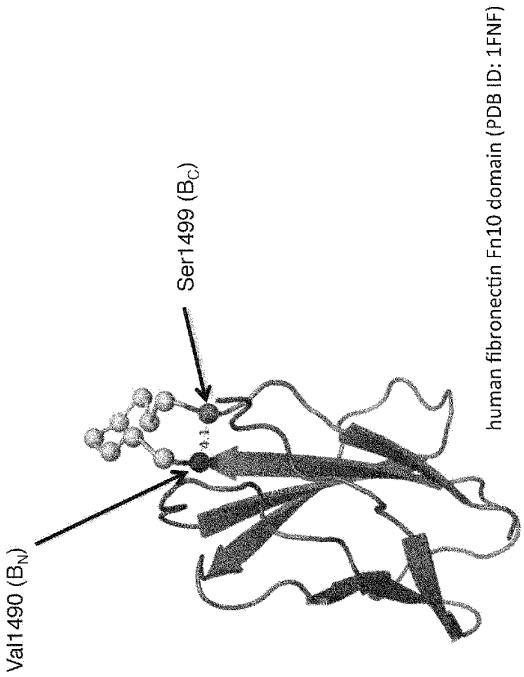
[図1]

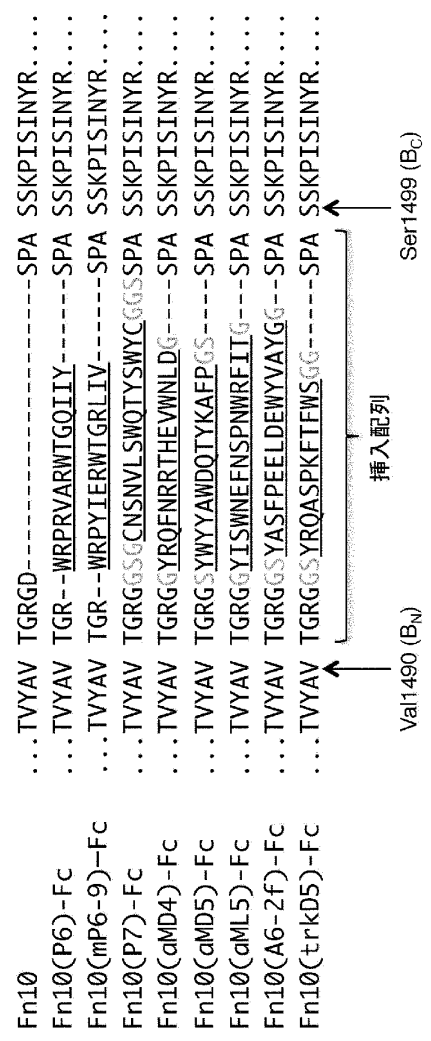


環状ペプチド名	結合分子	可変領域のアミノ酸配列
P6	ヒトプレキシンB1	wRPRVARWTGQIIY
mP6-9	ヒトプレキシンB1	wRPYIERWTGRLIV
P7	ヒトプレキシンB1	wNSNVLSWQTYSWY
aMD4	ヒトMet受容体	yRQFNRRTHEVWNLD
aMD5	ヒトMet受容体	yWYYAWDQTYKAFP
aML5	ヒトMet受容体	YISWNEFNSPNWRFTT
A6-2f	ヒトEGF受容体	yASFPEELDEWYVAYG
trkD5	ヒトTrkB	yRQASPKFTFWS

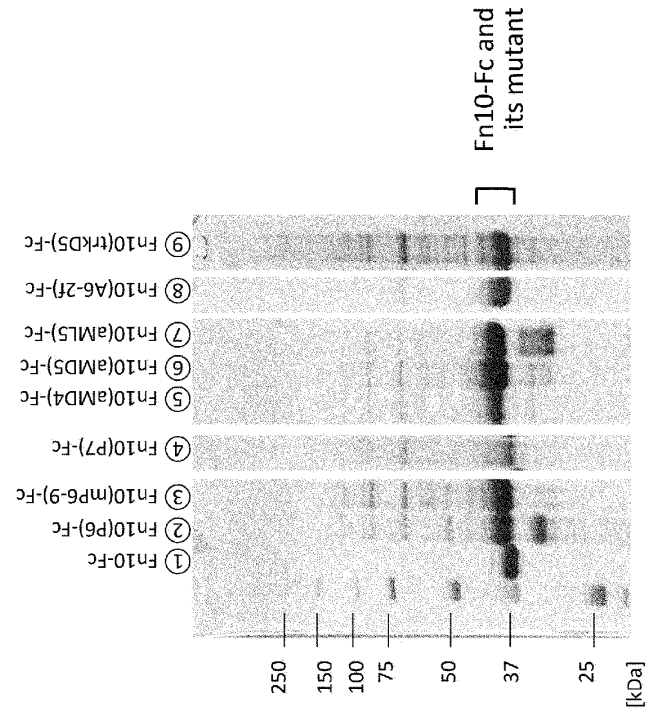
[図2]


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

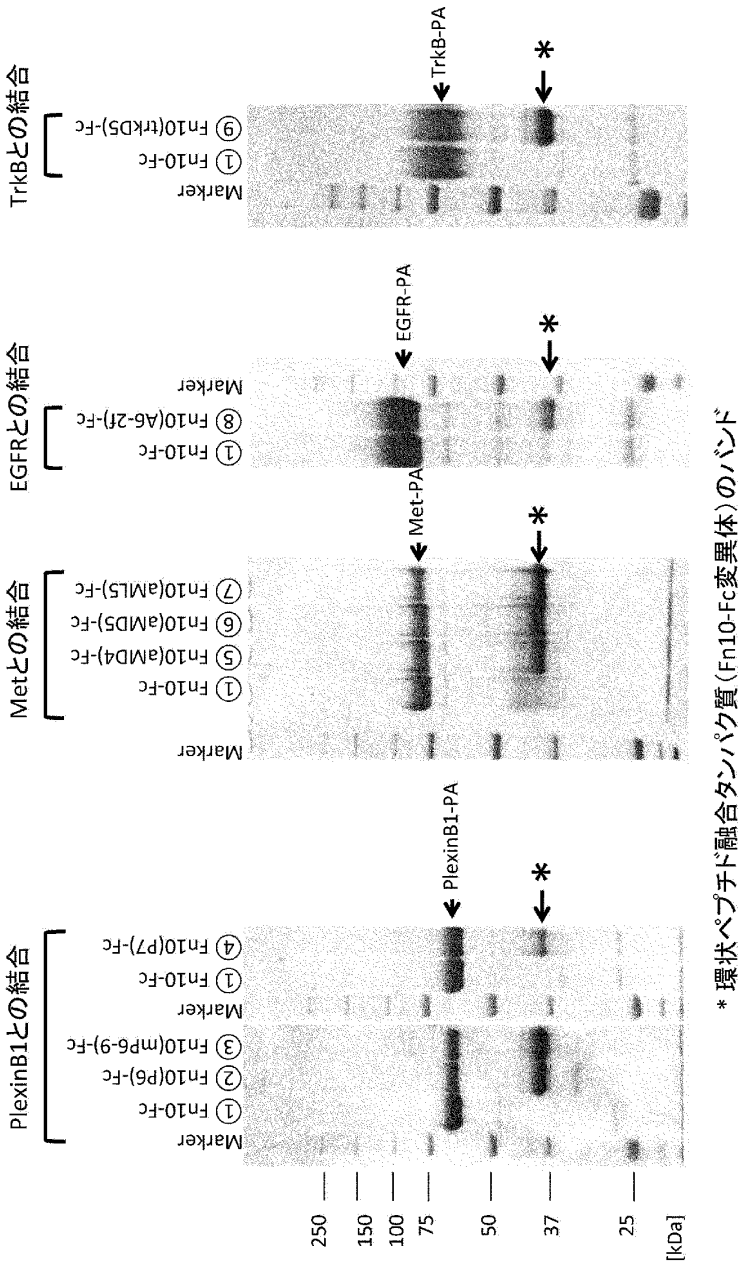
[図3]


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....
挿入配列
Val1490 (BN)
Ser1499 (BC)

