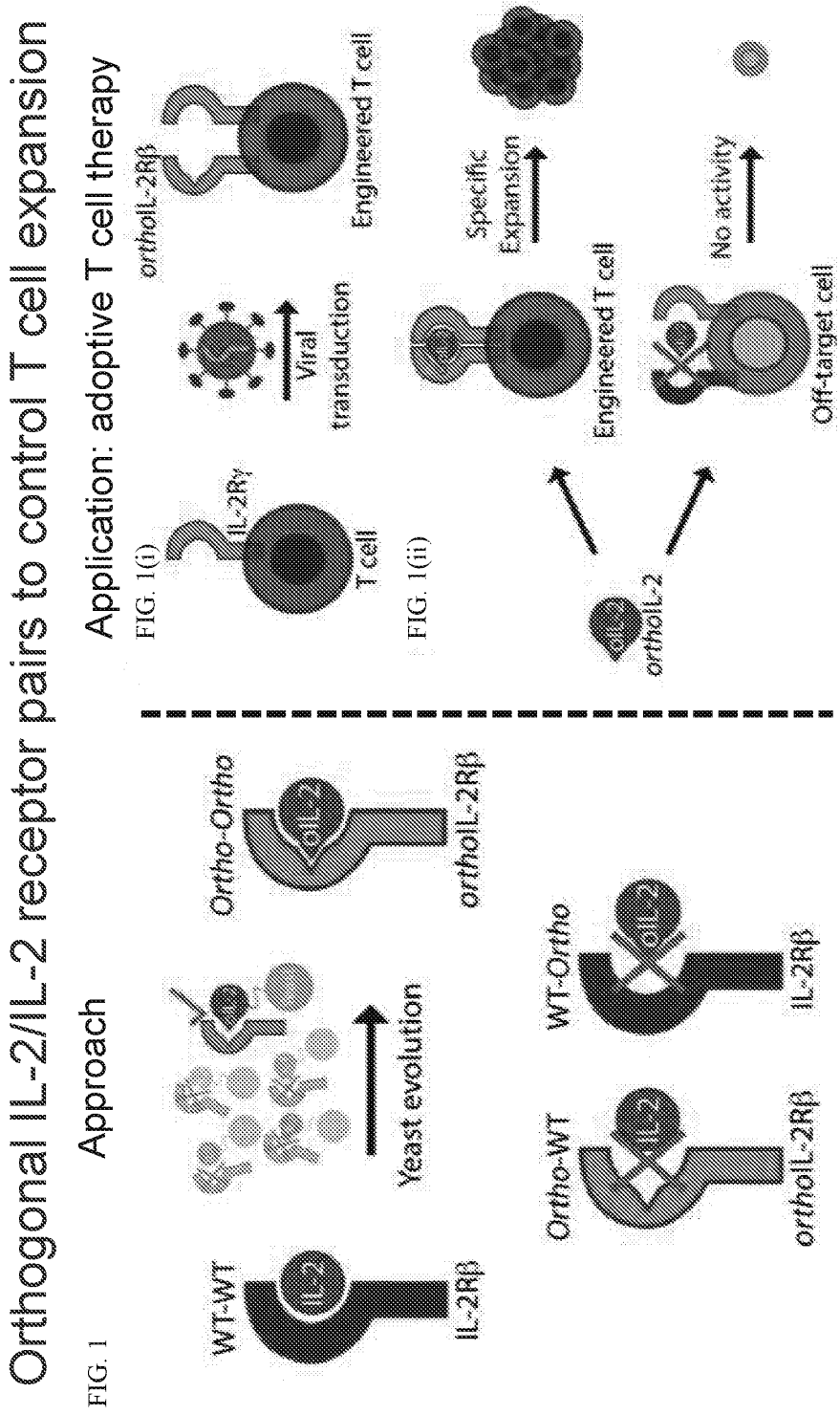
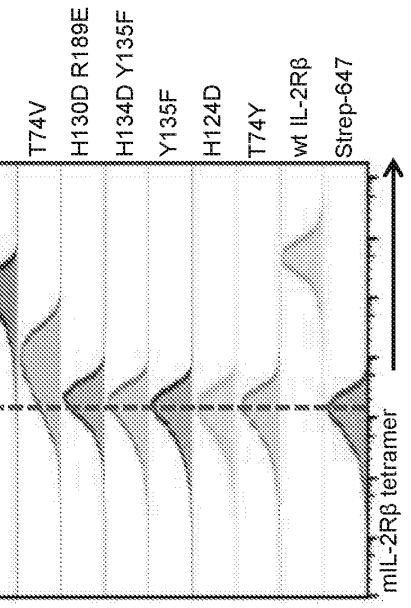

Orthogonal IL-2/IL-2 receptor pairs to control T cell expansion
Approach
FIG. 1
WT-WT
IL-2
IL-2Rβ
Yeast evolution
Ortho-Ortho
orthoIL-2
orthoIL-2Rβ
Ortho-WT
orthoIL-2Rβ
WT-Ortho
IL-2Rβ
Application: adoptive T cell therapy
FIG. 1(i)
IL-2Rγ
T cell
Viral transduction
orthoIL-2Rβ
Engineered T cell
FIG. 1(ii)
orthoIL-2
Engineered T cell
Specific Expansion
Off-target cell
No activity


R189E
T74V
H130D R189E
H134D Y135F
Y135F
H124D
T74Y
wt IL-2Rβ
Strep-647
mIL-2Rβ tetramer

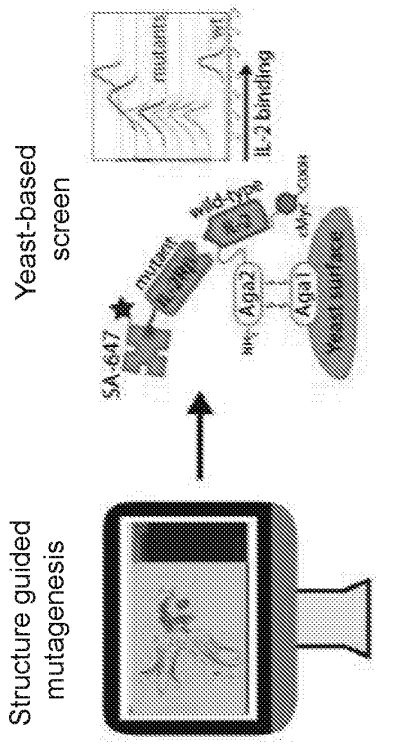

Structure guided mutagenesis
Yeast-based screen
SA-647
mutant
wild-type
Aga2
Aga1
IL-2 binding

FIG. 2

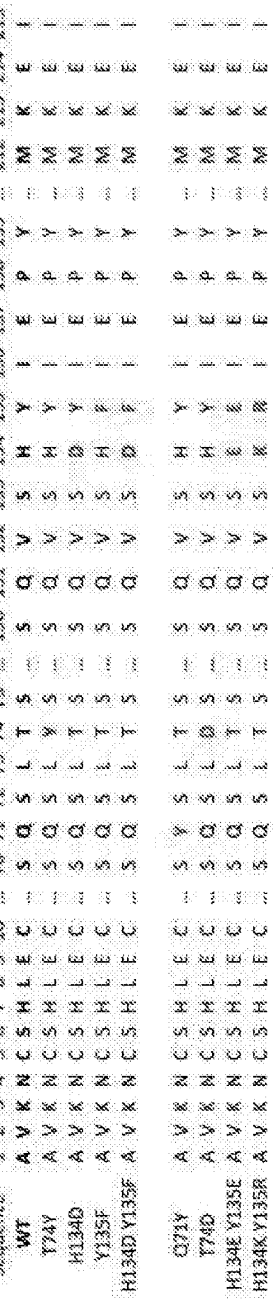

Validated to disrupt wild-type IL-2 binding
Sequence
WT
T74Y
H134D
Y135F
H134D Y135F
Q71Y
T74D
H134E Y135E
H134K Y135R

