Figure 1

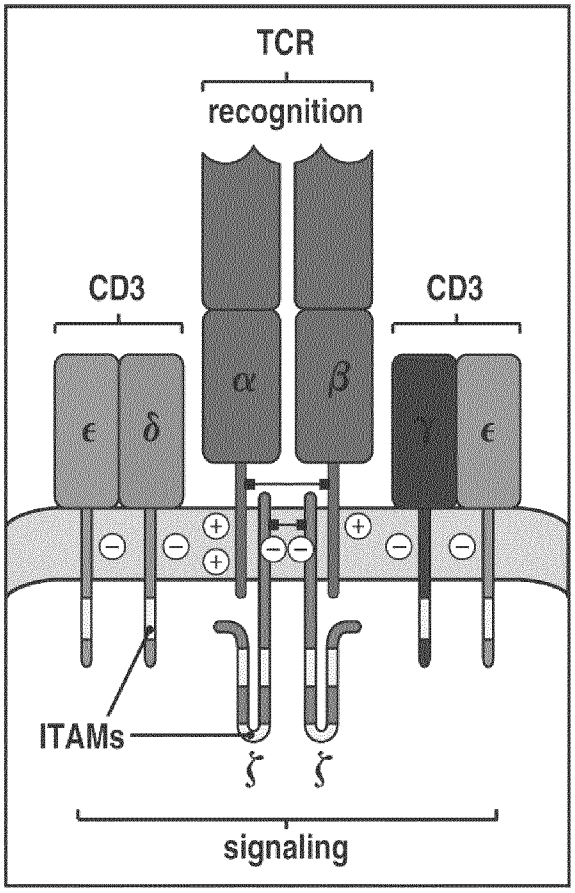


Figure 6-9 Immunobiology, 6/e. (© Garland Science 2005)

Figure 2A

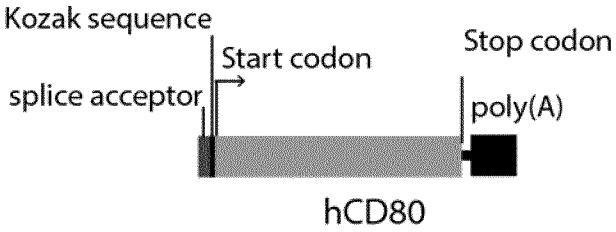


Figure 2B

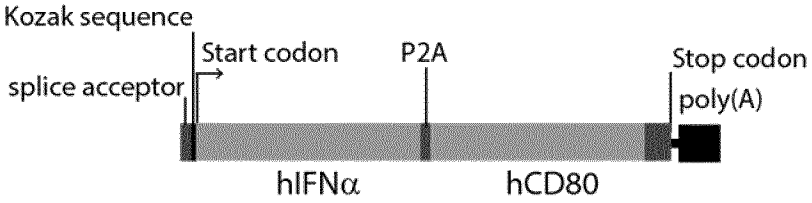


Figure 2C

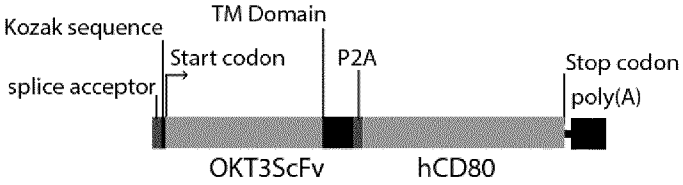


Figure 2D

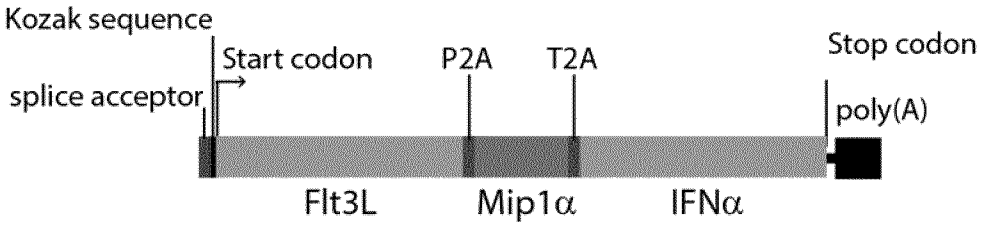


Figure 2E

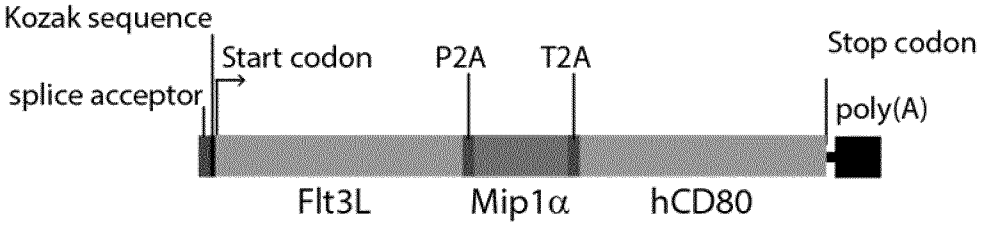


Figure 2F

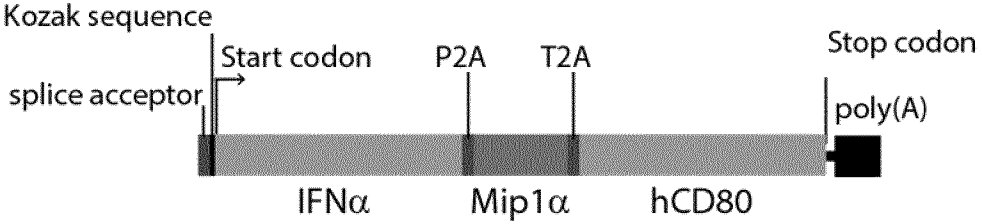


Figure 2G **ORF cassette for scFv antibody**

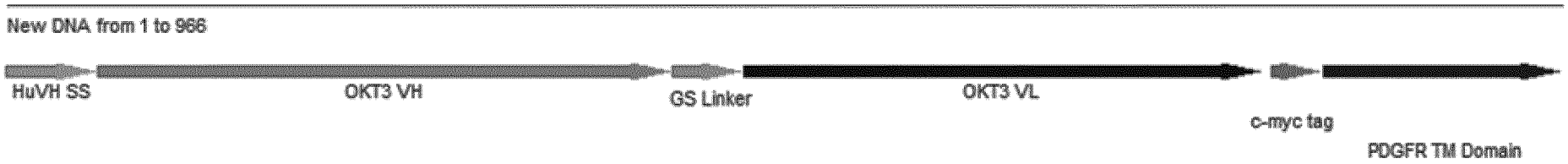


Figure 3

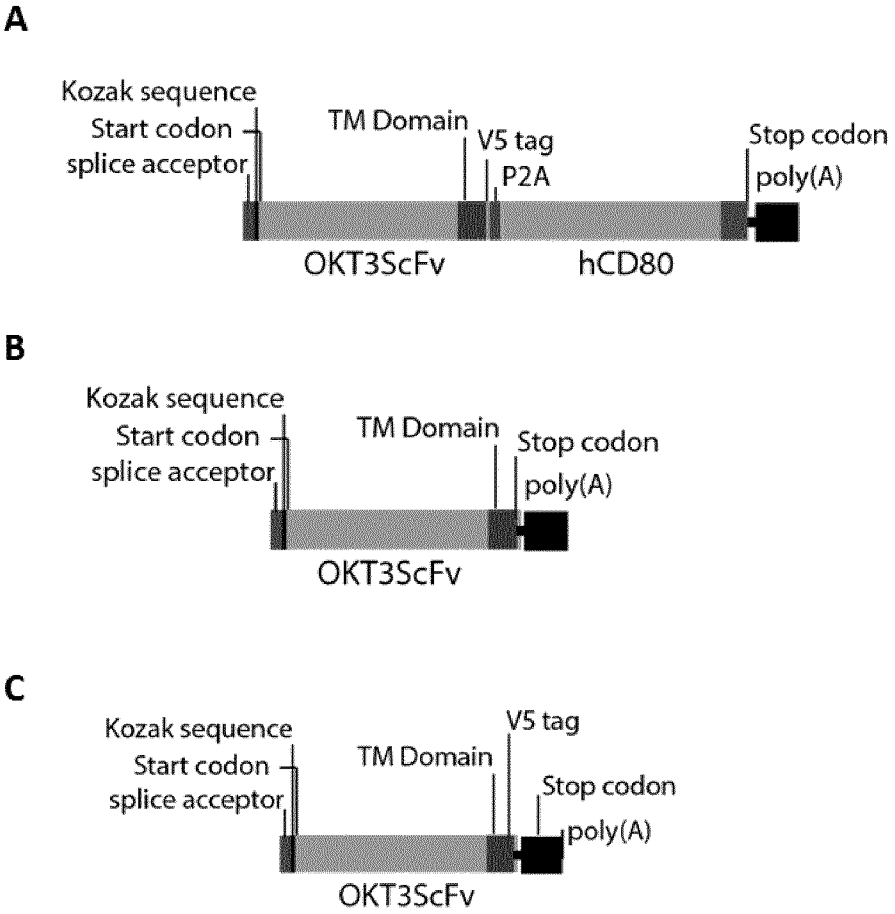

A
Kozak sequence
Start codon
splice acceptor
TM Domain
V5 tag
P2A
Stop codon
poly(A)
OKT3ScFv
hCD80
B
Kozak sequence
Start codon
splice acceptor
TM Domain
Stop codon
poly(A)
OKT3ScFv
C
Kozak sequence
Start codon
splice acceptor
TM Domain
V5 tag
Stop codon
poly(A)
OKT3ScFv

