SUBSTITUTE SHEET (RULE 26)

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

SUBSTITUTE SHEET (RULE 26)

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

SUBSTITUTE SHEET (RULE 26)

Figure 2

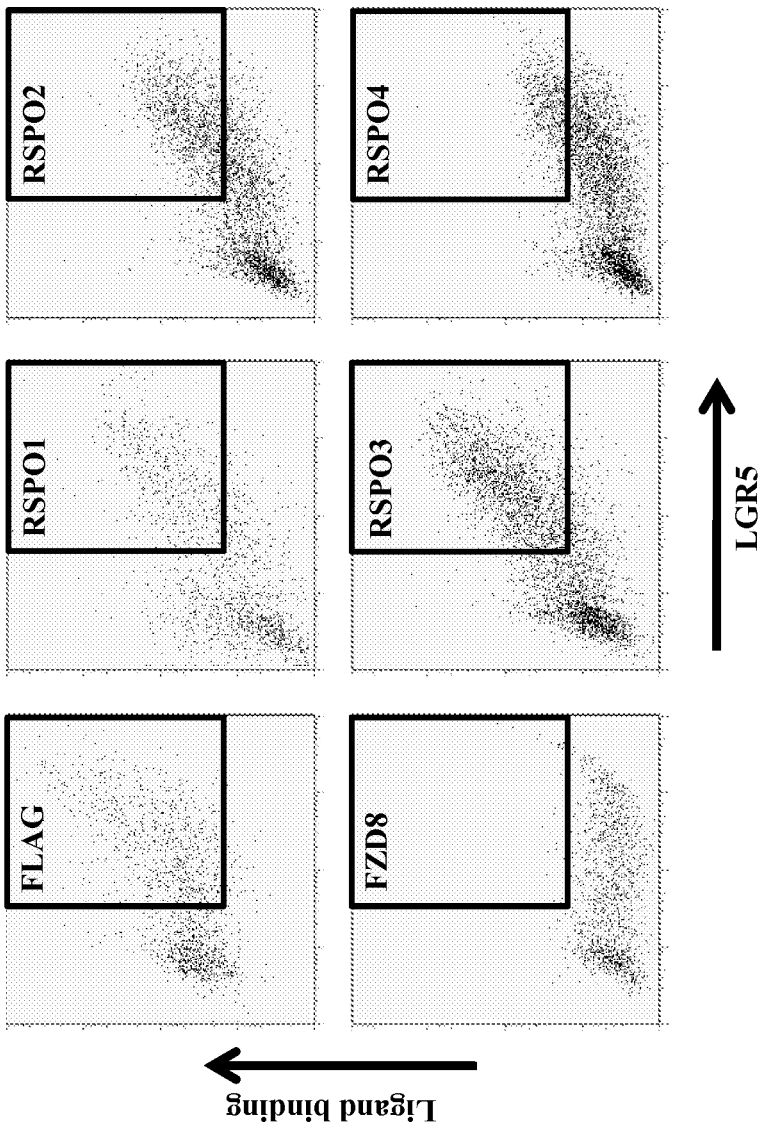


Figure 3

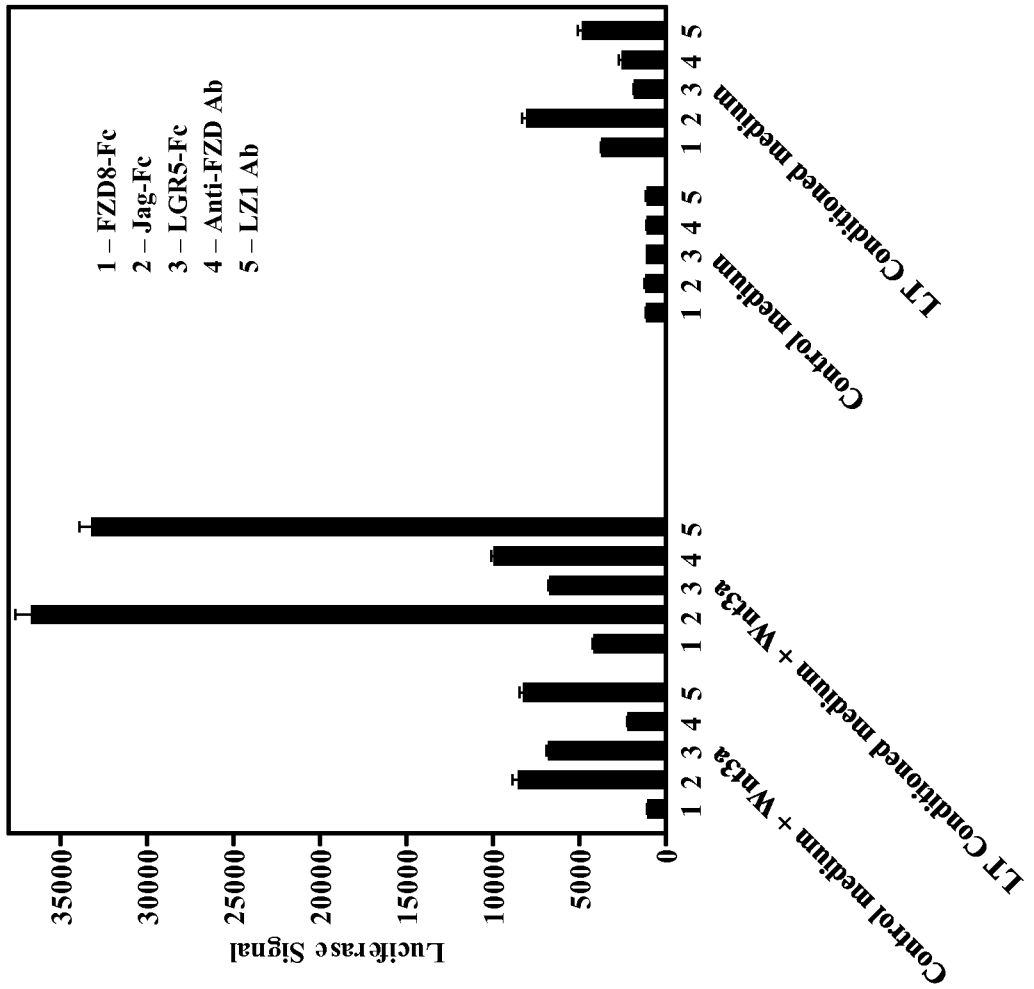

Luciferase Signal
35000
30000
25000
20000
15000
10000
5000
0
1 – FZD8-Fc
2 – Jag-Fc
3 – LGR5-Fc
4 – Anti-FZD Ab
5 – LZ1 Ab
1 2 3 4 5
Control medium + Wnt3a
LT Conditioned medium + Wnt3a
Control medium
LT Conditioned medium

