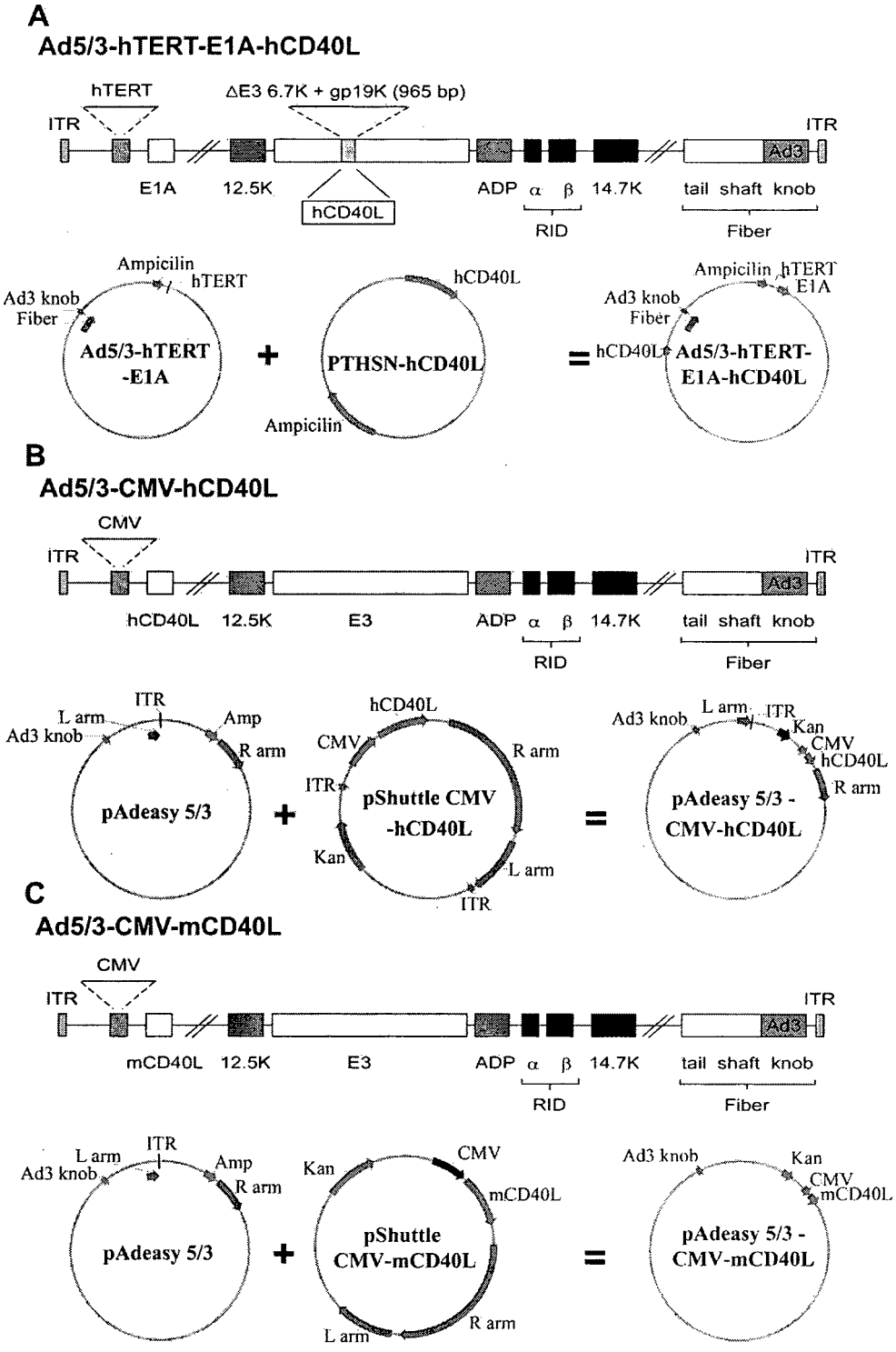

A
Ad5/3-hTERT-E1A-hCD40L
hTERT
ΔE3 6.7K + gp19K (965 bp)
ITR
ITR
Ad3
E1A
12.5K
hCD40L
ADP
α
β
14.7K
tail shaft knob
RID
Fiber
Ampicilin
hTERT
Ad3 knob
Fiber
Ad5/3-hTERT
-E1A
hCD40L
PTHSN-hCD40L
Ampicilin
Ampicilin
hTERT
E1A
Ad3 knob
Fiber
hCD40L
Ad5/3-hTERT-
E1A-hCD40L
B
Ad5/3-CMV-hCD40L
CMV
ITR
ITR
Ad3
hCD40L
12.5K
E3
ADP
α
β
14.7K
tail shaft knob
RID
Fiber
ITR
L arm
Amp
Ad3 knob
R arm
pAdeasy 5/3
hCD40L
CMV
R arm
ITR
pShuttle CMV
-hCD40L
Kan
L arm
ITR
Ad3 knob
L arm
ITR
Kan
CMV
hCD40L
R arm
pAdeasy 5/3 -
CMV-hCD40L
C
Ad5/3-CMV-mCD40L
CMV
ITR
ITR
Ad3
mCD40L
12.5K
E3
ADP
α
β
14.7K
tail shaft knob
RID
Fiber
ITR
L arm
Amp
Ad3 knob
R arm
pAdeasy 5/3
Kan
CMV
mCD40L
pShuttle
CMV-mCD40L
L arm
R arm
Ad3 knob
Kan
CMV
mCD40L
pAdeasy 5/3 -
CMV-mCD40L

