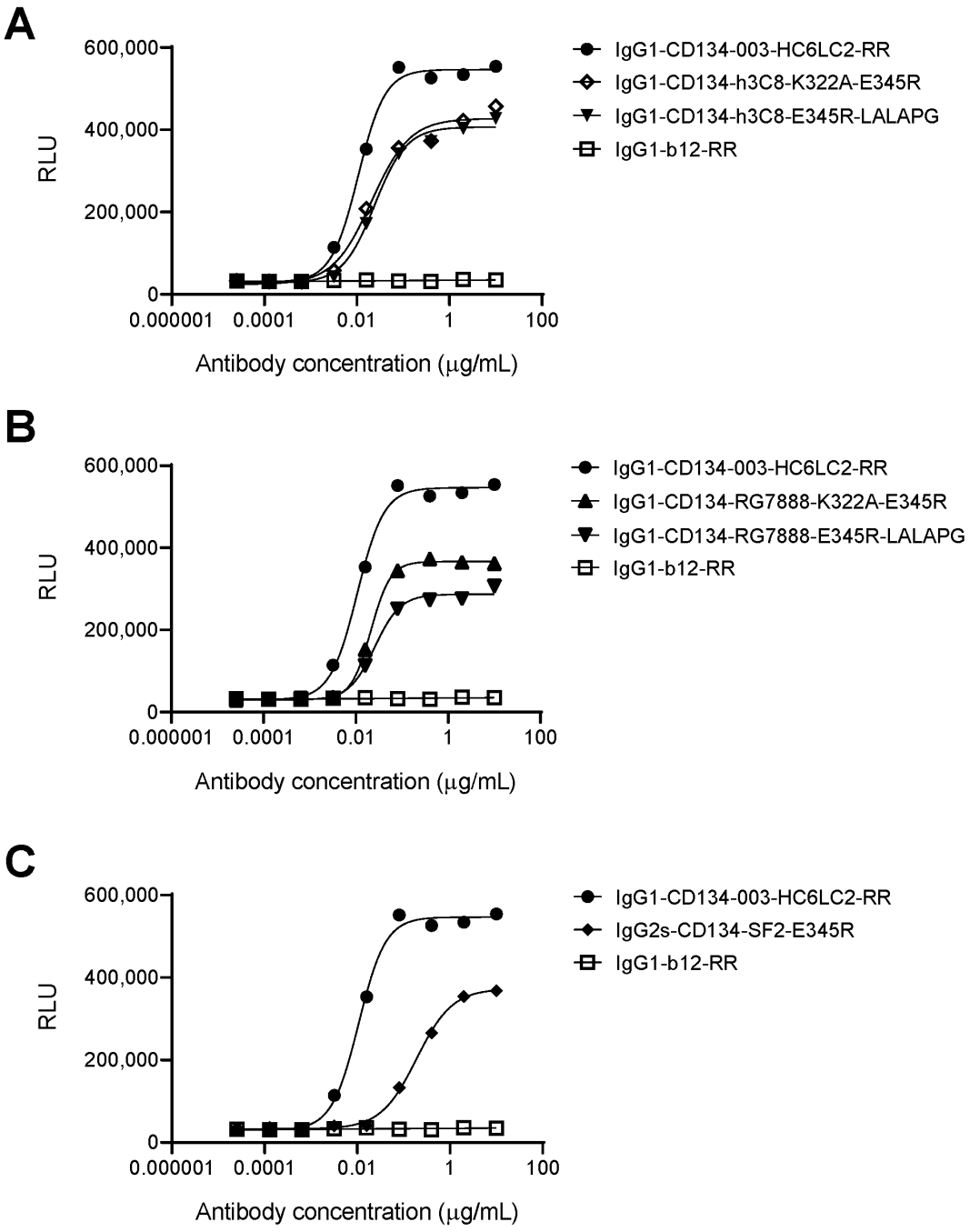

A
600,000
400,000
200,000
0
RLU
0.000001
0.0001
0.01
1
100
Antibody concentration (μg/mL)
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-h3C8-K322A-E345R
IgG1-CD134-h3C8-E345R-LALAPG
IgG1-b12-RR
B
600,000
400,000
200,000
0
RLU
0.000001
0.0001
0.01
1
100
Antibody concentration (μg/mL)
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-RG7888-K322A-E345R
IgG1-CD134-RG7888-E345R-LALAPG
IgG1-b12-RR
C
600,000
400,000
200,000
0
RLU
0.000001
0.0001
0.01
1
100
Antibody concentration (μg/mL)
IgG1-CD134-003-HC6LC2-RR
IgG2s-CD134-SF2-E345R
IgG1-b12-RR

Figure 11 - continued

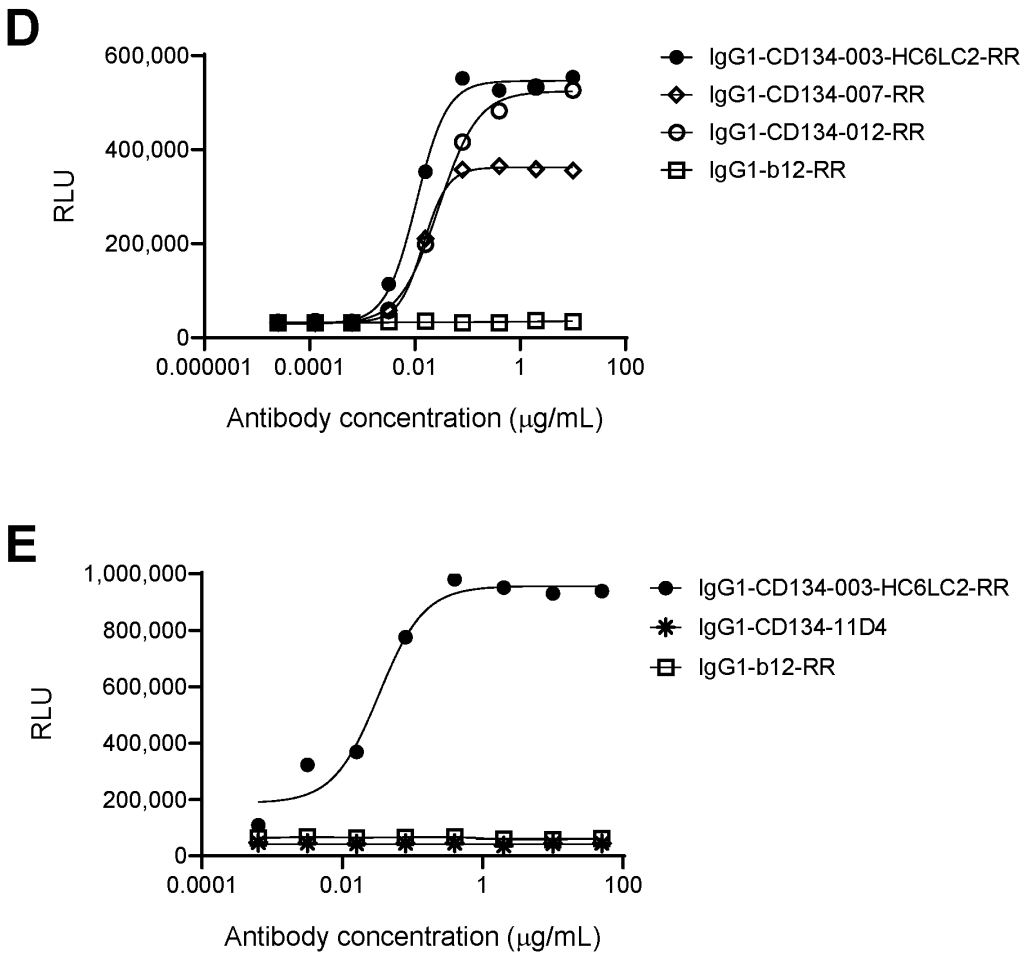


Figure 12

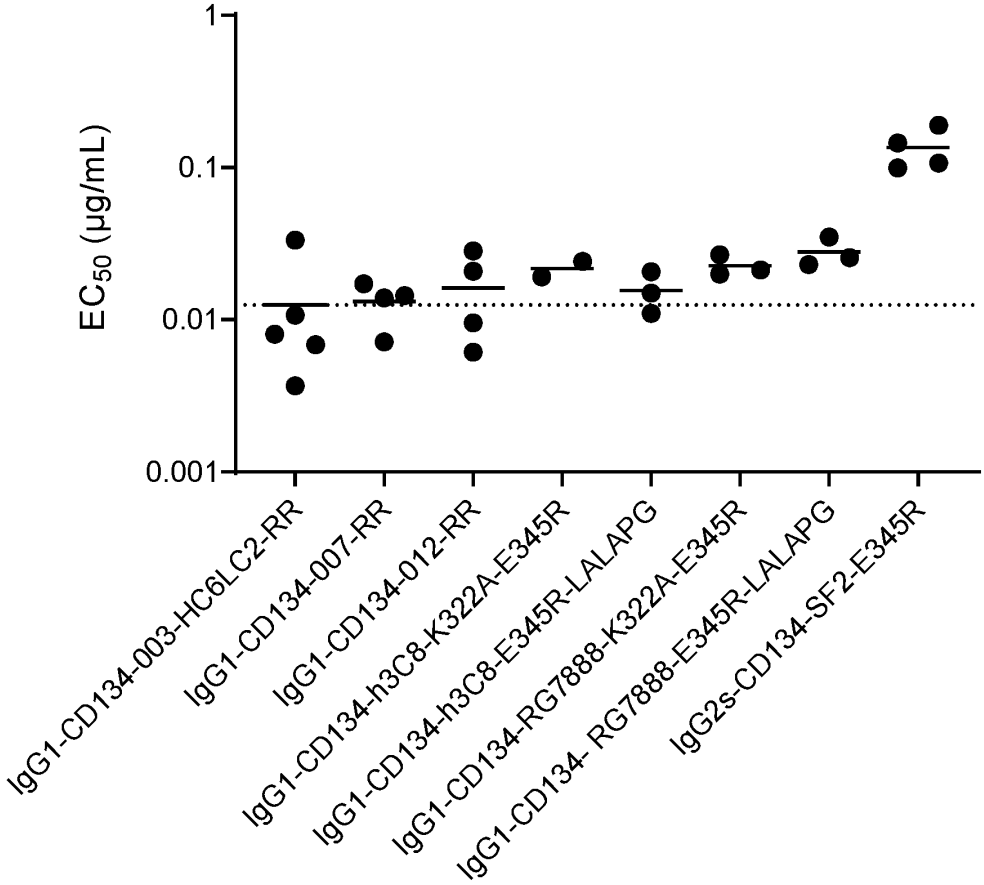

1
0.1
0.01
0.001
EC50 (µg/mL)
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-007-RR
IgG1-CD134-012-RR
IgG1-CD134-h3C8-K322A-E345R
IgG1-CD134-h3C8-E345R-LALAPG
IgG1-CD134-RG7888-K322A-E345R
IgG1-CD134- RG7888-E345R-LALAPG
IgG2s-CD134-SF2-E345R

Figure 13

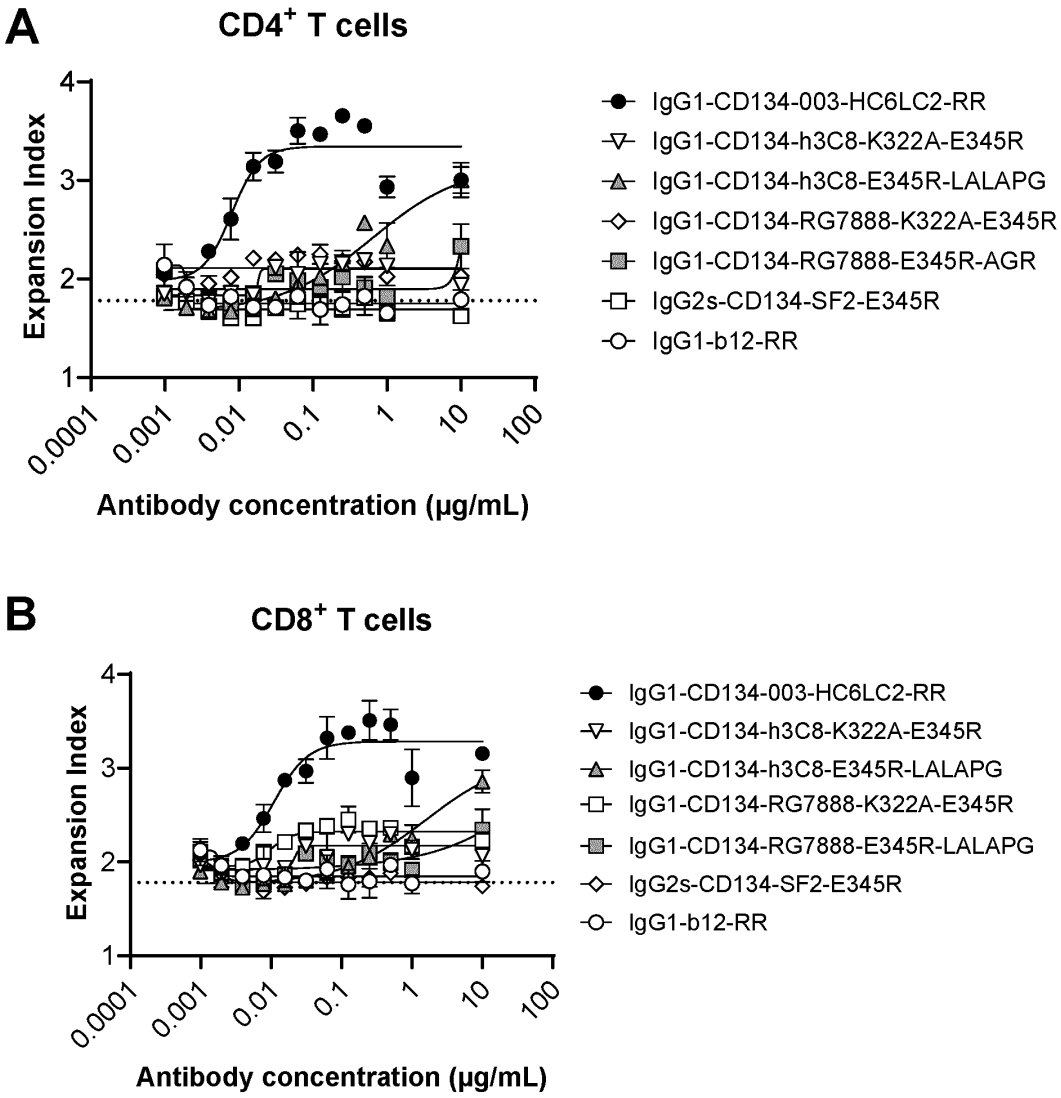

A
CD4+ T cells
Expansion Index
Antibody concentration (µg/mL)
B
CD8+ T cells
Expansion Index
Antibody concentration (µg/mL)
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-h3C8-K322A-E345R
IgG1-CD134-h3C8-E345R-LALAPG
IgG1-CD134-RG7888-K322A-E345R
IgG1-CD134-RG7888-E345R-AGR
IgG2s-CD134-SF2-E345R
IgG1-b12-RR

Figure 13 – continued

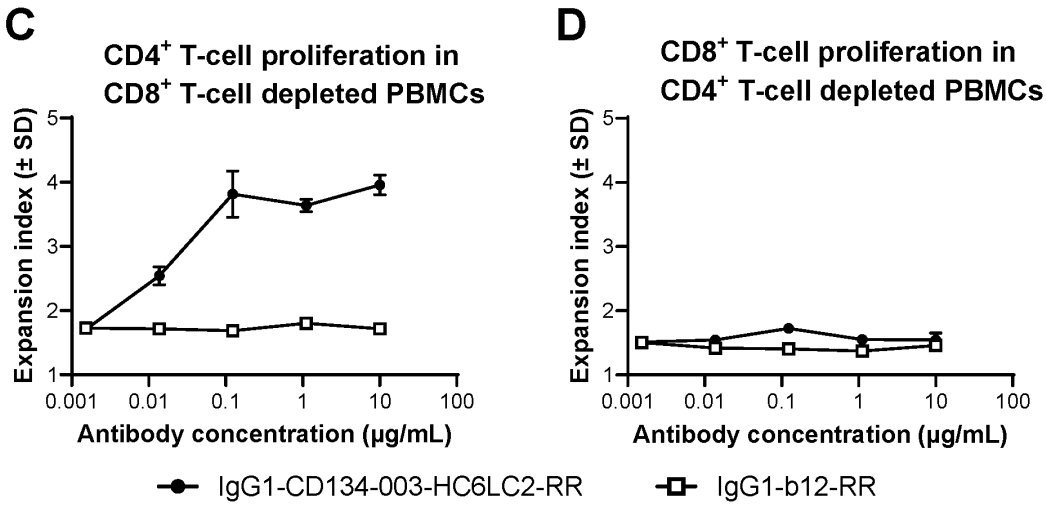


Figure 14

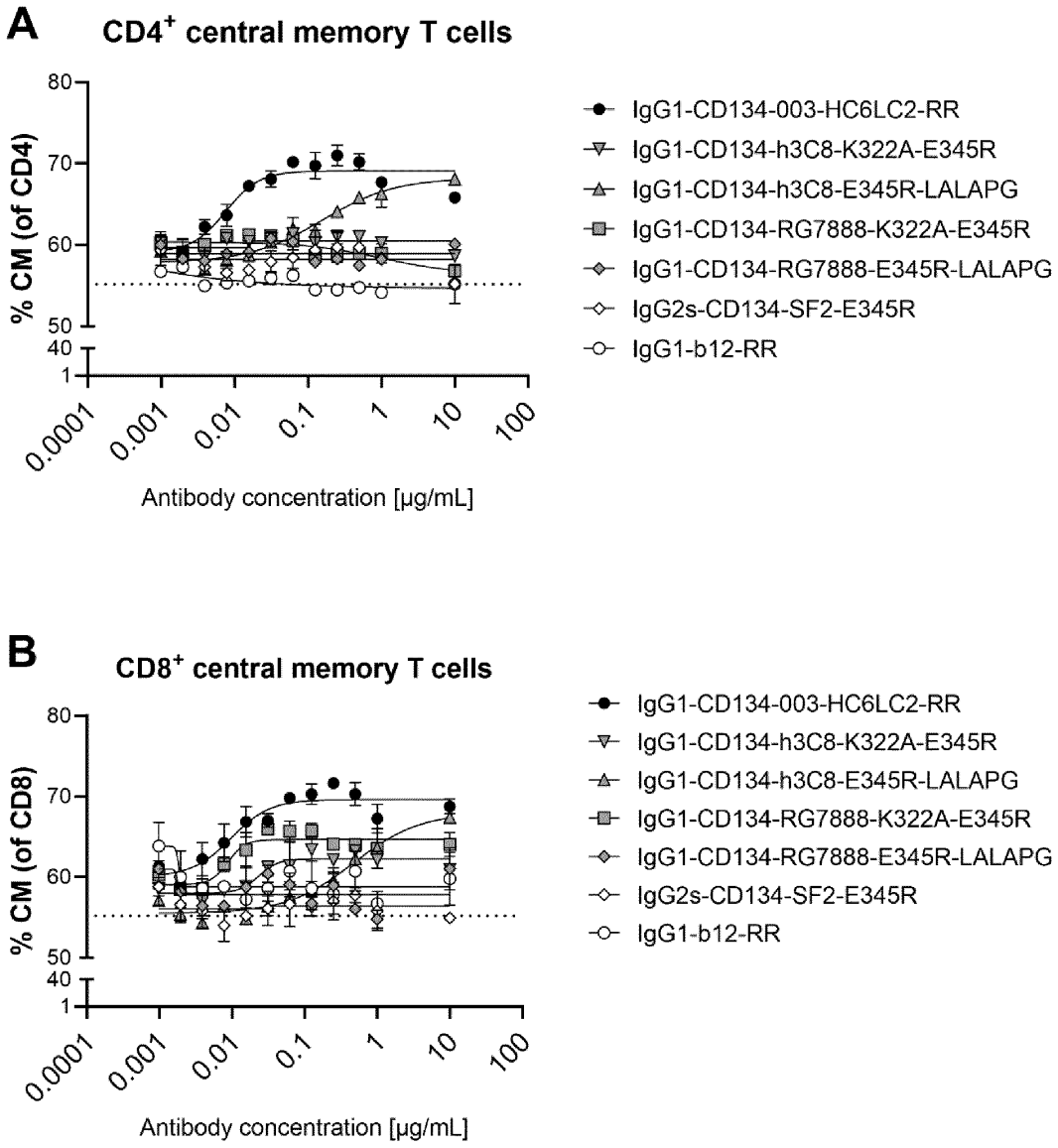

A
CD4+ central memory T cells
% CM (of CD4)
80
70
60
50
40
1
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [µg/mL]
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-h3C8-K322A-E345R
IgG1-CD134-h3C8-E345R-LALAPG
IgG1-CD134-RG7888-K322A-E345R
IgG1-CD134-RG7888-E345R-LALAPG
IgG2s-CD134-SF2-E345R
IgG1-b12-RR
B
CD8+ central memory T cells
% CM (of CD8)
80
70
60
50
40
1
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [µg/mL]
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-h3C8-K322A-E345R
IgG1-CD134-h3C8-E345R-LALAPG
IgG1-CD134-RG7888-K322A-E345R
IgG1-CD134-RG7888-E345R-LALAPG
IgG2s-CD134-SF2-E345R
IgG1-b12-RR

