CCAGCGGTGTGGGTTCC
*Bst*XI

hAng2
(F281-F496)

TTCAGAGACTGTGCTGAAGTATTCAAATCAGGACACACCACGAATGGCATCTA
CACGTTAACATTCCCTAATTCTACAGAAGAGATCAAGGCCTACTGTGACATGG
AAGCTGGAGGAGGCGGGTGGACAATTATTCAGCGACGTGAGGATGGCAGCGTT
GATTTTCAGAGGACTTGGAAAGAATATAAAGTGGGATTTGGTAACCCTTCAG
GAGAATATTGGCTGGGAAATGAGTTTGTTTCGCAACTGACTAATCAGCAACGC
TATGTGCTTAAAATACACCTTAAAGACTGGGAAGGGAATGAGGCTTACTCAT
TGTATGAACATTTCTATCTCTCAAGTGAAGAACTCAATTATAGGATTCACCT
TAAAGGACTTACAGGGACAGCCGGCAAAATAAGCAGCATCAGCCAACCAGGAA
ATGATTTTAGCACAAAGGATGGAGACAACGACAAATGTATCTGCAAATGTTC
ACAAATGCTAACAGGAGGCTGGTGGTTTGATGCATGTGGTCCTTCCAACTTGA
ACGGAATGTACTATCCACAGAGGCAGAACACAAATAAGTTCAACGGCATTAAA
TGGTACTACTGGAAAGGCTCAGGCTATTCGCTCAAGGCCACAACCATGATGAT
CCGACCAGCAGATTTC

TCAGGAGGCGGAGGTAGTGGCGGAGGAGGCTCCGGTTCC
Linker

6A6 V_L

AATTTTATGCTGACTCAGCCCCCCTCAGTGTCAGTGTCCCCAGGAAAGACGGC
CAGGATCACTTGTAGGGGAGATAACCTTGGAGATGTAAATGTTCACTGGTACC
AGCAGCGGCCAGGCCAGGCCCCTGTATTGGTCATGTATTATGATGCCGACCGGC
CCTCAGGGATCCCTGAGCGATTCTCTGGCTCCAACTCTGGGAACACGGCCACAC
TGACCATCAGCGGAGTCGAAGCCGGGGATGAGGCCGACTACTATTGTCAGGTG
TGGGATAGGACTAGTGAGTATGTCTTCGGAACTGGGACCAAGGTCACCGTCCT
AGGT

