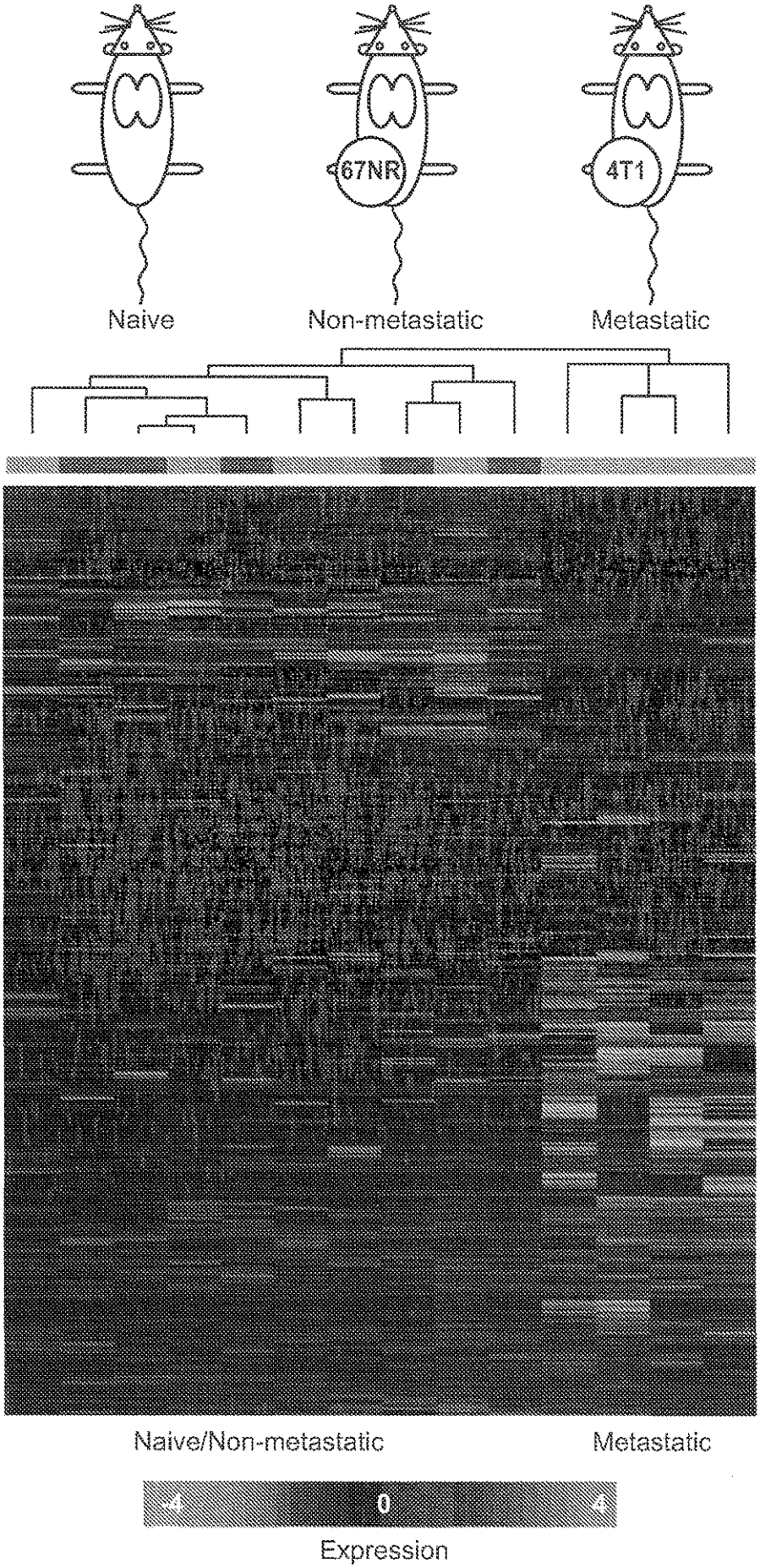
FIG. 1A


67NR
4T1
Naive
Non-metastatic
Metastatic
Naive/Non-metastatic
Metastatic
-4
0
4
Expression

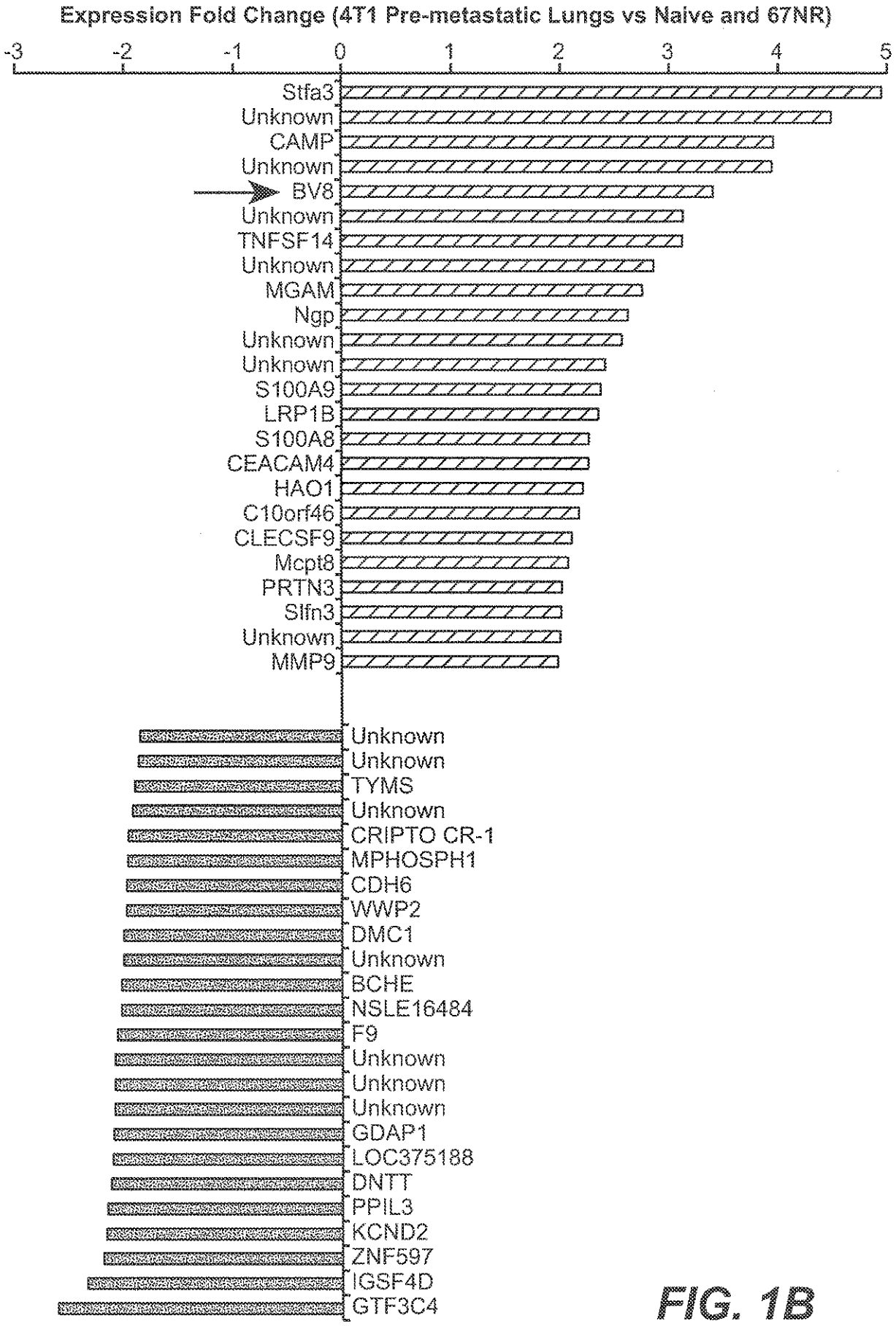

Expression Fold Change (4T1 Pre-metastatic Lungs vs Naive and 67NR)
-3
-2
-1
0
1
2
3
4
5
Stfa3
Unknown
CAMP
Unknown
BV8
Unknown
TNFSF14
Unknown
MGAM
Ngp
Unknown
Unknown
S100A9
LRP1B
S100A8
CEACAM4
HAO1
C10orf46
CLECSF9
Mcpt8
PRTN3
Slfn3
Unknown
MMP9
Unknown
Unknown
TYMS
Unknown
CRIPTO CR-1
MPHOSPH1
CDH6
WWP2
DMC1
Unknown
BCHE
NSLE16484
F9
Unknown
Unknown
Unknown
GDAP1
LOC375188
DNTT
PPIL3
KCND2
ZNF597
IGSF4D
GTF3C4

FIG. 1B

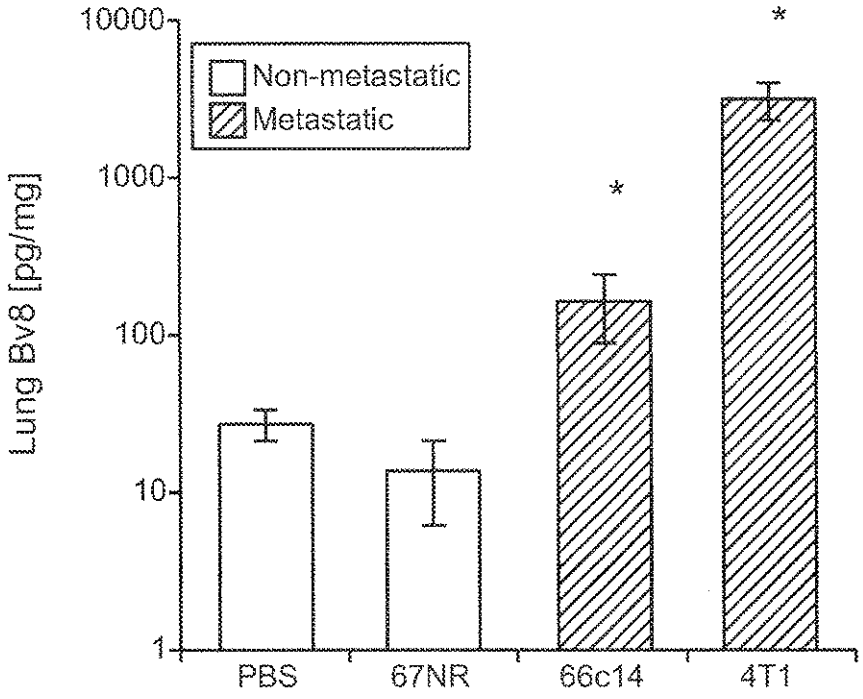

10000
1000
100
10
1
Lung Bv8 [pg/mg]
Non-metastatic
Metastatic
*
*
PBS
67NR
66c14
4T1

FIG. 1C

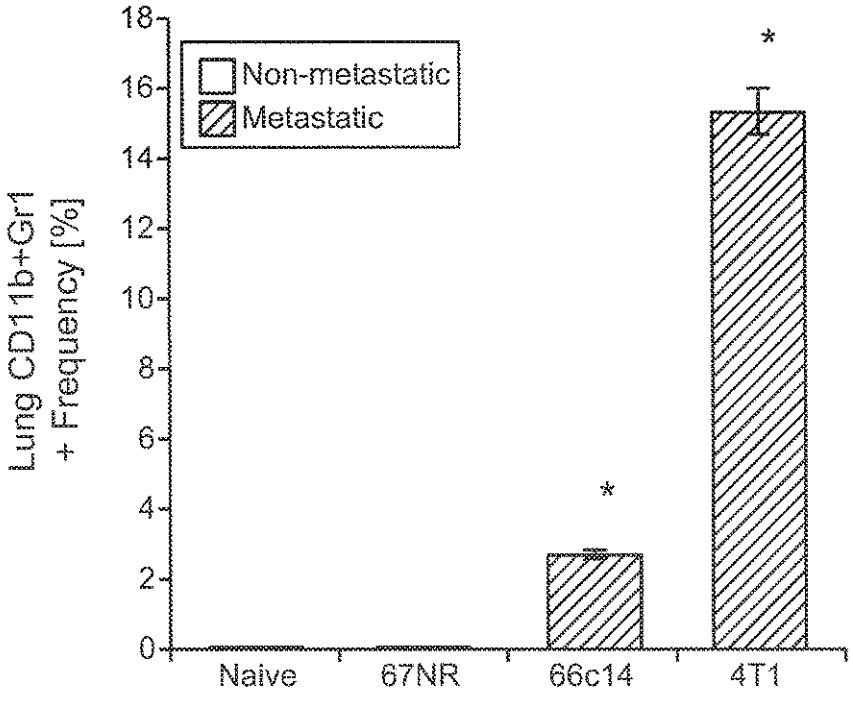

18
16
14
12
10
8
6
4
2
0
Lung CD11b+Gr1 + Frequency [%]
Non-metastatic
Metastatic
*
*
Naive
67NR
66c14
4T1

FIG. 1D

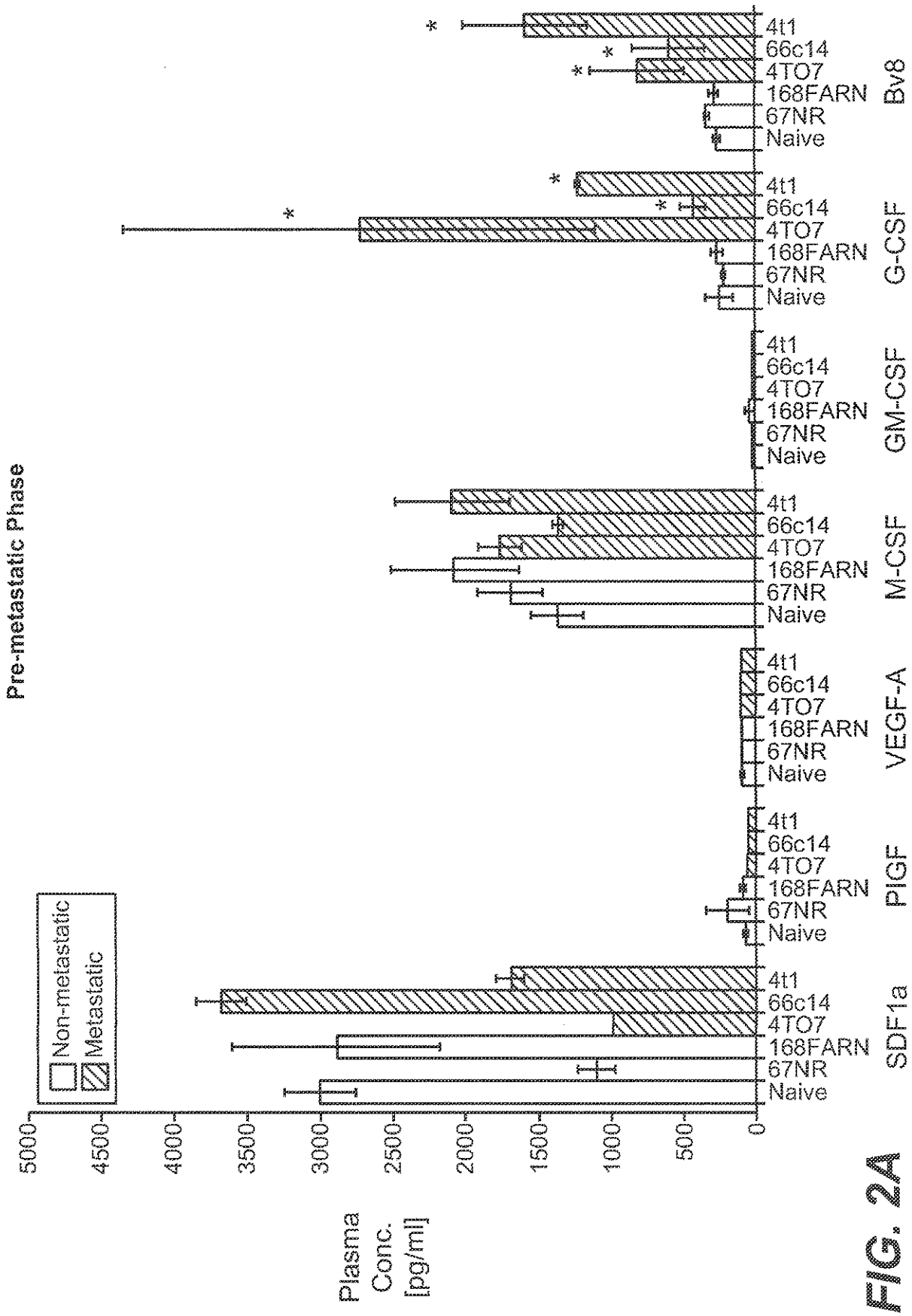

Pre-metastatic Phase
Non-metastatic
Metastatic
Plasma Conc. [pg/ml]
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
SDF1a
PlGF
VEGF-A
M-CSF
GM-CSF
G-CSF
Bv8
4t1
66c14
4TO7
168FARN
67NR
Naive

FIG. 2A

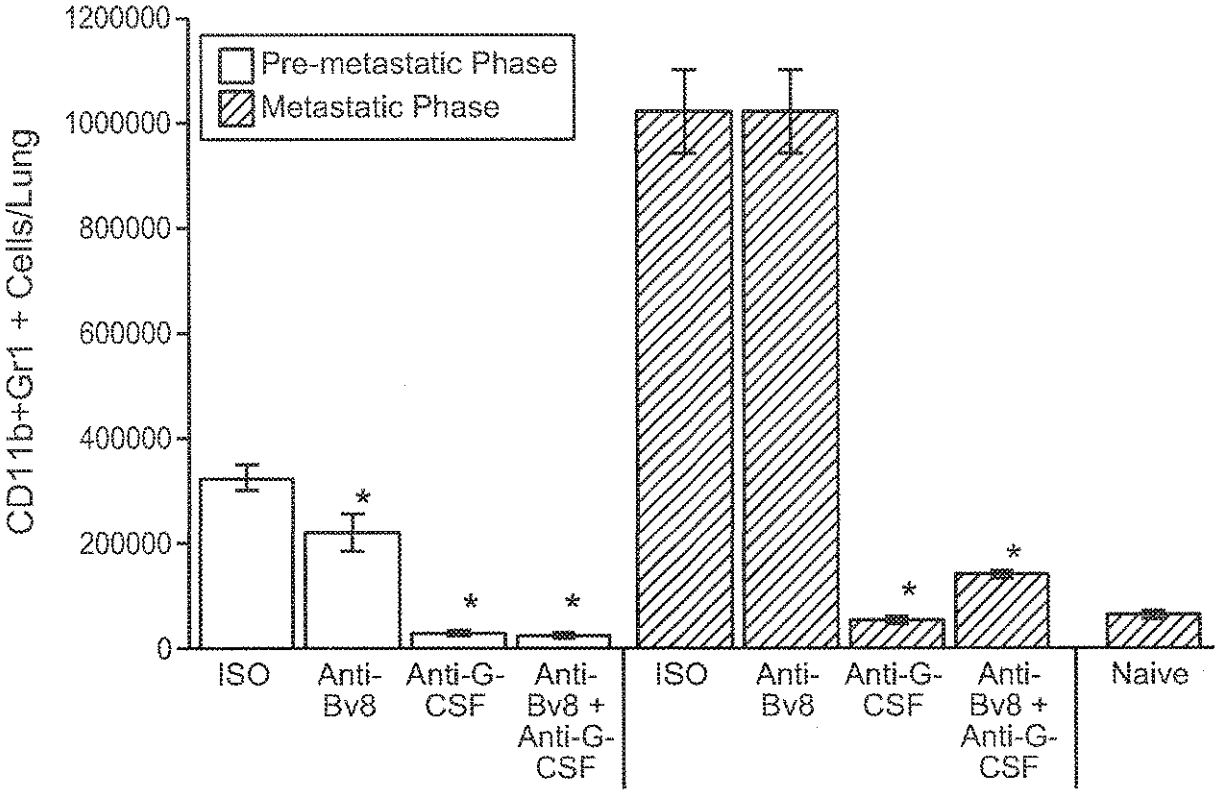

CD11b+Gr1 + Cells/Lung
1200000
1000000
800000
600000
400000
200000
0
Pre-metastatic Phase
Metastatic Phase
*
ISO
Anti-Bv8
Anti-G-CSF
Anti-Bv8 + Anti-G-CSF
Naive

FIG. 2B

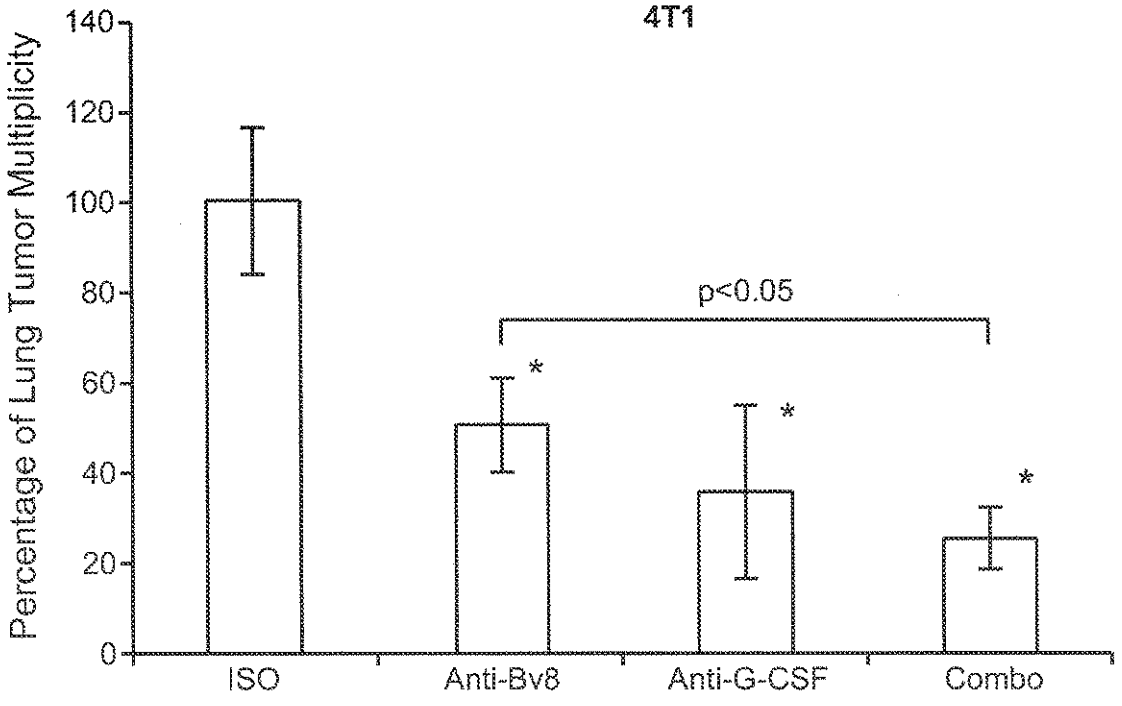

4T1
Percentage of Lung Tumor Multiplicity
140
120
100
80
60
40
20
0
p<0.05
*
ISO
Anti-Bv8
Anti-G-CSF
Combo

FIG. 2C

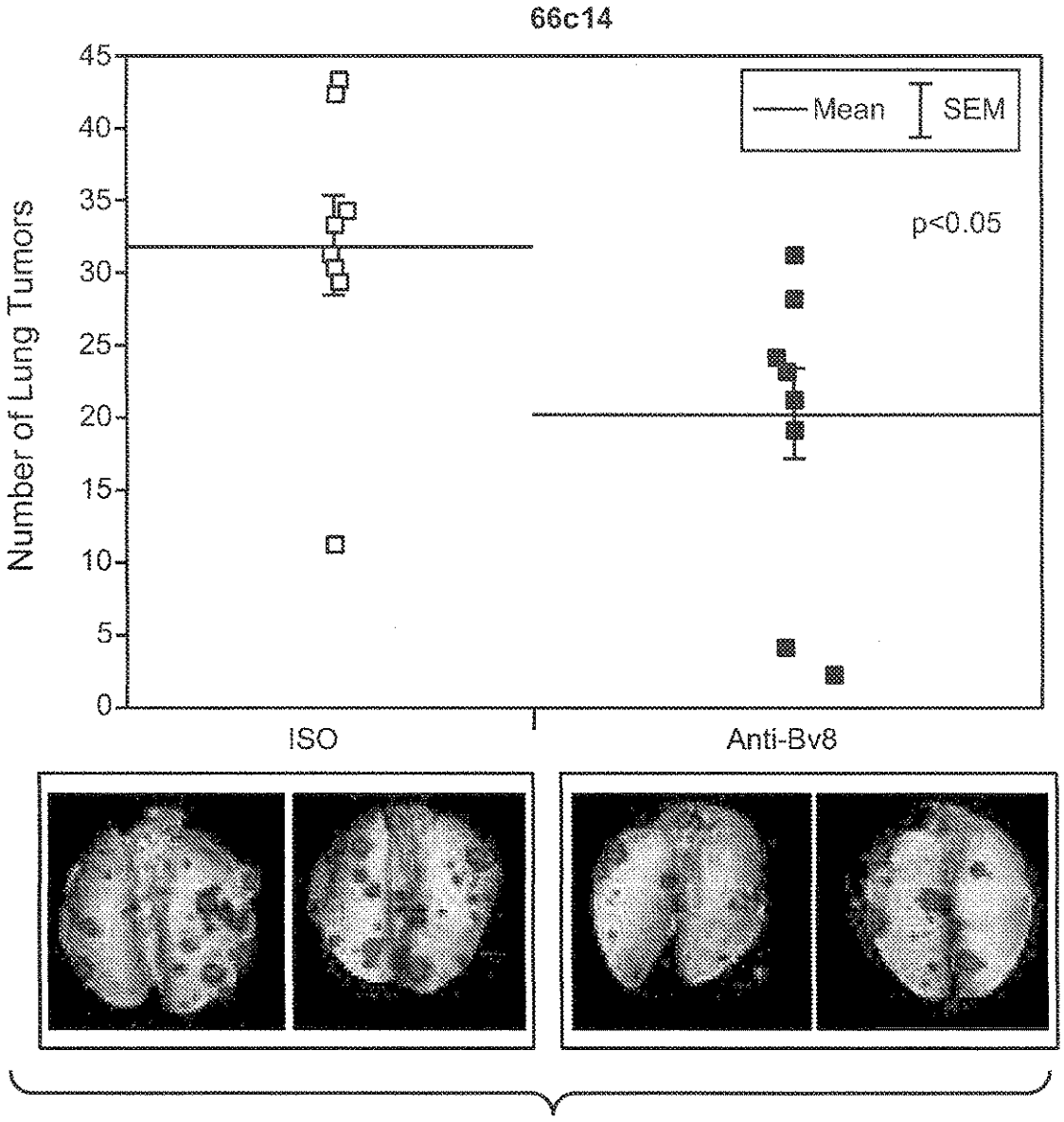

66c14
Number of Lung Tumors
45
40
35
30
25
20
15
10
5
0
Mean
SEM
p<0.05
ISO
Anti-Bv8

