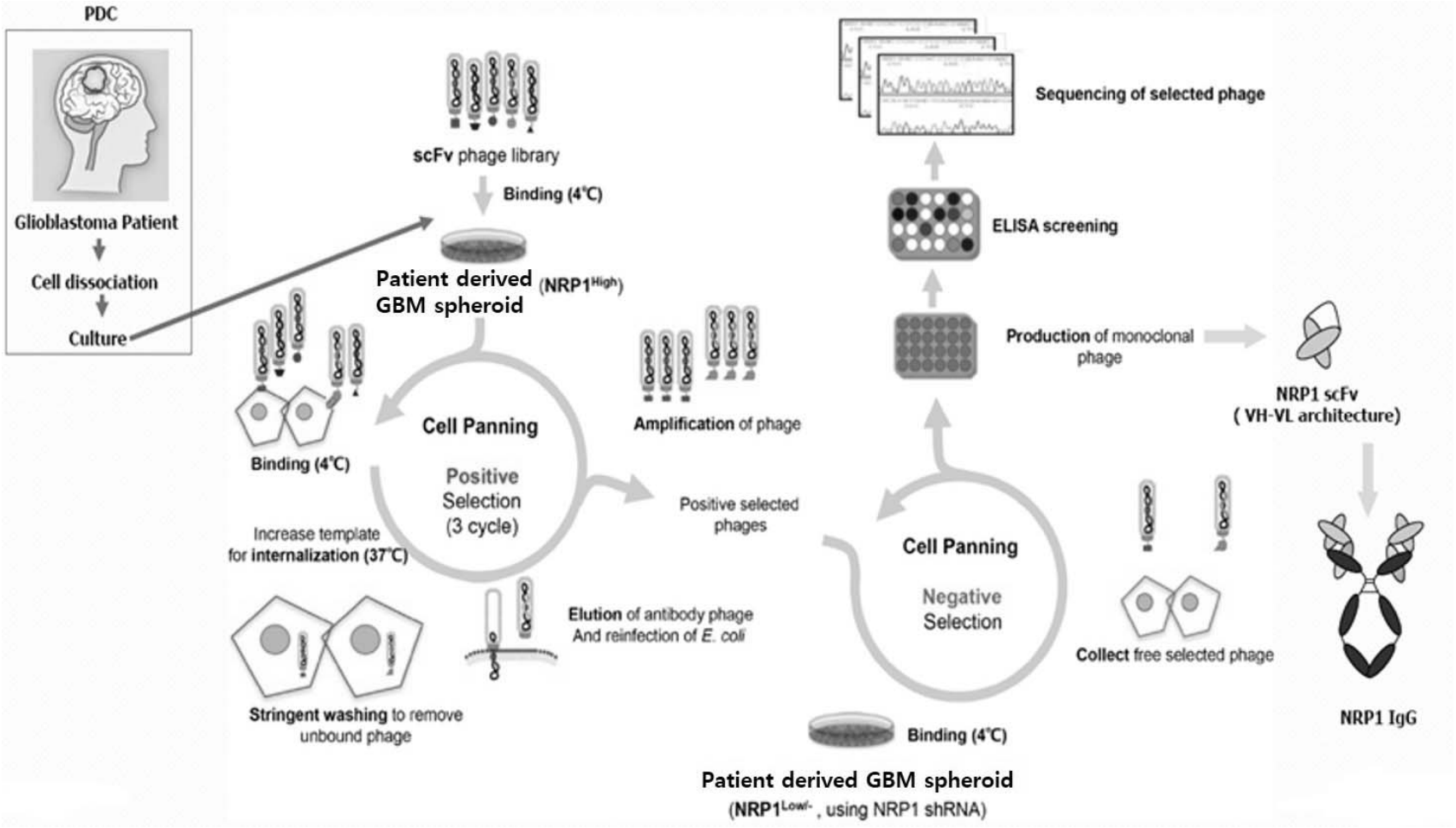
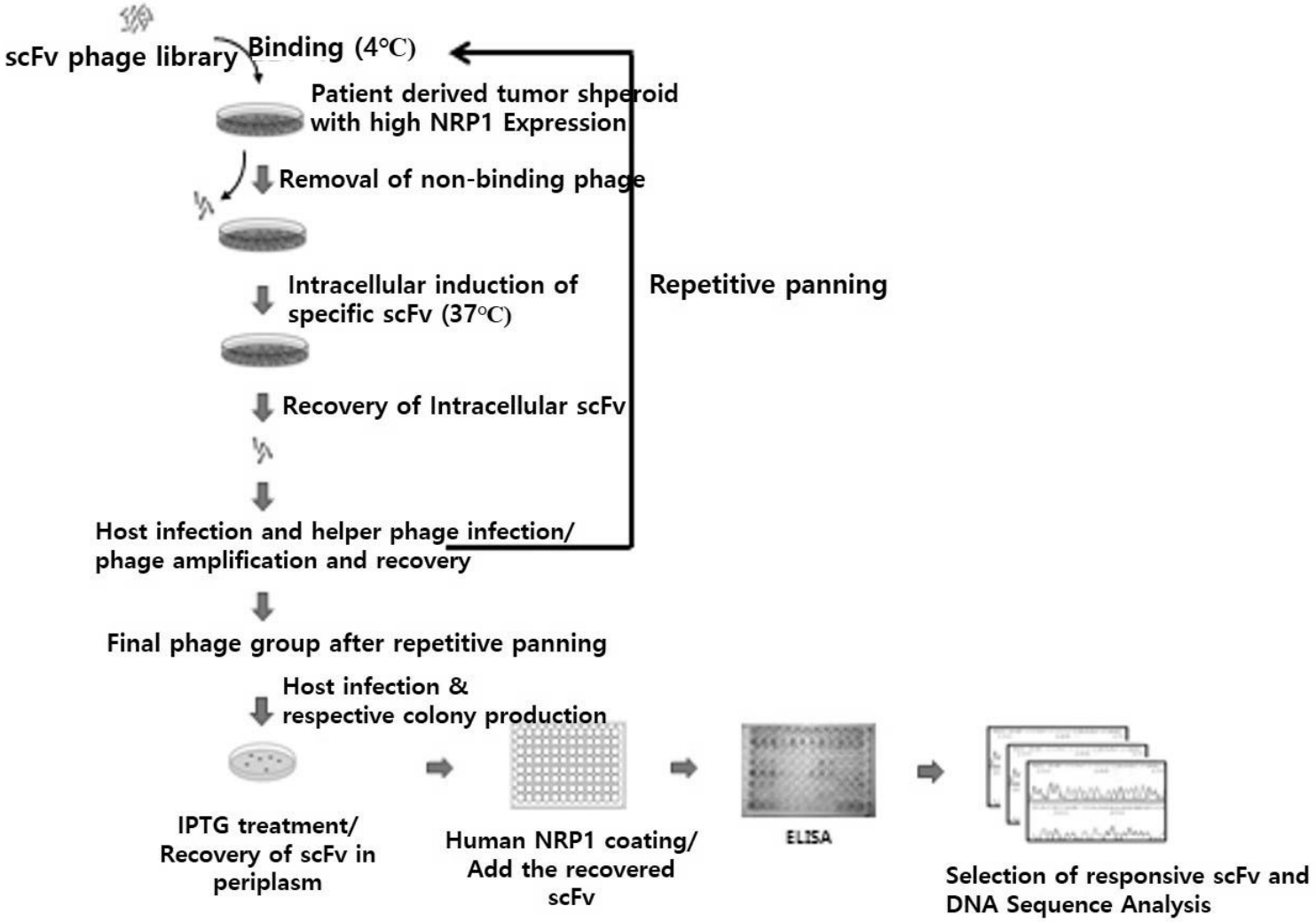
DRAWINGS

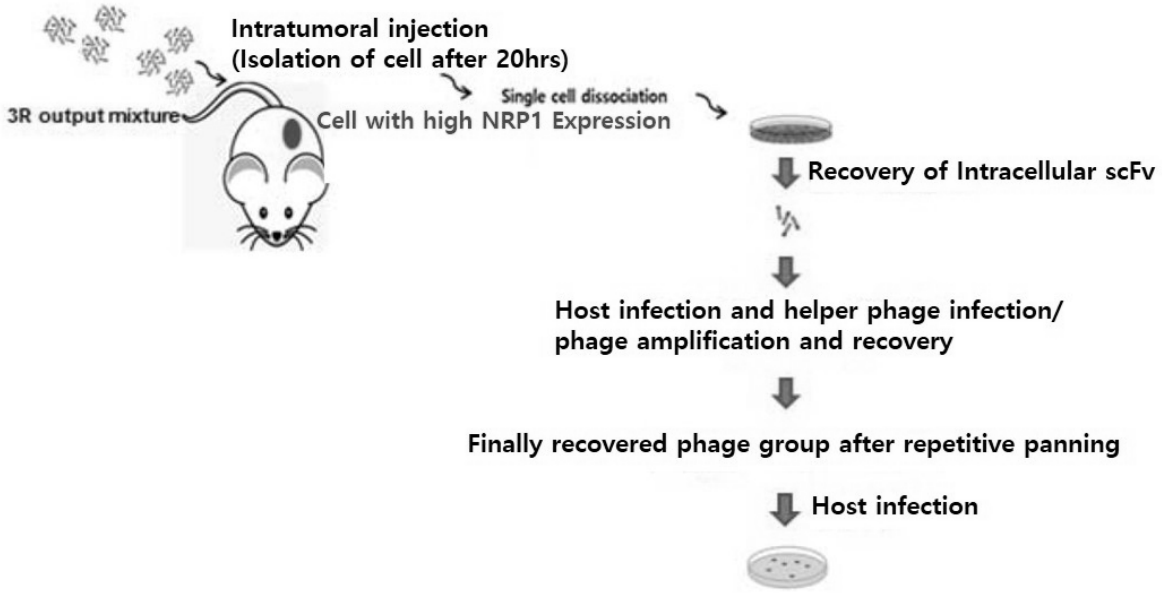
【Fig. 1】



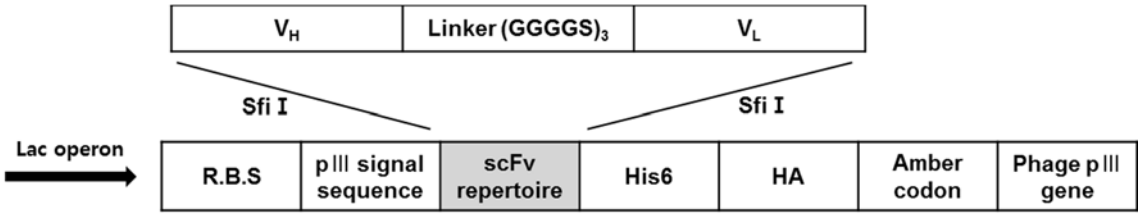
【Fig. 2】


scFv phage library
Binding (4℃)
Patient derived tumor shperoid with high NRP1 Expression
Removal of non-binding phage
Intracellular induction of specific scFv (37℃)
Repetitive panning
Recovery of Intracellular scFv
Host infection and helper phage infection/ phage amplification and recovery
Final phage group after repetitive panning
Host infection & respective colony production
IPTG treatment/ Recovery of scFv in periplasm
Human NRP1 coating/ Add the recovered scFv
ELISA
Selection of responsive scFv and DNA Sequence Analysis

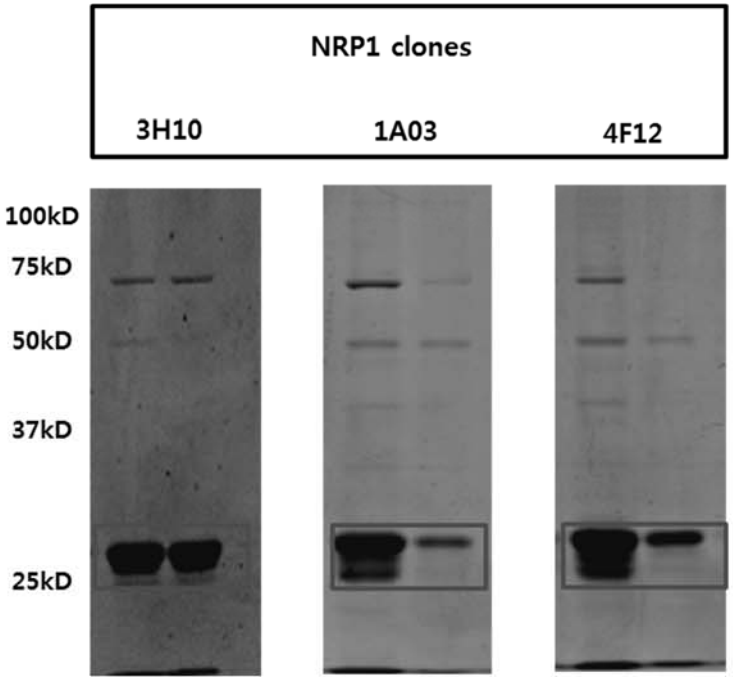
【Fig. 3】


Intratumoral injection
(Isolation of cell after 20hrs)
3R output mixture
Single cell dissociation
Cell with high NRP1 Expression
Recovery of Intracellular scFv
Host infection and helper phage infection/
phage amplification and recovery
Finally recovered phage group after repetitive panning
Host infection

