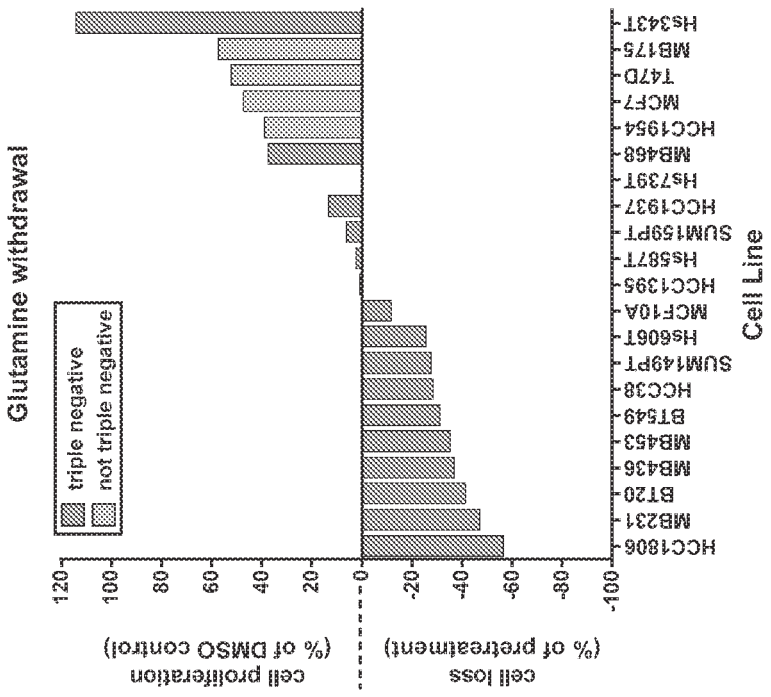

Glutamine withdrawal
triple negative
not triple negative
cell proliferation (% of DMSO control)
cell loss (% of pretreatment)
Cell Line
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
HCC1806
MB231
BT20
MB436
MB453
BT549
HCC38
SUM149PT
Hs578T
MCF10A
HCC1395
SUM159PT
HCC1937
Hs739T
MB468
HCC1954
MCF7
T47D
MB175
Hs343T

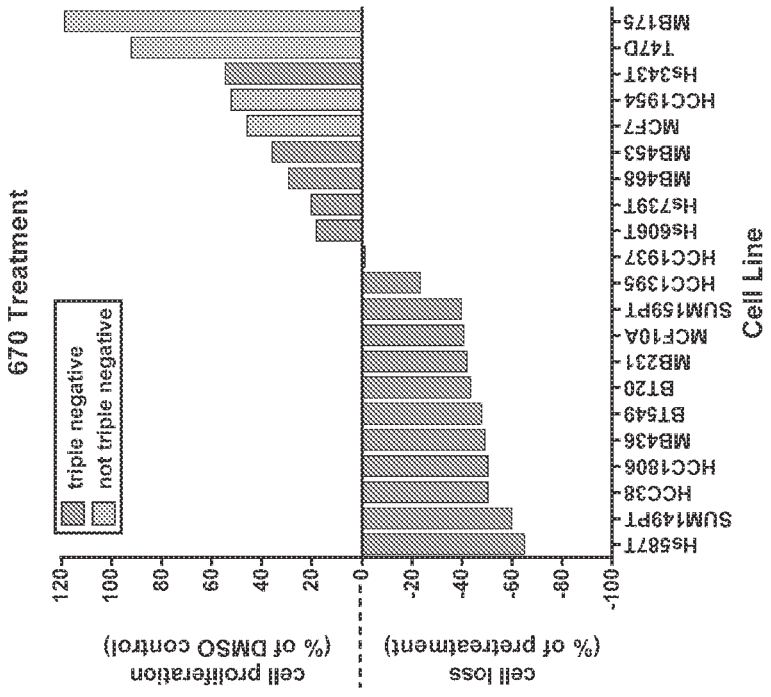

670 Treatment
triple negative
not triple negative
cell proliferation (% of DMSO control)
cell loss (% of pretreatment)
Cell Line
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
Hs578T
SUM149PT
HCC38
HCC1806
MB436
BT549
BT20
MB231
MCF10A
SUM159PT
HCC1395
HCC1937
Hs578T
Hs739T
MB468
MB453
MCF7
HCC1954
Hs343T
T47D
MB175

Figure 1

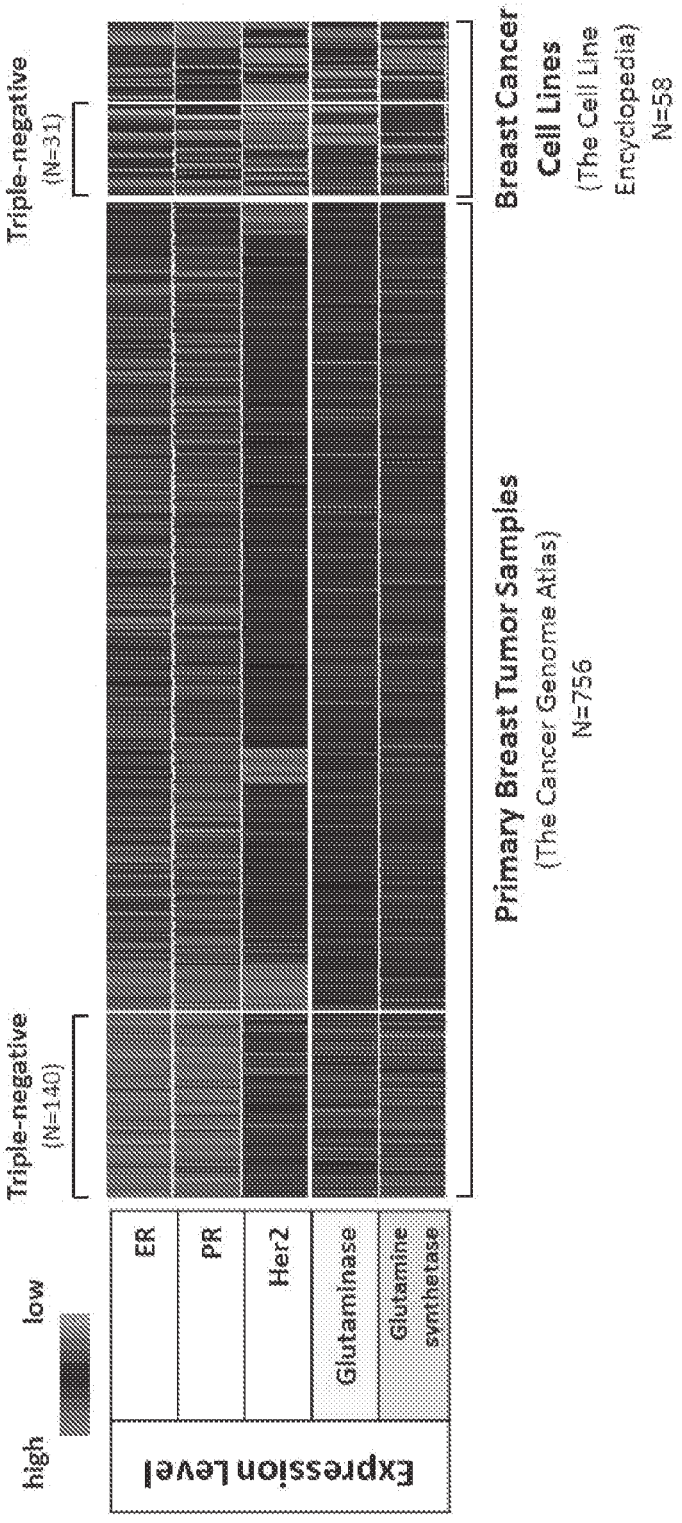

Expression Level
high
low
ER
PR
Her2
Glutaminase
Glutamine synthetase
Triple-negative (N=140)
Triple-negative (N=31)
Primary Breast Tumor Samples
(The Cancer Genome Atlas)
N=756
Breast Cancer Cell Lines
(The Cell Line Encyclopedia)
N=58

