Stage	Principle	Advantages	Disadvantages	Examples	Ref
1. Pre-transplantation stage: terminally differentiate hESC or selectively remove undifferentiated hESC					
Induced differentiation of remaining undifferentiated cells	Extended differentiation or additional differentiated steps by chemical induction	Easy to perform, Inexpensive;	Differentiation efficiency is crucial, Unclear about the effect of chemicals on desired cell;	Direct hESC differentiation to motor neuron progenitors in the presence of retinoic acid (RA) and sonic hedgehog (SHH)	21,32
Removal by specific antibodies or inhibitors	Purification of desired cell or removal of undesired cells with specific antibodies or inhibitors	High specificity;	Antibody and inhibitor specificity is crucial, Costs,	MACS or FACS sorting of well-characterized surface antigens, such as SSEA-4 and TRA-1-60, or inducing hESC death by cytotoxic mAb84, scFv84-HTH, or inhibitor	37,38,47-51
Selection by transgenic fluorescent marker	Purification of desired cell or removal of undesired cells with transgenic fluorescent marker	High specificity, High yield;	Genetic manipulation introduce another safety hurdle;	Genetic selection of sox1GFP-expressing neural precursors against undifferentiated hESC or removal of Oct-4 GFP-expressing undifferentiated hESC	52,53
Purification on physical properties	Division by differences in cell adherence or density gradient centrifugation	Easy to perform, Fast, Inexpensive;	Low specificity, Low yields;	Percoll density centrifugation enrich hESC-derived Cardiomyocytes	54,55
2. Early post-transplantation stage: interrupt tumor progression					
Prevention of tumor formation by cytotoxic drugs	Selectively targeting teratoma-forming cells with high proliferation rate	Easy to perform, Inexpensive, Fast;	Only active teratoma-forming cells can be targeted, Dormant cells cannot be eliminated;	Induction of apoptosis in undifferentiated cells expressing Oct-4/PAR-4 by ceramide analogues	56
Interruption of tumor formation by genetic engineering	Transfection with a fusion gene consisting a "suicide gene" and a fluorescent marker	Able to trace the kinetics of teratoma formation, Timely, High yields;	All cells carrying the "suicide gene" are eliminated, Genetically modified cells are prone to transformation;	Termination of undergoing teratomas formation by applying ganciclovir to hESC carrying thymidine kinase (TK) and fluorescent marker to induce cell death	57-59
3. Late post-transplantation stage: remove detected tumor					
Removal of detected tumor by genetic engineering	Transfection of hESC with transgenic viral gene and induce cell death with specific drugs	High yields;	All cells carrying the "suicide gene" are eliminated, Genetically modified cells are prone to transformation	Removal of detected tumors by applying ganciclovir to hESC carrying thymidine kinase (TK) to induce cell death	60

Figure 1

Antibody	Target cell	Target antigen	Mode of cell death
MEM-59	Hematopoietic progenitor cells	CD43	Apoptosis
Rituximab	Non-hodgkin's lymphoma	CD20	Apoptosis
GA101	Non-hodgkin's lymphoma	CD20	Oncosis
Tositumomab	Human lymphoma cells	CD20	Oncosis
anti-HLA-DR mAbs	Primary chronic lymphocytic leukemia cells	HLA-DR	Oncosis
RAV12	Adenocarcinoma	RAAG12	Oncosis
Anti-porimin	Jurkat cell	Porimin	Oncosis
RE2	Interleukin 2-dependent T cell	n.a.	Oncosis
Anti-NeuGcGM3 14F7	Breast carcinoma and melanoma cells	NeuGcGM3	Oncosis
Anti-NeuGcGM3 Abs	Lung tumor cells	NeuGcGM3	Oncosis

Figure 2

Hallmarks	Assays
Cell shrinkage and apoptotic body formation	Electron microscope (TEM or SEM)
Caspase activation	Measure caspase activity
Phosphatidylserine externalization	Annexin V detection
DNA fragmentation	TUNEL assay

Figure 3

Hallmarks	Assays
Rapid cell death (within seconds to minutes)	Flow cytometry
Membrane damage	Uptake of trypan blue/propidium iodide/Dextran beads; Scanning electron microscope
Apparent swelling of cell and organelles	Transmission electron microscope
Cytoskeleton proteins degradation	Western Blot
Leakage of intracellular components	Change in lactate dehydrogenase concentration

Figure 4A

	Oncosis	Apoptosis
Cell death time	Fast (seconds to minutes)	Slow (12-24hrs)
Volume changes	**Swelling** of cytoplasm and mitochondria	**Shrinking** of cytoplasm and condensation of nucleus
Membrane damage	Yes	No
Cytoplasm	Released	Retained in apoptotic bodies
Degradation of Cytoskeleton-associated proteins	Involved	Not involved
Caspases activity	No	Yes
DNA fragmentation	Random	Ordered
Inflammation	Yes	No

Figure 4B

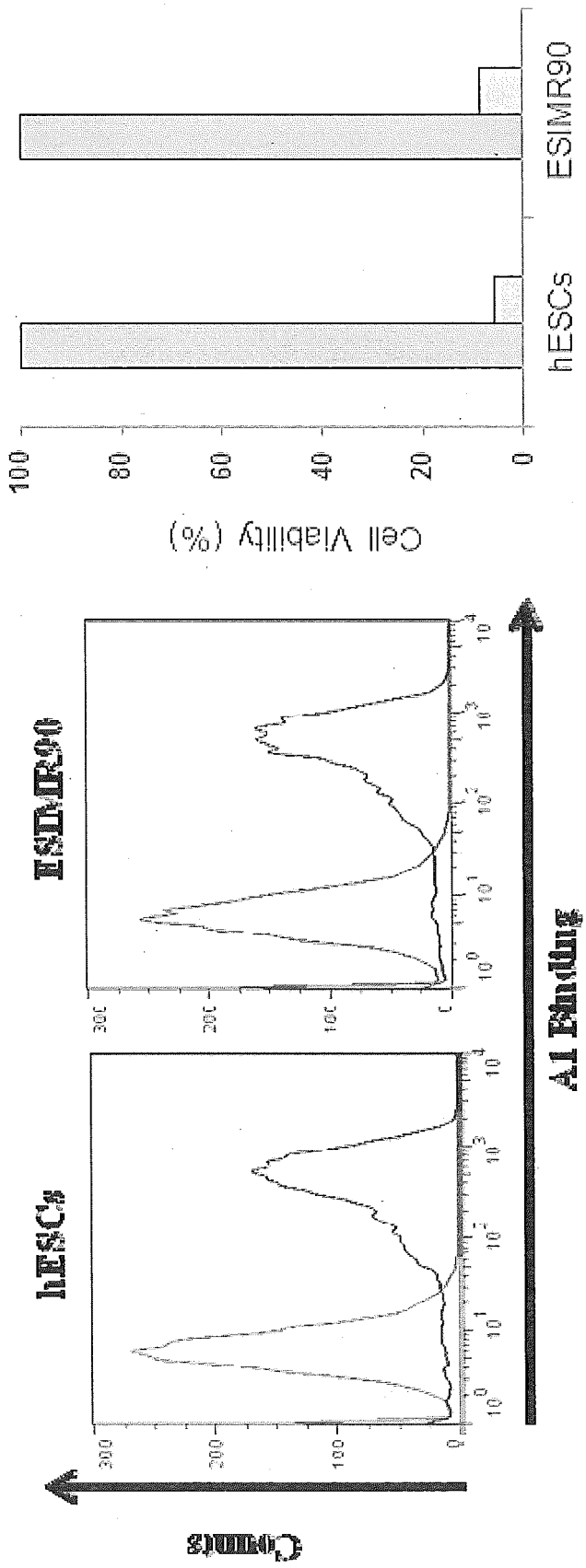

Cell Viability (%)
100
80
60
40
20
0
hESCs
ESIMR90
Counts
hESCs
ESIMR90
A1 Binding

