FIG. 1

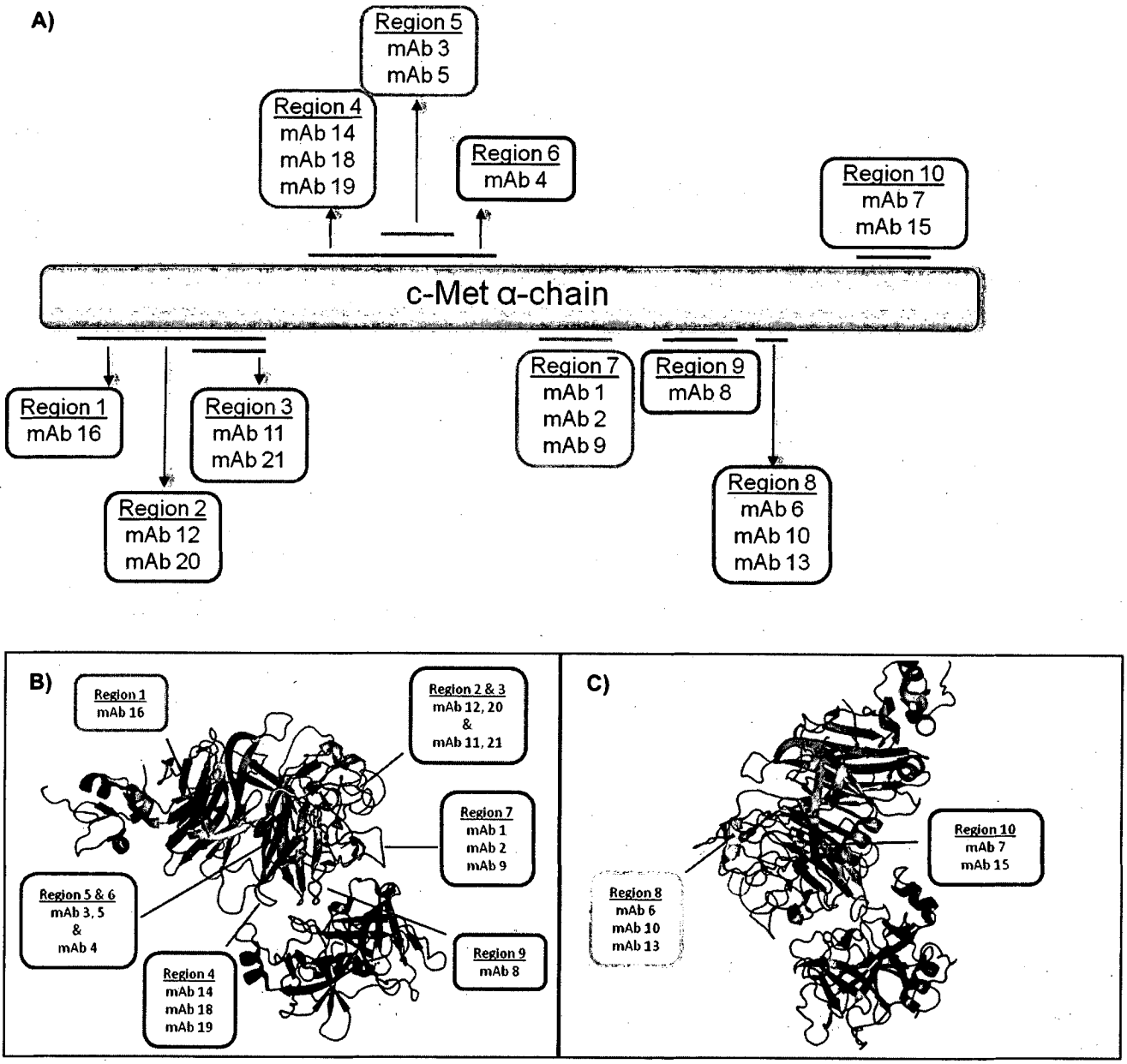

A)
Region 5
mAb 3
mAb 5
Region 4
mAb 14
mAb 18
mAb 19
Region 6
mAb 4
Region 10
mAb 7
mAb 15
c-Met α-chain
Region 1
mAb 16
Region 3
mAb 11
mAb 21
Region 2
mAb 12
mAb 20
Region 7
mAb 1
mAb 2
mAb 9
Region 9
mAb 8
Region 8
mAb 6
mAb 10
mAb 13
B)
Region 1
mAb 16
Region 2 & 3
mAb 12, 20
&
mAb 11, 21
Region 7
mAb 1
mAb 2
mAb 9
Region 5 & 6
mAb 3, 5
&
mAb 4
Region 9
mAb 8
Region 4
mAb 14
mAb 18
mAb 19
C)
Region 10
mAb 7
mAb 15
Region 8
mAb 6
mAb 10
mAb 13

FIG. 1 (cont'd)

D)

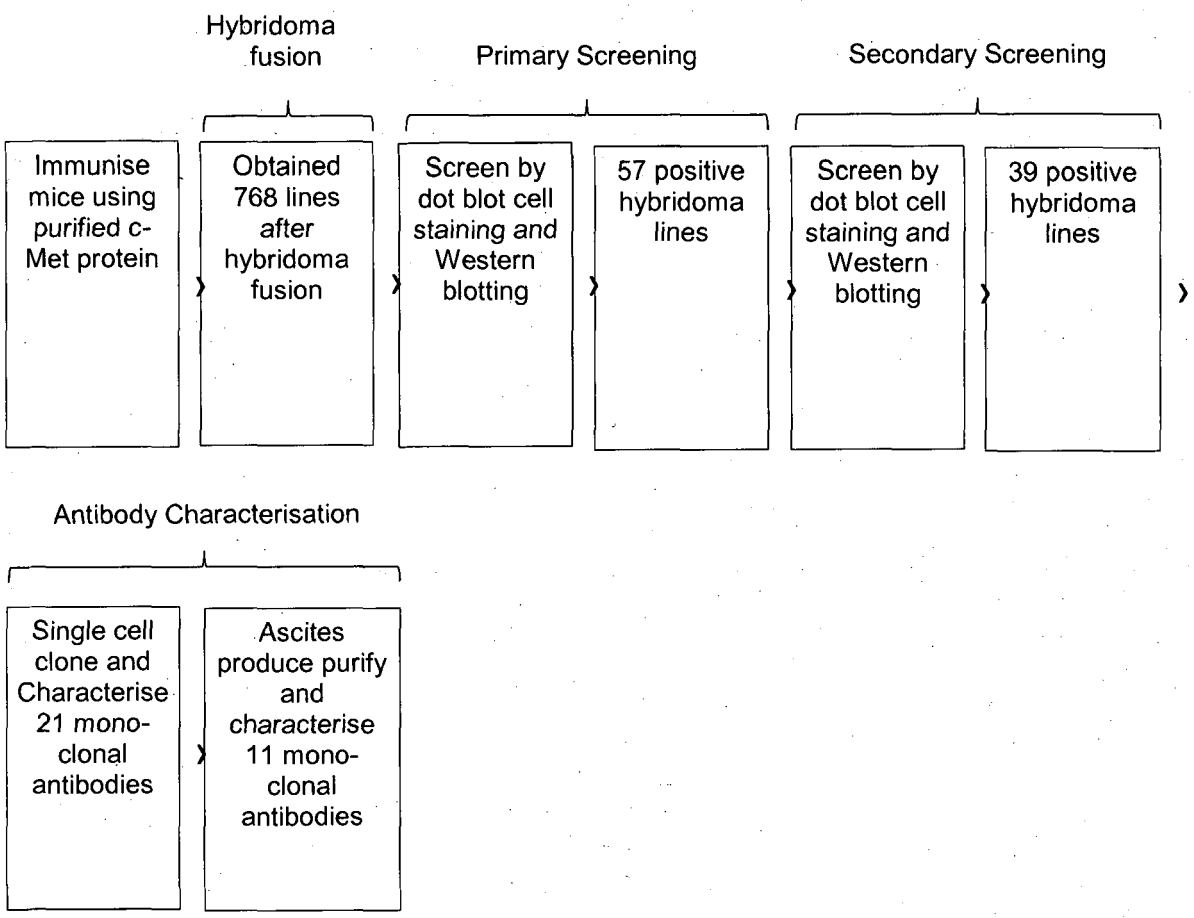

Hybridoma fusion
Primary Screening
Secondary Screening
Immunise mice using purified c-Met protein
Obtained 768 lines after hybridoma fusion
Screen by dot blot cell staining and Western blotting
57 positive hybridoma lines
Screen by dot blot cell staining and Western blotting
39 positive hybridoma lines
Antibody Characterisation
Single cell clone and Characterise 21 mono-clonal antibodies
Ascites produce purify and characterise 11 mono-clonal antibodies