Figure 1

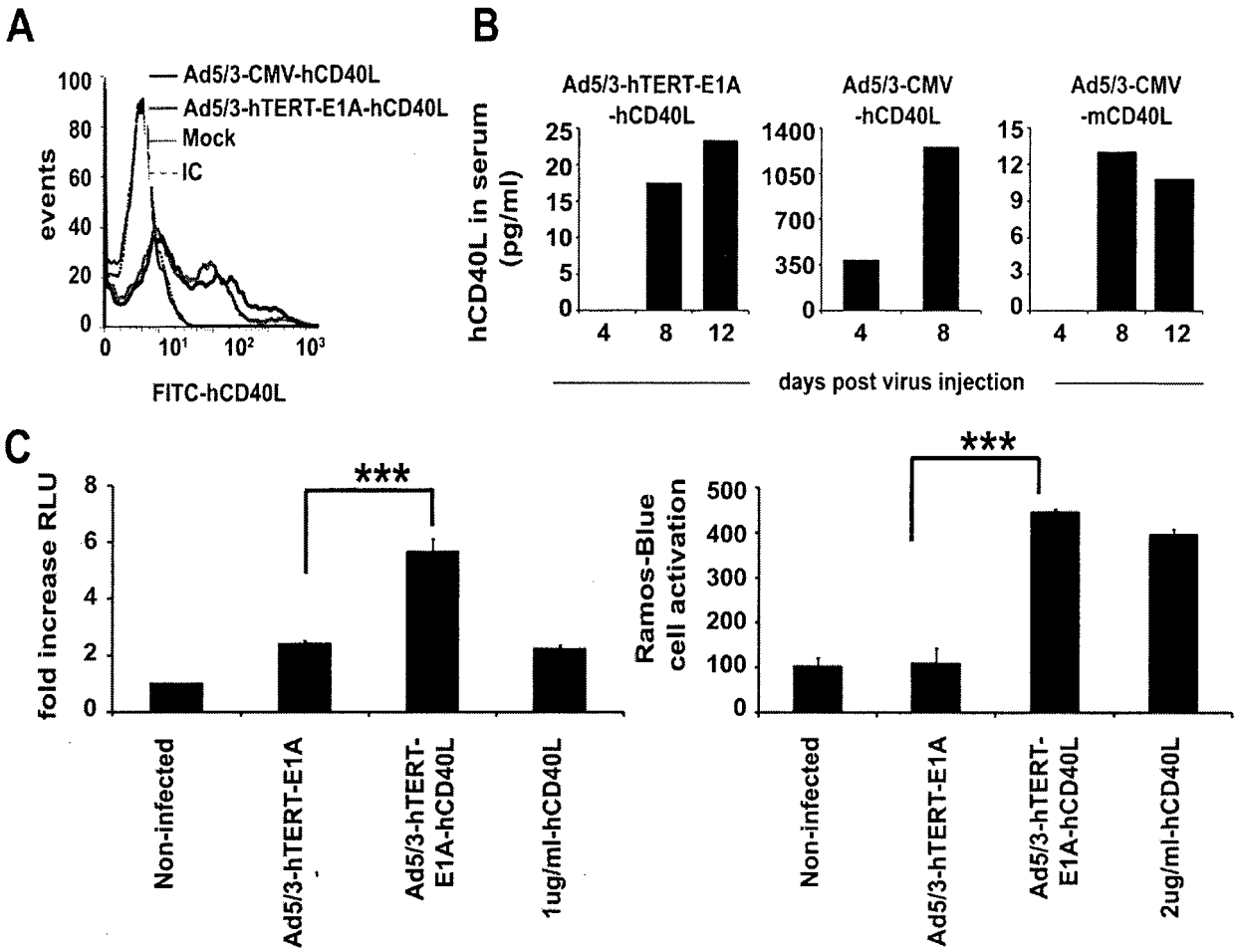

A
Ad5/3-CMV-hCD40L
Ad5/3-hTERT-E1A-hCD40L
Mock
IC
events
FITC-hCD40L
B
Ad5/3-hTERT-E1A -hCD40L
Ad5/3-CMV -hCD40L
Ad5/3-CMV -mCD40L
hCD40L in serum (pg/ml)
days post virus injection
C
fold increase RLU
***
Non-infected
Ad5/3-hTERT-E1A
Ad5/3-hTERT-E1A-hCD40L
1ug/ml-hCD40L
Ramos-Blue cell activation
***
Non-infected
Ad5/3-hTERT-E1A
Ad5/3-hTERT-E1A-hCD40L
2ug/ml-hCD40L