Figure 15

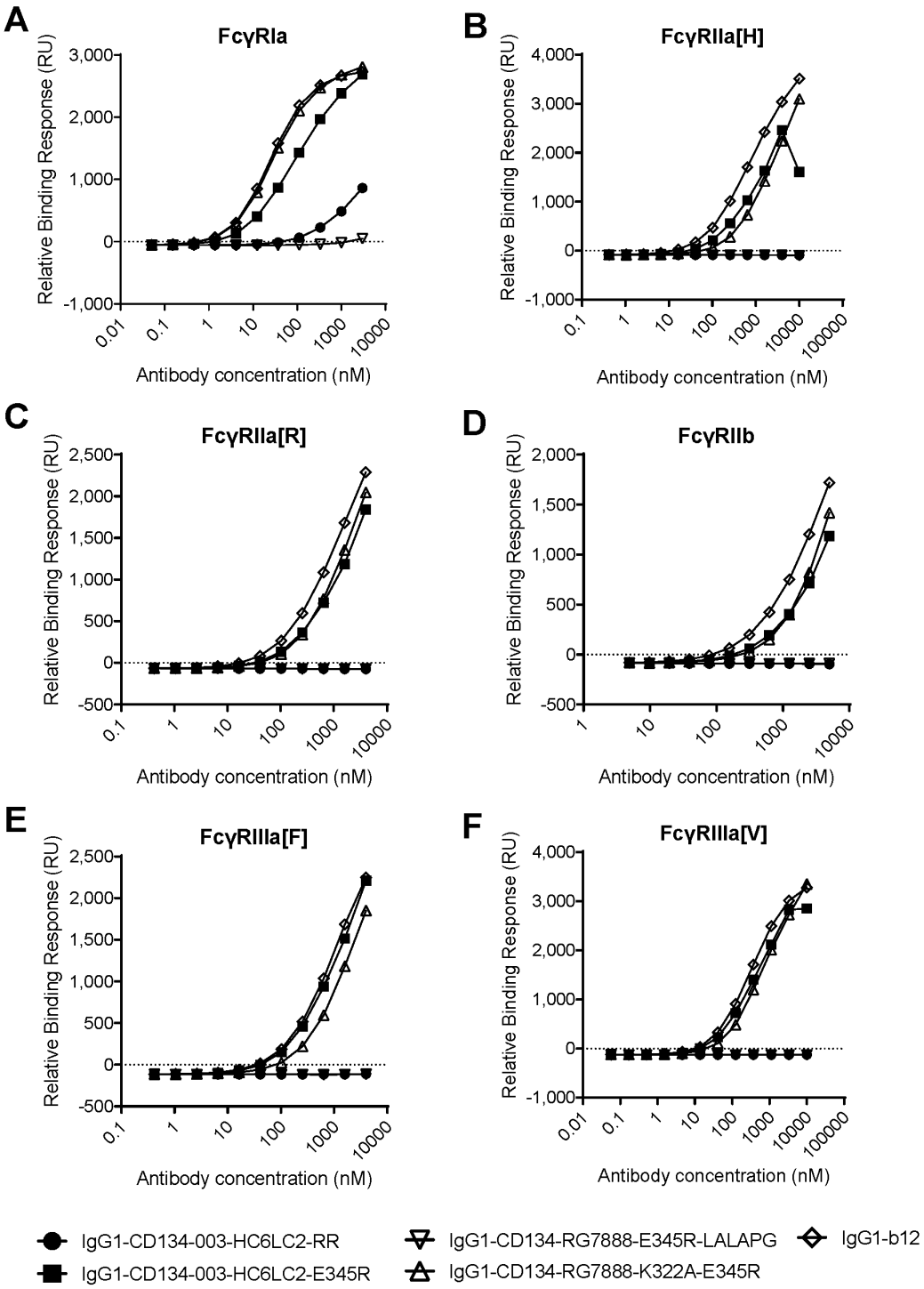

A
FcγRIa
Relative Binding Response (RU)
3,000
2,000
1,000
0
-1,000
0.01
0.1
1
10
100
1000
10000
Antibody concentration (nM)
B
FcγRIIa[H]
Relative Binding Response (RU)
4,000
3,000
2,000
1,000
0
-1,000
0.1
1
10
100
1000
10000
100000
Antibody concentration (nM)
C
FcγRIIa[R]
Relative Binding Response (RU)
2,500
2,000
1,500
1,000
500
0
-500
0.1
1
10
100
1000
10000
Antibody concentration (nM)
D
FcγRIIb
Relative Binding Response (RU)
2,000
1,500
1,000
500
0
-500
1
10
100
1000
10000
Antibody concentration (nM)
E
FcγRIIIa[F]
Relative Binding Response (RU)
2,500
2,000
1,500
1,000
500
0
-500
0.1
1
10
100
1000
10000
Antibody concentration (nM)
F
FcγRIIIa[V]
Relative Binding Response (RU)
4,000
3,000
2,000
1,000
0
-1,000
0.01
0.1
1
10
100
1000
10000
100000
Antibody concentration (nM)
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-003-HC6LC2-E345R
IgG1-CD134-RG7888-E345R-LALAPG
IgG1-CD134-RG7888-K322A-E345R
IgG1-b12

Figure 16

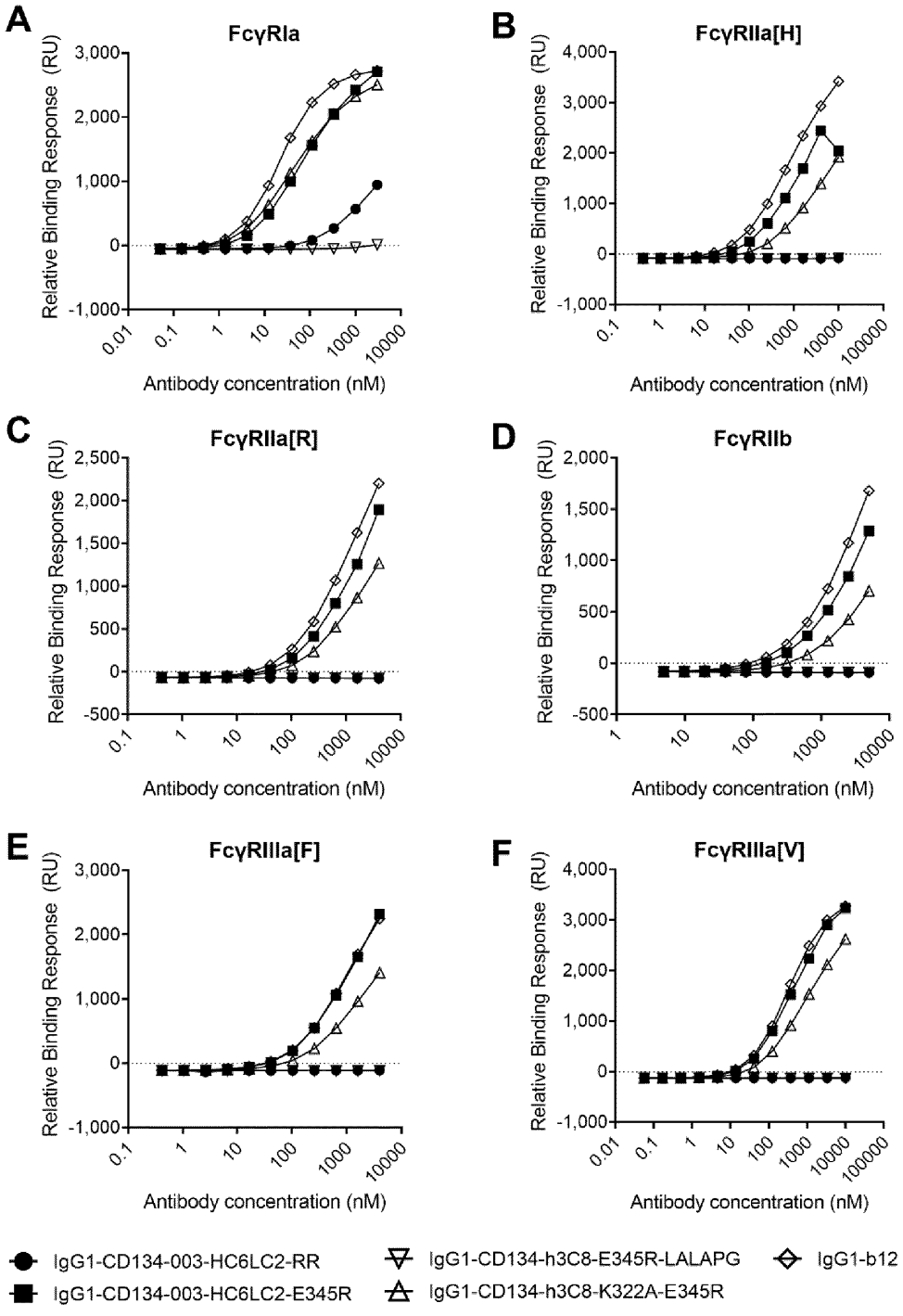

A
FcγRIa
Relative Binding Response (RU)
Antibody concentration (nM)
B
FcγRIIa[H]
C
FcγRIIa[R]
D
FcγRIIb
E
FcγRIIIa[F]
F
FcγRIIIa[V]
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-003-HC6LC2-E345R
IgG1-CD134-h3C8-E345R-LALAPG
IgG1-CD134-h3C8-K322A-E345R
IgG1-b12

Figure 17

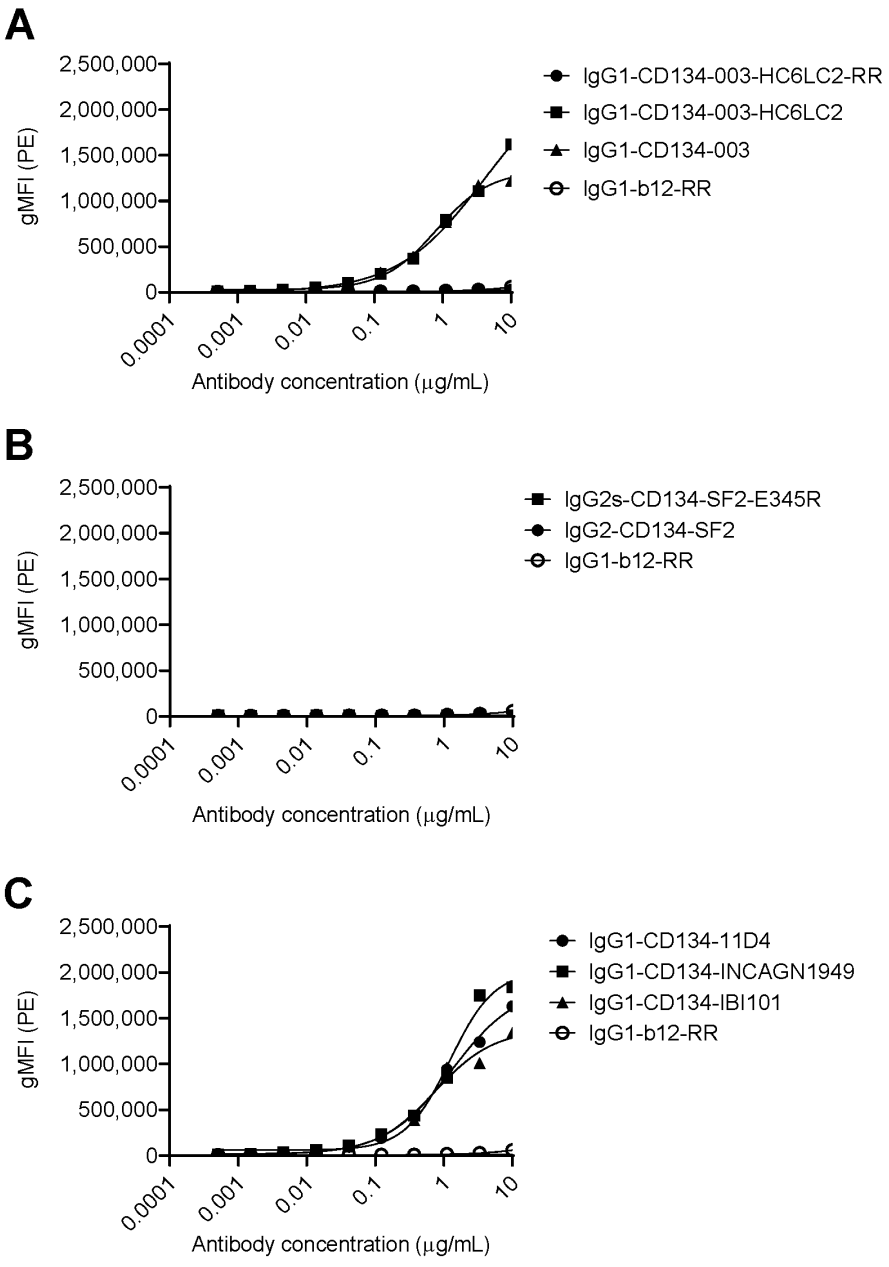

A
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
gMFI (PE)
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (µg/mL)
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-003-HC6LC2
IgG1-CD134-003
IgG1-b12-RR
B
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
gMFI (PE)
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (µg/mL)
IgG2s-CD134-SF2-E345R
IgG2-CD134-SF2
IgG1-b12-RR
C
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
gMFI (PE)
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (µg/mL)
IgG1-CD134-11D4
IgG1-CD134-INCAGN1949
IgG1-CD134-IBI101
IgG1-b12-RR

Figure 17 - continued

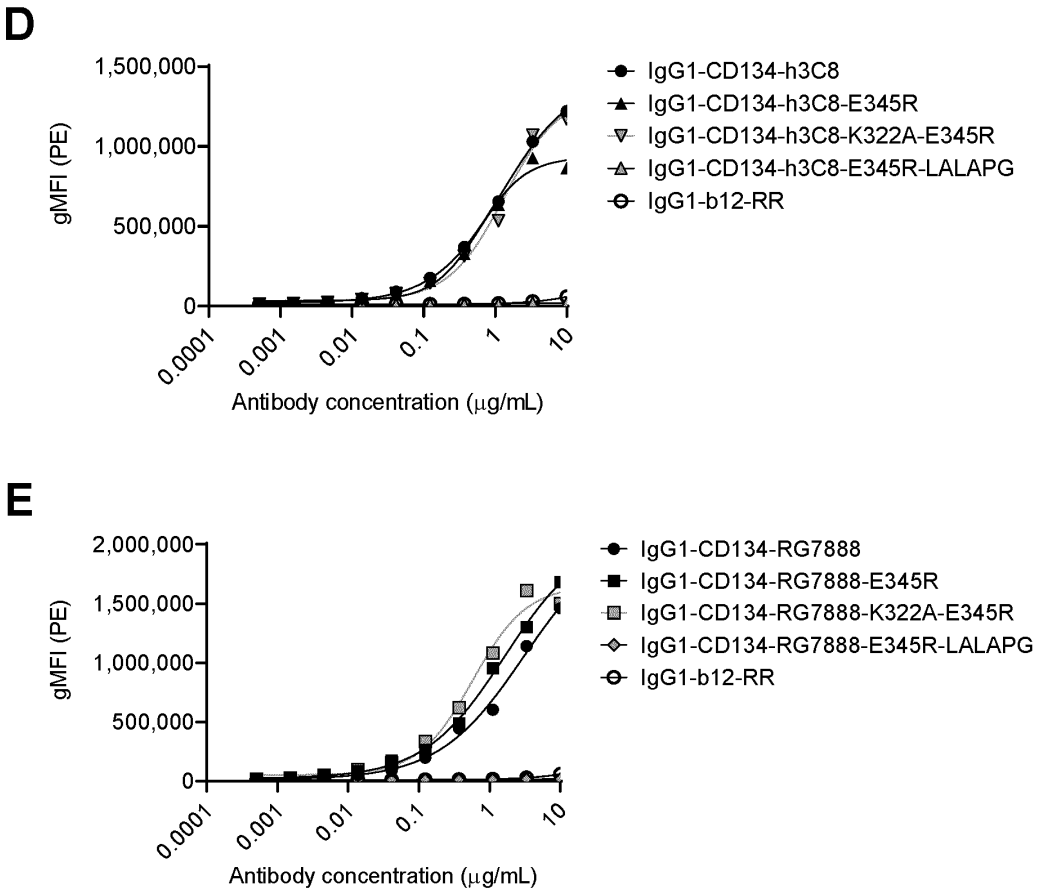


Figure 18

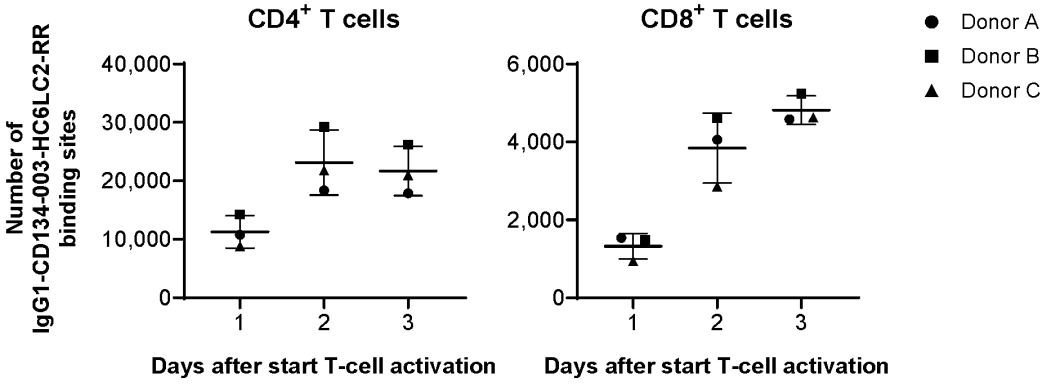


Figure 19

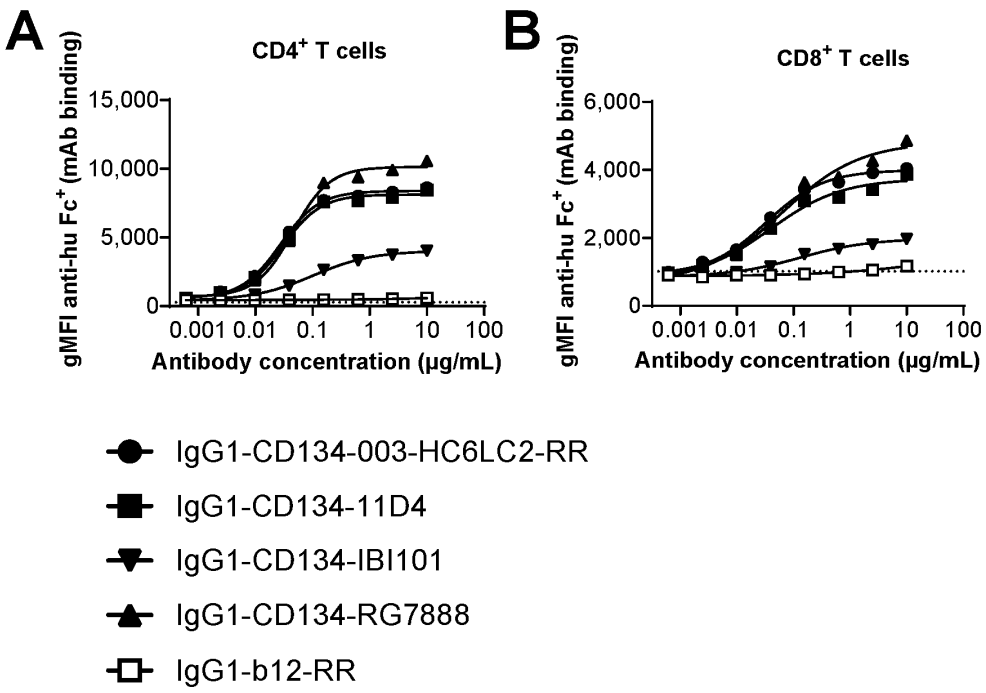


Figure 20

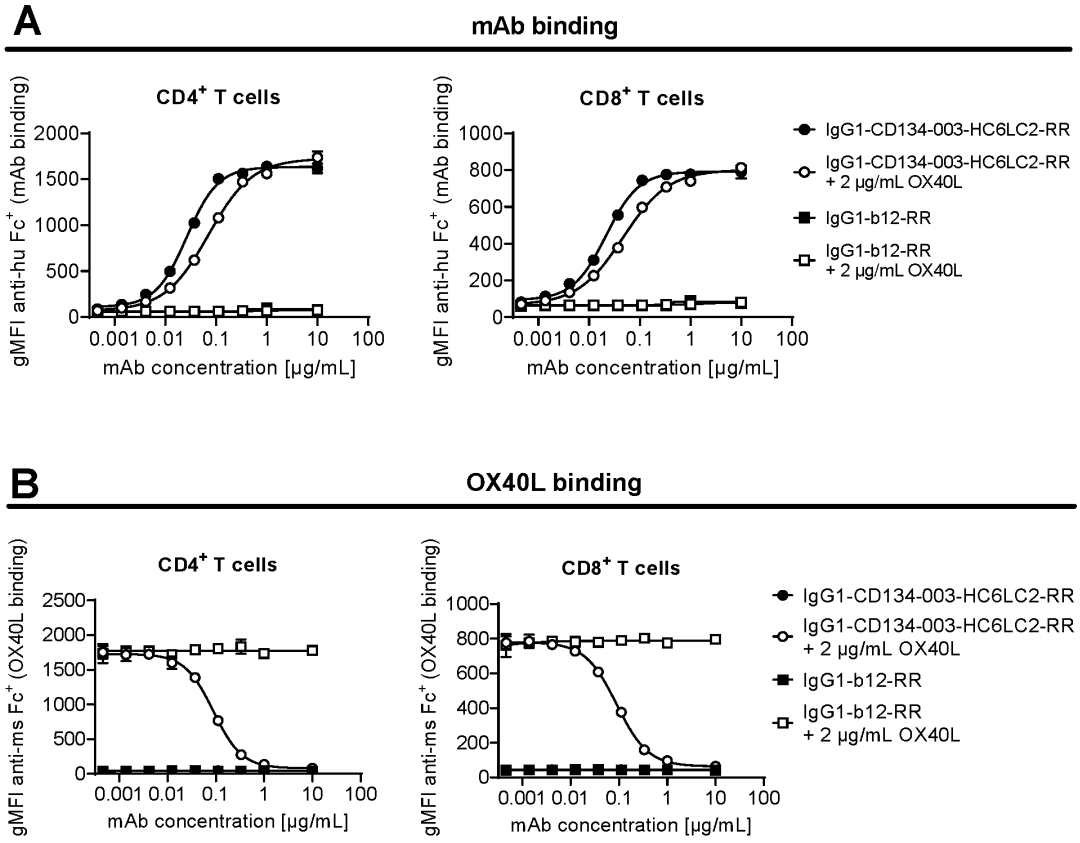

A
mAb binding
CD4+ T cells
CD8+ T cells
gMFI anti-hu Fc+ (mAb binding)
mAb concentration [µg/mL]
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-003-HC6LC2-RR + 2 µg/mL OX40L
IgG1-b12-RR
IgG1-b12-RR + 2 µg/mL OX40L
B
OX40L binding
CD4+ T cells
CD8+ T cells
gMFI anti-ms Fc+ (OX40L binding)
mAb concentration [µg/mL]
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-003-HC6LC2-RR + 2 µg/mL OX40L
IgG1-b12-RR
IgG1-b12-RR + 2 µg/mL OX40L

