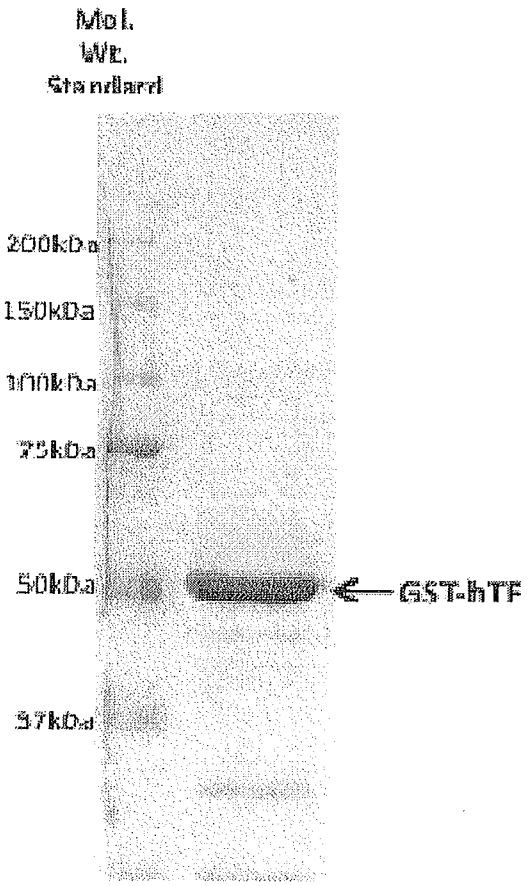

Mol.
Wt.
Standard
200kDa
150kDa
100kDa
75kDa
50kDa
37kDa
GST-hTF

FIG. 1

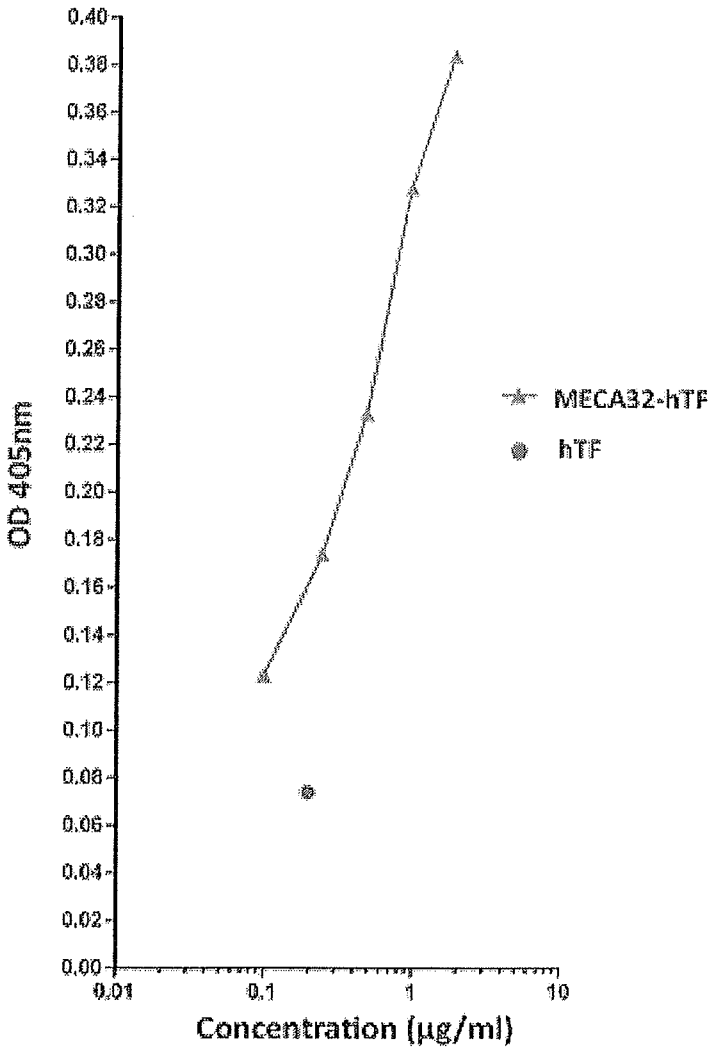

0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
OD 405nm
0.01
0.1
1
10
Concentration (µg/ml)
MECA32-hTF
hTF

FIG. 2

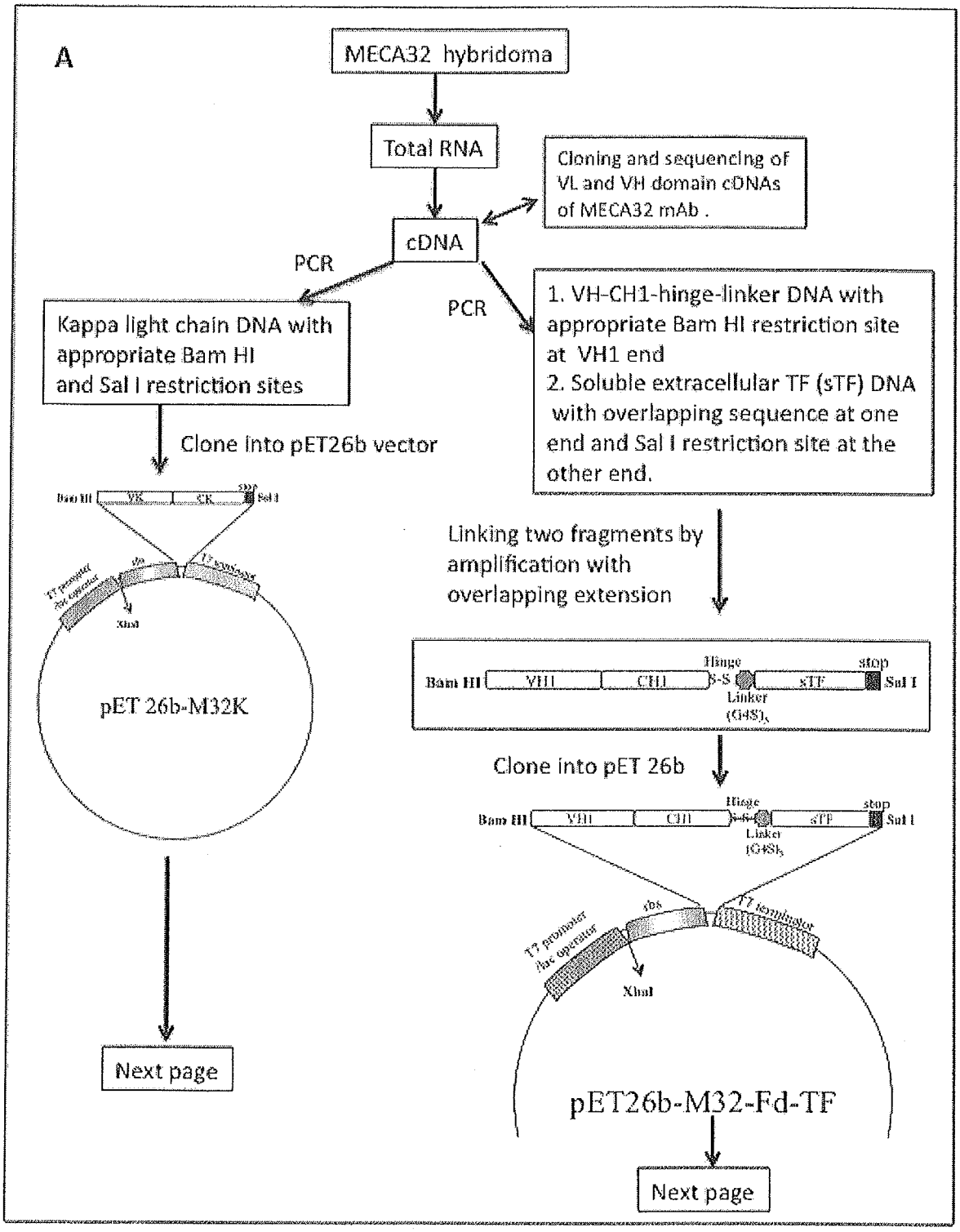

A
MECA32 hybridoma
Total RNA
Cloning and sequencing of VL and VH domain cDNAs of MECA32 mAb .
cDNA
PCR
PCR
Kappa light chain DNA with appropriate Bam HI and Sal I restriction sites
1. VH-CH1-hinge-linker DNA with appropriate Bam HI restriction site at VH1 end
2. Soluble extracellular TF (sTF) DNA with overlapping sequence at one end and Sal I restriction site at the other end.
Clone into pET26b vector
Linking two fragments by amplification with overlapping extension
pET 26b-M32K
Bam HI
VH
CH1
Hinge
S-S
Linker
(G4S)x
sTF
stop
Sal I
Clone into pET 26b
rbs
XhoI
T7 terminator
T7 promoter /lac operator
pET26b-M32-Fd-TF
Next page
Next page

