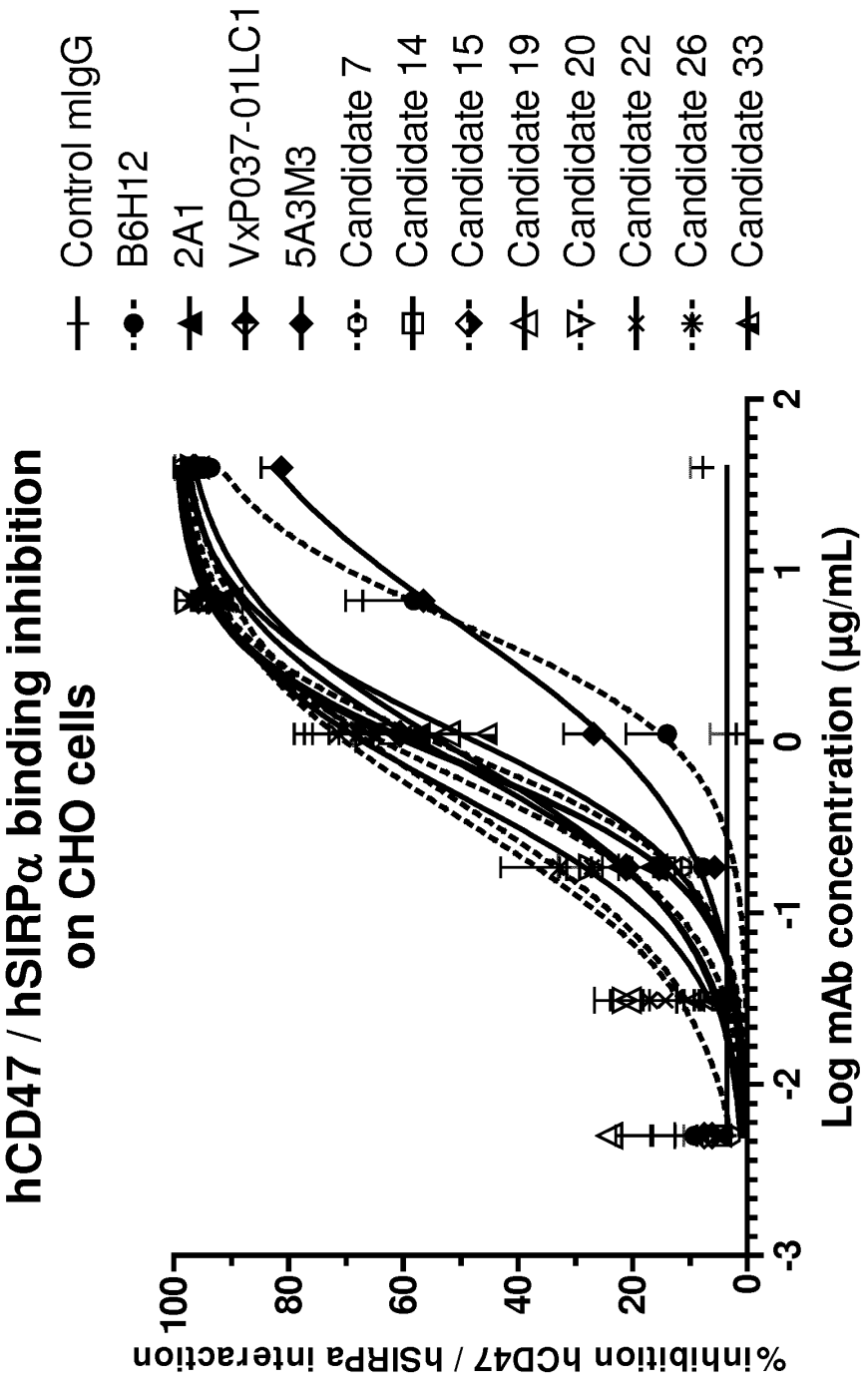

hCD47 / hSIRPα binding inhibition on CHO cells
Control mIgG
B6H12
2A1
VxP037-01LC1
5A3M3
Candidate 7
Candidate 14
Candidate 15
Candidate 19
Candidate 20
Candidate 22
Candidate 26
Candidate 33
%inhibition hCD47 / hSIRPa interaction
0
20
40
60
80
100
-3
-2
-1
0
1
2
Log mAb concentration (µg/mL)

Figure 1

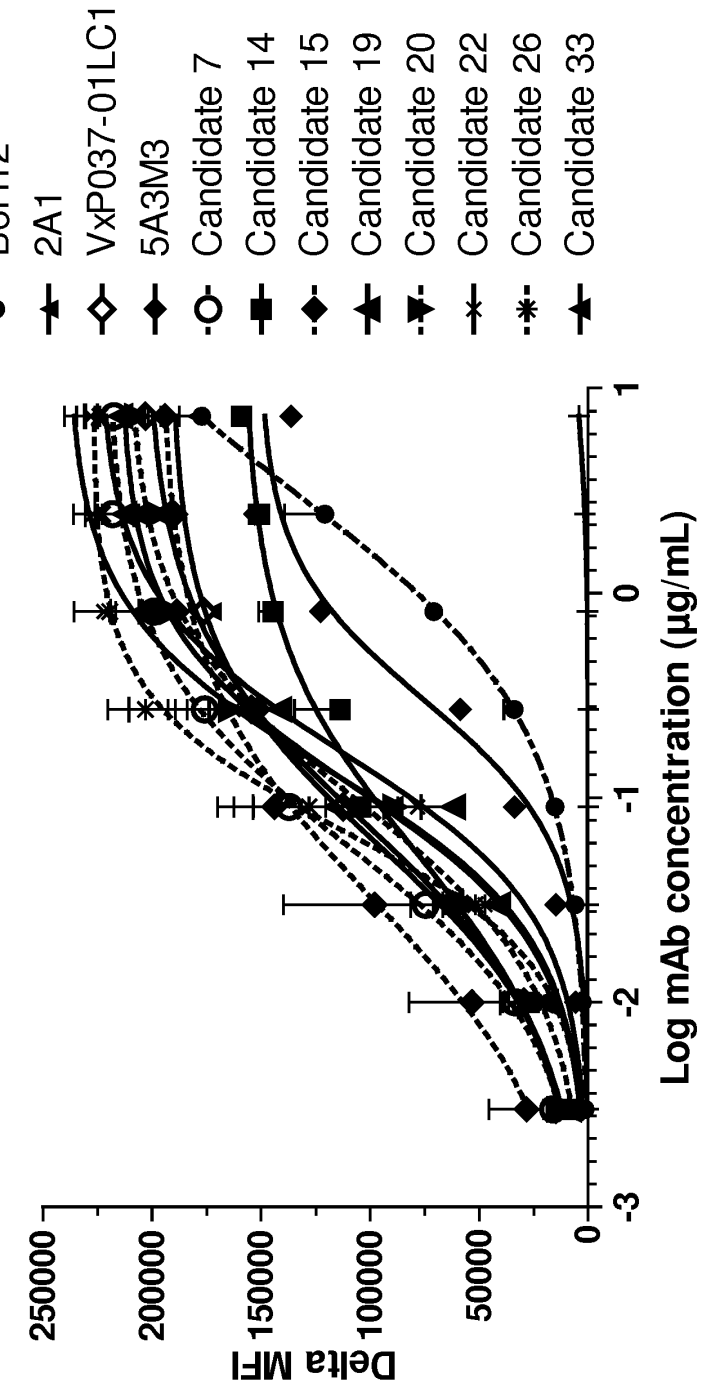

Binding on Raji cells
Control mAb
B6H12
2A1
VxP037-01LC1
5A3M3
Candidate 7
Candidate 14
Candidate 15
Candidate 19
Candidate 20
Candidate 22
Candidate 26
Candidate 33
Delta MFI
250000
200000
150000
100000
50000
0
-3
-2
-1
0
1
Log mAb concentration (µg/mL)

Figure 2

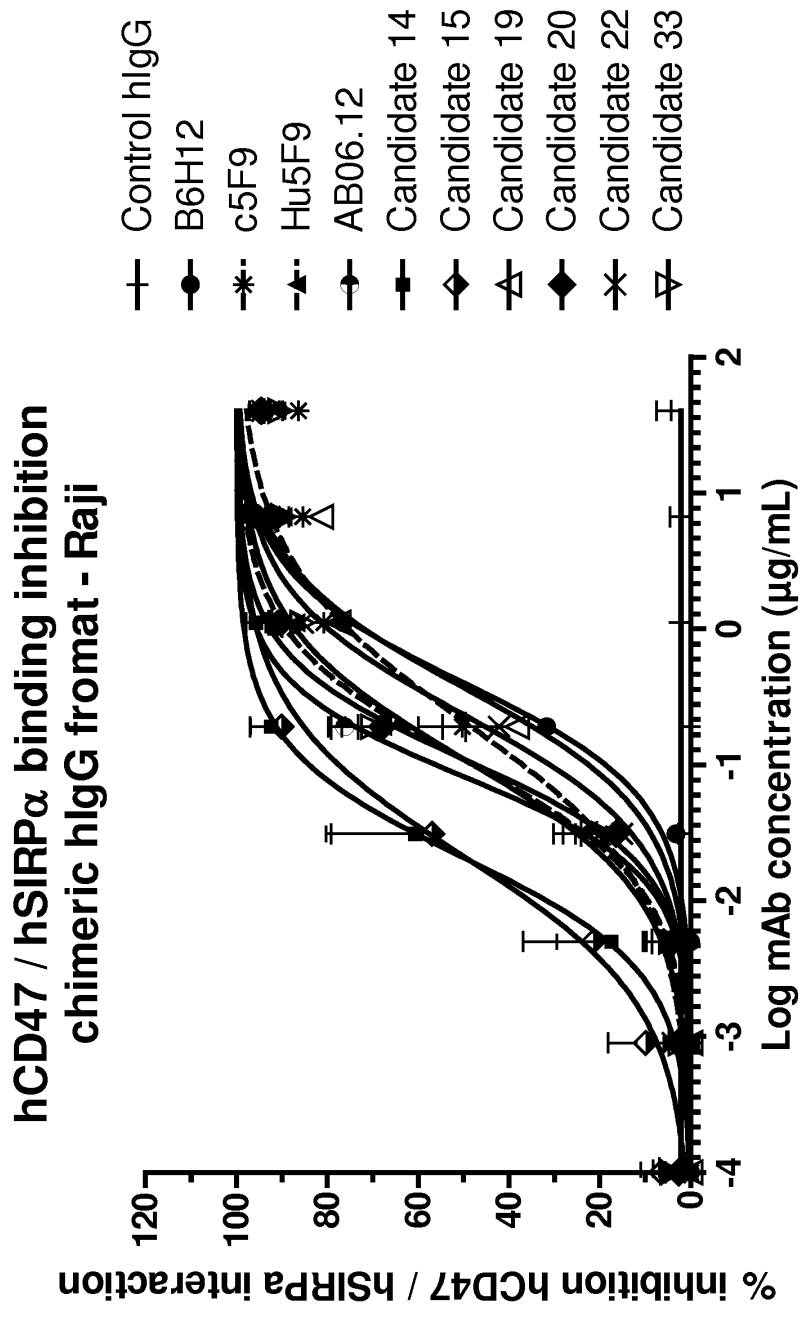

hCD47 / hSIRPα binding inhibition
chimeric hIgG fromat - Raji
% inhibition hCD47 / hSIRPa interaction
Log mAb concentration (µg/mL)
Control hIgG
B6H12
c5F9
Hu5F9
AB06.12
Candidate 14
Candidate 15
Candidate 19
Candidate 20
Candidate 22
Candidate 33

