
A
Sequence numbering
NAME
SEQ ID
Wild Type CTLA-4 35 MHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD
Variant 1315 36 MHVAQPAVVLASSRGIASFVCEYAMPGKATEVRVTVLRQADSQVTEVCAATYMMGNEGTFLDDSICTGTASGNQVNLTIKGLRAMDTGLYICQVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD
Variant 1322 37 MHVAQPAVVLASSRGIASFVCEYAMPSKATEVRVTVLRQADSQVTEVCAATYMIGSELSTQDYPNCTGTSSGNQVNLTIQGLRAMDTGLYICQVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD
Variant 1321 38 MHVAQPAVVLASSRGIASFVCEYAMPGKATEVRVTVLRQADSQVTEVCAATYMIGNEGTFLDDSICTGTASGNQVNLTIKGLRAMDTGLYICQVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD
Variant 0943 39 MHVAQPAVVLASSRGIASFVCEYAMPGKATEVRVTVLRQADSQVTEVCAATYMMGNEGTFLDDSICTGTSSGNQVNLTIKGLRAMDTGLYICQVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD
Variant 0898 40 MHVAQPAVVLASSRGVASFVCEYAMPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFPDDSICTGTSSGNQVNLTIQGLRAMDTGLYICQVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD
Variant 1319 41 MHVAQPAVVLASSRGIASFVCEYAMPGKATEVRVTVLRQADSQVTEVCAATYMMGSELSTQDYPNCTGTASGNQVNLTIQGLRAMDTGLYICQVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD
Variant 1115 42 MHVAQPAVVLASSRGVASFVCEYAMPGKATEVRVTVLRQADSQVTEVCAATYMMGNEGTFLDDSICTGTASGNQVNLTIQGLRAQDTGLYICQVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD
Variant 1299 43 MHVAQPAVVLASSRGRASFVCEYTMPSKATEVRVTVLRQADSQVTEVCAATYMMGNEATFLDDSICTGTASGNQVNLTIQGLRAQDTGLYICQVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD
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Variant 1227 47 MHVAQPAVVLASSRGEASFVCEYAMPSKATEVRVTVLRQADSQVTEVCAATYMMGNEATFLDDSICTGTASGNQVNLTIQGLRAQDTGLYICHVELMYPPPYYLGIGNGTQIYVIDPEPCPDSD
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Sequence numbering (top), and numbering according to Swiss-Prot entry P16410 (bottom)
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Fc Variant-2 58 QEPKSSDKTHTSPPAPAPELLGGSSVFLFPPKPKDTLAITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
Fc Variant-3 59 VEPKSCDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLAITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEK
Fc Variant-4 60 VEAKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLAITREPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
Name
SEQ ID
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Fc Variant-4 60 TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
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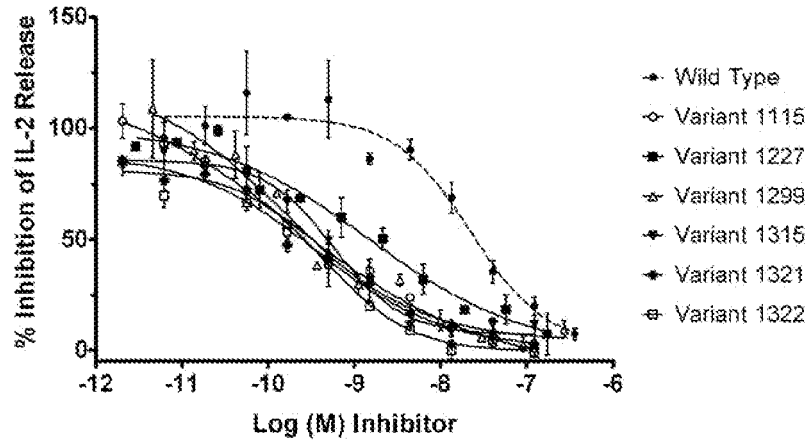
Figure 1