GGAGGAGCCAGCCTCGTGG
*Bst*XI

FIG.2

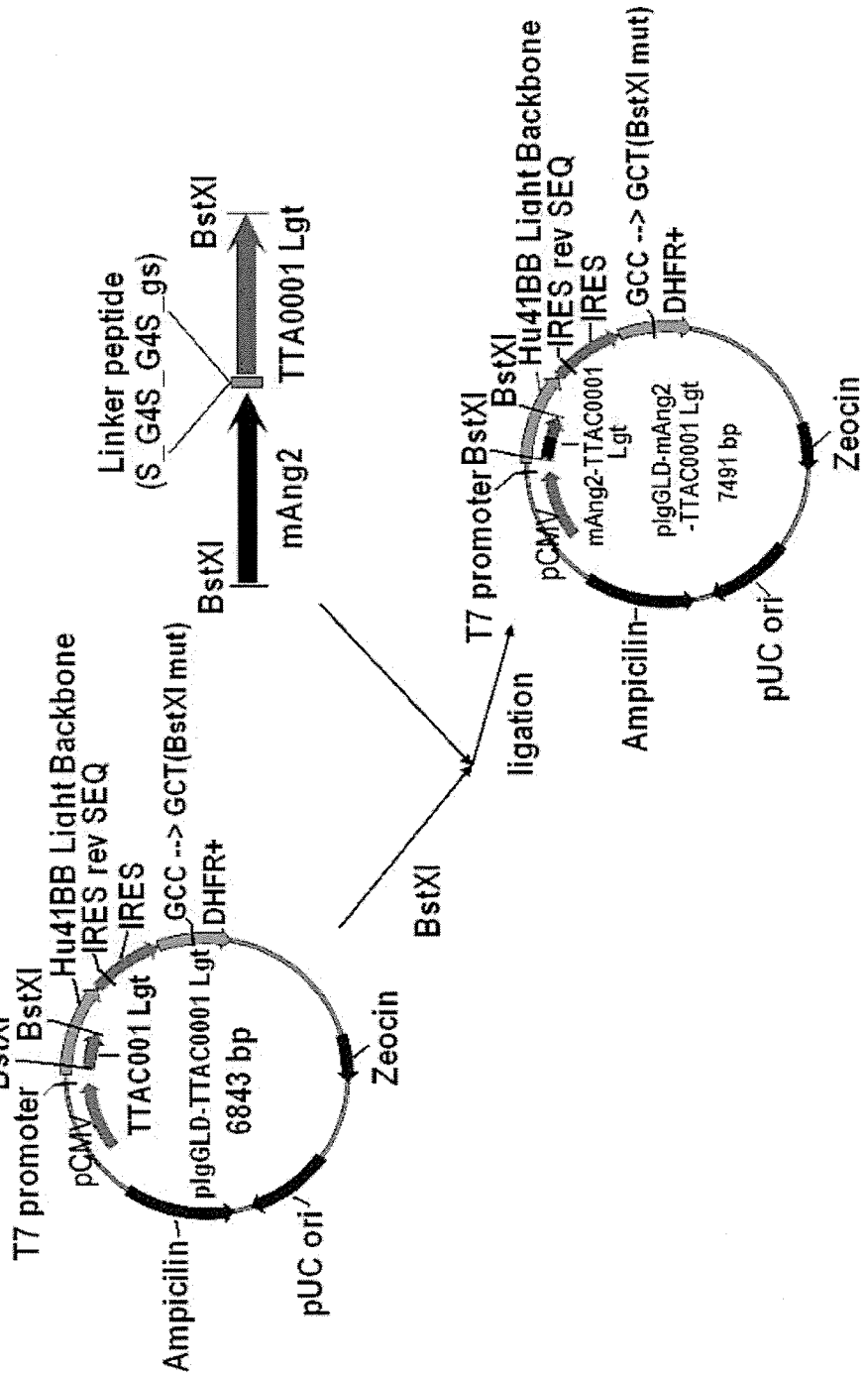

BstXI
BstXI
T7 promoter
pCMV
TTAC001 Lgt
Hu41BB Light Backbone
IRES rev SEQ
IRES
GCC --> GCT(BstXI mut)
DHFR+
plgGLD-TTAC0001 Lgt
6843 bp
Ampicilin
pUC ori
Zeocin
BstXI
Linker peptide
(S_G4S_G4S_gs)
BstXI
mAng2
TTA0001 Lgt
BstXI
ligation
T7 promoter
BstXI
pCMV
mAng2-TTAC0001
Lgt
Hu41BB Light Backbone
IRES rev SEQ
IRES
GCC --> GCT(BstXI mut)
DHFR+
plgGLD-mAng2
-TTAC0001 Lgt
7491 bp
Ampicilin
pUC ori
Zeocin