Figure 4A

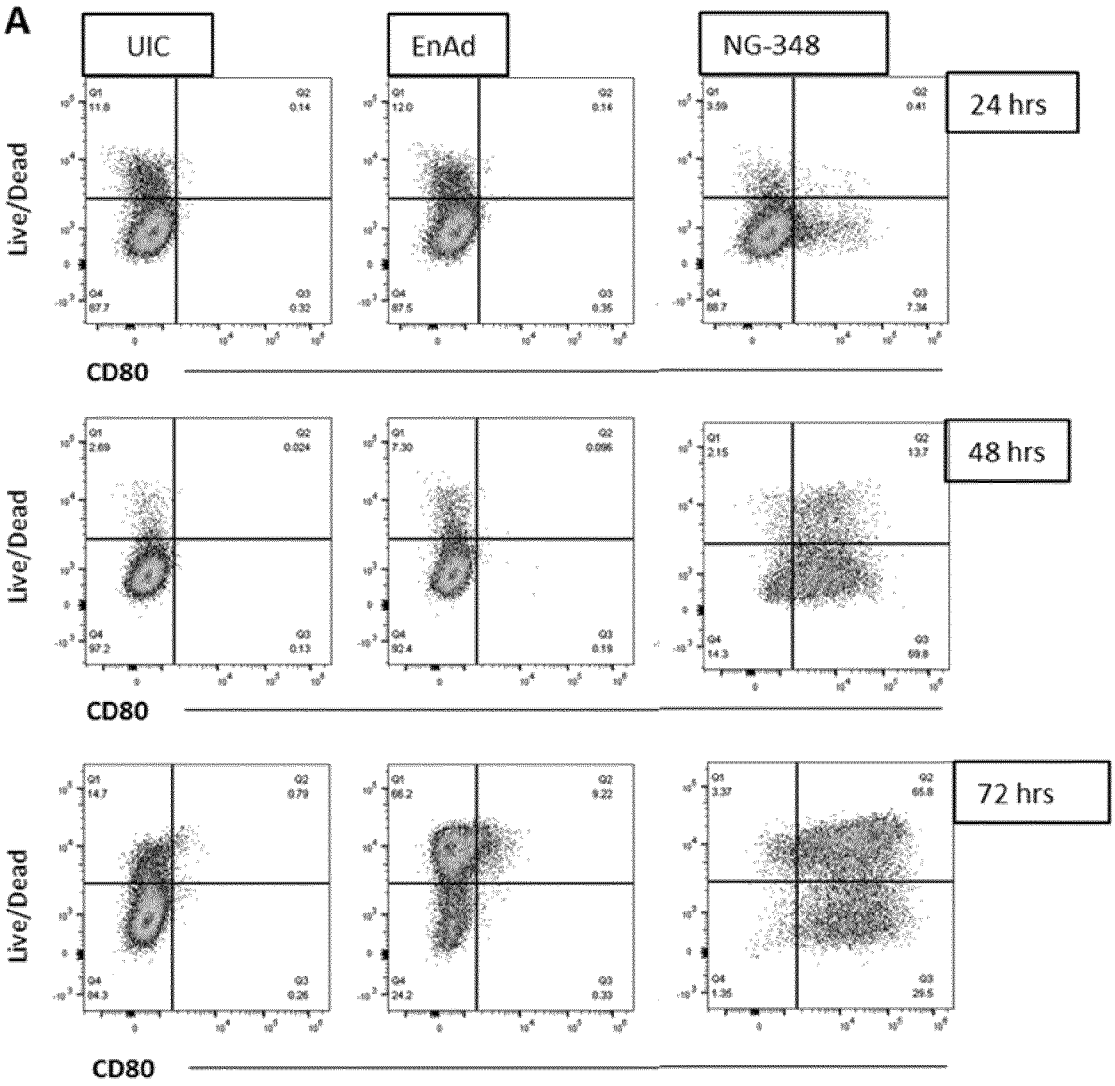


Figure 4B

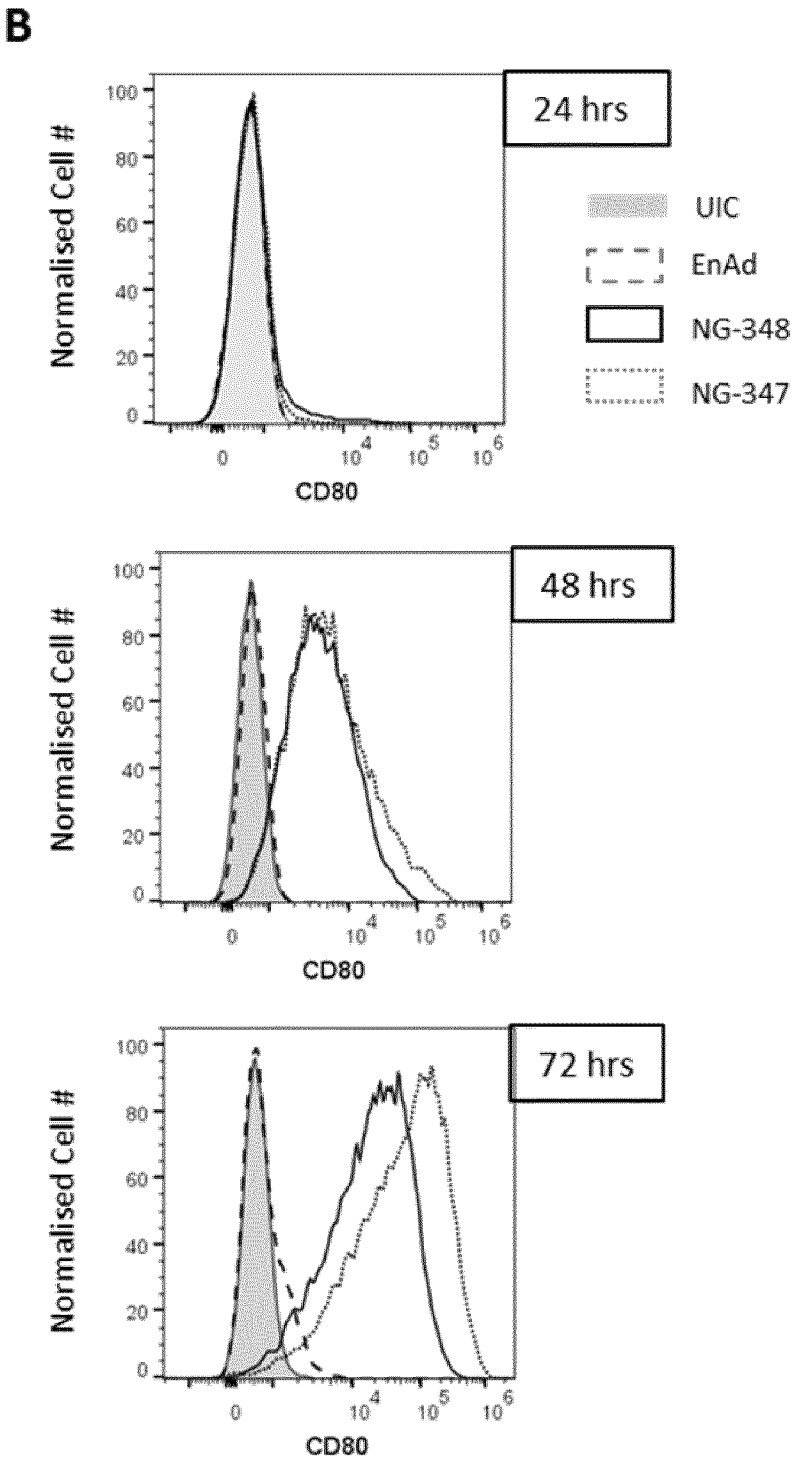

B
24 hrs
48 hrs
72 hrs
Normalised Cell #
CD80
UIC
EnAd
NG-348
NG-347

Figure 5A

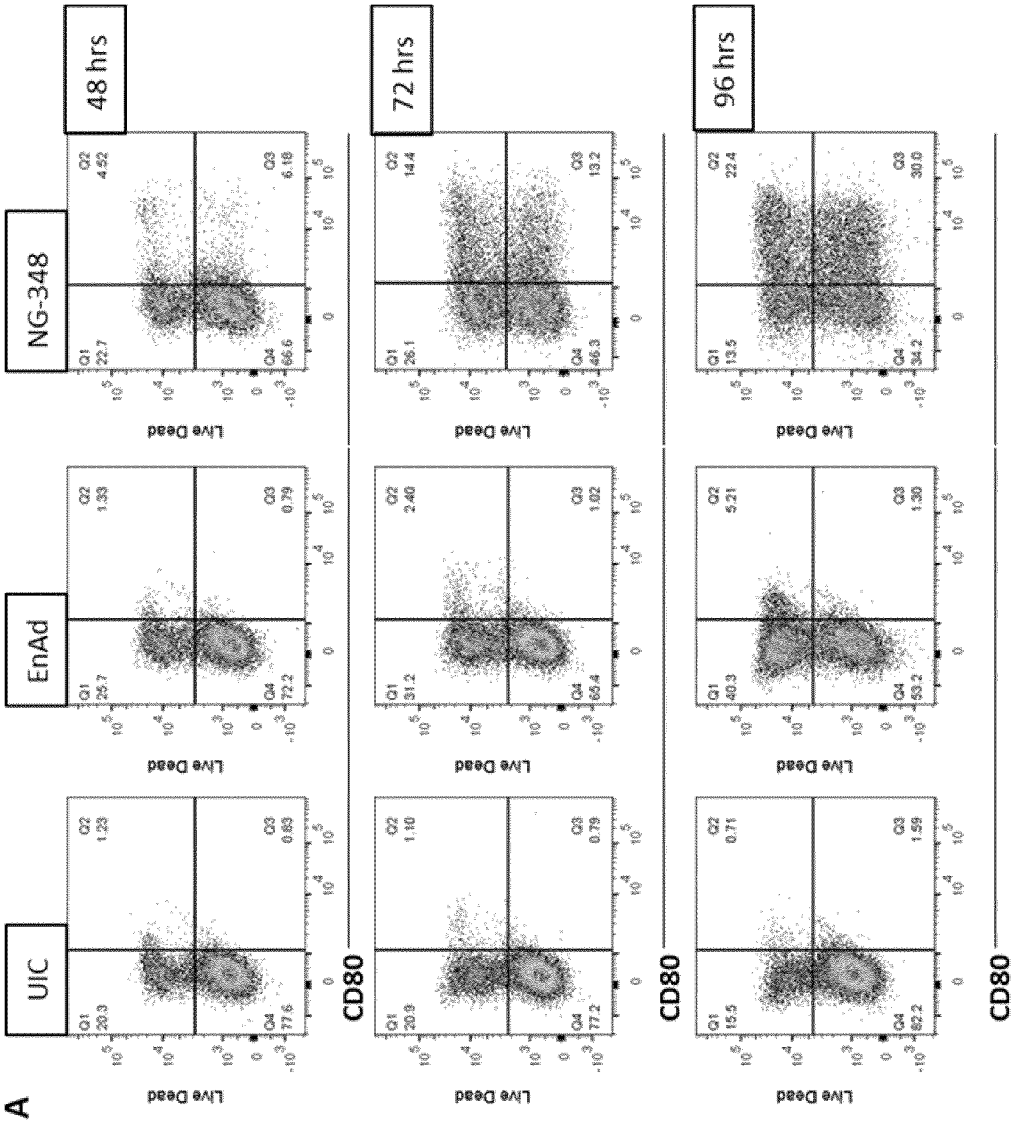

A
UIC
EnAd
NG-348
48 hrs
72 hrs
96 hrs
Live Dead
CD80
Q1 20.3
Q2 1.23
Q3 0.83
Q4 77.6
Q1 20.9
Q2 1.10
Q3 0.79
Q4 77.2
Q1 15.5
Q2 0.71
Q3 1.59
Q4 82.2
Q1 25.7
Q2 1.33
Q3 0.79
Q4 72.2
Q1 31.2
Q2 2.40
Q3 1.02
Q4 65.4
Q1 40.3
Q2 5.21
Q3 1.30
Q4 53.2
Q1 22.7
Q2 4.52
Q3 6.18
Q4 66.6
Q1 26.1
Q2 14.4
Q3 13.2
Q4 46.3
Q1 13.5
Q2 22.4
Q3 30.6
Q4 34.2

Figure 5B

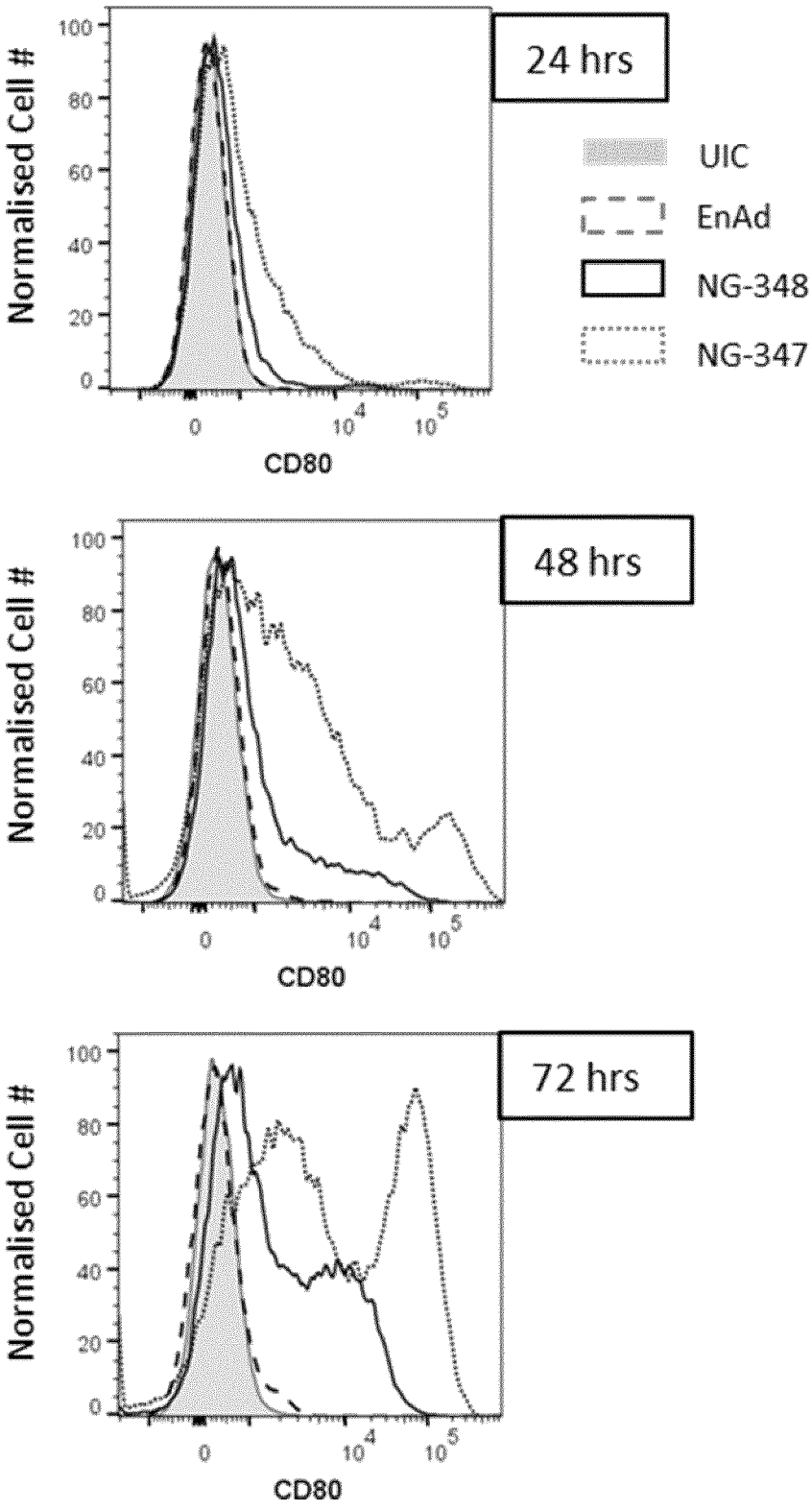

24 hrs
48 hrs
72 hrs
UIC
EnAd
NG-348
NG-347
Normalised Cell #
CD80
0
20
40
60
80
100
10^4
10^5

