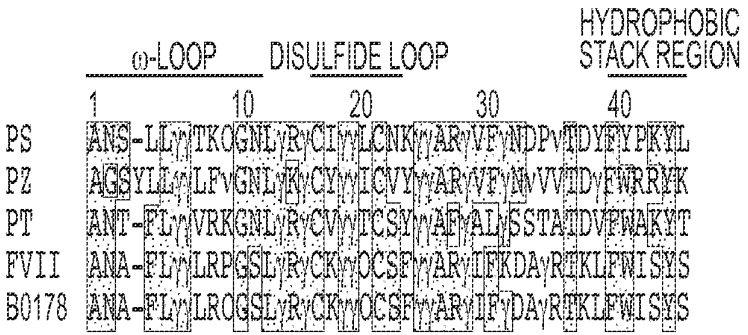
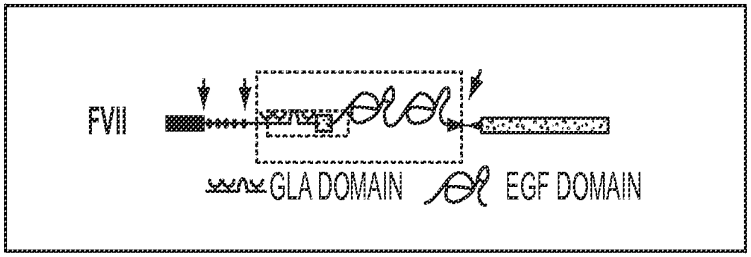


