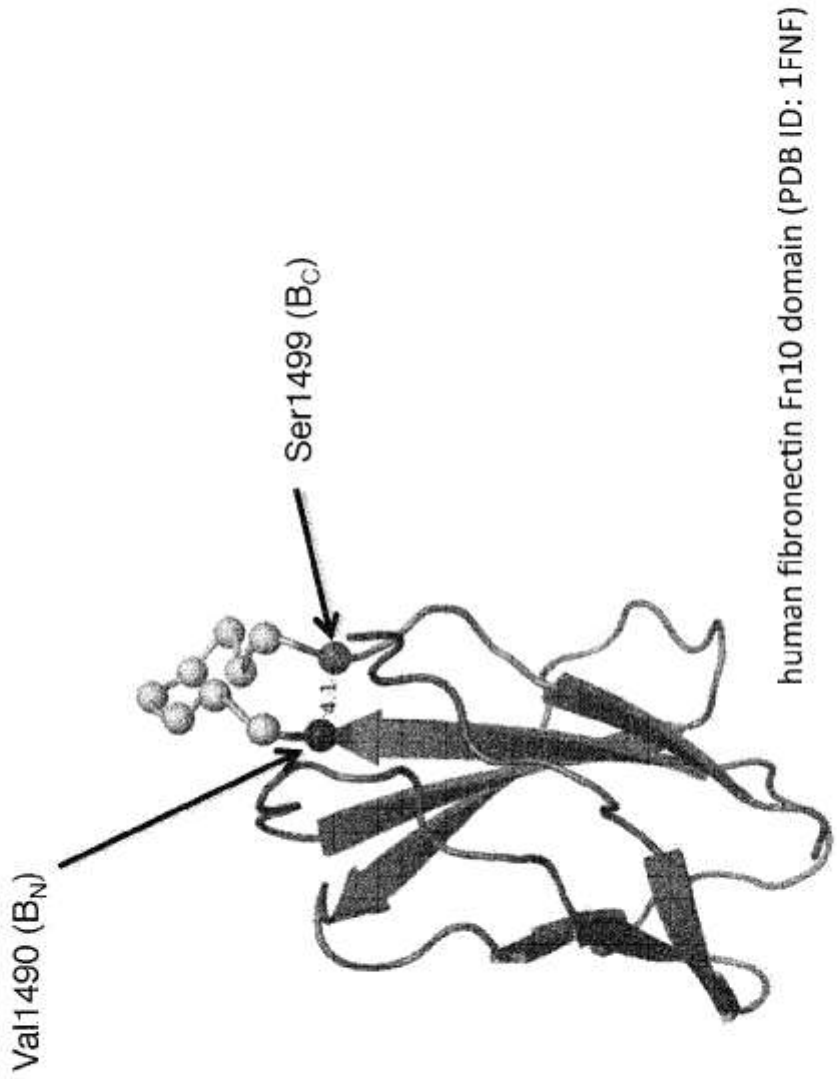
Figures

[Fig.1]

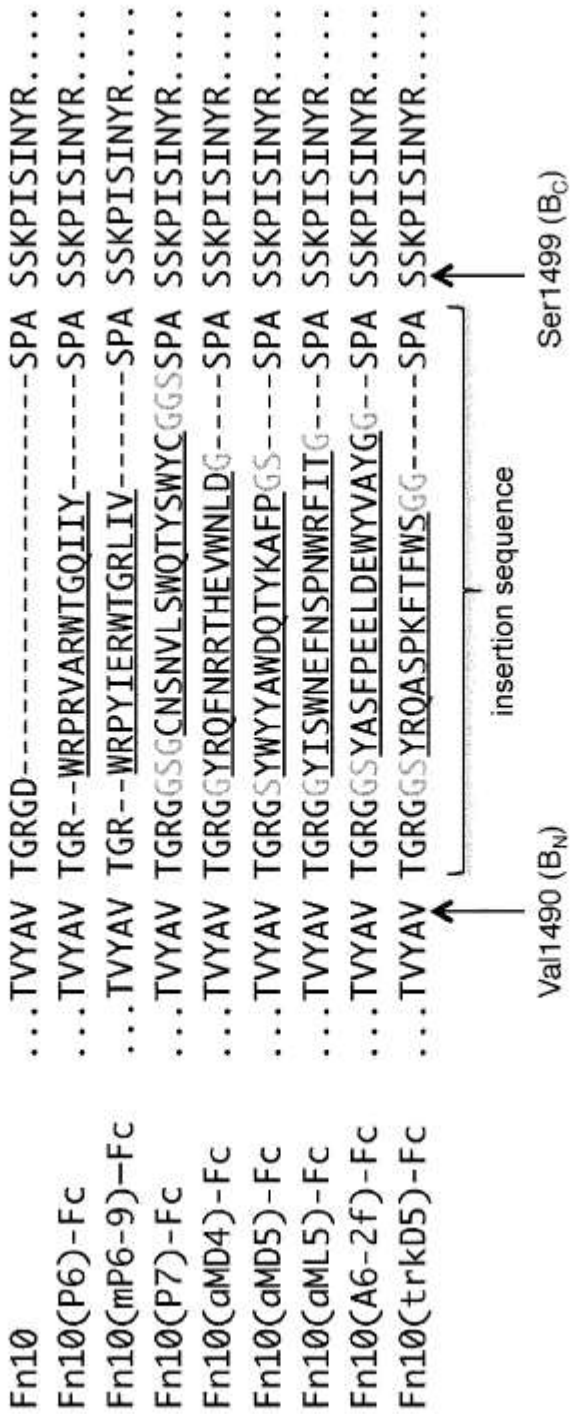


name of cyclic peptide	binding molecule	amino acid sequence of variable region
P6	human Plexin B1	wRPRVARWTGQIIY
mP6-9	human Plexin B1	wRPYIERWTGRLIV
P7	human Plexin B1	wNSNVLSWQTYSWY
aMD4	human Met receptor	yRQFNRRTHEVWNLD
aMD5	human Met receptor	yWYYAWDQTYKAFP
aML5	human Met receptor	YISWNEFNSPNWRFIT
A6-2f	human EGF receptor	yASFPEELDEWYVAYG
trkD5	human TrkB	yRQASPKFTFWS

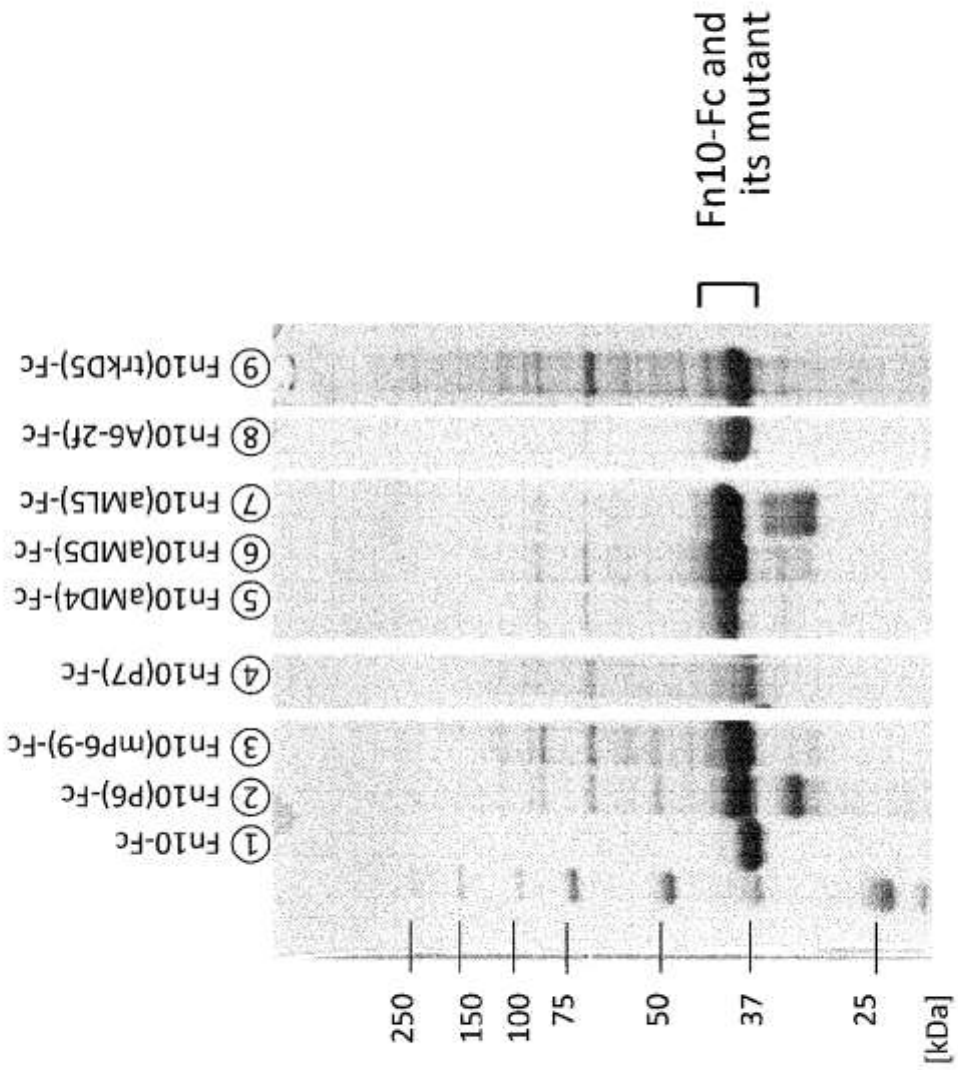
[Fig.2]


Val1490 (B_N)
Ser1499 (B_C)
4.1
human fibronectin Fn10 domain (PDB ID: 1FNF)

