A. RSPO1

| ID | Original Source | Purity | Treatment | Tissue Type | Avg Sig | STDev | PA Calls P | PA Calls A | PA Calls M | 139 278 417 556 |
|---|---|---|---|---|---|---|---|---|---|---|
| Colon Diseased | Colon | DISEASED | Colon::DISE... | | 4.86 | 0.14 | 0 | 21 | 0 | |
| Colon Benign | Colon | BENIGN | Colon::BENI... | | 4.92 | 0.09 | 0 | 24 | 0 | |
| Breast Normal | Breast | NORMAL | Breast::NOR... | | 11.25 | 13.18 | 5 | 17 | 0 | |
| Breast Malignant | Breast | MALIGNANT | Breast::MAL... | | 4.96 | 0.87 | 1 | 160 | 0 | |
| Breast Benign | Breast | BENIGN | Breast::BEN... | | 42.26 | 72.70 | 2 | 7 | 0 | |
| Brain Benign | Brain | BENIGN | Brain::BENI... | | 4.82 | 0.05 | 0 | 16 | 0 | |
| Brain Malignant | Brain | MALIGNANT | Brain::MALI... | | 4.82 | 0.16 | 0 | 23 | 0 | |
| Liver Benign | Liver | BENIGN | Liver::BENI... | | 4.91 | 0.14 | 0 | 4 | 0 | |
| Kidney Normal | Kidney | NORMAL | Cortex of k... | | 4.88 | 0.12 | 0 | 61 | 0 | |
| Kidney Malignant | Kidney | MALIGNANT | Kidney::MAL... | | 5.70 | 5.24 | 3 | 88 | 0 | |
| Kidney Benign | Kidney | BENIGN | Kidney::BEN... | | 4.94 | 0.12 | 0 | 15 | 0 | |
| Endometrium Malignant | Endometrium | MALIGNANT | Endometrium... | | 11.76 | 22.09 | 5 | 52 | 0 | |
| Endometrium Benign | Endometrium | BENIGN | Endometrium... | | 16.09 | 23.46 | 3 | 7 | 0 | |
| Colon Normal | Colon | NORMAL | Ascending c... | | 4.92 | 0.39 | 0 | 74 | 0 | |
| Colon Malignant | Colon | MALIGNANT | Colon::MALI... | | 4.93 | 0.85 | 1 | 140 | 0 | |
| Ovary Malignant | Ovary | MALIGNANT | Ovary::MALI... | | 33.80 | 85.09 | 32 | 105 | 1 | |
| Ovary Normal | Ovary | NORMAL | Ovary::NORM... | | 5.61 | 1.28 | 0 | 7 | 0 | |
| Lung Normal | Lung | NORMAL | Lung::NORMA... | | 5.33 | 0.95 | 1 | 63 | 0 | |
| Ovary Benign | Ovary | BENIGN | Ovary::BENI... | | 20.32 | 38.55 | 5 | 30 | 0 | |
| Lung Malignant | Lung | MALIGNANT | Lung::MALIG... | | 4.94 | 0.73 | 0 | 124 | 0 | |
| Lung Benign | Lung | BENIGN | Lung::BENIG... | | 4.80 | 0.08 | 0 | 5 | 0 | |
| Liver Diseased | Liver | DISEASED | Liver::DISE... | | 4.89 | 0.22 | 0 | 22 | 0 | |
| Liver Malignant | Liver | MALIGNANT | Liver::MALI... | | 4.92 | 0.15 | 0 | 25 | 0 | |
| Liver Normal | Liver | NORMAL | Liver::NORM... | | 4.95 | 0.13 | 0 | 6 | 0 | |
| Prostate Malignant | Prostate | MALIGNANT | Prostate::M... | | 5.00 | 0.69 | 0 | 73 | 0 | |
| Prostate Normal | Prostate | NORMAL | Prostate::N... | | 6.03 | 6.23 | 0 | 32 | 0 | |
| Pancreas Normal | Pancreas | NORMAL | Pancreas::N... | | 4.99 | 0.11 | 0 | 13 | 0 | |
| Prostate Diseased | Prostate | DISEASED | Prostate::D... | | 5.97 | 2.20 | 2 | 18 | 0 | |
| Pancreas Benign | Pancreas | BENIGN | Pancreas::B... | | 4.93 | 0.13 | 0 | 5 | 0 | |
| Pancreas Malignant | Pancreas | MALIGNANT | Pancreas::M... | | 4.79 | 0.11 | 0 | 66 | 0 | |
| ID | Original Source | Purity | Treatment | Tissue Type | Avg Sig | STDev | P | A | M | 139 278 417 556 |

Ovary

FIG. 1

B. RSPO2

| ID | Original Source | Purity | Treatment | Tissue Type | Avg Sig | STDev | PA Calls P | PA Calls A | PA Calls M | 152 304 456 609 |
|---|---|---|---|---|---|---|---|---|---|---|
| Colon Diseased | Colon | DISEASED | Colon::DISE... | | 28.82 | 51.62 | 16 | 5 | 0 | |
| Colon Benign | Colon | BENIGN | Colon::BENI... | | 7.40 | 6.58 | 3 | 21 | 0 | |
| Breast Normal | Breast | NORMAL | Breast::NOR... | | 7.04 | 7.00 | 1 | 21 | 0 | |
| Breast Malignant | Breast | MALIGNANT | Breast::MAL... | | 5.82 | 2.16 | 5 | 156 | 0 | |
| Breast Benign | Breast | BENIGN | Breast::BEN... | | 5.59 | 0.12 | 1 | 8 | 0 | |
| Brain Benign | Brain | BENIGN | Brain::BENI... | | 5.66 | 0.15 | 0 | 16 | 0 | |
| Brain Malignant | Brain | MALIGNANT | Brain::MALI... | | 16.70 | 30.11 | 11 | 12 | 0 | |
| Liver Benign | Liver | BENIGN | Liver::BENI... | | 6.00 | 0.43 | 0 | 4 | 0 | |
| Kidney Normal | Kidney | NORMAL | Cortex of k... | | 5.76 | 0.22 | 0 | 61 | 0 | |
| Kidney Malignant | Kidney | MALIGNANT | Kidney::MAL... | | 5.61 | 0.20 | 1 | 90 | 0 | |
| Kidney Benign | Kidney | BENIGN | Kidney::BEN... | | 18.30 | 34.71 | 5 | 10 | 0 | |
| Endometrium Malignant | Endometrium | MALIGNANT | Endometrium... | | 6.31 | 3.17 | 7 | 49 | 1 | |
| Endometrium Benign | Endometrium | BENIGN | Endometrium... | | 5.54 | 0.10 | 0 | 10 | 0 | |
| Colon Normal | Colon | NORMAL | Ascending c... | | 14.65 | 25.57 | 43 | 28 | 3 | |
| Colon Malignant | Colon | MALIGNANT | Colon::MALI... | | 6.67 | 11.61 | 10 | 130 | 1 | |
| Ovary Malignant | Ovary | MALIGNANT | Ovary::MALI... | | 10.33 | 50.15 | 7 | 131 | 0 | |
| Ovary Normal | Ovary | NORMAL | Ovary::NORM... | | 5.64 | 0.14 | 0 | 7 | 0 | |
| Lung Normal | Lung | NORMAL | Lung::NORMA... | | 16.18 | 14.17 | 53 | 11 | 0 | |
| Ovary Benign | Ovary | BENIGN | Ovary::BENI... | | 5.66 | 0.41 | 4 | 31 | 0 | |
| Lung Malignant | Lung | MALIGNANT | Lung::MALIG... | | 7.00 | 5.27 | 26 | 96 | 2 | |
| Lung Benign | Lung | BENIGN | Lung::BENIG... | | 5.61 | 0.15 | 0 | 5 | 0 | |
| Liver Diseased | Liver | DISEASED | Liver::DISE... | | 6.30 | 2.71 | 1 | 21 | 0 | |
| Liver Malignant | Liver | MALIGNANT | Liver::MALI... | | 5.91 | 0.42 | 0 | 24 | 1 | |
| Liver Normal | Liver | NORMAL | Liver::NORM... | | 5.96 | 0.27 | 0 | 6 | 0 | |
| Prostate Malignant | Prostate | MALIGNANT | Prostate::M... | | 18.11 | 61.02 | 40 | 31 | 2 | |
| Prostate Normal | Prostate | NORMAL | Prostate::N... | | 18.65 | 23.89 | 22 | 9 | 1 | |
| Pancreas Normal | Pancreas | NORMAL | Pancreas::N... | | 5.98 | 0.23 | 0 | 13 | 0 | |
| Prostate Diseased | Prostate | DISEASED | Prostate::D... | | 23.87 | 18.90 | 17 | 3 | 0 | |
| Pancreas Benign | Pancreas | BENIGN | Pancreas::B... | | 156.95 | 337.67 | 1 | 4 | 0 | |
| Pancreas Malignant | Pancreas | MALIGNANT | Pancreas::M... | | 5.57 | 0.17 | 1 | 65 | 0 | |
| ID | Original Source | Purity | Treatment | Tissue Type | Avg Sig | STDev | P | A | M | 152 304 456 609 |

