FIGURE 1

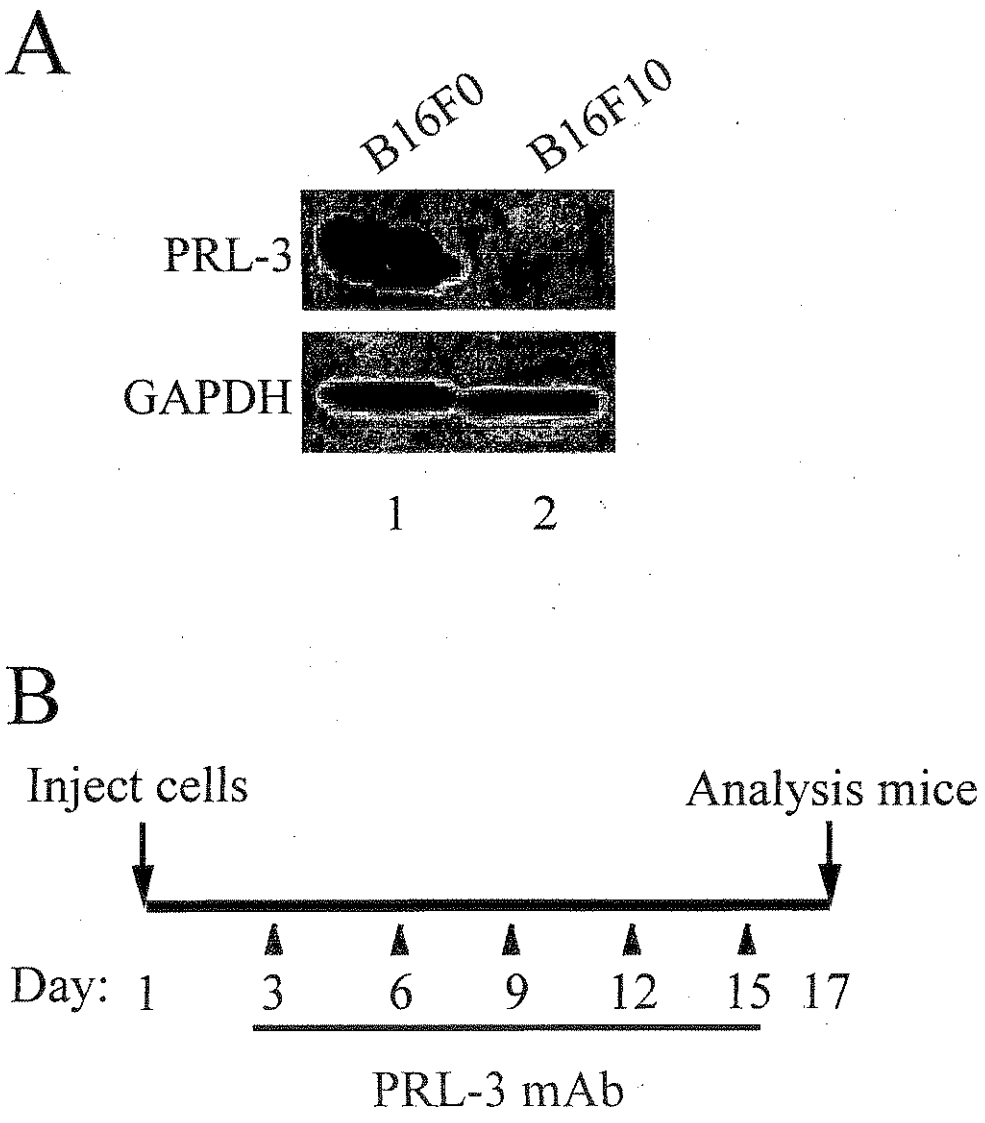

A
B16F0
B16F10
PRL-3
GAPDH
1
2
B
Inject cells
Analysis mice
Day: 1
3
6
9
12
15
17
PRL-3 mAb