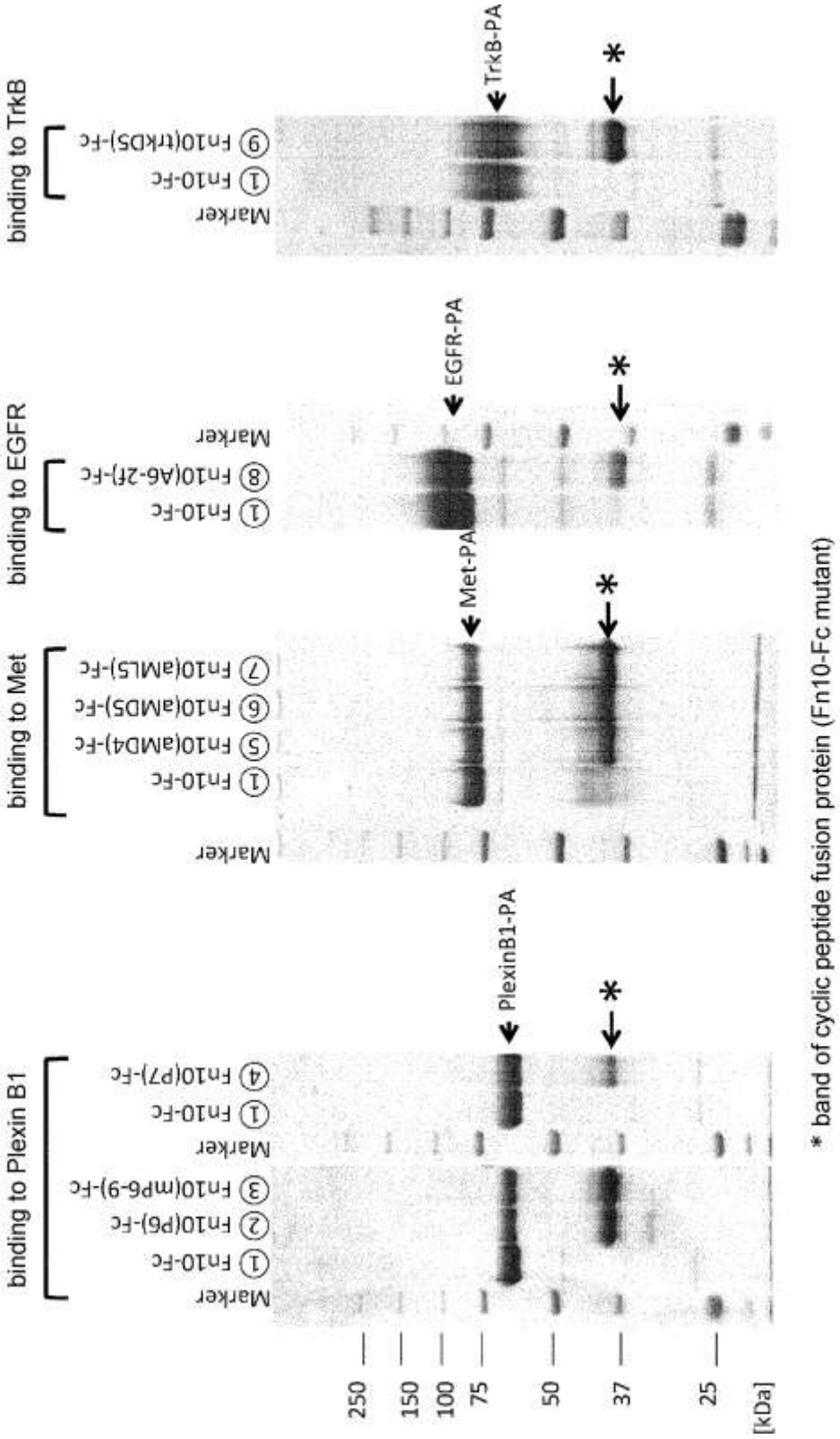
[Fig.3]


Fn10 ...TVYAV TGRGD----------SPA SSKPISINYR....
Fn10(P6)-Fc ...TVYAV TGR--WRPRVARWTGQIIY-----SPA SSKPISINYR....
Fn10(mP6-9)-Fc ...TVYAV TGR--WRPYIERWTGRLIV-----SPA SSKPISINYR....
Fn10(P7)-Fc ...TVYAV TGRGGSGCNSNVLSWQTYSWYCGGSSPA SSKPISINYR....
Fn10(aMD4)-Fc ...TVYAV TGRGGYRQFNRRTHEVWNLDG----SPA SSKPISINYR....
Fn10(aMD5)-Fc ...TVYAV TGRGSYWYYAWDQTYKAFPGS----SPA SSKPISINYR....
Fn10(aML5)-Fc ...TVYAV TGRGGYISWNEFNSPNWRFITG---SPA SSKPISINYR....
Fn10(A6-2f)-Fc ...TVYAV TGRGGSYASFPEELDEWYVAYGG--SPA SSKPISINYR....
Fn10(trkD5)-Fc ...TVYAV TGRGGSYRQASPKFTFWSGG----SPA SSKPISINYR....
Val1490 (B_N)
insertion sequence
Ser1499 (B_C)

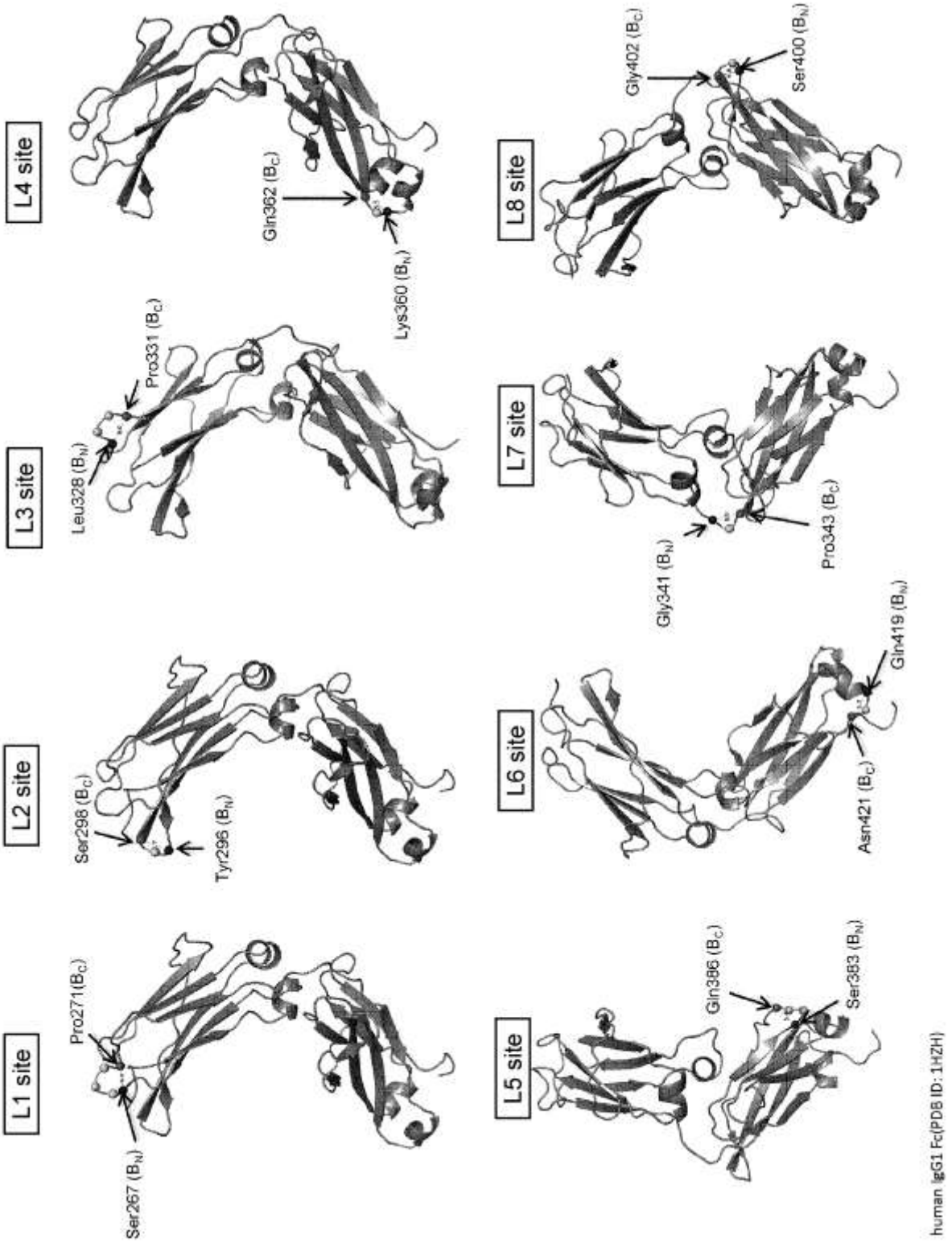
[Fig.4]


Fn10-Fc and its mutant
250
150
100
75
50
37
25
[kDa]
① Fn10-Fc
② Fn10(P6)-Fc
③ Fn10(mP6-9)-Fc
④ Fn10(P7)-Fc
⑤ Fn10(aMD4)-Fc
⑥ Fn10(aMD5)-Fc
⑦ Fn10(aML5)-Fc
⑧ Fn10(A6-2f)-Fc
⑨ Fn10(trkD5)-Fc

[Fig.5]


binding to Plexin B1
Marker
① Fn10-Fc
② Fn10(P6)-Fc
③ Fn10(mP6-9)-Fc
Marker
① Fn10-Fc
④ Fn10(P7)-Fc
PlexinB1-PA
*
binding to Met
Marker
① Fn10-Fc
⑤ Fn10(aMD4)-Fc
⑥ Fn10(aMD5)-Fc
⑦ Fn10(aML5)-Fc
Met-PA
*
binding to EGFR
Marker
① Fn10-Fc
⑧ Fn10(A6-2f)-Fc
EGFR-PA
*
binding to TrkB
Marker
① Fn10-Fc
⑨ Fn10(trkD5)-Fc
TrkB-PA
*
250
150
100
75
50
37
25
[kDa]
* band of cyclic peptide fusion protein (Fn10-Fc mutant)

[Fig.6]


L1 site
Pro271 (B_C)
Ser267 (B_N)
L2 site
Ser298 (B_C)
Tyr296 (B_N)
L3 site
Leu328 (B_N)
Pro331 (B_C)
L4 site
Gln362 (B_C)
Lys360 (B_N)
L5 site
Gln386 (B_C)
Ser383 (B_N)
L6 site
Gln419 (B_N)
Asn421 (B_C)
L7 site
Gly341 (B_N)
Pro343 (B_C)
L8 site
Gly402 (B_C)
Ser400 (B_N)
human IgG1 Fc(PDB ID: 1HZH)

[Fig.7]

1. L1 site mutant
Fc ...VVDVS HE----------D PEVKFN....
Fc(mP6-9_L1) ...VVDVS HGWRPYIERWTGRLIVGG--D PEVKFN....
Fc(αMD4_L1) ...VVDVS HGGYRQFNRRTHEVWNLDGGD PEVKFN....
Fc(αMD5_L1) ...VVDVS HGSYWYYAWDQTYKAFPGS-D PEVKFN....
Ser267 (BN)
insertion sequence
Pro271 (BC)
2. L2 site mutant
Fc ...REEQY ----------N STYRVV....
Fc(mP6-9_L2) ...REEQY GWRPYIERWTGRLIVG---N STYRVV....
Fc(αMD4_L2) ...REEQY GGYRQFNRRTHEVWNLDGSN STYRVV....
Fc(αMD5_L2) ...REEQY GSYWYYAWDQTYKAFPGS-N STYRVV....
Tyr296 (BN)
insertion sequence
Ser298 (BC)
3. L3 site mutant
Fc ...SNKAL PA---------- PIEKTI....
Fc(mP6-9_L3) ...SNKAL PGWRPYIERWTGRLIVG--- PIEKTI....
Fc(αMD4_L3) ...SNKAL PGGYRQFNRRTHEVWNLDGS PIEKTI....
Fc(αMD5_L3) ...SNKAL PGSYWYYAWDQTYKAFPGS- PIEKTI....
Leu328 (BN)
insertion sequence
Pro331 (BC)

4. L4 site mutant

```
Fc                ...DELTK ---------N---------- QVSLTC....
Fc(mP6-9_L4)      ...DELTK -GWRPYIERWTGRLIVGG-- QVSLTC....
Fc(αMD4_L4)       ...DELTK GGYRQFNRRTHEVWNLDGG- QVSLTC....
Fc(αMD5_L4)       ...DELTK SGGYWYYAWDQTYKAFPGGS QVSLTC....
```