FIG. 2

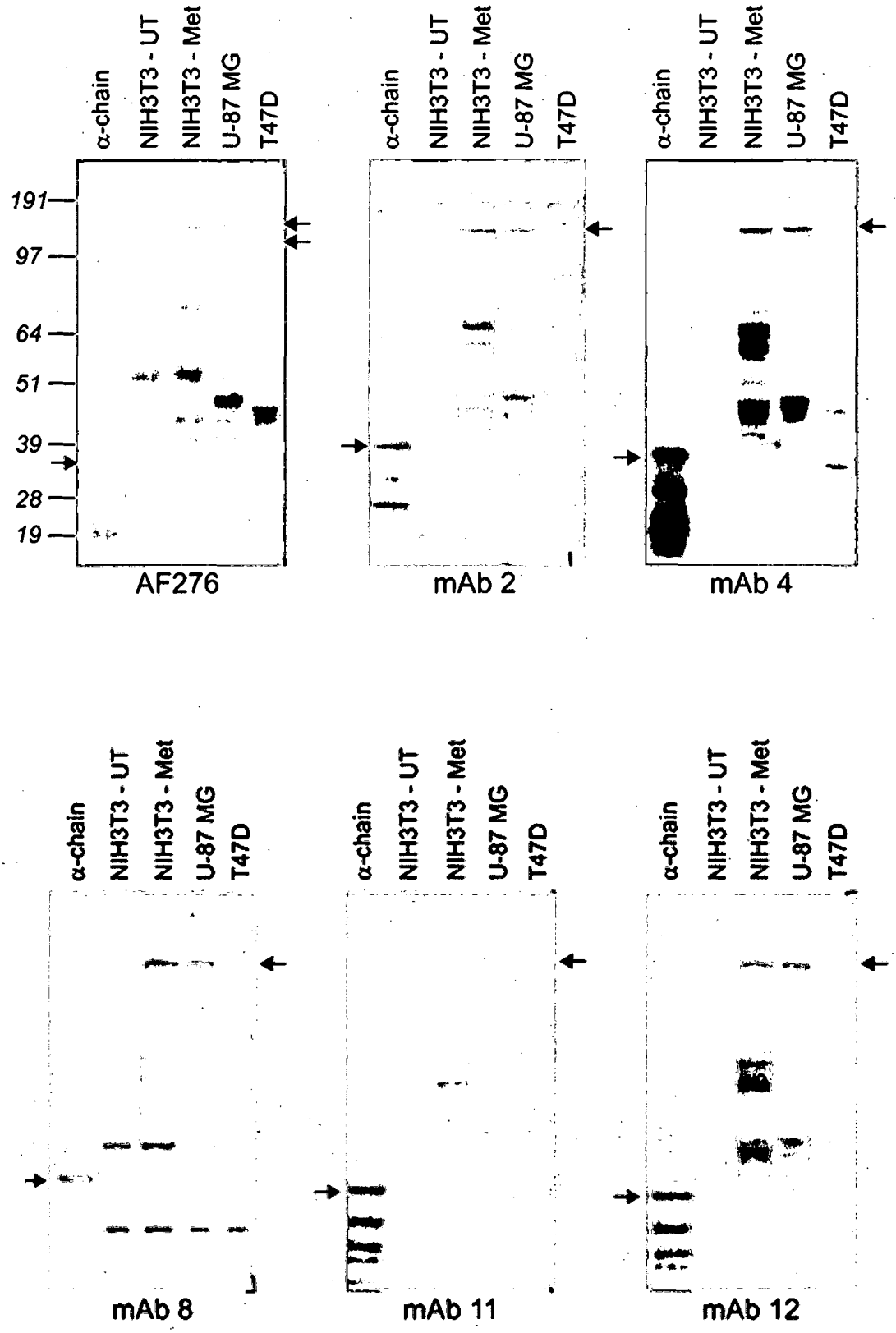

α-chain
NIH3T3 - UT
NIH3T3 - Met
U-87 MG
T47D
191
97
64
51
39
28
19
AF276
mAb 2
mAb 4
mAb 8
mAb 11
mAb 12

FIG. 2 (cont'd)

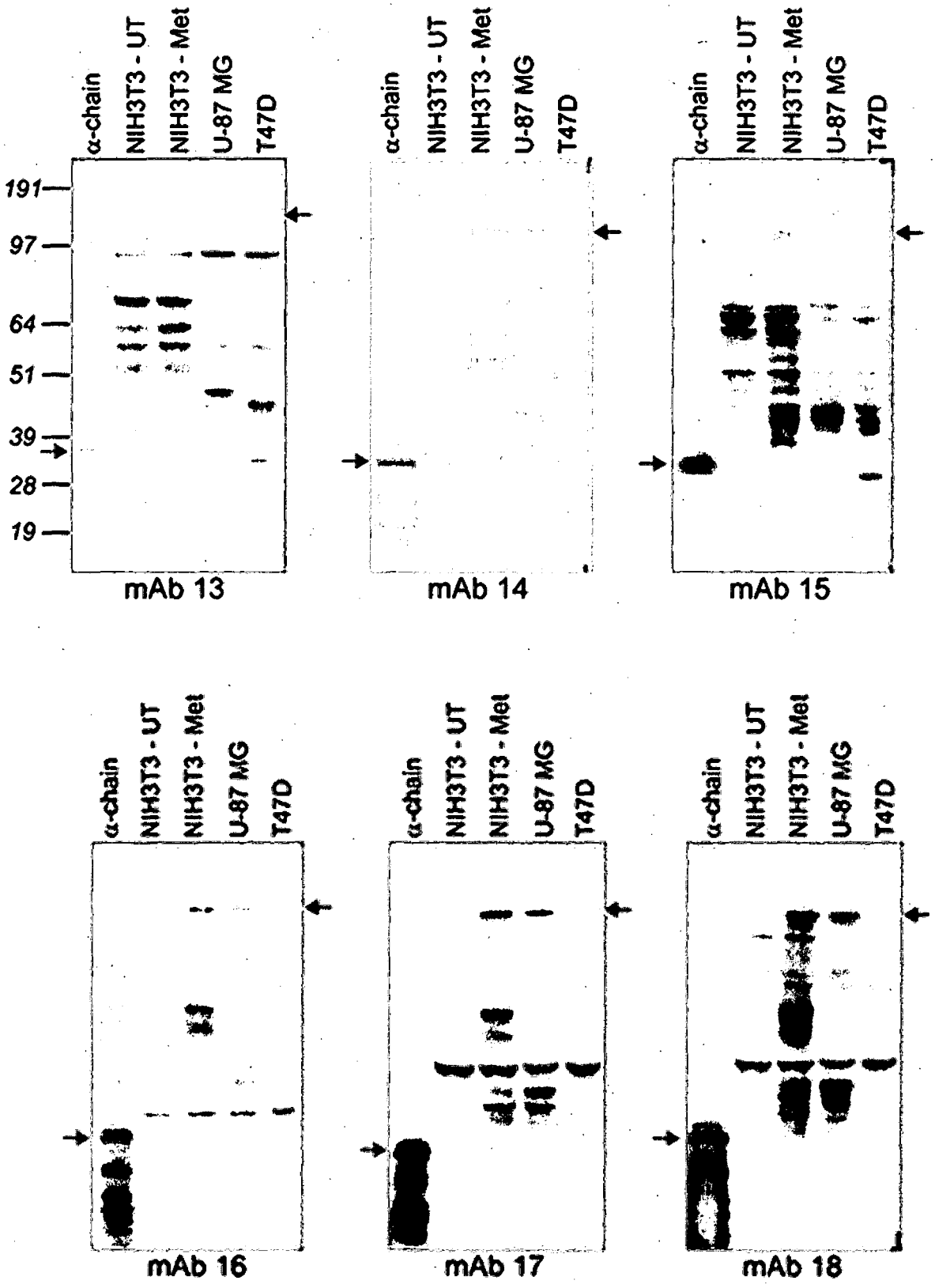

α-chain
NIH3T3 - UT
NIH3T3 - Met
U-87 MG
T47D
191
97
64
51
39
28
19
mAb 13
mAb 14
mAb 15
mAb 16
mAb 17
mAb 18