**FIGURE 1 (CONTINUED)**

C

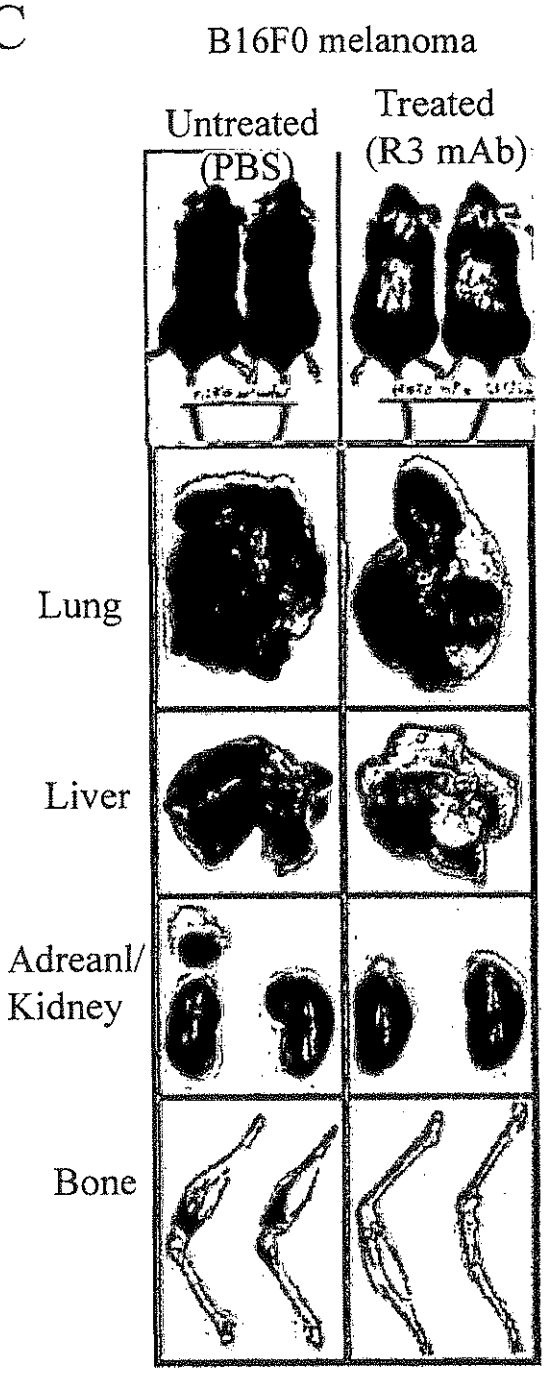


**FIGURE 1 (CONTINUED)**

D

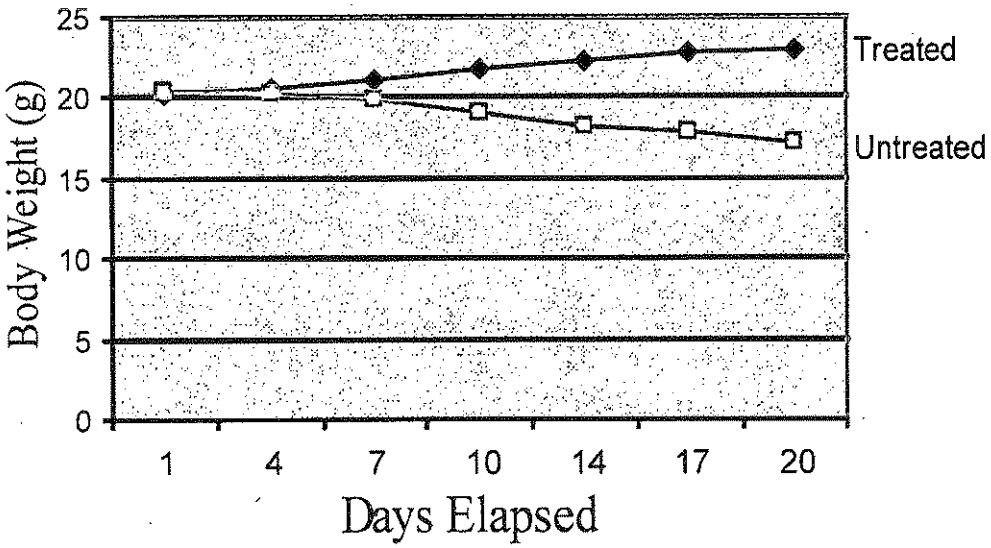


**FIGURE 1 (CONTINUED)**

E

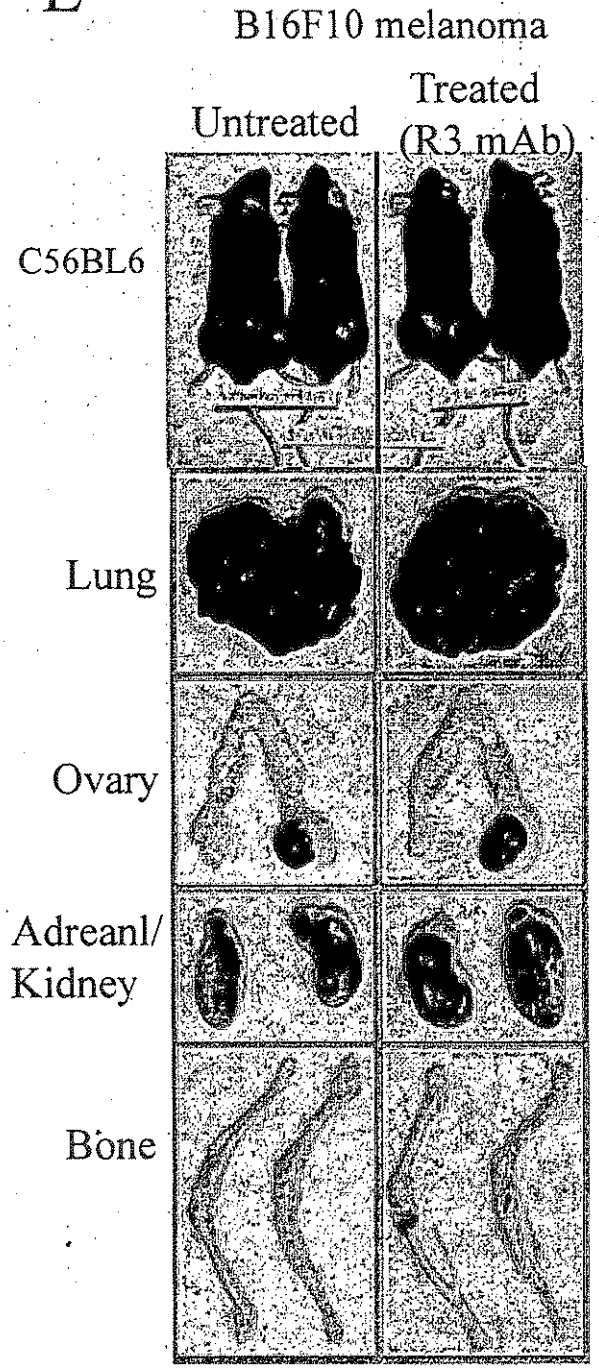


**FIGURE 1 (CONTINUED)**

F

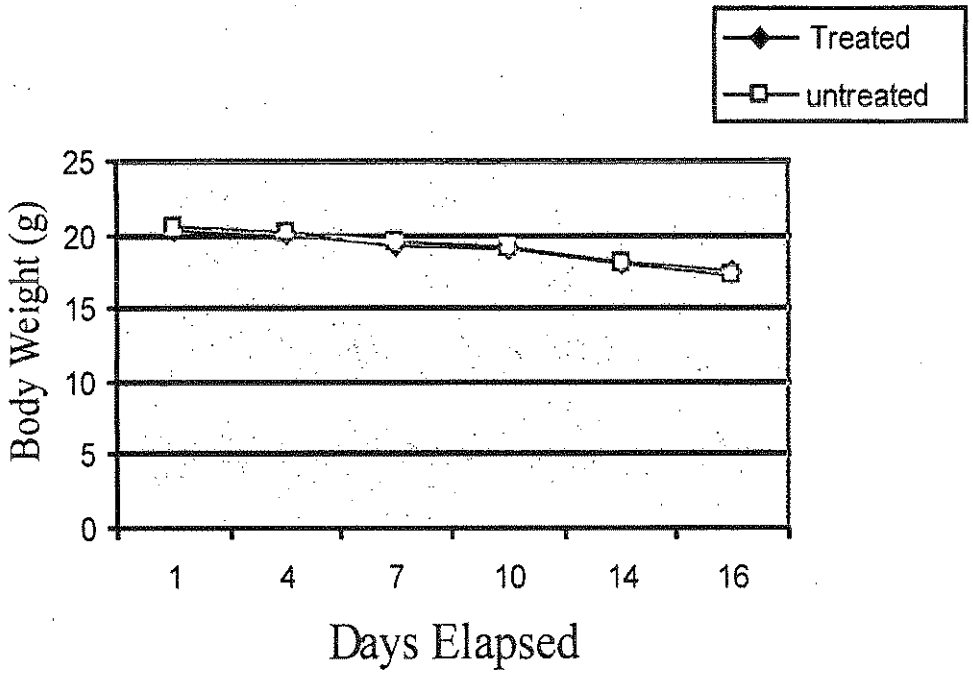


**FIGURE 2**

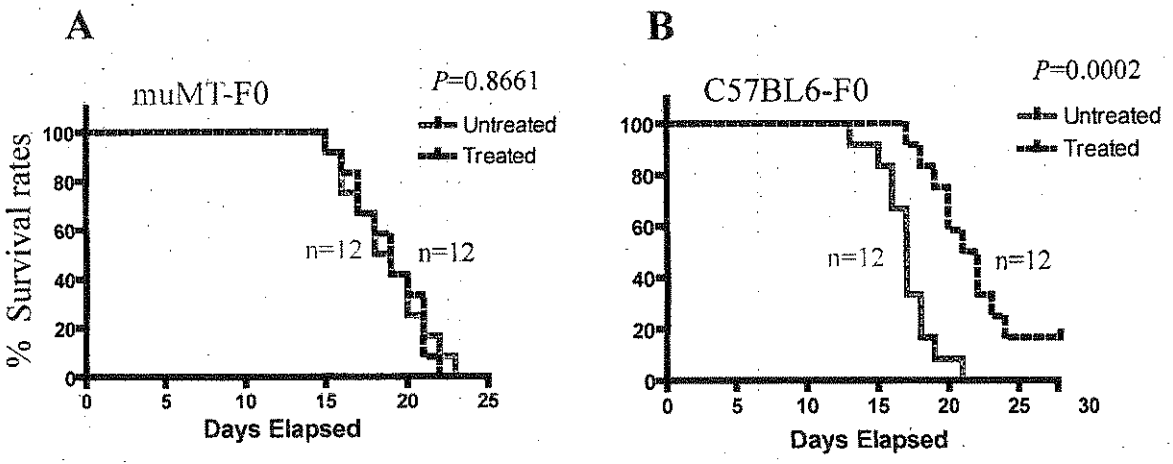
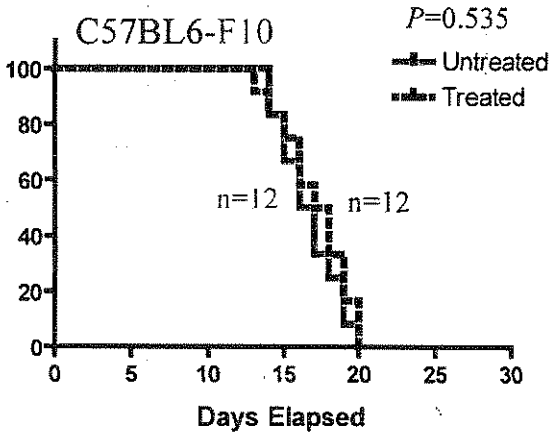

A
muMT-F0
P=0.8661
Untreated
Treated
% Survival rates
100
80
60
40
20
0
n=12
n=12
0
5
10
15
20
25
Days Elapsed
B
C57BL6-F0
P=0.0002
Untreated
Treated
100
80
60
40
20
0
n=12
n=12
0
5
10
15
20
25
30
Days Elapsed

