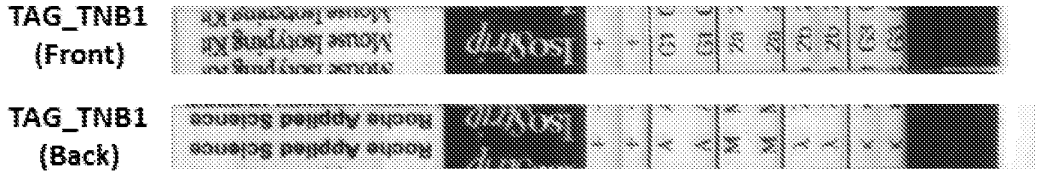
FIG. 1



Gene Sequence (Heavy Chain) (SEQ ID NO: 12)
CAGGTCAAACTGCAGGAGTCAGGGGCTGAACTGGTGAAGCCTGGGGCTTCAGT
GAAGTTGTCCTGTAAGGCTTCTGGCTTCACCTTCACCAGCTACTATATGTACTGG
GTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGGGGGGTTAGTCCTAG
CAATGGTGGTGCTAACTTCAATGAGAAGTTCAAGACCAAGGCCACACTGACTGT
AGACAAATCCTCCAGCACAGCCTACATGCAACTCAGCAGCCTGACATCTGAGGA
CTCTGCGGTCTATTACTGTACAAGATTCCTTTATGGTCCGAGGTACTTCGATGTC
TGGGGCCAAGGGACCACGGTCACCGTCTCCTCA

Gene Sequence (Light Chain) (SEQ ID NO: 13)
GACATTGAGCTCACCCAGTCTCCAGCTTCTTTGGCTGTGTCTCTAGGGCAGAGG
GCCACCATCTCCTGCAGAGCCAGCGAAAGTGTTGATAATTATGGCATTAGTTTTA
TGAACTGGTTCCAACAGAAACCAGGACAGCCACCCAAACTCCTCATTTATGGTG
CATCCAACCAGGGATCCGGGGTCCCTGCCAGGTTTAGTGGCAGTGGGTCTGGG
ACAGACTTCAGCCTCAACATCCATCCTATGGAGGAGGATGATACTGCAATGTAT
TTCTGTCAGCAAAGTAAGGAGGTTCCGTACACGTTCGGAGGGGGGACCAAGCT
GGAAATAAAAC

Protein Sequence (Heavy Chain) (SEQ ID NO: 1)
QVKLQESGAELVKPGASVKLSCKASGFTFTSYYMYWVKQRPGQGLEWIGGVSPSN
GGANFNEKFKTKATLTVDKSSSTAYMQLSSLTSEDSAVYYCTRFLYGPRYFDVWG
QGTTVTVSS

Protein Sequence (Light Chain) (SEQ ID NO: 2)
DIELTQSPASLAVSLGQRATISCRASESVDNYGISFMNWFQQKPGQPPKLLIYGASN
QGSGVPARFSGSGSGTDFSLNIHPMEEDDTAMYFCQQSKEVPYTFGGGTKLEIK

FIG. 2

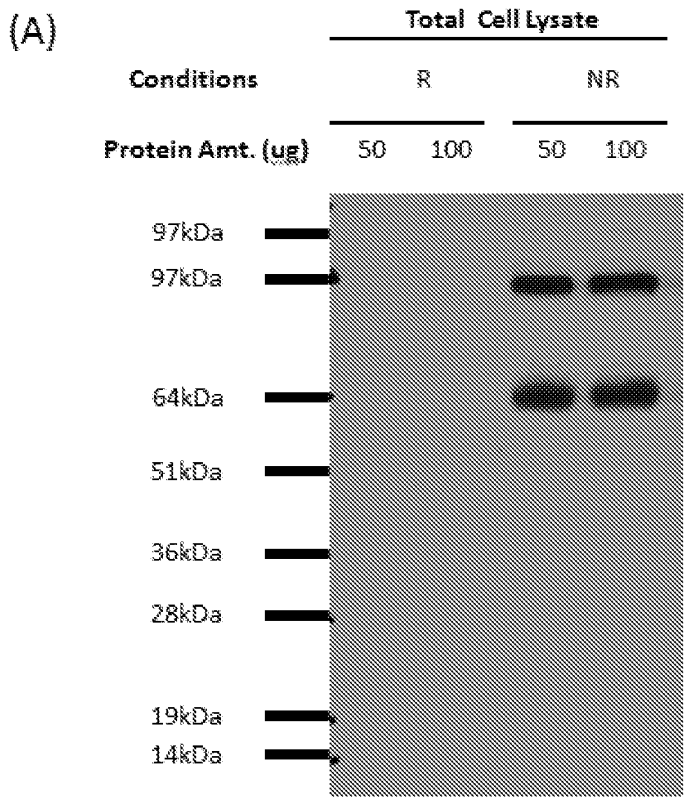

(A)
Total Cell Lysate
Conditions
R
NR
Protein Amt. (ug)
50
100
50
100
97kDa
97kDa
64kDa
51kDa
36kDa
28kDa
19kDa
14kDa

FIG. 2

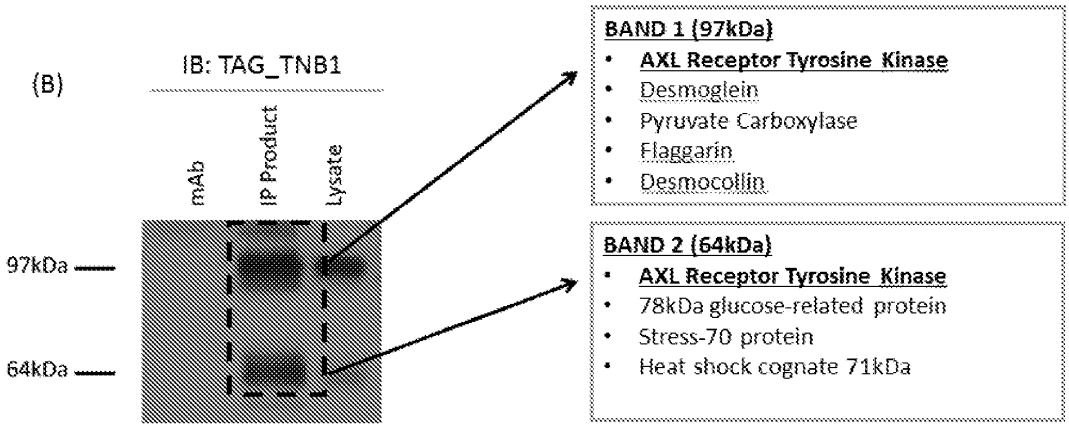

(B)
IB: TAG_TNB1
mAb
IP Product
Lysate
97kDa
64kDa
BAND 1 (97kDa)
AXL Receptor Tyrosine Kinase
Desmoglein
Pyruvate Carboxylase
Flaggarin
Desmocollin
BAND 2 (64kDa)
AXL Receptor Tyrosine Kinase
78kDa glucose-related protein
Stress-70 protein
Heat shock cognate 71kDa

FIG. 2

(C)

BT 549

AXL receptor tyrosine kinase peptide sequence- homo sapiens (Uniprot)