Lys360(B_N) — insertion sequence — Gln362(B_C)

5. L5 site mutant

```
Fc                ...VEWES --------N----------G QPENNY....
Fc(mP6-9_L5)      ...VEWES -GWRPYIERWTGRLIVGG--G QPENNY....
Fc(αMD4_L5)       ...VEWES GGYRQFNRRTHEVWNLDGG-G QPENNY....
Fc(αMD5_L5)       ...VEWES SGGYWYYAWDQTYKAFPGGSG QPENNY....
```

Ser383(B_N) — insertion sequence — Gln386 (B_C)

6. L6 site mutant

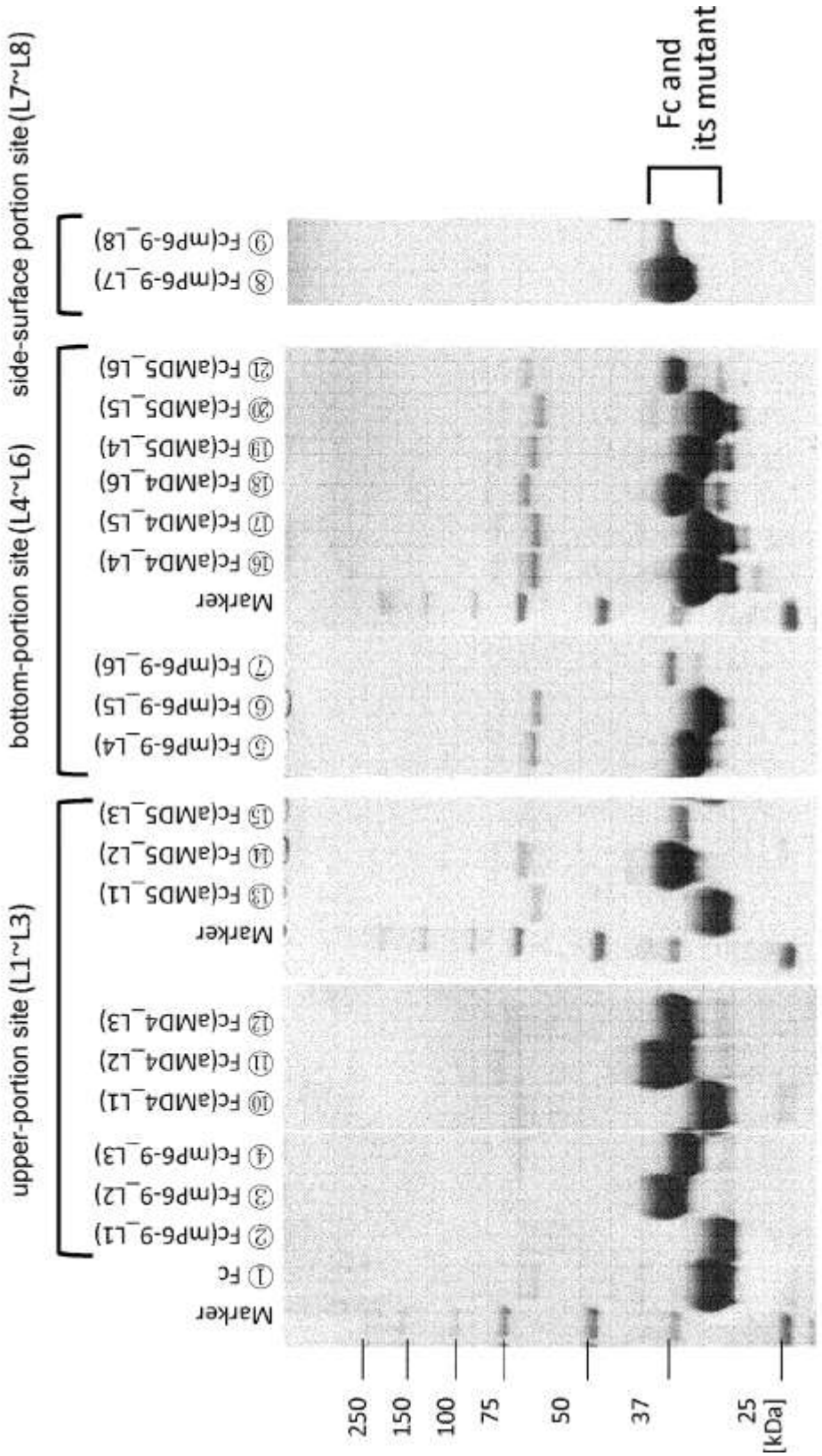
```
Fc                ...SRWQQ G------------------- NVFSCS....
Fc(mP6-9_L6)      ...SRWQQ GWRPYIERWTGRLIVGG--- NVFSCS....
Fc(αMD4_L6)       ...SRWQQ GGYRQFNRRTHEVWNLDGG- NVFSCS....
Fc(αMD5_L6)       ...SRWQQ GSGYWYYAWDQTYKAFPGSG NVFSCS....
```

Gln419 (B_N) — insertion sequence — Asn421 (B_C)

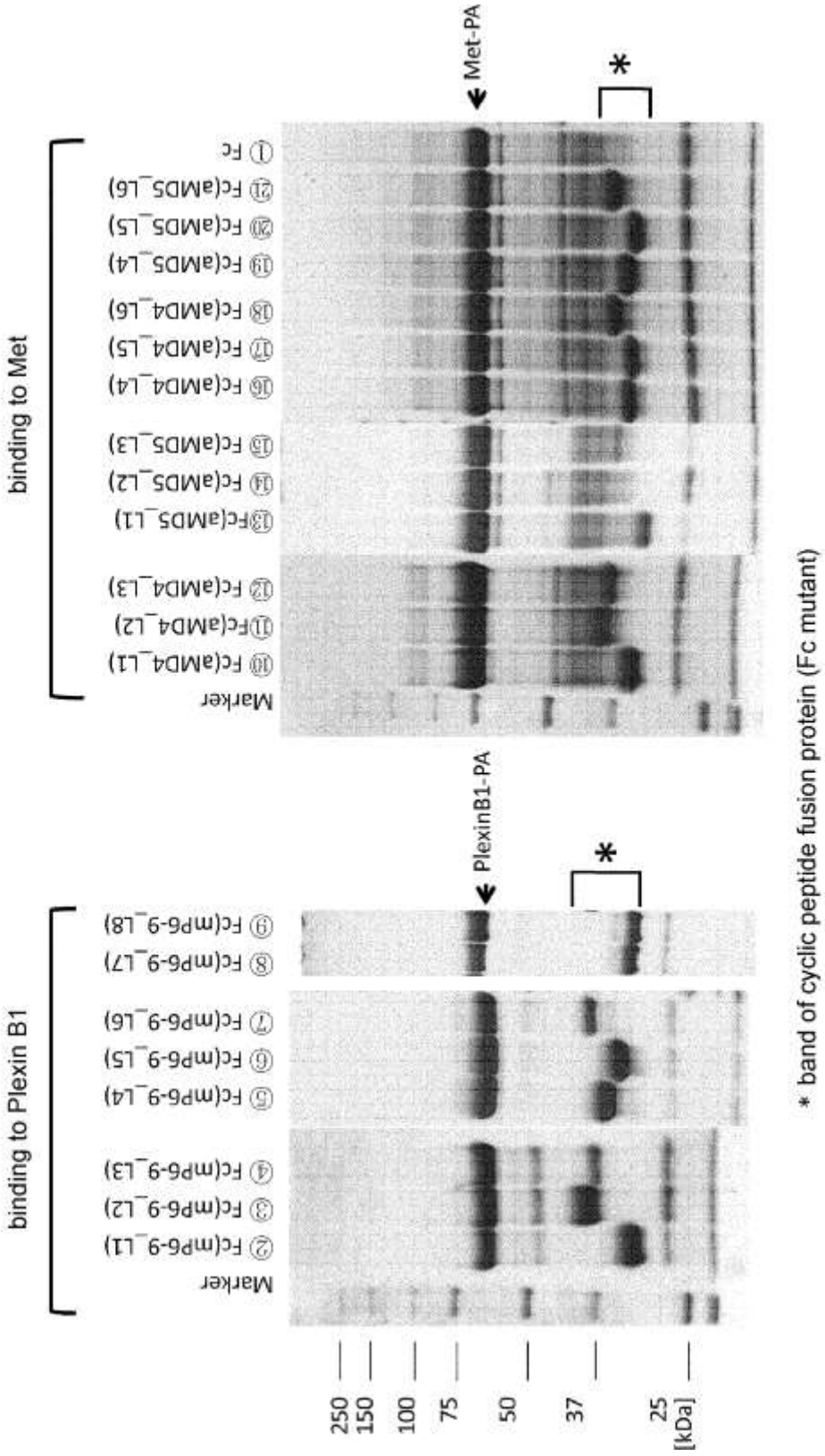
[Fig.9]

7. L7 site mutant
Fc
Fc(mP6-9_L7)
...SKSKG Q---------- PREPQV....
...SKSKG WRPYIERWTGRLIVGGQ PREPQV....
Gly341 (BN)
insertion sequence
Pro343 (BC)
8. L8 site mutant
Fc
Fc(mP6-9_L8)
...PVLDS D---------- GSFFLY....
...PVLDS GWRPYIERWTGRLIVGG GSFFLY....
Ser400 (BN)
insertion sequence
Gly402 (BC)

