Figure 1A

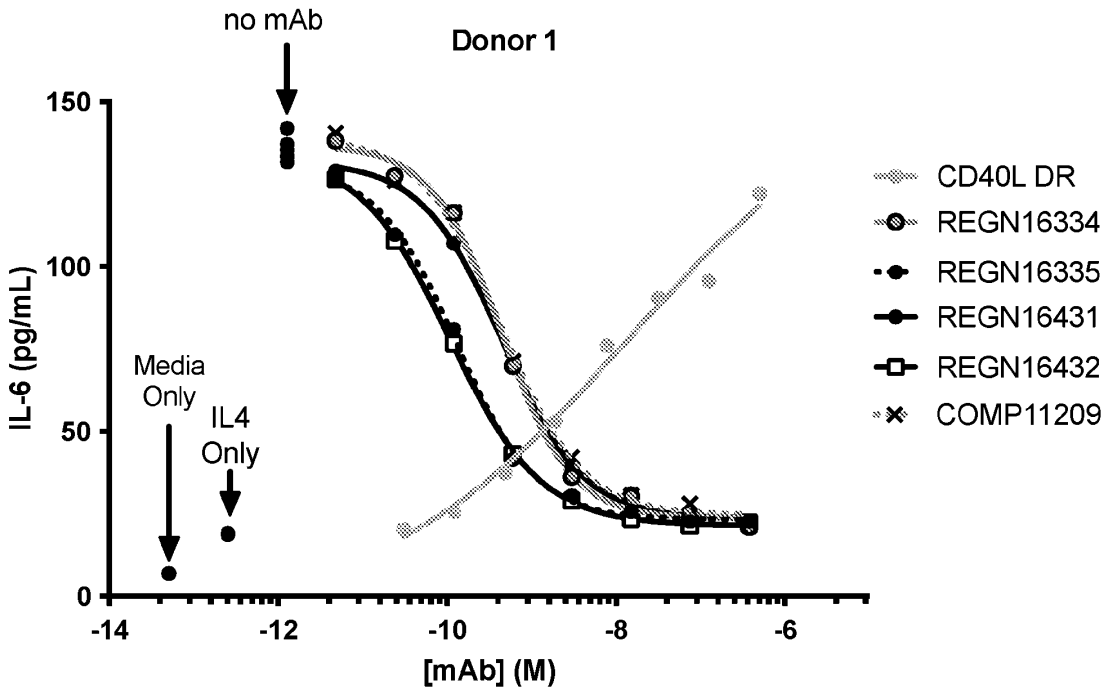

no mAb
Donor 1
150
100
50
0
IL-6 (pg/mL)
Media Only
IL4 Only
-14
-12
-10
-8
-6
[mAb] (M)
CD40L DR
REGN16334
REGN16335
REGN16431
REGN16432
COMP11209

Figure 1B

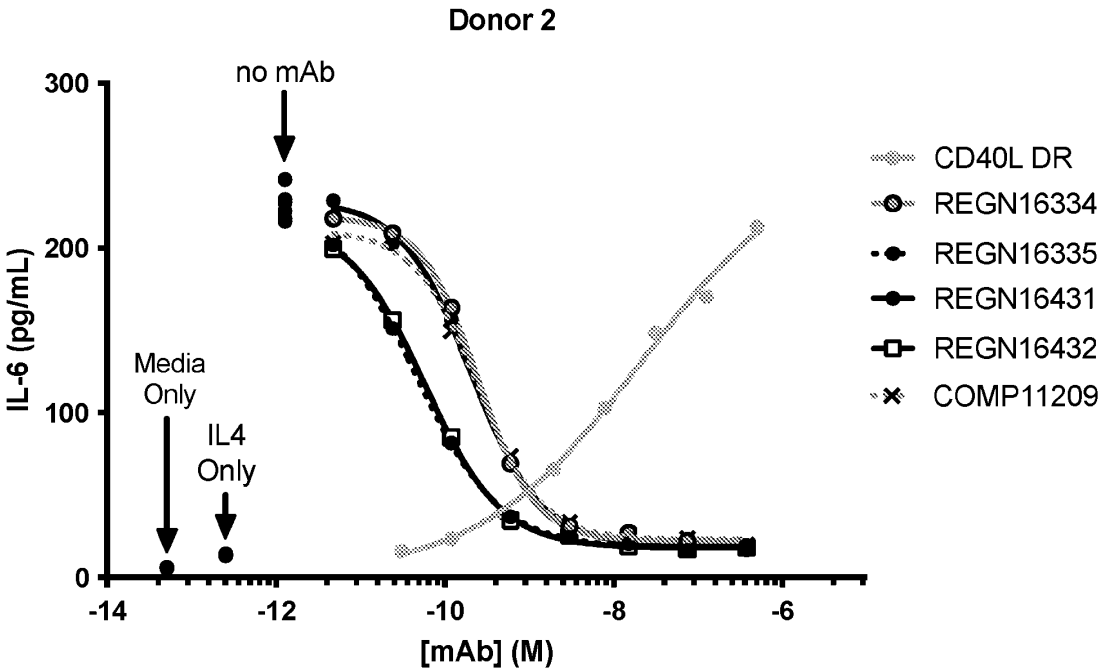

Donor 2
no mAb
Media Only
IL4 Only
IL-6 (pg/mL)
300
200
100
0
-14
-12
-10
-8
-6
[mAb] (M)
CD40L DR
REGN16334
REGN16335
REGN16431
REGN16432
COMP11209

Figure 1C

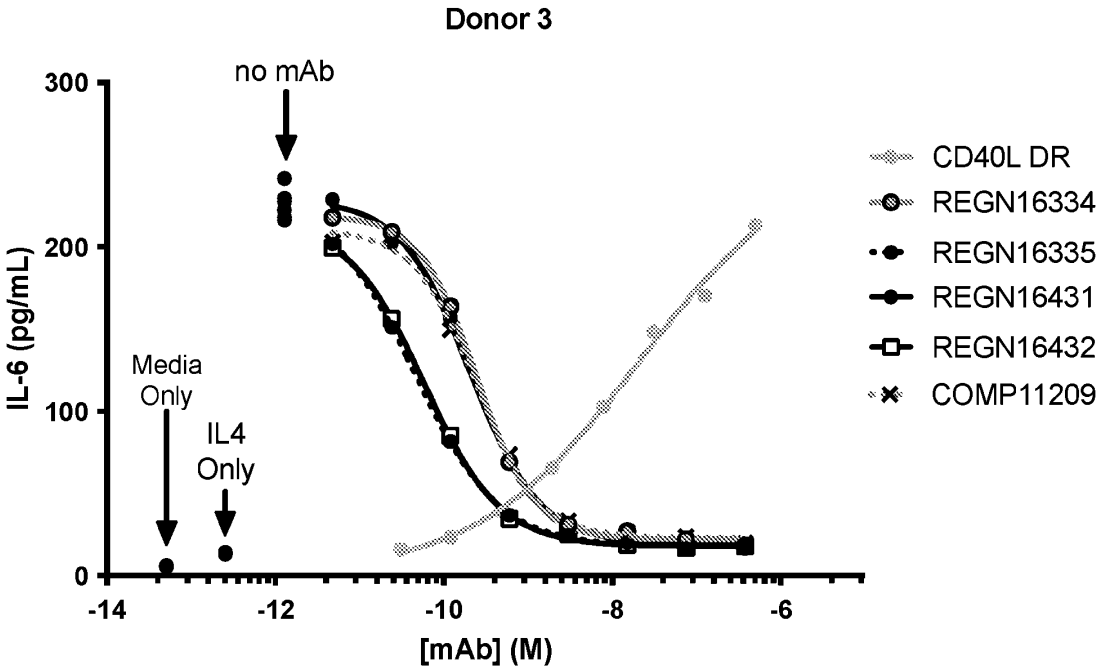

Donor 3
no mAb
Media Only
IL4 Only
IL-6 (pg/mL)
300
200
100
0
-14
-12
-10
-8
-6
[mAb] (M)
CD40L DR
REGN16334
REGN16335
REGN16431
REGN16432
COMP11209

Figure 1D

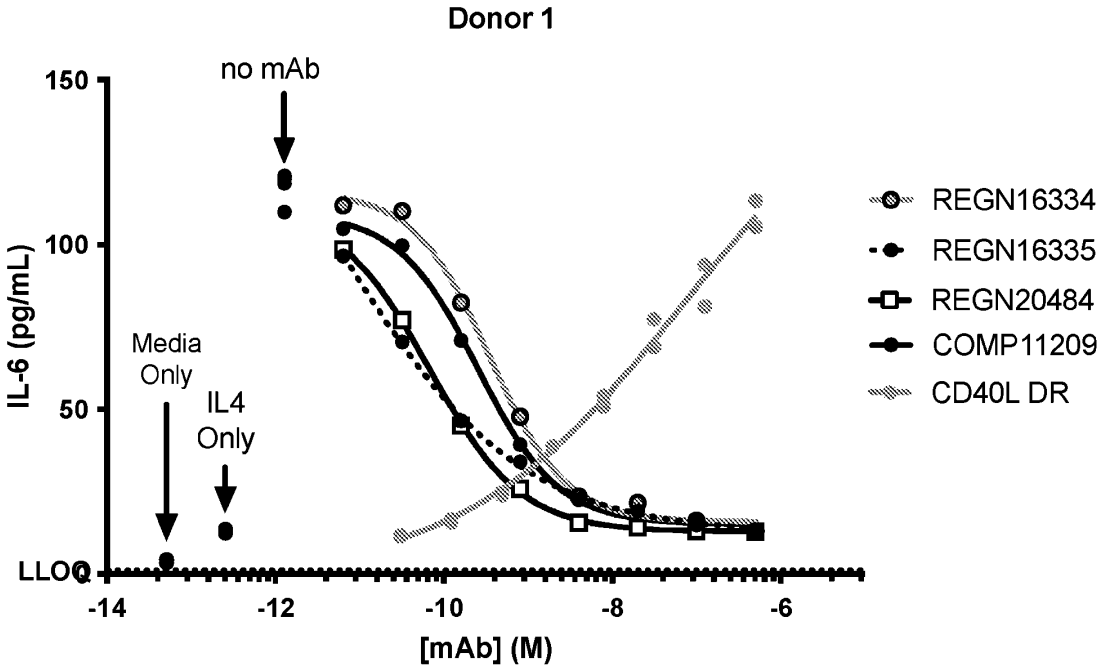

Donor 1
no mAb
Media Only
IL4 Only
IL-6 (pg/mL)
150
100
50
LLOQ
-14
-12
-10
-8
-6
[mAb] (M)
REGN16334
REGN16335
REGN20484
COMP11209
CD40L DR

Figure 1E

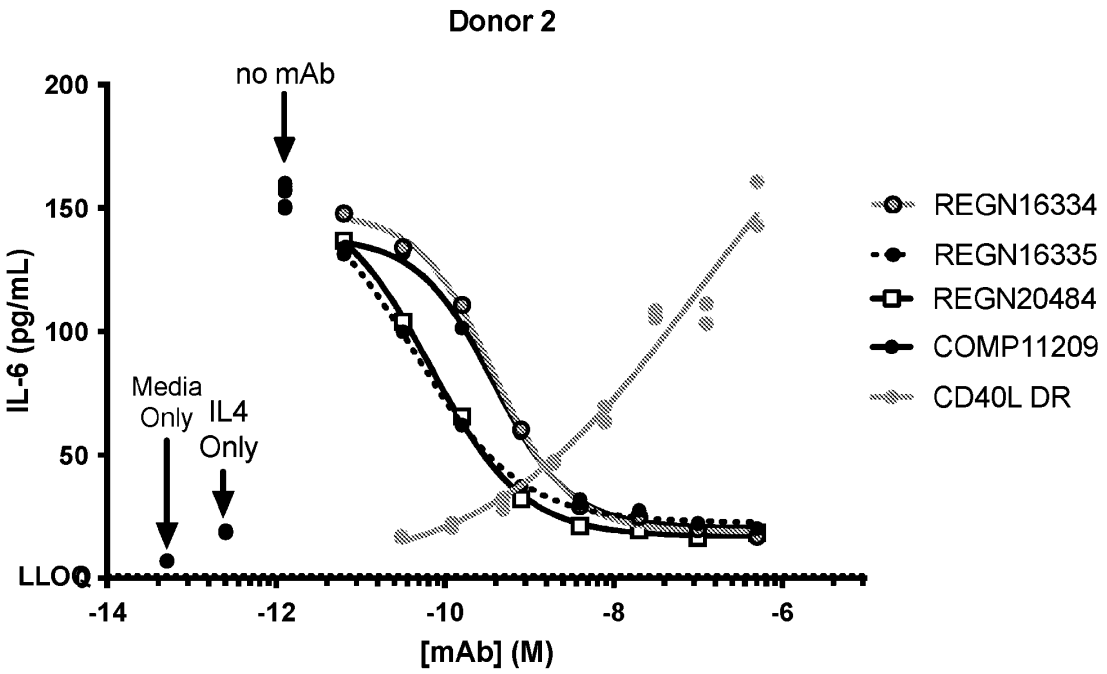

Donor 2
no mAb
Media Only
IL4 Only
IL-6 (pg/mL)
200
150
100
50
LLOQ
-14
-12
-10
-8
-6
[mAb] (M)
REGN16334
REGN16335
REGN20484
COMP11209
CD40L DR

Figure 2A

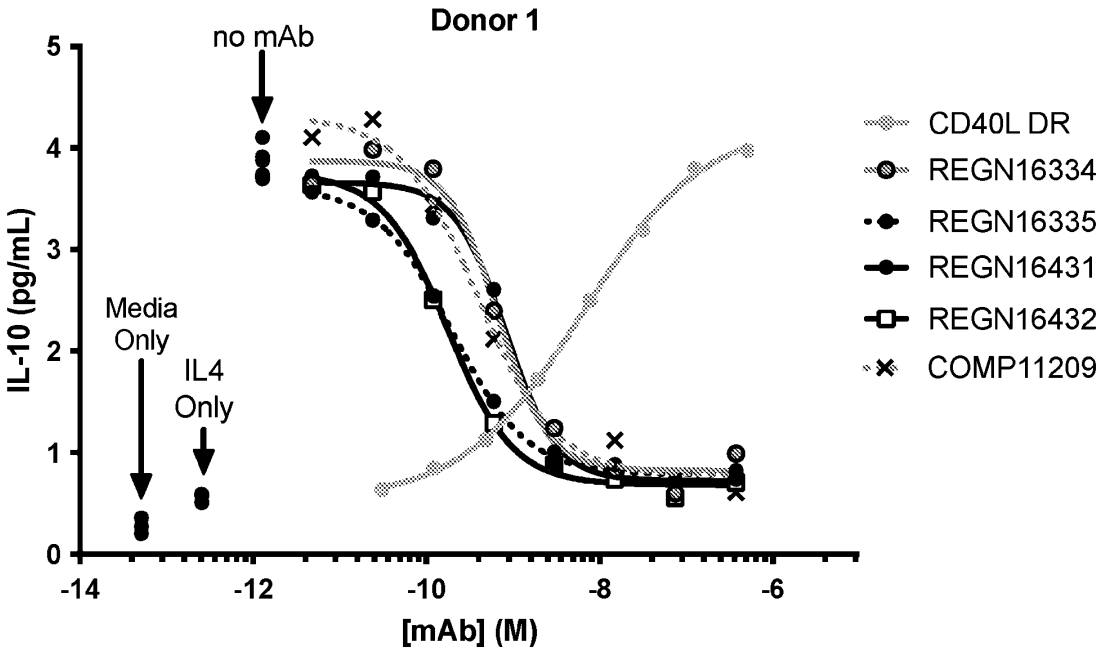

Donor 1
no mAb
Media Only
IL4 Only
IL-10 (pg/mL)
[mAb] (M)
5
4
3
2
1
0
-14
-12
-10
-8
-6
CD40L DR
REGN16334
REGN16335
REGN16431
REGN16432
COMP11209

Figure 2B

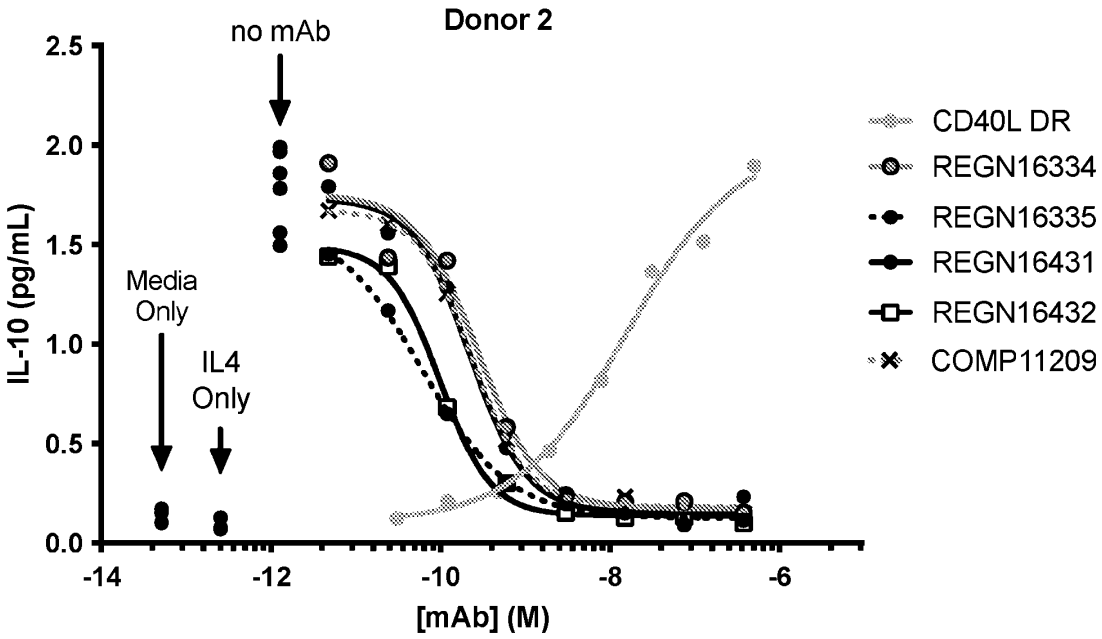

Donor 2
no mAb
Media Only
IL4 Only
IL-10 (pg/mL)
2.5
2.0
1.5
1.0
0.5
0.0
-14
-12
-10
-8
-6
[mAb] (M)
CD40L DR
REGN16334
REGN16335
REGN16431
REGN16432
COMP11209

Figure 2C

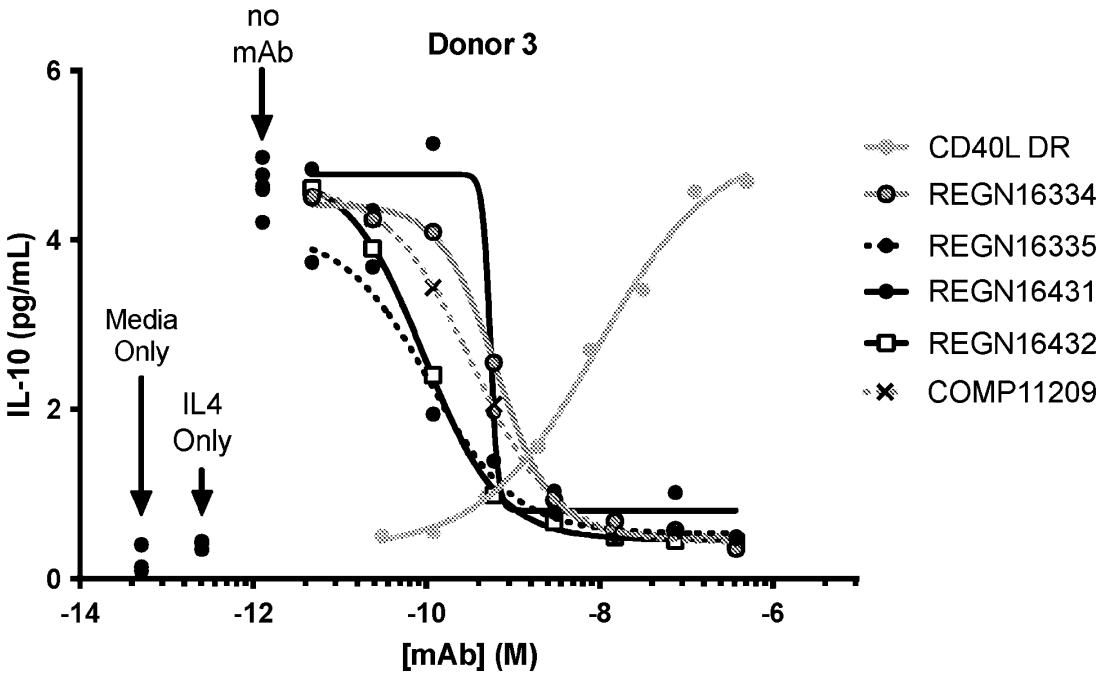

no
mAb
Donor 3
Media
Only
IL4
Only
IL-10 (pg/mL)
6
4
2
0
-14
-12
-10
-8
-6
[mAb] (M)
CD40L DR
REGN16334
REGN16335
REGN16431
REGN16432
COMP11209

Figure 2D

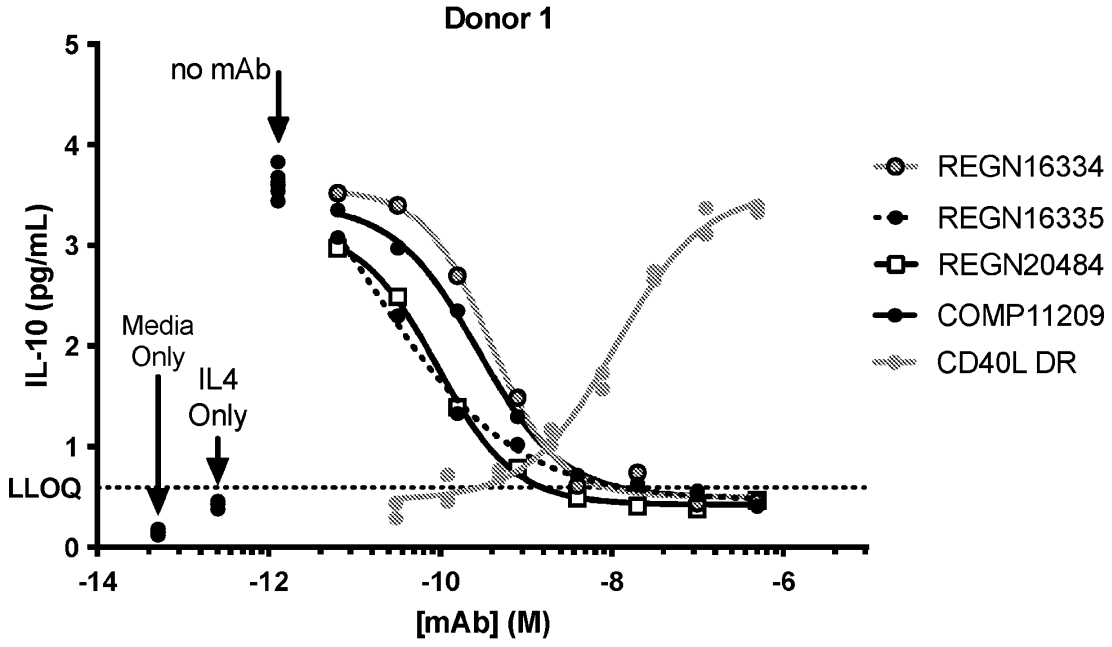

Donor 1
no mAb
Media Only
IL4 Only
LLOQ
IL-10 (pg/mL)
[mAb] (M)
REGN16334
REGN16335
REGN20484
COMP11209
CD40L DR

Figure 2E

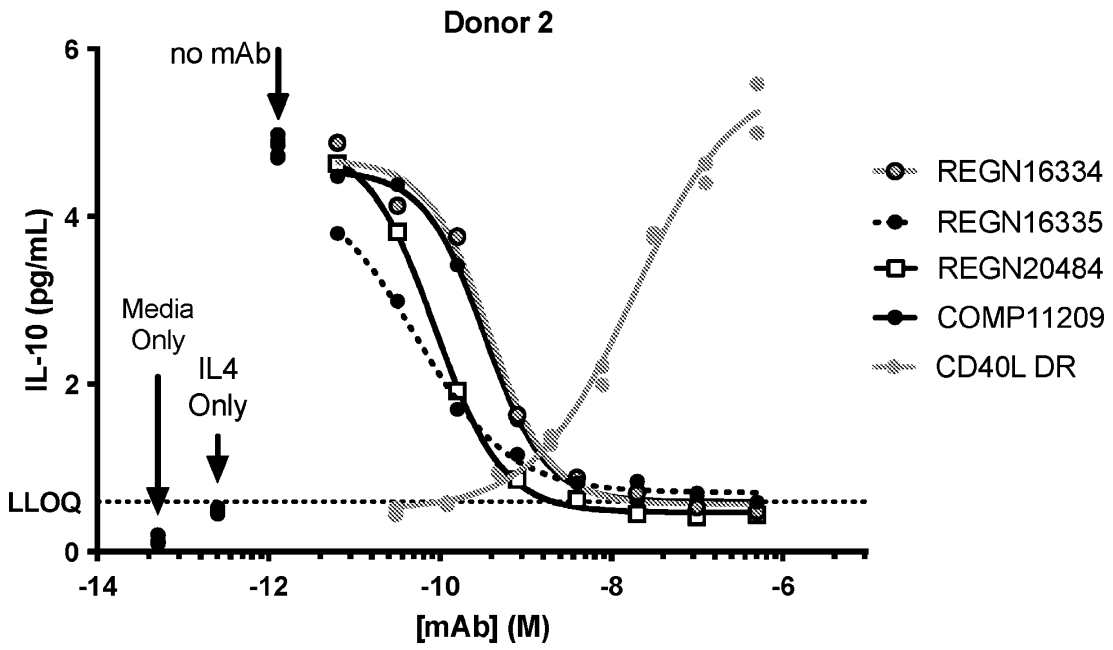

Donor 2
no mAb
Media Only
IL4 Only
LLOQ
IL-10 (pg/mL)
6
4
2
0
-14
-12
-10
-8
-6
[mAb] (M)
REGN16334
REGN16335
REGN20484
COMP11209
CD40L DR

Figure 3A

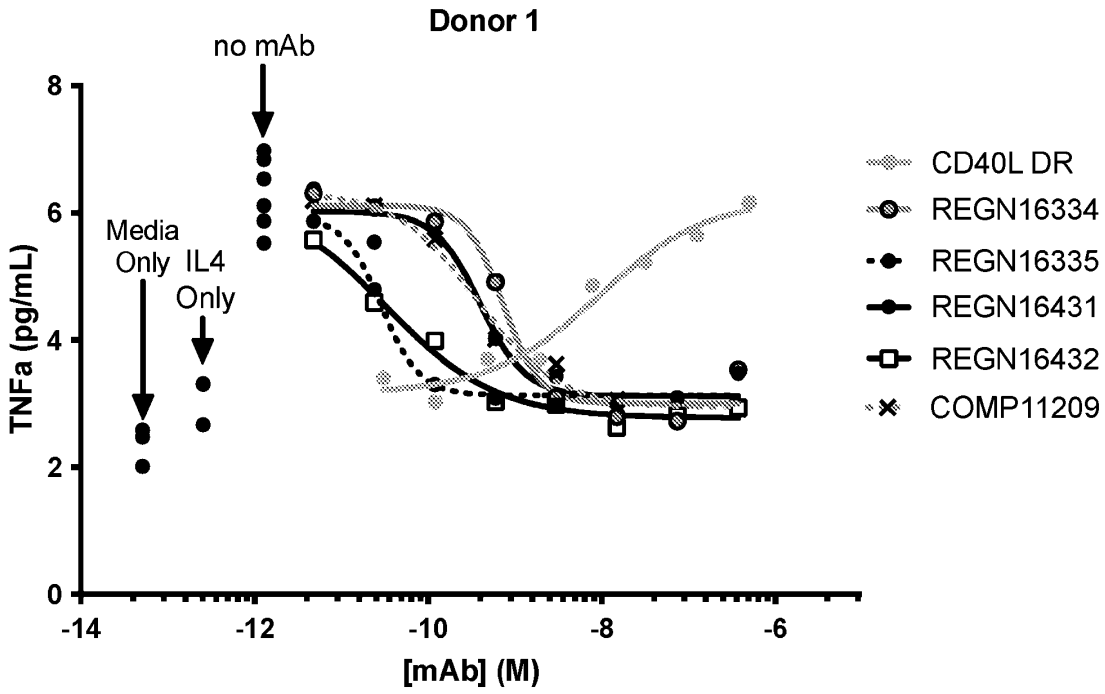

Donor 1
no mAb
Media Only
IL4 Only
TNFa (pg/mL)
8
6
4
2
0
-14
-12
-10
-8
-6
[mAb] (M)
CD40L DR
REGN16334
REGN16335
REGN16431
REGN16432
COMP11209

Figure 3B

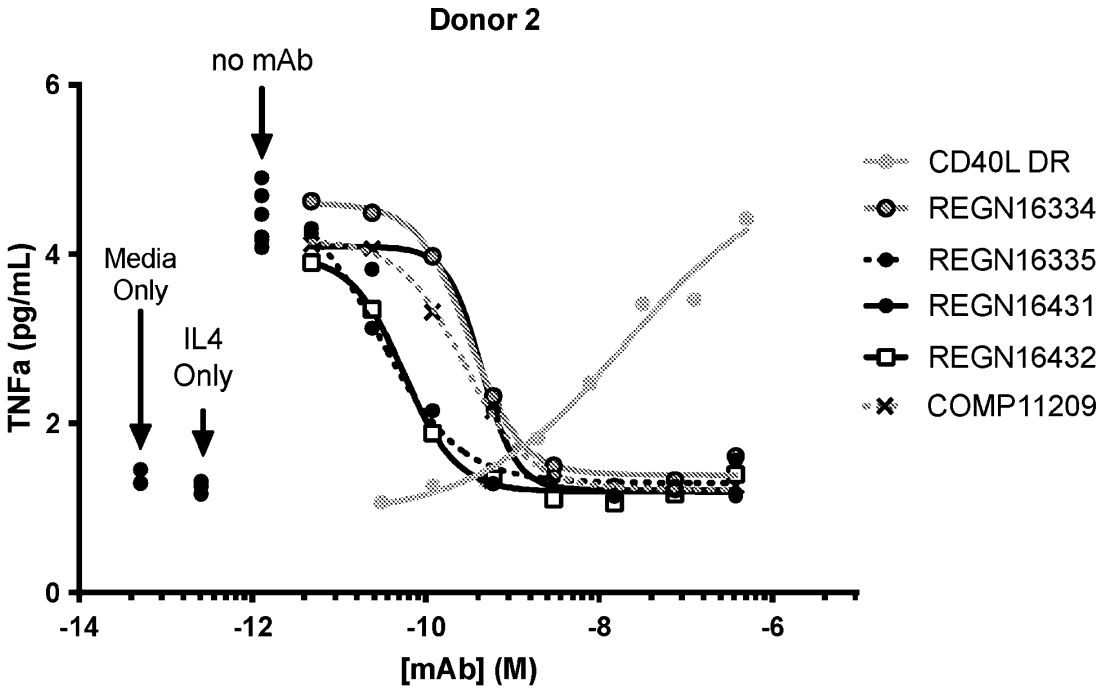

Donor 2
no mAb
Media Only
IL4 Only
TNFa (pg/mL)
6
4
2
0
-14
-12
-10
-8
-6
[mAb] (M)
CD40L DR
REGN16334
REGN16335
REGN16431
REGN16432
COMP11209