Figure 2

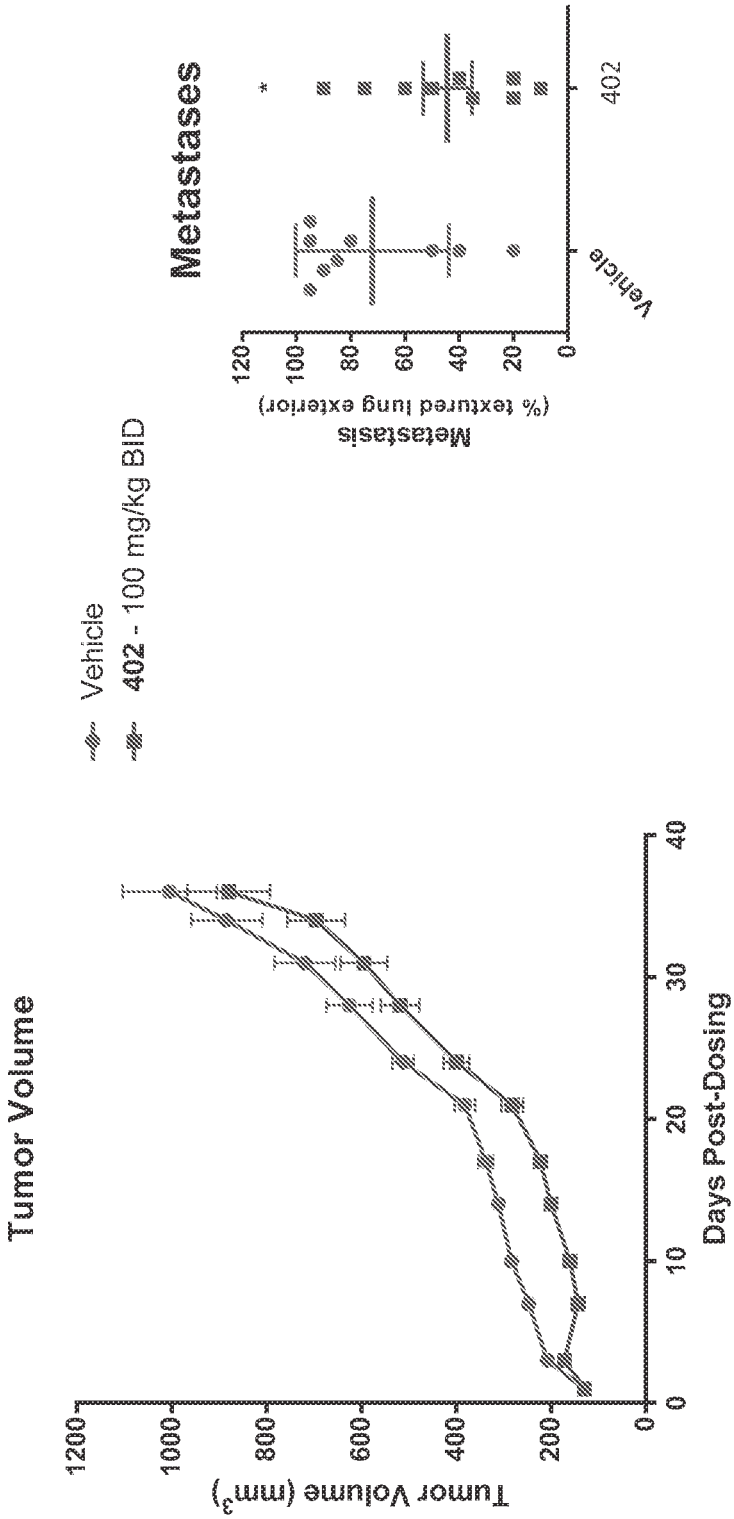

Tumor Volume
Vehicle
402 - 100 mg/kg BID
Tumor Volume (mm³)
1200
1000
800
600
400
200
0
0
10
20
30
40
Days Post-Dosing
Metastases
Metastasis
(% textured lung exterior)
120
100
80
60
40
20
0
*
Vehicle
402

Figure 3

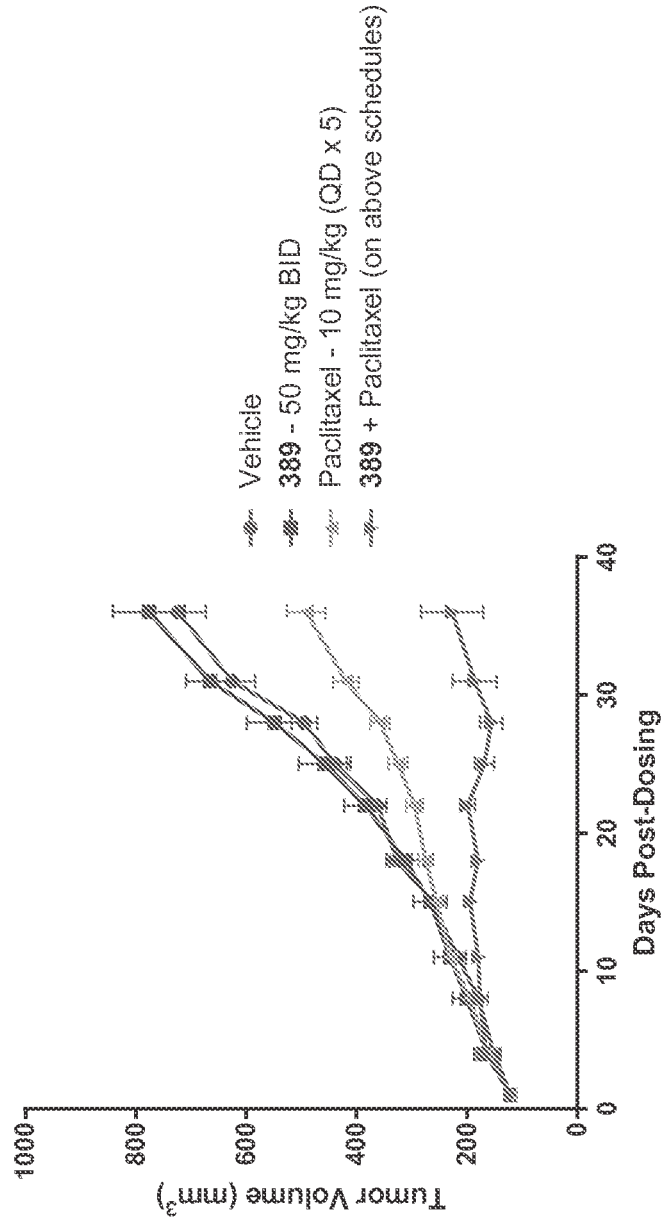

Vehicle
389 - 50 mg/kg BID
Paclitaxel - 10 mg/kg (QD x 5)
389 + Paclitaxel (on above schedules)
Tumor Volume (mm3)
1000
800
600
400
200
0
0
10
20
30
40
Days Post-Dosing

Figure 4

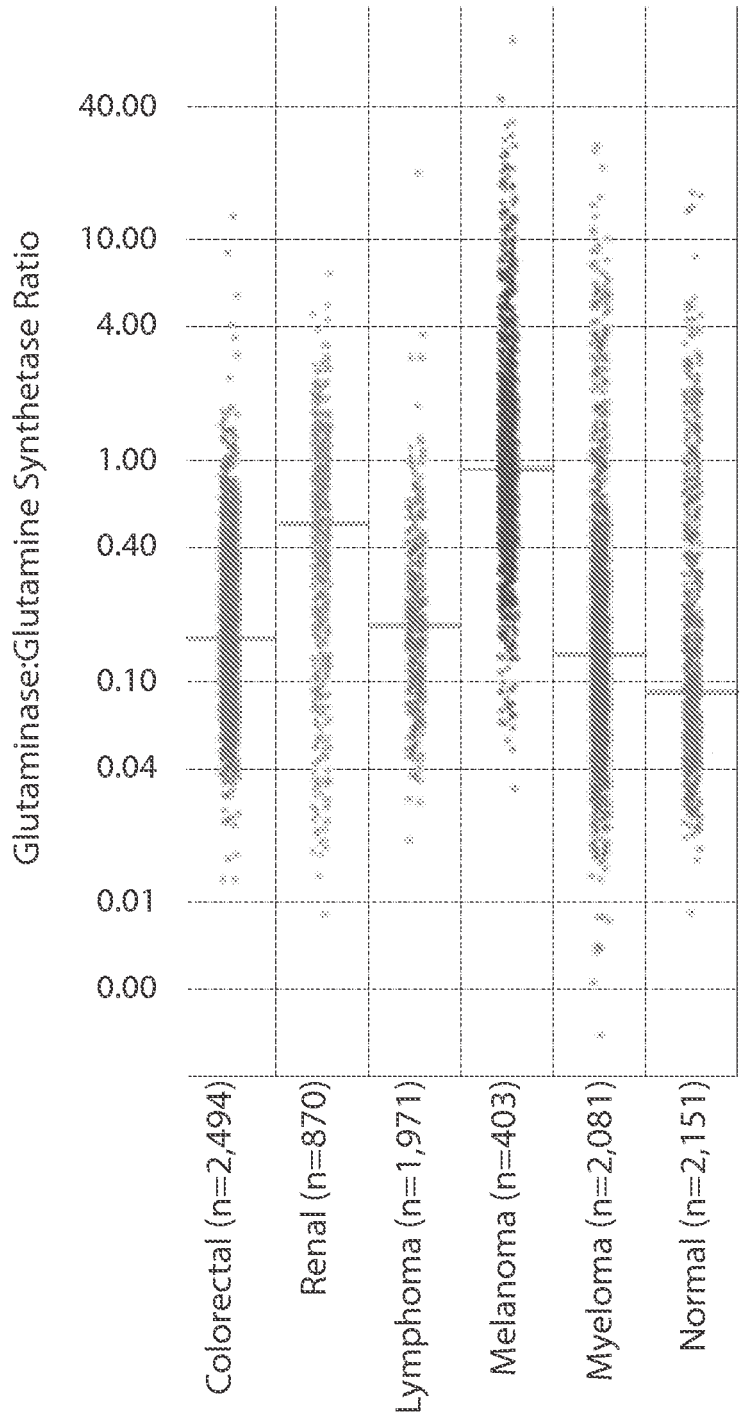

Glutaminase:Glutamine Synthetase Ratio
40.00
10.00
4.00
1.00
0.40
0.10
0.04
0.01
0.00
Colorectal (n=2,494)
Renal (n=870)
Lymphoma (n=1,971)
Melanoma (n=403)
Myeloma (n=2,081)
Normal (n=2,151)

