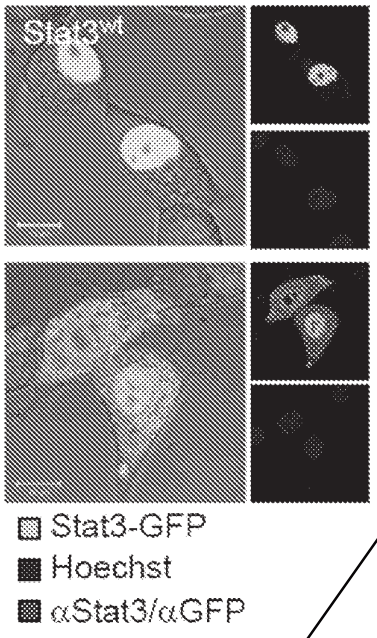

Stat3-GFP
Hoechst
αStat3/αGFP

FIG. 1A

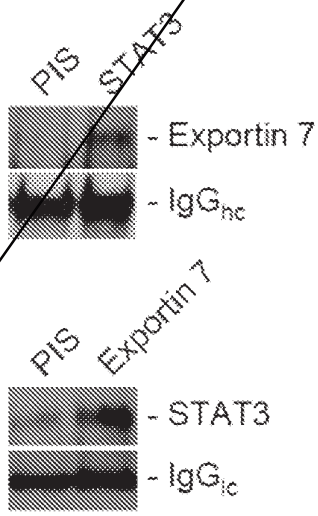

PIS
STAT3
- Exportin 7
- IgG hc
PIS
Exportin 7
- STAT3
- IgG lc

FIG. 1B

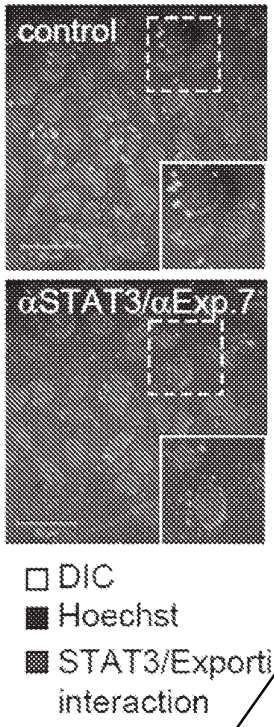

control
αSTAT3/αExp.7
DIC
Hoechst
STAT3/Exportin 7 interaction

FIG. 1C

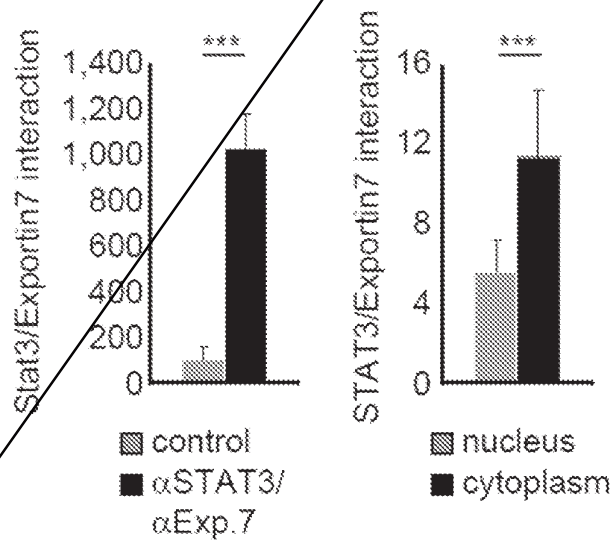

Stat3/Exportin7 interaction
1,400
1,200
1,000
800
600
400
200
0
***
control
αSTAT3/ αExp.7
STAT3/Exportin7 interaction
16
12
8
4
0
***
nucleus
cytoplasm

FIG.1D

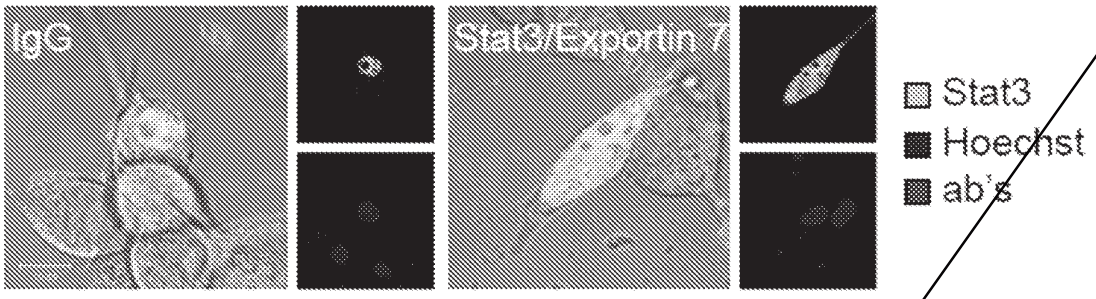


FIG. 1E

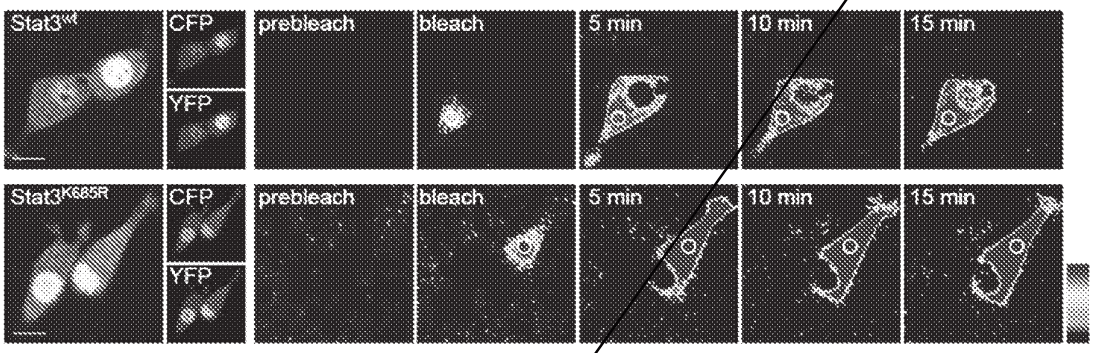


FIG. 2A

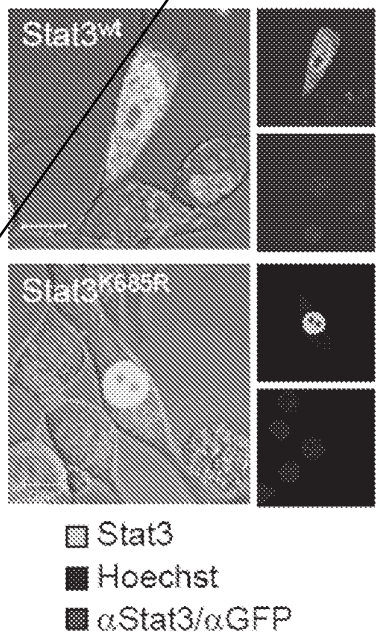


FIG. 2B

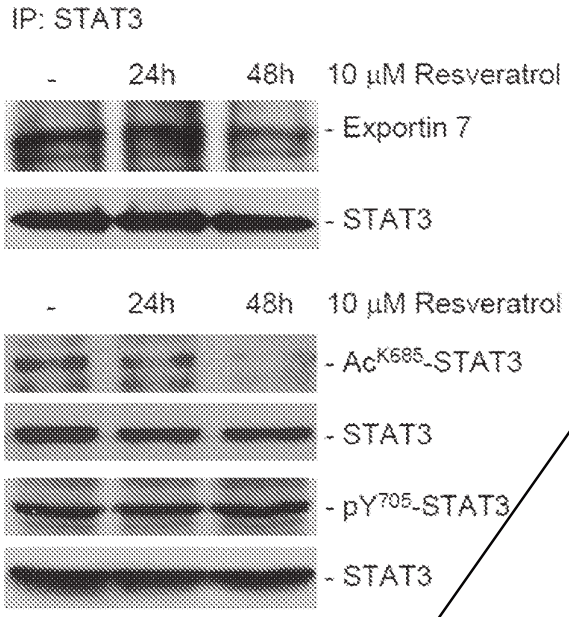

IP: STAT3
- 24h 48h 10 μM Resveratrol
- Exportin 7
- STAT3
- 24h 48h 10 μM Resveratrol
- Ac^{K685}-STAT3
- STAT3
- pY^{705}-STAT3
- STAT3

FIG. 2C

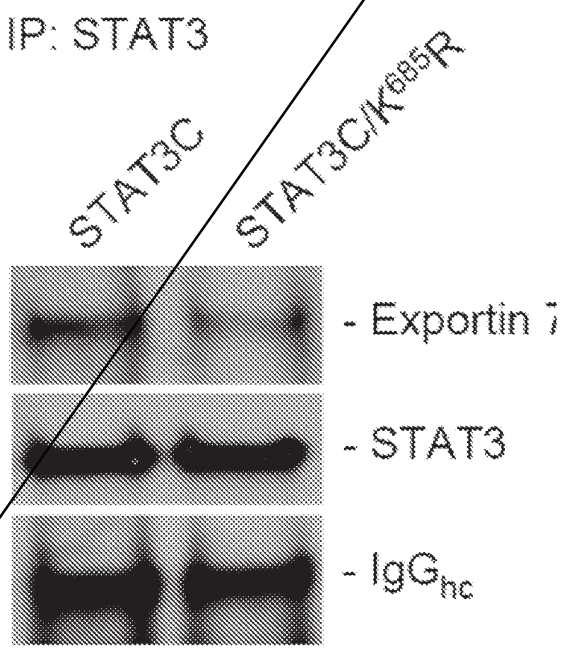

IP: STAT3
STAT3C
STAT3C/K^{685}R
- Exportin 7
- STAT3
- IgG$_{hc}$

FIG. 2D

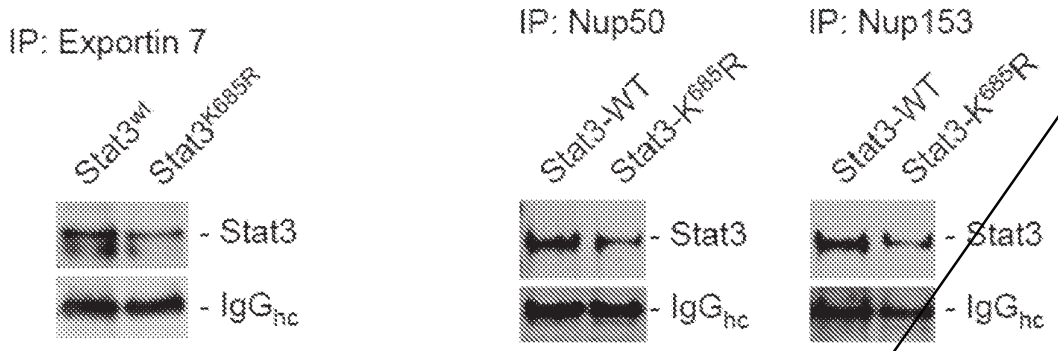

IP: Exportin 7
Stat3wt
Stat3K685R
- Stat3
- IgGhc
IP: Nup50
Stat3-WT
Stat3-K685R
- Stat3
- IgGhc
IP: Nup153
Stat3-WT
Stat3-K685R
- Stat3
- IgGhc

FIG. 2E

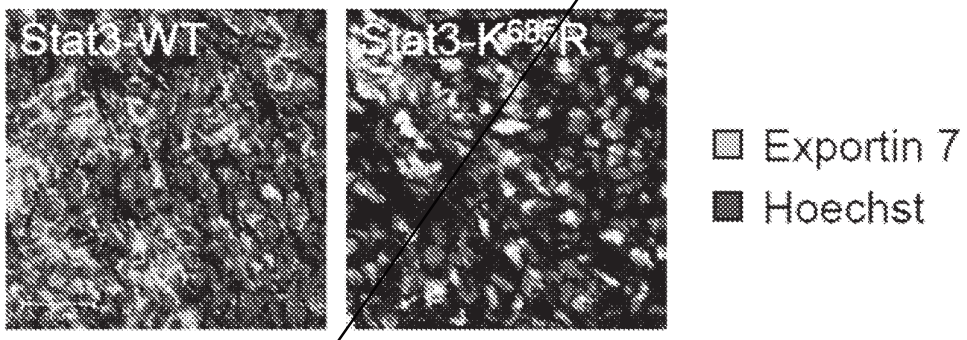

Stat3-WT
Stat3-K685R
Exportin 7
Hoechst

FIG. 2F

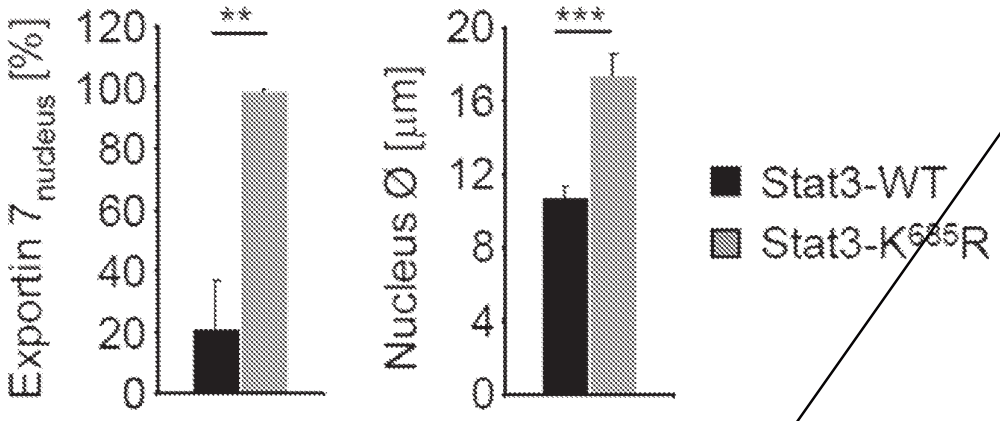

Exportin 7 nucleus [%]
120
100
80
60
40
20
0
**
Nucleus Ø [µm]
20
16
12
8
4
0
***
Stat3-WT
Stat3-K685R

FIG. 2G

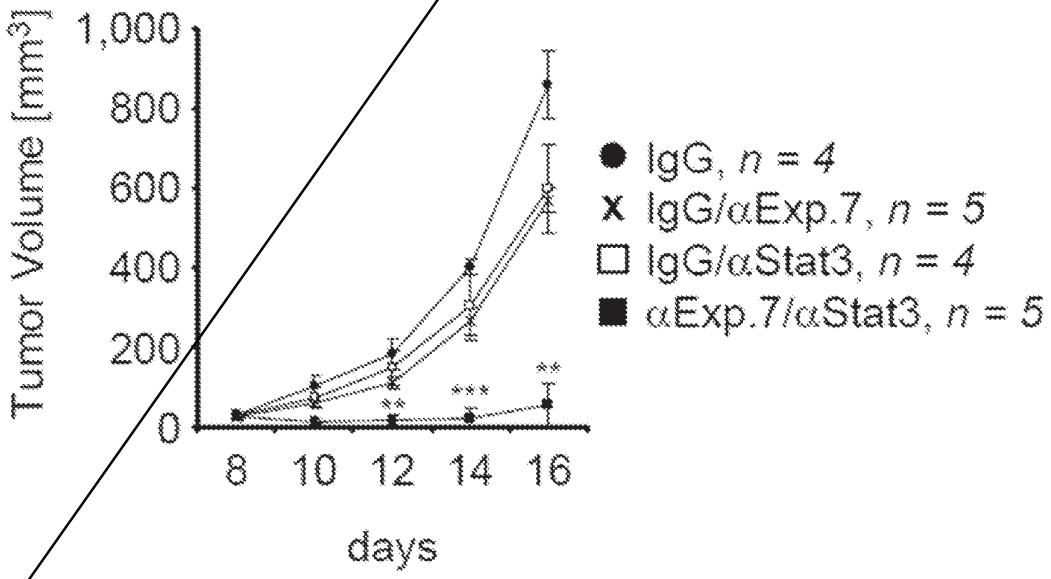

Tumor Volume [mm3]
1,000
800
600
400
200
0
8
10
12
14
16
days
IgG, n = 4
IgG/αExp.7, n = 5
IgG/αStat3, n = 4
αExp.7/αStat3, n = 5

FIG. 3A

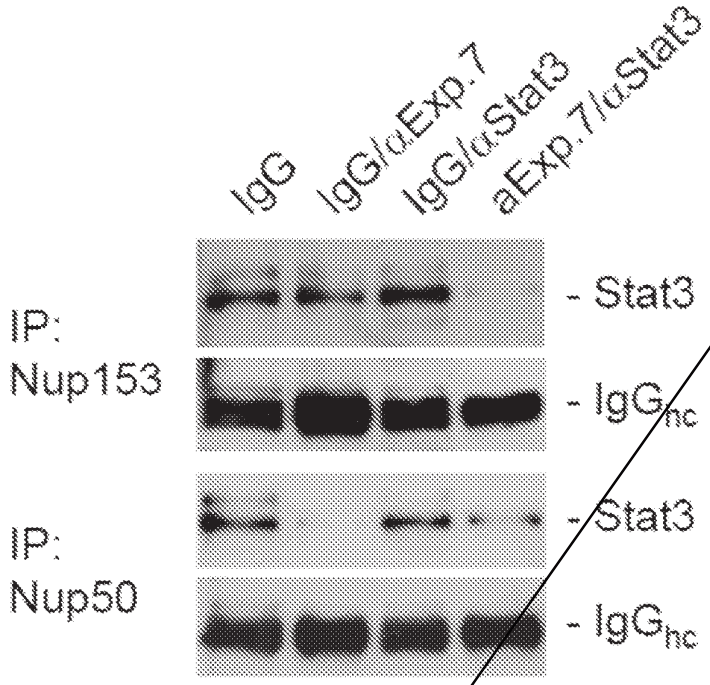

IgG
IgG/αExp.7
IgG/αStat3
αExp.7/αStat3
IP:
Nup153
- Stat3
- IgGhc
IP:
Nup50
- Stat3
- IgGhc

FIG. 3B

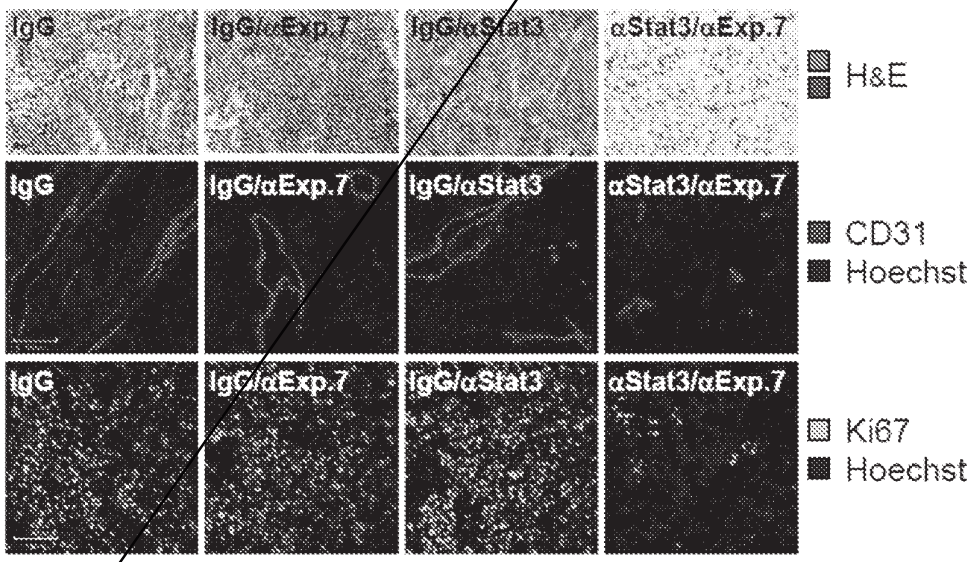

IgG
IgG/αExp.7
IgG/αStat3
αStat3/αExp.7
H&E
CD31
Hoechst
Ki67
Hoechst

FIG. 3C

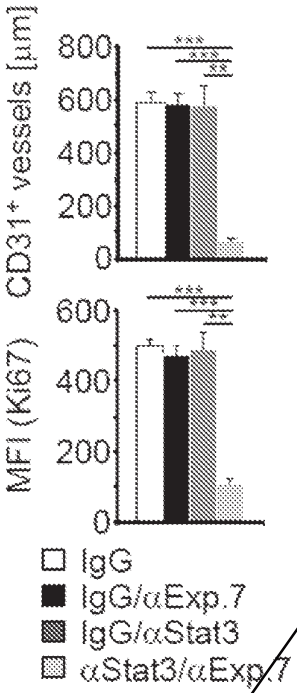

CD31+ vessels [μm]
800
600
400
200
0
***
***
**
MFI (Ki67)
600
400
200
0
***
***
**
IgG
IgG/αExp.7
IgG/αStat3
αStat3/αExp.7

FIG. 3D

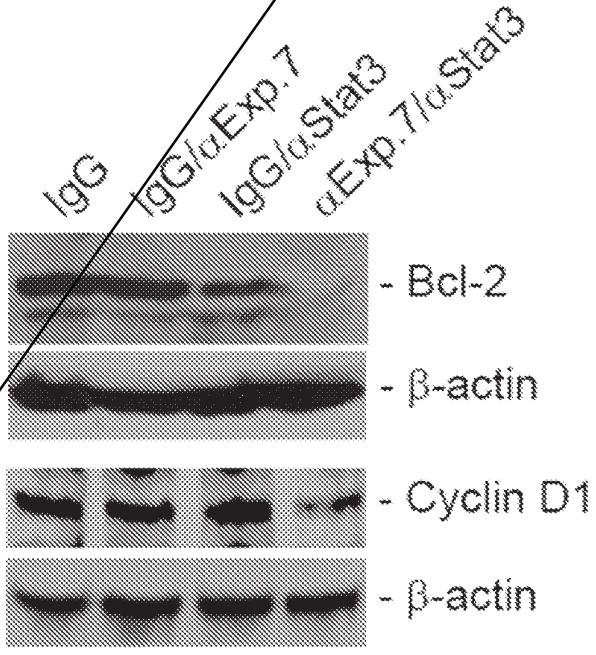

IgG
IgG/αExp.7
IgG/αStat3
αExp.7/αStat3
- Bcl-2
- β-actin
- Cyclin D1
- β-actin

FIG. 3E

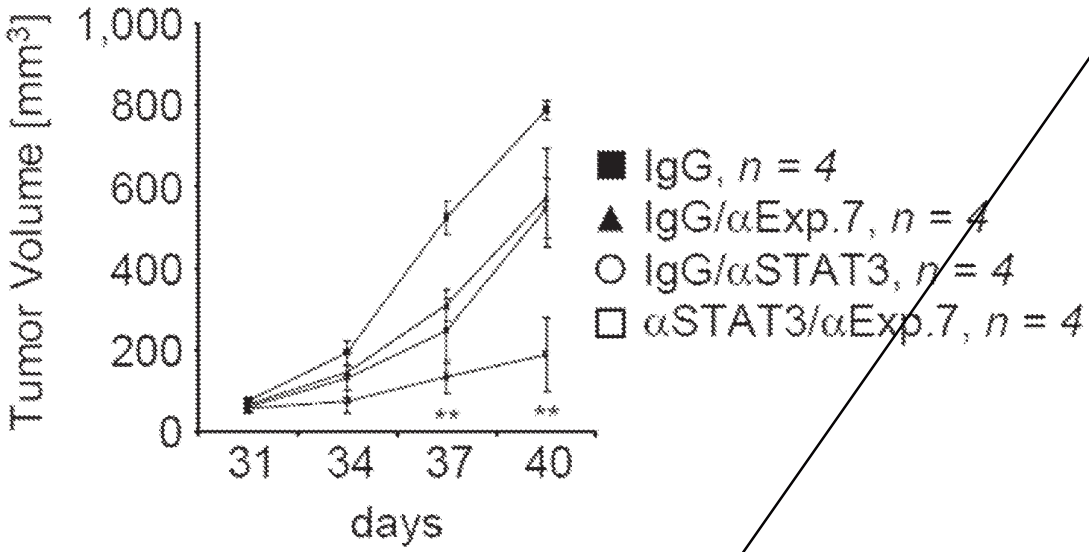

Tumor Volume [mm3]
1,000
800
600
400
200
0
31
34
37
40
days
**
**
IgG, n = 4
IgG/αExp.7, n = 4
IgG/αSTAT3, n = 4
αSTAT3/αExp.7, n = 4

FIG. 3F

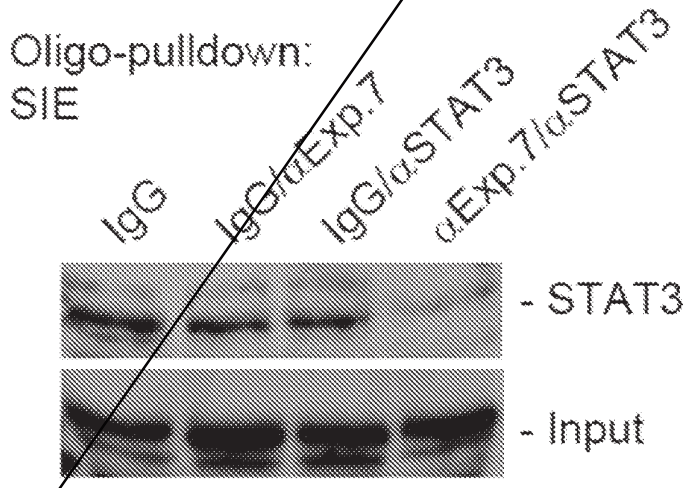

Oligo-pulldown:
SIE
IgG
IgG/αExp.7
IgG/αSTAT3
αExp.7/αSTAT3
- STAT3
- Input

FIG. 3G

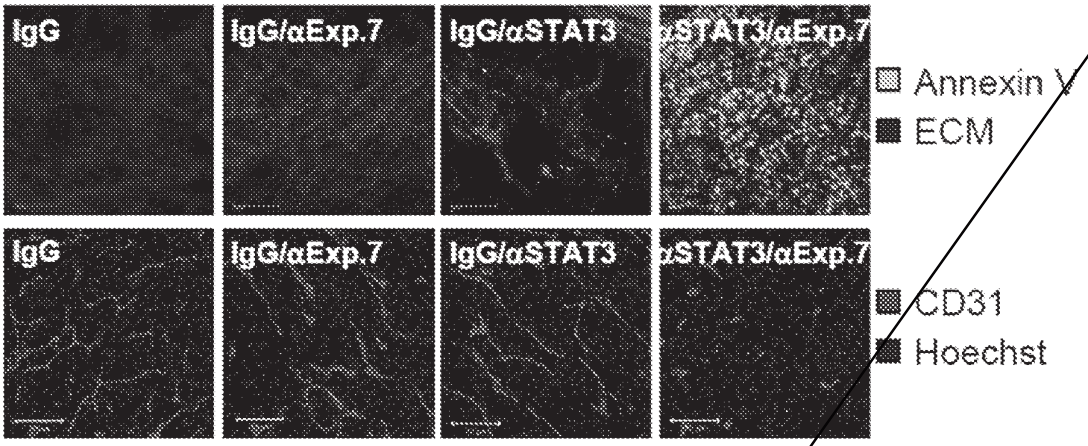


FIG. 3H

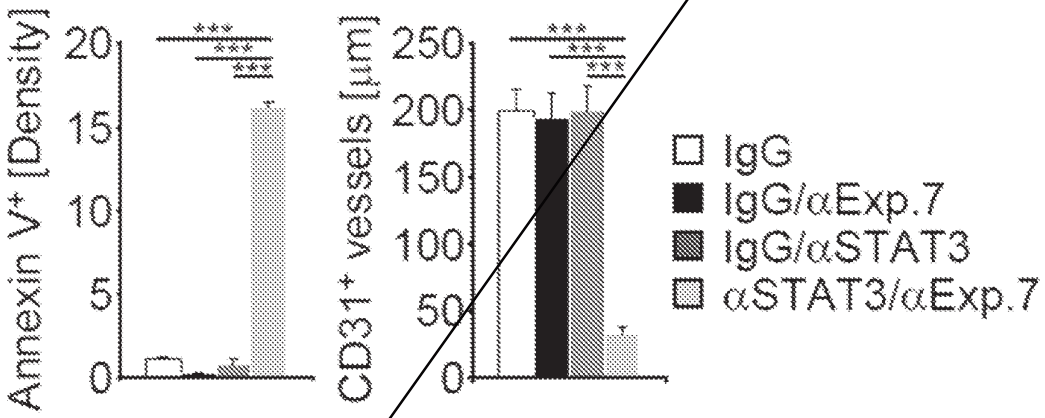


FIG. 3I

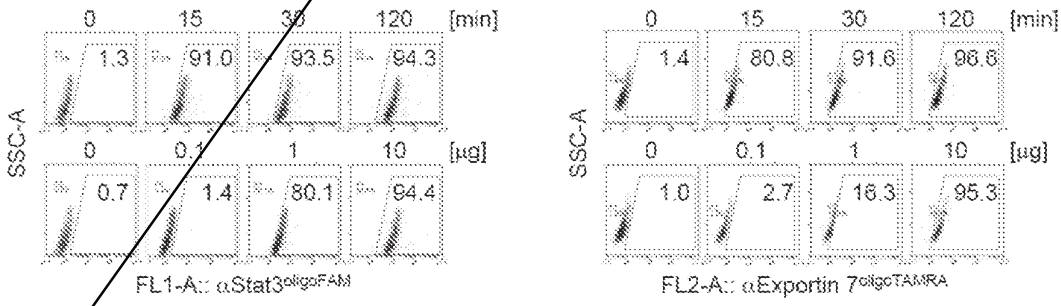


FIG. 4A

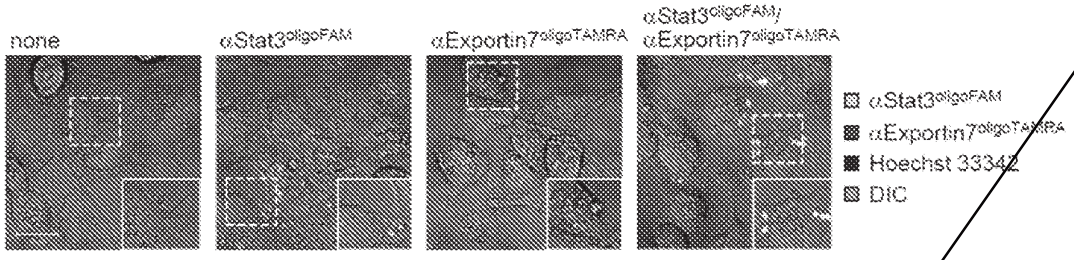

none
αStat3oligoFAM
αExportin7oligoTAMRA
αStat3oligoFAM/
αExportin7oligoTAMRA
αStat3oligoFAM
αExportin7oligoTAMRA
Hoechst 33342
DIC

FIG. 4B

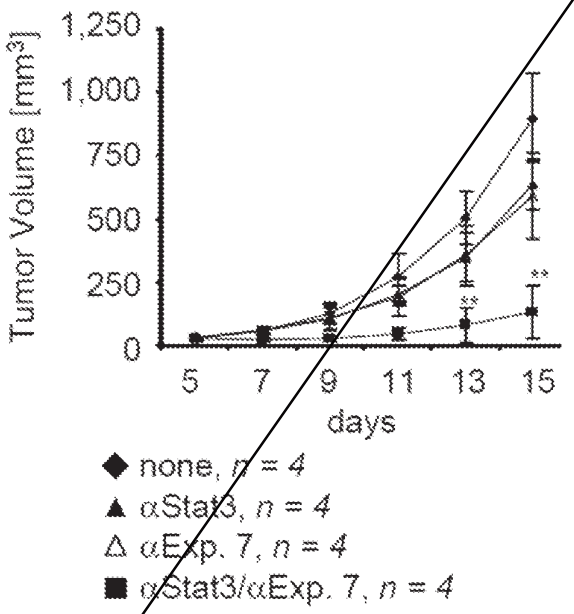

Tumor Volume [mm3]
1,250
1,000
750
500
250
0
5
7
9
11
13
15
days
◆ none, n = 4
▲ αStat3, n = 4
Δ αExp. 7, n = 4
■ αStat3/αExp. 7, n = 4

FIG. 4C

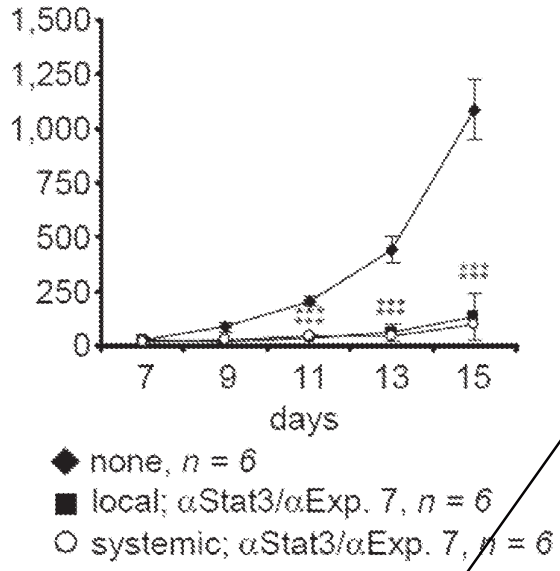

1,500
1,250
1,000
750
500
250
0
7
9
11
13
15
days
none, n = 6
local; αStat3/αExp. 7, n = 6
systemic; αStat3/αExp. 7, n = 6

FIG. 4D

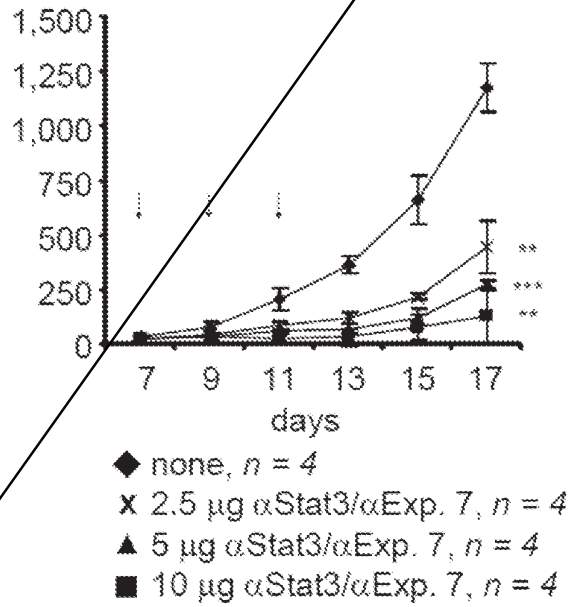

1,500
1,250
1,000
750
500
250
0
7
9
11
13
15
17
days
none, n = 4
2.5 µg αStat3/αExp. 7, n = 4
5 µg αStat3/αExp. 7, n = 4
10 µg αStat3/αExp. 7, n = 4

FIG. 4E

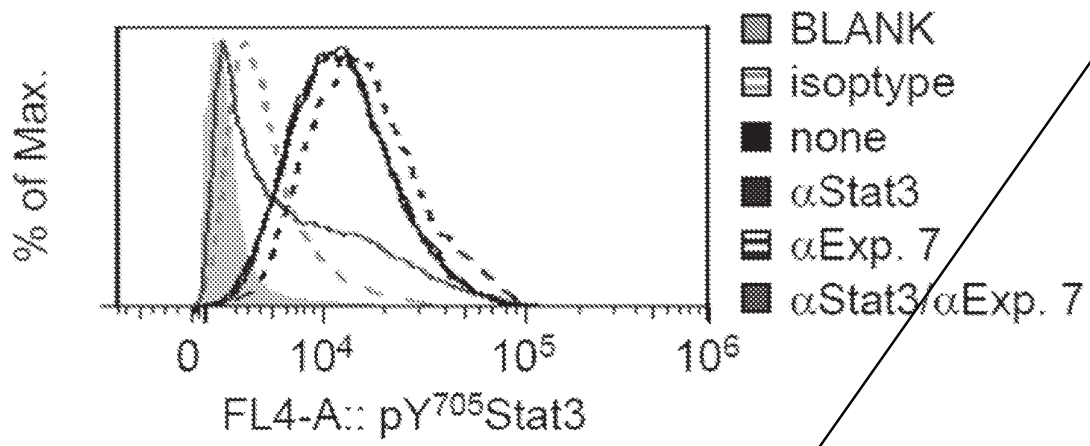

% of Max.
BLANK
isoptype
none
αStat3
αExp. 7
αStat3/αExp. 7
0
10^4
10^5
10^6
FL4-A:: pY705Stat3

FIG. 4F

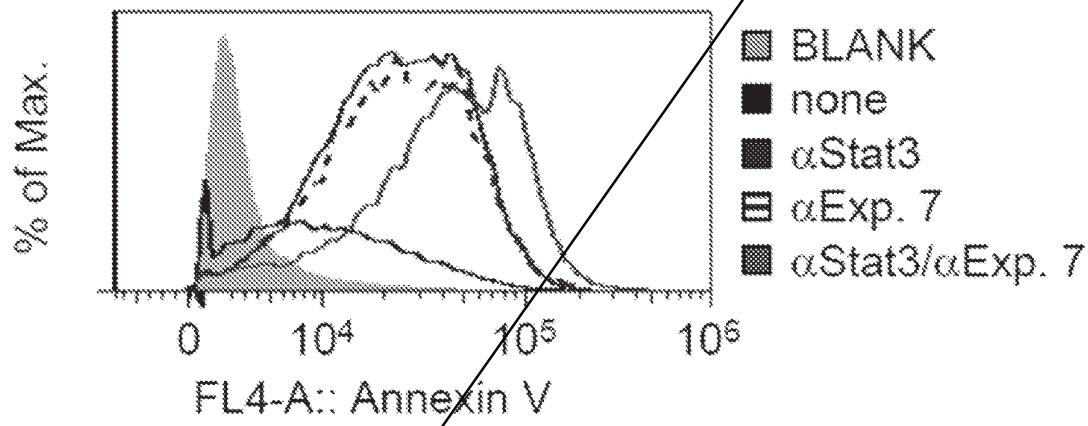

% of Max.
BLANK
none
αStat3
αExp. 7
αStat3/αExp. 7
0
10^4
10^5
10^6
FL4-A:: Annexin V

FIG. 4G

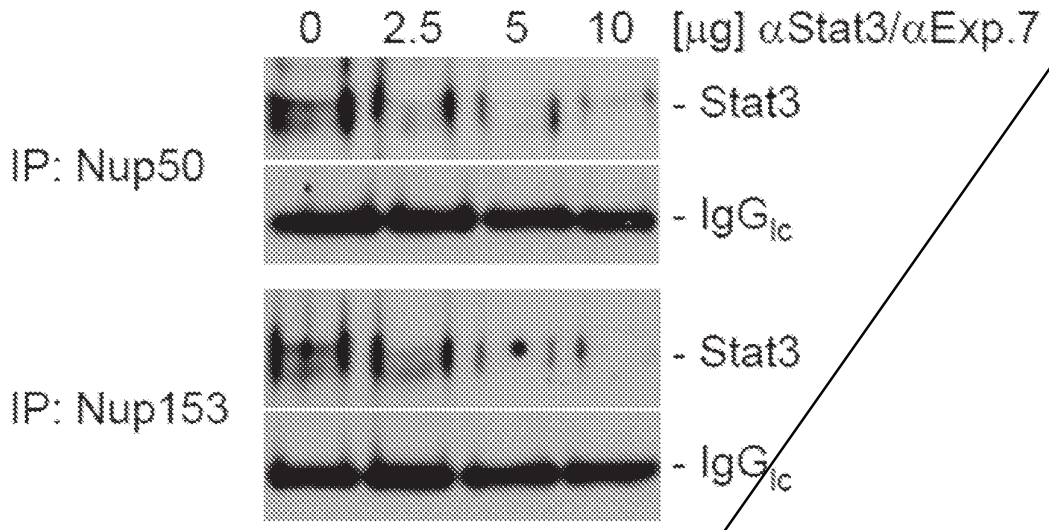

0 2.5 5 10 [µg] αStat3/αExp.7
IP: Nup50
- Stat3
- IgGlc
IP: Nup153
- Stat3
- IgGlc

FIG. 4H

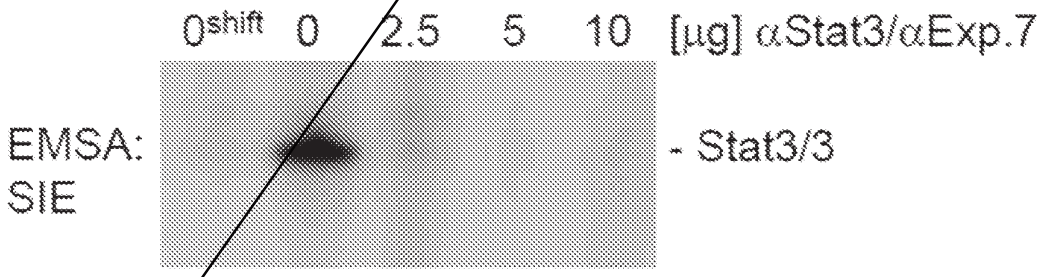

0shift 0 2.5 5 10 [µg] αStat3/αExp.7
EMSA:
SIE
- Stat3/3

FIG. 4I

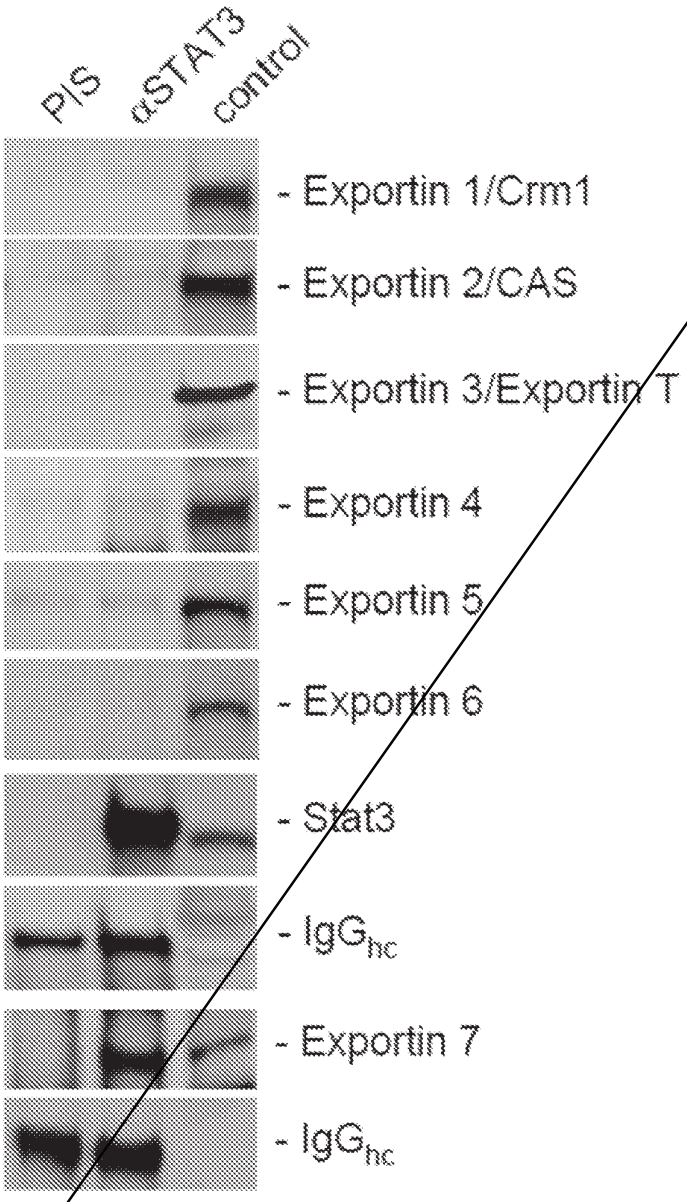

PIS
αSTAT3
control
- Exportin 1/Crm1
- Exportin 2/CAS
- Exportin 3/Exportin T
- Exportin 4
- Exportin 5
- Exportin 6
- Stat3
- IgG$_{hc}$
- Exportin 7
- IgG$_{hc}$

FIG. 5A

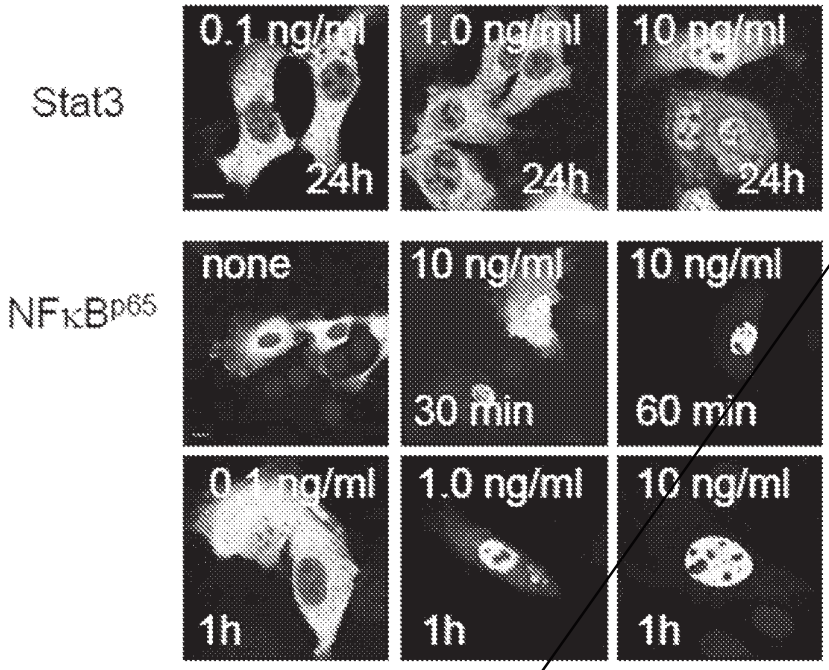
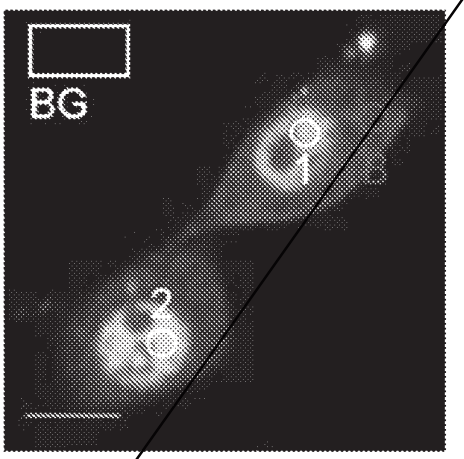