Figure 3C

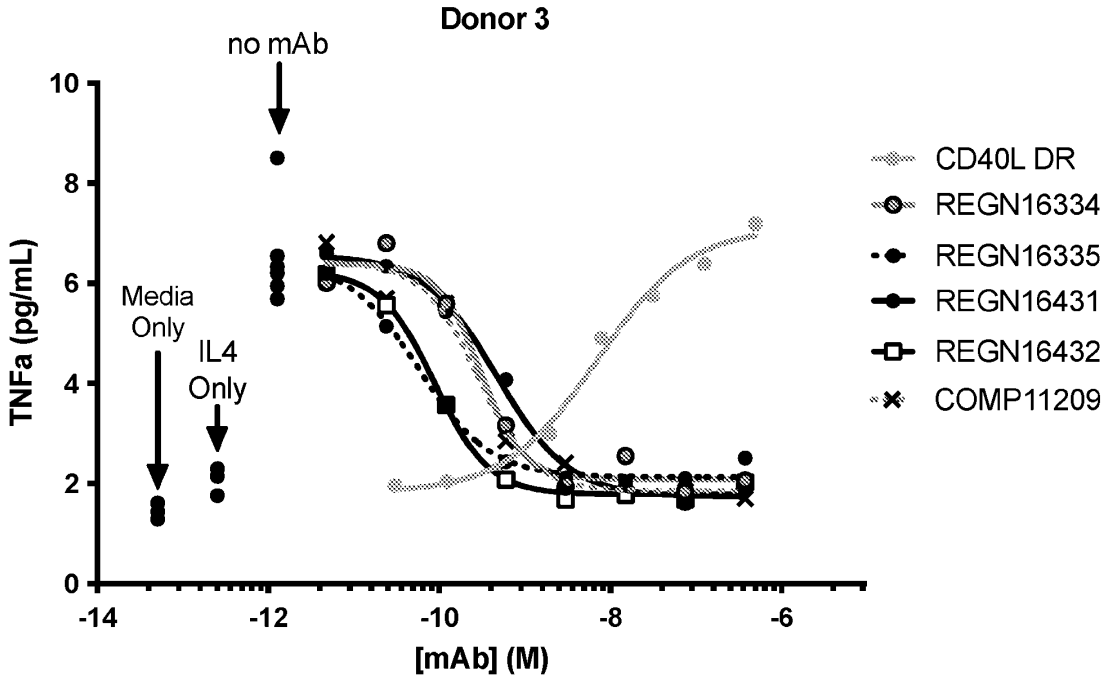

Donor 3
no mAb
Media Only
IL4 Only
TNFa (pg/mL)
10
8
6
4
2
0
-14
-12
-10
-8
-6
[mAb] (M)
CD40L DR
REGN16334
REGN16335
REGN16431
REGN16432
COMP11209

Figure 3D

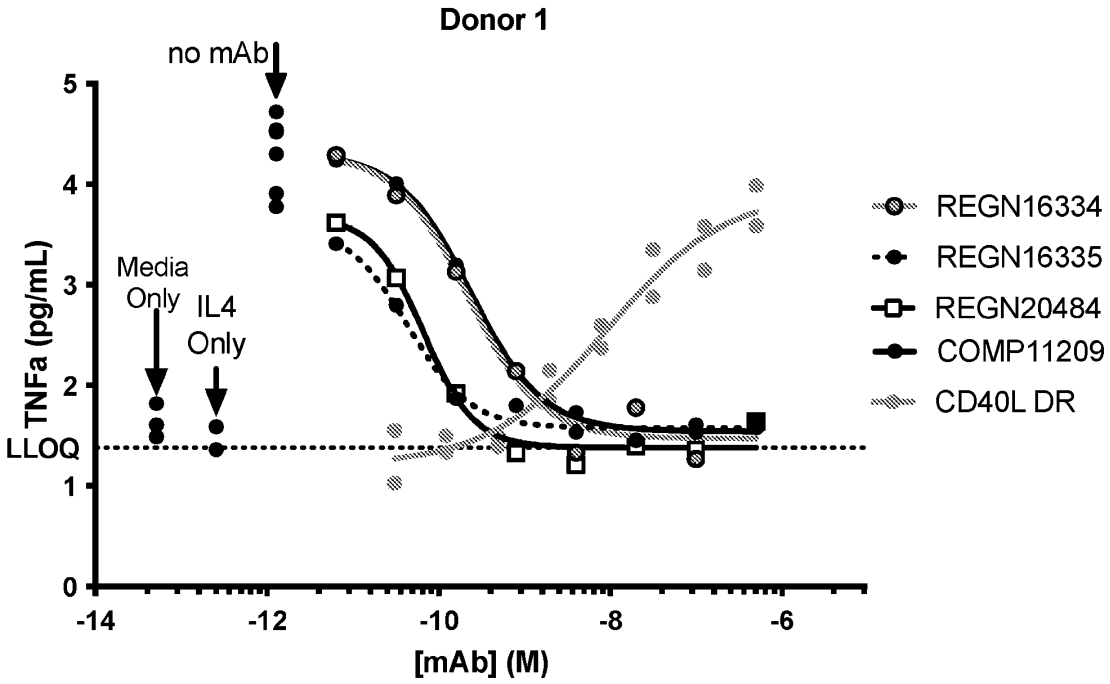

Donor 1
no mAb
Media Only
IL4 Only
LLOQ
TNFa (pg/mL)
[mAb] (M)
REGN16334
REGN16335
REGN20484
COMP11209
CD40L DR

Figure 3E

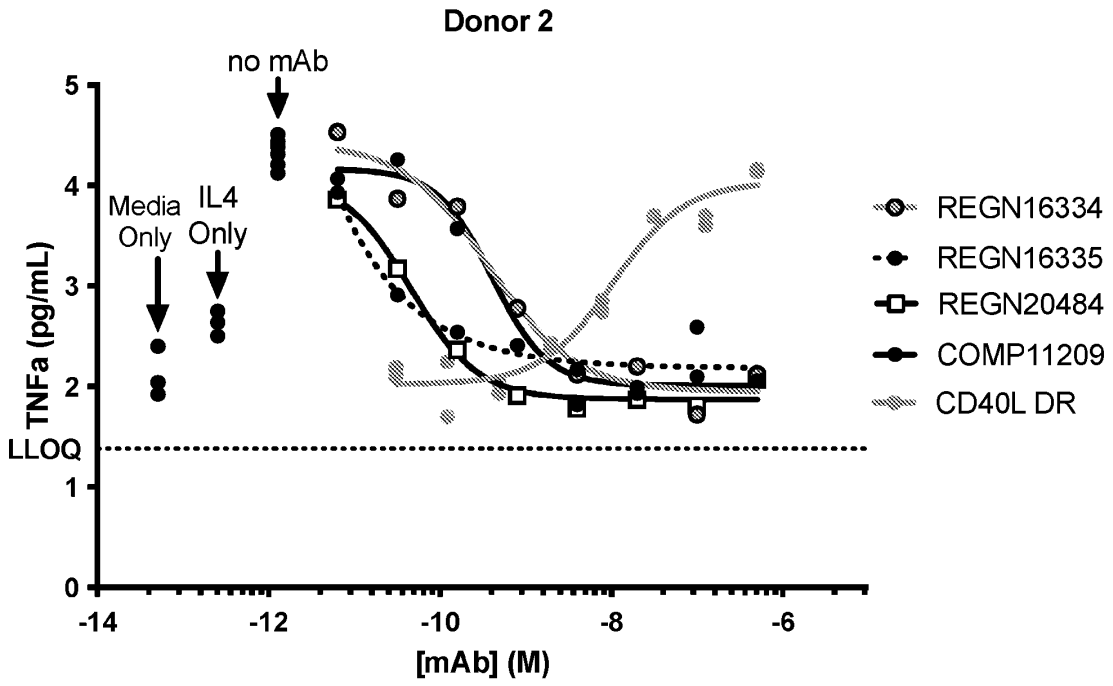

Donor 2
no mAb
Media Only
IL4 Only
TNFa (pg/mL)
LLOQ
5
4
3
2
1
0
-14
-12
-10
-8
-6
[mAb] (M)
REGN16334
REGN16335
REGN20484
COMP11209
CD40L DR

Figure 3F

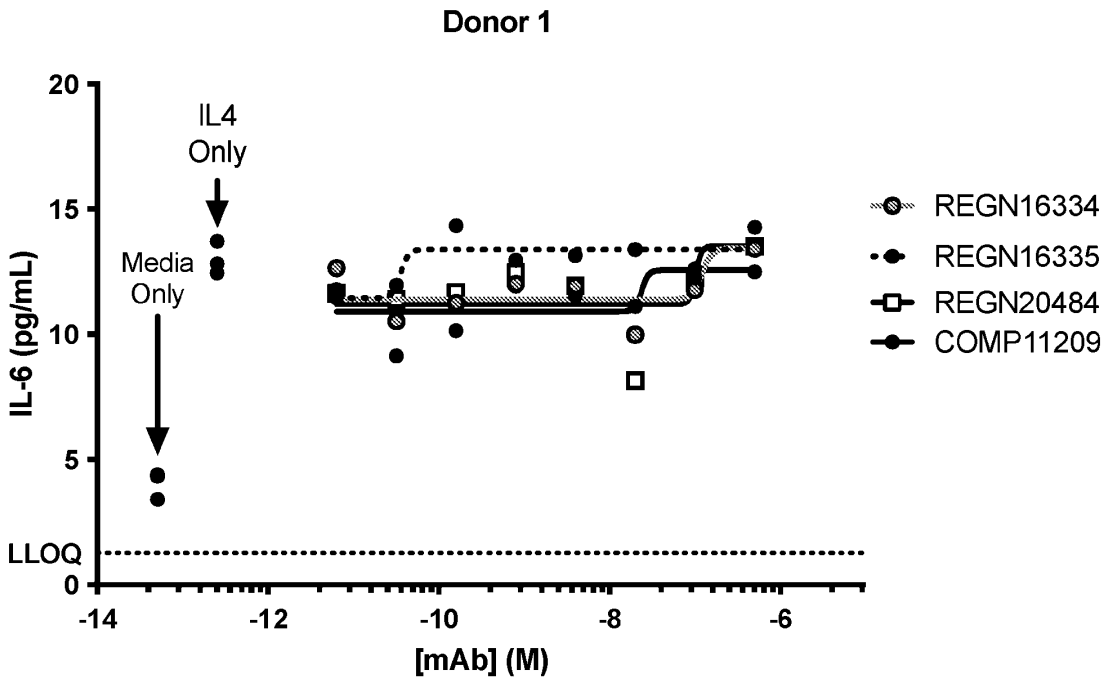

Donor 1
IL-6 (pg/mL)
20
15
10
5
LLOQ
0
IL4
Only
Media
Only
-14
-12
-10
-8
-6
[mAb] (M)
REGN16334
REGN16335
REGN20484
COMP11209

Figure 3G

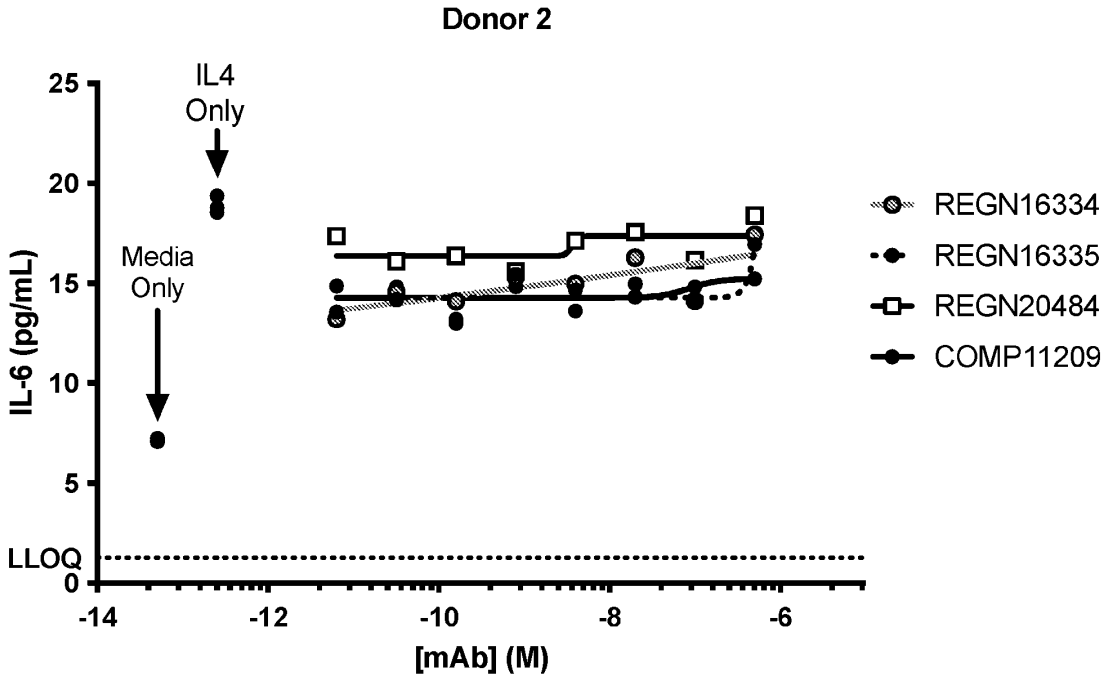

Donor 2
IL4
Only
Media
Only
IL-6 (pg/mL)
25
20
15
10
5
LLOQ
0
-14
-12
-10
-8
-6
[mAb] (M)
REGN16334
REGN16335
REGN20484
COMP11209

Figure 4A

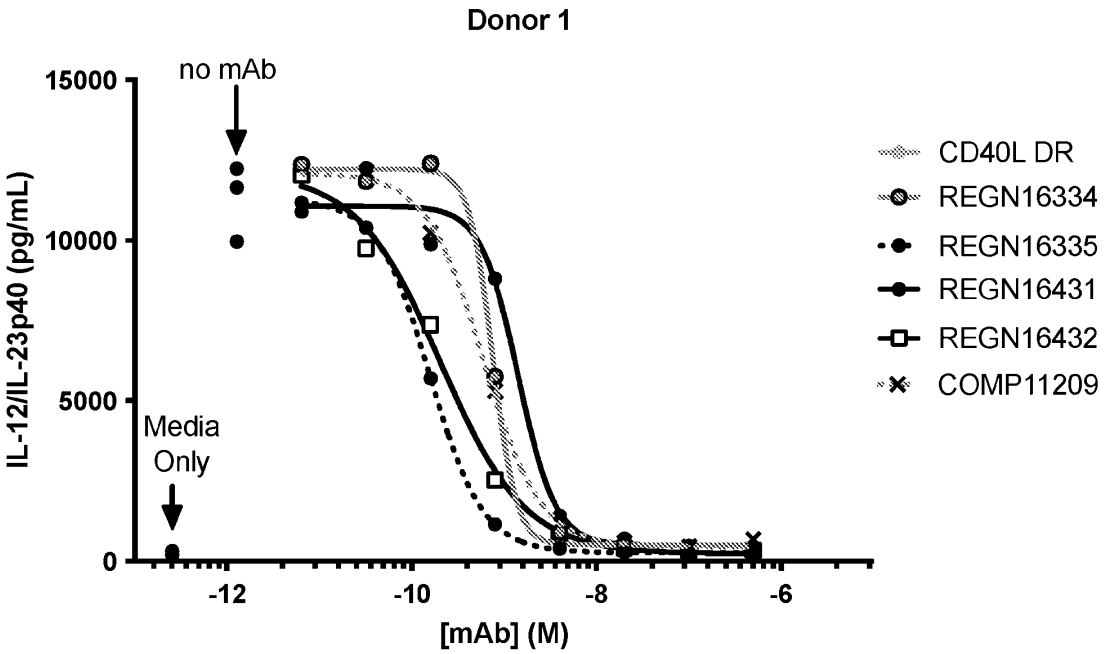

Donor 1
15000
10000
5000
0
IL-12/IL-23p40 (pg/mL)
no mAb
Media
Only
-12
-10
-8
-6
[mAb] (M)
CD40L DR
REGN16334
REGN16335
REGN16431
REGN16432
COMP11209

Figure 4B

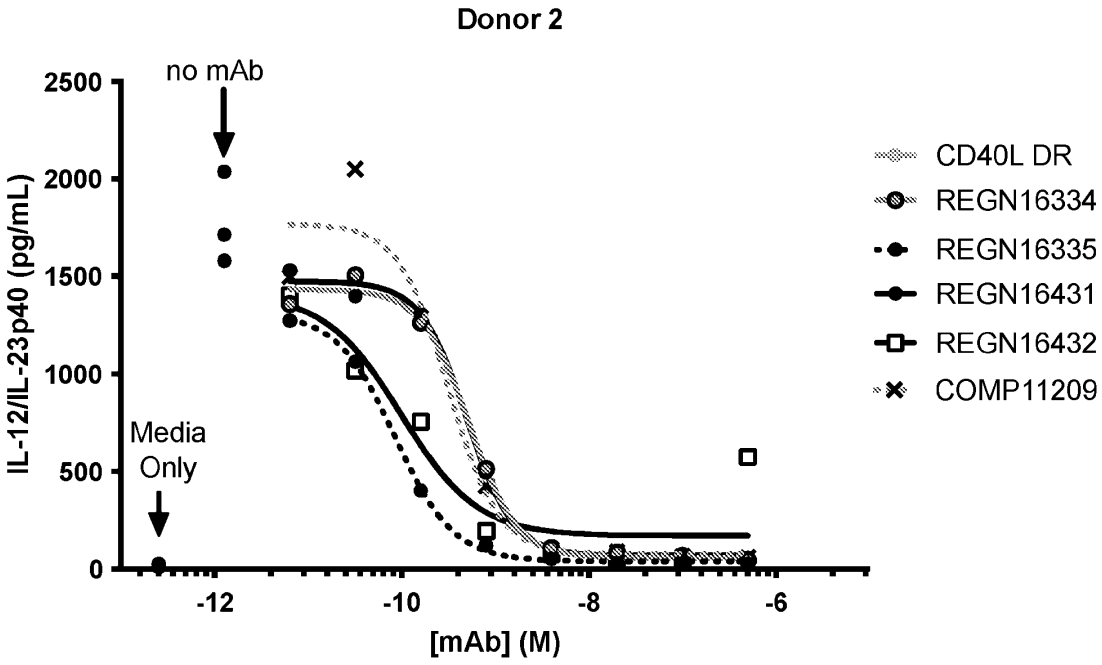

Donor 2
no mAb
Media Only
IL-12/IL-23p40 (pg/mL)
2500
2000
1500
1000
500
0
-12
-10
-8
-6
[mAb] (M)
CD40L DR
REGN16334
REGN16335
REGN16431
REGN16432
COMP11209

Figure 5A

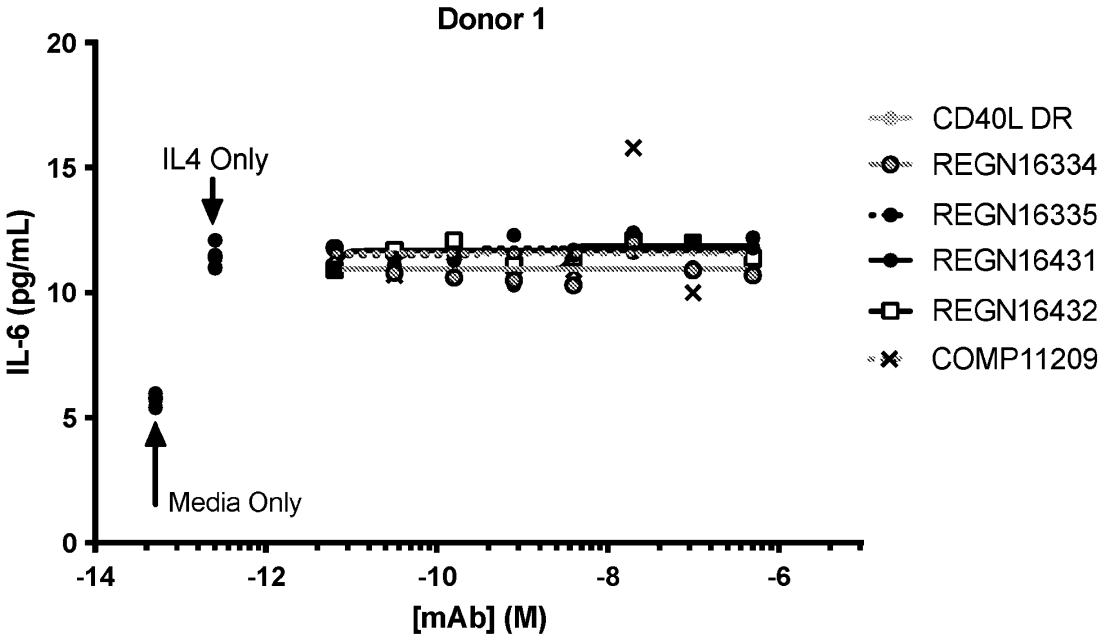

Donor 1
IL-6 (pg/mL)
20
15
10
5
0
IL4 Only
Media Only
-14
-12
-10
-8
-6
[mAb] (M)
CD40L DR
REGN16334
REGN16335
REGN16431
REGN16432
COMP11209

Figure 5B

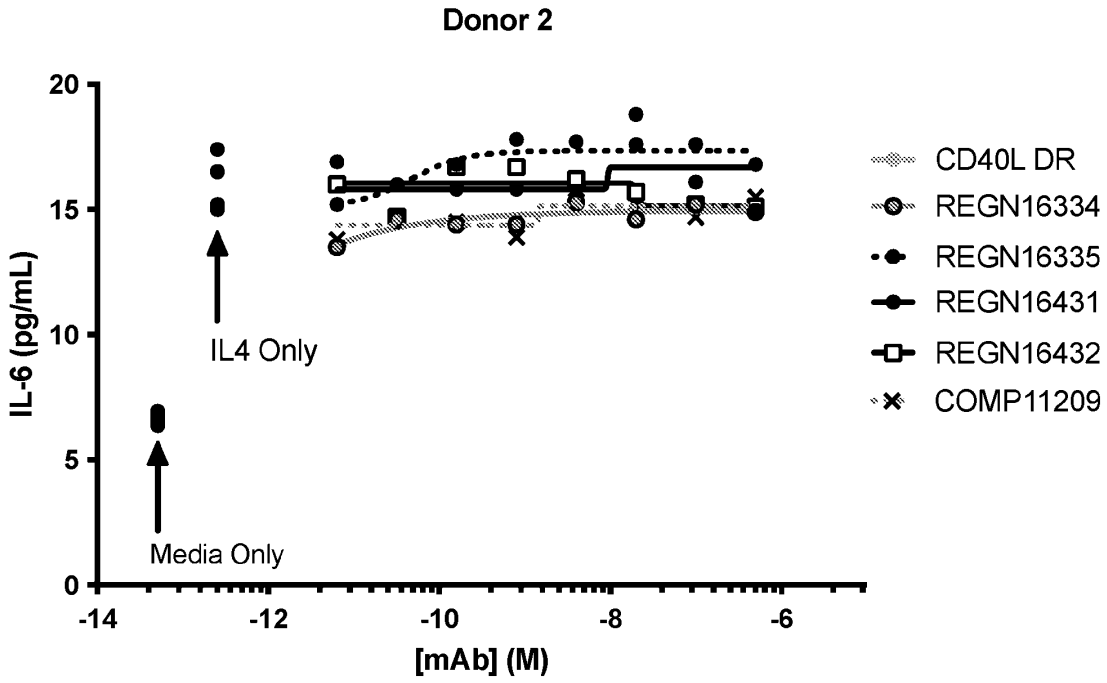

Donor 2
IL-6 (pg/mL)
20
15
10
5
0
IL4 Only
Media Only
-14
-12
-10
-8
-6
[mAb] (M)
CD40L DR
REGN16334
REGN16335
REGN16431
REGN16432
COMP11209

Figure 6A

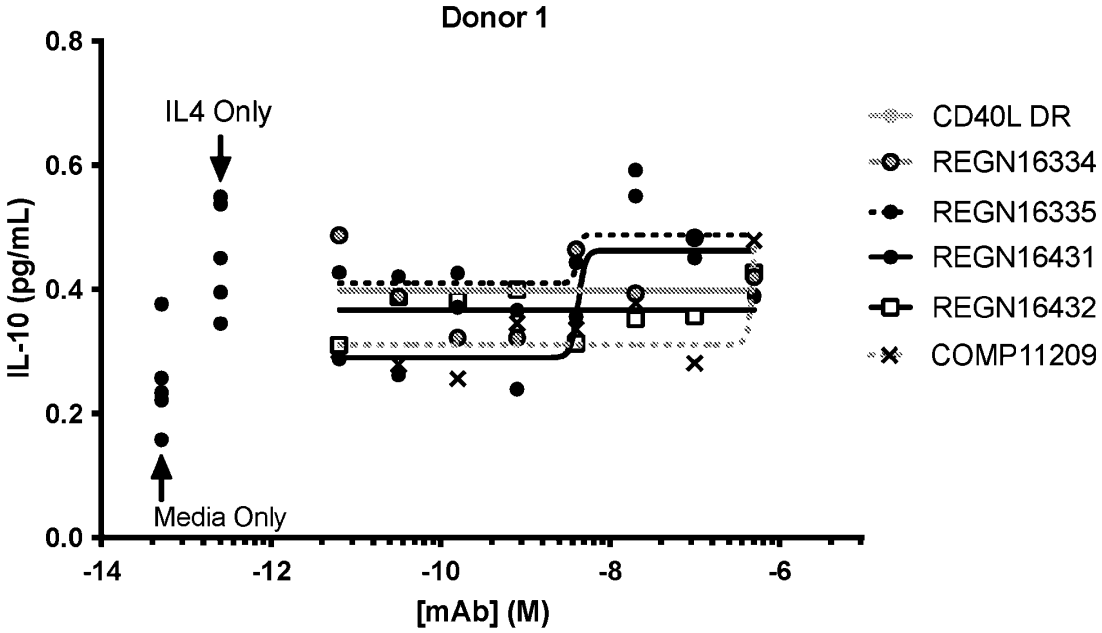

Donor 1
IL-10 (pg/mL)
0.8
0.6
0.4
0.2
0.0
IL4 Only
Media Only
-14
-12
-10
-8
-6
[mAb] (M)
CD40L DR
REGN16334
REGN16335
REGN16431
REGN16432
COMP11209

Figure 6B

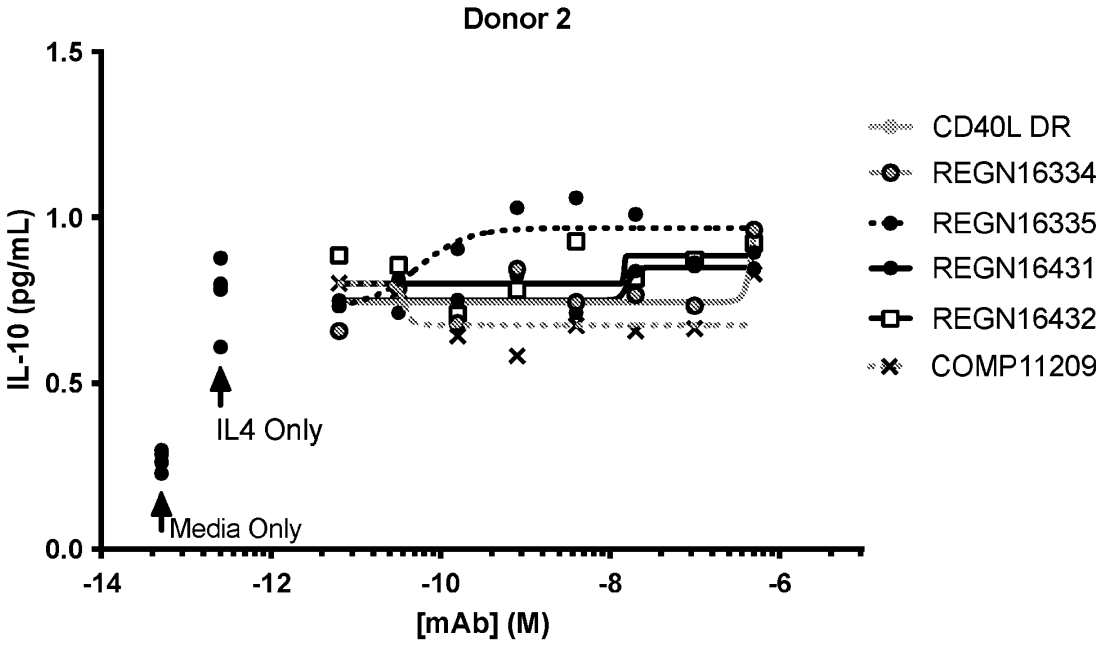

Donor 2
IL-10 (pg/mL)
1.5
1.0
0.5
0.0
-14
-12
-10
-8
-6
[mAb] (M)
IL4 Only
Media Only
CD40L DR
REGN16334
REGN16335
REGN16431
REGN16432
COMP11209

Figure 7

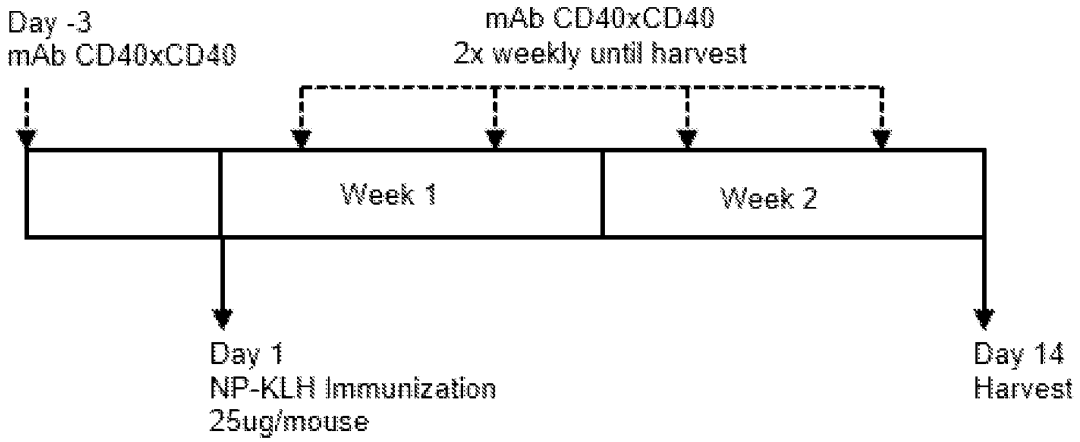

Day -3
mAb CD40xCD40
mAb CD40xCD40
2x weekly until harvest
Week 1
Week 2
Day 1
NP-KLH Immunization
25ug/mouse
Day 14
Harvest