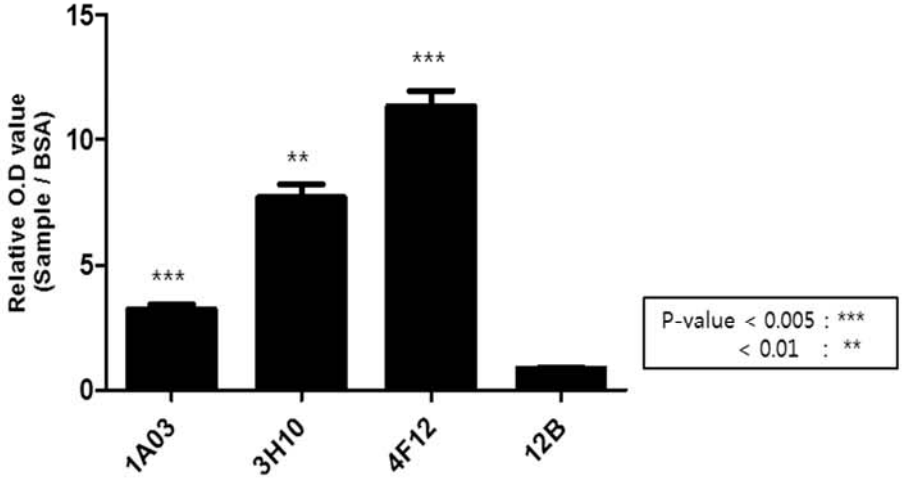
【Fig. 4】


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

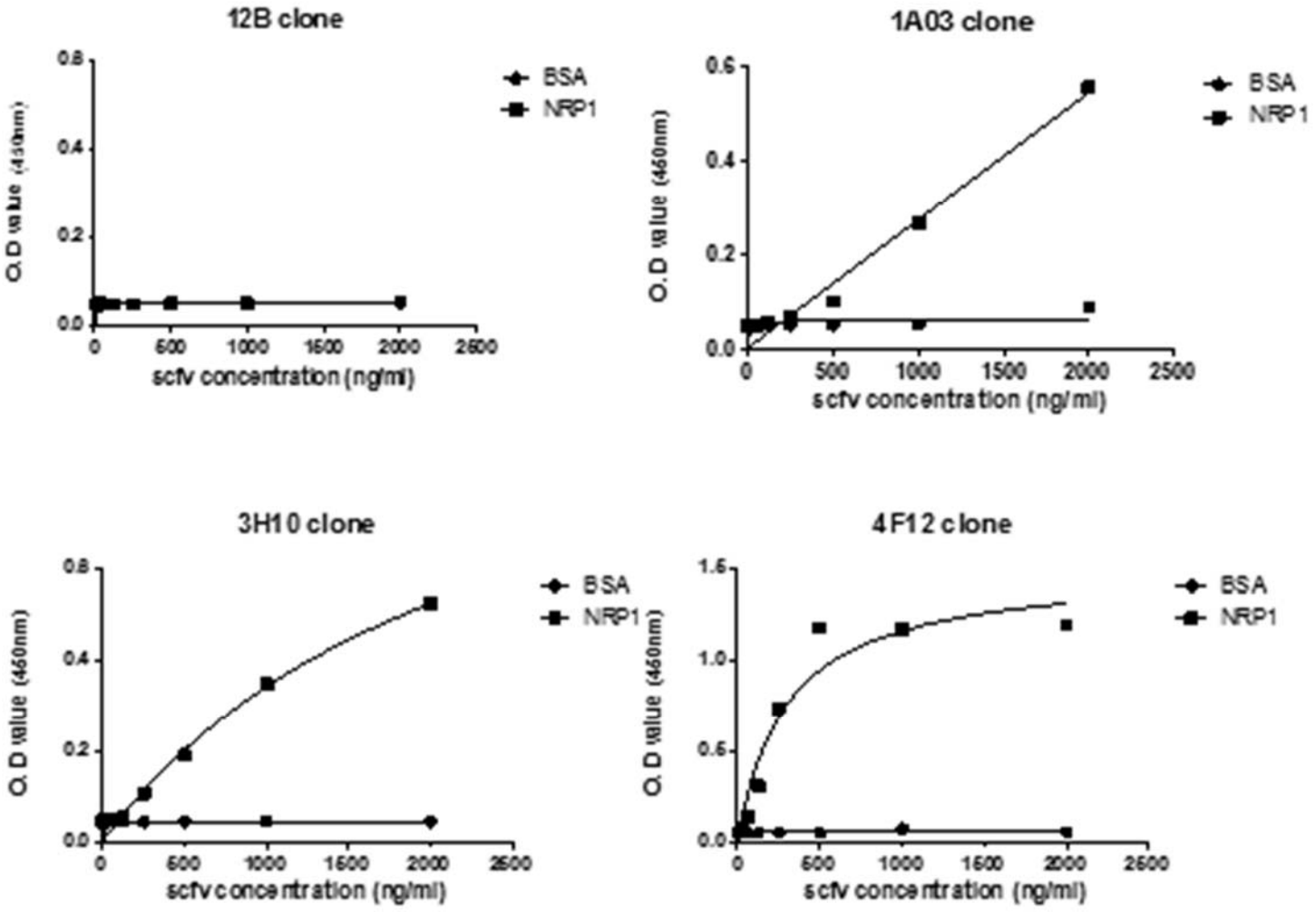
【Fig. 5】


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

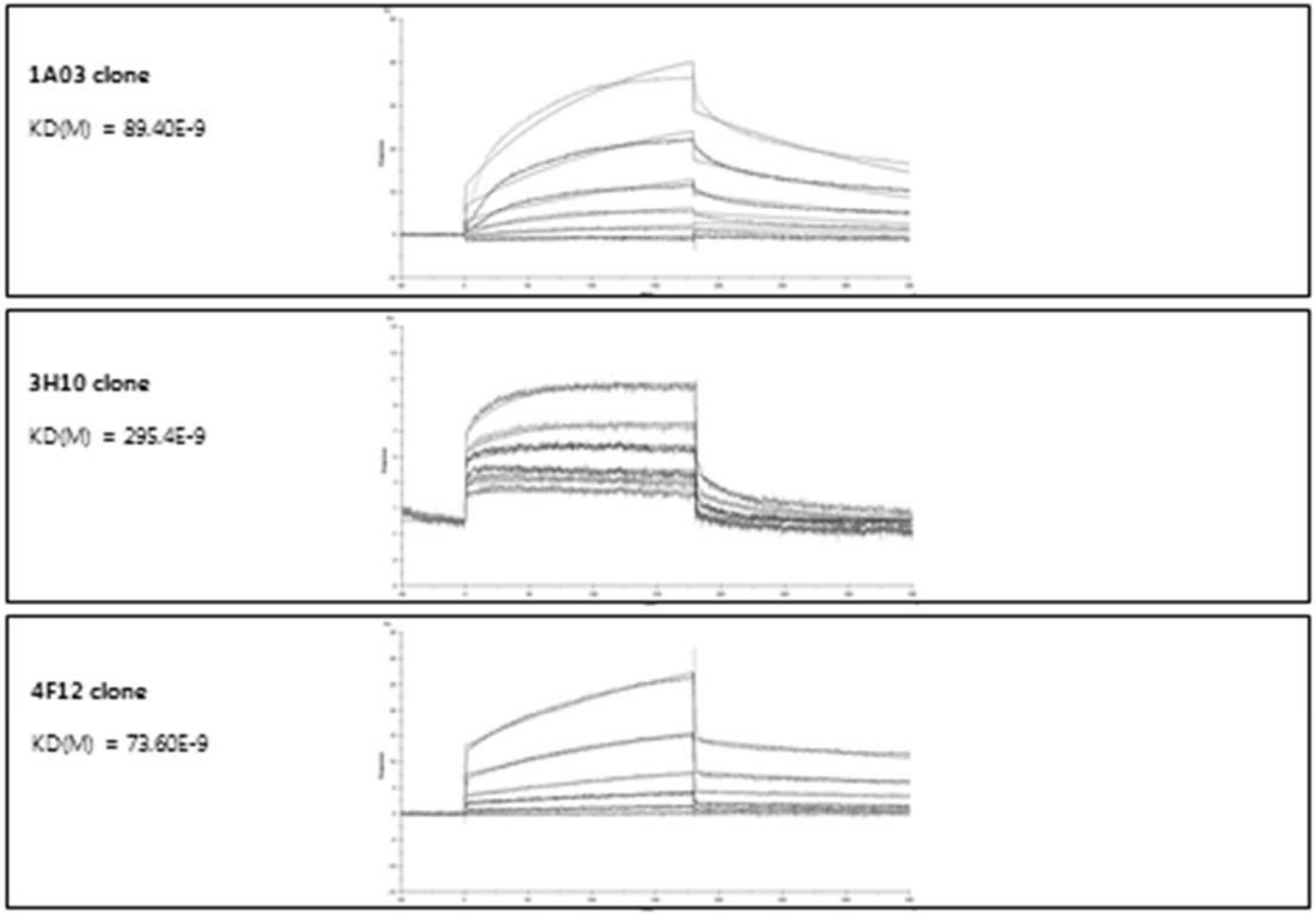
【Fig. 6】


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

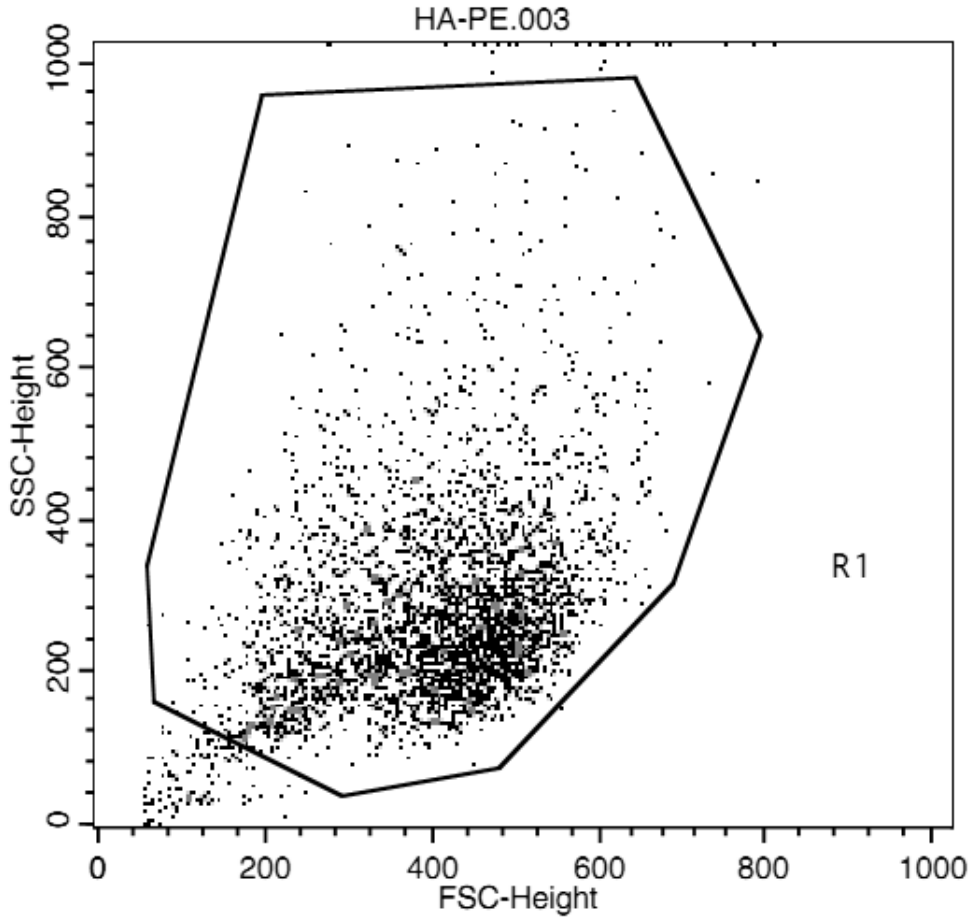
【Fig. 7】


12B clone
1A03 clone
3H10 clone
4F12 clone
BSA
NRP1
O.D value (460nm)
scfv concentration (ng/ml)

【Fig. 8】

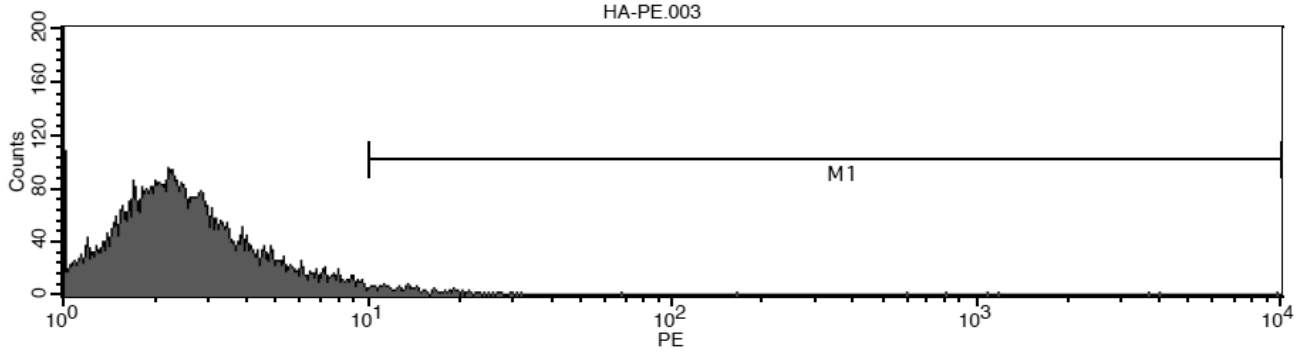

1A03 clone
KD(M) = 89.40E-9
3H10 clone
KD(M) = 295.4E-9
4F12 clone
KD(M) = 73.60E-9

【Fig. 9】

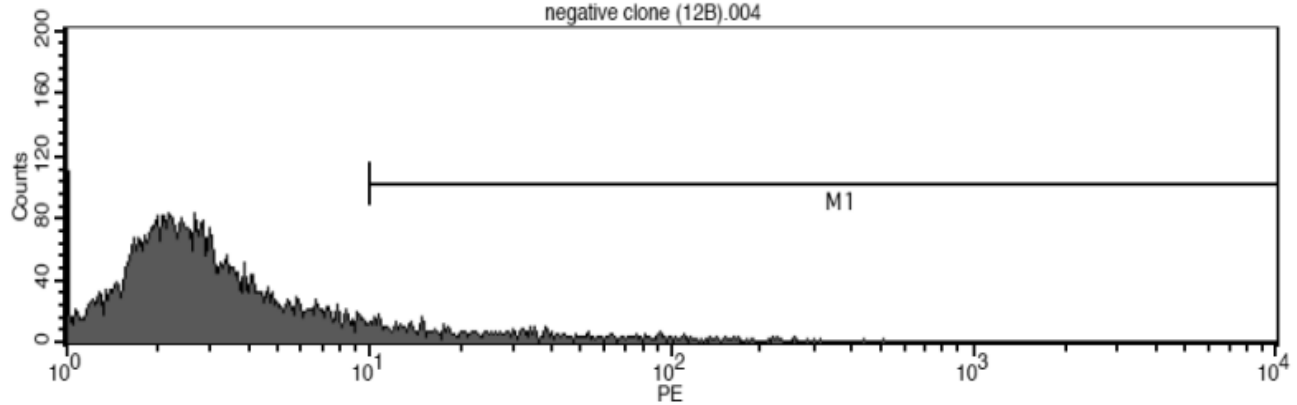


[Fig. 9】(cont.)

HA-PE

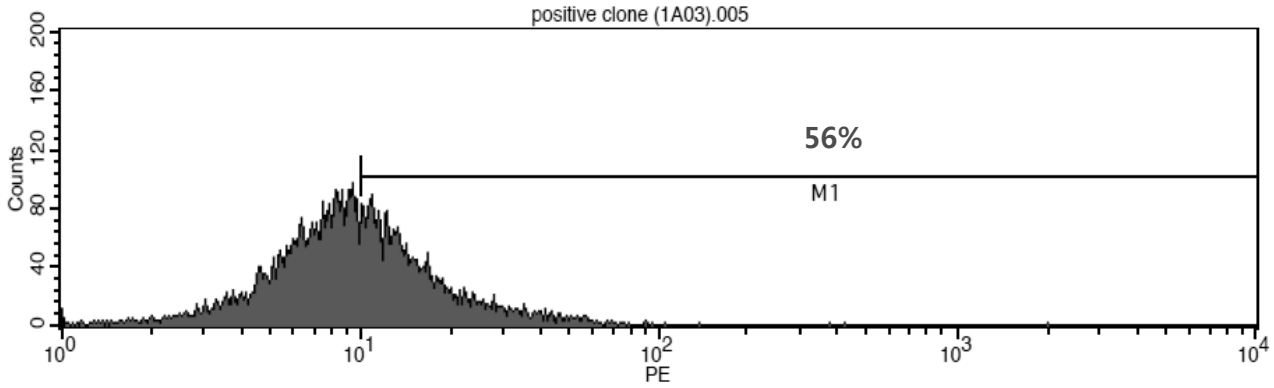


Negative control (12B)

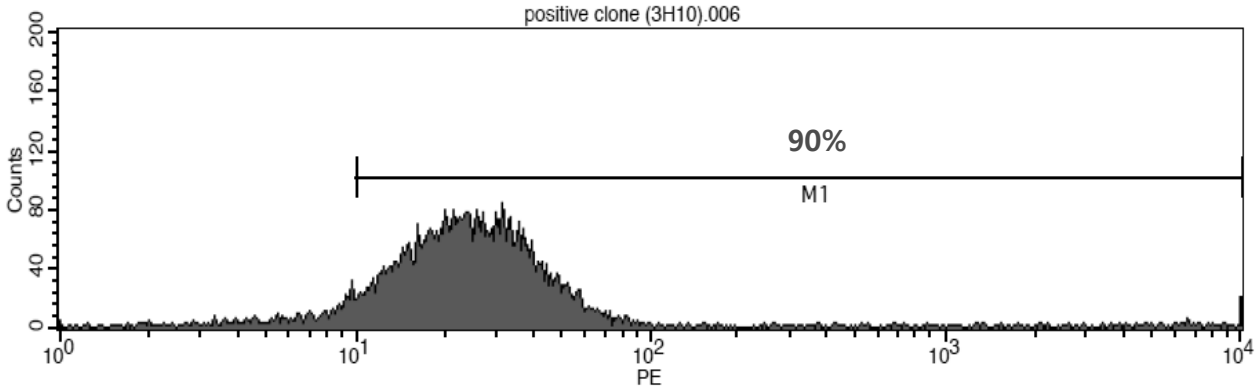


[Fig. 9】(cont.)

Positive control (1A03)

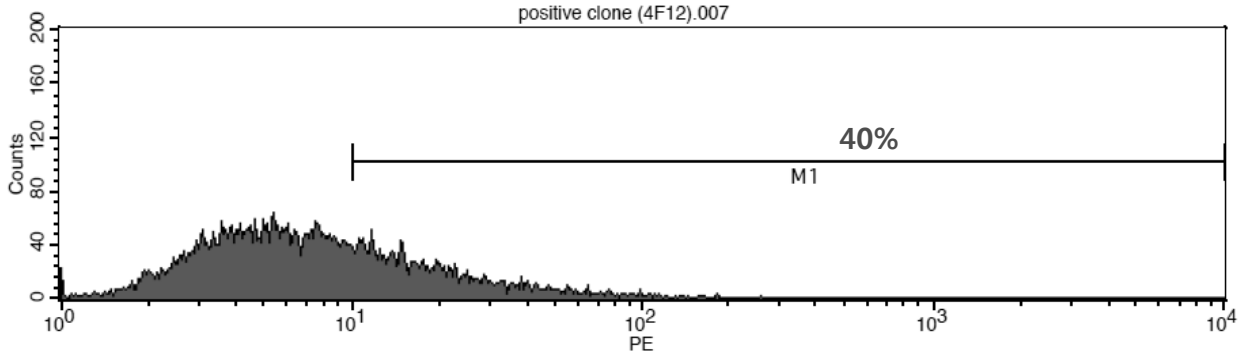


Positive control (3H10)

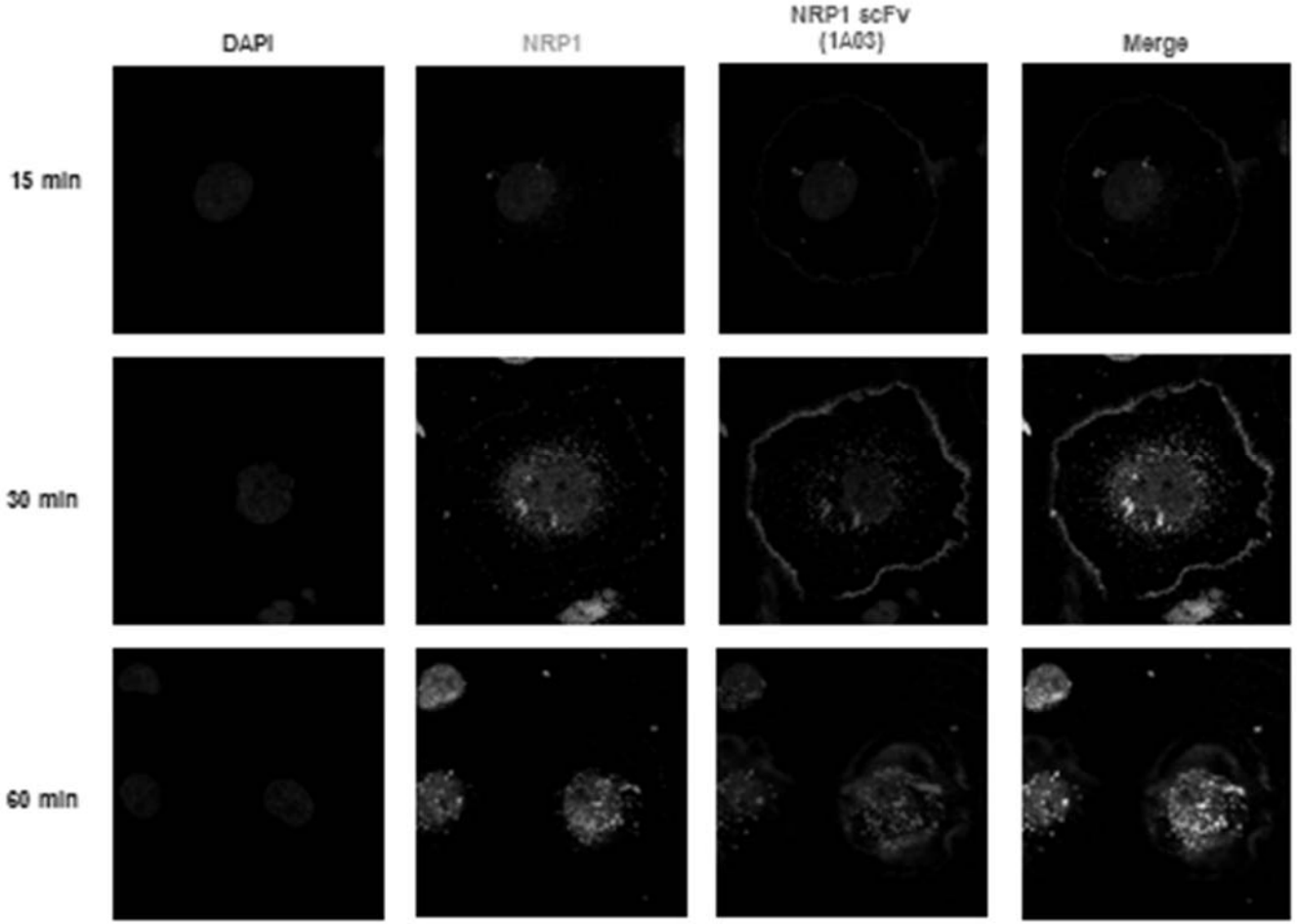


[Fig. 9】(cont.)