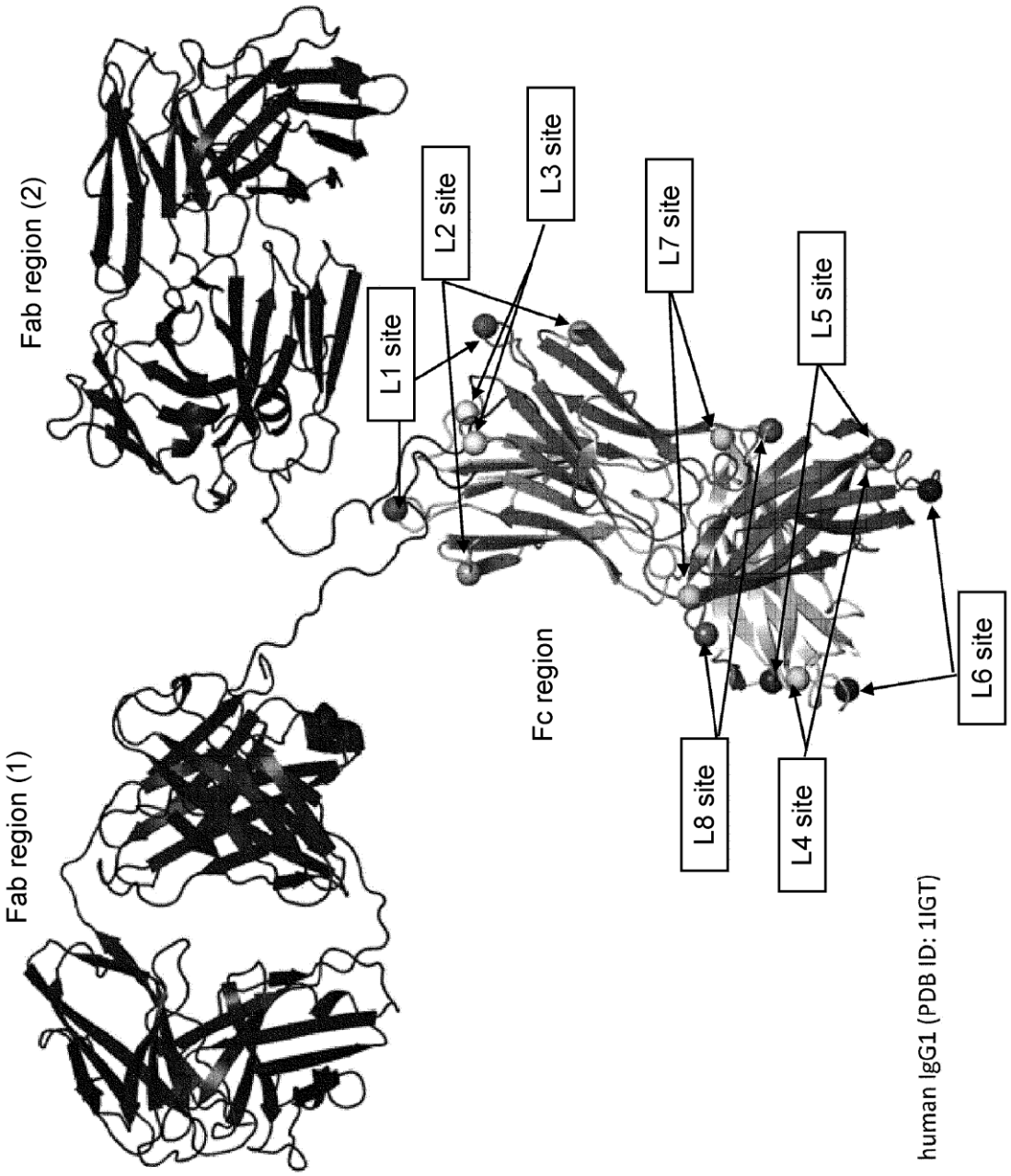
[Fig.10]


Fc and its mutant
250
150
100
75
50
37
25
[kDa]
Marker
① Fc
② Fc(mP6-9_L1)
③ Fc(mP6-9_L2)
④ Fc(mP6-9_L3)
⑩ Fc(aMD4_L1)
⑪ Fc(aMD4_L2)
⑫ Fc(aMD4_L3)
Marker
⑬ Fc(aMD5_L1)
⑭ Fc(aMD5_L2)
⑮ Fc(aMD5_L3)
upper-portion site (L1~L3)
⑤ Fc(mP6-9_L4)
⑥ Fc(mP6-9_L5)
⑦ Fc(mP6-9_L6)
Marker
⑯ Fc(aMD4_L4)
⑰ Fc(aMD4_L5)
⑱ Fc(aMD4_L6)
⑲ Fc(aMD5_L4)
⑳ Fc(aMD5_L5)
㉑ Fc(aMD5_L6)
bottom-portion site (L4~L6)
⑧ Fc(mP6-9_L7)
⑨ Fc(mP6-9_L8)
side-surface portion site (L7~L8)

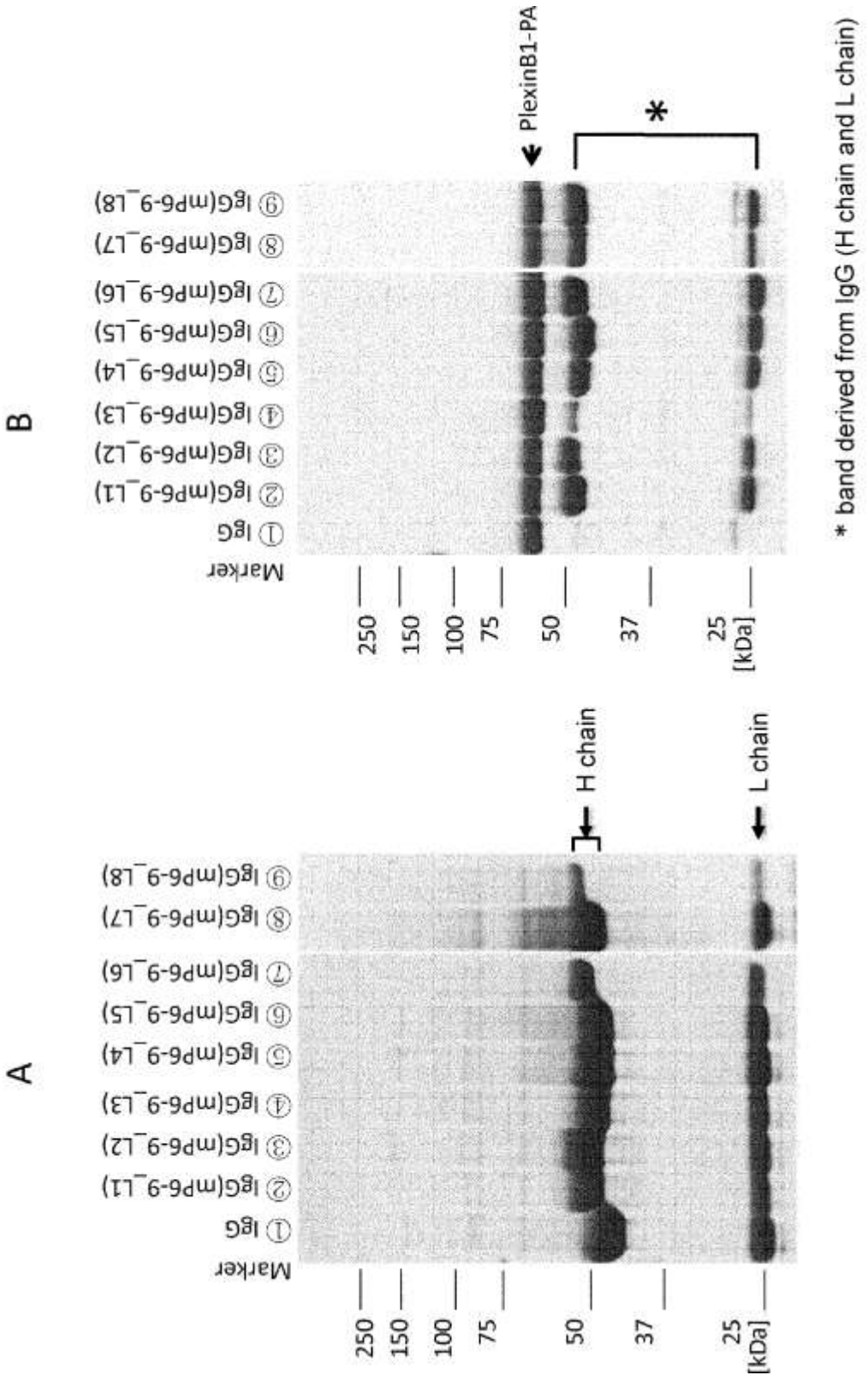
[Fig.11]


binding to Plexin B1
Marker
② Fc(mP6-9_L1)
③ Fc(mP6-9_L2)
④ Fc(mP6-9_L3)
⑤ Fc(mP6-9_L4)
⑥ Fc(mP6-9_L5)
⑦ Fc(mP6-9_L6)
⑧ Fc(mP6-9_L7)
⑨ Fc(mP6-9_L8)
PlexinB1-PA
*
binding to Met
Marker
⑩ Fc(aMD4_L1)
⑪ Fc(aMD4_L2)
⑫ Fc(aMD4_L3)
⑬ Fc(aMD5_L1)
⑭ Fc(aMD5_L2)
⑮ Fc(aMD5_L3)
⑯ Fc(aMD4_L4)
⑰ Fc(aMD4_L5)
⑱ Fc(aMD4_L6)
⑲ Fc(aMD5_L4)
⑳ Fc(aMD5_L5)
㉑ Fc(aMD5_L6)
① Fc
Met-PA
*
250
150
100
75
50
37
25
[kDa]
* band of cyclic peptide fusion protein (Fc mutant)

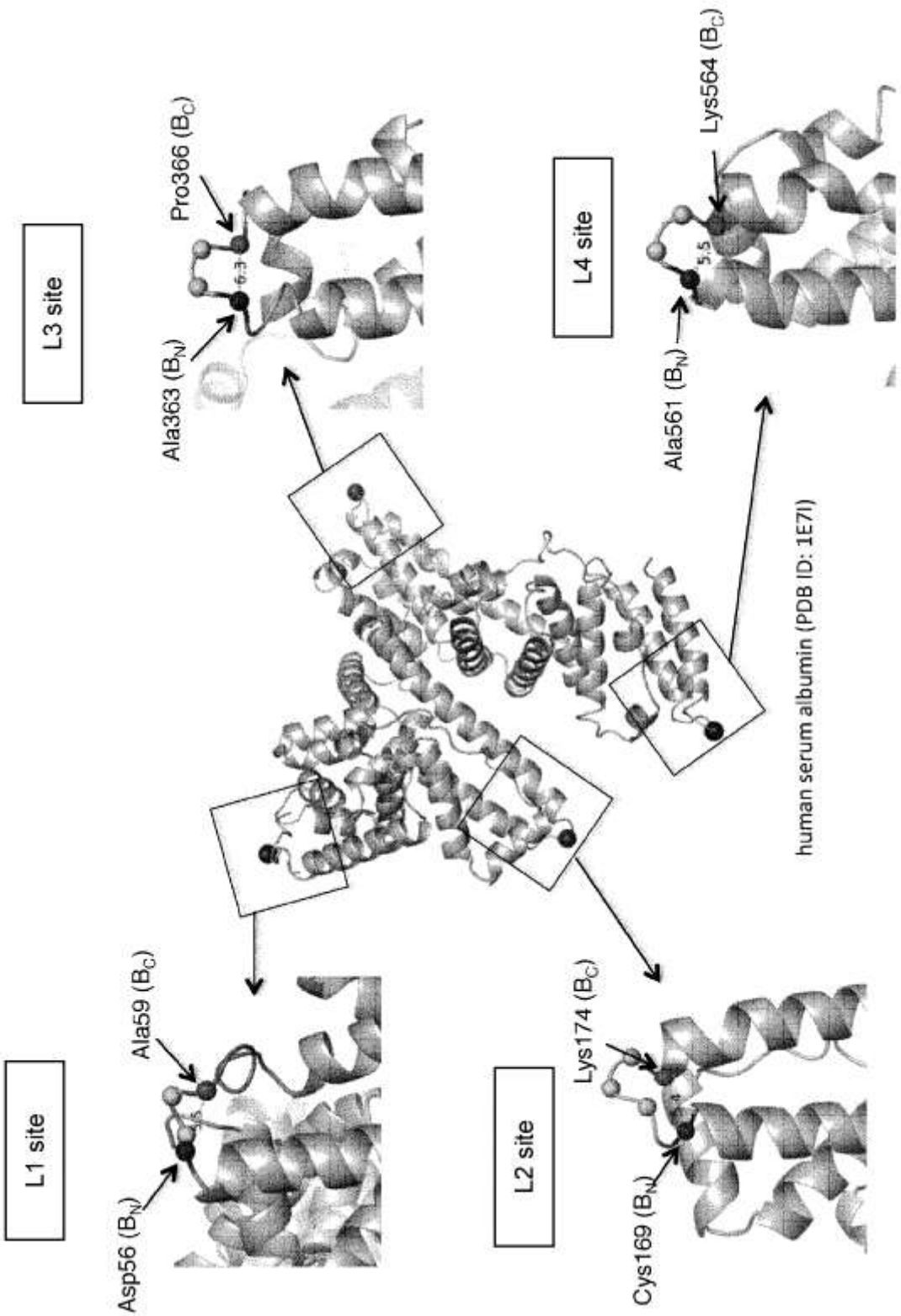
[Fig.12]


