Sindbis Virus
(SV)
wild-type
SV glycoprotein
(SVG)
SVG recognizes two viral receptors
E3
E2
6K
E1
heparan sulfate
recognition site
DC-SIGN
recognition site
envelope
lentivector
FUGW/SVG
transduce
multiple
cell types
Engineer SVG
to be SVGmut
SVGmut: recognizes one viral receptor
E3
E2
6K
E1
alterations to disable
its recognition to
heparan sulfate
recognition to
DC-SIGN retained
envelope
lentivector
FUGW/SVGmu
selectively
transduce
DC

Figure 1

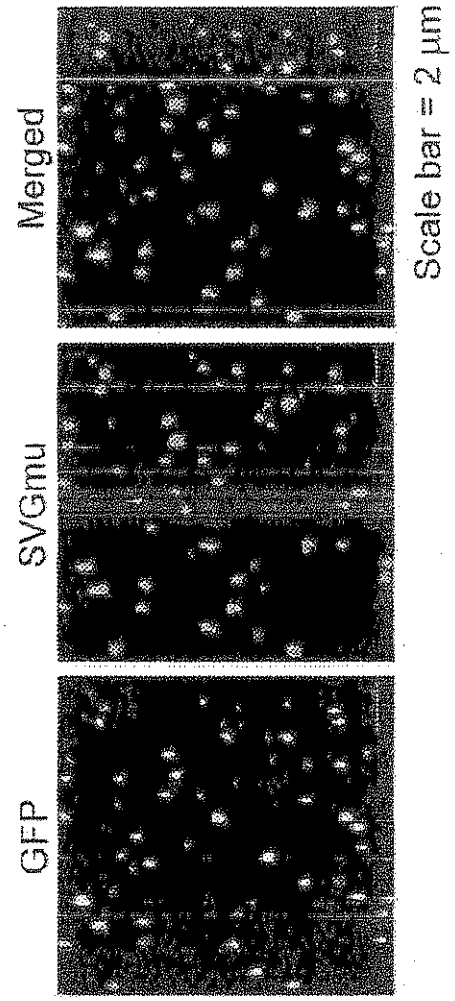


Figure 2

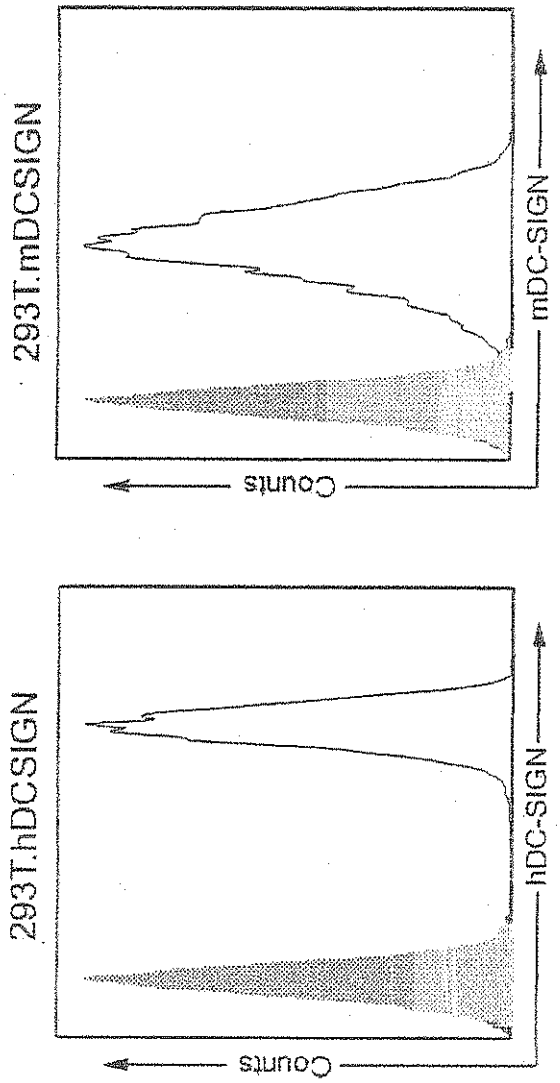


Figure 3A

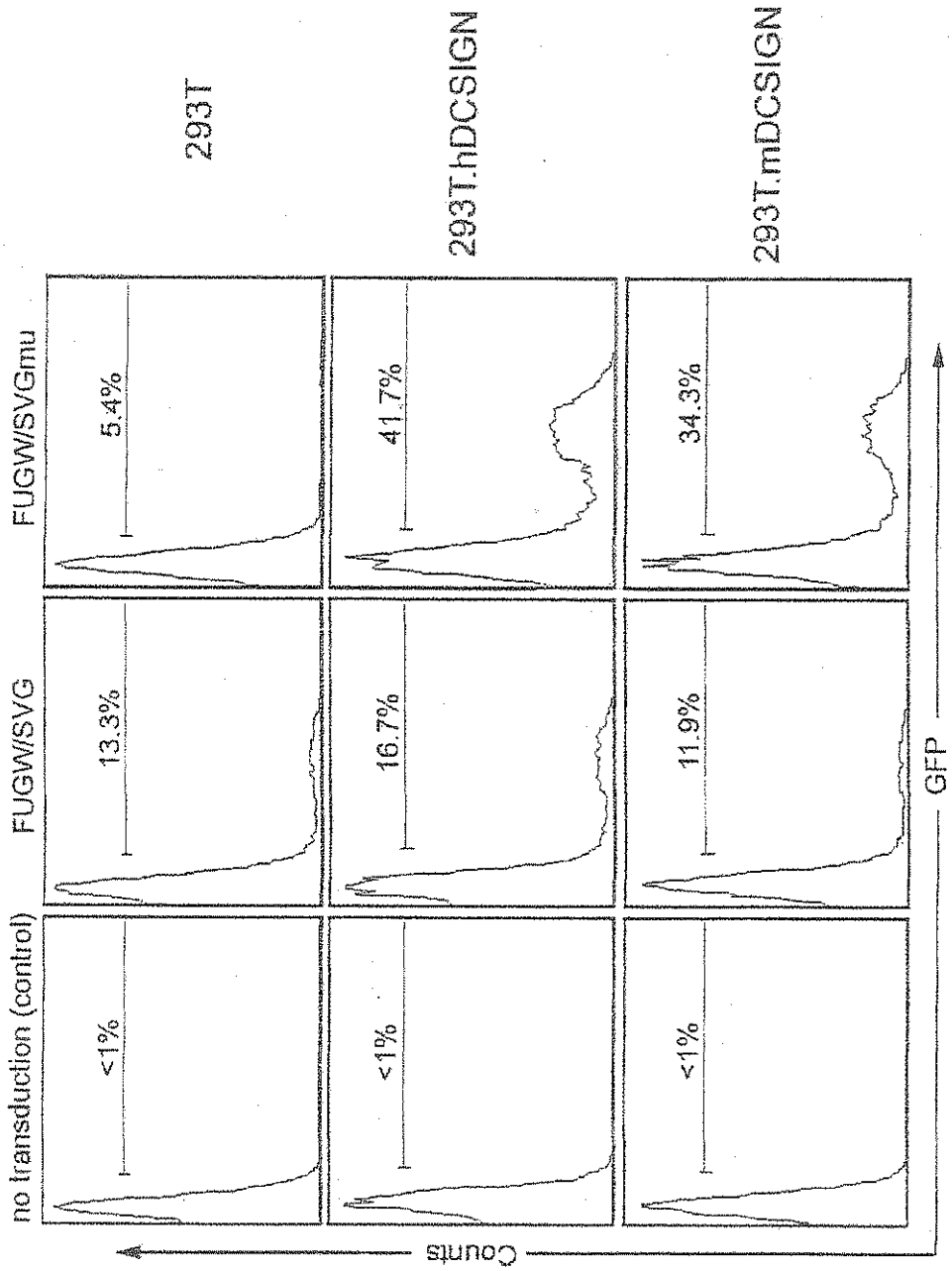

no transduction (control)
FUGW/SVG
FUGW/SVGmu
293T
293T.hDCSIGN
293T.mDCSIGN
<1%
13.3%
5.4%
<1%
16.7%
41.7%
<1%
11.9%
34.3%
Counts
GFP