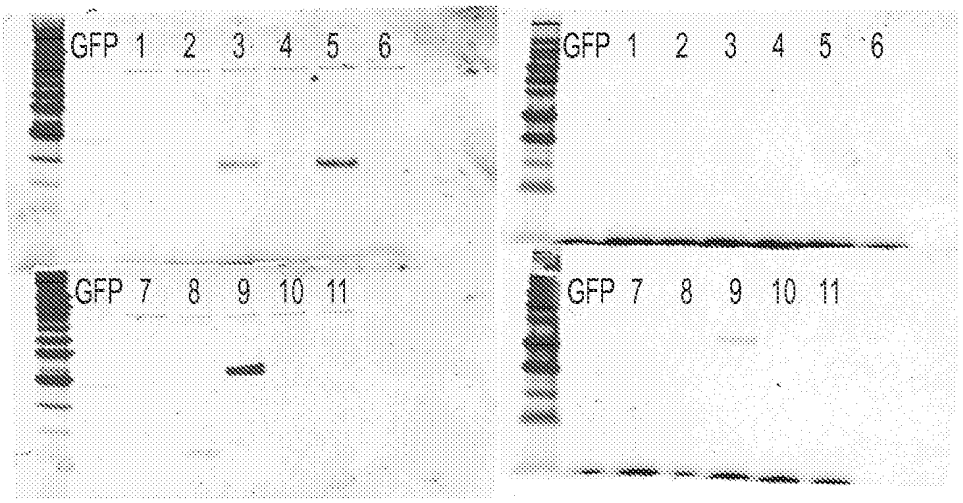
MODIFIED FROM HANSSON AND STENNO, JOURNAL OF THROMBOSIS AND HAEMOSTASIS



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

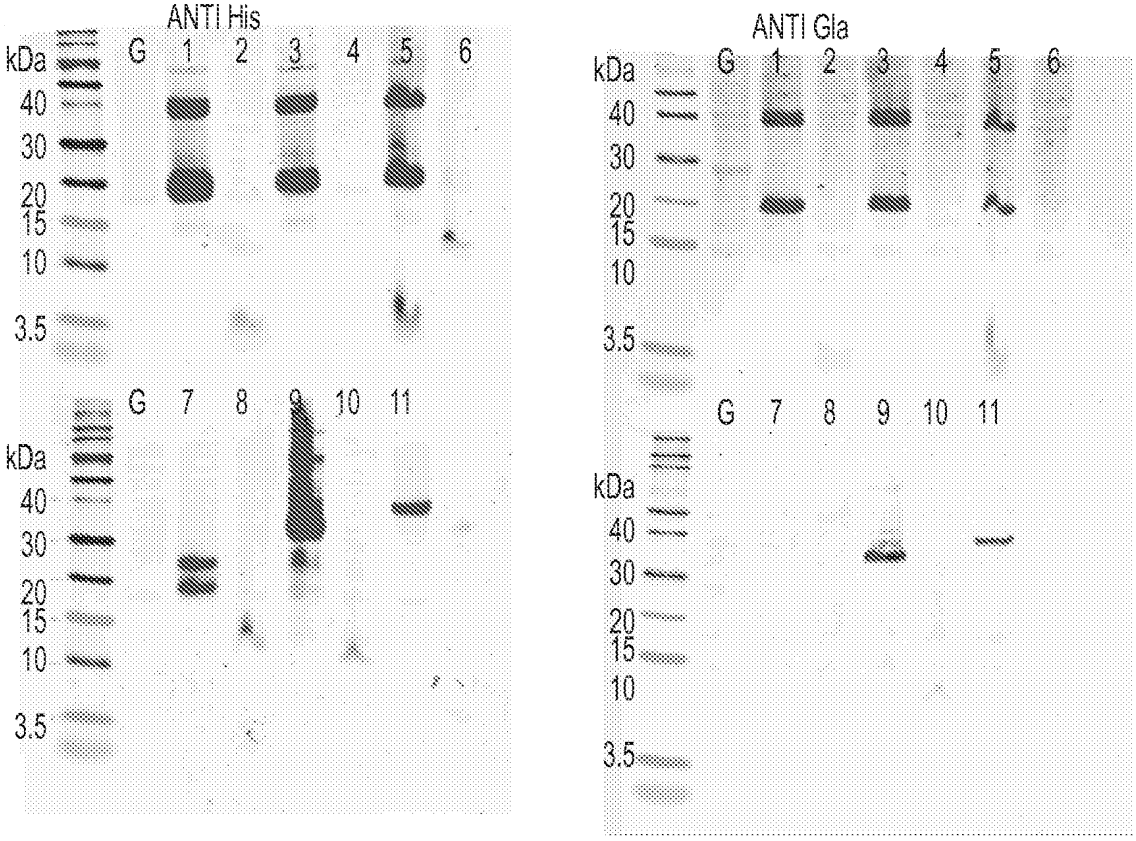


ANTI-Gla ANTI-His

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

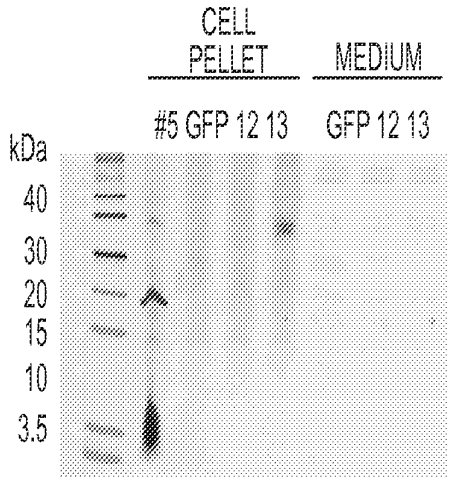
Gla PROTEIN	Gla DOMAIN ONLY (kDa)	Gla+EGF/KRINGLE (kDa)
B0178	6.5	15.4
FVII	6.5	15.4
PROTEIN Z	6.7	15.3
PROTEIN S	6.4	28
PROTHROMBIN	6.3	28.5