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
M	H	V	A	Q	P	A	V	V	L	A	S	S	R	G	R_{16}	A	S	F	V
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
C	E	Y	R_{24}	N	P	R_{27}	K	A	T	E	V	R	V	T	V	L	R	Q	A
41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
D	S	Q	V	T	E	V	C	A	A	T	Y	M	R_{54}	G	R_{56}	E	R_{58}	R_{59}	R_{60}
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
R_{61}	D	R_{63}	R_{64}	R_{65}	C	T	G	T	R_{70}	S	G	N	Q	V	N	L	T	I	R_{80}
81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
G	L	R	A	R_{85}	D	T	G	L	Y	I	C	R_{93}	V	E	L	M	Y	P	P
101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
P	Y	Y	L	G	I	G	N	G	T	Q	I	Y	V	I	D	P	E	P	C
121	122	123	124																
P	D	S	D																

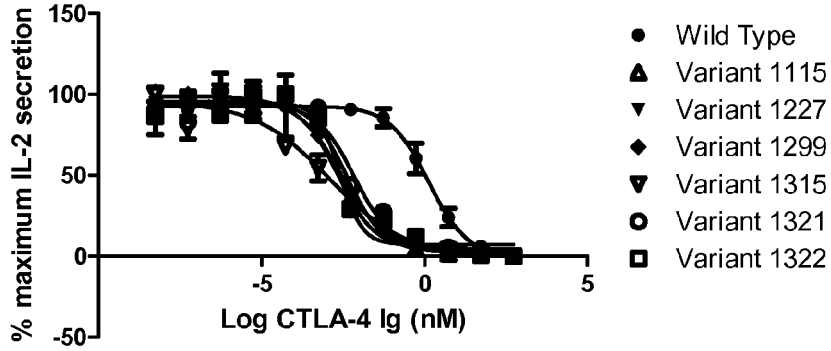
R_{16} = R, I, S or V
R_{24} = T or A
R_{27} = S or G
R_{54} = M or K
R_{56} = N or S
R_{58} = A, L or G
R_{59} = T or S
R_{60} = F or T
R_{61} = L or Q
R_{63} = D or Y
R_{64} = S or P
R_{65} = I, N or D
R_{70} = A or S
R_{80} = Q or R
R_{85} = Q, M or S
R_{93} = Q or H

Figure 2

A


% Inhibition of IL-2 Release
150
100
50
0
-12
-11
-10
-9
-8
-7
-6
Log (M) Inhibitor
Wild Type
Variant 1115
Variant 1227
Variant 1299
Variant 1315
Variant 1321
Variant 1322

B


% maximum IL-2 secretion
150
100
50
0
-50
-5
0
5
Log CTLA-4 Ig (nM)
Wild Type
Variant 1115
Variant 1227
Variant 1299
Variant 1315
Variant 1321
Variant 1322

C

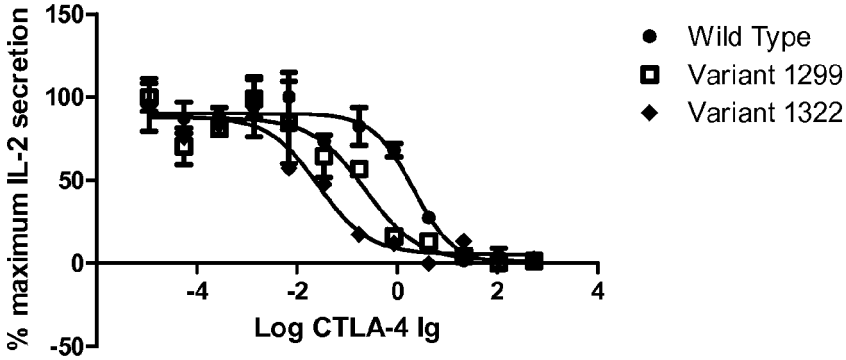
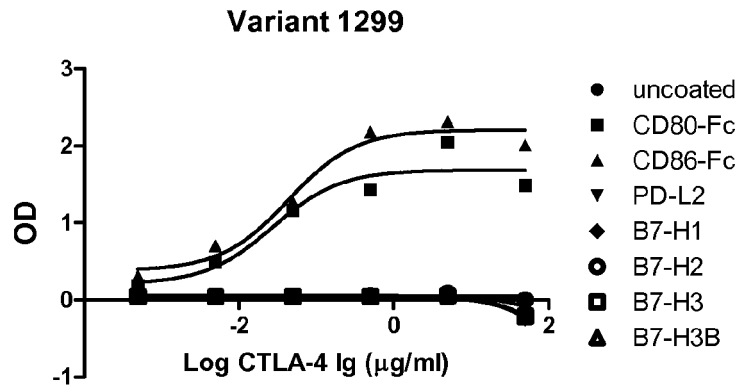