FIG. 3

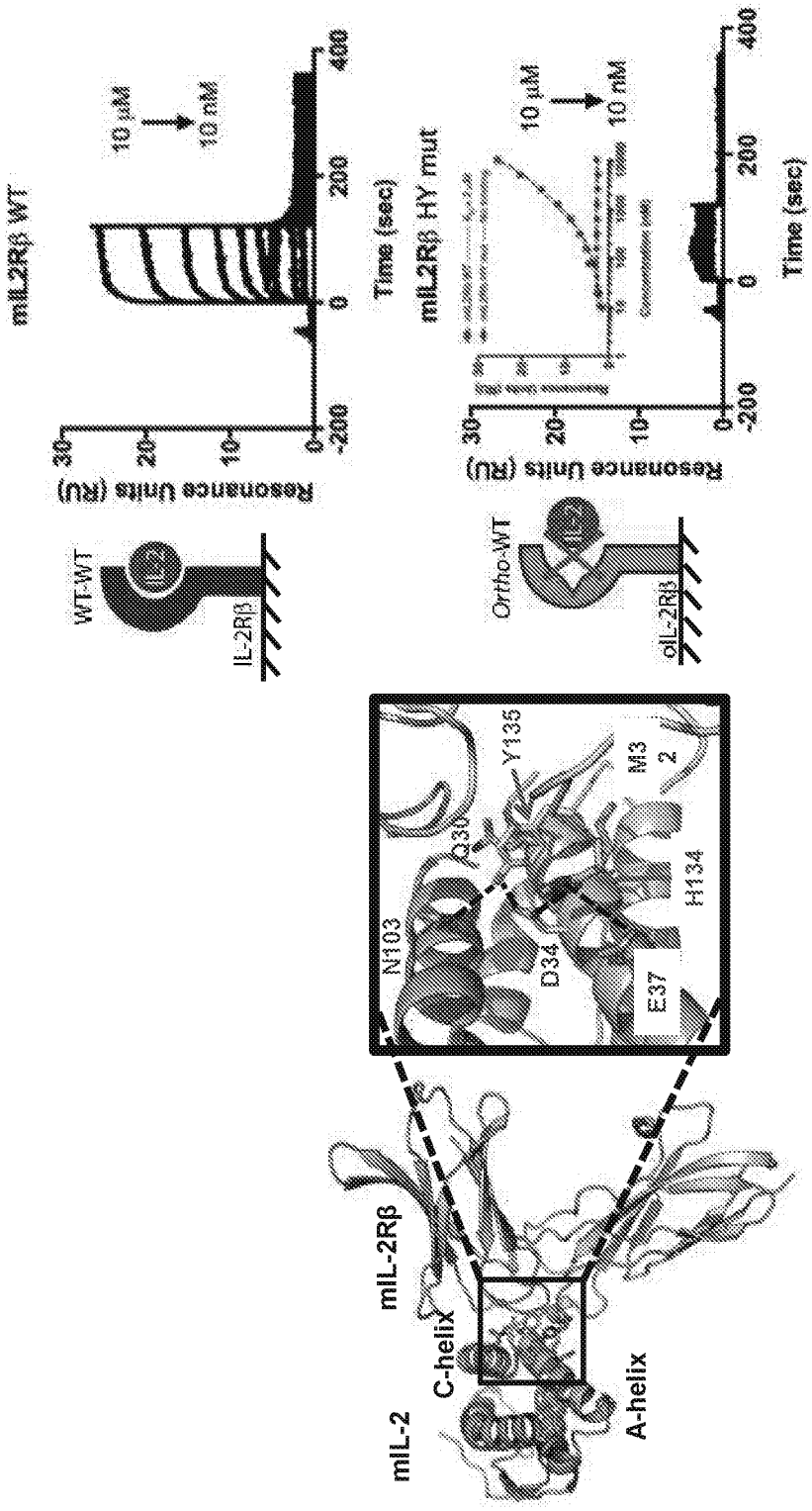

mIL2Rβ WT
10 μM
10 nM
Resonance Units (RU)
Time (sec)
WT-WT
IL-2Rβ
mIL2Rβ HY mut
Ortho-WT
oIL-2Rβ
mIL-2Rβ
C-helix
mIL-2
A-helix
N103
D34
E37
Q30
Y135
H134
M3

FIG. 4

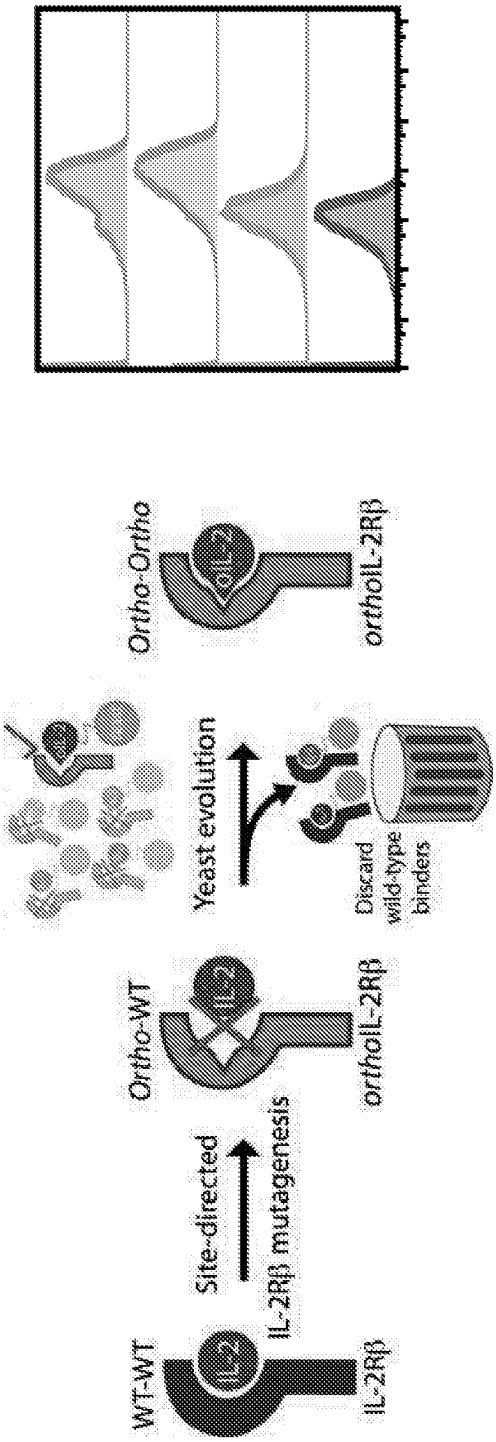
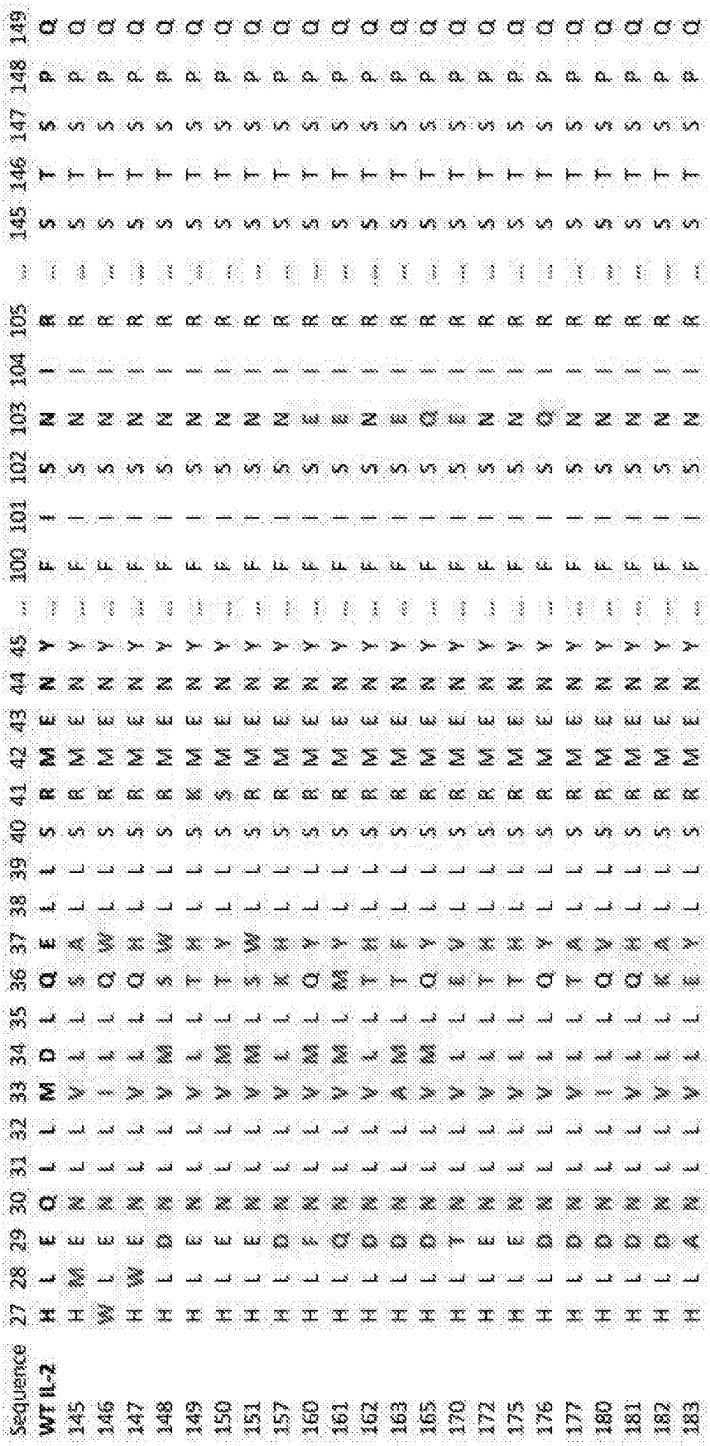

WT-WT
IL-2
IL-2Rβ
Site-directed
IL-2Rβ mutagenesis
Ortho-WT
IL-2
orthoIL-2Rβ
Yeast evolution
Discard
wild-type
binders
Ortho-Ortho
IL-2
orthoIL-2Rβ

