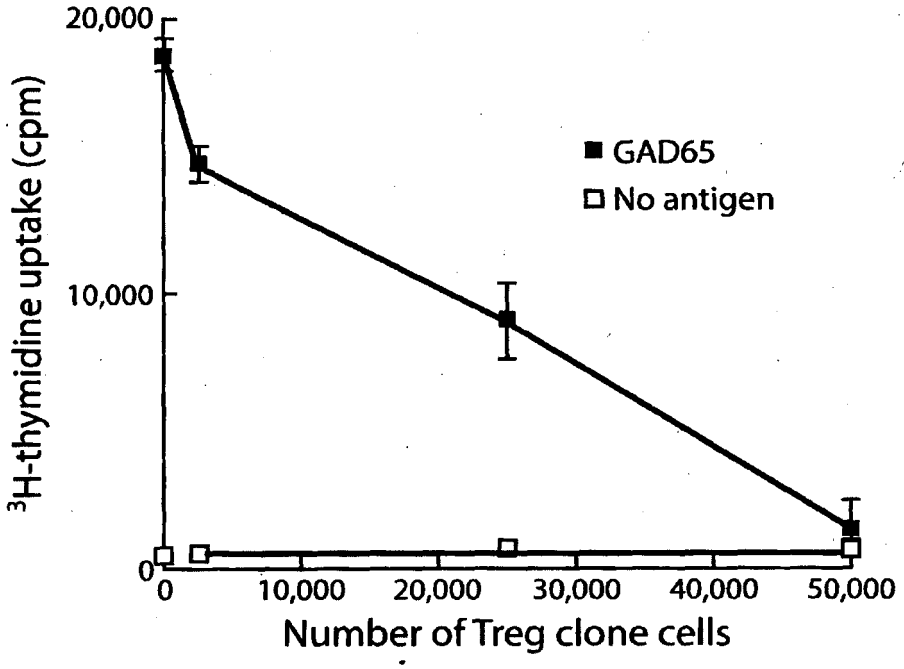
A


20,000
10,000
0
^{3}H-thymidine uptake (cpm)
GAD65
No antigen
0
10,000
20,000
30,000
40,000
50,000
Number of Treg clone cells

B

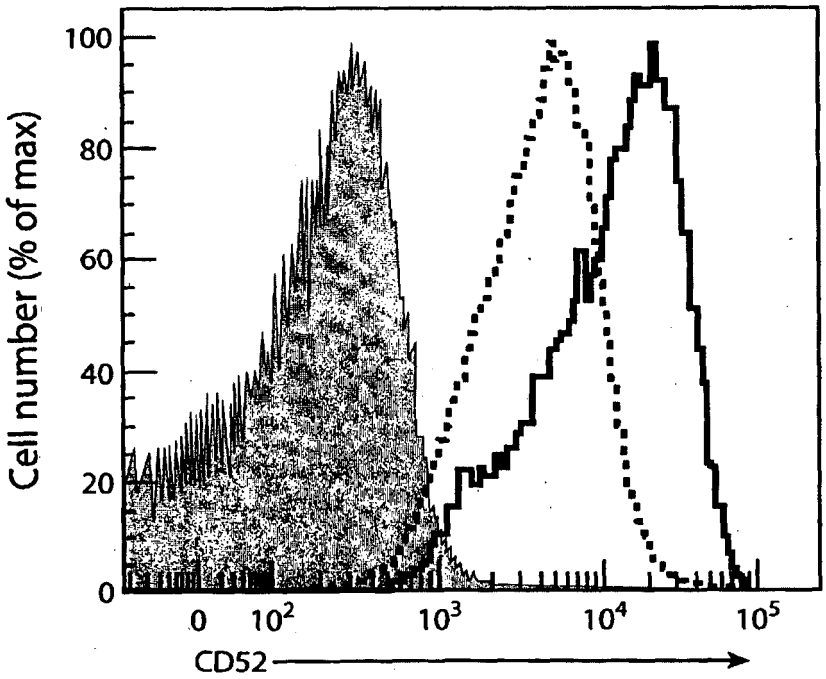
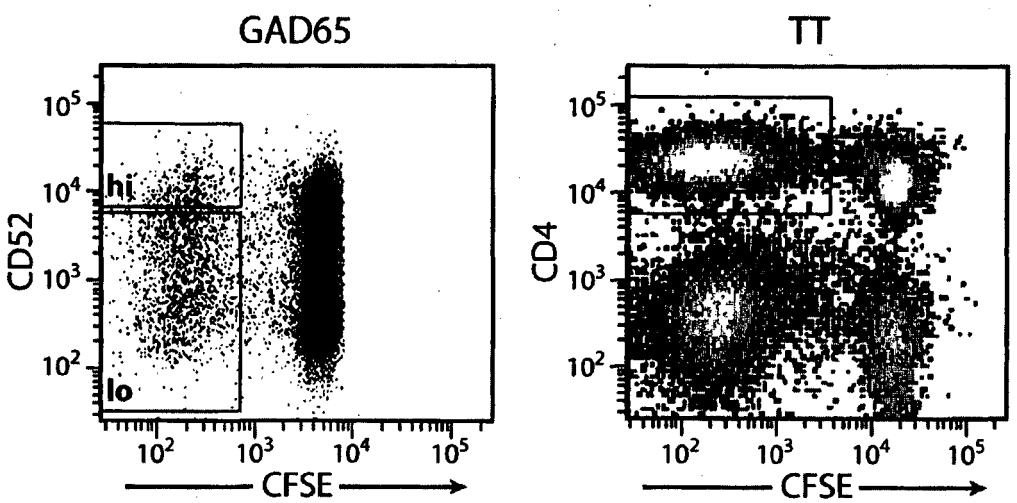
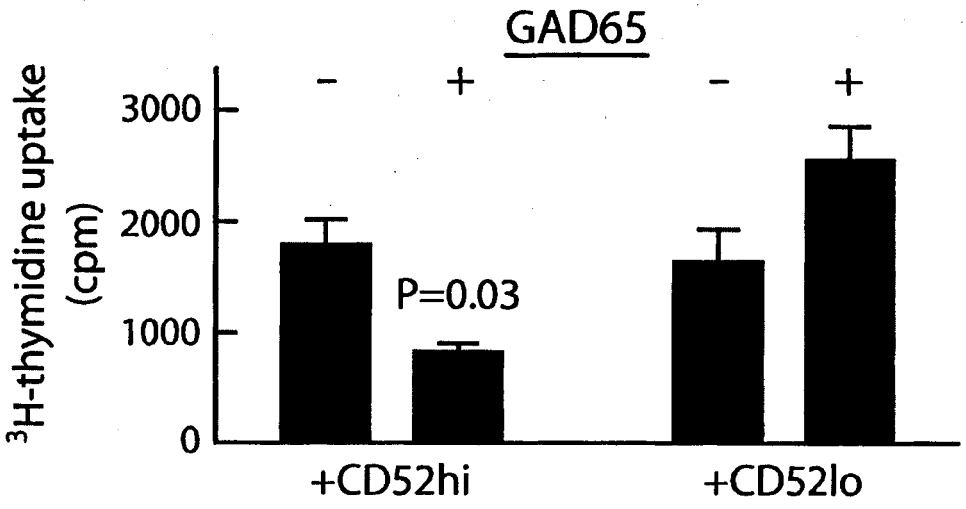

