FIG. 1

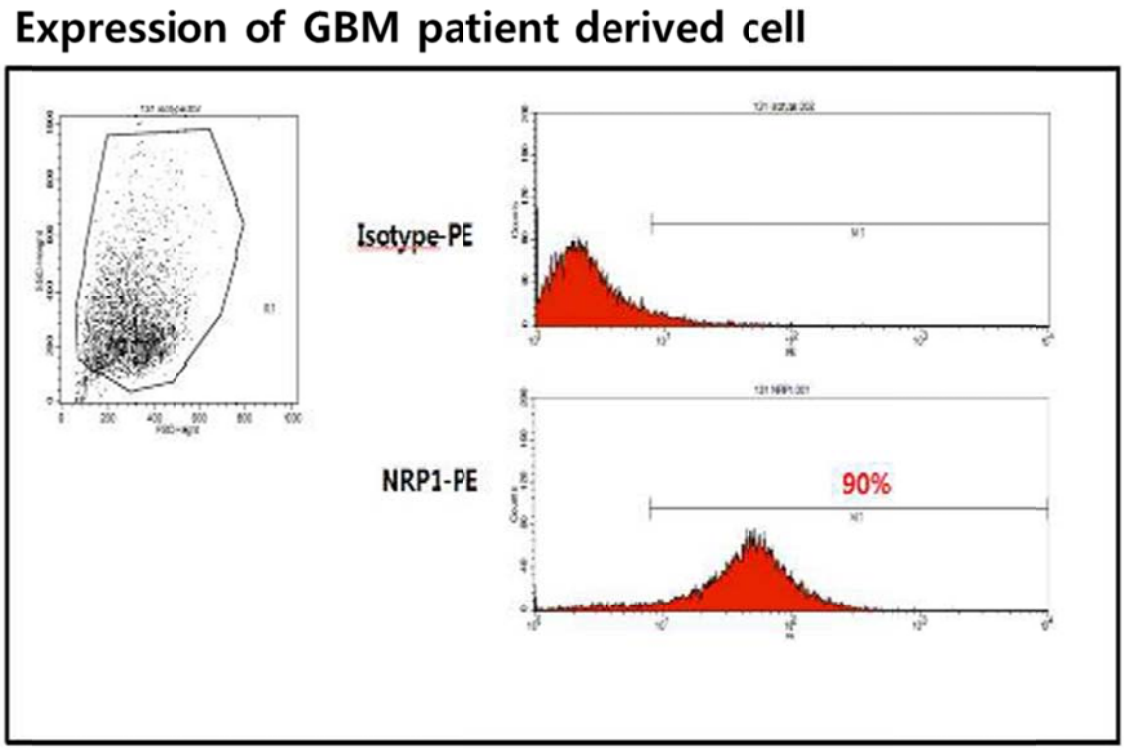

Expression of GBM patient derived cell
Isotype-PE
NRP1-PE
90%

FIG. 2

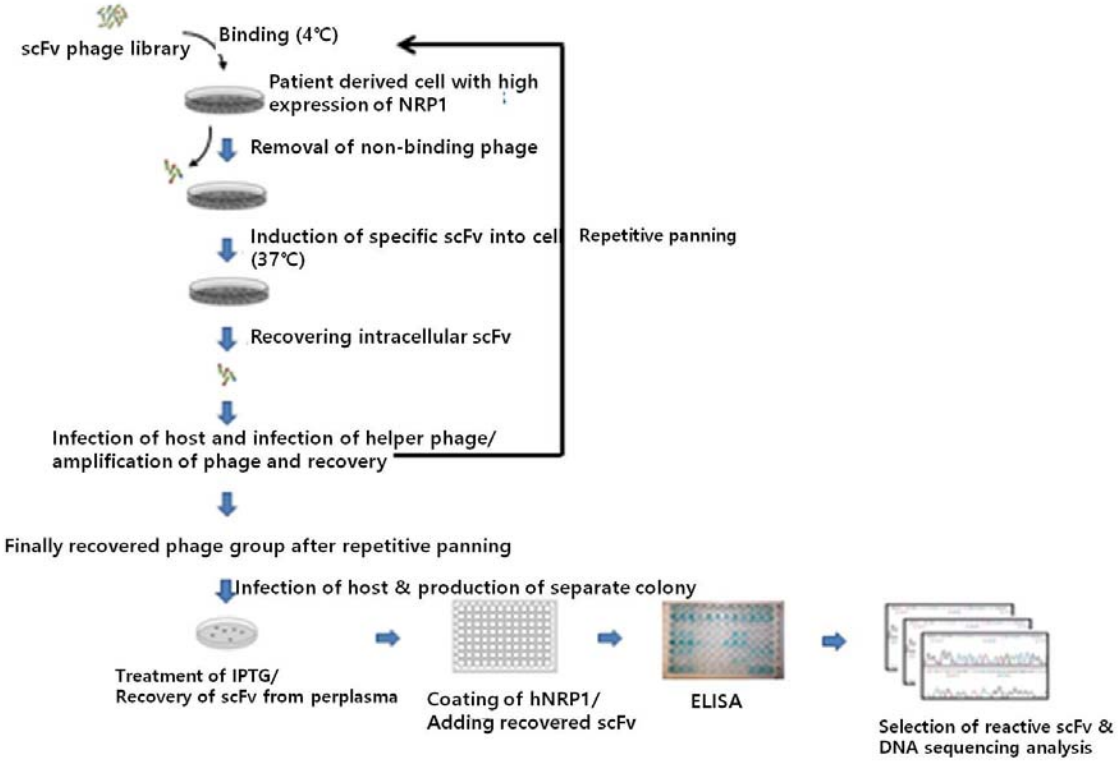

scFv phage library
Binding (4℃)
Patient derived cell with high expression of NRP1
Removal of non-binding phage
Induction of specific scFv into cell (37℃)
Repetitive panning
Recovering intracellular scFv
Infection of host and infection of helper phage/ amplification of phage and recovery
Finally recovered 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

