

FIG. 1

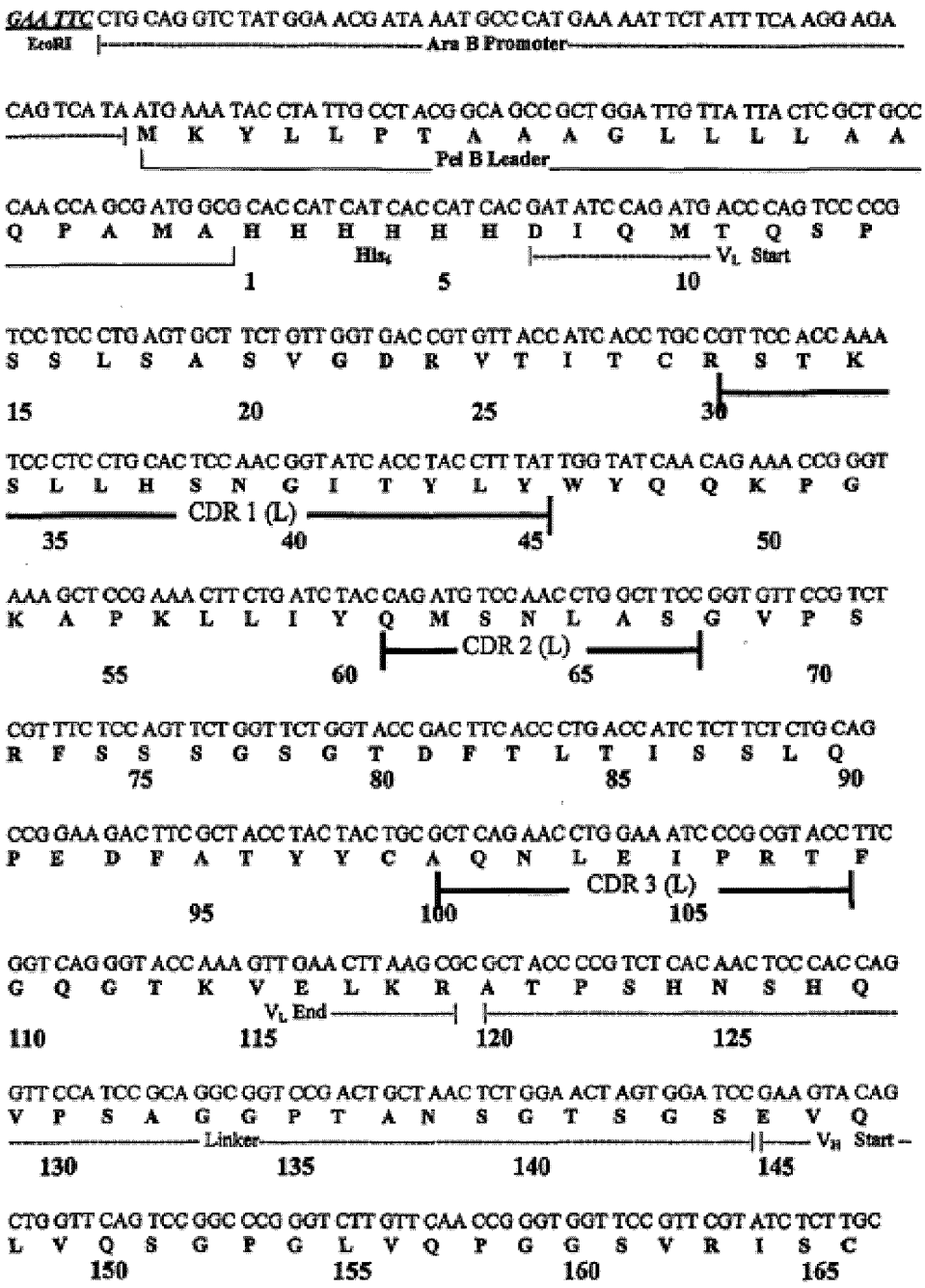

GAA TTC CTG CAG GTC TAT GGA ACG ATA AAT GCC CAT GAA AAT TCT ATT TCA AGG AGA
EcoRI
Ara B Promoter
CAG TCA TA ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCT GCC
M K Y L L P T A A A G L L L L A A
Pel B Leader
CAA CCA GCG ATG GCG CAC CAT CAT CAC CAT CAC GAT ATC CAG ATG ACC CAG TCC CCG
Q P A M A H H H H H H D I Q M T Q S P
His6
VL Start
1 5 10
TCC TCC CTG AGT GCT TCT GTT GGT GAC CGT GTT ACC ATC ACC TGC CGT TCC ACC AAA
S S L S A S V G D R V T I T C R S T K
15 20 25 30
TCC CTC CTG CAC TCC AAC GGT ATC ACC TAC CTT TAT TGG TAT CAA CAG AAA CCG GGT
S L L H S N G I T Y L Y W Y Q Q K P G
CDR 1 (L)
35 40 45 50
AAA GCT CCG AAA CTT CTG ATC TAC CAG ATG TCC AAC CTG GCT TCC GGT GTT CCG TCT
K A P K L L I Y Q M S N L A S G V P S
CDR 2 (L)
55 60 65 70
CGT TTC TCC AGT TCT GGT TCT GGT ACC GAC TTC ACC CTG ACC ATC TCT TCT CTG CAG
R F S S S G S G T D F T L T I S S L Q
75 80 85 90
CCG GAA GAC TTC GCT ACC TAC TAC TGC GCT CAG AAC CTG GAA ATC CCG CGT ACC TTC
P E D F A T Y Y C A Q N L E I P R T F
CDR 3 (L)
95 100 105
GGT CAG GGT ACC AAA GTT GAA CTT AAG CGC GCT ACC CCG TCT CAC AAC TCC CAC CAG
G Q G T K V E L K R A T P S H N S H Q
VL End
110 115 120 125
GTT CCA TCC GCA GGC GGT CCG ACT GCT AAC TCT GGA ACT AGT GGA TCC GAA GTA CAG
V P S A G G P T A N S G T S G S E V Q
Linker
VH Start
130 135 140 145
CTG GTT CAG TCC GGC CCG GGT CTT GTT CAA CCG GGT GGT TCC GTT CGT ATC TCT TGC
L V Q S G P G L V Q P G G S V R I S C
150 155 160 165