Figure 5

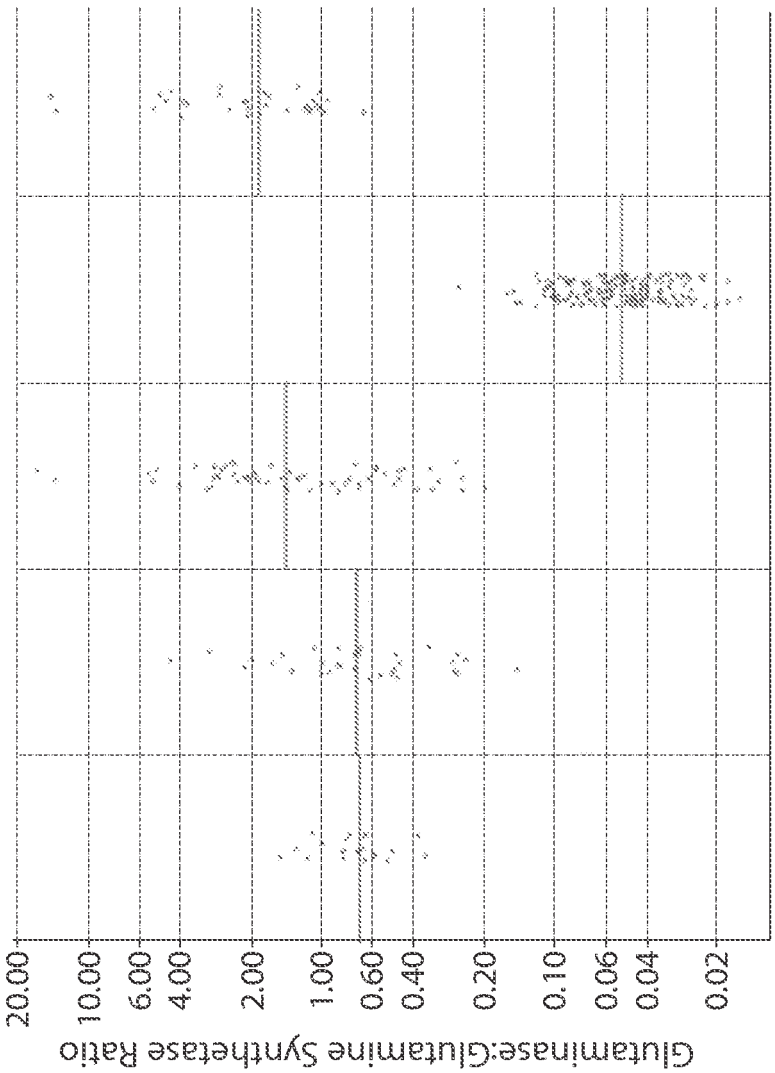

Glutaminase:Glutamine Synthetase Ratio
20.00
10.00
6.00
4.00
2.00
1.00
0.60
0.40
0.20
0.10
0.06
0.04
0.02
Adrenal cortex adenoma (n=22)
Adrenal cortex carcinoma (n=33)
Adrenal gland pheochromocytoma (n=33)
Endometrial endometrioid adenocarcinoma (n=298)
Parathyroid gland adenoma (n=19)

Figure 6

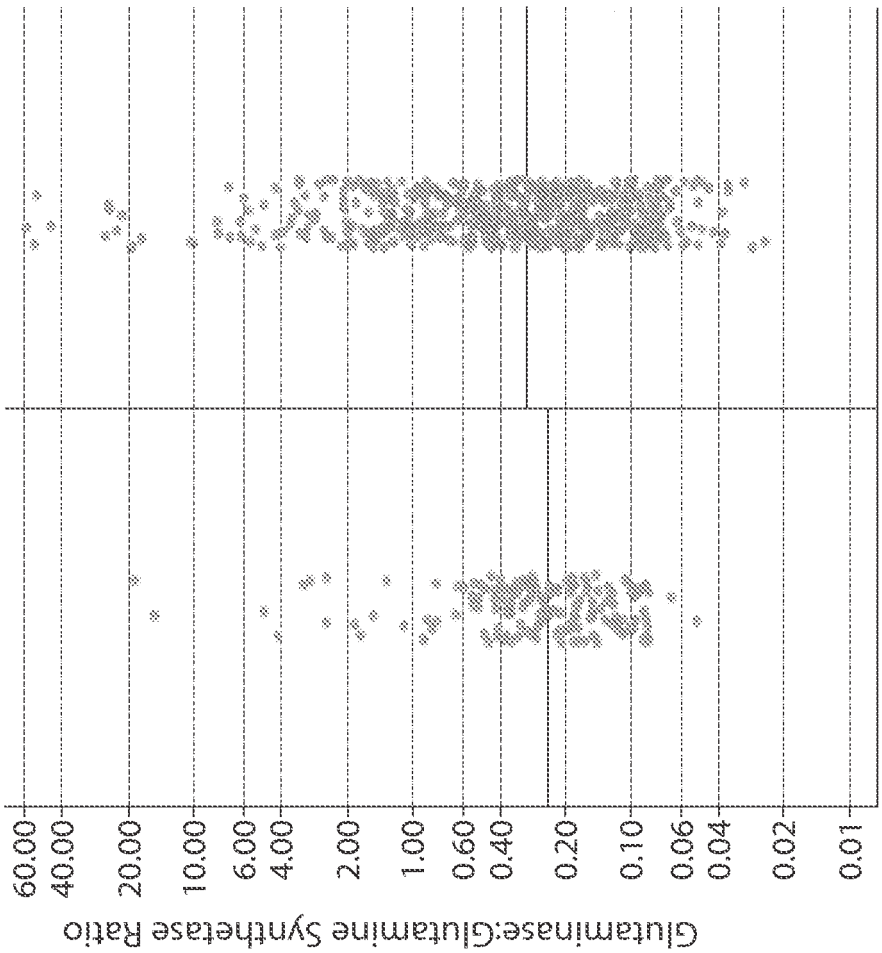

Glutaminase:Glutamine Synthetase Ratio
60.00
40.00
20.00
10.00
6.00
4.00
2.00
1.00
0.60
0.40
0.20
0.10
0.06
0.04
0.02
0.01
Acute lymphoblastic leukemia (n=161)
Chronic lymphocytic leukemia (n=588)