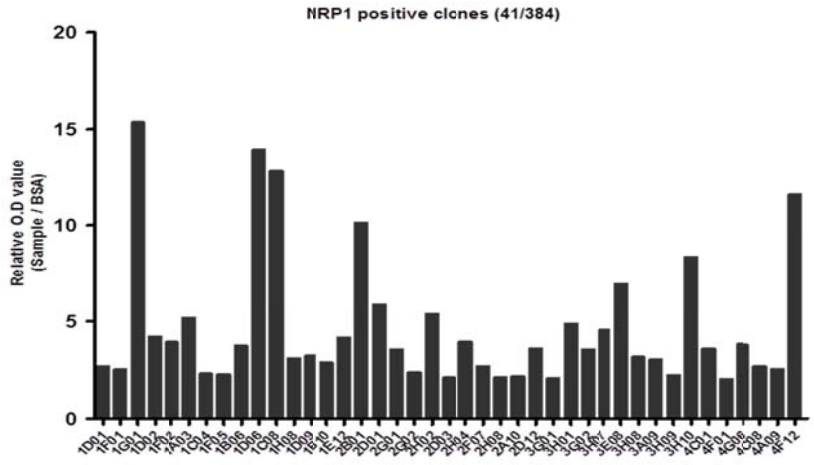

NRP1 positive clones (41/384)
Relative O.D value
(Sample / BSA)
20
15
10
5
0
1D01
1F01
1G01
1D02
1F02
1A03
1C04
1F05
1B06
1D06
1C08
1H08
1D09
1B10
1E12
2B01
2D01
2G01
2G02
2H02
2B03
2H04
2F07
2H08
2A10
2D12
3G01
3H01
3G02
3H07
3E08
3H08
3A09
3H09
3H10
4C01
4F01
4C06
4C08
4A09
4F12

FIG. 4

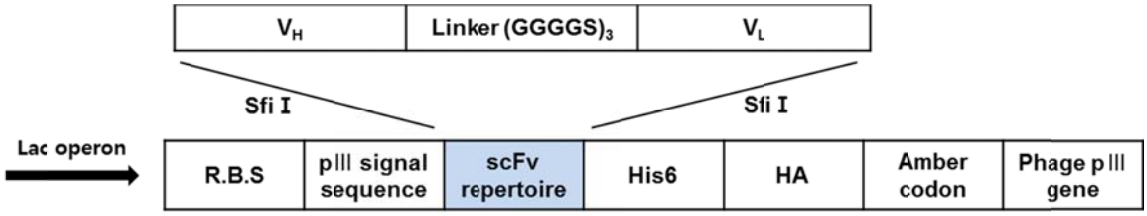

V_H
Linker $(GGGGS)_3$
V_L
Sfi I
Sfi I
Lac operon
R.B.S
pIII signal sequence
scFv repertoire
His6
HA
Amber codon
Phage pIII gene

FIG. 5

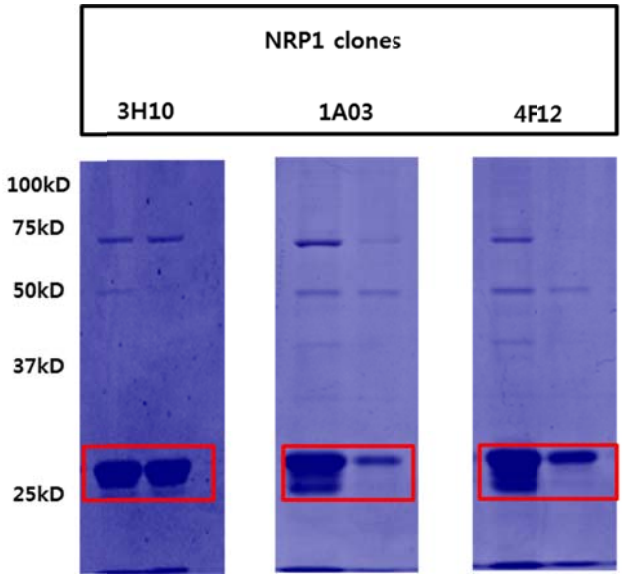

NRP1 clones
3H10
1A03
4F12
100kD
75kD
50kD
37kD
25kD

FIG. 6

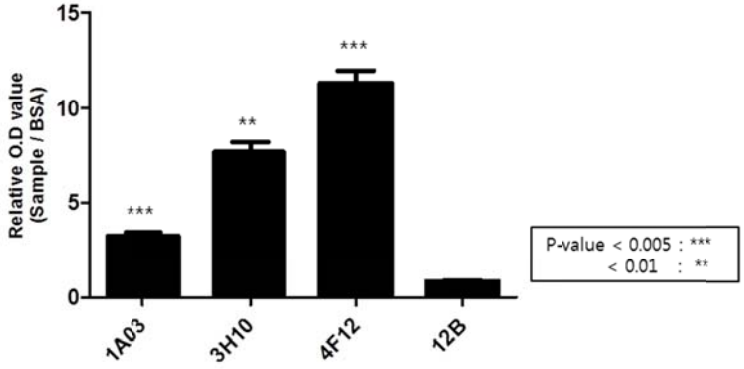

Relative O.D value
(Sample / BSA)
15
10
5
0
***
**
***
1A03
3H10
4F12
12B
P-value < 0.005 : ***
< 0.01 : **

FIG. 7

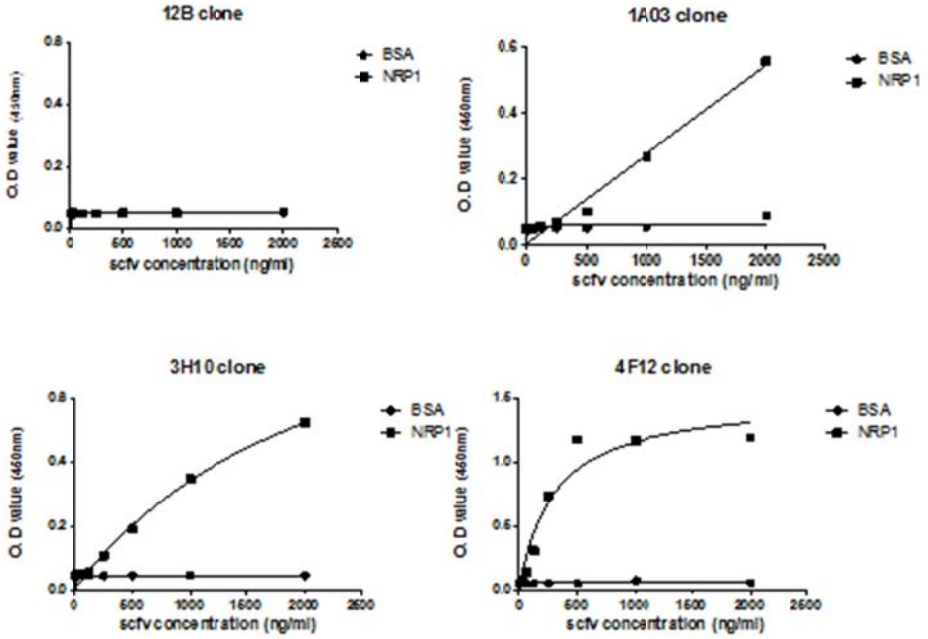

12B clone
1A03 clone
3H10 clone
4F12 clone
BSA
NRP1
O.D value (450nm)
scfv concentration (ng/ml)

