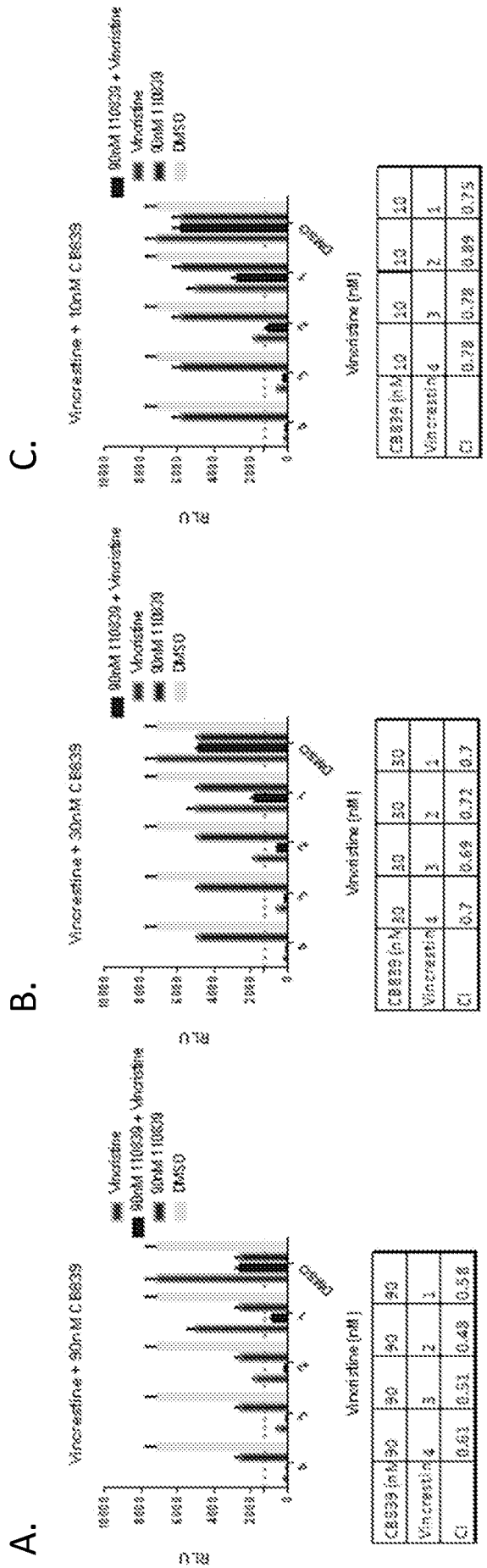

A.
Vincristine + 90nM CB839
Vincristine
90nM 116239 + Vincristine
90nM 116239
DMSO
RLU
Vincristine (nM)
CB839 (nM) 90 90 90 90
Vincristine 4 3 2 1
CI 0.61 0.53 0.48 0.58
B.
Vincristine + 30nM CB839
Vincristine
30nM 116239 + Vincristine
30nM 116239
DMSO
RLU
Vincristine (nM)
CB839 (nM) 30 30 30 30
Vincristine 4 3 2 1
CI 0.7 0.69 0.72 0.7
C.
Vincristine + 10nM CB839
Vincristine
10nM 116239 + Vincristine
10nM 116239
DMSO
RLU
Vincristine (nM)
CB839 (nM) 10 10 10 10
Vincristine 4 3 2 1
CI 0.78 0.78 0.89 0.73

FIG. 1

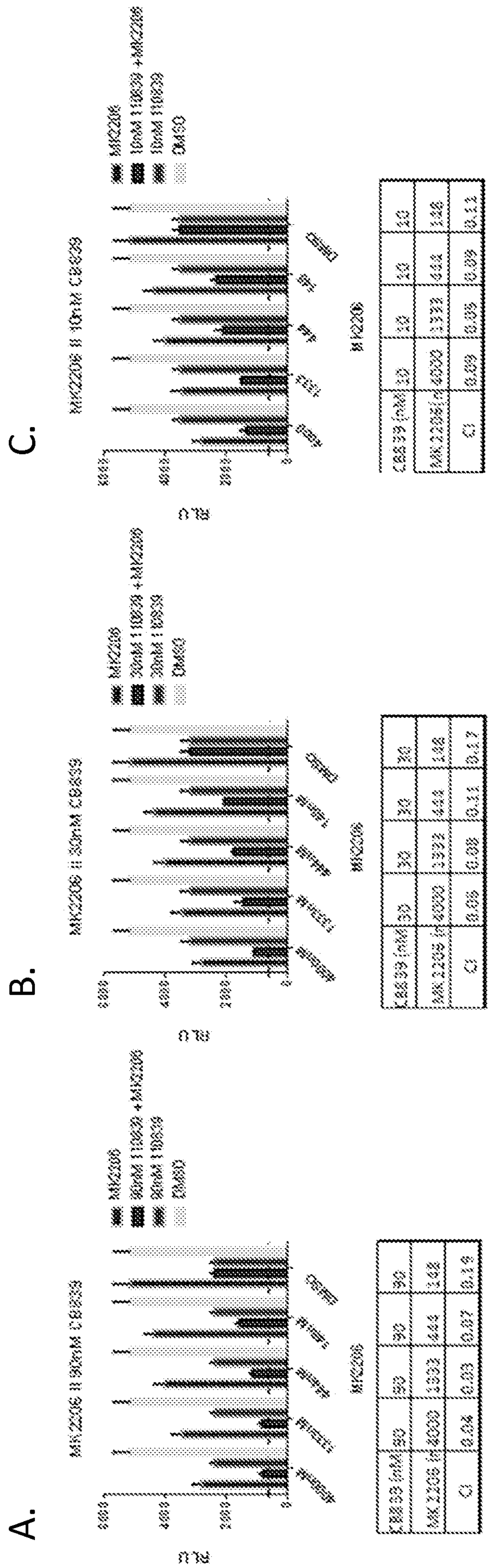

A.
MK2206 ± 90nM CB839
RLU
MK2206
DMSO
CB839 (nM) 90 90 90 90
MK 2206 (nM) 4000 1333 444 148
CI 0.04 0.03 0.07 0.19
B.
MK2206 ± 30nM CB839
RLU
MK2206
DMSO
CB839 (nM) 30 30 30 30
MK 2206 (nM) 4000 1333 444 148
CI 0.06 0.08 0.11 0.17
C.
MK2206 ± 10nM CB839
RLU
MK2206
DMSO
CB839 (nM) 10 10 10 10
MK 2206 (nM) 4000 1333 444 148
CI 0.09 0.05 0.09 0.11

FIG. 2

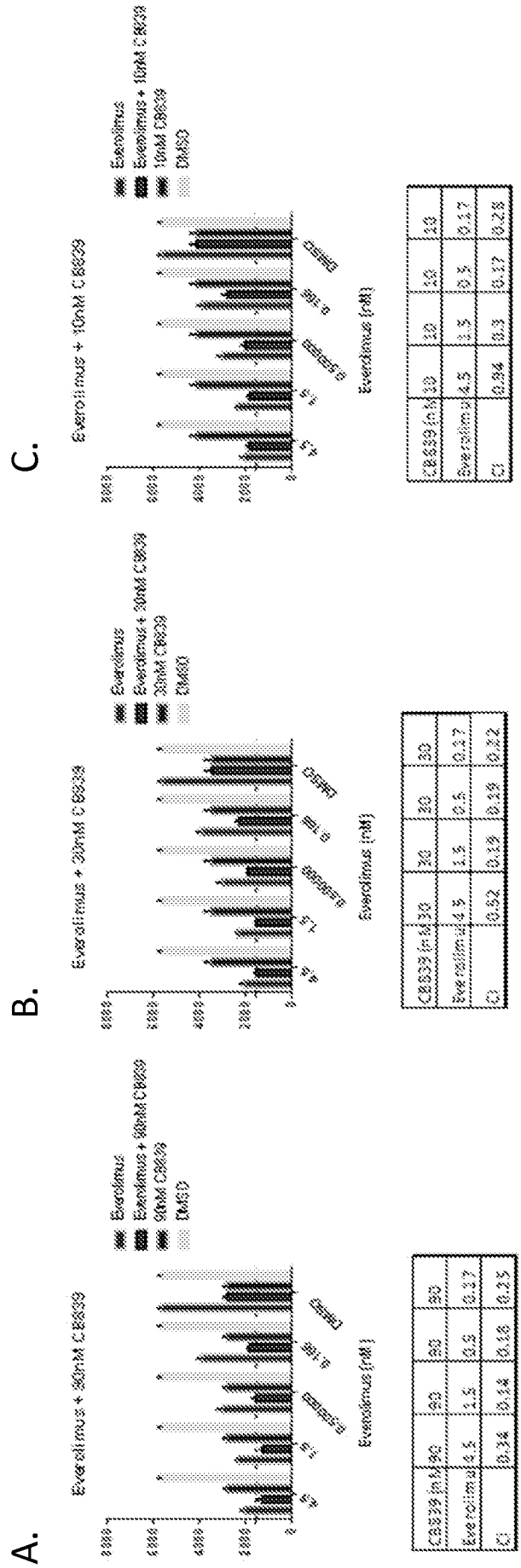

A.
Everolimus + 30nM CB639
Everolimus
Everolimus + 60nM CB639
60nM CB639
DMSO
Everolimus (nM)
CB639 (nM) 60 60 60 60
Everolimus 4.5 1.5 0.5 0.17
CI 0.34 0.18 0.25
B.
Everolimus + 20nM CB639
Everolimus
Everolimus + 30nM CB639
20nM CB639
DMSO
Everolimus (nM)
CB639 (nM) 30 30 30 30
Everolimus 4.5 1.5 0.5 0.17
CI 0.62 0.19 0.19 0.32
C.
Everolimus + 10nM CB639
Everolimus
Everolimus + 10nM CB639
10nM CB639
DMSO
Everolimus (nM)
CB639 (nM) 10 10 10 10
Everolimus 4.5 1.5 0.5 0.17
CI 0.84 0.3 0.17 0.28