Figure 6

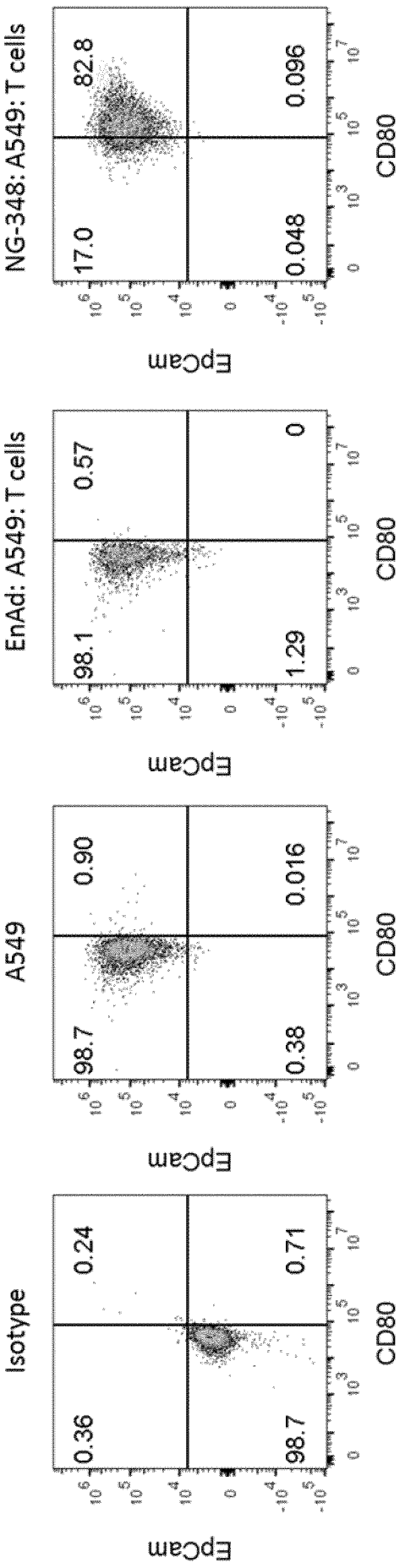
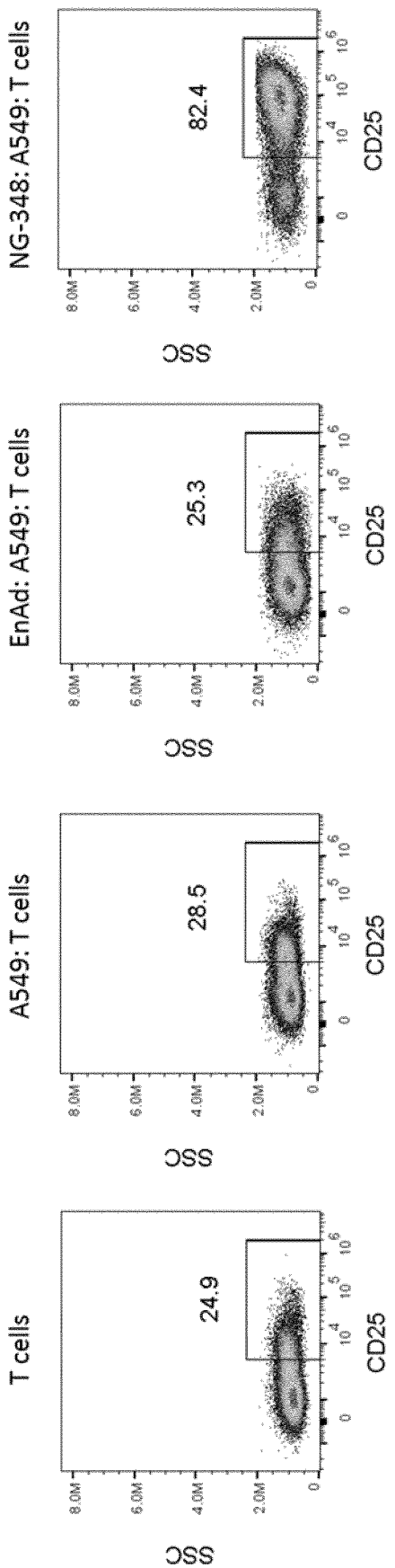

Isotype
A549
EnAd: A549: T cells
NG-348: A549: T cells
EpCam
CD80
0.36
0.24
98.7
0.71
98.7
0.90
0.38
0.016
98.1
0.57
1.29
0
17.0
82.8
0.048
0.096

Figure 7A


T cells
24.9
A549: T cells
28.5
EnAd: A549: T cells
25.3
NG-348: A549: T cells
82.4
SSC
CD25
8.0M
6.0M
4.0M
2.0M
0
10^4
10^5
10^6

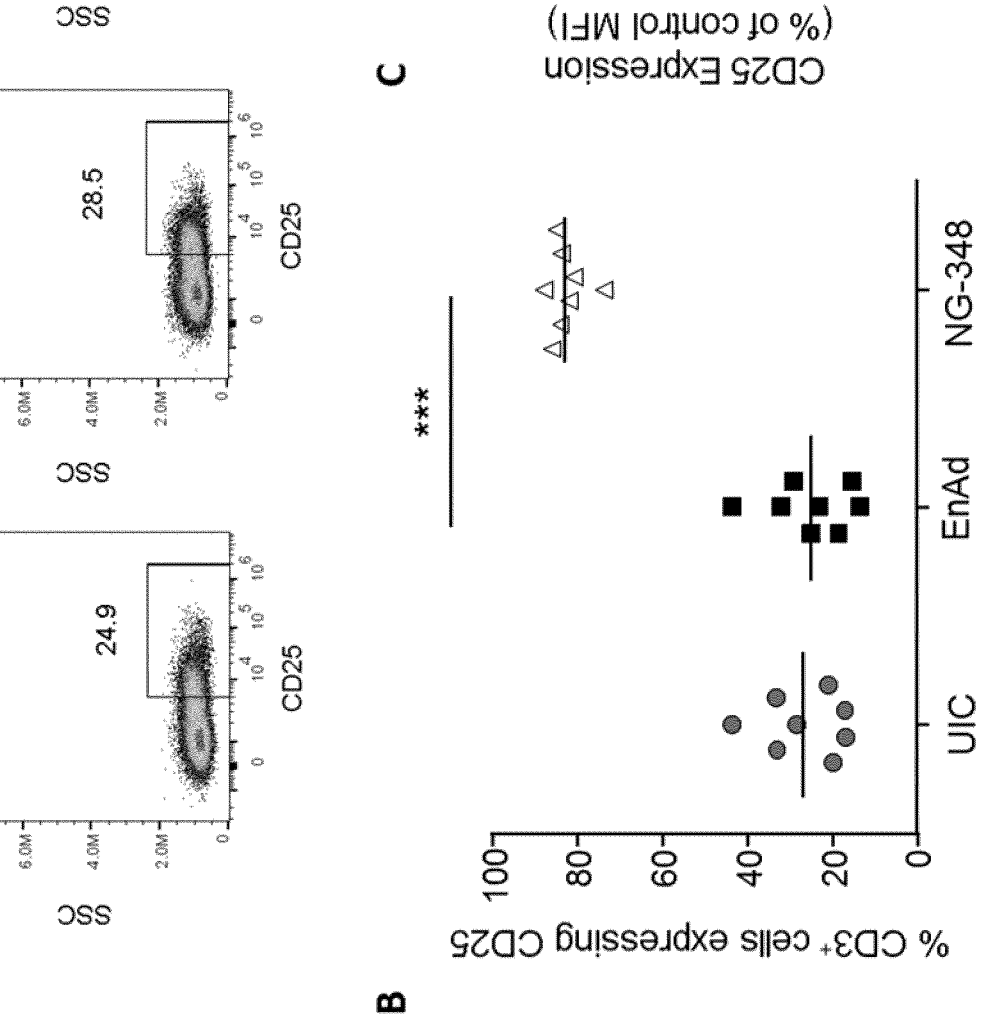
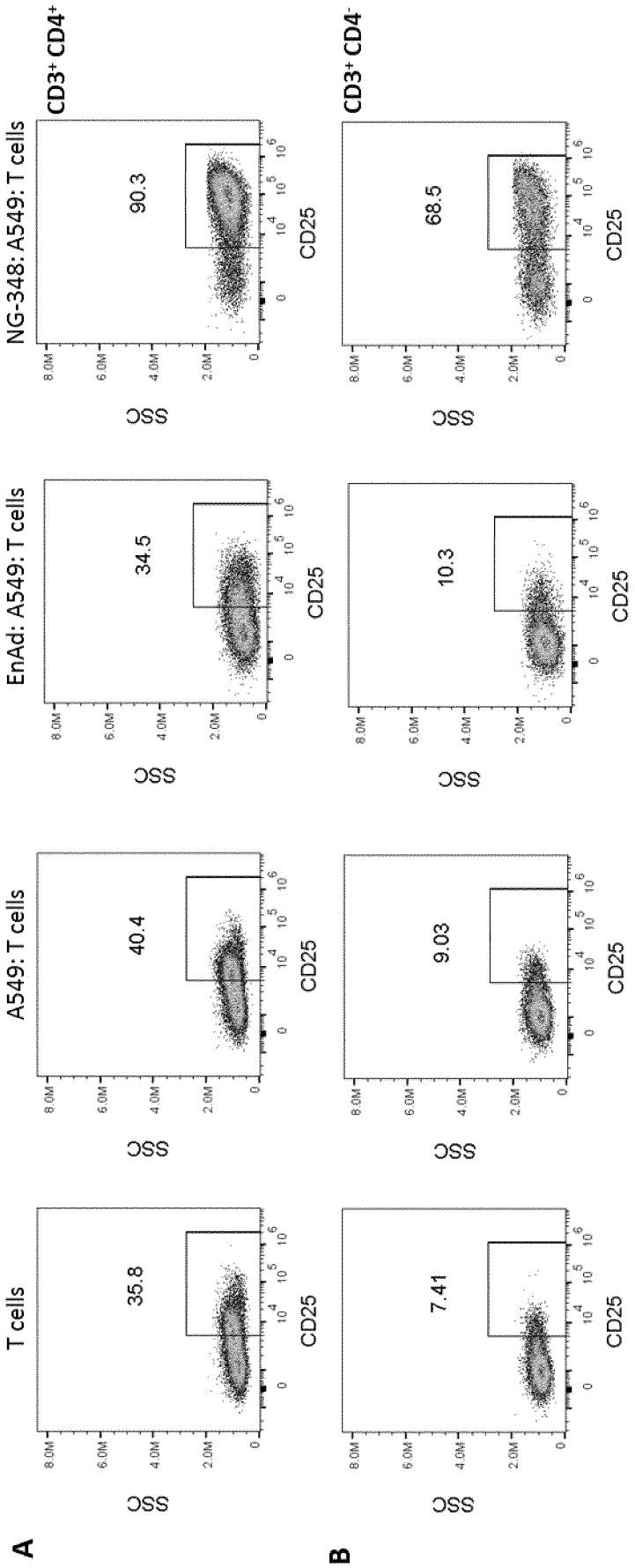

B
% CD3+ cells expressing CD25
100
80
60
40
20
0
***
UIC
EnAd
NG-348
C
CD25 Expression
(% of control MFI)
1000
800
600
400
200
0
***
EnAd
NG-348

Figure 8


A
B
T cells
A549: T cells
EnAd: A549: T cells
NG-348: A549: T cells
CD3+ CD4+
CD3+ CD4-
35.8
40.4
34.5
90.3
7.41
9.03
10.3
68.5
SSC
CD25

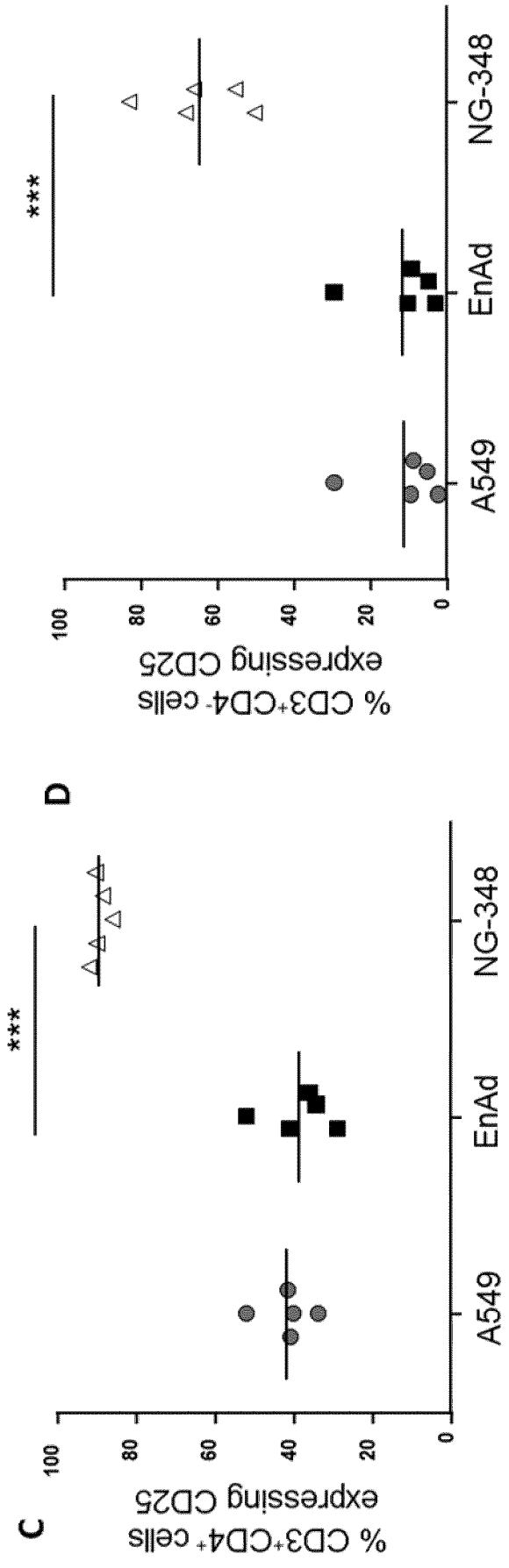

C
% CD3+CD4+ cells expressing CD25
100
80
60
40
20
0
***
A549
EnAd
NG-348
D
% CD3+CD4- cells expressing CD25
100
80
60
40
20
0
***
A549
EnAd
NG-348