Long form **(SEQ ID NO: 14)**

MAWRCPRMGRVPLAWCLALCGWACMAPRGTQAEESPFVGNPGNITGARGLTGTLRCQLQV

QGEPPEVHWLRDGQILELADSTQTQVPLGEDEQDDWIVVSQLRITSLQLSDTGQYQCLVF

LGHQTFVSQPGYVGLEGLPYFLEEPEDRTVAANTPFNLSCQAQGPPEPVDLLWLQDAVPL

ATAPGHGPQRSLHVPGLNKTSSFSCEAHNAKGVTTSRTATITVLPQQPRNLHLVSRQPTE

LEVAWTPGLSGIYPLTHCTLQAVLSNDGMGIQAGEPDPPEEPLTSQASVPPHQLRLGSLH

PHTPYHIRVACTSSQGPSSWTHWLPVETPEGVPLGPPENISATRNGSQAFVHWQEPRAPL

QGTLLGYRLAYQGQDTPEVLMDIGLRQEVTLELQGDGSVSNLTVCVAAYTAAGDGPWSLP

VPLEAWRP***GQAQPVHQL***VKEPSTPAFSWPWWYVLLGAVVAAACVLILALFLVHRRKKETR

YGEVFEPTVERGELVVRYRVRKSYSRRTTEATLNSLGISEELKEKLRDVMVDRHKVALGK

TLGEGEFGAVMEGQLNQDDSILKVAVKTMKIAICTRSELEDFLSEAVCMKEFDHPNVMRL

IGVCFQGSERESFPAPVVILPFMKHGDLHSFLLYSRLGDQPVYLPTQMLVKFMADIASGM

EYLSTKRFIHRDLAARNCMLNENMSVCVADFGLSKKIYNGDYYRQGRIAKMPVKWIAIES

LADRVYTSKSDVWSFGVTMWEIATRGQTPYPGVENSEIYDYLRQGNRLKQPADCLDGLYA

LMSRCWELNPQDRPSFTELREDLENTLKALPPAQEPDEILYVNMDEGGGYPEPPGAAGGA

DPPTQPDPKDSCSCLTAAEVHPAGRYVLCPSTTPSPAQPADRGSPAAPGQEDGA

Note:

1. ***GQAQPVHQL*** - difference between short and long isoforms of AXL

2. Results is a total of 10 Mass Spectrometry (MS) analysis (Both in-gel and in-solution digestion post IP); Underlined sequences are sequence that were identified by MS.

3. Approximately, **34.6% of the peptide were identified** from the combined MS results.

FIG. 2

(cont'd)

(C)

H1299

AXL receptor tyrosine kinase peptide sequence- homo sapiens (Uniprot)

Long form **(SEQ ID NO: 15)**

MAWRCPRMGRVPLAWCLALCGWACMAPRGTQAEESPFVGNPGNITGARGLTGTLRCQLQV

QGEPPEVHWLRDGQILELADSTQTQVPLGEDEQDDWIVVSQLRITSLQLSDTGQYQCLVF

LGHQTFVSQPGYVGLEGLPYFLEEPEDRTVAANTPFNLSCQAQGPPEPVDLLWLQDAVPL

ATAPGHGPQRSLHVPGLNKTSSFSCEAHNAKGVTTSRTATITVLPQQPRNLHLVSRQPTE

LEVAWTPGLSGIYPLTHCTLQAVLSNDGMGIQAGEPDPPEEPLTSQASVPPHQLRLGSLH

PHTPYHIRVACTSSQGPSSWTHWLPVETPEGVPLGPPENISATRNGSQAFVHWQEPRAPL

QGTLLGYRLAYQGQDTPEVLMDIGLRQEVTLELQGDGSVSNLTVCVAAYTAAGDGPWSLP

VPLEAWRP***GQAQPVHQL***VKEPSTPAFSWPWWYVLLGAVVAAACVLILALFLVHRRKKETR

YGEVFEPTVERGELVVRYRVRKSYSRRTTEATLNSLGISEELKEKLRDVMVDRHKVALGK

TLGEGEFGAVMEGQLNQDDSILKVAVKTMKIAICTRSELEDFLSEAVCMKEFDHPNVMRL

IGVCFQGSERESFPAPVVILPFMKHGDLHSFLLYSRLGDQPVYLPTQMLVKFMADIASGM

EYLSTKRFIHRDLAARNCMLNENMSVCVADFGLSKKIYNGDYYRQGRIAKMPVKWIAIES

LADRVYTSKSDVWSFGVTMWEIATRGQTPYPGVENSEIYDYLRQGNRLKQPADCLDGLYA

LMSRCWELNPQDRPSFTELREDLENTLKALPPAQEPDEILYVNMDEGGGYPEPPGAAGGA

DPPTQPDPKDSCSCLTAAEVHPAGRYVLCPSTTPSPAQPADRGSPAAPGQEDGA

Note:

1. ***GQAQPVHQL*** - difference between short and long isoforms of AXL

2. Results is a total of 5 Mass Spectrometry (MS) analysis (Both in-gel and in-solution digestion post IP); Underlined sequences are sequence that were identified by MS.

3. Approximately, **41.8% of the peptide were identified** from the combined MS results.

Fig. 3

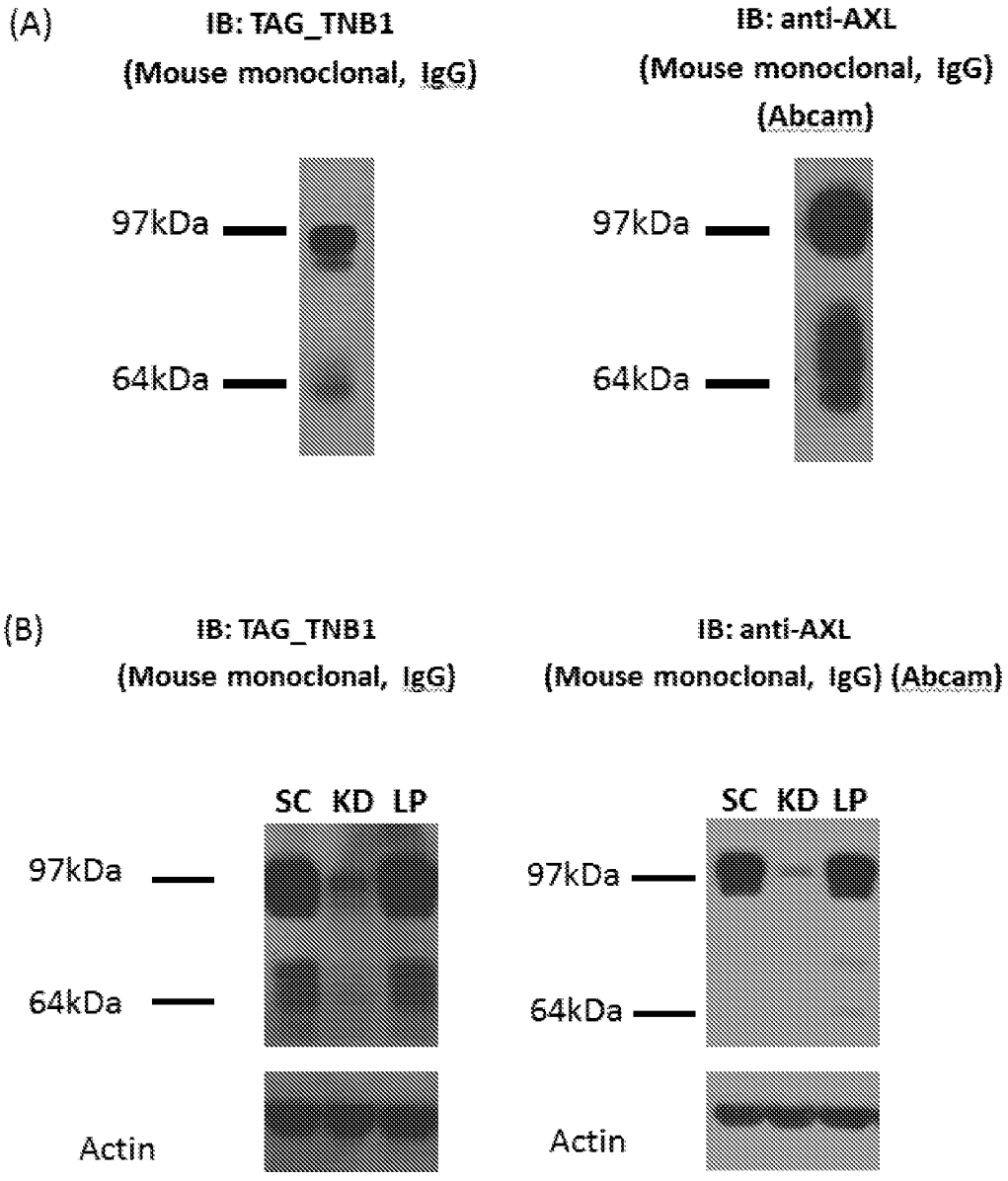

(A)
IB: TAG_TNB1
(Mouse monoclonal, IgG)
IB: anti-AXL
(Mouse monoclonal, IgG)
(Abcam)
97kDa
64kDa
97kDa
64kDa
(B)
IB: TAG_TNB1
(Mouse monoclonal, IgG)
IB: anti-AXL
(Mouse monoclonal, IgG) (Abcam)
SC KD LP
SC KD LP
97kDa
64kDa
Actin
97kDa
64kDa
Actin