FIG. 3

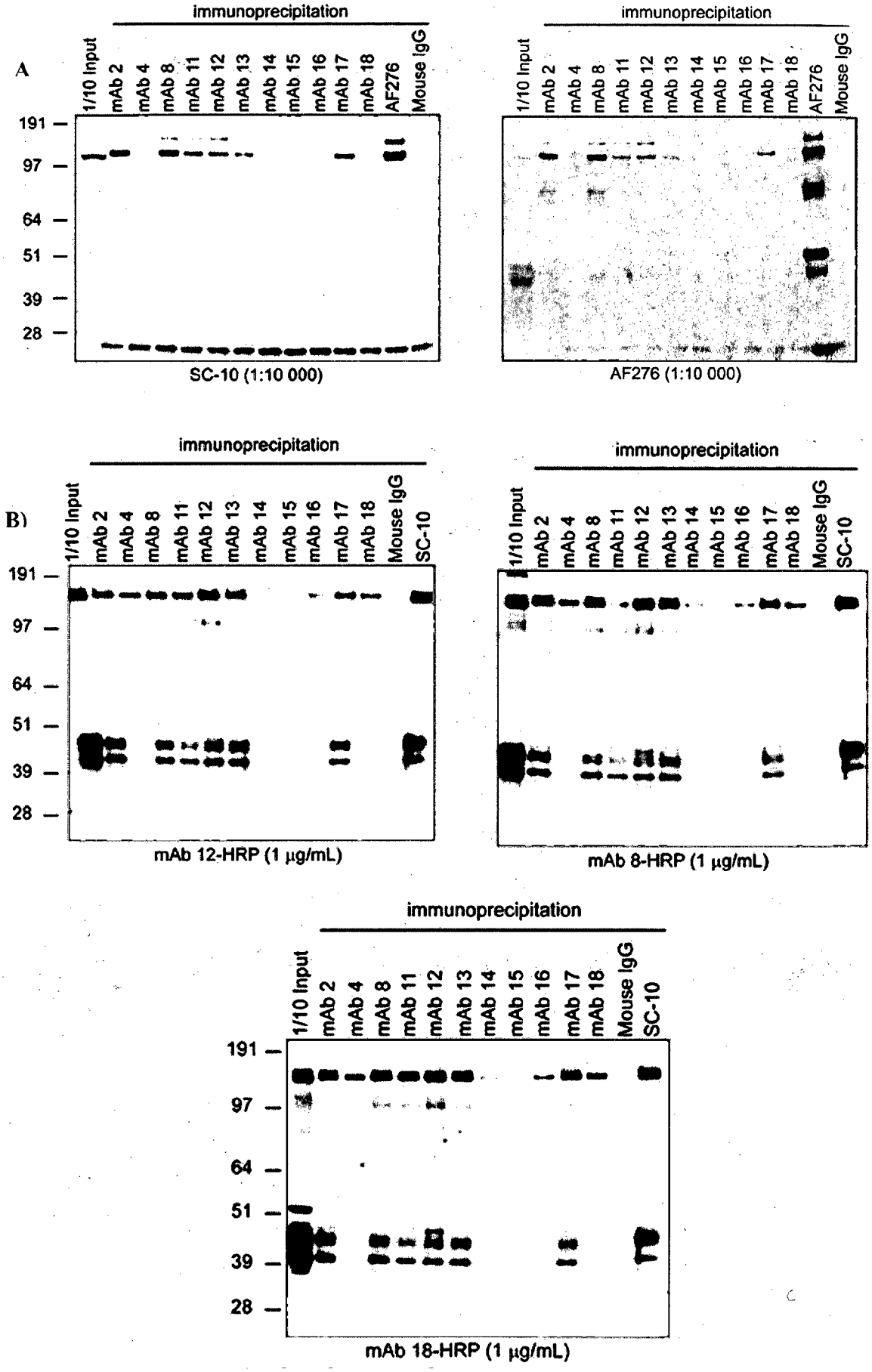

A
immunoprecipitation
1/10 Input
mAb 2
mAb 4
mAb 8
mAb 11
mAb 12
mAb 13
mAb 14
mAb 15
mAb 16
mAb 17
mAb 18
AF276
Mouse IgG
191
97
64
51
39
28
SC-10 (1:10 000)
AF276 (1:10 000)
B)
SC-10
mAb 12-HRP (1 µg/mL)
mAb 8-HRP (1 µg/mL)
mAb 18-HRP (1 µg/mL)

FIG. 4

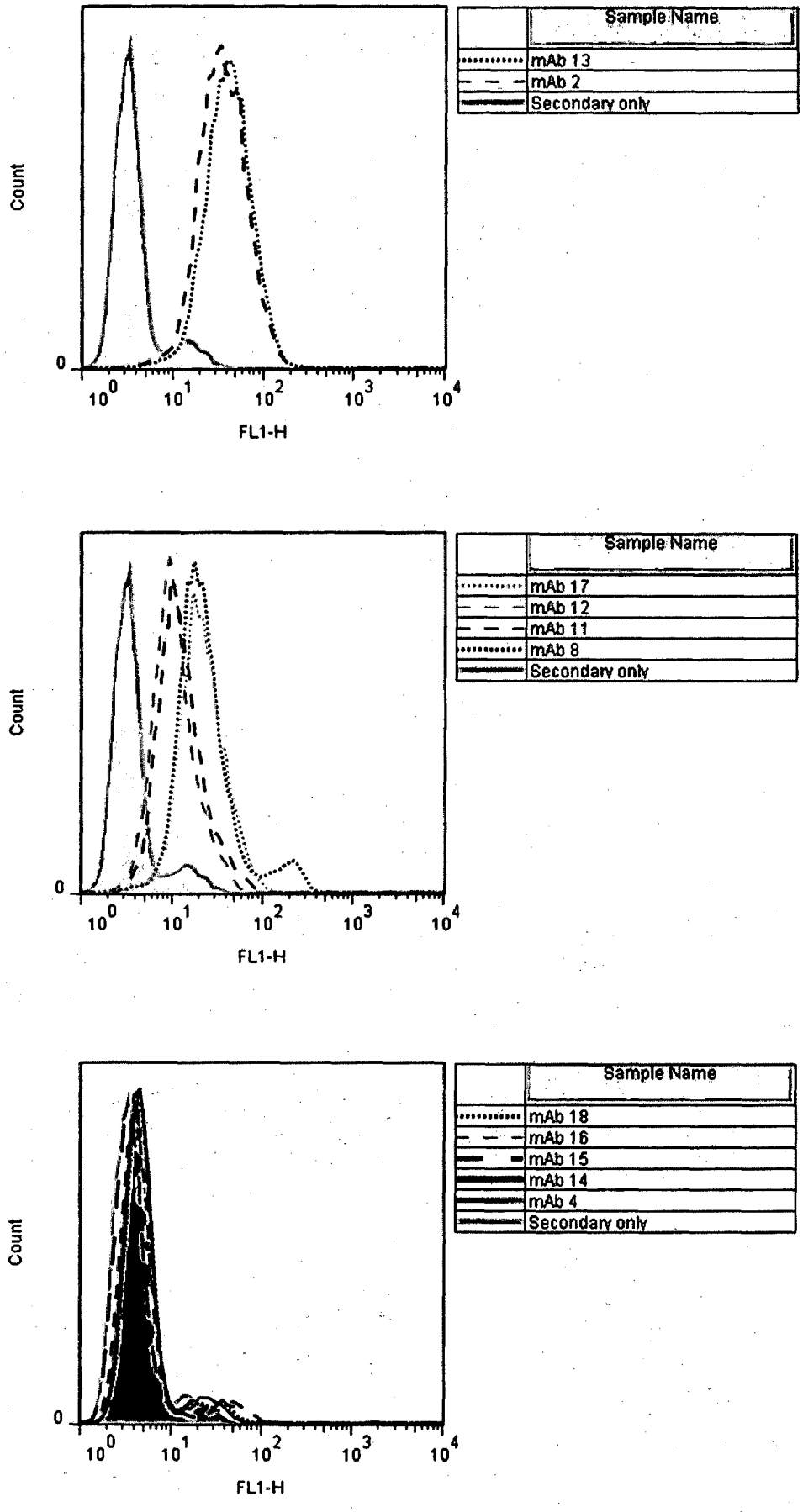

Count
0
10^0 10^1 10^2 10^3 10^4
FL1-H
Sample Name
mAb 13
mAb 2
Secondary only
Count
0
10^0 10^1 10^2 10^3 10^4
FL1-H
Sample Name
mAb 17
mAb 12
mAb 11
mAb 8
Secondary only
Count
0
10^0 10^1 10^2 10^3 10^4
FL1-H
Sample Name
mAb 18
mAb 16
mAb 15
mAb 14
mAb 4
Secondary only

FIG. 5

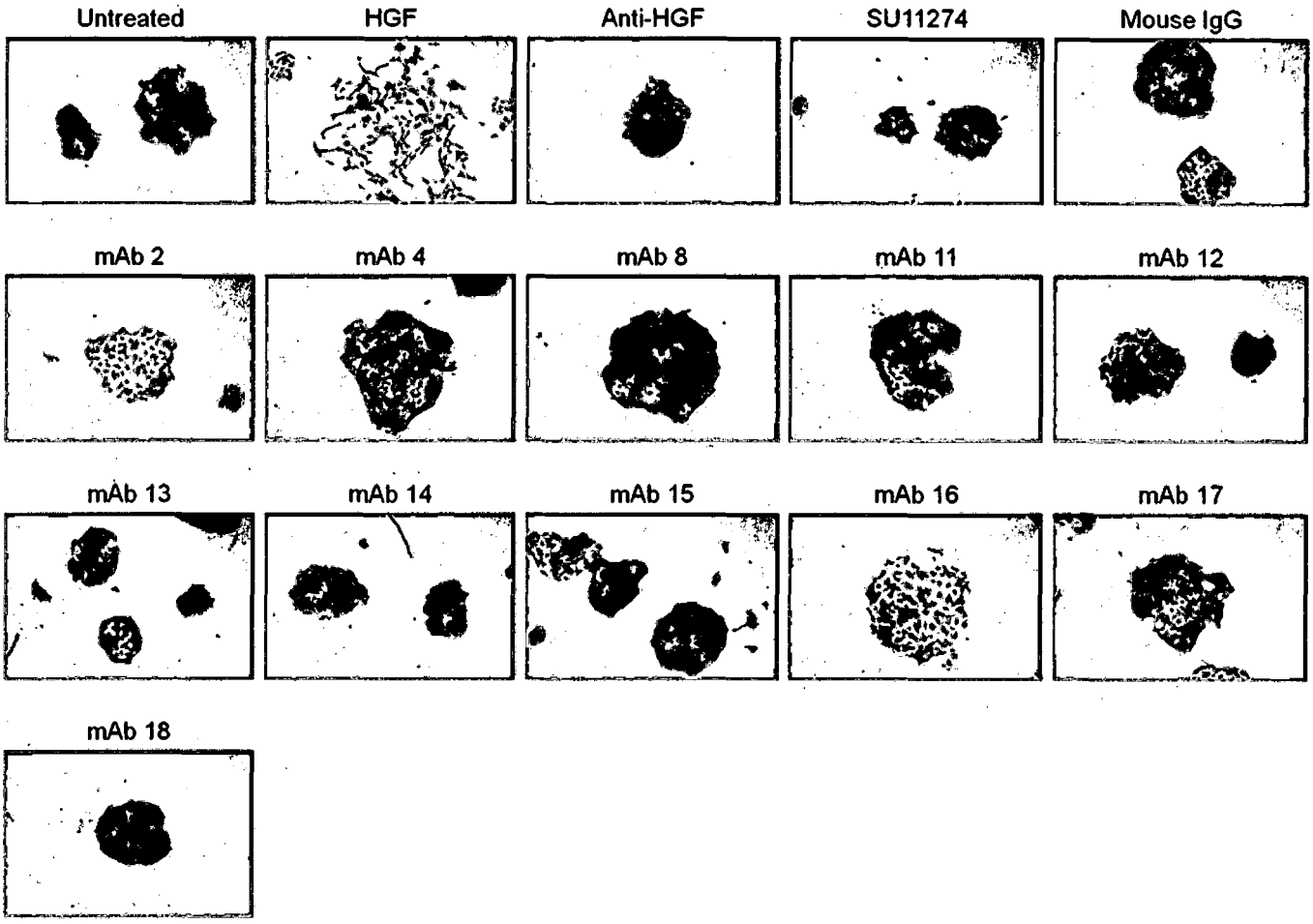
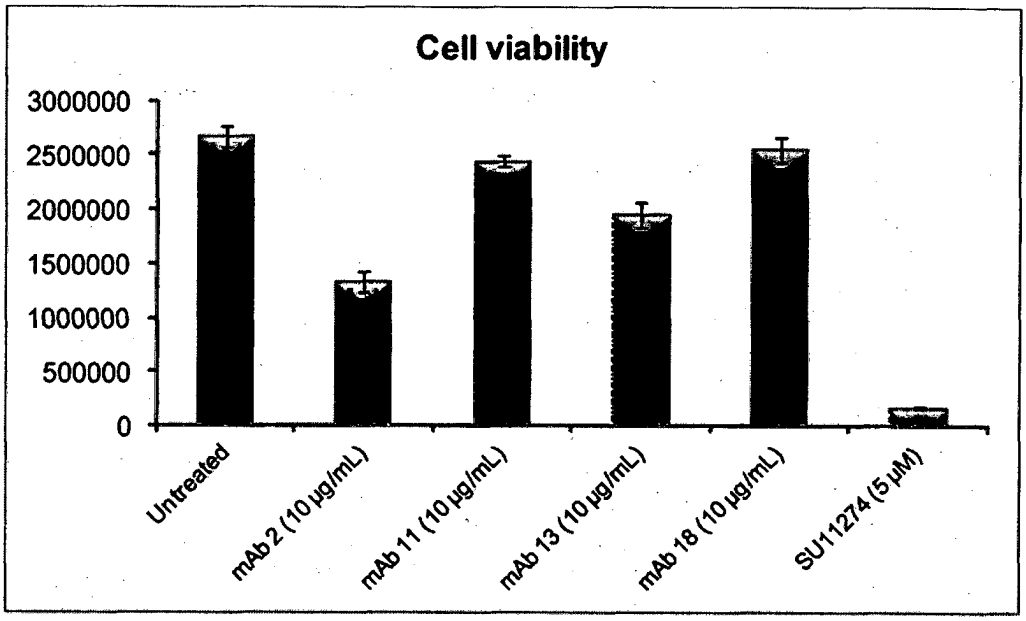

