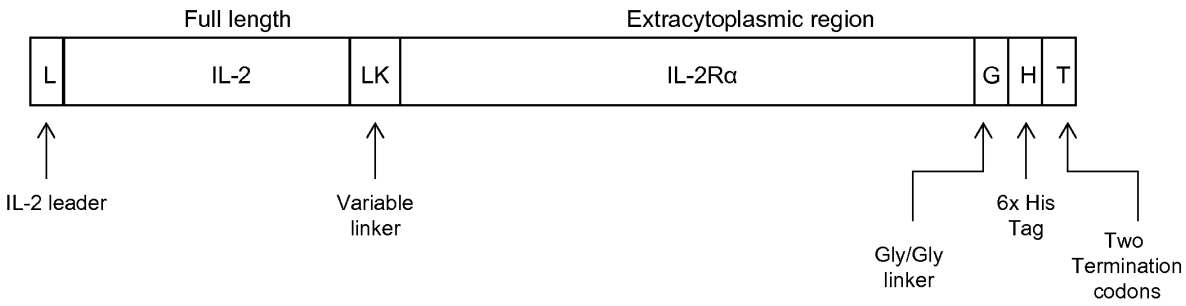

Full length
Extracytoplasmic region
L
IL-2
LK
IL-2Rα
G
H
T
IL-2 leader
Variable linker
Gly/Gly linker
6x His Tag
Two Termination codons