Fig. 4

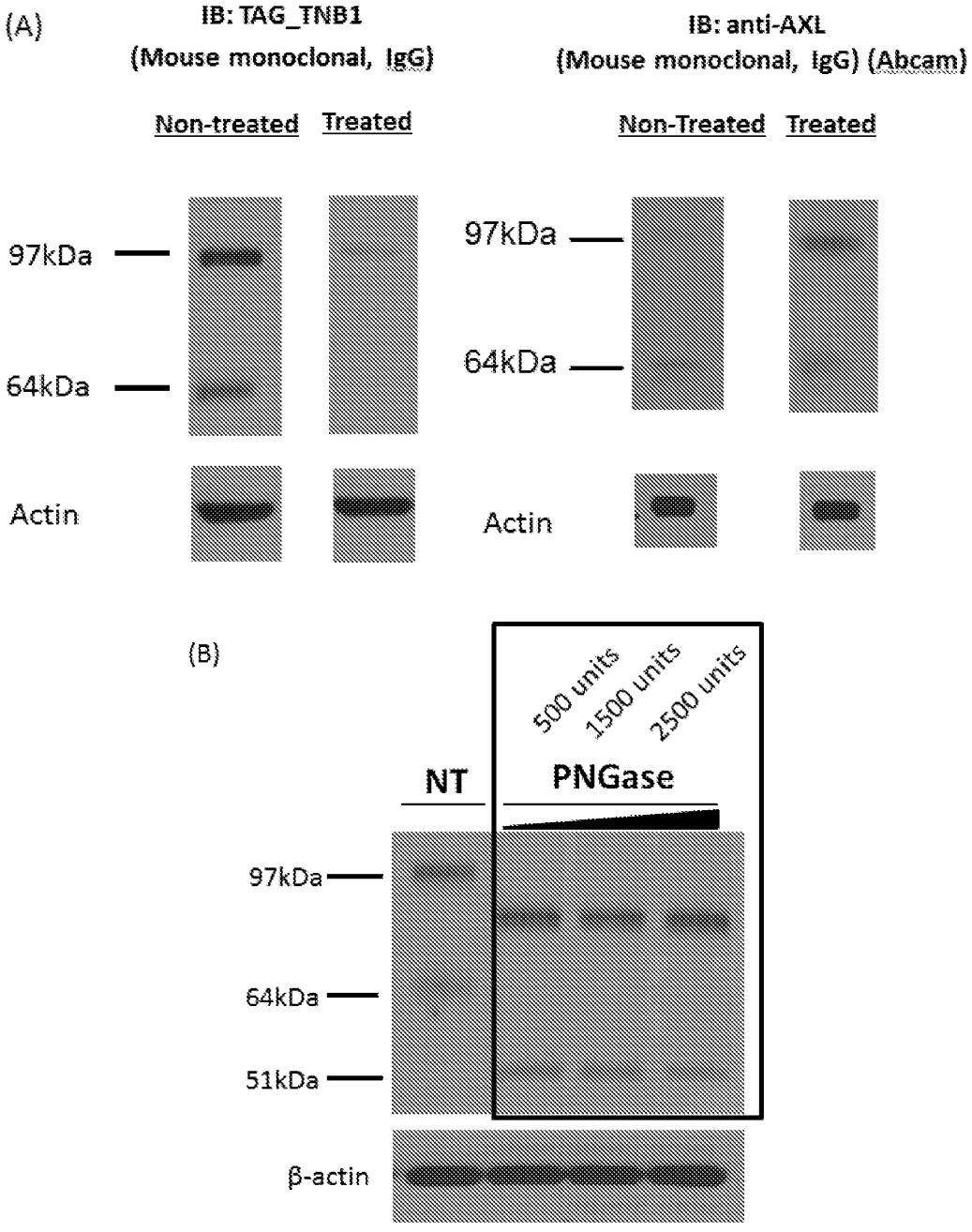

(A)
IB: TAG_TNB1
(Mouse monoclonal, IgG)
Non-treated
Treated
97kDa
64kDa
Actin
IB: anti-AXL
(Mouse monoclonal, IgG) (Abcam)
Non-Treated
Treated
97kDa
64kDa
Actin
(B)
500 units
1500 units
2500 units
NT
PNGase
97kDa
64kDa
51kDa
β-actin

FIG. 4

(C)

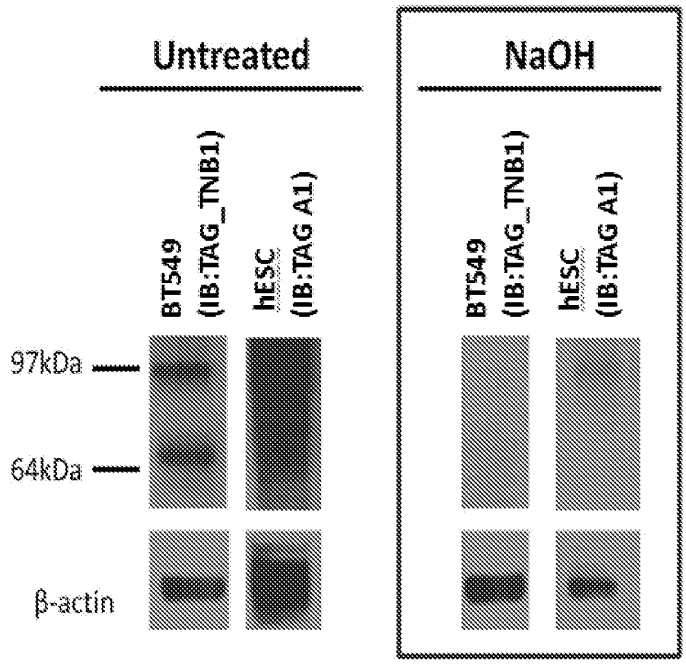

Untreated
NaOH
BT549 (IB:TAG_TNB1)
hESC (IB:TAG A1)
BT549 (IB:TAG_TNB1)
hESC (IB:TAG A1)
97kDa
64kDa
β-actin

FIG. 4

(D)