Figure 5

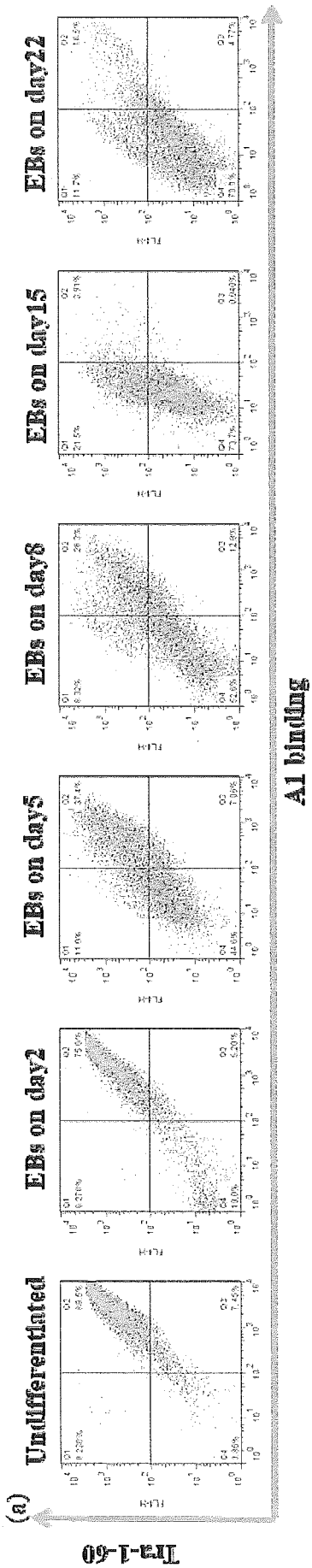


Figure 6A

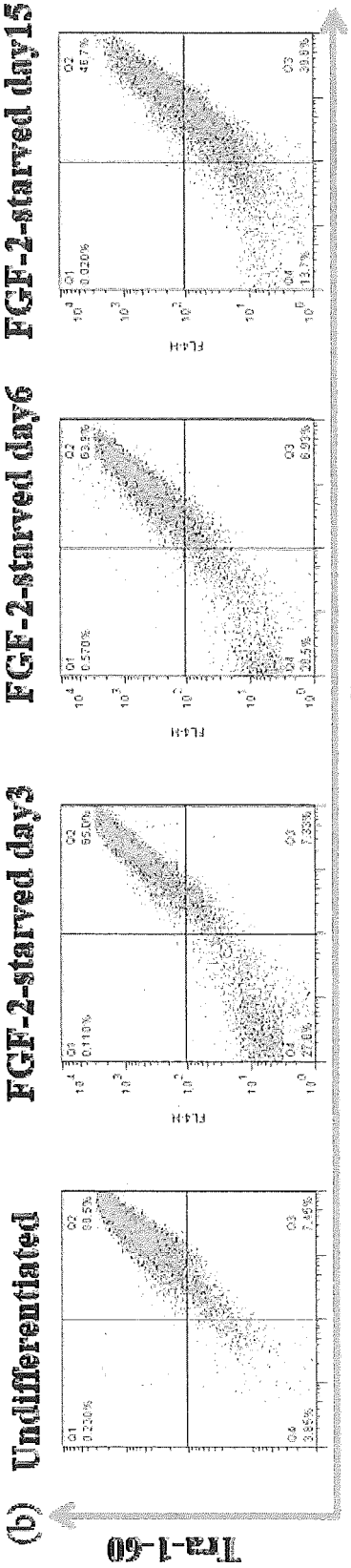


Figure 6B

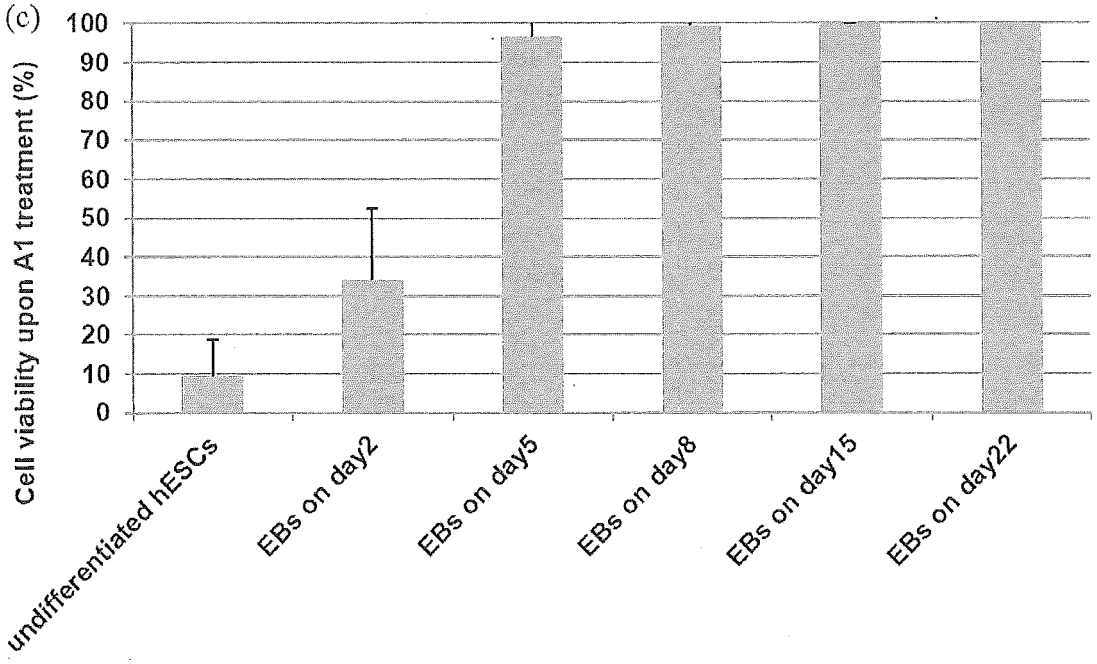


Figure 6C

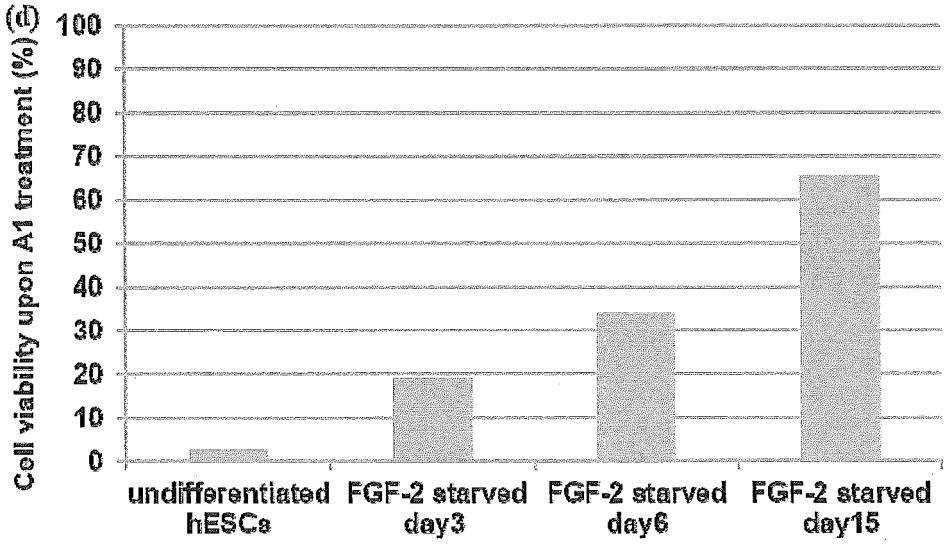


Figure 6D

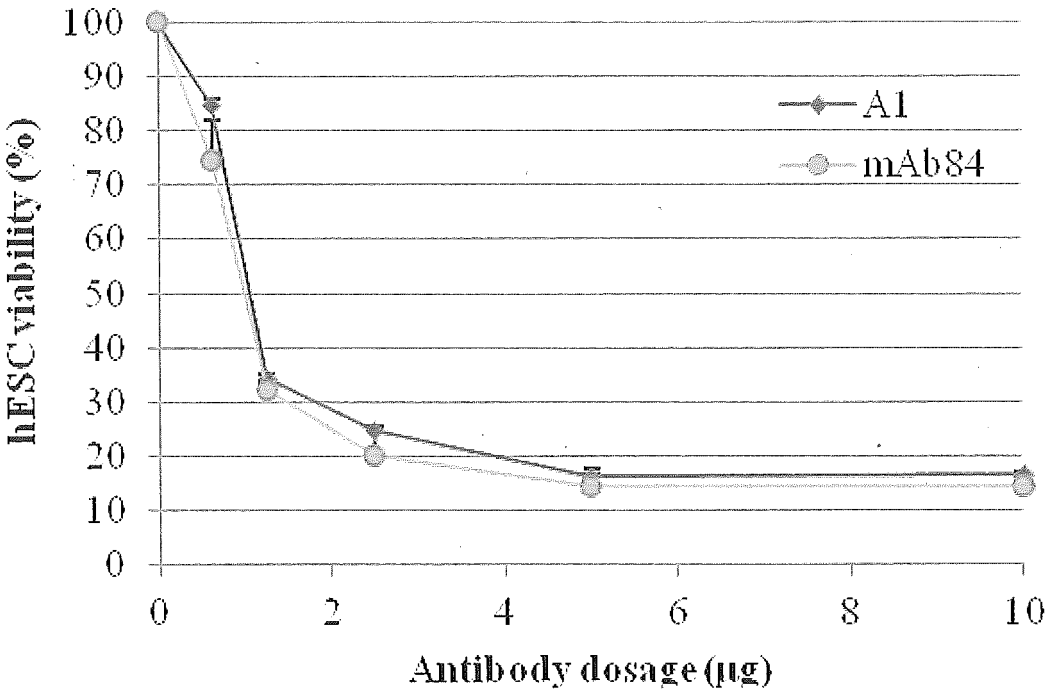


Figure 7

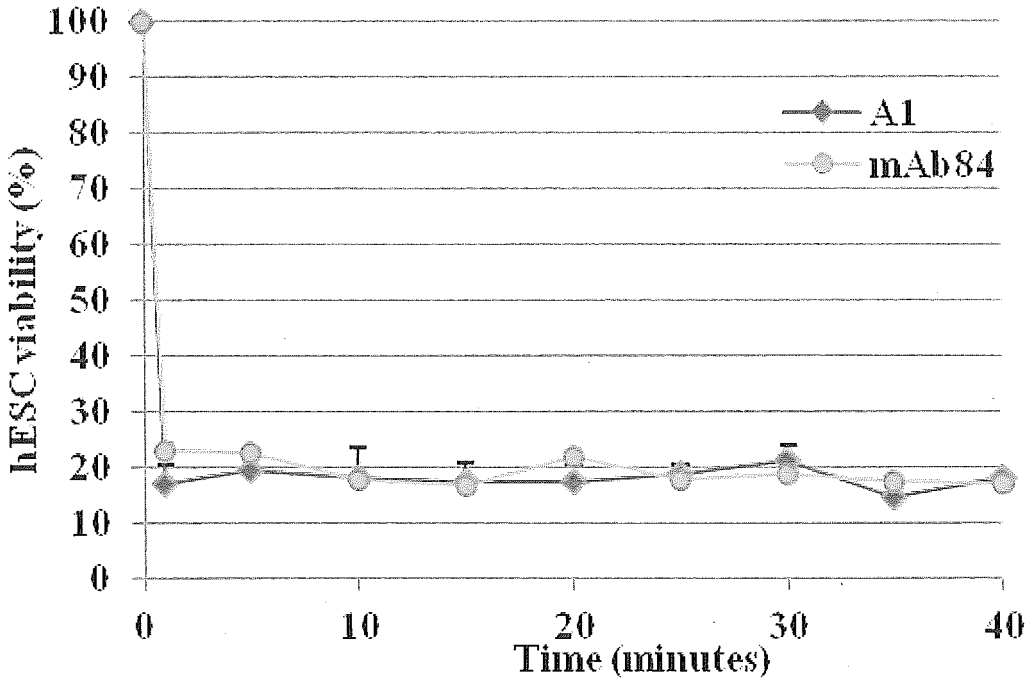


Figure 8

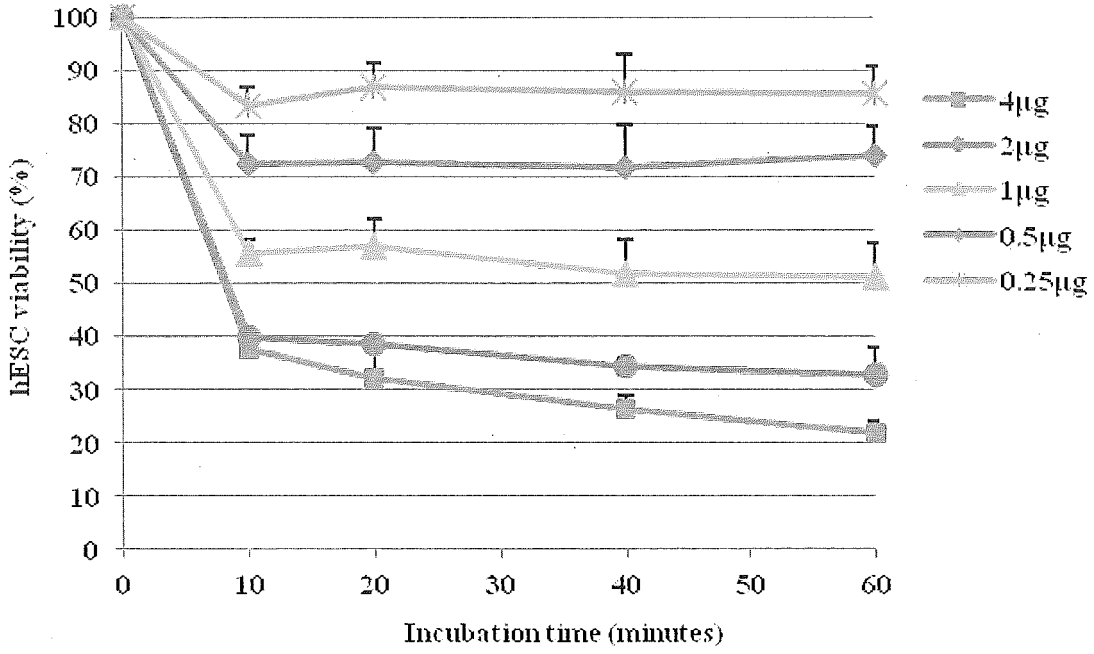


Figure 9A

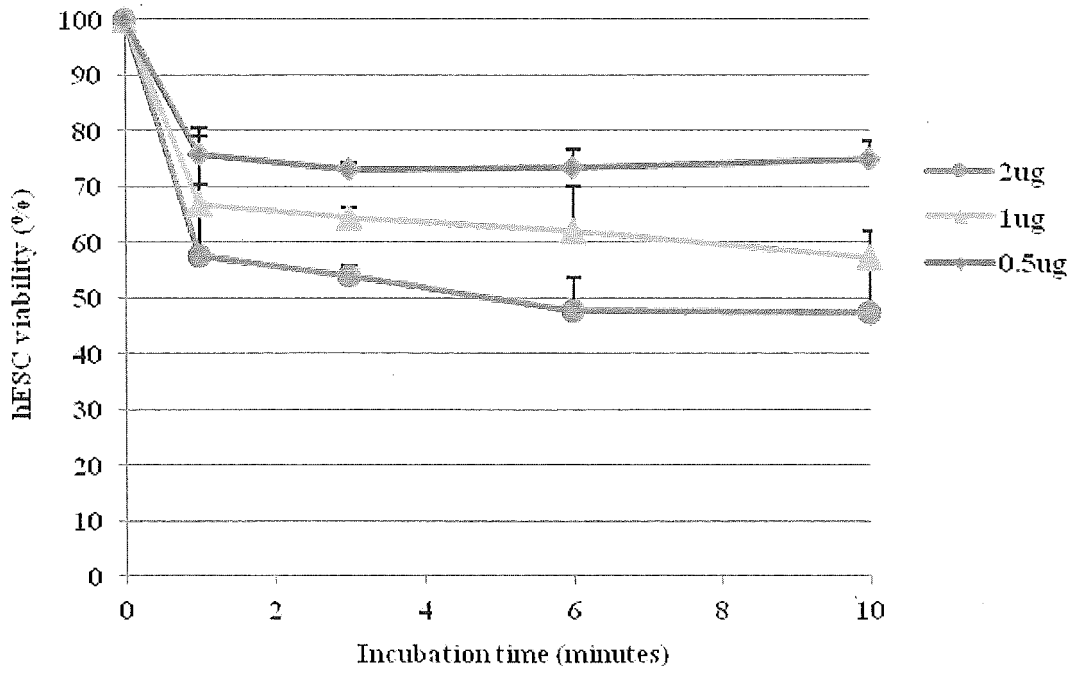


Figure 9B

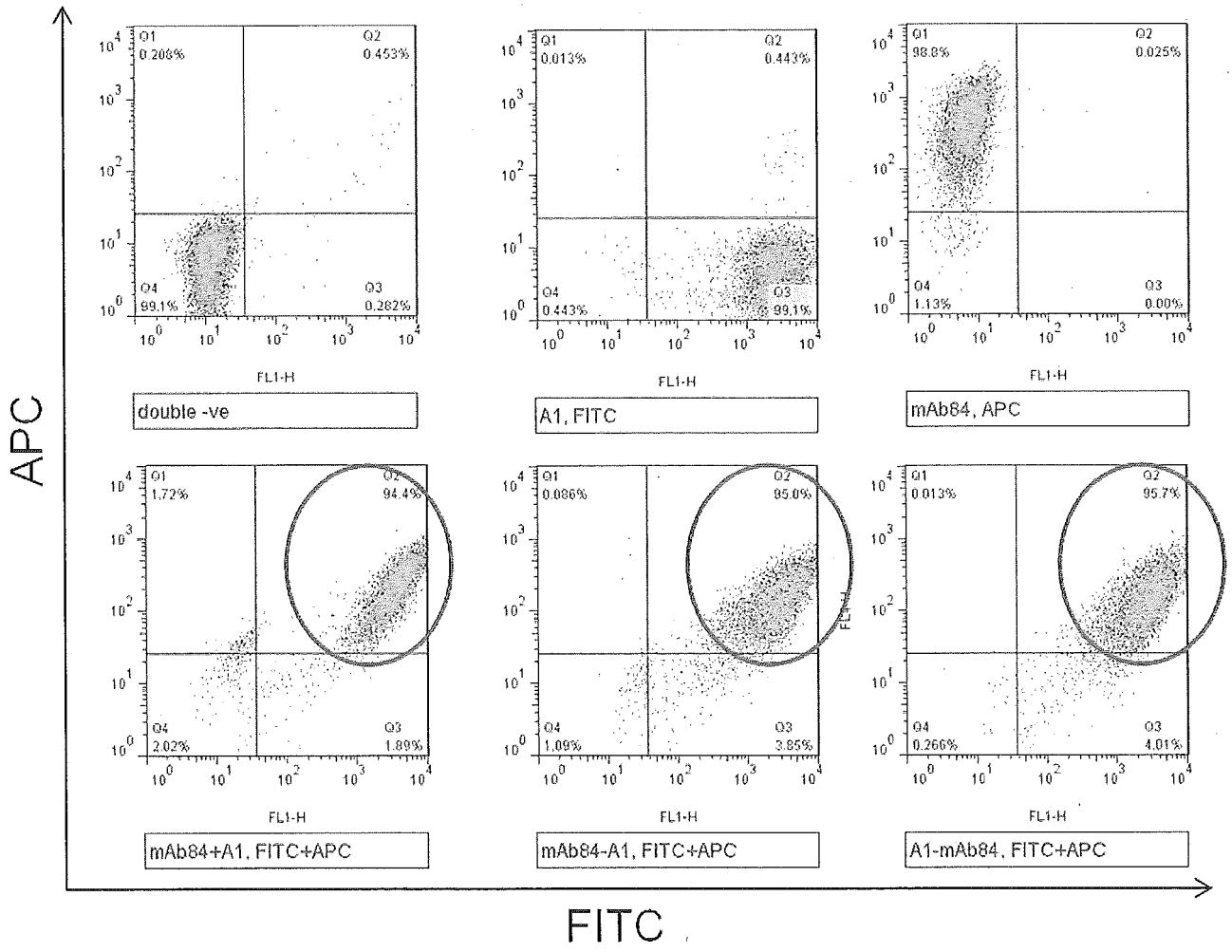

APC
FITC
Q1 0.208%
Q2 0.453%
Q4 99.1%
Q3 0.282%
FL1-H
double -ve
Q1 0.013%
Q2 0.443%
Q4 0.443%
Q3 99.1%
FL1-H
A1, FITC
Q1 98.8%
Q2 0.025%
Q4 1.13%
Q3 0.00%
FL1-H
mAb84, APC
Q1 1.72%
Q2 94.4%
Q4 2.02%
Q3 1.89%
FL1-H
mAb84+A1, FITC+APC
Q1 0.086%
Q2 95.0%
Q4 1.09%
Q3 3.85%
FL1-H
mAb84-A1, FITC+APC
Q1 0.013%
Q2 95.7%
Q4 0.266%
Q3 4.01%
FL1-H
A1-mAb84, FITC+APC