Figure 4

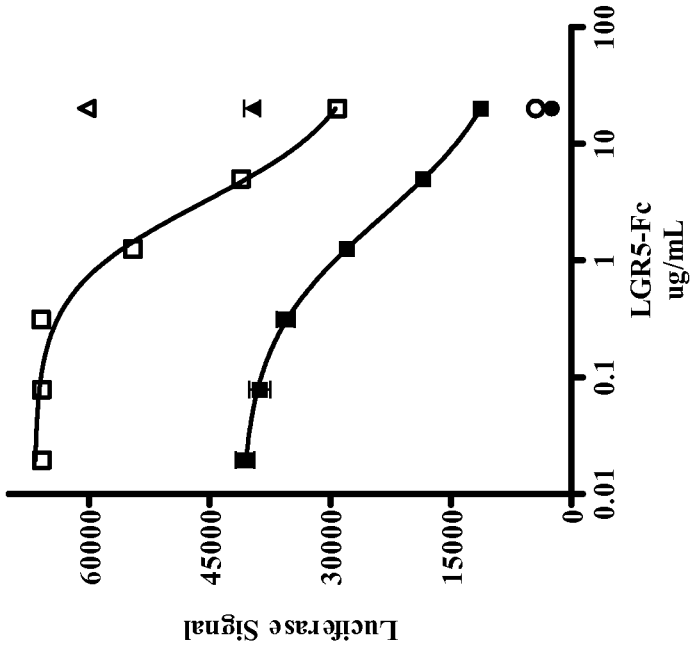

Luciferase Signal
60000
45000
30000
15000
0
0.01
0.1
1
10
100
LGR5-Fc
ug/mL

Figure 5

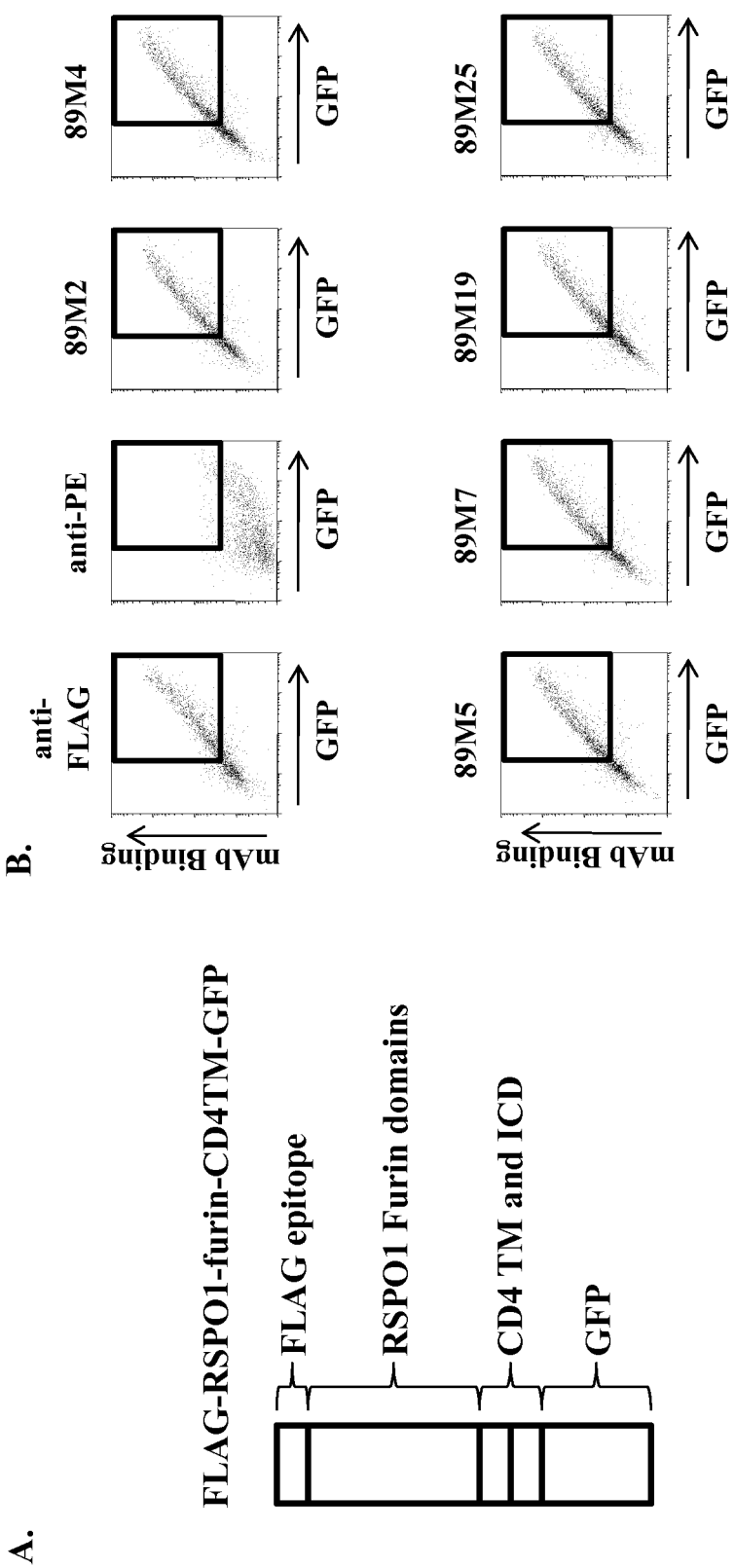

A.
FLAG-RSPO1-furin-CD4TM-GFP
FLAG epitope
RSPO1 Furin domains
CD4 TM and ICD
GFP
B.
anti-FLAG
anti-PE
89M4
89M2
89M5
89M7
89M19
89M25
mAb Binding
GFP

Figure 6

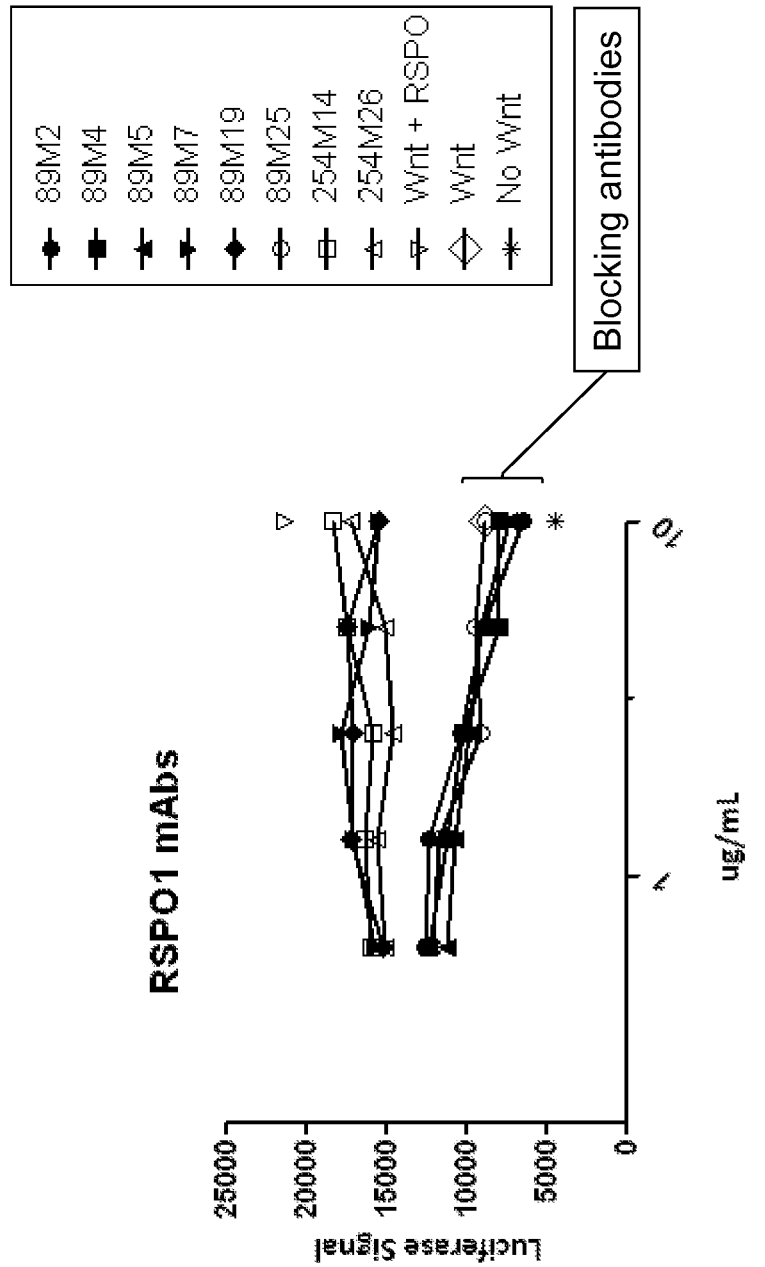

89M2
89M4
89M5
89M7
89M19
89M25
254M14
254M26
Wnt + RSPO
Wnt
No Wnt
Blocking antibodies
RSPO1 mAbs
Luciferase Signal
25000
20000
15000
10000
5000
0
10
1
ug/mL