FIG. 8

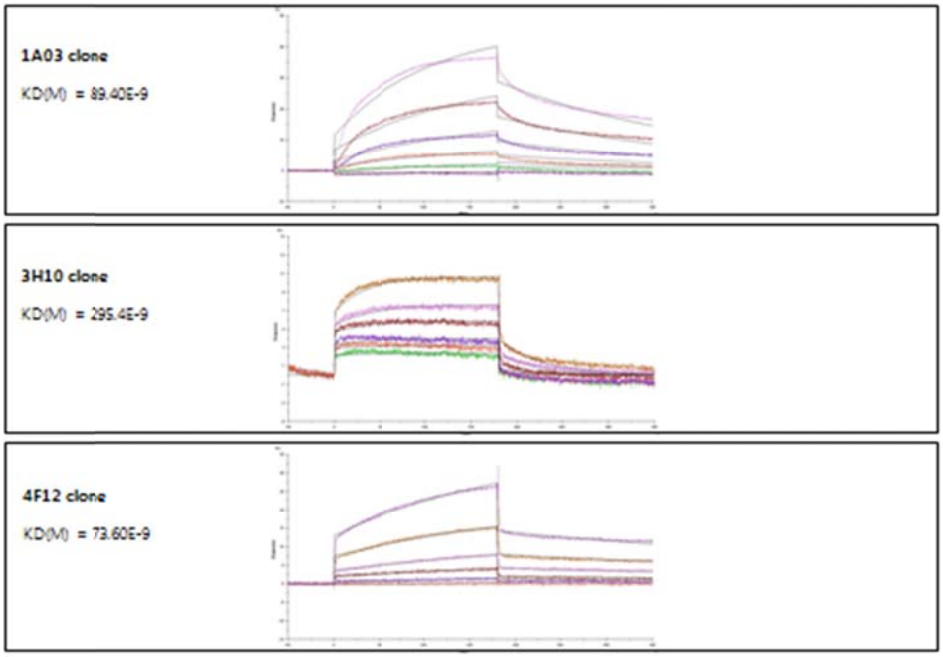

1A03 clone
KD(M) = 89.40E-9
3H10 clone
KD(M) = 295.4E-9
4F12 clone
KD(M) = 73.60E-9

FIG. 9

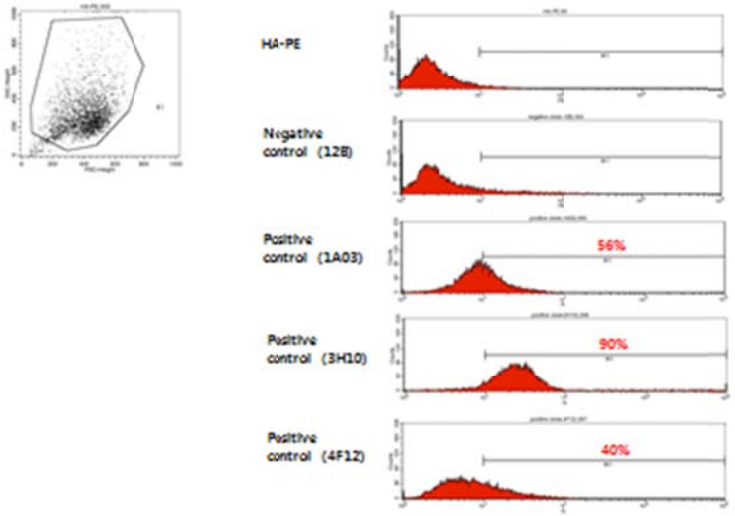

HA-PE
Negative control (12B)
Positive control (1A03)
56%
Positive control (3H10)
90%
Positive control (4F12)
40%