FIG.3

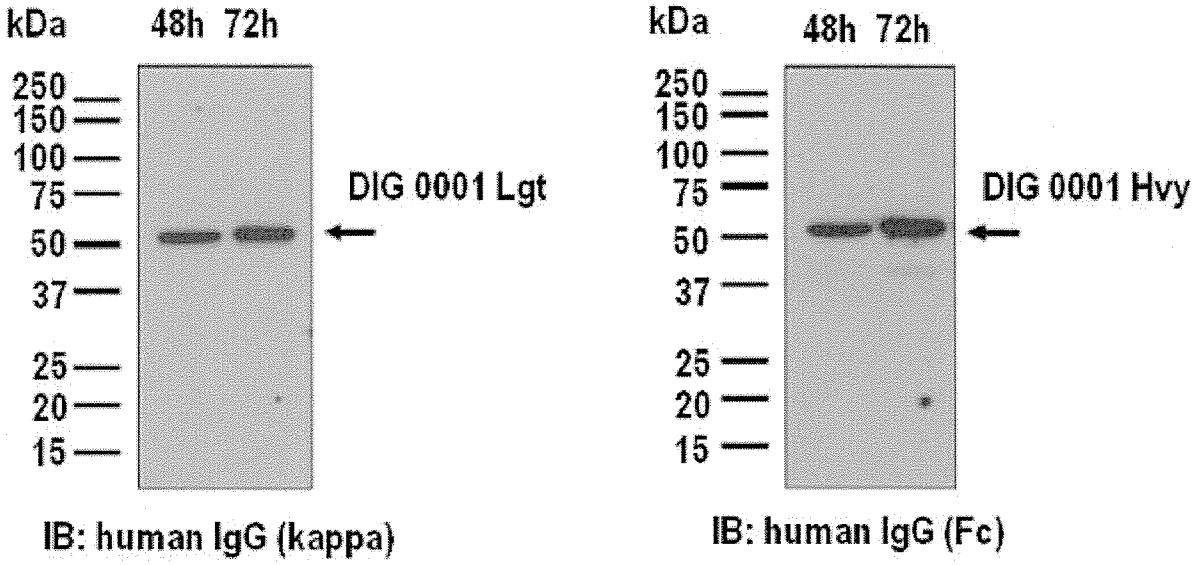

kDa
48h 72h
250
150
100
75
50
37
25
20
15
DIG 0001 Lgt
IB: human IgG (kappa)
kDa
48h 72h
250
150
100
75
50
37
25
20
15
DIG 0001 Hvy
IB: human IgG (Fc)

