FIG. 1

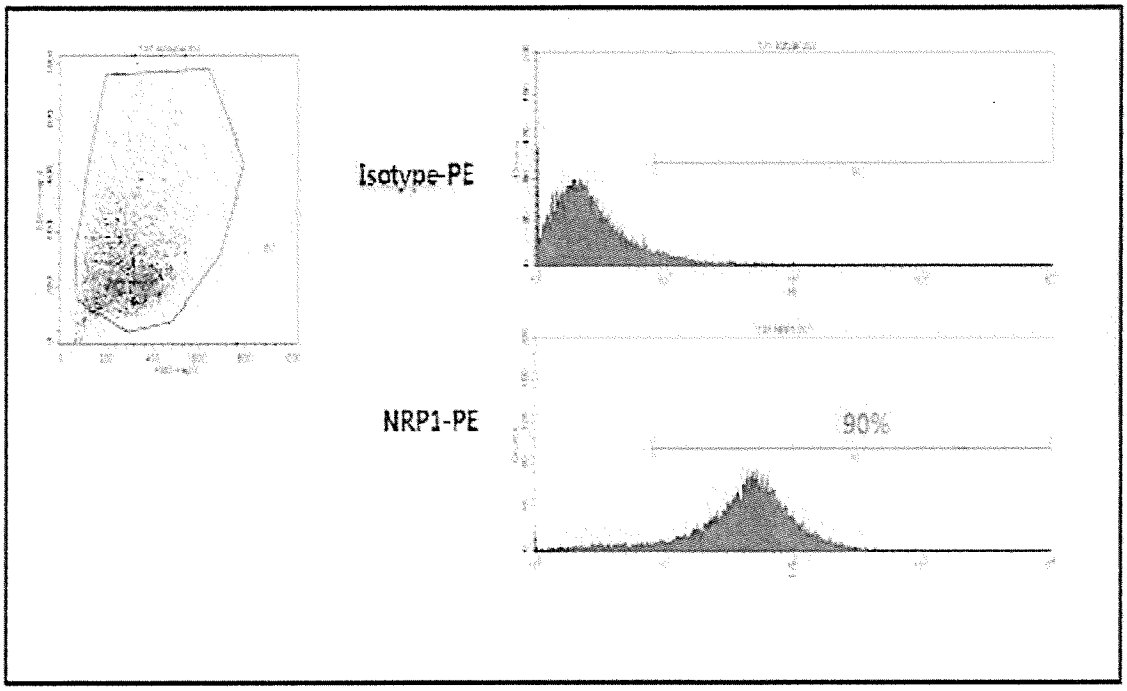

Expression of GBM patient derived cell
Isotype-PE
NRP1-PE
90%

FIG. 2

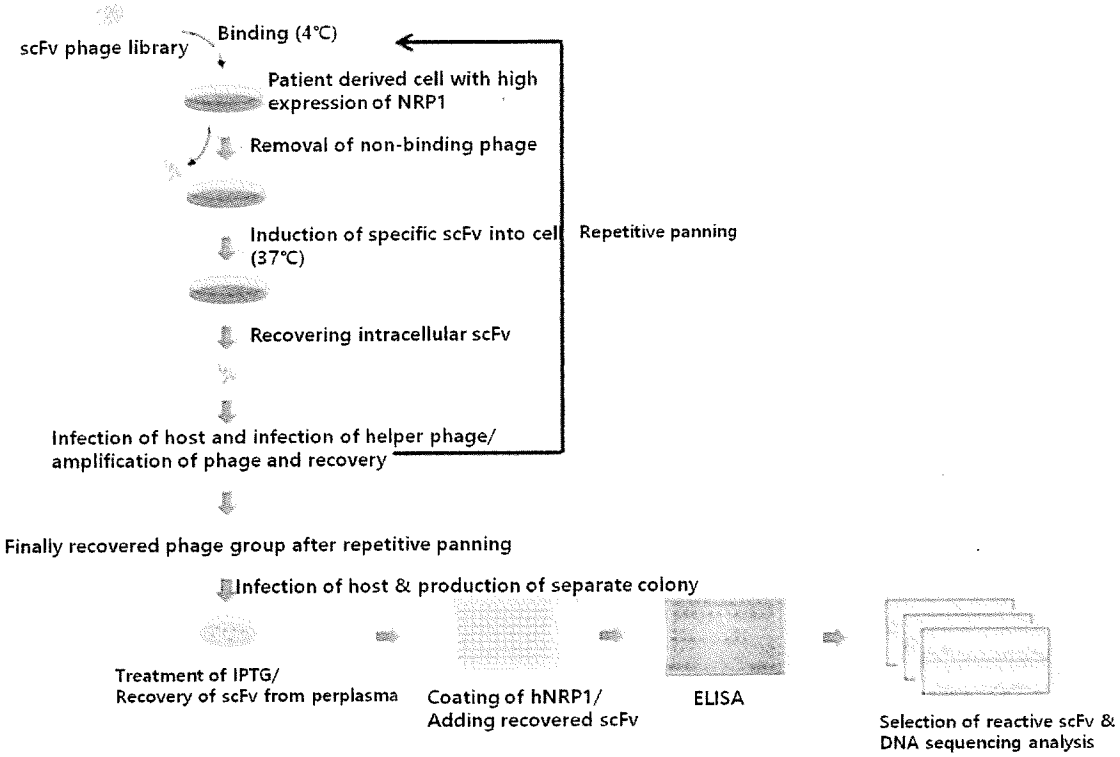

scFv phage library
Binding (4℃)
Patient derived cell with high expression of NRP1
Removal of non-binding phage
Induction of specific scFv into cell (37℃)
Recovering intracellular scFv
Infection of host and infection of helper phage/ amplification of phage and recovery
Repetitive panning
Finally recovered phage group after repetitive panning
Infection of host & production of separate colony
Treatment of IPTG/ Recovery of scFv from perplasma
Coating of hNRP1/ Adding recovered scFv
ELISA
Selection of reactive scFv & DNA sequencing analysis