FIG. 1

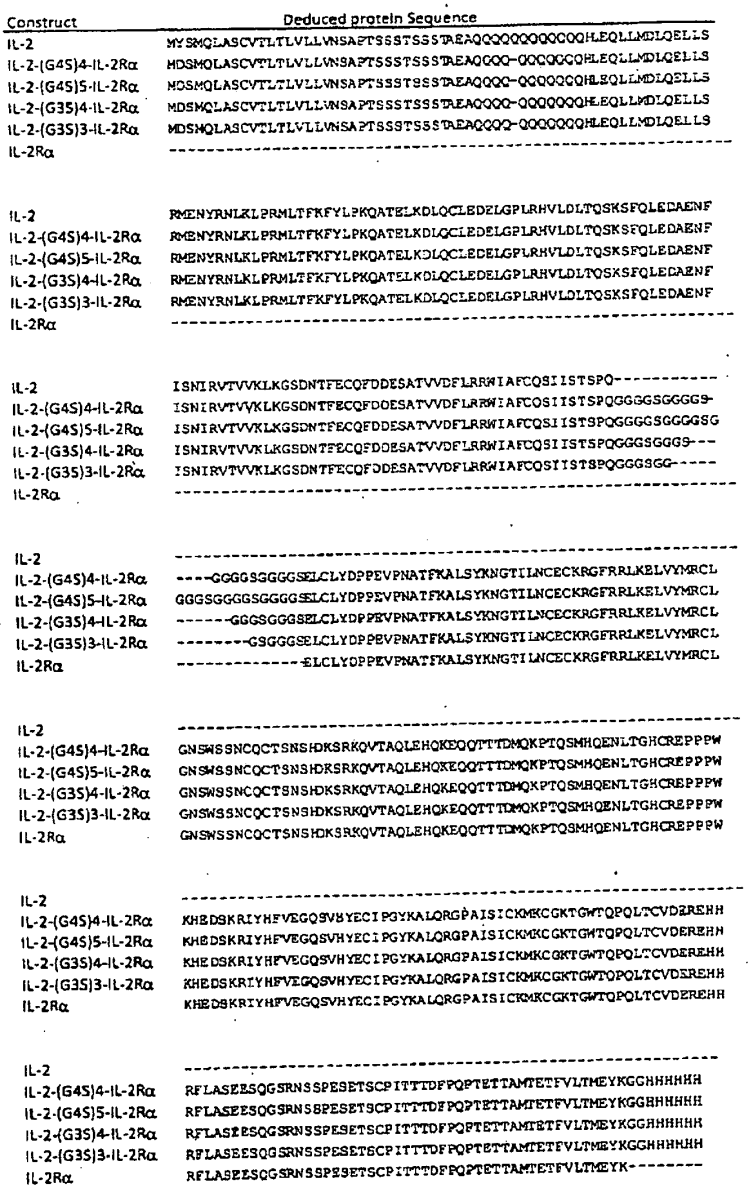

Construct
Deduced protein Sequence
IL-2
IL-2-(G4S)4-IL-2Rα
IL-2-(G4S)5-IL-2Rα
IL-2-(G3S)4-IL-2Rα
IL-2-(G3S)3-IL-2Rα
IL-2Rα
MYSMQLASCVTLTLVLLVNSAPTSSSTSSSTAEAQQQQQQQQQQQQHLEQLLMDLQELLS
MDSMQLASCVTLTLVLLVNSAPTSSSTSSSTAEAQQQ-QQQQQQQHLEQLLMDLQELLS
MDSMQLASCVTLTLVLLVNSAPTSSSTSSSTAEAQQQ-QQQQQQQHLEQLLMDLQELLS
MDSMQLASCVTLTLVLLVNSAPTSSSTSSSTAEAQQQ-QQQQQQQHLEQLLMDLQELLS
MDSMQLASCVTLTLVLLVNSAPTSSSTSSSTAEAQQQ-QQQQQQQHLEQLLMDLQELLS
RMENYRNLKLPRMLTFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQSKSFQLEDAENF
ISNIRVTVVKLKGSDNTFECQFDDESATVVDFLRRWIAFCQSIISTSPQ----------
ISNIRVTVVKLKGSDNTFECQFDDESATVVDFLRRWIAFCQSIISTSPQGGGGSGGGGS-
ISNIRVTVVKLKGSDNTFECQFDDESATVVDFLRRWIAFCQSIISTSPQGGGGSGGGGSG
ISNIRVTVVKLKGSDNTFECQFDDESATVVDFLRRWIAFCQSIISTSPQGGGGSGGGS---
ISNIRVTVVKLKGSDNTFECQFDDESATVVDFLRRWIAFCQSIISTSPQGGGGSGG-----
----GGGGSGGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKELVYMRCL
GGGSGGGGSGGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKELVYMRCL
------GGGSGGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKELVYMRCL
--------GSGGGGSELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKELVYMRCL
----------ELCLYDPPEVPNATFKALSYKNGTILNCECKRGFRRLKELVYMRCL
GNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTTTDMQKPTQSMHQENLTGHCREPPPW
GNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTTTDMQKPTQSMHQENLTGHCREPPPW
GNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTTTDMQKPTQSMHQENLTGHCREPPPW
GNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTTTDMQKPTQSMHQENLTGHCREPPPW
GNSWSSNCQCTSNSHDKSRKQVTAQLEHQKEQQTTTDMQKPTQSMHQENLTGHCREPPPW
KHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCGKTGWTQPQLTCVDEREHH
KHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCGKTGWTQPQLTCVDEREHH
KHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCGKTGWTQPQLTCVDEREHH
KHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCGKTGWTQPQLTCVDEREHH
KHEDSKRIYHFVEGQSVHYECIPGYKALQRGPAISICKMKCGKTGWTQPQLTCVDEREHH
RFLASEESQGSRNSSPESETSCPITTTDFPQPTETTAMTETFVLTMEYKGGHHHHHH
RFLASEESQGSRNSSPESETSCPITTTDFPQPTETTAMTETFVLTMEYKGGHHHHHH
RFLASEESQGSRNSSPESETSCPITTTDFPQPTETTAMTETFVLTMEYKGGHHHHHH
RFLASEESQGSRNSSPESETSCPITTTDFPQPTETTAMTETFVLTMEYKGGHHHHHH
RFLASEESQGSRNSSPESETSCPITTTDFPQPTETTAMTETFVLTMEYK--------