FIG. 2D

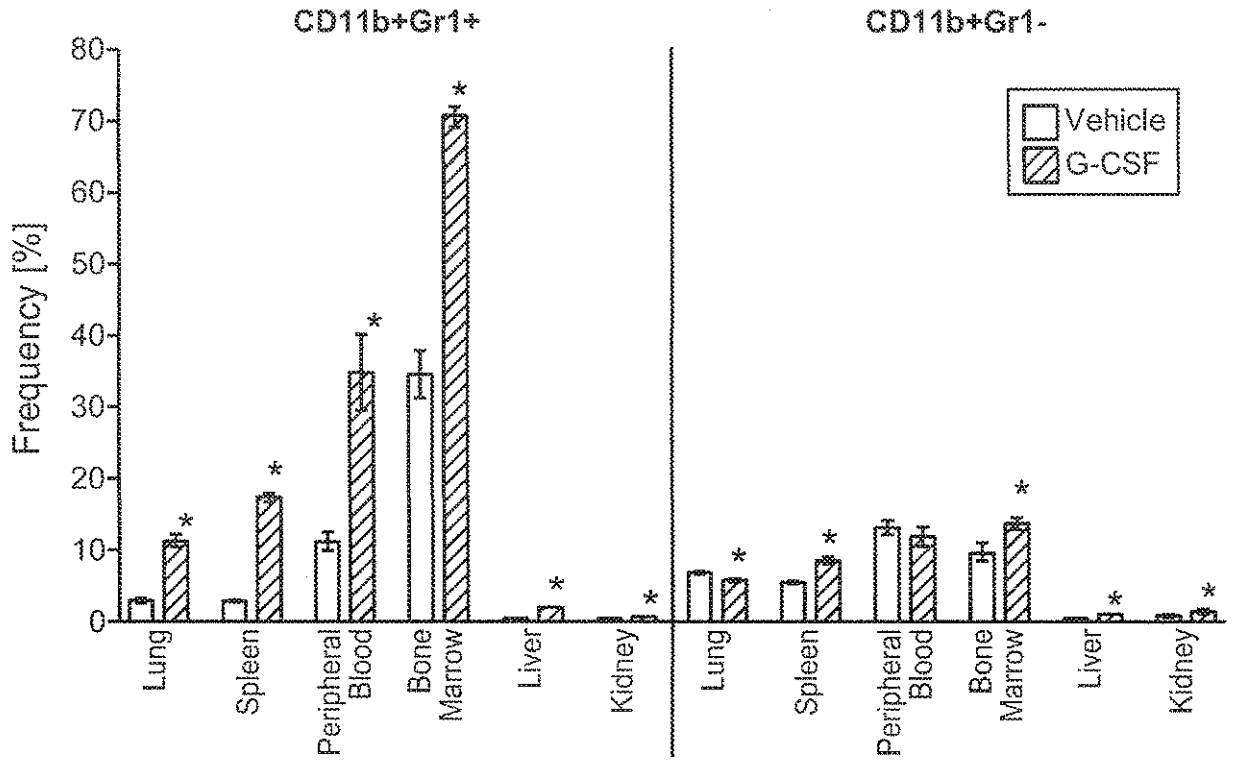

CD11b+Gr1+
CD11b+Gr1-
Frequency [%]
80
70
60
50
40
30
20
10
0
Vehicle
G-CSF
Lung
Spleen
Peripheral Blood
Bone Marrow
Liver
Kidney
*

FIG. 3A

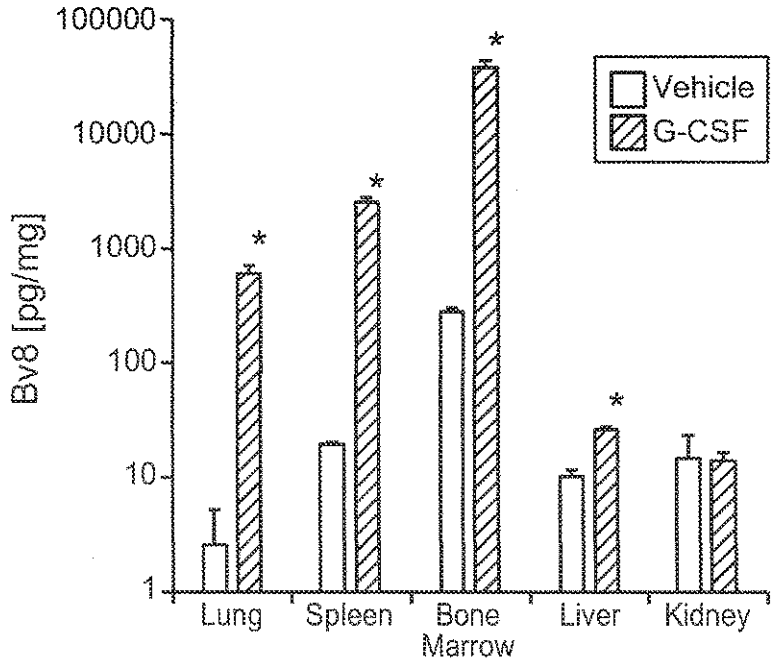

Bv8 [pg/mg]
100000
10000
1000
100
10
1
Vehicle
G-CSF
Lung
Spleen
Bone Marrow
Liver
Kidney
*

FIG. 3B

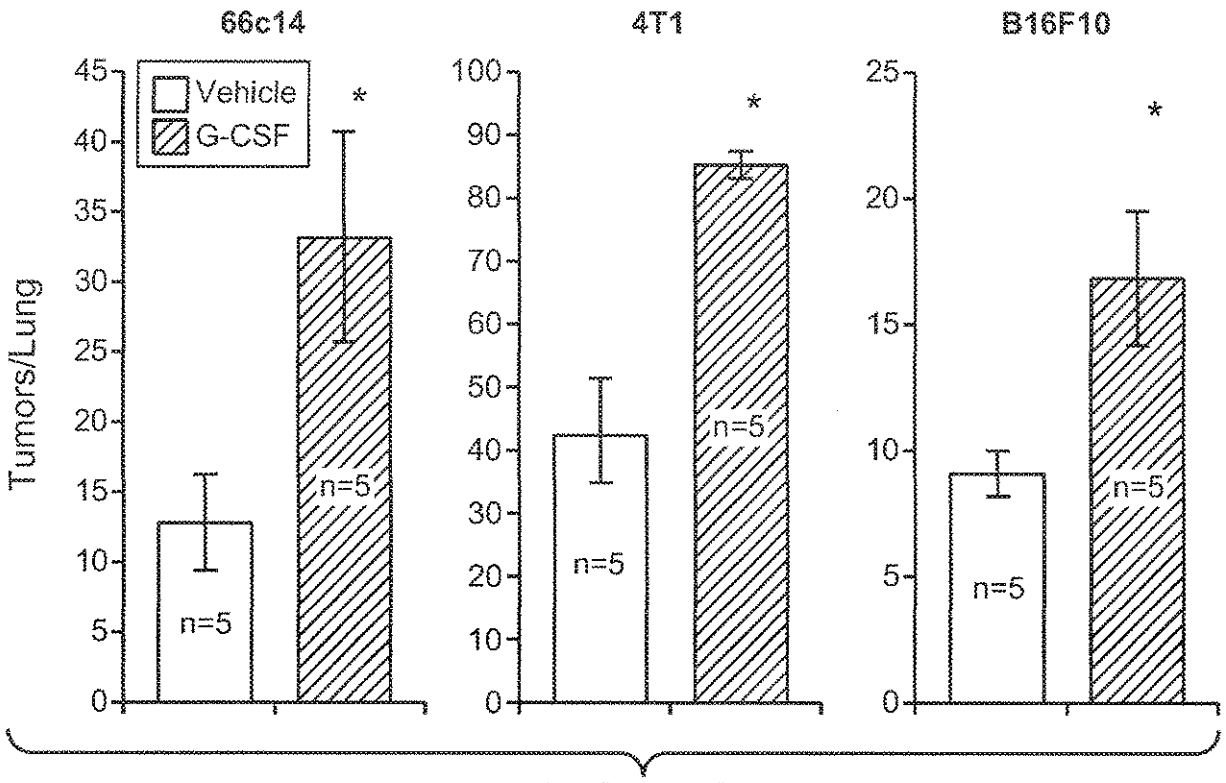

66c14
4T1
B16F10
Vehicle
G-CSF
Tumors/Lung
n=5
n=5
n=5
n=5
n=5
n=5
*
*
*
0
5
10
15
20
25
30
35
40
45
50
60
70
80
90
100

FIG. 3C

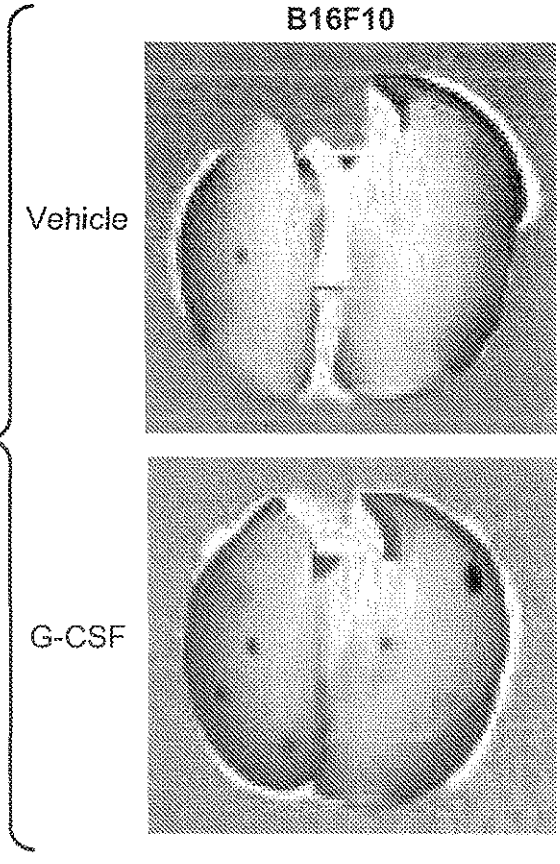

B16F10
Vehicle
G-CSF

FIG. 3D

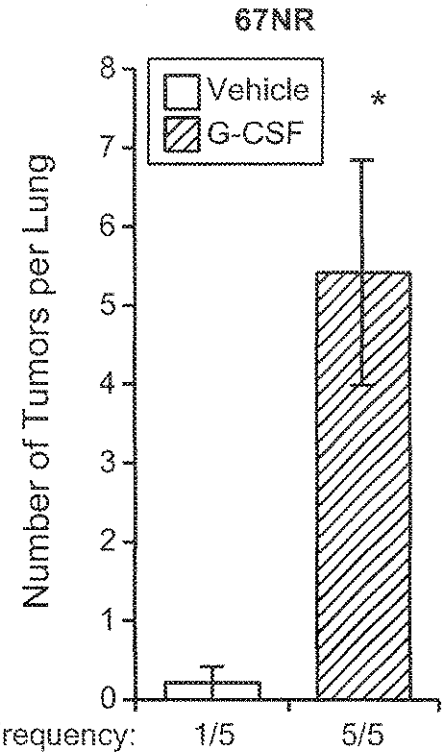

67NR
Vehicle
G-CSF
*
Number of Tumors per Lung
0
1
2
3
4
5
6
7
8
Frequency:
1/5
5/5

FIG. 3E

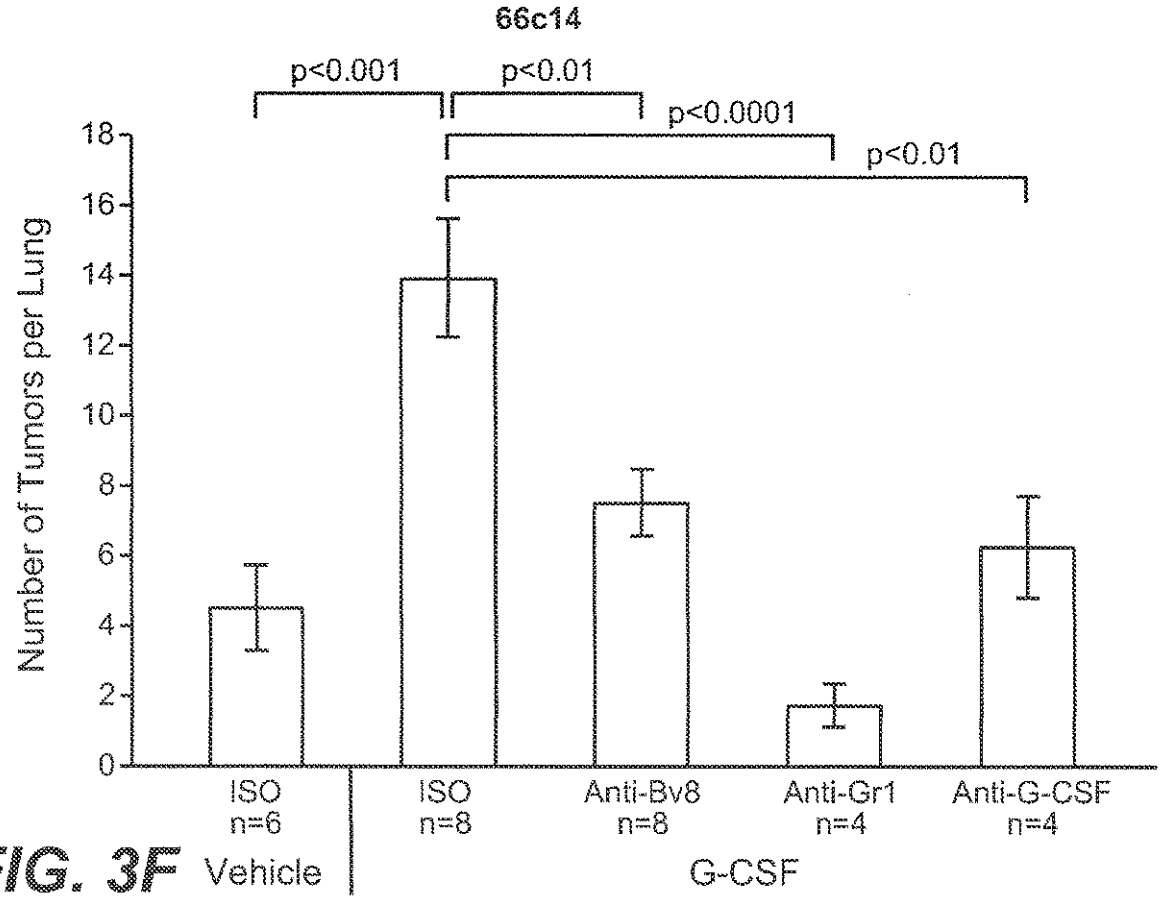

66c14
p<0.001
p<0.01
p<0.0001
p<0.01
Number of Tumors per Lung
0
2
4
6
8
10
12
14
16
18
ISO
n=6
ISO
n=8
Anti-Bv8
n=8
Anti-Gr1
n=4
Anti-G-CSF
n=4
Vehicle
G-CSF

FIG. 3F

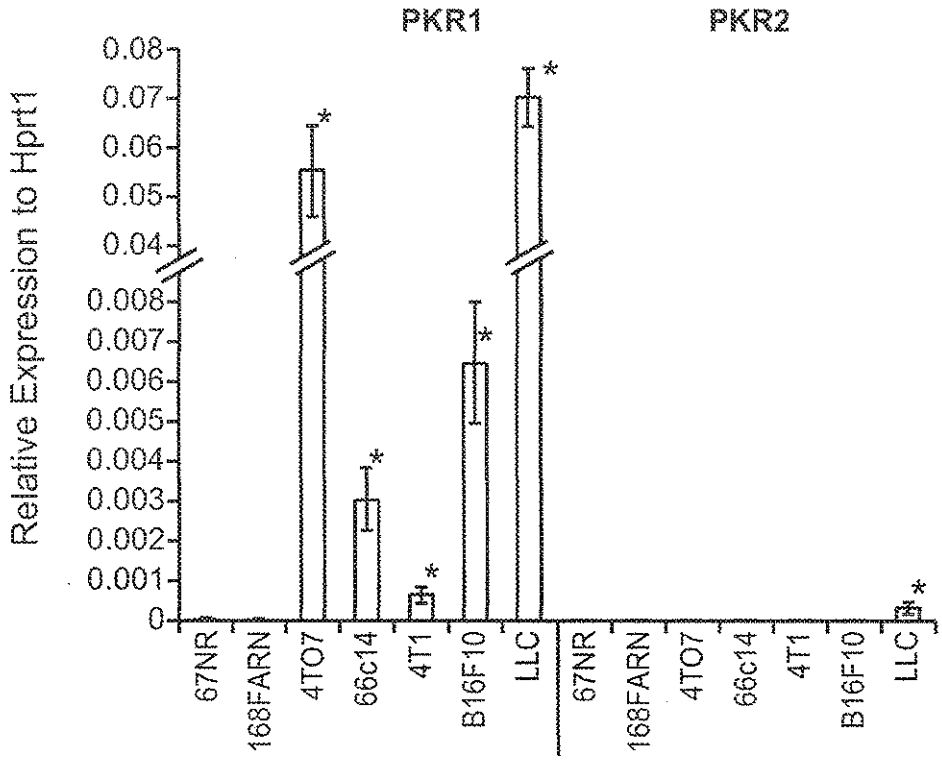

PKR1
PKR2
Relative Expression to Hprt1
0.08
0.07
0.06
0.05
0.04
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
67NR
168FARN
4TO7
66c14
4T1
B16F10
LLC
67NR
168FARN
4TO7
66c14
4T1
B16F10
LLC

FIG. 4A

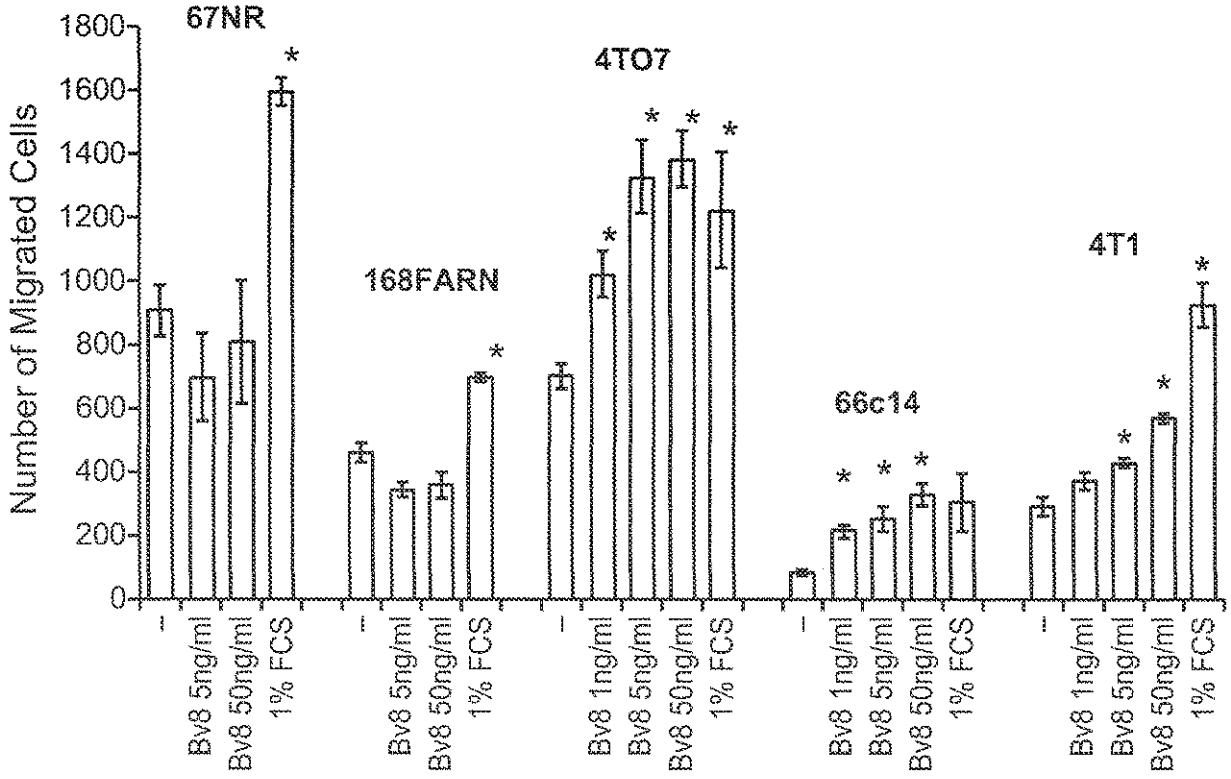

Number of Migrated Cells
1800
1600
1400
1200
1000
800
600
400
200
0
67NR
168FARN
4TO7
66c14
4T1
–
Bv8 5ng/ml
Bv8 50ng/ml
1% FCS
–
Bv8 5ng/ml
Bv8 50ng/ml
1% FCS
–
Bv8 1ng/ml
Bv8 5ng/ml
Bv8 50ng/ml
1% FCS
–
Bv8 1ng/ml
Bv8 5ng/ml
Bv8 50ng/ml
1% FCS
–
Bv8 1ng/ml
Bv8 5ng/ml
Bv8 50ng/ml
1% FCS

FIG. 4B

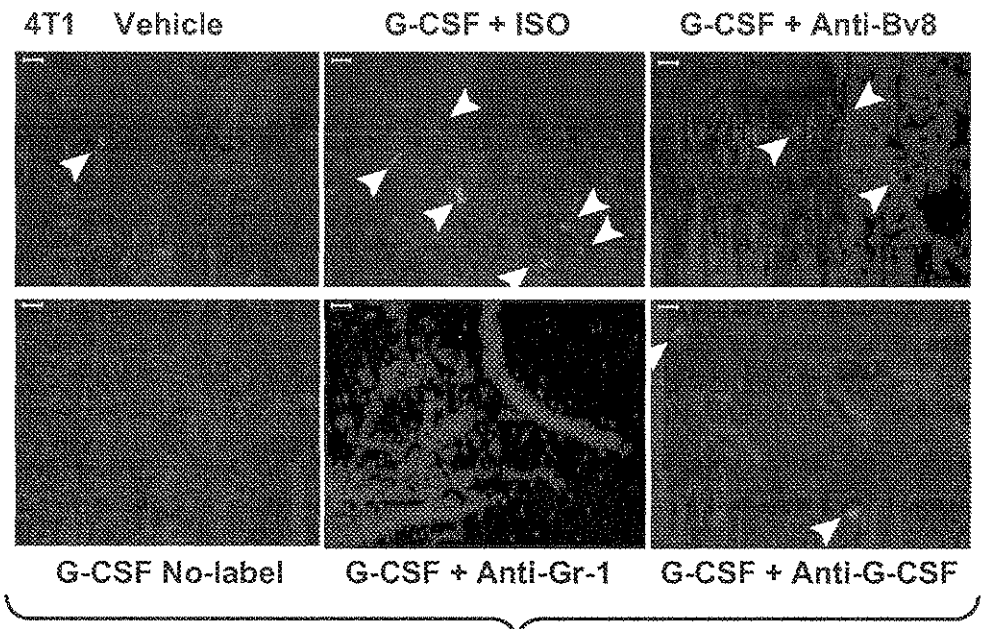

4T1
Vehicle
G-CSF + ISO
G-CSF + Anti-Bv8
G-CSF No-label
G-CSF + Anti-Gr-1
G-CSF + Anti-G-CSF