Figure 10

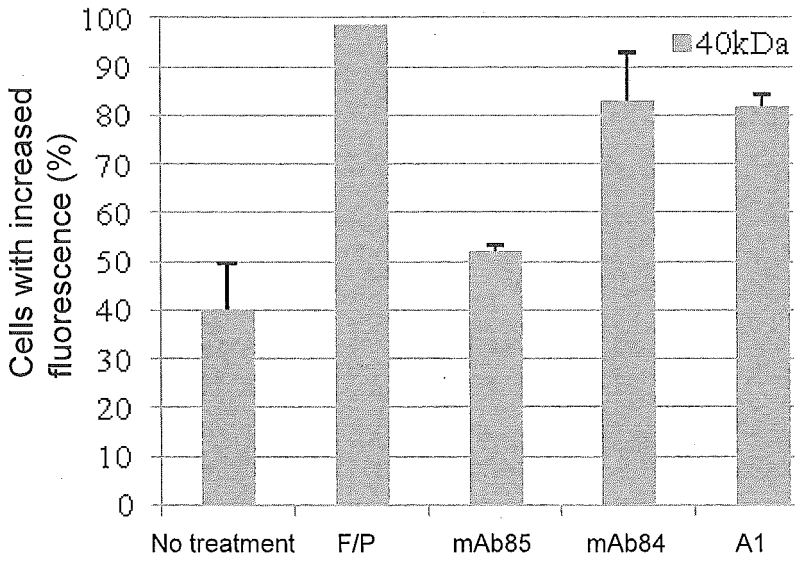


Figure 11A

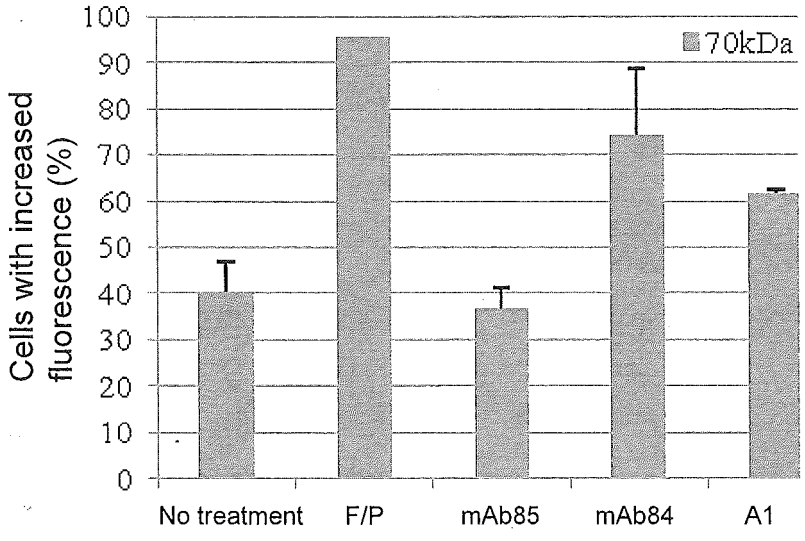


Figure 11B

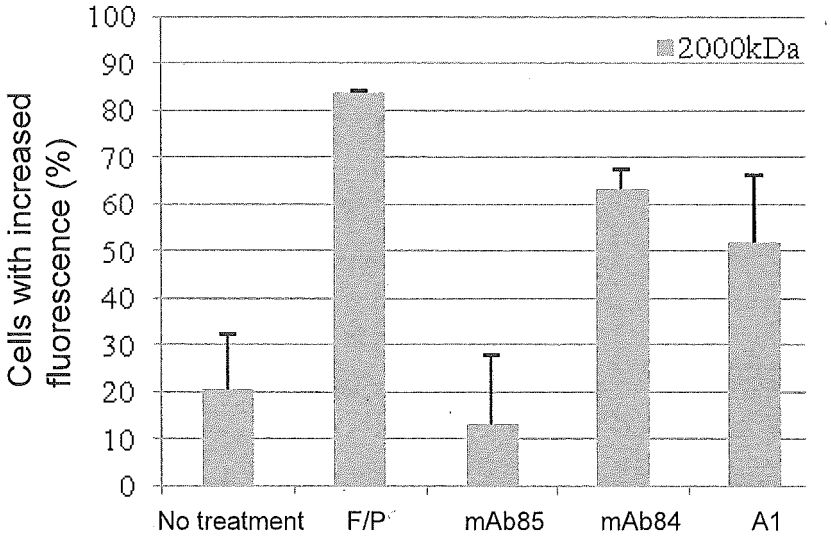

Cells with increased fluorescence (%)
100
90
80
70
60
50
40
30
20
10
0
2000kDa
No treatment
F/P
mAb85
mAb84
A1

Figure 11C

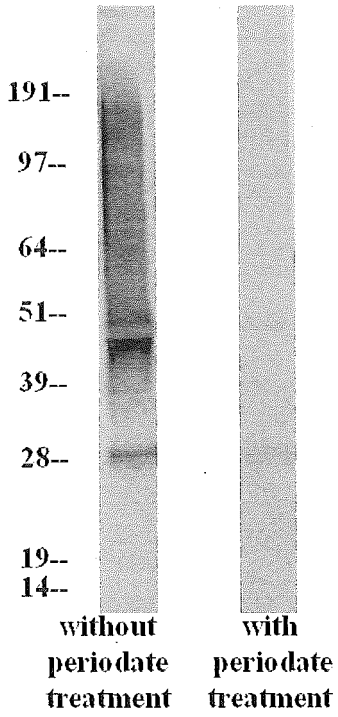


Figure 12A

Cell Adhesion proteins

PODXL, integrin α-6/α-5/α-1, integrinβ-1/β-5, SorLA-1, CD44, Protocadherin-1, Na/H exchanger, CD166, Neuroligin-4, Laminin γ-1, Neuroplastin, CD239, CD326, CD56, CD36, Cell adhesion molecule 1, CD111, CD113, Calsyntenin-1, Tubulin-specific chaperone D, Catenin α-1/β -1/Δ-1,

Cytoskeleton-associated proteins

Trans-Golgi network integral membrane protein 2, Prominin-1, Syndecan-1, Plexin-B2, CD100, Leucine-rich repeat neuronal protein 1, KIF 11/12, Dynamin-2, Alpha-actinin-1, NHERF-1, TCP-1-epsilon, TCP-1-delta, Filamin B

ATPase proteins

Na/K-transporting ATPase α-1/α-2/α-3/β-3, ATP5F1, CD340, E-NPP 1, Tyrosine-protein kinase Kit, CD331, SR Ca(2+)-ATPase 2, LACS 3, Hexokinase-1, V-ATPase subunit A, CTP synthase 1, PFK-A

Transporter proteins

CD98, GLUT-3, GLUT-2, GLUT-1, Sodium bicarbonate cotransporter 3, MRP46, K-Cl cotransporter 4, LTC4 transporter, Zinc transporter ZIP14, MOAT-C, MOAT-B, NPC1, SLC12A4, SLC5A6, Potassium-chloride transporter 9, MCT-1, Alpha-adaptin C, Beta-1-adaptin, Beta-COP, Alpha-COP, Gamma-2-COP, Exportin-2

Figure 12B

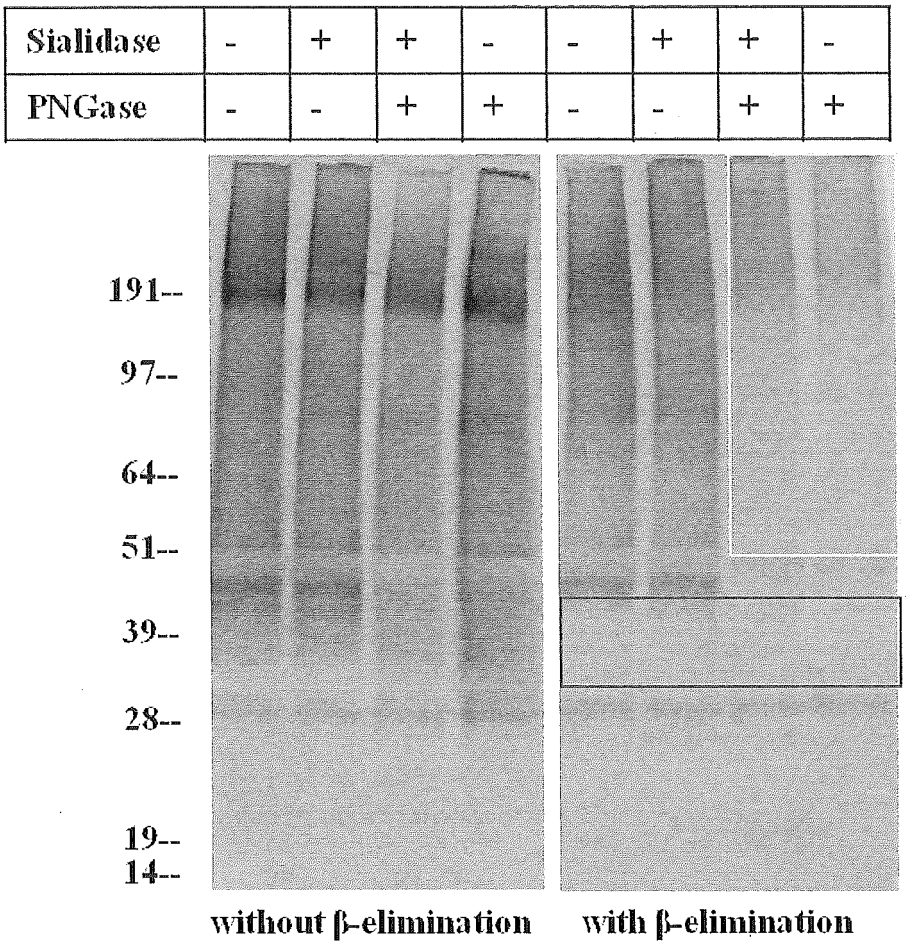
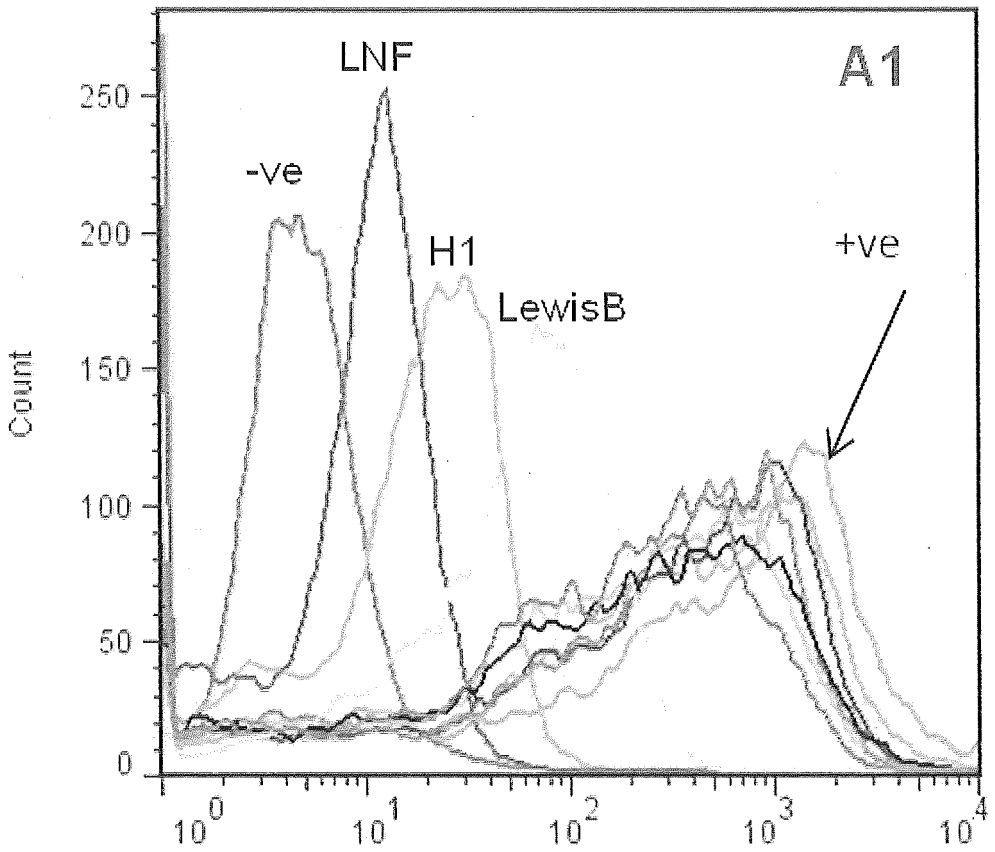

Sialidase - + + - - + + -
PNGase - - + + - - + +
191--
97--
64--
51--
39--
28--
19--
14--
without β-elimination
with β-elimination

Figure 12C



	TUBE NAME
	A1/LewisY
	A1/LewisX
	A1/LewisB
	A1/LewisA
	A1/bgB
	A1/bgA
	A1/LNF
	A1/H2
	A1/H1
	A1 only
	no treatment

