FIGURE 1

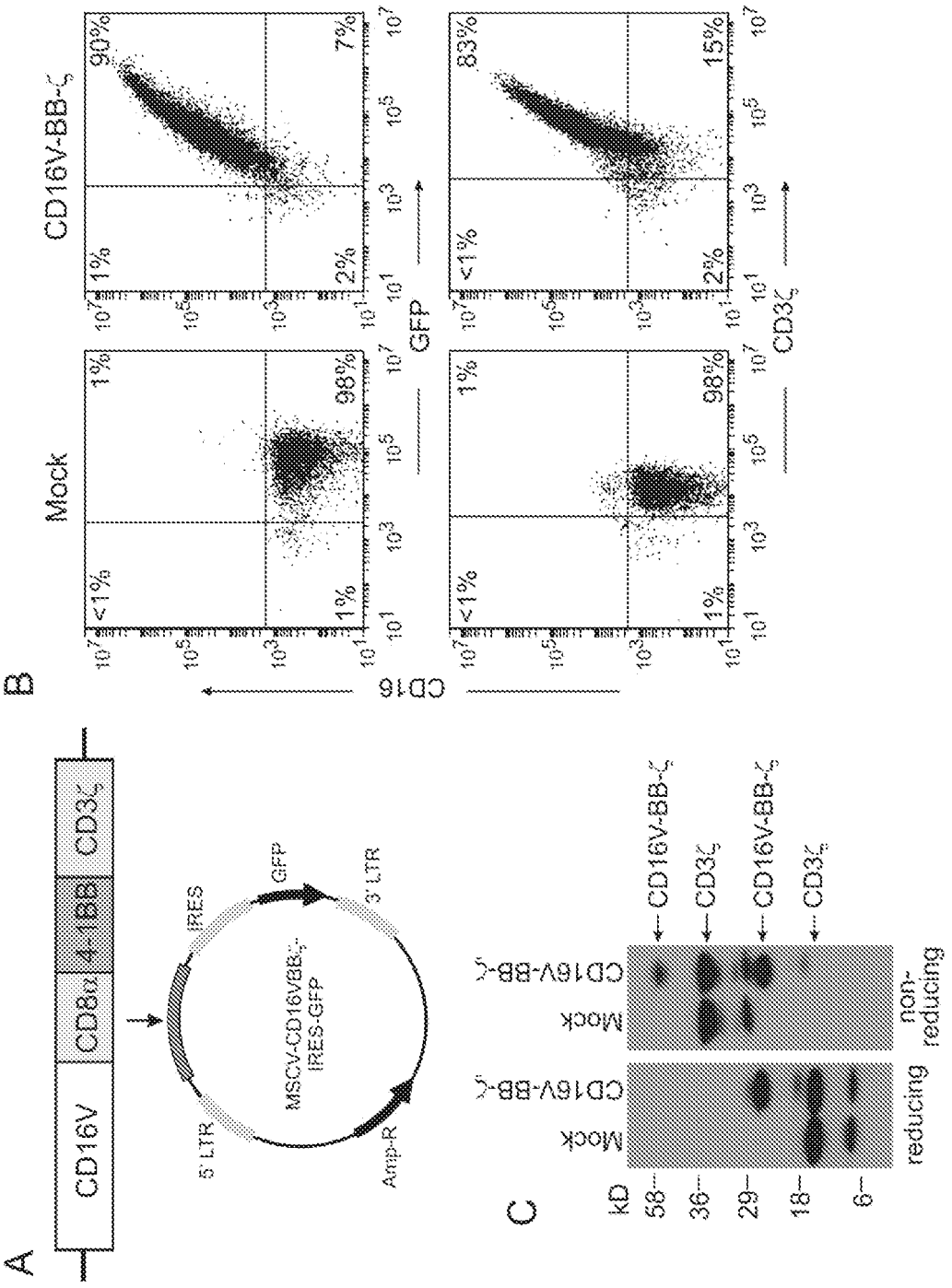

A
CD16V
CD8α
4-1BB
CD3ζ
5' LTR
IRES
GFP
MSCV-CD16VBBζ-IRES-GFP
3' LTR
Amp-R
B
Mock
CD16V-BB-ζ
CD16
GFP
CD3ζ
<1%
1%
98%
90%
7%
2%
83%
15%
12%
C
kD
58
36
29
18
6
Mock
CD16V-BB-ζ
CD16V-BB-ζ
CD3ζ
CD16V-BB-ζ
CD3ζ
reducing
non-reducing

FIGURE 2

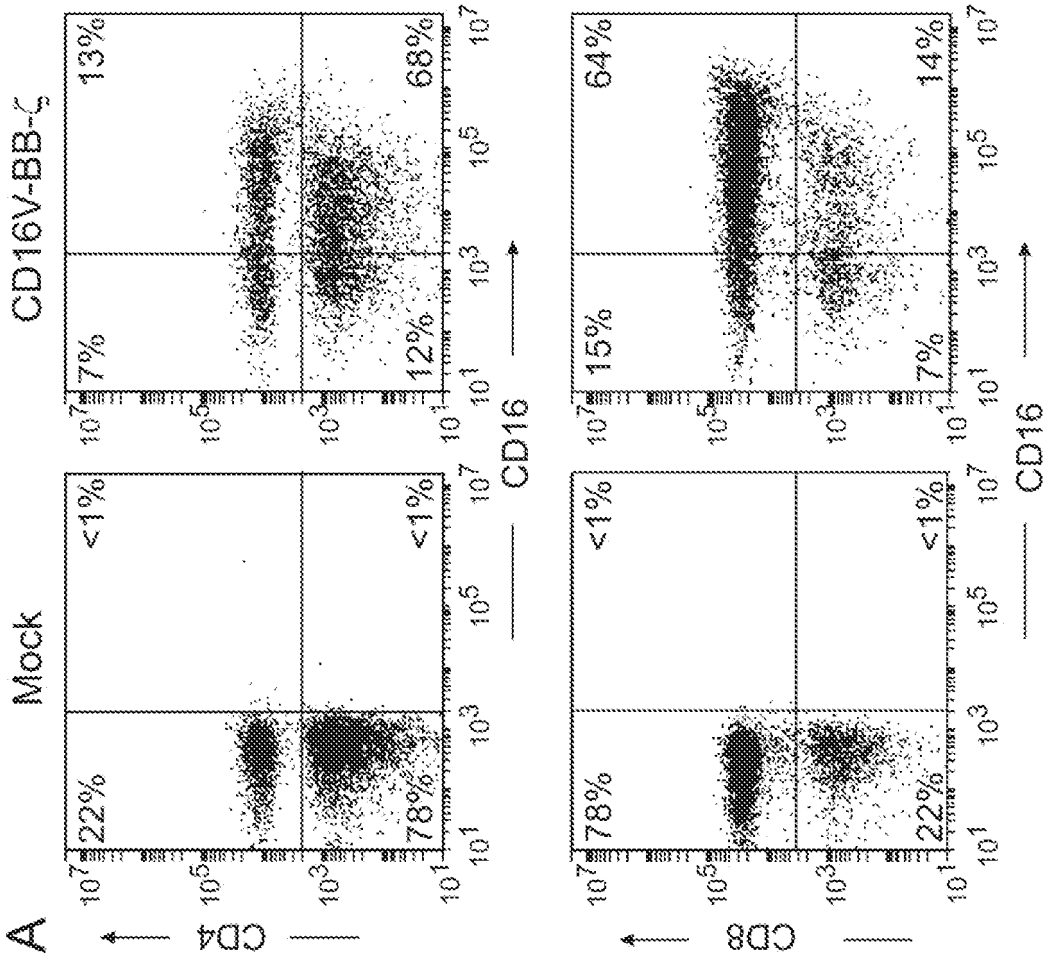

A
Mock
CD16V-BB-ζ
CD4
CD8
CD16
22%
<1%
78%
<1%
7%
13%
12%
68%
78%
<1%
22%
<1%
15%
64%
7%
14%
B
%CD16
100
80
60
40
20
0
CD4
CD8