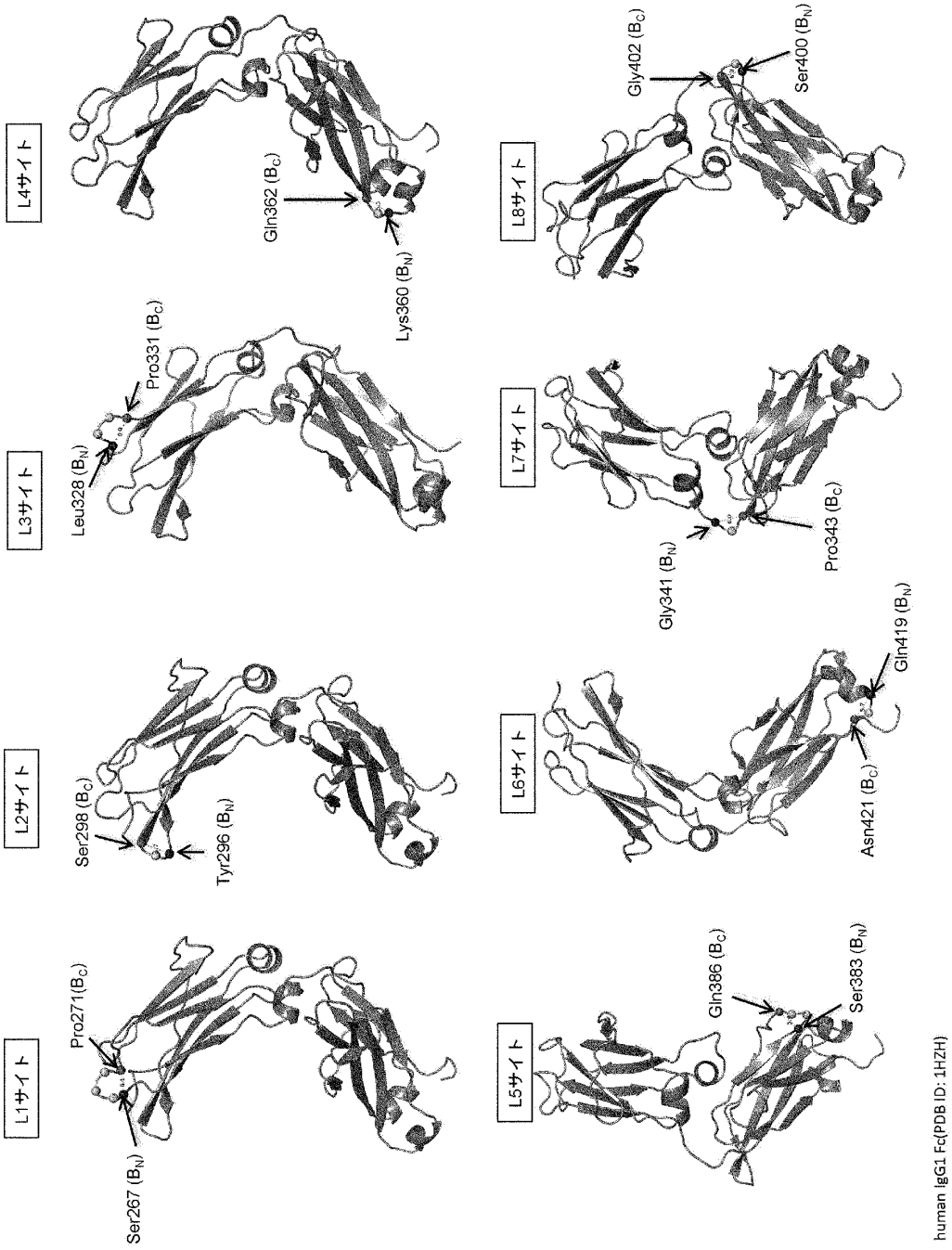
[図4]


Fn10-Fc and its mutant
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
250
150
100
75
50
37
25
[kDa]

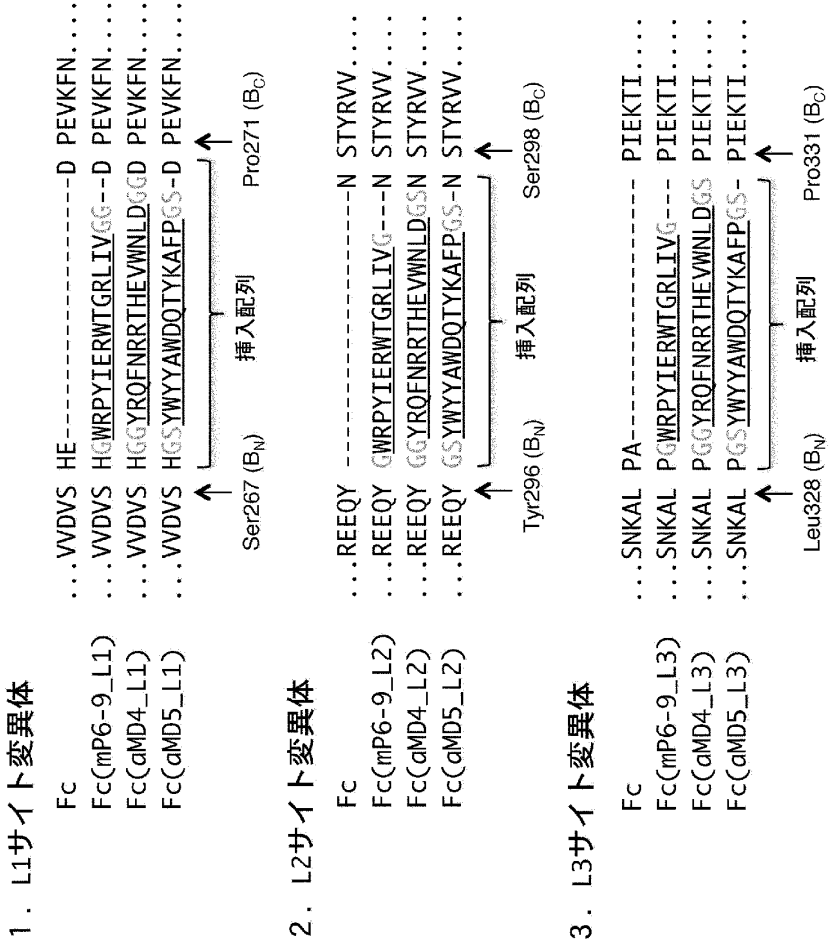
[図5]


PlexinB1との結合
Marker
① Fn10-Fc
② Fn10(P6)-Fc
③ Fn10(mP6-9)-Fc
Marker
① Fn10-Fc
④ Fn10(P7)-Fc
PlexinB1-PA
Metとの結合
Marker
① Fn10-Fc
⑤ Fn10(aMD4)-Fc
⑥ Fn10(aMD5)-Fc
⑦ Fn10(aML5)-Fc
Met-PA
EGFRとの結合
Marker
① Fn10-Fc
⑧ Fn10(A6-2f)-Fc
EGFR-PA
TrkBとの結合
Marker
① Fn10-Fc
⑨ Fn10(trkD5)-Fc
TrkB-PA
250
150
100
75
50
37
25
[kDa]
* 環状ペプチド融合タンパク質（Fn10-Fc変異体）のバンド

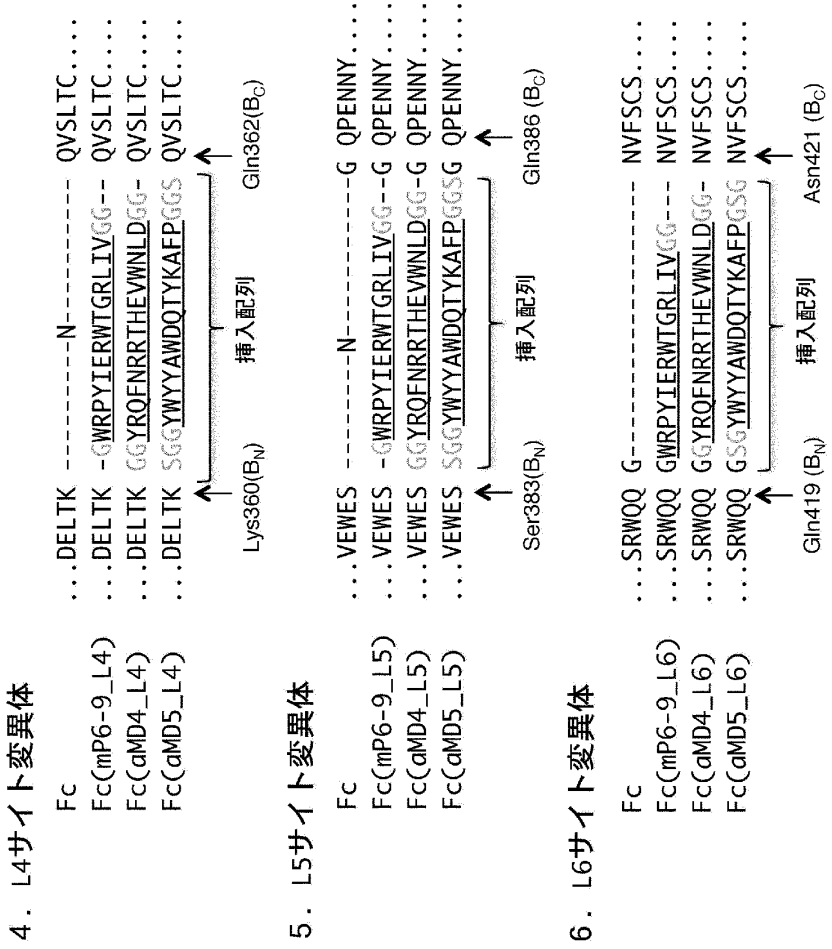
[図6]