FIG. 4C

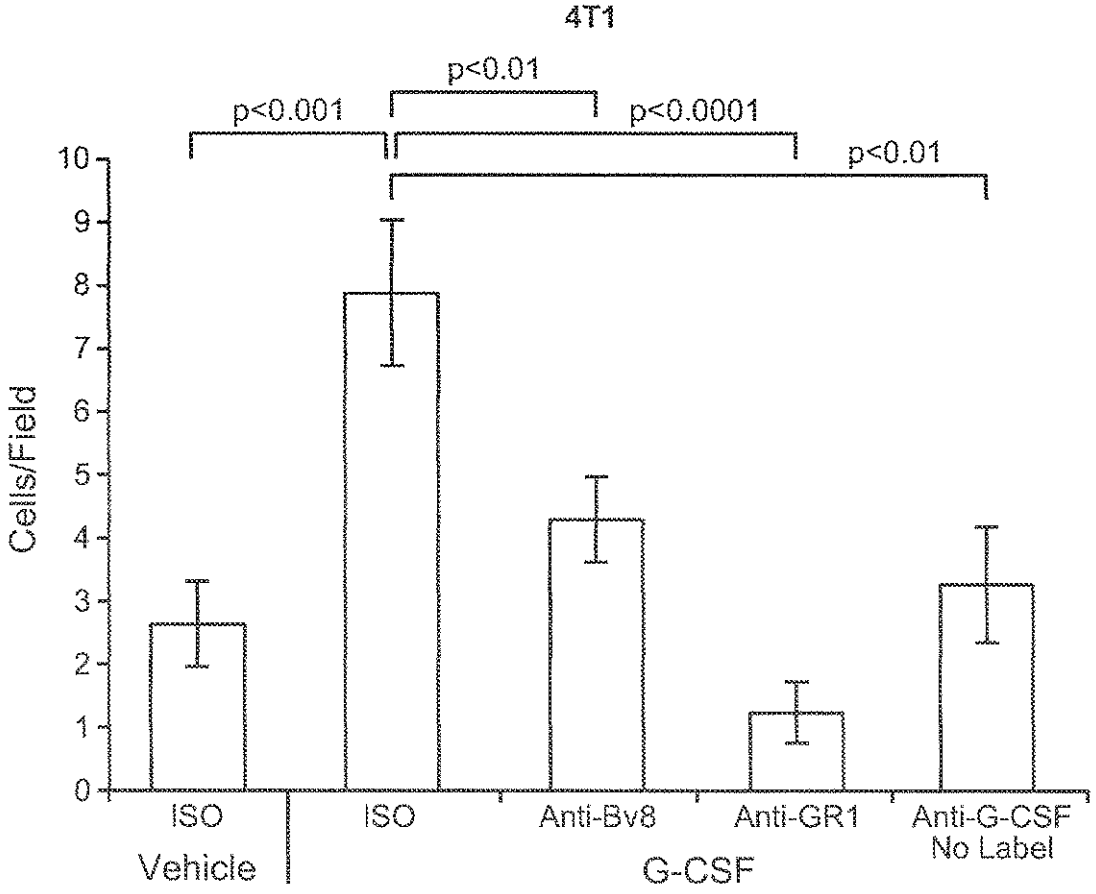

4T1
p<0.01
p<0.001
p<0.0001
p<0.01
Cells/Field
0
1
2
3
4
5
6
7
8
9
10
ISO
Vehicle
ISO
Anti-Bv8
Anti-GR1
Anti-G-CSF
No Label
G-CSF

FIG. 4D

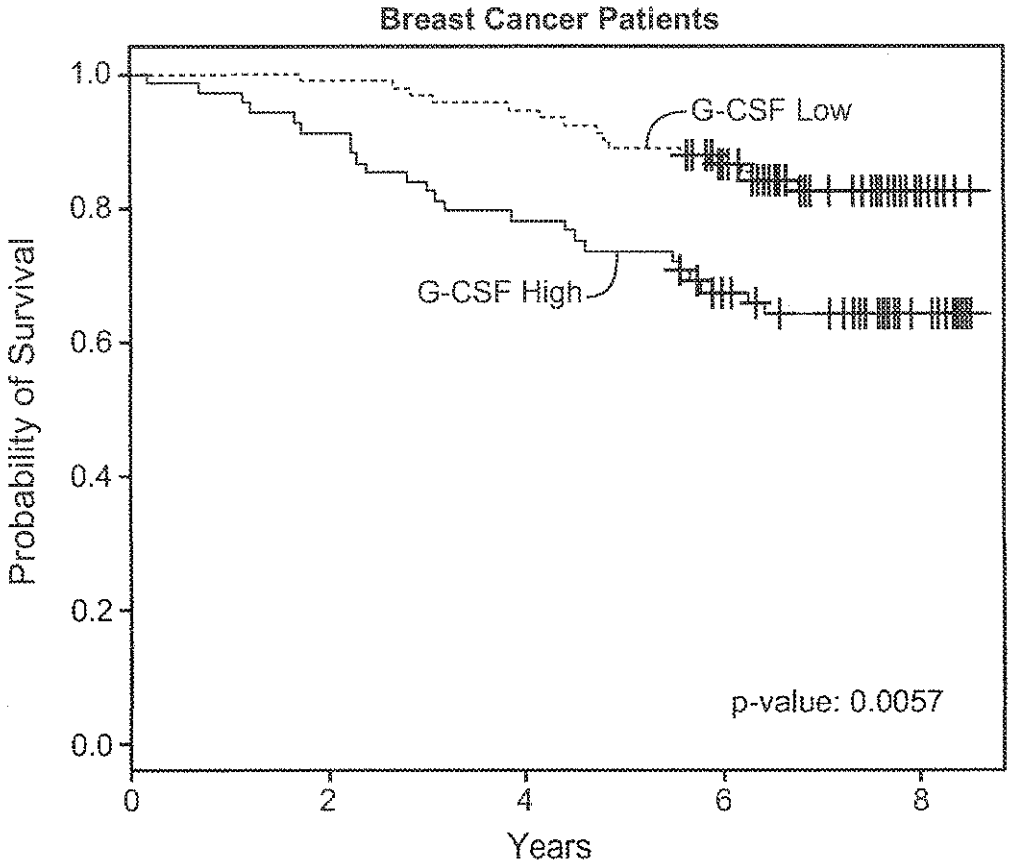

Breast Cancer Patients
Probability of Survival
1.0
0.8
0.6
0.4
0.2
0.0
G-CSF Low
G-CSF High
p-value: 0.0057
0
2
4
6
8
Years

FIG. 4E

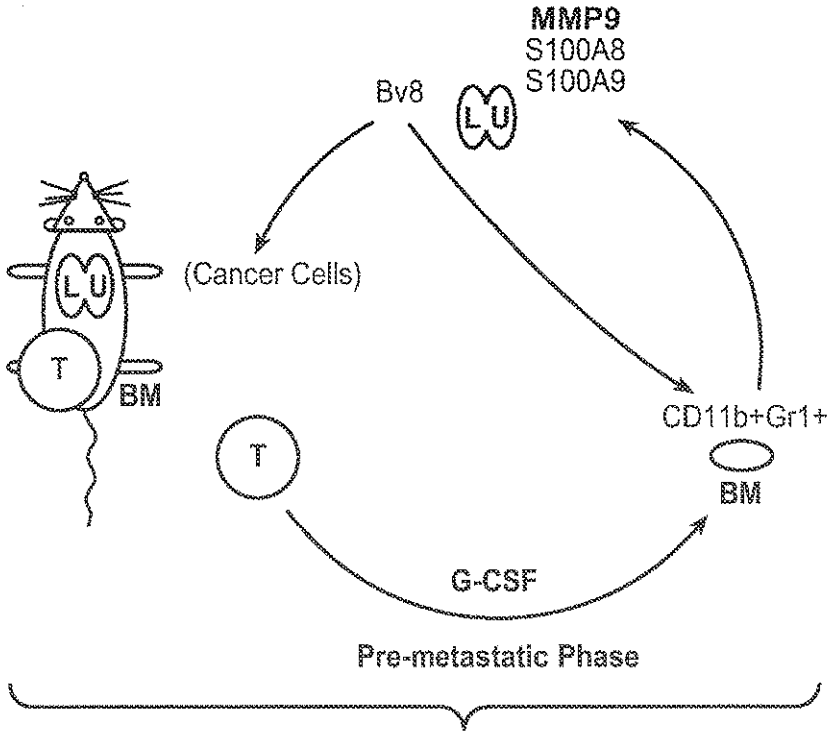

MMP9
S100A8
S100A9
Bv8
LU
(Cancer Cells)
LU
T
BM
T
CD11b+Gr1+
BM
G-CSF
Pre-metastatic Phase

FIG. 4F

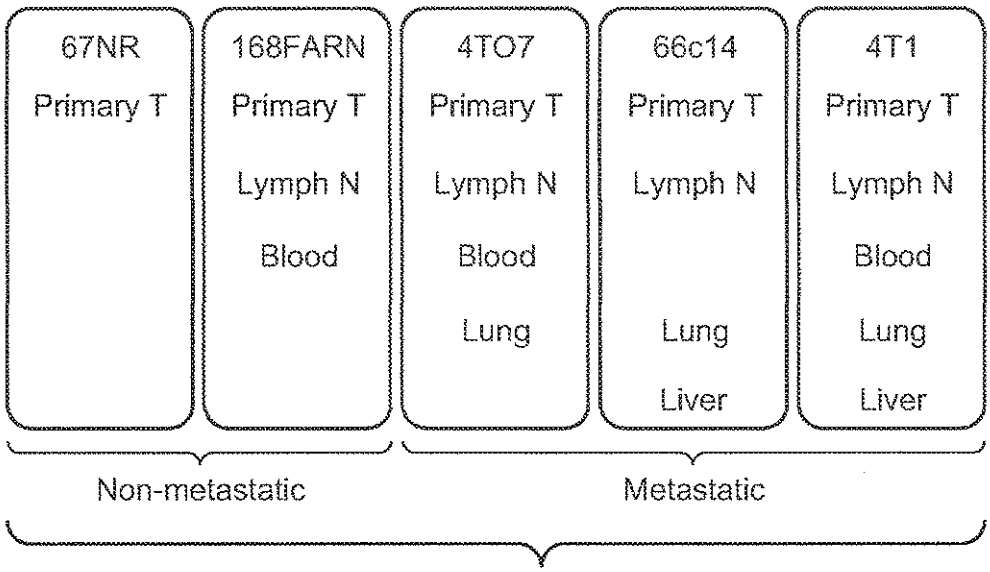

67NR
Primary T
168FARN
Primary T
Lymph N
Blood
4TO7
Primary T
Lymph N
Blood
Lung
66c14
Primary T
Lymph N
Lung
Liver
4T1
Primary T
Lymph N
Blood
Lung
Liver
Non-metastatic
Metastatic

FIG. 5A

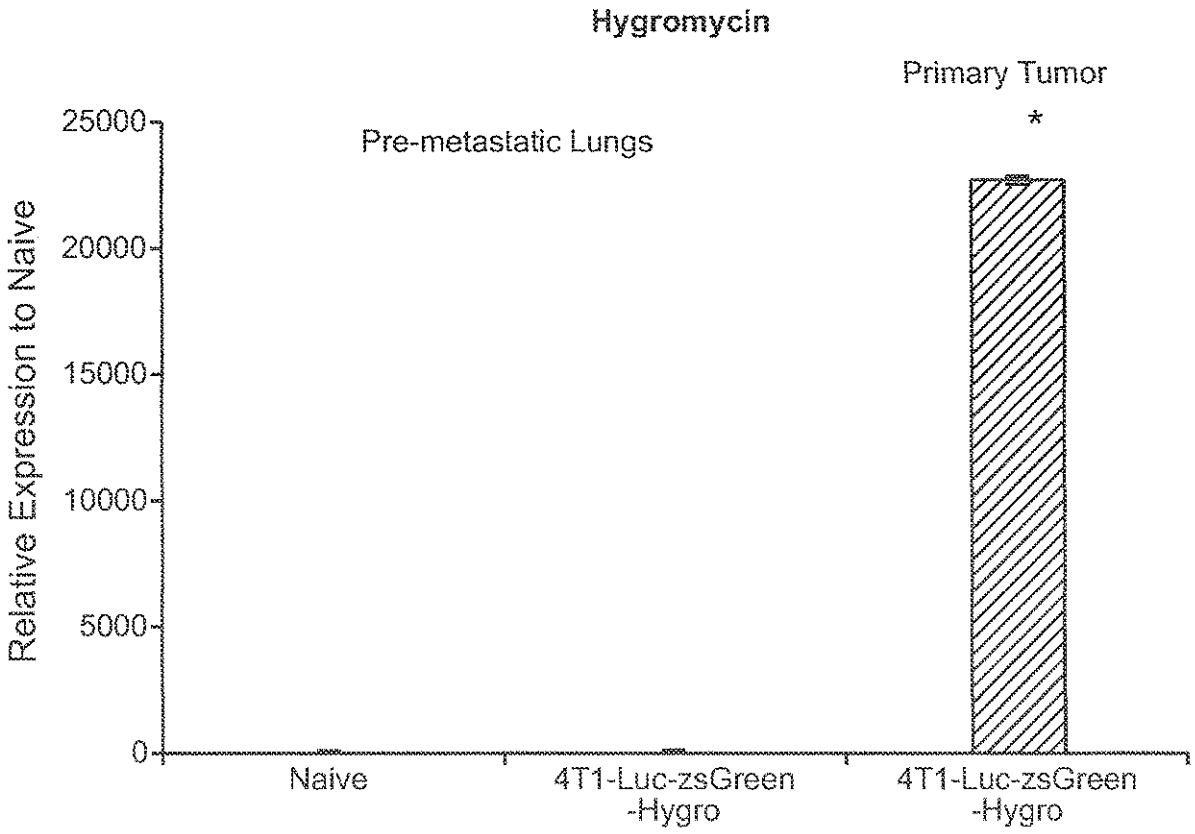

Hygromycin
Primary Tumor
*
Pre-metastatic Lungs
Relative Expression to Naive
25000
20000
15000
10000
5000
0
Naive
4T1-Luc-zsGreen
-Hygro
4T1-Luc-zsGreen
-Hygro

FIG. 5B

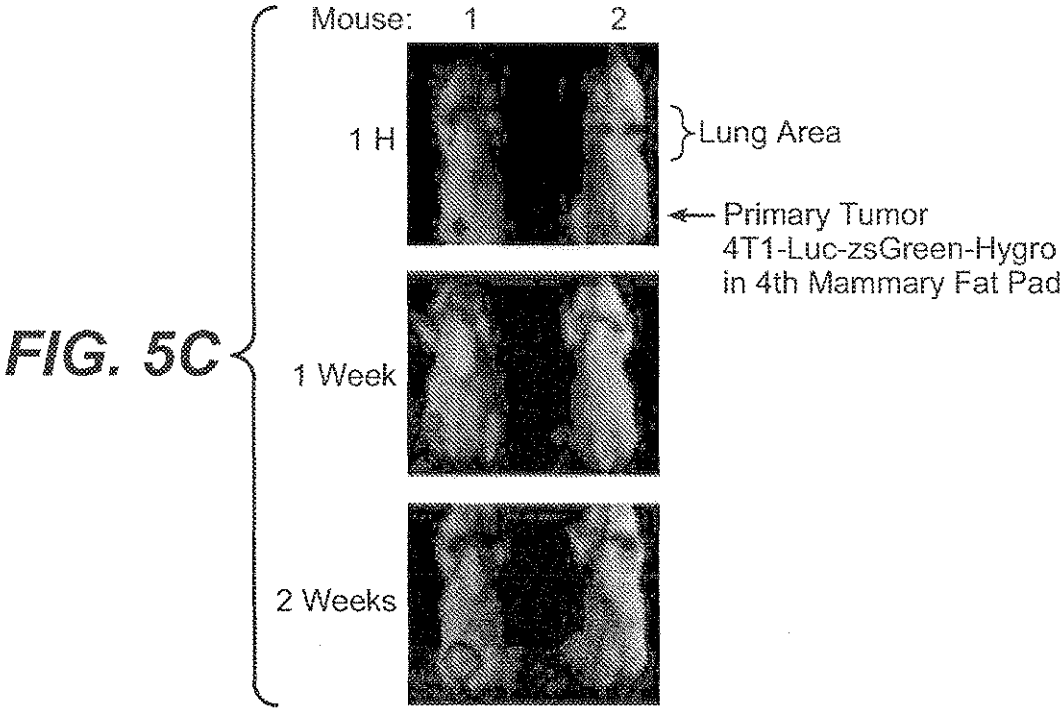

Mouse: 1 2
1 H
Lung Area
Primary Tumor
4T1-Luc-zsGreen-Hygro
in 4th Mammary Fat Pad
1 Week
2 Weeks

FIG. 5C

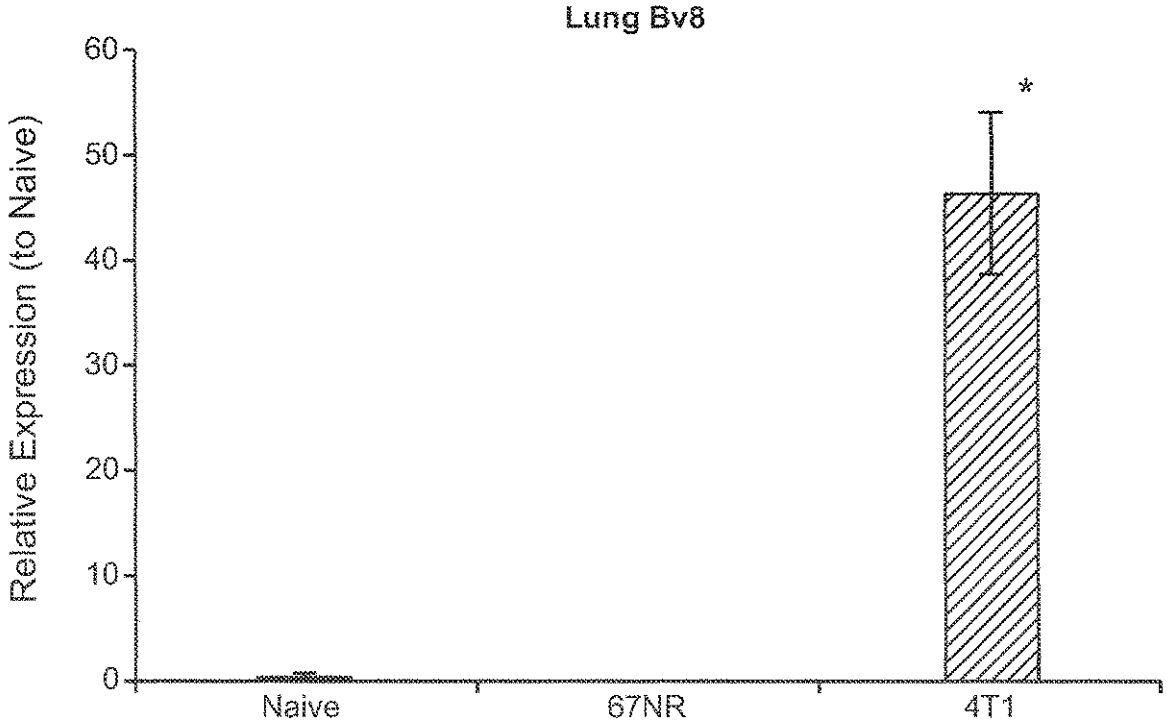

Lung Bv8
Relative Expression (to Naive)
60
50
40
30
20
10
0
*
Naive
67NR
4T1

FIG. 5D

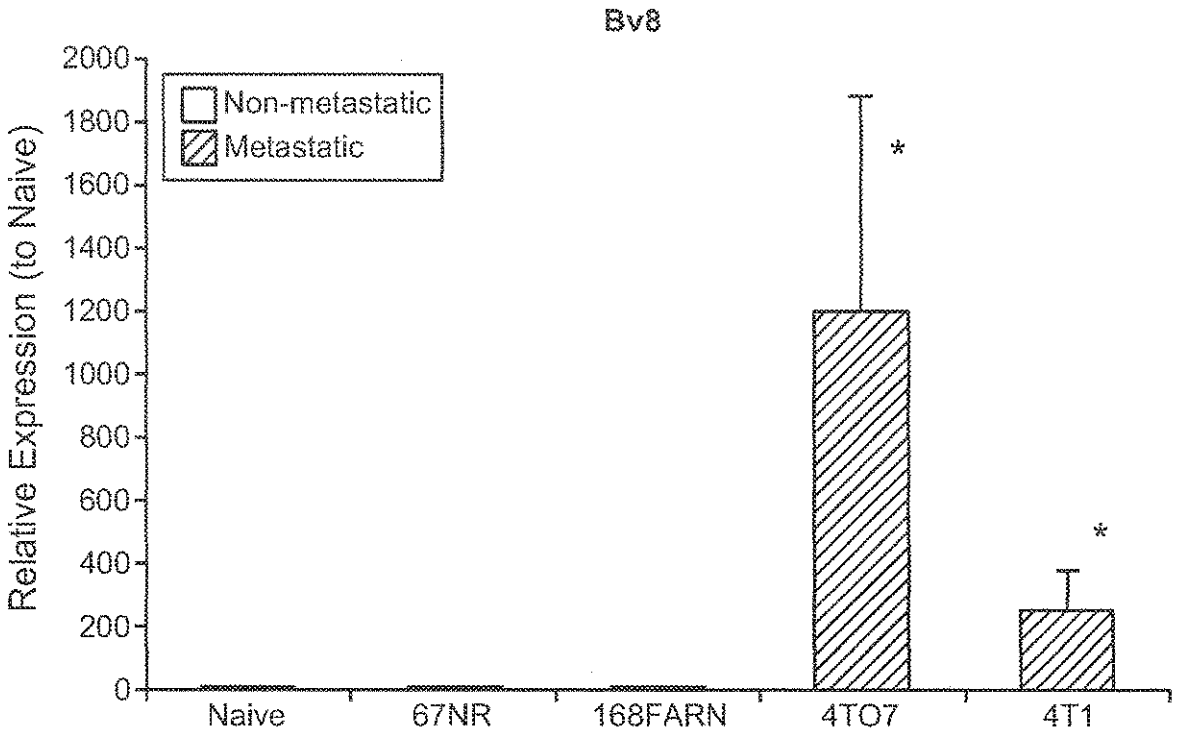

Bv8
Relative Expression (to Naive)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Non-metastatic
Metastatic
*
*
Naive
67NR
168FARN
4TO7
4T1

FIG. 5E

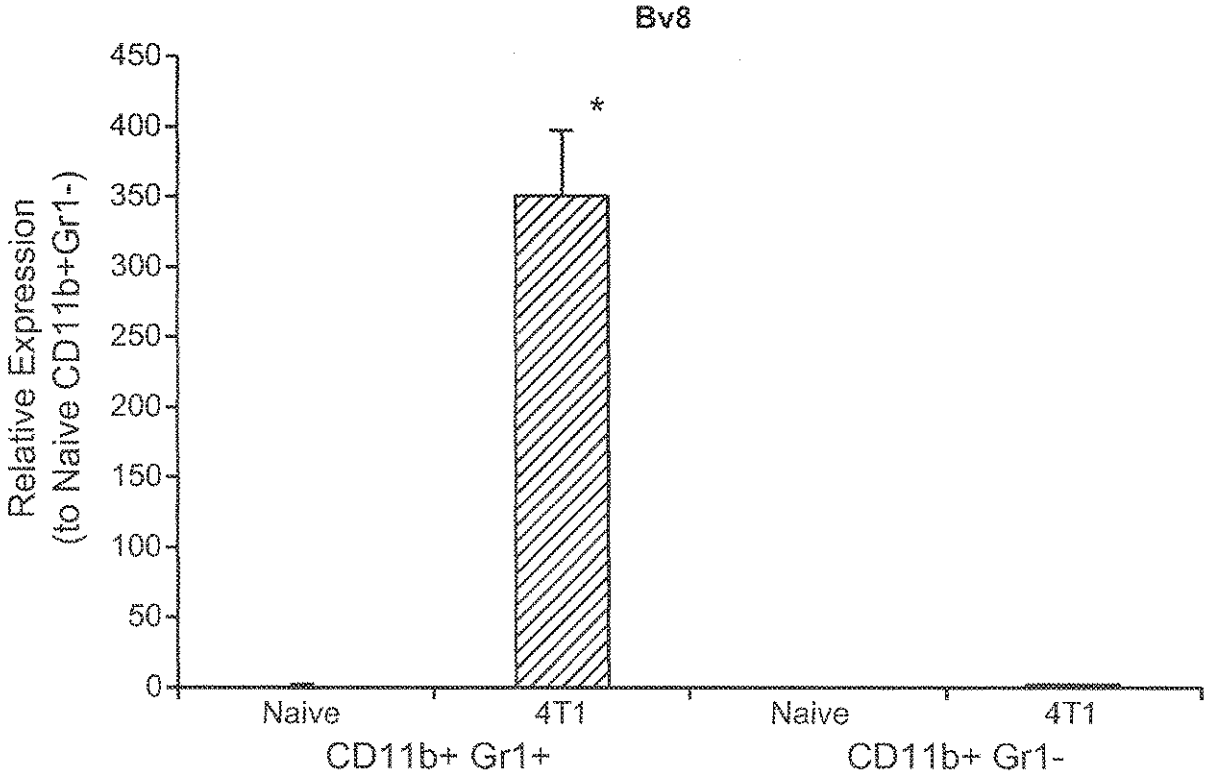

Bv8
Relative Expression
(to Naive CD11b+Gr1-)
450
400
350
300
250
200
150
100
50
0
*
Naive
4T1
Naive
4T1
CD11b+ Gr1+
CD11b+ Gr1-