FIGURE 3

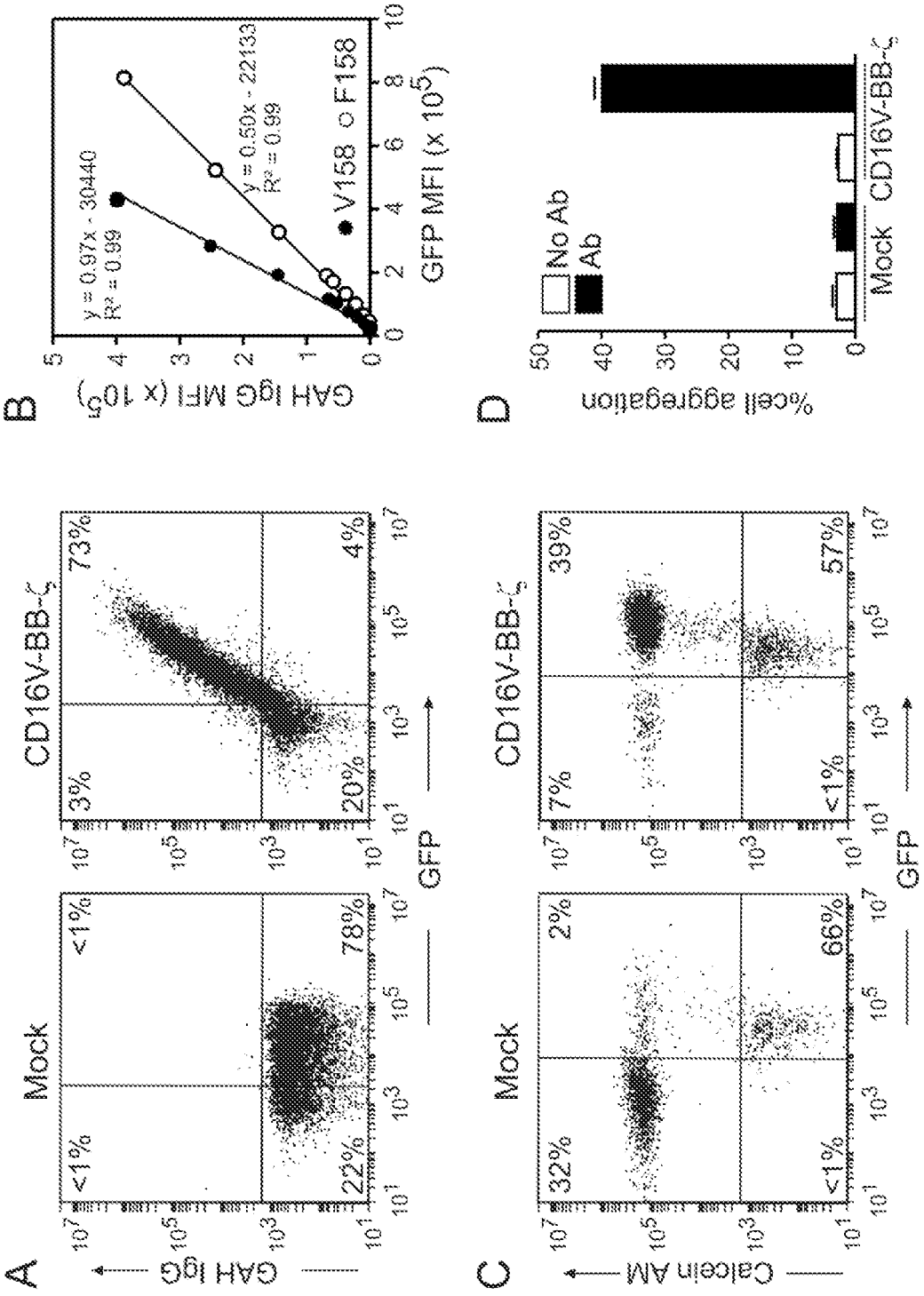

A
Mock
CD16V-BB-ζ
GAH IgG
GFP
<1%
<1%
22%
78%
3%
20%
73%
4%
B
GAH IgG MFI (x 10^5)
GFP MFI (x 10^5)
y = 0.97x - 30440
R² = 0.99
y = 0.50x - 22133
R² = 0.99
V158 ○ F158
C
Mock
CD16V-BB-ζ
Calcein AM
GFP
32%
2%
66%
<1%
7%
39%
57%
<1%
D
%cell aggregation
No Ab
Ab
Mock
CD16V-BB-ζ

FIGURE 4

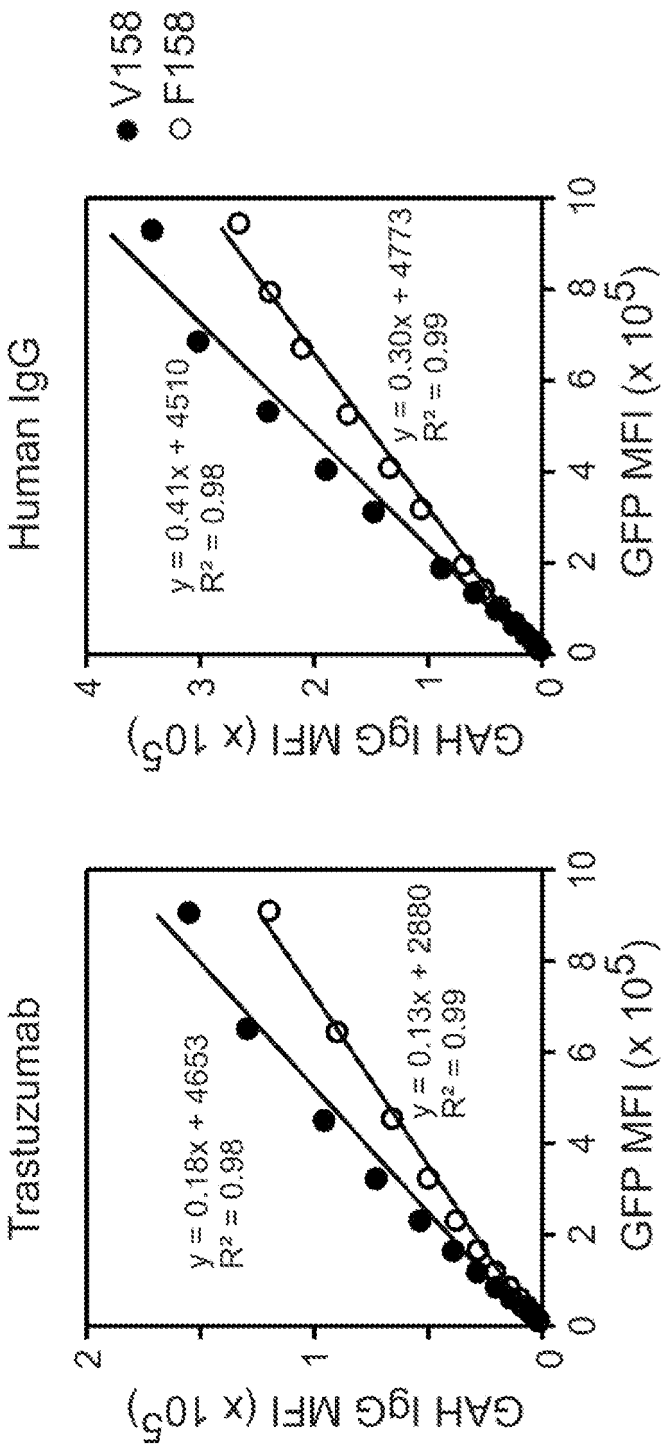

Human IgG
Trastuzumab
V158
F158
y = 0.41x + 4510
R² = 0.98
y = 0.30x + 4773
R² = 0.99
y = 0.18x + 4653
R² = 0.98
y = 0.13x + 2880
R² = 0.99
GAH IgG MFI (x 10⁵)
GFP MFI (x 10⁵)

FIGURE 5

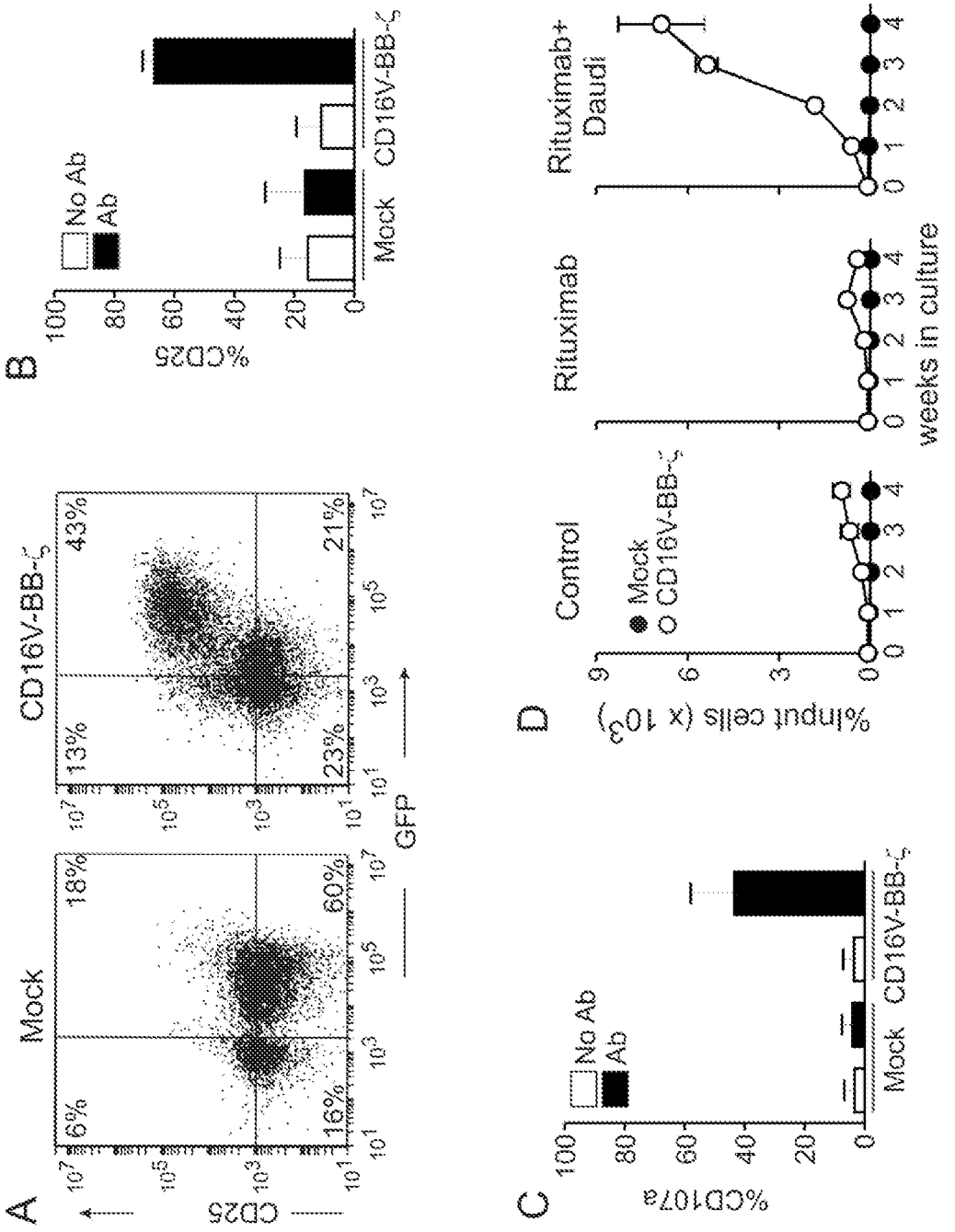

A
Mock
CD16V-BB-ζ
6%
18%
16%
60%
13%
43%
23%
21%
CD25
GFP
B
No Ab
Ab
%CD25
Mock
CD16V-BB-ζ
C
No Ab
Ab
%CD107a
Mock
CD16V-BB-ζ
D
Control
Rituximab
Rituximab+ Daudi
Mock
CD16V-BB-ζ
%Input cells (x 10³)
weeks in culture