Figure 3

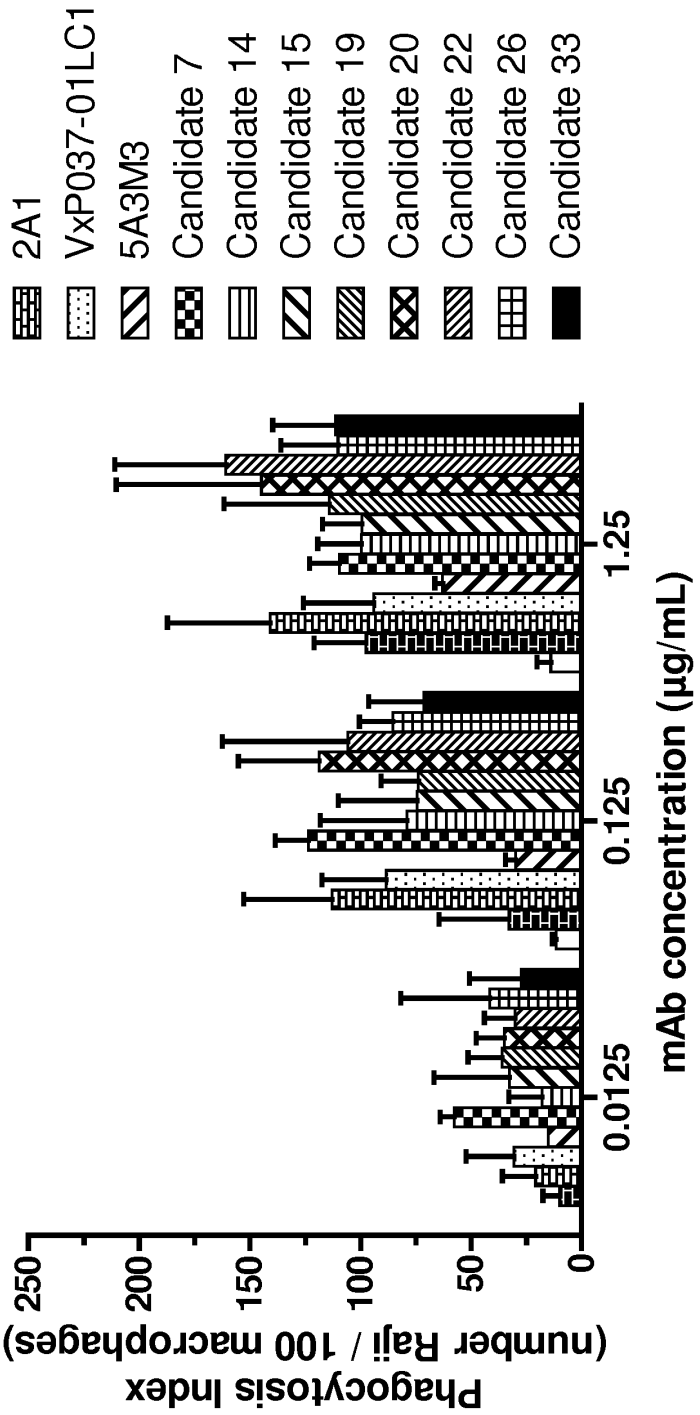

Phagocytosis of Raji cells
by human macrophages
Control mAb
B6H12
2A1
VxP037-01LC1
5A3M3
Candidate 7
Candidate 14
Candidate 15
Candidate 19
Candidate 20
Candidate 22
Candidate 26
Candidate 33
Phagocytosis Index
(number Raji / 100 macrophages)
250
200
150
100
50
0
0.0125
0.125
1.25
mAb concentration (µg/mL)

Figure 4

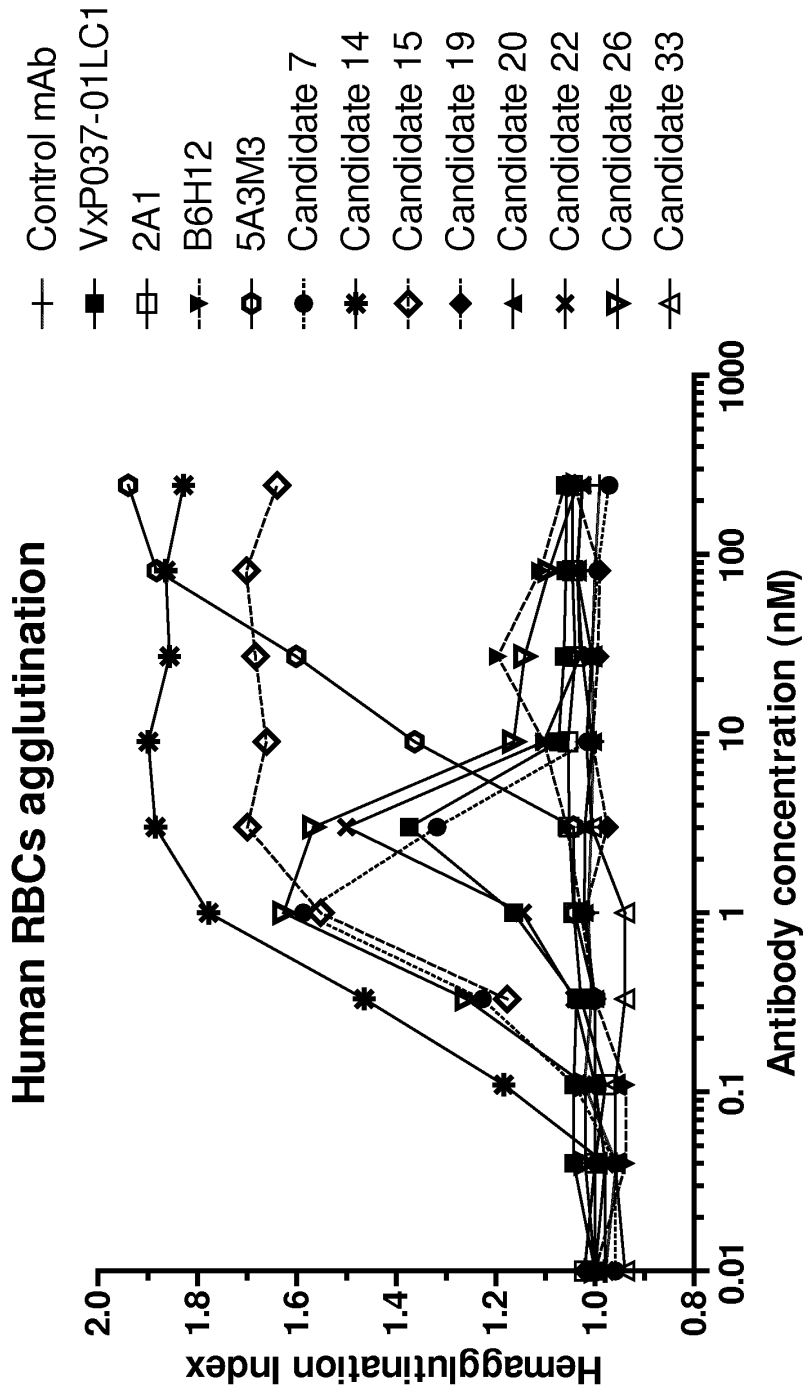
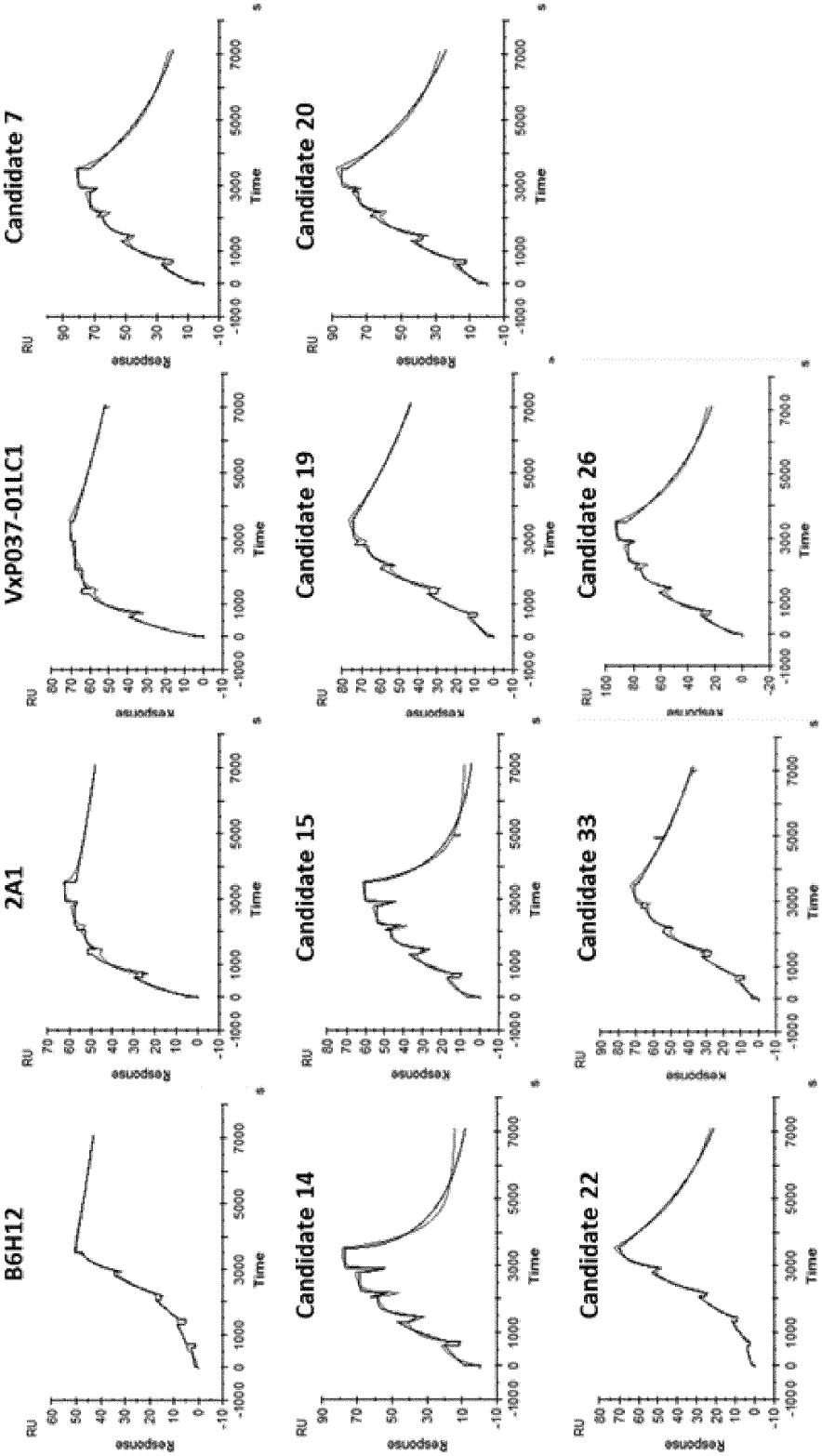

Human RBCs agglutination
Hemagglutination Index
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.01
0.1
1
10
100
1000
Antibody concentration (nM)
Control mAb
VxP037-01LC1
2A1
B6H12
5A3M3
Candidate 7
Candidate 14
Candidate 15
Candidate 19
Candidate 20
Candidate 22
Candidate 26
Candidate 33

Figure 5

Figure 6


B6H12
2A1
VxP037-011C1
Candidate 7
Candidate 14
Candidate 15
Candidate 19
Candidate 20
Candidate 22
Candidate 33
Candidate 26
RU
Response
Time
s