FIG. 3A

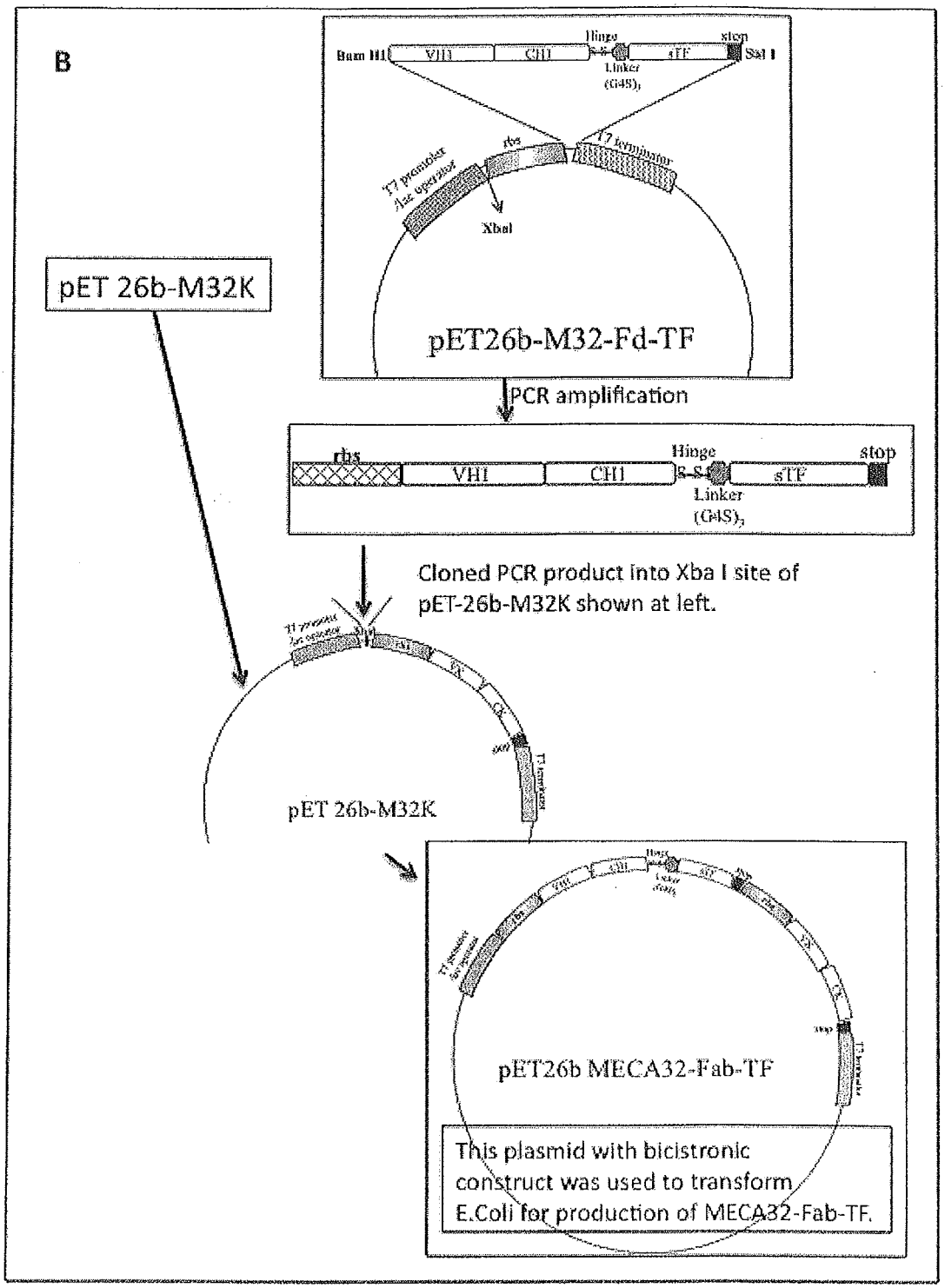

B
pET 26b-M32K
pET26b-M32-Fd-TF
PCR amplification
Cloned PCR product into Xba I site of pET-26b-M32K shown at left.
pET 26b-M32K
pET26b MECA32-Fab-TF
This plasmid with bicistronic construct was used to transform E.Coli for production of MECA32-Fab-TF.

FIG. 3B

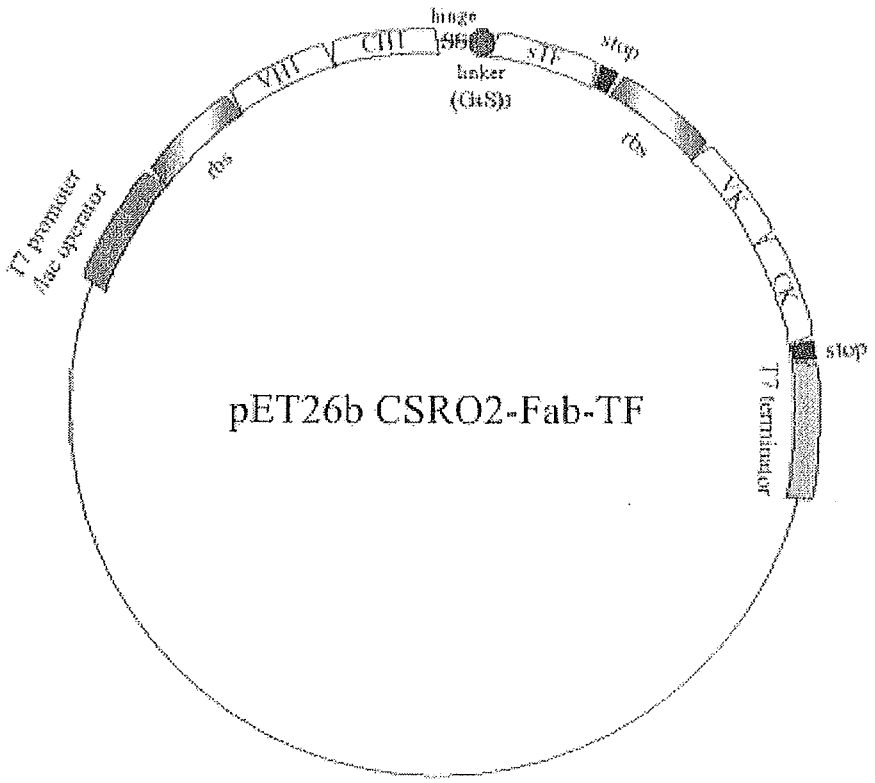

pET26b CSRO2-Fab-TF
T7 promoter
lac operator
rbs
VHH
CH1
hinge
linker
(GnS)3
stop
rbs
VL
CL
stop
T7 terminator