Figure 7

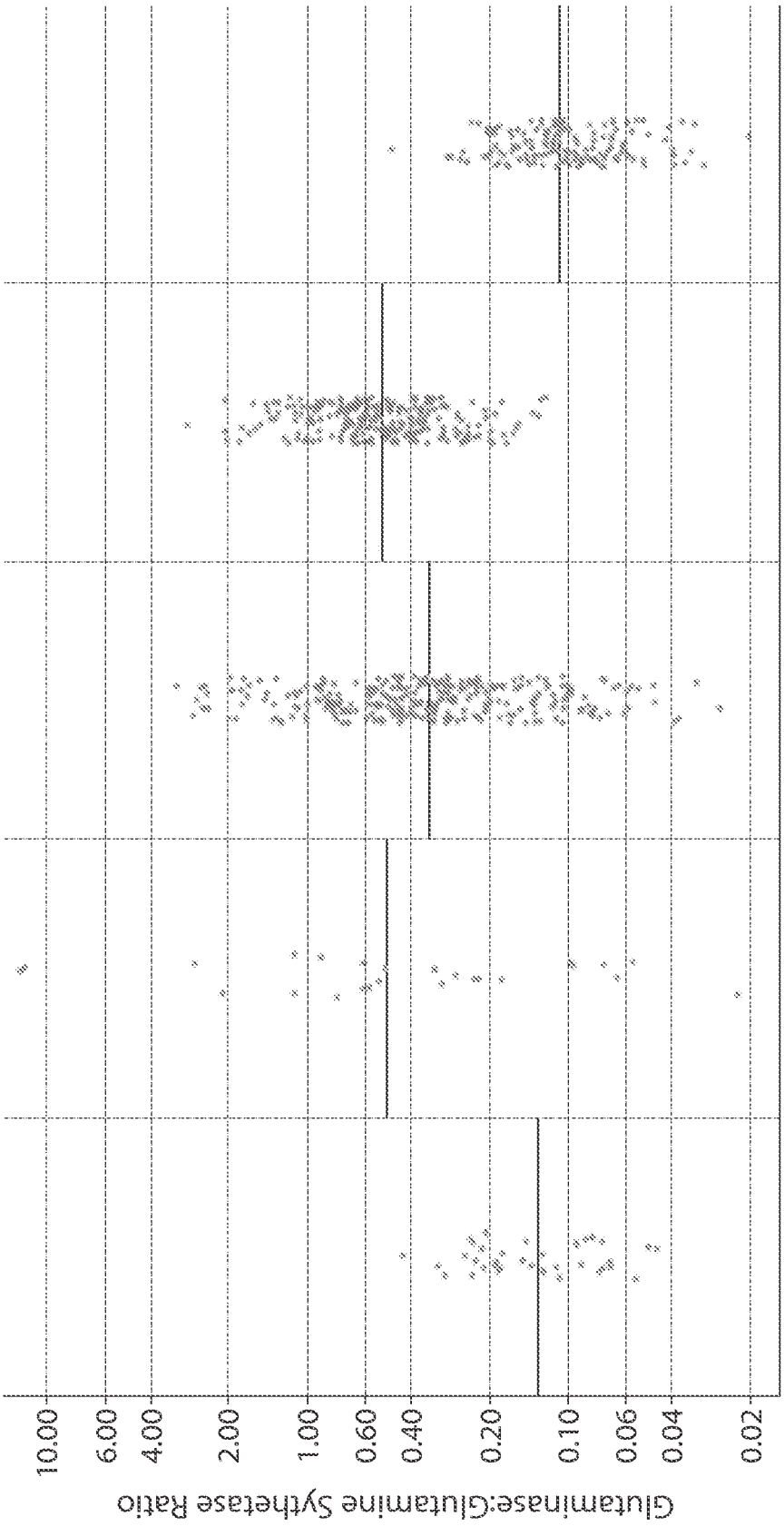

Glutaminase:Glutamine Sythetase Ratio
10.00
6.00
4.00
2.00
1.00
0.60
0.40
0.20
0.10
0.06
0.04
0.02
Anaplastic large cell lymphoma (n=38)
Burkitt's lymphoma (n=25)
Diffuse large B-cell lymphoma (n=294)
Follicular Lymphoma (n=242)
Hodgkin's lymphoma (n=146)

Figure 8

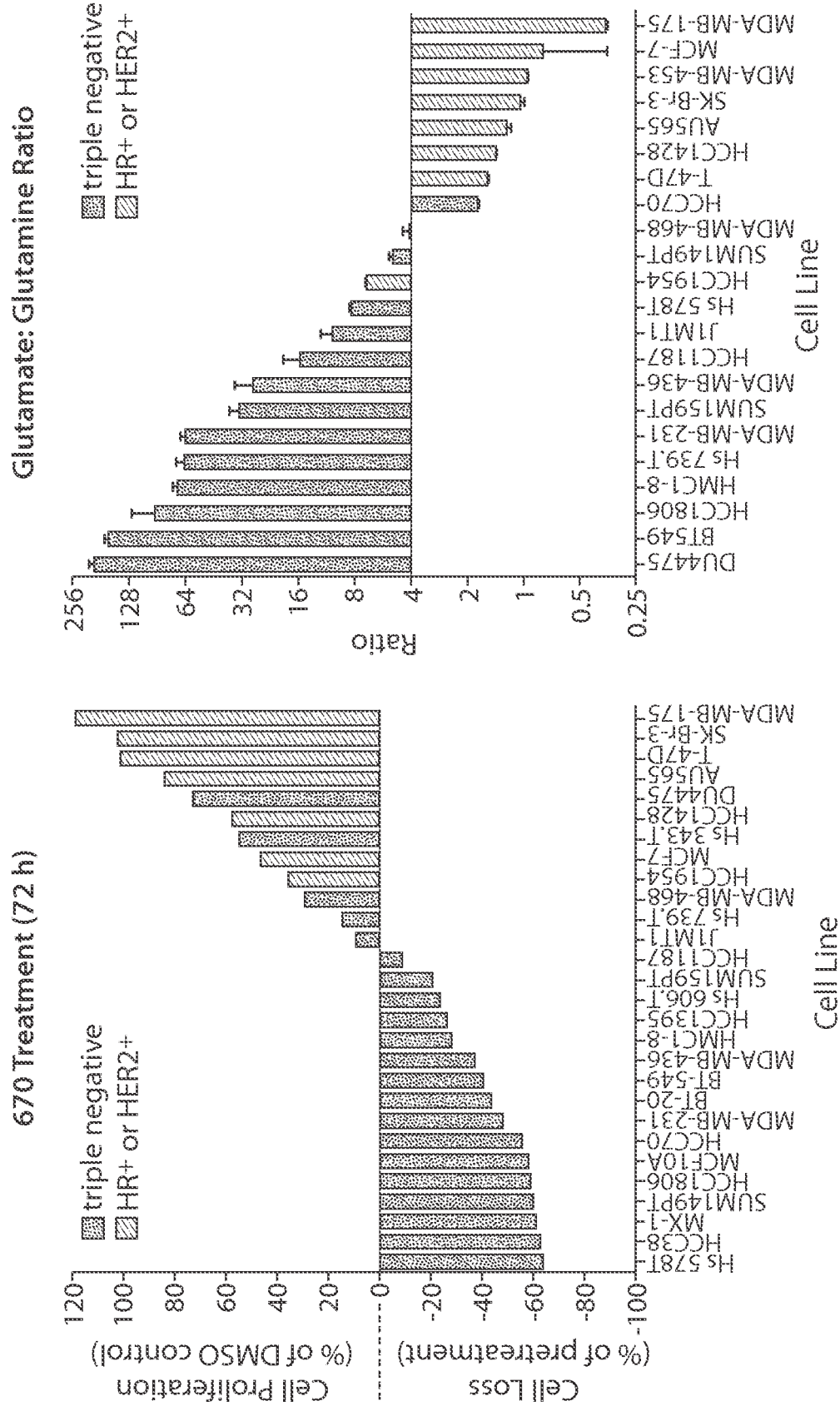

Glutamate: Glutamine Ratio
triple negative
HR+ or HER2+
Ratio
256
128
64
32
16
8
4
2
1
0.5
0.25
DU4475
BT549
HCC1806
HMC1-8
Hs 739.T
MDA-MB-231
SUM159PT
MDA-MB-436
HCC1187
J1MT1
Hs 578T
HCC1954
SUM149PT
MDA-MB-468
HCC70
T-47D
HCC1428
AU565
SK-Br-3
MDA-MB-453
MCF-7
MDA-MB-175
Cell Line
670 Treatment (72 h)
triple negative
HR+ or HER2+
Cell Proliferation
(% of DMSO control)
120
100
80
60
40
20
0
Cell Loss
(% of pretreatment)
-20
-40
-60
-80
-100
Hs 578T
HCC38
MX-1
SUM149PT
HCC1806
MCF10A
HCC70
MDA-MB-231
BT-20
BT-549
MDA-MB-436
HMC1-8
HCC1395
Hs 606.T
SUM159PT
HCC1187
J1MT1
Hs 739.T
MDA-MB-468
HCC1954
MCF7
Hs 343.T
HCC1428
DU4475
AU565
T-47D
SK-Br-3
MDA-MB-175
Cell Line