% maximum IL-2 secretion
150
100
50
0
-50
-4
-2
0
2
4
Log CTLA-4 Ig
Wild Type
Variant 1299
Variant 1322

Figure 3

A


Variant 1299
OD
3
2
1
0
-1
-2
0
2
Log CTLA-4 Ig (μg/ml)
uncoated
CD80-Fc
CD86-Fc
PD-L2
B7-H1
B7-H2
B7-H3
B7-H3B

B

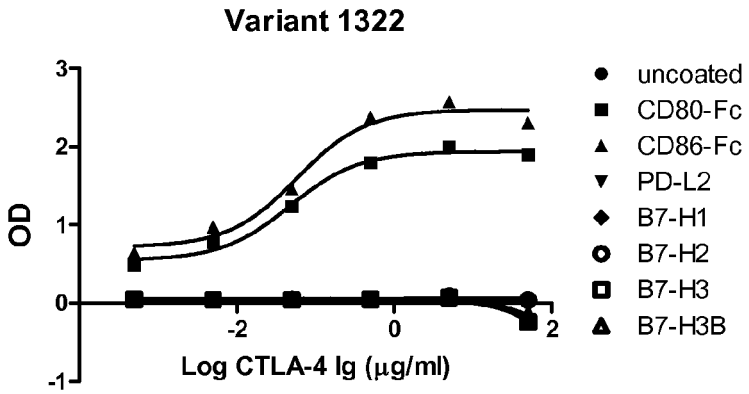
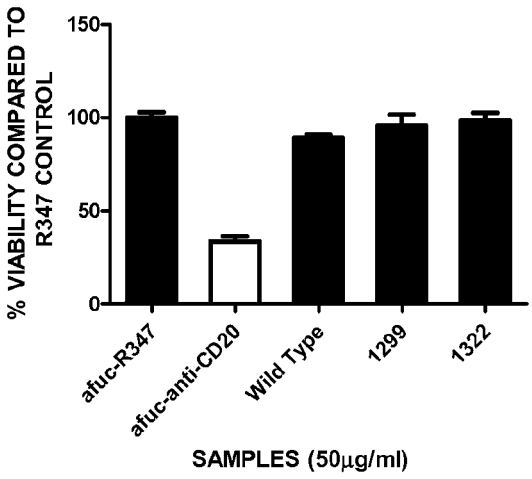

Variant 1322
OD
3
2
1
0
-1
-2
0
2
Log CTLA-4 Ig (μg/ml)
uncoated
CD80-Fc
CD86-Fc
PD-L2
B7-H1
B7-H2
B7-H3
B7-H3B

Figure 4

A


% VIABILITY COMPARED TO R347 CONTROL
150
100
50
0
afuc-R347
afuc-anti-CD20
Wild Type
1299
1322
SAMPLES (50µg/ml)

B

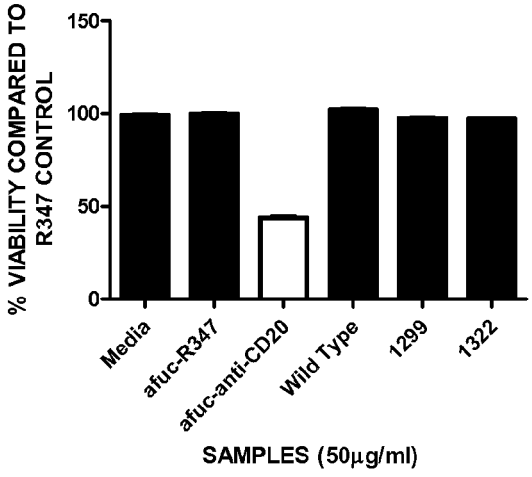

% VIABILITY COMPARED TO R347 CONTROL
150
100
50
0
Media
afuc-R347
afuc-anti-CD20
Wild Type
1299
1322
SAMPLES (50µg/ml)