FIG. 4

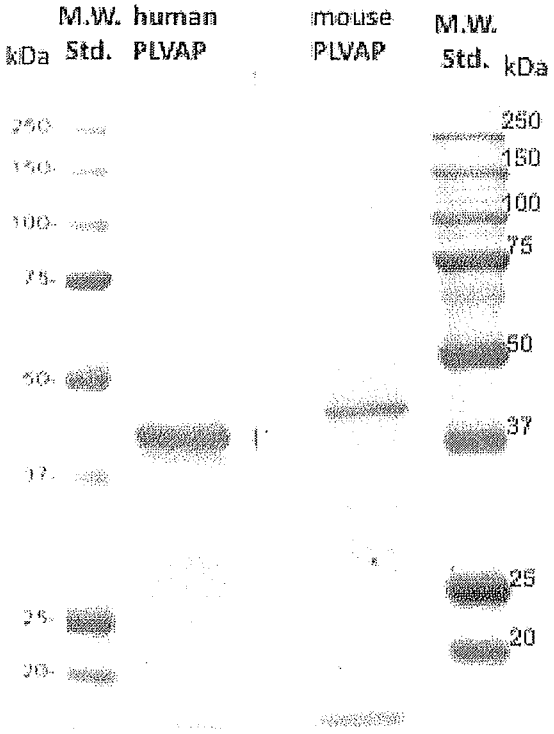

M.W.
kDa Std.
human
PLVAP
mouse
PLVAP
M.W.
Std.
kDa
250
150
100
75
50
37
25
20

FIG. 5

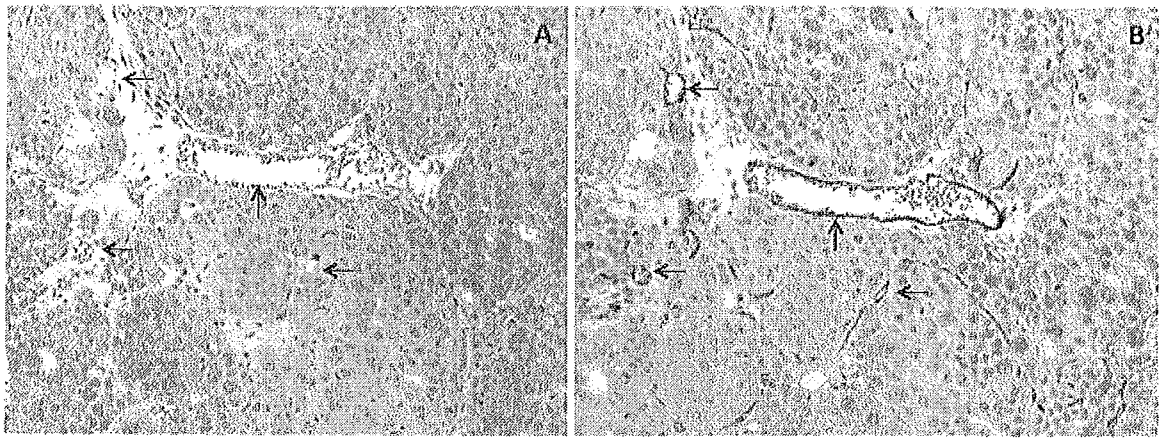


FIG. 6A

FIG. 6B

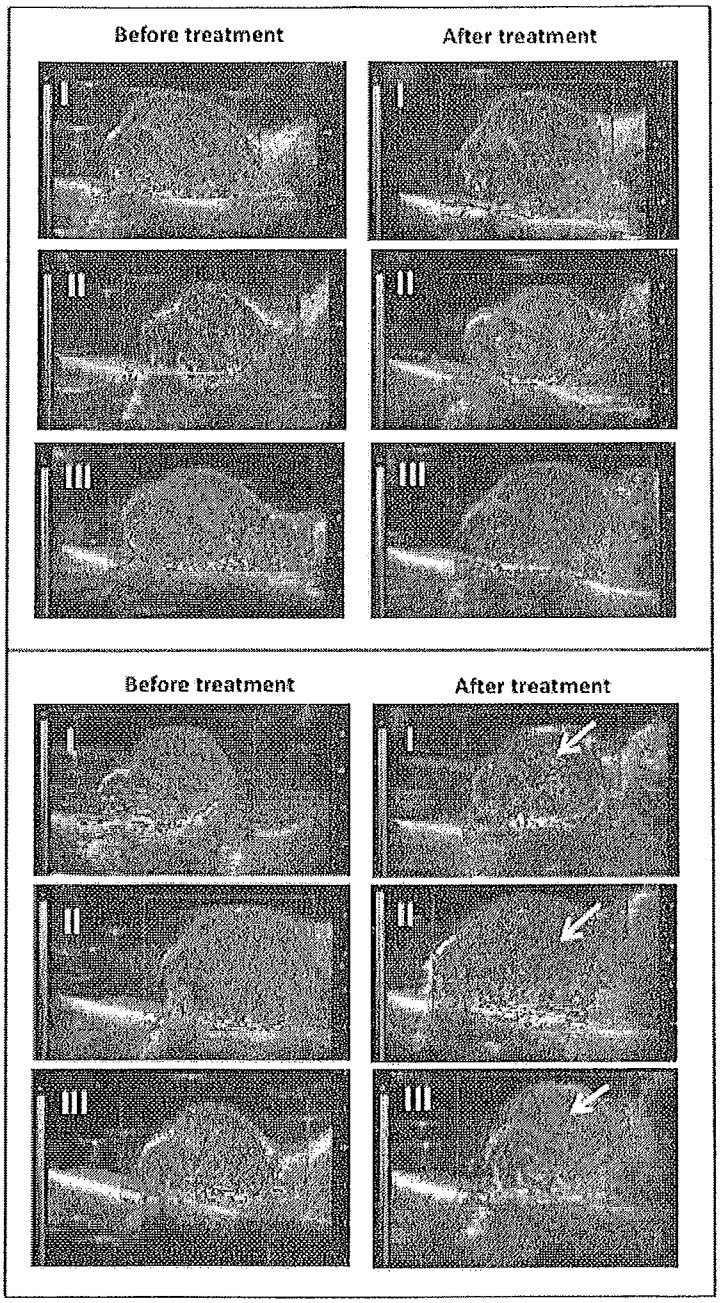

Before treatment
After treatment
I
II
III
Control group
Before treatment
After treatment
I
II
III
Treatment group