Figure 3B

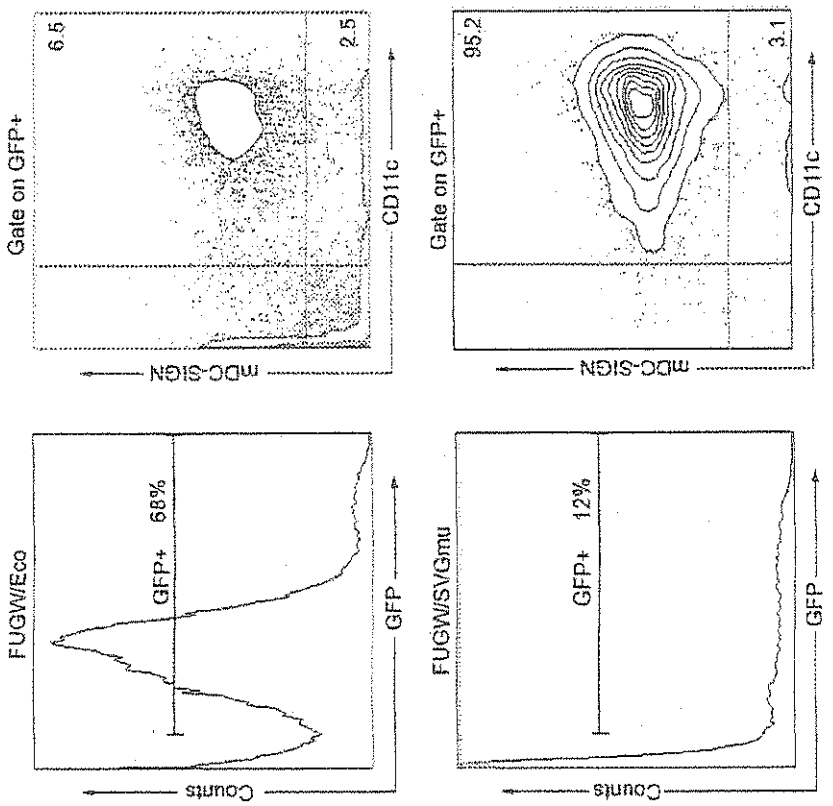

Gate on GFP+
6.5
2.5
mDC-SIGN
CD11c
95.2
3.1
FUGW/Eco
GFP+ 68%
Counts
GFP
FUGW/SV/Gmu
GFP+ 12%

Figure 4A

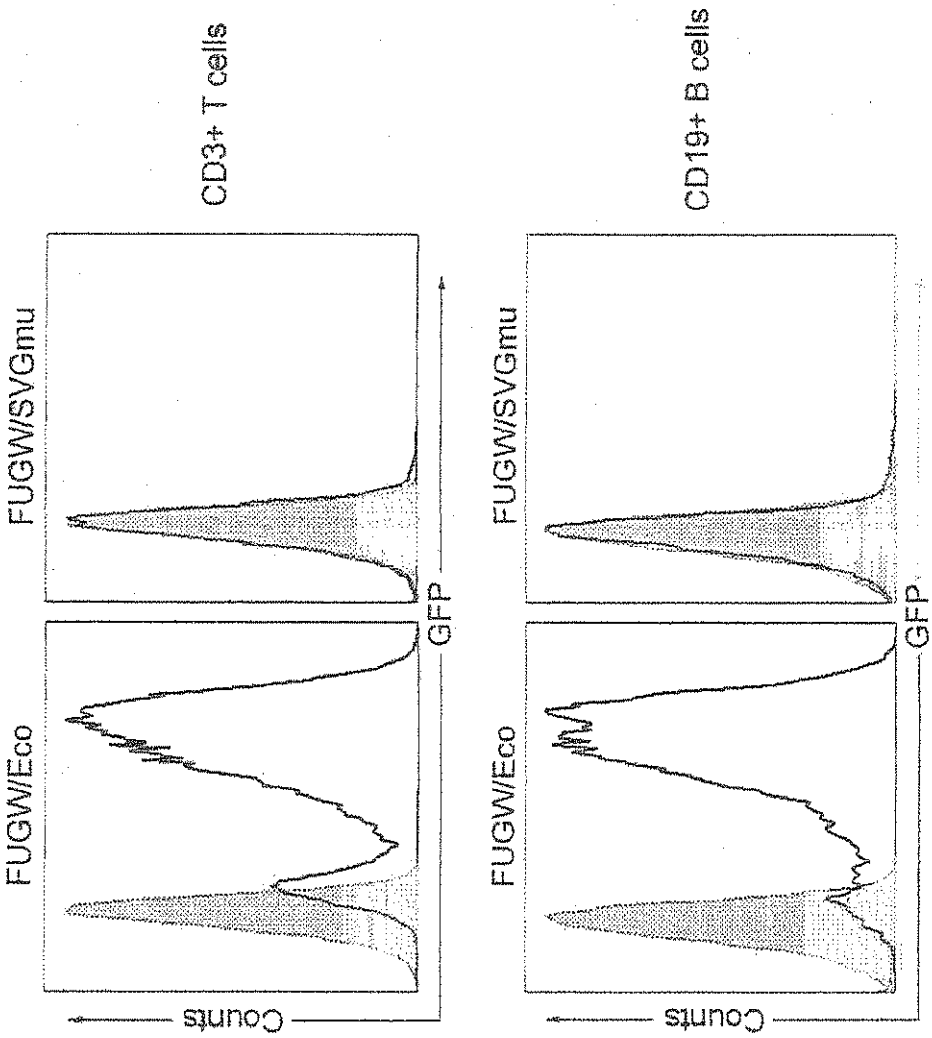


Figure 4B

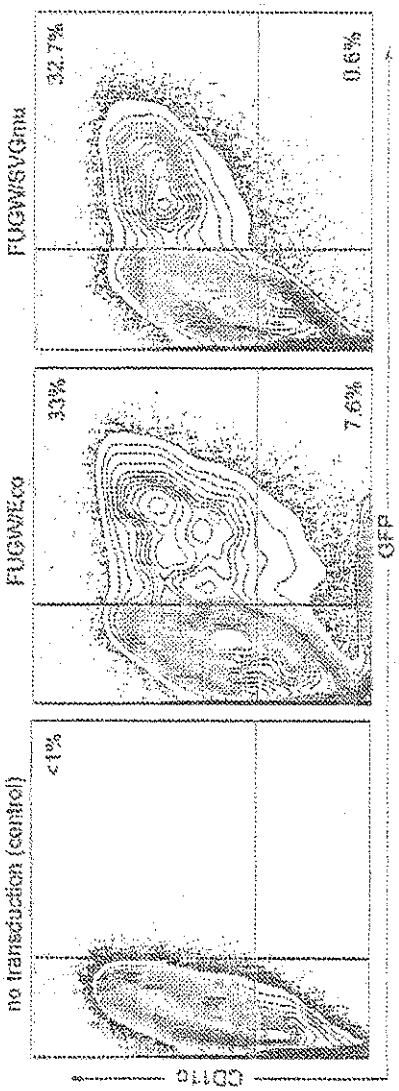
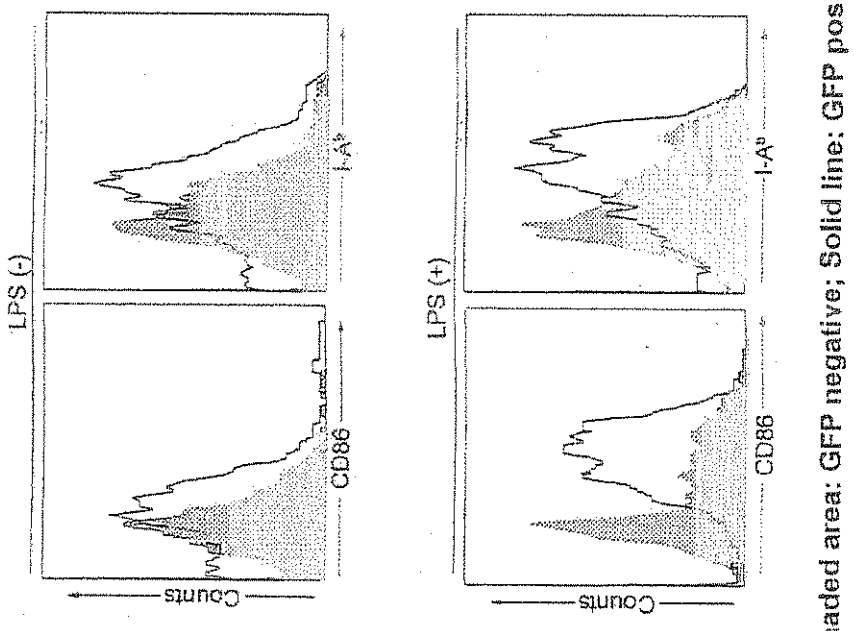