FIG. 3

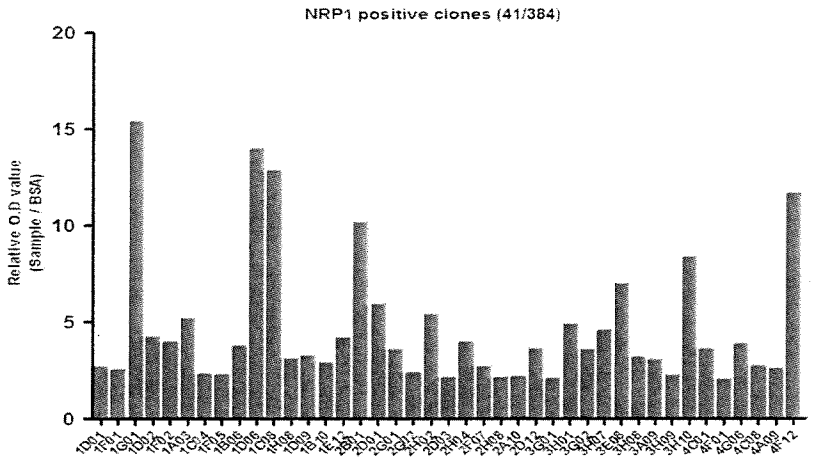

NRP1 positive clones (41/384)
Relative O.D value
(Sample / BSA)
20
15
10
5
0

FIG. 4

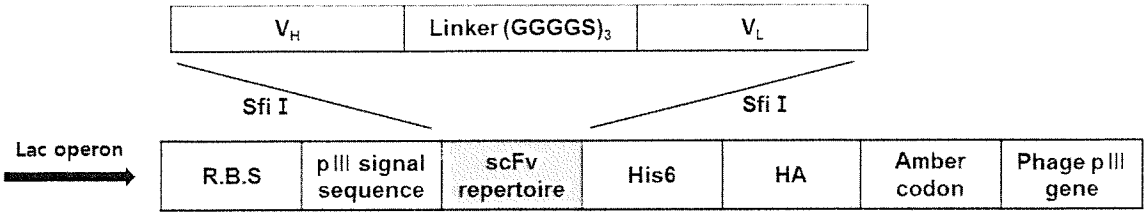

V_H
Linker $(GGGGS)_3$
V_L
Sfi I
Sfi I
Lac operon
R.B.S
pIII signal sequence
scFv repertoire
His6
HA
Amber codon
Phage pIII gene

FIG. 5

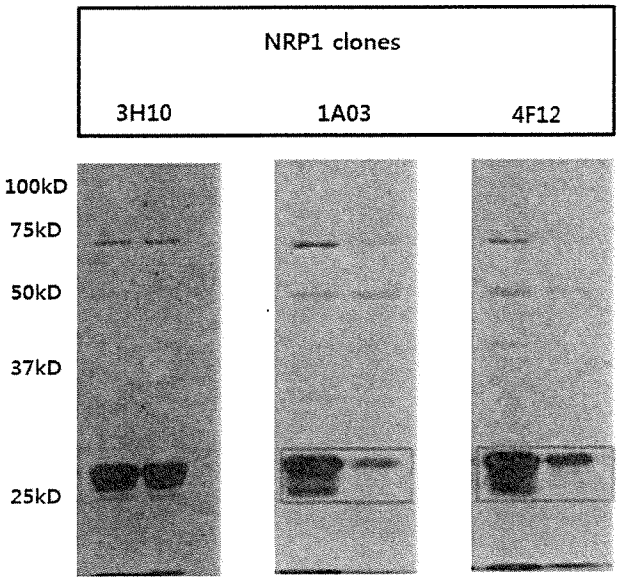

NRP1 clones
3H10
1A03
4F12
100kD
75kD
50kD
37kD
25kD

FIG. 6

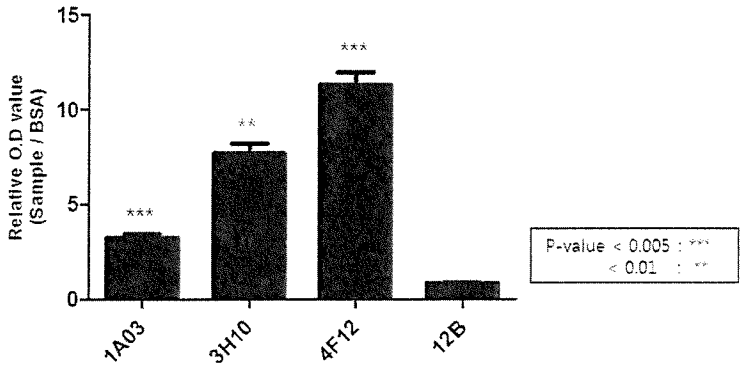

Relative O.D value
(Sample / BSA)
15
10
5
0
***
**
***
1A03
3H10
4F12
12B
P-value < 0.005 : ***
< 0.01 : **

FIG. 7

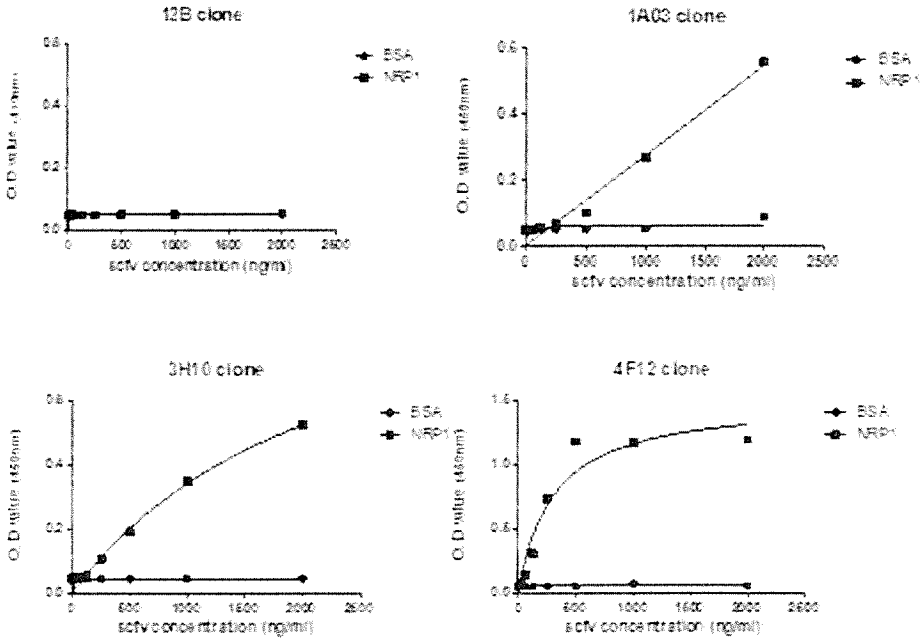

12B clone
1A03 clone
3H10 clone
4F12 clone
BSA
NRP1
scfv concentration (ng/ml)