FIG. 3

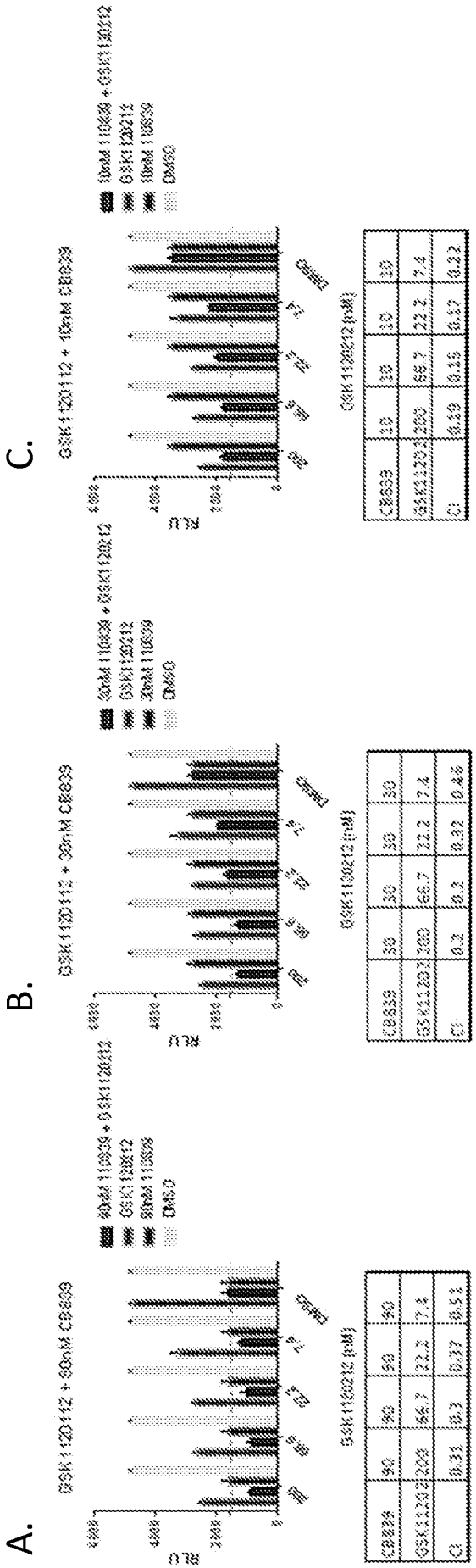

A.
B.
C.
GSK1120212 + 90nM CB839
GSK1120212 + 30nM CB839
GSK1120212 + 10nM CB839
90nM 115839 + GSK1120212
GSK1120212
90nM 115839
30nM 115839
10nM 115839
DMSO
RLU
CB839
GSK1120212
CI

FIG. 4

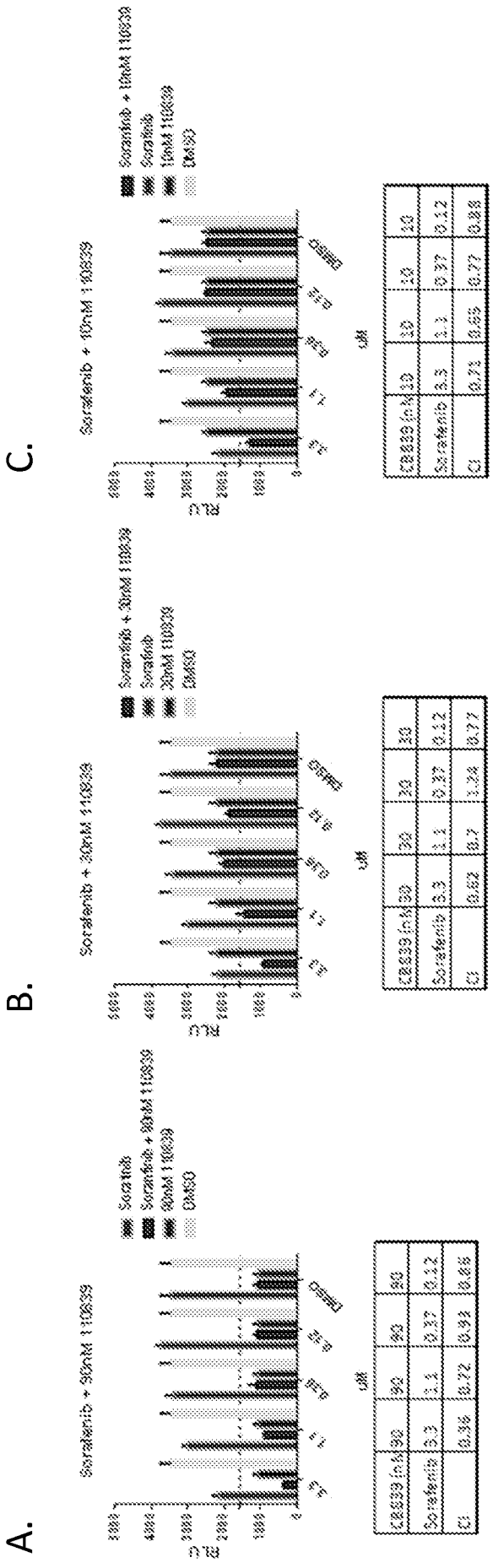

A.
Sorafenib + 90nM 110839
Sorafenib
Sorafenib + 90nM 110839
90nM 110839
DMSO
RLU
0
1000
2000
3000
4000
5000
DMSO
0.5
1.1
3.3
10
30
90
μM
CB839 (nM)
Sorafenib
CI
90
3.3
0.36
90
1.1
0.72
90
0.37
0.94
90
0.12
0.86
B.
Sorafenib + 30nM 110839
Sorafenib
Sorafenib + 30nM 110839
30nM 110839
DMSO
RLU
0
1000
2000
3000
4000
5000
DMSO
0.5
1.1
3.3
10
30
μM
CB839 (nM)
Sorafenib
CI
30
3.3
0.62
30
1.1
0.7
30
0.37
1.28
30
0.12
0.77
C.
Sorafenib + 10nM 110839
Sorafenib
Sorafenib + 10nM 110839
10nM 110839
DMSO
RLU
0
1000
2000
3000
4000
5000
DMSO
0.5
1.1
3.3
10
μM
CB839 (nM)
Sorafenib
CI
10
3.3
0.71
10
1.1
0.69
10
0.37
0.77
10
0.12
0.86

