Figure 1

Specific Binding of anti-DLL4 21M18 Antibodies to Native Cell-Surface DLL4 Protein

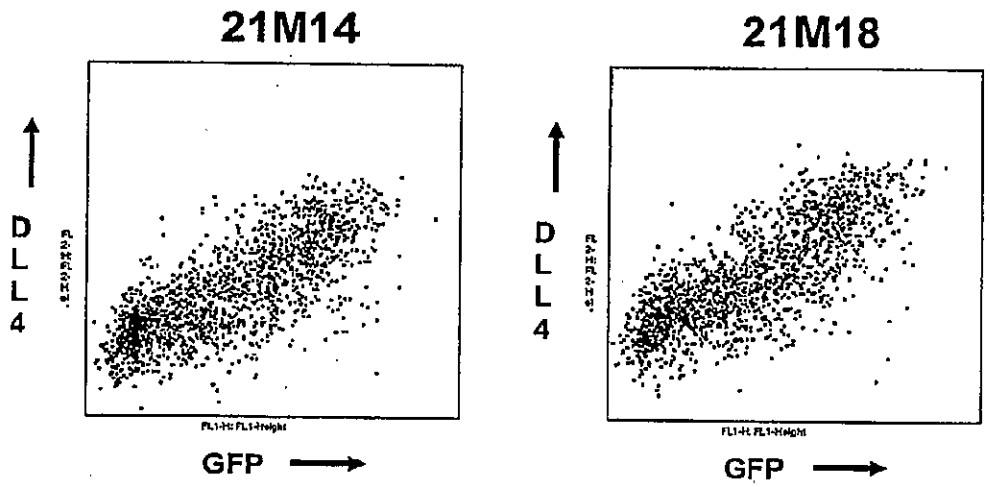
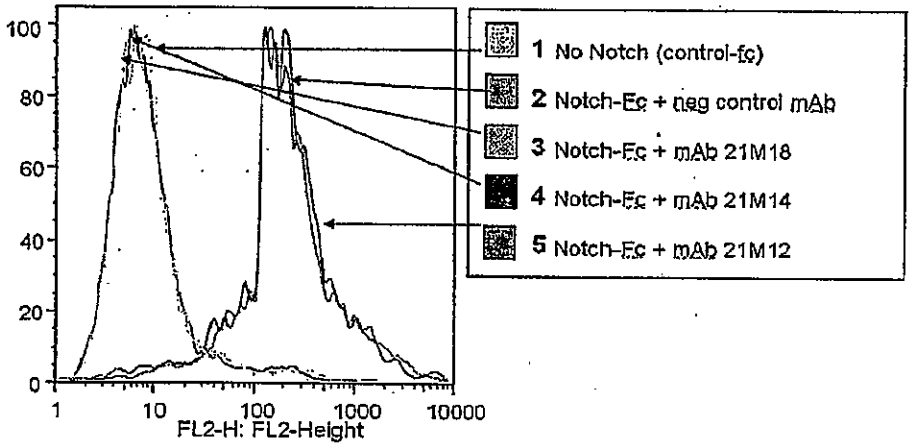