Figure 7

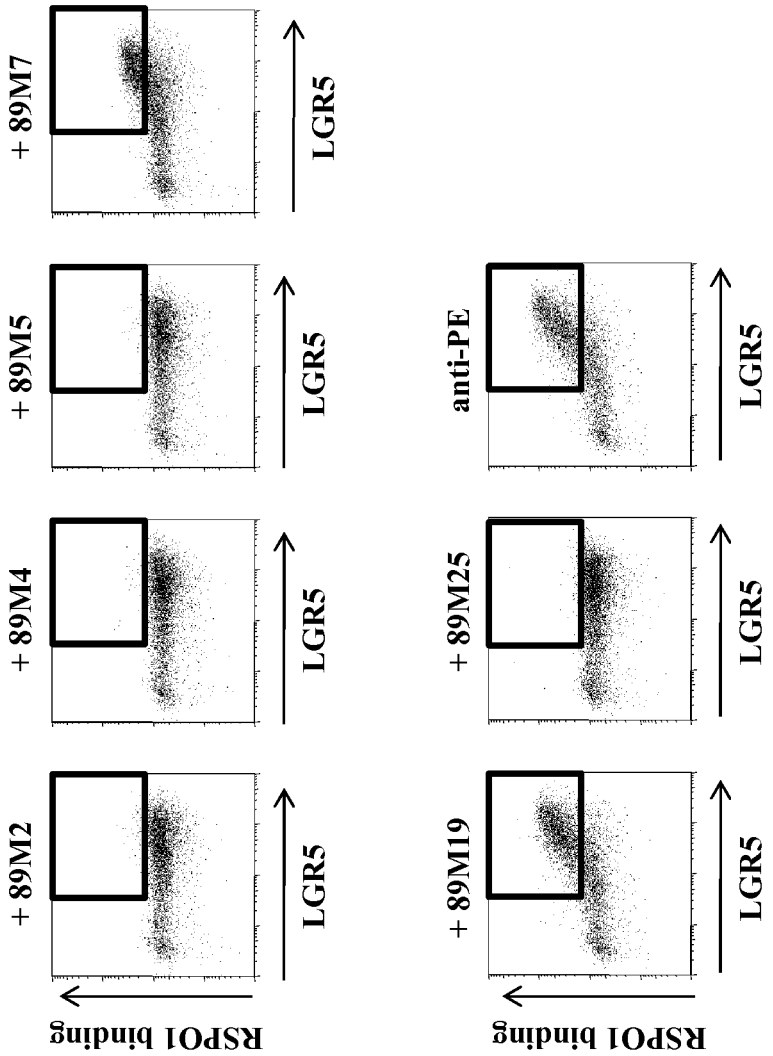


Figure 8

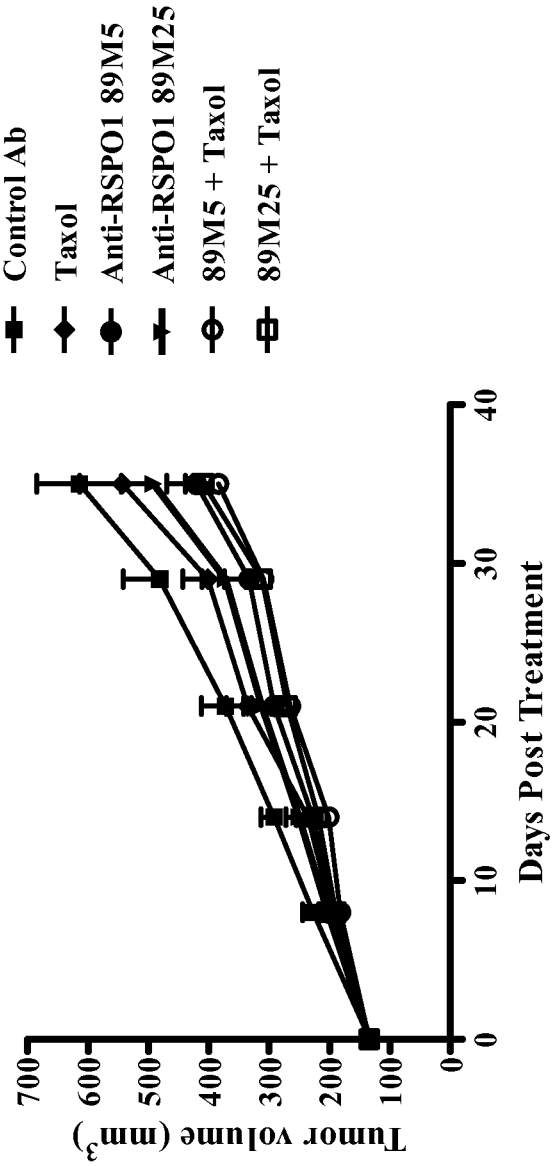

Control Ab
Taxol
Anti-RSPO1 89M5
Anti-RSPO1 89M25
89M5 + Taxol
89M25 + Taxol
Tumor volume (mm3)
0
100
200
300
400
500
600
700
0
10
20
30
40
Days Post Treatment