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

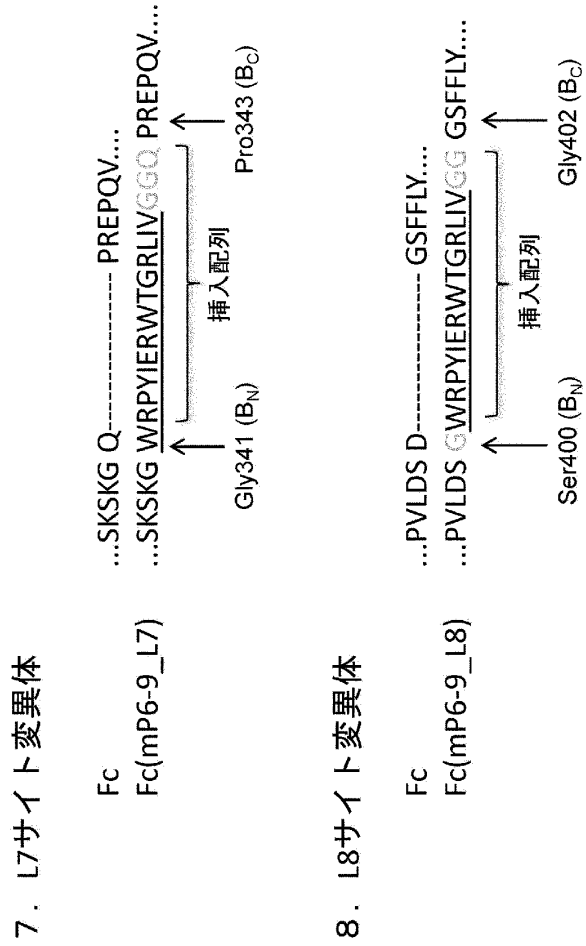
[図7]


1．L1サイト変異体
Fc ...VVDVS HE----------D PEVKFN....
Fc(mP6-9_L1) ...VVDVS HGWRPYIERWTGRLIVGG--D PEVKFN....
Fc(aMD4_L1) ...VVDVS HGGYRQFNRRTHEVWNLDGGD PEVKFN....
Fc(aMD5_L1) ...VVDVS HGSYWYYAWDQTYKAFPGS-D PEVKFN....
Ser267 (BN)
挿入配列
Pro271 (BC)
2．L2サイト変異体
Fc ...REEQY ----------N STYRVV....
Fc(mP6-9_L2) ...REEQY GWRPYIERWTGRLIVG---N STYRVV....
Fc(aMD4_L2) ...REEQY GGYRQFNRRTHEVWNLDGSN STYRVV....
Fc(aMD5_L2) ...REEQY GSYWYYAWDQTYKAFPGS-N STYRVV....
Tyr296 (BN)
挿入配列
Ser298 (BC)
3．L3サイト変異体
Fc ...SNKAL PA---------- PIEKTI....
Fc(mP6-9_L3) ...SNKAL PGWRPYIERWTGRLIVG--- PIEKTI....
Fc(aMD4_L3) ...SNKAL PGGYRQFNRRTHEVWNLDGS PIEKTI....
Fc(aMD5_L3) ...SNKAL PGSYWYYAWDQTYKAFPGS- PIEKTI....
Leu328 (BN)
挿入配列
Pro331 (BC)

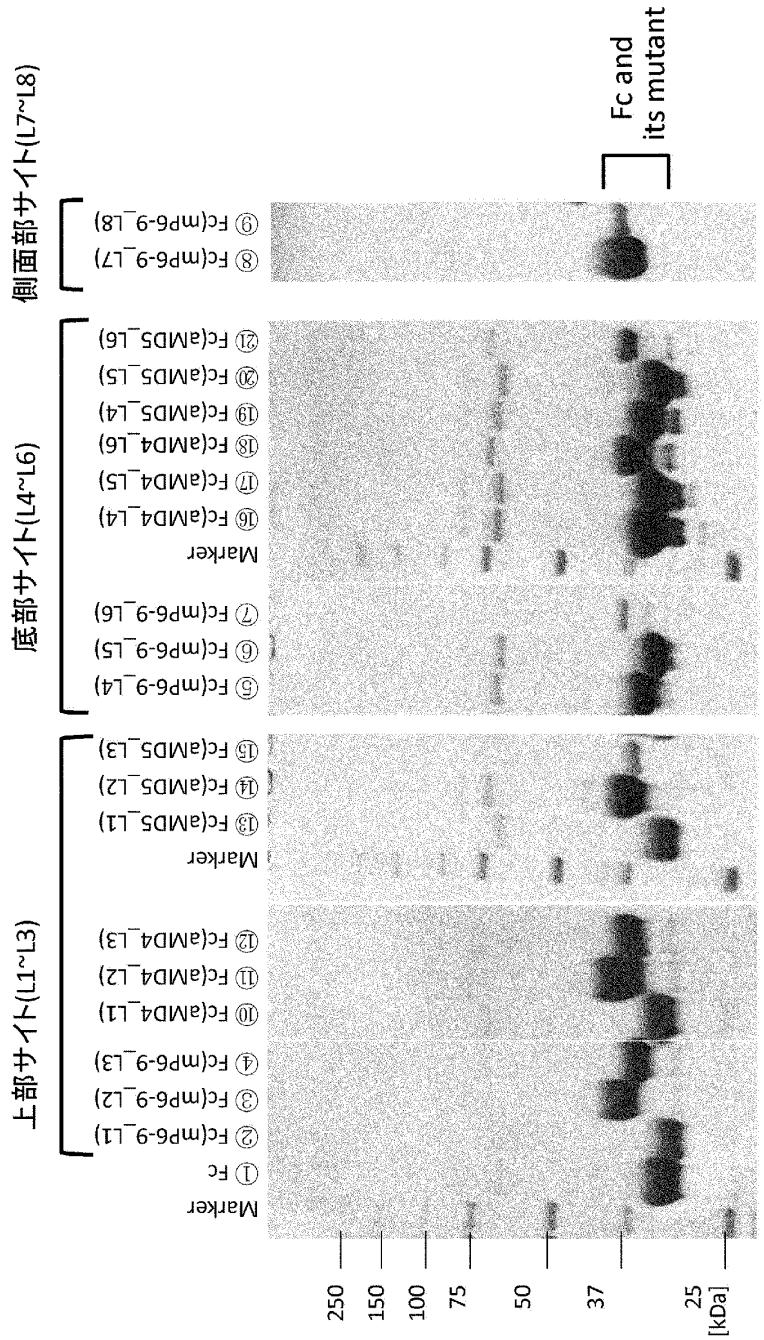
[図8]


4．L4サイト変異体
Fc ...DELTK ---------N--------- QVSLTC....
Fc(mP6-9_L4) ...DELTK -GWRPYIERWTGRLIVGG-- QVSLTC....
Fc(aMD4_L4) ...DELTK GGYRQFNRRTHEVWNLDGG- QVSLTC....
Fc(aMD5_L4) ...DELTK SGGYWYYAWDQTYKAFPGGS QVSLTC....
Lys360(BN)
挿入配列
Gln362(BC)
5．L5サイト変異体
Fc ...VEWES ---------N----------G QPENNY....
Fc(mP6-9_L5) ...VEWES -GWRPYIERWTGRLIVGG--G QPENNY....
Fc(aMD4_L5) ...VEWES GGYRQFNRRTHEVWNLDGG-G QPENNY....
Fc(aMD5_L5) ...VEWES SGGYWYYAWDQTYKAFPGGSG QPENNY....
Ser383(BN)
挿入配列
Gln386 (BC)
6．L6サイト変異体
Fc ...SRWQQ G---------- NVFSCS....
Fc(mP6-9_L6) ...SRWQQ GWRPYIERWTGRLIVGG--- NVFSCS....
Fc(aMD4_L6) ...SRWQQ GGYRQFNRRTHEVWNLDGG- NVFSCS....
Fc(aMD5_L6) ...SRWQQ GSGYWYYAWDQTYKAFPGSG NVFSCS....
Gln419 (BN)
挿入配列
Asn421 (BC)