Figure 2

DLL4 Antibodies Block the Interaction of DLL4 with the Human Notch Receptor

A



B

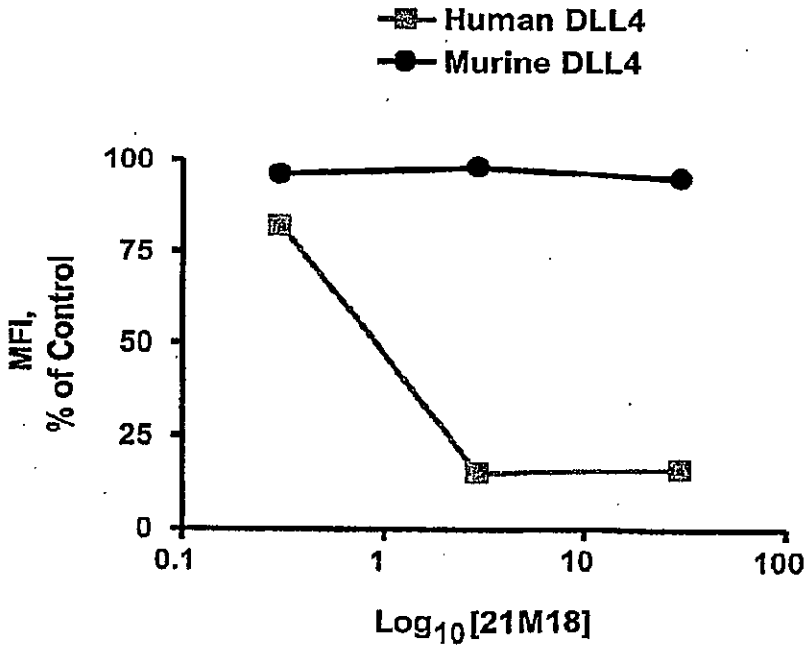


Figure 3

Epitope Mapping of Anti-DLL4 Antibodies

A

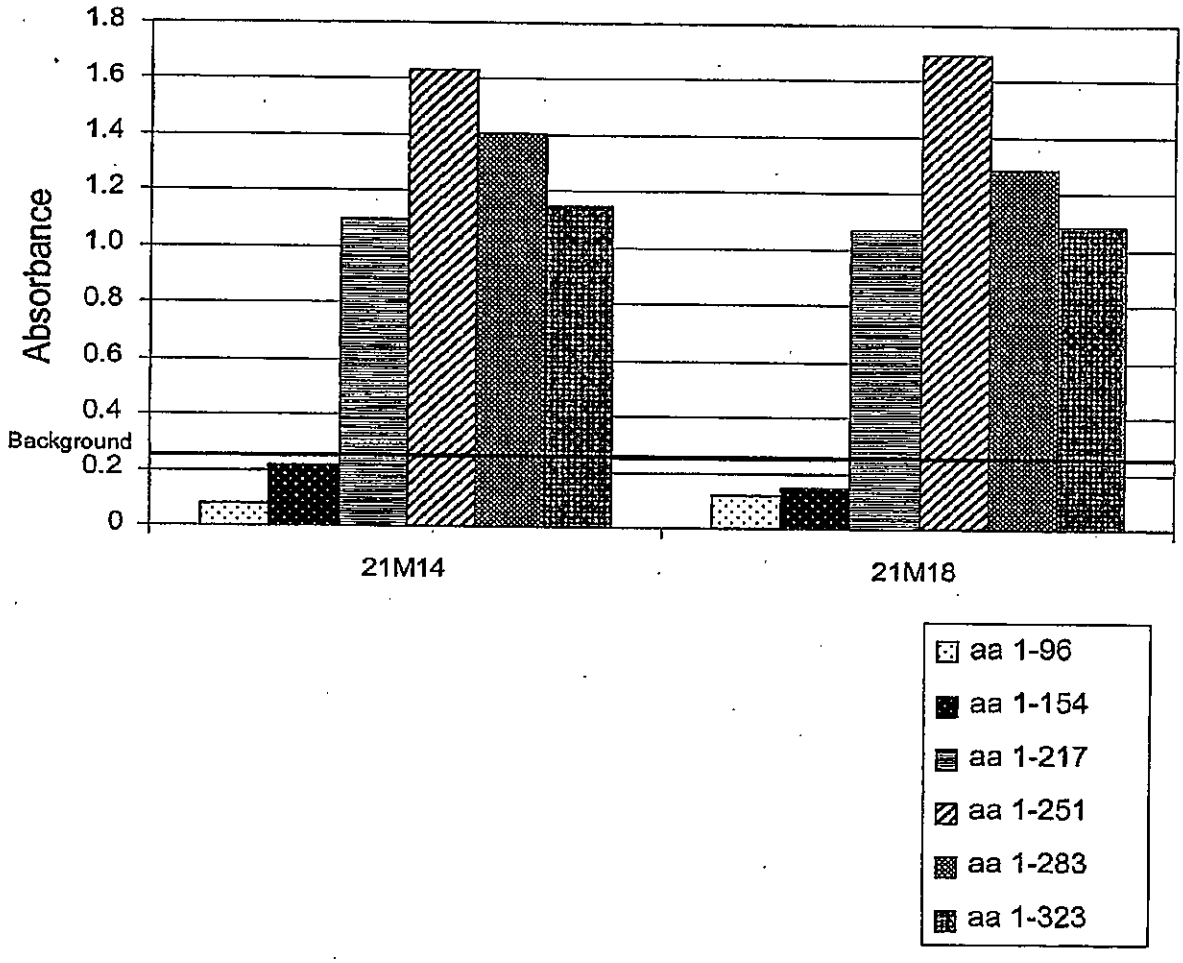


Figure 3B

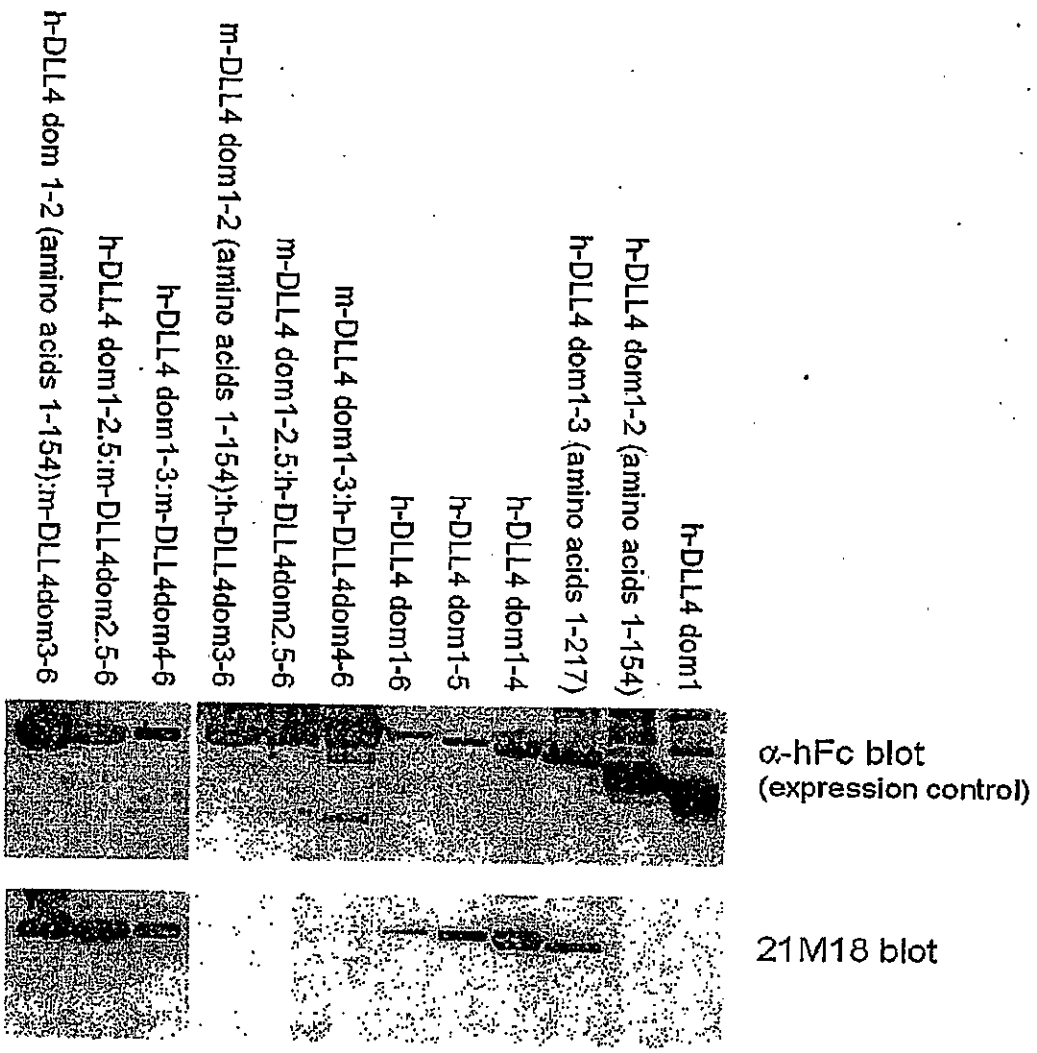

h-DLL4 dom1
h-DLL4 dom1-2 (amino acids 1-154)
h-DLL4 dom1-3 (amino acids 1-217)
h-DLL4 dom1-4
h-DLL4 dom1-5
h-DLL4 dom1-6
m-DLL4 dom1-3:h-DLL4dom4-6
m-DLL4 dom1-2.5:h-DLL4dom2.5-6
m-DLL4 dom1-2 (amino acids 1-154):h-DLL4dom3-6
h-DLL4 dom1-3:m-DLL4dom4-6
h-DLL4 dom1-2.5:m-DLL4dom2.5-6
h-DLL4 dom 1-2 (amino acids 1-154):m-DLL4dom3-6
α-hFc blot
(expression control)
21M18 blot