FIGURE 6

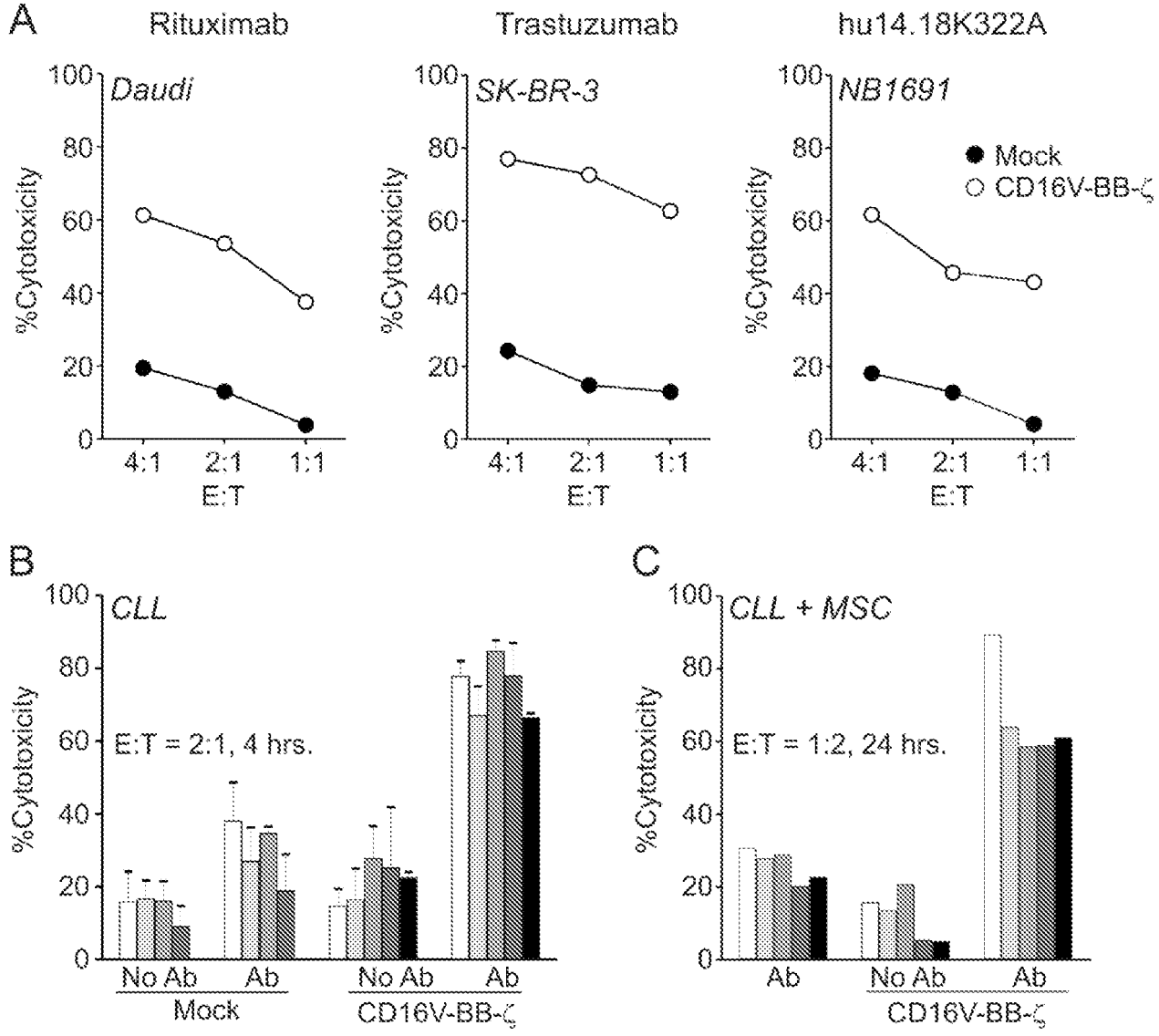

A
Rituximab
Trastuzumab
hu14.18K322A
Daudi
SK-BR-3
NB1691
Mock
CD16V-BB-ζ
%Cytotoxicity
E:T
4:1
2:1
1:1
B
CLL
E:T = 2:1, 4 hrs.
No Ab
Ab
Mock
CD16V-BB-ζ
C
CLL + MSC
E:T = 1:2, 24 hrs.
Ab
No Ab
CD16V-BB-ζ

FIGURE 7

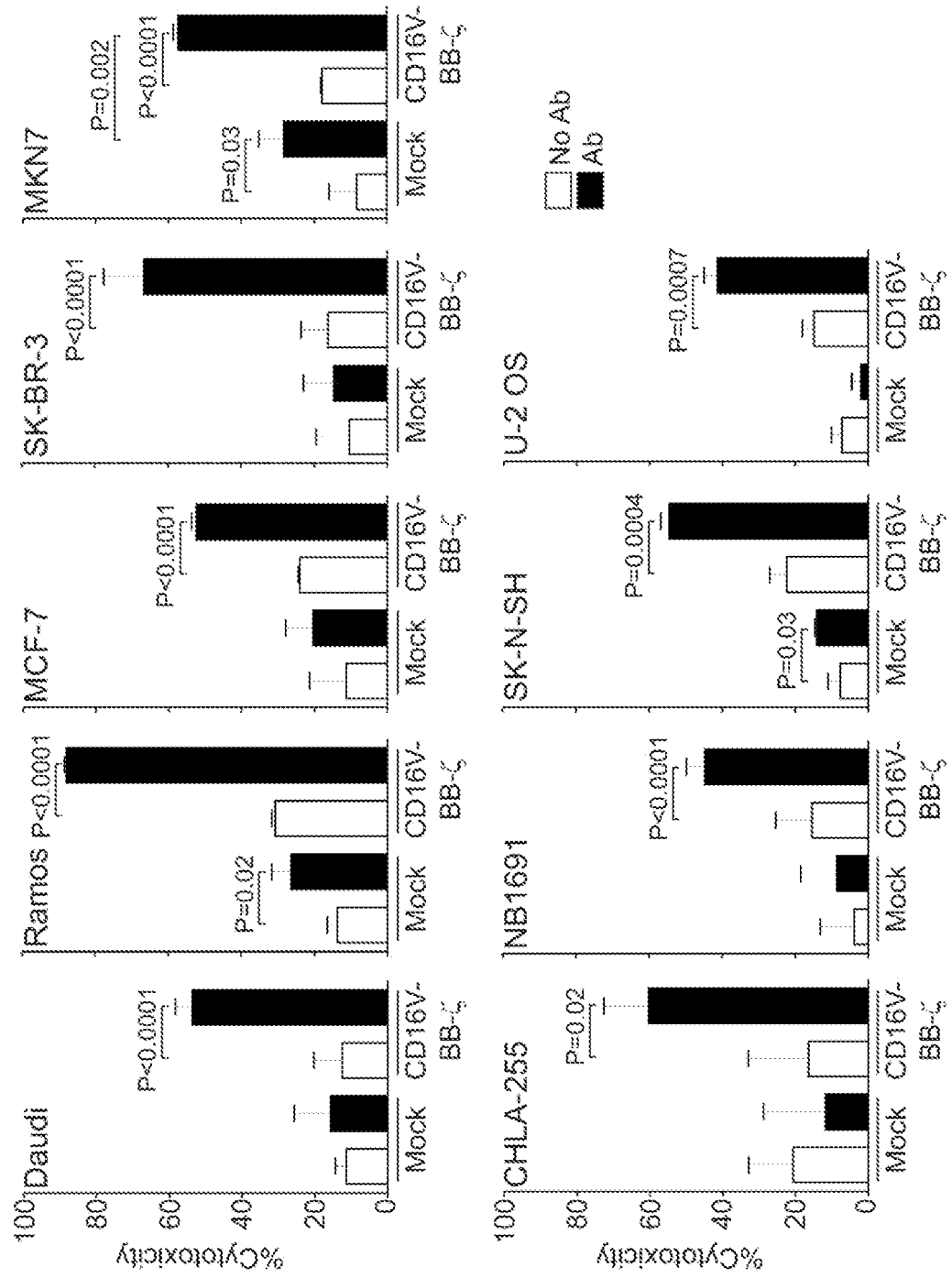

Daudi
Ramos
MCF-7
SK-BR-3
MKN7
CHLA-255
NB1691
SK-N-SH
U-2 OS
No Ab
Ab
%Cytotoxicity
Mock
CD16V-BB-ζ
P<0.0001
P=0.02
P=0.03
P=0.002
P=0.0004
P=0.0007