Figure 8

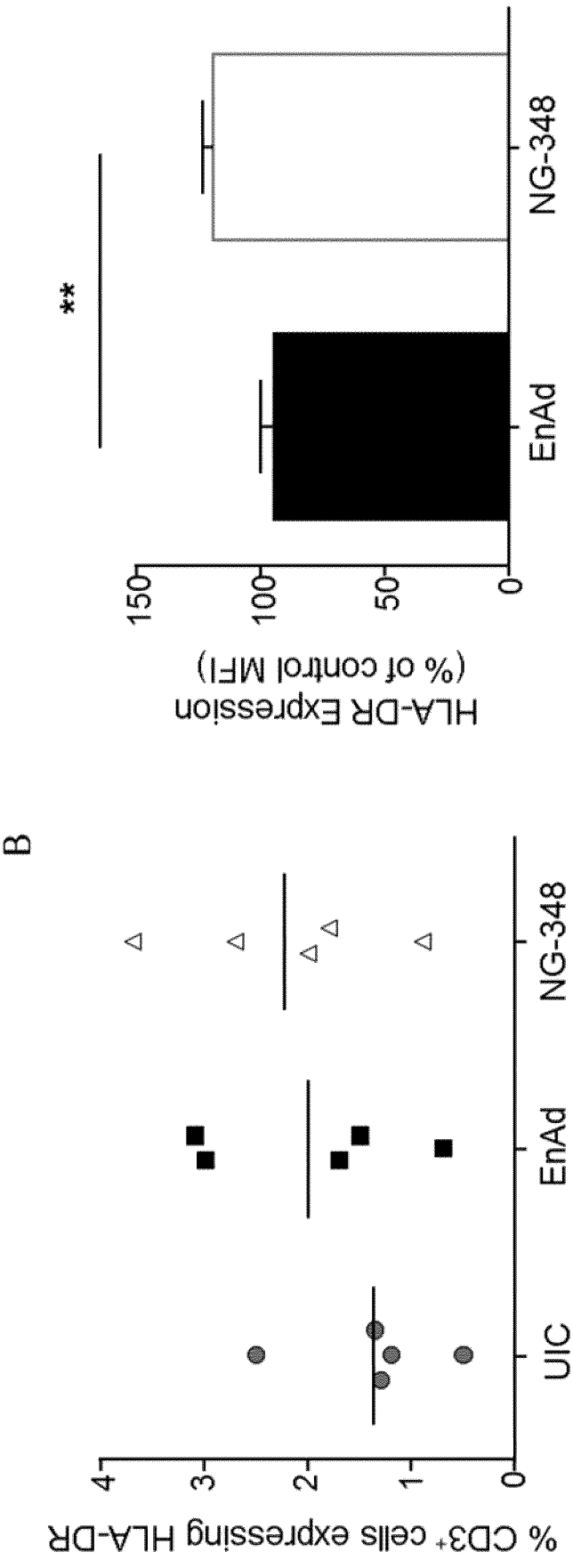

A
% CD3+ cells expressing HLA-DR
0
1
2
3
4
UIC
EnAd
NG-348
B
HLA-DR Expression
(% of control MFI)
0
50
100
150
**
EnAd
NG-348

Figure 9

Figure 10

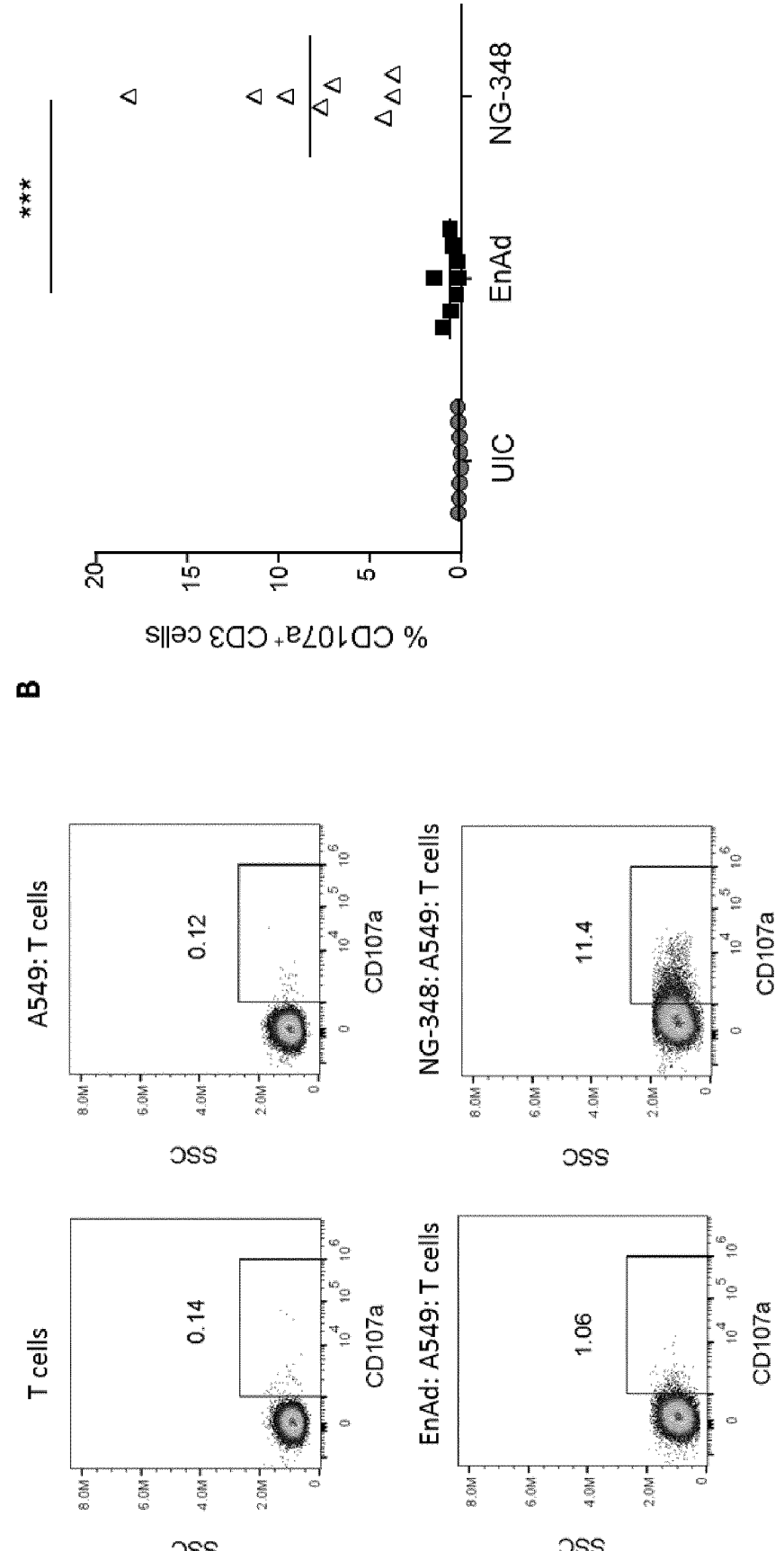
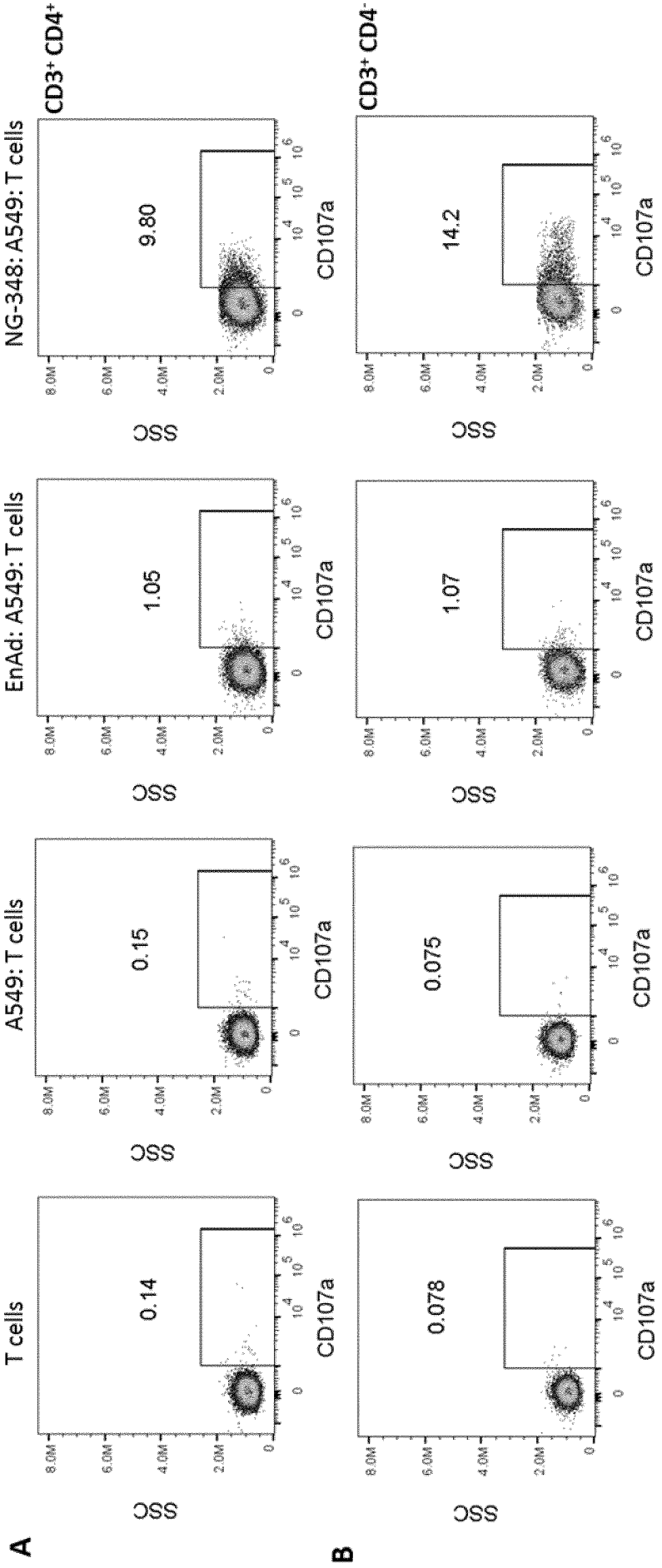

A
T cells
0.14
A549: T cells
0.12
EnAd: A549: T cells
1.06
NG-348: A549: T cells
11.4
SSC
CD107a
B
% CD107a+ CD3 cells
UIC
EnAd
NG-348
***

Figure 11


A
B
T cells
A549: T cells
EnAd: A549: T cells
NG-348: A549: T cells
CD3+ CD4+
CD3+ CD4-
0.14
0.15
1.05
9.80
0.078
0.075
1.07
14.2
SSC
CD107a

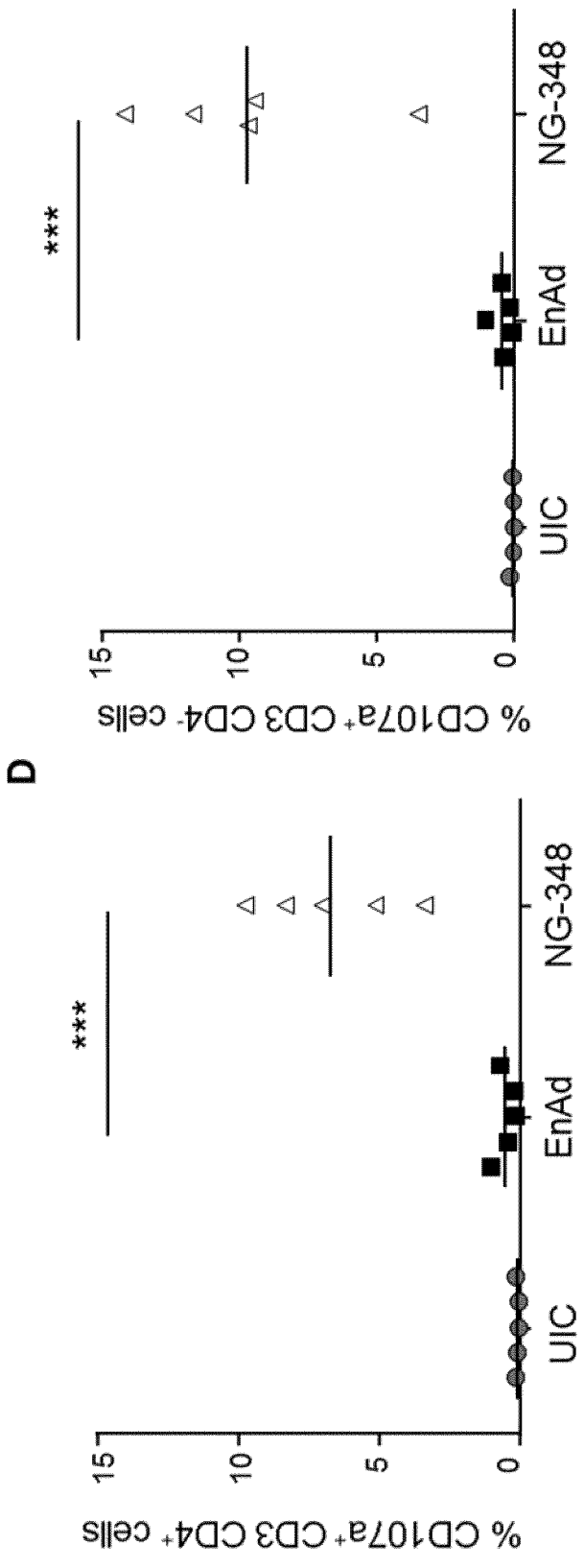
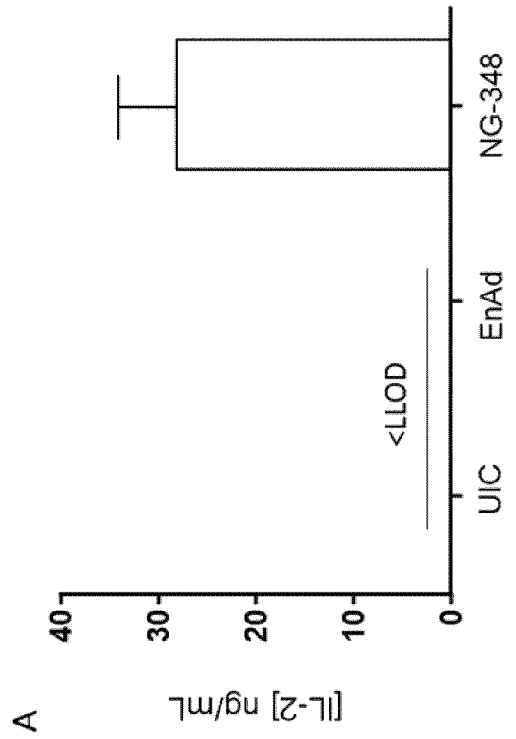

C
% CD107a+ CD3 CD4+ cells
15
10
5
0
***
UIC
EnAd
NG-348
D
% CD107a+ CD3 CD4- cells
15
10
5
0
***
UIC
EnAd
NG-348