FIG. 5



Sequence	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	...	100	101	102	103	104	105	...	145	146	147	148	149
WT IL-2	**H**	**L**	**E**	**Q**	**L**	**L**	**M**	**D**	**L**	**Q**	**E**	**L**	**L**	**S**	**R**	**M**	**E**	**N**	**Y**	...	**F**	**I**	**S**	**N**	**I**	**R**	...	**S**	**T**	**S**	**P**	**Q**
145	H	M	E	M	L	L	V	L	L	S	A	L	L	S	R	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q
146	W	L	E	M	L	L	I	L	L	Q	W	L	L	S	R	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q
147	H	W	E	M	L	L	V	L	L	Q	M	L	L	S	R	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q
148	H	L	D	M	L	L	V	M	L	S	W	L	L	S	R	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q
149	H	L	E	M	L	L	V	L	L	T	M	L	L	S	K	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q
150	H	L	E	M	L	L	V	M	L	T	Y	L	L	S	S	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q
151	H	L	E	M	L	L	V	M	L	S	W	L	L	S	R	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q
157	H	L	D	M	L	L	V	L	L	K	H	L	L	S	R	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q
160	H	L	F	M	L	L	V	M	L	Q	Y	L	L	S	R	M	E	N	Y	...	F	I	S	E	I	R	...	S	T	S	P	Q
161	H	L	Q	M	L	L	V	M	L	M	Y	L	L	S	R	M	E	N	Y	...	F	I	S	E	I	R	...	S	T	S	P	Q
162	H	L	D	M	L	L	V	L	L	T	H	L	L	S	R	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q
163	H	L	D	M	L	L	A	M	L	T	F	L	L	S	R	M	E	N	Y	...	F	I	S	E	I	R	...	S	T	S	P	Q
165	H	L	D	M	L	L	V	M	L	Q	Y	L	L	S	R	M	E	N	Y	...	F	I	S	Q	I	R	...	S	T	S	P	Q
170	H	L	T	M	L	L	V	L	L	E	V	L	L	S	R	M	E	N	Y	...	F	I	S	E	I	R	...	S	T	S	P	Q
172	H	L	E	M	L	L	V	L	L	T	H	L	L	S	R	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q
175	H	L	E	M	L	L	V	L	L	T	H	L	L	S	R	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q
176	H	L	D	M	L	L	V	L	L	Q	Y	L	L	S	R	M	E	N	Y	...	F	I	S	Q	I	R	...	S	T	S	P	Q
177	H	L	D	M	L	L	V	L	L	T	A	L	L	S	R	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q
180	H	L	D	M	L	L	I	L	L	Q	V	L	L	S	R	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q
181	H	L	D	M	L	L	V	L	L	Q	H	L	L	S	R	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q
182	H	L	D	M	L	L	V	K	L	K	A	L	L	S	R	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q
183	H	L	A	M	L	L	V	L	L	E	Y	L	L	S	R	M	E	N	Y	...	F	I	S	N	I	R	...	S	T	S	P	Q

FIG. 6

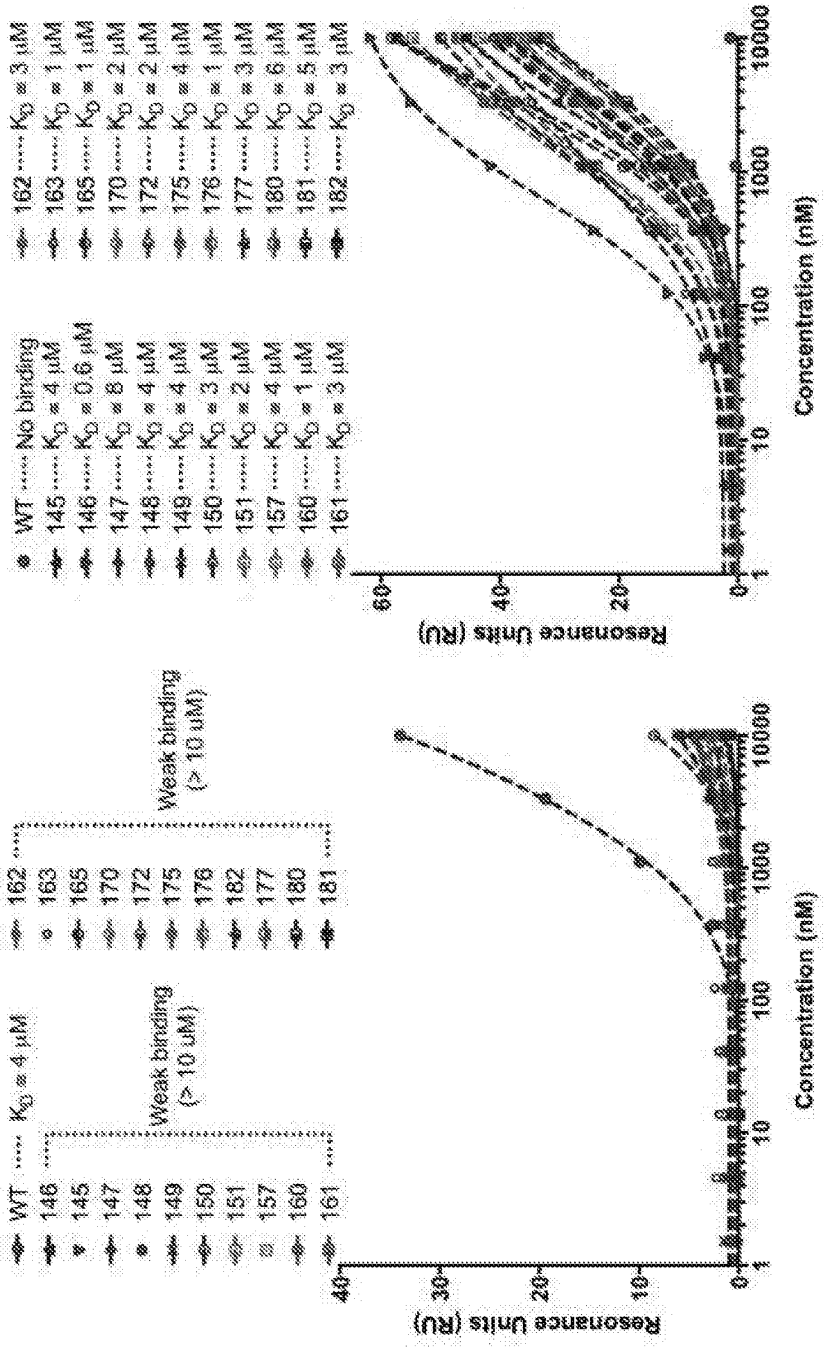

WT ..... No binding
145 ..... K_D ≈ 4 μM
146 ..... K_D ≈ 0.6 μM
147 ..... K_D ≈ 8 μM
148 ..... K_D ≈ 4 μM
149 ..... K_D ≈ 4 μM
150 ..... K_D ≈ 3 μM
151 ..... K_D ≈ 2 μM
157 ..... K_D ≈ 4 μM
160 ..... K_D ≈ 1 μM
161 ..... K_D ≈ 3 μM
162 ..... K_D ≈ 3 μM
163 ..... K_D ≈ 1 μM
165 ..... K_D ≈ 1 μM
170 ..... K_D ≈ 2 μM
172 ..... K_D ≈ 2 μM
175 ..... K_D ≈ 4 μM
176 ..... K_D ≈ 1 μM
177 ..... K_D ≈ 3 μM
180 ..... K_D ≈ 6 μM
181 ..... K_D ≈ 5 μM
182 ..... K_D ≈ 3 μM
Resonance Units (RU)
Concentration (nM)
WT ..... K_D ≈ 4 μM
146 .....
145
147
148
149
150
151
157
160
161 .....
Weak binding (> 10 uM)
162 .....
163
165
170
172
175
176
182
177
180
181 .....
Weak binding (> 10 uM)
Resonance Units (RU)
Concentration (nM)