FIG. 5

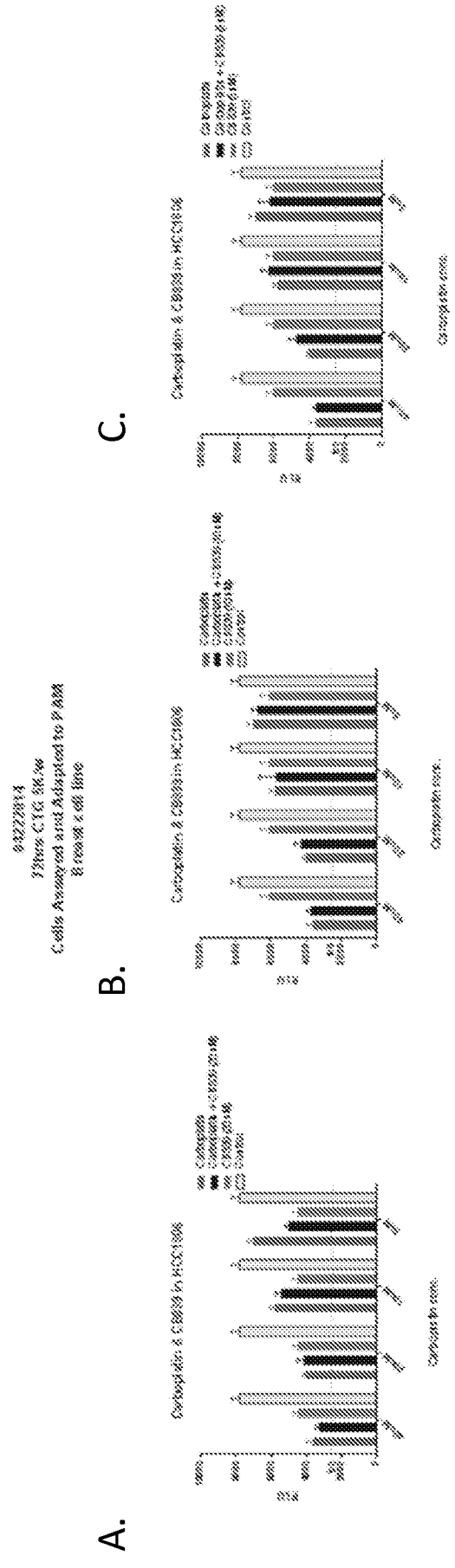


FIG. 6

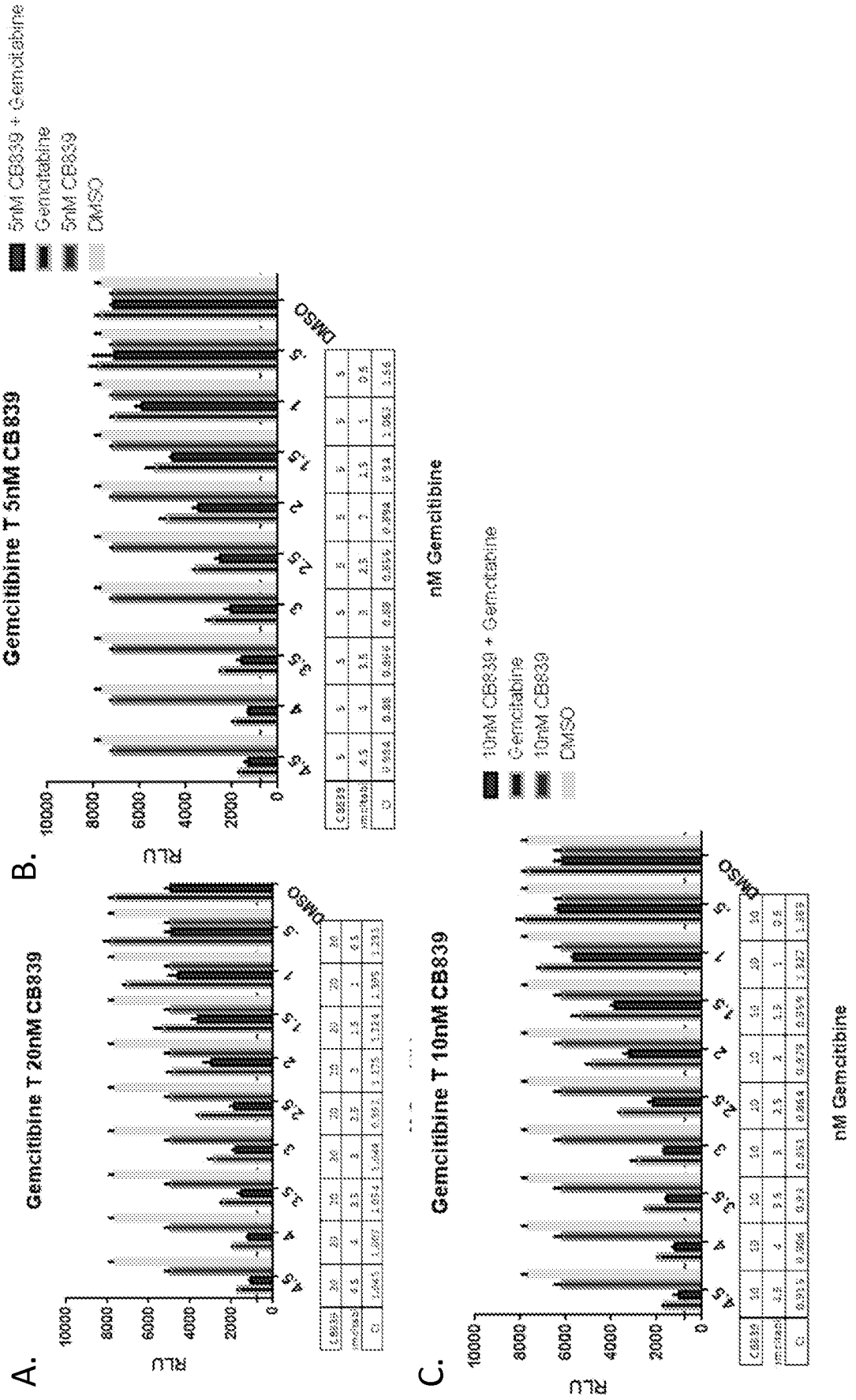

A.
Gemcitibine T 20nM CB839
RLU
nM Gemcitibine
DMSO
B.
Gemcitibine T 5nM CB839
5nM CB839 + Gemcitabine
Gemcitabine
5nM CB839
DMSO
C.
Gemcitibine T 10nM CB839
10nM CB839 + Gemcitabine
Gemcitabine
10nM CB839
DMSO

FIG. 7

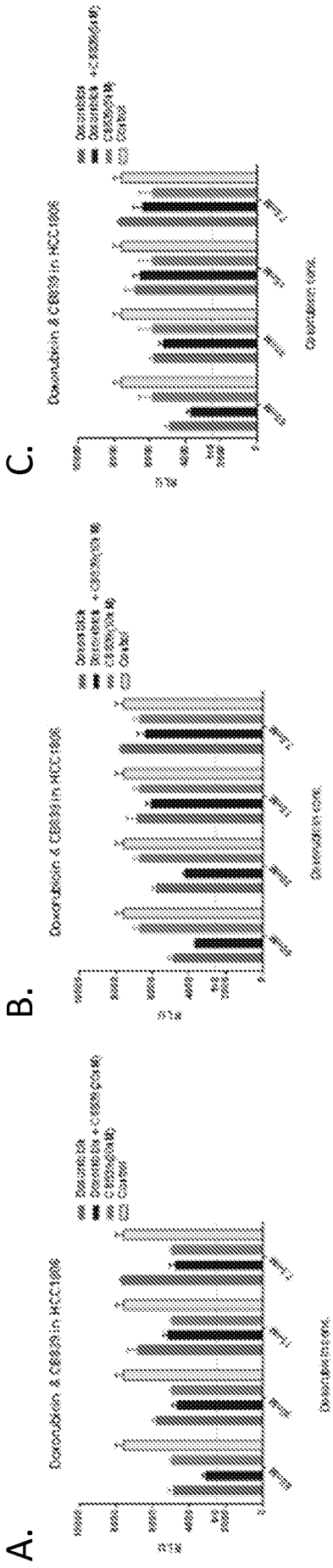
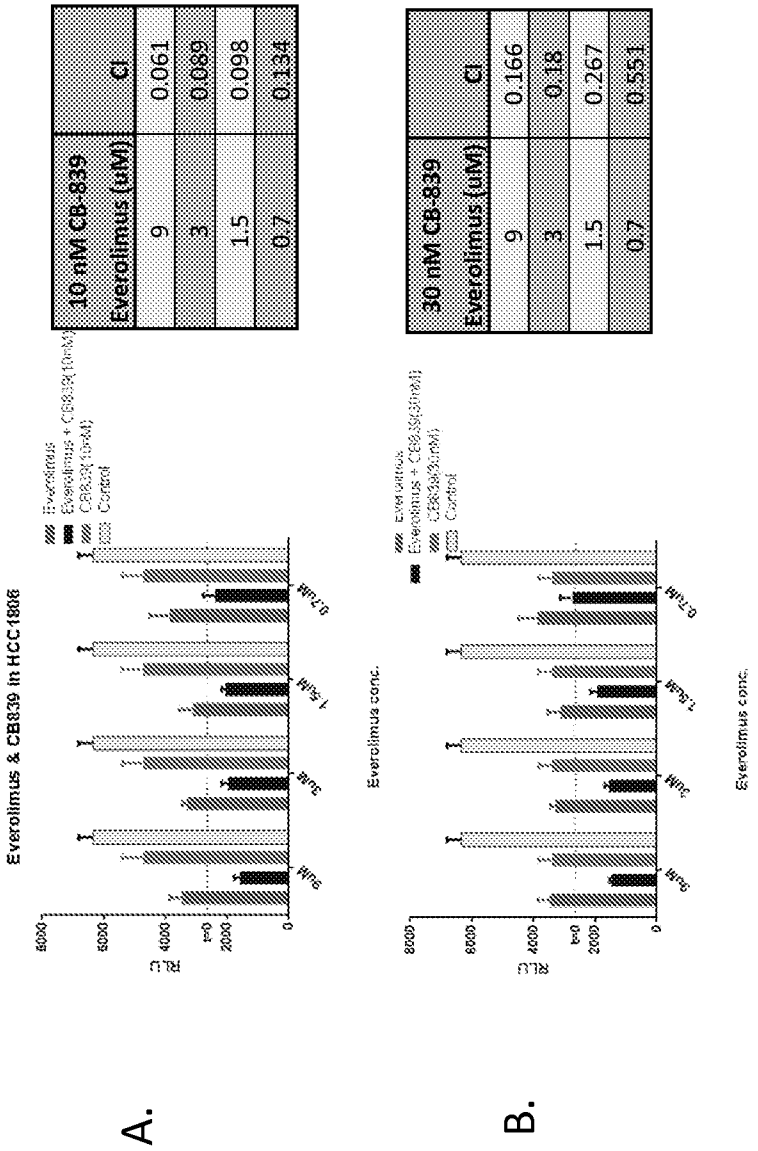