Figure 21

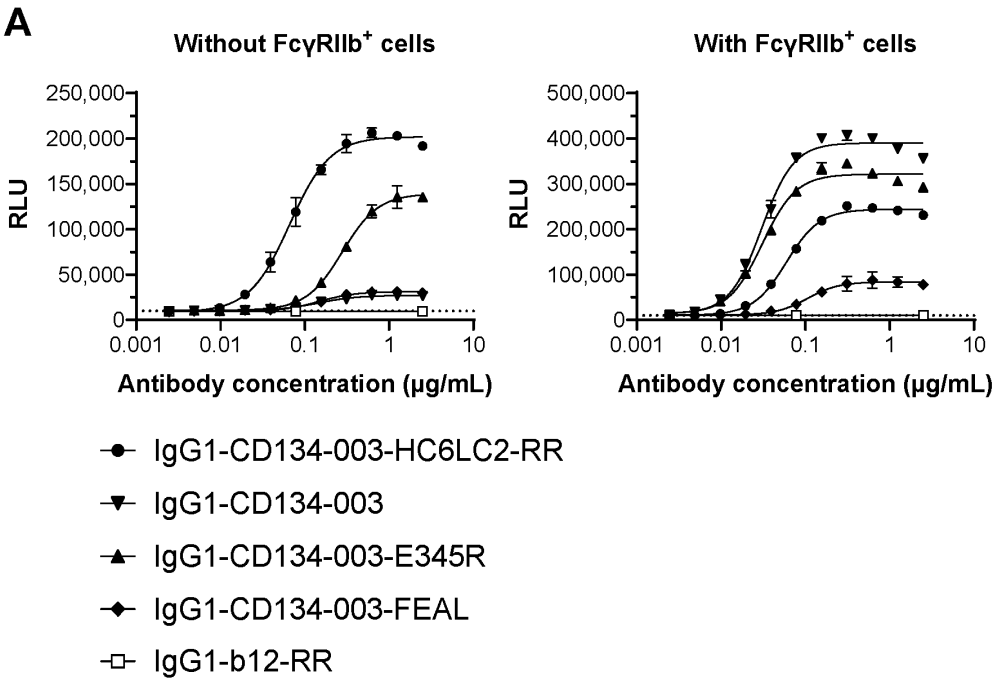

A
Without FcγRIIb+ cells
With FcγRIIb+ cells
RLU
250,000
200,000
150,000
100,000
50,000
0
500,000
400,000
300,000
200,000
100,000
0.001
0.01
0.1
1
10
Antibody concentration (µg/mL)
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-003
IgG1-CD134-003-E345R
IgG1-CD134-003-FEAL
IgG1-b12-RR

Figure 21 - *continued*

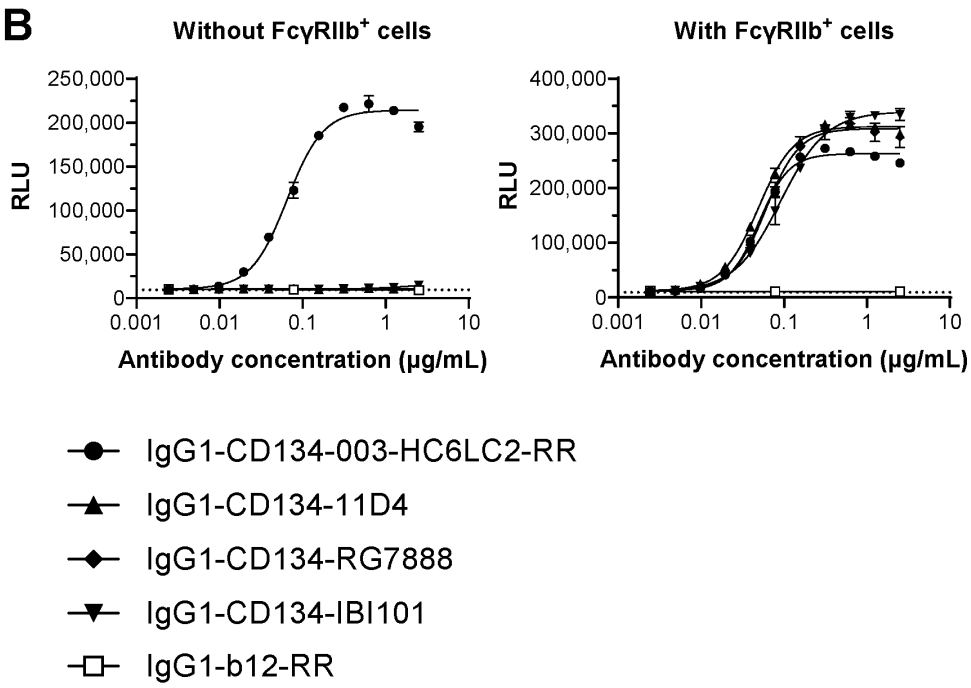


Figure 22

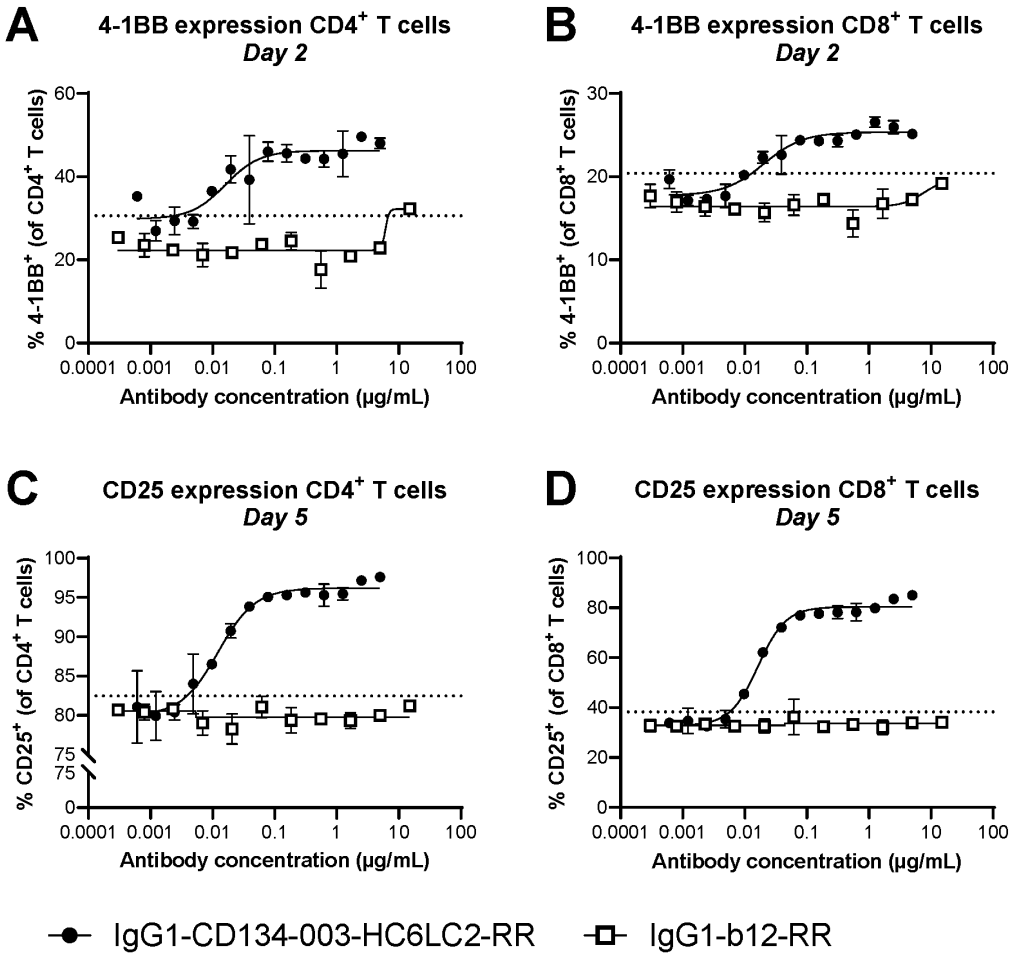

A
4-1BB expression CD4+ T cells
Day 2
% 4-1BB+ (of CD4+ T cells)
Antibody concentration (µg/mL)
B
4-1BB expression CD8+ T cells
Day 2
% 4-1BB+ (of CD8+ T cells)
Antibody concentration (µg/mL)
C
CD25 expression CD4+ T cells
Day 5
% CD25+ (of CD4+ T cells)
Antibody concentration (µg/mL)
D
CD25 expression CD8+ T cells
Day 5
% CD25+ (of CD8+ T cells)
Antibody concentration (µg/mL)
IgG1-CD134-003-HC6LC2-RR
IgG1-b12-RR

Figure 22 – *continued*

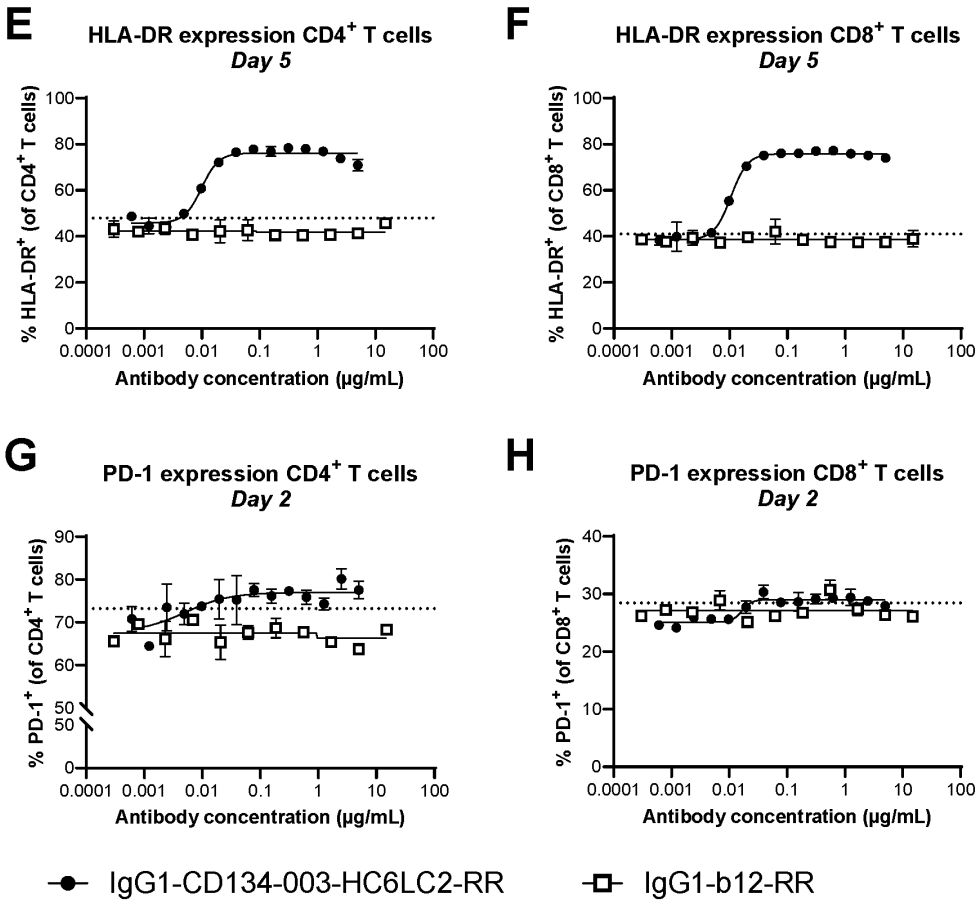


Figure 22 – *continued*

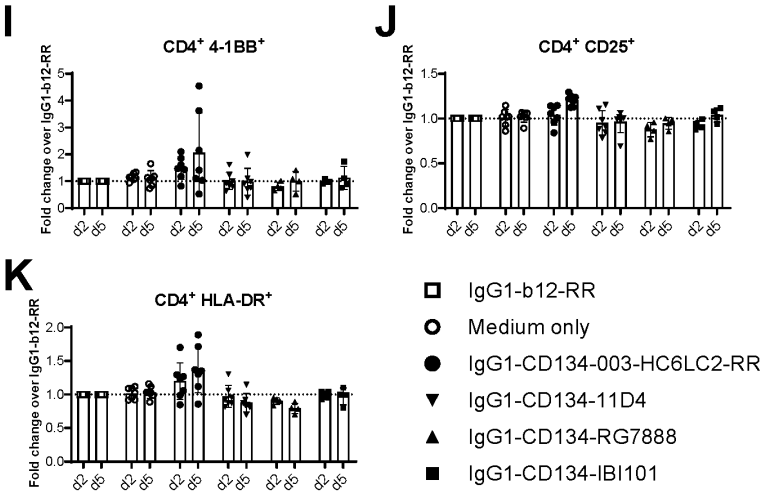


Figure 22 – *continued*

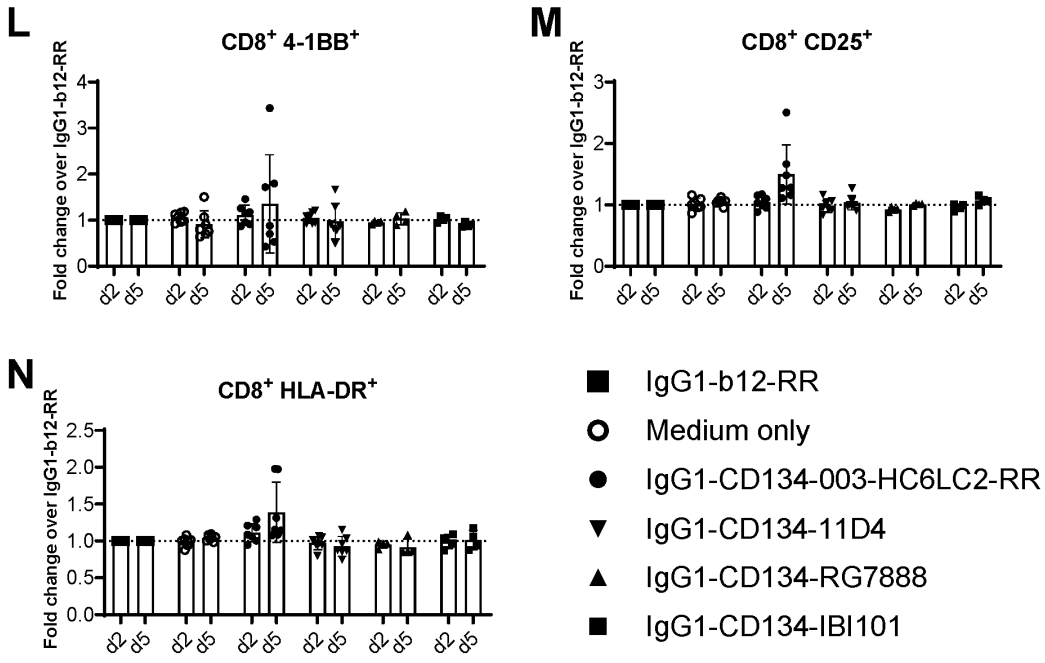


Figure 23

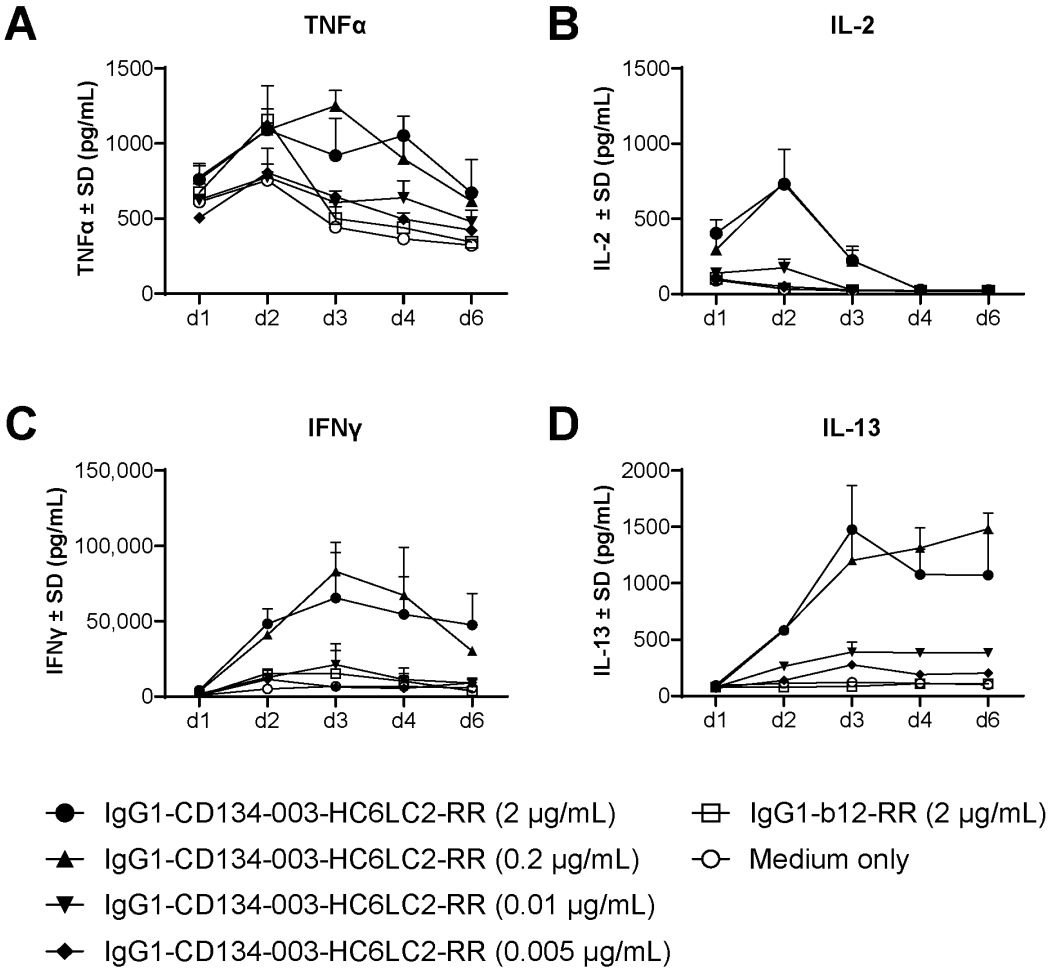

A
TNFα
TNFα ± SD (pg/mL)
1500
1000
500
0
d1
d2
d3
d4
d6
B
IL-2
IL-2 ± SD (pg/mL)
1500
1000
500
0
d1
d2
d3
d4
d6
C
IFNγ
IFNγ ± SD (pg/mL)
150,000
100,000
50,000
0
d1
d2
d3
d4
d6
D
IL-13
IL-13 ± SD (pg/mL)
2000
1500
1000
500
0
d1
d2
d3
d4
d6
IgG1-CD134-003-HC6LC2-RR (2 µg/mL)
IgG1-CD134-003-HC6LC2-RR (0.2 µg/mL)
IgG1-CD134-003-HC6LC2-RR (0.01 µg/mL)
IgG1-CD134-003-HC6LC2-RR (0.005 µg/mL)
IgG1-b12-RR (2 µg/mL)
Medium only