Figure 9

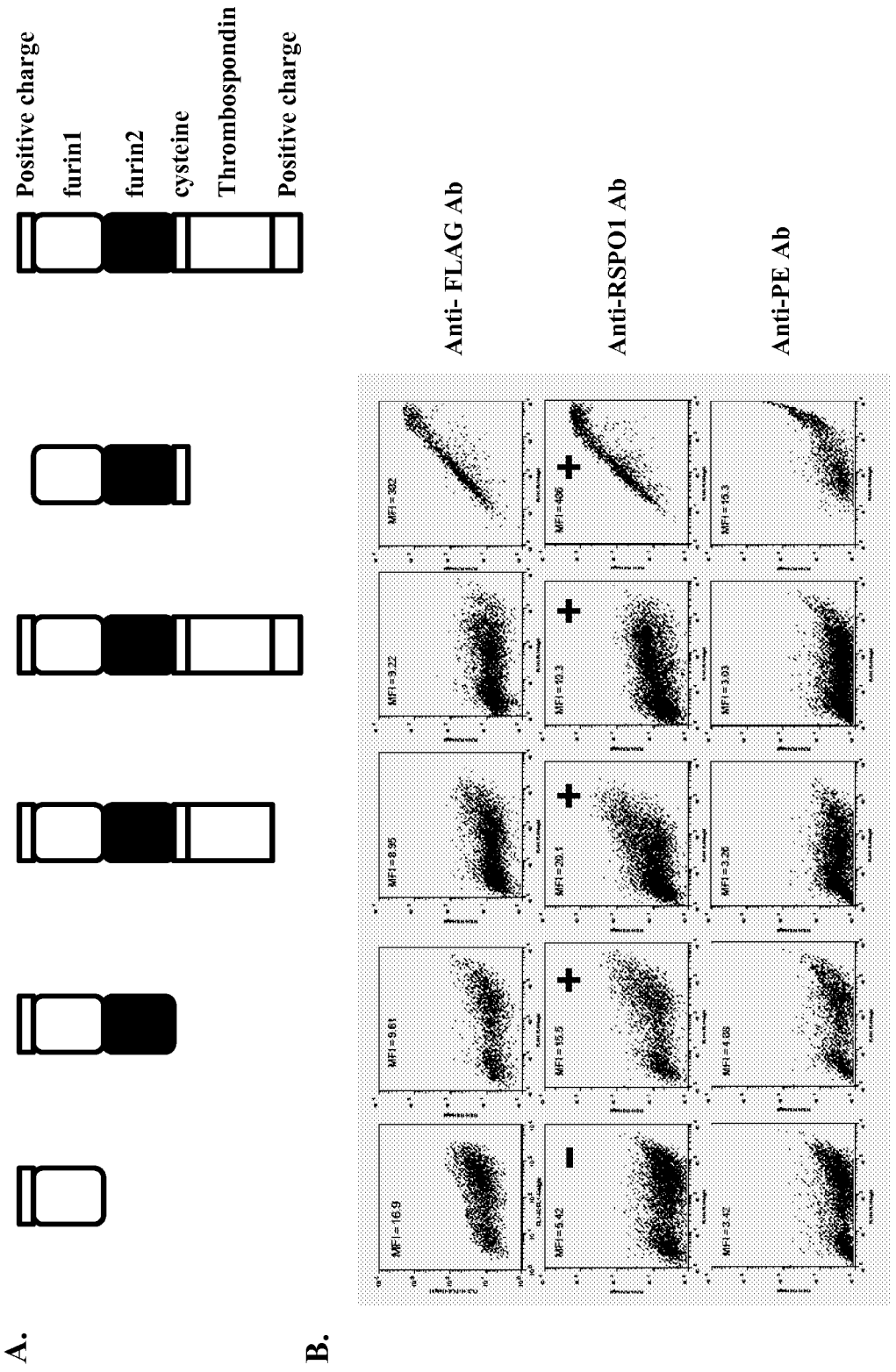

A.
Positive charge
furin1
furin2
cysteine
Thrombospondin
Positive charge
B.
Anti- FLAG Ab
Anti-RSPO1 Ab
Anti-PE Ab
MFI=16.9
MFI=9.61
MFI=8.99
MFI=9.22
MFI=322
MFI=5.42
MFI=15.5
MFI=20.1
MFI=10.3
MFI=486
MFI=3.42
MFI=4.68
MFI=3.26
MFI=3.03
MFI=16.3

Figure 10

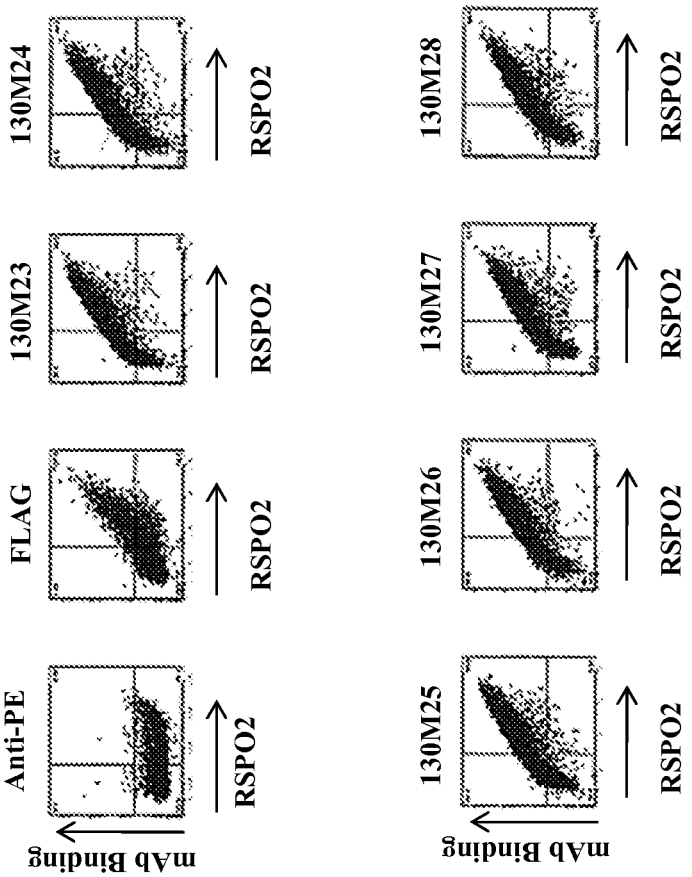

Anti-PE
FLAG
130M23
130M24
130M25
130M26
130M27
130M28
mAb Binding
RSPO2

Figure 11

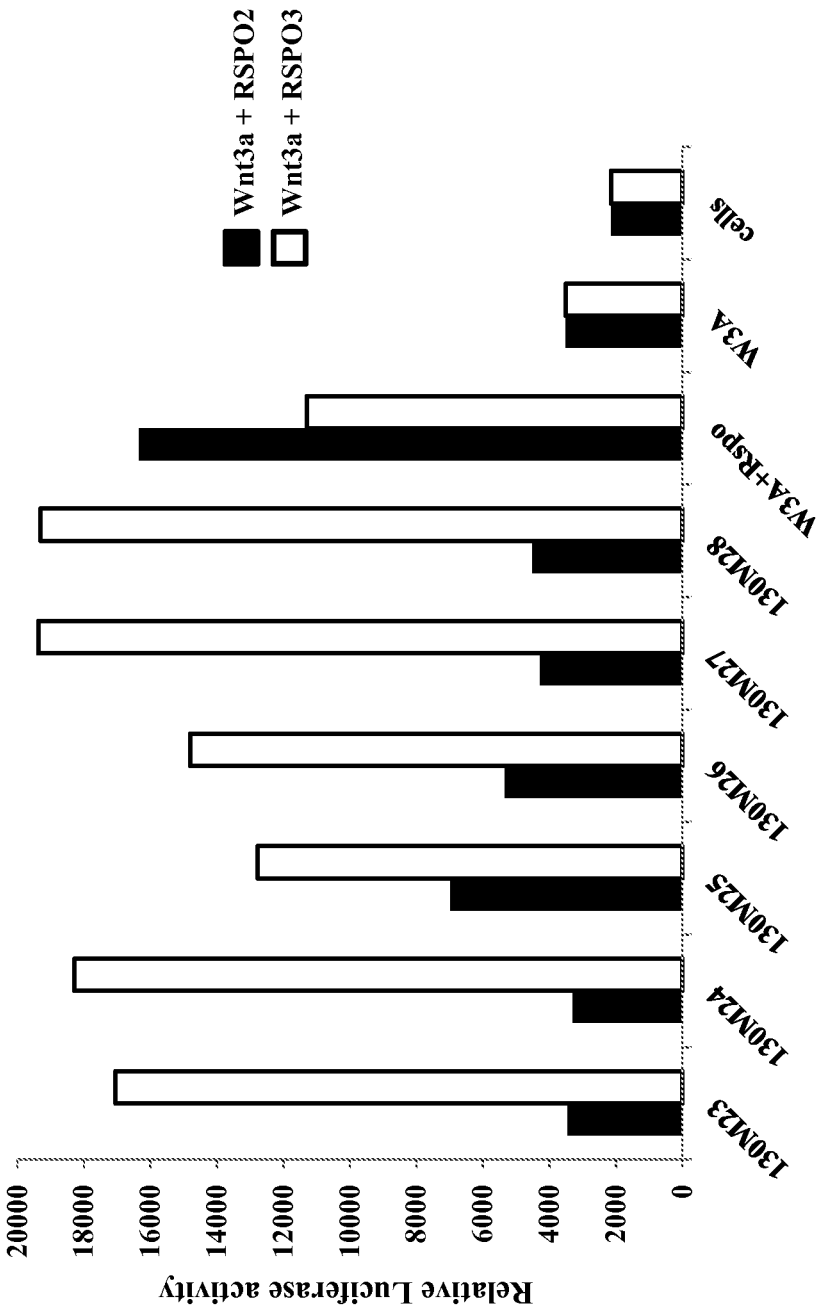

Wnt3a + RSPO2
Wnt3a + RSPO3
Relative Luciferase activity
0
2000
4000
6000
8000
10000
12000
14000
16000
18000
20000
130N123
130N124
130N125
130N126
130N127
130N128
W3A+Rspo
W3A
cells