FIG. 5F

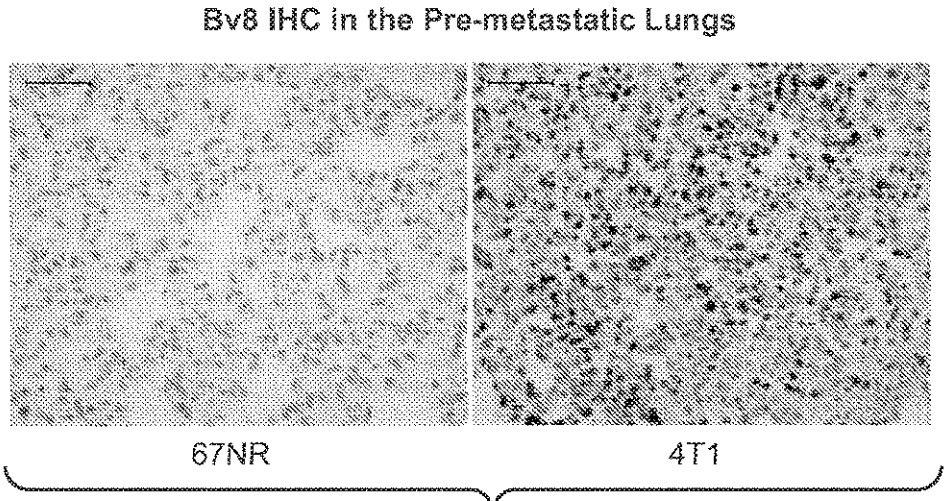

Bv8 IHC in the Pre-metastatic Lungs
67NR
4T1

FIG. 5G

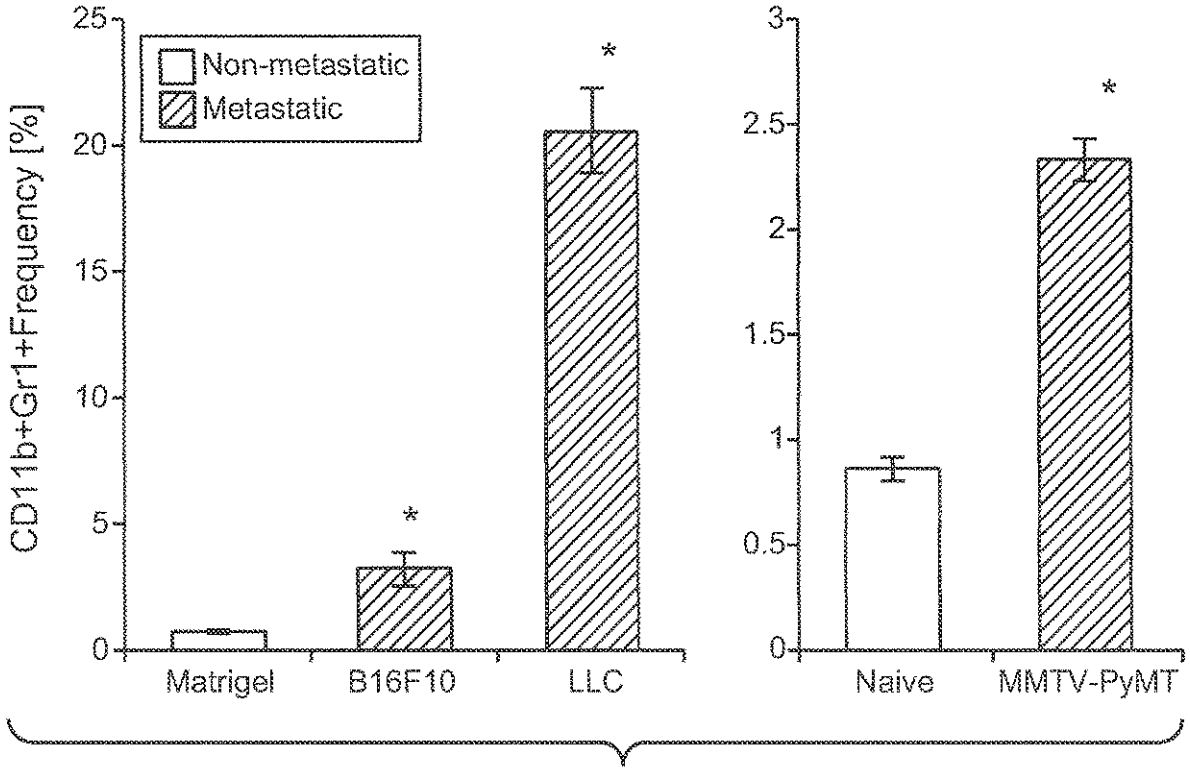

CD11b+Gr1+Frequency [%]
Non-metastatic
Metastatic
25
20
15
10
5
0
*
*
Matrigel
B16F10
LLC
3
2.5
2
1.5
1
0.5
0
*
Naive
MMTV-PyMT

FIG. 5H

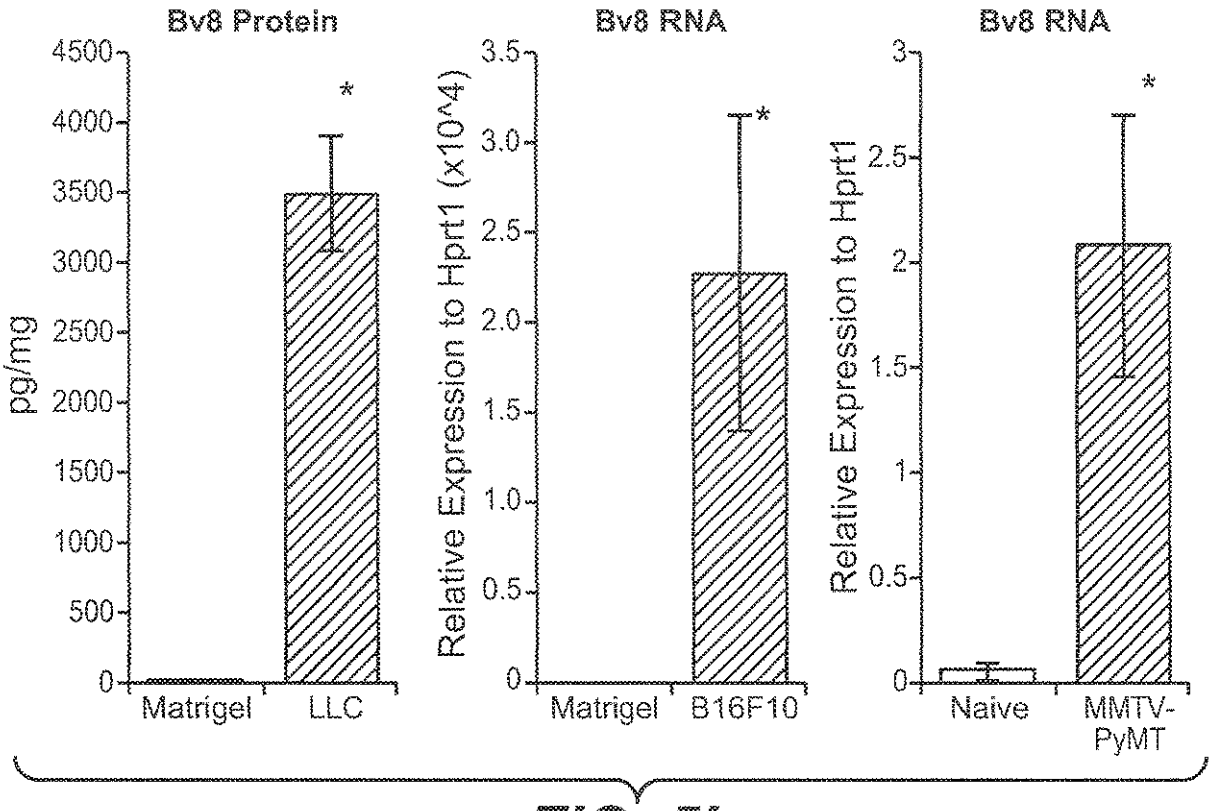

Bv8 Protein
pg/mg
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Matrigel
LLC
Bv8 RNA
Relative Expression to Hprt1 (x10^4)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Matrigel
B16F10
Bv8 RNA
Relative Expression to Hprt1
3
2.5
2
1.5
1
0.5
0
Naive
MMTV-PyMT

FIG. 5I

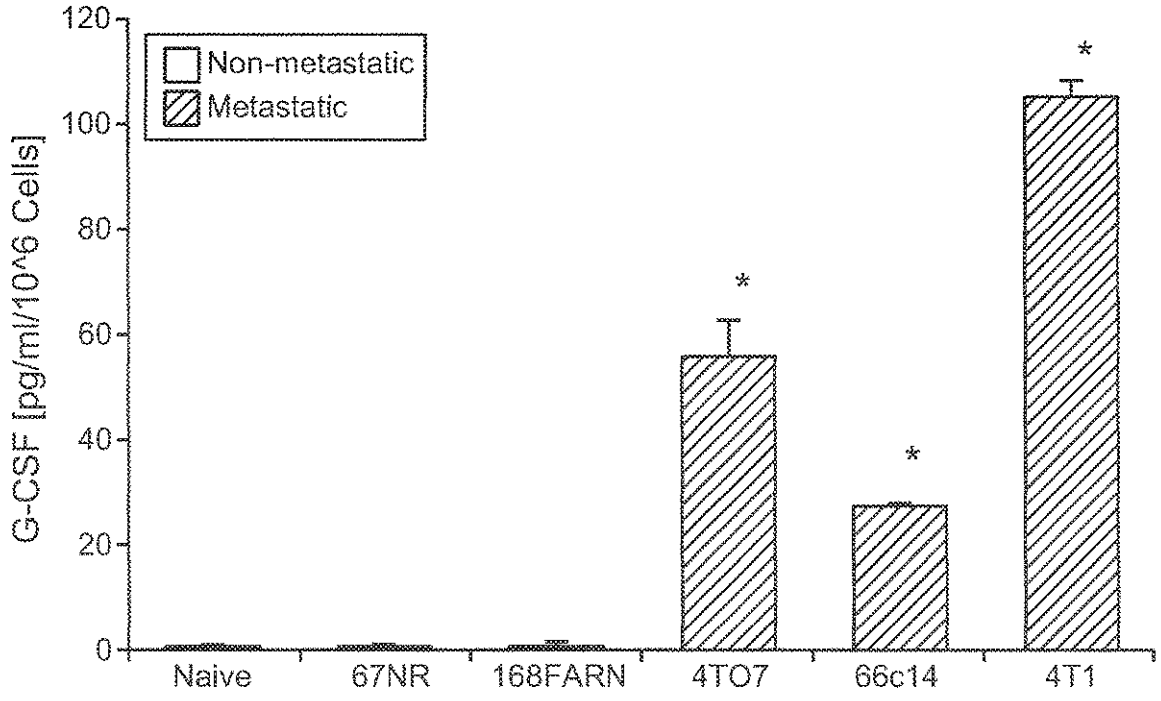

Non-metastatic
Metastatic
G-CSF [pg/ml/10^6 Cells]
120
100
80
60
40
20
0
Naive
67NR
168FARN
4TO7
66c14
4T1

FIG. 6A

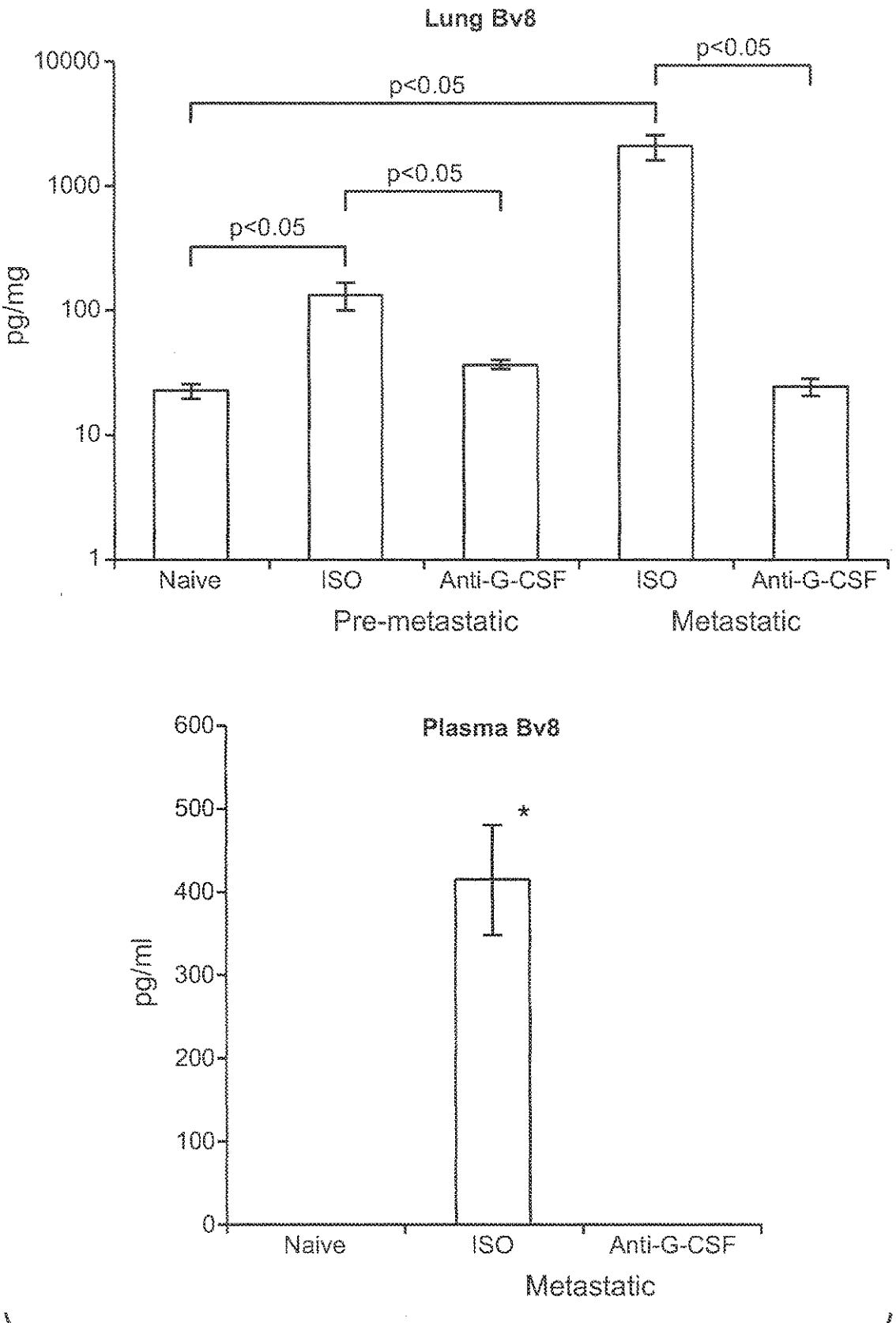

Lung Bv8
pg/mg
10000
1000
100
10
1
p<0.05
p<0.05
p<0.05
Naive
ISO
Anti-G-CSF
ISO
Anti-G-CSF
Pre-metastatic
Metastatic
Plasma Bv8
pg/ml
600
500
400
300
200
100
0
*
Naive
ISO
Anti-G-CSF
Metastatic

FIG. 6B

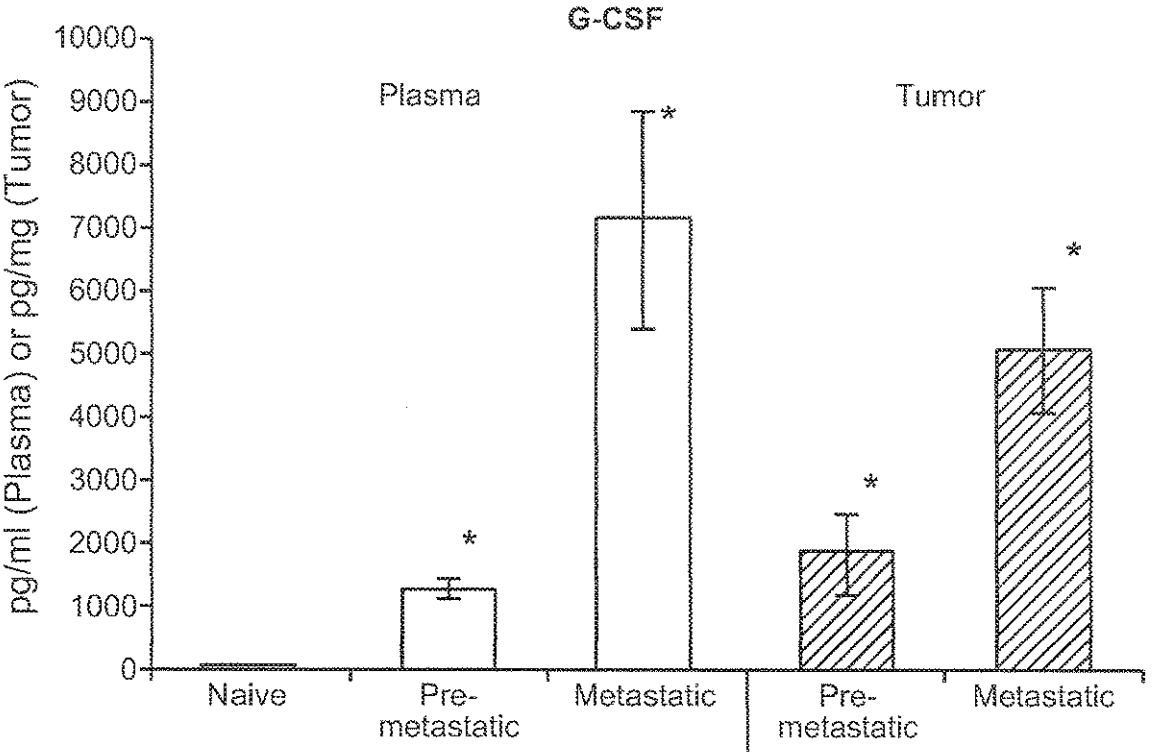

G-CSF
Plasma
Tumor
pg/ml (Plasma) or pg/mg (Tumor)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
*
Naive
Pre-metastatic
Metastatic
Pre-metastatic
Metastatic

FIG. 6C

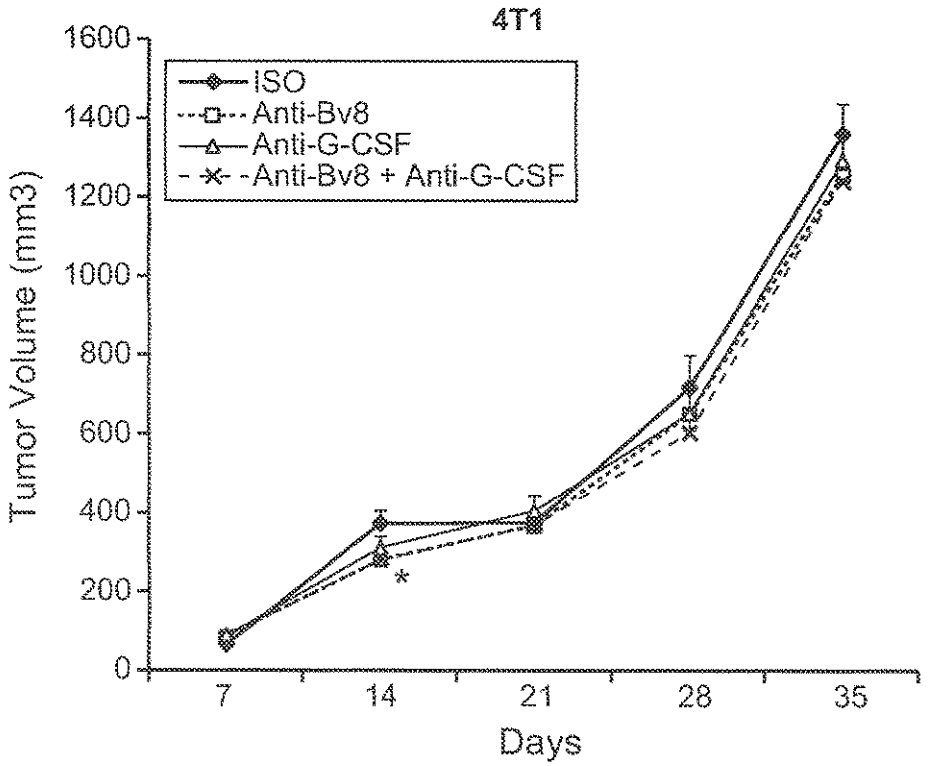

4T1
ISO
Anti-Bv8
Anti-G-CSF
Anti-Bv8 + Anti-G-CSF
Tumor Volume (mm3)
1600
1400
1200
1000
800
600
400
200
0
*
7
14
21
28
35
Days

FIG. 6D

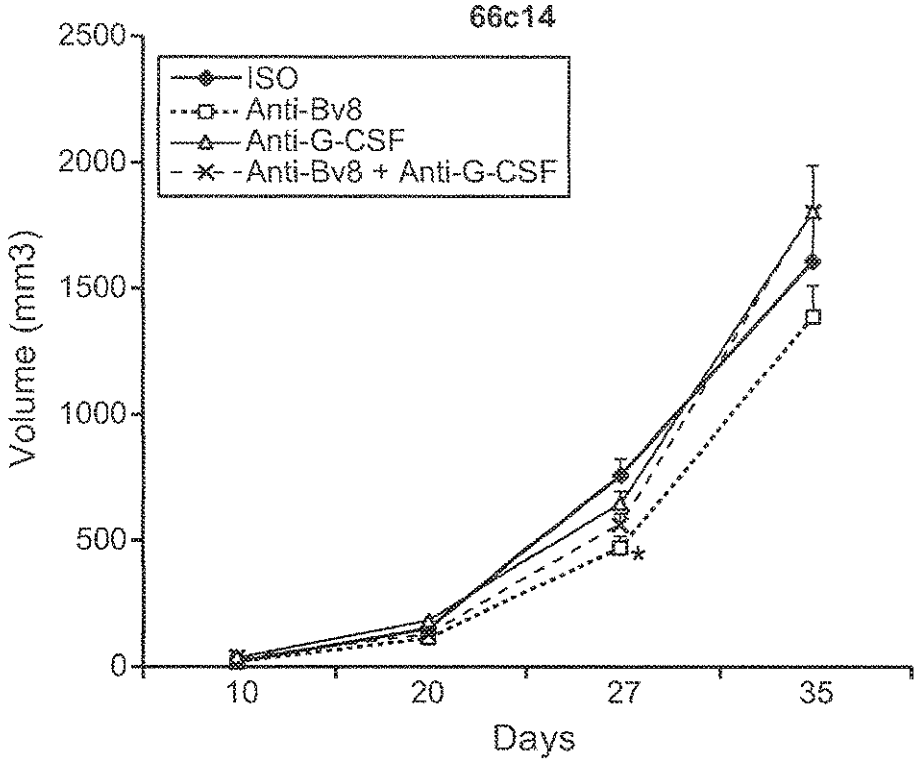

66c14
2500
2000
1500
1000
500
0
Volume (mm3)
ISO
Anti-Bv8
Anti-G-CSF
Anti-Bv8 + Anti-G-CSF
*
10
20
27
35
Days

FIG. 6E

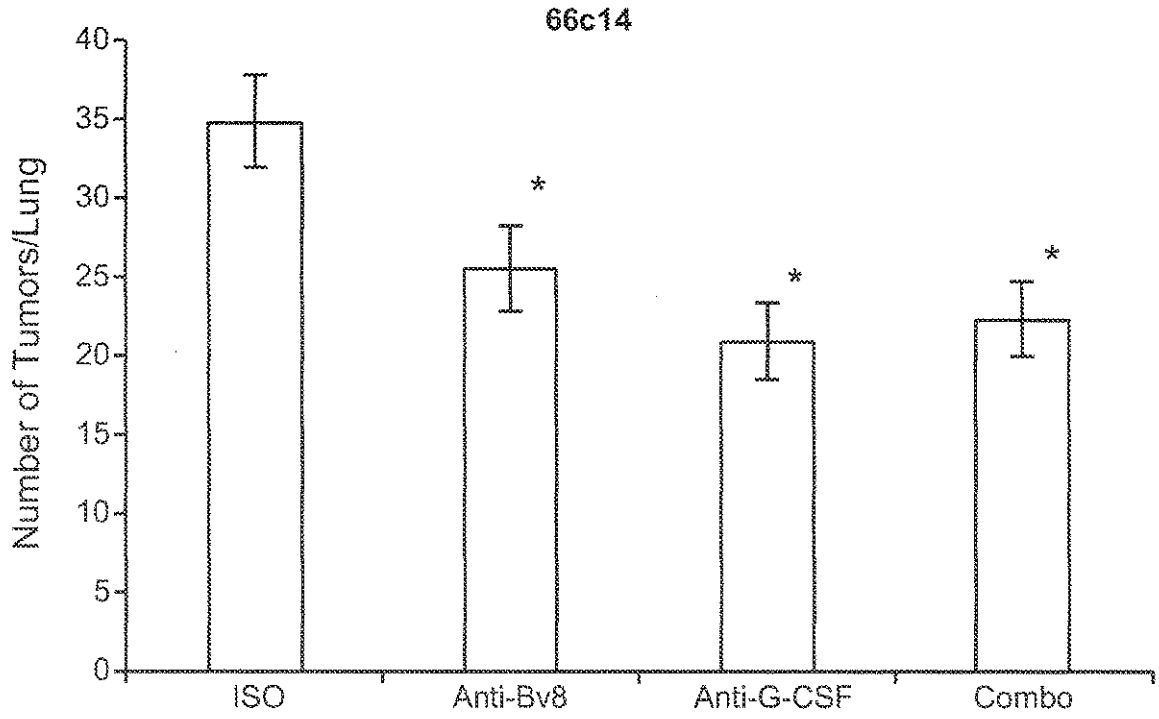

66c14
40
35
30
25
20
15
10
5
0
Number of Tumors/Lung
*
*
*
ISO
Anti-Bv8
Anti-G-CSF
Combo