FIGURE 8

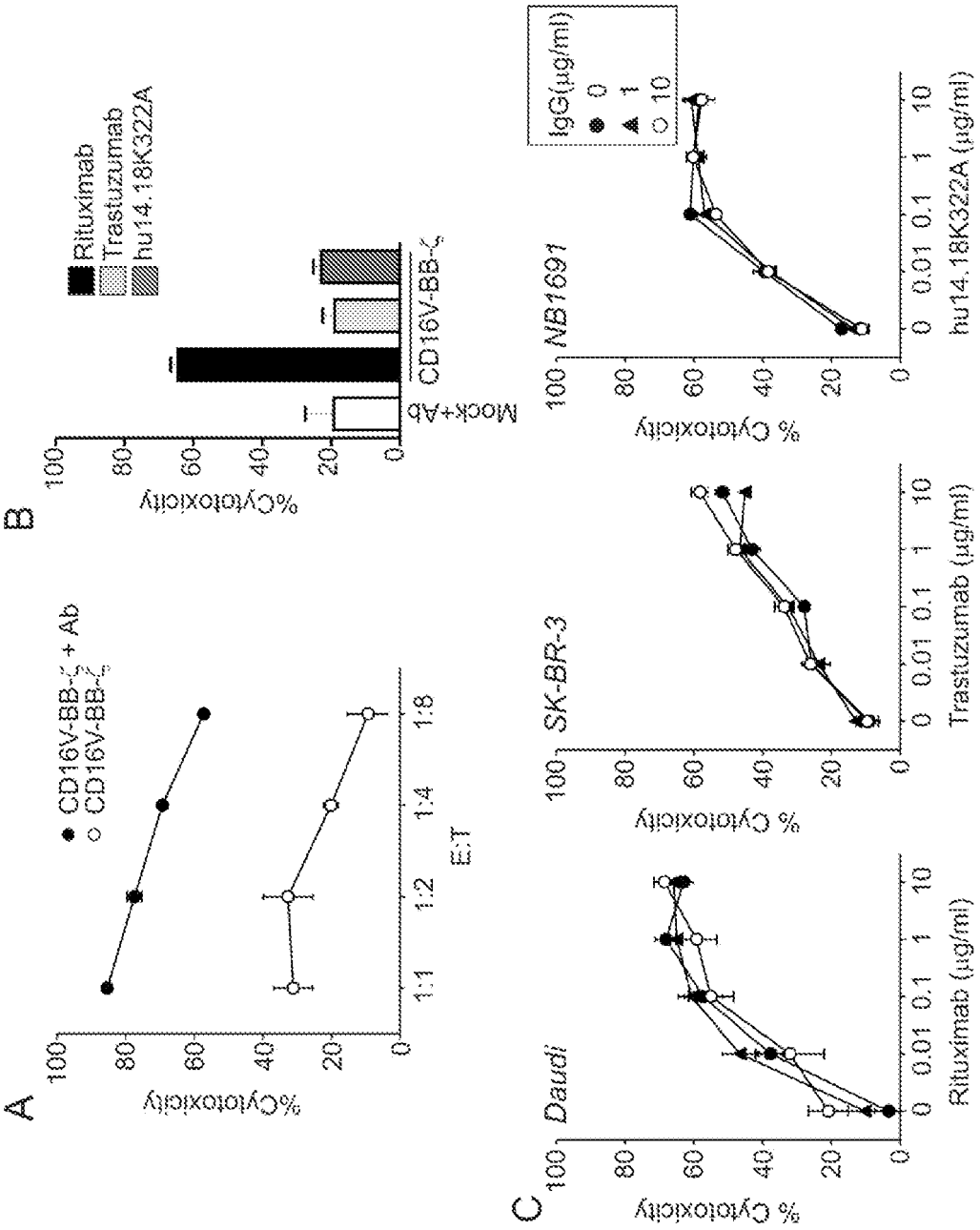

A
% Cytotoxicity
CD16V-BB-ζ + Ab
CD16V-BB-ζ
E:T
1:1
1:2
1:4
1:8
B
% Cytotoxicity
Rituximab
Trastuzumab
hu14.18K322A
Mock+Ab
CD16V-BB-ζ
C
Daudi
SK-BR-3
NB1691
% Cytotoxicity
IgG(μg/ml)
0
1
10
Rituximab (μg/ml)
Trastuzumab (μg/ml)
hu14.18K322A (μg/ml)

FIGURE 9

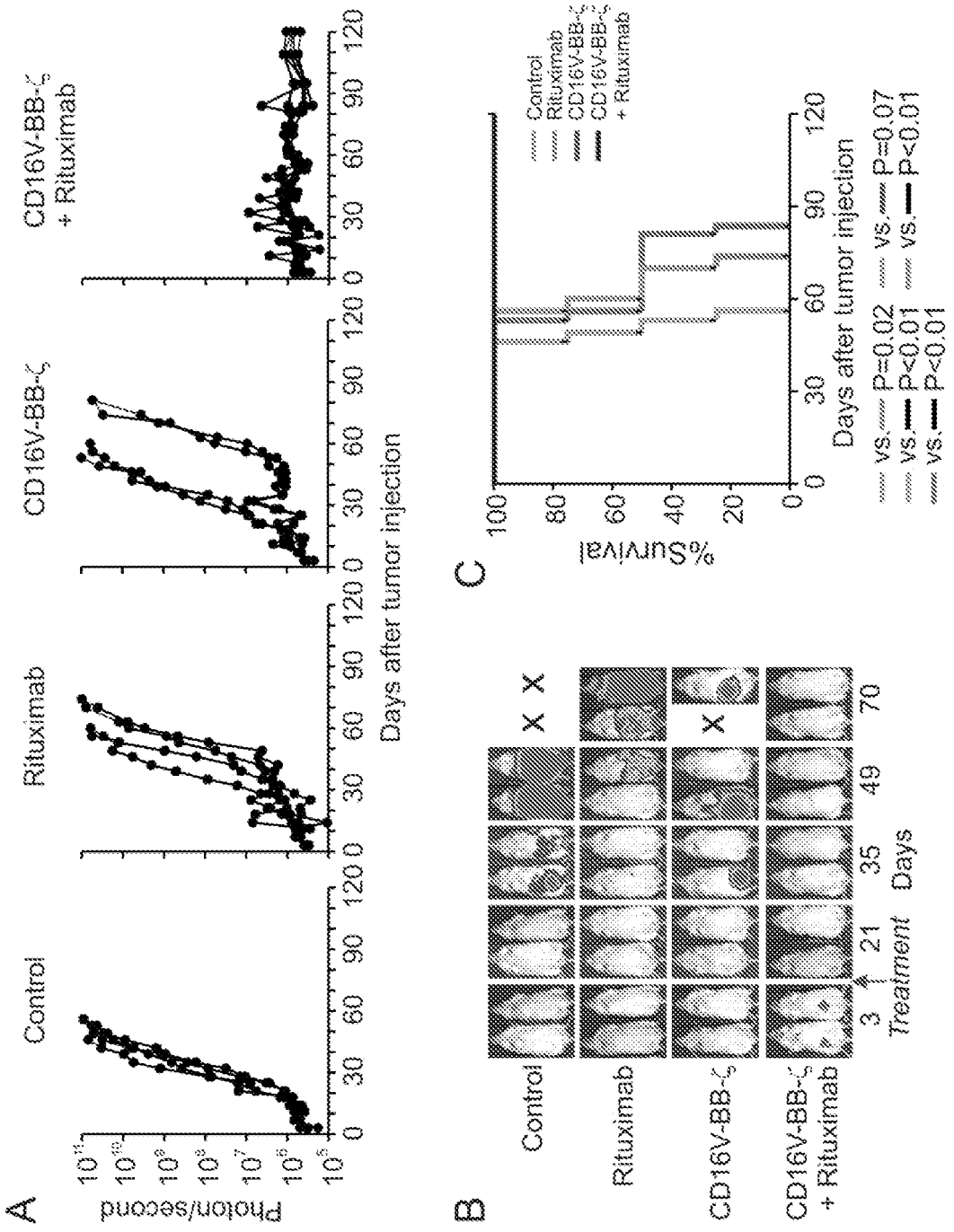

A
Control
Rituximab
CD16V-BB-ζ
CD16V-BB-ζ + Rituximab
Photon/second
Days after tumor injection
B
Control
Rituximab
CD16V-BB-ζ
CD16V-BB-ζ + Rituximab
Treatment
Days
C
%Survival
Days after tumor injection
Control
Rituximab
CD16V-BB-ζ
CD16V-BB-ζ + Rituximab
vs. P=0.02
vs. P=0.07
vs. P<0.01
vs. P<0.01
vs. P<0.01

FIGURE 10

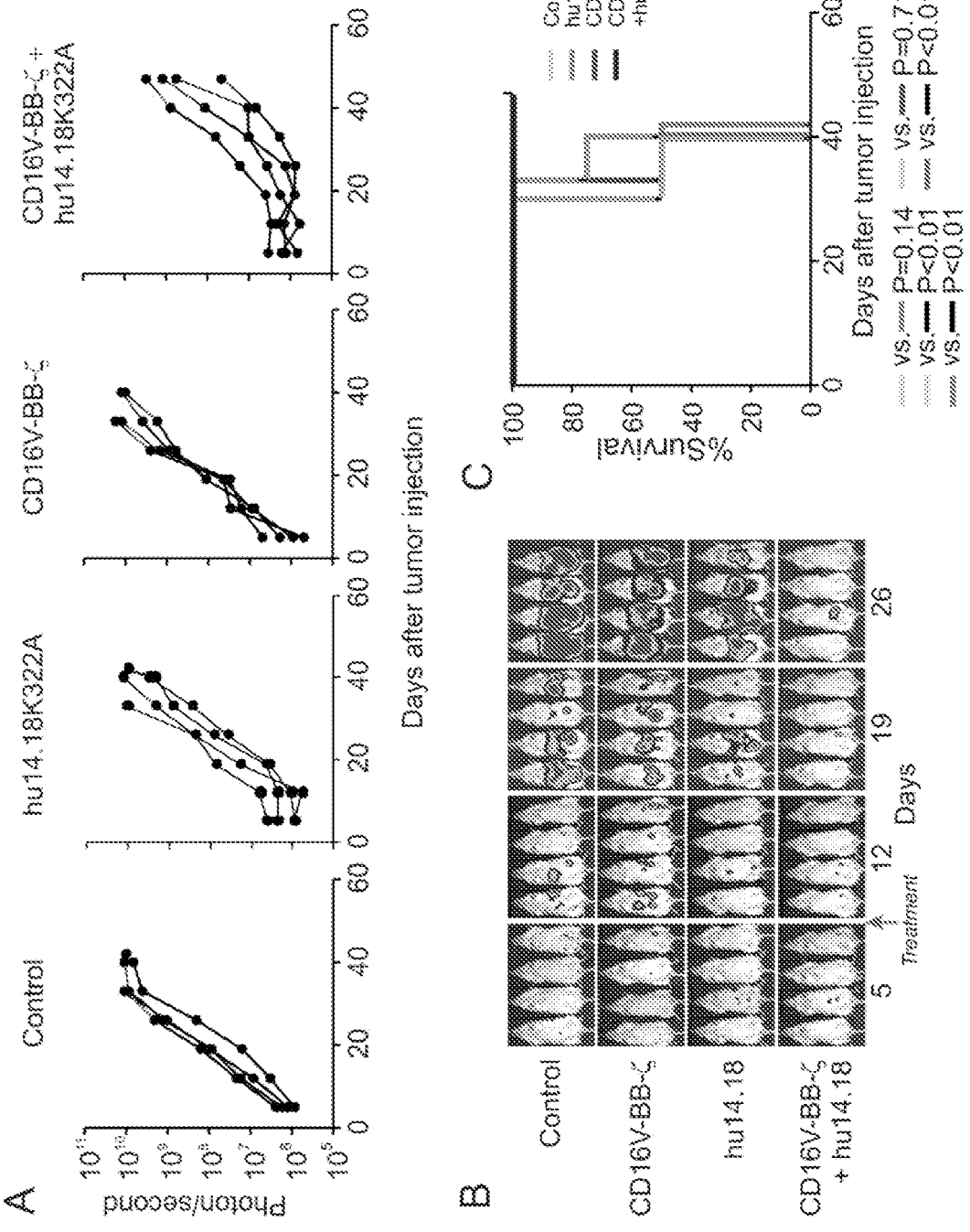

A
Control
hu14.18K322A
CD16V-BB-ζ
CD16V-BB-ζ + hu14.18K322A
Photon/second
Days after tumor injection
B
Control
CD16V-BB-ζ
hu14.18
CD16V-BB-ζ + hu14.18
5
12
19
26
Days
Treatment
C
%Survival
Days after tumor injection
Control
hu14.18
CD16V-BB-ζ
CD16V-BB-ζ +hu14.18
vs. P=0.14
vs. P<0.01
vs. P<0.01
vs. P=0.71
vs. P<0.01