Figure 12

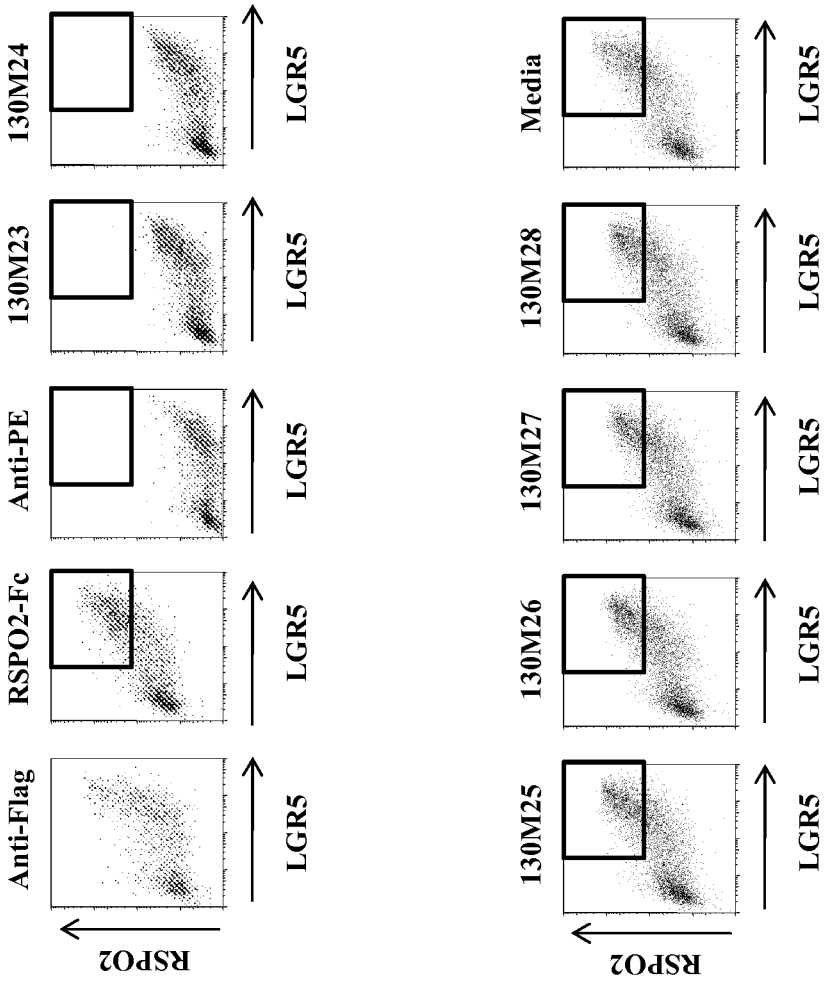

Anti-Flag
RSPO2-Fc
Anti-PE
130M23
130M24
Media
130M28
130M27
130M26
130M25
LGR5
RSPO2

Figure 13

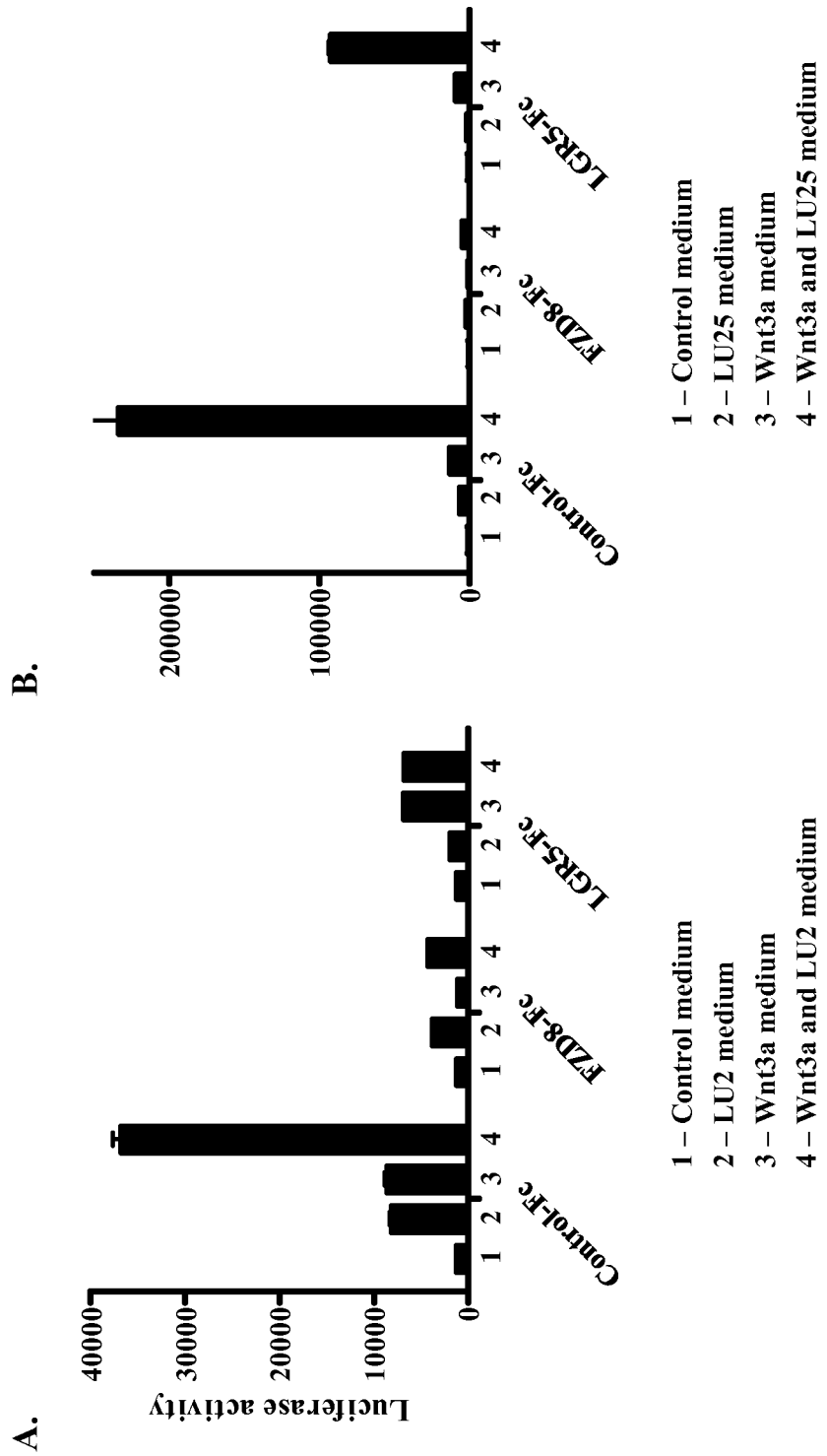

A.
Luciferase activity
40000
30000
20000
10000
0
1 2 3 4
Control-Fc
FZD8-Fc
LGR5-Fc
1 – Control medium
2 – LU2 medium
3 – Wnt3a medium
4 – Wnt3a and LU2 medium
B.
200000
100000
0
1 2 3 4
Control-Fc
FZD8-Fc
LGR5-Fc
1 – Control medium
2 – LU25 medium
3 – Wnt3a medium
4 – Wnt3a and LU25 medium