FIG. 2

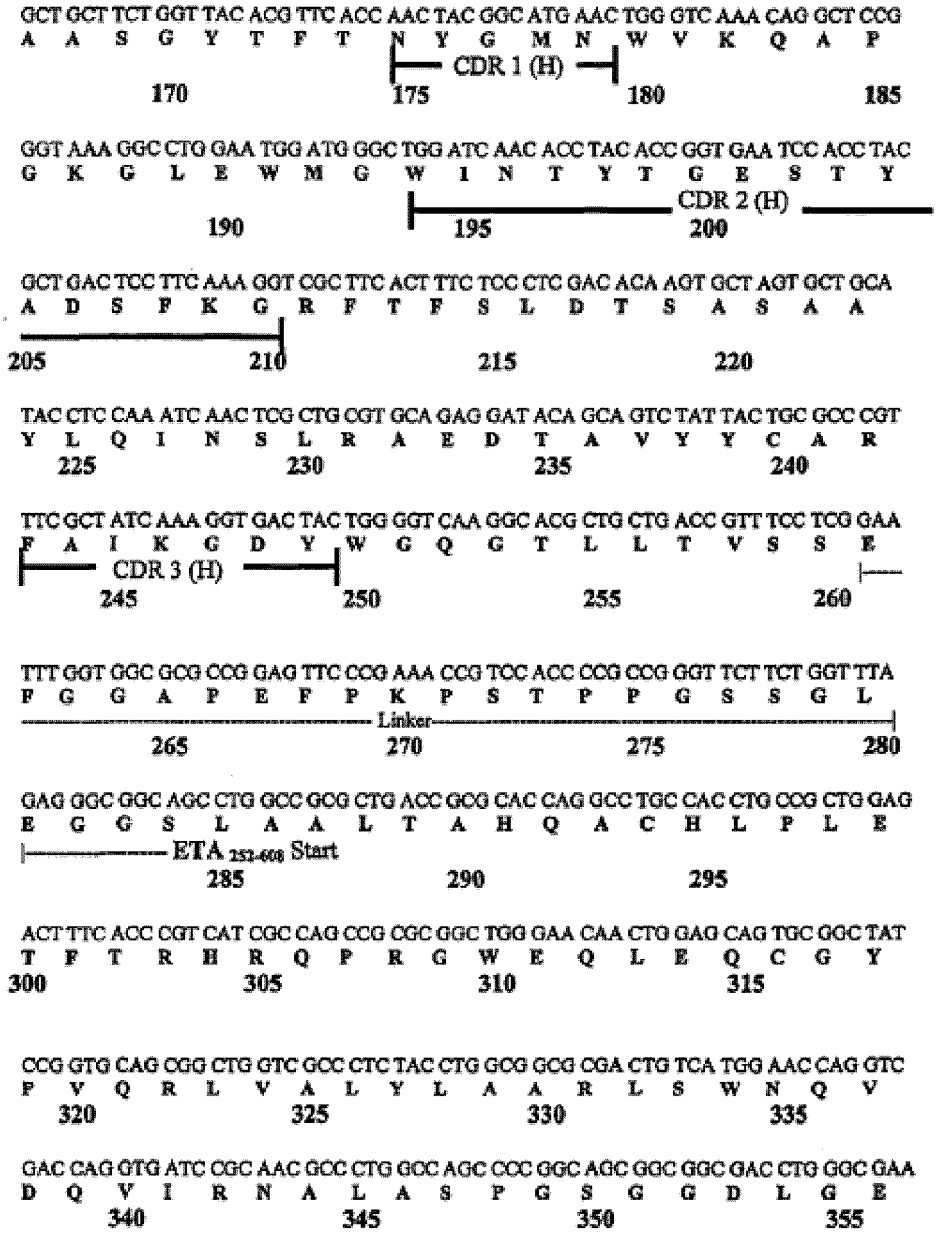

GCT GCT TCT GGT TAC ACG TTC ACC AAC TAC GGC ATG AAC TGG GTC AAA CAG GCT CCG
A A S G Y T F T N Y G M N W V K Q A P
CDR 1 (H)
170 175 180 185
GGT AAA GGC CTG GAA TGG ATG GGC TGG ATC AAC ACC TAC ACC GGT GAA TCC ACC TAC
G K G L E W M G W I N T Y T G E S T Y
CDR 2 (H)
190 195 200
GCT GAC TCC TTC AAA GGT CGC TTC ACT TTC TCC CTC GAC ACA AGT GCT AGT GCT GCA
A D S F K G R F T F S L D T S A S A A
205 210 215 220
TAC CTC CAA ATC AAC TCG CTG CGT GCA GAG GAT ACA GCA GTC TAT TAC TGC GCC CGT
Y L Q I N S L R A E D T A V Y Y C A R
225 230 235 240
TTC GCT ATC AAA GGT GAC TAC TGG GGT CAA GGC ACG CTG CTG ACC GTT TCC TCG GAA
F A I K G D Y W G Q G T L L T V S S E
CDR 3 (H)
245 250 255 260
TTT GGT GGC GCG CCG GAG TTC CCG AAA CCG TCC ACC CCG CCG GGT TCT TCT GGT TTA
F G G A P E F P K P S T P P G S S G L
Linker
265 270 275 280
GAG GGC GGC AGC CTG GCC GCG CTG ACC GCG CAC CAG GCC TGC CAC CTG CCG CTG GAG
E G G S L A A L T A H Q A C H L P L E
ETA 252-608 Start
285 290 295
ACT TTC ACC CGT CAT CGC CAG CCG CGC GGC TGG GAA CAA CTG GAG CAG TGC GGC TAT
T F T R H R Q P R G W E Q L E Q C G Y
300 305 310 315
CCG GTG CAG CGG CTG GTC GCC CTC TAC CTG GCG GCG CGA CTG TCA TGG AAC CAG GTC
P V Q R L V A L Y L A A R L S W N Q V
320 325 330 335
GAC CAG GTG ATC CGC AAC GCC CTG GCC AGC CCC GGC AGC GGC GGC GAC CTG GGC GAA
D Q V I R N A L A S P G S G G D L G E
340 345 350 355

FIG. 2 contd.

