Figure 1

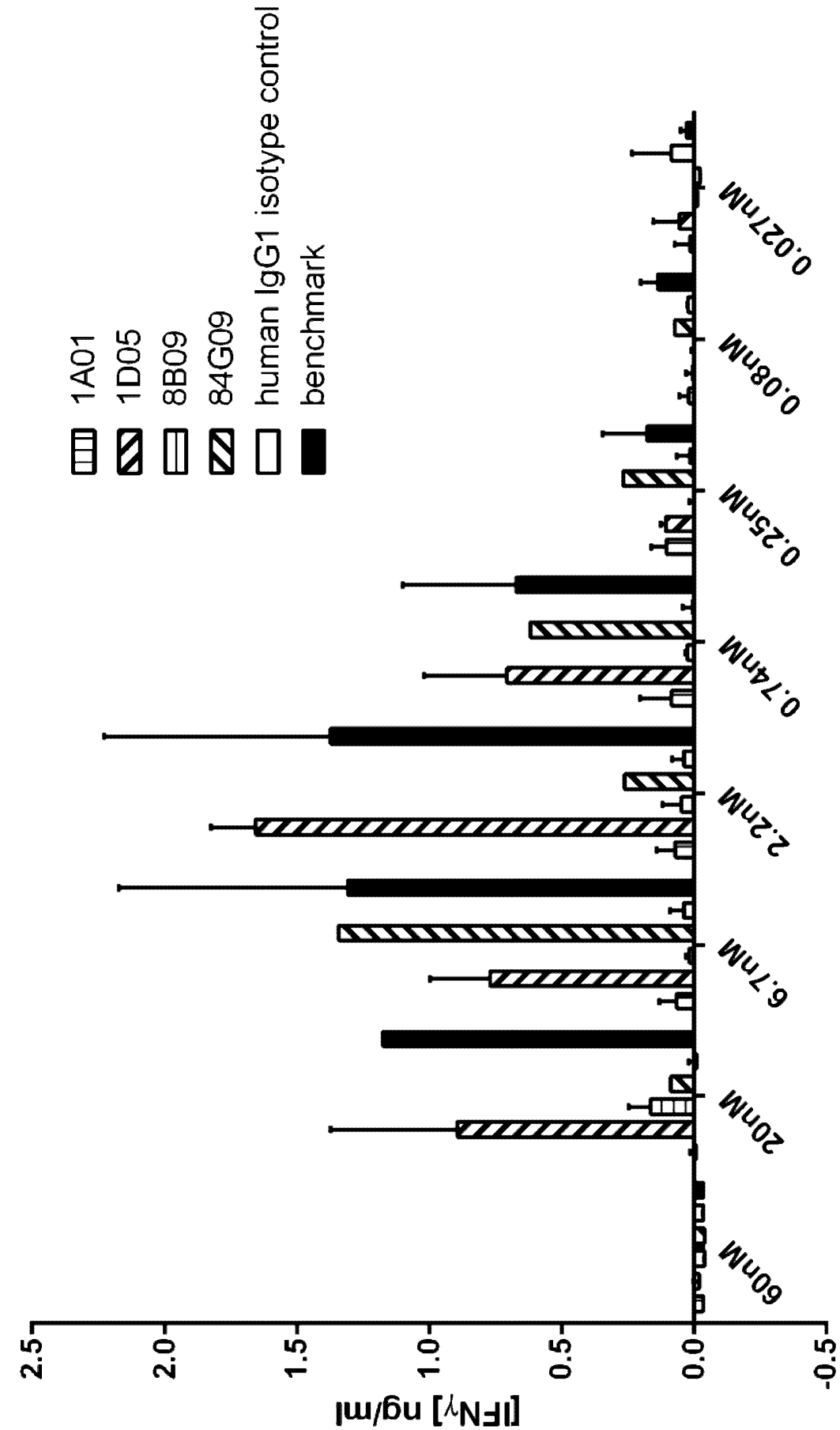

1A01
1D05
8B09
84G09
human IgG1 isotype control
benchmark
[IFNγ] ng/ml
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
60nM
20nM
6.7nM
2.2nM
0.74nM
0.25nM
0.08nM
0.027nM

Figure 2

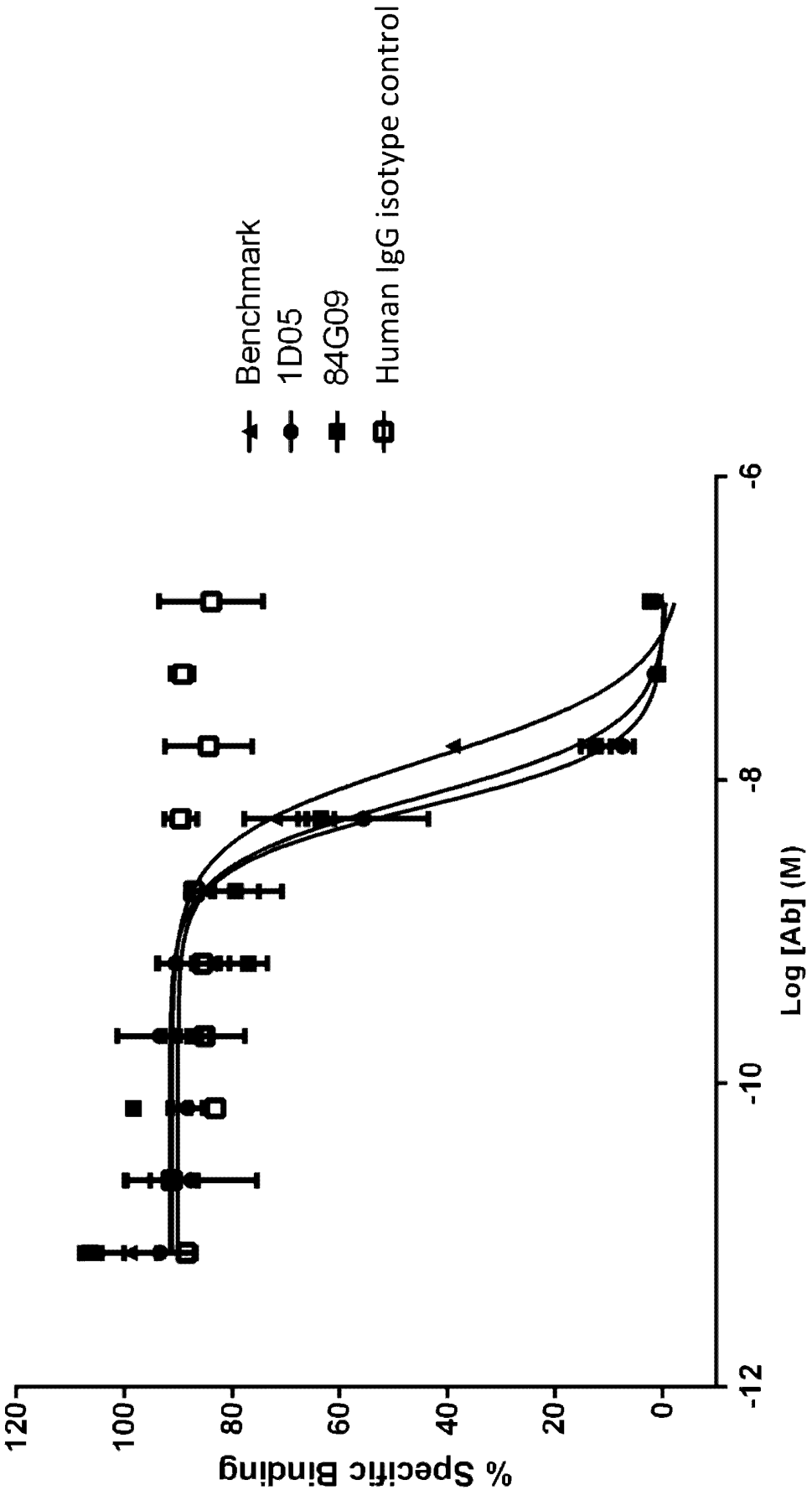

Benchmark
1D05
84G09
Human IgG isotype control
% Specific Binding
120
100
80
60
40
20
0
-12
-10
-8
-6
Log [Ab] (M)

Figure 3

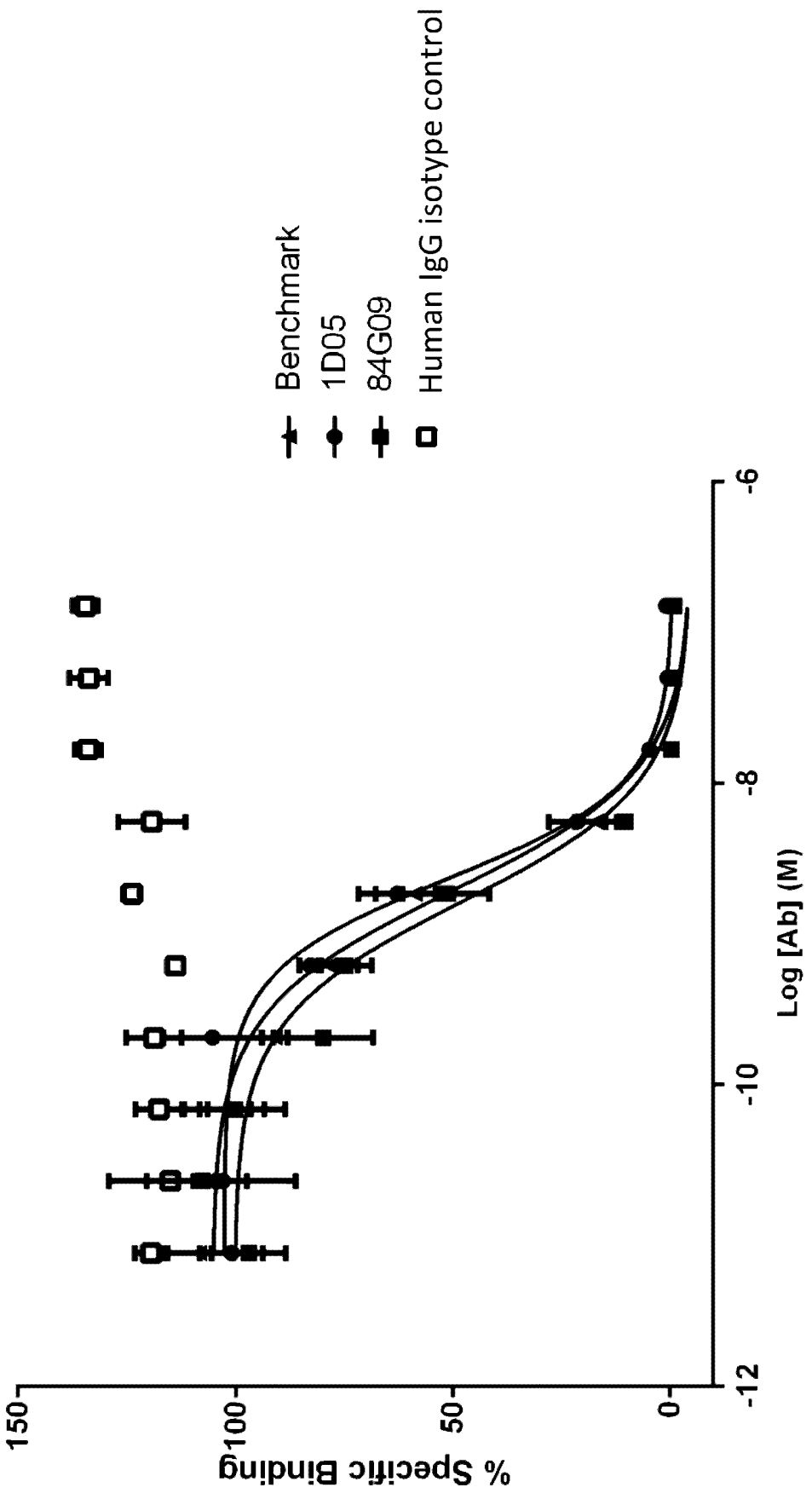

Benchmark
1D05
84G09
Human IgG isotype control
% Specific Binding
150
100
50
0
-12
-10
-8
-6
Log [Ab] (M)

Figure 4

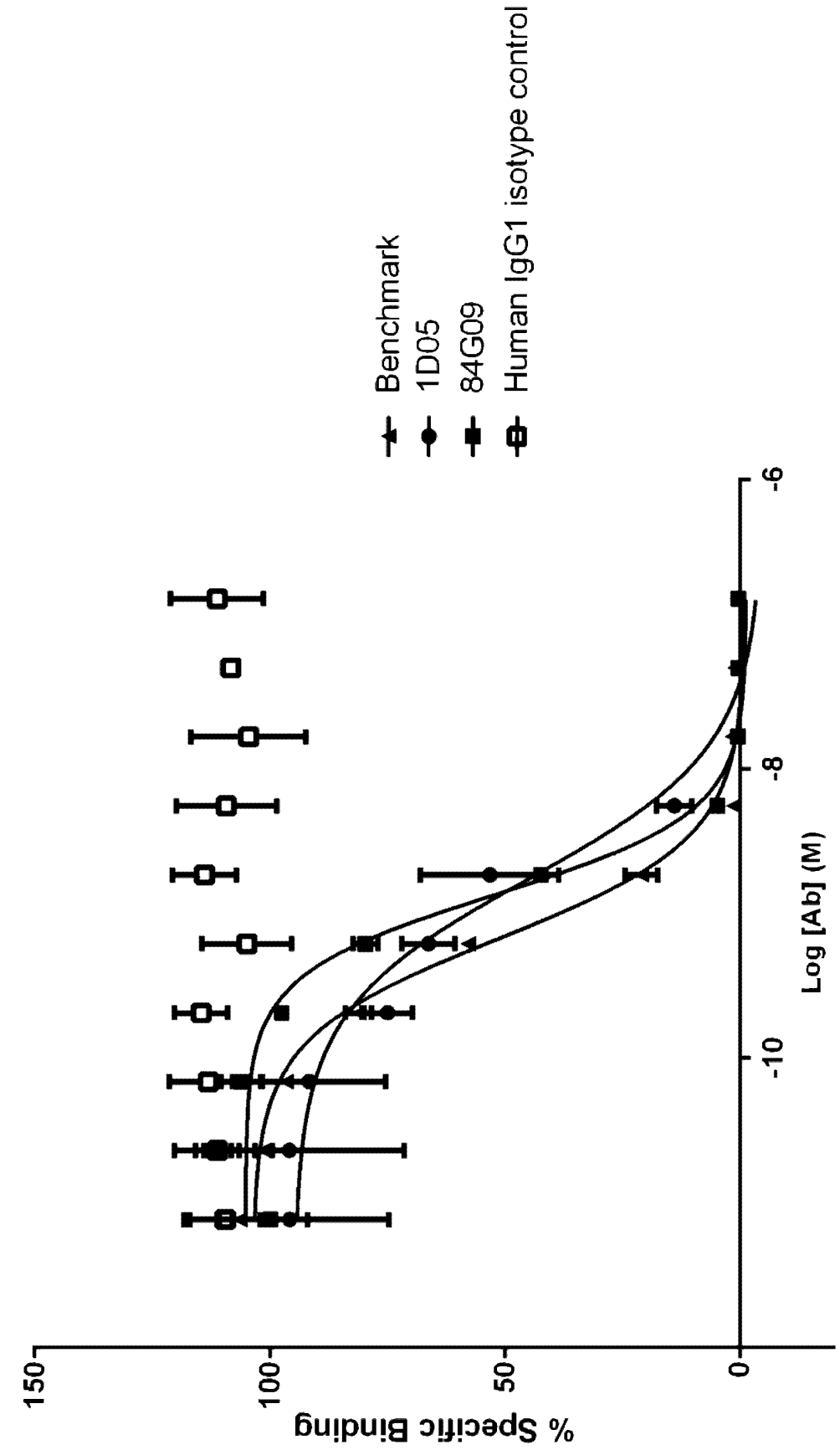

% Specific Binding
150
100
50
0
-6
-8
-10
Log [Ab] (M)
Benchmark
1D05
84G09
Human IgG1 isotype control

Figure 5a

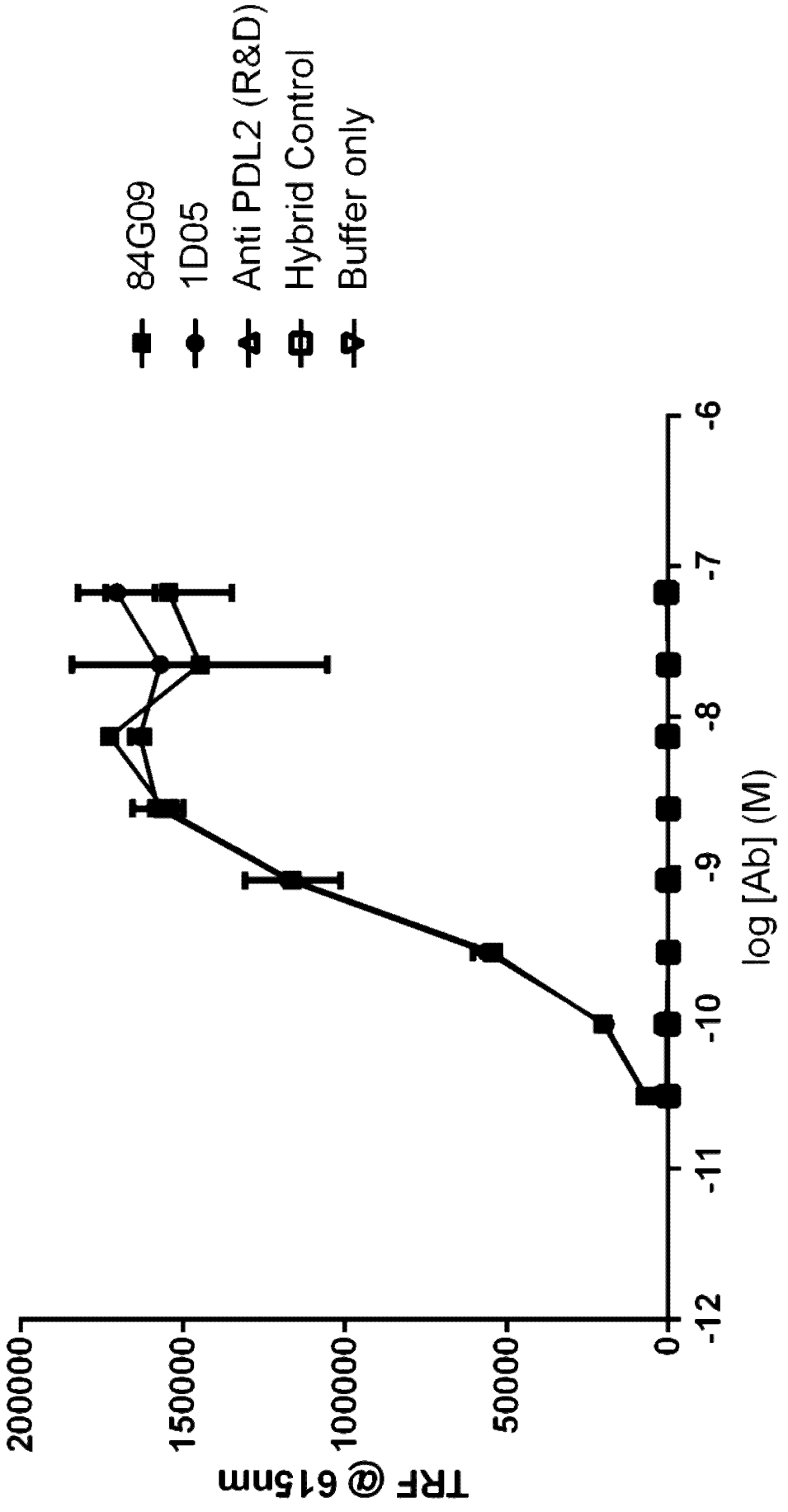

84G09
1D05
Anti PDL2 (R&D)
Hybrid Control
Buffer only
TRF @ 615nm
200000
150000
100000
50000
0
-12
-11
-10
-9
-8
-7
-6
log [Ab] (M)

Figure 5b

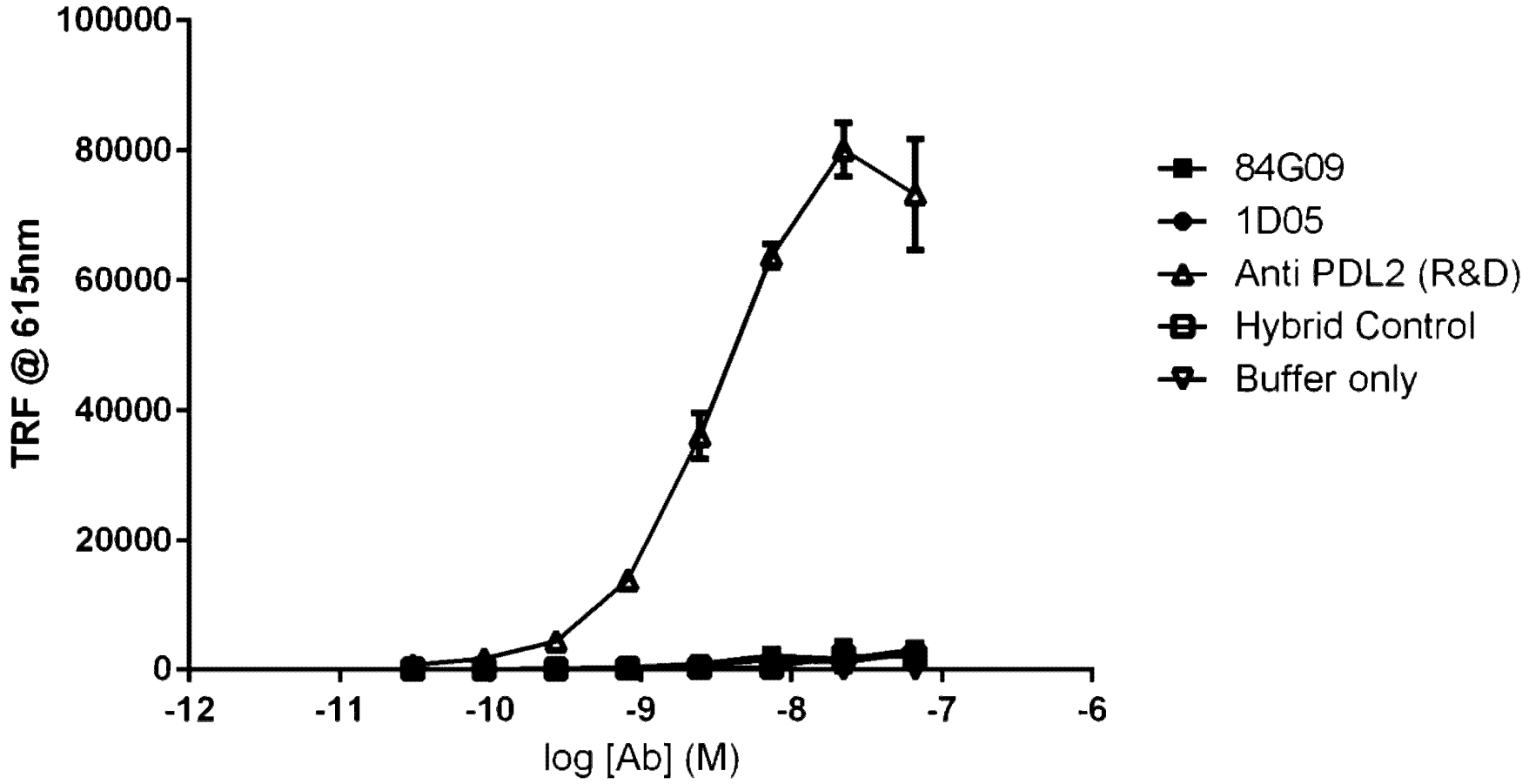

100000
80000
60000
40000
20000
0
TRF @ 615nm
-12
-11
-10
-9
-8
-7
-6
log [Ab] (M)
84G09
1D05
Anti PDL2 (R&D)
Hybrid Control
Buffer only

Figure 6

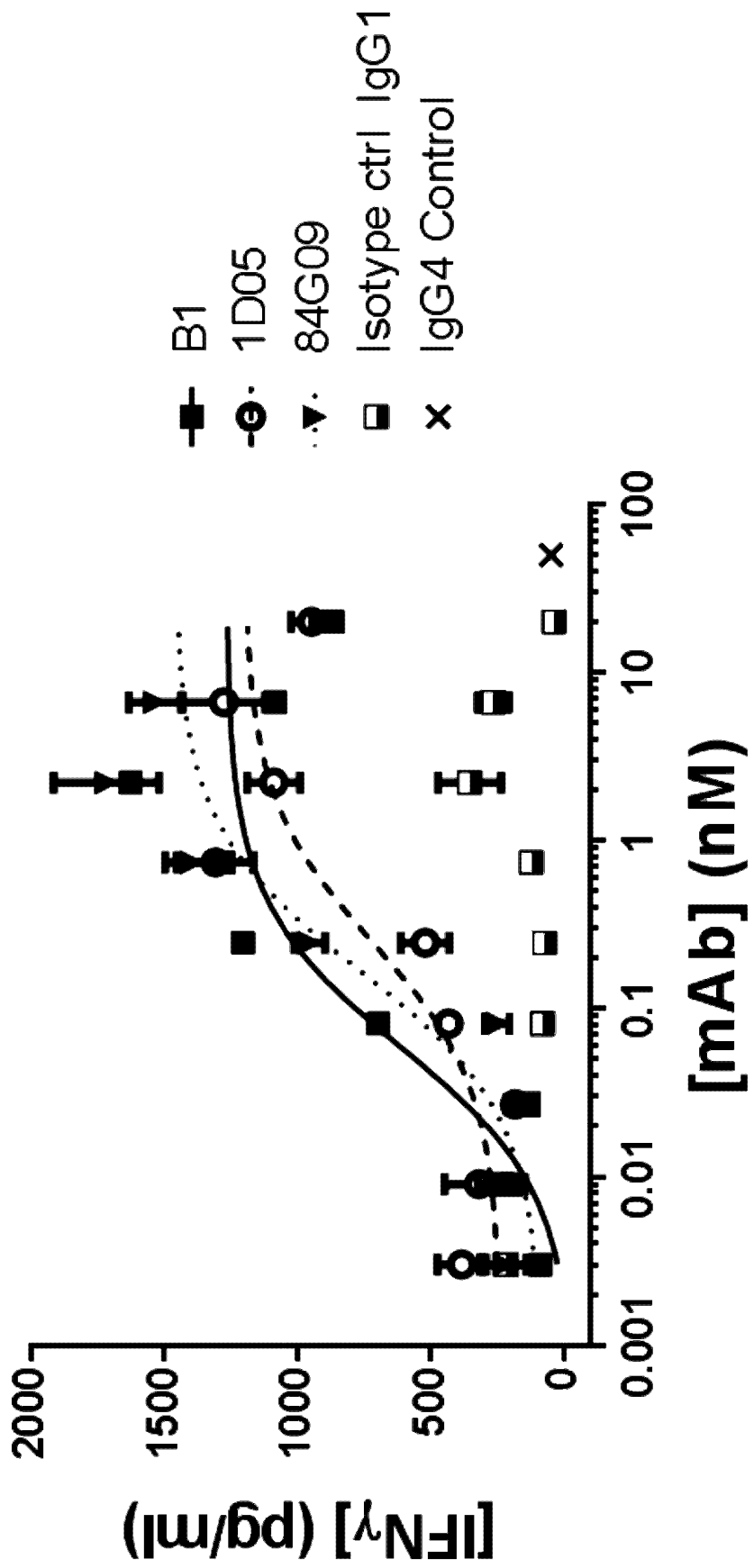
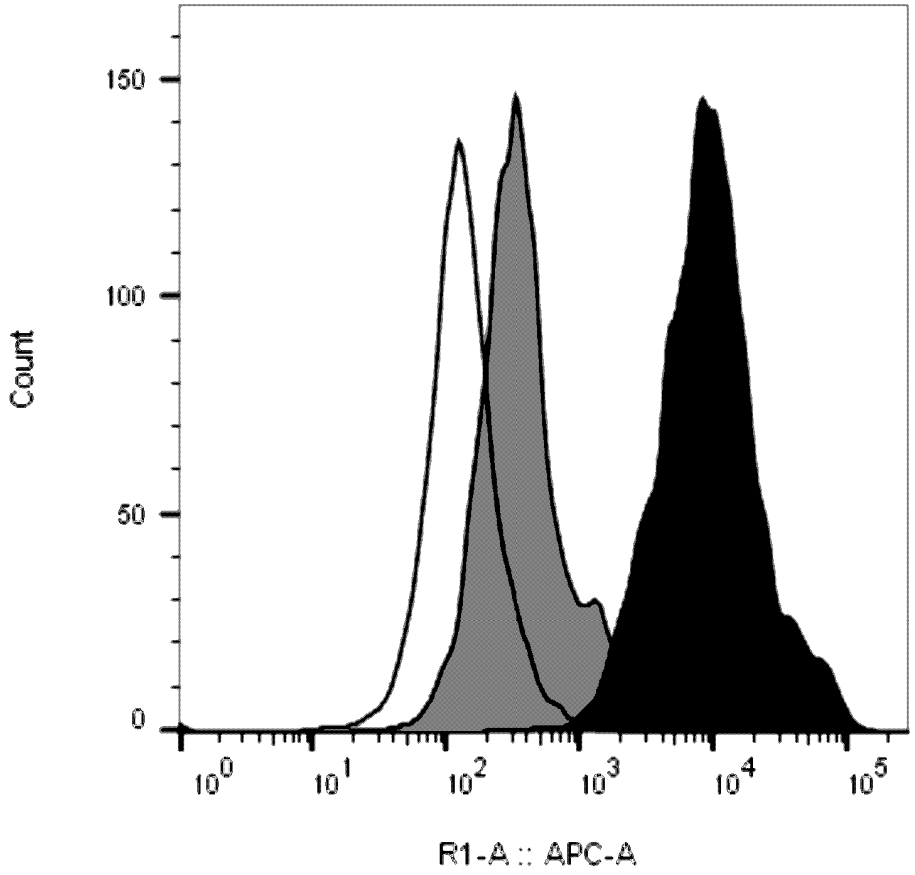
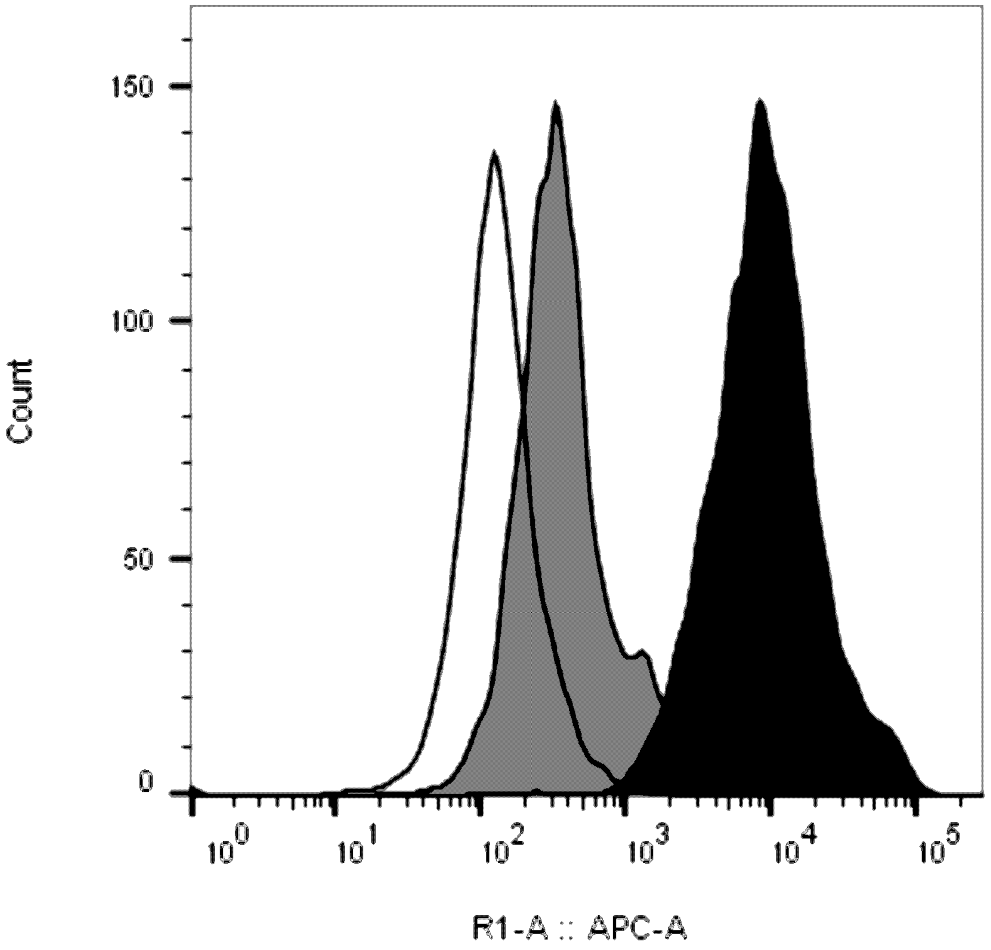

[IFNγ] (pg/ml)
2000
1500
1000
500
0
0.001
0.01
0.1
1
10
100
[mAb] (nM)
B1
1D05
84G09
Isotype ctrl IgG1
IgG4 Control

Figure 7a



	SampleID	Geometric Mean : R1-A
	Isotype hu IgG1 (D107)	379
	1D05 (D107)	8531
	unstained (D107)	127

