Fig. 1A
NME1 (~17KDa)

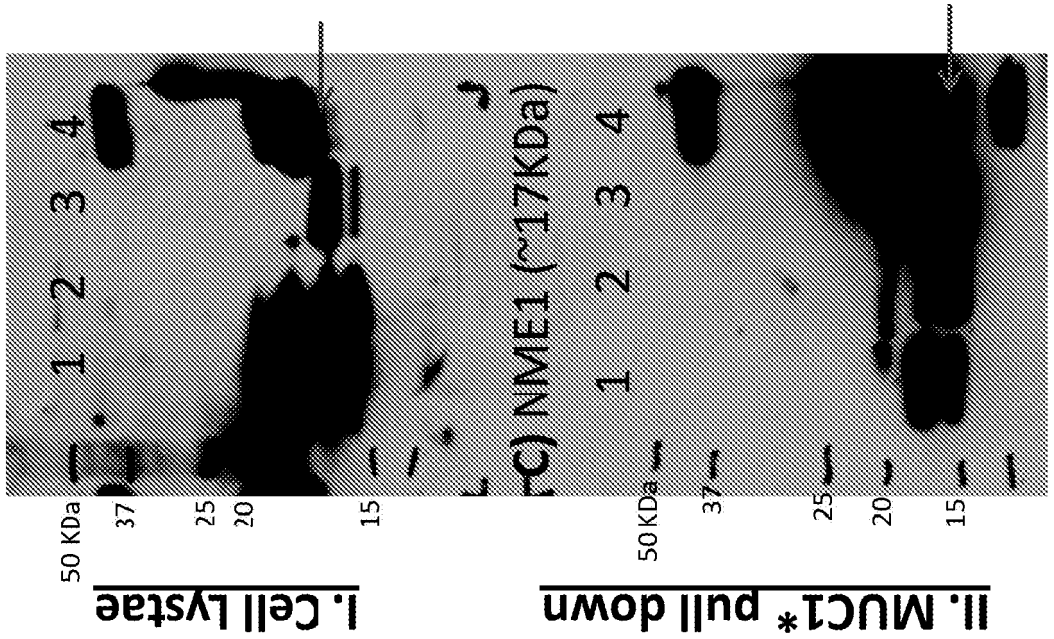


Fig. 1B
NME7 (~42KDa)

Western Blot Analysis of Cancer and Stem Cells, Probing for Presence of NME1, NME6 and NME7 in the Cell Lysates

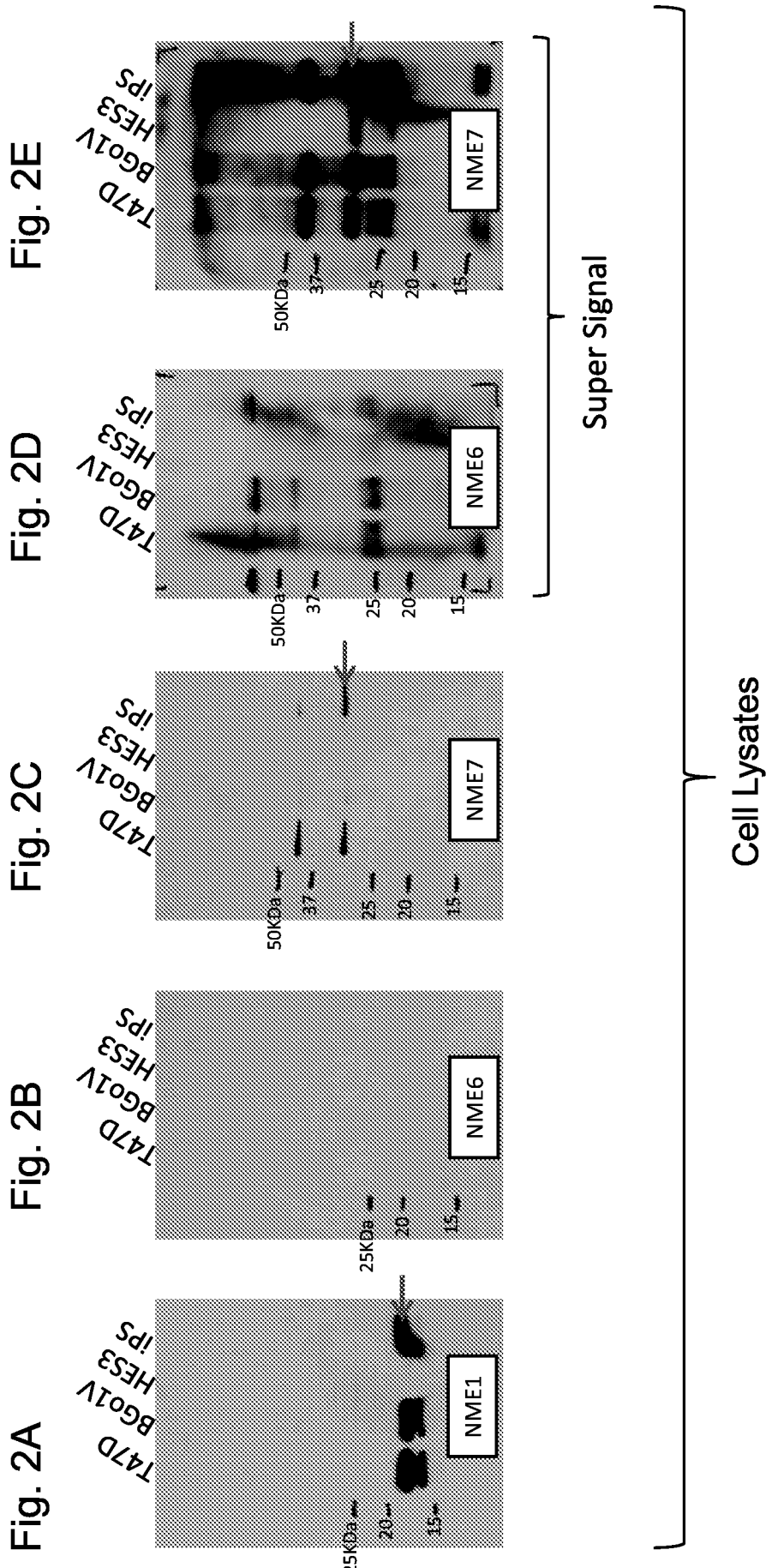
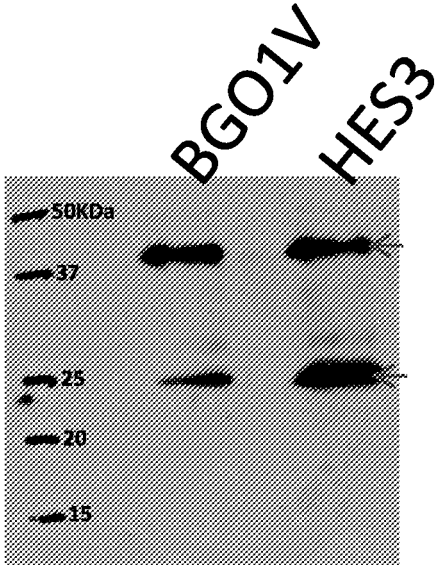


Fig. 3A

Detection of NME7 in cell lysate by western blot

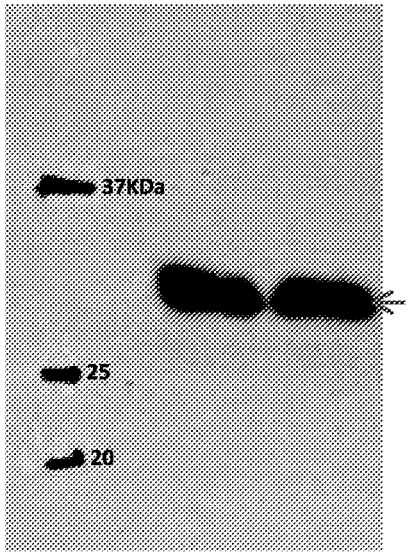


Reducing western blot

1- lyse cells with RIPA buffer
2- run reducing SDS-PAGE (20ul per lane)
3- transfer protein to PVDF membrane
4- probe membrane with anti NM23-H7 (B-9, Santa Cruz Biotechnlogy)
5- use goat anti mouse-HRP as secondary
6- detect protein by chemiluminescence

Fig. 3B

Detection of NME7 in conditioned media of iPS cells (SC101-A1) by western blot

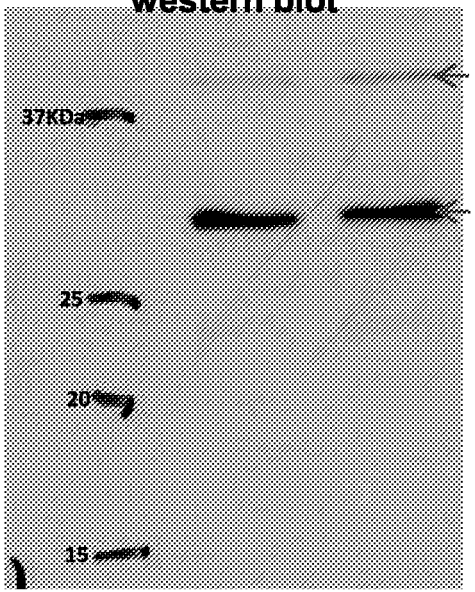


Reducing western blot

1- run reducing SDS-PAGE (20ul of CM per lane)
2- transfer protein to PVDF membrane
3- probe membrane with anti NM23-H7 (B-9, Santa Cruz Biotechnlogy)
4- use goat anti mouse-HRP as secondary
5- detect protein by chemiluminescence

Fig. 3C

Detection of NME7 in lysate of iPS cells (SC101-A1) by western blot



Reducing western blot

1- lyse cells with RIPA buffer
2- run reducing SDS-PAGE (20ul per lane)
3- transfer protein to PVDF membrane
4- probe membrane with anti NM23-H7 (B-9, Santa Cruz Biotechnlogy)
5- use goat anti mouse-HRP as secondary
6- detect protein by chemiluminescence

Recombinant NME7 novel variant containing NDPK domains A and B, "NME7-AB", expresses well with high yield in E. *coli* and as the soluble protein

Fig. 4A

FPLC purification of NME7-AB

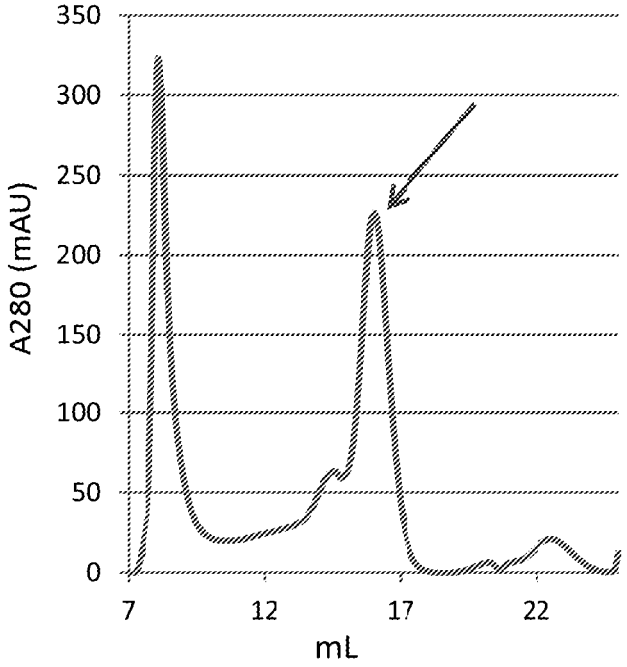
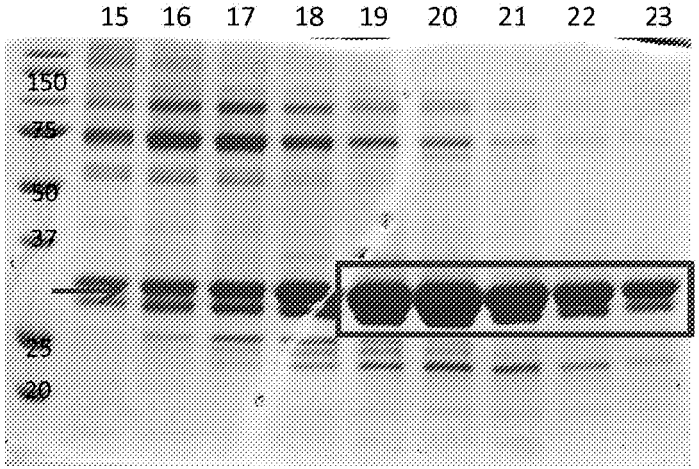


Fig. 4B

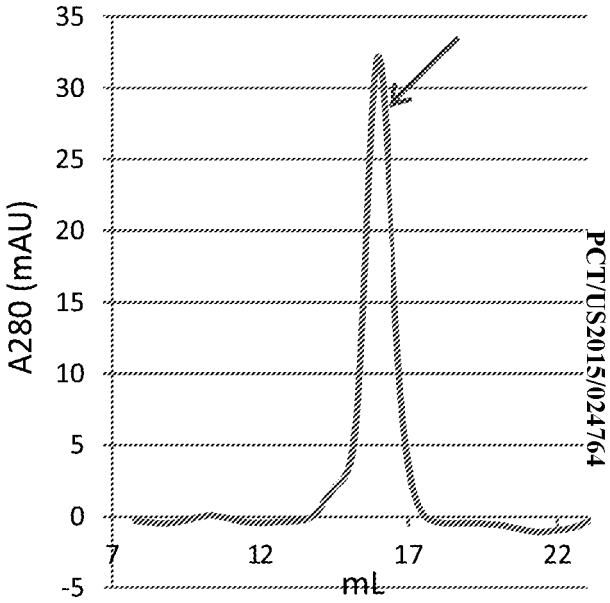
SDS-PAGE of NME7-AB



12% non-reducing SDS-PAGE

Fig. 4C

NME7-AB is purified



ELISA shows NME7 Dimerizes MUC1*

MUC1* extra cellular domain peptide immobilized on plate was bound by NME7 to saturation; a second MUC1* peptide with a C-terminal His-tag or Biotin tag was added and visualized by HRP labeled antibody to either His-tag or HRP labeled streptavidin

Fig. 5A

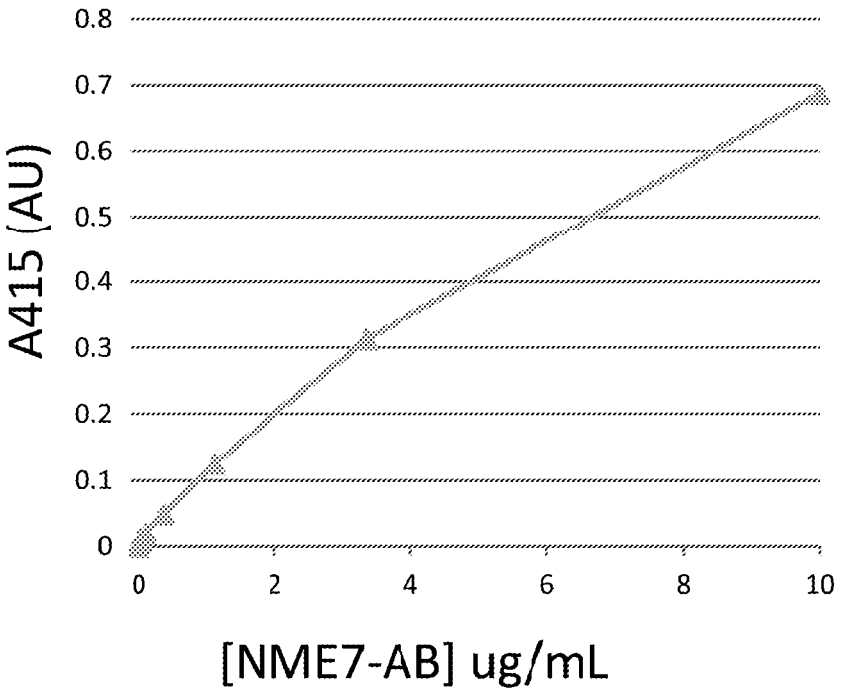
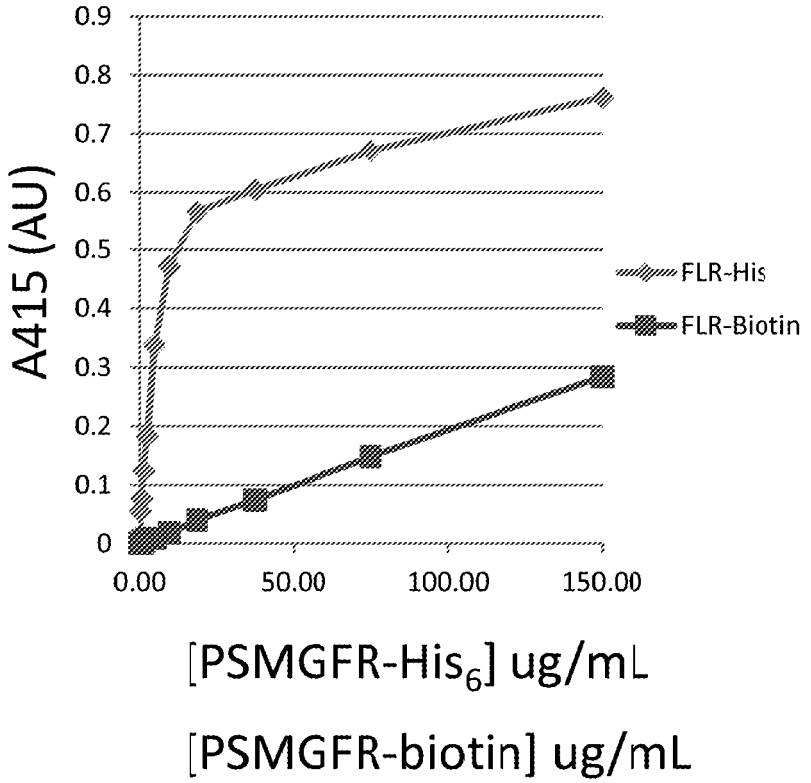