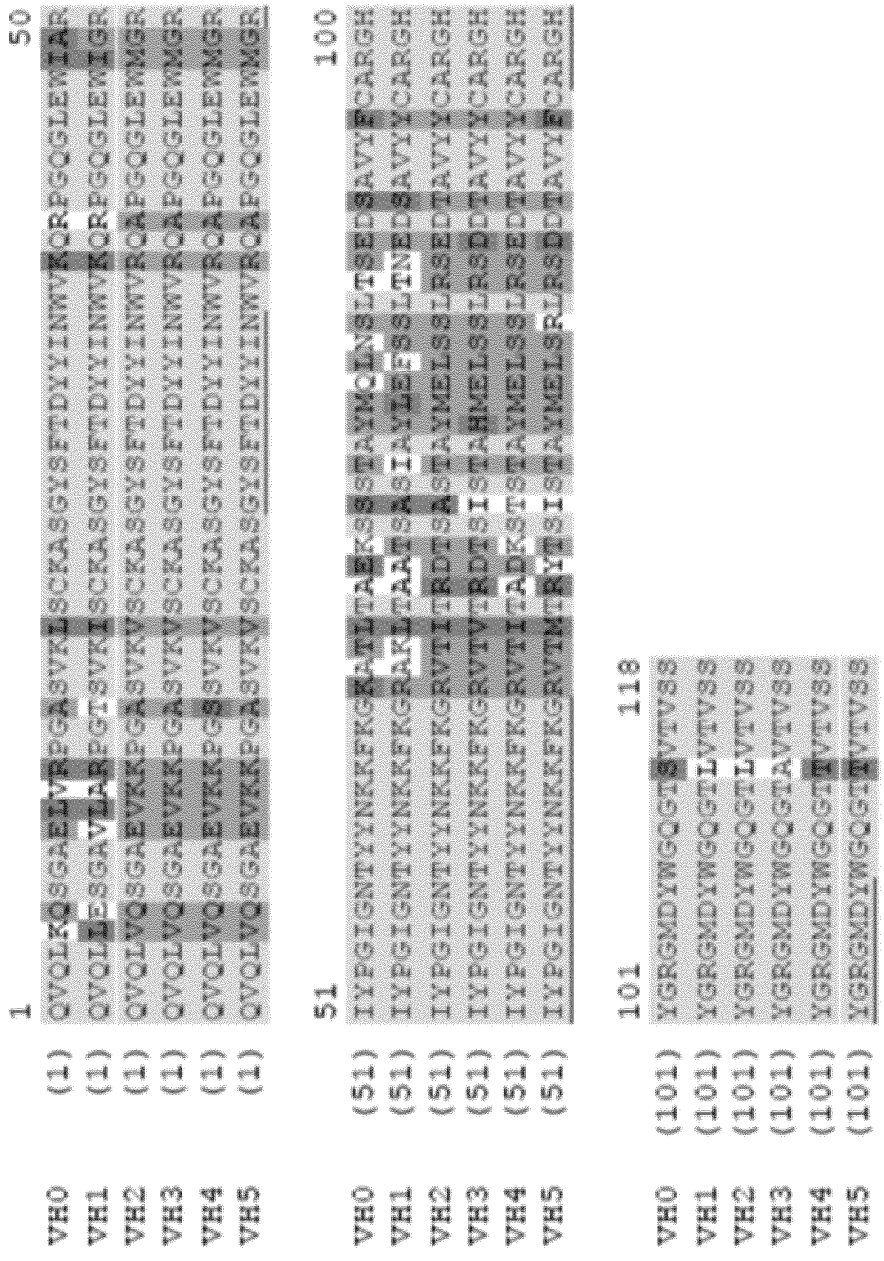
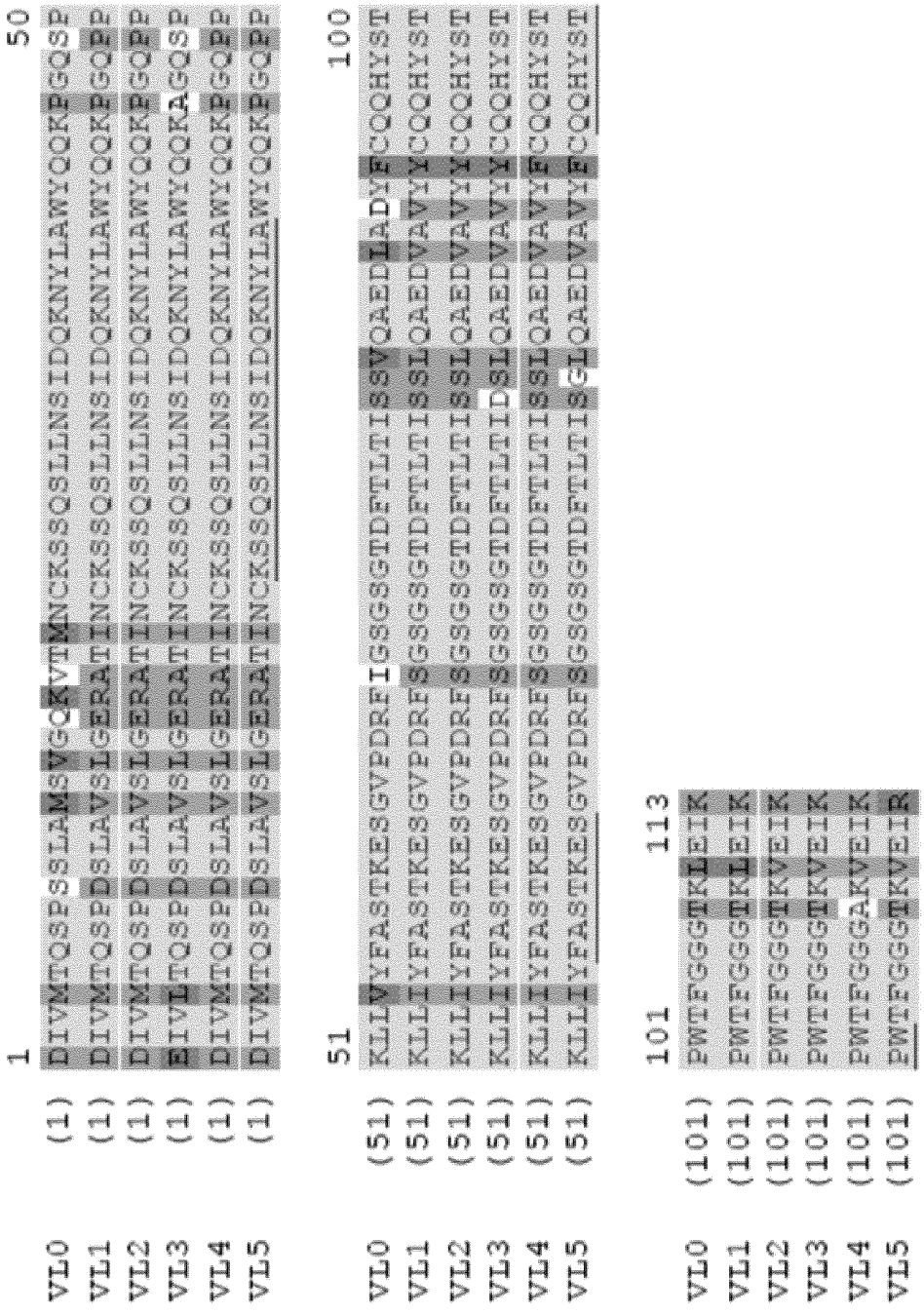

1 50
VH0 (1) QVQLKQSGAELVRPGASVKLSCKASGYSFTDYYINWVKQRPGQGLEWIA
VH1 (1) QVQLLESGAVLARPGTSVKISCKASGYSFTDYYINWVKQRPGQGLEWIGR
VH2 (1) QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGR
VH3 (1) QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGR
VH4 (1) QVQLVQSGAEVKKPGSSVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGR
VH5 (1) QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGR
51 100
VH0 (51) IYPGIGNTYYNKKFKGKATLTAEKSSSTAYMQLNSLTSEDSAVYFCARGH
VH1 (51) IYPGIGNTYYNKKFKGRARLTARTSASIAYLELESLTNEDSAVYYCARGH
VH2 (51) IYPGIGNTYYNKKFKGRVTITRDTSASTAYMELSSLRSEDTAVYYCARGH
VH3 (51) IYPGIGNTYYNKKFKGRVTVTRDTSISTAYMELSSLRSDDTAVYYCARGH
VH4 (51) IYPGIGNTYYNKKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGH
VH5 (51) IYPGIGNTYYNKKFKGRVTMTRYTSISTAYMELSRLRSDDTAVYFCARGH
101 118
VH0 (101) YGRGMDYWGQGTSVTVSS
VH1 (101) YGRGMDYWGQGTLVTVSS
VH2 (101) YGRGMDYWGQGTLVTVSS
VH3 (101) YGRGMDYWGQGTAVTVSS
VH4 (101) YGRGMDYWGQGTTVTVSS
VH5 (101) YGRGMDYWGQGTTVTVSS

Figure 7

Figure 8


1 50
51 100
101 113
VL0 (1) DIVMTQSPSSLAMSVGQKVTMNCKSSQSLLNSIDQKNYLAWYQQKPGQSP
VL1 (1) DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPP
VL2 (1) DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPP
VL3 (1) EIVLTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKAGQSP
VL4 (1) DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPP
VL5 (1) DIVMTQSPDSLAVSLGERATINCKSSQSLLNSIDQKNYLAWYQQKPGQPP
VL0 (51) KLLVYFASTKESGVPDRFIGSGSGTDFTLTISSVQAEDLADYFCQQHYST
VL1 (51) KLLIYFASTKESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYST
VL2 (51) KLLIYFASTKESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYST
VL3 (51) KLLIYFASTKESGVPDRFSGSGSGTDFTLTIDSLQAEDVAVYYCQQHYST
VL4 (51) KLLIYFASTKESGVPDREGSGSGTDFTLTISSLQAEDVAVYFCQQHYST
VL5 (51) KLLIYFASTKESGVPDRFSGSGSGTDFTLTISGLQAEDVAVYFCQQHYST
VL0 (101) PWTFGGGTKLEIK
VL1 (101) PWTFGGGTKLEIK
VL2 (101) PWTFGGGTKVEIK
VL3 (101) PWTFGGGTKVEIK
VL4 (101) PWTFGGGAKVEIK
VL5 (101) PWTFGGGTKVEIR

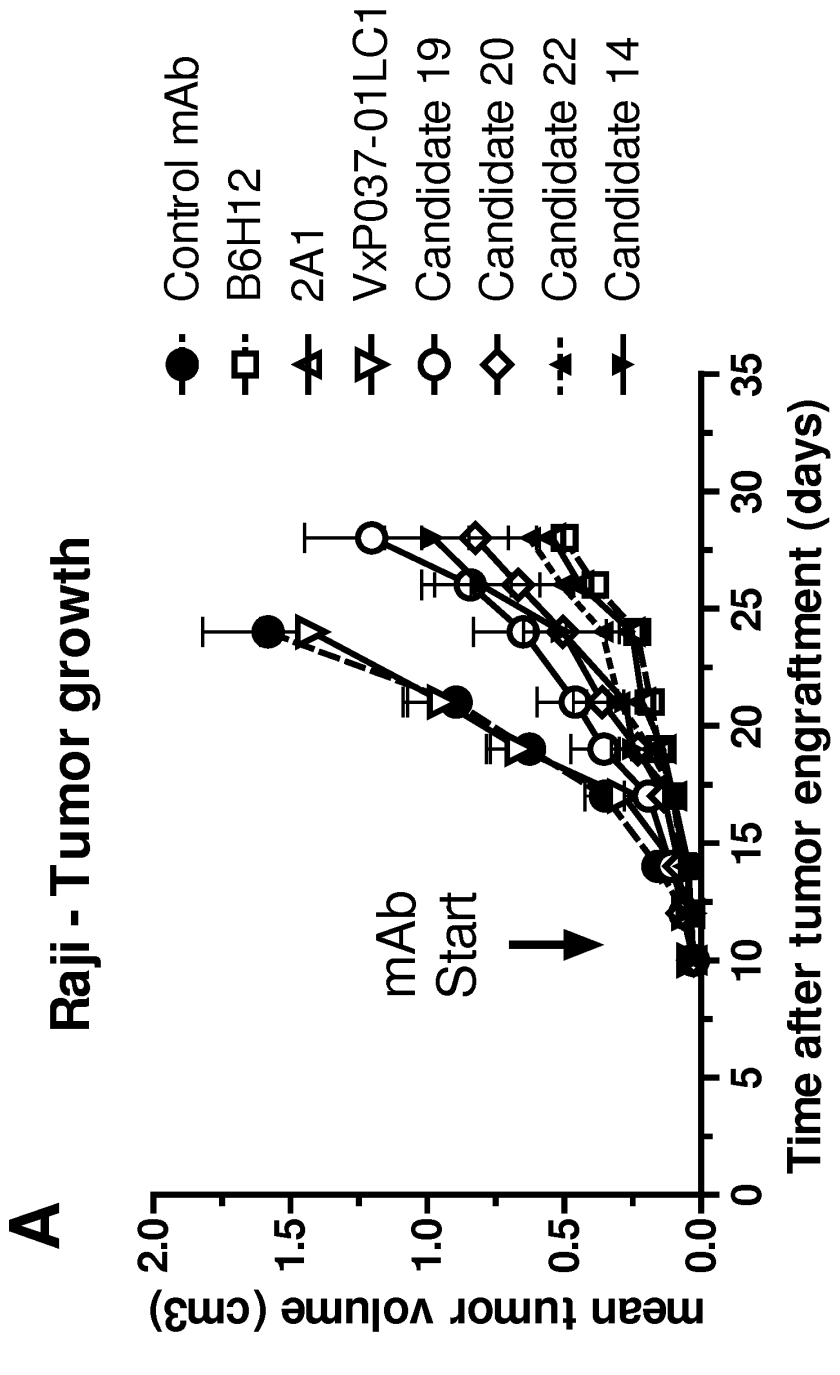

A
Raji - Tumor growth
mean tumor volume (cm3)
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
35
Time after tumor engraftment (days)
mAb
Start
Control mAb
B6H12
2A1
VxP037-01LC1
Candidate 19
Candidate 20
Candidate 22
Candidate 14