FIG. 5B



BG - Background-ROI

B - Bleach-ROI

1 – detection ROI 1 – nucleus bleached cell

2 – detection ROI 2 – nucleus unbleached cell

FIG. 6A

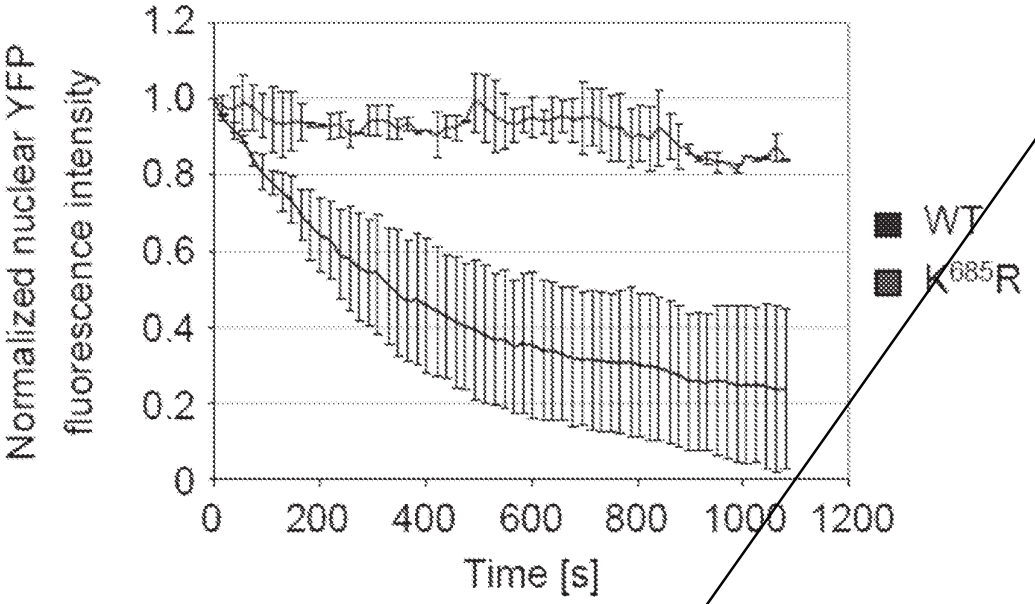

Normalized nuclear YFP fluorescence intensity
1.2
1.0
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
1200
Time [s]
WT
K685R

FIG. 6B

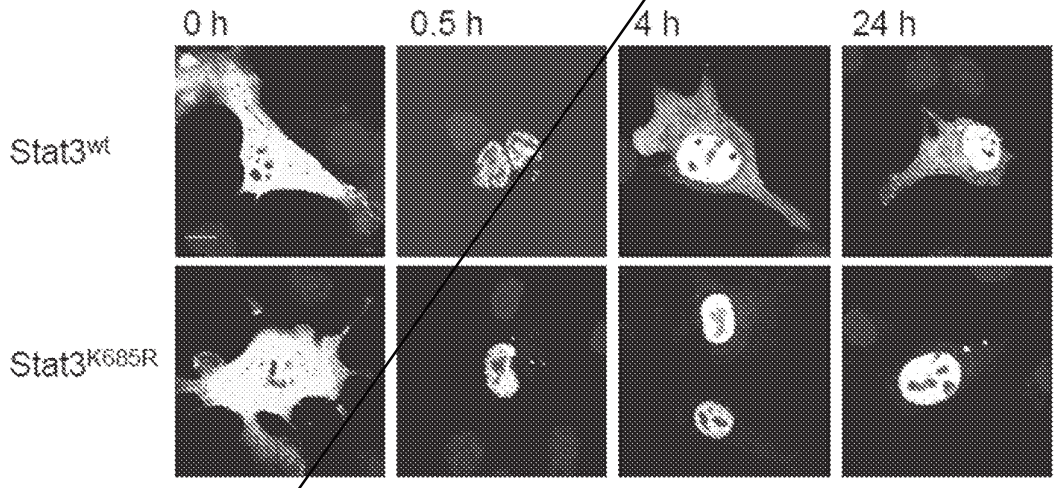

0 h
0.5 h
4 h
24 h
Stat3wt
Stat3K685R

FIG. 7A

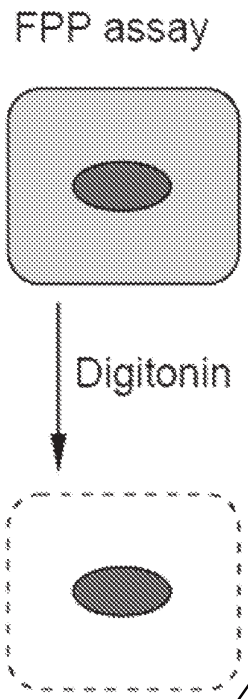

FPP assay
Digitonin

FIG. 7B

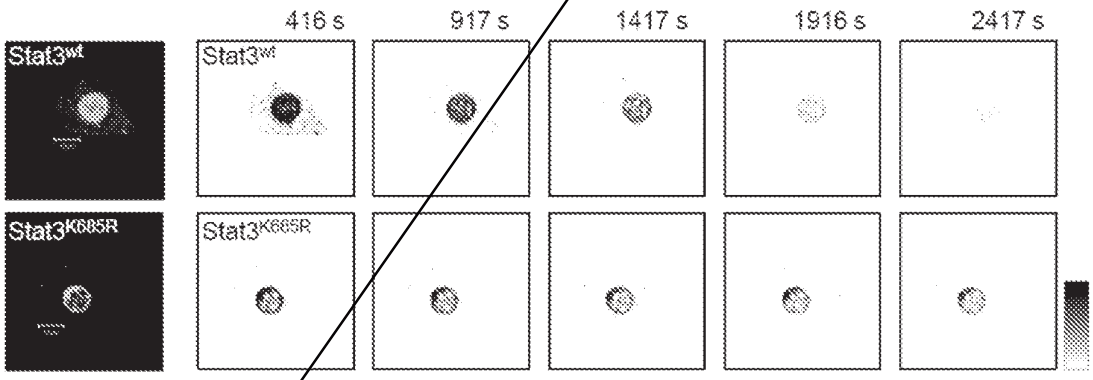

416 s
917 s
1417 s
1916 s
2417 s
Stat3wt
Stat3K685R

FIG. 7C

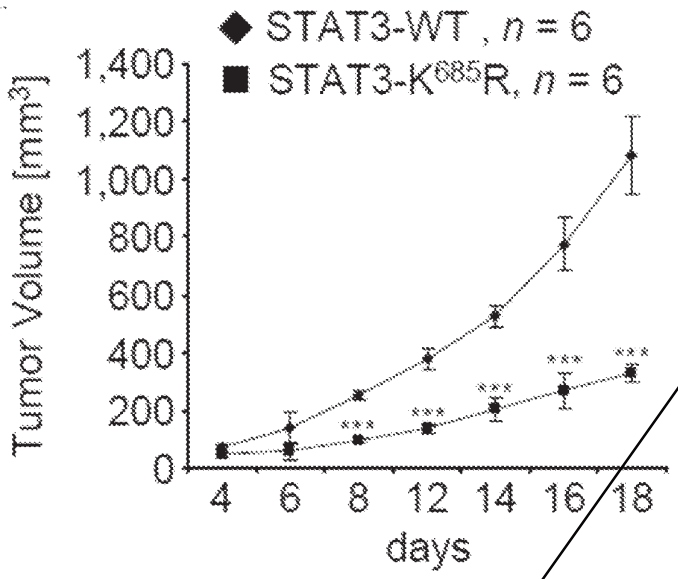

STAT3-WT , n = 6
STAT3-K685R, n = 6
Tumor Volume [mm3]
1,400
1,200
1,000
800
600
400
200
0
4
6
8
12
14
16
18
days

FIG. 8A


Cytoplasmic filaments
Nuclear basket
Nup50
Nup153
Stat3/Exportin 7

FIG. 8B

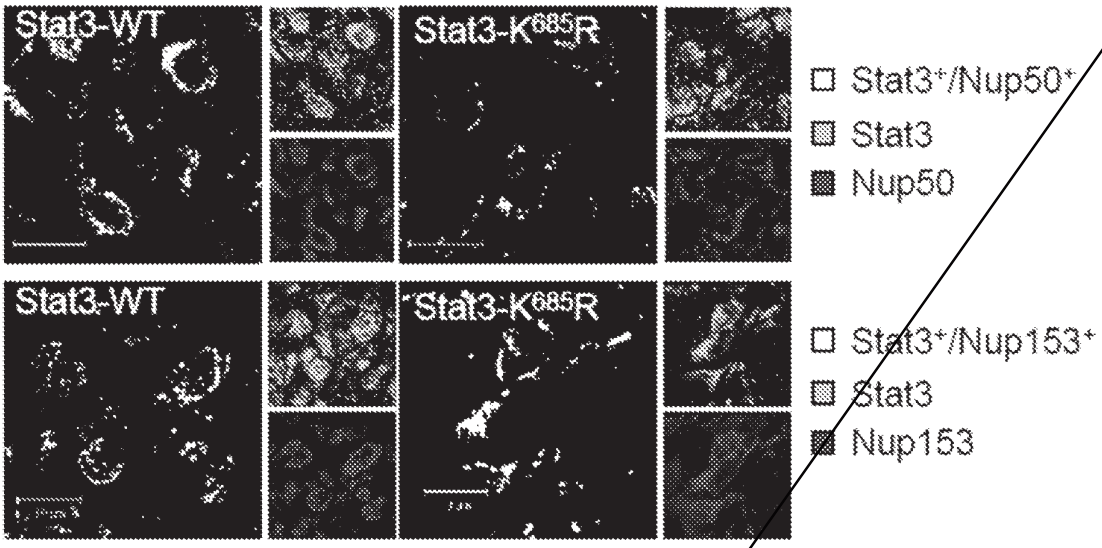

Stat3-WT
Stat3-K685R
Stat3+/Nup50+
Stat3
Nup50
Stat3-WT
Stat3-K685R
Stat3+/Nup153+
Stat3
Nup153

FIG. 8C

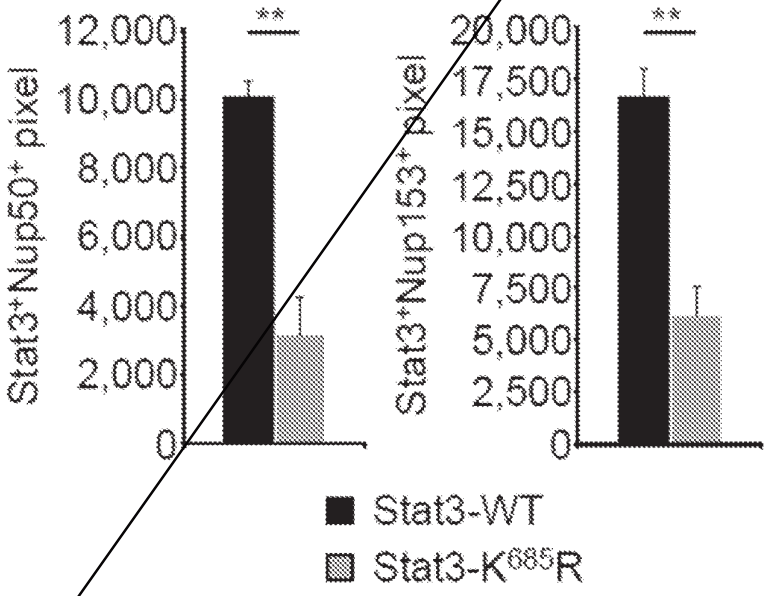

Stat3+Nup50+ pixel
12,000
10,000
8,000
6,000
4,000
2,000
0
**
Stat3+Nup153+ pixel
20,000
17,500
15,000
12,500
10,000
7,500
5,000
2,500
0
**
Stat3-WT
Stat3-K685R

FIG. 8D

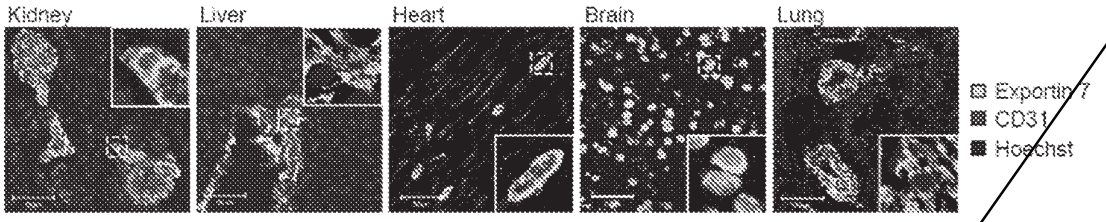

Kidney
Liver
Heart
Brain
Lung
Exportin 7
CD31
Hoechst

FIG. 9

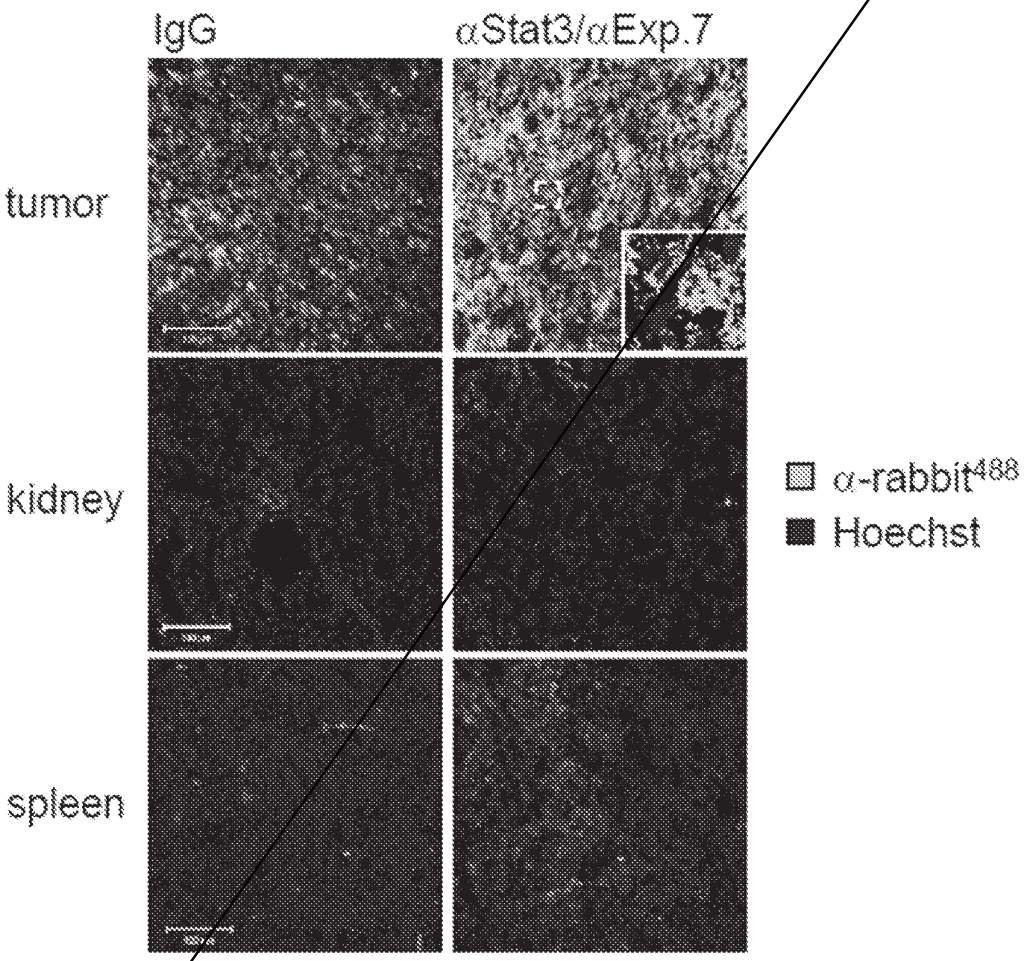

IgG
αStat3/αExp.7
tumor
kidney
spleen
α-rabbit488
Hoechst

FIG. 10A

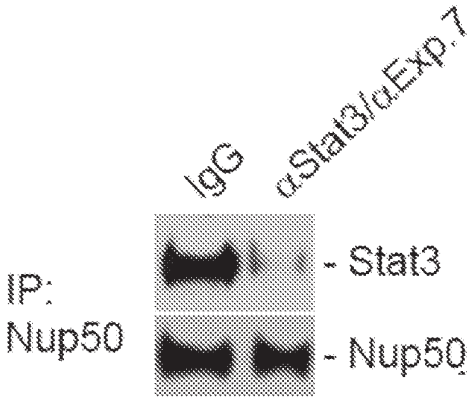


FIG. 10B

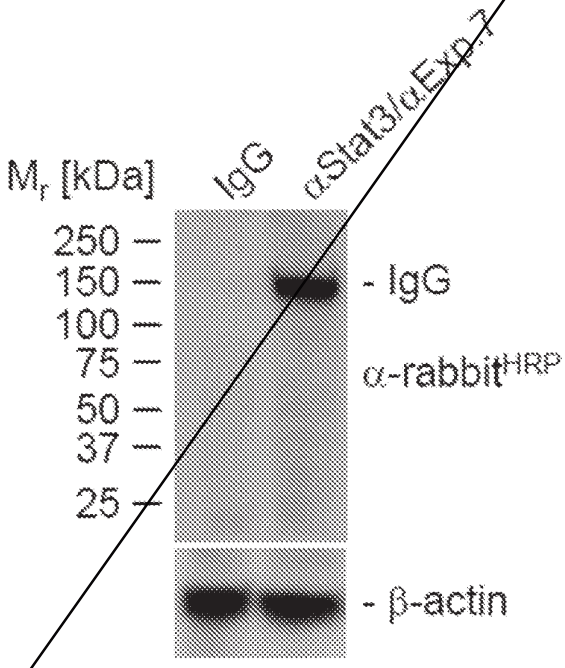


FIG. 10C

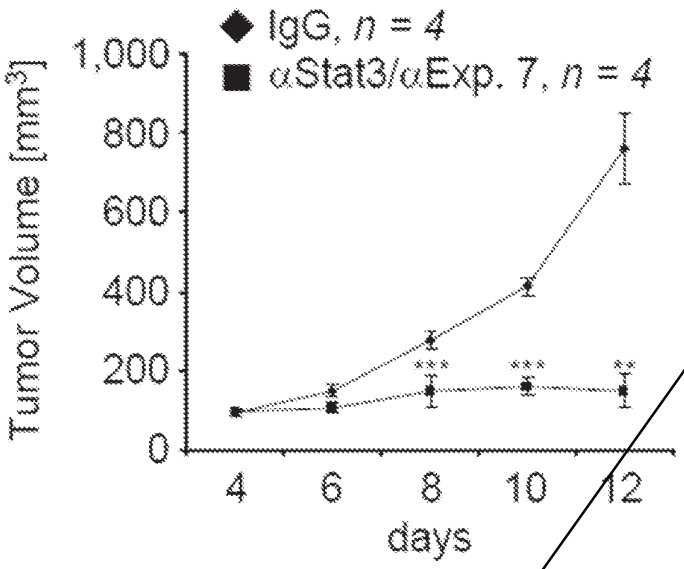


FIG. 11A

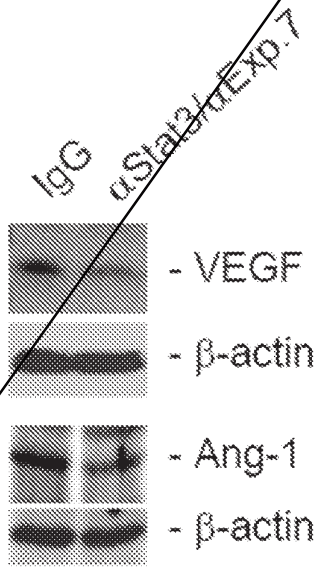


FIG. 11B

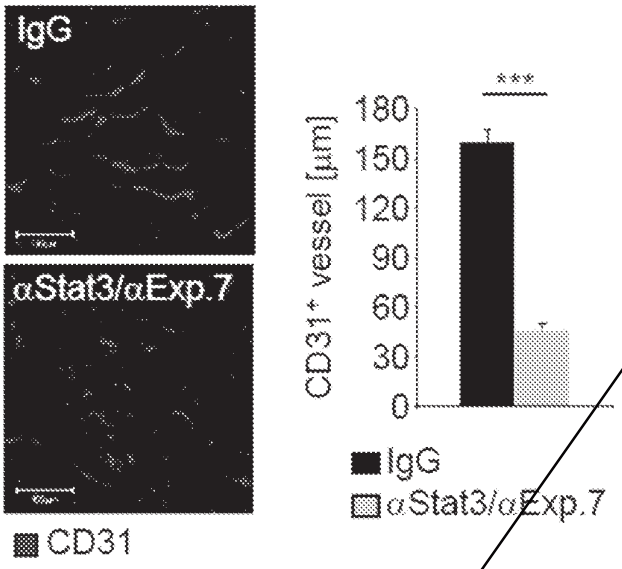


FIG. 11C

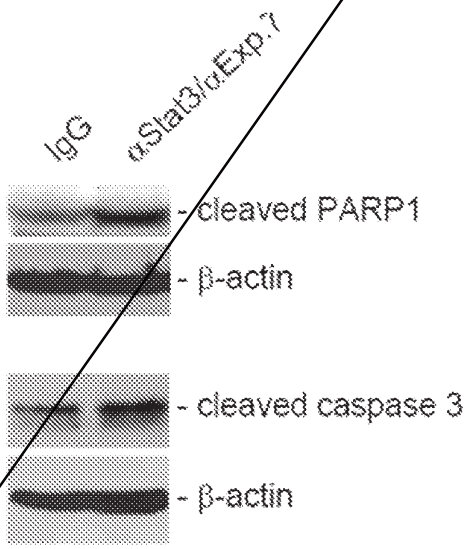


FIG. 11D



FIG. 12A

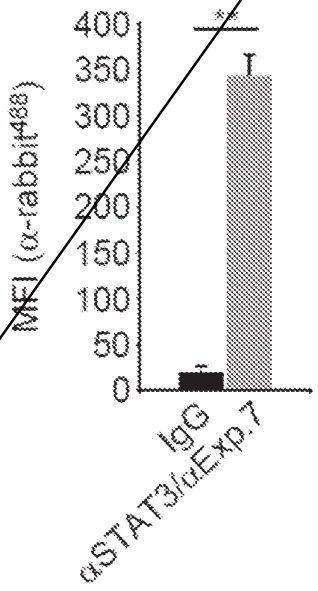


FIG. 12B

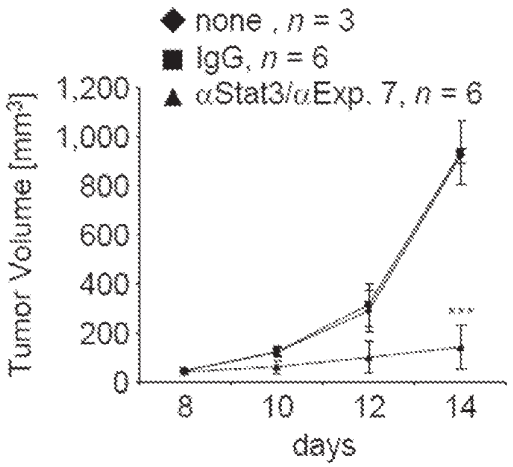


FIG. 12C

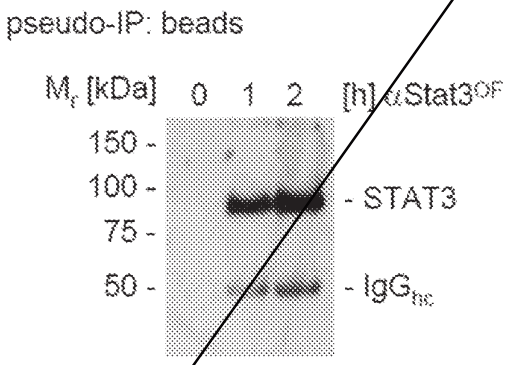


FIG. 13A

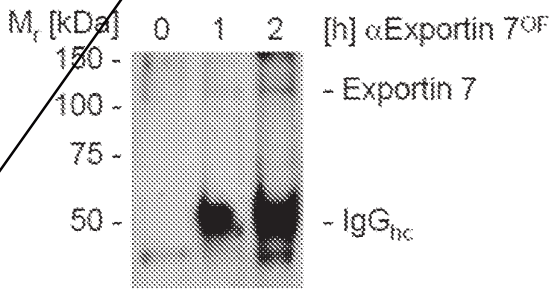


FIG. 13B

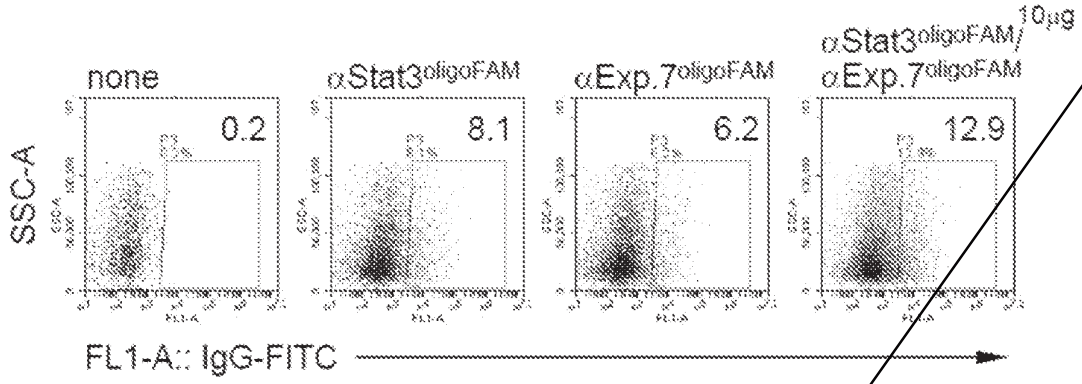


FIG. 14

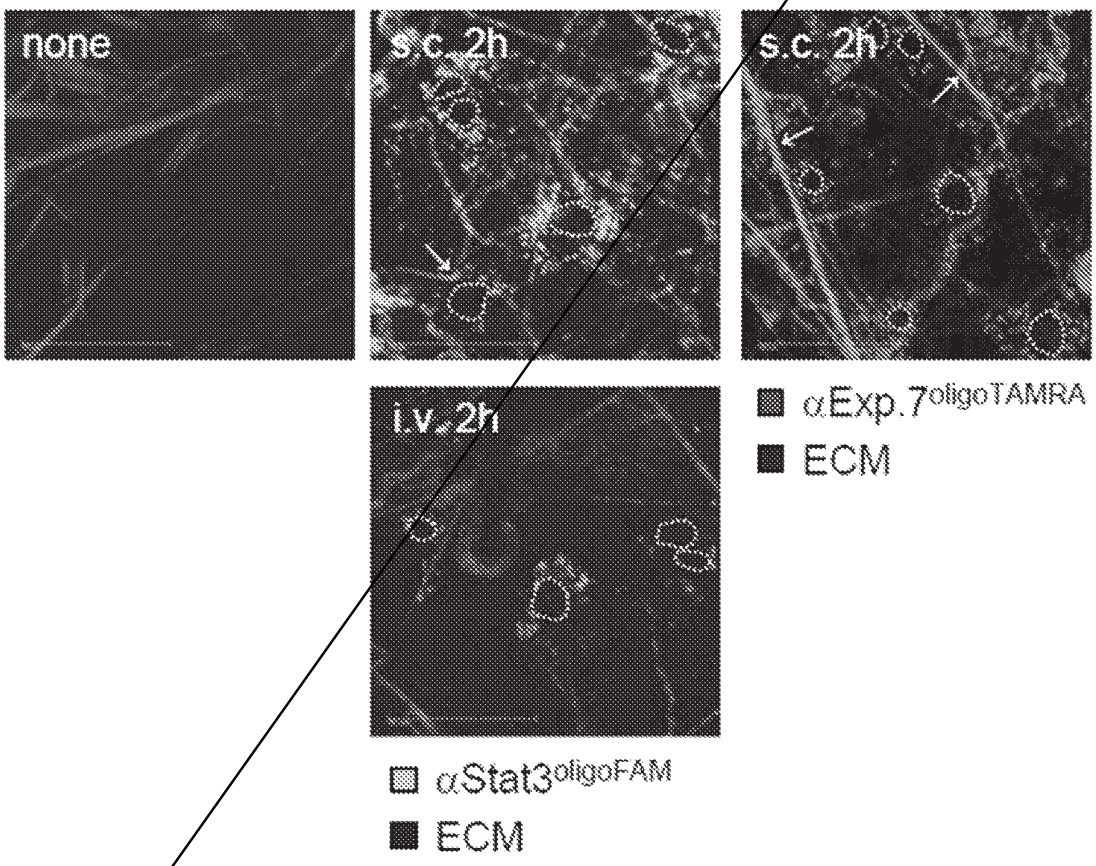


FIG. 15A

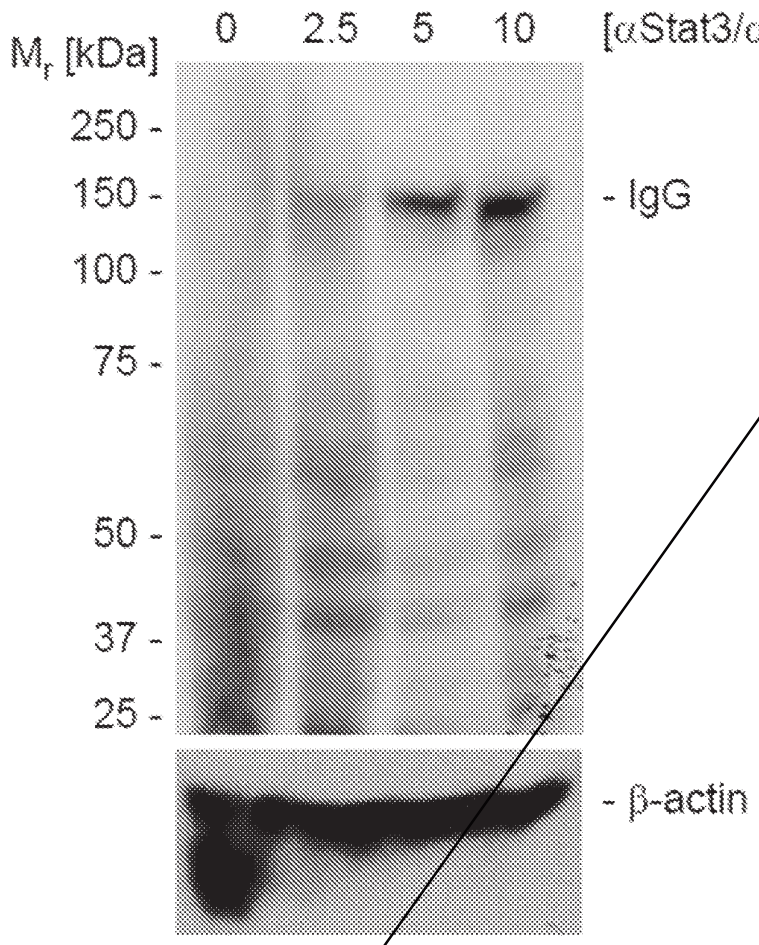


FIG. 15B

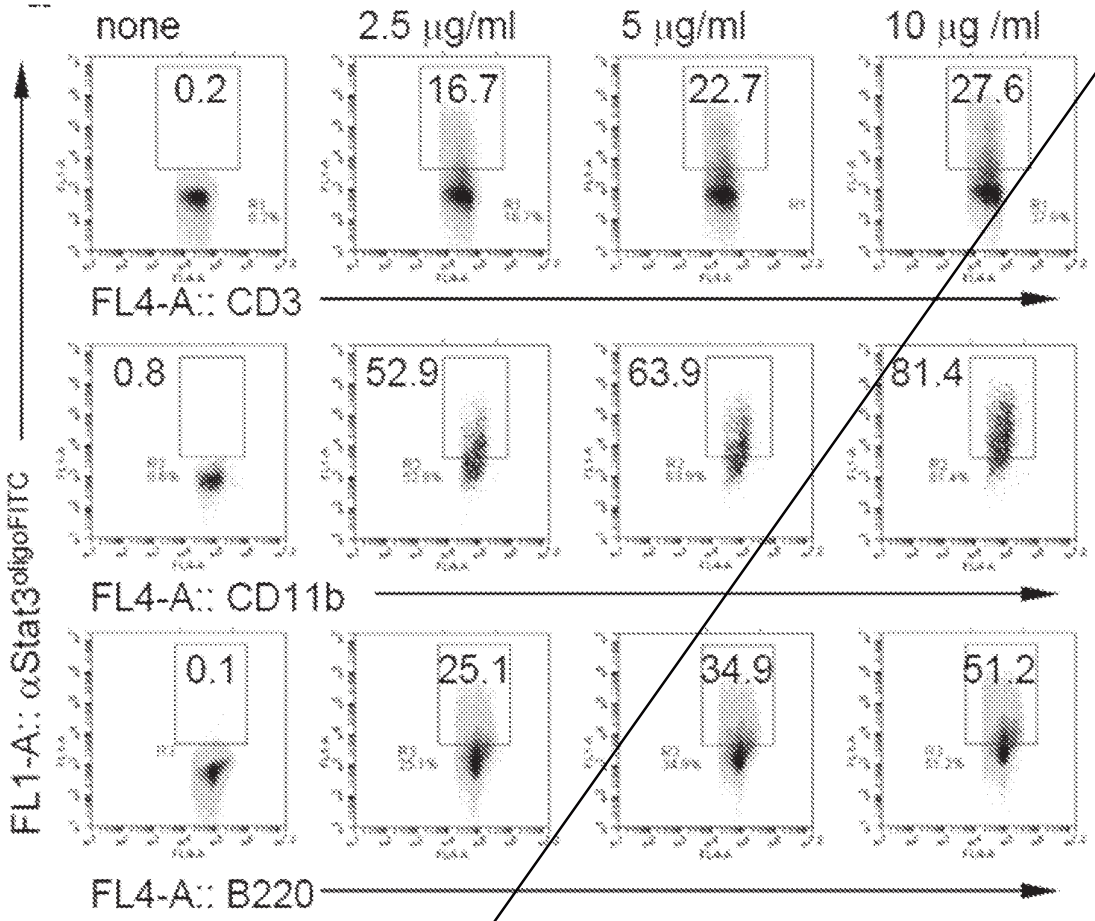

none
2.5 μg/ml
5 μg/ml
10 μg /ml
0.2
16.7
22.7
27.6
FL4-A:: CD3
0.8
52.9
63.9
81.4
FL4-A:: CD11b
0.1
25.1
34.9
51.2
FL4-A:: B220
FL1-A:: αStat3pTyrFITC

FIG. 16A

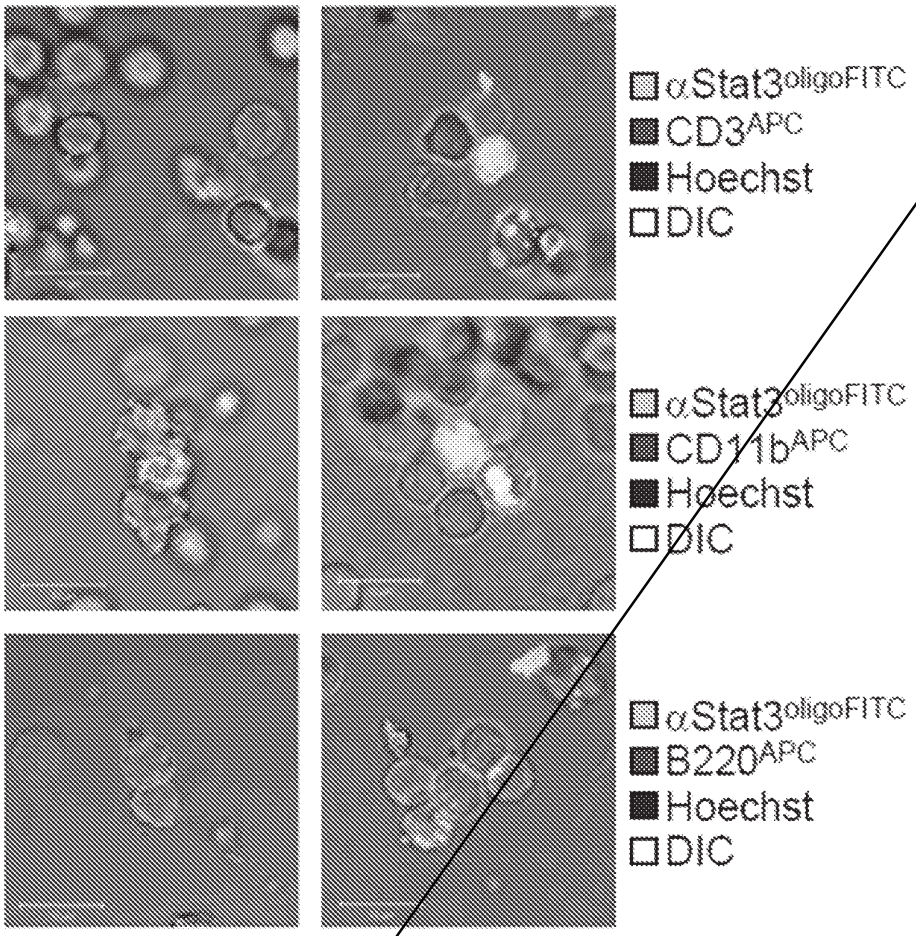

αStat3oligoFITC
CD3APC
Hoechst
DIC
αStat3oligoFITC
CD11bAPC
Hoechst
DIC
αStat3oligoFITC
B220APC
Hoechst
DIC

FIG. 16B

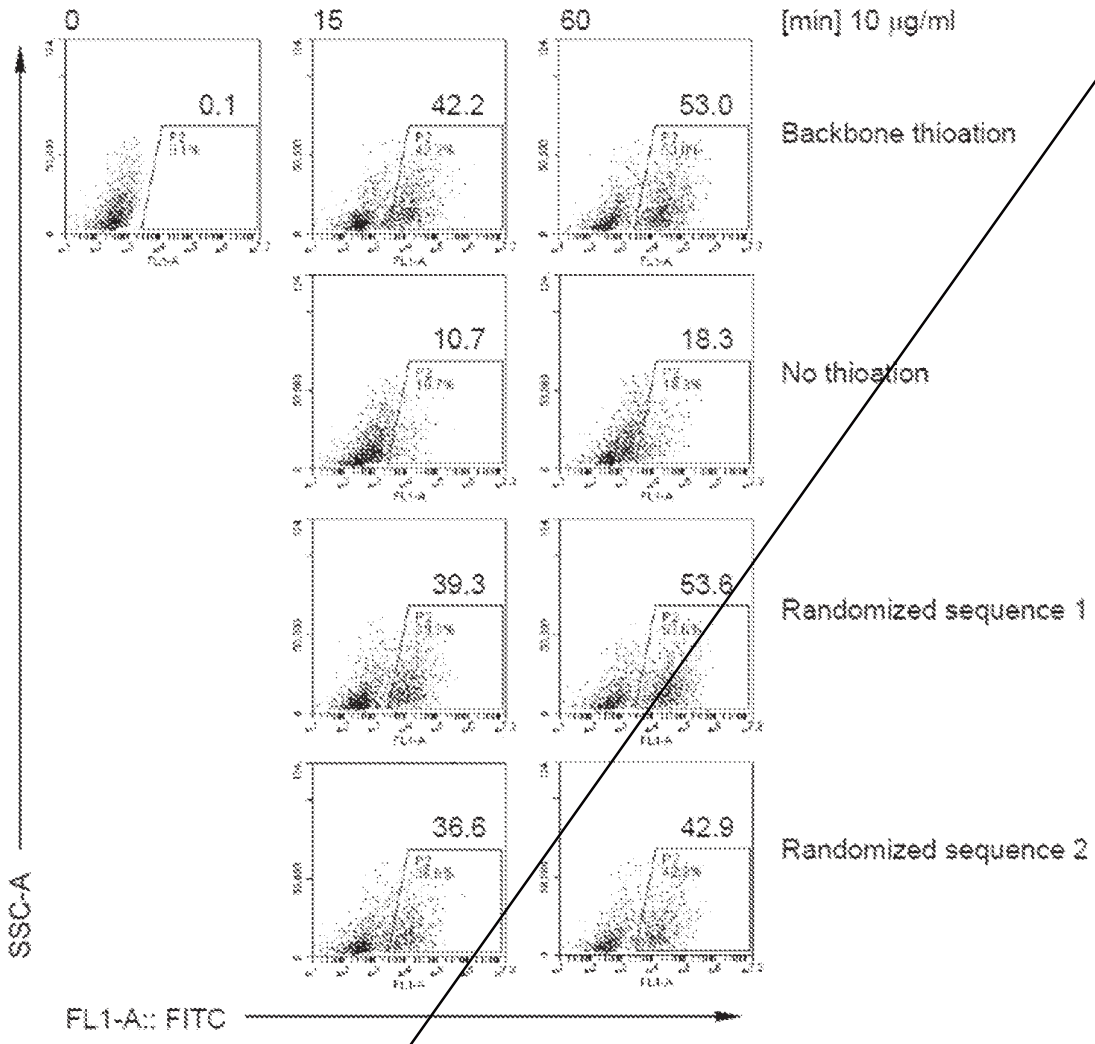


FIG. 17A

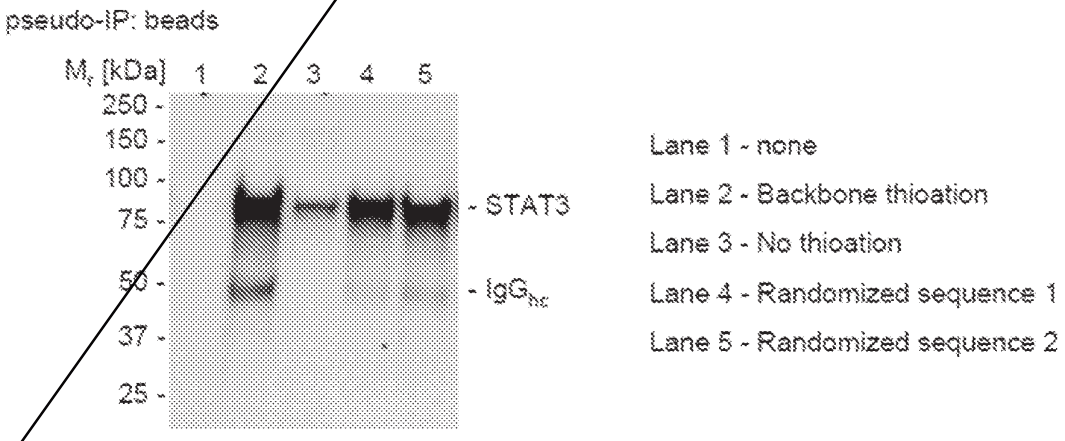


FIG. 17B

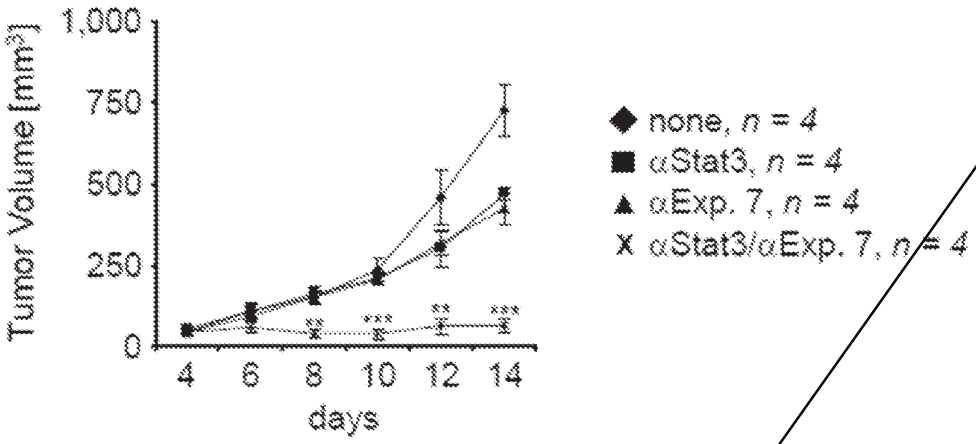

Tumor Volume [mm³]
1,000
750
500
250
0
4
6
8
10
12
14
days
none, n = 4
αStat3, n = 4
αExp. 7, n = 4
αStat3/αExp. 7, n = 4