Figure 3C

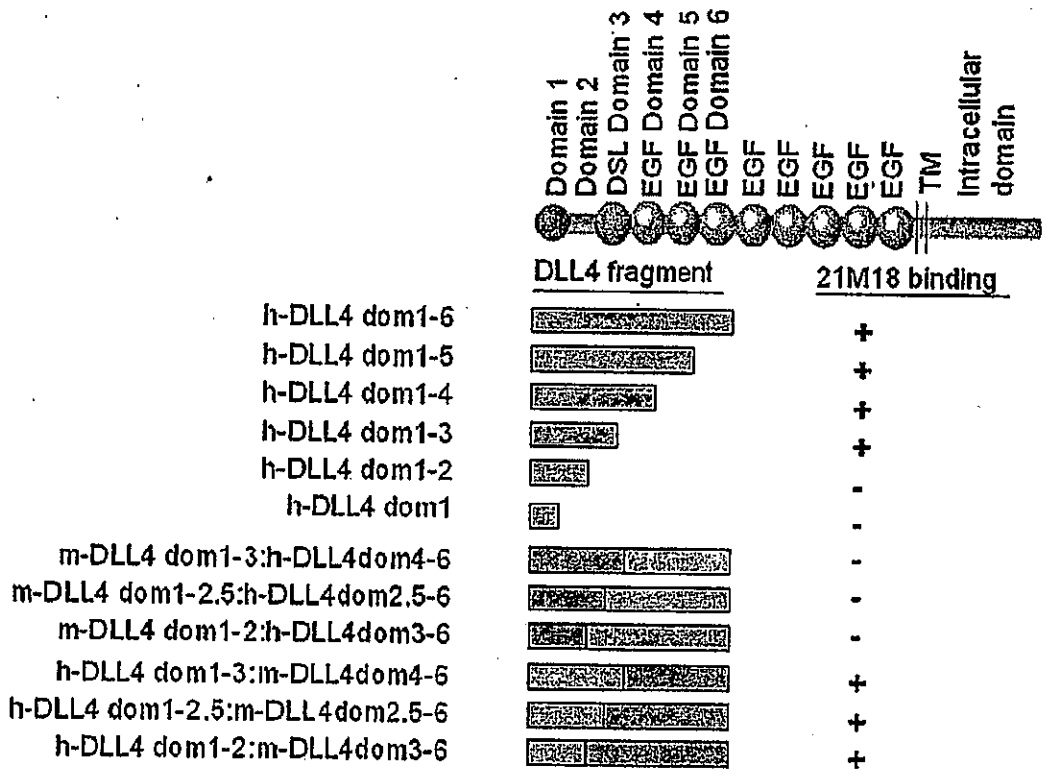

Domain 1
Domain 2
DSL Domain 3
EGF Domain 4
EGF Domain 5
EGF Domain 6
EGF
EGF
EGF
EGF
EGF
TM
Intracellular domain
DLL4 fragment
21M18 binding
h-DLL4 dom1-6
h-DLL4 dom1-5
h-DLL4 dom1-4
h-DLL4 dom1-3
h-DLL4 dom1-2
h-DLL4 dom1
m-DLL4 dom1-3:h-DLL4dom4-6
m-DLL4 dom1-2.5:h-DLL4dom2.5-6
m-DLL4 dom1-2:h-DLL4dom3-6
h-DLL4 dom1-3:m-DLL4dom4-6
h-DLL4 dom1-2.5:m-DLL4dom2.5-6
h-DLL4 dom1-2:m-DLL4dom3-6
+
+
+
+
-
-
-
-
-
+
+
+

Figure 3D

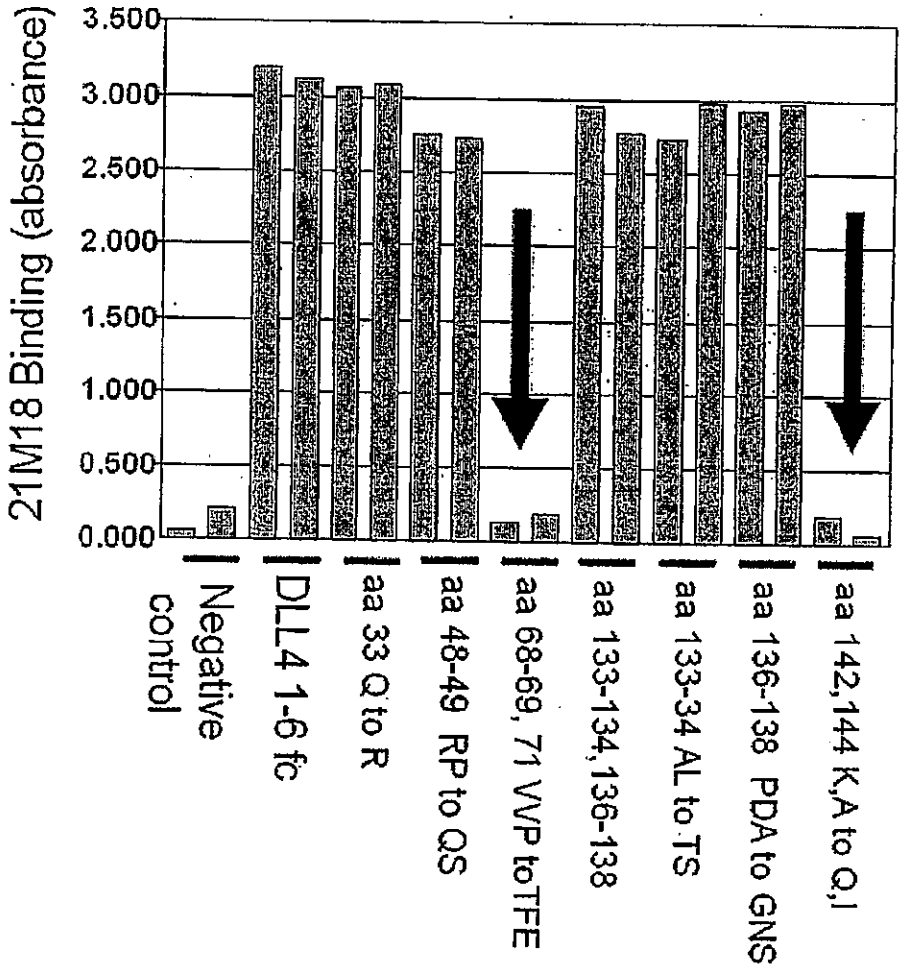

21M18 Binding (absorbance)
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
Negative control
DLL4 1-6 fc
aa 33 Q to R
aa 48-49 RP to QS
aa 68-69, 71 VVP toTFE
aa 133-134,136-138
aa 133-34 AL to TS
aa 136-138 PDA to GNS
aa 142,144 K,A to Q,I

Figure 3E

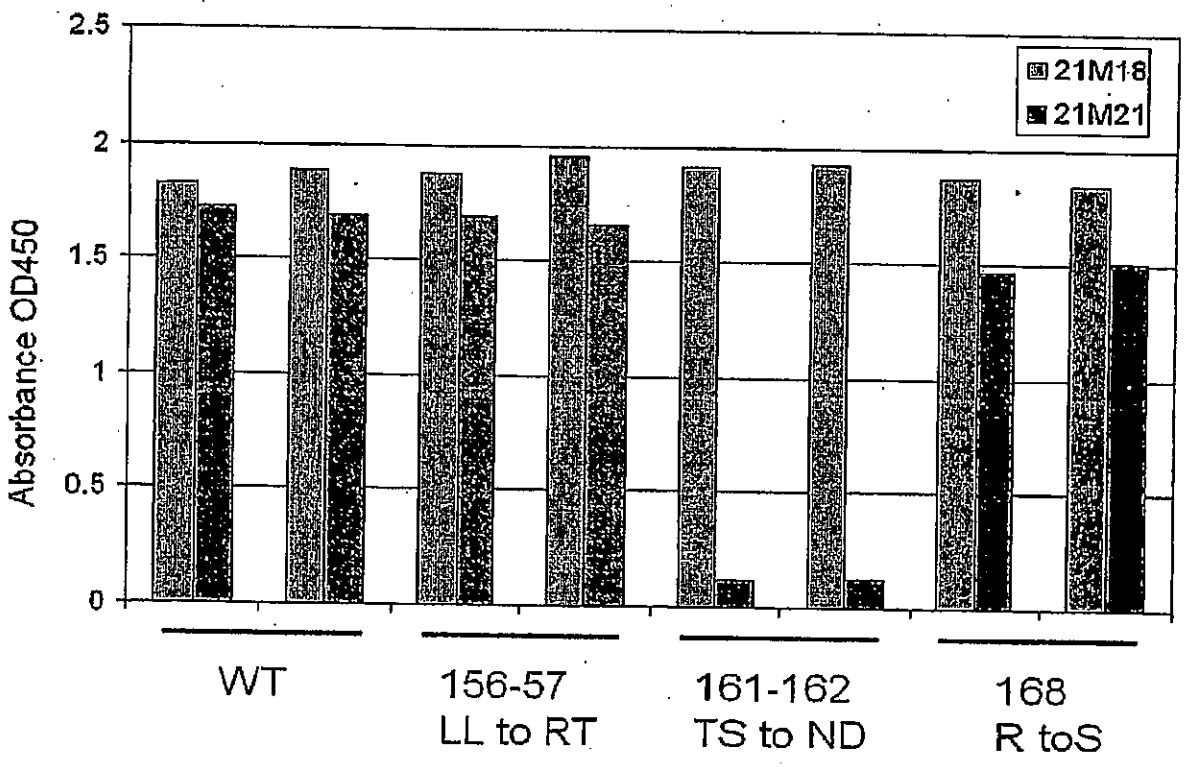

2.5
2
1.5
1
0.5
0
Absorbance OD450
21M18
21M21
WT
156-57
LL to RT
161-162
TS to ND
168
R toS