FIG. 7

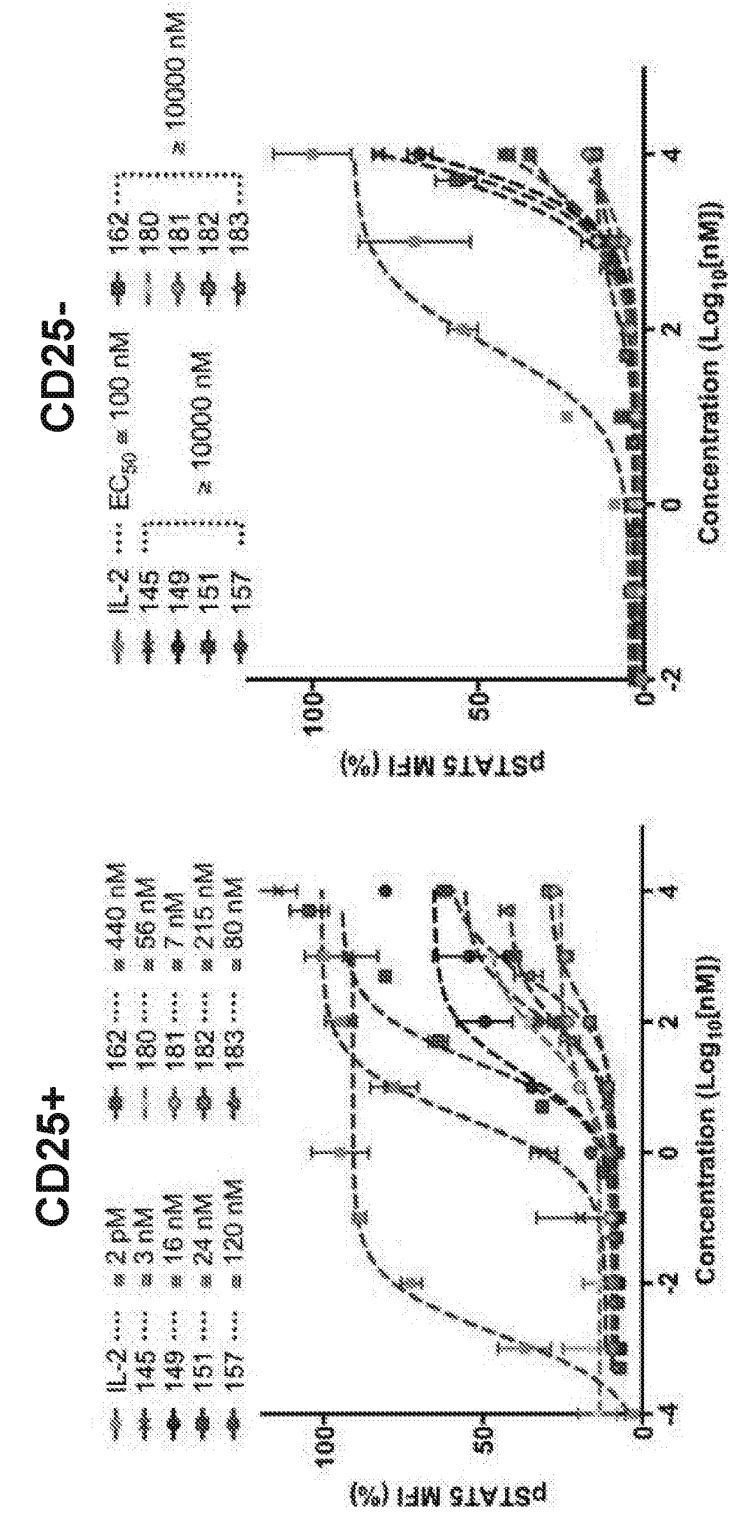

CD25+
IL-2 ≈ 2 pM
145 ≈ 3 nM
149 ≈ 16 nM
151 ≈ 24 nM
157 ≈ 120 nM
162 ≈ 440 nM
180 ≈ 56 nM
181 ≈ 7 nM
182 ≈ 215 nM
183 ≈ 80 nM
pSTAT5 MFI (%)
100
50
0
-4
-2
0
2
4
Concentration (Log10[nM])
CD25-
IL-2 EC50 ≈ 100 nM
145
149
151
157
≥ 10000 nM
162
180
181
182
183
≥ 10000 nM
pSTAT5 MFI (%)
100
50
0
-2
0
2
4
Concentration (Log10[nM])

FIG. 8

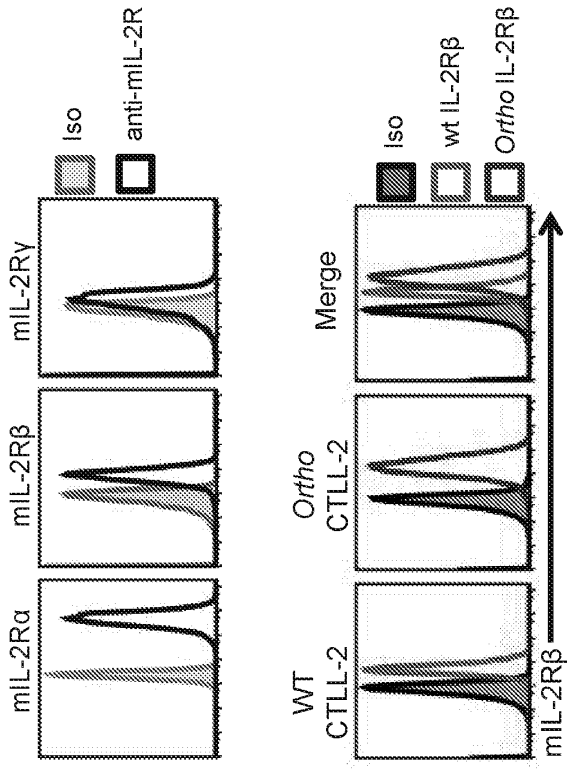

Iso
anti-mIL-2R
mIL-2Rγ
mIL-2Rβ
mIL-2Rα
Iso
wt IL-2Rβ
Ortho IL-2Rβ
Merge
Ortho CTLL-2
WT CTLL-2
mIL-2Rβ

FIG. 9

	CTLL-2 T cell line			
IL-2 clone	EC_{50} wild-type (pM)	EC_{50} ortho (pM)	WT / ortho	
WT	1.0	0.6	2	
145	1021	20	51	
146	40	2	17	
147	15	1	14	
148	4618	369	13	
149	971	15	67	
150	140	11	12	
151	3382	53	64	
157	129400	1543	84	
160	16000	2021	8	
161	19270	45760	0	
162	80640	248	325	
163	2104	1080	2	
165	39780	21720	2	
170	304	470	1	
172	555	44	13	
173	32830	9067	4	
175	831	80	10	
176	79220	15020	5	
177	89150	345	258	
180	1625	18	89	
181	4555	67	68	
182	5524	813	7	
183	15400	1121	14	

FIG. 10

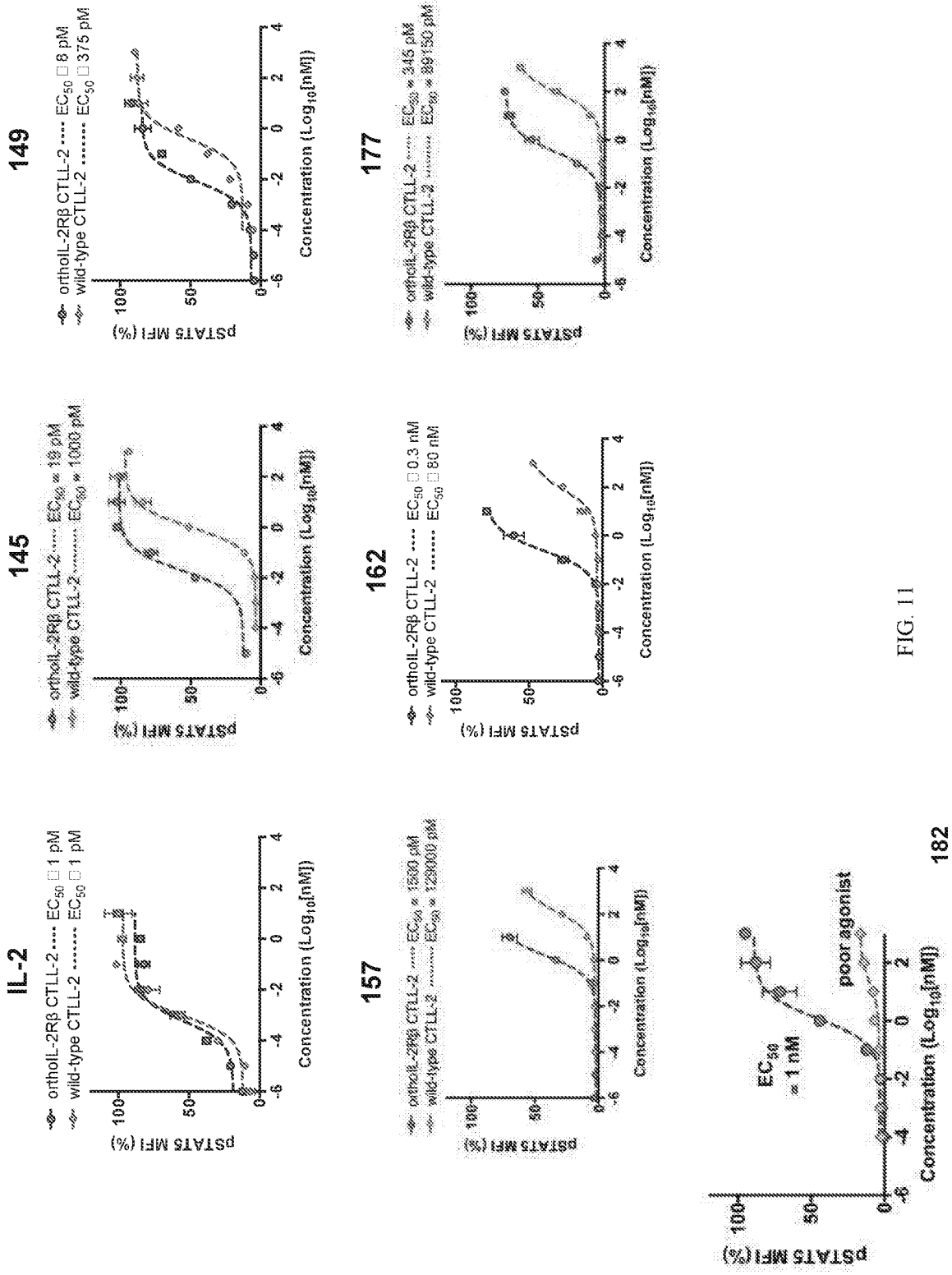

IL-2
orthoIL-2Rβ CTLL-2 ···· EC50 ≈ 1 pM
wild-type CTLL-2 ····· EC50 ≈ 1 pM
145
orthoIL-2Rβ CTLL-2 ···· EC50 ≈ 19 pM
wild-type CTLL-2 ····· EC50 ≈ 1000 pM
149
orthoIL-2Rβ CTLL-2 ···· EC50 ≈ 8 pM
wild-type CTLL-2 ····· EC50 ≈ 375 pM
157
orthoIL-2Rβ CTLL-2 ···· EC50 ≈ 1500 pM
wild-type CTLL-2 ····· EC50 ≈ 1280000 pM
162
orthoIL-2Rβ CTLL-2 ···· EC50 ≈ 0.3 nM
wild-type CTLL-2 ····· EC50 ≈ 80 nM
177
orthoIL-2Rβ CTLL-2 ···· EC50 ≈ 345 pM
wild-type CTLL-2 ····· EC50 ≈ 89150 pM
182
EC50 ≈ 1 nM
poor agonist
pSTAT5 MFI (%)
Concentration (Log10[nM])