Figure 12D

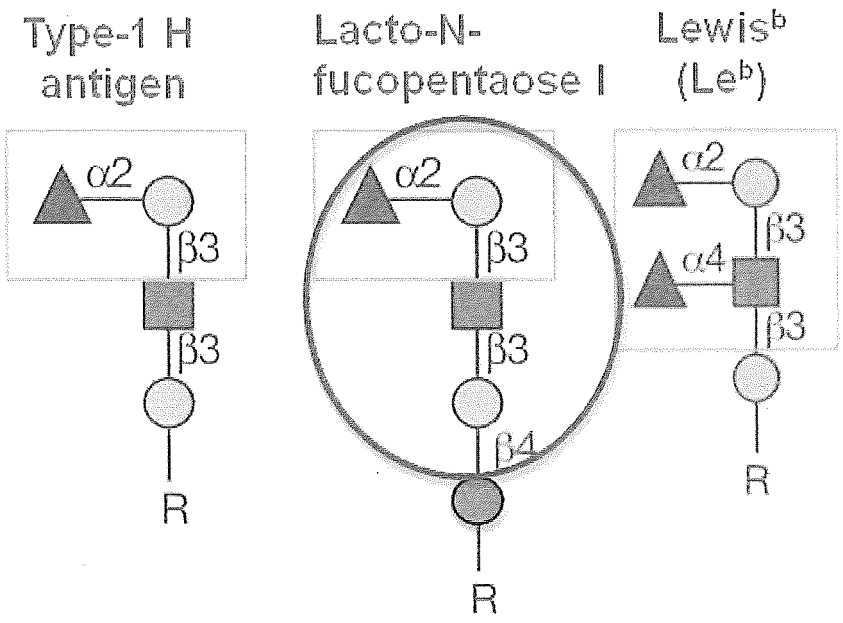


Figure 12E

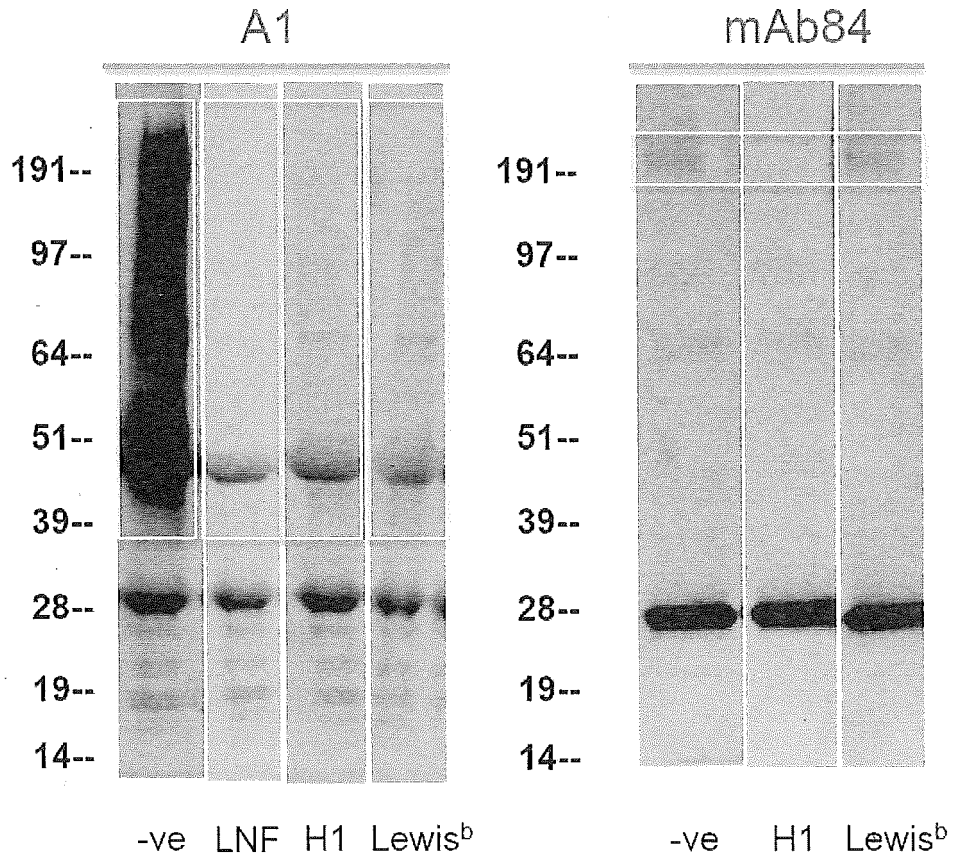


Figure 12F

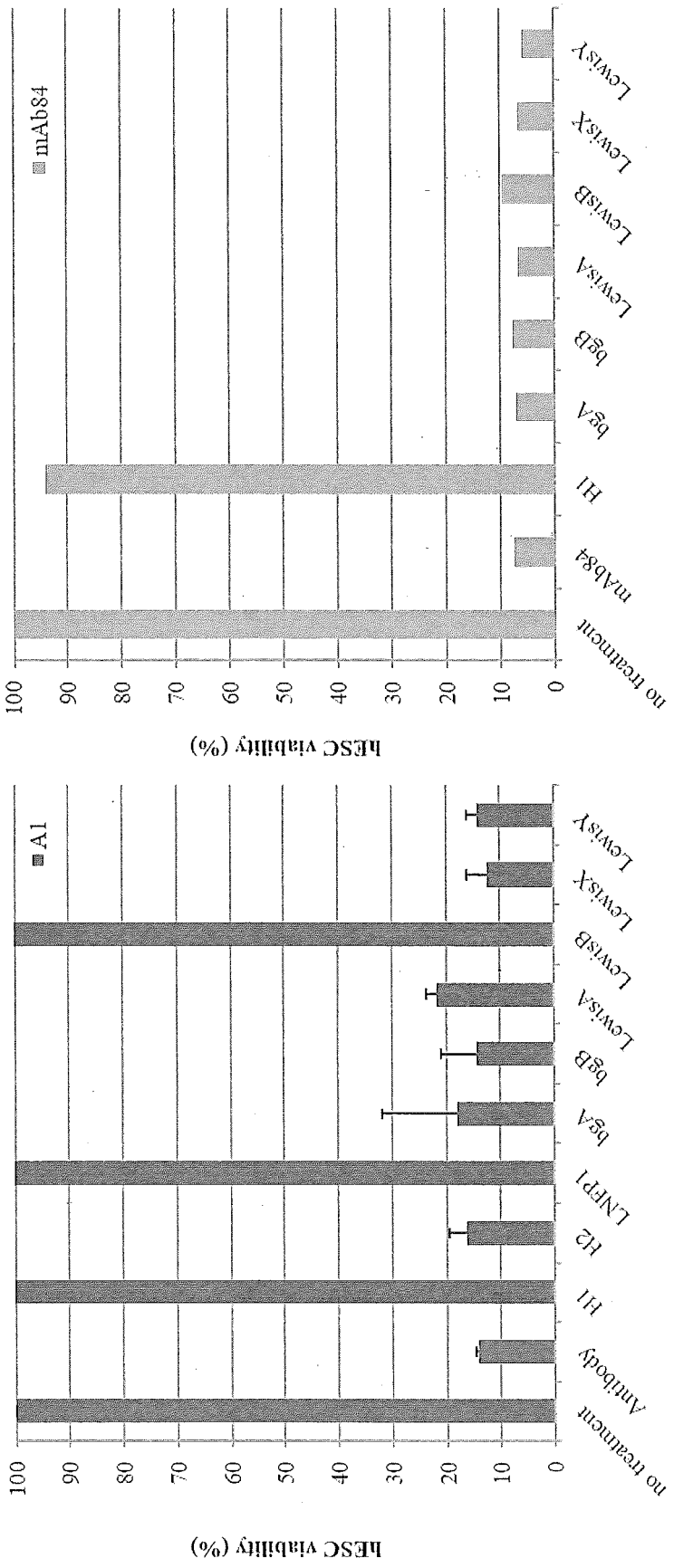
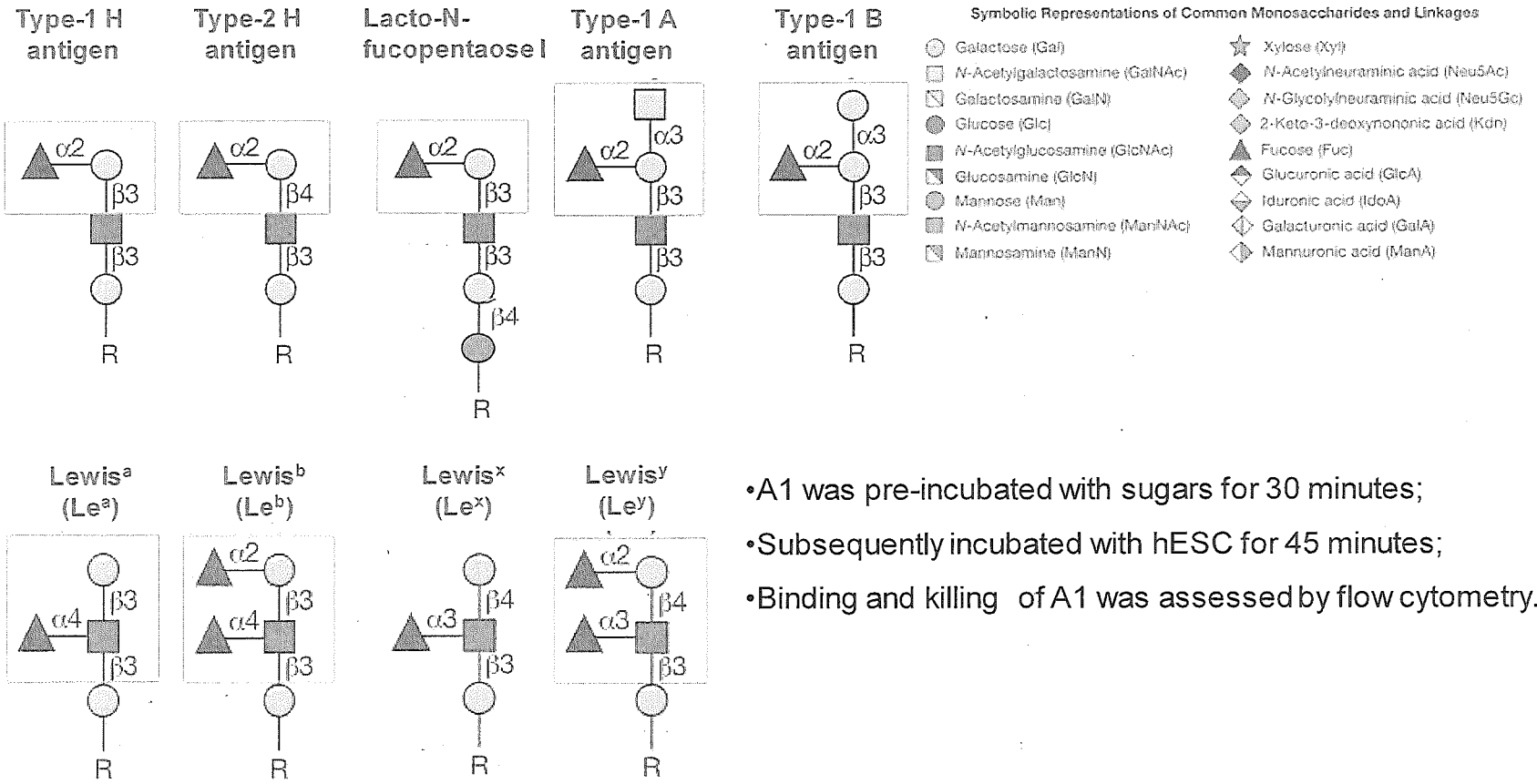

hESC viability (%)
100
90
80
70
60
50
40
30
20
10
0
A1
no treatment
Antibody
H1
H2
LNEP1
bgA
bgB
LewisA
LewisB
LewisX
LewisY
hESC viability (%)
100
90
80
70
60
50
40
30
20
10
0
mAb84
no treatment
mAb84
H1
bgA
bgB
LewisA
LewisB
LewisX
LewisY

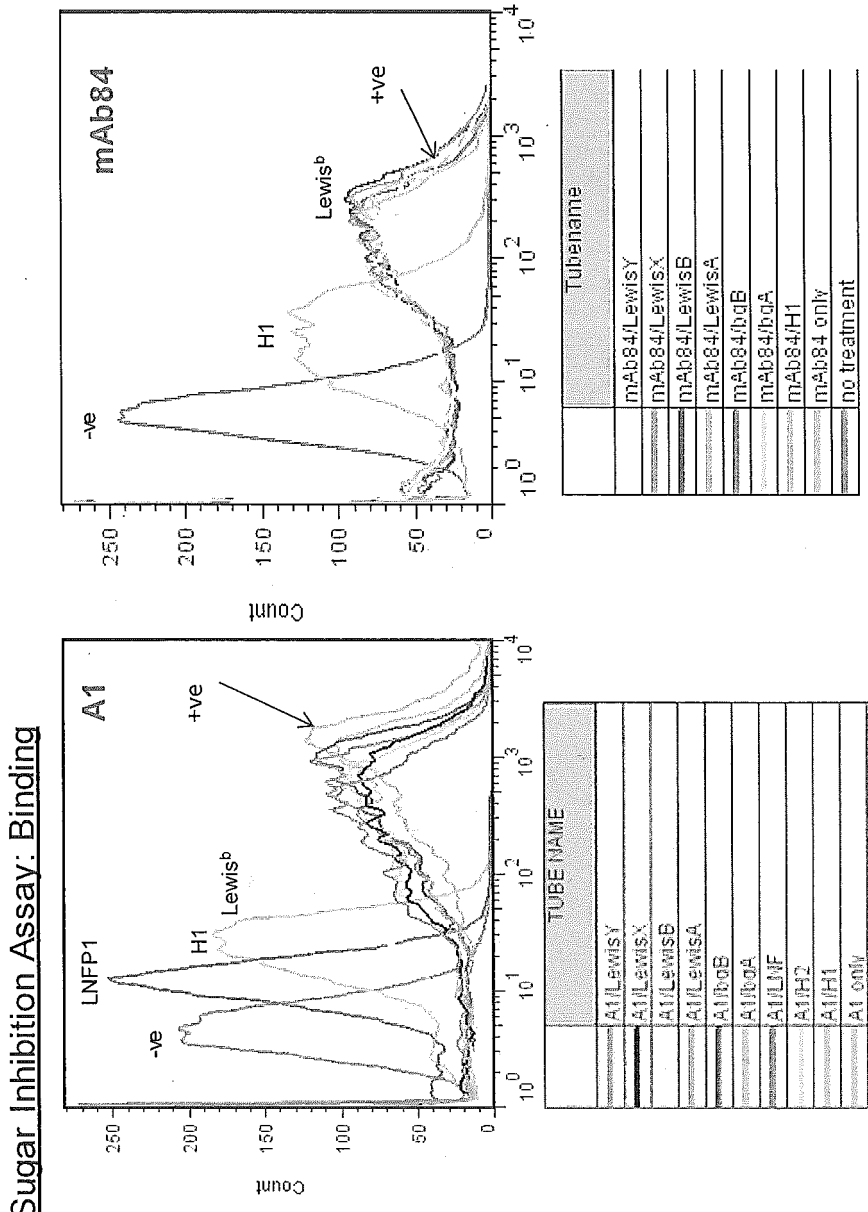
Figure 12G

✓Sugar Inhibition Assay



- A1 was pre-incubated with sugars for 30 minutes;
- Subsequently incubated with hESC for 45 minutes;
- Binding and killing of A1 was assessed by flow cytometry.

Figure 13



- **A1 binding to hESC can be blocked by H1, LNFP1, and Lewis^b;**
- **mAb84 binding to hESC can be blocked by H1, LNFP1 (not shown) but not Lewis^b**

Figure 14

Heavy chain: **CDR H1** **CDR H2**

84VH QVQLQQSGGGLVQPGGSMKLSCVAS**GFTFSNYWMN**WVRQSPEKGLEWVA**EIRLKSNNYATHYAESVKG**RFTISRDDSKSSVYL

A1H QVKLQQSGGGLVQPGGSMKLSCVAS**GFTFSNYYMN**WVRQSPEKGLE WVA**EIRLKSNNYATHYAESVKG**RFTISRDDSKSSVYL

Heavy chain: **CDR H3**

84VH QMNNLRAEDTGIYYCTG**ERA**WGQGTTVTVSS (SEQ ID NO: 7)

A1H QMNNLRAEDTGIYYCEG**FGY**WGQGTTVTVSS (SEQ ID NO: 8)

Figure 15A

Light chain: **CDR L1** **CDR L2**

84L DIELTQSPAI MSASPGEKVTMTC**SASSSVNYMY**WYQQKPGSSPRLLIY**DTSNLAS**GVPVRFSGSGSGTSYSLTISR

A1L DIELTQSPALMSASPGEKVTMTC**SASSSV<u>S</u>YM<u>F</u>**WYQQKPRSSPKPWIH**<u>L</u>TSNLAS**GVPARFSGSGSGTSYSLTISS

Light chain: **CDR L3**

84L MEAEDAATYYC**QQWSSYPYT**FGGGTKLEIKR (SEQ ID NO: 9)

A1L MEAEDAATYYC**QQWSS<u>N</u>PYT**FGGGTKLEIKR (SEQ ID NO: 10)

