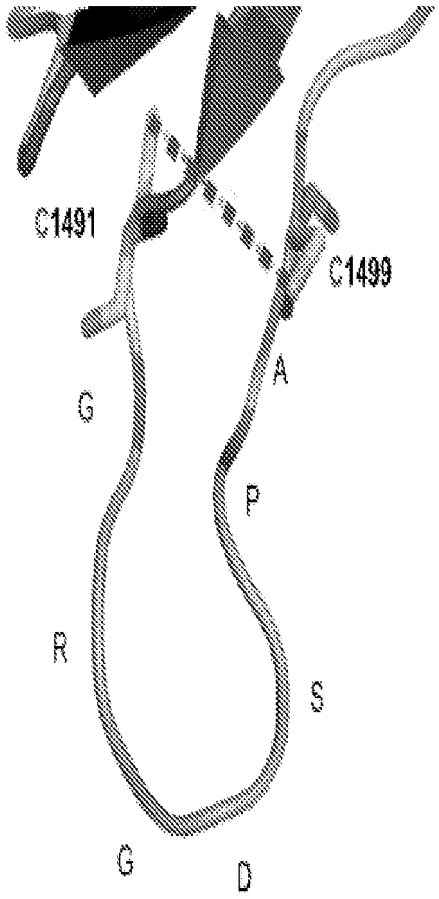
FN-III$_{10}$ wild type: ^{1490}VTGRGDSPASSK (SEQ ID NO:2)
FN-III$_{10}$ S-S : ^{1490}VCGRGDSPACSK (SEQ ID NO:12)



Hydrogen bond pattern of FN-III$_{10}$

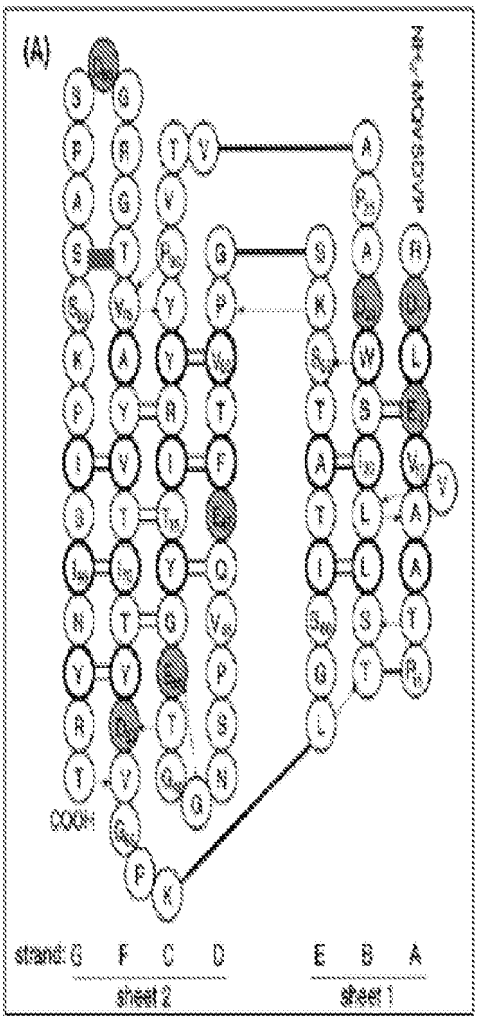


Figure 1

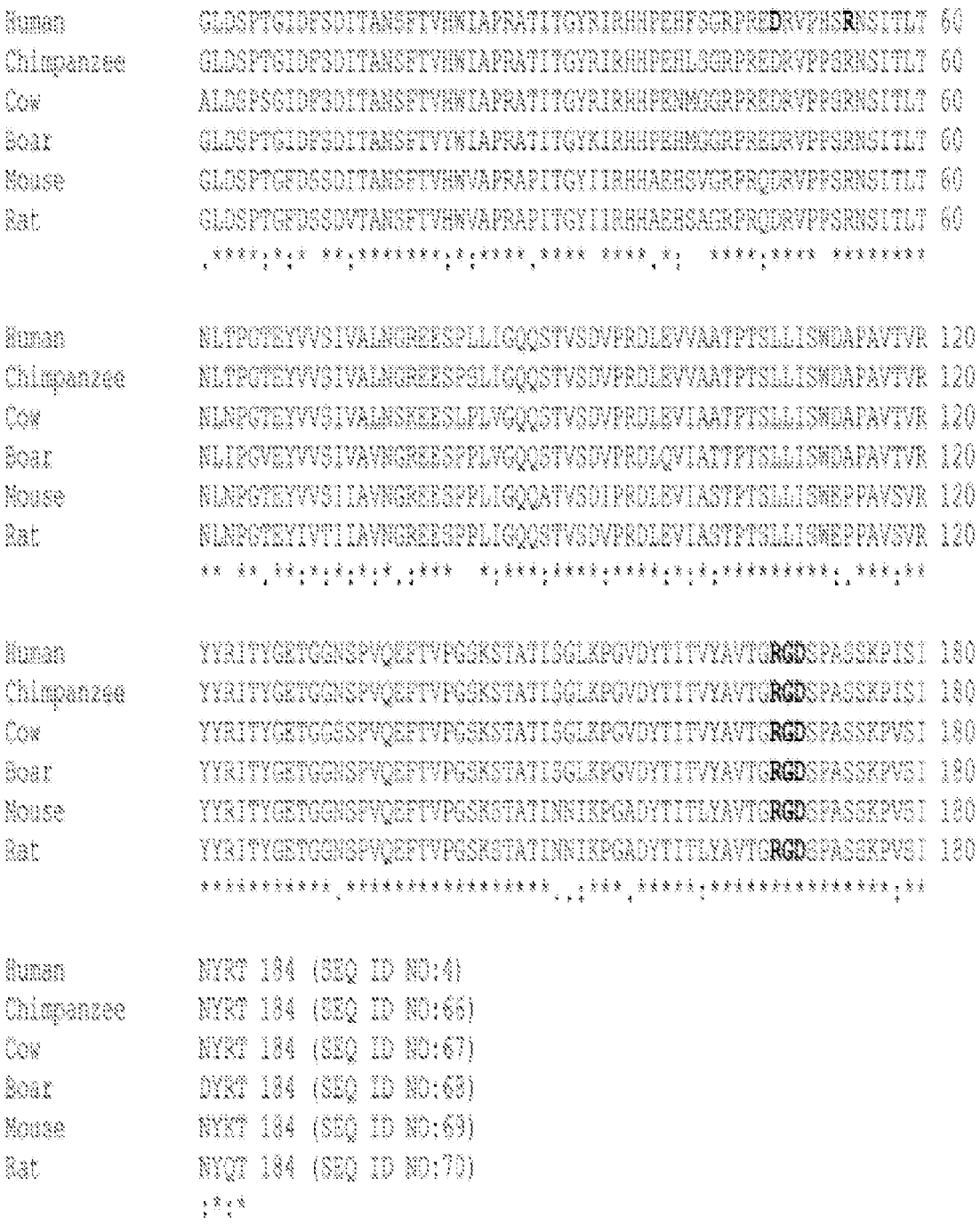

Human GLDSPTGIDFSDITANSFTVHWIAPRATITGYRIRHHPEHFSGRPREDRVPHSRNSITLT 60
Chimpanzee GLDSPTGIDFSDITANSFTVHWIAPRATITGYRIRHHPEHLSGRPREDKVPFSRNSITLT 60
Cow ALDSPSGIDFSDITANSFTVHWIAPRATITGYRIRHHPEHMSGRPREDRVPFSRNSITLT 60
Boar GLDSPTGIDFSDITANSFTVYWIAPRATITGYKIRHHPEHMSGRPREDRVPFSRNSITLT 60
Mouse GLDSPTGFDSSDITANSFTVHWVAPRAPITGYIIRHHAEHSVSGRPRQDRVPFSRNSITLT 60
Rat GLDSPTGFDSSDVTANSFTVHWVAPRAPITGYIIRHHAEHSASGRPRQDRVPFSRNSITLT 60
NLTPGTEYVVSIVALNGREESPLLIGQQSTVSDVPRDLEVVAATPTSLLISWDAPAVTVR 120
NLTPGTEYVVSIVALNGREESPGLIGQQSTVSDVPRDLEVVAATPTSLLISWDAPAVTVR 120
NLNPGTEYVVSIVALMSREESLPLVGQQSTVSDVPRDLEVIAATPTSLLISWDAPAVTVR 120
NLIPGVEYVVSIVAVNGREESPPLVGQQSTVSDVPRDLQVIATTPTSLLISWDAPAVTVR 120
NLNPGTEYVVSIIAVNGREESPPLIGQQATVSDIPRDLEVIASTPTSLLISWEPPAVSVR 120
NLNPGTEYIVTIIAVNGREESPPLIGQQSTVSDVPRDLEVIASTPTSLLISWEPPAVSVR 120
YYRITYGETGGNSPVQEFTVPGSKSTATISGLKPGVDYTITVYAVTGRGDSPASSKPISI 180
YYRITYGETGGNSPVQEFTVPGSKSTATISGLKPGVDYTITVYAVTGRGDSPASSKPISI 180
YYRITYGETGGSSPVQEFTVPGSKSTATISGLKPGVDYTITVYAVTGRGDSPASSKPVSI 180
YYRITYGETGGNSPVQEFTVPGSKSTATISGLKPGVDYTITVYAVTGRGDSPASSKPVSI 180
YYRITYGETGGNSPVQEFTVPGSKSTATINNIKPGADYTITLYAVTGRGDSPASSEPVSI 180
YYRITYGETGGNSPVQEFTVPGSKSTATINNIKPGADYTITLYAVTGRGDSPASSKPVSI 180
NYKT 184 (SEQ ID NO:4)
NYKT 184 (SEQ ID NO:66)
NYKT 184 (SEQ ID NO:67)
DYKT 184 (SEQ ID NO:68)
NYST 184 (SEQ ID NO:69)
NYQT 184 (SEQ ID NO:70)

