A
Sequance numbering
NAME
SEQ ID
Wild Type CTLA-4 35
Variant 1315 36
Variant 1322 37
Variant 1321 38
Variant 0943 39
Variant 0898 40
Variant 1319 41
Variant 1115 42
Variant 1299 43
Variant 1249 44
Variant 1303 45
Variant 1114 46
Variant 1227 47
Variant 0722 48
Variant 0645 49
Variant 0636 50
Variant 0745 51
Variant 0673 52
Variant 0788 53
Variant 0701 54
Variant 0439 55
Sequence numbering (top), and numbering according to Swiss-Prot entry P16410 (bottom)
B
Name SEQ ID
IgG1 Fc 56
Fc Variant-1 57
Fc Variant-2 58
Fc Variant-3 59
Fc Variant-4 60
Name SEQ ID
IgG1 Fc 56
Fc Variant-1 57
Fc Variant-2 58
Fc Variant-3 59
Fc Variant-4 60
Sequence numbering (top), and numbering according to Swiss-Prot entry P01857 (bottom)

Figure 1

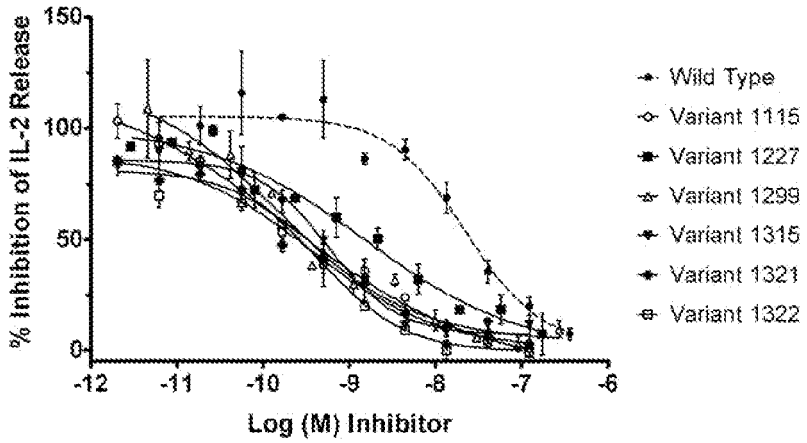
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
M	H	V	A	Q	P	A	V	V	L	A	S	S	R	G	R_{16}	A	S	F	V
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
C	E	Y	R_{24}	N	P	R_{27}	K	A	T	E	V	R	V	T	V	L	R	Q	A
41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
D	S	Q	V	T	E	V	C	A	A	T	Y	M	R_{54}	G	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

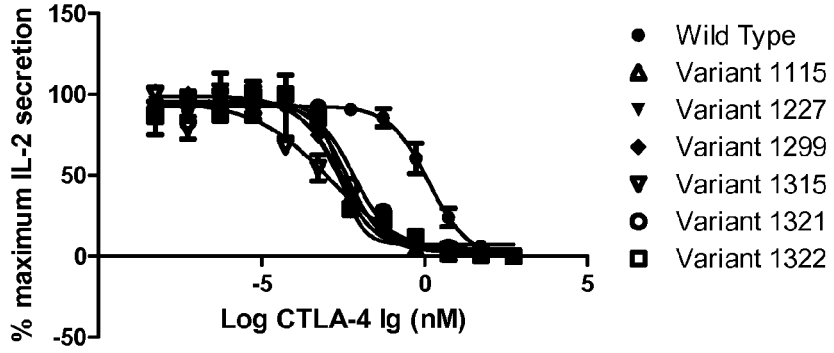
R_{61} = L or Q
R_{63} = D or Y
R_{64} = S or P
R_{65} = I, N or D
R_{70} = A or S
R_{80} = Q or R
R_{85} = Q, M or S
R_{93} = Q or H