Figure 15B

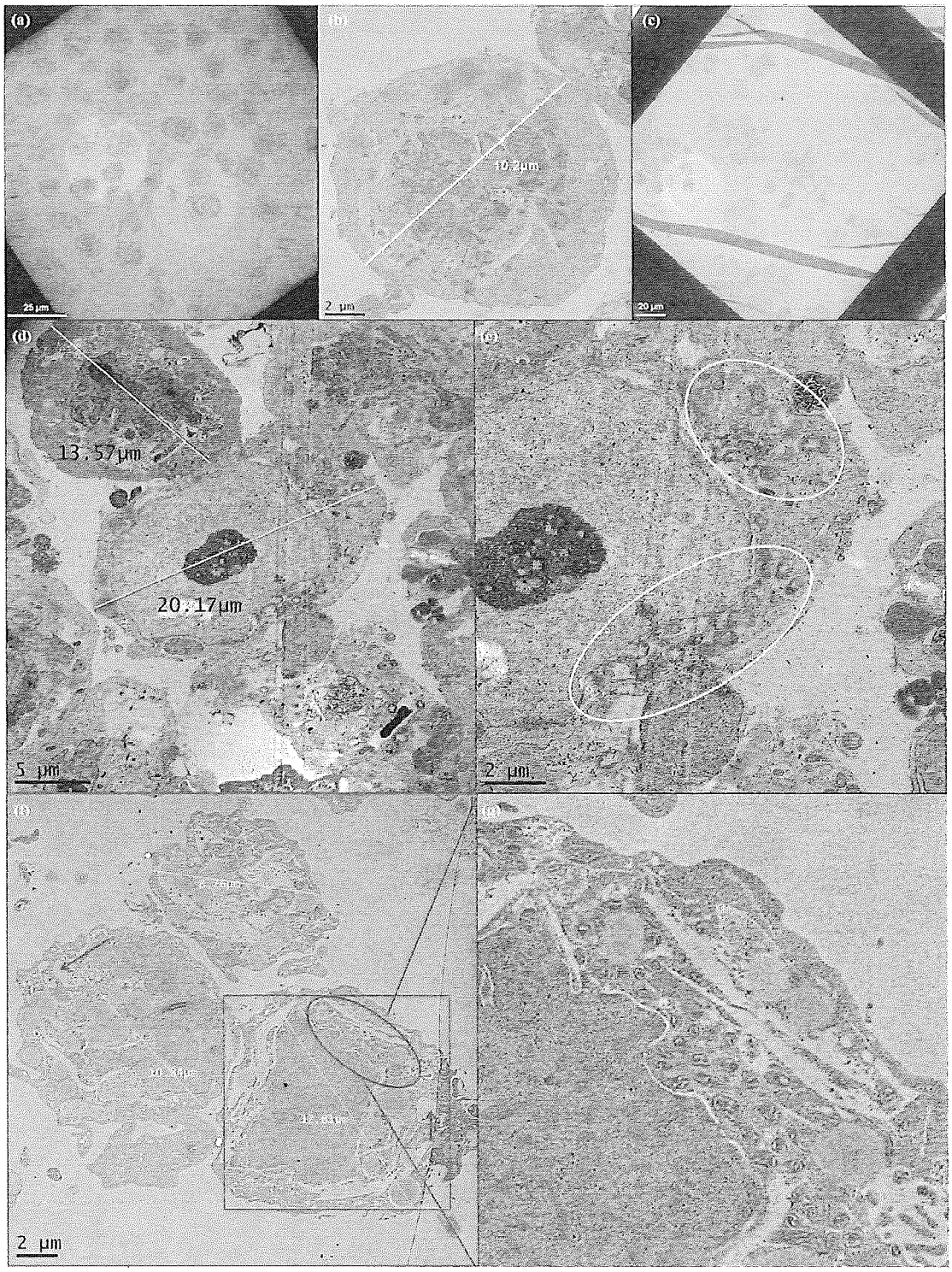


Figure 16

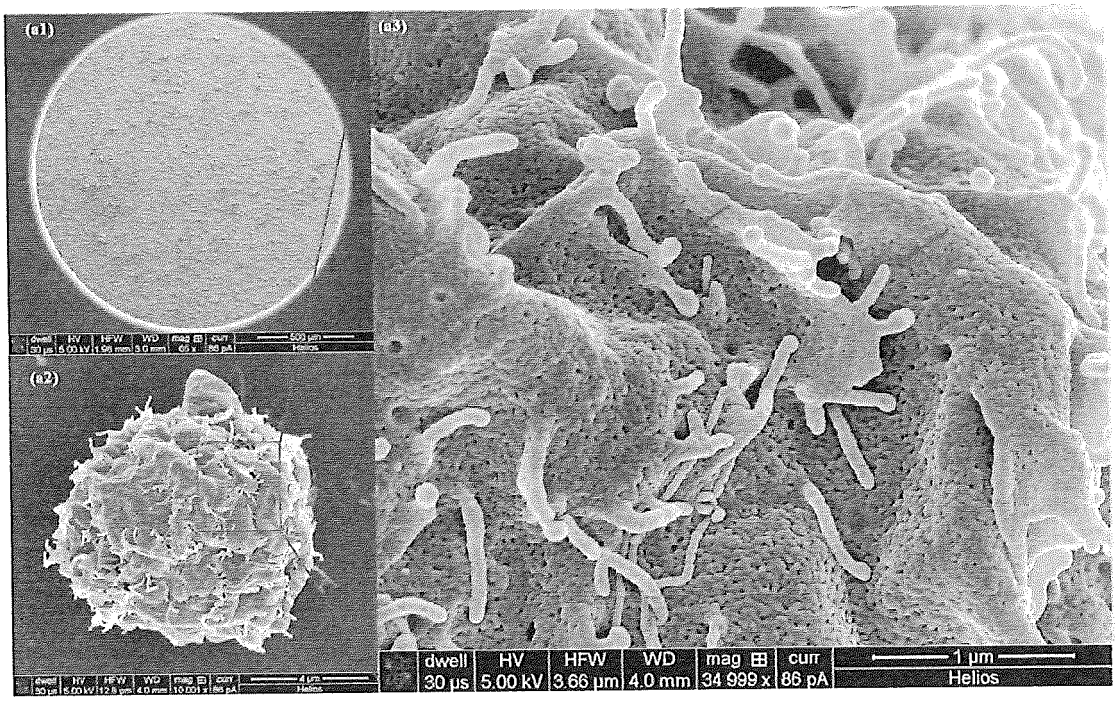

(a1)
(a2)
(a3)
dwell
30 µs
HV
5.00 kV
HFW
3.66 µm
WD
4.0 mm
mag
34 999 x
curr
86 pA
1 µm
Helios

Figure 17

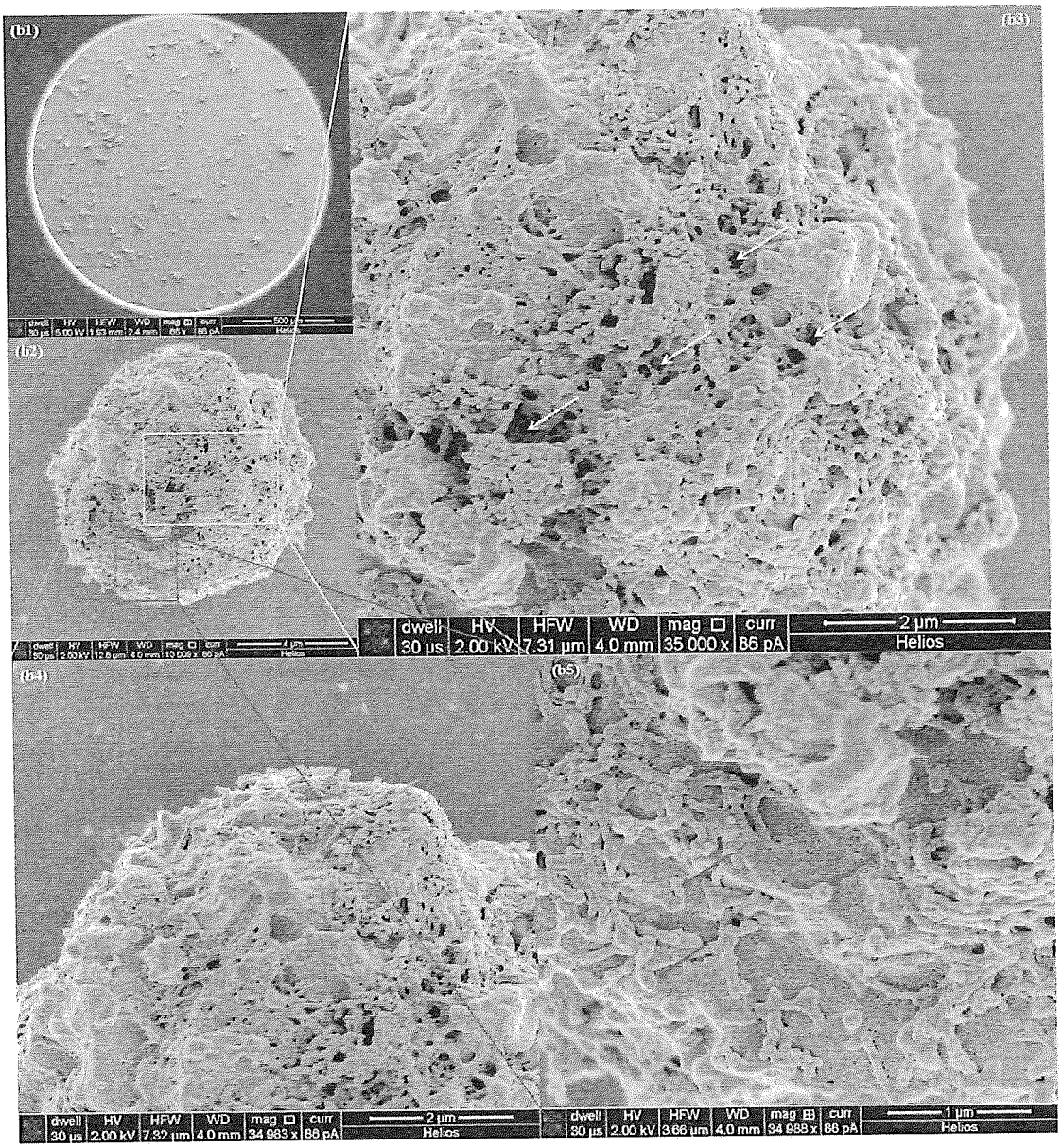

(b1)
(b2)
(b3)
dwell 30 µs HV 2.00 kV HFW 7.31 µm WD 4.0 mm mag 35 000 x curr 86 pA 2 µm Helios
(b4)
dwell 30 µs HV 2.00 kV HFW 7.32 µm WD 4.0 mm mag 34 983 x curr 88 pA 2 µm Helios
(b5)
dwell 30 µs HV 2.00 kV HFW 3.66 µm WD 4.0 mm mag 34 988 x curr 88 pA 1 µm Helios

Figure 18

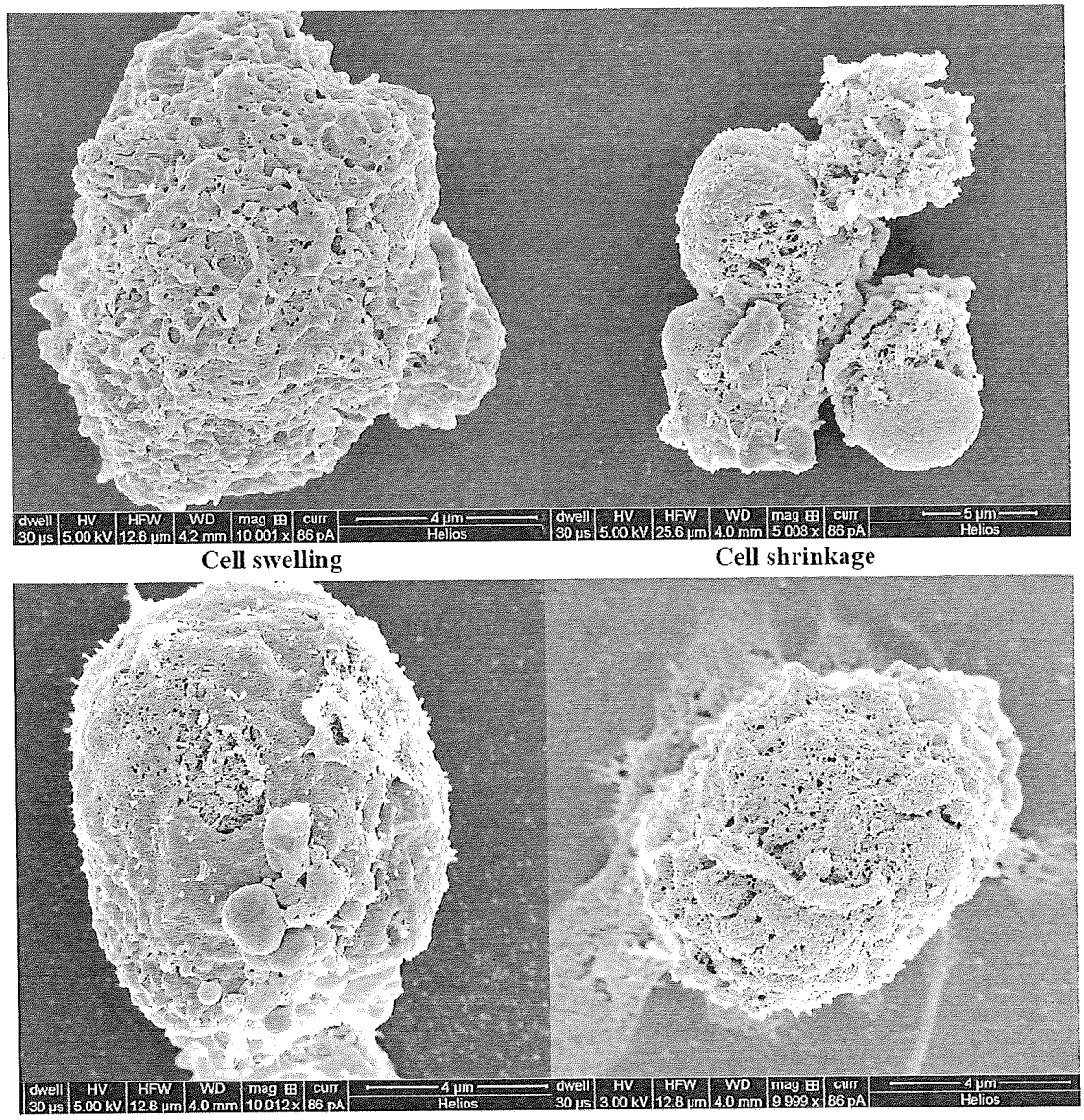

dwell HV HFW WD mag curr
30 µs 5.00 kV 12.8 µm 4.2 mm 10 001 x 86 pA
4 µm
Helios
dwell HV HFW WD mag curr
30 µs 5.00 kV 25.6 µm 4.0 mm 5 008 x 86 pA
5 µm
Helios
Cell swelling
Cell shrinkage
dwell HV HFW WD mag curr
30 µs 5.00 kV 12.8 µm 4.0 mm 10 012 x 86 pA
4 µm
Helios
dwell HV HFW WD mag curr
30 µs 3.00 kV 12.8 µm 4.0 mm 9 999 x 86 pA
4 µm
Helios

Figure 19

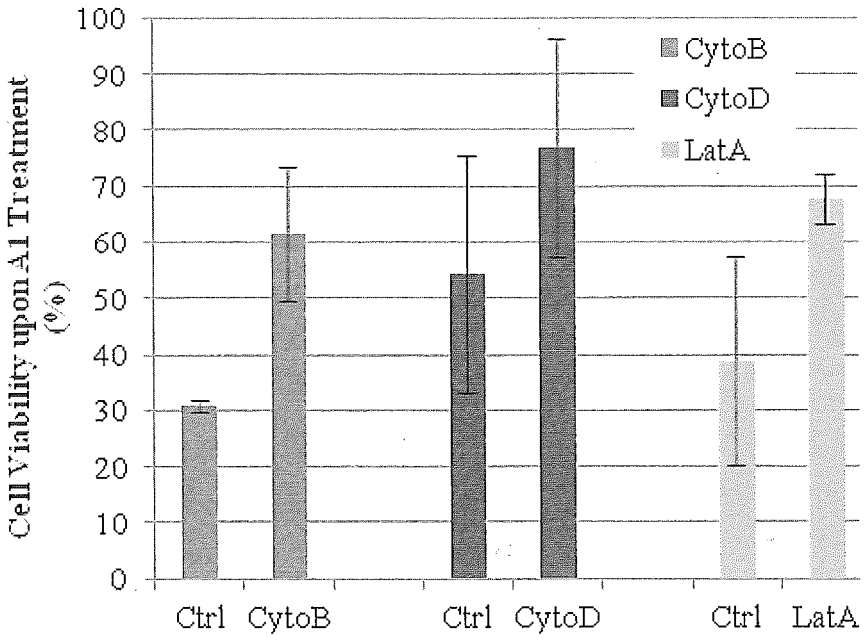


Figure 20A

Reduction in intensity

$$=(I_x/I_{x\text{-}GAPDH}-I_{-ve}/I_{-ve\text{-}GAPDH})/(I_{-ve}/I_{-ve\text{-}GAPDH})$$