no transduction (control)
<1%
FUGWEco
33%
7.6%
FUGWVSVGmut
32.7%
0.6%
CD11b
GFP

Figure 5



Shaded area: GFP negative; Solid line: GFP positive

Figure 6

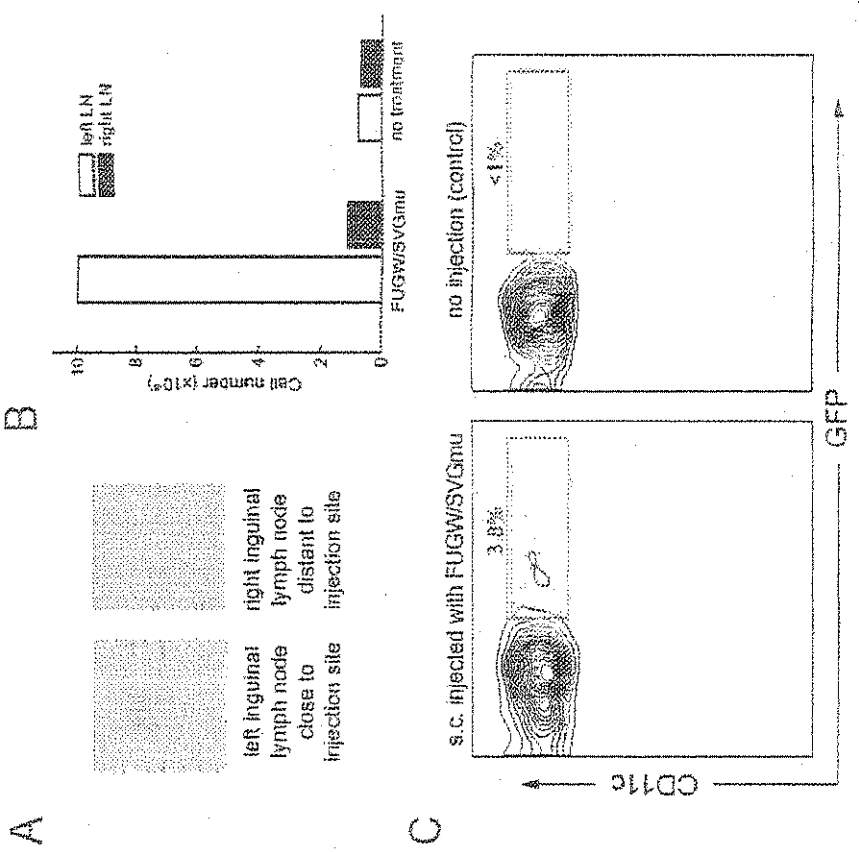

A
left inguinal lymph node close to injection site
right inguinal lymph node distant to injection site
B
left LN
right LN
Cell number (x10⁶)
10
8
6
4
2
0
FUGW/SVGmu
no treatment
C
s.c. injected with FUGW/SVGmu
3.8%
no injection (control)
<1%
CD11c
GFP