Fig. 5B



T47D + anti-NM23-H7 rabbit polyclonal – t=48h

Fig. 6A

Fig. 6B

Fig. 6C

Fig. 6D

Fig. 6E

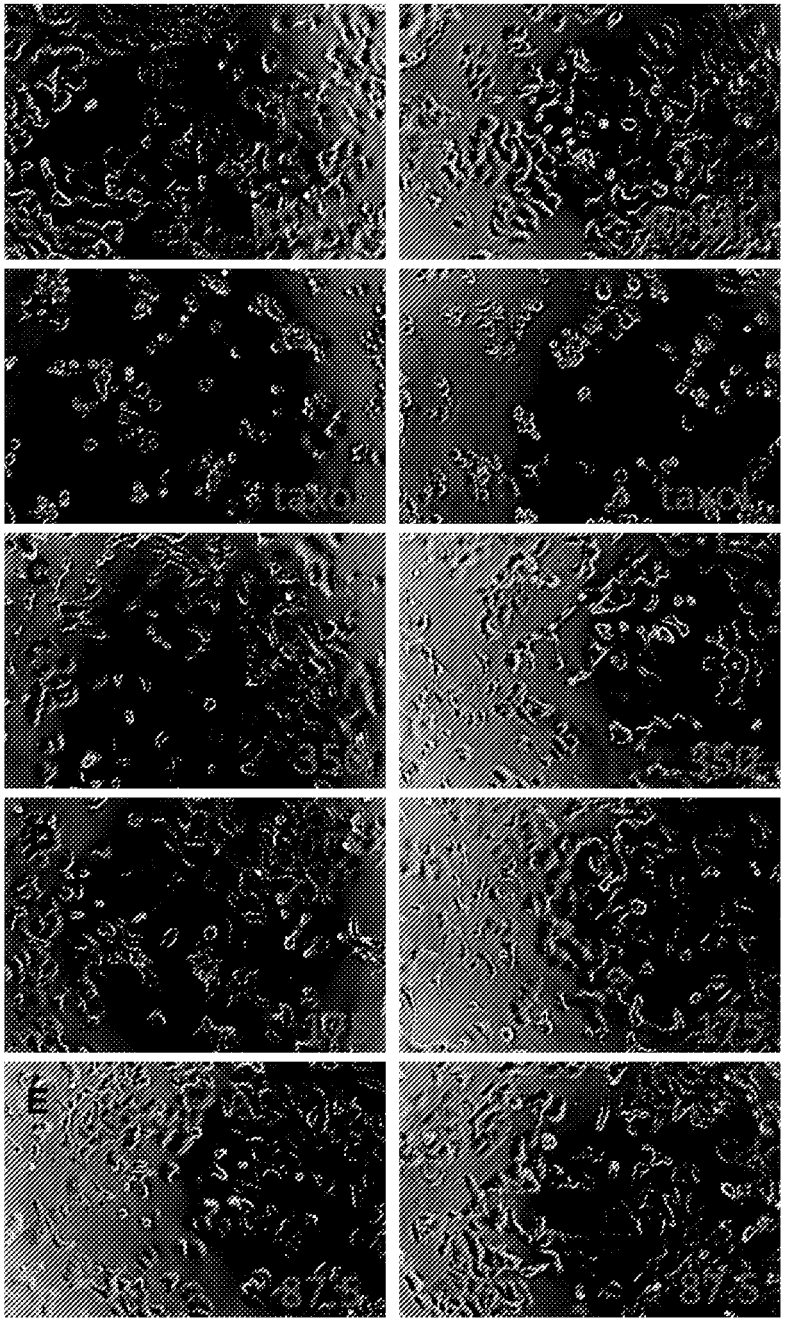


Fig. 6F

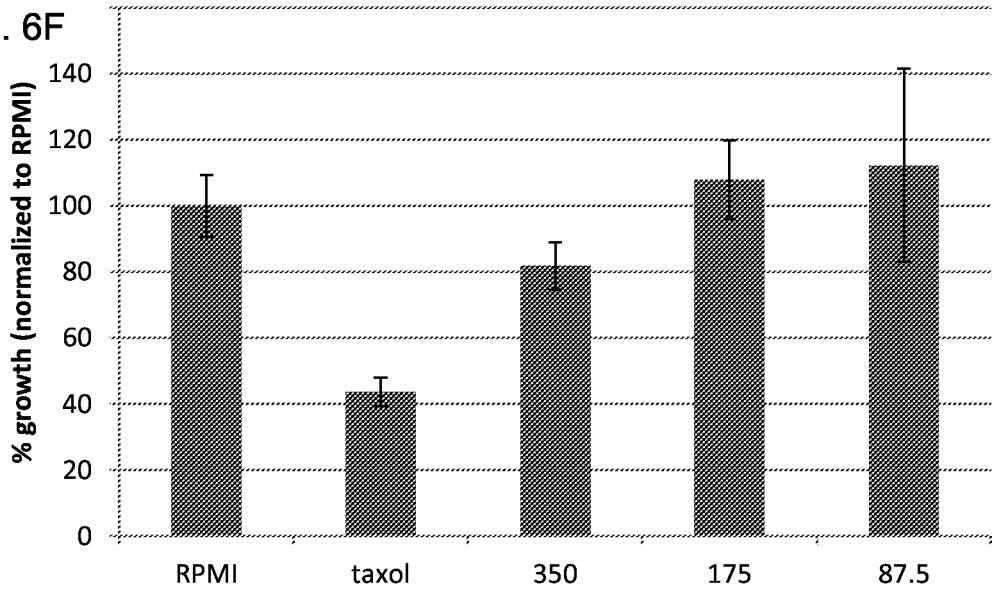
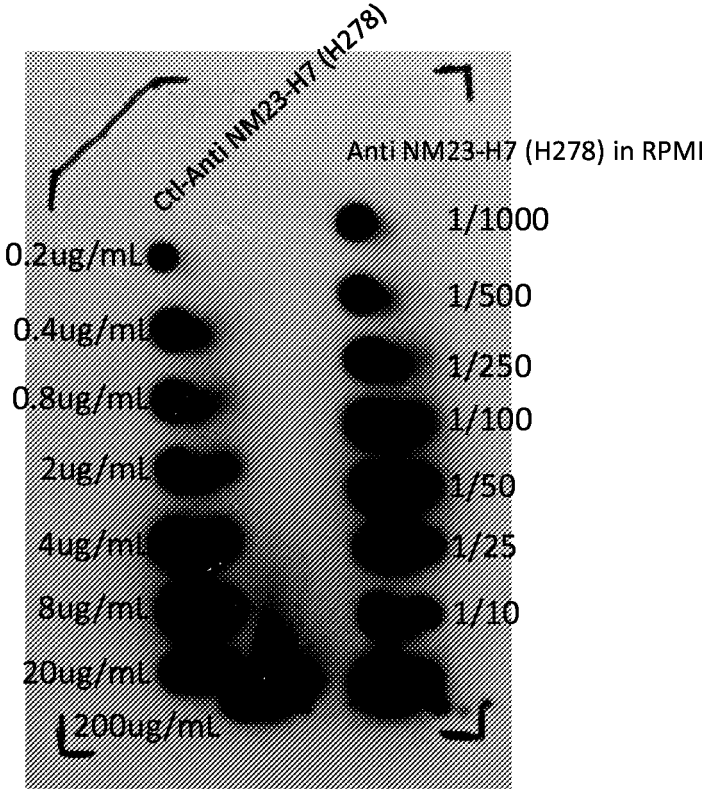
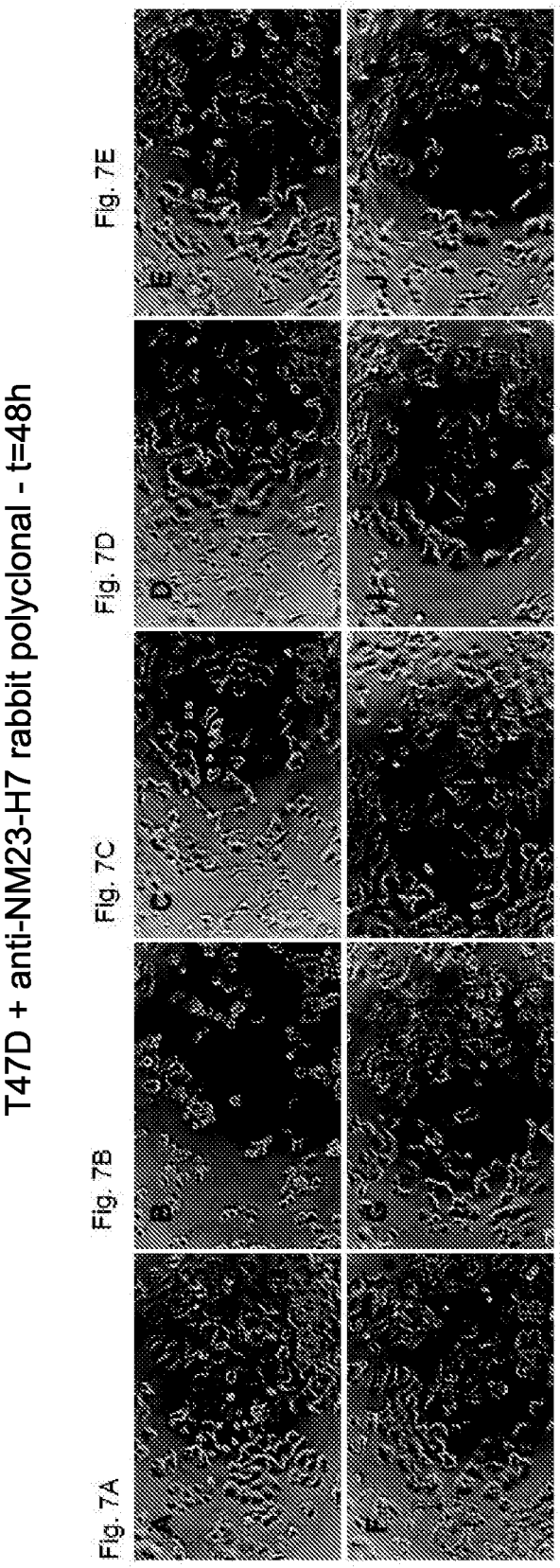


Fig. 6G




T47D + anti-NM23-H7 rabbit polyclonal - t=48h
Fig. 7A
Fig. 7B
Fig. 7C
Fig. 7D
Fig. 7E
Fig. 7F
Fig. 7G
Fig. 7H
Fig. 7I
Fig. 7J

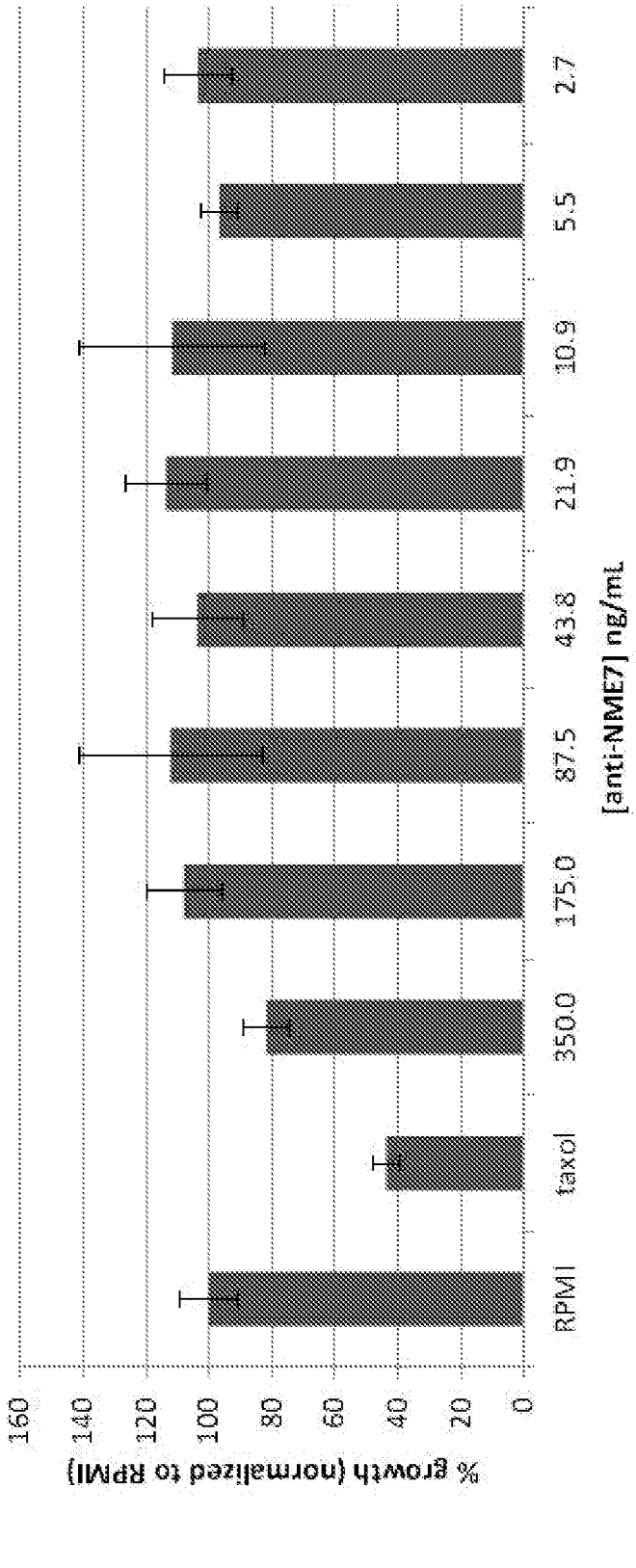
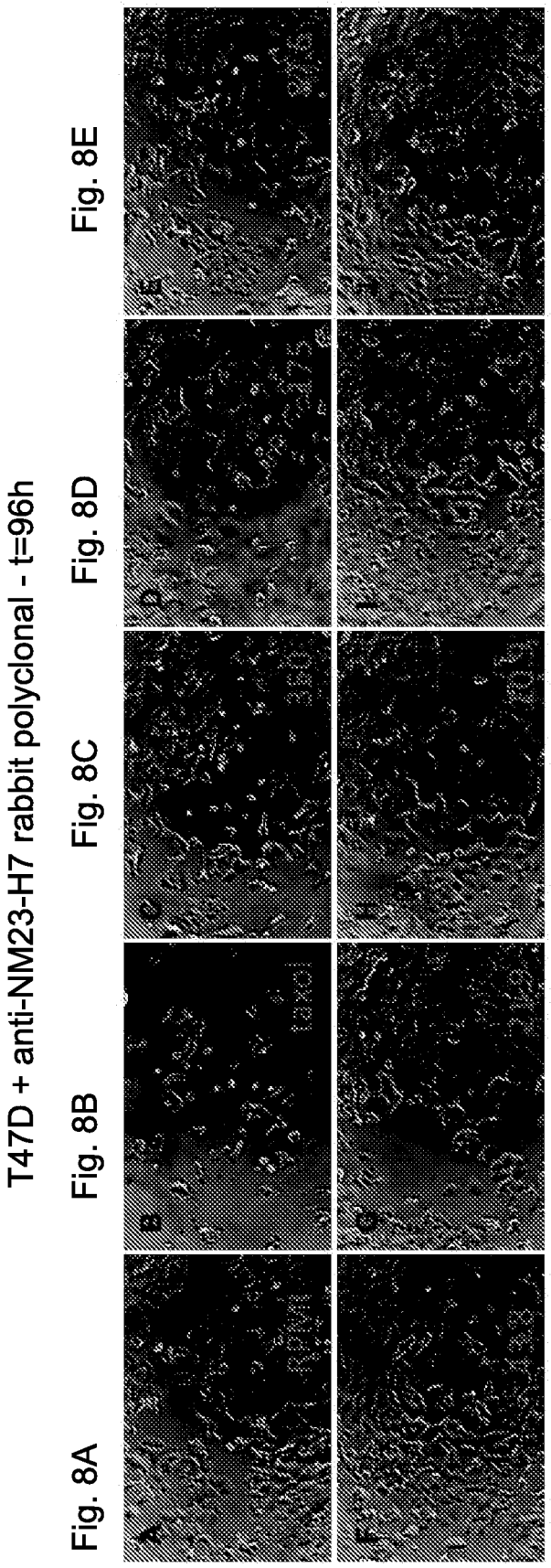

% growth (normalized to RPMI)
0
20
40
60
80
100
120
140
160
RPMI
taxol
350.0
175.0
87.5
43.8
21.9
10.9
5.5
2.7
[anti-NME7] ng/mL

Fig. 7K


T47D + anti-NM23-H7 rabbit polyclonal - t=96h
Fig. 8A
Fig. 8B
Fig. 8C
Fig. 8D
Fig. 8E
Fig. 8F
Fig. 8G
Fig. 8H
Fig. 8I
Fig. 8J
A
B
taxol
C
D
E
F
G
H
I
J

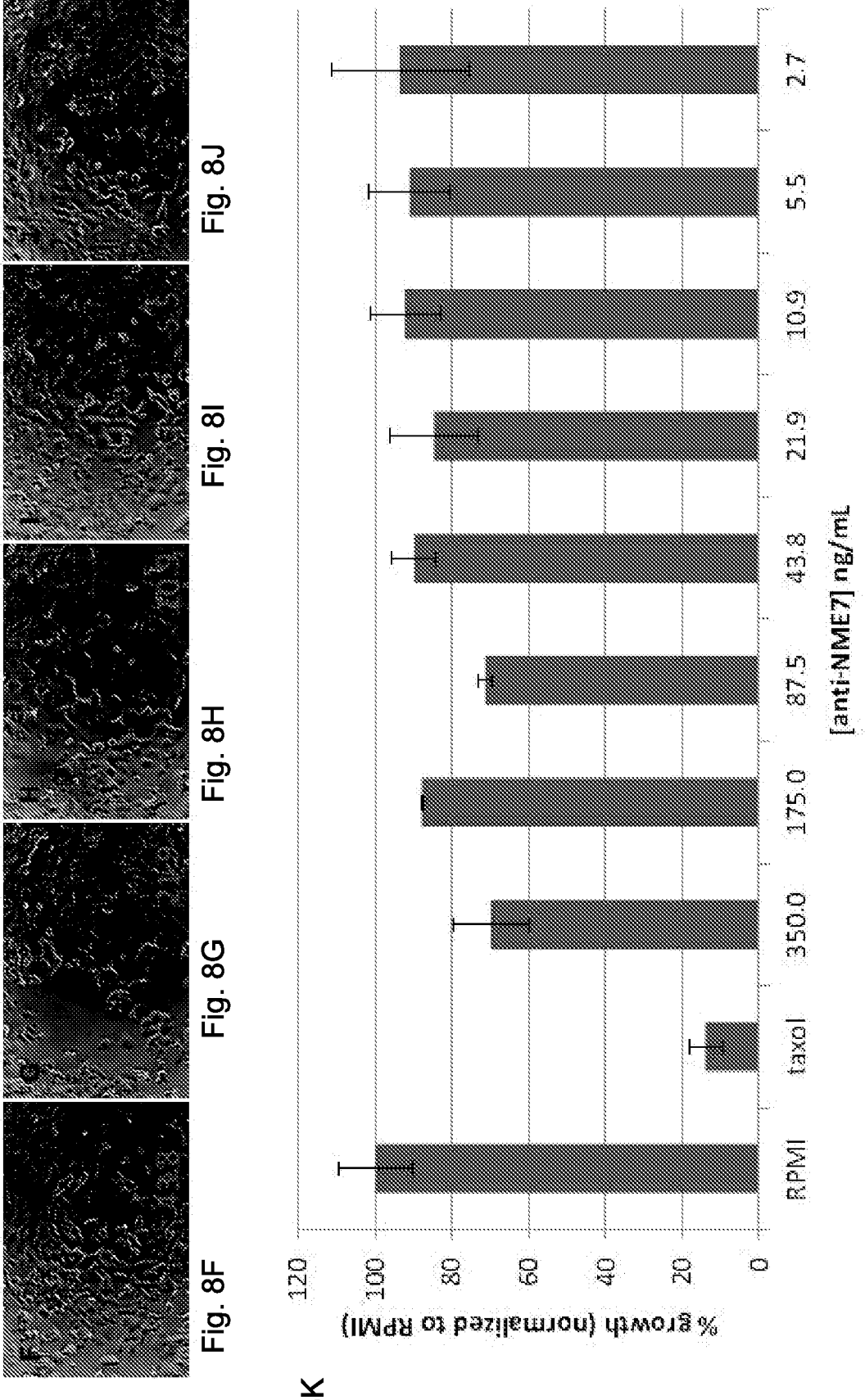

% growth (normalized to RPMI)
120
100
80
60
40
20
0
RPMI
taxol
350.0
175.0
87.5
43.8
21.9
10.9
5.5
2.7
[anti-NME7] ng/mL

Fig. 8K

Stem Cell Lysates + Corresponding Conditioned Media Probed for Presence of NME-7

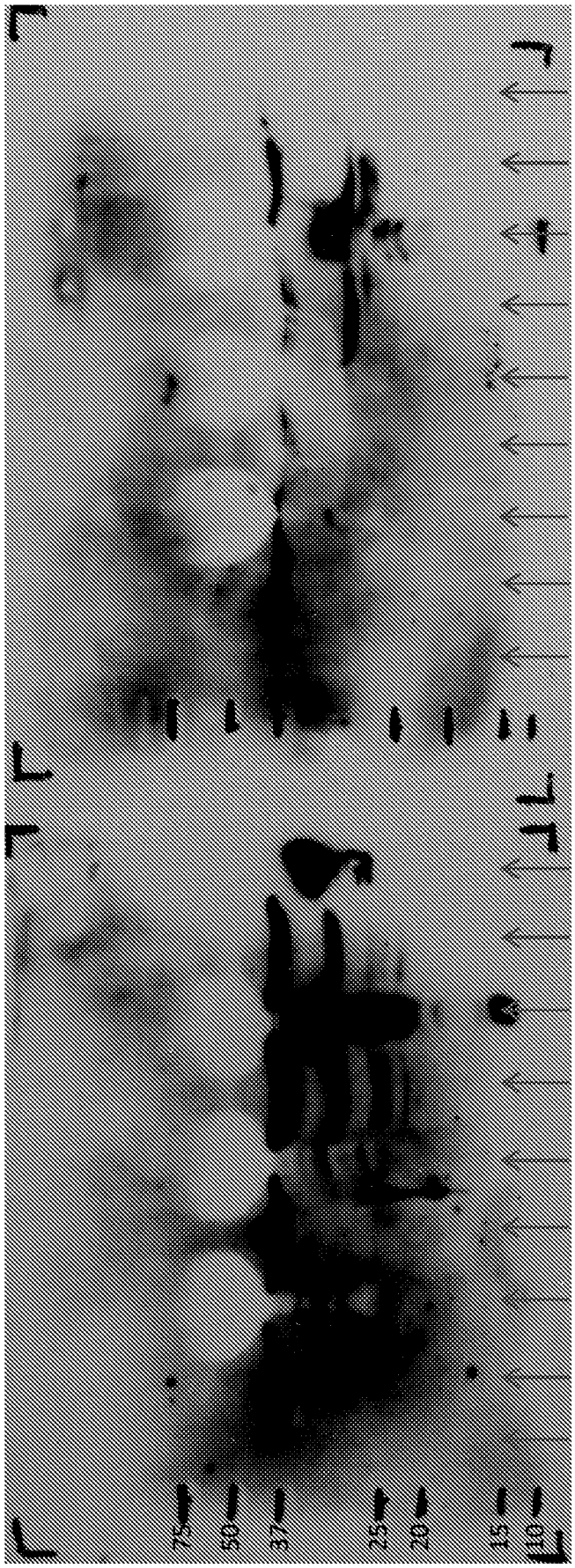


Fig. 9

Fig. 10B

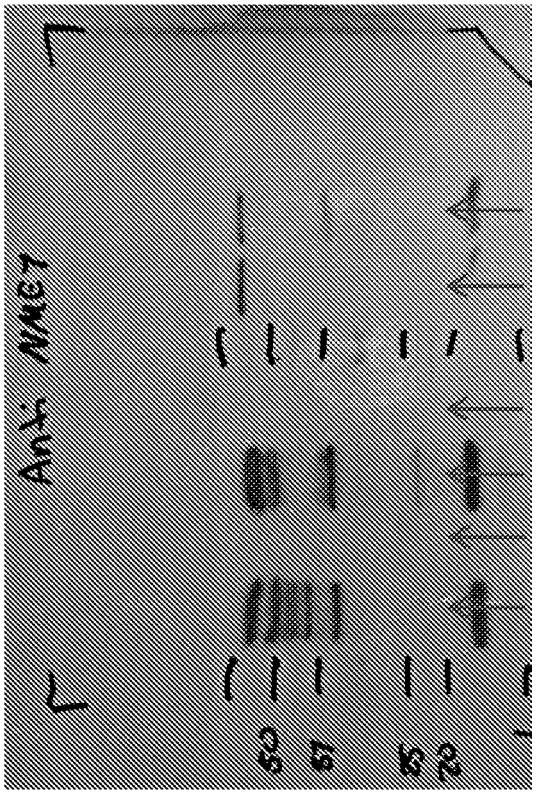
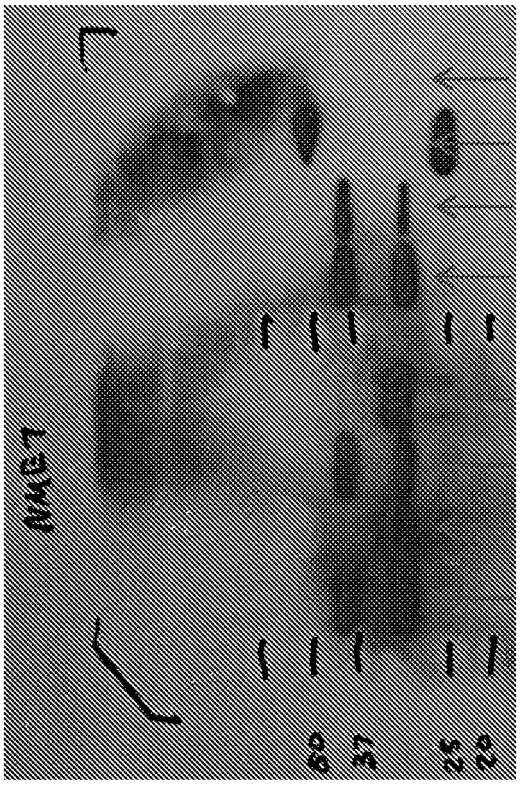