GCG ATC CGC GAG CAG CCG GAG CAG GCC CGT CTG GCC CTG ACC CTG GCC GCC GCC GAG
A I R E Q P E Q A R L A L T L A A A E
360 365 370 375

AGC GAG CGC TTC GTC CGG CAG GGC ACC GGC AAC GAC GAG GCC GGC GCG GCC AGC GCC
S E R F V R Q G T G N D E A G A A S A
380 385 390

GAC GTG GTG AGC CTG ACC TGC CCG GTC GCC GCC GGT GAA TGC GCG GGC CCG GCG GAC
D V V S L T C P V A A G E C A G P A D
395 400 405 410

AGC GGC GAC GCC CTG CTG GAG CGC AAC TAT CCC ACT GGC GCG GAG TTC CTC GGC GAC
S G D A L L E R N Y P T G A E F L G D
415 420 425 430

GGT GGC GAC GTC AGC TTC AGC ACC CGC GGC ACG CAG AAC TGG ACG GTG GAG CGG CTG
G G D V S F S T R G T Q N W T V E R L
435 440 445 450

CTC CAG GCG CAC CGC CAA CTG GAG GAG CGC GGC TAT GTG TTC GTC GGC TAC CAC GGC
L Q A H R Q L E E R G Y V F V G Y H G
455 460 465 470

ACC TTC CTC GAA GCG GCG CAA AGC ATC GTC TTC GGC GGG GTG CGC GCG CGC AGC CAG
T F L E A A Q S I V F G G V R A R S Q
475 480 485

GAT CTC GAC GCG ATC TGG CGC GGT TTC TAT ATC GCC GGC GAT CCG GCG CTG GCC TAC
D L D A I W R G F Y I A G D P A L A Y
490 495 500 505

GGC TAC GCC CAG GAC CAG GAA CCC GAC GCG CGC GGC CGG ATC CGC AAC GGT GCC CTG
G Y A Q D Q E P D A R G R I R N G A L
510 515 520 525

CTG CGG GTC TAT GTG CCG CGC TCC AGC CTG CCG GGC TTC TAC CGC ACC GGC CTG ACC
L R V Y V P R S S L P G F Y R T G L T
530 535 540 545

CTG GCC GCG CCG GAG GCG GCG GGC GAG GTC GAA CGG CTG ATC GGC CAT CCG CTG CCG
L A A P E A A G E V E R L I G H P L P
550 555 560 565

FIG. 2 contd.