**FIGURE 2 (CONTINUED)**

**C**




**D**

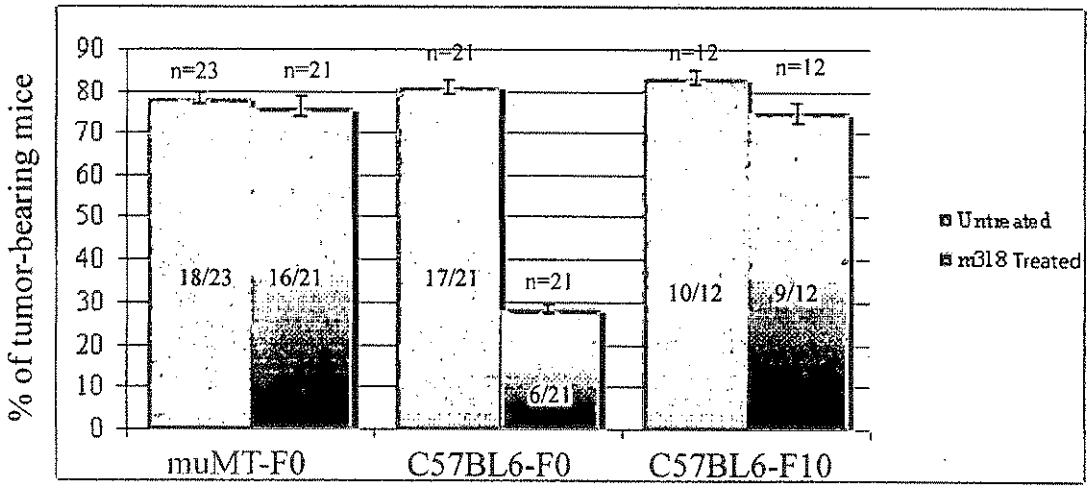


**FIGURE 3**

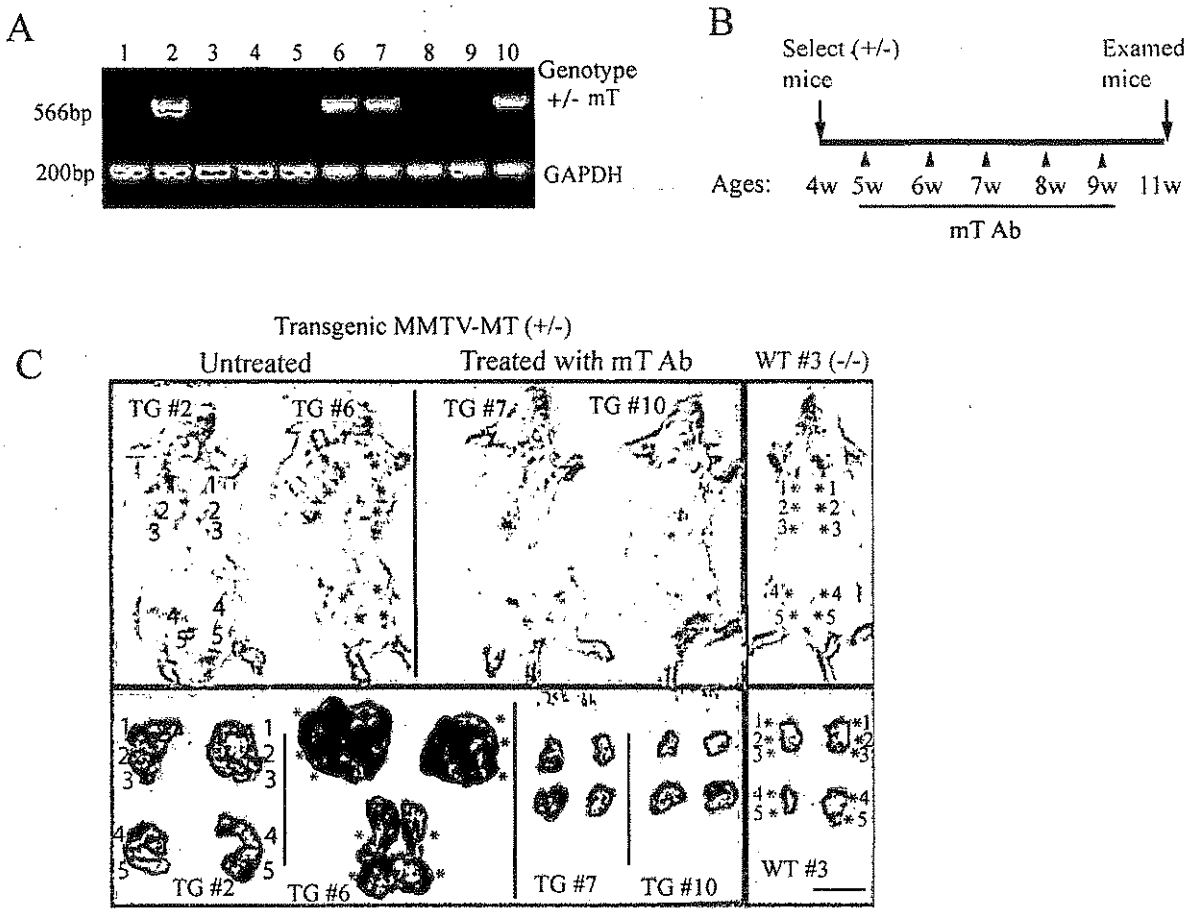
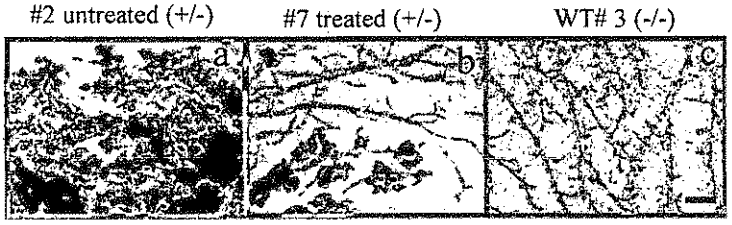
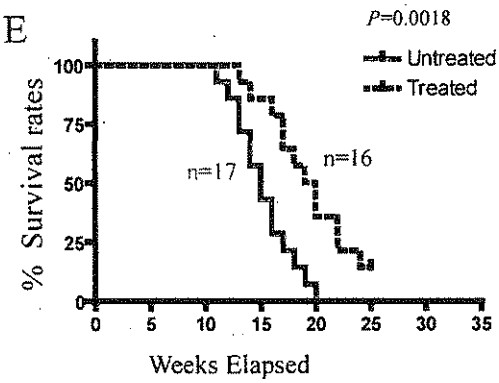

A
1 2 3 4 5 6 7 8 9 10
Genotype
+/- mT
566bp
200bp
GAPDH
B
Select (+/-)
mice
Examed
mice
Ages: 4w 5w 6w 7w 8w 9w 11w
mT Ab
C
Transgenic MMTV-MT (+/-)
Untreated
Treated with mT Ab
WT #3 (-/-)
TG #2
TG #6
TG #7
TG #10
WT #3

**FIGURE 3 (CONTINUED)**

D




E




F

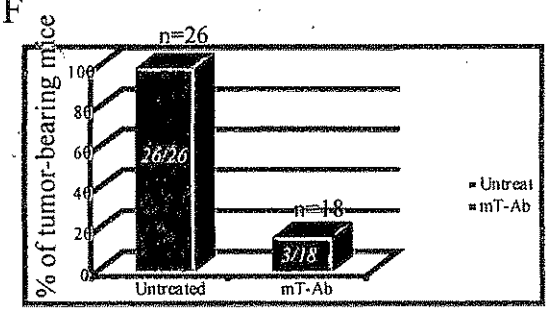


**FIGURE 4**

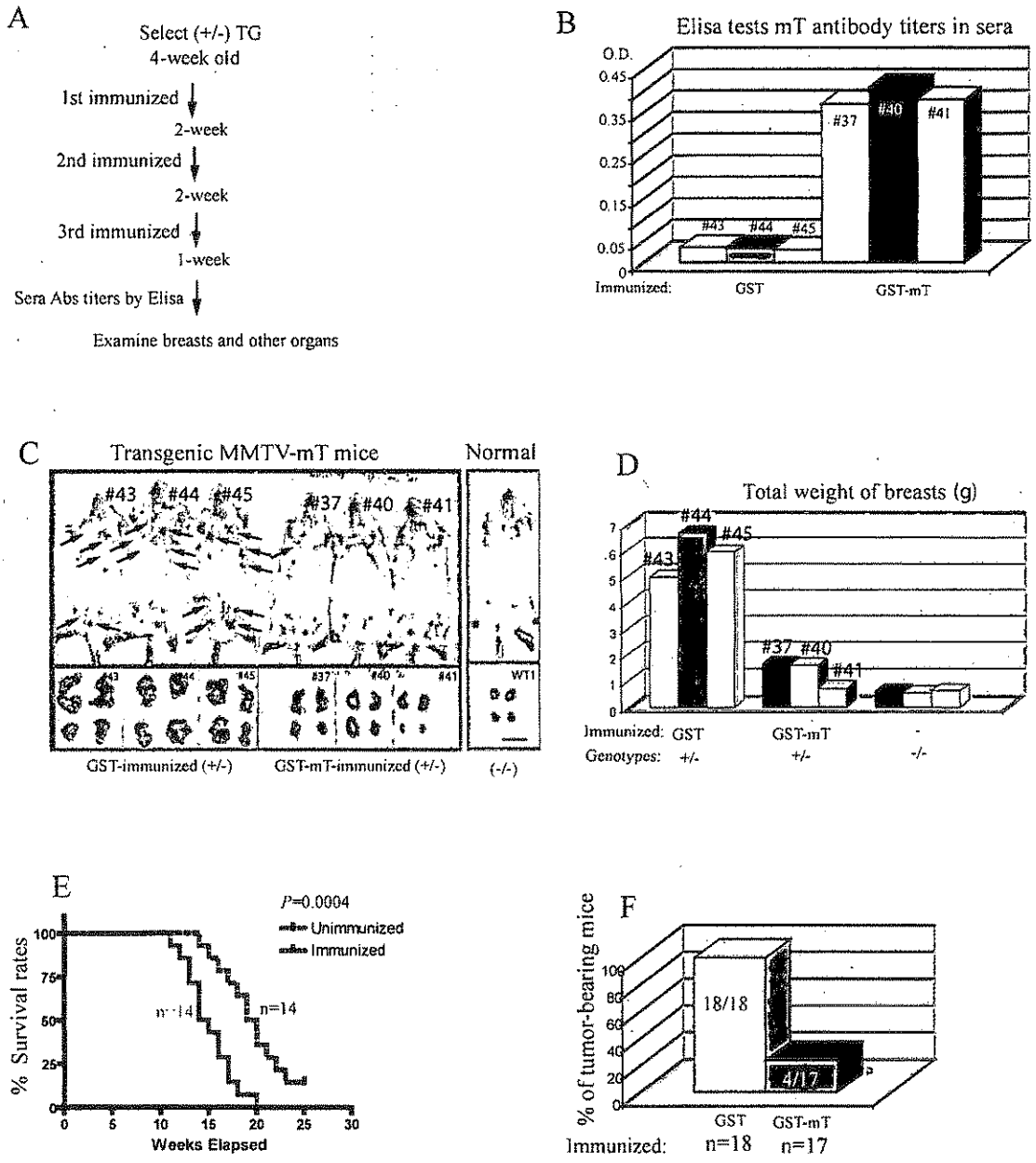

A
Select (+/-) TG
4-week old
1st immunized
2-week
2nd immunized
2-week
3rd immunized
1-week
Sera Abs titers by Elisa
Examine breasts and other organs
B
Elisa tests mT antibody titers in sera
O.D.
0.45
0.35
0.25
0.15
0.05
0
#43
#44
#45
#37
#40
#41
Immunized:
GST
GST-mT
C
Transgenic MMTV-mT mice
Normal
#43
#44
#45
#37
#40
#41
WT1
GST-immunized (+/-)
GST-mT-immunized (+/-)
(-/-)
D
Total weight of breasts (g)
#43
#44
#45
#37
#40
#41
Immunized:
GST
GST-mT
Genotypes:
+/-
+/-
-/-
E
P=0.0004
Unimmunized
Immunized
% Survival rates
100
75
50
25
0
n=14
n=14
0
5
10
15
20
25
30
Weeks Elapsed
F
% of tumor-bearing mice
100
80
60
40
20
0
18/18
4/17
GST
GST-mT
Immunized:
n=18
n=17