FIG. 1

C. RSPO3

| ID | Original Source | Purity | Treatment | Tissue Type | Avg Sig | STDev | PA Calls P | PA Calls A | PA Calls M | 1405 2810 4216 5621 |
|---|---|---|---|---|---|---|---|---|---|---|
| Colon Diseased | Colon | DISEASED | Colon::DISE... | | 424.06 | 396.34 | 21 | 0 | 0 | |
| Colon Benign | Colon | BENIGN | Colon::BENI... | | 20.73 | 33.58 | 9 | 15 | 0 | |
| Breast Normal | Breast | NORMAL | Breast::NOR... | | 176.15 | 140.69 | 21 | 1 | 0 | |
| Breast Malignant | Breast | MALIGNANT | Breast::MAL... | | 75.62 | 435.84 | 133 | 26 | 2 | |
| Breast Benign | Breast | BENIGN | Breast::BEN... | | 237.01 | 342.37 | 9 | 0 | 0 | |
| Brain Benign | Brain | BENIGN | Brain::BENI... | | 803.28 | 1306.74 | 13 | 2 | 1 | |
| Brain Malignant | Brain | MALIGNANT | Brain::MALI... | | 10.15 | 8.10 | 5 | 17 | 1 | |
| Liver Benign | Liver | BENIGN | Liver::BENI... | | 87.84 | 68.68 | 4 | 0 | 0 | |
| Kidney Normal | Kidney | NORMAL | Cortex of k... | | 24.72 | 27.21 | 39 | 22 | 0 | |
| Kidney Malignant | Kidney | MALIGNANT | Kidney::MAL... | | 99.48 | 283.90 | 54 | 33 | 4 | |
| Kidney Benign | Kidney | BENIGN | Kidney::BEN... | | 1032.08 | 1889.99 | 7 | 8 | 0 | |
| Endometrium Malignant | Endometrium | MALIGNANT | Endometrium... | | 176.43 | 285.60 | 43 | 14 | 0 | |
| Endometrium Benign | Endometrium | BENIGN | Endometrium... | | 3288.94 | 1998.11 | 10 | 0 | 0 | |
| Colon Normal | Colon | NORMAL | Ascending c... | | 118.87 | 137.80 | 73 | 1 | 0 | |
| Colon Malignant | Colon | MALIGNANT | Colon::MALI... | | 108.15 | 360.84 | 119 | 20 | 2 | |
| Ovary Malignant | Ovary | MALIGNANT | Ovary::MALI... | | 154.28 | 556.65 | 76 | 61 | 1 | |
| Ovary Normal | Ovary | NORMAL | Ovary::NORM... | | 23.20 | 31.46 | 2 | 5 | 0 | |
| Lung Normal | Lung | NORMAL | Lung::NORMA... | | 60.79 | 43.83 | 62 | 2 | 0 | |
| Ovary Benign | Ovary | BENIGN | Ovary::BENI... | | 226.13 | 736.49 | 8 | 26 | 1 | |
| Lung Malignant | Lung | MALIGNANT | Lung::MALIG... | | 111.01 | 340.16 | 103 | 20 | 1 | |
| Lung Benign | Lung | BENIGN | Lung::BENIG... | | 189.94 | 406.63 | 1 | 4 | 0 | |
| Liver Diseased | Liver | DISEASED | Liver::DISE... | | 67.81 | 46.12 | 22 | 0 | 0 | |
| Liver Malignant | Liver | MALIGNANT | Liver::MALI... | | 48.36 | 128.64 | 14 | 11 | 0 | |
| Liver Normal | Liver | NORMAL | Liver::NORM... | | 58.22 | 18.95 | 6 | 0 | 0 | |
| Prostate Malignant | Prostate | MALIGNANT | Prostate::M... | | 53.33 | 76.76 | 64 | 8 | 1 | |
| Prostate Normal | Prostate | NORMAL | Prostate::N... | | 101.58 | 188.93 | 30 | 2 | 0 | |
| Pancreas Normal | Pancreas | NORMAL | Pancreas::N... | | 50.23 | 47.53 | 12 | 1 | 0 | |
| Prostate Diseased | Prostate | DISEASED | Prostate::D... | | 43.30 | 45.82 | 17 | 2 | 1 | |
| Pancreas Benign | Pancreas | BENIGN | Pancreas::B... | | 31.14 | 27.97 | 3 | 2 | 0 | |
| Pancreas Malignant | Pancreas | MALIGNANT | Pancreas::M... | | 66.48 | 83.74 | 54 | 10 | 2 | |
| ID | Original Source | Purity | Treatment | Tissue Type | Avg Sig | STDev | P | A | M | 1405 2810 4216 5621 |