Figure 7

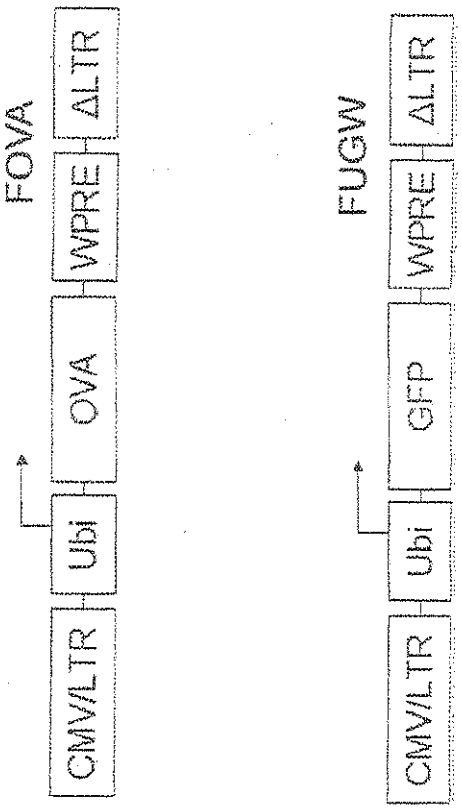


Figure 8

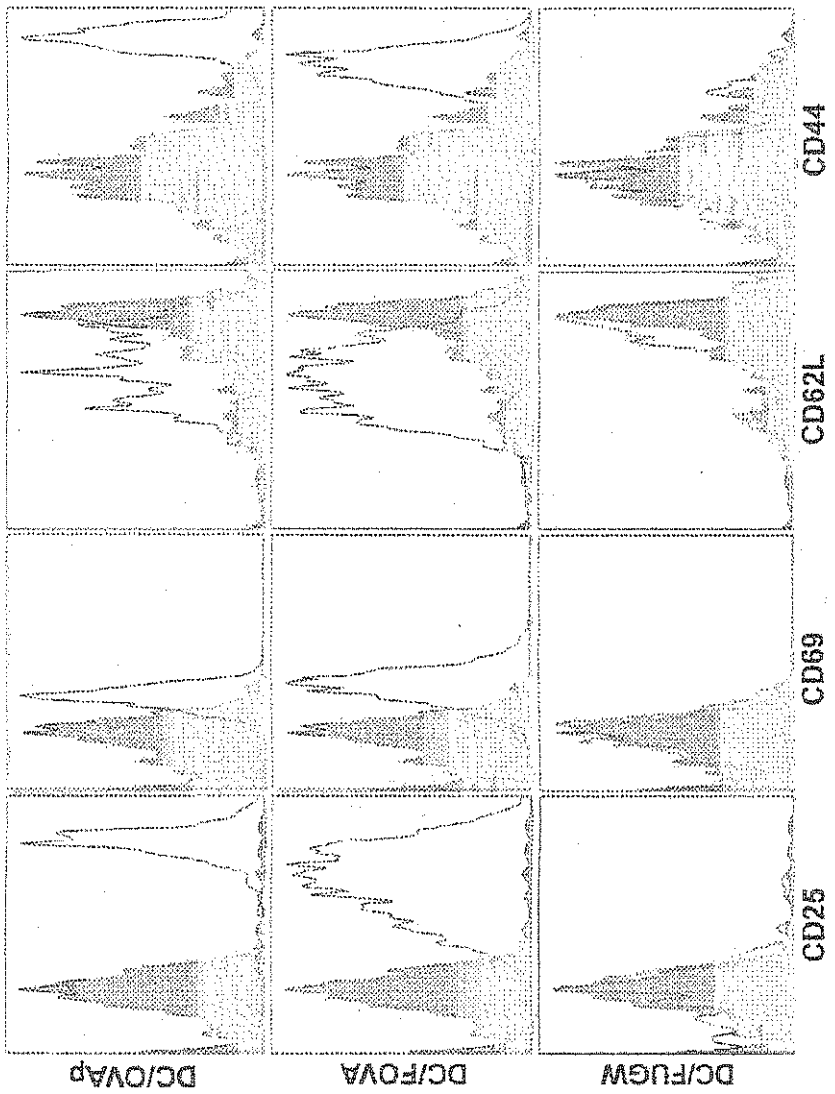


Figure 9

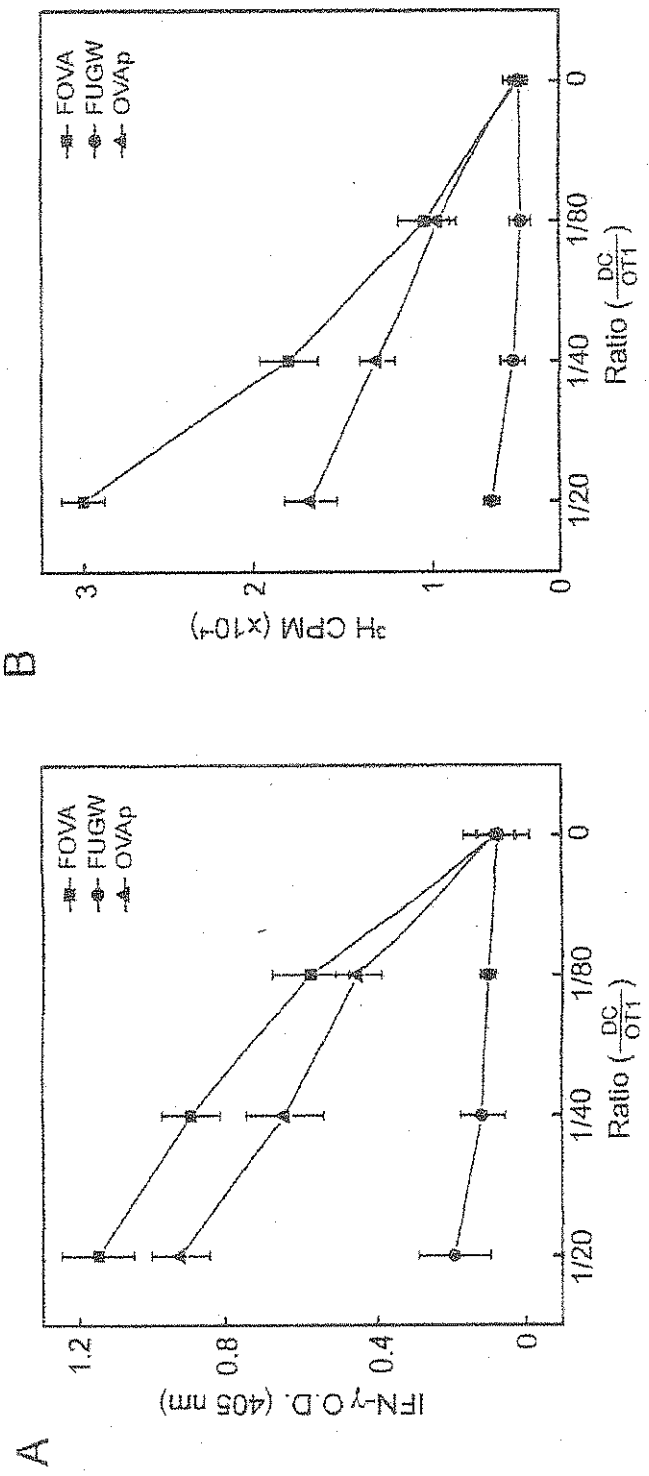

B
FOVA
FUGW
OVAp
3H CPM (x10-4)
3
2
1
0
1/20
1/40
1/80
0
Ratio (DC/OT1)
A
FOVA
FUGW
OVAp
IFN-γ O.D. (405 nm)
1.2
0.8
0.4
0
1/20
1/40
1/80
0
Ratio (DC/OT1)

Figure 10

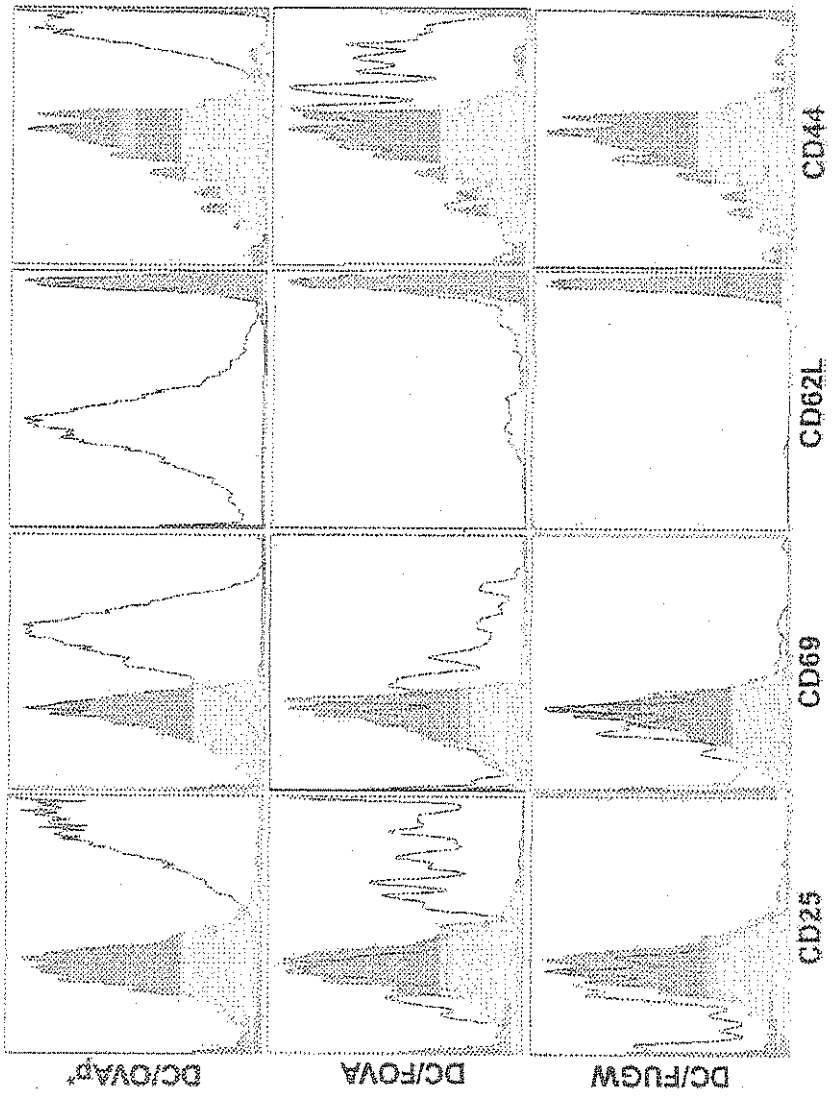


Figure 11

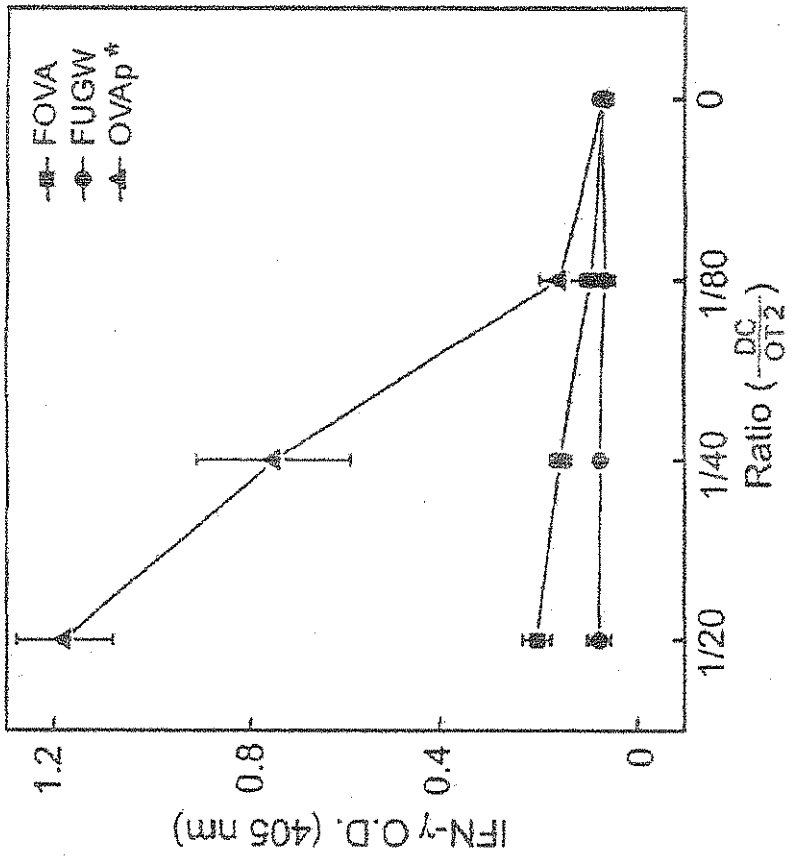

FOVA
FUGW
OVAp*
IFN-γ O.D. (405 nm)
1.2
0.8
0.4
0
1/20
1/40
1/80
0
Ratio ($\frac{DC}{OT2}$)