100
80
60
40
20
0
Cell number (% of max)
0
10^2
10^3
10^4
10^5
CD52

Figure 1

A1



A2



A3

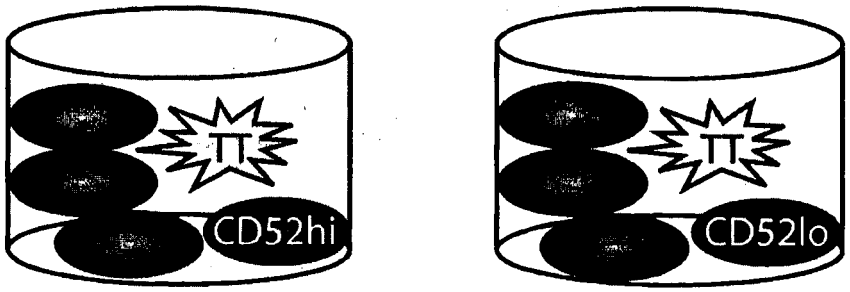


Figure 2A

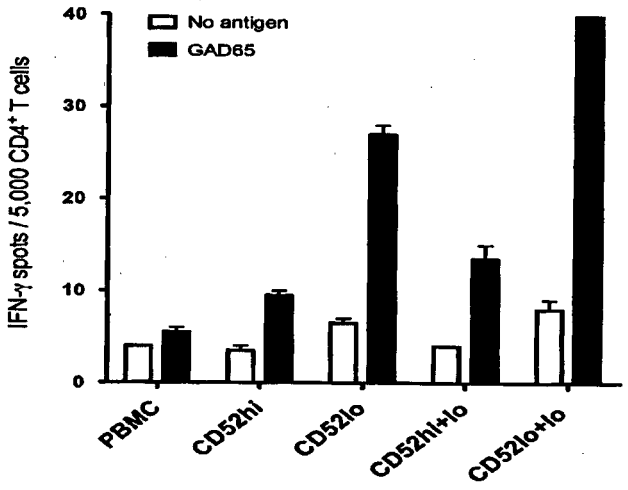


Figure 2B

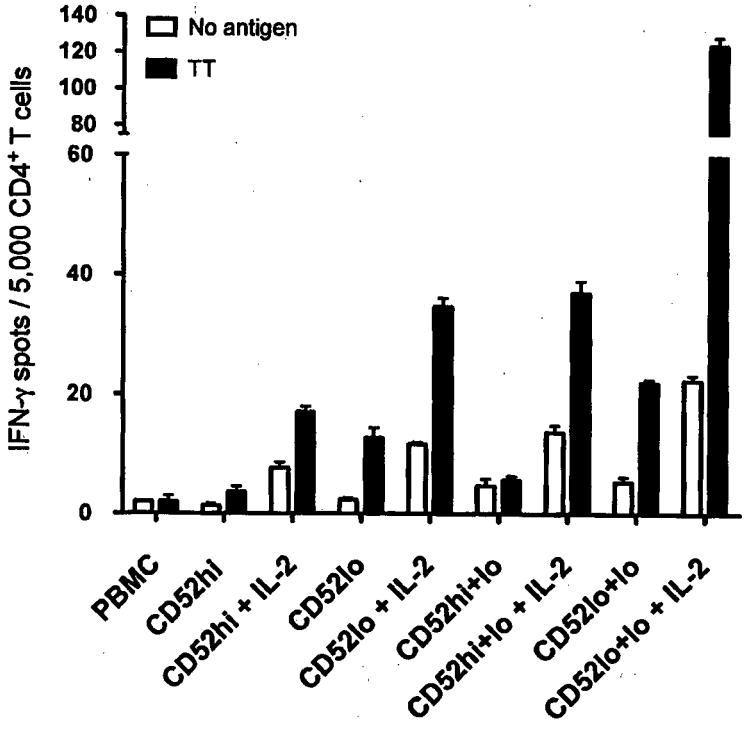


Figure 2C

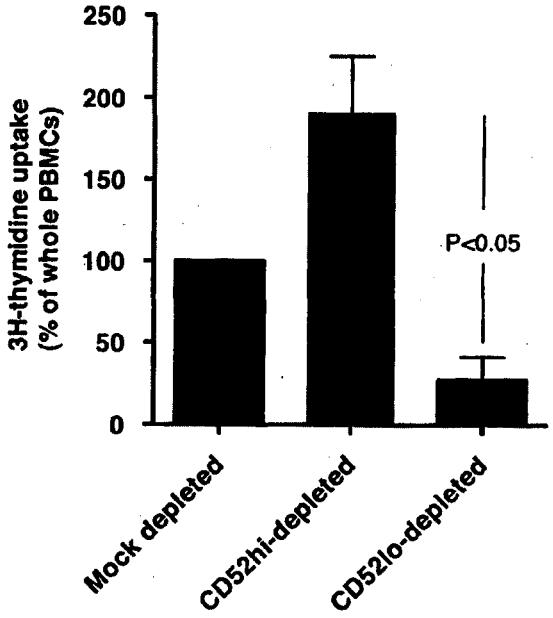


Figure 2D

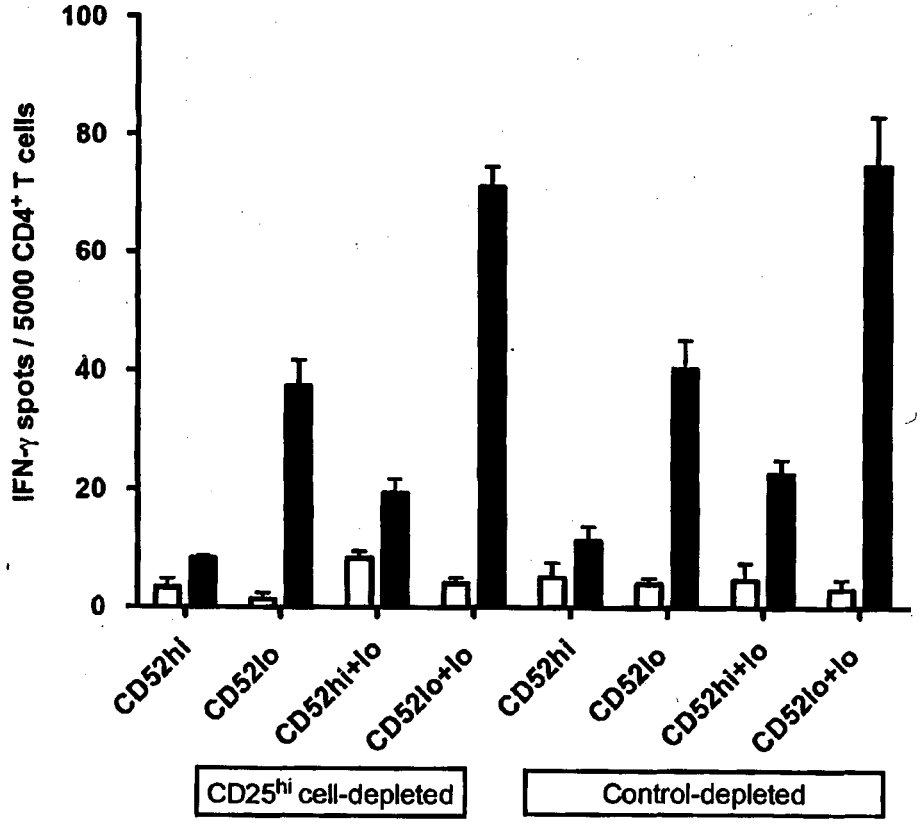


Figure 3

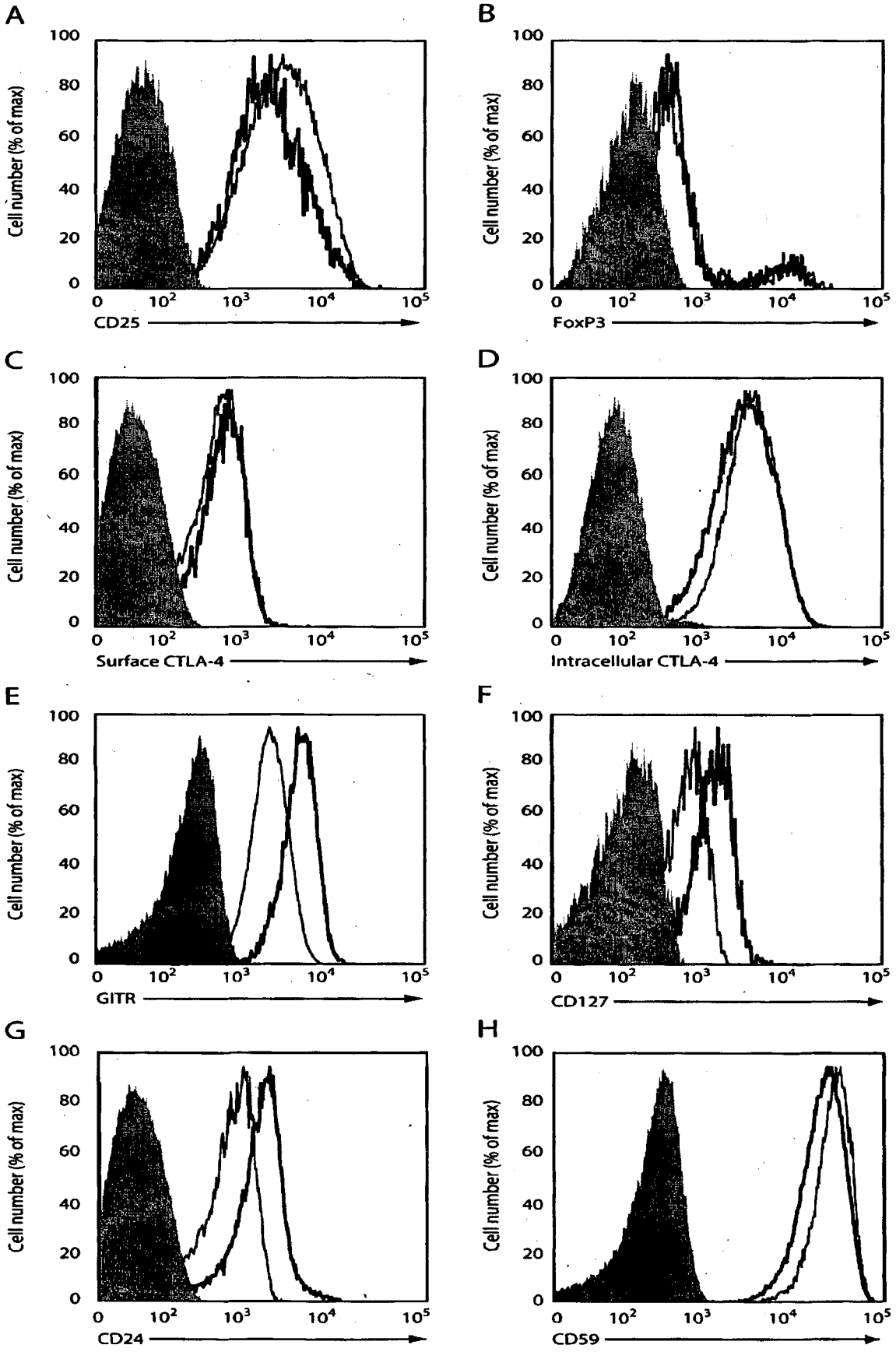

A
B
C
D
E
F
G
H
Cell number (% of max)
CD25
FoxP3
Surface CTLA-4
Intracellular CTLA-4
GITR
CD127
CD24
CD59
0
20
40
60
80
100
0
10²
10³
10⁴
10⁵

Figure 4

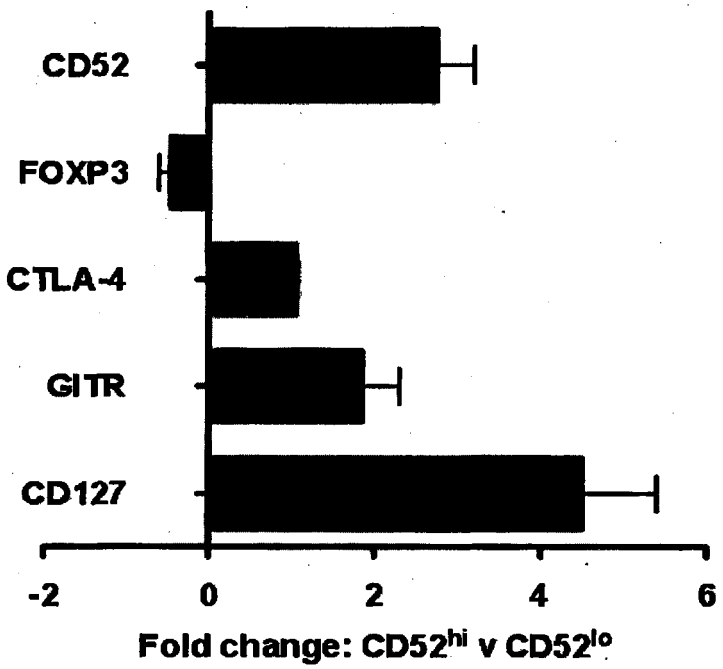


Figure 5

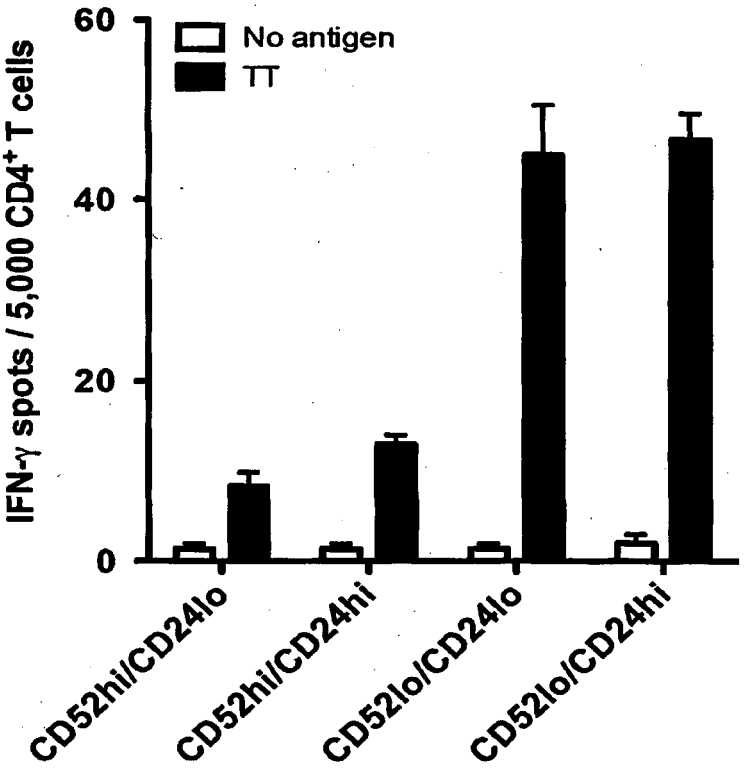


Figure 6

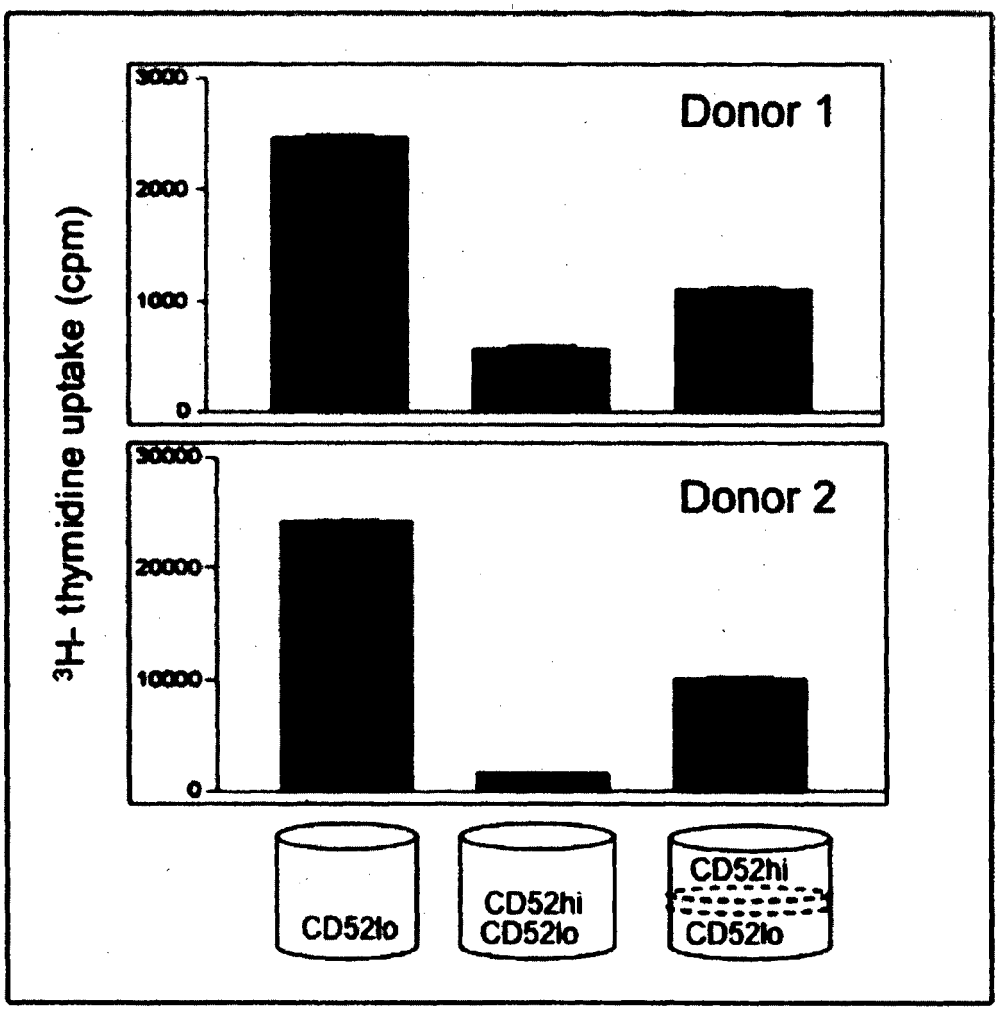

Donor 1
3000
2000
1000
0
Donor 2
30000
20000
10000
0
^{3}H- thymidine uptake (cpm)
CD52lo
CD52hi
CD52lo
CD52hi
CD52lo