FIG.4

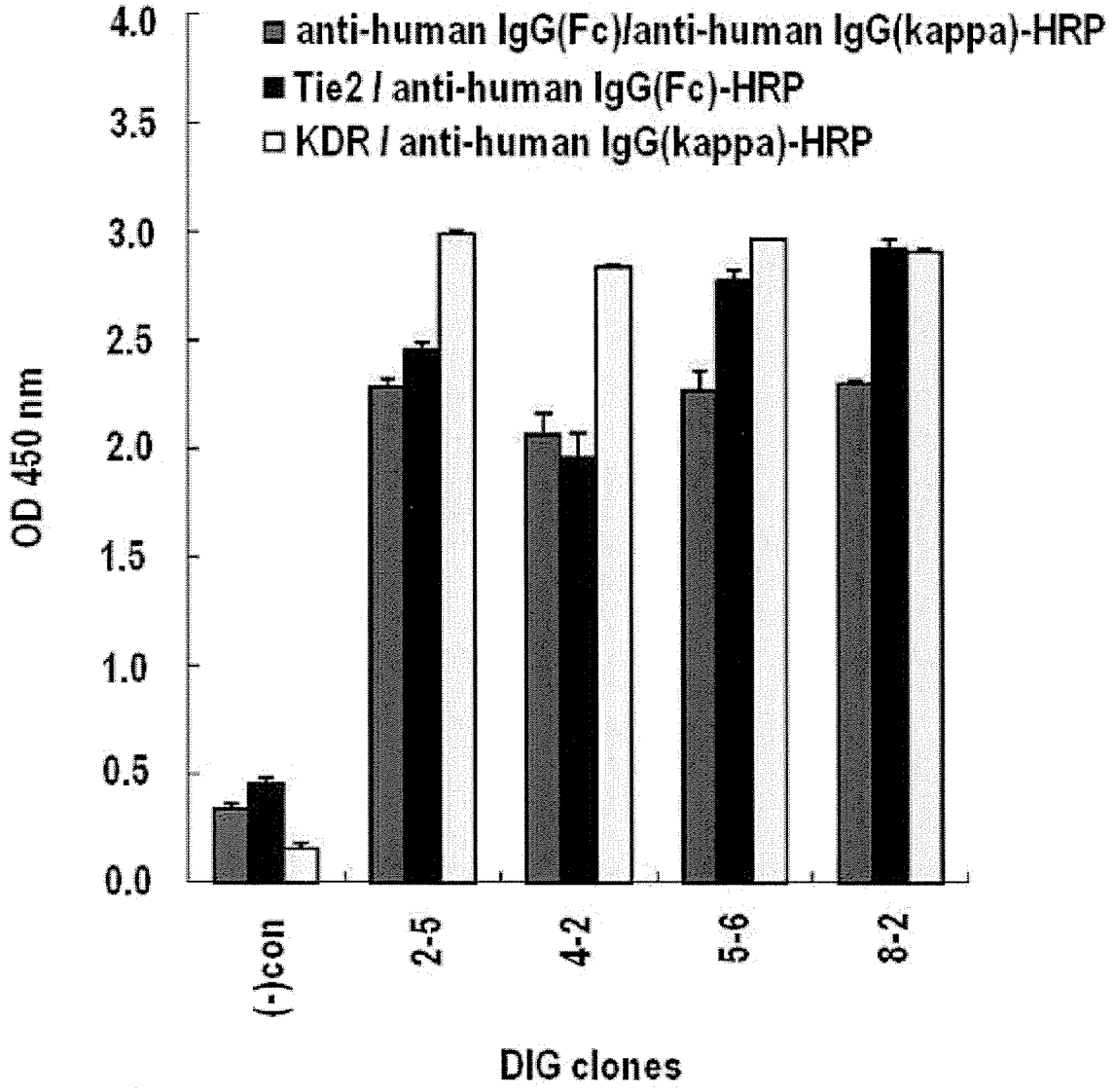

anti-human IgG(Fc)/anti-human IgG(kappa)-HRP
Tie2 / anti-human IgG(Fc)-HRP
KDR / anti-human IgG(kappa)-HRP
OD 450 nm
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(-)con
2-5
4-2
5-6
8-2
DIG clones

FIG.5

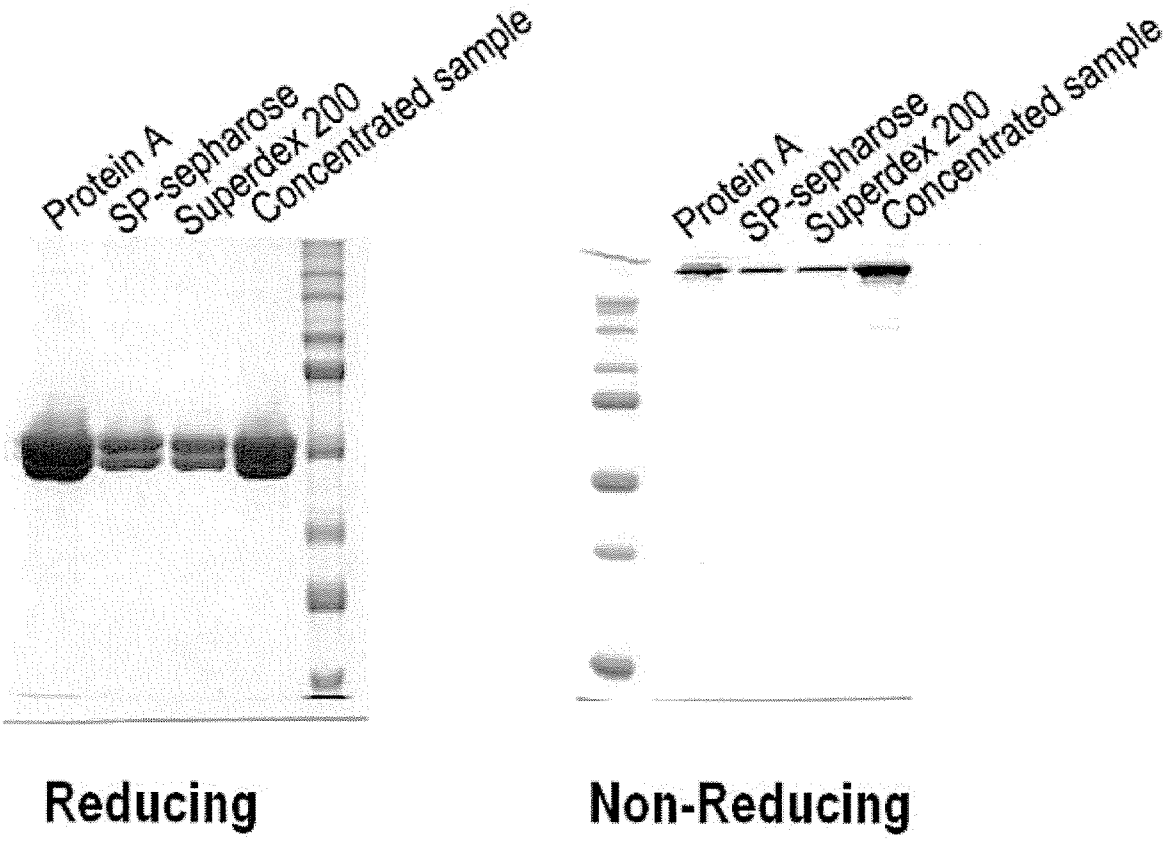

Protein A
SP-sepharose
Superdex 200
Concentrated sample
Protein A
SP-sepharose
Superdex 200
Concentrated sample
Reducing
Non-Reducing