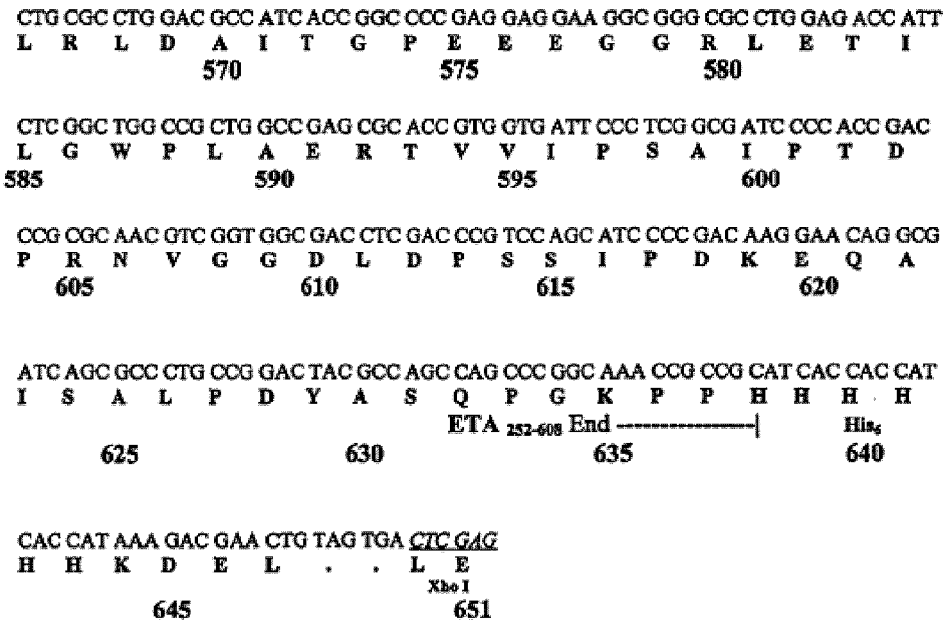

CTG CGC CTG GAC GCC ATC ACC GGC CCC GAG GAG GAA GGC GGG CGC CTG GAG ACC ATT
L R L D A I T G P E E E G G R L E T I
570 575 580
CTC GGC TGG CCG CTG GCC GAG CGC ACC GTG GTG ATT CCC TCG GCG ATC CCC ACC GAC
L G W P L A E R T V V I P S A I P T D
585 590 595 600
CCG CGC AAC GTC GGT GGC GAC CTC GAC CCG TCC AGC ATC CCC GAC AAG GAA CAG GCG
P R N V G G D L D P S S I P D K E Q A
605 610 615 620
ATC AGC GCC CTG CCG GAC TAC GCC AGC CAG CCC GGC AAA CCG CCG CAT CAC CAC CAT
I S A L P D Y A S Q P G K P P H H H H
ETA 252-608 End
His6
625 630 635 640
CAC CAT AAA GAC GAA CTG TAG TGA CTC GAG
H H K D E L . . L E
Xho I
645 651

FIG. 2 contd.

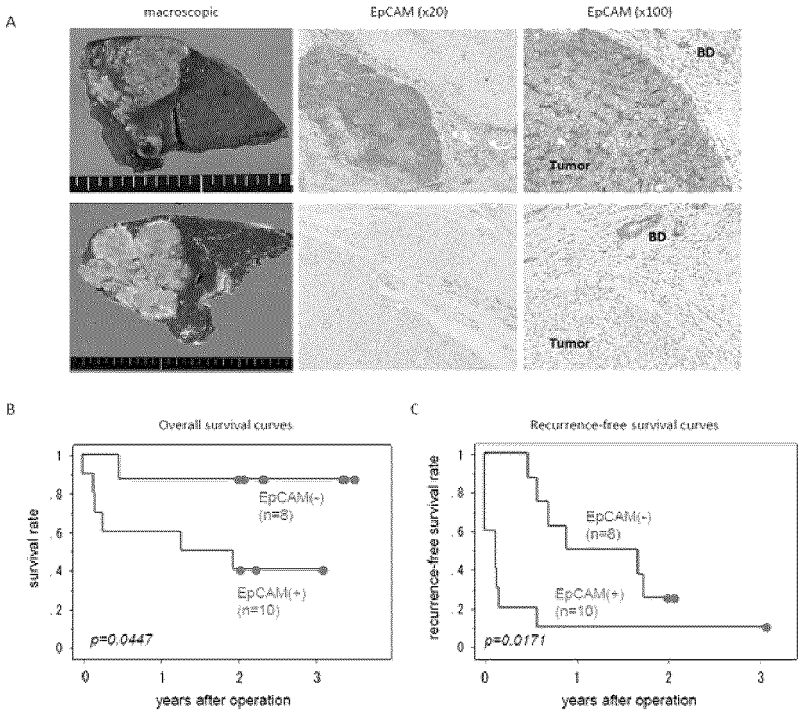

A
macroscopic
EpCAM (x20)
EpCAM (x100)
BD
Tumor
BD
Tumor
B
Overall survival curves
survival rate
EpCAM(-)
(n=8)
EpCAM(+)
(n=10)
p=0.0447
years after operation
C
Recurrence-free survival curves
recurrence-free survival rate
EpCAM(-)
(n=8)
EpCAM(+)
(n=10)
p=0.0171
years after operation