FIG. 18

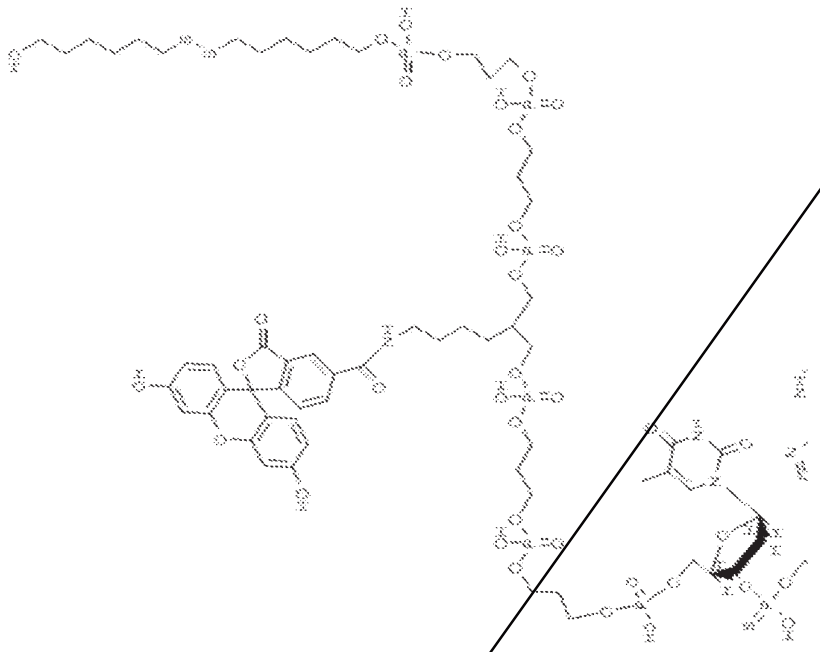


FIG. 19

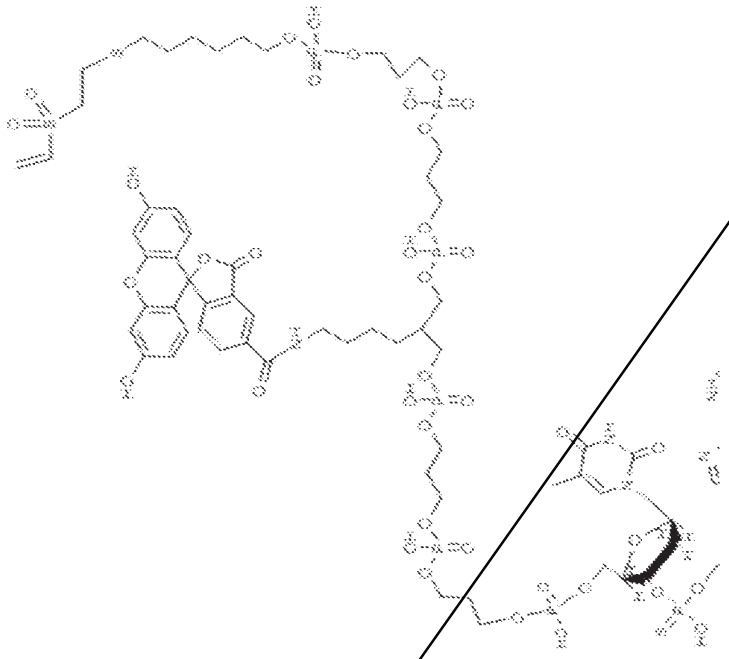


FIG. 20

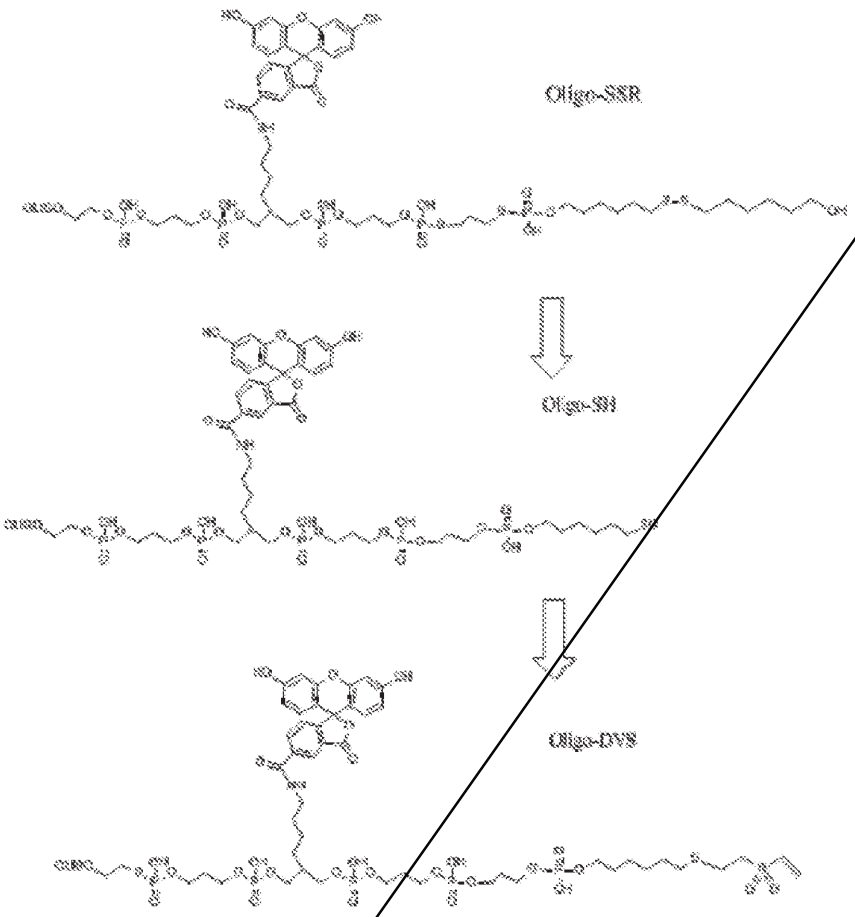


FIG. 21

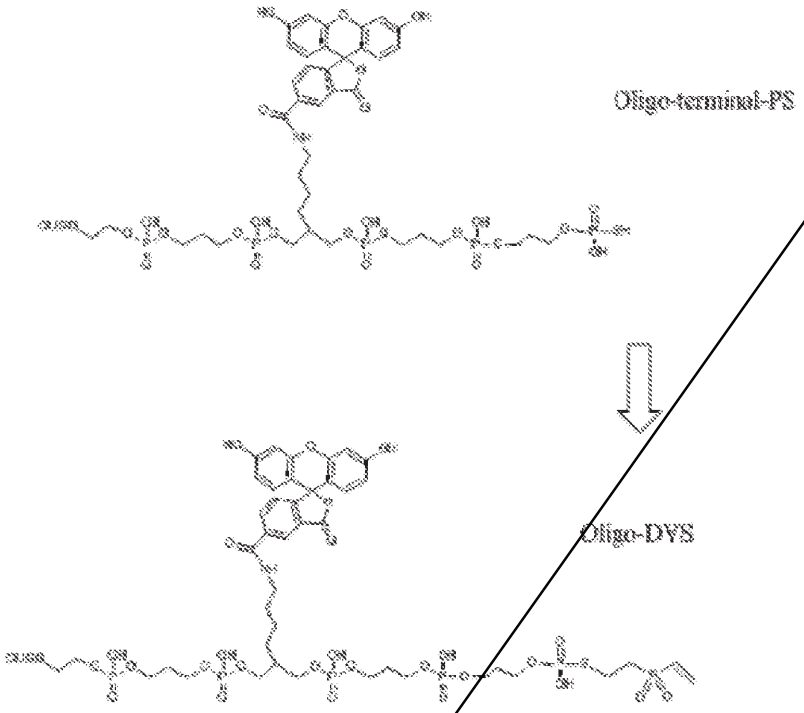


FIG. 22

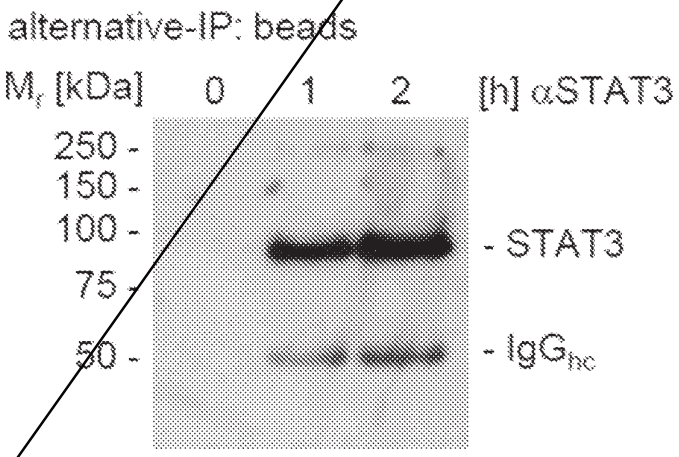


FIG. 23A

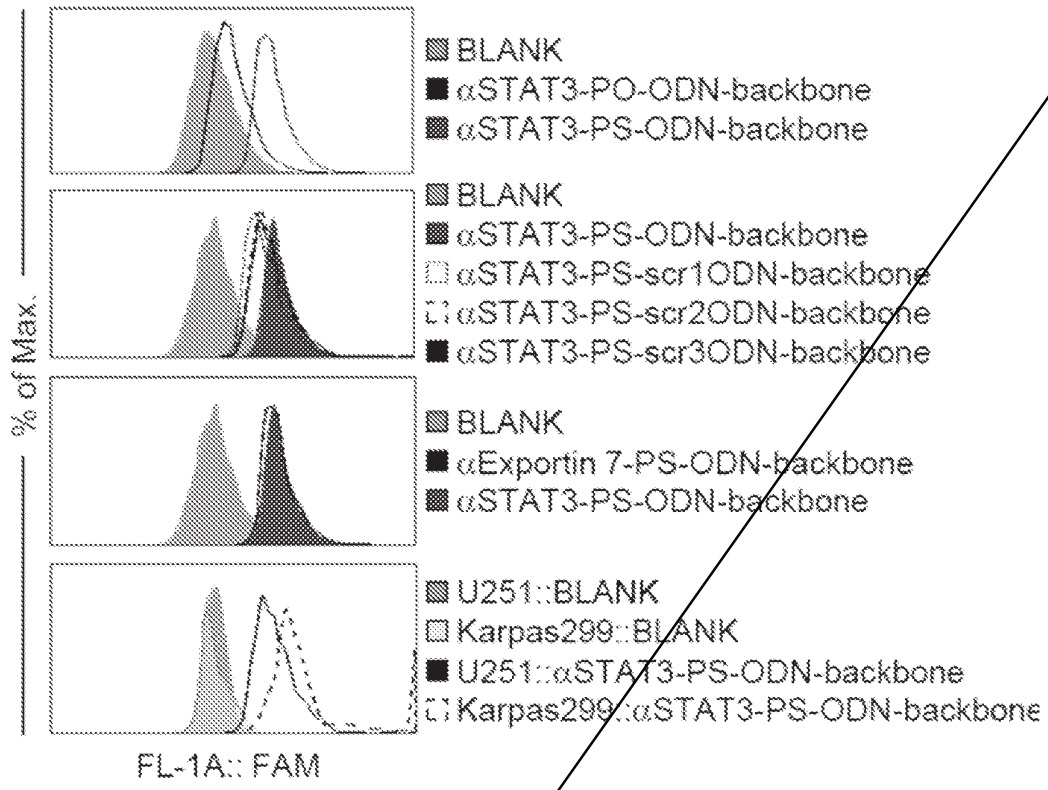


FIG. 23B

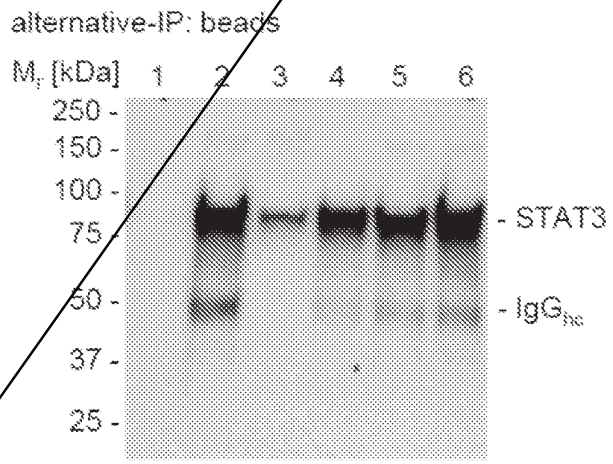


FIG. 23C

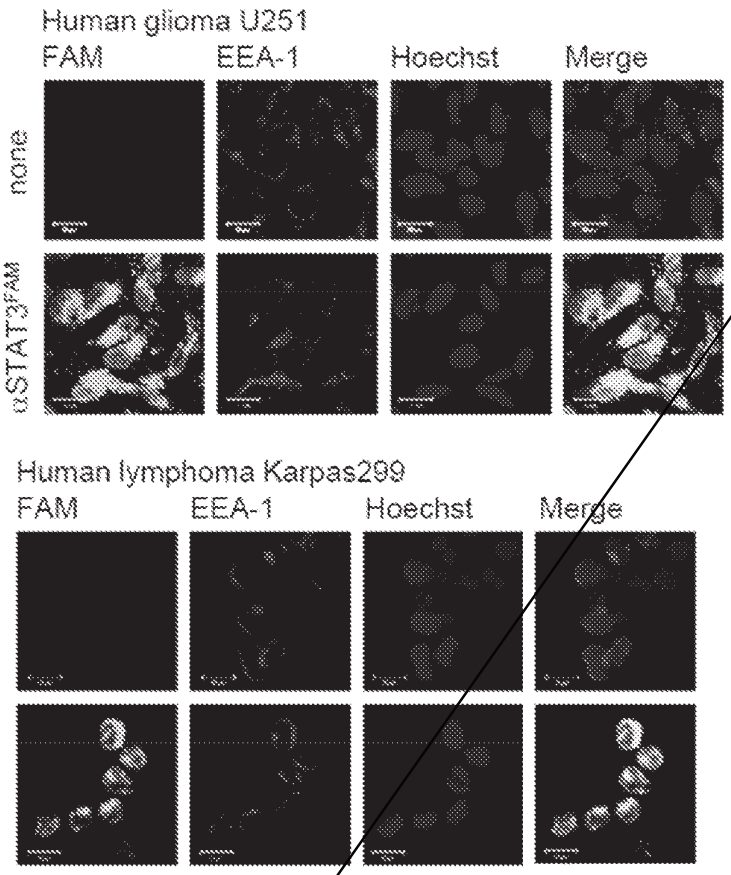


FIG. 23D

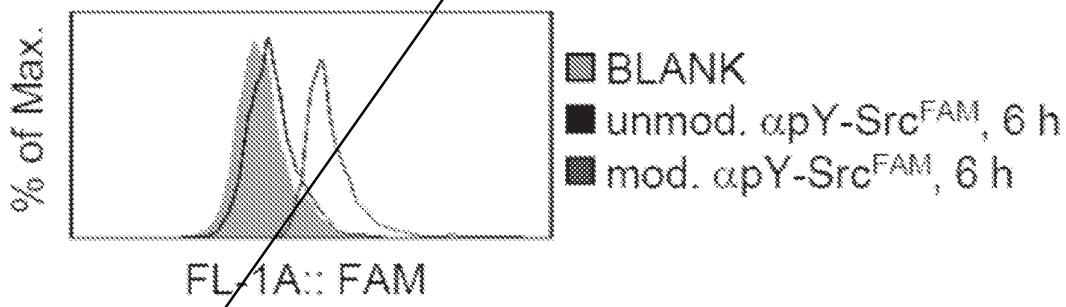


FIG. 23E

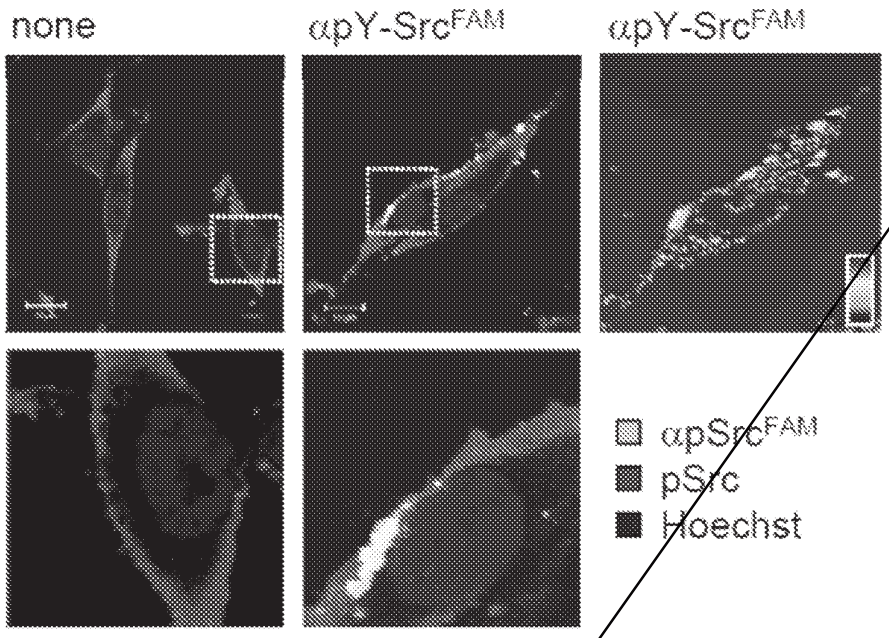


FIG. 23F

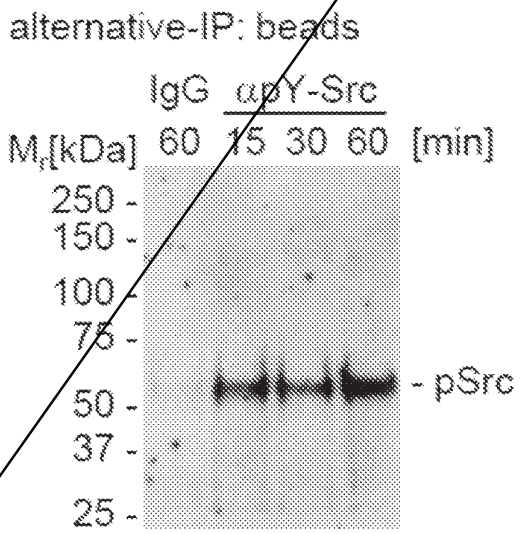


FIG. 23G

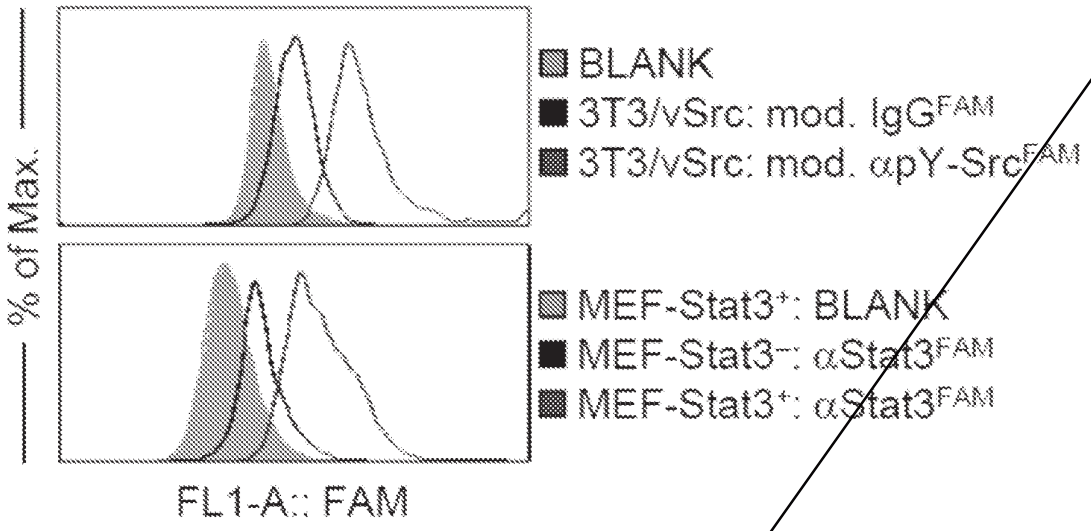


FIG. 24

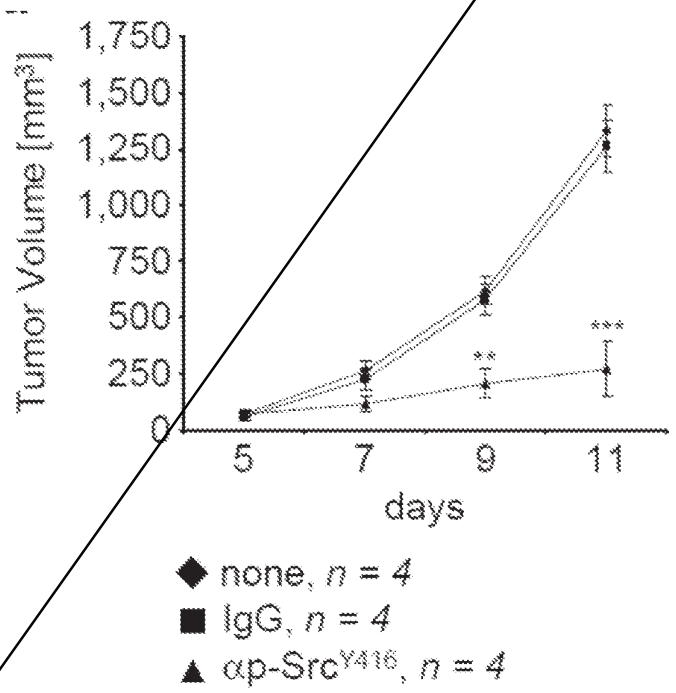


FIG. 25A

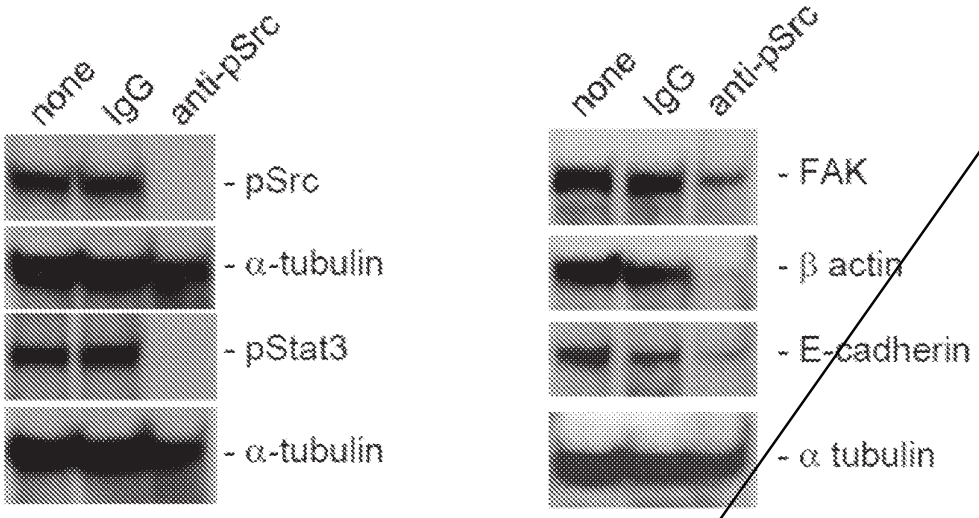


FIG. 25B

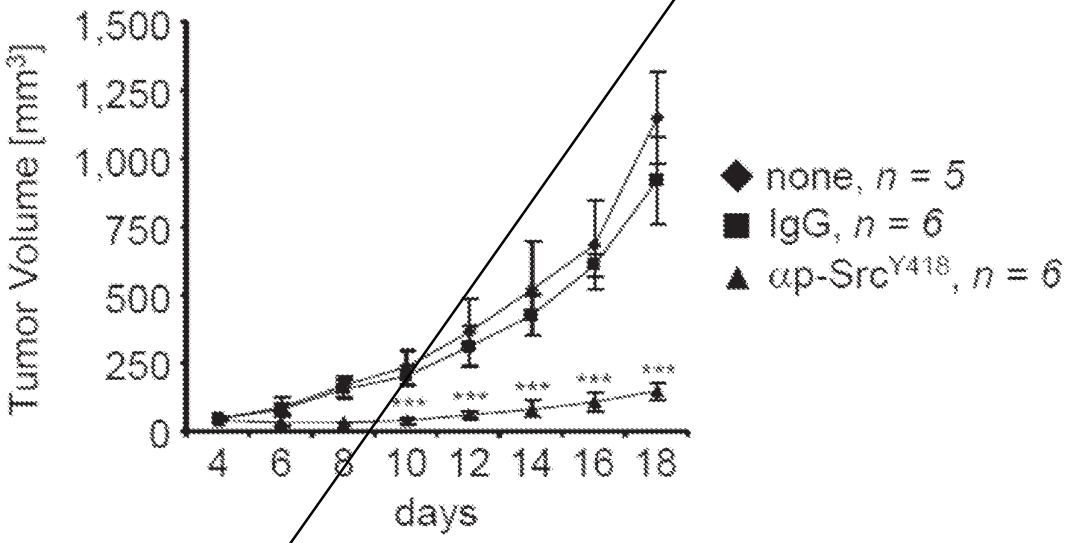


FIG. 25C

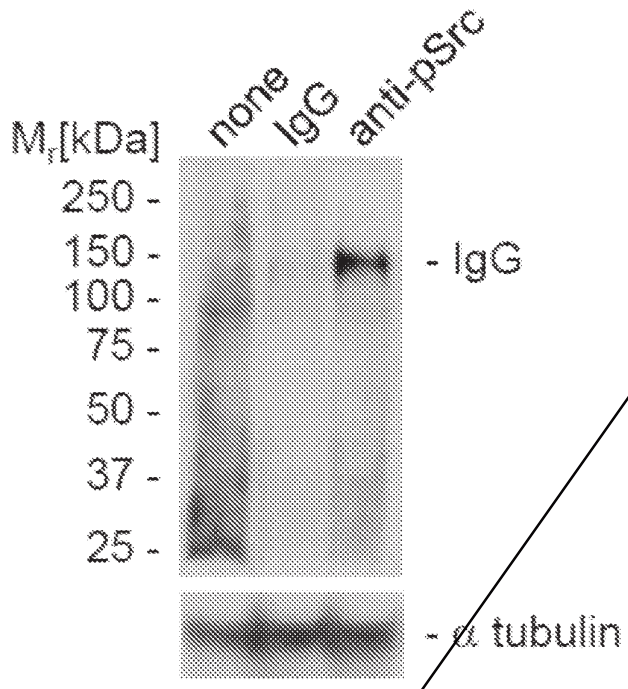


FIG. 25D

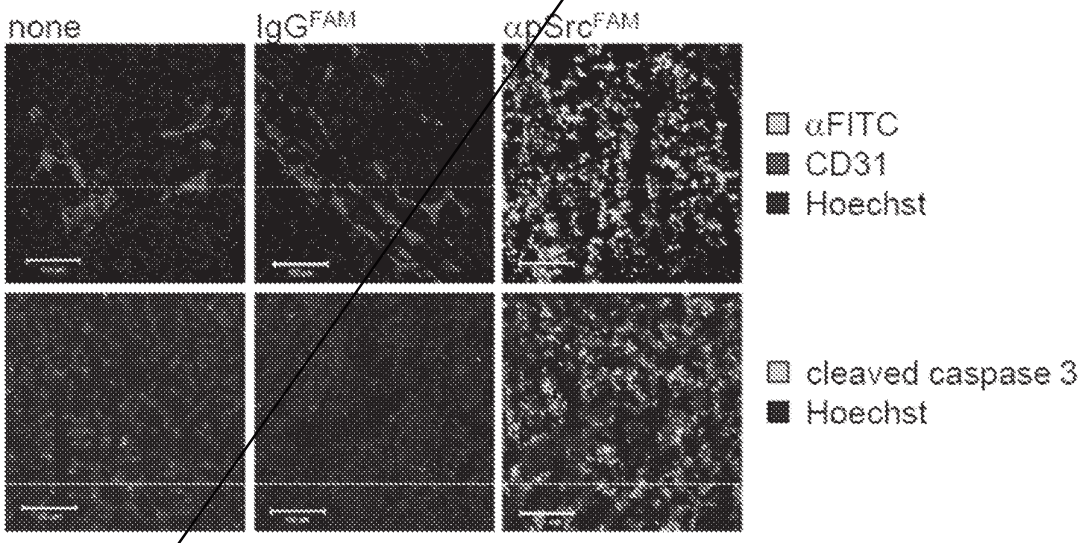


FIG. 25E

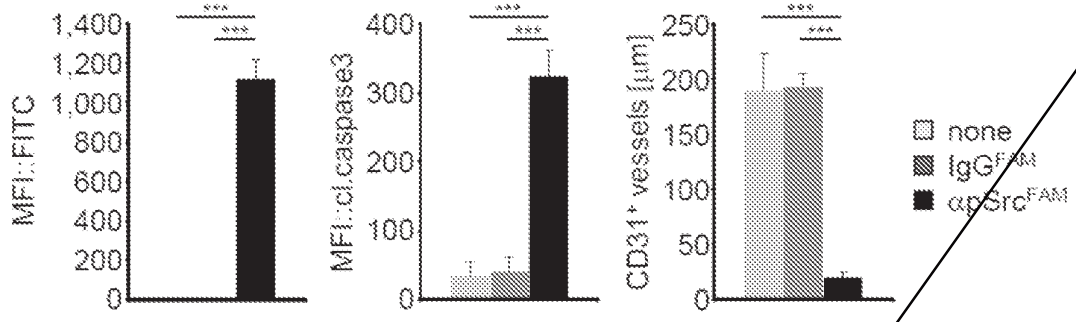


FIG. 25F

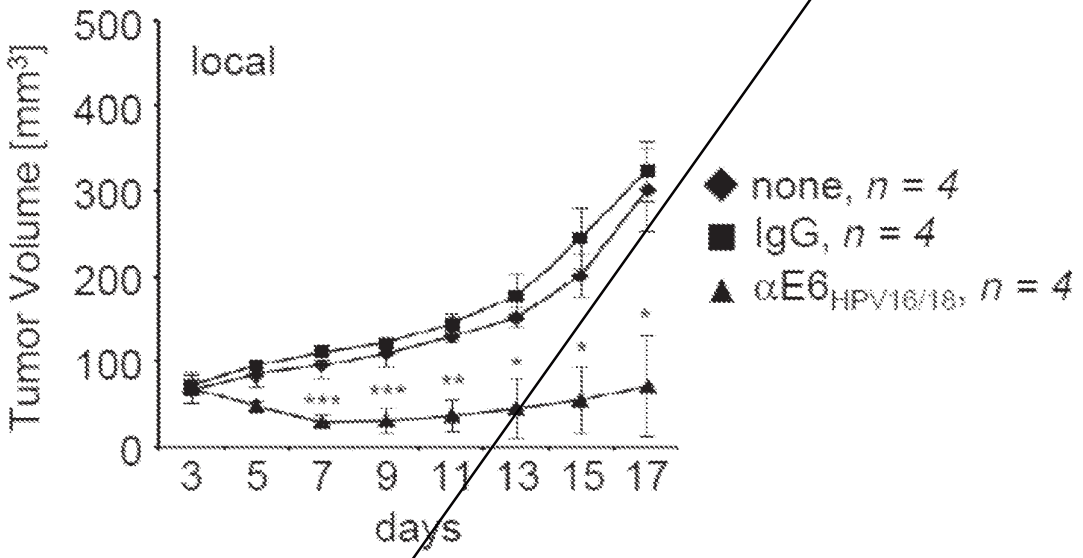


FIG. 26A

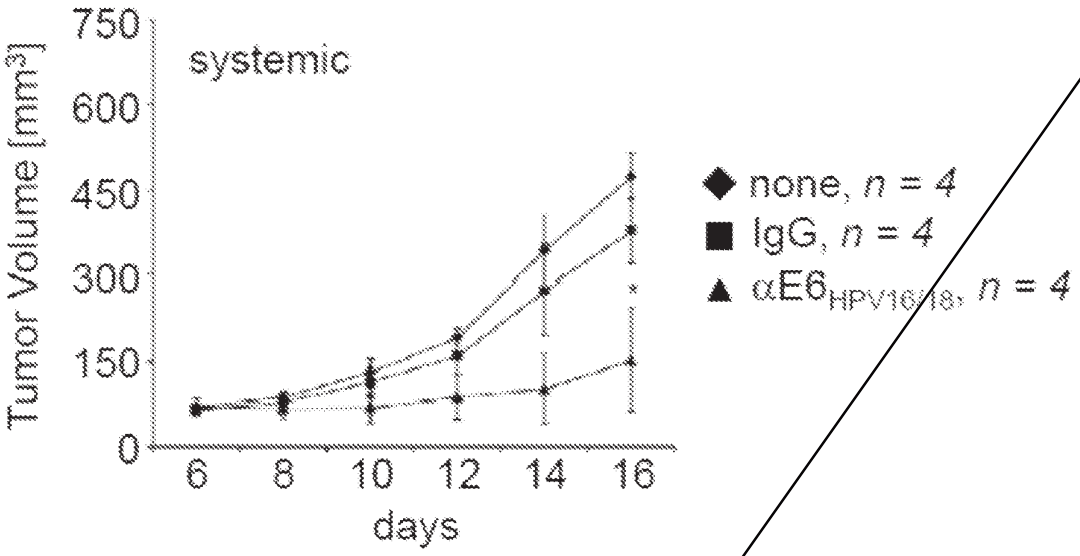


FIG. 26B

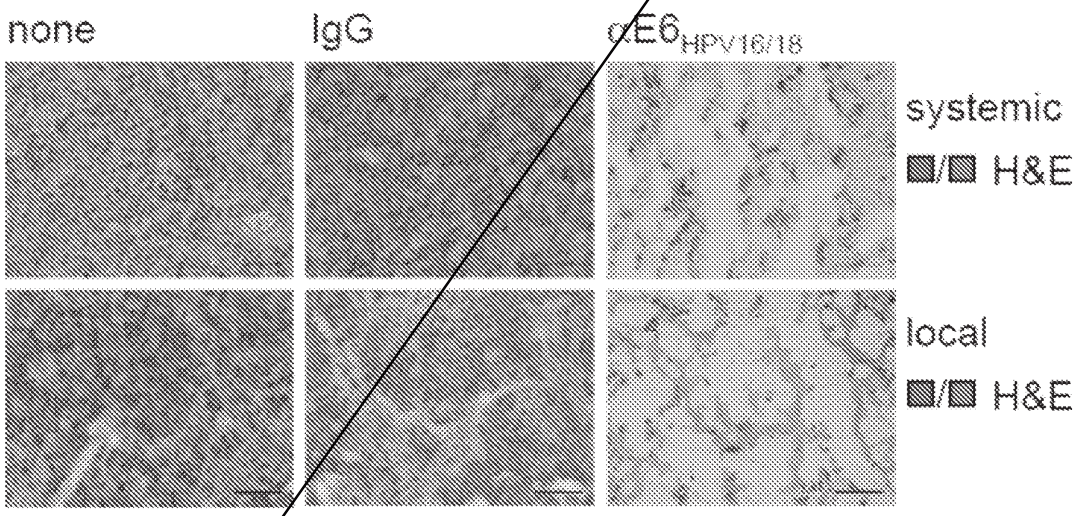


FIG. 26C

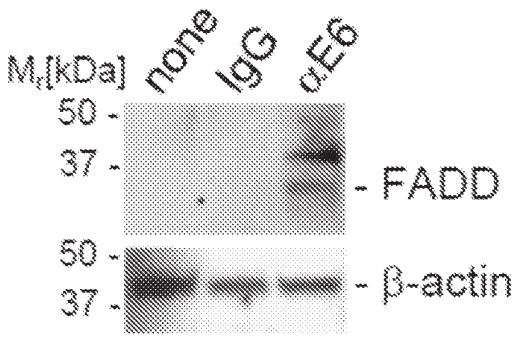


FIG. 26D

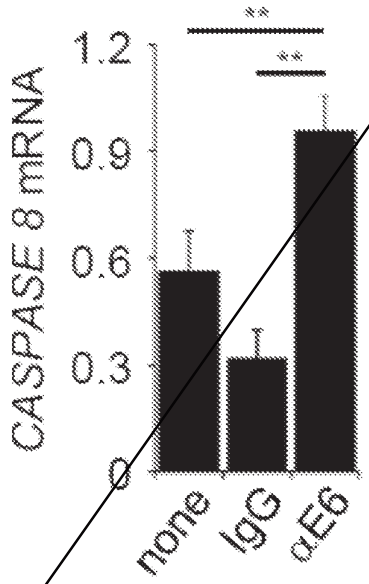


FIG. 26E

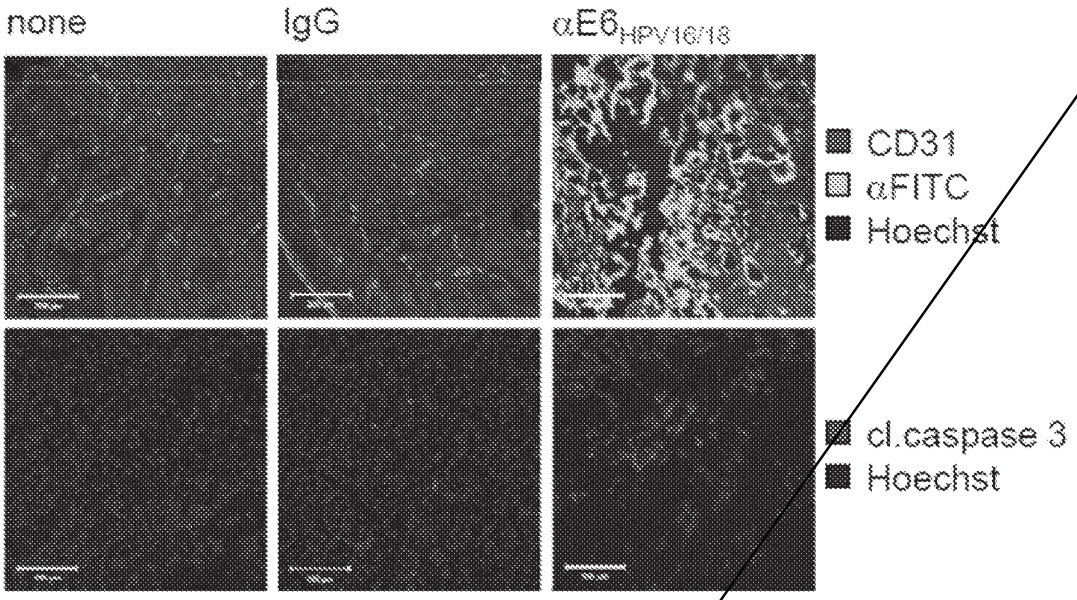


FIG. 26F

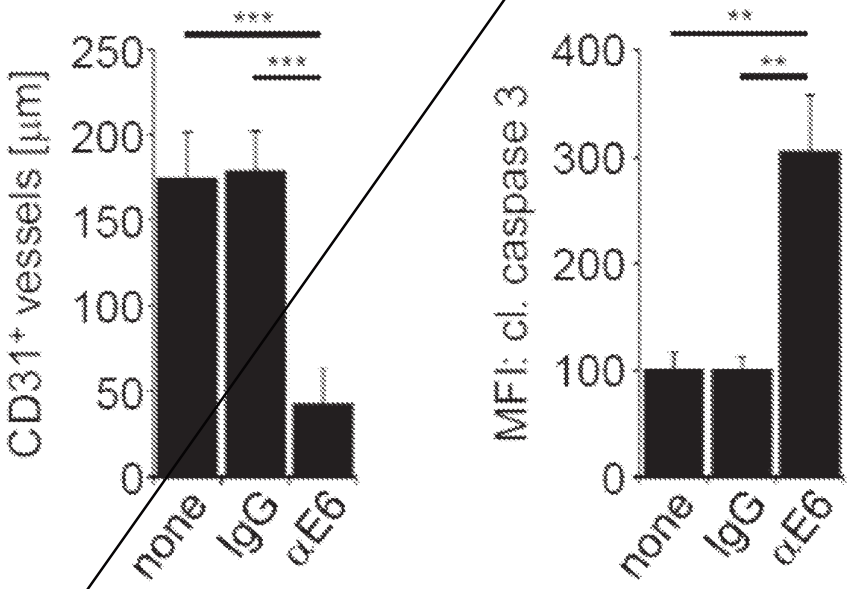


FIG. 26G

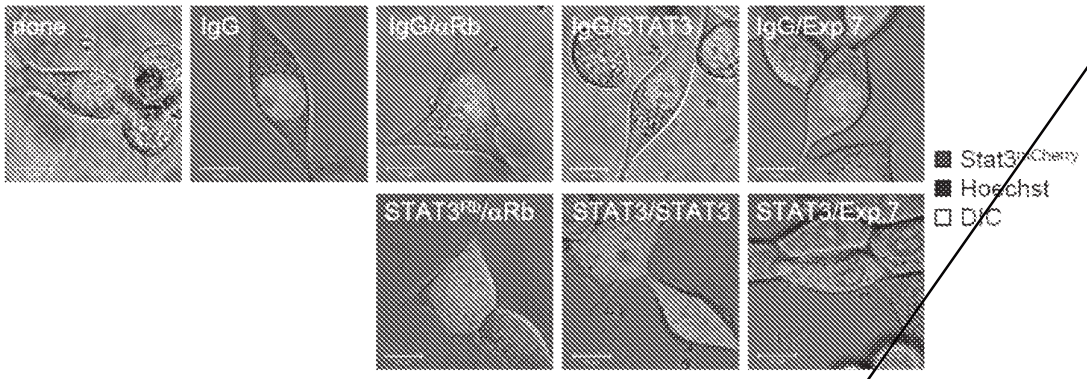


FIG. 27A

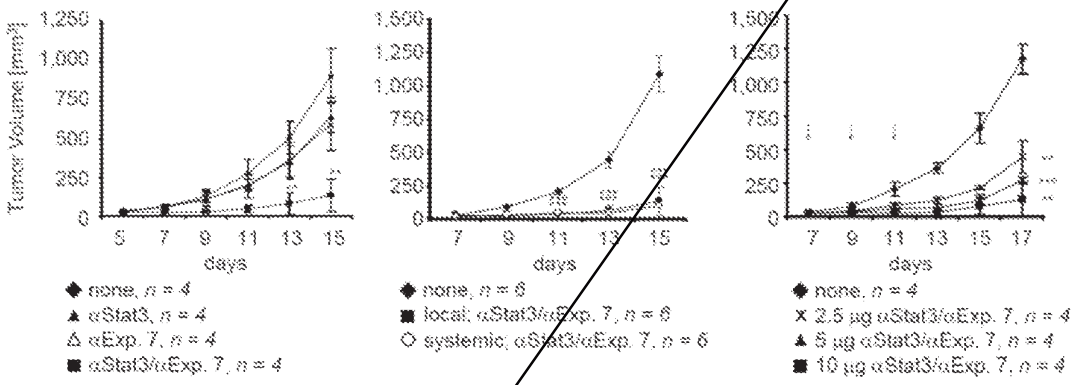


FIG. 27B

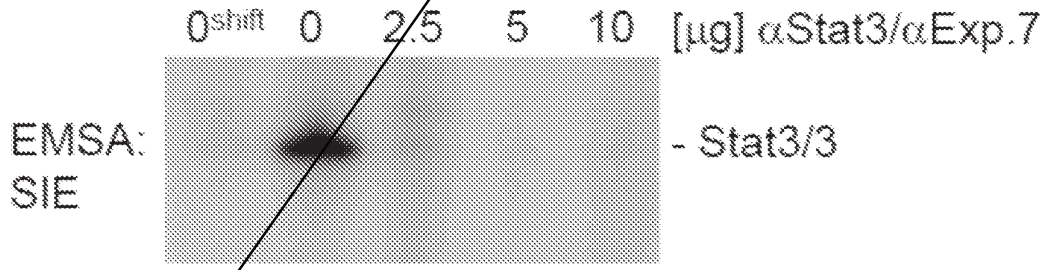


FIG. 27C

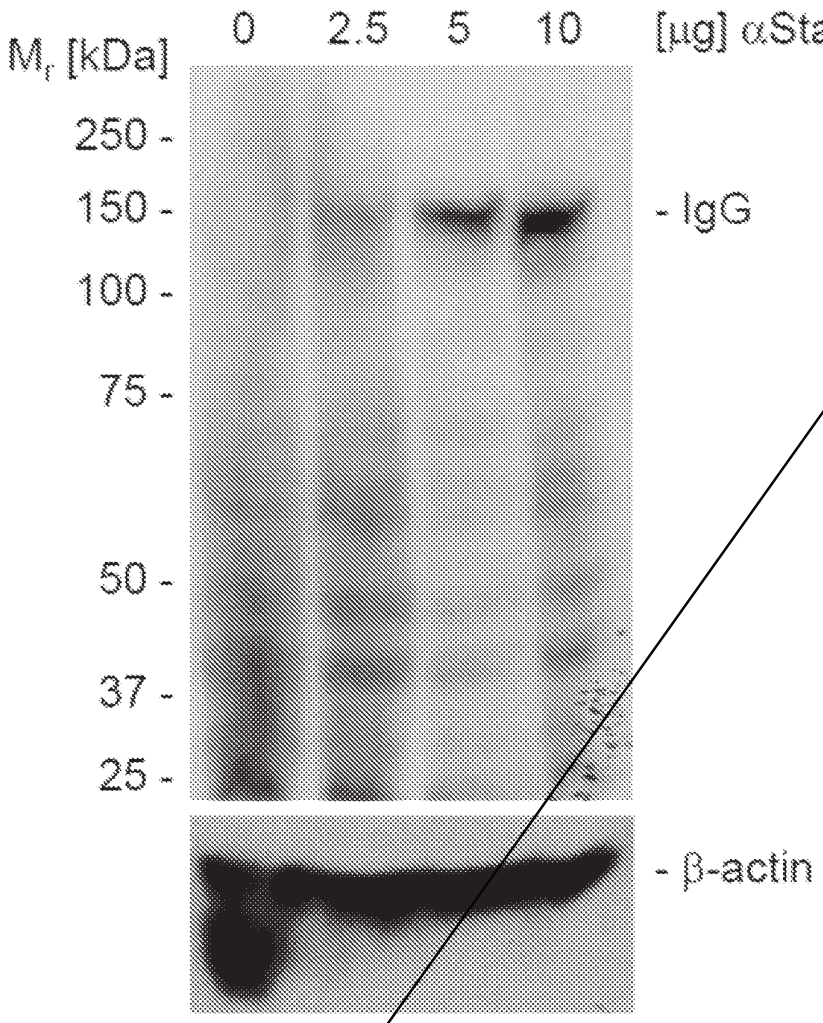

0
2.5
5
10
[µg] αStat3/αExp.7
M$_r$ [kDa]
250 -
150 -
100 -
75 -
50 -
37 -
25 -
- IgG
- β-actin

FIG. 27D

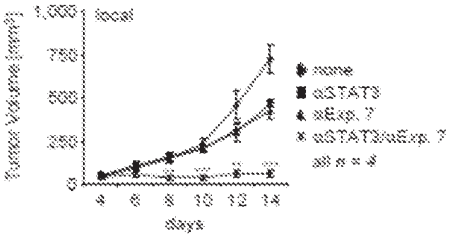


FIG. 27E

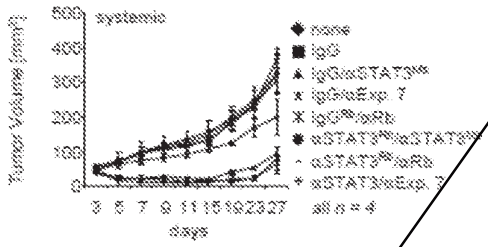


FIG. 27F

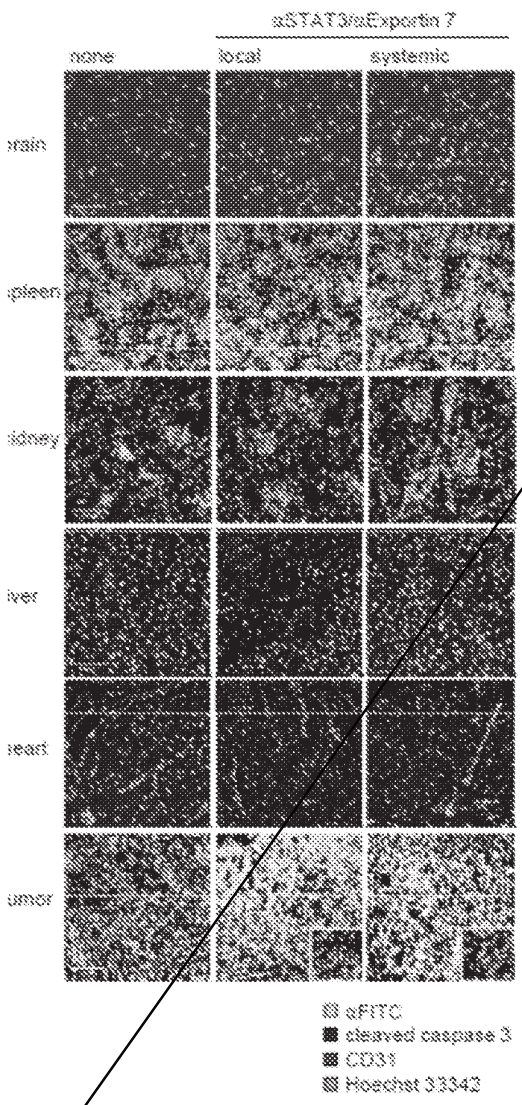

aSTAT3/aExportin 1
none
local
systemic
brain
spleen
kidney
liver
heart
tumor
aFITC
cleaved caspase 3
CD31
Hoechst 33342