Figure 9A

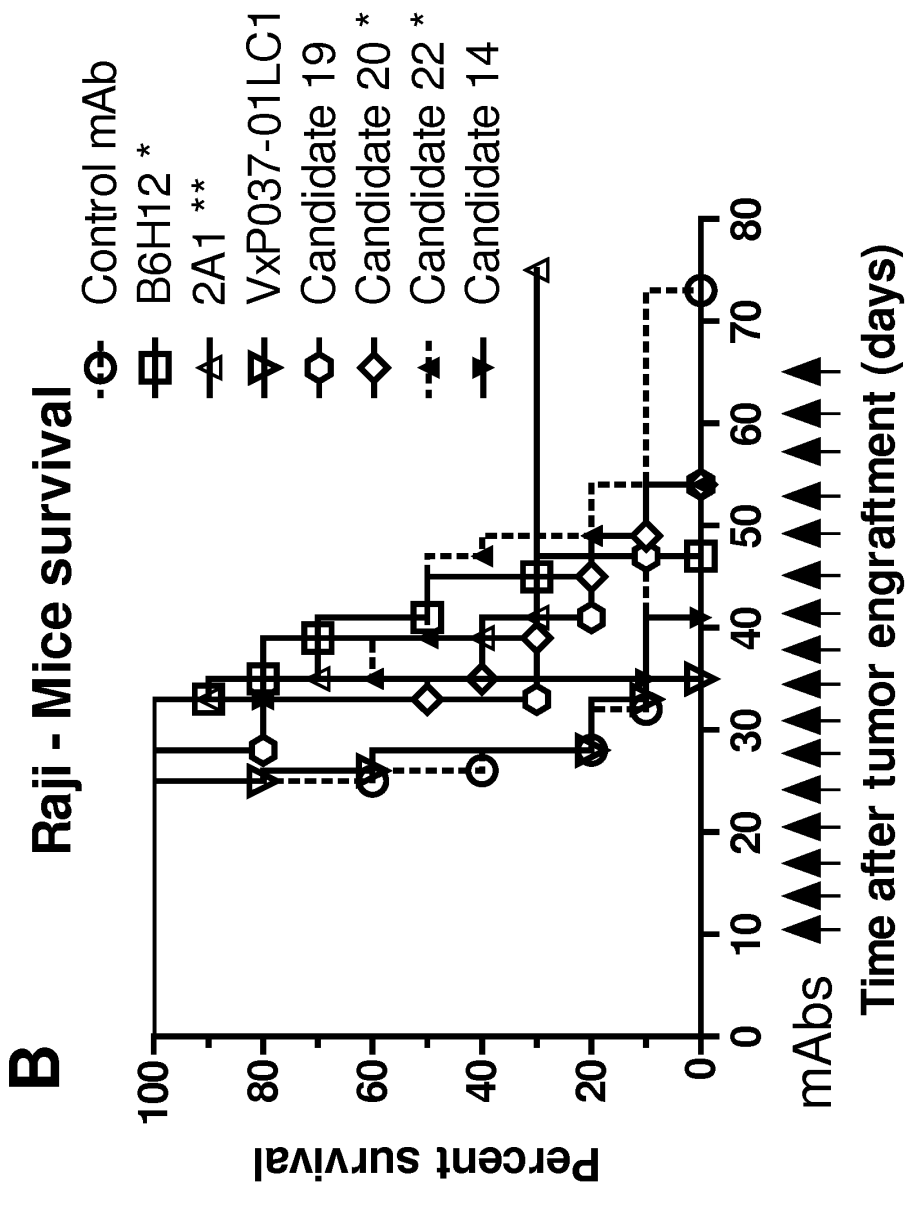

B
Raji - Mice survival
Control mAb
B6H12 *
2A1 **
VxP037-01LC1
Candidate 19
Candidate 20 *
Candidate 22 *
Candidate 14
Percent survival
100
80
60
40
20
0
mAbs
0
10
20
30
40
50
60
70
80
Time after tumor engraftment (days)

Figure 9B

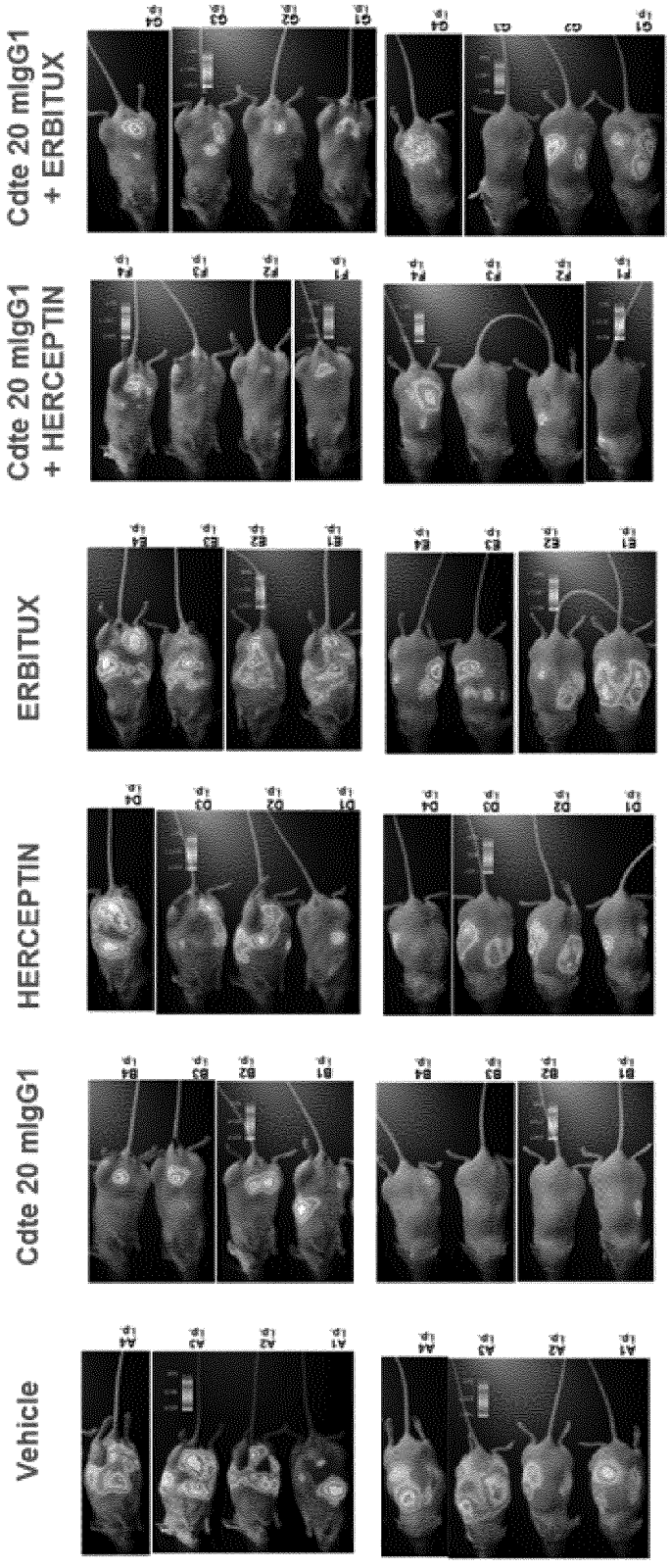

Cdte 20 mIgG1 + ERBITUX
Cdte 20 mIgG1 + HERCEPTIN
ERBITUX
HERCEPTIN
Cdte 20 mIgG1
Vehicle

Figure 10A

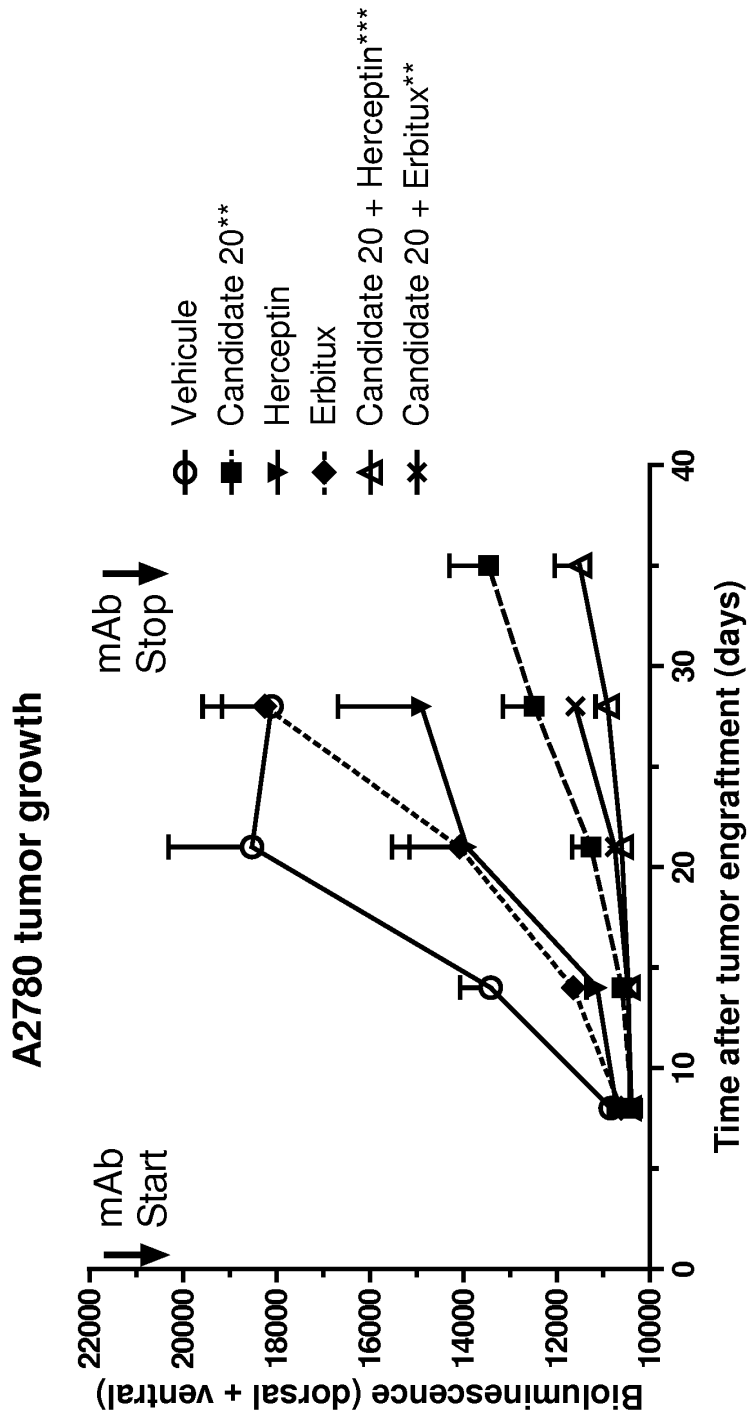

A2780 tumor growth
mAb Start
mAb Stop
Vehicule
Candidate 20**
Herceptin
Erbitux
Candidate 20 + Herceptin***
Candidate 20 + Erbitux**
Bioluminescence (dorsal + ventral)
22000
20000
18000
16000
14000
12000
10000
0
10
20
30
40
Time after tumor engraftment (days)

Figure 10B

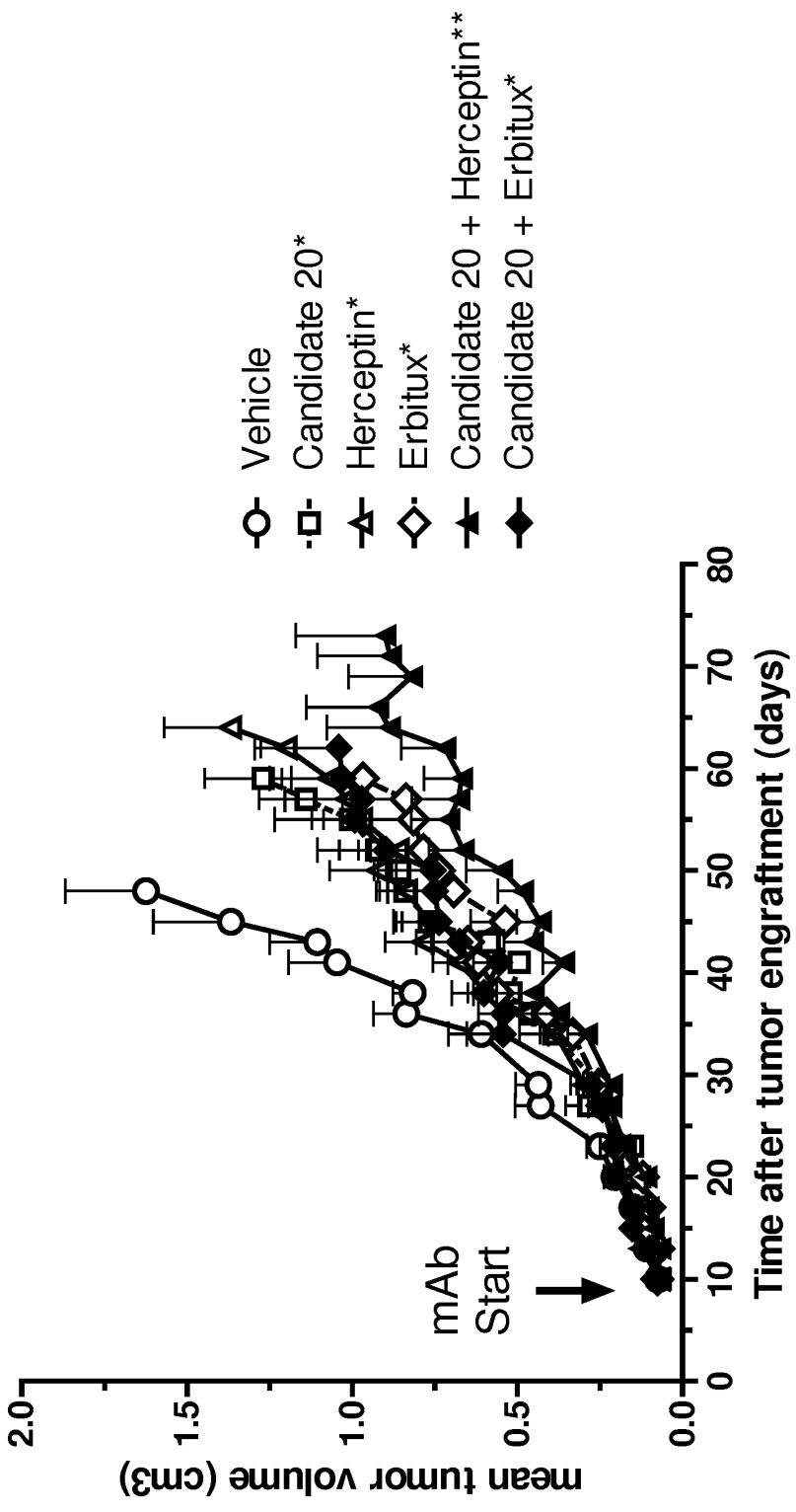

A549 tumor growth
mean tumor volume (cm3)
Time after tumor engraftment (days)
mAb Start
Vehicle
Candidate 20*
Herceptin*
Erbitux*
Candidate 20 + Herceptin**
Candidate 20 + Erbitux*
0.0
0.5
1.0
1.5
2.0
0
10
20
30
40
50
60
70
80