Figure 7

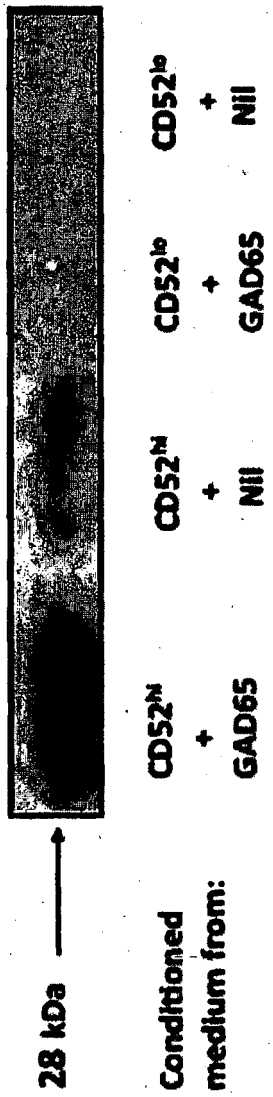


Figure 8A

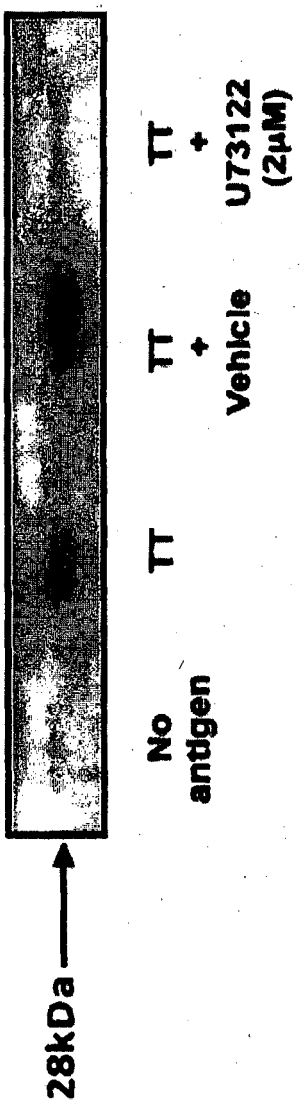


Figure 8B

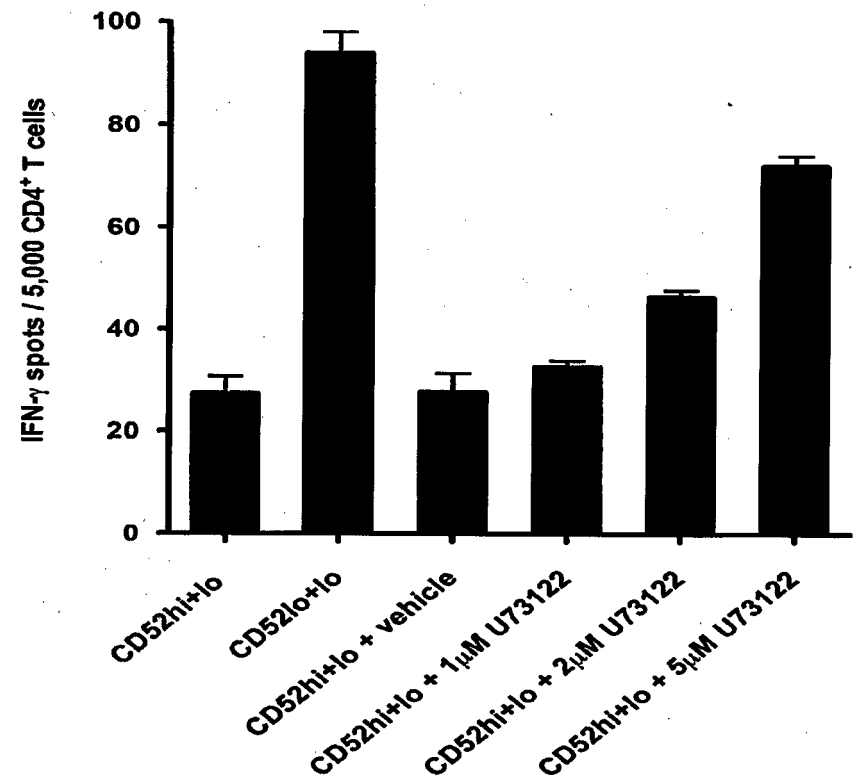


Figure 8C

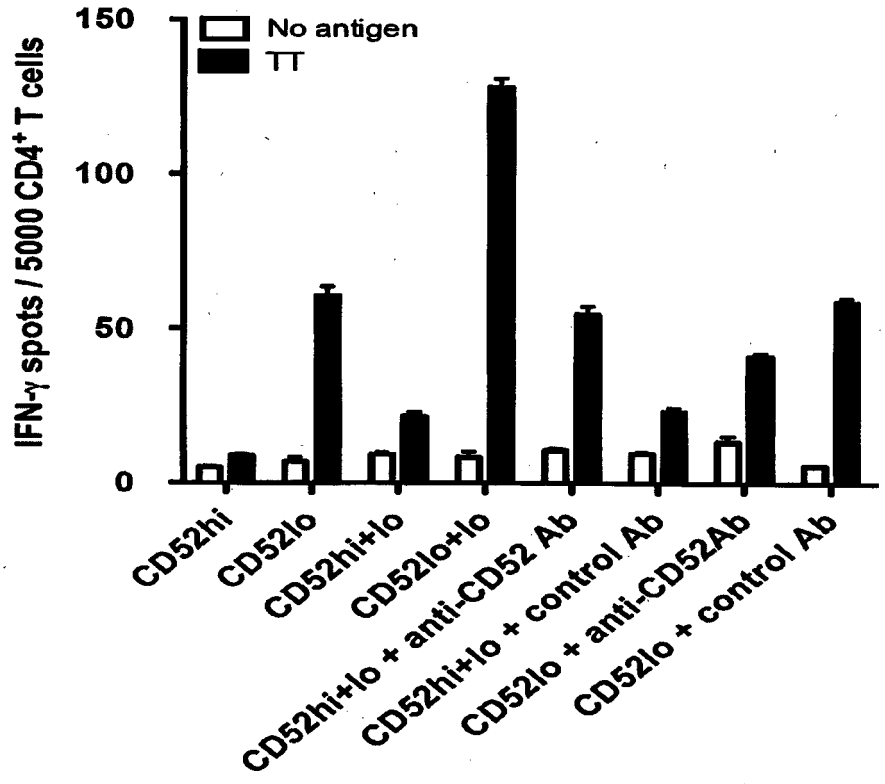


Figure 8D

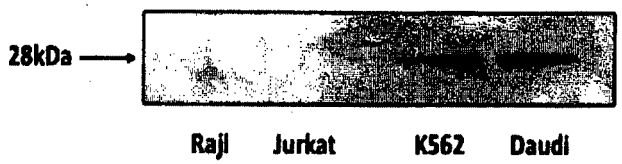


Figure 9A

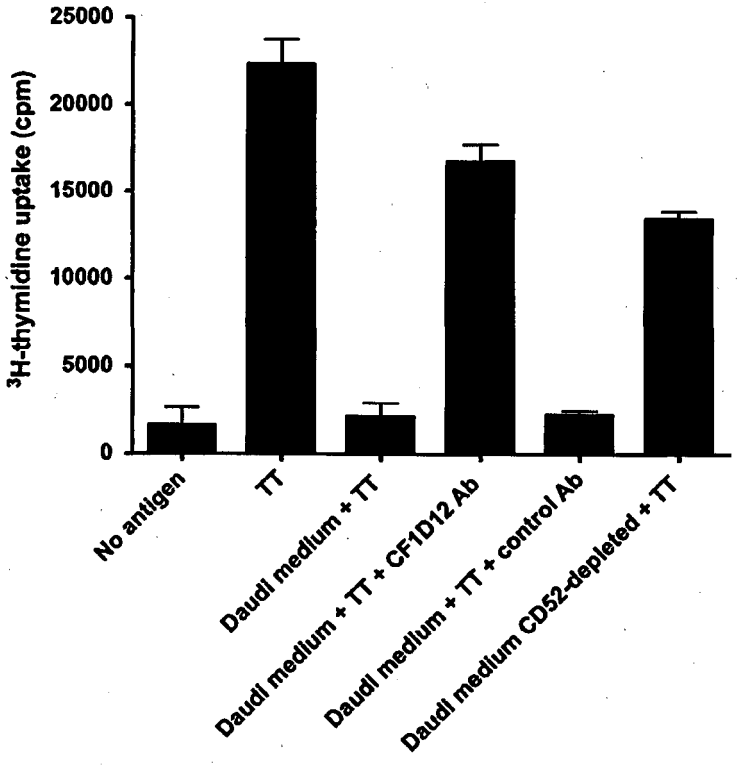


Figure 9B

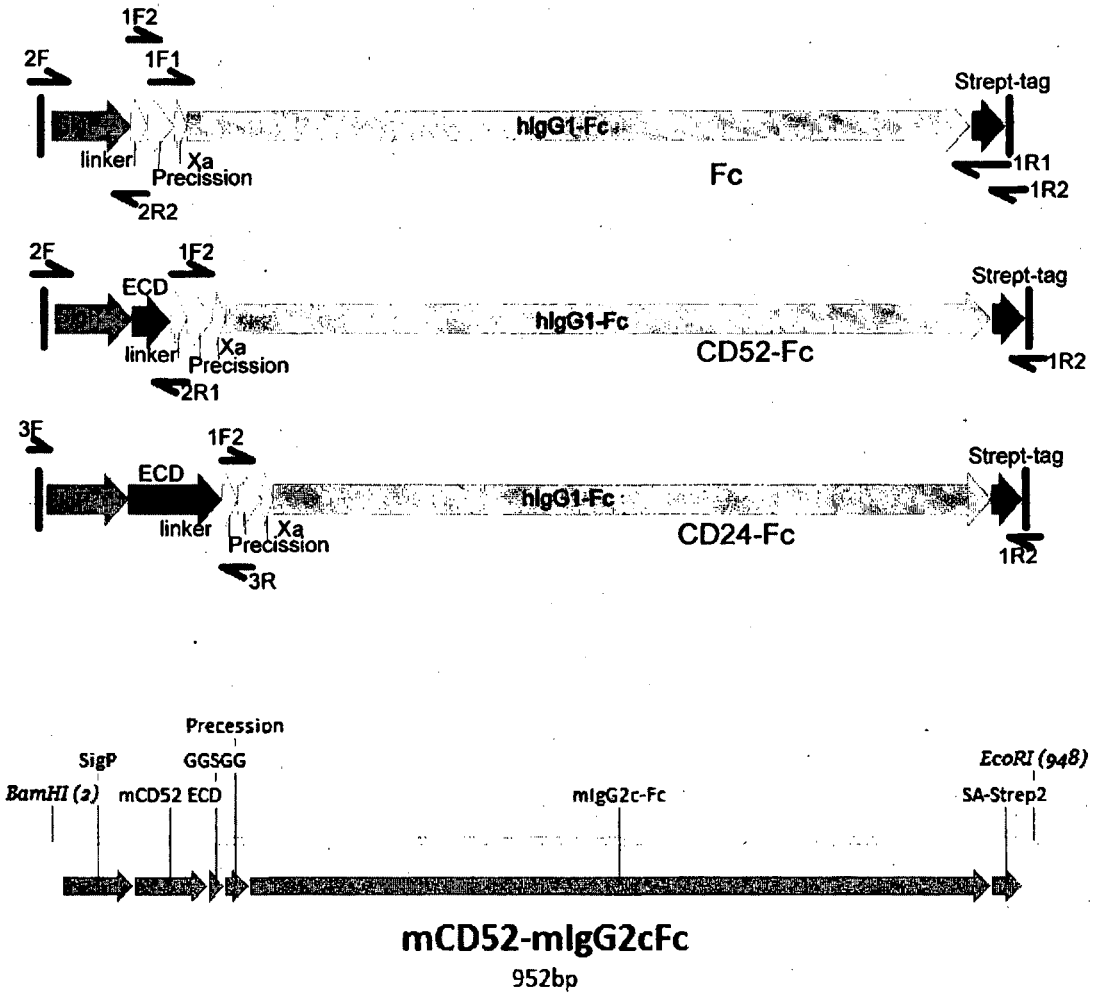

1F2
2F
1F1
Strept-tag
hIgG1-Fc
linker
Xa
Precission
Fc
1R1
1R2
2R2
2F
1F2
ECD
Strept-tag
hIgG1-Fc
linker
Xa
Precission
CD52-Fc
1R2
2R1
3F
1F2
ECD
Strept-tag
hIgG1-Fc
linker
Xa
Precission
CD24-Fc
1R2
3R
Precession
SigP
GGSGG
EcoRI (948)
BamHI (2)
mCD52 ECD
mIgG2c-Fc
SA-Strep2
mCD52-mIgG2cFc
952bp