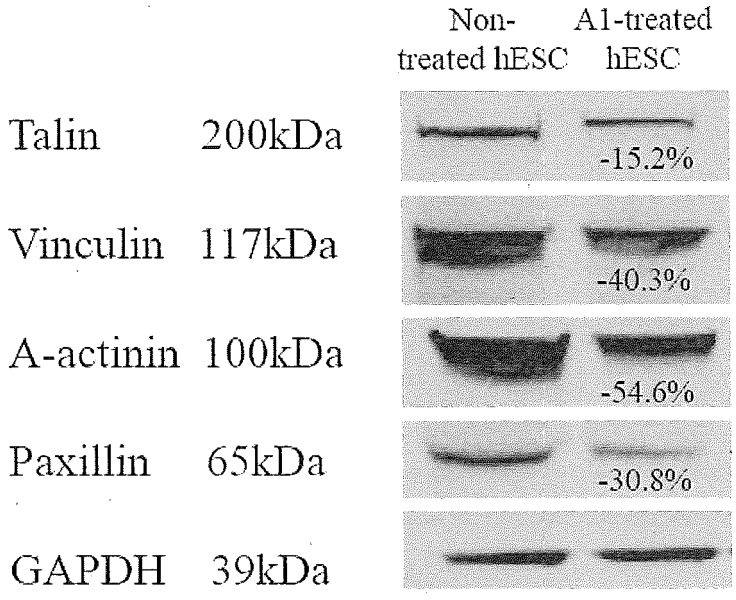


Figure 20B

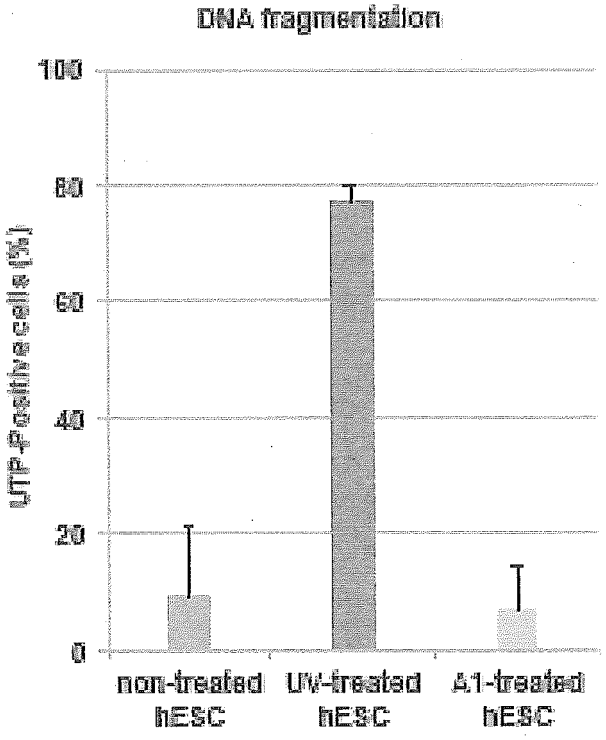


Figure 21A

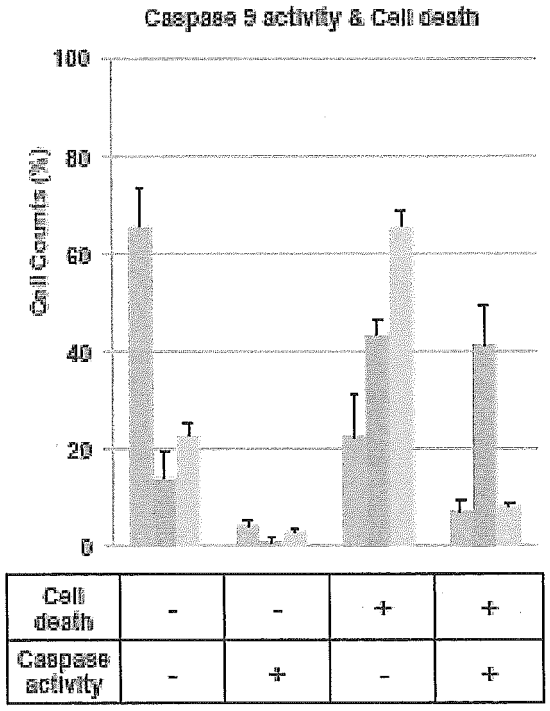


Figure 21B

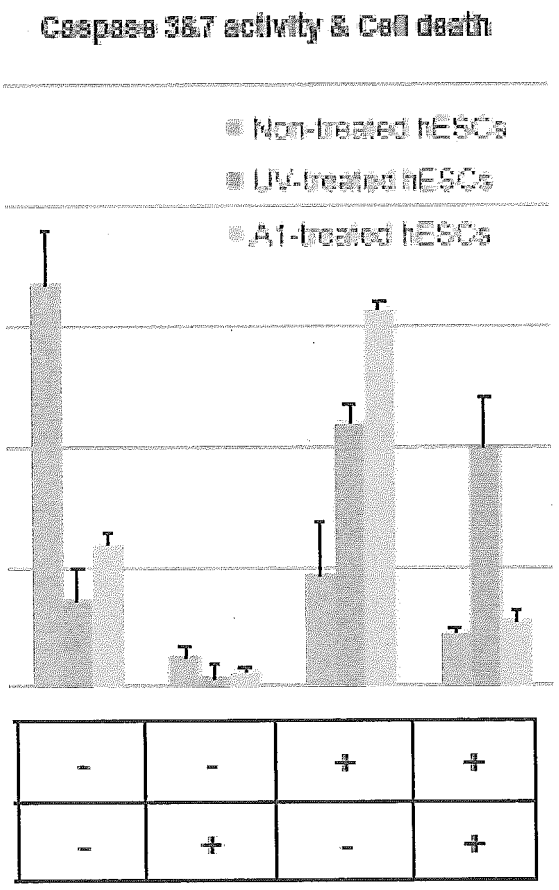

Caspase 3&7 activity & Cell death
Non-treated hESCs
UV-treated hESCs
A1-treated hESCs
-
-
+
+
-
+
-
+

Figure 21C

Non-treated hESC, control

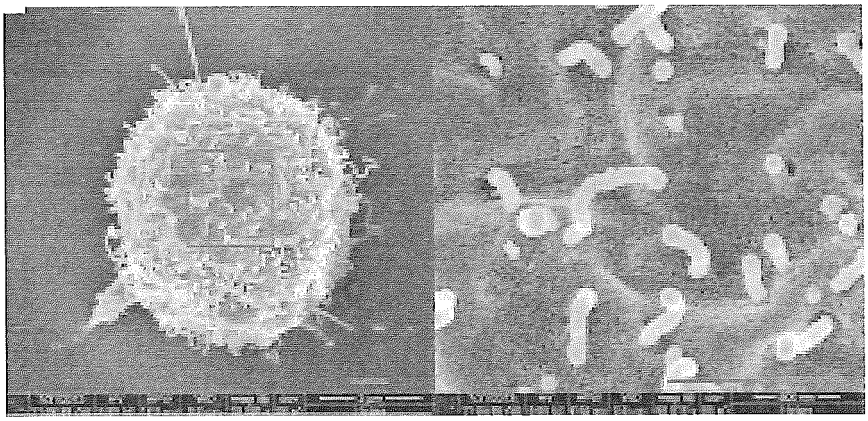


Figure 22A

A1-treated hESC, stage 1

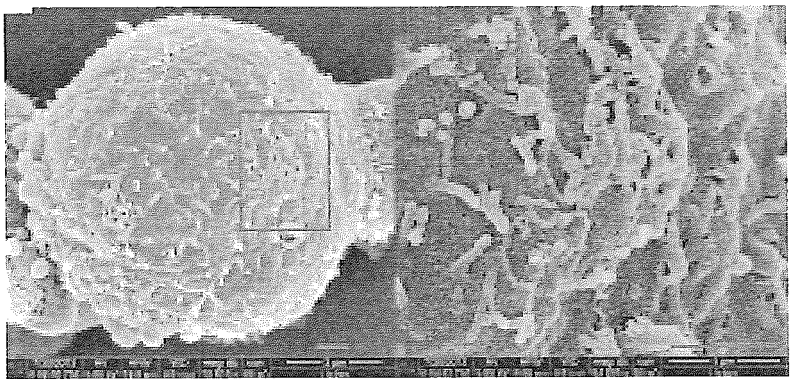


Figure 22B

A1-treated hESC, stage 2

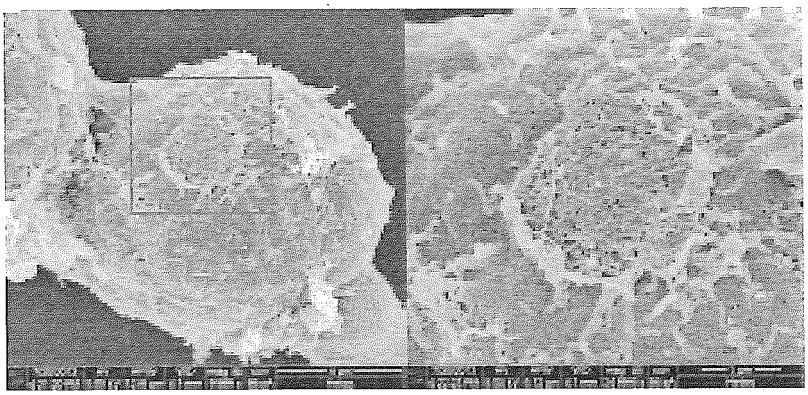


Figure 22C

A1-treated hESC, stage 3

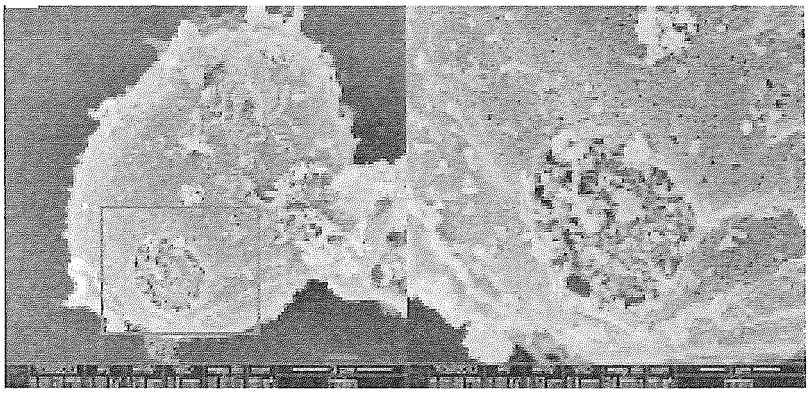


Figure 22D

A1-treated hESC, stage 4

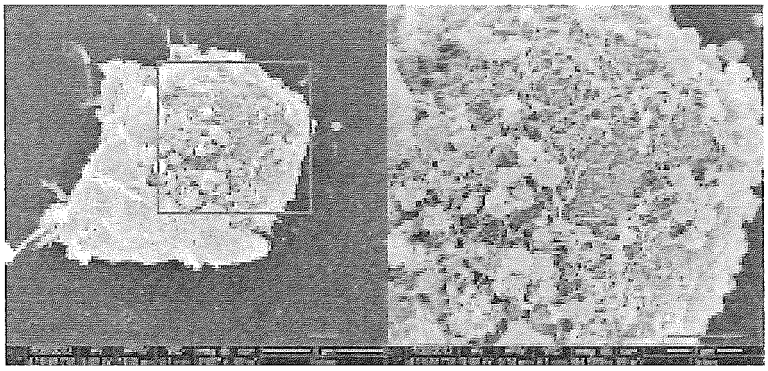


Figure 22E

A1-treated hESC, stage 5

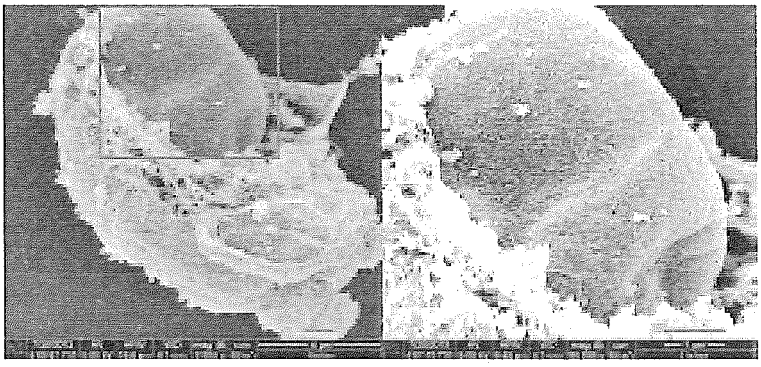


Figure 22F

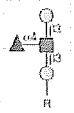
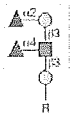
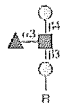
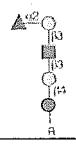
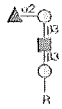
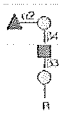
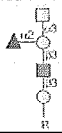
Sugars	Structure	Sequence	Block A1 binding on hESC	Block A1 killing on hESC
Lewis^a trisaccharide		Galβ1-3(Fucα1-4)GlcNAc	-	-
Lewis^b tetrasaccharide		Fucα1-2Galβ1-3(Fucα1-4)GlcNAc	+	+
Lewis^x trisaccharide		Galβ1-4(Fucα1-3)GlcNAc	-	-
Lewis^y tetrasaccharide		(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAc	-	-
Lacto-N-fucopentaose I (LNFP I)		(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glc	+	+
Blood Group H type I trisaccharide		Fucα1-2Galβ1-3GlcNAc	+	+
Blood Group H type II trisaccharide		Fucα1-2Galβ1-4GlcNAc	-	-
Blood Group A trisaccharide		GalNAcα1-3(Fucα1-2)Gal	-	-
Blood Group B trisaccharide		Galα1-3(Fucα1-2)Gal	-	-