FIG. 8

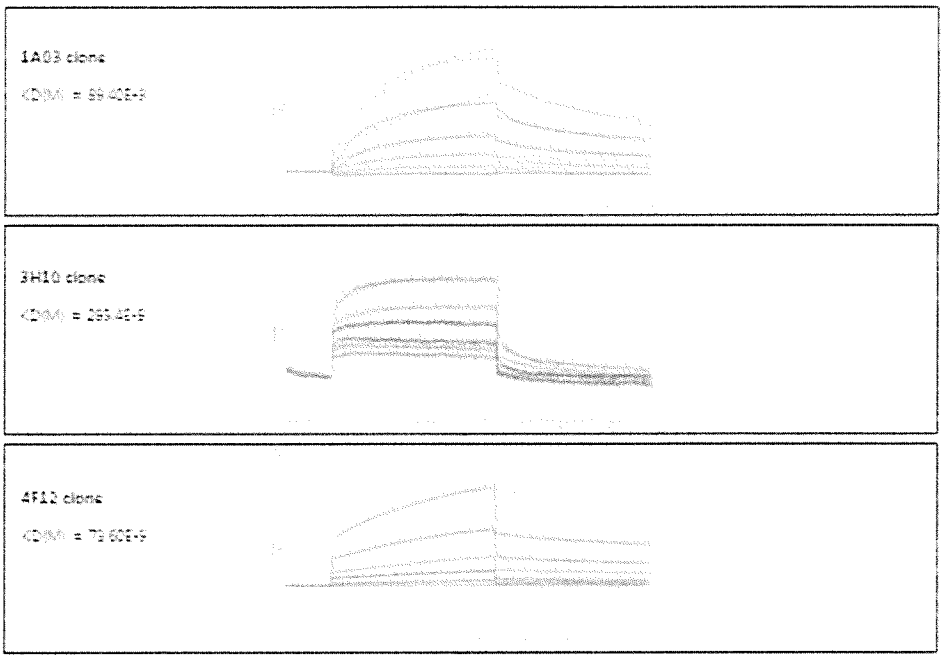

1A03 clone
3H10 clone
4F12 clone

FIG. 9

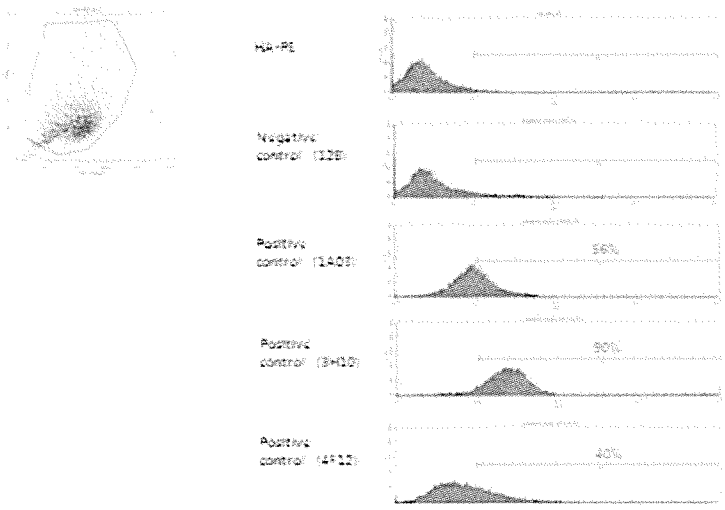

Negative control (1E8)
Positive control (1A03)
56%
Positive control (3H10)
50%
Positive control (4F12)
40%