FIG. 7

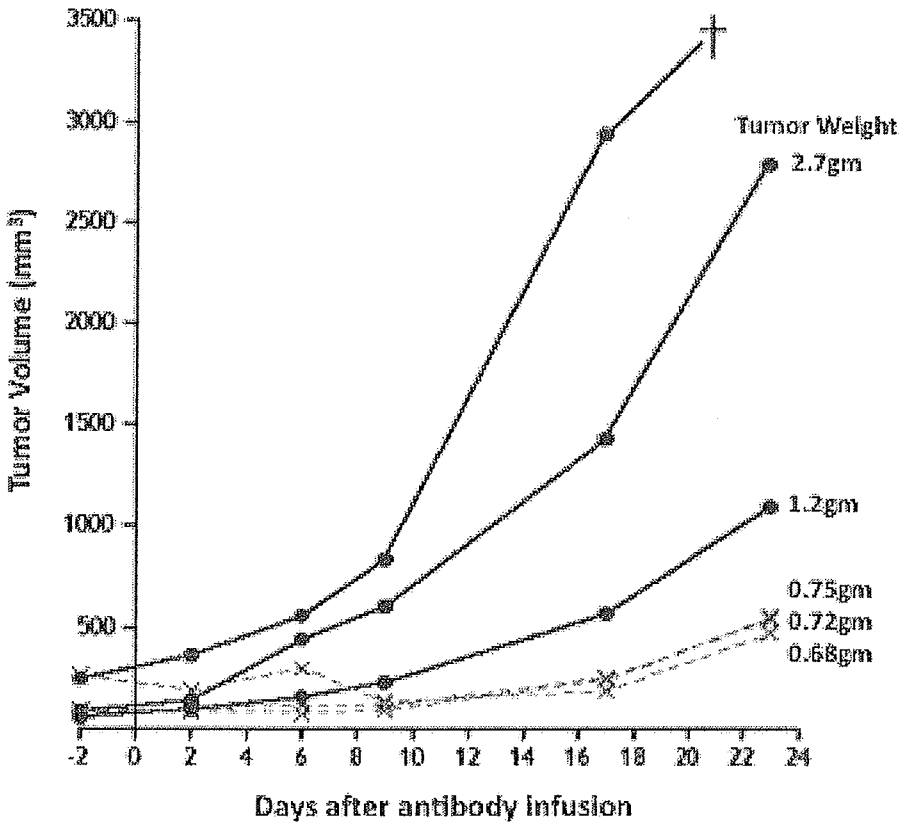
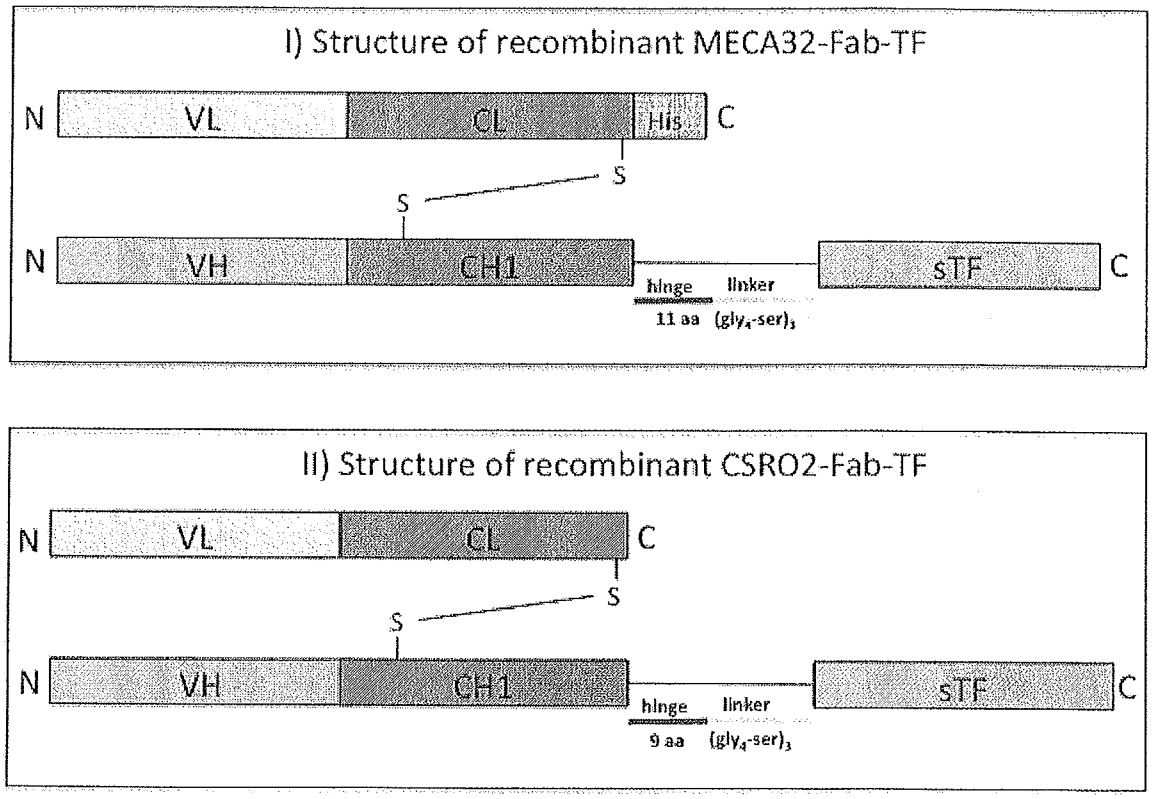

3500
3000
2500
2000
1500
1000
500
Tumor Volume (mm³)
Tumor Weight
2.7gm
1.2gm
0.75gm
0.72gm
0.68gm
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
Days after antibody infusion

FIG. 8



sTF: soluble extracellular domain of tissue factor
VL: Variable domain of antibody light chain
CL: Constant domain of antibody light chain
VH: Variable domain of antibody heavy chain
CH1: Constant domain 1 of antibody heavy chain
His: Histidine tag
N: N-terminus **C:** C-terminus

FIG. 9

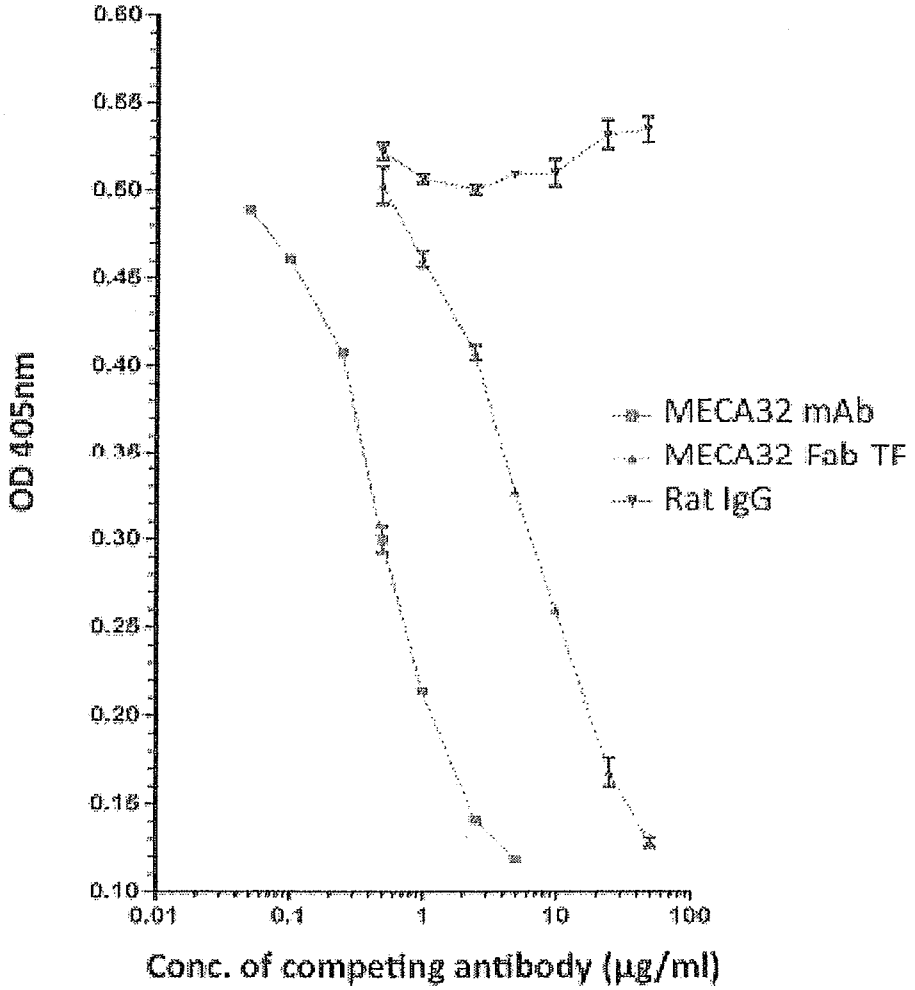

0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
OD 405nm
0.01
0.1
1
10
100
Conc. of competing antibody (µg/ml)
MECA32 mAb
MECA32 Fab TF
Rat IgG