FIG. 8



10 nM CB-839 Everolimus (uM)	CI
9	0.061
3	0.089
1.5	0.098
0.7	0.134

30 nM CB-839 Everolimus (uM)	CI
9	0.166
3	0.18
1.5	0.267
0.7	0.551

FIG. 9

10 nM CB-839 Everolimus (uM)	CI
9	0.061
3	0.089
1.5	0.098
0.7	0.134

30 nM CB-839 Everolimus (uM)	CI
9	0.166
3	0.18
1.5	0.267
0.7	0.551

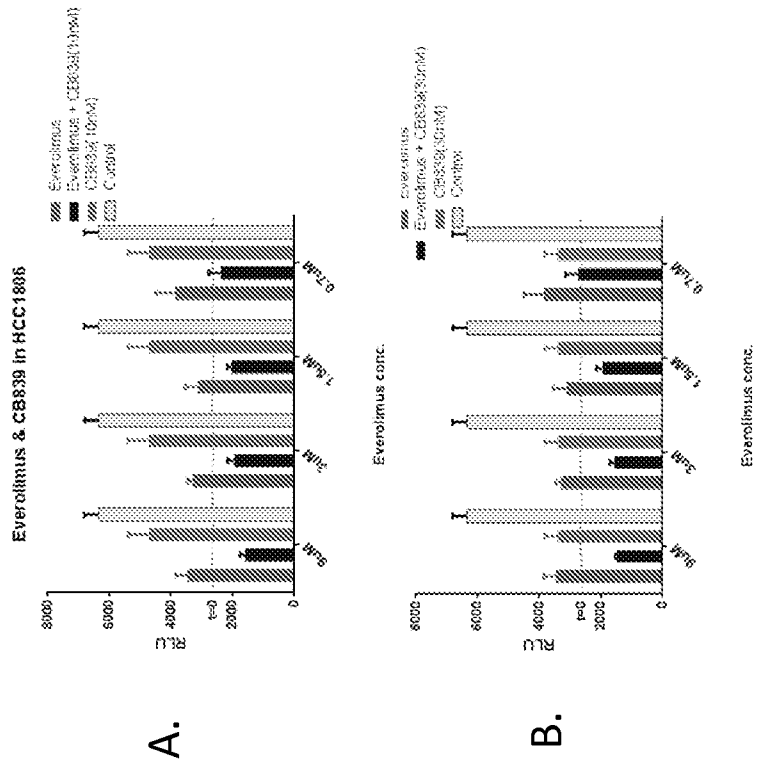


FIG. 10

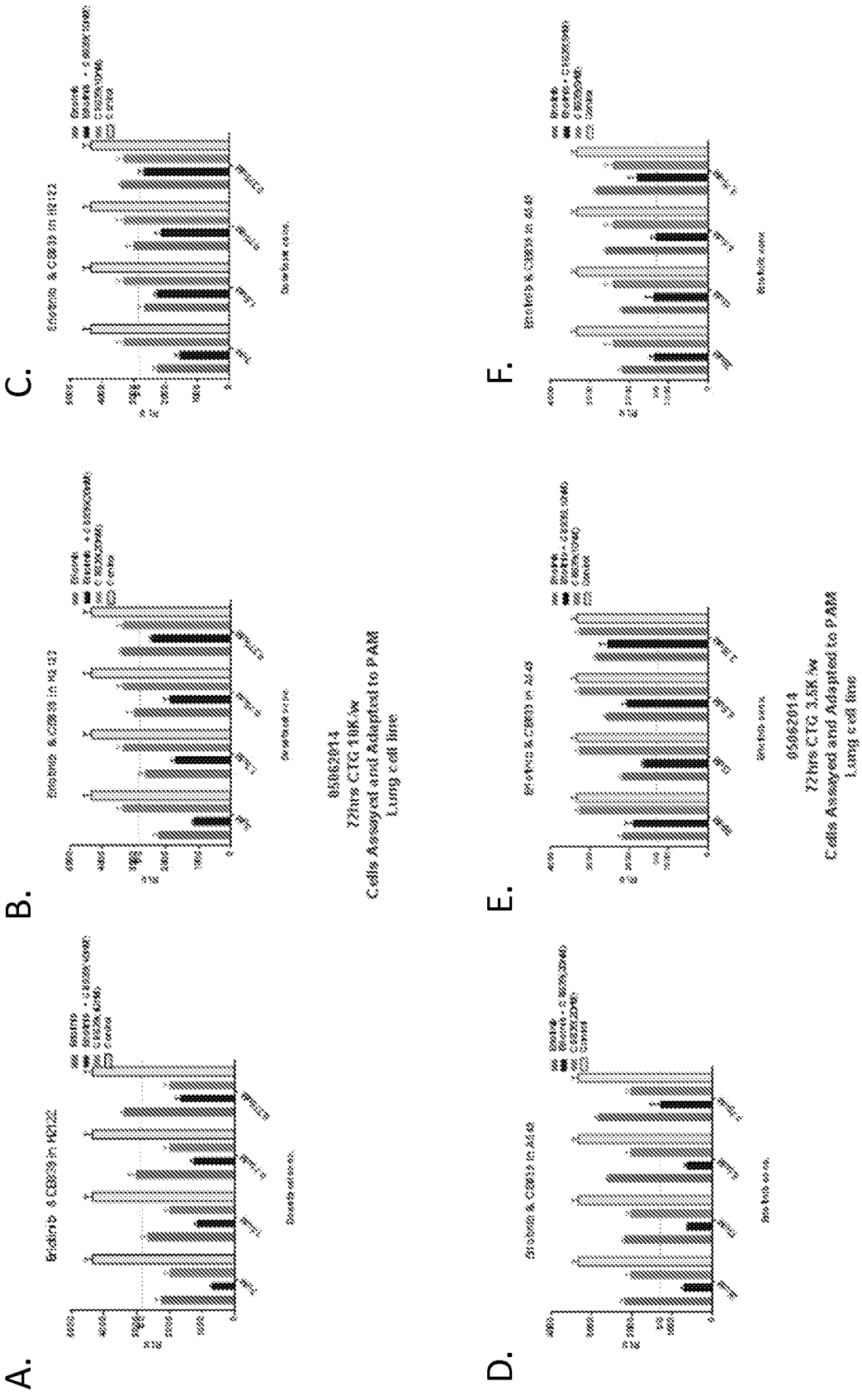
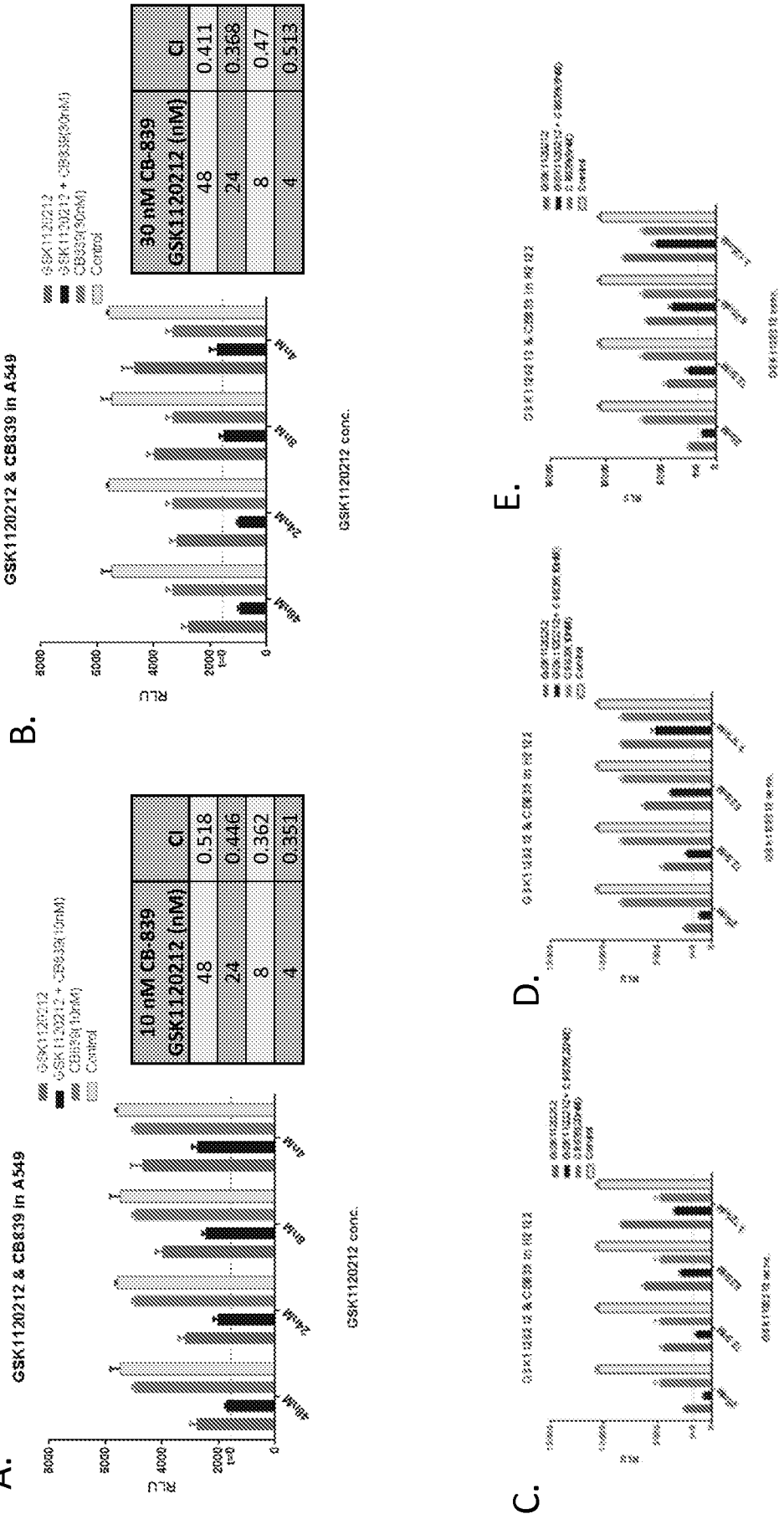


FIG. 11



10 nM CB-839 GSK1120212 (nM)	CI
48	0.518
24	0.446
8	0.362
4	0.351

30 nM CB-839 GSK1120212 (nM)	CI
48	0.411
24	0.368
8	0.47
4	0.513

FIG. 12

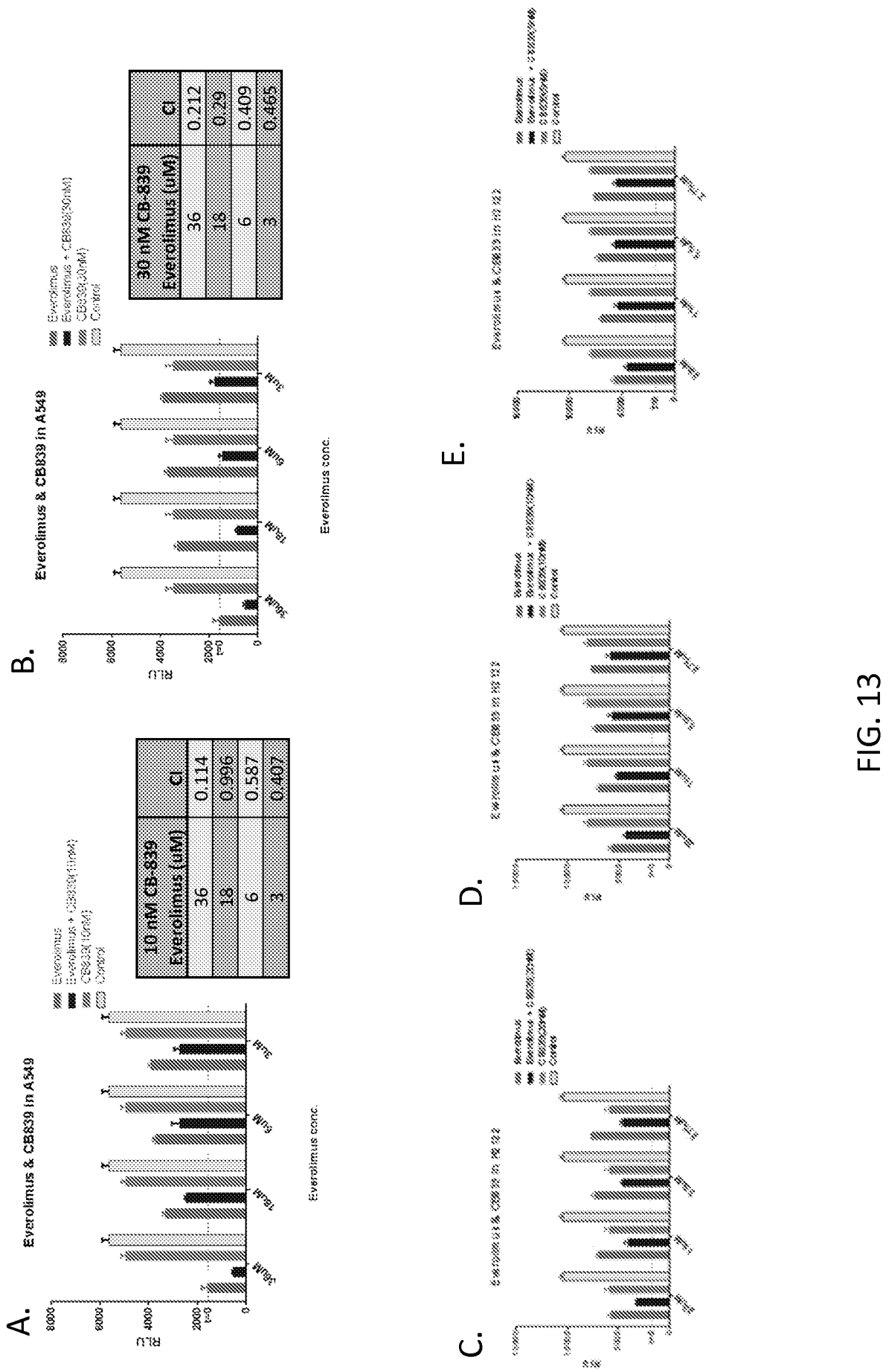

A.
Everolimus & CB839 in A549
Everolimus
Everolimus + CB839(10nM)
CB839(10nM)
Control
RLU
Everolimus conc.
10 nM CB-839
Everolimus (uM)
CI
36
0.114
18
0.996
6
0.587
3
0.407
B.
Everolimus & CB839 in A549
Everolimus
Everolimus + CB839(30nM)
CB839(30nM)
Control
RLU
Everolimus conc.
30 nM CB-839
Everolimus (uM)
CI
36
0.212
18
0.29
6
0.409
3
0.465
C.
D.
E.