FIG. 2A

```
IL-2                MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRML
IL-2-(G3S)2-IL-2Rα  MDRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRML
IL-2-(G3S)3-IL-2Rα  MDRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRML
IL-2-(G3S)4-IL-2Rα  MDRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRML
IL-2-(G4S)4-IL-2Rα  MDRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRML
IL-2Rα              ------------------------------------------------------------

IL-2                TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE
IL-2-(G3S)2-IL-2Rα  TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQEKNFHLRPRDLISNINVIVLELKGSE
IL-2-(G3S)3-IL-2Rα  TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE
IL-2-(G3S)4-IL-2Rα  TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE
IL-2-(G4S)4-IL-2Rα  TFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE
IL-2Rα              ------------------------------------------------------------

IL-2                TTFMCEYADETATIVEFLNRWITFCQSIISTLT----------------------------
IL-2-(G3S)2-IL-2Rα  TTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGS -----------GGGSELCDDDP
IL-2-(G3S)3-IL-2Rα  TTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGSGGG ----SGGGSGGGSELCDDDP
IL-2-(G3S)4-IL-2Rα  TTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGSGGG ----SGGGSGGGSELCDDDP
IL-2-(G4S)4-IL-2Rα  TTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGGSGGGGSGGGGSELCDDDP
IL-2Rα              ------------------------------------------------ ELCDDDP

IL-2                ------------------------------------------------------------
IL-2-(G3S)2-IL-2Rα  PEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR
IL-2-(G3S)3-IL-2Rα  PEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR
IL-2-(G3S)4-IL-2Rα  PEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR
IL-2-(G4S)4-IL-2Rα  PEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR
IL-2Rα              PEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATR

IL-2                ------------------------------------------------------------
IL-2-(G3S)2-IL-2Rα  NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERIYHFVVGQMV
IL-2-(G3S)3-IL-2Rα  NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERIYHFVVGQMV
IL-2-(G3S)4-IL-2Rα  NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERIYHFVVGQMV
IL-2-(G4S)4-IL-2Rα  NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERIYHFVVGQMV
IL-2Rα              NTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERIYHFVVGQMV

IL-2                ------------------------------------------------------------
IL-2-(G3S)2-IL-2Rα  YYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQFPGEEKPQASPEGRPE
IL-2-(G3S)3-IL-2Rα  YYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQFPGEEKPQASPEGRPE
IL-2-(G3S)4-IL-2Rα  YYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQFPGEEKPQASPEGRPE
IL-2-(G4S)4-IL-2Rα  YYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQFPGEEKPQASPEGRPE
IL-2Rα              YYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGEMETSQFPGEEKPQASPEGRPE

IL-2                ----------------------------------------
IL-2-(G3S)2-IL-2Rα  SETSCLVTTTDFQIQTEMAATMETSIFTTEYQGGHHHHHH
IL-2-(G3S)3-IL-2Rα  SETSCLVTTTDFQIQTEMAATMETSIFTTEYQGGHHHHHH
IL-2-(G3S)4-IL-2Rα  SETSCLVTTTDFQIQTEMAATMETSIFTTEYQGGHHHHHH
IL-2-(G4S)4-IL-2Rα  SETSCLVTTTDFQIQTEMAATMETSIFTTEYQGGHHHHHH
IL-2Rα              SETSCLVTTTDFQIQTEMAATMETSIFTTEYQ --------
```

FIG. 2B

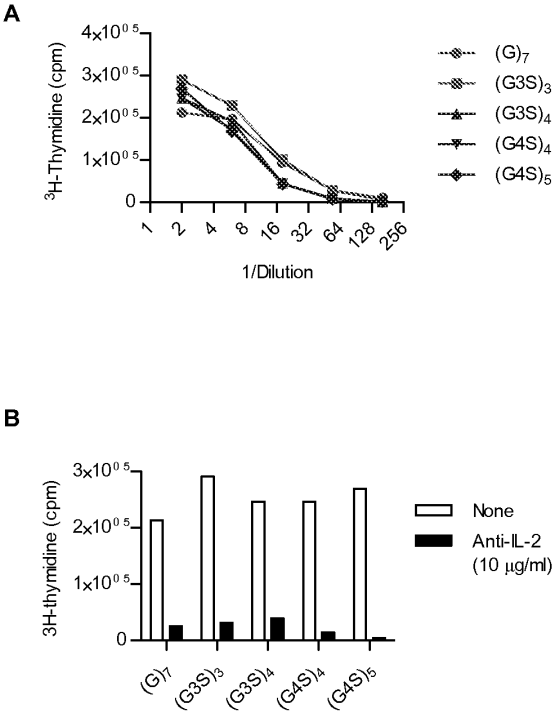

A
^{3}H-Thymidine (cpm)
4x10^{05}
3x10^{05}
2x10^{05}
1x10^{05}
0
1
2
4
8
16
32
64
128
256
1/Dilution
(G)$_7$
(G3S)$_3$
(G3S)$_4$
(G4S)$_4$
(G4S)$_5$
B
3H-thymidine (cpm)
3x10^{05}
2x10^{05}
1x10^{05}
0
(G)$_7$
(G3S)$_3$
(G3S)$_4$
(G4S)$_4$
(G4S)$_5$
None
Anti-IL-2
(10 μg/ml)