Fab region (2)
Fab region (1)
Fc region
L1 site
L2 site
L3 site
L7 site
L5 site
L6 site
L8 site
L4 site
human IgG1 (PDB ID: 1IGT)

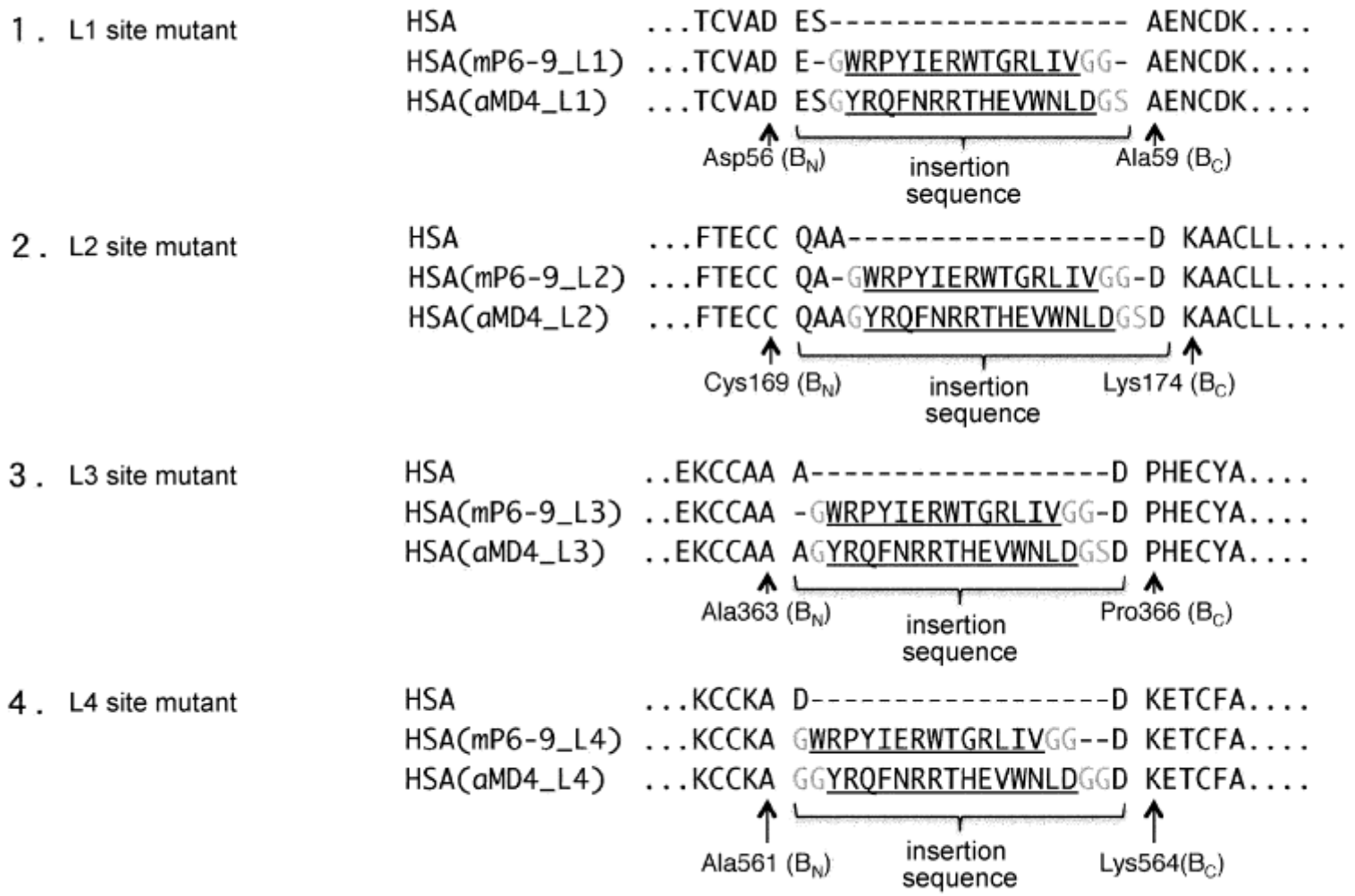
[Fig.13]


A
B
Marker
① IgG
② IgG(mP6-9_L1)
③ IgG(mP6-9_L2)
④ IgG(mP6-9_L3)
⑤ IgG(mP6-9_L4)
⑥ IgG(mP6-9_L5)
⑦ IgG(mP6-9_L6)
⑧ IgG(mP6-9_L7)
⑨ IgG(mP6-9_L8)
250
150
100
75
50
37
25
[kDa]
H chain
L chain
PlexinB1-PA
*
* band derived from IgG (H chain and L chain)

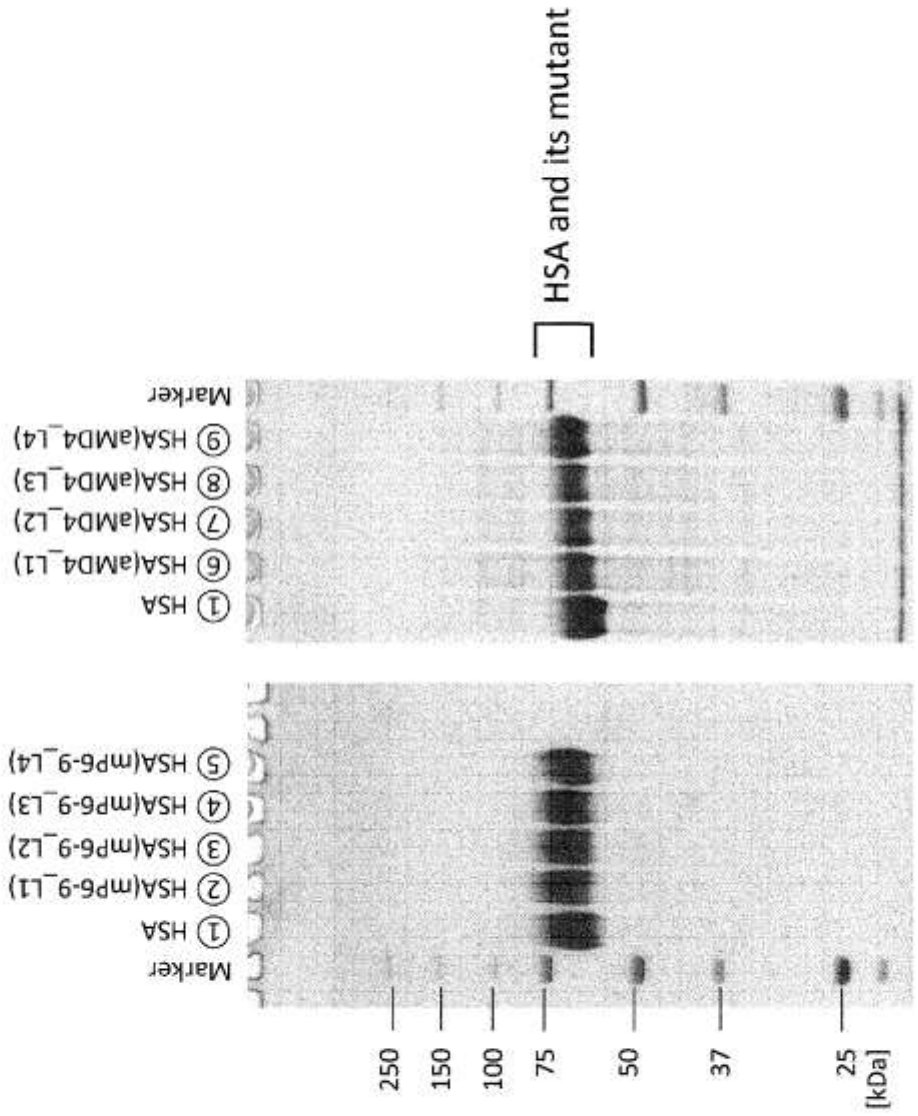
[Fig.14]


L1 site
L2 site
L3 site
L4 site
Asp56 (B_N)
Ala59 (B_C)
Cys169 (B_N)
Lys174 (B_C)
Ala363 (B_N)
Pro366 (B_C)
Ala561 (B_N)
Lys564 (B_C)
human serum albumin (PDB ID: 1E7I)

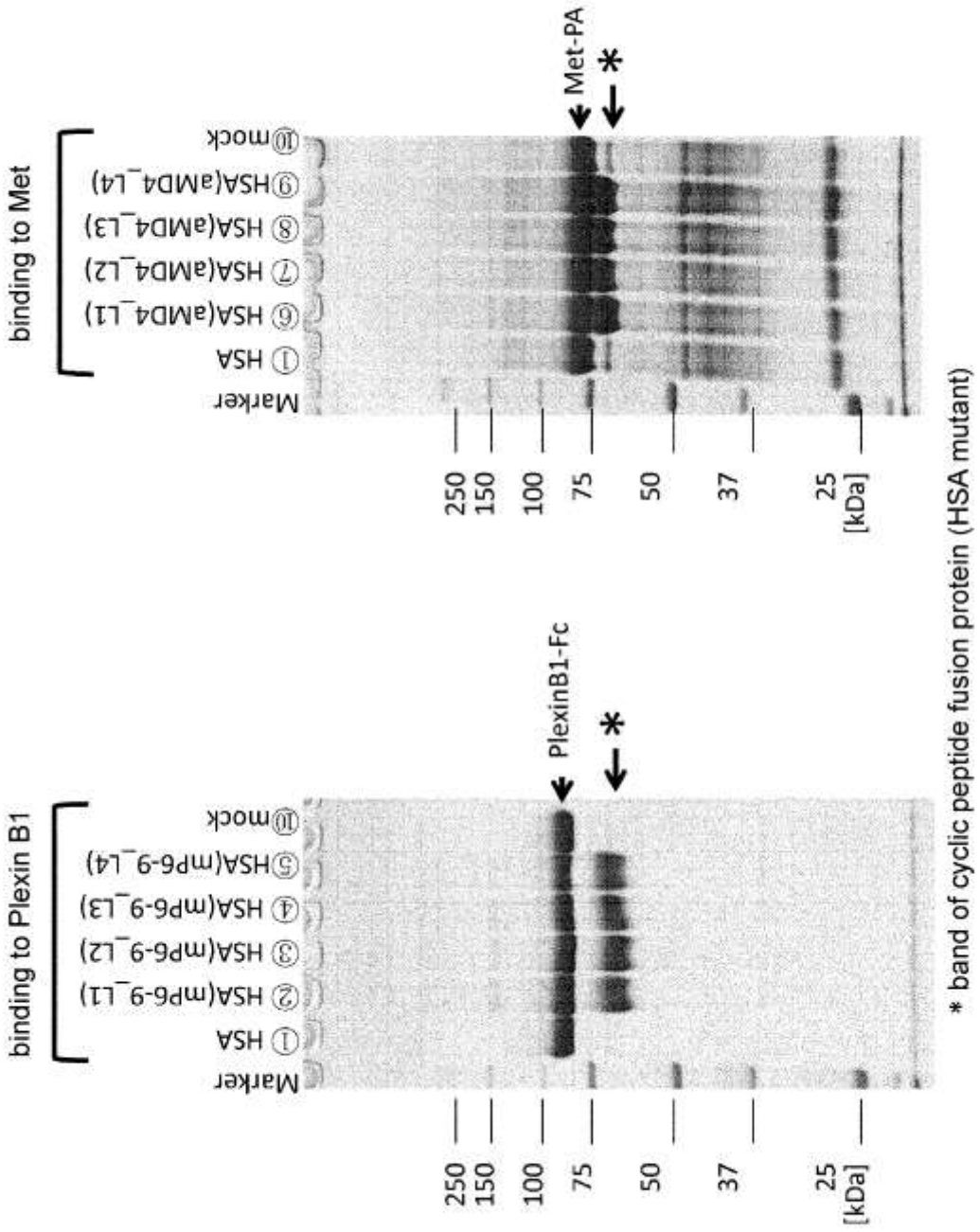
[Fig.15]