Figure 9

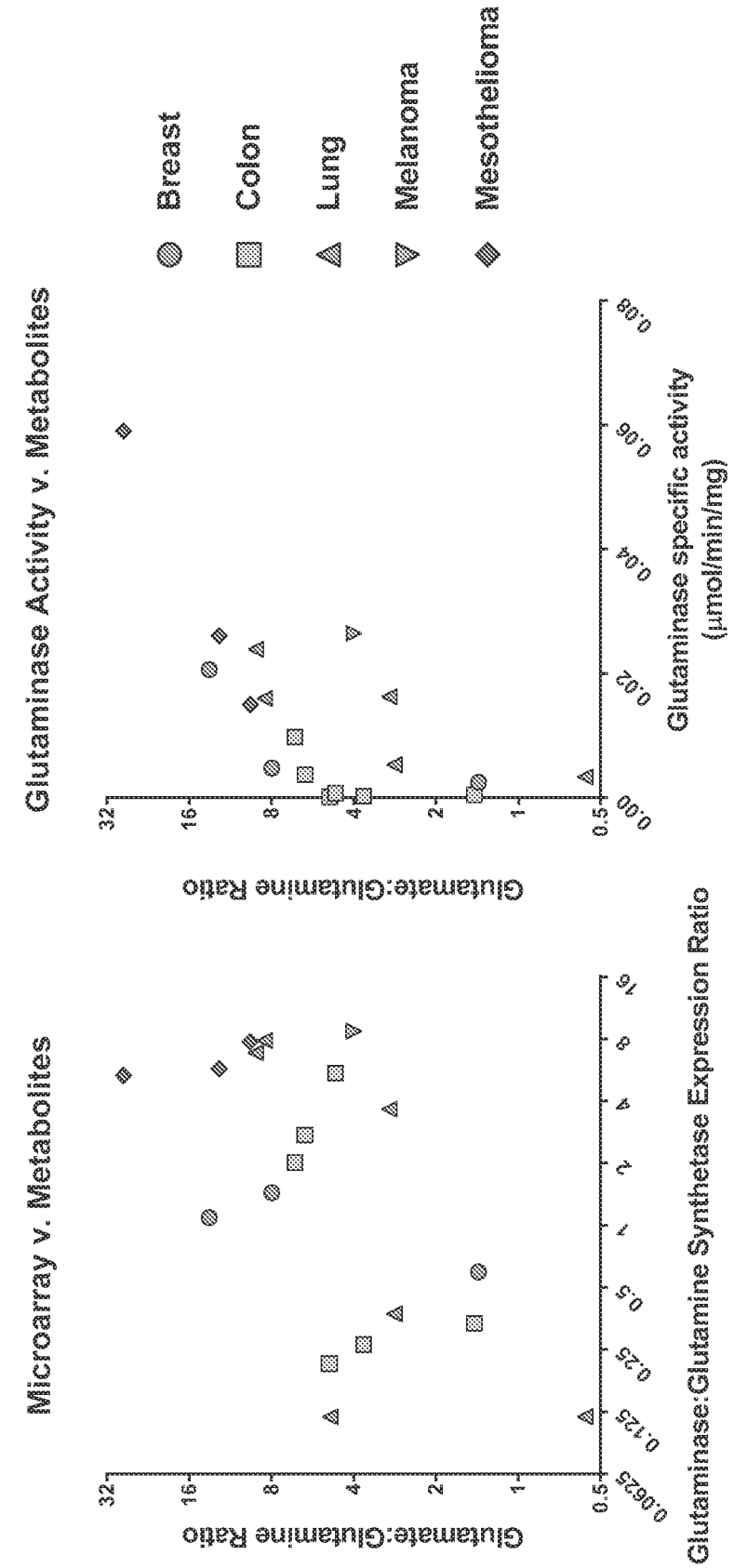

Glutaminase Activity v. Metabolites
Breast
Colon
Lung
Melanoma
Mesothelioma
Glutamate:Glutamine Ratio
32
16
8
4
2
1
0.5
0.00
0.02
0.04
0.06
0.08
Glutaminase specific activity
(µmol/min/mg)
Microarray v. Metabolites
Glutamate:Glutamine Ratio
Glutaminase:Glutamine Synthetase Expression Ratio
0.0625
0.125
0.25
0.5
1
2
4
8
16

Figure 10

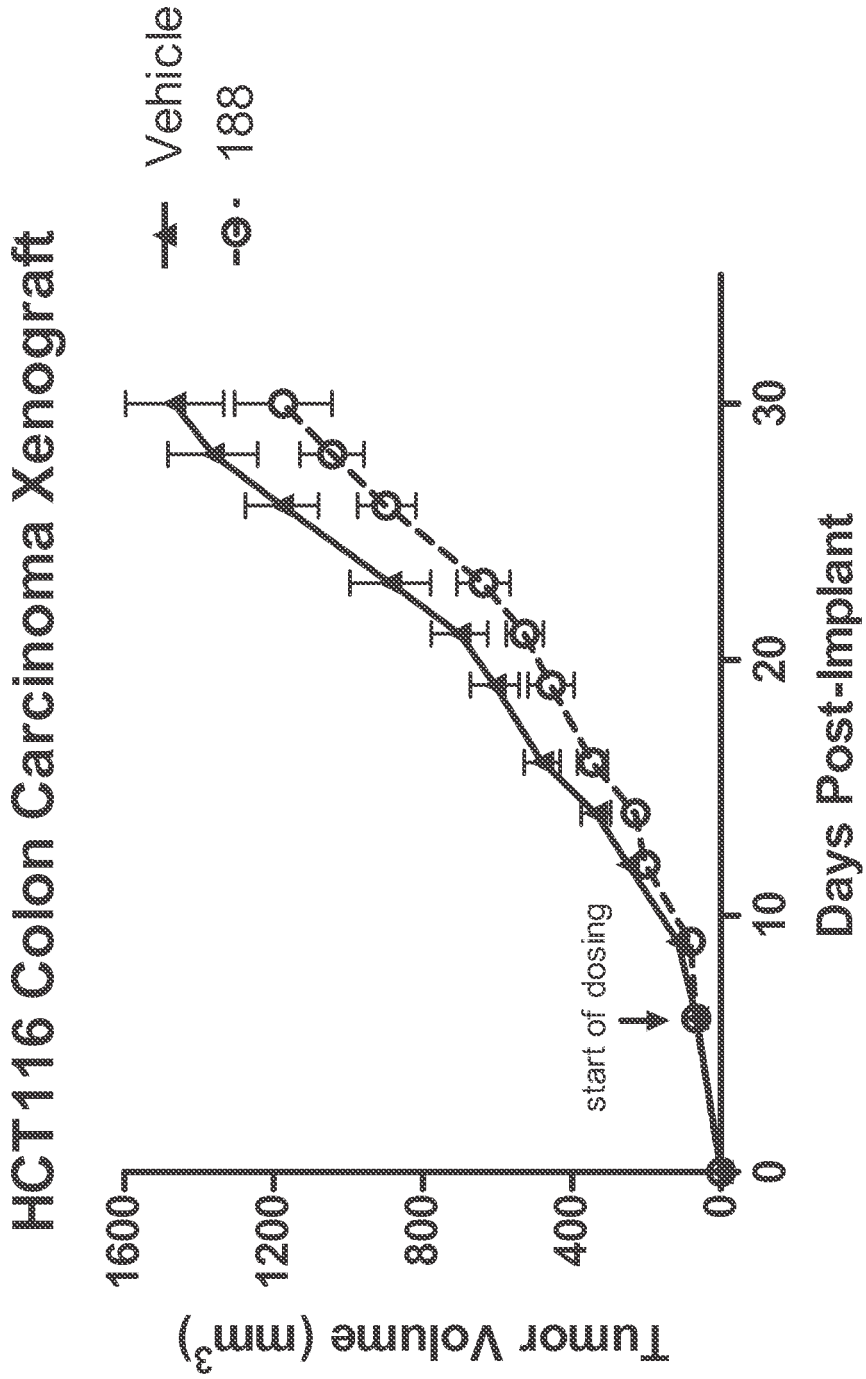

HCT116 Colon Carcinoma Xenograft
Vehicle
188
Tumor Volume (mm3)
1600
1200
800
400
0
start of dosing
0
10
20
30
Days Post-Implant