FIG. 10

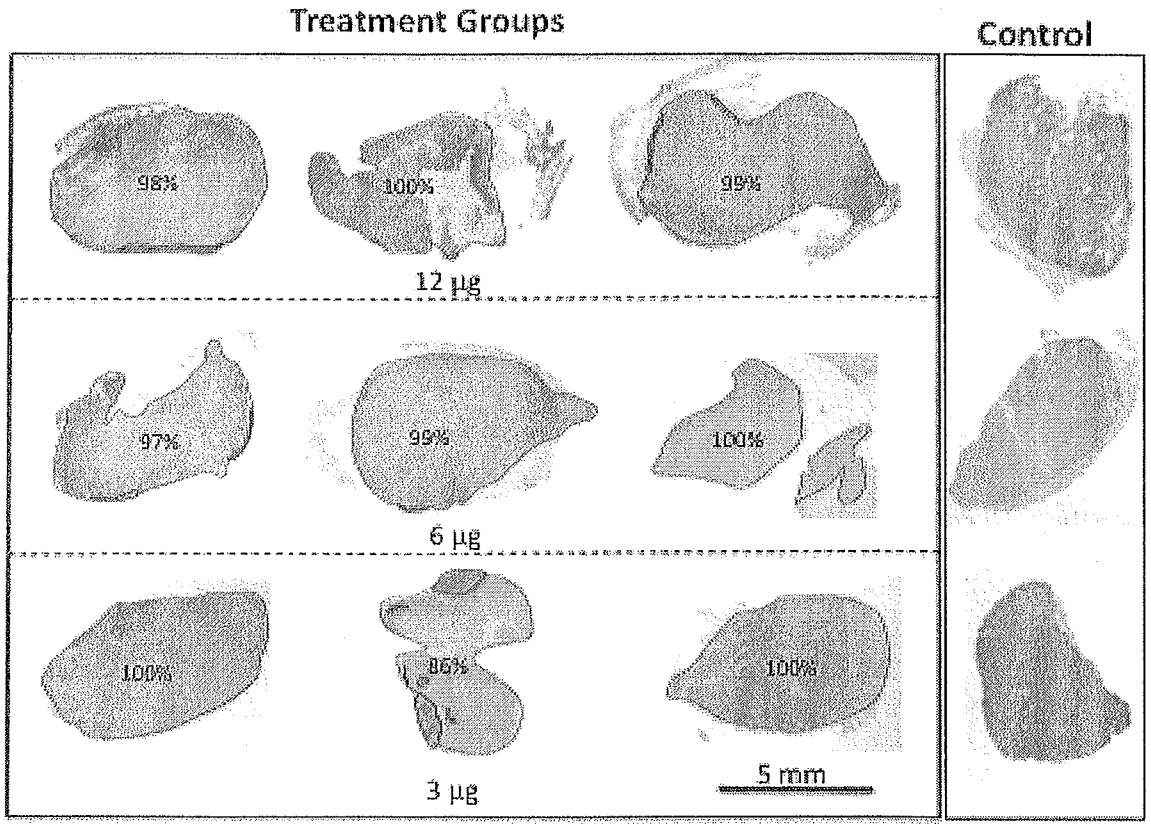

Treatment Groups
Control
98%
100%
99%
12 µg
97%
99%
100%
6 µg
100%
86%
100%
3 µg
5 mm