Figure 8A

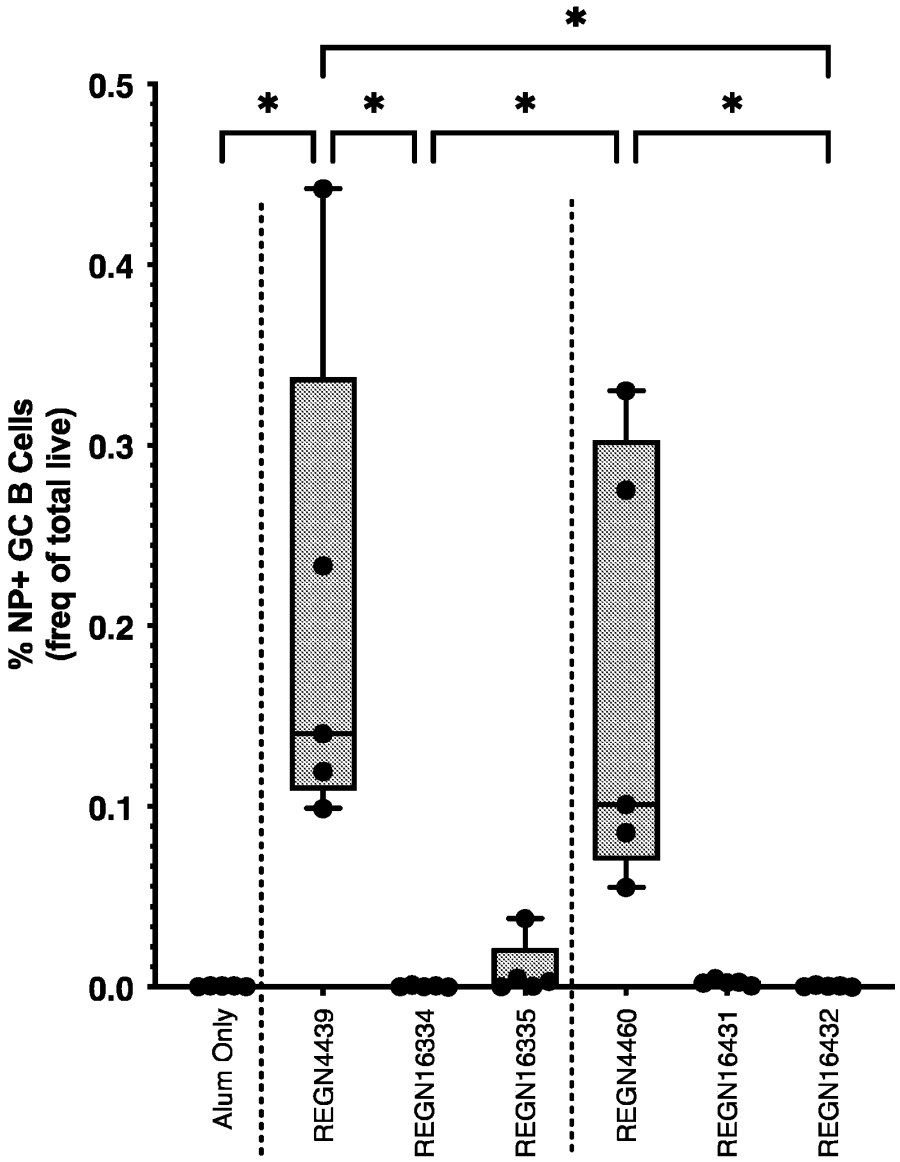

*
*
*
*
*
0.5
0.4
0.3
0.2
0.1
0.0
% NP+ GC B Cells
(freq of total live)
Alum Only
REGN4439
REGN16334
REGN16335
REGN4460
REGN16431
REGN16432

Figure 8B

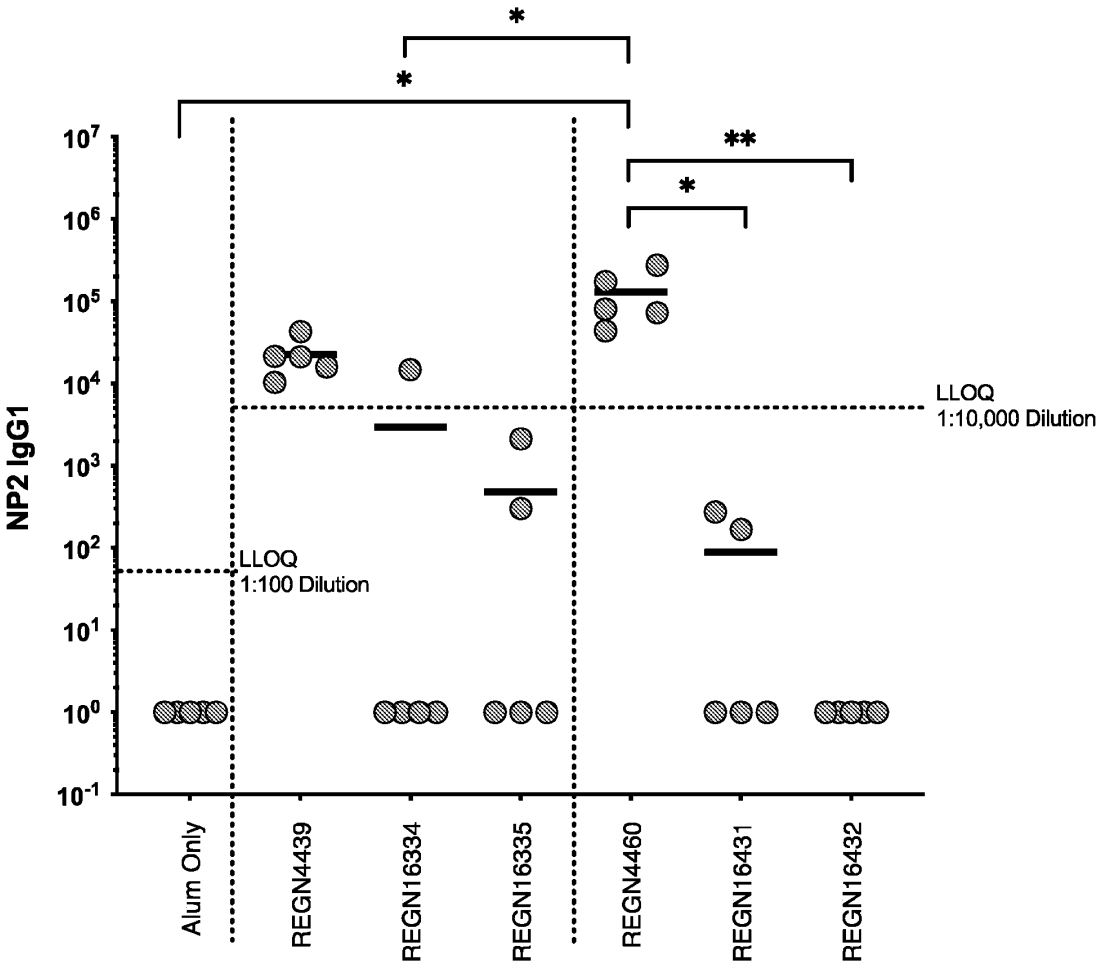

*
*
**
*
NP2 IgG1
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
LLOQ
1:10,000 Dilution
LLOQ
1:100 Dilution
Alum Only
REGN4439
REGN16334
REGN16335
REGN4460
REGN16431
REGN16432

Figure 9A

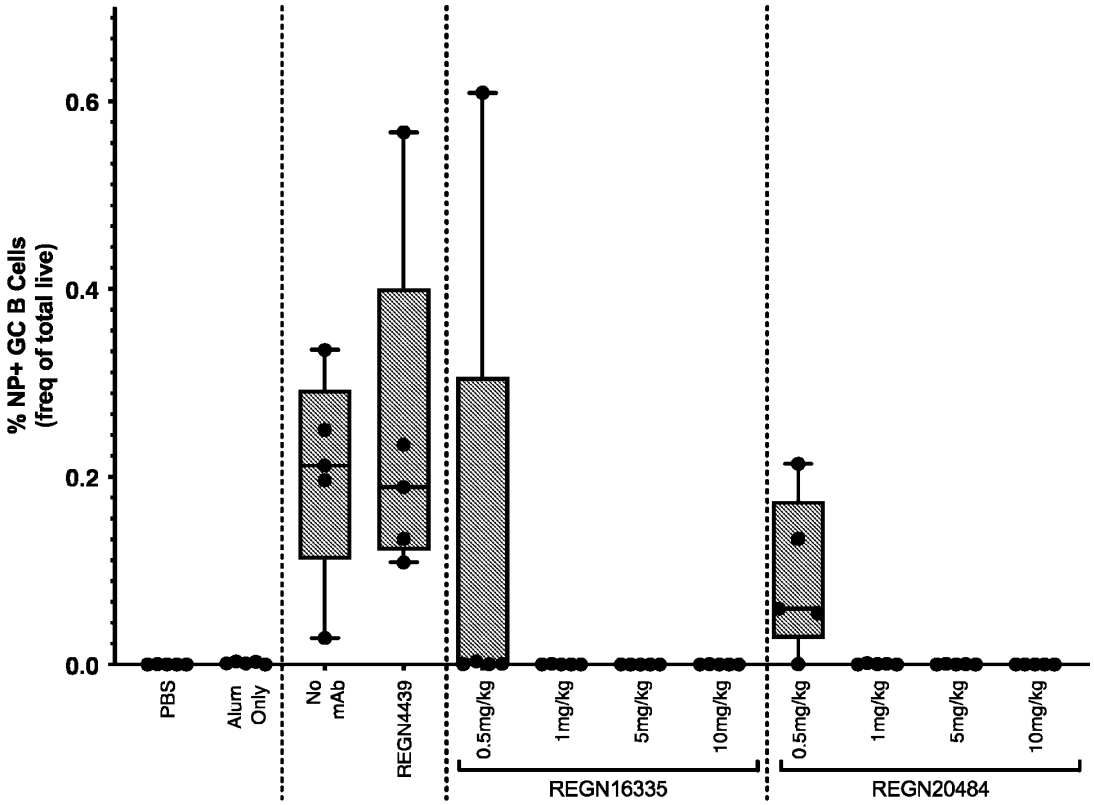

% NP+ GC B Cells
(freq of total live)
0.6
0.4
0.2
0.0
PBS
Alum Only
No mAb
REGN4439
0.5mg/kg
1mg/kg
5mg/kg
10mg/kg
REGN16335
0.5mg/kg
1mg/kg
5mg/kg
10mg/kg
REGN20484

Figure 9B

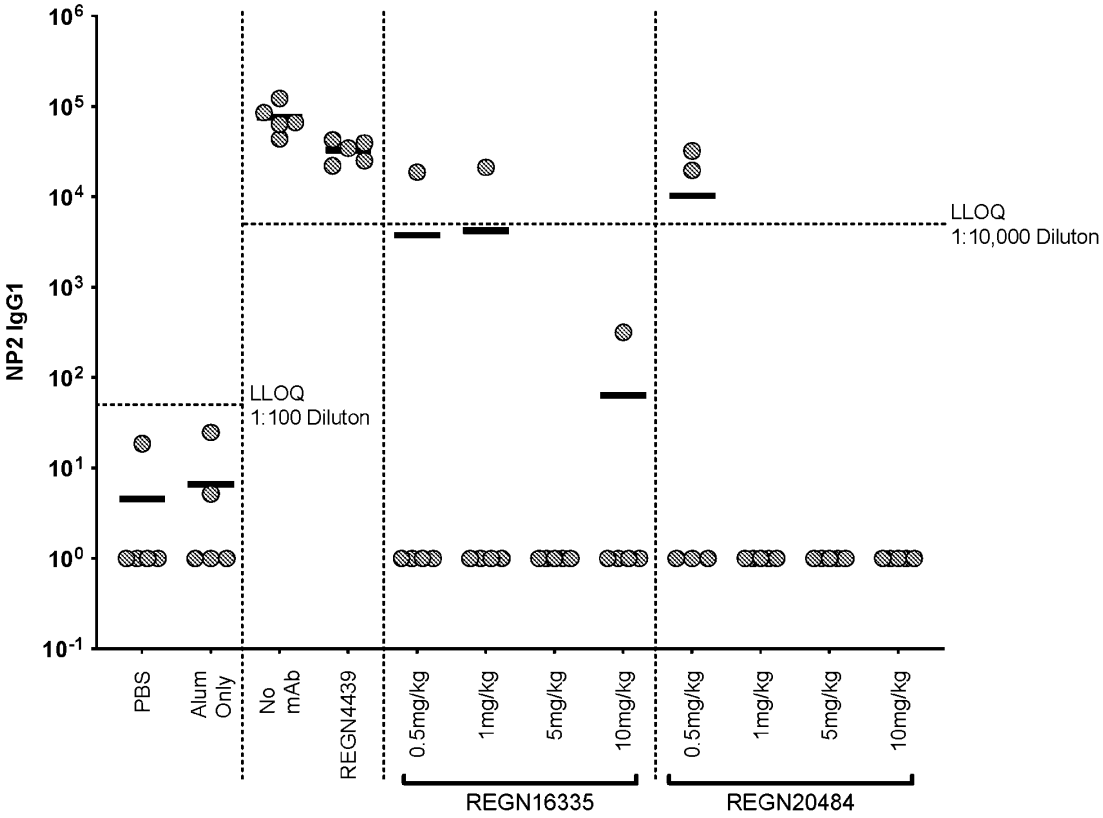

NP2 IgG1
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
LLOQ
1:100 Diluton
LLOQ
1:10,000 Diluton
PBS
Alum Only
No mAb
REGN4439
0.5mg/kg
1mg/kg
5mg/kg
10mg/kg
0.5mg/kg
1mg/kg
5mg/kg
10mg/kg
REGN16335
REGN20484

Figure 10

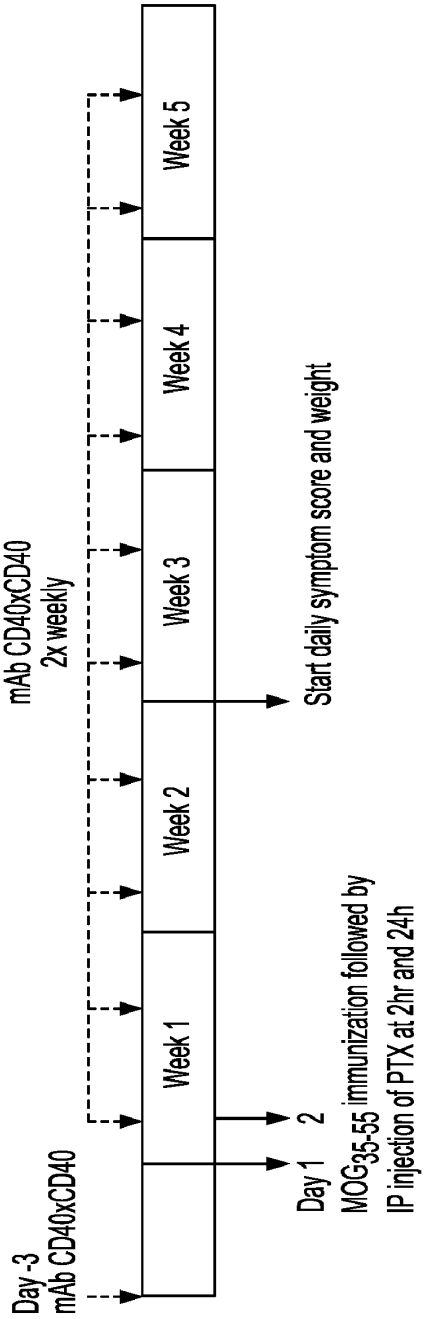

Day -3
mAb CD40xCD40
Week 1
Week 2
Week 3
Week 4
Week 5
mAb CD40xCD40
2x weekly
Start daily symptom score and weight
Day 1 2
MOG35-55 immunization followed by
IP injection of PTX at 2hr and 24h

Figure 11A

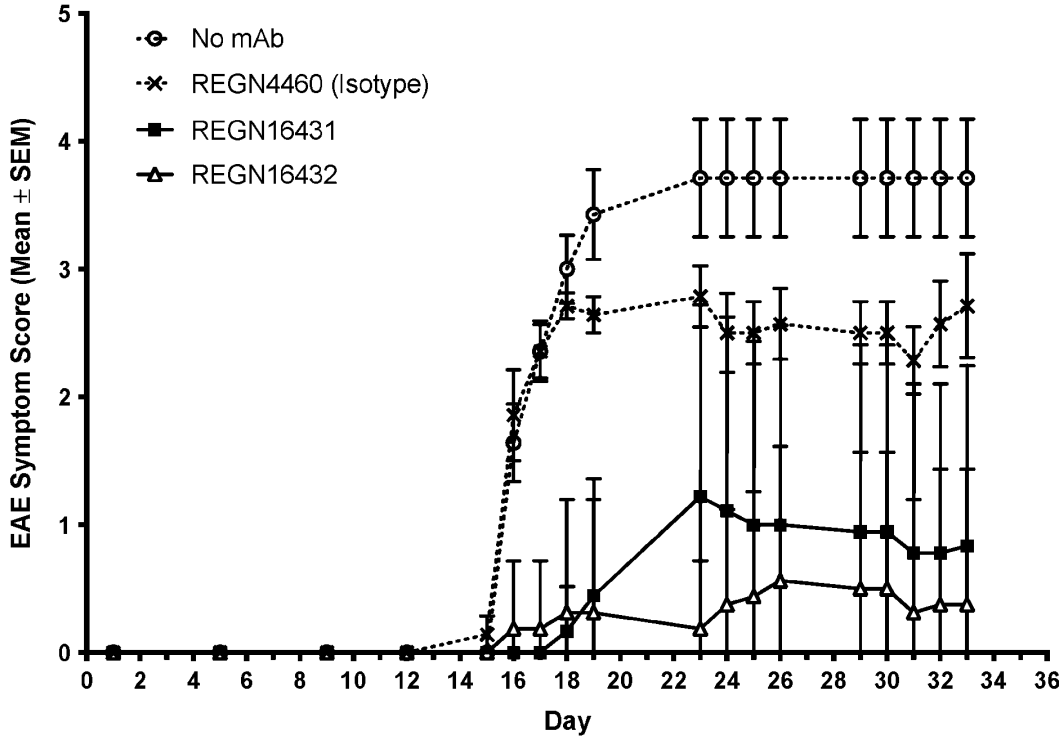

No mAb
REGN4460 (Isotype)
REGN16431
REGN16432
EAE Symptom Score (Mean ± SEM)
Day
0
1
2
3
4
5
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36

Figure 11B

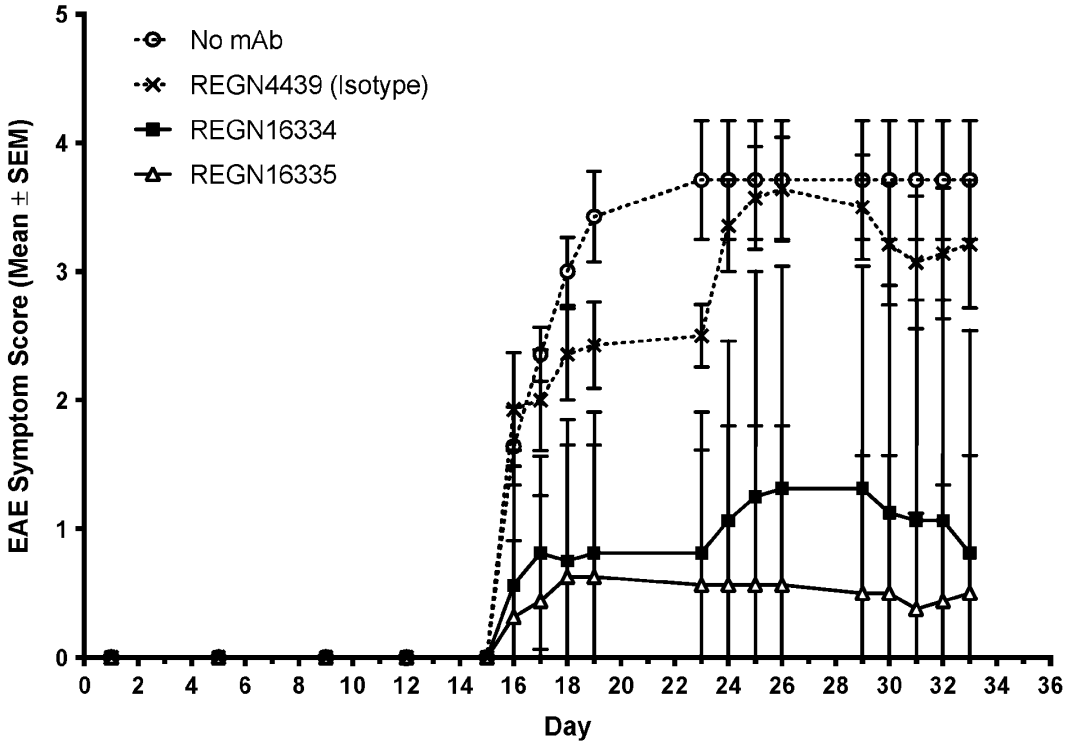

No mAb
REGN4439 (Isotype)
REGN16334
REGN16335
EAE Symptom Score (Mean ± SEM)
Day
0
1
2
3
4
5
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36

Figure 11C

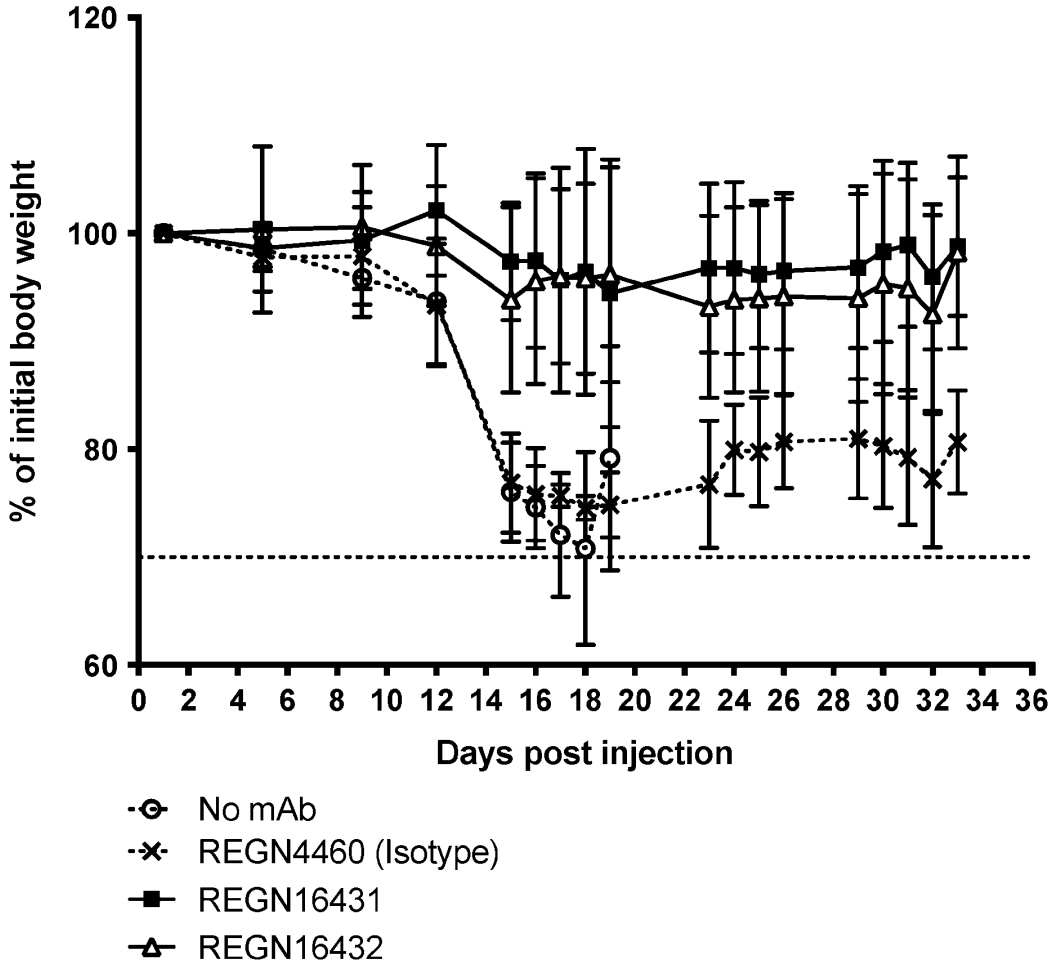

120
100
80
60
% of initial body weight
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Days post injection
No mAb
REGN4460 (Isotype)
REGN16431
REGN16432

Figure 11D

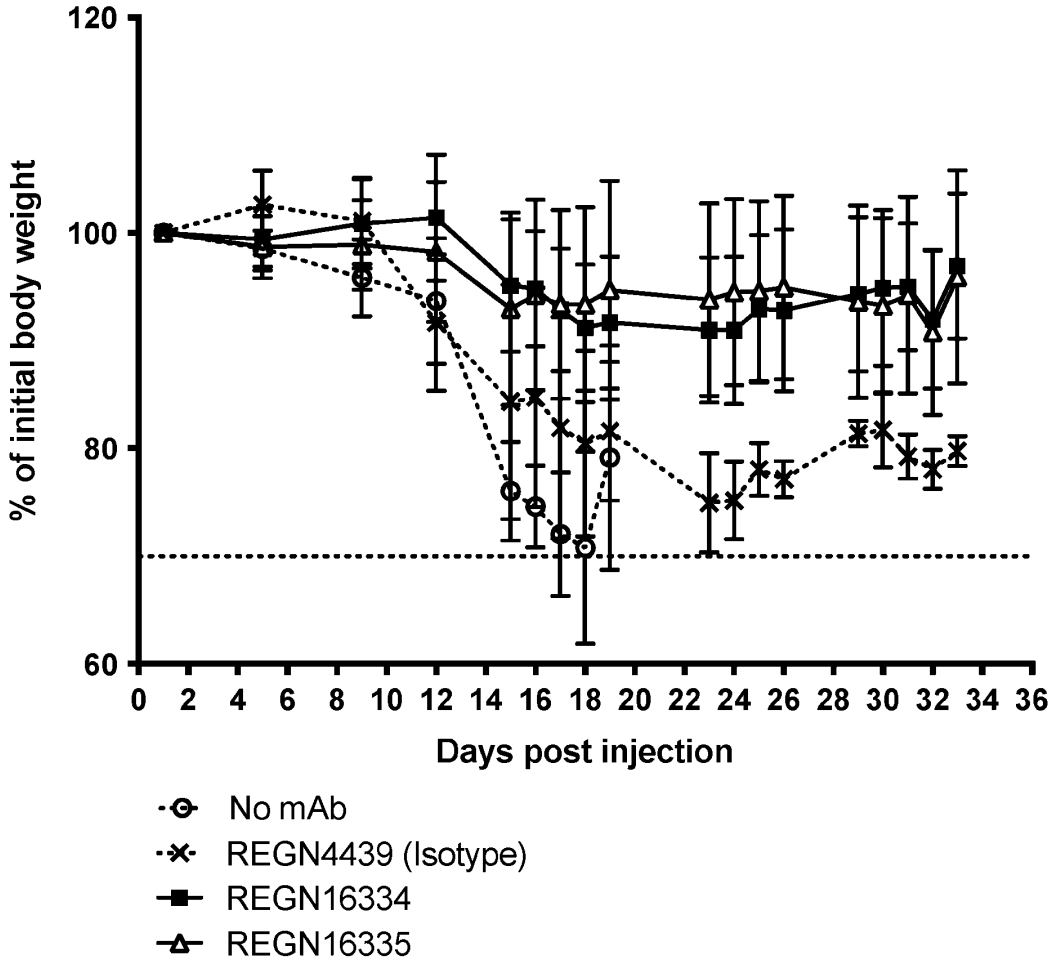

120
100
80
60
% of initial body weight
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Days post injection
No mAb
REGN4439 (Isotype)
REGN16334
REGN16335

Figure 12A

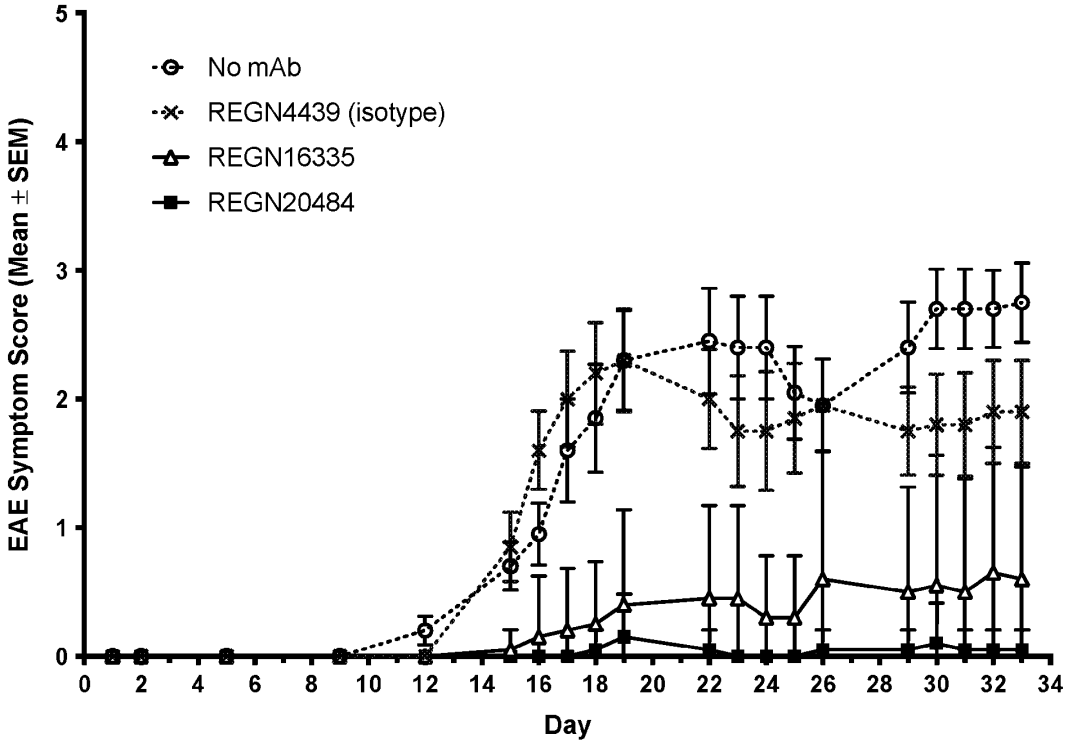

No mAb
REGN4439 (isotype)
REGN16335
REGN20484
EAE Symptom Score (Mean ± SEM)
Day
0
1
2
3
4
5
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34

Figure 12B

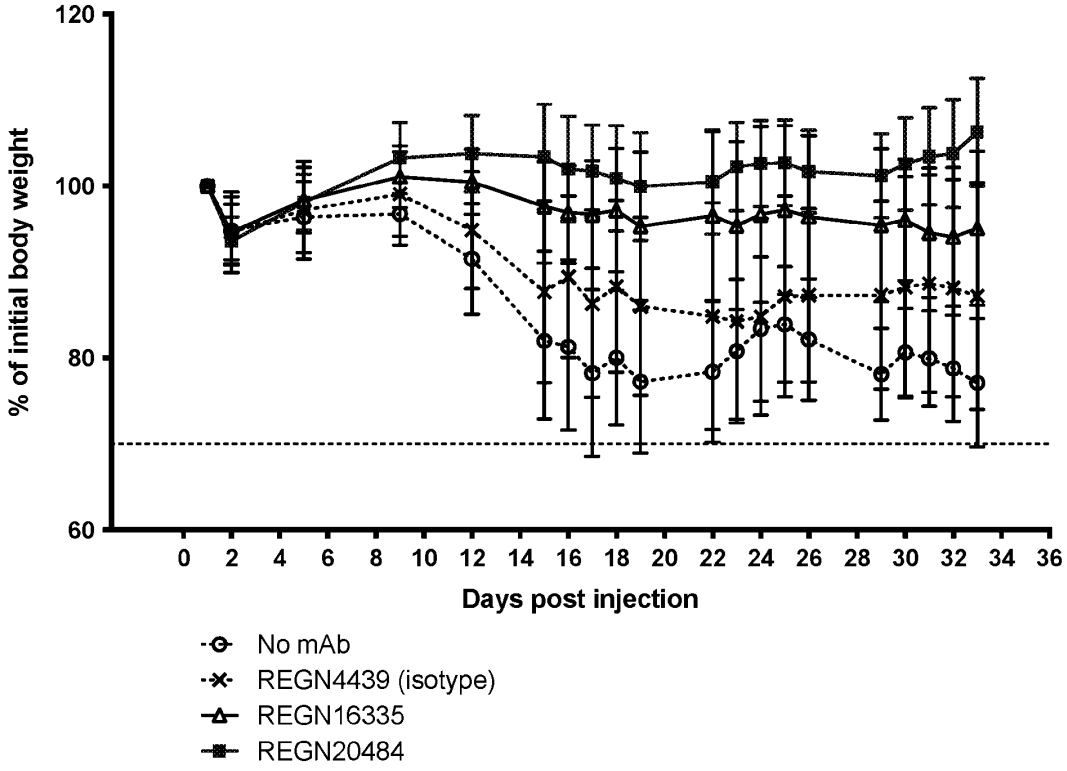

120
100
80
60
% of initial body weight
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Days post injection
No mAb
REGN4439 (isotype)
REGN16335
REGN20484