Figure 4

Sequence alignment of the heavy chain variable region

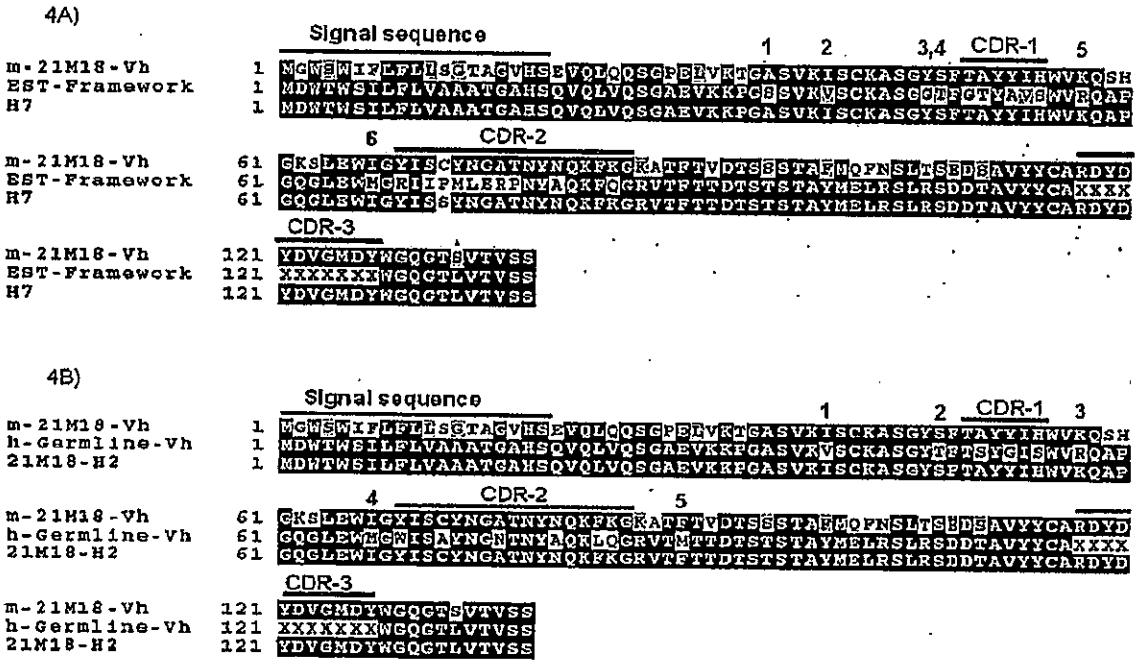


Figure 5

Sequence alignment of the light chain variable region

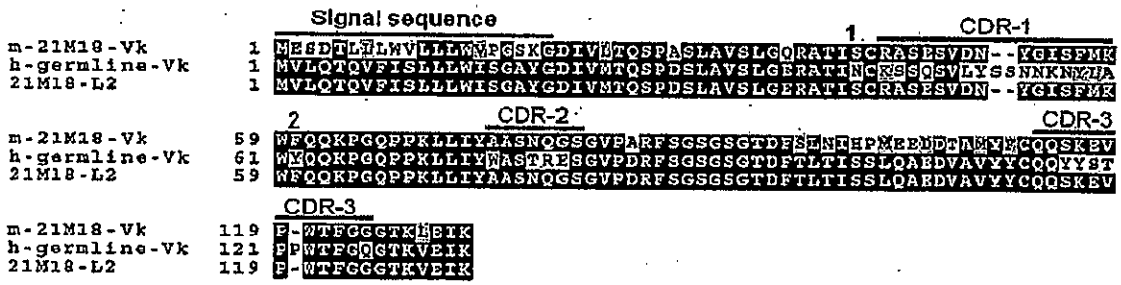


Figure 6

21M14 and 21M18 Block DLL4 Mediated Notch signaling

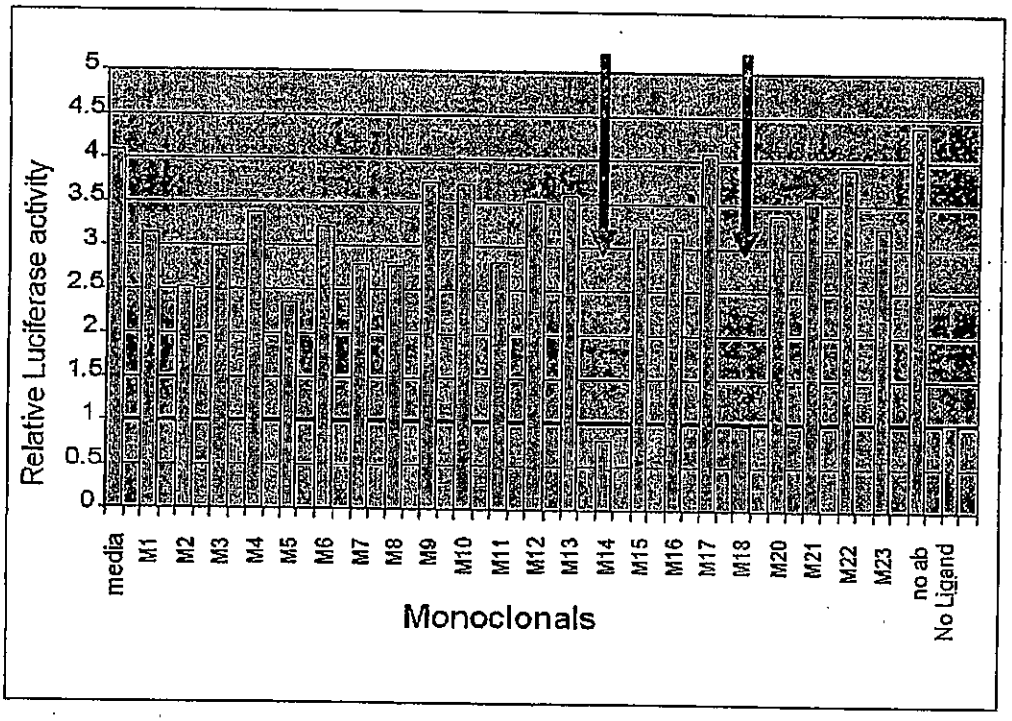
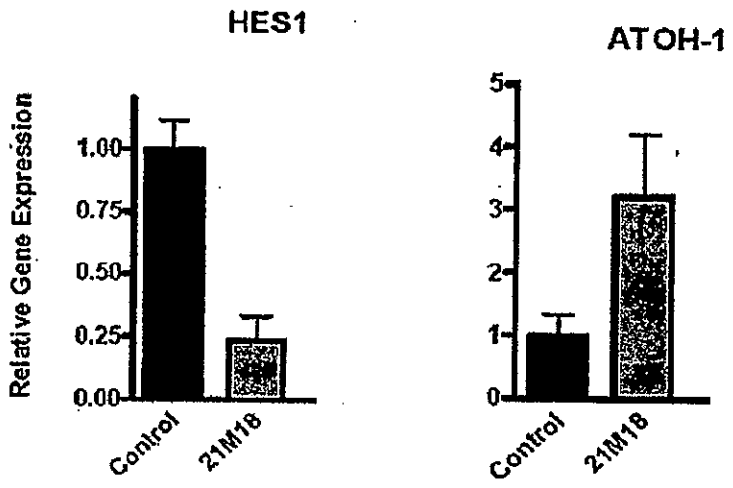