FIG. 10a

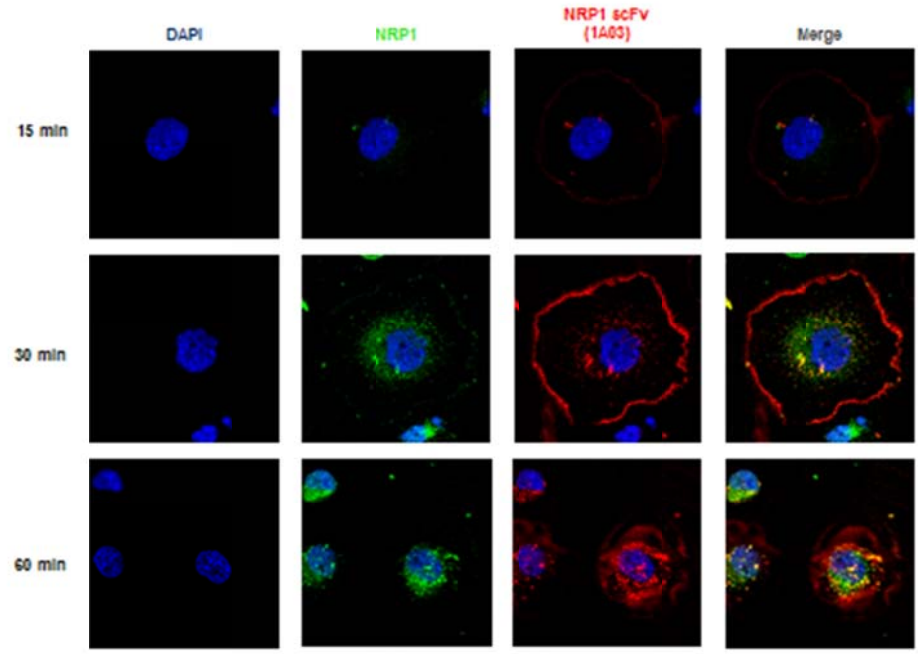


FIG. 10b

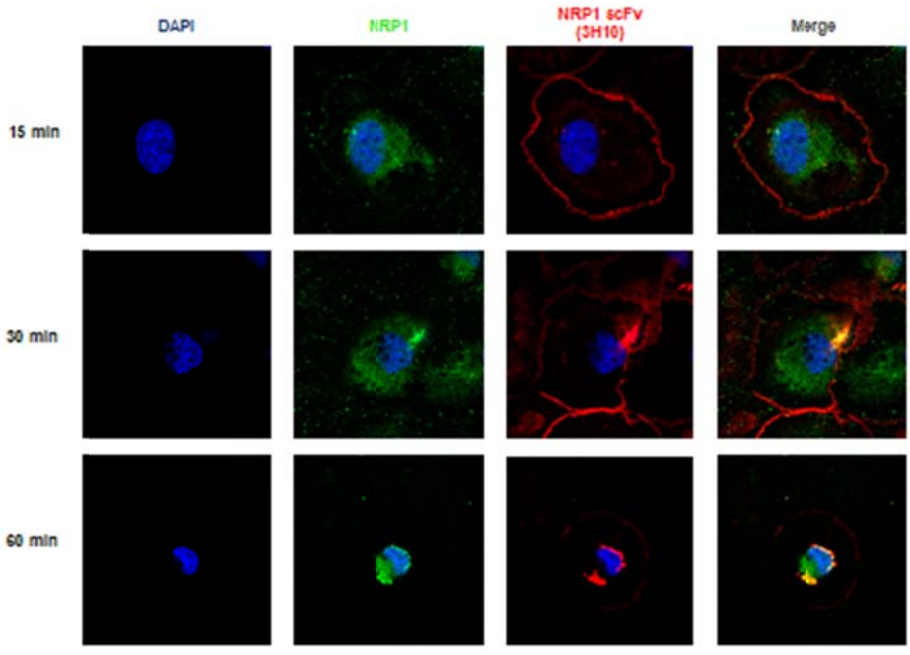


FIG. 10c

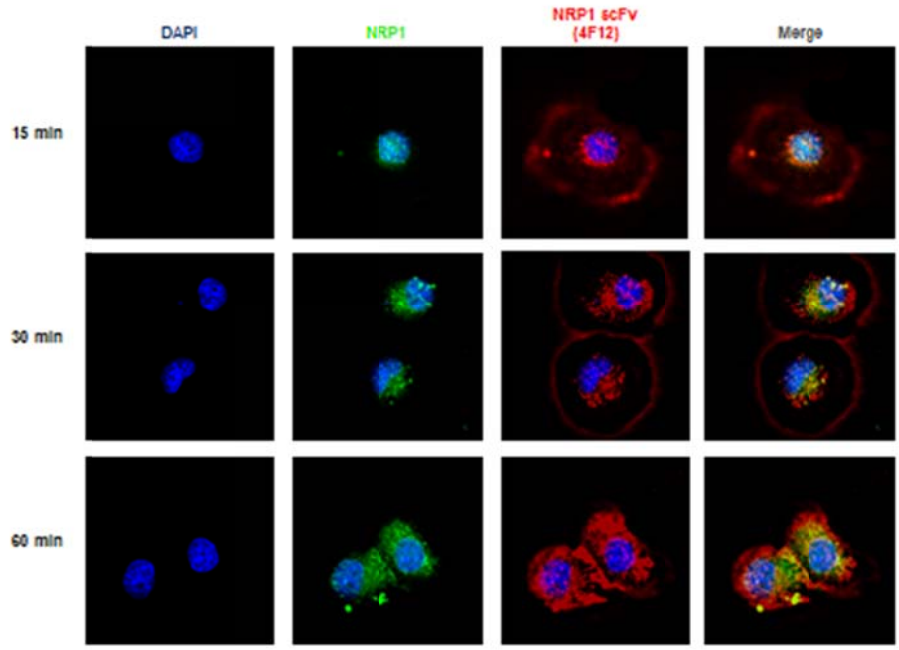


FIG. 11

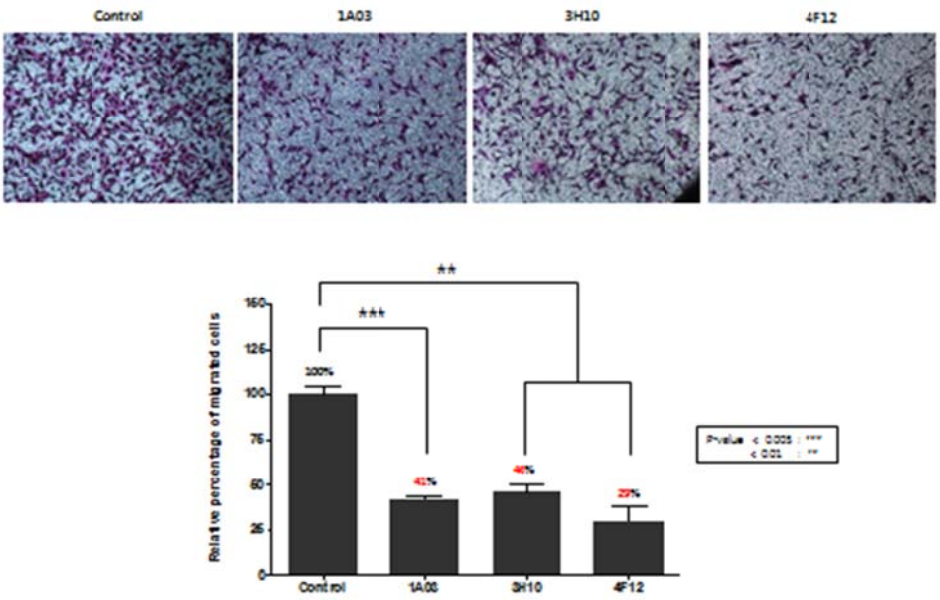


FIG. 12

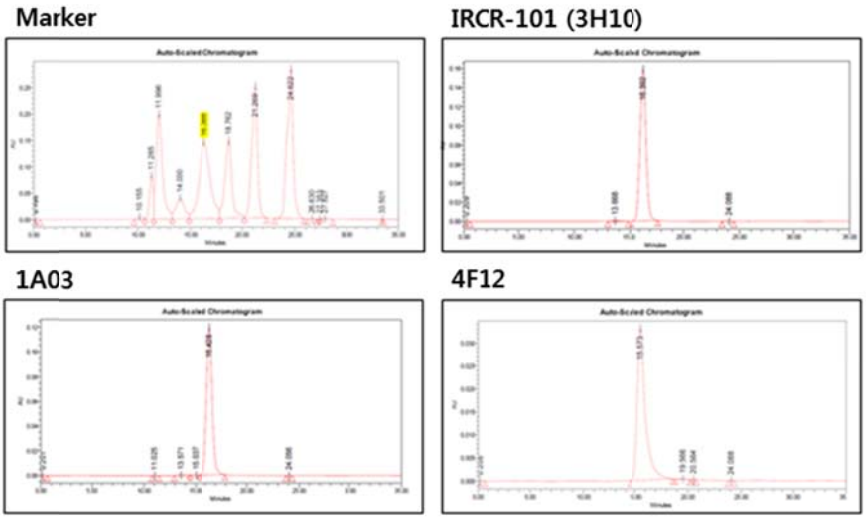

Marker
IRCR-101 (3H10)
1A03
4F12

FIG. 13

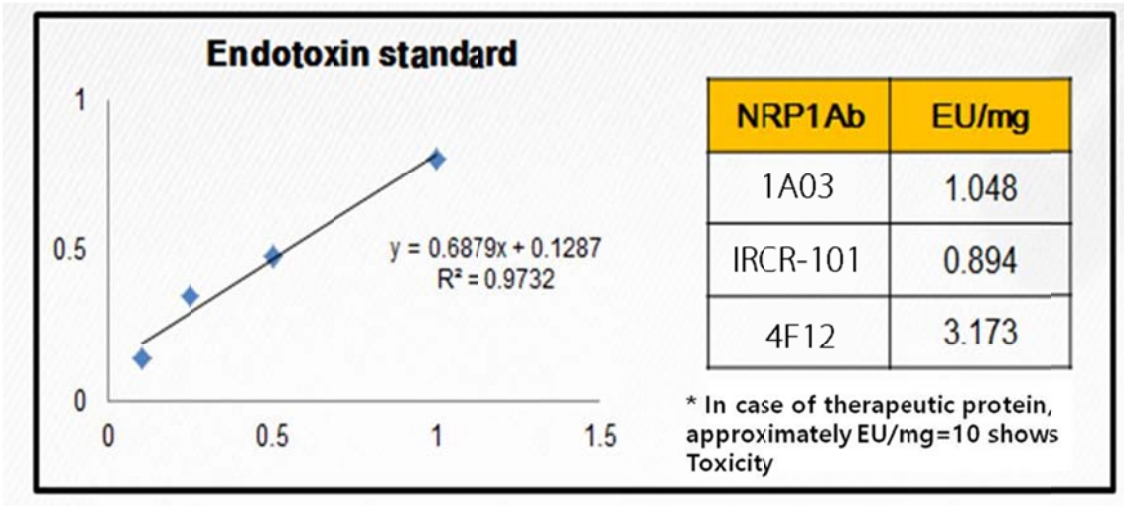

Endotoxin standard
y = 0.6879x + 0.1287
R² = 0.9732
0