Figure 13

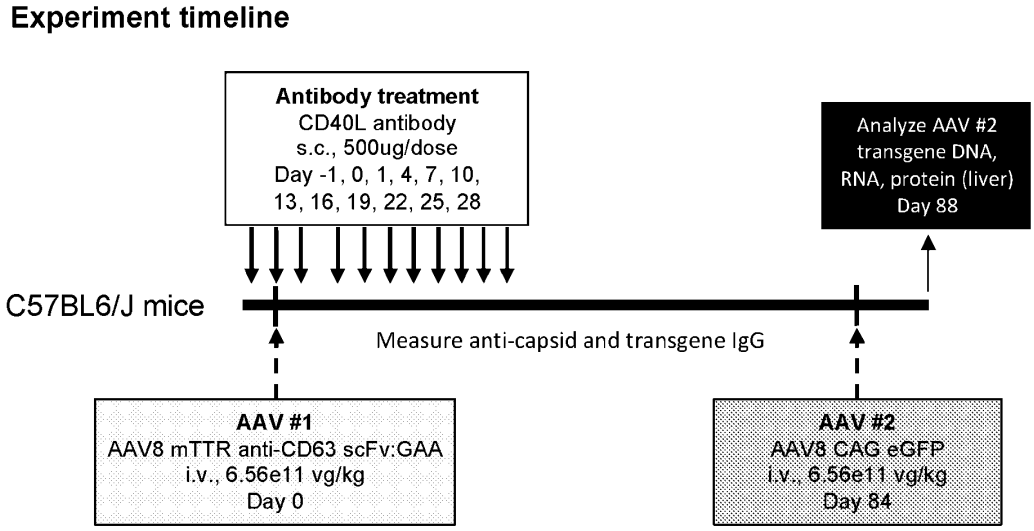

Experiment timeline
Antibody treatment
CD40L antibody
s.c., 500ug/dose
Day -1, 0, 1, 4, 7, 10,
13, 16, 19, 22, 25, 28
Analyze AAV #2
transgene DNA,
RNA, protein (liver)
Day 88
C57BL6/J mice
Measure anti-capsid and transgene IgG
AAV #1
AAV8 mTTR anti-CD63 scFv:GAA
i.v., 6.56e11 vg/kg
Day 0
AAV #2
AAV8 CAG eGFP
i.v., 6.56e11 vg/kg
Day 84

Figure 14

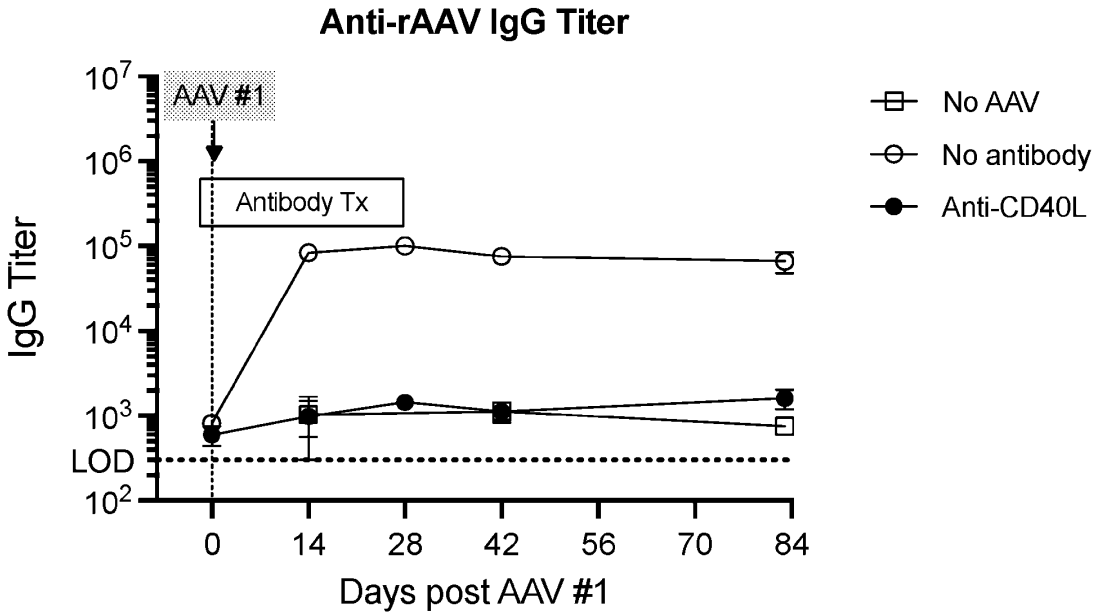

Anti-rAAV IgG Titer
IgG Titer
10^7
10^6
10^5
10^4
10^3
LOD
10^2
AAV #1
Antibody Tx
0
14
28
42
56
70
84
Days post AAV #1
No AAV
No antibody
Anti-CD40L

Figure 15A

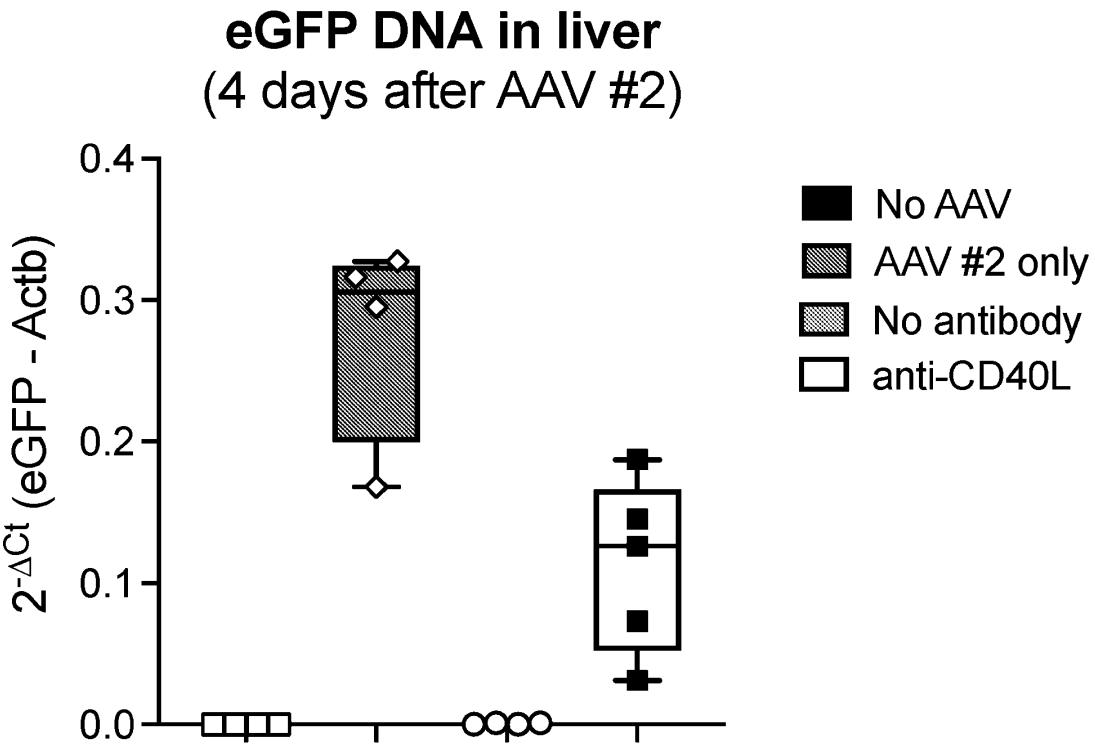

eGFP DNA in liver
(4 days after AAV #2)
0.4
0.3
0.2
0.1
0.0
2-ΔCt (eGFP - Actb)
No AAV
AAV #2 only
No antibody
anti-CD40L

Figure 15B

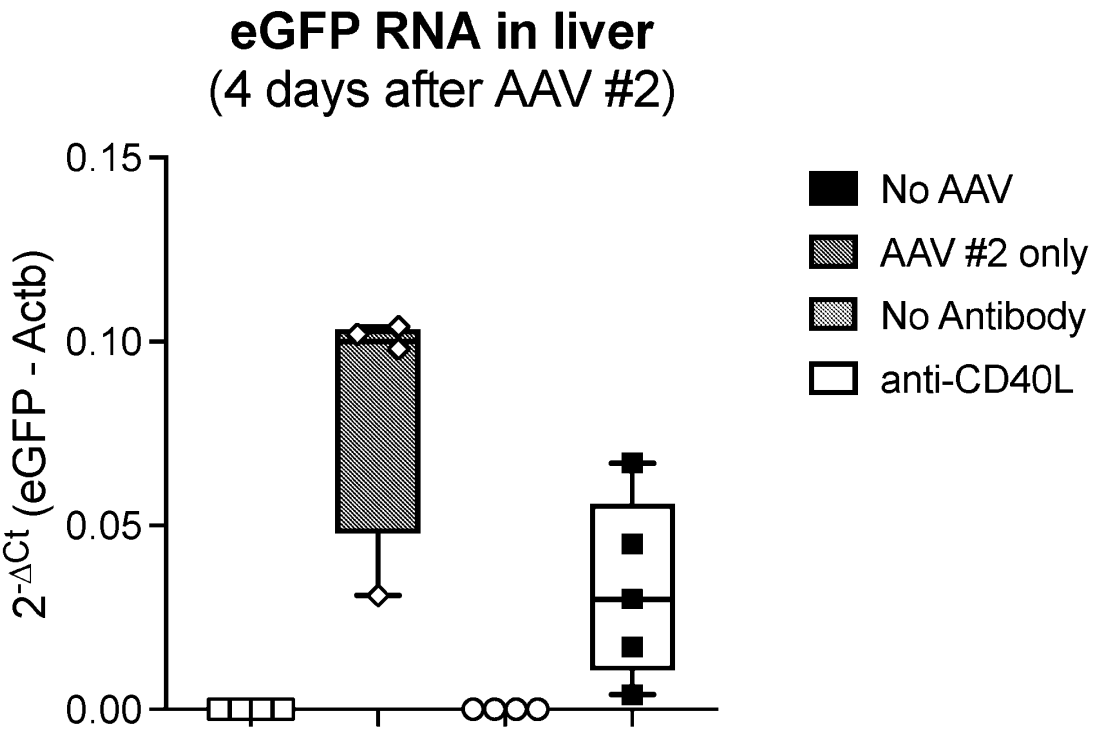

eGFP RNA in liver
(4 days after AAV #2)
0.15
0.10
0.05
0.00
2-ΔCt (eGFP - Actb)
No AAV
AAV #2 only
No Antibody
anti-CD40L

Figure 15C

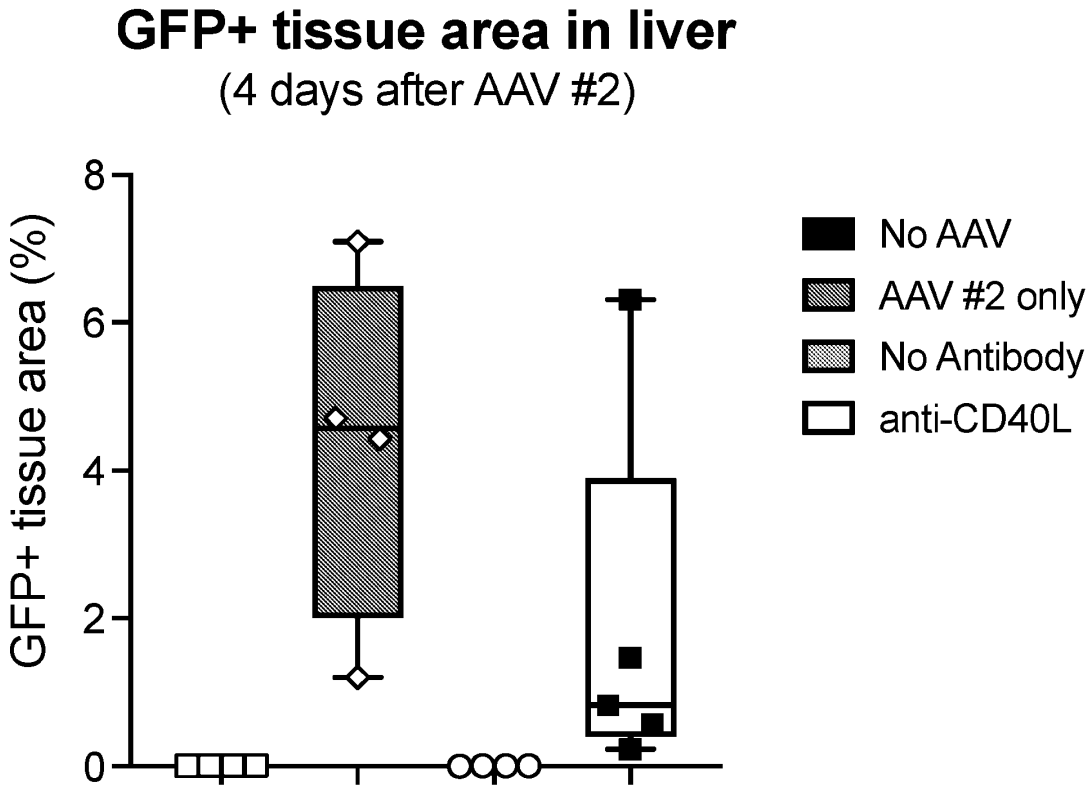

GFP+ tissue area in liver
(4 days after AAV #2)
GFP+ tissue area (%)
8
6
4
2
0
No AAV
AAV #2 only
No Antibody
anti-CD40L

Figure 16

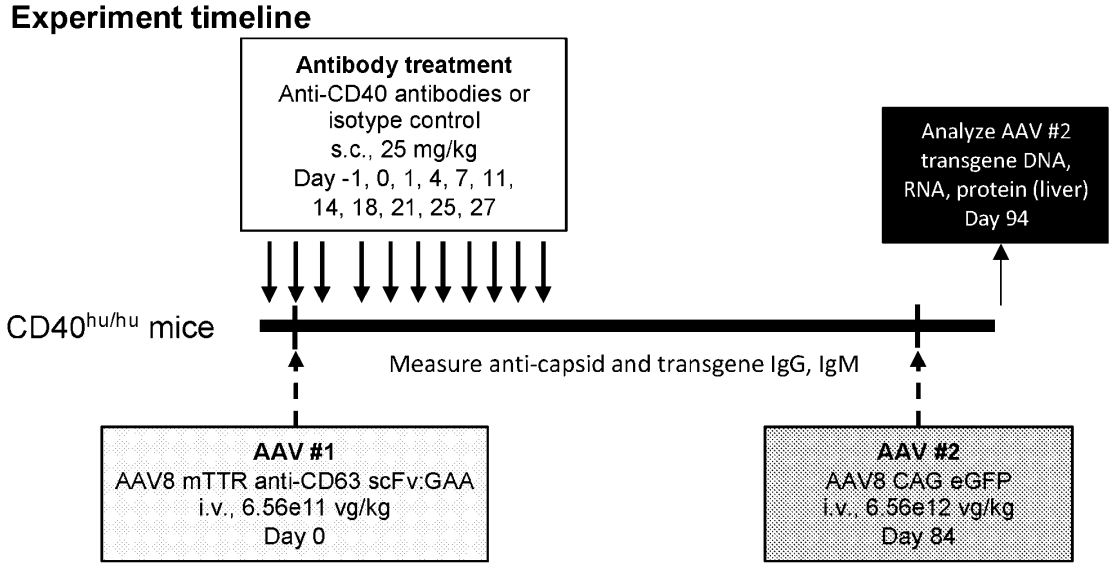

Experiment timeline
Antibody treatment
Anti-CD40 antibodies or isotype control
s.c., 25 mg/kg
Day -1, 0, 1, 4, 7, 11, 14, 18, 21, 25, 27
Analyze AAV #2 transgene DNA, RNA, protein (liver)
Day 94
CD40hu/hu mice
Measure anti-capsid and transgene IgG, IgM
AAV #1
AAV8 mTTR anti-CD63 scFv:GAA
i.v., 6.56e11 vg/kg
Day 0
AAV #2
AAV8 CAG eGFP
i.v., 6.56e12 vg/kg
Day 84

Figure 17A

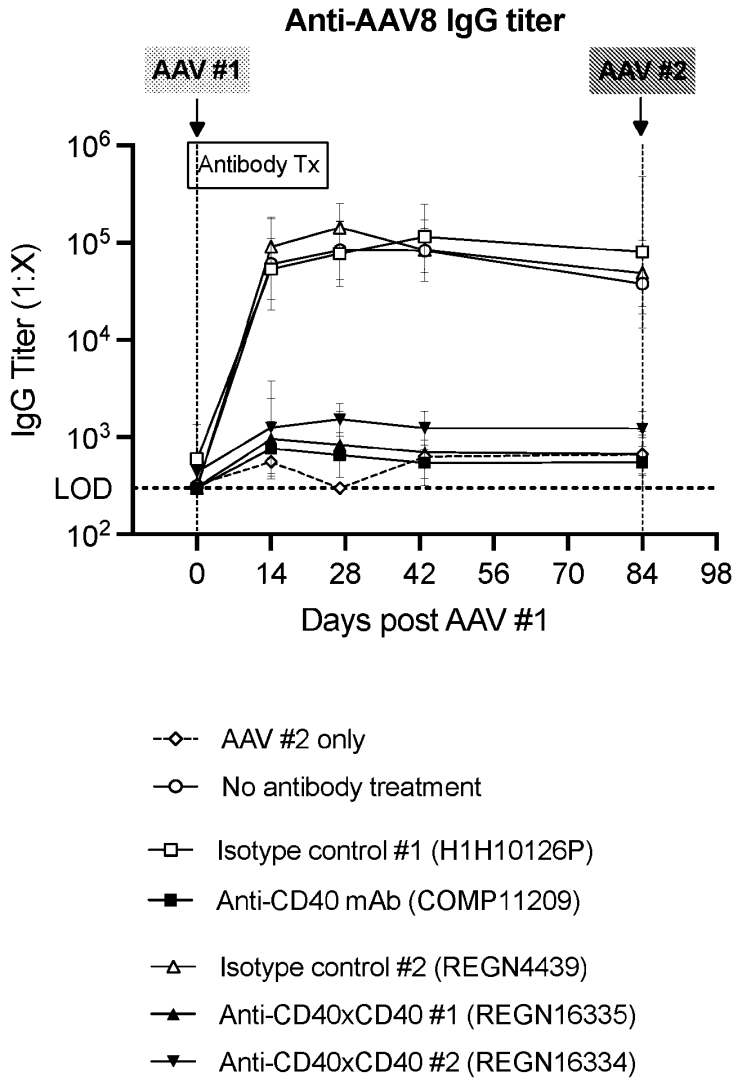

Anti-AAV8 IgG titer
AAV #1
AAV #2
Antibody Tx
IgG Titer (1:X)
10^6
10^5
10^4
10^3
LOD
10^2
0
14
28
42
56
70
84
98
Days post AAV #1
AAV #2 only
No antibody treatment
Isotype control #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Isotype control #2 (REGN4439)
Anti-CD40xCD40 #1 (REGN16335)
Anti-CD40xCD40 #2 (REGN16334)

Figure 17B

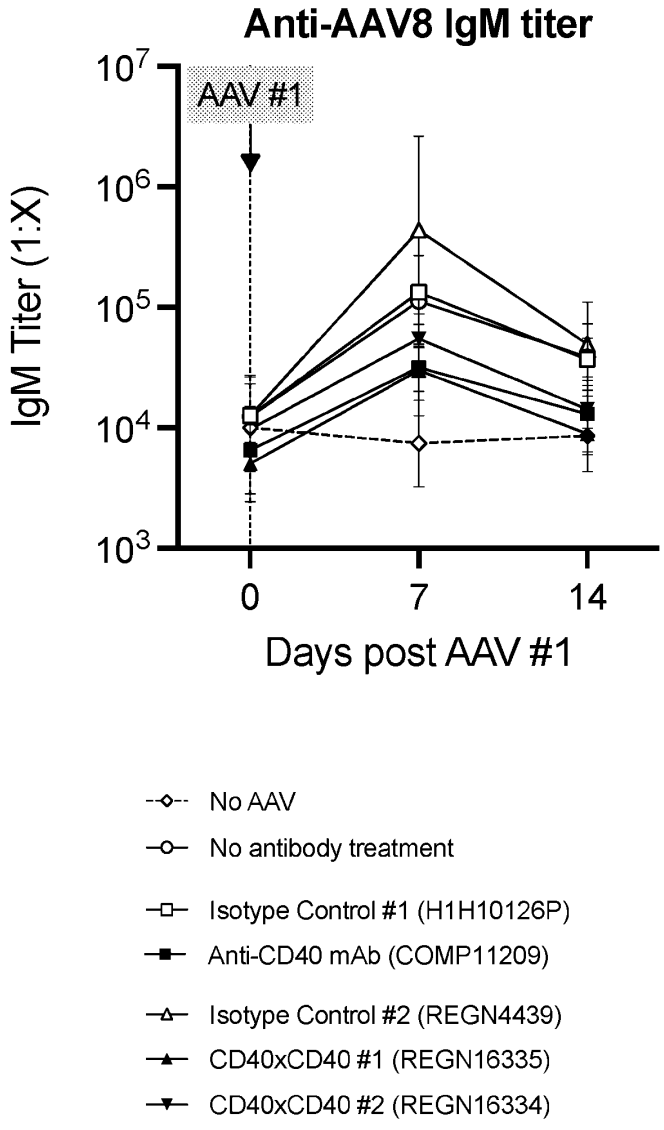

Anti-AAV8 IgM titer
AAV #1
IgM Titer (1:X)
10^7
10^6
10^5
10^4
10^3
0
7
14
Days post AAV #1
No AAV
No antibody treatment
Isotype Control #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Isotype Control #2 (REGN4439)
CD40xCD40 #1 (REGN16335)
CD40xCD40 #2 (REGN16334)

Figure 18A

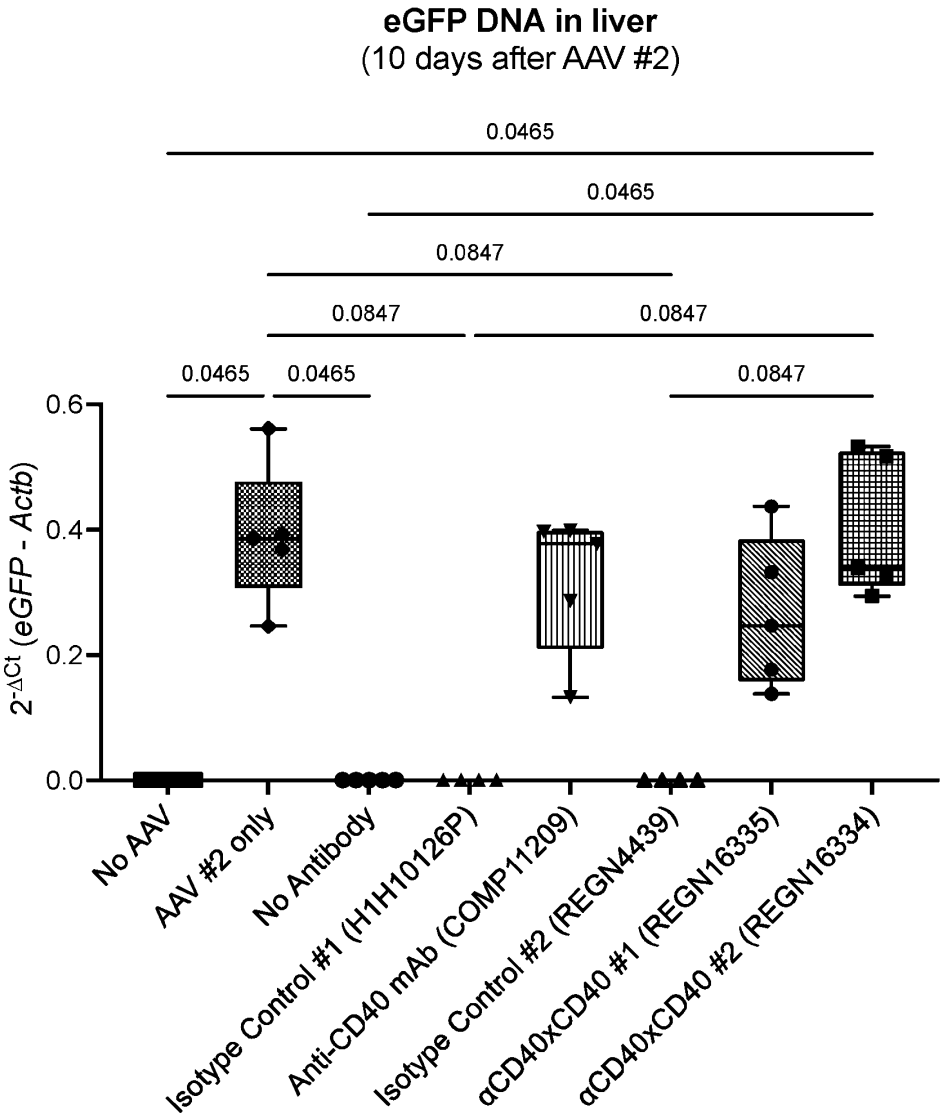

eGFP DNA in liver
(10 days after AAV #2)
0.0465
0.0465
0.0847
0.0847
0.0847
0.0465
0.0465
0.0847
0.6
0.4
0.2
0.0
2-ΔCt (eGFP - Actb)
No AAV
AAV #2 only
No Antibody
Isotype Control #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Isotype Control #2 (REGN4439)
αCD40xCD40 #1 (REGN16335)
αCD40xCD40 #2 (REGN16334)

Figure 18B

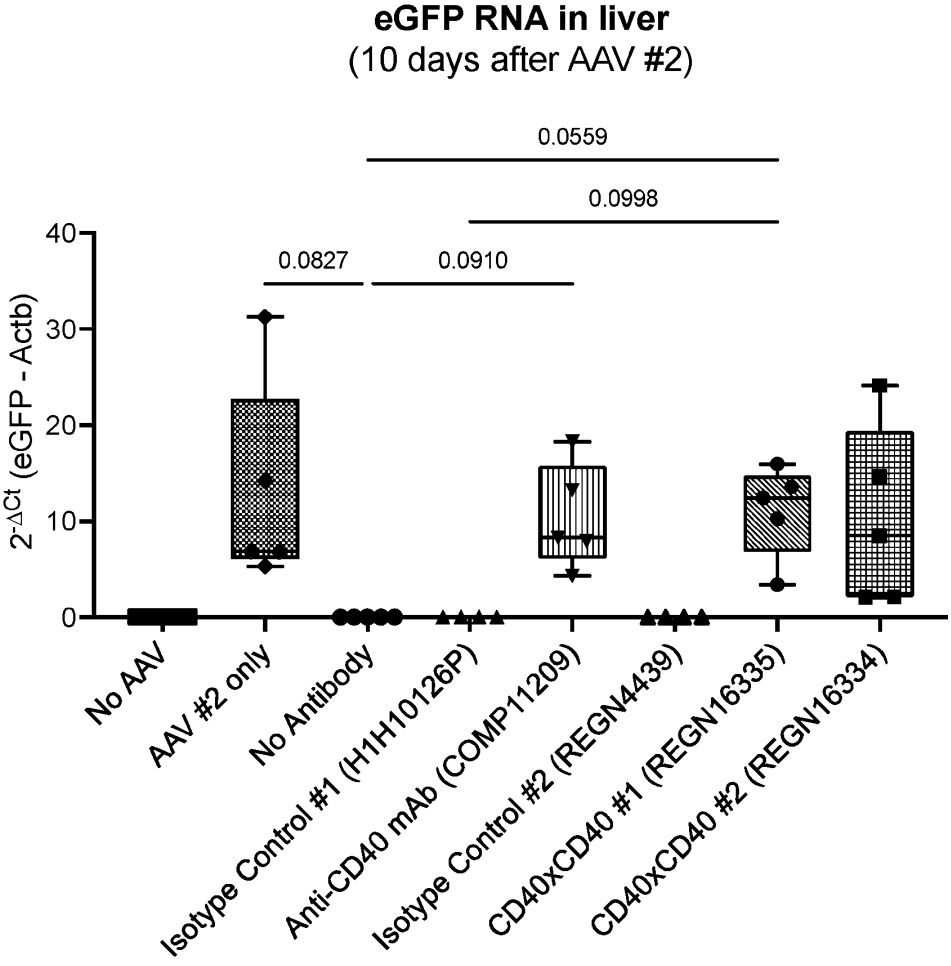

eGFP RNA in liver
(10 days after AAV #2)
0.0559
0.0998
0.0827
0.0910
40
30
20
10
0
2-ΔCt (eGFP - Actb)
No AAV
AAV #2 only
No Antibody
Isotype Control #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Isotype Control #2 (REGN4439)
CD40xCD40 #1 (REGN16335)
CD40xCD40 #2 (REGN16334)

Figure 18C

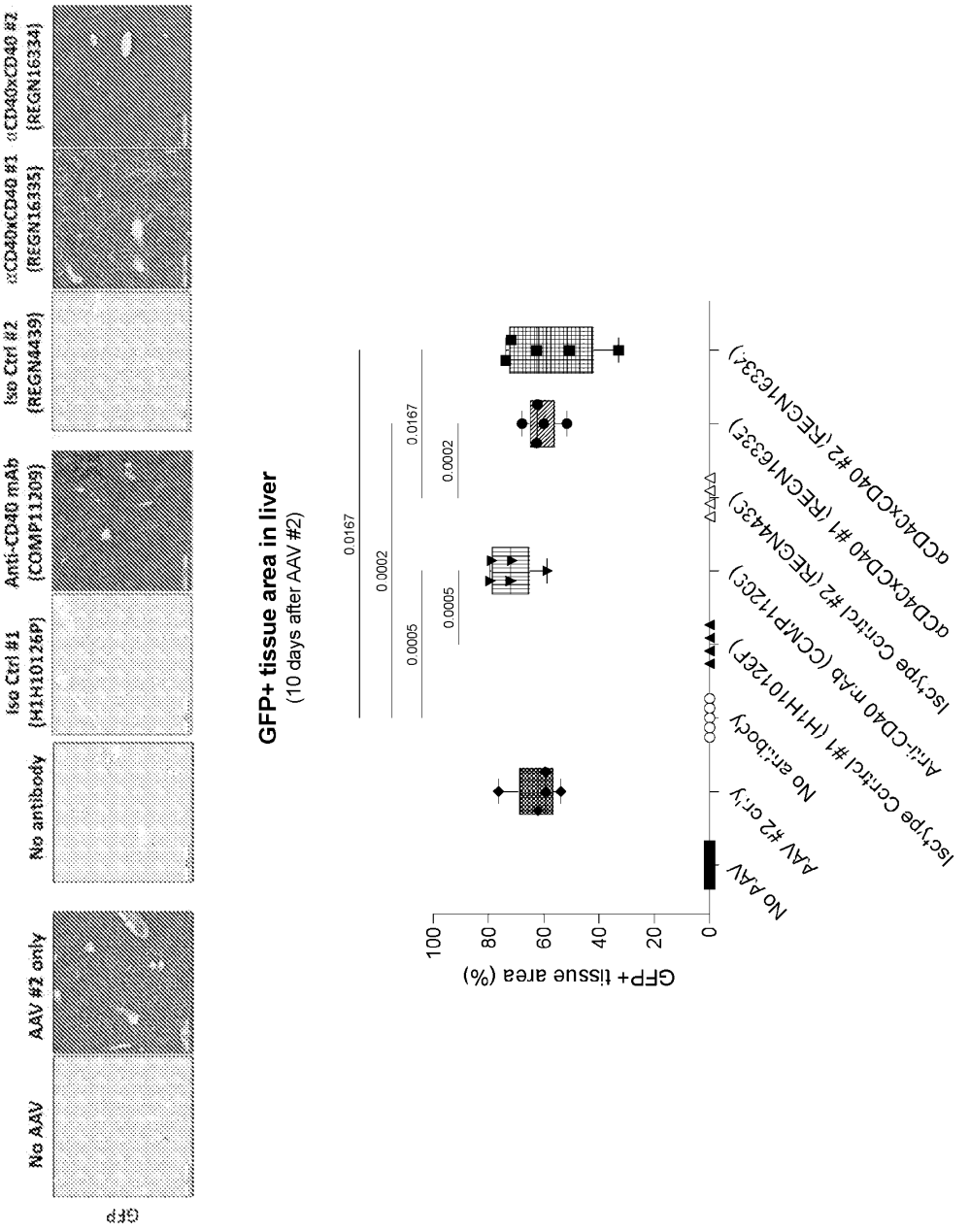

No AAV
AAV #2 only
No antibody
Iso Ctrl #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Iso Ctrl #2 (REGN4439)
αCD40xCD40 #1 (REGN16335)
αCD40xCD40 #2 (REGN16334)
GFP
GFP+ tissue area in liver
(10 days after AAV #2)
0.0167
0.0002
0.0005
0.0167
0.0005
0.0002
GFP+ tissue area (%)
100
80
60
40
20
0
No AAV
AAV #2 only
No antibody
Iso Ctrl #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Iso Ctrl #2 (REGN4439)
αCD40xCD40 #1 (REGN16335)
αCD40xCD40 #2 (REGN16332)