FIG. 3

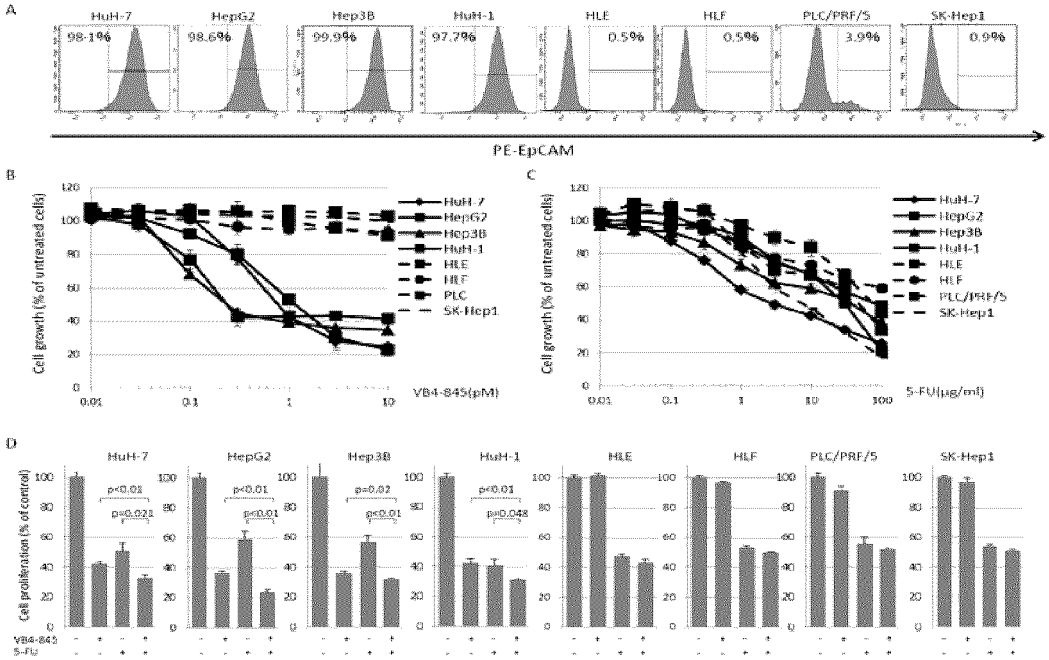

A
HuH-7
HepG2
Hep3B
HuH-1
HLE
HLF
PLC/PRF/5
SK-Hep1
98.1%
98.6%
99.9%
97.7%
0.5%
0.5%
3.9%
0.9%
PE-EpCAM
B
Cell growth (% of untreated cells)
VB4-845(pM)
C
5-FU(μg/ml)
D
Cell proliferation (% of control)
VB4-845
5-FU