Figure 11

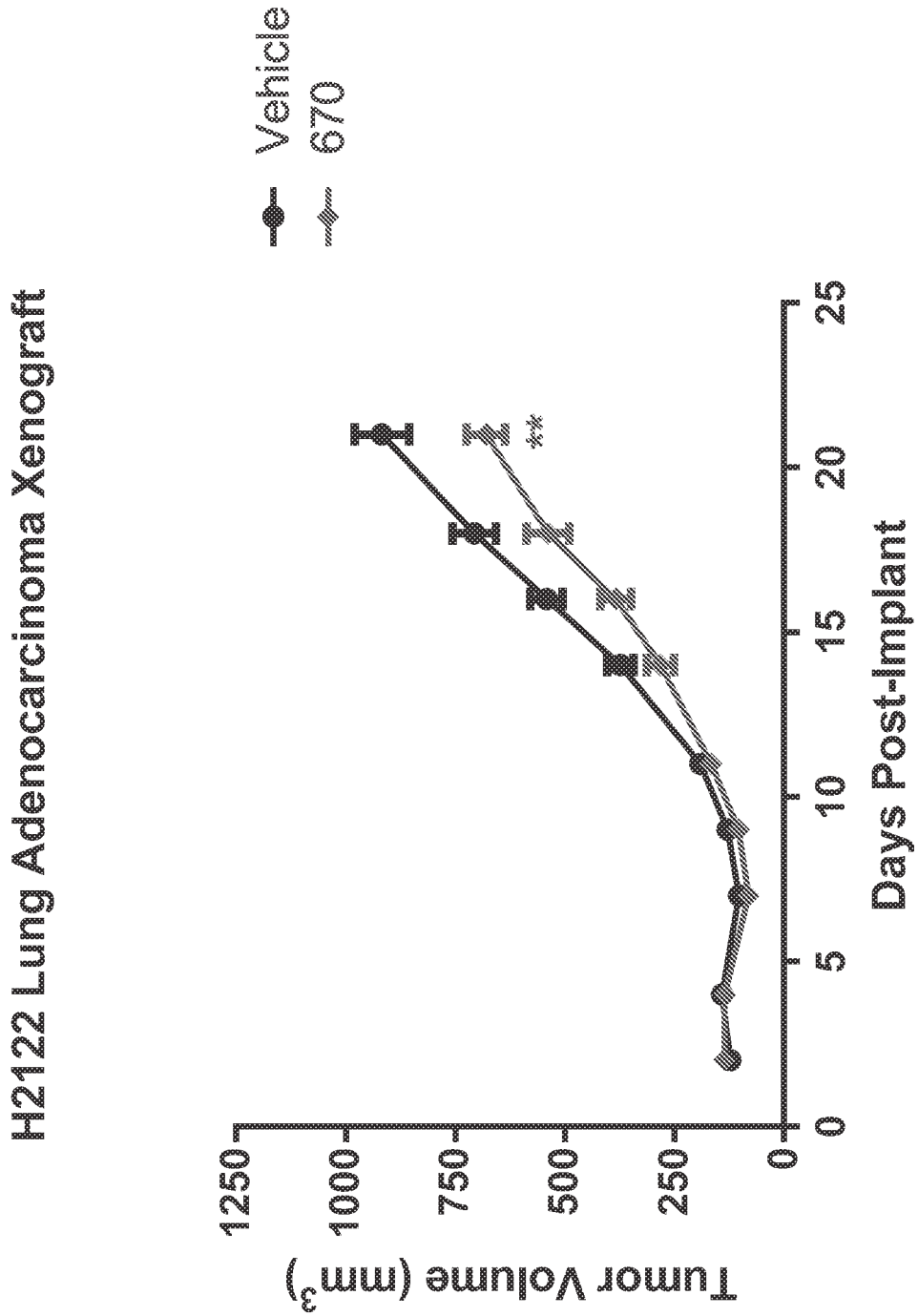

H2122 Lung Adenocarcinoma Xenograft
Vehicle
670
**
Tumor Volume (mm3)
1250
1000
750
500
250
0
0
5
10
15
20
25
Days Post-Implant

Figure 12

Figure 13

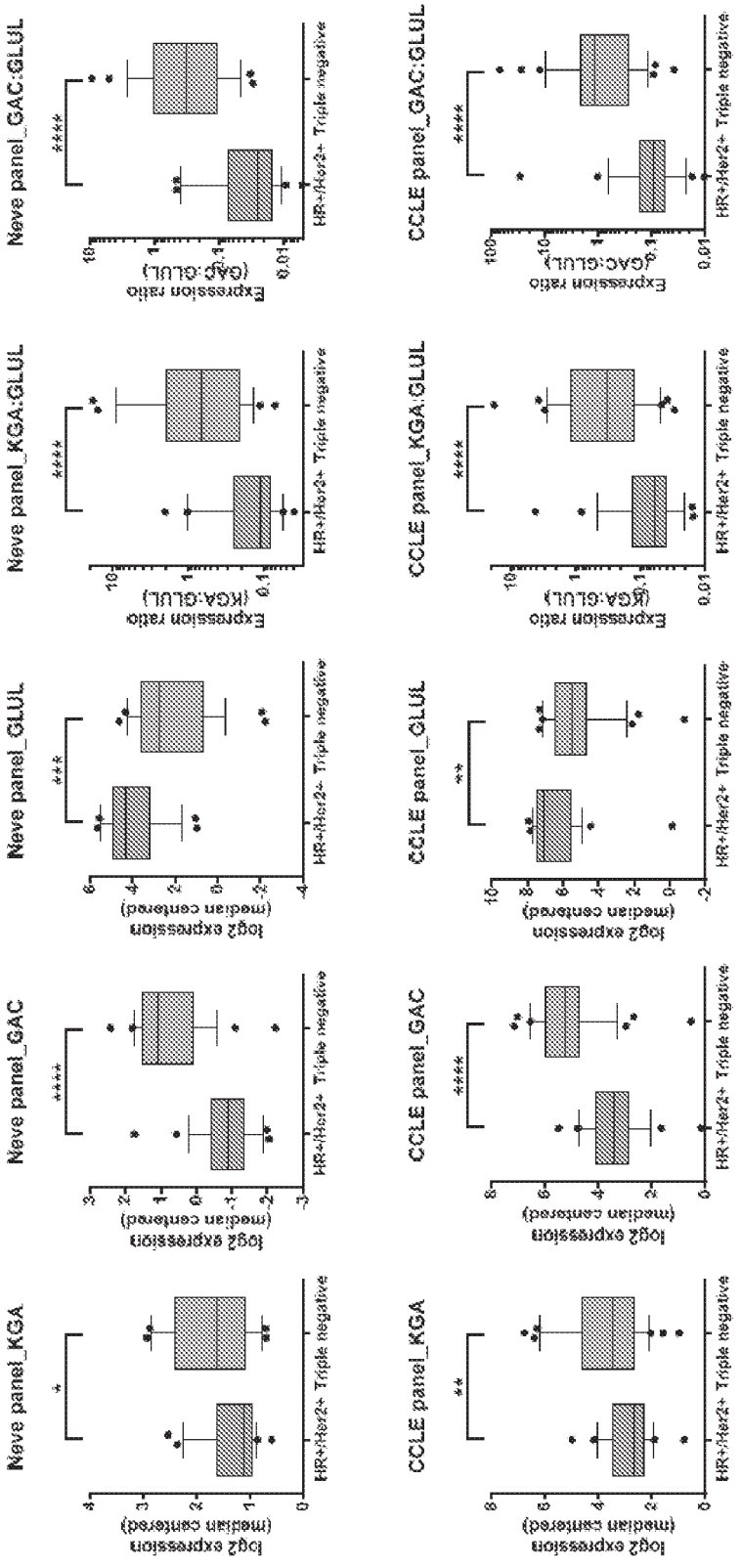
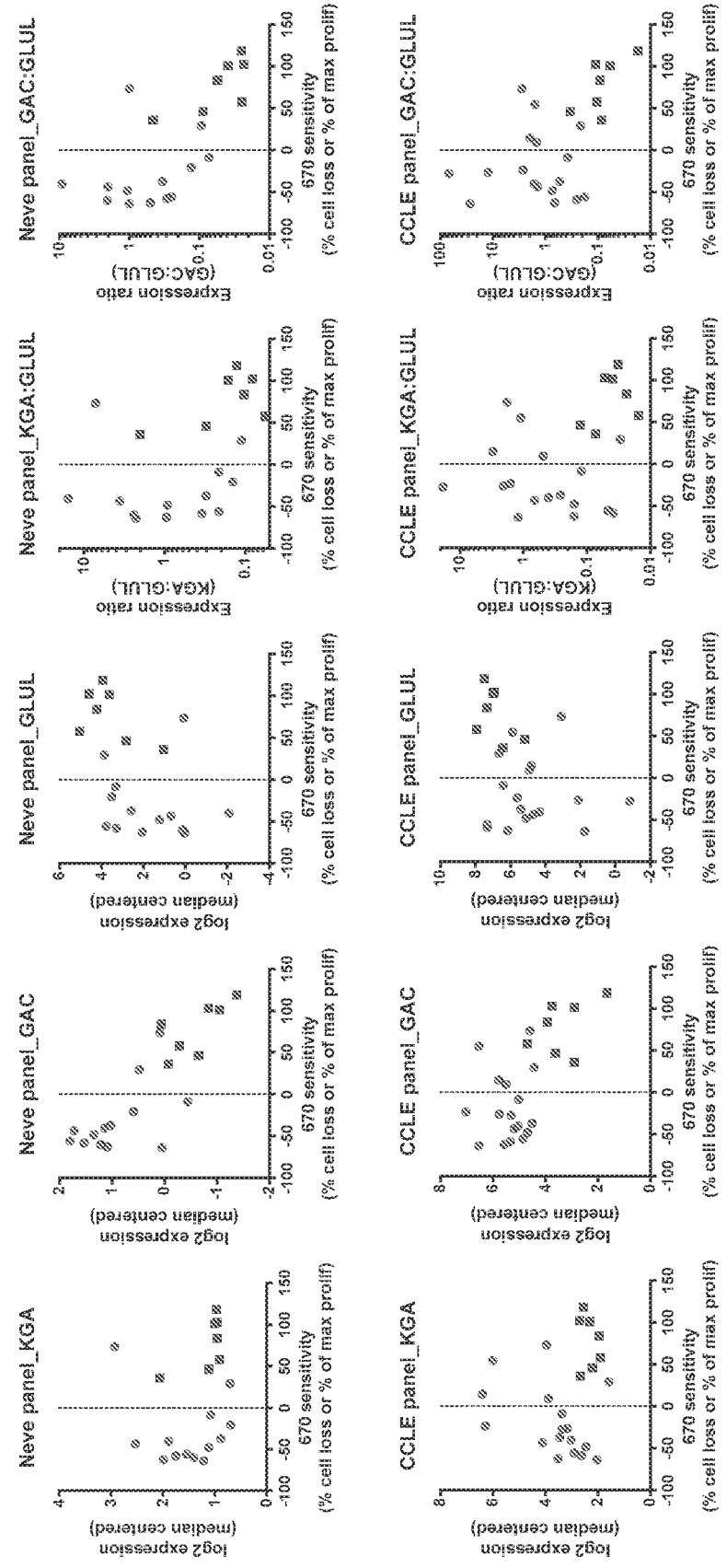