Figure 7b



	SampleID	Geometric Mean : R1-A
	Isotype hu IgG1 (D107)	379
	84G09 (D107)	8218
	unstained (D107)	127

Figure 8a

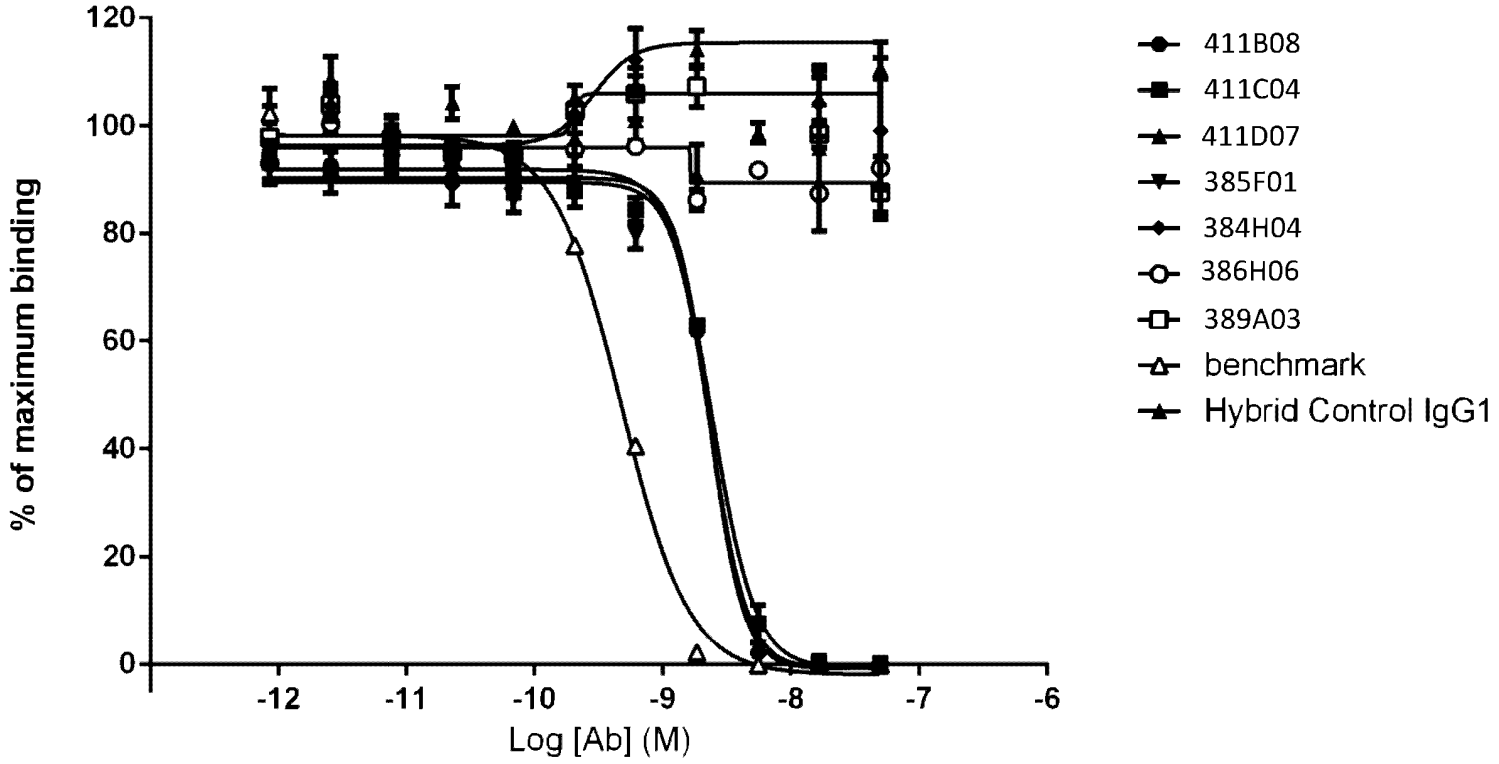

% of maximum binding
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
Log [Ab] (M)
411B08
411C04
411D07
385F01
384H04
386H06
389A03
benchmark
Hybrid Control IgG1

Figure 8b

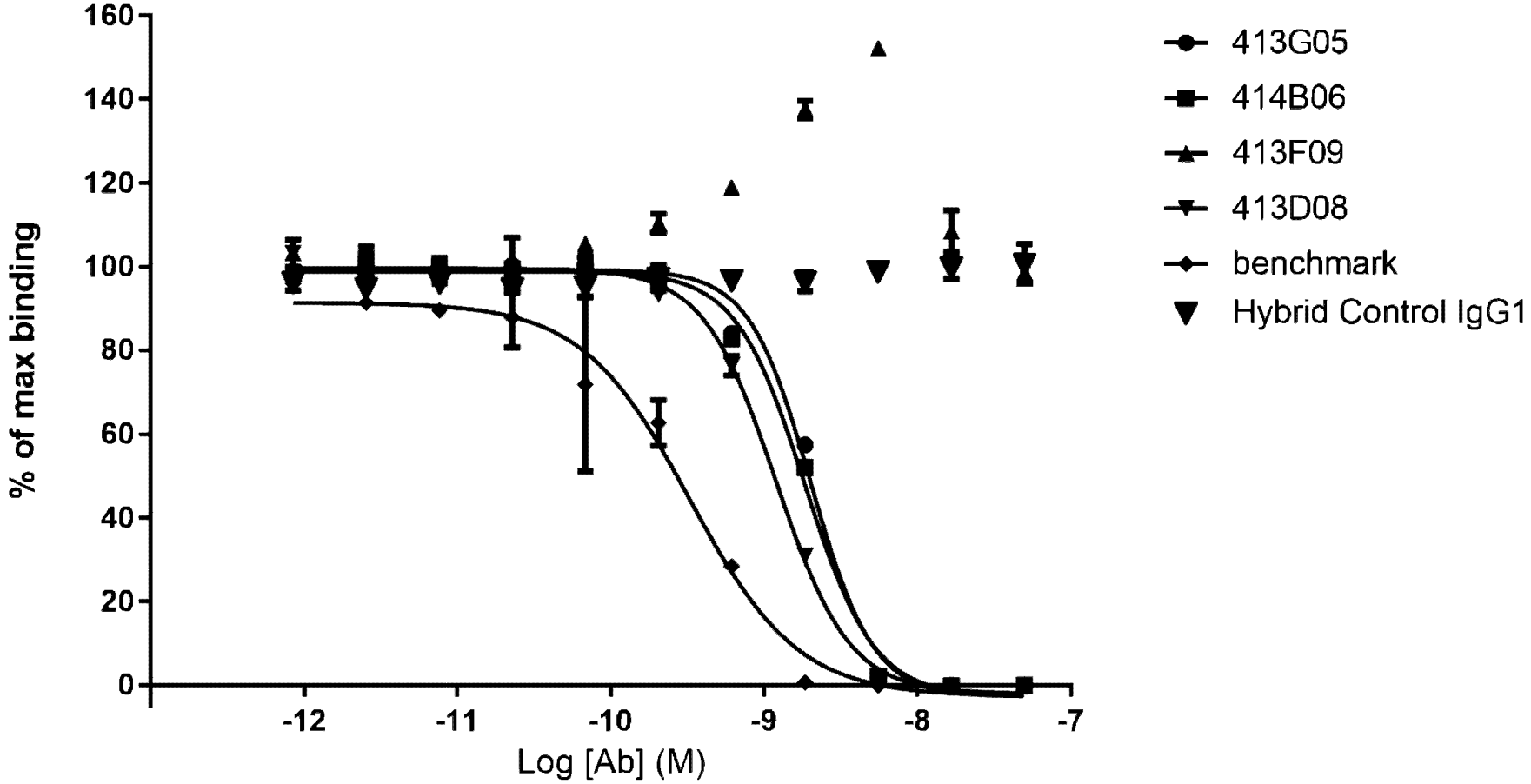

160
140
120
100
80
60
40
20
0
% of max binding
-12
-11
-10
-9
-8
-7
Log [Ab] (M)
413G05
414B06
413F09
413D08
benchmark
Hybrid Control IgG1

Figure 8c

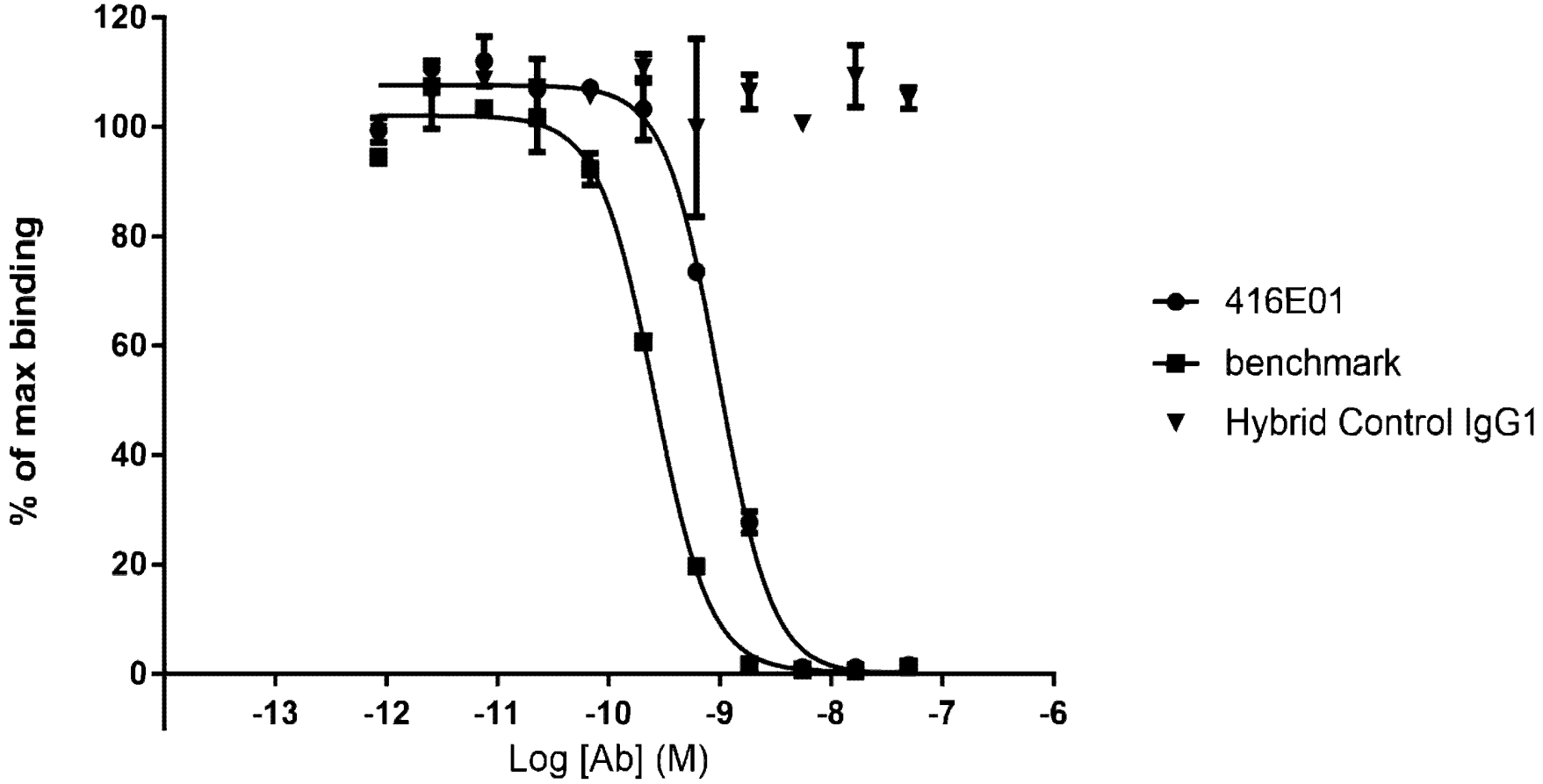

120
100
80
60
40
20
0
% of max binding
-13
-12
-11
-10
-9
-8
-7
-6
Log [Ab] (M)
416E01
benchmark
Hybrid Control IgG1

Figure 9a

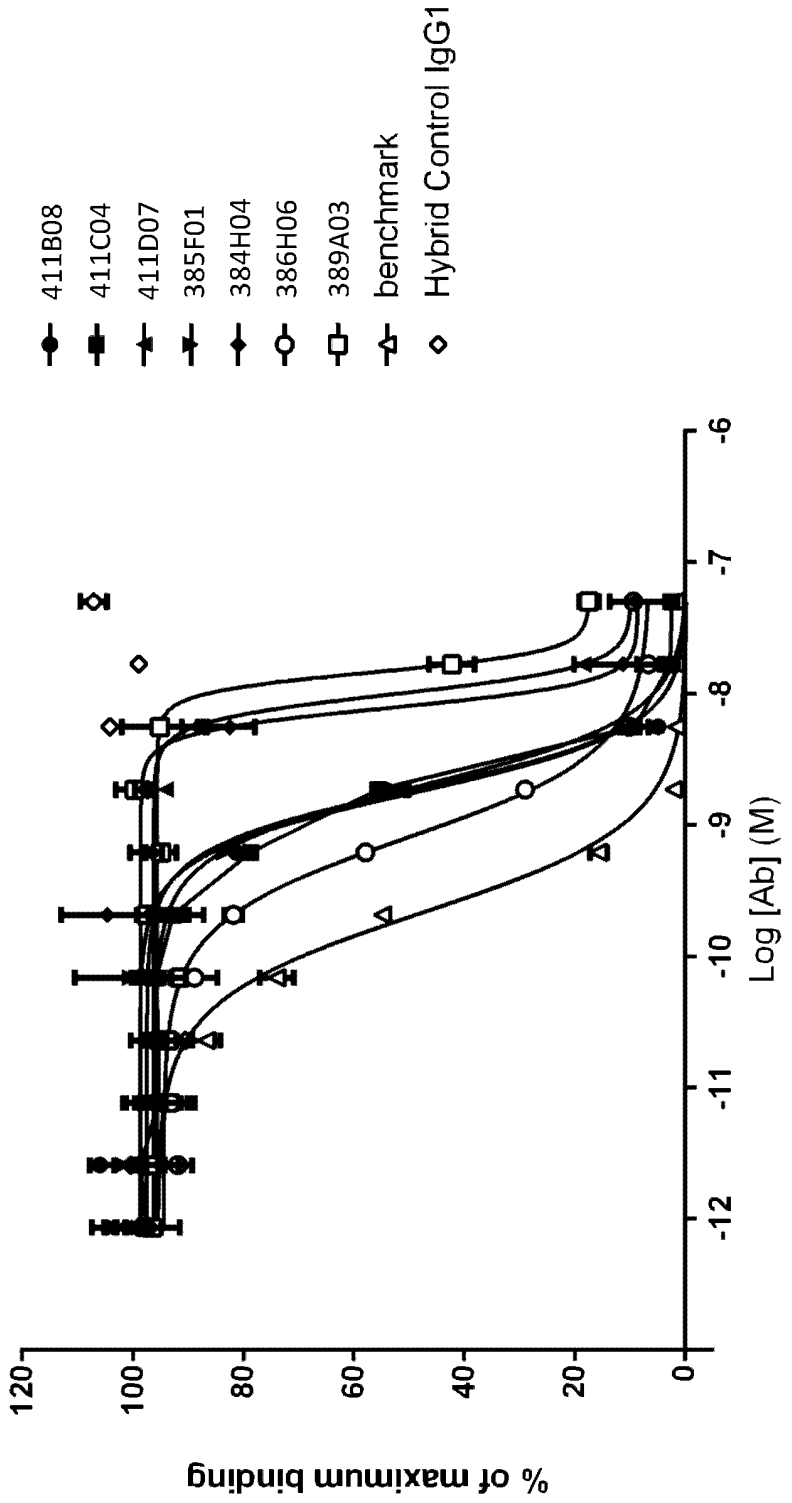

411B08
411C04
411D07
385F01
384H04
386H06
389A03
benchmark
Hybrid Control IgG1
% of maximum binding
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
Log [Ab] (M)

Figure 9b

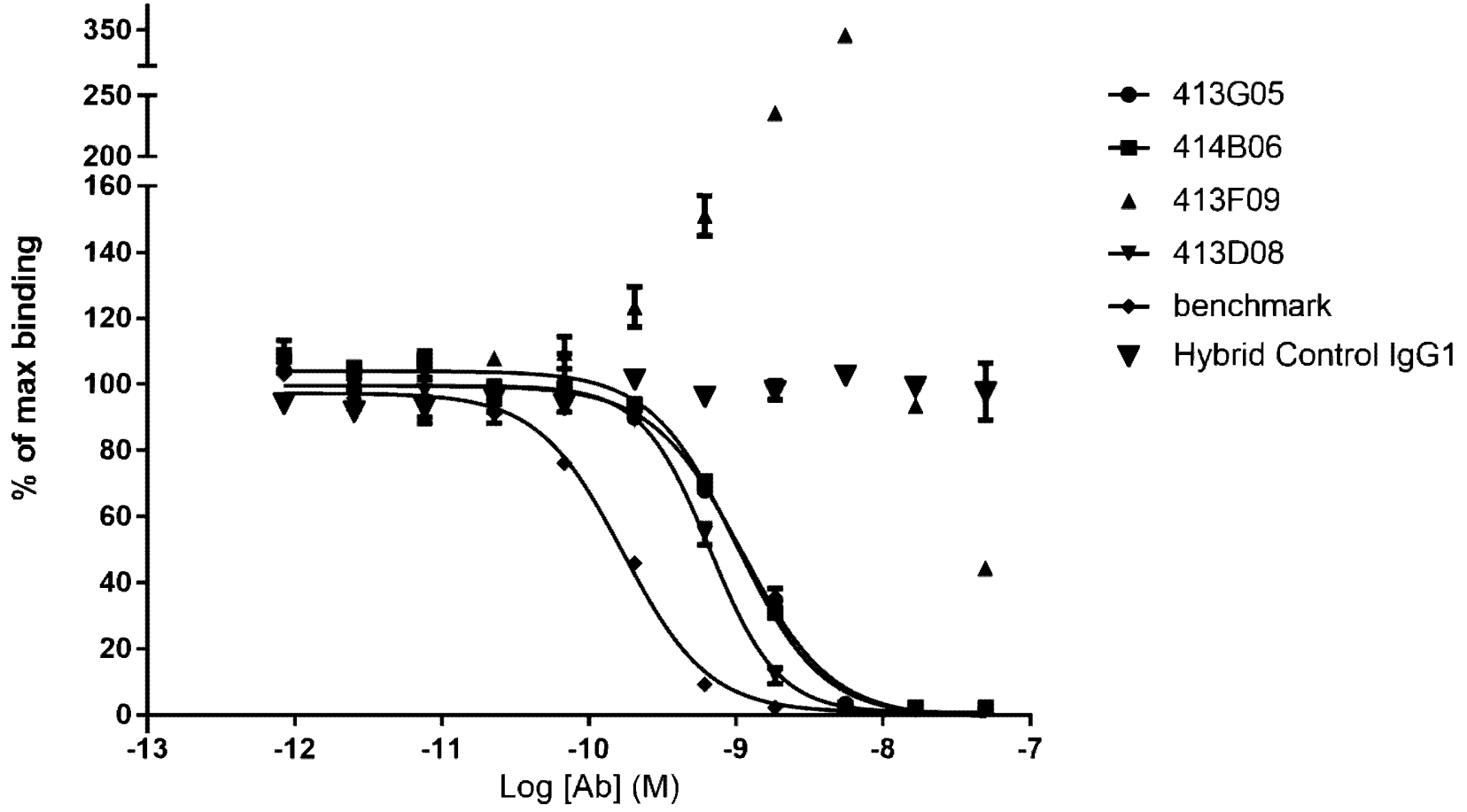

350
250
200
160
140
120
100
80
60
40
20
0
% of max binding
-13
-12
-11
-10
-9
-8
-7
Log [Ab] (M)
413G05
414B06
413F09
413D08
benchmark
Hybrid Control IgG1

Figure 9c

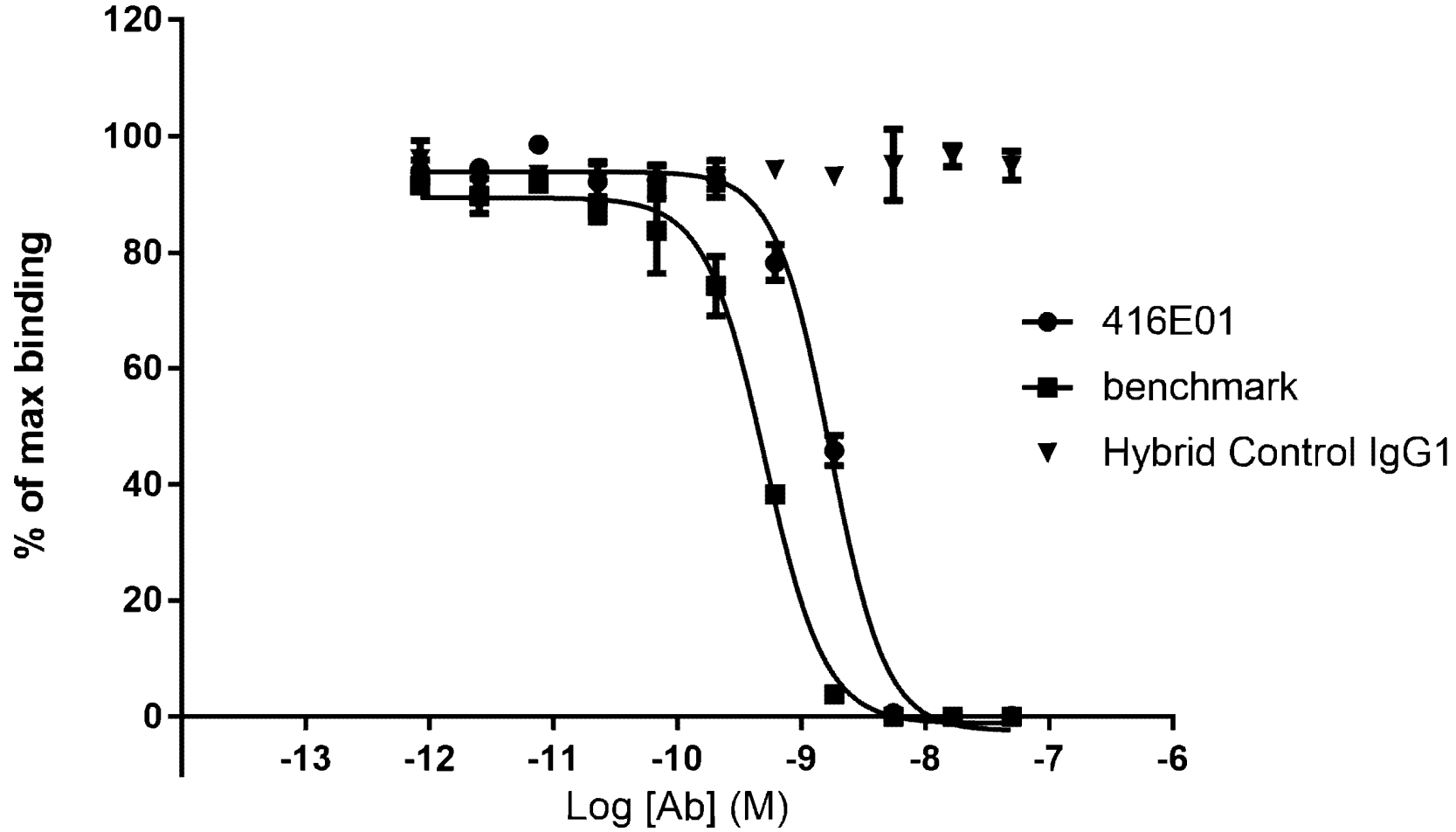

120
100
80
60
40
20
0
% of max binding
-13
-12
-11
-10
-9
-8
-7
-6
Log [Ab] (M)
416E01
benchmark
Hybrid Control IgG1

Figure 10

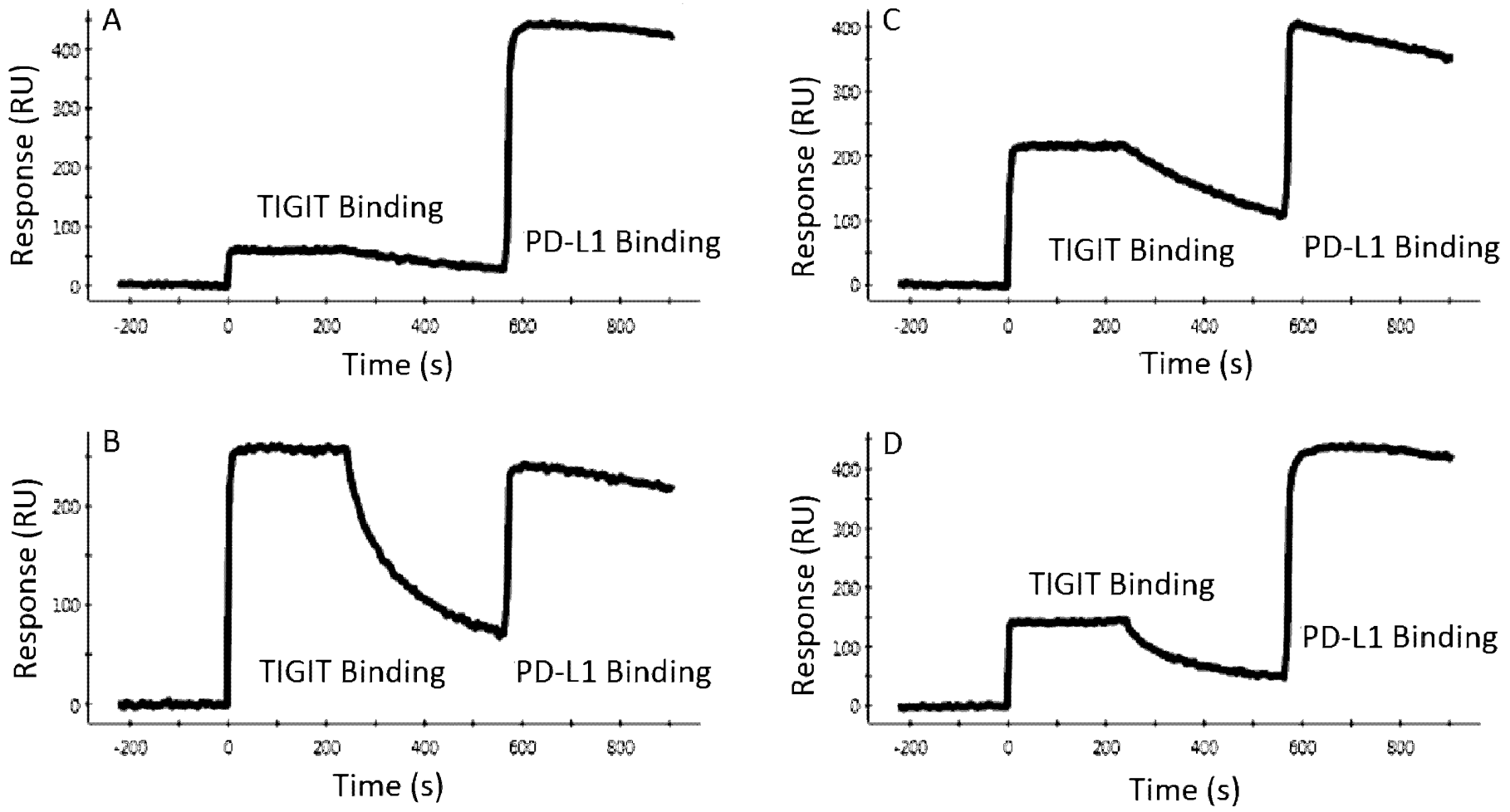

A
Response (RU)
TIGIT Binding
PD-L1 Binding
Time (s)
B
Response (RU)
TIGIT Binding
PD-L1 Binding
Time (s)
C
Response (RU)
TIGIT Binding
PD-L1 Binding
Time (s)
D
Response (RU)
TIGIT Binding
PD-L1 Binding
Time (s)

Figure 11

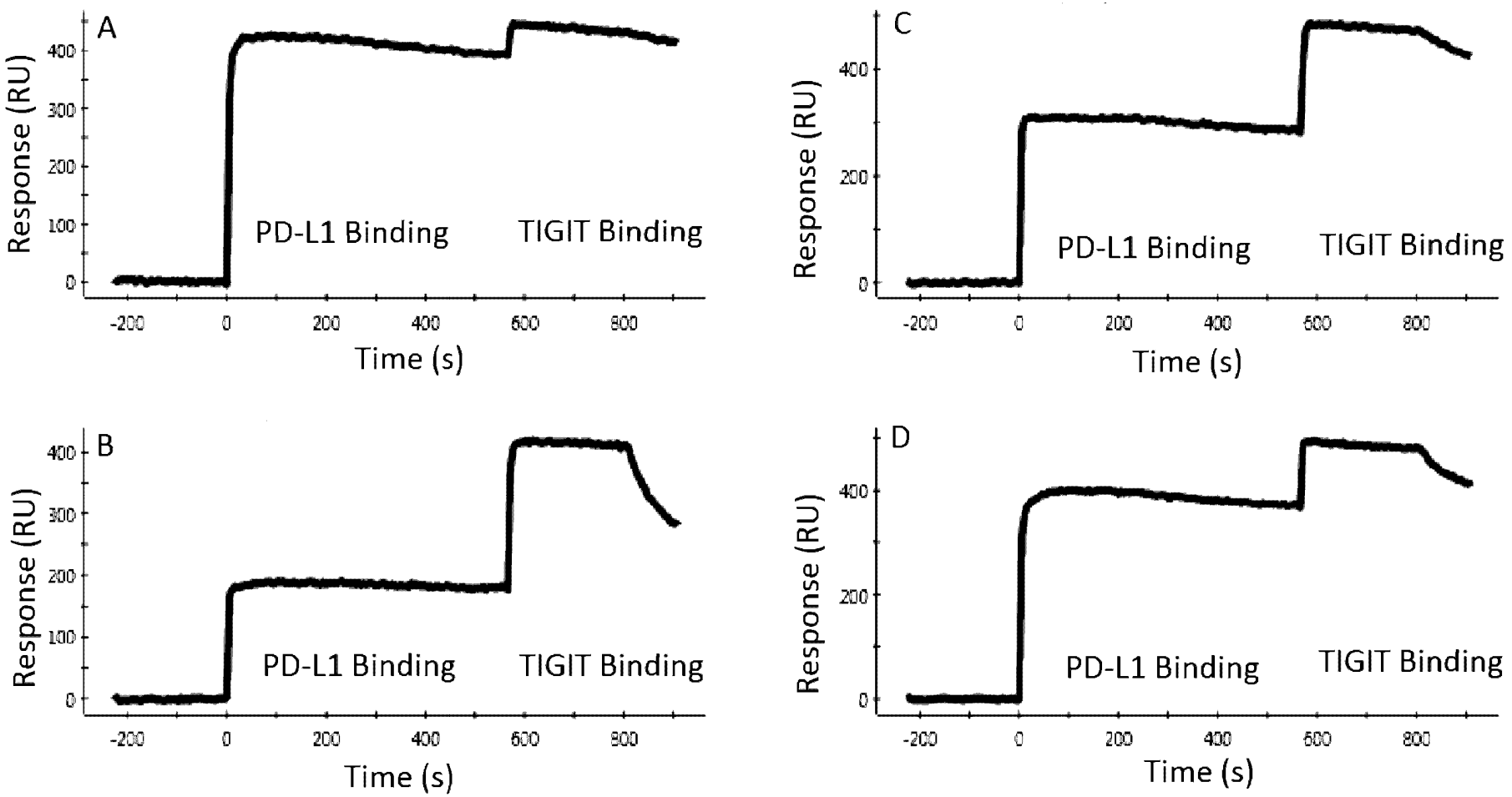
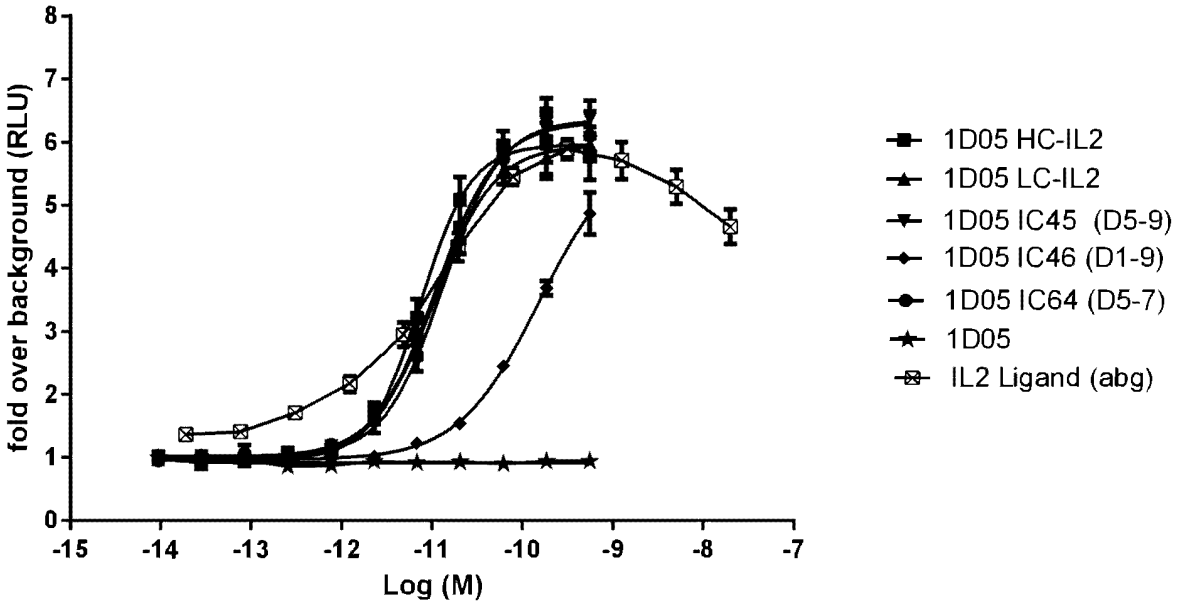

A
Response (RU)
400
300
200
100
0
PD-L1 Binding
TIGIT Binding
-200
0
200
400
600
800
Time (s)
C
Response (RU)
400
200
0
PD-L1 Binding
TIGIT Binding
-200
0
200
400
600
800
Time (s)
B
Response (RU)
400
300
200
100
0
PD-L1 Binding
TIGIT Binding
-200
0
200
400
600
800
Time (s)
D
Response (RU)
400
200
0
PD-L1 Binding
TIGIT Binding
-200
0
200
400
600
800
Time (s)

Figure 12(a)


fold over background (RLU)
Log (M)
1D05 HC-IL2
1D05 LC-IL2
1D05 IC45 (D5-9)
1D05 IC46 (D1-9)
1D05 IC64 (D5-7)
1D05
IL2 Ligand (abg)

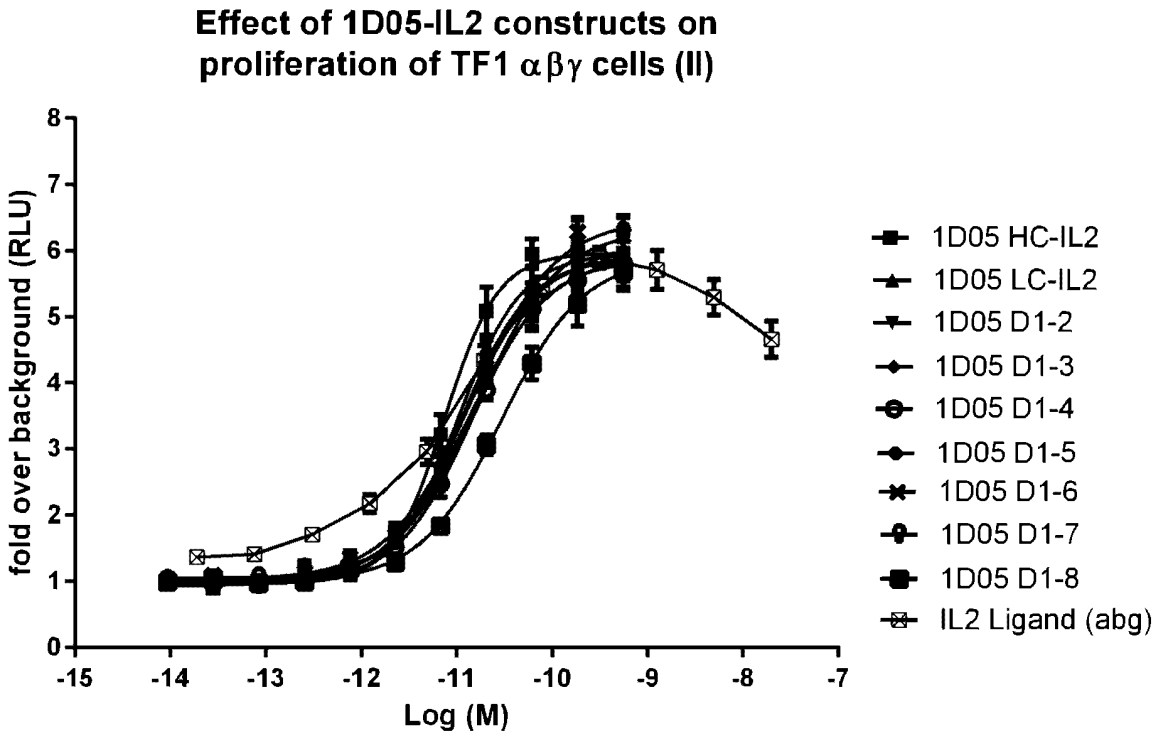

Effect of 1D05-IL2 constructs on proliferation of TF1 αβγ cells (II)
fold over background (RLU)
Log (M)
1D05 HC-IL2
1D05 LC-IL2
1D05 D1-2
1D05 D1-3
1D05 D1-4
1D05 D1-5
1D05 D1-6
1D05 D1-7
1D05 D1-8
IL2 Ligand (abg)

Figure 12(a) cont...