FIGURE 11

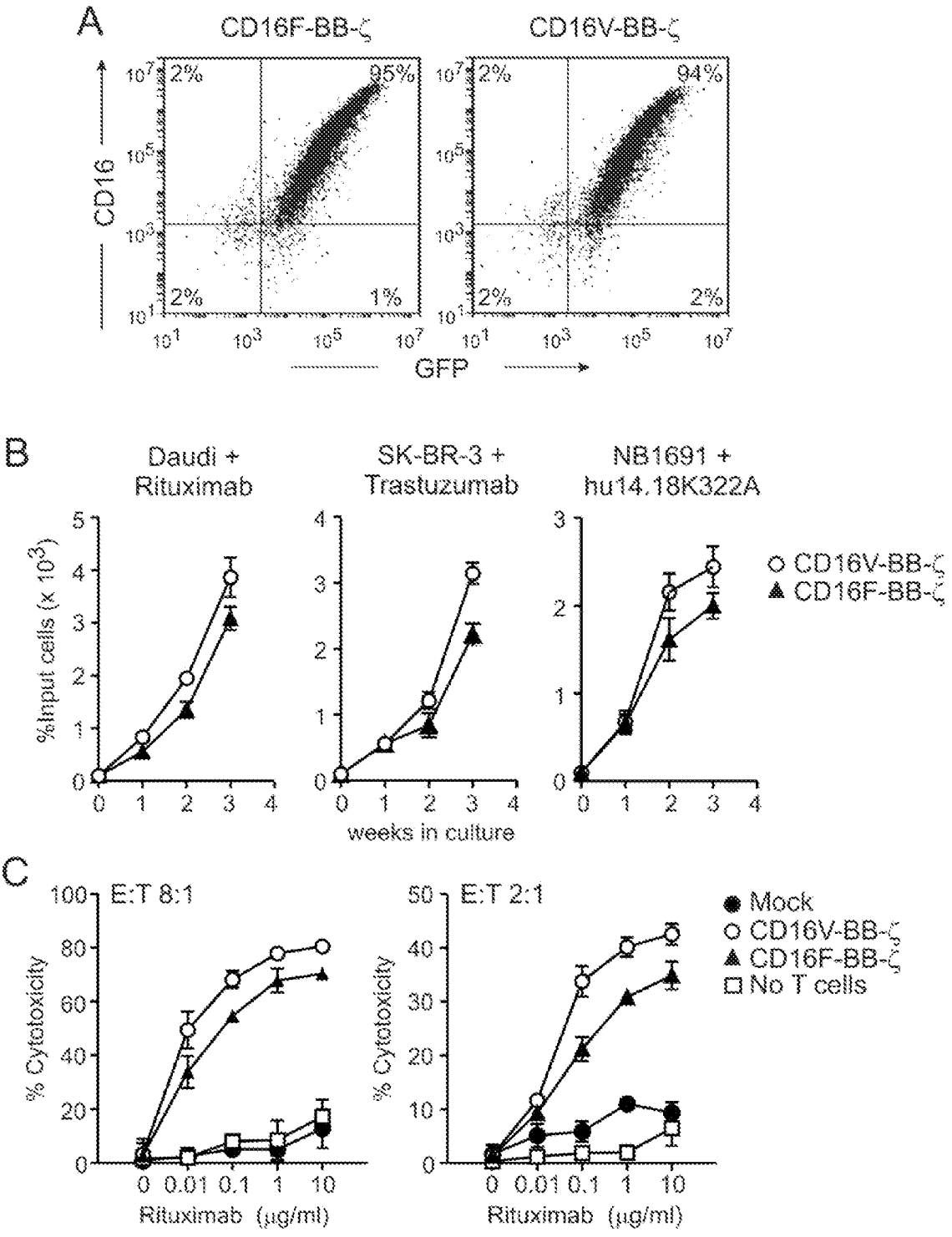

A
CD16F-BB-ζ
CD16V-BB-ζ
CD16
GFP
2%
95%
2%
1%
2%
94%
2%
2%
B
Daudi + Rituximab
SK-BR-3 + Trastuzumab
NB1691 + hu14.18K322A
%Input cells (x 10^3)
weeks in culture
CD16V-BB-ζ
CD16F-BB-ζ
C
E:T 8:1
E:T 2:1
% Cytotoxicity
Rituximab (μg/ml)
Mock
CD16V-BB-ζ
CD16F-BB-ζ
No T cells

FIGURE 12

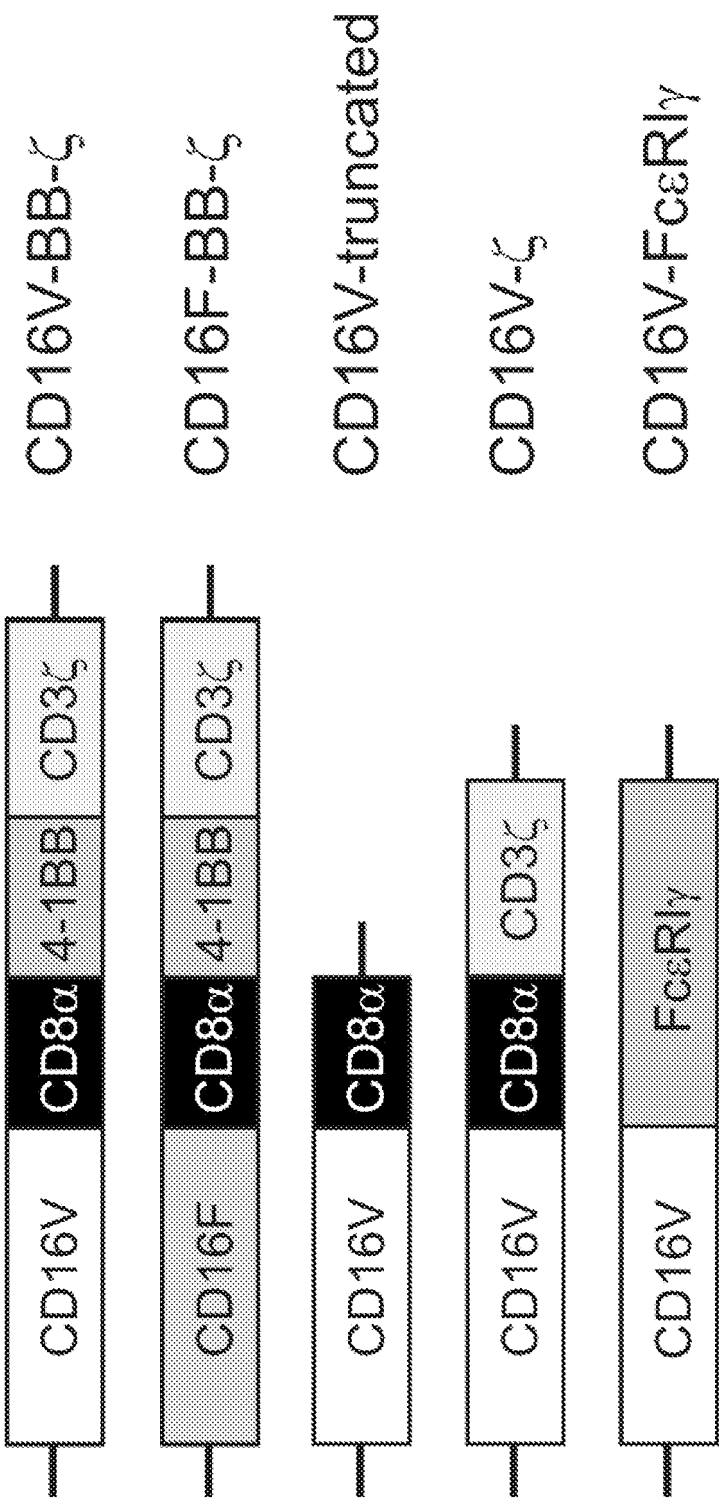

CD16V-BB-ζ
CD16V
CD8α
4-1BB
CD3ζ
CD16F-BB-ζ
CD16F
CD8α
4-1BB
CD3ζ
CD16V-truncated
CD16V
CD8α
CD16V-ζ
CD16V
CD8α
CD3ζ
CD16V-FcεRIγ
CD16V
FcεRIγ