Figure 7

DLL4 Antibodies Modulate Expression of Notch Target Genes in Colon Tumors

A



B

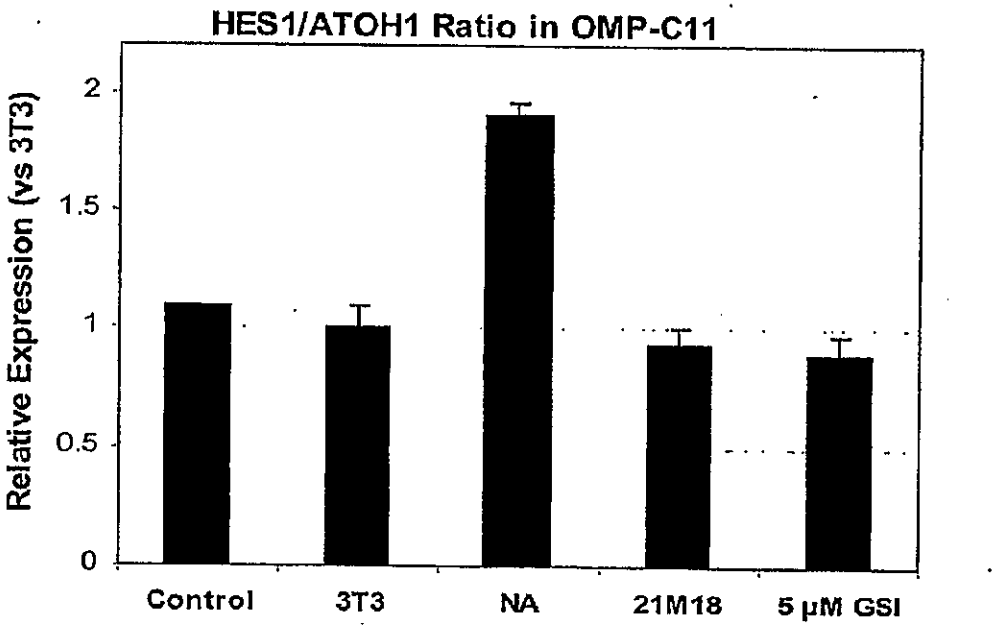
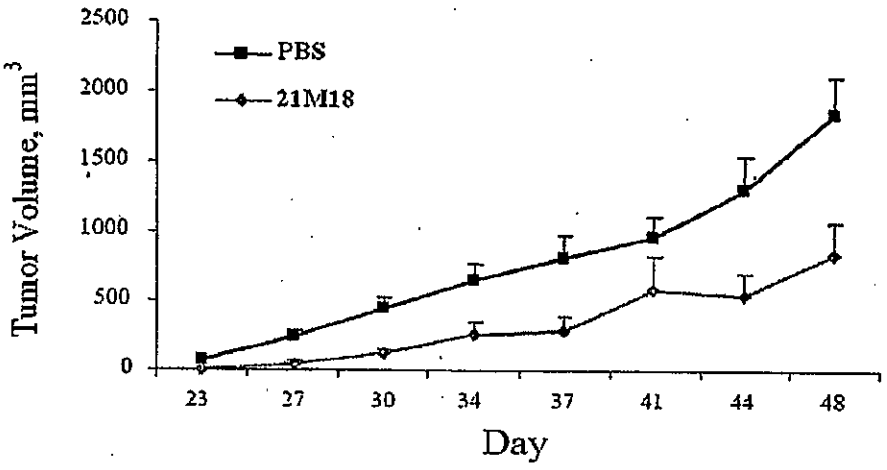


Figure 8

21M18 (Anti-DLL4) Reduces UM-C4 Colon Tumor Growth



Tumor reduction = 54% p = 0.03

Figure 9

Anti-DLL4 21M18 Antibodies Reduces the Number of Proliferating Tumor Cells in Vivo

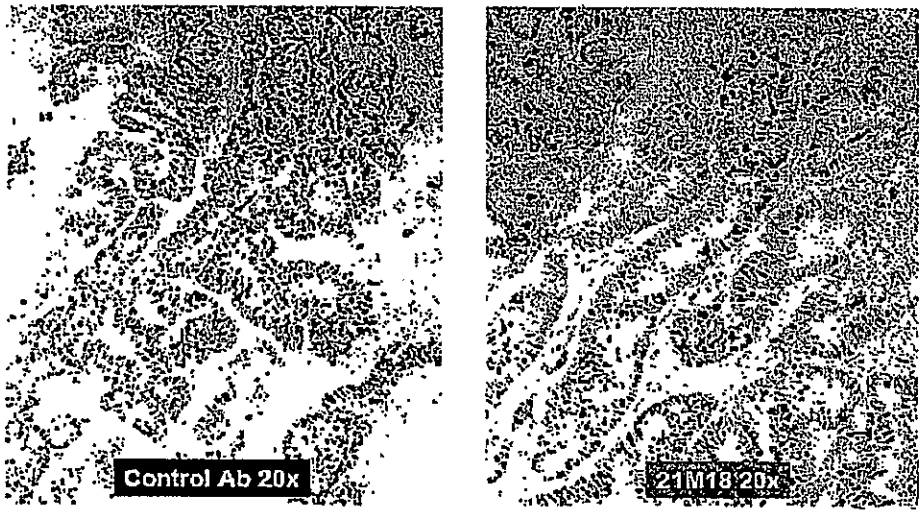
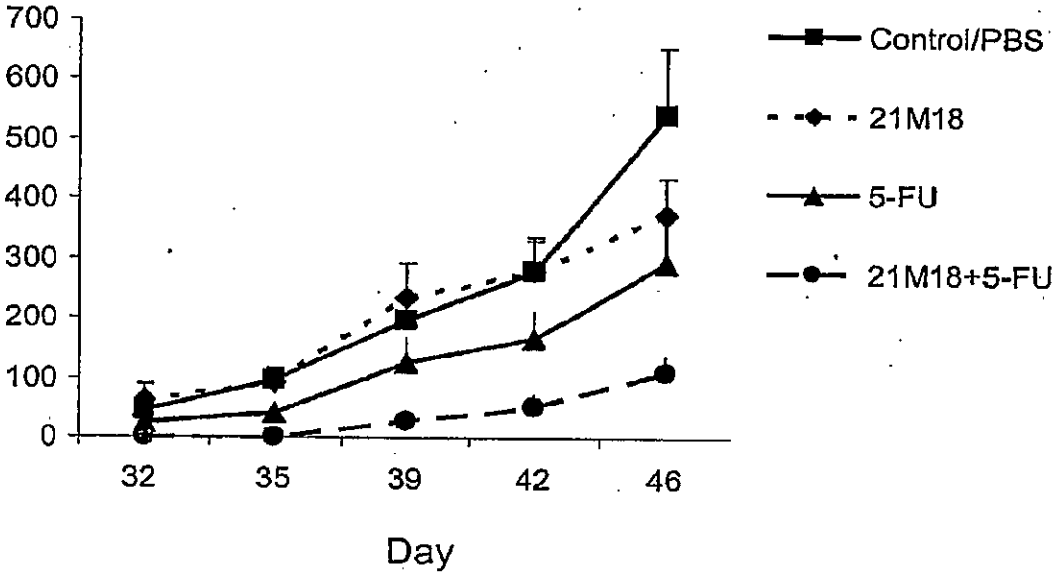