FIG. 6F

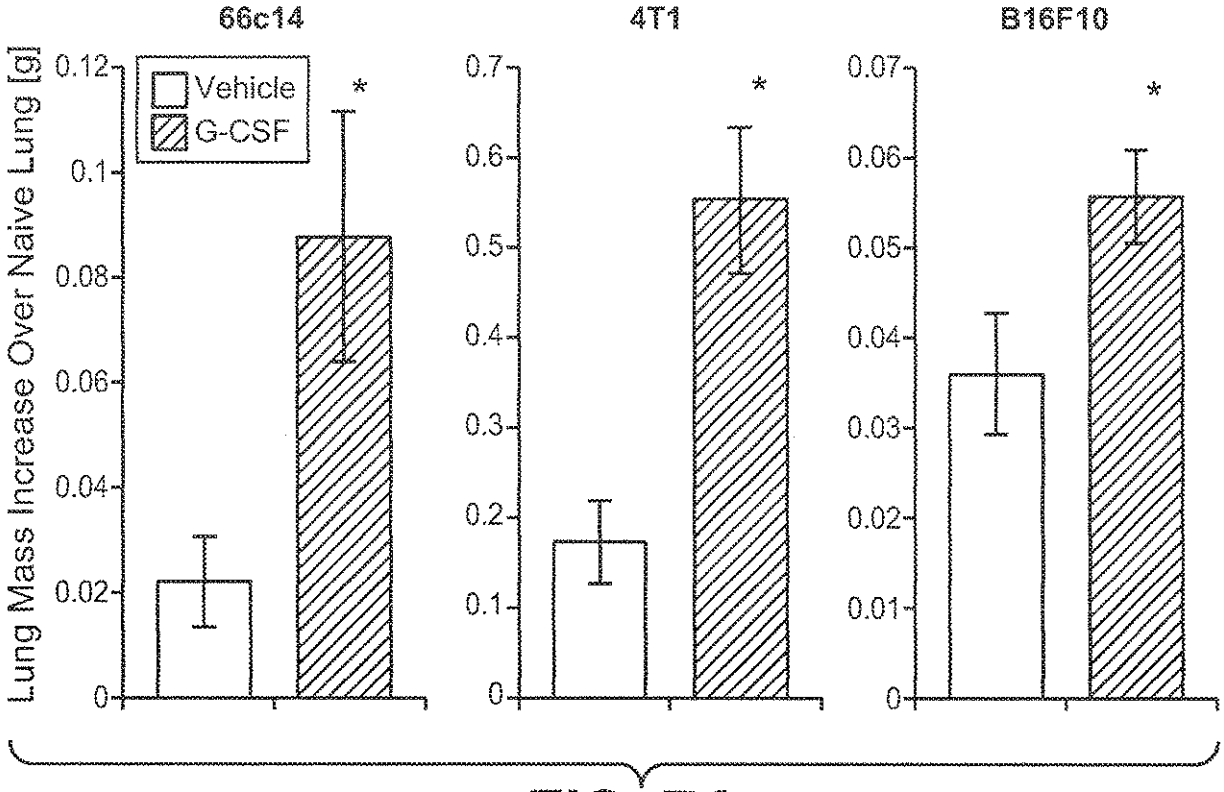

66c14
4T1
B16F10
Lung Mass Increase Over Naive Lung [g]
Vehicle
G-CSF
0.12
0.1
0.08
0.06
0.04
0.02
0
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
*
*
*

FIG. 7A

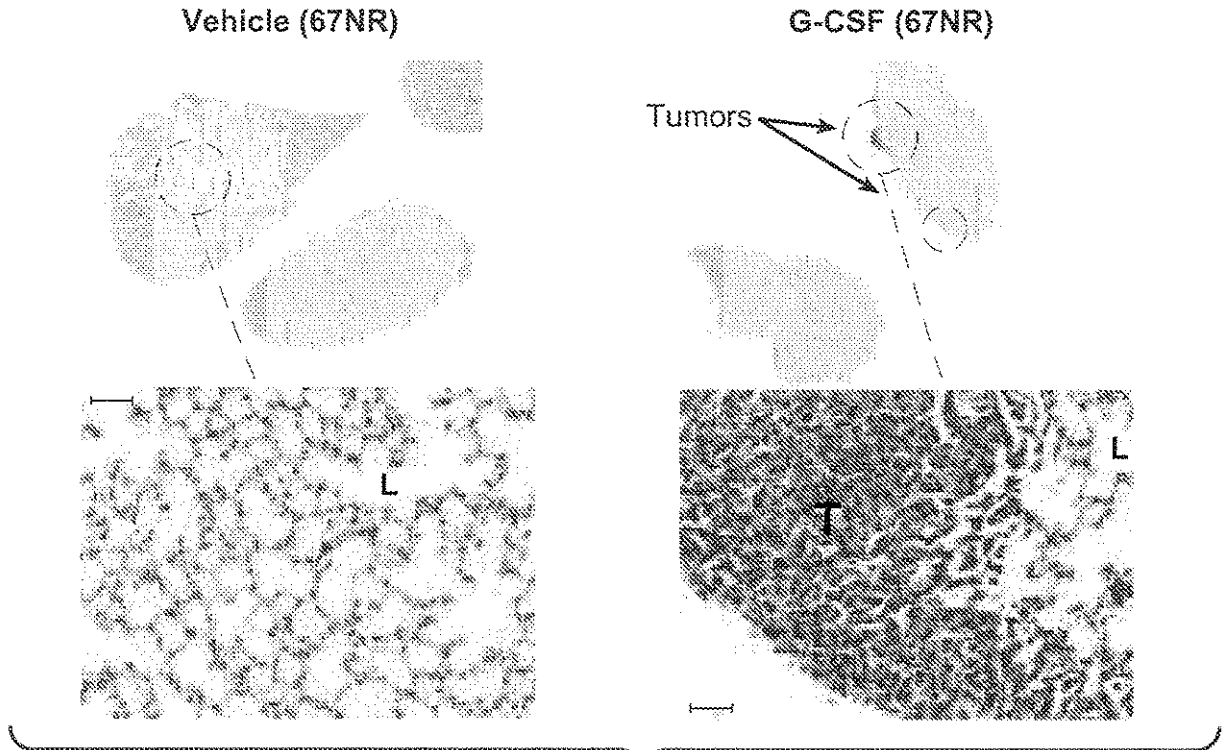

Vehicle (67NR)
G-CSF (67NR)
Tumors
L
L
T

FIG. 7B

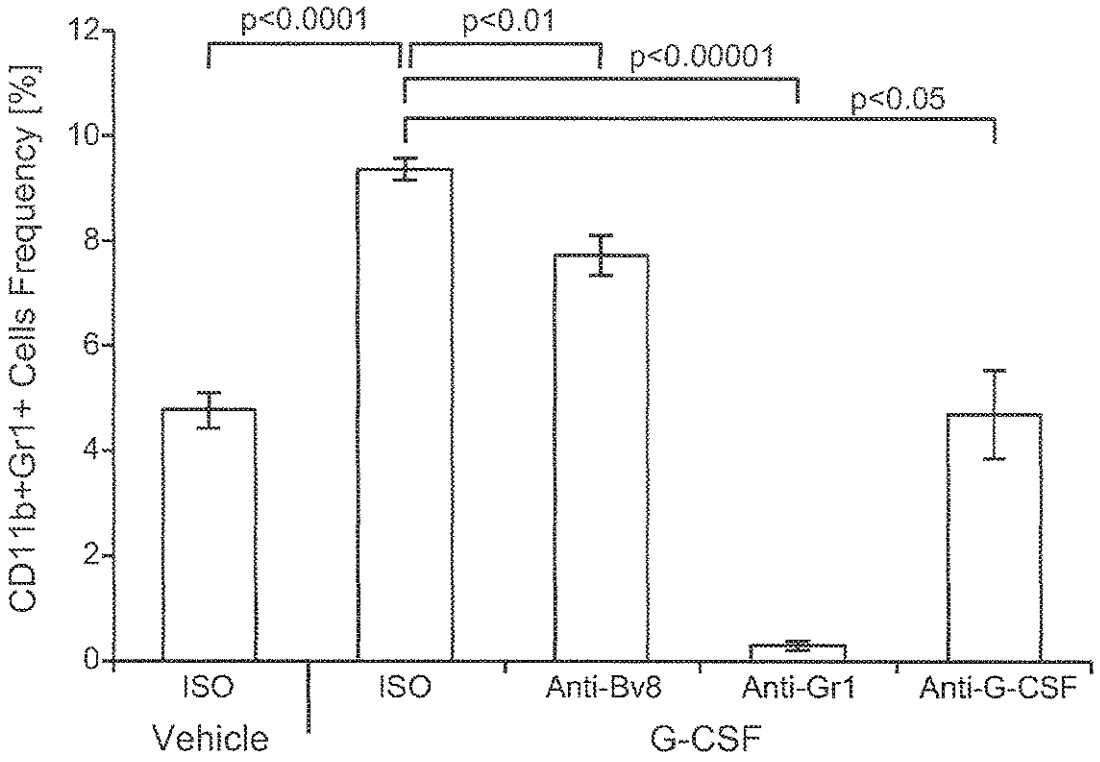

p<0.0001
p<0.01
p<0.00001
p<0.05
CD11b+Gr1+ Cells Frequency [%]
12
10
8
6
4
2
0
ISO
ISO
Anti-Bv8
Anti-Gr1
Anti-G-CSF
Vehicle
G-CSF

FIG. 7C

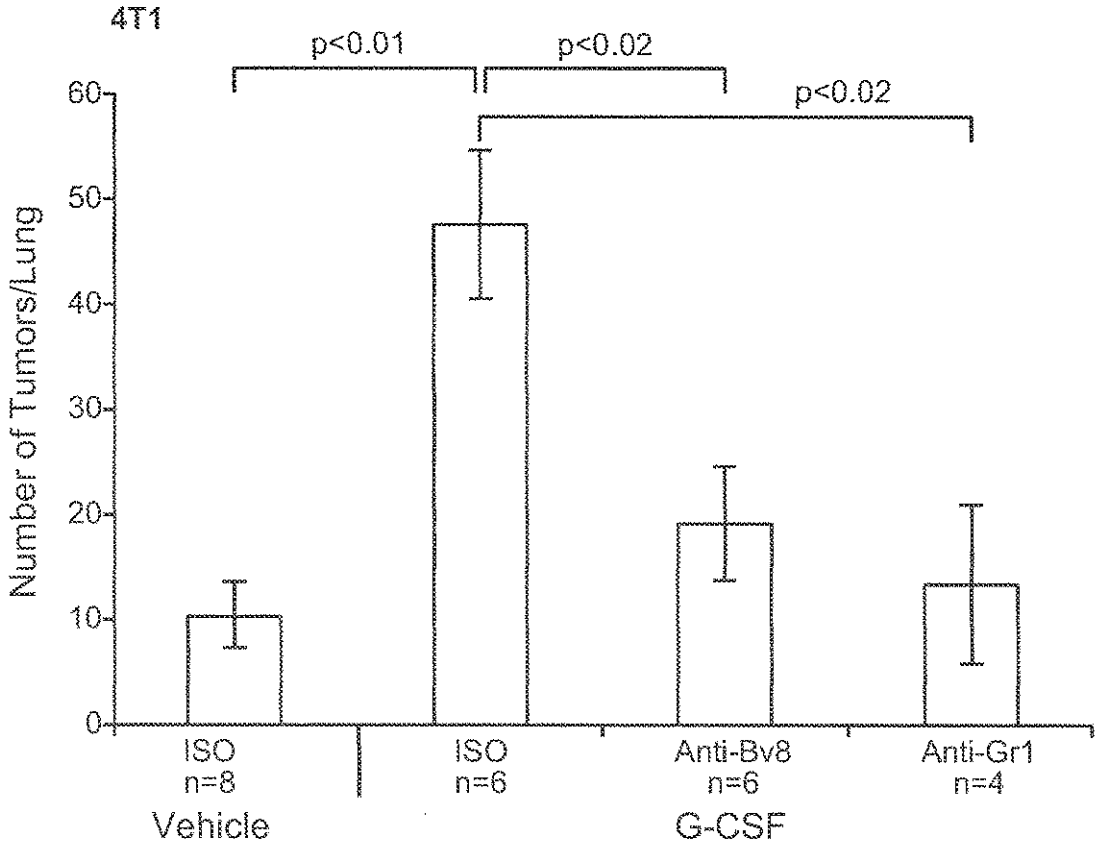

4T1
p<0.01
p<0.02
p<0.02
Number of Tumors/Lung
60
50
40
30
20
10
0
ISO
n=8
ISO
n=6
Anti-Bv8
n=6
Anti-Gr1
n=4
Vehicle
G-CSF

FIG. 7D

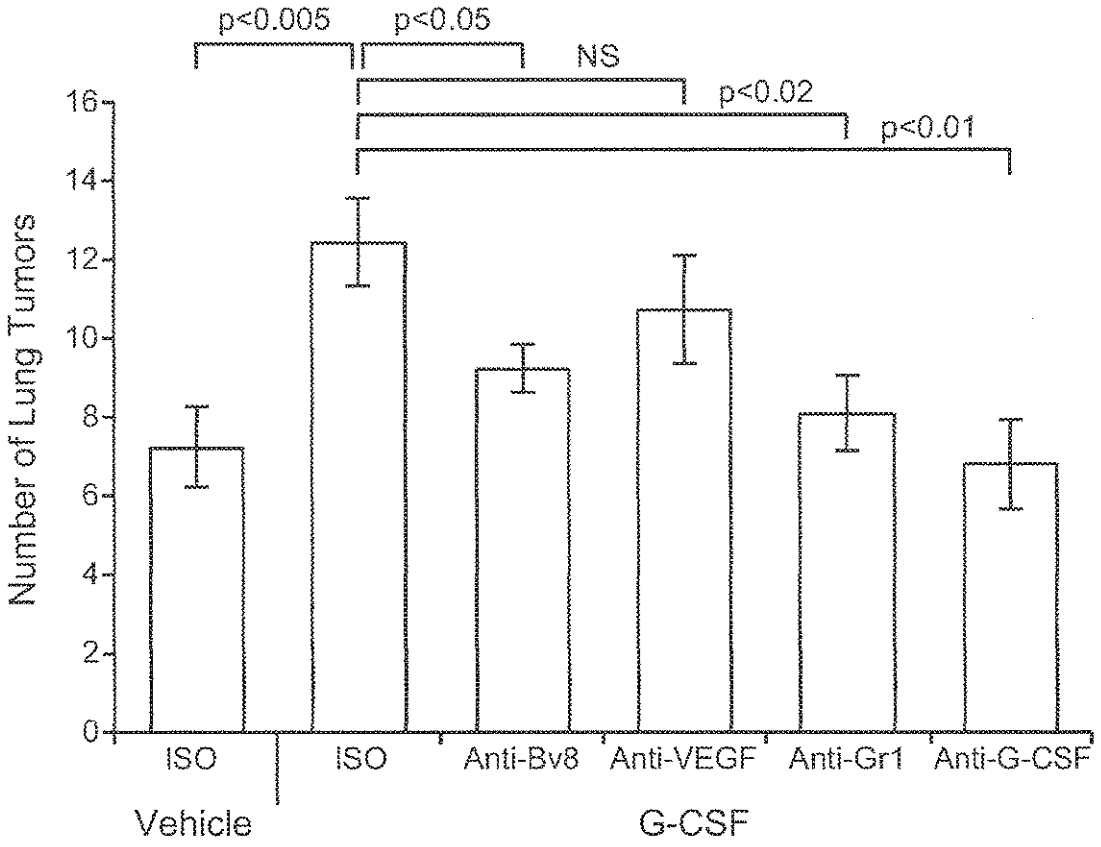

p<0.005
p<0.05
NS
p<0.02
p<0.01
Number of Lung Tumors
16
14
12
10
8
6
4
2
0
ISO
ISO
Anti-Bv8
Anti-VEGF
Anti-Gr1
Anti-G-CSF
Vehicle
G-CSF

FIG. 7E

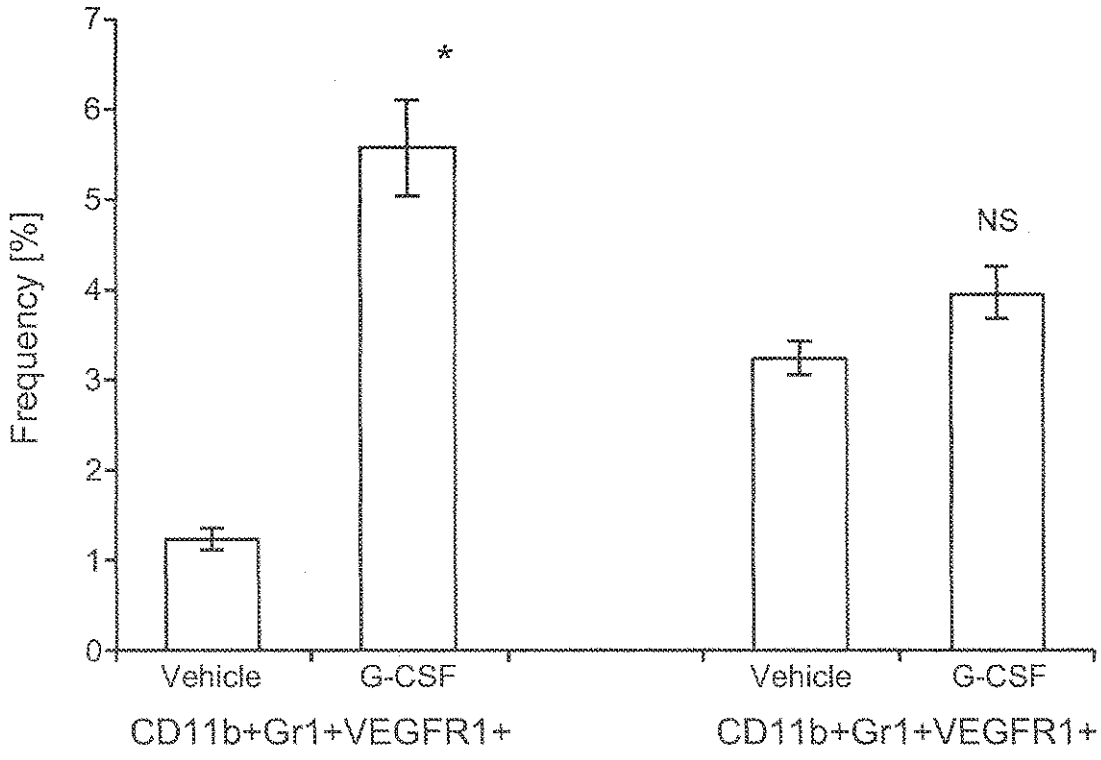

Frequency [%]
7
6
5
4
3
2
1
0
*
NS
Vehicle
G-CSF
Vehicle
G-CSF
CD11b+Gr1+VEGFR1+
CD11b+Gr1+VEGFR1+

FIG. 7F

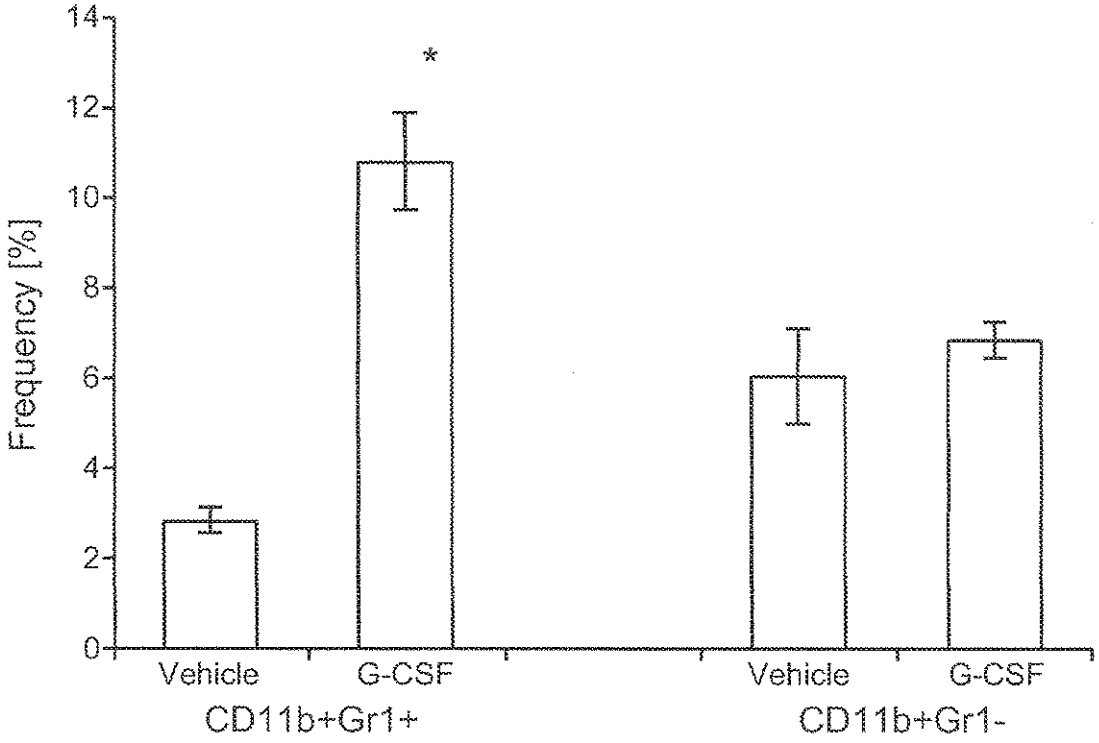

14
12
10
8
6
4
2
0
Frequency [%]
*
Vehicle
G-CSF
Vehicle
G-CSF
CD11b+Gr1+
CD11b+Gr1-

FIG. 7G

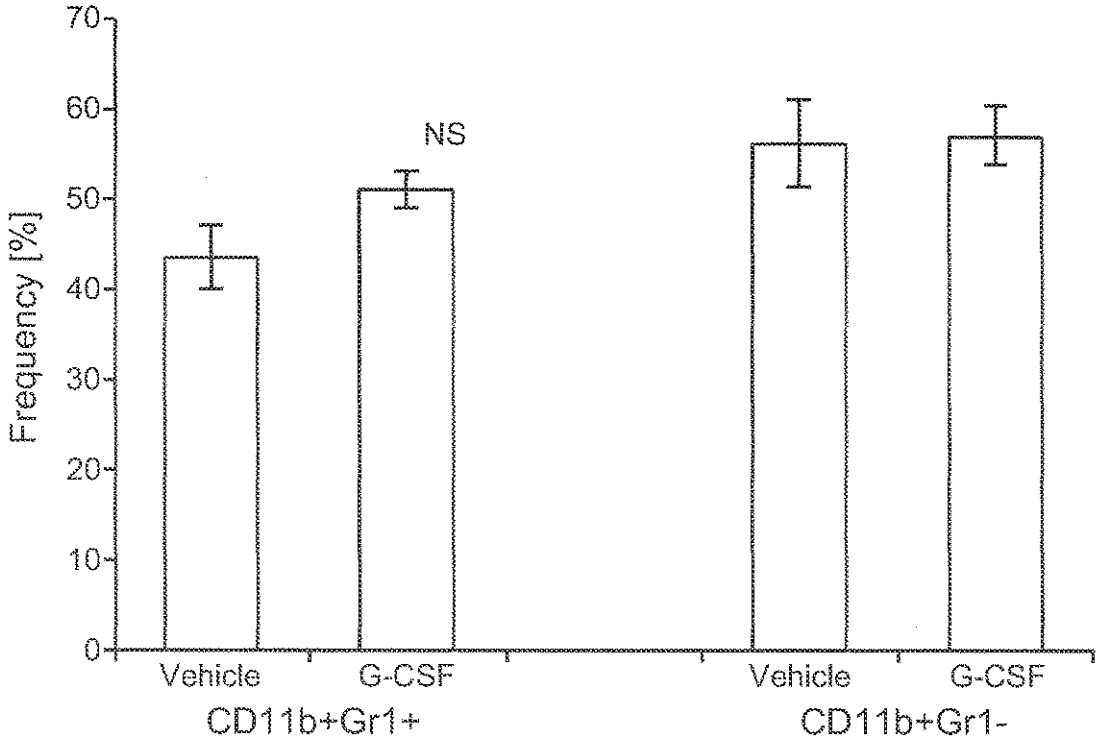

70
60
50
40
30
20
10
0
Frequency [%]
NS
Vehicle
G-CSF
Vehicle
G-CSF
CD11b+Gr1+
CD11b+Gr1-