FIGURE 13

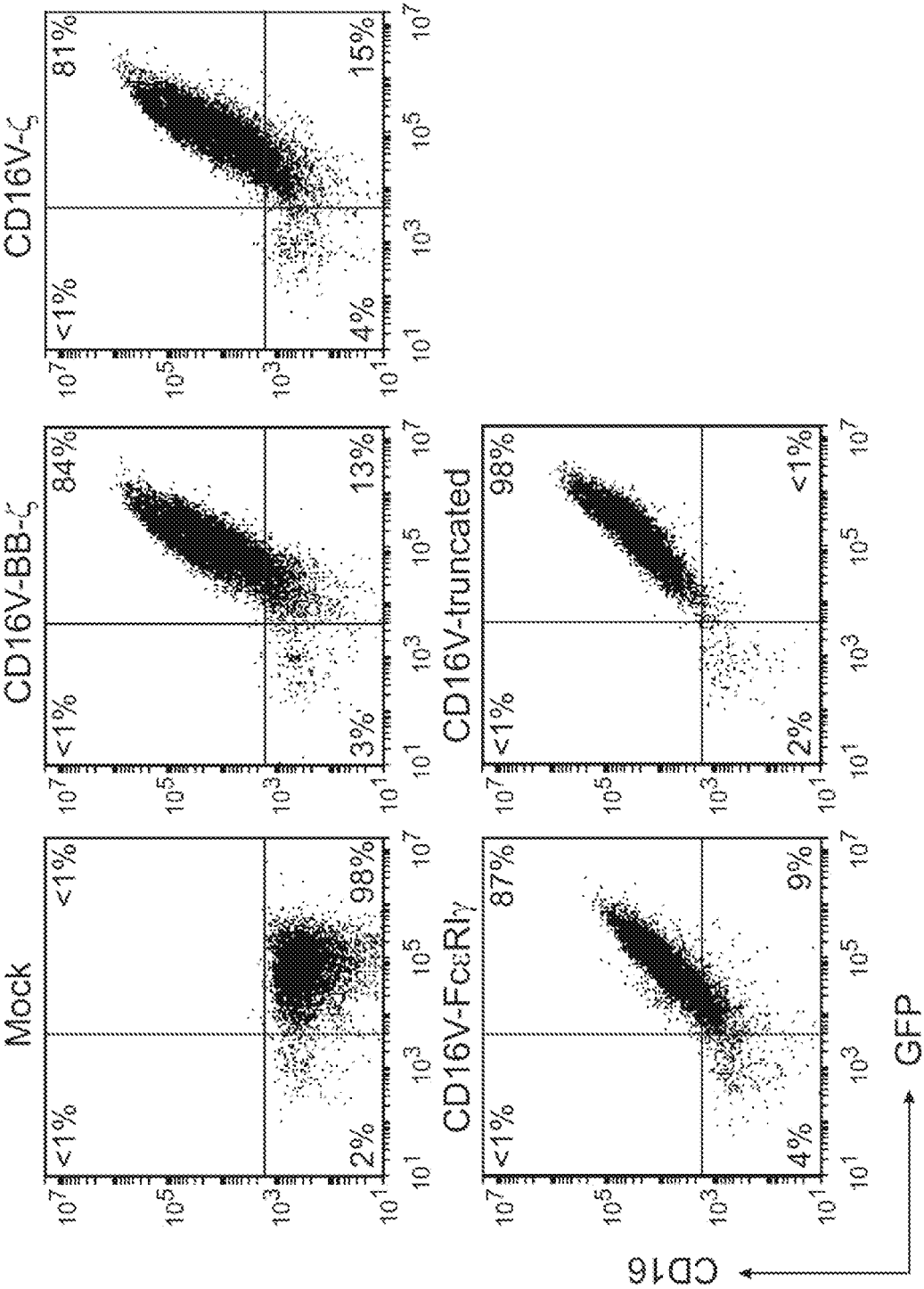

Mock
CD16V-BB-ζ
CD16V-ζ
CD16V-truncated
CD16V-FcεRIγ
<1%
98%
2%
84%
13%
3%
81%
15%
4%
98%
<1%
2%
87%
9%
4%
CD16
GFP

FIGURE 14

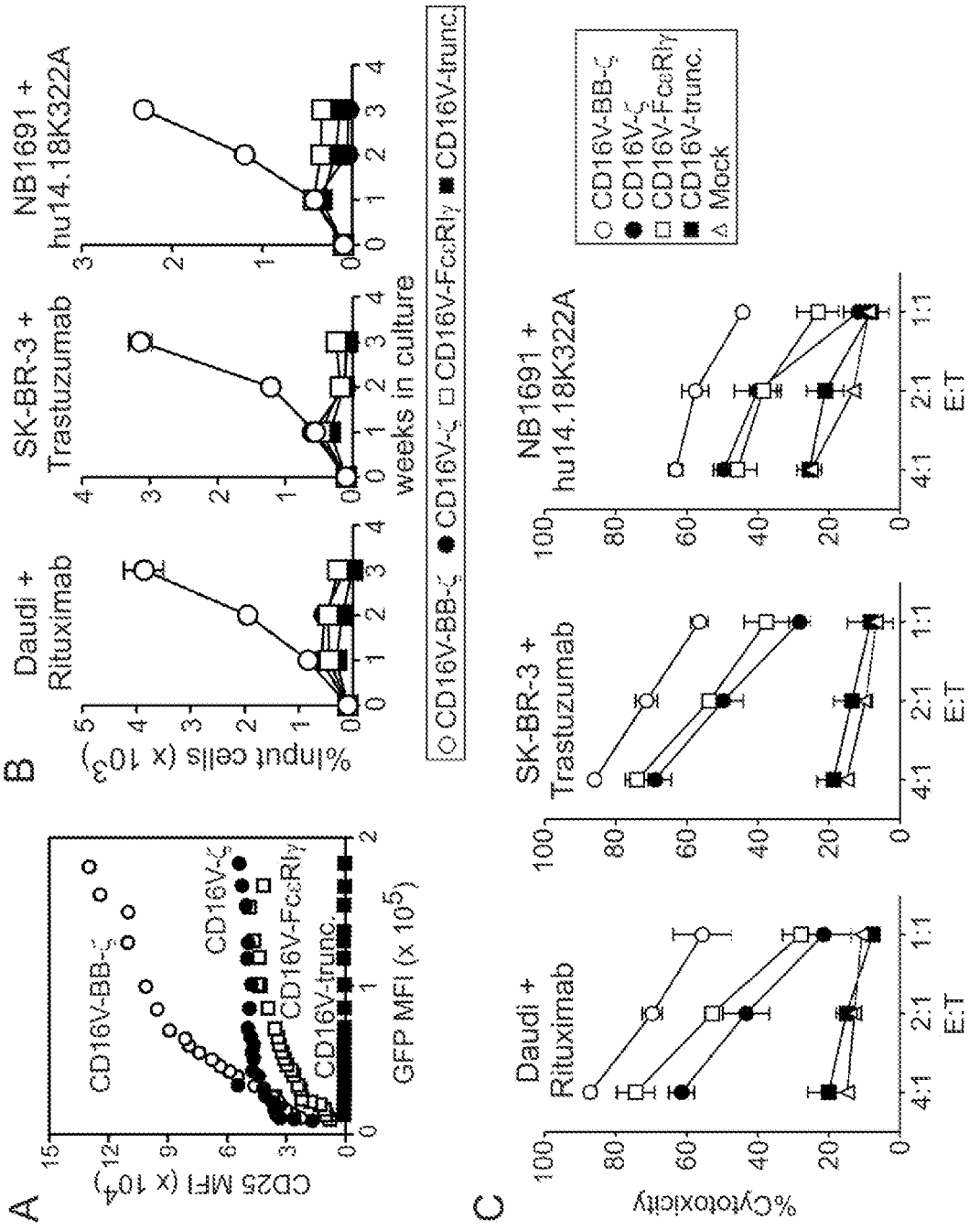

A
CD25 MFI (x 10^4)
GFP MFI (x 10^5)
CD16V-BB-ζ
CD16V-ζ
CD16V-FcεRIγ
CD16V-trunc.
B
Daudi + Rituximab
SK-BR-3 + Trastuzumab
NB1691 + hu14.18K322A
%Input cells (x 10^3)
weeks in culture
CD16V-BB-ζ CD16V-ζ CD16V-FcεRIγ CD16V-trunc
C
Daudi + Rituximab
SK-BR-3 + Trastuzumab
NB1691 + hu14.18K322A
%Cytotoxicity
E:T
4:1 2:1 1:1
CD16V-BB-ζ
CD16V-ζ
CD16V-FcεRIγ
CD16V-trunc.
Mock

FIGURE 15

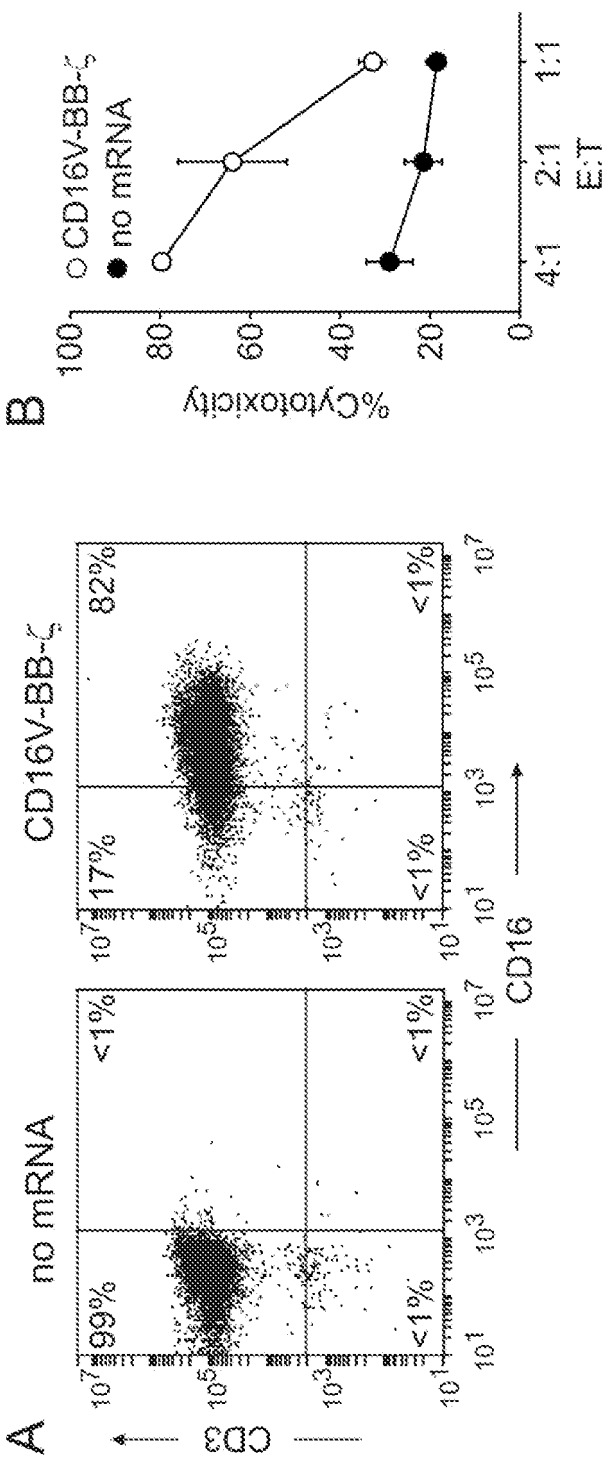

A
no mRNA
CD16V-BB-ζ
99%
<1%
17%
82%
CD3
CD16
B
%Cytotoxicity
CD16V-BB-ζ
no mRNA
E:T
4:1
2:1
1:1