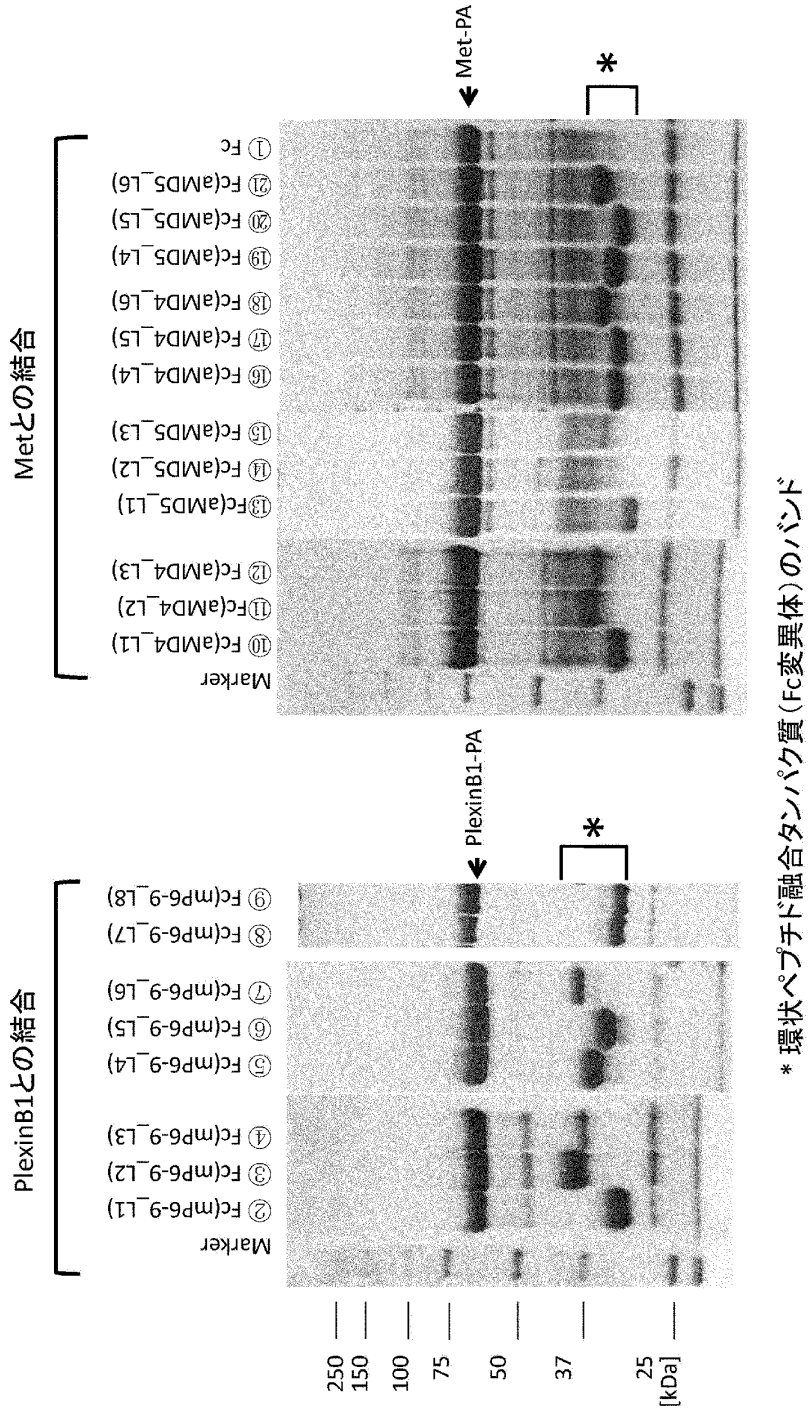
[図9]


7．L7サイト変異体
Fc
Fc(mP6-9_L7)
...SKSKG Q---------- PREPQV....
...SKSKG WRPYIERWTGRLIVGGQ PREPQV....
Gly341 (BN)
挿入配列
Pro343 (BC)
8．L8サイト変異体
Fc
Fc(mP6-9_L8)
...PVLDS D---------- GSFFLY....
...PVLDS GWRPYIERWTGRLIVGG GSFFLY....
Ser400 (BN)
挿入配列
Gly402 (BC)

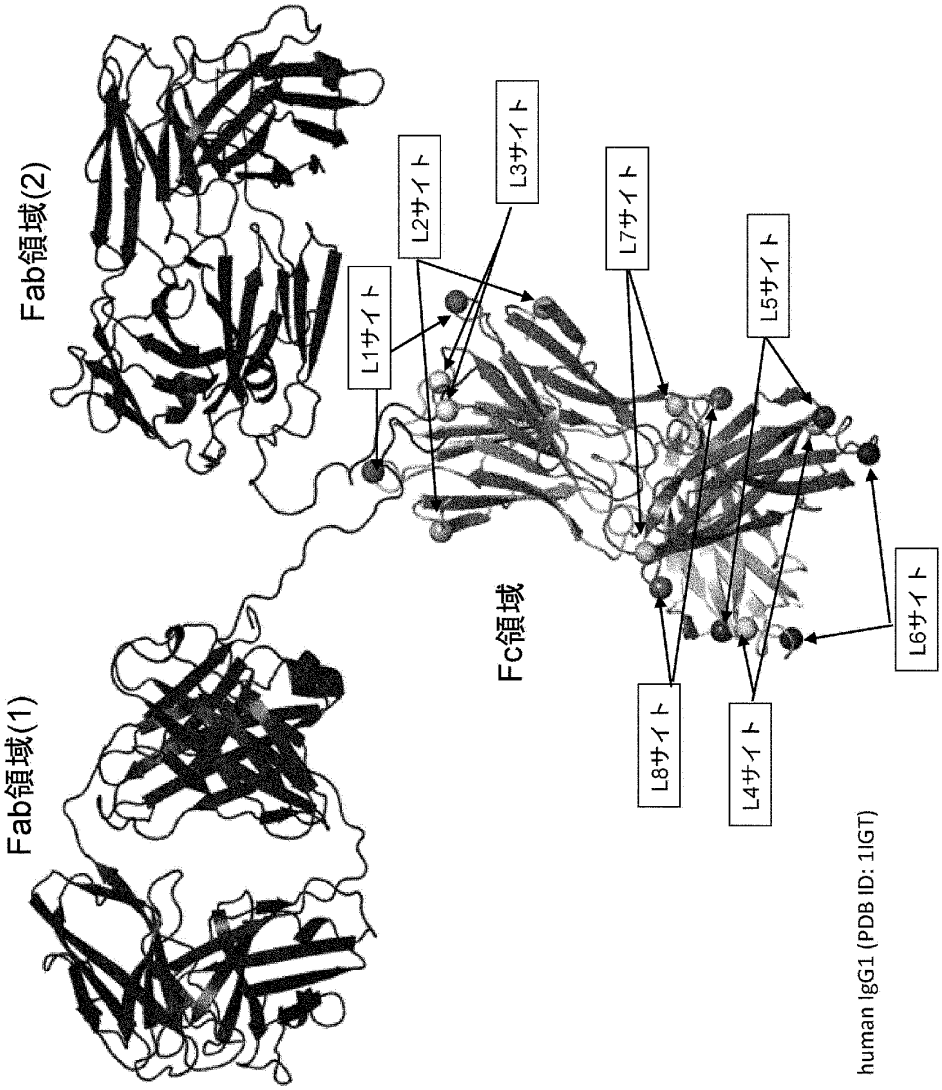
[図10]


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)
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)
⑧ Fc(mP6-9_L7)
⑨ Fc(mP6-9_L8)
上部サイト(L1~L3)
底部サイト(L4~L6)
側面部サイト(L7~L8)

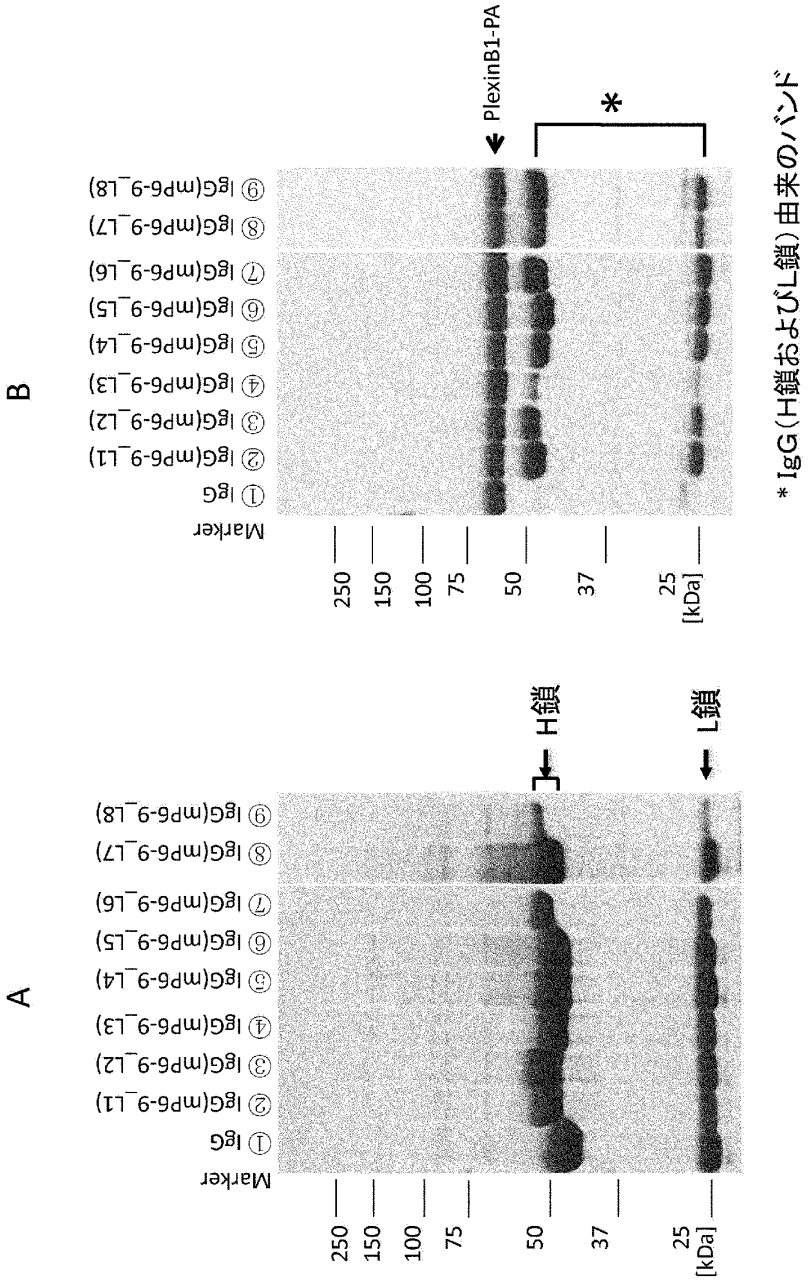
[図11]