FIG. 11

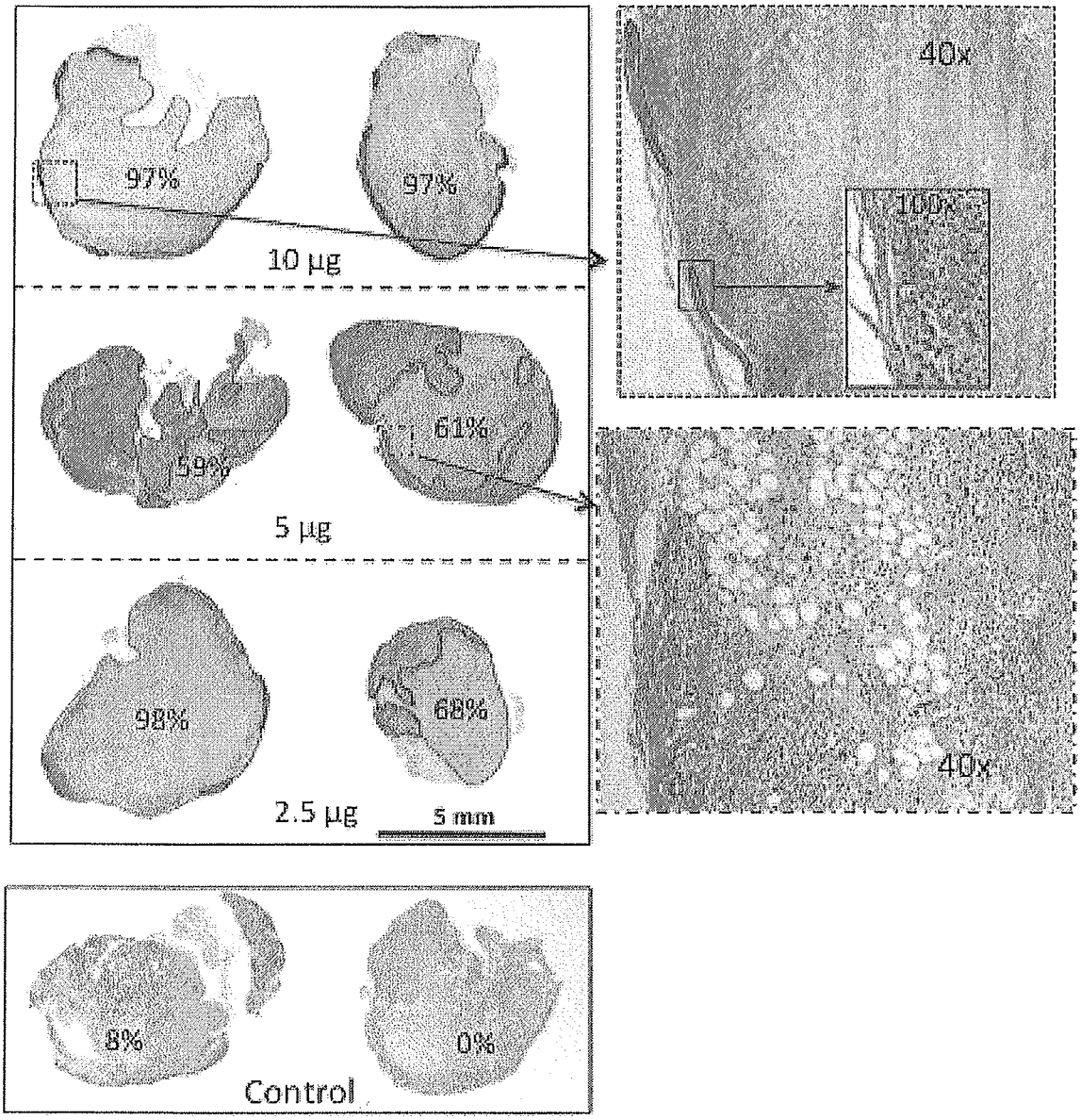


FIG. 12

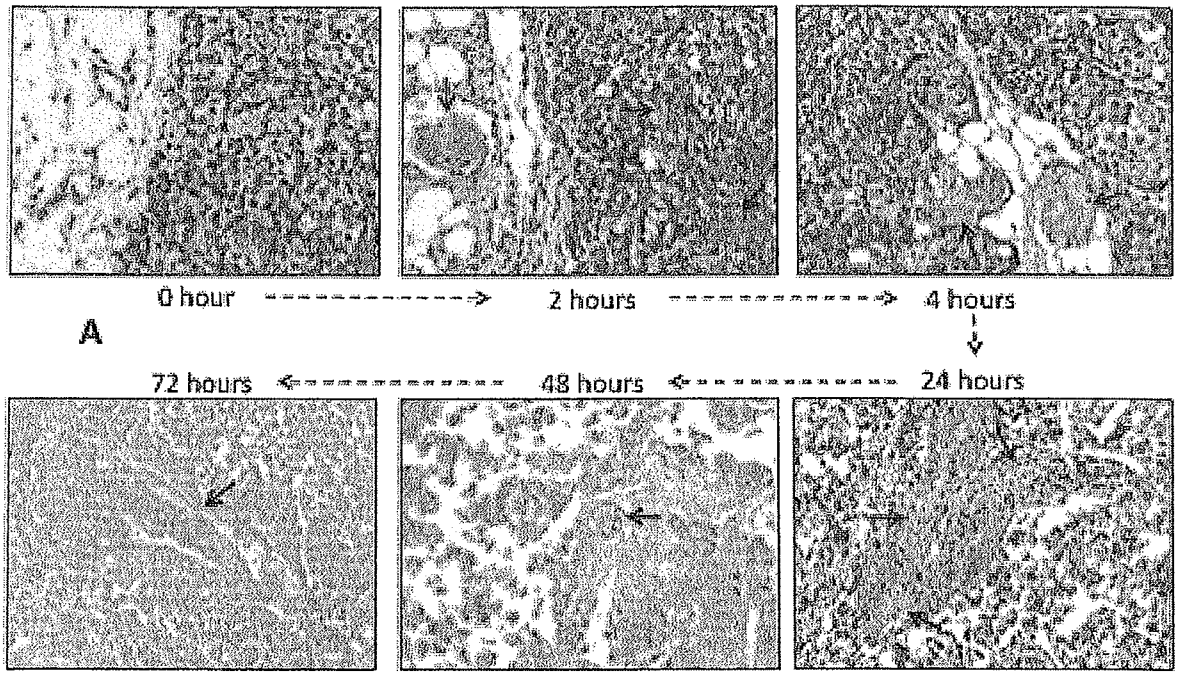


FIG. 13A

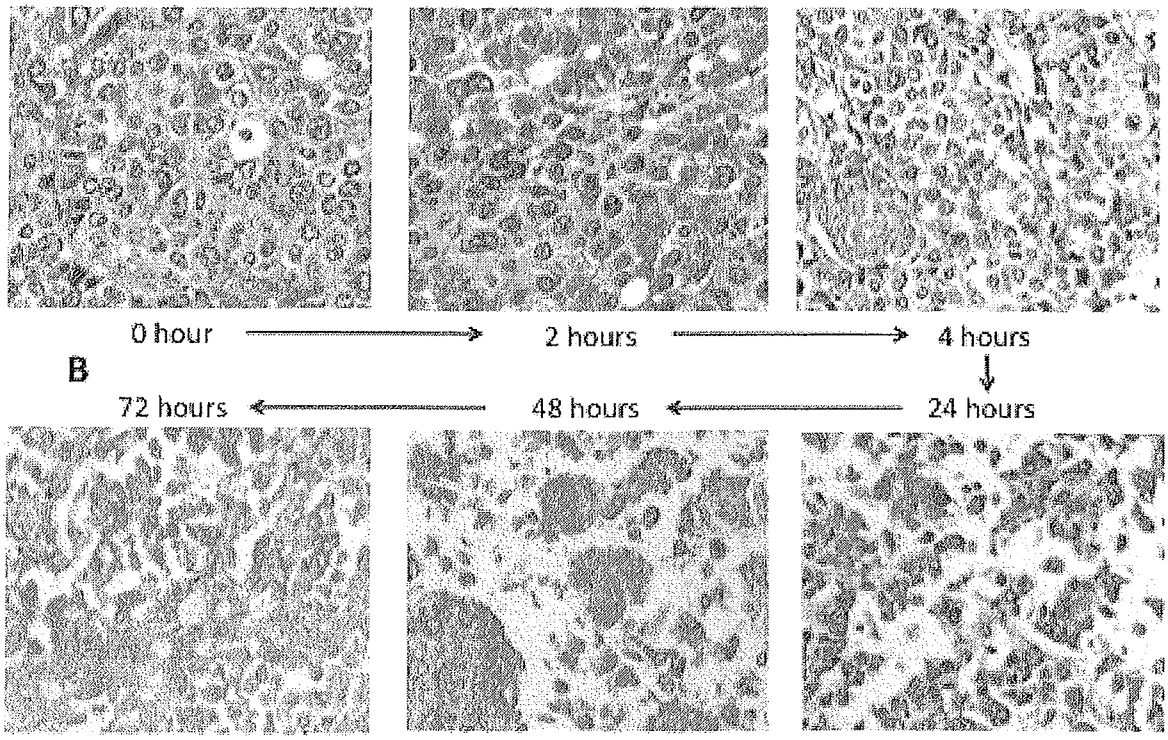


FIG. 13B

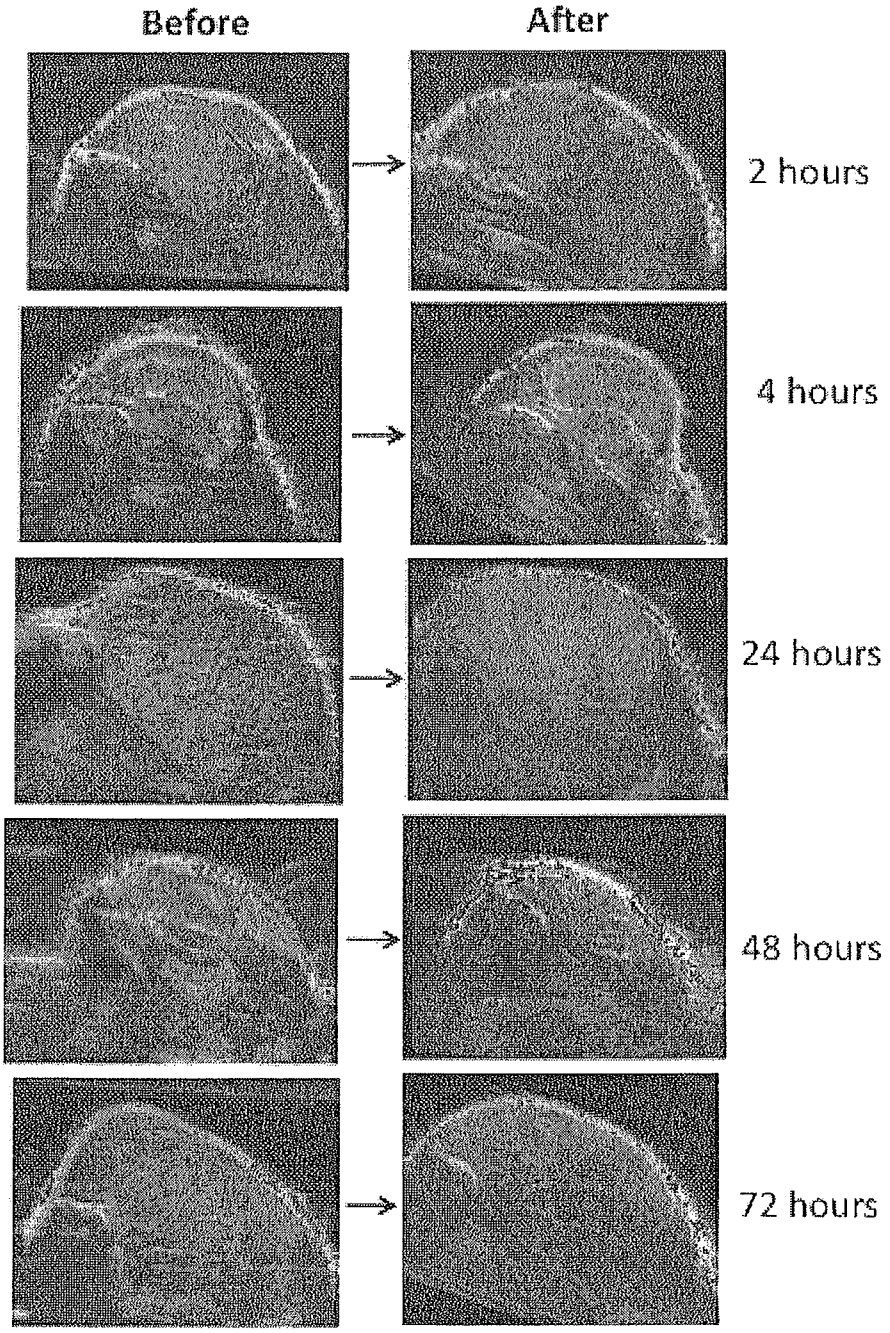


FIG. 14

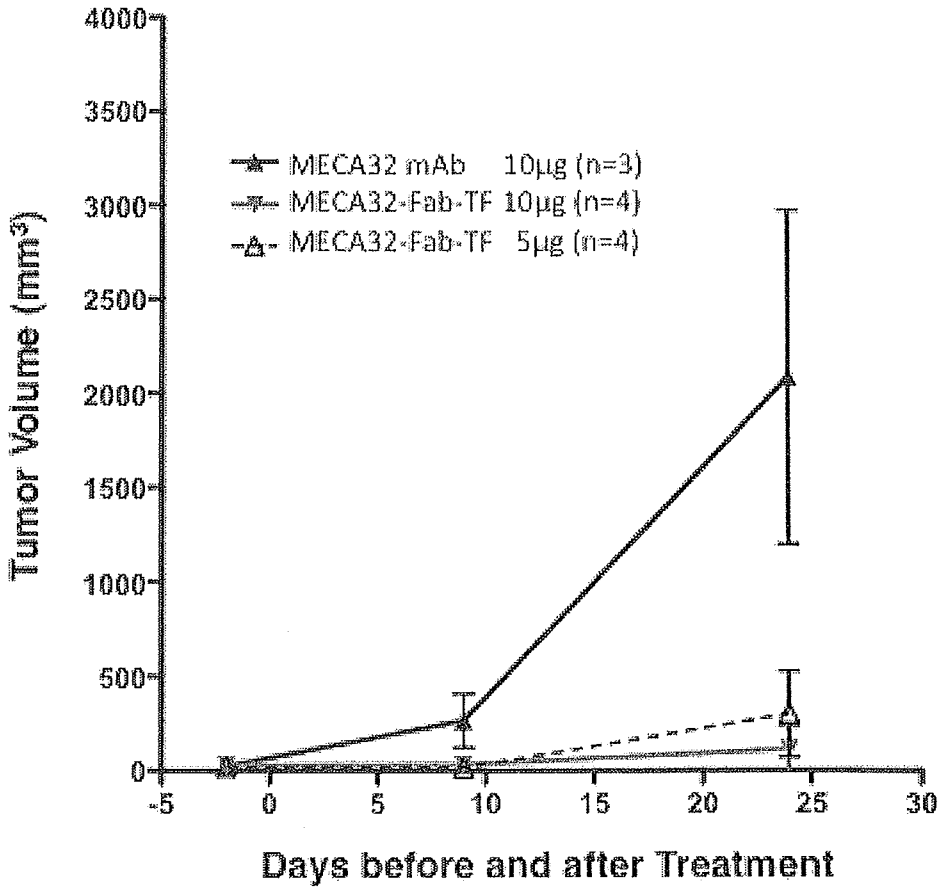
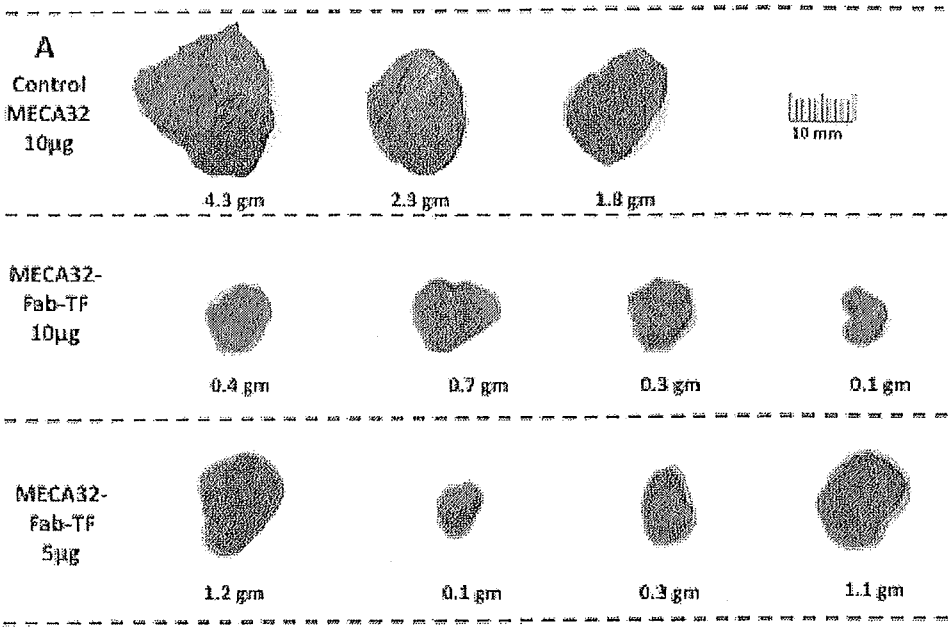

4000
3500
3000
2500
2000
1500
1000
500
0
Tumor Volume (mm³)
MECA32 mAb 10µg (n=3)