**FIGURE 5**

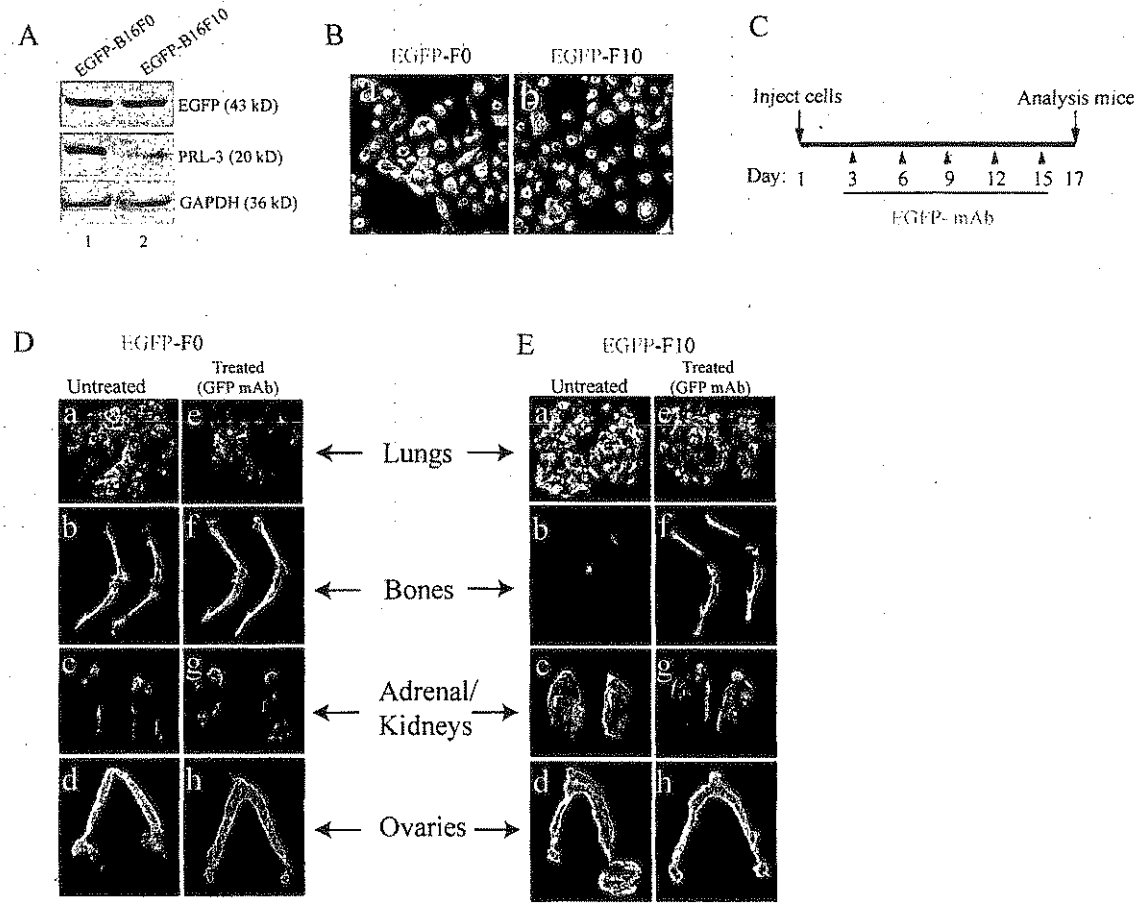
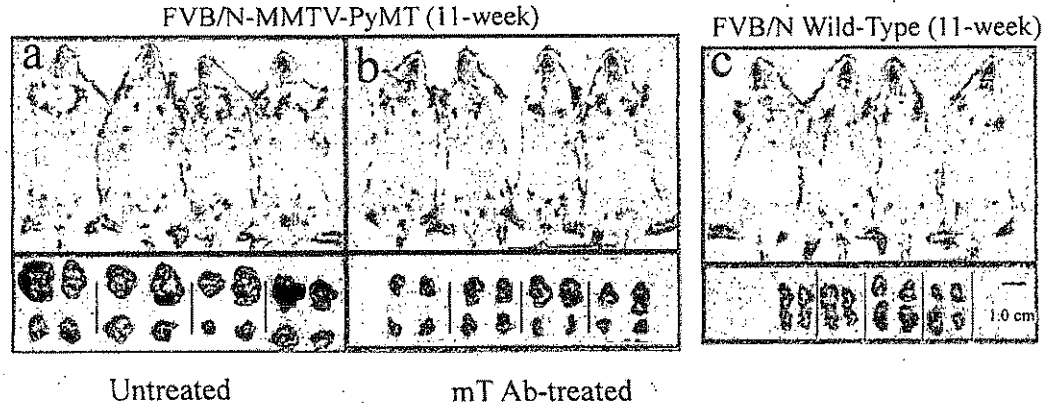

A
EGFP-B16F0
EGFP-B16F10
EGFP (43 kD)
PRL-3 (20 kD)
GAPDH (36 kD)
1
2
B
EGFP-F0
EGFP-F10
a
b
C
Inject cells
Analysis mice
Day: 1 3 6 9 12 15 17
EGFP- mAb
D
EGFP-F0
Untreated
Treated (GFP mAb)
E
EGFP-F10
Untreated
Treated (GFP mAb)
Lungs
Bones
Adrenal/ Kidneys
Ovaries

**FIGURE 5 (CONTINUED)**

F




G

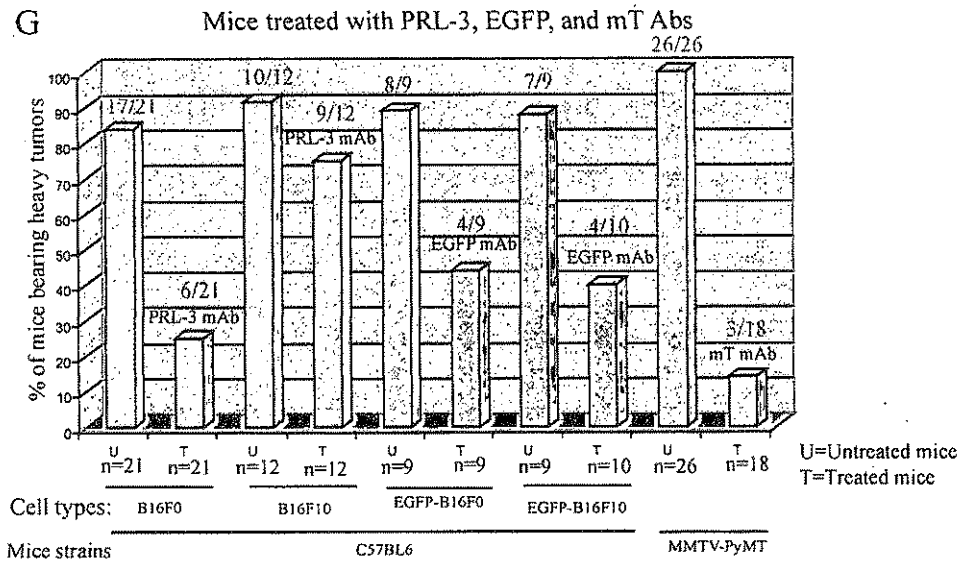
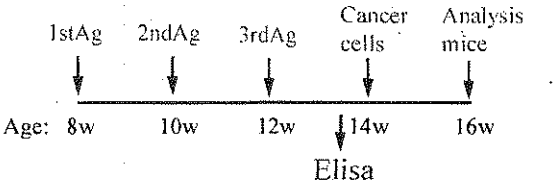