FIG. 7H

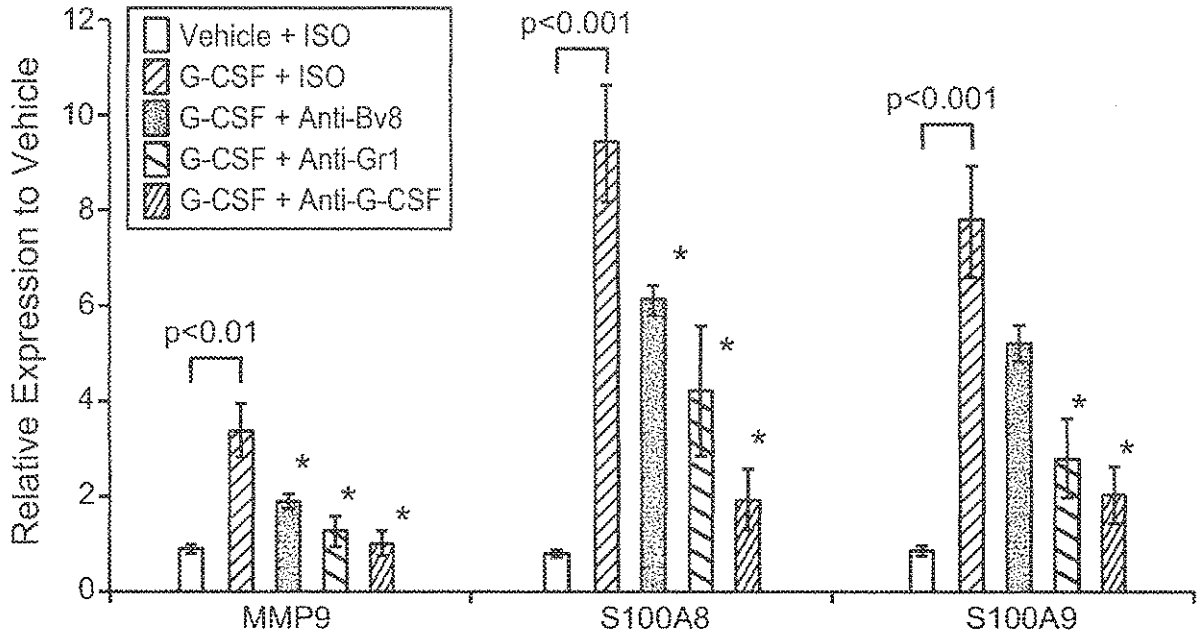

Relative Expression to Vehicle
Vehicle + ISO
G-CSF + ISO
G-CSF + Anti-Bv8
G-CSF + Anti-Gr1
G-CSF + Anti-G-CSF
p<0.01
p<0.001
p<0.001
MMP9
S100A8
S100A9

FIG. 8A

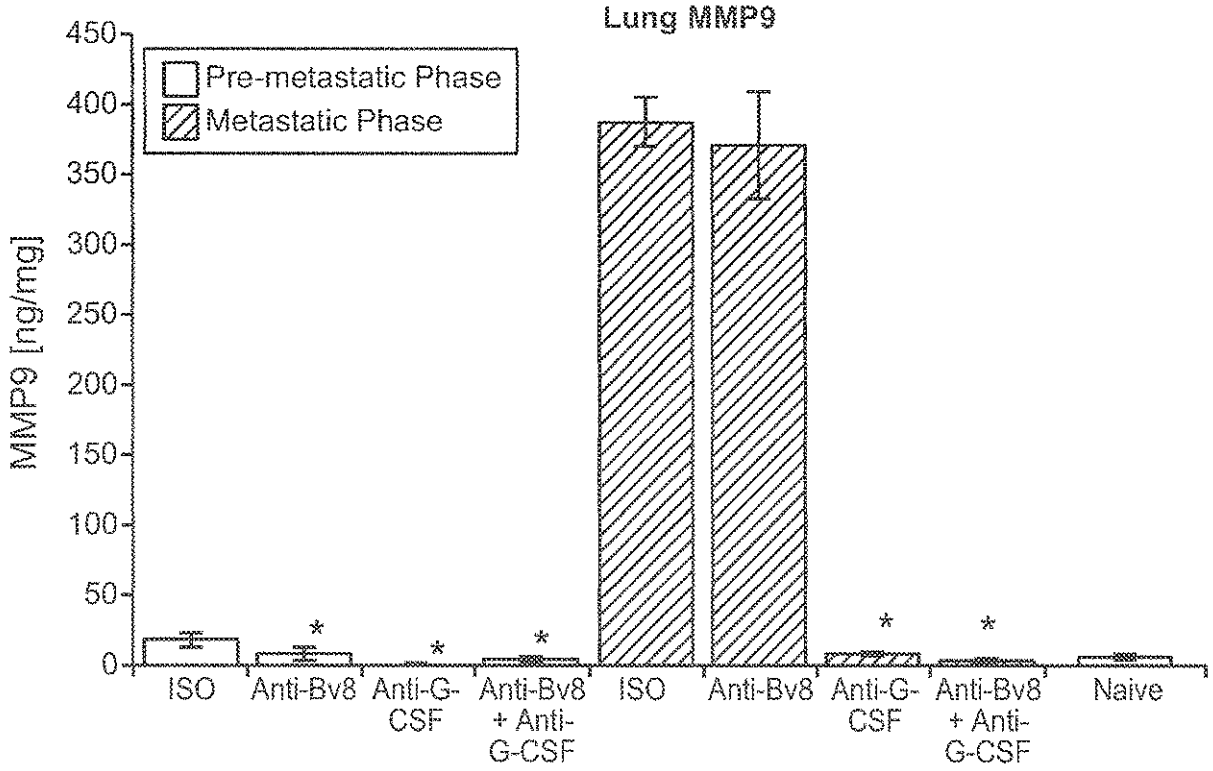

Lung MMP9
Pre-metastatic Phase
Metastatic Phase
MMP9 [ng/mg]
ISO
Anti-Bv8
Anti-G-CSF
Anti-Bv8 + Anti-G-CSF
ISO
Anti-Bv8
Anti-G-CSF
Anti-Bv8 + Anti-G-CSF
Naive

FIG. 8C

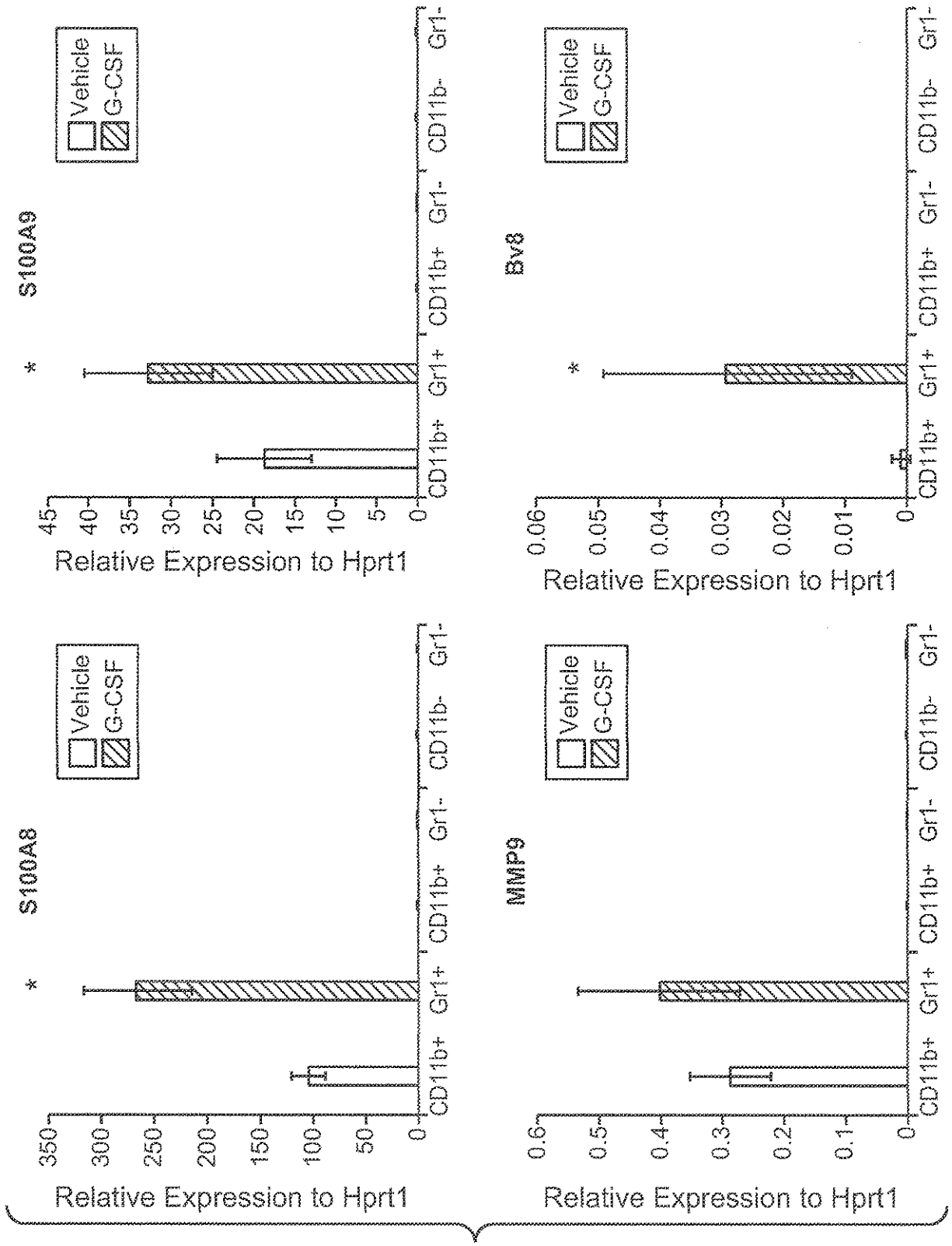

S100A9
Vehicle
G-CSF
*
45
40
35
30
25
20
15
10
5
0
CD11b+ Gr1+
CD11b+ Gr1-
CD11b- Gr1-
Relative Expression to Hprt1
Bv8
Vehicle
G-CSF
*
0.06
0.05
0.04
0.03
0.02
0.01
0
CD11b+ Gr1+
CD11b+ Gr1-
CD11b- Gr1-
Relative Expression to Hprt1
S100A8
Vehicle
G-CSF
*
350
300
250
200
150
100
50
0
CD11b+ Gr1+
CD11b+ Gr1-
CD11b- Gr1-
Relative Expression to Hprt1
MMP9
Vehicle
G-CSF
0.6
0.5
0.4
0.3
0.2
0.1
0
CD11b+ Gr1+
CD11b+ Gr1-
CD11b- Gr1-
Relative Expression to Hprt1

FIG. 8B

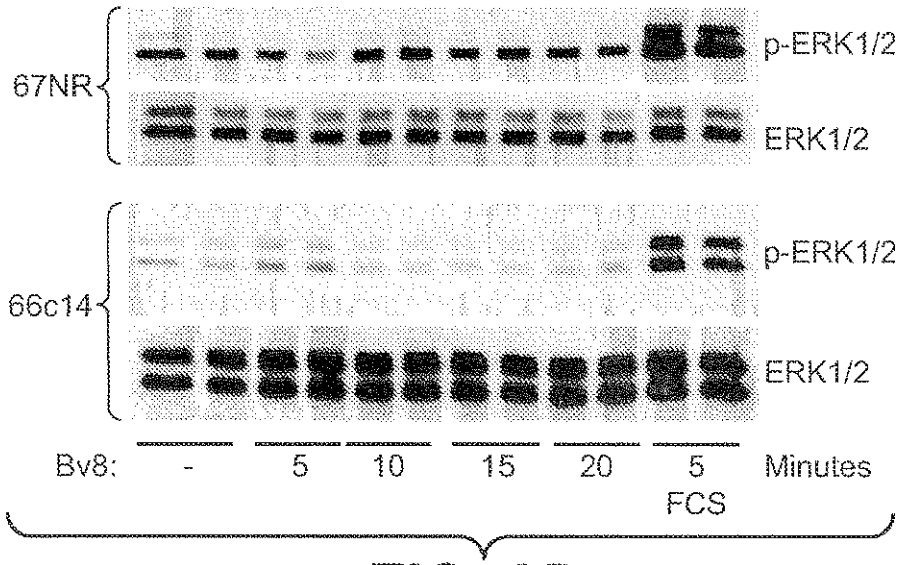

67NR
p-ERK1/2
ERK1/2
66c14
p-ERK1/2
ERK1/2
Bv8:
-
5
10
15
20
5
FCS
Minutes

FIG. 8D

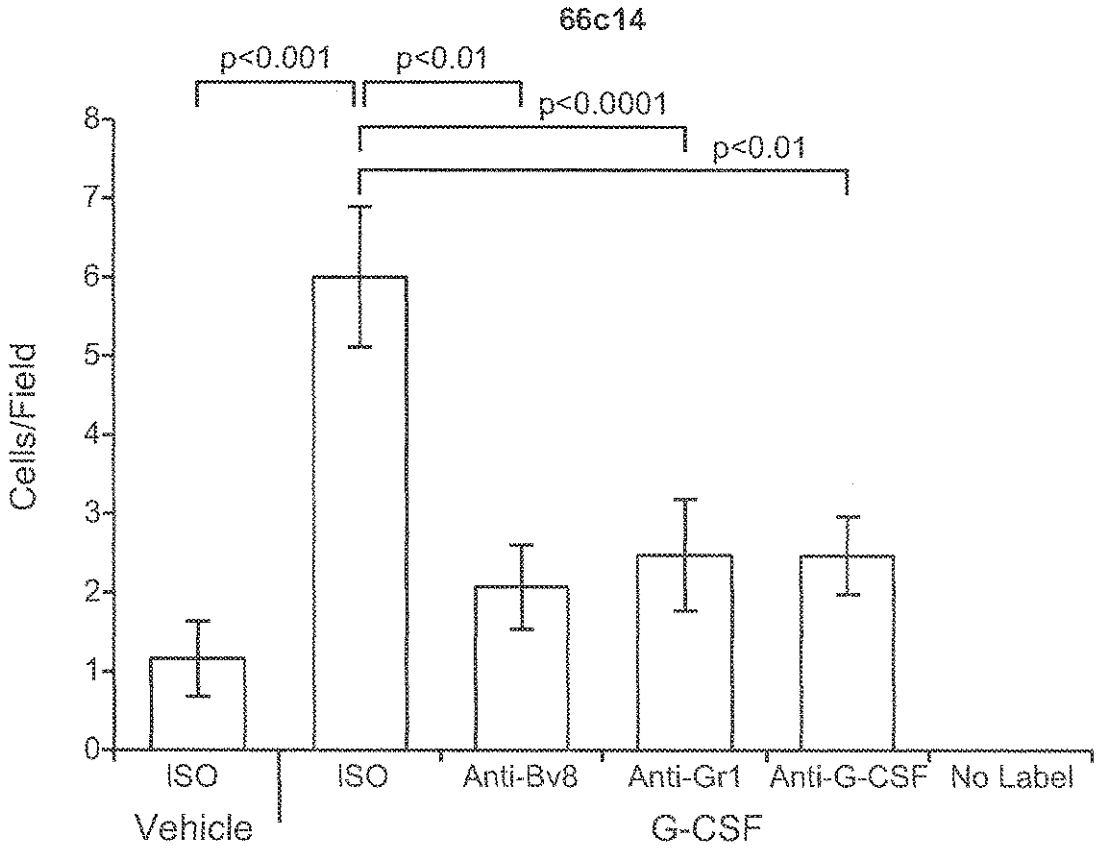

66c14
p<0.001
p<0.01
p<0.0001
p<0.01
Cells/Field
0
1
2
3
4
5
6
7
8
ISO
Vehicle
ISO
Anti-Bv8
Anti-Gr1
Anti-G-CSF
No Label
G-CSF

FIG. 8E

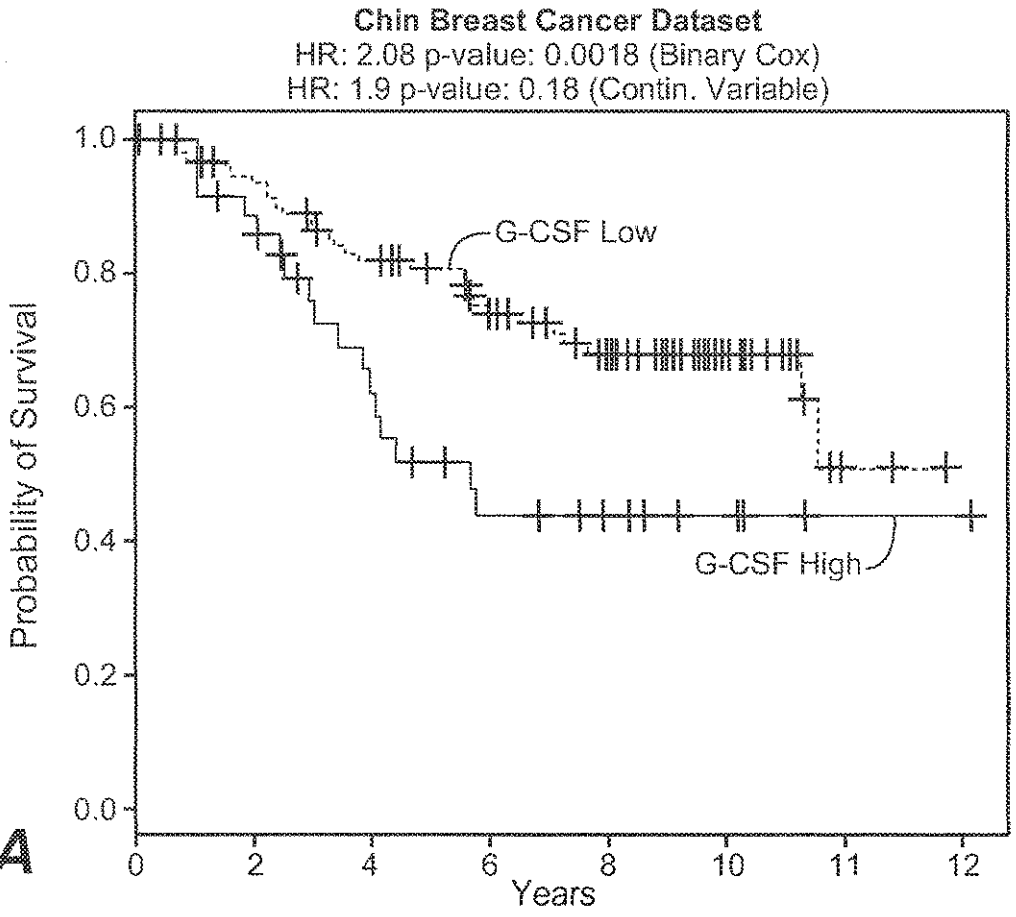

Chin Breast Cancer Dataset
HR: 2.08 p-value: 0.0018 (Binary Cox)
HR: 1.9 p-value: 0.18 (Contin. Variable)
Probability of Survival
1.0
0.8
0.6
0.4
0.2
0.0
G-CSF Low
G-CSF High
0
2
4
6
8
10
11
12
Years

FIG. 9A

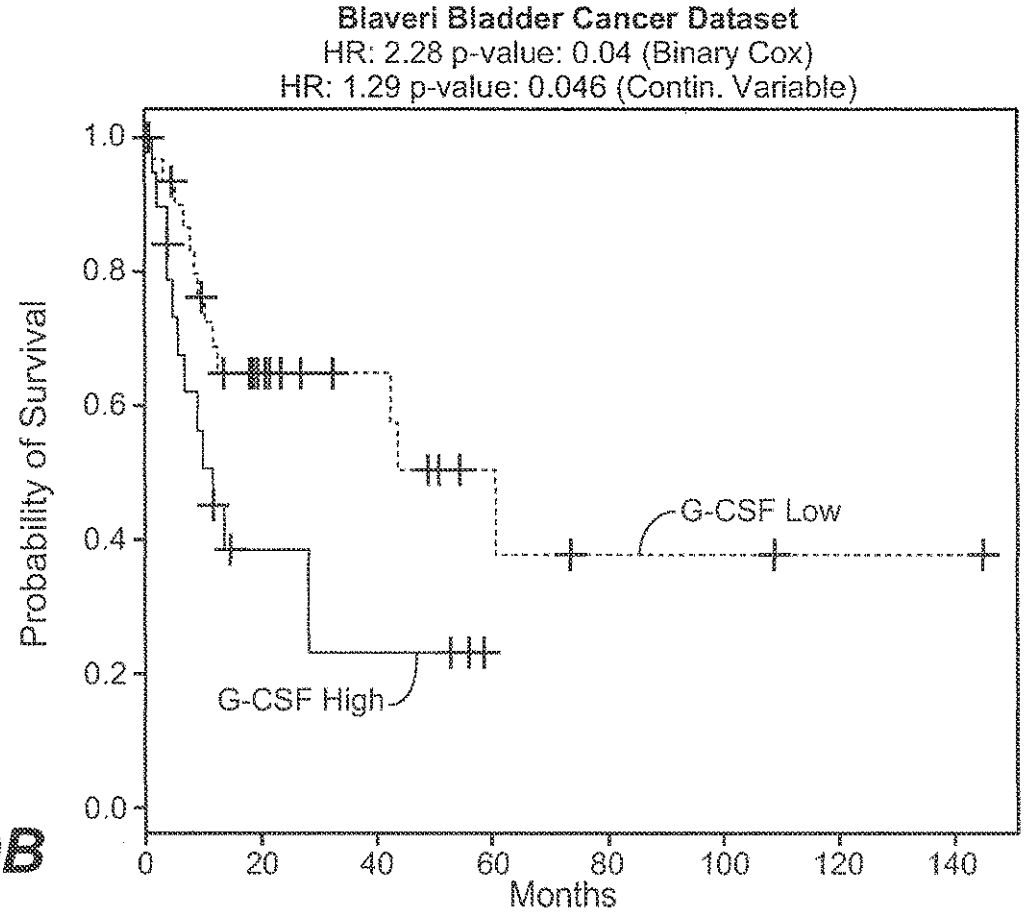

Blaveri Bladder Cancer Dataset
HR: 2.28 p-value: 0.04 (Binary Cox)
HR: 1.29 p-value: 0.046 (Contin. Variable)
Probability of Survival
1.0
0.8
0.6
0.4
0.2
0.0
G-CSF Low
G-CSF High
0
20
40
60
80
100
120
140
Months

FIG. 9B

	No. of Patients	HR[1]	P-value[1]	HR[2]	P-value[2]
Blaveri Bladder	98	2.28	0.04	1.29	0.046
Pawitan BRC	159	2.48	0.0057	1.7	0.059
Chin BRC	118	2.08	0.018	1.9	0.18

1: The hazard rations were estimated using the Cox model between the high and low G-CSF groups, where the cutoff was determined using the log rank statistics.
2: G-CSF expression was treated as a continuous variable in the Cox model