1. L1 site mutant
HSA ...TCVAD ES---------- AENCDK....
HSA(mP6-9_L1) ...TCVAD E-GWRPYIERWTGRLIVGG- AENCDK....
HSA(αMD4_L1) ...TCVAD ESGYRQFNRRTHEVWNLDGS AENCDK....
Asp56 (B_N)
insertion sequence
Ala59 (B_C)
2. L2 site mutant
HSA ...FTECC QAA----------D KAACLL....
HSA(mP6-9_L2) ...FTECC QA-GWRPYIERWTGRLIVGG-D KAACLL....
HSA(αMD4_L2) ...FTECC QAAGYRQFNRRTHEVWNLDGSD KAACLL....
Cys169 (B_N)
insertion sequence
Lys174 (B_C)
3. L3 site mutant
HSA ..EKCCAA A----------D PHECYA....
HSA(mP6-9_L3) ..EKCCAA -GWRPYIERWTGRLIVGG-D PHECYA....
HSA(αMD4_L3) ..EKCCAA AGYRQFNRRTHEVWNLDGSD PHECYA....
Ala363 (B_N)
insertion sequence
Pro366 (B_C)
4. L4 site mutant
HSA ...KCCKA D----------D KETCFA....
HSA(mP6-9_L4) ...KCCKA GWRPYIERWTGRLIVGG--D KETCFA....
HSA(αMD4_L4) ...KCCKA GGYRQFNRRTHEVWNLDGGD KETCFA....
Ala561 (B_N)
insertion sequence
Lys564(B_C)

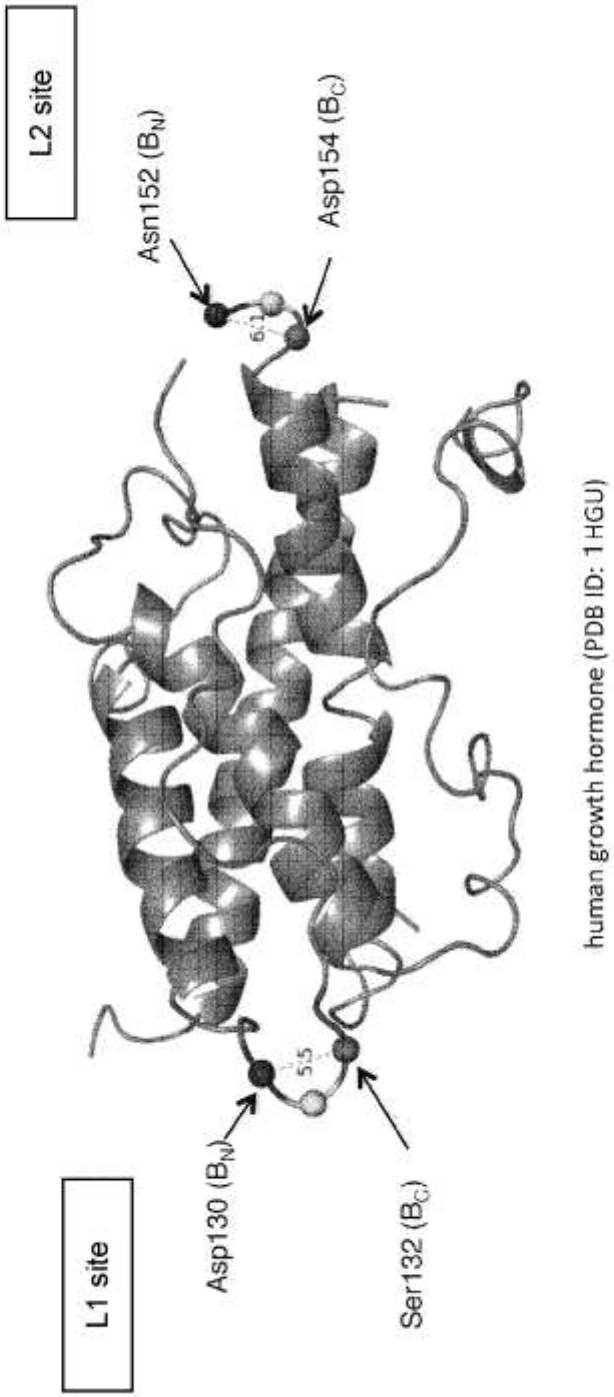
[Fig.16]


HSA and its mutant
Marker
① HSA
② HSA(mP6-9_L1)
③ HSA(mP6-9_L2)
④ HSA(mP6-9_L3)
⑤ HSA(mP6-9_L4)
Marker
① HSA
⑥ HSA(aMD4_L1)
⑦ HSA(aMD4_L2)
⑧ HSA(aMD4_L3)
⑨ HSA(aMD4_L4)
250
150
100
75
50
37
25
[kDa]

[Fig.17]


binding to Met
⑩mock
⑨HSA(aMD4_L4)
⑧HSA(aMD4_L3)
⑦HSA(aMD4_L2)
⑥HSA(aMD4_L1)
① HSA
Marker
Met-PA
*
250
150
100
75
50
37
25
[kDa]
binding to Plexin B1
⑩mock
⑤HSA(mP6-9_L4)
④HSA(mP6-9_L3)
③HSA(mP6-9_L2)
②HSA(mP6-9_L1)
① HSA
Marker
PlexinB1-Fc
*
250
150
100
75
50
37
25
[kDa]
* band of cyclic peptide fusion protein (HSA mutant)

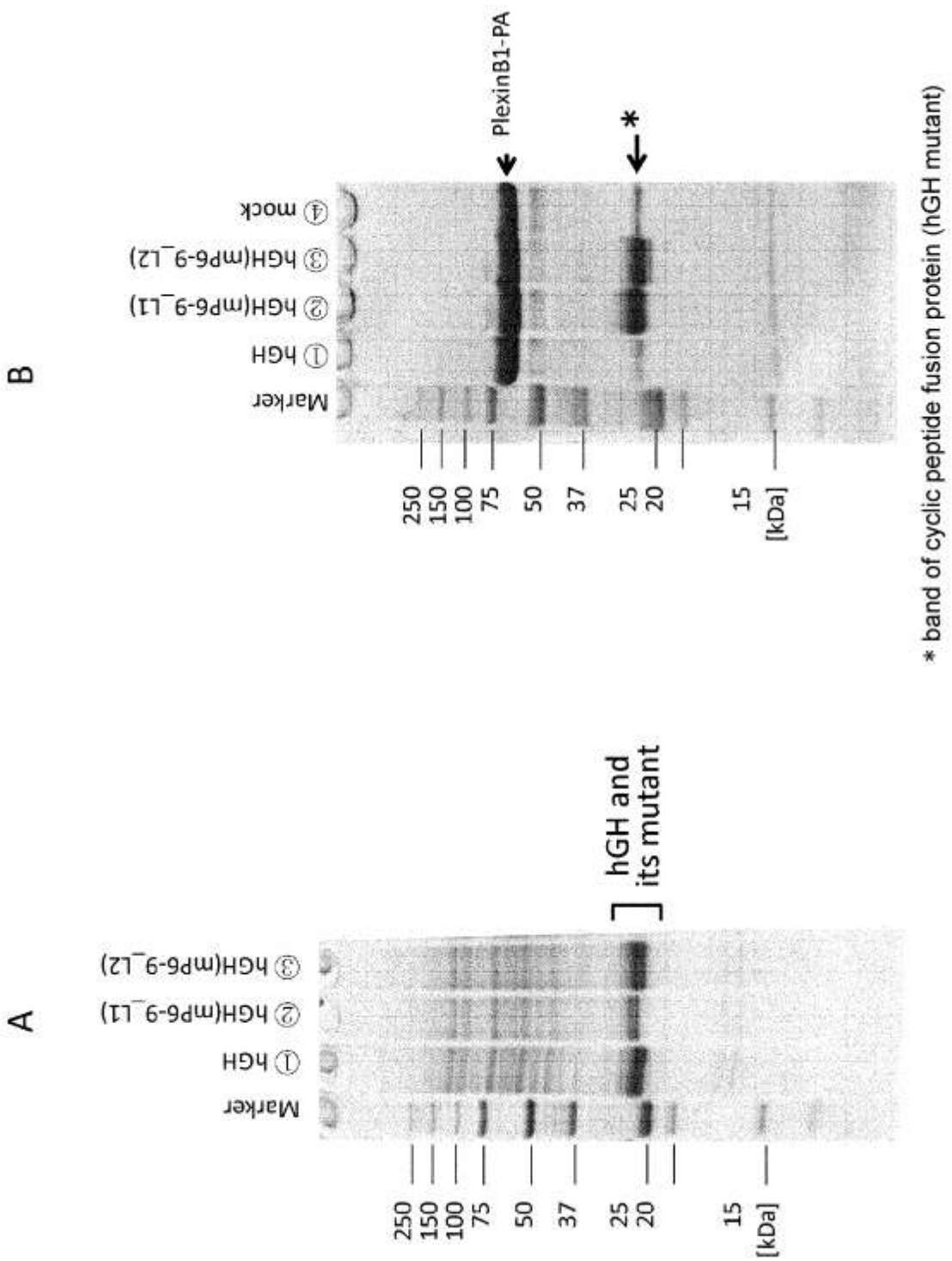
[Fig.18]


L2 site
Asn152 (B_N)
Asp154 (B_C)
6:1
L1 site
Asp130 (B_N)
Ser132 (B_C)
5:5
human growth hormone (PDB ID: 1 HGU)

