



FIGURE 1A

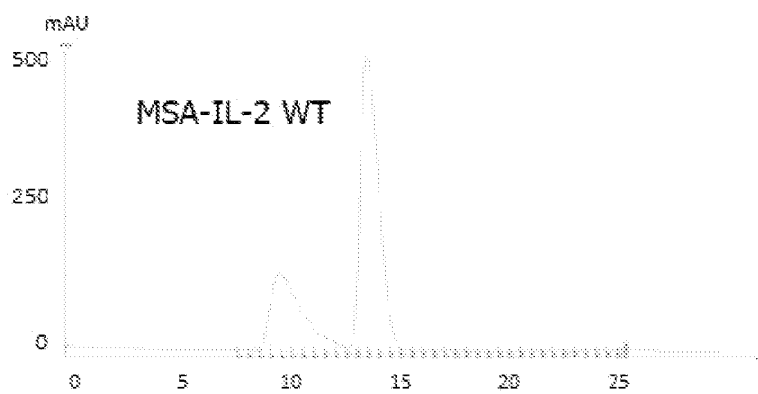


FIGURE 1B

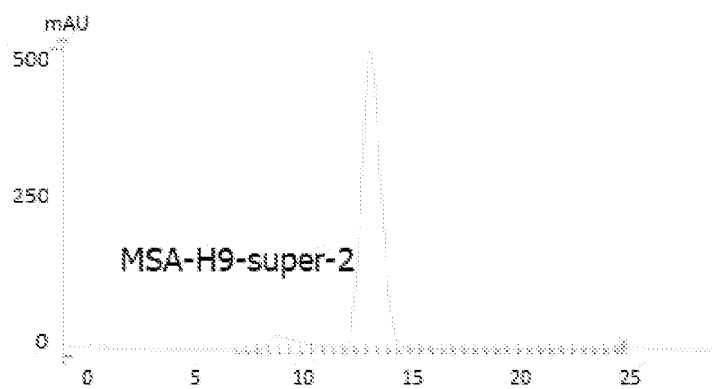


FIGURE 1C

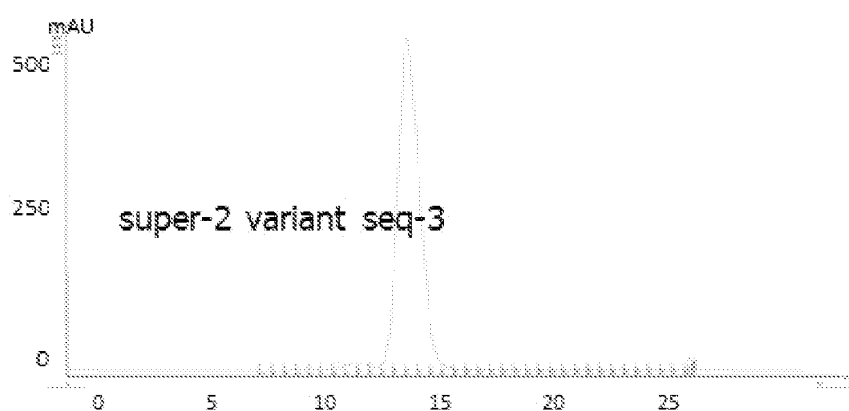


FIGURE 1D

MSA-H9-super-2 MSA-IL2 WT
Fraction 12-16 Fraction 12-16

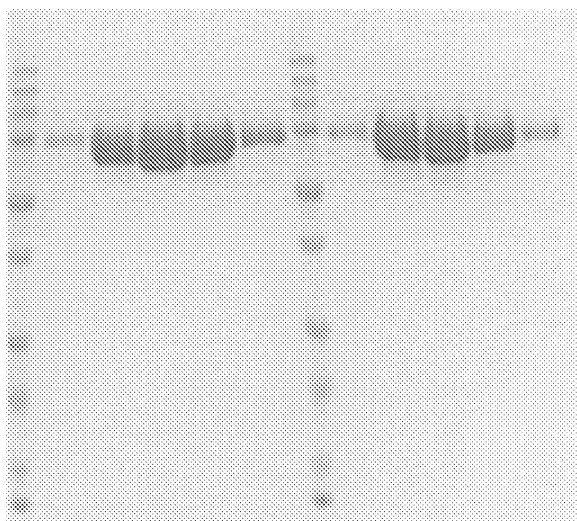


FIGURE 1E

Super-2 variant seq3
Fraction 12-16