FIG. 1

Figure 2

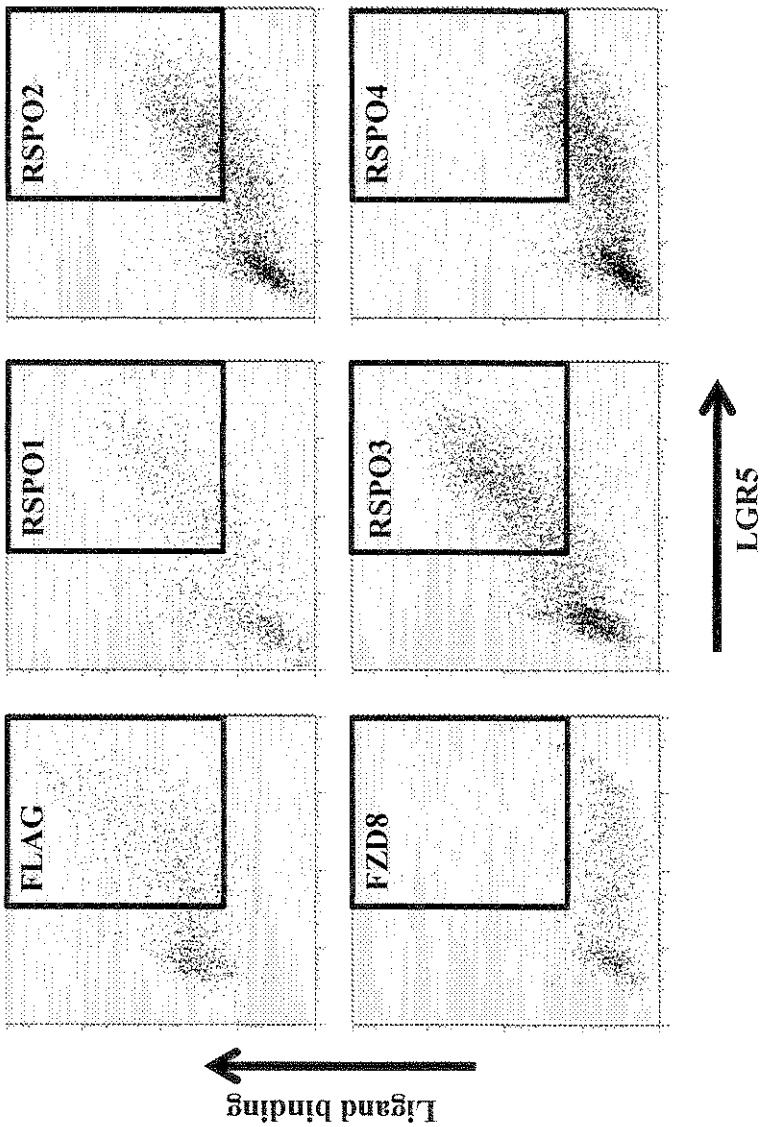


Figure 3

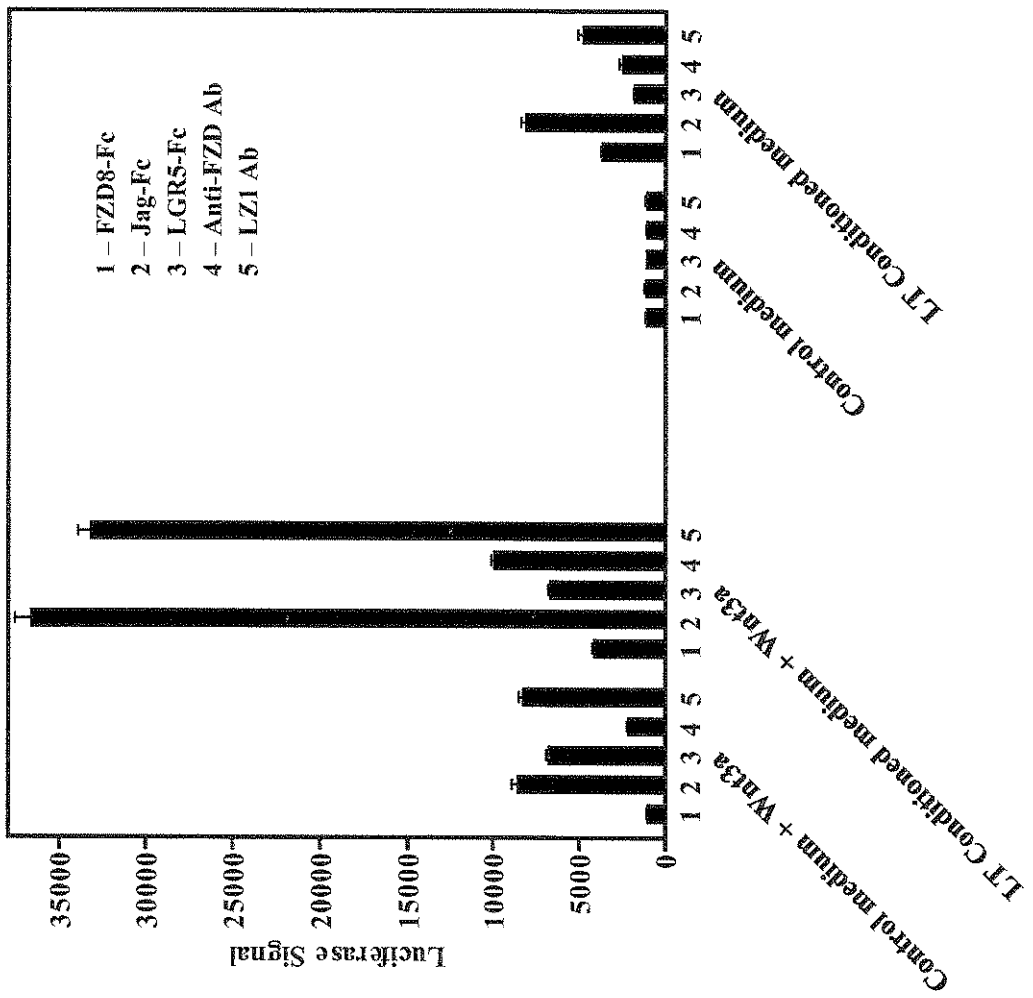

Luciferase Signal
35000
30000
25000
20000
15000
10000
5000
0
1 – FZD8-Fc
2 – Jag-Fc
3 – LGR5-Fc
4 – Anti-FZD Ab
5 – LZ1 Ab
1 2 3 4 5
1 2 3 4 5
1 2 3 4 5
1 2 3 4 5
Control medium + Wnt3a
LT Conditioned medium + Wnt3a
Control medium
LT Conditioned medium

Figure 4

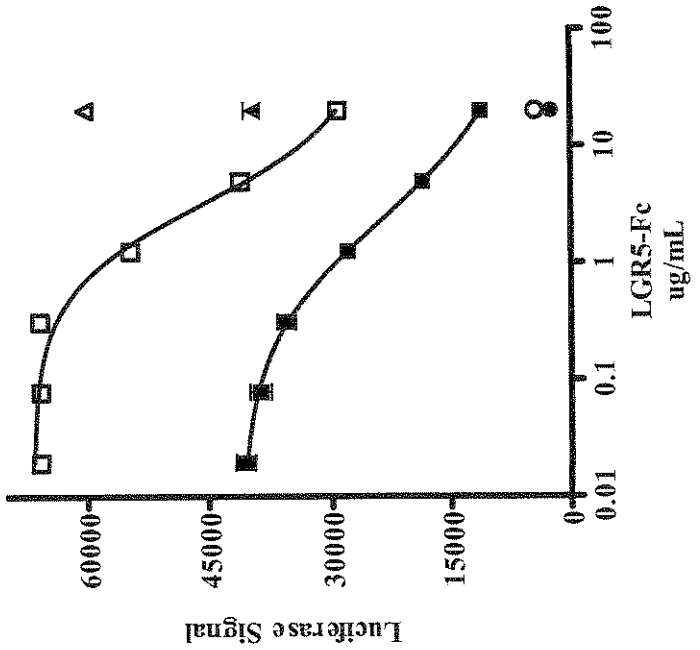

Luciferase Signal
60000
45000
30000
15000
0
0.01
0.1
1
10
100
LGR5-Fc
ug/mL

Figure 5

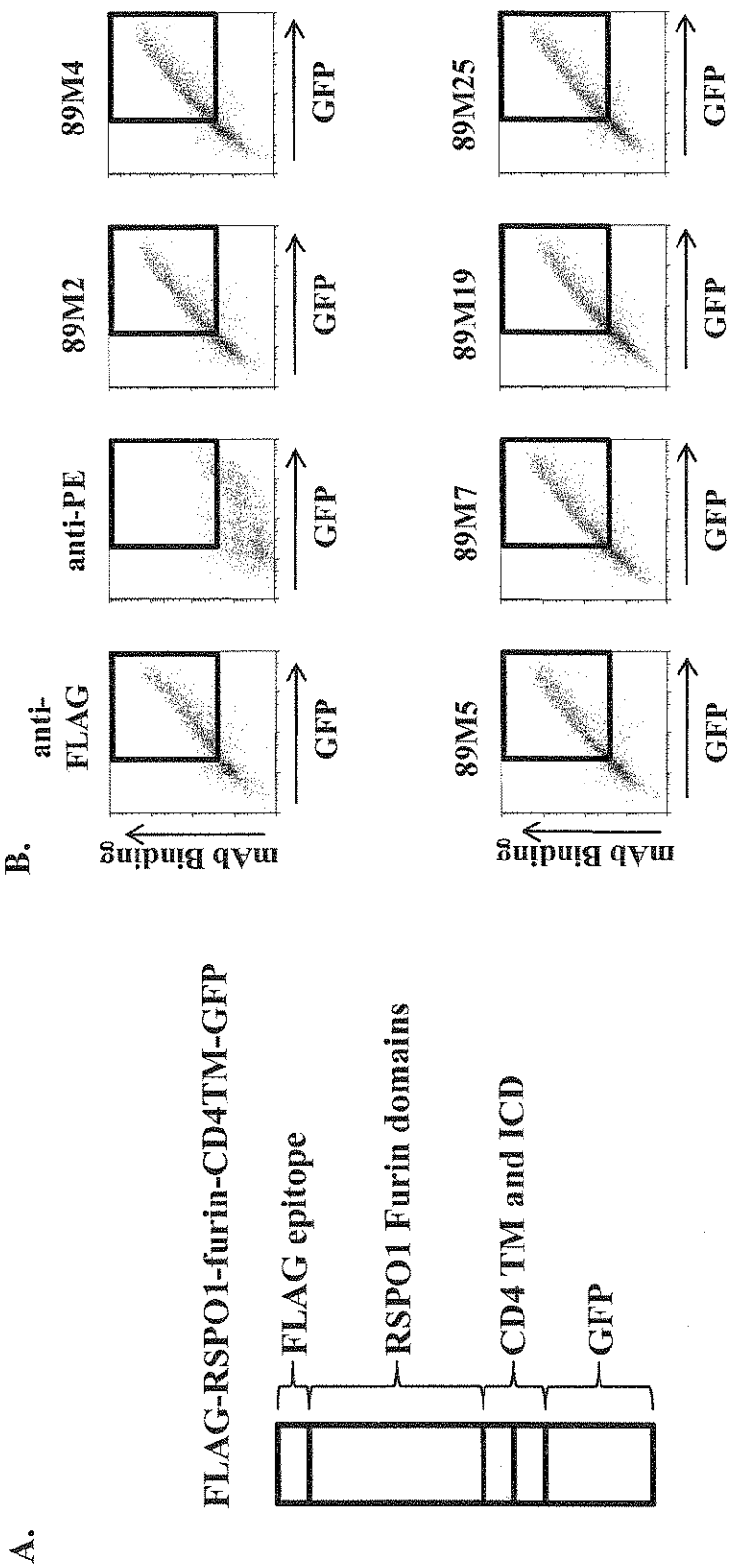

A.
FLAG-RSPO1-furin-CD4TM-GFP
FLAG epitope
RSPO1 Furin domains
CD4 TM and ICD
GFP
B.
anti-FLAG
anti-PE
89M2
89M4
89M5
89M7
89M19
89M25
mAb Binding
GFP

Figure 6

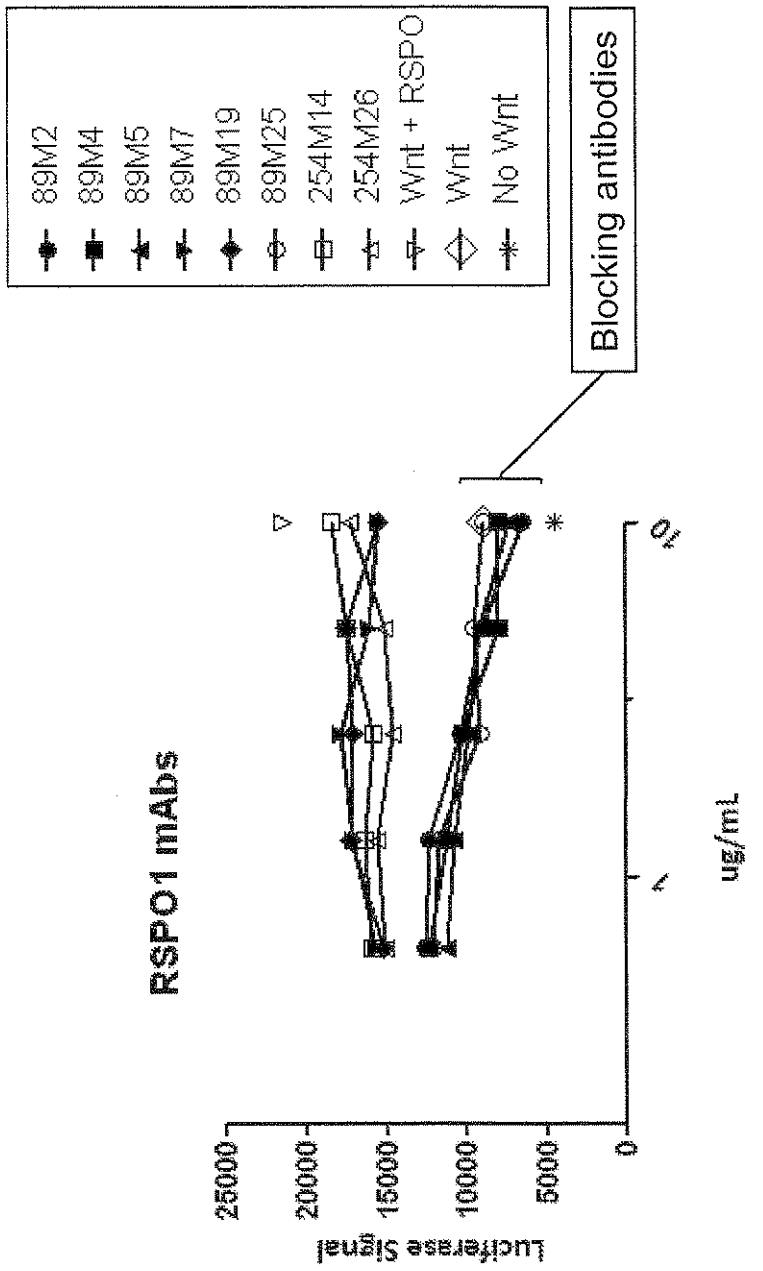

89M2
89M4
89M5
89M7
89M19
89M25
254M14
254M26
Wnt + RSPO
Wnt
No Wnt
Blocking antibodies
RSPO1 mAbs
Luciferase Signal
25000
20000
15000
10000
5000
0
10
1
ug/mL