FIG. 13

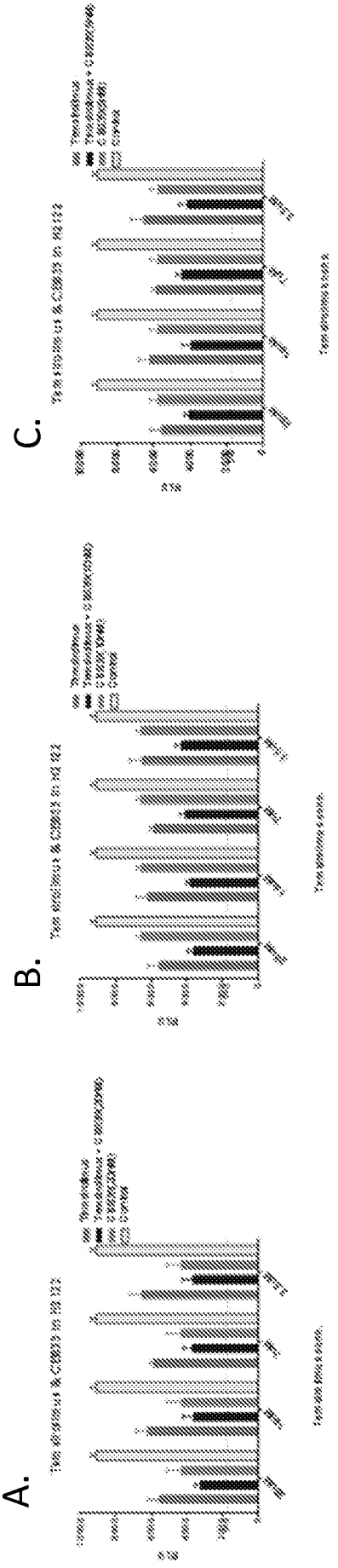


FIG. 14

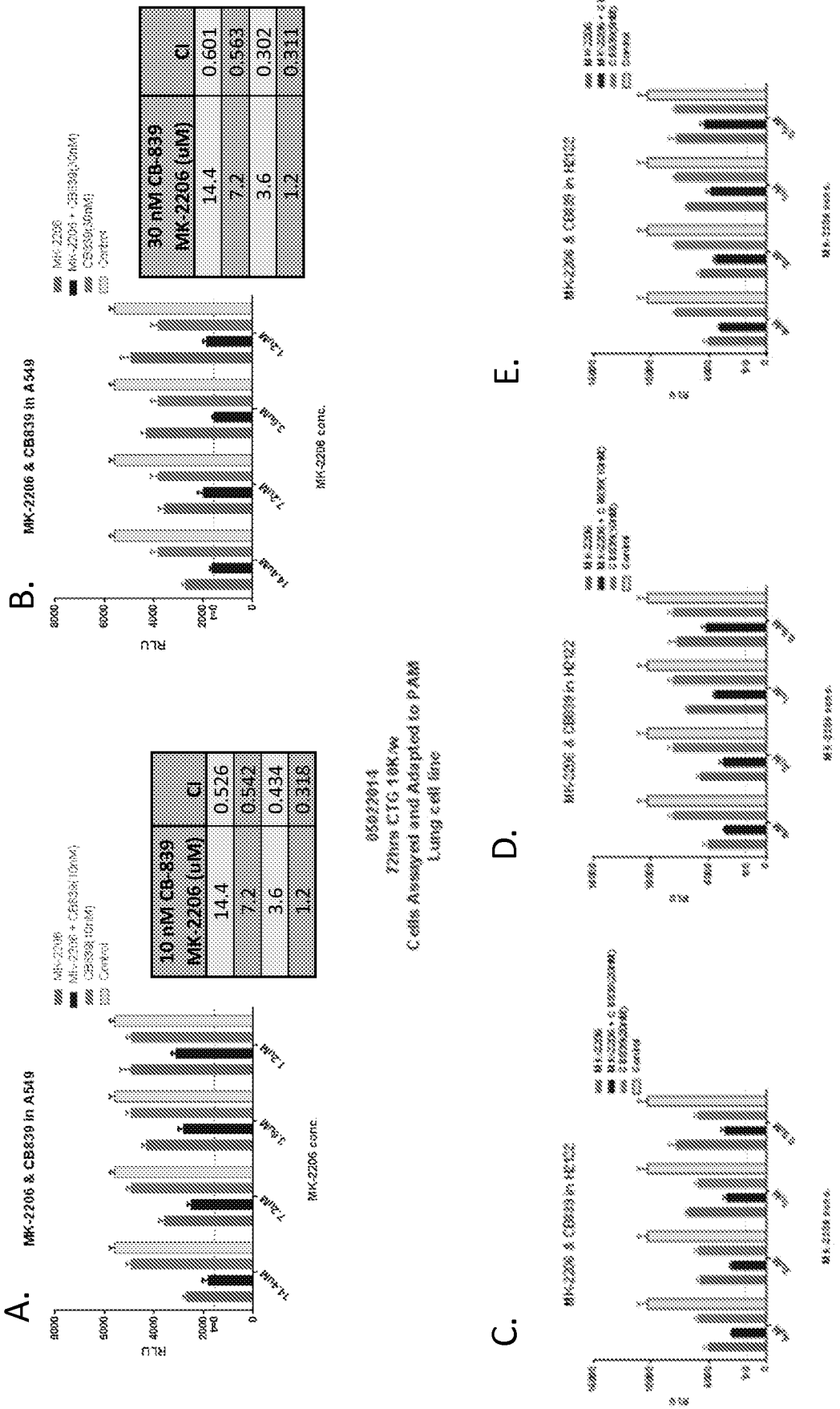

A.
MK-2206 & CB839 in A549
MK-2206
MK-2206 + CB839(10nM)
CB839(10nM)
Control
RLU
MK-2206 conc.
10 nM CB-839
MK-2206 (uM) CI
14.4 0.526
7.2 0.542
3.6 0.434
1.2 0.318
B.
MK-2206 & CB839 in A549
MK-2206
MK-2206 + CB839(30nM)
CB839(30nM)
Control
RLU
MK-2206 conc.
30 nM CB-839
MK-2206 (uM) CI
14.4 0.601
7.2 0.563
3.6 0.302
1.2 0.311
05/27/2014
72hrs CTG 10K/w
Cells Assayed and Adapted to PAM
Lung cell line
C.
D.
E.

FIG. 15

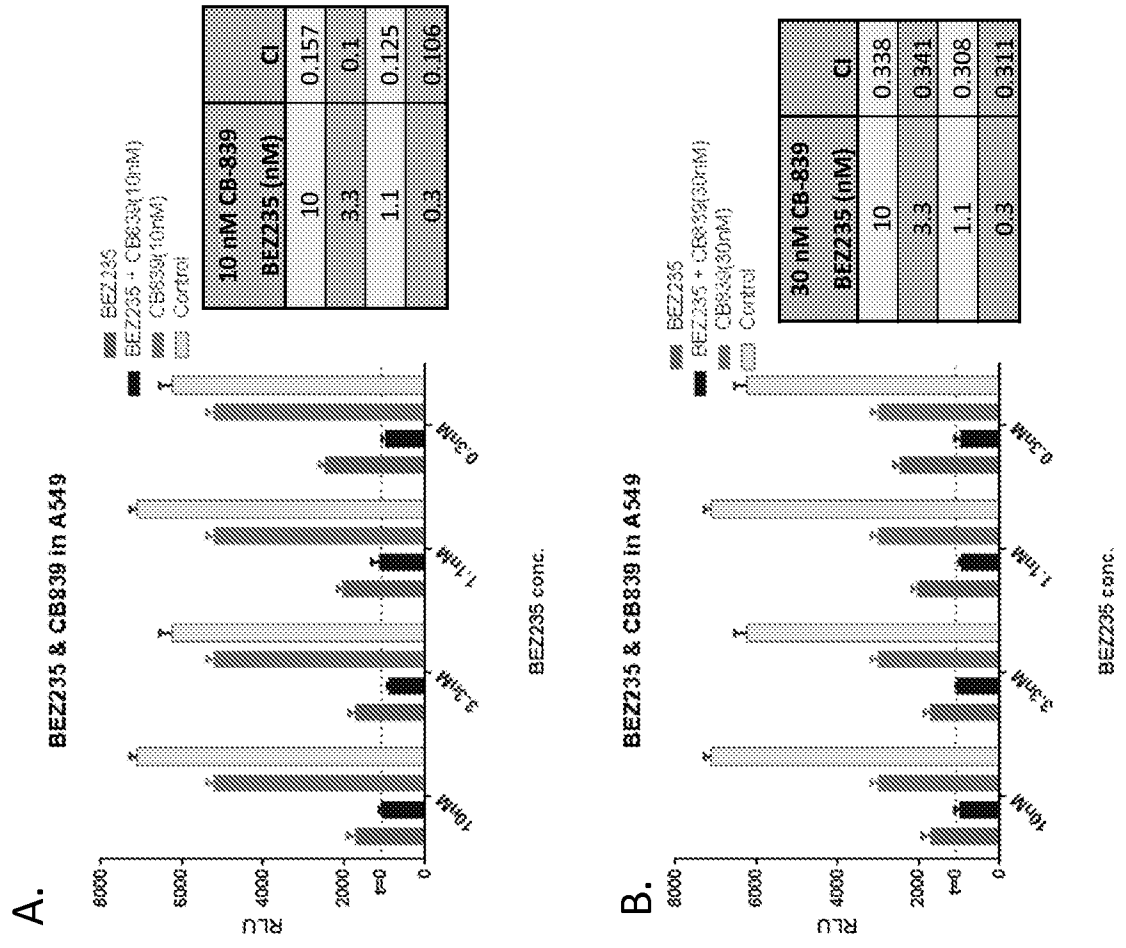

A.
BEZ235 & CB839 in A549
BEZ235
BEZ235 + CB839(10nM)
CB839(10nM)
Control
RLU
8000
6000
4000
2000
0
10nM
3.3nM
1.1nM
0.3nM
BEZ235 conc.
10 nM CB-839
BEZ235 (nM) CI
10 0.157
3.3 0.1
1.1 0.125
0.3 0.106
B.
BEZ235 & CB839 in A549
BEZ235
BEZ235 + CB839(30nM)
CB839(30nM)
Control
RLU
8000
6000
4000
2000
0
10nM
3.3nM
1.1nM
0.3nM
BEZ235 conc.
30 nM CB-839
BEZ235 (nM) CI
10 0.338
3.3 0.341
1.1 0.308
0.3 0.311