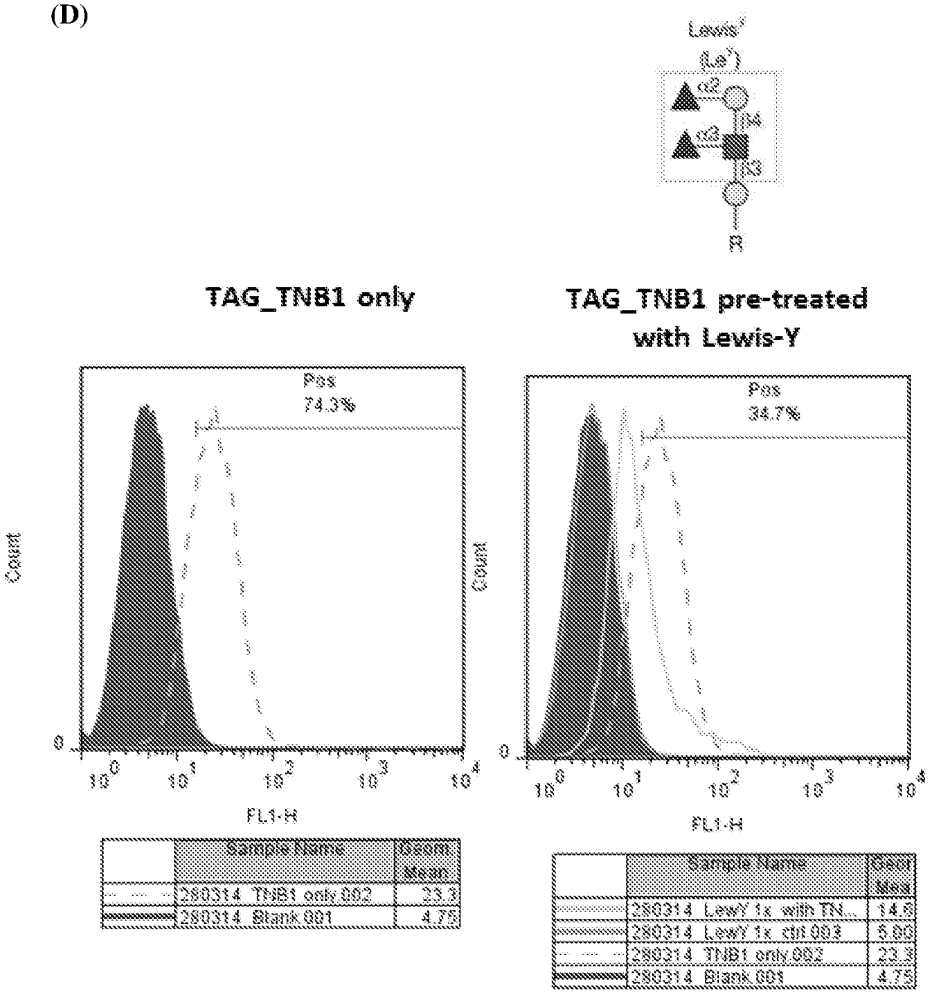

Lewis^y
(Le^y)
α2
α3
β4
β3
R
TAG_TNB1 only
TAG_TNB1 pre-treated with Lewis-Y
Pos
74.3%
Pos
34.7%
Count
Count
0
0
10^0 10^1 10^2 10^3 10^4
10^0 10^1 10^2 10^3 10^4
FL1-H
FL1-H
Sample Name
0 Count Mean
280314_TNB1 only 002 23.3
280314_Blank 001 4.75
Sample Name
Geo Mea
280314_LewY 1x_with TN... 14.6
280314_LewY 1x_ctrl 003 5.05
280314_TNB1 only 002 23.3
280314_Blank 001 4.75

FIG. 4

(cont'd)

(D)

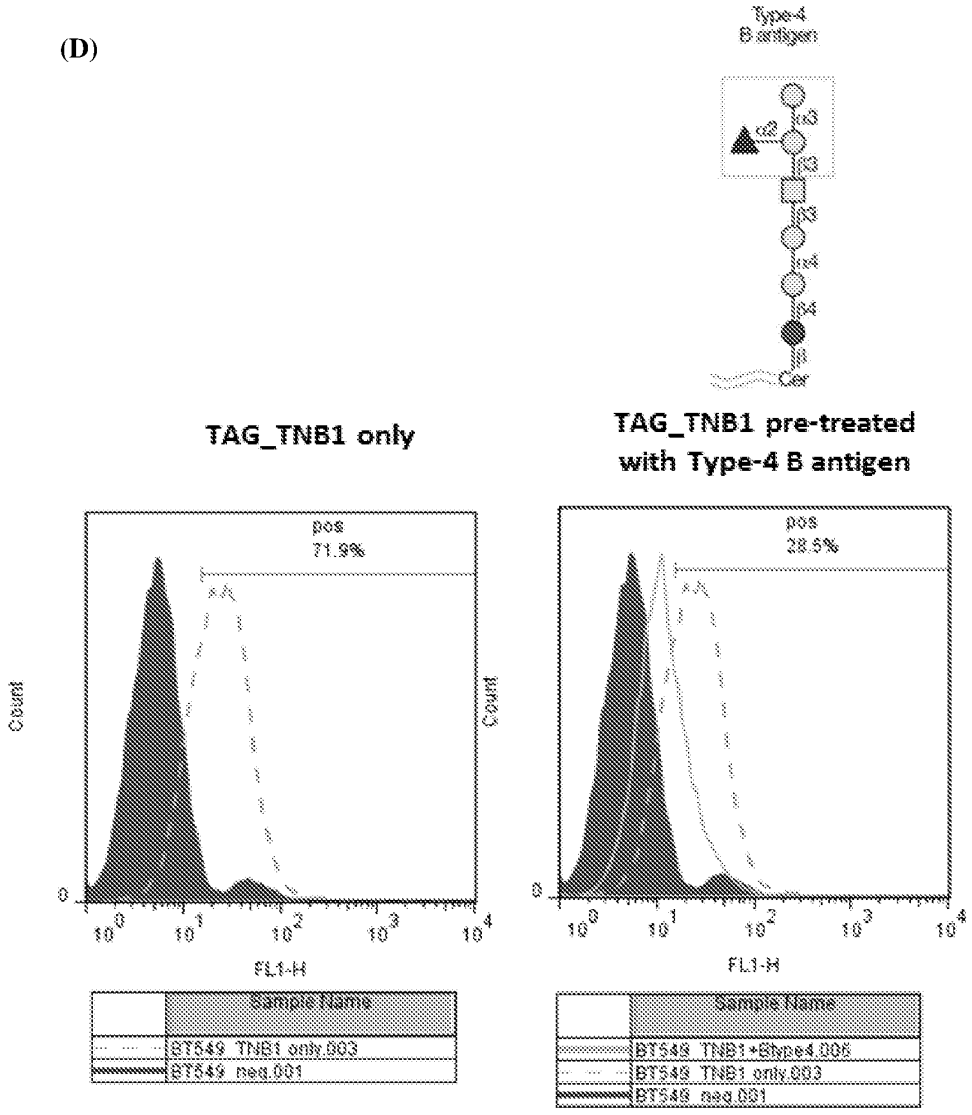

Type-4
B antigen
TAG_TNB1 only
TAG_TNB1 pre-treated
with Type-4 B antigen
pos
71.9%
pos
28.5%
Count
FL1-H
Sample Name
BT549_TNB1 only.003
BT549_neg.001
BT549_TNB1+Btype4.006
BT549_TNB1 only.003
BT549_neg.001

