

1/17

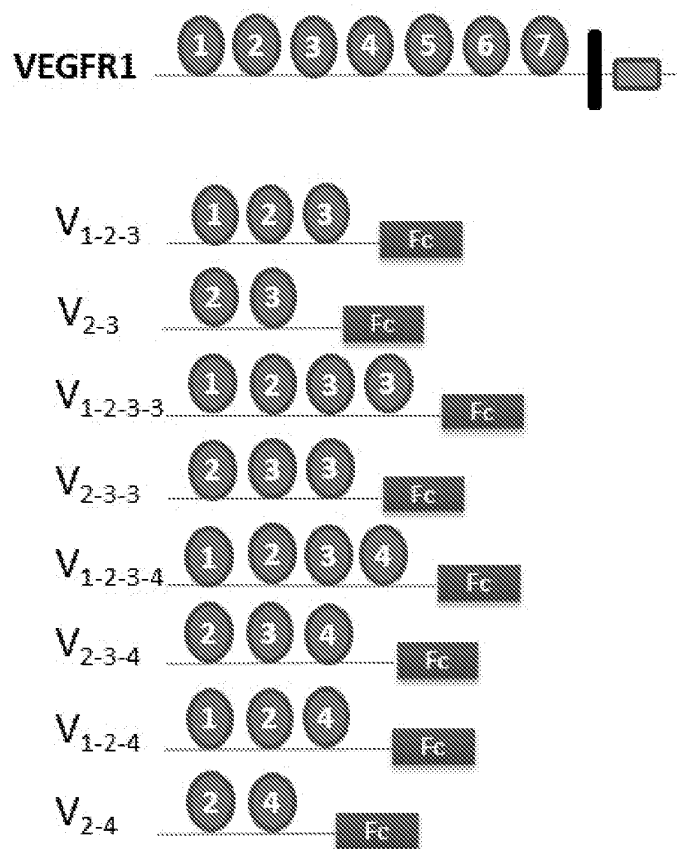


FIGURE 1

Strategy of plasmid construction and expression

Synthesis of the interested ECD fragments of VEGFR1 with enzyme sites of EcoRI/BglII



Sub-clone into mammalian expression vector pFUSE-hlgG1-Fc1 in sites EcoRI/BglII



Site mutagenesis to remove the interval BgIII site



Select positive clones and confirmation by sequencing



Small scale of expression in 293T cells and confirmation by ELISA and western blot



Expand the transient expression using Expi293 Expression System to prepare conditioned media



Protein purification

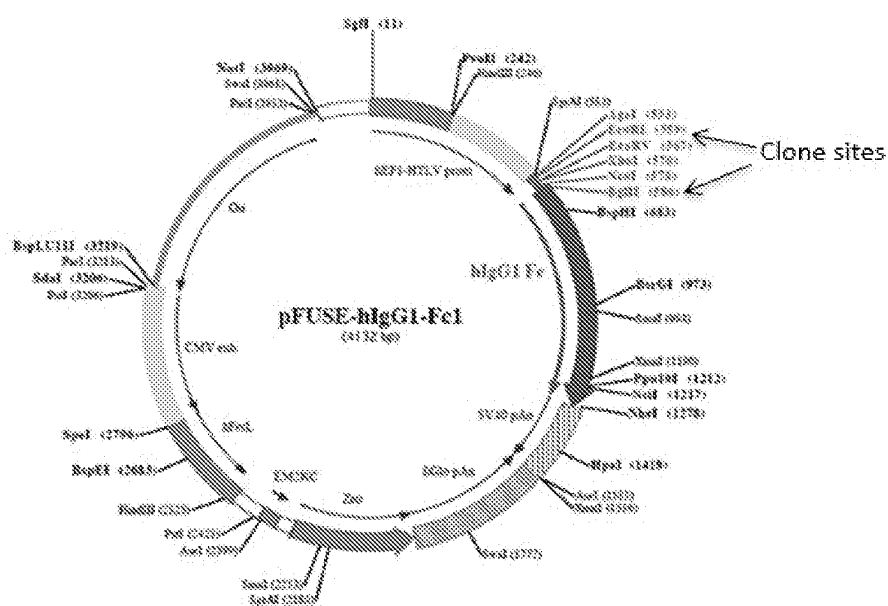


FIGURE 2

3/17

V₁₂₃

Amino acid sequence

MYSDTGVLLCAILLSCILLTSSSSGSLKDFELSLNGTQHIMQAGQTLHLQCRGEAAHKNWSPPEMVS
 KESERLSITKSAQGRNGQFCSTLILNTAQANHTGFYSCKYLAVFTSKKNETESAIIYIFISDTGRFVY
 ENYSRIPEIIMTGRNELVTPKRVTSNTIVLLKTFPLDTLIPDGKRIIWDSSRGFIISNATYKEIGL
 LTCEAIVNGHLYKTNLYLTHRQINTIIIDVQISTFRPVKLLRGHTLVNLCTATTPINTRVQMTWSYFDEK
 NKRASVRRRIDQSNSHANIYISVLTIDKMNHDKELYTCRVRSGPSFKSVNTSVHIYSKDKHTCTPPC
 PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAAKTKPREEQYNS
 TYRVVSVLTIVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL
 VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNH
 YTKSLSLSPGK

Nucleic acid sequence

ATGGTCAGCTACTGGGACACCGGGGGTCTGCTGCTGGGCGGCTGCTCAGCTGTCTGCTTCTCACAGGAT
 TAGTTTCAAGGTTCARAAATTAAAGATCCTGAACTGAGTTTAAAGGGCACCCAGCACATCATGCAAGCAG
 GCCAGACACTGCATCTCCAATGCAGGGGGGAAGCAGCCCATAAATGGTCTTTGCGCTGAAATGGTGAGT
 AAGGAAAGCGAAAGGCTGAGCATAACTAAATCTGCGCTGTGGAAGAAATGGCAACCAATTCTGCAGTAC
 TTAACTTGAACACAGCTCAAGCAARCCACACTGGCTTCTACAGCTGCCAATATCTAGCTGTACCTA
 CTTCARAGAGAGGAAGCAACAGAACTCTGCAATCTATATATTTATITAGTGATACAGGTAGAACCTTTCSTA
 GAGATGTACAGTGAATCCCGCAAAATTATACACATGACTGAAGGAAGGGAGCTCGTTCATTCCTTGGCG
 GGTACCTCACCTAACATCACTGTTACTTTAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAA
 AACGCTAATCTGGGACAGTAGAAAGGGCTTCATCATATCAAAATGCACAGTACAAAGAAATAGGGCTT
 CTGACTGTGAAGCAACAGTCAATGGGCAATTGTATAAGACAACCTATCTCACACATGCACAAACCAA
 TACAATCATAGATGTCCAAATAAGCACACCAAGCCAGTCAAAATTACTTAGAGGCCATACTCTTGTCC
 TCATTTGACTTGCTACCACTGCCCTTGAAACACGGAGTTCAAAATGACCTGGAGTTACCTGATGAAAAA
 AATAAGAGAGCTTTCGTAAAGCGGACCAATTGACCAAGCAATTCCCATGCCCCAACAATTCTACAGTGT
 TCTTACTATTGACAAATGCAAGACAAAGCAAGGCACTTTATACCTTGTCTGTGAAGAGTGGACCAT
 CAITCAAAATCTGTTAACAGCTCAGTGCATATATATGATAAAGACAAAACCTCACACATGCCCAACCGTGC
 CCAGCACCTGAACTCCTGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCCCAAGGACACCCCTCAT
 GATCTCCCGGACCCCTGAGGTCAATGCGTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGT
 TCAACTGGTACGTGGACGGCGTGGAGGTGCAATAATGCCAAGACAAAGCCCGGGAGGAGCAGTACAAC
 AGCACGTACCGTGTGCTCAGCGTCTCACCGTCTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA
 GTGCAAGGTCTCCAAACAAGCCCTCCAGGCCCATCGAGAAACCACTCTCCAAAGCCAAAGGGCAGC
 CCCGAGAACCAAGGTGTACACCTGCCCCCATCCCGGAGGAGATGACCAAGAACCAAGGTCAAGCTG
 ACCTGCTGGTCAAAGGCTTCTATCCAGCGACATCCCGTGGAGTGGGAGAGCAATGGGCAGCCGGA
 GAACAACTACAAGACACGCTCCCGTGTGGAGTCCGAGCGGCTCTTCTCTCTACAGCAAGCTCA
 CCGTGGACAAGAGCAGGTGCGACAGGGGAAAGCTCTCTCATGCTCCGTGATGCAACGAGGCTCTGCA
 AACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGTAAA

Grey with italic: signal peptide; Yellow: Ig-like domain 1; Blue: Ig-like domain 2

Grey: Ig-like domain 3; Black with underline: Human IgG1-Fc fragment

FIGURE 3

4/17

V₂₃ Amino acid sequence

MVSYWDTGVLLCAILSCILLTGSSEGI FISDTGRPFVEMYSEIPEIIHMTGRELVIPCRVTSFNITV
 TLEKFFLDTLIPDGRRIIWDSENGFIISNATYFEIGLLTCEATVNGHLYKTNFLTHRQTNTIISVQIS
 IPRFVRLLAGHTLVNCTATTPLNTRVQMTWSYPDEFNKRASVERRIDCSNSEANIFYSVLTIDEMQN
 KDKSLYTICRVRSGPSEKSVNTSVHIYDKDKTHICPPCPAPELLGGPSVFLFPKPKDILMISRTPEVT
 CVVVDSHEDPEVKFNWYVDGVEVHNATKEREQYNSTYRVVSVLTVLHQDNLNGKEYKCNVSNKAL
 PAPIEKTISKANGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPF
 VLDSGSEFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLS PGK

Nucleic acid sequence

ATGGTCAGCTACTGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGTGTCTGCTTCTCACAGGATC
 TAGTTCAGGTATATTTATTAGTGATACAGGTAGACCTTTCGTAGAGATGTACAGTGAATCCCGGAA
 TTATACACATGACTGAAGGAAGGGAGCTCTCTATTCCCTGCGGGTTACGTCACTTAACATCACTGT
 ACTTTAAAAAGTTTCCTCTTGACACTTIGATCCCTGATGGAAAAACGCATAATCTGGACAGTAGAAA
 GGGCTTCATCATATCAAAATGCACGTACAAAGAAATAGGGCTTCTGACCTGTGAAGCAACAGTCAATG
 GGCATTGTATAGACAACTATCTCACACATCGACAAACCAATACAAATCATAGATGTCCAAATAAGC
 ACACCCGCGCCAGTCAAATTACTTAGAGGCCATACTCTTGTCTCAATGTACTGCTACCCCTCCCT
 GAACACGAGAGTTCAAATGACCTGGAGTTACCTTGATGAATAAATAAGAGAGCTTCCGTAAAGCGGAC
 GAATTGACCAAGCAATTCOCATGCCACATATTCTACAGTGTCTTACTATTGACAAATGCAGAAC
 AAGACAAAGGACTTATATCTTGTGGTGAAGGAGTGGACCATCAATCAAAATCTGTTAAACCTCAGT
 GCATATATATGATAAAACAAACTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGAC
 CGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTACAC
 TGCGTGGTGGTGGAGCTGAGCCACGAAGACCTGAGGTCAAGTTCACCTGGTACGTGGAAGCGTGGG
 GGTGCATAATGCCAAGACAAAGCCCGGGAGGAGCAGTACAAACAGCACGTACCGTGTGCTCAGCGTCC
 TCACCGTCTTGCAACAGGACTGGCTGAATGGCAAGGAGTACAGTGCAGGCTCTCCAAACAAAGCCCTC
 CCAGCCCCATCGAGAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACACAGGTGTACACCTC
 GCCCCATCCCGGAGGAGATGACCAAGAACAGGTGACGCTGACCTGACCTGGTCAAGGGCTTCTATC
 CCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAAGCGGAGAACAACTACAAAGACCGGCTCC
 GTGCTGGACTCCGAGCGCTCTTCTTCTCTACAGCAAGCTCACCGTGGACAAAGAGGAGTGGAGCA
 GGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGACAAACCACTACACGAGAGAGCCCTCT
 CCTGTCTCCGGTAAA

Grey with *italic*: signal peptide; Blue: Ig-like domain 2; Grey: Ig-like domain 3

Black with underline: Human IgG1-Fc fragment

FIGURE 4

5/17

V₁₂₃₃

Amino acid sequence

NTVYWDTEGVLLCALESCELLTGGSSGSKLDPELSLNGTQHMQAGQTLHLQCRGERAAHNSLPENVS
 KESERLSITKSACGRNGKQFCSTILTNTAQANHTGFYSCNYLAVPTSKNNEETESAIIYIFISDTGRPEV
 EMYSEIPEIILHMTGKELVIPCWVTSNITVTLKKYPLDTLIPDGKRIIWUSRRGFIISNATYKEIGL
 LTCEATVNGHLYKINYLHKQINTI IDVQISTPFRVYKLLAGHTIVLWSTAITPLNIRVQMTNSYFEEK
 NERASVRRRIDQSNSSHANIFYSVLTIDKMQNDKGLTICRVKSGSPFKSVNTSVHIYDAVQISTPFR
 VKLLRGHTIVLWSTAITPLNIRVQMTNSYFSENNKRAVSRRRIDQSNSSHANIFYSVLTIDKMQNDKGL
 LITCRVRSQSPFKSVNTSVHIYDMDKTHTCPPCPAPPELLGGPSVFLFPFKPKDTLMISRTPEVTCVVV
 DVSHEDEPVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTIVLHQQDNLNGKEYKCNVSNKALPABI
 EMTISKAKGQPREPQVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDL
 DGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALNNHYTKQSLSLSPGK

Nucleic acid sequence

ATGGTCACTACTGGGACACCGGGGTCCTGCTGTGGCGGCTGCTCAGCTGTCTGCTTCTCAGAGGATC
 TACTTCAGGTTCAAATTAAGATCCTGAACTGAGTTTAAAGGCACCCAGCACATCATGCAAGCAG
 GCCAGACACTGCATCTCCAAATGCAAGGGGGAAGCAGCCCAATAATGGTCTTTGCCGTGAATGGTGAAT
 AAGGAAGCGAAAGGCTGAGCATAACTAAATCTGCCCTGTGGAAGAAATGGCAACAAATCTTCAGTAC
 TTTAACTTGAACACAGCTCAAGCAACCACTGCTTCTACAGCTGCAAAATATCTAGCTGTACCTA
 CTTCRAAGAAAGGAACAGAACTCTGCAATCTATATATTATTAGTGTATACAGGTAGACCTTTGTA
 GAGATGTACAGTGAATATCCCGAAATTATACATGACTGAAAGGAGGGAGCTGCTCATTCCTGCGG
 GGTATCGTCACTTACATCACTGTTACTTTAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAA
 AACGCATAATCTGGGACAGTAGAAAGGGCTTCATCATATCAAAATGCAACGTACAAAGAAATAGGGCTT
 CTGACCTGTGAGGCAACAGTCAATGGGCAATTTGTATAAGCAAACTATCTCACACATCGACAAACCAA
 TACAATCATAGATCTCCAAATAGCCACACAGCCCGAGTCAAAATTAATTAGAGGCCACTACTCTTTGTCC
 TCAATTTGATCTGCTACACTGCTTGAACAGAGAGTTCAAAATGAGCTGGAGTTACCTTGATGAAAAA
 AATAAGAGAGCTTCCGTAAGGCGACGAATTAACCAAGCAATTCATGCAACATATTTCTACAGTGT
 TCTTACTATTGACAAAATGCAAGACAAAGCAAAAGGACTTTATACCTTGTGTTAAGGAGTGAACCAT
 CATTCAAATCTGTTAAACCTCAGTGCATATATATGATAAAGCACTCCAAATAAGCACACCGGCCCA
 GTCAAAATTAATTAGAGGCATATCTCTGCTCAATTTGACTGTACACTGCTTGAACAGAGAGT
 TCAAAATGAGCTGGAGTTACCTGATGAAAAAATAAGAGAGCTTCCGTAAGGCGAGCAATTTGACCAA
 GCAATTTGCAAGCAACATATTTACAGTGTCTTACTATTGACAAAATGCAAGACAAAGCAAAAGGA
 CTTATACCTTGTGCTGTAAGGCTGAGCACTCAATCAAAATCTGTACACCTCAGTGCATATATATGA
 TAAATACAAAATCACACATGCCACCGGTGCCAGCACCTGAACCTCTGGGGGAGCCGTGAGTCTTCC
 TCTTCCCCCAAAACCCCAAGGCAACCTCATGATCTCCCGGACCCCTGAGGTCAATGCGTGGTGGTG
 GACGTGAGCCACGAGGACCTGAGGTCAAGTTCAACTGGTACCTGGACGCGGTGGAGGTGCATAATGC
 CAAGCAAAAGCGCGGAGGAGCAGTACAACAGCAGCTACGCTGTGGTCAAGCTCCTCACCGTCTCTGC
 ACCAGCACTGGCTGAATGGCARGGAGTACAAGTGCAGGCTCTCCAAACAAAGCCCTCCAGCCCTCATC
 GAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCGAGAAACACAGGTGTACACCTGCCCCCATCCCG
 GGAGGAGATGAACCAAGAACAGGTCAAGCTGACCTGCCCTGGTCAAAGGCTTCTATCCAGCGACATCG
 CCGTGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCTCCCGTGTGGACTCC
 GACGGCTCTCTTCTTCTCTACAGCAAGCTCACCGTGGACAAAGAGCAGGTGGCAGCAGGGGAACGTCTT
 CTCATGCTCGTGTATGCAGAGGCTCTGCACAACTACACGCAGAGAGGCTCTCCTGTCTCCGG
 GTAAA

Grey with (ital): signal peptide; Yellow: Ig-like domain 1; Blue: Ig-like domain 2

Grey: Ig-like domain 3; Black with underline: Human IgG1-Fc fragment

FIGURE 5

6/17

V₂₃₃

Amino acid sequence

MCVYFDTGVLLCALLSCILLTGSSEGGIFISDTRGPFVEMYSEIPEIITHMTEGRELVIPCRVTSFNITY
 TLEKFFELDTLLPDGRRIINDSREGFTITSNATYIEIGLTLCEATVNGHLYKINYLIHQRTINIIIDVQIS
 TPKEPVKLLRGHTILVNLCTAITPLNTRVNTWNSYDPEKNKRASVRRRIQSNSHANIFYSVLTITDKMQN
 KGLGYTCRVRSGPSFKSVNTSVHYIDKAVQISTPRPVKLLRGHTILVNLCTAITPLNTRVNTWNSATPE
 EKNKRASVRRRIQSNSHANIFYSVLTITDKMQNDELGLYTCRVRSGPSFKSVNTSVHYIDKDKTHITCF
 PCPAPPELLGGPSVFLFPFKPKDILMISRTPEVTCVVVSVSHEDPEVKFNWVVDGGEVHVHAKTPREEQ
 YNSTYRVSVSLTVLQDQWLNGKEYKCKVSNKALPAPTEKTIKAKGQFPREPQVYTLPPSRDELTKNQV
 FLNCTVKGFTVPSIAQWVESNGQPENNVTTPPVVLSDSGSFFLYSKLTVDKSRWQQGNVFSCSVWHEA
 LHNHYTQKSLSLSPGK

Nucleic acid sequence

ATGGTCAGCTACTGCGACACCGGGGTCTCTCTGTGGCGCGCTGCTCAGCTGTCTGTTCTCACAGGATG
TACTCTCAGGTAATATTTATGTATACAGGCTAGACCTTTTCGTASAGATGTACAGTGAATTC CCGCGAAA
TTATACACACTGACTGAAGGAGGAGGCTCGTCACTTCCTCGCGGGTACGTCACCTTAACATCACTGTT
ACTTTAAAAAGATTTCACCTTTGACACTTTGATCCTTGATGAAAACCCATAATCTGGGACAGTAGTAAA
GGGCTTCATCATATCAAATGCAACGTACAAAGAAATAGGGCTTCTGAOCTGTGAAGCAACASTCAAT
GGCTTTGTATAGACAAACTATCTACACACTGCACAAACCAATACAACTATAGATGTCCAAATAGAG
ACACCTCGCCCGAGTCAAAATTACTTTAGAGGCCATCTCTTGCTCCTCAATTGTACTGCTACCACTCCCTT
GAACACGAGAGTTCAAATGACCTGGAGTTACCTGTATGAAAATAATAGAGAGGCTTCGGTAAAGGGCGC
GAATTGACCAAAGCAATTCACATGCCAACATATTTACAGTGTCTTACTATTGACAAAATGCGAAGC
AAAGACAAAGGACTTTATCTTTGCTGTGAAGGAGTGGACCACTCAATCAAATCTGTATACCACTTCAGT
GCATATATATGATAAAGCACTCCAAATAACACACACAGCCCGAGTCAAATTACTTTAGAGGCCCTACTC
TGTGCTCAATTTGACTGTCTACCACTCCCTTGAACACGAGAGTTCAAATGACCTGGAGTTACCTGAT
GAAAAAATAAGAGAGCTTCCTGTAAAGGCGACGAATTGACCAAAGCAATTCOCATGCCAACATATTTCTA
CAGTGTCTTACTATTGACAAAATGCGAAGCAAGGACGACTTTATGCTTTGTGCTGTAAAGGAGTG
GACCACTCAATCAAATCTGTATACACCTCAGTGCATATATGATAAAGCAAAACCTCACACATGCCCA
CCGTGCCAGCACTGAACTCTGGGGGACCGTCACTTCTCTTCCCGCCAAAACCCGAGGACAC
CCTCATGATCTCTCGGACCCCTGGAGGTACATGTGCTGTGGTGTGAGCTGAGCCACCGAAGACCTGAG
TCAAGTTCACTGGTACGTGGACGGCTGGAGGTGCATTAATGCCAGACAAAGCTCCGGGAGGAGCACG
TACAACAGGCACCTACCTGTGGTCAGCGTCTCCACCTGCTCTGCTGACCCAGGAGCTGGCTGAATGGCAGGA
GTCAAGTGCAGAGTCTCCACAAAGCCCTCCGACCTCCCATCGAGAGAAACCATCTCCAAAGCCAGAG
GGCAGCCCGGAGAAACACAGGTGTACACCTGCCCCCATCCGCGGAGSAGATGACCAAGAAACGAGGTC
AGCCTGACCTGCCCTGGTCAAAAGGCTTCTATCCAGCGACATCGCGGTGGAGTGGGAGAGCAATGGGCA
GCCGGAGAGAACCACTACAAAGACCGCTCCCGGTGCTGGACTCCGACGGCTCTCTTCTCTCTCTACAGCA
ACCTCACCGTGGACAAAGAGCAGGTGGCACAGGGGAACGCTCTTCTACCTGCTGATGTCACGAGGCT
CTGCACAACCACTACACGCGASAGAGGCTCTCTCTGCTCCGGGTAAA