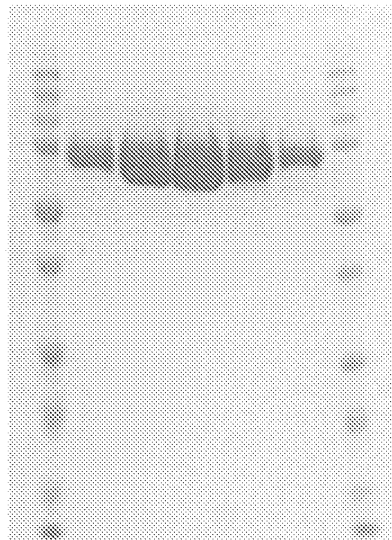


FIGURE 1F

FIG. 2A

packing between
helix1 and loop3

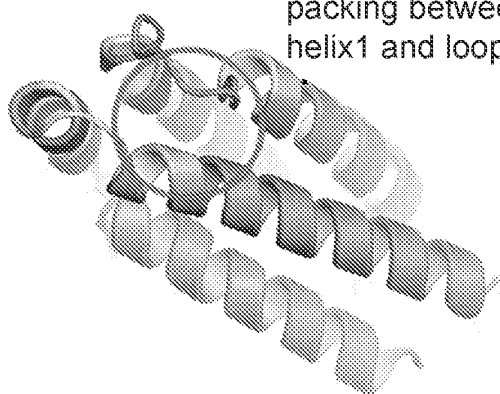


FIG. 2B

packing between
helix2 and helix3

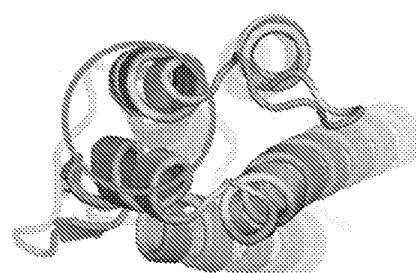


FIG. 2C

packing between
helix1 and helix2

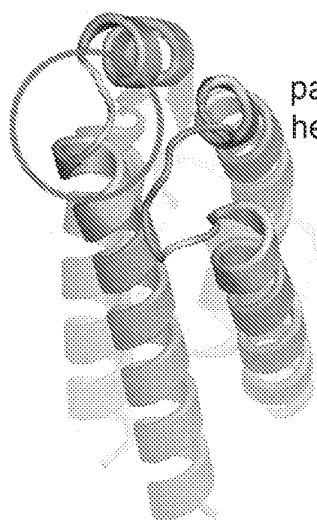
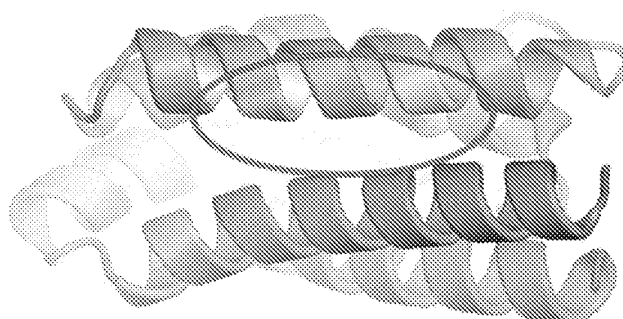