Figure 11

Figure 12



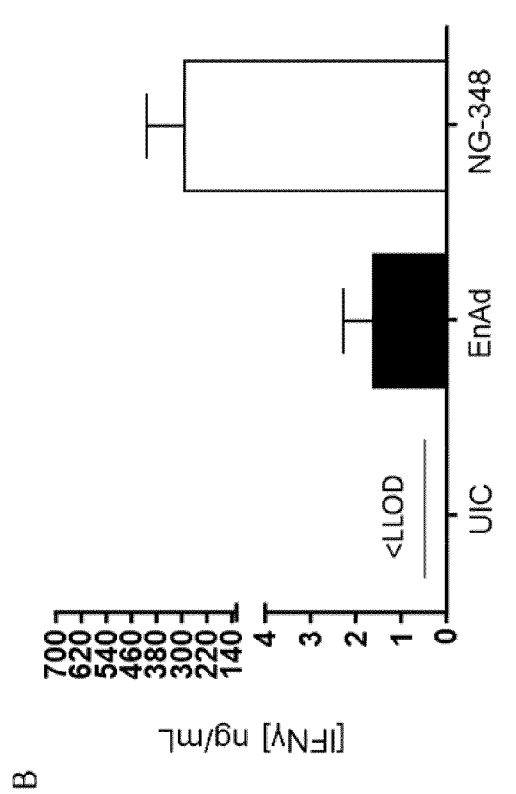
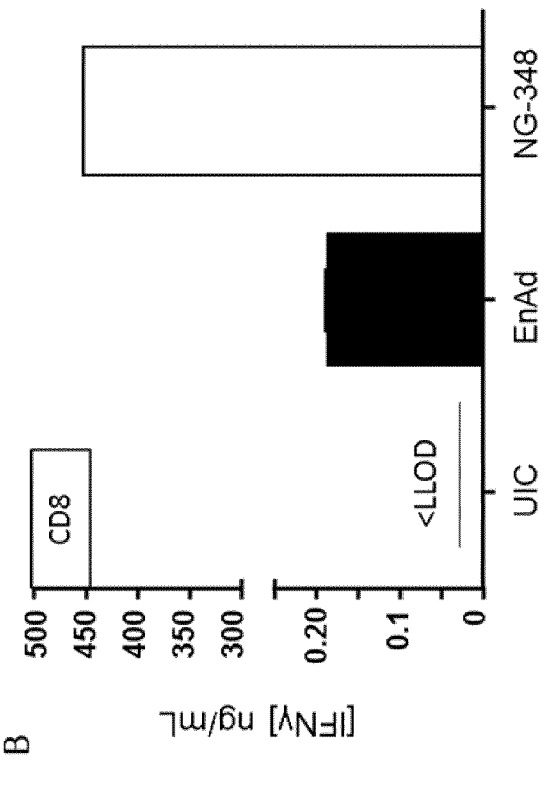


Figure 13



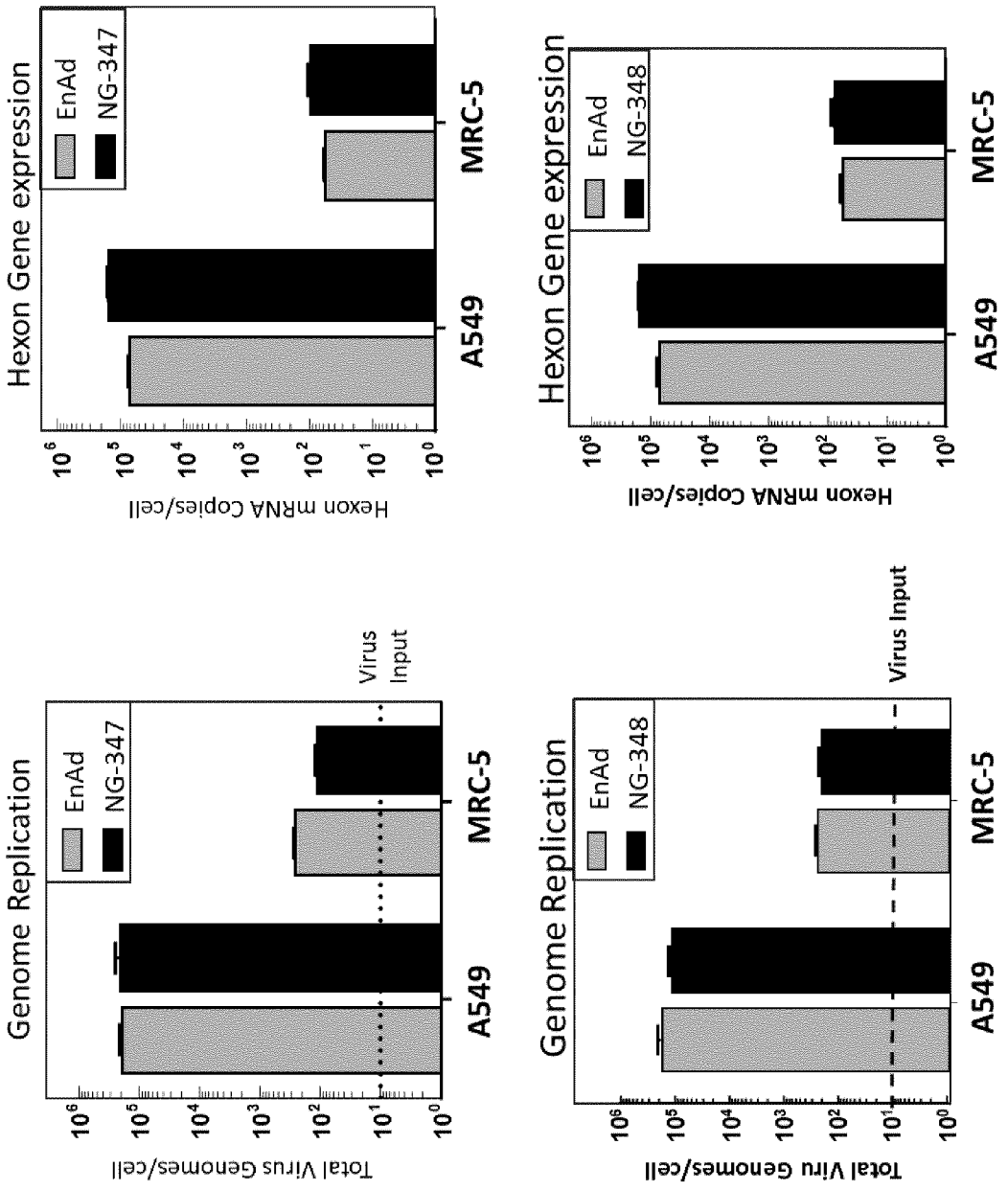

Hexon Gene expression
EnAd
NG-347
Hexon mRNA Copies/cell
A549
MRC-5
Hexon Gene expression
EnAd
NG-348
Hexon mRNA Copies/cell
A549
MRC-5
Genome Replication
EnAd
NG-347
Total Virus Genomes/cell
Virus Input
A549
MRC-5
Genome Replication
EnAd
NG-348
Total Viru Genomes/cell
Virus Input
A549
MRC-5

Figure 14

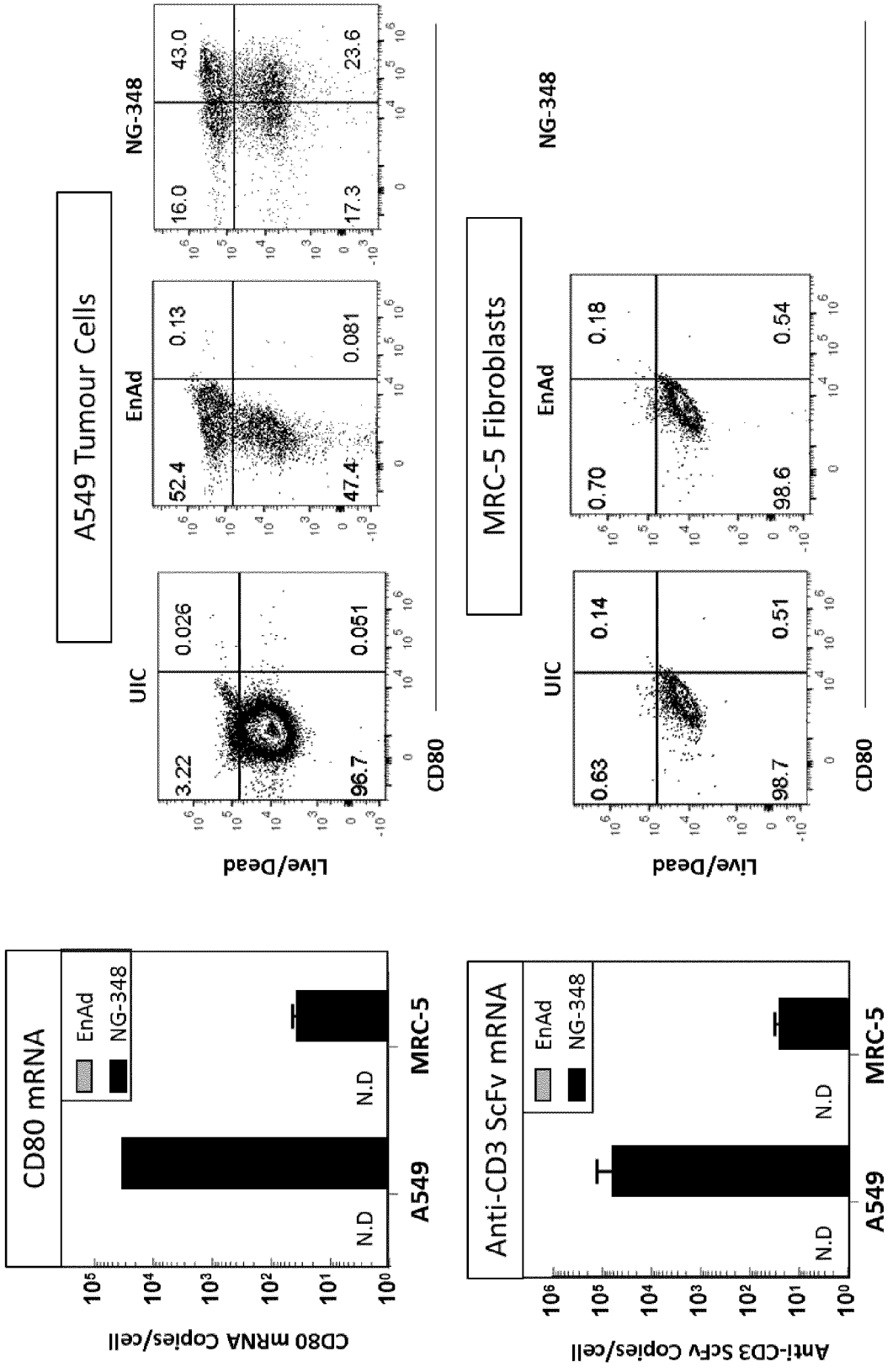
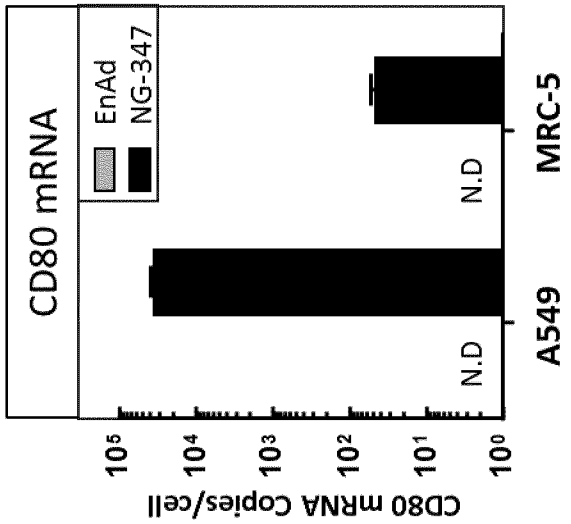

A549 Tumour Cells
MRC-5 Fibroblasts
UIC
EnAd
NG-348
Live/Dead
CD80
3.22
0.026
96.7
0.051
52.4
0.13
47.4
0.081
16.0
43.0
17.3
23.6
0.63
0.14
98.7
0.51
0.70
0.18
98.6
0.54
CD80 mRNA
EnAd
NG-348
CD80 mRNA Copies/cell
N.D
A549
MRC-5
Anti-CD3 ScFv mRNA
Anti-CD3 ScFv Copies/cell