FIG. 3

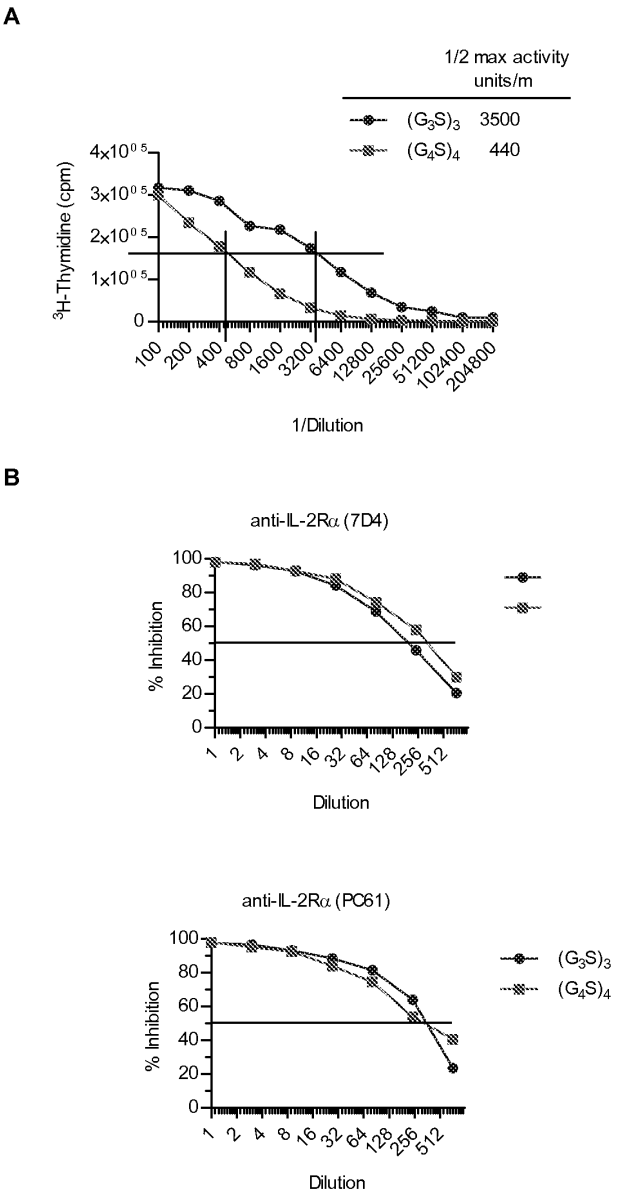

A
1/2 max activity units/m
$(G_3S)_3$ 3500
$(G_4S)_4$ 440
^{3}H-Thymidine (cpm)
4x10^05
3x10^05
2x10^05
1x10^05
0
100
200
400
800
1600
3200
6400
12800
25600
51200
102400
204800
1/Dilution
B
anti-IL-2Rα (7D4)
% Inhibition
100
80
60
40
20
0
1
2
4
8
16
32
64
128
256
512
Dilution
anti-IL-2Rα (PC61)
% Inhibition
100
80
60
40
20
0
1
2
4
8
16
32
64
128
256
512
Dilution
$(G_3S)_3$
$(G_4S)_4$

FIG. 4

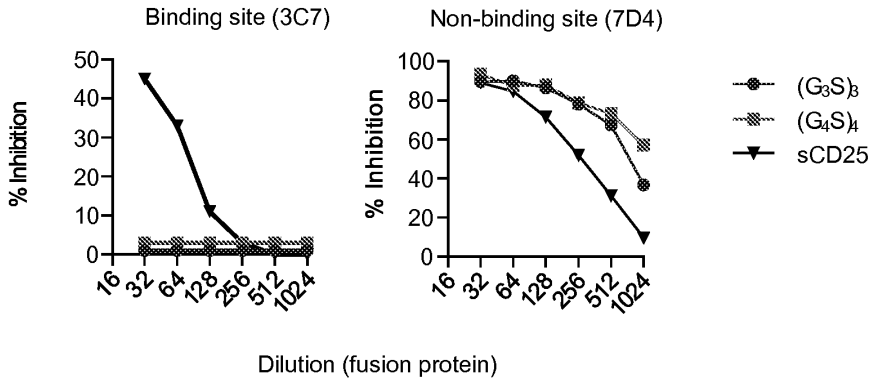

Binding site (3C7)
Non-binding site (7D4)
% Inhibition
% Inhibition
50
40
30
20
10
0
100
80
60
40
20
0
16
32
64
128
256
512
1024
Dilution (fusion protein)
(G3S)3
(G4S)4
sCD25