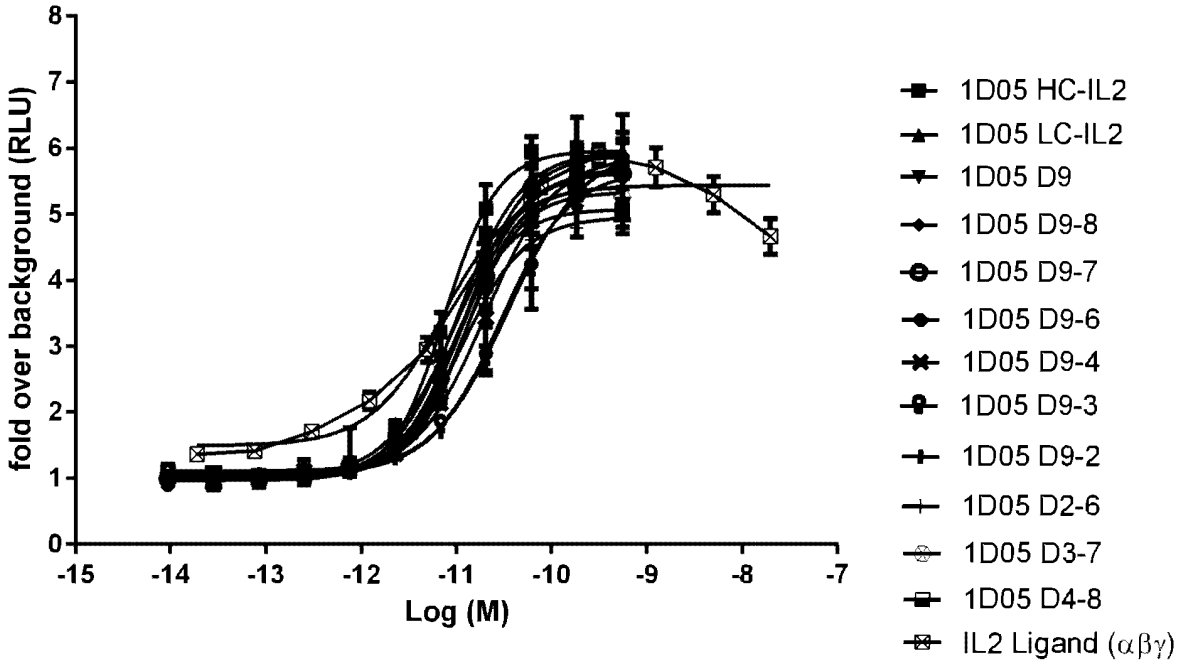
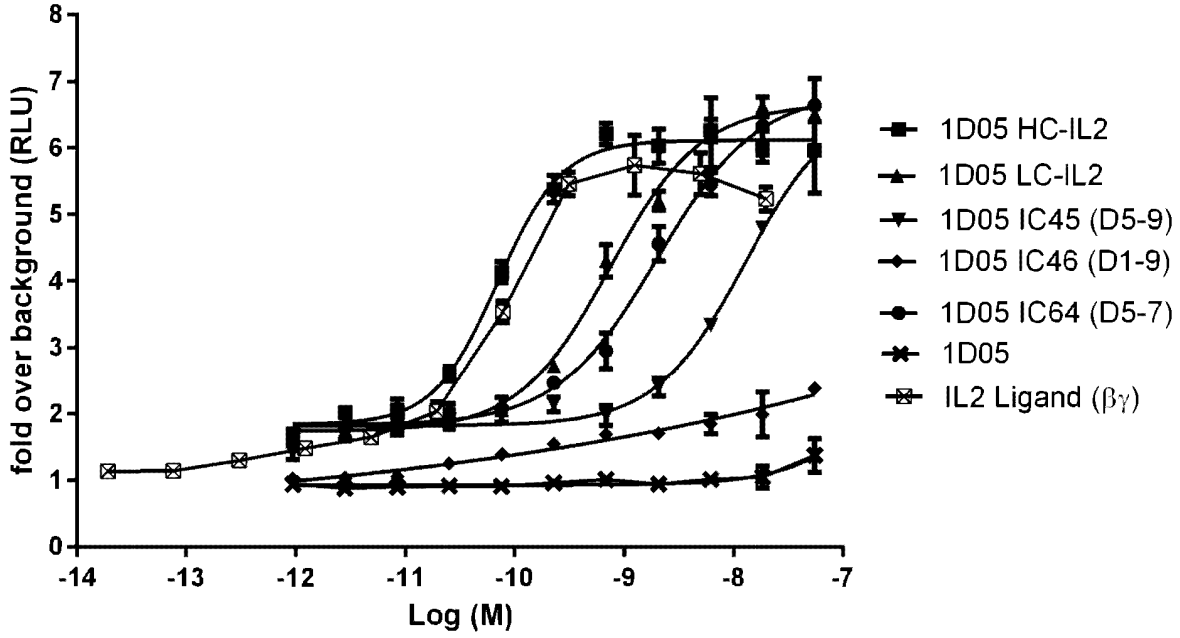


Figure 12(b)


fold over background (RLU)
Log (M)
1D05 HC-IL2
1D05 LC-IL2
1D05 IC45 (D5-9)
1D05 IC46 (D1-9)
1D05 IC64 (D5-7)
1D05
IL2 Ligand (βγ)

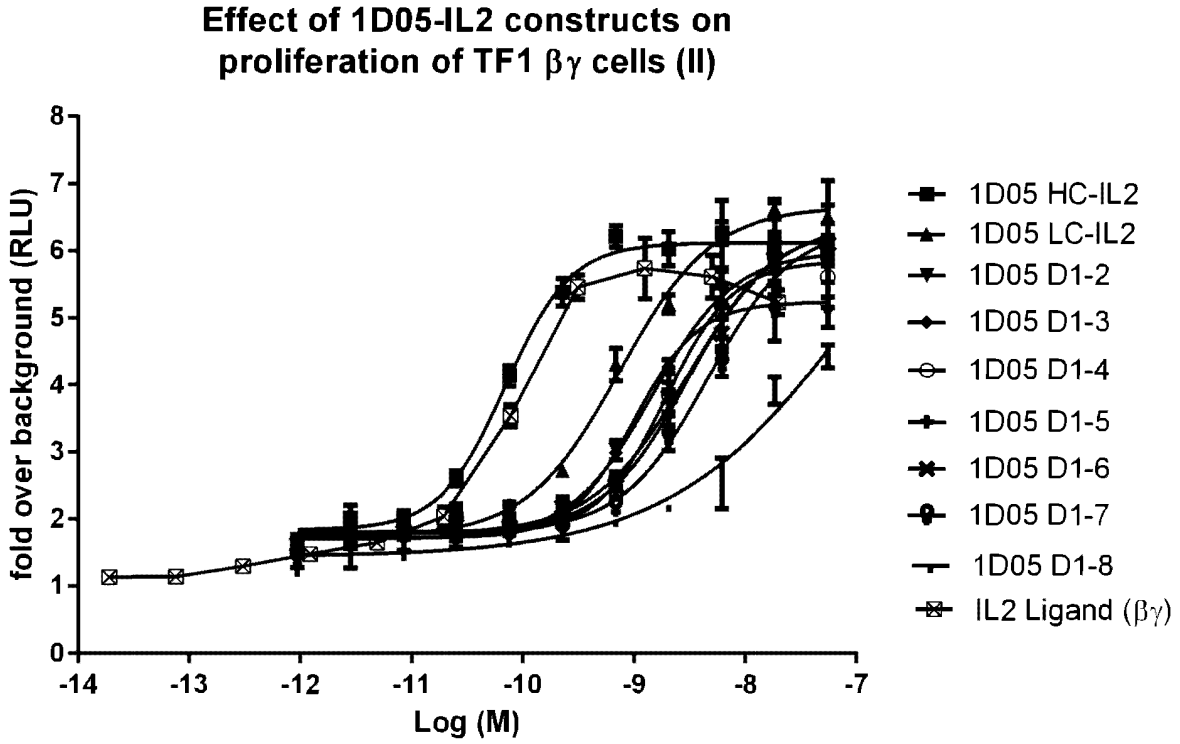

Effect of 1D05-IL2 constructs on proliferation of TF1 βγ cells (II)
fold over background (RLU)
Log (M)
1D05 HC-IL2
1D05 LC-IL2
1D05 D1-2
1D05 D1-3
1D05 D1-4
1D05 D1-5
1D05 D1-6
1D05 D1-7
1D05 D1-8
IL2 Ligand (βγ)

Figure 12(b) cont

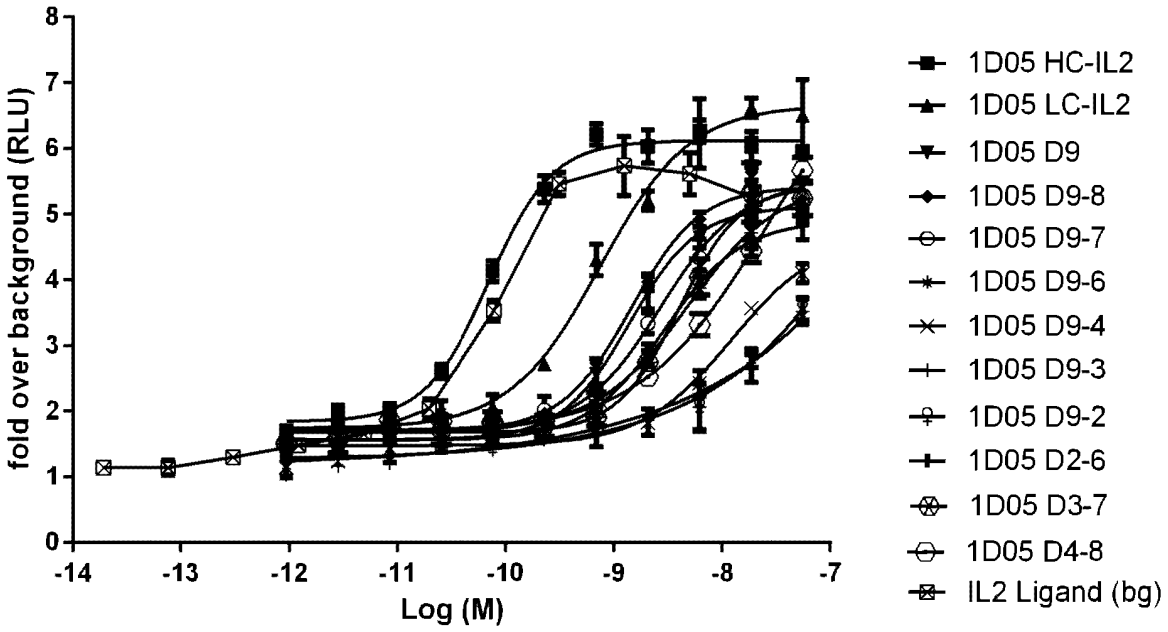
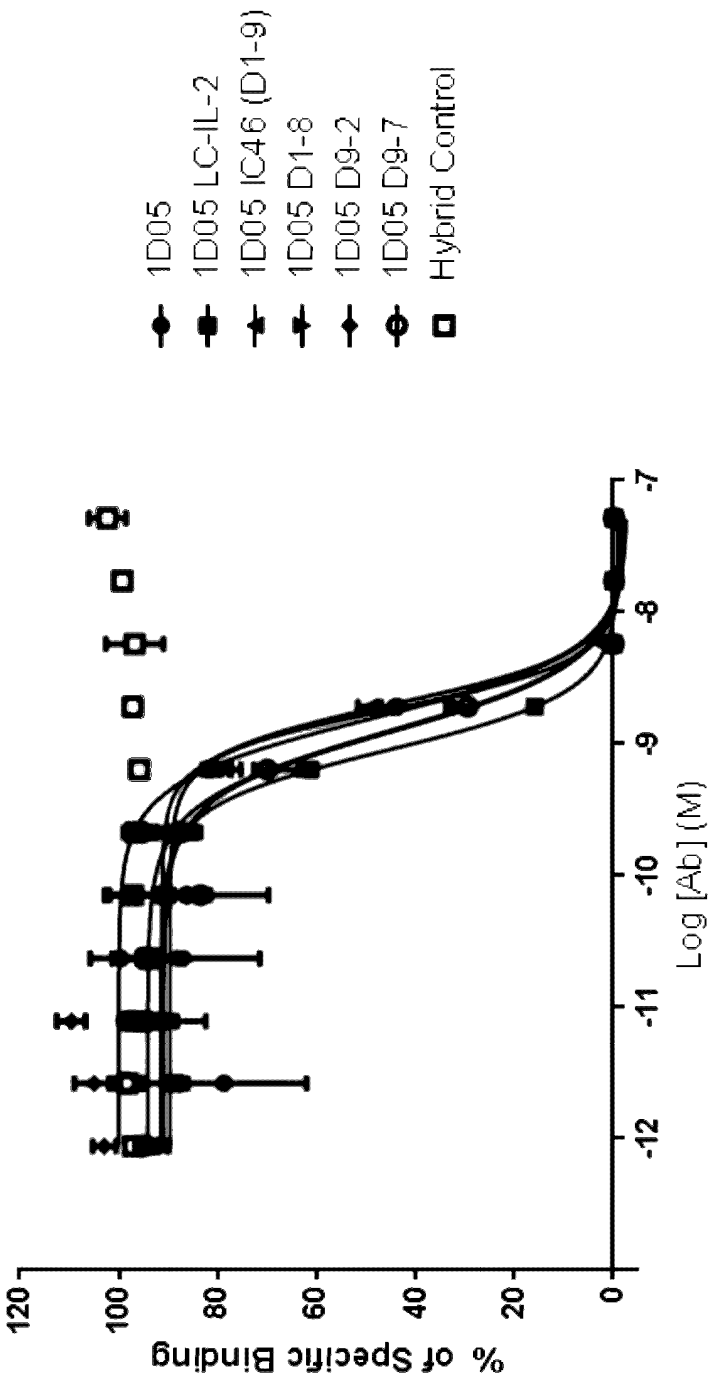


Figure 13(a)


1D05
1D05 LC-IL-2
1D05 IC46 (D1-9)
1D05 D1-8
1D05 D9-2
1D05 D9-7
Hybrid Control
% of Specific Binding
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
Log [Ab] (M)

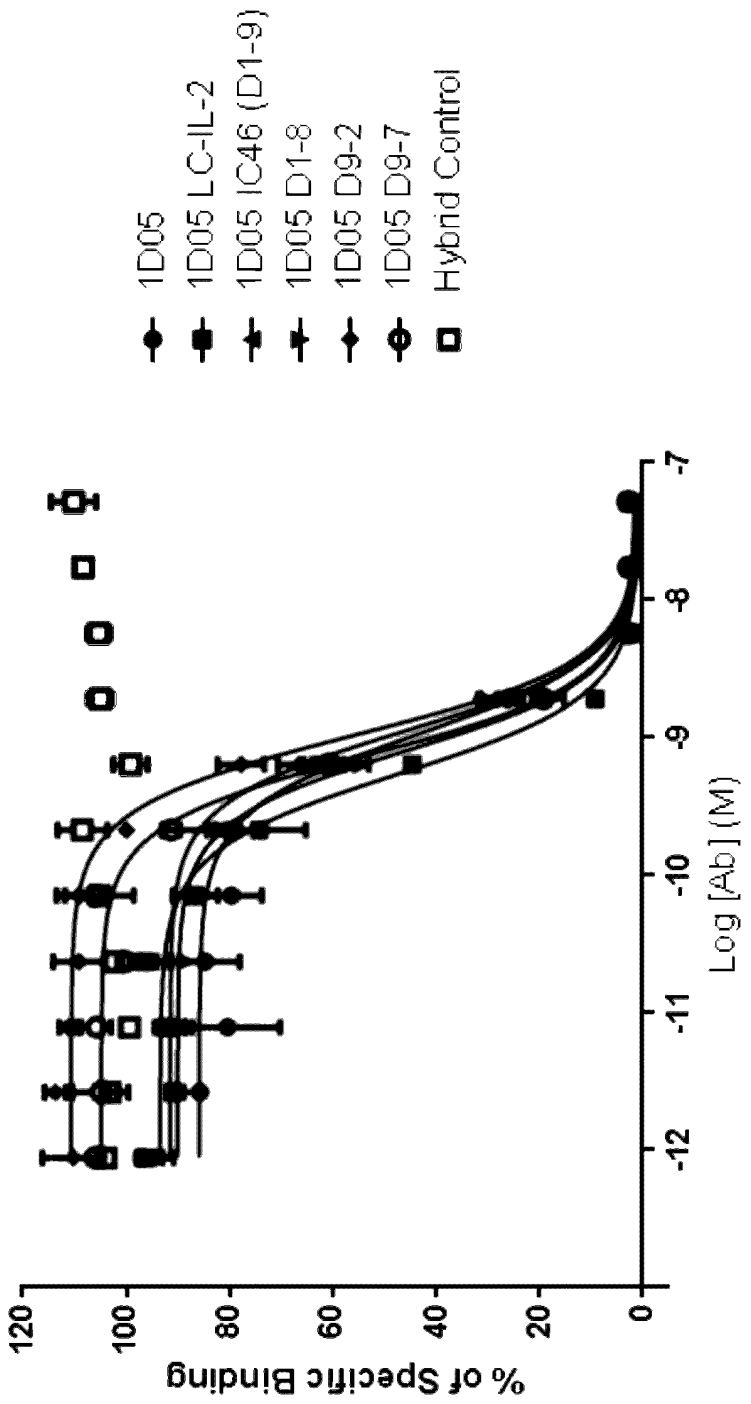

1D05
1D05 LC-IL-2
1D05 IC46 (D1-9)
1D05 D1-8
1D05 D9-2
1D05 D9-7
Hybrid Control
% of Specific Binding
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
Log [Ab] (M)

Figure 13(b)

Figure 14

a)

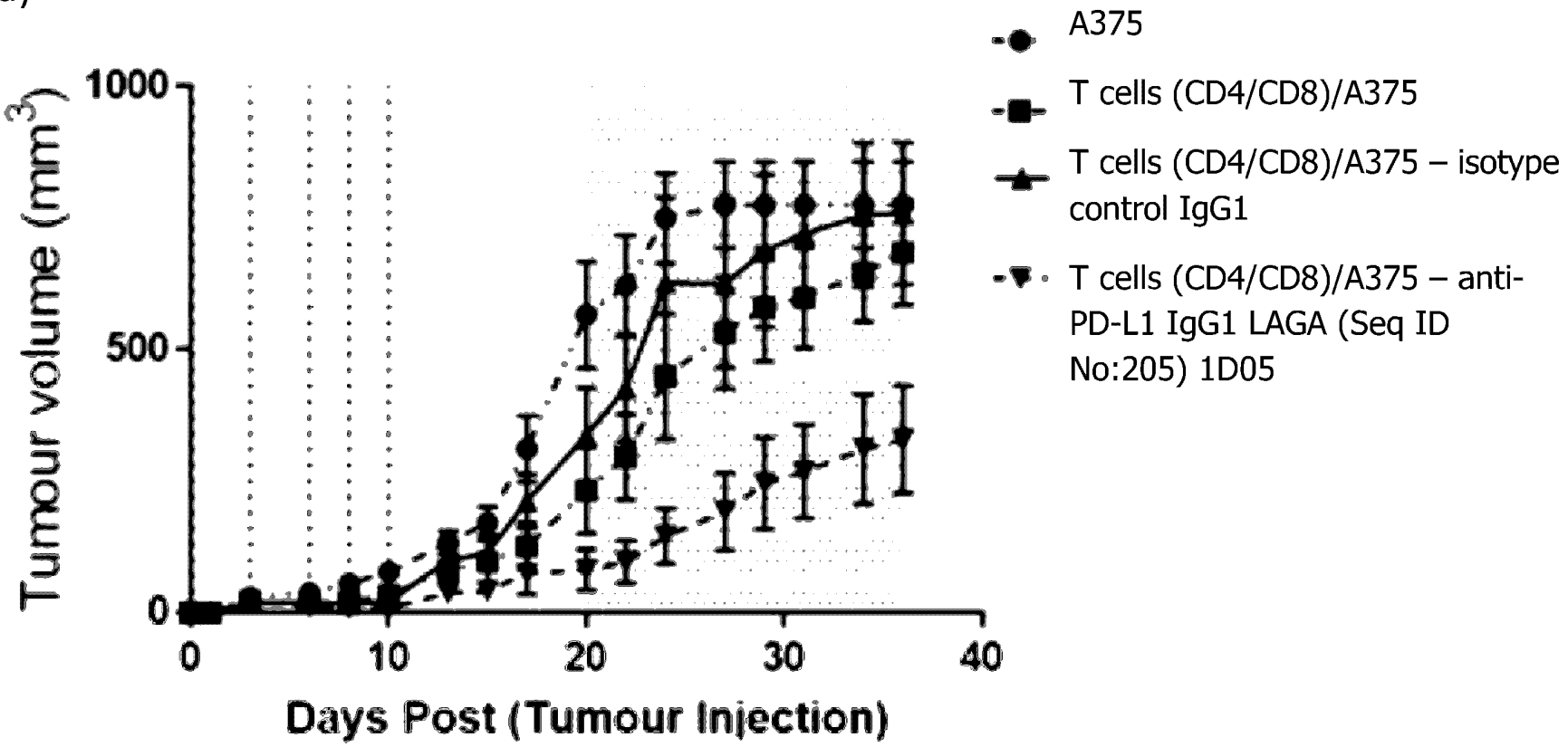
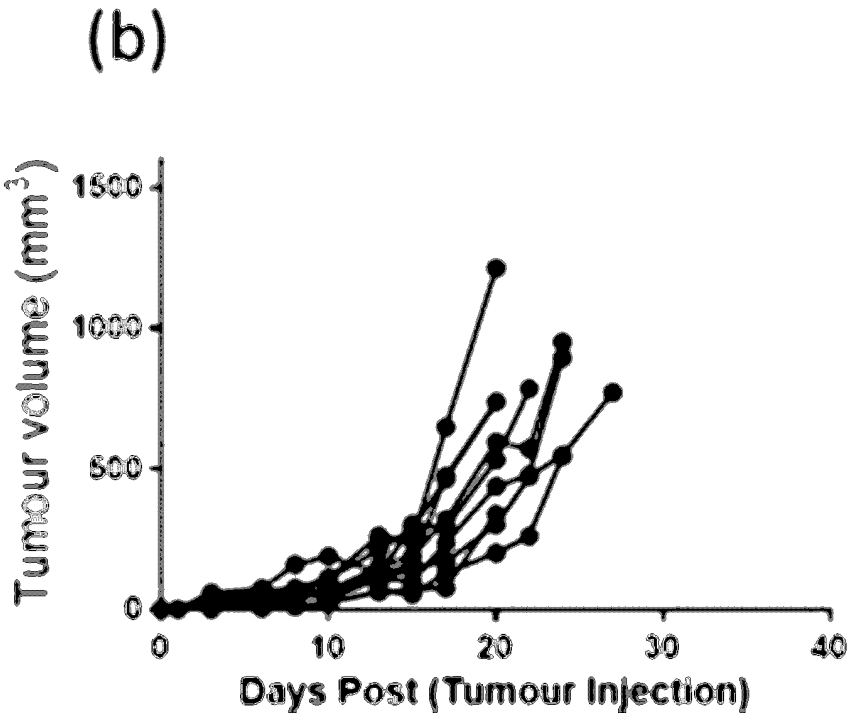

Tumour volume (mm3)
1000
500
0
0
10
20
30
40
Days Post (Tumour Injection)
A375
T cells (CD4/CD8)/A375
T cells (CD4/CD8)/A375 – isotype control IgG1
T cells (CD4/CD8)/A375 – anti-PD-L1 IgG1 LAGA (Seq ID No:205) 1D05

Figure 14 continued…



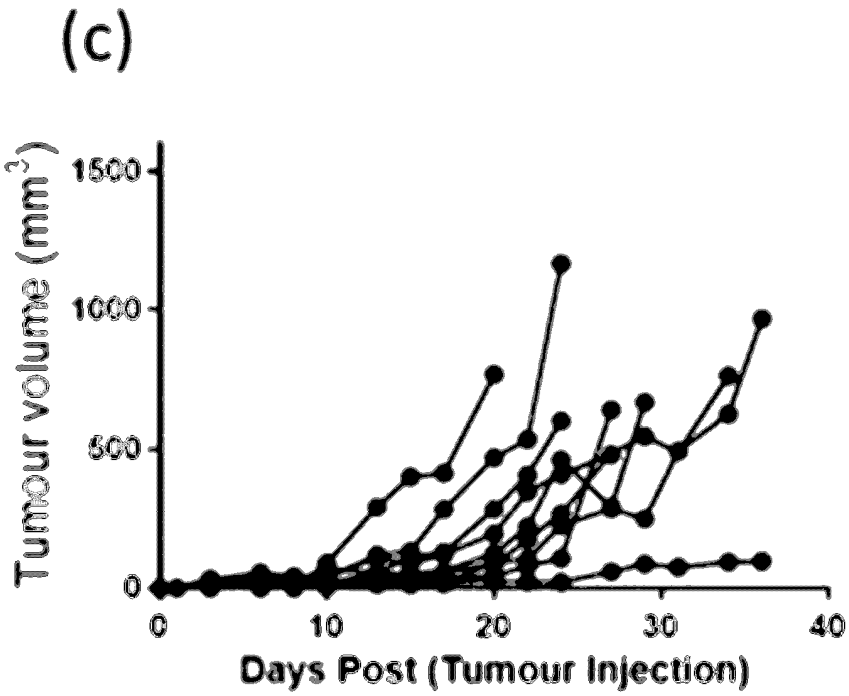


Figure 14 continued…

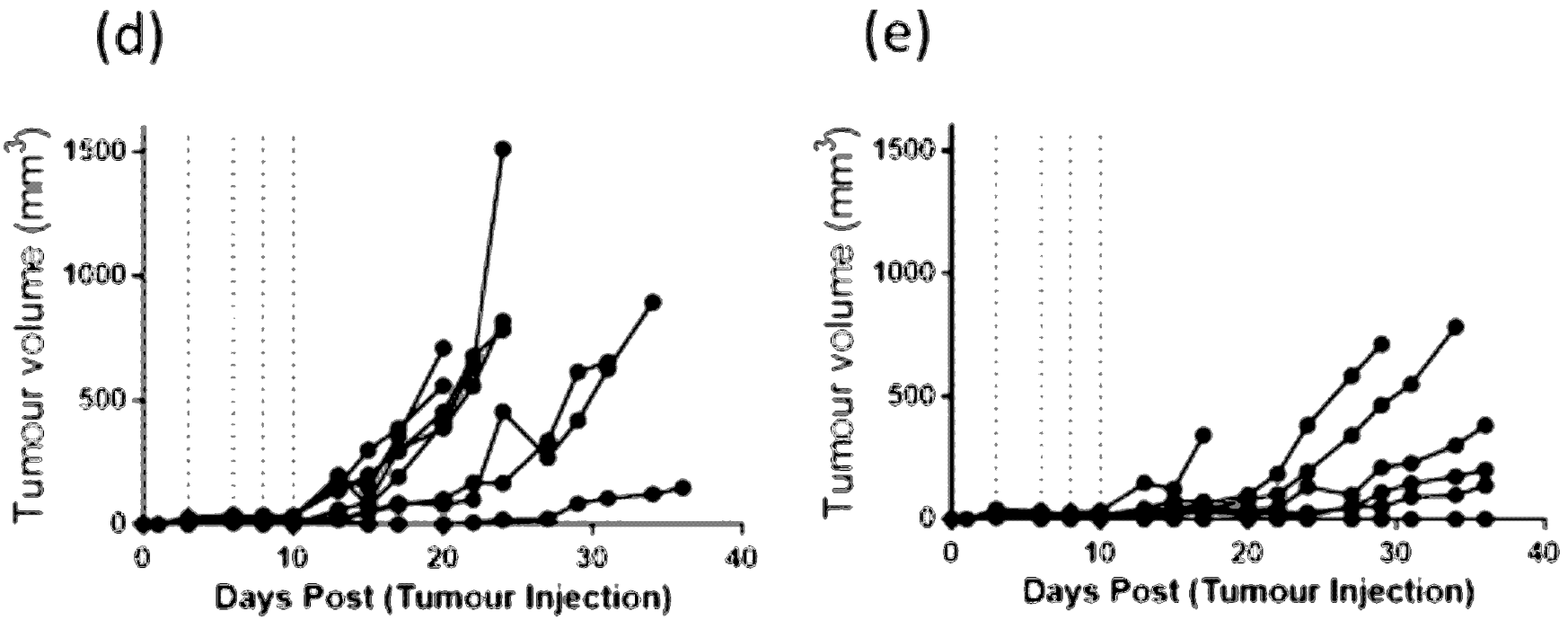


Figure 15

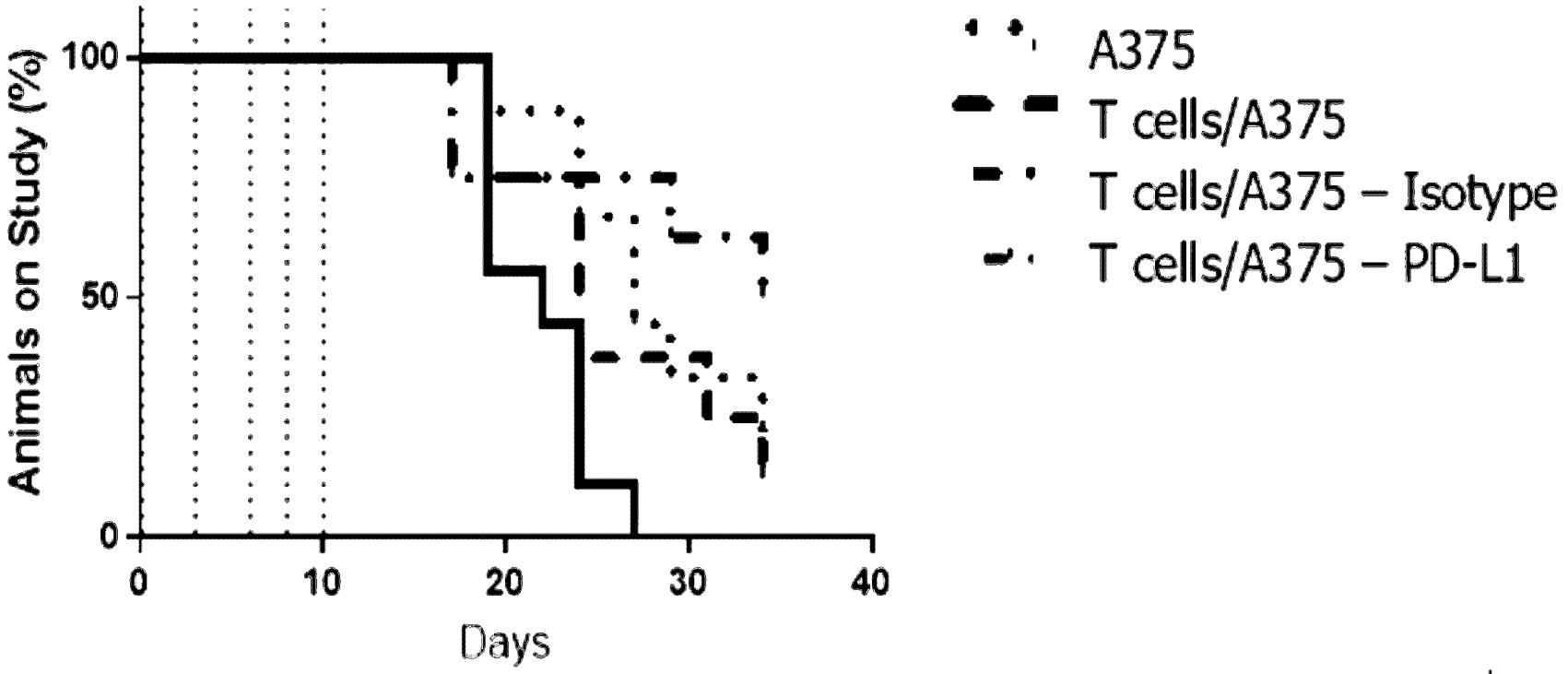

Animals on Study (%)
100
50
0
0
10
20
30
40
Days
A375
T cells/A375
T cells/A375 – Isotype
T cells/A375 – PD-L1