Figure 15


CD80 mRNA
EnAd
NG-347
CD80 mRNA Copies/cell
N.D
N.D
A549
MRC-5

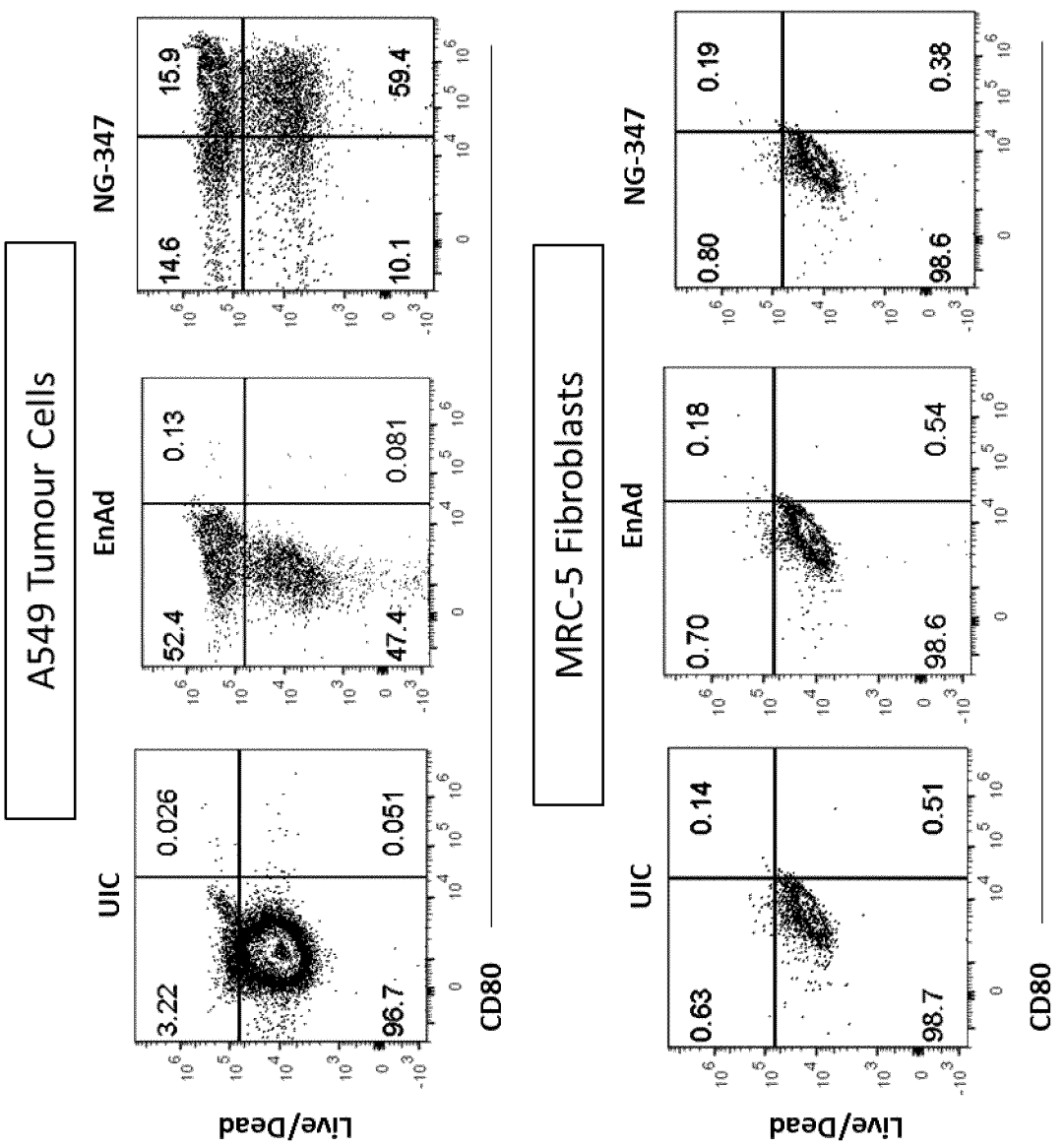

A549 Tumour Cells
MRC-5 Fibroblasts
UIC
EnAd
NG-347
Live/Dead
CD80
3.22
0.026
96.7
0.051
52.4
0.13
47.4
0.081
14.6
15.9
10.1
59.4
0.63
0.14
98.7
0.51
0.70
0.18
98.6
0.54
0.80
0.19
98.6
0.38

Figure 16

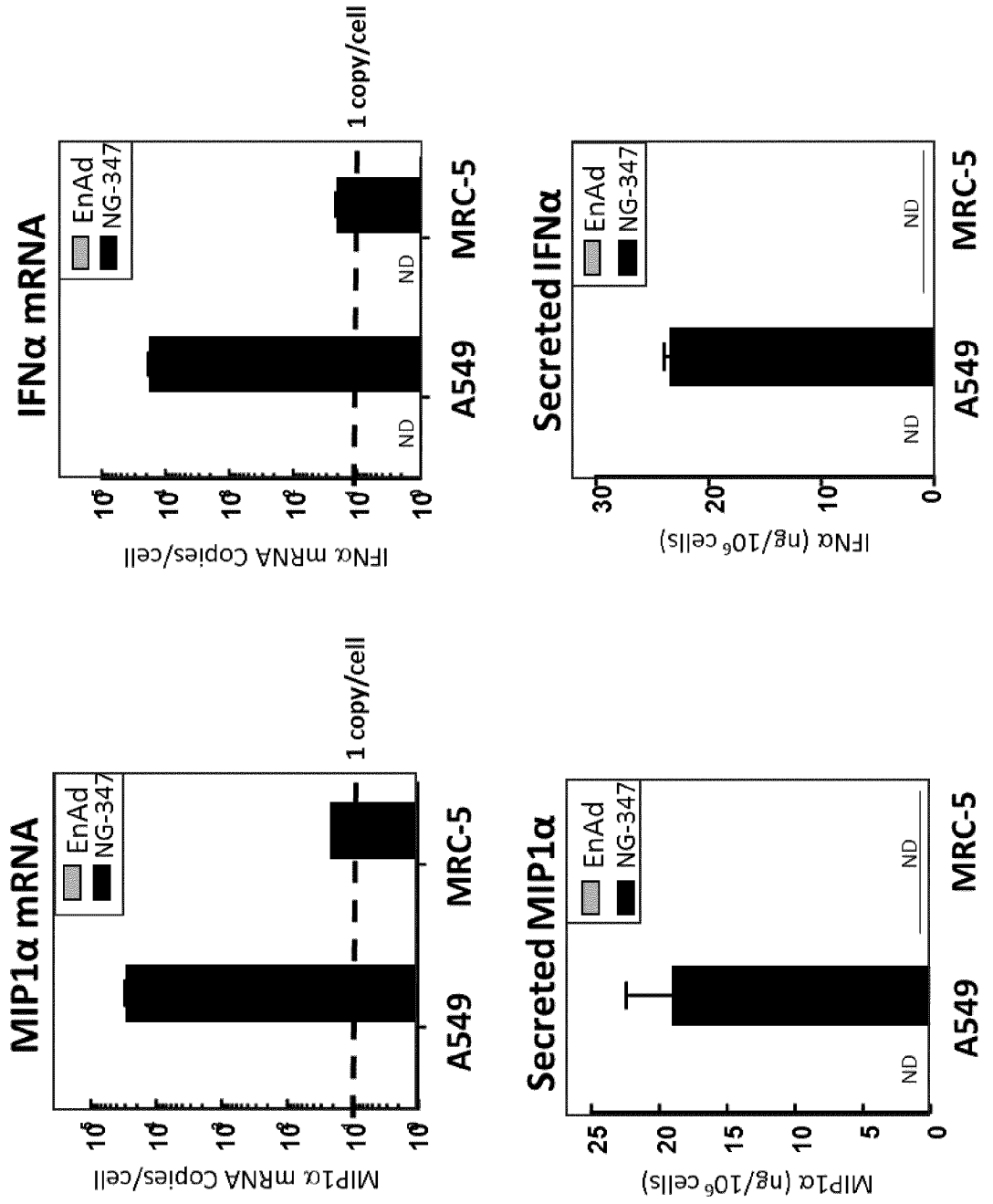

MIP1α mRNA
EnAd
NG-347
1 copy/cell
MIP1α mRNA Copies/cell
A549
MRC-5
Secreted MIP1α
MIP1α (ng/10⁶ cells)
ND
IFNα mRNA
IFNα mRNA Copies/cell
Secreted IFNα
IFNα (ng/10⁶ cells)

Figure 17

Figure 18

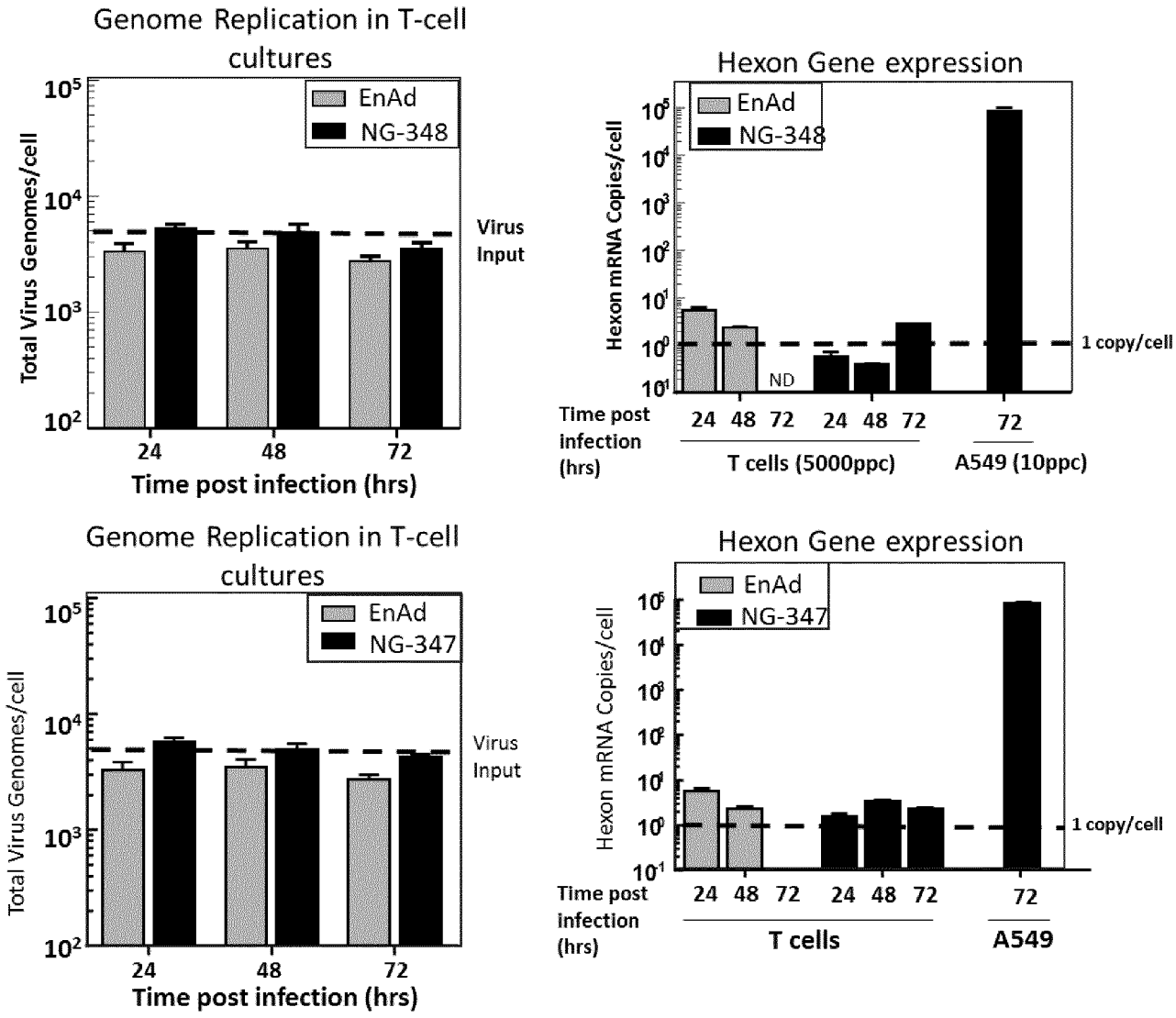
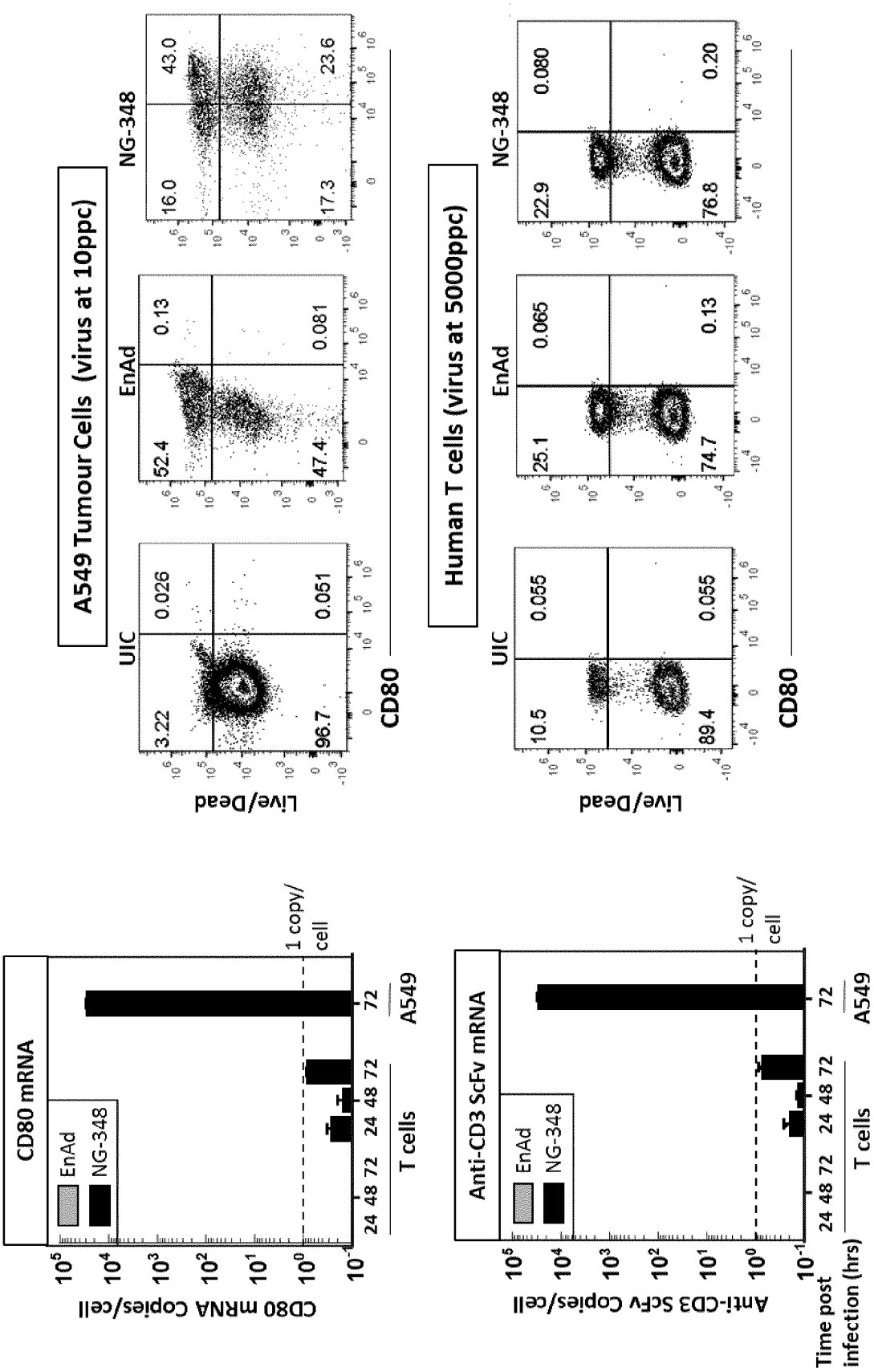