FIG. 4

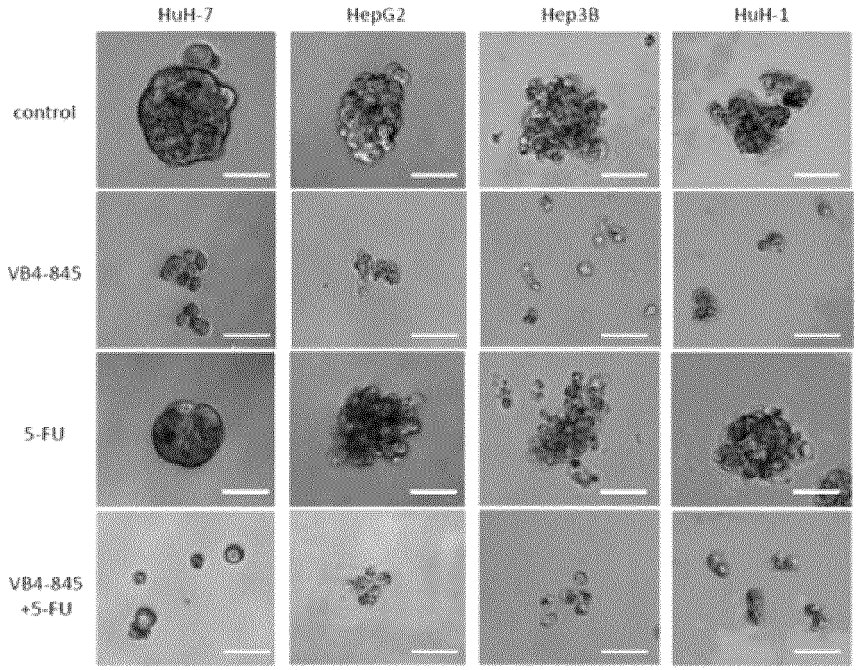


FIG. 5

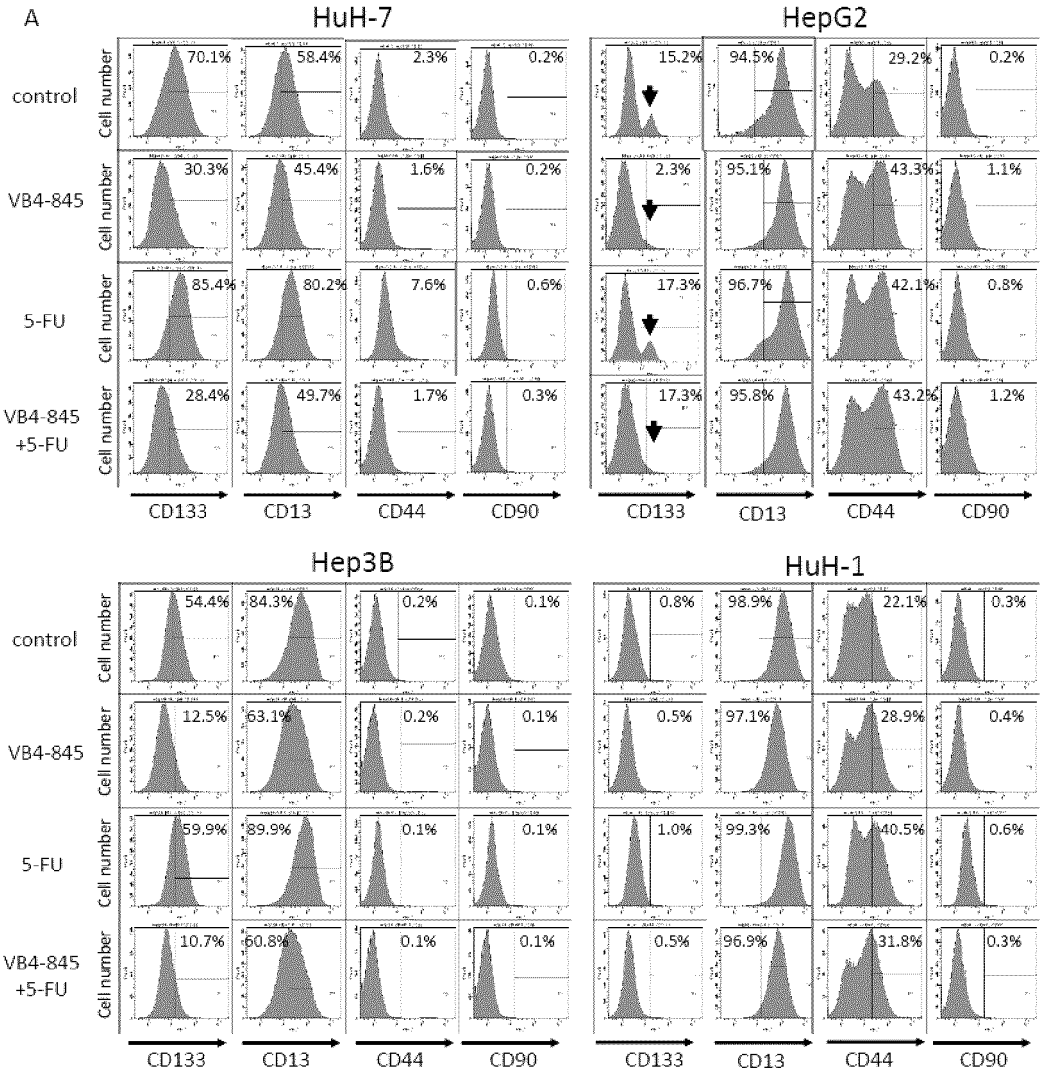

A
HuH-7
HepG2
Hep3B
HuH-1
control
VB4-845
5-FU
VB4-845 +5-FU
Cell number
CD133
CD13
CD44
CD90
70.1%
58.4%
2.3%
0.2%
15.2%
94.5%
29.2%
0.2%
30.3%
45.4%
1.6%
0.2%
2.3%
95.1%
43.3%
1.1%
85.4%
80.2%
7.6%
0.6%
17.3%
96.7%
42.1%
0.8%
28.4%
49.7%
1.7%
0.3%
17.3%
95.8%
43.2%
1.2%
54.4%
84.3%
0.2%
0.1%
0.8%
98.9%
22.1%
0.3%
12.5%
63.1%
0.2%
0.1%
0.5%
97.1%
28.9%
0.4%
59.9%
89.9%
0.1%
0.1%
1.0%
99.3%
40.5%
0.6%
10.7%
60.8%
0.1%
0.1%
0.5%
96.9%
31.8%
0.3%