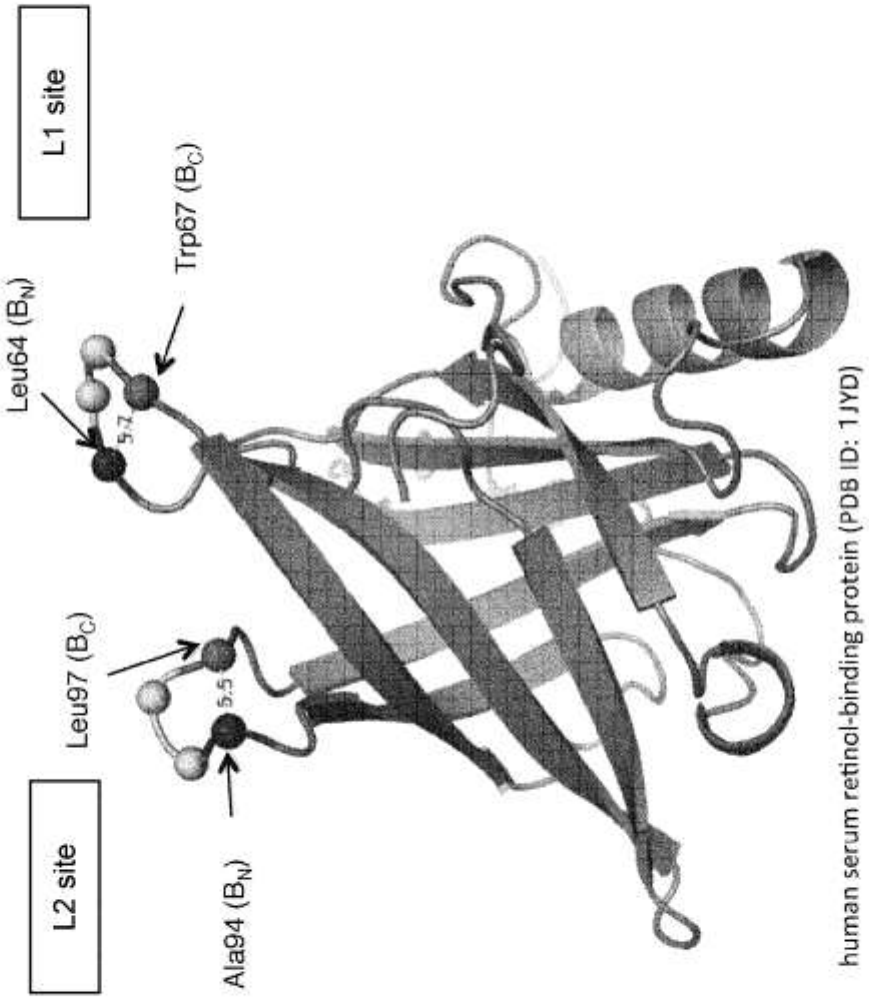
[Fig.19]

1. L1 site mutant
insertion sequence
hGH ...MGRLED G---------- SPRTQA....
hGH(mP6-9_L1) ...MGRLED GWRPYIERWTGRLIVG SPRTQA....
Asp130 (B_N)
insertion sequence
Ser132 (B_C)
2. L2 site mutant
hGH ...DANSHN ------D--------- DALLKNY....
hGH(mP6-9_L2) ...DANSHN GWRPYIERWTGRLIVG DALLKNY....
Asn152 (B_N)
insertion sequence
Asp154 (B_C)

[Fig.20]


A
Marker
① hGH
② hGH(mP6-9_L1)
③ hGH(mP6-9_L2)
hGH and its mutant
250
150
100
75
50
37
25
20
15
[kDa]
B
Marker
① hGH
② hGH(mP6-9_L1)
③ hGH(mP6-9_L2)
④ mock
PlexinB1-PA
*
250
150
100
75
50
37
25
20
15
[kDa]
* band of cyclic peptide fusion protein (hGH mutant)

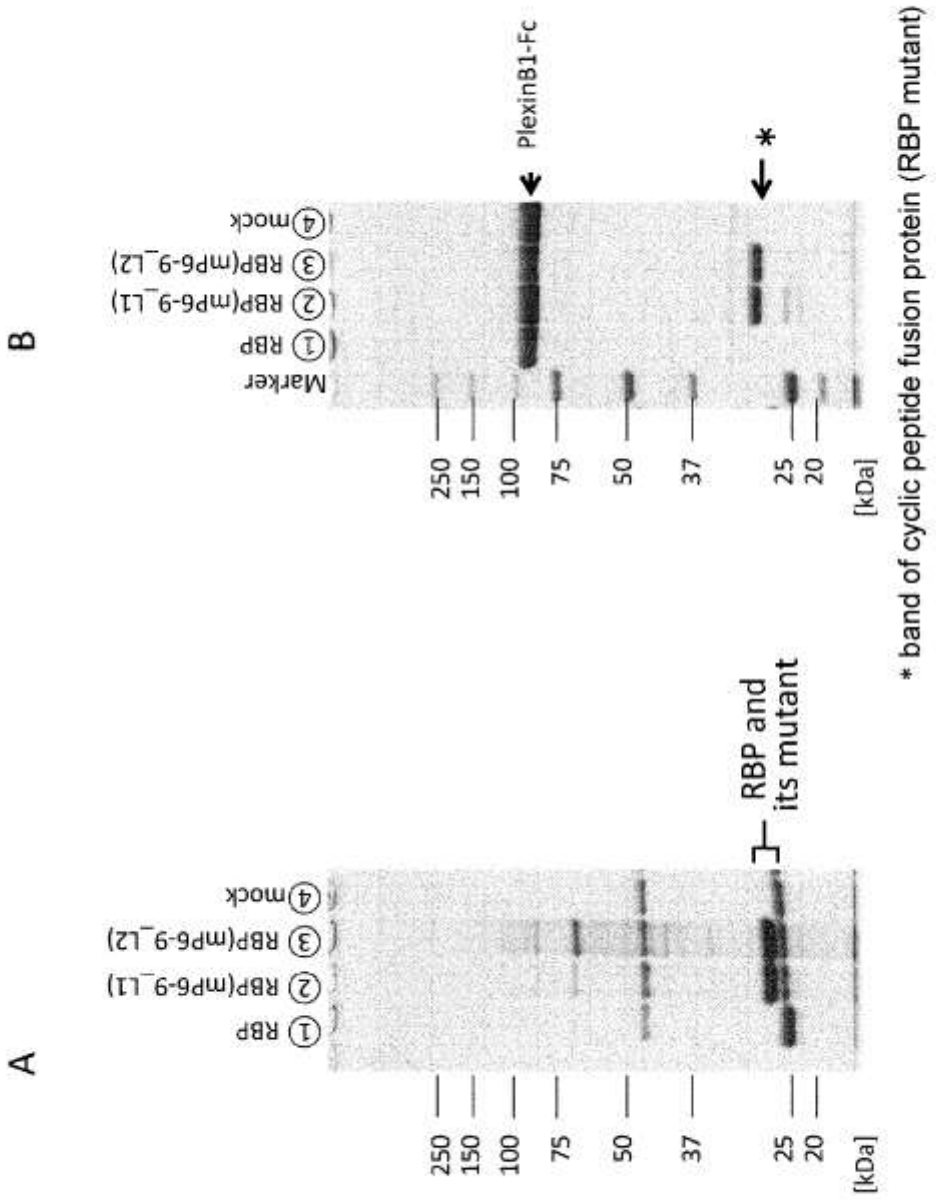
[Fig.21]


L1 site
Trp67 (B_C)
Leu64 (B_N)
5.7
L2 site
Leu97 (B_C)
5.5
Ala94 (B_N)
human serum retinol-binding protein (PDB ID: 1JYD)

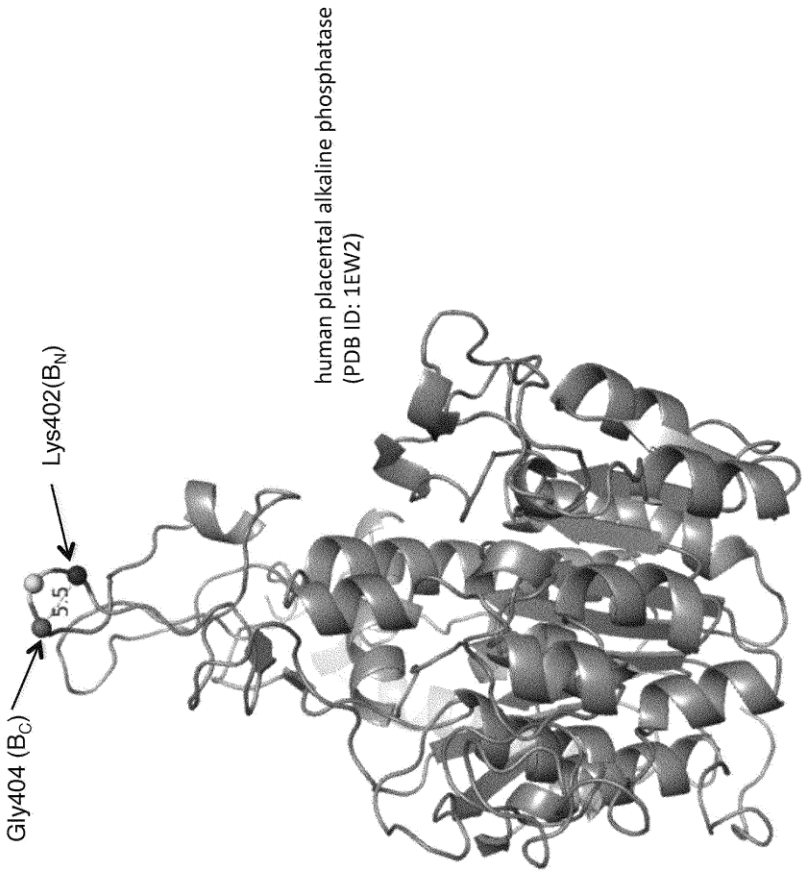
[Fig.22]

1. L1 site mutant
insertion sequence
RBP ...GRVRLL ----------NN WDVCAD....
RBP(mP6-9_L1) ...GRVRLL GWRPYIERWTGRLIVGNN WDVCAD....
Leu64 (B_N)
insertion sequence
Trp67 (B_C)
2. L2 site mutant
RBP ...KYWGVA ----------SF LQKGNDD....
RBP(mP6-9_L2) ...KYWGVA GWRPYIERWTGRLIVGSF LQKGNDD....
Ala94 (B_N)
insertion sequence
Leu97 (B_C)

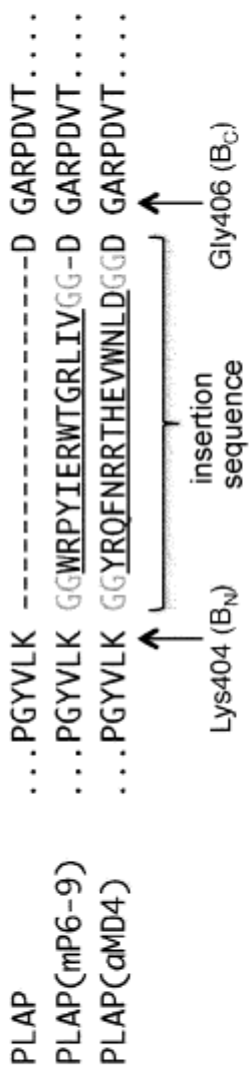
[Fig.23]