Genome Replication in T-cell cultures
EnAd
NG-348
Total Virus Genomes/cell
Virus Input
Time post infection (hrs)
Hexon Gene expression
Hexon mRNA Copies/cell
1 copy/cell
ND
Time post infection (hrs)
T cells (5000ppc)
A549 (10ppc)
Genome Replication in T-cell cultures
EnAd
NG-347
Total Virus Genomes/cell
Virus Input
Time post infection (hrs)
Hexon Gene expression
Hexon mRNA Copies/cell
1 copy/cell
Time post infection (hrs)
T cells
A549

Figure 19


A549 Tumour Cells (virus at 10ppc)
Human T cells (virus at 5000ppc)
UIC
EnAd
NG-348
Live/Dead
CD80
3.22
96.7
0.026
0.051
52.4
47.4
0.13
0.081
16.0
17.3
43.0
23.6
10.5
89.4
0.055
0.055
25.1
74.7
0.065
0.13
22.9
76.8
0.080
0.20
CD80 mRNA
Anti-CD3 ScFv mRNA
EnAd
NG-348
1 copy/ cell
CD80 mRNA Copies/cell
Anti-CD3 ScFv Copies/cell
Time post infection (hrs)
24 48 72 24 48 72
T cells
72
A549

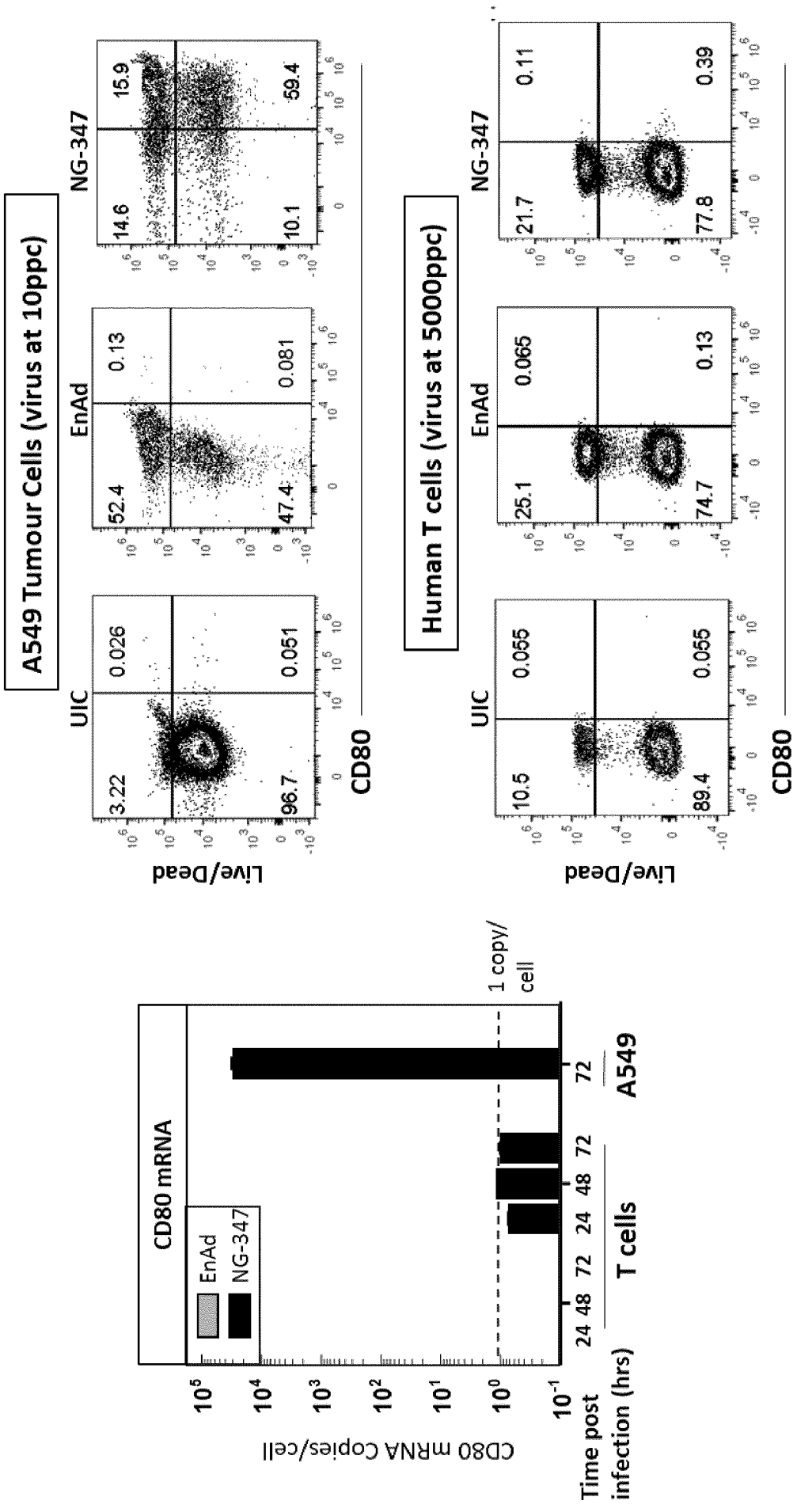

A549 Tumour Cells (virus at 10ppc)
Human T cells (virus at 5000ppc)
UIC
EnAd
NG-347
Live/Dead
CD80
3.22
96.7
0.026
0.051
52.4
47.4
0.13
0.081
14.6
10.1
15.9
59.4
10.5
89.4
0.055
0.055
25.1
74.7
0.065
0.13
21.7
77.8
0.11
0.39
CD80 mRNA
EnAd
NG-347
1 copy/ cell
CD80 mRNA Copies/cell
Time post infection (hrs)
24 48 72 24 48 72
T cells
72
A549

Figure 20

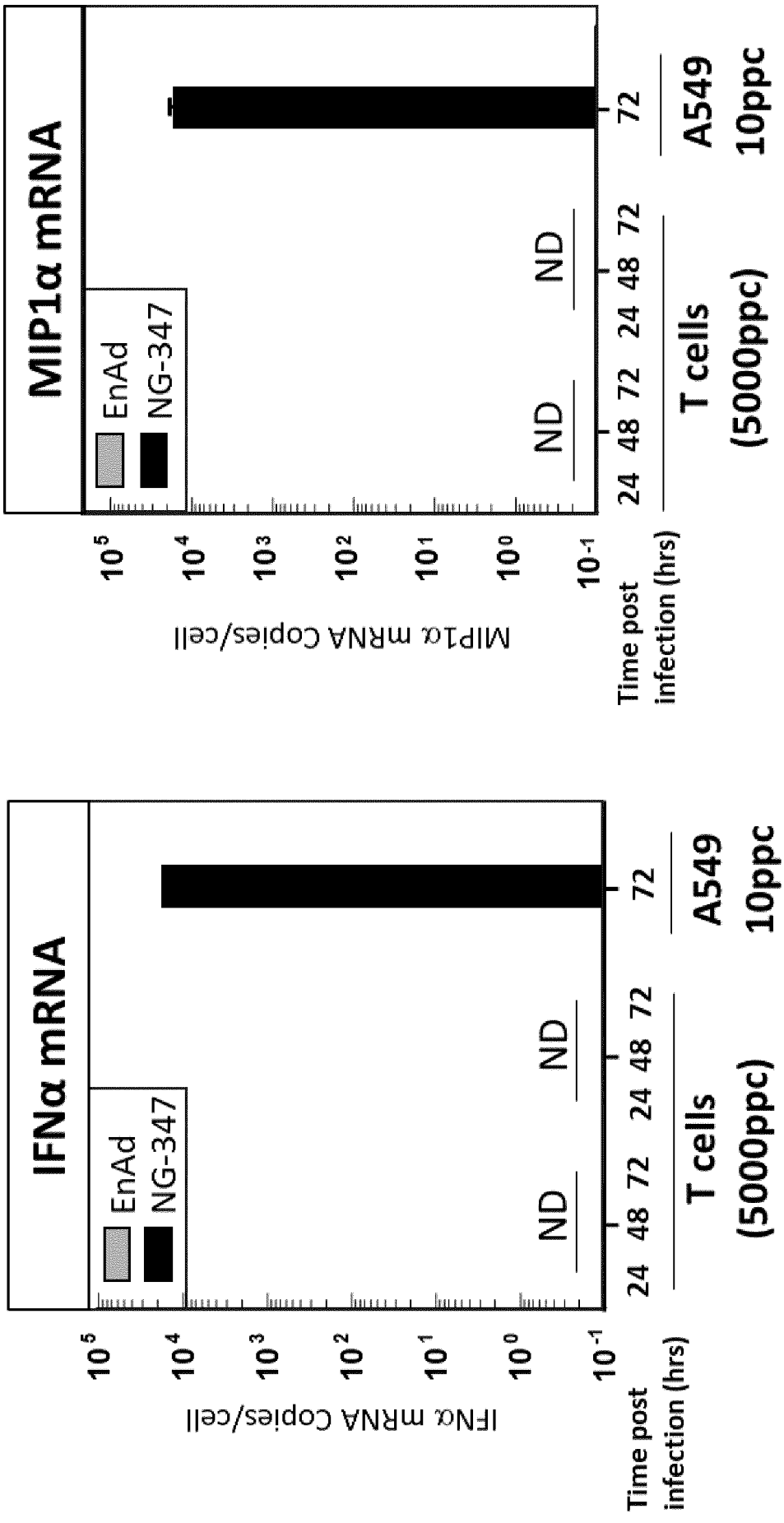

MIP1α mRNA
EnAd
NG-347
MIP1α mRNA Copies/cell
10^5
10^4
10^3
10^2
10^1
10^0
10^-1
ND
ND
24 48 72 24 48 72 72
Time post infection (hrs)
T cells (5000ppc)
A549 10ppc
IFNα mRNA
EnAd
NG-347
IFNα mRNA Copies/cell
10^5
10^4
10^3
10^2
10^1
10^0
10^-1
ND
ND
24 48 72 24 48 72 72
Time post infection (hrs)
T cells (5000ppc)
A549 10ppc

Figure 21

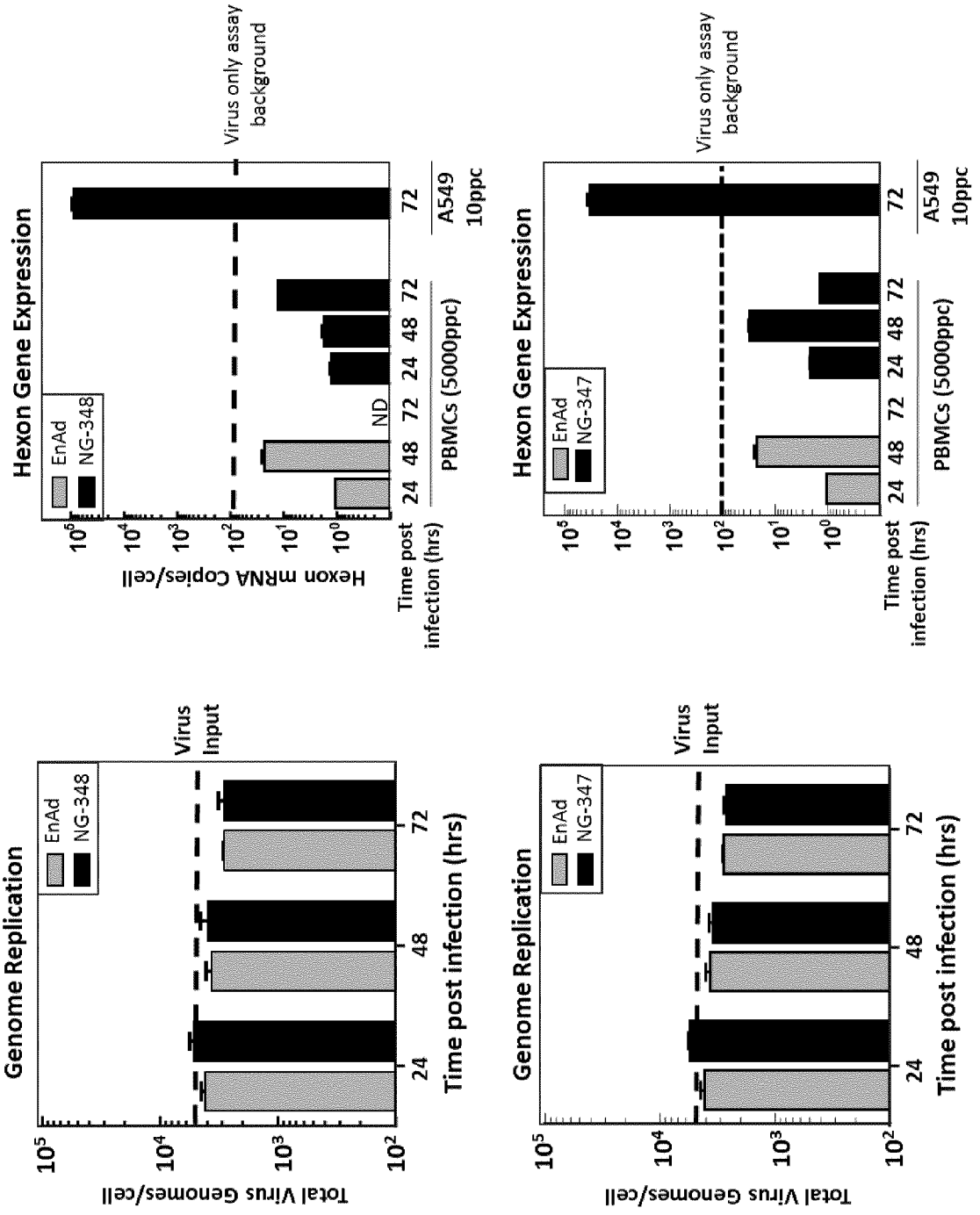

Hexon Gene Expression
EnAd
NG-348
Hexon mRNA Copies/cell
Virus only assay background
ND
Time post infection (hrs)
24 48 72 24 48 72
PBMCs (5000ppc)
72
A549 10ppc
Hexon Gene Expression
EnAd
NG-347
Virus only assay background
Time post infection (hrs)
24 48 72 24 48 72
PBMCs (5000ppc)
72
A549 10ppc
Genome Replication
EnAd
NG-348
Virus Input
Total Virus Genomes/cell
Time post infection (hrs)
24
48
72
Genome Replication
EnAd
NG-347
Virus Input
Total Virus Genomes/cell
Time post infection (hrs)
24
48
72