FIG. 2D

packing between helix1 and
helix3 - testing mutations
from super 2



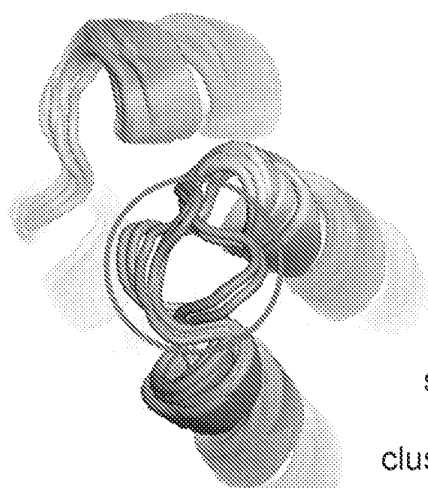


FIG. 3A

shortened and idealized
Loop3, showing two
clusters tested experimentally

FIG. 3B



resample and try to
improve the native loop
by design

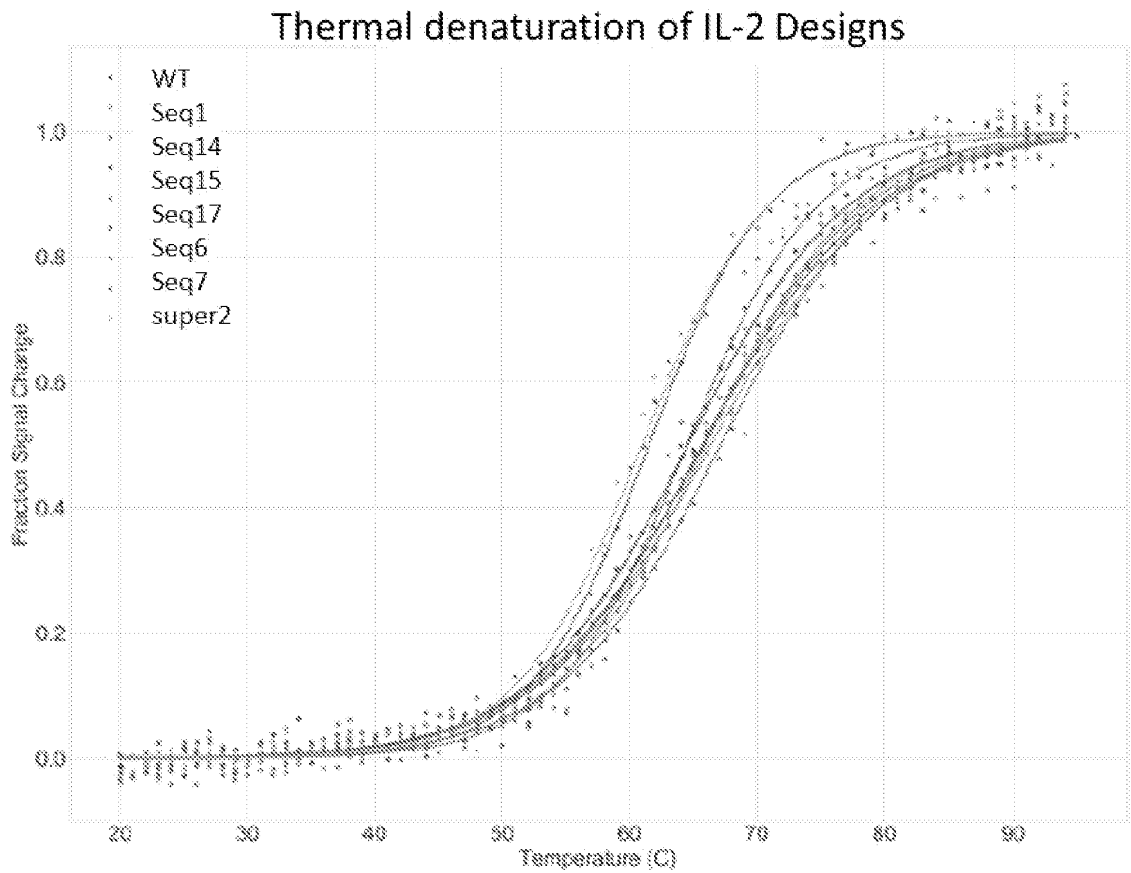


FIGURE 4

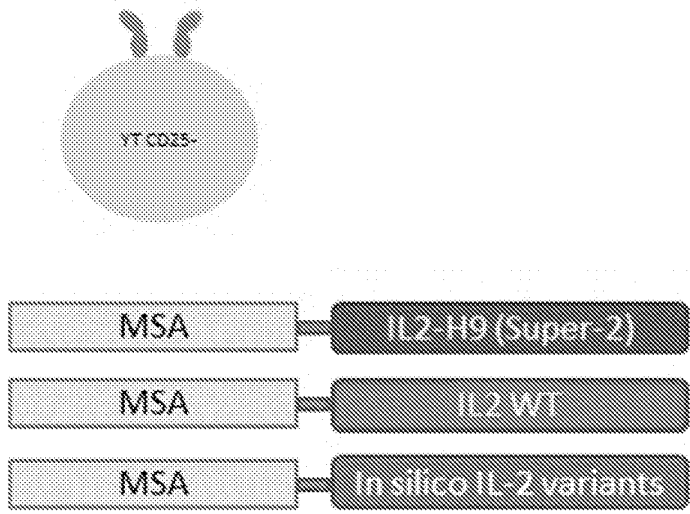


FIGURE 5A

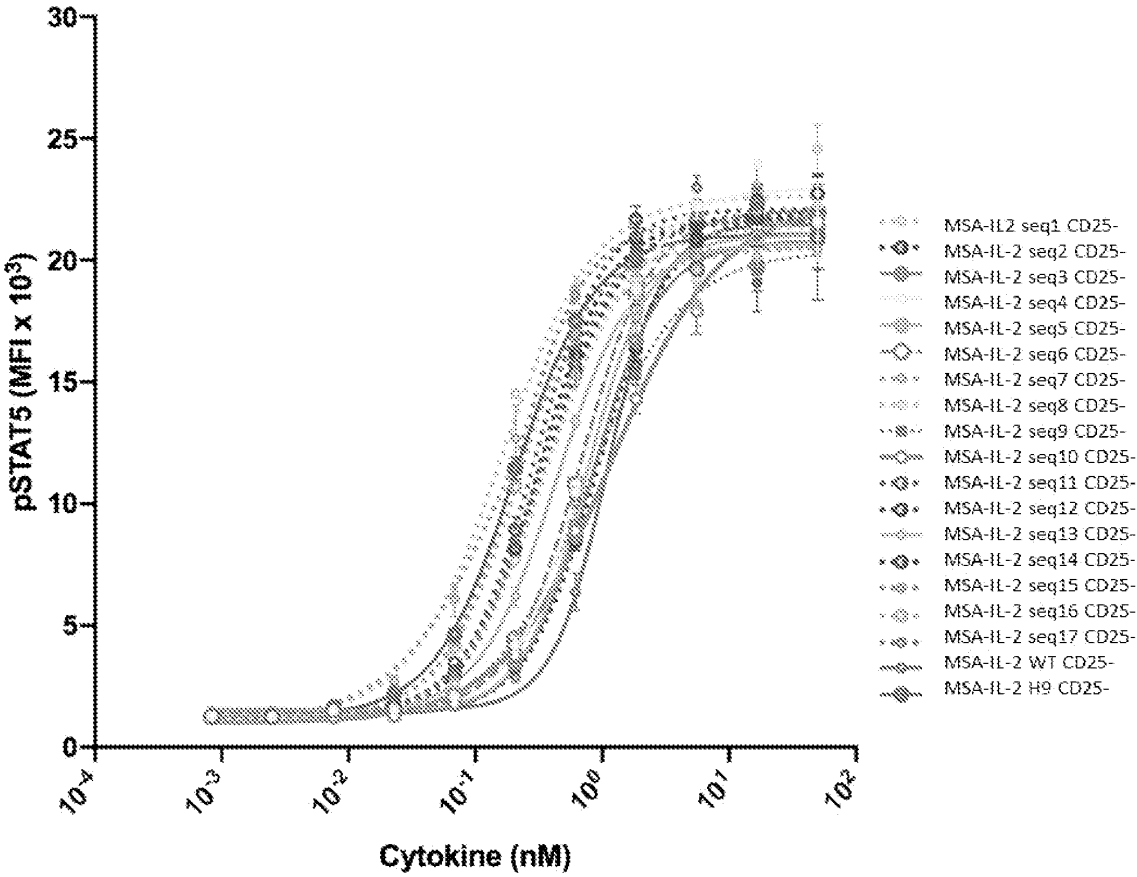


FIGURE 5B

FIGURE 6A

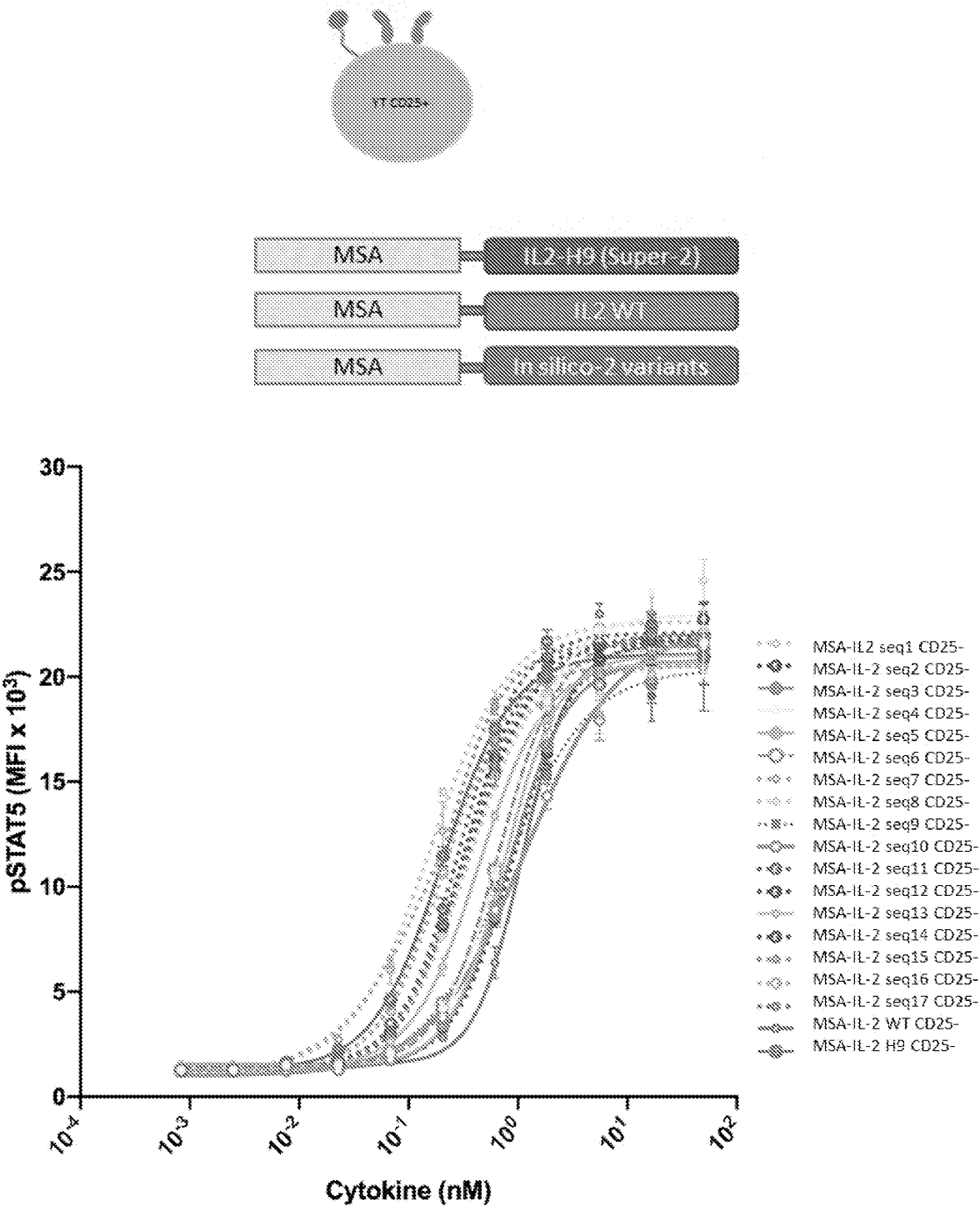


FIGURE 6B

CD25- YT

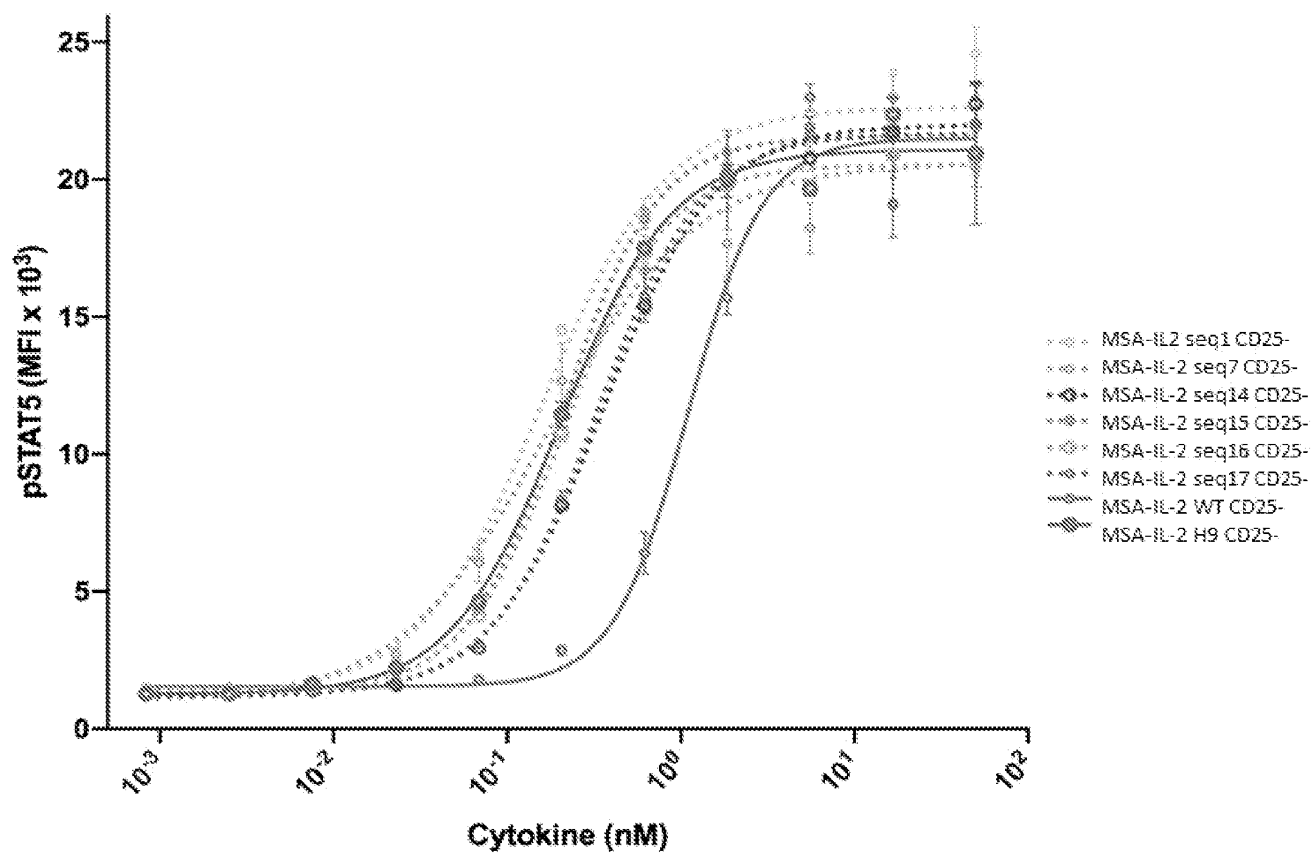


FIGURE 7A

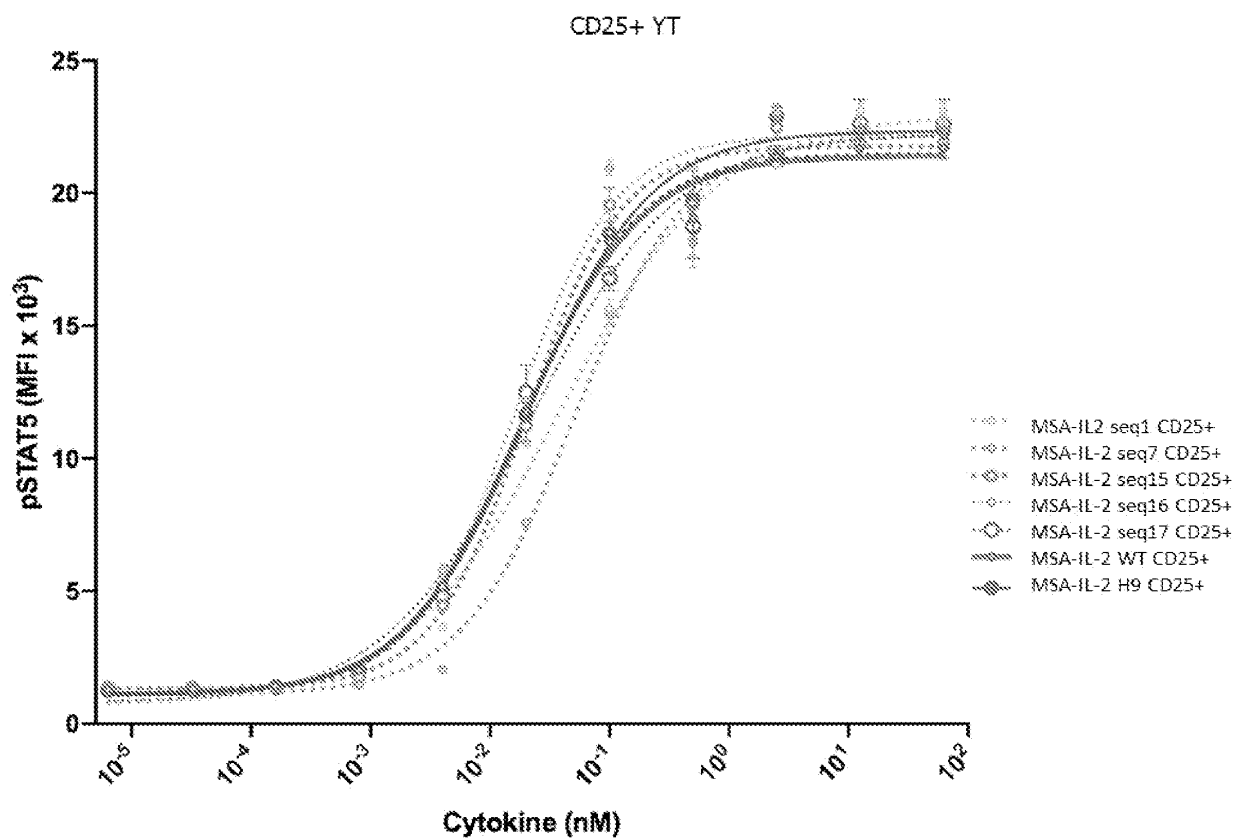


FIGURE 7B

Residue table with EC50

residue #	27	28	31	32	35	39	92	69	72	keep	80	81	82	85	86	92	115	EC50 in CD25+ VT (nM)	EC50 in CD25+ VT (nM)
WT 1-2	G	F	Y	K	K	R	E	Y	L	AQNNHRL	L	R	P	L	Y	F	Y	0.01337	0.01337
Subst 2	S	F	Y	K	K	R	E	Y	L	AQNNHRL	F	D	P	L	Y	F	Y	0.02062	0.02062