Figure 11A

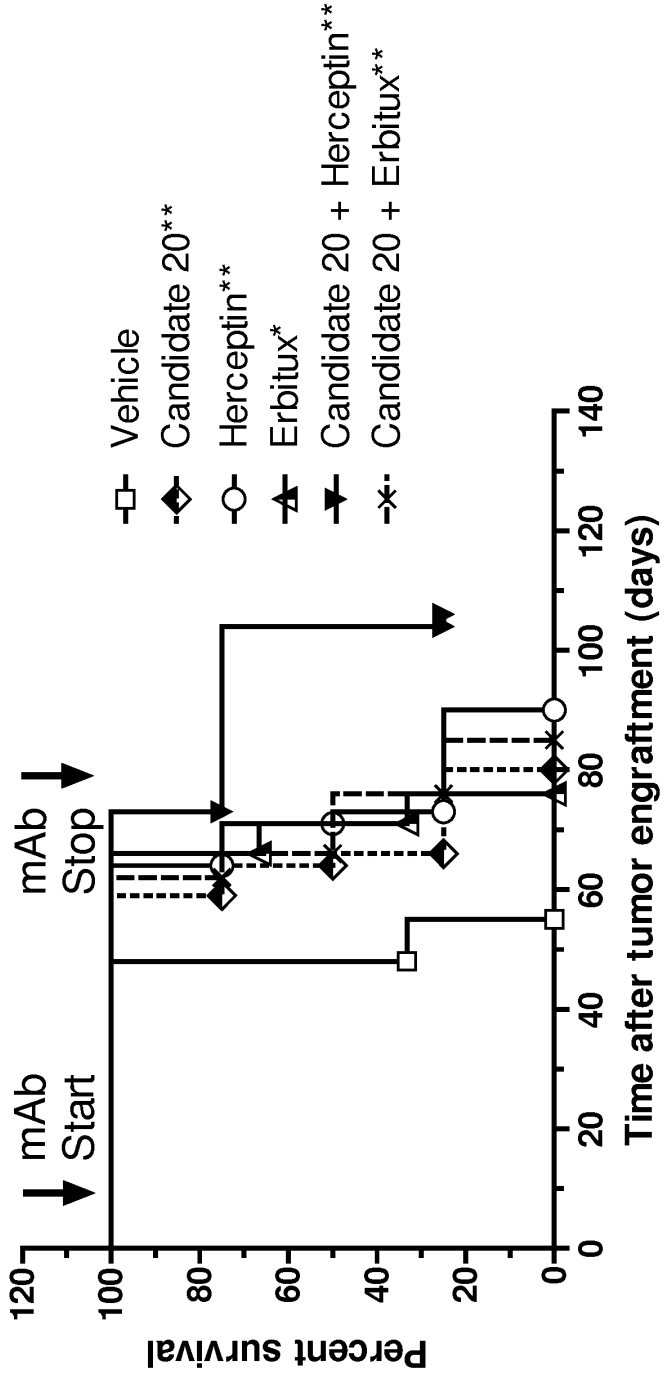

A549 tumor - Mice Survival
mAb Start
mAb Stop
Vehicle
Candidate 20**
Herceptin**
Erbitux*
Candidate 20 + Herceptin**
Candidate 20 + Erbitux**
Percent survival
Time after tumor engraftment (days)
0
20
40
60
80
100
120
140

Figure 11B

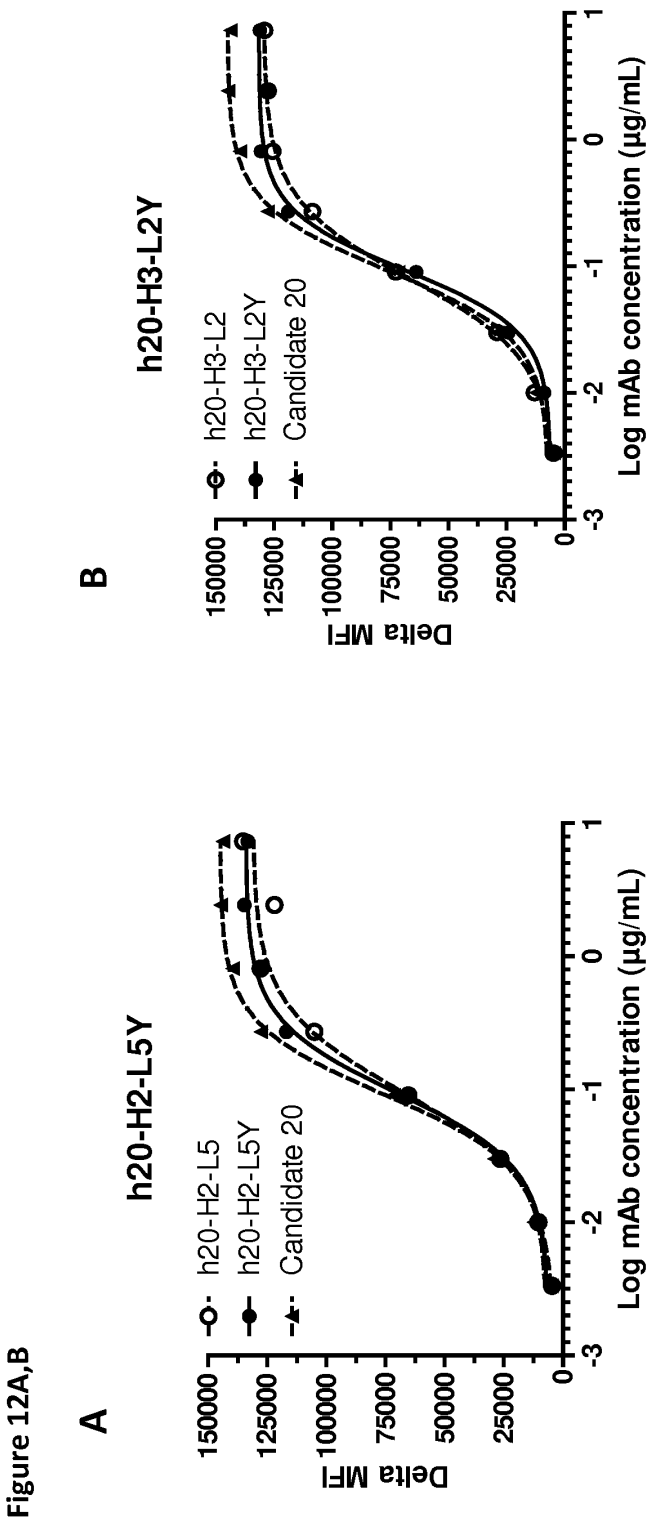

A
h20-H2-L5Y
h20-H2-L5
h20-H2-L5Y
Candidate 20
Delta MFI
150000
125000
100000
75000
50000
25000
0
-3
-2
-1
0
1
Log mAb concentration (µg/mL)
B
h20-H3-L2Y
h20-H3-L2
h20-H3-L2Y
Candidate 20
Delta MFI
150000
125000
100000
75000
50000
25000
0
-3
-2
-1
0
1
Log mAb concentration (µg/mL)

Figure 12A,B

Figure 12C,D

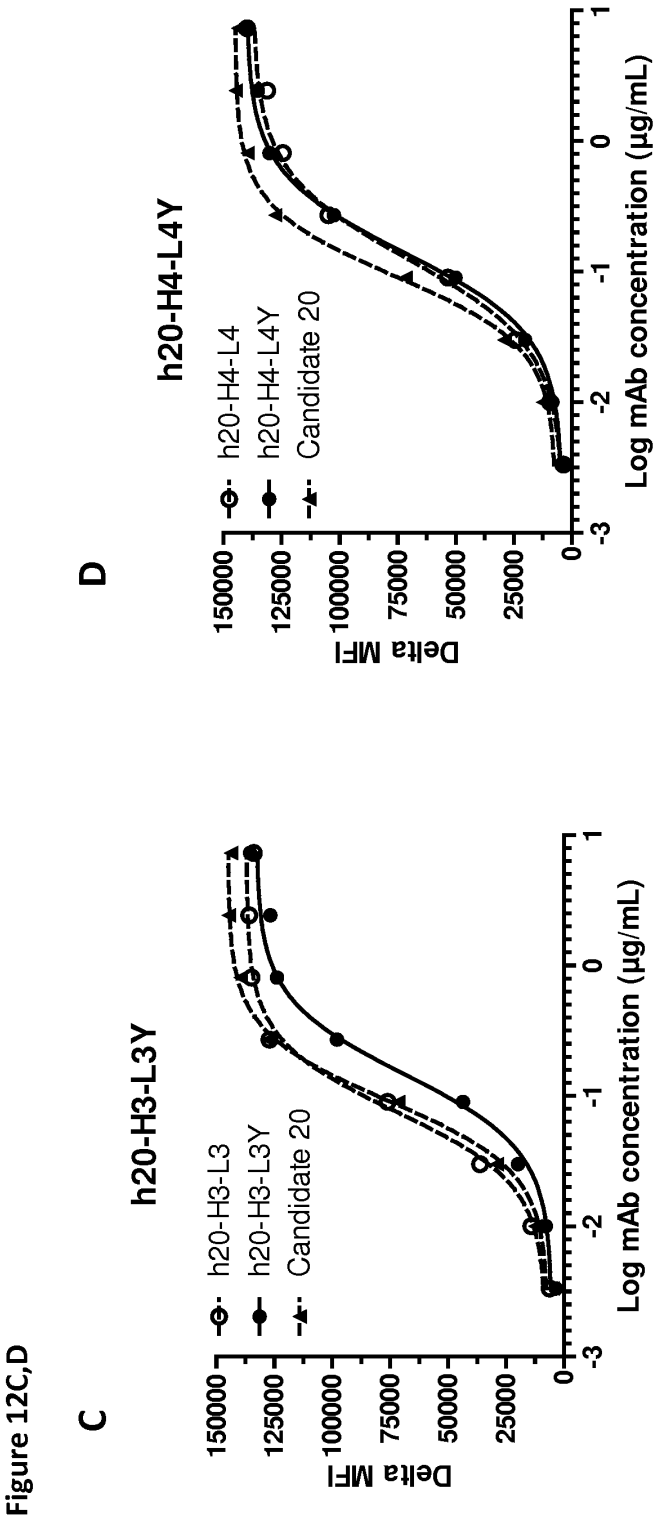
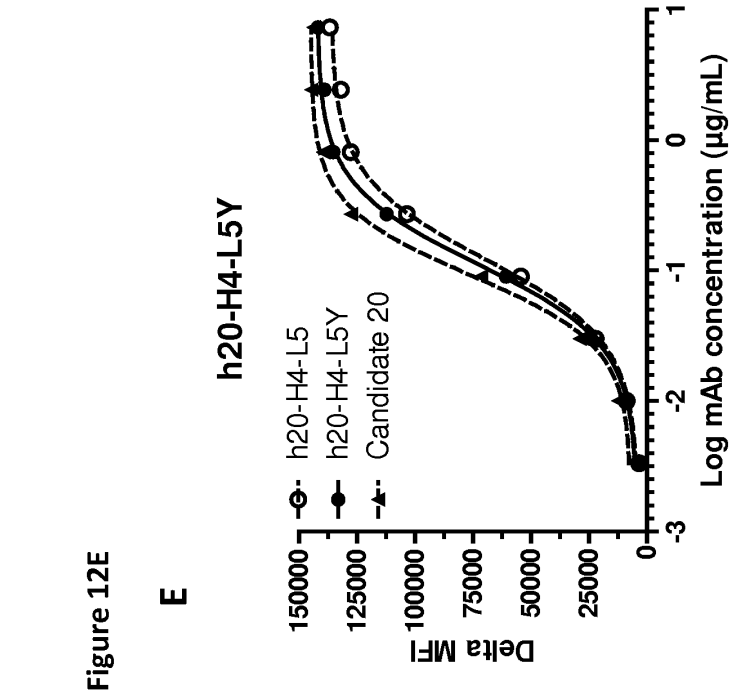
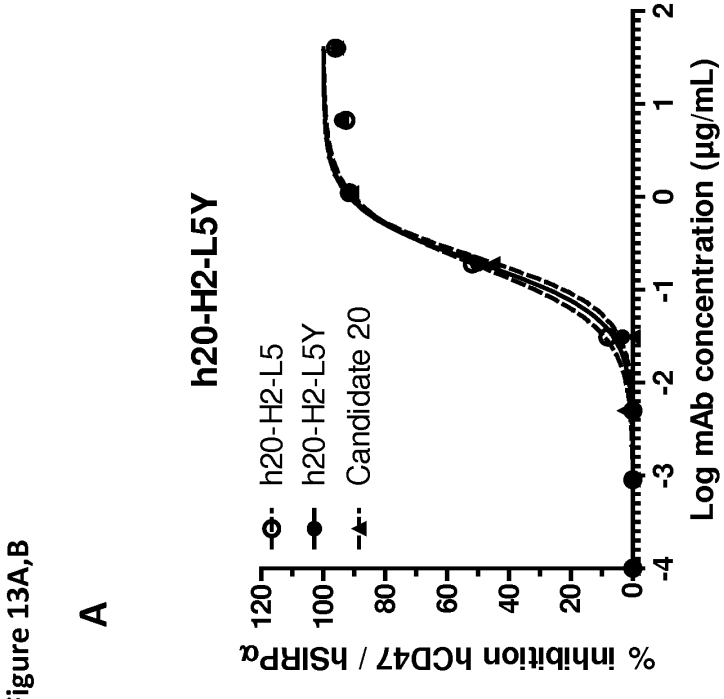