FIG. 16

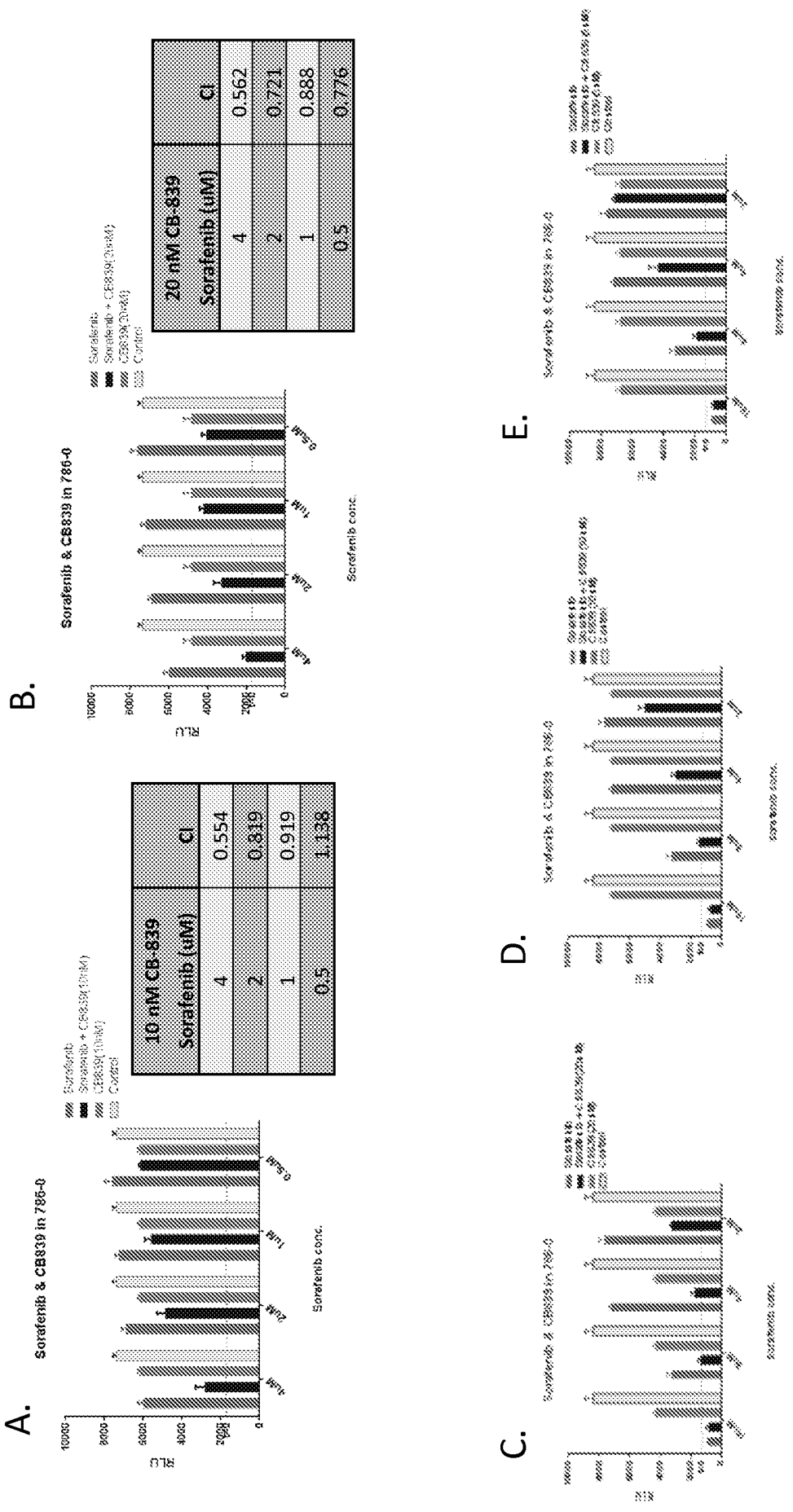

A.
Sorafenib & CB839 in 786-0
Sorafenib
Sorafenib + CB839(10nM)
CB839(10nM)
Control
RLU
Sorafenib conc.
10 nM CB-839
Sorafenib (uM) CI
4 0.554
2 0.819
1 0.919
0.5 1.138
B.
Sorafenib & CB839 in 786-0
Sorafenib
Sorafenib + CB839(20nM)
CB839(20nM)
Control
RLU
Sorafenib conc.
20 nM CB-839
Sorafenib (uM) CI
4 0.562
2 0.721
1 0.888
0.5 0.776
C.
D.
E.

FIG. 17

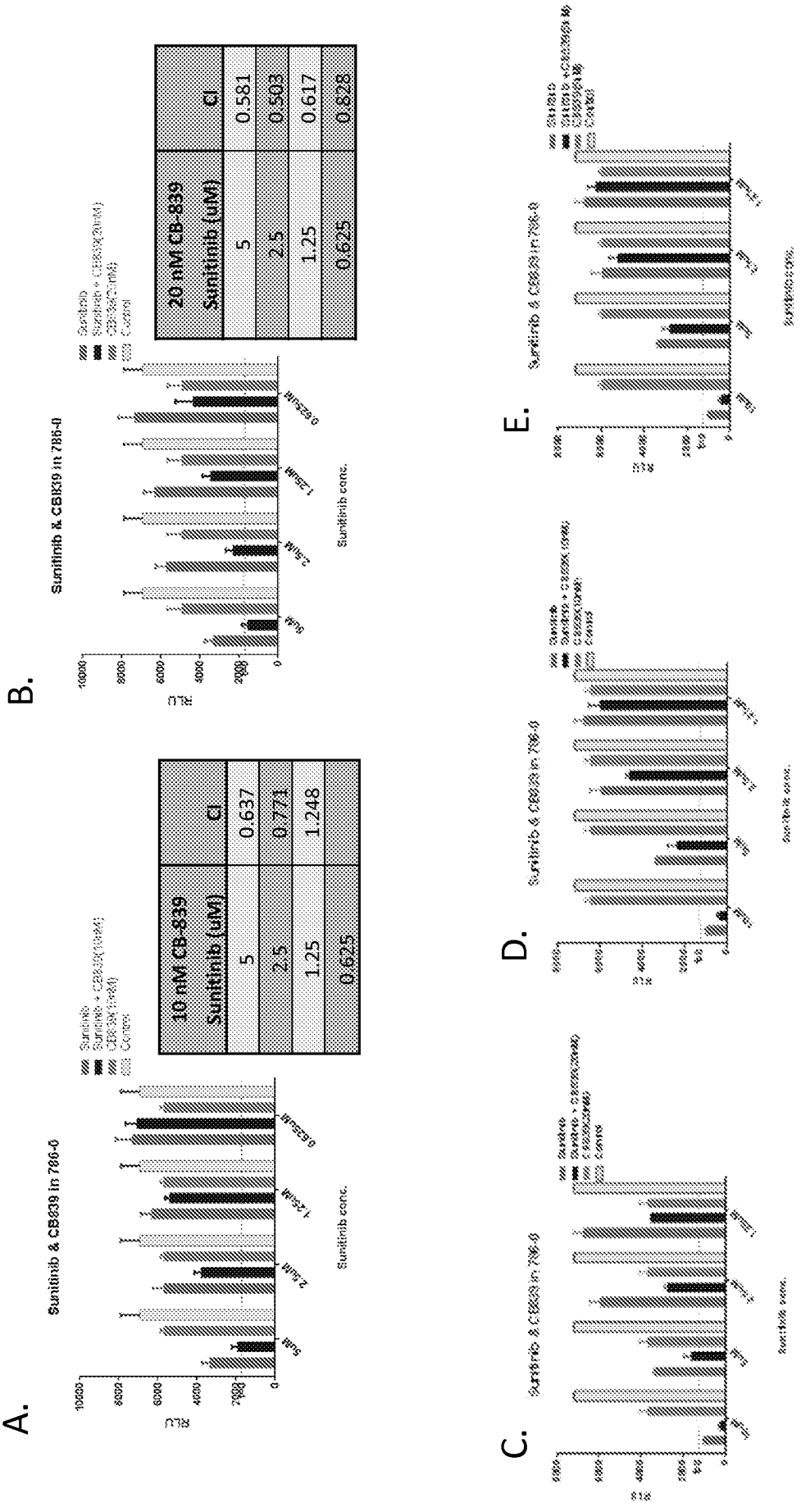
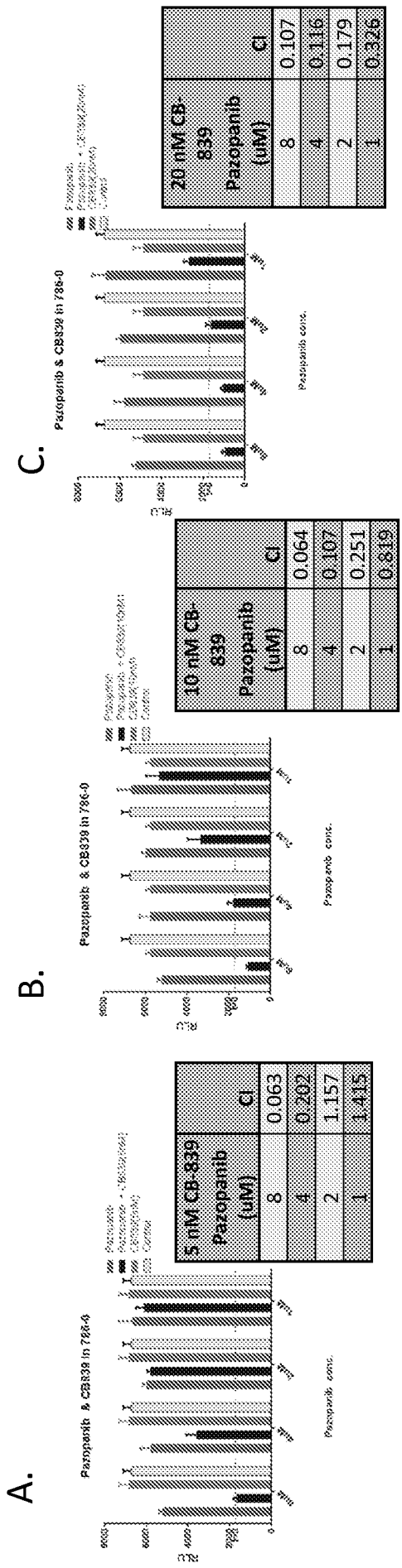