Figure 24

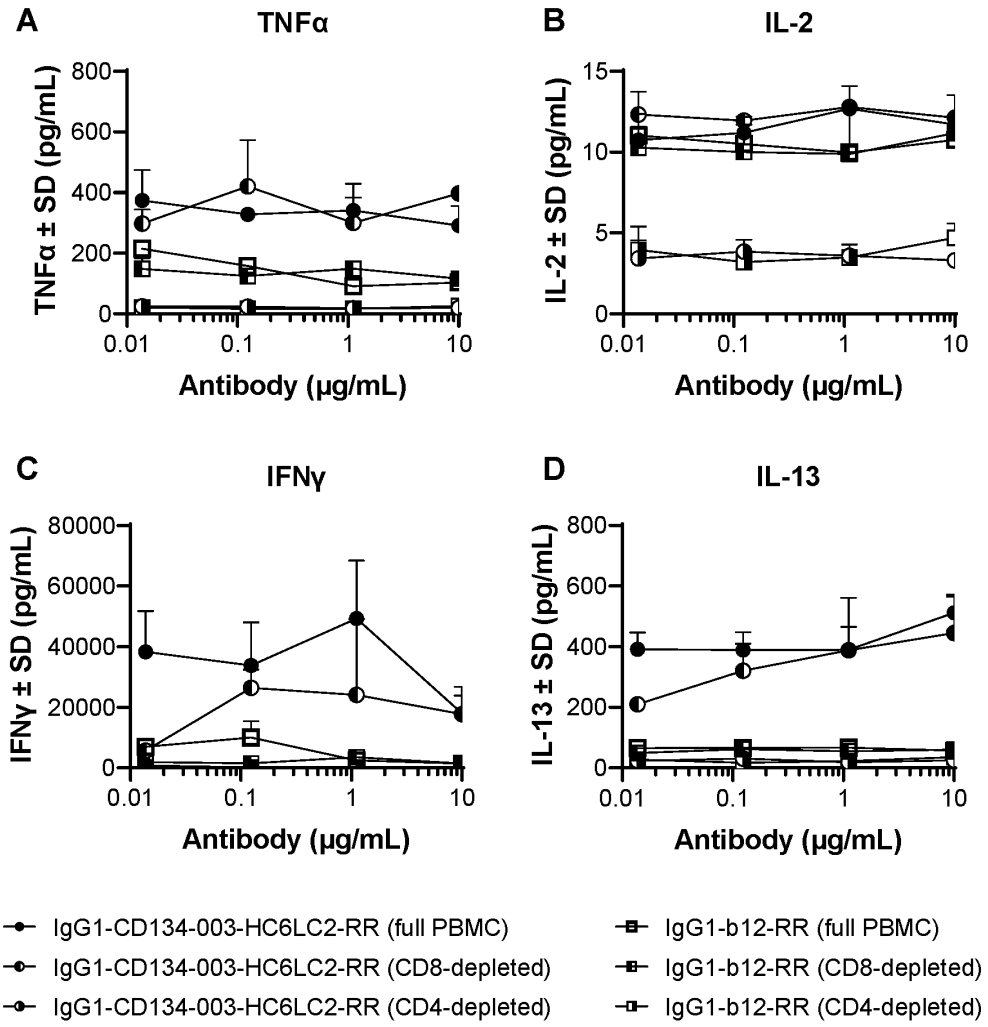

A
TNFα
TNFα ± SD (pg/mL)
800
600
400
200
0
0.01
0.1
1
10
Antibody (µg/mL)
B
IL-2
IL-2 ± SD (pg/mL)
15
10
5
0
0.01
0.1
1
10
Antibody (µg/mL)
C
IFNγ
IFNγ ± SD (pg/mL)
80000
60000
40000
20000
0
0.01
0.1
1
10
Antibody (µg/mL)
D
IL-13
IL-13 ± SD (pg/mL)
800
600
400
200
0
0.01
0.1
1
10
Antibody (µg/mL)
IgG1-CD134-003-HC6LC2-RR (full PBMC)
IgG1-CD134-003-HC6LC2-RR (CD8-depleted)
IgG1-CD134-003-HC6LC2-RR (CD4-depleted)
IgG1-b12-RR (full PBMC)
IgG1-b12-RR (CD8-depleted)
IgG1-b12-RR (CD4-depleted)

Figure 25

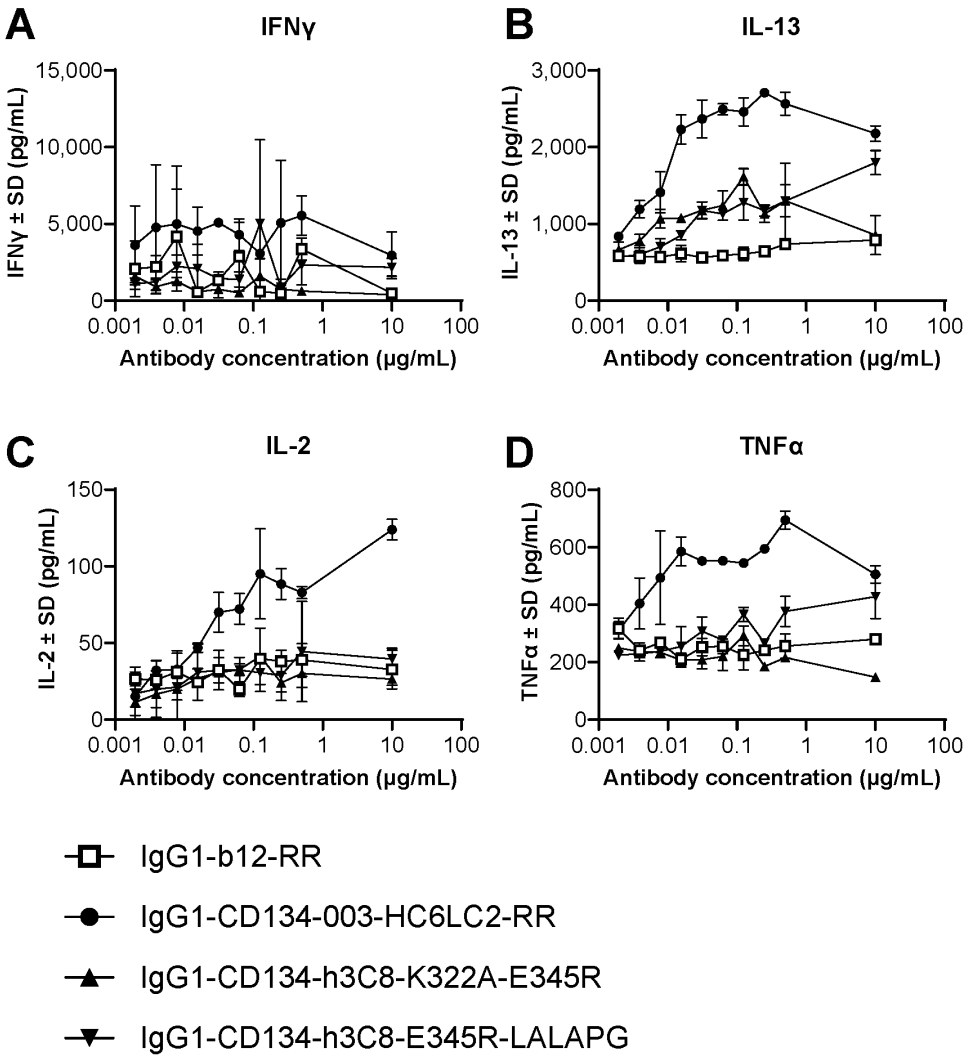

A
IFNγ
IFNγ ± SD (pg/mL)
15,000
10,000
5,000
0
0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
B
IL-13
IL-13 ± SD (pg/mL)
3,000
2,000
1,000
0
0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
C
IL-2
IL-2 ± SD (pg/mL)
150
100
50
0
0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
D
TNFα
TNFα ± SD (pg/mL)
800
600
400
200
0
0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
IgG1-b12-RR
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-h3C8-K322A-E345R
IgG1-CD134-h3C8-E345R-LALAPG

Figure 26

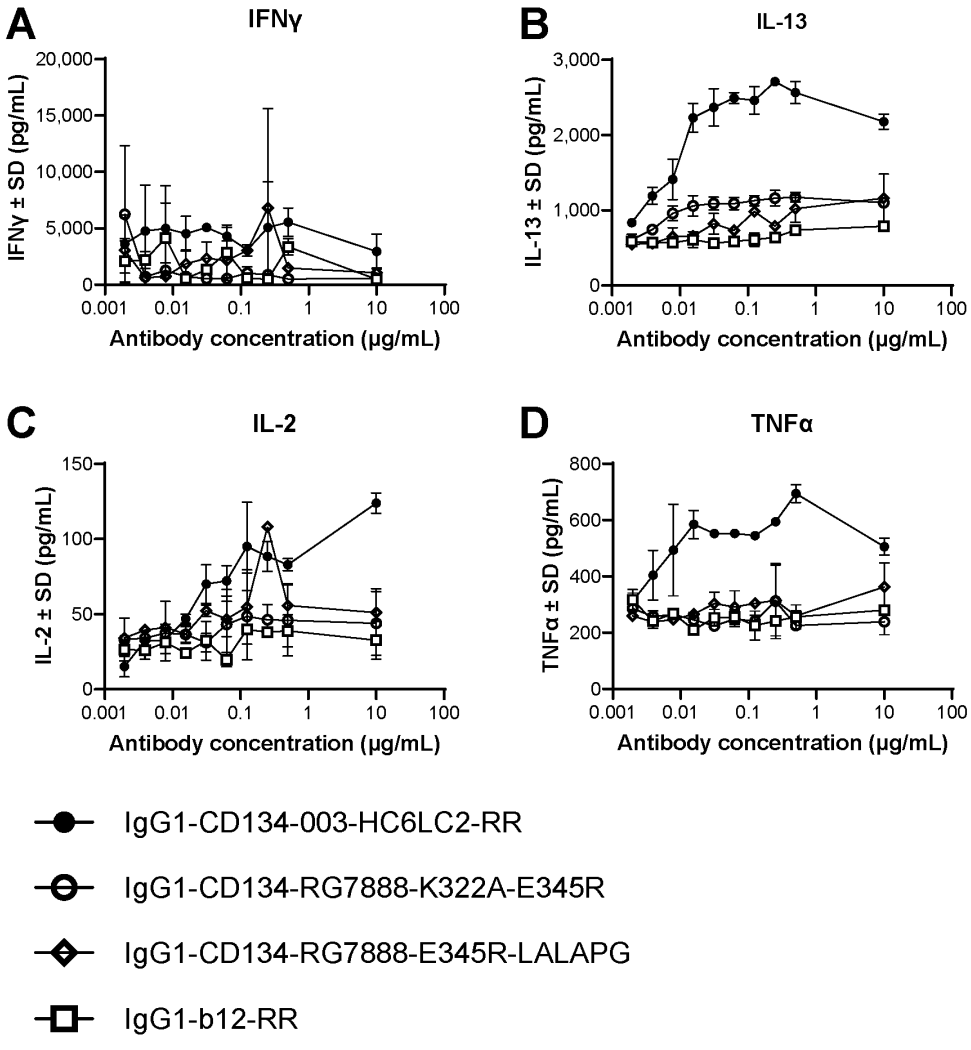

A
IFNγ
IFNγ ± SD (pg/mL)
20,000
15,000
10,000
5,000
0
0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
B
IL-13
IL-13 ± SD (pg/mL)
3,000
2,000
1,000
0
0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
C
IL-2
IL-2 ± SD (pg/mL)
150
100
50
0
0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
D
TNFα
TNFα ± SD (pg/mL)
800
600
400
200
0
0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-RG7888-K322A-E345R
IgG1-CD134-RG7888-E345R-LALAPG
IgG1-b12-RR

Figure 27

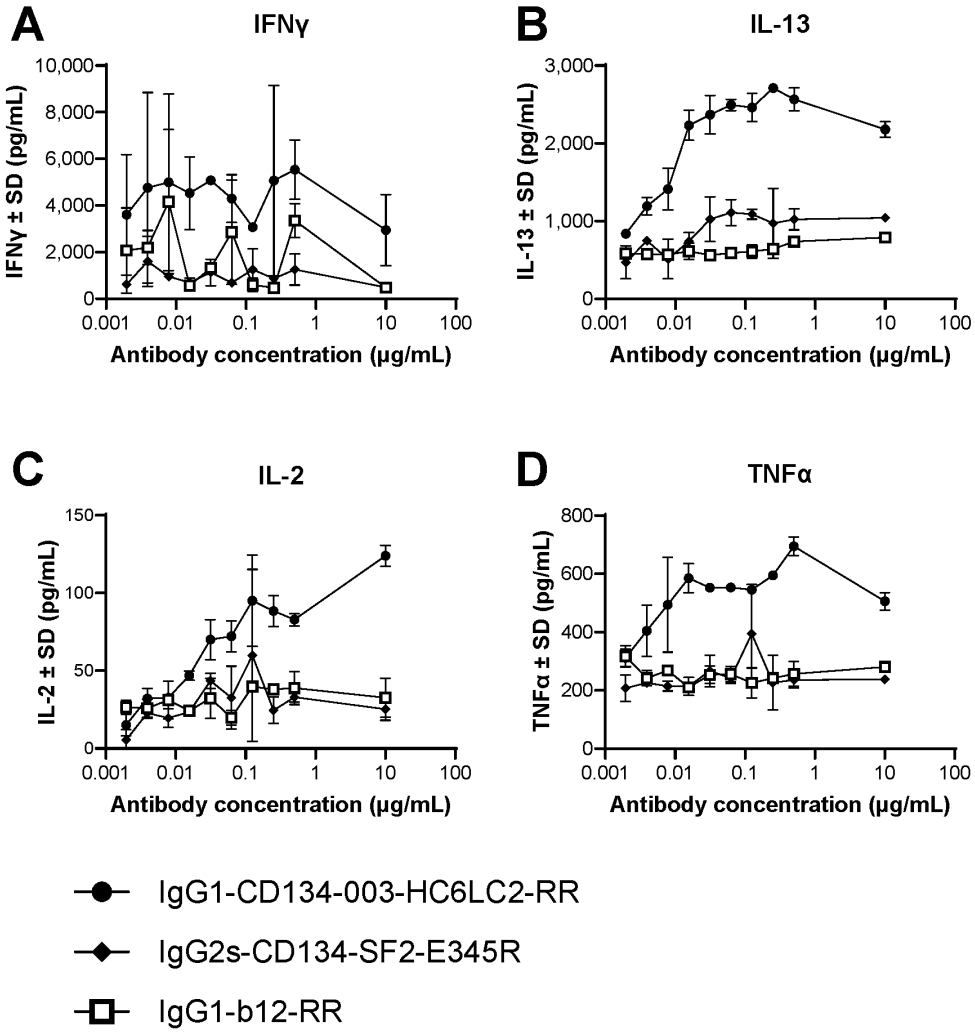

A
IFNγ
IFNγ ± SD (pg/mL)
10,000
8,000
6,000
4,000
2,000
0
0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
B
IL-13
IL-13 ± SD (pg/mL)
3,000
2,000
1,000
0
0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
C
IL-2
IL-2 ± SD (pg/mL)
150
100
50
0
0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
D
TNFα
TNFα ± SD (pg/mL)
800
600
400
200
0
0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
IgG1-CD134-003-HC6LC2-RR
IgG2s-CD134-SF2-E345R
IgG1-b12-RR

Figure 28

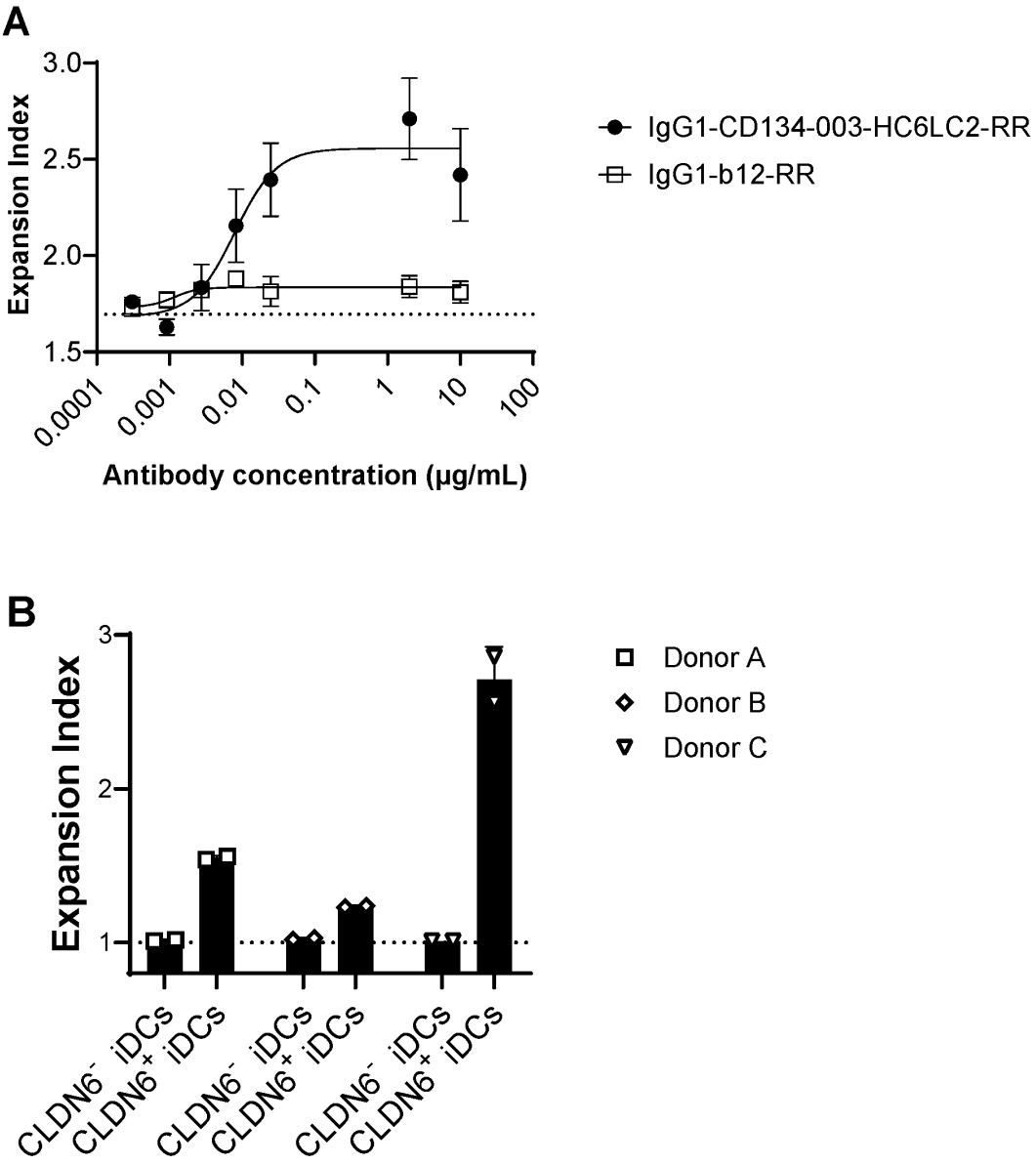

A
Expansion Index
3.0
2.5
2.0
1.5
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration (µg/mL)
IgG1-CD134-003-HC6LC2-RR
IgG1-b12-RR
B
Expansion Index
3
2
1
CLDN6- iDCs
CLDN6+ iDCs
CLDN6- iDCs
CLDN6+ iDCs
CLDN6- iDCs
CLDN6+ iDCs
Donor A
Donor B
Donor C

Figure 29

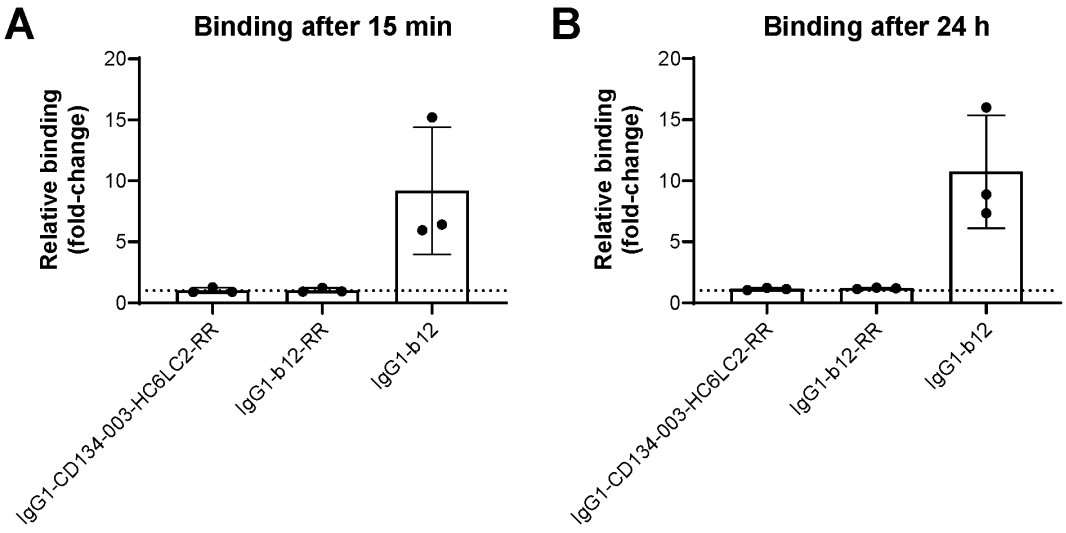

A
Binding after 15 min
Relative binding (fold-change)
20
15
10
5
0
IgG1-CD134-003-HC6LC2-RR
IgG1-b12-RR
IgG1-b12
B
Binding after 24 h
Relative binding (fold-change)
20
15
10
5
0
IgG1-CD134-003-HC6LC2-RR
IgG1-b12-RR
IgG1-b12