Figure 16

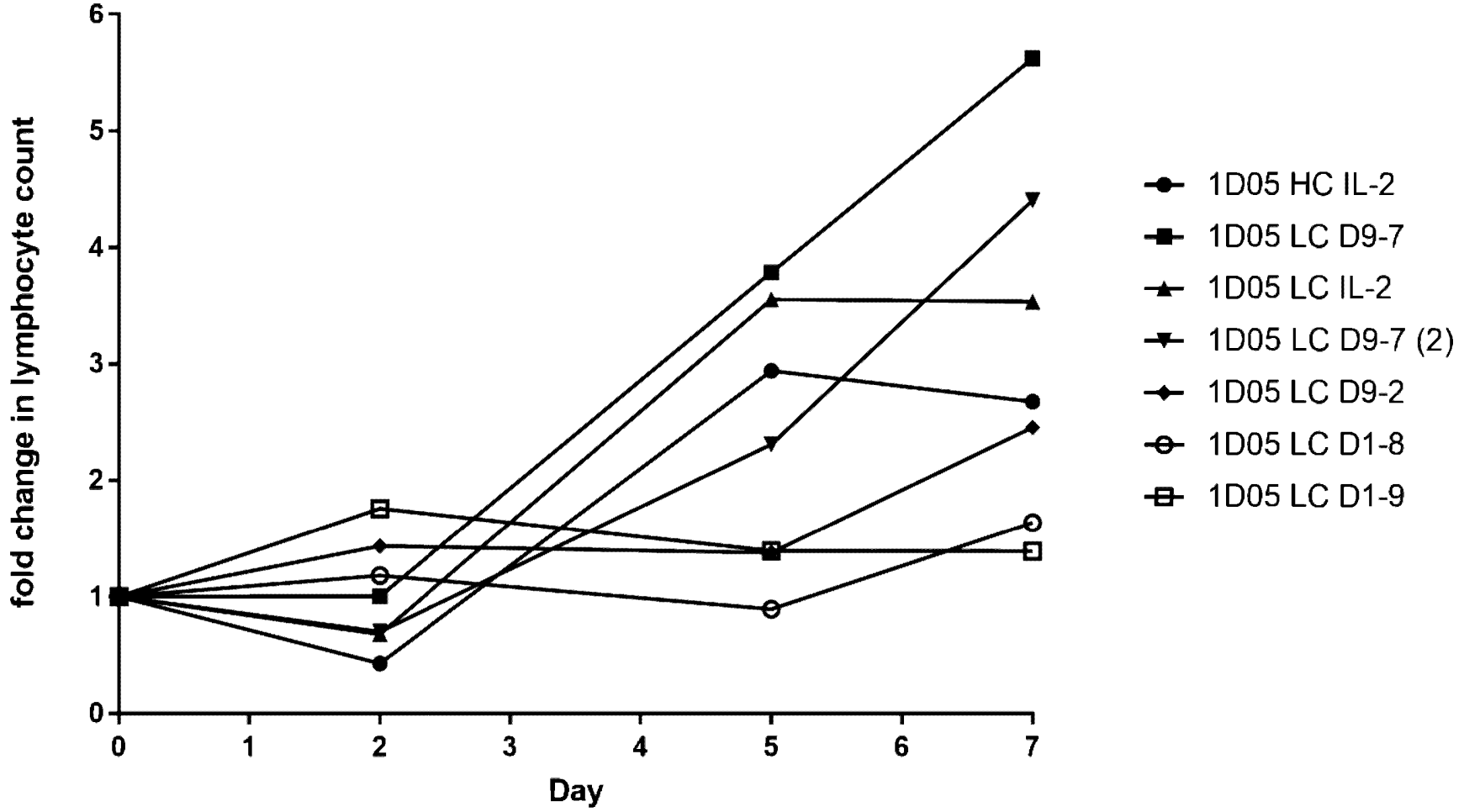

fold change in lymphocyte count
0
1
2
3
4
5
6
0
1
2
3
4
5
6
7
Day
1D05 HC IL-2
1D05 LC D9-7
1D05 LC IL-2
1D05 LC D9-7 (2)
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D1-9

Figure 17

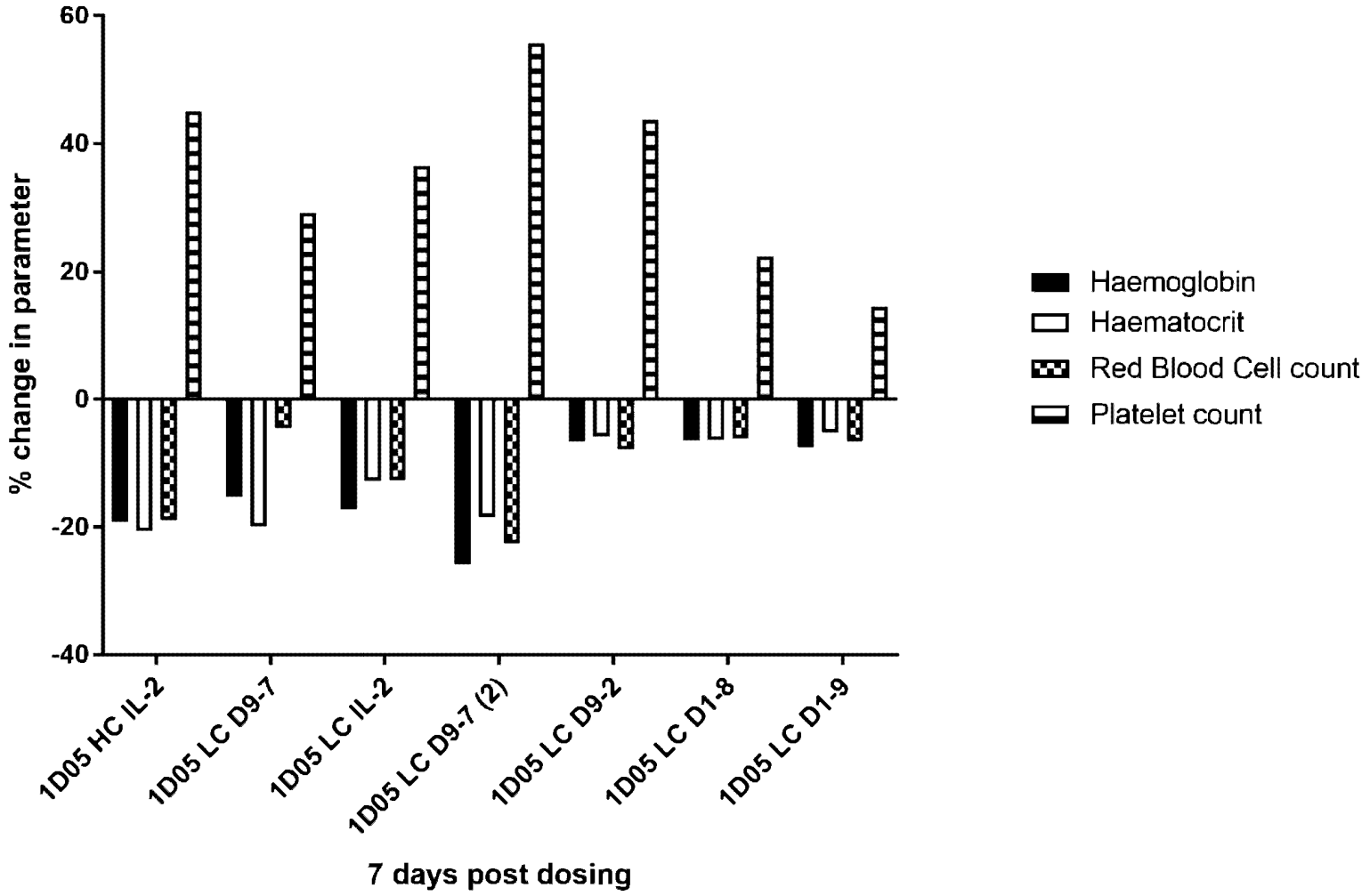

60
40
20
0
-20
-40
% change in parameter
1D05 HC IL-2
1D05 LC D9-7
1D05 LC IL-2
1D05 LC D9-7 (2)
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D1-9
7 days post dosing
Haemoglobin
Haematocrit
Red Blood Cell count
Platelet count

Figure 18

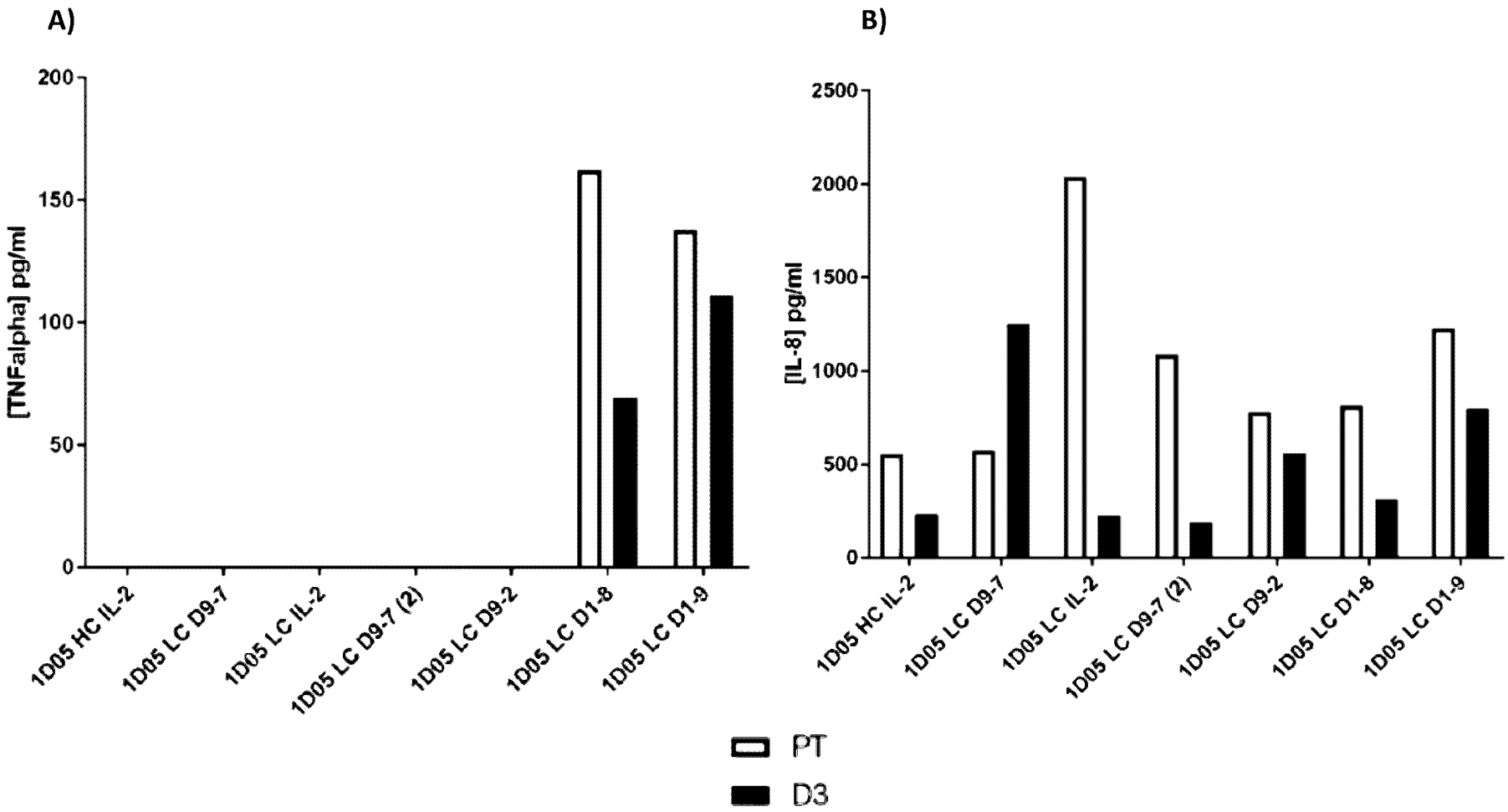

A)
[TNFalpha] pg/ml
200
150
100
50
0
1D05 HC IL-2
1D05 LC D9-7
1D05 LC IL-2
1D05 LC D9-7 (2)
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D1-9
B)
[IL-8] pg/ml
2500
2000
1500
1000
500
0
1D05 HC IL-2
1D05 LC D9-7
1D05 LC IL-2
1D05 LC D9-7 (2)
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D1-9
PT
D3

Figure 18 cont

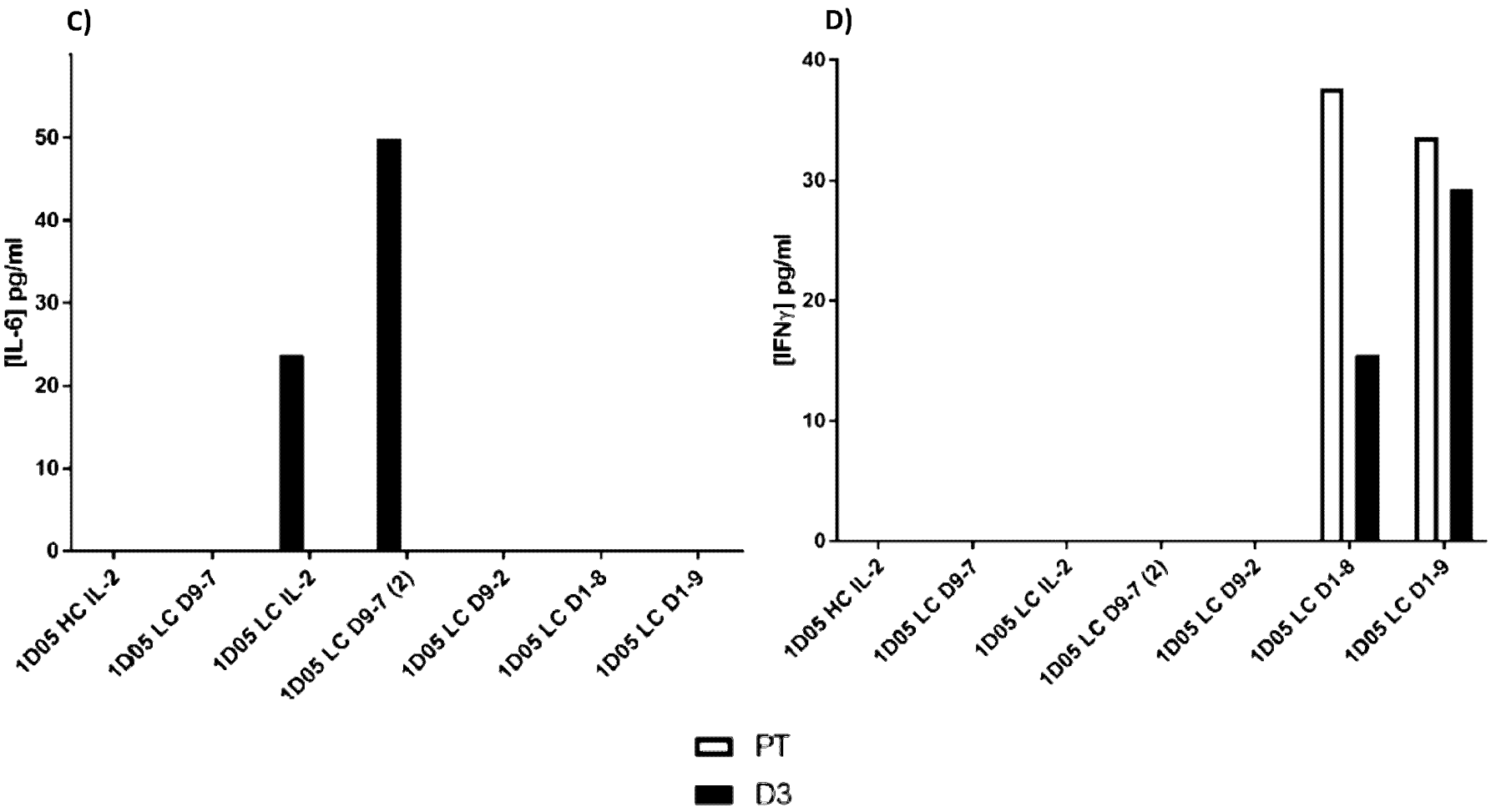


Figure 18 cont

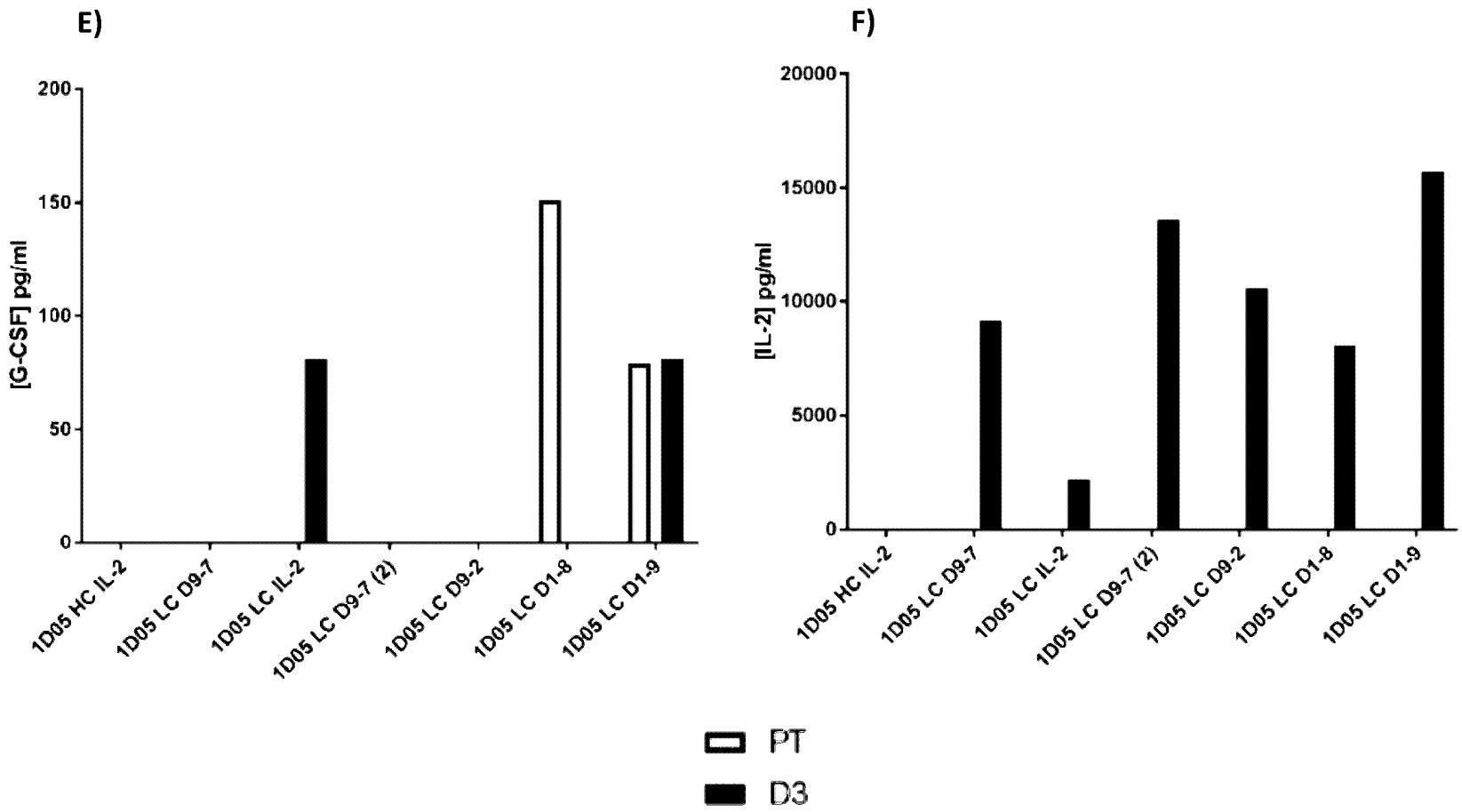


Figure 19

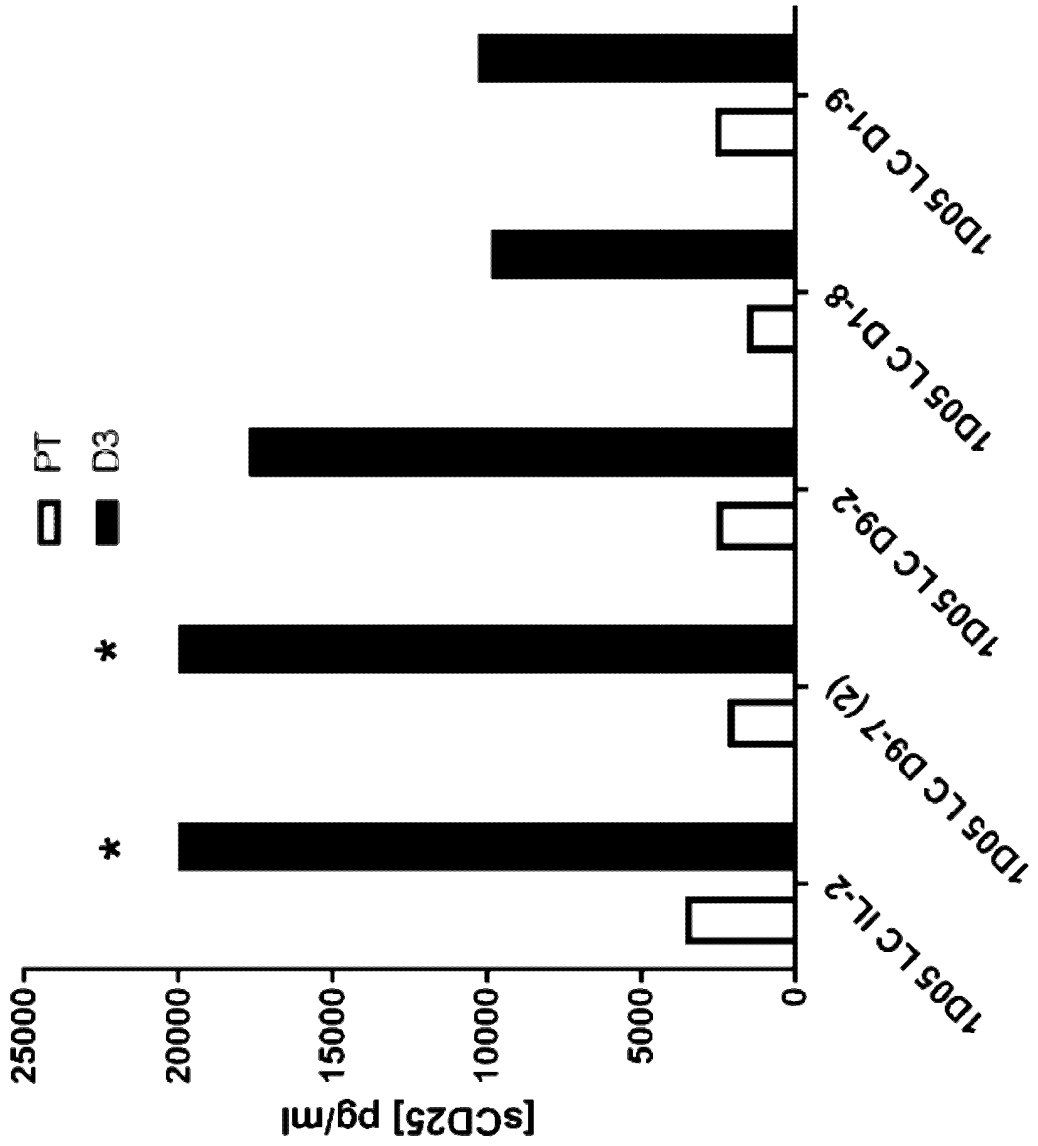

[sCD25] pg/ml
25000
20000
15000
10000
5000
0
PT
D3
*
*
1D05 LC IL-2
1D05 LC D9-7 (2)
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D1-9

Figure 20a

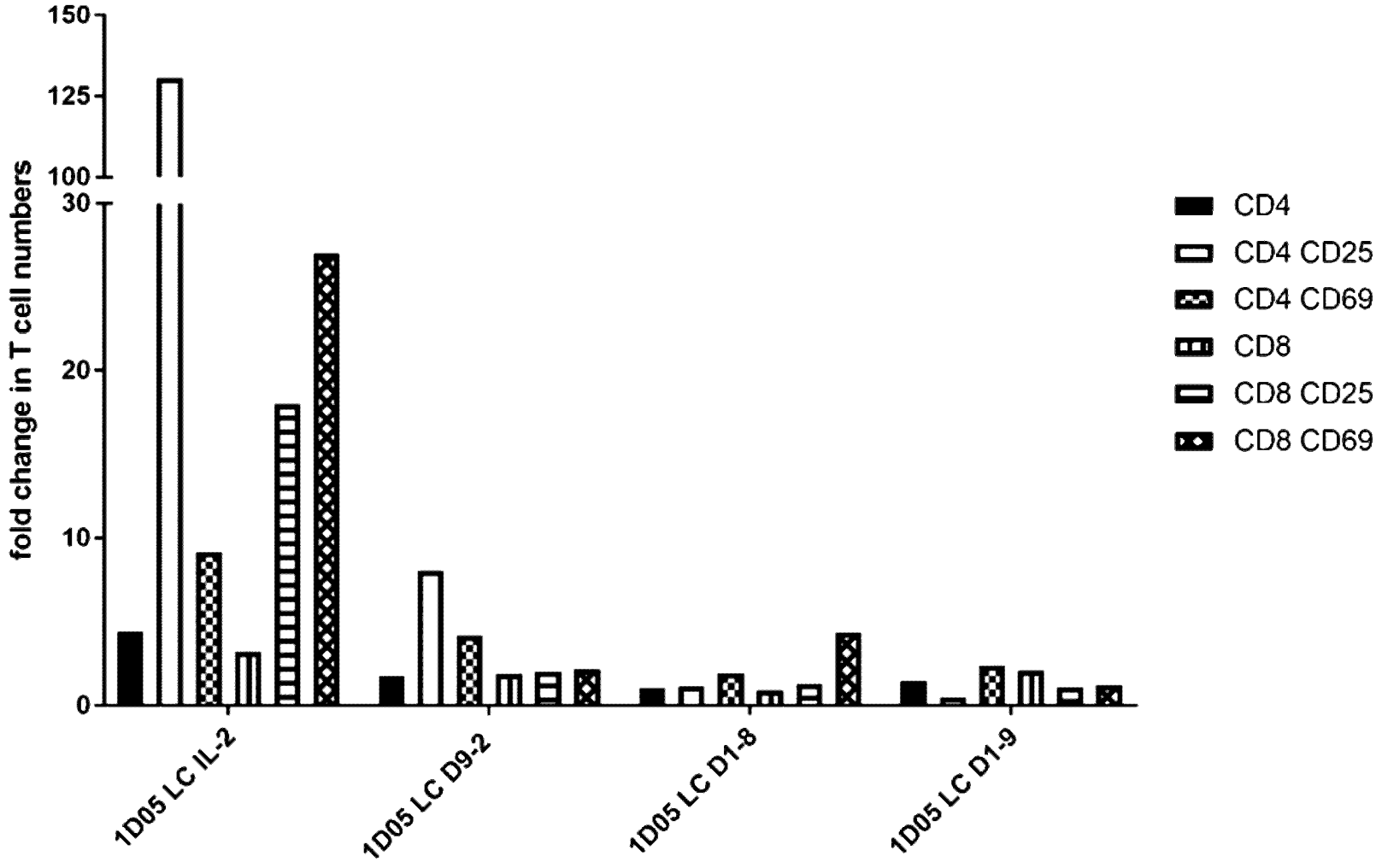

150
125
100
30
20
10
0
fold change in T cell numbers
1D05 LC IL-2
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D1-9
CD4
CD4 CD25
CD4 CD69
CD8
CD8 CD25
CD8 CD69

Figure 20b

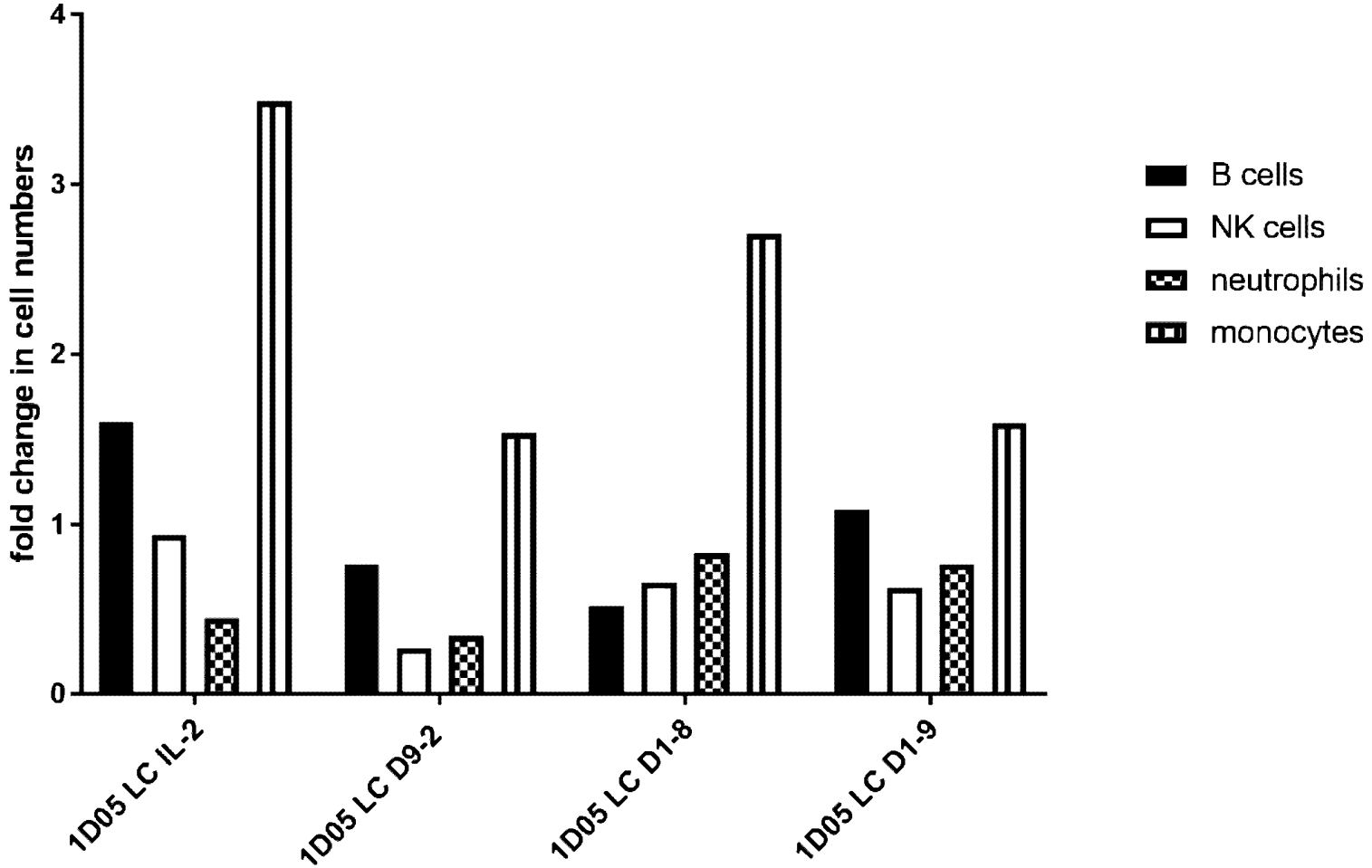

fold change in cell numbers
4
3
2
1
0
1D05 LC IL-2
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D1-9
B cells
NK cells
neutrophils
monocytes