FIGURE 16

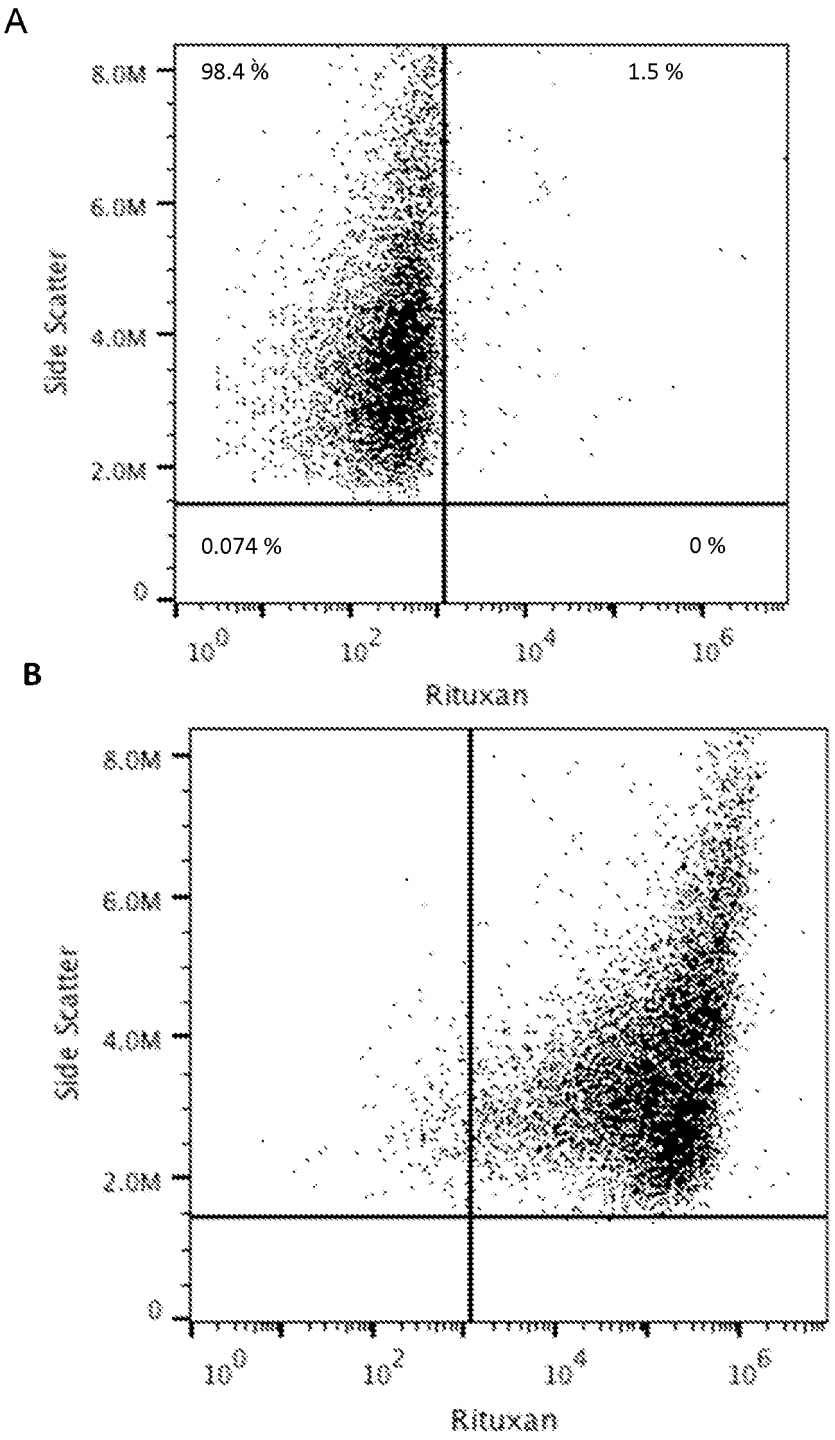

A
98.4 %
1.5 %
0.074 %
0 %
8.0M
6.0M
4.0M
2.0M
0
Side Scatter
10^0
10^2
10^4
10^6
Rituxan
B
8.0M
6.0M
4.0M
2.0M
0
Side Scatter
10^0
10^2
10^4
10^6
Rituxan

FIGURE 16 (CONT'D)

C

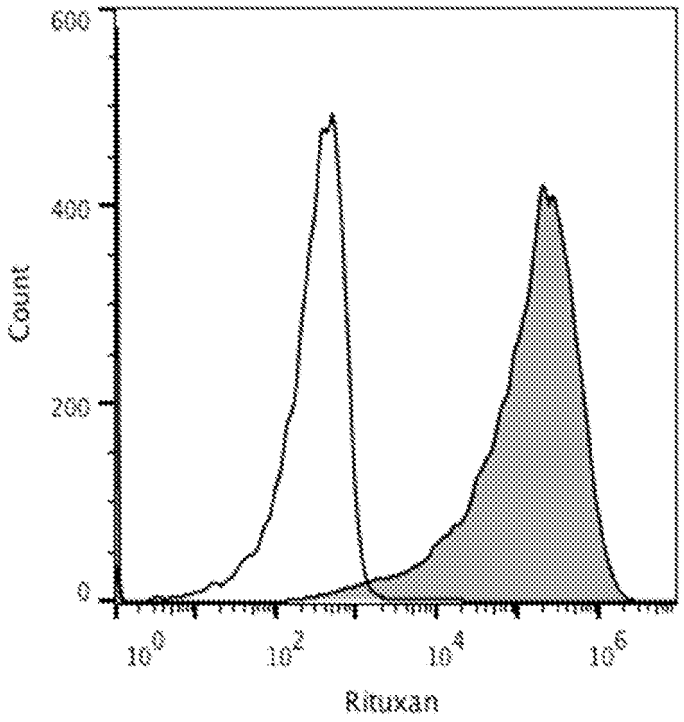


FIGURE 17

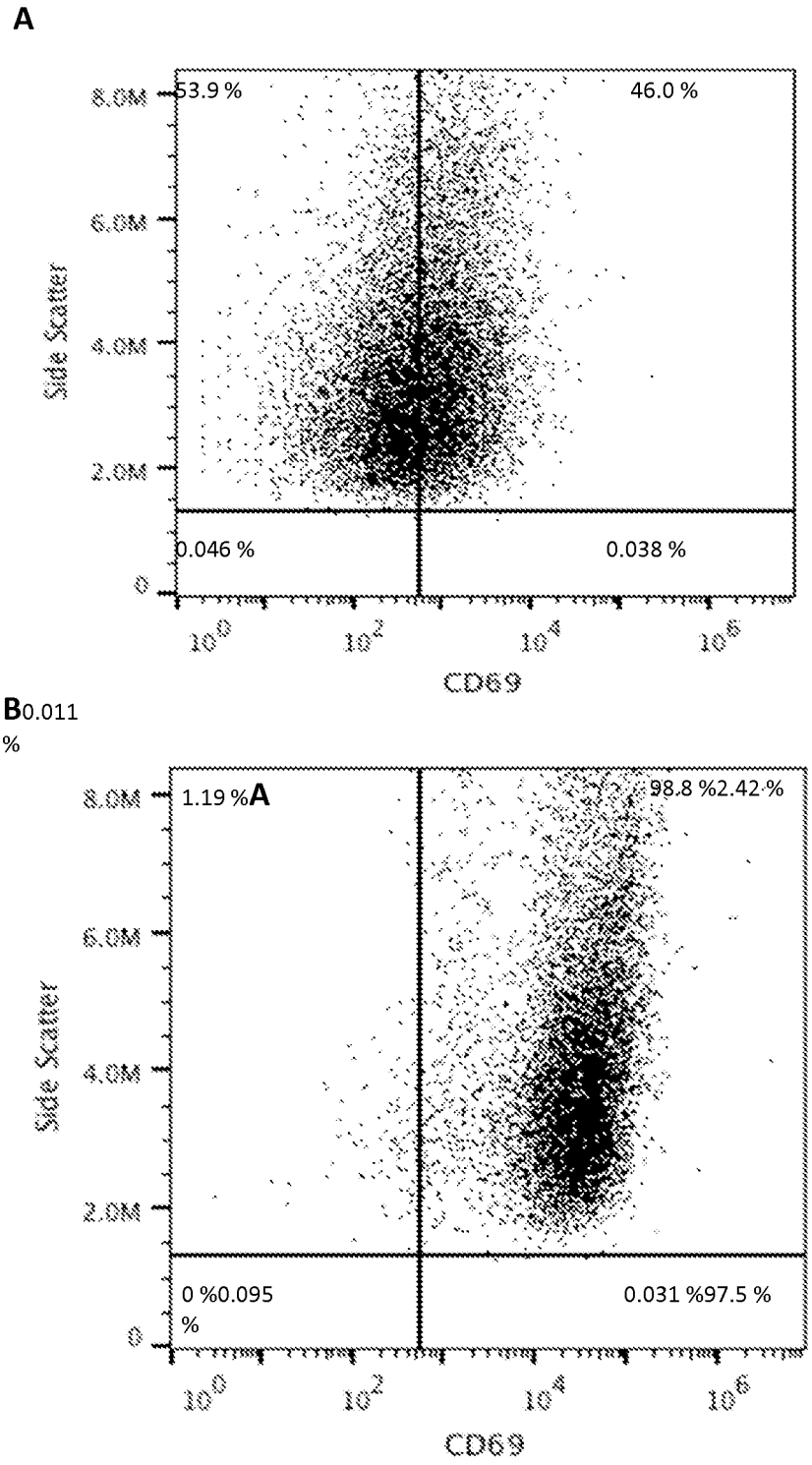

A
53.9 %
46.0 %
0.046 %
0.038 %
8.0M
6.0M
4.0M
2.0M
0
Side Scatter
10^0
10^2
10^4
10^6
CD69
B0.011 %
1.19 %A
98.8 %2.42 %
0 %0.095 %
0.031 %97.5 %
8.0M
6.0M
4.0M
2.0M
0
Side Scatter
10^0
10^2
10^4
10^6
CD69