FIG. 11

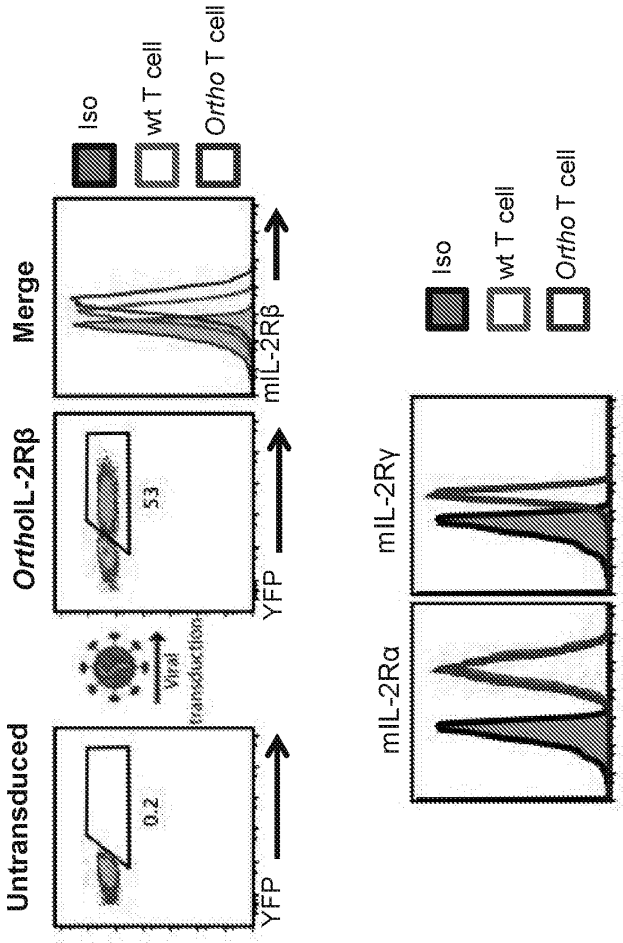

Untransduced
0.2
YFP
Viral transductions
OrthoIL-2Rβ
53
YFP
Merge
mIL-2Rβ
Iso
wt T cell
Ortho T cell
mIL-2Rα
mIL-2Rγ
Iso
wt T cell
Ortho T cell

FIG. 12

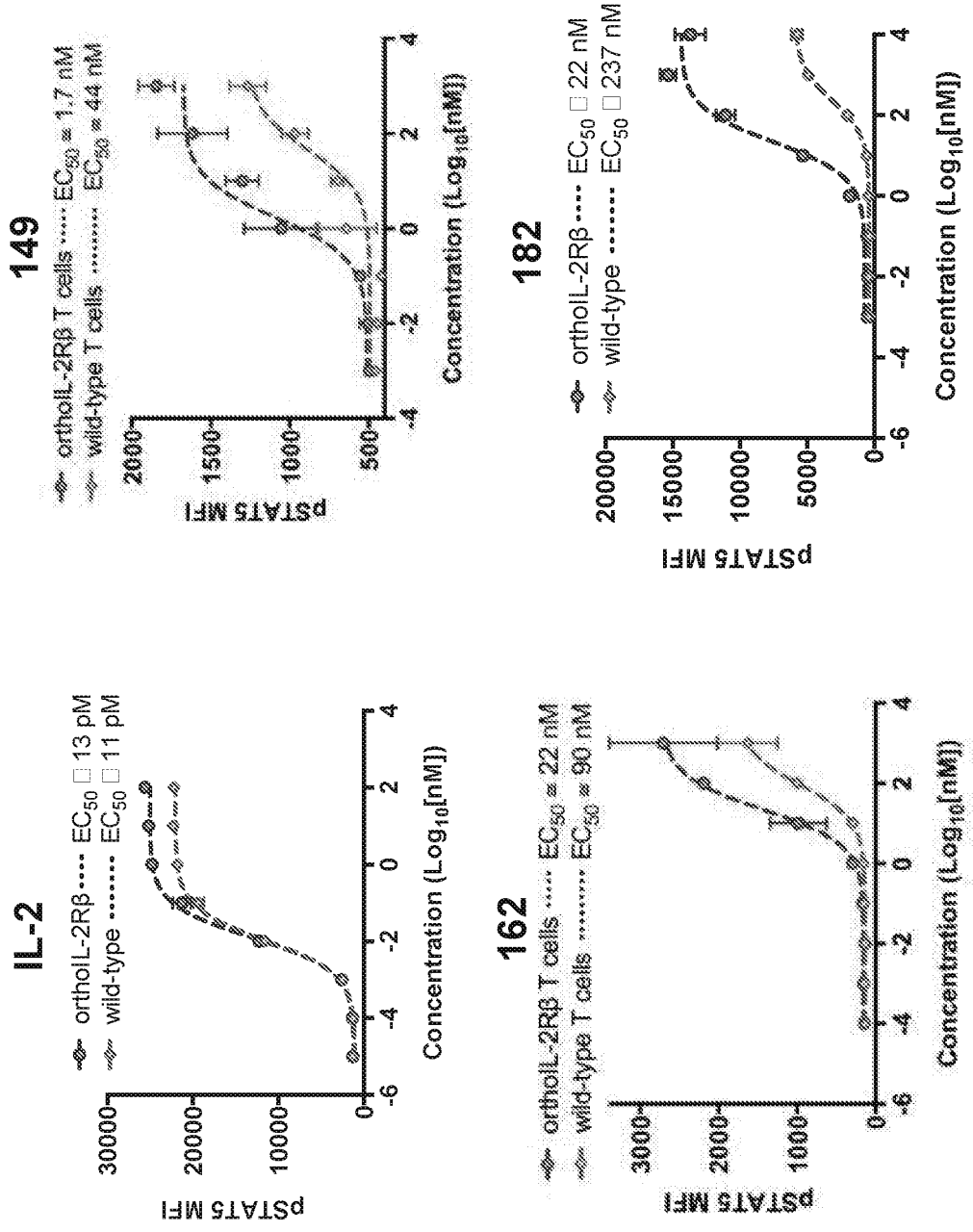

IL-2
orthoIL-2Rβ ···· EC50 ≈ 13 pM
wild-type ····· EC50 ≈ 11 pM
pSTAT5 MFI
Concentration (Log10[nM])
149
orthoIL-2Rβ T cells ····· EC50 ≈ 1.7 nM
wild-type T cells ····· EC50 ≈ 44 nM
pSTAT5 MFI
Concentration (Log10[nM])
182
orthoIL-2Rβ ···· EC50 ≈ 22 nM
wild-type ····· EC50 ≈ 237 nM
pSTAT5 MFI
Concentration (Log10[nM])
162
orthoIL-2Rβ T cells ····· EC50 ≈ 22 nM
wild-type T cells ····· EC50 ≈ 90 nM
pSTAT5 MFI
Concentration (Log10[nM])

FIG. 13

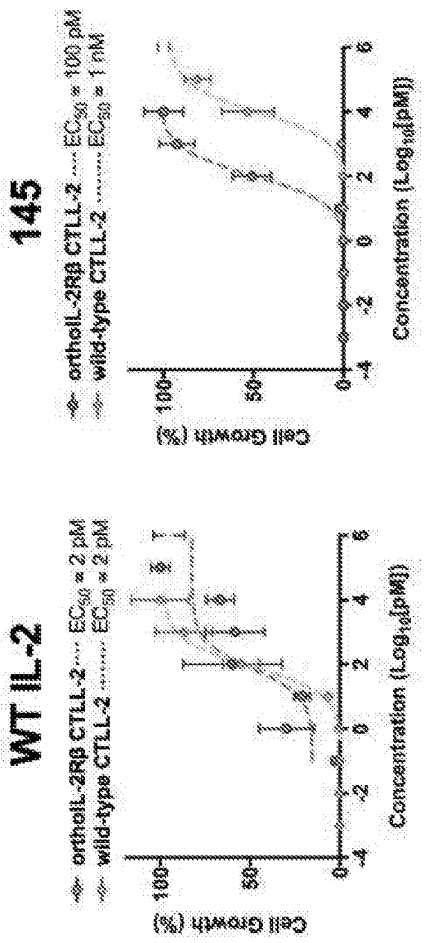

WT IL-2
orthoIL-2Rβ CTLL-2 ···· EC50 = 2 pM
wild-type CTLL-2 ····· EC50 = 2 pM
Cell Growth (%)
100
50
0
-4
-2
0
2
4
6
Concentration (Log10[pM])
145
orthoIL-2Rβ CTLL-2 ···· EC50 = 100 pM
wild-type CTLL-2 ····· EC50 = 1 nM
Cell Growth (%)
100
50
0
-4
-2
0
2
4
6
Concentration (Log10[pM])

FIG. 14

```
IL2RB_HUMAN       1    AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWAC   60
IL2RB_MOUSE       1    AVKNCSHLECFYNSRANVSCMWSHEEALNVTTCHVHAKSNLRHWNKTCELTLVRQASWAC   60
                       **:  *:: ********:**:**:: **: *:*:***  : *:**:****  * ******

IL2RB_HUMAN      61    NLILGA-PDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVET  119
IL2RB_MOUSE      61    NLILGSFPESQSLTSVDLLDINVVCWEEKGWRRVKTCDFHPFDNLRLVAPRSLQVLHIDT  120
                       *****: *:**.**:**:: :.*:* *   ** :   **:**:****:** ****:*::*

IL2RB_HUMAN      120   HRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYE  179
IL2RB_MOUSE      121   QRCNISWKVSQVSHYIEPYLEFEARRRLLGHSWEDASVLSLKQRQQWLFLEMLIPSTSYE  180
                       :*******::**.***:* :******    **:**:* :*:***:*:*: ** * *.*.**

IL2RB_HUMAN      180   FQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDT--IPWLGHLLVGLSGAFGFIILVYL  237
IL2RB_MOUSE      181   VQVRVKAQRNNTGTWSPWSQPLTFRTRPADPMKEILPMSWLRYLLLVLGCFSGFFSCVYI  240
                       .*****  : :  *********:***:**   *:   : ** :**: *.   **:  **:


IL2_MOUSE         1    APTSSSTSSSTAEAQQQQQQQQQQQQHLEQLLMDLQELLSRMENYRNLKLPRMLTFKFYL   60
IL2_HUMAN         1    APTSS----------------STKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYM   46
                       *****               . :: * :**:**:*** :*. ::***:* ** ********:

IL2_MOUSE        61    PKQATELKDLQCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTVVKLKGSDNTFEC  120
IL2_HUMAN        47    PKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRP-RDLISNINVIVLELKGSETTFMC  105
                       **:*****.*****:** **..**:*:***.*:*.  .::****.* *::****:.** *

IL2_MOUSE        121   QFDDESATVVDFLRRWIAFCQSIISTSPQ                                149
IL2_HUMAN        106   EYADETATIVEFLNRWITFCQSIISTLT-                                133
                       :: **:**:*:**.***:********
```