Figure 21a

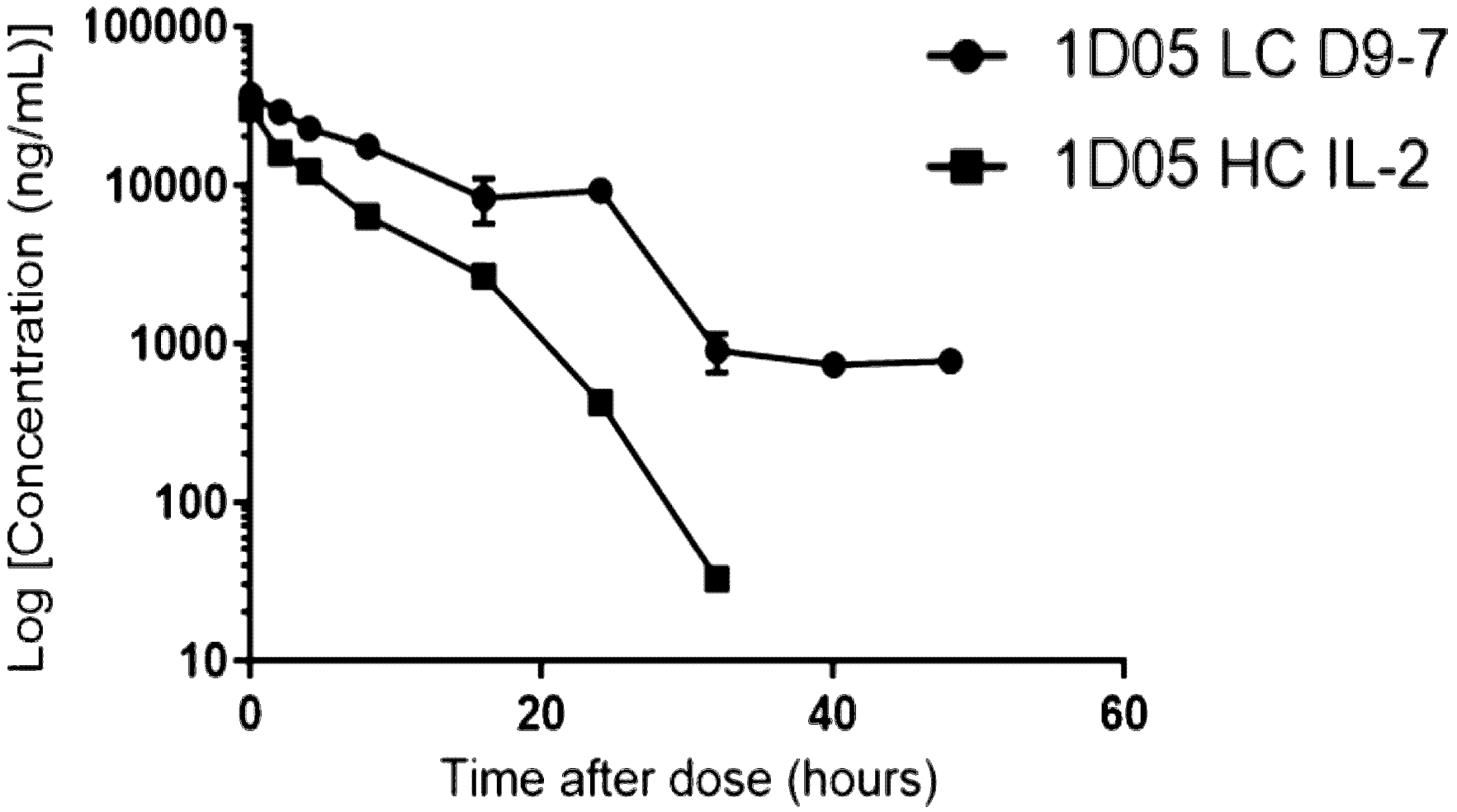
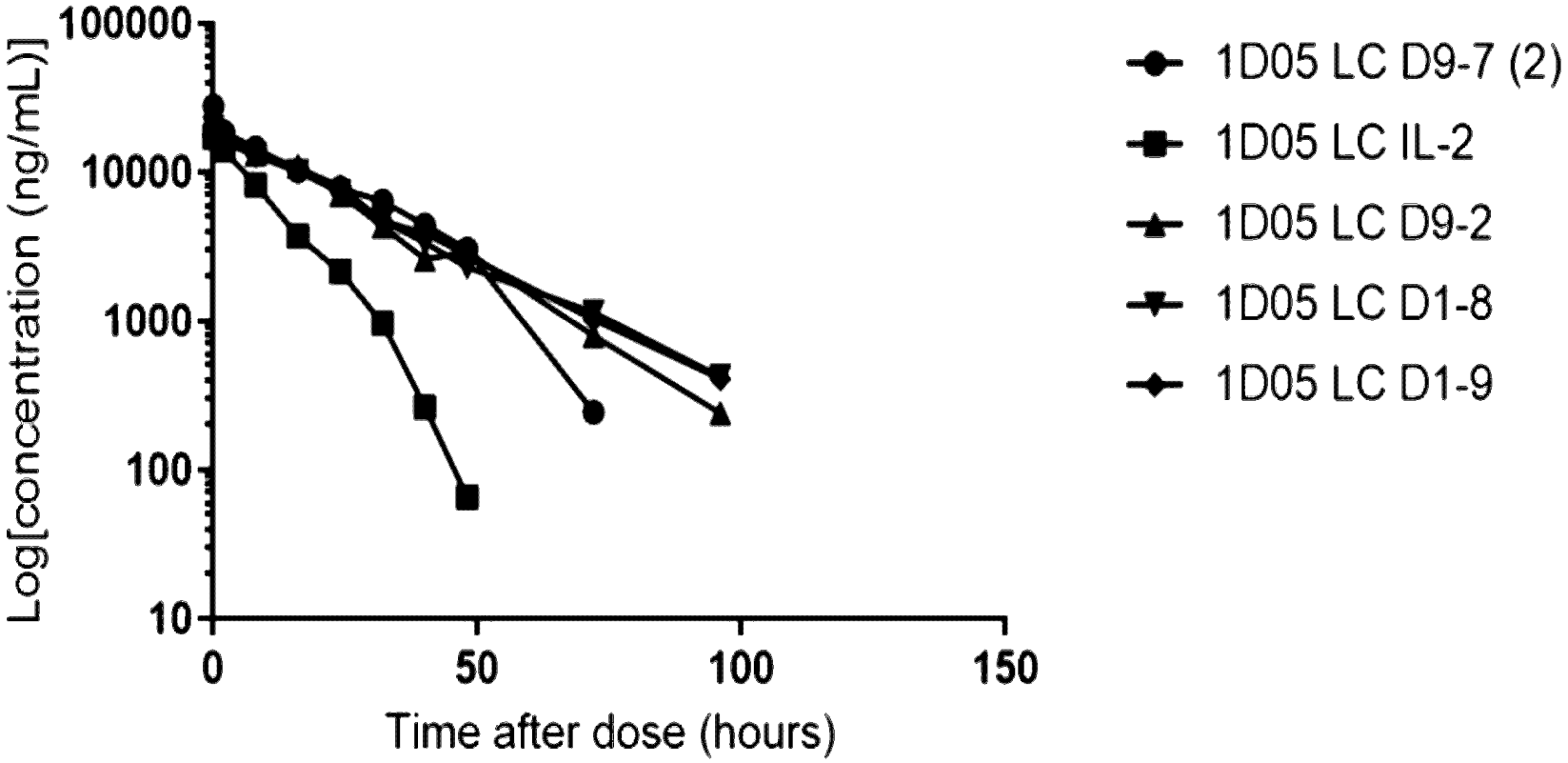

100000
10000
1000
100
10
Log [Concentration (ng/mL)]
0
20
40
60
Time after dose (hours)
1D05 LC D9-7
1D05 HC IL-2

Figure 21b


100000
10000
1000
100
10
Log[concentration (ng/mL)]
0
50
100
150
Time after dose (hours)
1D05 LC D9-7 (2)
1D05 LC IL-2
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D1-9

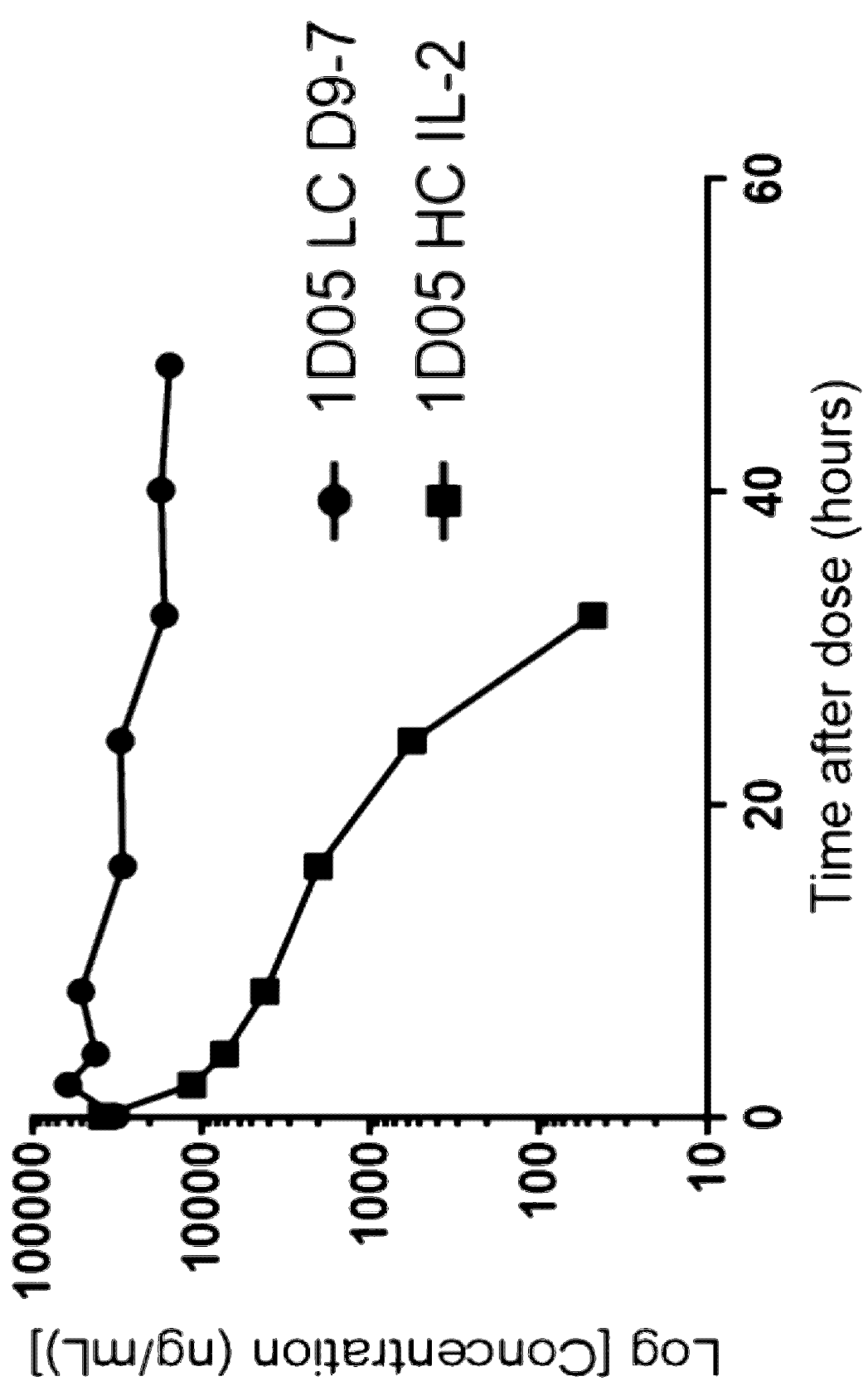

Log [Concentration (ng/mL)]
100000
10000
1000
100
10
0
20
40
60
Time after dose (hours)
1D05 LC D9-7
1D05 HC IL-2

Figure 21c

Figure 21d

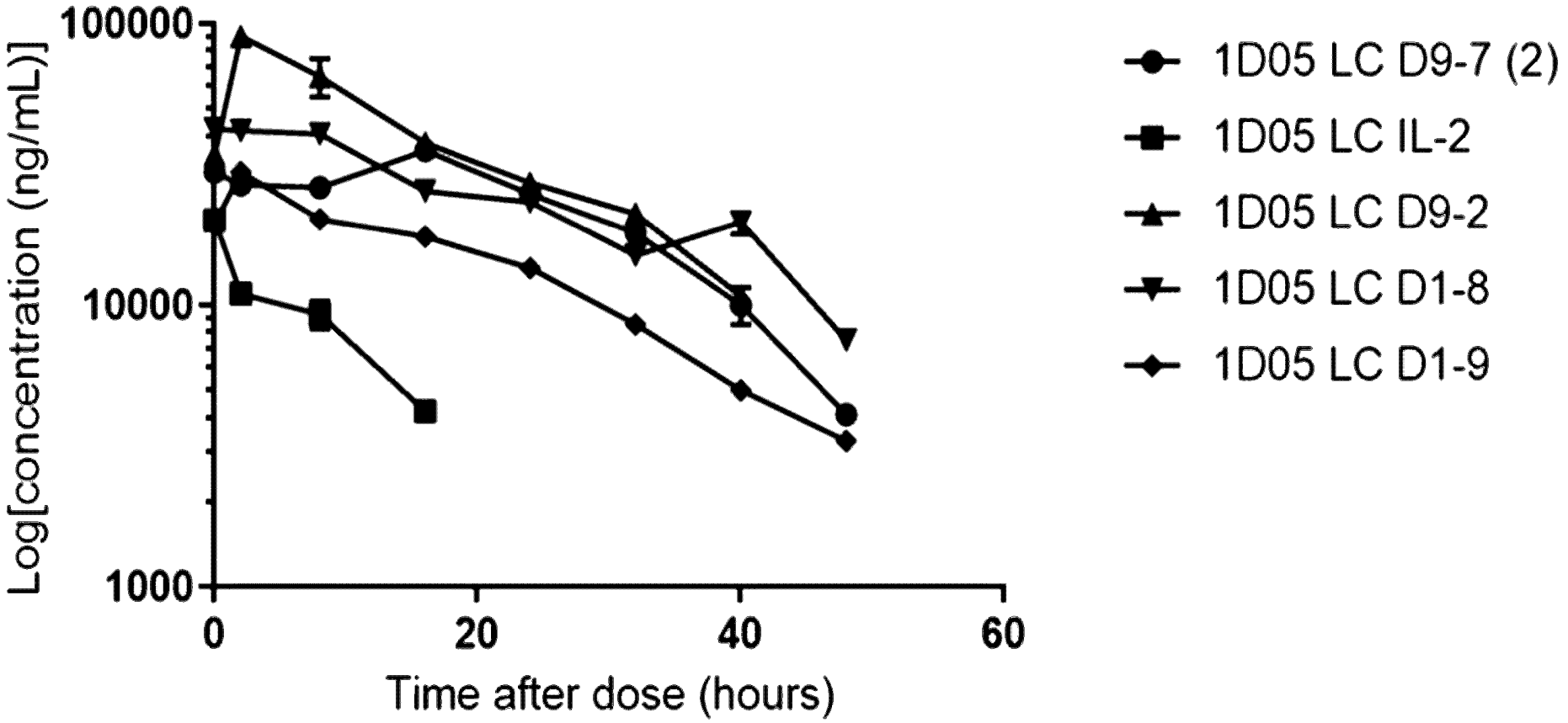
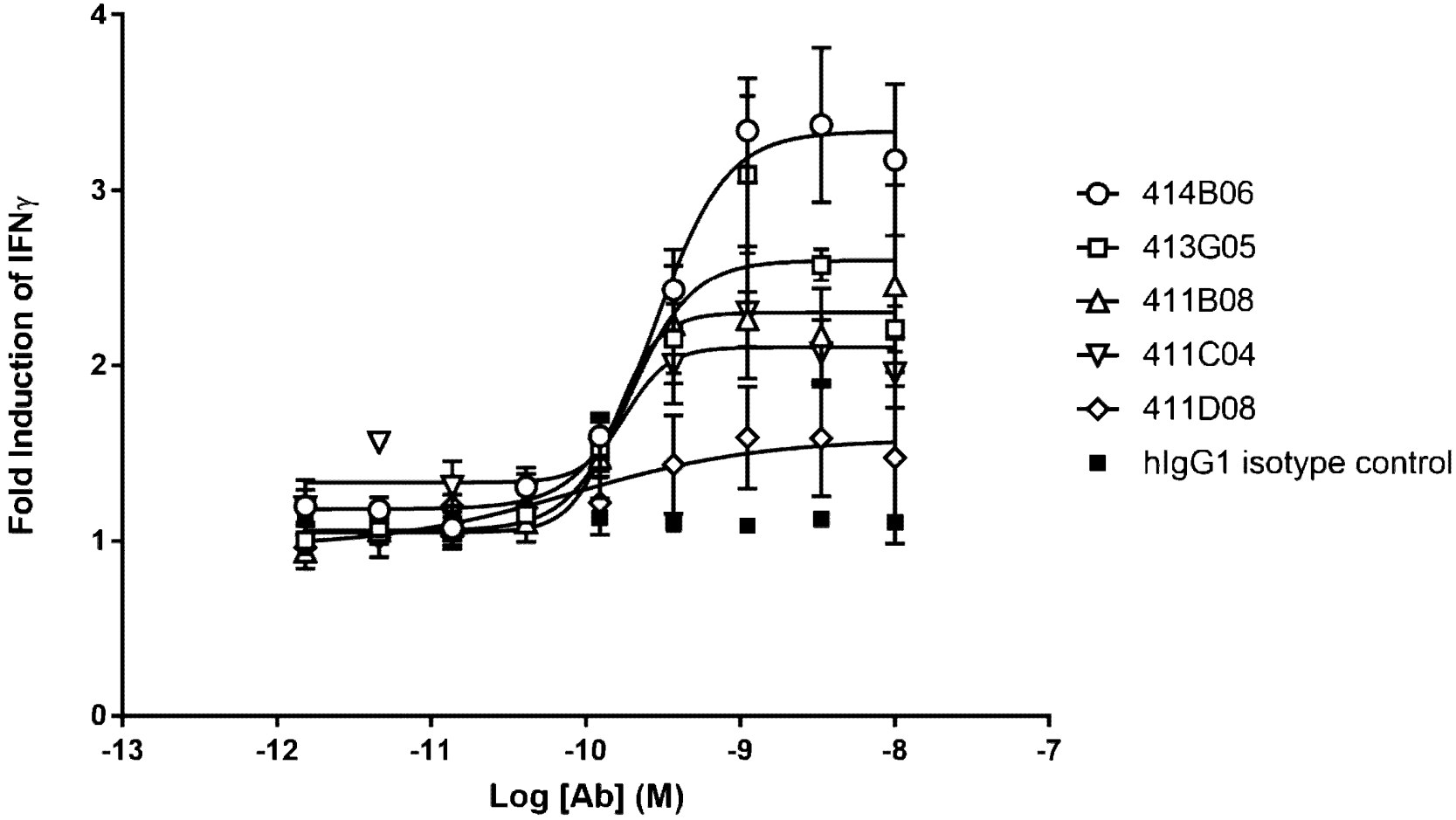

100000
10000
1000
Log[concentration (ng/mL)]
0
20
40
60
Time after dose (hours)
1D05 LC D9-7 (2)
1D05 LC IL-2
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D1-9

Figure 22a


Fold Induction of IFNγ
4
3
2
1
0
-13
-12
-11
-10
-9
-8
-7
Log [Ab] (M)
414B06
413G05
411B08
411C04
411D08
hIgG1 isotype control

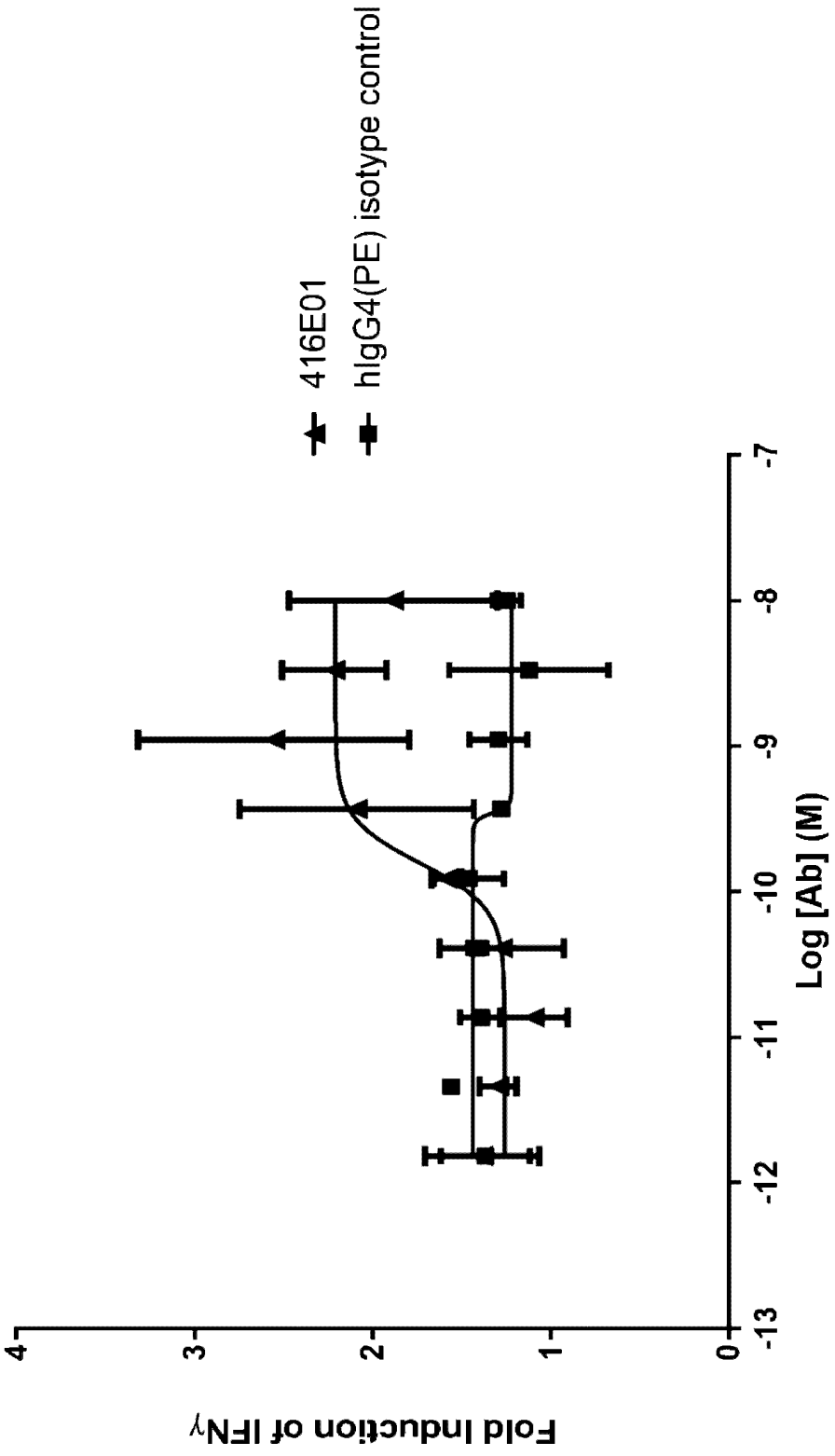

416E01
hIgG4(PE) isotype control
Fold Induction of IFNγ
Log [Ab] (M)
0
1
2
3
4
-13
-12
-11
-10
-9
-8
-7

Figure 22b

Figure 23(a)

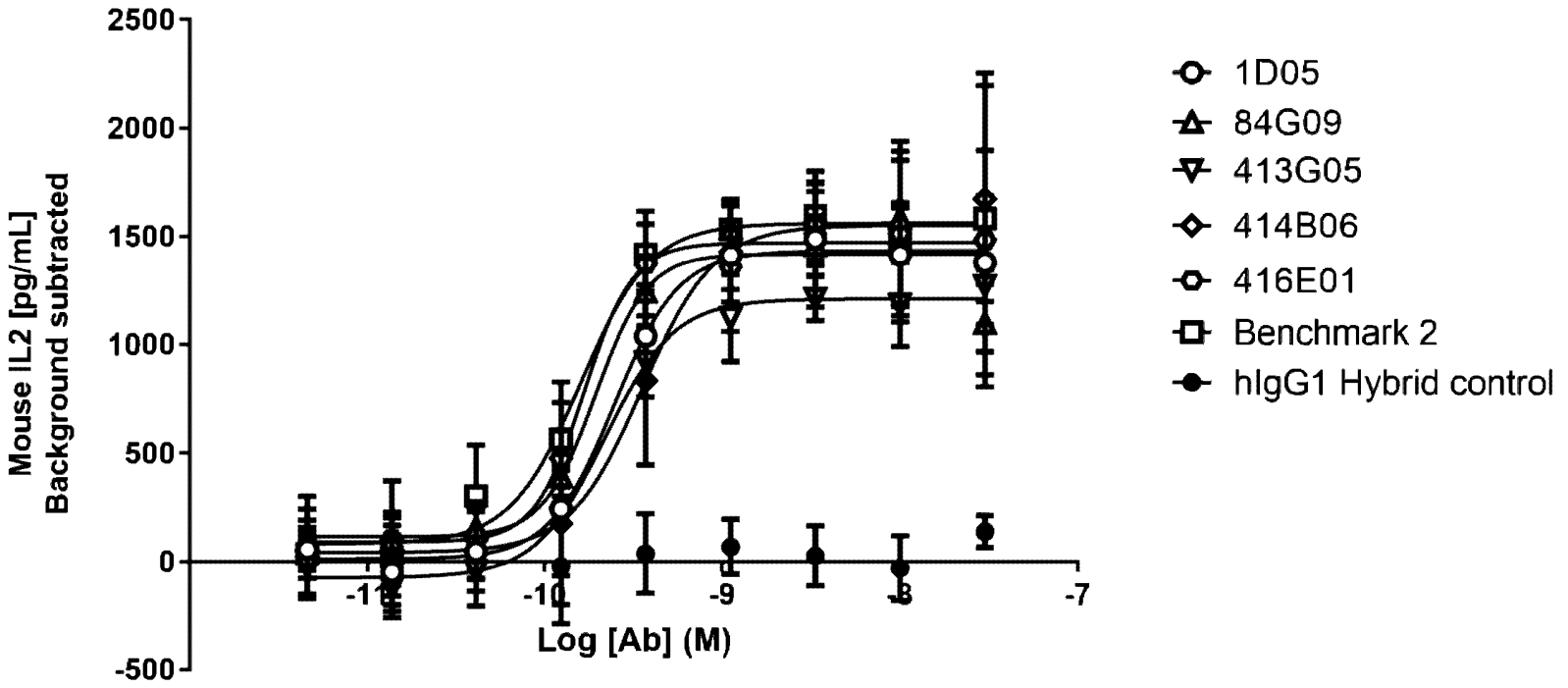

Mouse IL2 [pg/mL]
Background subtracted
2500
2000
1500
1000
500
0
-500
-11
-10
-9
-8
-7
Log [Ab] (M)
1D05
84G09
413G05
414B06
416E01
Benchmark 2
hIgG1 Hybrid control

Figure 23(b)

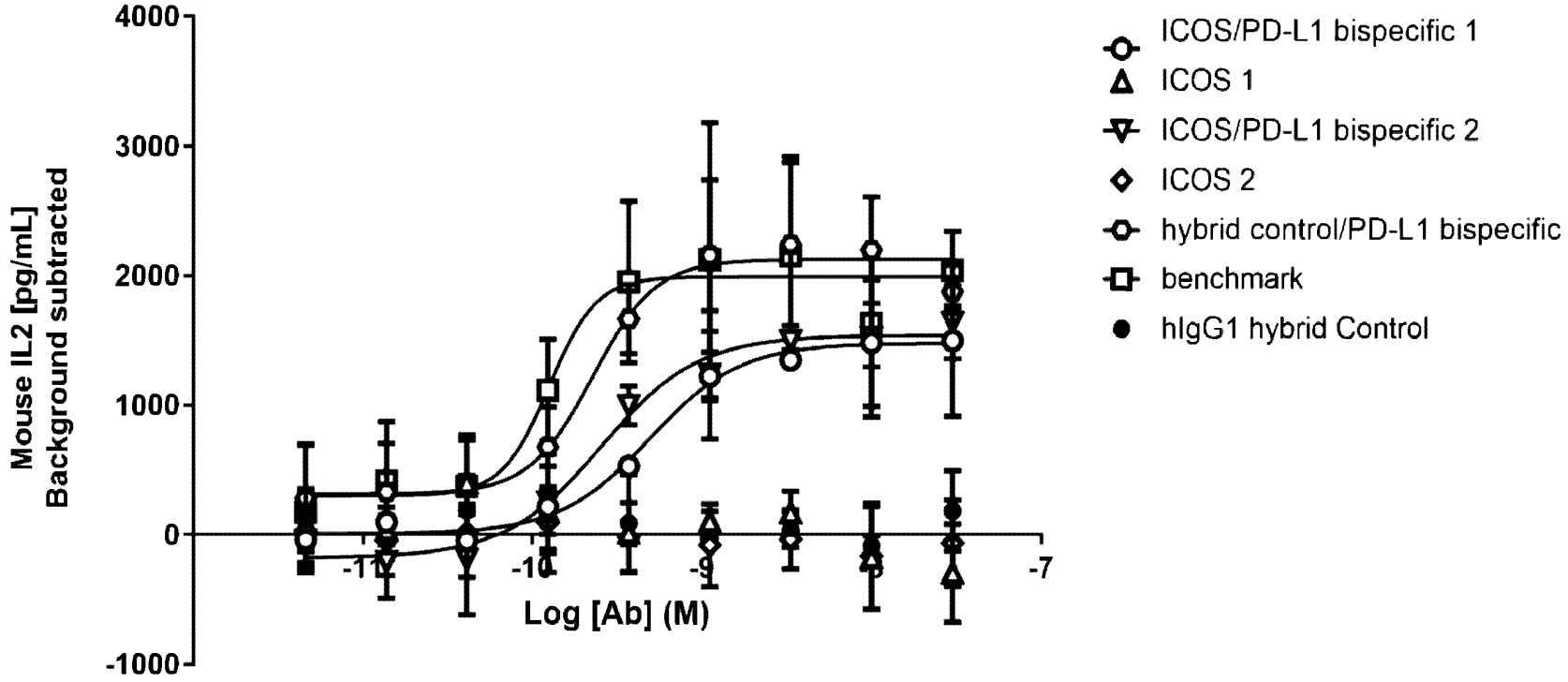

4000
3000
2000
1000
0
-1000
Mouse IL2 [pg/mL]
Background subtracted
-11
-10
-9
-7
Log [Ab] (M)
ICOS/PD-L1 bispecific 1
ICOS 1
ICOS/PD-L1 bispecific 2
ICOS 2
hybrid control/PD-L1 bispecific
benchmark
hIgG1 hybrid Control

Figure 24(a)