FIG. 5

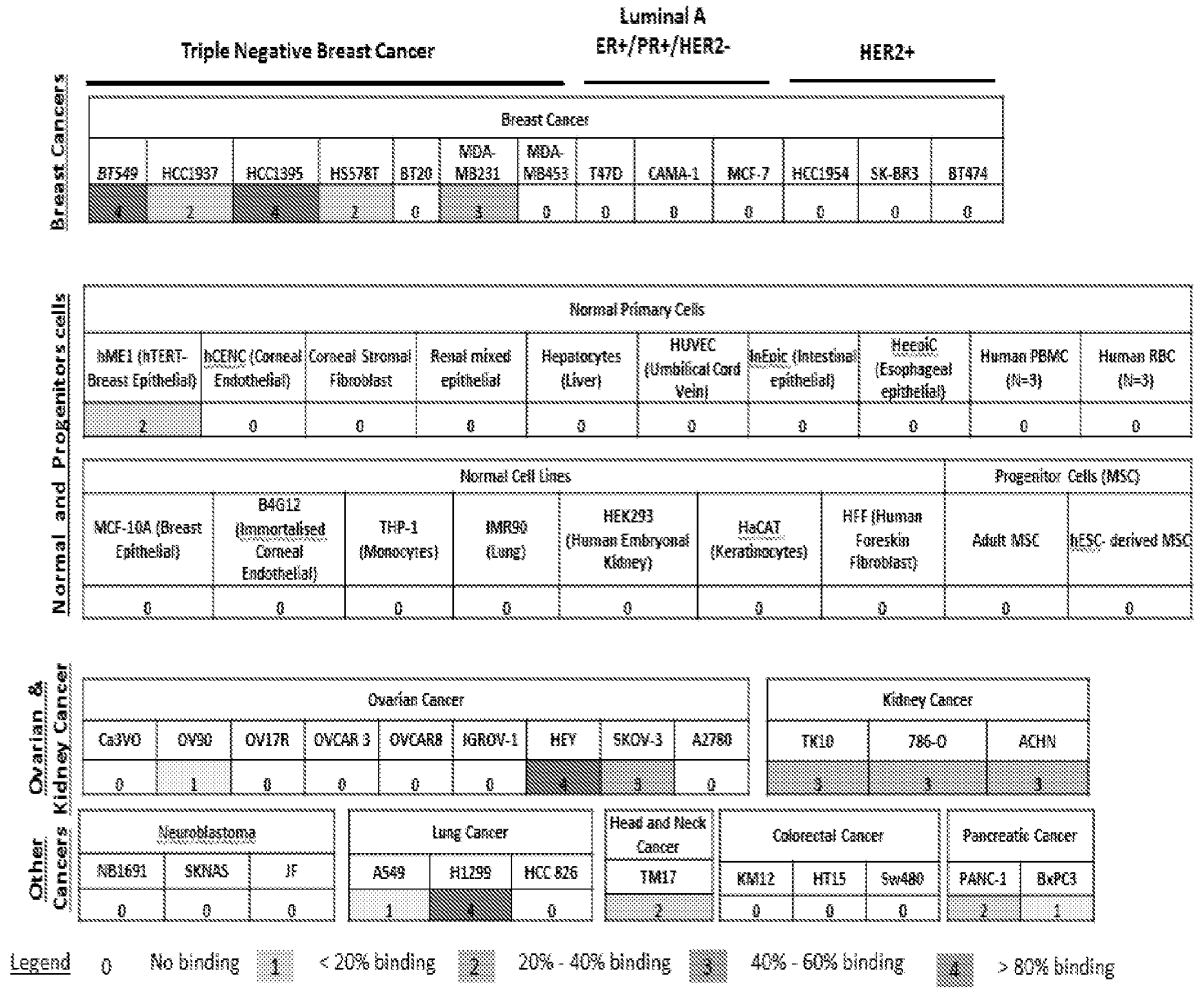
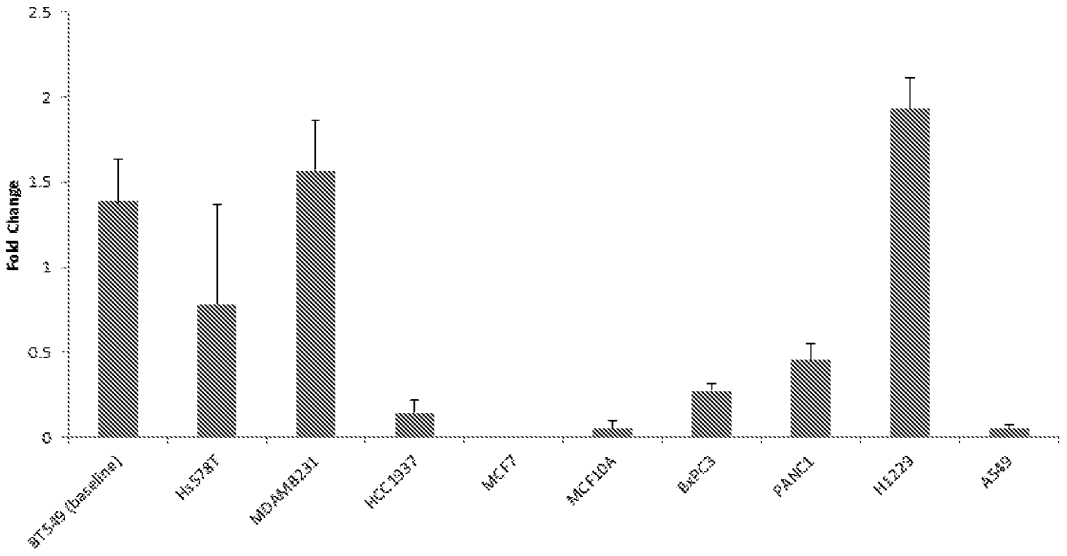

Triple Negative Breast Cancer
Luminal A ER+/PR+/HER2-
HER2+
Breast Cancers
Breast Cancer
BT549 HCC1937 HCC1395 HS578T BT20 MDA-MB231 MDA-MB453 T47D CAMA-1 MCF-7 HCC1954 SK-BR3 BT474
4 2 4 2 0 3 0 0 0 0 0 0 0
Normal and Progenitors cells
Normal Primary Cells
hME1 (hTERT-Breast Epithelial) hCENC (Corneal Endothelial) Corneal Stromal Fibroblast Renal mixed epithelial Hepatocytes (Liver) HUVEC (Umbilical Cord Vein) InEpic (Intestinal epithelial) HeesiC (Esophageal epithelial) Human PBMC (N=3) Human RBC (N=3)
2 0 0 0 0 0 0 0 0 0
Normal Cell Lines
Progenitor Cells (MSC)
MCF-10A (Breast Epithelial) B4G12 (Immortalised Corneal Endothelial) THP-1 (Monocytes) IMR90 (Lung) HEK293 (Human Embryonal Kidney) HaCAT (Keratinocytes) HFF (Human Foreskin Fibroblast) Adult MSC hESC- derived MSC
0 0 0 0 0 0 0 0 0
Ovarian & Kidney Cancer
Ovarian Cancer
Ca3VO OV90 OV17R OVCAR 3 OVCAR8 NGROV-1 HEY SKOV-3 A2780
0 1 0 0 0 0 3 3 0
Kidney Cancer
TK10 786-O ACHN
3 3 3
Other Cancers
Neuroblastoma
NB1691 SKNAS JF
0 0 0
Lung Cancer
A549 H1299 HCC 826
1 4 0
Head and Neck Cancer
TM17
2
Colorectal Cancer
KM12 HT15 Sw480
0 0 0
Pancreatic Cancer
PANC-1 BxPC3
2 1
Legend 0 No binding 1 < 20% binding 2 20% - 40% binding 3 40% - 60% binding 4 > 80% binding

Fig. 6

(A)


Fold Change
2.5
2
1.5
1
0.5
0
BT549 (baseline)
Hs578T
MDAMB231
HCC1937
MCF7
MCF10A
BxPC3
PANC1
H1229
A549

(B)