FIG. 27G

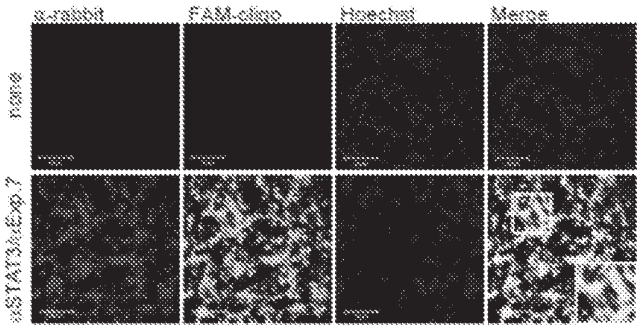


FIG. 28

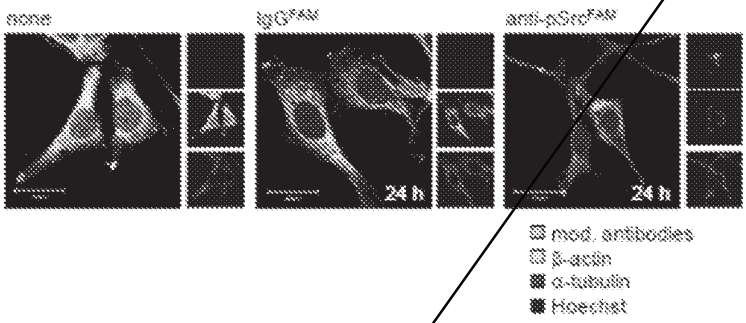


FIG. 29

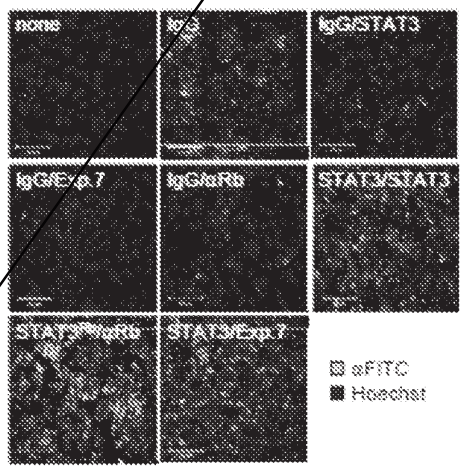


FIG. 30A

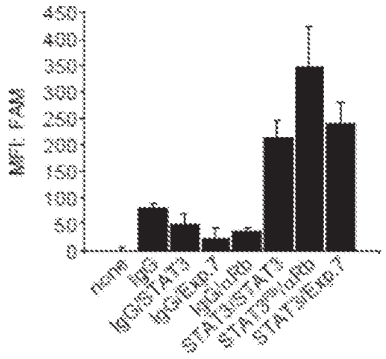

MFI PAM
450
400
350
300
250
200
150
100
50
0
none
IgG
IgGSTAT3
IgGSIENED.7
IgGpSTAT3
STAT3pSTAT3
STAT3pSTAT3
STAT3SIENED.7

FIG. 30B

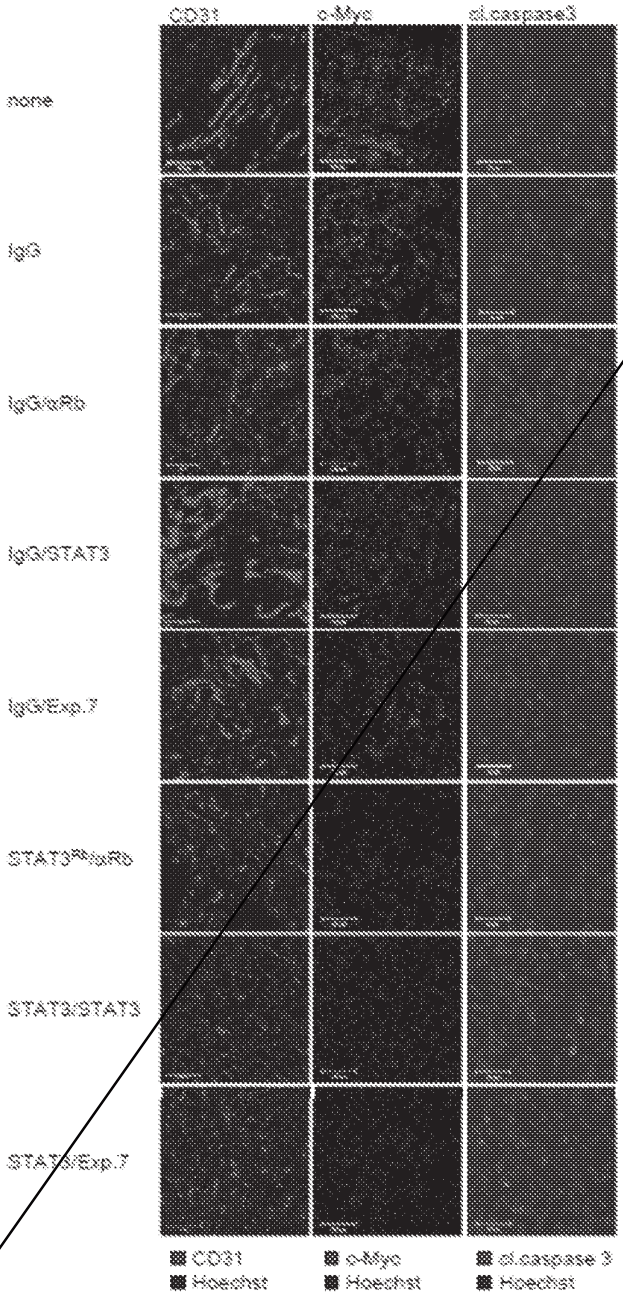

CD31
c-Myc
cl.caspase3
none
IgG
IgG/αRb
IgG/STAT3
IgG/Exp.7
STAT3PA/αRb
STAT3/STAT3
STAT3/Exp.7
CD31
Hoechst
c-Myc
Hoechst
cl.caspase 3
Hoechst

FIG. 31A

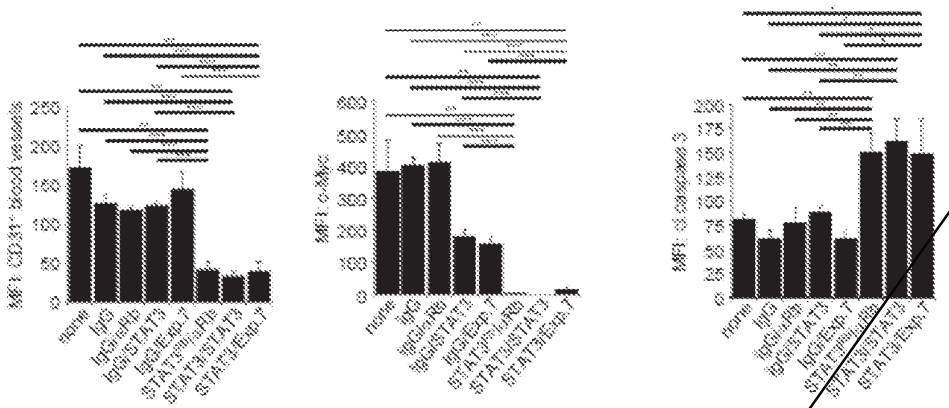


FIG. 31B

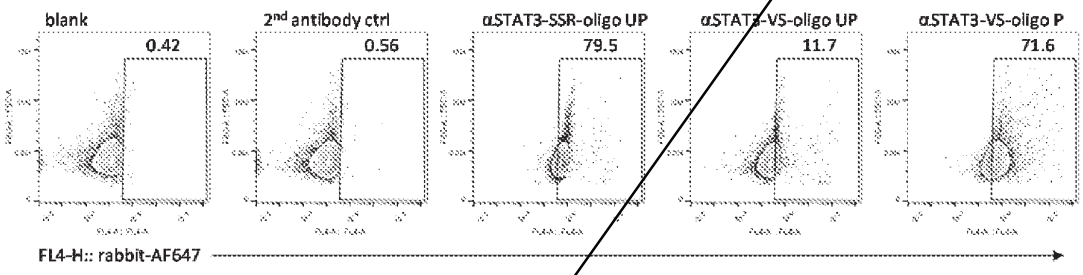


FIG. 32

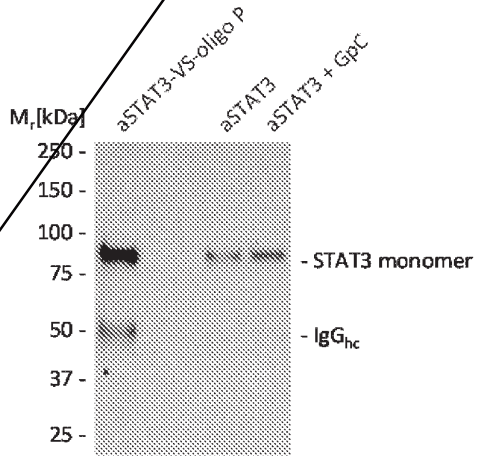


FIG. 33A

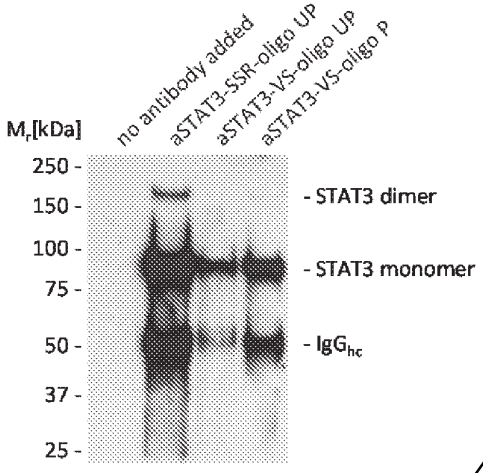

no antibody added
aSTAT3-SSR-oligo UP
aSTAT3-VS-oligo UP
aSTAT3-VS-oligo P
M$_r$[kDa]
250 -
150 -
100 -
75 -
50 -
37 -
25 -
- STAT3 dimer
- STAT3 monomer
- IgG$_{hc}$

FIG. 33B

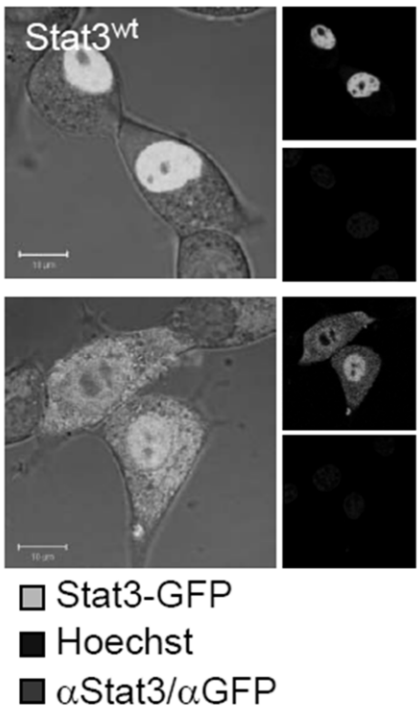

Stat3wt
Stat3-GFP
Hoechst
αStat3/αGFP

FIG. 1A


PIS
STAT3
- Exportin 7
- IgGhc
PIS
Exportin 7
- STAT3
- IgGlc

FIG. 1B

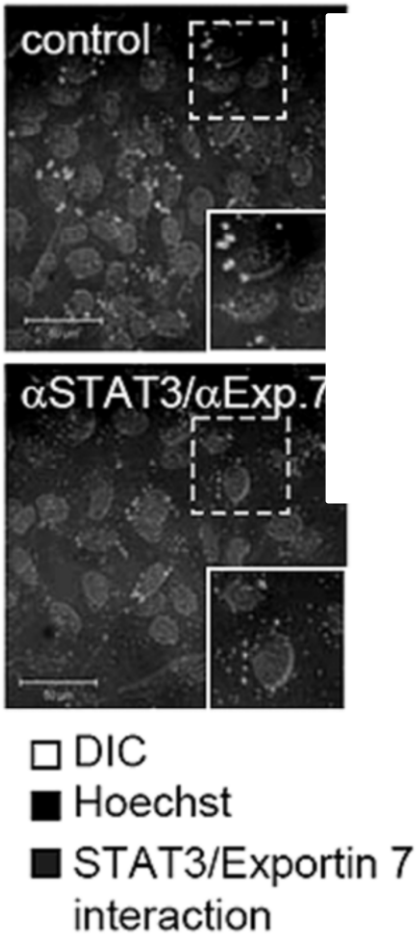

control
αSTAT3/αExp.7
DIC
Hoechst
STAT3/Exportin 7 interaction

FIG. 1C

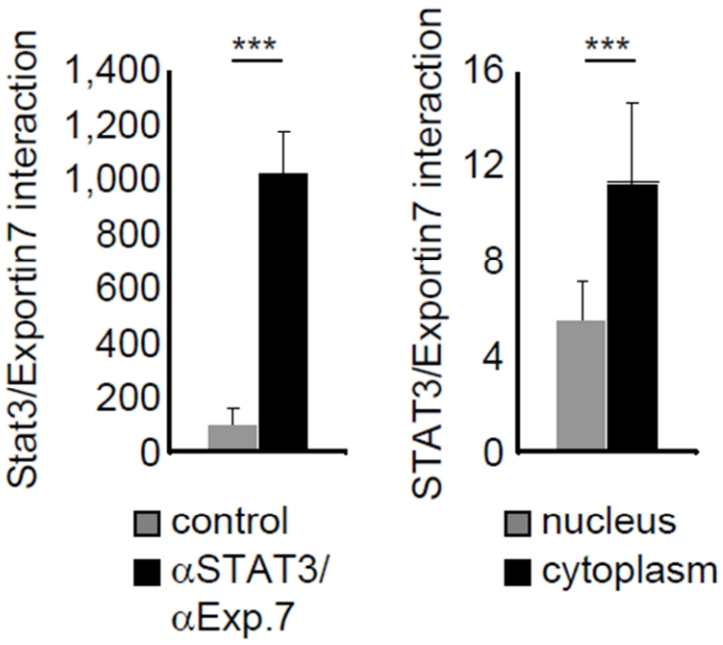

Stat3/Exportin7 interaction
1,400
1,200
1,000
800
600
400
200
0
***
control
αSTAT3/ αExp.7
STAT3/Exportin7 interaction
16
12
8
4
0
***
nucleus
cytoplasm

FIG.1D

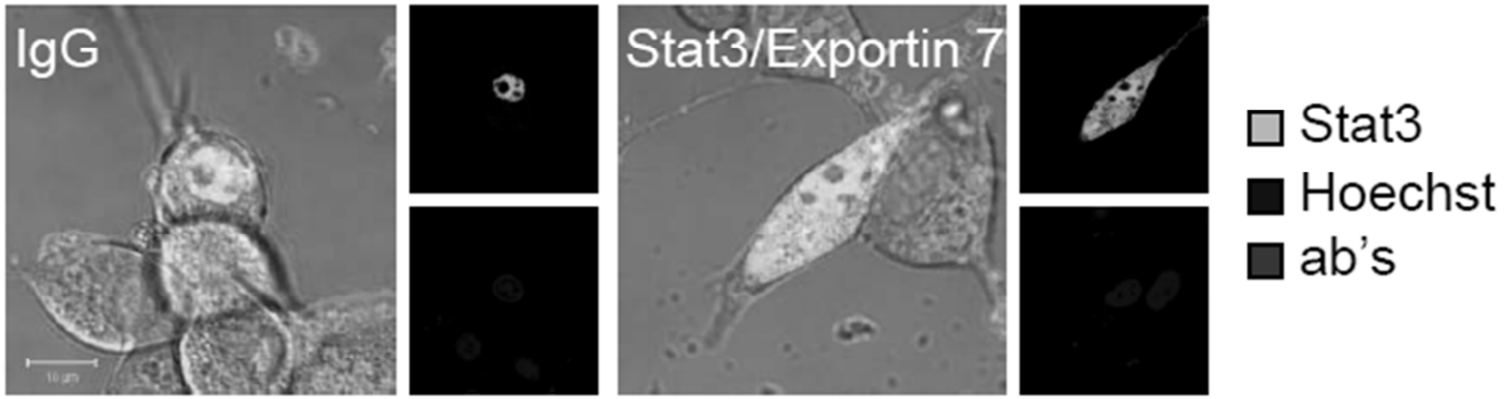

IgG
Stat3/Exportin 7
Stat3
Hoechst
ab's

FIG. 1E

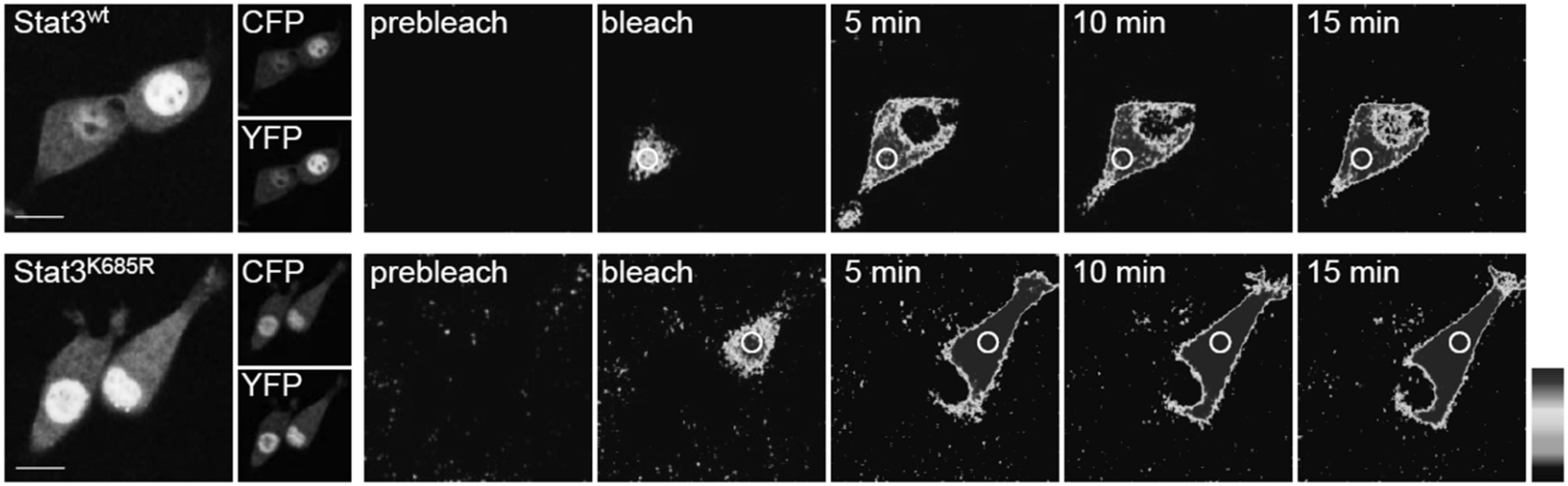

Stat3wt
CFP
YFP
prebleach
bleach
5 min
10 min
15 min
Stat3K685R
CFP
YFP
prebleach
bleach
5 min
10 min
15 min

FIG. 2A

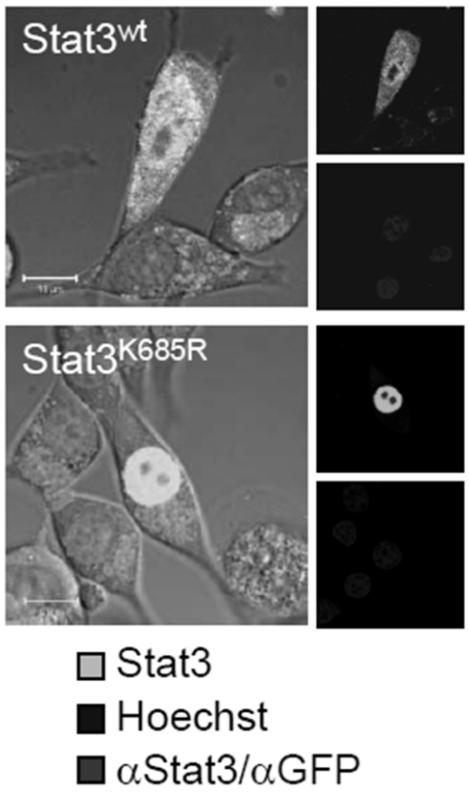

Stat3wt
Stat3K685R
Stat3
Hoechst
αStat3/αGFP

FIG. 2B

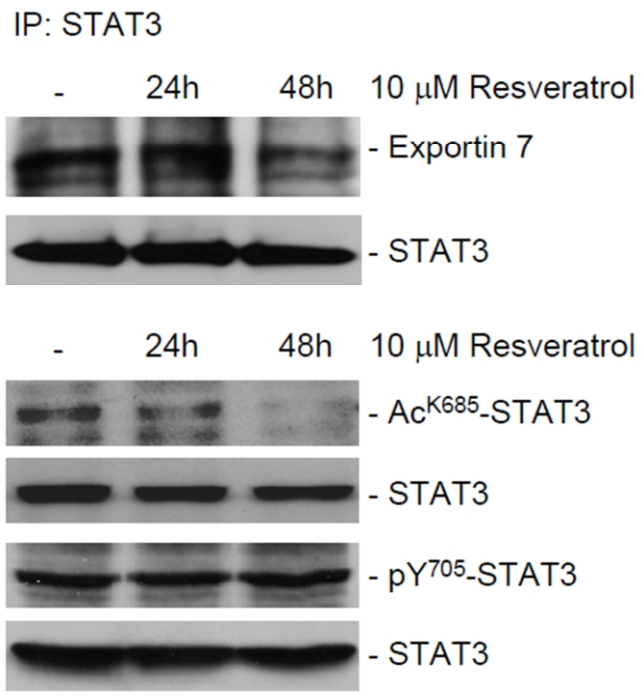

IP: STAT3
- 24h 48h 10 μM Resveratrol
- Exportin 7
- STAT3
- 24h 48h 10 μM Resveratrol
- AcK685-STAT3
- STAT3
- pY705-STAT3
- STAT3

FIG. 2C

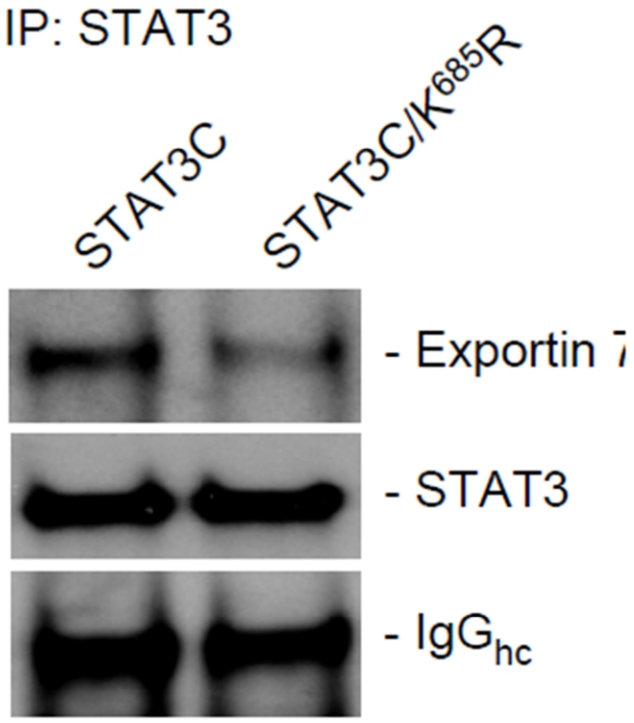

IP: STAT3
STAT3C
STAT3C/K685R
- Exportin 7
- STAT3
- IgGhc

FIG. 2D

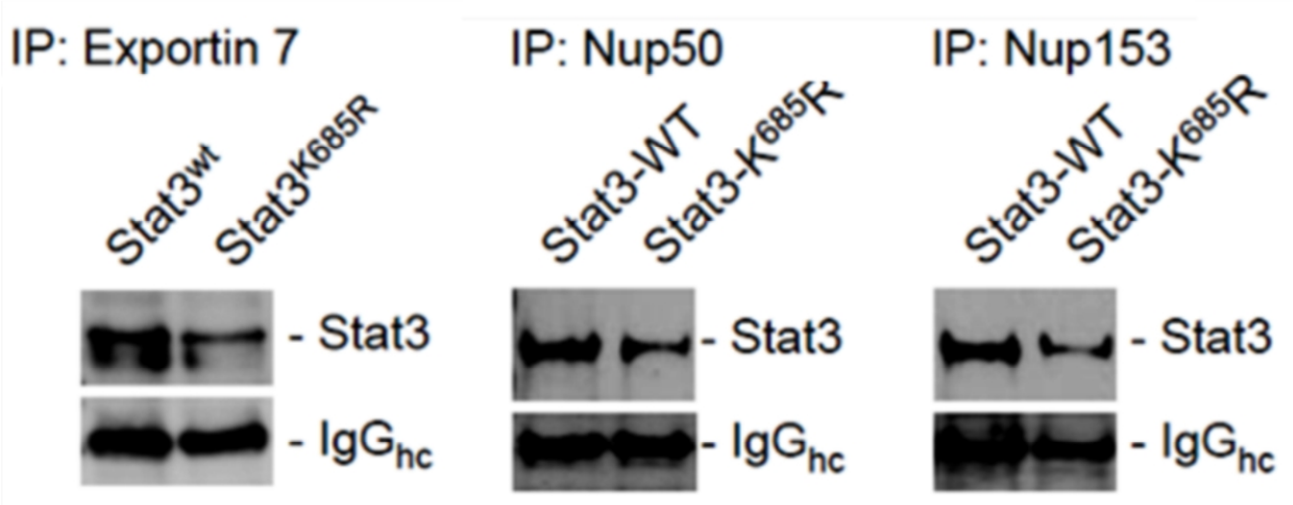

IP: Exportin 7
Stat3wt
Stat3K685R
- Stat3
- IgGhc
IP: Nup50
Stat3-WT
Stat3-K685R
- Stat3
- IgGhc
IP: Nup153
Stat3-WT
Stat3-K685R
- Stat3
- IgGhc

FIG. 2E

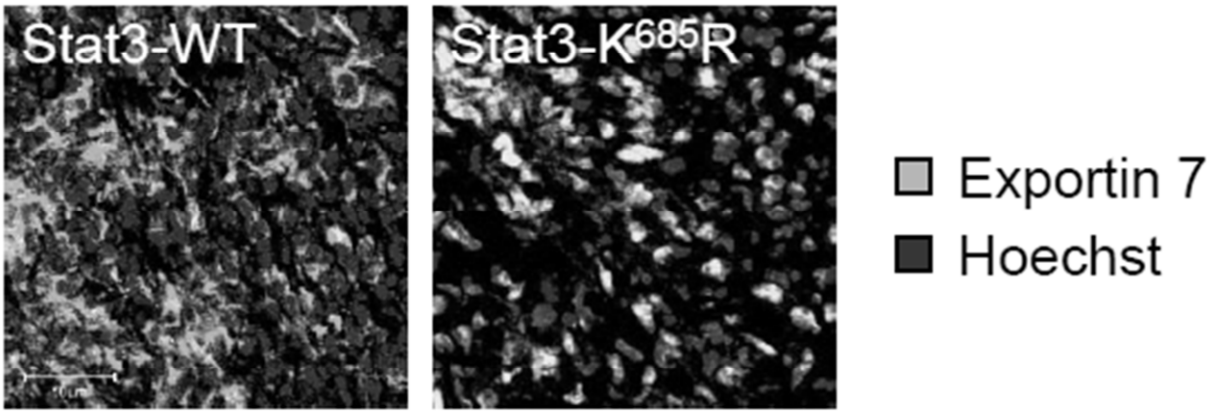

Stat3-WT
Stat3-K685R
Exportin 7
Hoechst

FIG. 2F

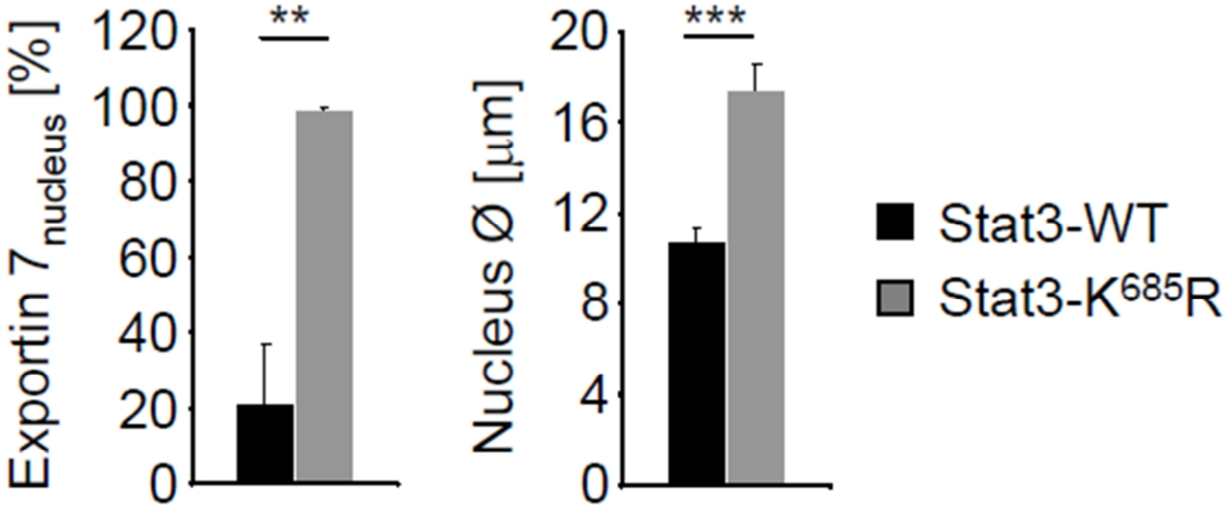

Exportin 7nucleus [%]
120
100
80
60
40
20
0
**
Nucleus Ø [µm]
20
16
12
8
4
0
***
Stat3-WT
Stat3-K685R

FIG. 2G

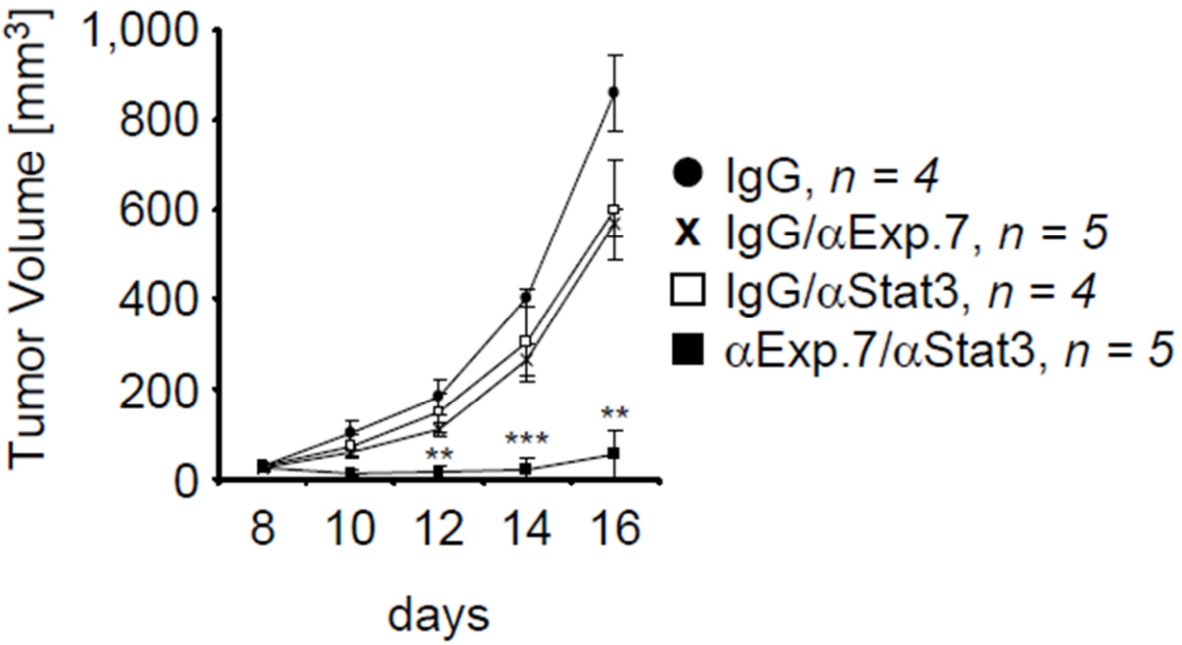

Tumor Volume [mm³]
1,000
800
600
400
200
0
8
10
12
14
16
days
**
***
**
IgG, n = 4
IgG/αExp.7, n = 5
IgG/αStat3, n = 4
αExp.7/αStat3, n = 5

FIG. 3A

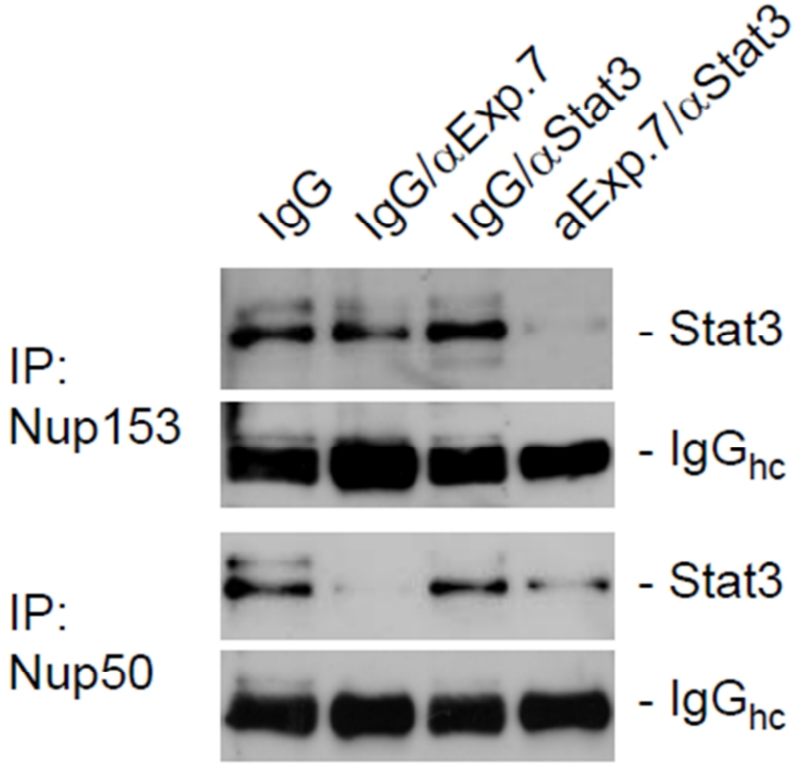

IgG
IgG/αExp.7
IgG/αStat3
aExp.7/αStat3
IP:
Nup153
- Stat3
- IgGhc
IP:
Nup50
- Stat3
- IgGhc

FIG. 3B

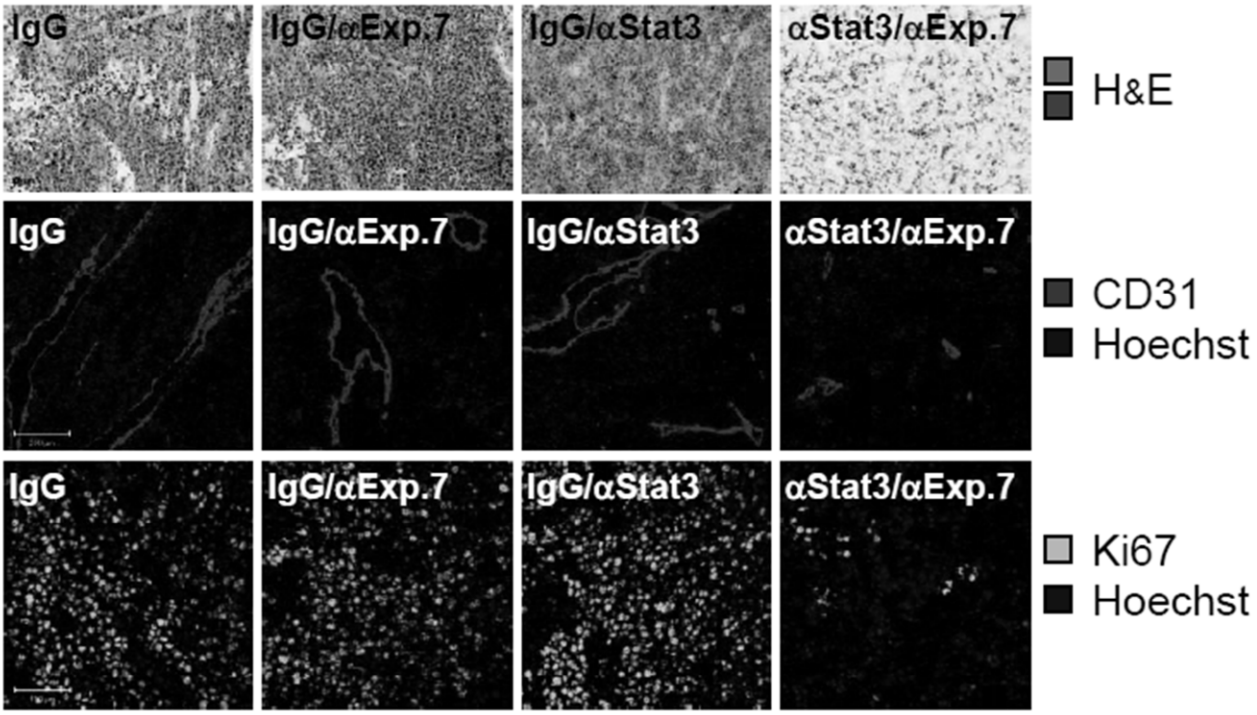

IgG
IgG/αExp.7
IgG/αStat3
αStat3/αExp.7
H&E
CD31
Hoechst
Ki67
Hoechst

FIG. 3C

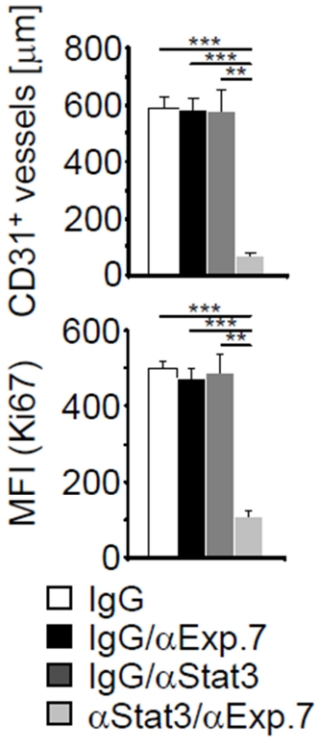

CD31⁺ vessels [µm]
MFI (Ki67)
800
600
400
200
0
***
***
**
IgG
IgG/αExp.7
IgG/αStat3
αStat3/αExp.7

FIG. 3D

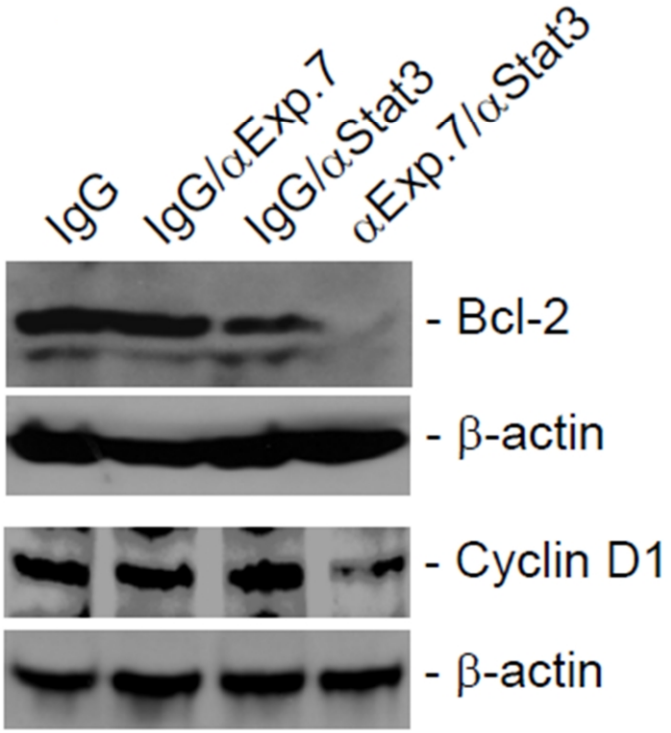

IgG
IgG/αExp.7
IgG/αStat3
αExp.7/αStat3
- Bcl-2
- β-actin
- Cyclin D1
- β-actin

FIG. 3E

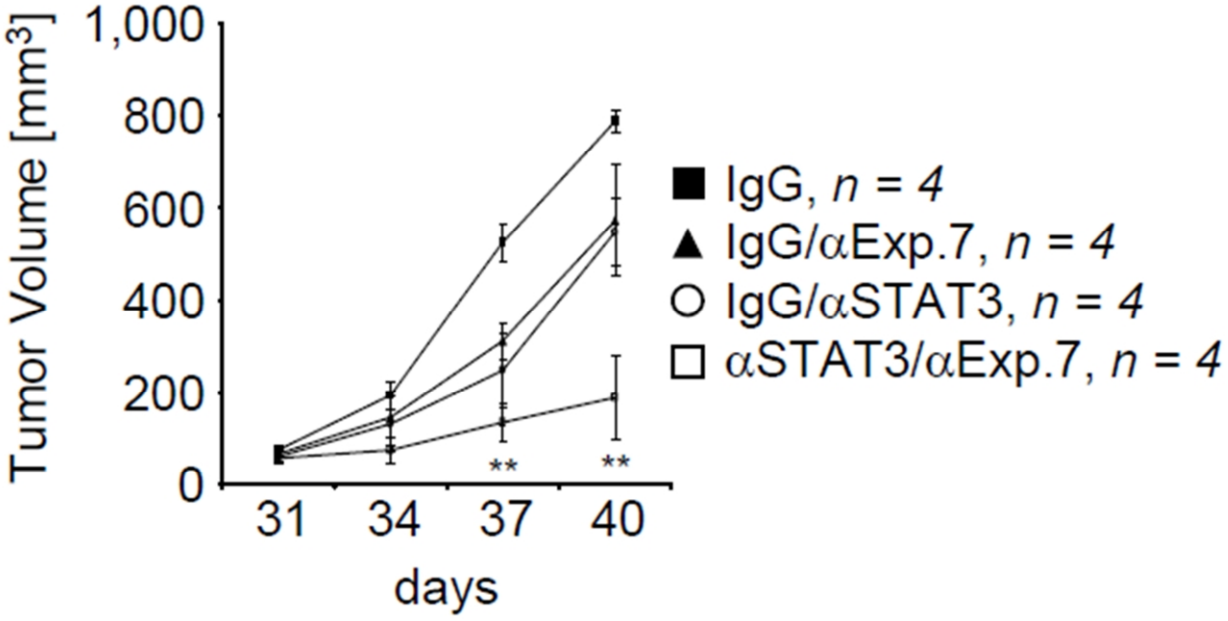

Tumor Volume [mm³]
1,000
800
600
400
200
0
31
34
37
40
days
**
**
IgG, n = 4
IgG/αExp.7, n = 4
IgG/αSTAT3, n = 4
αSTAT3/αExp.7, n = 4

FIG. 3F

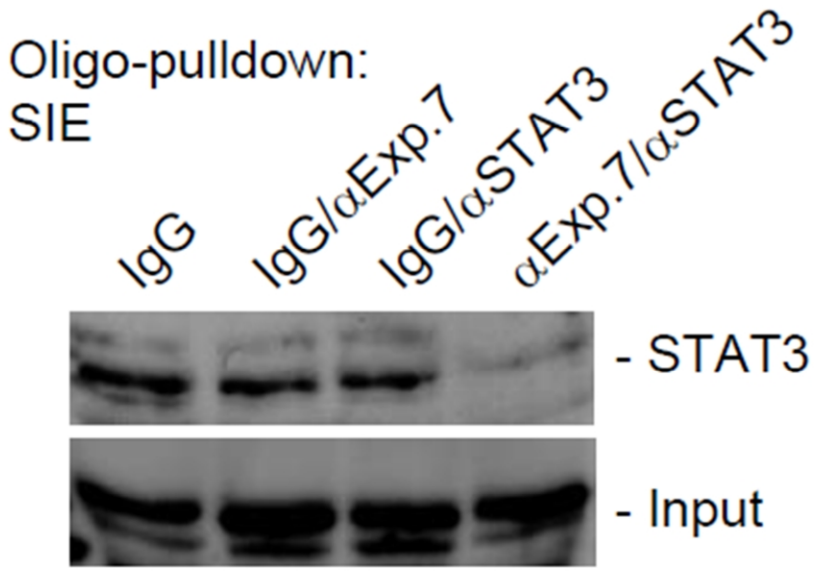

Oligo-pulldown:
SIE
IgG
IgG/αExp.7
IgG/αSTAT3
αExp.7/αSTAT3
- STAT3
- Input

FIG. 3G

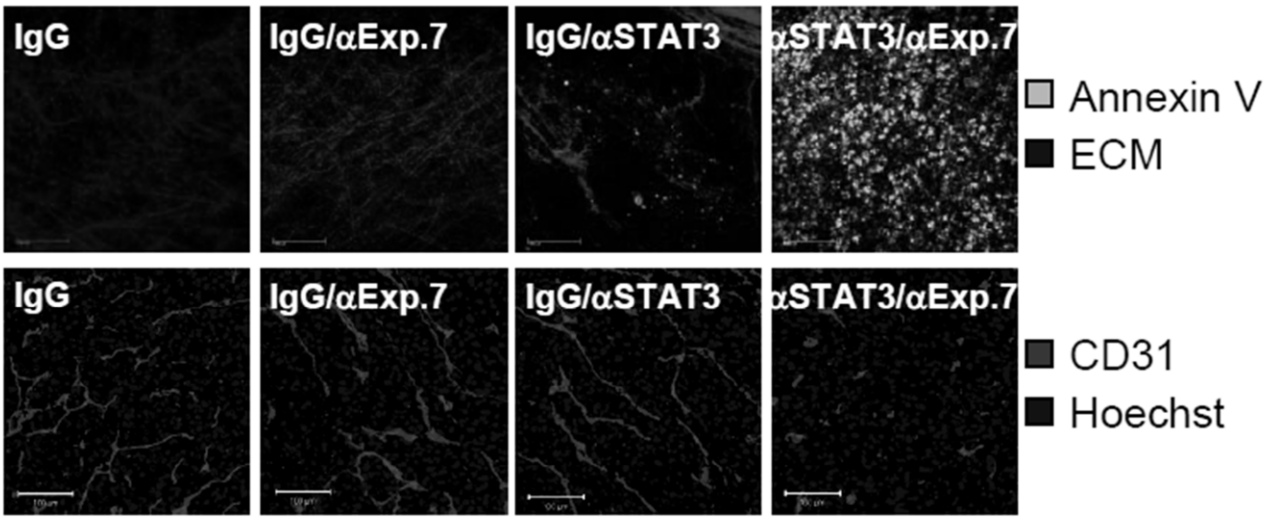

IgG
IgG/αExp.7
IgG/αSTAT3
αSTAT3/αExp.7
Annexin V
ECM
IgG
IgG/αExp.7
IgG/αSTAT3
αSTAT3/αExp.7
CD31
Hoechst