0.5
1
1.5
0
0.5
1
NRP1Ab
EU/mg
1A03
1.048
IRCR-101
0.894
4F12
3.173
* In case of therapeutic protein, approximately EU/mg=10 shows Toxicity

FIG. 14

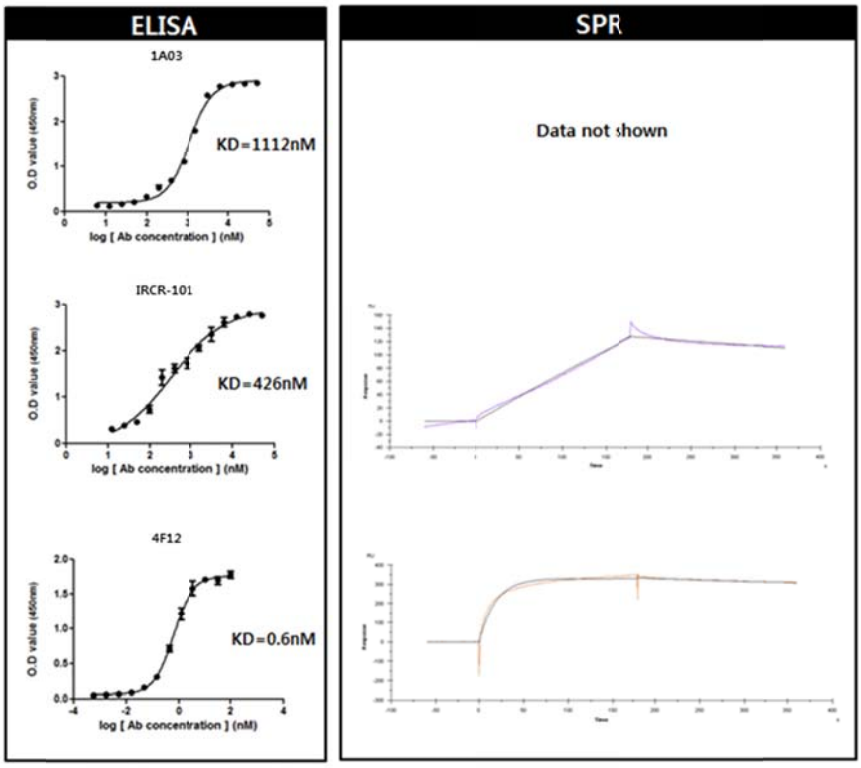

ELISA
SPR
1A03
KD=1112nM
O.D value (450nm)
log [Ab concentration] (nM)
IRCR-101
KD=426nM
O.D value (450nm)
log [Ab concentration] (nM)
4F12
KD=0.6nM
O.D value (450nm)
log [Ab concentration] (nM)
Data not shown