Figure 30

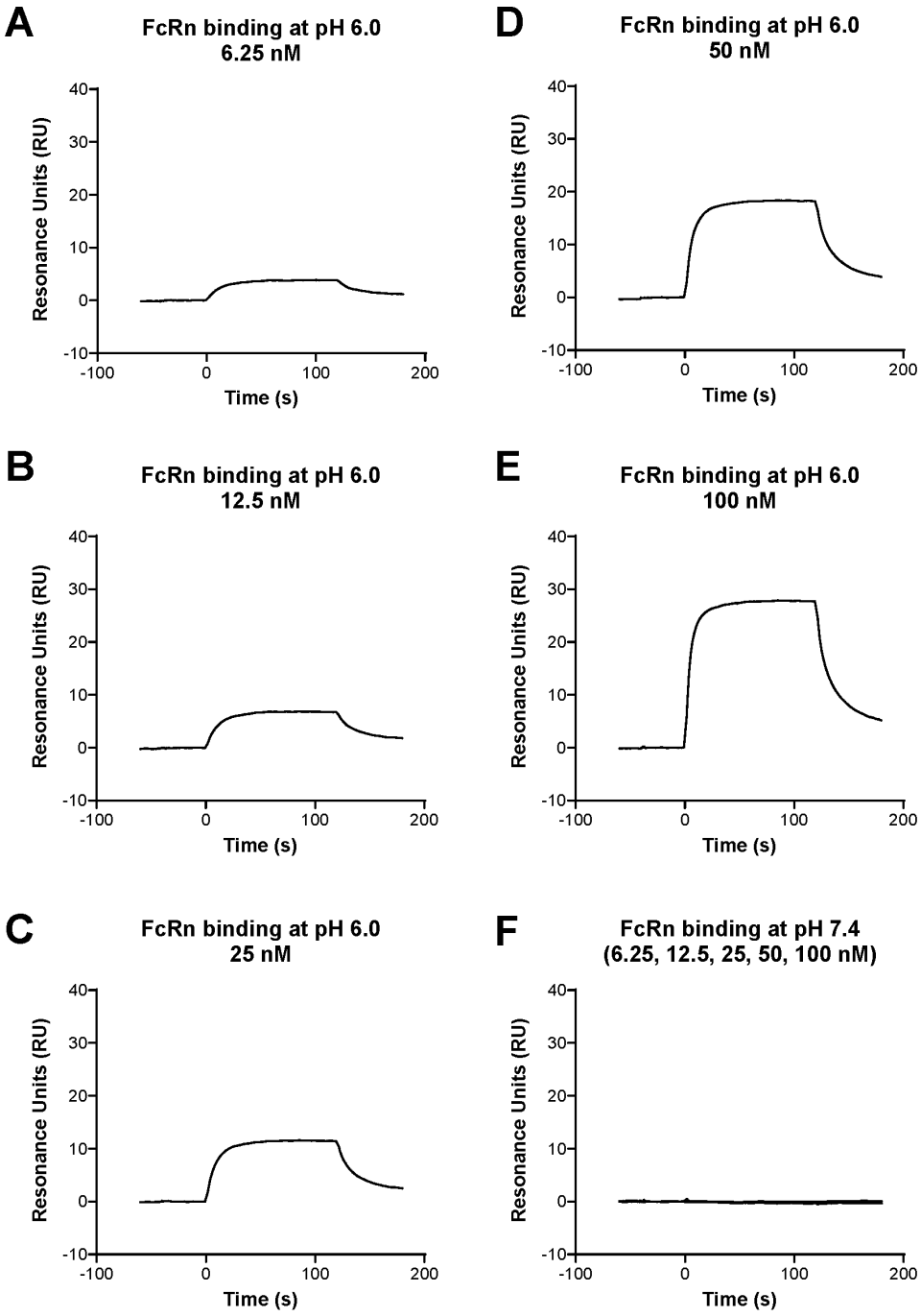

A
FcRn binding at pH 6.0
6.25 nM
D
FcRn binding at pH 6.0
50 nM
B
FcRn binding at pH 6.0
12.5 nM
E
FcRn binding at pH 6.0
100 nM
C
FcRn binding at pH 6.0
25 nM
F
FcRn binding at pH 7.4
(6.25, 12.5, 25, 50, 100 nM)
Resonance Units (RU)
Time (s)
40
30
20
10
0
-10
-100
0
100
200

Figure 31

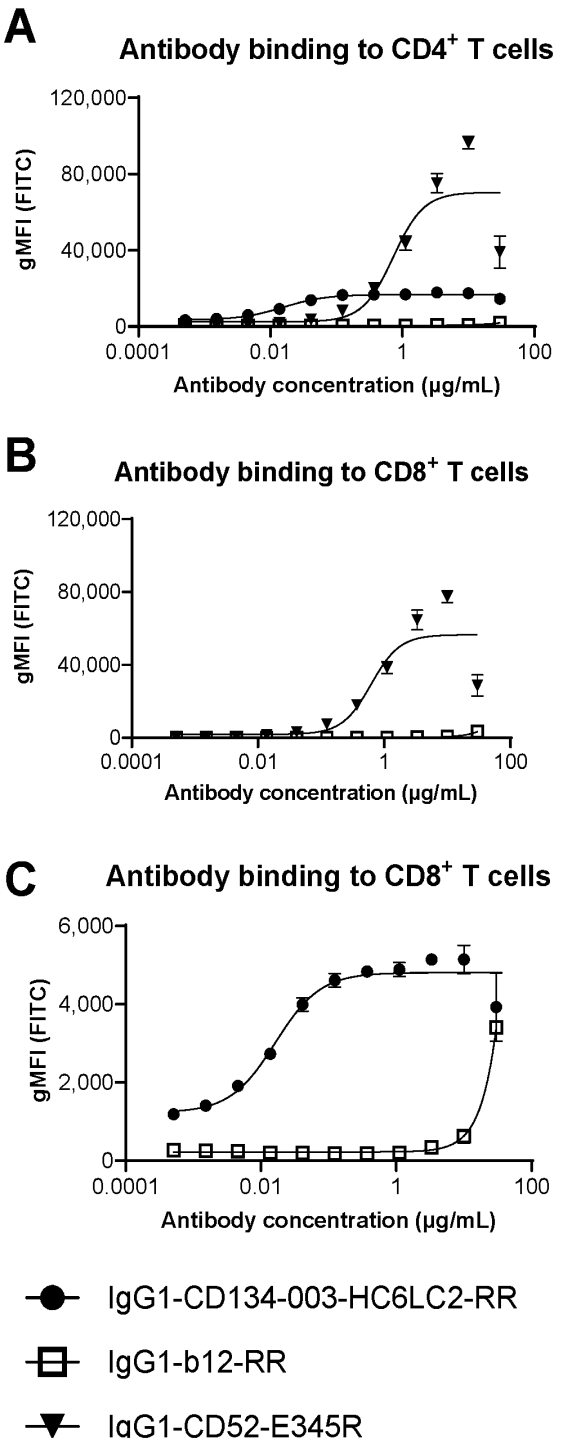

A
Antibody binding to CD4+ T cells
gMFI (FITC)
120,000
80,000
40,000
0
0.0001
0.01
1
100
Antibody concentration (µg/mL)
B
Antibody binding to CD8+ T cells
gMFI (FITC)
120,000
80,000
40,000
0
0.0001
0.01
1
100
Antibody concentration (µg/mL)
C
Antibody binding to CD8+ T cells
gMFI (FITC)
6,000
4,000
2,000
0
0.0001
0.01
1
100
Antibody concentration (µg/mL)
IgG1-CD134-003-HC6LC2-RR
IgG1-b12-RR
IgG1-CD52-E345R

Figure 31 ***continued***

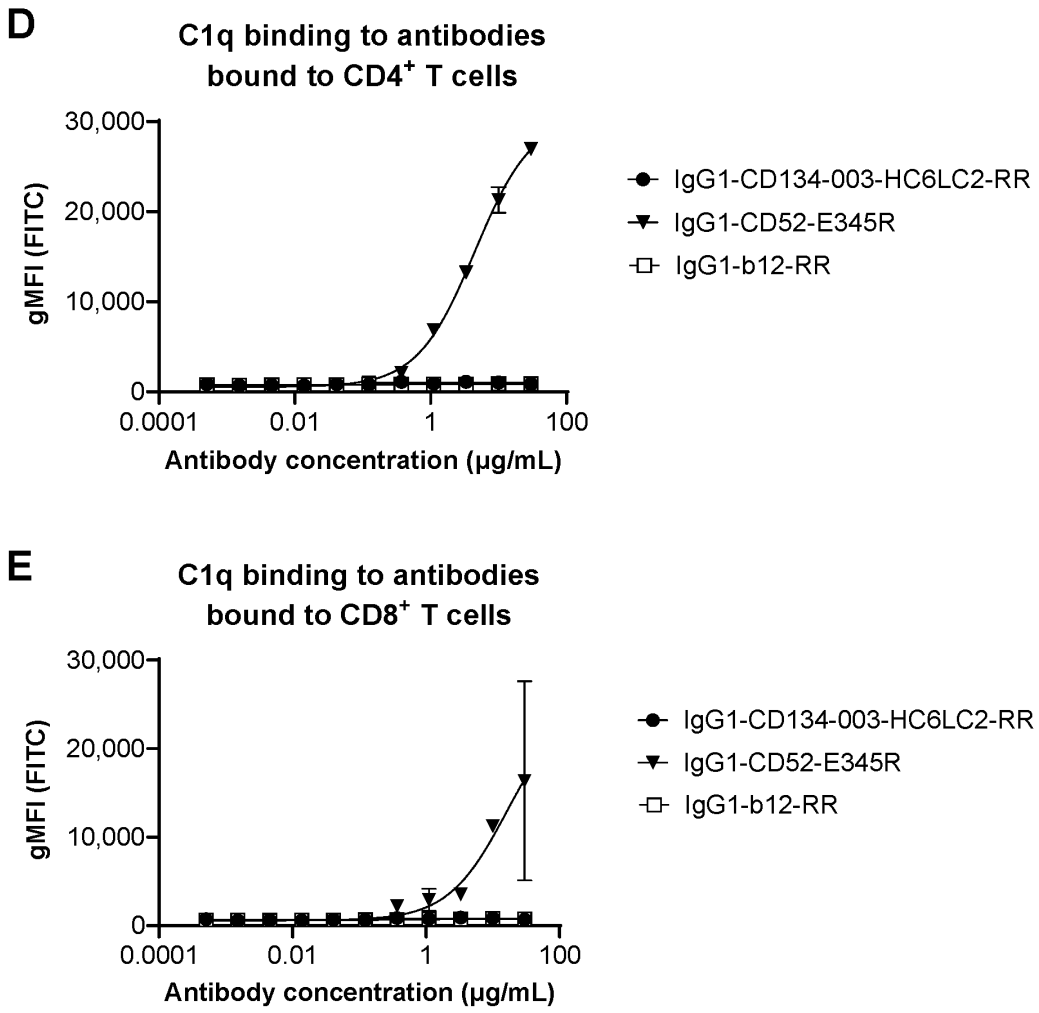


Figure 32

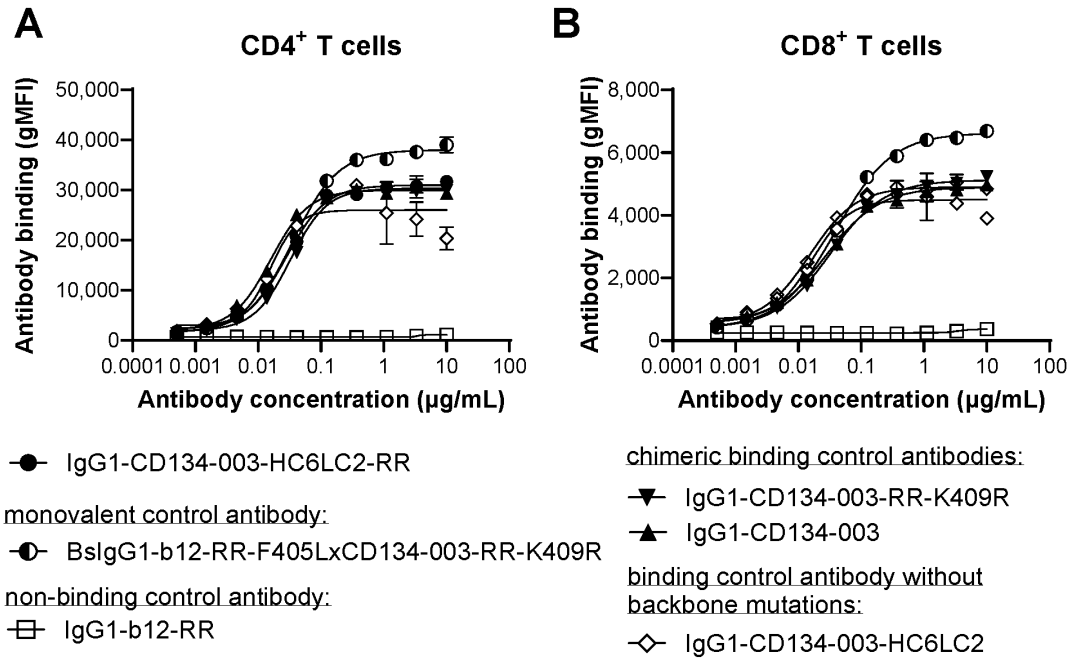

A
CD4+ T cells
Antibody binding (gMFI)
50,000
40,000
30,000
20,000
10,000
0
0.0001 0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
B
CD8+ T cells
Antibody binding (gMFI)
8,000
6,000
4,000
2,000
0
0.0001 0.001 0.01 0.1 1 10 100
Antibody concentration (µg/mL)
IgG1-CD134-003-HC6LC2-RR
monovalent control antibody:
BsIgG1-b12-RR-F405LxCD134-003-RR-K409R
non-binding control antibody:
IgG1-b12-RR
chimeric binding control antibodies:
IgG1-CD134-003-RR-K409R
IgG1-CD134-003
binding control antibody without backbone mutations:
IgG1-CD134-003-HC6LC2

Figure 33

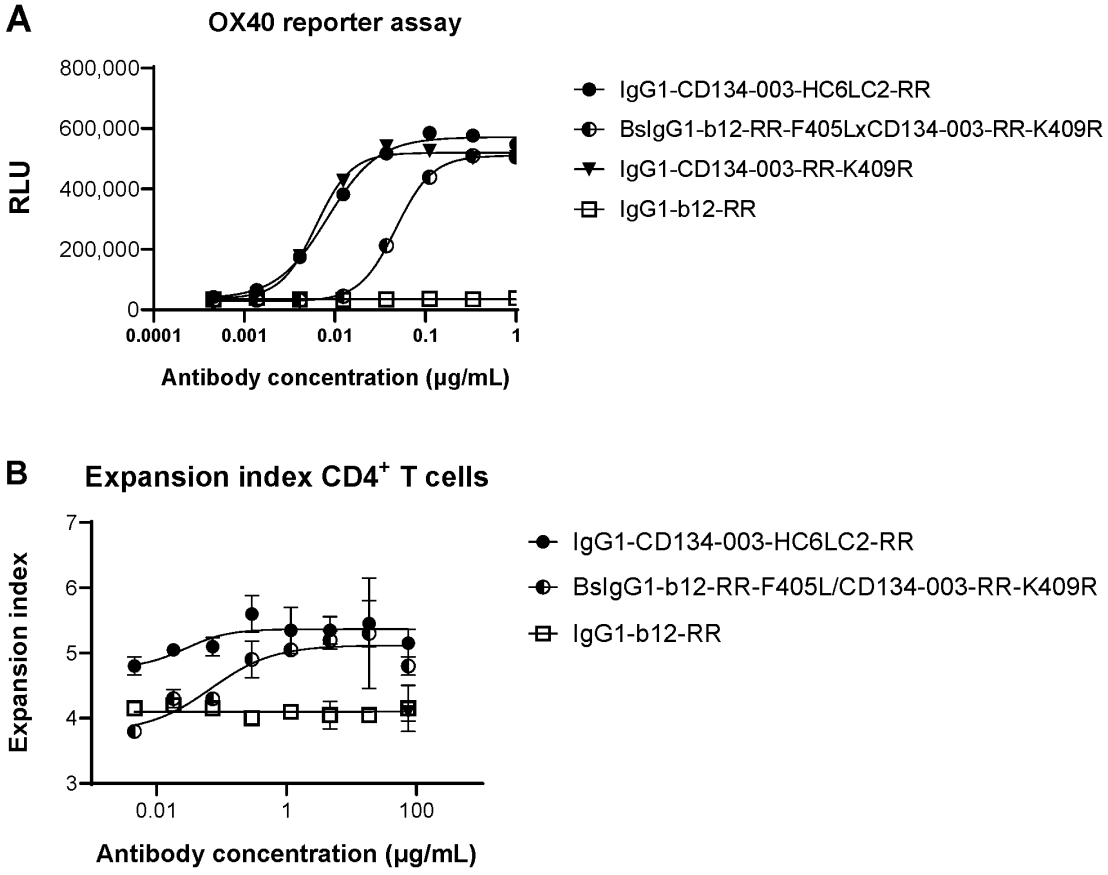

A
OX40 reporter assay
RLU
800,000
600,000
400,000
200,000
0
0.0001
0.001
0.01
0.1
1
Antibody concentration (µg/mL)
IgG1-CD134-003-HC6LC2-RR
BsIgG1-b12-RR-F405LxCD134-003-RR-K409R
IgG1-CD134-003-RR-K409R
IgG1-b12-RR
B
Expansion index CD4+ T cells
Expansion index
7
6
5
4
3
0.01
1
100
Antibody concentration (µg/mL)
IgG1-CD134-003-HC6LC2-RR
BsIgG1-b12-RR-F405L/CD134-003-RR-K409R
IgG1-b12-RR

Figure 34

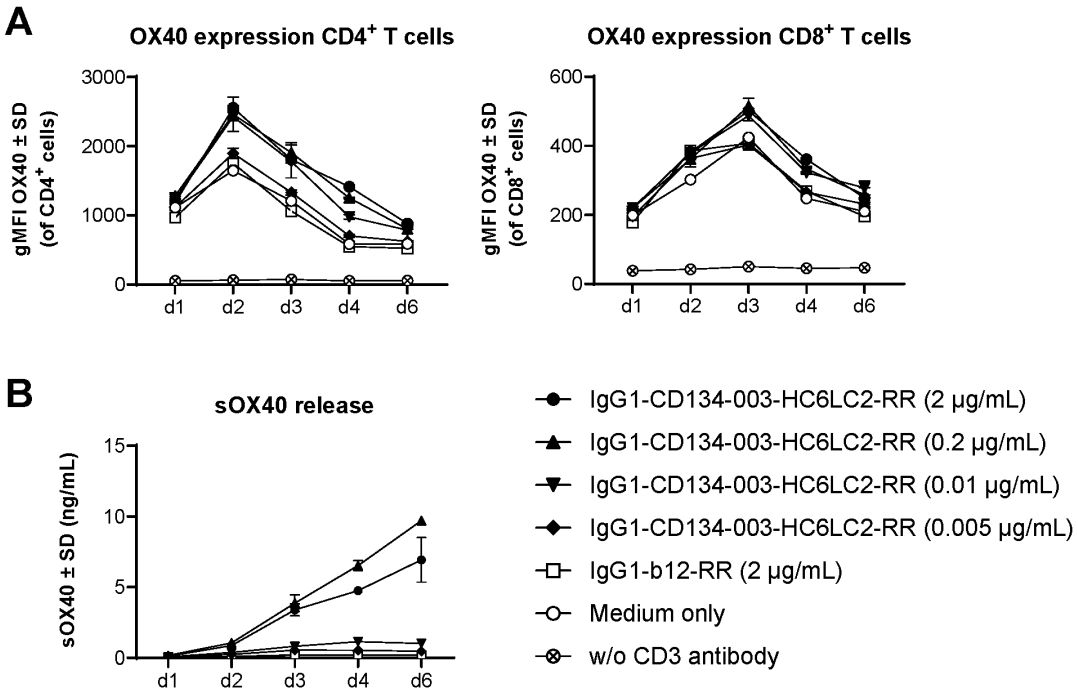

A
OX40 expression CD4+ T cells
gMFI OX40 ± SD (of CD4+ cells)
3000
2000
1000
0
d1
d2
d3
d4
d6
OX40 expression CD8+ T cells
gMFI OX40 ± SD (of CD8+ cells)
600
400
200
0
d1
d2
d3
d4
d6
B
sOX40 release
sOX40 ± SD (ng/mL)
15
10
5
0
d1
d2
d3
d4
d6
IgG1-CD134-003-HC6LC2-RR (2 µg/mL)
IgG1-CD134-003-HC6LC2-RR (0.2 µg/mL)
IgG1-CD134-003-HC6LC2-RR (0.01 µg/mL)
IgG1-CD134-003-HC6LC2-RR (0.005 µg/mL)
IgG1-b12-RR (2 µg/mL)
Medium only
w/o CD3 antibody

Figure 35

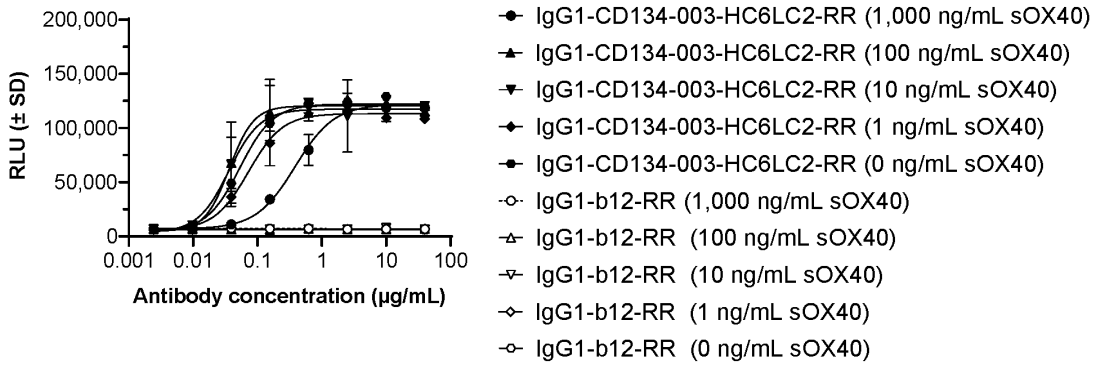

200,000
150,000
100,000
50,000
0
RLU (± SD)
0.001
0.01
0.1
1
10
100
Antibody concentration (µg/mL)
IgG1-CD134-003-HC6LC2-RR (1,000 ng/mL sOX40)
IgG1-CD134-003-HC6LC2-RR (100 ng/mL sOX40)
IgG1-CD134-003-HC6LC2-RR (10 ng/mL sOX40)
IgG1-CD134-003-HC6LC2-RR (1 ng/mL sOX40)
IgG1-CD134-003-HC6LC2-RR (0 ng/mL sOX40)
IgG1-b12-RR (1,000 ng/mL sOX40)
IgG1-b12-RR (100 ng/mL sOX40)
IgG1-b12-RR (10 ng/mL sOX40)
IgG1-b12-RR (1 ng/mL sOX40)
IgG1-b12-RR (0 ng/mL sOX40)