FIG. 5

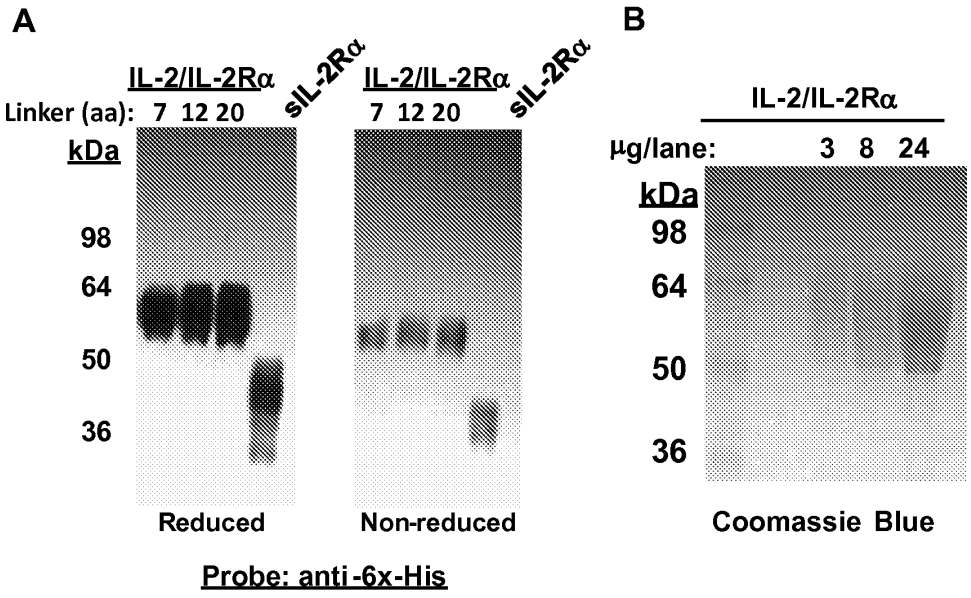

A
IL-2/IL-2Rα
sIL-2Rα
IL-2/IL-2Rα
sIL-2Rα
Linker (aa): 7 12 20
7 12 20
kDa
98
64
50
36
Reduced
Non-reduced
Probe: anti-6x-His
B
IL-2/IL-2Rα
µg/lane: 3 8 24
kDa
98
64
50
36
Coomassie Blue