FIG. 9C

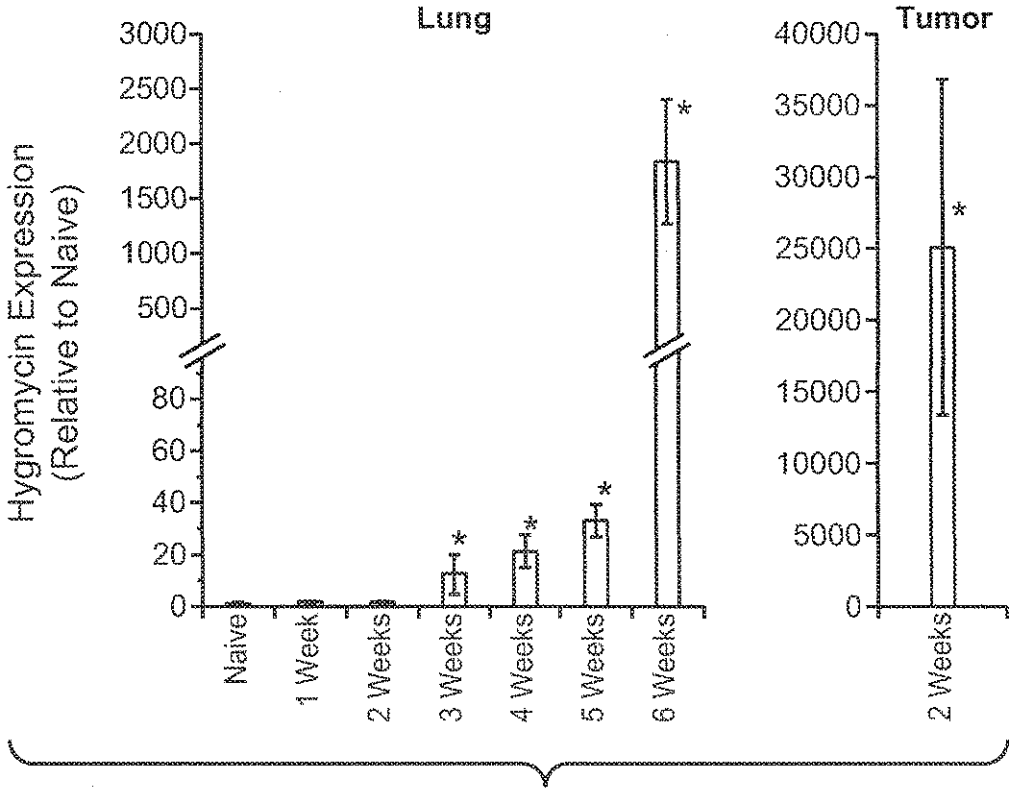


FIG. 10

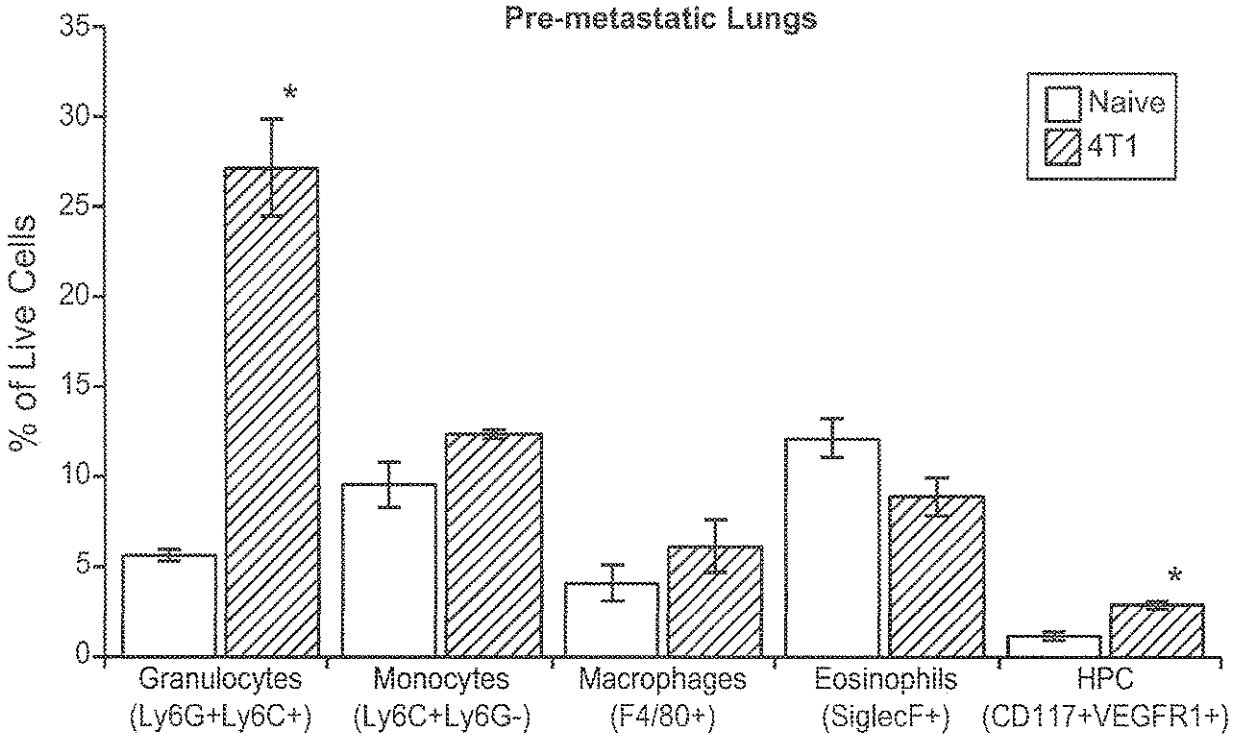


FIG. 11A

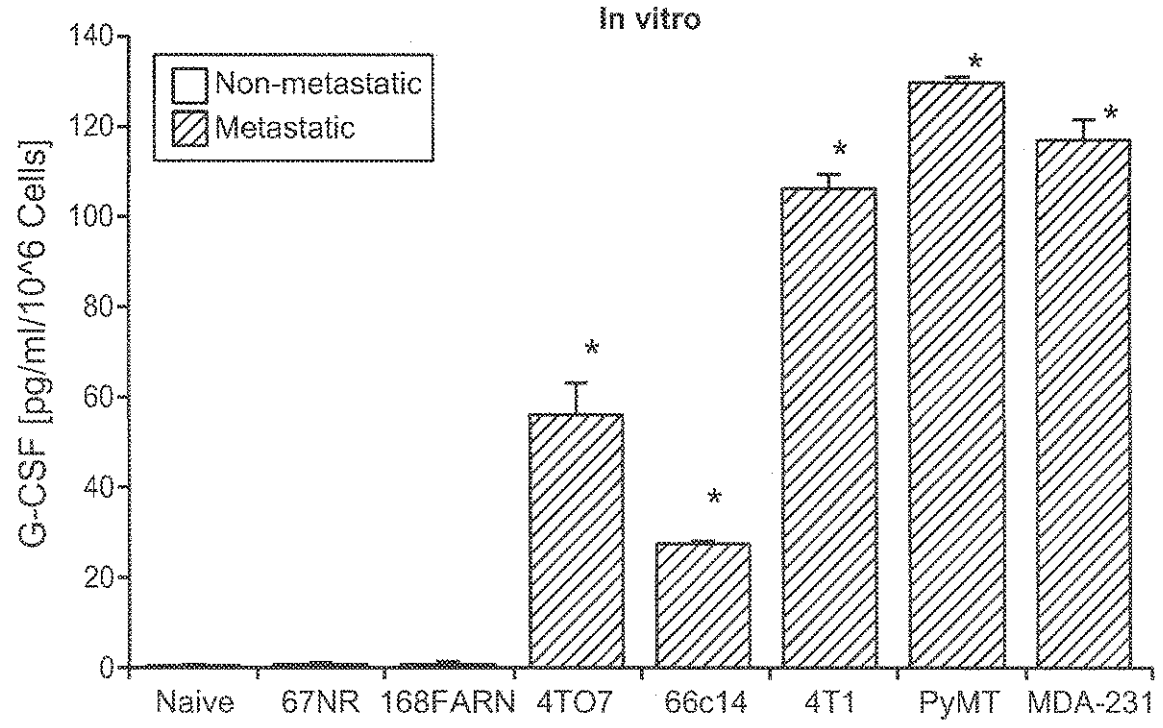


FIG. 11B

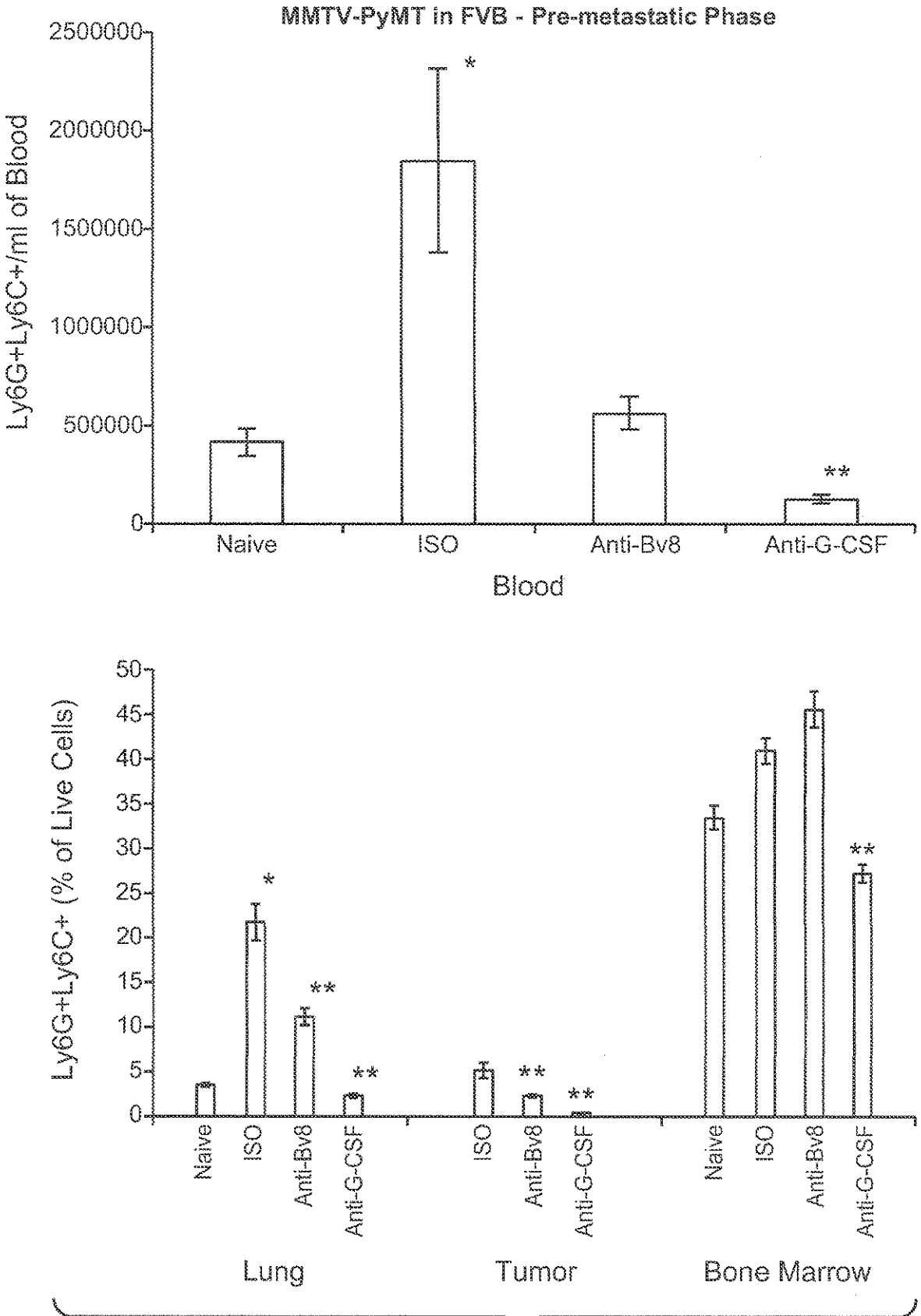

MMTV-PyMT in FVB - Pre-metastatic Phase
Ly6G+Ly6C+/ml of Blood
2500000
2000000
1500000
1000000
500000
0
*
**
Naive
ISO
Anti-Bv8
Anti-G-CSF
Blood
Ly6G+Ly6C+ (% of Live Cells)
50
45
40
35
30
25
20
15
10
5
0
Naive
ISO
Anti-Bv8
Anti-G-CSF
Lung
Tumor
Bone Marrow