Figure 10

Anti-DLL4 and 5-FU Reduce UM-C4 Colon Tumor Growth

A



B

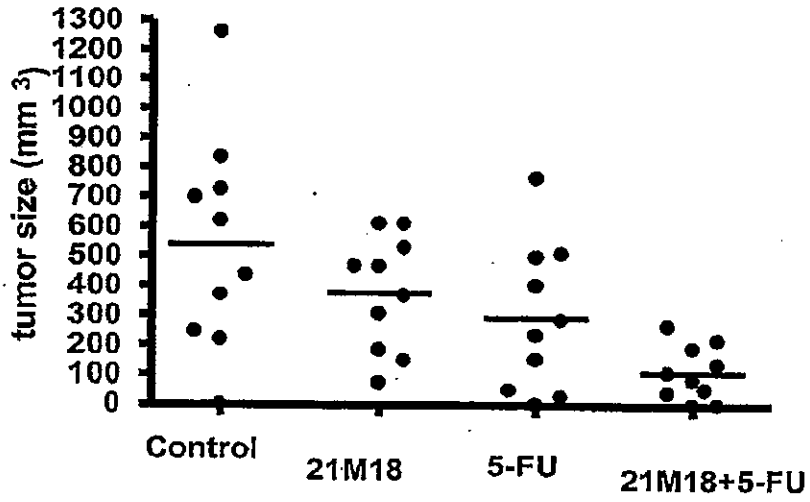


Figure 11

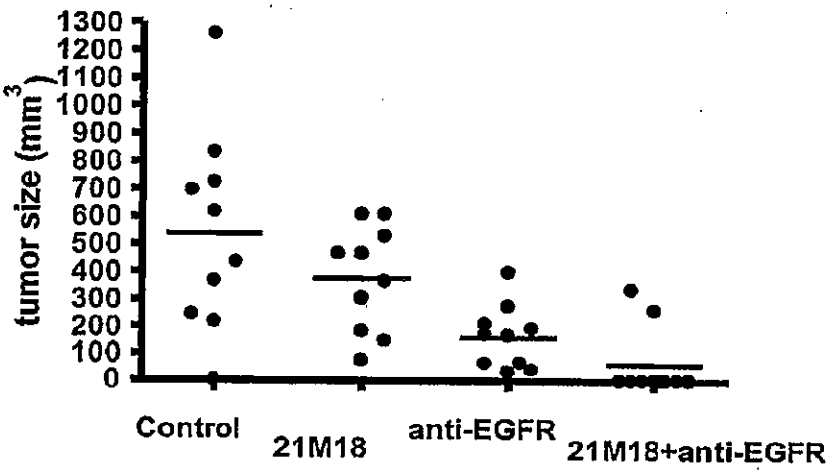
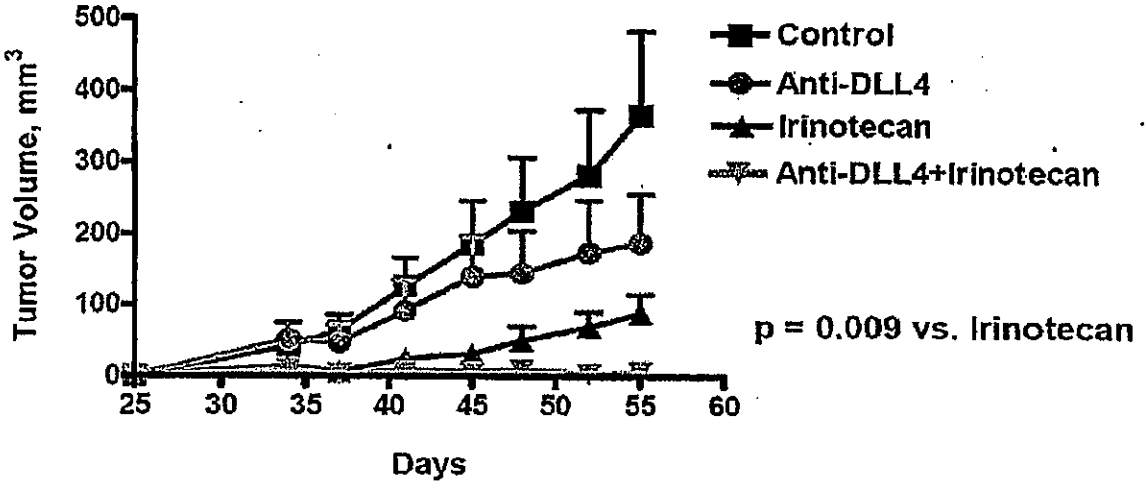

Combination of anti-DLL4 and anti-EGFR Inhibits UM-C4 Tumor Growth
tumor size (mm3)
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
Control
21M18
anti-EGFR
21M18+anti-EGFR

Figure 12

Anti-DLL4 mAb 21M18 and Irinotecan Act Synergistically to Inhibit Colon Tumor Growth

A



B

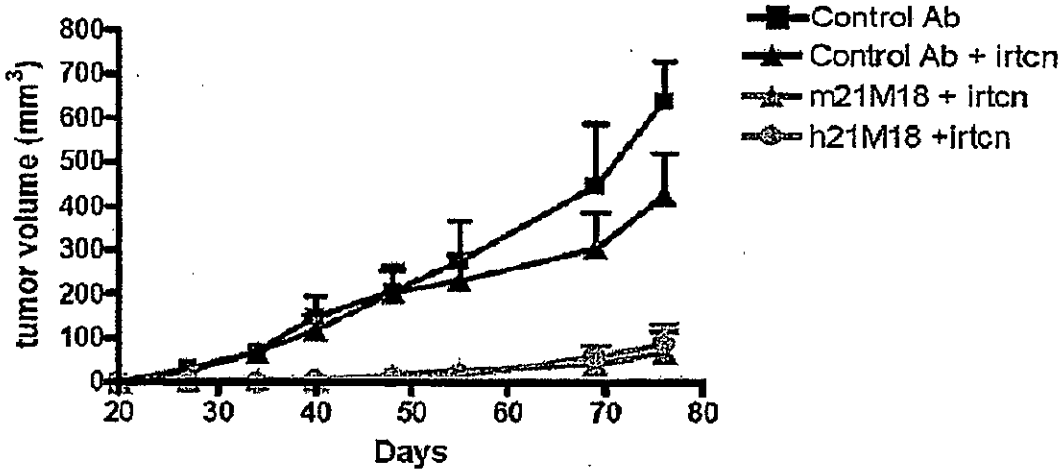
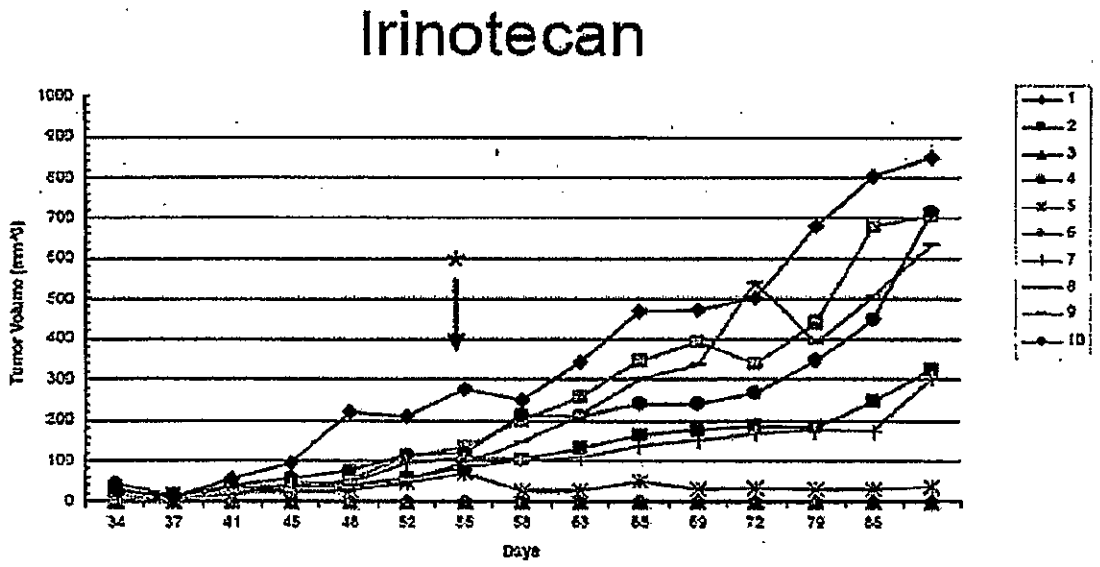