C
h20-H3-L3Y
h20-H3-L3
h20-H3-L3Y
Candidate 20
Delta MFI
150000
125000
100000
75000
50000
25000
0
-3
-2
-1
0
1
Log mAb concentration (µg/mL)
D
h20-H4-L4Y
h20-H4-L4
h20-H4-L4Y
Candidate 20
Delta MFI
150000
125000
100000
75000
50000
25000
0
-3
-2
-1
0
1
Log mAb concentration (µg/mL)

Figure 12E


E
h20-H4-L5Y
h20-H4-L5
h20-H4-L5Y
Candidate 20
Delta MFI
150000
125000
100000
75000
50000
25000
0
-3
-2
-1
0
1
Log mAb concentration (µg/mL)


A
h20-H2-L5Y
h20-H2-L5
h20-H2-L5Y
Candidate 20
% inhibition hCD47 / hSIRPα
Log mAb concentration (µg/mL)
120
100
80
60
40
20
0
-4
-3
-2
-1
0
1
2

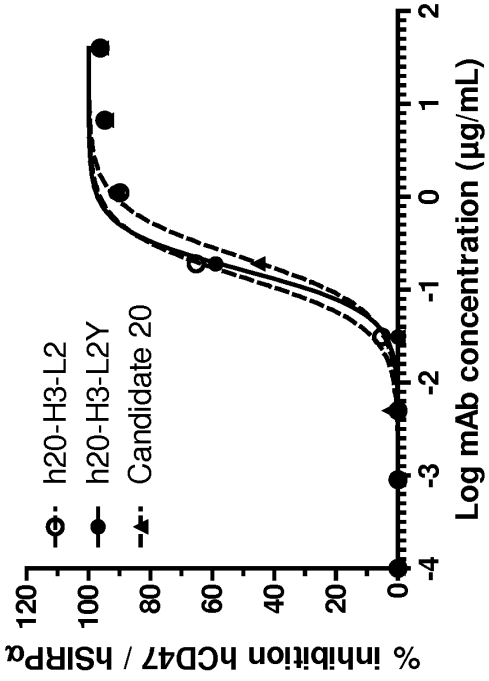

B
h20-H3-L2Y
h20-H3-L2
h20-H3-L2Y
Candidate 20
% inhibition hCD47 / hSIRPα
Log mAb concentration (µg/mL)
120
100
80
60
40
20
0
-4
-3
-2
-1
0
1
2

Figure 13A,B

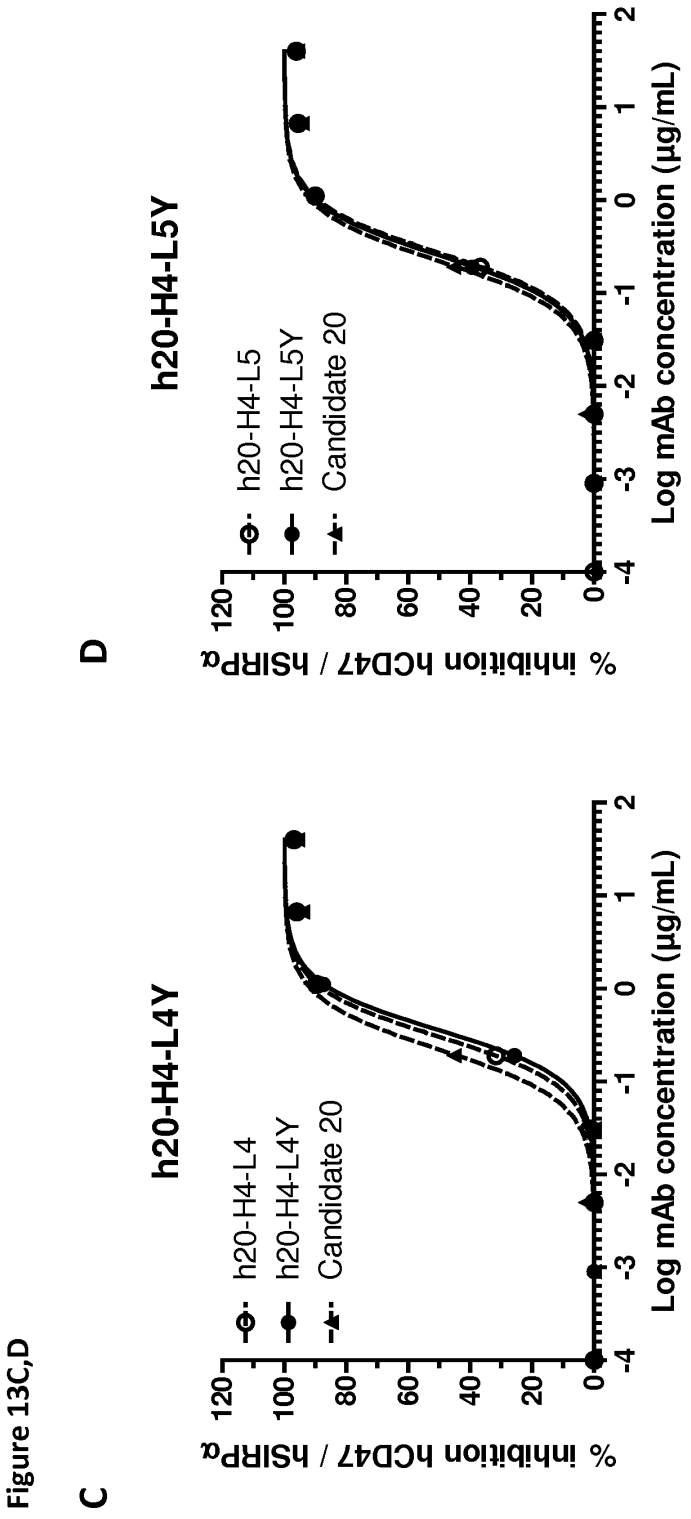

C
h20-H4-L4Y
h20-H4-L4
h20-H4-L4Y
Candidate 20
% inhibition hCD47 / hSIRPα
Log mAb concentration (µg/mL)
D
h20-H4-L5Y
h20-H4-L5
h20-H4-L5Y
Candidate 20
% inhibition hCD47 / hSIRPα
Log mAb concentration (µg/mL)

Figure 13C,D

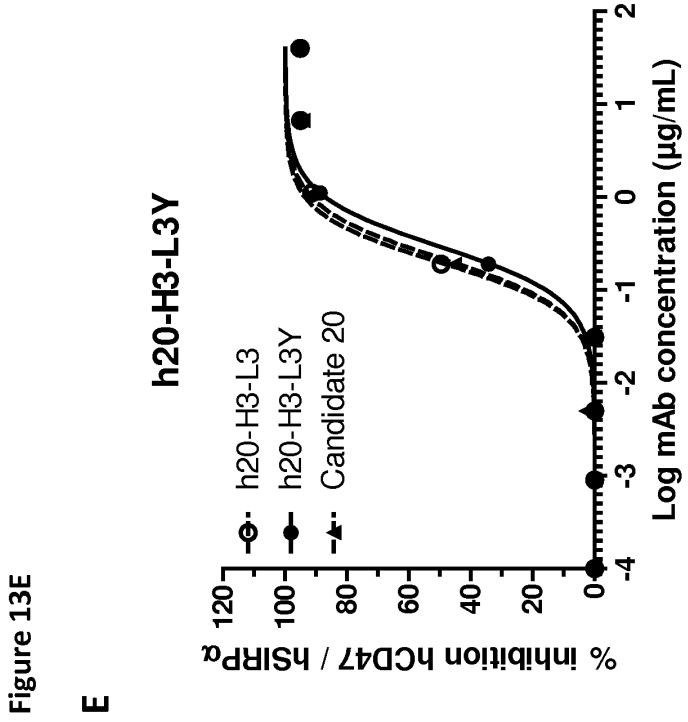
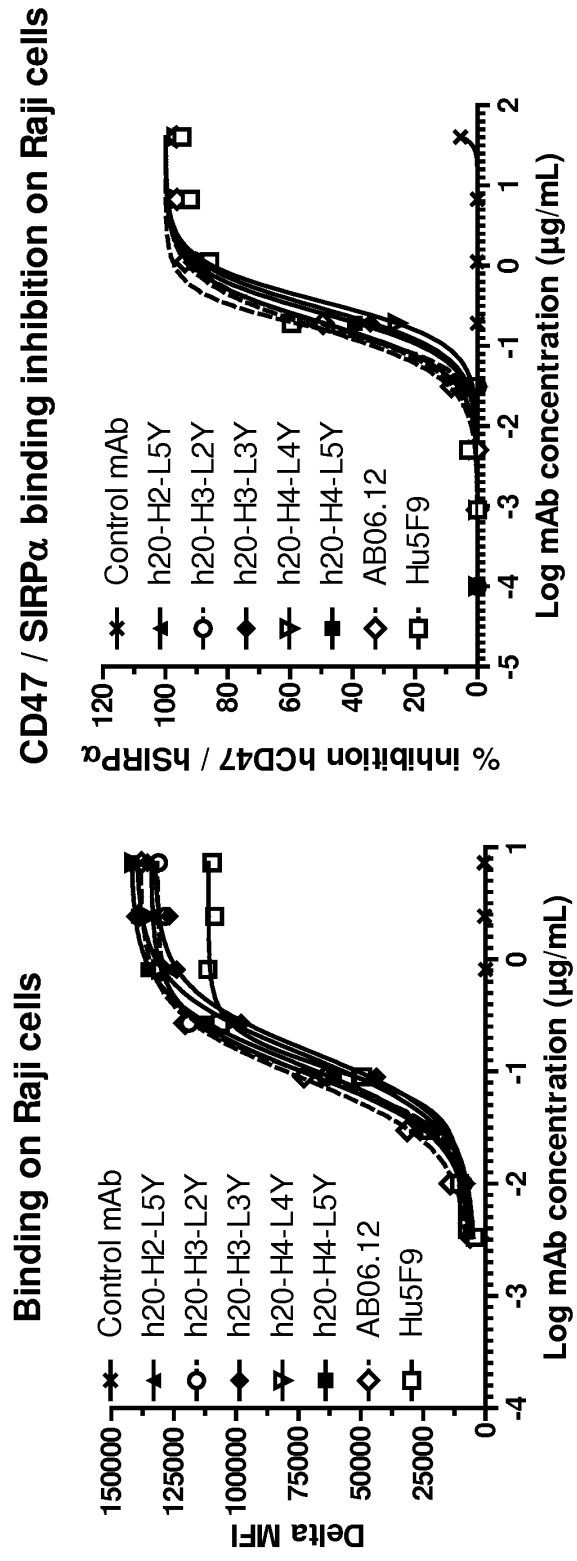

E
h20-H3-L3Y
h20-H3-L3
h20-H3-L3Y
Candidate 20
% inhibition hCD47 / hSIRPα
120
100
80
60
40
20
0
-4
-3
-2
-1
0
1
2
Log mAb concentration (µg/mL)

Figure 13E

Figure 14


Binding on Raji cells
Delta MFI
150000
125000
100000
75000
50000
25000
0
-4
-3
-2
-1
0
1
Log mAb concentration (µg/mL)
Control mAb
h20-H2-L5Y
h20-H3-L2Y
h20-H3-L3Y
h20-H4-L4Y
h20-H4-L5Y
AB06.12
Hu5F9
CD47 / SIRPα binding inhibition on Raji cells
% inhibition hCD47 / hSIRPα
120
100
80
60
40
20
0
-5
-4
-3
-2
-1
0
1
2
Log mAb concentration (µg/mL)
Control mAb
h20-H2-L5Y
h20-H3-L2Y
h20-H3-L3Y
h20-H4-L4Y
h20-H4-L5Y
AB06.12
Hu5F9