Untreated
HGF
Anti-HGF
SU11274
Mouse IgG
mAb 2
mAb 4
mAb 8
mAb 11
mAb 12
mAb 13
mAb 14
mAb 15
mAb 16
mAb 17
mAb 18

FIG. 6

A)


Cell viability
3000000
2500000
2000000
1500000
1000000
500000
0
Untreated
mAb 2 (10 µg/mL)
mAb 11 (10 µg/mL)
mAb 13 (10 µg/mL)
mAb 18 (10 µg/mL)
SU11274 (5 µM)

B)

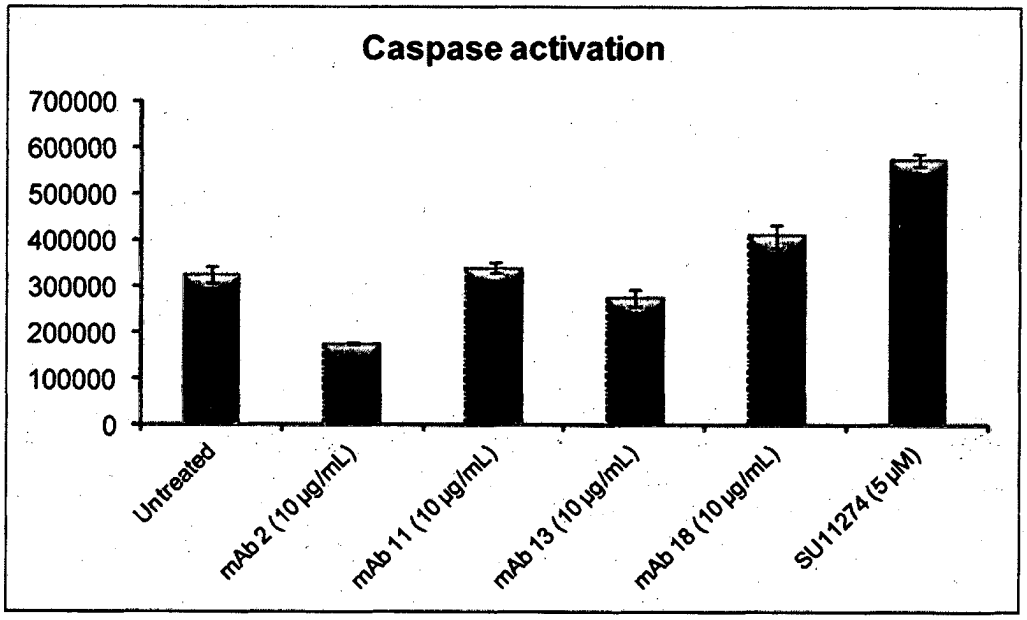
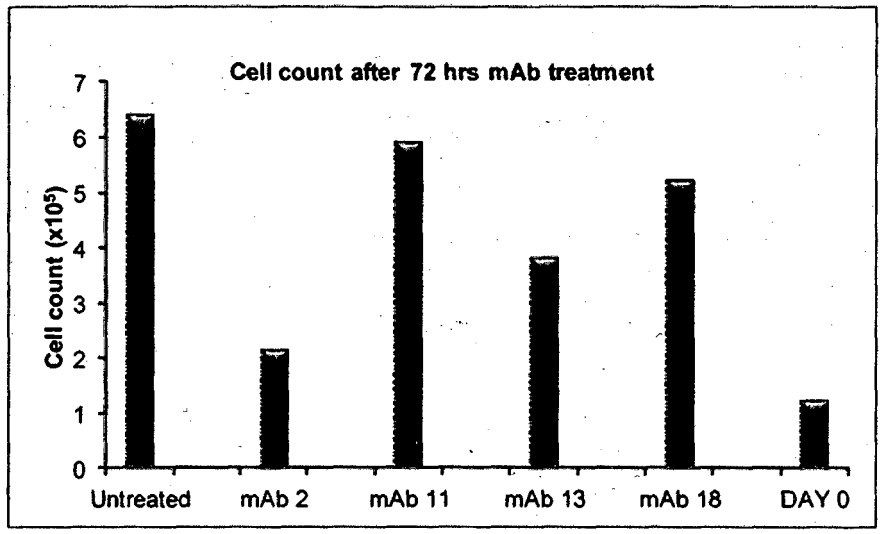
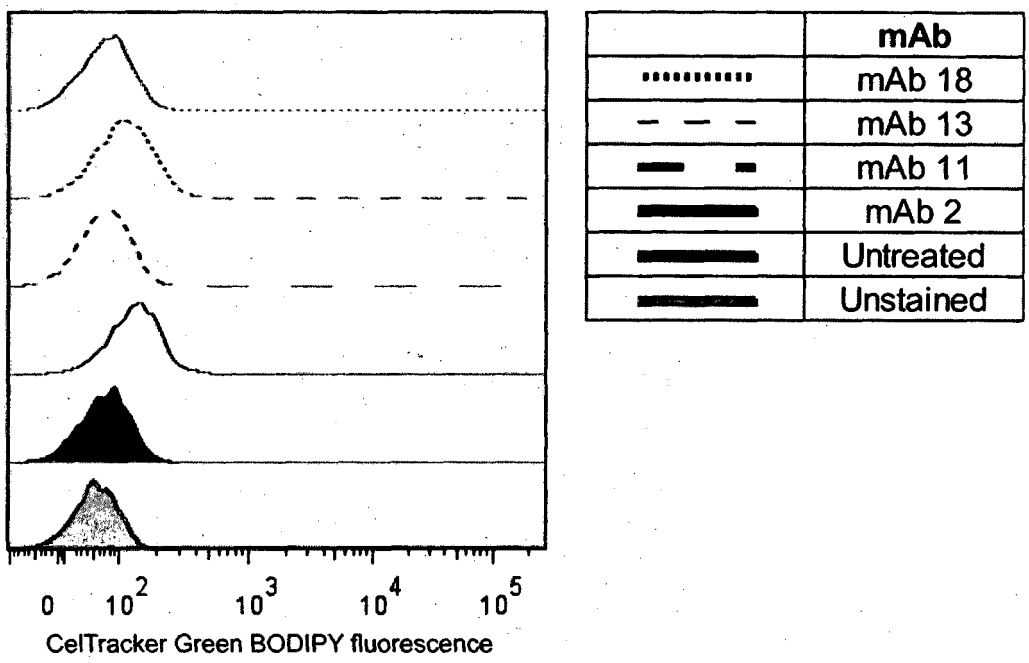

Caspase activation
700000
600000
500000
400000
300000
200000
100000
0
Untreated
mAb 2 (10 µg/mL)
mAb 11 (10 µg/mL)
mAb 13 (10 µg/mL)
mAb 18 (10 µg/mL)
SU11274 (5 µM)

FIG. 7

A)


Cell count after 72 hrs mAb treatment
Cell count (x10⁵)
0
1
2
3
4
5
6
7
Untreated
mAb 2
mAb 11
mAb 13
mAb 18
DAY 0