Figure 2

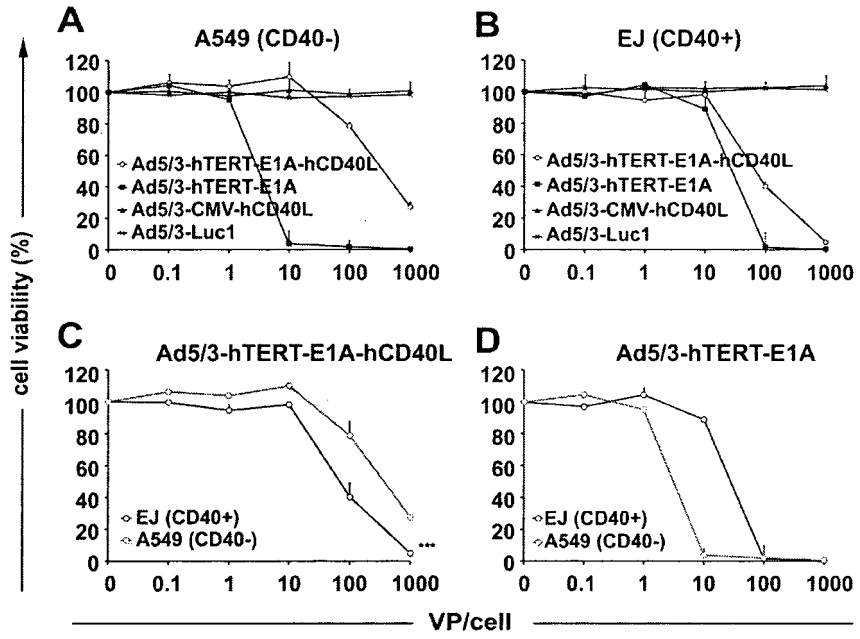


Figure 3

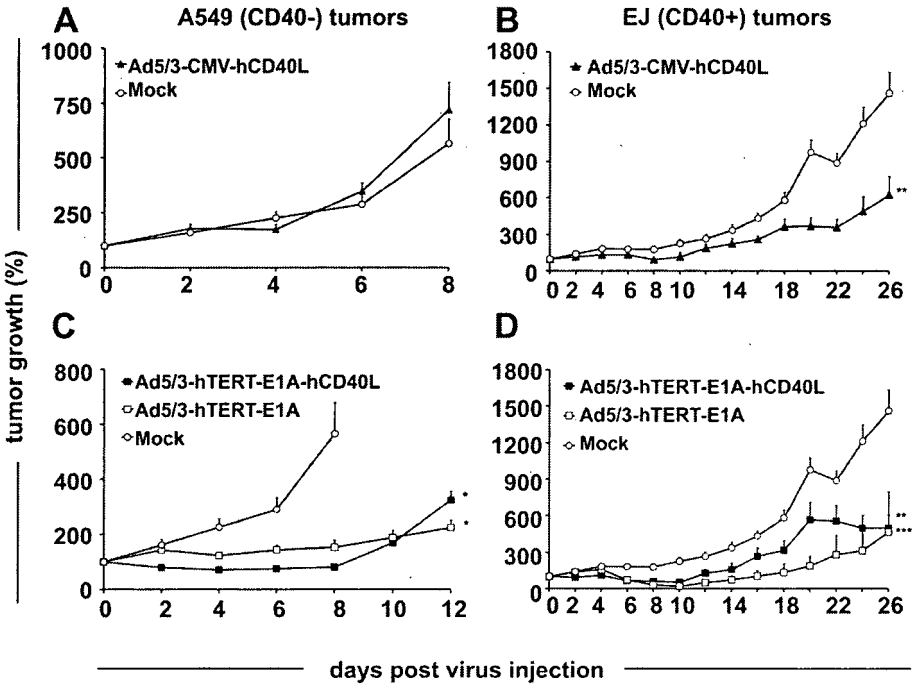


Figure 4

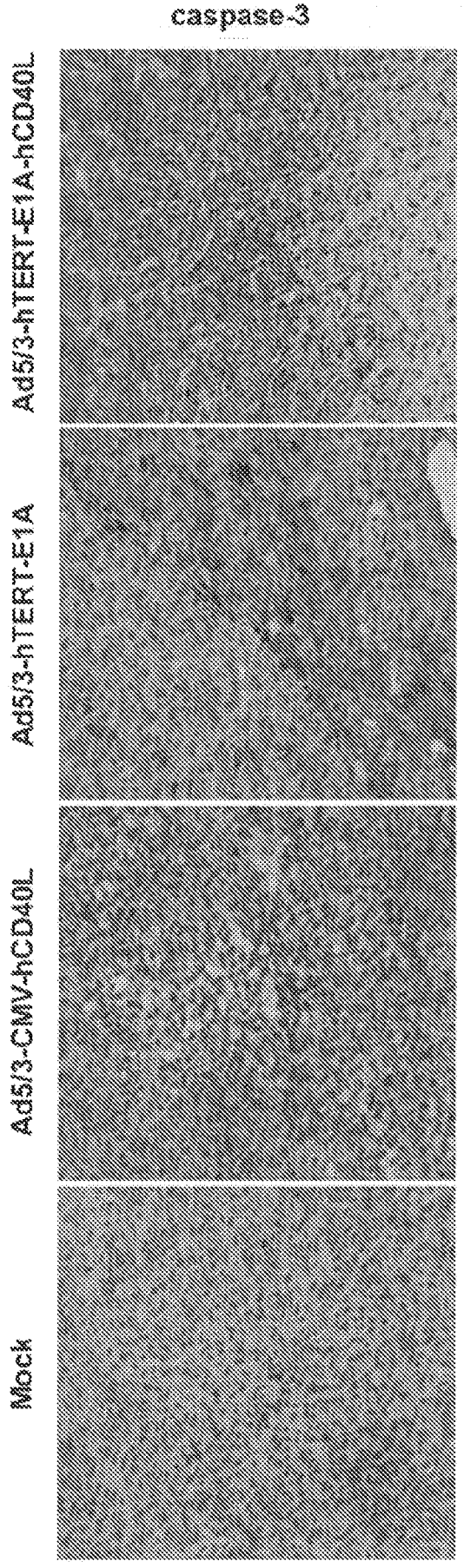

caspase-3
Ad5/3-hTERT-E1A-hCD40L
Ad5/3-hTERT-E1A
Ad5/3-CMV-hCD40L
Mock

Figure 5

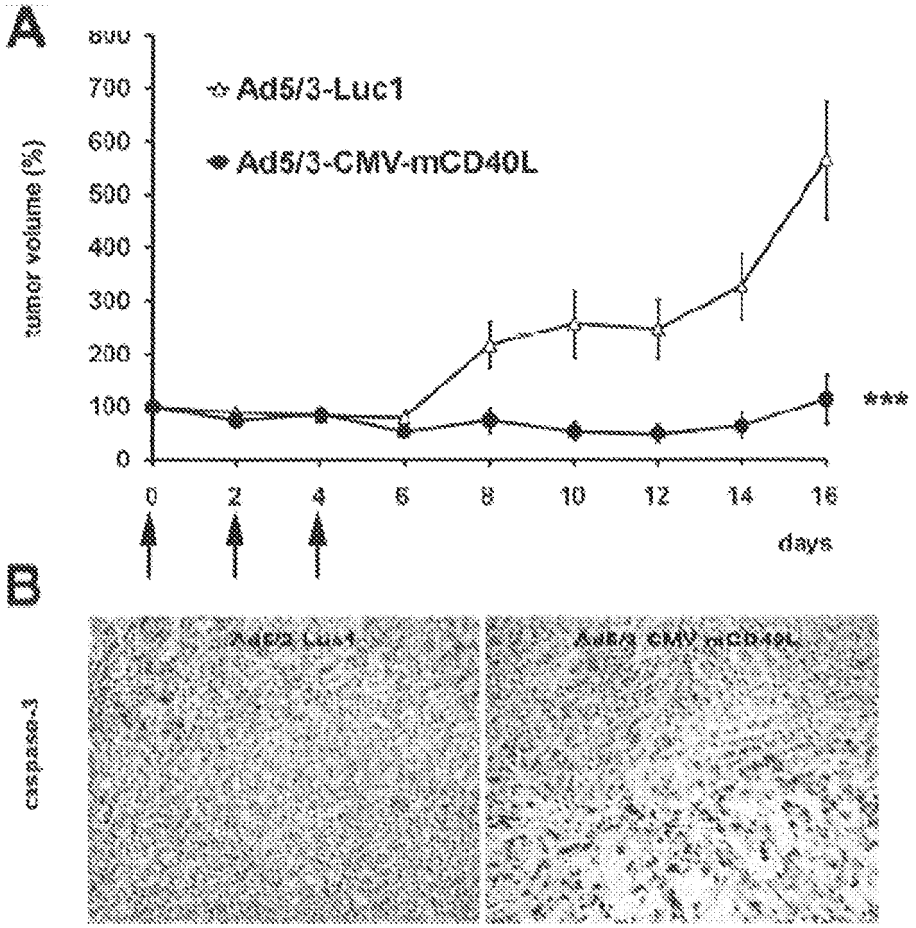

A
tumor volume (%)
800
700
600
500
400
300
200
100
0
Ad5/3-Luc1
Ad5/3-CMV-mCD40L
***
0
2
4
6
8
10
12
14
16
days
B
caspase-3
Ad5/3 Luc1
Ad5/3 CMV-mCD40L

Figure 6

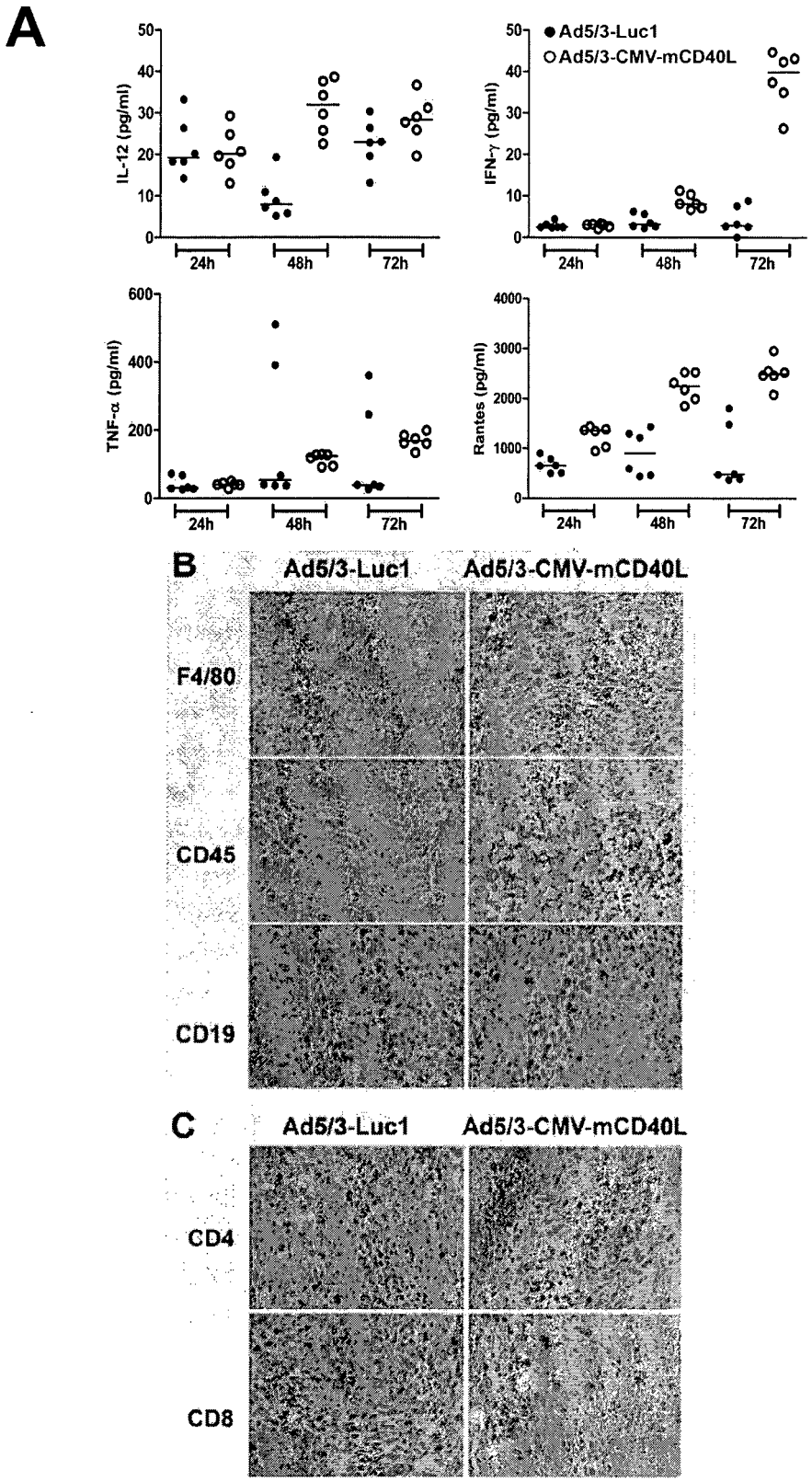

A
IL-12 (pg/ml)
IFN-γ (pg/ml)
TNF-α (pg/ml)
Rantes (pg/ml)
Ad5/3-Luc1
Ad5/3-CMV-mCD40L
24h
48h
72h
B
Ad5/3-Luc1
Ad5/3-CMV-mCD40L
F4/80
CD45
CD19
C
Ad5/3-Luc1
Ad5/3-CMV-mCD40L
CD4
CD8