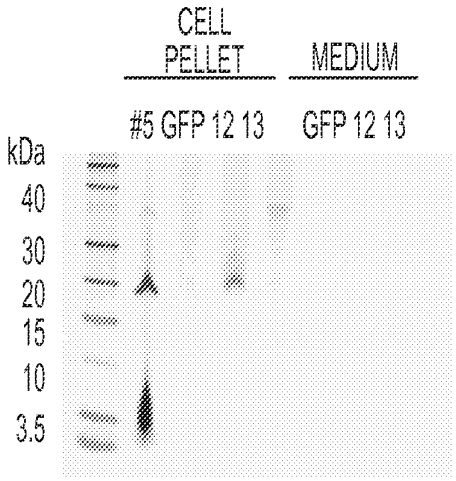
20ul OF SAMPLE (1/100 OF TOTAL CELL PELLET) LOADED

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



ANTI-Gla



ANTI-His

13.3ul OF EACH SAMPLE LOADED
10% BIS-TRIS GEL, REDUCING CONDITIONS

#	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

#5=Gla PROTEIN CONTROL (FV11 g+e FROM 3/2011 BHK21exp)
GFP=GFP TRANSFECTION CONTROL
#12=ProteinZ g+e WITH ProtS SIGNAL SEQUENCE AND PROPEPTIDE
#13=PROTHROMBIN g+k WITH ProtS SIGNAL SEQUENCE AND PROPEPTIDE