FIG. 15

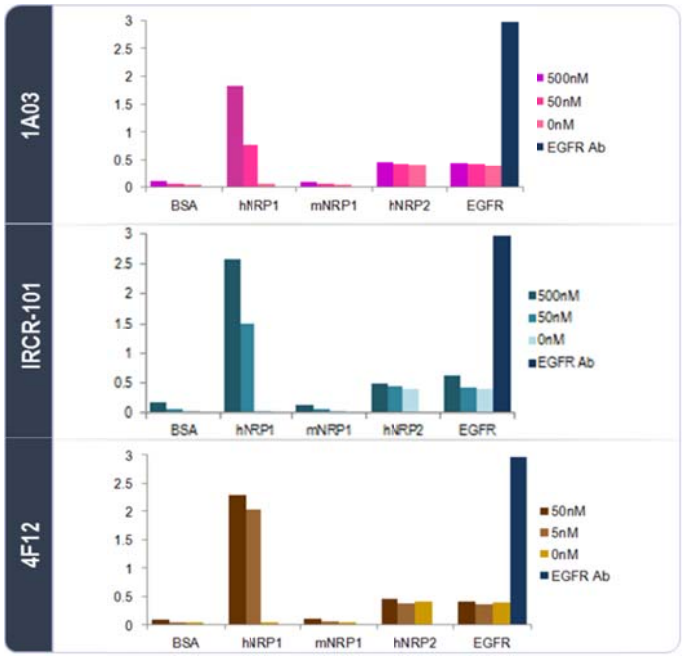

1A03
IRCR-101
4F12
BSA
hNRP1
mNRP1
hNRP2
EGFR
500nM
50nM
0nM
EGFR Ab
5nM

FIG. 16

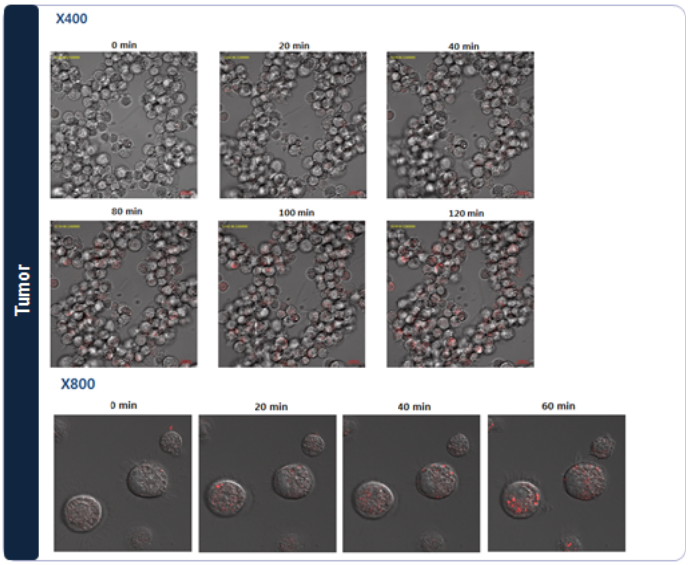

Tumor
X400
0 min
20 min
40 min
80 min
100 min
120 min
X800
0 min
20 min
40 min
60 min