Figure 13

Combination Anti-DLL4 mAb 21M18 and Irinotecan Treatment Prevents Colon Tumor Re-Growth

A



B

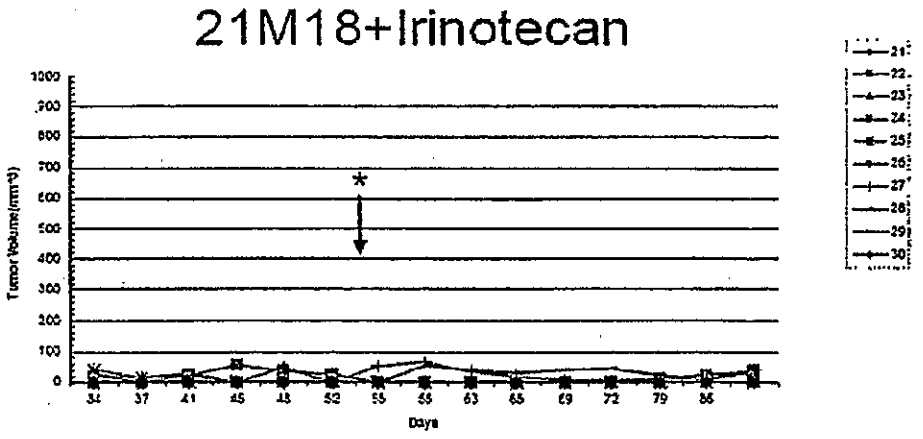


Figure 14

Combination Anti-DLL4 21M18 and Irinotecan Treatment Inhibits Growth of Established Colon Tumors More Effectively than Single Therapy Treatment

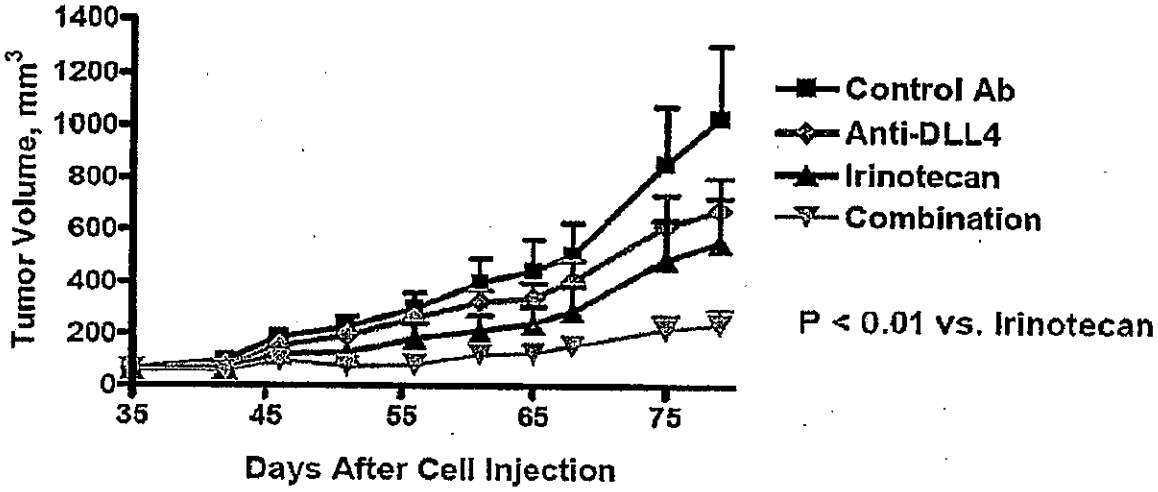


Figure 15A

Combination Anti-DLL4 21M18 and Irinotecan Treatment Reduces Cancer Stem Cell Frequency

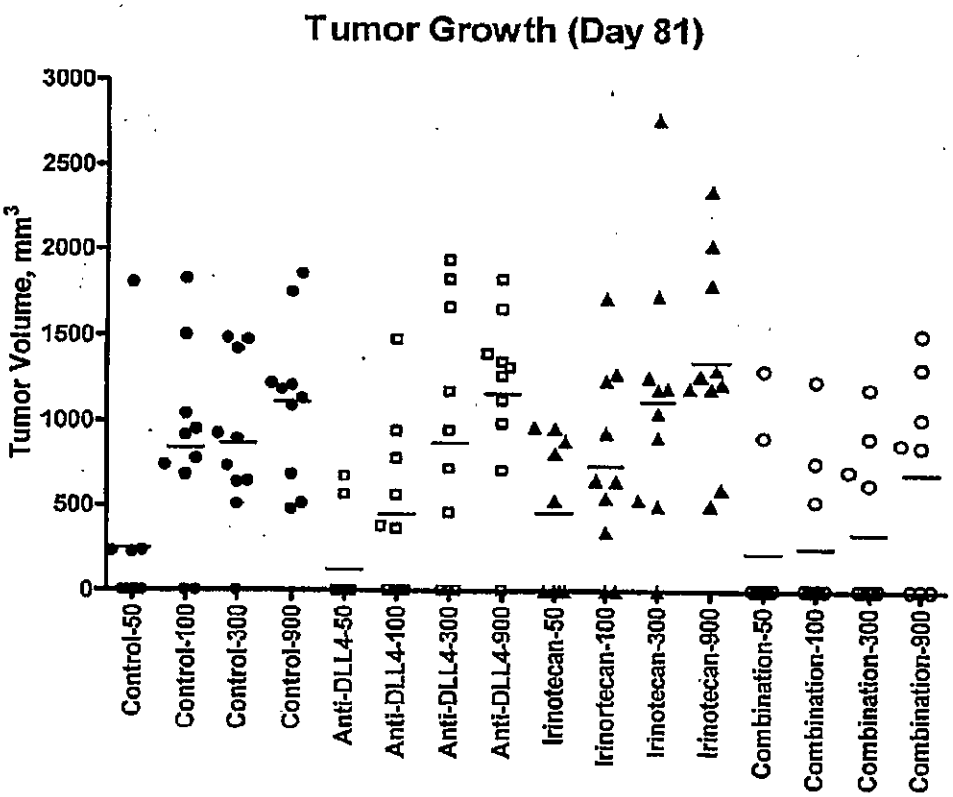


Figure 15B

Combination Anti-DLL4 21M18 and Irinotecan Treatment Reduces Cancer Stem Cell Frequency