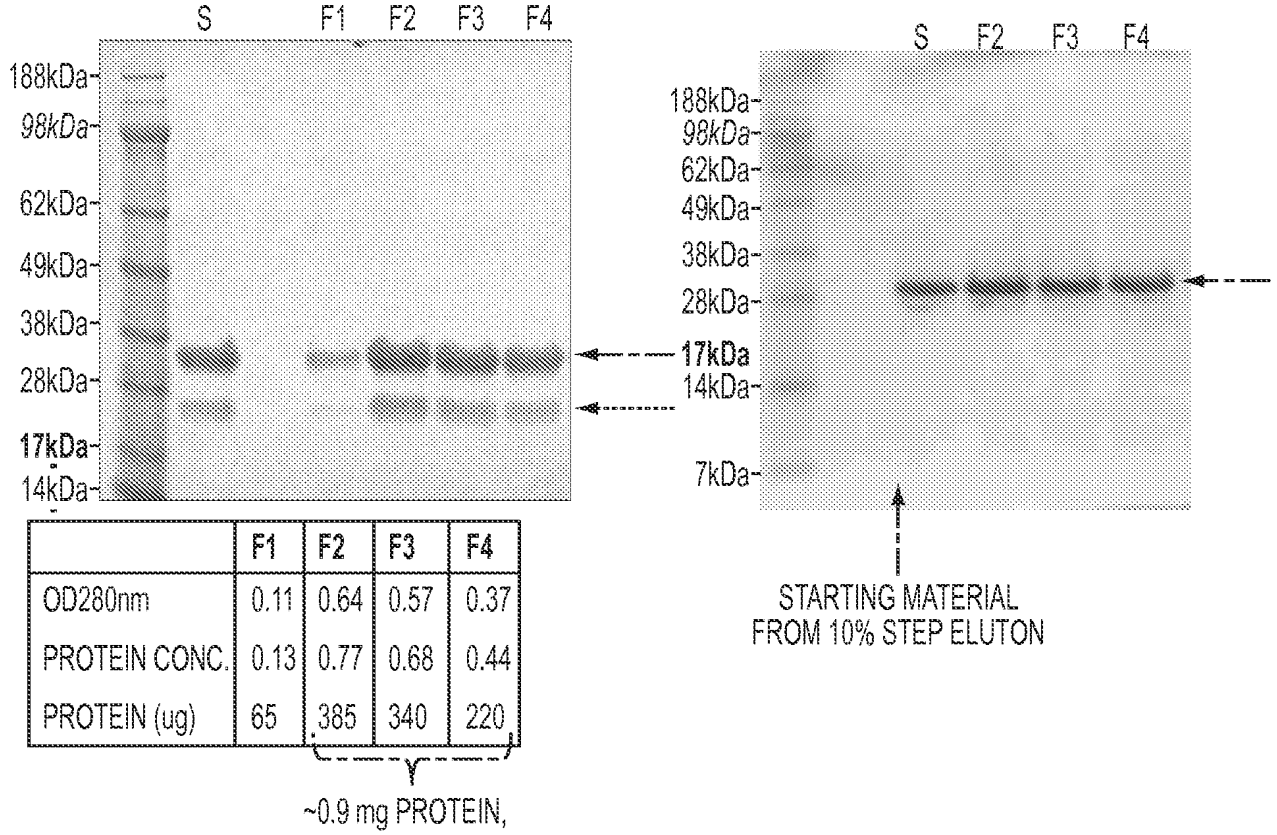
FIG. 4

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

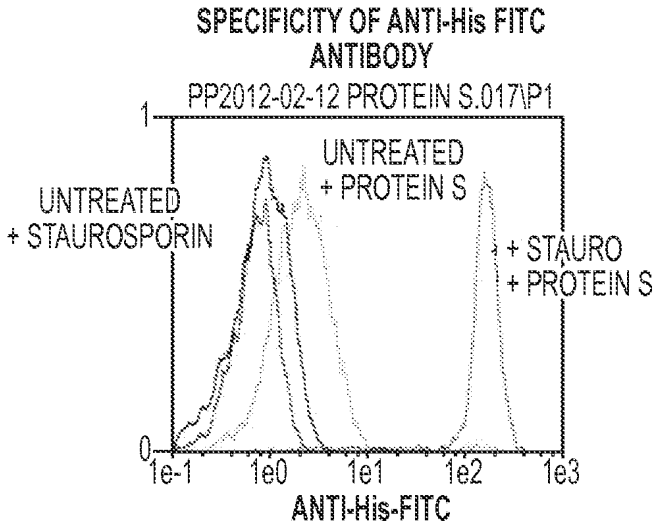
FIG. 5



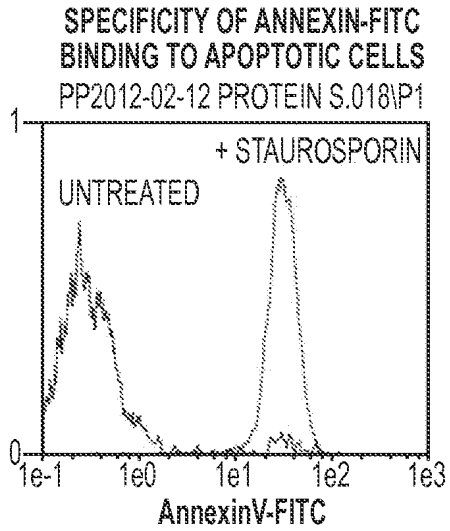
	F1	F2	F3	F4
OD280nm	0.11	0.64	0.57	0.37
PROTEIN CONC.	0.13	0.77	0.68	0.44
PROTEIN (ug)	65	385	340	220