FIG. 6A

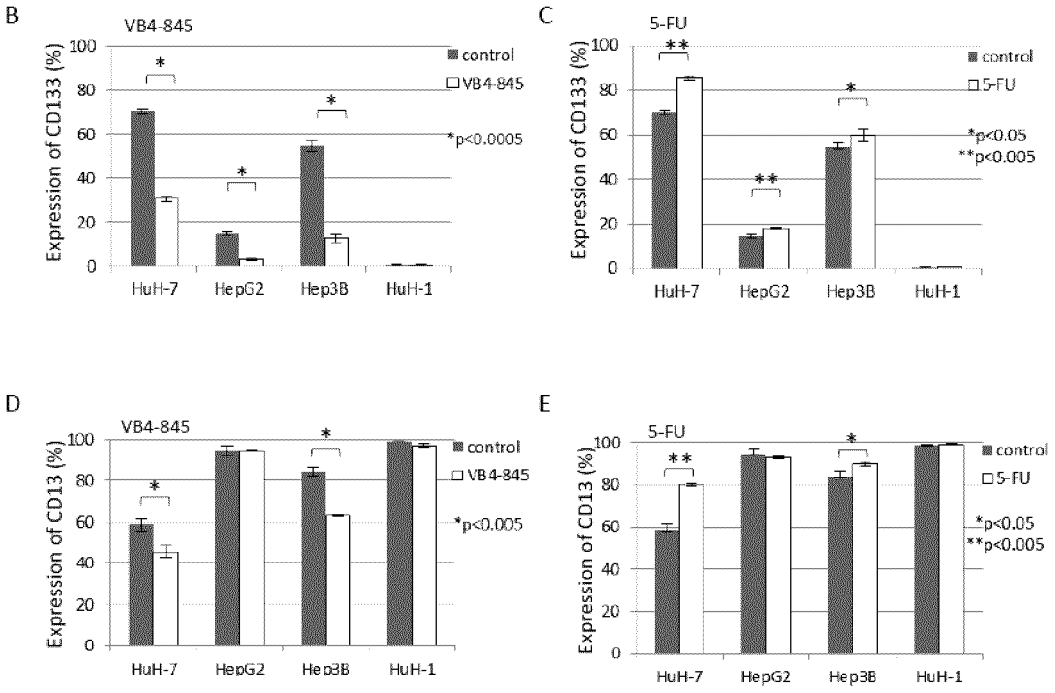

B
VB4-845
Expression of CD133 (%)
control
VB4-845
*p<0.0005
HuH-7
HepG2
Hep3B
HuH-1
C
5-FU
Expression of CD133 (%)
control
5-FU
*p<0.05
**p<0.005
HuH-7
HepG2
Hep3B
HuH-1
D
VB4-845
Expression of CD13 (%)
control
VB4-845
*p<0.005
HuH-7
HepG2
Hep3B
HuH-1
E
5-FU
Expression of CD13 (%)
control
5-FU
*p<0.05
**p<0.005
HuH-7
HepG2
Hep3B
HuH-1

FIG. 6B-E

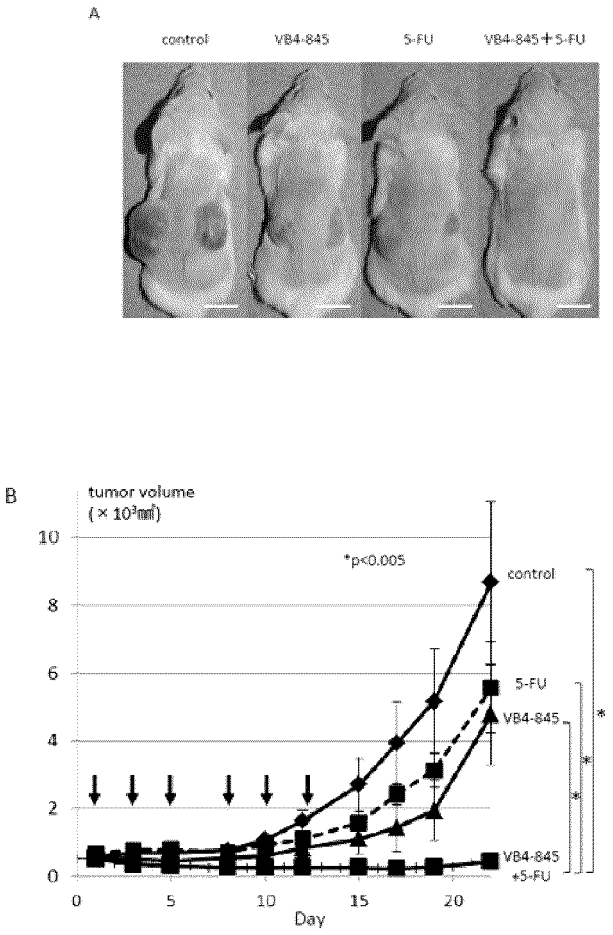

A
control
VB4-845
5-FU
VB4-845+5-FU
B
tumor volume
(×10³㎣)
10
8
6
4
2
0
*p<0.005
control
5-FU
VB4-845
VB4-845
+5-FU
*
*
*
0
5
10
15
20
Day