Figure 7

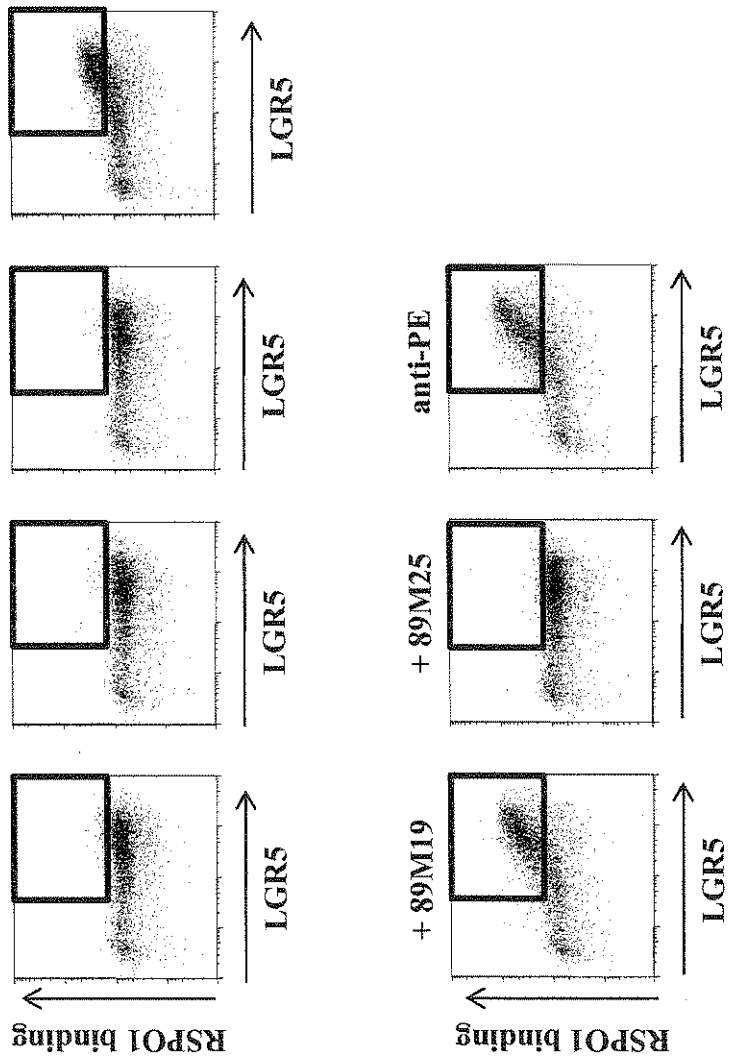


Figure 8

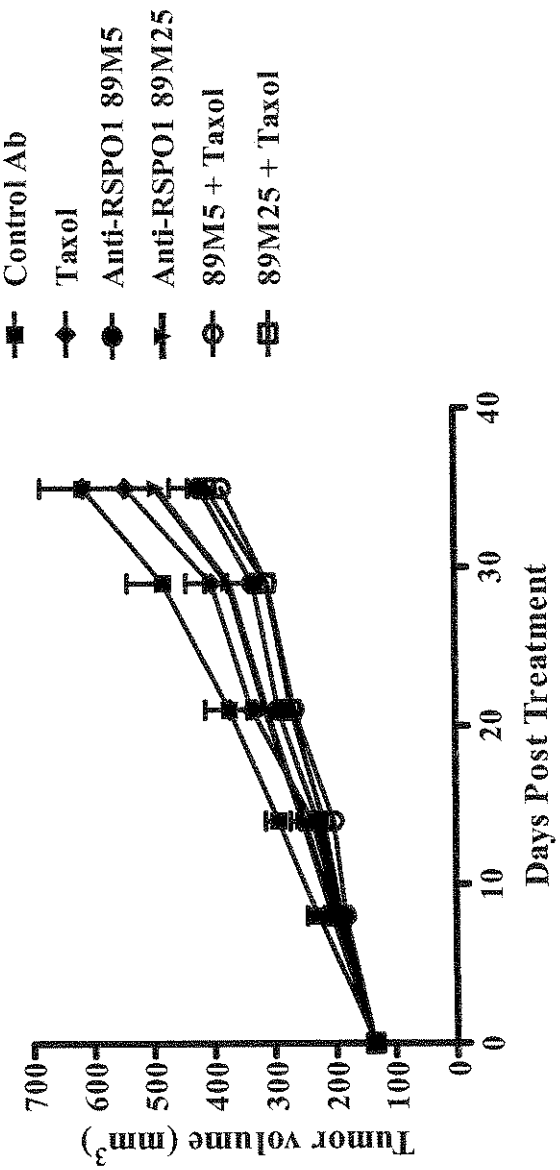

Control Ab
Taxol
Anti-RSPO1 89M5
Anti-RSPO1 89M25
89M5 + Taxol
89M25 + Taxol
Tumor volume (mm3)
700
600
500
400
300
200
100
0
0
10
20
30
40
Days Post Treatment