A
① RBP
② RBP(mP6-9_L1)
③ RBP(mP6-9_L2)
④ mock
RBP and its mutant
250
150
100
75
50
37
25
20
[kDa]
B
Marker
① RBP
② RBP(mP6-9_L1)
③ RBP(mP6-9_L2)
④ mock
PlexinB1-Fc
*
250
150
100
75
50
37
25
20
[kDa]
* band of cyclic peptide fusion protein (RBP mutant)

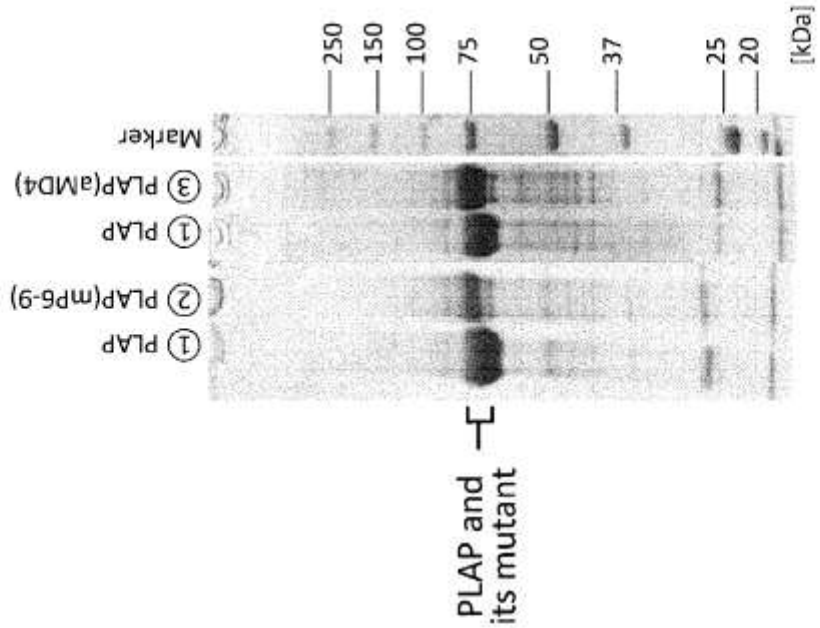
[Fig.24]


human placental alkaline phosphatase
(PDB ID: 1EW2)
Lys402(B_N)
5.5
Gly404 (B_C)

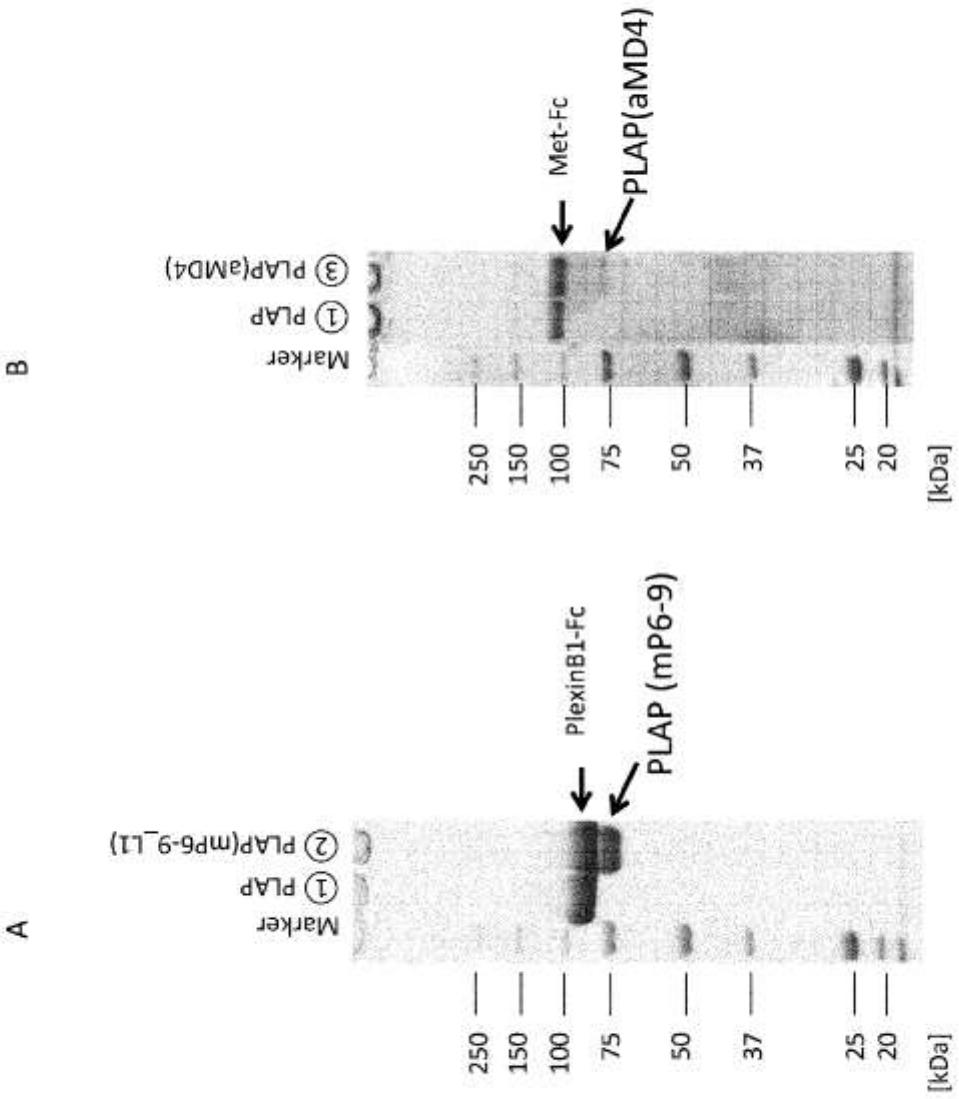
[Fig.25]


PLAP ...PGYVLK ----------D GARPDVT....
PLAP(mP6-9) ...PGYVLK GGWRPYIERWTGRLIVGG-D GARPDVT....
PLAP(αMD4) ...PGYVLK GGYRQFNRRTHEVWNLDGGD GARPDVT....
Lys404 (B_N)
insertion sequence
Gly406 (B_C)

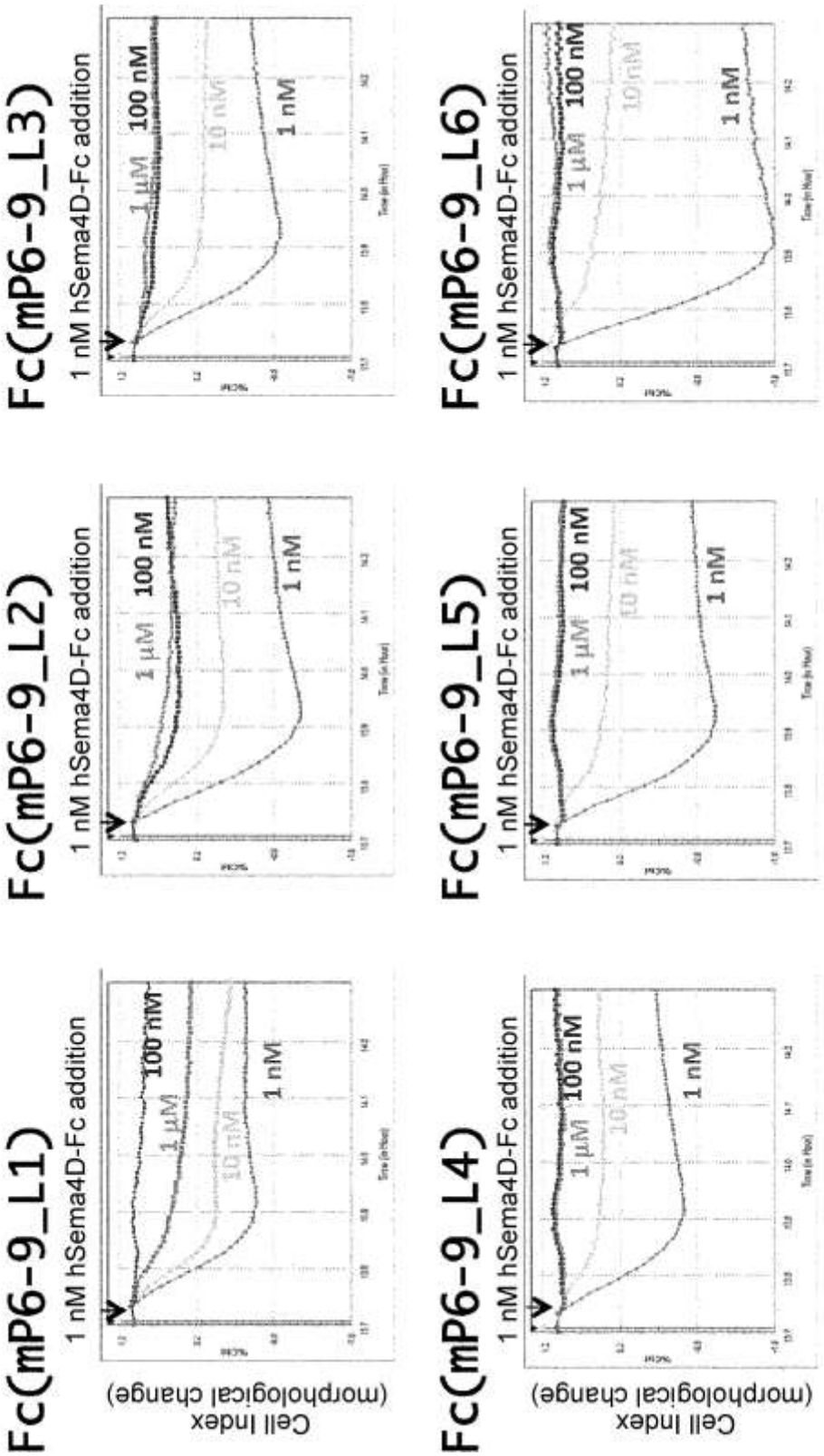
[Fig.26]


250
150
100
75
50
37
25
20
[kDa]
Marker
③ PLAP(aMD4)
① PLAP
② PLAP(mP6-9)
① PLAP
PLAP and its mutant

[Fig.27]


A
Marker
① PLAP
② PLAP(mP6-9_L1)
PlexinB1-Fc
PLAP (mP6-9)
250
150
100
75
50
37
25
20
[kDa]
B
Marker
① PLAP
③ PLAP(aMD4)
Met-Fc
PLAP(aMD4)
250
150
100
75
50
37
25
20
[kDa]

[Fig.28]


Fc(mP6-9_L1)
Fc(mP6-9_L2)
Fc(mP6-9_L3)
Fc(mP6-9_L4)
Fc(mP6-9_L5)
Fc(mP6-9_L6)
1 nM hSema4D-Fc addition
Cell Index
(morphological change)
1 μM
100 nM
10 nM
1 nM