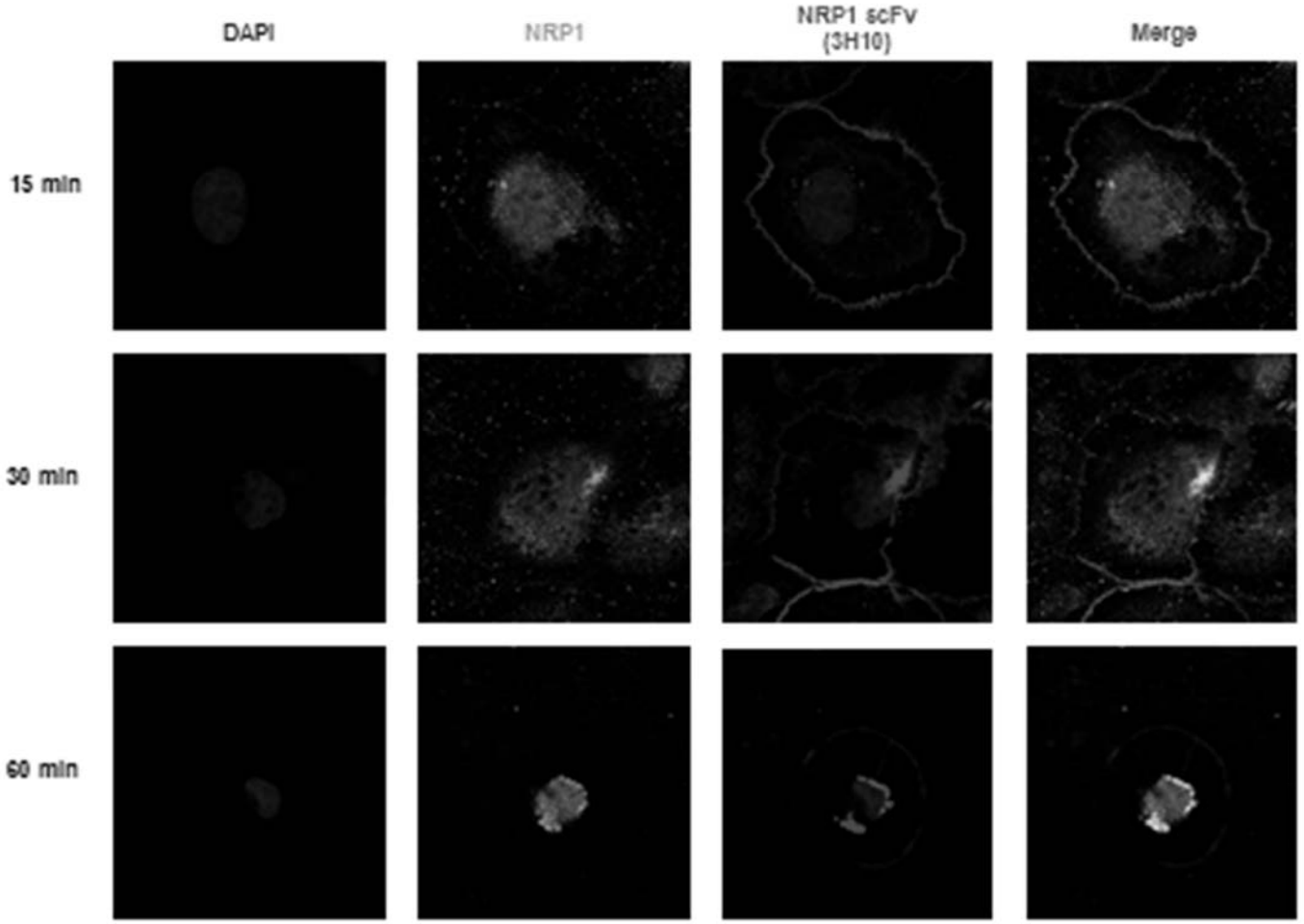
Positive control (4F12)



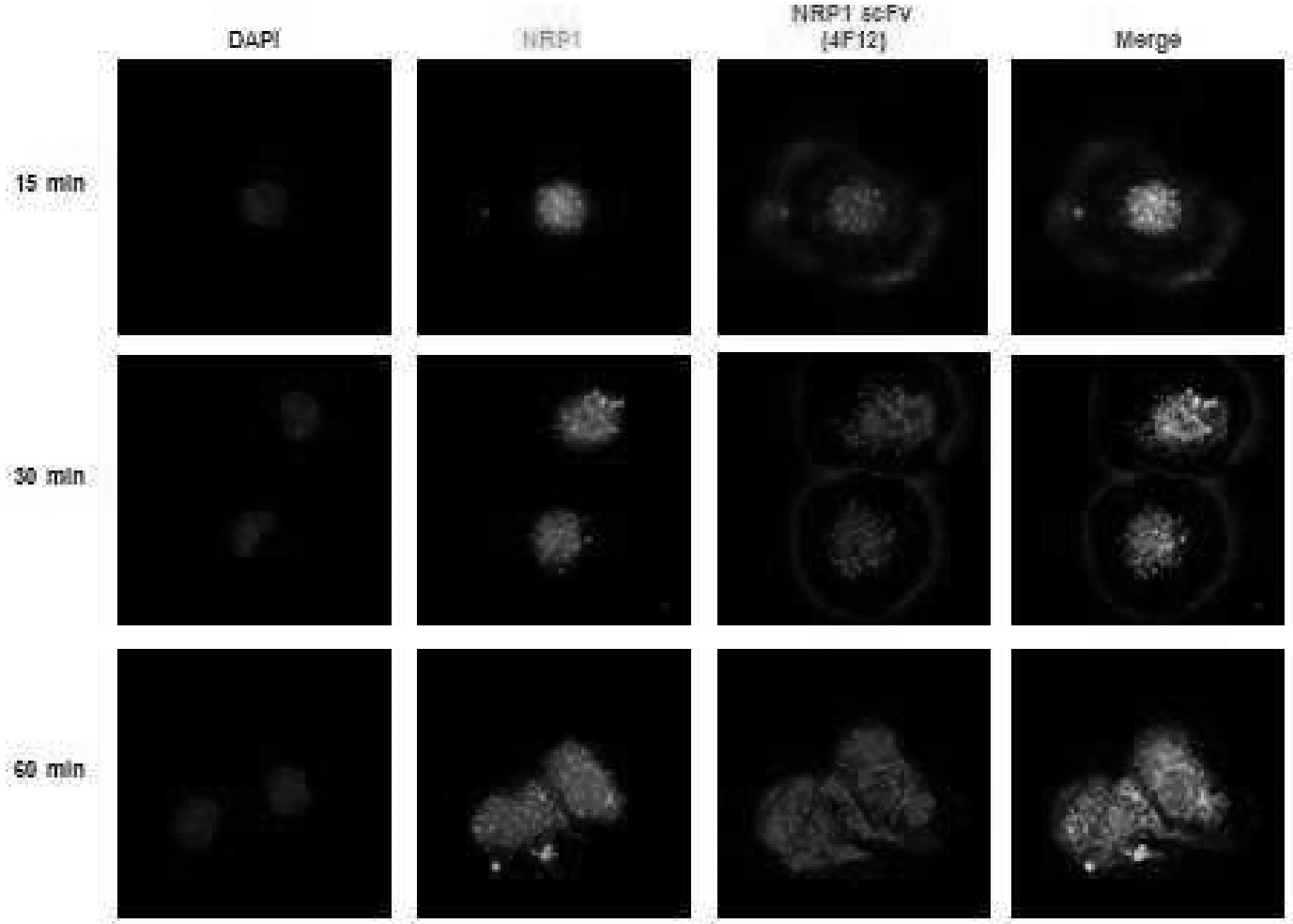
【Fig. 10a】



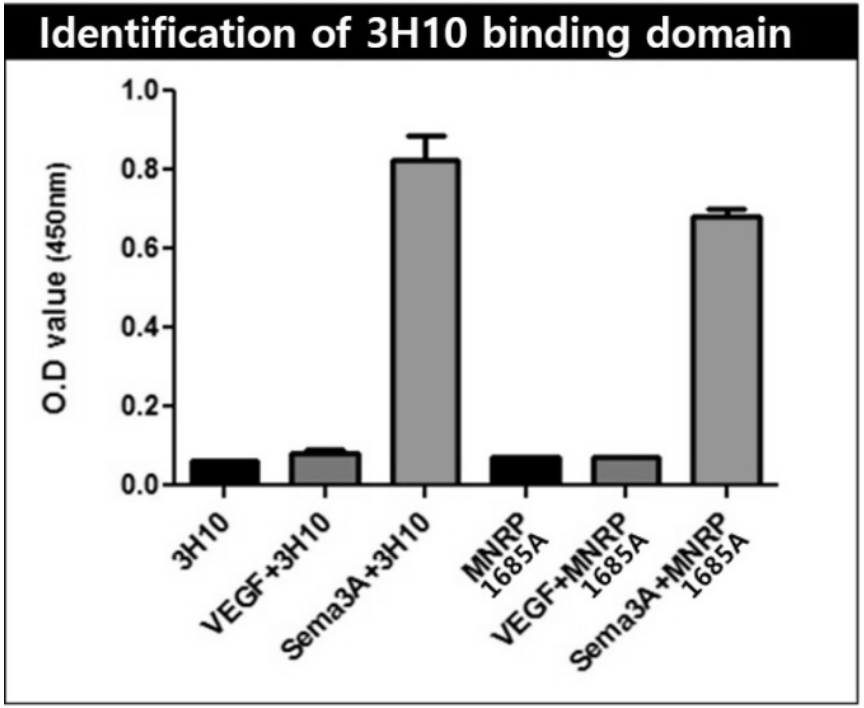
【Fig. 10b】

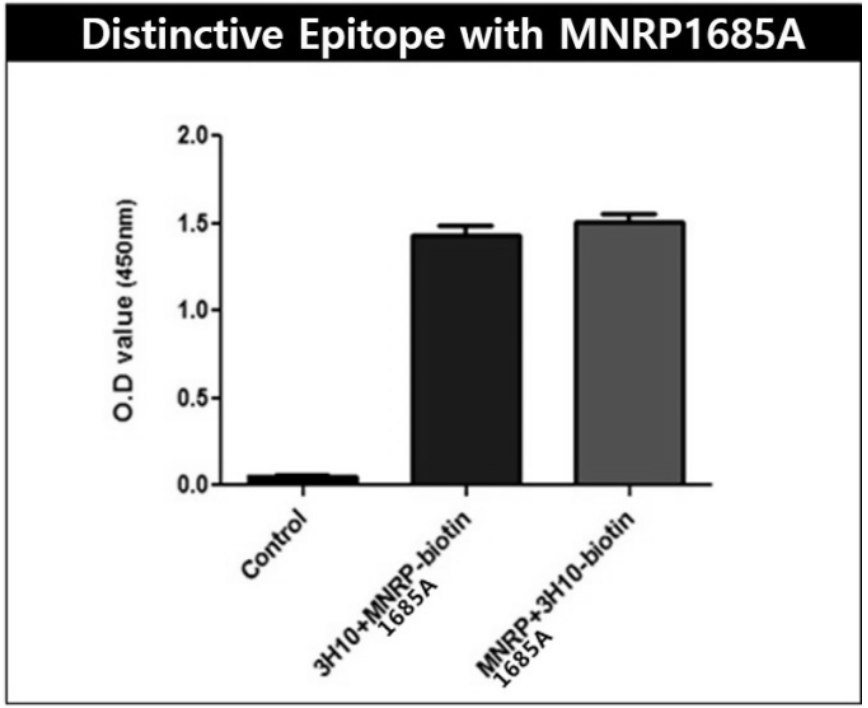


【Fig. 10c】

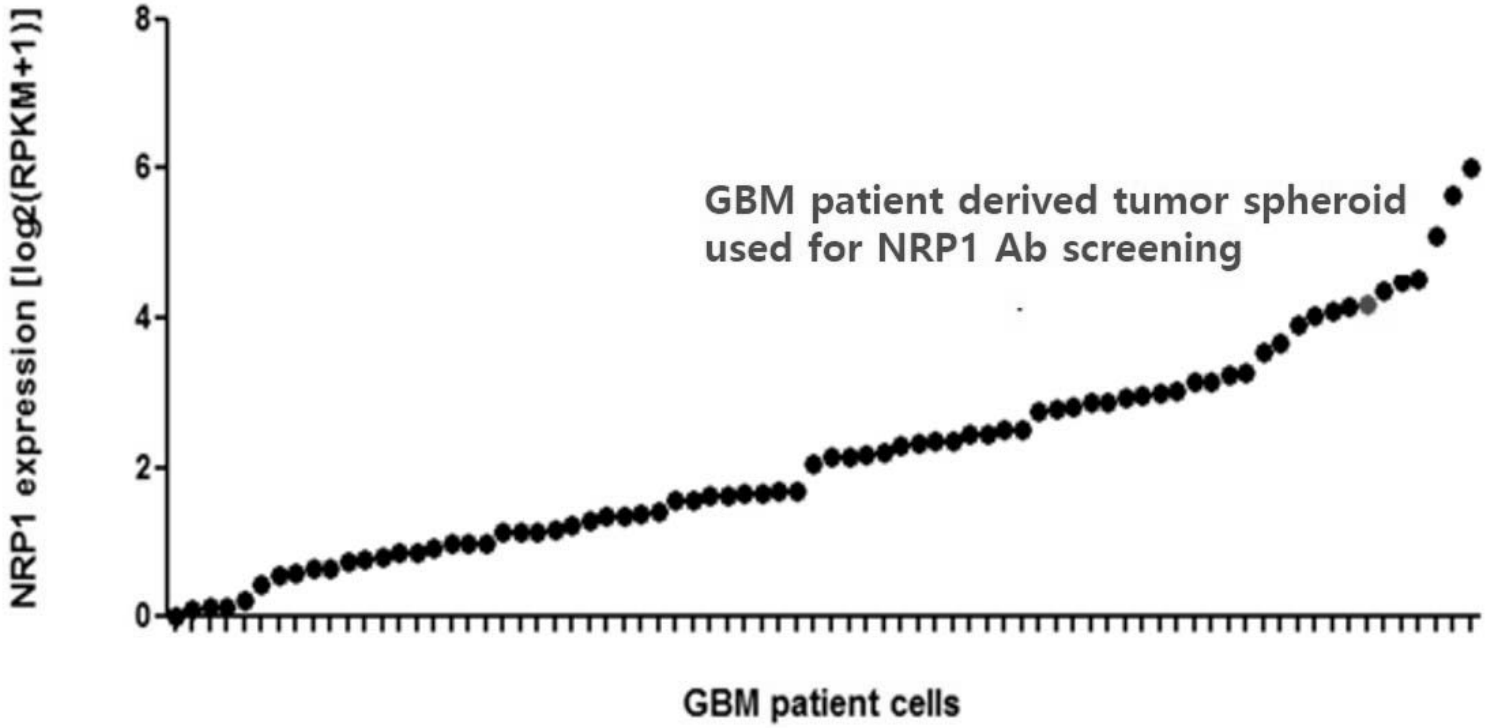


【Fig. 11】


Identification of 3H10 binding domain
O.D value (450nm)
1.0
0.8
0.6
0.4
0.2
0.0
3H10
VEGF+3H10
Sema3A+3H10
MNRP 1685A
VEGF+MNRP 1685A
Sema3A+MNRP 1685A