Figure 9

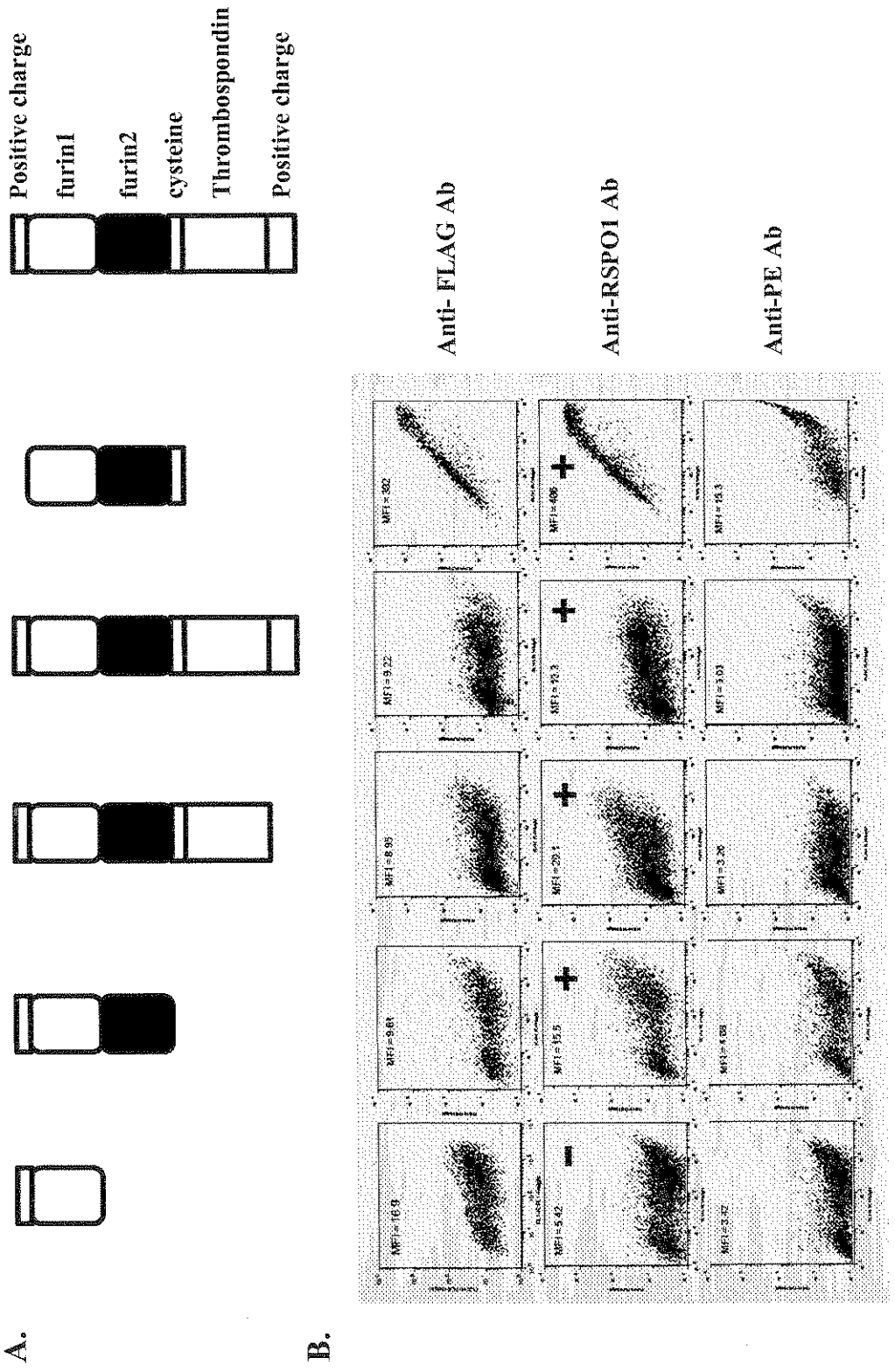

A.
Positive charge
furin1
furin2
cysteine
Thrombospondin
Positive charge
B.
Anti- FLAG Ab
Anti-RSPO1 Ab
Anti-PE Ab

Figure 10

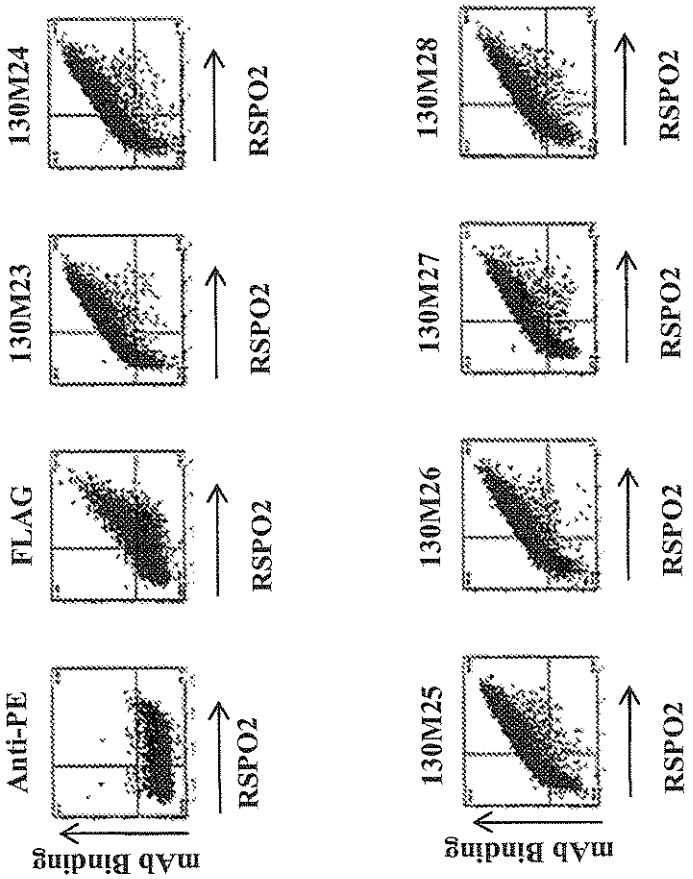

Anti-PE
FLAG
130M23
130M24
130M25
130M26
130M27
130M28
mAb Binding
RSPO2

Figure 11

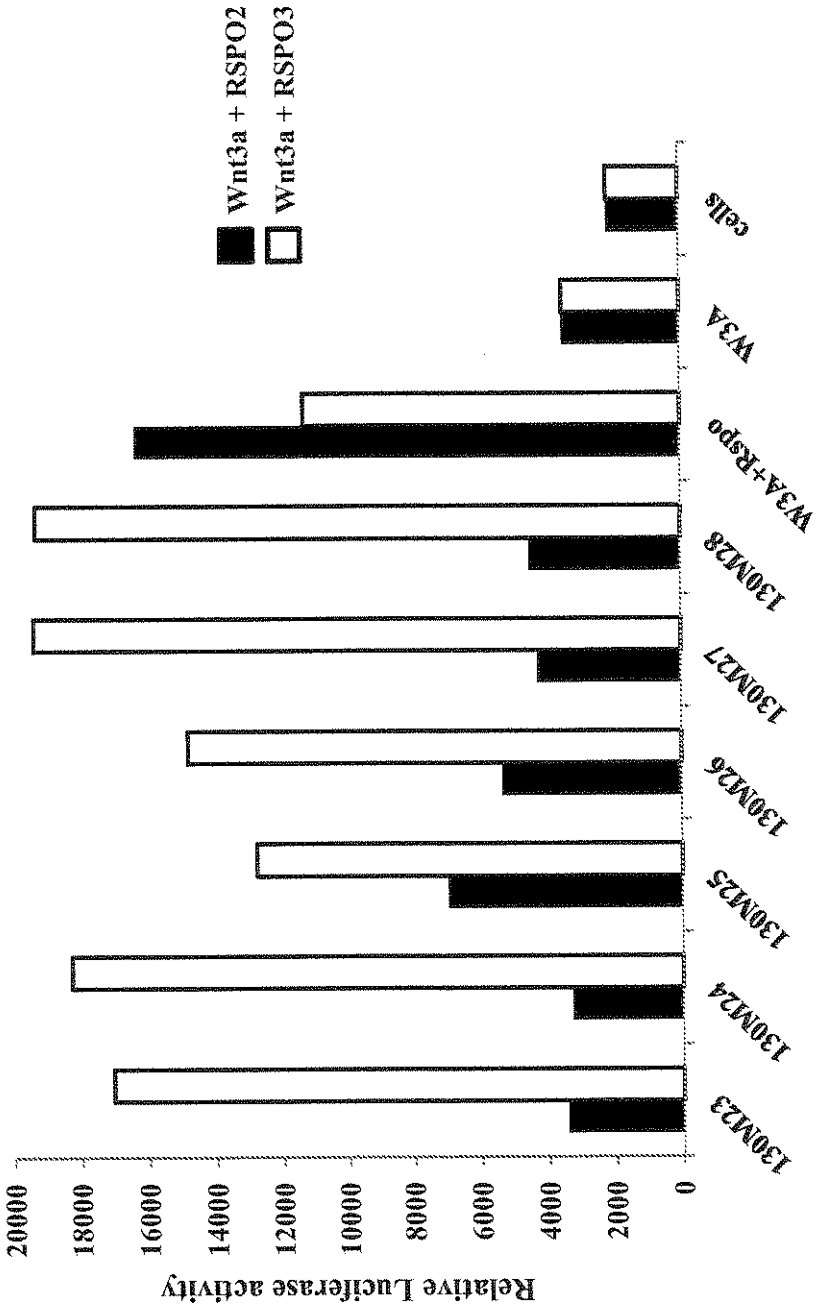

Wnt3a + RSPO2
Wnt3a + RSPO3
Relative Luciferase activity
0
2000
4000
6000
8000
10000
12000
14000
16000
18000
20000
cells
W3A
W3A+Rspo
130M28
130M27
130M26
130M25
130M24
130M23

Figure 12

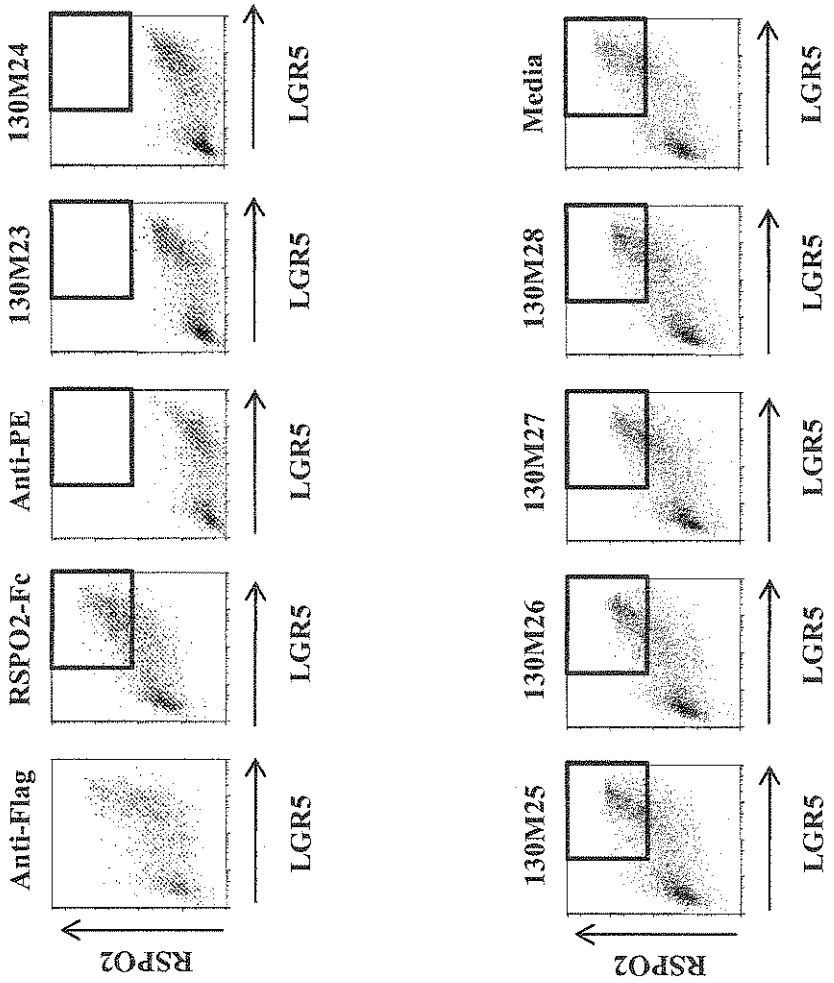

Anti-Flag
RSPO2-Fc
Anti-PE
130M23
130M24
130M25
130M26
130M27
130M28
Media
LGR5
RSPO2