FIG. 3H

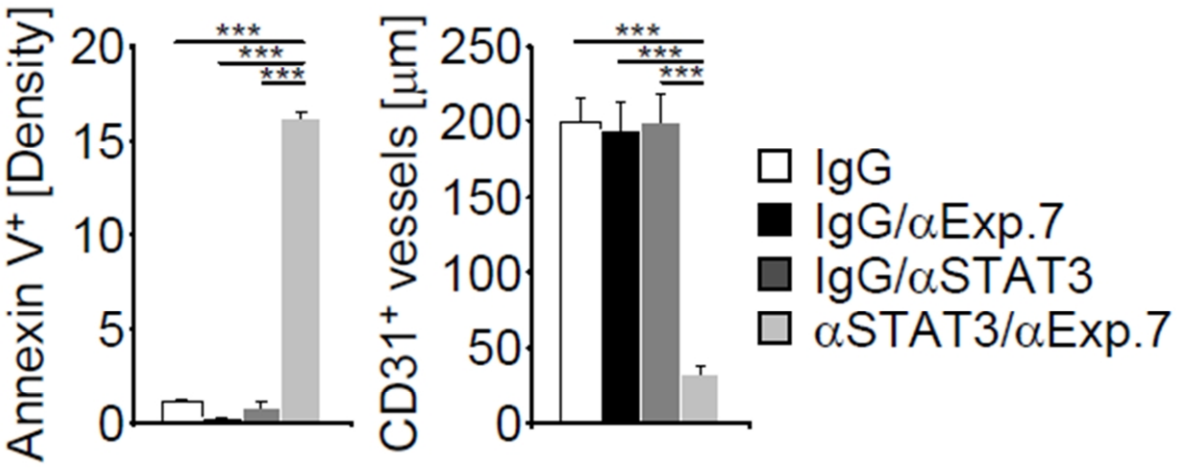

Annexin V+ [Density]
20
15
10
5
0
***
***
***
CD31+ vessels [µm]
250
200
150
100
50
0
***
***
***
IgG
IgG/αExp.7
IgG/αSTAT3
αSTAT3/αExp.7

FIG. 3I

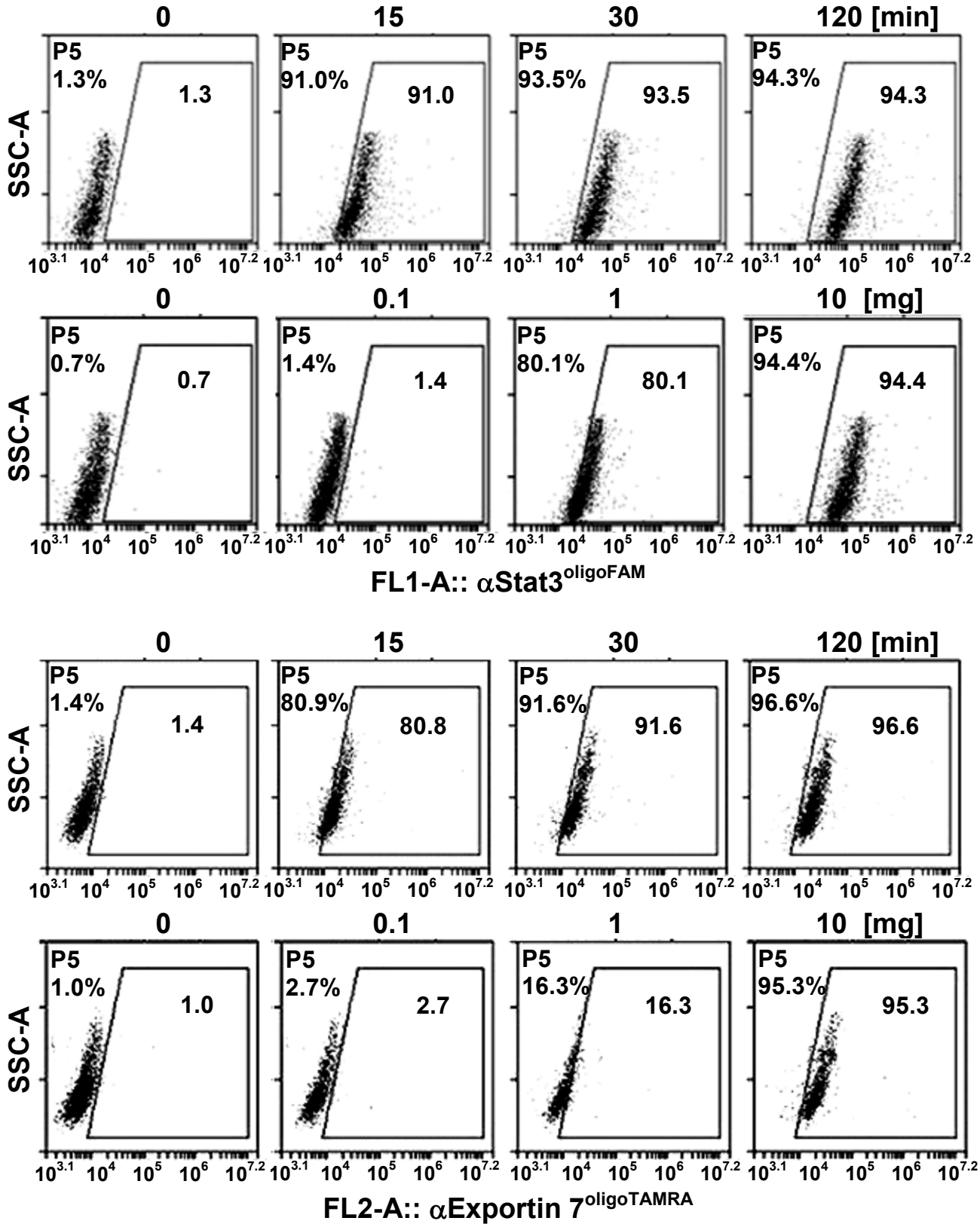

0
15
30
120 [min]
P5
1.3%
1.3
P5
91.0%
91.0
P5
93.5%
93.5
P5
94.3%
94.3
SSC-A
0
0.1
1
10 [mg]
P5
0.7%
0.7
P5
1.4%
1.4
P5
80.1%
80.1
P5
94.4%
94.4
FL1-A:: αStat3oligoFAM
0
15
30
120 [min]
P5
1.4%
1.4
P5
80.9%
80.8
P5
91.6%
91.6
P5
96.6%
96.6
SSC-A
0
0.1
1
10 [mg]
P5
1.0%
1.0
P5
2.7%
2.7
P5
16.3%
16.3
P5
95.3%
95.3
FL2-A:: αExportin 7oligoTAMRA

FIG. 4A

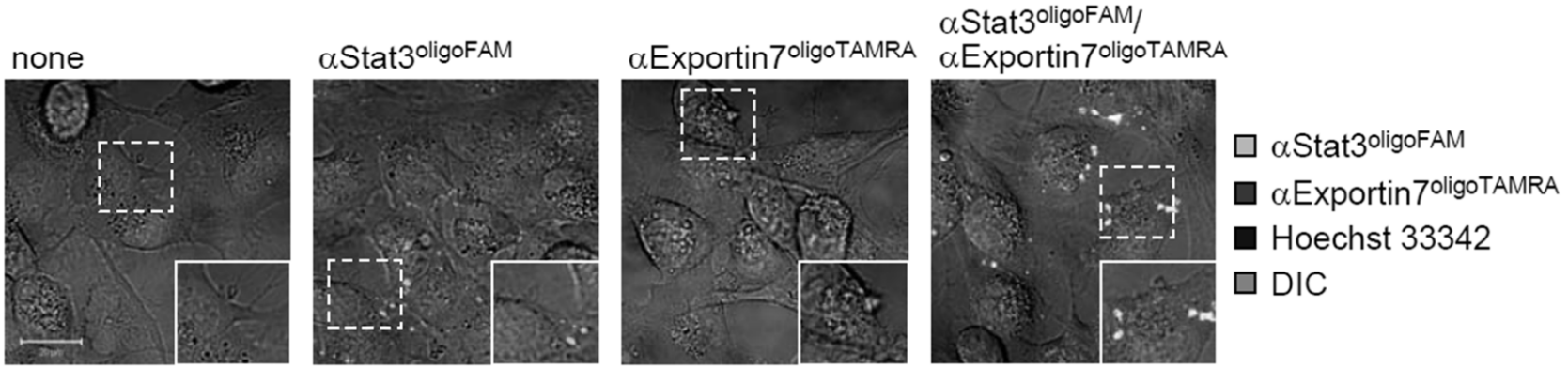

none
αStat3oligoFAM
αExportin7oligoTAMRA
αStat3oligoFAM/
αExportin7oligoTAMRA
αStat3oligoFAM
αExportin7oligoTAMRA
Hoechst 33342
DIC

FIG. 4B

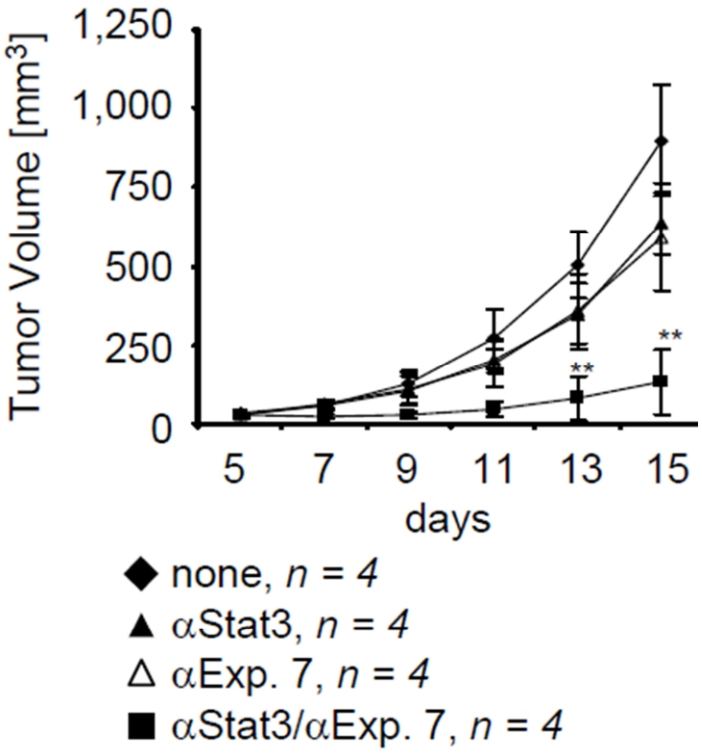

Tumor Volume [mm³]
1,250
1,000
750
500
250
0
5
7
9
11
13
15
days
**
**
◆ none, n = 4
▲ αStat3, n = 4
△ αExp. 7, n = 4
■ αStat3/αExp. 7, n = 4

FIG. 4C

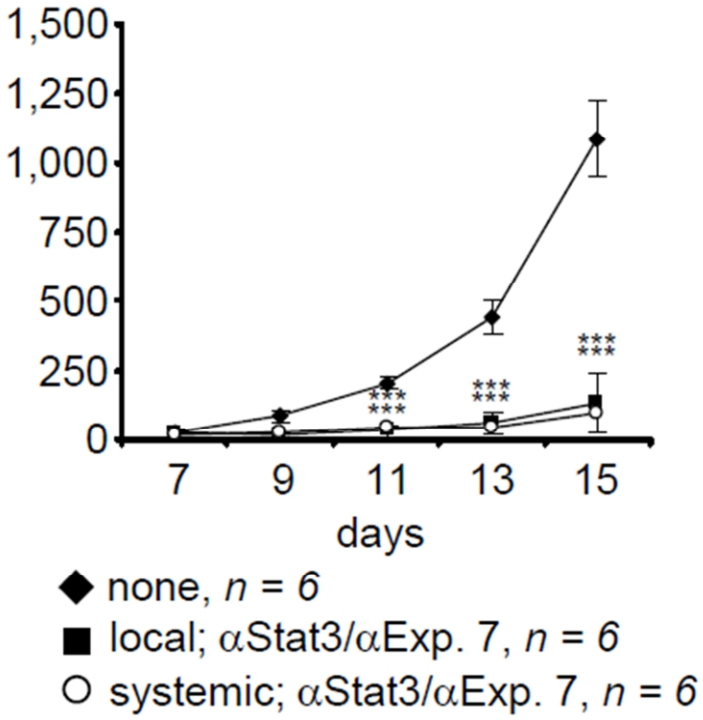

1,500
1,250
1,000
750
500
250
0
7
9
11
13
15
days
***
***
***
***
***
***
◆ none, n = 6
■ local; αStat3/αExp. 7, n = 6
○ systemic; αStat3/αExp. 7, n = 6

FIG. 4D

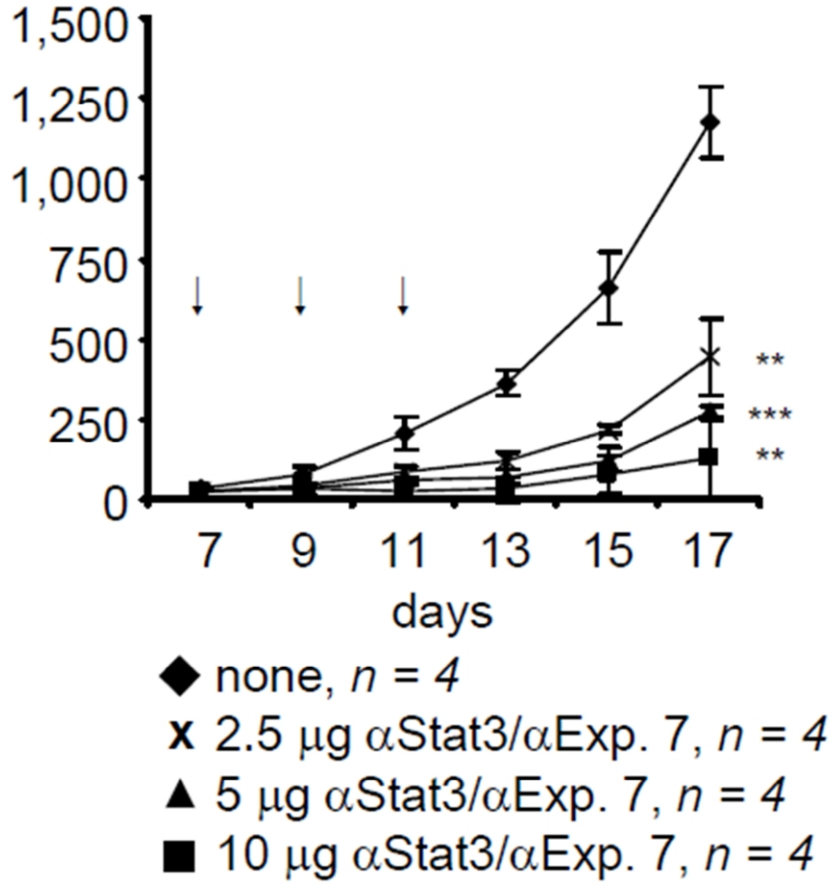

1,500
1,250
1,000
750
500
250
0
7
9
11
13
15
17
days
**
***
**
◆ none, n = 4
x 2.5 μg αStat3/αExp. 7, n = 4
▲ 5 μg αStat3/αExp. 7, n = 4
■ 10 μg αStat3/αExp. 7, n = 4

FIG. 4E

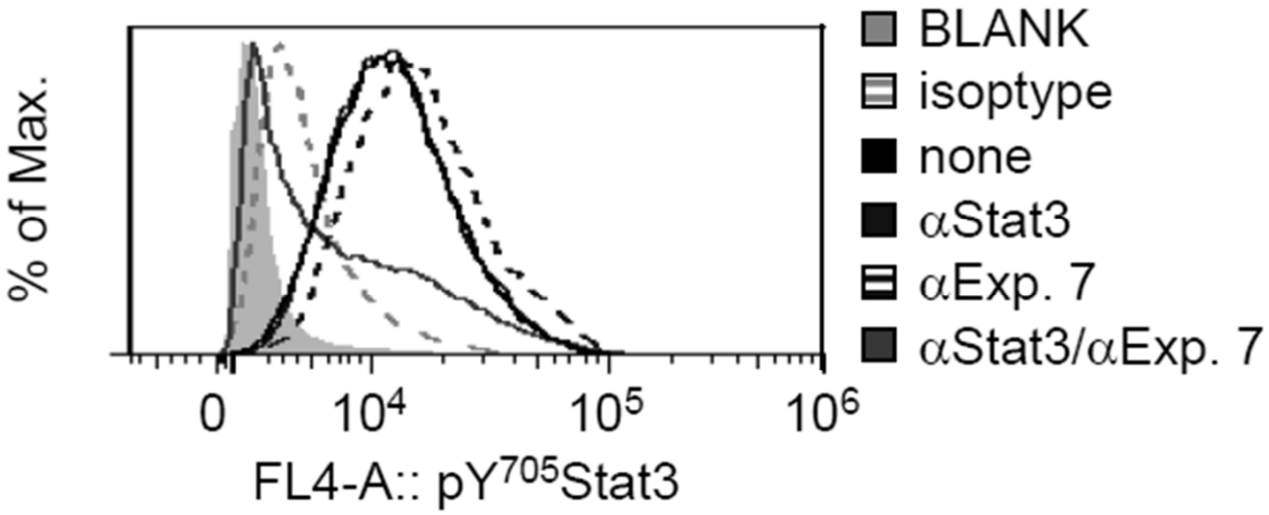

% of Max.
BLANK
isoptype
none
αStat3
αExp. 7
αStat3/αExp. 7
0
10^4
10^5
10^6
FL4-A:: pY705Stat3

FIG. 4F

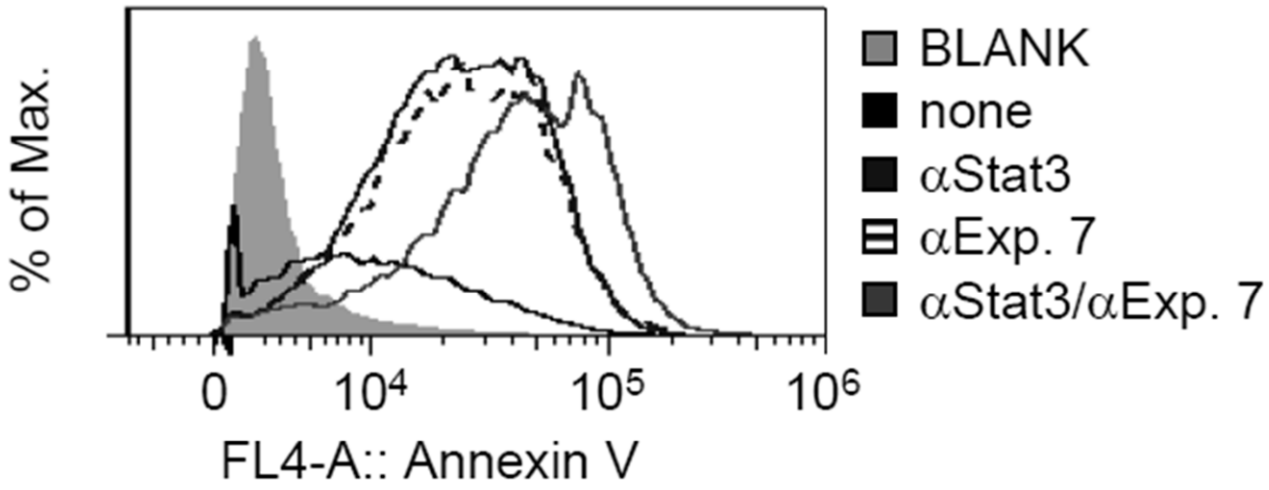

% of Max.
0
10^4
10^5
10^6
FL4-A:: Annexin V
BLANK
none
αStat3
αExp. 7
αStat3/αExp. 7

FIG. 4G

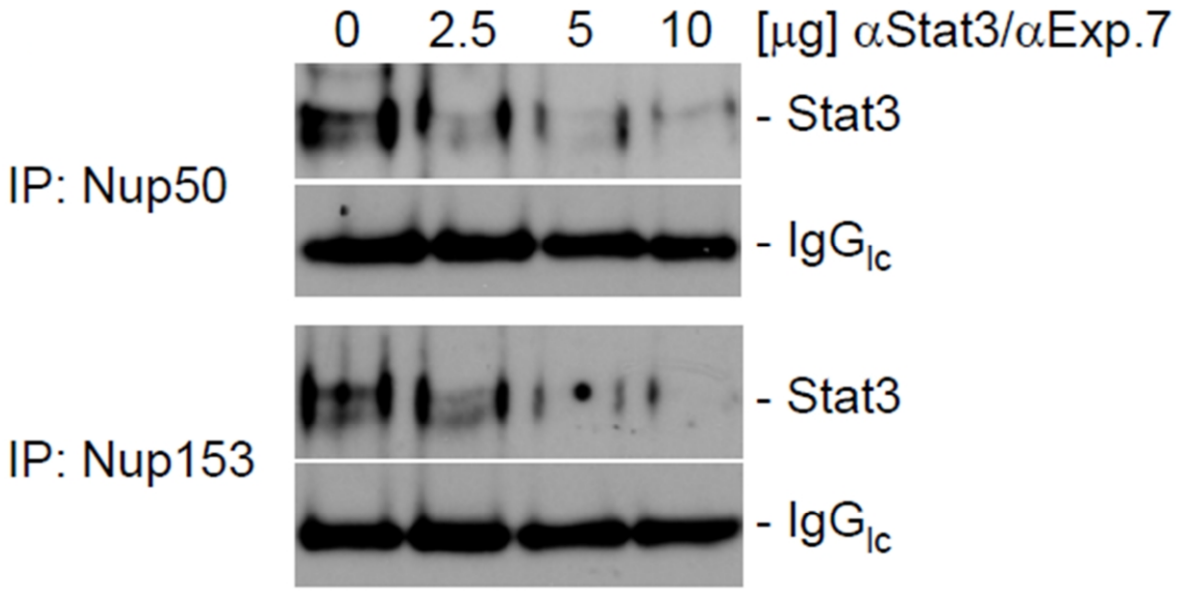

0
2.5
5
10
[μg] αStat3/αExp.7
IP: Nup50
- Stat3
- IgG_lc
IP: Nup153
- Stat3
- IgG_lc

FIG. 4H

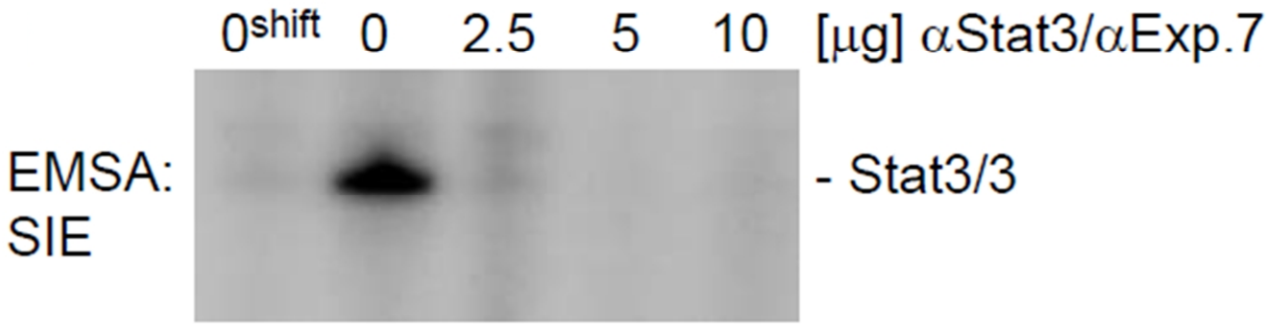


FIG. 4I

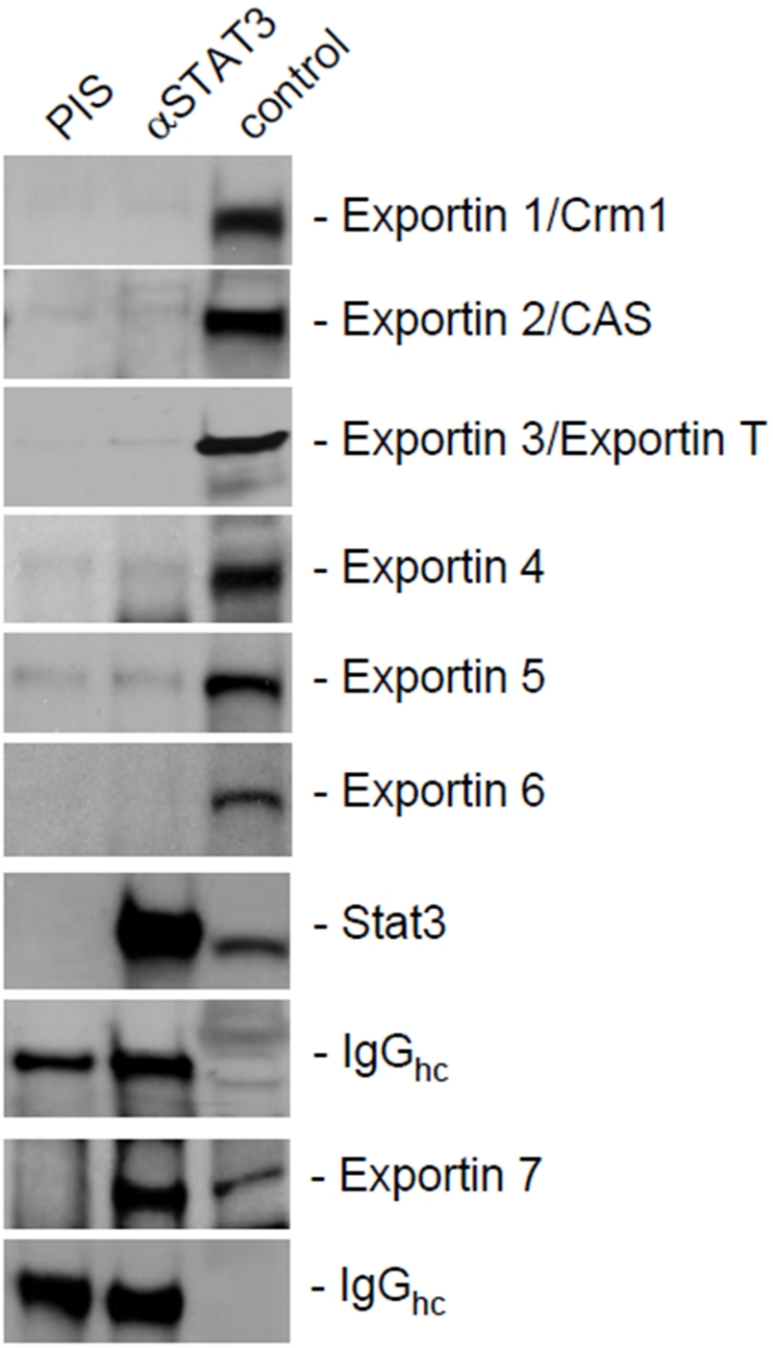

PIS
αSTAT3
control
- Exportin 1/Crm1
- Exportin 2/CAS
- Exportin 3/Exportin T
- Exportin 4
- Exportin 5
- Exportin 6
- Stat3
- IgG$_{hc}$
- Exportin 7
- IgG$_{hc}$

FIG. 5A

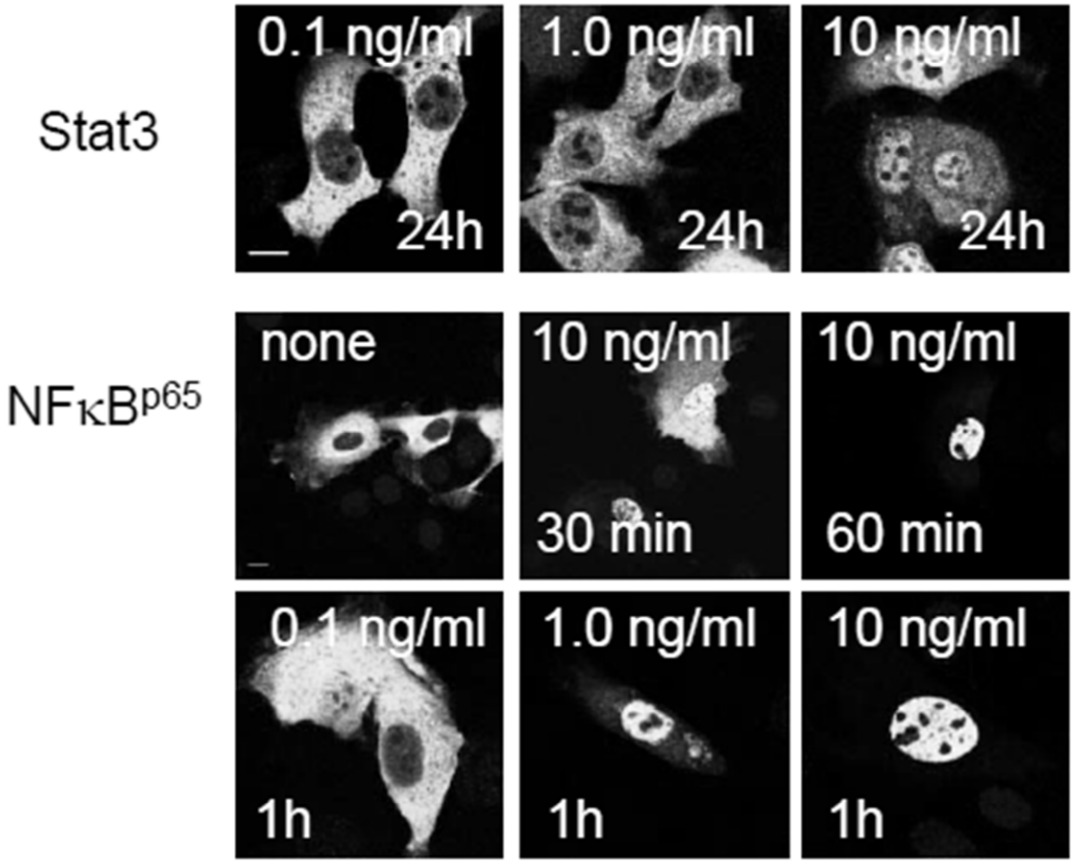
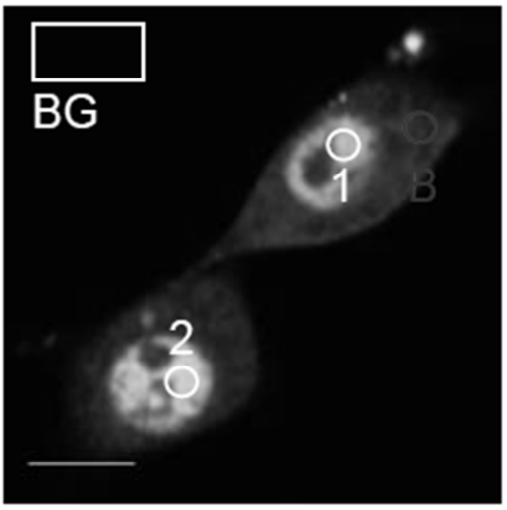


FIG. 5B



BG - Background-ROI

B - Bleach-ROI

1 – detection ROI 1 – nucleus bleached cell

2 – detection ROI 2 – nucleus unbleached cell

FIG. 6A

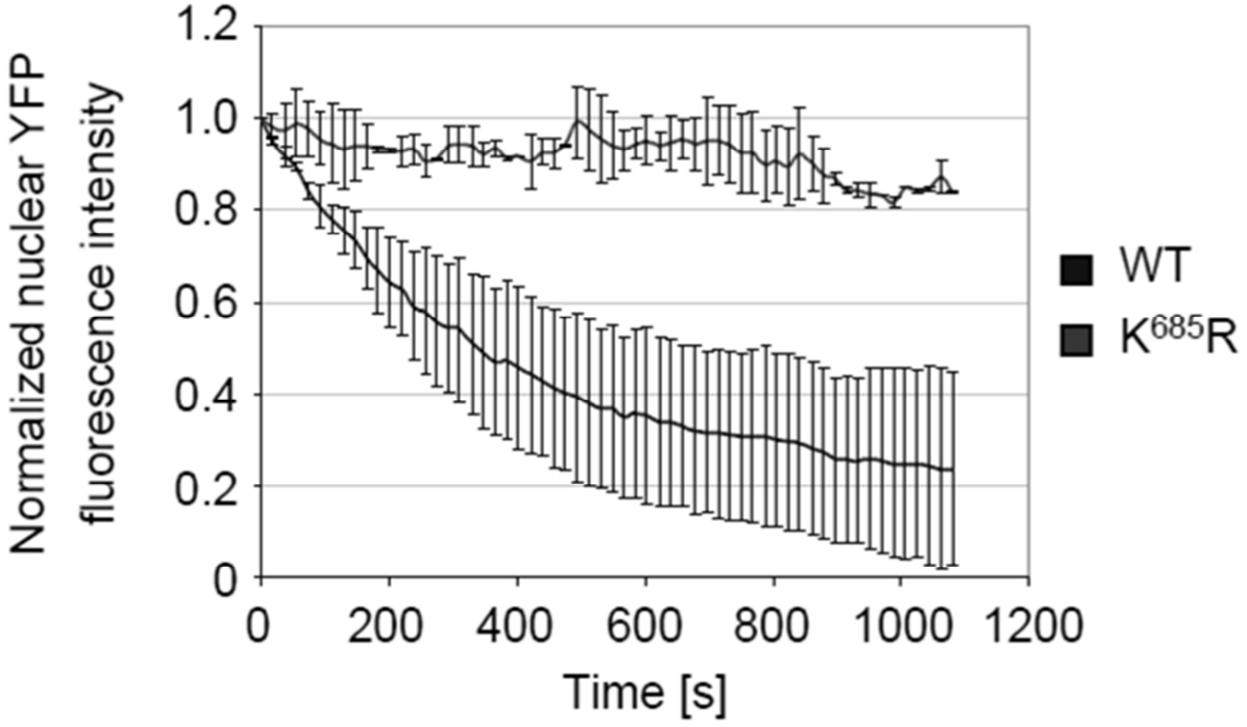

Normalized nuclear YFP fluorescence intensity
1.2
1.0
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
1200
Time [s]
WT
K685R

FIG. 6B

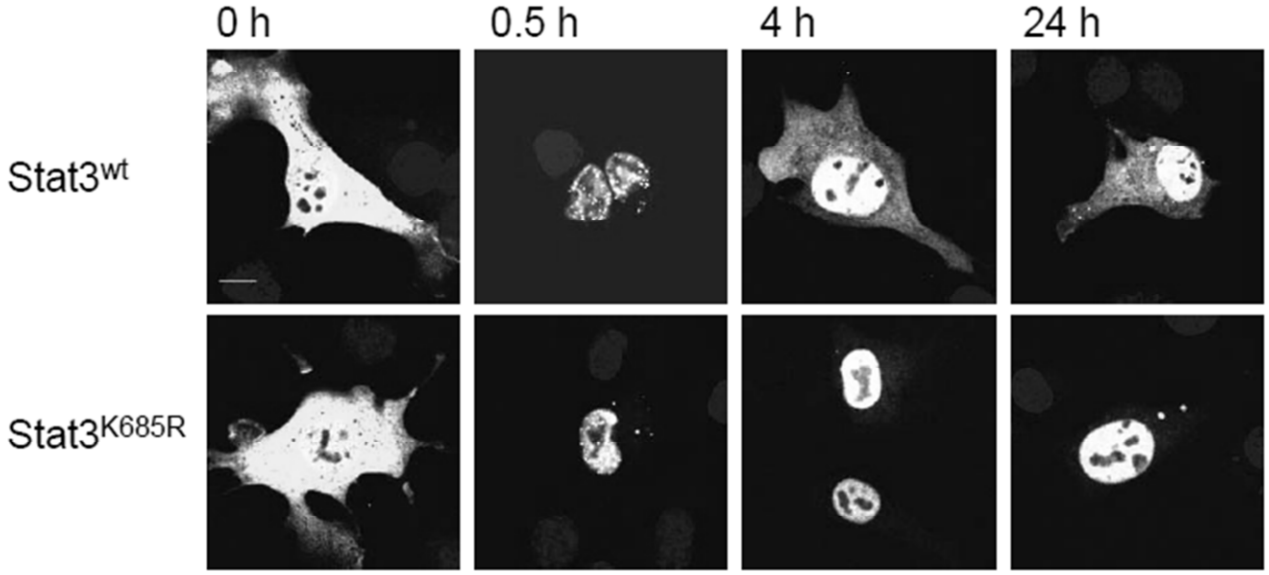

0 h
0.5 h
4 h
24 h
Stat3wt
Stat3K685R

FIG. 7A

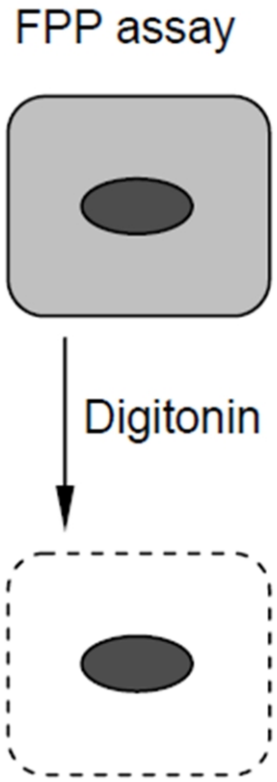

FPP assay
Digitonin

FIG. 7B

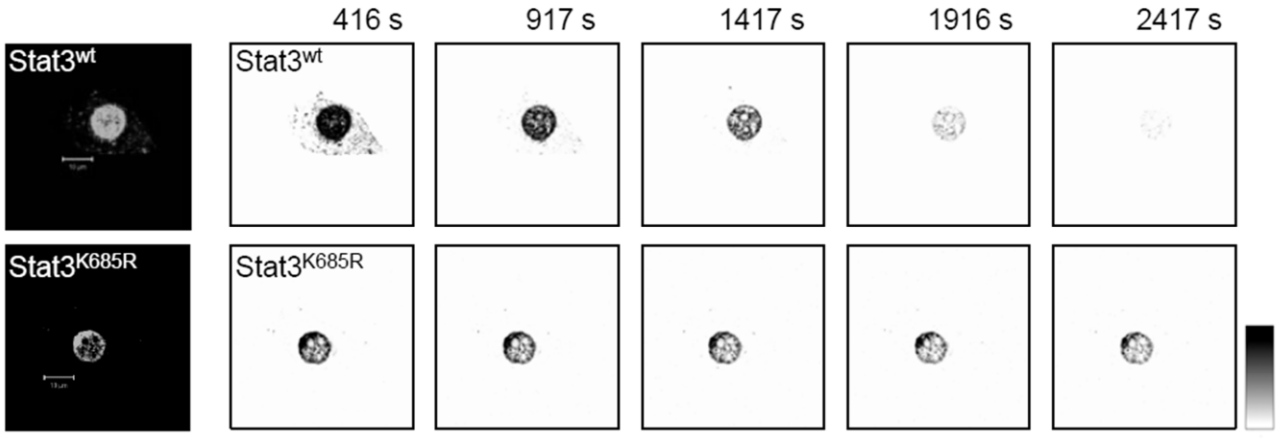

416 s
917 s
1417 s
1916 s
2417 s
Stat3wt
Stat3wt
Stat3K685R
Stat3K685R

FIG. 7C

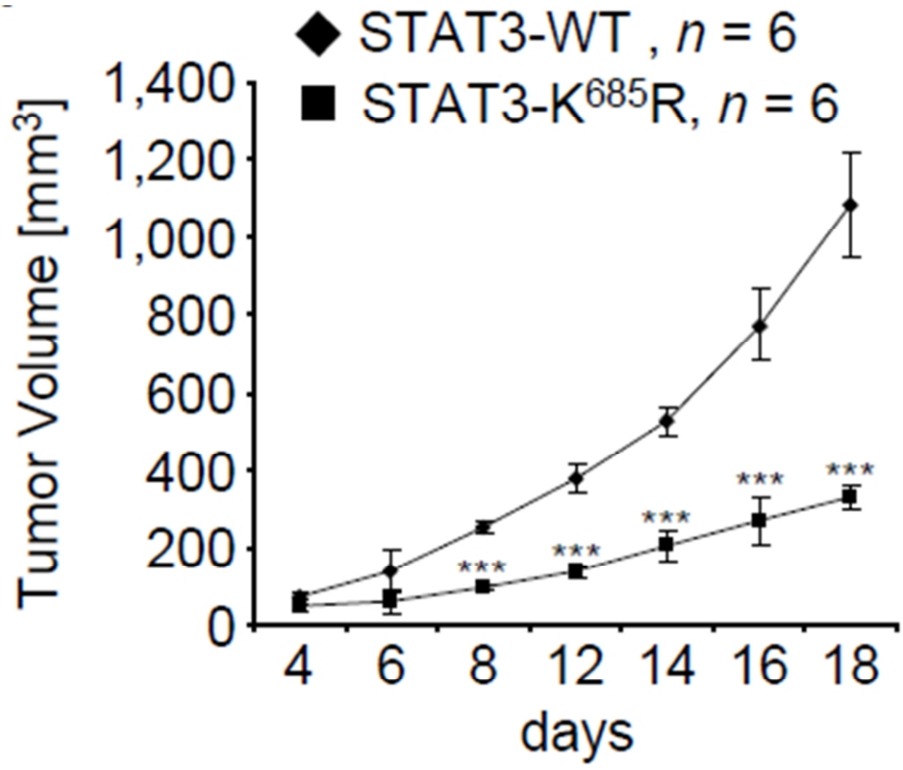

STAT3-WT , n = 6
STAT3-K685R, n = 6
Tumor Volume [mm3]
1,400
1,200
1,000
800
600
400
200
0
4
6
8
12
14
16
18
days
***
***
***
***
***

FIG. 8A

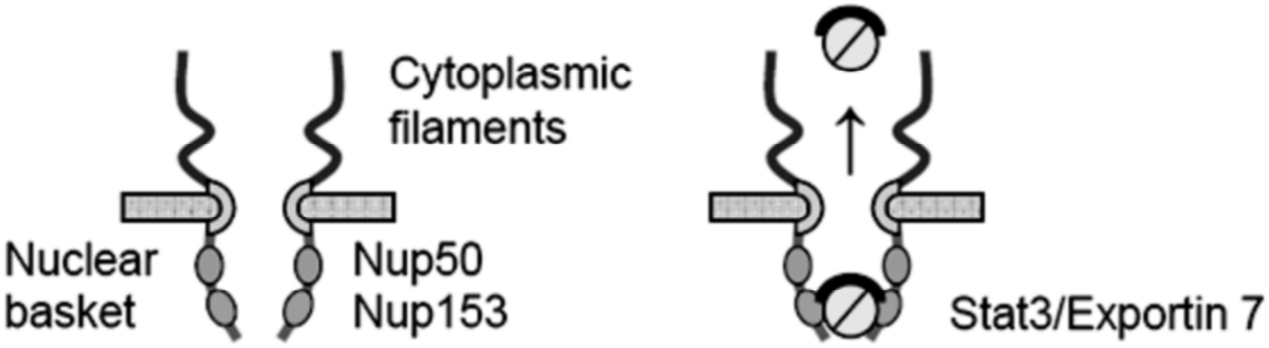

Cytoplasmic
filaments
Nuclear
basket
Nup50
Nup153
Stat3/Exportin 7

FIG. 8B

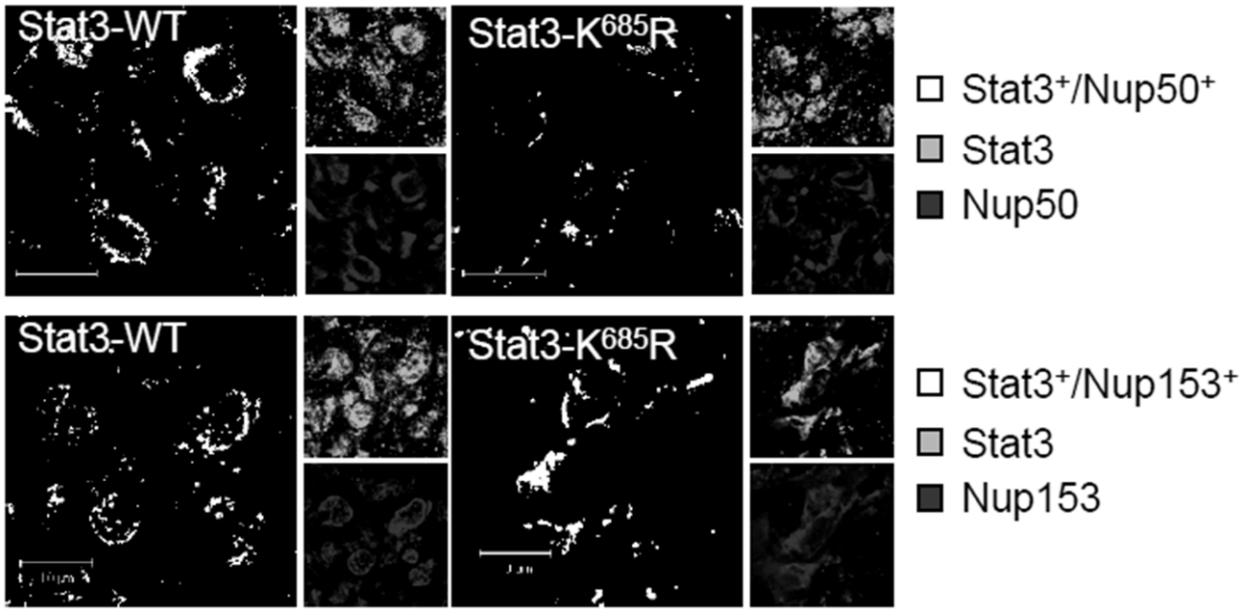

Stat3-WT
Stat3-K685R
Stat3+/Nup50+
Stat3
Nup50
Stat3-WT
Stat3-K685R
Stat3+/Nup153+
Stat3
Nup153