FIG. 6

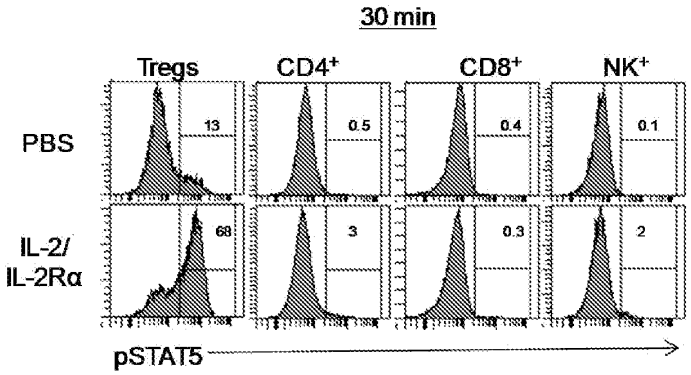


FIG. 7

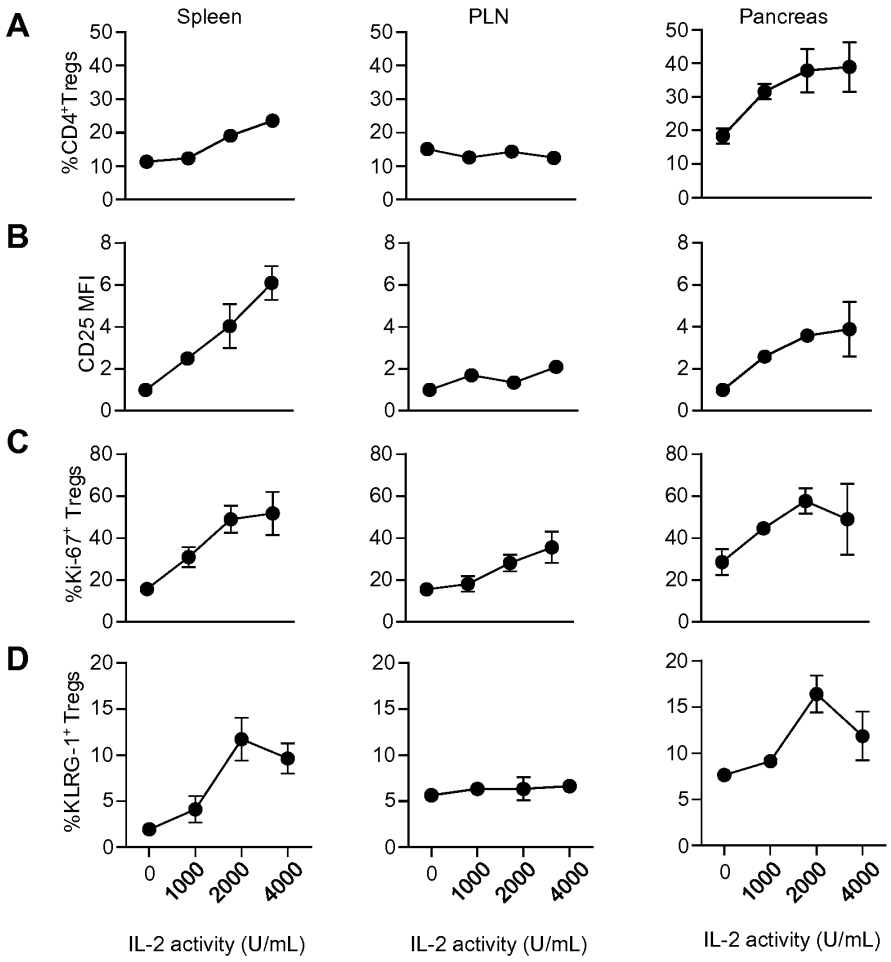

A
B
C
D
Spleen
PLN
Pancreas
%CD4+Tregs
CD25 MFI
%Ki-67+ Tregs
%KLRG-1+ Tregs
0
1000
2000
4000
IL-2 activity (U/mL)
IL-2 activity (U/mL)
IL-2 activity (U/mL)

FIG. 8

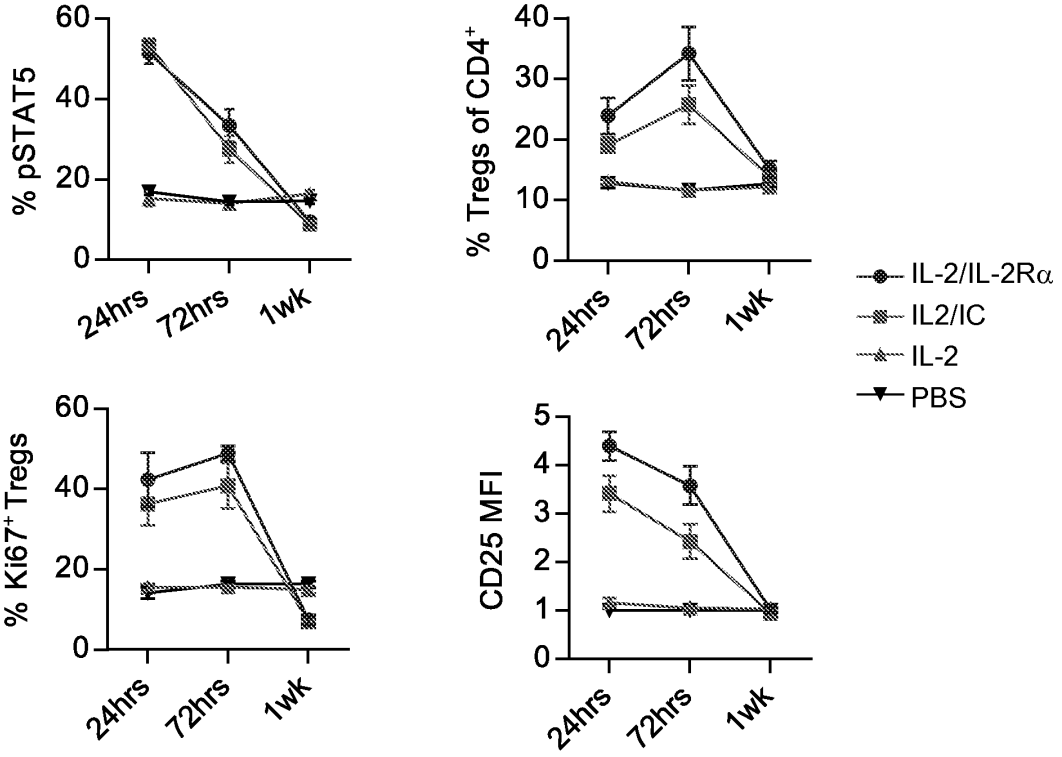

% pSTAT5
% Tregs of CD4+
% Ki67+ Tregs
CD25 MFI
24hrs
72hrs
1wk
IL-2/IL-2Rα
IL2/IC
IL-2
PBS