Fig. 10A



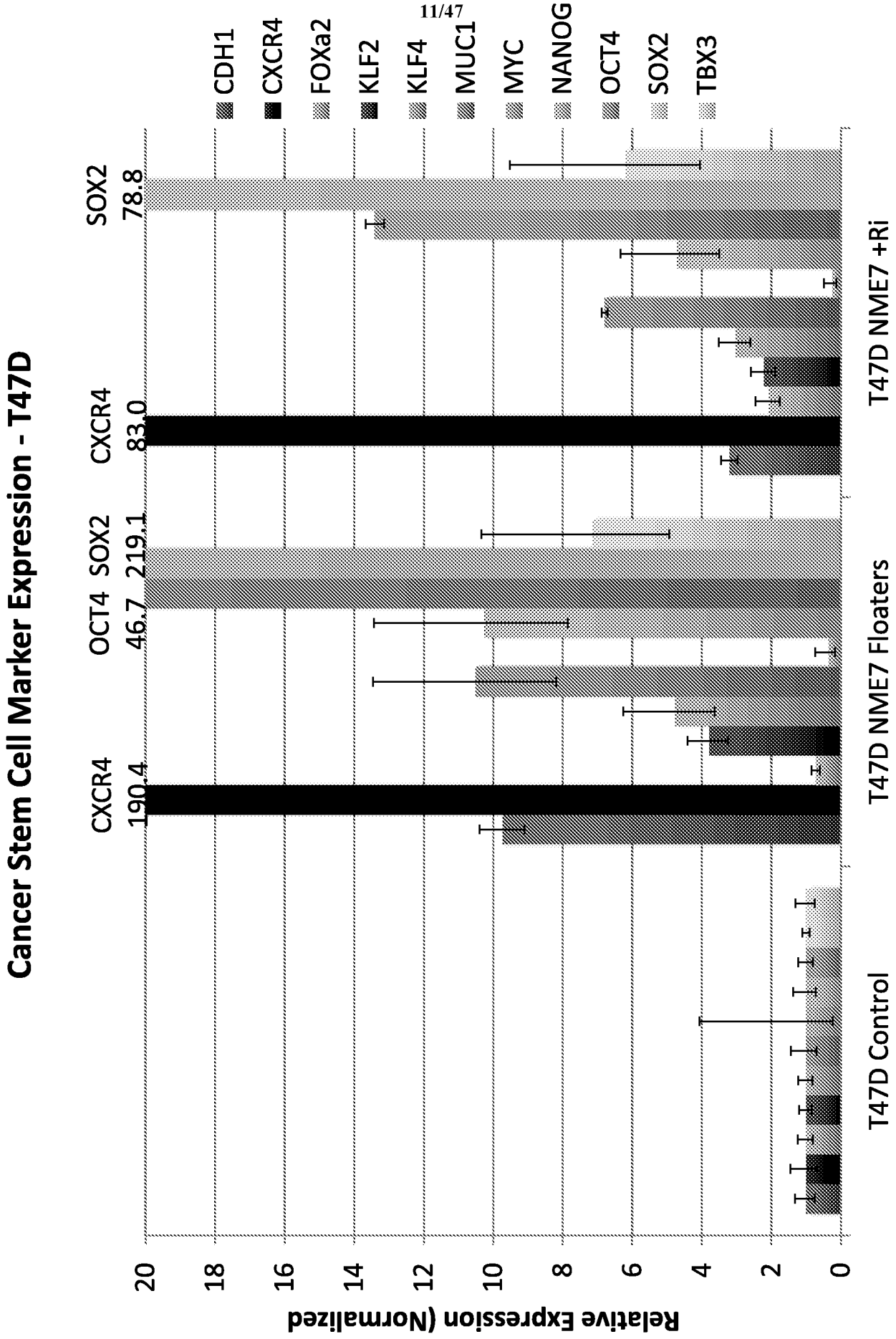

Cancer Stem Cell Marker Expression - T47D
Relative Expression (Normalized)
0
2
4
6
8
10
12
14
16
18
20
CDH1
CXCR4
FOXa2
KLF2
KLF4
MUC1
MYC
NANOG
OCT4
SOX2
TBX3
CXCR4 190.4
OCT4 46.7
SOX2 219.1
CXCR4 83.0
SOX2 78.8
T47D Control
T47D NME7 Floaters
T47D NME7 +Ri

Fig. 11

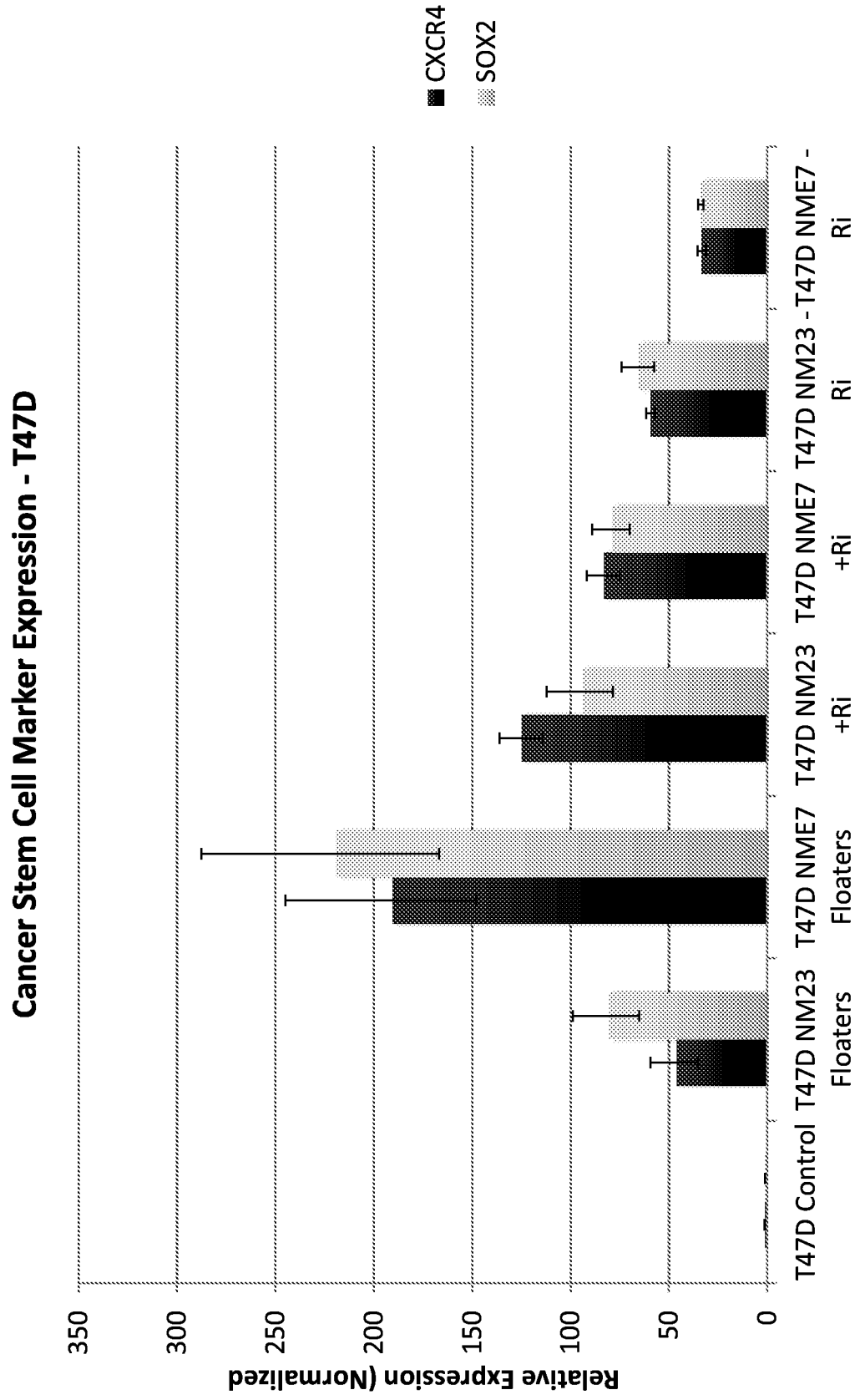

Cancer Stem Cell Marker Expression - T47D
CXCR4
SOX2
Relative Expression (Normalized)
350
300
250
200
150
100
50
0
T47D Control
T47D NM23 Floaters
T47D NME7 Floaters
T47D NM23 +Ri
T47D NME7 +Ri
T47D NM23 - Ri
T47D NME7 - Ri

Fig. 12

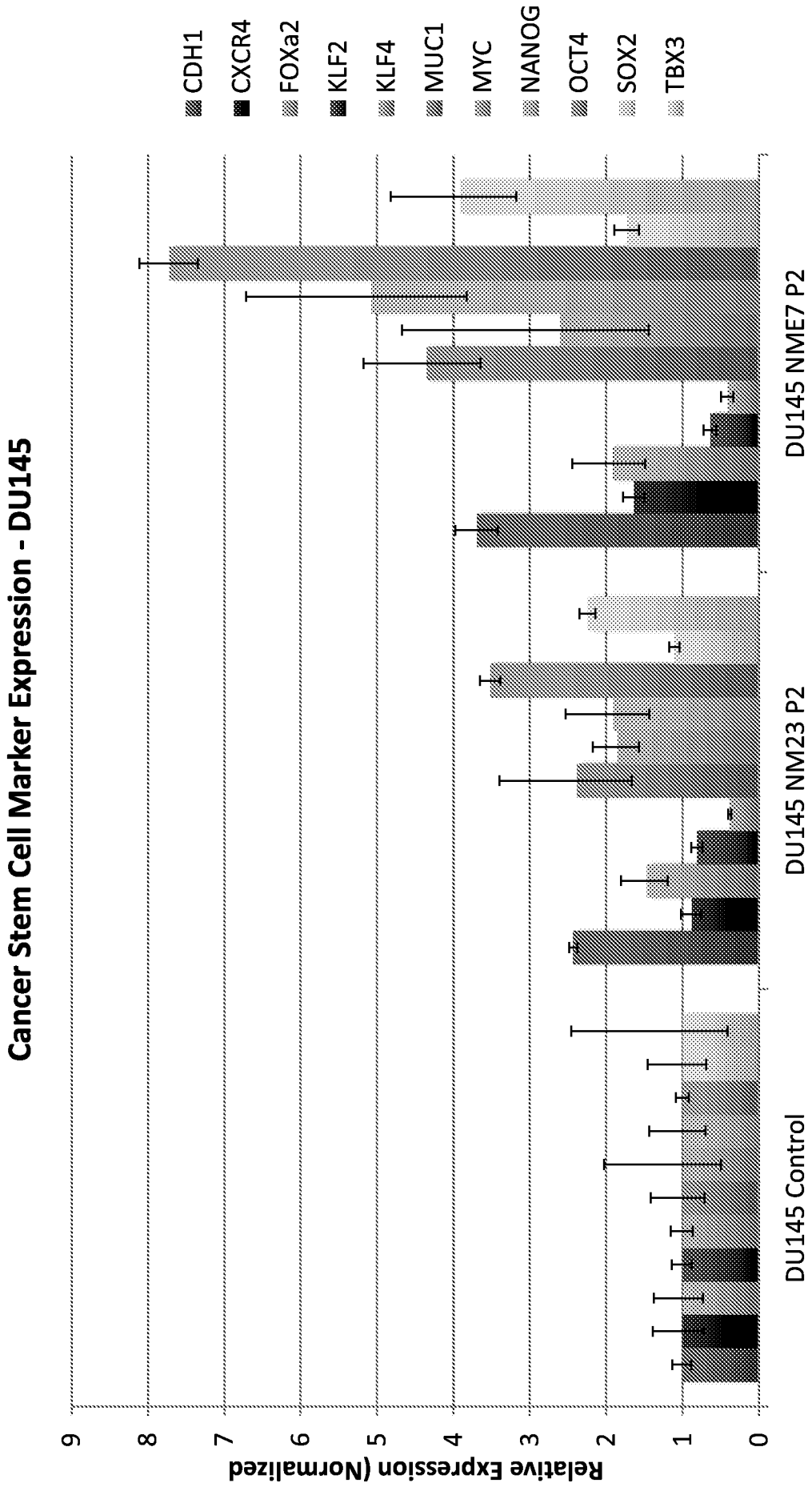

Cancer Stem Cell Marker Expression - DU145
Relative Expression (Normalized
0
1
2
3
4
5
6
7
8
9
CDH1
CXCR4
FOXa2
KLF2
KLF4
MUC1
MYC
NANOG
OCT4
SOX2
TBX3
DU145 Control
DU145 NM23 P2
DU145 NME7 P2

Fig. 13

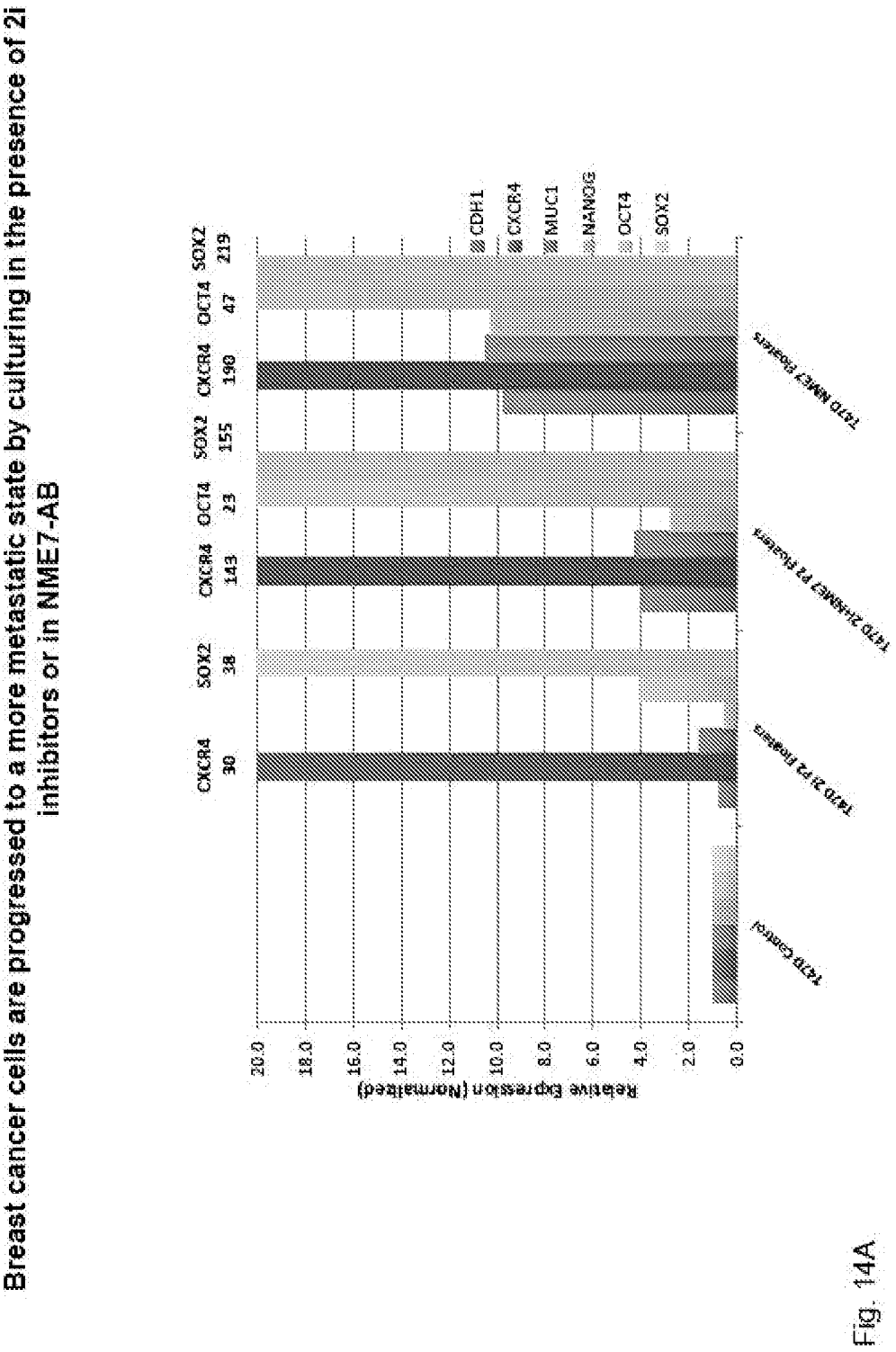

Breast cancer cells are progressed to a more metastatic state by culturing in the presence of 2i inhibitors or in NME7-AB
Relative Expression (Normalized)
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
CDH1
CXCR4
MUC1
NANOG
OCT4
SOX2
CXCR4 30
SOX2 38
CXCR4 143
OCT4 23
SOX2 155
CXCR4 190
OCT4 47
SOX2 219
T47D Control
T47D 2i 72 Hours
T47D 2i+NME7 72 Hours
T47D NME7 Isolates

Fig. 14A

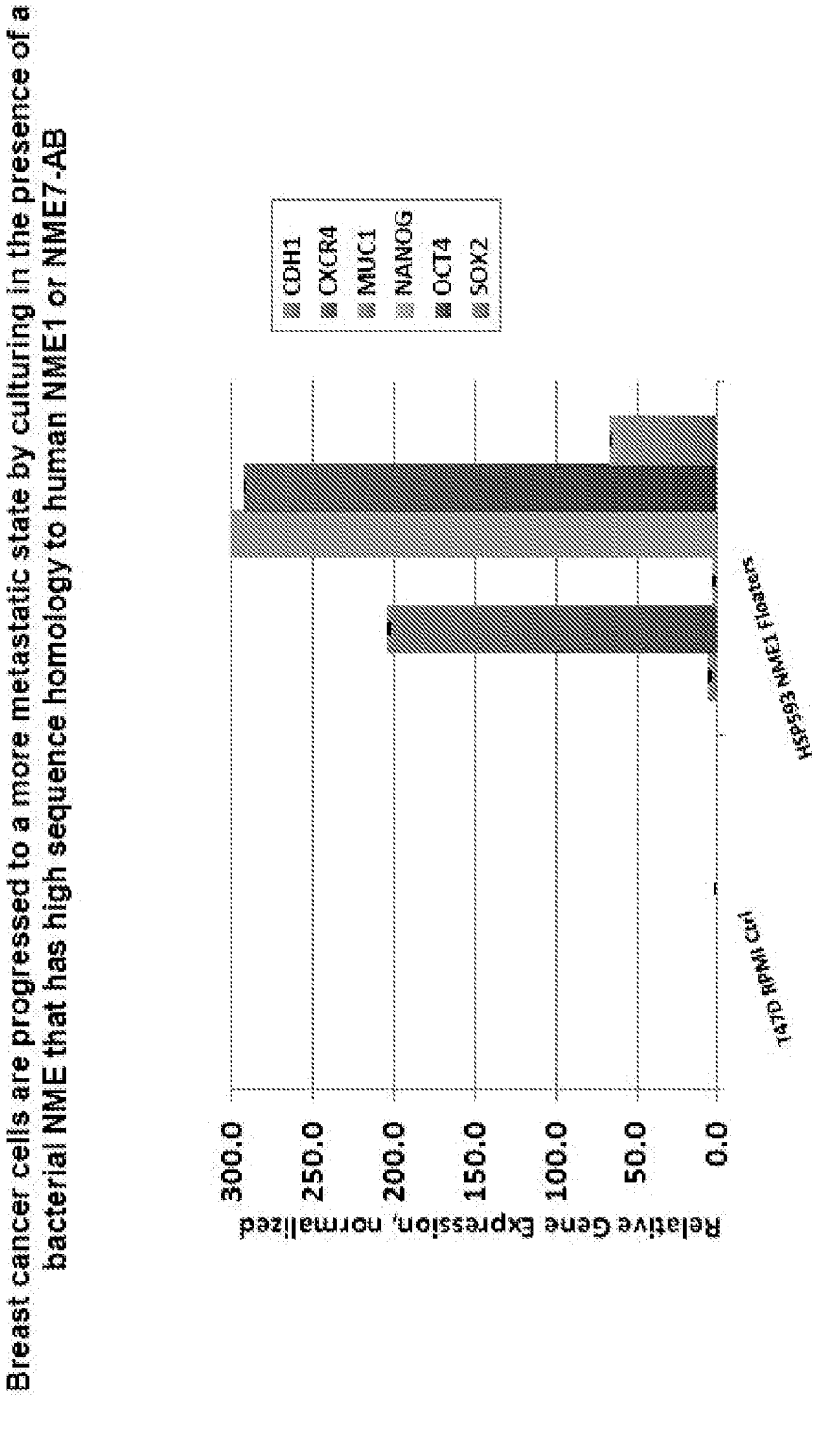

Breast cancer cells are progressed to a more metastatic state by culturing in the presence of a bacterial NME that has high sequence homology to human NME1 or NME7-AB
CDH1
CXCR4
MUC1
NANOG
OCT4
SOX2
Relative Gene Expression, normalized
300.0
250.0
200.0
150.0
100.0
50.0
0.0
T47D RPMI Ctrl
HSP593 NME1 Floaters