FIG. 17

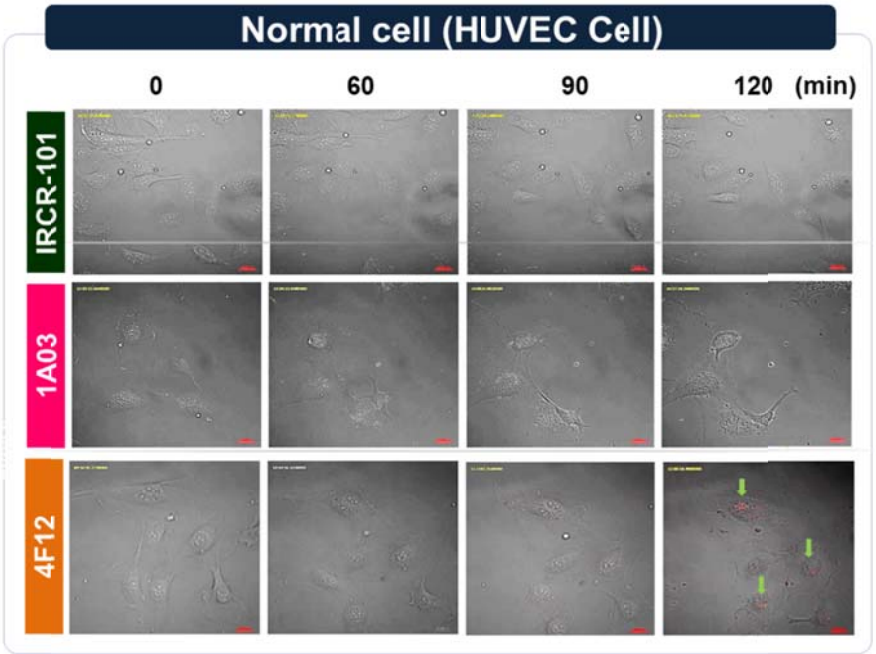

Normal cell (HUVEC Cell)
0
60
90
120 (min)
IRCR-101
1A03
4F12

FIG. 18

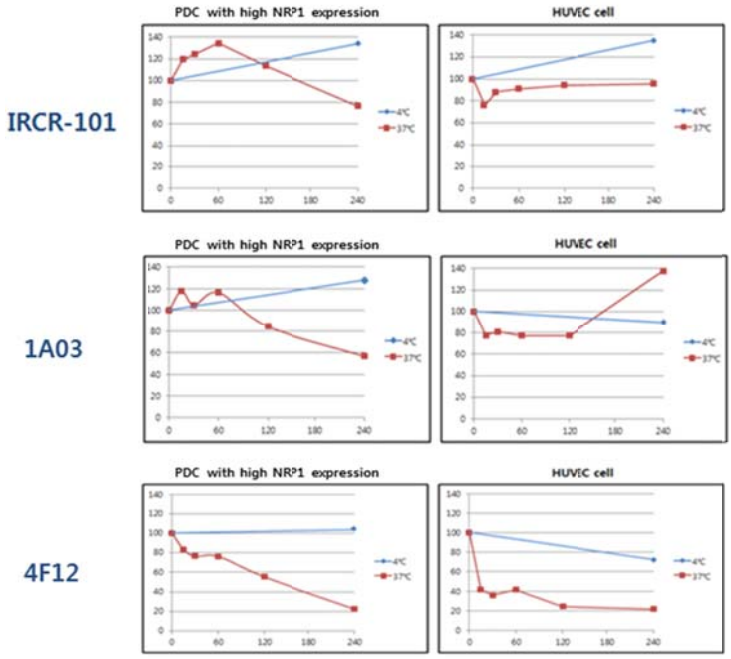

IRCR-101
1A03
4F12
PDC with high NRP1 expression
HUVEC cell
4℃
37℃

FIG. 19

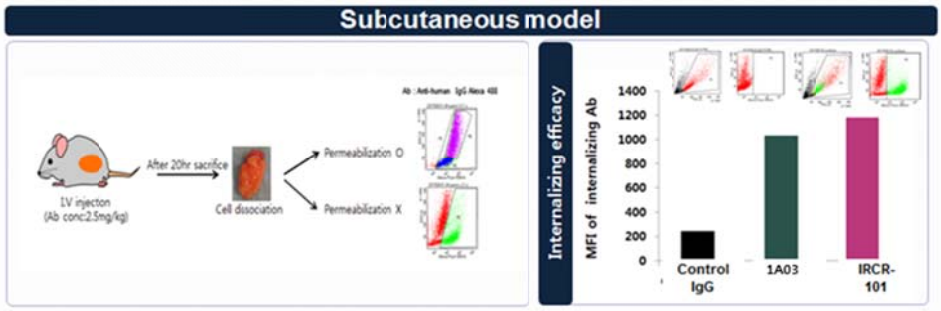

Subcutaneous model
IV injecton
(Ab conc:2.5mg/kg)
After 20hr sacrifice
Cell dissocation
Permeabilization O
Permeabilization X
Internalizing efficacy
MFI of internalizing Ab
1400
1200
1000
800
600
400
200
0
Control IgG
1A03
IRCR-101

FIG. 20

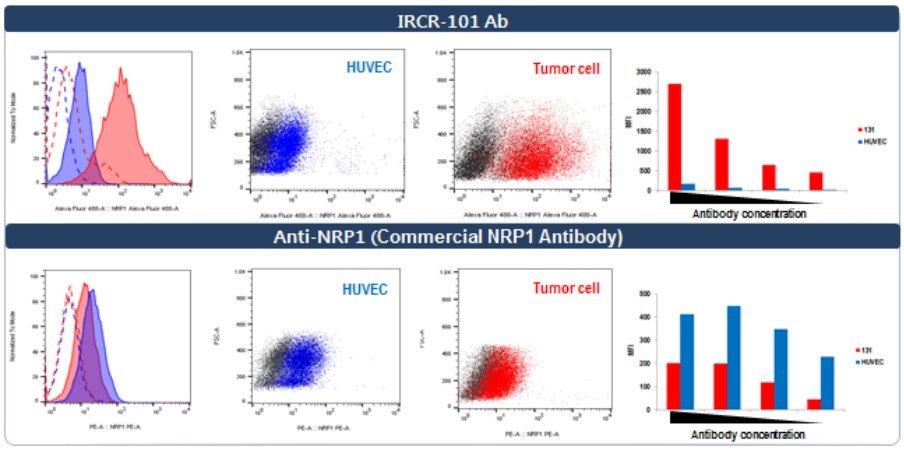

IRCR-101 Ab
HUVEC
Tumor cell
Antibody concentration
Anti-NRP1 (Commercial NRP1 Antibody)
HUVEC
Tumor cell
Antibody concentration

FIG. 21

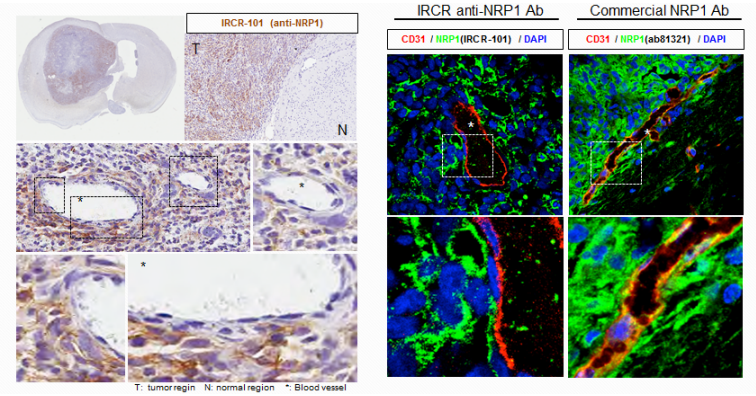

IRCR-101 (anti-NRP1)
T
N
T: tumor regin N: normal region *: Blood vessel
IRCR anti-NRP1 Ab
Commercial NRP1 Ab
CD31 / NRP1(IRCR-101) / DAPI
CD31 / NRP1(ab81321) / DAPI