Figure 19

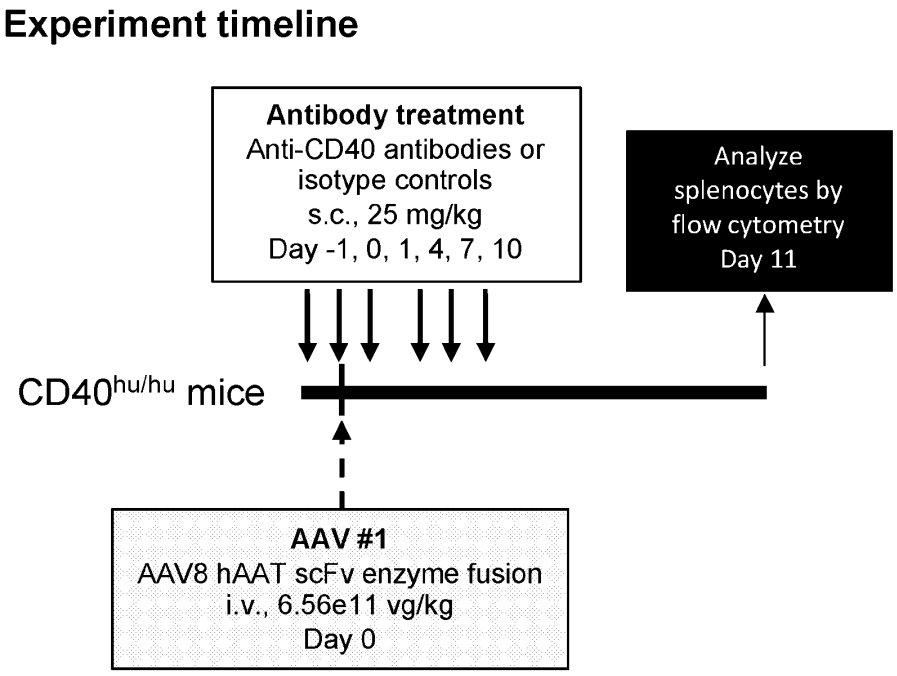

Experiment timeline
Antibody treatment
Anti-CD40 antibodies or isotype controls
s.c., 25 mg/kg
Day -1, 0, 1, 4, 7, 10
Analyze splenocytes by flow cytometry
Day 11
CD40hu/hu mice
AAV #1
AAV8 hAAT scFv enzyme fusion
i.v., 6.56e11 vg/kg
Day 0

Figure 20A

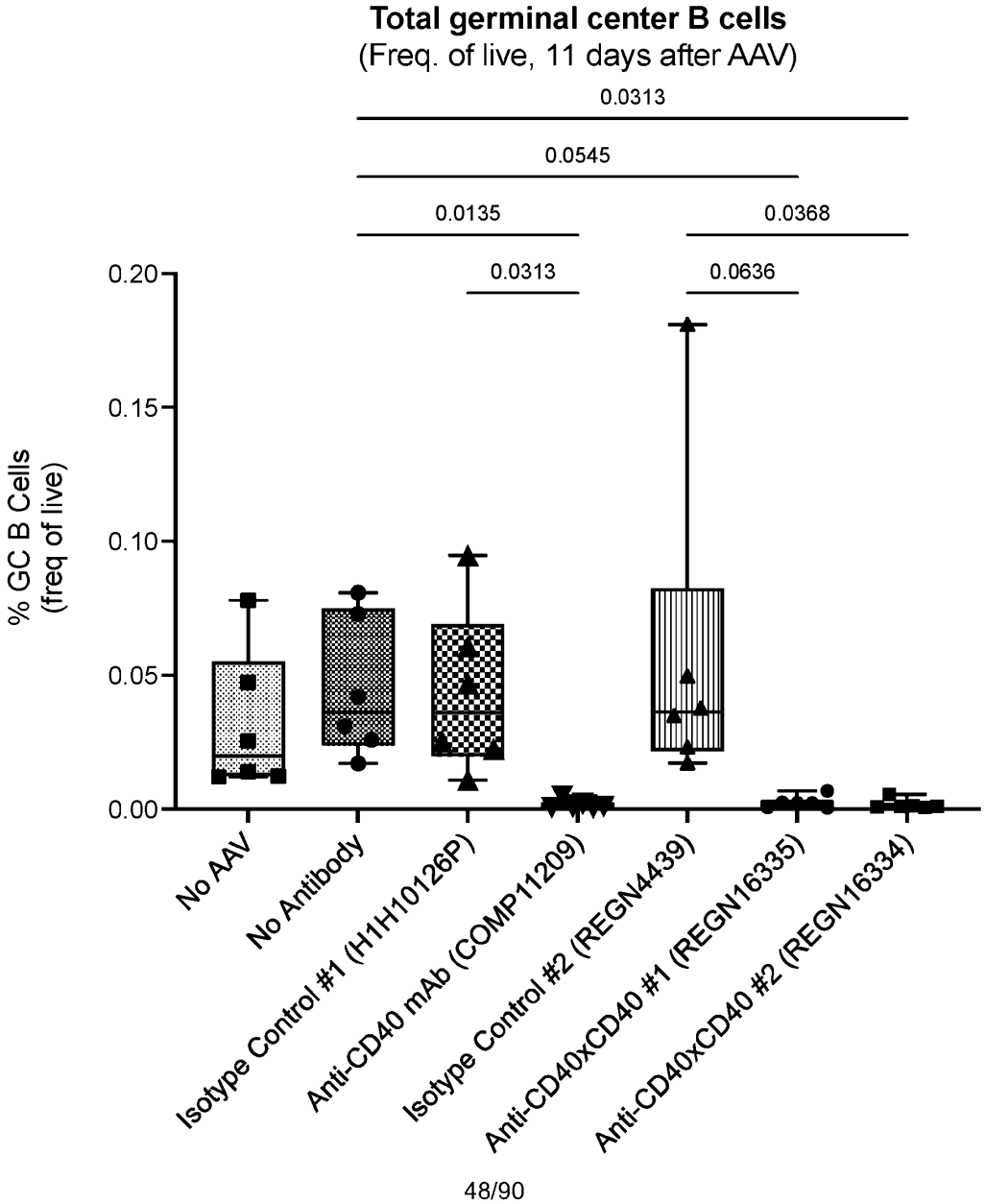

Total germinal center B cells
(Freq. of live, 11 days after AAV)
0.0313
0.0545
0.0135
0.0368
0.0313
0.0636
% GC B Cells
(freq of live)
0.20
0.15
0.10
0.05
0.00
No AAV
No Antibody
Isotype Control #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Isotype Control #2 (REGN4439)
Anti-CD40xCD40 #1 (REGN16335)
Anti-CD40xCD40 #2 (REGN16334)

Figure 20B

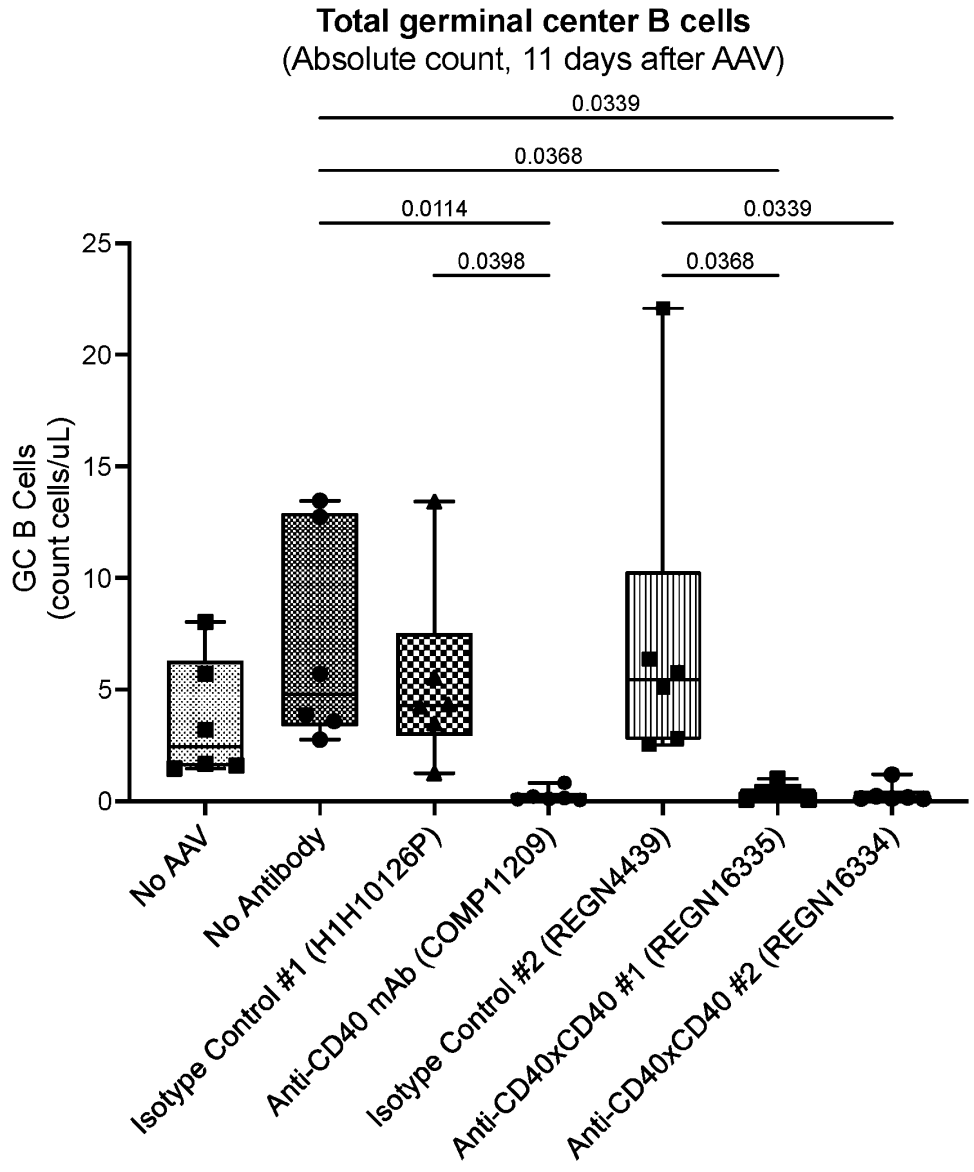

Total germinal center B cells
(Absolute count, 11 days after AAV)
0.0339
0.0368
0.0114
0.0339
0.0398
0.0368
GC B Cells
(count cells/uL)
25
20
15
10
5
0
No AAV
No Antibody
Isotype Control #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Isotype Control #2 (REGN4439)
Anti-CD40xCD40 #1 (REGN16335)
Anti-CD40xCD40 #2 (REGN16334)

Figure 20C

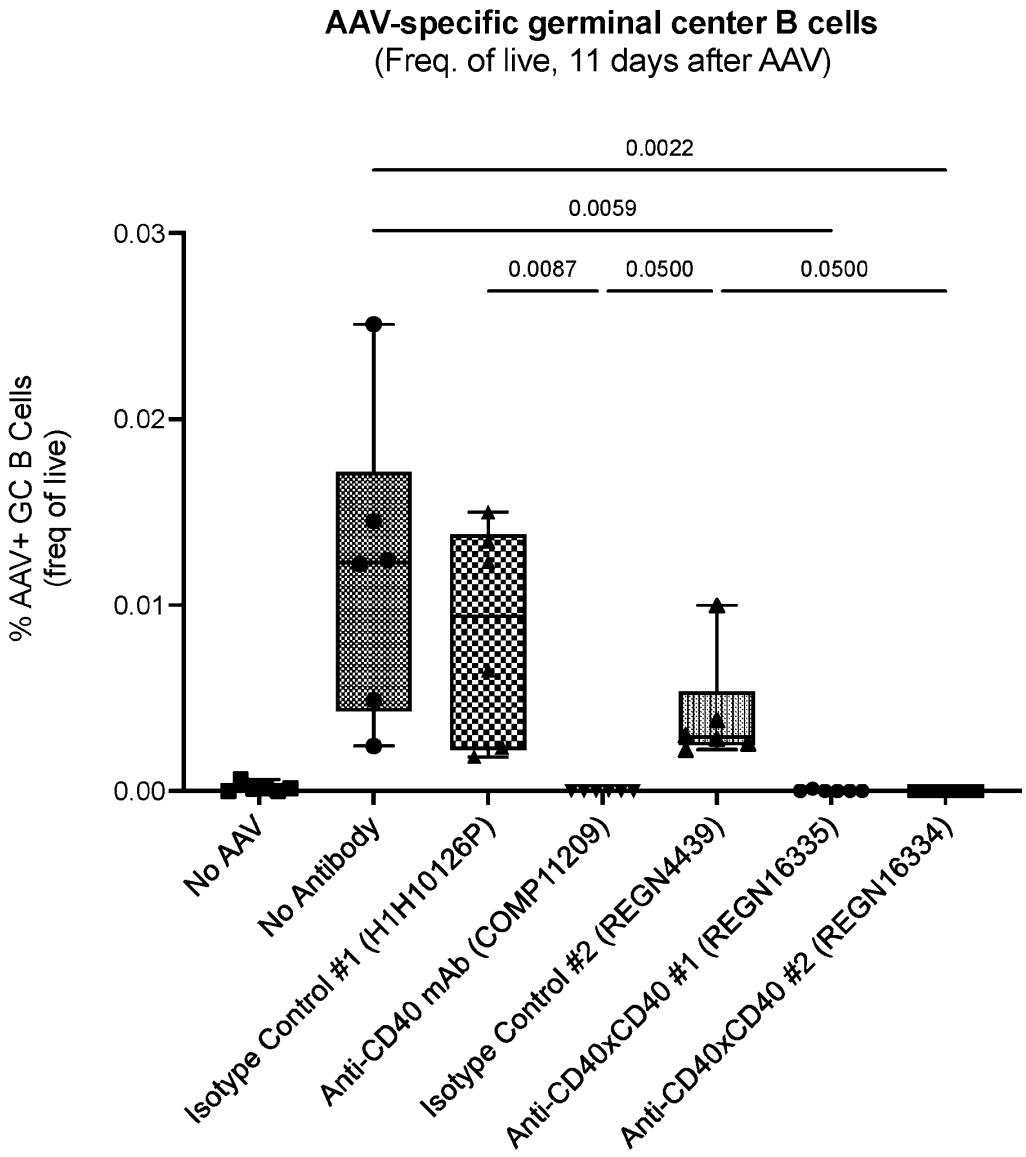

AAV-specific germinal center B cells
(Freq. of live, 11 days after AAV)
0.0022
0.0059
0.0087
0.0500
0.0500
% AAV+ GC B Cells
(freq of live)
0.03
0.02
0.01
0.00
No AAV
No Antibody
Isotype Control #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Isotype Control #2 (REGN4439)
Anti-CD40xCD40 #1 (REGN16335)
Anti-CD40xCD40 #2 (REGN16334)

Figure 20D

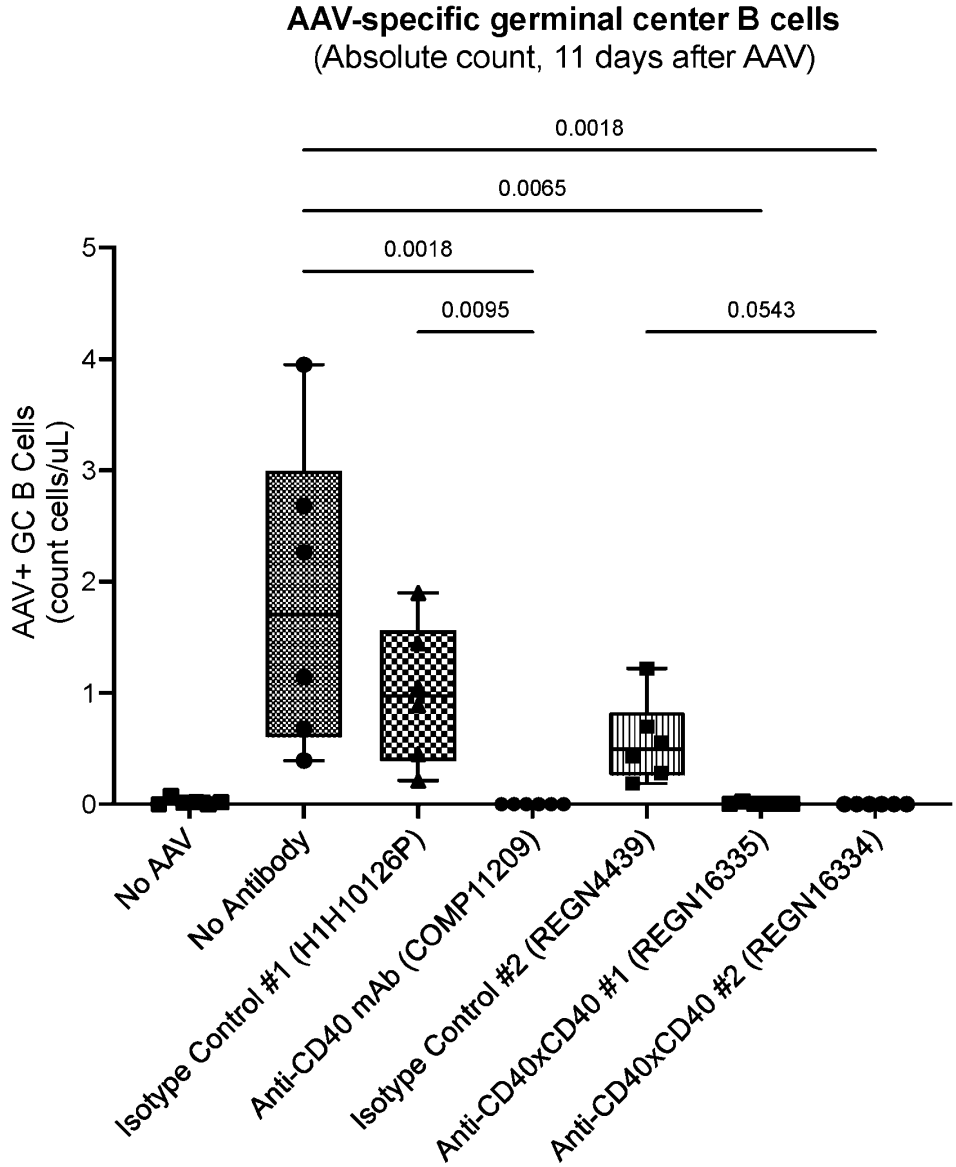

AAV-specific germinal center B cells
(Absolute count, 11 days after AAV)
0.0018
0.0065
0.0018
0.0095
0.0543
AAV+ GC B Cells
(count cells/uL)
5
4
3
2
1
0
No AAV
No Antibody
Isotype Control #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Isotype Control #2 (REGN4439)
Anti-CD40xCD40 #1 (REGN16335)
Anti-CD40xCD40 #2 (REGN16334)

Figure 21A

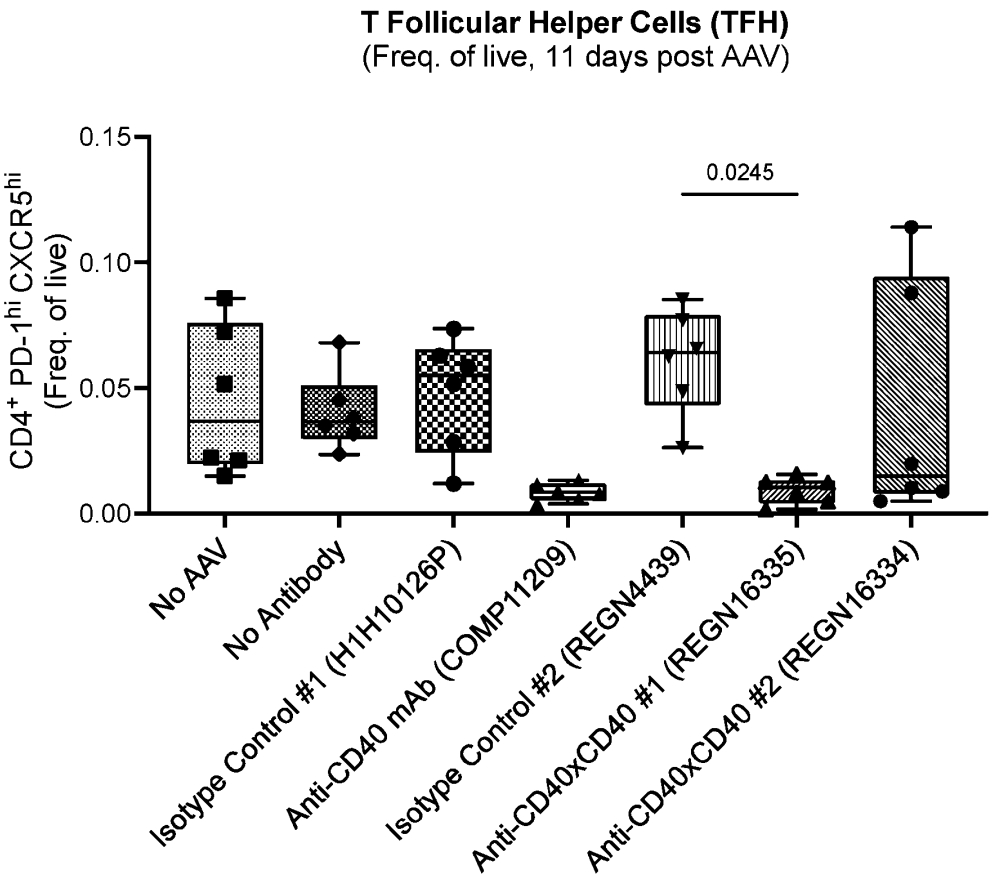

T Follicular Helper Cells (TFH)
(Freq. of live, 11 days post AAV)
CD4+ PD-1hi CXCR5hi
(Freq. of live)
0.15
0.10
0.05
0.00
0.0245
No AAV
No Antibody
Isotype Control #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Isotype Control #2 (REGN4439)
Anti-CD40xCD40 #1 (REGN16335)
Anti-CD40xCD40 #2 (REGN16334)

Figure 21B

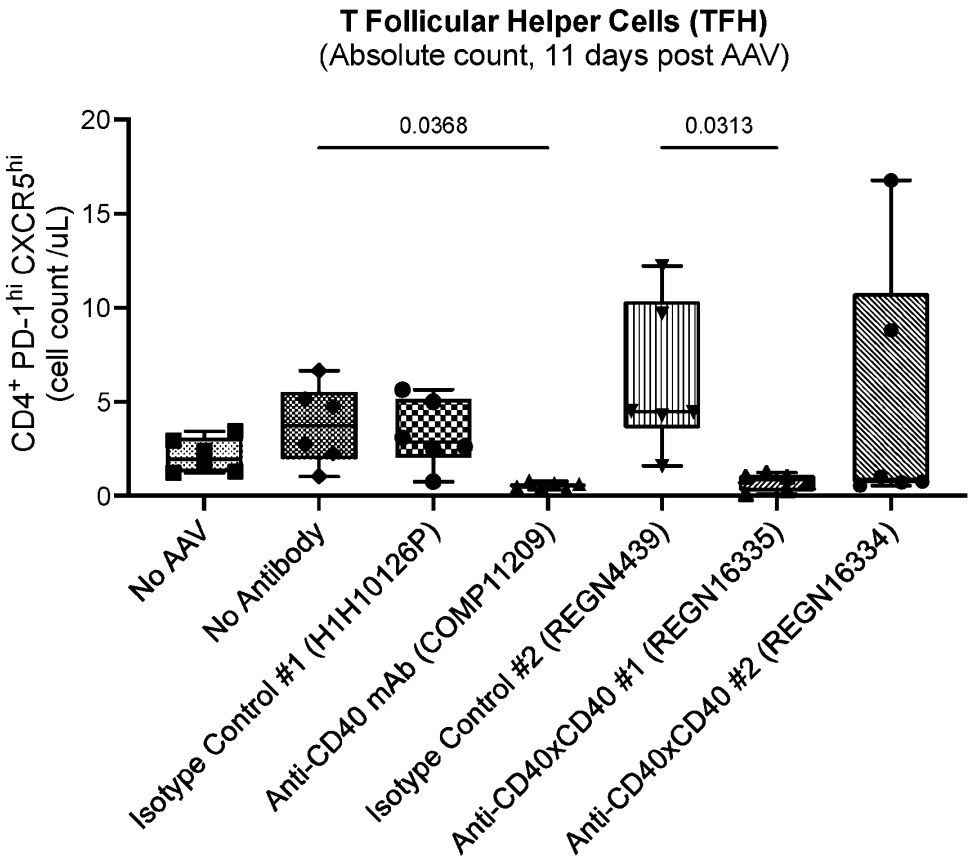

T Follicular Helper Cells (TFH)
(Absolute count, 11 days post AAV)
CD4+ PD-1hi CXCR5hi
(cell count /uL)
20
15
10
5
0
0.0368
0.0313
No AAV
No Antibody
Isotype Control #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Isotype Control #2 (REGN4439)
Anti-CD40xCD40 #1 (REGN16335)
Anti-CD40xCD40 #2 (REGN16334)

Figure 21C

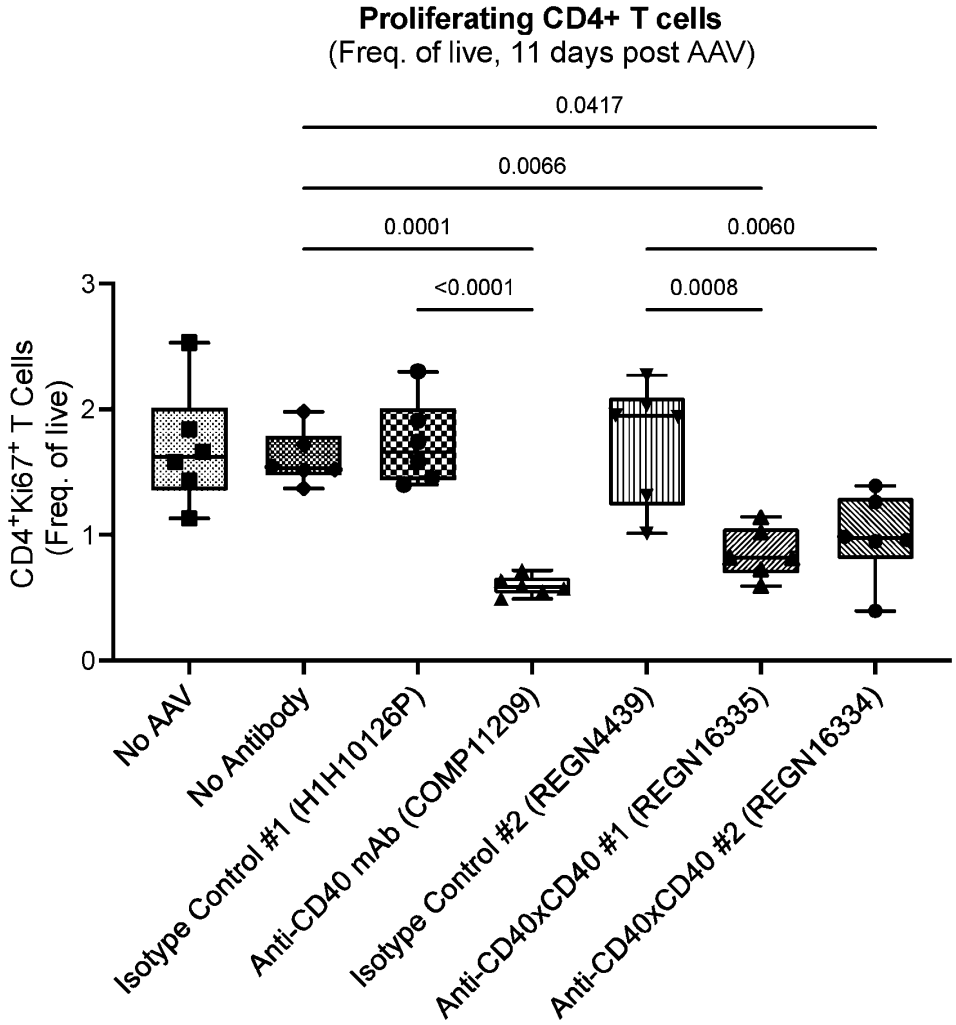

Proliferating CD4+ T cells
(Freq. of live, 11 days post AAV)
0.0417
0.0066
0.0001
0.0060
<0.0001
0.0008
CD4+Ki67+ T Cells
(Freq. of live)
3
2
1
0
No AAV
No Antibody
Isotype Control #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Isotype Control #2 (REGN4439)
Anti-CD40xCD40 #1 (REGN16335)
Anti-CD40xCD40 #2 (REGN16334)

Figure 21D

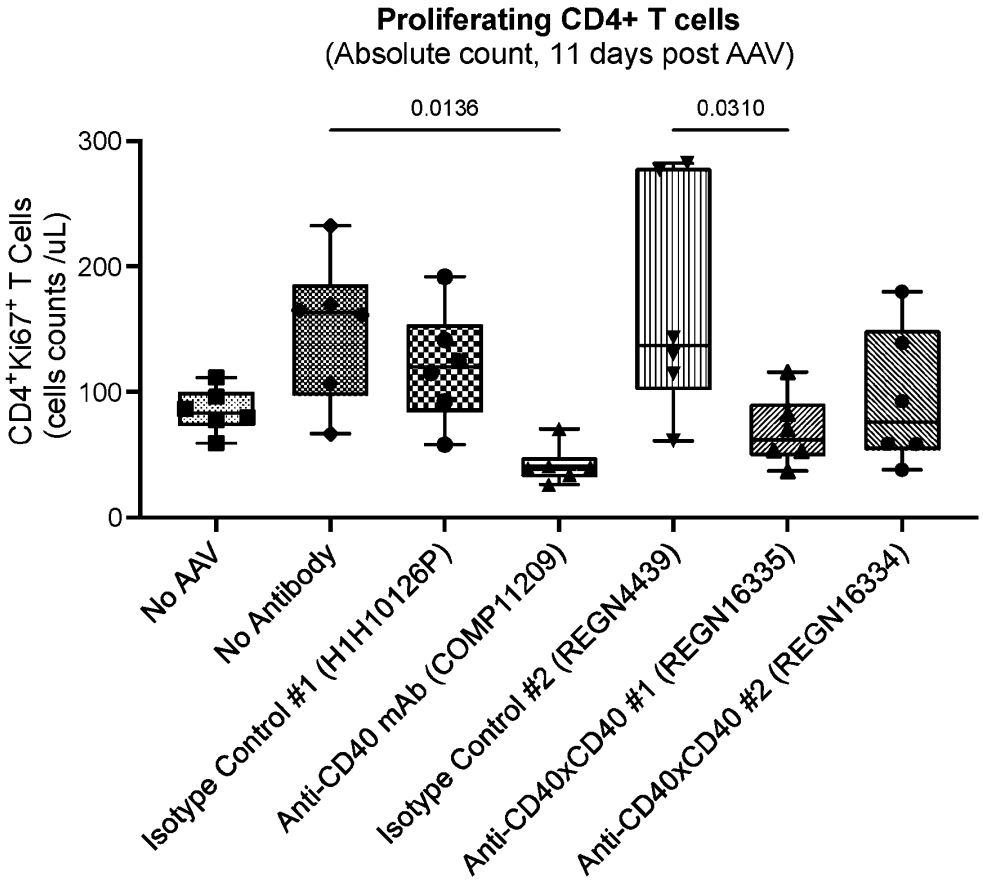

Proliferating CD4+ T cells
(Absolute count, 11 days post AAV)
0.0136
0.0310
CD4+Ki67+ T Cells
(cells counts /uL)
300
200
100
0
No AAV
No Antibody
Isotype Control #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Isotype Control #2 (REGN4439)
Anti-CD40xCD40 #1 (REGN16335)
Anti-CD40xCD40 #2 (REGN16334)