Fig. 14B

Fig. 15

Sequence alignment of human NME1 to human NME7-A and –B domains

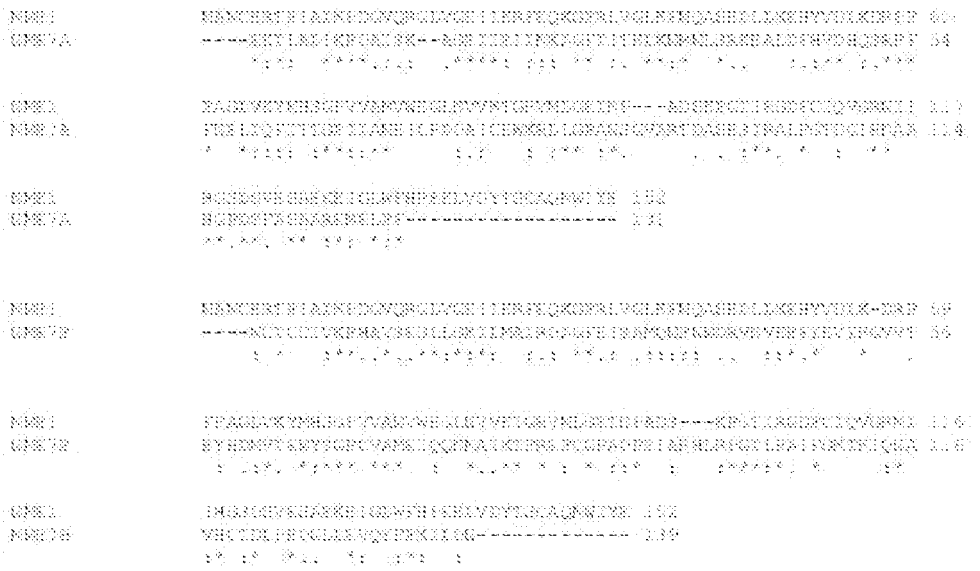


Fig. 16

NME7 specific peptides for generating antibodies to inhibit NME7 for the treatment or prevention of cancers.

The following peptide sequences are identified as being immunogenic peptides giving rise to antibodies that target human NME7 but not human NME1. The sequences were chosen for their lack of sequence homology to human NME1.

1. LALIKPDA
2. MMMLSRKEALDFHVDHQS
3. ALDFHVDHQS
4. EILRDDAICEWKRL
5. FNELIQFITTGP
6. RDDAICEW
7. SGVARTDASESIRALFGTDGIRNAA
8. ELFFPSSGG
9. KFTNCTCCIVKPHAVSEGLLGKILMA
10. LMAIRDAGFEISAMQMFNMDRVNVEEFYEVYKGVVT
11. EFYEVYKGVVTEYHD
12. EIQQNNATKTFREFCGPADPEIARHLRPGTLRAIFGKTKIQNA
13. YSGPCVAM
14. FREFCGP
15. VHCTDLPEDGLLEVQYFFKILDN
16. IQNAVHCTD
17. TDLPEDGLLEVQYFFKILDN
18. PEDGLLEVQYFFK
19. EIINKAGFTITK
20. MLSRKEALDFHVDHQS
21. NELIQFITT
22. EILRDDAICEWKRL
23. SGVARTDASESIRALFGTDGI
24. SGVARTDASES

Fig. 16 (Continued)

25. ALFGTDGI

26. NCTCCIVKPHAVSE

27. LGKILMAIRDA

28. EISAMQMFNMDRVNVE

29. EVYKGVVT

30. EYHDMVTE

31. EFCGPADPEIARHLR

G T N

Fig. 17

NME7 specific peptides for generating antibodies to inhibit NME7 for the treatment or prevention of cancers.

The following are preferred as they are likely areas that are important for structural integrity or for binding to the MUC1* peptide. Bivalent antibodies wherein each variable region would bind to each one of a pair are preferred.

35. ICEWKRL
36. LGKILMAIRDA

37. HAVSEGLLGK
38. VTEMYSGP

39. NATKTFREF
40. AIRDAGFEI

41. AICEWKRLLGPAN
42. DHQSRPFF

43. AICEWKRLLGPAN
44. VDHQSRPF
45. PDSFAS
46. KAGEIIEIINKAGFTITK

Fig. 18

The following peptide sequences are from human NME1 and were selected for their high homology to human NME7 as well as for their homology to other bacterial NME proteins that are able to mimic its function.

47. MANCERTFIAIKPDGVQRGLVGEIIKRFE
48. VDLKDRPF
49. HGSDSVESAEKEIGLWF

Especially preferred for their high homology to human NME7-A or -B and also to HSP 593 are:

50. ERTFIAIKPDGVQRGLVGEIIKRFE
51. VDLKDRPFFAGLVKYMHSGPVVAMVWEGLN
52. NIIHGSDSVESAEKEIGLWFHPEELV
53. KPDGVQRGLVGEII

Fig. 19

NME7-AB specific peptides preferred for generating antibodies for the treatment or prevention of cancer.

NME7A peptide 1
MLSRKEALDFHVDHQSC

NME7A peptide 2
SGVARTDASESC

NME7B peptide 1
DAGFEISAMQMFNMDRVNVEC

NME7B peptide 2
EVYKGVVTEYHDMVTEC

NME7B peptide 3
AIFGKTKIQNAVHCTDLPEDGLLEVQYFFC

The Test of Whether or not a Cancer Cell is Metastatic or a 'Cancer Stem Cell' is if 200 or less are able to Form a Tumor in a test Animal; Regular Cancer Cells Require 6M or more.
As Few as 50 NME-p-Induced Metastatic Cancer Cells are Sufficient to Form Tumors in test Animals. Animals that were also injected Daily with Human NME-p Developed Metastatic Cancer rather than just Localized Tumors

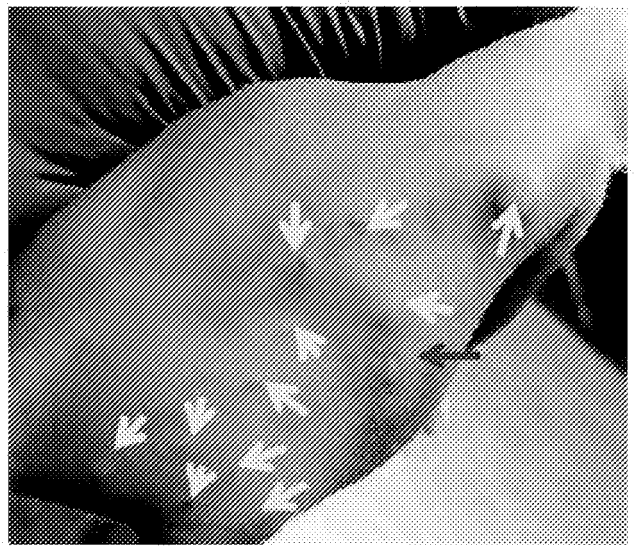


Figure 20

MOUSE #	# CELLS IMPLANTED	cMET	TUMOR	METS	SIZE
1	50	Y	Y	Y	M
2	50	N	Y	Y	S
3	50	Y	Y	Y	M
4	50	N	Y	N	M
5	50	Y	Y	Y	M
6	50	N	N	N	No visible tumor
7	100	Y	Y	Y	S
8	100	N	N	N	N
9	100	Y	Y	Y	S
10	100	N	N	N	No visible tumor
11	100	Y	Y	Y	S
12	1,000	Y	Y	Y	S/M
13	1,000	N	Y	N	S/M
14	1,000	Y	N	N	No visible tumor
15	1,000	N	Y	N	L
16	1,000	Y	Y	N	No visible tumor
17	1,000	N	N	N	No visible tumor
18	10,000	Y	Y	Y	S
19	10,000	N	Y	N	S
20	10,000	Y	Y	Y	L
21	10,000	N	Y	N	L
22	10,000	Y	Y	N	L

Figure 21

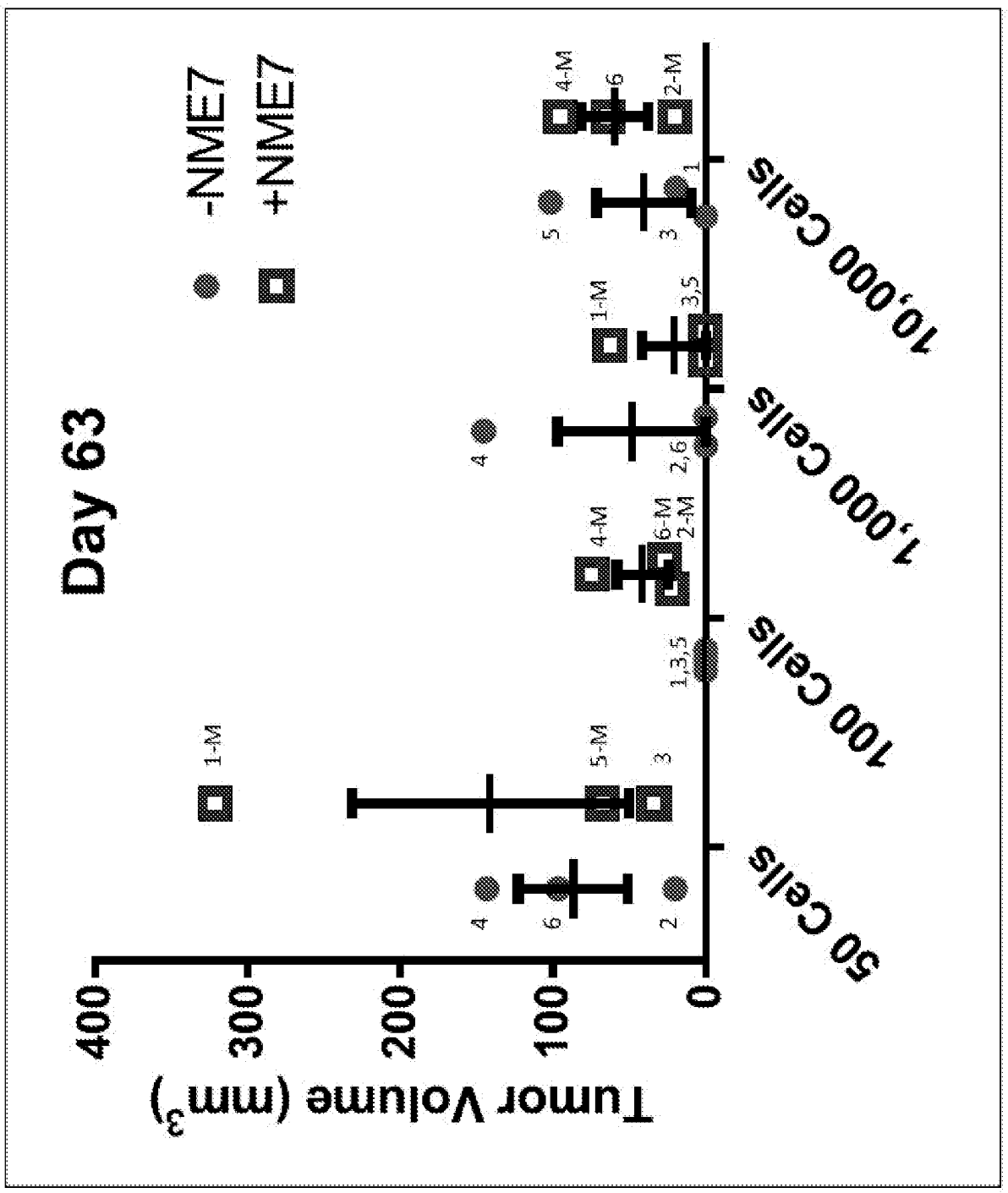

Day 63
-NME7
+NME7
Tumor Volume (mm³)
400
300
200
100
0
50 Cells
100 Cells
1,000 Cells
10,000 Cells
1-M
5-M
4-M
6-M
2-M
1,3,5
2,6
3,5

Fig. 22A

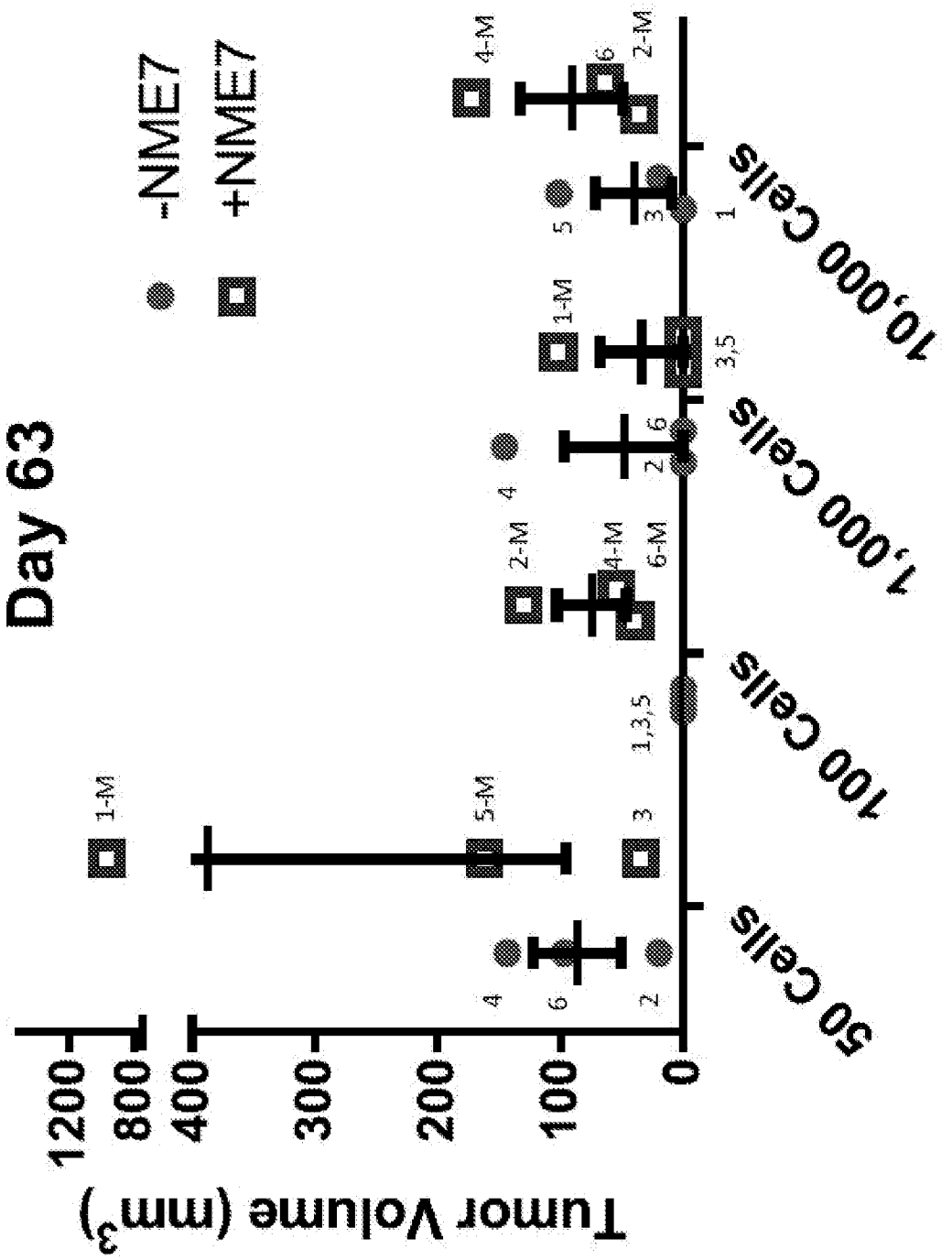
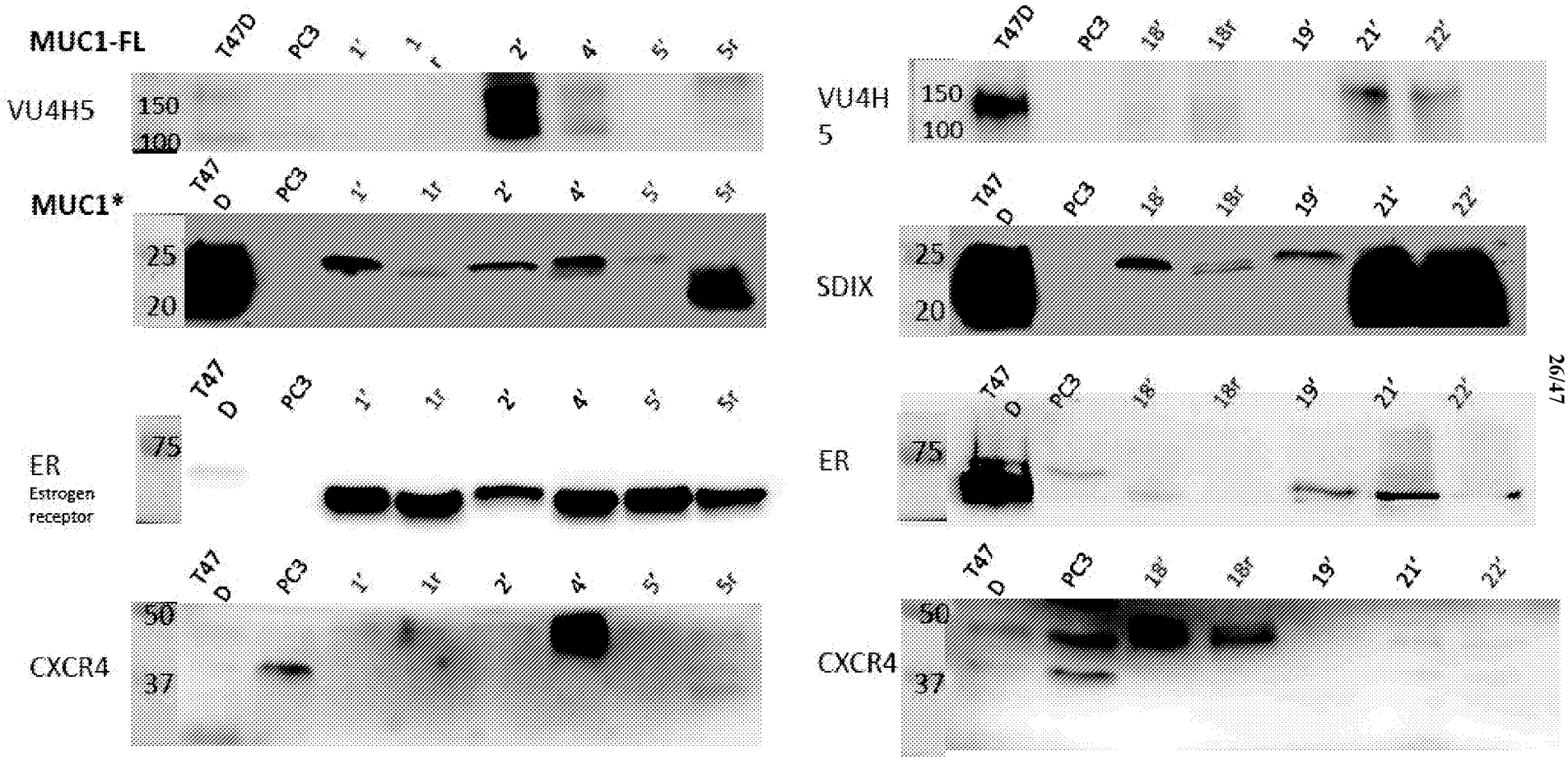

Day 63
-NME7
+NME7
Tumor Volume (mm³)
1200
800
400
300
200
100
0
50 Cells
100 Cells
1,000 Cells
10,000 Cells
1-M
5-M
2-M
4-M
6-M
1,3,5
3,5

Fig. 22B

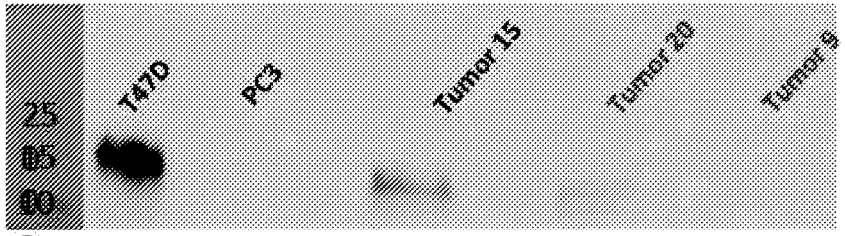
Tumor Analysis: Off-Injection-Site 'Bumps' are Human Breast Tumors