FIG. 9

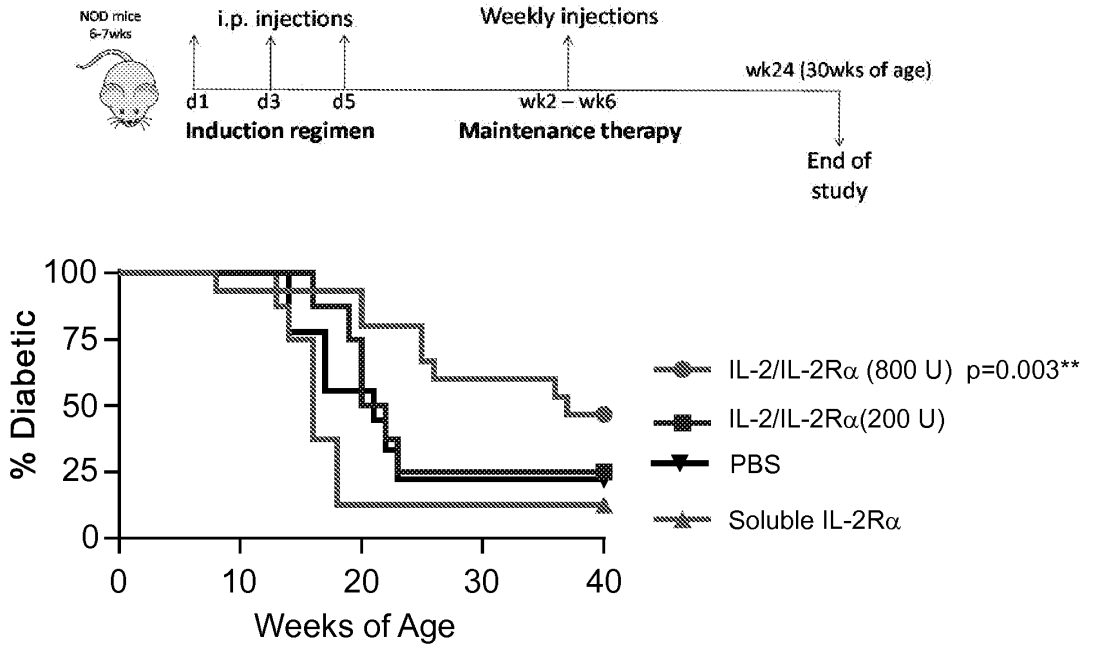

NOD mice
6-7wks
i.p. injections
Weekly injections
wk24 (30wks of age)
d1
d3
d5
wk2 – wk6
Induction regimen
Maintenance therapy
End of
study
% Diabetic
100
75
50
25
0
0
10
20
30
40
Weeks of Age
IL-2/IL-2Rα (800 U) p=0.003**
IL-2/IL-2Rα(200 U)
PBS
Soluble IL-2Rα

FIG. 10

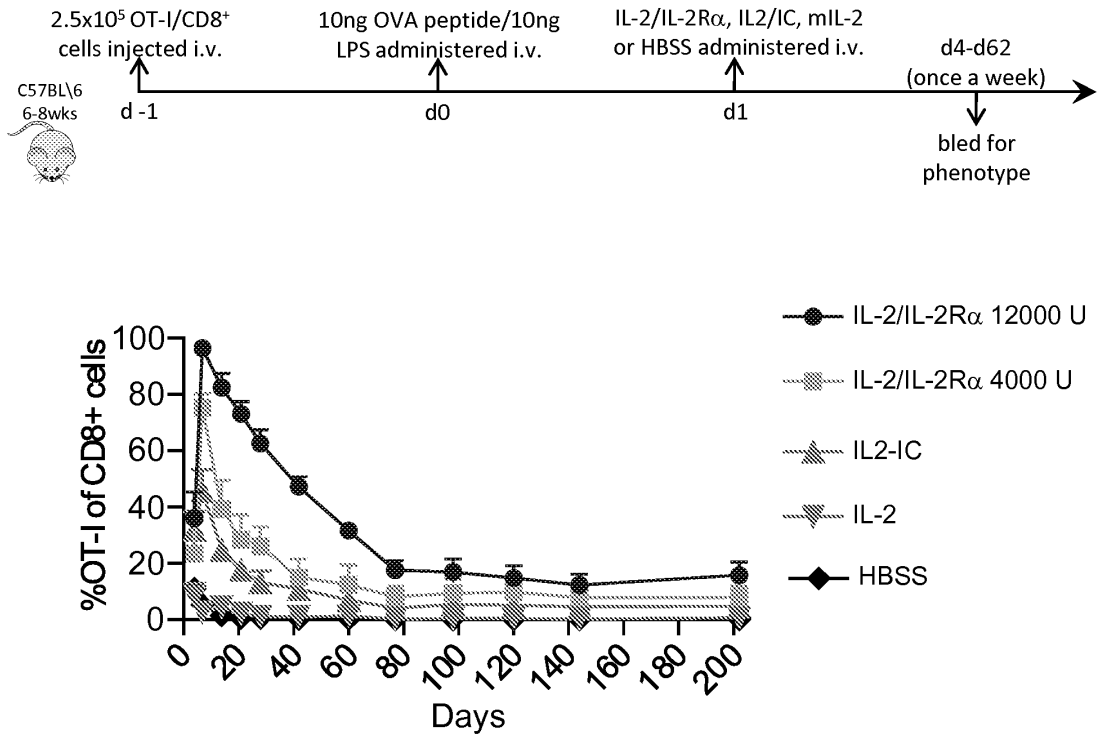

2.5x10⁵ OT-I/CD8⁺ cells injected i.v.
10ng OVA peptide/10ng LPS administered i.v.
IL-2/IL-2Rα, IL2/IC, mIL-2 or HBSS administered i.v.
d4-d62 (once a week)
C57BL\6 6-8wks
d -1
d0
d1
bled for phenotype
%OT-I of CD8+ cells
Days
IL-2/IL-2Rα 12000 U
IL-2/IL-2Rα 4000 U
IL2-IC
IL-2
HBSS