Grey with italic: signal peptide; Blue: Ig-like domain 2; Grey: Ig-like domain 3

Black with underline: Human IgG1-Fc fragment

FIGURE 6

7/17

V₁₂₃₄

Amino acid sequence

MPTIWDTCVLLCALLSCILLTGSSESGSKLKDPELSLKGTOHINOAGQTLHLQCRGEAAHFWSLPEMVS
 KESERLSIINKSACGRNKKQFCSTLTLTNTAQANHTGFYSCKYLAVFTSKKKEZSAIYIIFISDTGRPFV
 EMYSEIPEIIMTEGRELVIPCRVTSNITVTLKKFPLDTLIPDGKRIINDSRKGFIIISNATYKEIGL
 LTCEATVNGHLYETINYLTHQINTIILVQISTPSPVLLRGHTLWLNCTATFLNIRVQMTWSYFEEK
 NKRAEVARRIDQENSHANIEYSVLTIENGNKNDKELYICRVASGSEFHSVNTSVHLIYDEAFITVKKHK
 QQVLETVAGKRSYRLSMKVTAFSPSEVWMLKDGILPATEKSARYILTSYSLIINKDVTEEDAGNYITLLS
 IKQENVFENLITAILRNVEFDKHTCFPCPAPELLGGFSEVFLFPPKPKDTLHISRTPEVTCVWVDSH
 EDPEVKFNWYVDGVEVHNKTEPREEQYNSTYRVSVLTVLHQDWLNGKEYECKVSNKALPAPIEKTI
 SKAKGQPPREFQVYITLTPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYETITPFLVDSGGSF
 FLYSKLTVDKSRNQQGNVSCSVMSHEALRNHYTKMSLSLSPPGK

Nucleic acid sequence

ATGCTTCAGCTACTGGACACCTGGGCTCTCTGCTGTCGGGCTCTCTGAGCTCTCTCTCTCTCACAGGAA
 TACTTCAGGTTCAAAATTAAAGGATCCTGAACTGAGTTTAAAGGACACCCAGCAGTCATGCAAGGAG
 GGCACACACTGCATCTCCARTGACGGGGGGAAGCAGGCCATRAATGGTCTTTGGCTGAAATGGTGGAGT
 AAGCAAGCGAAAGGCTGACCATAACTAAATCTGCTCTGCAAGAAATGGCAACCAATTCTGCAGTAC
 TTTAACCTTGAACACAGCTCAAGCAACCCCACTGGCTTTACAGCTGCCAATATCTAGCTGTACCTA
 CTTCAAAGGAGGGAACAGAAATCTGCAATCTATATATTATTAGTGATACAGGTAGACCTTTCTGTA
 GAGATGTACAGTGAATCCCGAAATTATACACATGACTGAAGGAGGGAGCTCTCTCAATTCCTGGCGG
 GGTTCAGCTCACTTAACATCACTGTTACTTTAAAGAGTTTCACTTGACACTTTGATCCTGATGGAA
 AAGGCATAATCTGGGACAGTAGAAAGGGCTTCATCATATCAAAATGCAAGCTACAAGAAATAGGGCTT
 CTGAGCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGCAAACTATCTCACACATCGACAAACCAA
 TACAATCATAGATGTCAATAGGCACACACGCGGCTGAAATTAATTAAGTGAAGGGCATACTCTTGTCG
 TCAATTTGACTGCTACACTCTCTTGAACACGAGAGTTCAAAATGAGCTGGAGTTTACCTGATGAAAG
 NATAGAGAGCTTCGTAAGGCGAGCAATTGACCAAGCAATTCGATGCGAATATCTCACTGCT
 TCTTACTATTGACAAATTCAGAACAAAGACAAAGGACTTTATATTGTGTGTGTAAGGATGAGACAT
 CATTCAAATCTGTTACACCTCACTGCATATATATGATAAAGCATTCATCACTGTGAAACATCGAAAG
 CAGCAGTCTCTTGAACCGTAGCTGGCAAGGGCTCTTACCGGCTCTCTATGAAAGTCAAGGCAATTTCG
 CTGGCGGAAAGTTGTATGGTTAAAGATGGGTTACCTGGGACTGAGAAATCTGCTGGCTATTITGACTC
 GTGGCTACTGCTTAATTATCAAGGACGTACTGAAAGAGATGCAAGCAATTATACATCTTGCTGAGG
 ATAAACAGTCAAAATGTGTTAAAAACCTCACTGCACTCTAATTGTCAATGTGAAGCGGACAAAC
 TCACACATGCGGACCTTGCCAGCACTGAACTCTCGGGGGGACGGTCACTCTTCTCTTCTCCGCCAA
 AAGCCAGGACACCTCATGATCTCCCGACCTCTGAGGTGACNTGGGTGGTGGTGGAGCTGAGCCAC
 GAAGACCTTGAGCTCAAGTTCAACTGCTACGTGGACGGGTGGAGGTGCATAATGCCAAGACAAAGCC
 CGGGAGGGAGCTACACAGCACTGACCTGTGTGTGAGGTCTCTACGCTCTCTGACACGAGCTGGC
 TGAATGGCAAGGAGTACAGTGTAGGTCTCTACACAAAGCCCTCCAGGCCCATCGAGAAACCATC
 TCCAAAGCCAAAGGGTAGCCCTGAGAACCCAGGTGTACACCTGCCCCCATCCCGGAGGAGATGAC
 CAAGAACCAAGTCAAGCTGACCTGCTGCTCAAGGCTTTCTATCCAGCGACATGCGCGTGGAGTGGG
 AGAGCAATGGGCGGGGAGAACTACAGAACCAAGCCCTCCCGTGTGTGACTCGGAGCGCTCTCTC
 TTCTCTACAGCAAGCTCACCGTGGACAGAACAGGTGGCAGCGGGGAGCTCTTCTCATGCTCGCT
 GATGCACAGGCTCTGCACAACTACTACAGCAGAGAGCTCTCTCTCTCTCCGGGTAAA