Figure 36

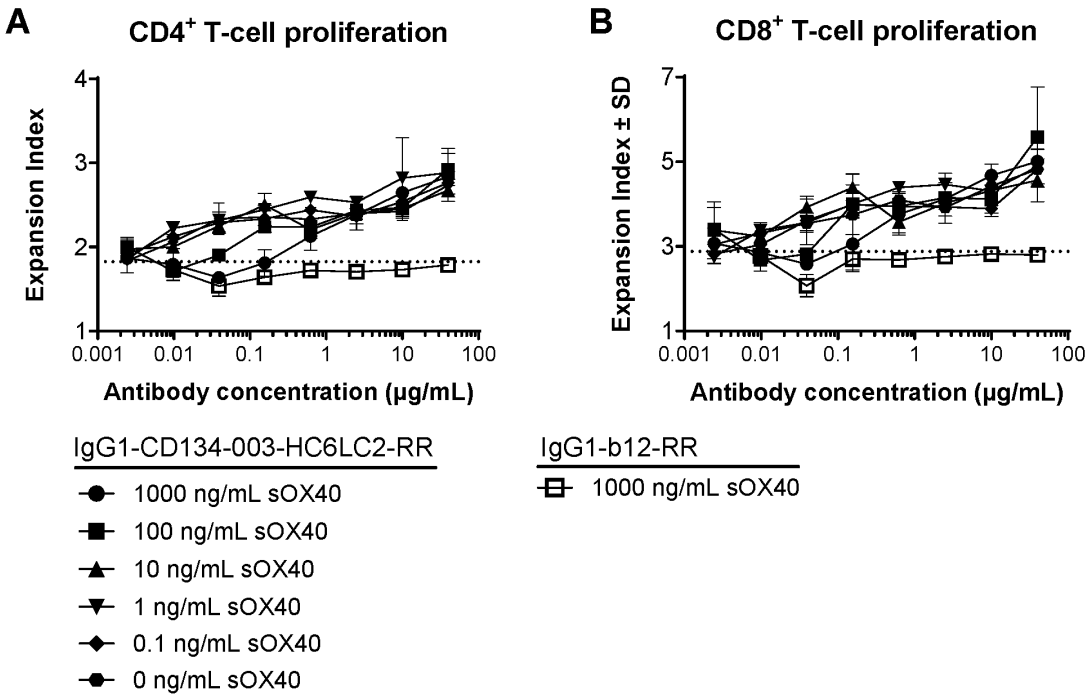

A
CD4+ T-cell proliferation
Expansion Index
Antibody concentration (µg/mL)
B
CD8+ T-cell proliferation
Expansion Index ± SD
Antibody concentration (µg/mL)
IgG1-CD134-003-HC6LC2-RR
1000 ng/mL sOX40
100 ng/mL sOX40
10 ng/mL sOX40
1 ng/mL sOX40
0.1 ng/mL sOX40
0 ng/mL sOX40
IgG1-b12-RR
1000 ng/mL sOX40

Figure 37

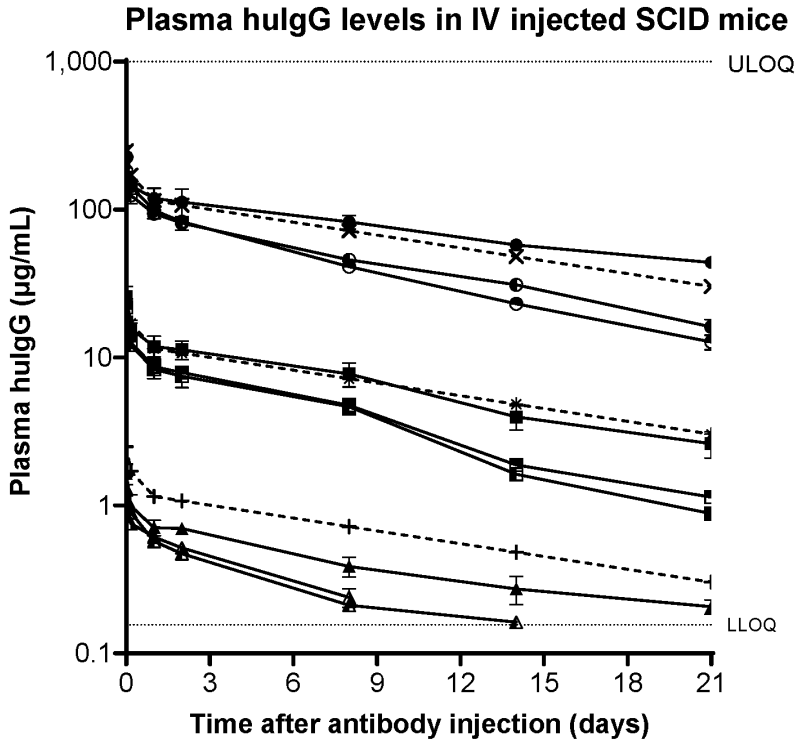

Plasma huIgG levels in IV injected SCID mice
1,000
100
10
1
0.1
ULOQ
LLOQ
Plasma huIgG (µg/mL)
0
3
6
9
12
15
18
21
Time after antibody injection (days)
12.5 mg/kg IgG1-CD134-003-HC6LC2-RR
12.5 mg/kg IgG1-CD134-003-FEAL
12.5 mg/kg IgG1-CD134-003-RR-K409R
12.5 mg/kg WT huIgG (predicted)
1.25 mg/kg IgG1-CD134-003-HC6LC2-RR
1.25 mg/kg IgG1-CD134-003-FEAL
1.25 mg/kg IgG1-CD134-003-RR-K409R
1.25 mg/kg WT huIgG (predicted)
0.125 mg/kg IgG1-CD134-003-HC6LC2-RR
0.125 mg/kg IgG1-CD134-003-FEAL
0.125 mg/kg IgG1-CD134-003-RR-K409R
0.125 mg/kg WT huIgG (predicted)

Figure 38

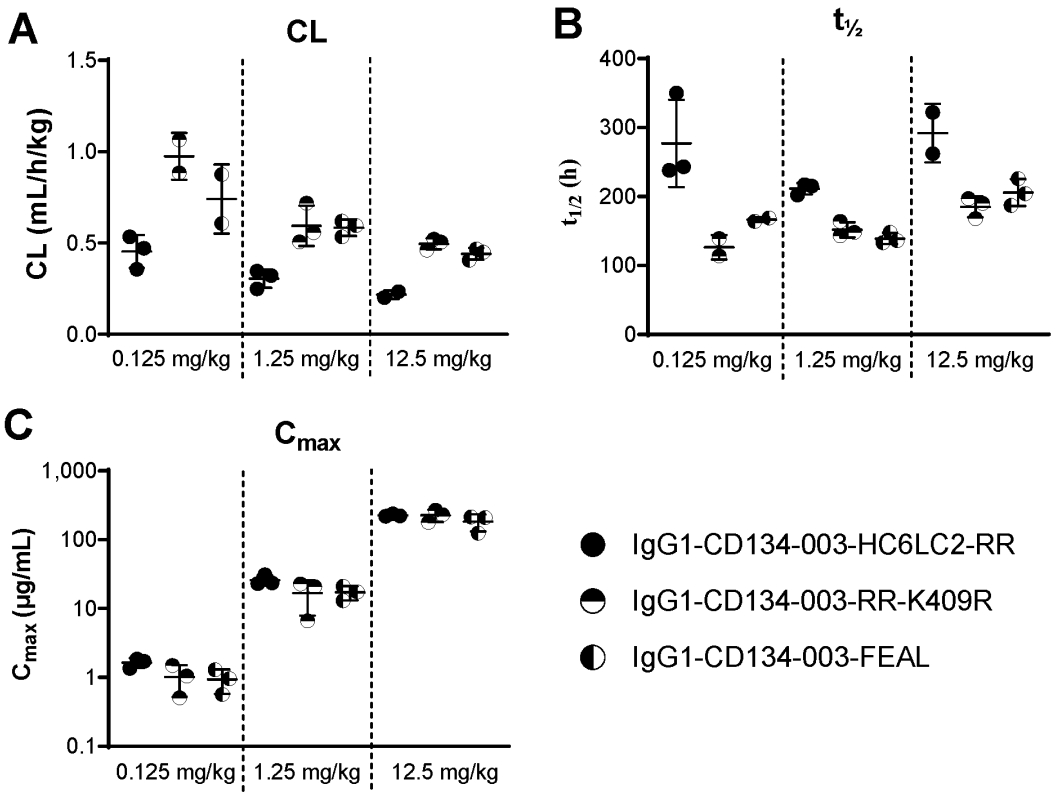

A
CL
CL (mL/h/kg)
1.5
1.0
0.5
0.0
0.125 mg/kg
1.25 mg/kg
12.5 mg/kg
B
t½
t1/2 (h)
400
300
200
100
0
0.125 mg/kg
1.25 mg/kg
12.5 mg/kg
C
Cmax
Cmax (µg/mL)
1,000
100
10
1
0.1
0.125 mg/kg
1.25 mg/kg
12.5 mg/kg
IgG1-CD134-003-HC6LC2-RR
IgG1-CD134-003-RR-K409R
IgG1-CD134-003-FEAL

Figure 39

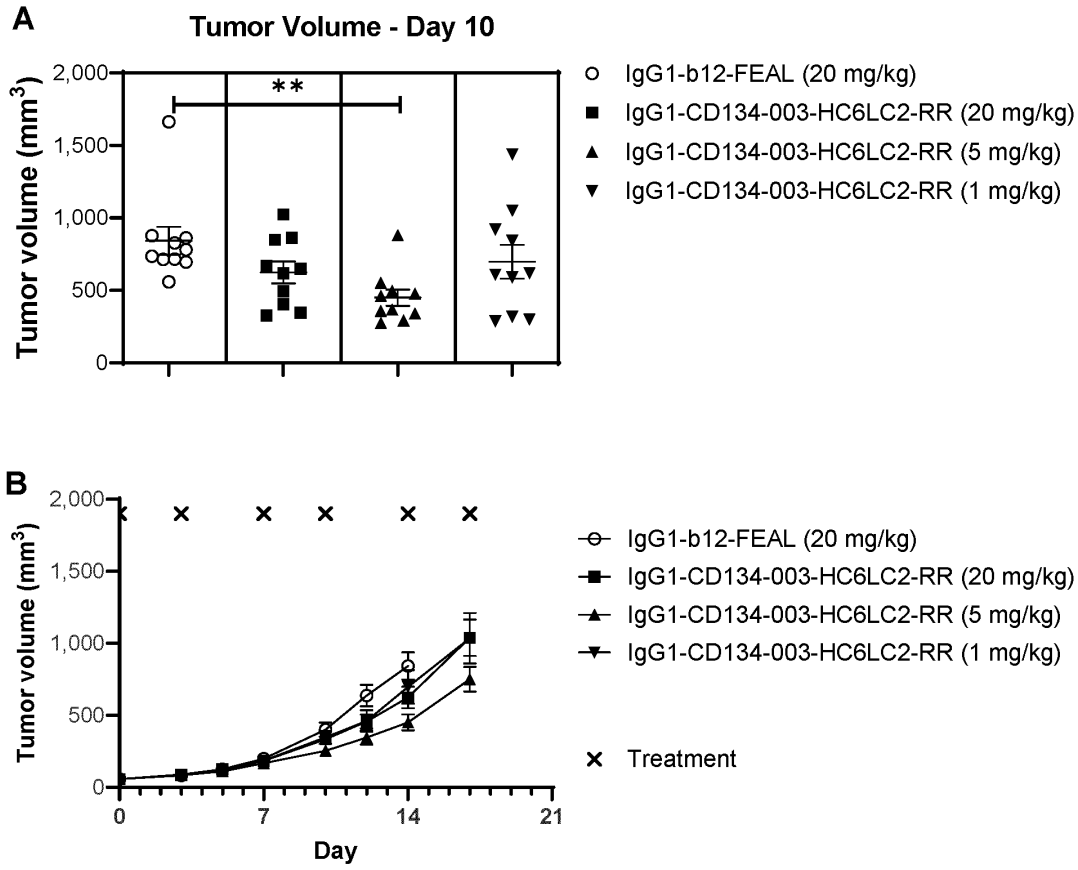

A
Tumor Volume - Day 10
Tumor volume (mm3)
2,000
1,500
1,000
500
0
**
IgG1-b12-FEAL (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (5 mg/kg)
IgG1-CD134-003-HC6LC2-RR (1 mg/kg)
B
Tumor volume (mm3)
2,000
1,500
1,000
500
0
0
7
14
21
Day
IgG1-b12-FEAL (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (5 mg/kg)
IgG1-CD134-003-HC6LC2-RR (1 mg/kg)
Treatment

Figure 39 - *continued*

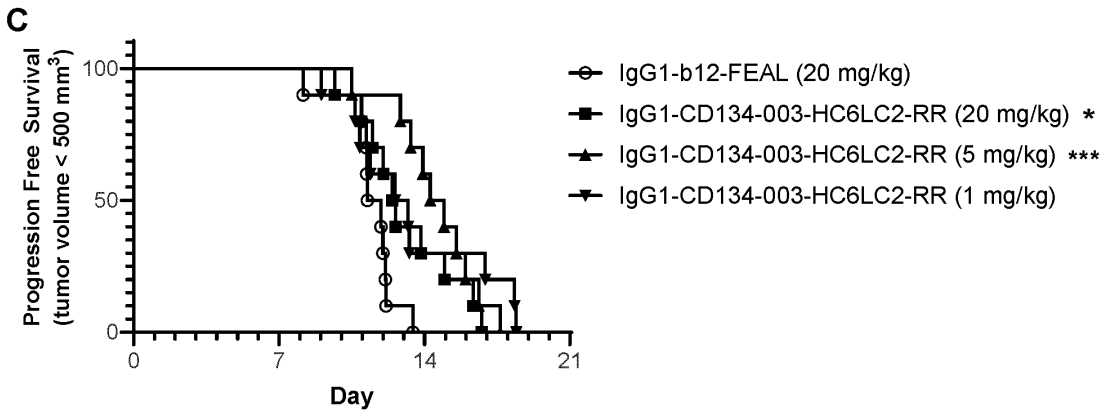


Figure 40

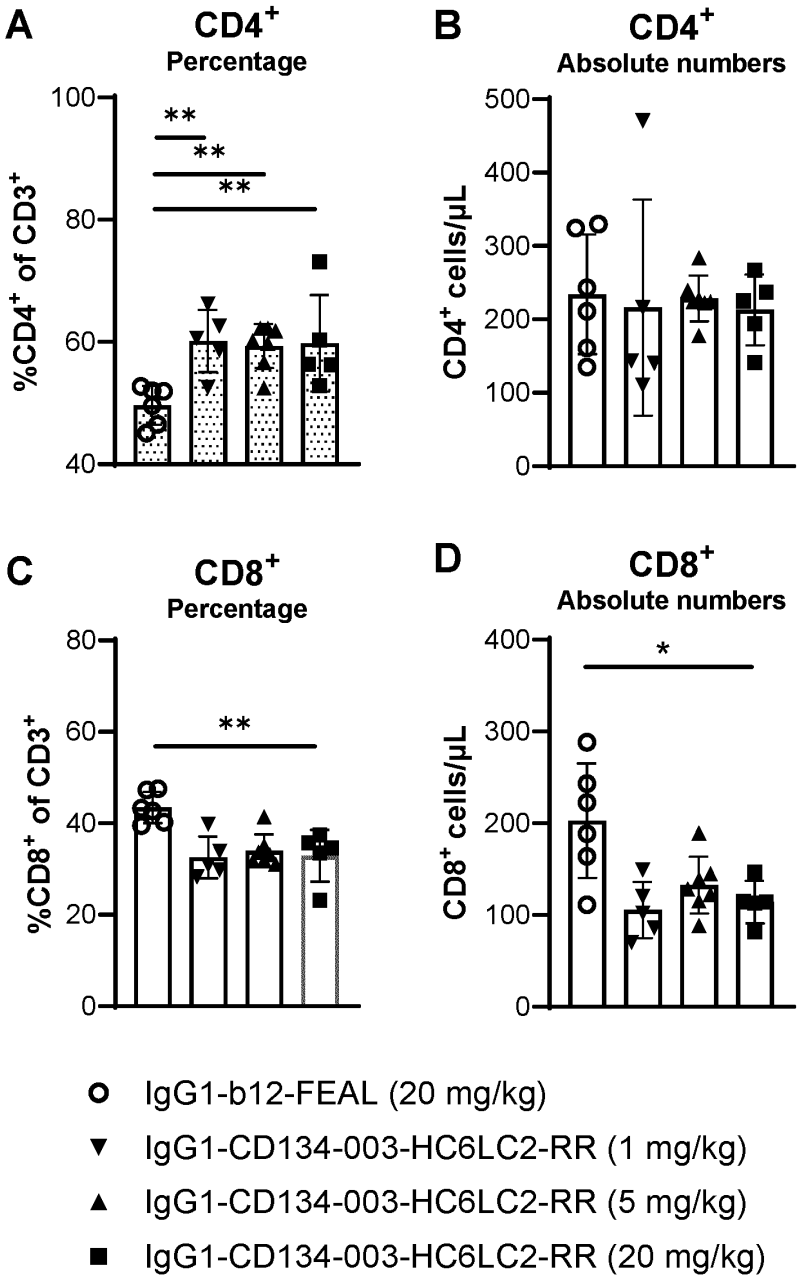

A
CD4+
Percentage
%CD4+ of CD3+
100
80
60
40
**
**
**
B
CD4+
Absolute numbers
CD4+ cells/µL
500
400
300
200
100
0
C
CD8+
Percentage
%CD8+ of CD3+
80
60
40
20
0
**
D
CD8+
Absolute numbers
CD8+ cells/µL
400
300
200
100
0
*
IgG1-b12-FEAL (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (1 mg/kg)
IgG1-CD134-003-HC6LC2-RR (5 mg/kg)
IgG1-CD134-003-HC6LC2-RR (20 mg/kg)

Figure 41

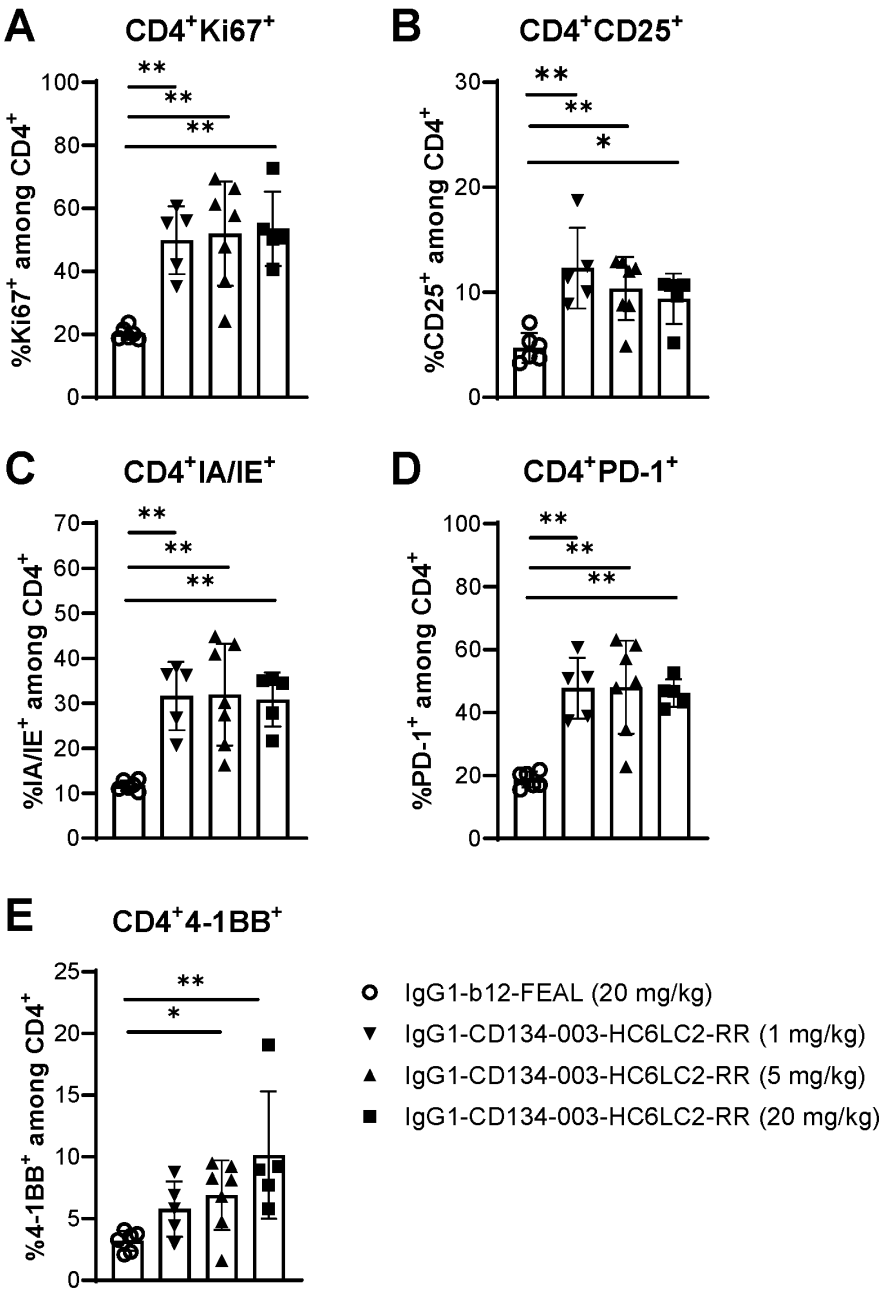

A
CD4+Ki67+
%Ki67+ among CD4+
B
CD4+CD25+
%CD25+ among CD4+
C
CD4+IA/IE+
%IA/IE+ among CD4+
D
CD4+PD-1+
%PD-1+ among CD4+
E
CD4+4-1BB+
%4-1BB+ among CD4+
IgG1-b12-FEAL (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (1 mg/kg)
IgG1-CD134-003-HC6LC2-RR (5 mg/kg)
IgG1-CD134-003-HC6LC2-RR (20 mg/kg)