Figure 2

A

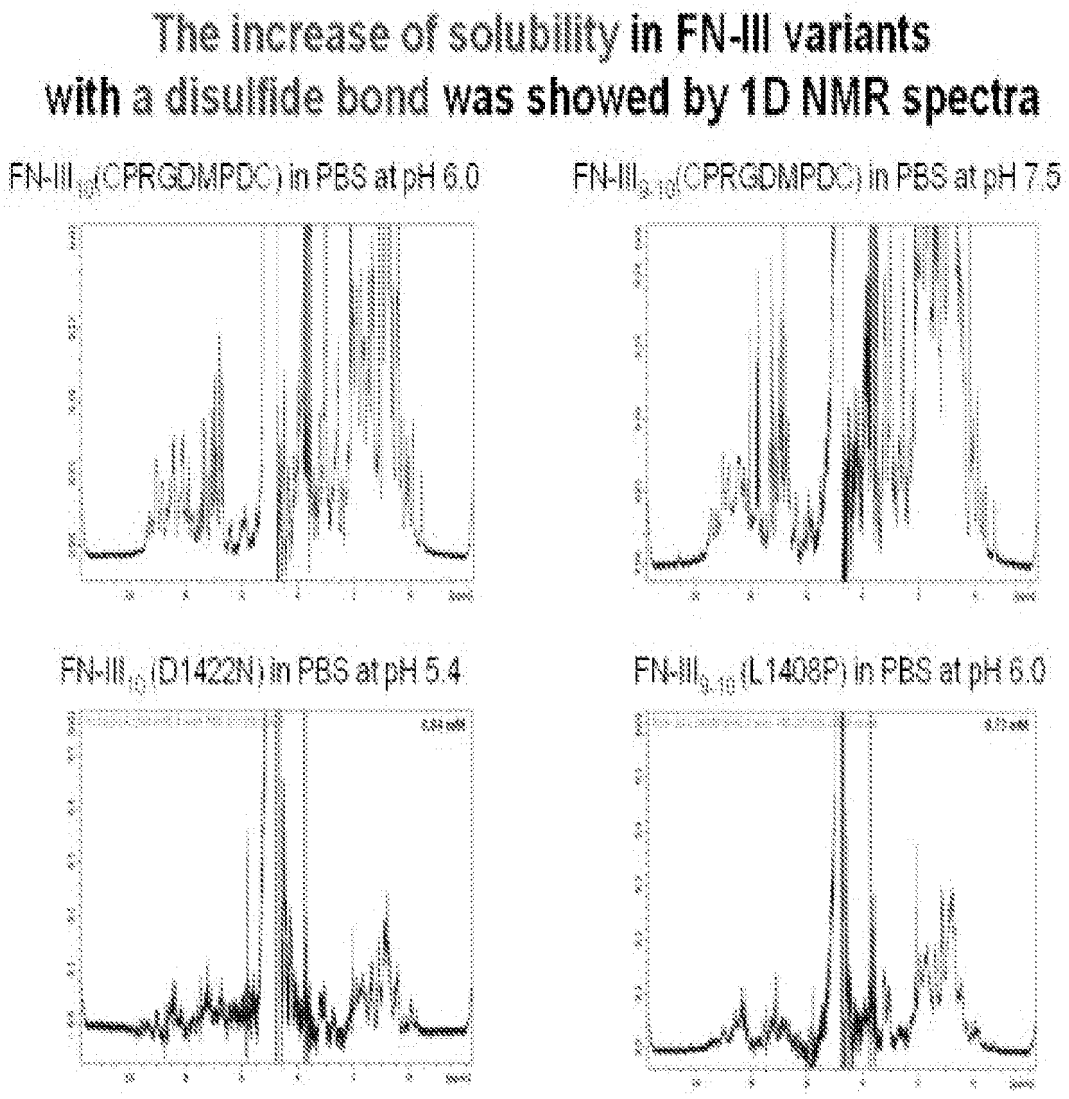
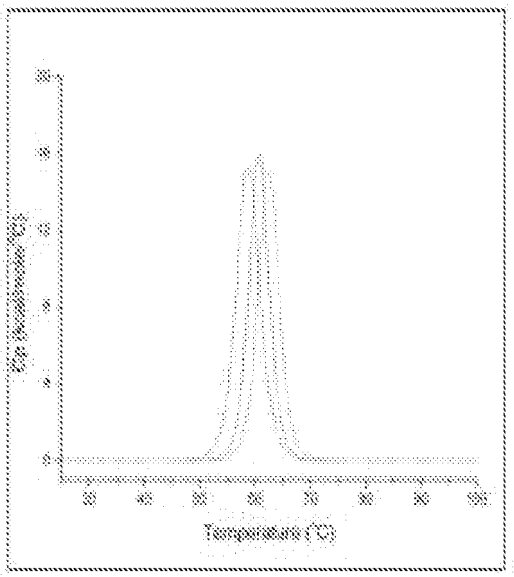

The increase of solubility in FN-III variants
with a disulfide bond was showed by 1D NMR spectra
FN-III10(CPRGDMPDC) in PBS at pH 6.0
FN-III8-10(CPRGDMPDC) in PBS at pH 7.5
FN-III10 (D1422N) in PBS at pH 5.4
FN-III8-10 (L1408P) in PBS at pH 6.0

Figure 3

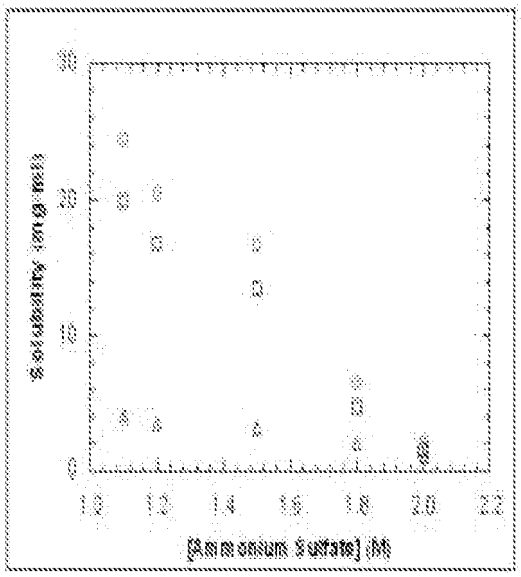
B

Thermostability and Solubility of FN-III$_{9-10}$ Variants

Thermostability determination by Differential Scanning Calorimetry



Solubility measurements by ammonium sulfate precipitation



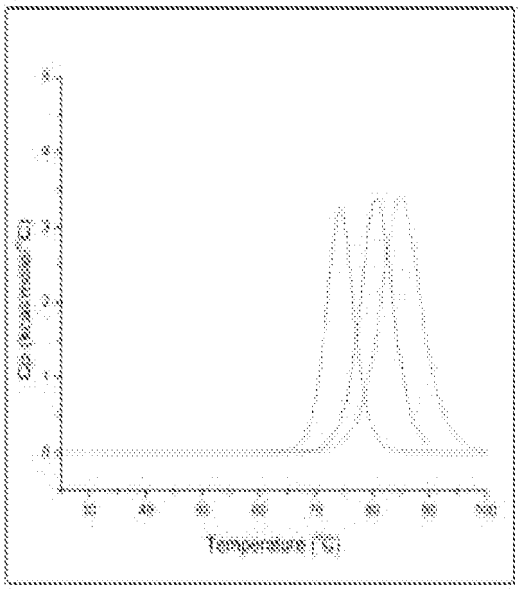
Protein	Thermostability (°C)	Solubility (mg/ml)
FN-III$_{9-10}$ L14GP	80.6	19.9
FN-III$_{9-10}$ L14GP (RAPGDIPD)	58.7	4.6
FN-III$_{9-10}$ L14GP (CRARGDIPDC)	62.4	24.4

Figure 3 (Continued)

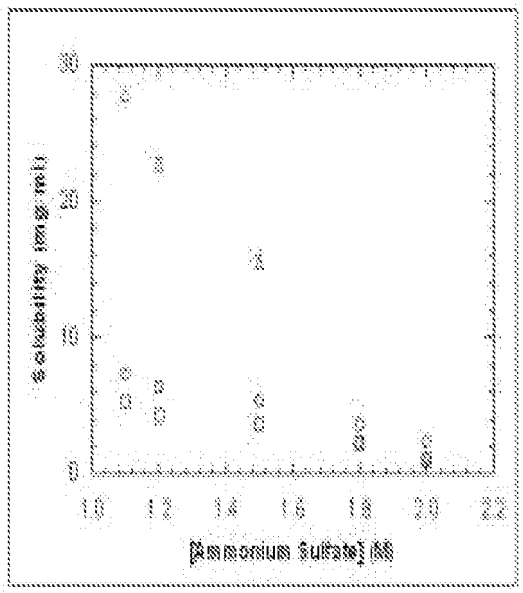
C

Thermostability and Solubility of FN-III$_{10}$ Variants

Thermostability determination by Differential Scanning Calorimetry



Solubility measurements by ammonium sulfate precipitation



Protein	Thermostability (°C)	Solubility (mg/ml)
FN-III$_{10}$	80.7	7.3
FN-III$_{10}$ (PRSOMFD)	74.3	5.3
FN-III$_{10}$ (CPRSOMFDC)	85.0	27.8

Figure 3 (Continued)

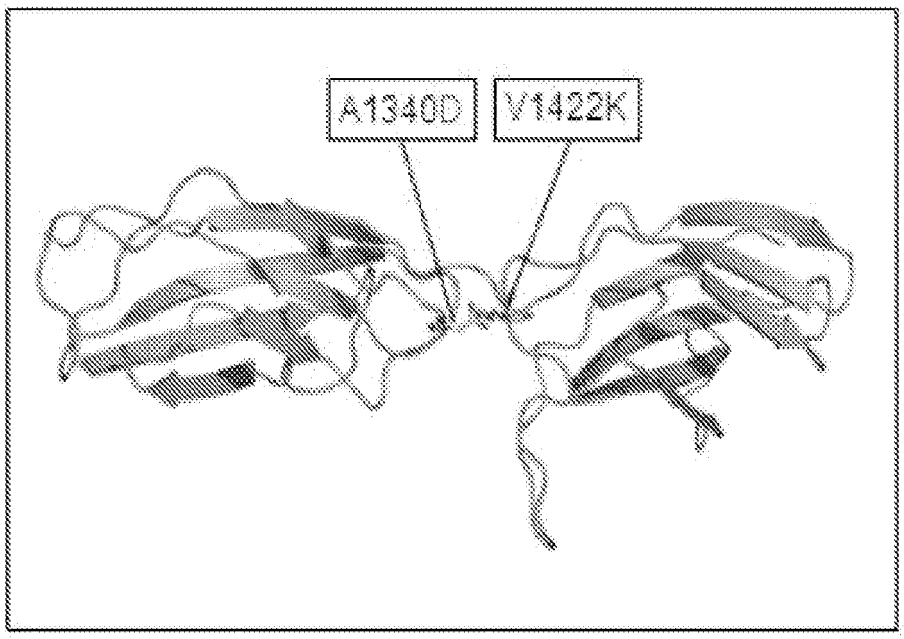


Figure 4

A.