Grey with line: signal peptide; Yellow: Ig-like domain 1; Blue: Ig-like domain 2

Grey: Ig-like domain 3; Green: Ig-like domain 4; Black with underline: Human IgG1-Fc fragment

FIGURE 7

8/17

V₂₃₄

Amino acid sequence

MVGVDTGVLLCALLSCLLITSSSGGI FISTDTGRPFVEMYSEI PEI IHNTGRELVI PCRVTS PNITV
 TLKNFFLDTLI PDGKRIIWESRNGFI ISNATYKEI GLLTCEATVNGHLYKTNYLTHRQTNTII ISWQIS
 IPREVMILRGHTIVLNLCTATIFLNTRVQMTWSYFEDENMKRASVRRISQSMSEANIFYSVLTIDKMQN
 EDKGLYICNVRSGPSFKSVNTSVHYIDNAFI TVNHRNQVLET VAGKPSYRLSMKVKAFFSPEVWVLK
 DGLPATEKSARYLTRGYSLIIKQVIEEDAGNYTILLISIKQSNVFKHLTALLIVNVKPDHITC PPCPA
PELLGGPSVFL FPEKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTIVLHQDWLNGKE INCKVSNKALPAPIEKTIISKARGQPREPQVYTLPPSRDELTKNQVSLTCL
LVKGFFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHAEALNNH
 YTQNSLSLSPGK

Nucleic acid sequence

ATGCTACGCTACTGGGACACGGGGGTCCTGCTGTCGCGCTGCTCAGCTGTCTCTCTCTCACAGGATC
 TAGTTCAGGTATATTTATTAGTGATACAGGTAGACCTTTCGTAGAGATGTACAGTGAATCCGCGAAA
 TTATACACATGACTGAGGGAAGGGAGCTCGTCATTCCCTGCGGGGTTACGTCCCTTAACATCACTGT
 ACTTTAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAACGCATAATCTGGGACAGTAGAAA
 GGGCTTCATCATATCAAAATGCAACGTACAAAGAAATAGGGCTTCTGACCTGTGAAGCAACAGTCAATG
 GGCATTGTATTAAGACAACTATCTCACACATCGACAAACCAATACAAATCATAGATGTCCAAATAAGC
 ACACACGCGCCAGTCAAAATTAATAGAGGCCATACCTTGTCTGCTCAATTTGACTGCTACCACTCCCTT
 GAACACGAGAGTTCAAAATGACCTGGAGTILACCTGATGAAAAAATAAGAGAGCTTCCGTAAAGCGGAC
 GAATTGACCAAGCAATTCATGCCAATATTTACAGTGTCTTACTATISACAAATGACAGAC
 AAGACAAAGGACTTTATACCTGTCTGTAAAGGAGTGGACCAATCATCAAAATCTGTTAAACCTCAGT
 GCATATATATGATAAGCAATTCATCACTGTGAACATCGAAAAACAGCAGGTGCTTGAACCGTAGCTG
 GCAGCGGTCTTACCGGCTCTCTATGAAGTGAAGGCATTTCCCTGCGCGGAAGTTGATGGTTAAAA
 GATGGGTTACCTGGGACTGAGAAATCTGCTGCTATTTGACTCTGGCTACTCGTAAATATCAAGGA
 CGTAACTGAGAGGATGACAGGAATTATACAAATCTTGCTGACATAAAACAGTCAAAATGTGTTAAAA
 AACTCACTGCGCACTCTAATTTGTCAATGTGAAACCGGACAAACTCACACATGCCACCGTGCCAGCA
 CCTGAACCTCTGGGGGACCGTCACTCTTCTCTTCCCCCAAAACCCAGGACACCTCATGATCTC
 CCGGACCCCTGAGGTCACTGCTGCTGGTGGAGCTGAGCCACGAGACCCCTGAGGTCAAGTTCAACT
 GGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGAGGAGCAGTACACAGCACG
 TACCGTGTGCTCAGCGTCTCACCCTCTGCAACAGGACTGCTGAATGGCAAGGAGTACAAAGTGCAA
 GGCTCTCAACAAAGCCCTCCAGCCCCATCGAGAAACCATCTCCAAGCCAAAGGGCAGCCCGAG
 AACCACAGGTGTACACCTGCCCATCCCGGAGGAGATGACCAAGAACAGGTCAAGCTGACCTGCTG
 CTGGTCAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGACGCGGAGACAA
 CTACAAGACCAAGCCCTCCGTGCTGGACTCCGACGGCTCTCTCTCTCTACAGCAAGCTCACCCTGG
 ACAGAGCAGGTGGCAGCAGGGGAACGCTTCTCATGCTCGGTGATGACAGAGGCTCTGACACACAC
 TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

Grey with italic: signal peptide; Blue: Ig-like domain 2; Grey: Ig-like domain 3; Green: Ig-like domain 4;

Black with underline: Human IgG1-Fc fragment

FIGURE 8

9/17

V₂₄

Amino acid sequence

M/GYWDGTVLLCALLSCILLTSSSSGI FTSDTGRPFVEMYSEIPEIIRHMEGRELVIPCRVTSFNIIV
 TLKRFPLDTLIPDGKRIINDSRKGFII SNATYKEIGLLTCEATVNGHLYKINYLTHAQTN I I DVFIT
 VHRKQVLEIVAGKRSYRLSMKVAFPSPEVWNLKDGLPATEKSAARYLTRGYSLIINDVITEEDAGNY
 IILLSTPQSNVFNLTATLIVNVKPKHTHCPCPAPELLGGF SVFLFPEKPKDTLMISRIPEVTCVV
 VDVSHELPEVKENWYVDGVEVHNATKPREEQNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAP
 IEKTIISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFPYSDIAVEWESNGQPENNYKTT PPVLD
 SDGSEFLYSKLTIVIKSRWQQGNVFSOSVMHEALHNHYTQKSLSLS PGK