Figure 23

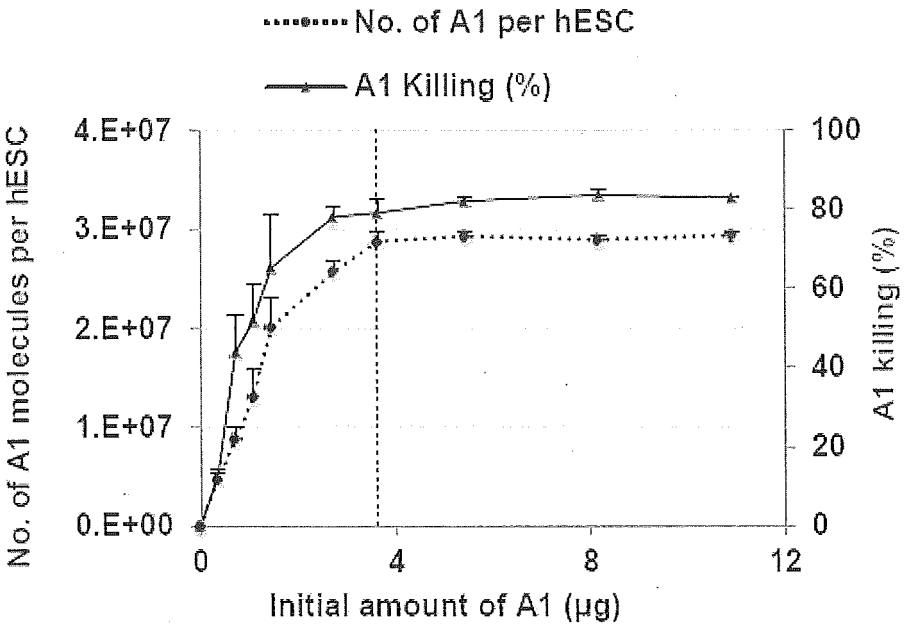


Figure 24A

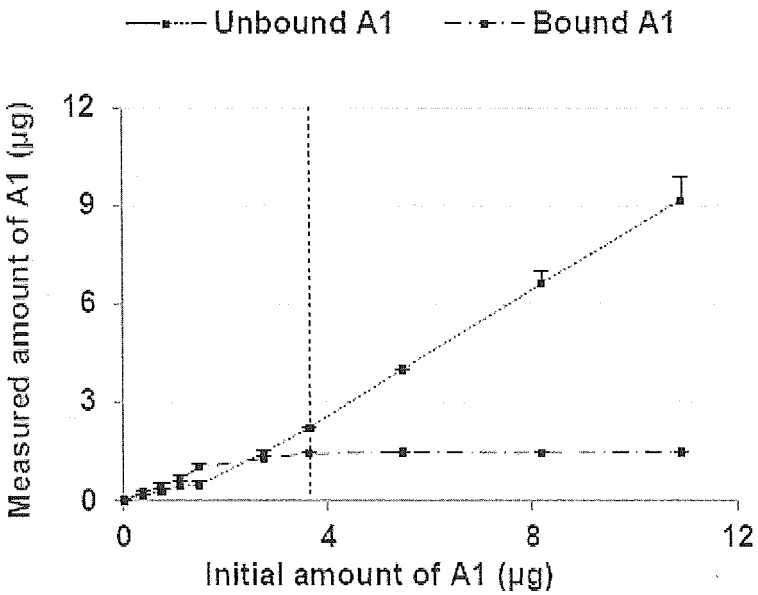


Figure 24B

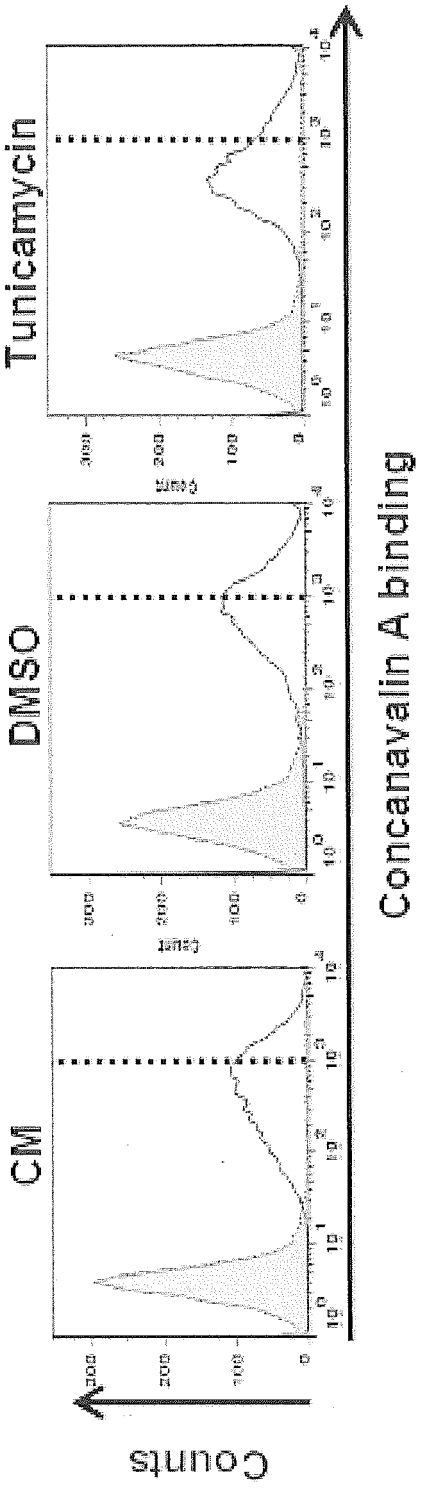


Figure 25A

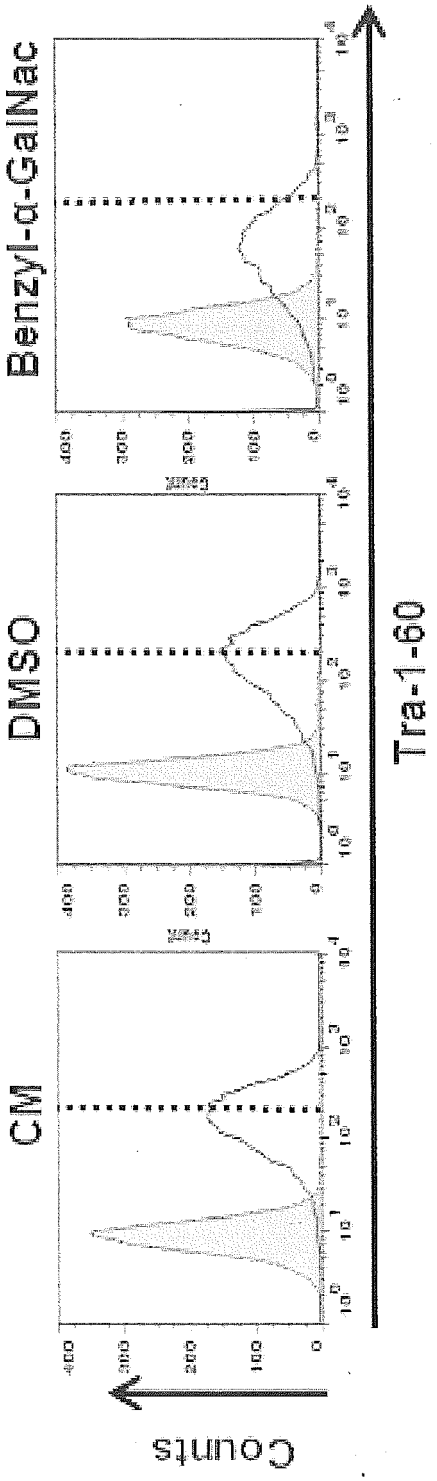


Figure 25B

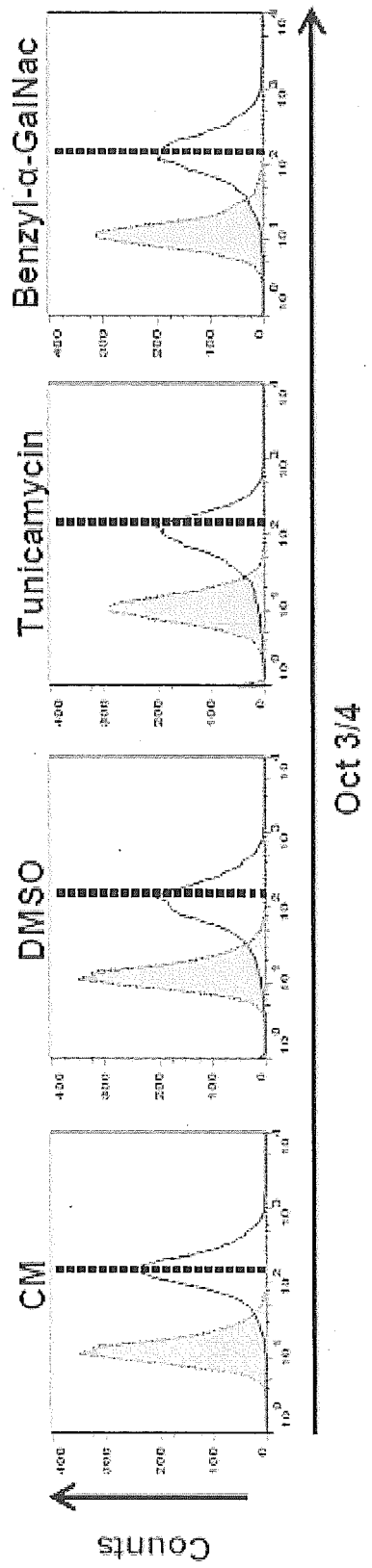


Figure 25C

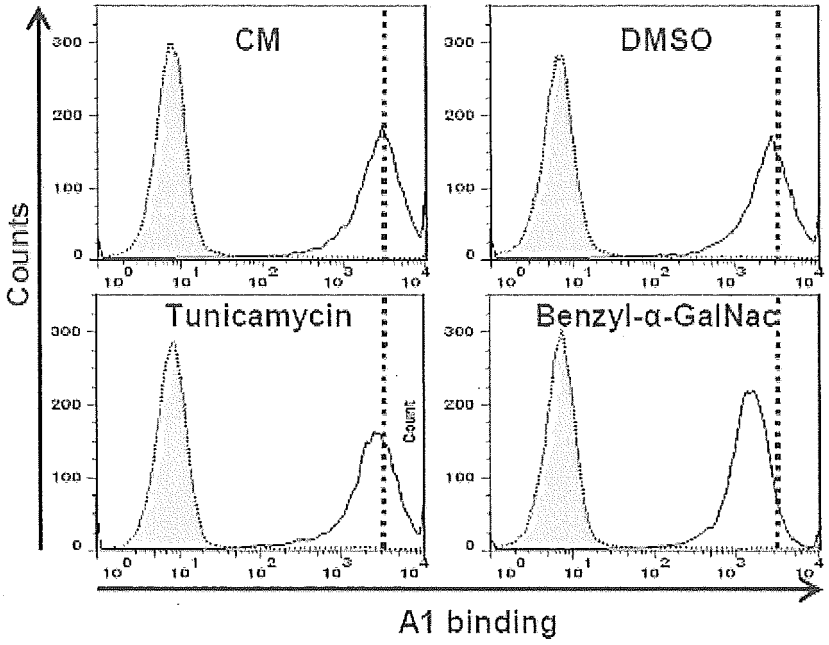


Figure 26A

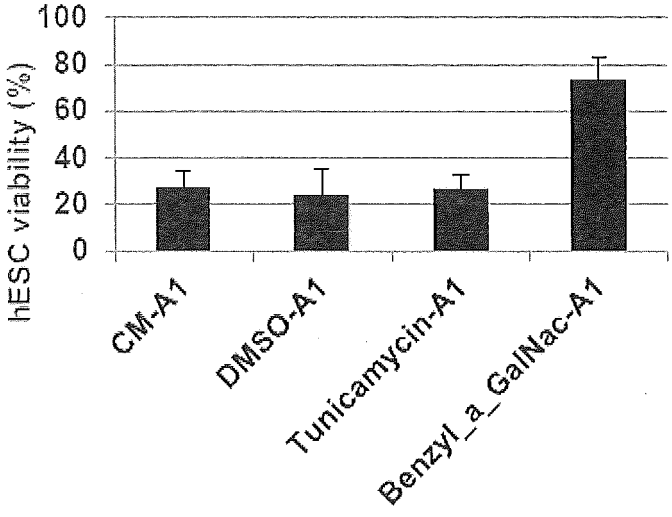


Figure 26B

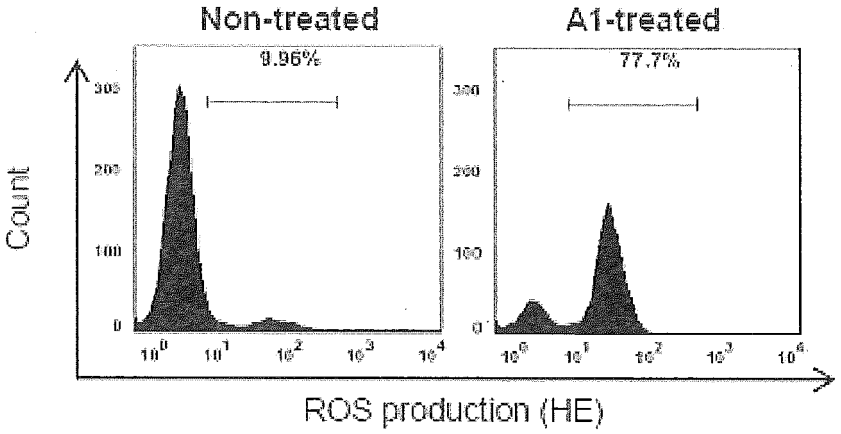


Figure 27A

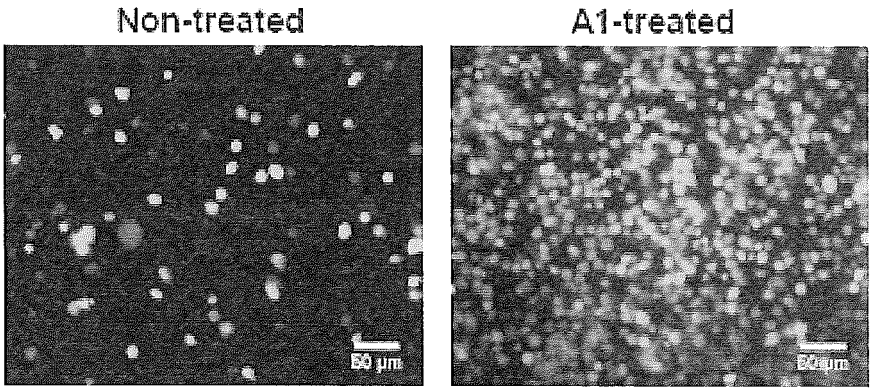


Figure 27B

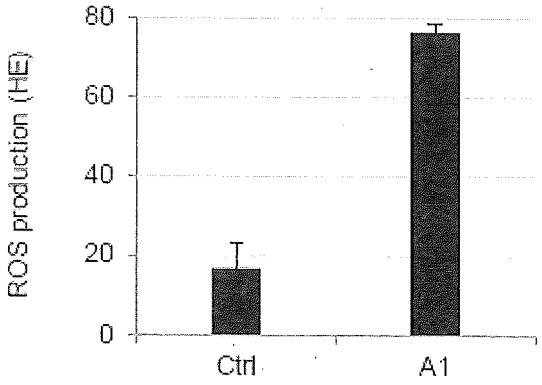


Figure 27C

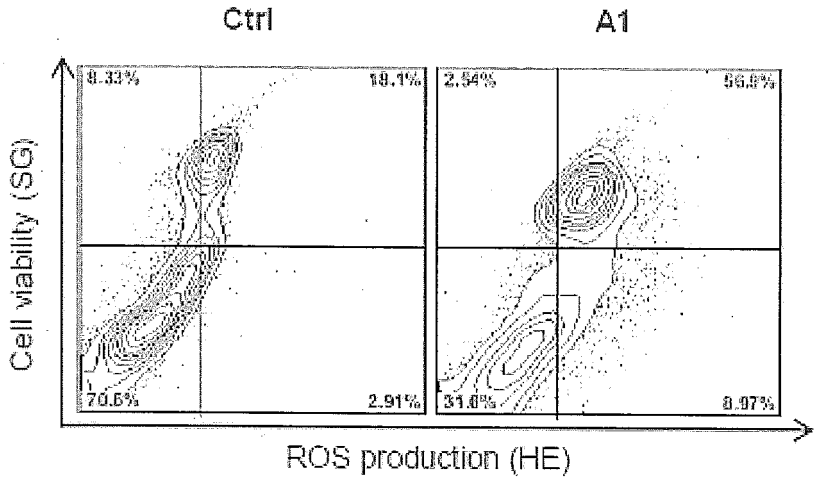


Figure 27D

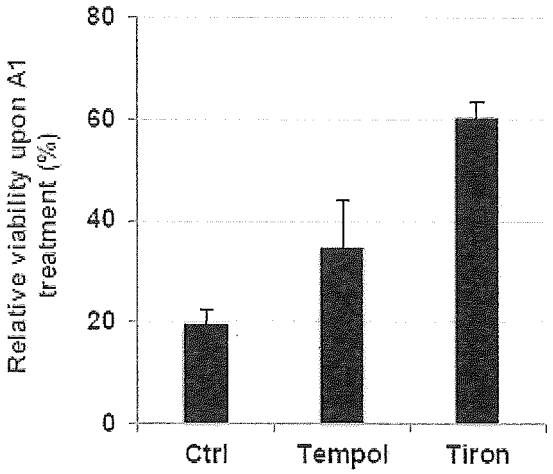


Figure 28A

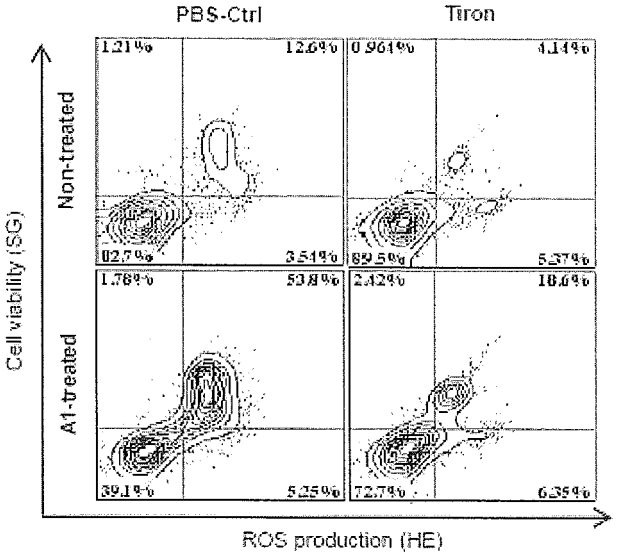


Figure 28B

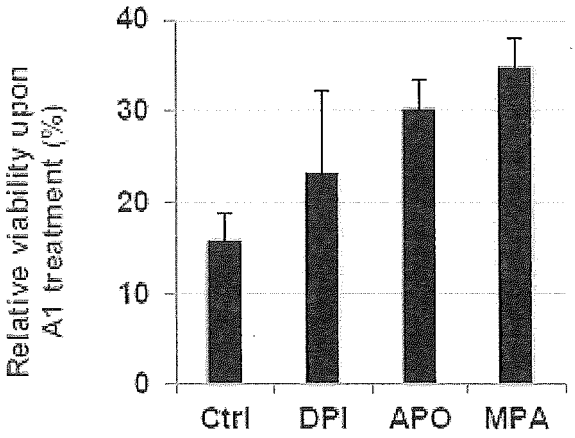