**FIGURE 6**

A


1stAg
2ndAg
3rdAg
Cancer cells
Analysis mice
Age: 8w
10w
12w
14w
16w
Elisa

B

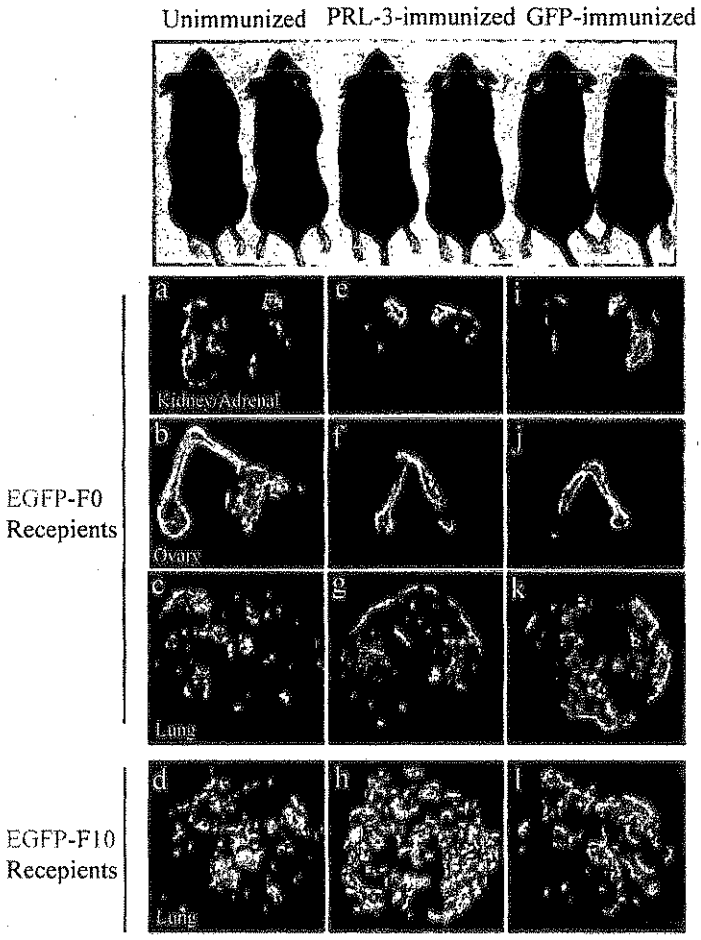

Unimmunized
PRL-3-immunized
GFP-immunized
a
e
i
Kidney/Adrenal
b
f
j
Ovary
c
g
k
Lung
d
h
l
Lung
EGFP-F0 Recepients
EGFP-F10 Recepients

**FIGURE 6 (CONTINUED)**

C

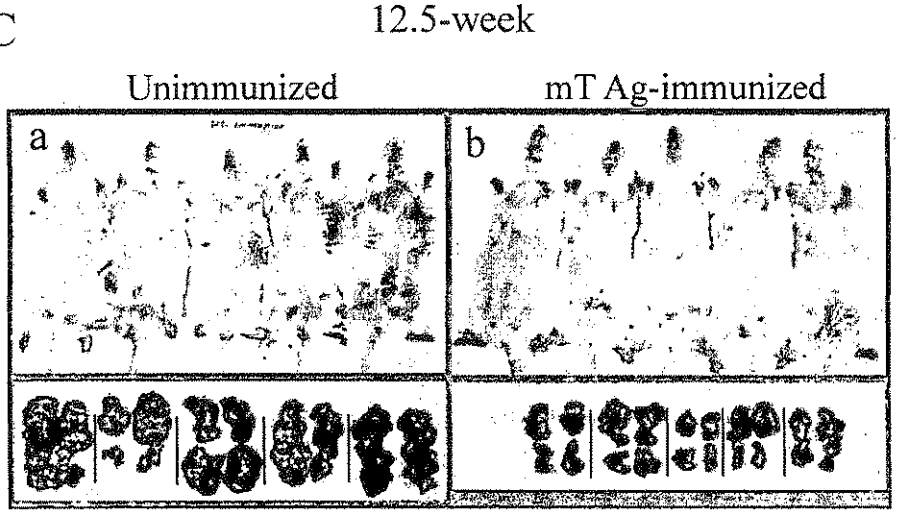
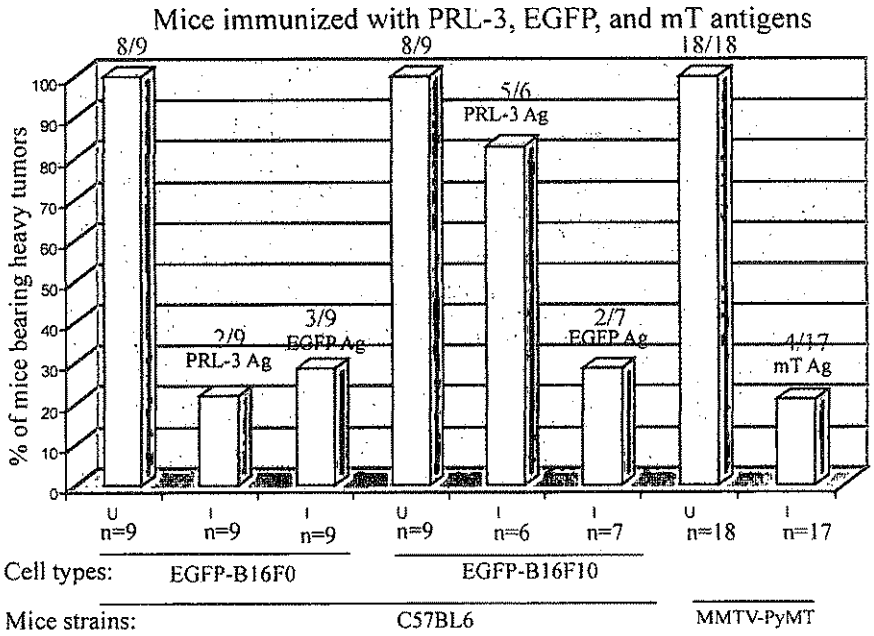