Nucleic acid sequence

ATGGTACGCTACTGGGACACCGGGTCTTCCTGTCGAGCTGCTAGCTGCTTGCTTTCACAGGAT
 TAGTTTACGGTATATTATAGTGATACAGGTAGACCTTTCTGTAGAGATGTACAGTGAATCCCGGAAA
 TTATACACATGACTGAAGGAAGGGAGCTCGTCTTCCCTGCCGGGTTACGTCACTAACATCACTGTT
 ACTTTAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAACGGCAATCTGGGACAGTAGAAA
 GGGCTTCATCATATCAAATGCAACGTACAAAAGAAATAGGGCTTCTGACCTGTGAAGCAACAGTCAATG
 GGCATTTGTATAAGACAAACTATCTCACACATGACAAACCAATACAATCATAGATGCTTCATCACT
 GTGAACATCGAAAACAGCAGGTGCTTGAACCGTAGCTGGCAAGCGGTCTTACCGGCTCTCTATGAA
 AGTGAAAGCATTTCCCTCGCCGGAAGTTGTATGGTTAAAGATGGGTATCTTGGACTGAGAAATCTG
 CTGGCTATTTGACTCGTGGCTACTCGTTAATTATCAAGGACGTAACTGAAGAGGATGCAAGGAATAT
 ACAATCTTGCTGAGCATAAACAGTCAATGTGTTTAAAAACCTCACTGCCACTCTAATTGTCAATGT
 GAAACCGGACAAACTCACACATGCGCACCGTGCCAGCACTGAACTCTGGGGGACCGTCACTCT
 TCCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTG
 GTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGAAGCGCTGGAGGTGCATAA
 TSCCAAGACAAAGCCGCGGGAGGAGCAGTACACAGCAGTACCGGTGTGGTTCAGGTCTCTCACCGTCC
 TGCACTAGGACTGGCTGAATGGCAAGGATCAAGTGTCAAGGTCTCAACCAAGCCCTCCAGCCCCC
 ATGAGAAAACCATCTCCAAAGCCAAAGGCGAGCCCGAGAAACACAGGTGTACACCTGCCCCATC
 CCGGGAGGAGATGACCAGAACCAGGTGAGCTGACCTGCTGGTCAAGGCTTCTATCCAGCGACA
 TCGCCCTGGAGTGGGAGAGCAATGGGCGCCCGAGAGACAACTACAAGACACGCCCTCCCGTCTGGAC
 TCCGACGGCTCTCTCTCTCTACAGCAGCTCACTGTGGACAGGAGCAGGTGGCAGCAGGGAGCGT
 CTCTCATGCTCCGTGATGCACGAGGCTCTGCACAAACACTACACGAGAGAGGCTCTCCCTGTCTC
 CGGGTAAA

Grey with italic: signal peptide; Blue: Ig-like domain 2; Green: Ig-like domain 4

Black with underline: Human IgG1-Fc fragment

FIGURE 9

Expression of VEGFR1 Constructs in 293 Cells: Effects of Heparin (0-1000 µg/ml) on Release