Figure 7

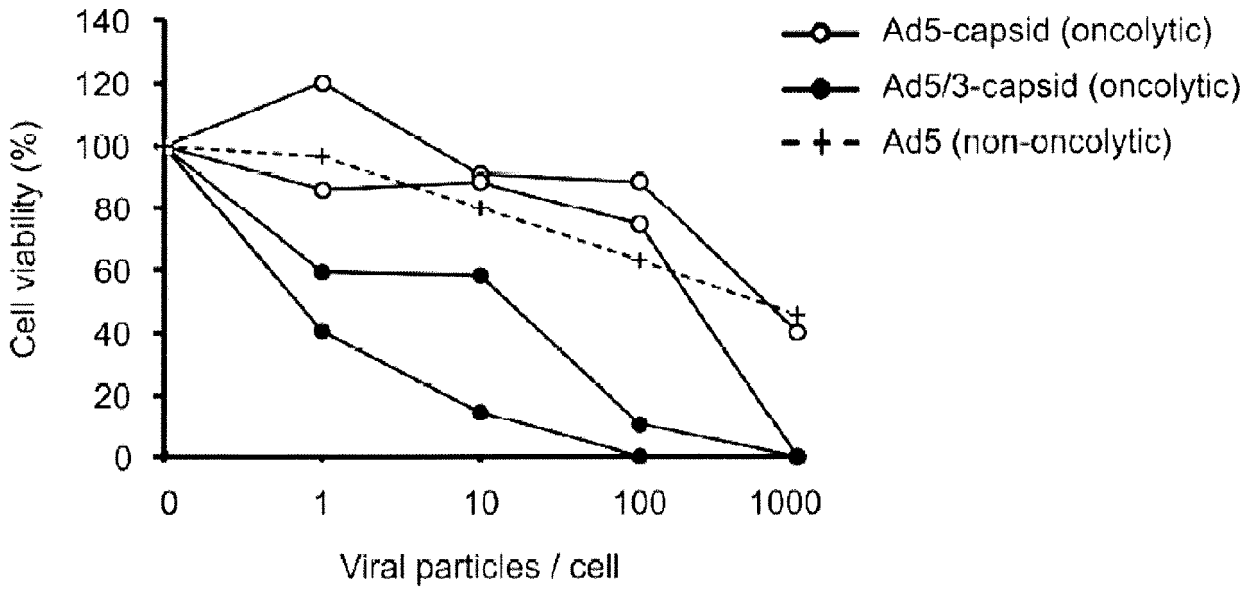


Figure 8

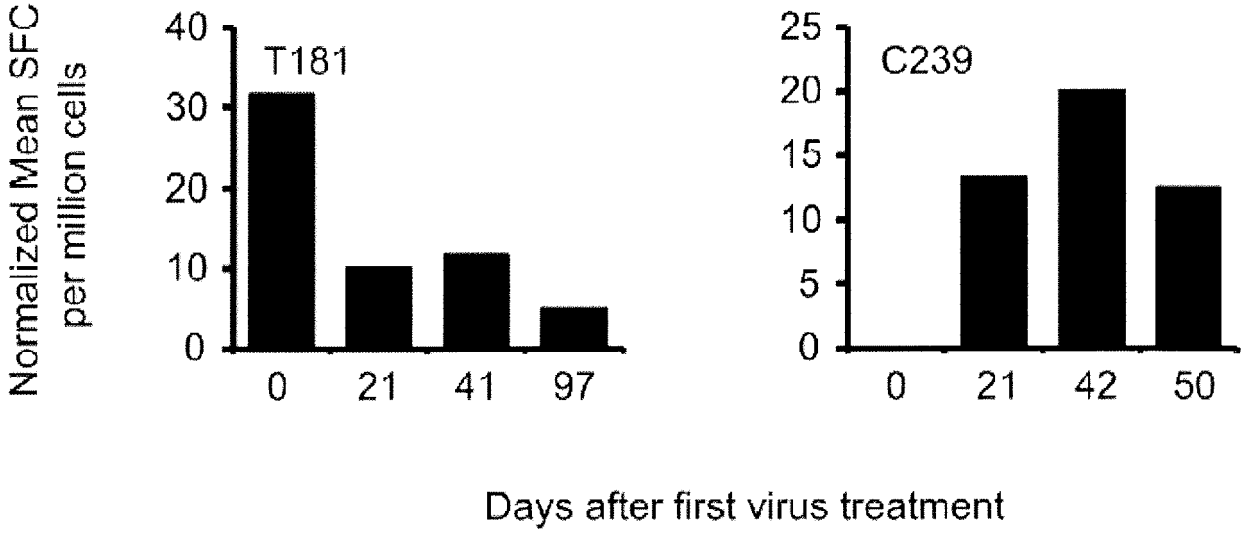


Figure 9

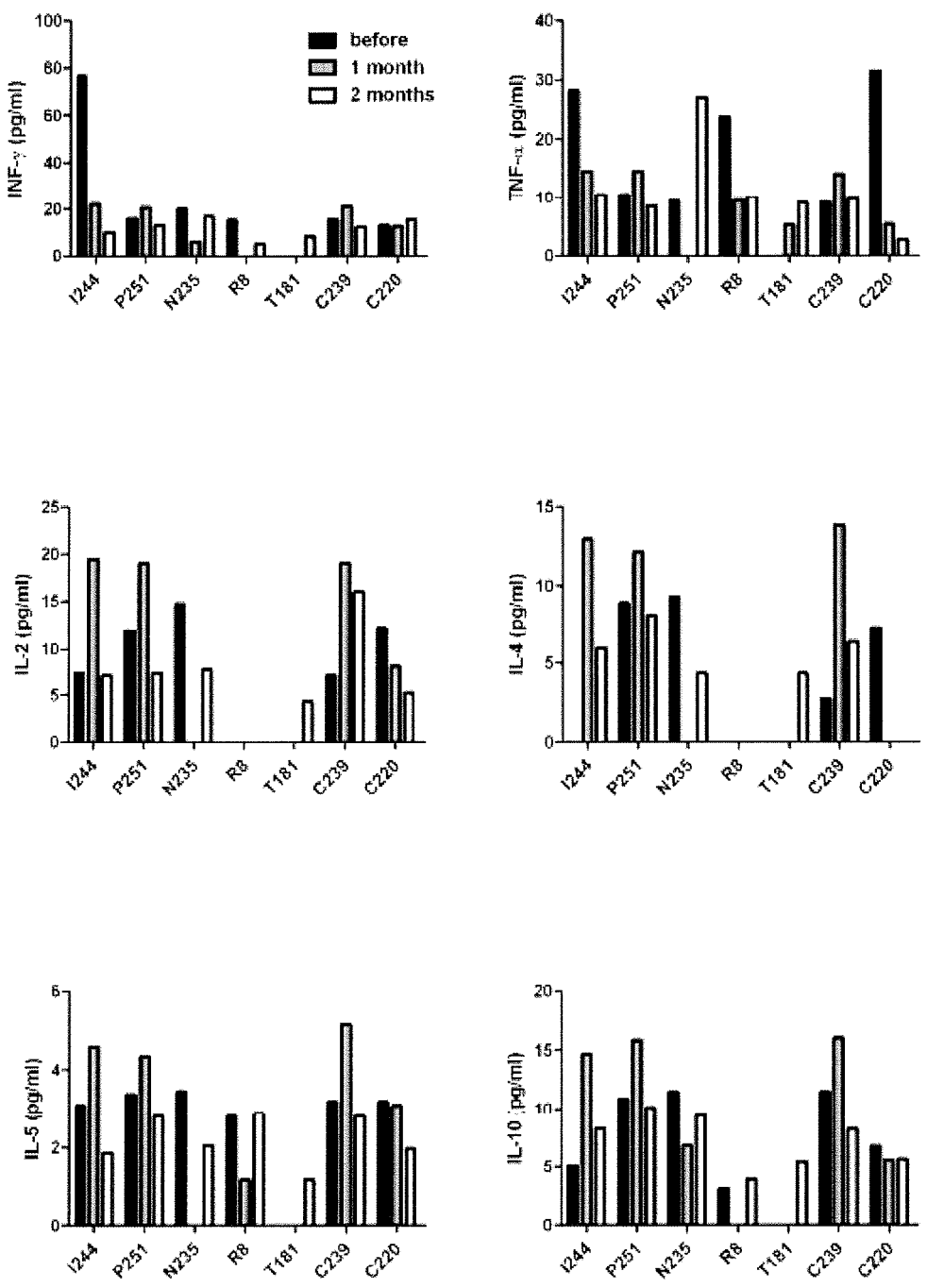

before
1 month
2 months
INF-γ (pg/ml)
TNF-α (pg/ml)
IL-2 (pg/ml)
IL-4 (pg/ml)
IL-5 (pg/ml)
IL-10 (pg/ml)
I244
P251
N235
R8
T181
C239
C220