Figure 13

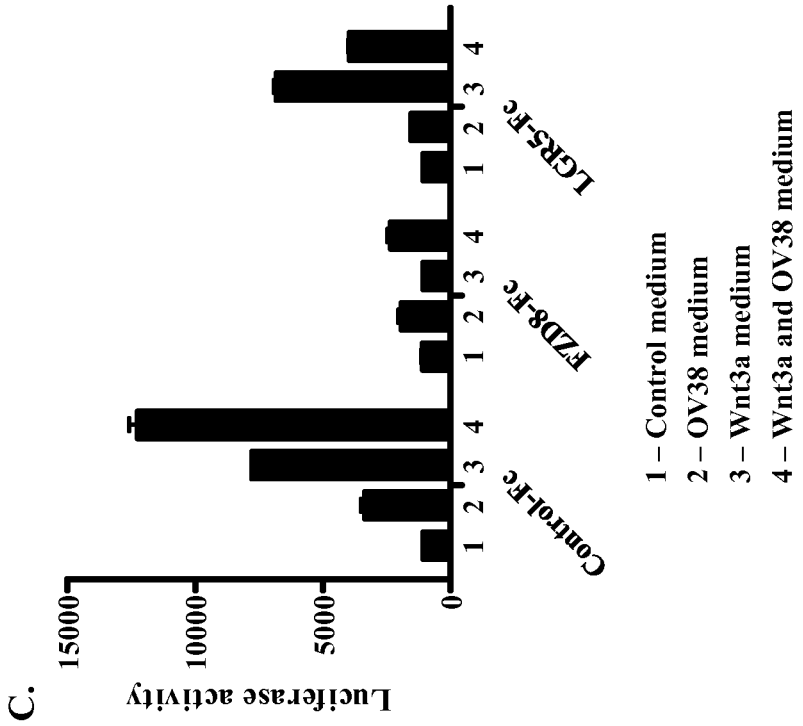

C.
Luciferase activity
15000
10000
5000
0
1 2 3 4
Control-Fc
1 2 3 4
FZD8-Fc
1 2 3 4
LGR5-Fc
1 – Control medium
2 – OV38 medium
3 – Wnt3a medium
4 – Wnt3a and OV38 medium

Figure 14

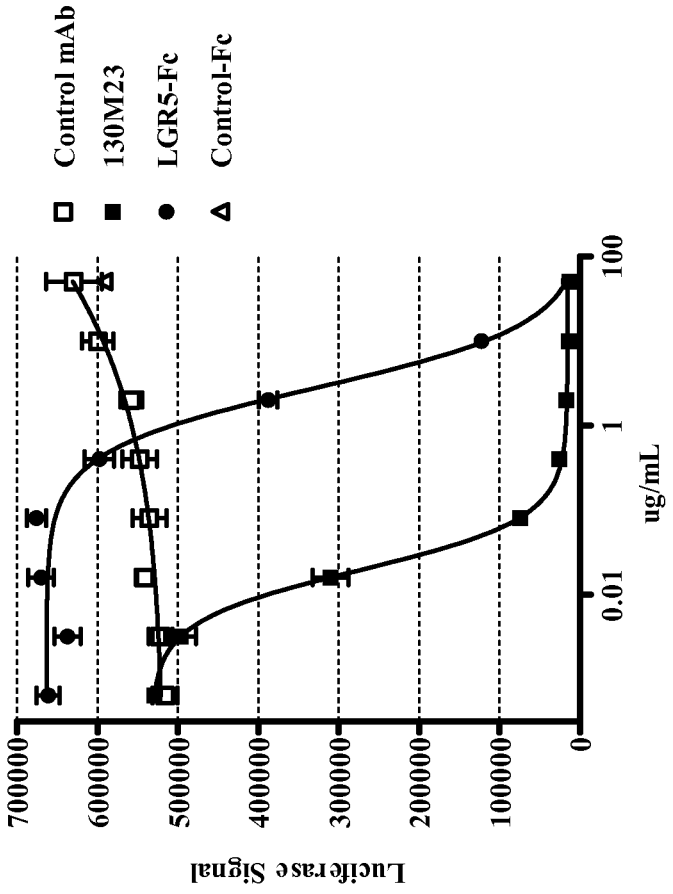

Control mAb
130M23
LGR5-Fc
Control-Fc
Luciferase Signal
700000
600000
500000
400000
300000
200000
100000
0
0.01
1
100
ug/mL

Figure 15

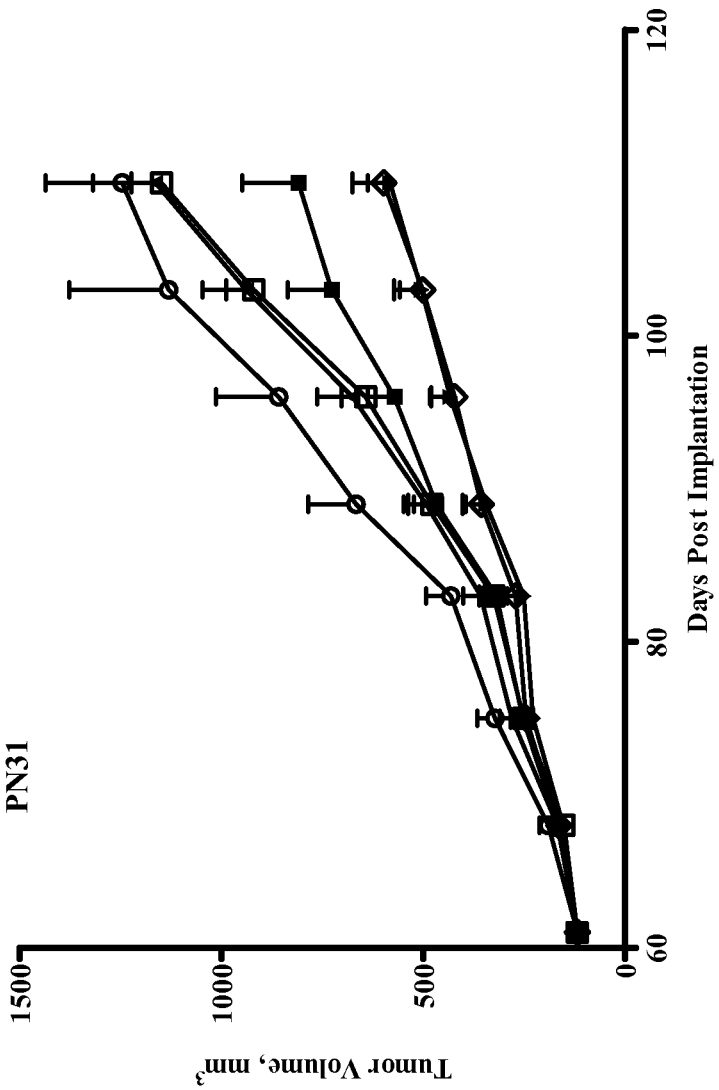

1B711
Gemcitabine
89M5
130M23
89M5 + Gem
130M23 + Gem
PN31
Tumor Volume, mm3
1500
1000
500
0
60
80
100
120
Days Post Implantation