KEY
Number is Mouse #
' = primary tumor
r = remote tumor
RED = injected daily with NME7-AB

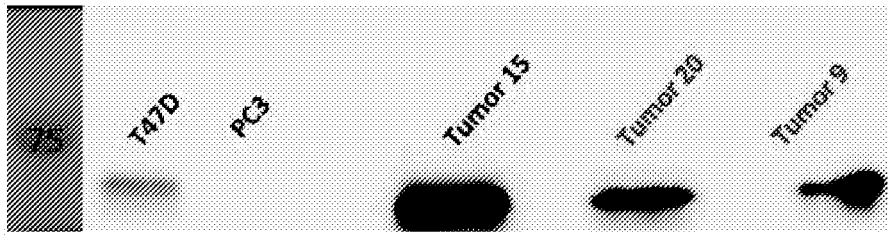
KEY MOUSE # AND NUMBER OF CANCER CELLS ENGRAFTED
50 cells: MICE # 1-6
100 cells: MICE # 7-11
1,000 cells: MICE # 12-17
10,000 cells: MICE # 18-22

Fig. 23

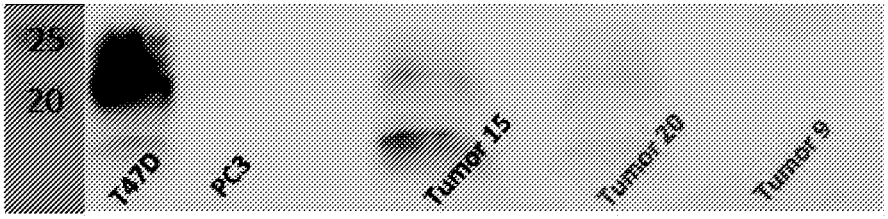


0

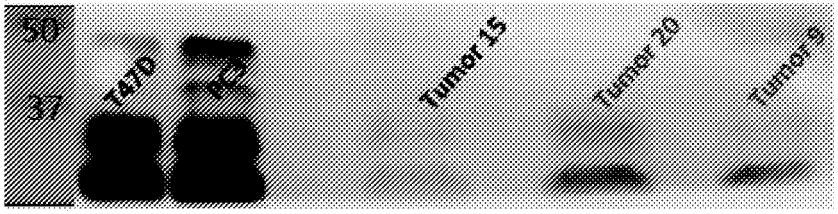
MUC1 FULL LENGTH



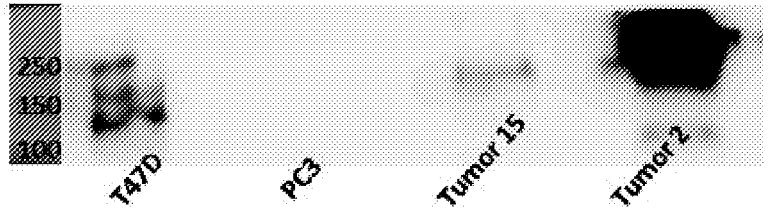
ESTROGEN RECEPTOR



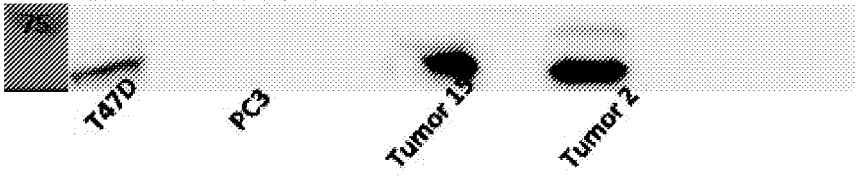
MUC1*

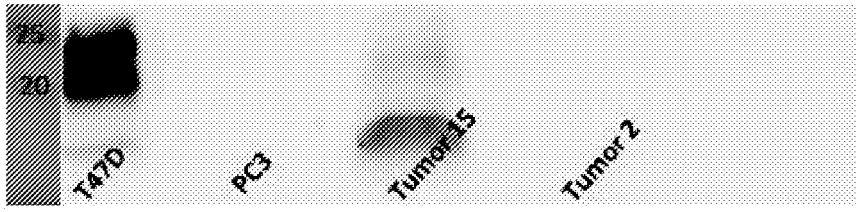


CXCR4



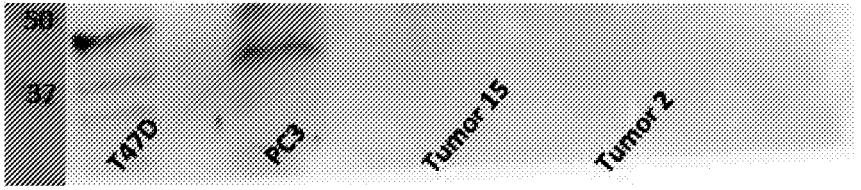
ESTROGEN RECEPTOR





MUC1*

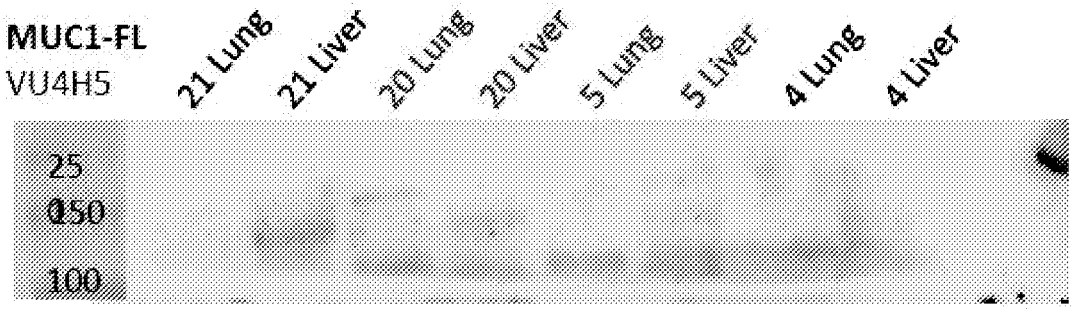
CXCR4



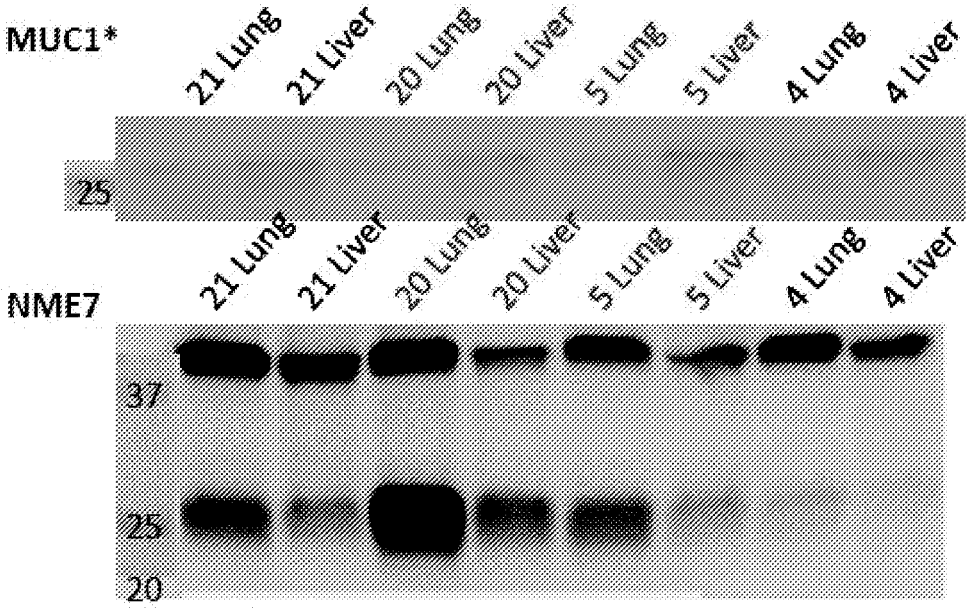
KEY
Number is Mouse #
' = primary tumor
r = remote tumor
RED = injected daily with NME7-AB

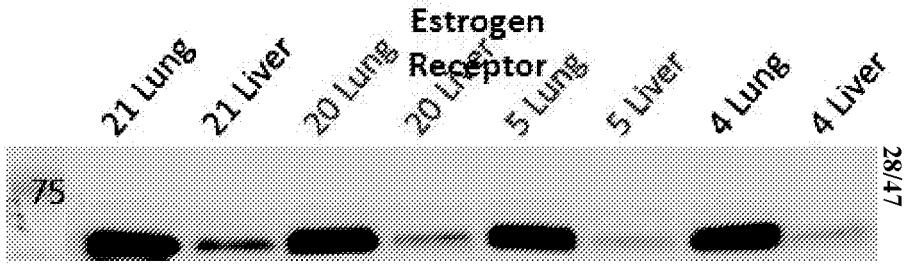
KEY MOUSE # AND NUMBER OF CANCER CELLS ENGRAFTED
50 cells: MICE # 1-6
100 cells: MICE # 7-11
1,000 cells: MICE # 12-17
10,000 cells: MICE # 18-22

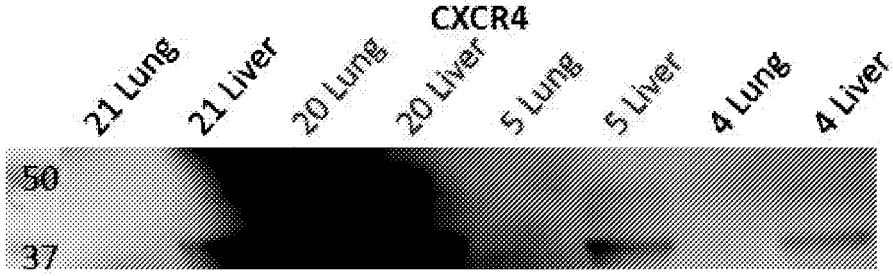
Fig. 24



Mouse#	+NME7	Mets	Size Tumor'
21	NO	NO	L
20	YES	YES	L
5	YES	YES	S
4	NO	NO	S







KEY
Number is Mouse #
' = primary tumor
r = remote tumor
RED = injected daily with NME7-AB

KEY MOUSE # AND NUMBER OF CANCER CELLS ENGRAFTED
50 cells: MICE # 1-6
100 cells: MICE # 7-11
1,000 cells: MICE # 12-17
10,000 cells: MICE # 18-22

Fig. 25

PCT/US2015/024764

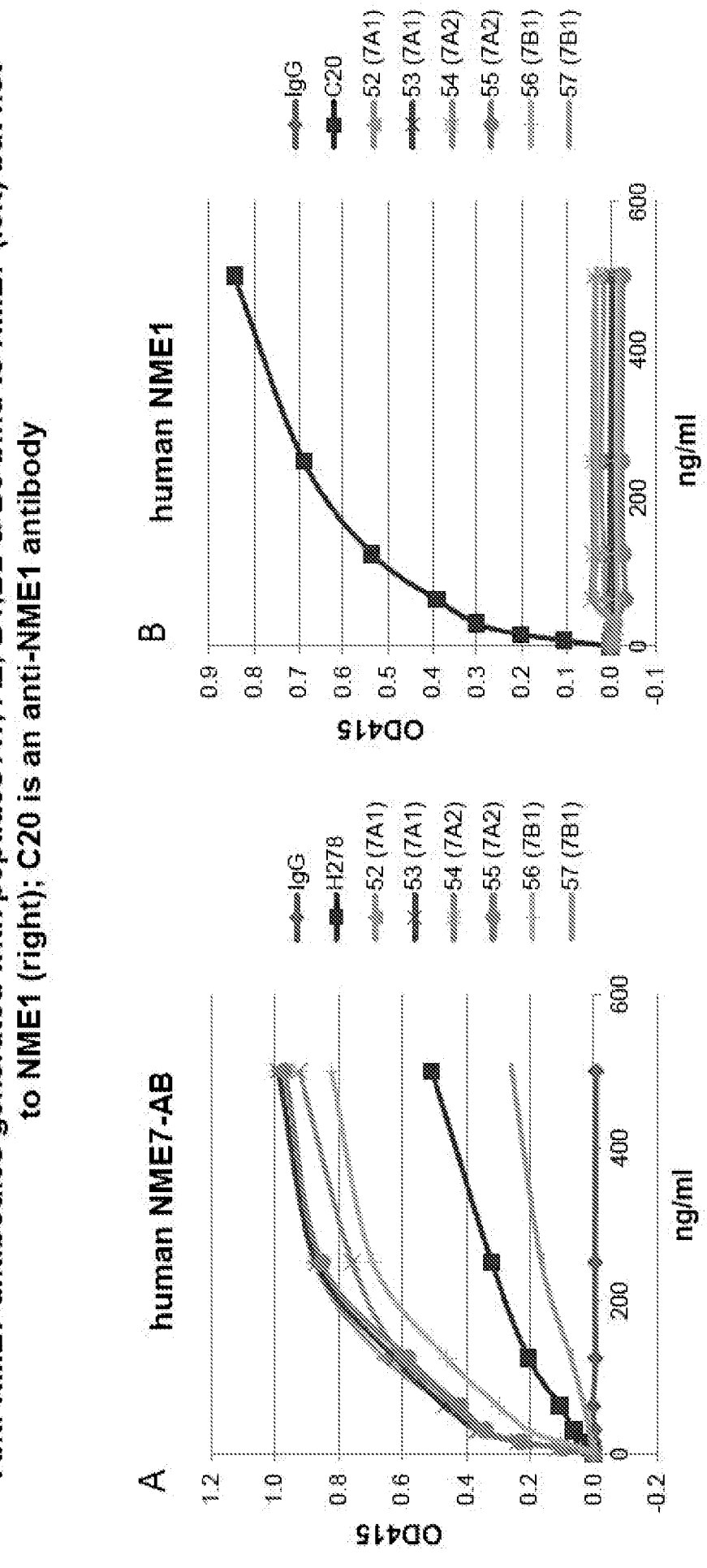

Anti-NME7 antibodies generated with peptides A1, A2, B1,B2 & B3 bind to NME7 (left) but not to NME1 (right); C20 is an anti-NME1 antibody
A
human NME7-AB
OD415
ng/ml
IgG
H278
52 (7A1)
53 (7A1)
54 (7A2)
55 (7A2)
56 (7B1)
57 (7B1)
B
human NME1
OD415
ng/ml
IgG
C20
52 (7A1)
53 (7A1)
54 (7A2)
55 (7A2)
56 (7B1)
57 (7B1)

Fig. 26

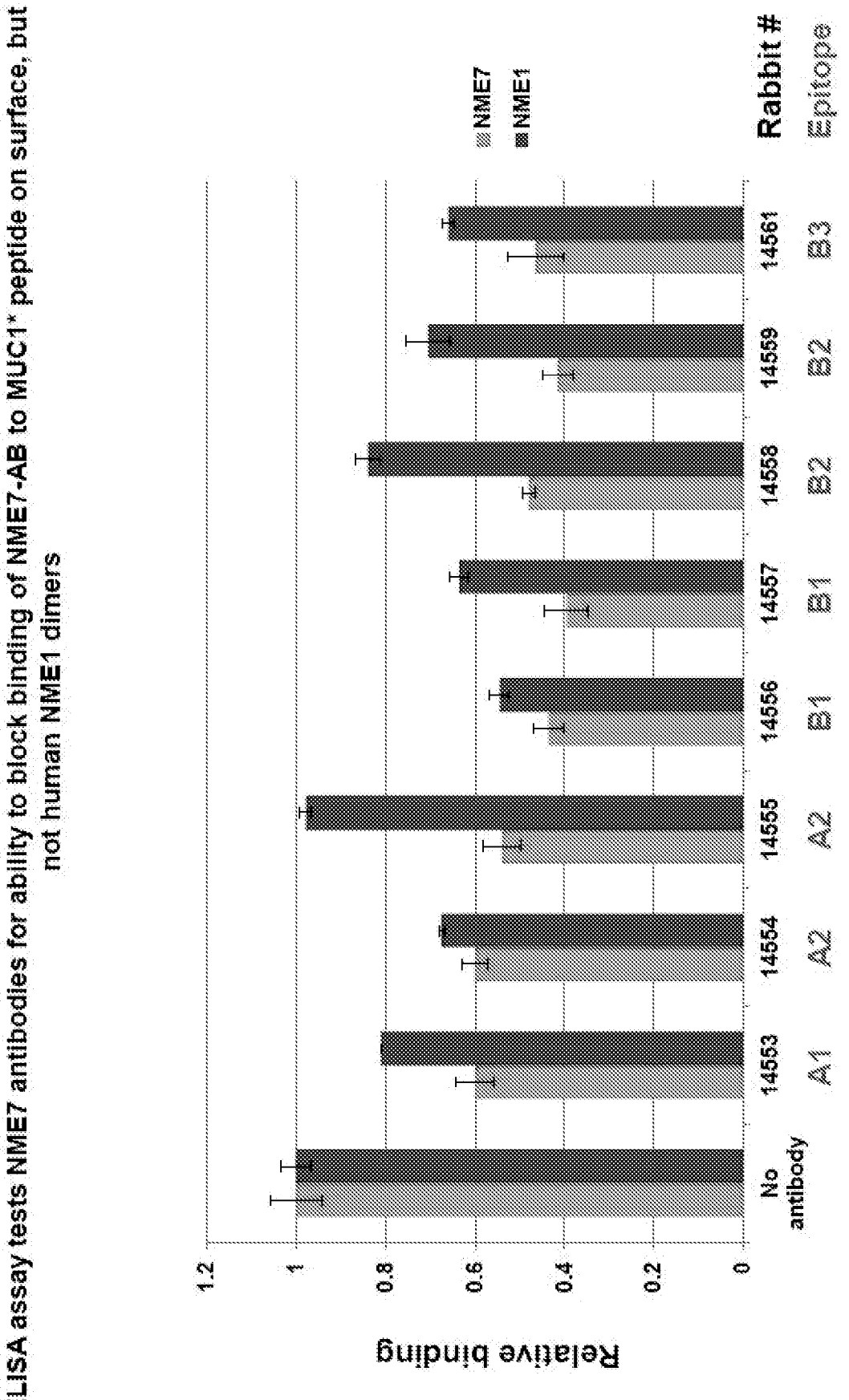

ELISA assay tests NME7 antibodies for ability to block binding of NME7-AB to MUC1* peptide on surface, but not human NME1 dimers
Relative binding
1.2
1
0.8
0.6
0.4
0.2
0
NME7
NME1
Rabbit #
Epitope
No antibody
14553
A1
14554
A2
14555
A2
14556
B1
14557
B1
14558
B2
14559
B2
14561
B3
Antibody Source

Fig. 27

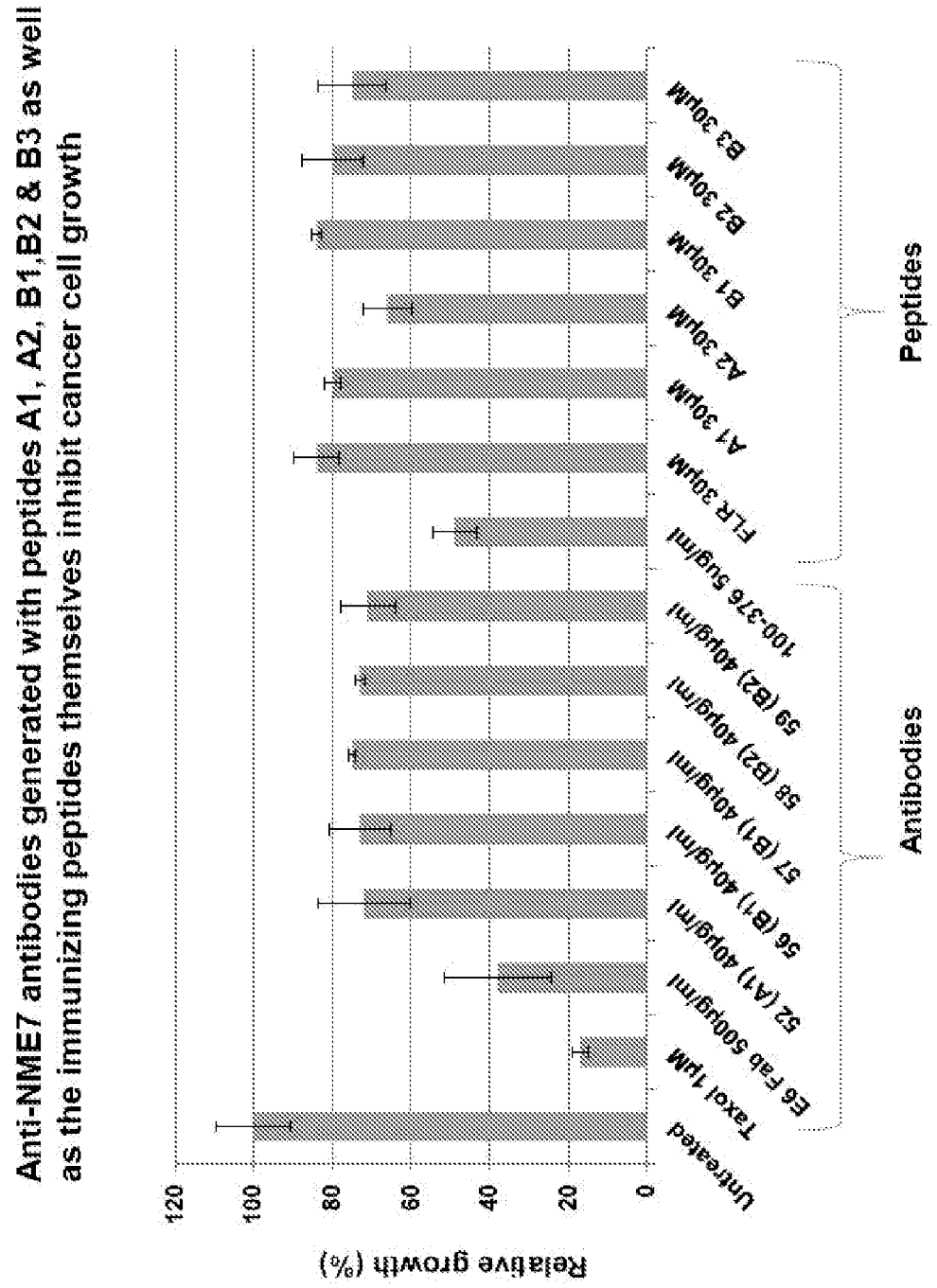

Anti-NME7 antibodies generated with peptides A1, A2, B1,B2 & B3 as well as the immunizing peptides themselves inhibit cancer cell growth
Relative growth (%)
120
100
80
60
40
20
0
Untreated
Taxol 1μM
E6 Fab 500μg/ml
52 (A1) 40μg/ml
56 (B1) 40μg/ml
57 (B1) 40μg/ml
58 (B2) 40μg/ml
59 (B2) 40μg/ml
100-376 5ug/ml
FLR 30μM
A1 30μM
A2 30μM
B1 30μM
B2 30μM
B3 30μM
Antibodies
Peptides