FIG. 15

FIG. 16A

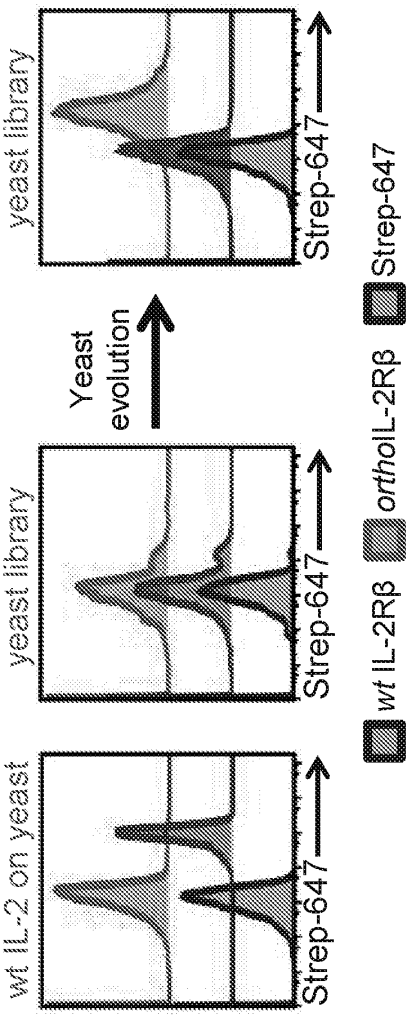


FIG. 16B

FIG. 16C

	15	16	19	20	22	23
WT	E	H	L	D	Q	M
C1	S	Q	V	T	K	L
C2	S	Q	V	S	K	L
C3	S	Q	V	S	K	L
C4	S	Q	I	S	K	L
C5	S		V	M	K	S
C6	T	Q	V	S		S
C7	Q		V	M	K	S
C8	Q	Q	V	T	K	V
C9	H	Q	I	S	K	L
C10	H	Q	I	L	K	T
C11			V	M	N	S

FIG. 16D

	15	16	19	20	23
WT	E	H	L	D	M
C1	H	Q	S	I	E
C2	T	Q	V	T	I
C3	T	Q	I	T	I
C4	T	Q	I	T	T
C5	D	N	I	M	F
C6	S	Q	V	L	Q
C7	K	Q	I	T	V
C8	I	Q	I	S	T
C9	K	Q	V	M	L
C10	K	Q	V	T	L
C11	V	Q	V	S	L
C12		N	V	M	A
C13	K		I	M	Q
C14	S	Q	V	L	V

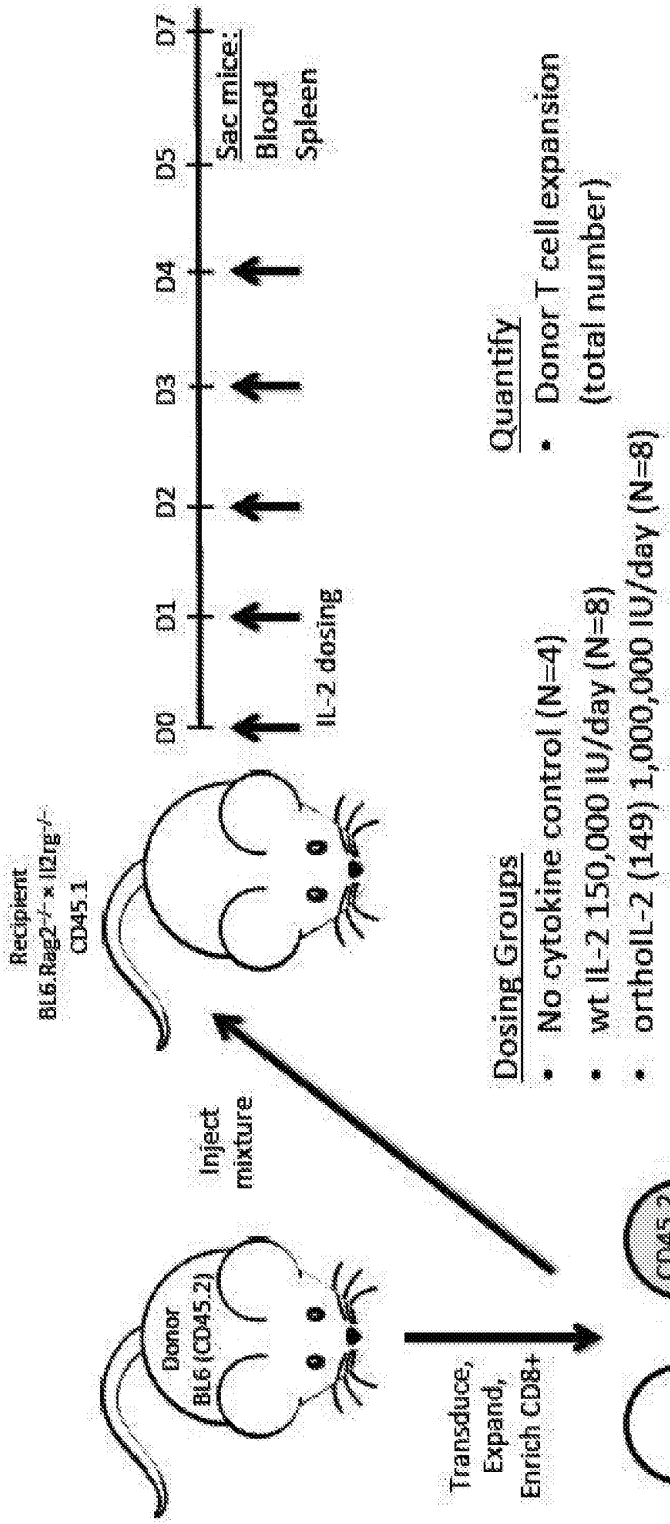

Recipient
BL6.Rag2$^{-/-}$ × Il2rg$^{-/-}$
CD45.1
D0
D1
D2
D3
D4
D5
D7
IL-2 dosing
Sac mice:
Blood
Spleen
Quantify
• Donor T cell expansion (total number)
Dosing Groups
• No cytokine control (N=4)
• wt IL-2 150,000 IU/day (N=8)
• ortholL-2 (149) 1,000,000 IU/day (N=8)
Inject mixture
Donor
BL6 (CD45.2)
Transduce, Expand, Enrich CD8+
CD45.2
wt T cell
(45%)
CD45.2
YFP+
ortholL-2Rβ T cell
(55%)