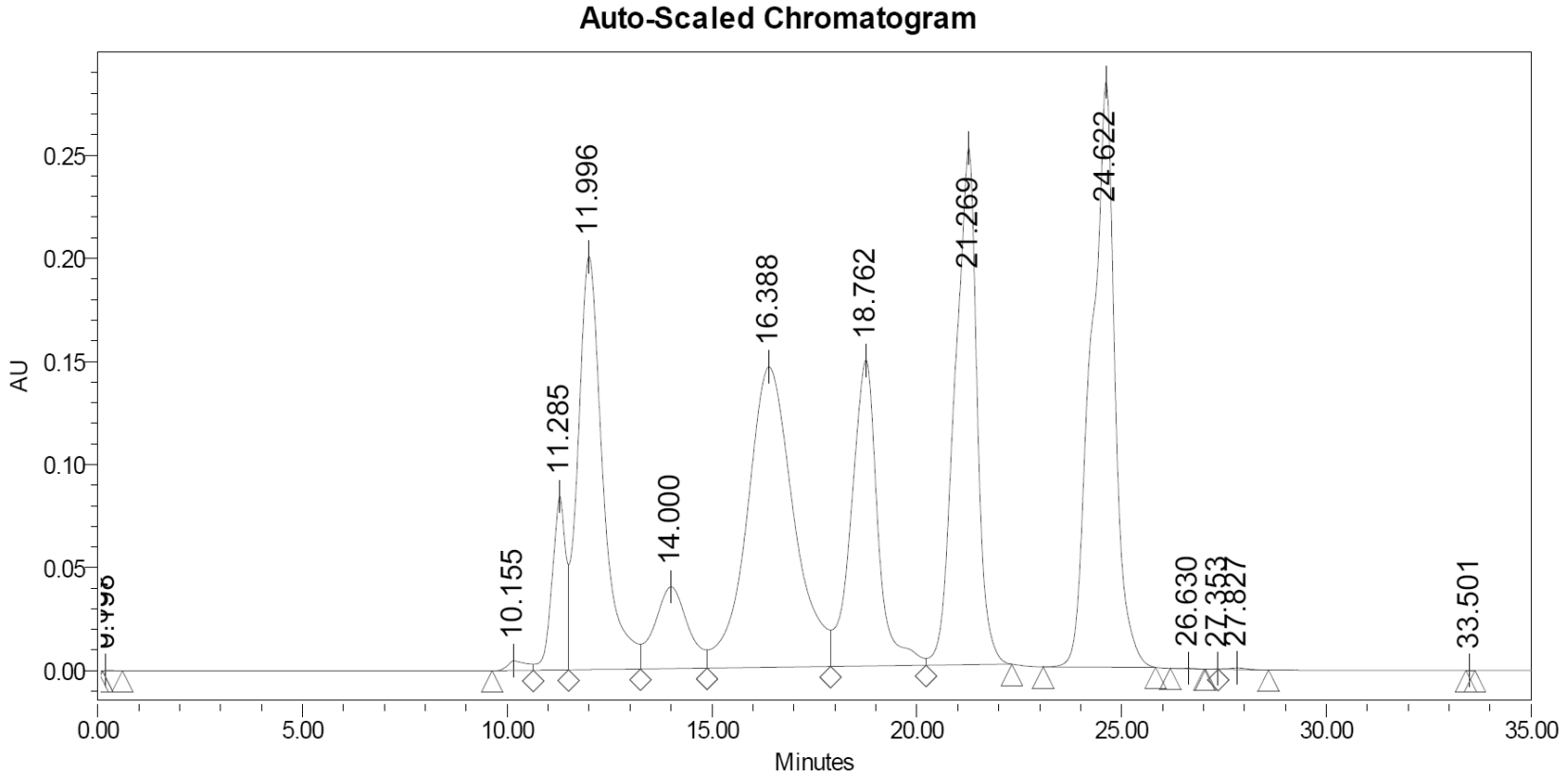

Distinctive Epitope with MNRP1685A
O.D value (450nm)
2.0
1.5
1.0
0.5
0.0
Control
3H10+MNRP-biotin 1685A
MNRP+3H10-biotin 1685A

【Fig. 12】


NRP1 expression [log2(RPKM+1)]
8
6
4
2
0
GBM patient derived tumor spheroid
used for NRP1 Ab screening
GBM patient cells

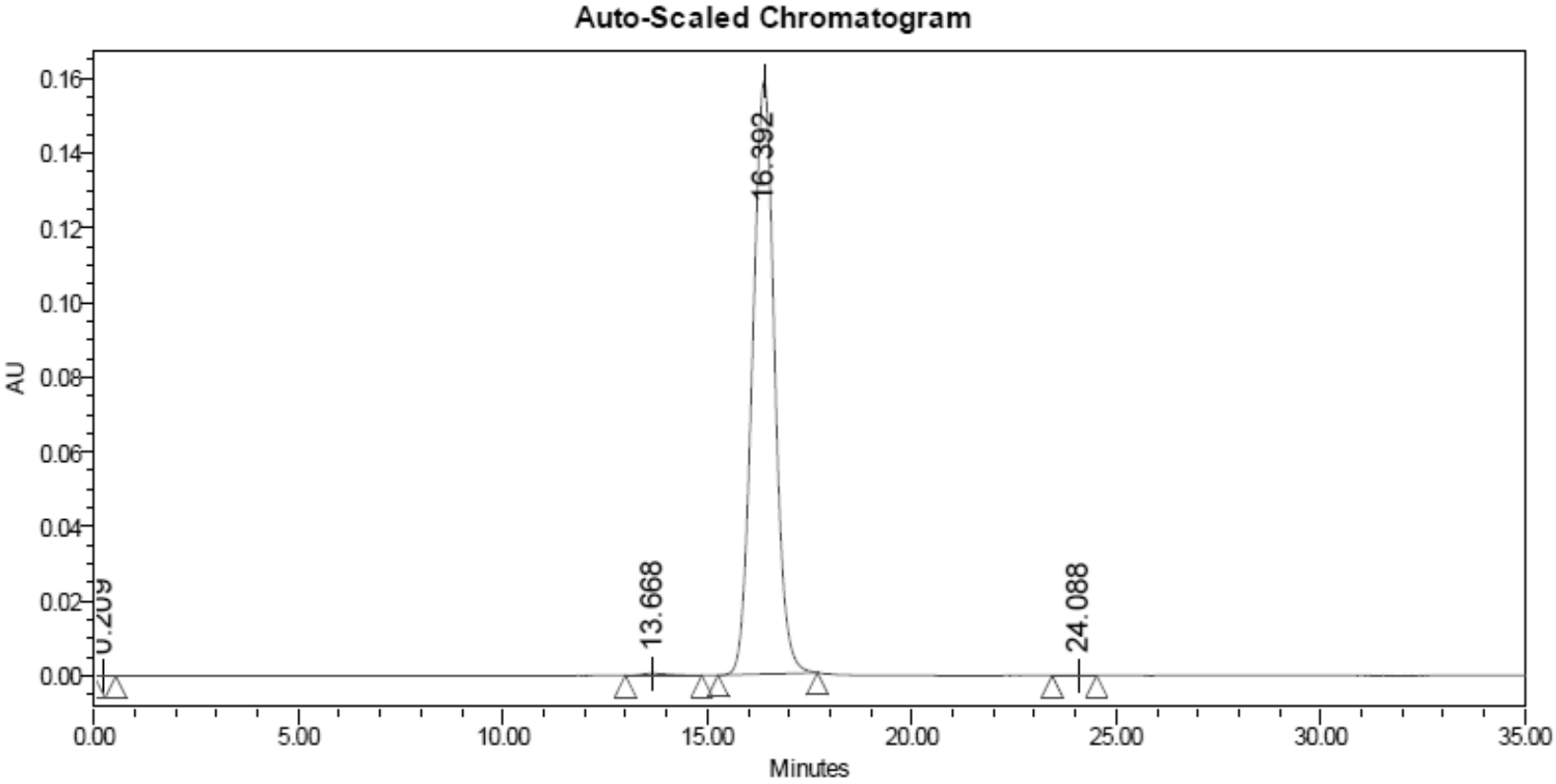
【Fig. 13】

Marker



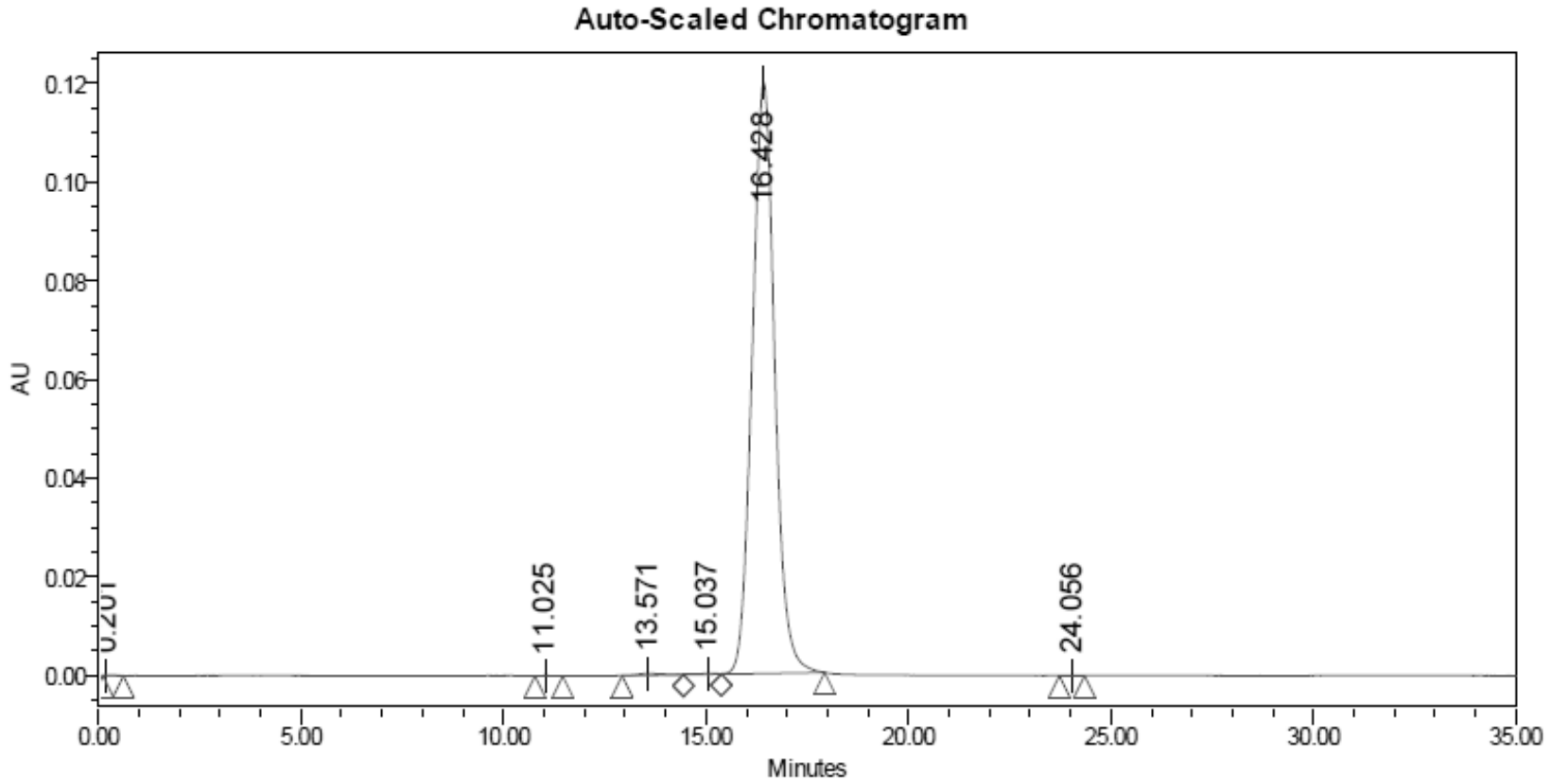
【Fig. 13】(cont.)

IRCR-101 (3H10)



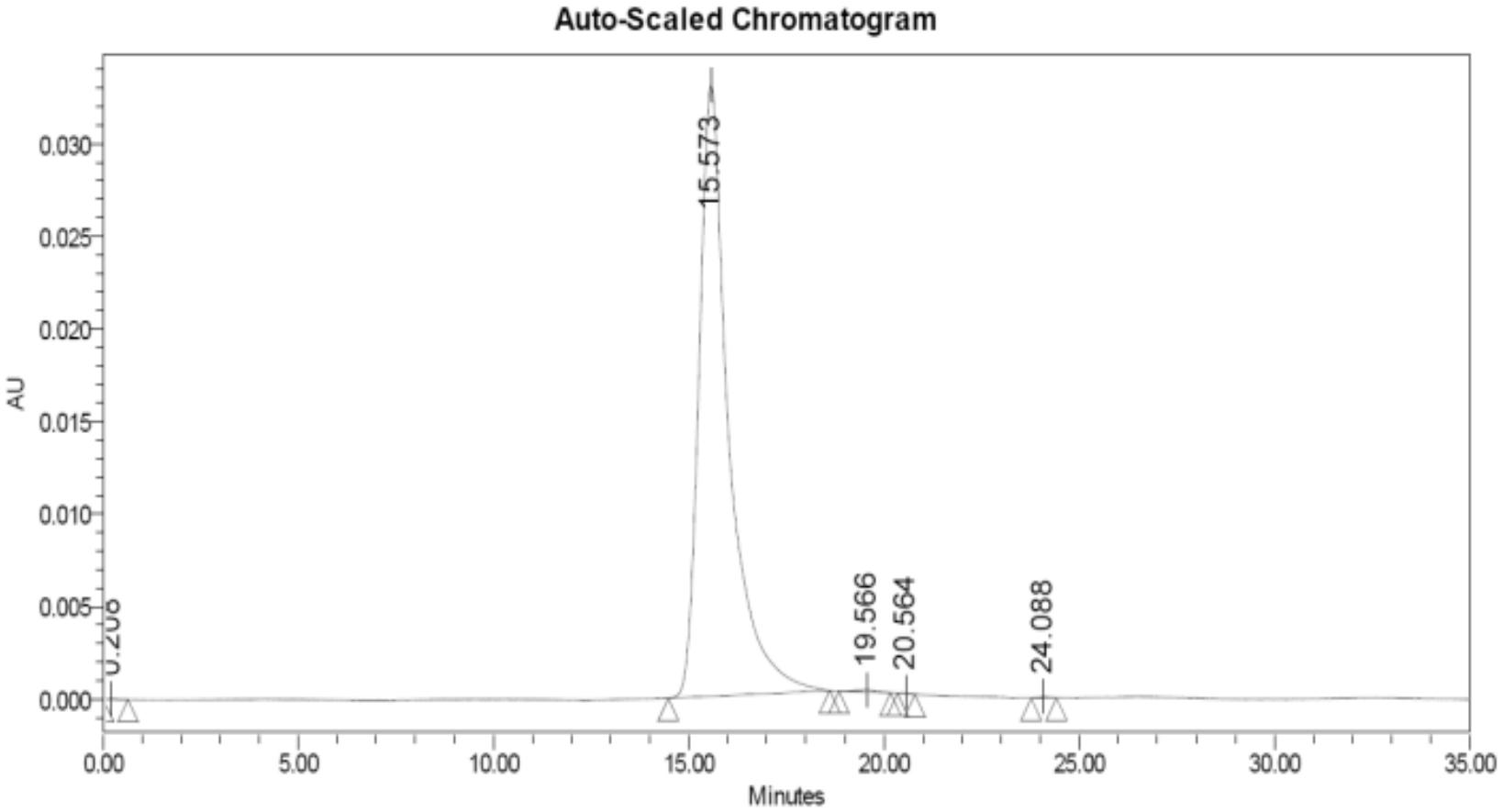
【Fig. 13】(cont.)

1A03

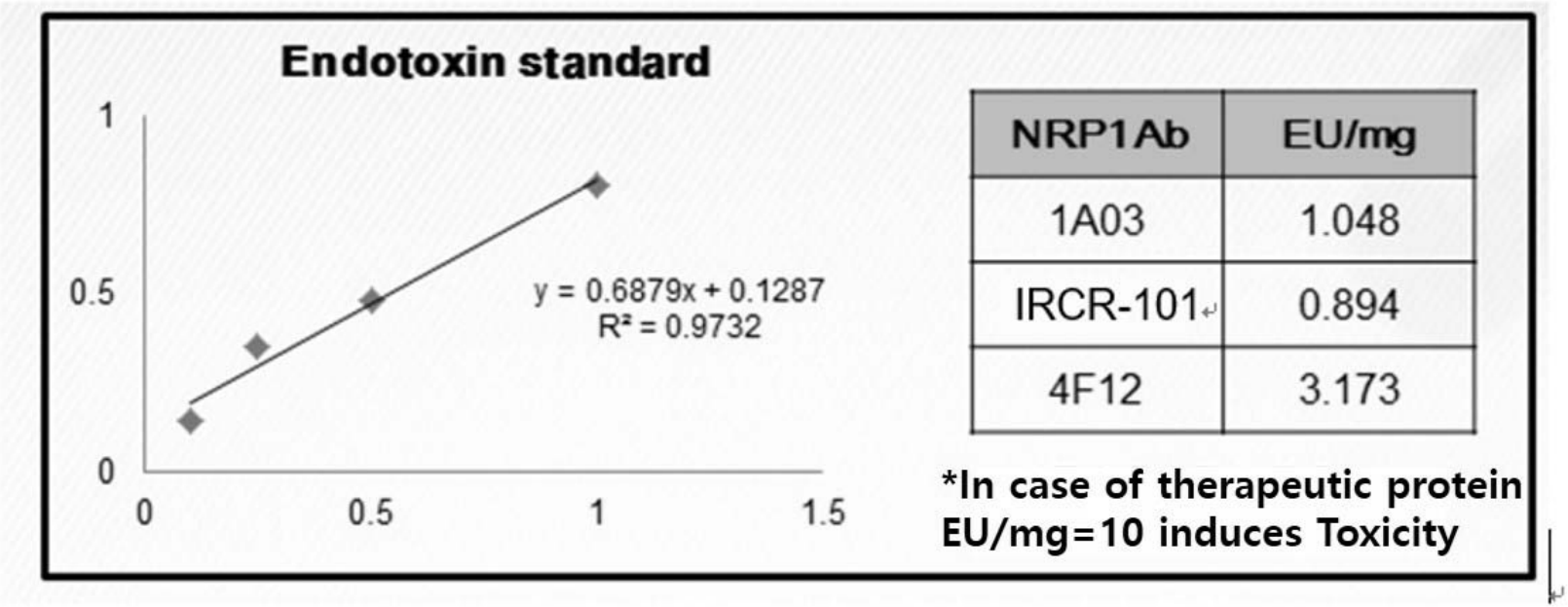


【Fig. 13】(cont.)