Fig. 28

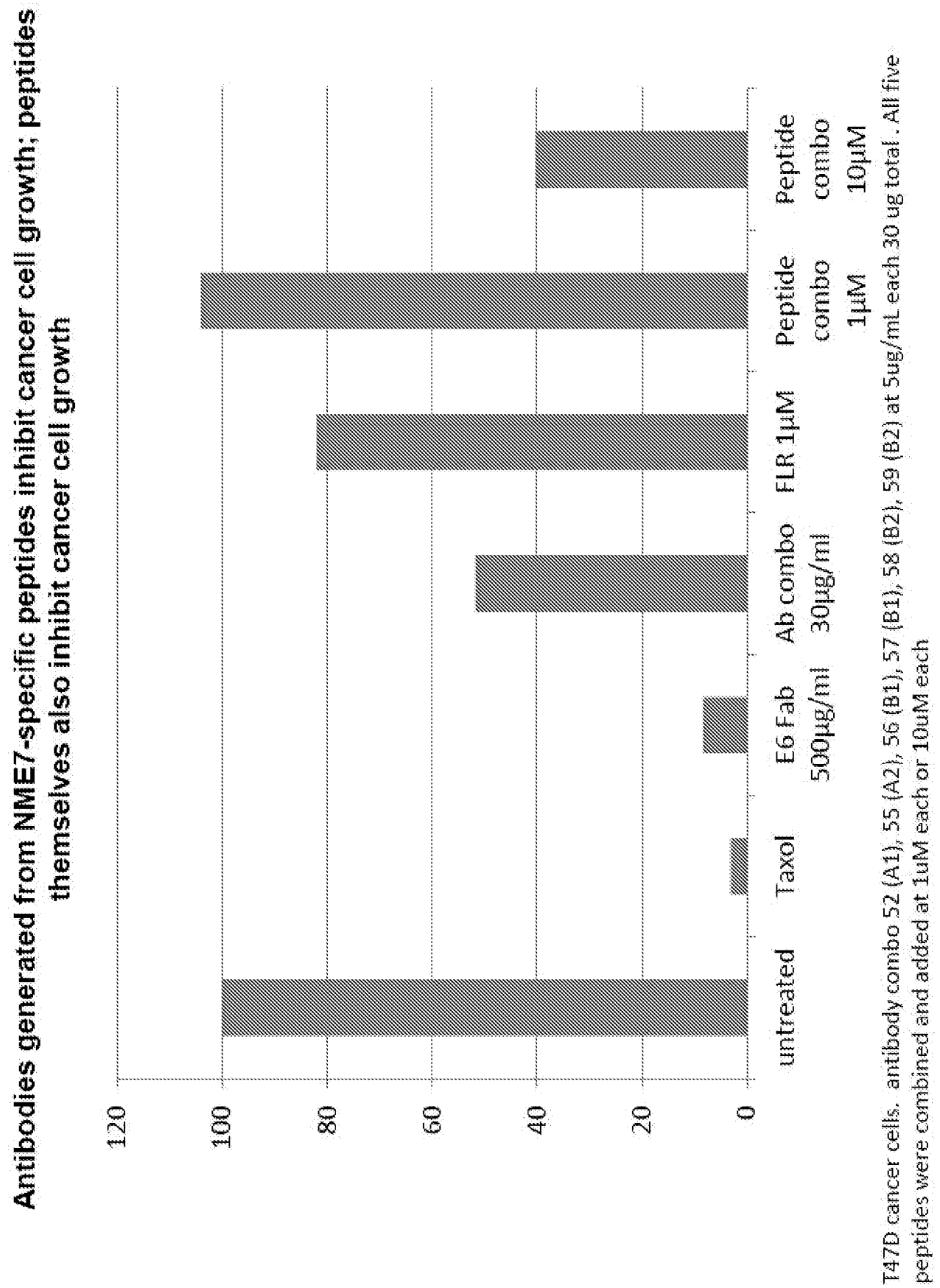

Antibodies generated from NME7-specific peptides inhibit cancer cell growth; peptides themselves also inhibit cancer cell growth
120
100
80
60
40
20
0
untreated
Taxol
E6 Fab 500µg/ml
Ab combo 30µg/ml
FLR 1µM
Peptide combo 1µM
Peptide combo 10µM
T47D cancer cells. antibody combo 52 (A1), 55 (A2), 56 (B1), 57 (B1), 58 (B2), 59 (B2) at 5ug/mL each 30 ug total . All five peptides were combined and added at 1uM each or 10uM each

Fig. 29

Treating cancer cells with anti-NME7 antibodies inhibits transition to "floater" cells, which PCR shows have greatly increased expression of metastatic markers such as CXCR4; xenograft experiments show that the floater cells form a tumor at extremely low copy number – 50 – and thus fulfill the requirement for being classified cancer stem cells or metastatic cancer cells.

Antibodies	Floater observation
Control IgG	100%
53,55,57 (A1,A2,B1)	70%
53,57 (A1,B1)	50%
61 (B3)	5%

JR observations

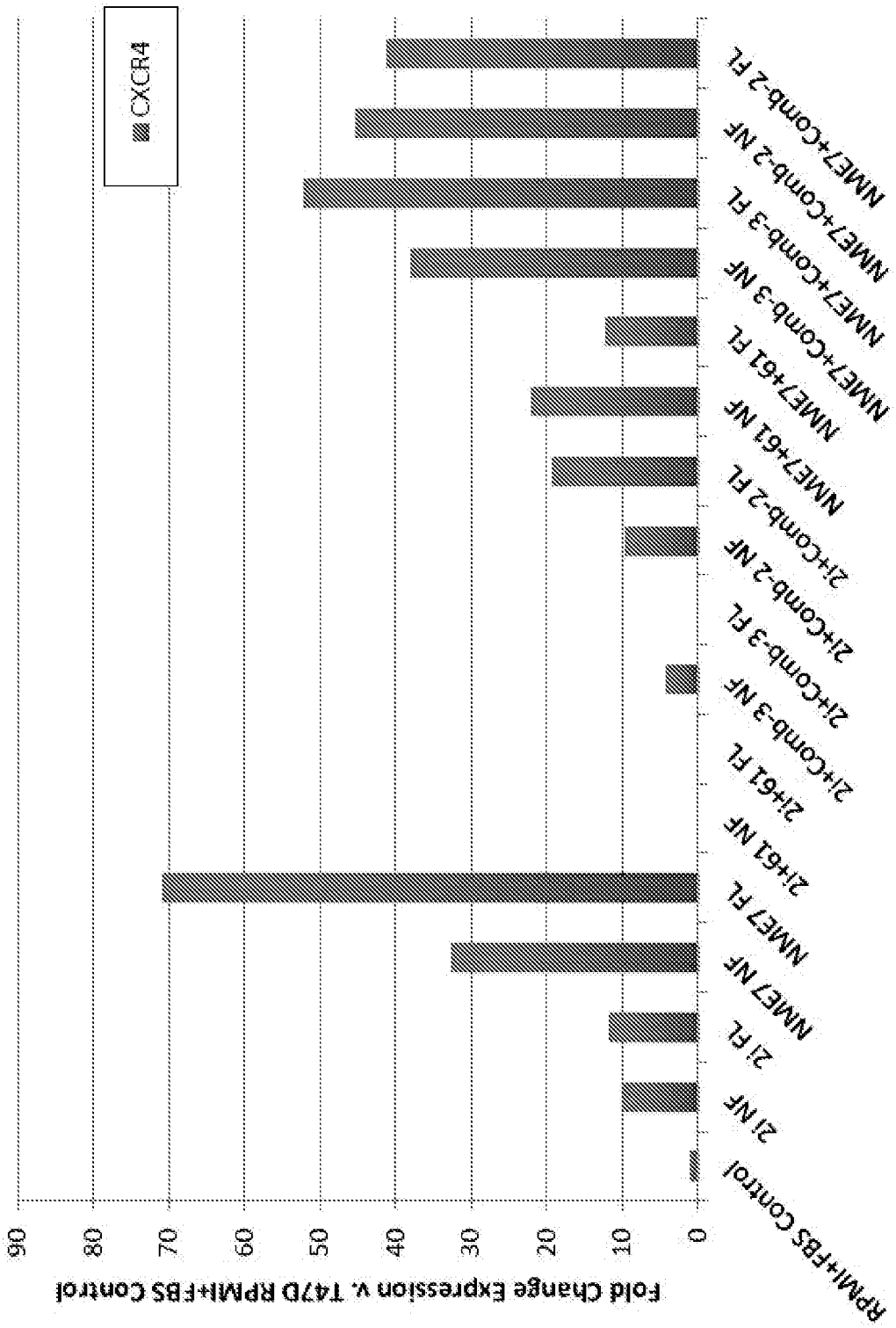
Antibodies	Floater observation
Control IgG	100%
53,55,57 (A1,A2,B1)	65%
53,57 (A1,B1)	40%
61 (B3)	5%

VH observations

The number of "Floater" cells, which are the ones that have higher expression of metastatic markers and that form tumors in animals at extremely low copy number is typically 20% of the amount of plated cells by Day 7. Here, we define 100% as the number of floater cells that results when a control antibody is added. Other percentages of floater cells is relative to the control in which a control IgG antibody was added.

Fig. 30

Fig. 31A



T47D breast cancer cells. 2i are MEK and GSK3-beta inhibitors. NF=non-floaters; FL=floaters. 61= rabbit #61 immunized with B3 peptide derived from NME7. Comb-2 = combination of antibodies from rabbits immunized with A1 and A2. Comb-3 = combination of antibodies from rabbits immunized with peptides A1, A2 and B1.

Fig. 31B

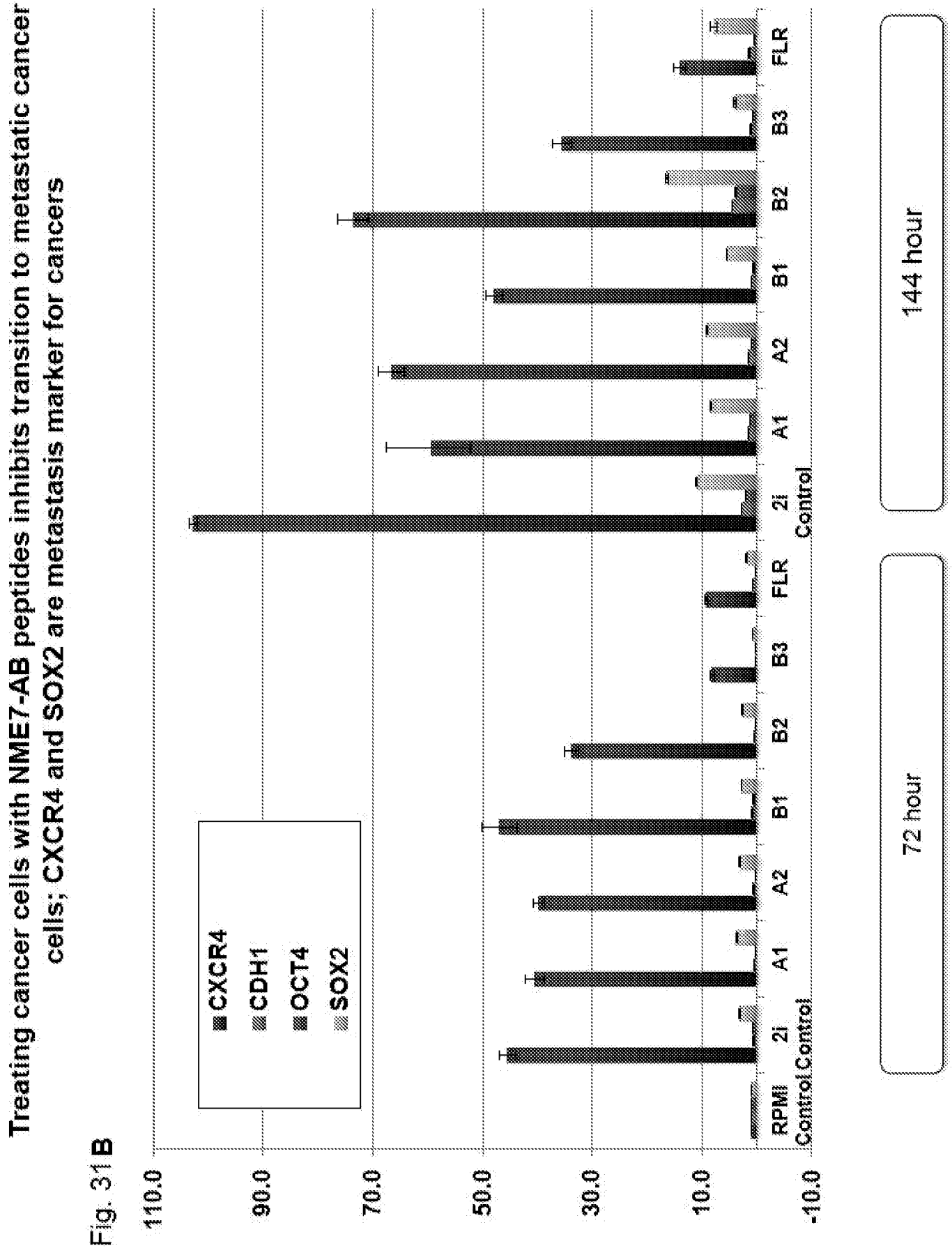
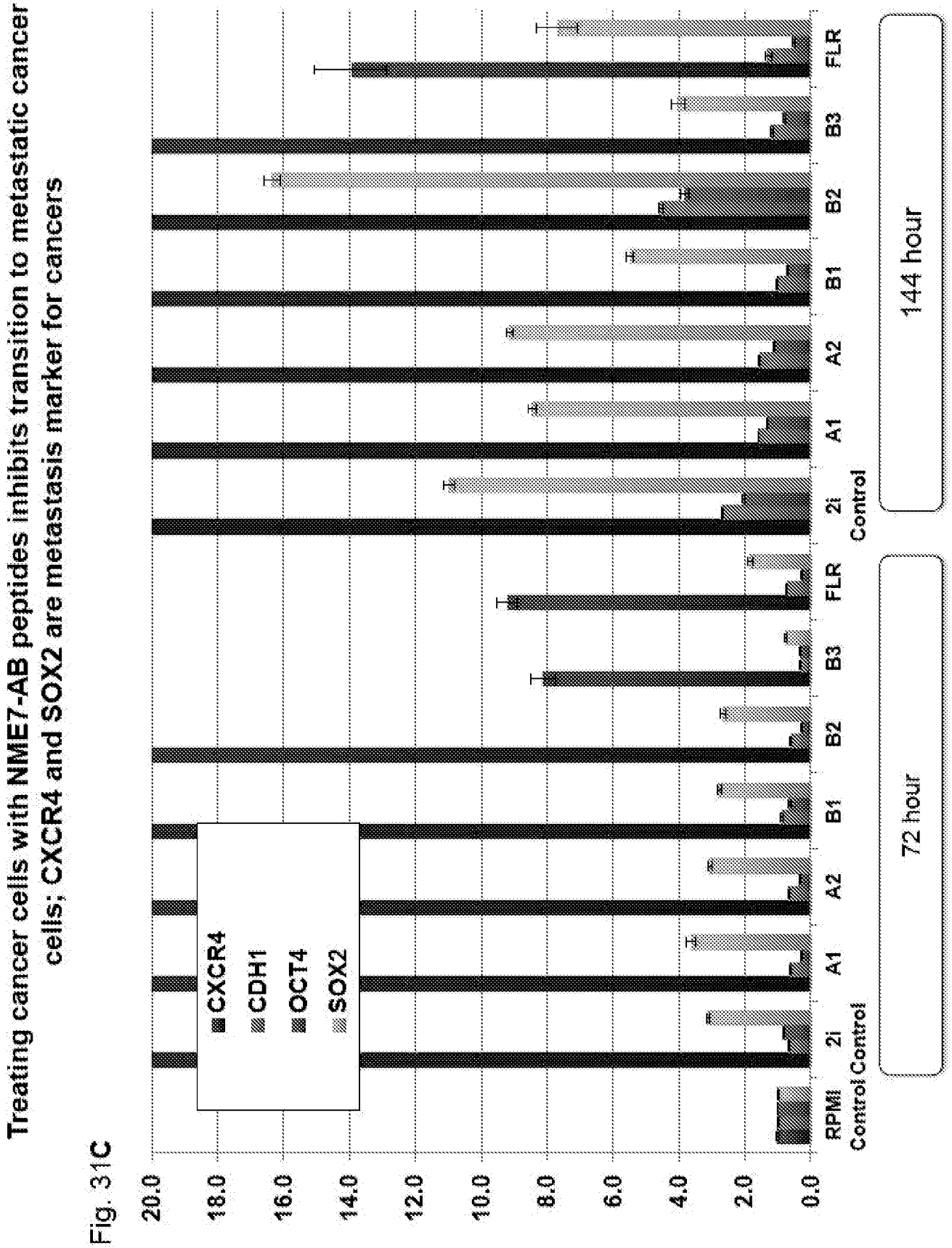

Treating cancer cells with NME7-AB peptides inhibits transition to metastatic cancer cells; CXCR4 and SOX2 are metastasis marker for cancers
CXCR4
CDH1
OCT4
SOX2
110.0
90.0
70.0
50.0
30.0
10.0
-10.0
RPMI
Control
Control
2i
A1
A2
B1
B2
B3
FLR
2i
A1
A2
B1
B2
B3
FLR
Control
72 hour
144 hour

Fig. 31C


Treating cancer cells with NME7-AB peptides inhibits transition to metastatic cancer cells; CXCR4 and SOX2 are metastasis marker for cancers
CXCR4
CDH1
OCT4
SOX2
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
RPMI Control
2i Control
A1
A2
B1
B2
B3
FLR
2i
A1
A2
B1
B2
B3
FLR
Control
72 hour
144 hour

	Cell Line T47D	Medium	Total RNA ng/µL	Yield (µg)	EEF1A1 Threshold Cycle
CONTROLS	Control	RPMI + 10% FBS	395.8	47.49	15.2
	2i attached	MM + 2i	387.8	11.64	14.5
	2i Floaters	MM + 2i	234.6	7.04	14.9
	NME7 attached	MM+NME7 4nM	334.8	10.04	14.7
	NME7 Floaters	MM+NME7 4nM	259.3	7.78	16.0
	2i+ Antibody B3 rabbit 61 attached	MM + 2i	2.7	0.08	25.9
	2i+ Antibody B3 rabbit 61 Floaters	MM + 2i	3.6	0.11	25.0
	2i + Antibody Combination 3 attached	MM + 2i	44.7	1.34	17.0
	2i+ Antibody Combination 3 Floaters	MM + 2i	39.0	1.17	15.9
	2i+ Antibody Combination 2 attached	MM + 2i	46.0	1.38	15.6
	2i+ Antibody Combination 2 Floaters	MM + 2i	77.8	2.33	16.3
	NME7+ Antibody B3 rabbit 61 attached	MM+NME7 4nM	65.7	1.97	17.0
	NME7+ Antibody B3 rabbit 61 Floaters	MM+NME7 4nM	15.8	0.47	19.9
	NME7+ Antibody Combination 3 attached	MM+NME7 4nM	32.1	0.96	17.0
	NME7+ Antibody Combination 3 Floaters	MM+NME7 4nM	109.3	3.28	16.1
	NME7+ Antibody Combination 2 attached	MM+NME7 4nM	134.5	4.03	16.1
	NME7+ Antibody Combination 2 Floaters	MM+NME7 4nM	139.5	4.19	18.6