**FIGURE 6 (CONTINUED)**

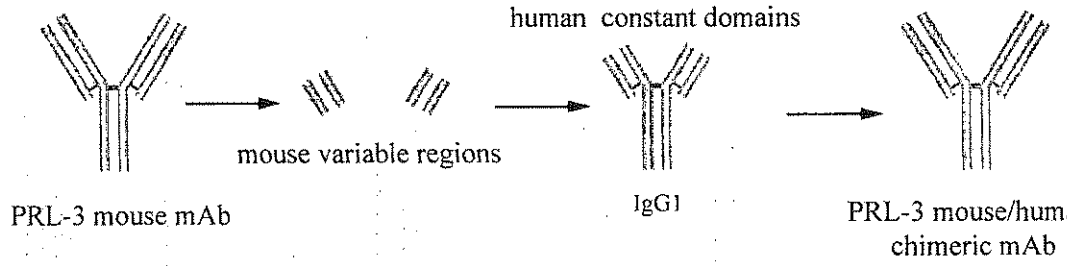
D




U=Unimmunized mice
I=Immunized mice

**FIGURE 7**

**A** Outline of PRL-3 chimeric antibody construction




**B**




**C**

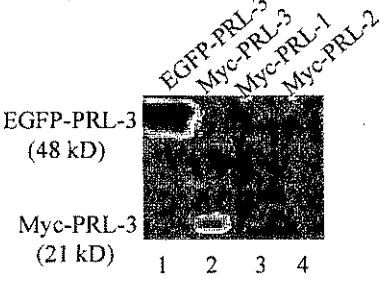


**FIGURE 8**

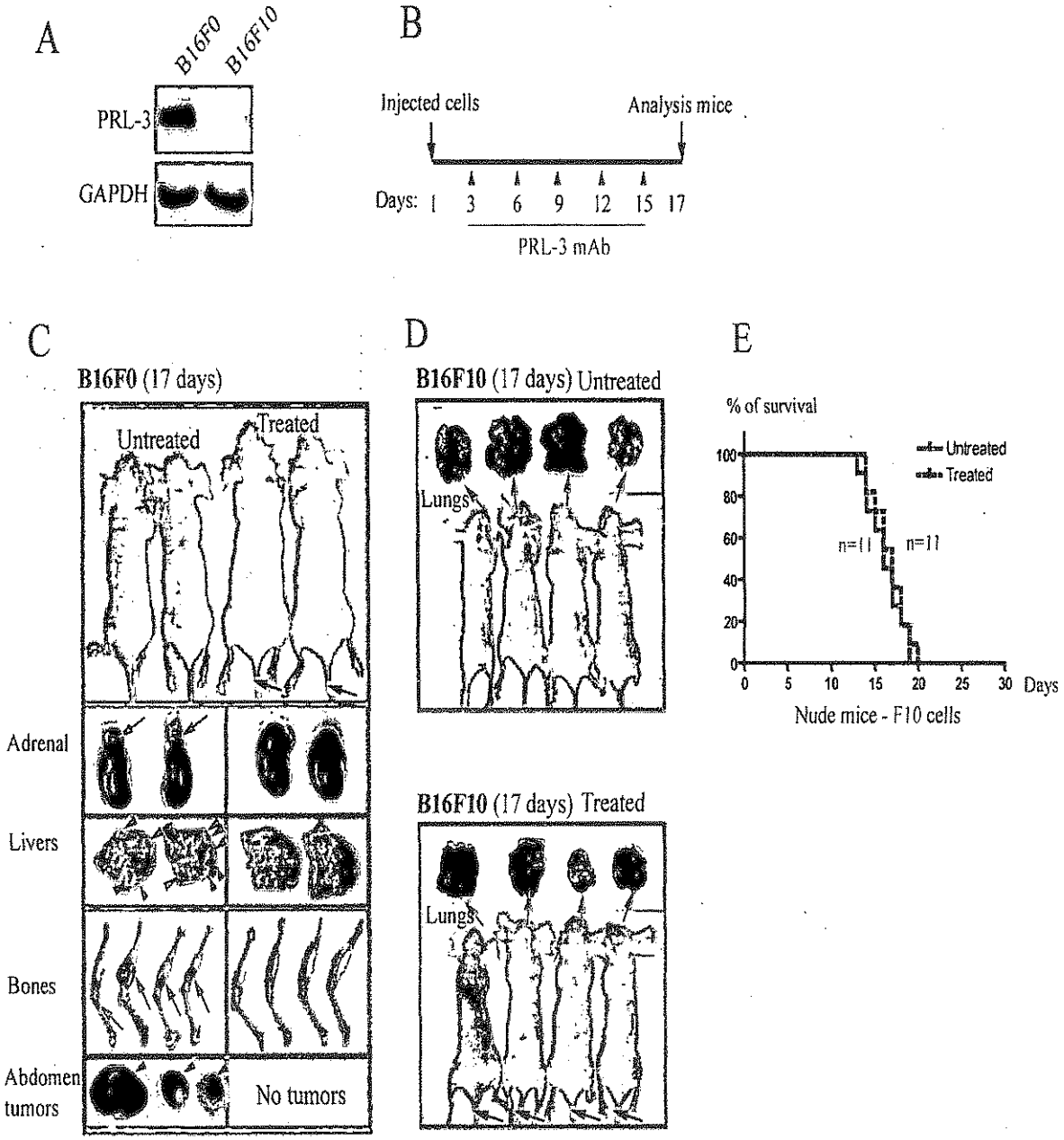

A
B16F0
B16F10
PRL-3
GAPDH
B
Injected cells
Analysis mice
Days: 1 3 6 9 12 15 17
PRL-3 mAb
C
B16F0 (17 days)
Untreated
Treated
Adrenal
Livers
Bones
Abdomen tumors
No tumors
D
B16F10 (17 days) Untreated
Lungs
B16F10 (17 days) Treated
Lungs
E
% of survival
Untreated
Treated
n=11
n=11
Days
Nude mice - F10 cells

**FIGURE 9**

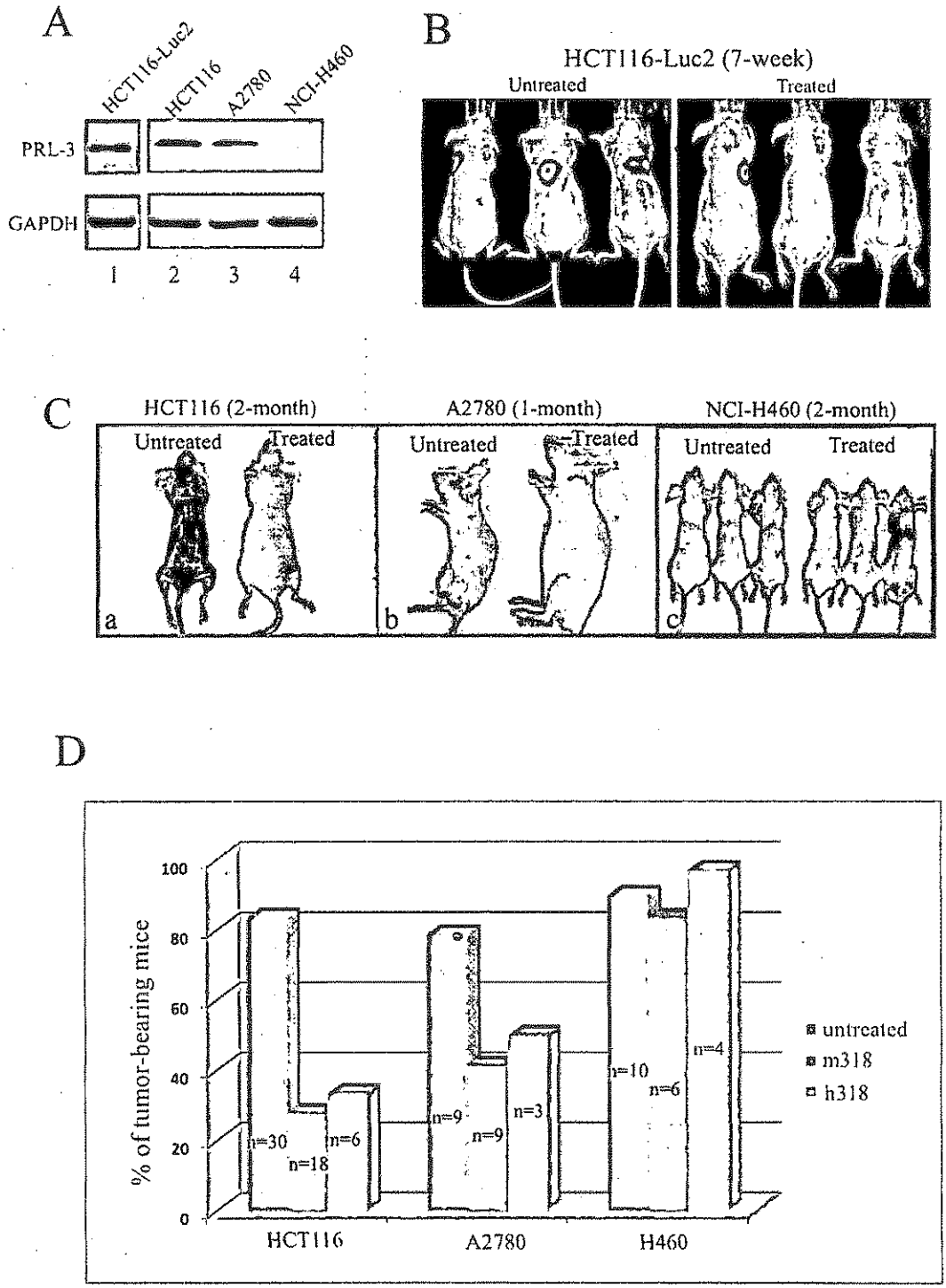
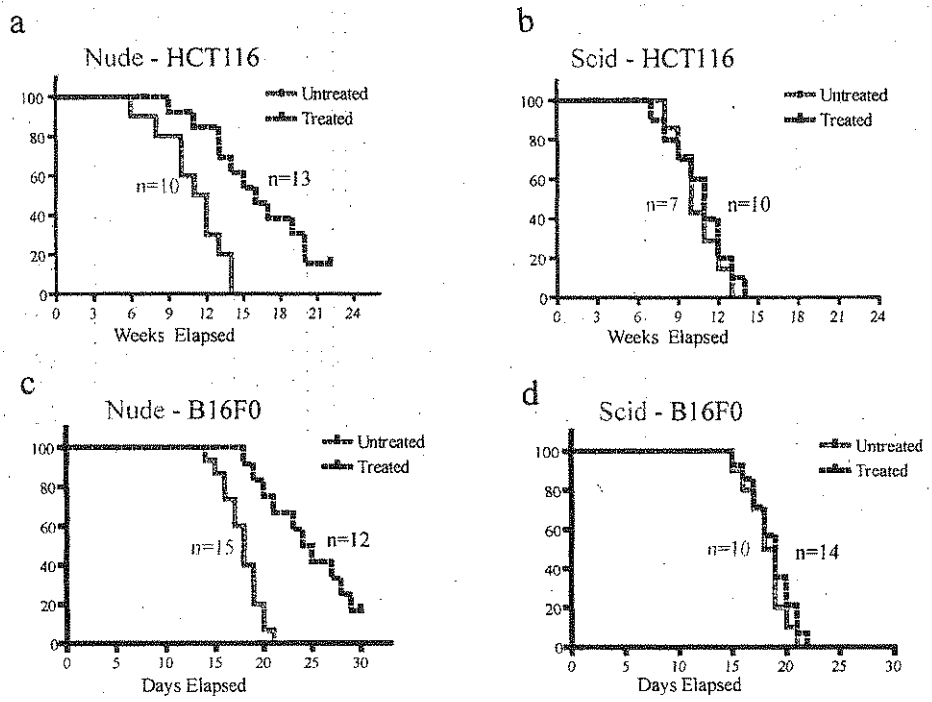

A
HCT116-Luc2
HCT116
A2780
NCI-H460
PRL-3
GAPDH
1
2
3
4
B
HCT116-Luc2 (7-week)
Untreated
Treated
C
HCT116 (2-month)
A2780 (1-month)
NCI-H460 (2-month)
Untreated
Treated
Untreated
Treated
Untreated
Treated
a
b
c
D
% of tumor-bearing mice
100
80
60
40
20
0
n=30
n=18
n=6
n=9
n=9
n=3
n=10
n=6
n=4
HCT116
A2780
H460
untreated
m318
h318