4F12



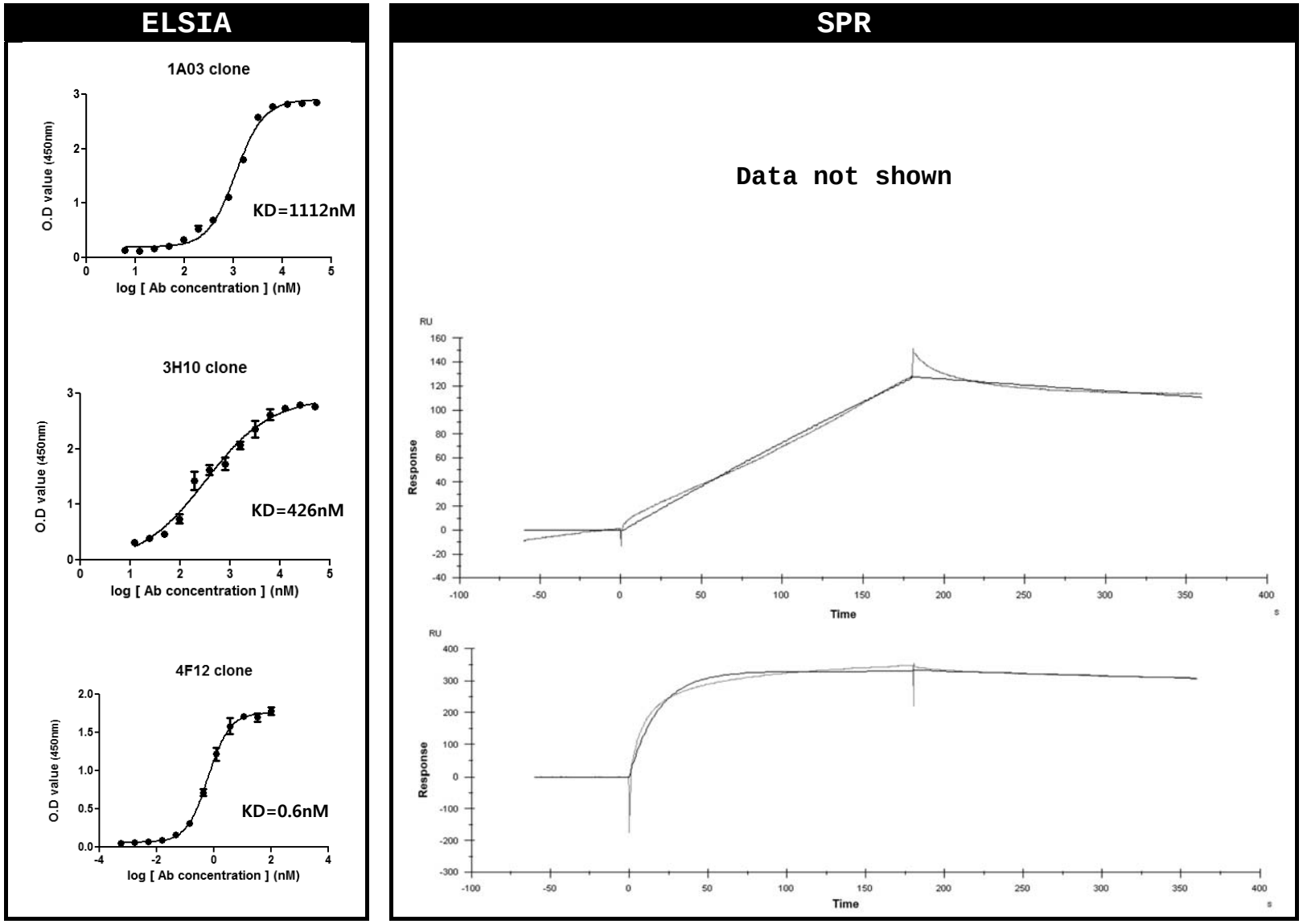
【Fig. 14】



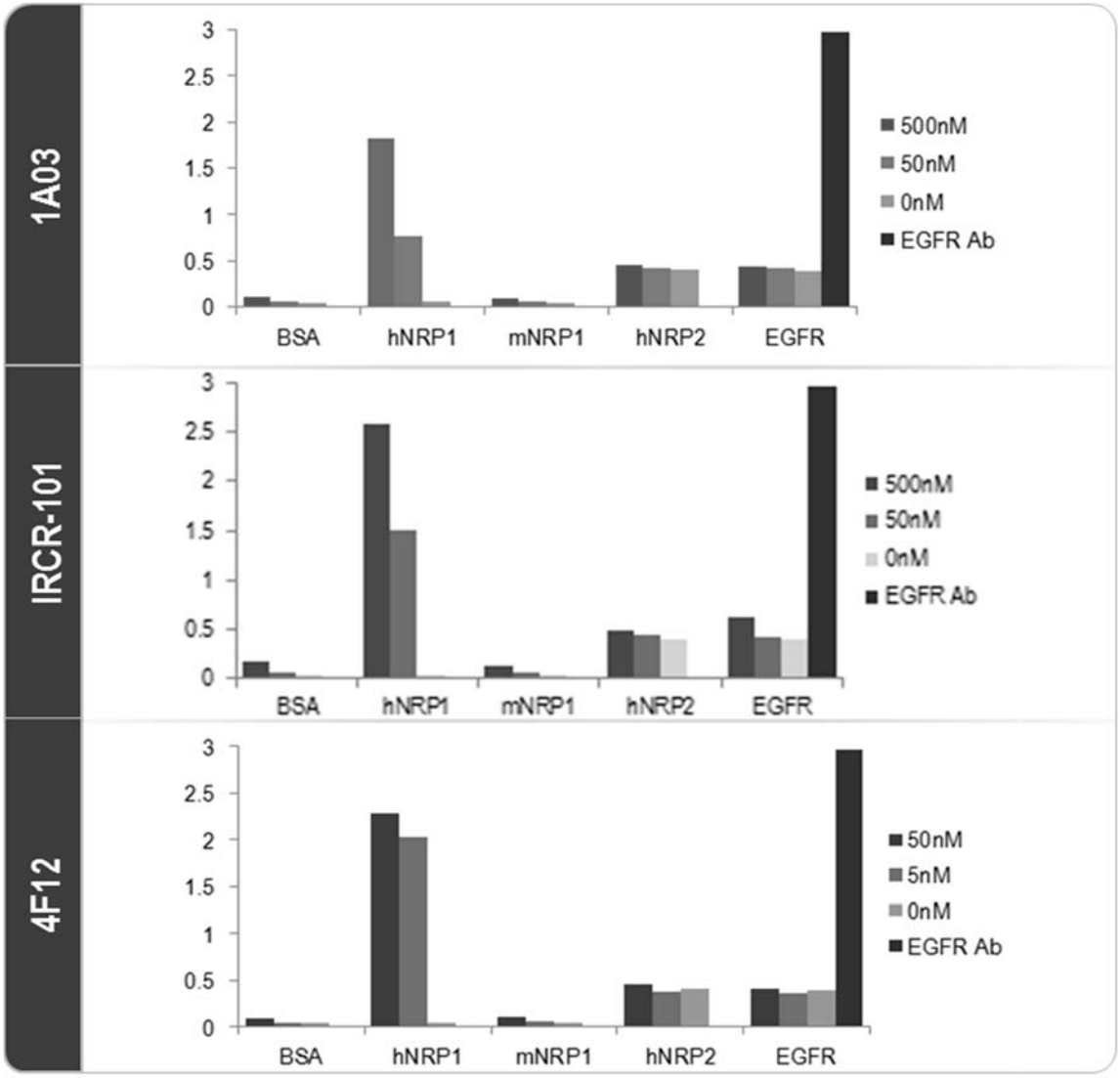
NRP1Ab	EU/mg
1A03	1.048
IRCR-101	0.894
4F12	3.173

*In case of therapeutic protein
EU/mg=10 induces Toxicity

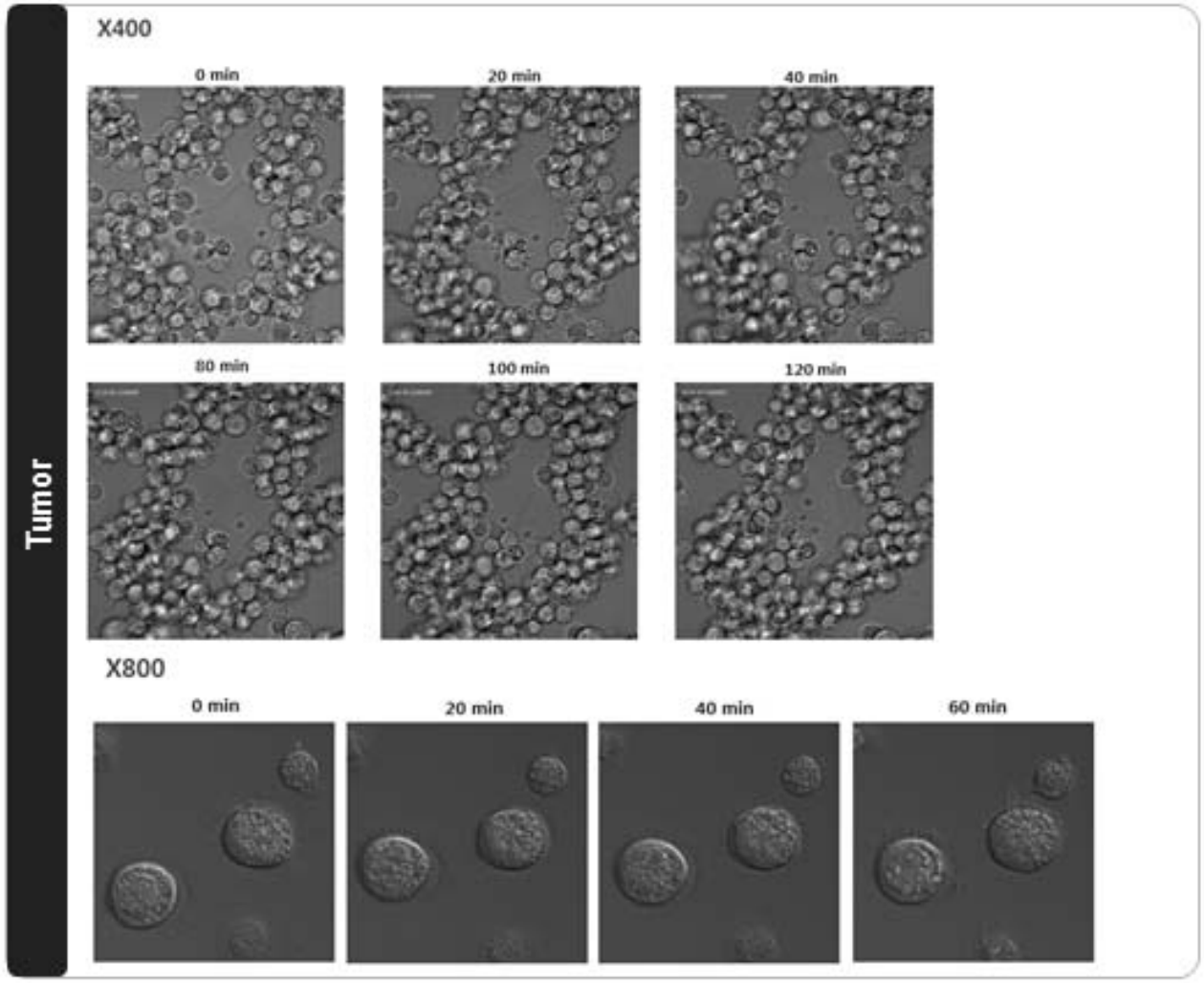
【Fig. 15】


ELSIA
1A03 clone
O.D value (450nm)
log [Ab concentration] (nM)
KD=1112nM
3H10 clone
O.D value (450nm)
log [Ab concentration] (nM)
KD=426nM
4F12 clone
O.D value (450nm)
log [Ab concentration] (nM)
KD=0.6nM
SPR
Data not shown
RU
Response
Time
RU
Response
Time

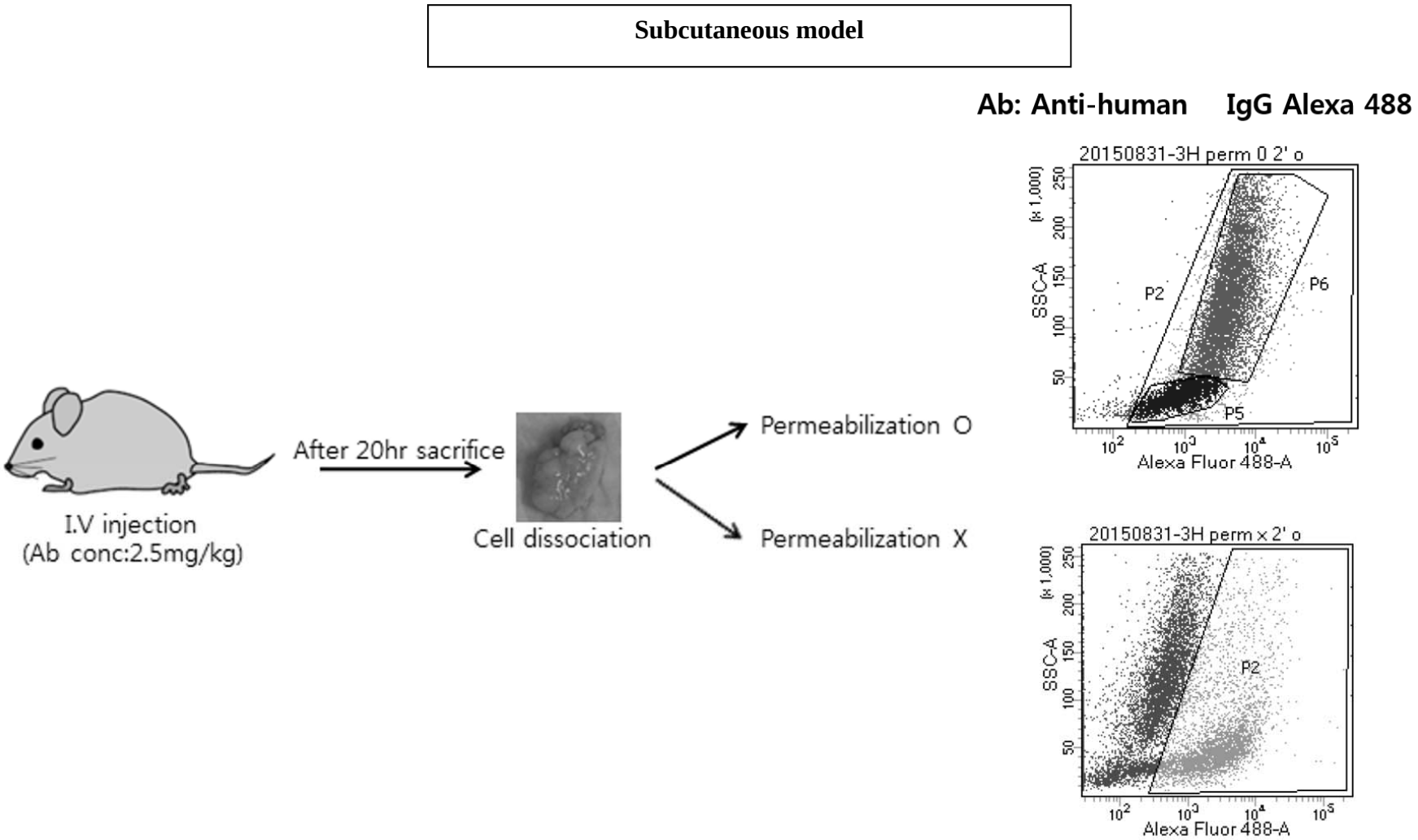
【Fig. 16】


1A03
3
2.5
2
1.5
1
0.5
0
BSA
hNRP1
mNRP1
hNRP2
EGFR
500nM
50nM
0nM
EGFR Ab
IRCR-101
3
2.5
2
1.5
1
0.5
0
BSA
hNRP1
mNRP1
hNRP2
EGFR
500nM
50nM
0nM
EGFR Ab
4F12
3
2.5
2
1.5
1
0.5
0
BSA
hNRP1
mNRP1
hNRP2
EGFR
50nM
5nM
0nM
EGFR Ab