Figure 32

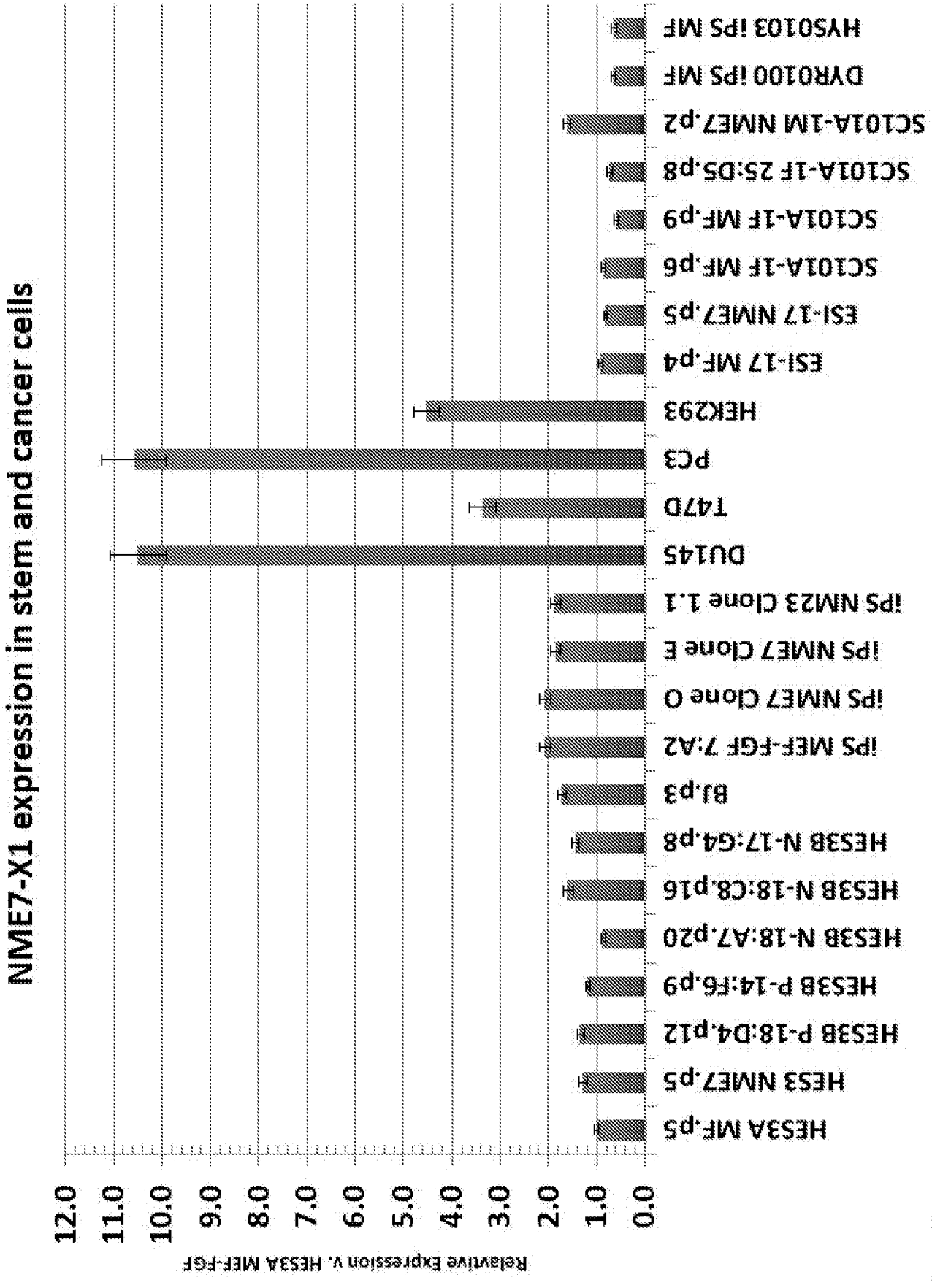

NME7-X1 expression in stem and cancer cells
Relative Expression v. HES3A MEF-FGF
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
HES3A MF.p5
HES3 NME7.p5
HES3B P-18:D4.p12
HES3B P-14:F6.p9
HES3B N-18:A7.p20
HES3B N-18:C8.p16
HES3B N-17:G4.p8
BJ.p3
iPS MEF-FGF 7:A2
iPS NME7 Clone O
iPS NME7 Clone E
iPS NM23 Clone 1.1
DU145
T47D
PC3
HEK293
ESI-17 MF.p4
ESI-17 NME7.p5
SC101A-1F MF.p6
SC101A-1F MF.p9
SC101A-1F 25:D5.p8
SC101A-1M NME7.p2
DYR0100 iPS MF
HYS0103 iPS MF

Figure 33

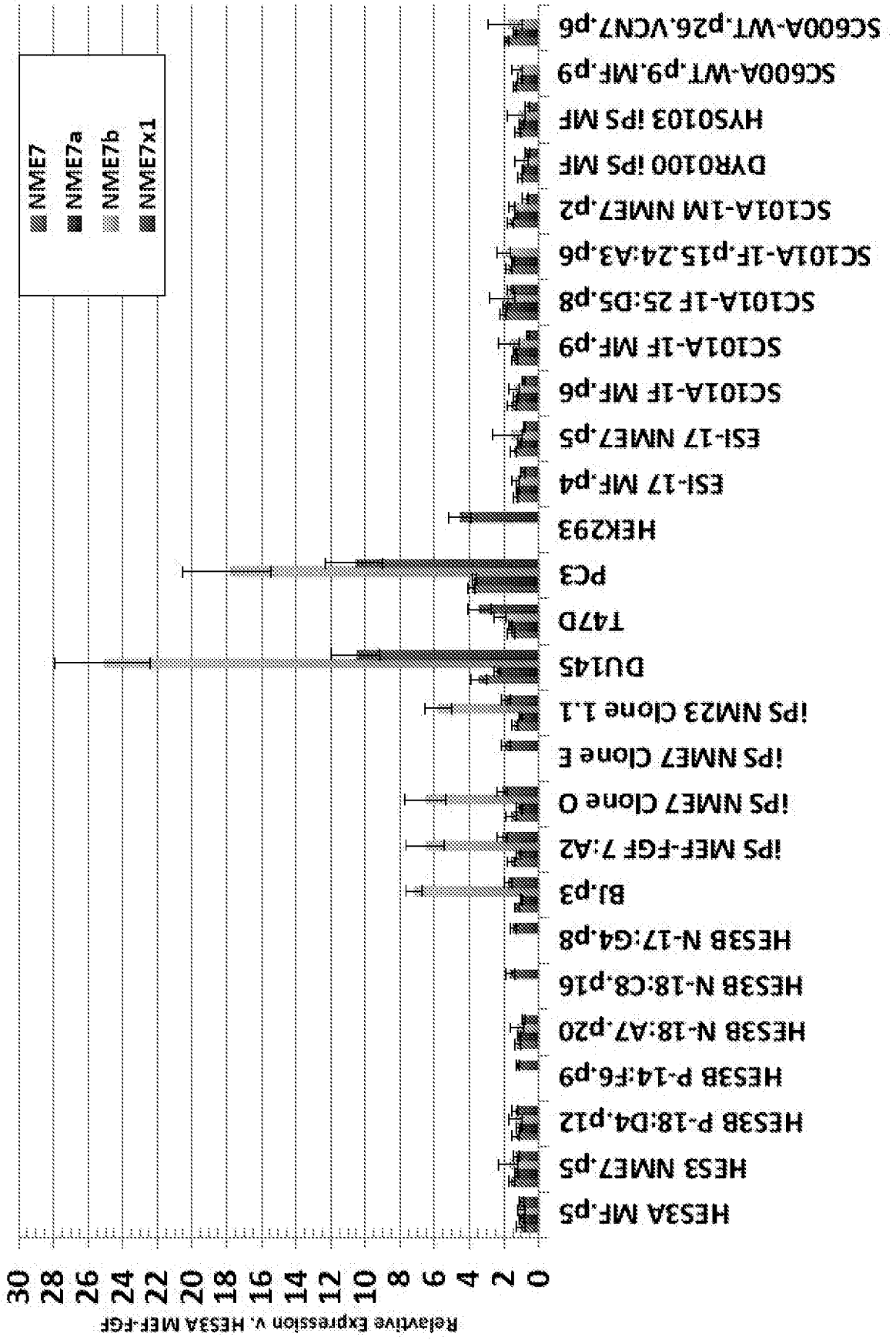

NME7 isoform expression in stem cells and cancer cells
NME7
NME7a
NME7b
NME7x1
Relative Expression v. HES3A MEF-FGF
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
HES3A MF.p5
HES3 NME7.p5
HES3B P-18:D4.p12
HES3B P-14:F6.p9
HES3B N-18:A7.p20
HES3B N-18:C8.p16
HES3B N-17:G4.p8
BJ.p3
iPS MEF-FGF 7:A2
iPS NME7 Clone O
iPS NME7 Clone E
iPS NM23 Clone 1.1
DU145
T47D
PC3
HEK293
ESI-17 MF.p4
ESI-17 NME7.p5
SC101A-1F MF.p6
SC101A-1F MF.p9
SC101A-1F 25:D5.p8
SC101A-1F.p15.24:A3.p6
SC101A-1M NME7.p2
DYR0100 iPS MF
HYS0103 iPS MF
SC600A-WT.p9.MF.p9
SC600A-WT.p26.VCN7.p6

Figure 34

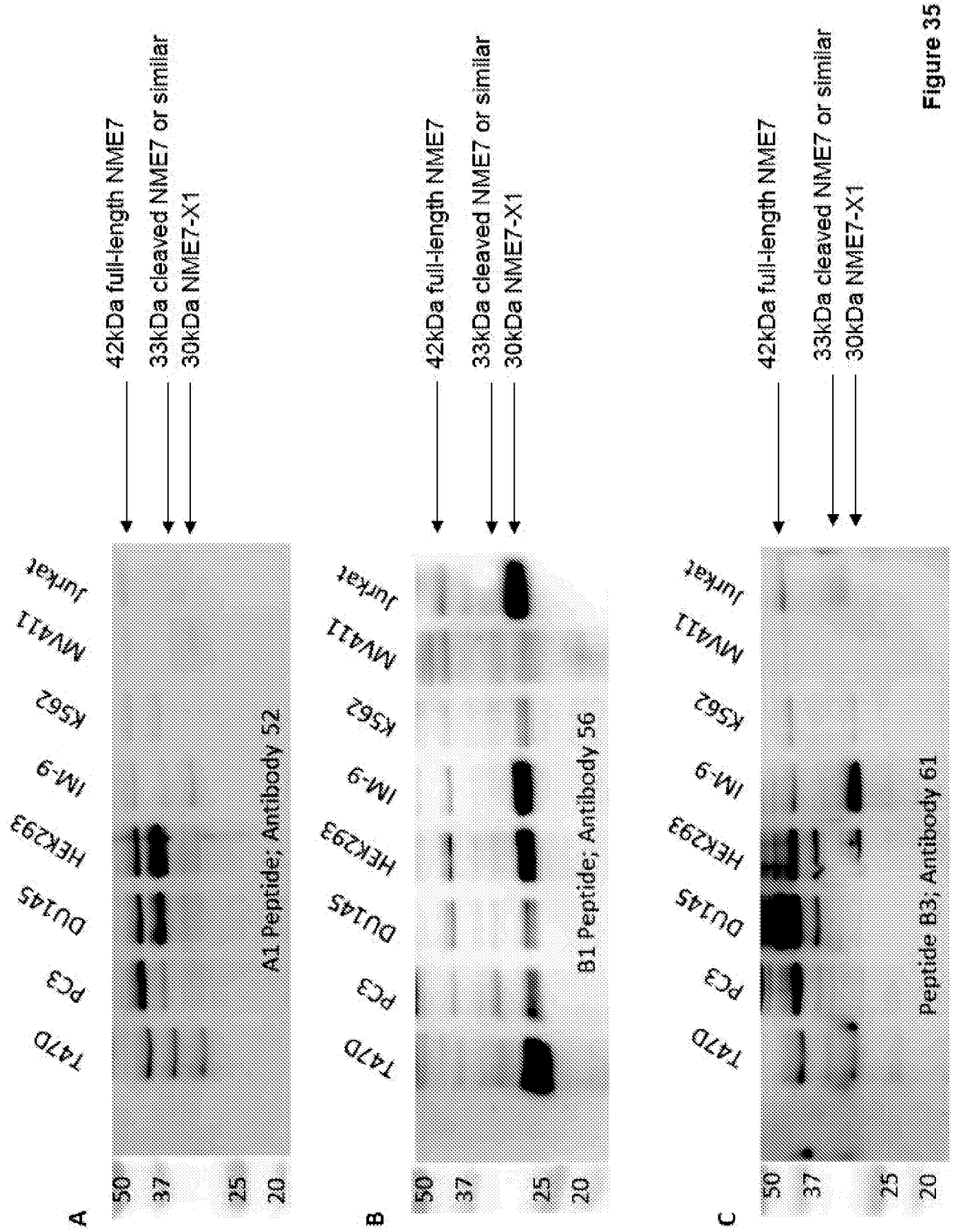

A
B
C
50
37
25
20
42kDa full-length NME7
33kDa cleaved NME7 or similar
30kDa NME7-X1
A1 Peptide; Antibody 52
B1 Peptide; Antibody 56
Peptide B3; Antibody 61
T47D
PC3
DU145
HEK293
IM-9
K562
MV411
Jurkat

Figure 35

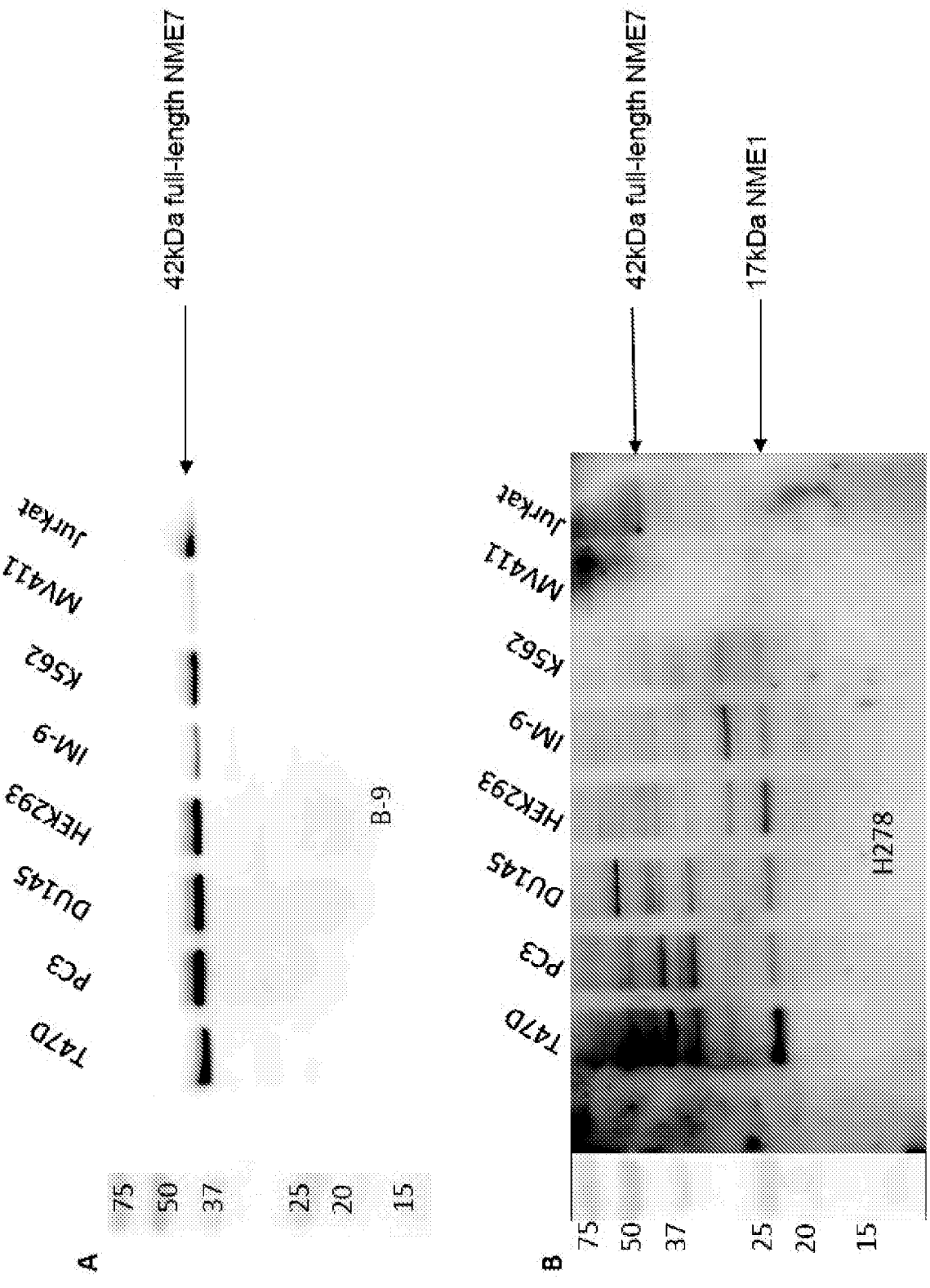

A
42kDa full-length NME7
T47D
PC3
DU145
HEK293
IM-9
K562
MV411
Jurkat
B-9
75
50
37
25
20
15
B
42kDa full-length NME7
17kDa NME1
T47D
PC3
DU145
HEK293
IM-9
K562
MV411
Jurkat
H278
75
50
37
25
20
15

Figure 36

Fig. 37A

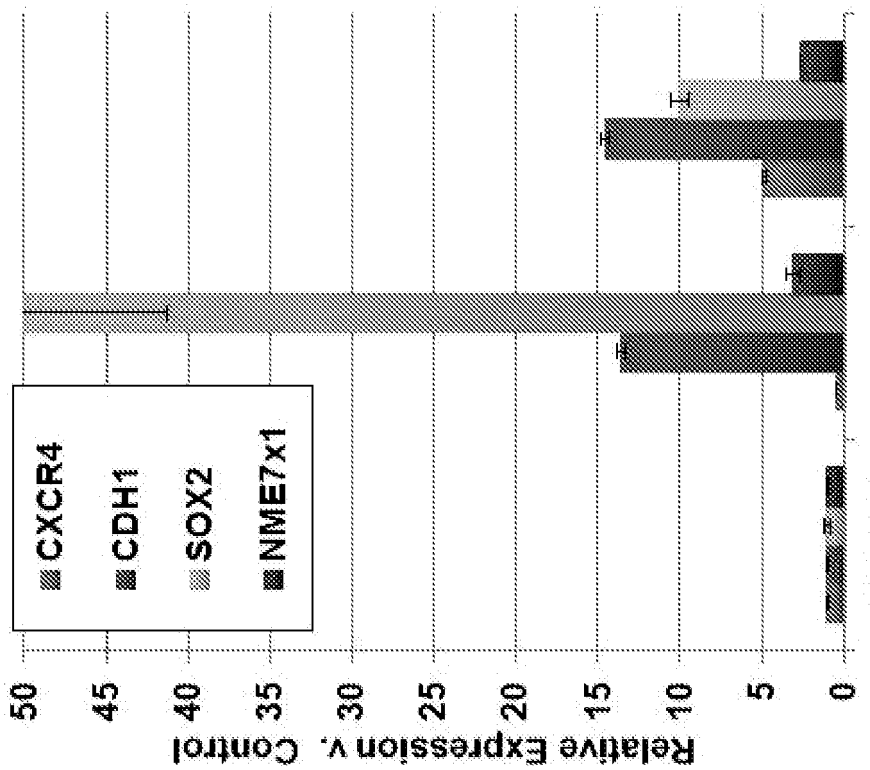


Fig. 37B

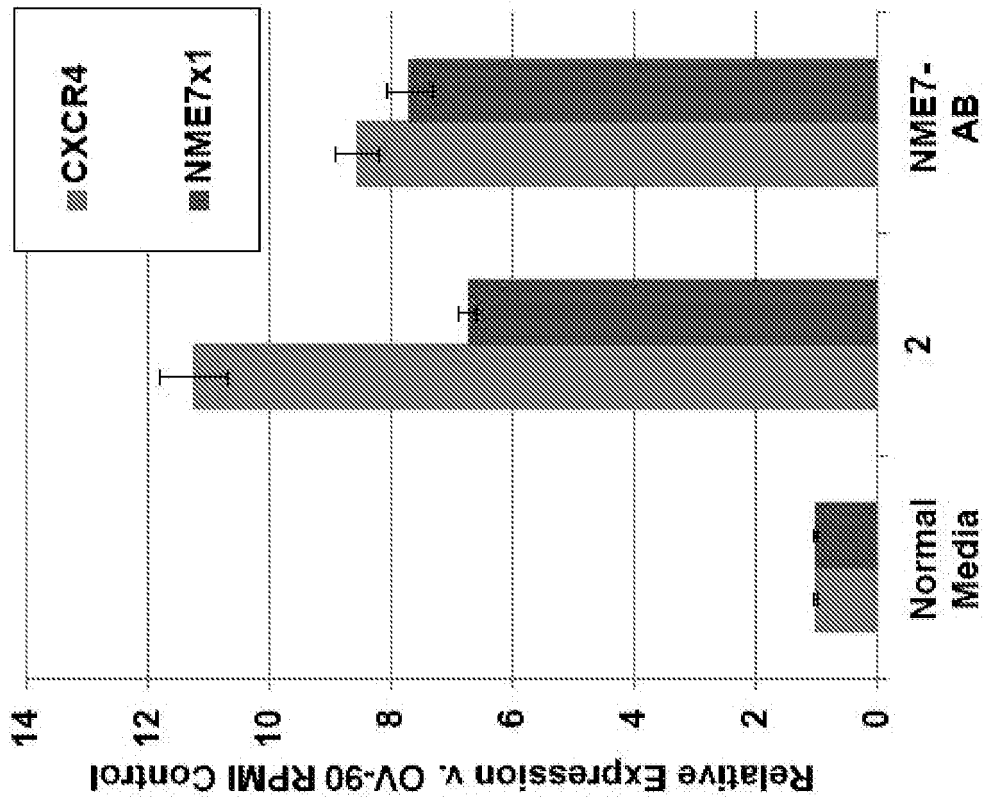
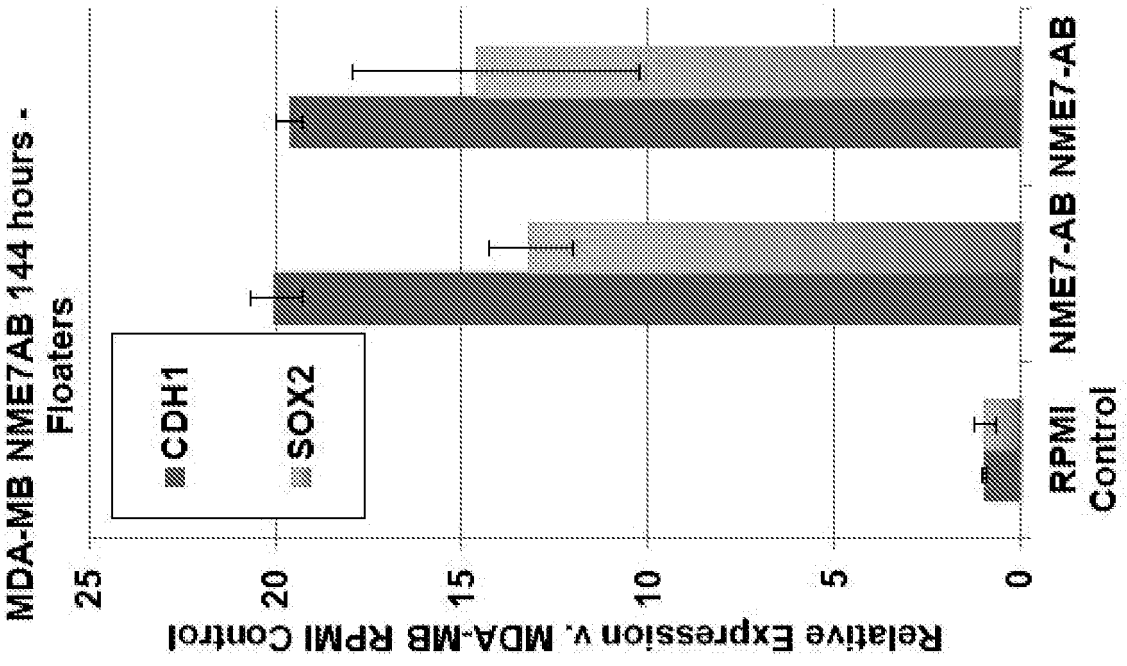