A.
Sunitinib & CB839 in 786-0
Sunitinib
Sunitinib + CB839(10nM)
CB839(10nM)
Control
RLU
Sunitinib conc.
10 nM CB-839
Sunitinib (uM) CI
5 0.637
2.5 0.771
1.25 1.248
0.625
B.
Sunitinib & CB839 in 786-0
20 nM CB-839
Sunitinib (uM) CI
5 0.581
2.5 0.503
1.25 0.617
0.625 0.828
C.
D.
E.
Sunitinib & CB839 in 786-0

FIG. 18



5 nM CB-839 Pazopanib (uM)	CI
8	0.063
4	0.202
2	1.157
1	1.415

10 nM CB-839 Pazopanib (uM)	CI
8	0.064
4	0.107
2	0.251
1	0.819

20 nM CB-839 Pazopanib (uM)	CI
8	0.107
4	0.116
2	0.179
1	0.326

FIG. 19

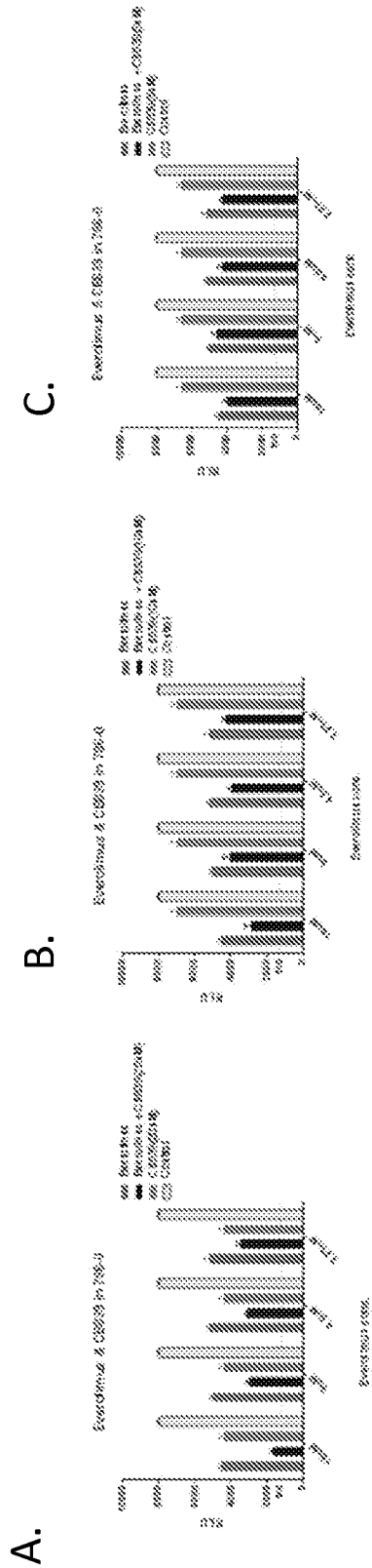
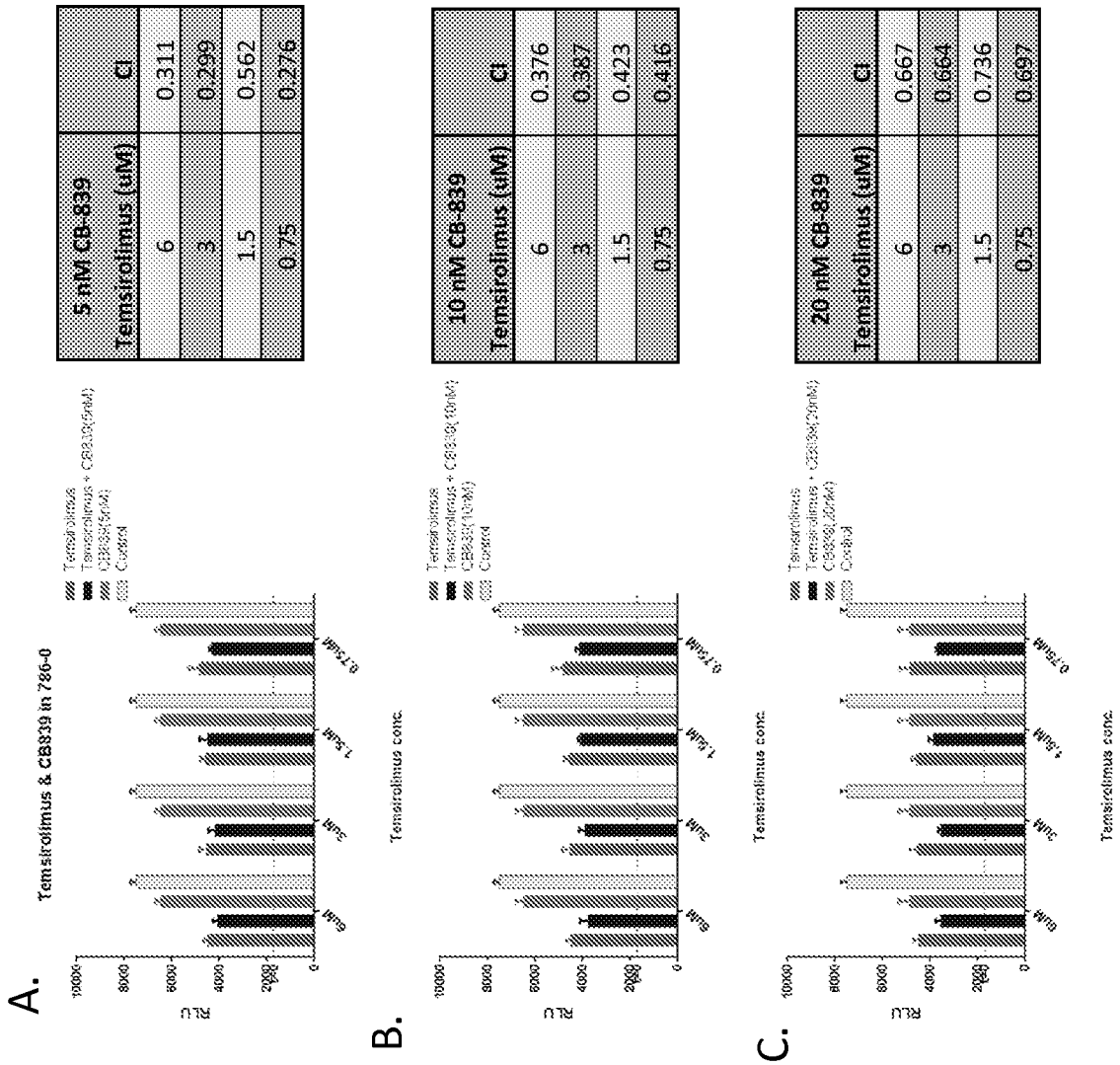