Figure 12

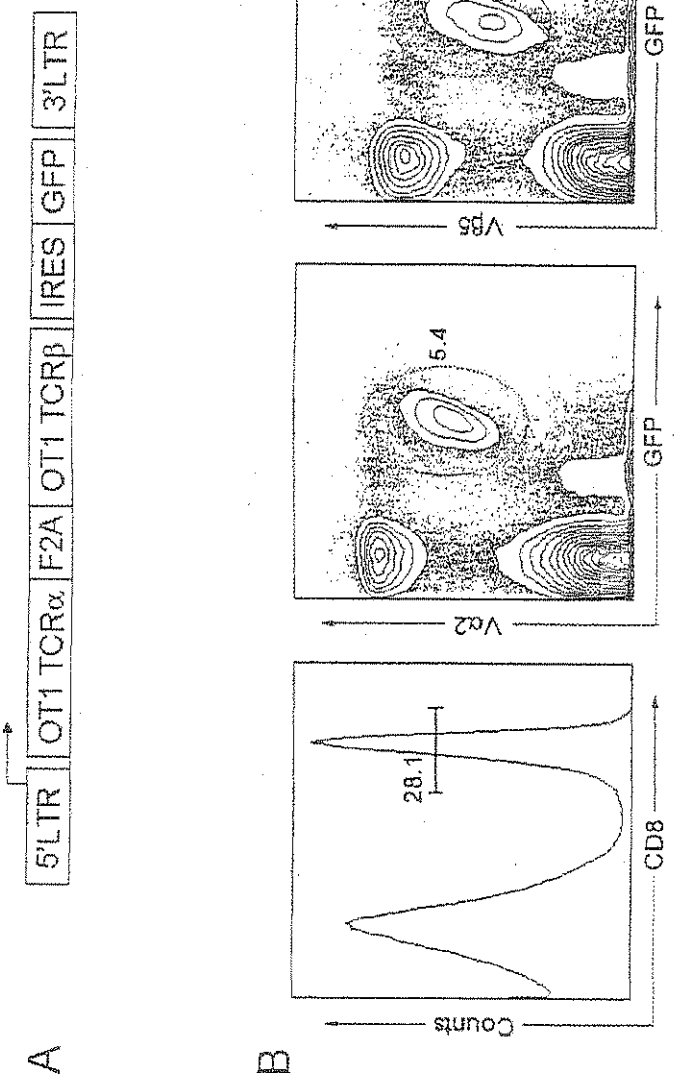

A
5'LTR
OT1 TCRα
F2A
OT1 TCRβ
IRES
GFP
3'LTR
B
Counts
CD8
28.1
Vα2
5.4
GFP
Vβ5
5.1
GFP

Figure 13

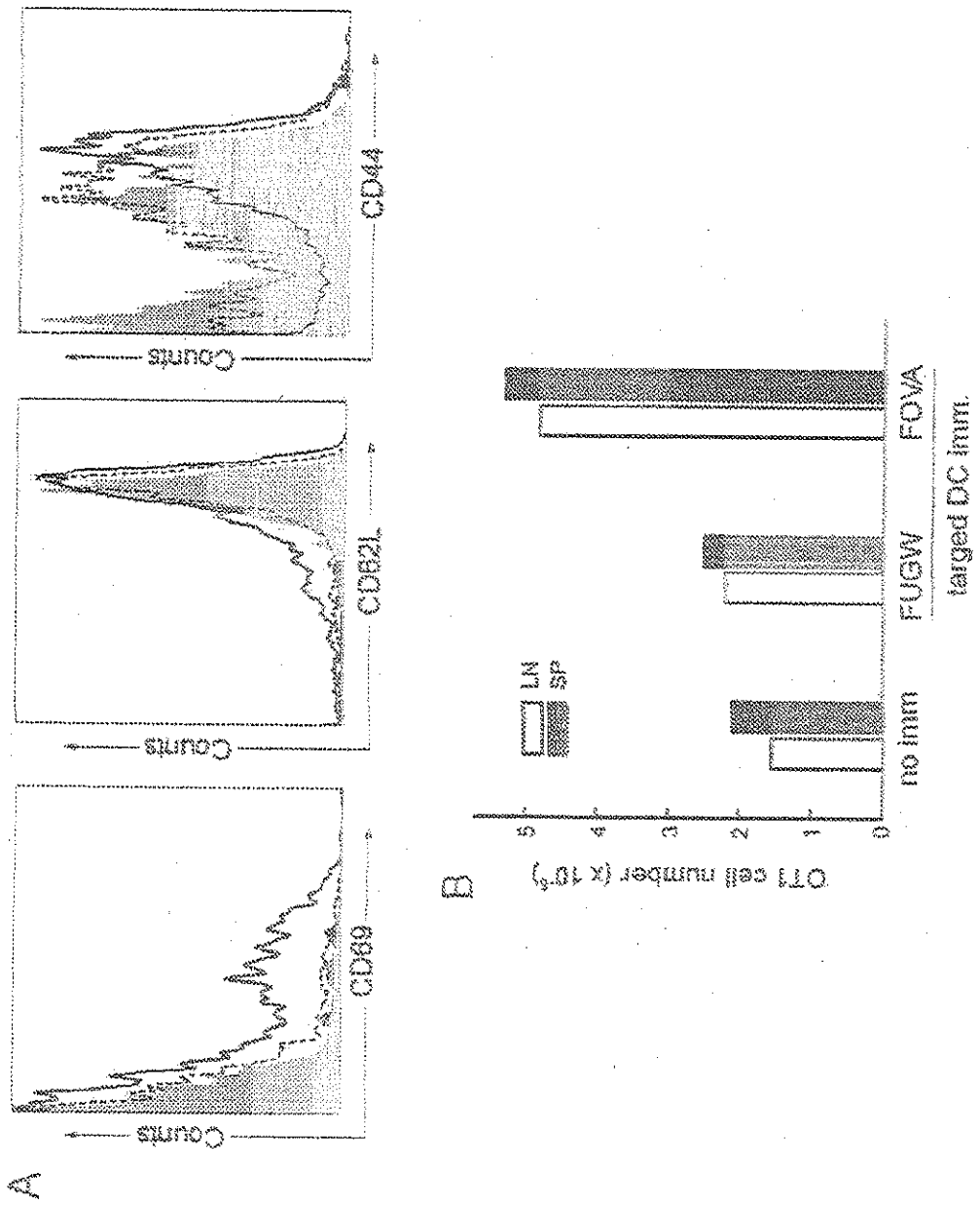

A
Counts
CD69
Counts
CD62L
Counts
CD44
B
OT1 cell number (x 10⁵)
0
1
2
3
4
5
LN
SP
no imm
FUGW
FOVA
targed DC imm.

Figure 14

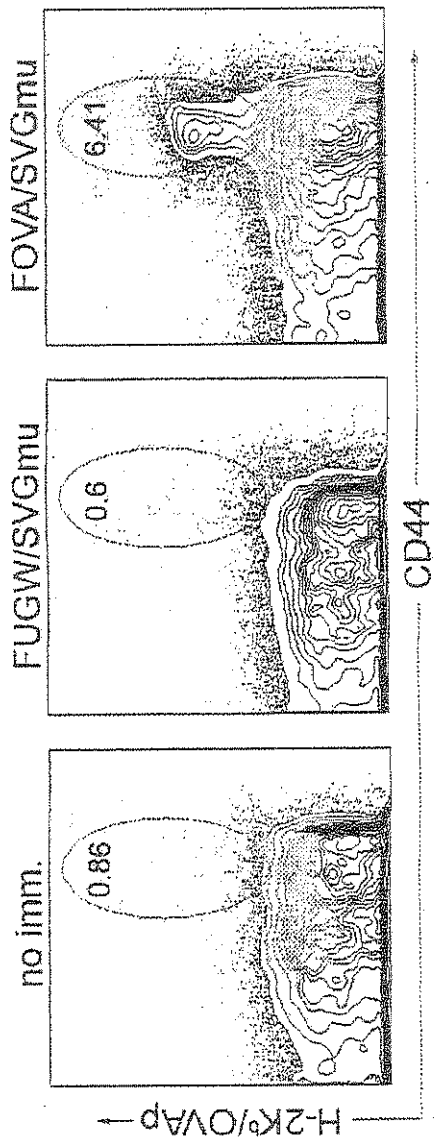


Figure 15

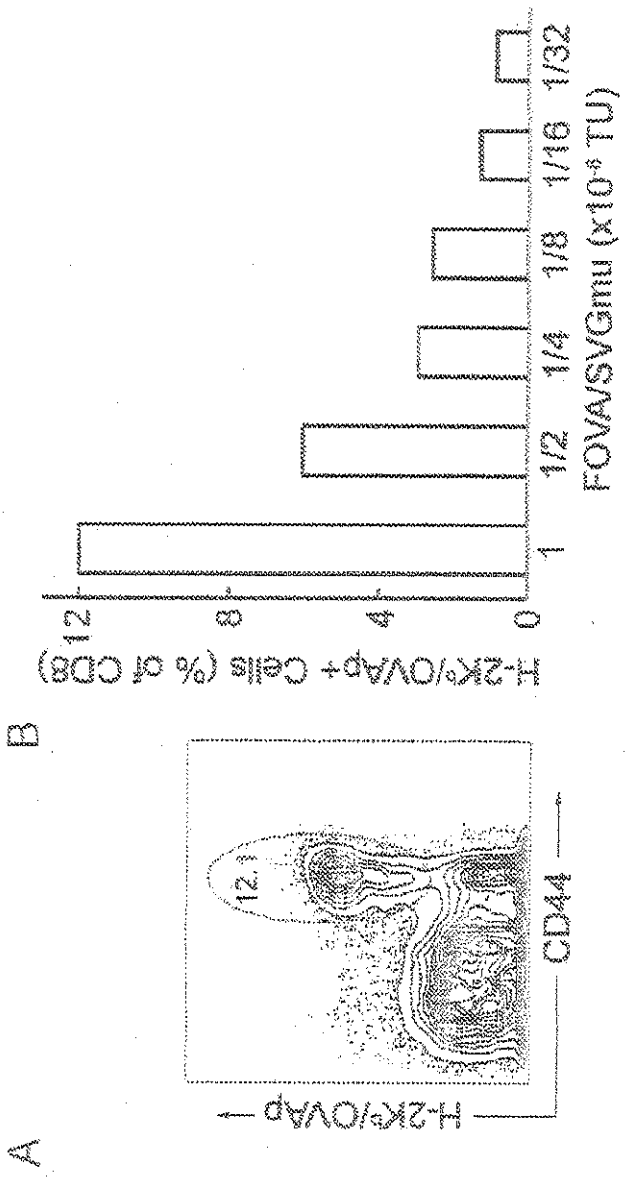

A
H-2Kb/OVAp
CD44
12.1
B
H-2Kb/OVAp+ Cells (% of CD8)
0
4
8
12
1
1/2
1/4
1/8
1/16
1/32
FOVA/SVGmu (x10⁴ TU)