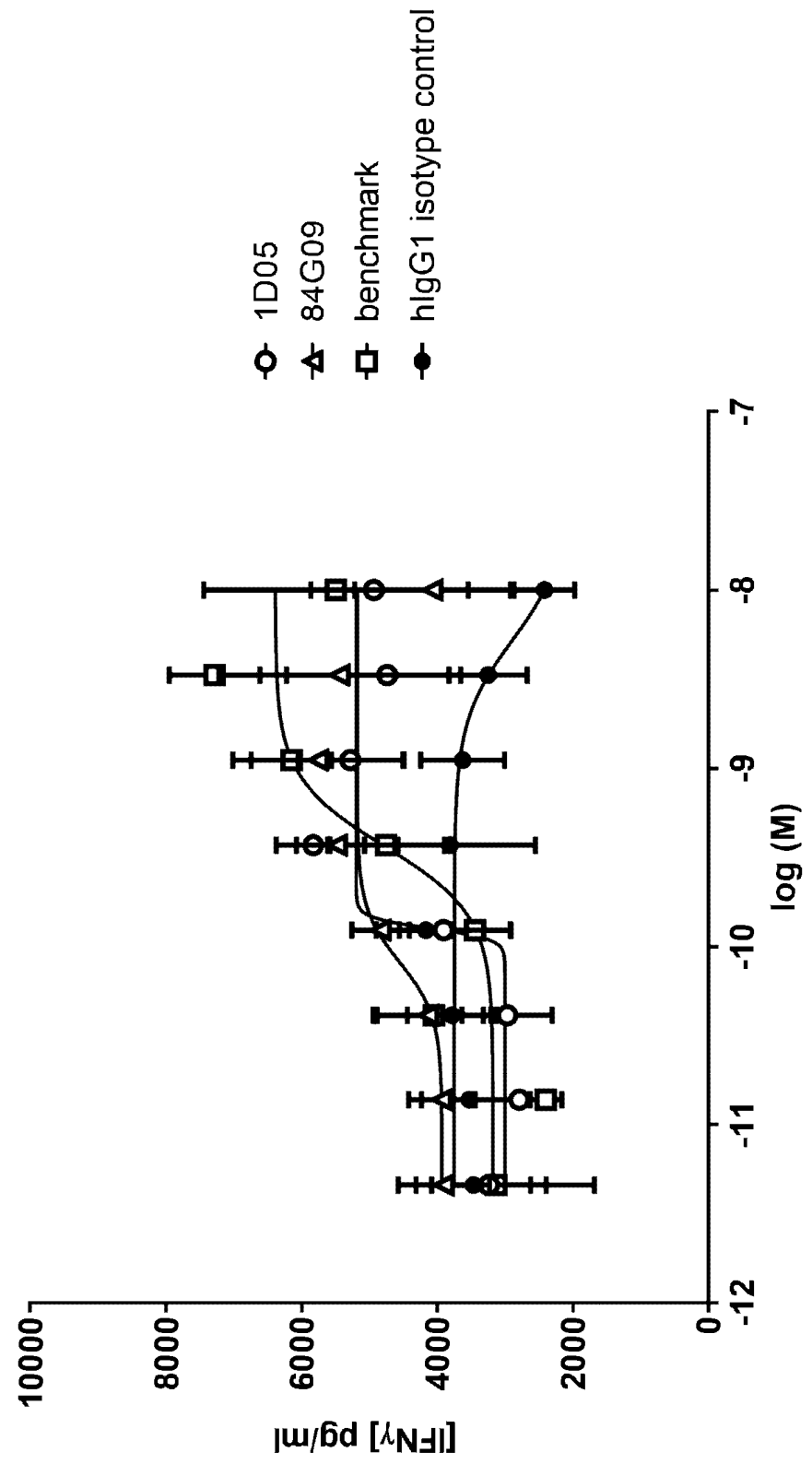

1D05
84G09
benchmark
hIgG1 isotype control
[IFNγ] pg/ml
10000
8000
6000
4000
2000
0
-12
-11
-10
-9
-8
-7
log (M)

Figure 24(b)

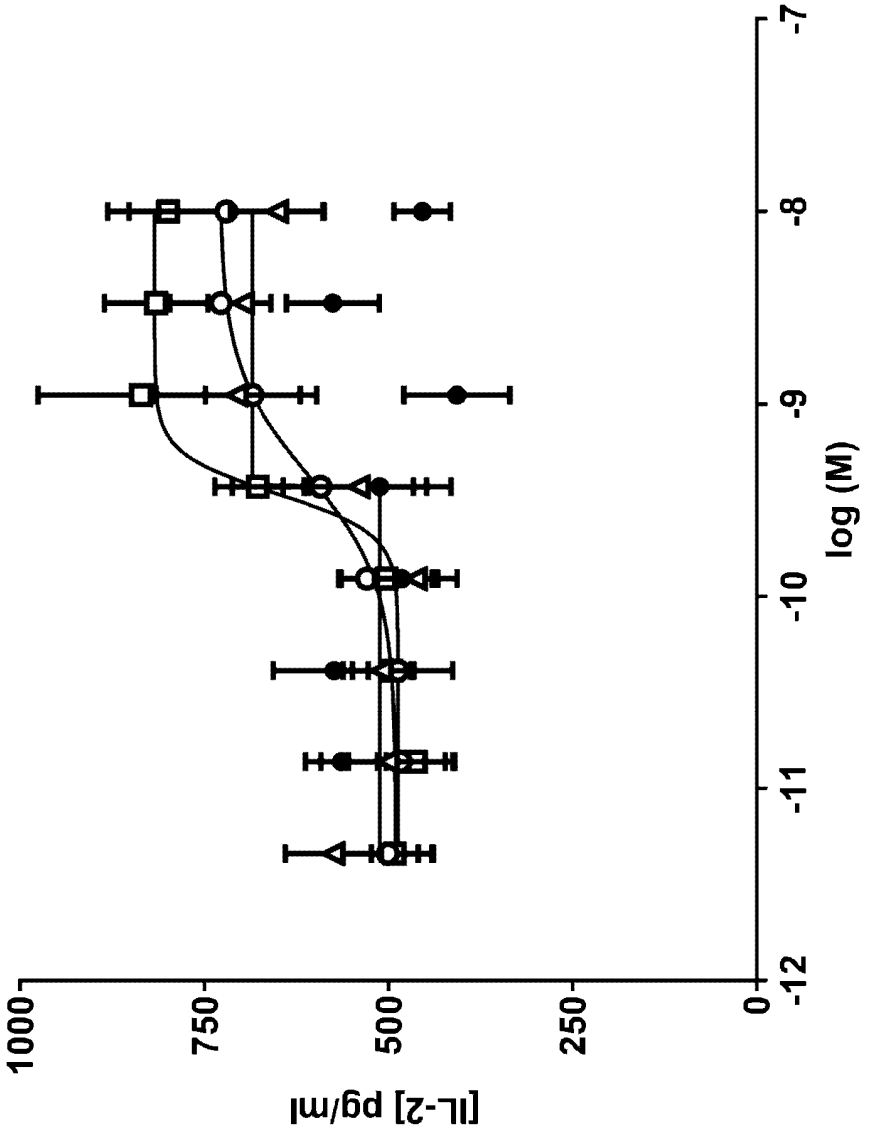

1D05
84G09
benchmark
hIgG1 isotype control
[IL-2] pg/ml
1000
750
500
250
0
-12
-11
-10
-9
-8
-7
log (M)

Figure 25(a)

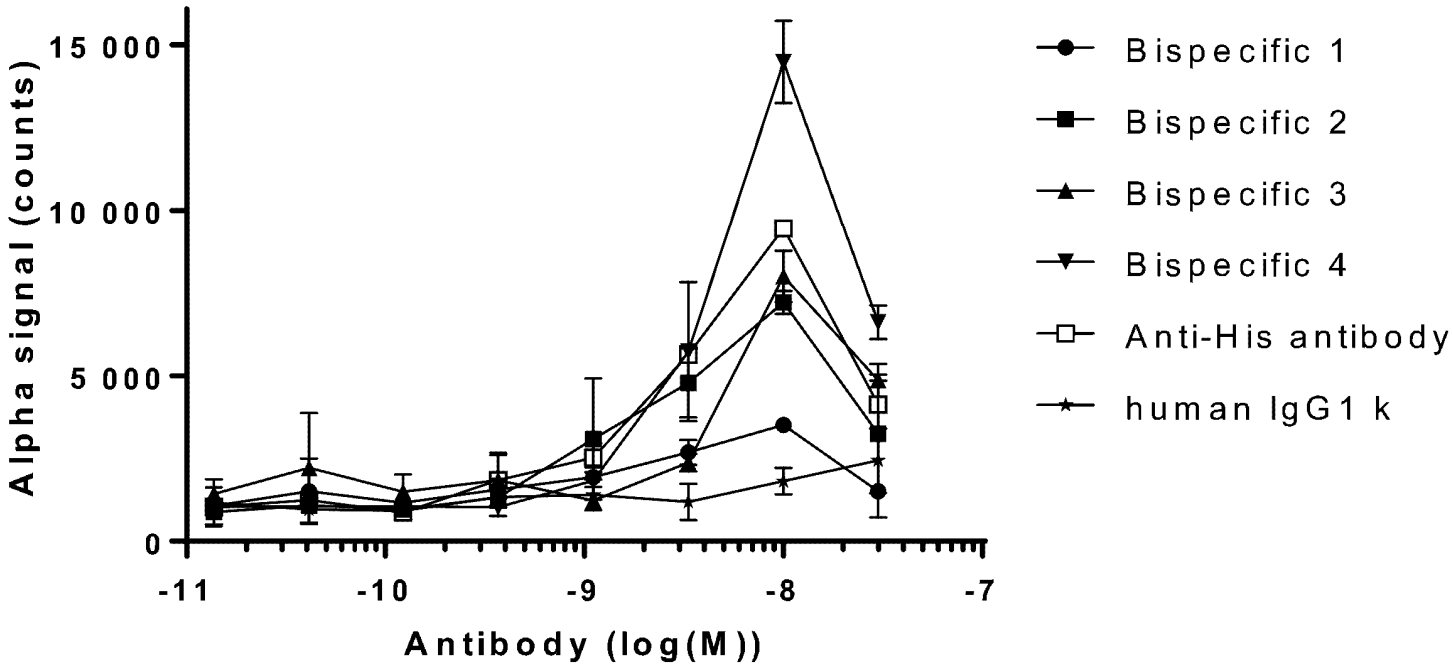

Alpha signal (counts)
15 000
10 000
5 000
0
-11
-10
-9
-8
-7
Antibody (log(M))
Bispecific 1
Bispecific 2
Bispecific 3
Bispecific 4
Anti-His antibody
human IgG1 k

Figure 25(b)

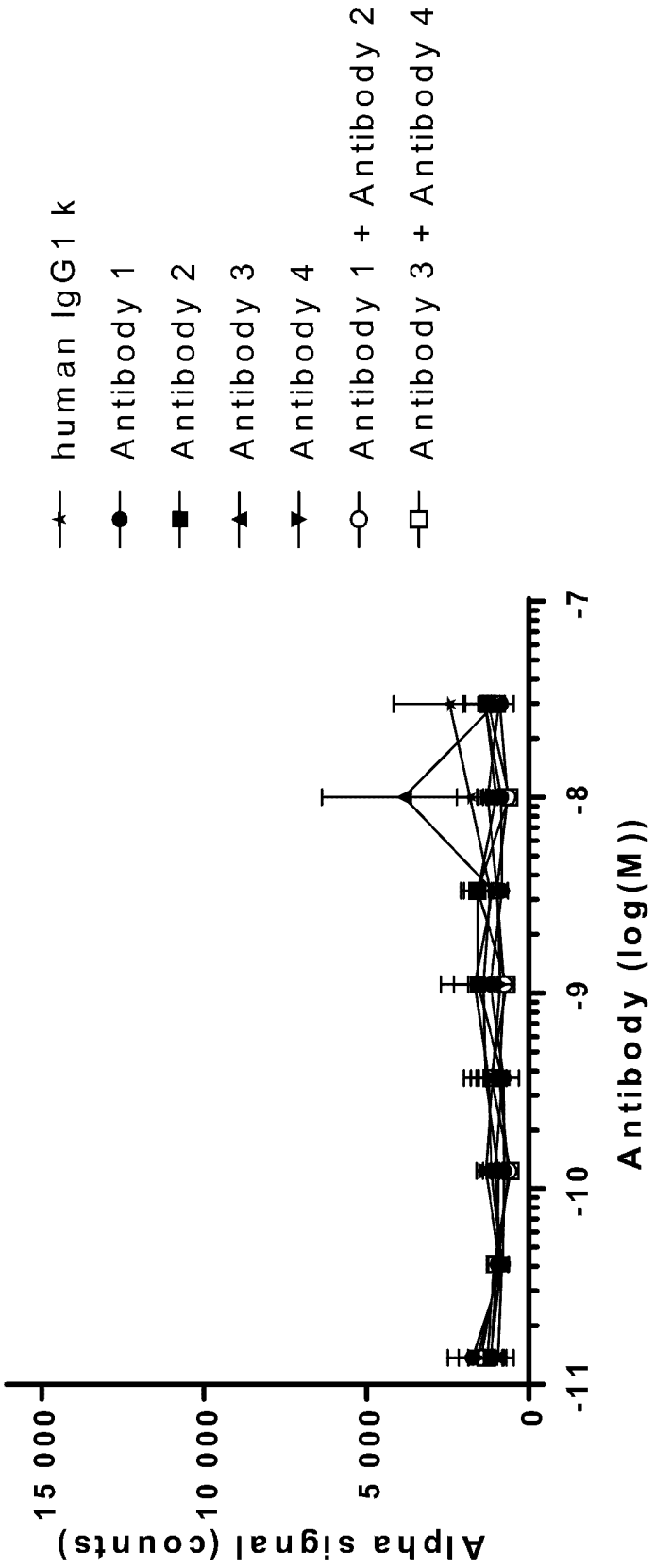

human IgG1 k
Antibody 1
Antibody 2
Antibody 3
Antibody 4
Antibody 1 + Antibody 2
Antibody 3 + Antibody 4
Alpha signal (counts)
15 000
10 000
5 000
0
-11
-10
-9
-8
-7
Antibody (log(M))

Figure 26(a)

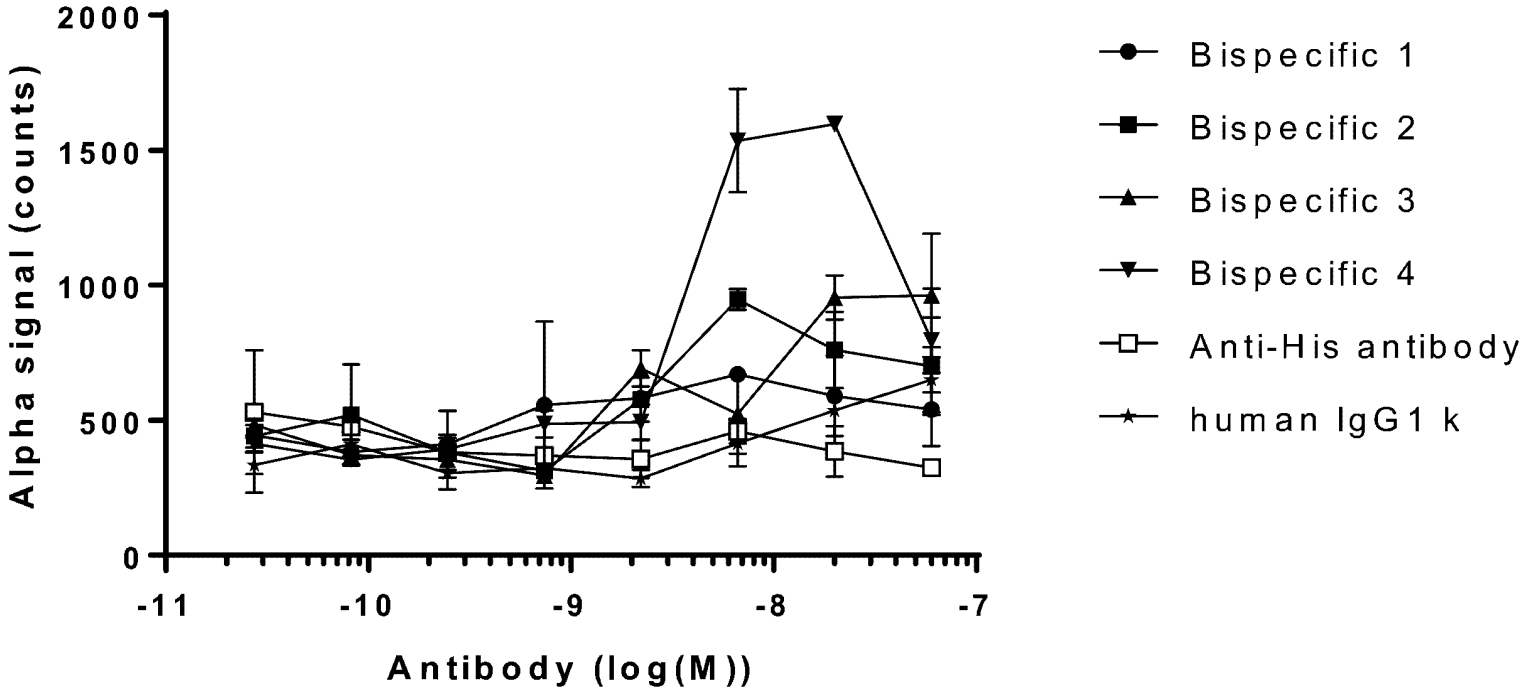

2000
1500
1000
500
0
Alpha signal (counts)
-11
-10
-9
-8
-7
Antibody (log(M))
Bispecific 1
Bispecific 2
Bispecific 3
Bispecific 4
Anti-His antibody
human IgG1 k

Figure 26(b)

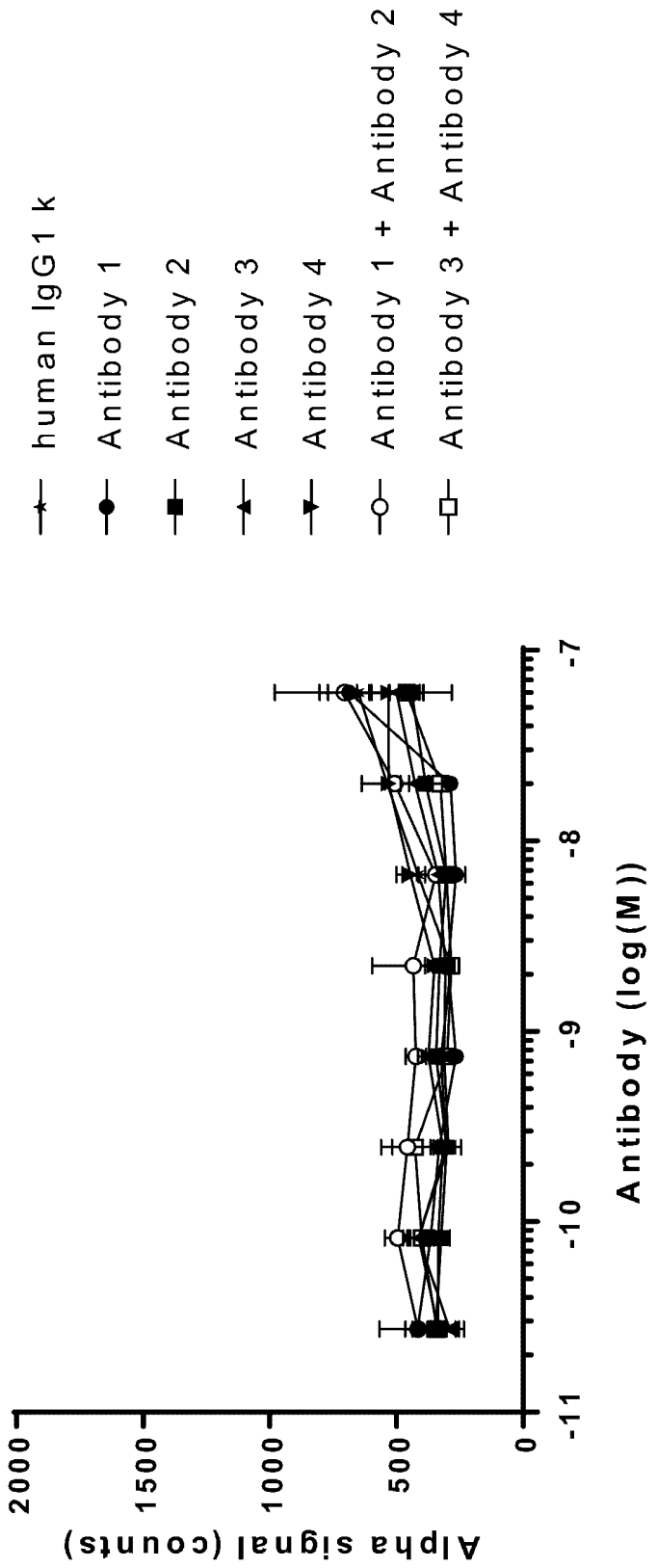

human IgG1 k
Antibody 1
Antibody 2
Antibody 3
Antibody 4
Antibody 1 + Antibody 2
Antibody 3 + Antibody 4
Alpha signal (counts)
2000
1500
1000
500
0
-11
-10
-9
-8
-7
Antibody (log(M))

Figure 27

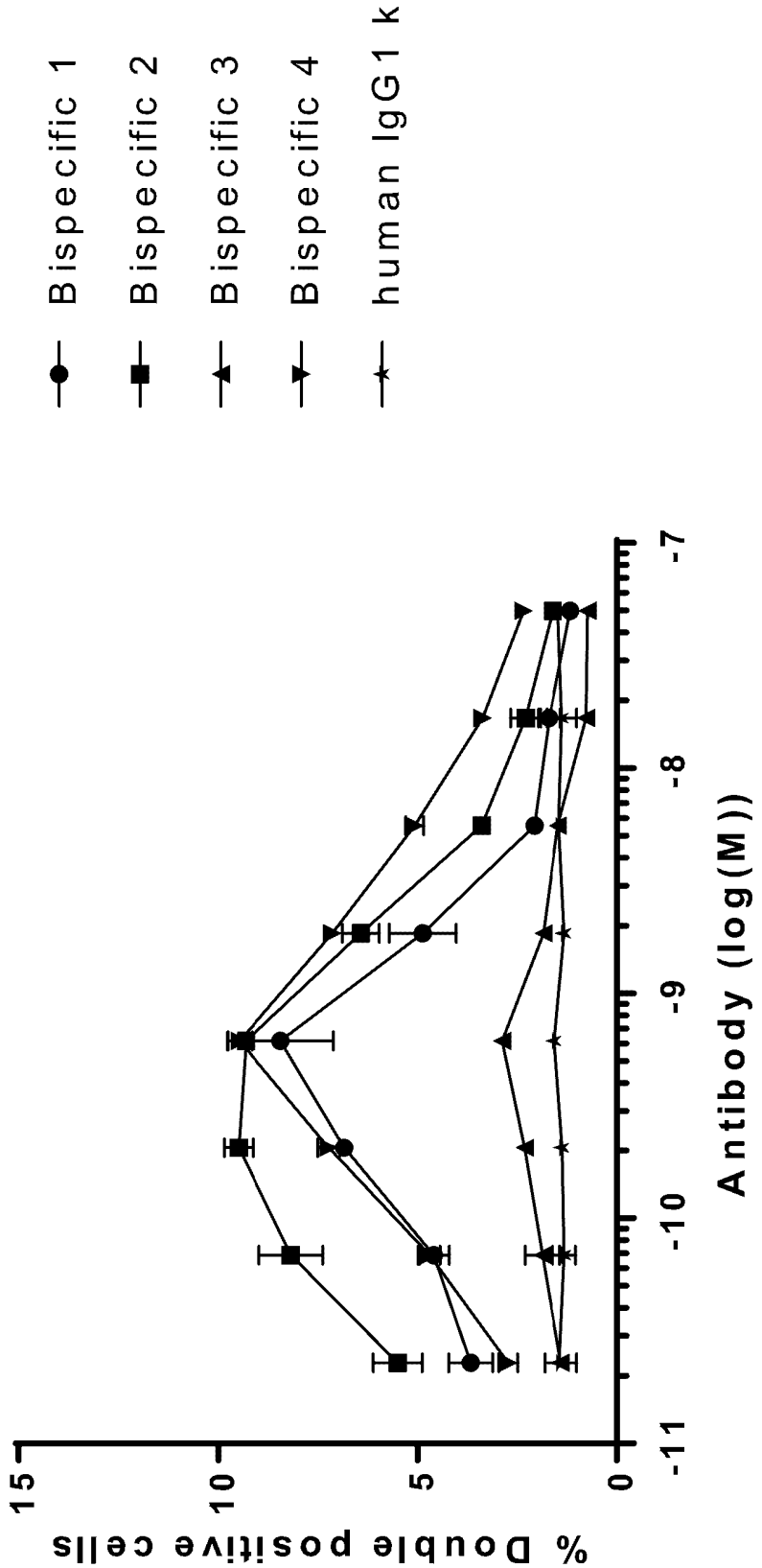

Bispecific 1
Bispecific 2
Bispecific 3
Bispecific 4
human IgG1 k
% Double positive cells
15
10
5
0
-11
-10
-9
-8
-7
Antibody (log(M))

Figure 28

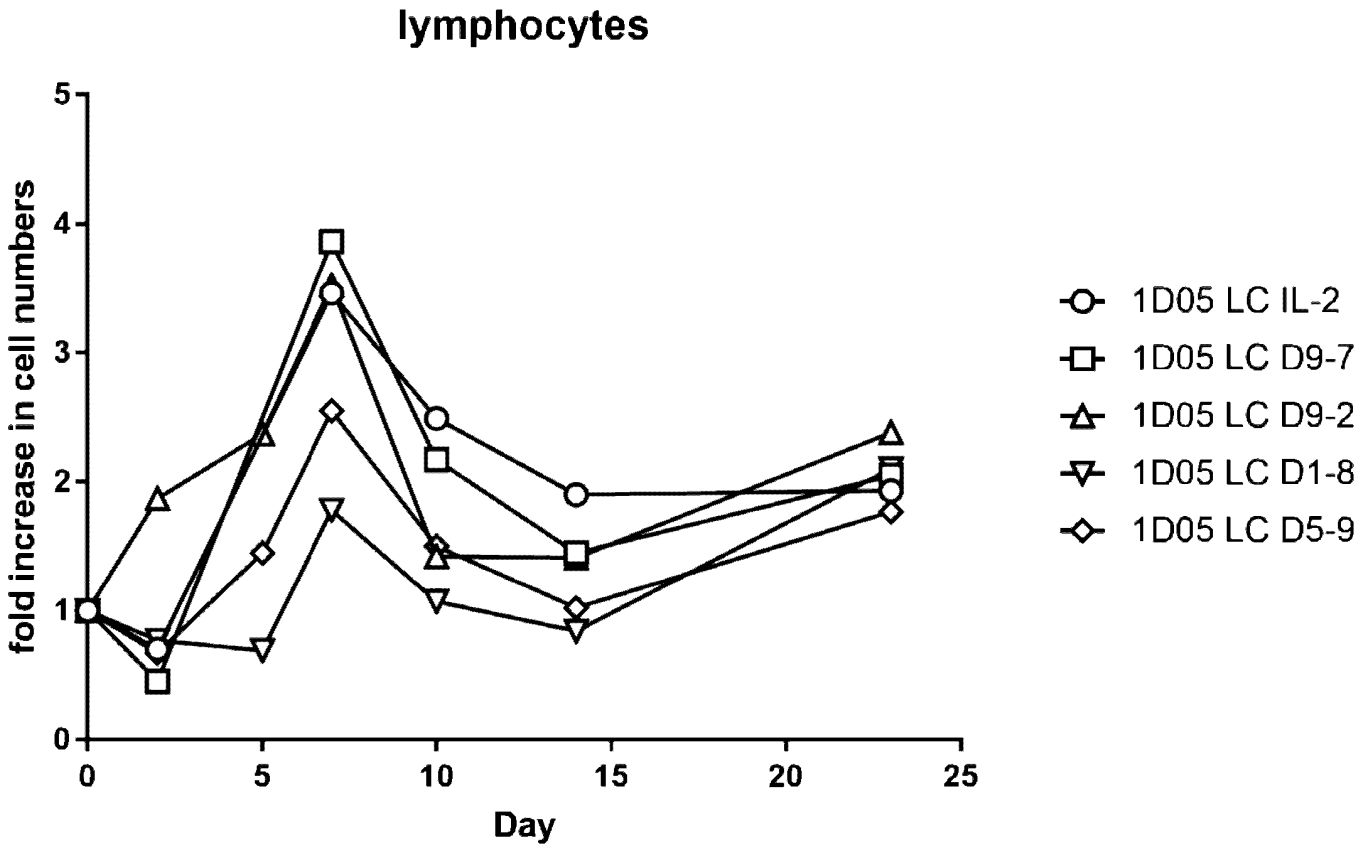

lymphocytes
fold increase in cell numbers
Day
0
1
2
3
4
5
0
5
10
15
20
25
1D05 LC IL-2
1D05 LC D9-7
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D5-9

Figure 29

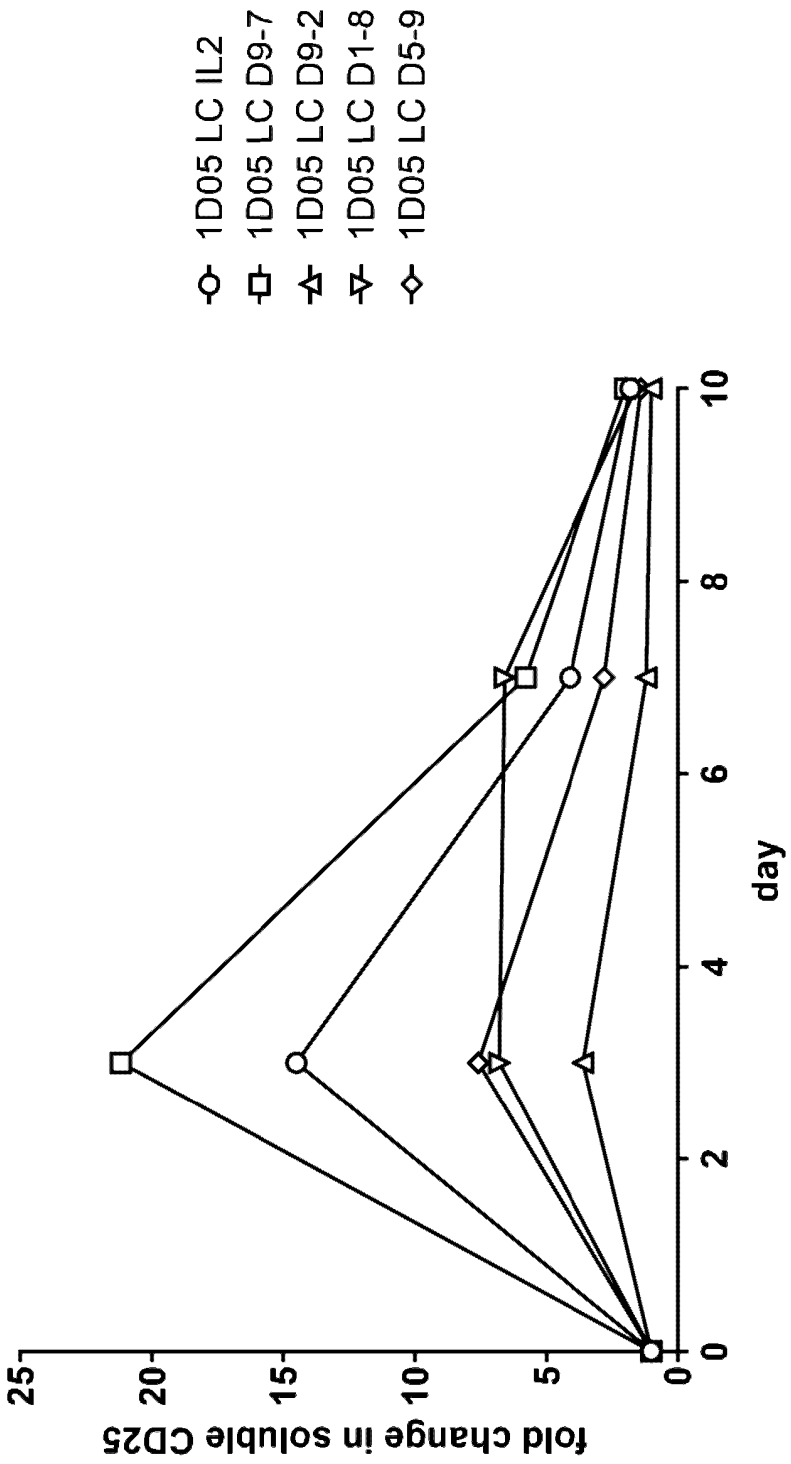

1D05 LC IL2
1D05 LC D9-7
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D5-9
fold change in soluble CD25
0
5
10
15
20
25
0
2
4
6
8
10
day

Figure 30(a)