【Fig. 17】


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

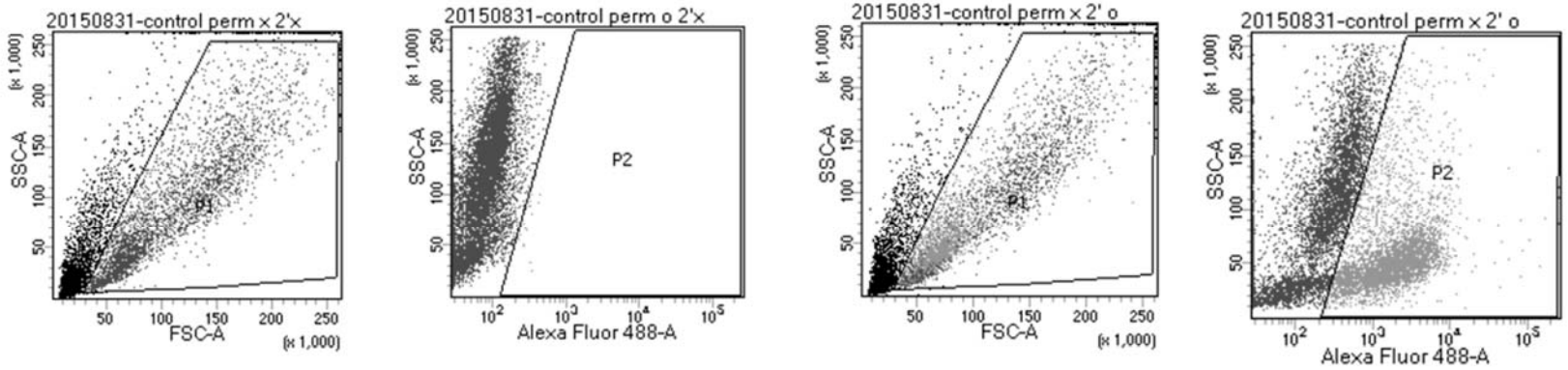
【Fig. 18】

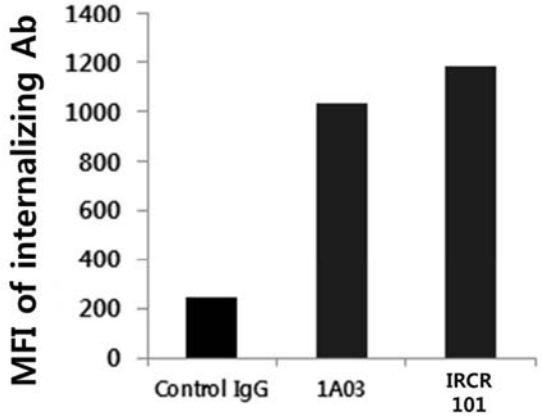

Subcutaneous model
Ab: Anti-human IgG Alexa 488
I.V injection
(Ab conc:2.5mg/kg)
After 20hr sacrifice
Cell dissociation
Permeabilization O
Permeabilization X
20150831-3H perm 0 2' o
SSC-A
(x 1,000)
P2
P6
P5
Alexa Fluor 488-A
20150831-3H perm x 2' o
SSC-A
(x 1,000)
P2
Alexa Fluor 488-A

【Fig. 18】(cont.)

Subcutaneous model

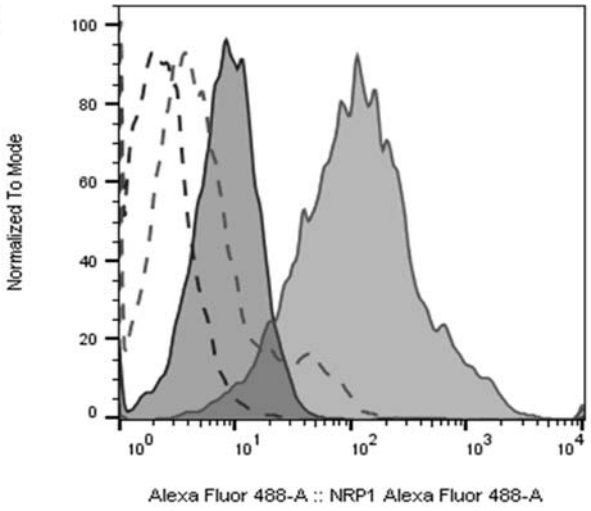
Internalizing efficacy

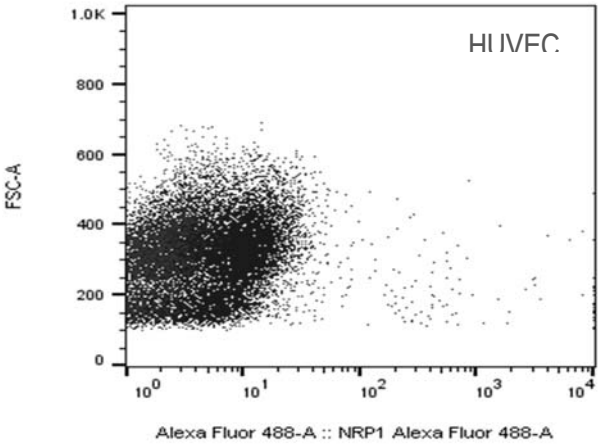


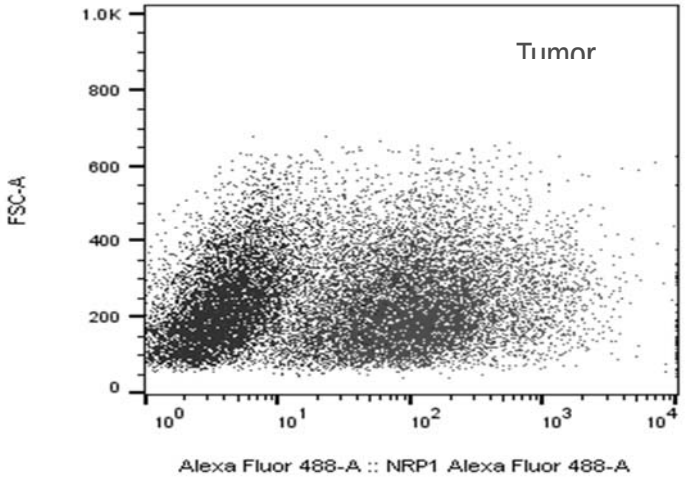


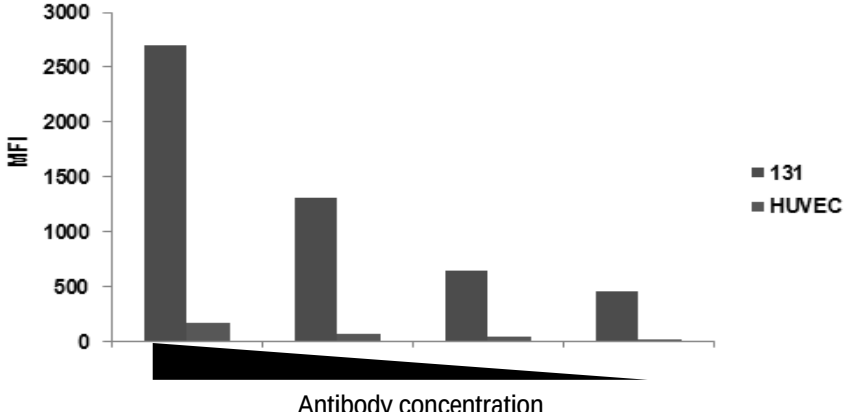
IRCR – 101 Ab

【Fig. 19】

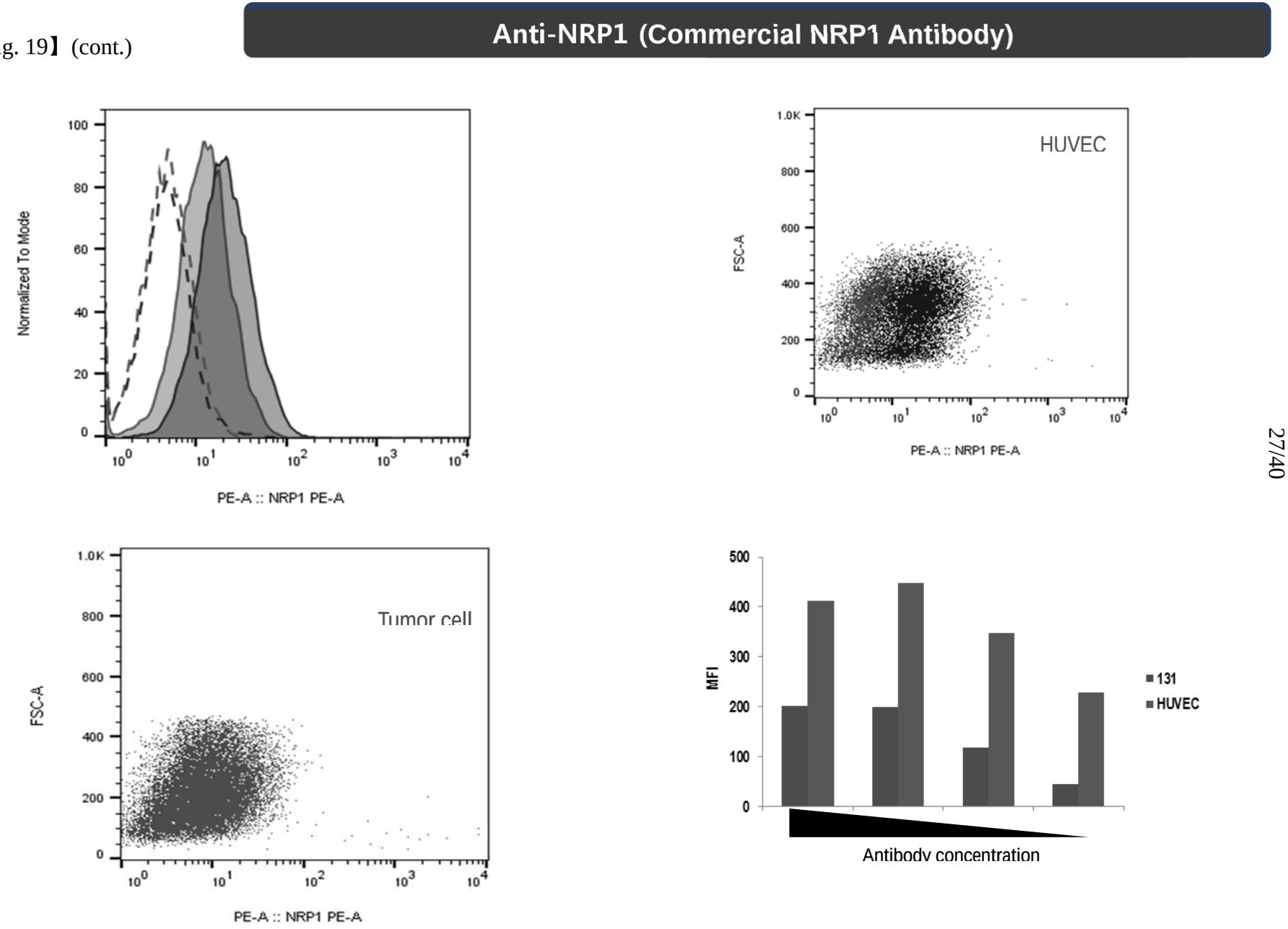




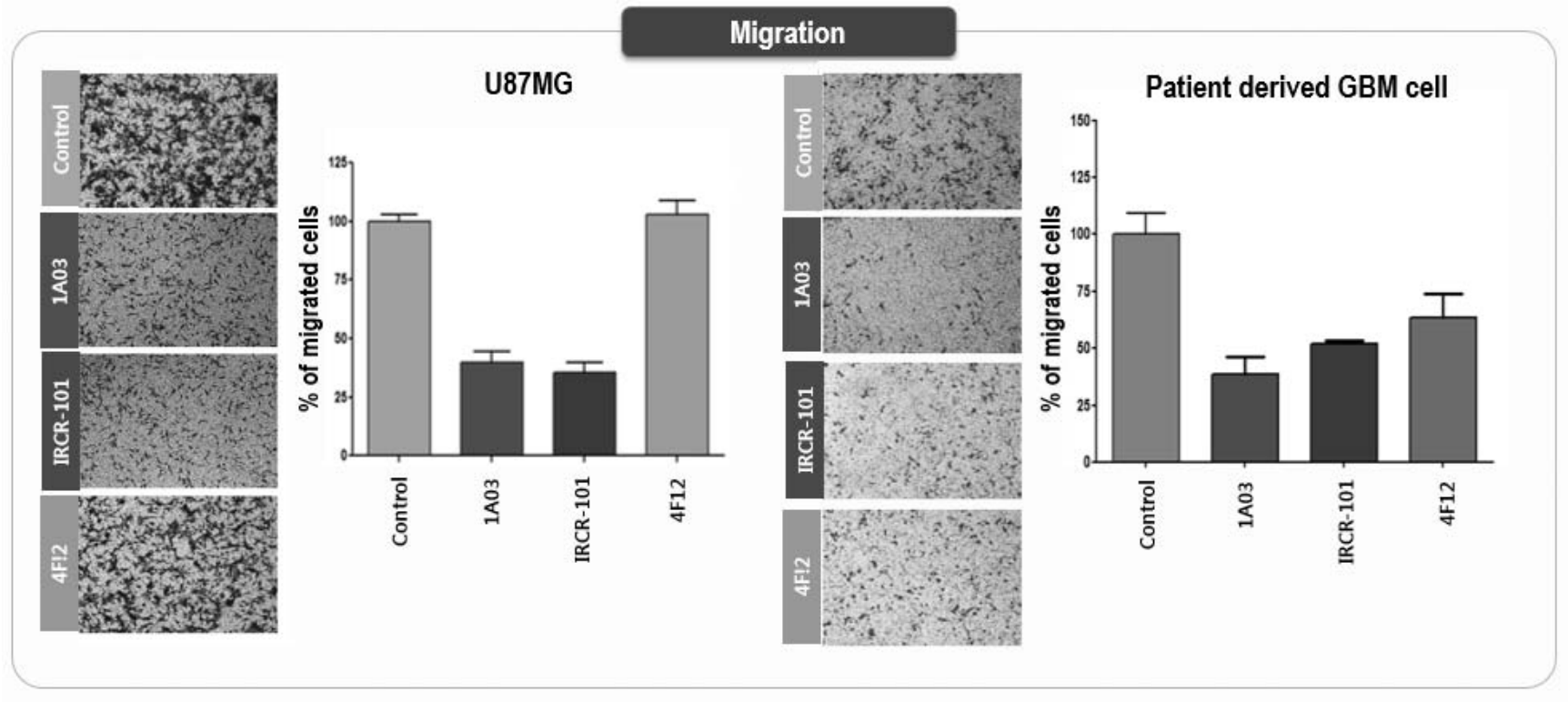




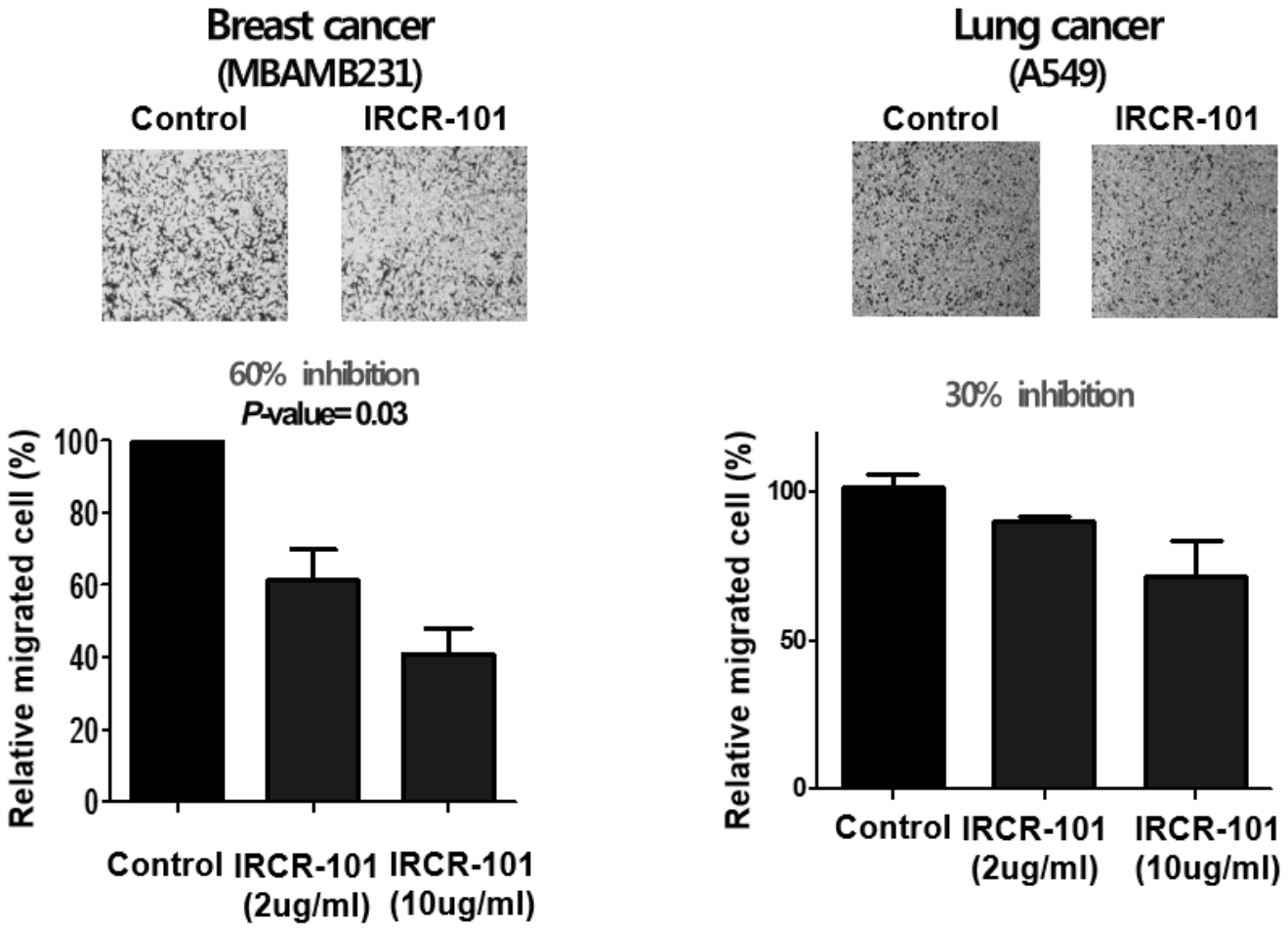
【Fig. 19】(cont.)