FIG. 10a

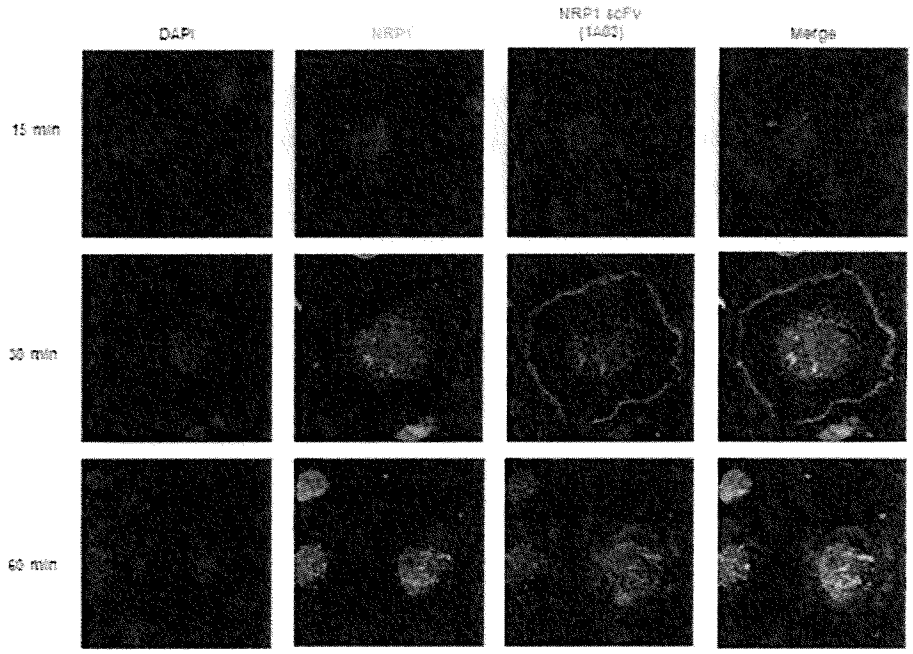


FIG. 10b

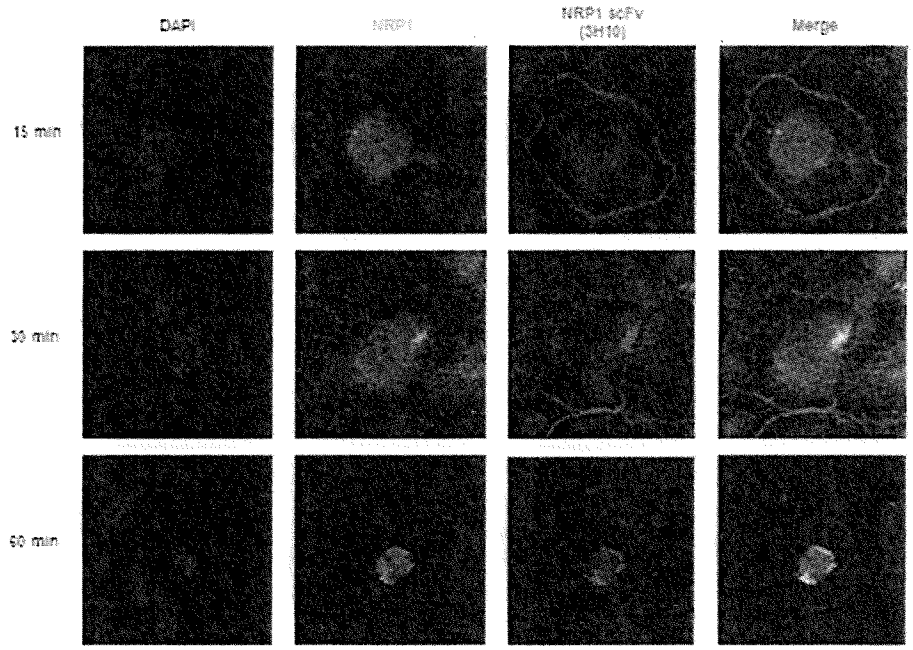


FIG. 10c

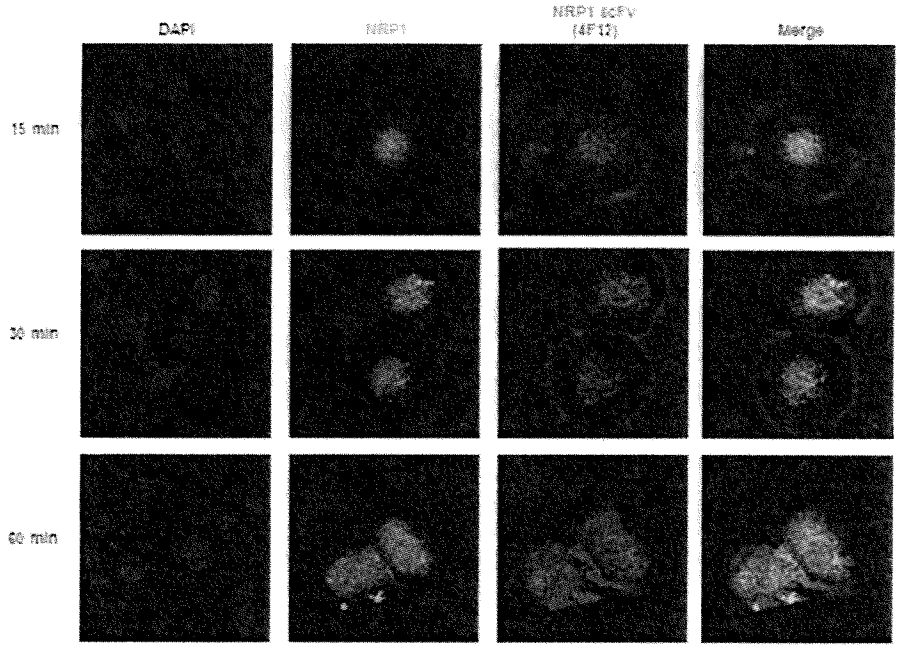


FIG. 11

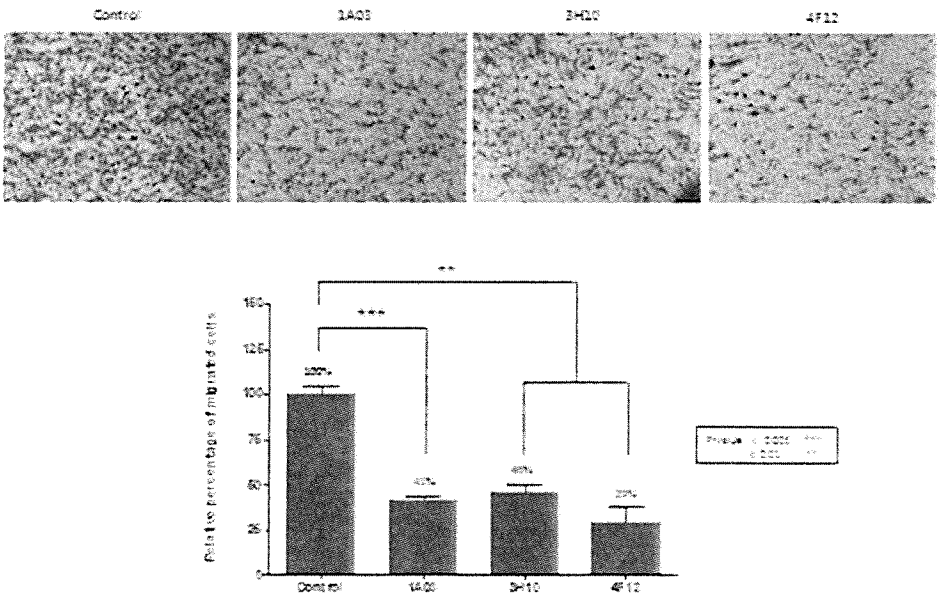


FIG. 12

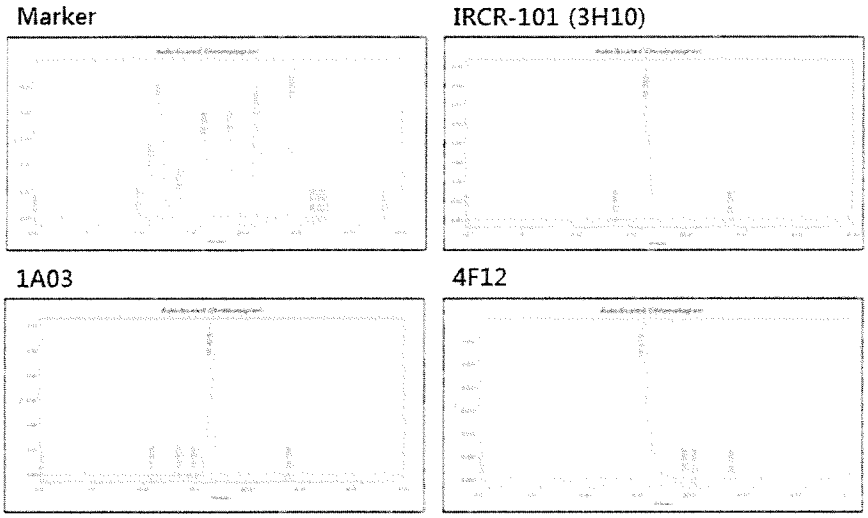
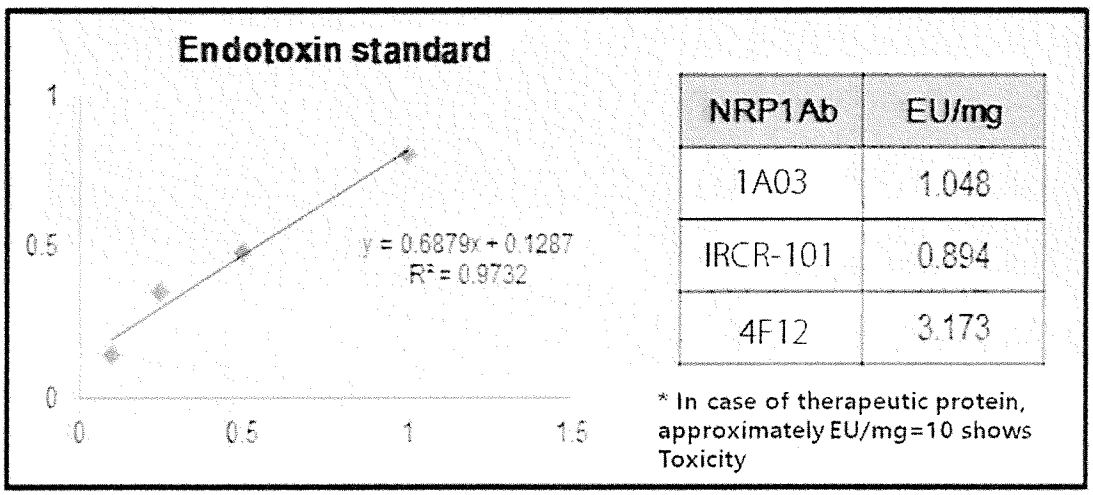


