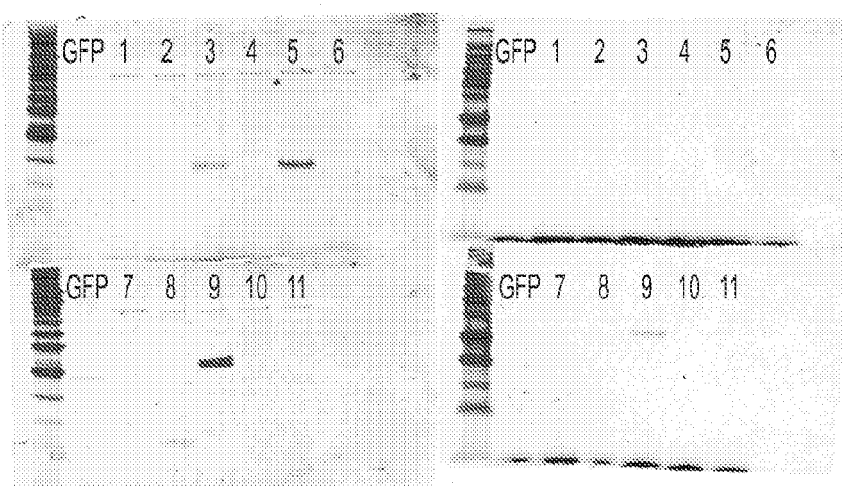
Gla PROTEIN	MEMBRANE AFFINITY	Gla RESIDUES	ADDITIONAL DOMAIN(S)	SIZE** (kD)
PROTEIN S	HIGH	11	EGF DOMAINS	4.95, 26.7
PROTEIN Z	MID	13	EGF DOMAINS	5.06, 13.9
PROTHROMBIN	MID	10	KRINGLE DOMAINS	5.06, 27.3
FVII	LOW	10	EGF DOMAIN ASSOCIATED WITH TF BINDING	4.95, 14.1
B0178	MID*	11	EGF DOMAIN ASSOCIATED WITH TF BINDING	4.95, 14.1