FIG. 8C

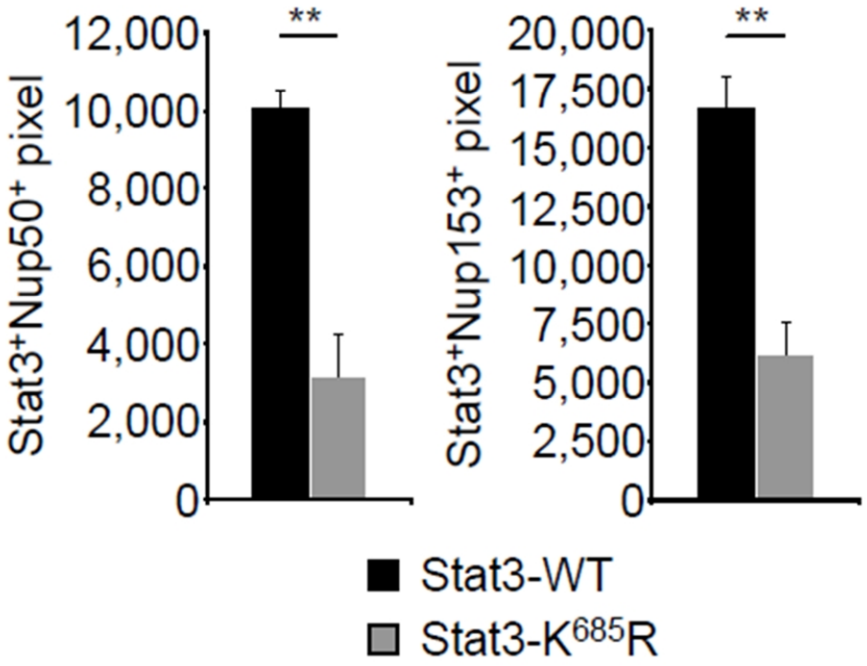

**
**
Stat3+Nup50+ pixel
Stat3+Nup153+ pixel
12,000
10,000
8,000
6,000
4,000
2,000
0
20,000
17,500
15,000
12,500
10,000
7,500
5,000
2,500
0
Stat3-WT
Stat3-K685R

FIG. 8D

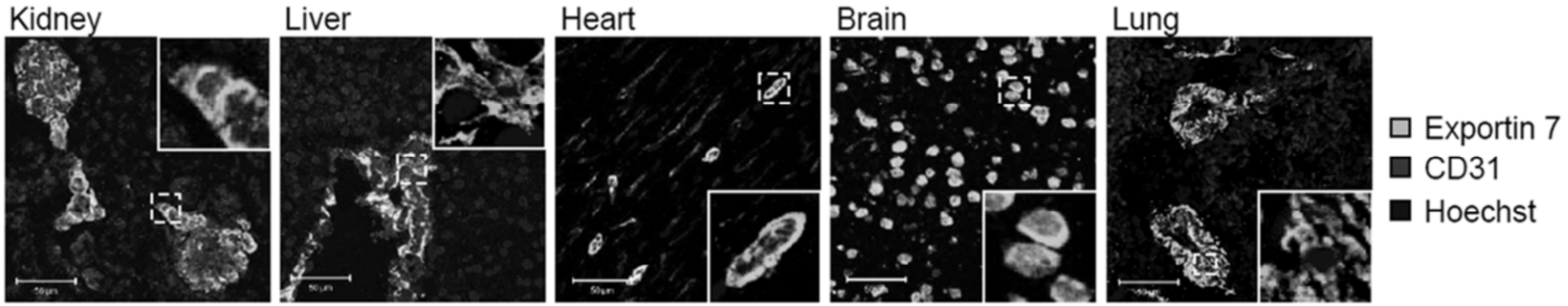


FIG. 9

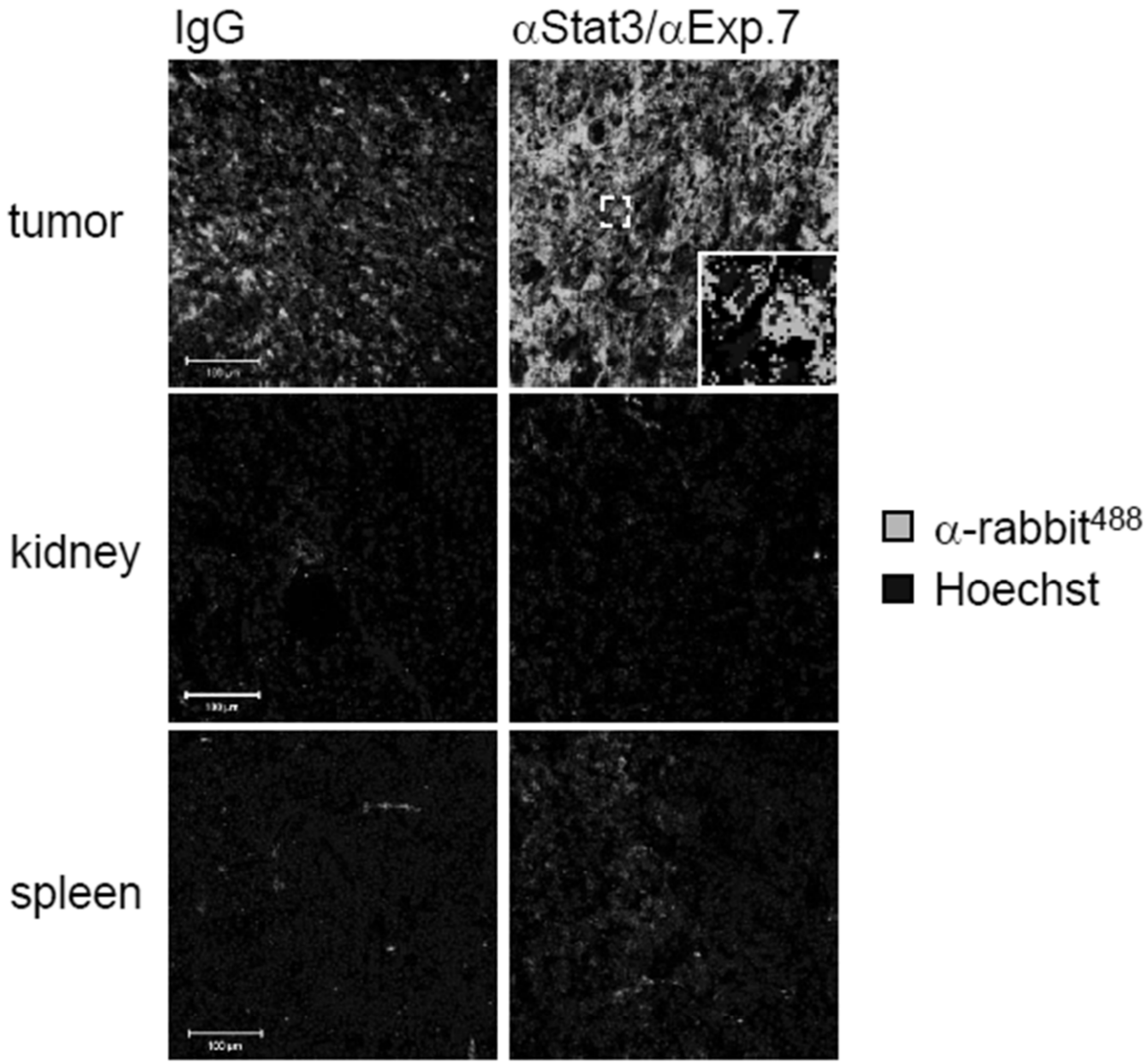


FIG. 10A

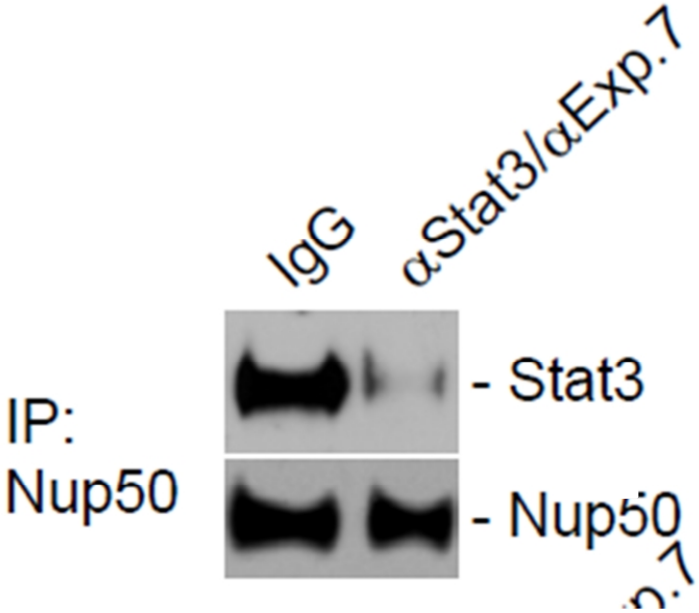


FIG. 10B

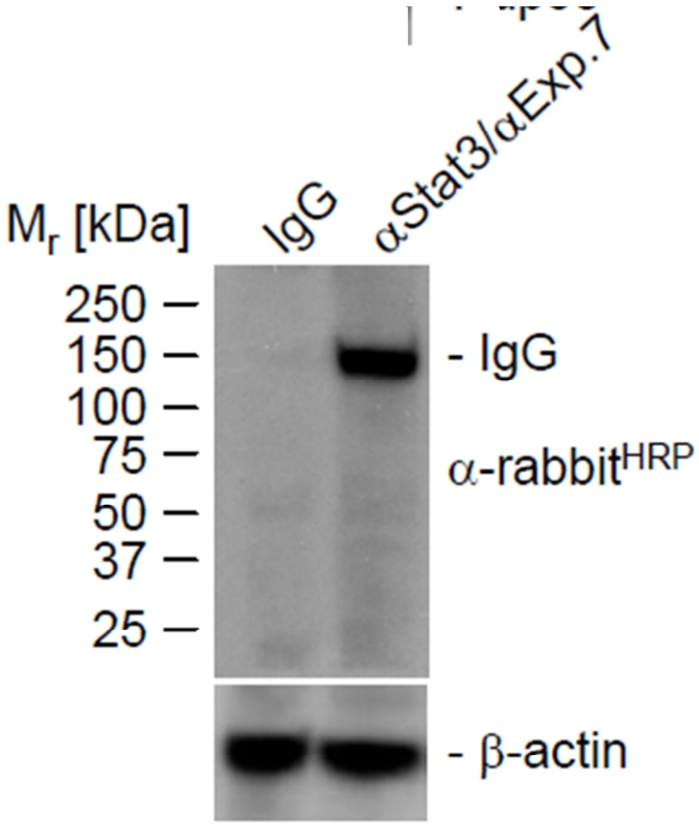


FIG. 10C

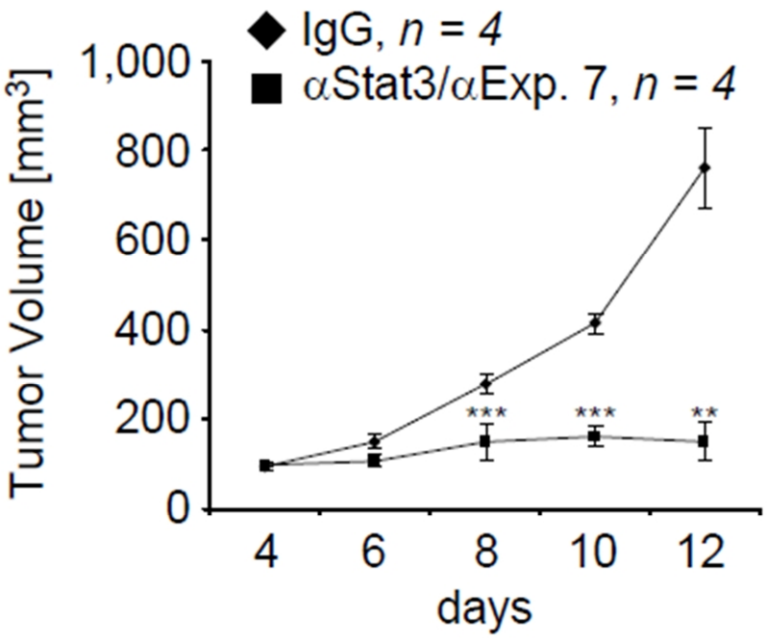


FIG. 11A

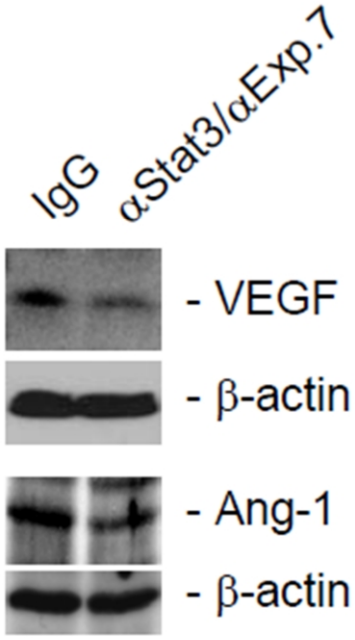


FIG. 11B

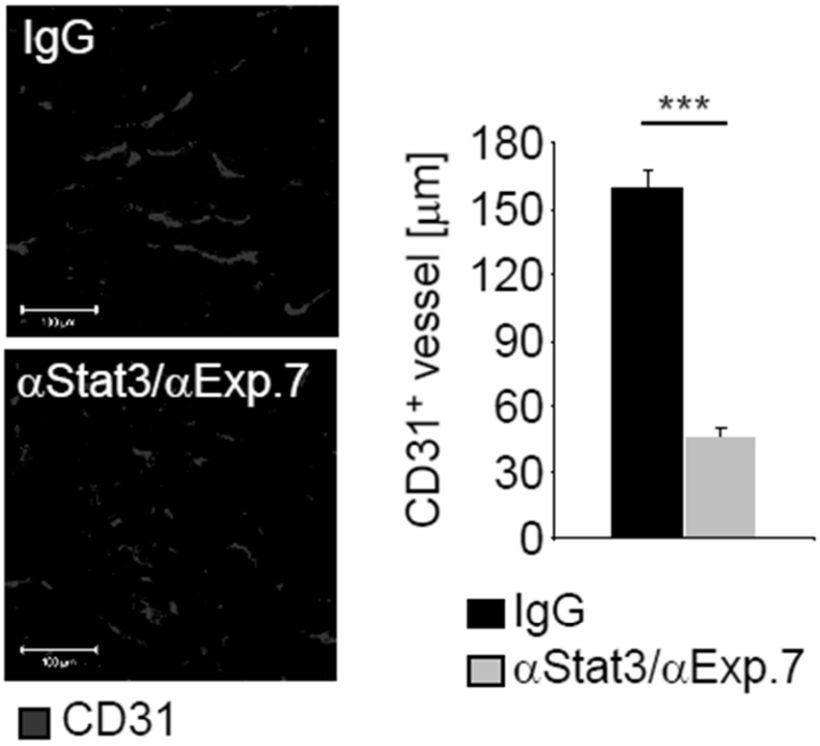


FIG. 11C

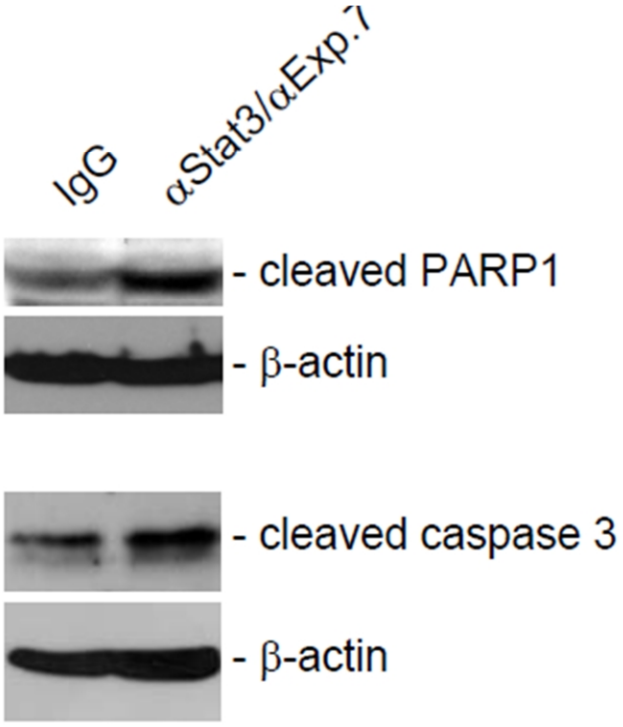


FIG. 11D

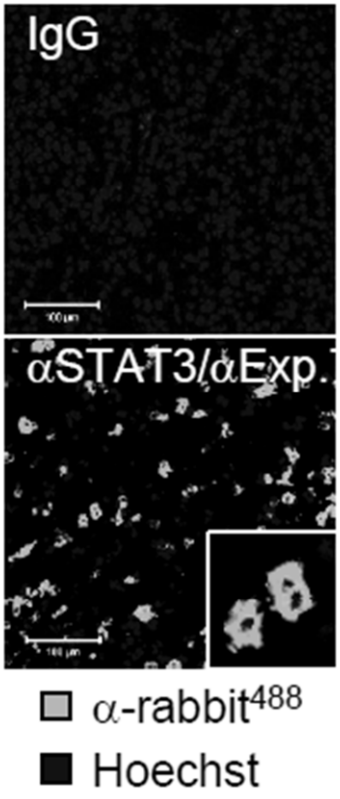


FIG. 12A

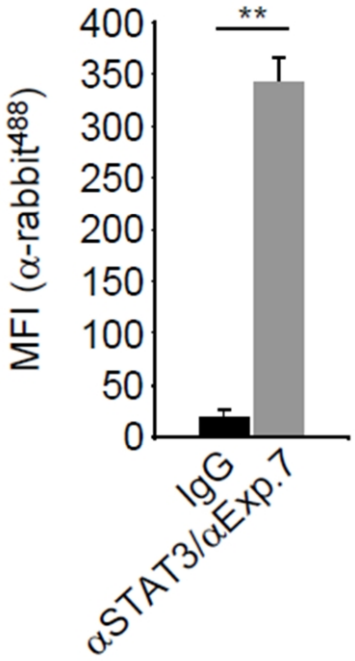


FIG. 12B

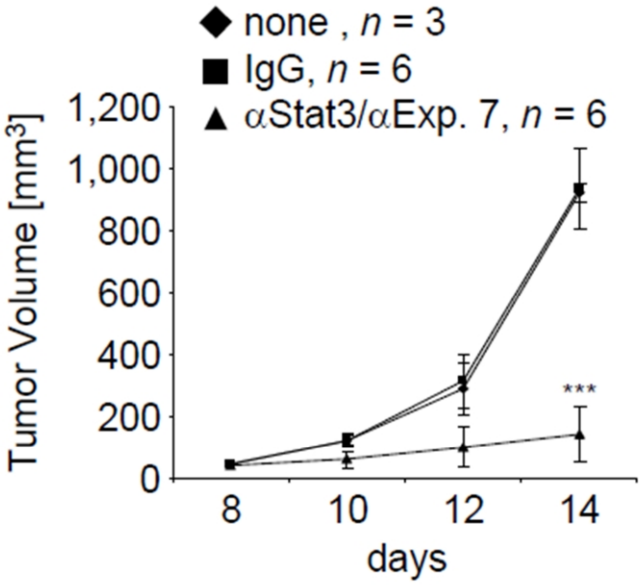


FIG. 12C

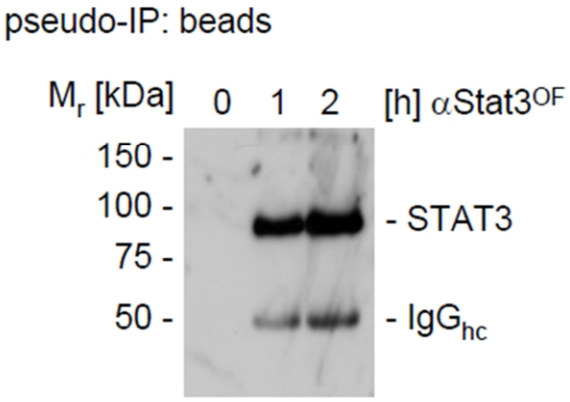


FIG. 13A

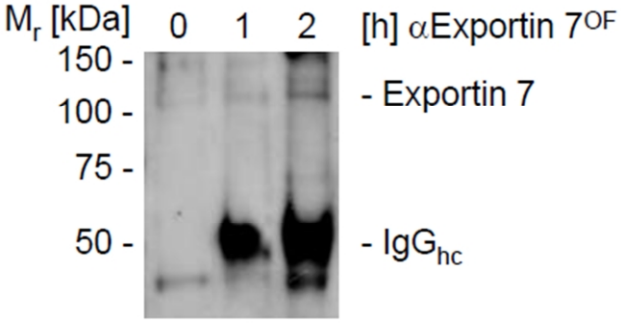


FIG. 13B

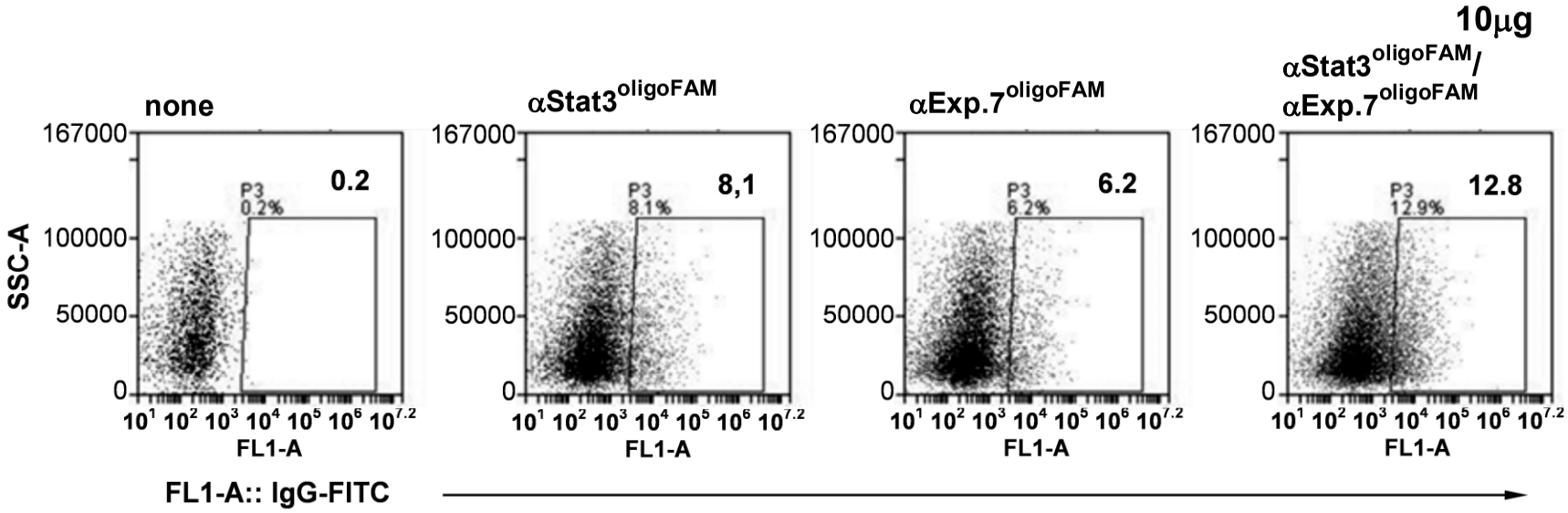

10μg
none
αStat3oligoFAM
αExp.7oligoFAM
αStat3oligoFAM/
αExp.7oligoFAM
SSC-A
167000
100000
50000
0
P3
0.2%
0.2
P3
8.1%
8,1
P3
6.2%
6.2
P3
12.9%
12.8
10¹ 10² 10³ 10⁴ 10⁵ 10⁶ 10⁷.²
FL1-A
FL1-A:: IgG-FITC

FIG. 14

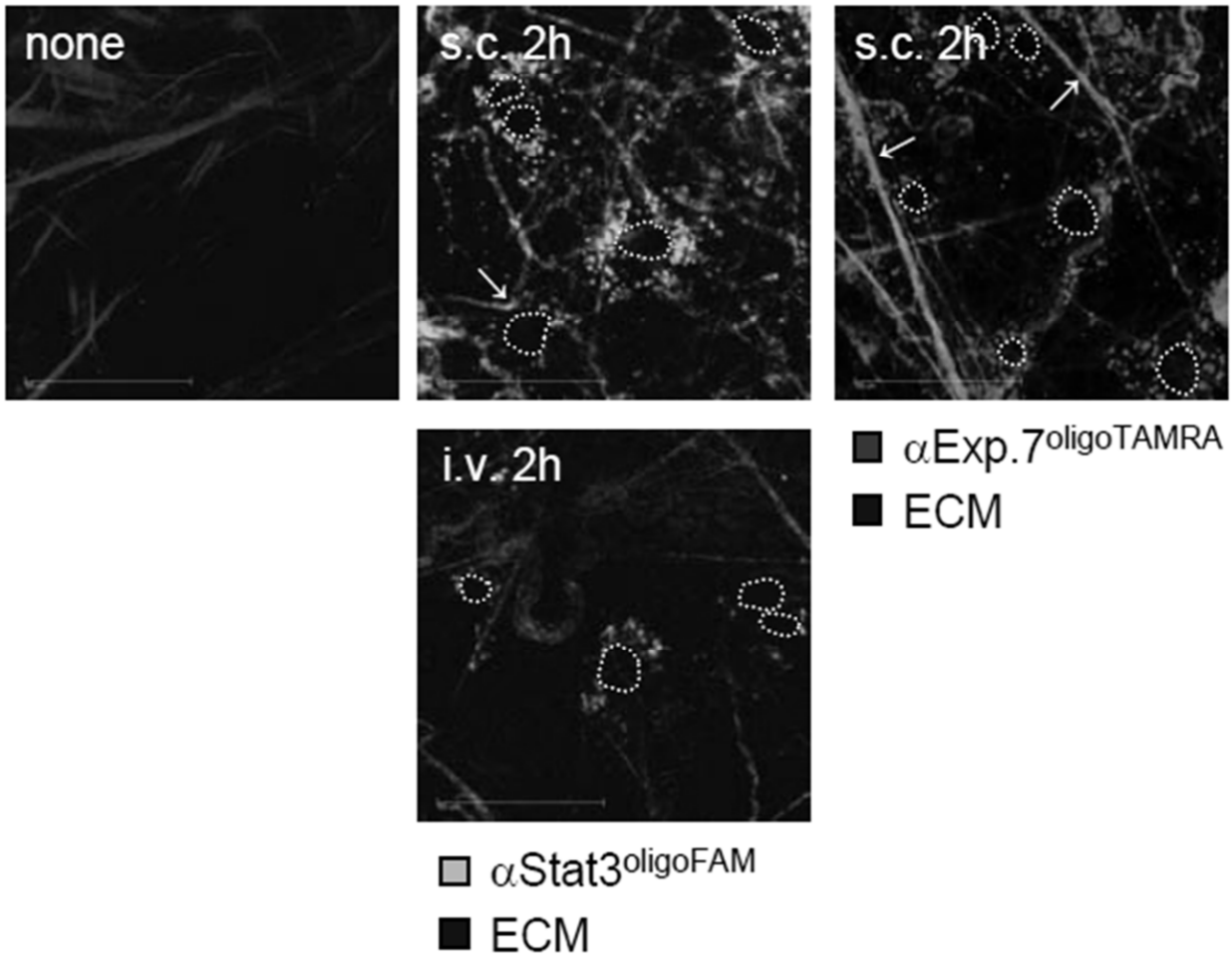

none
s.c. 2h
s.c. 2h
i.v. 2h
αExp.7oligoTAMRA
ECM
αStat3oligoFAM
ECM

FIG. 15A

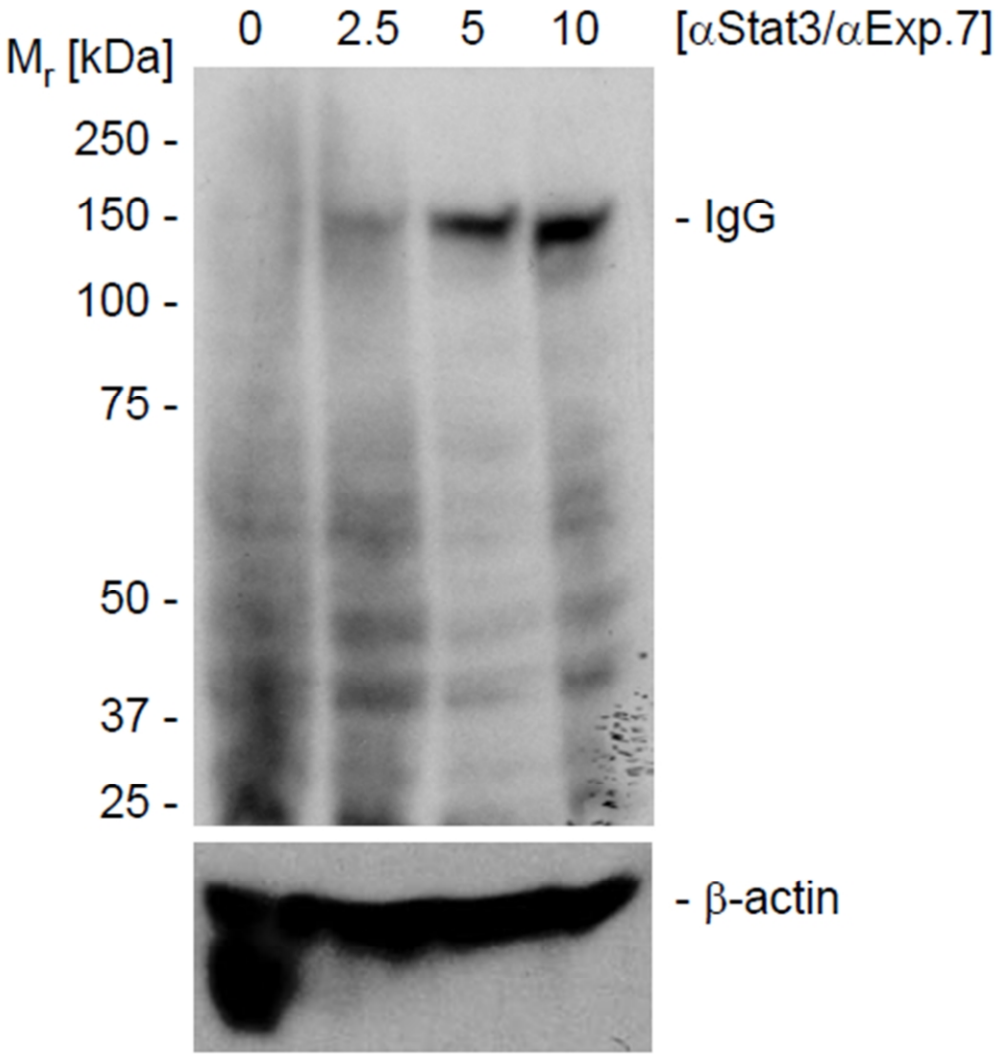


FIG. 15B

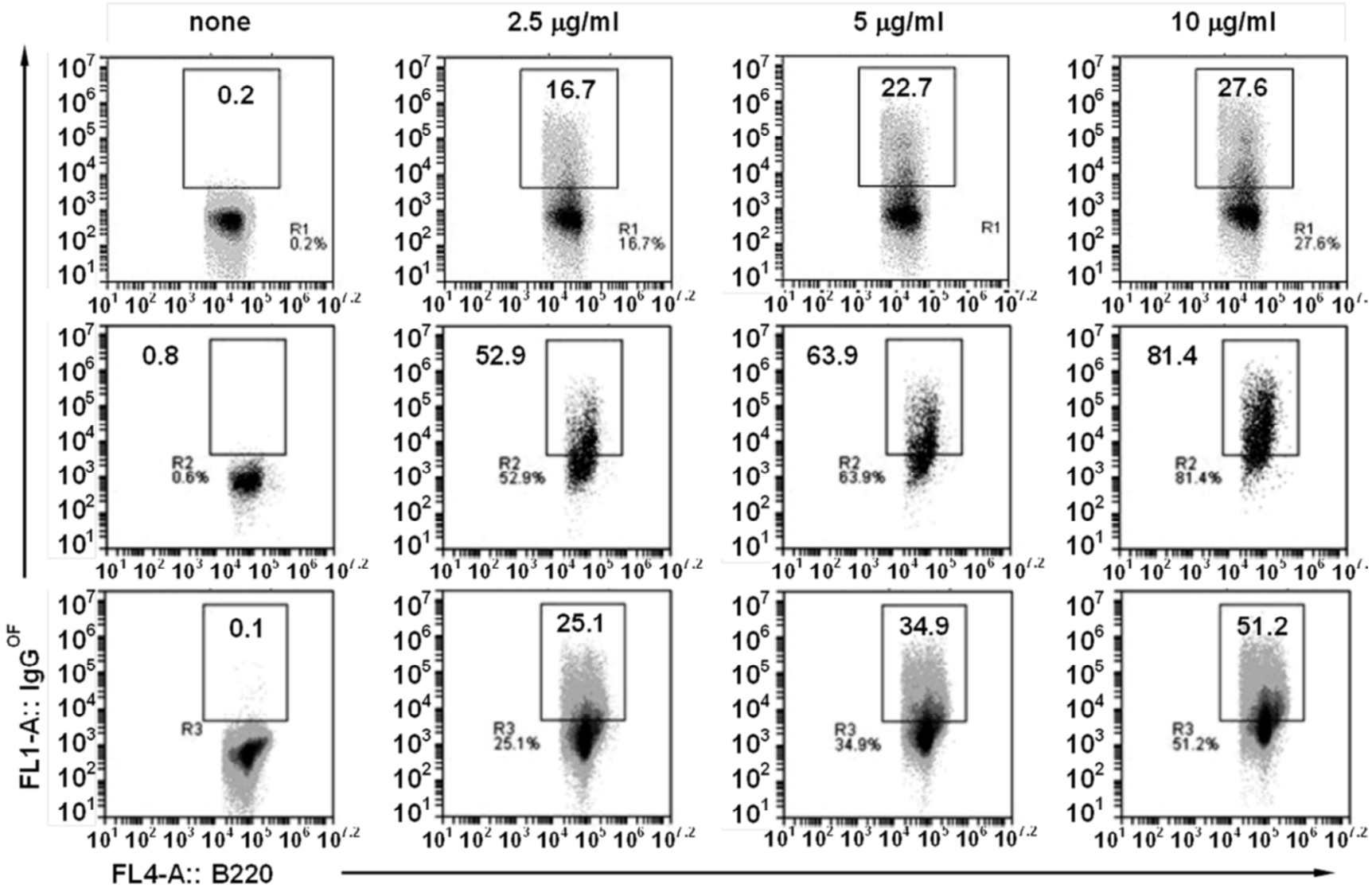

none
2.5 μg/ml
5 μg/ml
10 μg/ml
0.2
16.7
22.7
27.6
R1
0.2%
R1
16.7%
R1
R1
27.6%
0.8
52.9
63.9
81.4
R2
0.6%
R2
52.9%
R2
63.9%
R2
81.4%
0.1
25.1
34.9
51.2
R3
R3
25.1%
R3
34.9%
R3
51.2%
FL1-A:: IgG OF
FL4-A:: B220

FIG. 16A

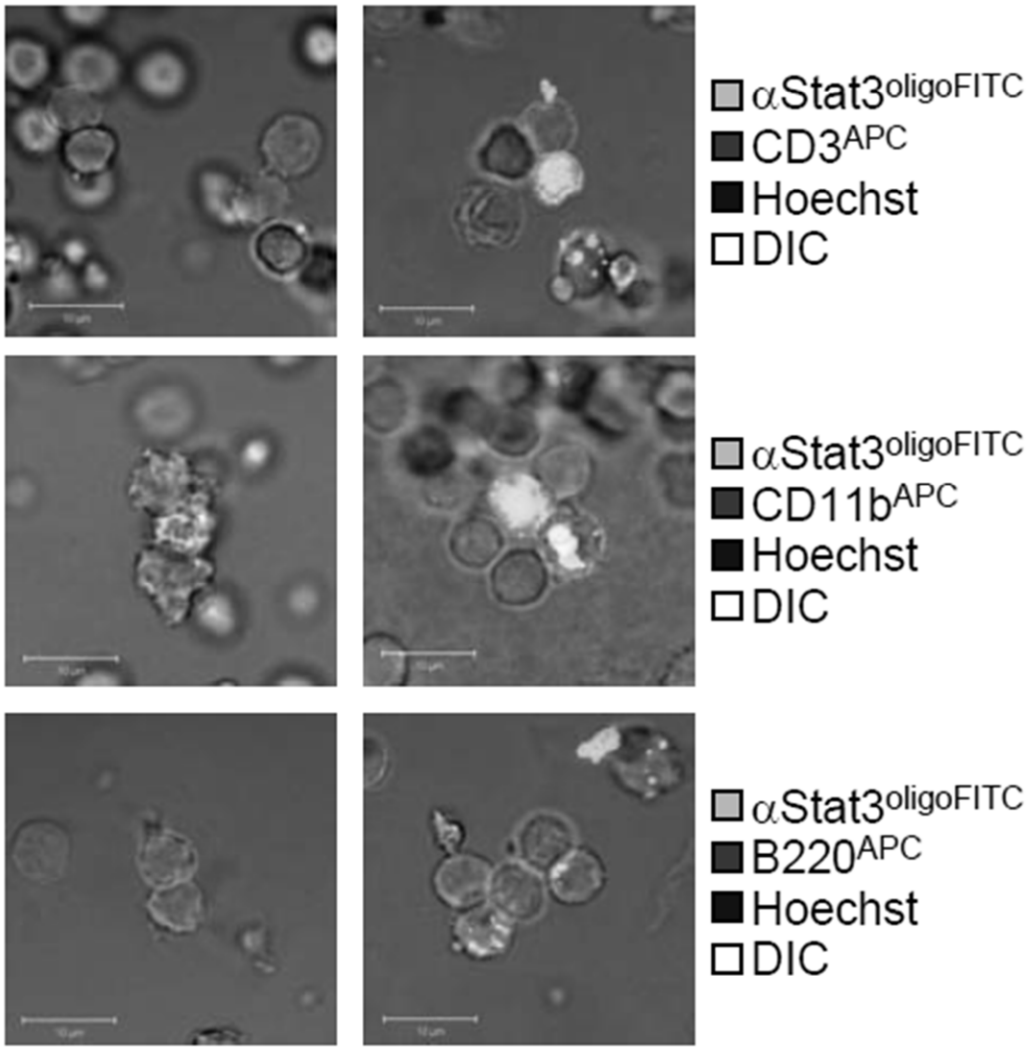

αStat3oligoFITC
CD3APC
Hoechst
DIC
αStat3oligoFITC
CD11bAPC
Hoechst
DIC
αStat3oligoFITC
B220APC
Hoechst
DIC

FIG. 16B

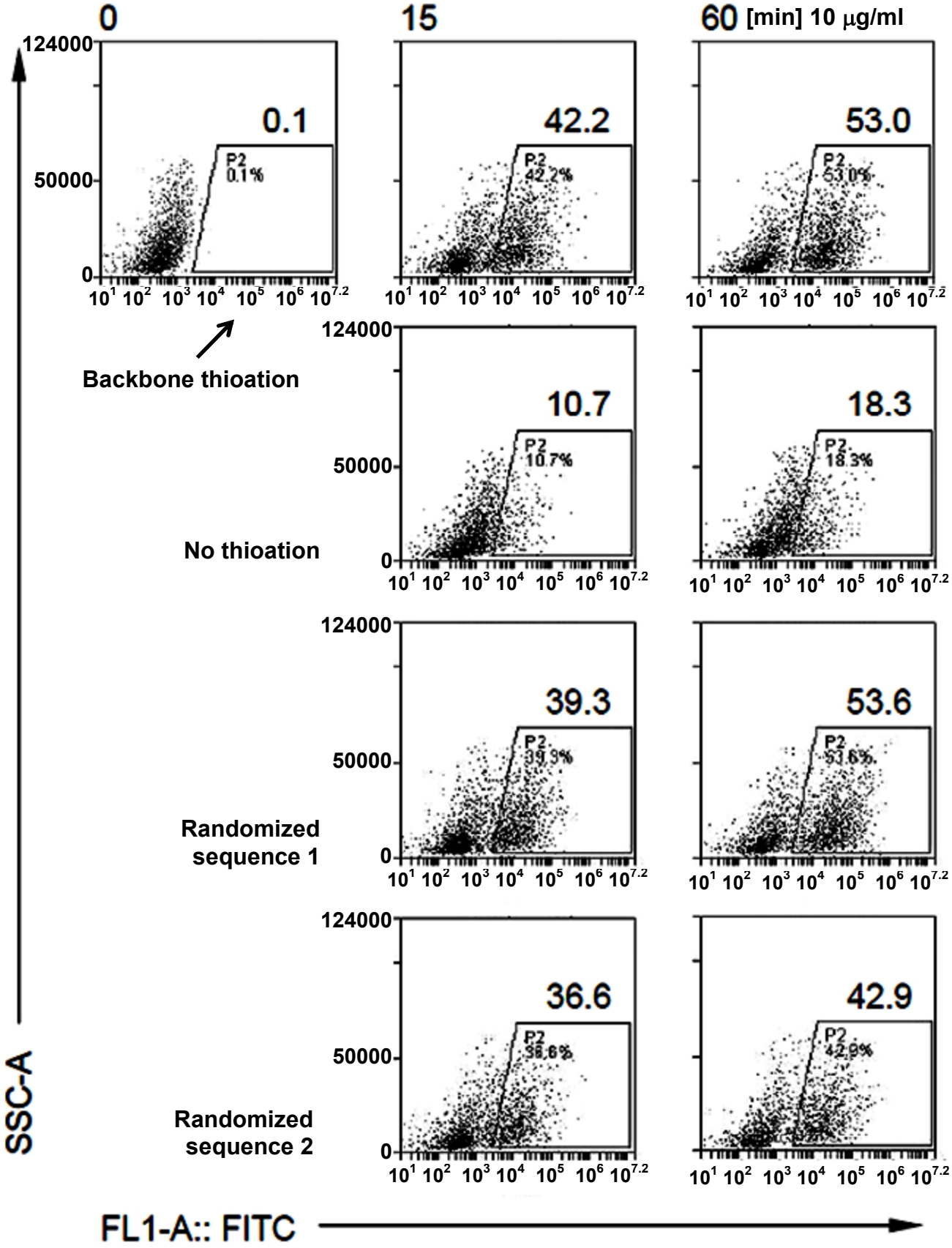

0
15
60 [min] 10 μg/ml
124000
50000
0
0.1
42.2
53.0
P2
0.1%
42.2%
53.0%
Backbone thioation
10.7
18.3
10.7%
18.3%
No thioation
39.3
53.6
39.3%
53.6%
Randomized sequence 1
36.6
42.9
36.6%
42.9%
Randomized sequence 2
SSC-A
FL1-A:: FITC