B)


mAb
mAb 18
mAb 13
mAb 11
mAb 2
Untreated
Unstained
0
10²
10³
10⁴
10⁵
CelTracker Green BODIPY fluorescence

C)

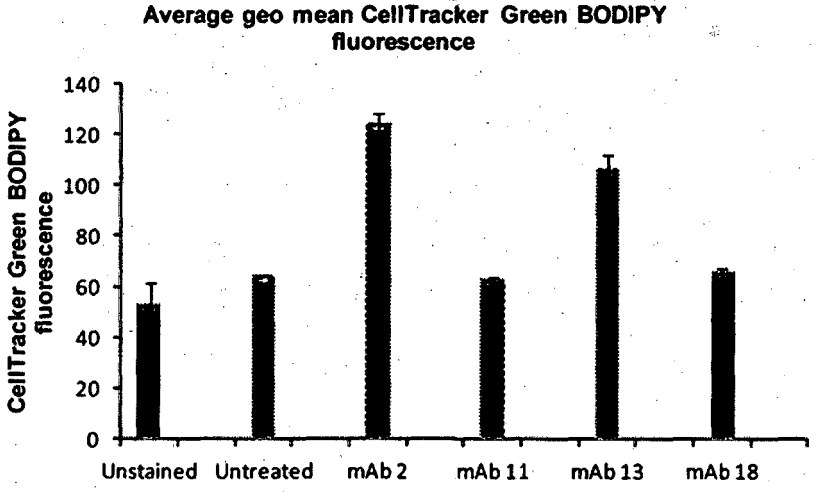

Average geo mean CellTracker Green BODIPY fluorescence
CellTracker Green BODIPY fluorescence
0
20
40
60
80
100
120
140
Unstained
Untreated
mAb 2
mAb 11
mAb 13
mAb 18

FIG. 8

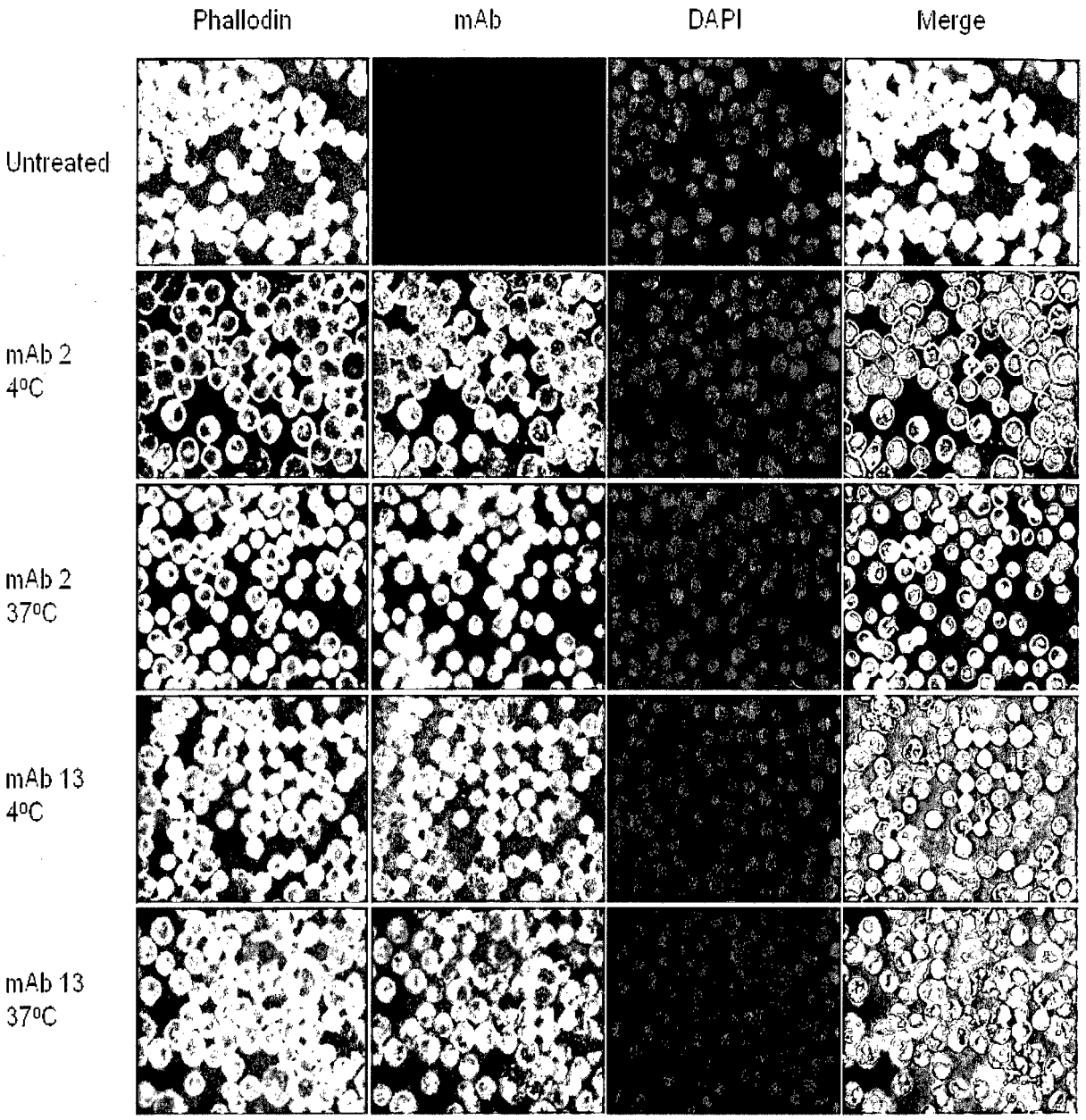

Phallodin
mAb
DAPI
Merge
Untreated
mAb 2
4°C
mAb 2
37°C
mAb 13
4°C
mAb 13
37°C

FIG. 9

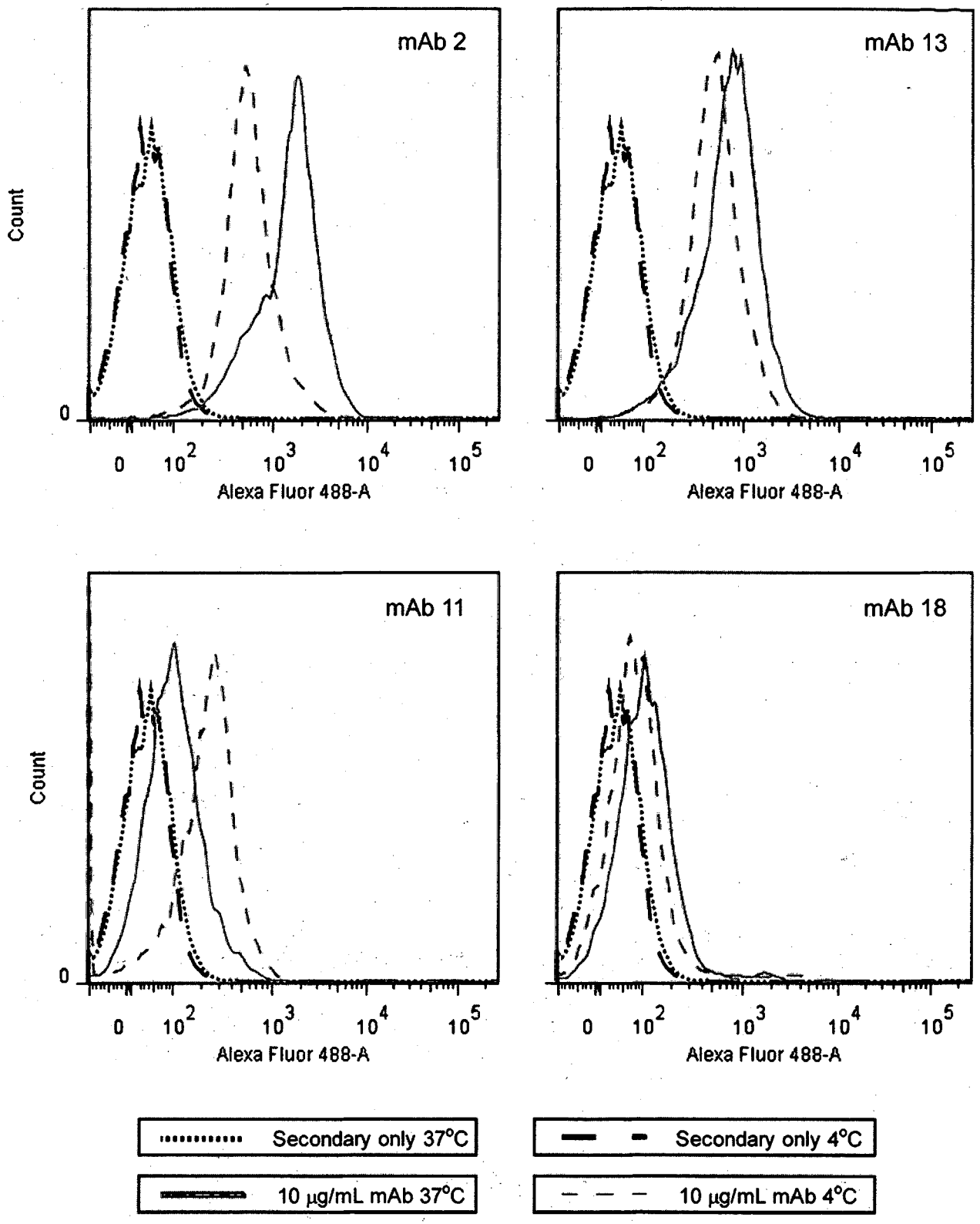

mAb 2
mAb 13
mAb 11
mAb 18
Count
0
10^2
10^3
10^4
10^5
Alexa Fluor 488-A
Secondary only 37°C
Secondary only 4°C
10 µg/mL mAb 37°C
10 µg/mL mAb 4°C