FIG. 22

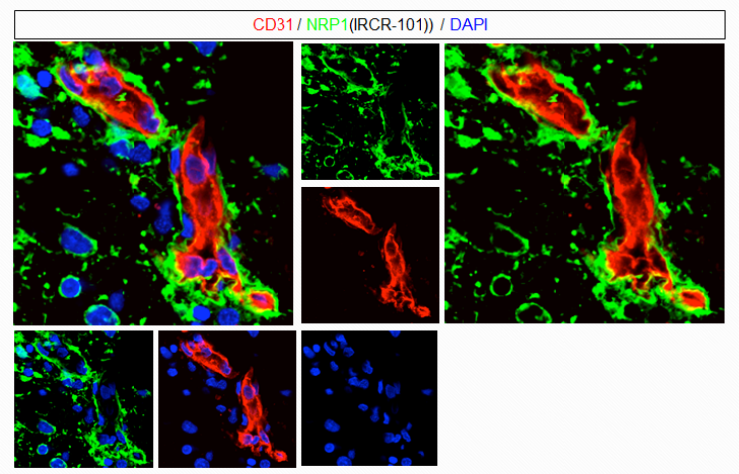

CD31 / NRP1(IRCR-101)) / DAPI

FIG. 23

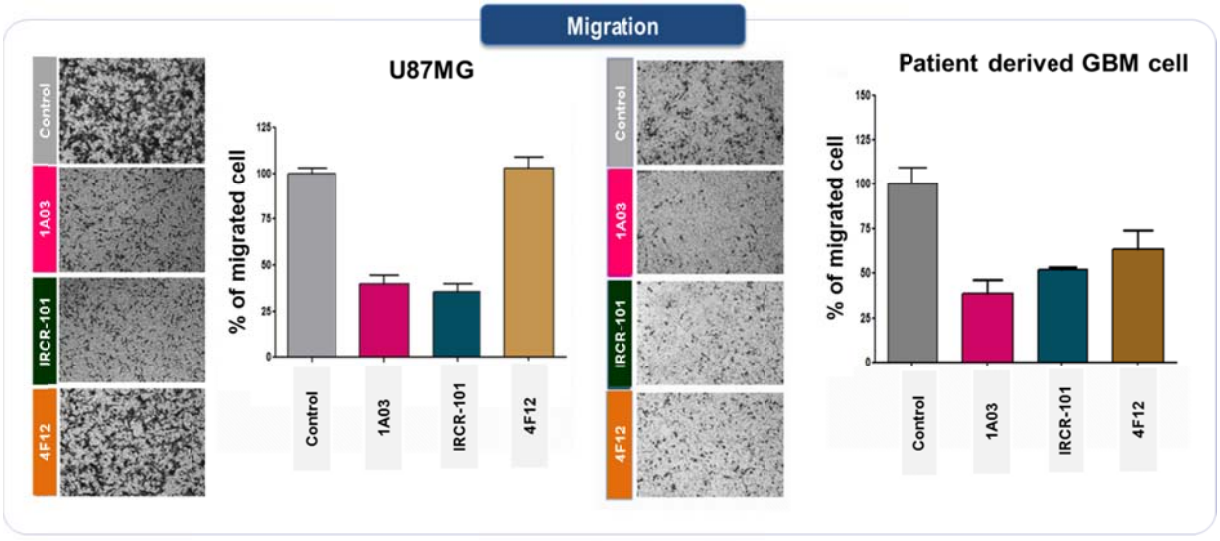

Migration
U87MG
Control
1A03
IRCR-101
4F12
% of migrated cell
Patient derived GBM cell

FIG. 24

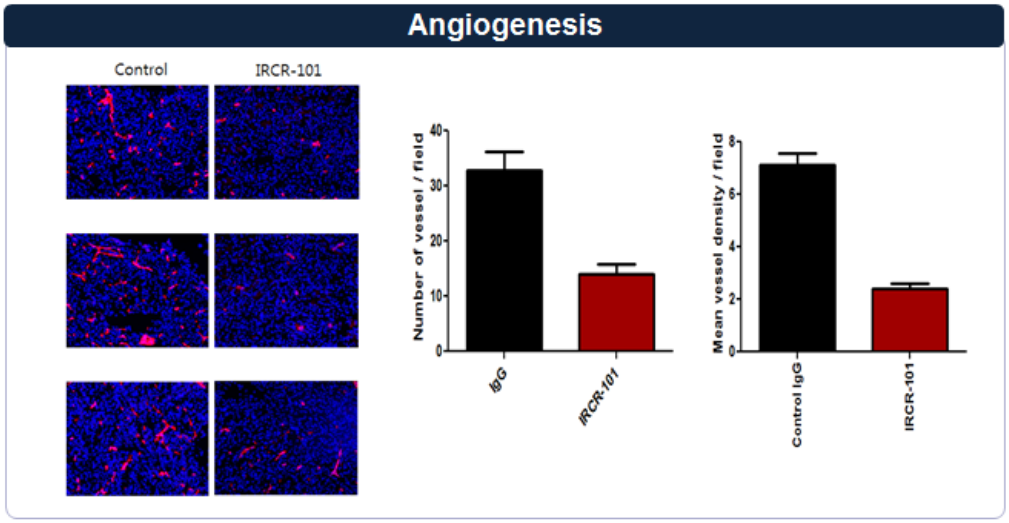

Angiogenesis
Control
IRCR-101
Number of vessel / field
40
30
20
10
0
IgG
IRCR-101
Mean vessel density / field
8
6
4
2
0
Control IgG
IRCR-101

FIG. 25

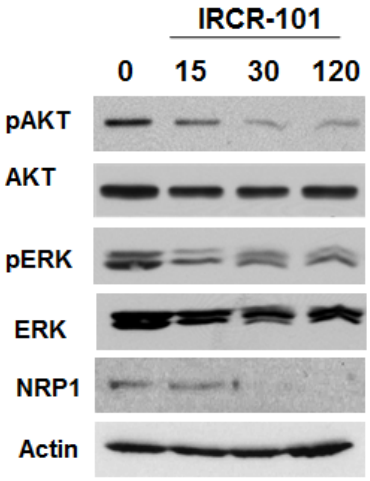

IRCR-101
0
15
30
120
pAKT
AKT
pERK
ERK
NRP1
Actin

FIG. 26

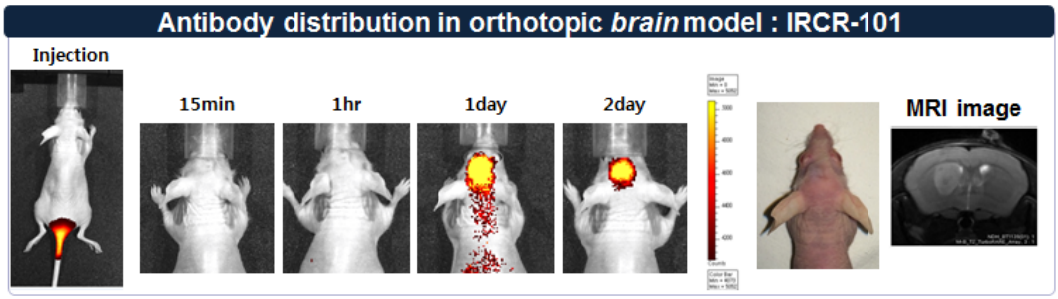


FIG. 27

	Control Human IgG	IRCR-101	Commercial NRP1 Ab (abcam ab81321)
aorta	-	-	+
cerebrum	-	-	+++++
cerebellum	-	-	+++++
cecum	-	-	+++++
colon	-	+	+++++
esophagus	-	+	+++++
heart	-	+	+++
ileum	-	+	+++
jejunum	-	+	++++
kidney	-	-	+++++
liver	-	+	+
lung	-	-	+
lymph node(median)	-	-	+++
pancreas	-	+ (islet)	+++
parathyroid gland	-	-	+
pituitary gland	-	+	+++
salivary gland	-	-	+++
nerve	-	-	+
muscle	-	-	-
spleen(cross section)	-	-	+++
spleen	-	-	+++
stomach	-	+	++++
tongue	-	-	+++
vena	-	+	++++
eye	-	+	+++
thyroid	-	+	++++
thymus	-	+	+++++
tonsil	-	++	+++++
adrenal gland	-	-	+++
prostate	-	+	+
urethra	-	+	++++
vesibular gland	-	+	+++++
testis	-	-	+++
epididymis	-	-	+++