FIG.6

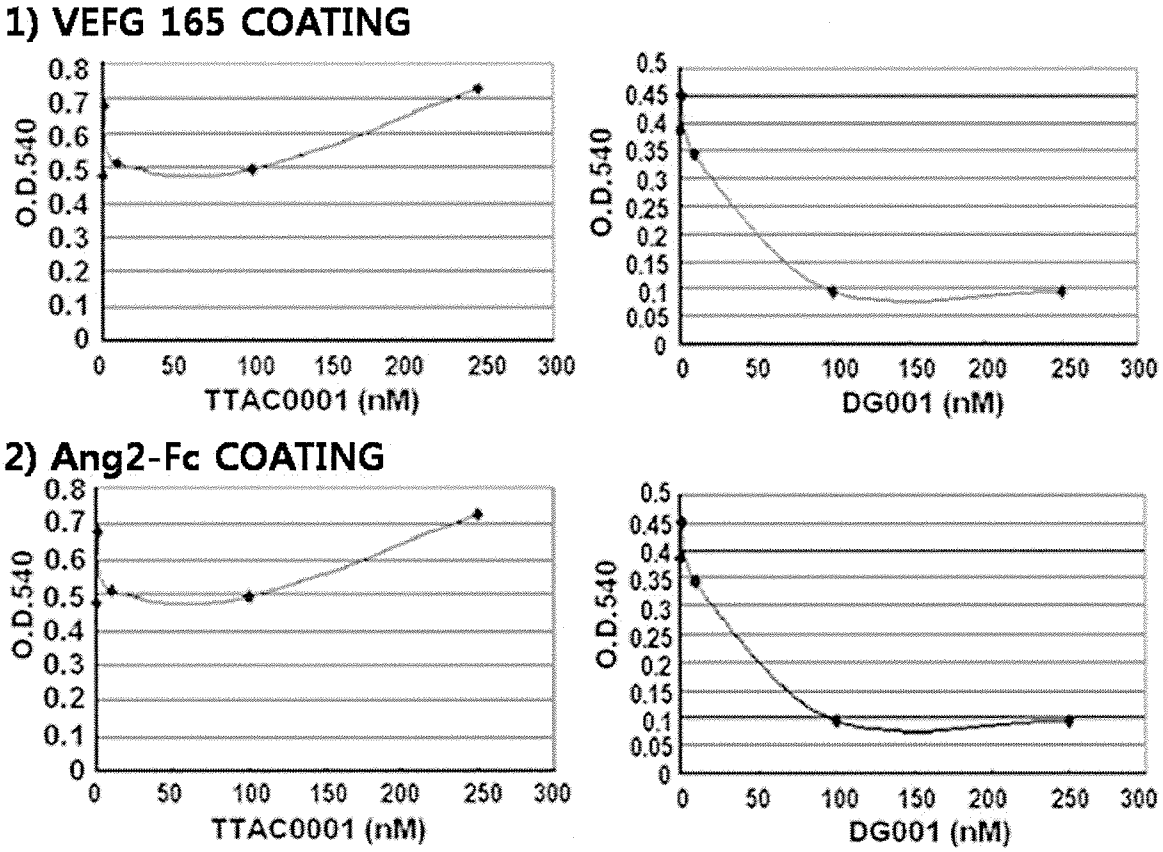

1) VEFG 165 COATING
O.D.540
TTAC0001 (nM)
DG001 (nM)
2) Ang2-Fc COATING
O.D.540
TTAC0001 (nM)
DG001 (nM)