Met-PA
PlexinB1-PA
*
250
150
100
75
50
37
25
[kDa]
PlexinB1との結合
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)
Metとの結合
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
* 環状ペプチド融合タンパク質（Fc変異体）のバンド

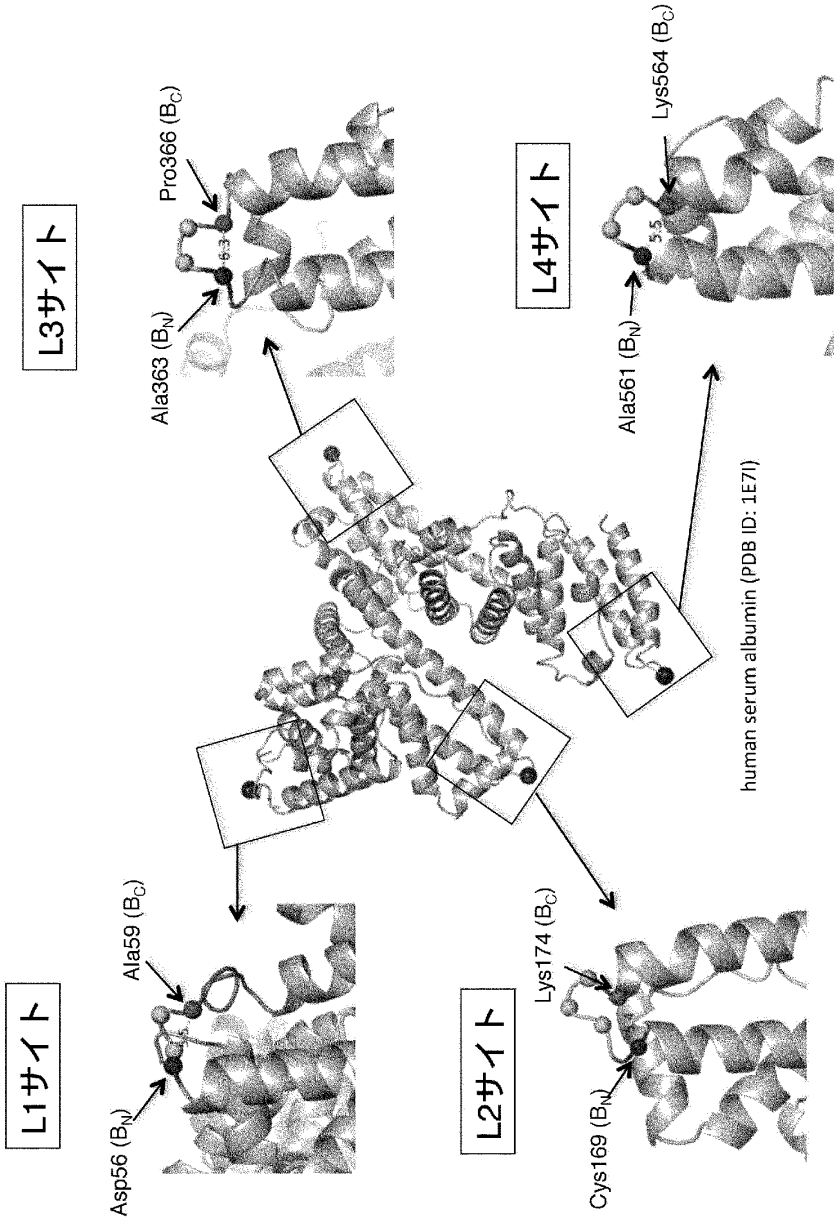
[図12]


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

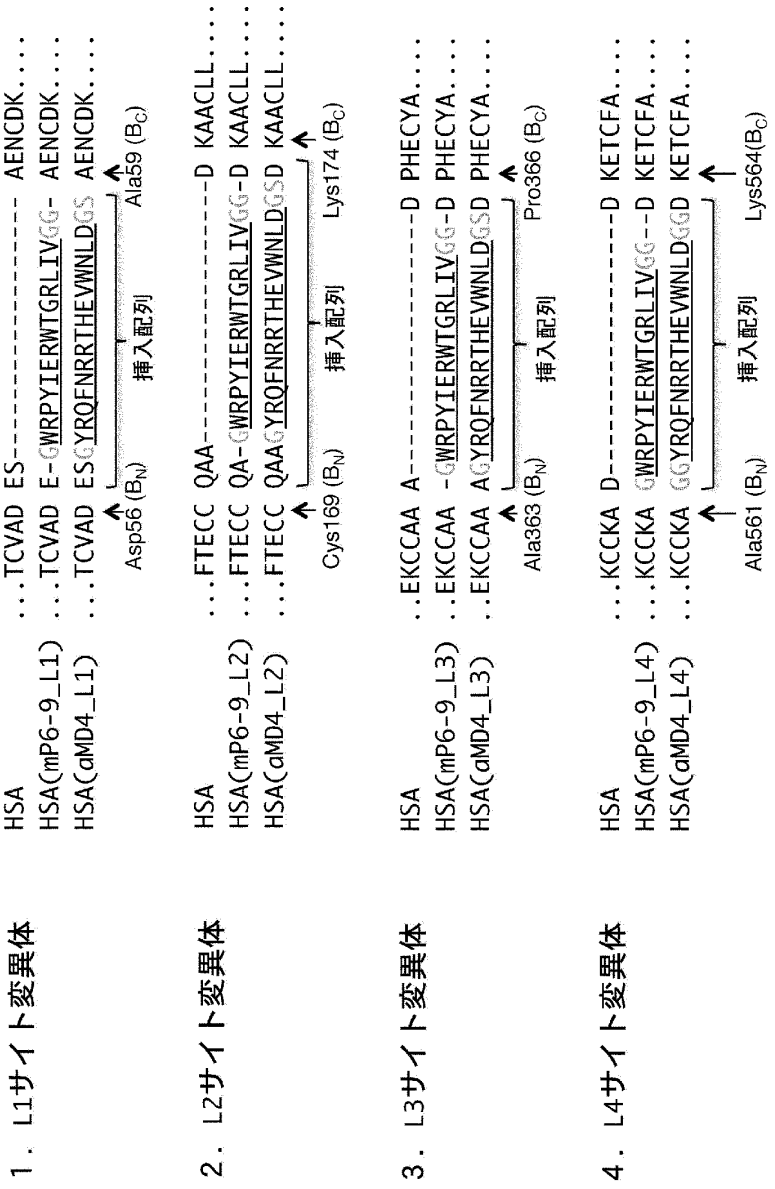
[図13]


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鎖
L鎖
PlexinB1-PA
*
* IgG（H鎖およびL鎖）由来のバンド

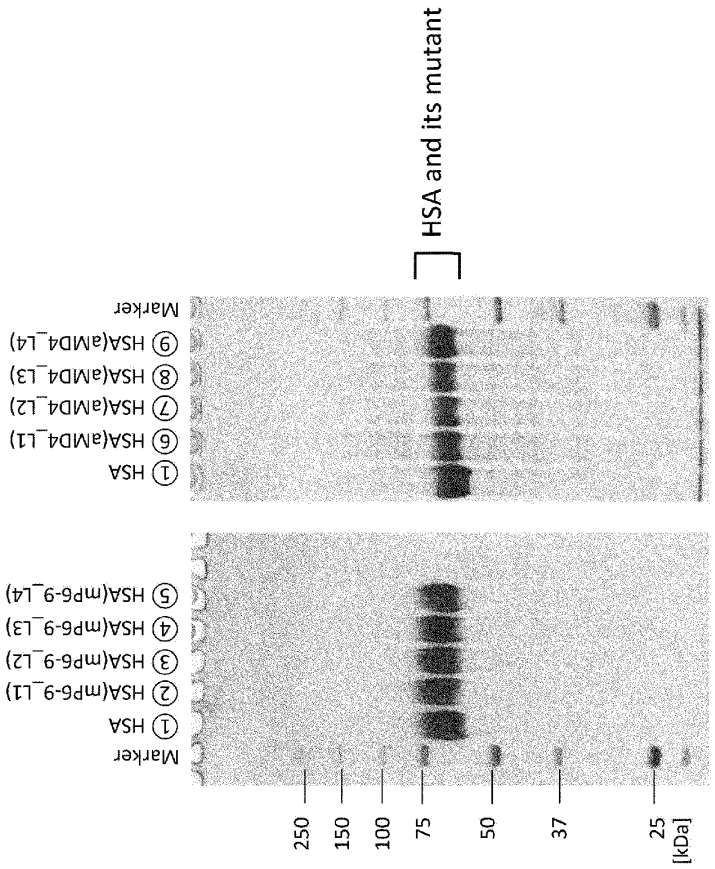
[図14]


L3サイト
Pro366 (B$_C$)
Ala363 (B$_N$)
6.3
L4サイト
Lys564 (B$_C$)
Ala561 (B$_N$)
5.5
L1サイト
Ala59 (B$_C$)
Asp56 (B$_N$)
L2サイト
Lys174 (B$_C$)
Cys169 (B$_N$)
human serum albumin (PDB ID: 1E7I)