Figure 10

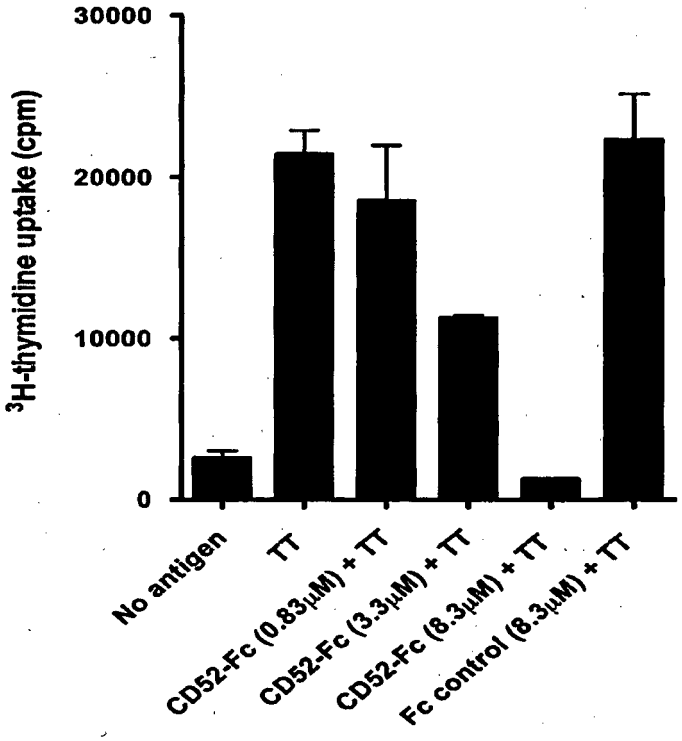


Figure 11A

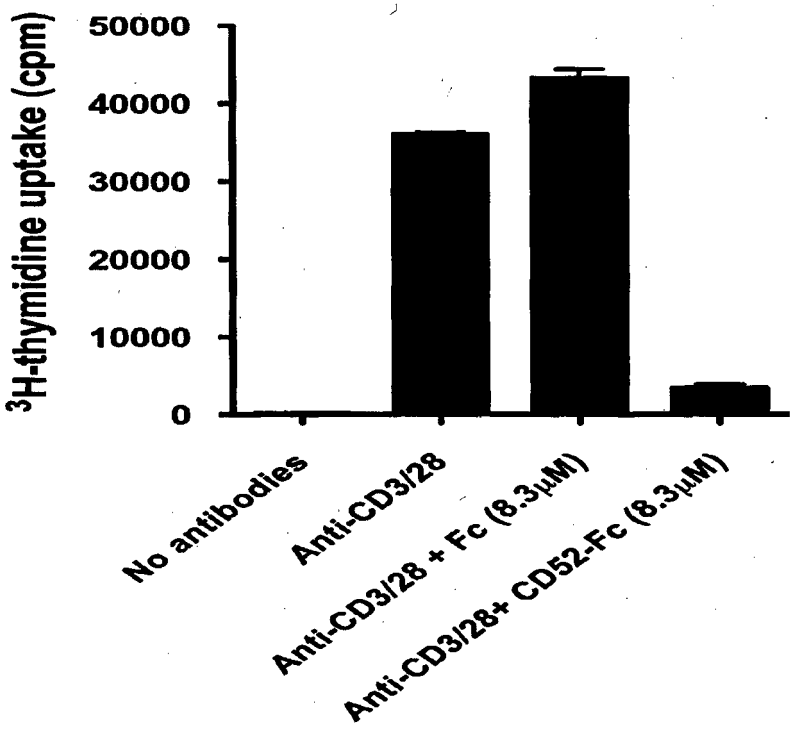


Figure 11B

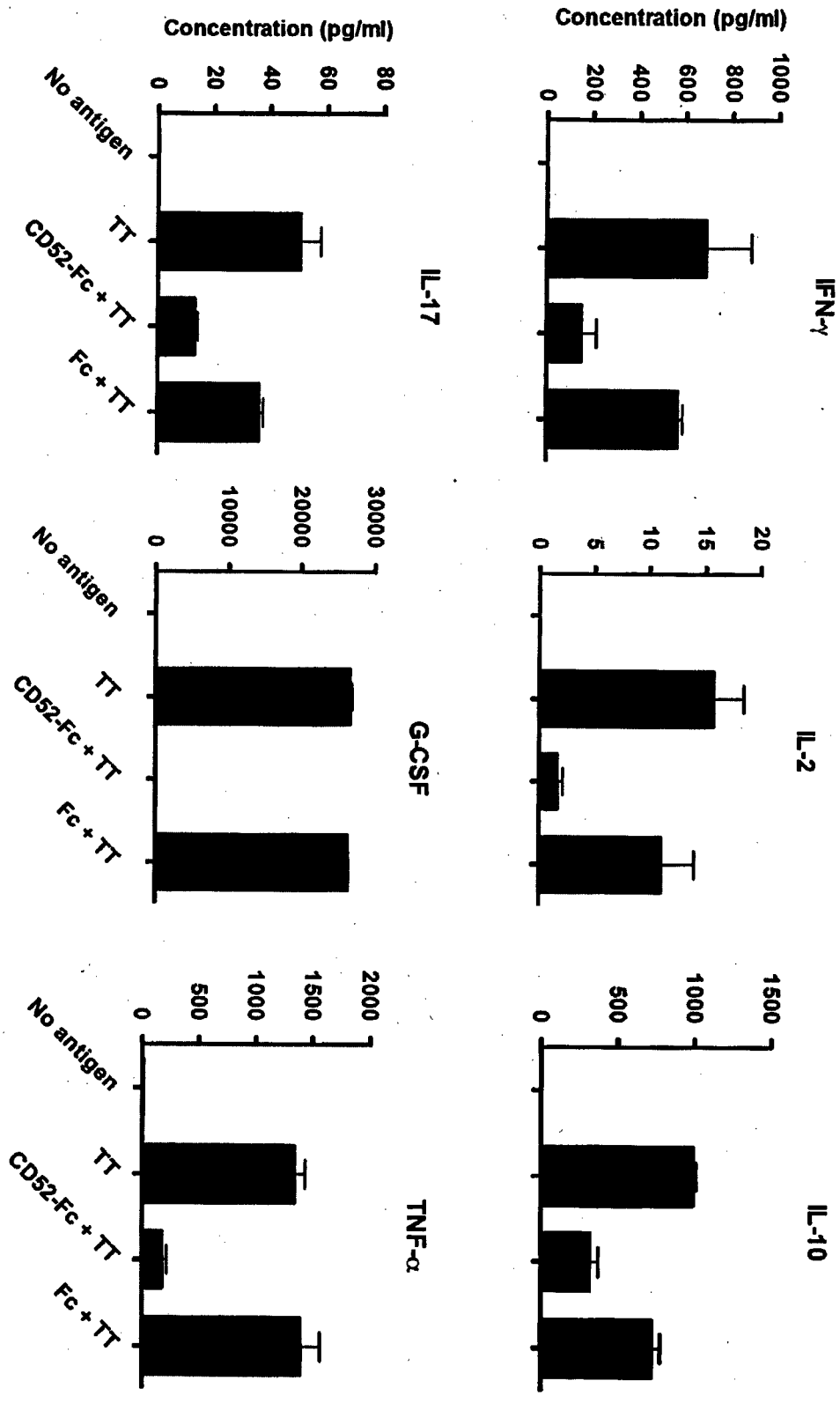

Concentration (pg/ml)
IFN-γ
IL-17
IL-2
G-CSF
IL-10
TNF-α
No antigen
TT
CD52-Fc + TT
Fc + TT

Figure 11C

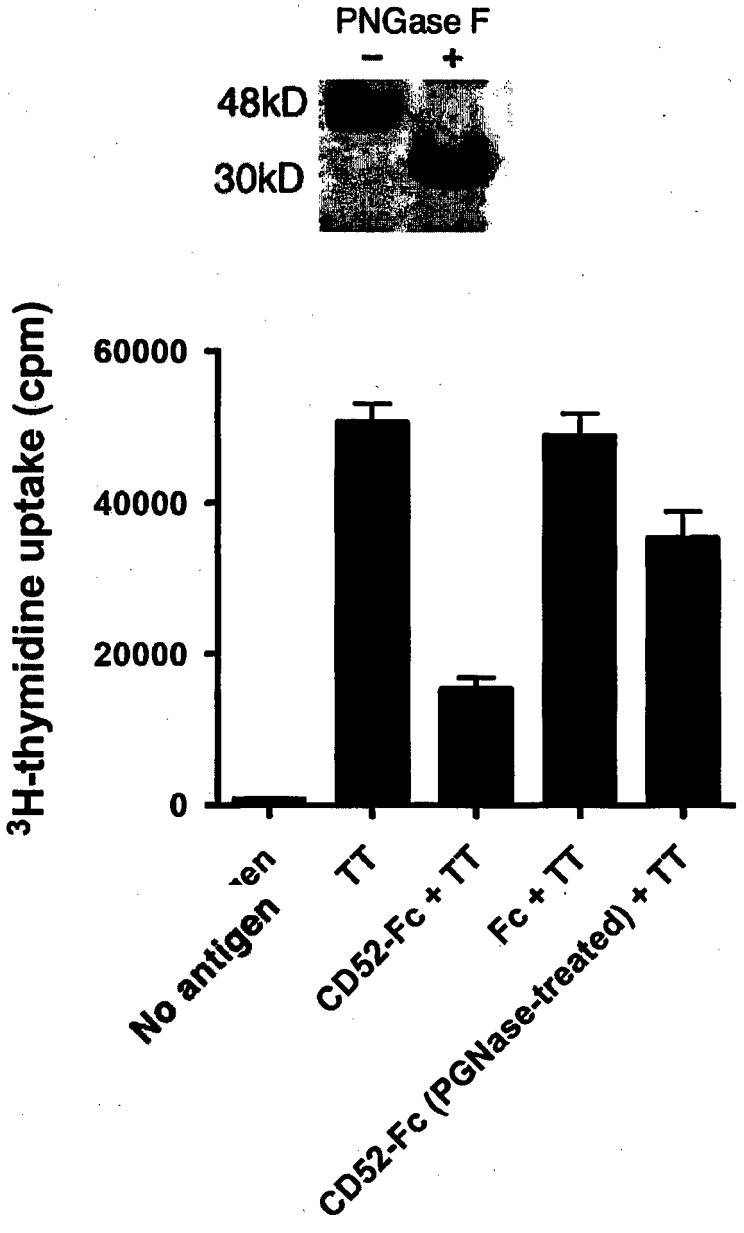

PNGase F
−
+
48kD
30kD
^{3}H-thymidine uptake (cpm)
60000
40000
20000
0
No antigen
TT
CD52-Fc + TT
Fc + TT
CD52-Fc (PGNase-treated) + TT