FIG. 13



NRP1Ab	EU/mg
1A03	1.048
IRCR-101	0.894
4F12	3.173

* In case of therapeutic protein, approximately EU/mg=10 shows Toxicity

FIG. 14

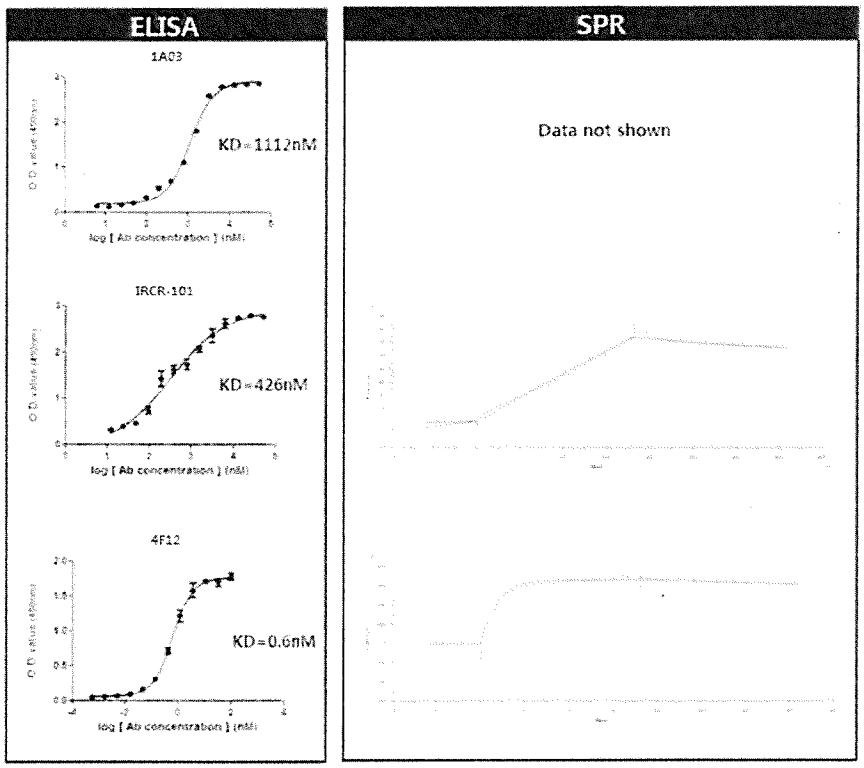

ELISA
SPR
1A03
KD=1112nM
IRCR-101
KD=426nM
4F12
KD=0.6nM
Data not shown

FIG. 15

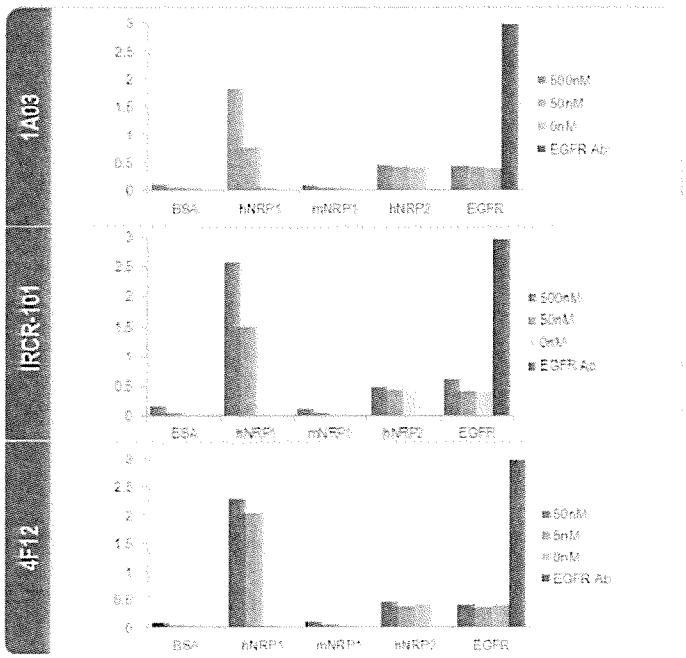

1A03
IRCR-101
4F12
BSA
hNRP1
mNRP1
hNRP2
EGFR
EGFR Ab

FIG. 16

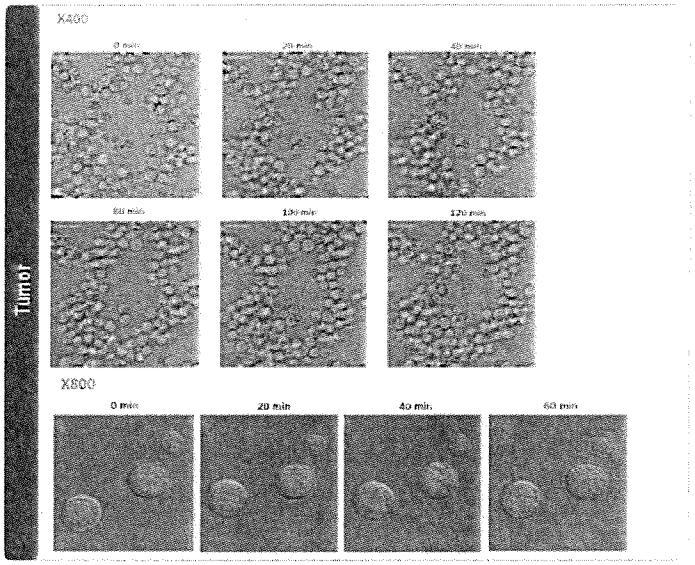

Tumor
X400
X800
0 min
20 min
40 min
60 min
120 min

FIG. 17

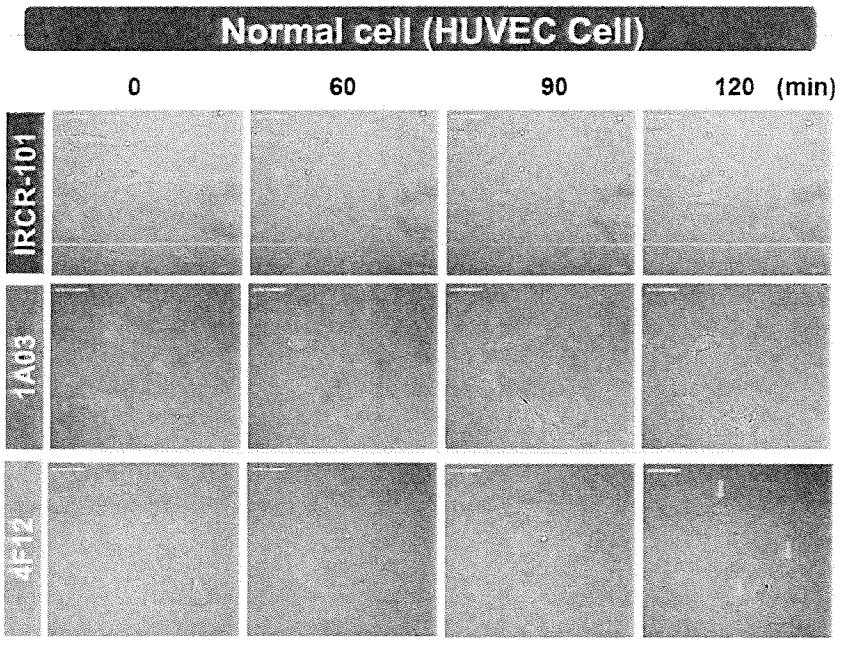

Normal cell (HUVEC Cell)
0
60
90
120 (min)
IRCR-101
1A03
4F12

FIG. 18

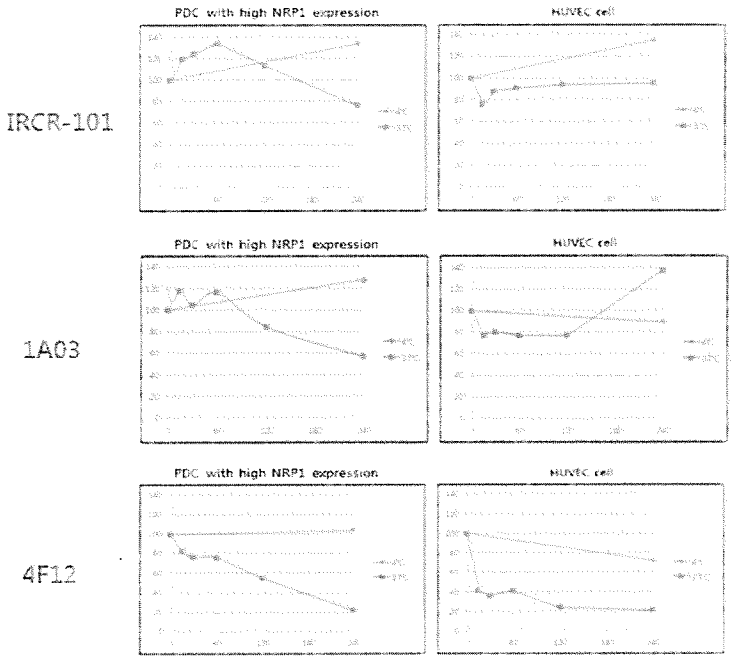

IRCR-101
1A03
4F12
PDC with high NRP1 expression
HUVEC cell

FIG. 19

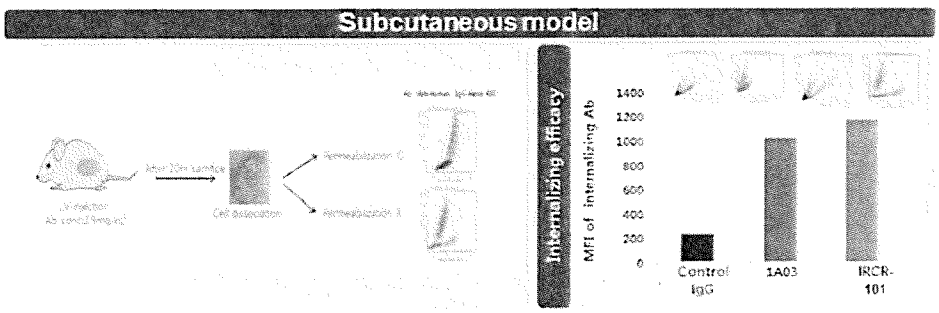