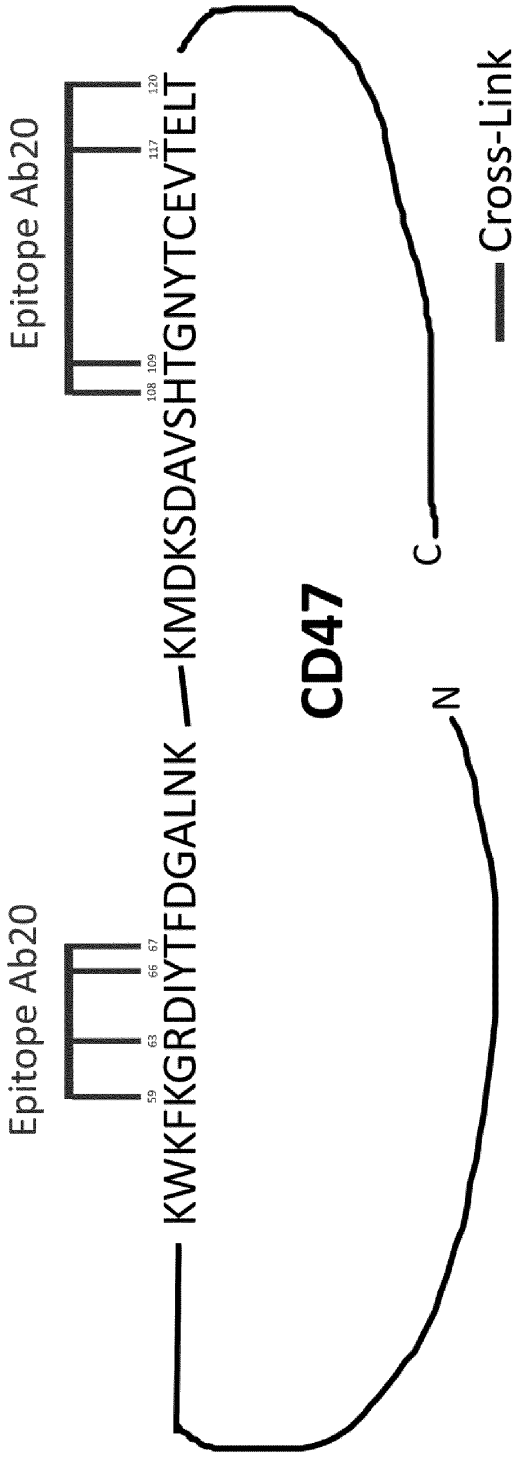

Epitope Ab20
Epitope Ab20
KWKFKGRDIYTFDGALNK
KMDKSDAVSHTGNYTCEVTELT
59
63
66
67
108
109
117
120
CD47
N
C
Cross-Link

Figure 15

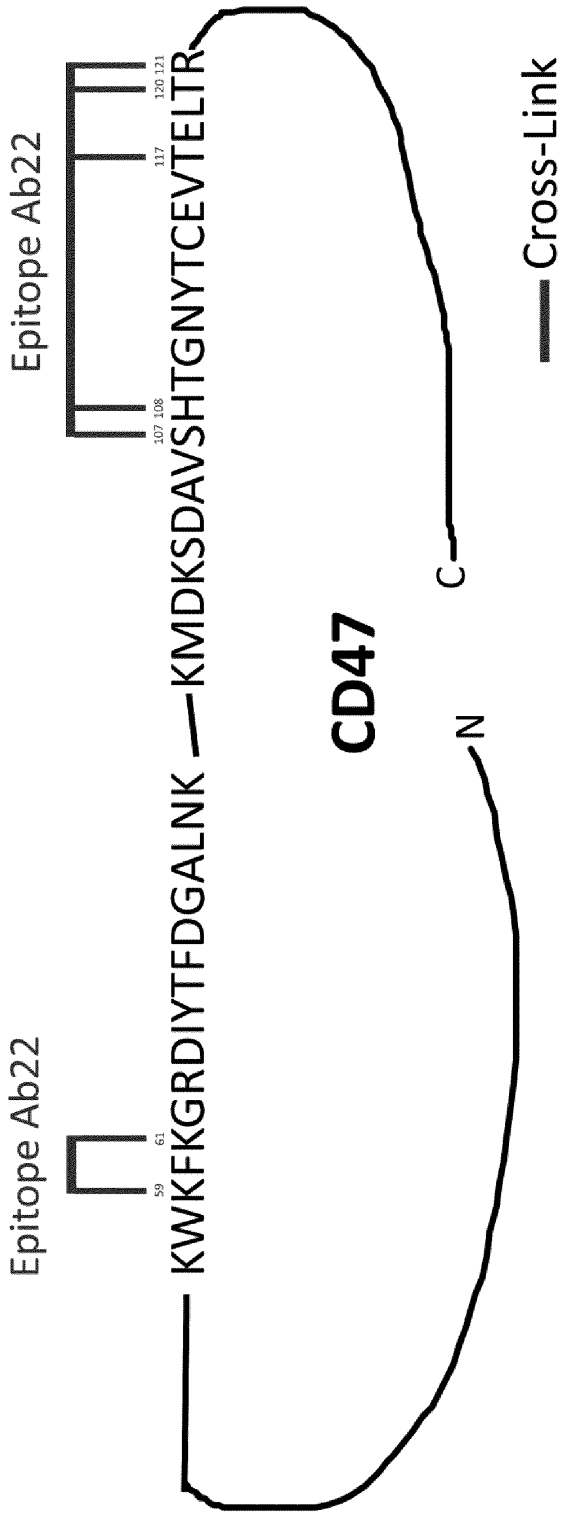

Epitope Ab22
59
61
KWKFKGRDIYTFDGALNK
Epitope Ab22
107
108
KMDKSDAVSHTGNYTCEVTELTR
117
120
121
CD47
N
C
Cross-Link

Figure 16

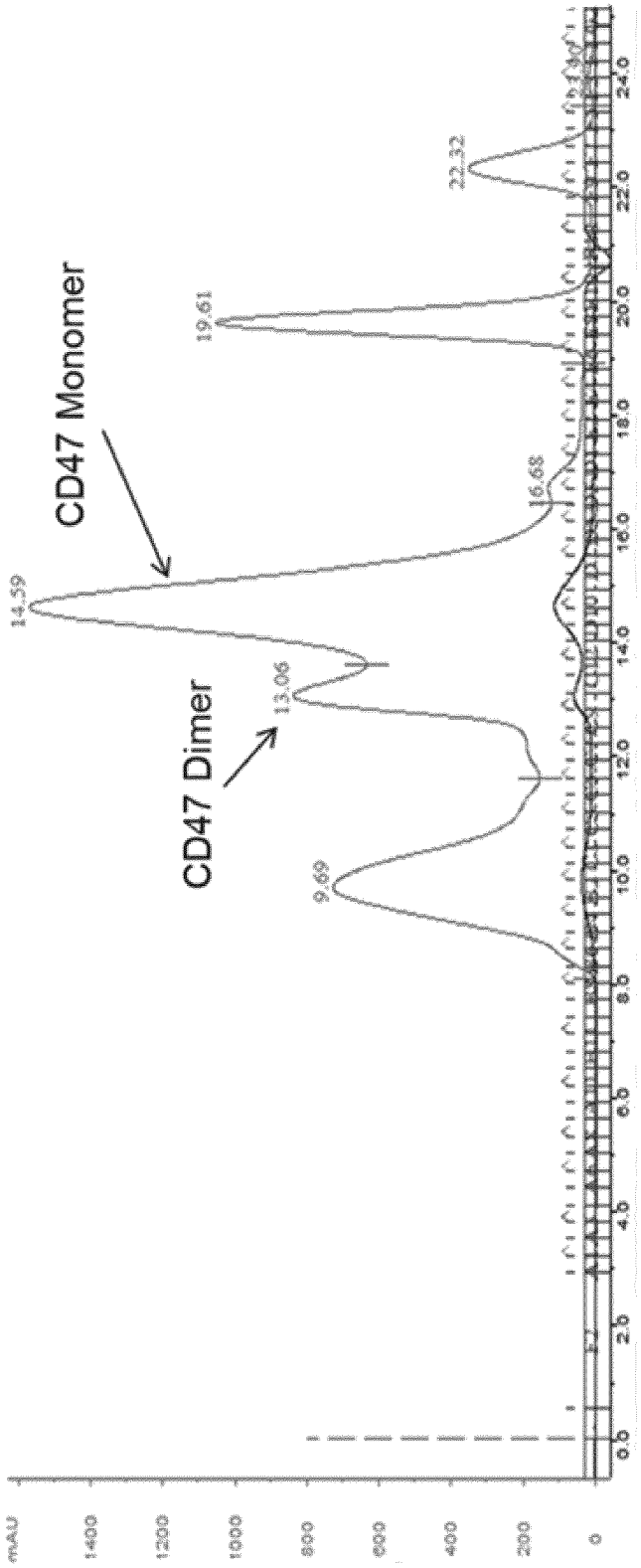

CD47 Monomer
CD47 Dimer
14.59
13.06
9.69
19.61
16.68
22.32
mAU
1400
1200
1000
800
600
400
200
0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
24.0

Figure 17

Figure 18

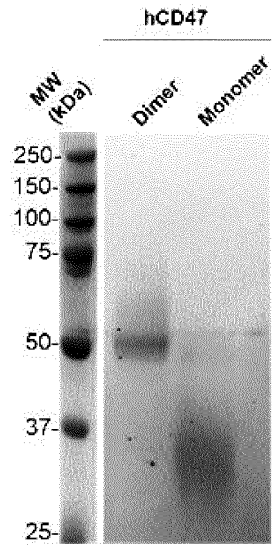


Figure 19

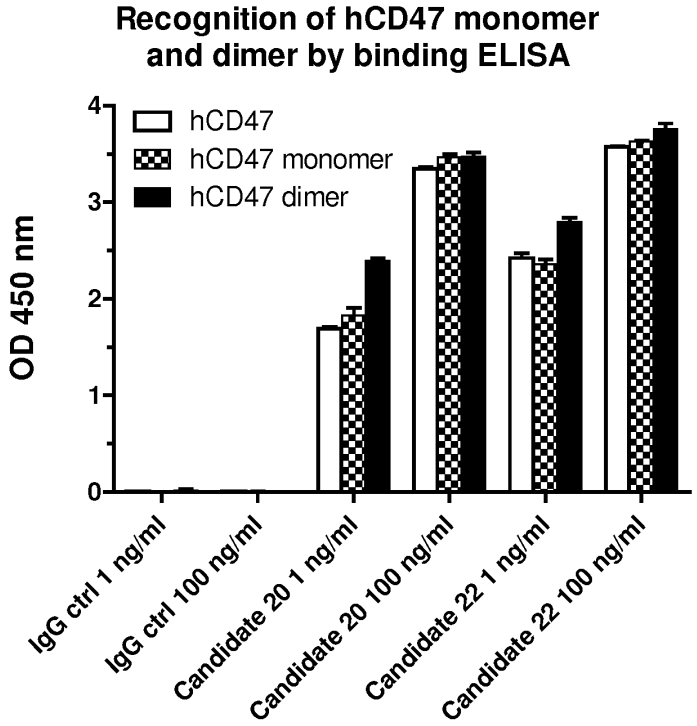


Figure 20

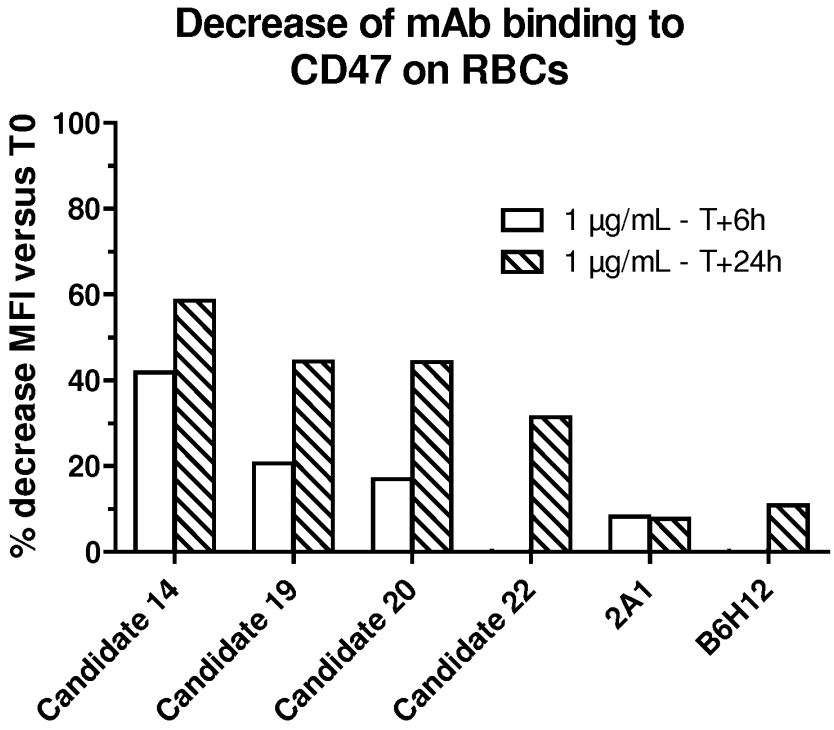

Decrease of mAb binding to CD47 on RBCs
% decrease MFI versus T0
100
80
60
40
20
0
1 µg/mL - T+6h
1 µg/mL - T+24h
Candidate 14
Candidate 19
Candidate 20
Candidate 22
2A1
B6H12