MECA32-Fab-TF 10µg (n=4)
MECA32-Fab-TF 5µg (n=4)
-5
0
5
10
15
20
25
30
Days before and after Treatment

FIG. 15

FIG. 16A



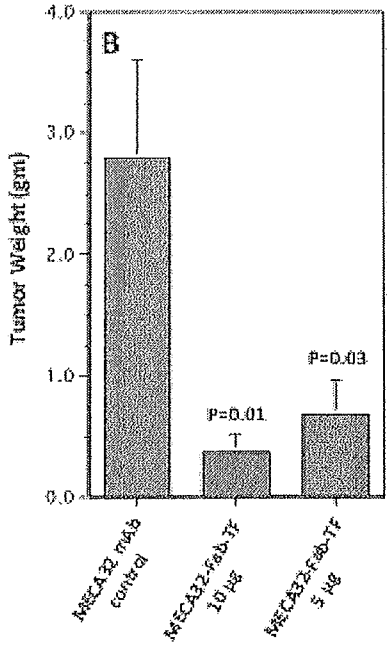


FIG. 16B

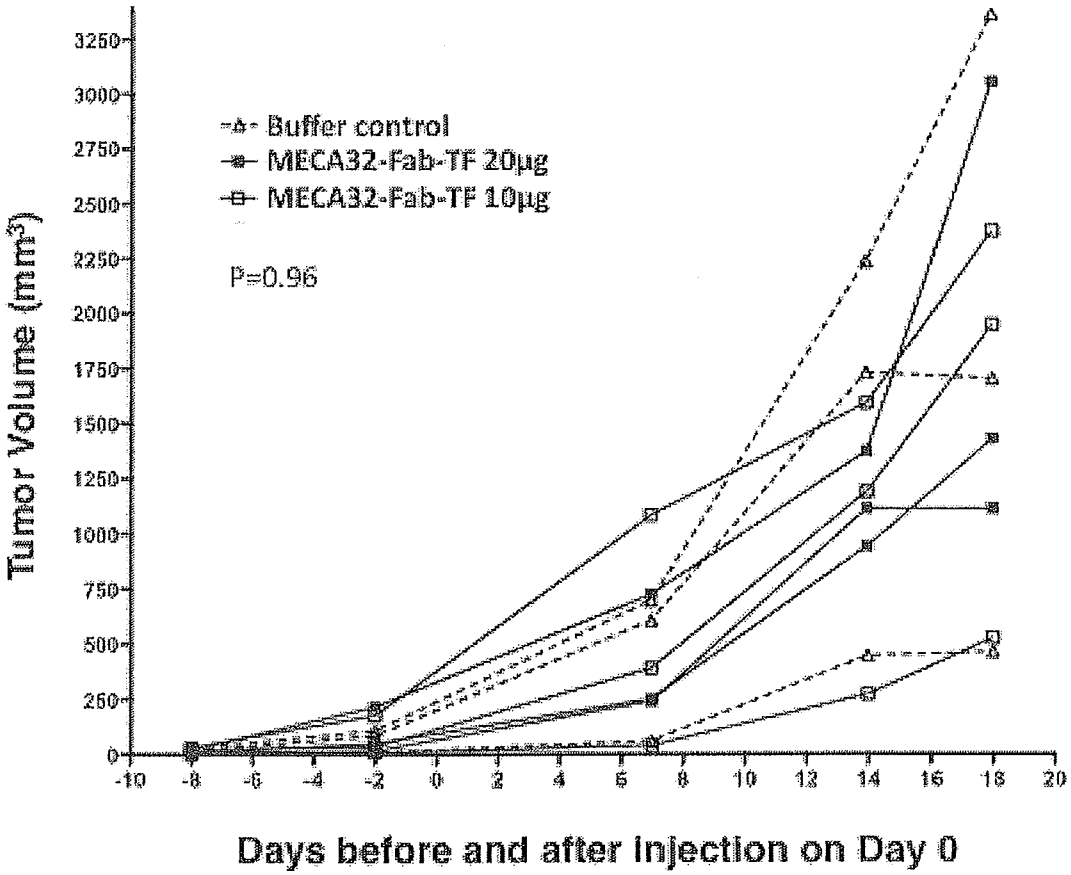

Buffer control
MECA32-Fab-TF 20µg
MECA32-Fab-TF 10µg
P=0.96
Tumor Volume (mm3)
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
Days before and after Injection on Day 0