Subcutaneous model
Internalizing efficacy
MFI of internalizing Ab
1400
1200
1000
800
600
400
200
0
Control IgG
1A03
IRCR-101

FIG. 20

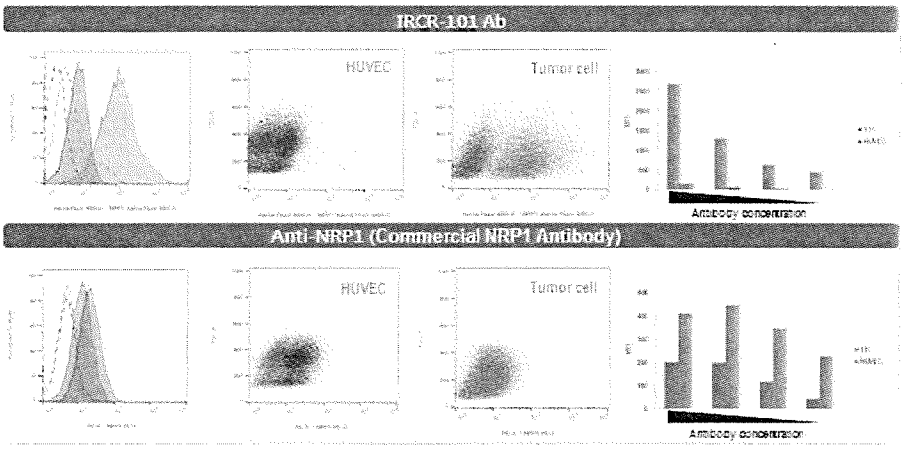

IRCR-101 Ab
HUVEC
Tumor cell
Antibody concentration
Anti-NRP1 (Commercial NRP1 Antibody)
HUVEC
Tumor cell
Antibody concentration

FIG. 21

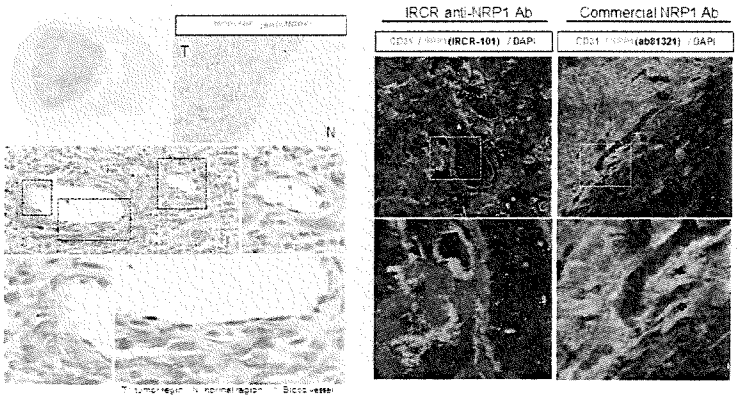

T
N
IRCR anti-NRP1 Ab
Commercial NRP1 Ab
(IRCR-101) / DAPI
(ab81321) / DAPI

FIG. 22

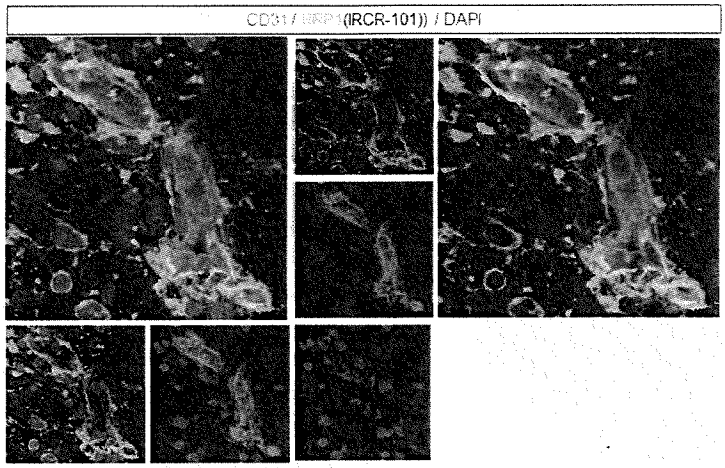

CD31 / (IRCR-101)) / DAPI

FIG. 23

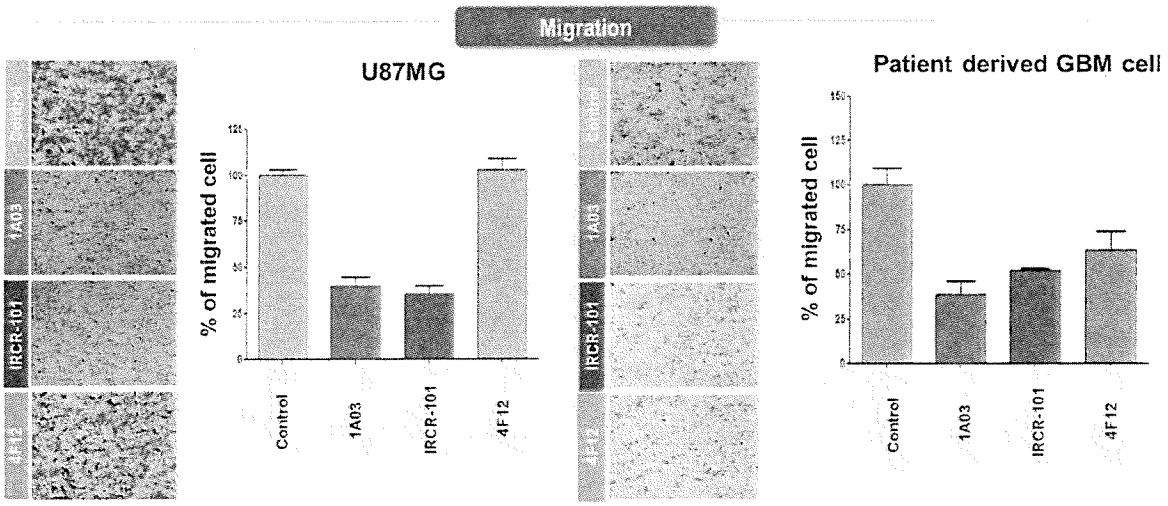

Migration
U87MG
Patient derived GBM cell
Control
1A03
IRCR-101
4F12
% of migrated cell
Control
1A03
IRCR-101
4F12
% of migrated cell
Control
1A03
IRCR-101
4F12