FIG.7

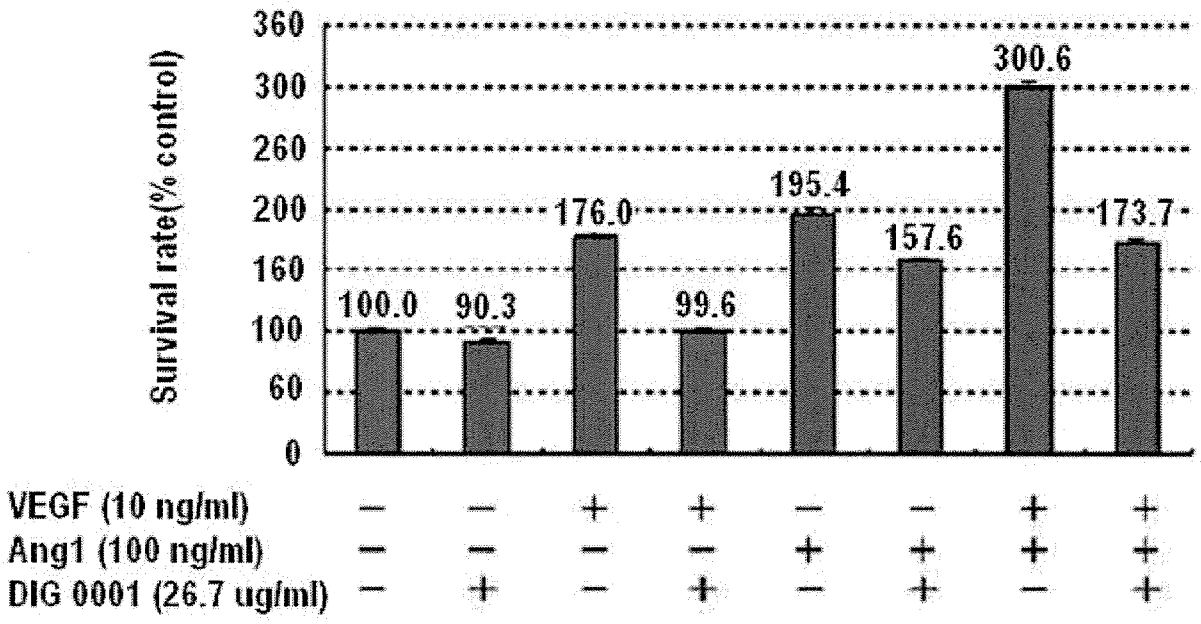

Survival rate(% control)
360
300
260
200
160
100
60
0
100.0
90.3
176.0
99.6
195.4
157.6
300.6
173.7
VEGF (10 ng/ml) − − + + − − + +
Ang1 (100 ng/ml) − − − − + + + +
DIG 0001 (26.7 ug/ml) − + − + − + − +