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

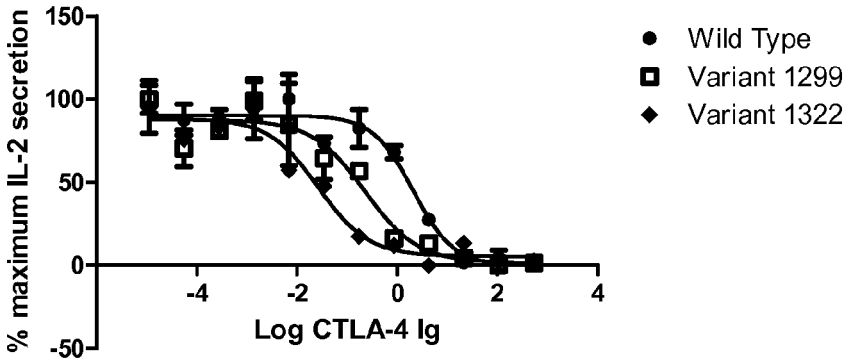
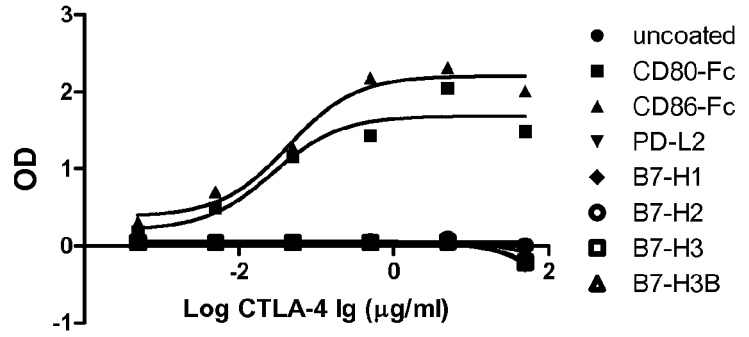

% maximum IL-2 secretion
150
100
50
0
-50
-4
-2
0
2
4
Log CTLA-4 Ig
Wild Type
Variant 1299
Variant 1322

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

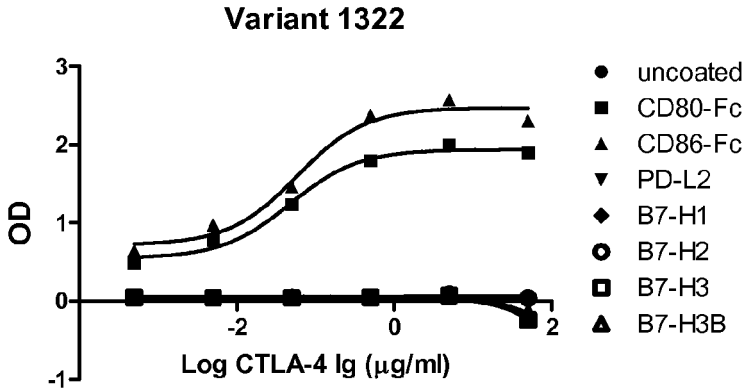
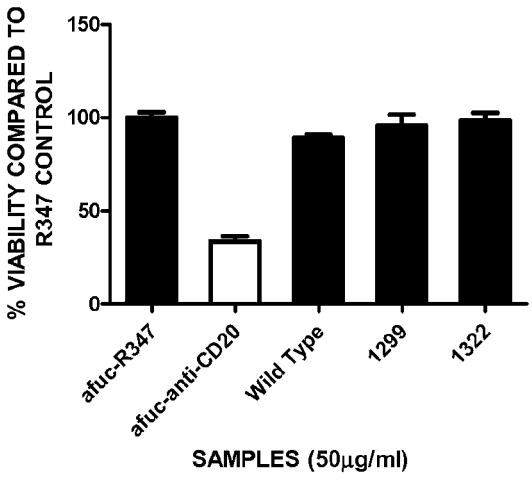

Variant 1322
OD
3
2
1
0
-1
-2
0
2
Log CTLA-4 Ig (μg/ml)
uncoated
CD80-Fc
CD86-Fc
PD-L2
B7-H1
B7-H2
B7-H3
B7-H3B

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

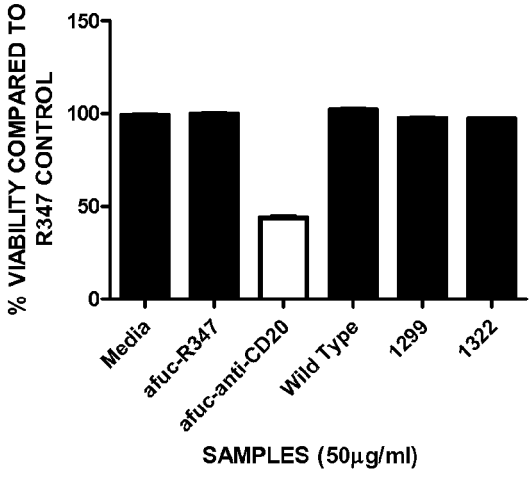

% VIABILITY COMPARED TO R347 CONTROL
150
100
50
0
Media
afuc-R347
afuc-anti-CD20
Wild Type
1299
1322
SAMPLES (50µg/ml)