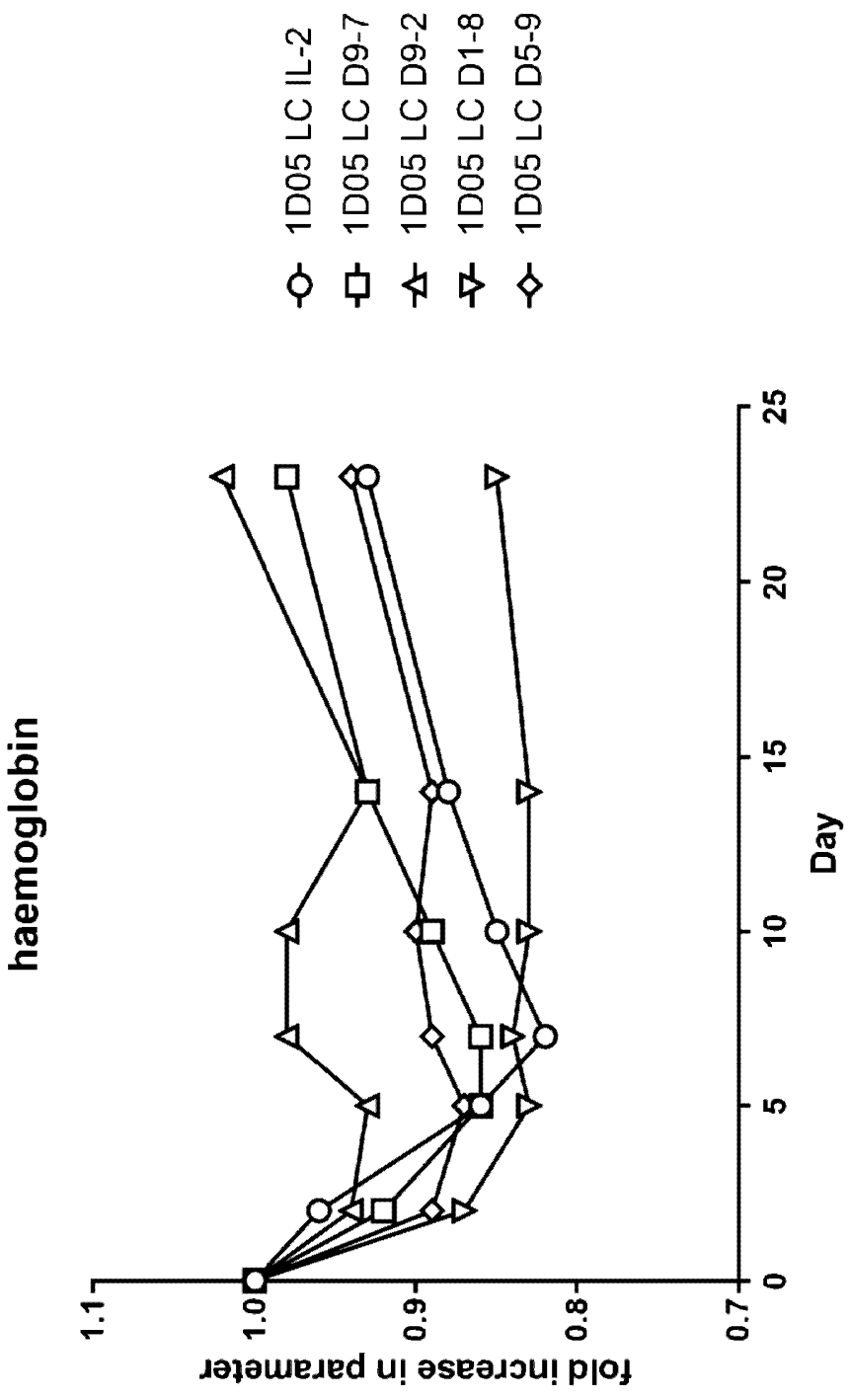

haemoglobin
1D05 LC IL-2
1D05 LC D9-7
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D5-9
fold increase in parameter
1.1
1.0
0.9
0.8
0.7
0
5
10
15
20
25
Day

Figure 30(b)

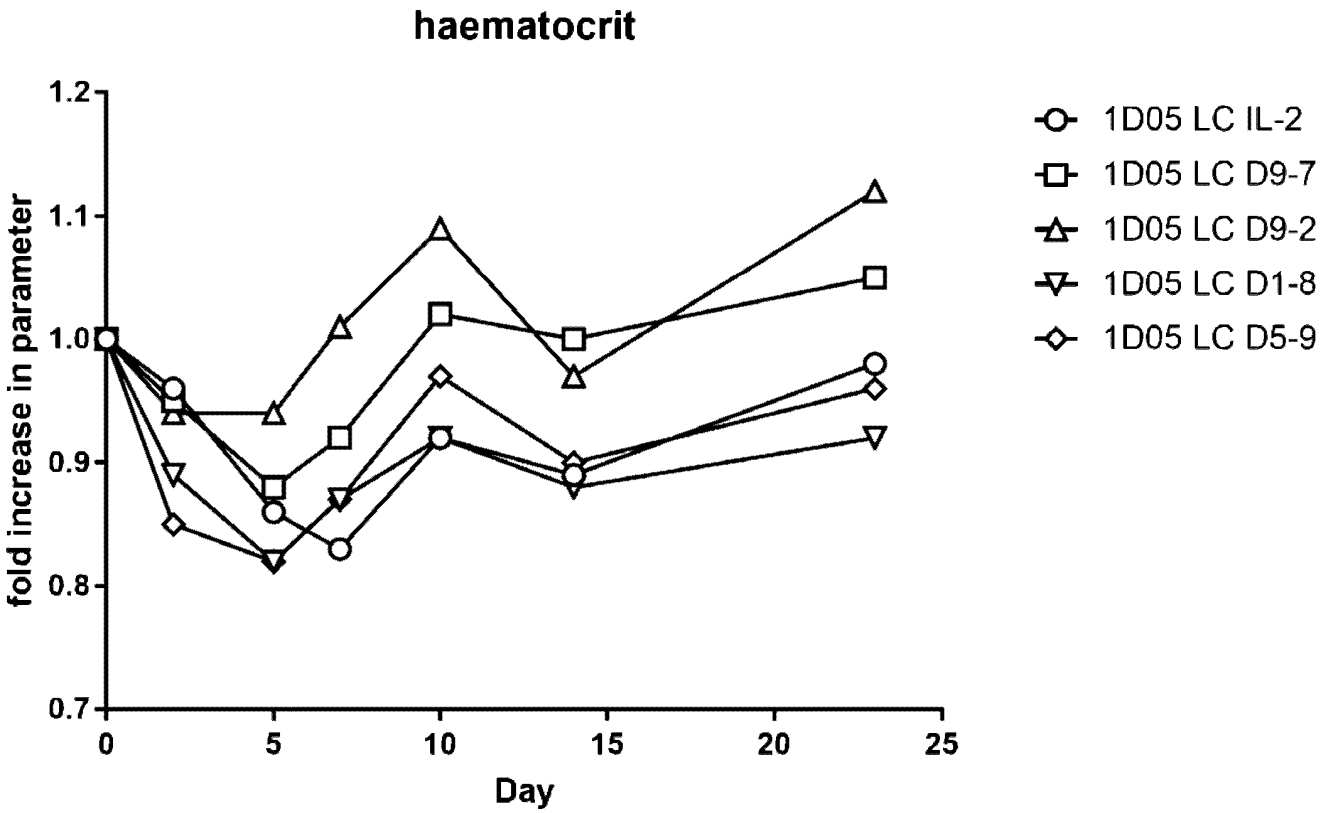

haematocrit
fold increase in parameter
1.2
1.1
1.0
0.9
0.8
0.7
0
5
10
15
20
25
Day
1D05 LC IL-2
1D05 LC D9-7
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D5-9

Figure 30(c)

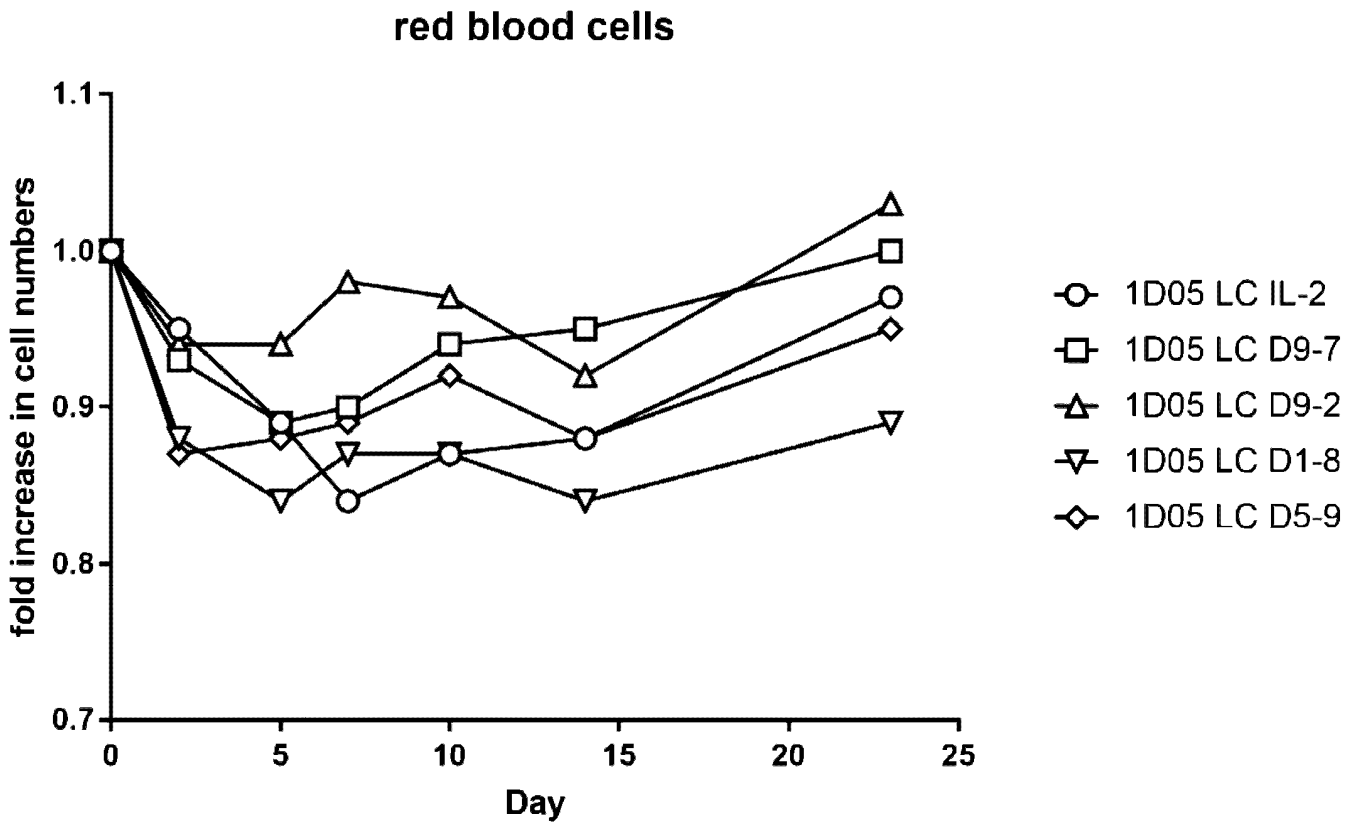

red blood cells
fold increase in cell numbers
1.1
1.0
0.9
0.8
0.7
0
5
10
15
20
25
Day
1D05 LC IL-2
1D05 LC D9-7
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D5-9

Figure 30(d)

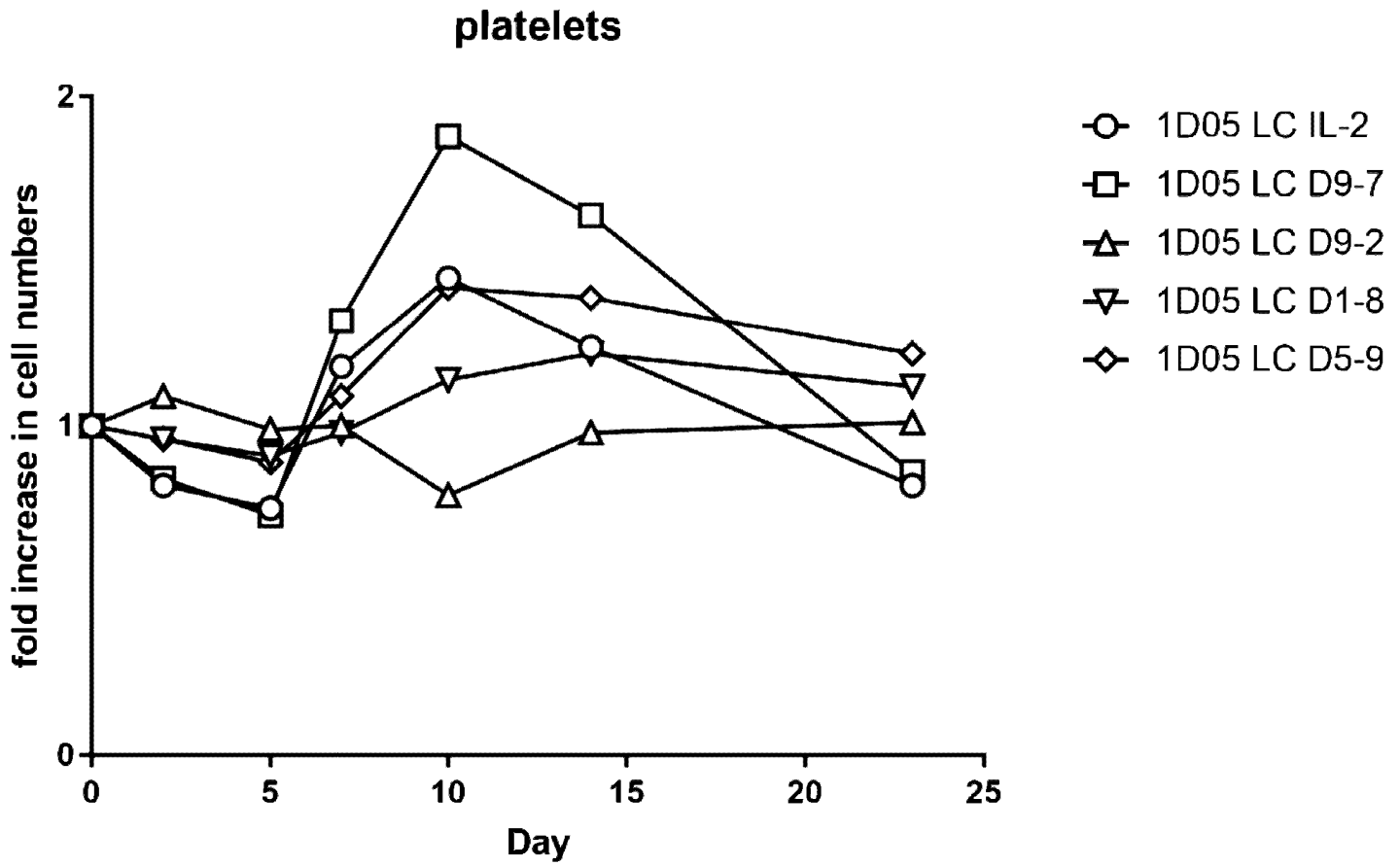

platelets
fold increase in cell numbers
2
1
0
0
5
10
15
20
25
Day
1D05 LC IL-2
1D05 LC D9-7
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D5-9

Figure 31(a)

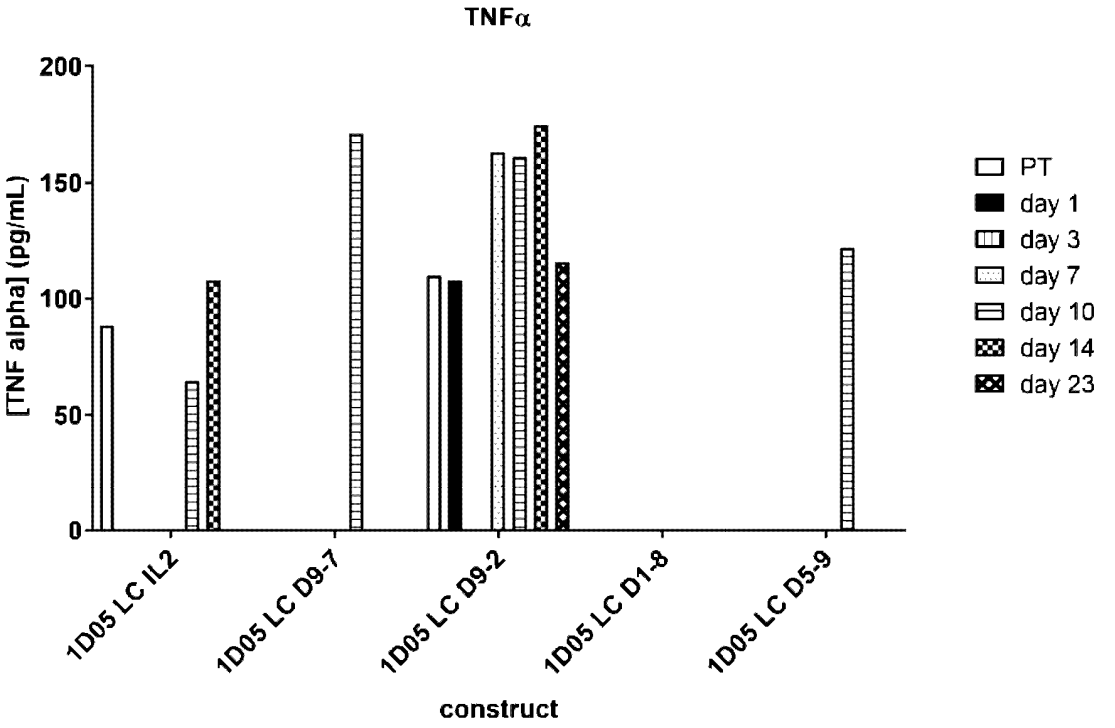


Figure 31(b)

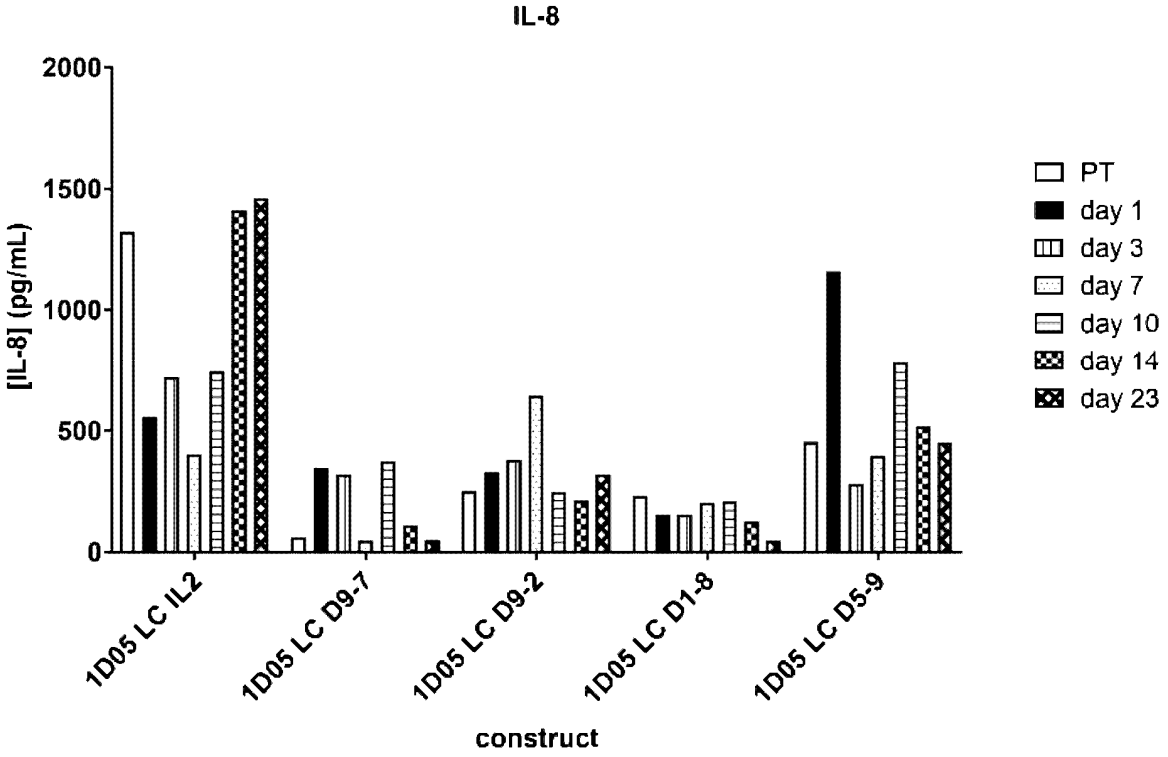


Figure 31(c)

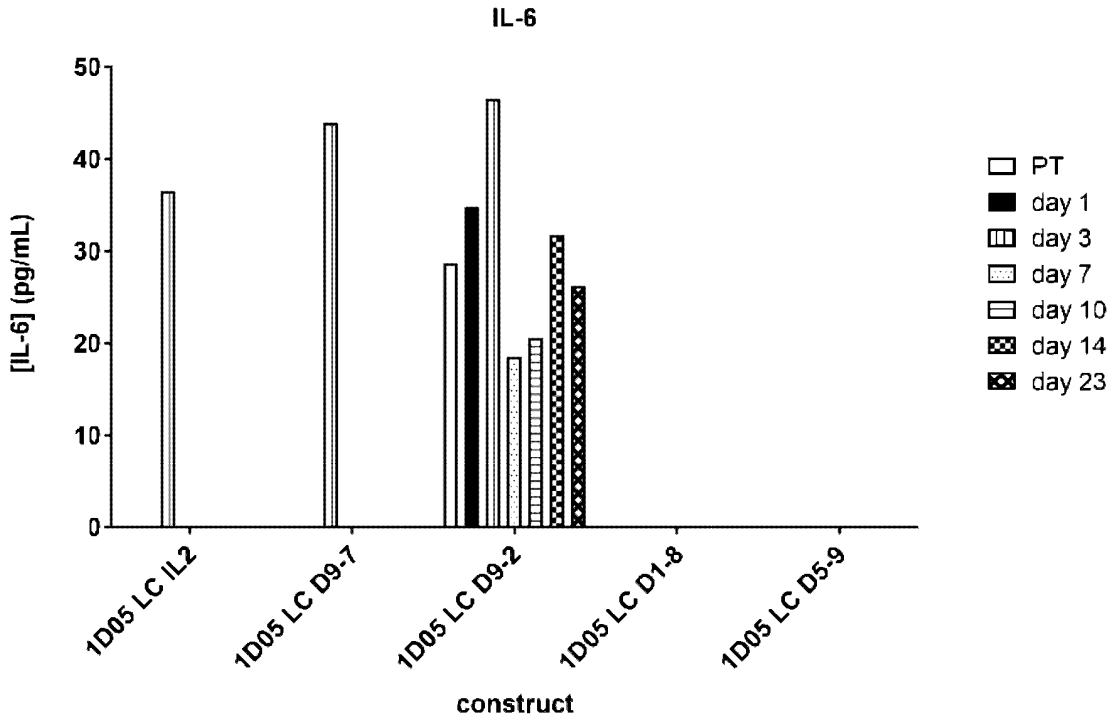


Figure 31(d)

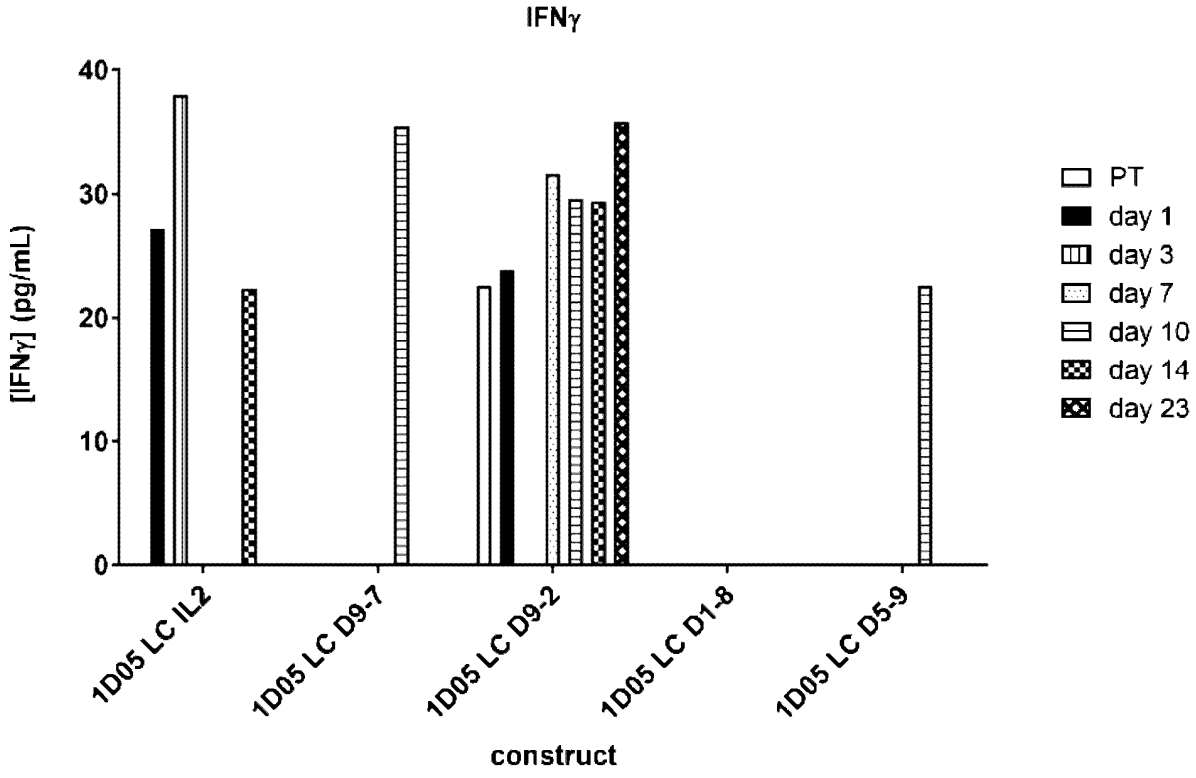


Figure 31 (e)

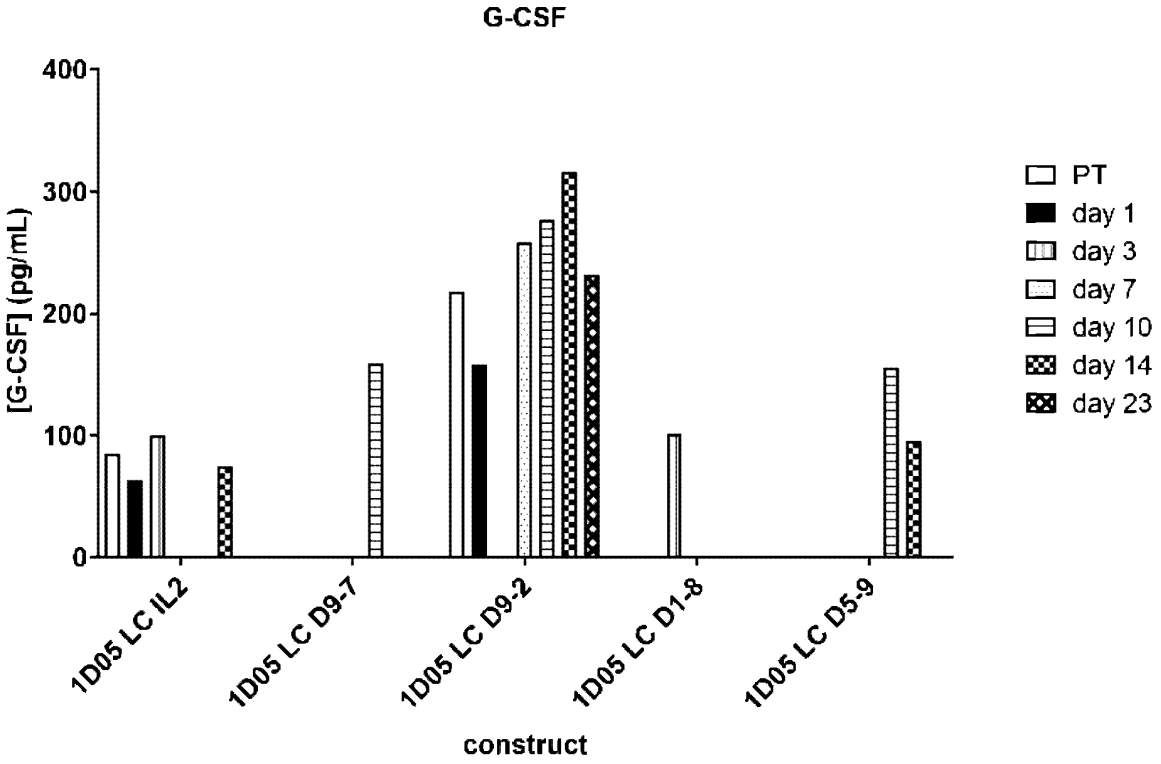


Figure 31(f)

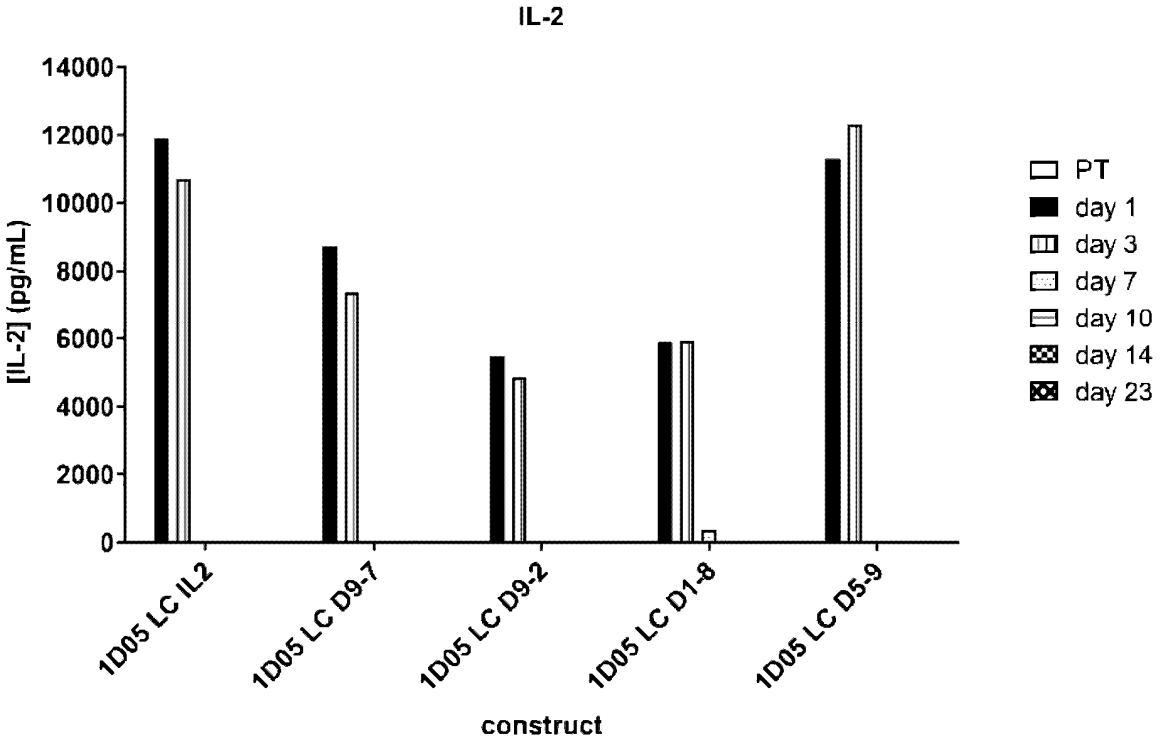


Figure 31(g)

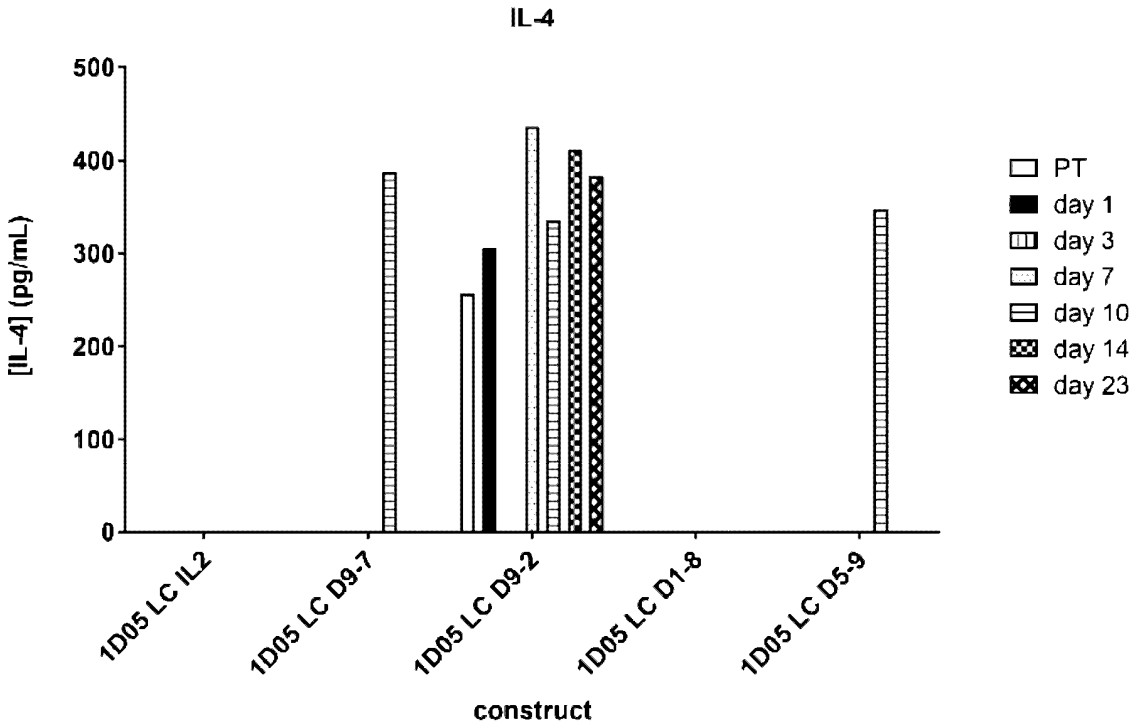


Figure 31(h)