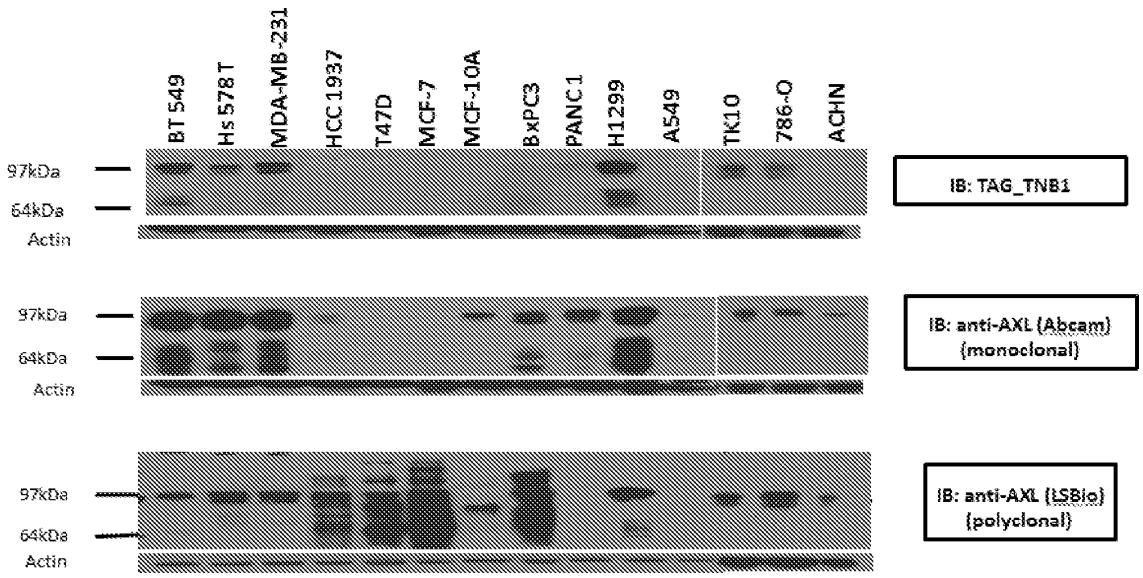

BT 549
Hs 578 T
MDA-MB-231
HCC 1937
T47D
MCF-7
MCF-10A
BxPC3
PANC 1
H1299
A549
TK10
786-O
ACHN
97kDa
64kDa
Actin
IB: TAG_TNB1
IB: anti-AXL (Abcam) (monoclonal)
IB: anti-AXL (LSBio) (polyclonal)

Fig. 7

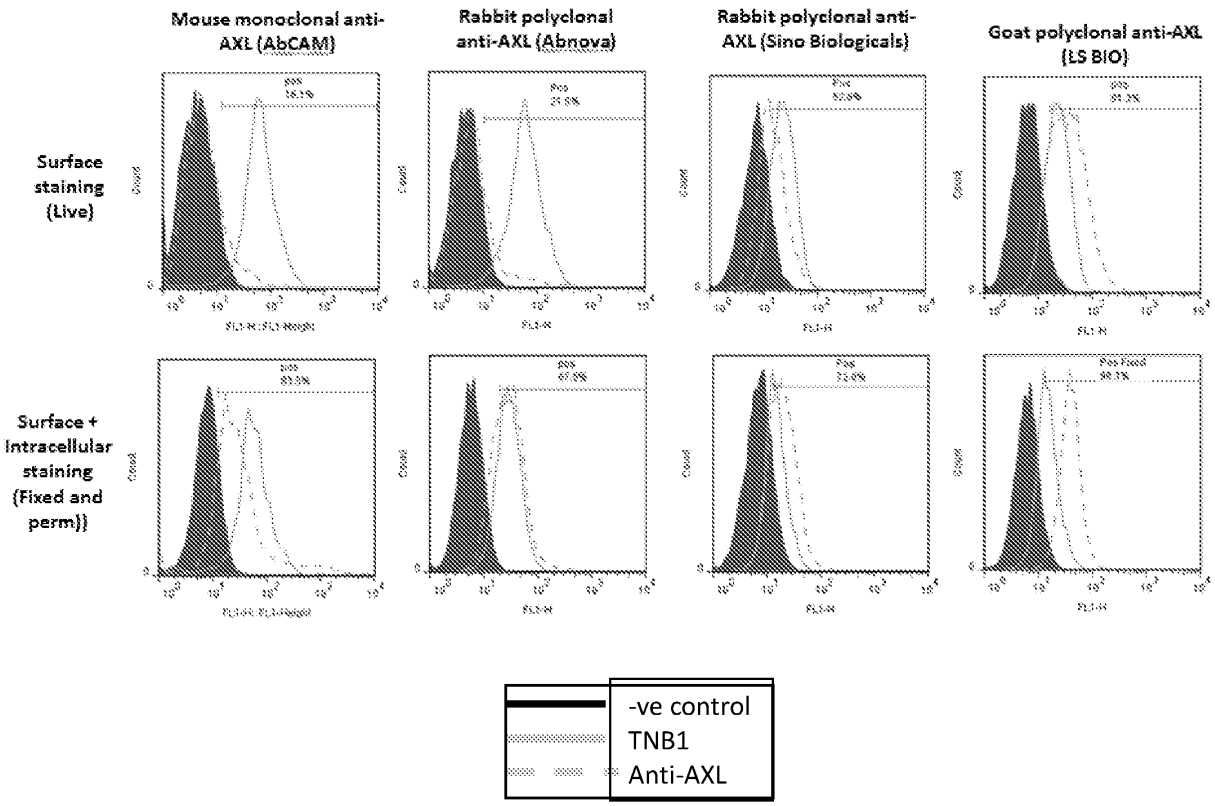

Mouse monoclonal anti-AXL (AbCAM)
Rabbit polyclonal anti-AXL (Abnova)
Rabbit polyclonal anti-AXL (Sino Biologicals)
Goat polyclonal anti-AXL (LS BIO)
Surface staining (Live)
Surface + Intracellular staining (Fixed and perm))
-ve control
TNB1
Anti-AXL

Fig. 8

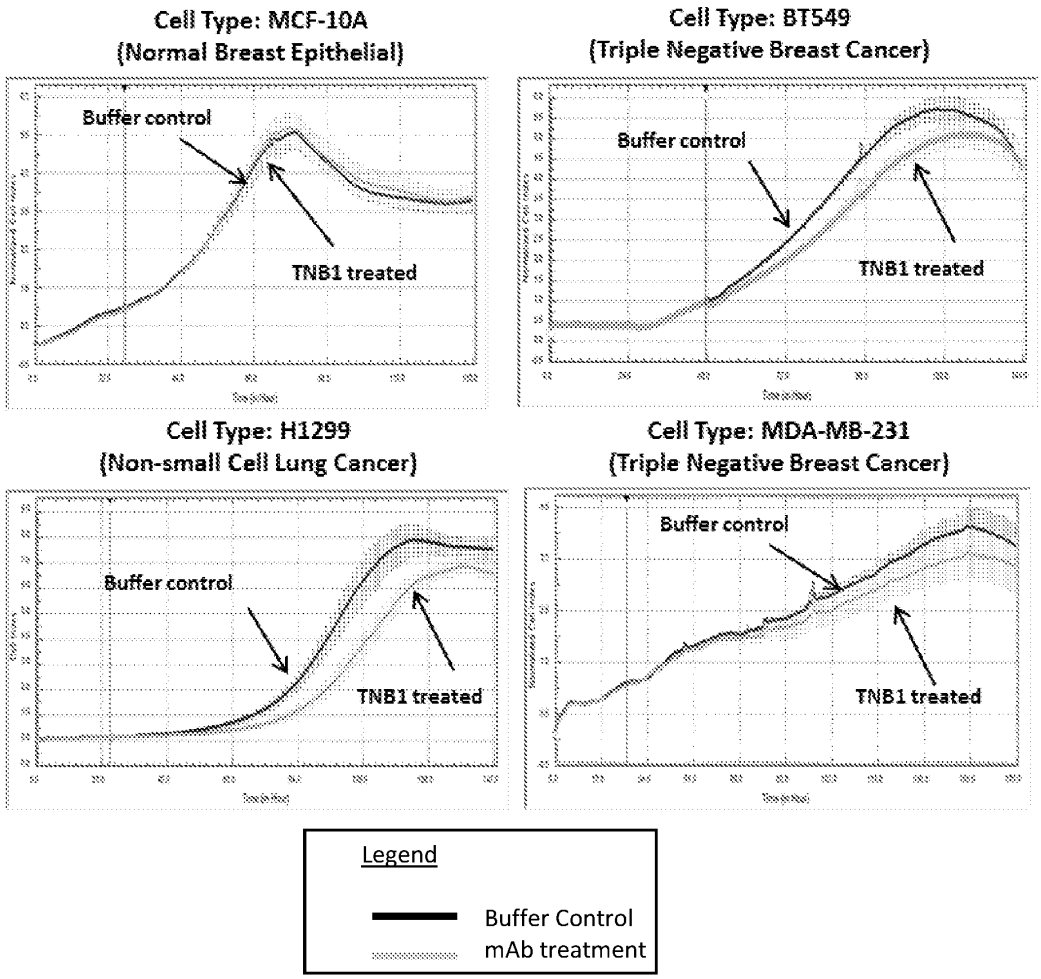

Cell Type: MCF-10A
(Normal Breast Epithelial)
Buffer control
TNB1 treated
Cell Type: BT549
(Triple Negative Breast Cancer)
Buffer control
TNB1 treated
Cell Type: H1299
(Non-small Cell Lung Cancer)
Buffer control
TNB1 treated
Cell Type: MDA-MB-231
(Triple Negative Breast Cancer)
Buffer control
TNB1 treated
Legend
Buffer Control
mAb treatment