FIG. 11

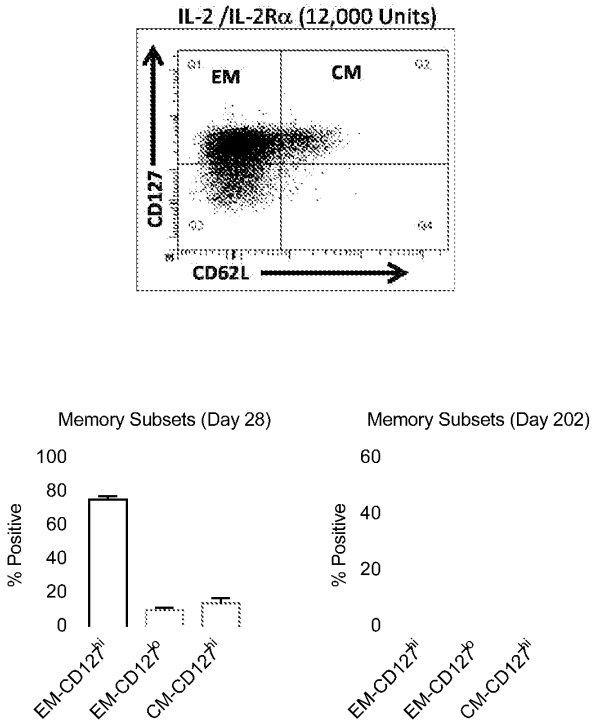
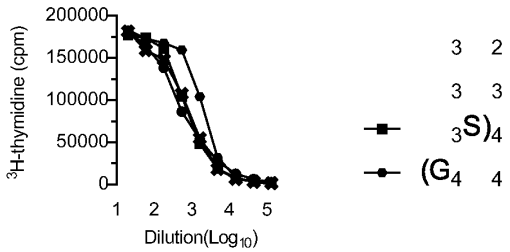

IL-2 /IL-2Rα (12,000 Units)
EM
CM
CD127
CD62L
Memory Subsets (Day 28)
% Positive
100
80
60
40
20
0
EM-CD127hi
EM-CD127lo
CM-CD127hi
Memory Subsets (Day 202)
% Positive
60
40
20
0
EM-CD127hi
EM-CD127lo
CM-CD127hi

FIG. 12


200000
150000
100000
50000
0
^{3}H-thymidine (cpm)
1 2 3 4 5
Dilution(Log$_{10}$)
3 2
3 3
$_3$S)$_4$
(G$_4$ 4

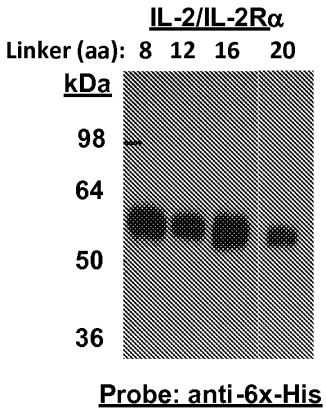

IL-2/IL-2Rα
Linker (aa): 8 12 16 20
kDa
98
64
50
36
Probe: anti-6x-His

FIG. 13

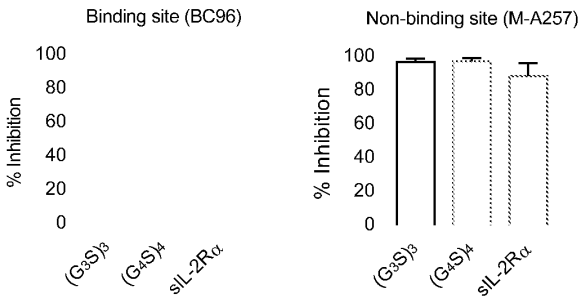

Binding site (BC96)
Non-binding site (M-A257)
% Inhibition
100
80
60
40
20
0
(G3S)3
(G4S)4
sIL-2Rα

FIG. 14

400000
300000
200000
100000
0
^{3}H-thymidine (cpm)
0 1 2 3 4 5 6 7 8
Dilution(Log$_{10}$)
$(G_3S)_2$
$(G_3S)_3$
$(G_3S)_4$
$(G_4S)_4$
$Mut(G_3S)_3$
$Mut(G_4S)_4$

FIG. 15