FIG. 20

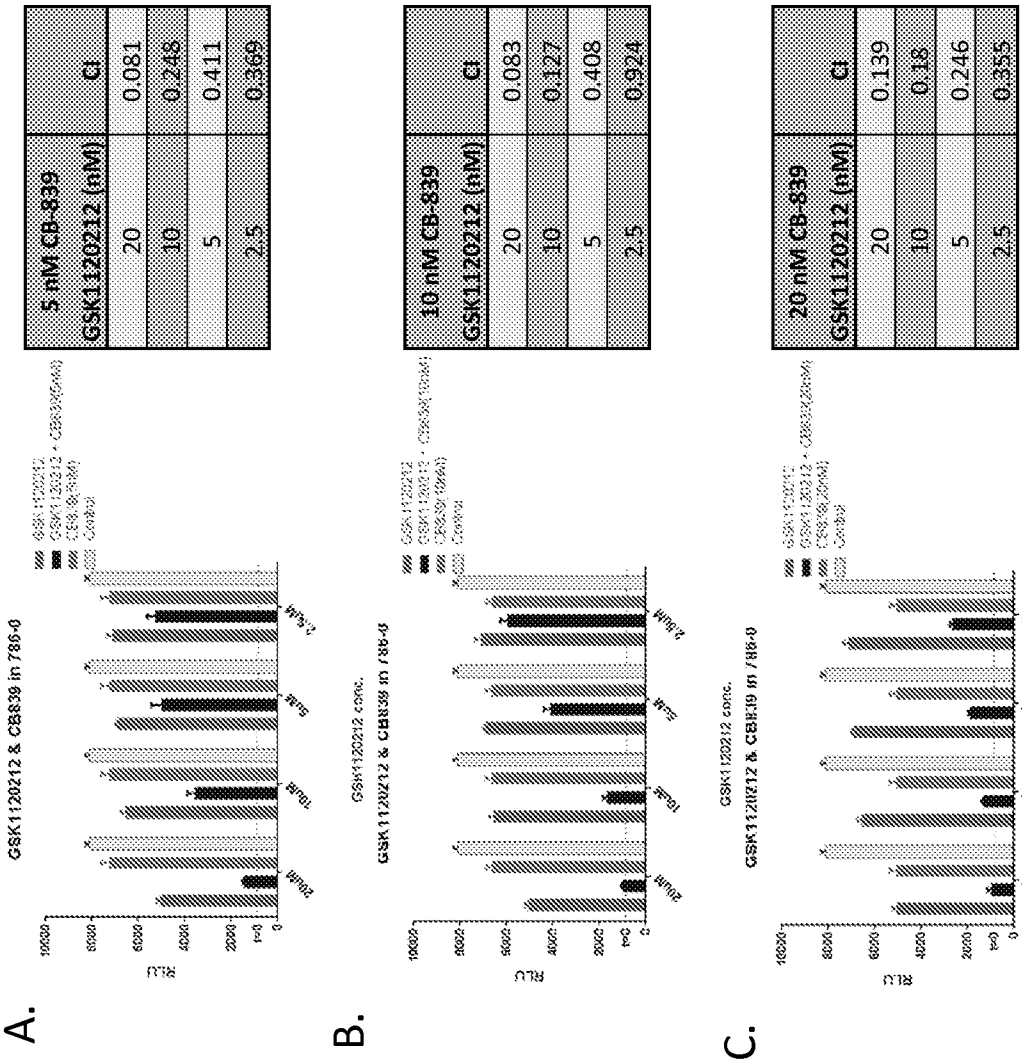


5 nM CB-839 Temsirolimus (uM)	CI
6	0.311
3	0.299
1.5	0.562
0.75	0.276

10 nM CB-839 Temsirolimus (uM)	CI
6	0.376
3	0.387
1.5	0.423
0.75	0.416

20 nM CB-839 Temsirolimus (uM)	CI
6	0.667
3	0.664
1.5	0.736
0.75	0.697

FIG. 21



5 nM CB-839 GSK1120212 (nM)	CI
20	0.081
10	0.248
5	0.411
2.5	0.369

10 nM CB-839 GSK1120212 (nM)	CI
20	0.083
10	0.127
5	0.408
2.5	0.924

20 nM CB-839 GSK1120212 (nM)	CI
20	0.139
10	0.18
5	0.246
2.5	0.355

FIG. 22

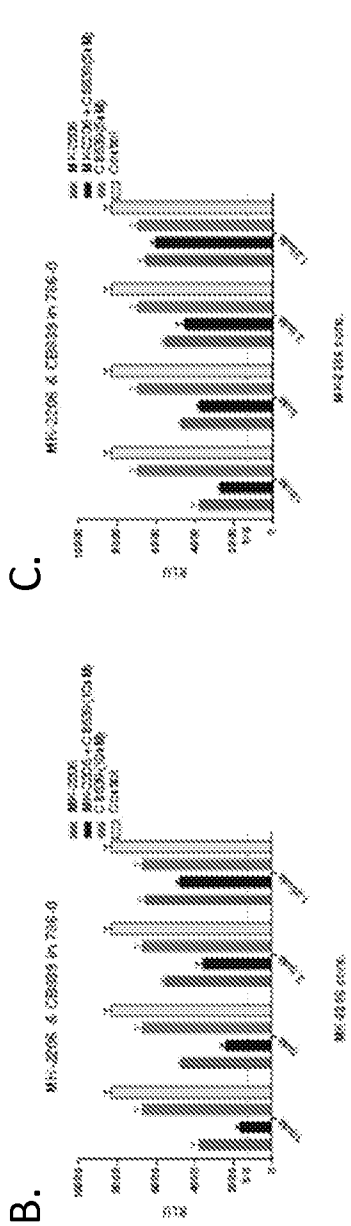


FIG. 23

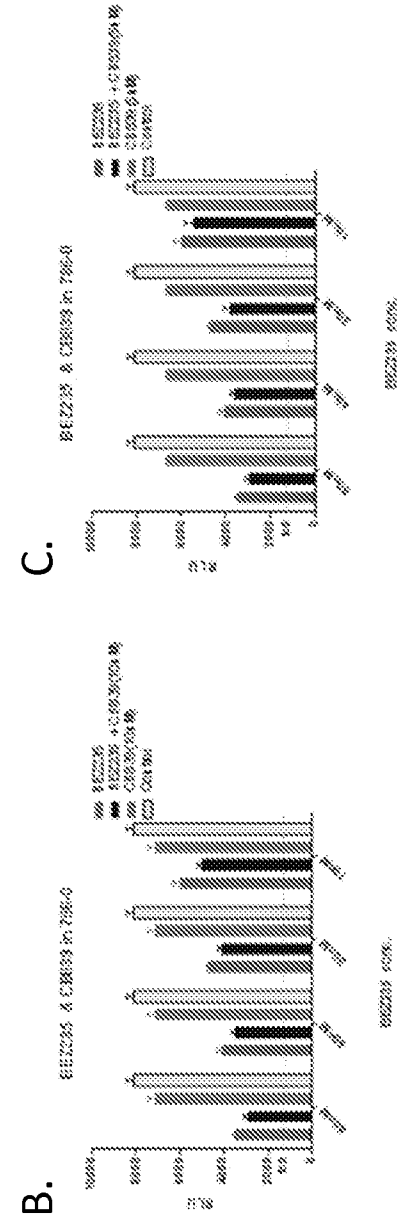


FIG. 24

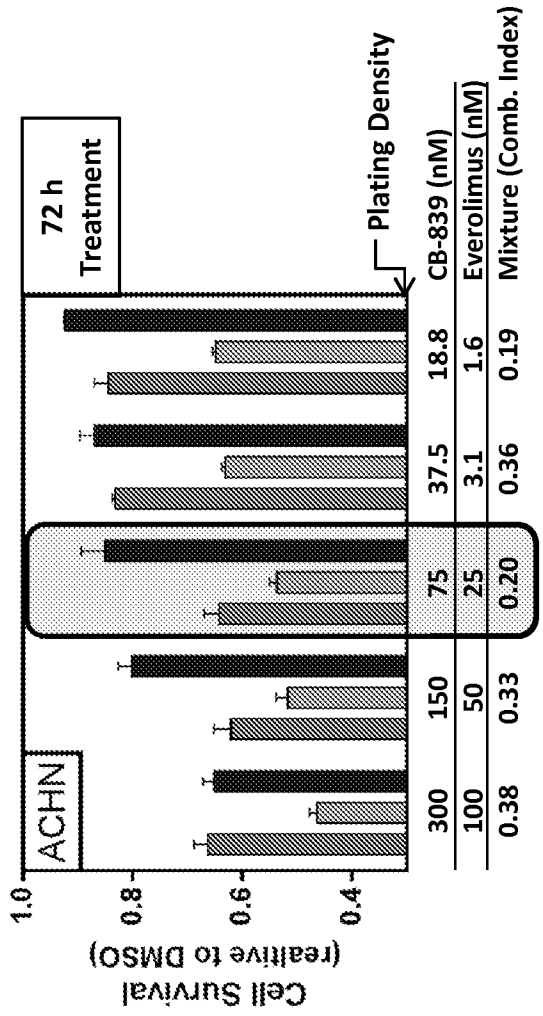
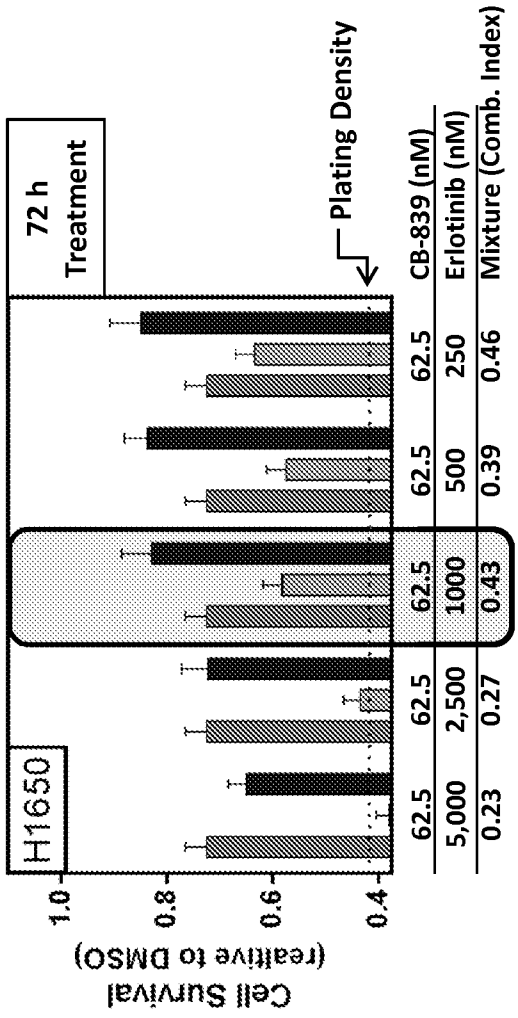

Cell Survival
(realtive to DMSO)
1.0
0.8
0.6
0.4
ACHN
72 h
Treatment
Plating Density
CB-839 (nM)
Everolimus (nM)
Mixture (Comb. Index)
300 150 75 37.5 18.8
100 50 25 3.1 1.6
0.38 0.33 0.20 0.36 0.19

FIG. 25


H1650
72 h Treatment
Plating Density
Cell Survival (realtive to DMSO)
1.0
0.8
0.6
0.4
CB-839 (nM)
Erlotinib (nM)
Mixture (Comb. Index)
62.5 62.5 62.5 62.5 62.5
5,000 2,500 1000 500 250
0.23 0.27 0.43 0.39 0.46

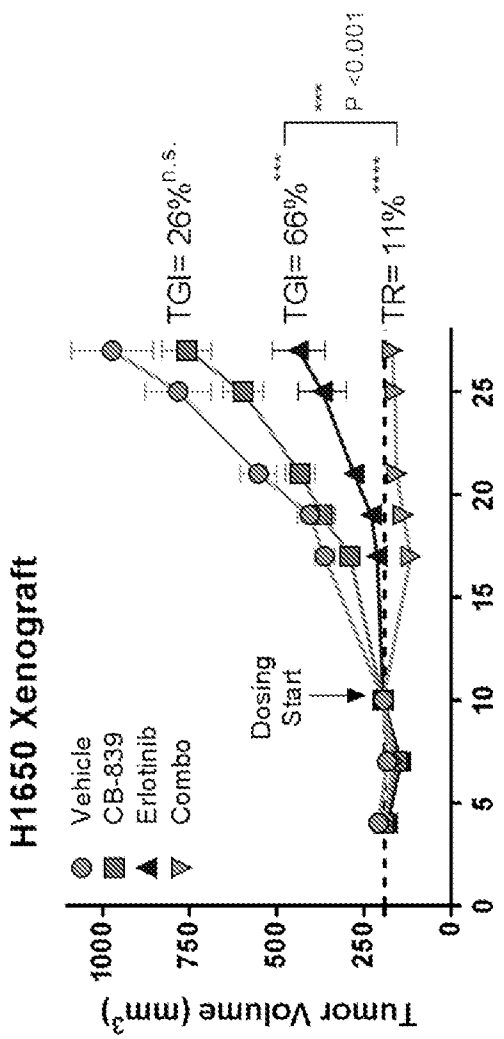

H1650 Xenograft
Vehicle
CB-839
Erlotinib
Combo
Dosing Start
TGI= 26% n.s.
TGI= 66% ***
TR= 11% ****
P <0.001
Tumor Volume (mm3)
1000
750
500
250
0
0 5 10 15 20 25

FIG. 26