Subst 3	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.03897	0.03897
Subst 4	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.04425	0.04425
Subst 5	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.03902	0.03902
Subst 6	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.10253	0.10253
Subst 7	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.11604	0.11604
Subst 8	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.009608	0.009608
Subst 9	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.03181	0.03181
Subst 10	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.0327	0.0327
Subst 11	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.07403	0.07403
Subst 12	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.0295	0.0295
Subst 13	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.01512	0.01512
Subst 14	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.03985	0.03985
Subst 15	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.03727	0.03727
Subst 16	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.03856	0.03856
Subst 17	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.02931	0.02931
Subst 18	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.0162	0.0162
Subst 19	S	F	Y	S	K	R	S	A	A	AQNNHRL	F	D	P	Y	Y	F	Y	0.03186	0.03186

FIGURE 8

Residue No.		37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
Consensus		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
wt N2		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 2		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 1		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 2		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 3		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 4		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 5		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 6		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 7		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 8		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 9		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 10		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 11		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 12		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 13		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 14		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 15		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 16		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																
Sequence 17		APTSSTKATQLEHLIDLOMIINLNNYDNPALTRLTKEYPKATSLKRLQCLEELPLEELN A B I--RLISINIVIVLEKOSSETPECEVADEATIEENMWTFCQIITLY																

FIGURE 9