Figure 16

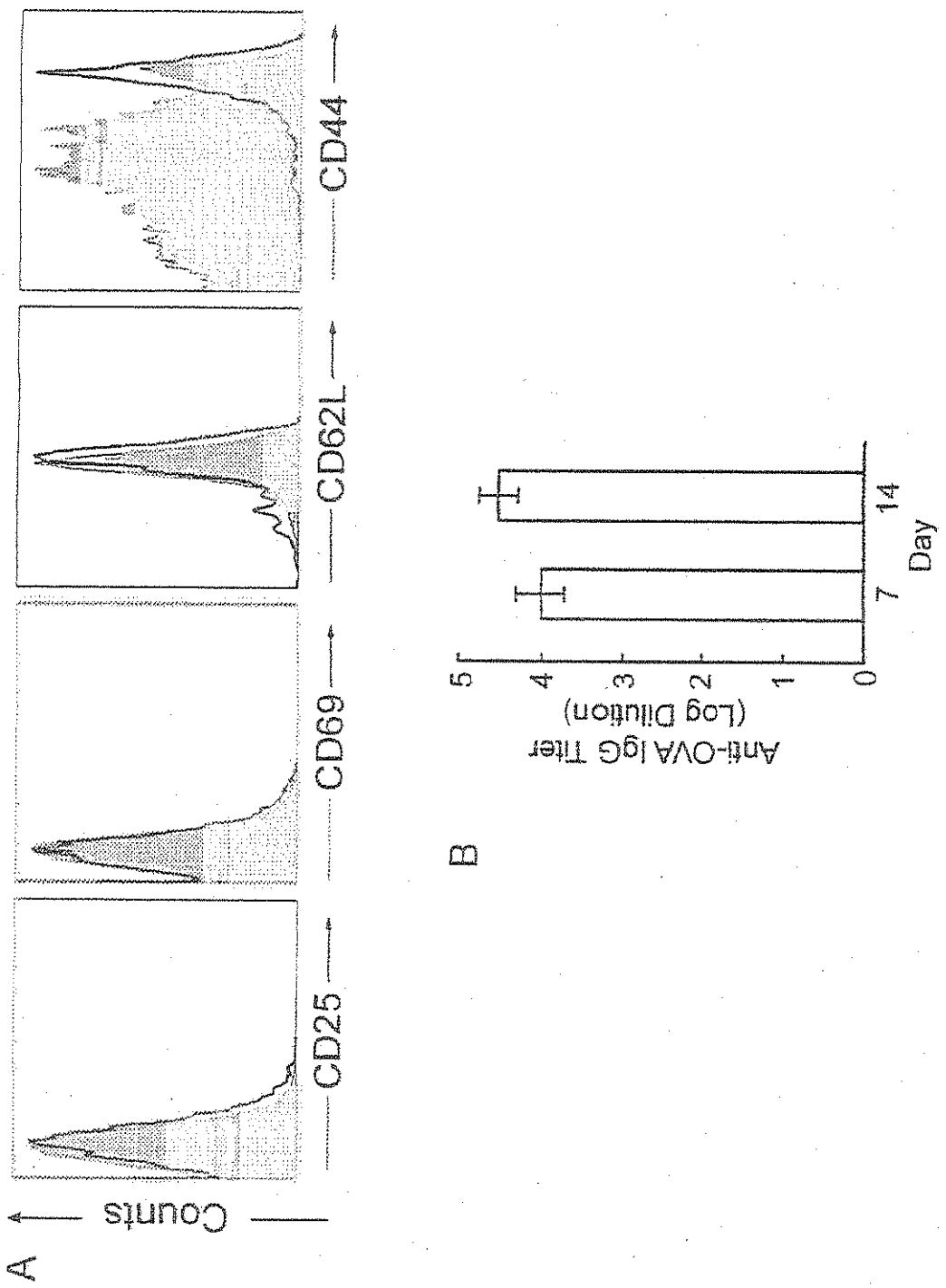

A
Counts
CD25
CD69
CD62L
CD44
B
Anti-OVA IgG Titer
(Log Dilution)
5
4
3
2
1
0
7
14
Day

Figure 17

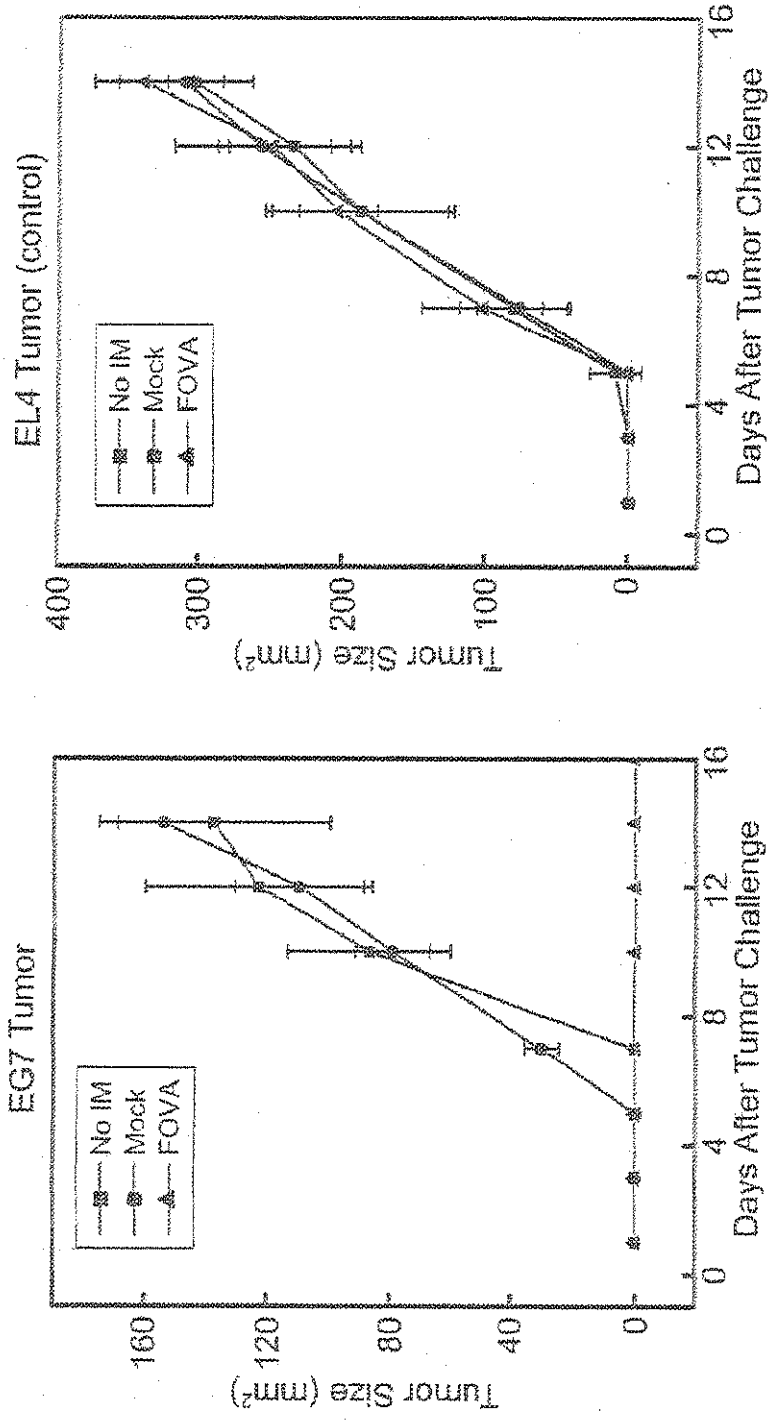
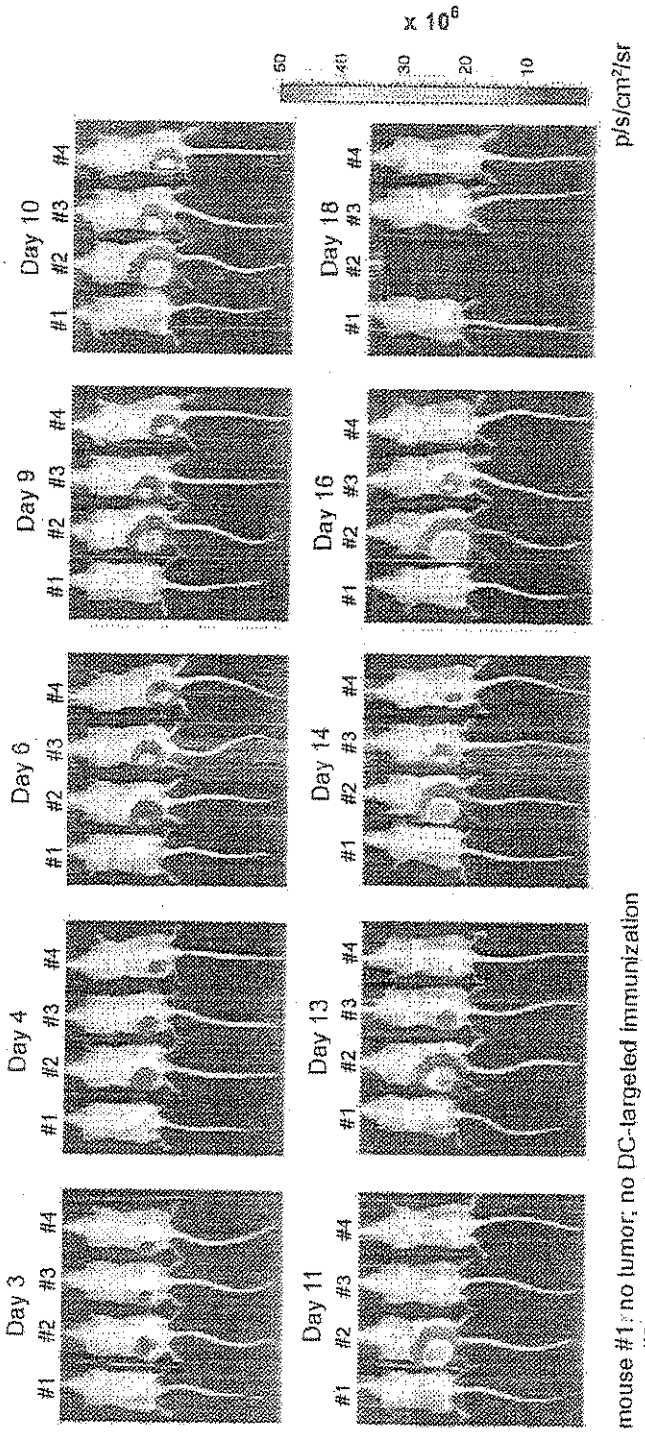