FIG. 10

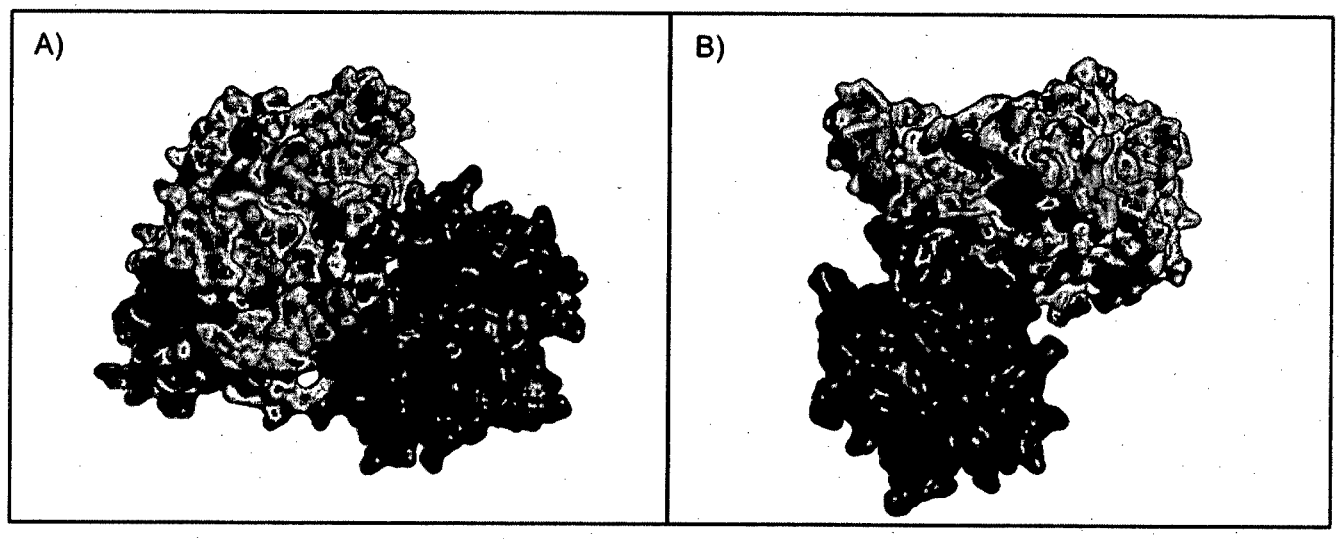


FIG. 11

a)

Peptide No.	Peptide sequence	ELISA reading
O1	ECKEALAKSEMNVNM	0.046
O27	QSEVHCIFSPQIEEP	0.016
O28	CIFSPQIEEPSQCPD	1.434
O29	QIEEPSQCPDCVVSA	1.290

b)

O1	O9	O17	O25	O33	O41	O49
O2	O10	O18	O26	O34	O42	O50
O3	O11	O19	O27	O35	O43	O51
O4	O12	O20	O28	O36	O44	O52
O5	O13	O21	O29	O37	O45	O53
O6	O14	O22	O30	O38	O46	O54
O7	O15	O23	O31	O39	O47	O55
O8	O16	O24	O32	O40	O48	O56

c)

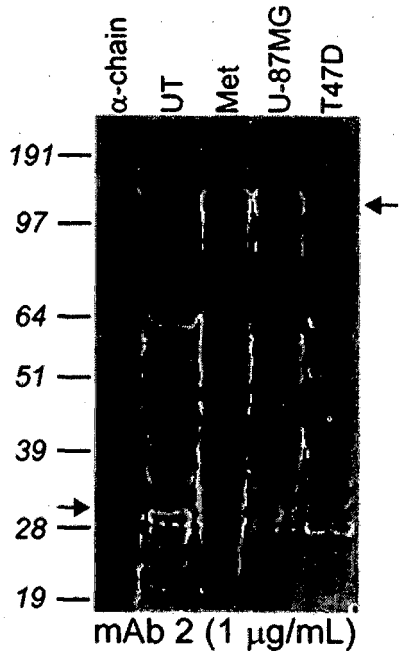
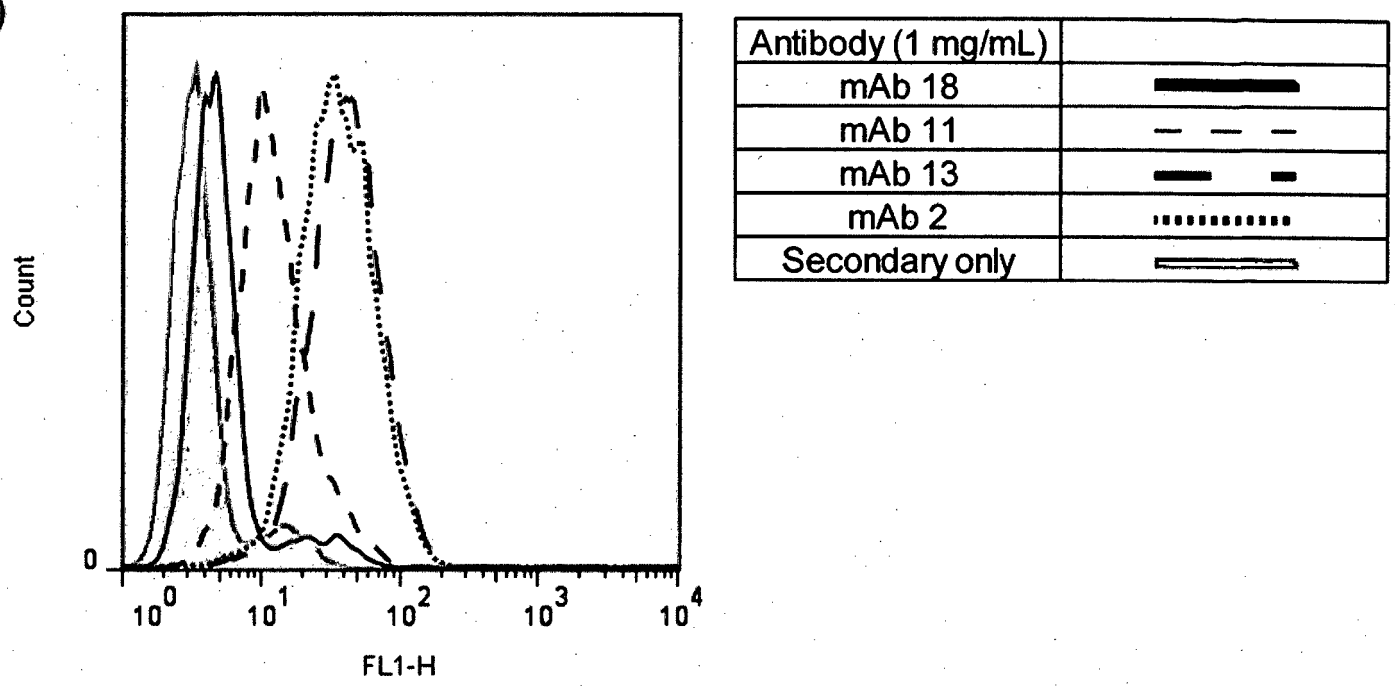


FIG. 11

d)


Count
0
10^0
10^1
10^2
10^3
10^4
FL1-H
Antibody (1 mg/mL)
mAb 18
mAb 11
mAb 13
mAb 2
Secondary only

e)