Figure 21E

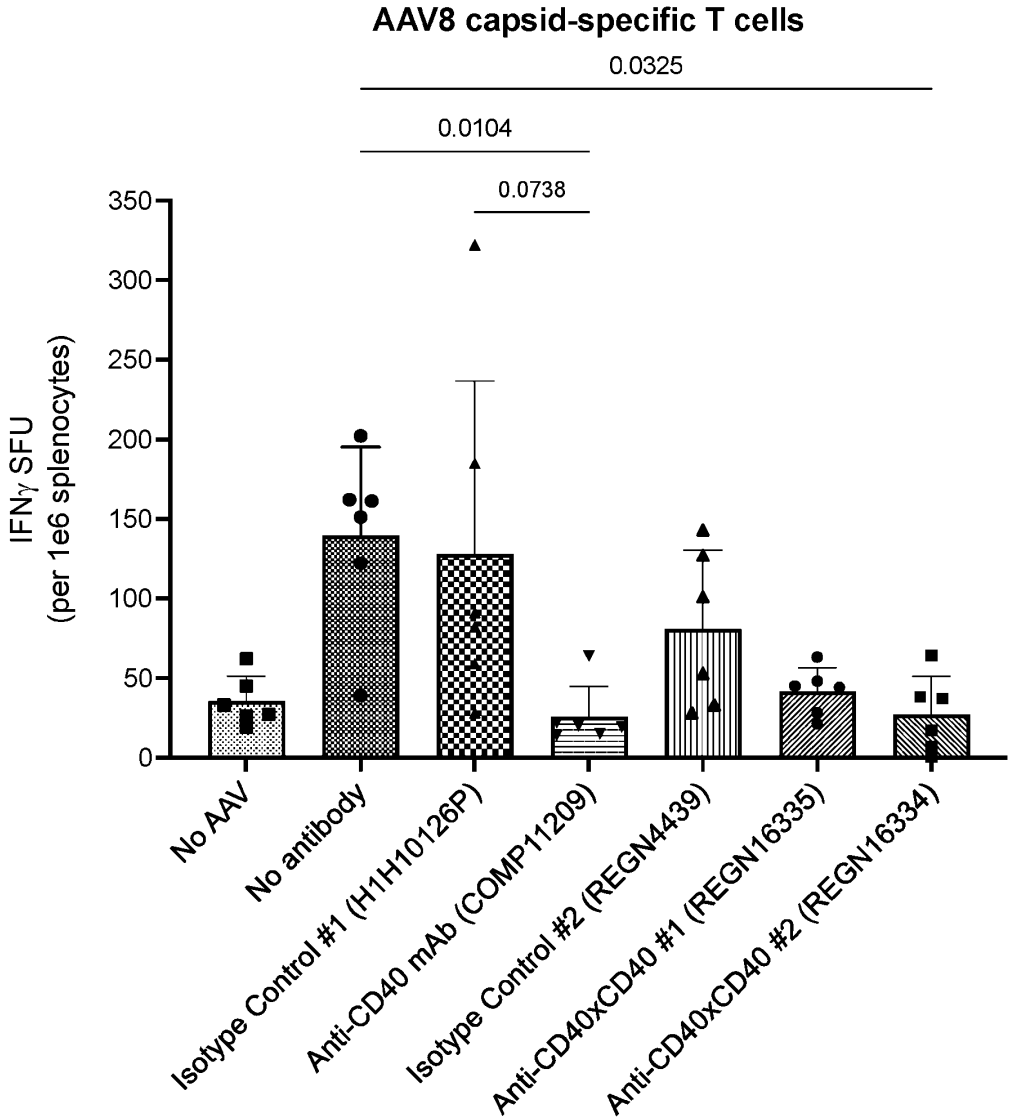

AAV8 capsid-specific T cells
0.0325
0.0104
0.0738
IFNγ SFU
(per 1e6 splenocytes)
350
300
250
200
150
100
50
0
No AAV
No antibody
Isotype Control #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Isotype Control #2 (REGN4439)
Anti-CD40xCD40 #1 (REGN16335)
Anti-CD40xCD40 #2 (REGN16334)

Figure 22

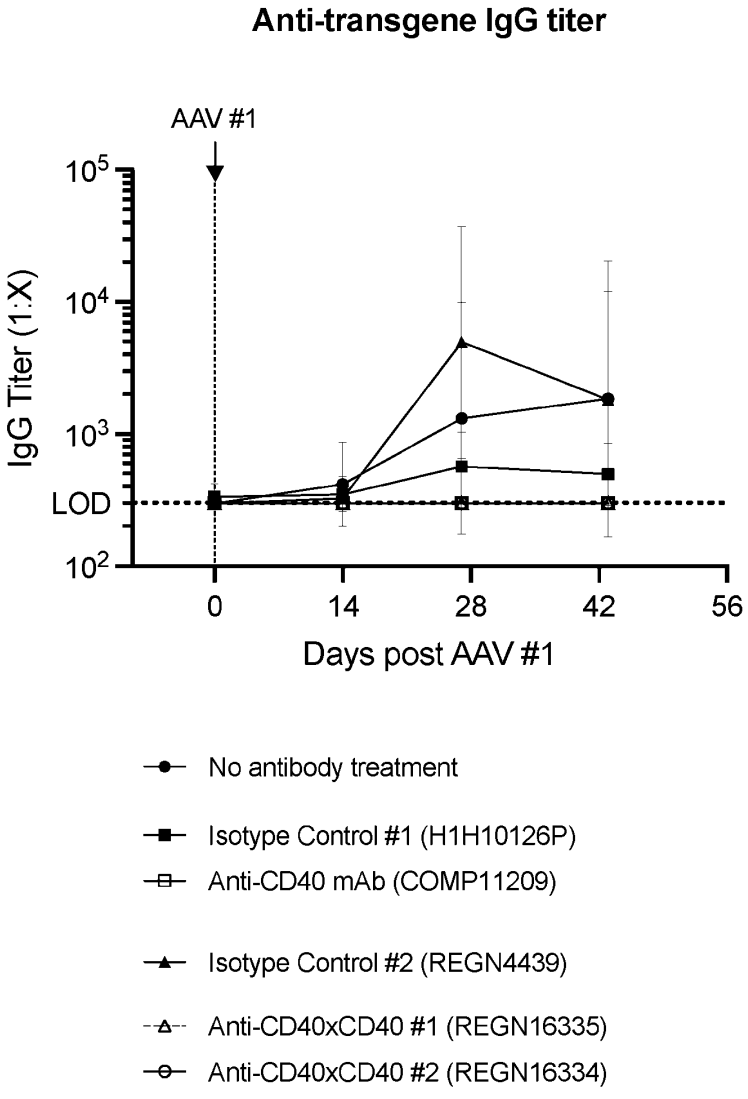

Anti-transgene IgG titer
AAV #1
IgG Titer (1:X)
10^5
10^4
10^3
LOD
10^2
0
14
28
42
56
Days post AAV #1
No antibody treatment
Isotype Control #1 (H1H10126P)
Anti-CD40 mAb (COMP11209)
Isotype Control #2 (REGN4439)
Anti-CD40xCD40 #1 (REGN16335)
Anti-CD40xCD40 #2 (REGN16334)

Figure 23

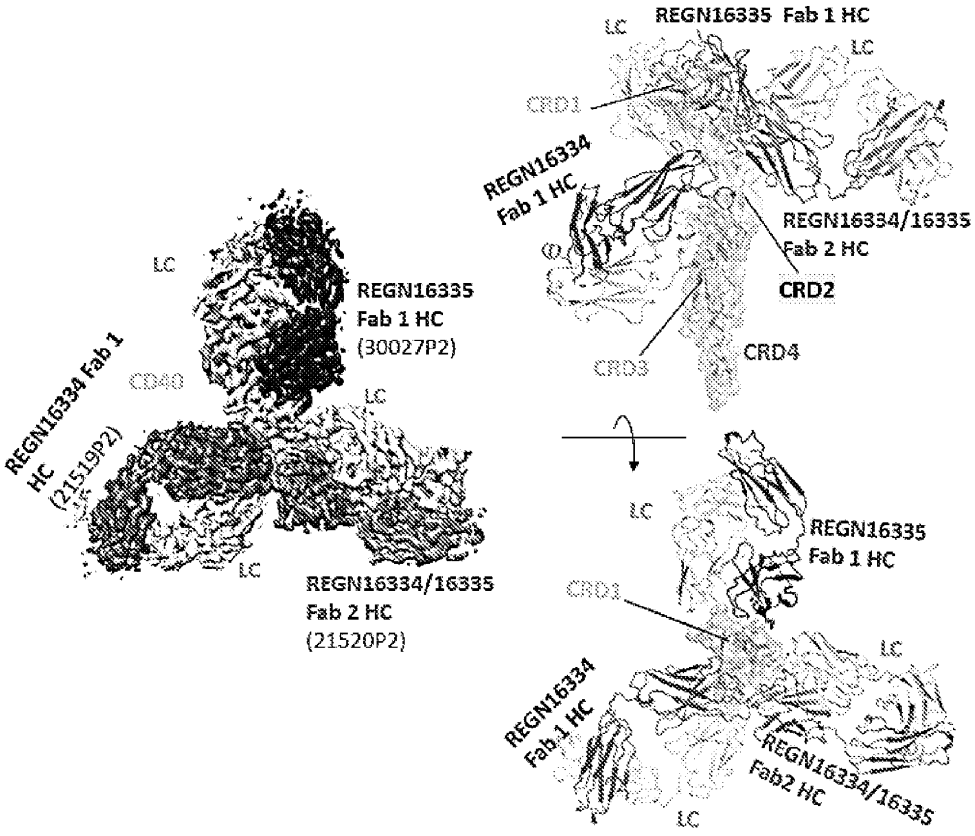

REGN16335 Fab 1 HC
LC
LC
CRD1
REGN16334
Fab 1 HC
REGN16334/16335
Fab 2 HC
CRD2
CRD3
CRD4
LC
REGN16335
Fab 1 HC
(30027P2)
REGN16334 Fab 1
HC
(21519P2)
CRD4
LC
LC
REGN16334/16335
Fab 2 HC
(21520P2)
LC
REGN16335
Fab 1 HC
CRD1
LC
REGN16334
Fab 1 HC
REGN16334/16335
Fab2 HC
LC

Figure 24

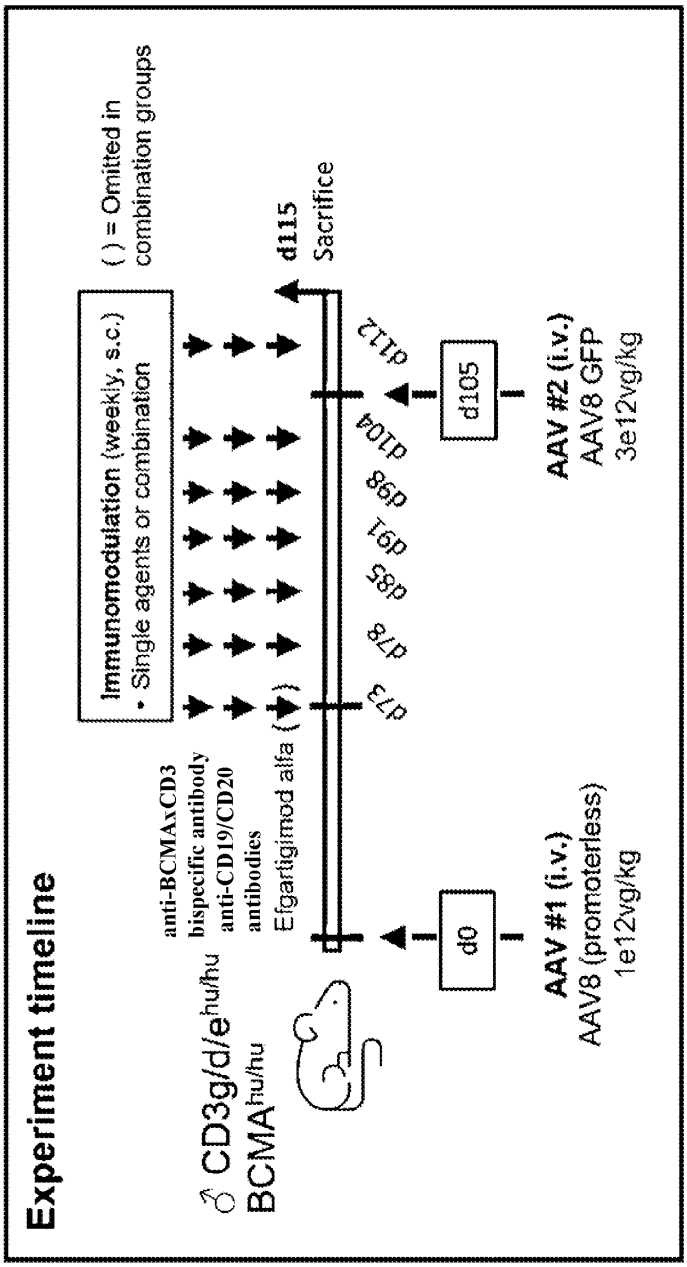

Experiment timeline
Immunomodulation (weekly, s.c.)
• Single agents or combination
( ) = Omitted in combination groups
anti-BCMAxCD3 bispecific antibody
anti-CD19/CD20 antibodies
Efgartigimod alfa (↓)
CD3g/d/e hu/hu
BCMA hu/hu
d0
d105
d115
Sacrifice
d73
d78
d85
d91
d98
d104
d112
AAV #1 (i.v.)
AAV8 (promoterless)
1e12vg/kg
AAV #2 (i.v.)
AAV8 GFP
3e12vg/kg

Figure 25

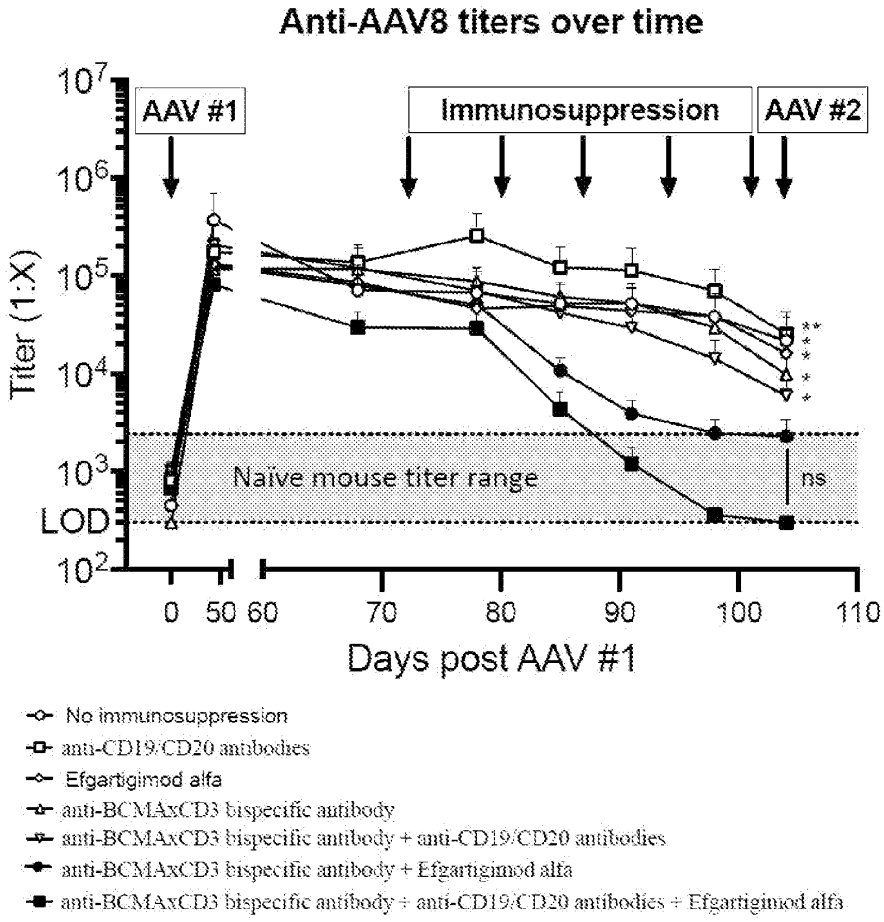

Anti-AAV8 titers over time
AAV #1
Immunosuppression
AAV #2
Titer (1:X)
10^7
10^6
10^5
10^4
10^3
LOD
10^2
Naive mouse titer range
ns
0 50 60 70 80 90 100 110
Days post AAV #1
No immunosuppression
anti-CD19/CD20 antibodies
Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies
anti-BCMAxCD3 bispecific antibody + Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies + Efgartigimod alfa

Figure 26

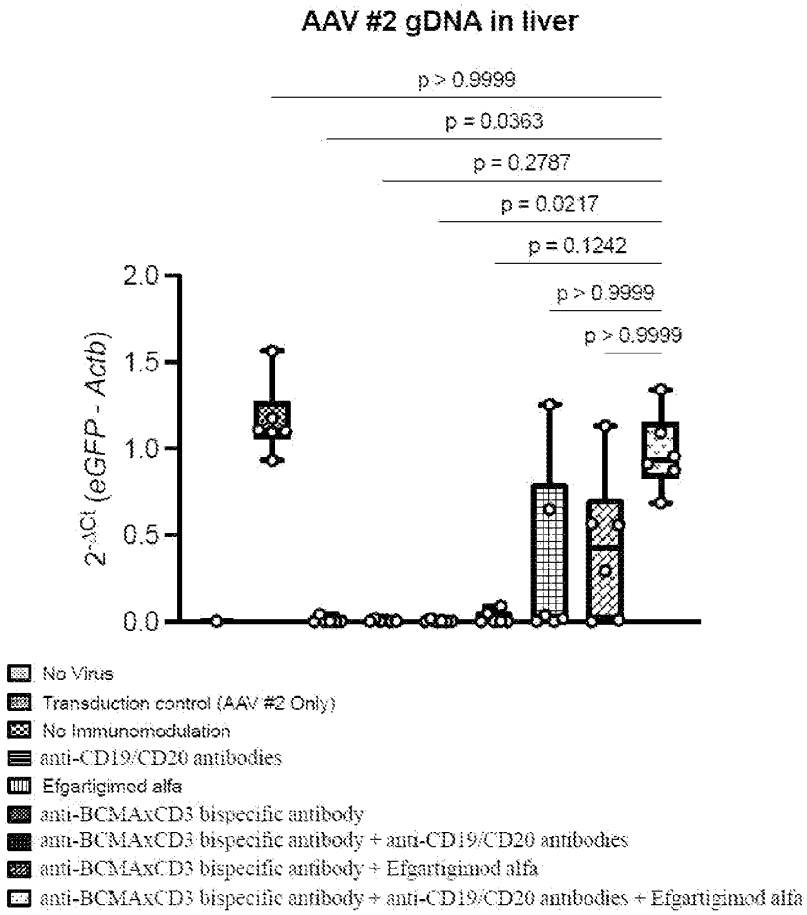

AAV #2 gDNA in liver
p > 0.9999
p = 0.0363
p = 0.2787
p = 0.0217
p = 0.1242
p > 0.9999
p > 0.9999
2^-ΔCt (eGFP - Actb)
2.0
1.5
1.0
0.5
0.0
No Virus
Transduction control (AAV #2 Only)
No Immunomodulation
anti-CD19/CD20 antibodies
Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies
anti-BCMAxCD3 bispecific antibody + Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies + Efgartigimod alfa

Figure 27

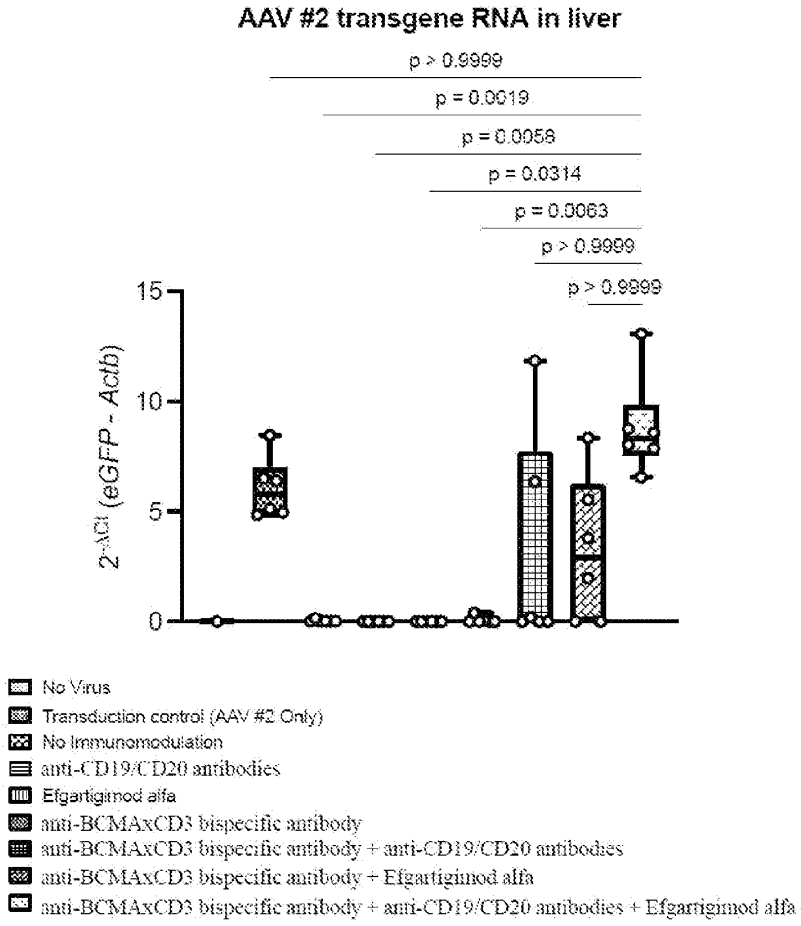

AAV #2 transgene RNA in liver
p > 0.9999
p = 0.0019
p = 0.0058
p = 0.0314
p = 0.0063
p > 0.9999
p > 0.9999
15
10
5
0
2^-ΔCt (eGFP - Actb)
No Virus
Transduction control (AAV #2 Only)
No Immunomodulation
anti-CD19/CD20 antibodies
Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies
anti-BCMAxCD3 bispecific antibody + Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies + Efgartigimod alfa

Figure 28A

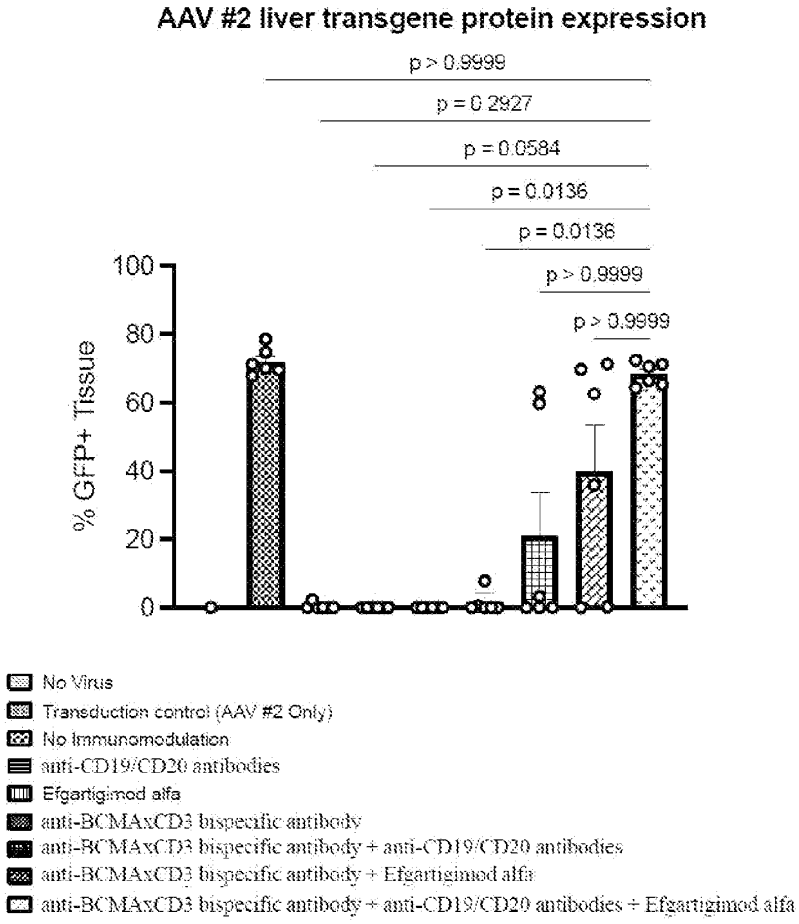

AAV #2 liver transgene protein expression
p > 0.9999
p = 0.2927
p = 0.0584
p = 0.0136
p = 0.0136
p > 0.9999
p > 0.9999
% GFP+ Tissue
100
80
60
40
20
0
No Virus
Transduction control (AAV #2 Only)
No Immunomodulation
anti-CD19/CD20 antibodies
Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies
anti-BCMAxCD3 bispecific antibody + Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies + Efgartigimod alfa

Figure 28B

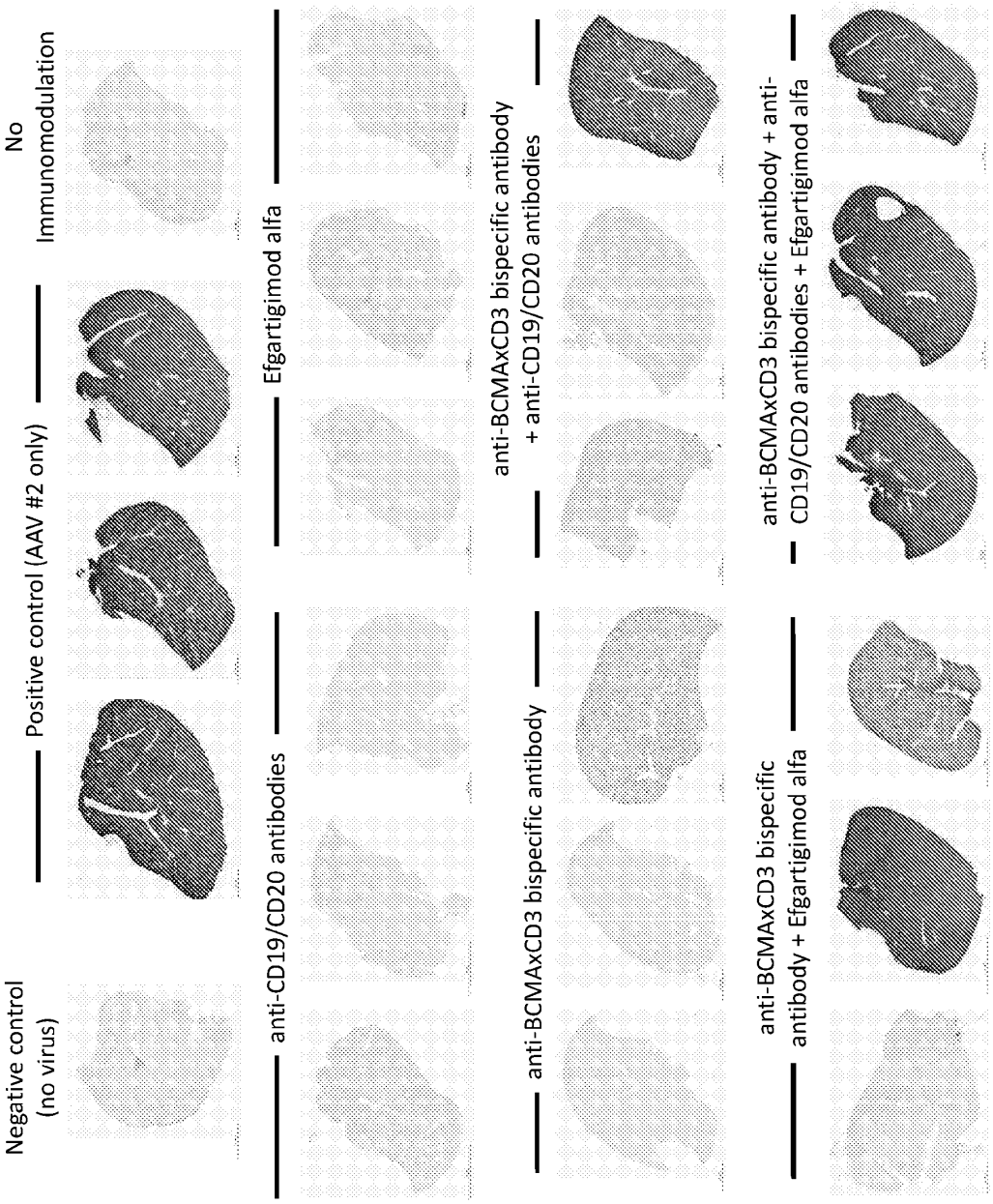

Negative control (no virus)
Positive control (AAV #2 only)
No Immunomodulation
anti-CD19/CD20 antibodies
Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies
anti-BCMAxCD3 bispecific antibody + Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies + Efgartigimod alfa

Figure 29A

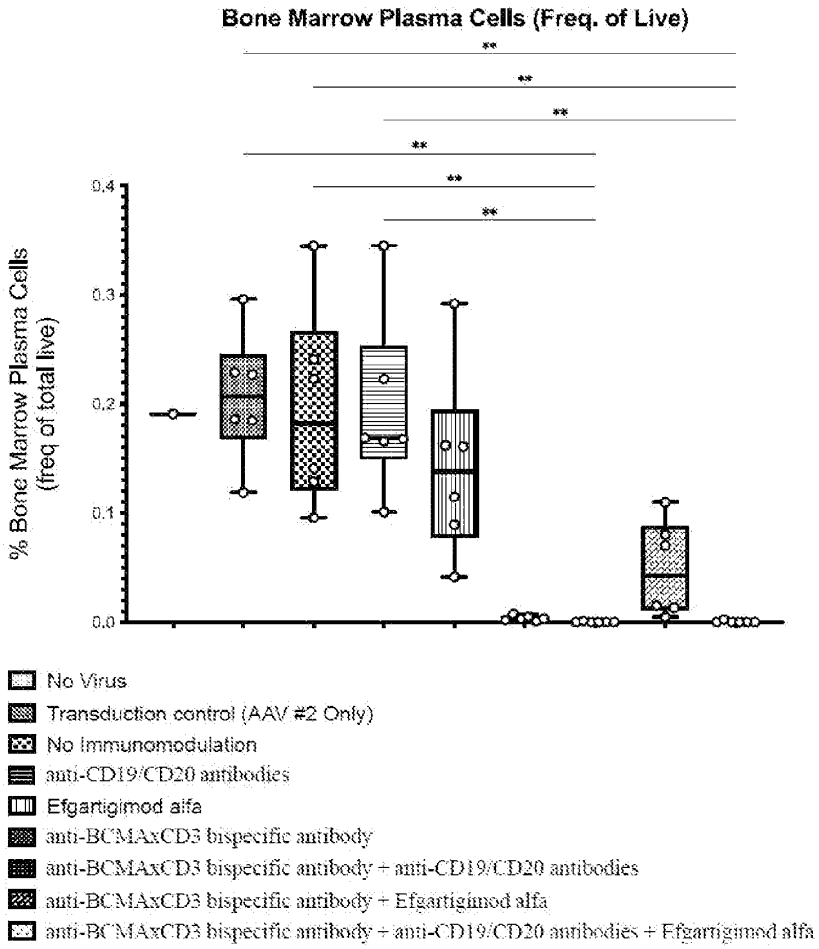

Bone Marrow Plasma Cells (Freq. of Live)
**
**
**
**
**
**
% Bone Marrow Plasma Cells (freq of total live)
0.4
0.3
0.2
0.1
0.0
No Virus
Transduction control (AAV #2 Only)
No Immunomodulation
anti-CD19/CD20 antibodies
Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies
anti-BCMAxCD3 bispecific antibody + Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies + Efgartigimod alfa