FIGURE 17 (CONT'D)

C

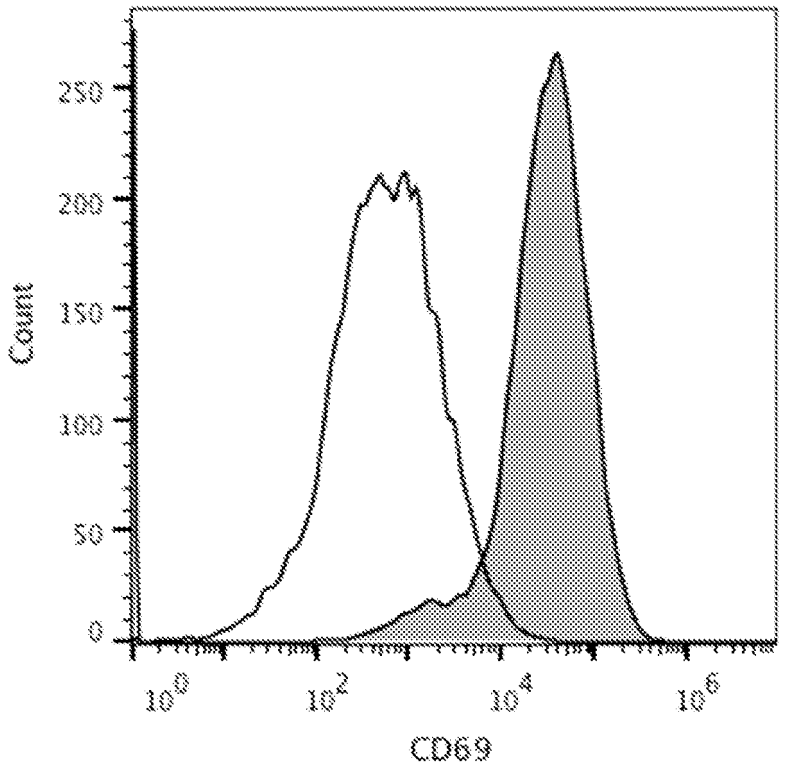
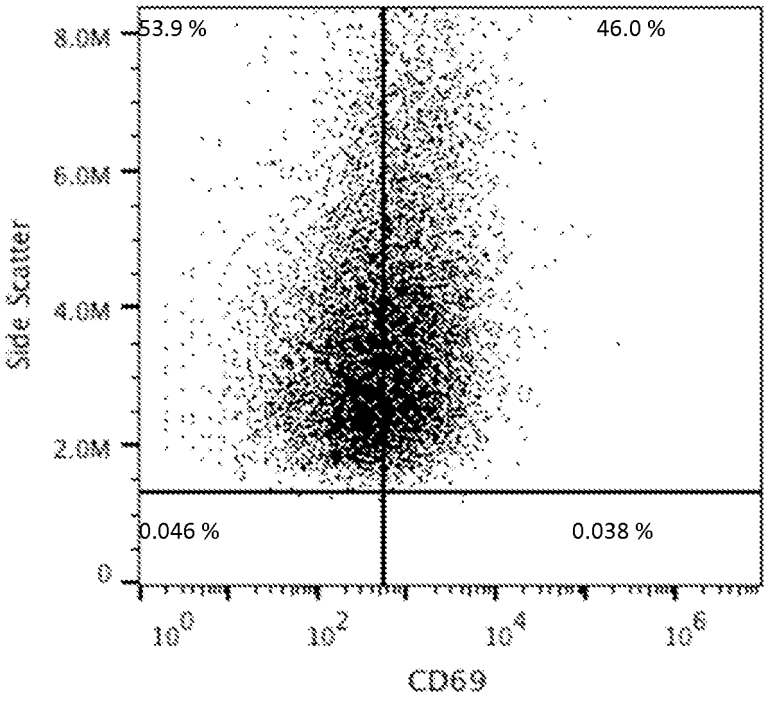


FIGURE 18

A


53.9 %
46.0 %
0.046 %
0.038 %
8.0M
6.0M
4.0M
2.0M
0
Side Scatter
10^0
10^2
10^4
10^6
CD69

B

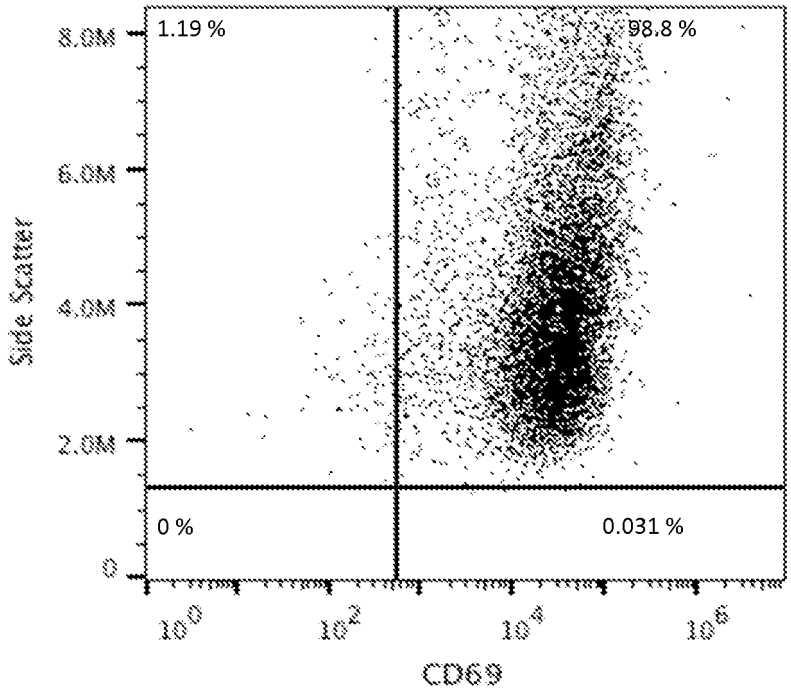

1.19 %
98.8 %
0 %
0.031 %
8.0M
6.0M
4.0M
2.0M
0
Side Scatter
10^0
10^2
10^4
10^6
CD69

FIGURE 18 (CONT'D)

C

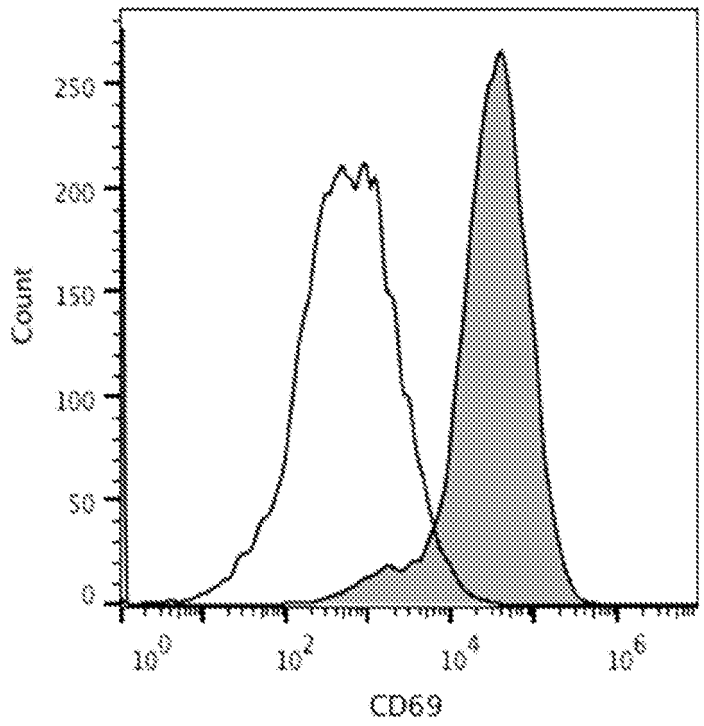
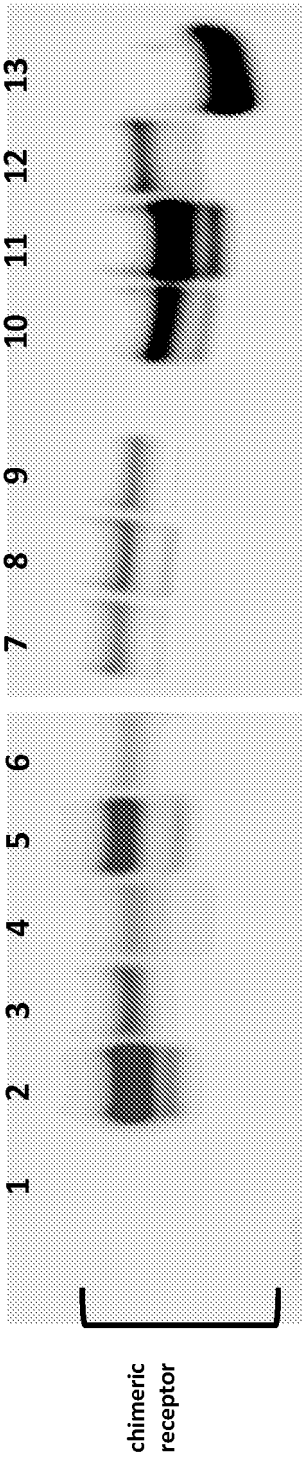



FIGURE 19