Figure 29A

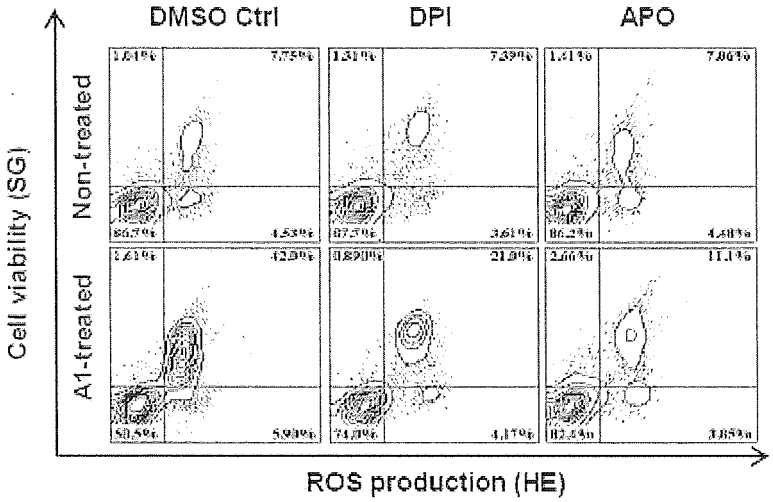


Figure 29B

Targets of NADPH oxidase inhibitors

	Inhibitors		
Isoforms	DPI	MPA	APO
Nox1	√	√	
Nox2	√	√	√
Nox3	√	?	
Nox4	√		
Nox5	√		
Duox1	√		
Duox2	√		

Figure 29C

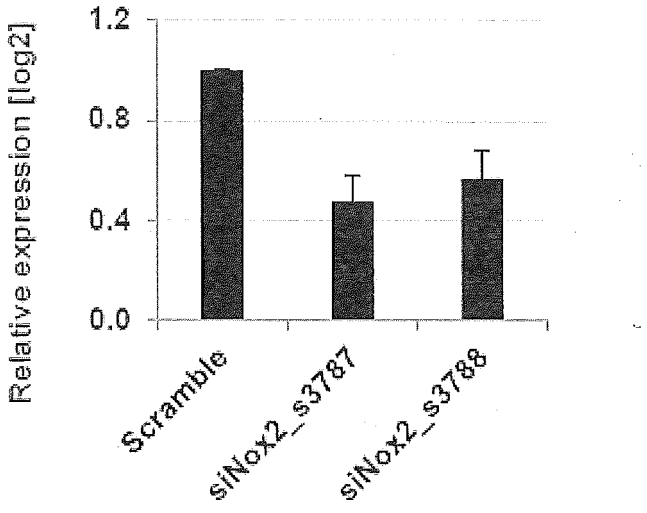


Figure 29D

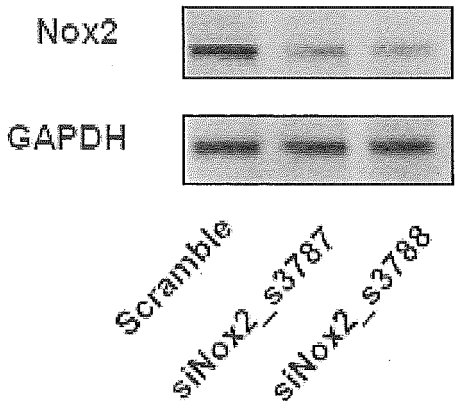


Figure 29E

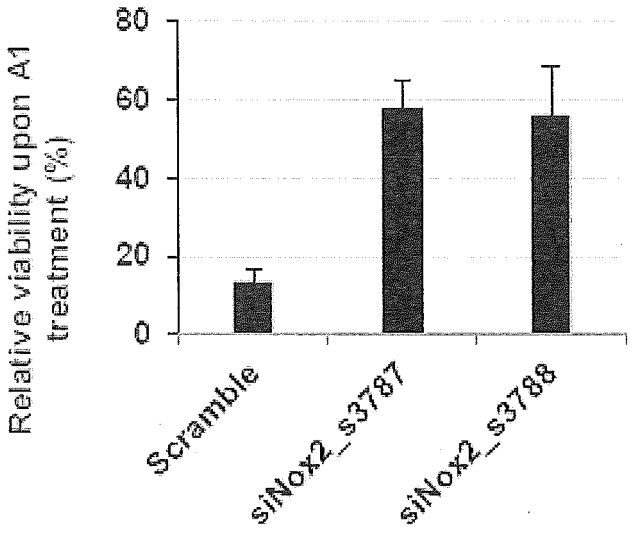


Figure 29F

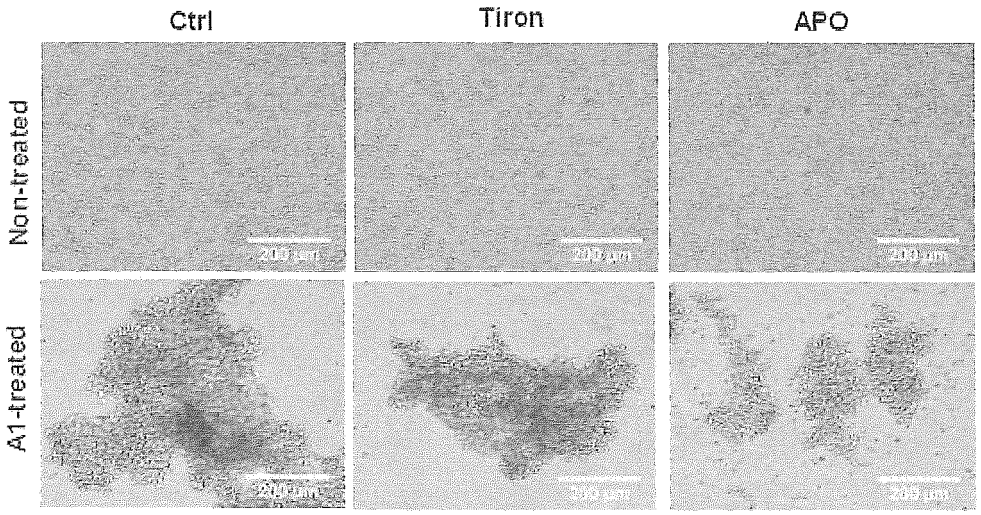


Figure 30A

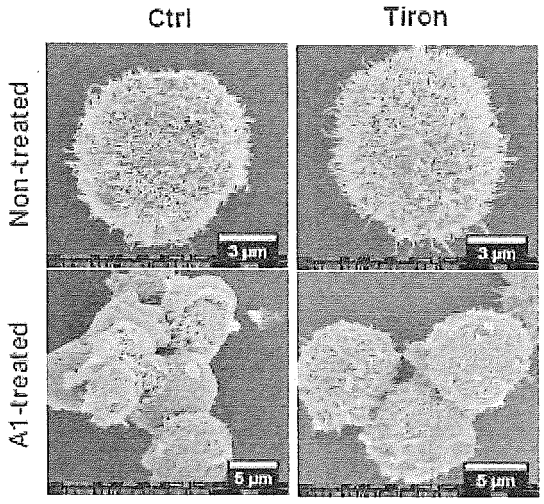


Figure 30B

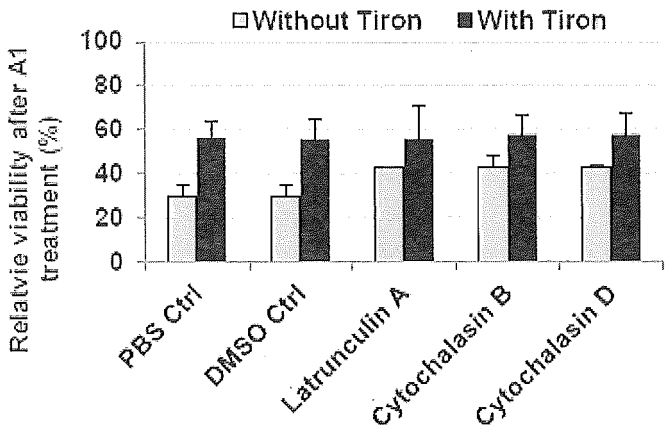


Figure 30C

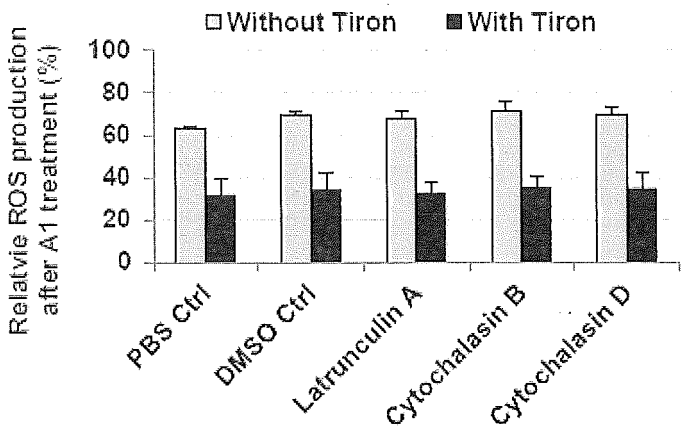


Figure 30D

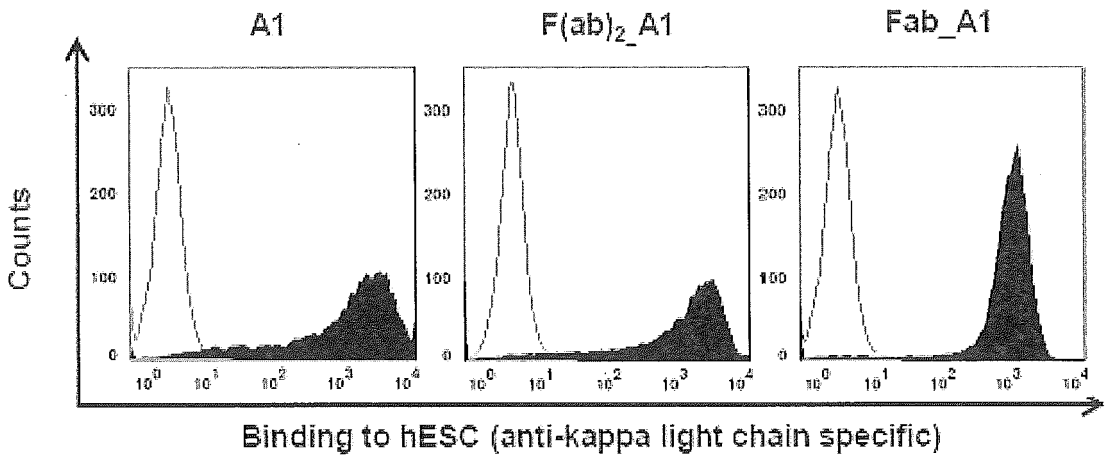


Figure 31A

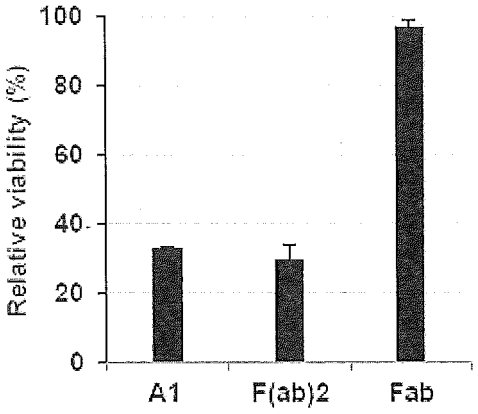


Figure 31B

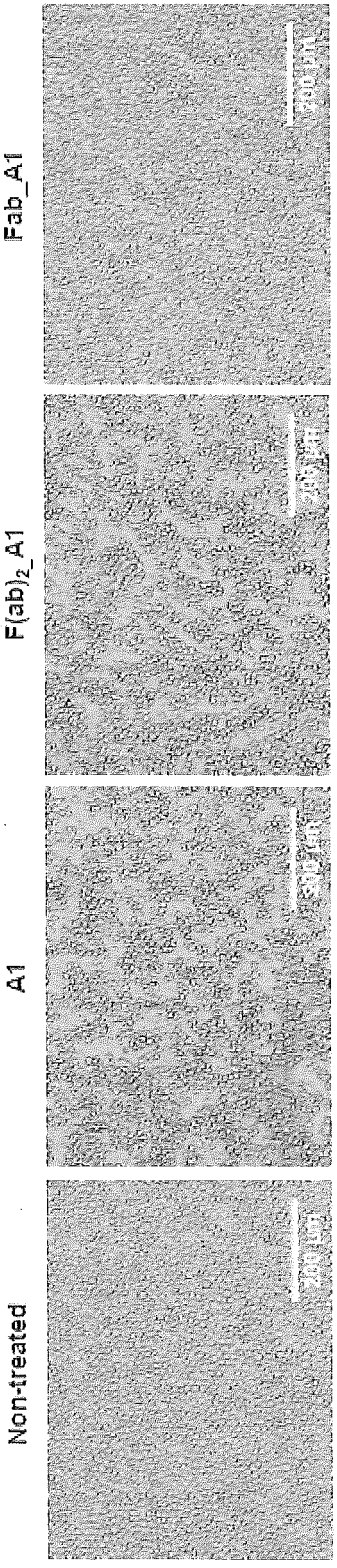


Figure 31C