Residue No.	27	28	32	39	52	69	72	81	115	115
Consensus	G	I	K	M	E	V	L	R	V	
WT IL2	G	I	K	M	E	V	L	R	V	
Super-2	M	L	D	M	E	A	Q	D	I	
Sequence 15	M	L	D	L	S	A	Q	D	I	
Sequence 16	M	L	D	L	S	A	Q	D	I	

APTSSSTKTKTQLEHLLDQIMILN...NNY NPRLTRMLTFKFPYMPKATELKHQCLERELKPLEE...LQ AOSKMPHIDPRDLISINIVIVLELQSSSTIFMCEVABETATI...EFLNRPVITFCQSIISTLY 115

APTSSSTKTKTQLEHLLDQIMILN...NNY NPRLTRMLTFKFPYMPKATELKHQCLERELKPLEE...LQ AOSKMPHIDPRDLISINIVIVLELQSSSTIFMCEVABETATI...EFLNRPVITFCQSIISTLY 115

APTSSSTKTKTQLEHLLDQIMILN...NNY NPRLTRMLTFKFPYMPKATELKHQCLERELKPLEE...LQ AOSKMPHIDPRDLISINIVIVLELQSSSTIFMCEVABETATI...EFLNRPVITFCQSIISTLY 115

APTSSSTKTKTQLEHLLDQIMILN...NNY NPRLTRMLTFKFPYMPKATELKHQCLERELKPLEE...LQ AOSKMPHIDPRDLISINIVIVLELQSSSTIFMCEVABETATI...EFLNRPVITFCQSIISTLY 115

APTSSSTKTKTQLEHLLDQIMILN...NNY NPRLTRMLTFKFPYMPKATELKHQCLERELKPLEE...LQ AOSKMPHIDPRDLISINIVIVLELQSSSTIFMCEVABETATI...EFLNRPVITFCQSIISTLY 115