Figure 10

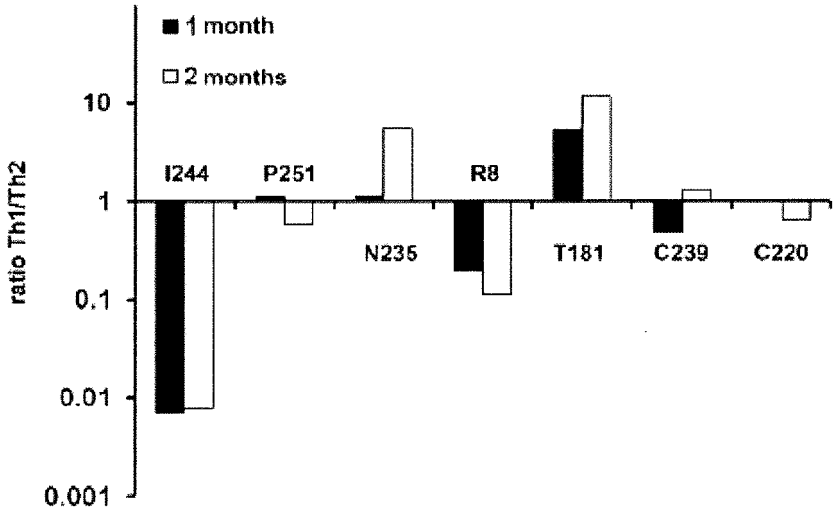

1 month
2 months
ratio Th1/Th2
10
1
0.1
0.01
0.001
I244
P251
N235
R8
T181
C239
C220

Figure 11

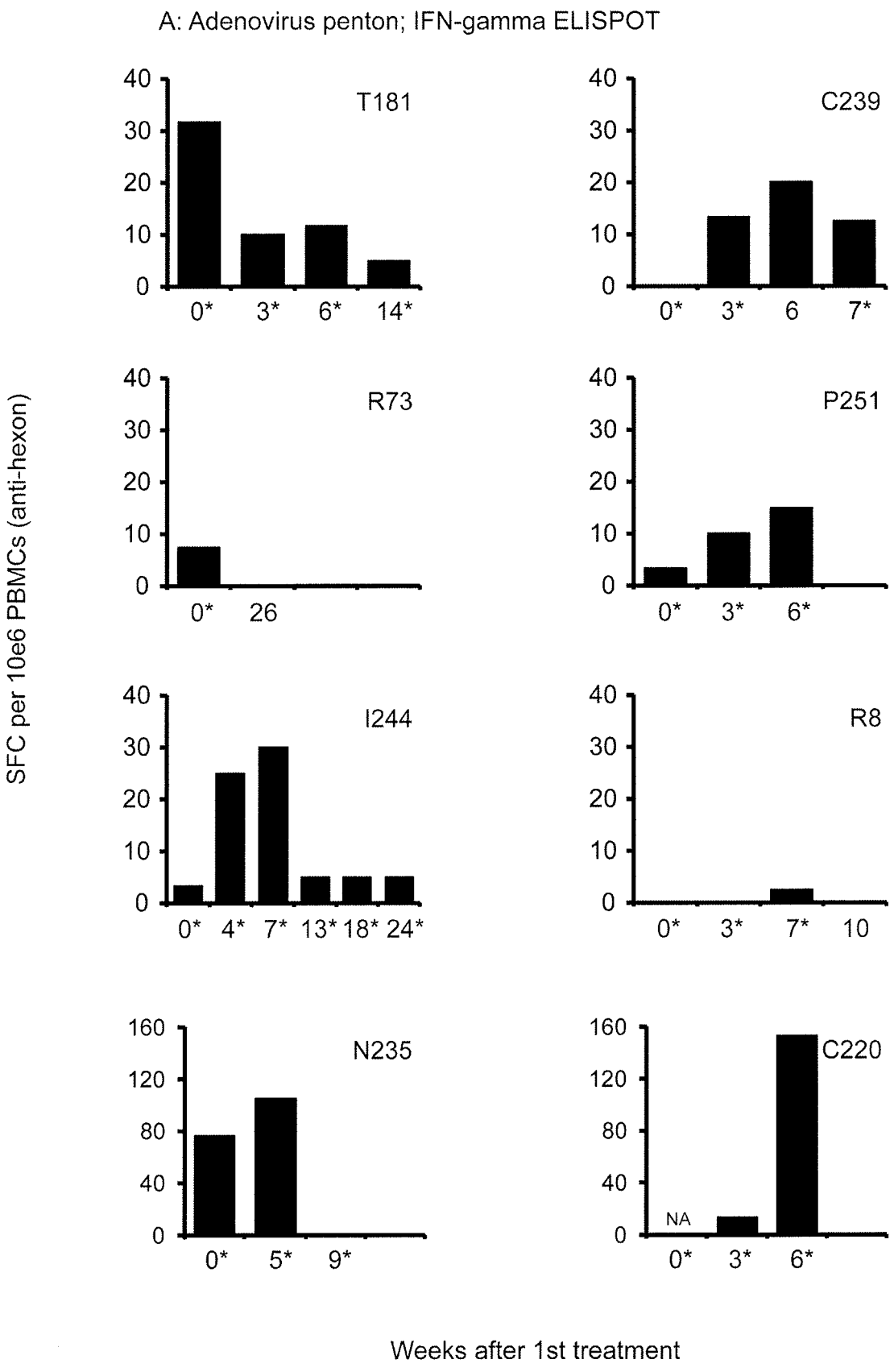

A: Adenovirus penton; IFN-gamma ELISPOT
SFC per 10e6 PBMCs (anti-hexon)
T181
0* 3* 6* 14*
C239
0* 3* 6 7*
R73
0* 26
P251
0* 3* 6*
I244
0* 4* 7* 13* 18* 24*
R8
0* 3* 7* 10
N235
0* 5* 9*
C220
NA
0* 3* 6*
Weeks after 1st treatment