*AS DETERMINED BY FACs ON ACTIVATED PLATELETS IN COMPARISON TO FVII
**WITHOUT TAG

FIG. 1

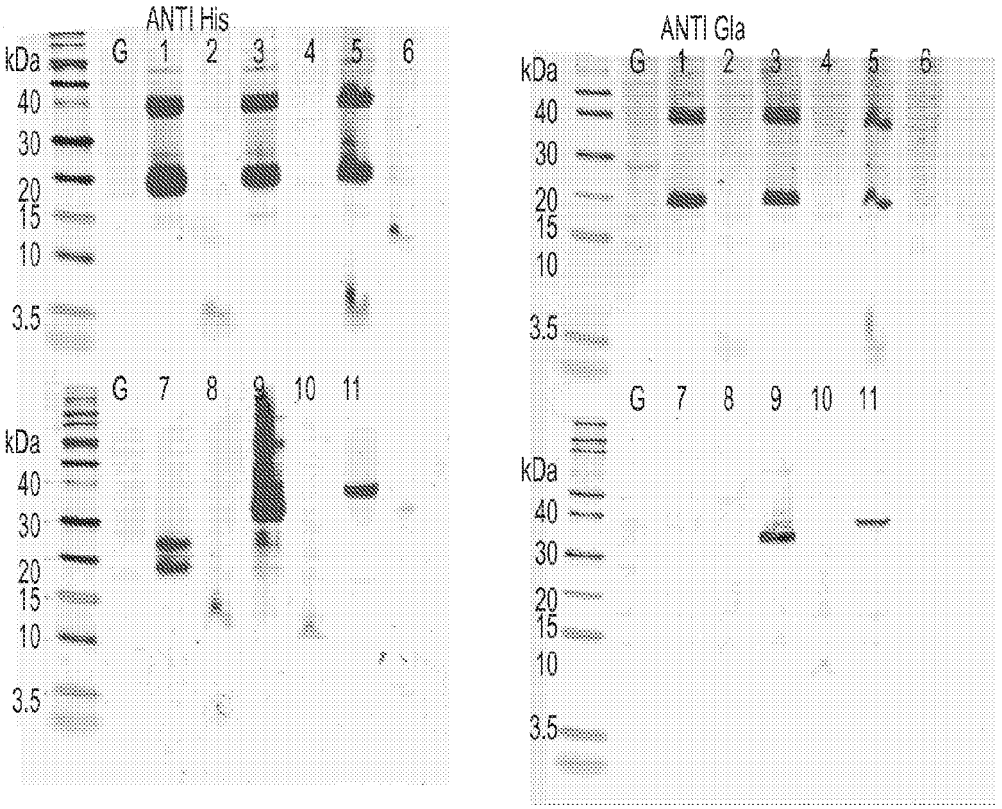


ANTI-Gla ANTI-His

PREP		DESCRIPTION
1	pEAKfl-CMV B0178 Gla+EGF	PROTHROMBIN SIGNAL SEQUENCE/PROPEPTIDE
2	pEAKfl-CMV B0178 Gla	
(3)	pEAKfl-CMV B0178 Gla+EGF	
4	pEAKfl-CMV FVII Gla	
(5)	pEAKfl-CMV FVII Gla+EGF	
6	pEAKfl-CMV ProteinZ Gla	
7	pEAKfl-CMV ProteinZ Gla+EGF	
(8)	pEAKfl-CMV ProteinS Gla	
(9)	pEAKfl-CMV ProteinS Gla+EGF	
10	pEAKfl-CMV PROTHROMBIN Gla	
11	pEAKfl-CMV PROTHROMBIN Gla+KRINGLE	

FIG. 2

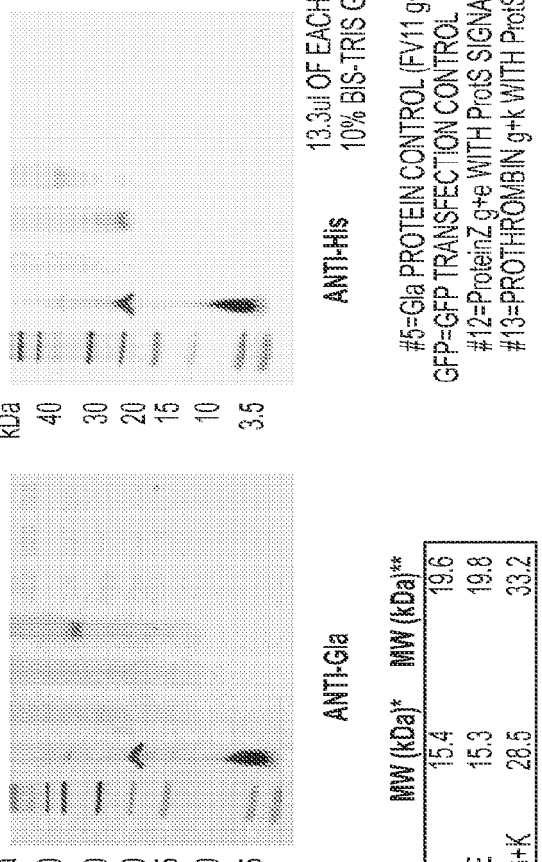
Gla PROTEIN	Gla DOMAIN ONLY (kDa)	Gla+EGF/KRINGLE (kDa)
B0178	6.5	15.4
FVII	6.5	15.4
PROTEIN Z	6.7	15.3
PROTEIN S	6.4	28
PROTHROMBIN	6.3	28.5



20ul OF SAMPLE (1/100 OF TOTAL CELL PELLET) LOADED

PREP		DESCRIPTION
1	pEAKfl-CMV B0178 Gla+EGF	PROTHROMBIN SIGNAL SEQUENCE/PROPEPTIDE
2	pEAKfl-CMV B0178 Gla	
3	pEAKfl-CMV B0178 Gla+EGF	
4	pEAKfl-CMV FVII Gla	
5	pEAKfl-CMV FVII Gla+EGF	
6	pEAKfl-CMV ProteinZ Gla	
7	pEAKfl-CMV ProteinZ Gla+EGF	
8	pEAKfl-CMV ProteinS Gla	
9	pEAKfl-CMV ProteinS Gla+EGF	
10	pEAKfl-CMV PROTHROMBIN Gla	
11	pEAKfl-CMV PROTHROMBIN Gla+KRINGLE	