APTSSSTKTKTQLEHLLDQIMILN...NNY NPRLTRMLTFKFPYMPKATELKHQCLERELKPLEE...LQ AOSKMPHIDPRDLISINIVIVLELQSSSTIFMCEVABETATI...EFLNRPVITFCQSIISTLY 115

FIGURE 10A

Residue no.	27	28	32	39	52	69	72	81	115	EC50 in CD25- YT (nM)	EC50 in CD25+ YT (nM)
WT IL2	G	I	K	M	E	V	L	R	V	1.104	0.01837
Seq 15	M	L	D	M	E	A	Q	D	I	0.2051	0.02011
Seq 16	M	L	D	L	S	A	Q	D	I	0.2137	0.01622

FIGURE 10B

Residue no.	80	81	85	86	92	EC50 in CD25- YT (nM)	EC50 in CD25+ YT (nM)
WT IL2	L	R	L	I	I	1.104	0.01837
Super-2	F	D	V	V	F	0.204	0.02088

FIGURE 10C

Residue no.	27	28	32	39	52	69	72	81	115	mutation number	ECS0 in CD25- Y1 (nM)
WT IL2	G	I	K	M	E	V	L	R	V	0	1.773
Seq 15	M	L	D	M	E	A	Q	D	I	7	0.2312
Seq 15 rev	M	L	D	M	E	V	Q	D	V	5	0.2241

FIGURE 11A

Residue no.	27	28	32	39	52	69	72	loop	115	mutation number	ECS0 in CD25- Y1 (nM)
WT IL2	G	I	K	M	E	V	L	AGSKNPHL	V	0	1.773
Seq 1	M	L	D	L	S	A	A	GDDPKTI	I	15	0.3545
Seq 1 rev	M	L	D	L	S	V	A	GDDPKTI	V	13	0.373

FIGURE 11B

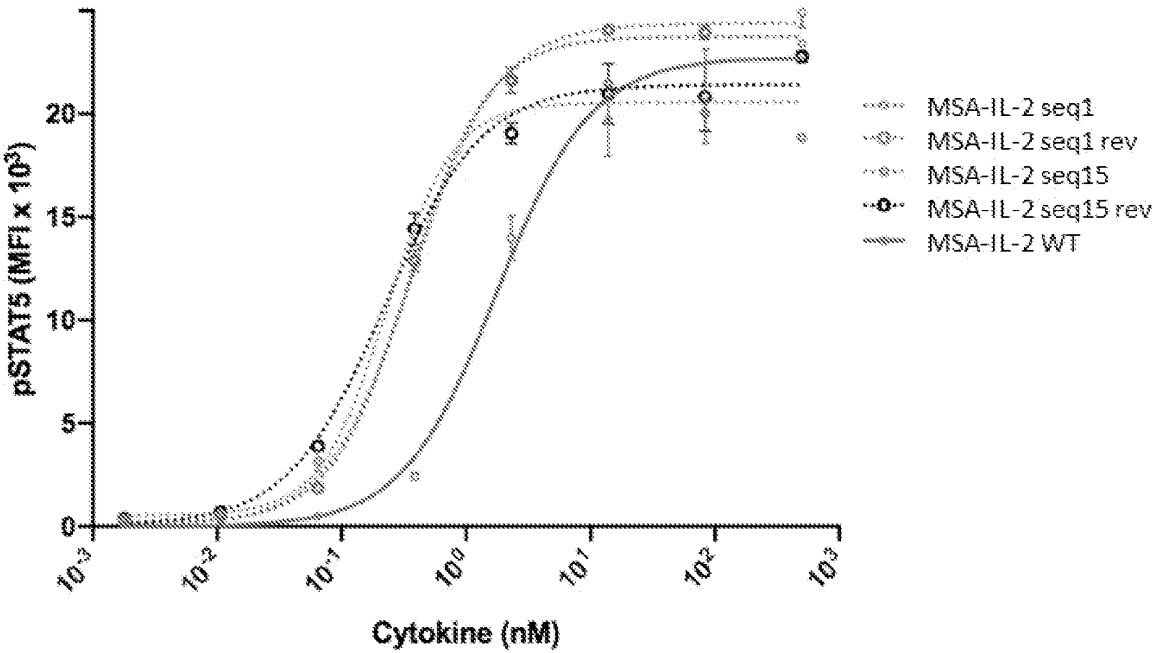


FIGURE 11C