Figure 29B

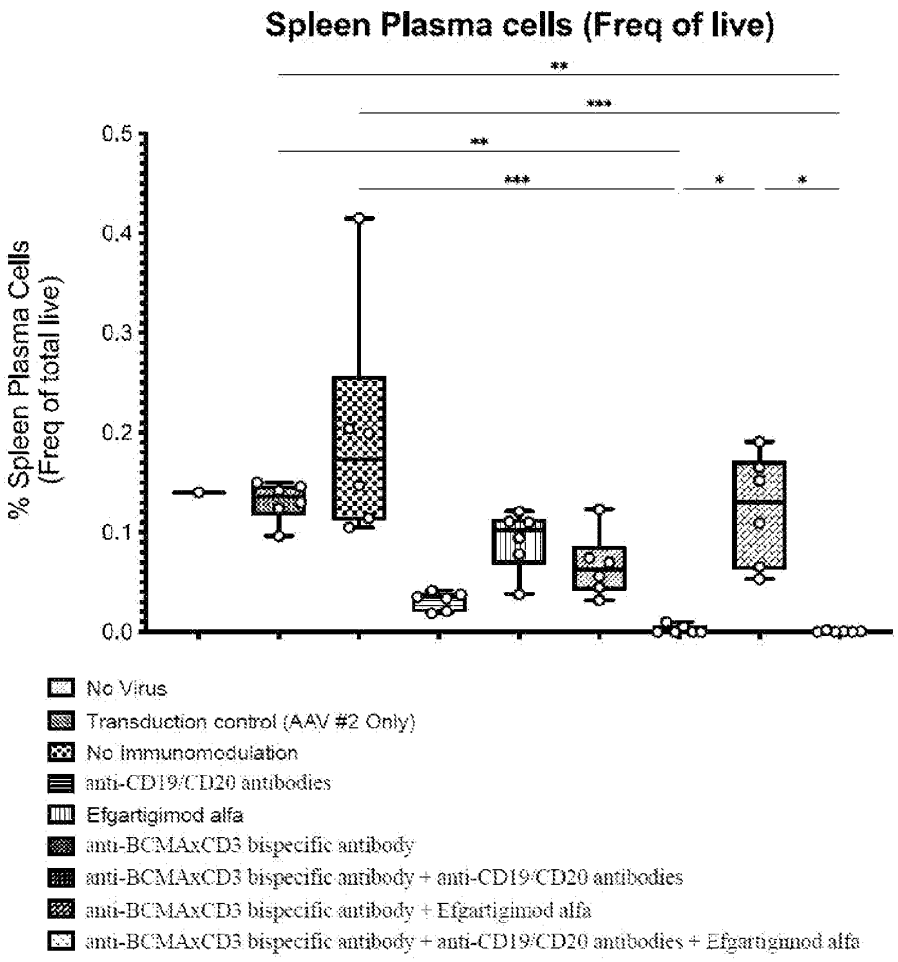

Spleen Plasma cells (Freq of live)
**
***
**
***
*
*
% Spleen Plasma Cells
(Freq of total live)
0.5
0.4
0.3
0.2
0.1
0.0
No Virus
Transduction control (AAV #2 Only)
No immunomodulation
anti-CD19/CD20 antibodies
Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies
anti-BCMAxCD3 bispecific antibody + Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies + Efgartigimod alfa

Figure 29C

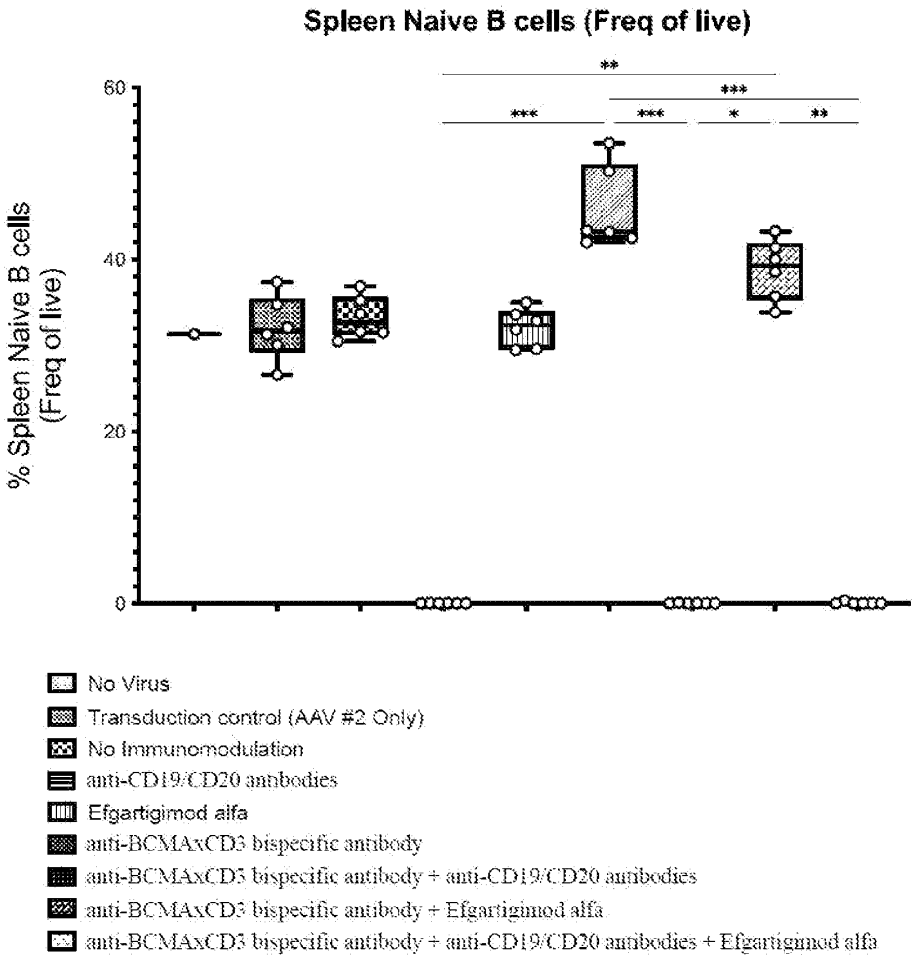

Spleen Naive B cells (Freq of live)
% Spleen Naive B cells (Freq of live)
60
40
20
0
**
***
***
***
*
**
No Virus
Transduction control (AAV #2 Only)
No Immunomodulation
anti-CD19/CD20 antibodies
Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies
anti-BCMAxCD3 bispecific antibody + Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies + Efgartigimod alfa

Figure 29D

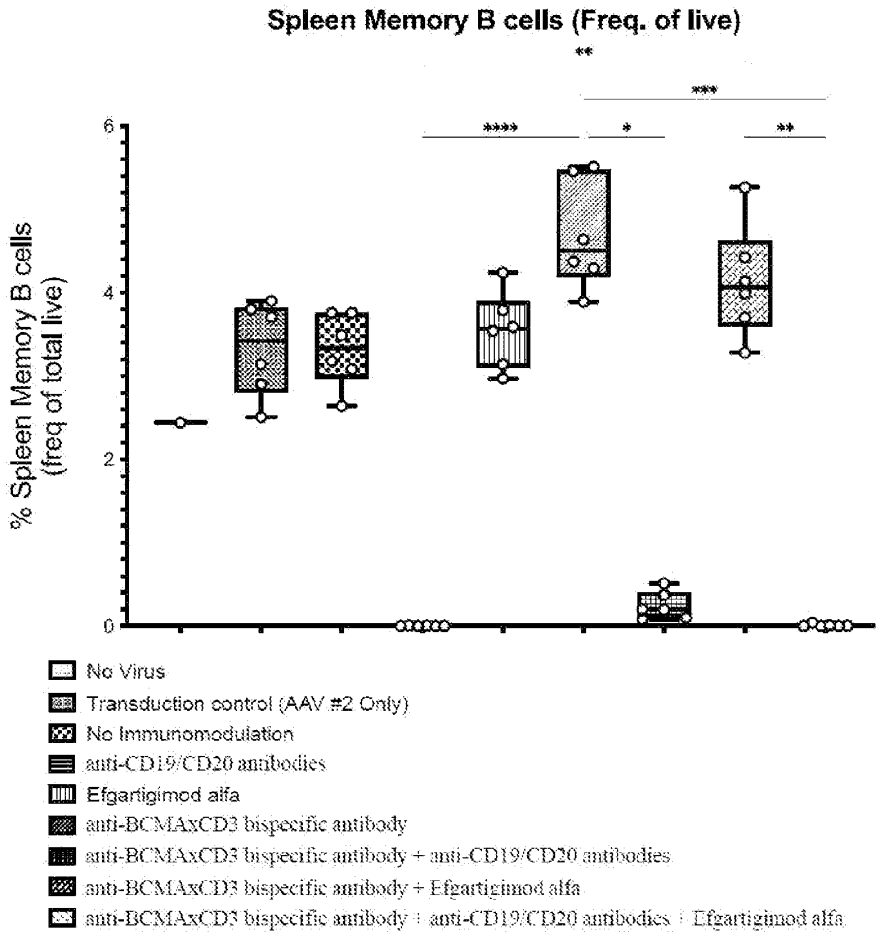

Spleen Memory B cells (Freq. of live)
**
***
****
*
**
% Spleen Memory B cells
(freq of total live)
6
4
2
0
No Virus
Transduction control (AAV #2 Only)
No Immunomodulation
anti-CD19/CD20 antibodies
Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies
anti-BCMAxCD3 bispecific antibody + Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies + Efgartigimod alfa

Figure 29E

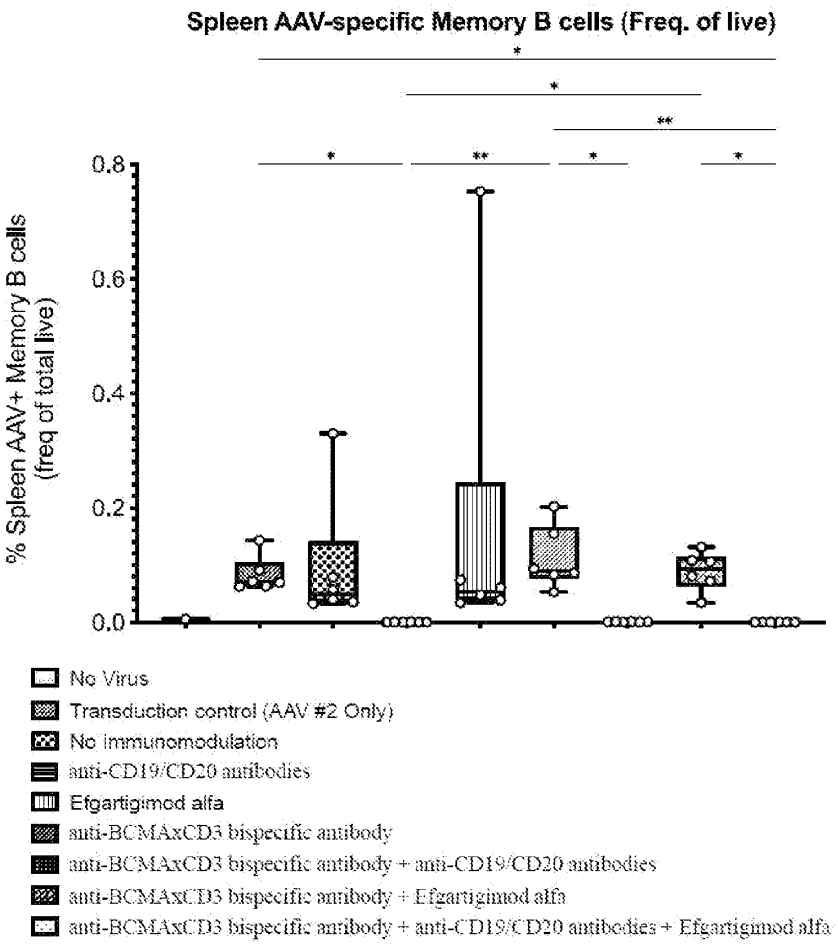

Spleen AAV-specific Memory B cells (Freq. of live)
% Spleen AAV+ Memory B cells (freq of total live)
0.0
0.2
0.4
0.6
0.8
*
*
**
*
**
*
*
No Virus
Transduction control (AAV #2 Only)
No immunomodulation
anti-CD19/CD20 antibodies
Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies
anti-BCMAxCD3 bispecific antibody + Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies + Efgartigimod alfa

Figure 29F

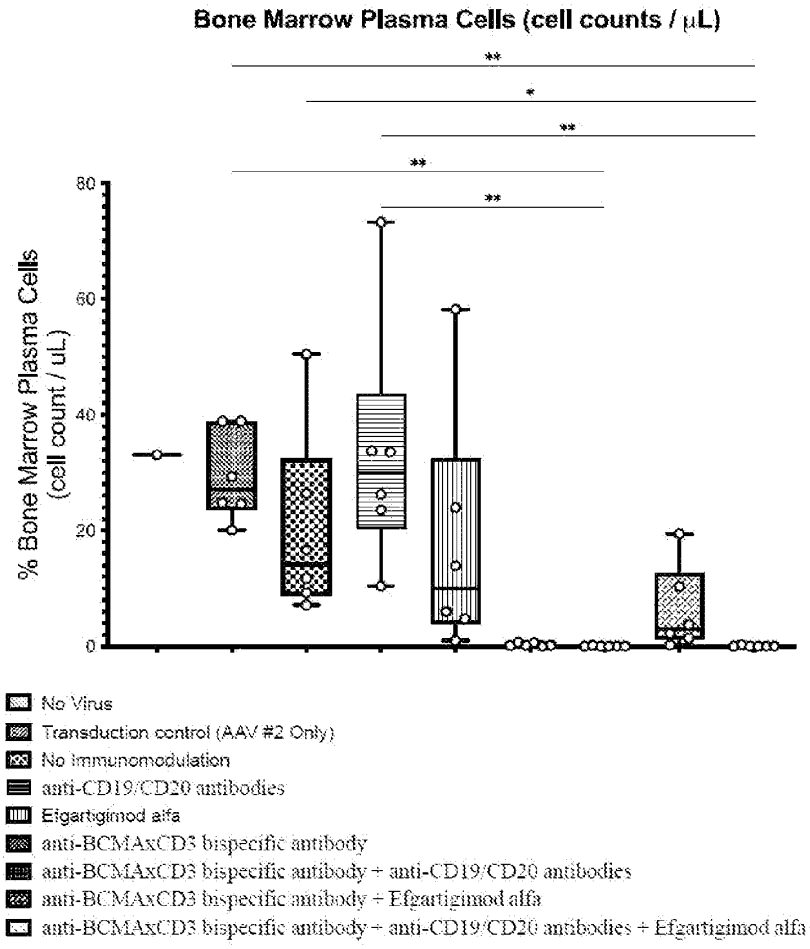

Bone Marrow Plasma Cells (cell counts / µL)
**
*
**
**
**
% Bone Marrow Plasma Cells (cell count / uL)
80
60
40
20
0
No Virus
Transduction control (AAV #2 Only)
No Immunomodulation
anti-CD19/CD20 antibodies
Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies
anti-BCMAxCD3 bispecific antibody + Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies + Efgartigimod alfa

Figure 29G

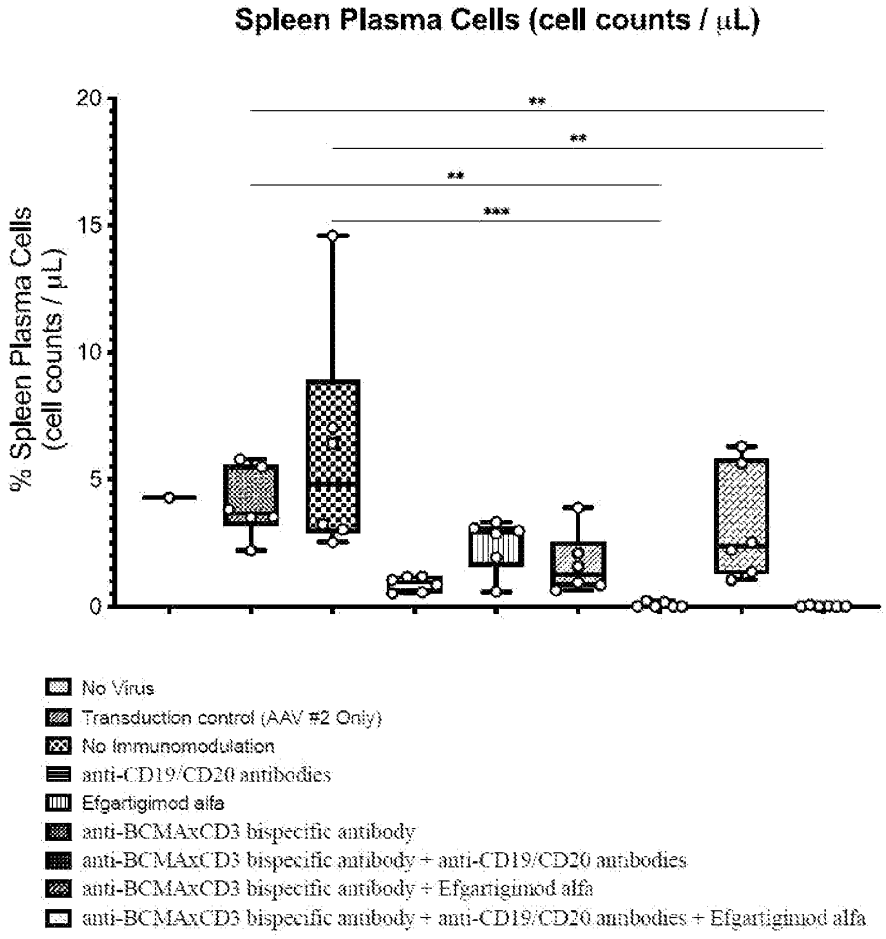

Spleen Plasma Cells (cell counts / μL)
% Spleen Plasma Cells (cell counts / μL)
20
15
10
5
0
**
**
**
***
No Virus
Transduction control (AAV #2 Only)
No Immunomodulation
anti-CD19/CD20 antibodies
Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies
anti-BCMAxCD3 bispecific antibody + Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies + Efgartigimod alfa

Figure 29H

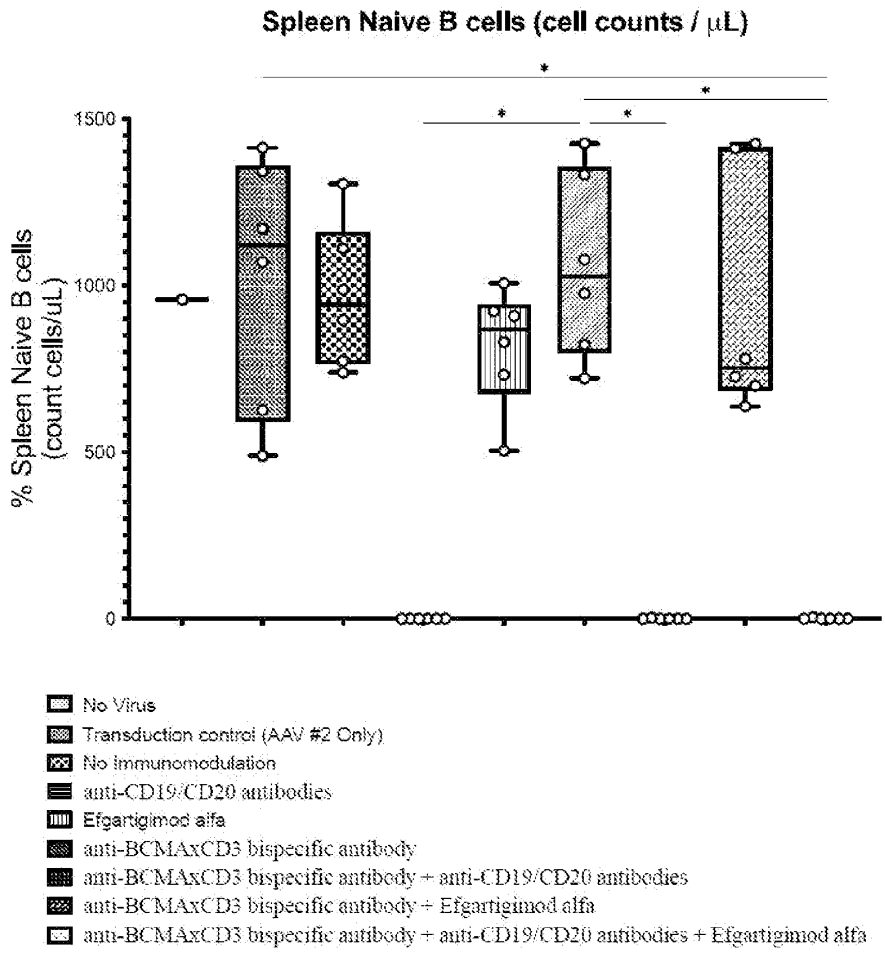

Spleen Naive B cells (cell counts / μL)
% Spleen Naive B cells (count cells/uL)
1500
1000
500
0
*
*
*
*
No Virus
Transduction control (AAV #2 Only)
No immunomodulation
anti-CD19/CD20 antibodies
Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies
anti-BCMAxCD3 bispecific antibody + Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies + Efgartigimod alfa

Figure 29I

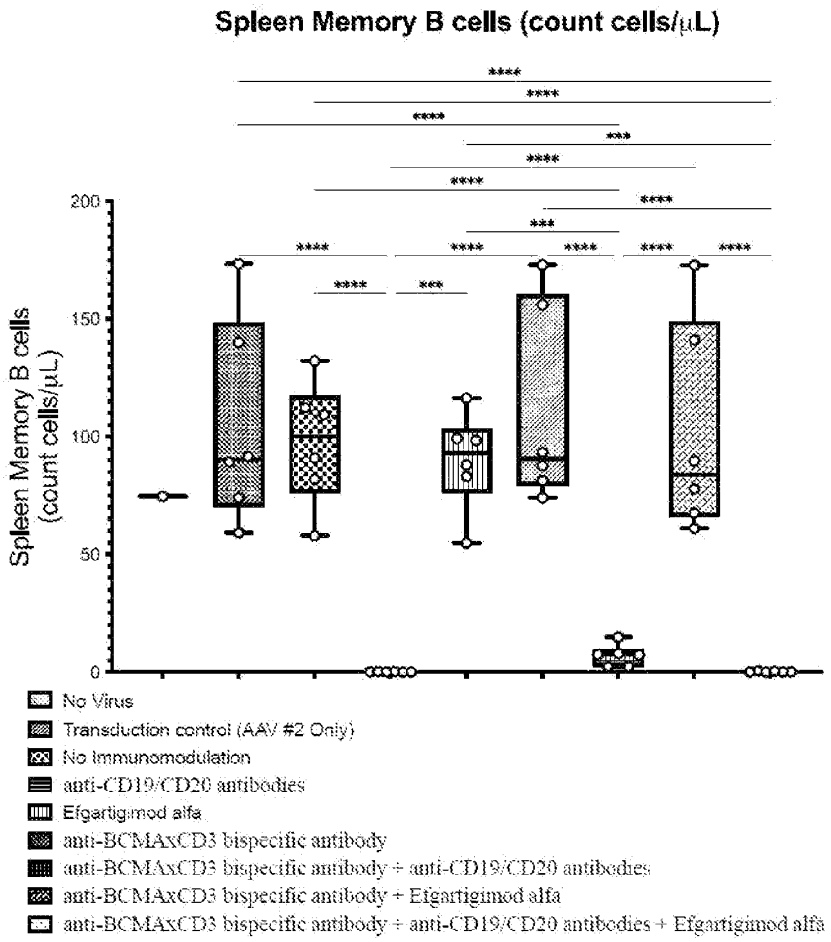

Spleen Memory B cells (count cells/μL)
Spleen Memory B cells (count cells/μL)
200
150
100
50
0
****
***
No Virus
Transduction control (AAV #2 Only)
No Immunomodulation
anti-CD19/CD20 antibodies
Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies
anti-BCMAxCD3 bispecific antibody + Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies + Efgartigimod alfa

Figure 29J

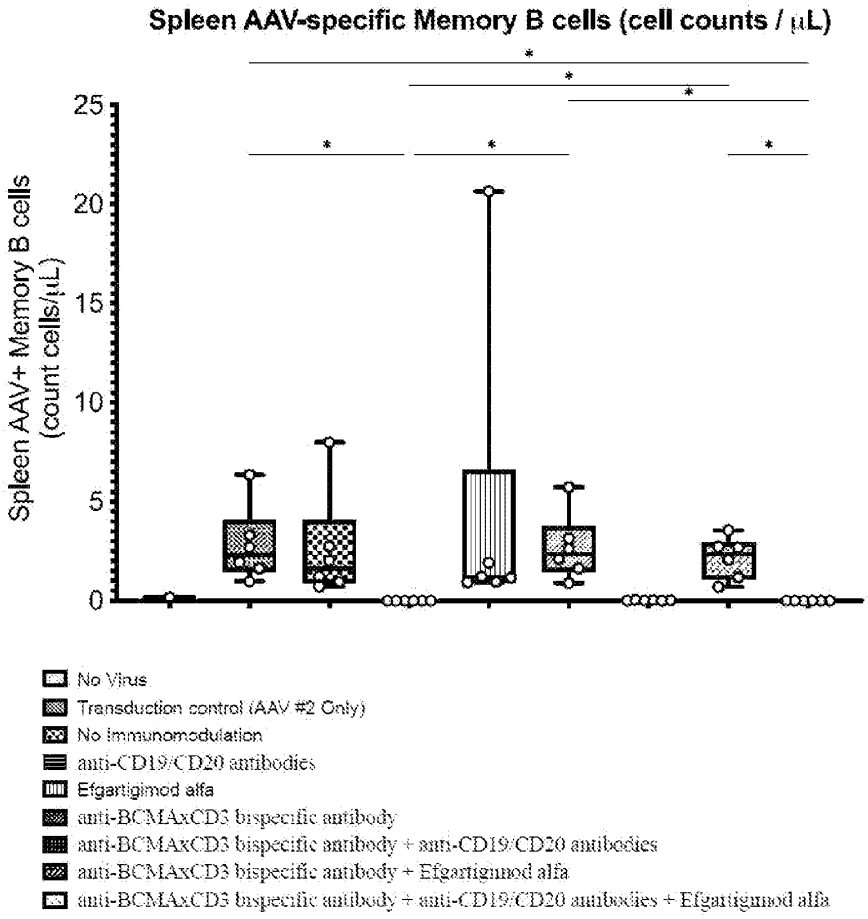

Spleen AAV-specific Memory B cells (cell counts / μL)
Spleen AAV+ Memory B cells (count cells/μL)
25
20
15
10
5
0
*
*
*
*
*
*
No Virus
Transduction control (AAV #2 Only)
No immunomodulation
anti-CD19/CD20 antibodies
Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies
anti-BCMAxCD3 bispecific antibody + Efgartigimod alfa
anti-BCMAxCD3 bispecific antibody + anti-CD19/CD20 antibodies + Efgartigimod alfa

Figure 30

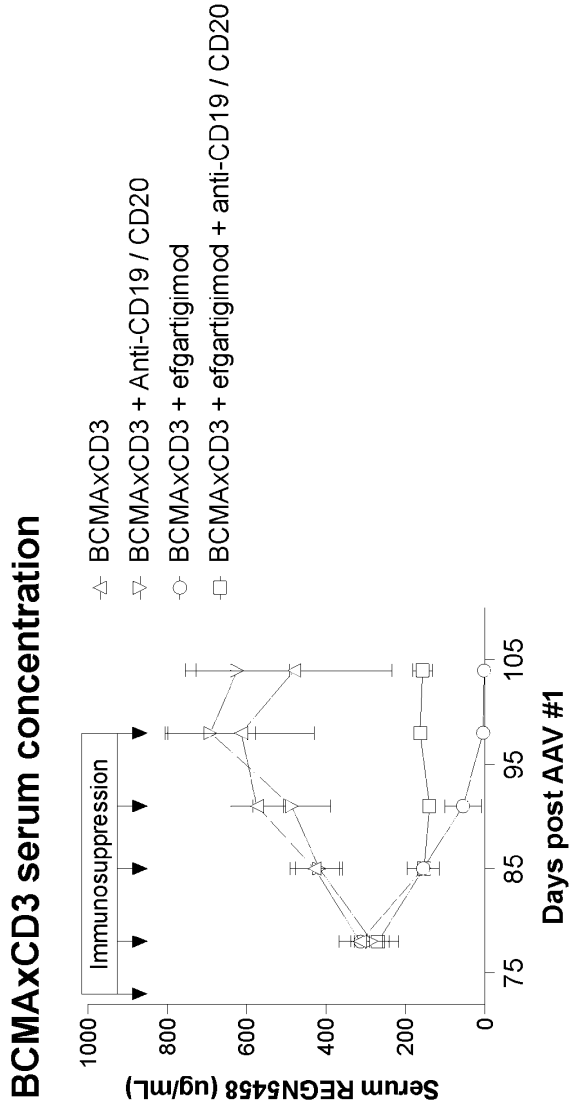

BCMAxCD3 serum concentration
BCMAxCD3
BCMAxCD3 + Anti-CD19 / CD20
BCMAxCD3 + efgartigimod
BCMAxCD3 + efgartigimod + anti-CD19 / CD20
Immunosuppression
Serum REGN5458 (ug/mL)
1000
800
600
400
200
0
75
85
95
105
Days post AAV #1

Figure 31

Study timeline
Analyze AAV Neutralizing antibodies (NAb)
Day: 1 4 8 15 22 29
Group #
G3,4,6 REGN5458 (20 mg/kg)
G3,5,6 REGN1979 (0.1 -> 1 mg/kg)
G4-6 Efgartigimod (20 mg/kg)
D11,12,13 D20 D27