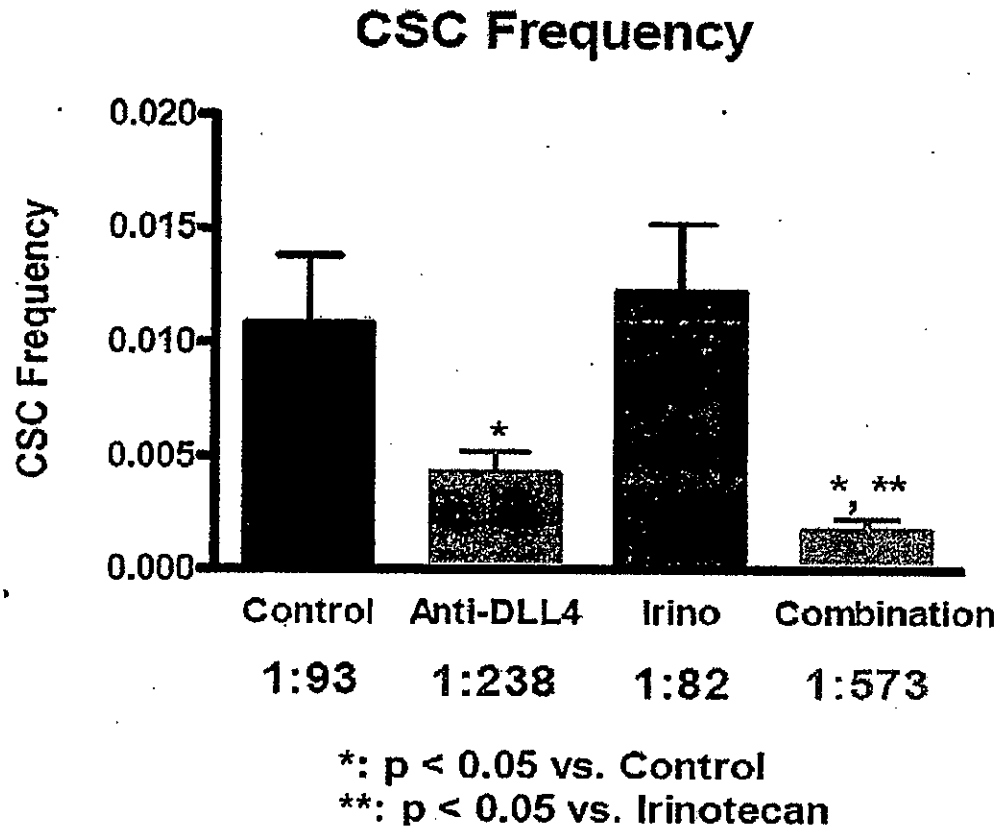


Figure 16

Anti-DLL4 21M18 Treatment Delays Tumor Recurrence after DLL-4 and Irinotecan Combination Treatment

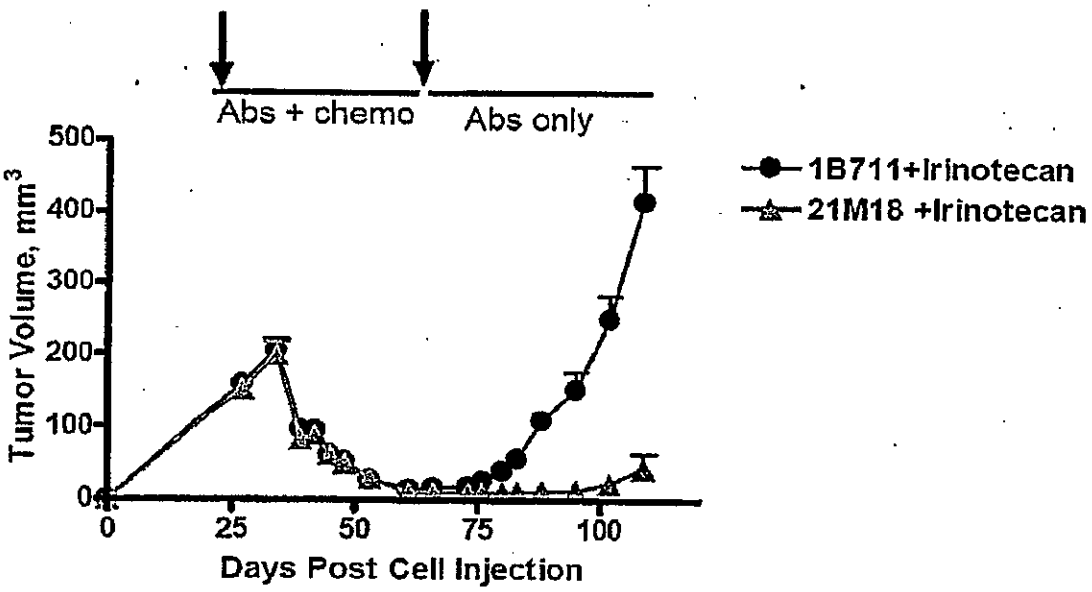


Figure 17

Combination Anti-DLL4 21M18-Irinotecan Treatment Reduces Tumor Volume 47 Days After Treatment Termination

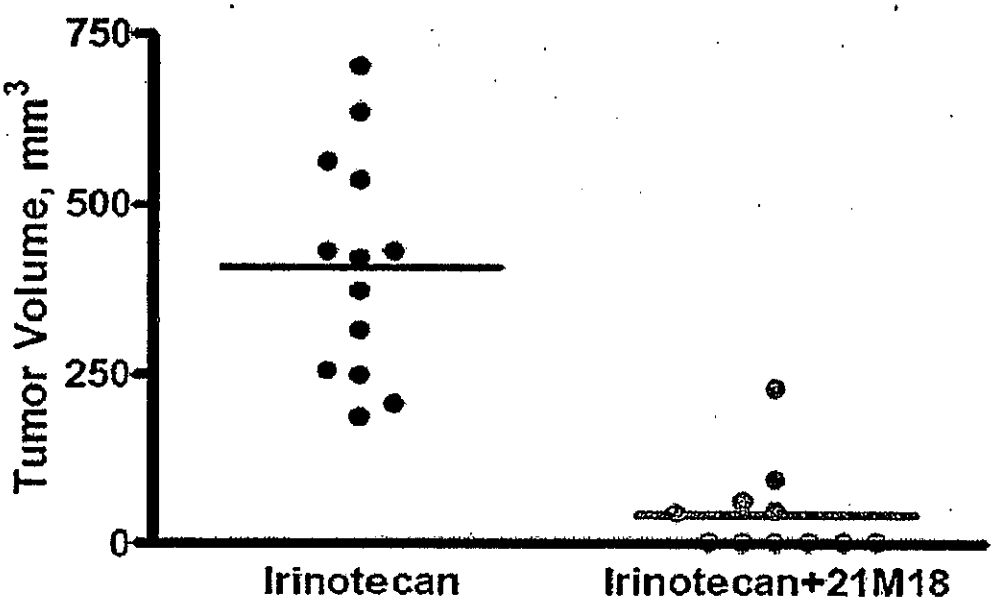


Figure 18

21M18 and Anti-VEGF Inhibit Colon Tumor Growth