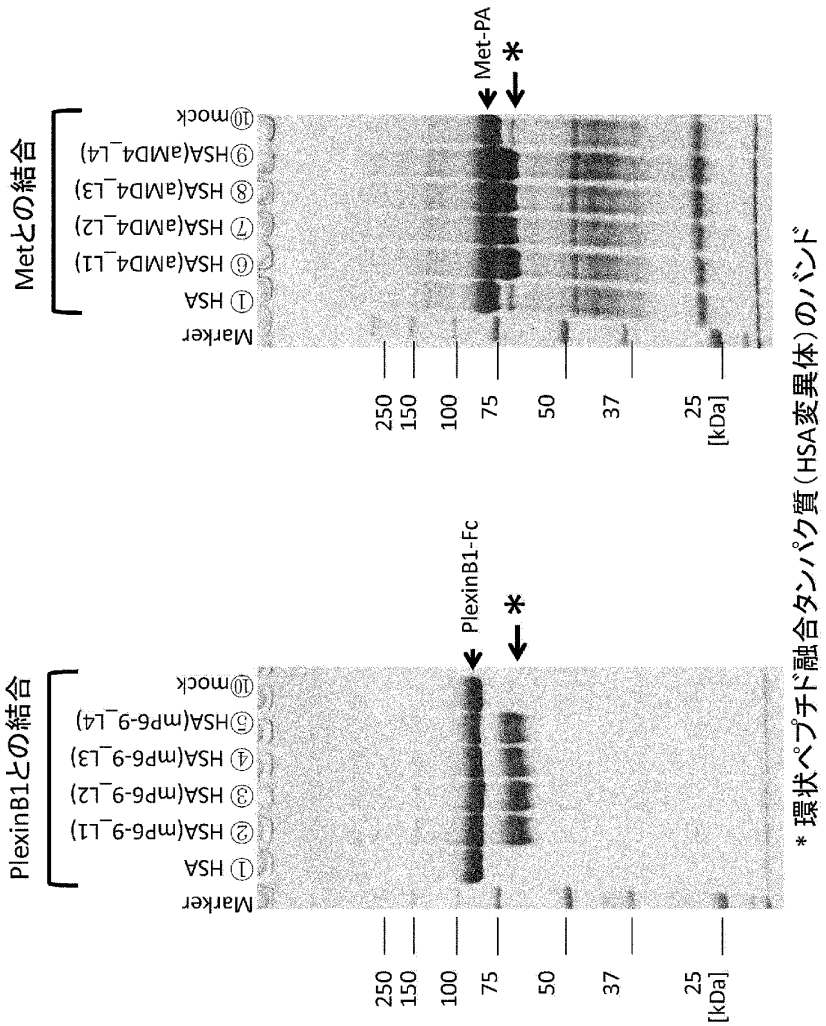
[図15]


1．L1サイト変異体
HSA ...TCVAD ES---------- AENCDK....
HSA(mP6-9_L1) ...TCVAD E-GWRPYIERWTGRLIVGG- AENCDK....
HSA(αMD4_L1) ...TCVAD ESGYRQFNRRTHEVWNLDGS AENCDK....
Asp56 (BN)
挿入配列
Ala59 (BC)
2．L2サイト変異体
HSA ...FTECC QAA----------D KAACLL....
HSA(mP6-9_L2) ...FTECC QA-GWRPYIERWTGRLIVGG-D KAACLL....
HSA(αMD4_L2) ...FTECC QAAGYRQFNRRTHEVWNLDGSD KAACLL....
Cys169 (BN)
挿入配列
Lys174 (BC)
3．L3サイト変異体
HSA ..EKCCAA A----------D PHECYA....
HSA(mP6-9_L3) ..EKCCAA -GWRPYIERWTGRLIVGG-D PHECYA....
HSA(αMD4_L3) ..EKCCAA AGYRQFNRRTHEVWNLDGSD PHECYA....
Ala363 (BN)
挿入配列
Pro366 (BC)
4．L4サイト変異体
HSA ...KCCKA D----------D KETCFA....
HSA(mP6-9_L4) ...KCCKA GWRPYIERWTGRLIVGG--D KETCFA....
HSA(αMD4_L4) ...KCCKA GGYRQFNRRTHEVWNLDGGD KETCFA....
Ala561 (BN)
挿入配列
Lys564(BC)

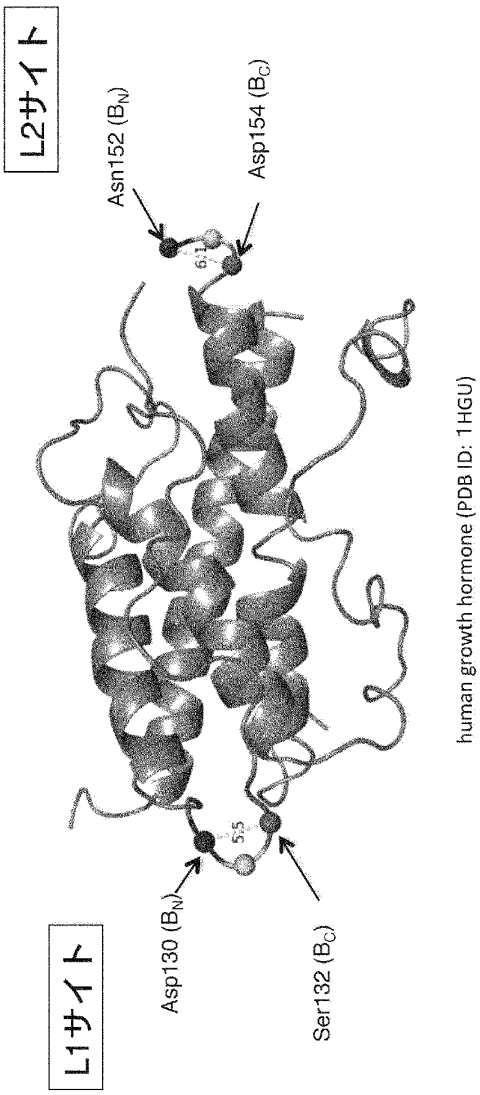
[図16]


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]

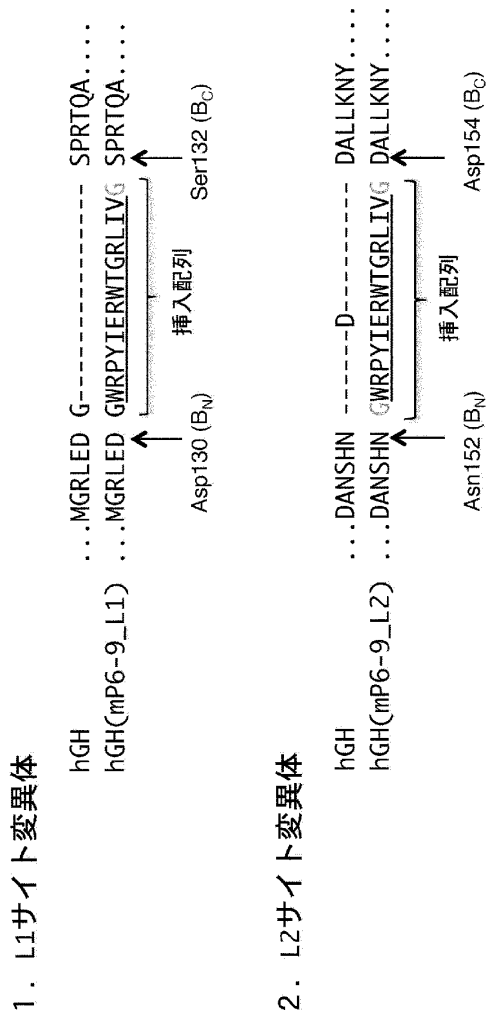
[図17]


PlexinB1との結合
Marker
① HSA
② HSA(mP6-9_L1)
③ HSA(mP6-9_L2)
④ HSA(mP6-9_L3)
⑤ HSA(mP6-9_L4)
⑩ mock
PlexinB1-Fc
*
250
150
100
75
50
37
25
[kDa]
Metとの結合
Marker
① HSA
⑥ HSA(aMD4_L1)
⑦ HSA(aMD4_L2)
⑧ HSA(aMD4_L3)
⑨ HSA(aMD4_L4)
⑩ mock
Met-PA
*
250
150
100
75
50
37
25
[kDa]
* 環状ペプチド融合タンパク質（HSA変異体）のバンド