Figure 11D

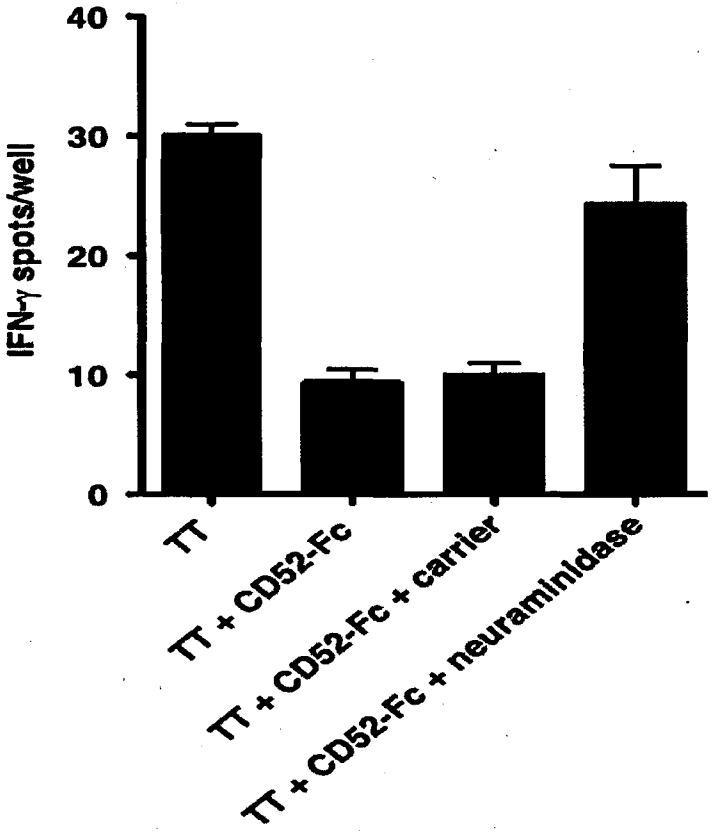


Figure 12A

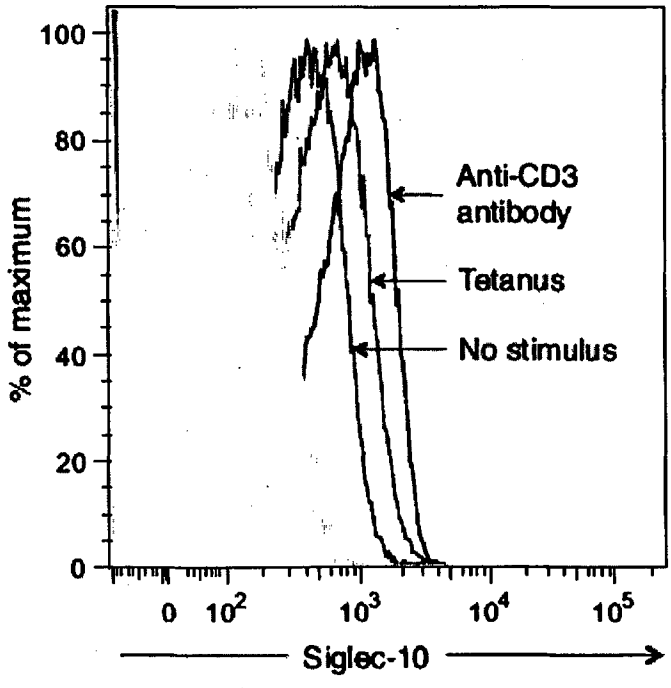


Figure 12B

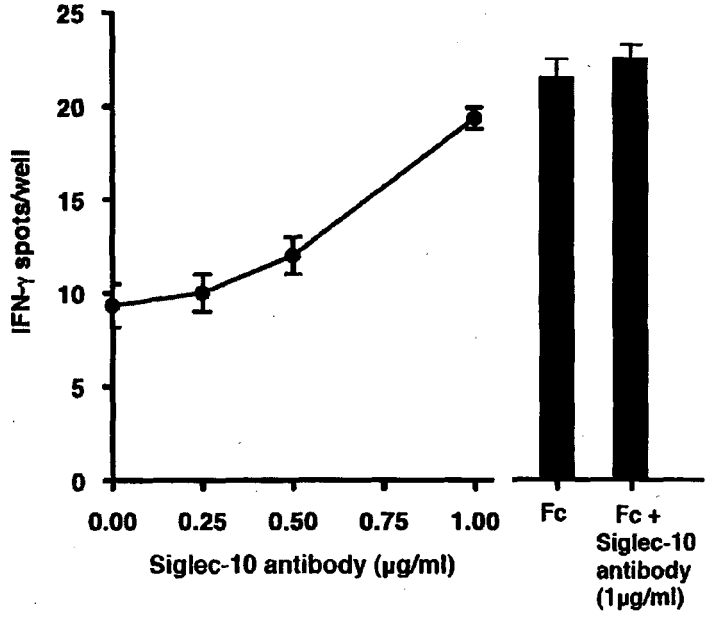


Figure 12C

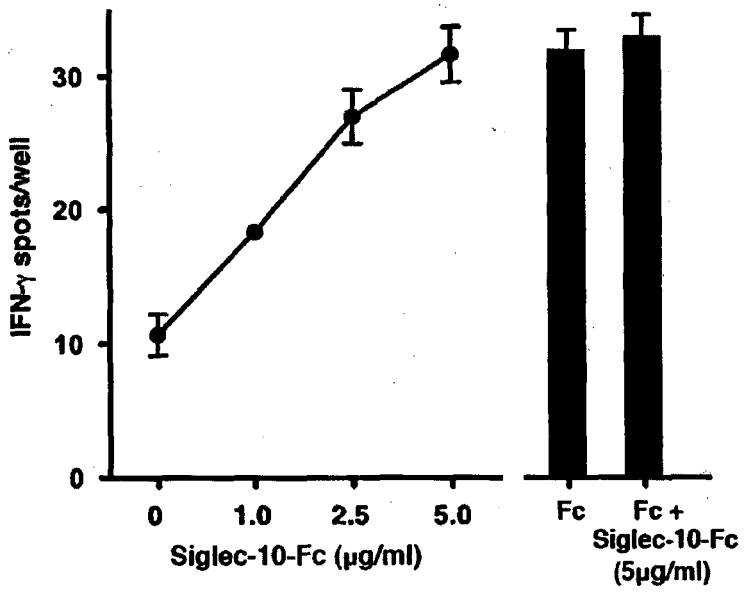


Figure 12D

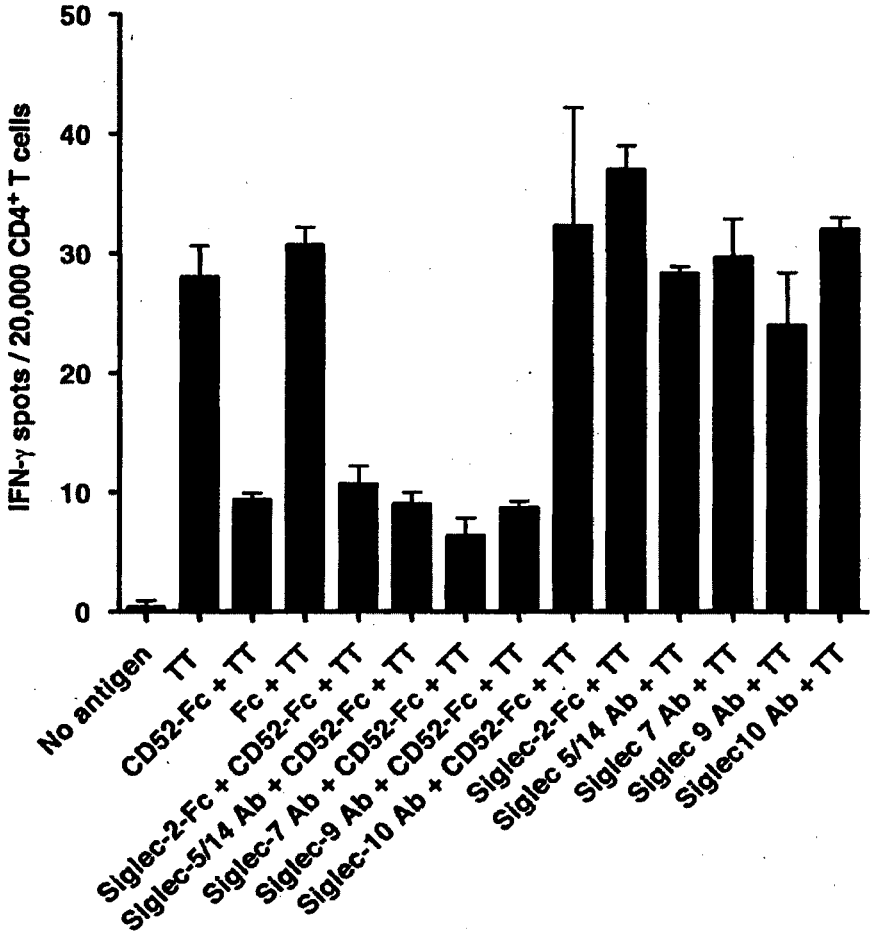


Figure 12E

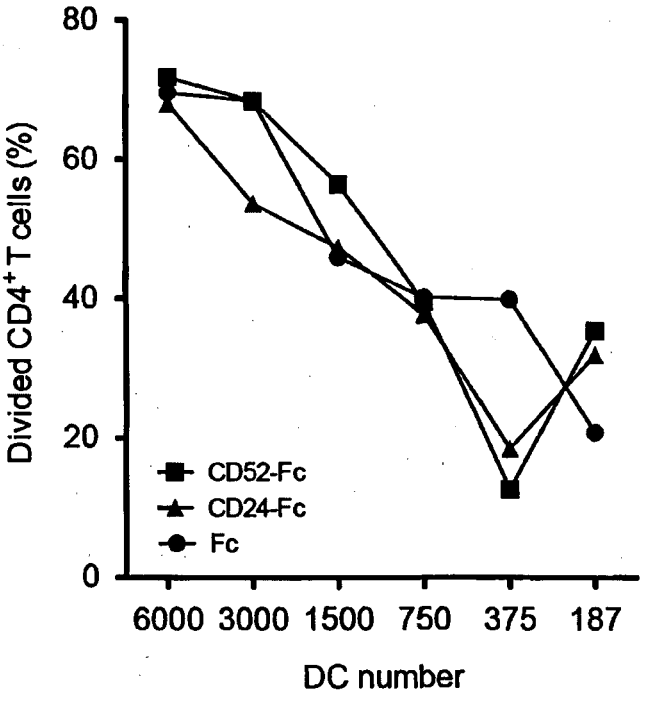
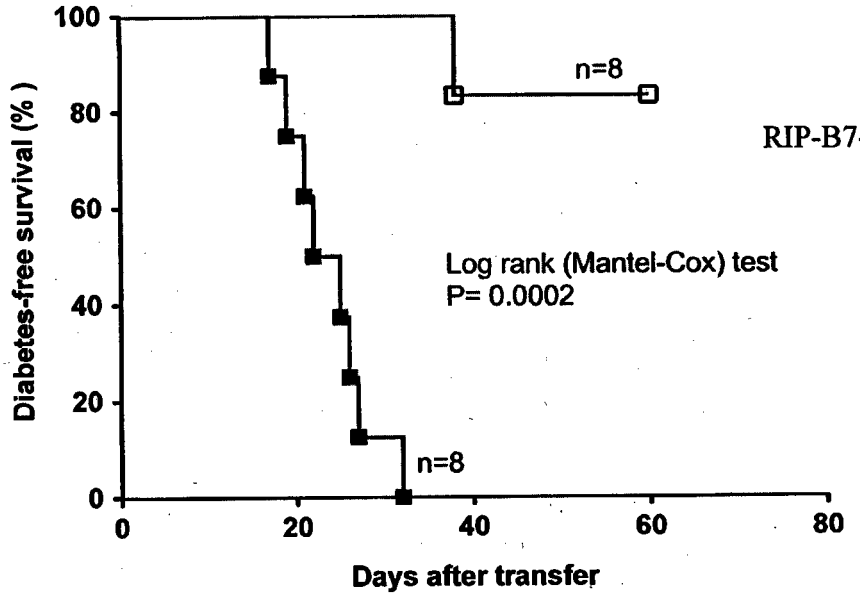


Figure 13

A


100
80
60
40
20
0
Diabetes-free survival (%)
n=8
RIP-B7-NOD.SCID
Log rank (Mantel-Cox) test
P= 0.0002
n=8
0
20
40
60
80
Days after transfer

B

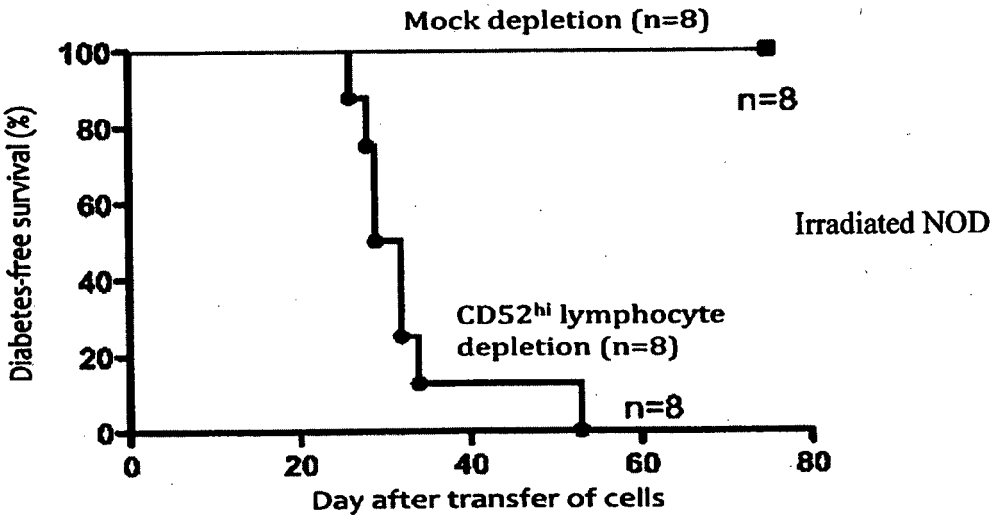
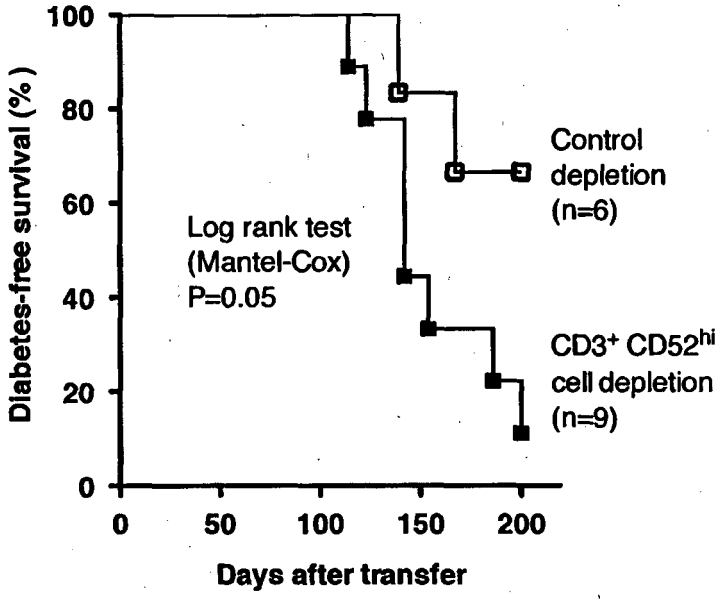

Mock depletion (n=8)
100
80
60
40
20
0
Diabetes-free survival (%)
n=8
Irradiated NOD
CD52hi lymphocyte
depletion (n=8)
n=8
0
20
40
60
80
Day after transfer of cells

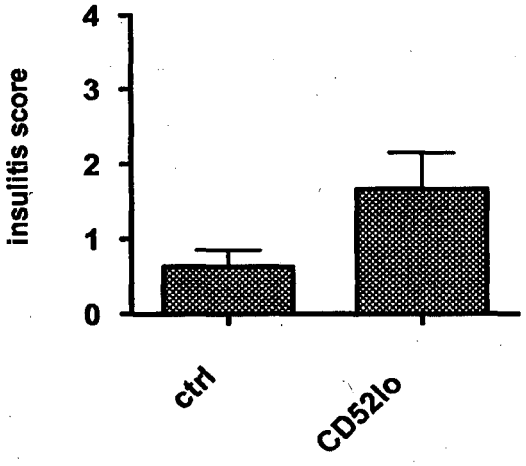
Figure 14

A



Insutitis score of male NOD mice injected with CD3+ T cells or CD52lo CD3+ T cells after 4 weeks

B



The insulitis score was determined as follows:
0= no infiltration,
1= peri-ductal infitrate,
2= peri-islet infiltrate,
3= intra-islet infiltrate,
4= beta cell destruction