Figure 5

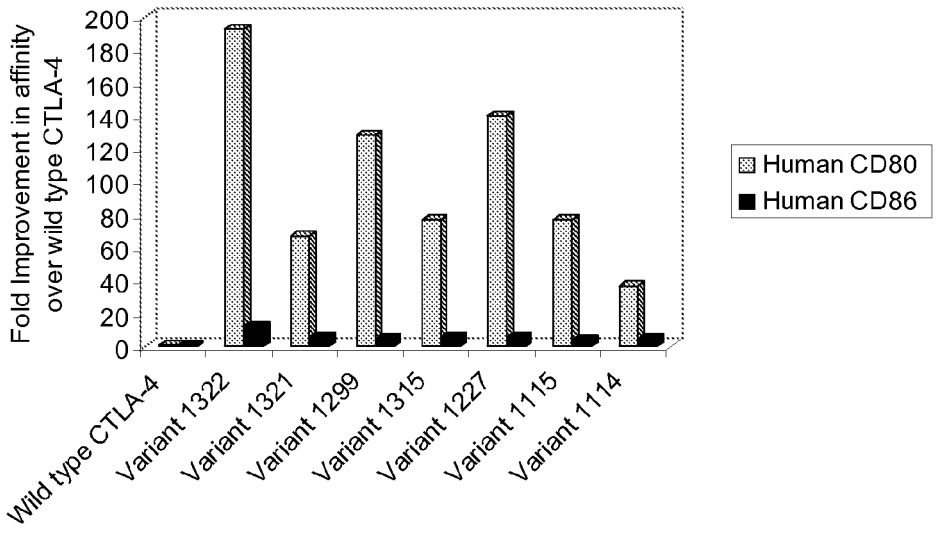
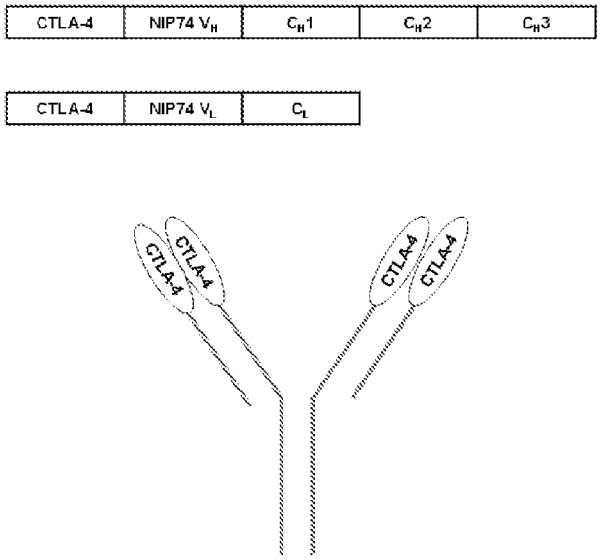

Fold Improvement in affinity over wild type CTLA-4
200
180
160
140
120
100
80
60
40
20
0
Wild type CTLA-4
Variant 1322
Variant 1321
Variant 1299
Variant 1315
Variant 1227
Variant 1115
Variant 1114
Human CD80
Human CD86

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

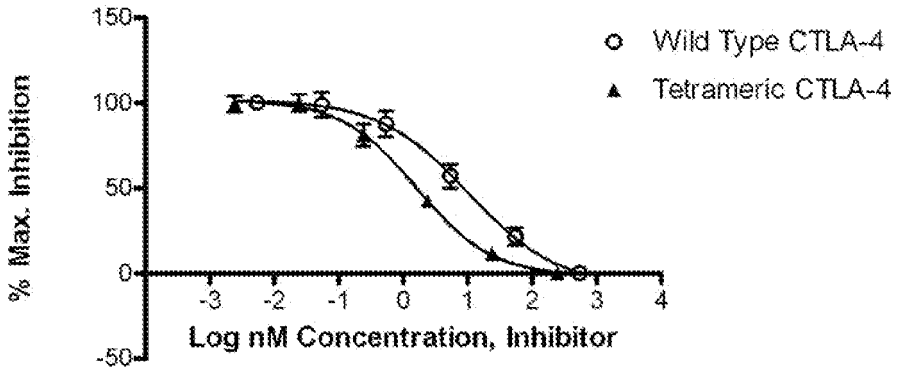

% Max. Inhibition
150
100
50
0
-50
-3
-2
-1
0
1
2
3
4
Log nM Concentration, Inhibitor
Wild Type CTLA-4
Tetrameric CTLA-4

Figure 7