D1373R , R1379D

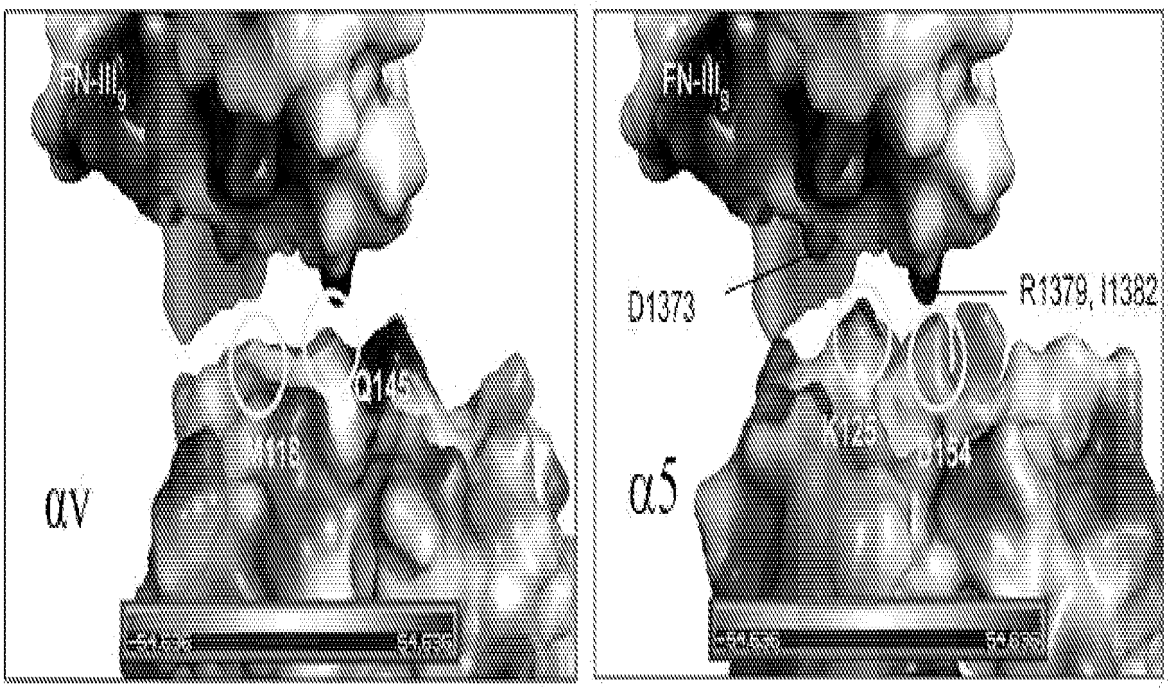
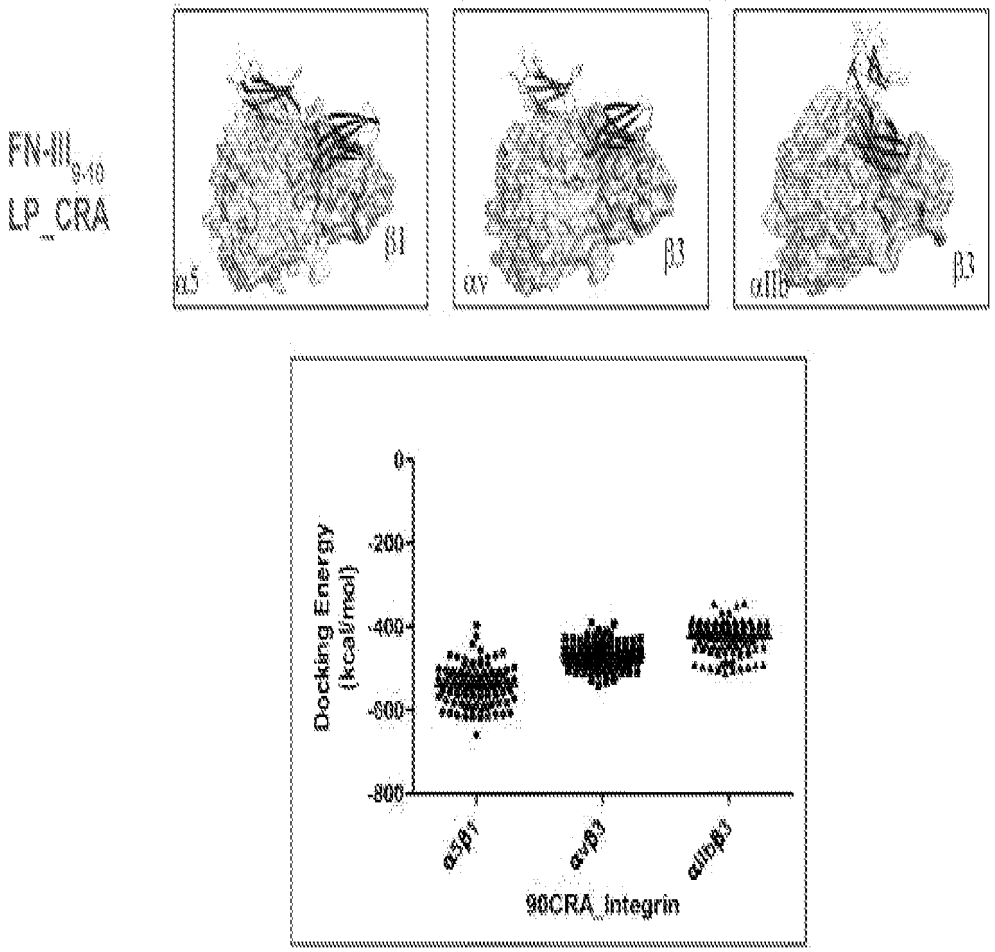


Figure 5

B.

Docking Model of FN-III$_{9-10}$LP_CRA – integrin Complexes



FN-III$_{9-10}$LP_CRA shows integrin α5β1-selectivity.

Figure 5 (Continued)

A Purification of FN-III$_{9-10}$(CPRGDMPDC)(SEQ ID NO:20) by Ni^{2+}-chelating chromatography

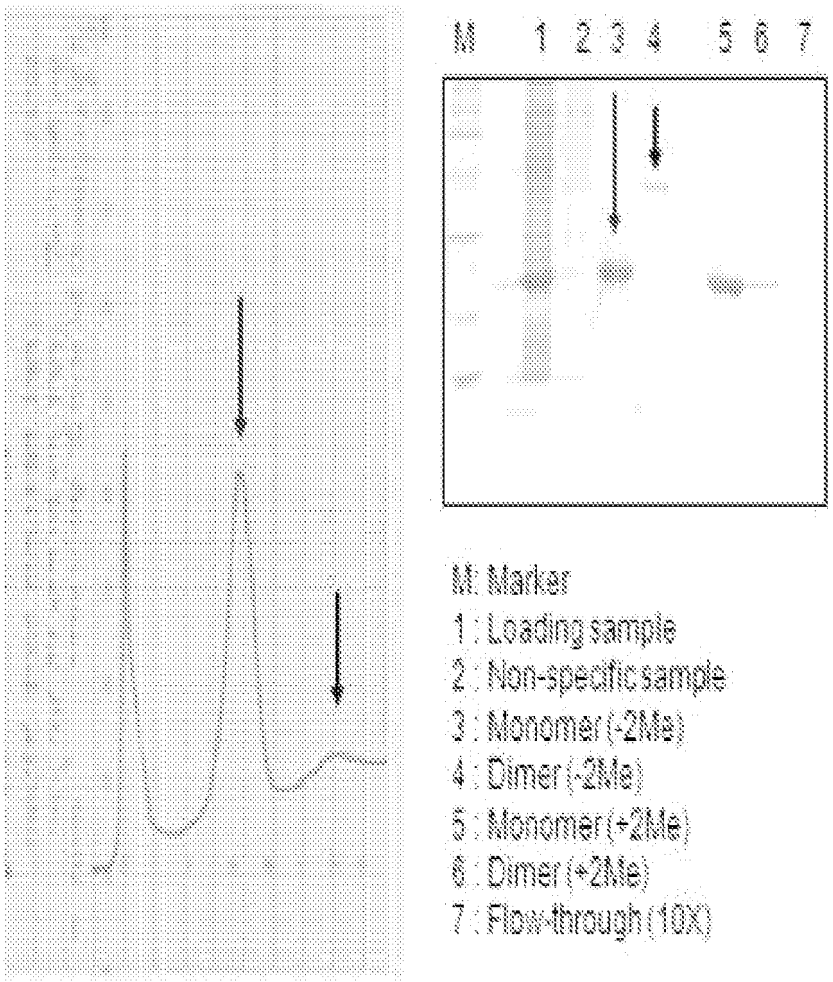
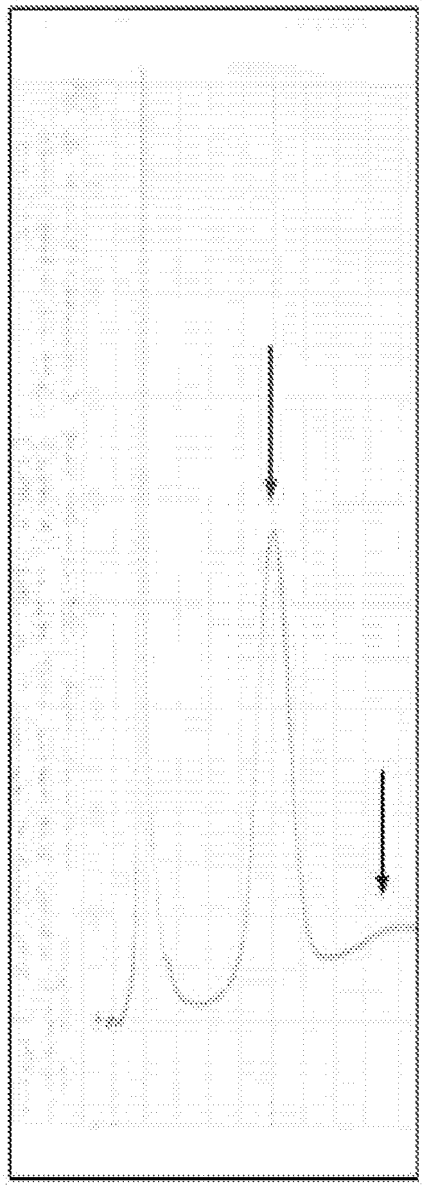
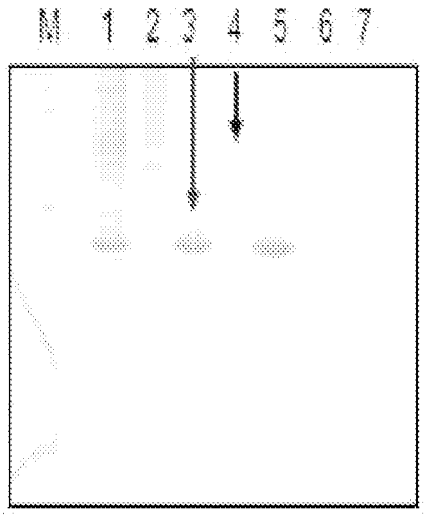