Figure 15

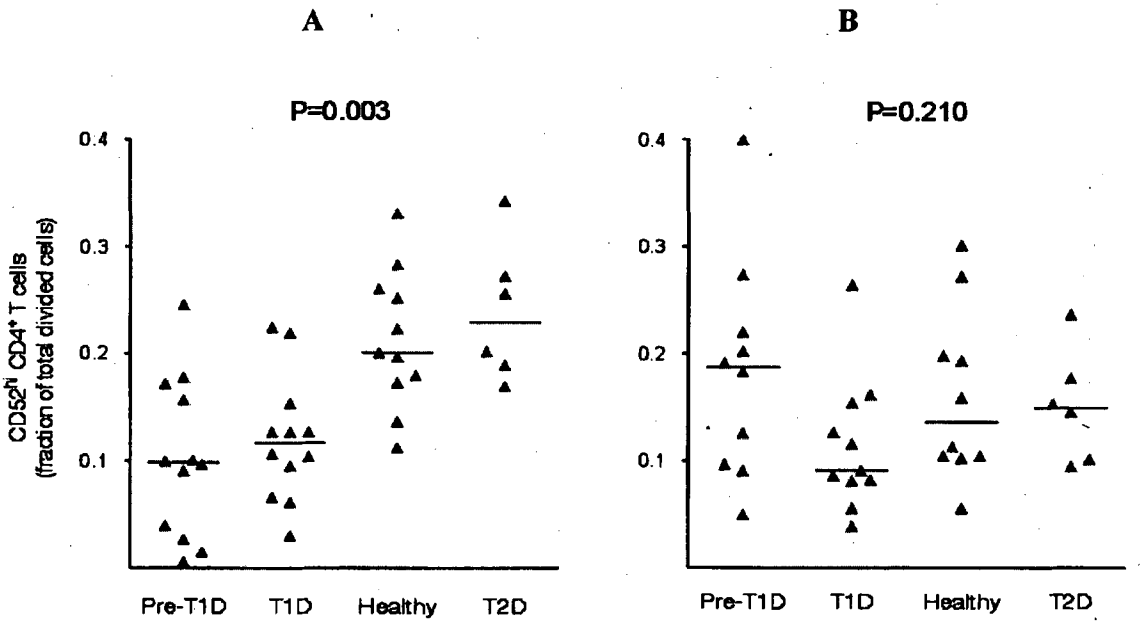


Figure 16

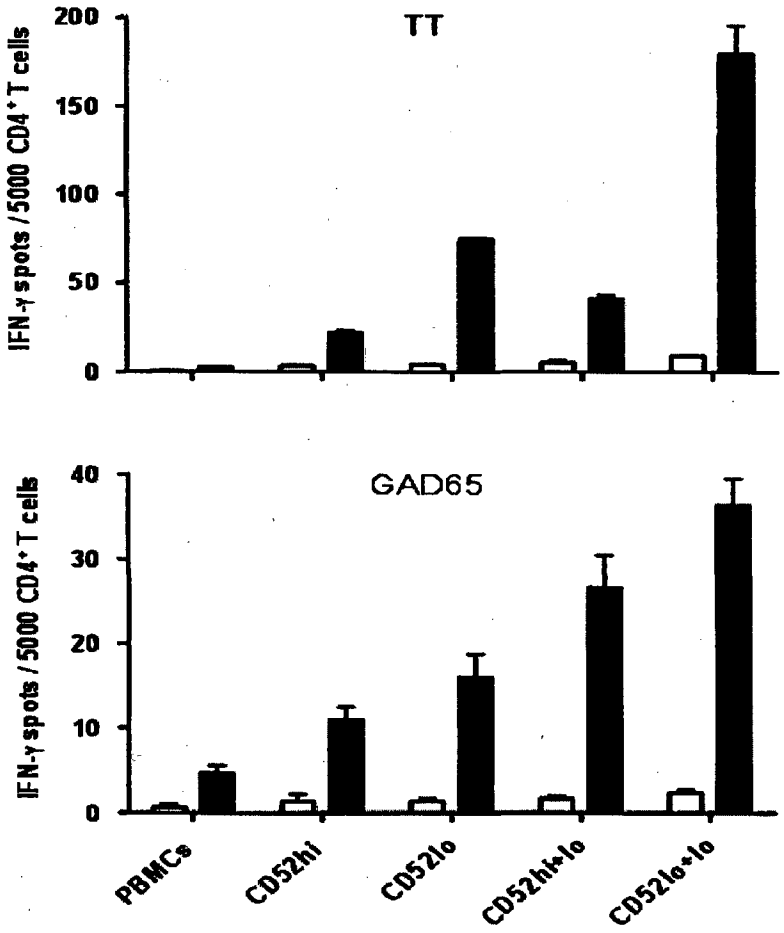
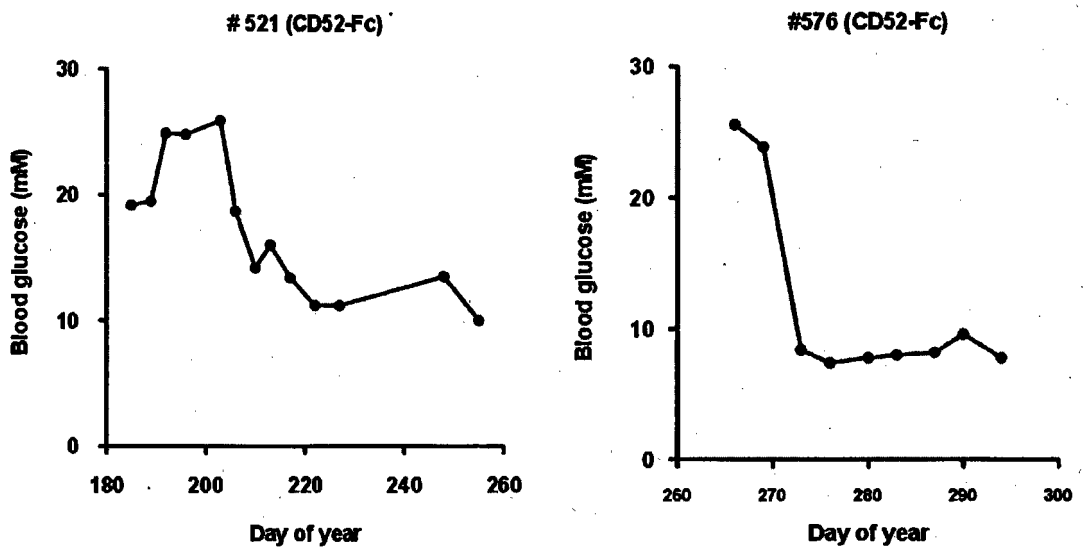


Figure 17

A



B

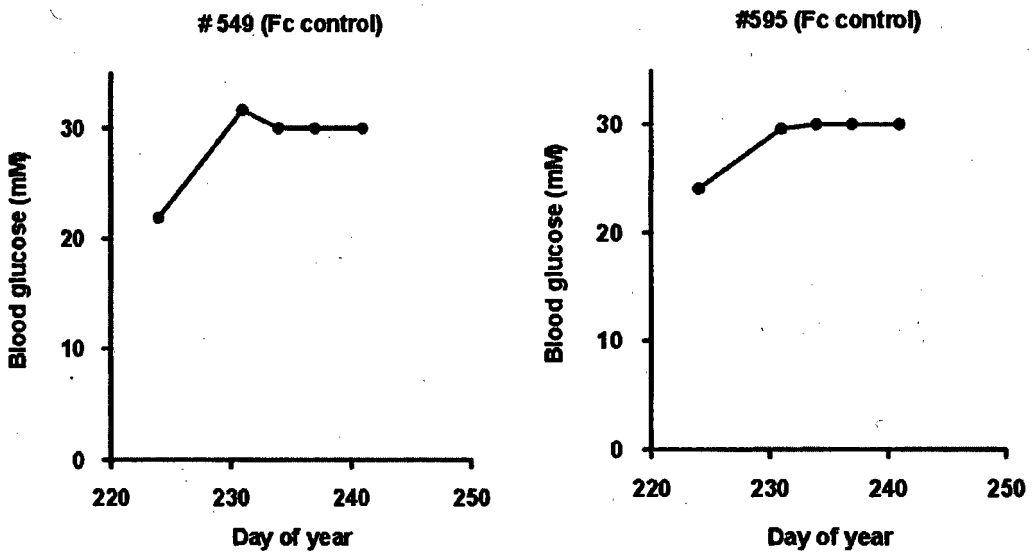


Figure 18

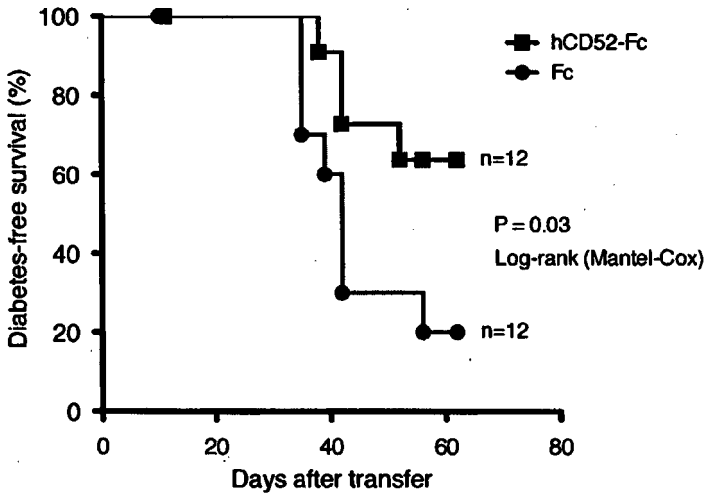


Figure 19

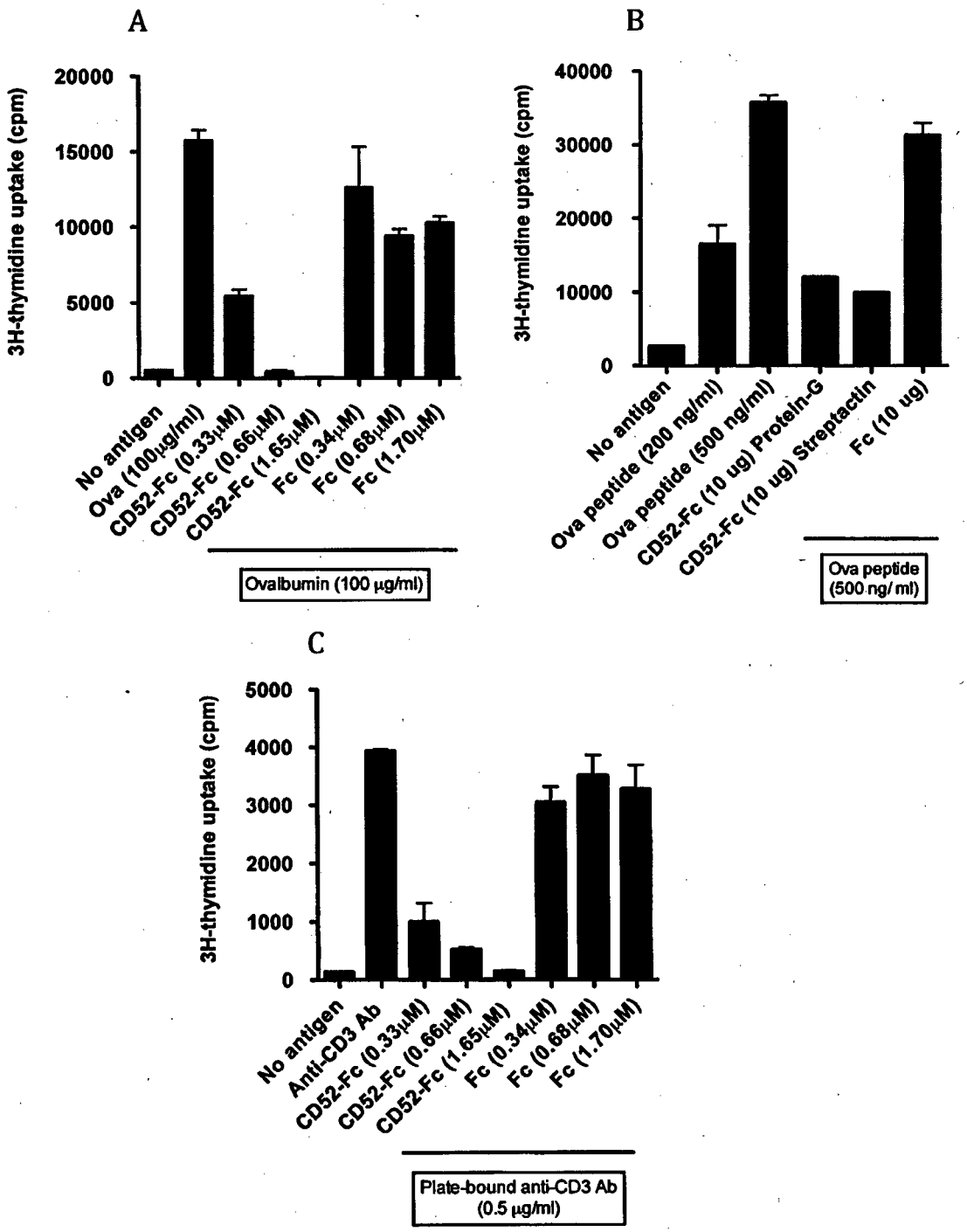

A
3H-thymidine uptake (cpm)
20000
15000
10000
5000
0
No antigen
Ova (100µg/ml)
CD52-Fc (0.33µM)
CD52-Fc (0.66µM)
CD52-Fc (1.65µM)
Fc (0.34µM)
Fc (0.68µM)
Fc (1.70µM)
Ovalbumin (100 µg/ml)
B
3H-thymidine uptake (cpm)
40000
30000
20000
10000
0
No antigen
Ova peptide (200 ng/ml)
Ova peptide (500 ng/ml)
CD52-Fc (10 ug) Protein-G
CD52-Fc (10 ug) Streptactin
Fc (10 ug)
Ova peptide (500 ng/ml)
C
3H-thymidine uptake (cpm)
5000
4000
3000
2000
1000
0
No antigen
Anti-CD3 Ab
CD52-Fc (0.33µM)
CD52-Fc (0.66µM)
CD52-Fc (1.65µM)
Fc (0.34µM)
Fc (0.68µM)
Fc (1.70µM)
Plate-bound anti-CD3 Ab (0.5 µg/ml)