Neve panel_KGA
Neve panel_GAC
Neve panel_GLUL
Neve panel_KGA:GLUL
Neve panel_GAC:GLUL
CCLE panel_KGA
CCLE panel_GAC
CCLE panel_GLUL
CCLE panel_KGA:GLUL
CCLE panel_GAC:GLUL
log2 expression (median centered)
Expression ratio (KGA:GLUL)
Expression ratio (GAC:GLUL)
HR+/Her2+ Triple negative
HR+/Her2+
Triple negative

Figure 14


Neve panel_KGA
Neve panel_GAC
Neve panel_GLUL
Neve panel_KGA:GLUL
Neve panel_GAC:GLUL
CCLE panel_KGA
CCLE panel_GAC
CCLE panel_GLUL
CCLE panel_KGA:GLUL
CCLE panel_GAC:GLUL
log2 expression (median centered)
Expression ratio (KGA:GLUL)
Expression ratio (GAC:GLUL)
670 sensitivity
(% cell loss or % of max proliff)
triple negative
HR+ or HER2+

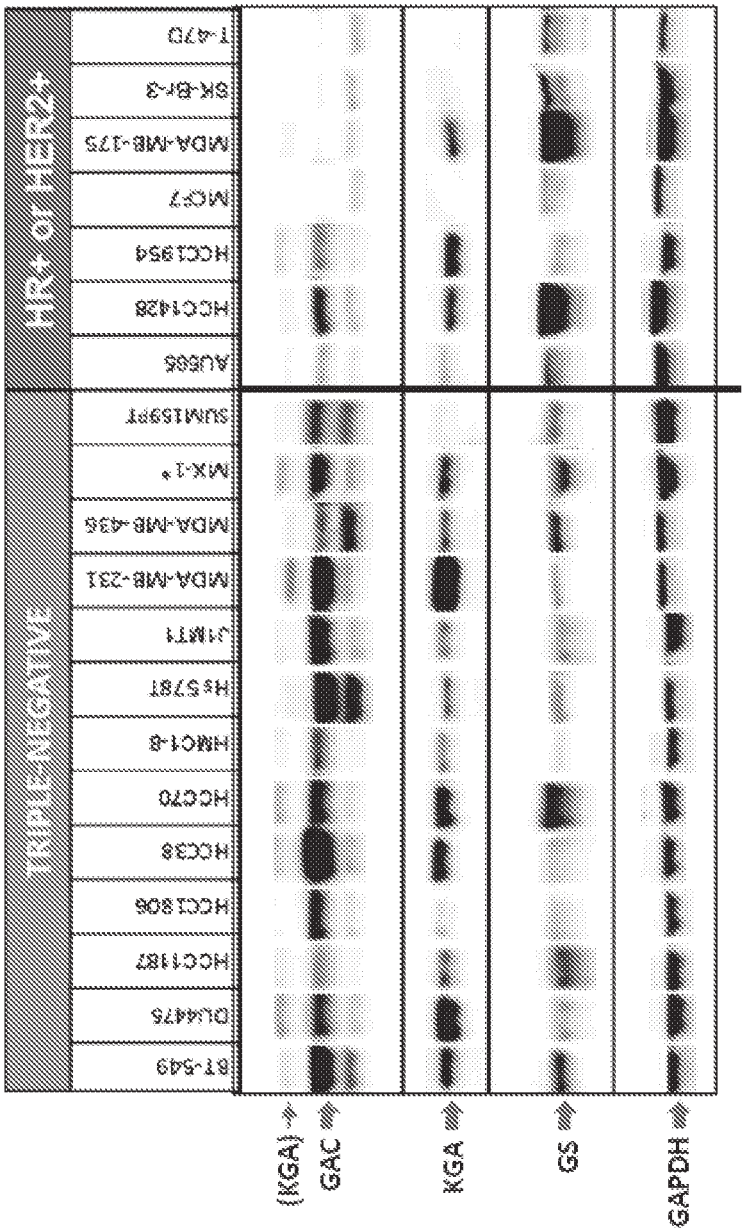

HR+ or HER2+
T-47D
BR-3
MDA-MB-175
MCF7
HCC1954
HCC1428
AU565
TRIPLE-NEGATIVE
SUM159PT
MX-1*
MDA-MB-435
MDA-MB-231
UMT1
Hs 578T
HMC1-8
HCC70
HCC38
HCC1806
HCC1187
DU4475
BT-549
(KGA) →
GAC
KGA
GS
GAPDH

Figure 15

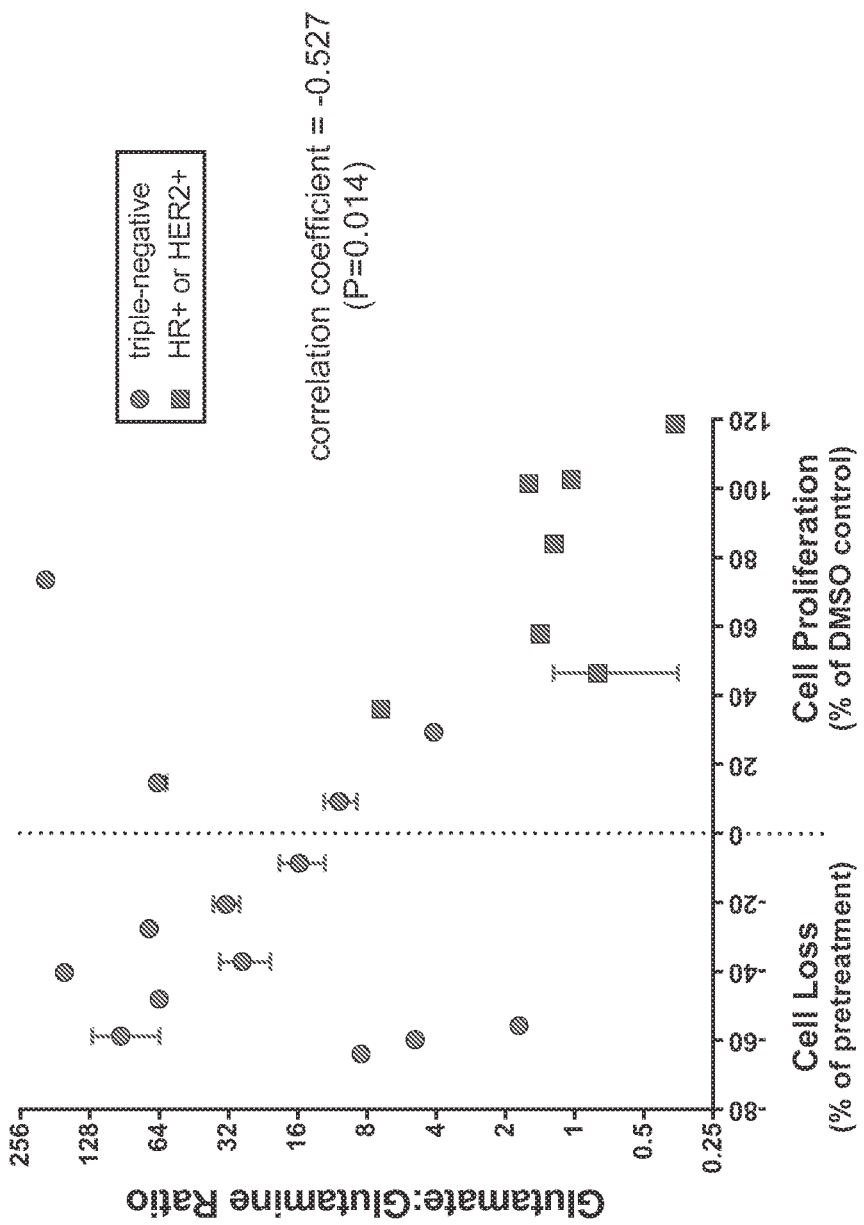

triple-negative
HR+ or HER2+
correlation coefficient = -0.527
(P=0.014)
Glutamate:Glutamine Ratio
256
128
64
32
16
8
4
2
1
0.5
0.25
Cell Loss
(% of pretreatment)
-80
-60
-40
-20
0
Cell Proliferation
(% of DMSO control)
20
40
60
80
100
120
670 Treatment (1 μM for 72 h)