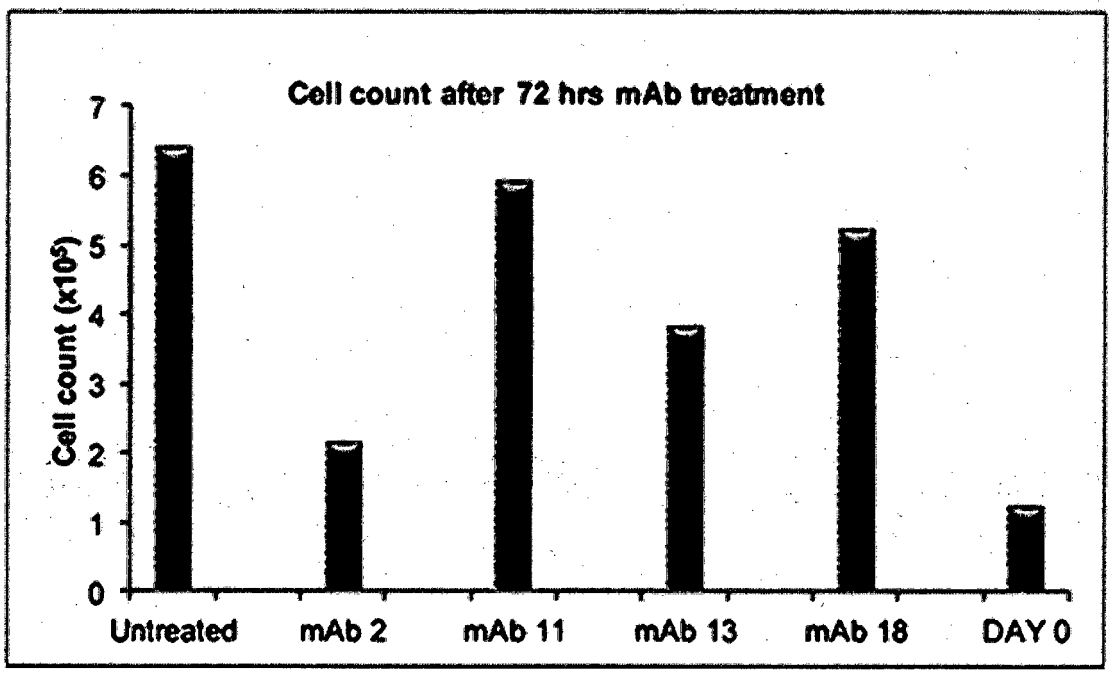
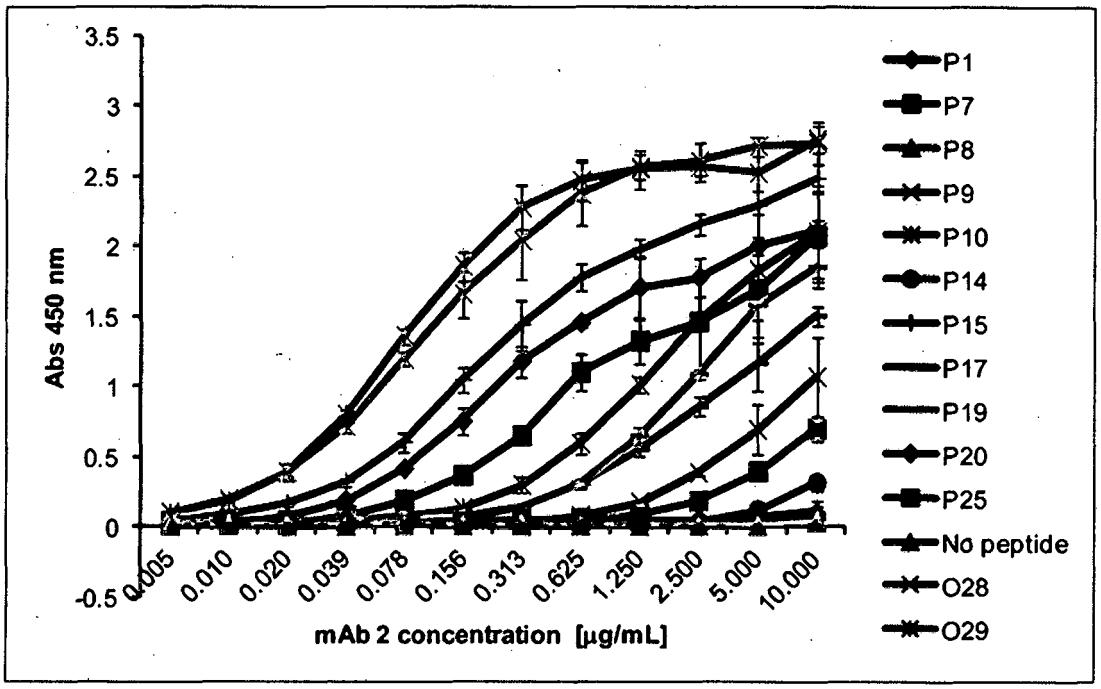

Cell count after 72 hrs mAb treatment
Cell count (x10⁵)
0
1
2
3
4
5
6
7
Untreated
mAb 2
mAb 11
mAb 13
mAb 18
DAY 0

FIG. 12

a)

Purified mAb 2 (0.05 µg/mL)					
	A	B	C	D	E
1	P1	P9	P17	P25	O1
2	P2	P10	P18	P26	O27
3	P3	P11	P19	P27	O28
4	P4	P12	P20	P28	O29
5	P5	P13	P21	P29	
6	P6	P14	P22	P30	
7	P7	P15	P23	P31	
8	P8	P16	P24	No peptide	

b)



c)

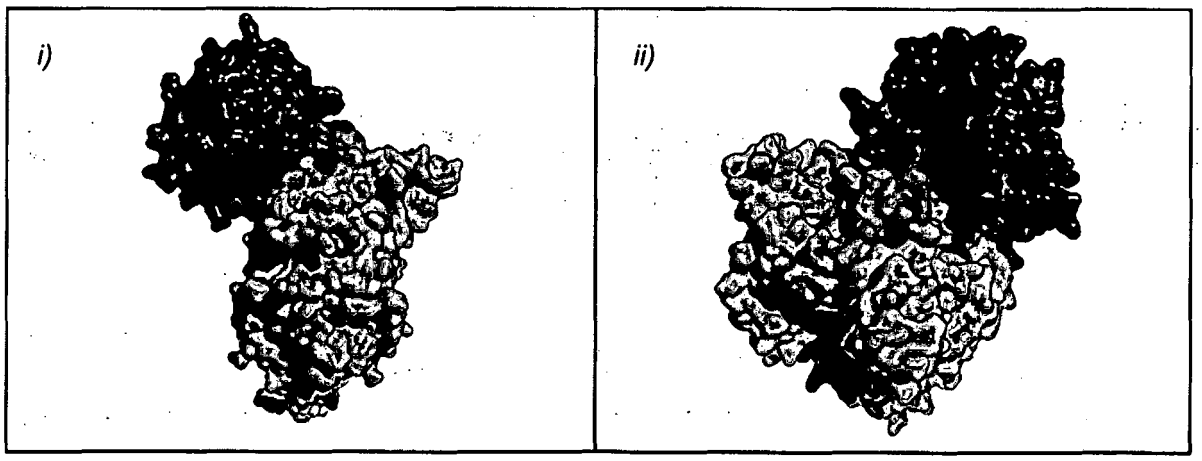


FIG. 13

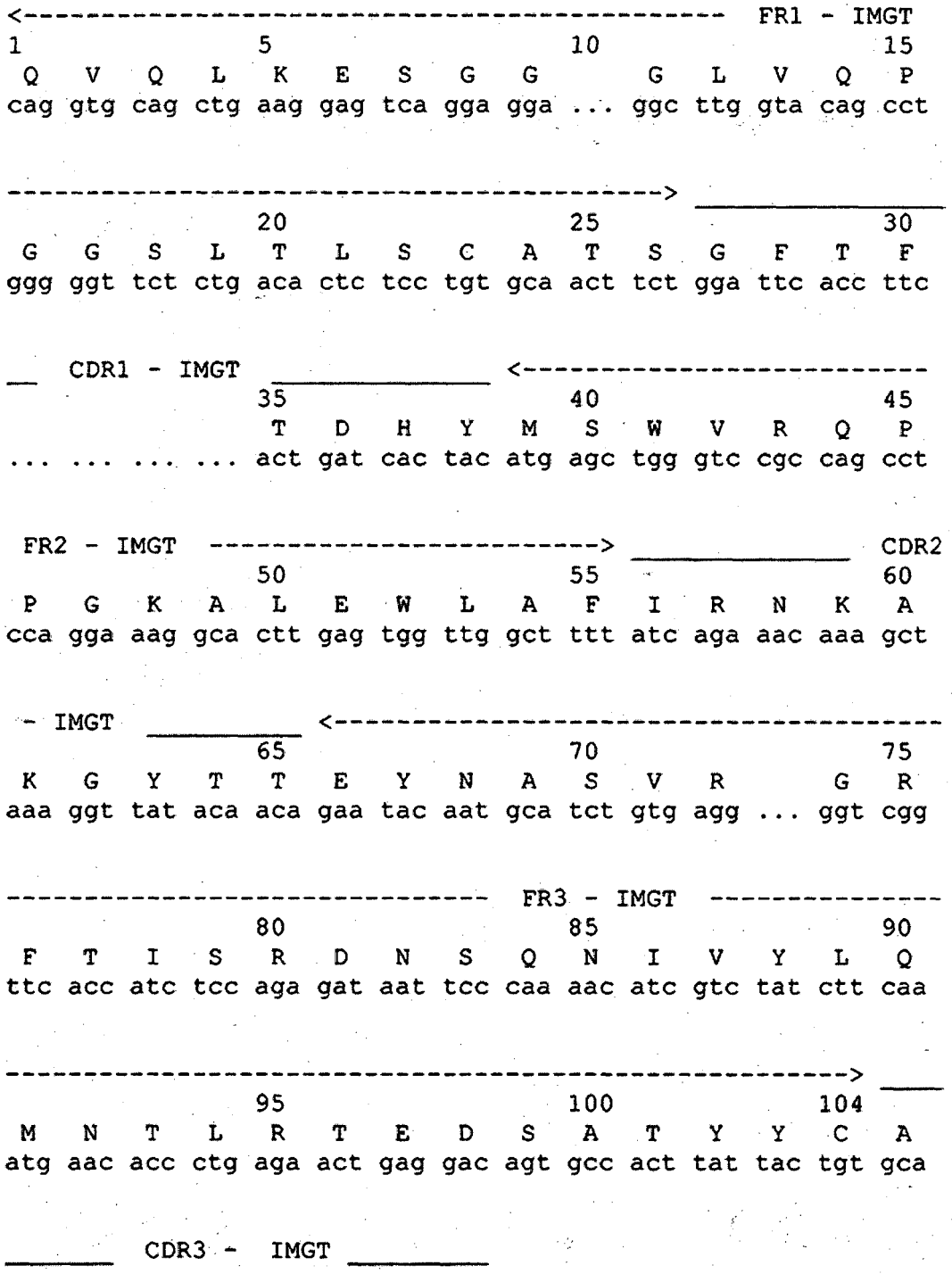

<---------- FR1 - IMGT
1 5 10 15
Q V Q L K E S G G G L V Q P
cag gtg cag ctg aag gag tca gga gga ... ggc ttg gta cag cct
----------> _____
20 25 30
G G S L T L S C A T S G F T F
ggg ggt tct ctg aca ctc tcc tgt gca act tct gga ttc acc ttc
__ CDR1 - IMGT _____ <----------
35 40 45
T D H Y M S W V R Q P
... ... act gat cac tac atg agc tgg gtc cgc cag cct
FR2 - IMGT ----------> _____ CDR2
50 55 60
P G K A L E W L A F I R N K A
cca gga aag gca ctt gag tgg ttg gct ttt atc aga aac aaa gct
- IMGT _____ <----------
65 70 75
K G Y T T E Y N A S V R G R
aaa ggt tat aca aca gaa tac aat gca tct gtg agg ... ggt cgg
---------- FR3 - IMGT ----------
80 85 90
F T I S R D N S Q N I V Y L Q
ttc acc atc tcc aga gat aat tcc caa aac atc gtc tat ctt caa
----------> ____
95 100 104
M N T L R T E D S A T Y Y C A
atg aac acc ctg aga act gag gac agt gcc act tat tac tgt gca
_____ CDR3 - IMGT _____
R D G V G I A Y W G Q G T L V
aga gat ggg gtg gga att gct tac tgg ggc caa ggg act ctg gtc
T V S A A K T T P P S V Y P L
act gtt tct gca gcc aaa acg aca ccc cca tct gtc tat cca ctg
A P
gcc cct