Figure 20

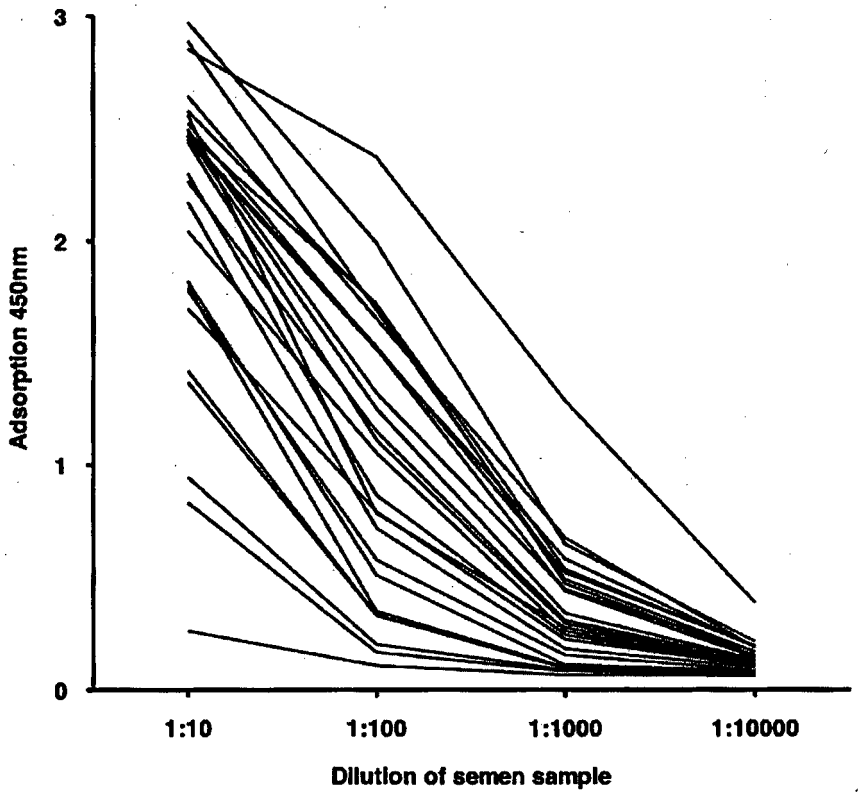


Figure 21

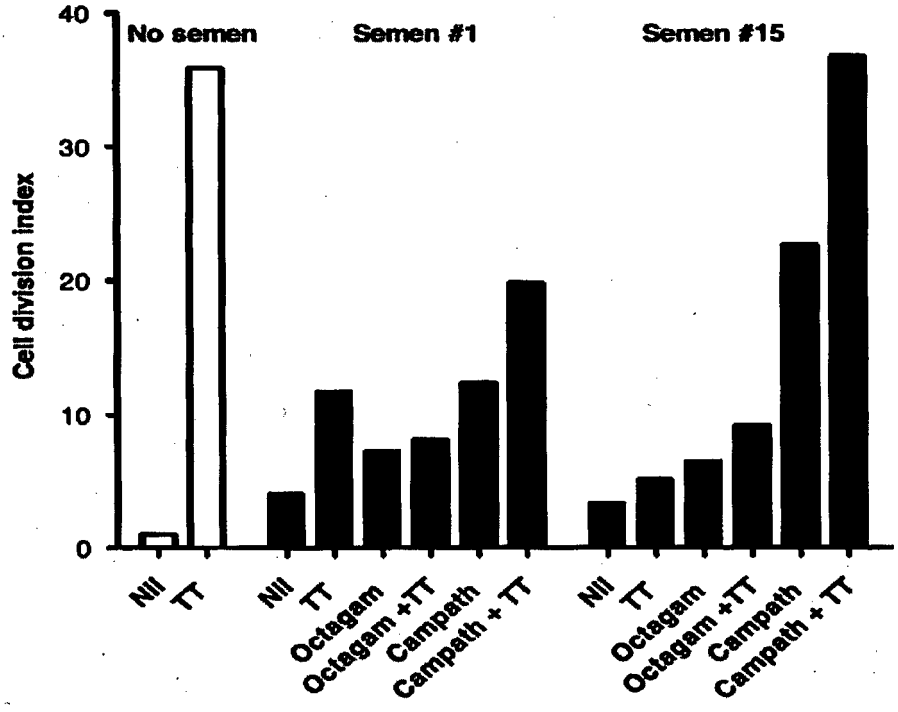


Figure 22

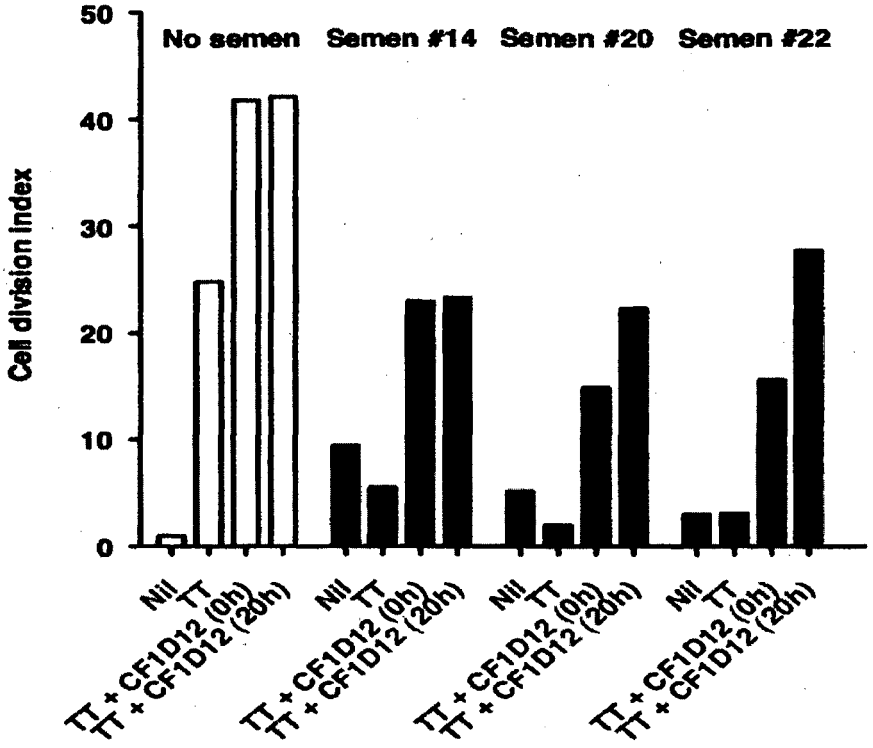


Figure 23

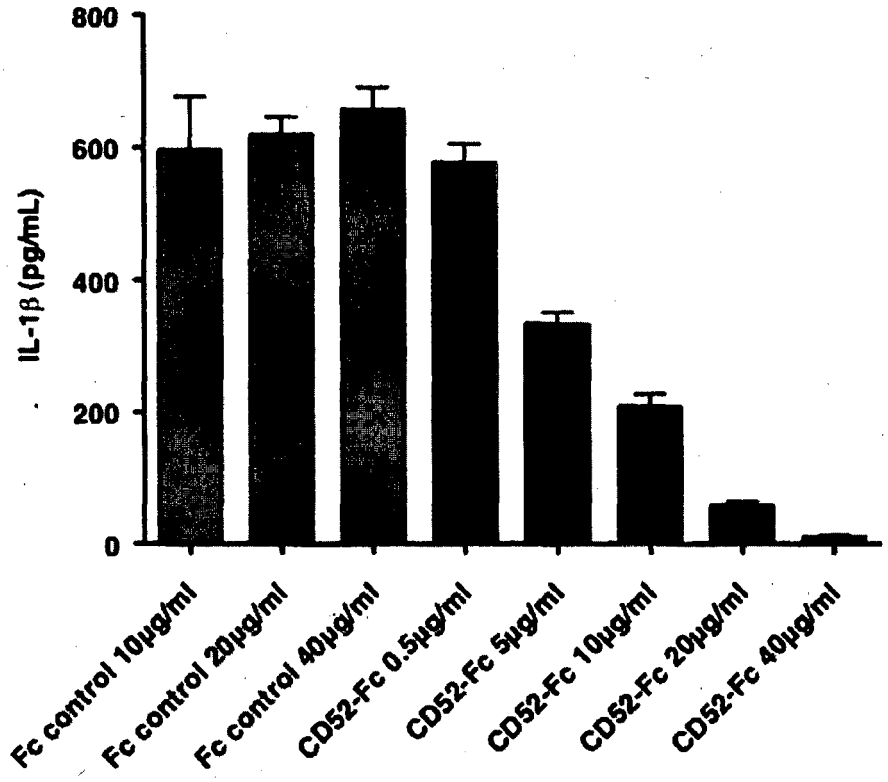


Figure 24

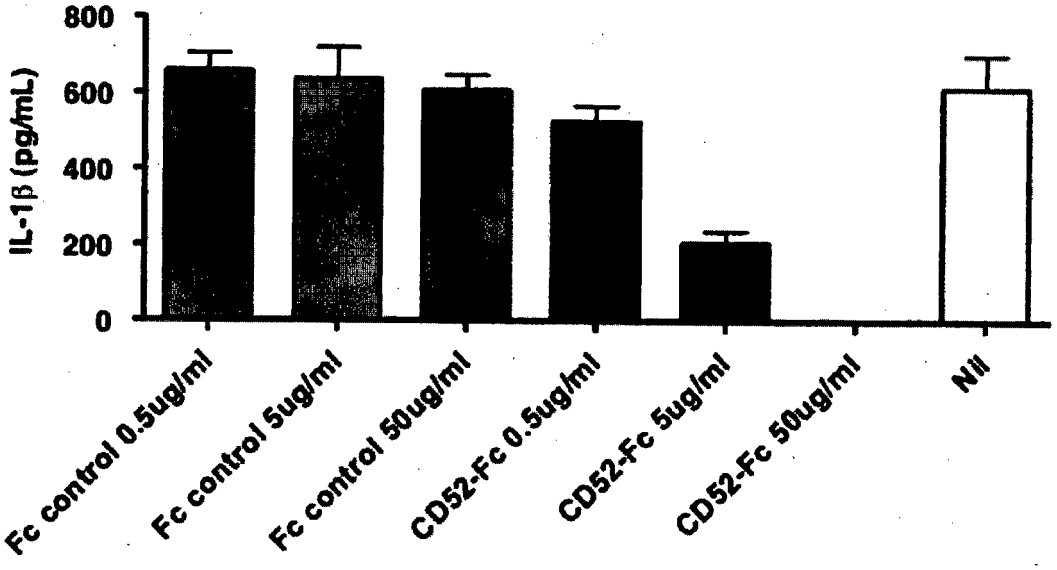


Figure 25

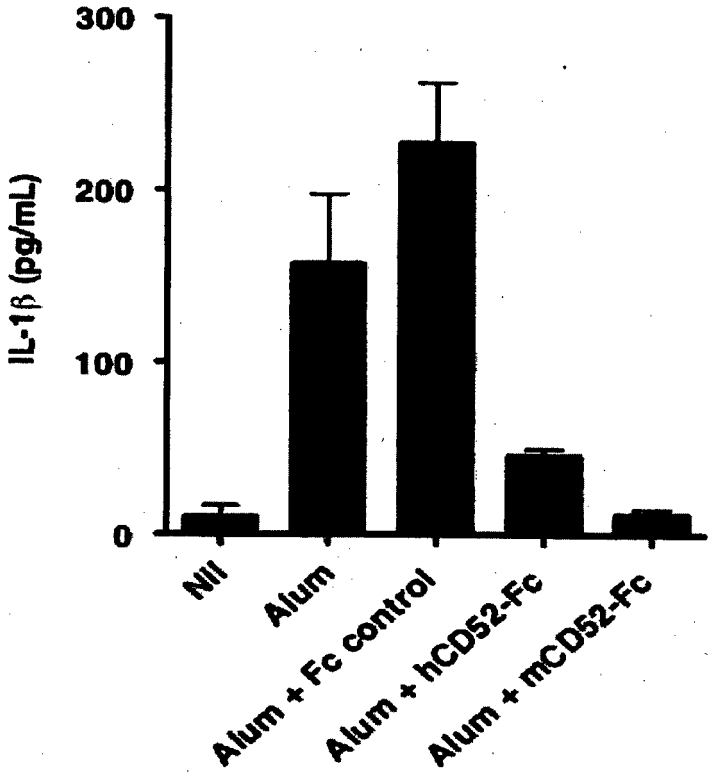


Figure 26

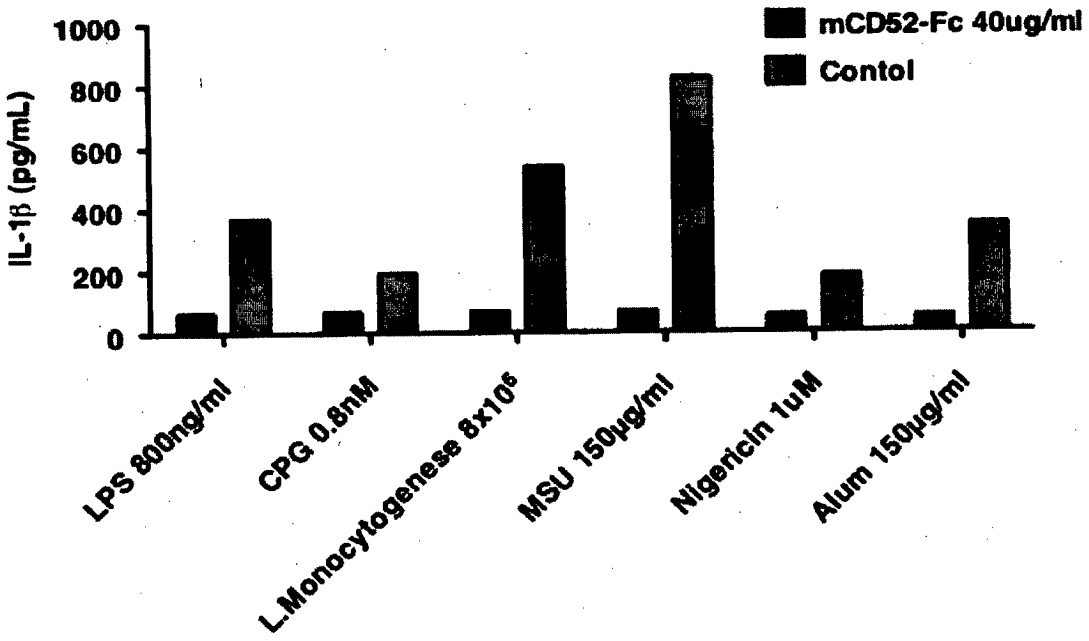


Figure 27

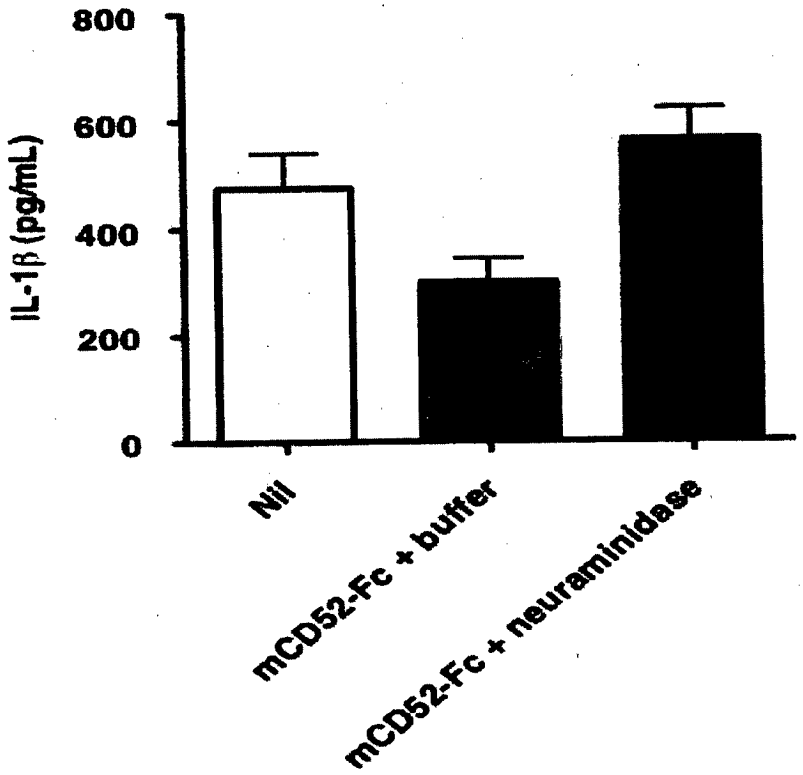


Figure 28

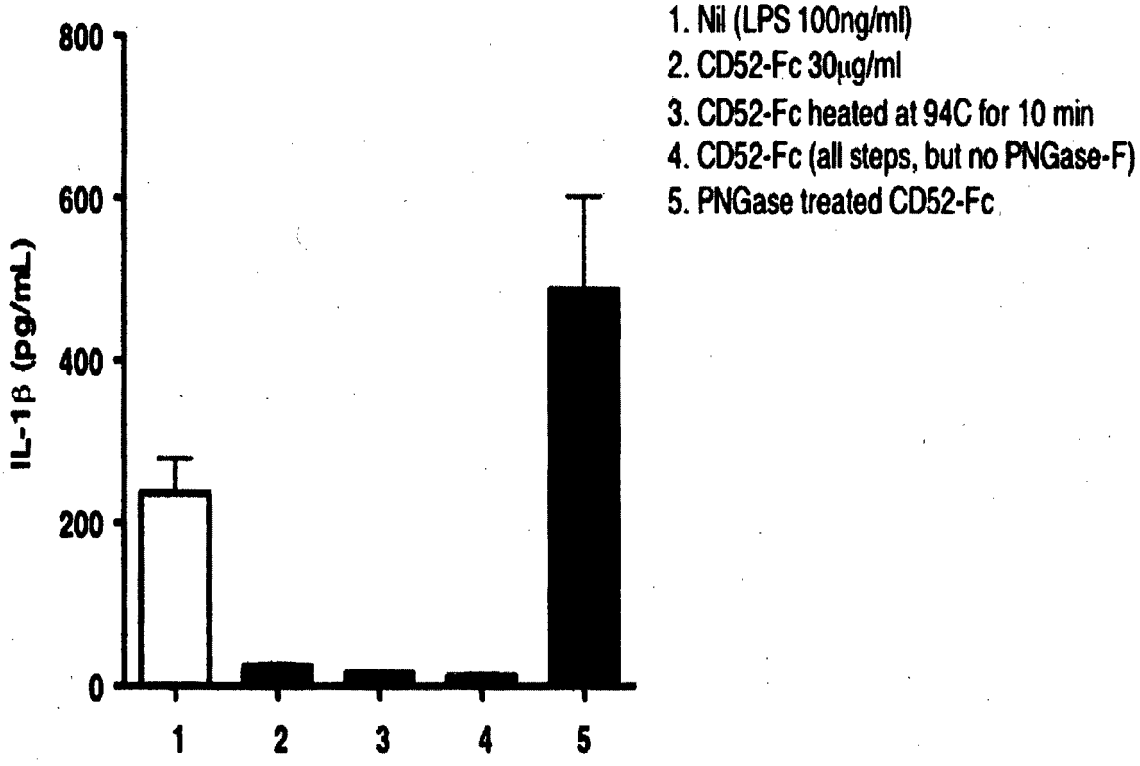

800
600
400
200
0
IL-1β (pg/mL)
1
2
3
4
5
1. Nil (LPS 100ng/ml)
2. CD52-Fc 30μg/ml
3. CD52-Fc heated at 94C for 10 min
4. CD52-Fc (all steps, but no PNGase-F)