Figure 12

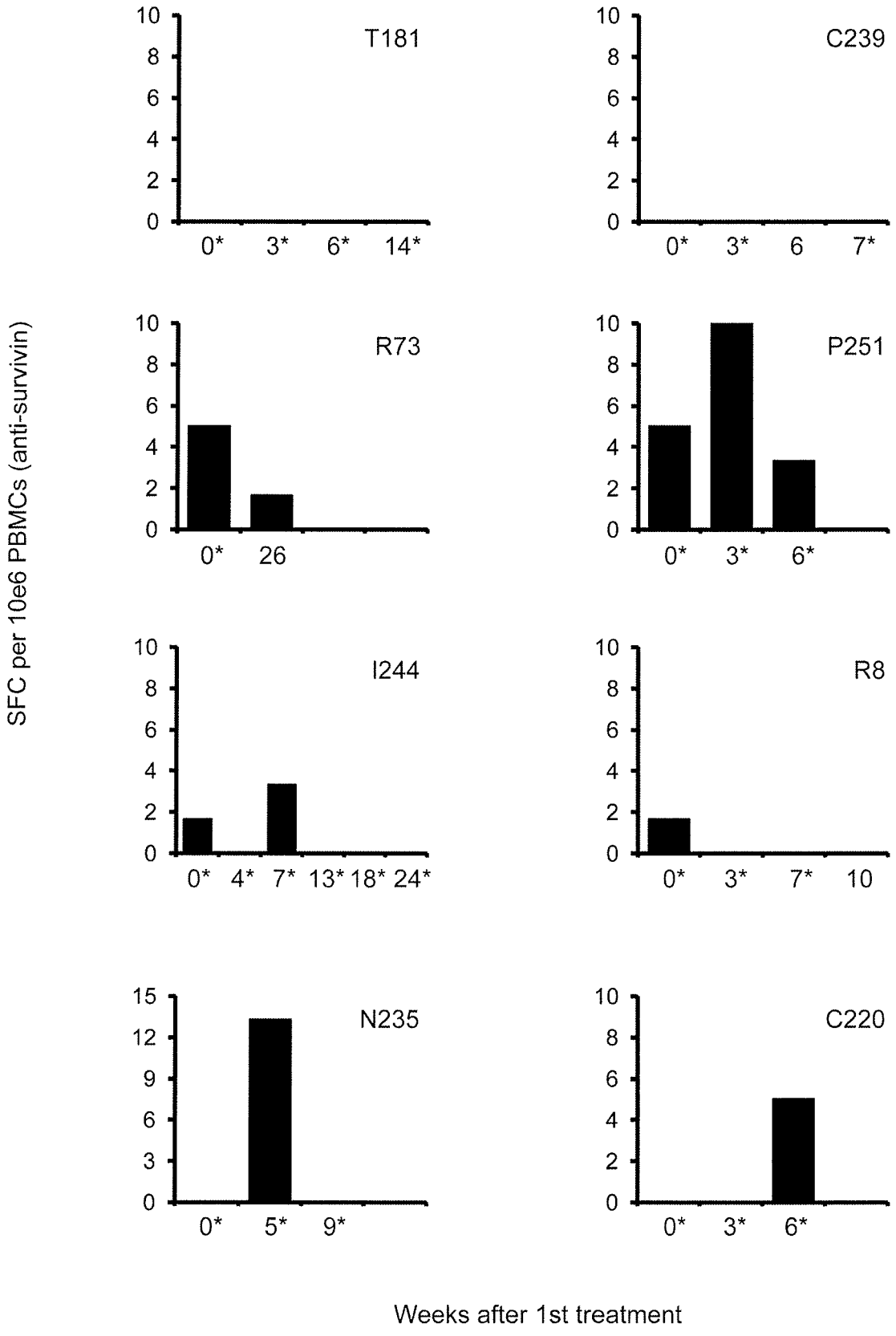
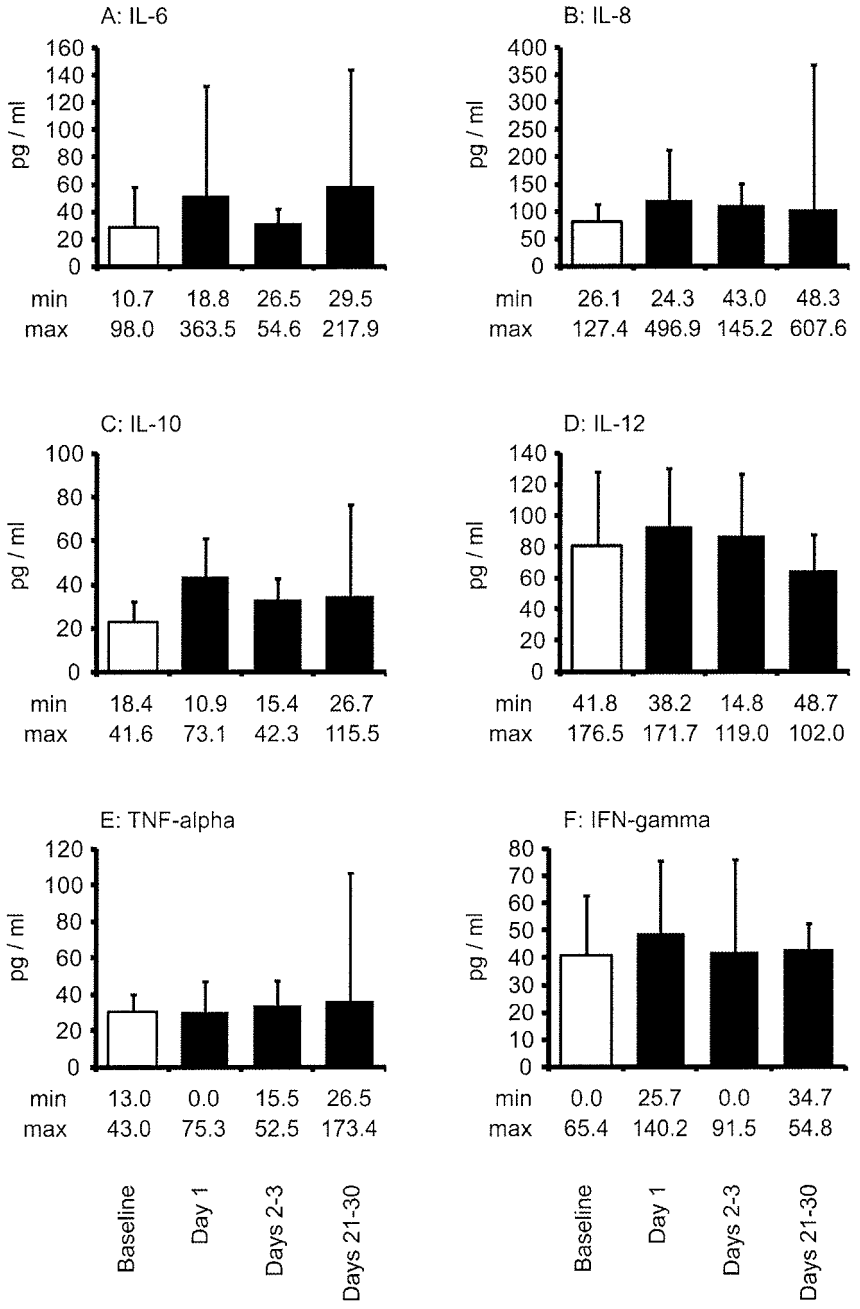
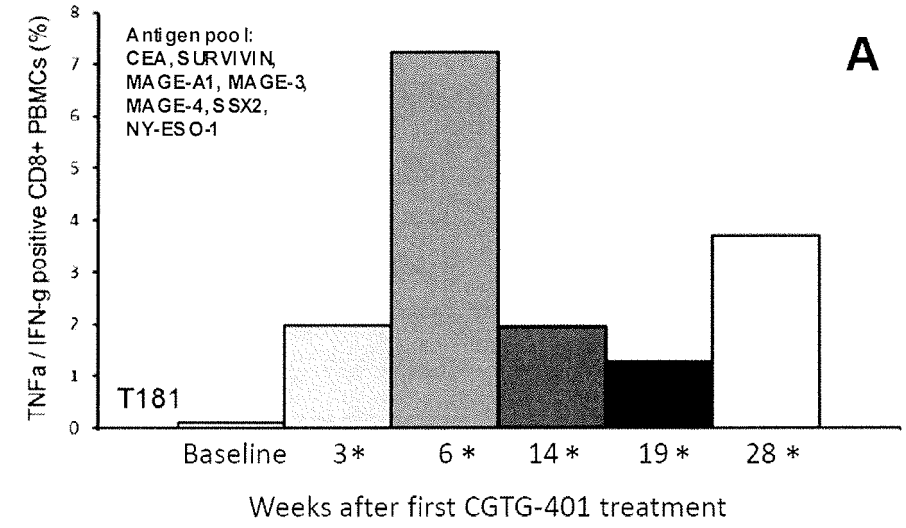

T181
C239
R73
P251
I244
R8
N235
C220
SFC per 10e6 PBMCs (anti-survivin)
Weeks after 1st treatment

Figure 12B



Supplementary Figure 2. Pesonen et al.

Figure 13


A
Antigen pool:
CEA, SURVIVIN,
MAGE-A1, MAGE-3,
MAGE-4, SSX2,
NY-ESO-1
TNFa / IFN-g positive CD8+ PBMCs (%)
T181
Baseline
3*
6 *
14 *
19 *
28 *
Weeks after first CGTG-401 treatment