Figure 6

B Purification of FN-III$_{9-10}$L1403P(CARGDNPDC)(SEQ ID NO:22) by Ni^{2+}-chelating chromatography





M: Marker
1 : Loading sample
2 : Non-specific sample
3 : Monomer (-2Me)
4 : Dimer (-2Me)
5 : Monomer (+2Me)
6 : Dimer (+2Me)
7 : Flow-through

Figure 6 (Continued)

C Purification of FN-III$_{8-10}$(L1406P V1442K)(SEQ ID NO:38) by Ni^{2+}-chelating chromatography

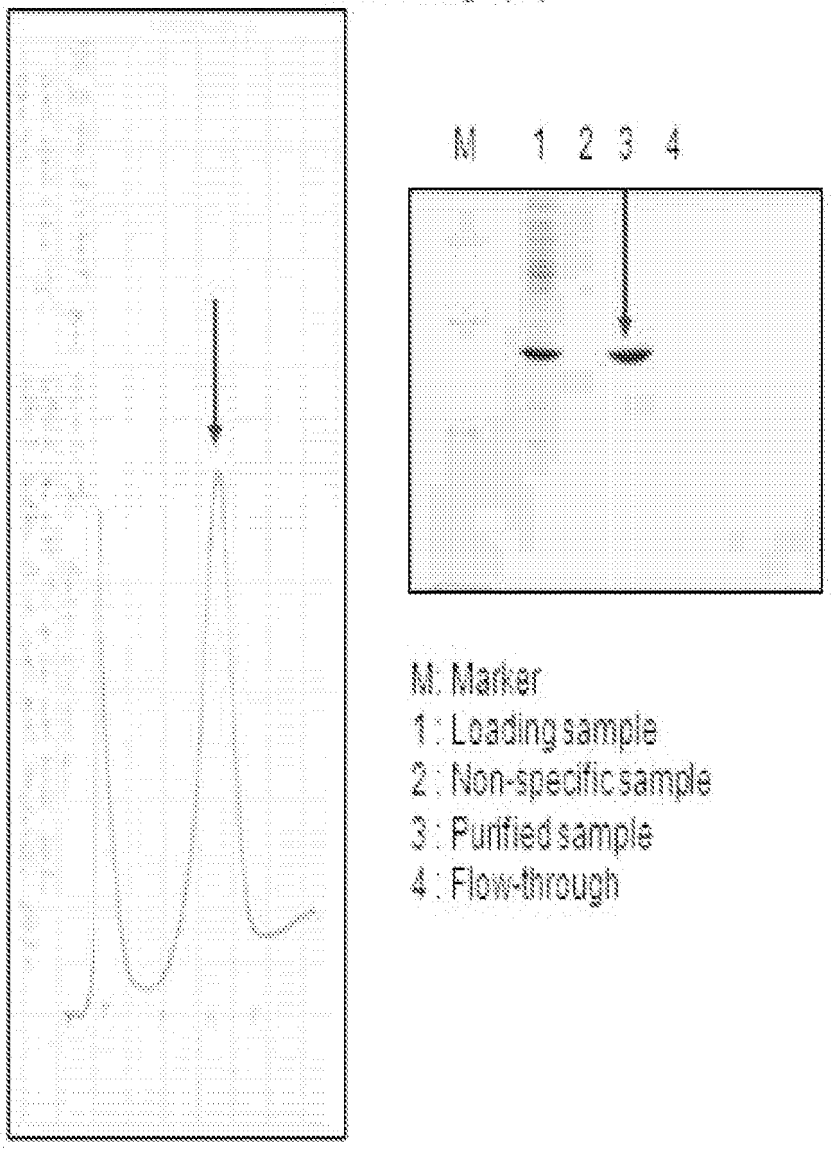


Figure 6 (Continued)

A.

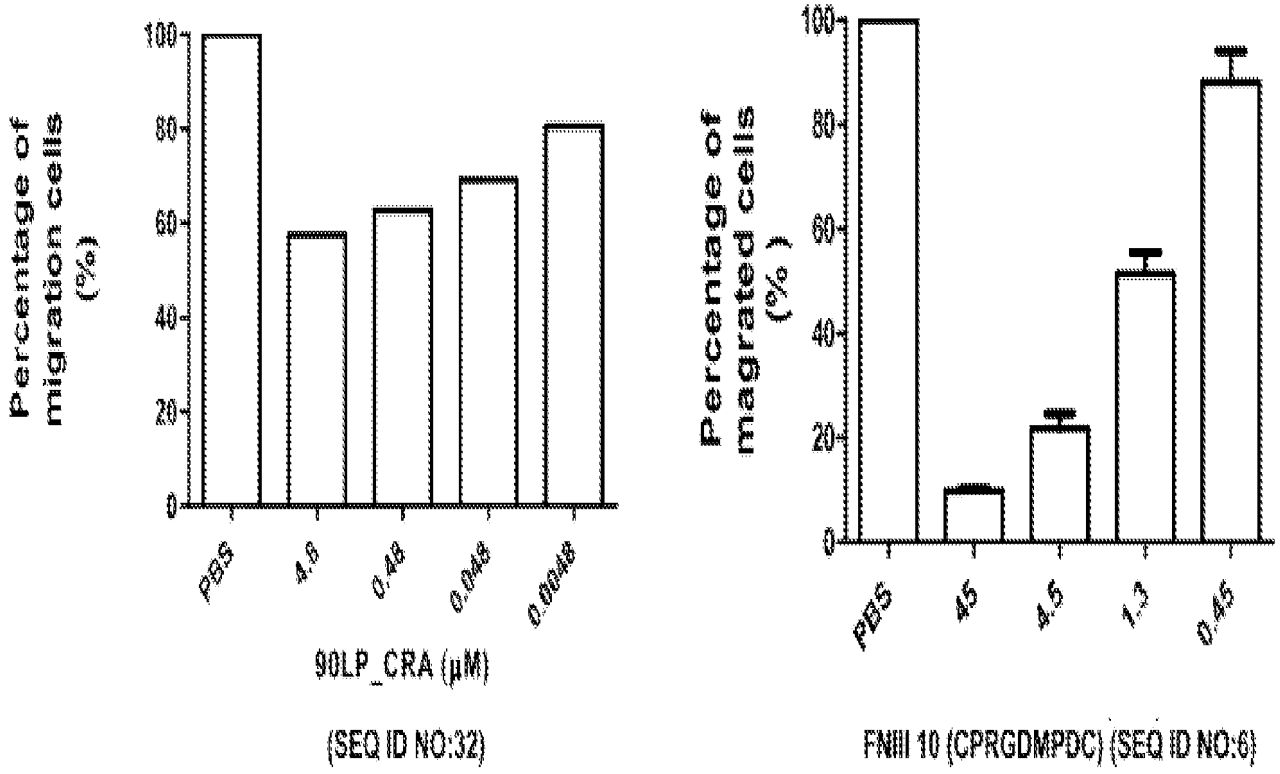

Percentage of migration cells (%)
100
80
60
40
20
0
PBS
4.5
0.48
0.048
0.0048
90LP_CRA (μM)
(SEQ ID NO:32)
Percentage of magrated cells (%)
100
80
60
40
20
0
PBS
45
4.5
1.3
0.45
FNIII 10 (CPRGDMPDC) (SEQ ID NO:6)

Figure 7

B

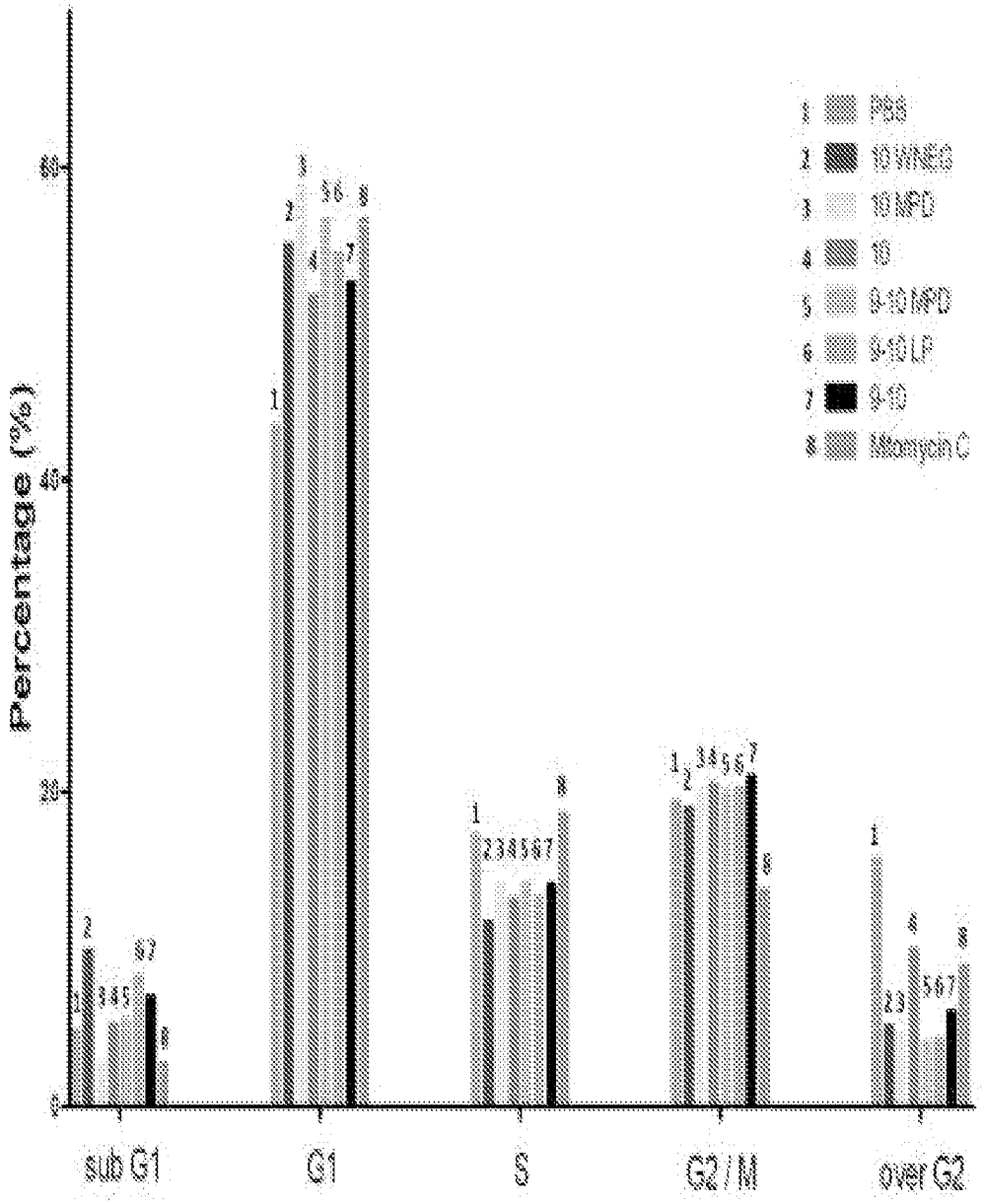


Figure 7 (Continued)