FIG. 17

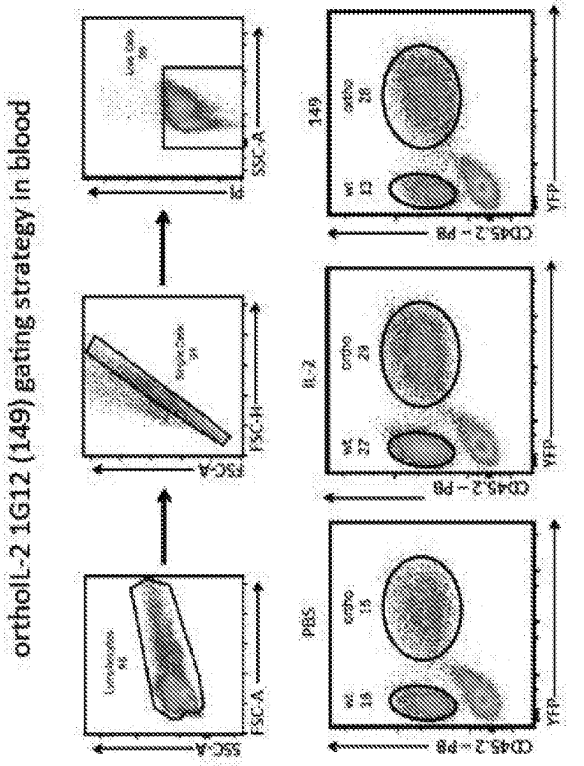


FIG. 18A

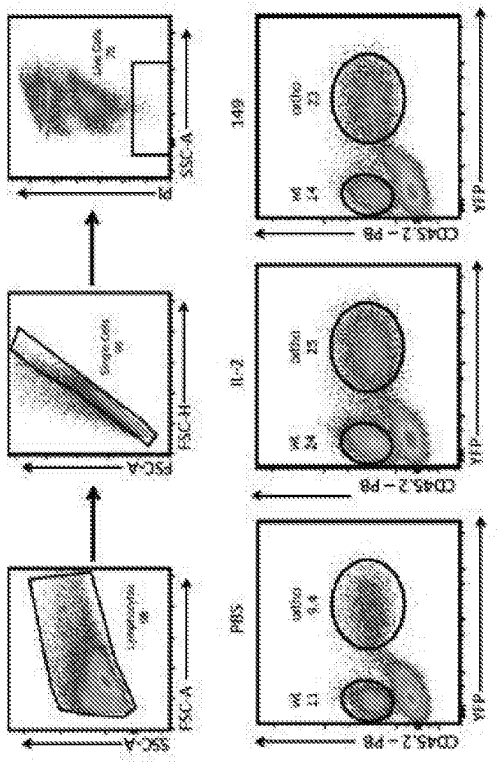


FIG. 18B

FIG. 19

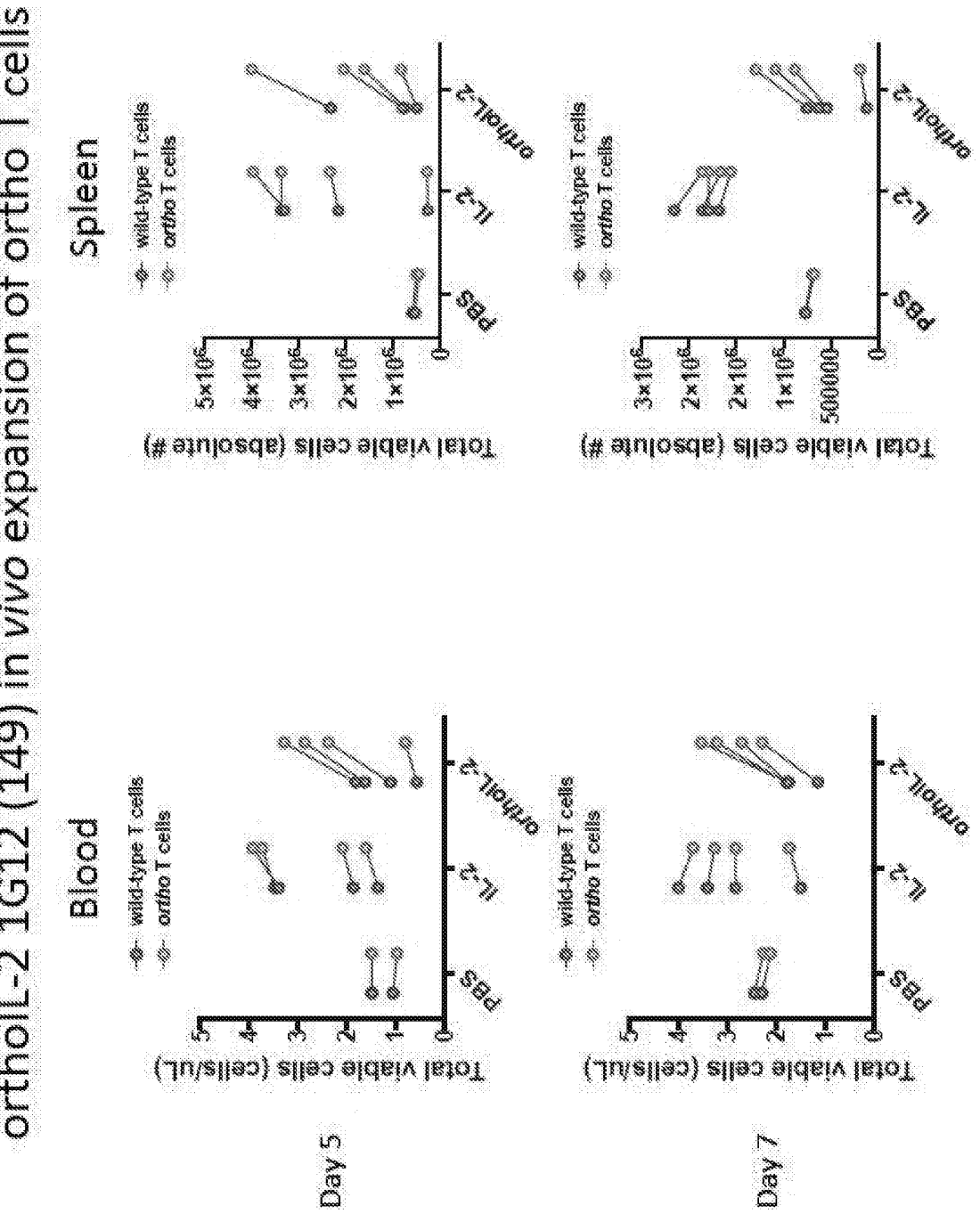

orthoIL-2 1G12 (149) in vivo expansion of ortho T cells
Blood
Spleen
wild-type T cells
ortho T cells
Total viable cells (cells/uL)
Total viable cells (absolute #)
Day 5
Day 7
PBS
IL-2
orthoIL-2