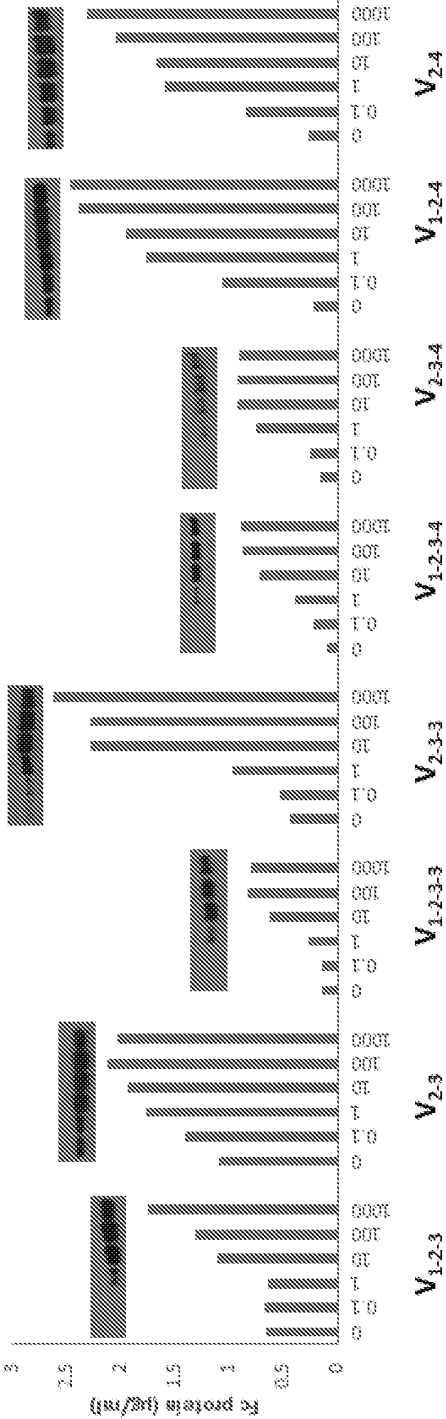


FIGURE 10

11/17

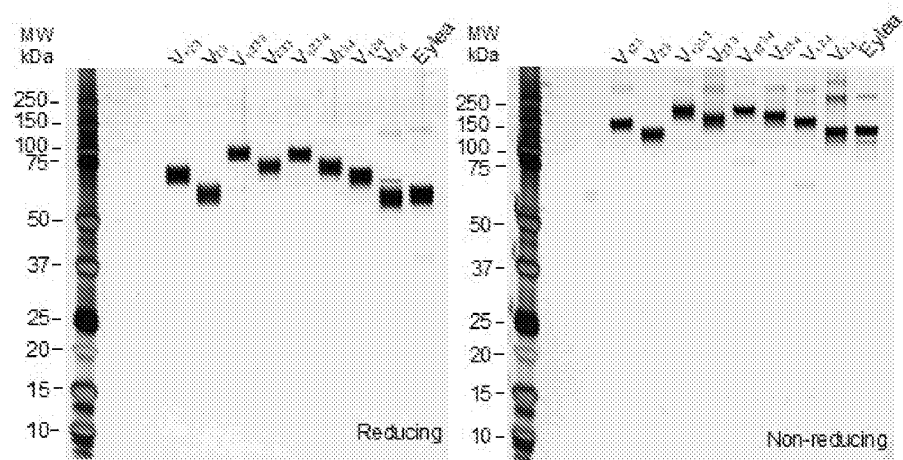


FIGURE 11

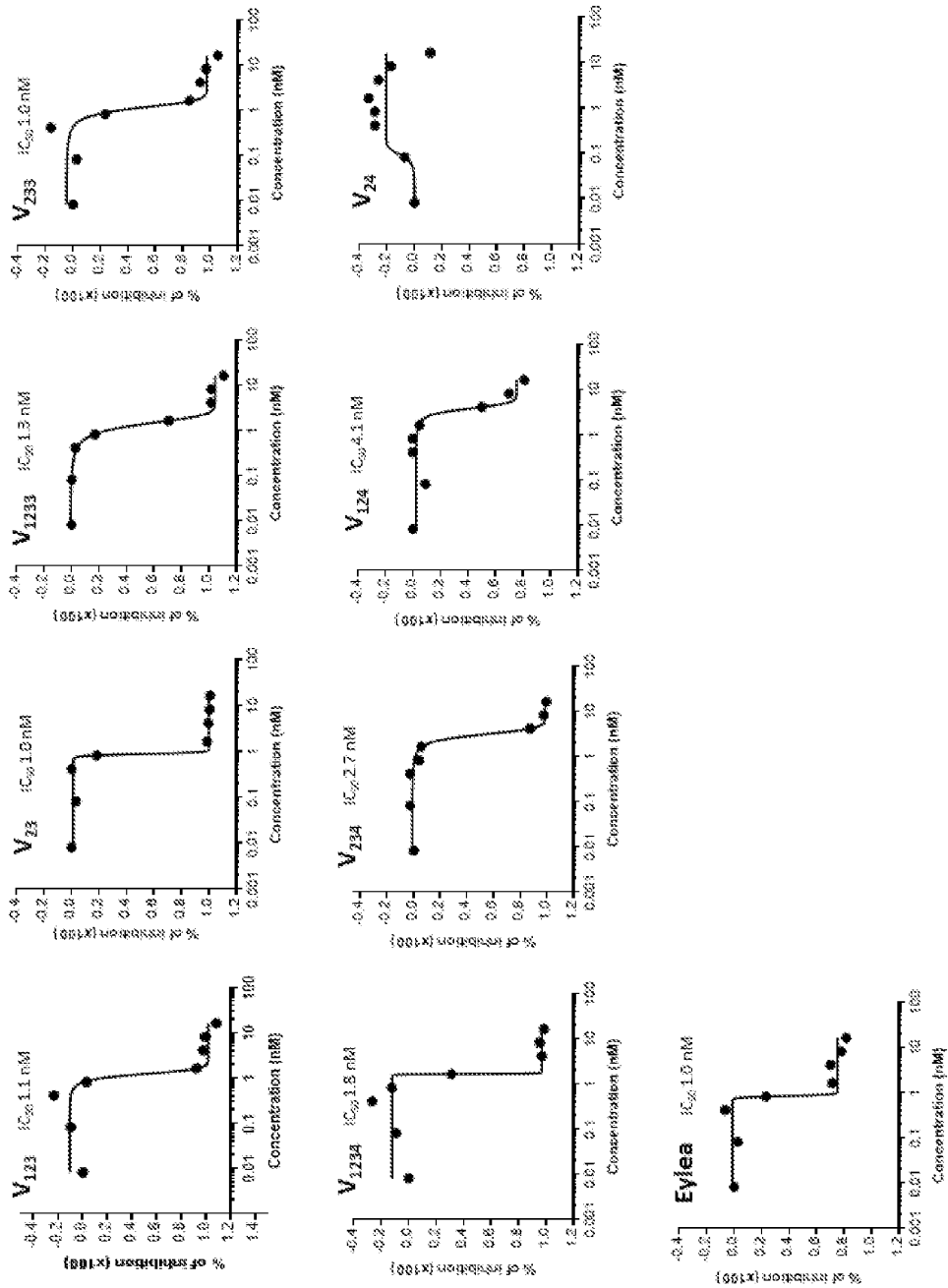
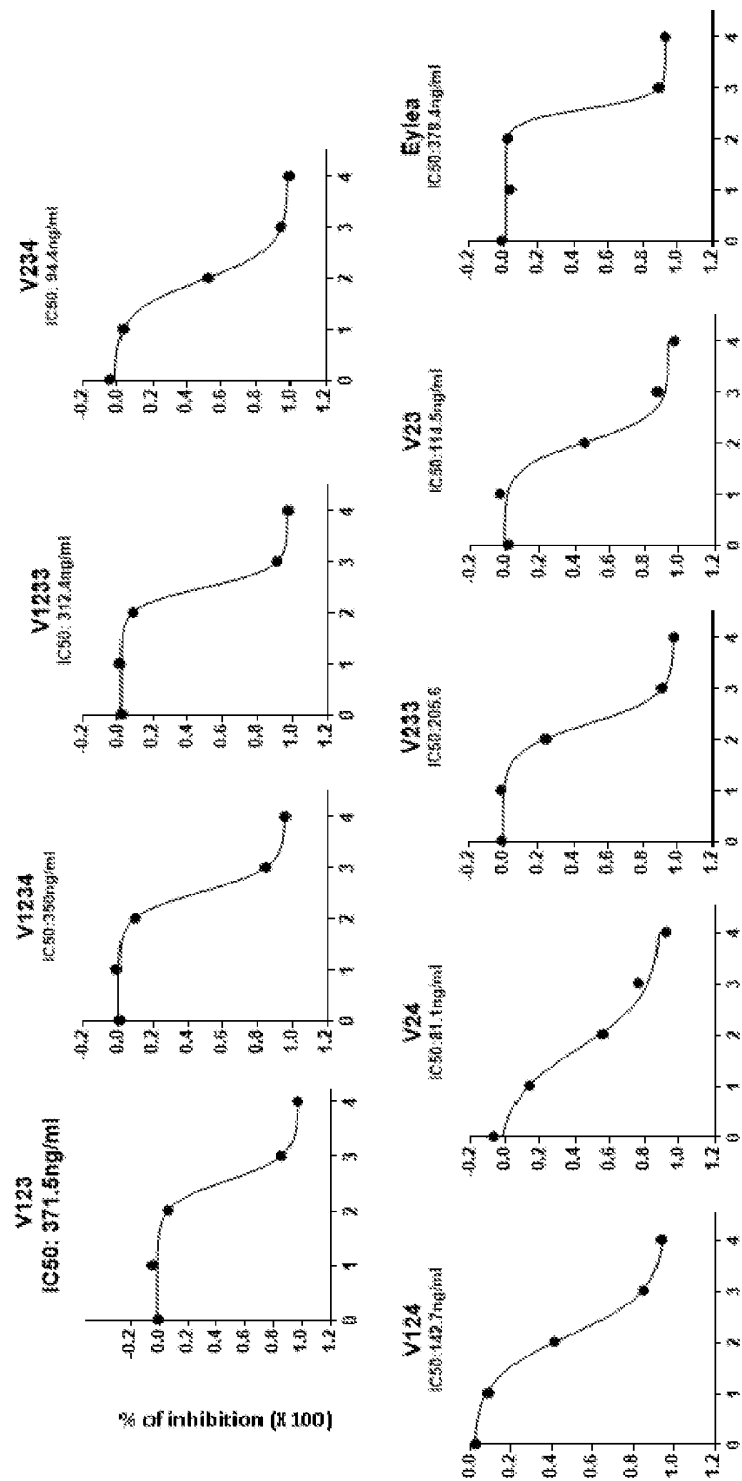


FIGURE 12



Log concentrations of VEGF (ng/ml)