FIG. 11C

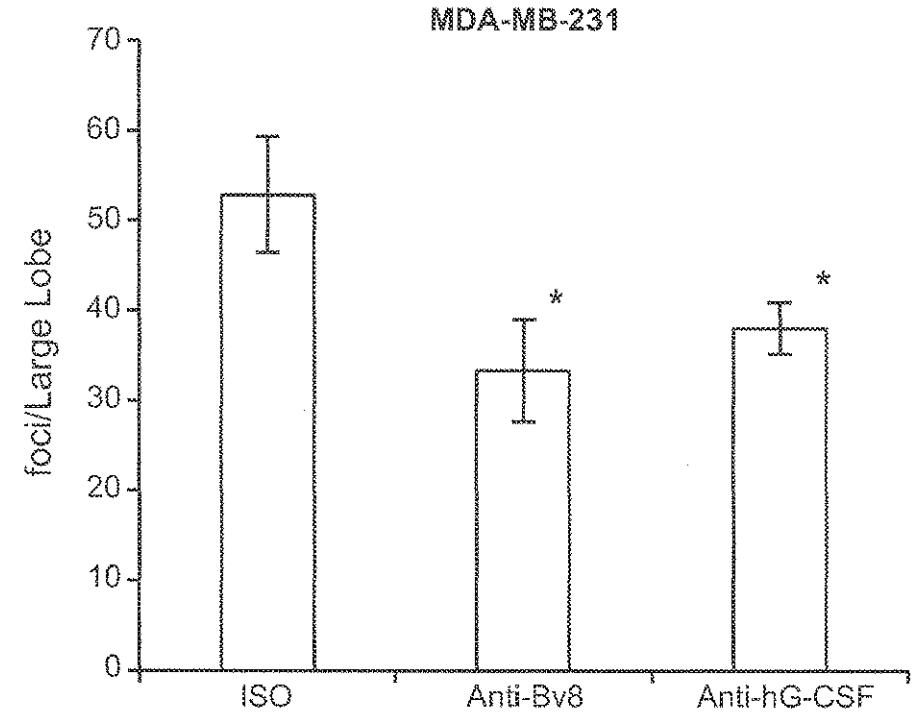


FIG. 12A

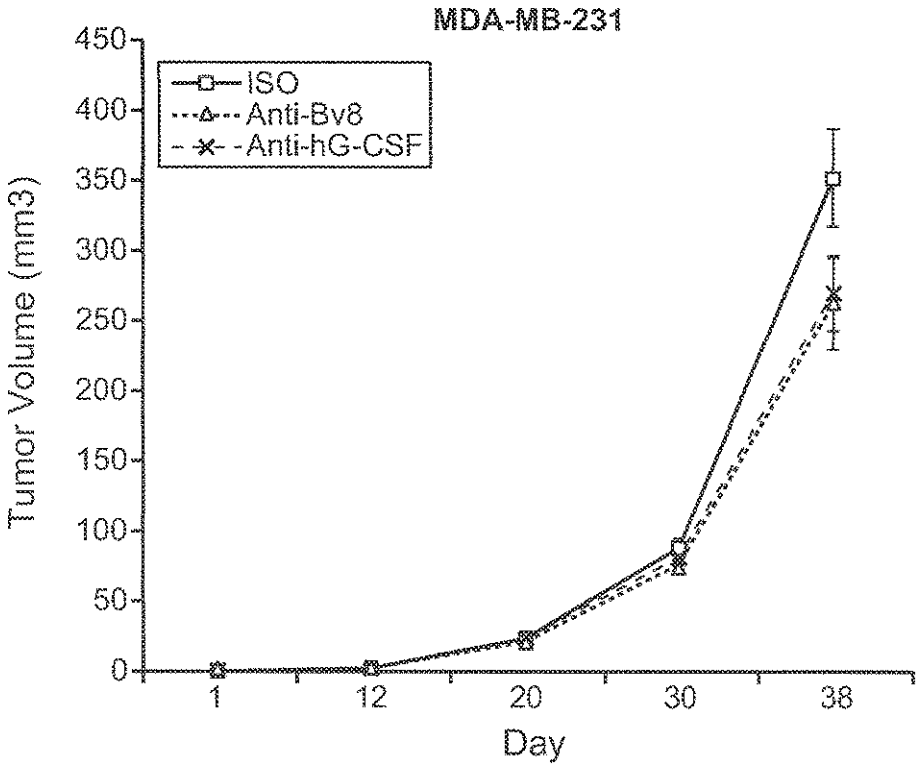


FIG. 12B

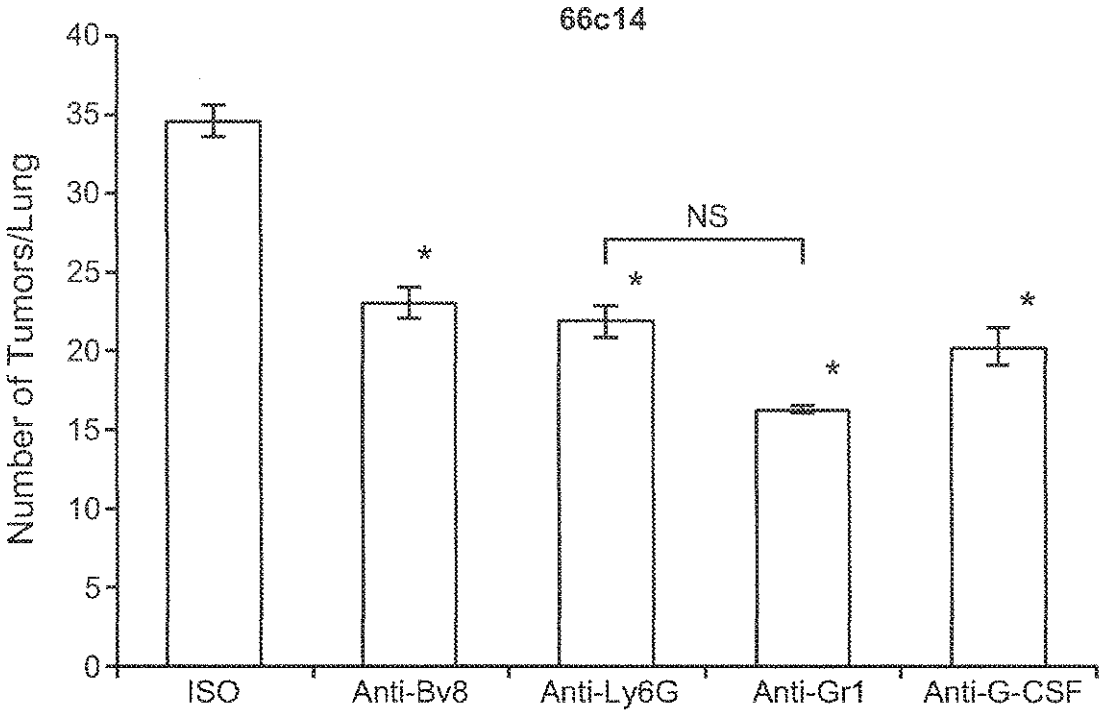


FIG. 12C

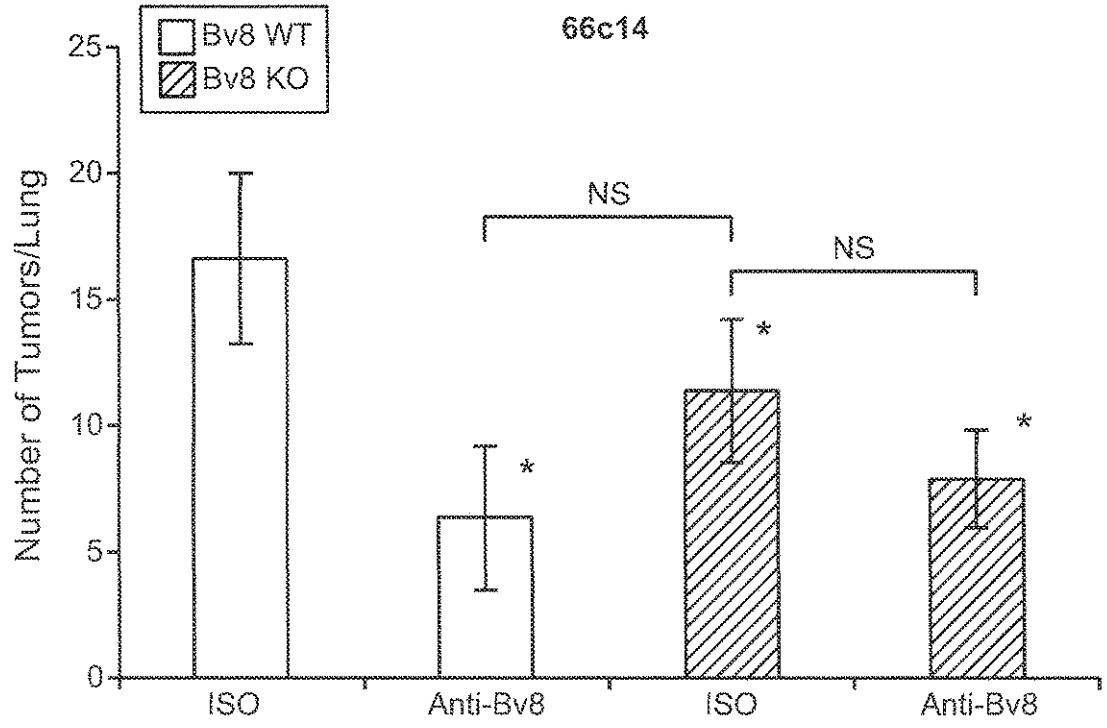


FIG. 13A

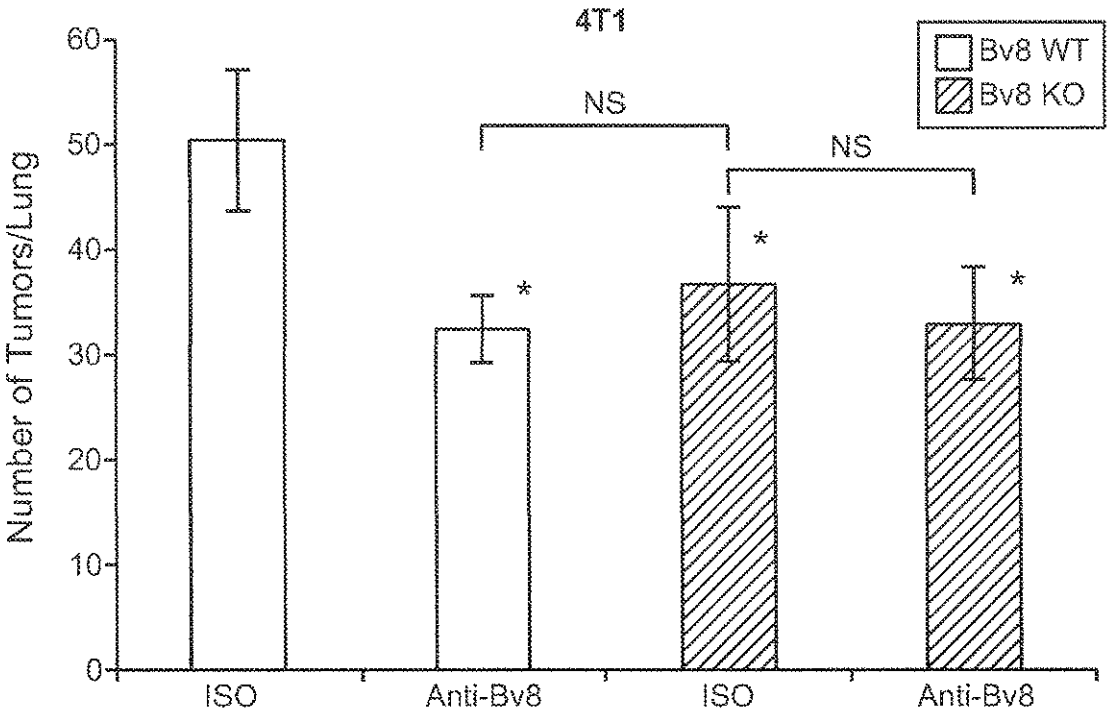


FIG. 13B

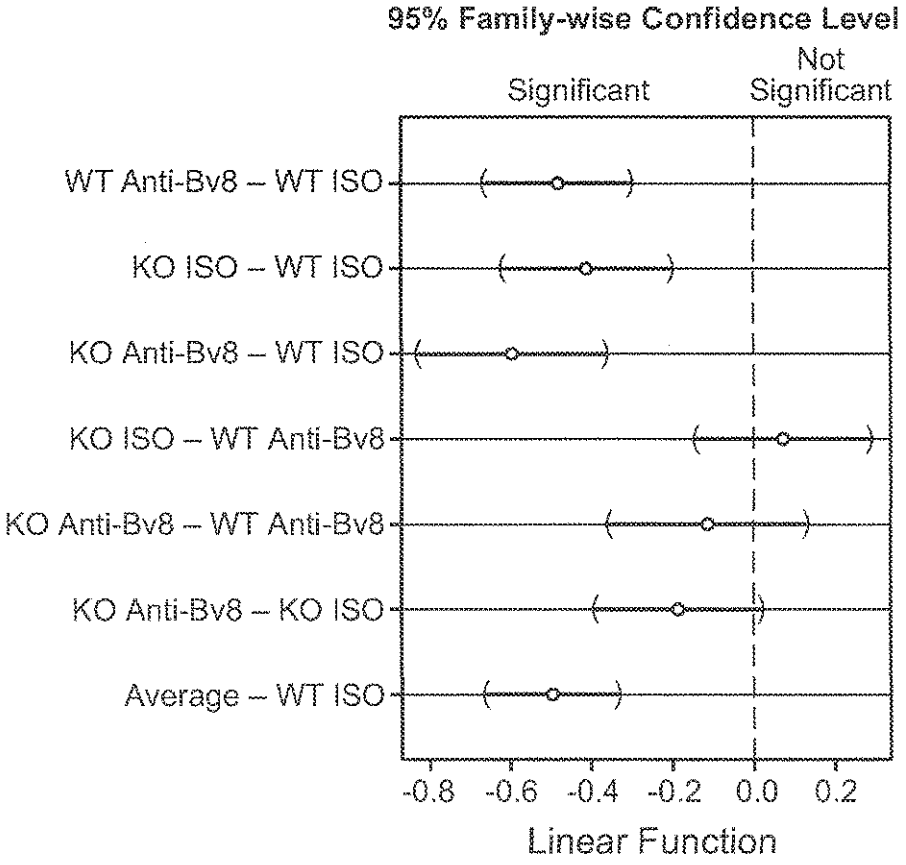


FIG. 13C

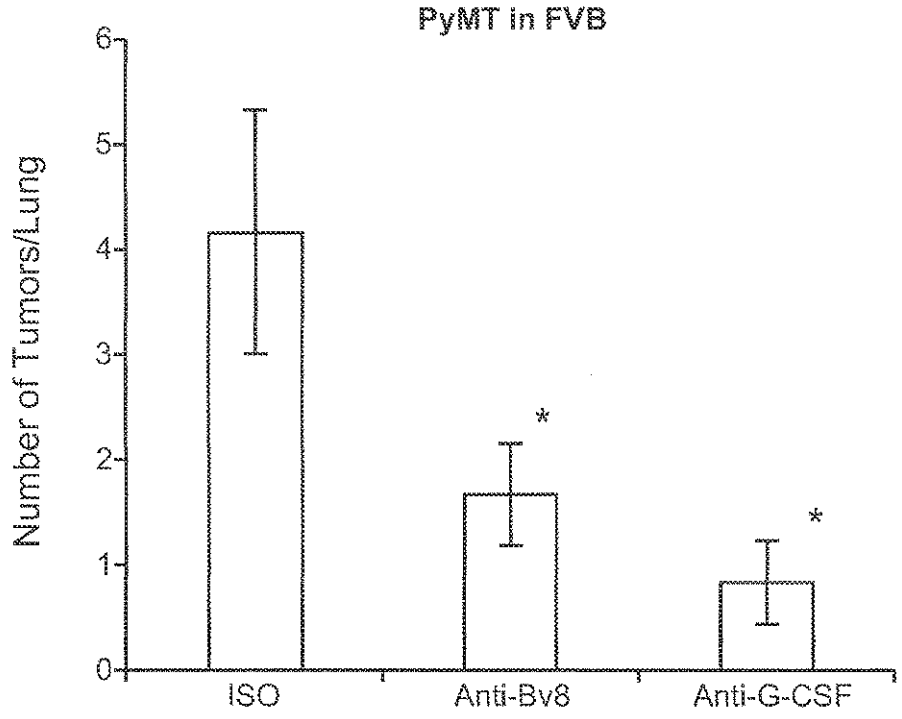


FIG. 13D

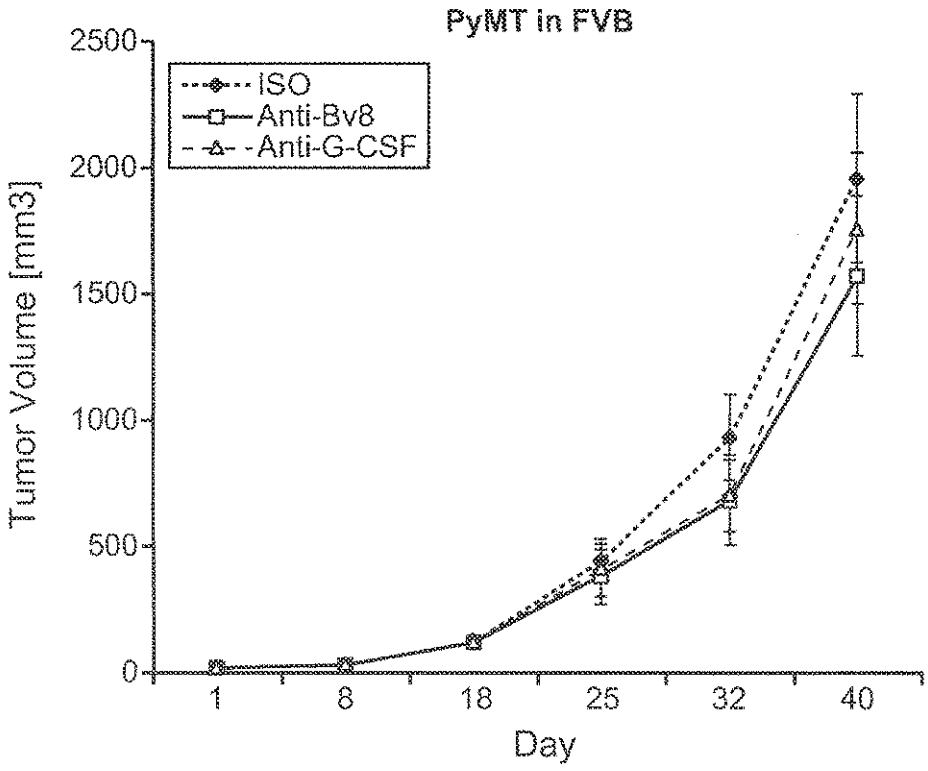


FIG. 13E

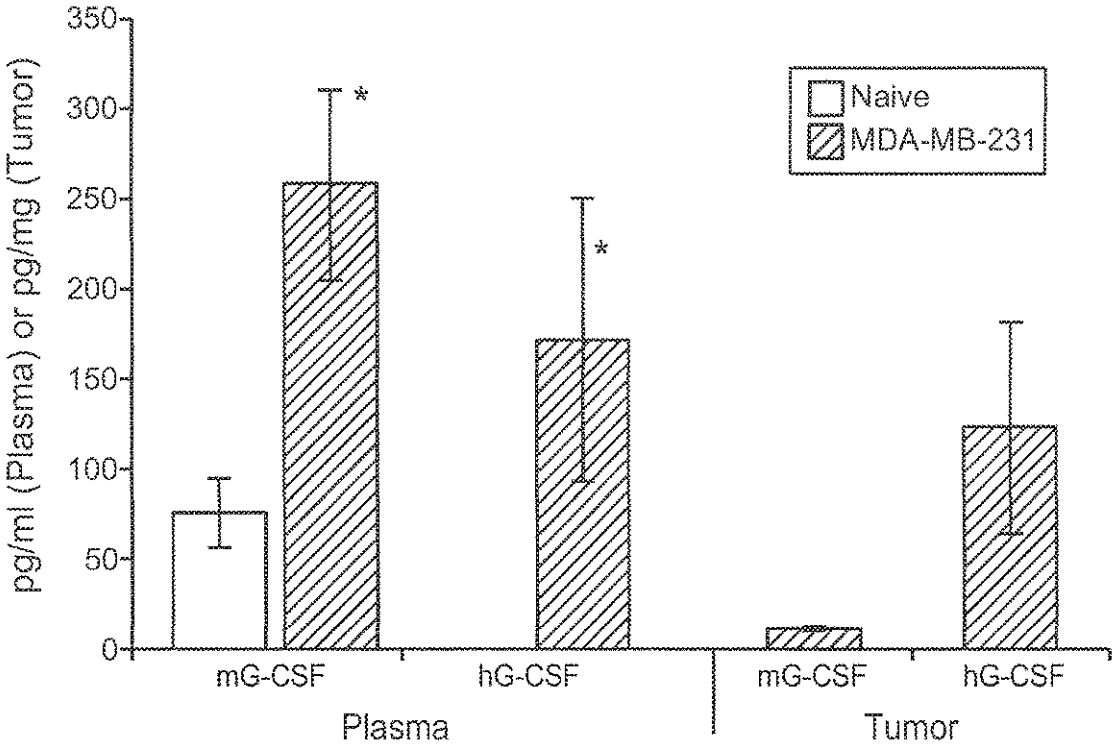


FIG. 13F

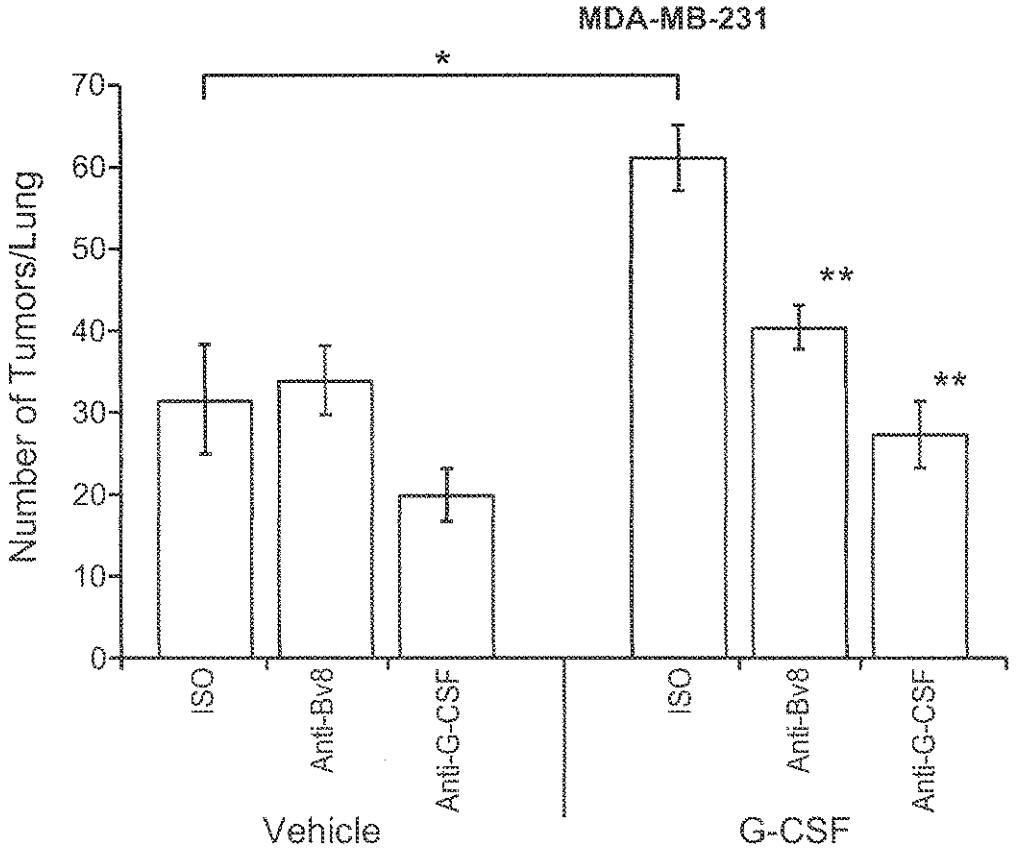


FIG. 14A

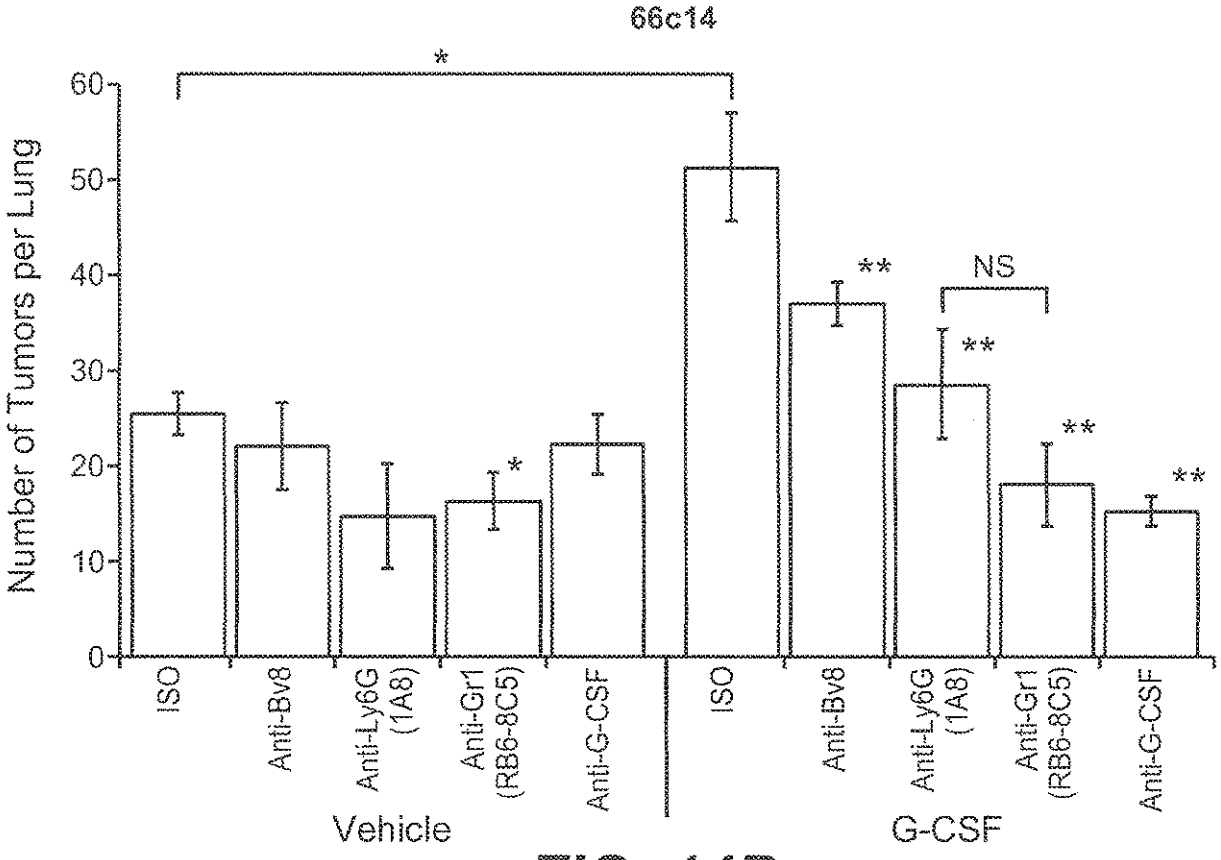


FIG. 14B

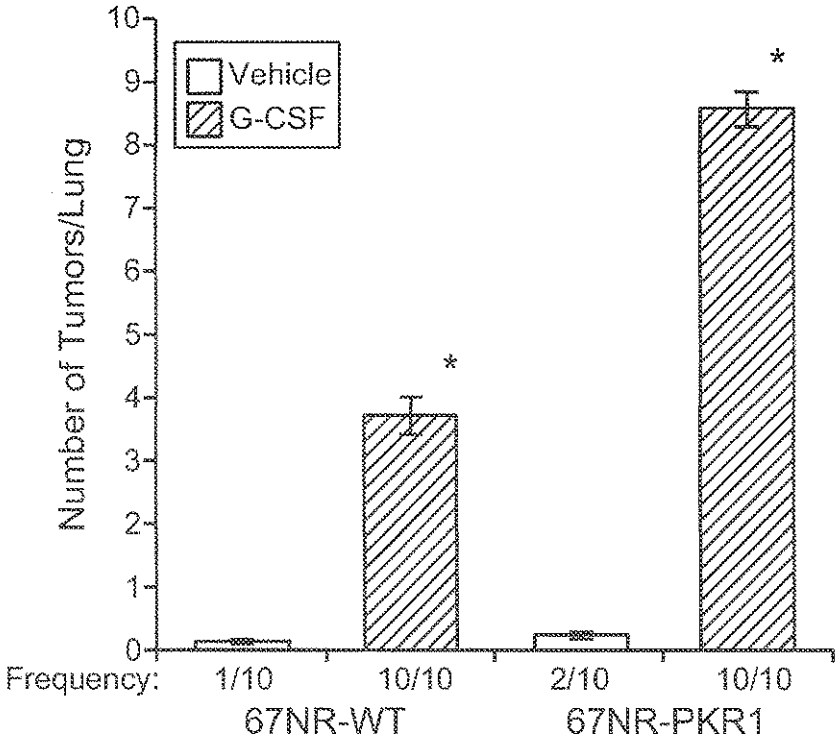


FIG. 15A

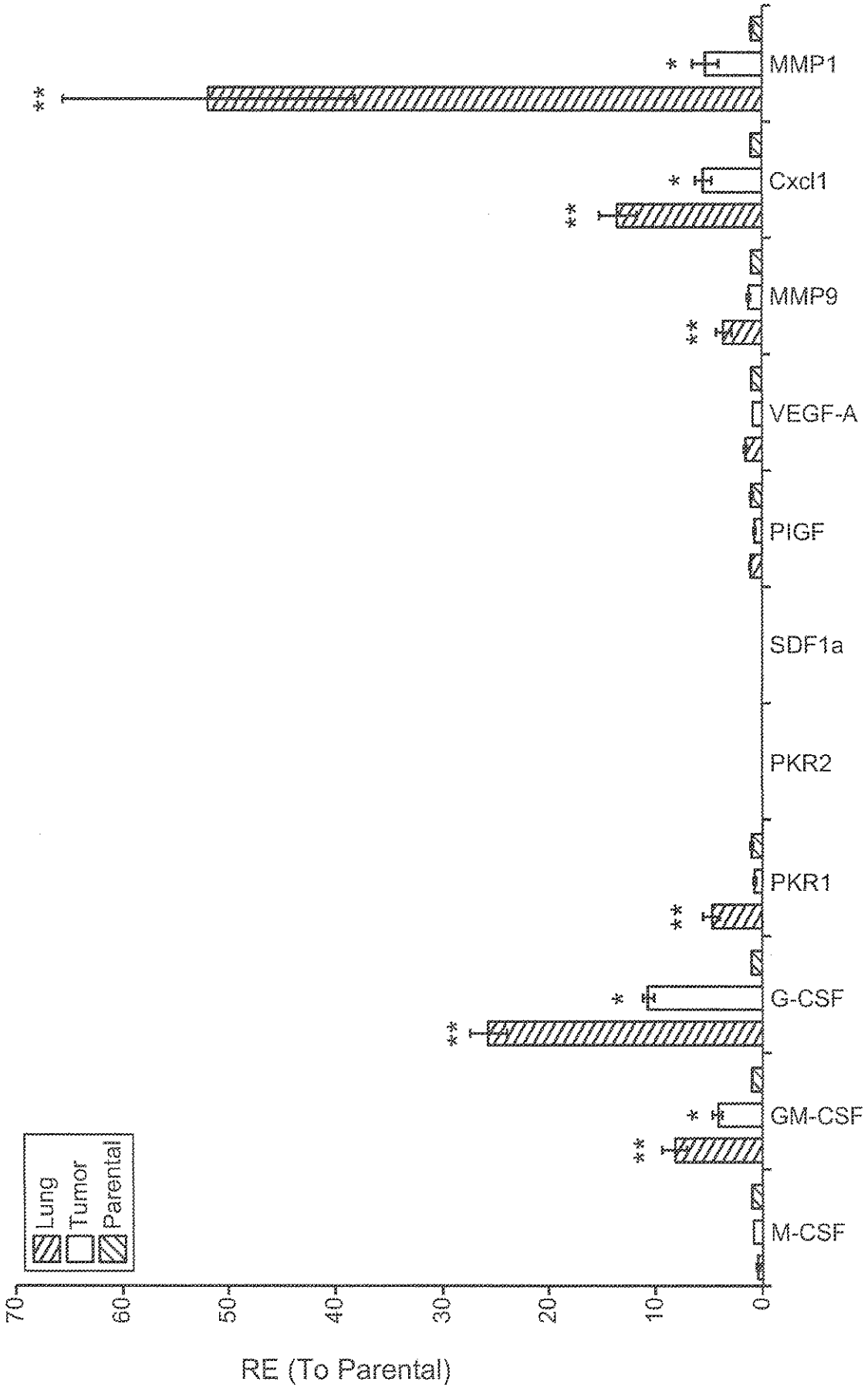

MMP1
Cxcl1
MMP9
VEGF-A
PlGF
SDF1a
PKR2
PKR1
G-CSF
GM-CSF
M-CSF
Lung
Tumor
Parental
70
60
50
40
30
20
10
0
RE (To Parental)

FIG. 15B

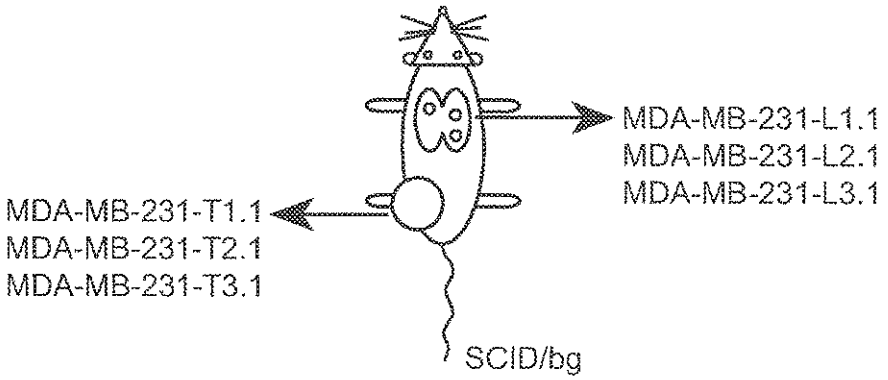


FIG. 15C

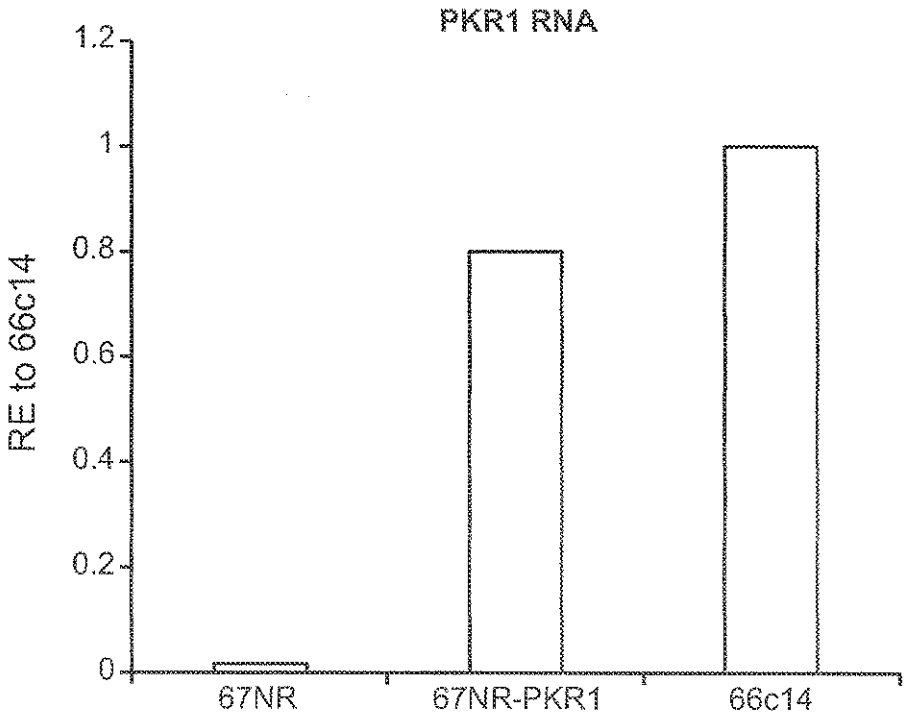


FIG. 16A

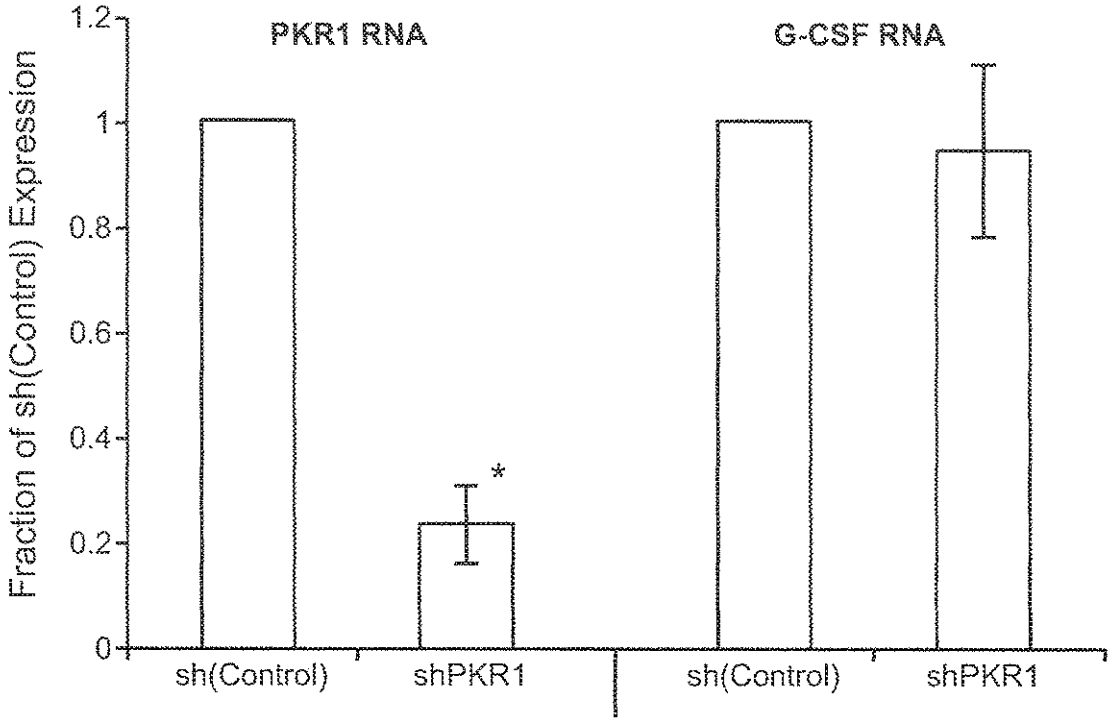


FIG. 16B

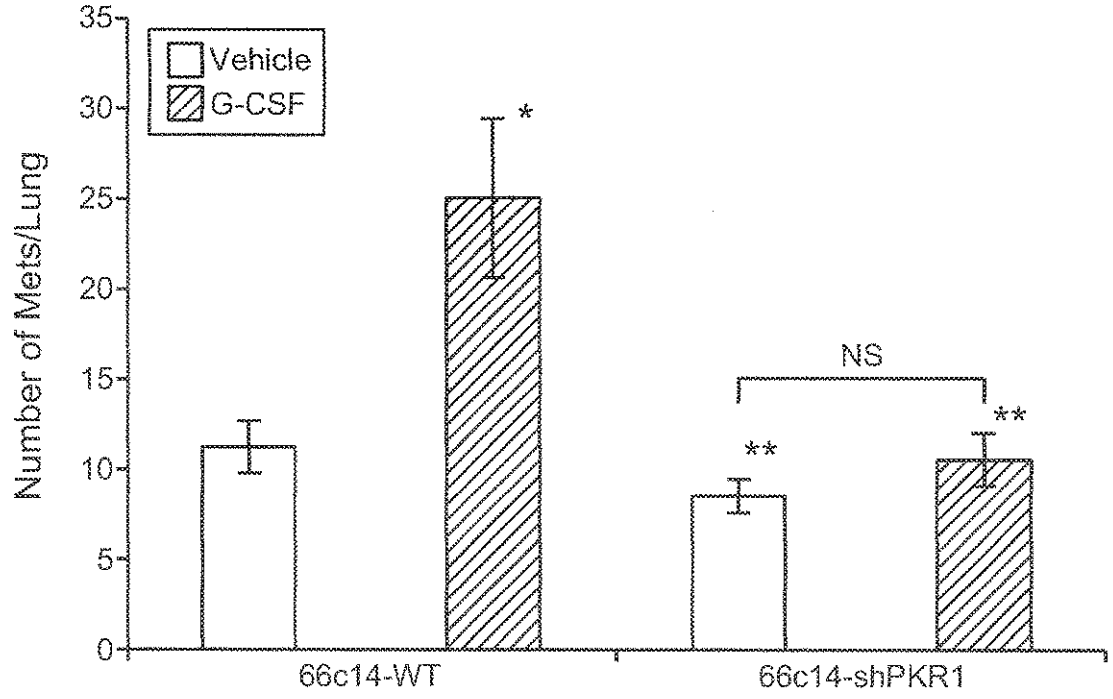


FIG. 16C