5. PNGase treated CD52-Fc

Figure 29

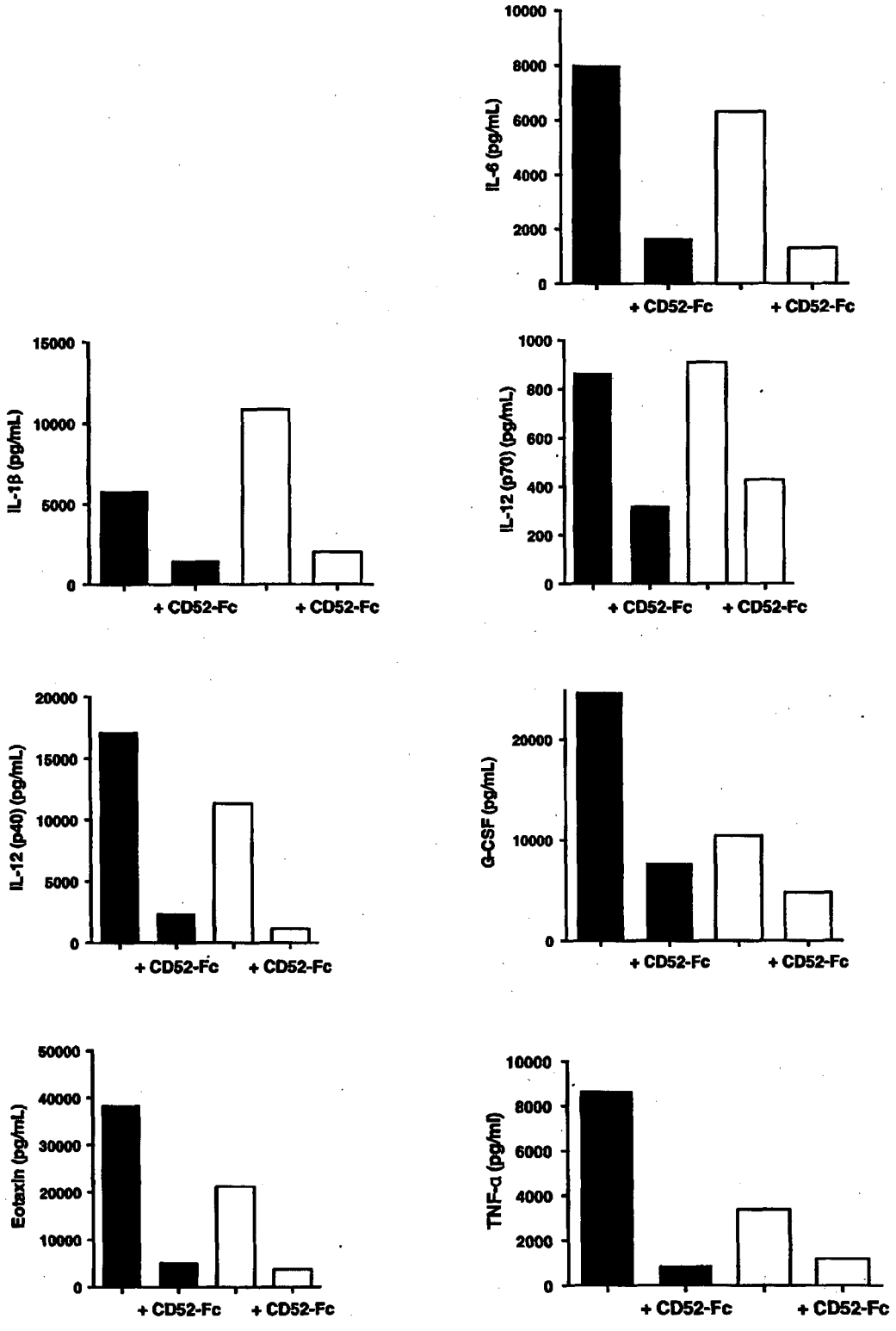

IL-6 (pg/mL)
10000
8000
6000
4000
2000
0
+ CD52-Fc
+ CD52-Fc
IL-1β (pg/mL)
15000
10000
5000
0
+ CD52-Fc
+ CD52-Fc
IL-12 (p70) (pg/mL)
1000
800
600
400
200
0
+ CD52-Fc
+ CD52-Fc
IL-12 (p40) (pg/mL)
20000
15000
10000
5000
0
+ CD52-Fc
+ CD52-Fc
G-CSF (pg/mL)
20000
10000
0
+ CD52-Fc
+ CD52-Fc
Eotaxin (pg/mL)
50000
40000
30000
20000
10000
0
+ CD52-Fc
+ CD52-Fc
TNF-α (pg/ml)
10000
8000
6000
4000
2000
0
+ CD52-Fc
+ CD52-Fc

Figure 30

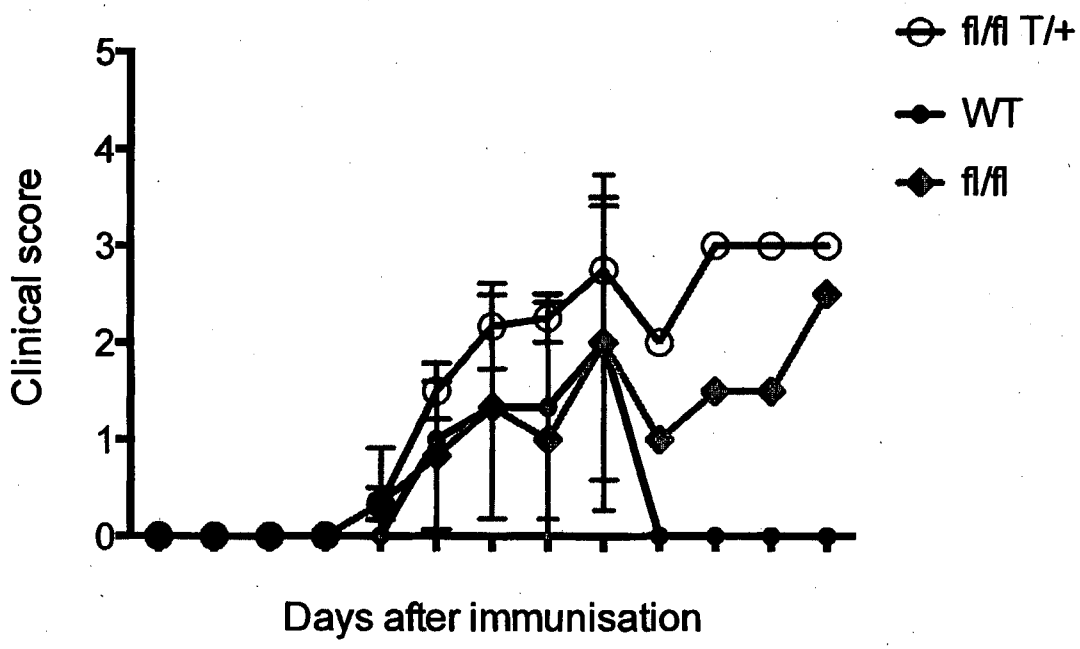

fl/fl T/+
WT
fl/fl
Clinical score
0
1
2
3
4
5
Days after immunisation

Figure 31