C

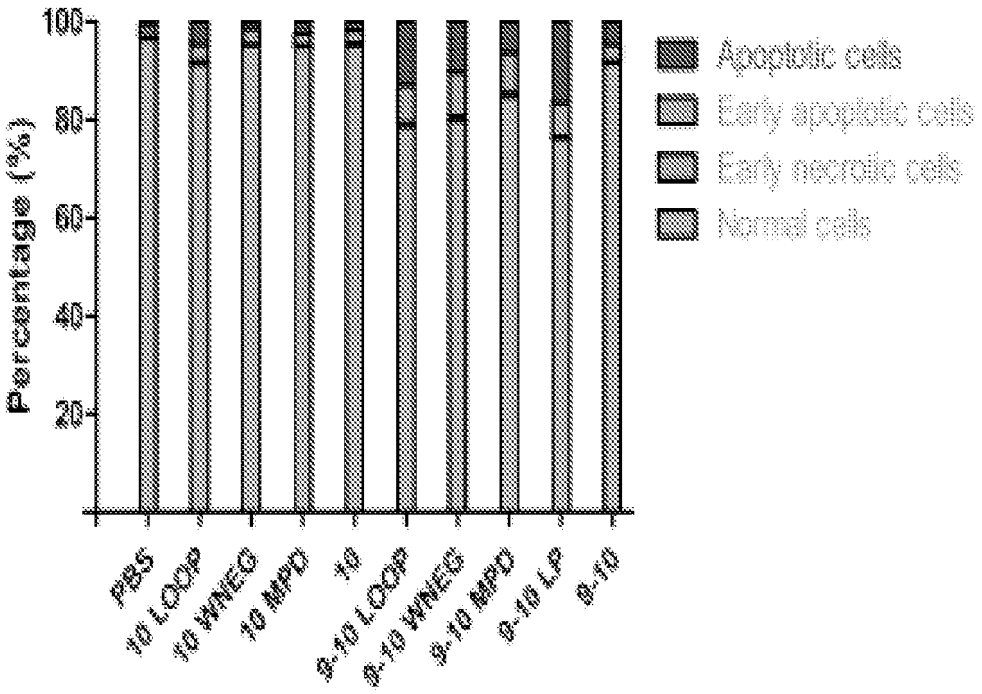


Figure 7 (Continued)

D

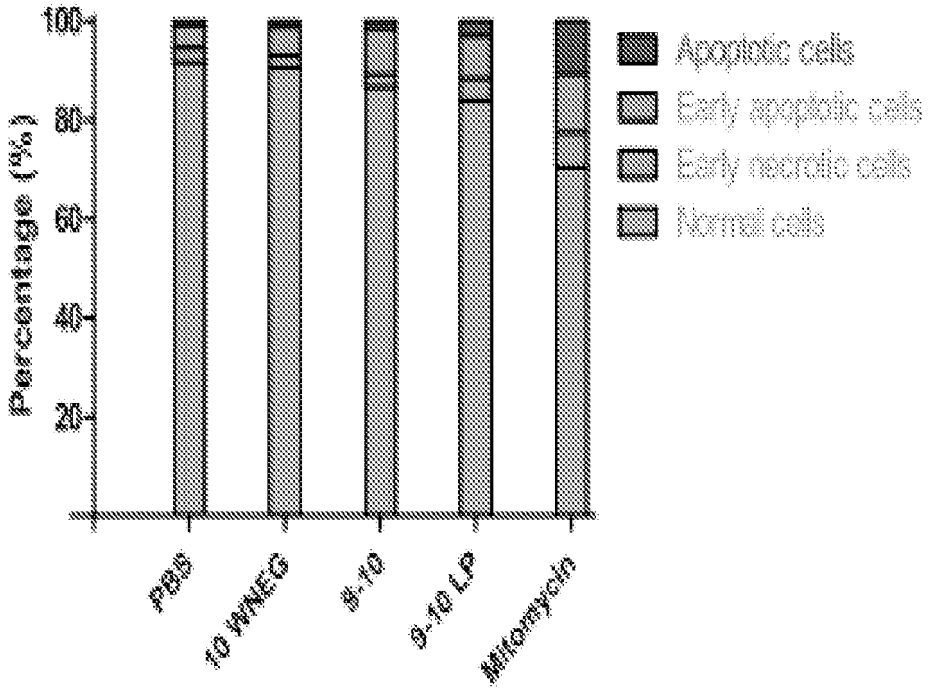
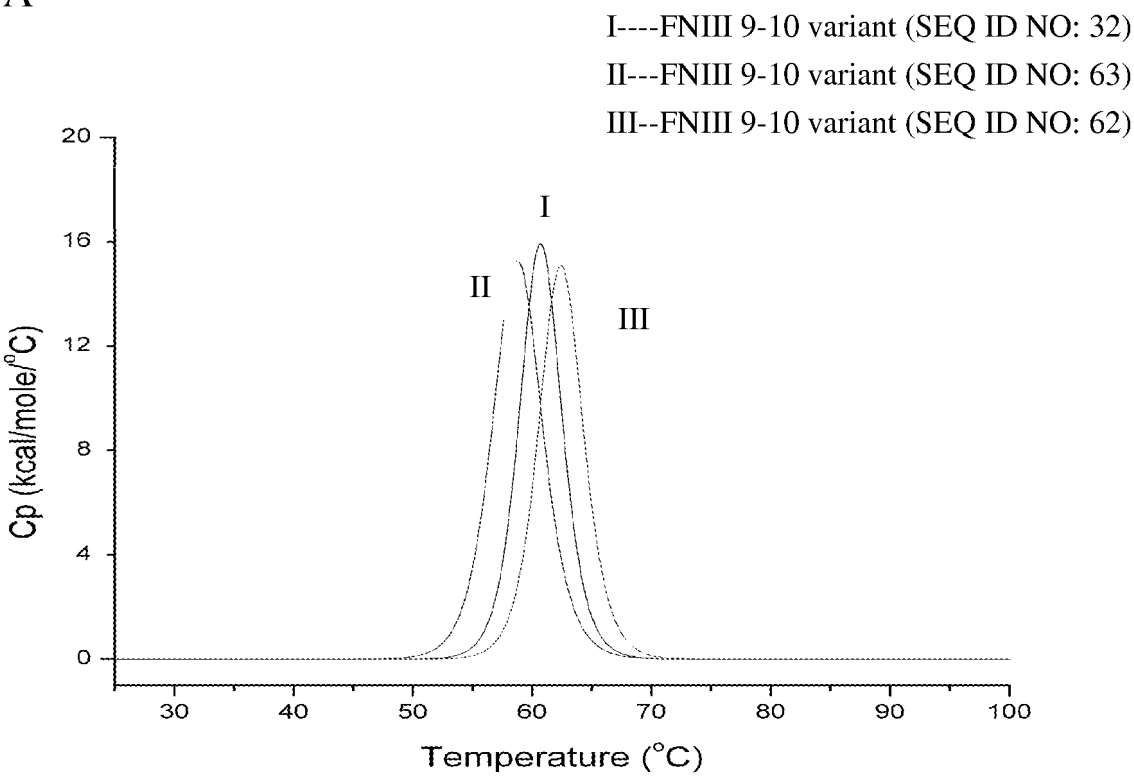


Figure 7 (Continued)

A


I----FNIII 9-10 variant (SEQ ID NO: 32)
II---FNIII 9-10 variant (SEQ ID NO: 63)
III--FNIII 9-10 variant (SEQ ID NO: 62)
I
II
III
Cp (kcal/mole/°C)
Temperature (°C)
0
4
8
12
16
20
30
40
50
60
70
80
90
100