FIG.8

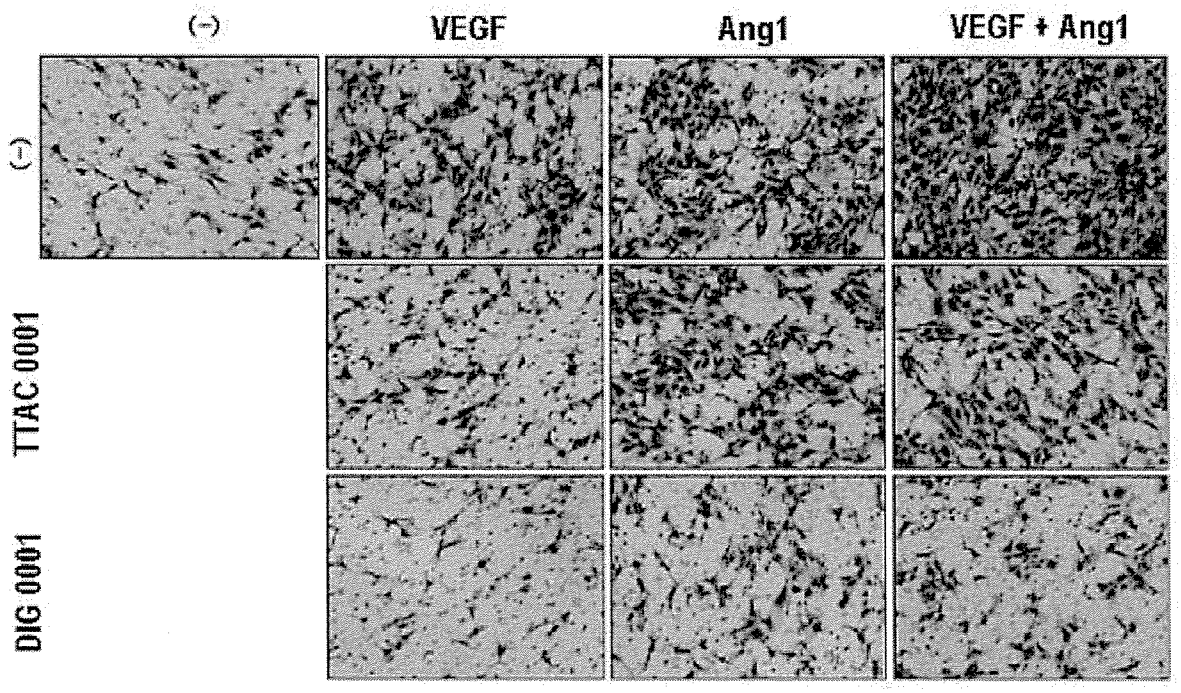

(-)
VEGF
Ang1
VEGF + Ang1
(-)
TTAC 0001
DIG 0001

FIG.9

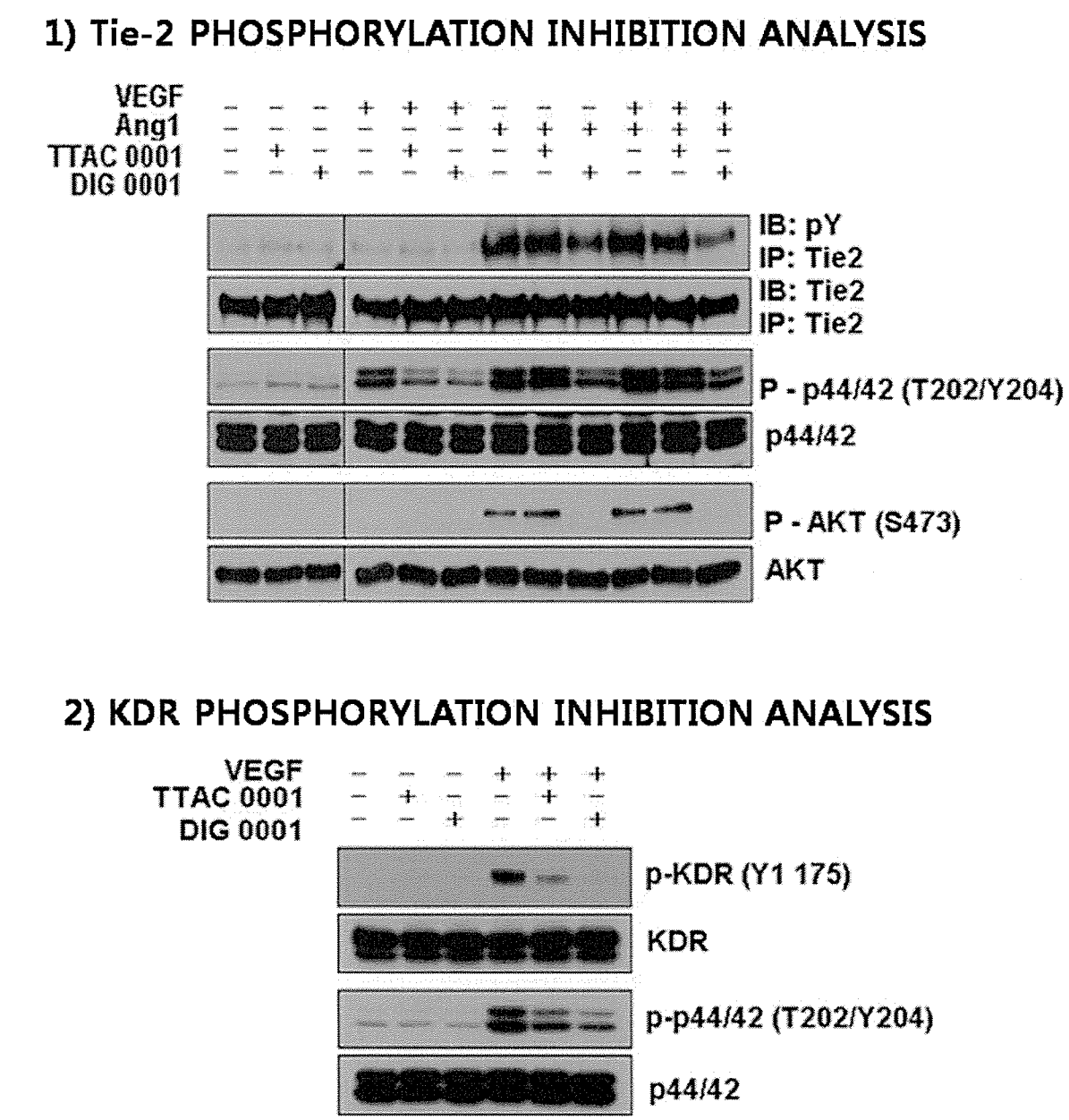
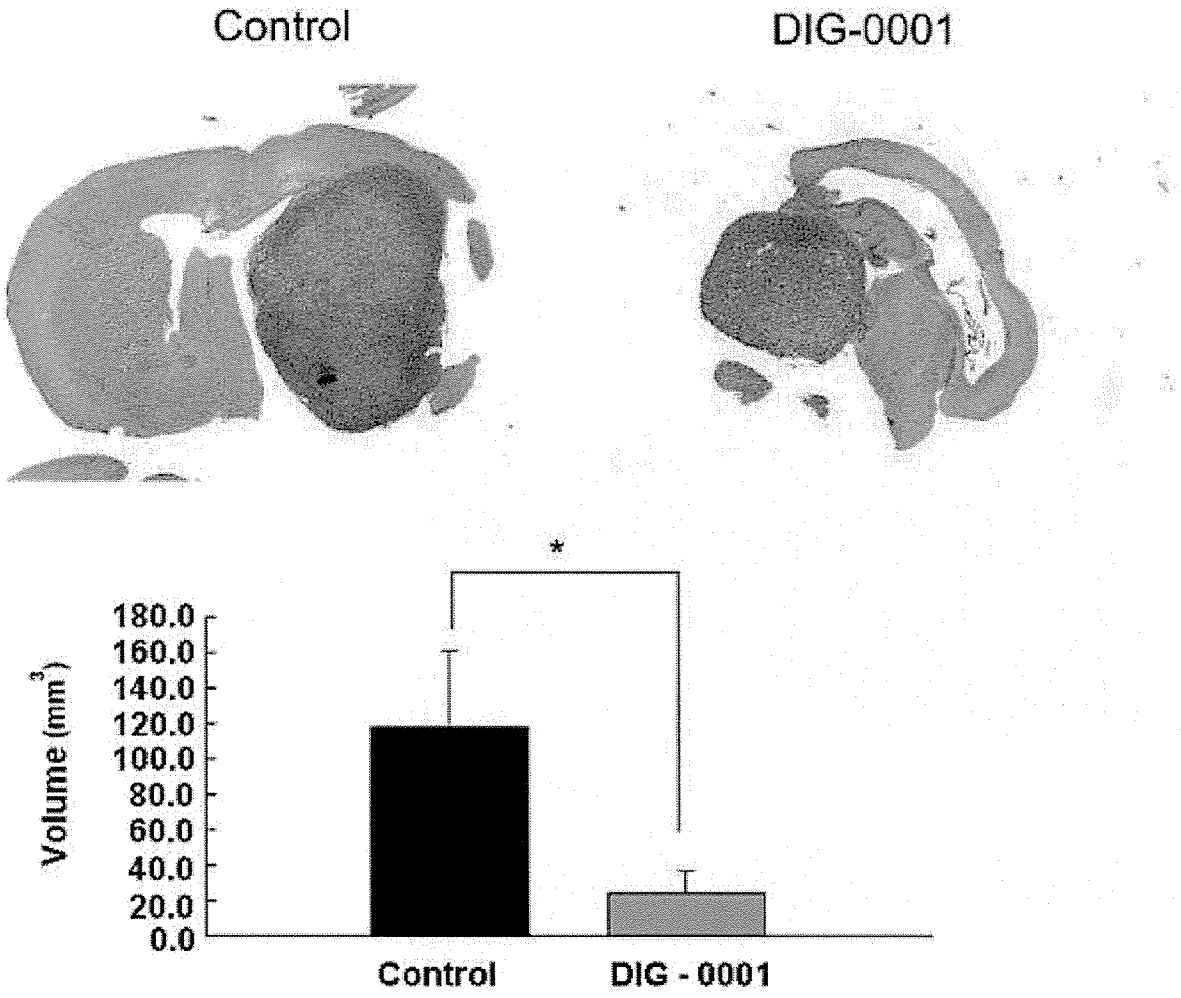

1) Tie-2 PHOSPHORYLATION INHIBITION ANALYSIS
VEGF
Ang1
TTAC 0001
DIG 0001
IB: pY
IP: Tie2
IB: Tie2
IP: Tie2
P - p44/42 (T202/Y204)
p44/42
P - AKT (S473)
AKT
2) KDR PHOSPHORYLATION INHIBITION ANALYSIS
VEGF
TTAC 0001
DIG 0001
p-KDR (Y1 175)
KDR
p-p44/42 (T202/Y204)
p44/42

FIG.10


Control
DIG-0001
*
Volume (mm3)
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Control
DIG - 0001