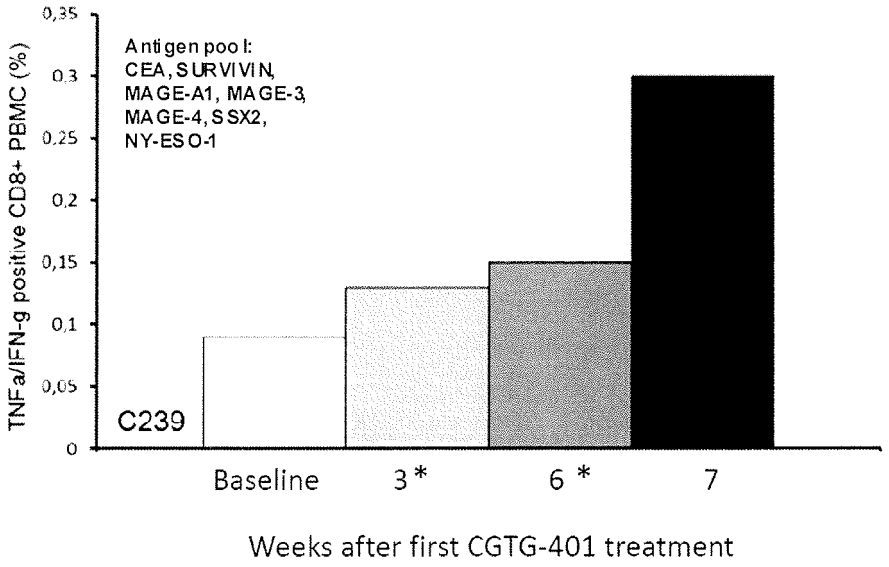
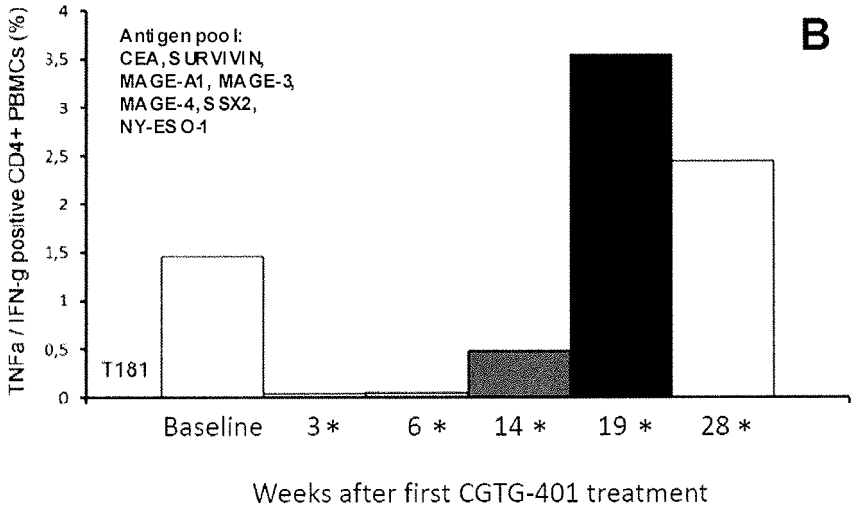

Antigen pool:
CEA, SURVIVIN,
MAGE-A1, MAGE-3,
MAGE-4, SSX2,
NY-ESO-1
TNFa/IFN-g positive CD8+ PBMC (%)
C239
Baseline
3 *
6 *
7
Weeks after first CGTG-401 treatment

Figure 14


B
Antigen pool:
CEA, SURVIVIN,
MAGE-A1, MAGE-3,
MAGE-4, SSX2,
NY-ESO-1
T181
TNFa / IFN-g positive CD4+ PBMCs (%)
4
3,5
3
2,5
2
1,5
1
0,5
0
Baseline
3 *
6 *
14 *
19 *
28 *
Weeks after first CGTG-401 treatment

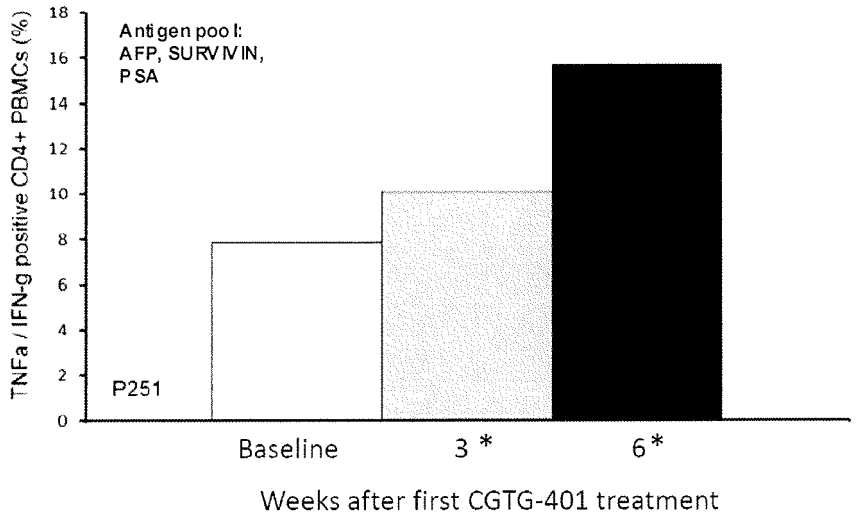

Antigen pool:
AFP, SURVIVIN,
PSA
P251
TNFa / IFN-g positive CD4+ PBMCs (%)
18
16
14
12
10
8
6
4
2
0
Baseline
3 *
6*
Weeks after first CGTG-401 treatment

Figure 14B

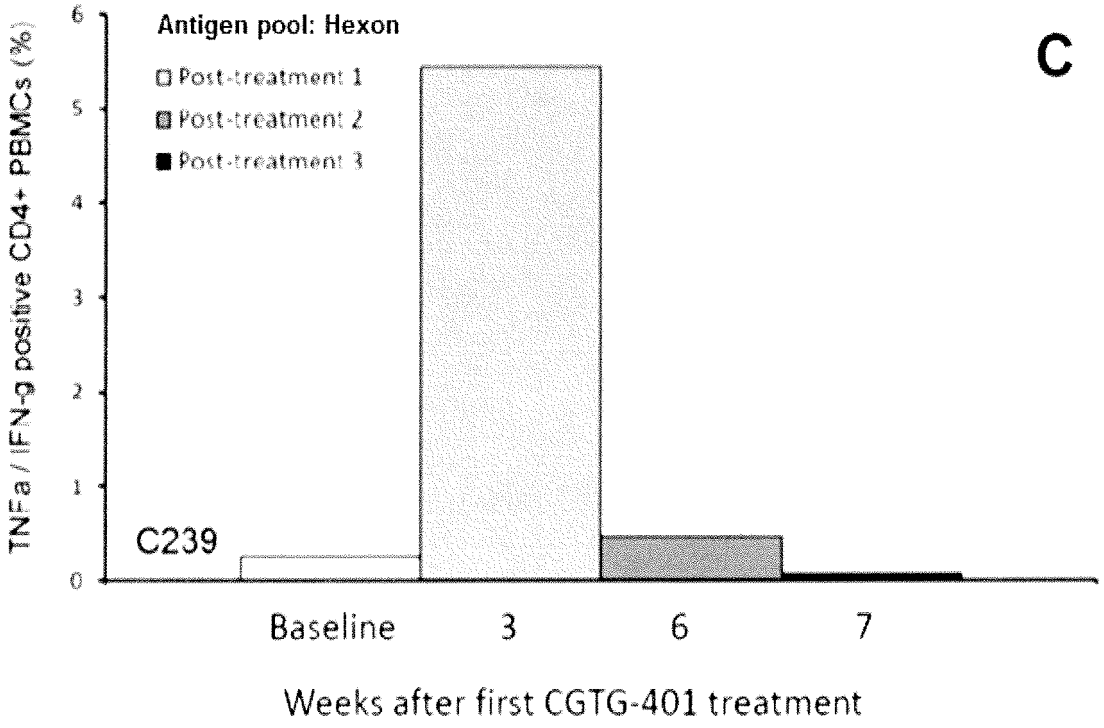

Antigen pool: Hexon
C
Post-treatment 1
Post-treatment 2
Post-treatment 3
TNFa / IFN-g positive CD4+ PBMCs (%)
0
1
2
3
4
5
6
C239
Baseline
3
6
7
Weeks after first CGTG-401 treatment