Figure 21

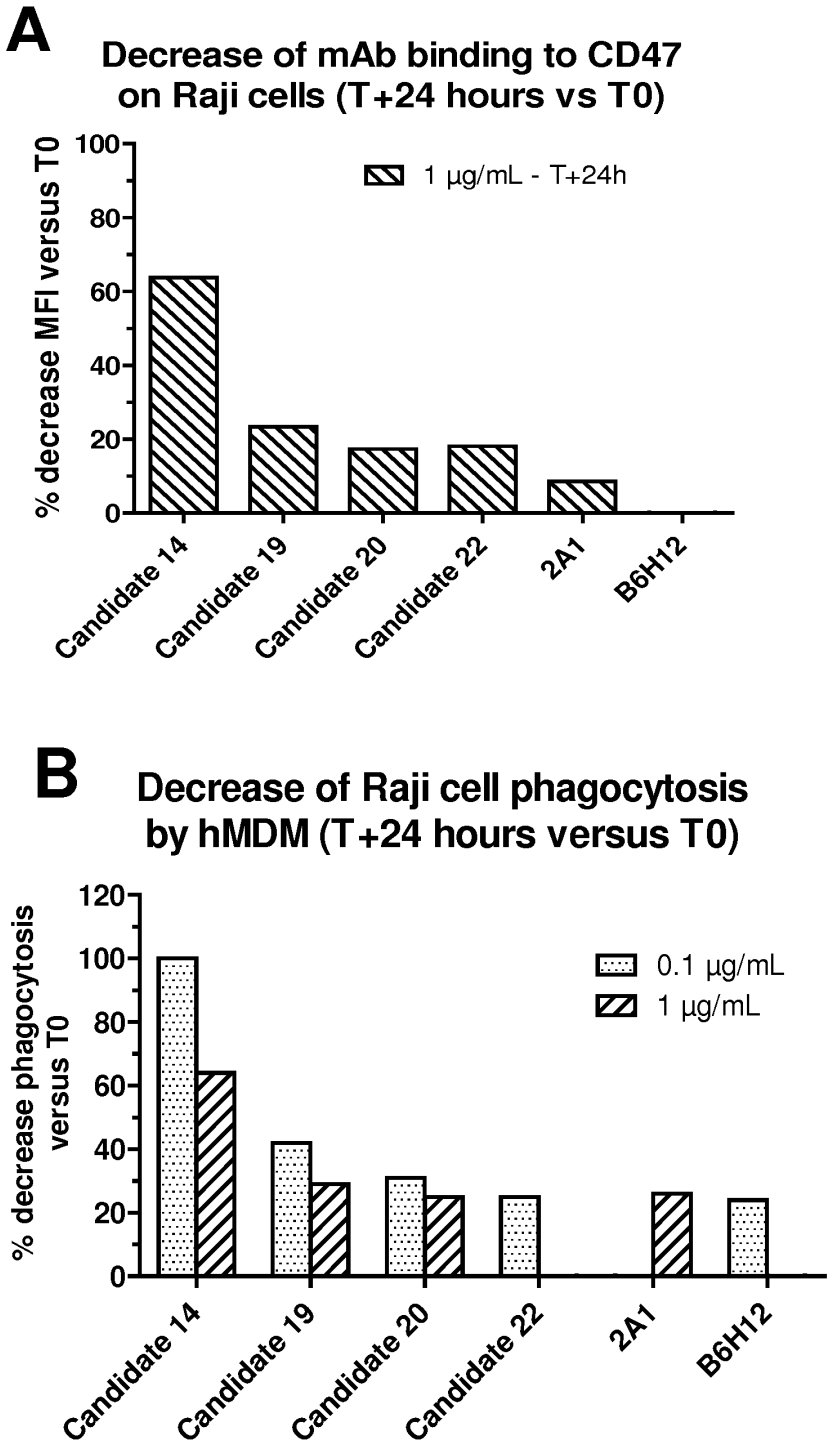

A
Decrease of mAb binding to CD47 on Raji cells (T+24 hours vs T0)
% decrease MFI versus T0
100
80
60
40
20
0
1 µg/mL - T+24h
Candidate 14
Candidate 19
Candidate 20
Candidate 22
2A1
B6H12
B
Decrease of Raji cell phagocytosis by hMDM (T+24 hours versus T0)
% decrease phagocytosis versus T0
120
100
80
60
40
20
0
0.1 µg/mL
1 µg/mL
Candidate 14
Candidate 19
Candidate 20
Candidate 22
2A1
B6H12

Figure 22

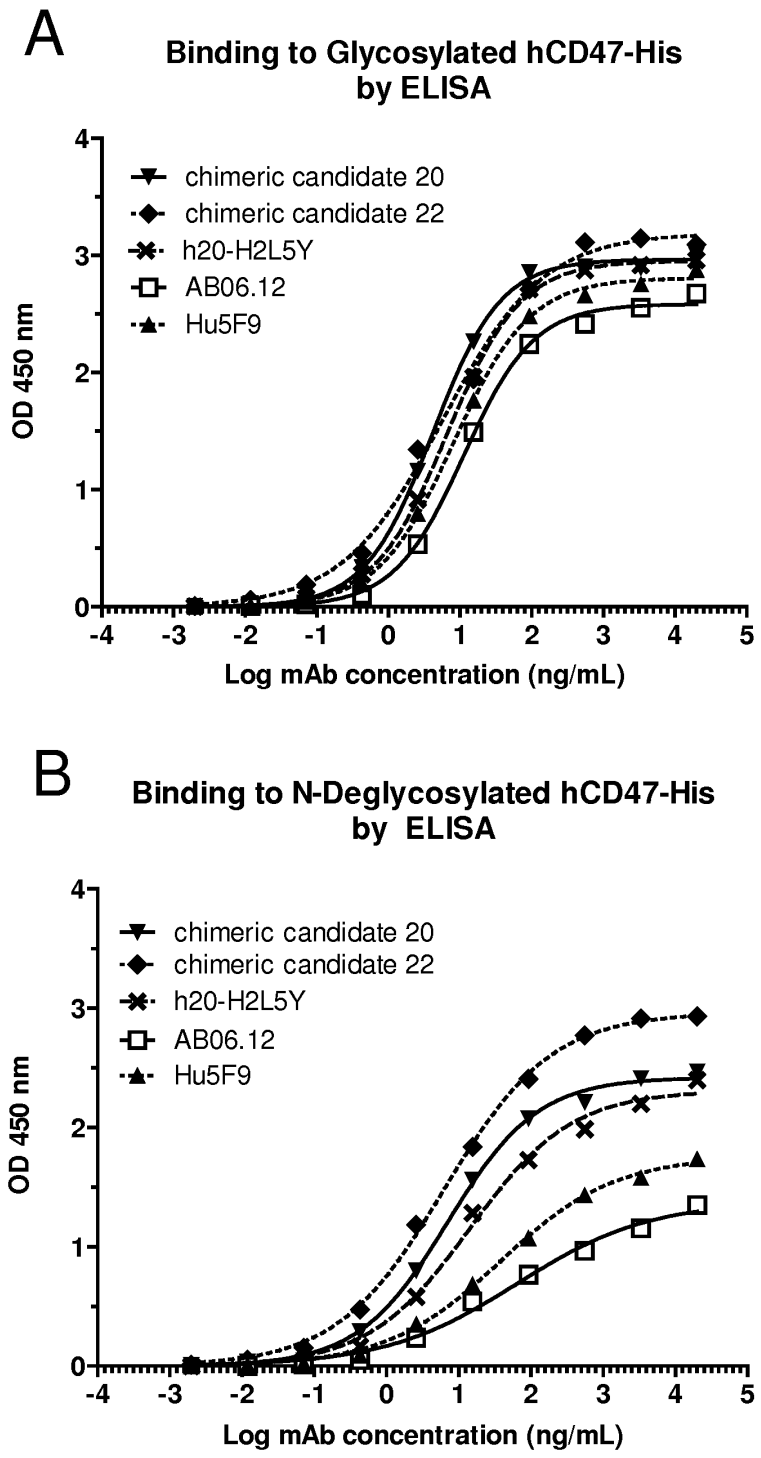

A
Binding to Glycosylated hCD47-His by ELISA
chimeric candidate 20
chimeric candidate 22
h20-H2L5Y
AB06.12
Hu5F9
OD 450 nm
Log mAb concentration (ng/mL)
B
Binding to N-Deglycosylated hCD47-His by ELISA
chimeric candidate 20
chimeric candidate 22
h20-H2L5Y
AB06.12
Hu5F9
OD 450 nm
Log mAb concentration (ng/mL)

Figure 23

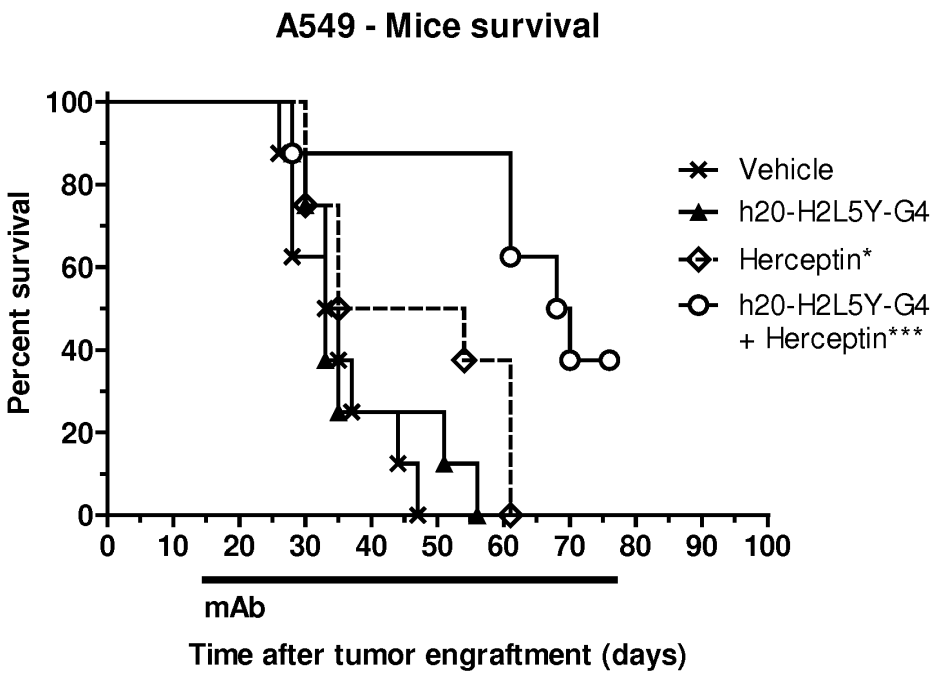
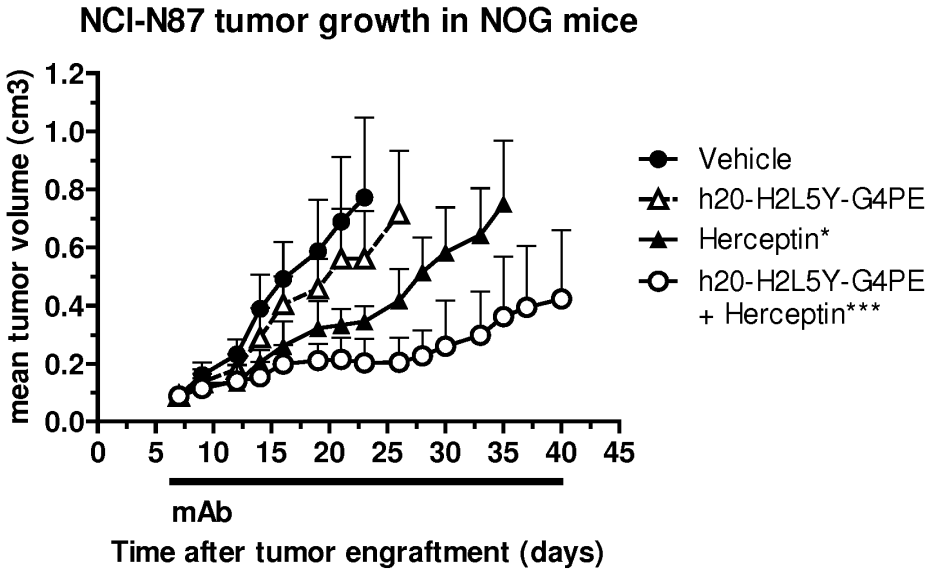

A549 - Mice survival
Percent survival
100
80
60
40
20
0
0 10 20 30 40 50 60 70 80 90 100
mAb
Time after tumor engraftment (days)
Vehicle
h20-H2L5Y-G4
Herceptin*
h20-H2L5Y-G4
+ Herceptin***

Figure 24


NCI-N87 tumor growth in NOG mice
mean tumor volume (cm3)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
35
40
45
mAb
Time after tumor engraftment (days)
Vehicle
h20-H2L5Y-G4PE
Herceptin*
h20-H2L5Y-G4PE
+ Herceptin***