Figure 32A

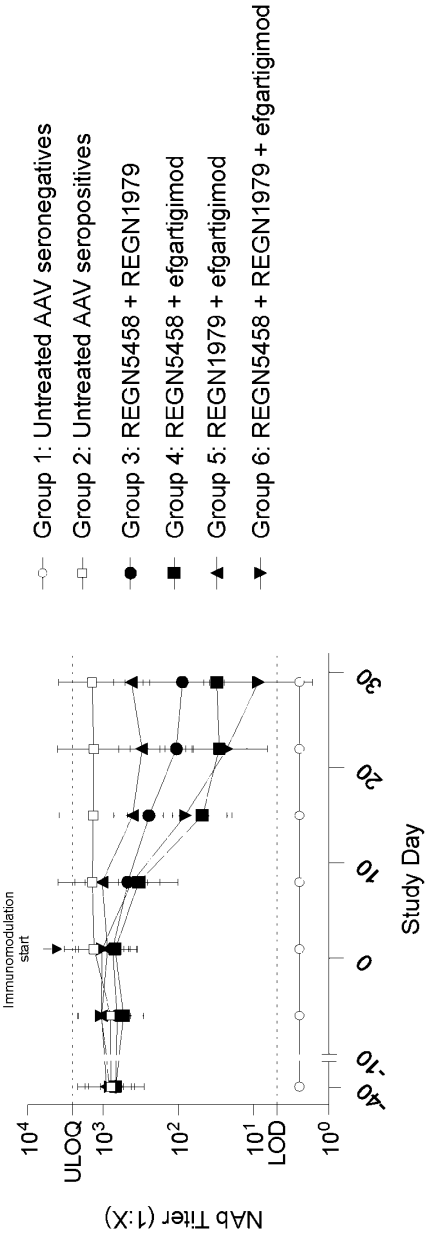


Figure 32B

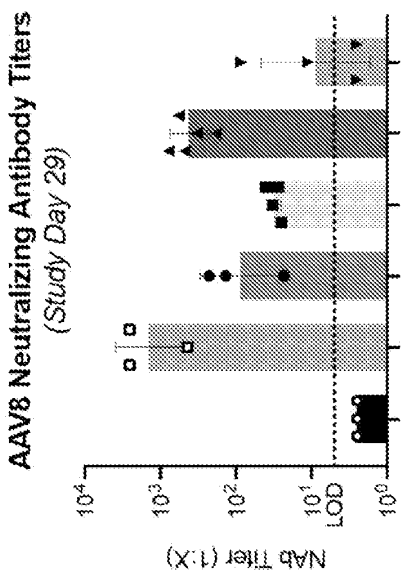


Figure 33

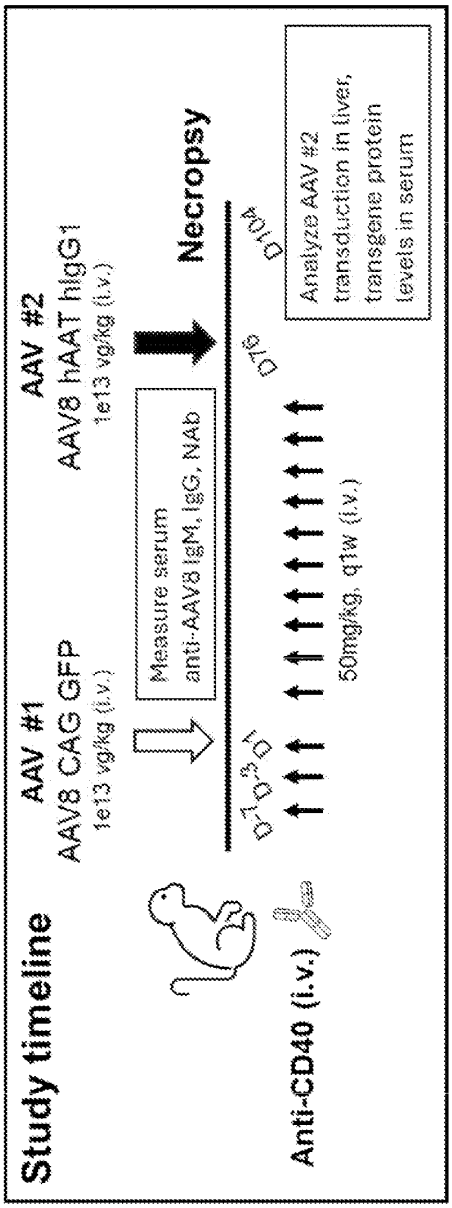

Study timeline
AAV #1
AAV8 CAG GFP
1e13 vg/kg (i.v.)
AAV #2
AAV8 hAAT hIgG1
1e13 vg/kg (i.v.)
Measure serum
anti-AAV8 IgM, IgG, NAb
Necropsy
Anti-CD40 (i.v.)
50mg/kg, q1w (i.v.)
Analyze AAV #2
transduction in liver,
transgene protein
levels in serum

Figure 34A

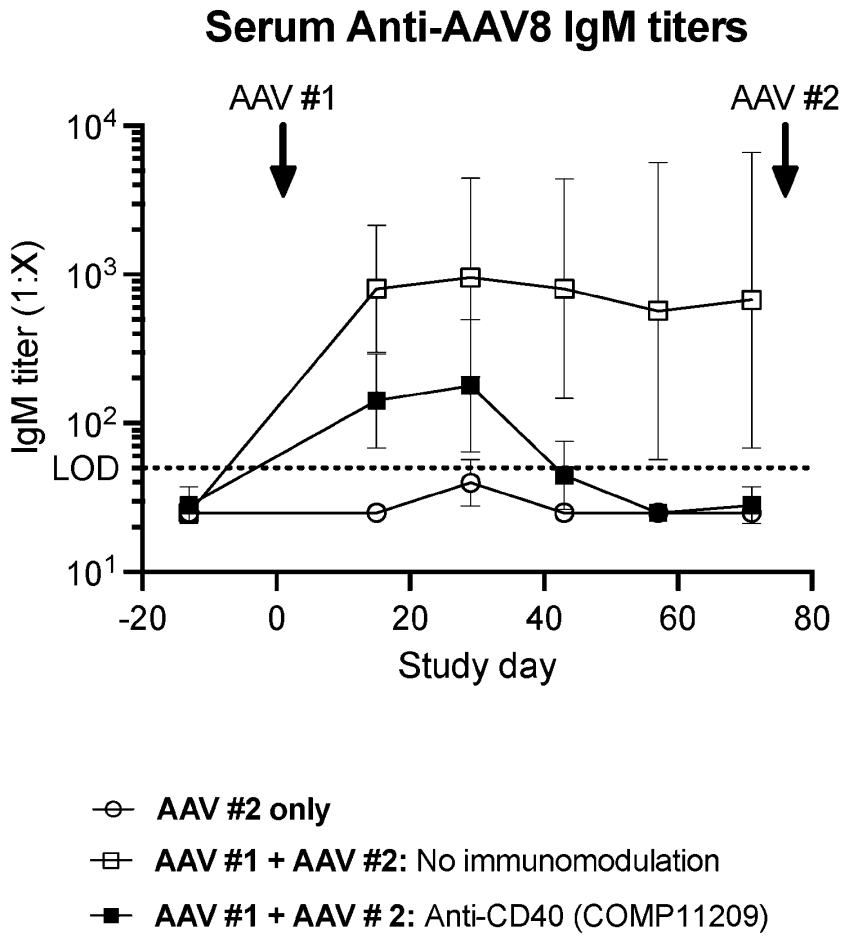

Serum Anti-AAV8 IgM titers
AAV #1
AAV #2
IgM titer (1:X)
10^4
10^3
10^2
LOD
10^1
-20
0
20
40
60
80
Study day
AAV #2 only
AAV #1 + AAV #2: No immunomodulation
AAV #1 + AAV # 2: Anti-CD40 (COMP11209)

Figure 34B

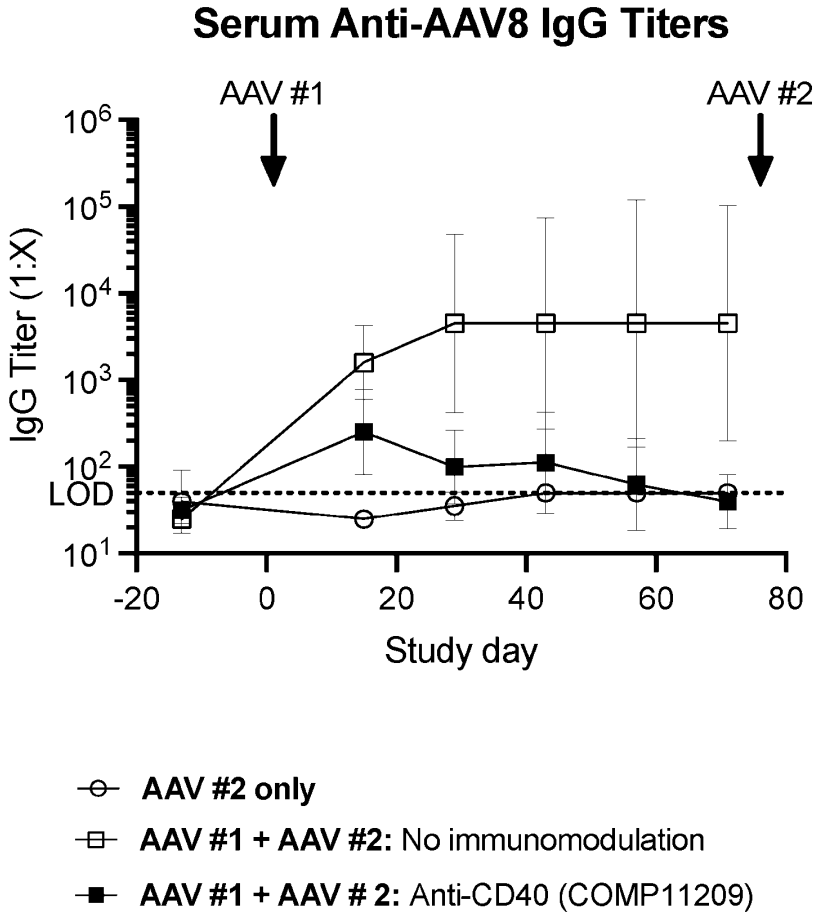

Serum Anti-AAV8 IgG Titers
AAV #1
AAV #2
IgG Titer (1:X)
10^6
10^5
10^4
10^3
10^2
LOD
10^1
-20
0
20
40
60
80
Study day
AAV #2 only
AAV #1 + AAV #2: No immunomodulation
AAV #1 + AAV # 2: Anti-CD40 (COMP11209)

Figure 34C

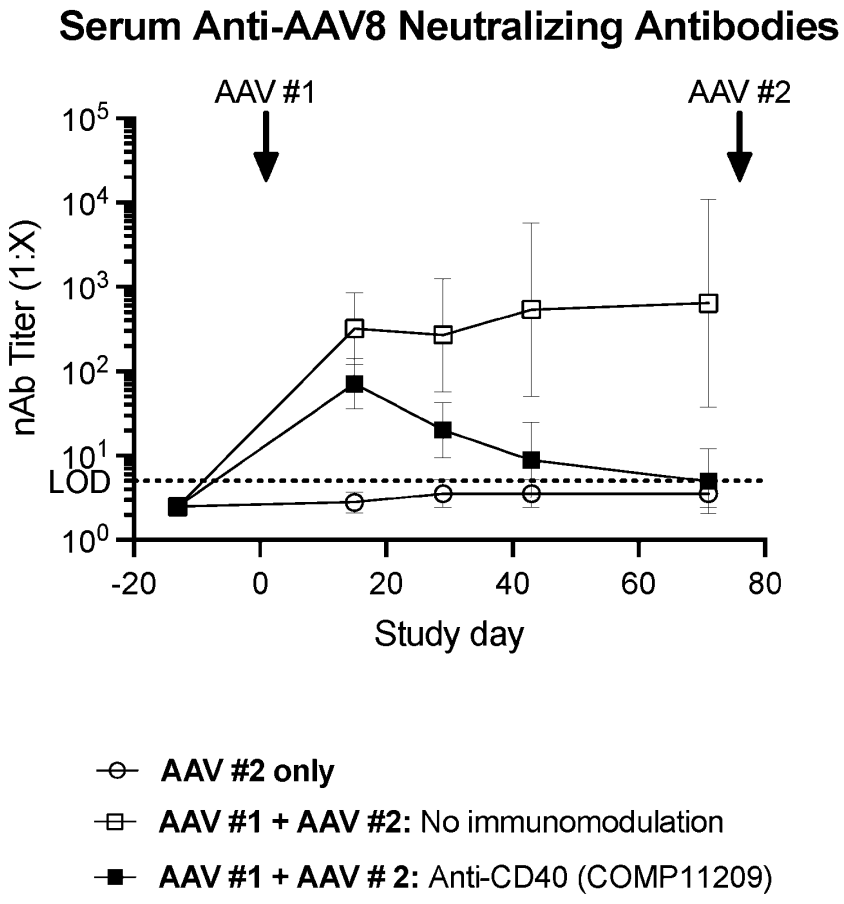

Serum Anti-AAV8 Neutralizing Antibodies
AAV #1
AAV #2
nAb Titer (1:X)
10^5
10^4
10^3
10^2
10^1
LOD
10^0
-20
0
20
40
60
80
Study day
AAV #2 only
AAV #1 + AAV #2: No immunomodulation
AAV #1 + AAV # 2: Anti-CD40 (COMP11209)

Figure 34D

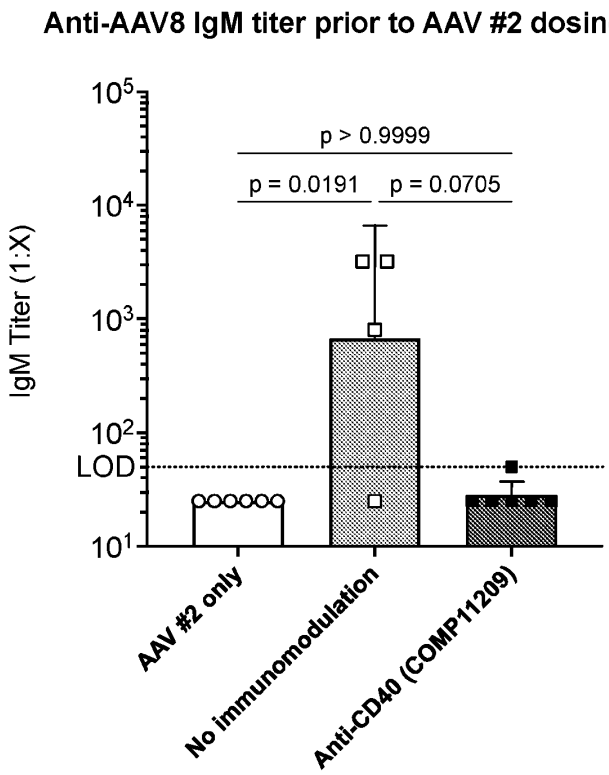

Anti-AAV8 IgM titer prior to AAV #2 dosing (D71)
IgM Titer (1:X)
10^5
10^4
10^3
10^2
LOD
10^1
p > 0.9999
p = 0.0191
p = 0.0705
AAV #2 only
No immunomodulation
Anti-CD40 (COMP11209)

Figure 34E

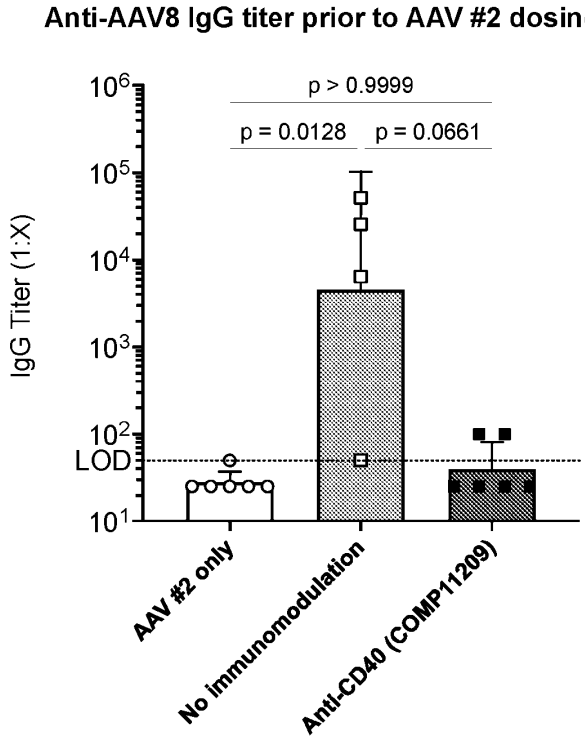

Anti-AAV8 IgG titer prior to AAV #2 dosing (D71)
p > 0.9999
p = 0.0128
p = 0.0661
IgG Titer (1:X)
10^6
10^5
10^4
10^3
10^2
LOD
10^1
AAV #2 only
No immunomodulation
Anti-CD40 (COMP11209)

Figure 34F

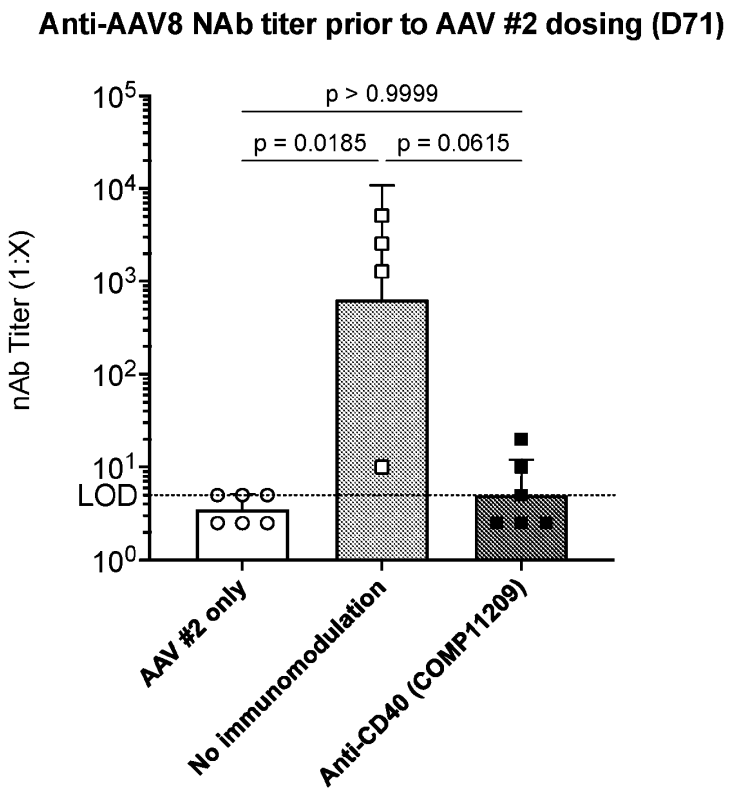

Anti-AAV8 NAb titer prior to AAV #2 dosing (D71)
p > 0.9999
p = 0.0185
p = 0.0615
nAb Titer (1:X)
10^5
10^4
10^3
10^2
10^1
LOD
10^0
AAV #2 only
No immunomodulation
Anti-CD40 (COMP11209)

Figure 35A

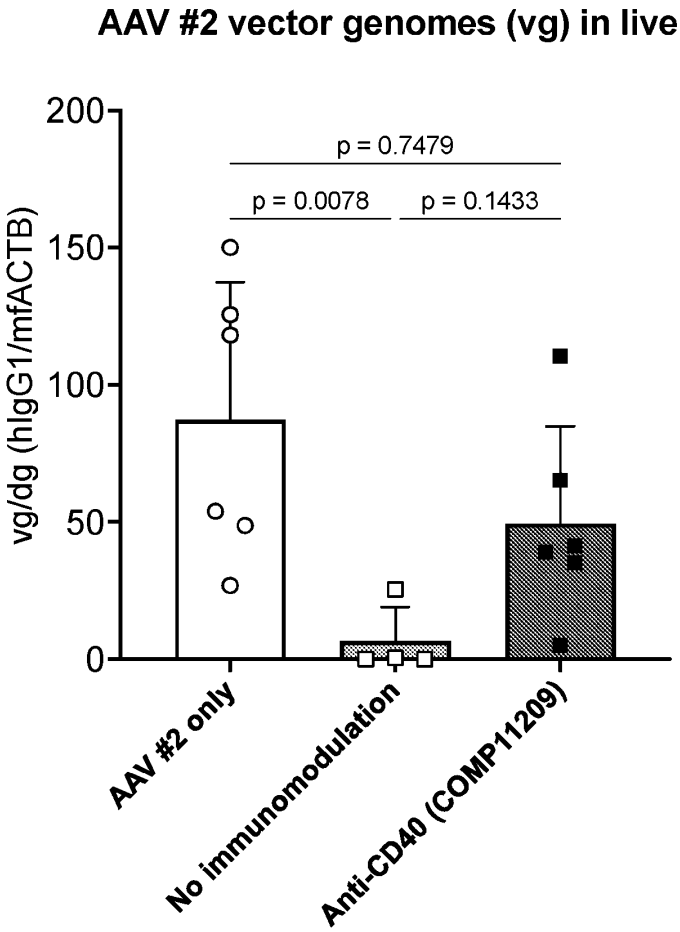

AAV #2 vector genomes (vg) in liver
vg/dg (hIgG1/mfACTB)
200
150
100
50
0
p = 0.7479
p = 0.0078
p = 0.1433
AAV #2 only
No immunomodulation
Anti-CD40 (COMP11209)

Figure 35B

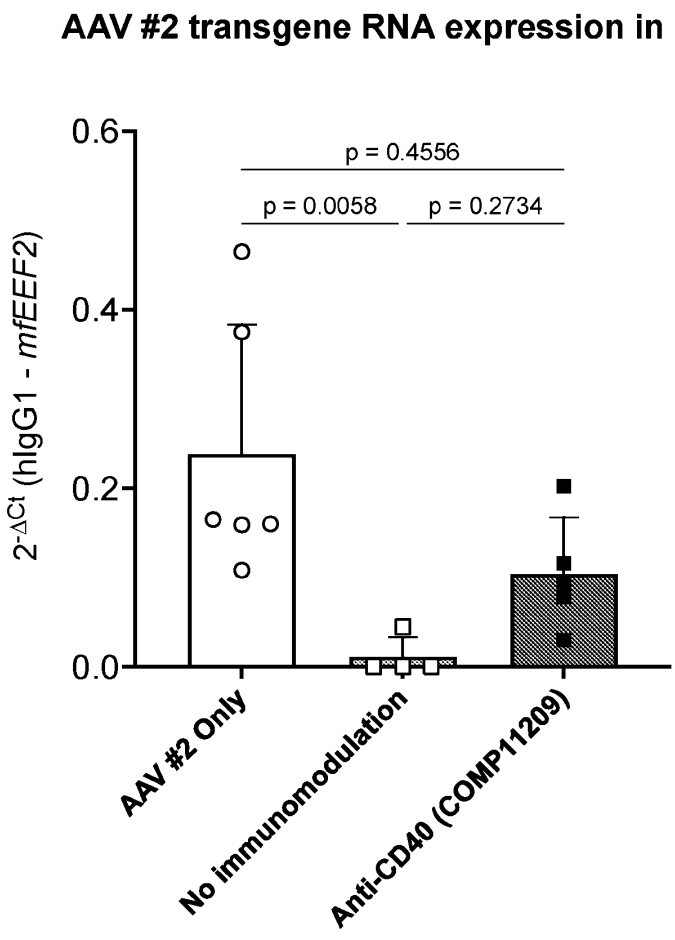

AAV #2 transgene RNA expression in liver
p = 0.4556
p = 0.0058
p = 0.2734
0.6
0.4
0.2
0.0
2-ΔCt (hIgG1 - mfEEF2)
AAV #2 Only
No immunomodulation
Anti-CD40 (COMP11209)

Figure 35C

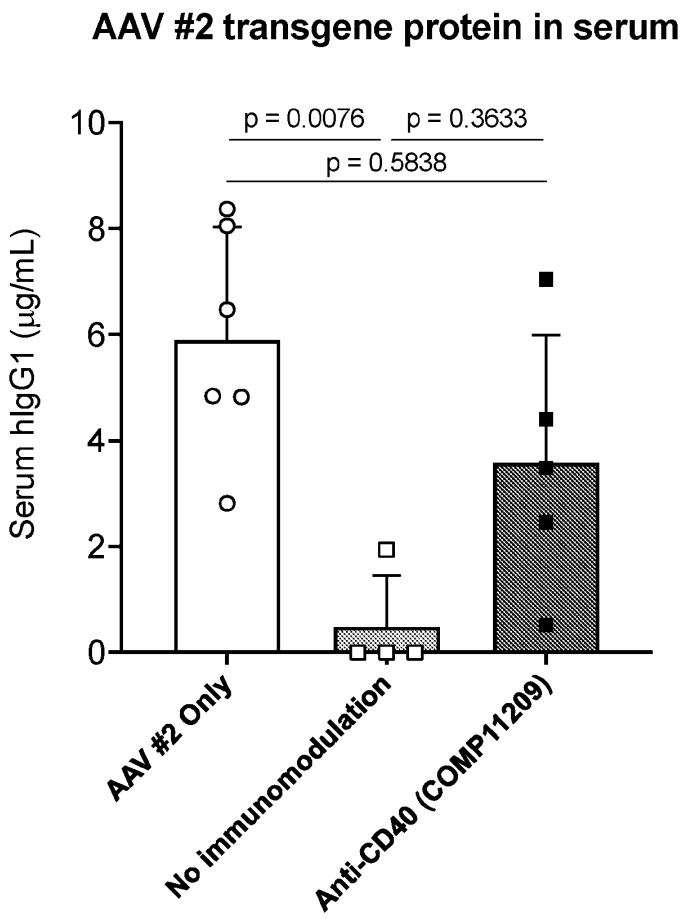

AAV #2 transgene protein in serum
p = 0.0076
p = 0.3633
p = 0.5838
Serum hIgG1 (µg/mL)
10
8
6
4
2
0
AAV #2 Only
No immunomodulation
Anti-CD40 (COMP11209)

Figure 36A

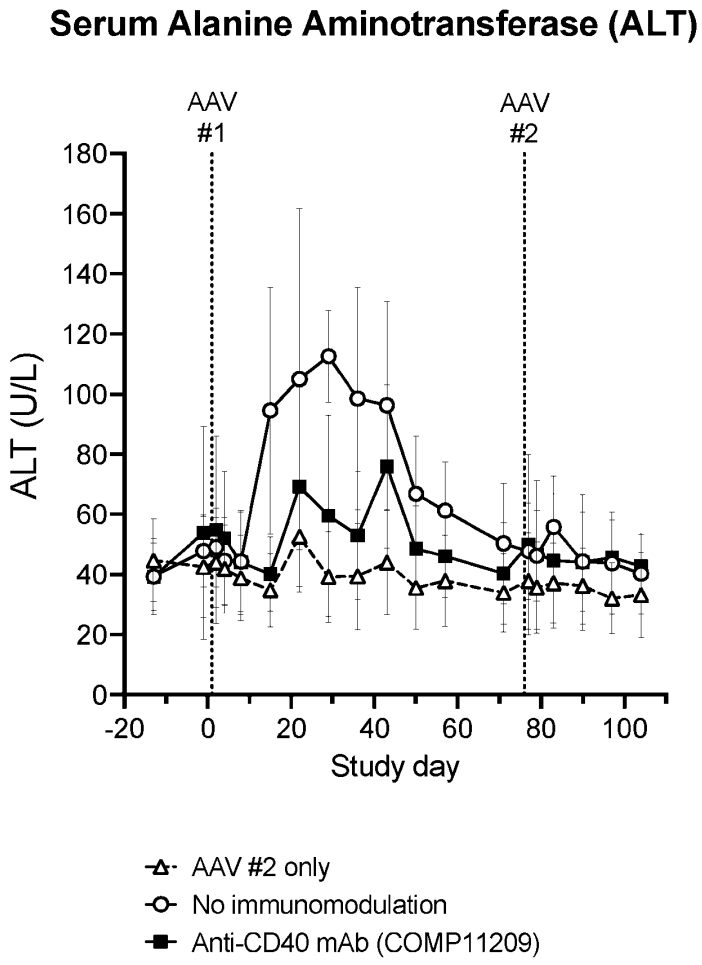

Serum Alanine Aminotransferase (ALT)
AAV #1
AAV #2
ALT (U/L)
180
160
140
120
100
80
60
40
20
0
-20
0
20
40
60
80
100
Study day
AAV #2 only
No immunomodulation
Anti-CD40 mAb (COMP11209)

Figure 36B

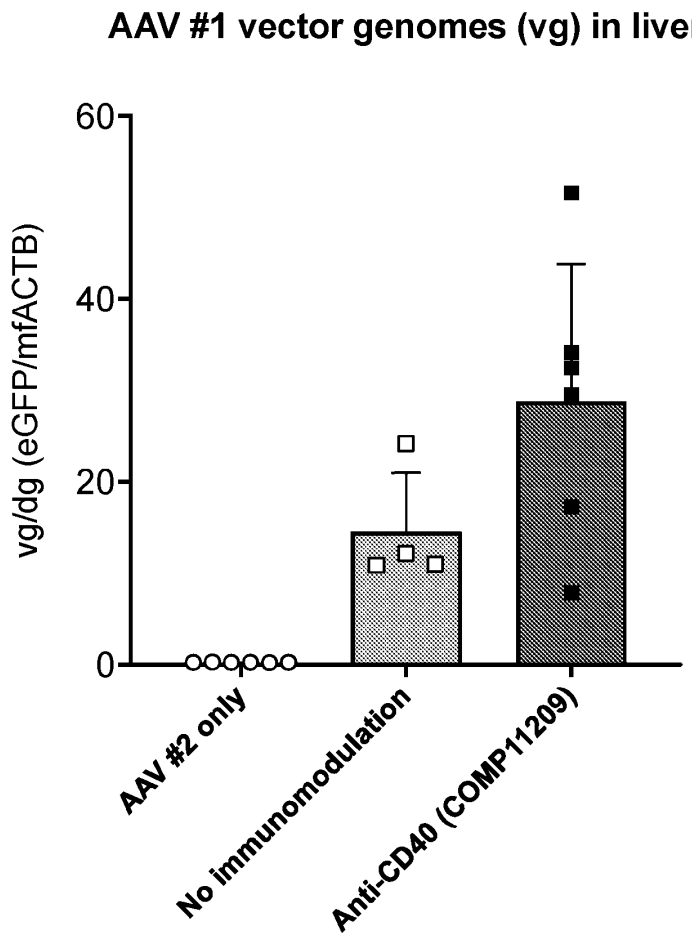
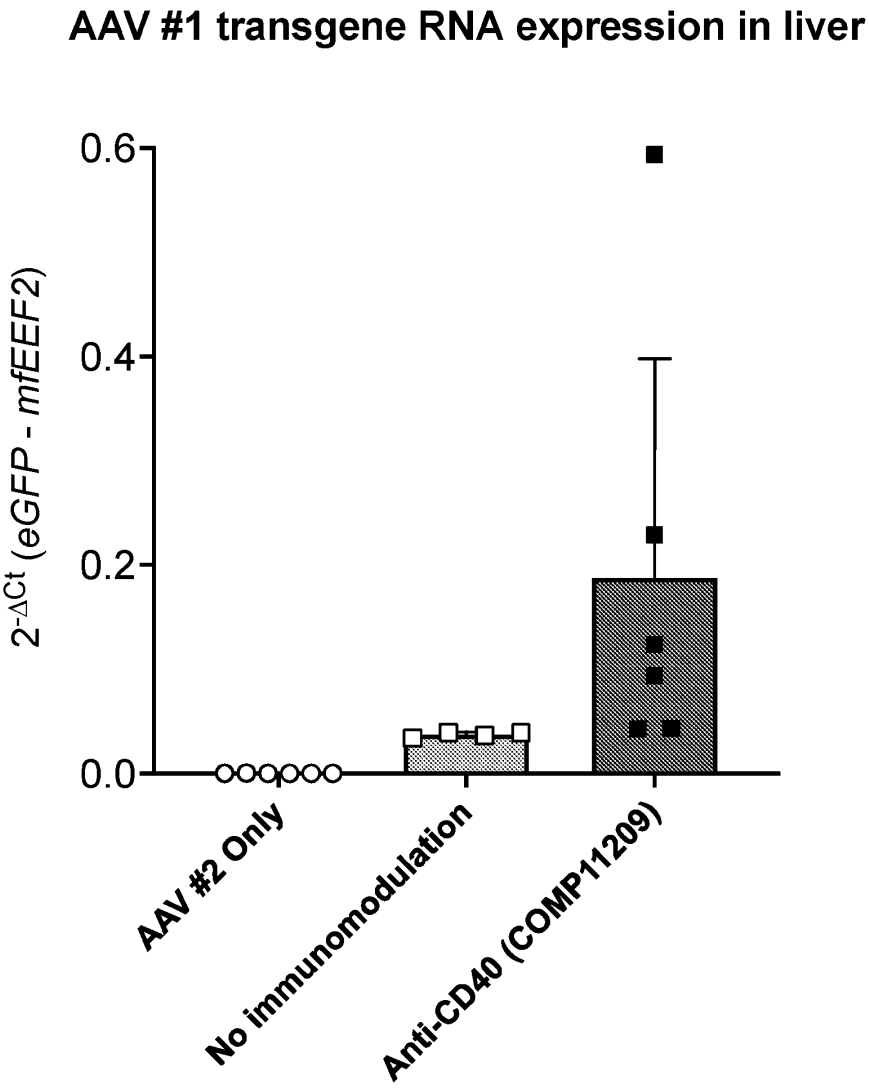

AAV #1 vector genomes (vg) in liver
vg/dg (eGFP/mfACTB)
60
40
20
0
AAV #2 only
No immunomodulation
Anti-CD40 (COMP11209)

Figure 36C


AAV #1 transgene RNA expression in liver
0.6
0.4
0.2
0.0
2-ΔCt (eGFP - mfEEF2)
AAV #2 Only
No immunomodulation
Anti-CD40 (COMP11209)