EL4 Tumor (control)
No IM
Mock
FOVA
Tumor Size (mm²)
0
100
200
300
400
0
4
8
12
16
Days After Tumor Challenge
EG7 Tumor
No IM
Mock
FOVA
Tumor Size (mm²)
0
40
80
120
160
0
4
8
12
16
Days After Tumor Challenge

Figure 18



mouse #1: no tumor; no DC-targeted immunization
mouse #2: tumor ($5x10^5$ EG.7luc cells); no DC-targeted immunization
mouse #3&4: tumor ($5x10^5$ EG.7luc cells); DC-targeted immunization with FOVA/SVGmu

Figure 19

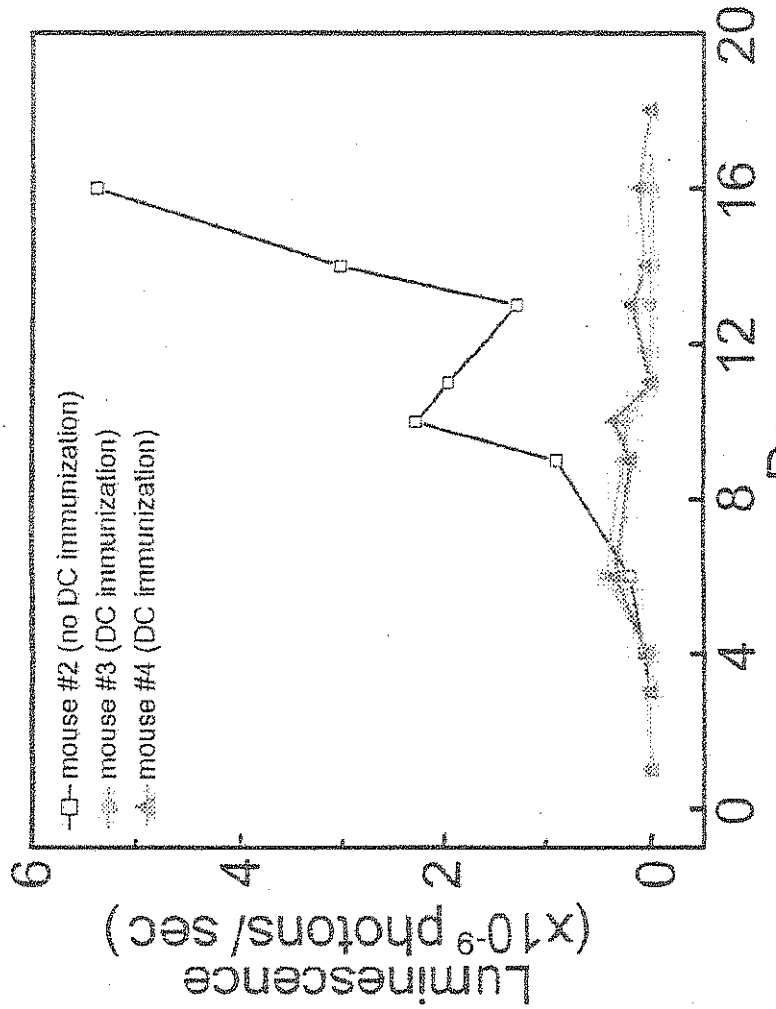

Luminescence
(x10-9 photons/ sec)
0
2
4
6
0
4
8
12
16
20
Days
-□- mouse #2 (no DC immunization)
mouse #3 (DC immunization)
mouse #4 (DC immunization)

Figure 20

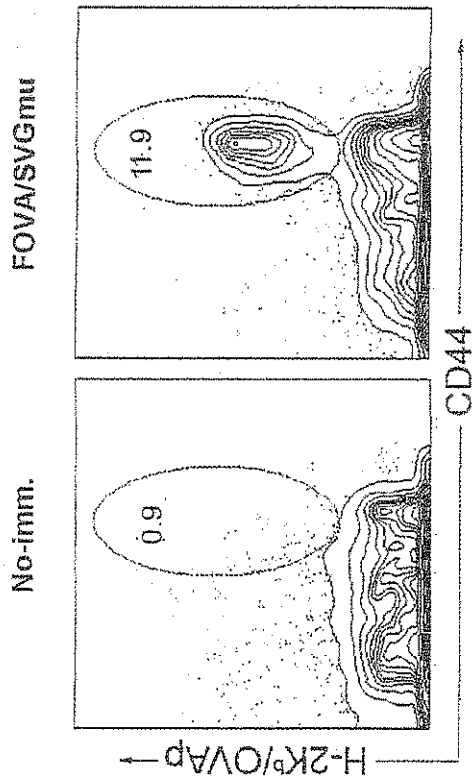


Figure 21

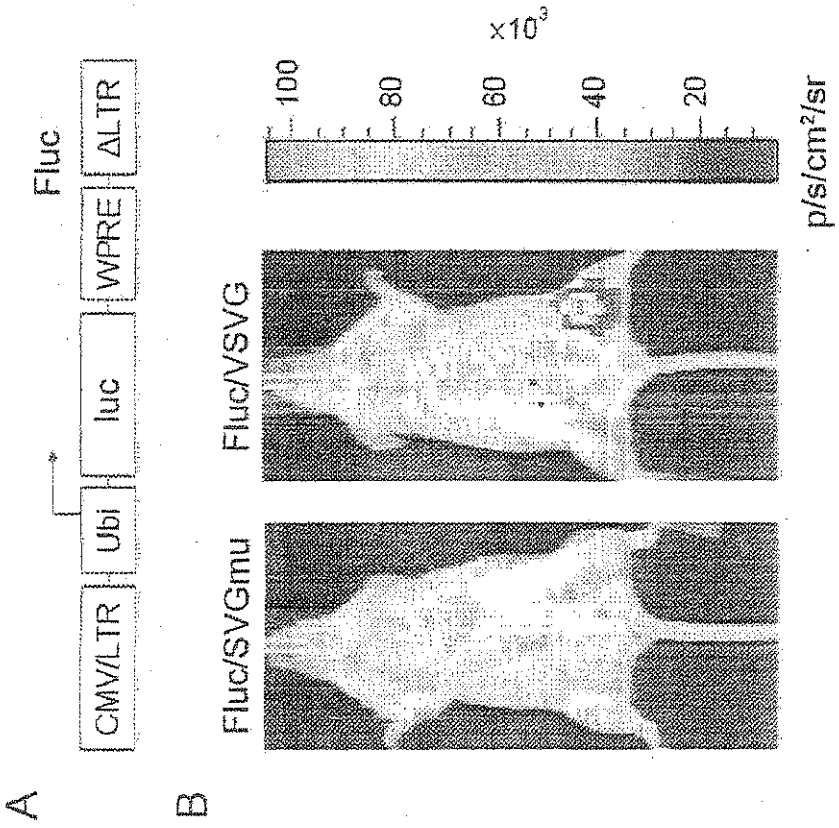

A
Fluc
CMV/LTR
Ubi
luc
WPRE
ΔLTR
B
Fluc/SVGmu
Fluc/VSVG
×10³
100
80
60
40
20
p/s/cm²/sr