**FIGURE 10**

A


a
Nude - HCT116
Untreated
Treated
n=10
n=13
Weeks Elapsed
b
Scid - HCT116
Untreated
Treated
n=7
n=10
Weeks Elapsed
c
Nude - B16F0
Untreated
Treated
n=15
n=12
Days Elapsed
d
Scid - B16F0
Untreated
Treated
n=10
n=14
Days Elapsed

B

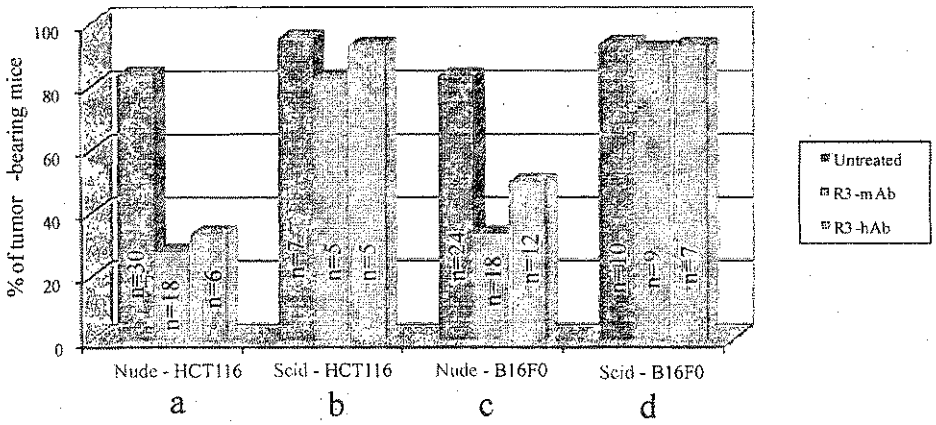
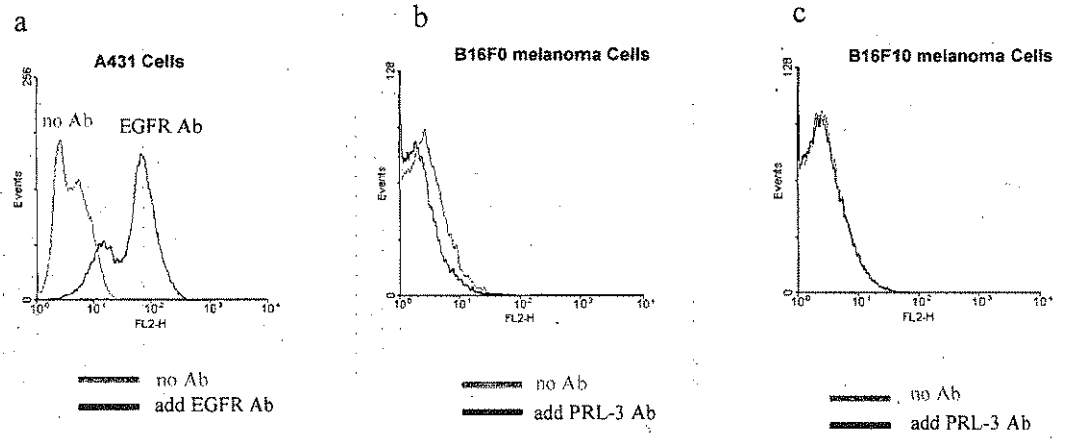

% of tumor -bearing mice
n=30
n=18
n=6
n=7
n=5
n=5
n=24
n=18
n=12
n=10
n=9
n=7
Nude - HCT116
Scid - HCT116
Nude - B16F0
Scid - B16F0
a
b
c
d
Untreated
R3 -mAb
R3 -hAb

**FIGURE 11**

A


a
A431 Cells
no Ab
EGFR Ab
Events
FL2-H
no Ab
add EGFR Ab
b
B16F0 melanoma Cells
Events
FL2-H
no Ab
add PRL-3 Ab
c
B16F10 melanoma Cells
Events
FL2-H
no Ab
add PRL-3 Ab

B

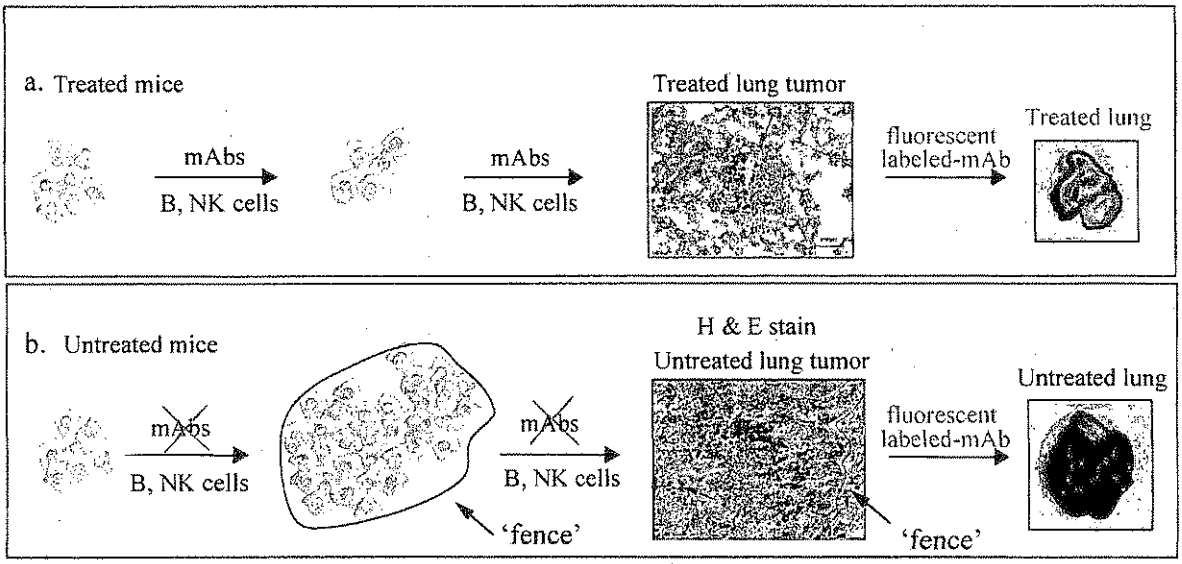

a. Treated mice
mAbs
B, NK cells
mAbs
B, NK cells
Treated lung tumor
fluorescent
labeled-mAb
Treated lung
b. Untreated mice
mAbs
B, NK cells
mAbs
B, NK cells
'fence'
H & E stain
Untreated lung tumor
fluorescent
labeled-mAb
'fence'
Untreated lung

FIGURE 12

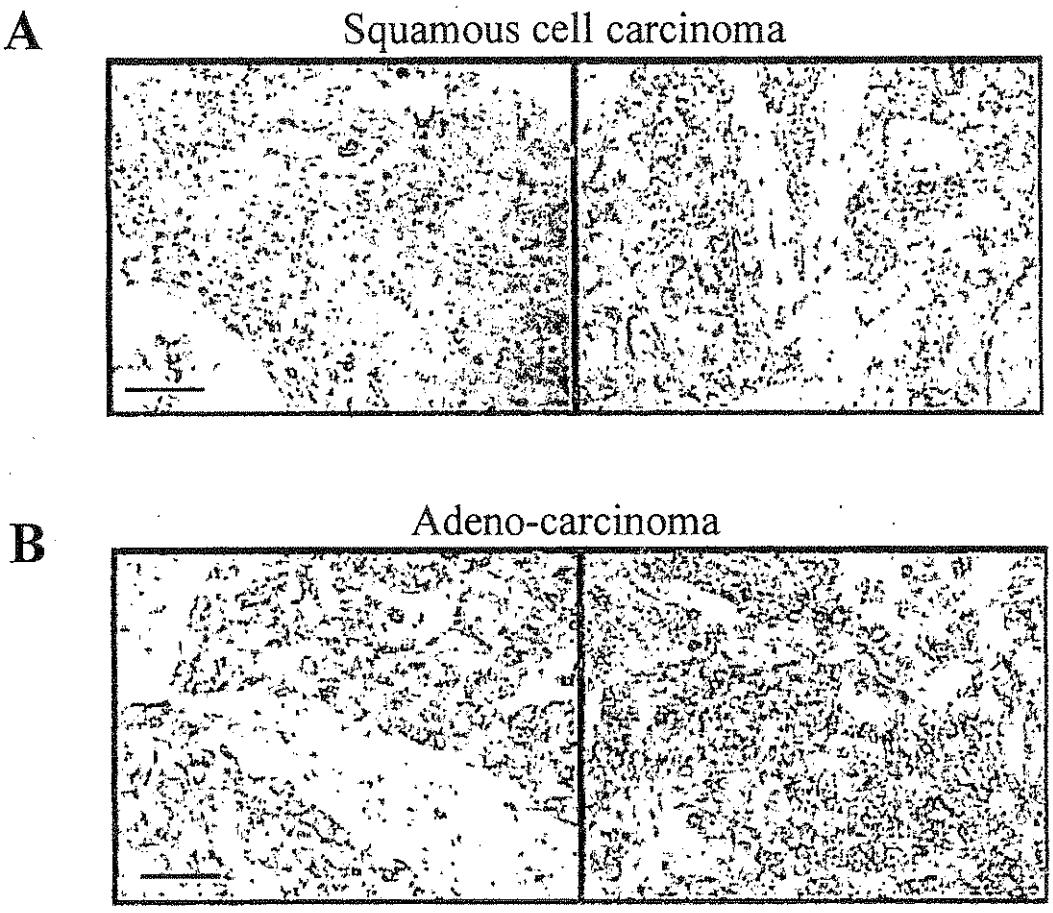


**FIGURE 12 (CONTINUED)**

**C** **Summary of PRL-3 expression in human lung cancers**

| Subtypes of lung cancers | Squamous cell carcinoma | Adeno-carcinoma | Small cell carcinoma | Large cell carcinoma | Carcinoid | Normal lung |
|---|---|---|---|---|---|---|
| Total samples | 74 | 68 | 12 | 5 | 4 | 8 |
| PRL-3 positive samples | 23 | 18 | 1 | 1 | 1 | 0 |
| % of PRL-3 positive samples | 31.1 % | 26.4 % | 8.3 % | 1/5 | 1/4 | 0/8 |