Figure 13

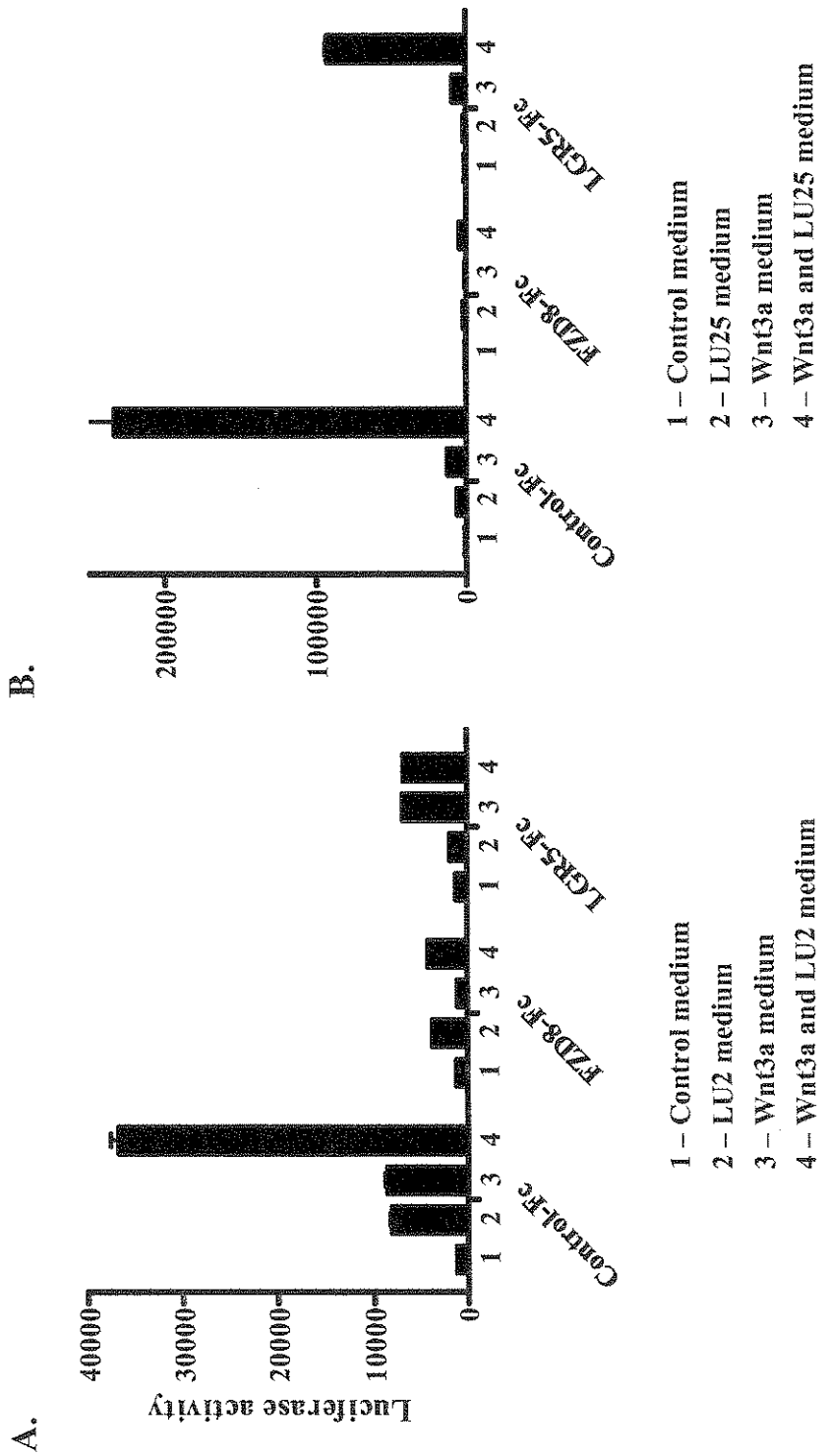

A.
Luciferase activity
40000
30000
20000
10000
0
1 2 3 4
Control-Fc
FZD8-Fc
LGR5-Fc
1 – Control medium
2 – LU2 medium
3 – Wnt3a medium
4 – Wnt3a and LU2 medium
B.
200000
100000
0
1 2 3 4
Control-Fc
FZD8-Fc
LGR5-Fc
1 – Control medium
2 – LU25 medium
3 – Wnt3a medium
4 – Wnt3a and LU25 medium

Figure 13

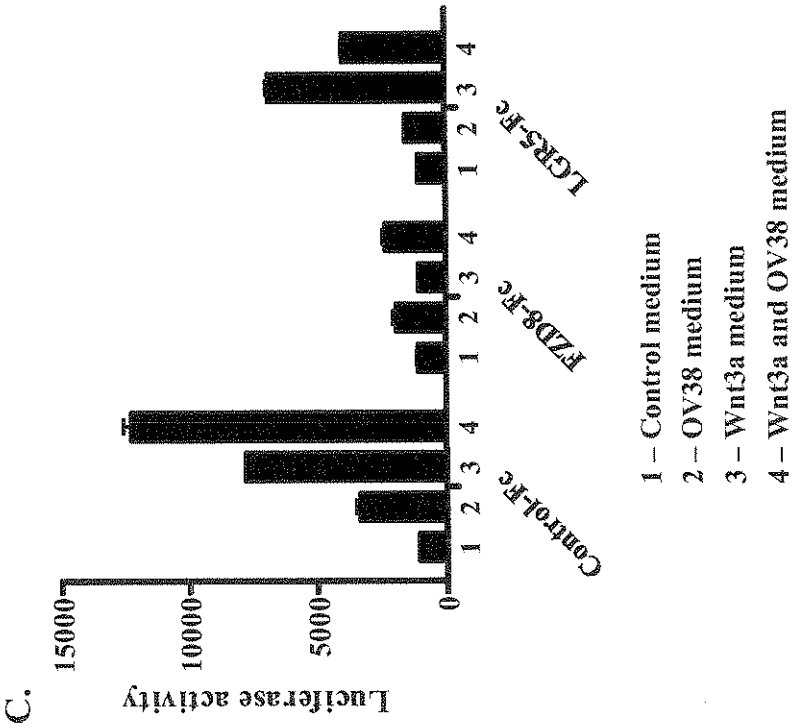

C
Luciferase activity
15000
10000
5000
0
1 2 3 4
Control-Fc
1 2 3 4
FZD8-Fc
1 2 3 4
LGR5-Fc
1 – Control medium
2 – OV38 medium
3 – Wnt3a medium
4 – Wnt3a and OV38 medium

Figure 14

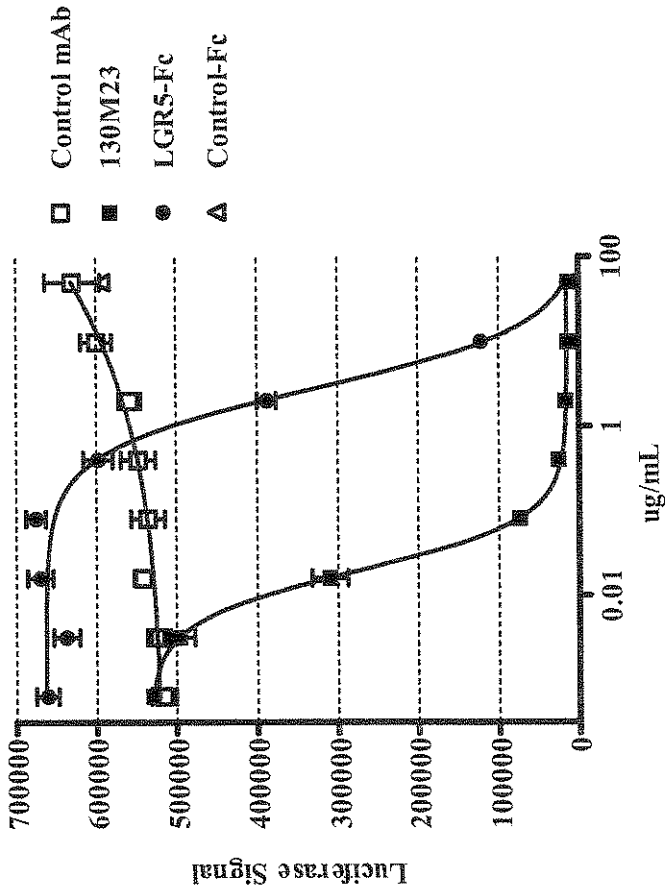

Control mAb
130M23
LGR5-Fc
Control-Fc
Luciferase Signal
700000
600000
500000
400000
300000
200000
100000
0
0.01
1
100
ug/mL