FIG. 17

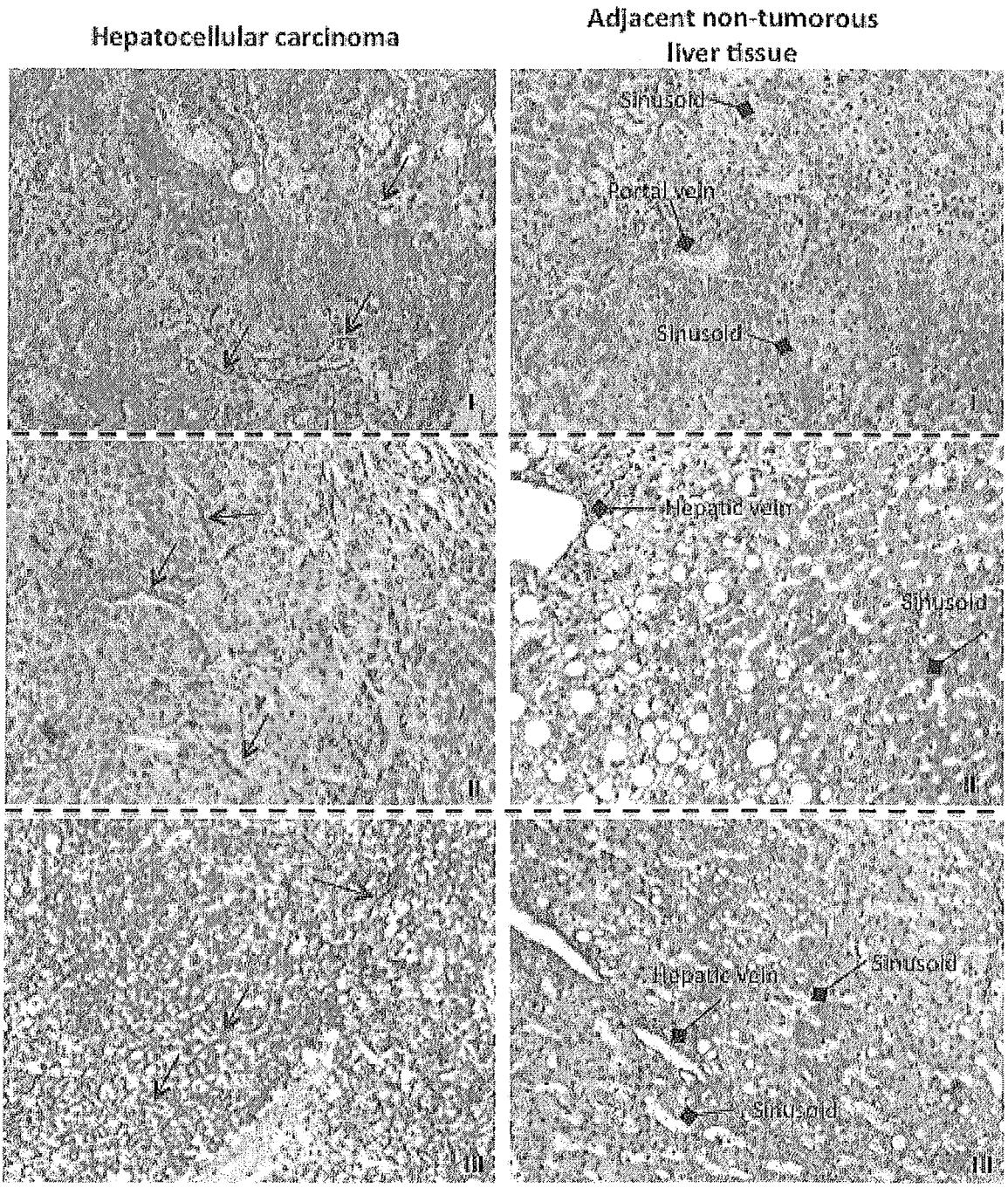

Hepatocellular carcinoma
Adjacent non-tumorous liver tissue
Sinusoid
Portal vein
Sinusoid
I
Hepatic vein
Sinusoid
II
Hepatic vein
Sinusoid
Sinusoid
III

FIG. 18

mglamehggs yaraggssrg cwyylryffl fvsliqflii lglvlfmvyg nvhvstesnl qaterraegl ysqllgltas qsnltkelnf ttrakdaimq mwlnarrdld rinasfrqcq gdrviytnnq rymaaiilse kqcrdqfkdm nkscdallfm lnqkvktlev eiakektict kdkesvllnk rvaeeqlvec vktrelqhqe rqlakeqlqk vqalclpldk dkfemdlrnl wrdsiiprsl dnlgynlyhp lgselasirr acdhmpslms skveelarsl radiervare nsdlqrqkle aqqglrasqe akqkvekeaq areaklqaec srqtqlalee kavlrkerdn lakeleekkr eaeqlrmela irnsaldtci ktksqpmmpv srpmgpvpnp qpidpaslee fkrkilesqr ppagipvaps sg (SEQ ID NO: 2)

FIG. 19

EVQLQQSGAEFVRSGASVKLSCTASGFNIKDYYIHWVKQRPEQGLEWIGWIDPENGDIEYAPKFQGK
ATMTADTSSNTAYLQFSSLTSEDTAVYYCLYQEGSWGQGTTLTVSSA (SEQ ID NO: 3)

FIG. 20

DVVMTQTPLTLSVTIGQPASISCKSSQSLLNSDGKTYLNWLLQRPGQSPKRLIYLVSKLDSGVPD
RFTGSGSGTDFTLKISRVEAEDLGVYYCWQGTHFPFTFGGGTKLEIK (SEQ ID NO: 4)

FIG. 21

QVQLQQPGAELVRPGASVKLSCKASGYTFTSNYINWVKQRPGQGLEWIGNIYPSDGFTNYNQKFKDR
ATLTVDKSSSTAYMQLSSPTSEDSAVYYCTRNFDVWGAGTTVTVSSA (SEQ ID NO: 5)

FIG. 22

DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLQWYLQKPGQSPKLLIYTVSNRFSGVPDRF
SGSGSGPDFTLKISRVEAEDLGVYFCSQSTHVPFTFGSGTKLEIK (SEQ ID NO: 6)

FIG. 23

MQVQLVQSGS ELKKPGASVK VSCKASGYTF TSNYINWVRQ APGQGLEWMG
NIYPSDGFTN YNQKFKDRVT ITVDKSTSTA YMELSSLRSE DTAVYYCTRN
FDVWGQGTTV TVSS**ASTKGP SVFPLAPCSR STSESTAALG CLVKDYFPEP**
VTVSWNSGAL TSGVHTFPAV LQSSGLYSLS SVVTVPSSSL GTKTYTCNVD
HKPSNTKVDK RVESKYGPPC PSCPGgggsg gggsggggs*S GTTNTVAAYN*
LTWKSTNFKT ILEWEPKPVN QVYTVQISTK SGDWKSKCFY TTDTECDLTD
EIVKDVKQTY LARVFSYPAG NVESTGSAGE PLYENSPEFT PYLETNLGQP
TIQSFEQVGT KVNVTVEDER TLVRRNNTFL SLRDVFGKDL IYTLYYWKSS
SSGKKTAKTN TNEFLIDVDK GENYCFSVQA VIPSRTVNRK STDSPVECMG QEKGEFRE
(SEQ ID NO: 23)

FIG. 24