Figure 42

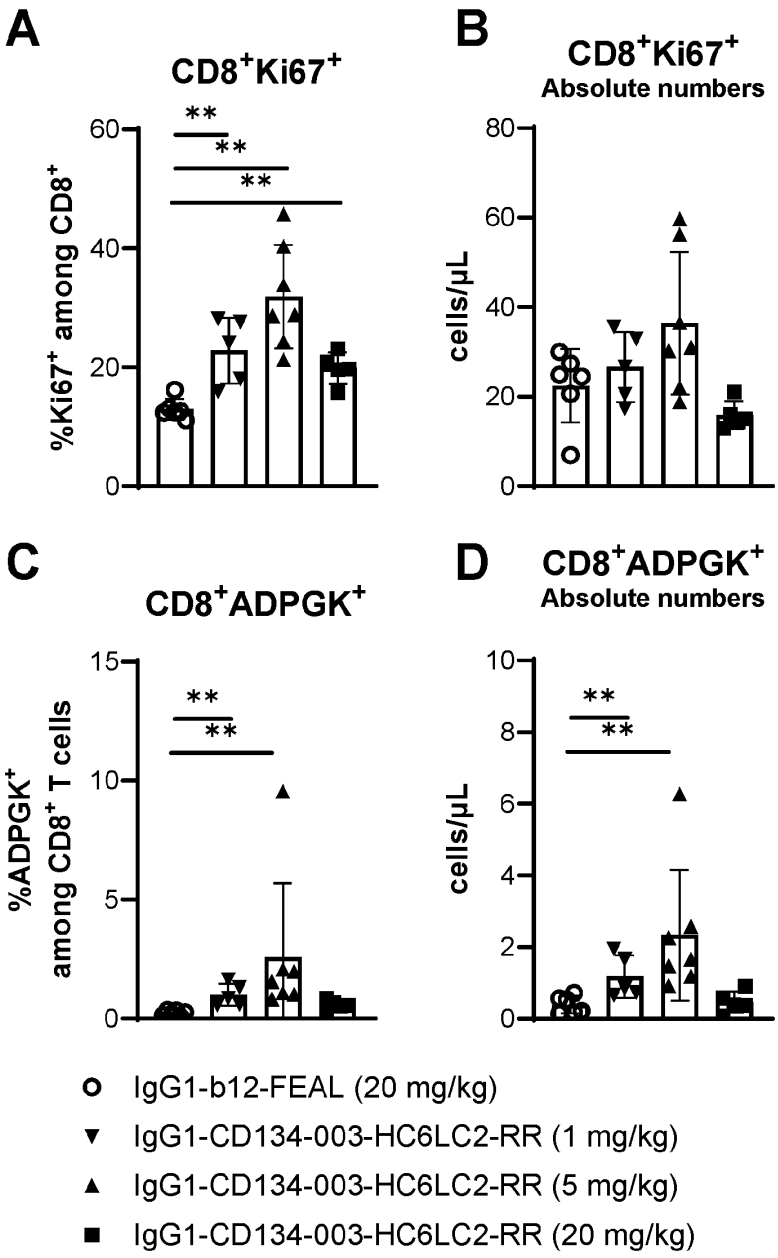

A
CD8+Ki67+
%Ki67+ among CD8+
60
40
20
0
**
**
**
B
CD8+Ki67+
Absolute numbers
cells/µL
80
60
40
20
0
C
CD8+ADPGK+
%ADPGK+ among CD8+ T cells
15
10
5
0
**
**
D
CD8+ADPGK+
Absolute numbers
cells/µL
10
8
6
4
2
0
**
**
IgG1-b12-FEAL (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (1 mg/kg)
IgG1-CD134-003-HC6LC2-RR (5 mg/kg)
IgG1-CD134-003-HC6LC2-RR (20 mg/kg)

Figure 43

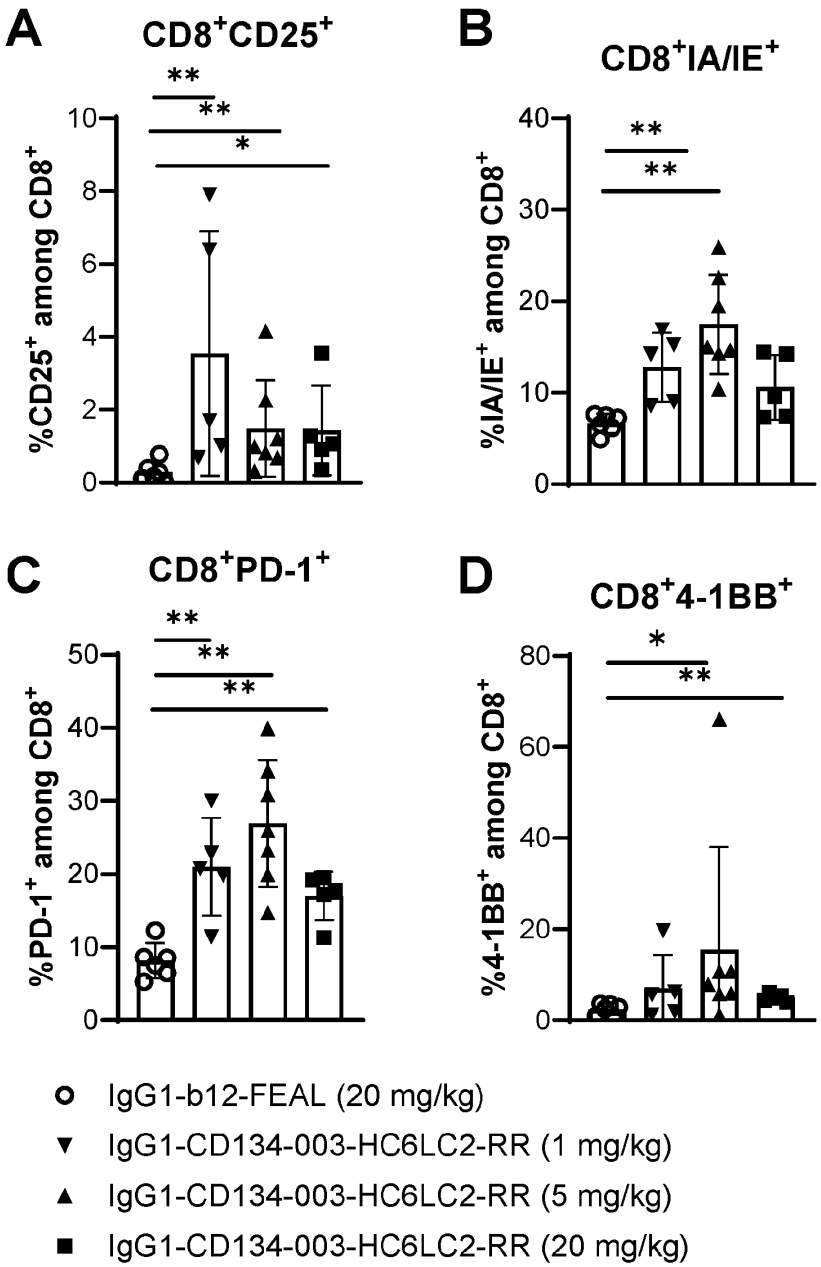

A
CD8+CD25+
%CD25+ among CD8+
B
CD8+IA/IE+
%IA/IE+ among CD8+
C
CD8+PD-1+
%PD-1+ among CD8+
D
CD8+4-1BB+
%4-1BB+ among CD8+
IgG1-b12-FEAL (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (1 mg/kg)
IgG1-CD134-003-HC6LC2-RR (5 mg/kg)
IgG1-CD134-003-HC6LC2-RR (20 mg/kg)

Figure 44

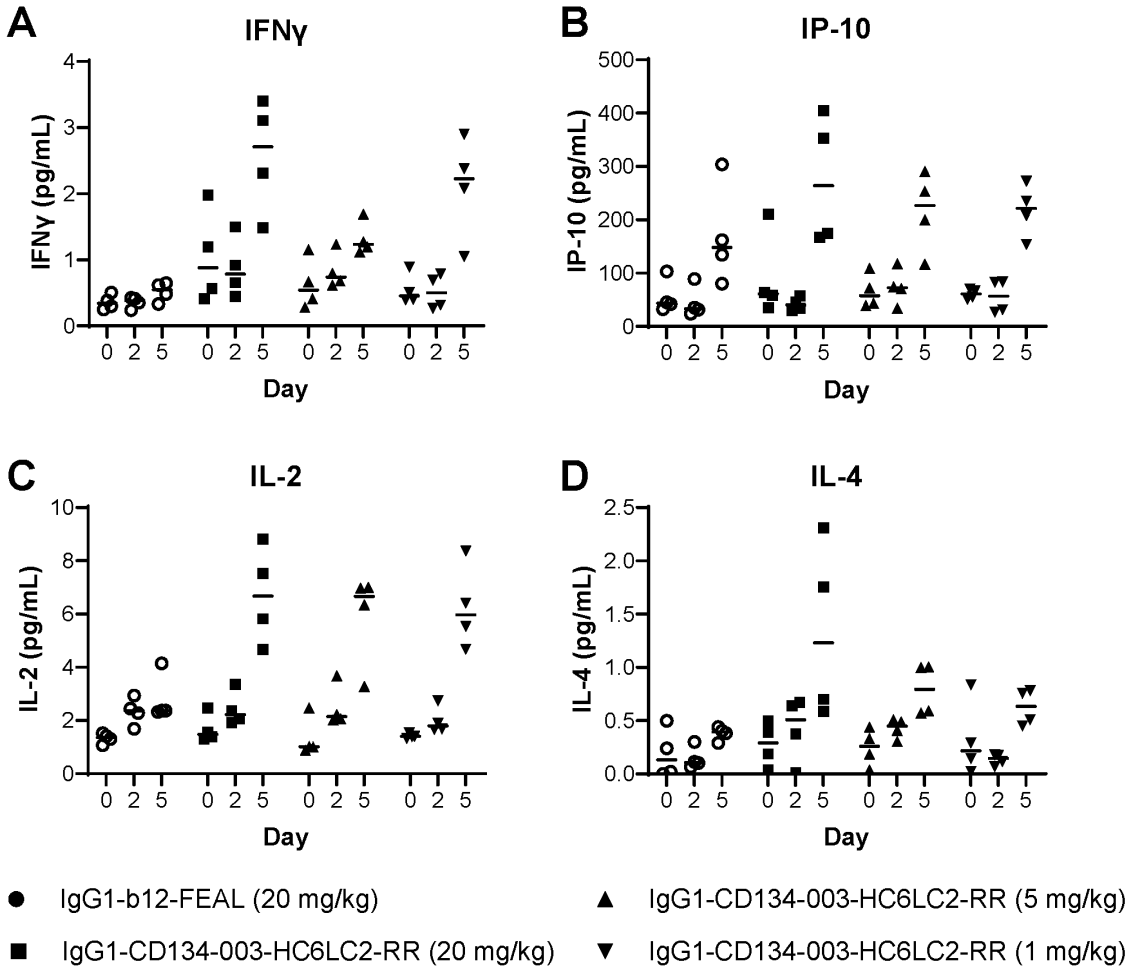

A
IFNγ
IFNγ (pg/mL)
Day
B
IP-10
IP-10 (pg/mL)
Day
C
IL-2
IL-2 (pg/mL)
Day
D
IL-4
IL-4 (pg/mL)
Day
IgG1-b12-FEAL (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (5 mg/kg)
IgG1-CD134-003-HC6LC2-RR (1 mg/kg)

Figure 44 – *continued*

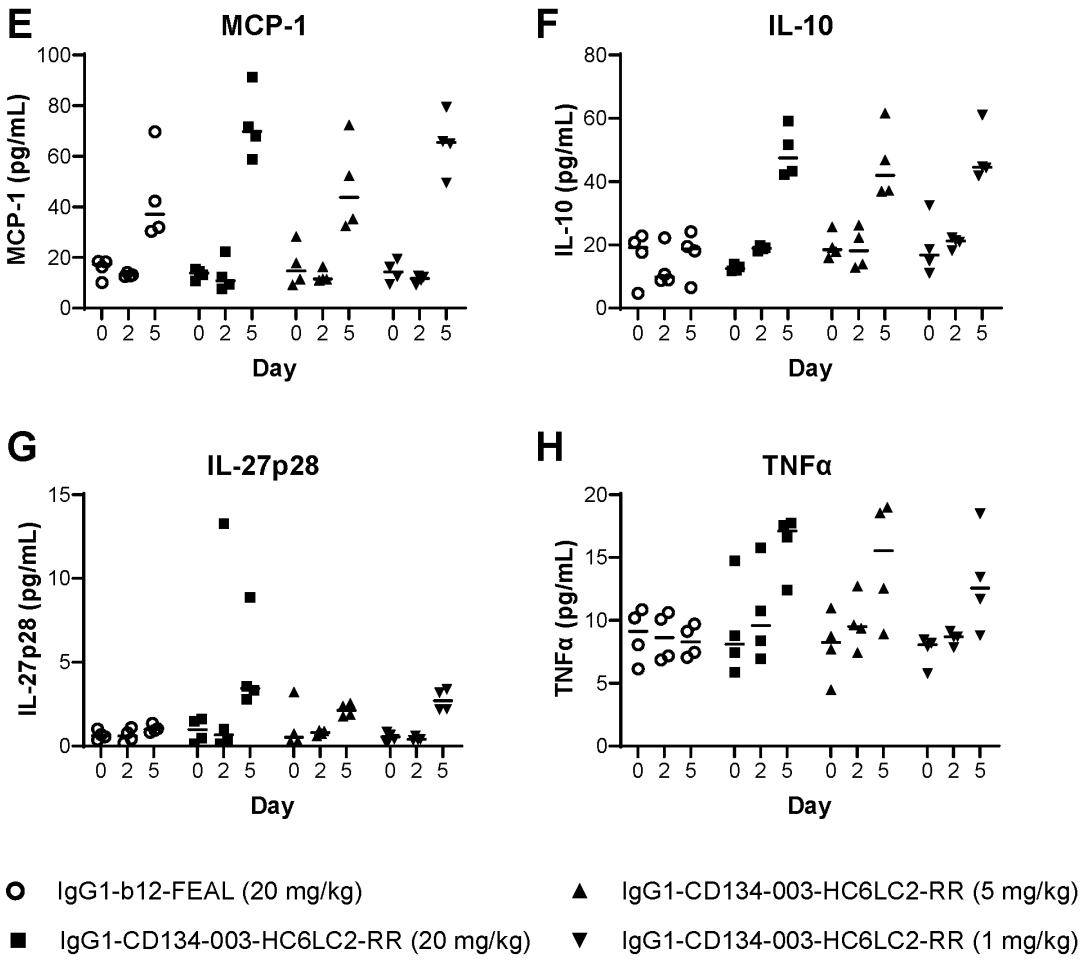


Figure 45

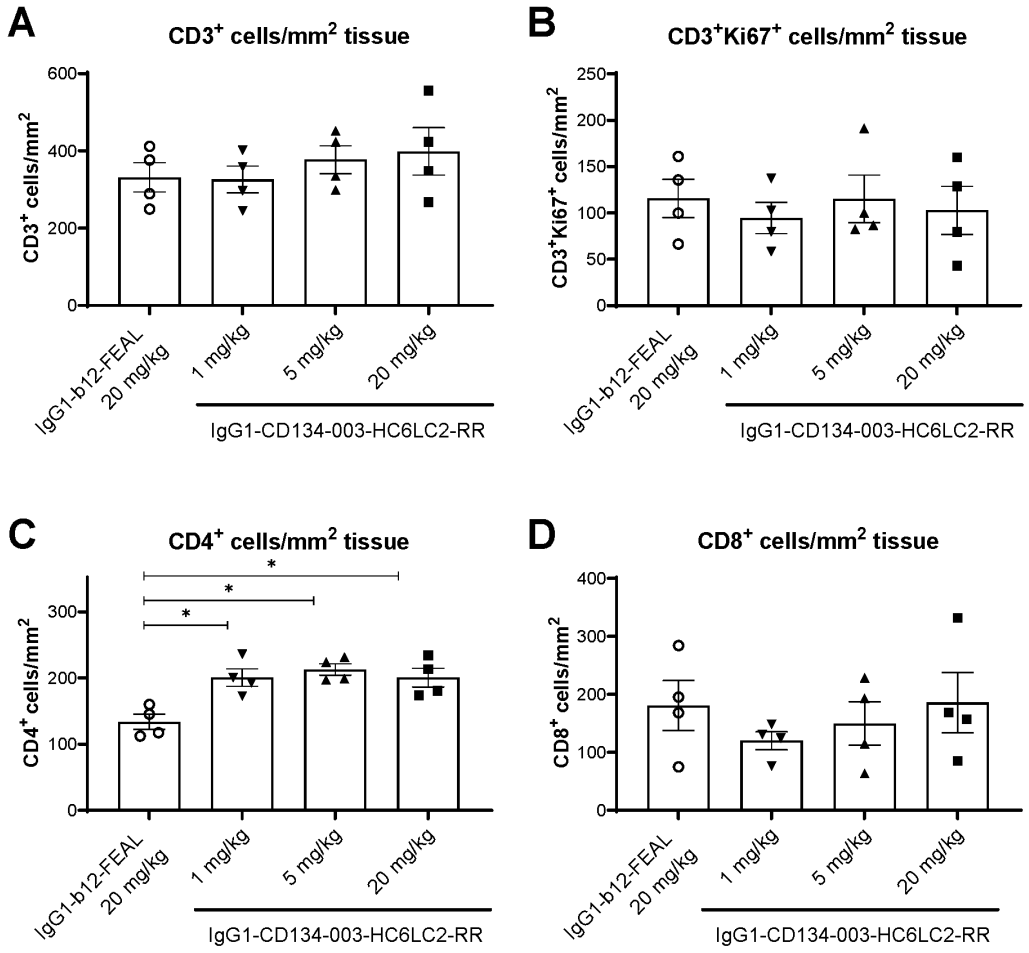

A
CD3+ cells/mm2 tissue
CD3+ cells/mm2
600
400
200
0
IgG1-b12-FEAL 20 mg/kg
1 mg/kg
5 mg/kg
20 mg/kg
IgG1-CD134-003-HC6LC2-RR
B
CD3+Ki67+ cells/mm2 tissue
CD3+Ki67+ cells/mm2
250
200
150
100
50
0
IgG1-b12-FEAL 20 mg/kg
1 mg/kg
5 mg/kg
20 mg/kg
IgG1-CD134-003-HC6LC2-RR
C
CD4+ cells/mm2 tissue
CD4+ cells/mm2
300
200
100
0
*
*
*
IgG1-b12-FEAL 20 mg/kg
1 mg/kg
5 mg/kg
20 mg/kg
IgG1-CD134-003-HC6LC2-RR
D
CD8+ cells/mm2 tissue
CD8+ cells/mm2
400
300
200
100
0
IgG1-b12-FEAL 20 mg/kg
1 mg/kg
5 mg/kg
20 mg/kg
IgG1-CD134-003-HC6LC2-RR

Figure 45 - *continued*

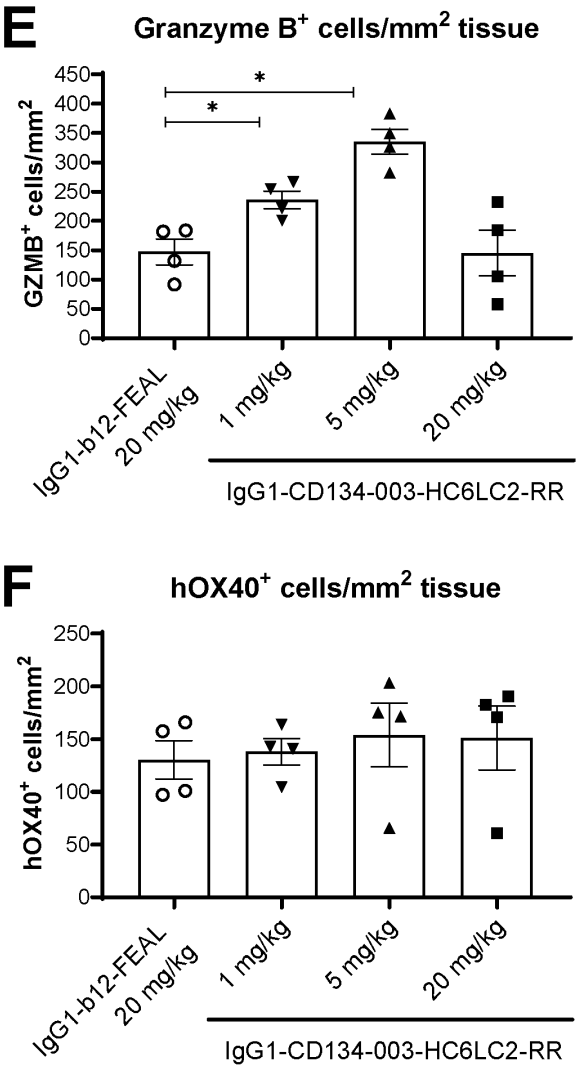


Figure 46

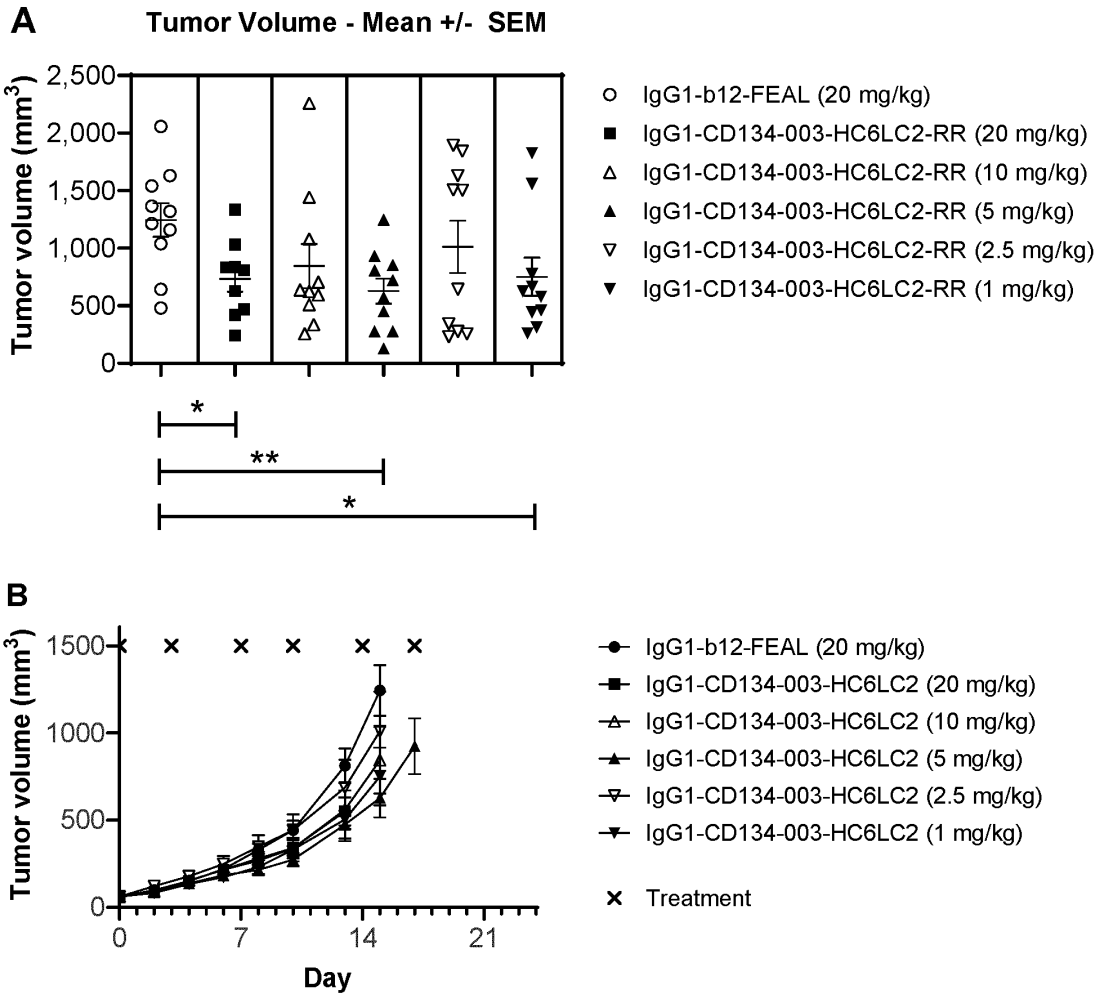

A
Tumor Volume - Mean +/- SEM
Tumor volume (mm3)
2,500
2,000
1,500
1,000
500
0
*
**
*
IgG1-b12-FEAL (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (10 mg/kg)
IgG1-CD134-003-HC6LC2-RR (5 mg/kg)
IgG1-CD134-003-HC6LC2-RR (2.5 mg/kg)
IgG1-CD134-003-HC6LC2-RR (1 mg/kg)
B
Tumor volume (mm3)
1500
1000
500
0
0
7
14
21
Day
IgG1-b12-FEAL (20 mg/kg)
IgG1-CD134-003-HC6LC2 (20 mg/kg)
IgG1-CD134-003-HC6LC2 (10 mg/kg)
IgG1-CD134-003-HC6LC2 (5 mg/kg)
IgG1-CD134-003-HC6LC2 (2.5 mg/kg)
IgG1-CD134-003-HC6LC2 (1 mg/kg)
Treatment