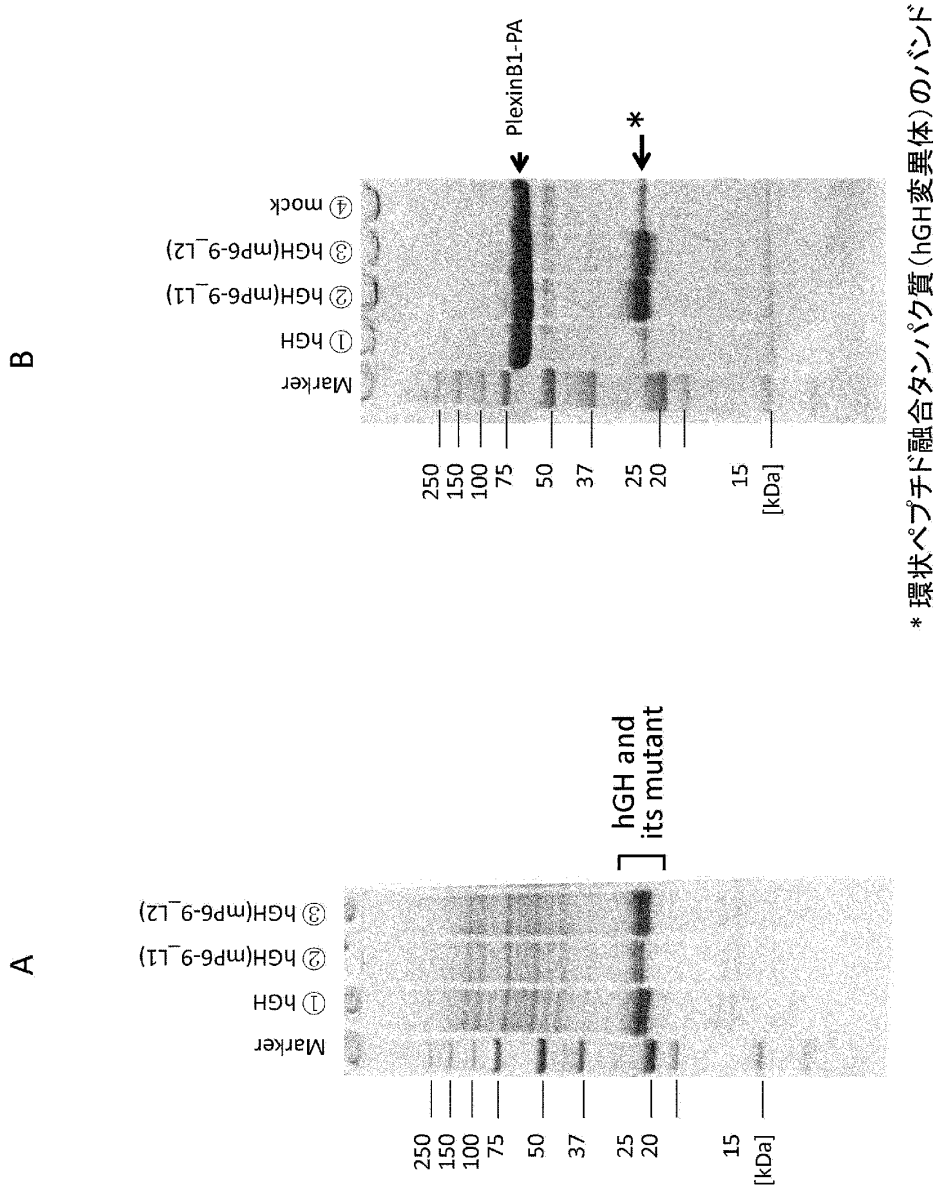
[図18]


L2サイト
Asn152 (B_N)
Asp154 (B_C)
6:11
L1サイト
Asp130 (B_N)
Ser132 (B_C)
5:5
human growth hormone (PDB ID: 1HGU)

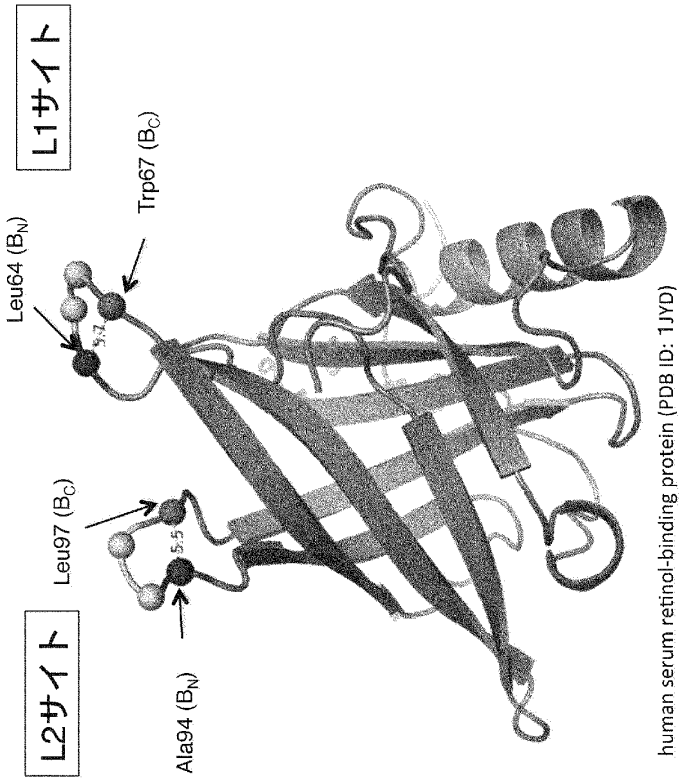
[図19]


1．L1サイト変異体
hGH ...MGRLED G---------- SPRTQA....
hGH(mP6-9_L1) ...MGRLED GWRPYIERWTGRLIVG SPRTQA....
挿入配列
Asp130 (BN)
Ser132 (BC)
2．L2サイト変異体
hGH ...DANSHN ------D--------- DALLKNY....
hGH(mP6-9_L2) ...DANSHN GWRPYIERWTGRLIVG DALLKNY....
挿入配列
Asn152 (BN)
Asp154 (BC)

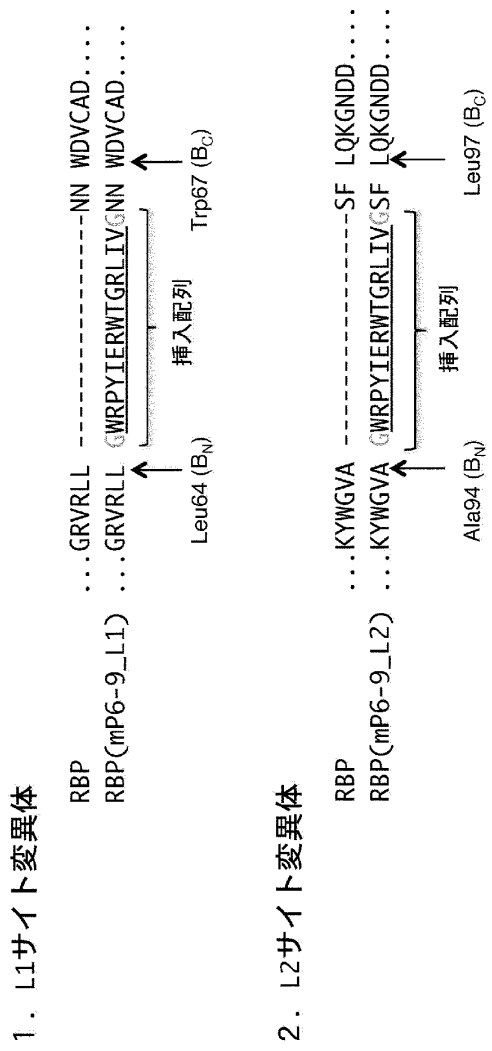
[図20]


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]
* 環状ペプチド融合タンパク質（hGH変異体）のバンド

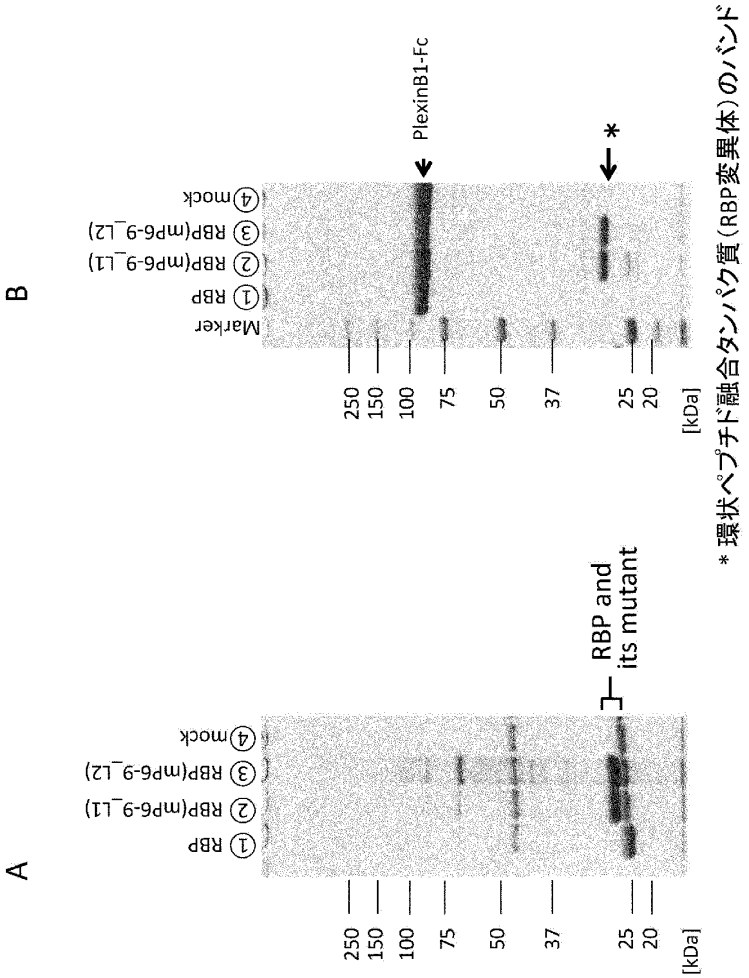
[図21]