FIG. 3



#	PROTEIN	MW (kDa)*	MW (kDa)**
5	FV11 G+E	15.4	19.6
12	PROTEIN Z G+E	15.3	19.8
13	PROTHROMBIN G+K	28.5	33.2

*PROCESSED FORM INCLUDING His TAG
**UNPROCESSED FORM INCLUDING His TAG

FIG. 4

1 11 21 31 41 51 61 71
ANSLLEETKQ GNLERECIEE LCNKEEAREV FENDPETDYF YPKYLVCLRS FQTGLFTAAR QSTNAYPDLR SCVNAIPDQC

81 91 101 111 121 131 141 151
SPLPCNEDGY MSCKDGKASF TCTCKPGWQG EKCEFDINEC KDPSNINGGC SQICDNTPGS YHCSCKNGFV MLSNKKDCKD

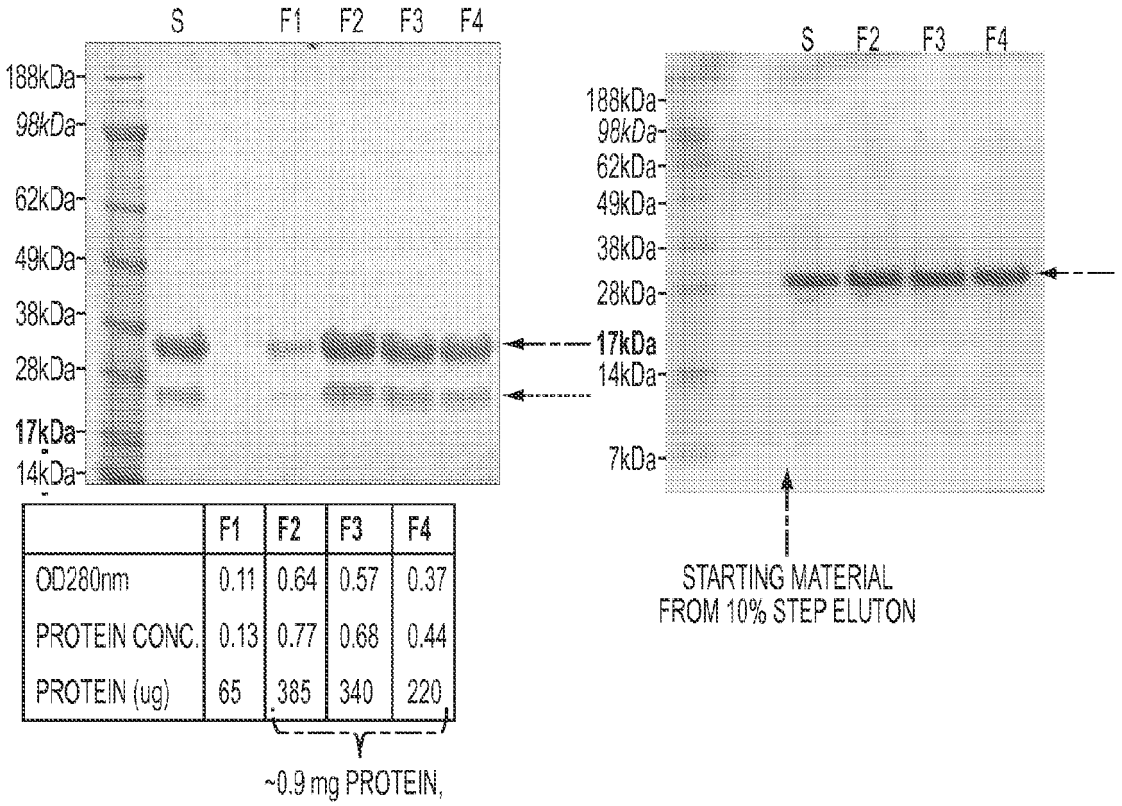
161 171 181 191 201 211 221 231
VDECSLKPSI CGTAVCKNIP GDFECECPEG YRYNLKSKSC EDIDECSENM CAQLCVNYPG GYTCYCDGKK GFKLAQDQKS

241
CESRHHHHHH

PTMs:

γ-CARBOXYLATION: 11Gla
D95: HYDROXYLATION
DISUFLIDE BOND: PSSIBLE 14, REPORTED 6
TAG: 6xHis

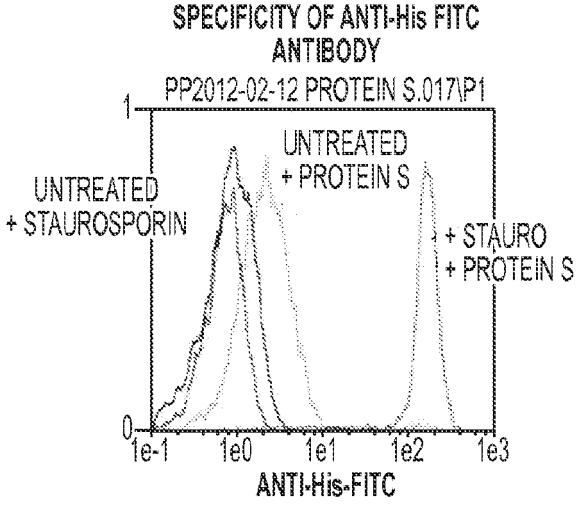
FIG. 5



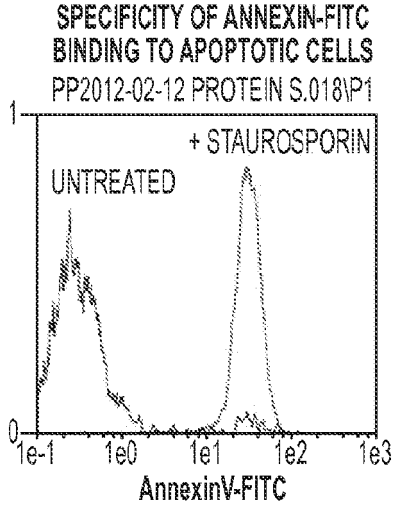
	F1	F2	F3	F4
OD280nm	0.11	0.64	0.57	0.37
PROTEIN CONC.	0.13	0.77	0.68	0.44
PROTEIN (ug)	65	385	340	220

~0.9 mg PROTEIN,

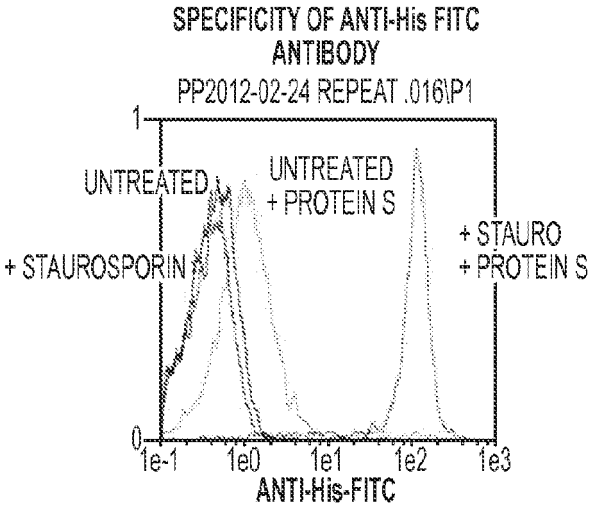
FIG. 6



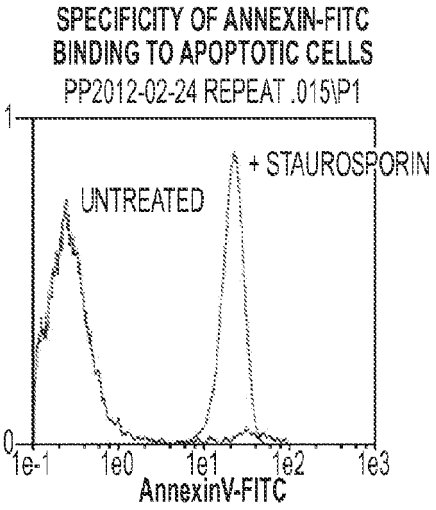
ID	UNTREATED	+ STAU	UNTREATED + PROT S	+ STAU +PROT S
MFI	0.91	0.79	4.72	159.3