Figure 14C

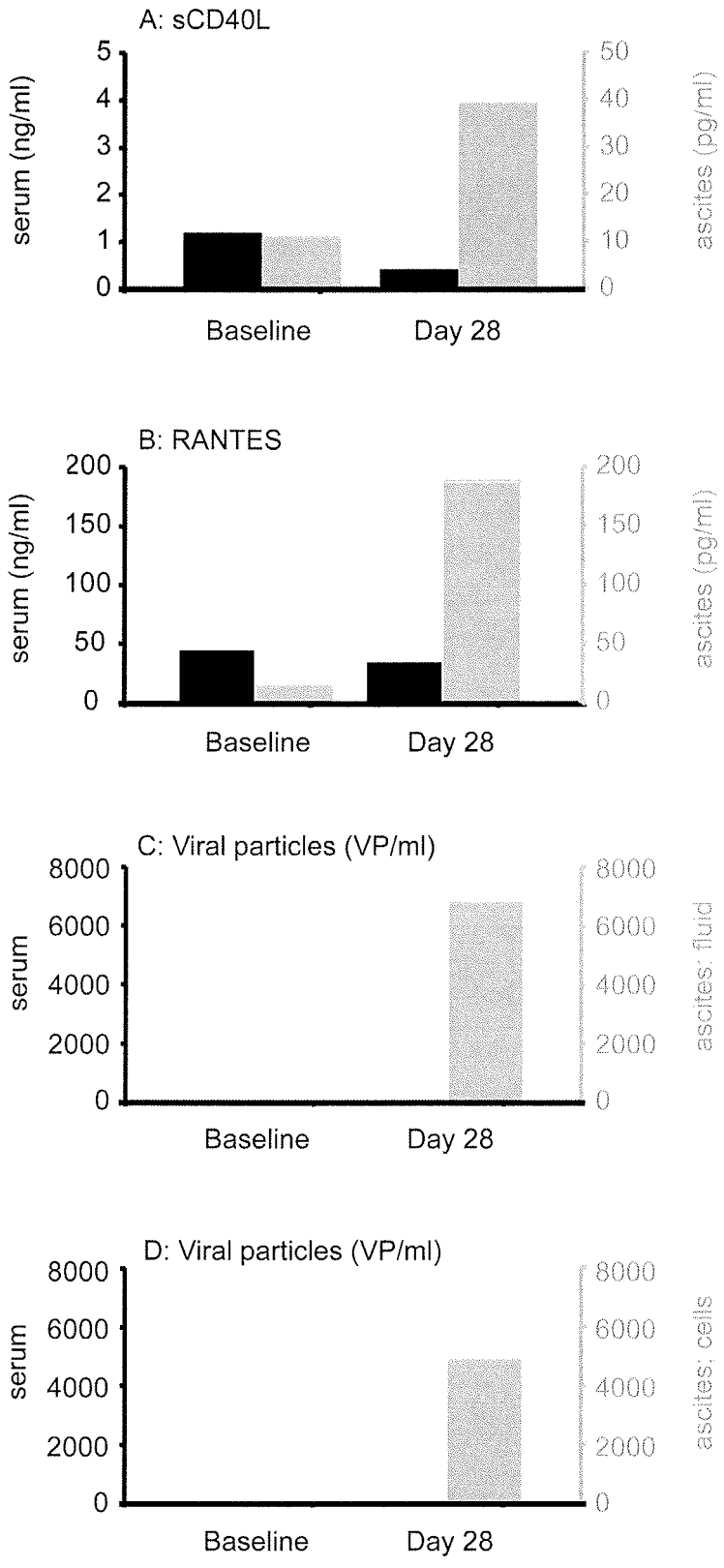

A: sCD40L
serum (ng/ml)
ascites (pg/ml)
5
4
3
2
1
0
50
40
30
20
10
0
Baseline
Day 28
B: RANTES
serum (ng/ml)
ascites (pg/ml)
200
150
100
50
0
200
150
100
50
0
Baseline
Day 28
C: Viral particles (VP/ml)
serum
ascites: fluid
8000
6000
4000
2000
0
8000
6000
4000
2000
0
Baseline
Day 28
D: Viral particles (VP/ml)
serum
ascites: cells
8000
6000
4000
2000
0
8000
6000
4000
2000
0
Baseline
Day 28

Figure 15

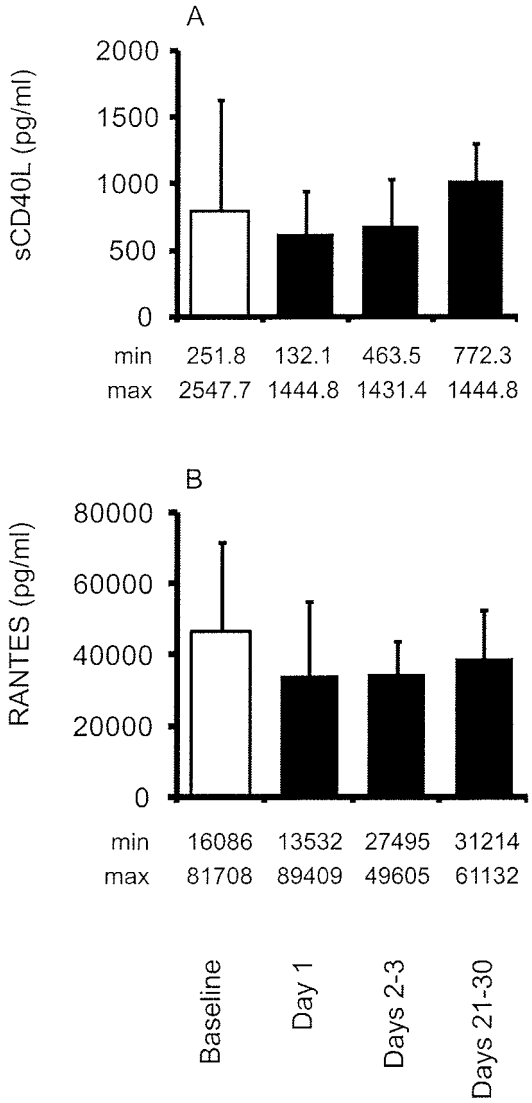

A
sCD40L (pg/ml)
2000
1500
1000
500
0
min 251.8 132.1 463.5 772.3
max 2547.7 1444.8 1431.4 1444.8
B
RANTES (pg/ml)
80000
60000
40000
20000
0
min 16086 13532 27495 31214
max 81708 89409 49605 61132
Baseline
Day 1
Days 2-3
Days 21-30

Figure 16

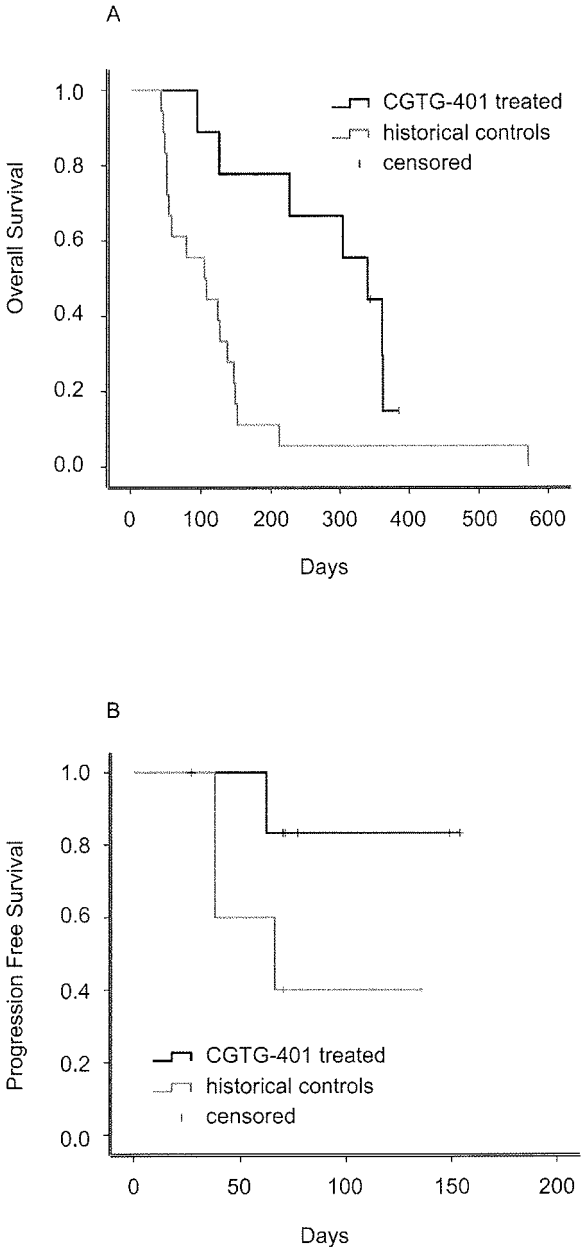

A
Overall Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
500
600
Days
CGTG-401 treated
historical controls
censored
B
Progression Free Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
Days
CGTG-401 treated
historical controls
censored

Figure 17