Figure 16

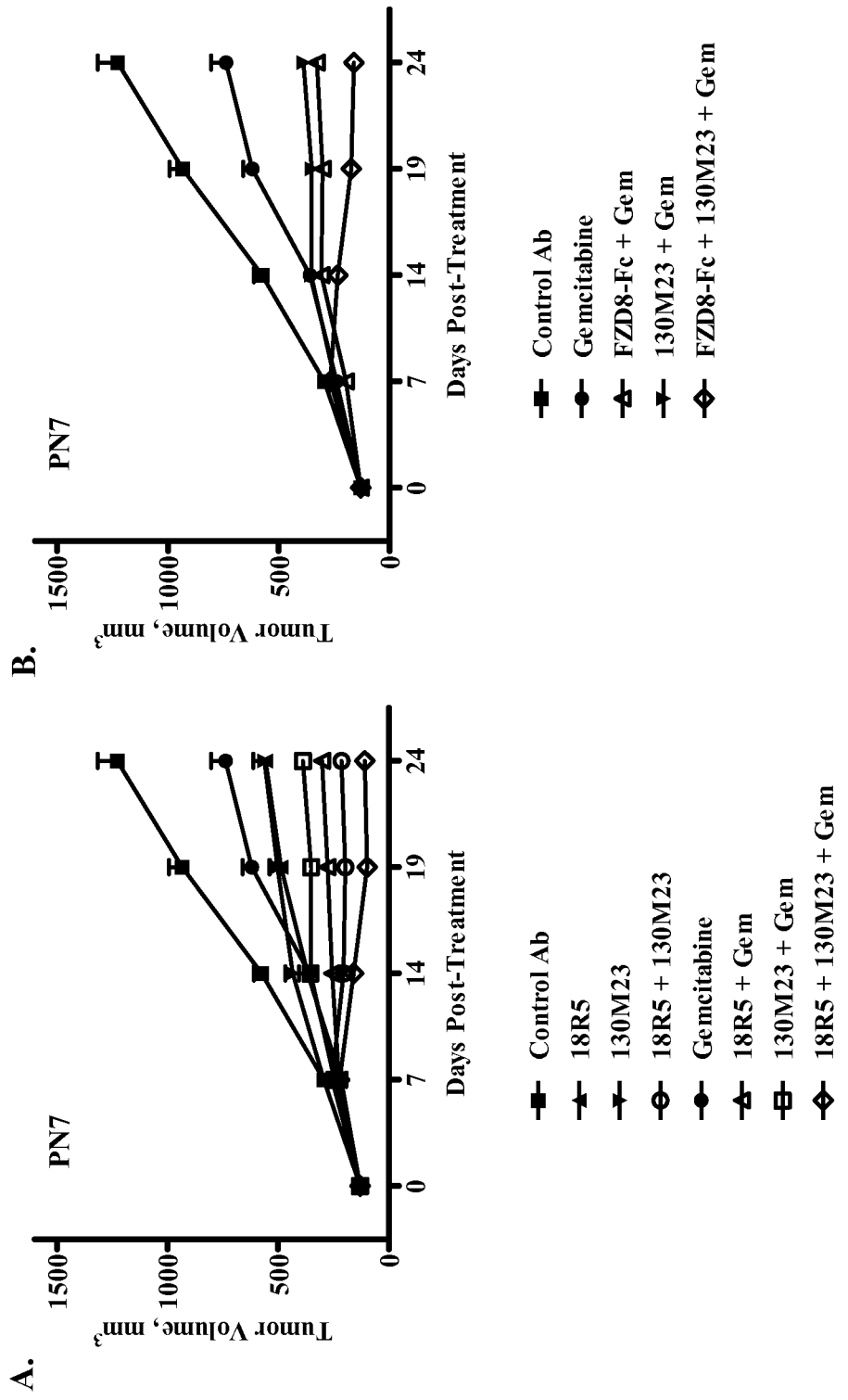

A.
PN7
Tumor Volume, mm3
1500
1000
500
0
0
7
14
19
24
Days Post-Treatment
Control Ab
18R5
130M23
18R5 + 130M23
Gemcitabine
18R5 + Gem
130M23 + Gem
18R5 + 130M23 + Gem
B.
PN7
Tumor Volume, mm3
1500
1000
500
0
0
7
14
19
24
Days Post-Treatment
Control Ab
Gemcitabine
FZD8-Fc + Gem
130M23 + Gem
FZD8-Fc +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

Figure 16

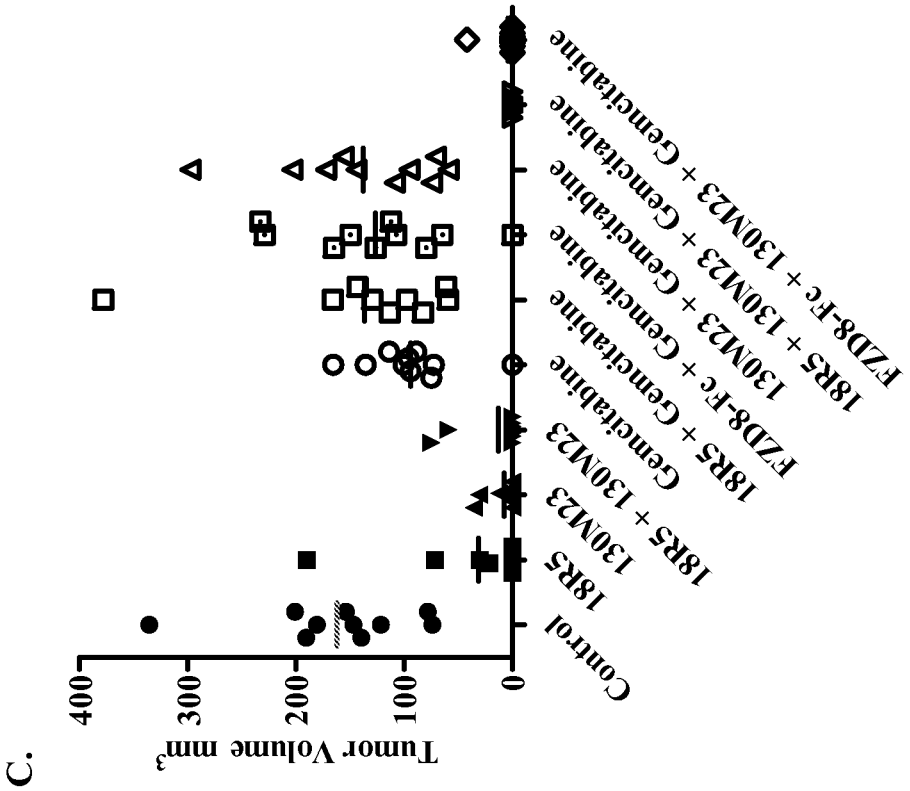

C.
Tumor Volume mm3
400
300
200
100
0
Control
18R5
13OM23
18R5 + 13OM23
Gemcitabine
18R5 + Gemcitabine
FZD8-Fc + Gemcitabine
13OM23 + Gemcitabine
18R5 + 13OM23 + Gemcitabine
FZD8-Fc + 13OM23 + Gemcitabine