Figure 15

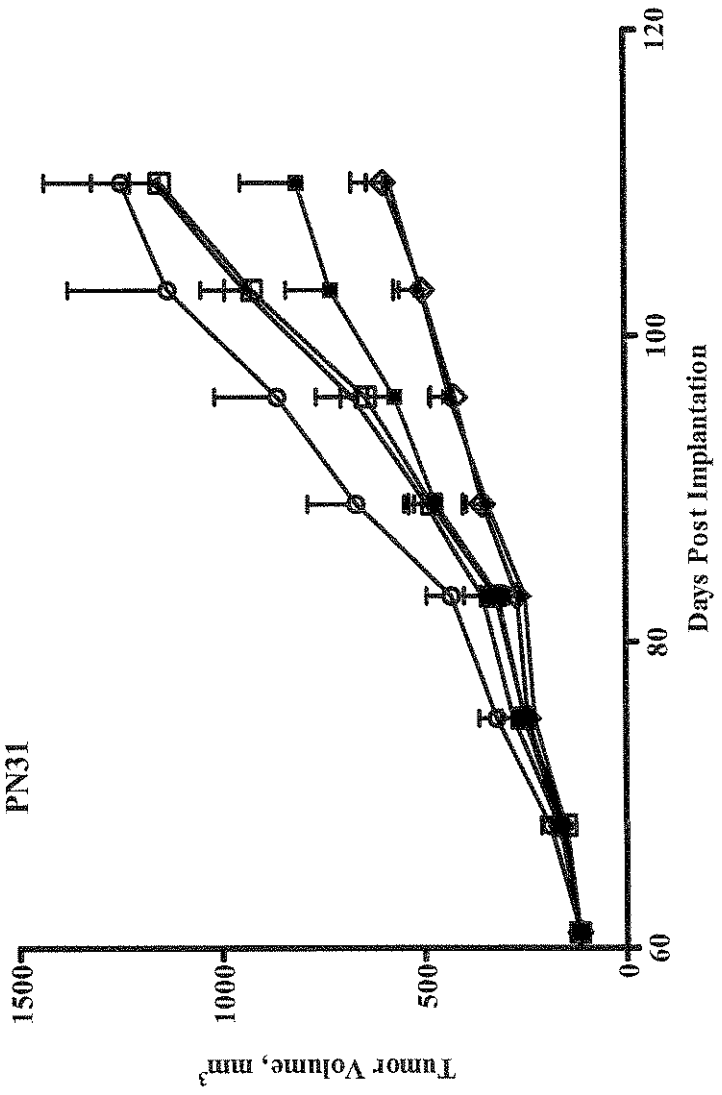

1B711
Gemcitabine
89M5
130M23
89M5 + Gem
130M23 + Gem
PN31
Tumor Volume, mm3
1500
1000
500
0
60
80
100
120
Days Post Implantation

Figure 16

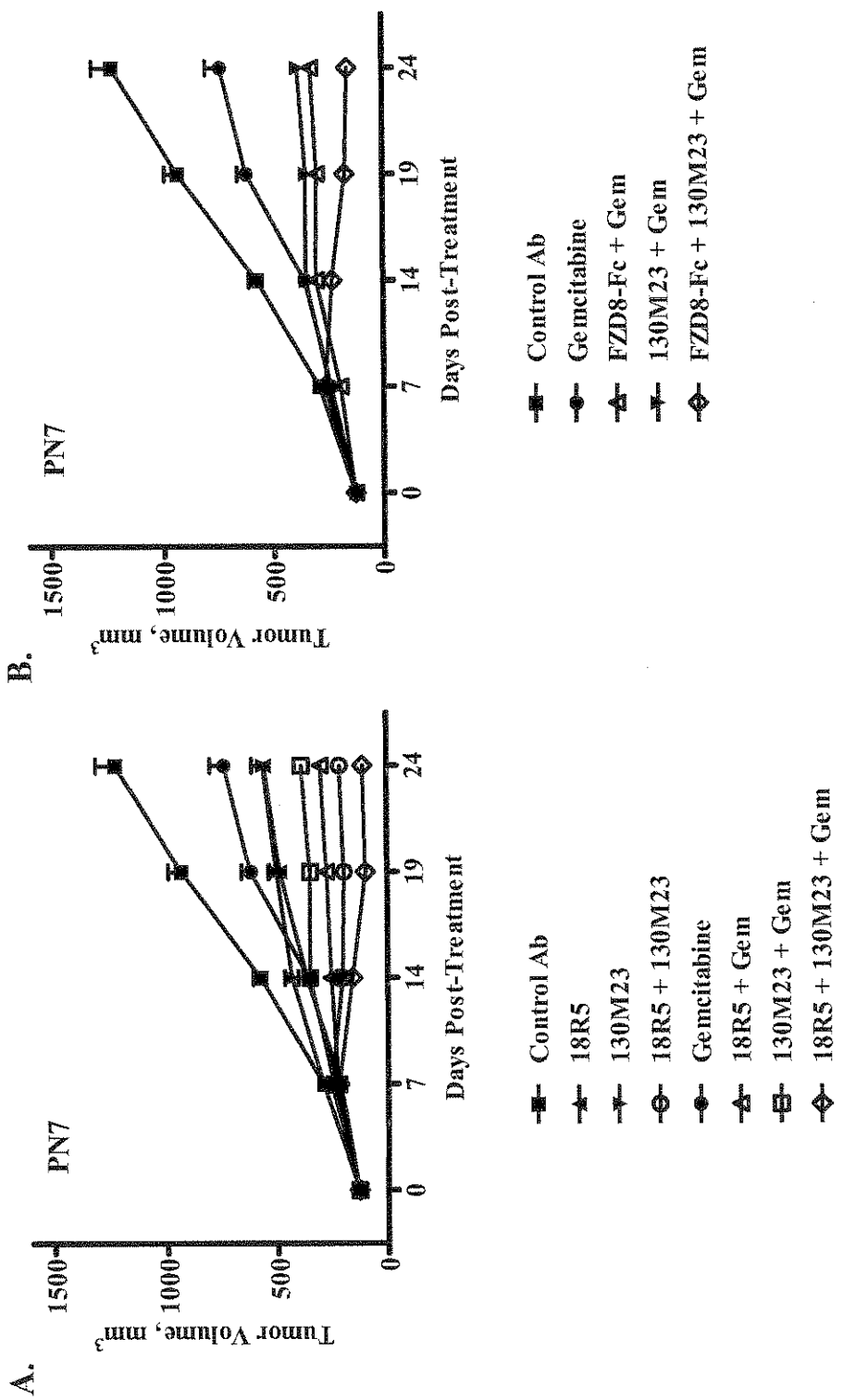

A.
PN7
Tumor Volume, mm3
1500
1000
500
0
0
7
14
19
24
Days Post-Treatment
Control Ab
18R5
130M23
18R5 + 130M23
Gemcitabine
18R5 + Gem
130M23 + Gem
18R5 + 130M23 + Gem
B.
PN7
Tumor Volume, mm3
1500
1000
500
0
0
7
14
19
24
Days Post-Treatment
Control Ab
Gemcitabine
FZD8-Fc + Gem
130M23 + Gem
FZD8-Fc + 130M23 + Gem

Figure 16

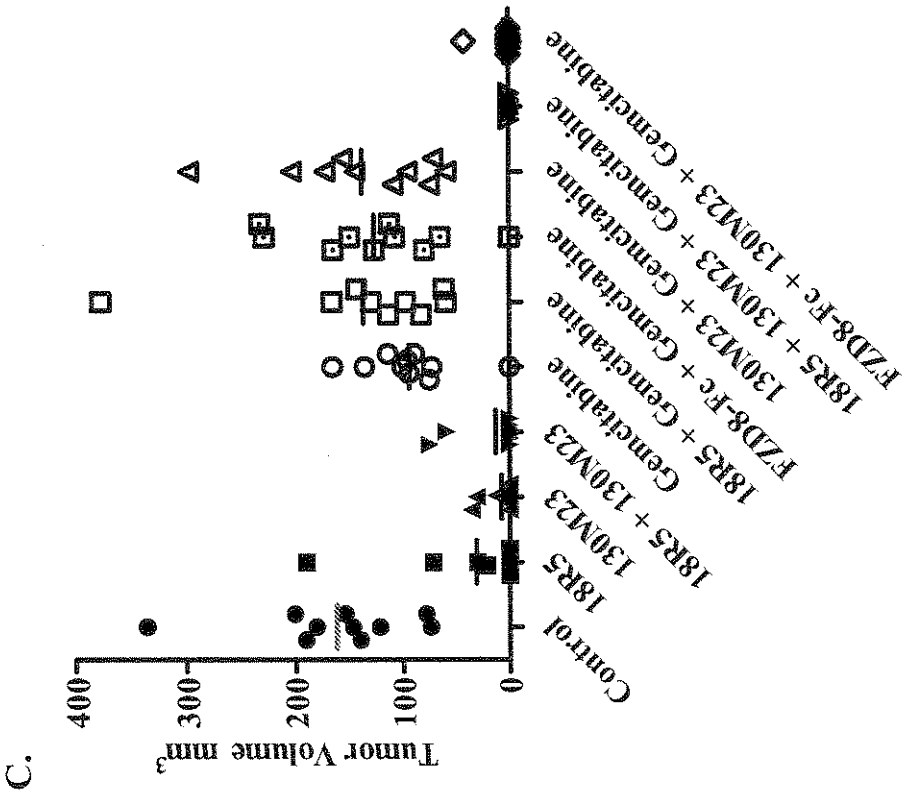

C.
Tumor Volume mm3
400
300
200
100
0
Control
18R5
130M23
18R5 + 130M23
Gemcitabine
18R5 + Gemcitabine
FZD8-Fc + Gemcitabine
130M23 + Gemcitabine
18R5 + 130M23 + Gemcitabine
FZD8-Fc + 130M23 + Gemcitabine