Figure 22

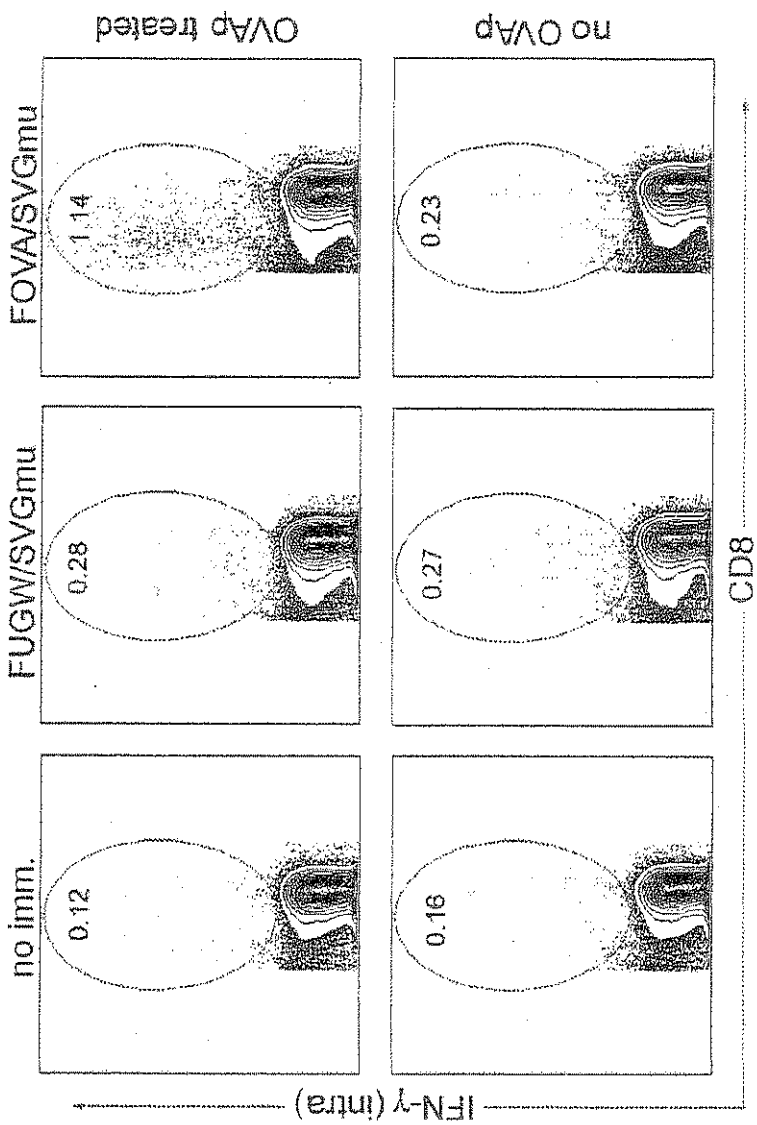

OVAp treated
no OVAp
FOVA/SVGmu
FUGW/SVGmu
no imm.
1.14
0.23
0.28
0.27
0.12
0.16
IFN-γ (intra)
CD8

Figure 23

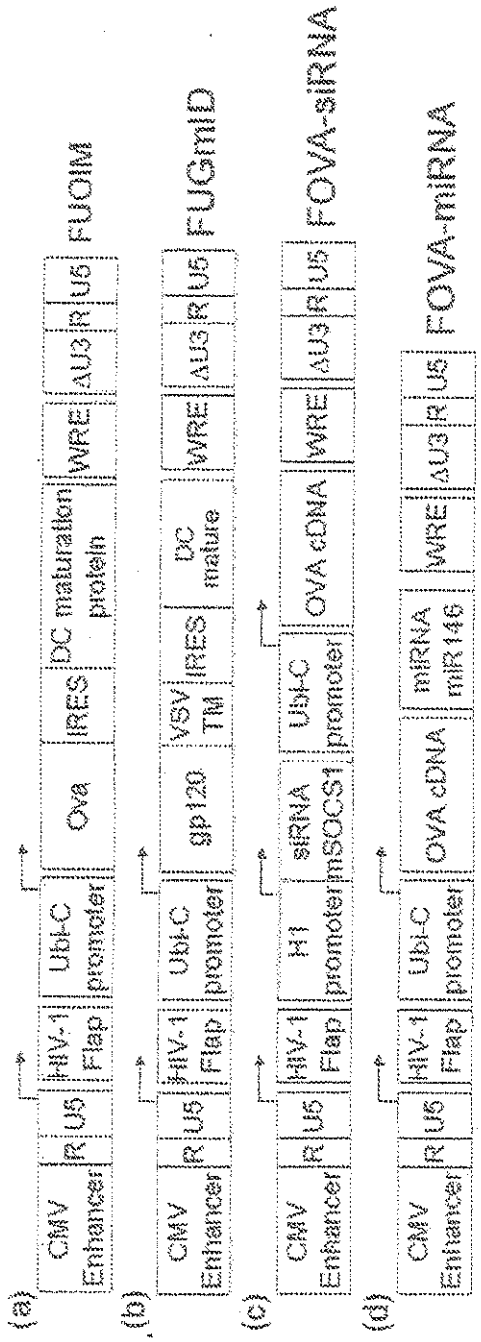

(a)
CMV Enhancer
R
U5
HIV-1 Flap
Ubi-C promoter
Ova
IRES
DC maturation protein
WRE
ΔU3
R
U5
FUOM
(b)
CMV Enhancer
R
U5
HIV-1 Flap
Ubi-C promoter
gp120
VSV TM
IRES
DC mature
WRE
ΔU3
R
U5
FUGmID
(c)
CMV Enhancer
R
U5
HIV-1 Flap
H1 promoter
siRNA mSOCS1
Ubi-C promoter
OVA cDNA
WRE
ΔU3
R
U5
FOVA-siRNA
(d)
CMV Enhancer
R
U5
HIV-1 Flap
Ubi-C promoter
OVA cDNA
miRNA miR146
WRE
ΔU3
R
U5
FOVA-miRNA

Figure 24

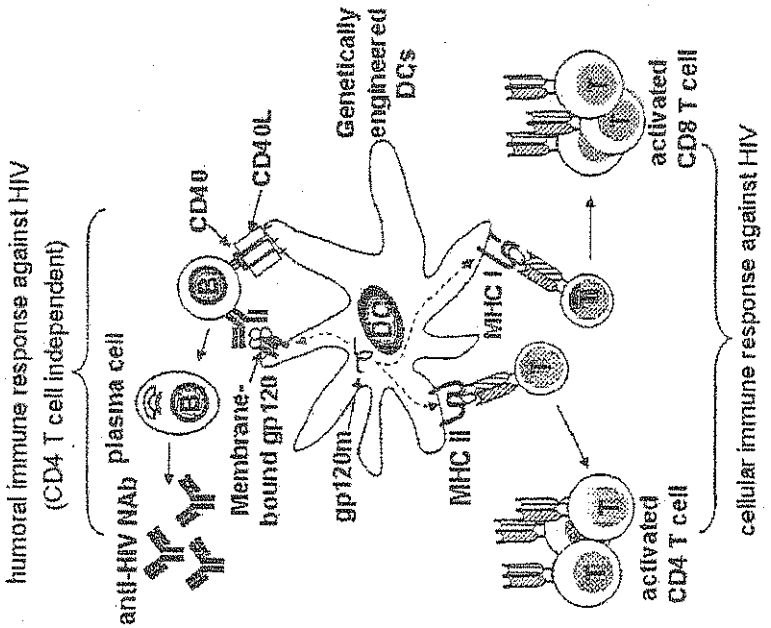

humoral immune response against HIV
(CD4 T cell independent)
anti-HIV NAb
plasma cell
CD40
CD40L
Membrane-bound gp120
gp120m
Genetically engineered DCs
MHC II
MHC I
activated CD4 T cell
activated CD8 T cell
cellular immune response against HIV

Figure 25