FIG. 24

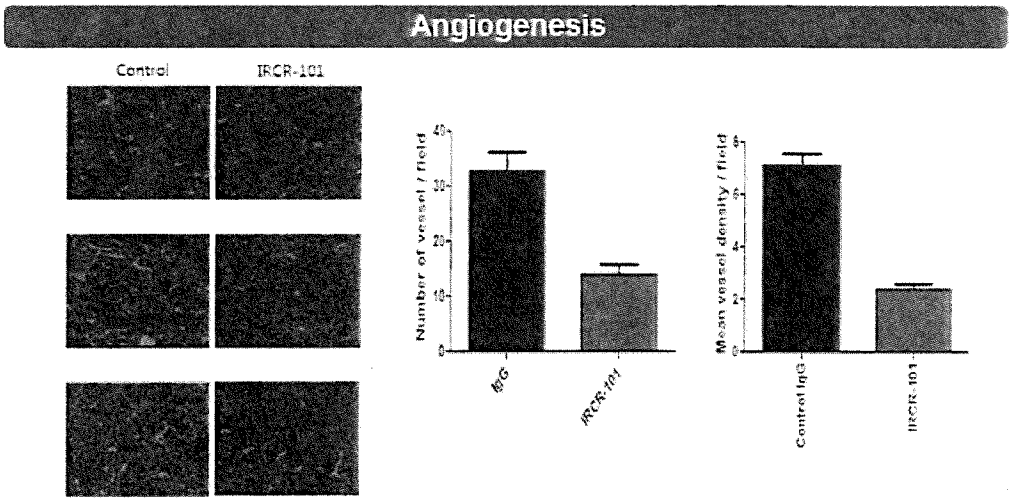

Angiogenesis
Control
IRCR-101
Number of vessel / field
IgG
IRCR-101
Mean vessel density / field
Control IgG
IRCR-101

FIG. 25

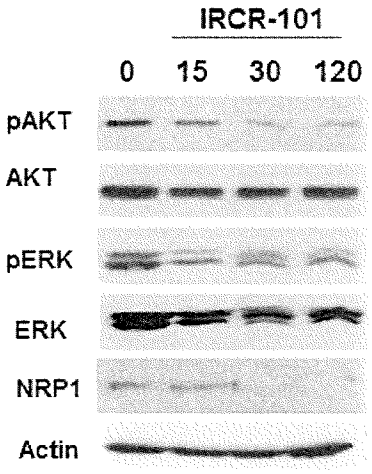

IRCR-101
0
15
30
120
pAKT
AKT
pERK
ERK
NRP1
Actin

FIG. 26

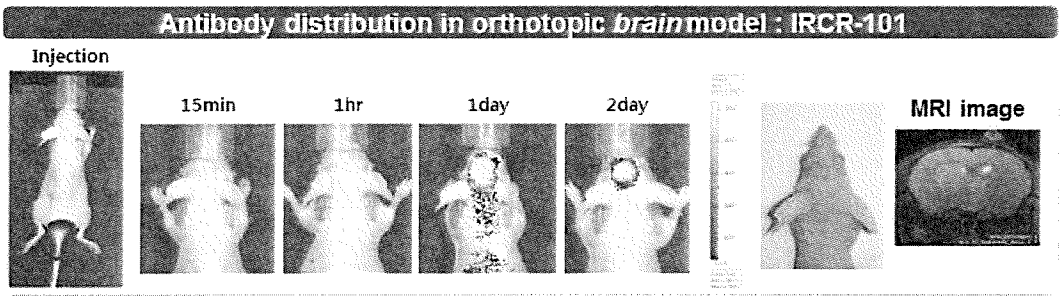


FIG. 27

	Control Human IgG	IRCR-101	Commercial NRP1 Ab (abcam, ab81321)
aorta	-	-	+ -
cerebrum	-	-	+ + + + +
cerebellum	-	-	+ + + + +
rectum	-	-	+ + + + +
colon	-	+	+ + + + +
esophagus	-	+	+ + + + +
heart	+	+	+ + +
ileum	+	+	+ + +
jejunum	+	+	+ + +
kidney	+	+	+ + + +
liver	+	+	+ +
lung	-	-	+
lymph node(mediastinum)	-	-	+ + +
pancreas	-	+ (islet)	+ + +
parathyroid gland	-	-	+ +
pituitary gland	+	-	+ + +
salivary gland	-	-	+ + +
nerve	-	-	+
muscle	-	+	+
spleen(frozen section)	+	+	+ + +
spleen	-	-	+ + +
stomach	-	+	+ + + +
tongue	-	-	+ + +
uterus	+	+	+ + + +
vagina	-	-	+ + +
thyroid	-	+	+ + + +
thymus	+	+	+ + + +
tonsil	-	-	+ + + +
adrenal gland	-	-	+ + +
prostate	-	-	+ +
urethra	-	-	+ + + +
vestibular gland	-	-	+ + + +
testis	-		+ + +
epididymis	-		+ + +

SEQUENCE LISTING

<110> SAMSUNG LIFE PUBLIC WELFARE FOUNDATION

<120> ANTIBODY BINDING TO NEUROPILIN 1 AND USE THEREOF

<130> PF-B1893-SG

<140> PCT/KR2015/013102
<141> 2015-12-03

<150> 10-2014-0171949
<151> 2014-12-03

<160> 42

<170> PatentIn version 3.5

<210> 1
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRH1 of 4F12

<400> 1

Gly Phe Thr Phe Ser Gly Tyr Ala
1 5

<210> 2
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRH2 of 4F12

<400> 2

Ile Ser Pro Gly Ser Gly Ser Thr
1 5

<210> 3
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRH3 of 4F12

<400> 3

Ala Lys Arg Lys Thr Arg Phe Asp Tyr
1 5

<210> 4
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRL1 of 4F12

<400> 4

Ser Ser Asn Ile Gly Asn Asn Ser
1 5

<210> 5
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRL2 of 4F12

<400> 5

Ala Asn Asn
1

<210> 6
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRL3 of 4F12

<400> 6

Ala Ala Trp Asp Ser Ser Leu Asn Gly Tyr Val
1 5 10

<210> 7
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRH1 of 1A03 or 3H10

<400> 7

Gly Phe Thr Phe Ser Ser Tyr Tyr
1 5

<210> 8
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRH2 of 1A03 or 3H10

<400> 8

Ile Ser Pro Gly Ser Ser Asn Lys
1 5

<210> 9
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRH3 of 1A03

<400> 9

Ala Arg Arg Lys Lys Ser Phe Asp Tyr
1 5

<210> 10
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRL1 of 1A03 or 3H10