B

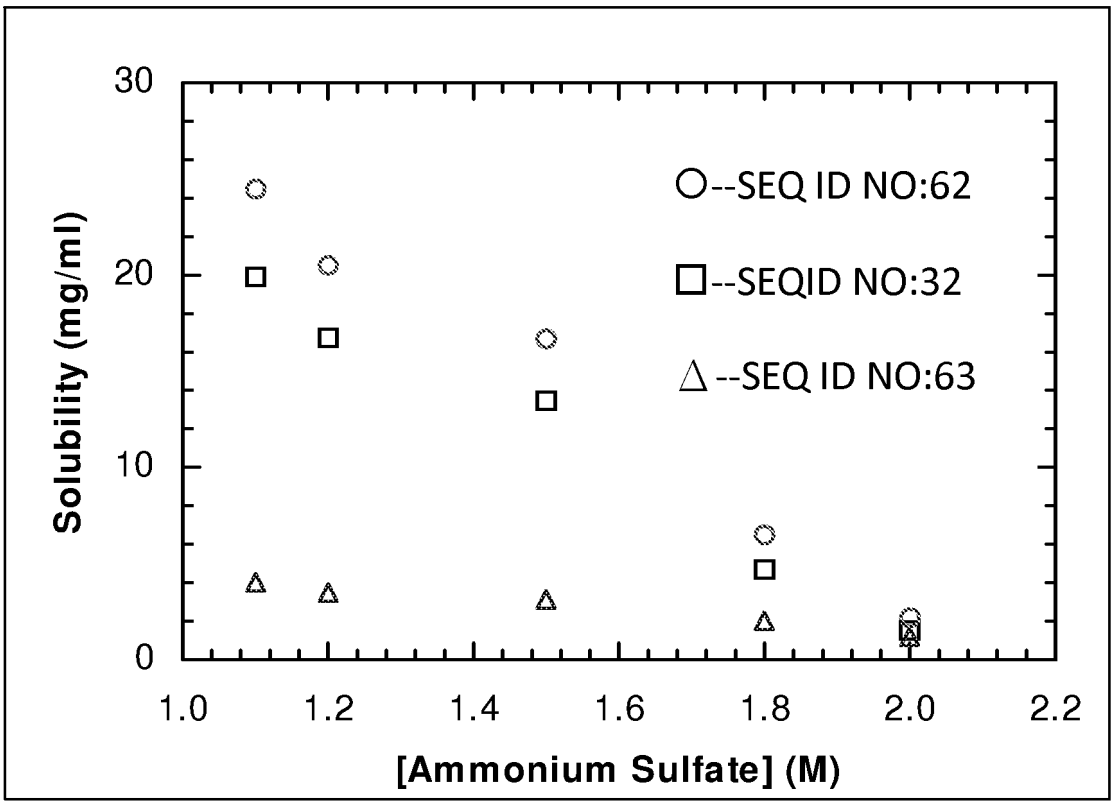
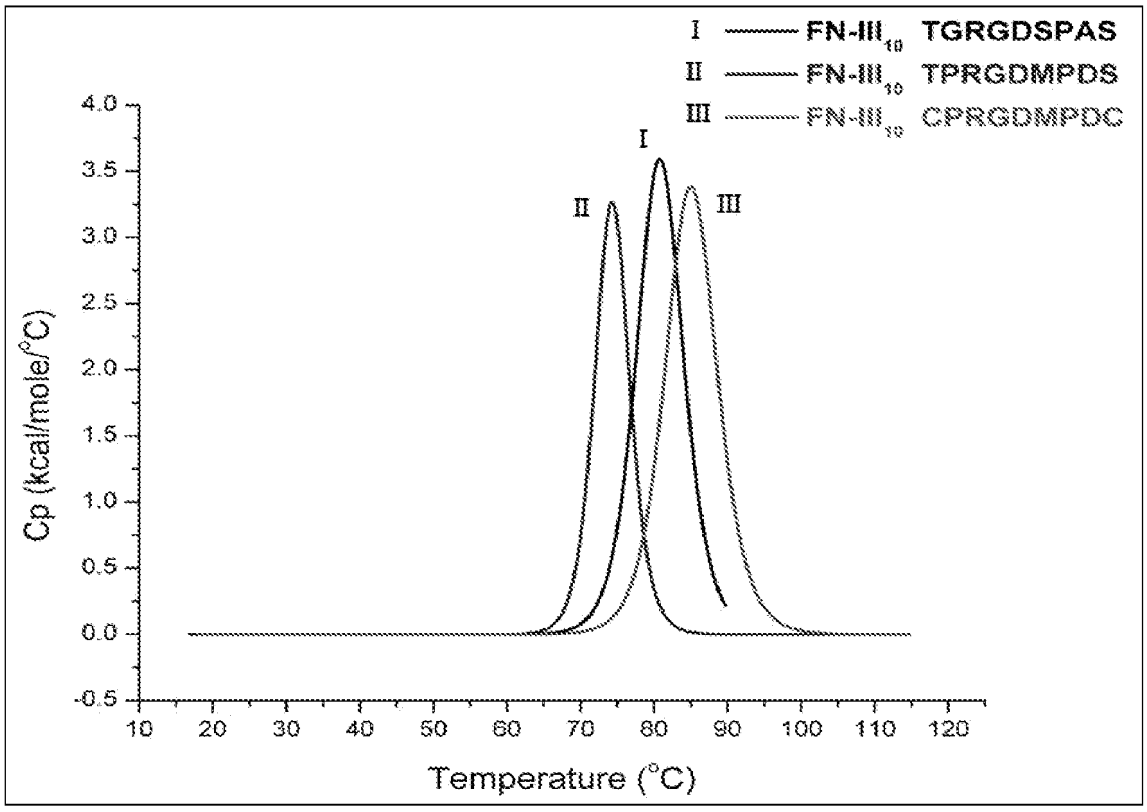

Solubility (mg/ml)
[Ammonium Sulfate] (M)
○--SEQ ID NO:62
□--SEQID NO:32
△--SEQ ID NO:63
0
10
20
30
1.0
1.2
1.4
1.6
1.8
2.0
2.2

Figure 8

A


I FN-III10 TGRGDSPAS
II FN-III10 TPRGDMPDS
III FN-III10 CPRGDMPDC
Cp (kcal/mole/°C)
Temperature (°C)

B

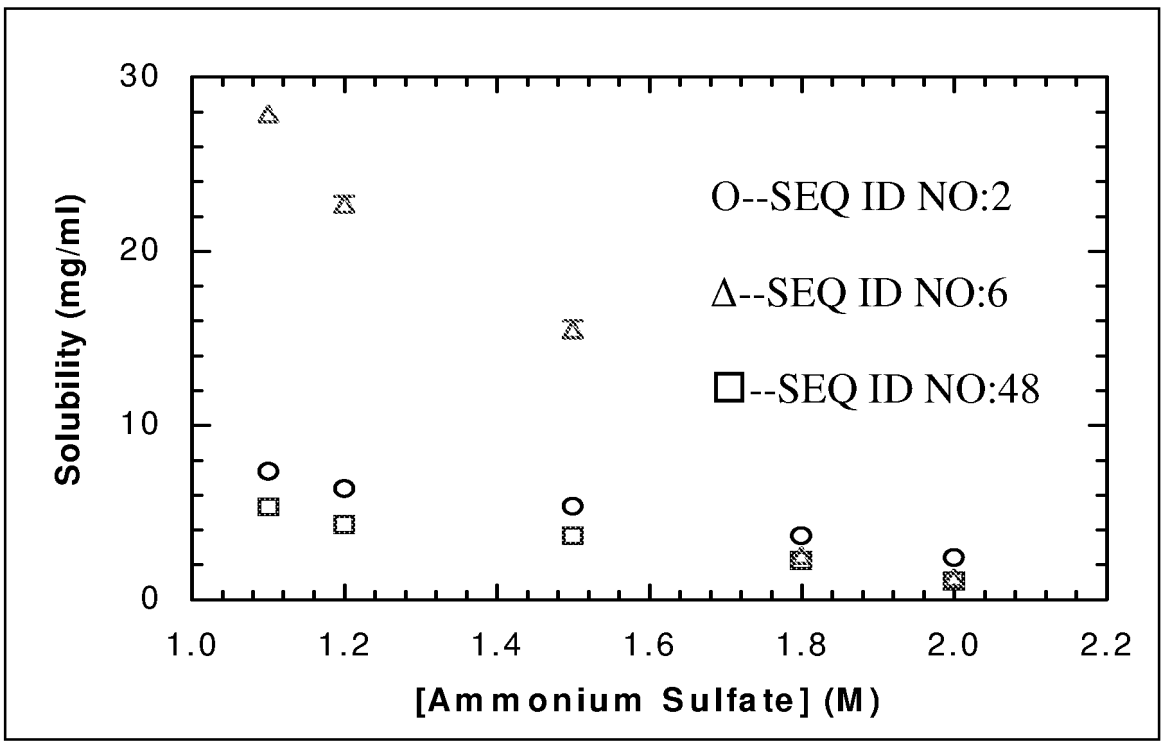

O--SEQ ID NO:2
Δ--SEQ ID NO:6
□--SEQ ID NO:48
Solubility (mg/ml)
[Ammonium Sulfate] (M)

Figure 9

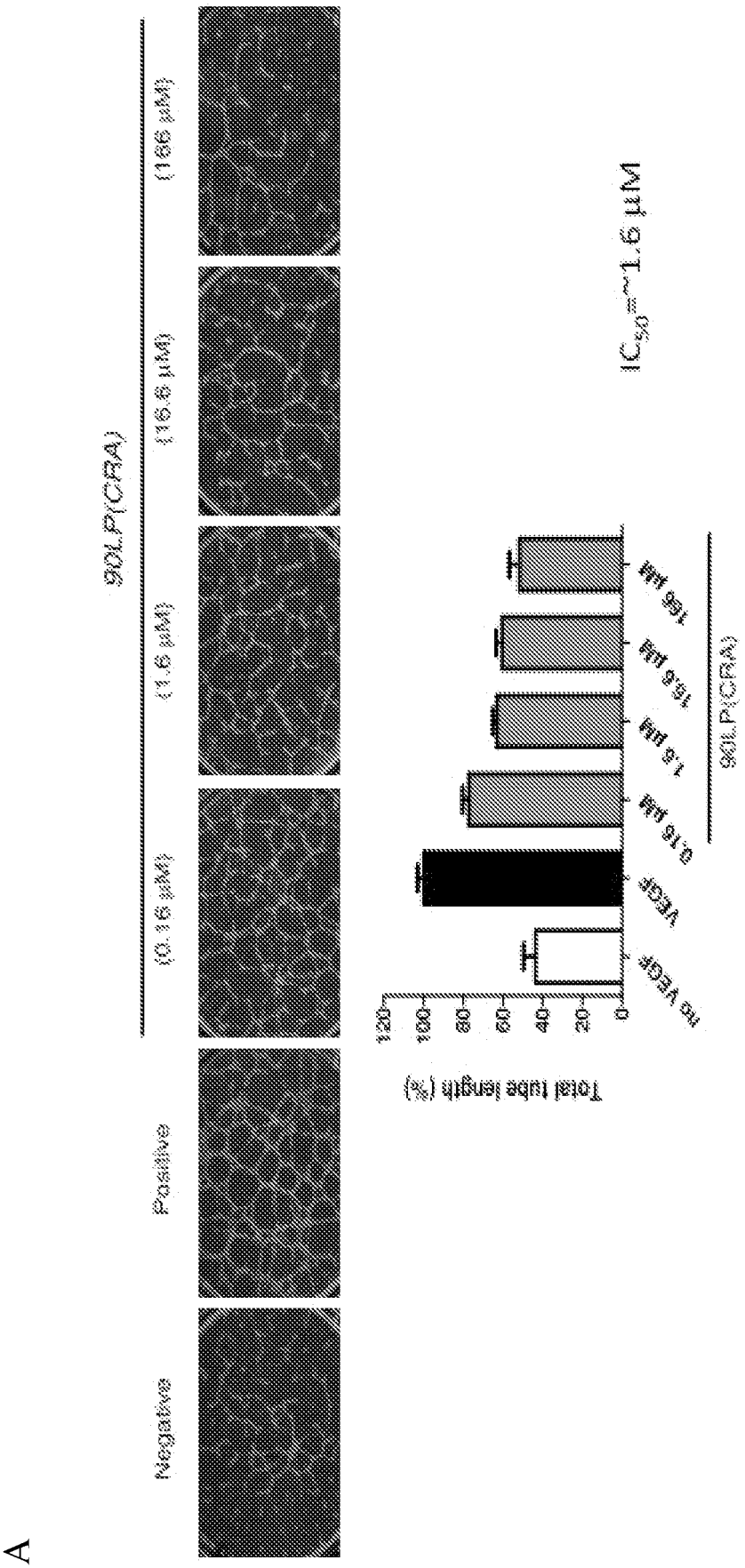

A
Negative
Positive
SOLP(CRA)
(0.16 μM)
(1.6 μM)
(16.6 μM)
(166 μM)
Total tube length (%)
120
100
80
60
40
20
0
no VEGF
VEGF
0.16 μM
1.6 μM
16.6 μM
166 μM
SOLP(CRA)
IC_{50}=~1.6 μM