Fig. 9

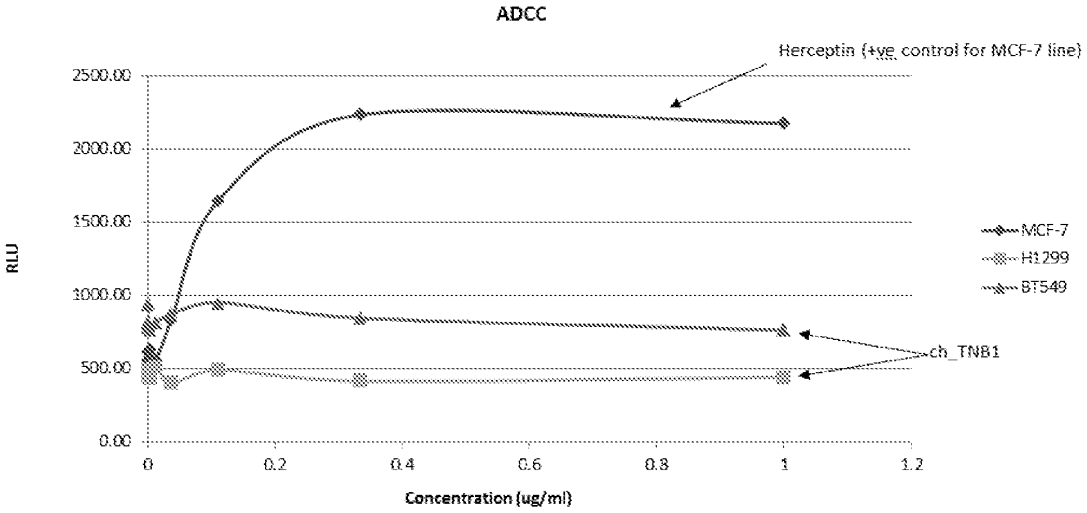

ADCC
Herceptin (+ve control for MCF-7 line)
2500.00
2000.00
1500.00
1000.00
500.00
0.00
RLU
0
0.2
0.4
0.6
0.8
1
1.2
Concentration (ug/ml)
MCF-7
H1299
BT549
ch_TNB1

Fig. 10

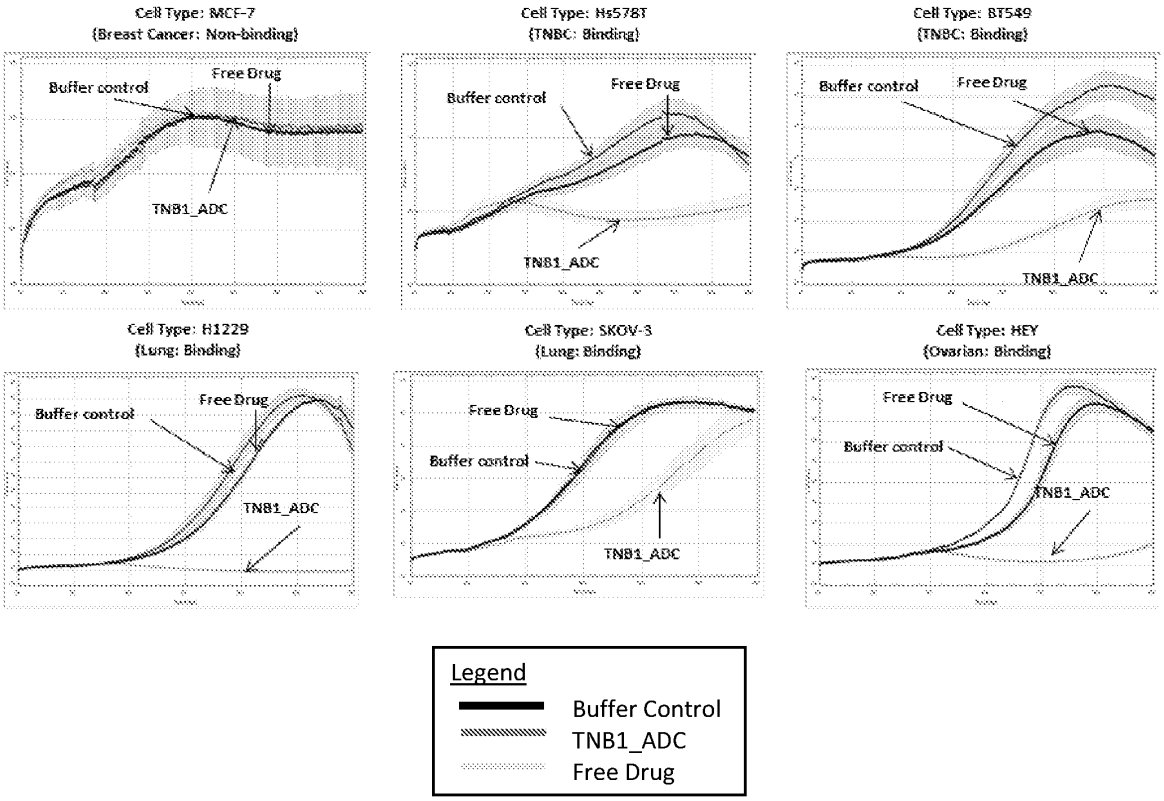
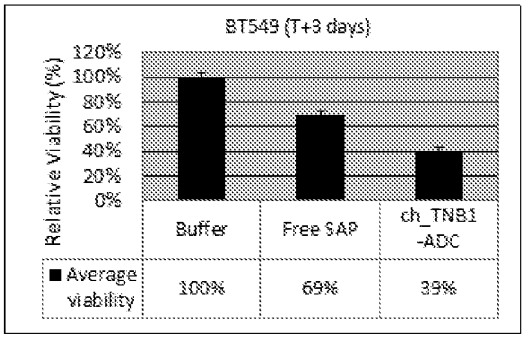
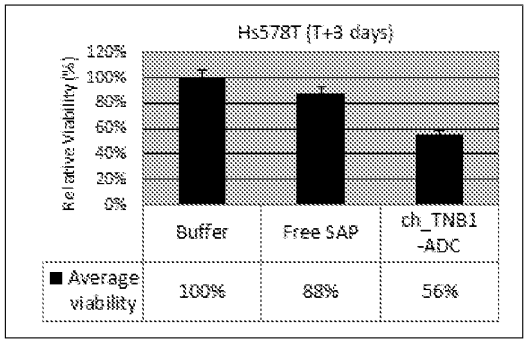

Cell Type: MCF-7
(Breast Cancer: Non-binding)
Buffer control
Free Drug
TNB1_ADC
Cell Type: Hs578T
(TNBC: Binding)
Buffer control
Free Drug
TNB1_ADC
Cell Type: BT549
(TNBC: Binding)
Buffer control
Free Drug
TNB1_ADC
Cell Type: H1229
(Lung: Binding)
Buffer control
Free Drug
TNB1_ADC
Cell Type: SKOV-3
(Lung: Binding)
Free Drug
Buffer control
TNB1_ADC
Cell Type: HEY
(Ovarian: Binding)
Free Drug
Buffer control
TNB1_ADC
Legend
Buffer Control
TNB1_ADC
Free Drug