Figure 16

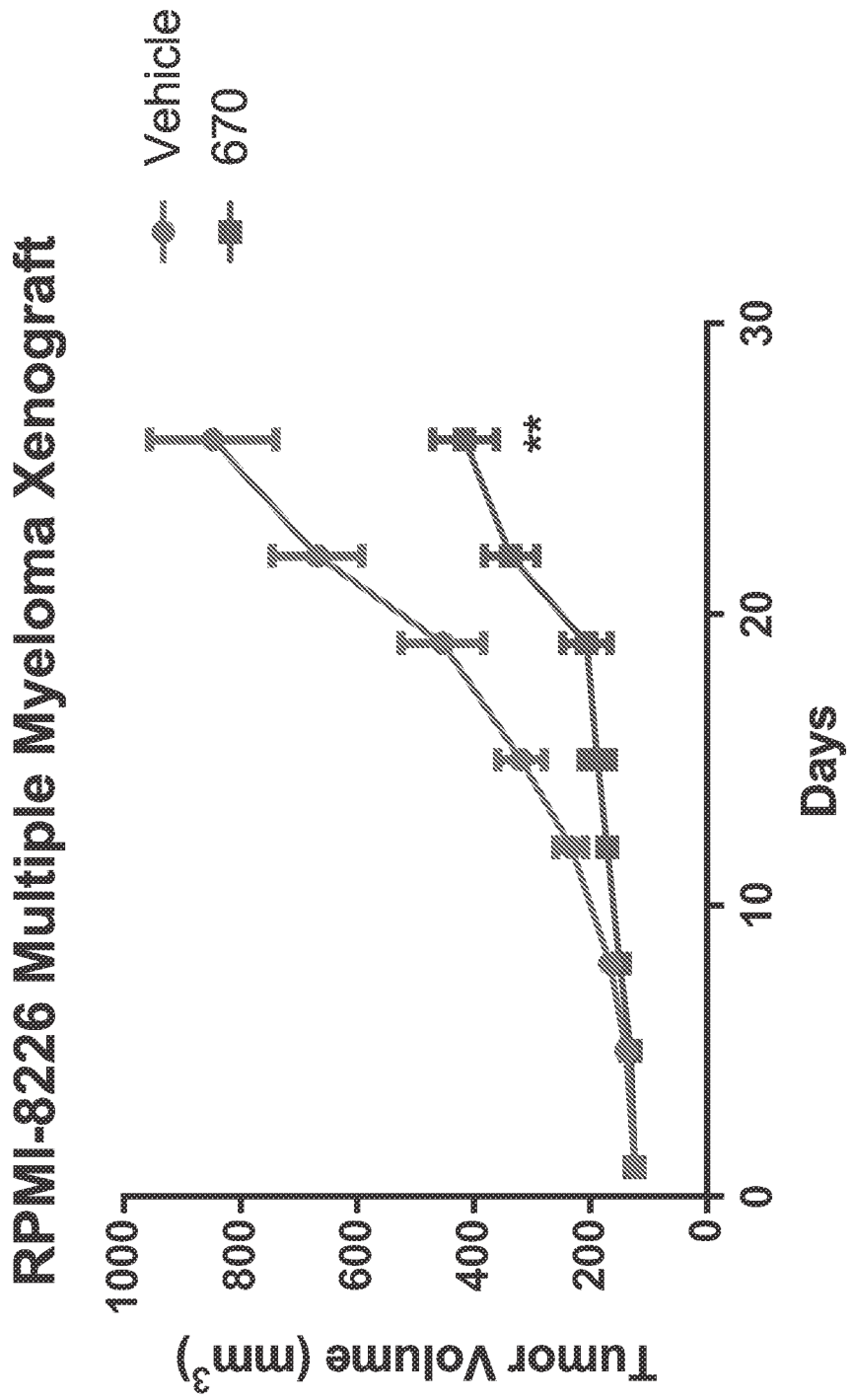

RPMI-8226 Multiple Myeloma Xenograft
Vehicle
670
**
Tumor Volume (mm3)
1000
800
600
400
200
0
0
10
20
30
Days

Figure 17

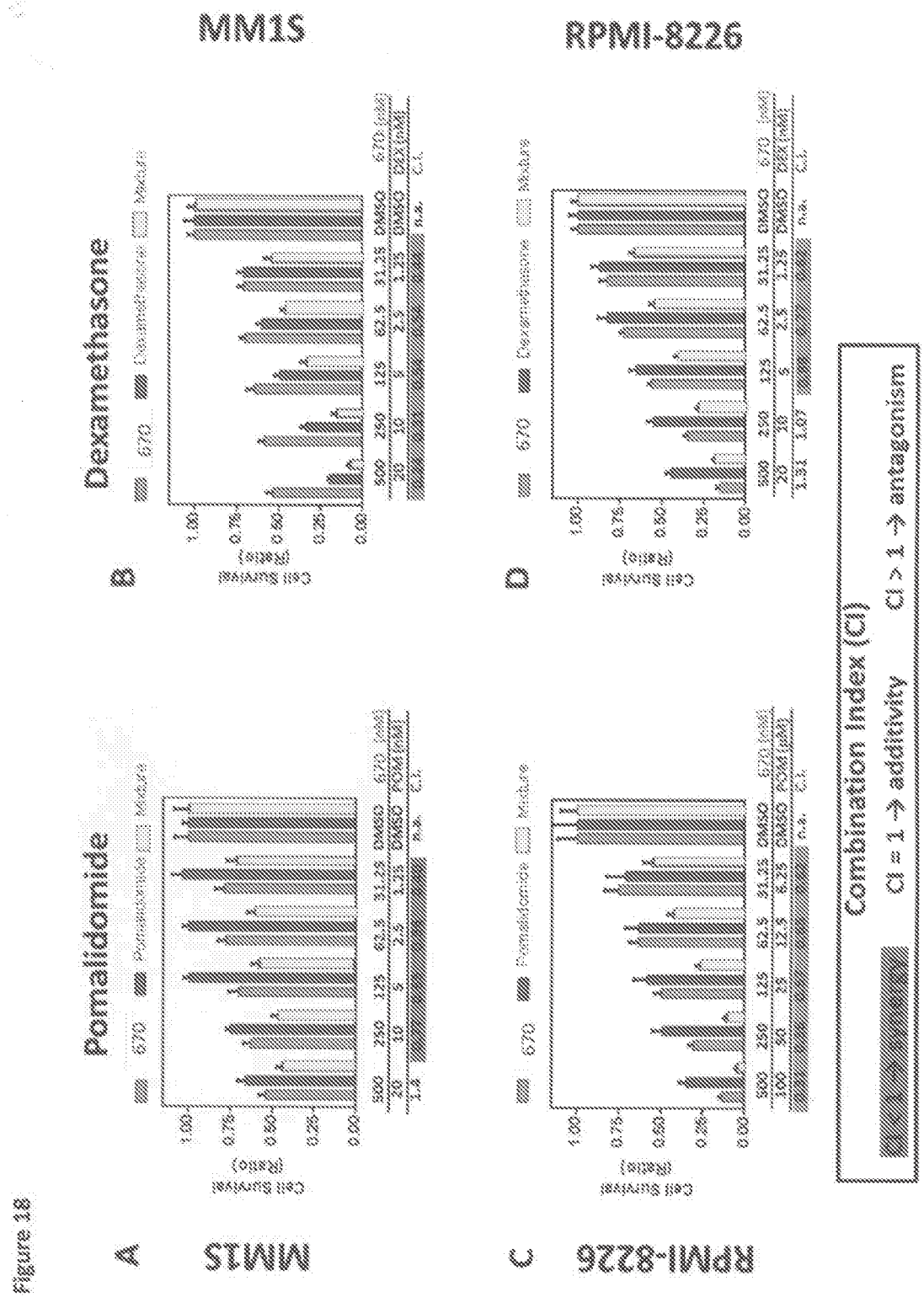

MM1S
RPMI-8226
A
MM1S
Pomalidomide
B
Dexamethasone
C
RPMI-8226
D
Cell Survival (Ratio)
670
Pomalidomide
Dexamethasone
Mixture
DMSO
n.a.
Combination Index (CI)
CI = 1 → additivity
CI > 1 → antagonism

Figure 18