Figure 46 - *continued*

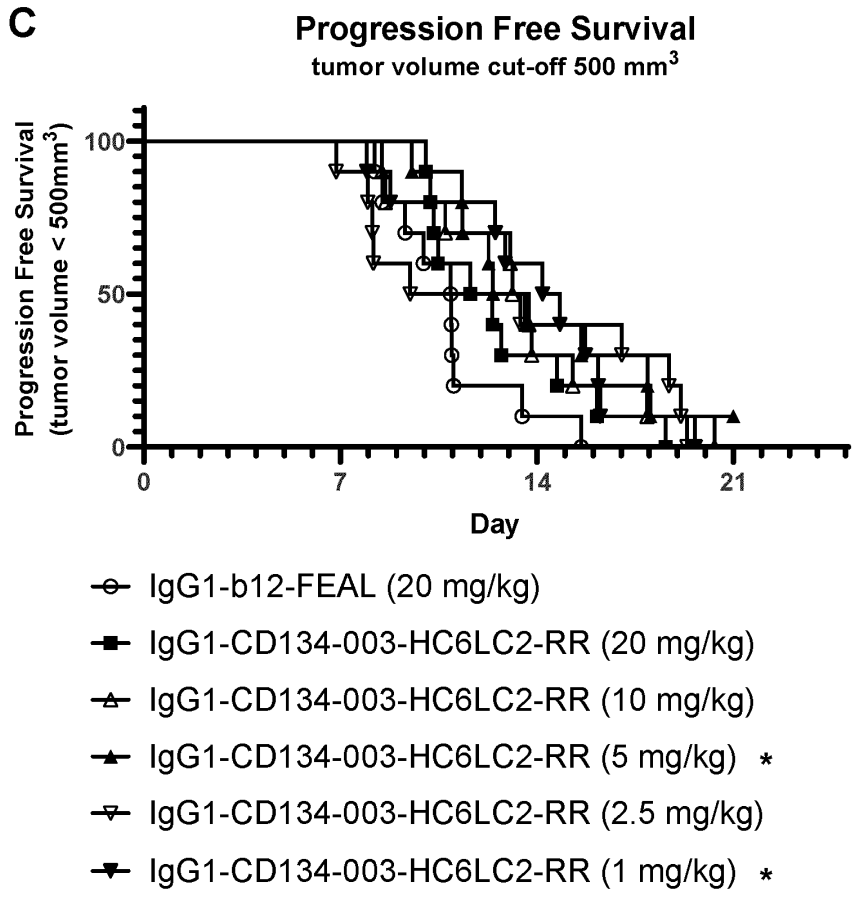


Figure 47

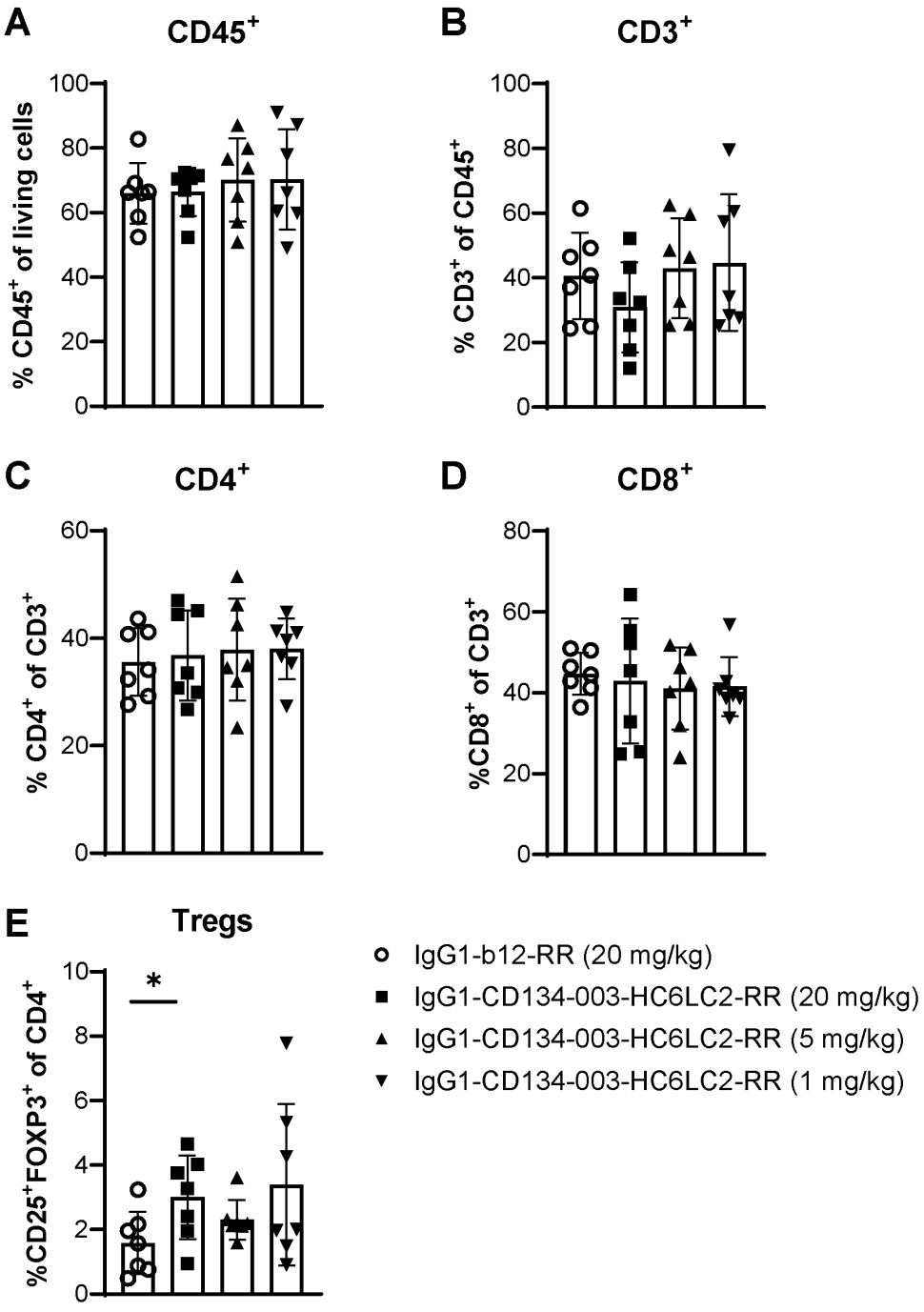

A
CD45+
% CD45+ of living cells
B
CD3+
% CD3+ of CD45+
C
CD4+
% CD4+ of CD3+
D
CD8+
%CD8+ of CD3+
E
Tregs
%CD25+FOXP3+ of CD4+
*
IgG1-b12-RR (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (5 mg/kg)
IgG1-CD134-003-HC6LC2-RR (1 mg/kg)

Figure 47 - *continued*

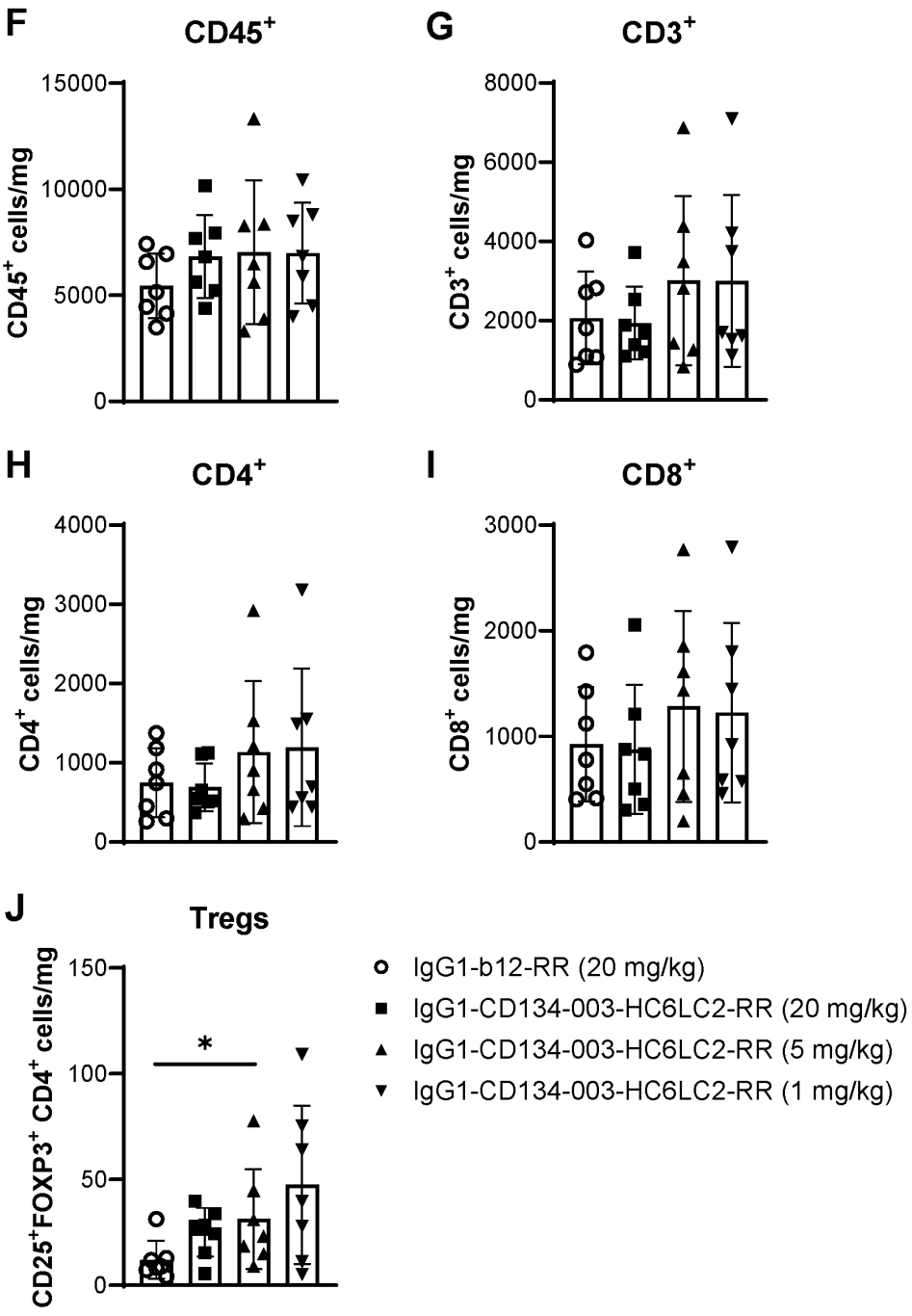


Figure 48

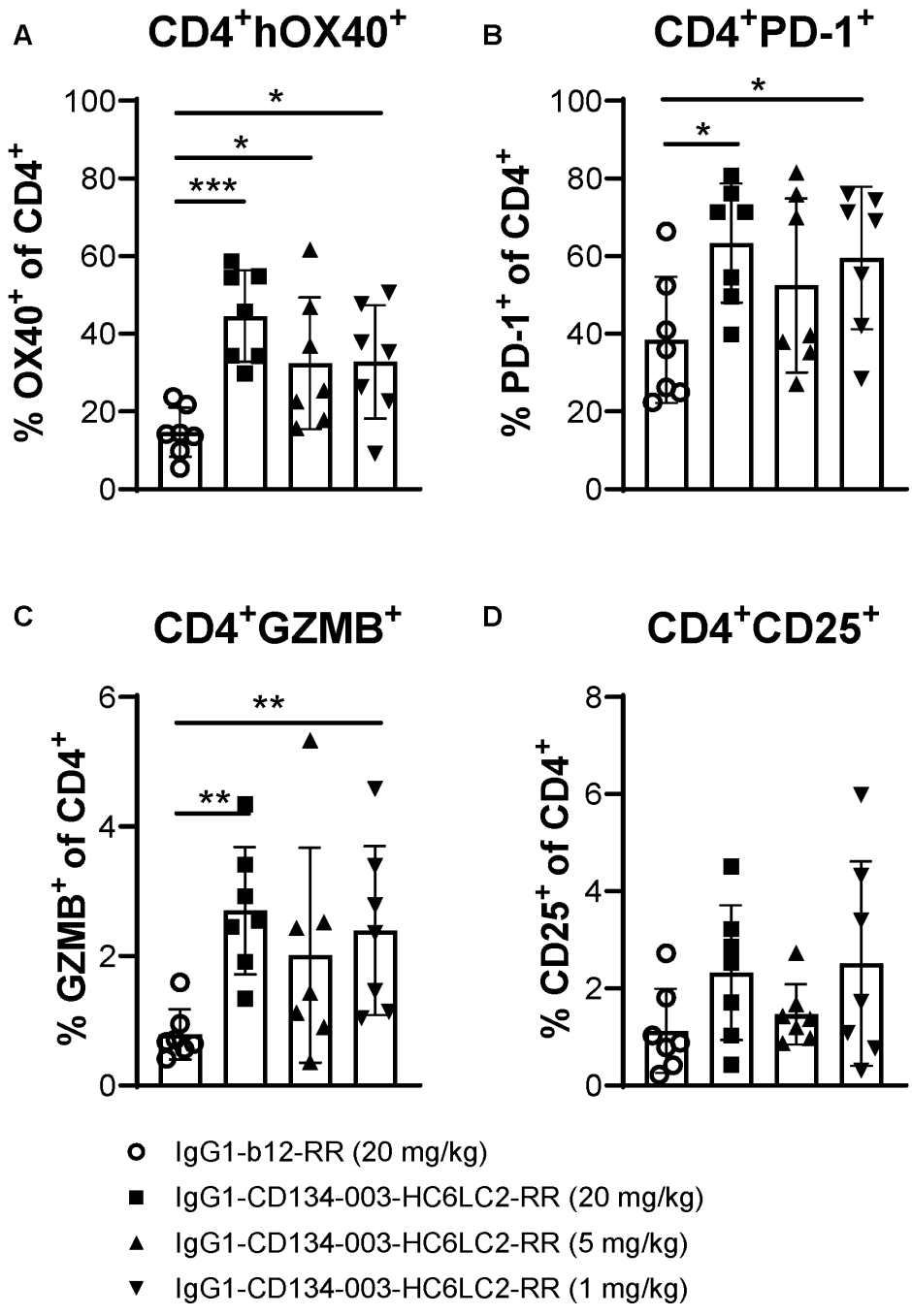
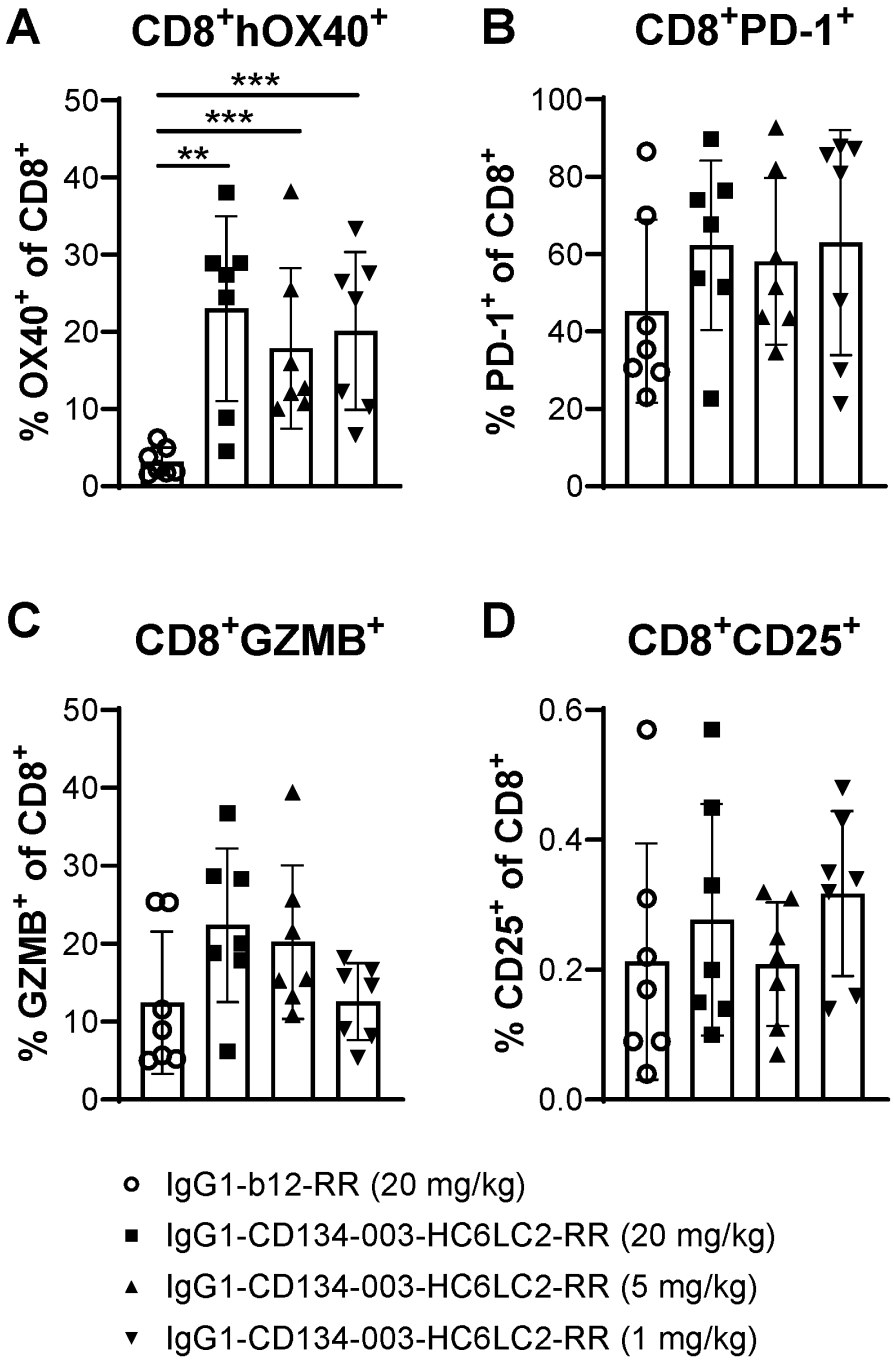

A
CD4+hOX40+
% OX40+ of CD4+
100
80
60
40
20
0
*
*
***
B
CD4+PD-1+
% PD-1+ of CD4+
100
80
60
40
20
0
*
*
C
CD4+GZMB+
% GZMB+ of CD4+
6
4
2
0
**
**
D
CD4+CD25+
% CD25+ of CD4+
8
6
4
2
0
IgG1-b12-RR (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (5 mg/kg)
IgG1-CD134-003-HC6LC2-RR (1 mg/kg)

Figure 49


A
CD8+hOX40+
% OX40+ of CD8+
***
***
**
B
CD8+PD-1+
% PD-1+ of CD8+
C
CD8+GZMB+
% GZMB+ of CD8+
D
CD8+CD25+
% CD25+ of CD8+
IgG1-b12-RR (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (20 mg/kg)
IgG1-CD134-003-HC6LC2-RR (5 mg/kg)
IgG1-CD134-003-HC6LC2-RR (1 mg/kg)