Anti-NRP1 (Commercial NRP1 Antibody)
Normalized To Mode
PE-A :: NRP1 PE-A
HUVEC
FSC-A
PE-A :: NRP1 PE-A
Tumor cell
FSC-A
PE-A :: NRP1 PE-A
MFI
131
HUVEC
Antibody concentration

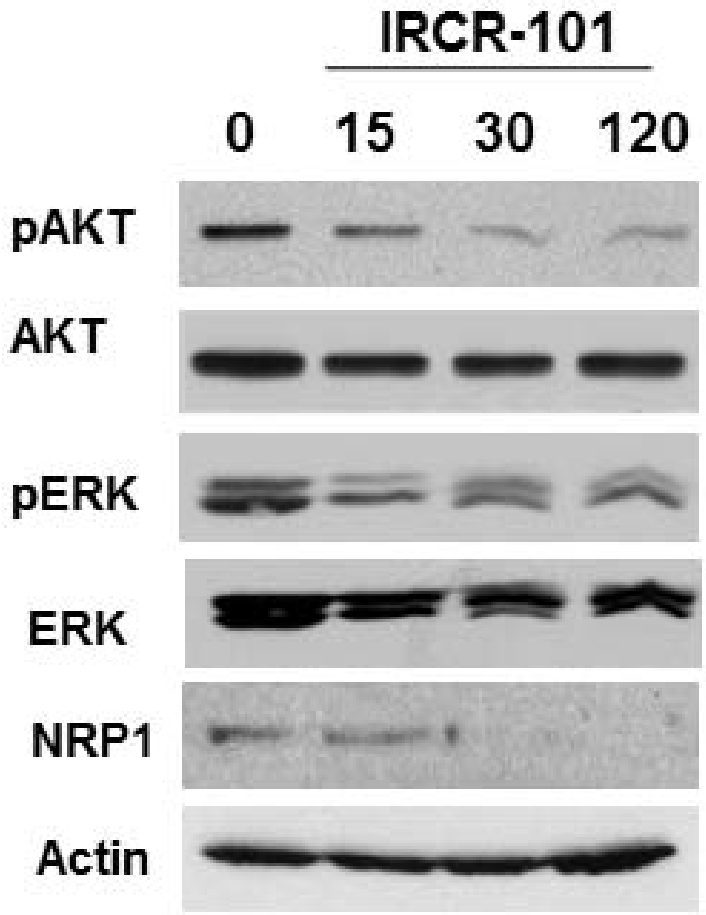
【Fig. 20】


Migration
U87MG
Control
1A03
IRCR-101
4F12
% of migrated cells
0
25
50
75
100
125
Control
1A03
IRCR-101
4F12
Patient derived GBM cell
Control
1A03
IRCR-101
4F12
% of migrated cells
0
25
50
75
100
125
150
Control
1A03
IRCR-101
4F12

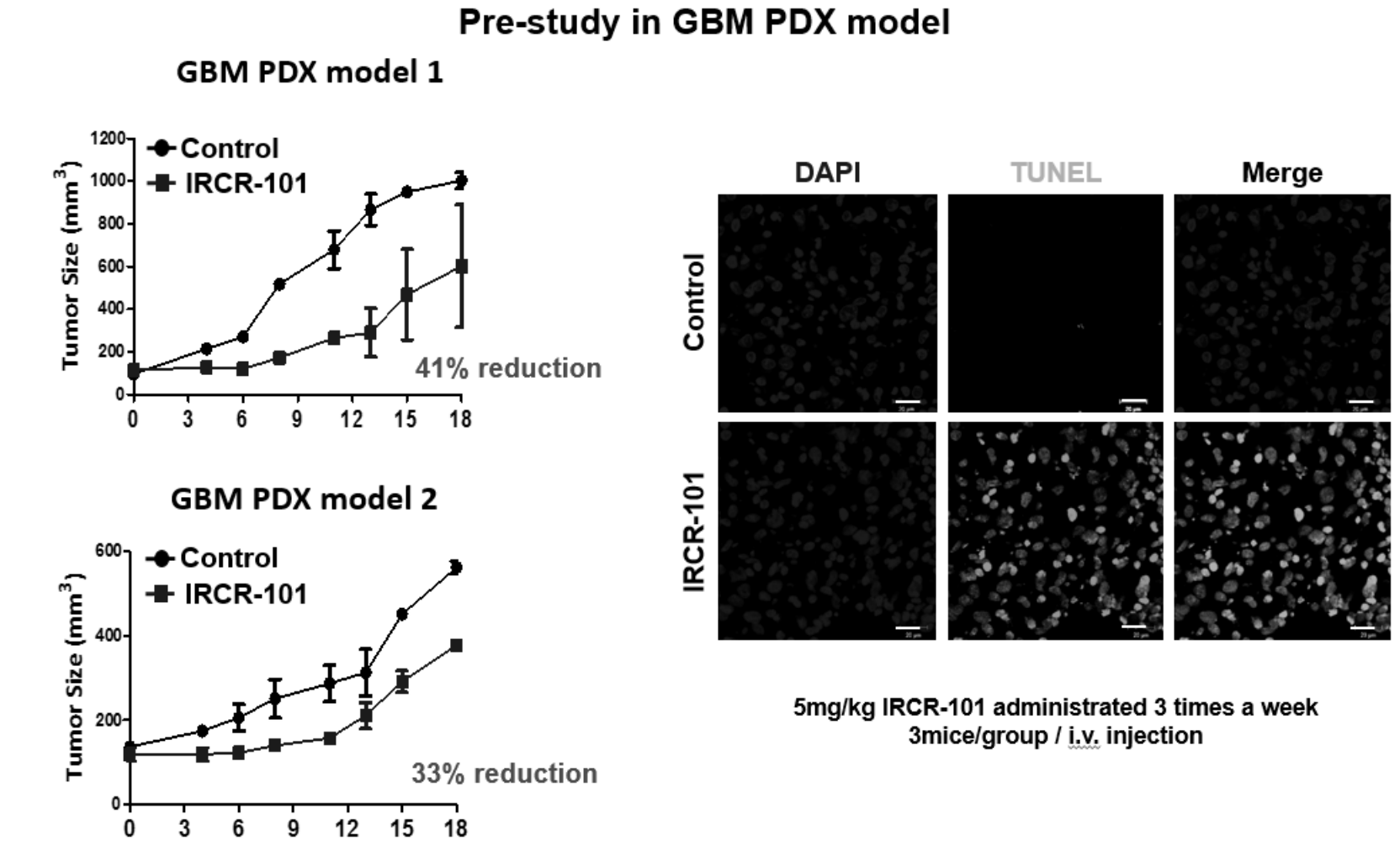
【Fig. 21】


Breast cancer
(MBAMB231)
Control
IRCR-101
60% inhibition
P-value= 0.03
Relative migrated cell (%)
100
80
60
40
20
0
Control
IRCR-101 (2ug/ml)
IRCR-101 (10ug/ml)
Lung cancer
(A549)
Control
IRCR-101
30% inhibition
Relative migrated cell (%)
100
50
0
Control
IRCR-101 (2ug/ml)
IRCR-101 (10ug/ml)

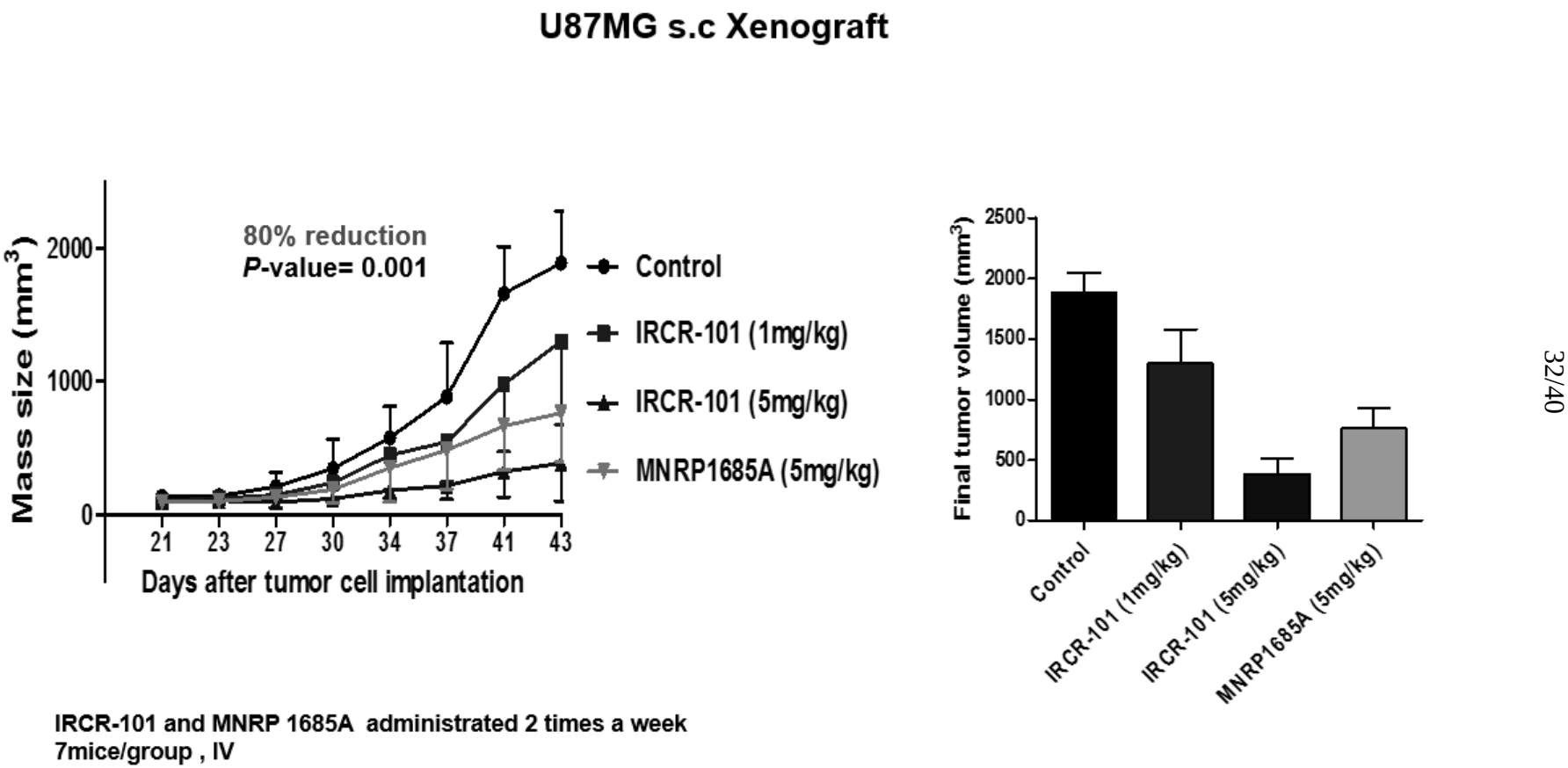
【Fig. 22】



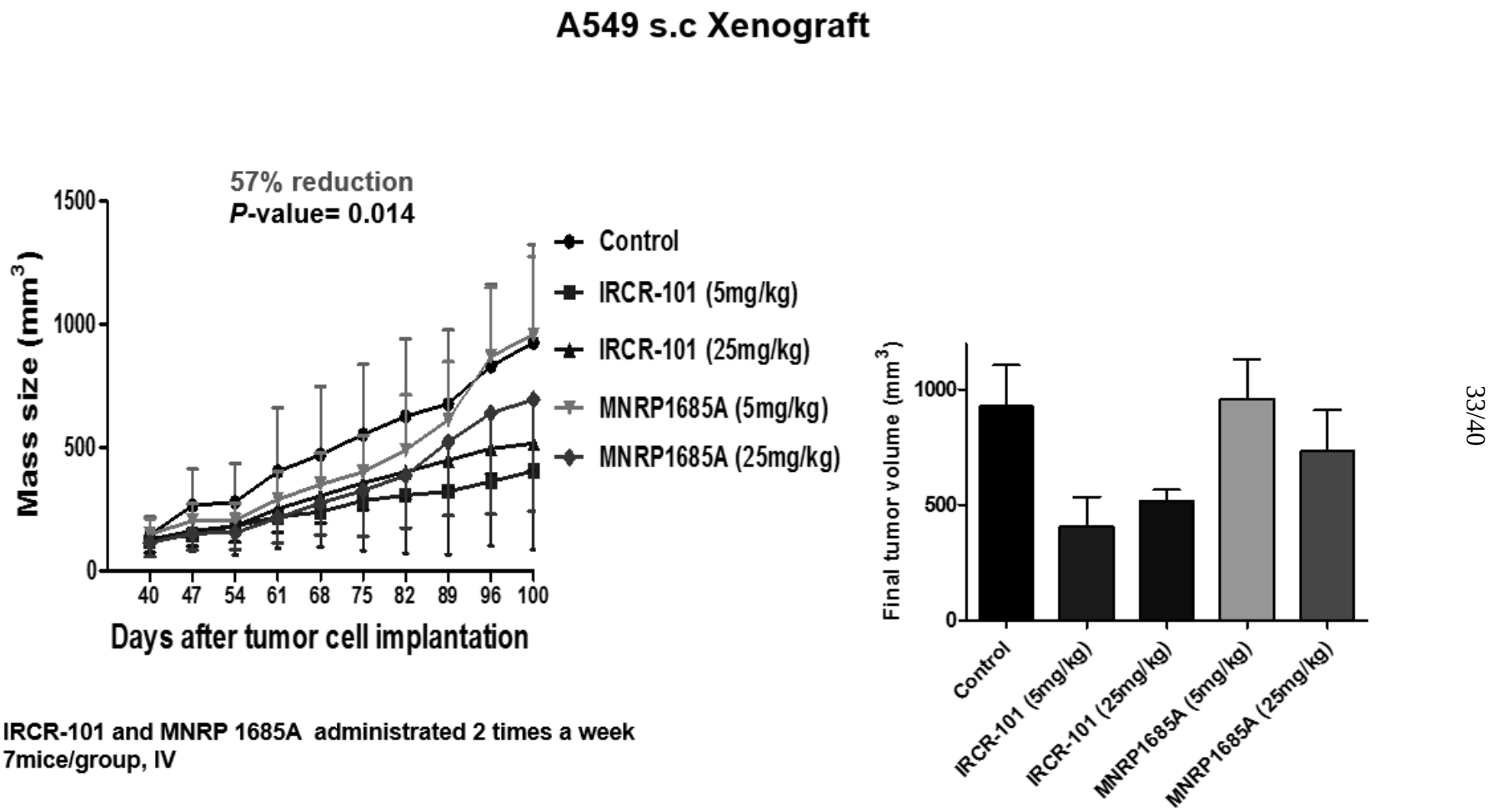
【Fig. 23】


Pre-study in GBM PDX model
GBM PDX model 1
Control
IRCR-101
Tumor Size (mm3)
41% reduction
GBM PDX model 2
Control
IRCR-101
Tumor Size (mm3)
33% reduction
DAPI
TUNEL
Merge
Control
IRCR-101
5mg/kg IRCR-101 administrated 3 times a week
3mice/group / i.v. injection

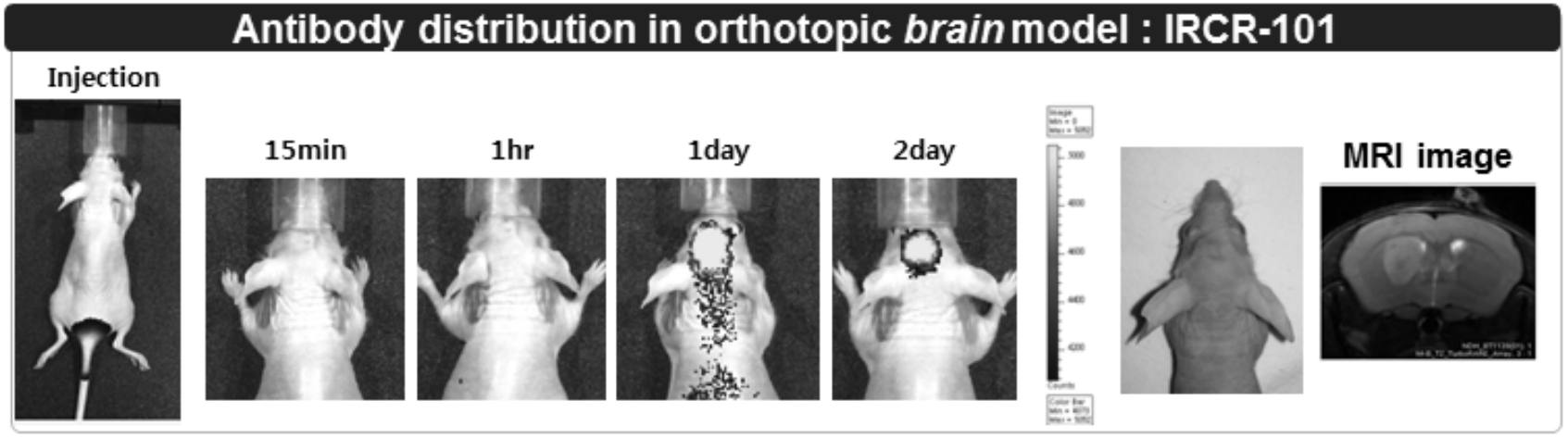
【Fig. 24】


U87MG s.c Xenograft
80% reduction
P-value= 0.001
Mass size (mm3)
2000
1000
0
21
23
27
30
34
37
41
43
Days after tumor cell implantation
Control
IRCR-101 (1mg/kg)
IRCR-101 (5mg/kg)
MNRP1685A (5mg/kg)
Final tumor volume (mm3)
2500
2000
1500
1000
500
0
Control
IRCR-101 (1mg/kg)
IRCR-101 (5mg/kg)
MNRP1685A (5mg/kg)
IRCR-101 and MNRP 1685A administrated 2 times a week
7mice/group , IV