Figure 10

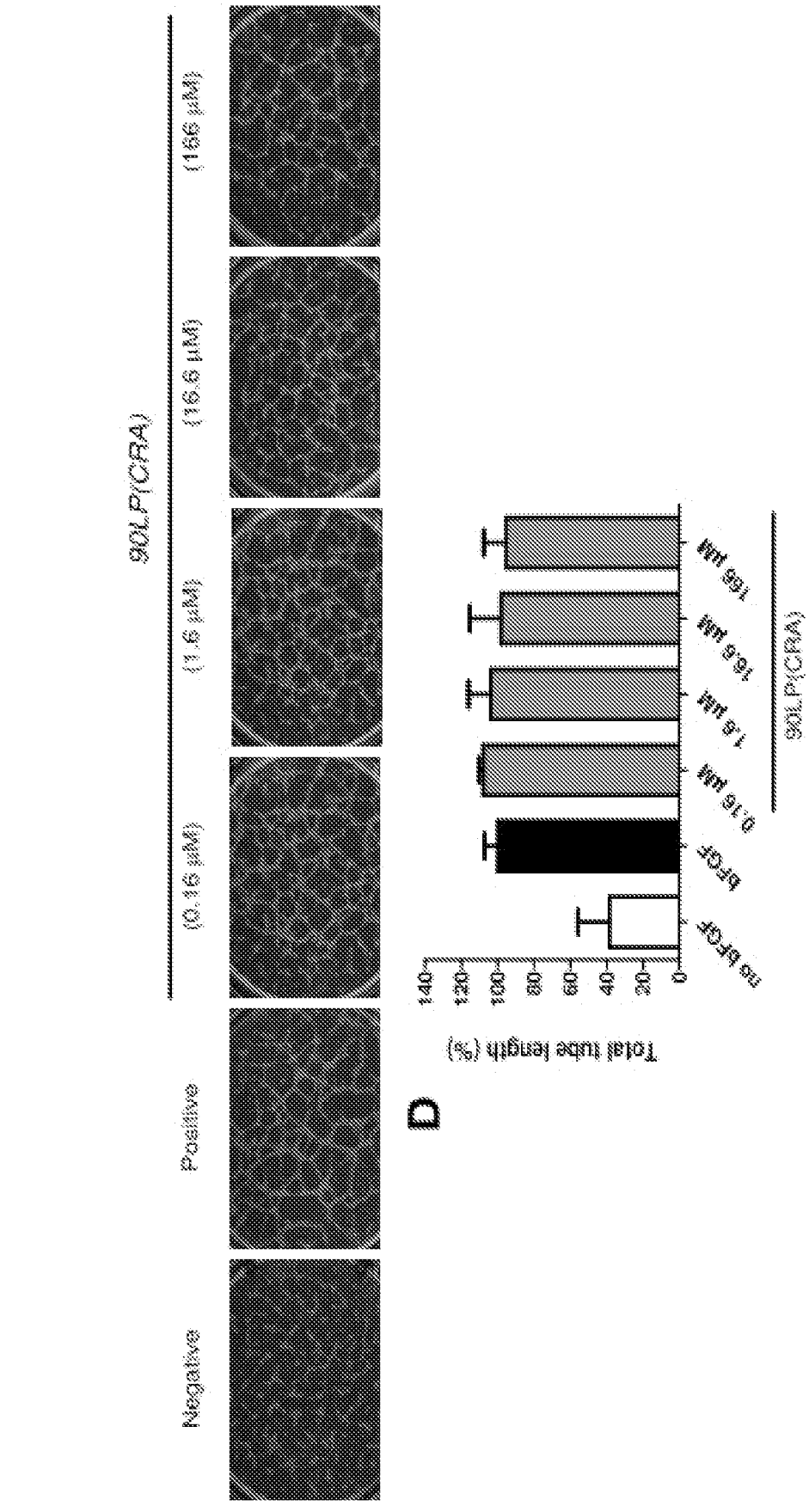
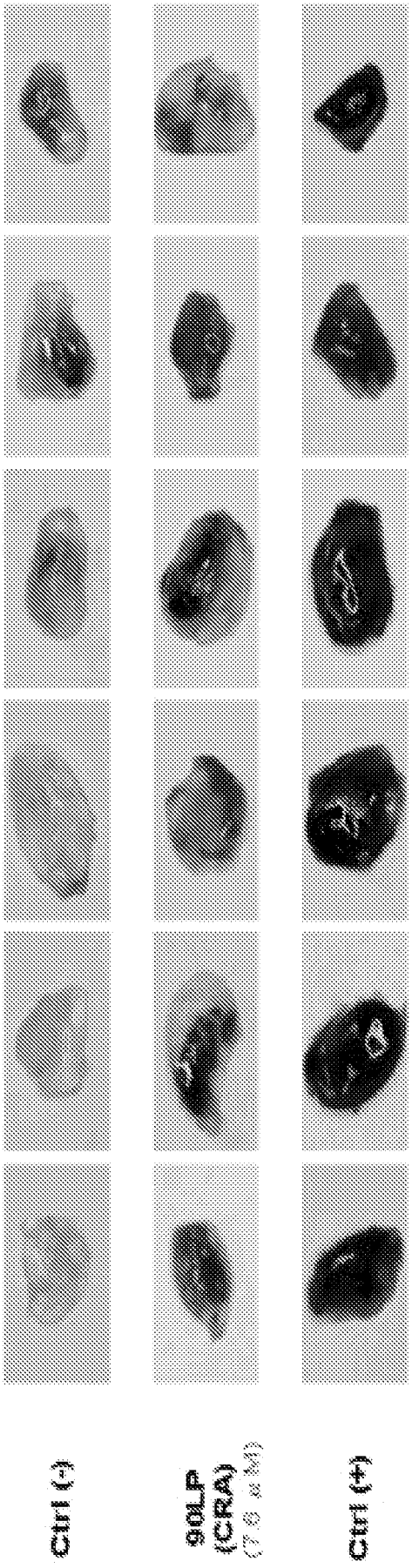


Figure 10 (Continued)