FIGURE 13

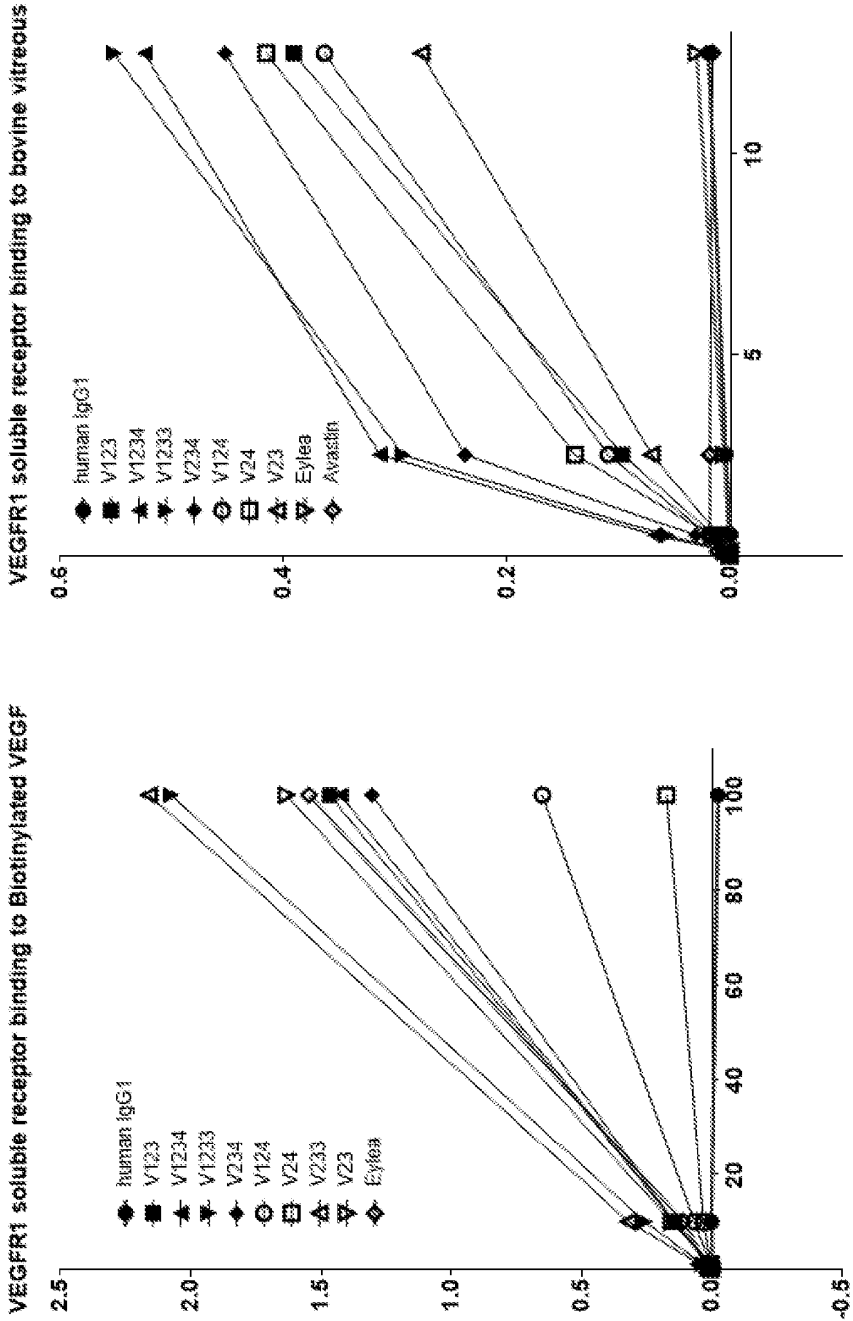


FIGURE 14

15/17

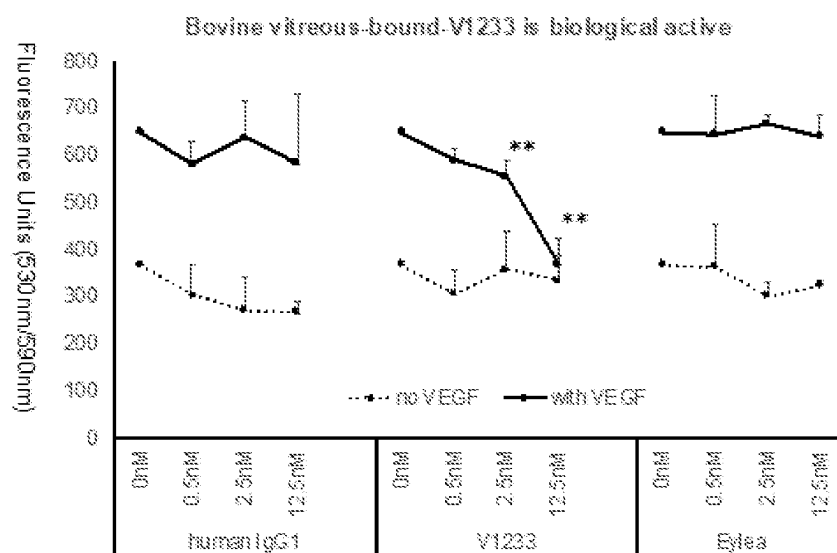
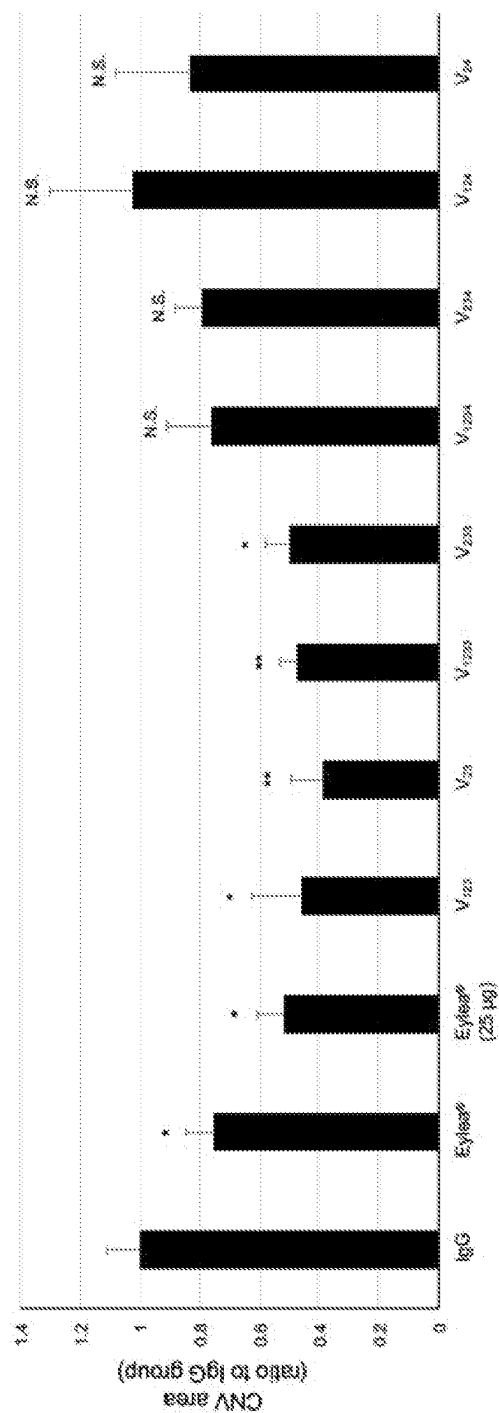


FIGURE 15



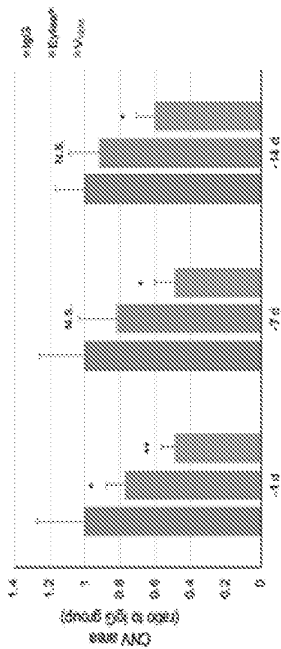


FIGURE 17A

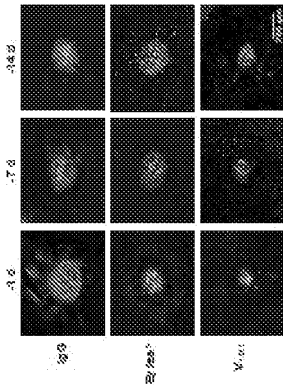


FIGURE 17B