FIG. 14

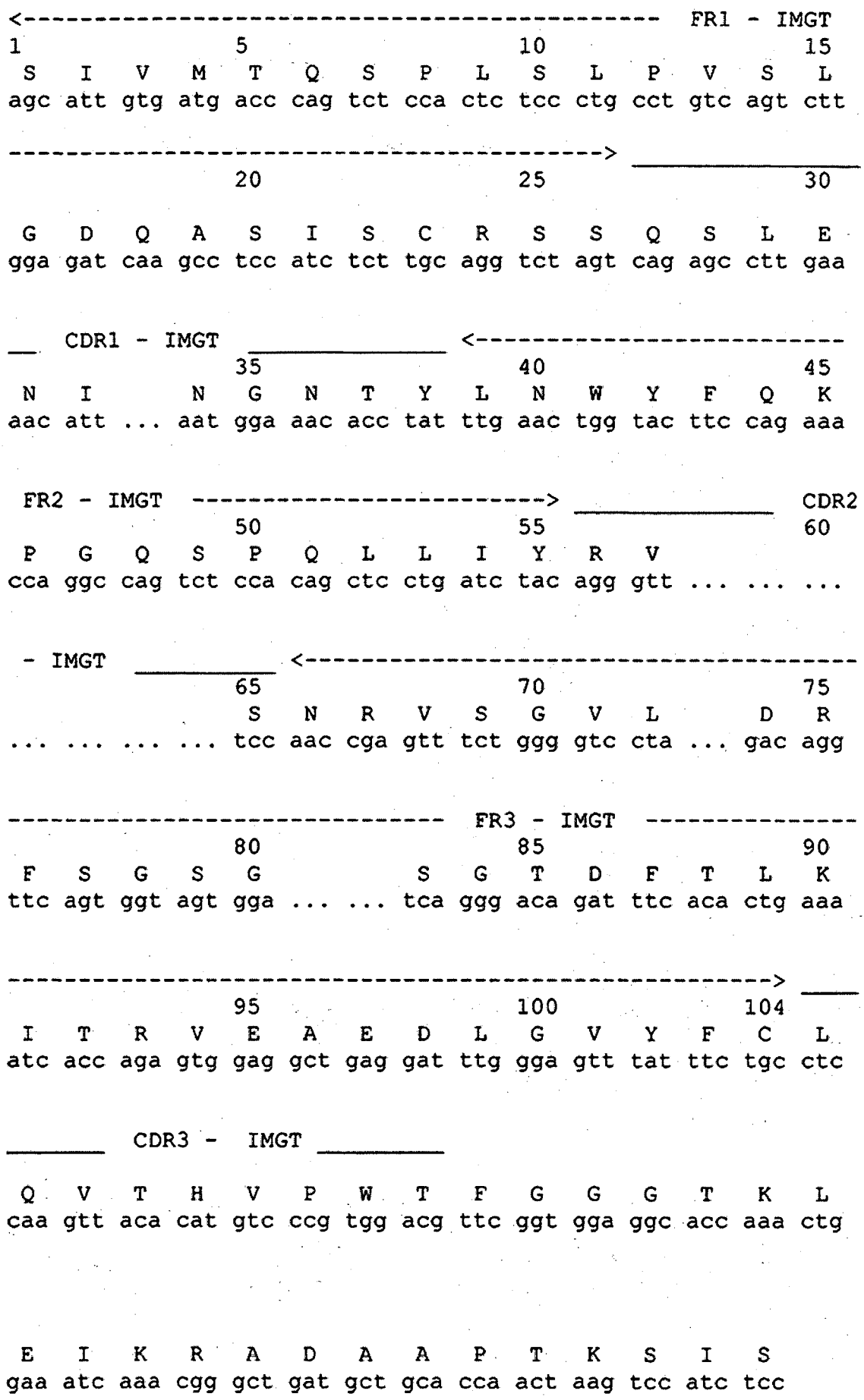
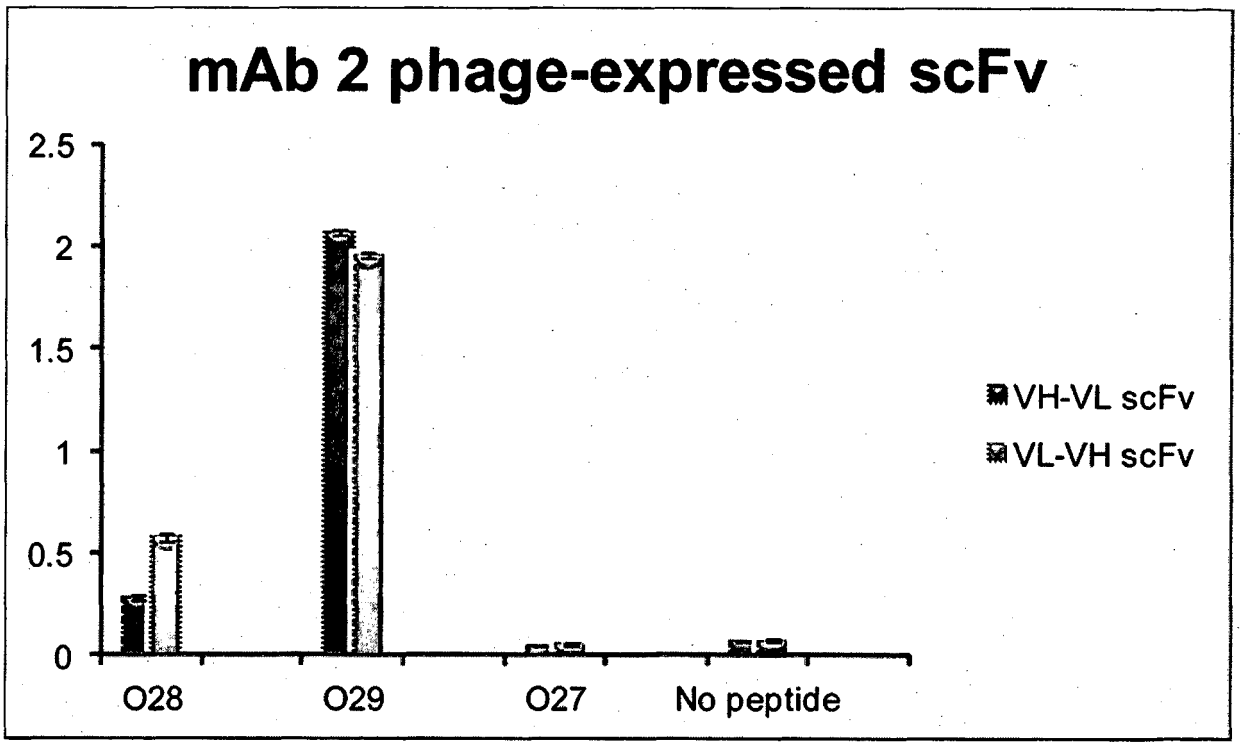

<---------- FR1 - IMGT
1 5 10 15
S I V M T Q S P L S L P V S L
agc att gtg atg acc cag tct cca ctc tcc ctg cct gtc agt ctt
----------> _____
20 25 30
G D Q A S I S C R S S Q S L E
gga gat caa gcc tcc atc tct tgc agg tct agt cag agc ctt gaa
__ CDR1 - IMGT _____ <----------
35 40 45
N I N G N T Y L N W Y F Q K
aac att ... aat gga aac acc tat ttg aac tgg tac ttc cag aaa
FR2 - IMGT ----------> _____ CDR2
50 55 60
P G Q S P Q L L I Y R V
cca ggc cag tct cca cag ctc ctg atc tac agg gtt ... ...
- IMGT _____ <----------
65 70 75
S N R V S G V L D R
... ... tcc aac cga gtt tct ggg gtc cta ... gac agg
---------- FR3 - IMGT ----------
80 85 90
F S G S G S G T D F T L K
ttc agt ggt agt gga ... ... tca ggg aca gat ttc aca ctg aaa
----------> ____
95 100 104
I T R V E A E D L G V Y F C L
atc acc aga gtg gag gct gag gat ttg gga gtt tat ttc tgc ctc
_____ CDR3 - IMGT _____
Q V T H V P W T F G G G T K L
caa gtt aca cat gtc ccg tgg acg ttc ggt gga ggc acc aaa ctg
E I K R A D A A P T K S I S
gaa atc aaa cgg gct gat gct gca cca act aag tcc atc tcc

FIG. 15


mAb 2 phage-expressed scFv
2.5
2
1.5
1
0.5
0
O28
O29
O27
No peptide
VH-VL scFv
VL-VH scFv