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

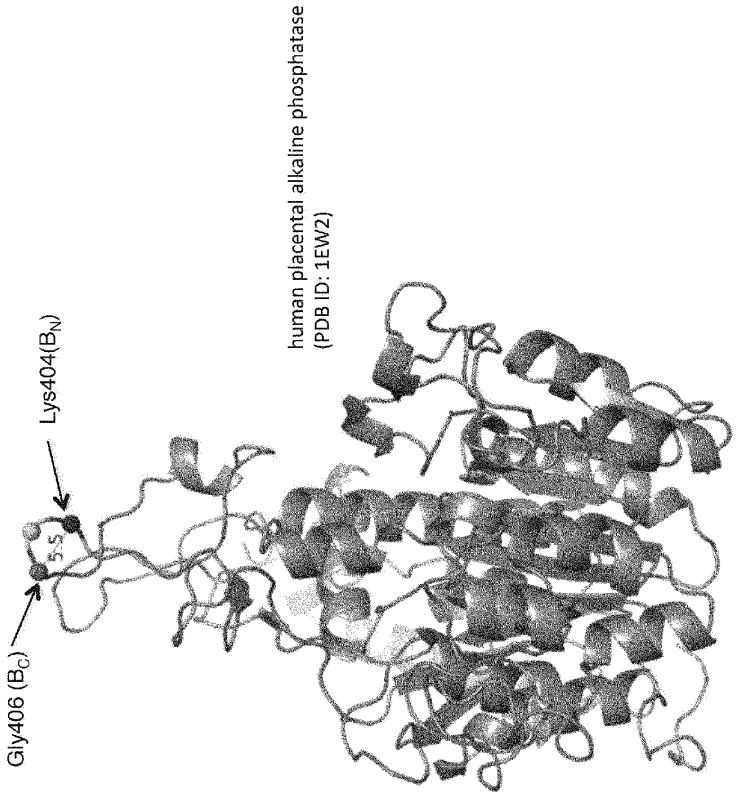
[図22]


1．L1サイト変異体
RBP ...GRVRLL ----------NN WDVCAD....
RBP(mP6-9_L1) ...GRVRLL GWRPYIERWTGRLIVGNN WDVCAD....
Leu64 (B_N)
挿入配列
Trp67 (B_C)
2．L2サイト変異体
RBP ...KYWGVA ----------SF LQKGNDD....
RBP(mP6-9_L2) ...KYWGVA GWRPYIERWTGRLIVGSF LQKGNDD....
Ala94 (B_N)
挿入配列
Leu97 (B_C)

[図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]
* 環状ペプチド融合タンパク質（RBP変異体）のバンド

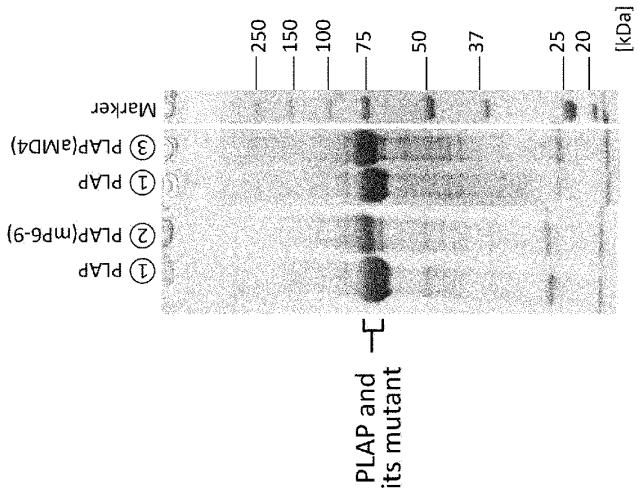
[図24]


human placental alkaline phosphatase
(PDB ID: 1EW2)
Lys404(B_N)
S-S
Gly406 (B_C)

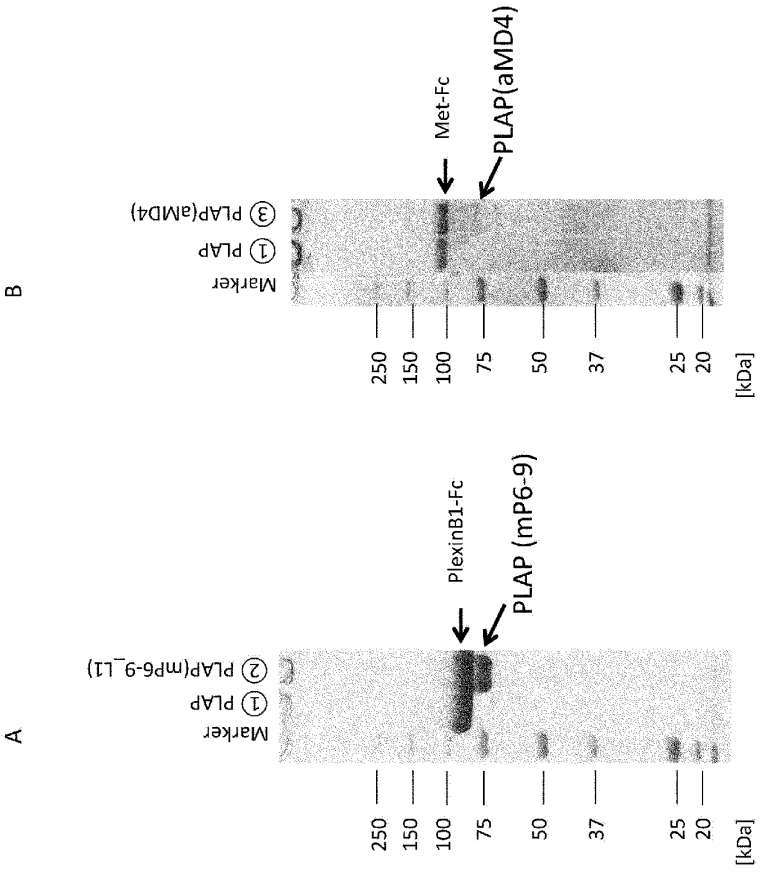
[図25]


PLAP ...PGYVLK ----------D GARPDVT....
PLAP(mP6-9) ...PGYVLK GGWRPYIERWTGRLIVGG-D GARPDVT....
PLAP(αMD4) ...PGYVLK GGYRQFNRRTHEVWNLDGGD GARPDVT....
Lys404 (B_N)
挿入配列
Gly406 (B_C)

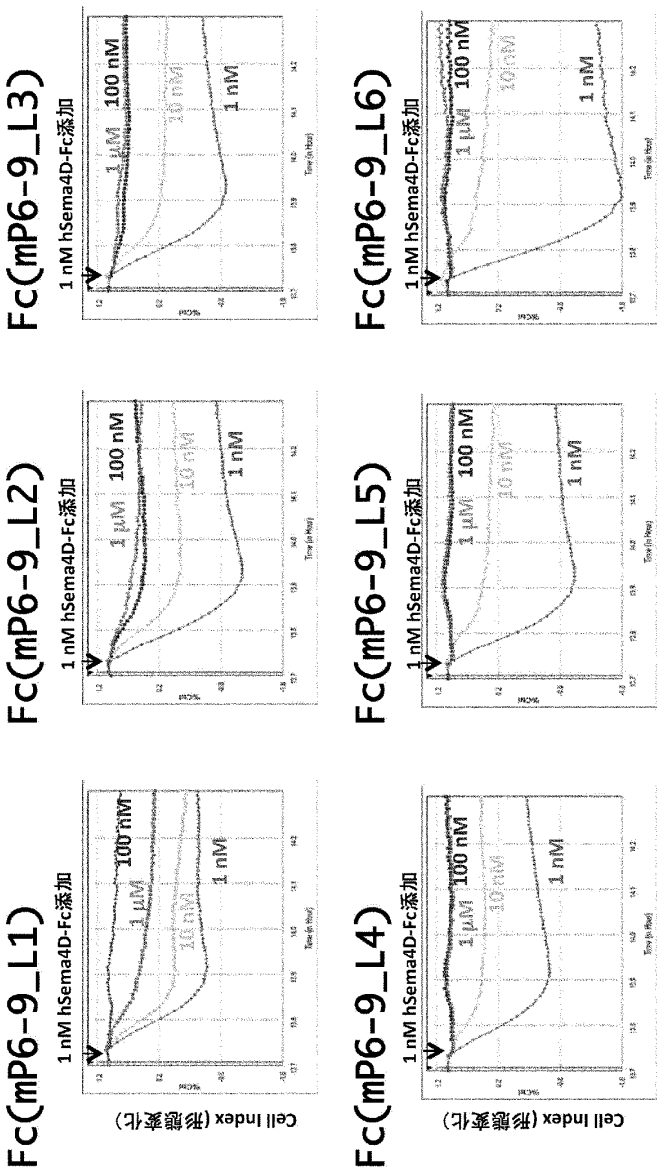
[図26]


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

[図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]

[図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添加
1 μM
100 nM
10 nM
1 nM
Cell Index (形態変化)