Fig. 11

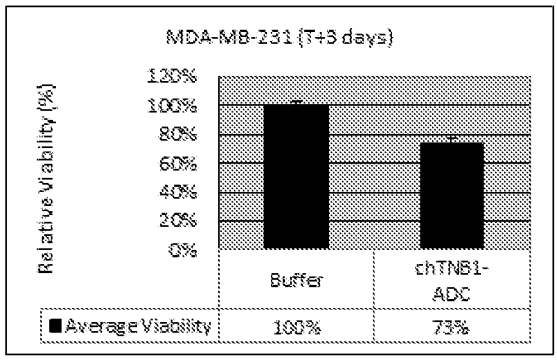
Cell Type: BT549
(TNBC: Binding)



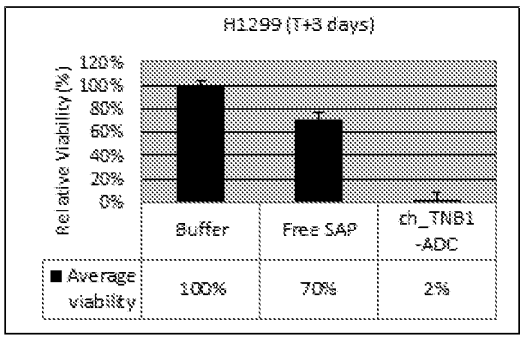
Cell Type: Hs578T
(TNBC: Binding)



Cell Type: MDA-MB-231
(TNBC: Binding)



Cell Type: H1299
(TNBC: Binding)



Cell Type: MCF-7
(TNBC: Non-binding)

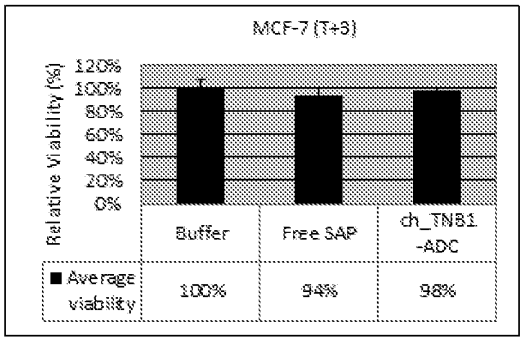
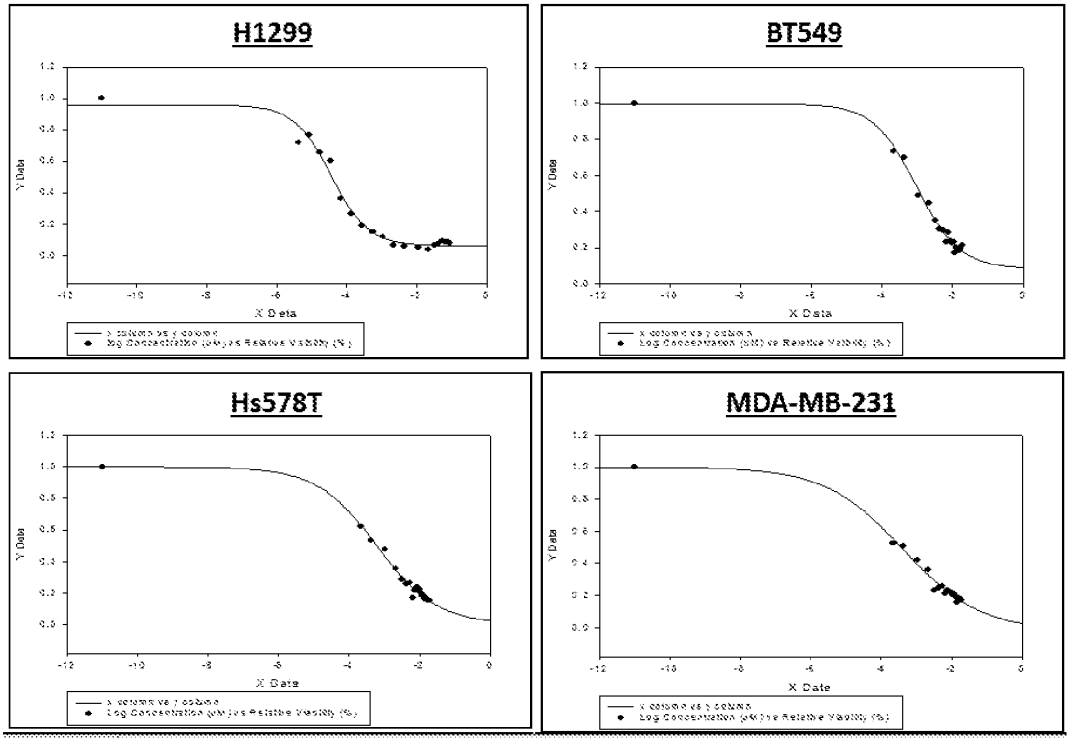
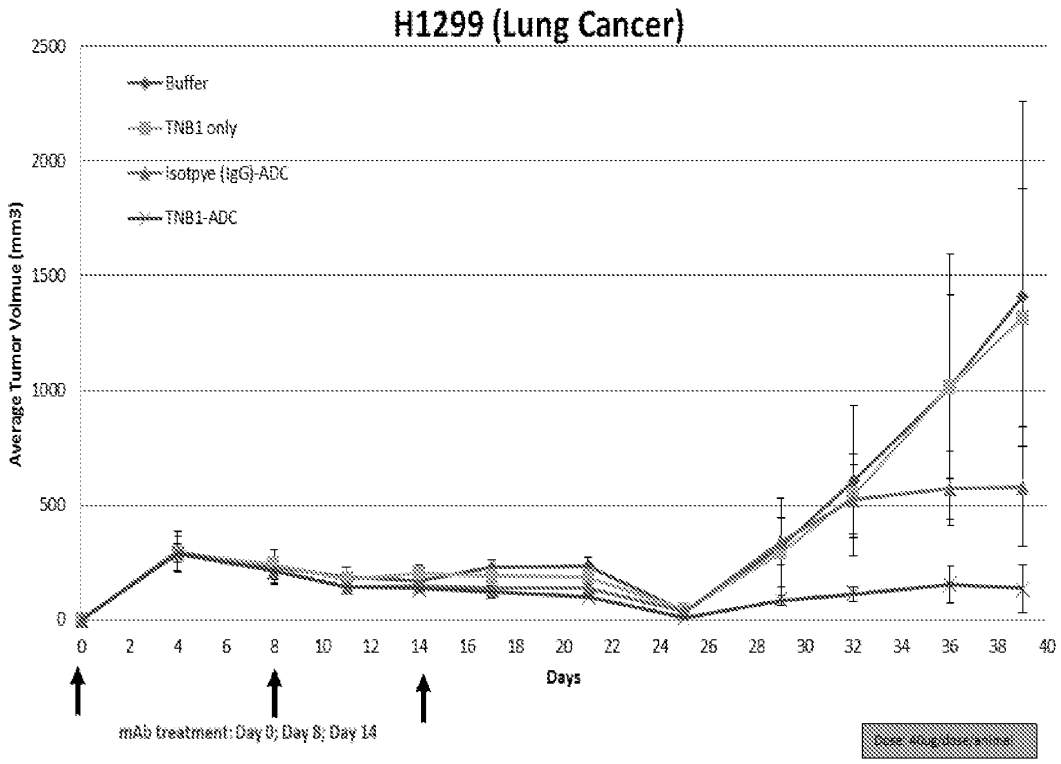


Fig. 12



Cell Line	IC50 (CTG)
H1299	35.33 pM
BT549	1240.80 pM
Hs578T	528.91 pM
MDA-MB-231	339.78 pM

Fig. 13


H1299 (Lung Cancer)
Buffer
TNB1 only
isotpye (IgG)-ADC
TNB1-ADC
Average Tumor Volmue (mm3)
2500
2000
1500
1000
500
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Days
mAb treatment: Day 0; Day 8; Day 14