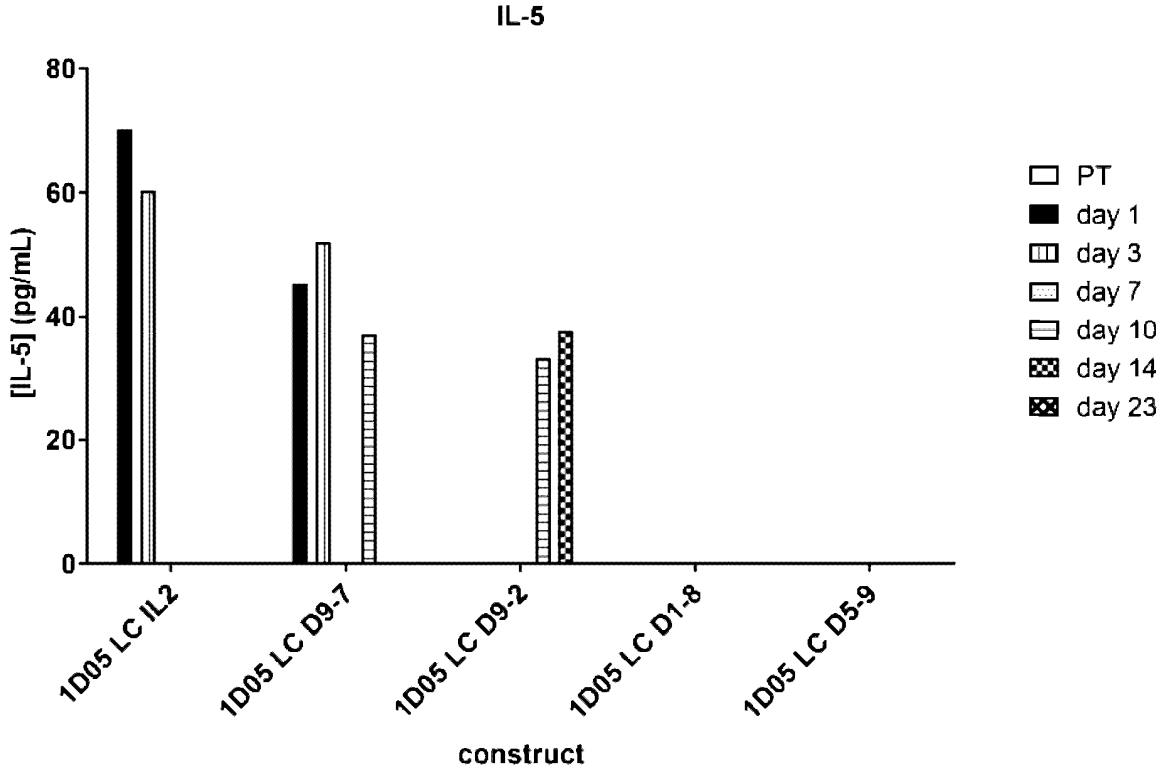


Figure 32

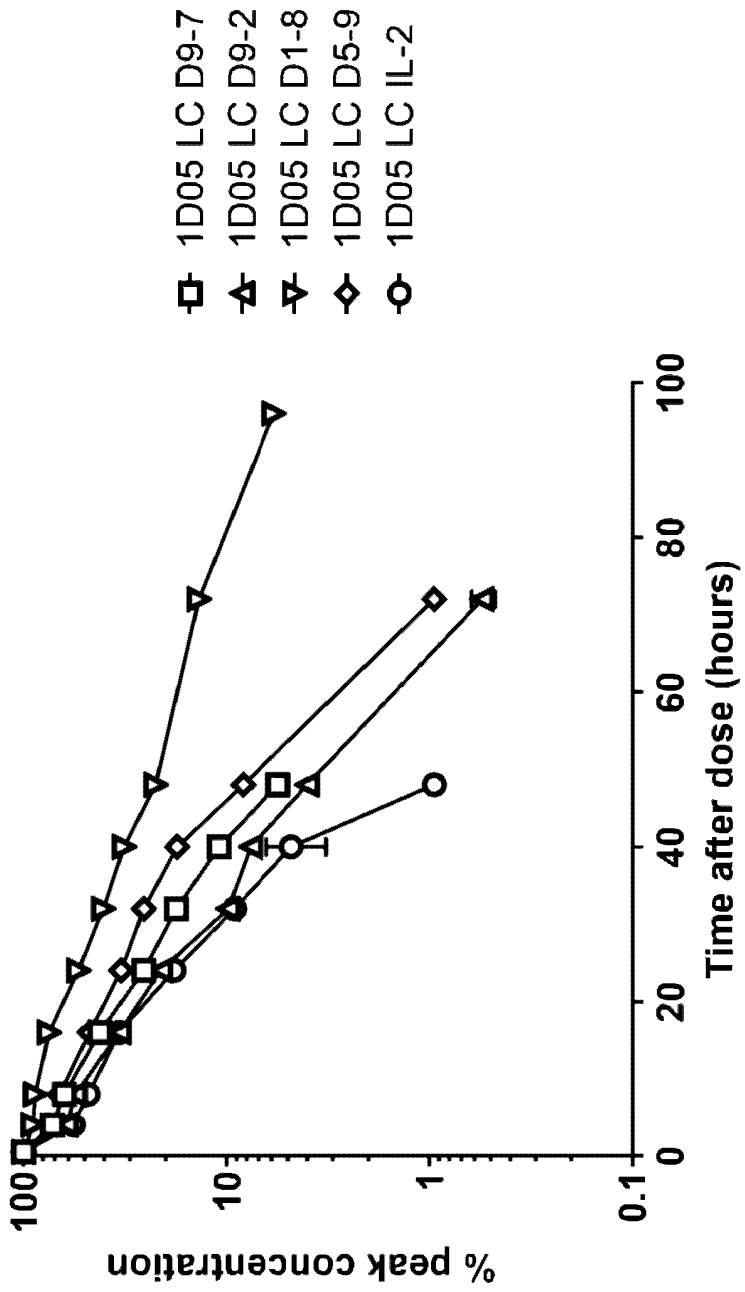

1D05 LC D9-7
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D5-9
1D05 LC IL-2
% peak concentration
100
10
1
0.1
0
20
40
60
80
100
Time after dose (hours)

Figure 33(a)

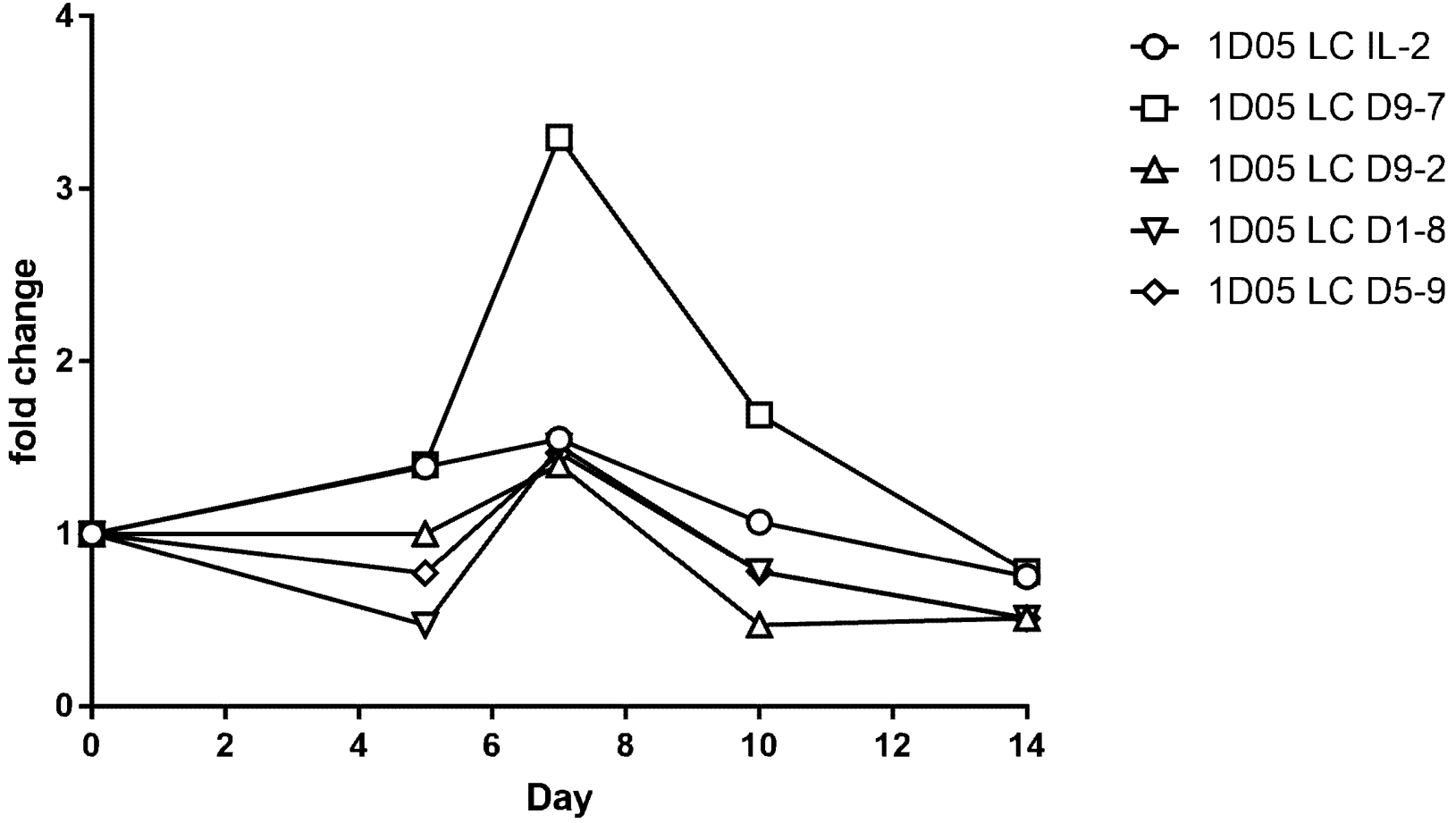

4
3
2
1
0
fold change
0
2
4
6
8
10
12
14
Day
1D05 LC IL-2
1D05 LC D9-7
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D5-9

Figure 33(b)

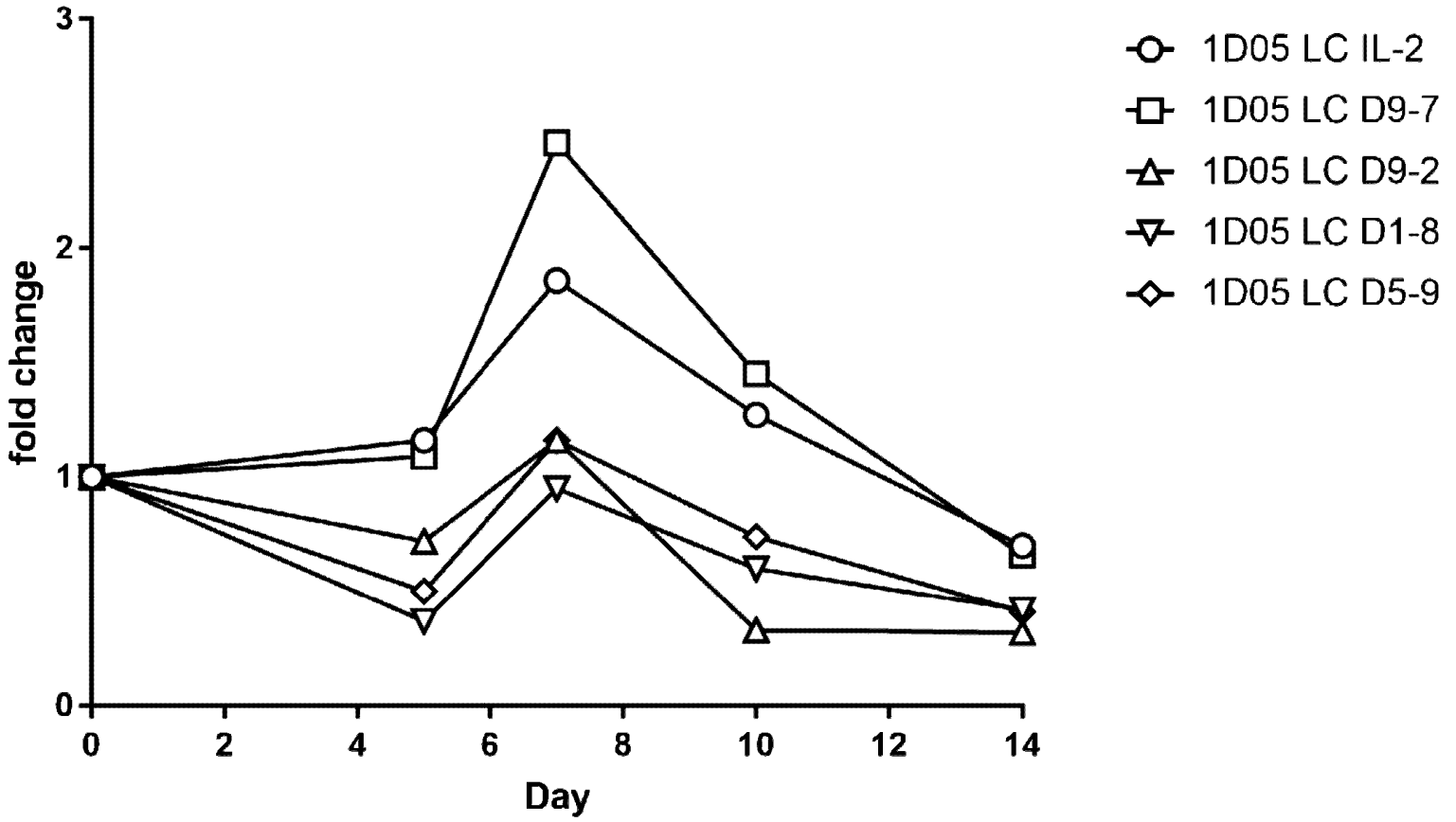

3
2
1
0
fold change
0
2
4
6
8
10
12
14
Day
1D05 LC IL-2
1D05 LC D9-7
1D05 LC D9-2
1D05 LC D1-8
1D05 LC D5-9

Figure 34

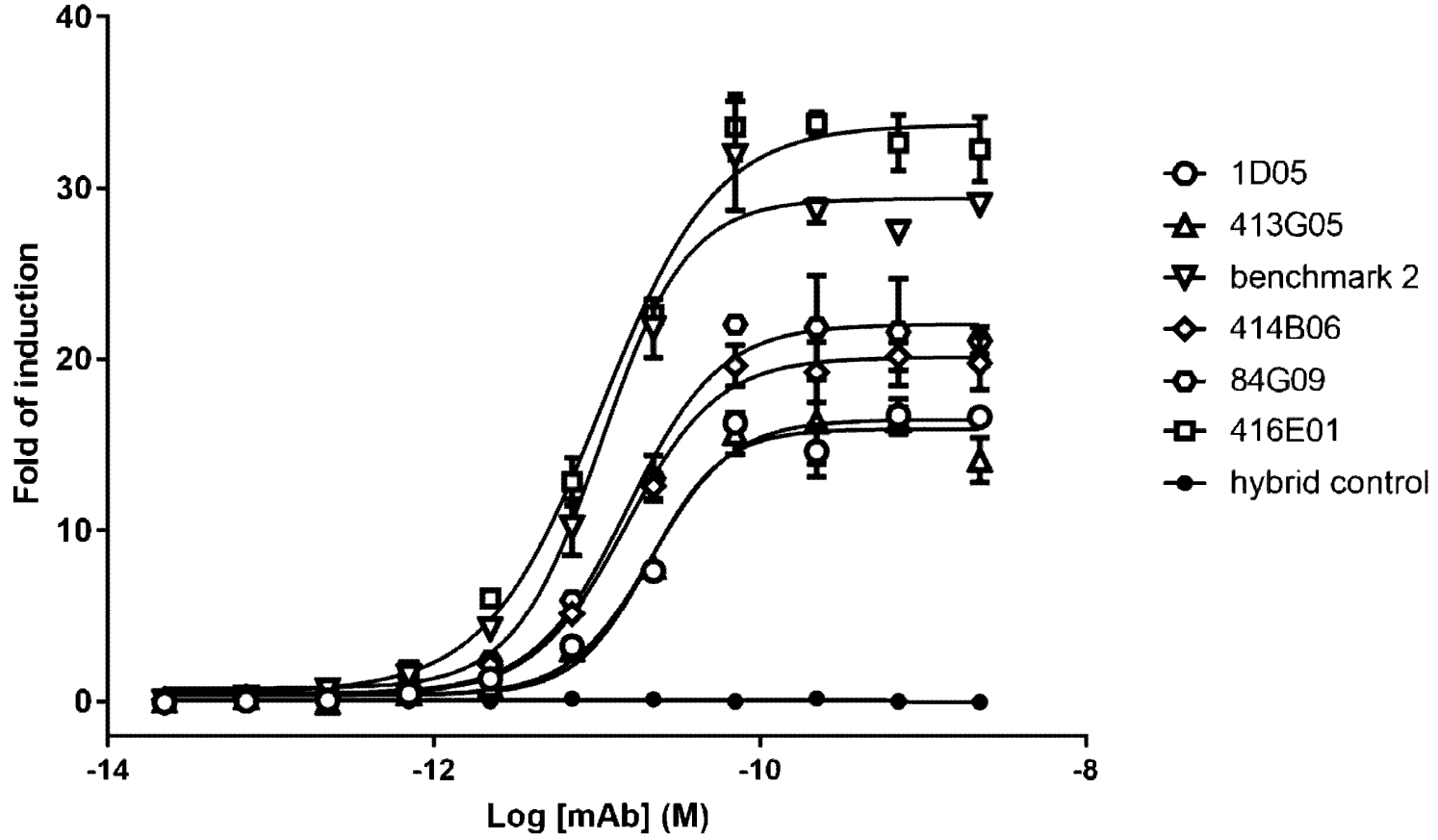

40
30
20
10
0
Fold of induction
-14
-12
-10
-8
Log [mAb] (M)
1D05
413G05
benchmark 2
414B06
84G09
416E01
hybrid control

Figure 35

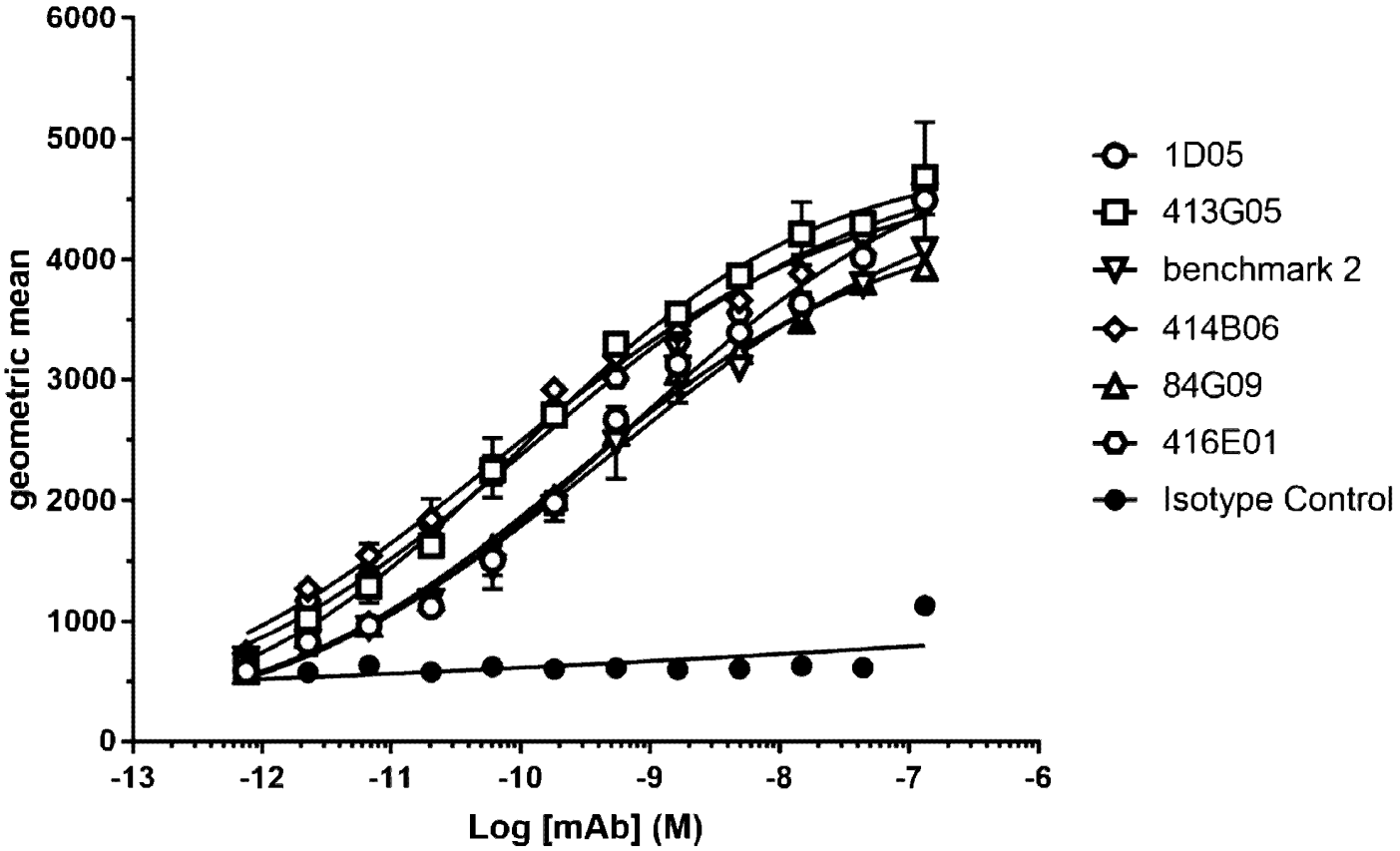

6000
5000
4000
3000
2000
1000
0
geometric mean
-13
-12
-11
-10
-9
-8
-7
-6
Log [mAb] (M)
1D05
413G05
benchmark 2
414B06
84G09
416E01
Isotype Control

Figure 36(a)

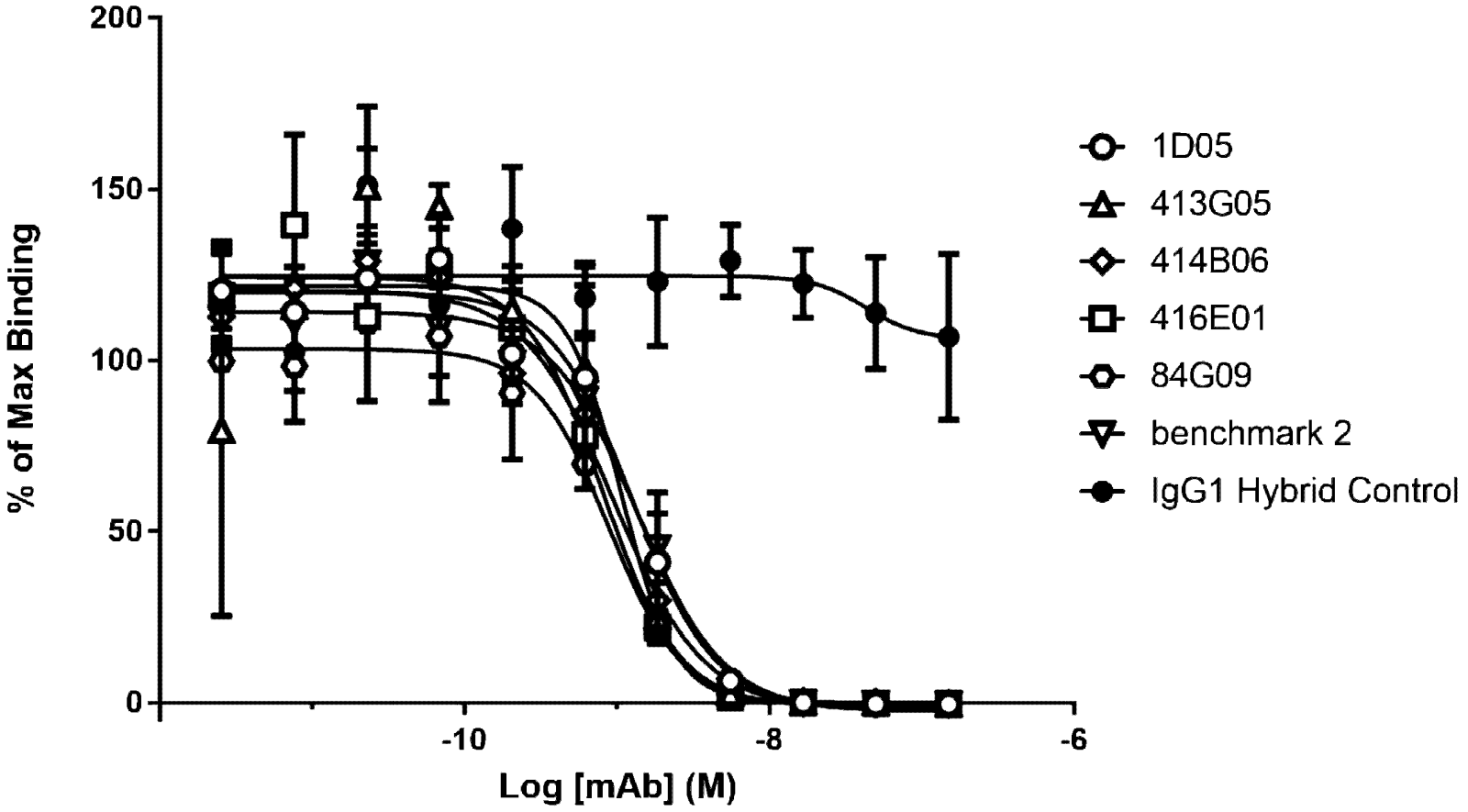

200
150
100
50
0
% of Max Binding
-10
-8
-6
Log [mAb] (M)
1D05
413G05
414B06
416E01
84G09
benchmark 2
IgG1 Hybrid Control

Figure 36(b)

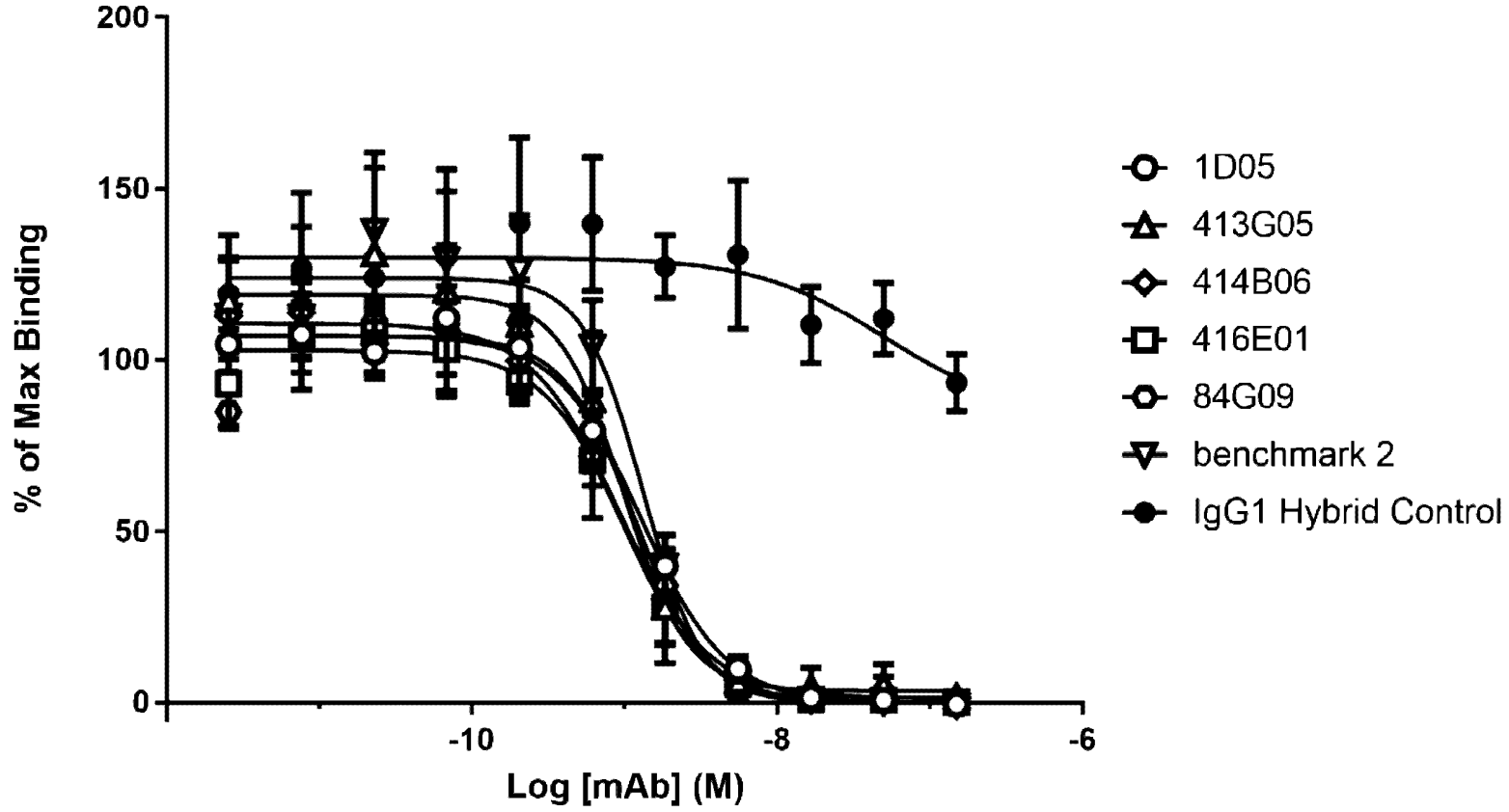
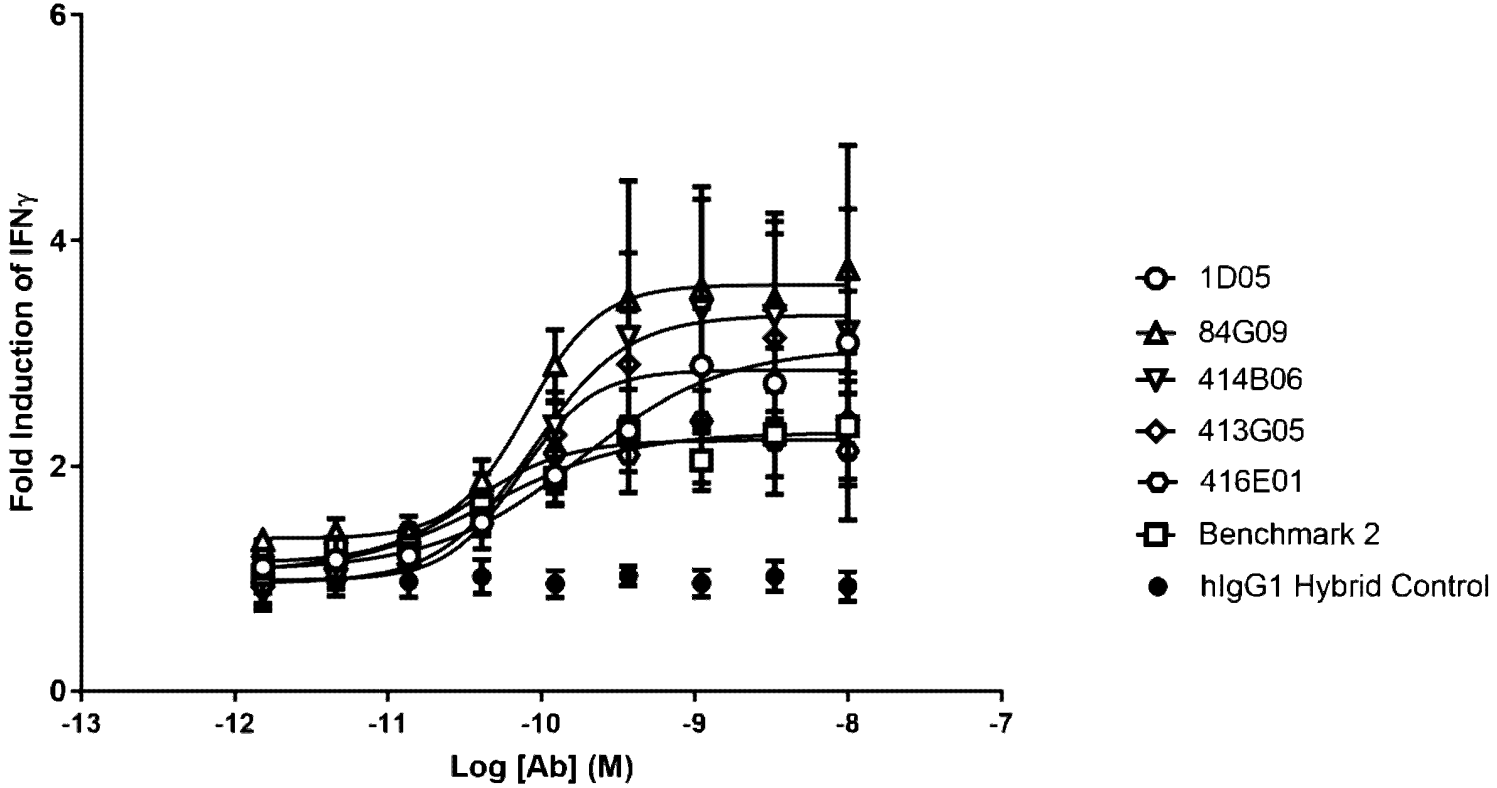

200
150
100
50
0
% of Max Binding
-10
-8
-6
Log [mAb] (M)
1D05
413G05
414B06
416E01
84G09
benchmark 2
IgG1 Hybrid Control

Figure 37


Fold Induction of IFNγ
6
4
2
0
-13
-12
-11
-10
-9
-8
-7
Log [Ab] (M)
1D05
84G09
414B06
413G05
416E01
Benchmark 2
hIgG1 Hybrid Control