FIG. 7

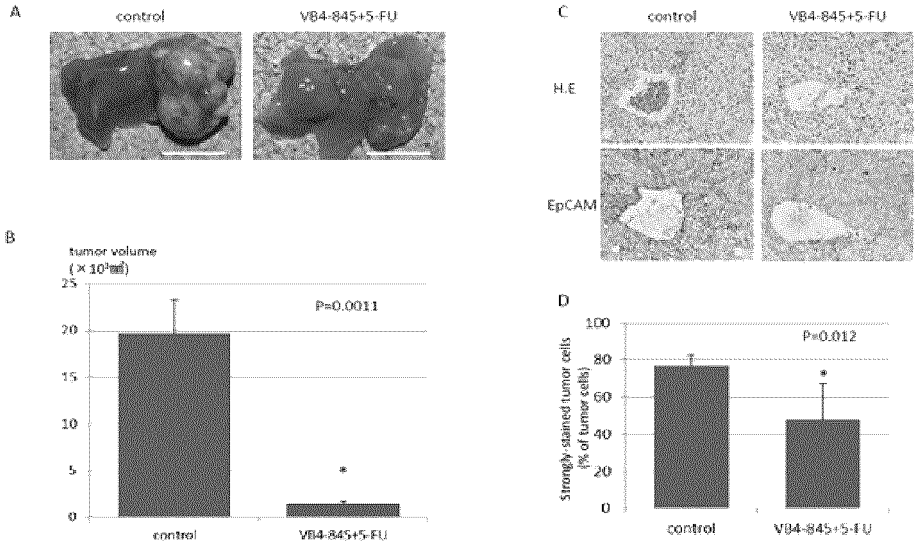

A
control
VB4-845+5-FU
B
tumor volume
(× 10³mm³)
25
20
15
10
5
0
P=0.0011
*
control
VB4-845+5-FU
C
control
VB4-845+5-FU
H.E
EpCAM
D
Strongly stained tumor cells
(% of tumor cells)
100
80
60
40
20
0
P=0.012
*
control
VB4-845+5-FU

FIG. 8

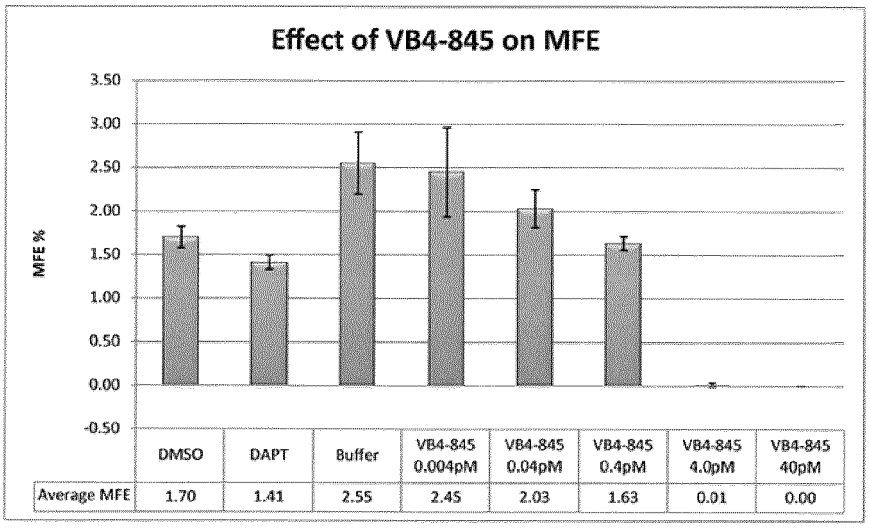

Effect of VB4-845 on MFE
MFE %
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
-0.50
DMSO
DAPT
Buffer
VB4-845 0.004pM
VB4-845 0.04pM
VB4-845 0.4pM
VB4-845 4.0pM
VB4-845 40pM
Average MFE
1.70
1.41
2.55
2.45
2.03
1.63
0.01
0.00

FIG.9

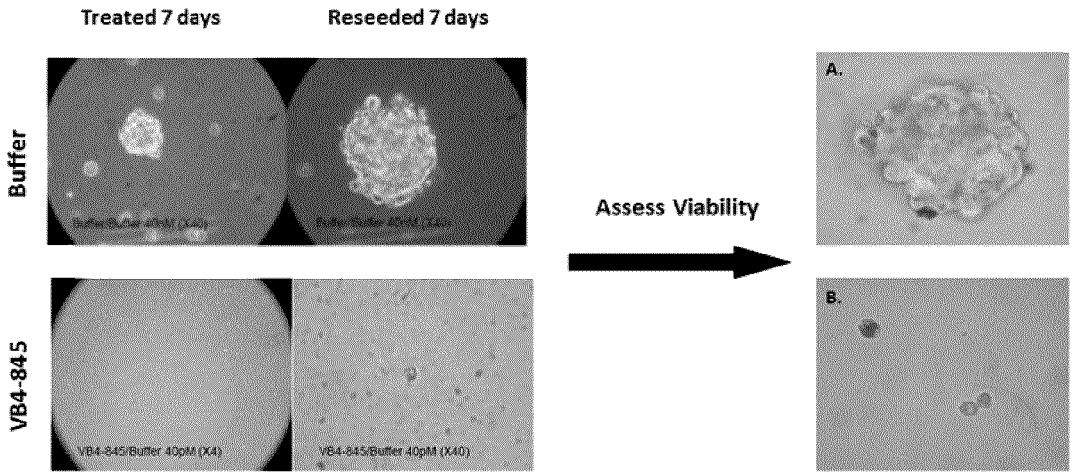

Treated 7 days
Reseeded 7 days
Buffer
VB4-845
Assess Viability
A.
B.
VB4-845/Buffer 40pM (X4)
VB4-845/Buffer 40pM (X40)

FIG. 10