Figure 5

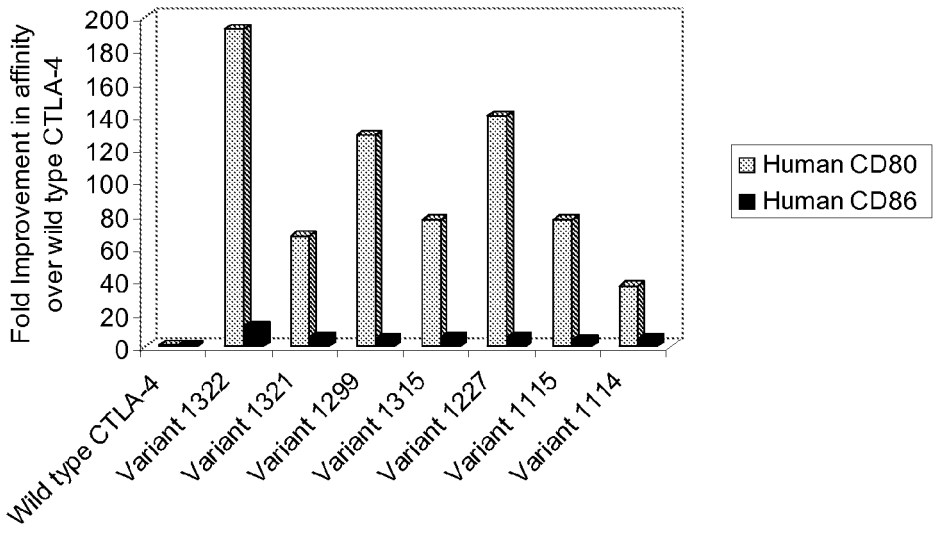
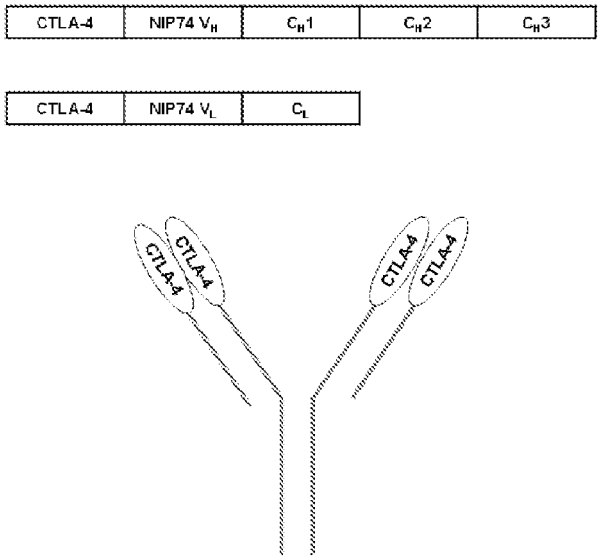

Fold Improvement in affinity over wild type CTLA-4
200
180
160
140
120
100
80
60
40
20
0
Wild type CTLA-4
Variant 1322
Variant 1321
Variant 1299
Variant 1315
Variant 1227
Variant 1115
Variant 1114
Human CD80
Human CD86

Figure 6

A


CTLA-4
NIP74 V_H
C_H1
C_H2
C_H3
CTLA-4
NIP74 V_L
C_L
CTLA-4
CTLA-4
CTLA-4
CTLA-4

B

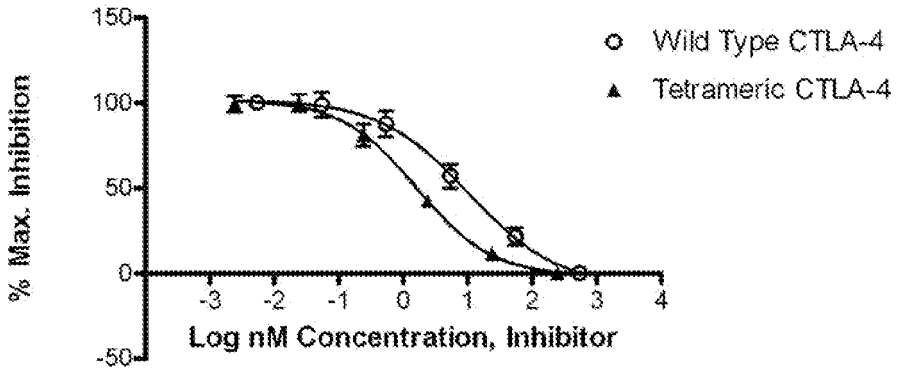

% Max. Inhibition
150
100
50
0
-50
-3
-2
-1
0
1
2
3
4
Log nM Concentration, Inhibitor
Wild Type CTLA-4
Tetrameric CTLA-4

Figure 7