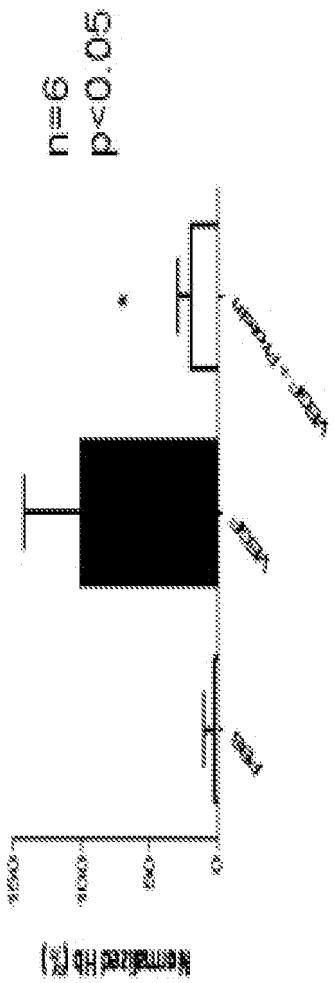


Figure 11

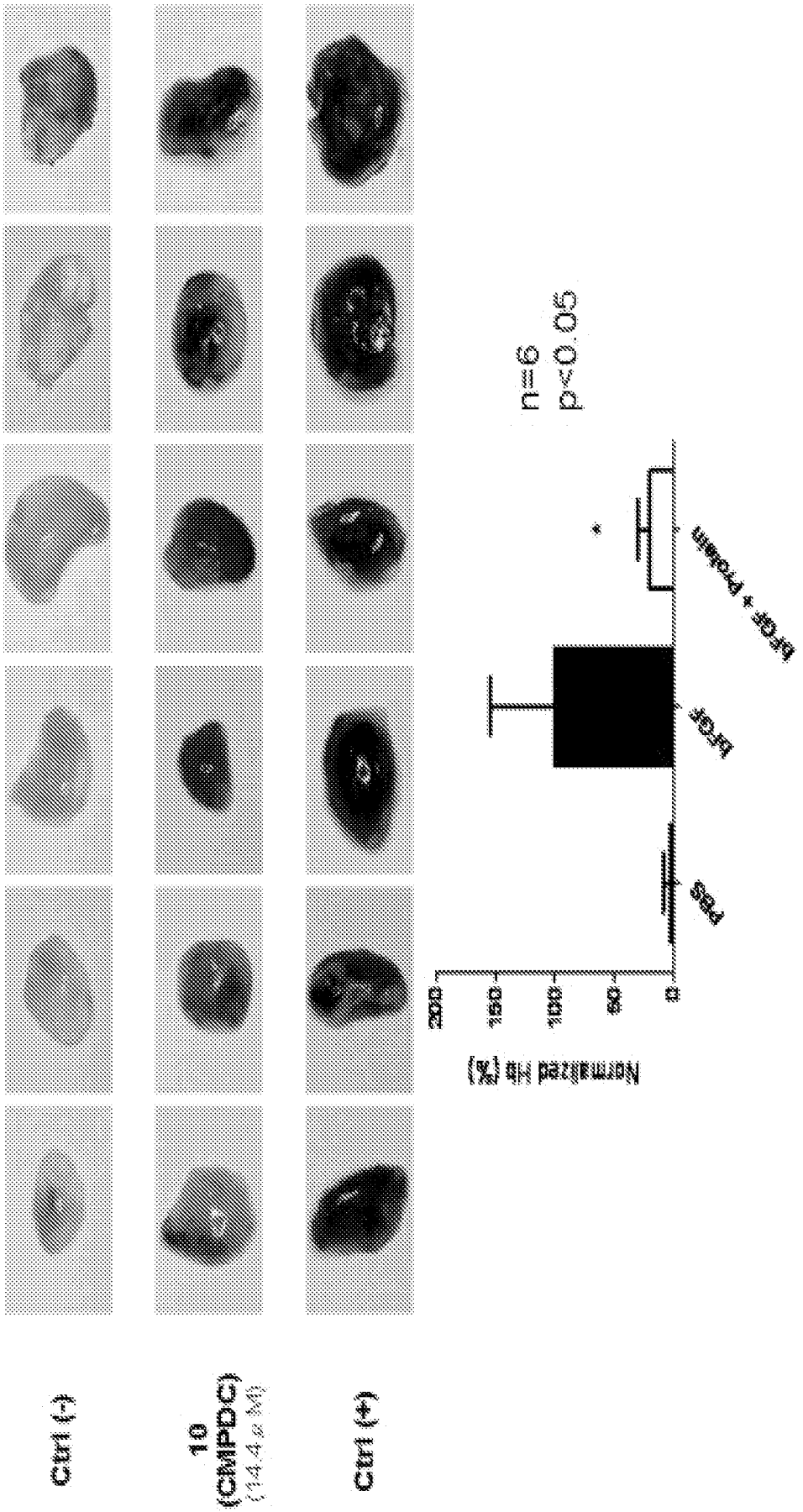

Ctrl (-)
10 (CMPDC) (14.4 μM)
Ctrl (+)
n=6
p<0.05
*
Normalized Nb (%)
200
150
100
50
0
PBS
VEGF
VEGF + Vitamin

Figure 12

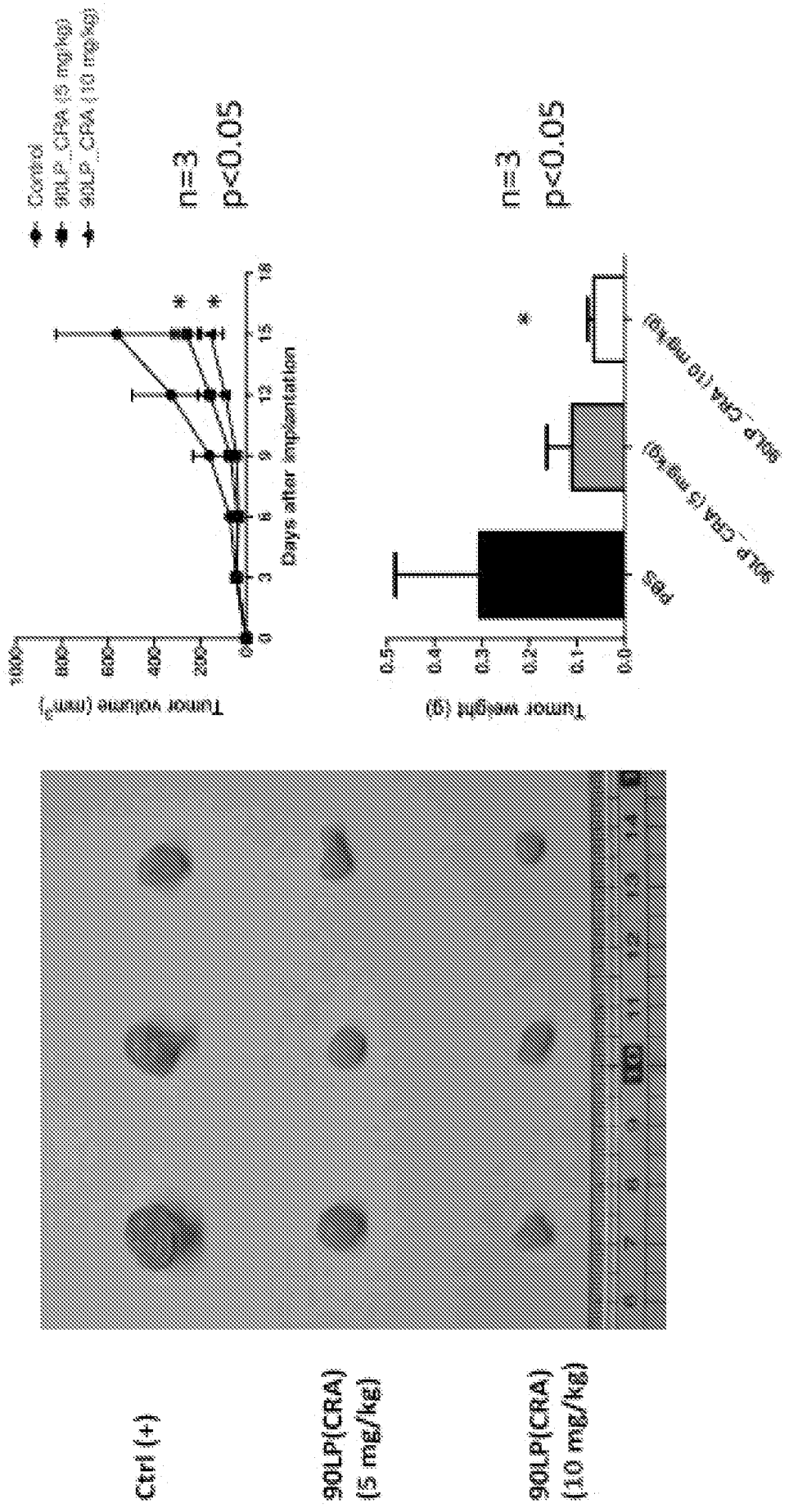

Control
90LP_CRA (5 mg/kg)
90LP_CRA (10 mg/kg)
n=3
p<0.05
Tumor volume (mm³)
Days after implantation
n=3
p<0.05
Tumor weight (g)
Saline
90LP_CRA (5 mg/kg)
90LP_CRA (10 mg/kg)
Ctrl (+)
90LP(CRA) (5 mg/kg)
90LP(CRA) (10 mg/kg)

Figure 13

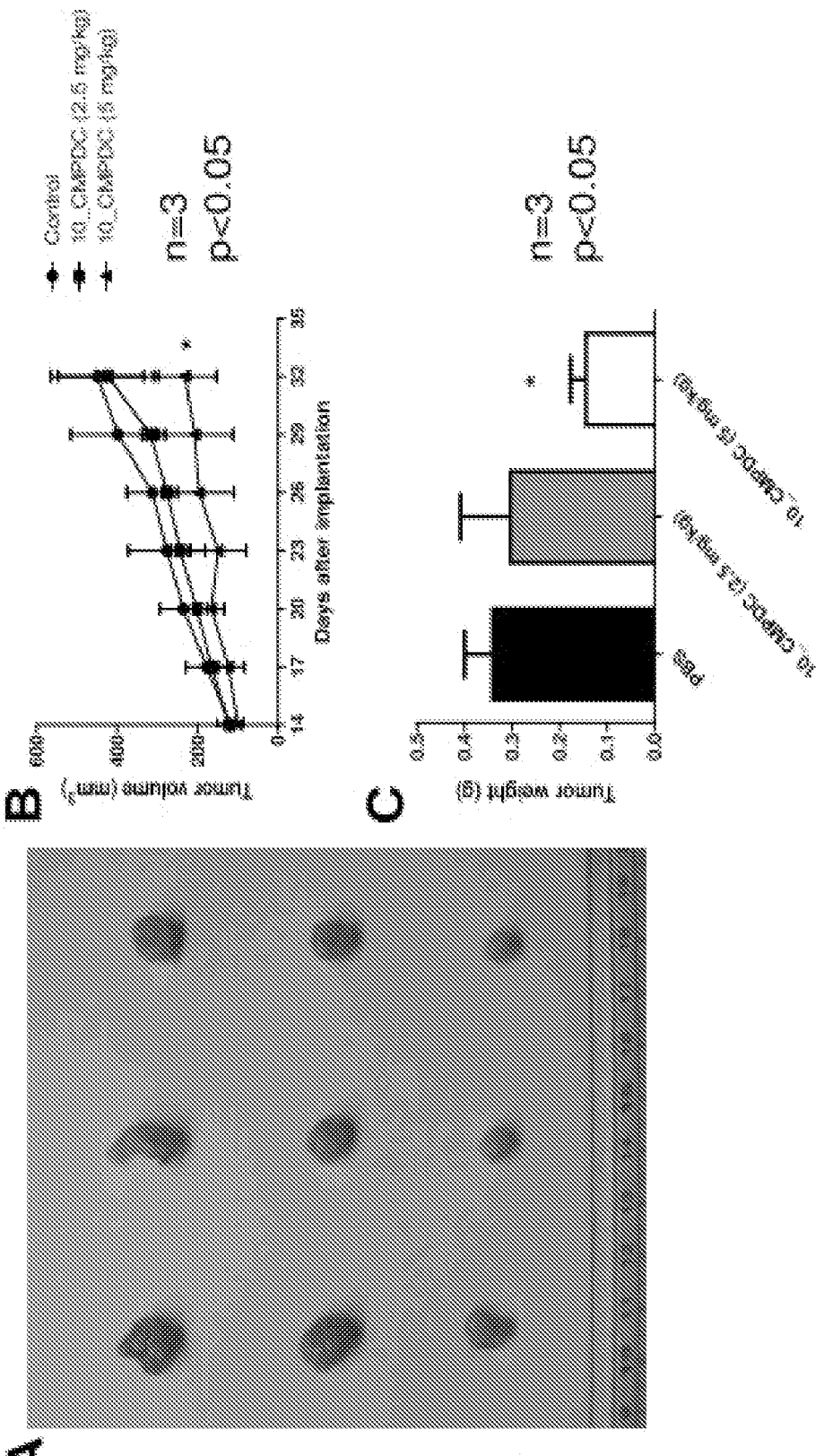

A
Ctrl (+)
10(CMPDC) (2.5 mg/kg)
10(CMPDC) (5 mg/kg)
B
Tumor volume (mm³)
Days after implantation
Control
10_CMPDC (2.5 mg/kg)
10_CMPDC (5 mg/kg)
n=3
p<0.05
C
Tumor weight (g)
PBS
10_CMPDC (2.5 mg/kg)
10_CMPDC (5 mg/kg)
n=3
p<0.05

Figure 14