**D** **PRL-3 overexpressed in 35 % of AML**

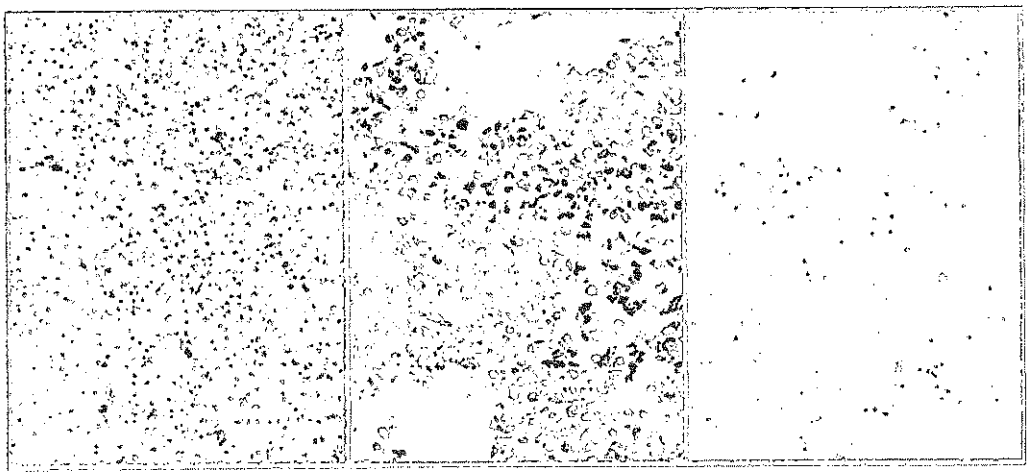


**FIGURE 13**



**FIGURE 14**

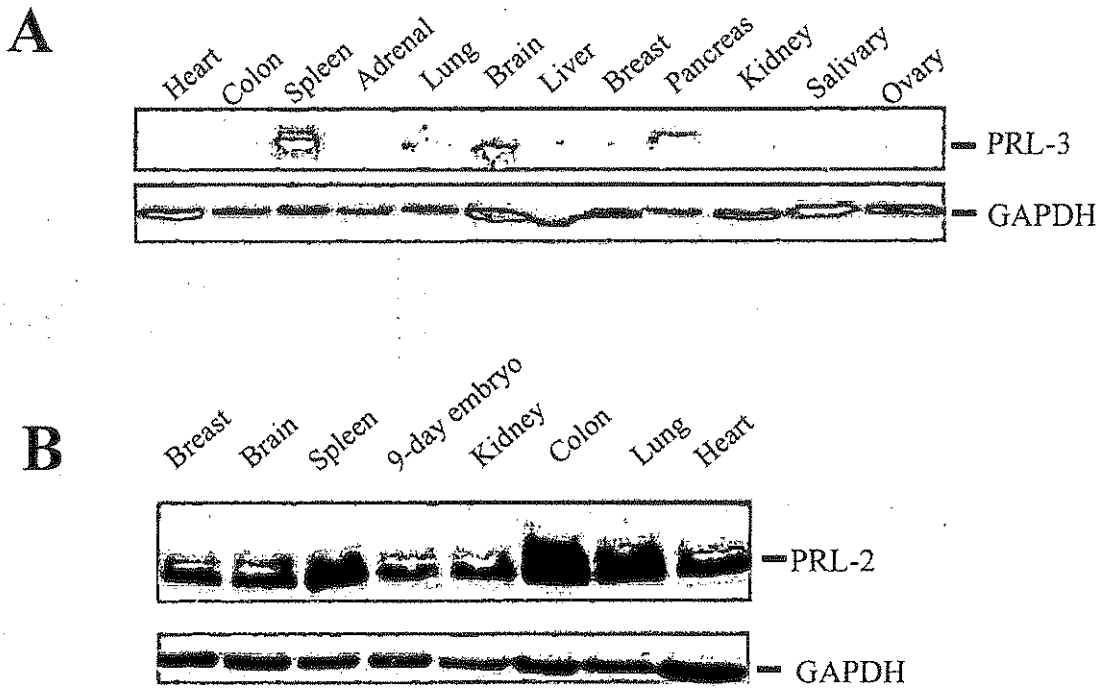
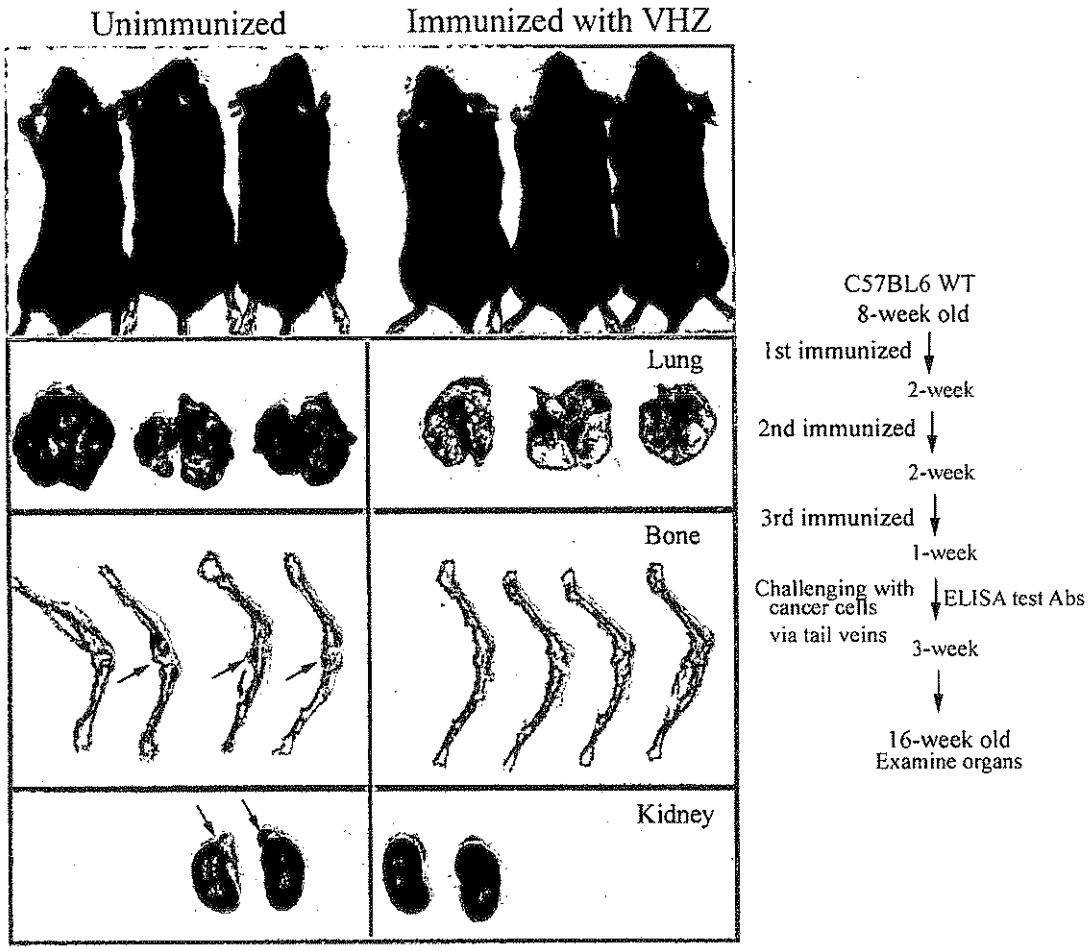

A
Heart
Colon
Spleen
Adrenal
Lung
Brain
Liver
Breast
Pancreas
Kidney
Salivary
Ovary
PRL-3
GAPDH
B
Breast
Brain
Spleen
9-day embryo
Kidney
Colon
Lung
Heart
PRL-2
GAPDH
C
PRL-2 Ab
Untreated
PRL-2 Ab
PRL-3 Ab
Died 1-week earlier

**FIGURE 15**




C57BL6 mice Challenging with
B16F0-VHZ-overexpressing melanoma cells

Arrows point to melanoma tumors