<400> 10

Ser Ser Asn Ile Gly Asn Asn Asp
1 5

<210> 11
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRL2 of 1A03

<400> 11

Ser Asp Asn
1

<210> 12
<211> 11

<212> PRT
<213> Artificial Sequence

<220>
<223> CDRL3 of 1A03

<400> 12

Gly Ala Trp Val Ala Ser Leu Ser Ala Tyr Val
1 5 10

<210> 13
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRH3 of 3H10

<400> 13

Ala Arg Arg Lys Tyr Met Phe Asp Tyr
1 5

<210> 14
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRL2 of 3H10

<400> 14

Ser Asp Ser
1

<210> 15
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> CDRL3 of 3H10

<400> 15

Ala Ser Trp Asp Ser Ser Leu Ser Gly Tyr Val
1 5 10

<210> 16
<211> 116
<212> PRT
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<220>
<223> VH of 4F12

<400> 16

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gly Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ser Pro Gly Ser Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Arg Lys Thr Arg Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 17
<211> 110
<212> PRT
<213> Artificial Sequence

<220>
<223> VL of 4F12

<400> 17

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Arg
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
20 25 30

Ser Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

```
Ile Tyr Ala Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65                  70                  75                  80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Ser Ser Leu
                85                  90                  95

Asn Gly Tyr Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105                 110
```

<210> 18
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> VH of 1A03

<400> 18

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Ala Ile Ser Pro Gly Ser Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Gln Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Arg Lys Lys Ser Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110

Thr Val Ser Ser
        115
```

<210> 19
<211> 110
<212> PRT
<213> Artificial Sequence

<220>
<223> VL of 1A03

<400> 19

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Pro Ser Ser Asn Ile Gly Asn Asn
20 25 30

Asp Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Ser Asp Asn Asn Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Gly Ala Trp Val Ala Ser Leu
85 90 95

Ser Ala Tyr Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 20
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> VH of 3H10

<400> 20

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

```
Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Ala Ile Ser Pro Gly Ser Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Arg Lys Tyr Met Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110

Thr Val Ser Ser
        115
```

<210> 21
<211> 110
<212> PRT
<213> Artificial Sequence

<220>
<223> VL of 3H10

<400> 21

```
Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1               5                   10                  15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Asn Asn
            20                  25                  30

Asp Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45

Ile Tyr Ser Asp Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65                  70                  75                  80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ser Trp Asp Ser Ser Leu
                85                  90                  95
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Ser Gly Tyr Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
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120

ccagggaagg ggctggagtg ggtctcaggg atctctcctg gtagtggtag tacatattac
180

gctgattctg taaaaggtcg gttcaccatc tccagagaca attccaagaa cacgctgtat
240

ctgcaaatga acagcctgag agccgaggac acggccgtgt attactgtgc gaaacgtaag
300

actaggttcg actactgggg ccagggtaca ctggtcaccg tgagctca
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<210> 23
<211> 330
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120

ccaggaacgg cccccaaact cctcatctat gctaataata agcggccaag cggggtccct
180

gaccgattct ctggctccaa gtctggcacc tcagcctccc tggccatcag tgggctccgg
240

tccgaggatg aggctgatta ttactgtgct gcttgggatt ctagcctgaa tggttatgtc
300

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<210> 24
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<212> DNA
<213> Artificial Sequence

<220>
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120

ccagggaagg ggctggagtg ggtctcagcg atctctcctg gtagtagtaa taaatattac
180

gctgattctg tacaaggtcg gttcaccatc tccagagaca attccaagaa cacgctgtat
240

ctgcaaatga acagcctgag agccgaggac acggccgtgt attactgtgc gagaaggaag
300

aagtcgttcg actactgggg ccagggtaca ctggtcaccg tgagctca
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<210> 25
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120

ccaggaacgg ctcccaaact cctcatctat tctgataata atcggccaag cggggtccct
180

gaccgattct ctggctccaa gtctggcacc tcagcctccc tggccatcag tgggctccgg
240

tccgaggatg aggctgatta ttactgtggt gcttgggttg ctagcctgag tgcttatgtc
300

ttcggcggag gcaccaagct gacggtccta
330

<210> 26
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ccagggaagg ggctggagtg ggtctcagcg atctctcctg gtagtagtaa taaatattac
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gctgattctg taaaaggtcg gttcaccatc tccagagaca attccaagaa cacgctgtat
240

ctgcaaatga acagcctgag agccgaggac acggccgtgt attactgtgc gagaaggaag
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tatatgttcg actactgggg ccagggtaca ctggtcaccg tgagctca
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<220>
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120

ccaggaacgg cacccaaact cctcatctat tctgatagta atcggccaag cggggtccct
180

gaccgattct ctggctccaa gtctggcacc tcagcctccc tggccatcag tgggctccgg
240

tccgaggatg aggctgatta ttactgtgct tcttgggatt ctagcctgag tggttatgtc
300

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<212> PRT
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<400> 28

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Ser Leu Arg Leu Ser Cys Ala Ala Ser
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<400> 29

Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
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Ala

<210> 30
<211> 17
<212> PRT
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<220>
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<400> 30

Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
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Gly

<210> 31
<211> 38
<212> PRT
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<220>

<223> 1A03 Hv FR3

<400> 31

Tyr Tyr Ala Asp Ser Val Gln Gly Arg Phe Thr Ile Ser Arg Asp Asn
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Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
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Thr Ala Val Tyr Tyr Cys
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<210> 32
<211> 38
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<400> 32

Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
1 5 10 15

Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
20 25 30

Thr Ala Val Tyr Tyr Cys
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<210> 33
<211> 11
<212> PRT
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<220>
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<400> 33

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
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<210> 34
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<220>

<223> 1A03 Lv FR1

<400> 34

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Pro
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<210> 35
<211> 25
<212> PRT
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<220>
<223> 3H10 Lv FR1

<400> 35

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser
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<210> 36
<211> 25
<212> PRT
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<220>
<223> 4F12 Lv FR1

<400> 36

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Arg
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Arg Val Thr Ile Ser Cys Ser Gly Ser
20 25

<210> 37
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<400> 37

Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile
1 5 10 15

Tyr

<210> 38
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> 3H10 Lv FR2

<400> 38

Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile
1 5 10 15

Tyr

<210> 39
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> 4F12 Lv FR2

<400> 39

Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile
1 5 10 15

Tyr

<210> 40
<211> 36
<212> PRT
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<220>
<223> 1A03 & 3H10 Lv FR3

<400> 40

Asn Arg Pro Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Lys Ser Gly
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Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg Ser Glu Asp Glu Ala
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Asp Tyr Tyr Cys
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<210> 41
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<212> PRT
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<220>
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<400> 41

Lys Arg Pro Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Lys Ser Gly
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Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg Ser Glu Asp Glu Ala
20 25 30

Asp Tyr Tyr Cys
35

<210> 42
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<223> 1A03, 3H10 & 4F12 Lv FR4

<400> 42

Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
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