FIG. 17A

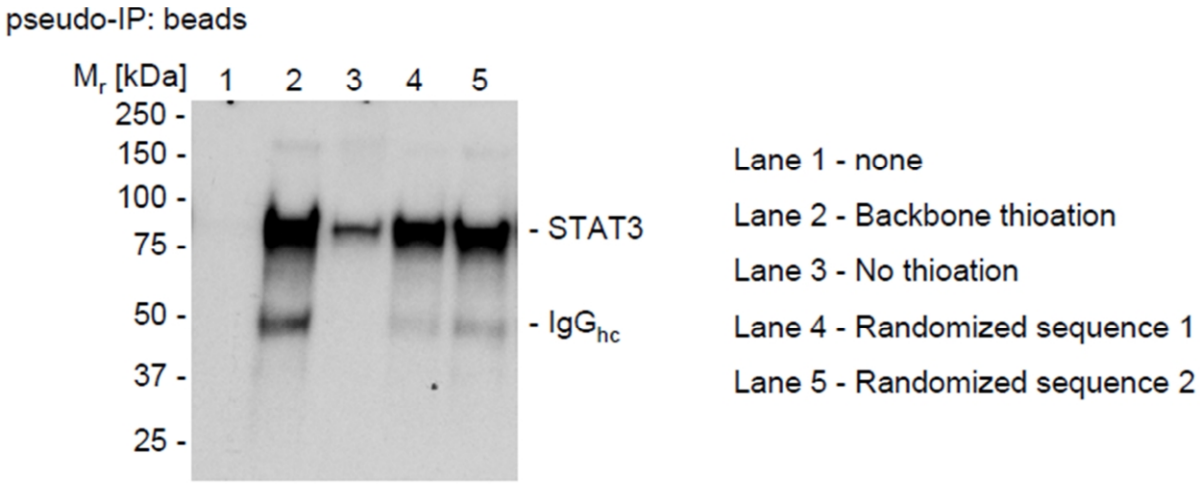


FIG. 17B

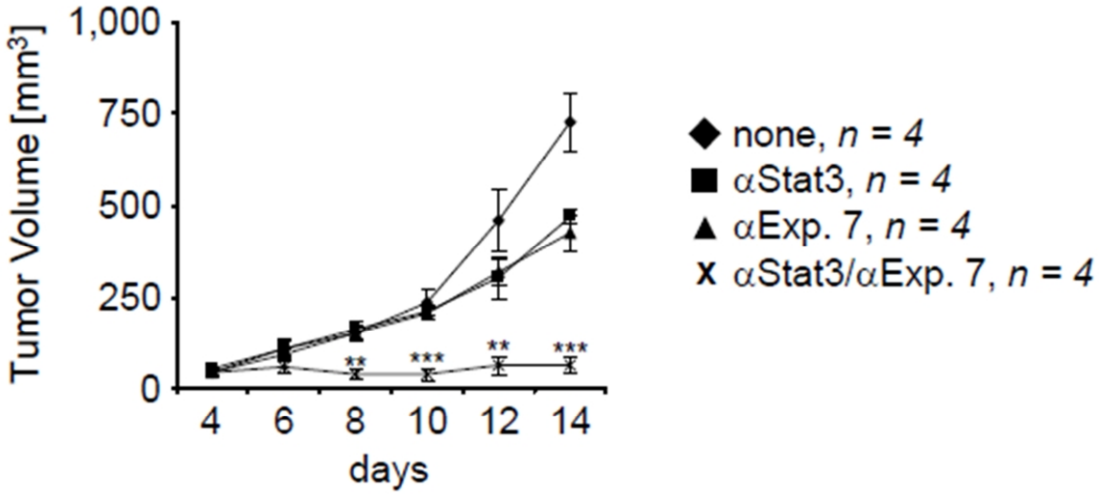


FIG. 18

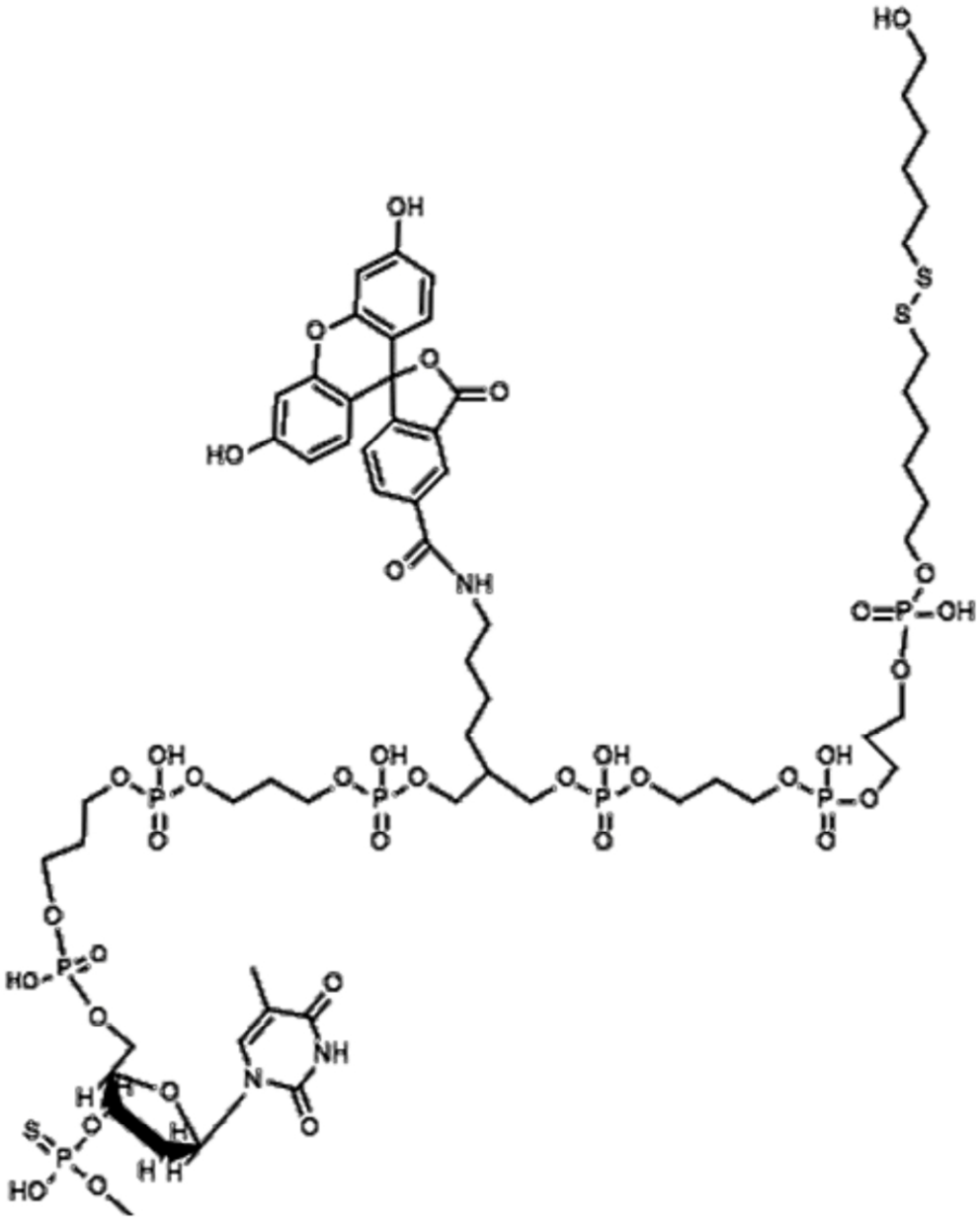


FIG. 19

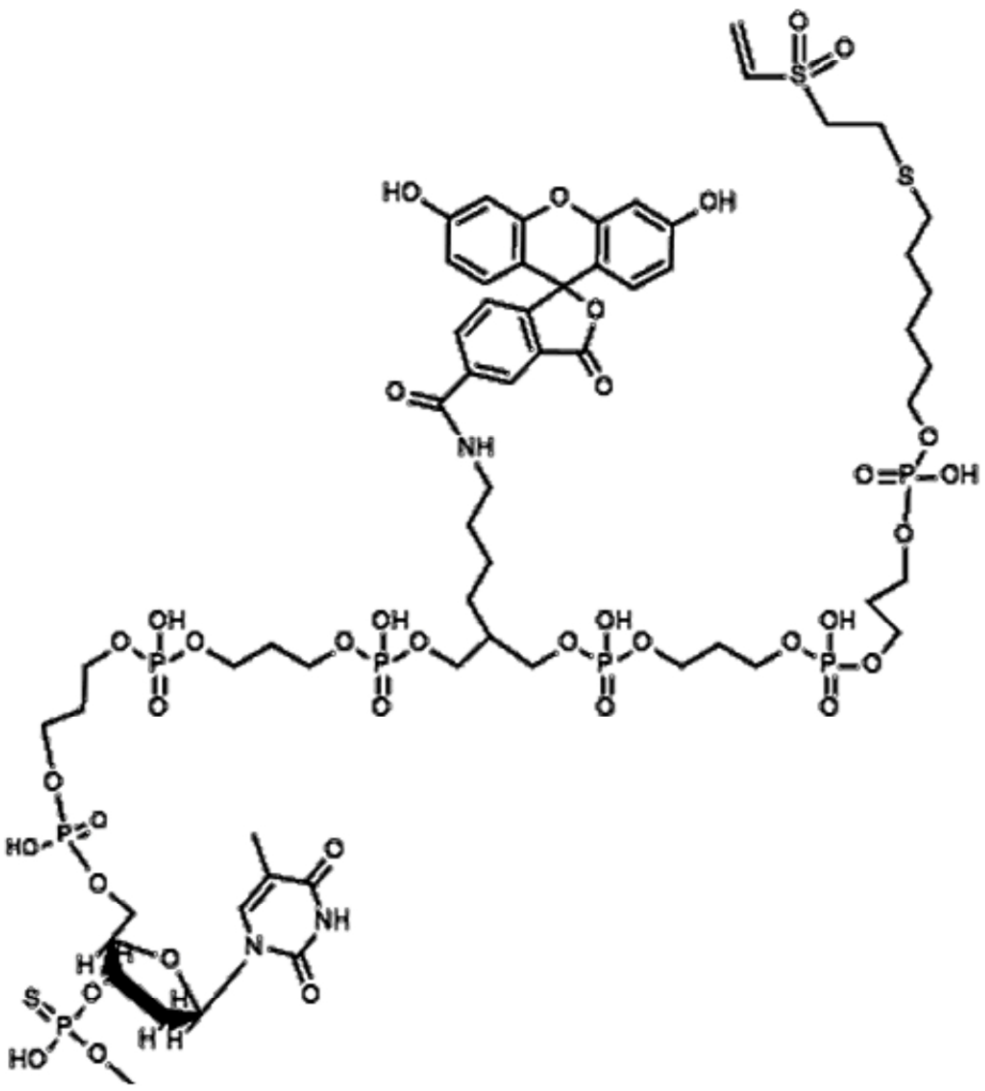


FIG. 20

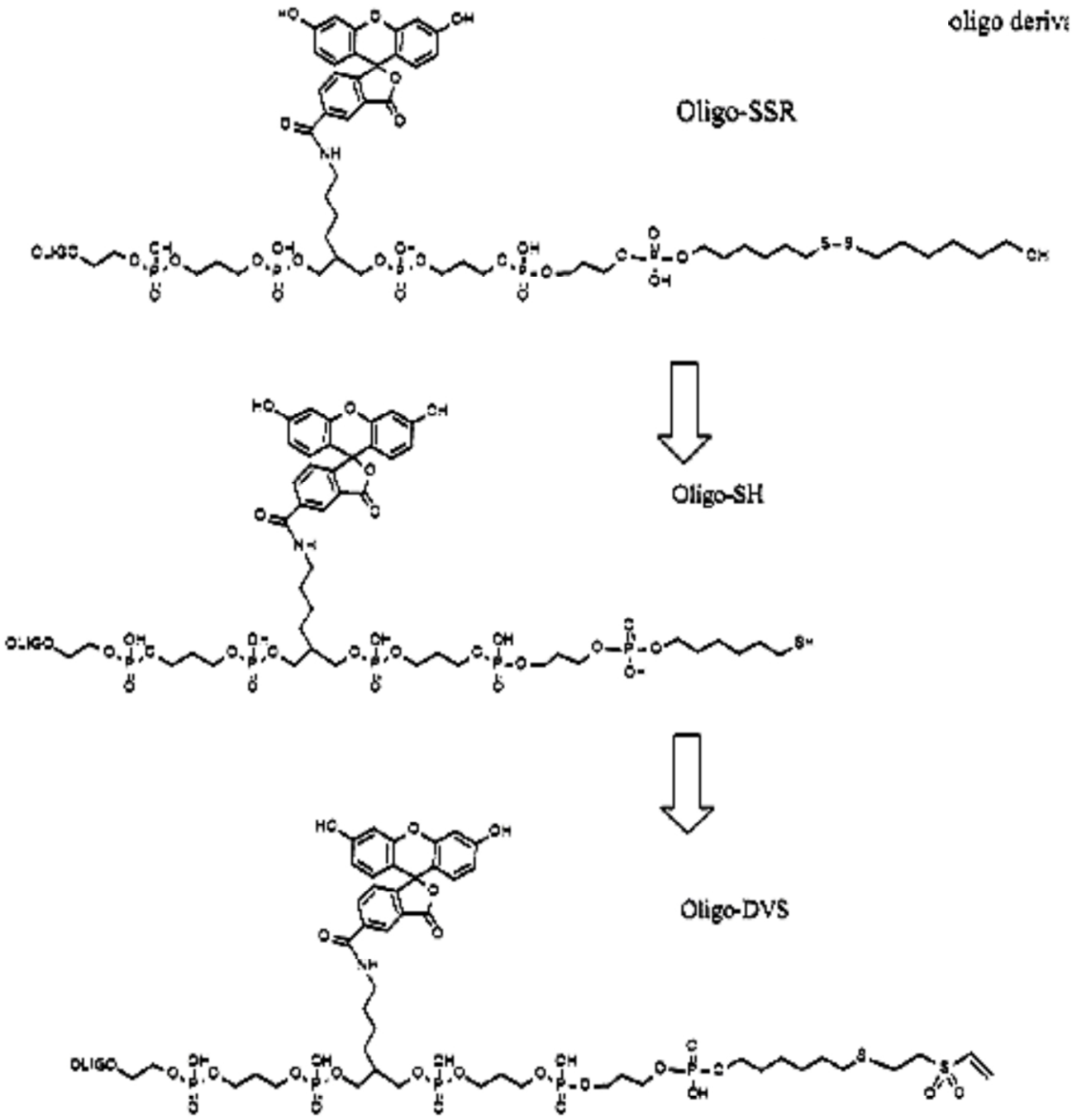


FIG. 21

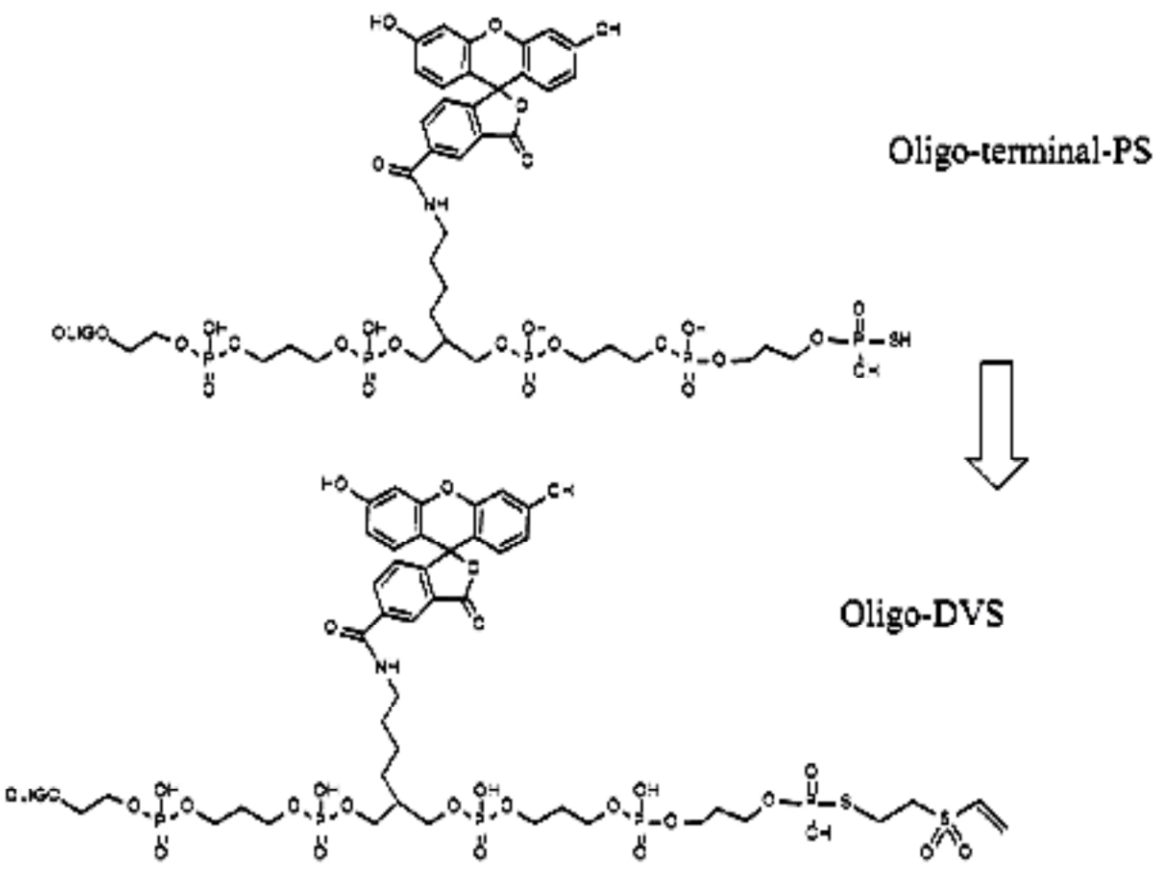


FIG. 22

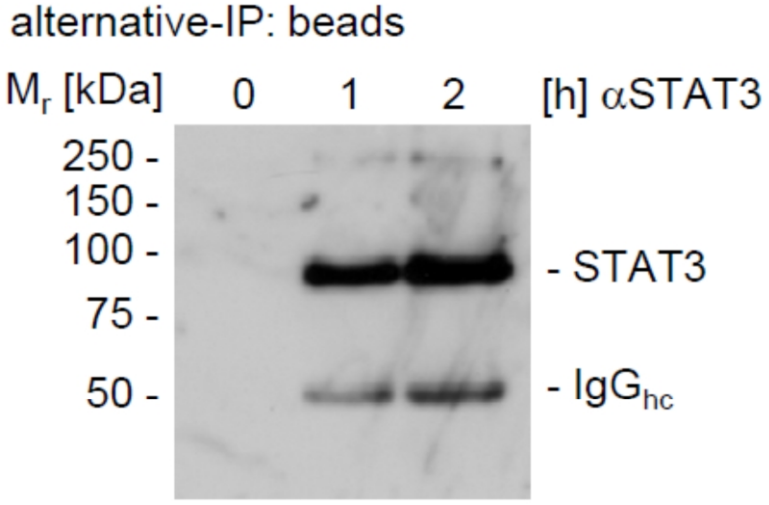


FIG. 23A

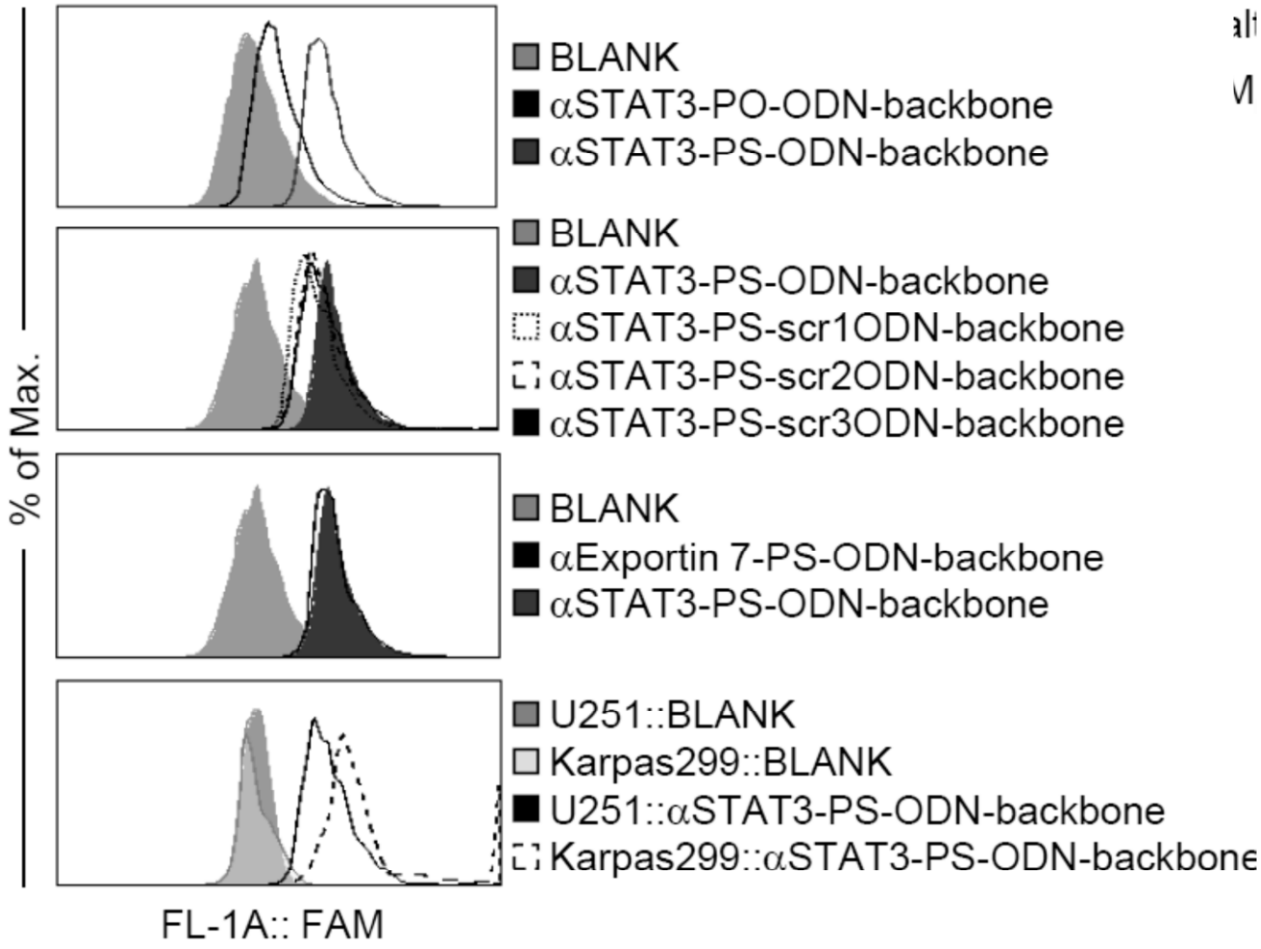


FIG. 23B

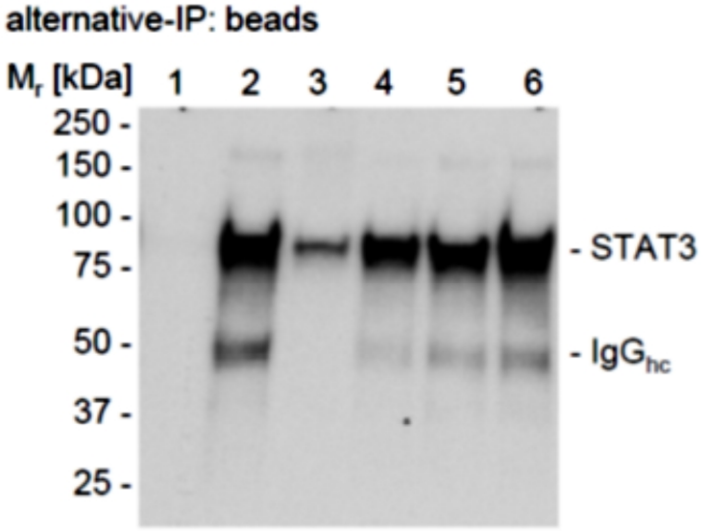


FIG. 23C

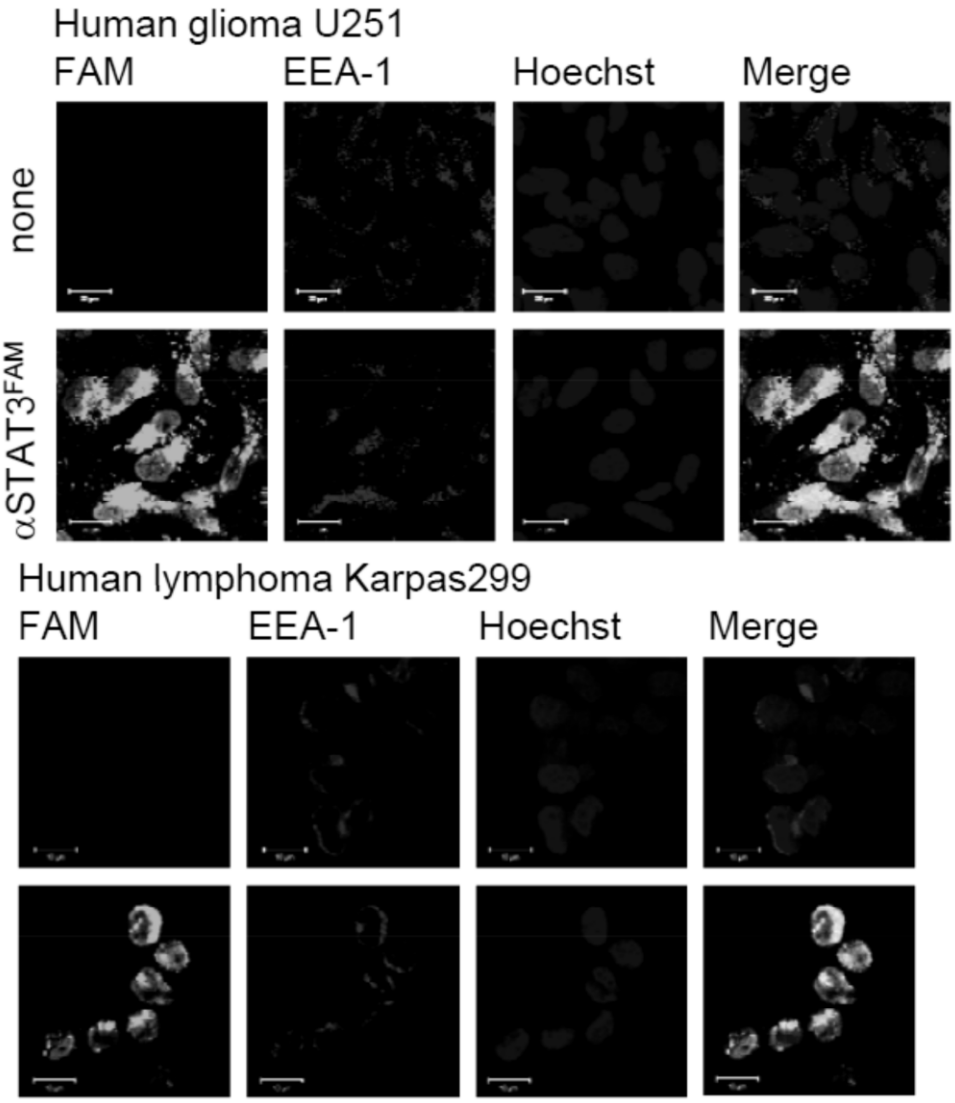


FIG. 23D

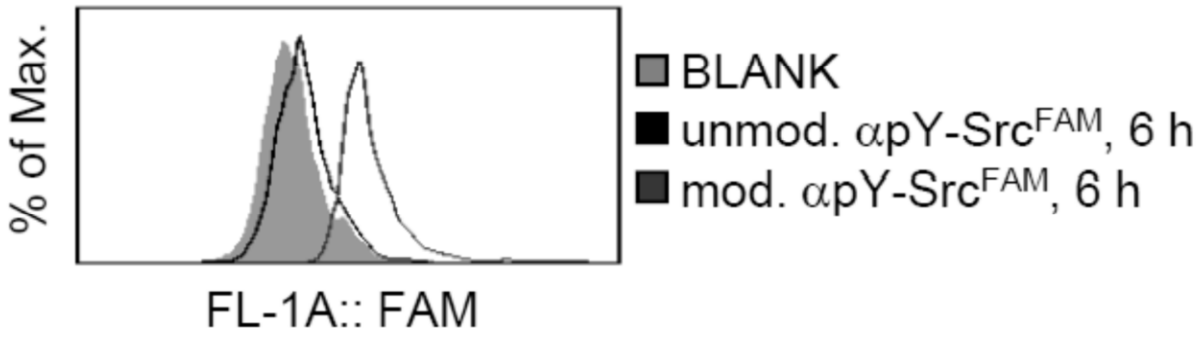


FIG. 23E

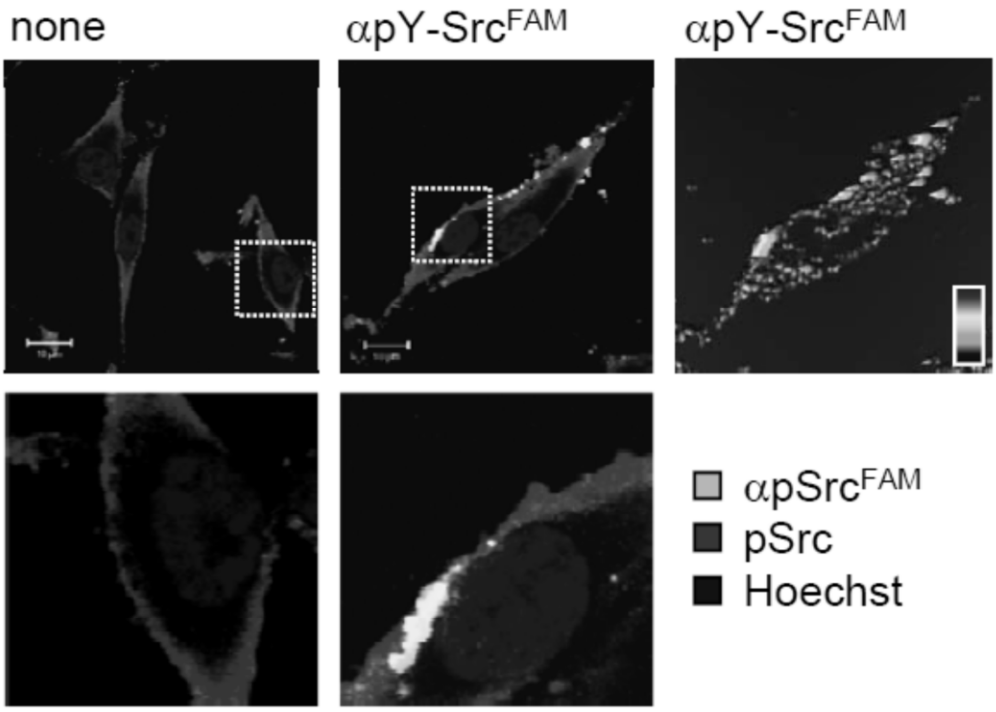


FIG. 23F

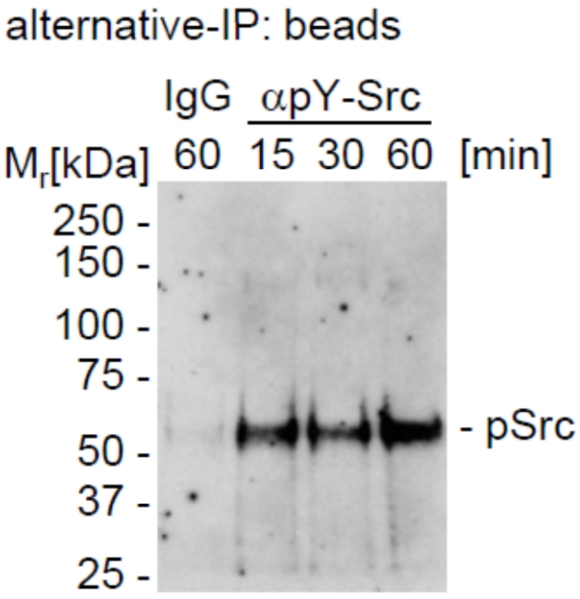


FIG. 23G

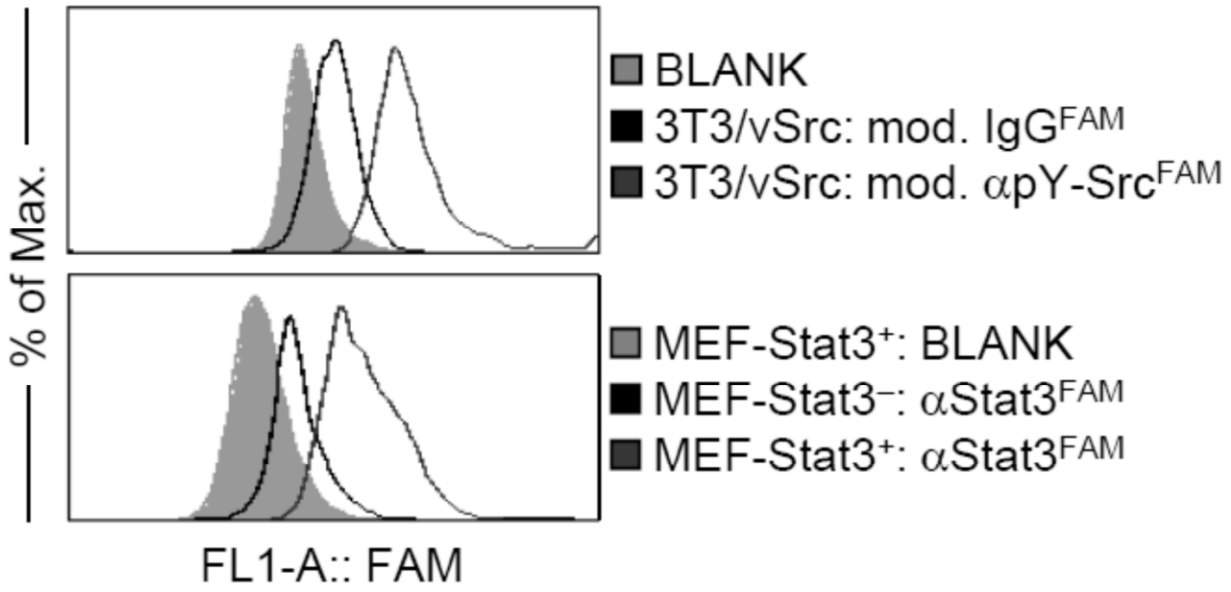
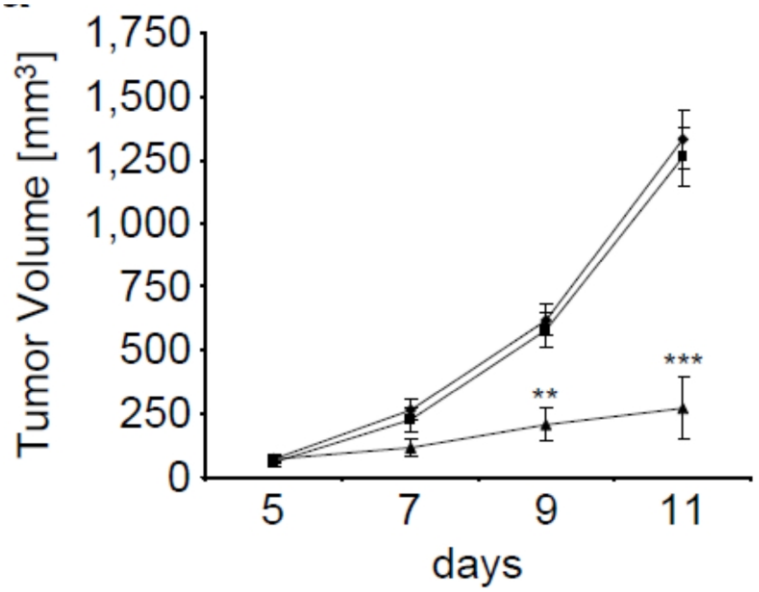