FIG. 20A

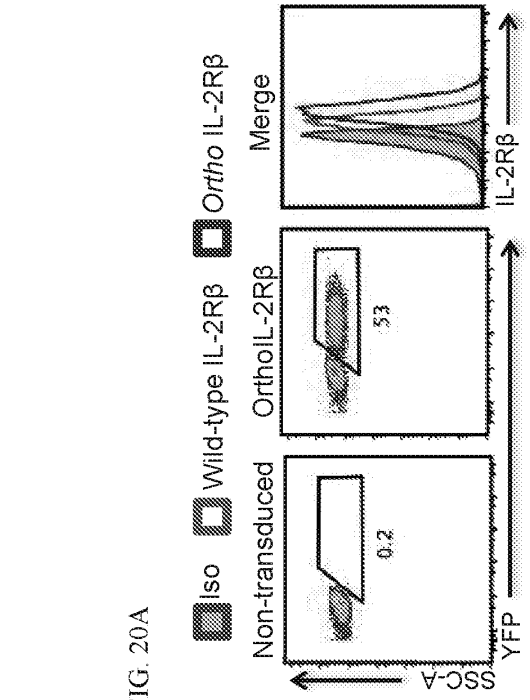


FIG. 20B

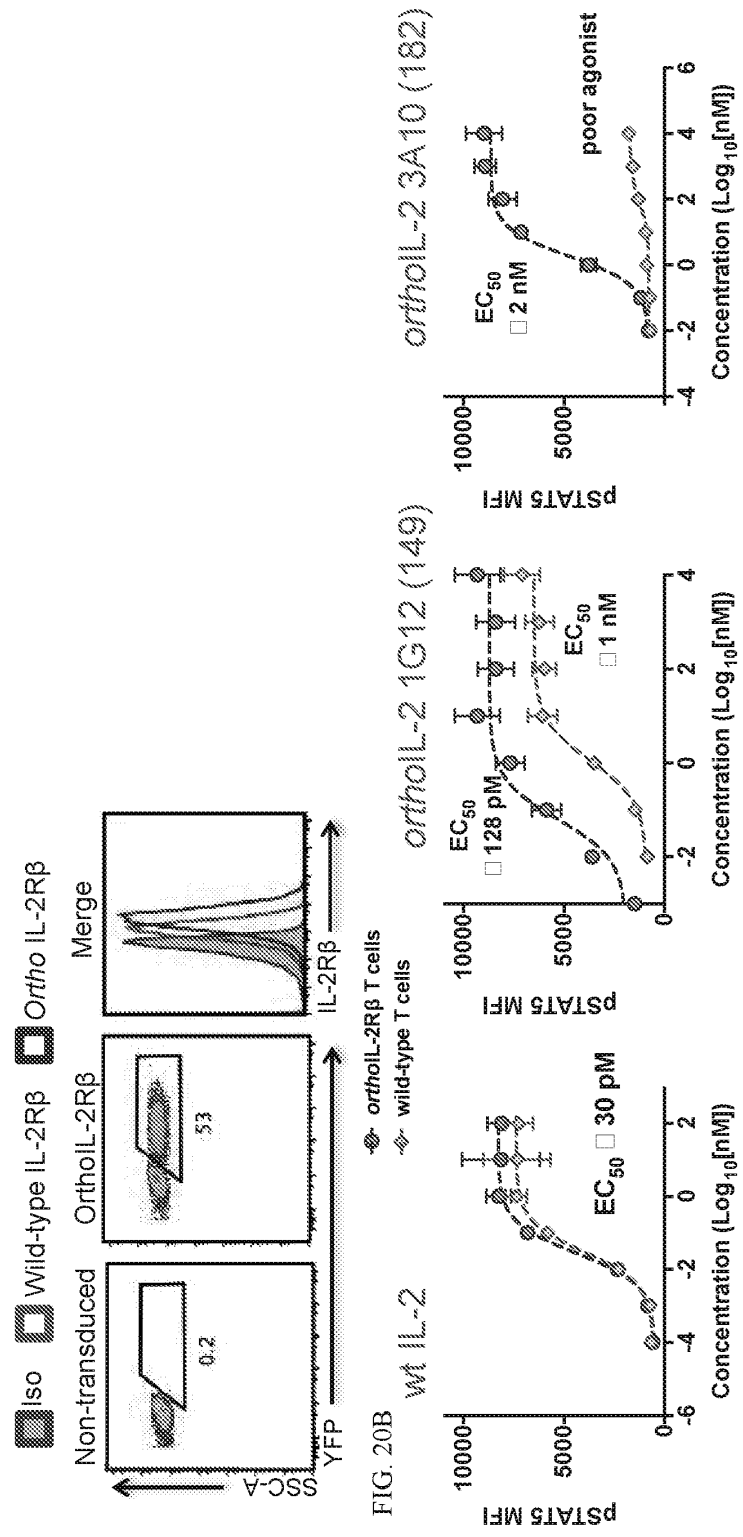


FIG. 21A

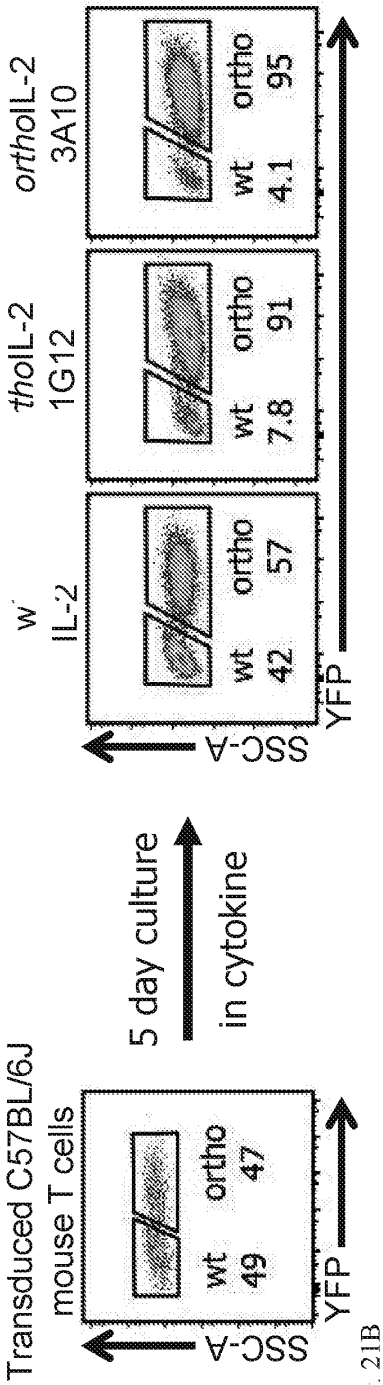


FIG. 21B

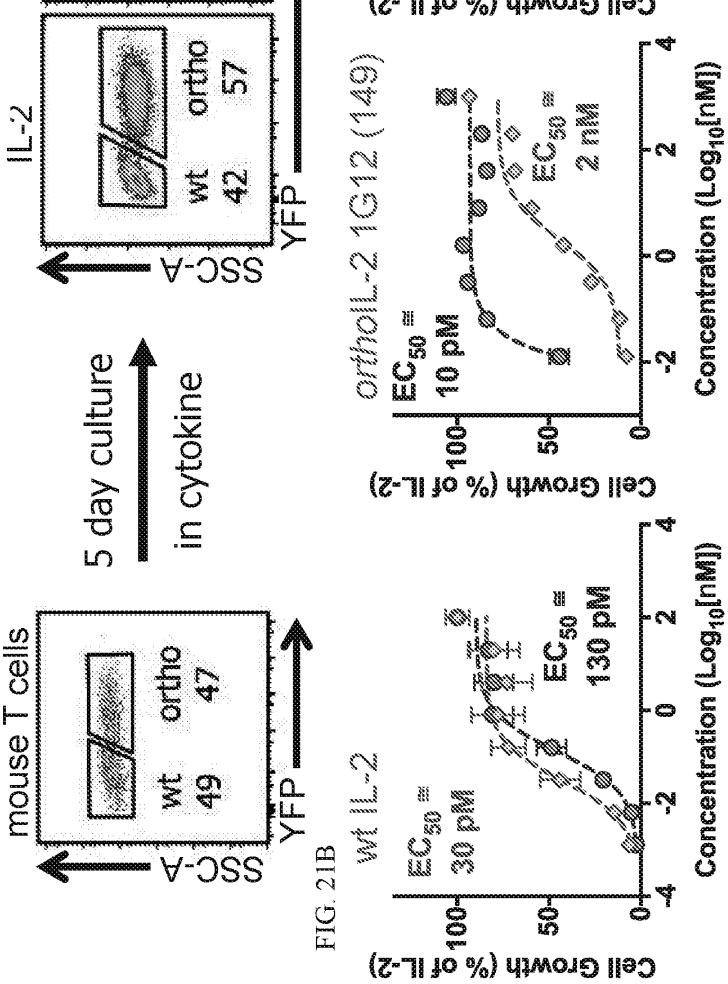
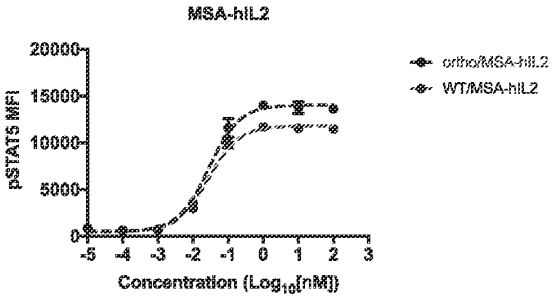
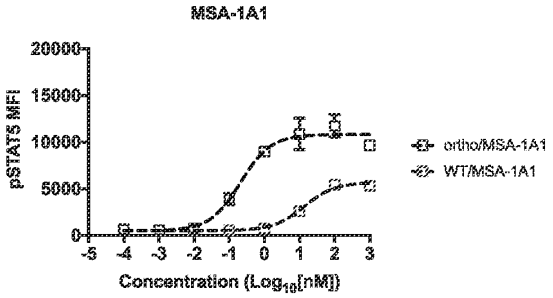


FIG. 22A



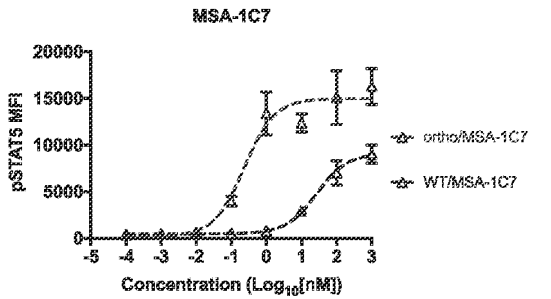
	ortho/MSA-hIL2	WT/MSA-hIL2
EC50	0.02812	0.02736

FIG. 22B



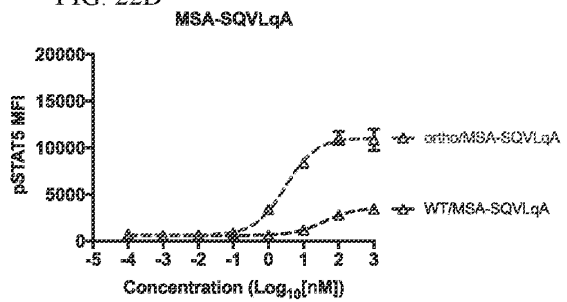
	ortho/MSA-1A1	WT/MSA-1A1
EC50	0.2092	13.65

FIG. 22C



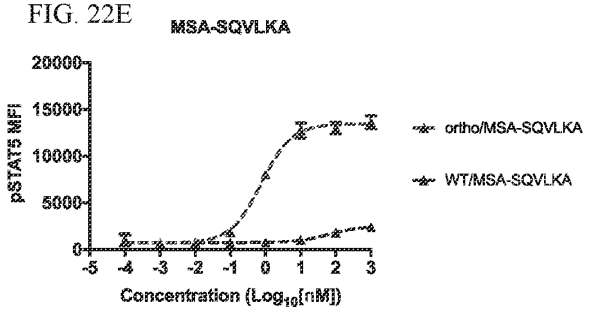
	ortho/MSA-1C7	WT/MSA-1C7
EC50	0.2348	27.28

FIG. 22D



	ortho/MSA-SQVLqA	WT/MSA-SQVLqA
EC50	3.041	38.11

FIG. 22E



	ortho/MSA-SQVLKA	WT/MSA-SQVLKA
EC50	0.76	62.05

FIG. 23A

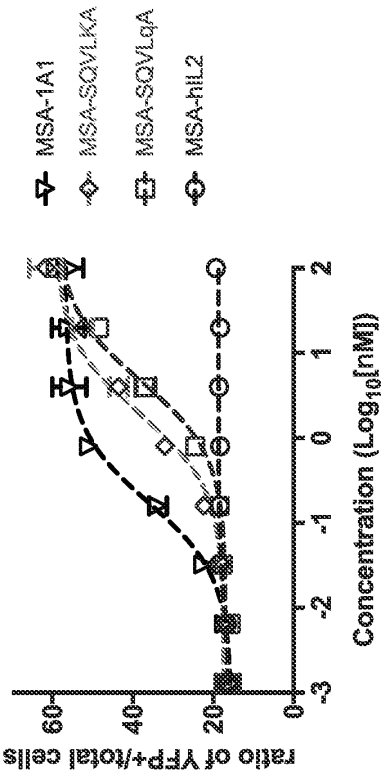
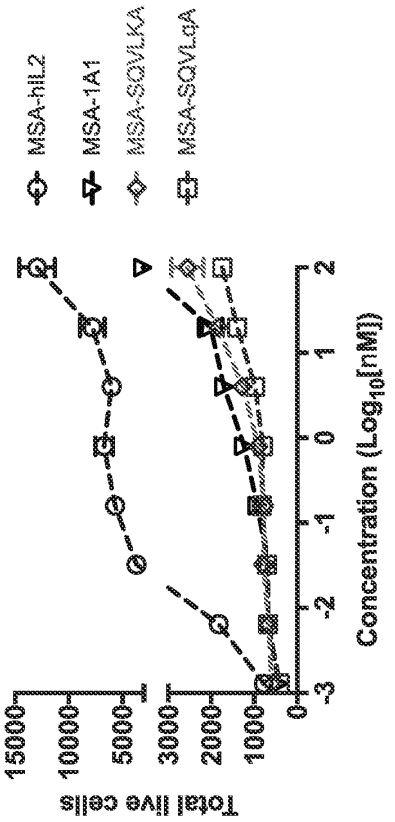



FIG. 23B