Figure 17

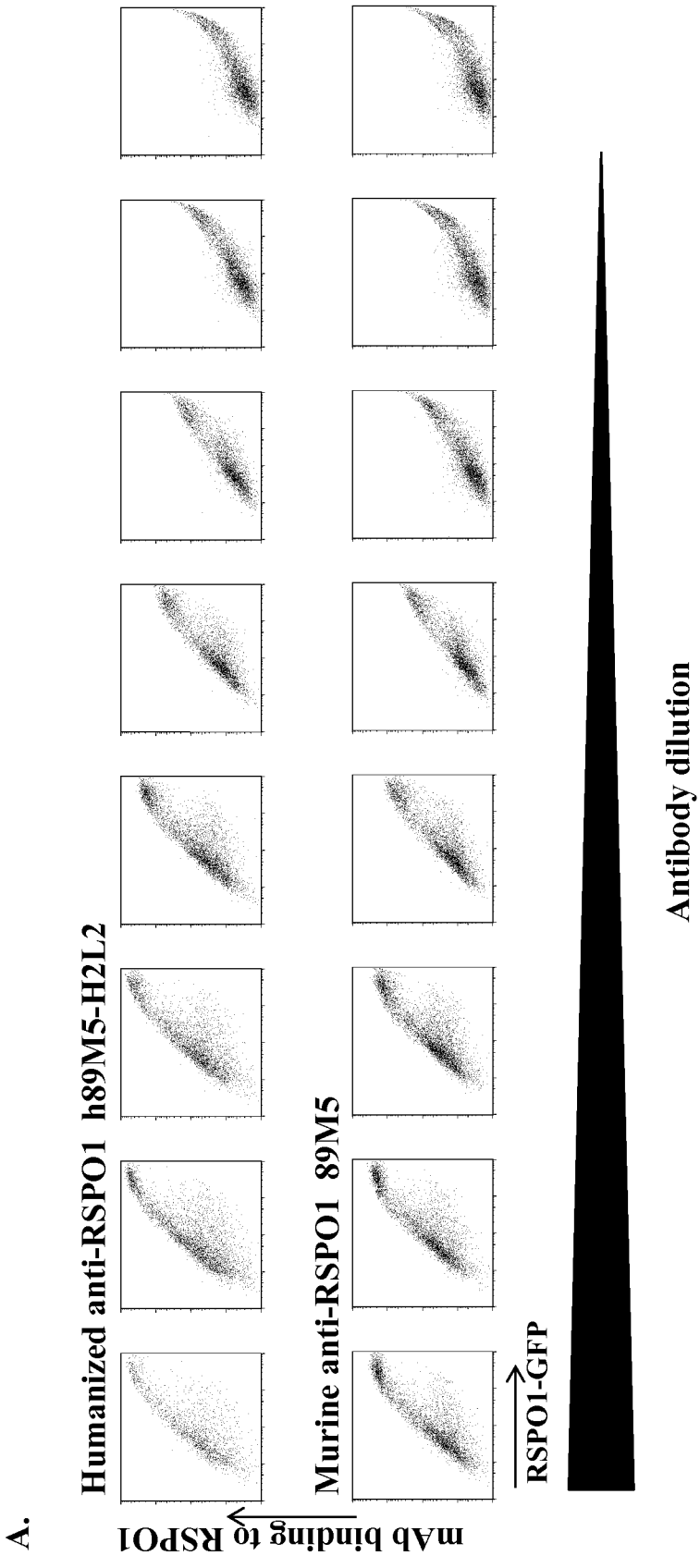

A.
Humanized anti-RSPO1 h89M5-H2L2
Murine anti-RSPO1 89M5
mAb binding to RSPO1
RSPO1-GFP
Antibody dilution

Figure 17

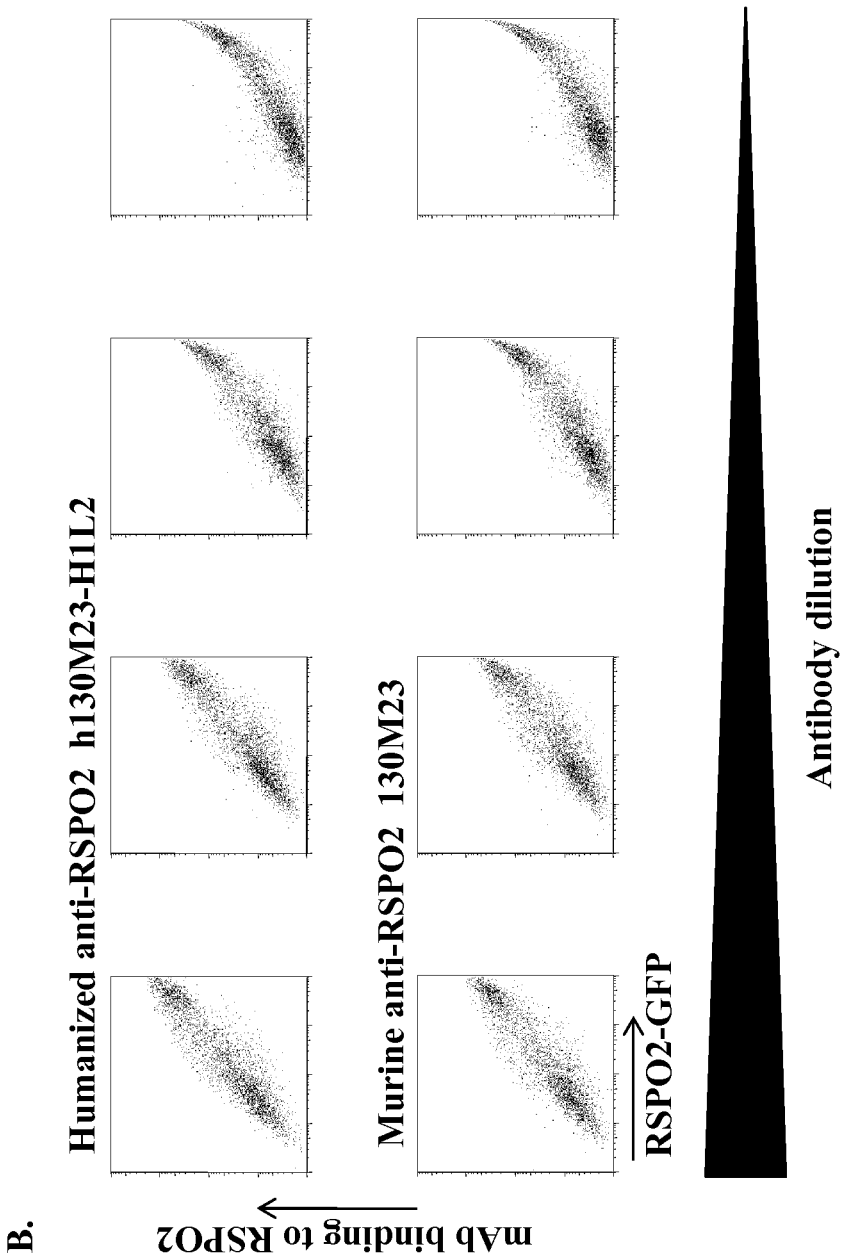
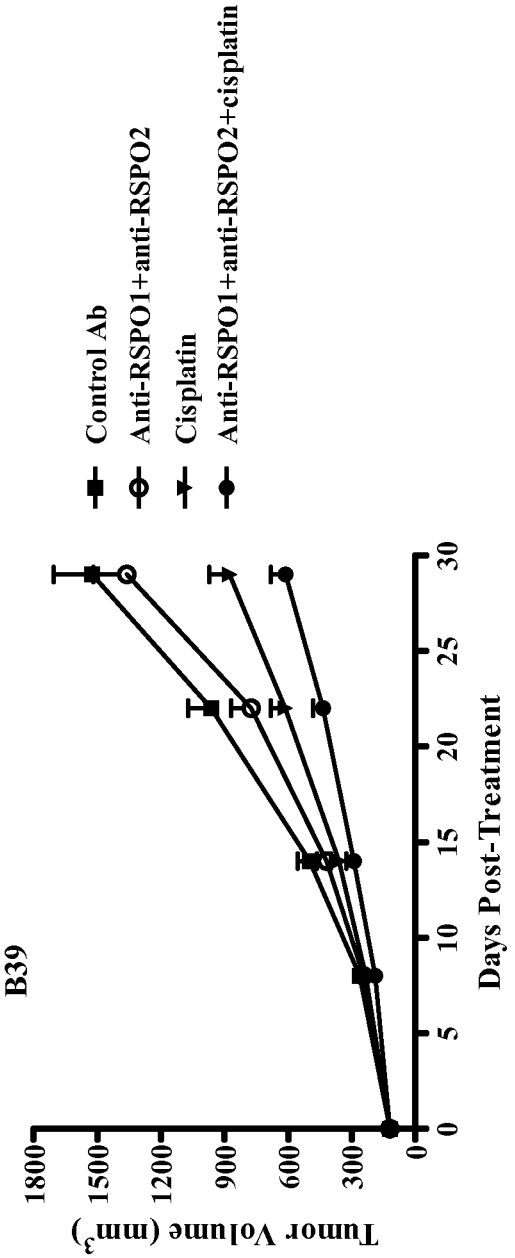

B.
mAb binding to RSPO2
Humanized anti-RSPO2 h130M23-H1L2
Murine anti-RSPO2 130M23
RSPO2-GFP
Antibody dilution

Figure 18


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