FIG. 24



◆ none, *n* = 4
■ IgG, *n* = 4
▲ αp-Src^{Y416}, *n* = 4

FIG. 25A

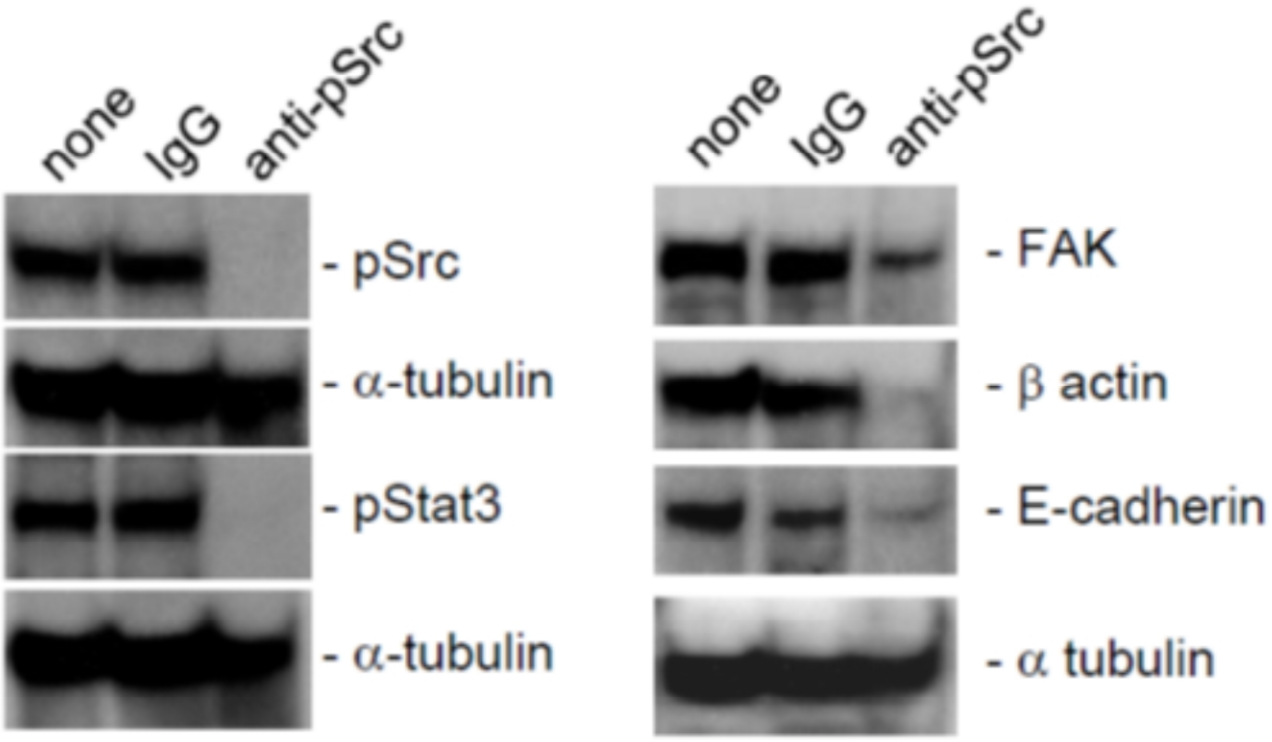


FIG. 25B

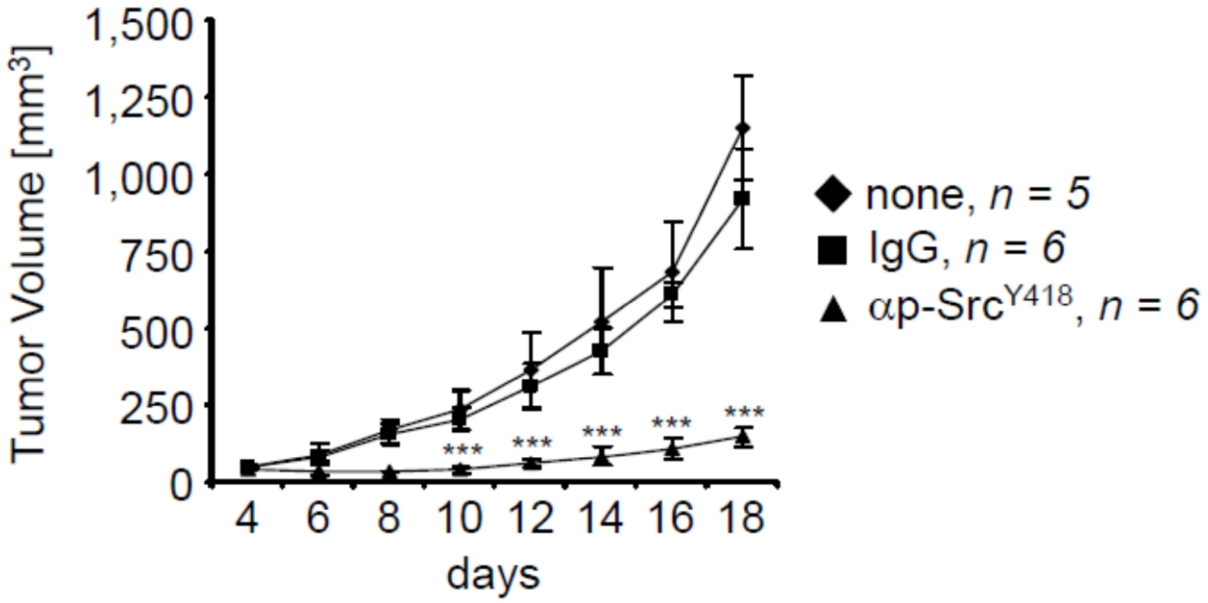


FIG. 25C

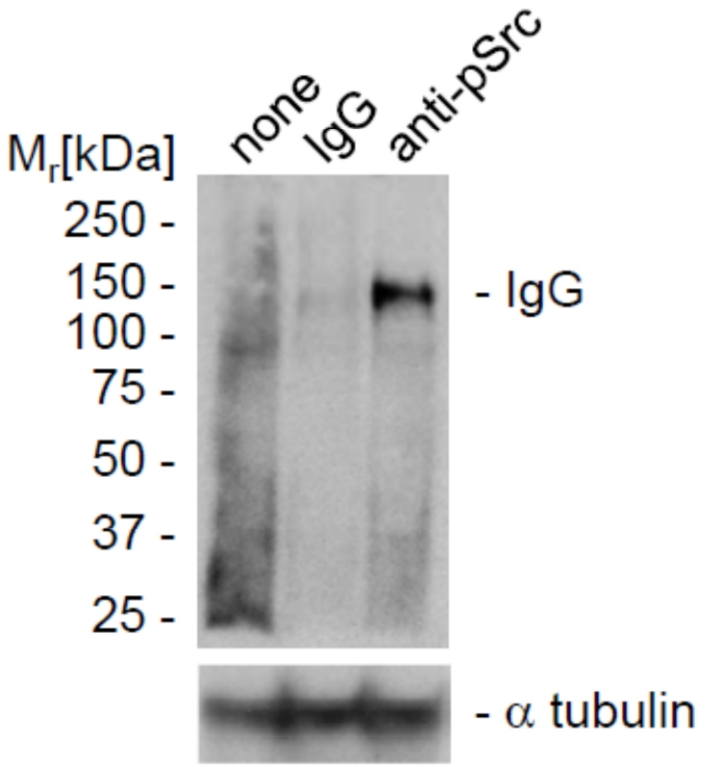


FIG. 25D

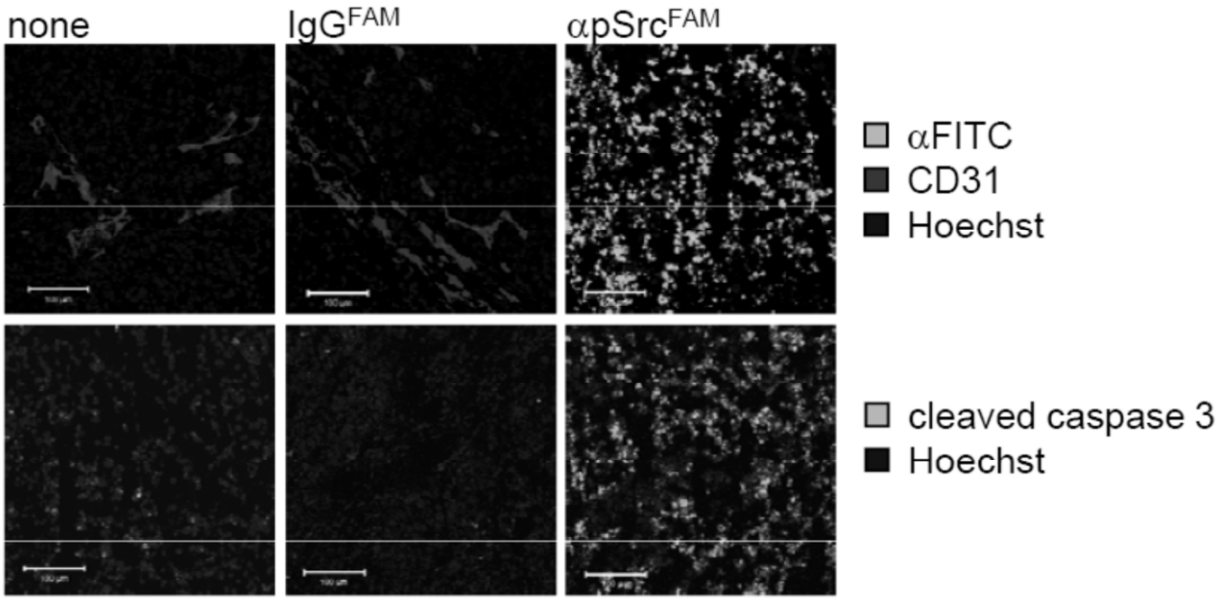


FIG. 25E

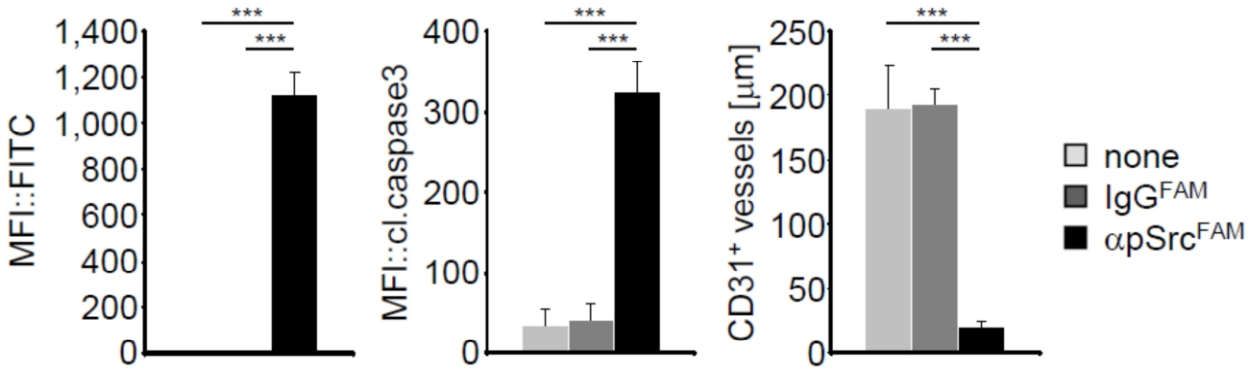


FIG. 25F

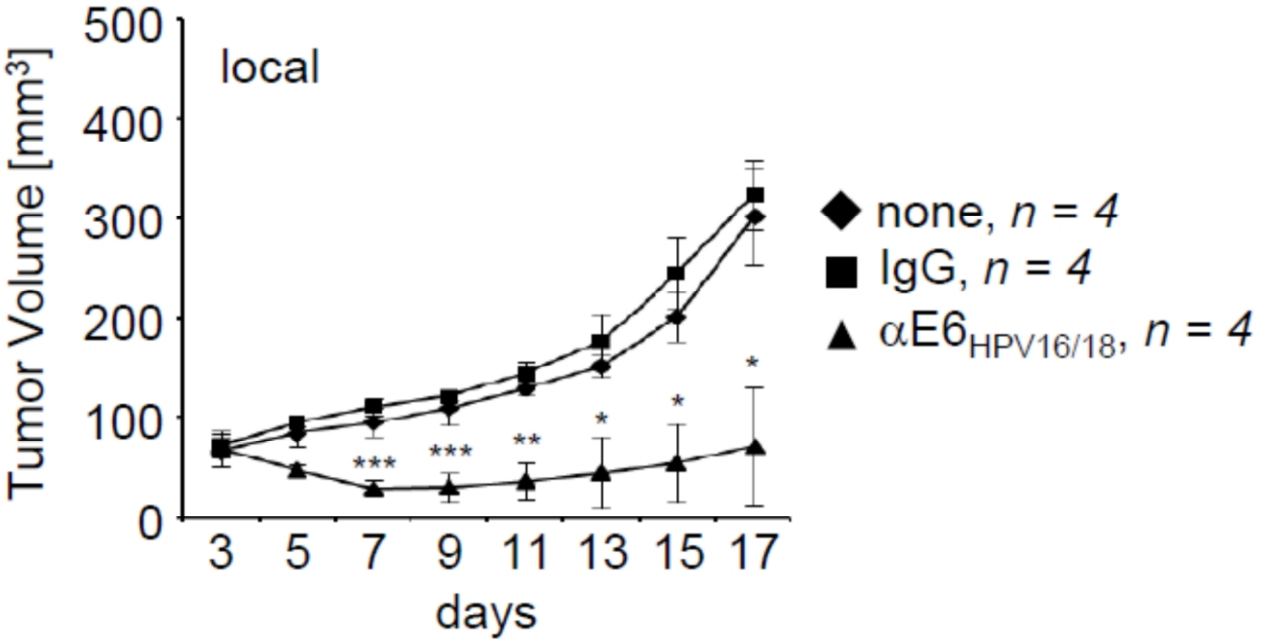


FIG. 26A

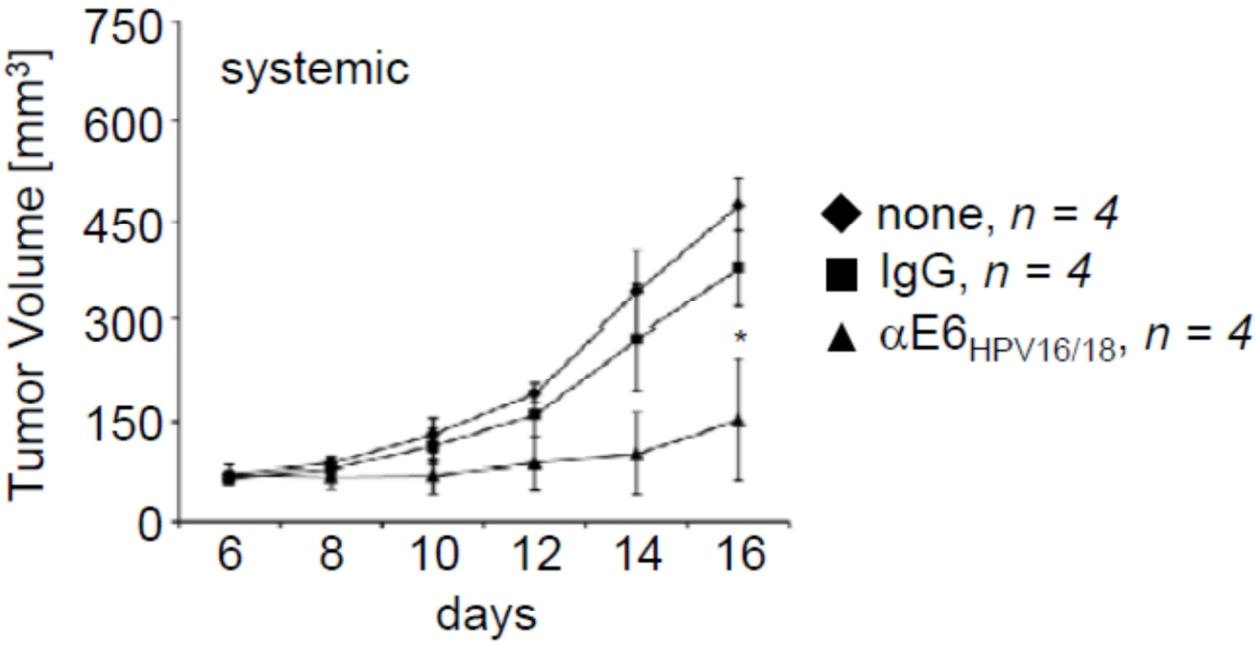


FIG. 26B

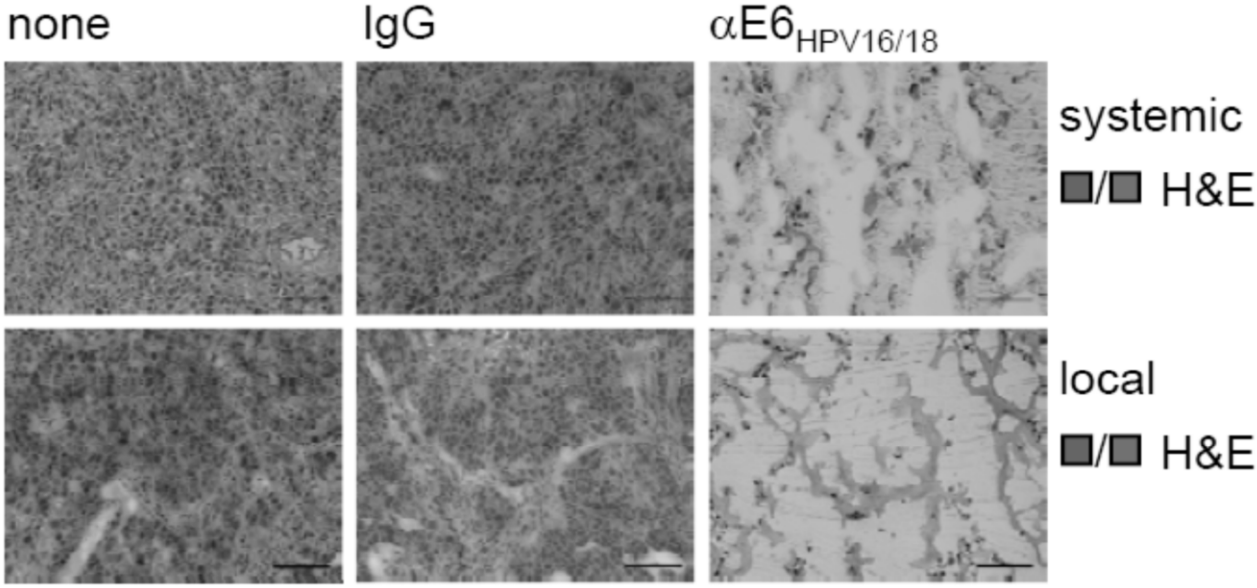


FIG. 26C

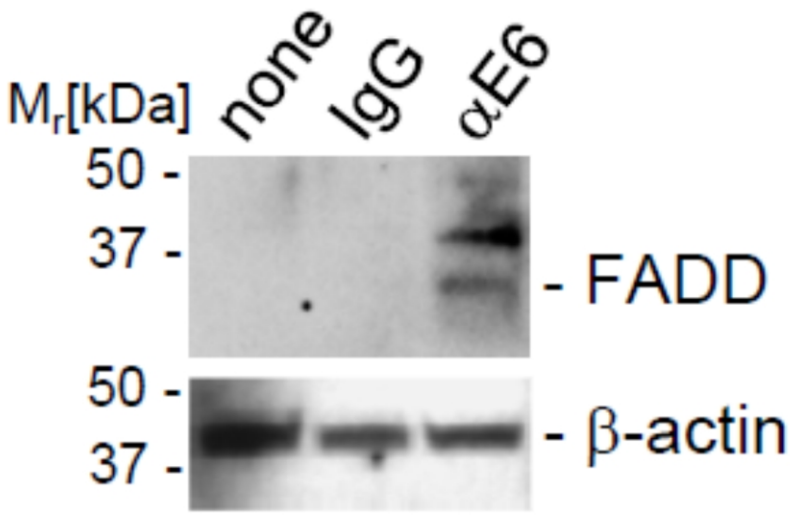


FIG. 26D

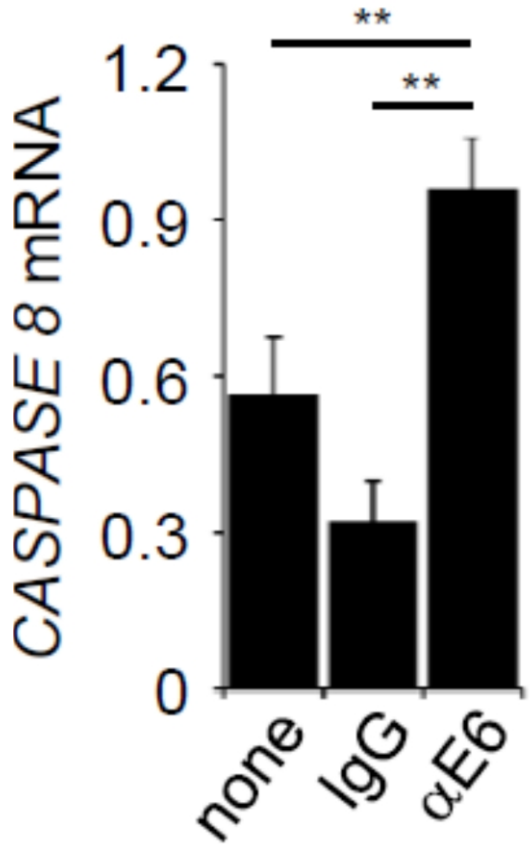


FIG. 26E

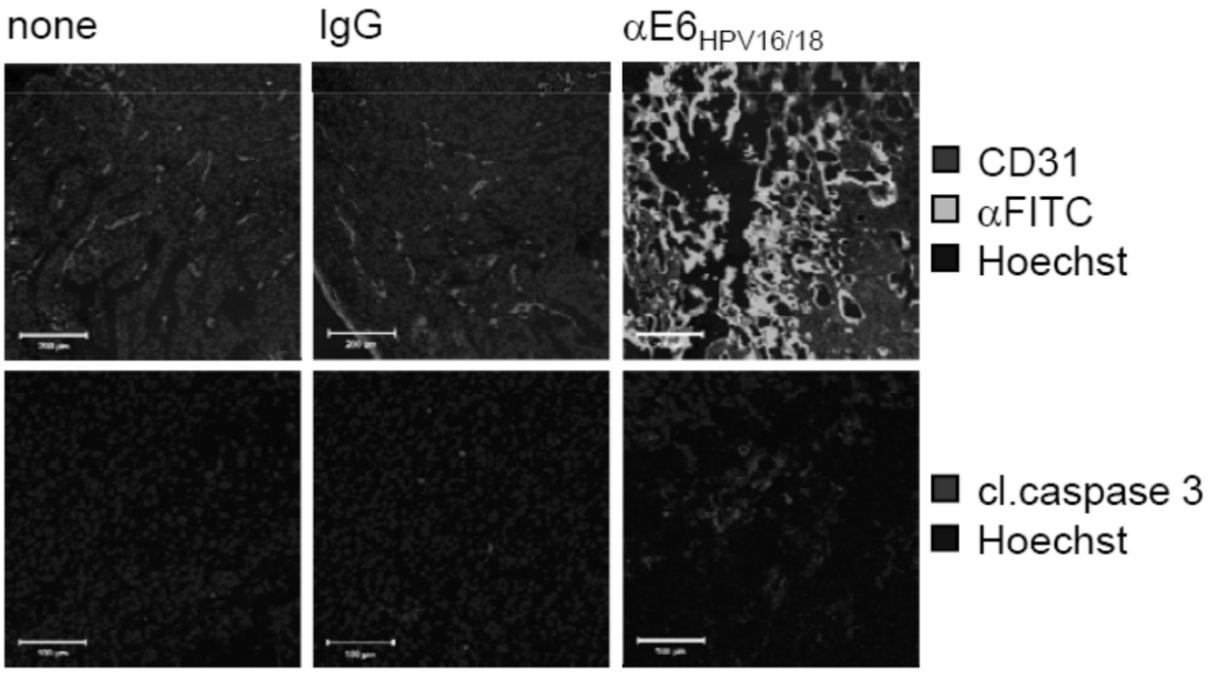


FIG. 26F

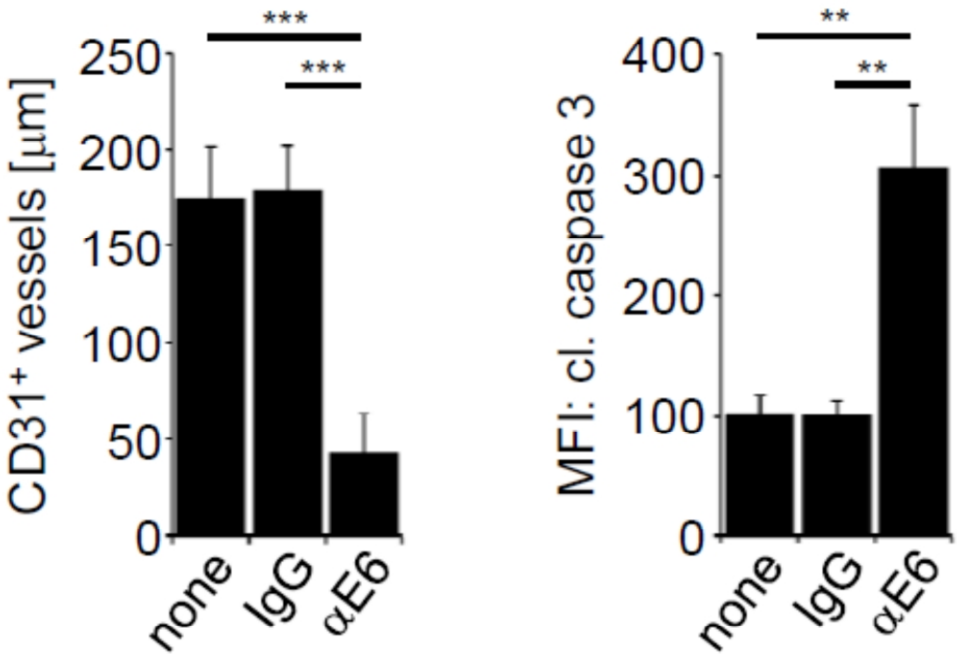


FIG. 26G

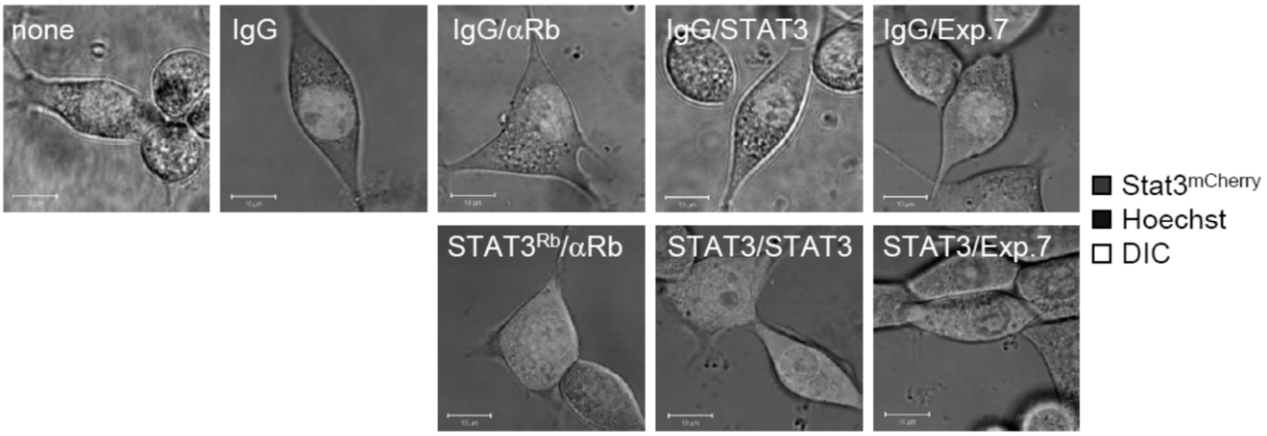


FIG. 27A

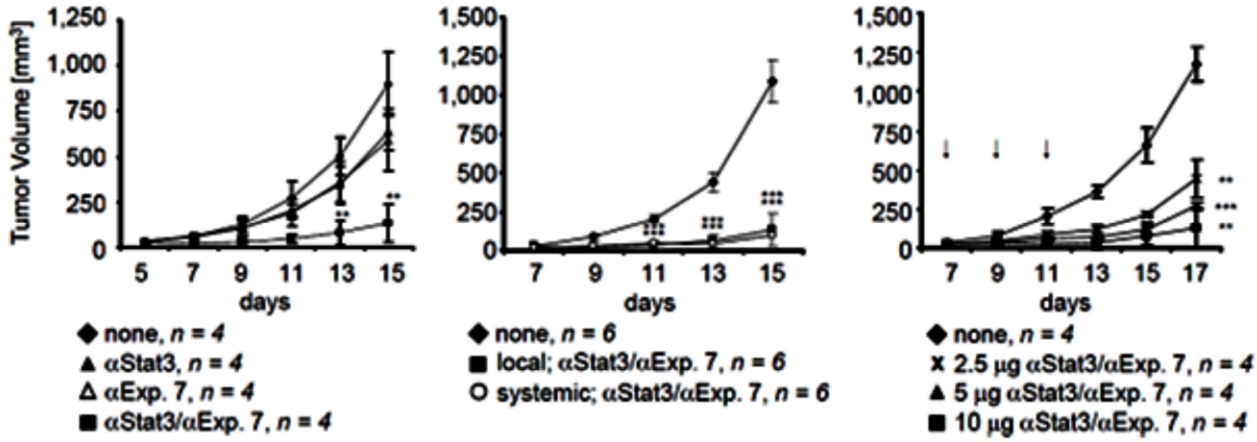


FIG. 27B

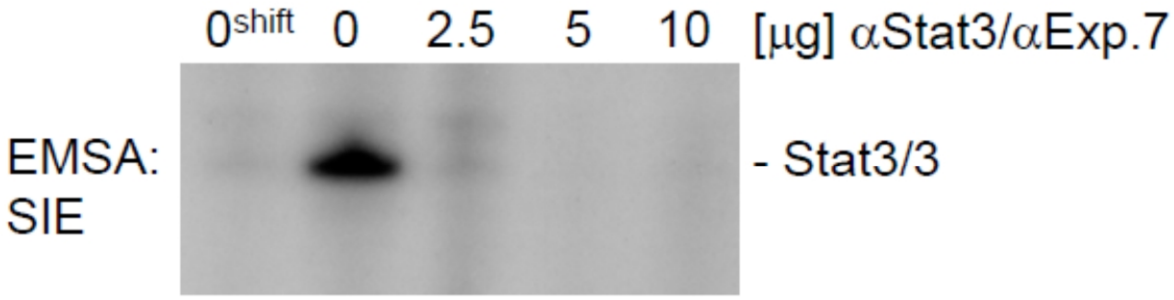


FIG. 27C

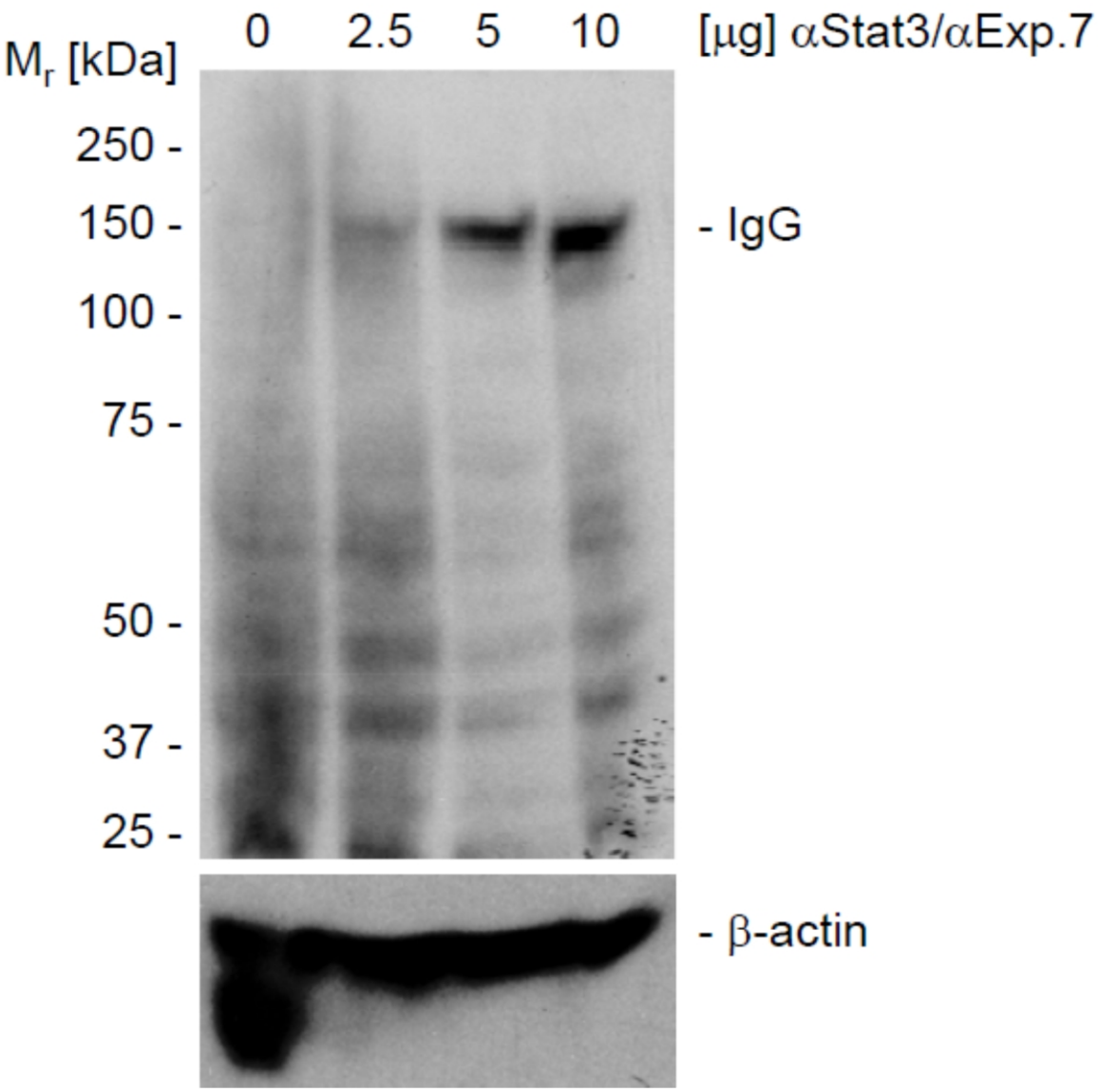

0
2.5
5
10
[µg] αStat3/αExp.7
Mr [kDa]
250 -
150 -
100 -
75 -
50 -
37 -
25 -
- IgG
- β-actin

FIG. 27D

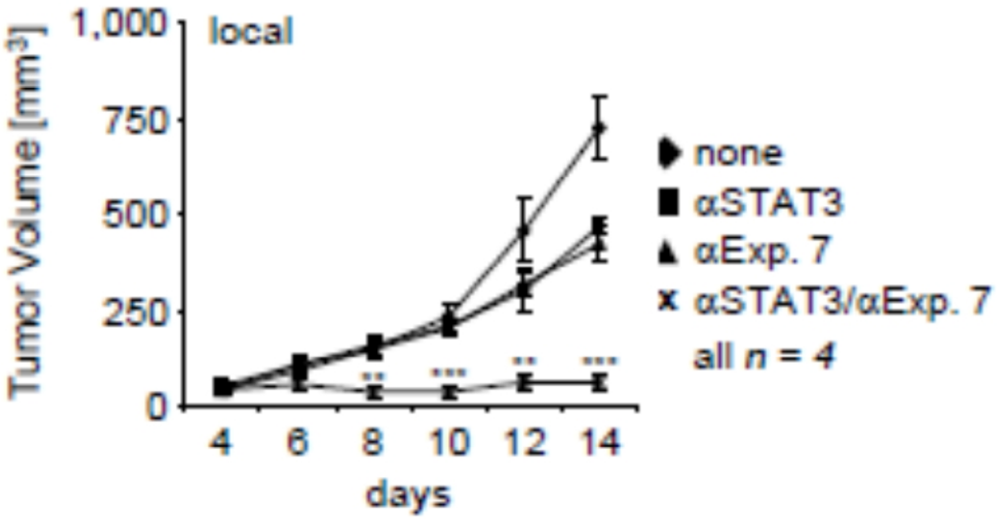


FIG. 27E

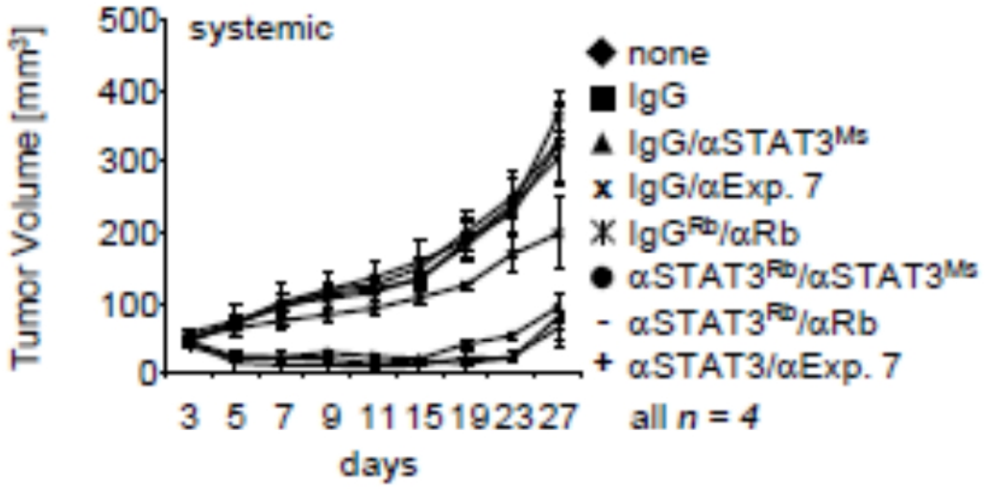


FIG. 27F

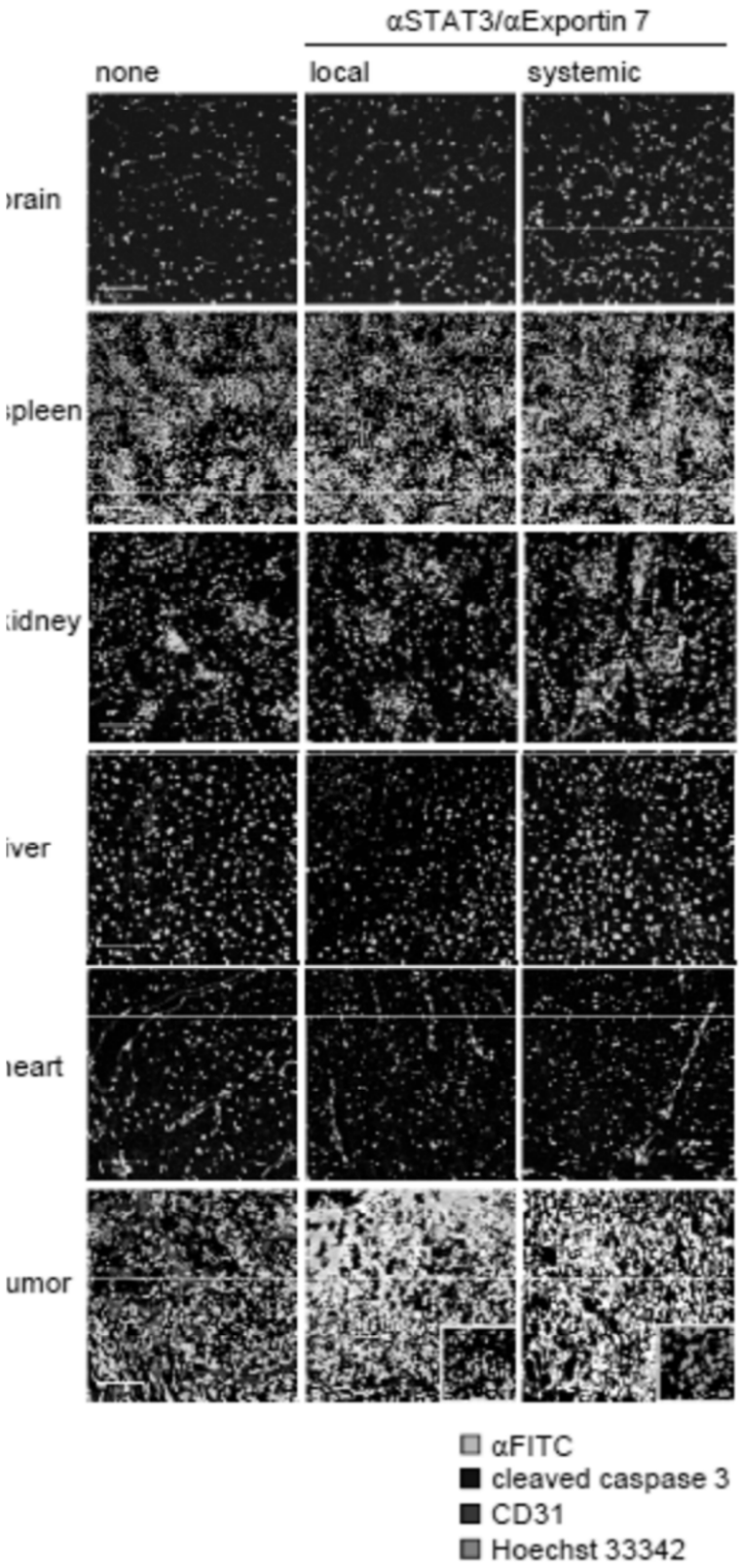

αSTAT3/αExportin 7
none
local
systemic
rain
pleen
idney
iver
eart
umor
αFITC
cleaved caspase 3
CD31
Hoechst 33342

FIG. 27G

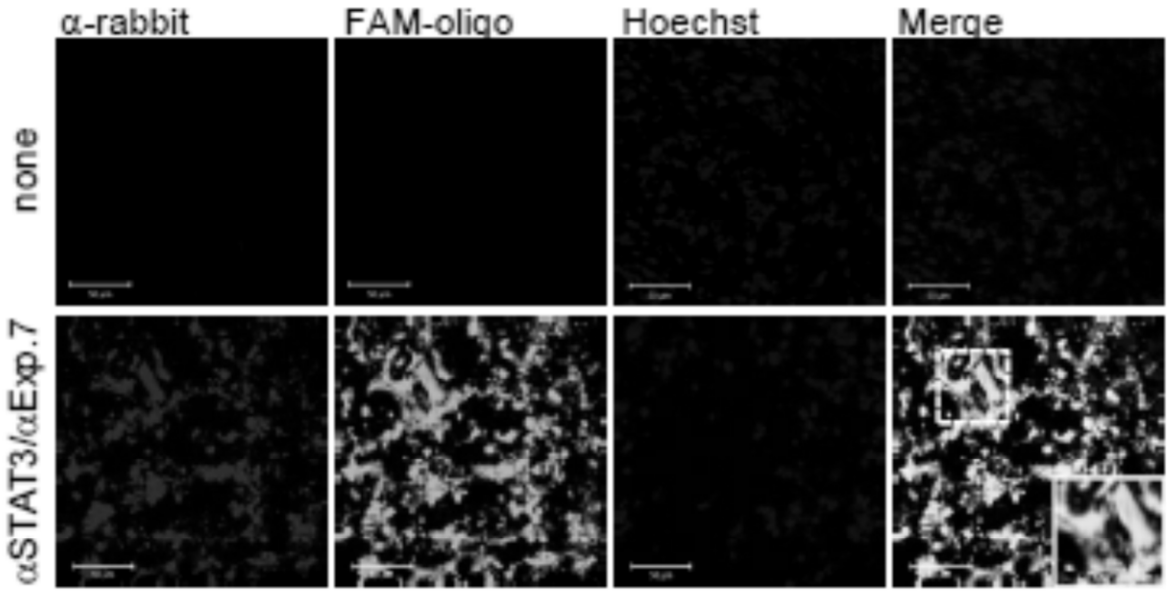

α-rabbit
FAM-oligo
Hoechst
Merge
none
αSTAT3/αExp.7

FIG. 28

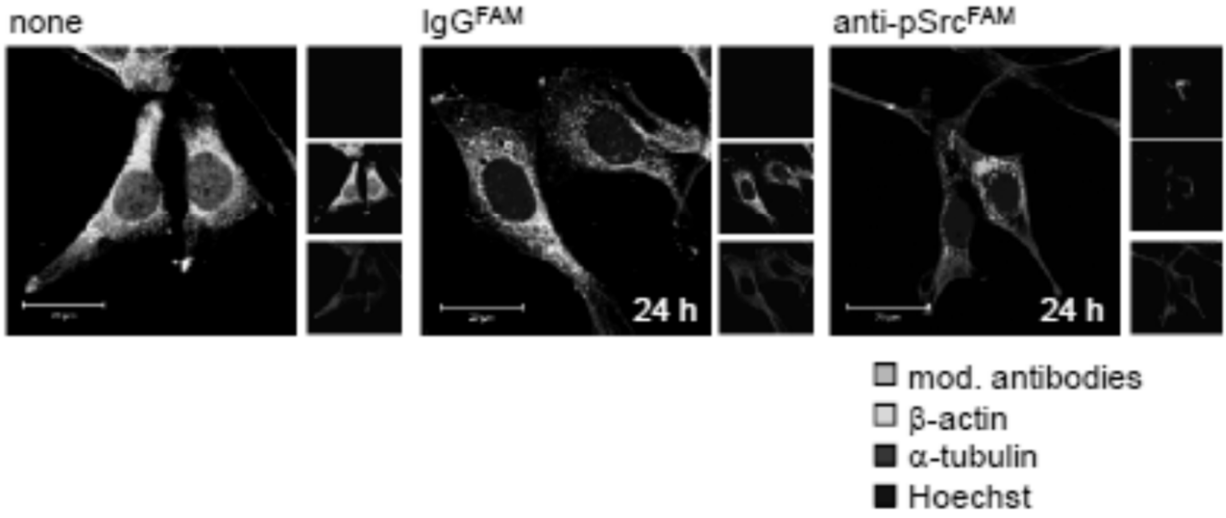

none
IgG^FAM
anti-pSrc^FAM
24 h
24 h
mod. antibodies
β-actin
α-tubulin
Hoechst

FIG. 29

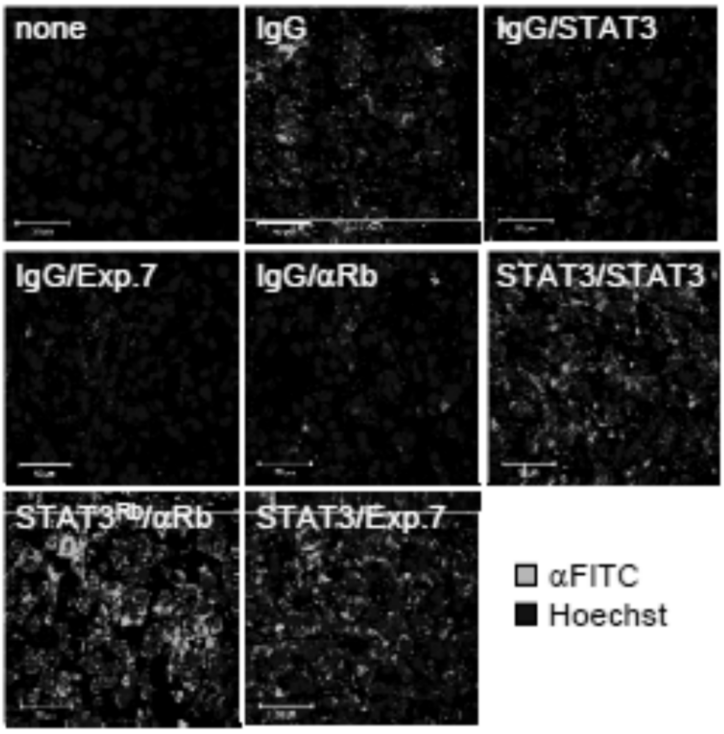


FIG. 30A

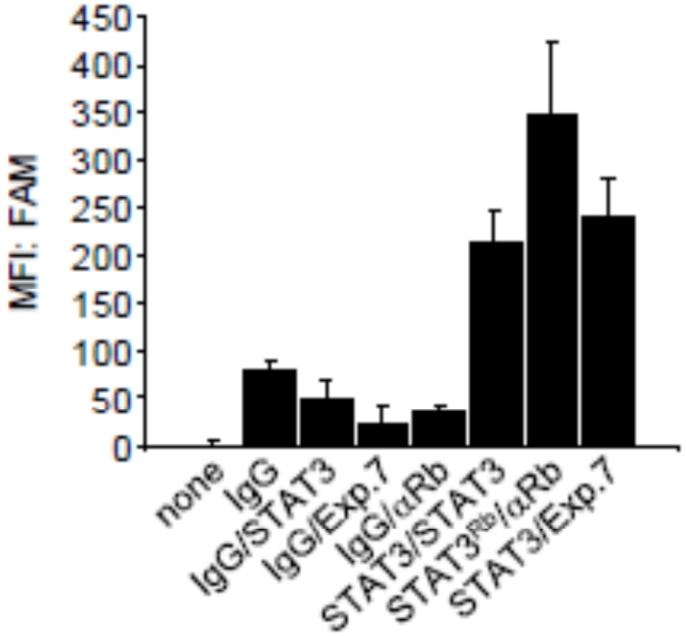


FIG. 30B

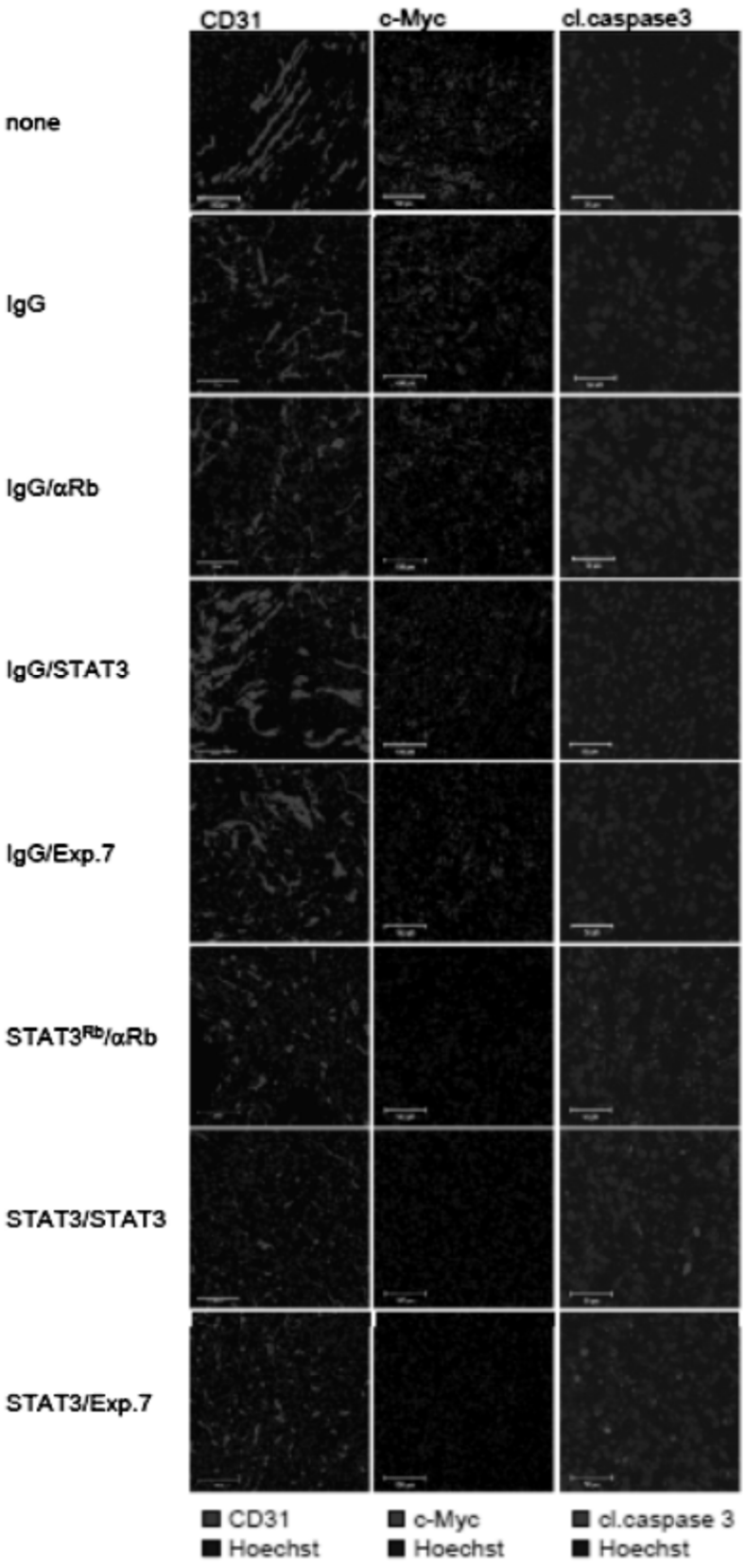

CD31
c-Myc
cl.caspase3
none
IgG
IgG/αRb
IgG/STAT3
IgG/Exp.7
STAT3Rb/αRb
STAT3/STAT3
STAT3/Exp.7
CD31
Hoechst
c-Myc
Hoechst
cl.caspase 3
Hoechst

FIG. 31A

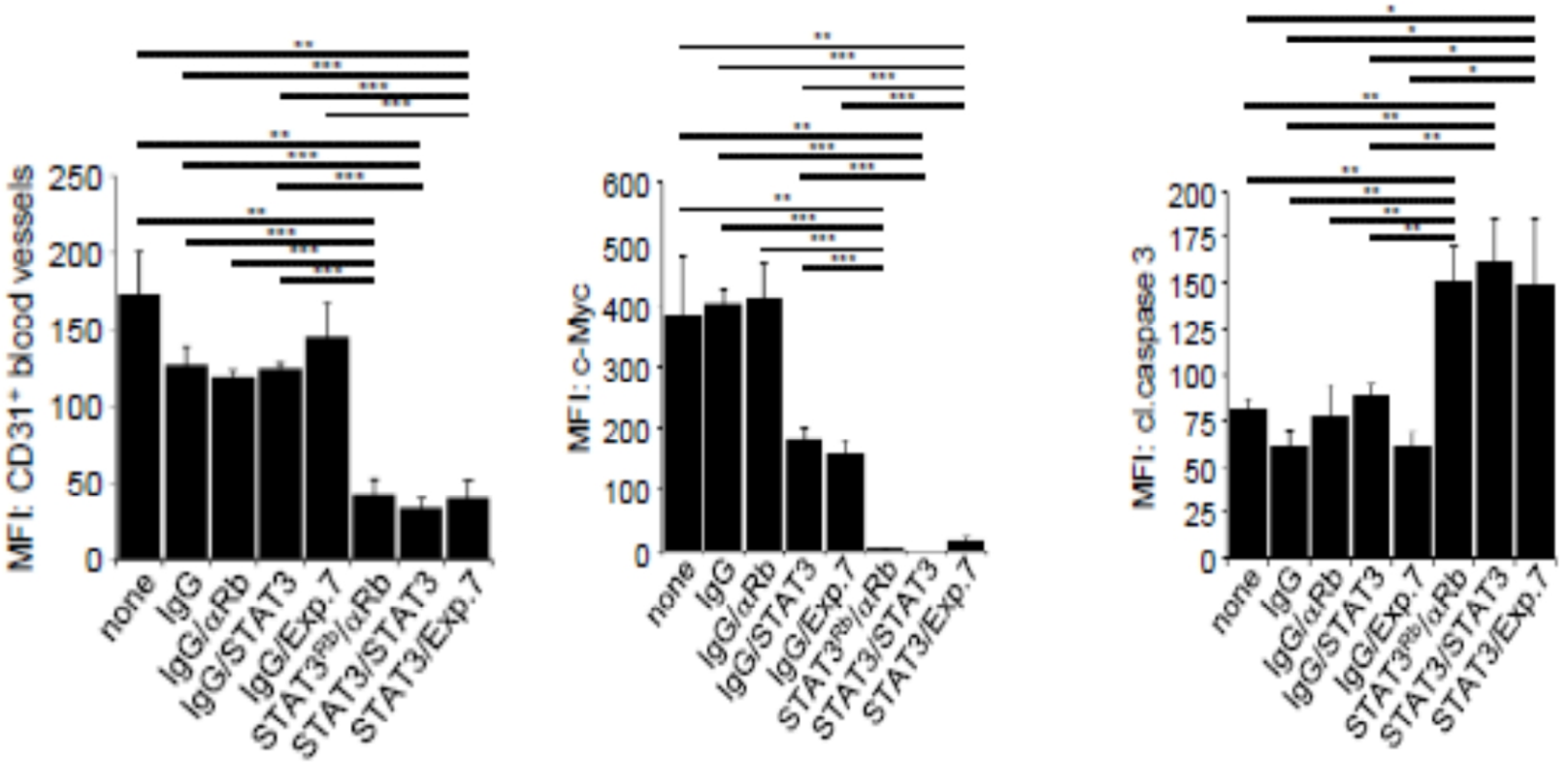

MFI: CD31+ blood vessels
MFI: c-Myc
MFI: cl.caspase 3
none
IgG
IgG/αRb
IgG/STAT3
IgG/Exp.7
STAT3Rb/αRb
STAT3/STAT3
STAT3/Exp.7

FIG. 31B

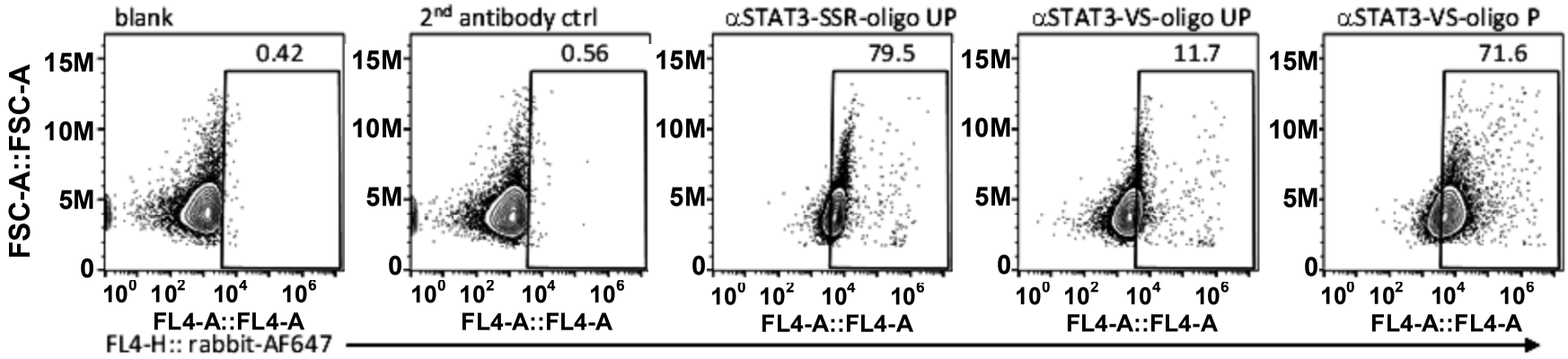

blank
2nd antibody ctrl
αSTAT3-SSR-oligo UP
αSTAT3-VS-oligo UP
αSTAT3-VS-oligo P
0.42
0.56
79.5
11.7
71.6
FSC-A::FSC-A
15M
10M
5M
0
10^0 10^2 10^4 10^6
FL4-A::FL4-A
FL4-H:: rabbit-AF647

FIG. 32

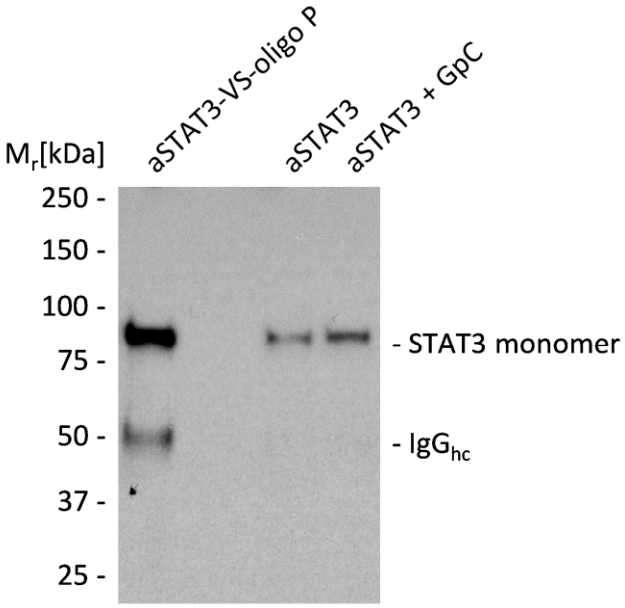


FIG. 33A

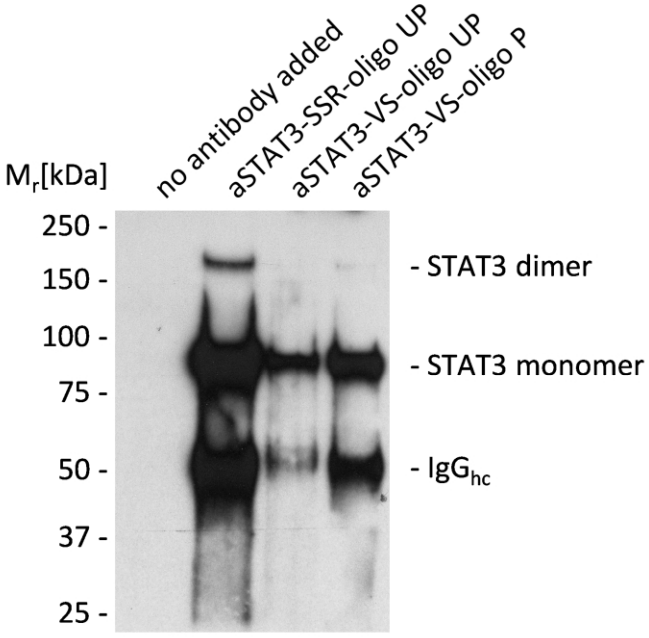


FIG. 33B