Fig. 37C


MDA-MB NME7AB 144 hours - Floaters
CDH1
SOX2
Relative Expression v. MDA-MB RPMI Control
25
20
15
10
5
0
RPMI Control
NME7-AB
NME7-AB

Various cancer cell lines that express MUC1* and NME7-AB-like species

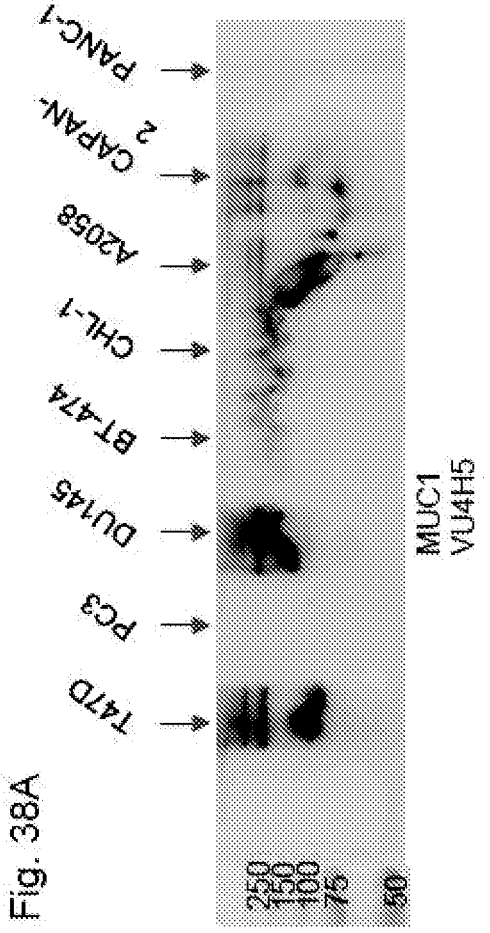


Fig. 38A

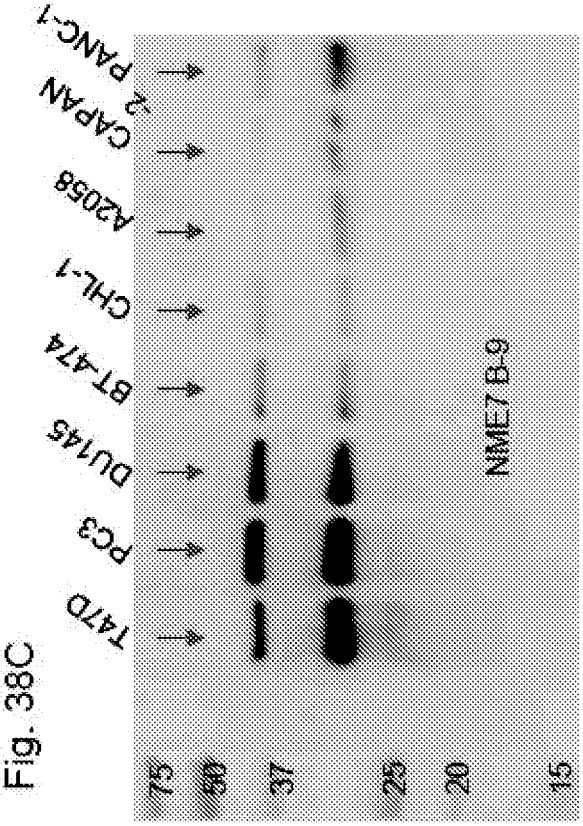


Fig. 38C

CHL-1 – Melanoma
A2058 – Melanoma
CAPAN-2 – Pancreatic (+)
PANC-1 – Pancreatic (-)

All samples normalized to 40µg/mL
SDIX – Goat anti-Rabbit
VU4H5 and B-9 – Goat anti Mouse

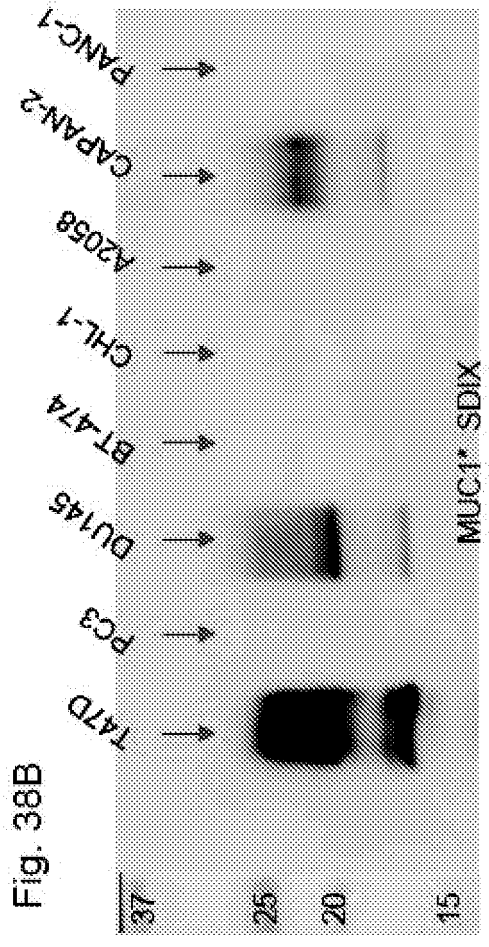


Fig. 38B

BT-474 HER2 positive breast cancer cells express almost no MUC1 or MUC1* until they become Metastatic, which is resistant to Herceptin and other chemotherapy drugs. Blocking MUC1* with siRNA or anti-MUC1* Fab reversed the metastatic transition

Fig. 38D

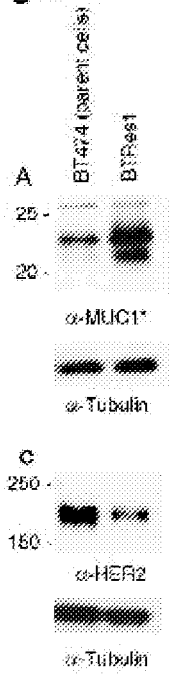


Fig. 38E

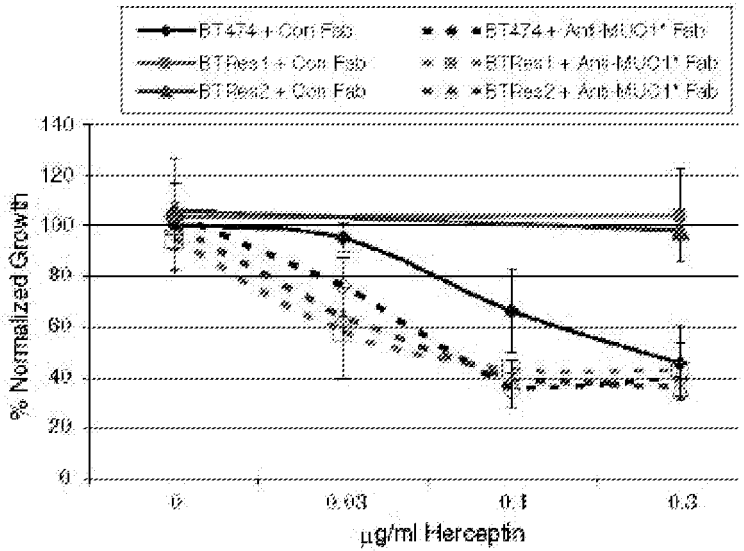
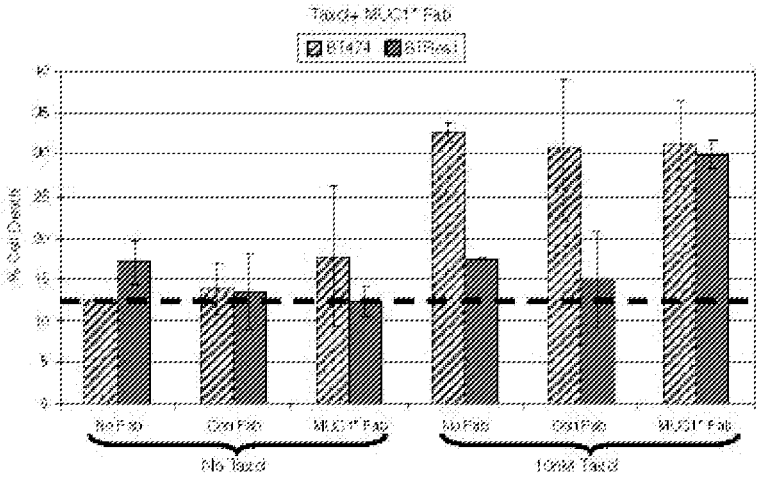
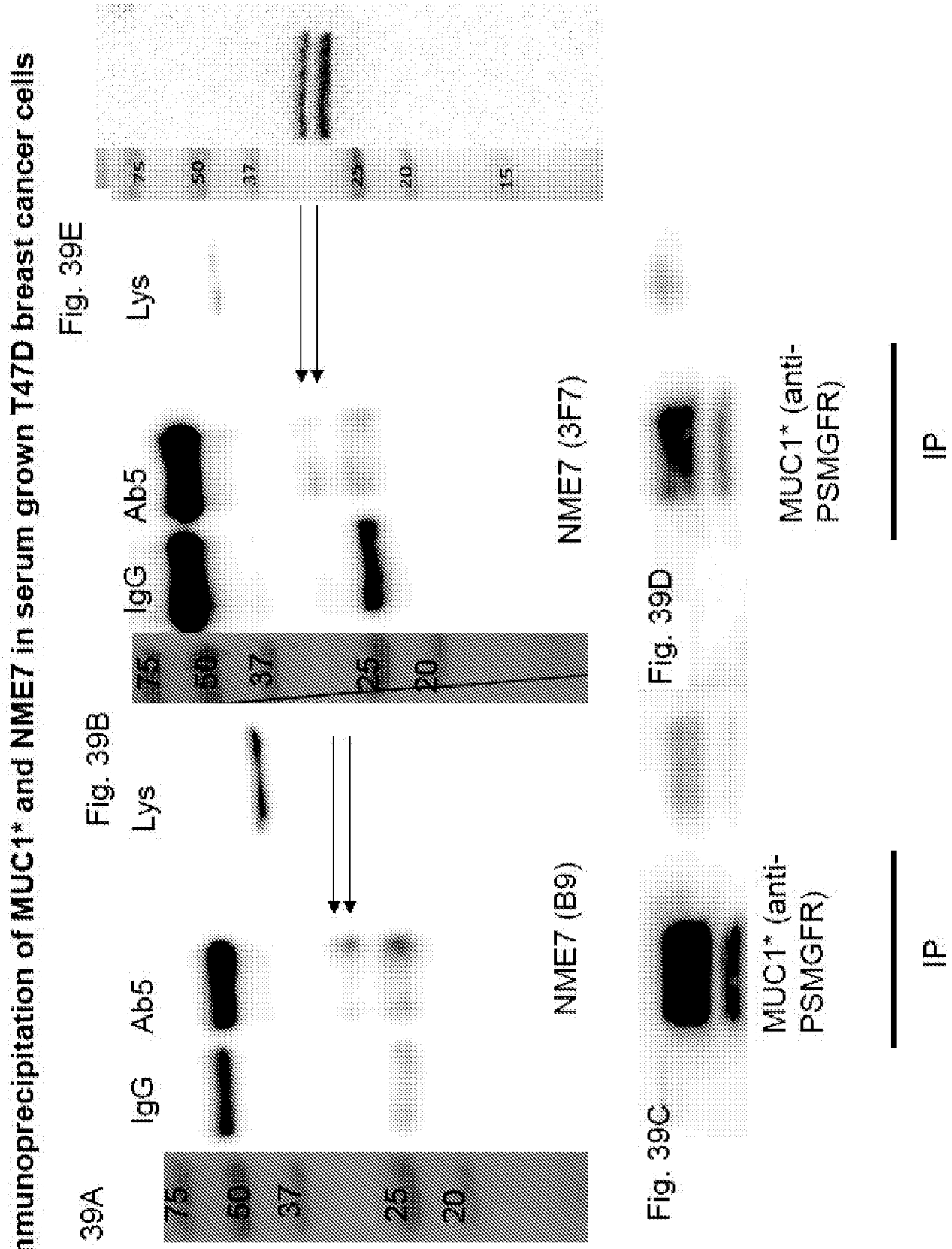


Fig. 38F



Fessler et al 2009, MUC1* is a Determinant of Herceptin Resistance in Breast Cancer Cells, 2009 Breast Cancer Res Treat


Co-Immunoprecipitation of MUC1* and NME7 in serum grown T47D breast cancer cells
Fig. 39A
IgG
Ab5
75
50
37
25
20
Fig. 39B
Lys
NME7 (B9)
Fig. 39C
MUC1* (anti-PSMGFR)
IP
Fig. 39D
Lys
NME7 (3F7)
Fig. 39E
75
50
37
25
20
15
MUC1* (anti-PSMGFR)
IP

Co-Immunoprecipitation of MUC1* and NME7 from human embryonic stem cells & iPS cells

Fig. 40A

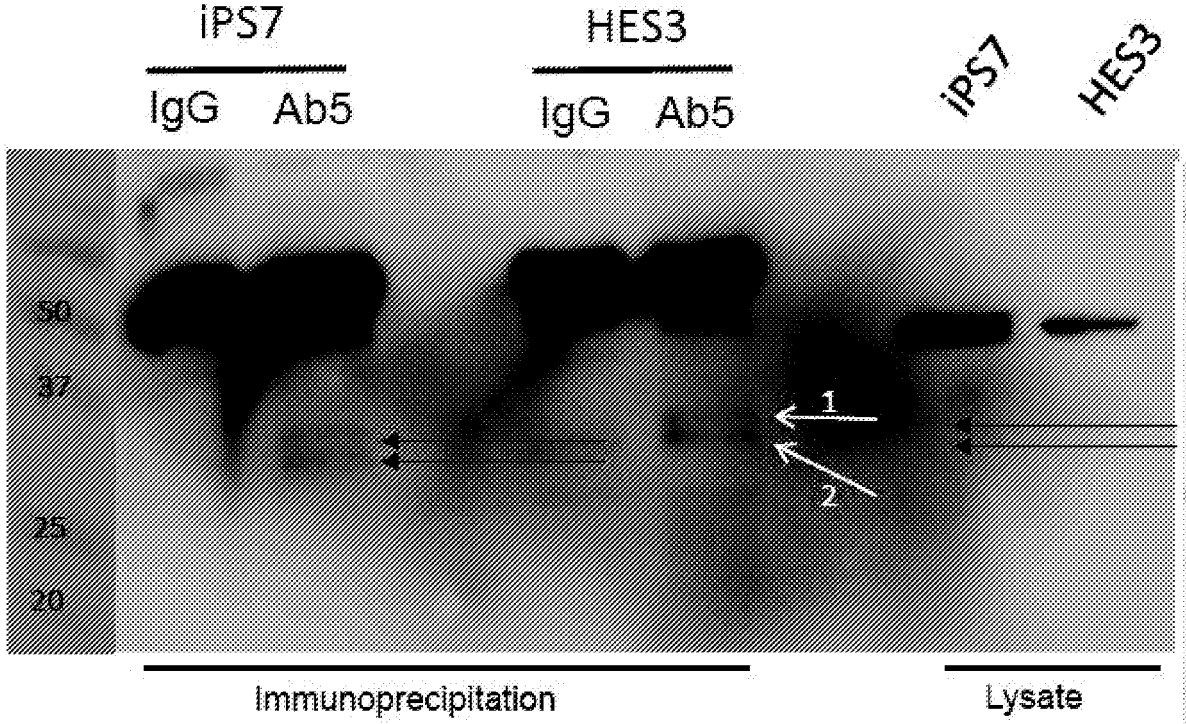


Fig. 40C

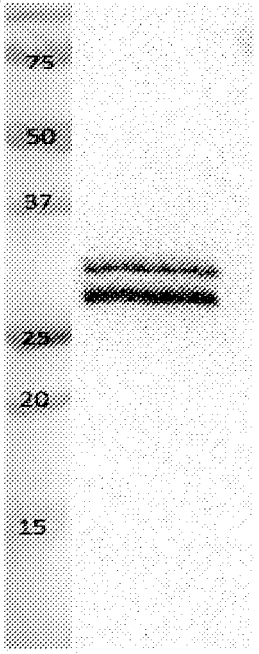
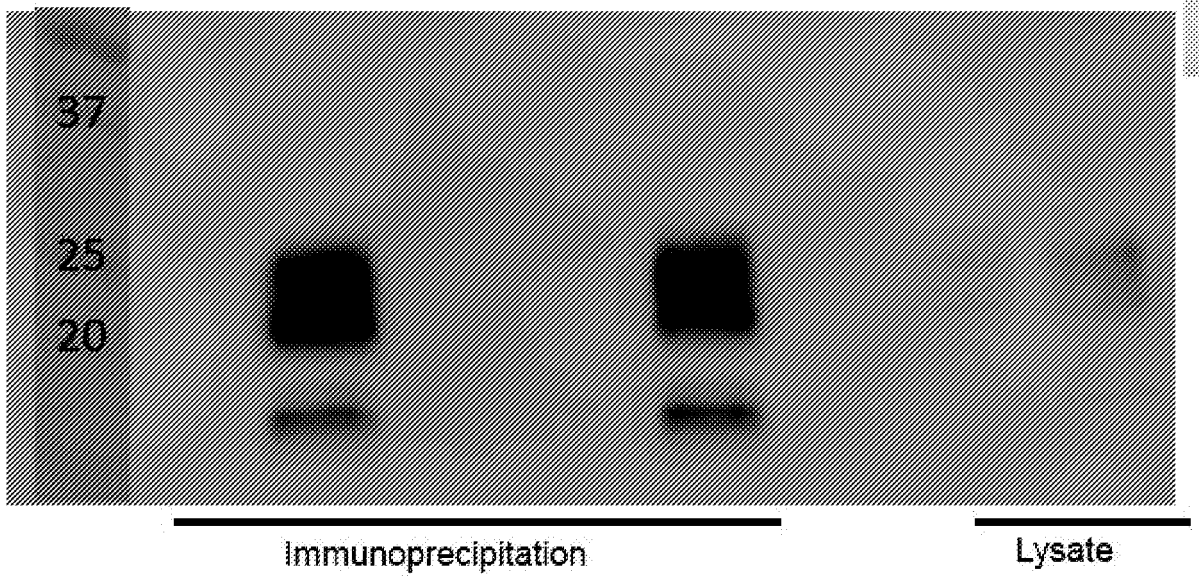



Fig. 40B