Figure 17

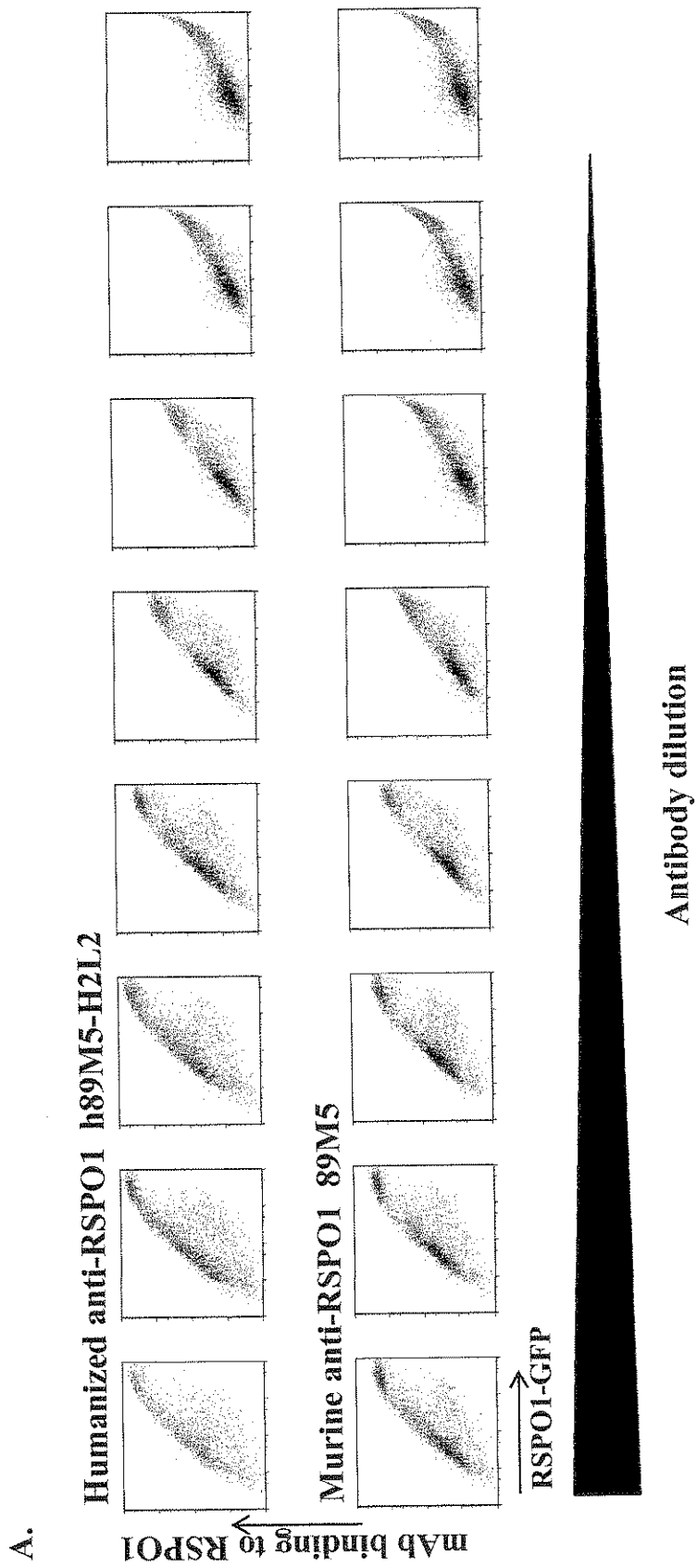

A.
Humanized anti-RSPO1 h89M5-H2L2
Murine anti-RSPO1 89M5
mAb binding to RSPO1
RSPO1-GFP
Antibody dilution

Figure 17

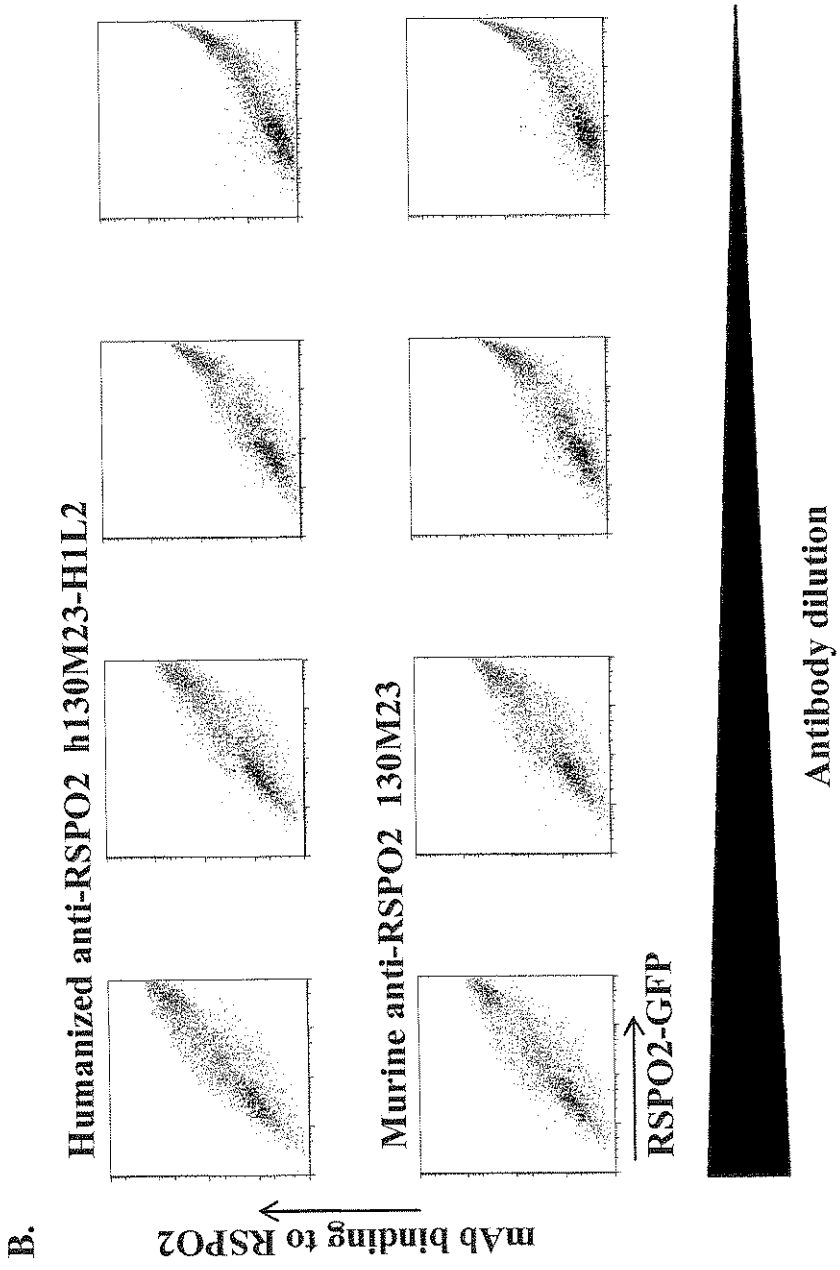
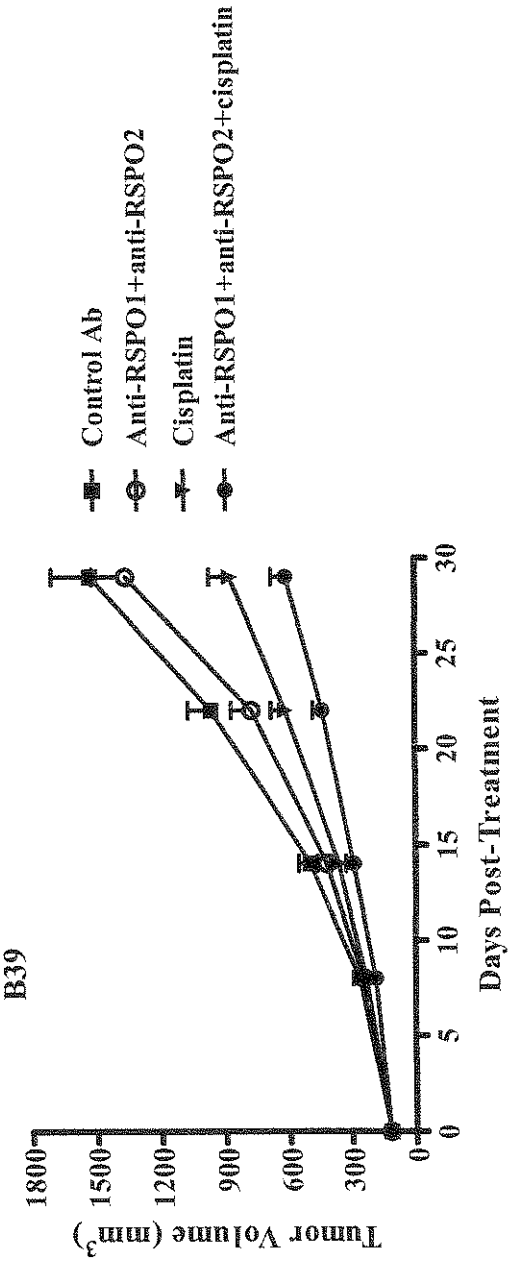

B.
mAb binding to RSPO2
Humanized anti-RSPO2 h130M23-H1L2
Murine anti-RSPO2 130M23
RSPO2-GFP
Antibody dilution

Figure 18


B39
Tumor Volume (mm3)
1800
1500
1200
900
600
300
0
0
5
10
15
20
25
30
Days Post-Treatment
Control Ab
Anti-RSPO1+anti-RSPO2
Cisplatin
Anti-RSPO1+anti-RSPO2+cisplatin