~0.9 mg PROTEIN,

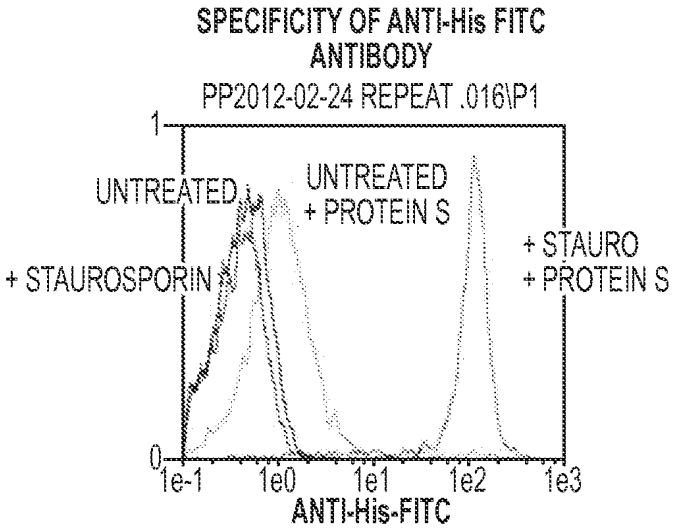
FIG. 6



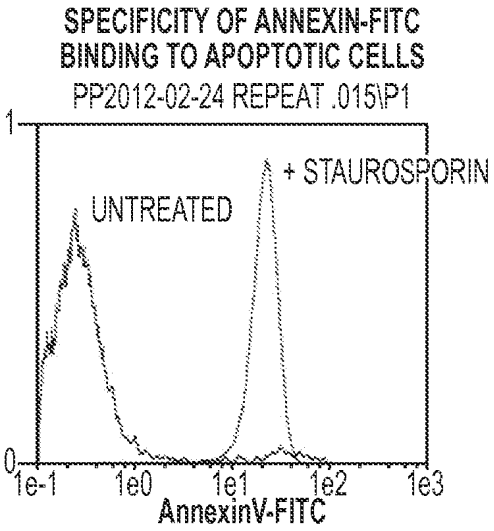
ID	UNTREATED	+ STAU	UNTREATED + PROT S	+ STAU +PROT S
MFI	0.91	0.79	4.72	159.3



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

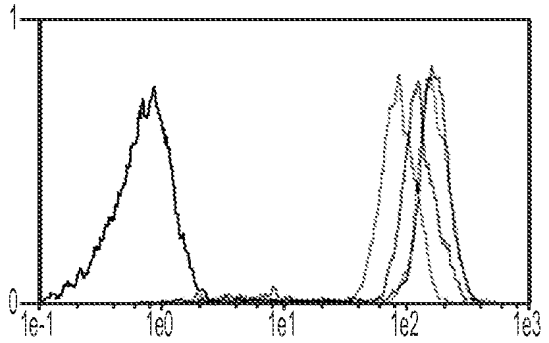


ID	UNTREATED	+ STAU
MFI	1.89	21.9

FIG. 7

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

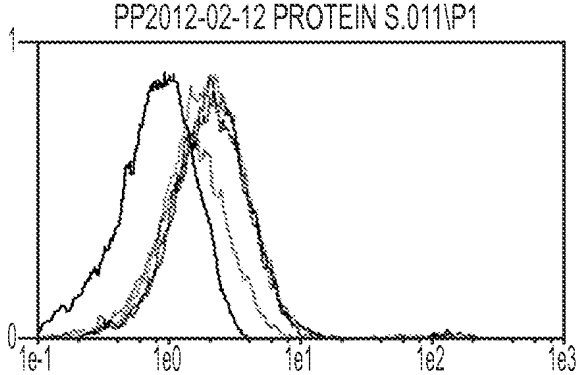
PP2012-02-12 PROTEIN S.001\P1



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

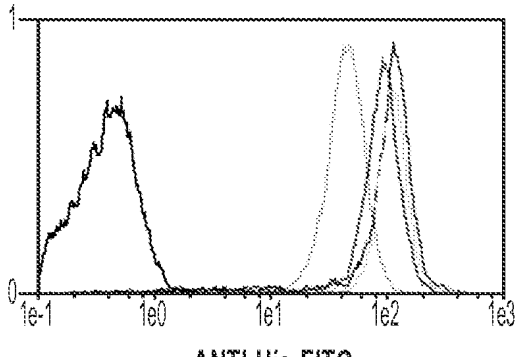
PP2012-02-12 PROTEIN S.011\P1



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

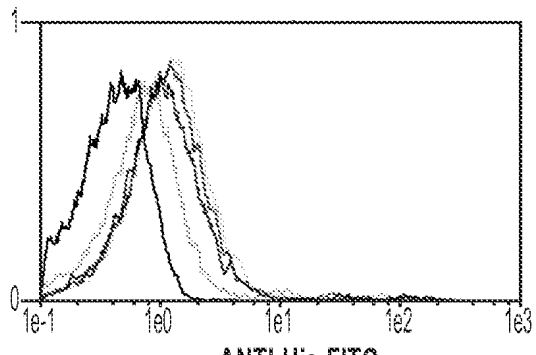
PP2012-02-24 REPEAT .006\P1



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