ID	UNTREATED	+ STAU
MFI	1.93	32.3



ID	UNTREATED	+ STAU	UNTREATED + PROT S	+ STAU +PROT S
MFI	0.38	0.38	3.23	109.5

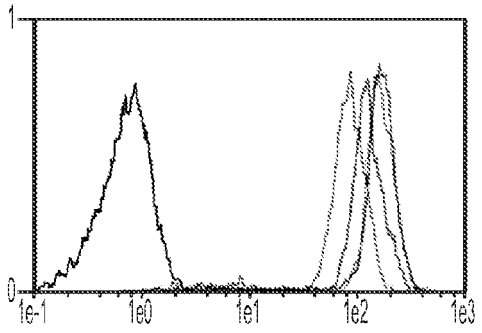


ID	UNTREATED	+ STAU
MFI	1.89	21.9

FIG. 7

ANNEXIN V COMPETES OFF PROTEIN S BINDING TO STAUROSPORIN TREATED JURKAT CELLS

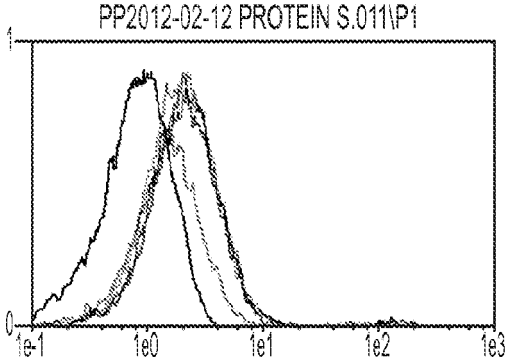
PP2012-02-12 PROTEIN S.001\P1



ID	NO PROT S	+ PROT S	+ PROT S + 0.2X Ann-V	+ PROT S + 2.0X Ann-V	+ PROT S + 5.0X Ann-V
MFI	0.79	159.3	163.1	119.9	82.2

WEAK BINDING OF PROTEIN S TO UNTREATED JURKAT IS ALSO COMPETED OFF BY ANNEXIN V

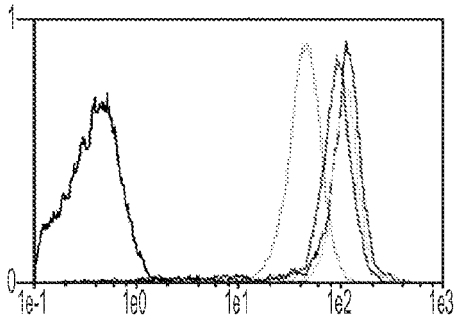
PP2012-02-12 PROTEIN S.011\P1



ID	NO PROT S	+ PROT S	+ PROT S + 0.2X Ann-V	+ PROT S + 2.0X Ann-V	+ PROT S + 5.0X Ann-V
MFI	0.91	4.72	3.84	3.94	2.65

ANNEXIN V COMPETES OFF PROTEIN S BINDING TO STAUROSPORIN TREATED JURKAT CELLS

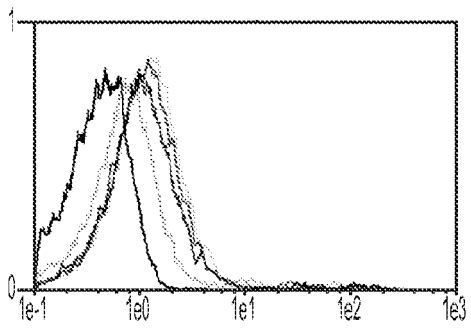
PP2012-02-24 REPEAT .006\P1



ID	NO PROT S	+ PROT S	+ PROT S + 0.4X Ann-V	+ PROT S + 4.0X Ann-V	+ PROT S + 10.0X Ann-V
MFI	0.38	109.5	110.3	89.8	45.3

WEAK BINDING OF PROTEIN S TO UNTREATED JURKAT IS ALSO COMPETED OFF BY ANNEXIN V

PP2012-02-24 REPEAT .016\P1



ID	NO PROT S	+ PROT S	+ PROT S +0.4X Ann-V	+ PROT S + 4.0X Ann-V	+ PROT S + 10.0X Ann-V
MFI	0.38	3.23	2.52	2.86	2.32

FIG. 8