【Fig. 25】


A549 s.c Xenograft
57% reduction
P-value= 0.014
Mass size (mm3)
1500
1000
500
0
40 47 54 61 68 75 82 89 96 100
Days after tumor cell implantation
Control
IRCR-101 (5mg/kg)
IRCR-101 (25mg/kg)
MNRP1685A (5mg/kg)
MNRP1685A (25mg/kg)
IRCR-101 and MNRP 1685A administrated 2 times a week
7mice/group, IV
Final tumor volume (mm3)
1000
500
0
Control
IRCR-101 (5mg/kg)
IRCR-101 (25mg/kg)
MNRP1685A (5mg/kg)
MNRP1685A (25mg/kg)

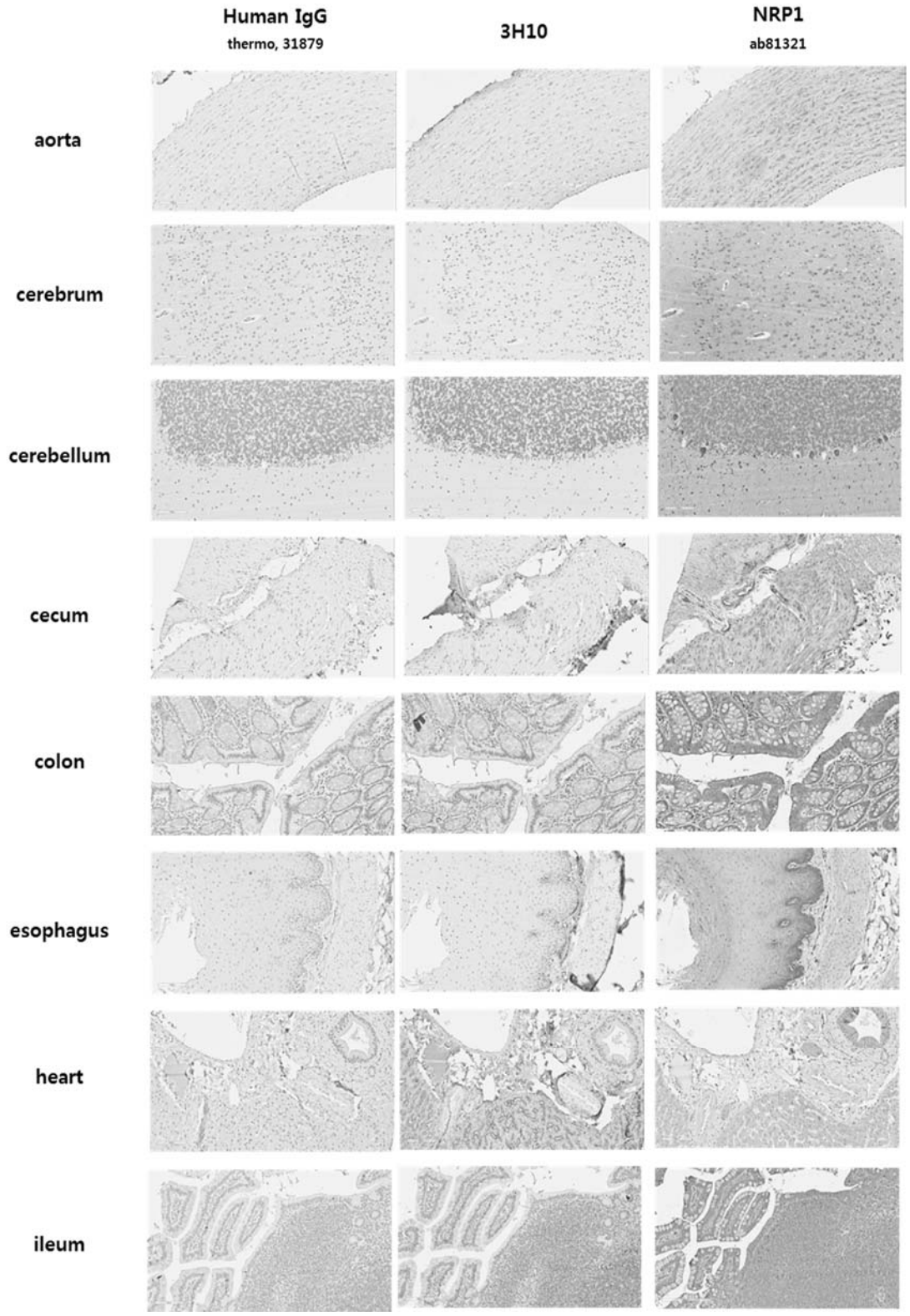
【Fig. 26】


Antibody distribution in orthotopic *brain* model : IRCR-101
Injection
15min
1hr
1day
2day
MRI image

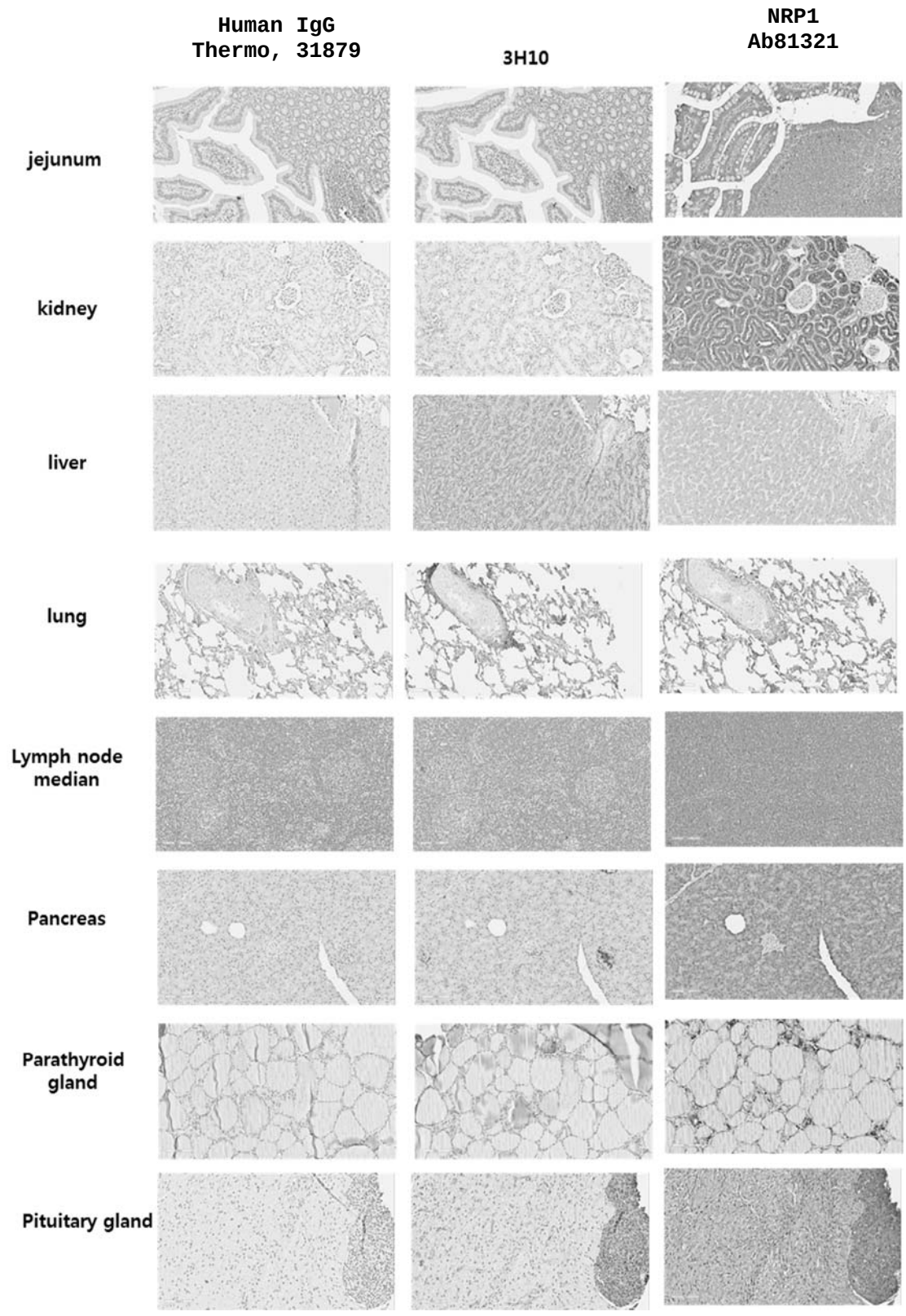
【Fig. 27】

	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	-	+	++++
Vestibular gland	-	+	+++++
Testis	-	-	+++
Epididymis	-	-	+++

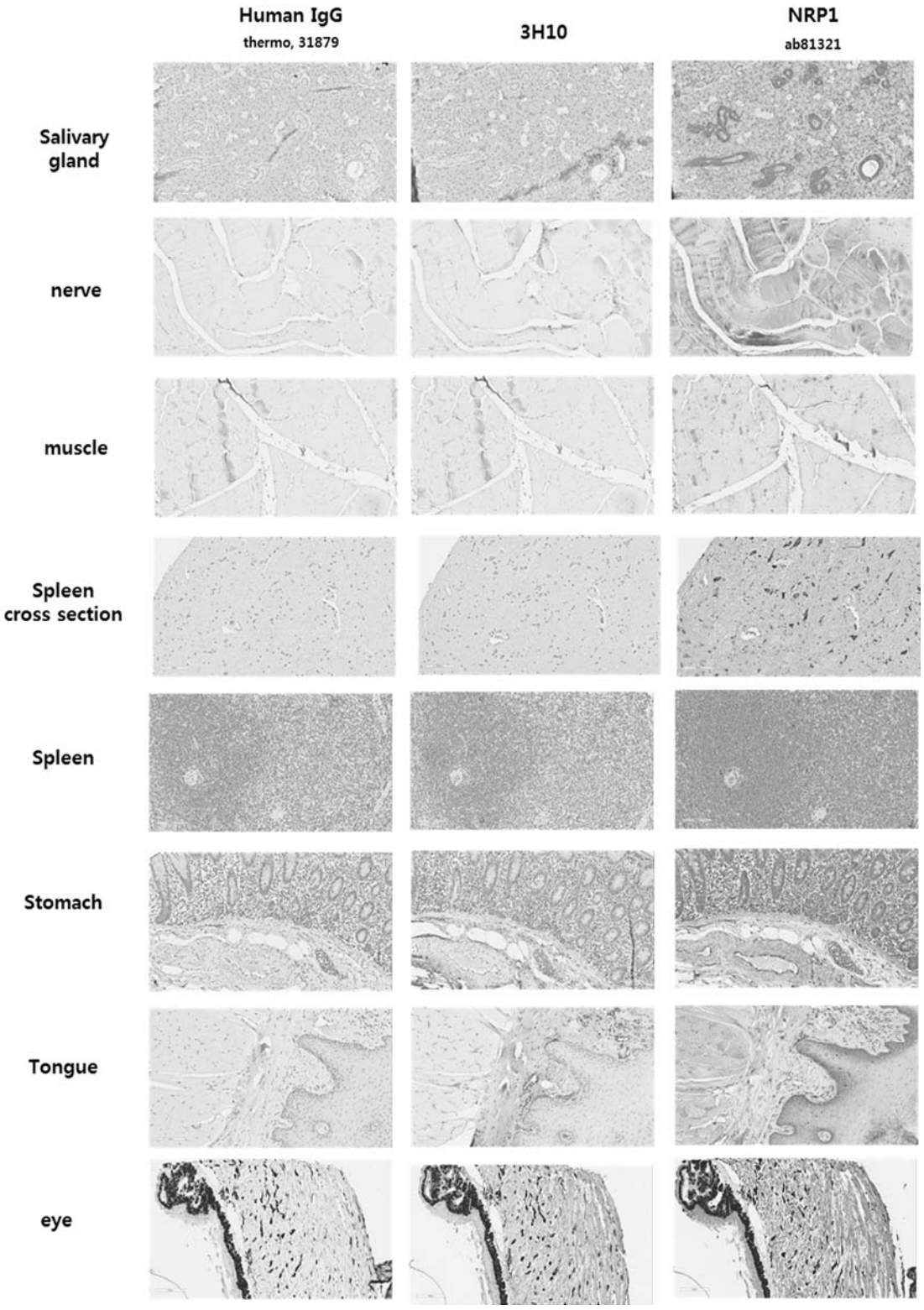
【Fig. 27】(cont.)


Human IgG
thermo, 31879
3H10
NRP1
ab81321
aorta
cerebrum
cerebellum
cecum
colon
esophagus
heart
ileum

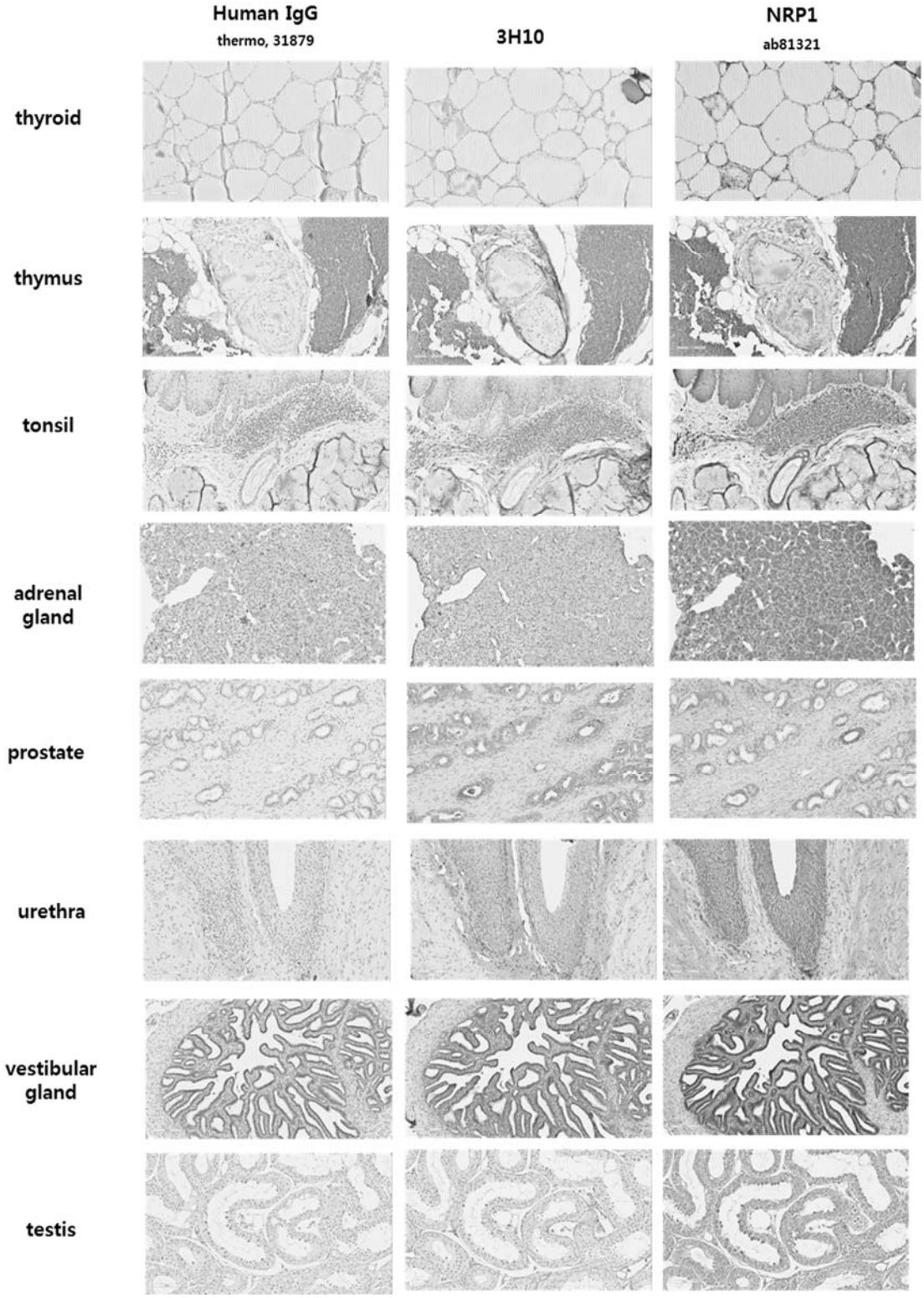
【Fig. 27】(cont.)


Human IgG
Thermo, 31879
3H10
NRP1
Ab81321
jejunum
kidney
liver
lung
Lymph node
median
Pancreas
Parathyroid
gland
Pituitary gland

【Fig. 27】(cont.)


Human IgG
thermo, 31879
3H10
NRP1
ab81321
Salivary gland
nerve
muscle
Spleen cross section
Spleen
Stomach
Tongue
eye

【Fig. 27】(cont.)


Human IgG
thermo, 31879
3H10
NRP1
ab81321
thyroid
thymus
tonsil
adrenal gland
prostate
urethra
vestibular gland
testis

【Fig. 27】(cont.)