Figure 22

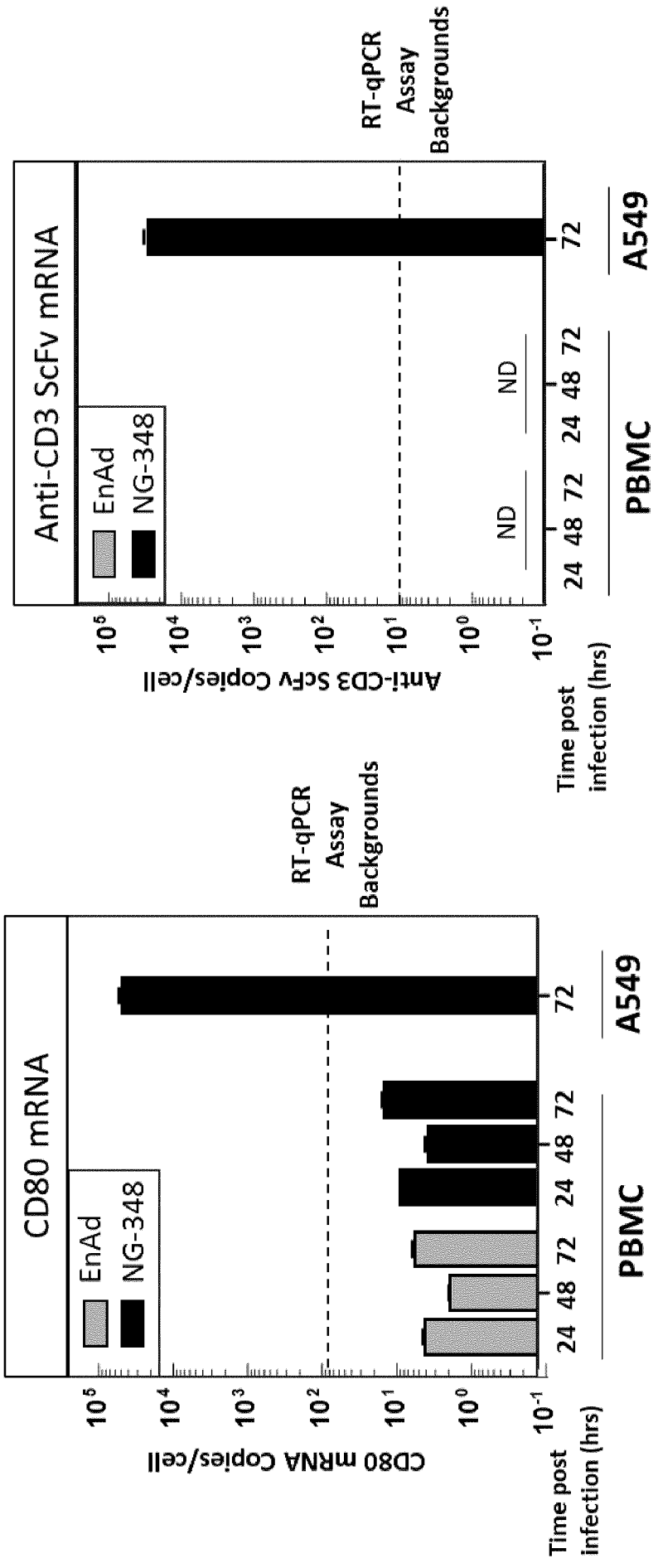
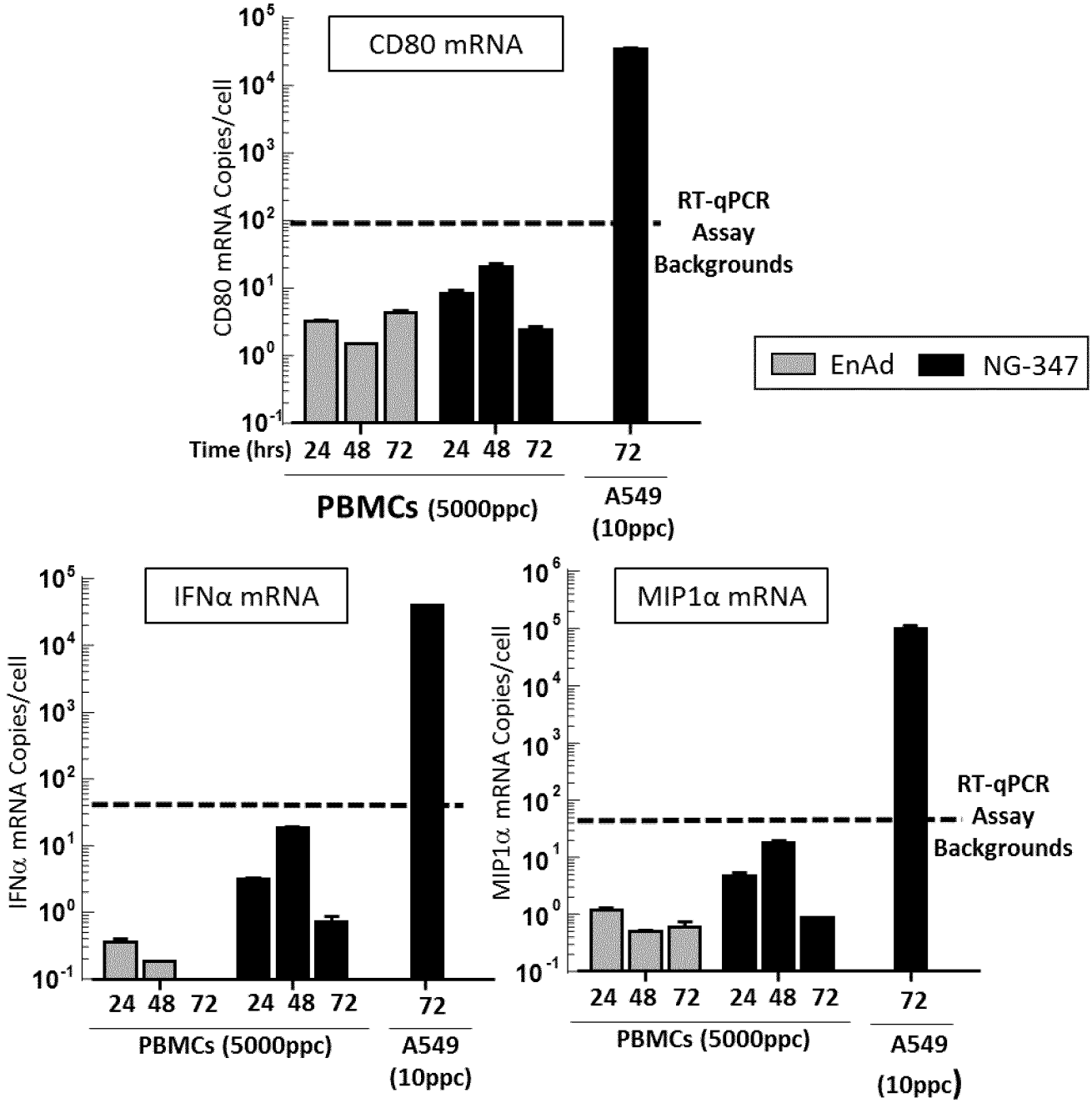

CD80 mRNA
EnAd
NG-348
CD80 mRNA Copies/cell
RT-qPCR Assay Backgrounds
Time post infection (hrs)
24 48 72 24 48 72
PBMC
A549
Anti-CD3 ScFv mRNA
EnAd
NG-348
Anti-CD3 ScFv Copies/cell
RT-qPCR Assay Backgrounds
ND
ND
Time post infection (hrs)
24 48 72 24 48 72
PBMC
A549

Figure 23

Figure 24


CD80 mRNA
CD80 mRNA Copies/cell
RT-qPCR Assay Backgrounds
EnAd
NG-347
Time (hrs) 24 48 72 24 48 72 72
PBMCs (5000ppc)
A549 (10ppc)
IFNα mRNA
IFNα mRNA Copies/cell
24 48 72 24 48 72 72
PBMCs (5000ppc)
A549 (10ppc)
MIP1α mRNA
MIP1α mRNA Copies/cell
RT-qPCR Assay Backgrounds
24 48 72 24 48 72 72
PBMCs (5000ppc)
A549 (10ppc)

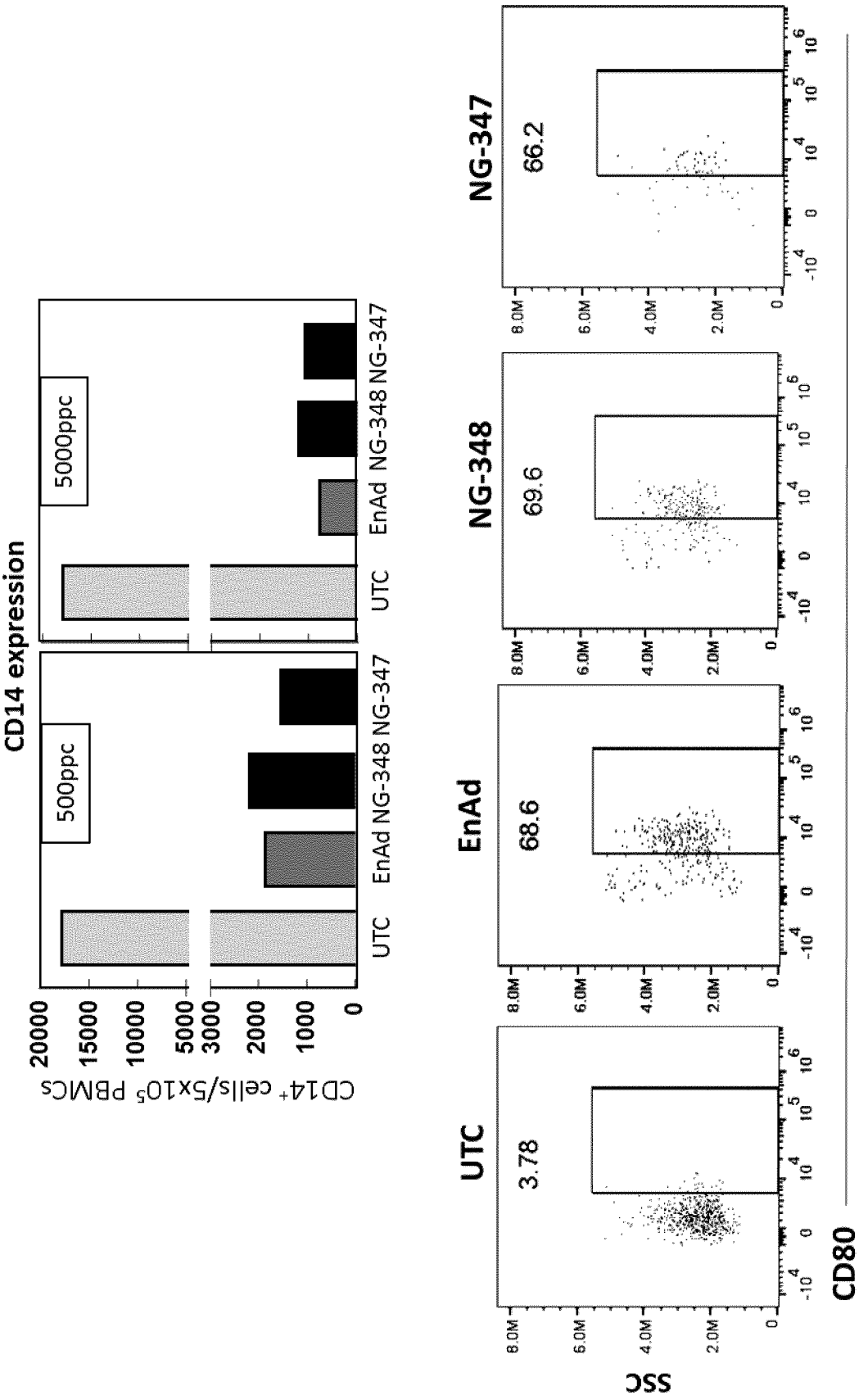

CD14 expression
CD14+ cells/5x10^5 PBMCs
20000
15000
10000
5000
3000
2000
1000
0
500ppc
5000ppc
UTC
EnAd
NG-348
NG-347
UTC
EnAd
NG-348
NG-347
UTC
EnAd
NG-348
NG-347
3.78
68.6
69.6
66.2
SSC
CD80
8.0M
6.0M
4.0M
2.0M
0
-10^4
0
10^4
10^5
10^6

Figure 25

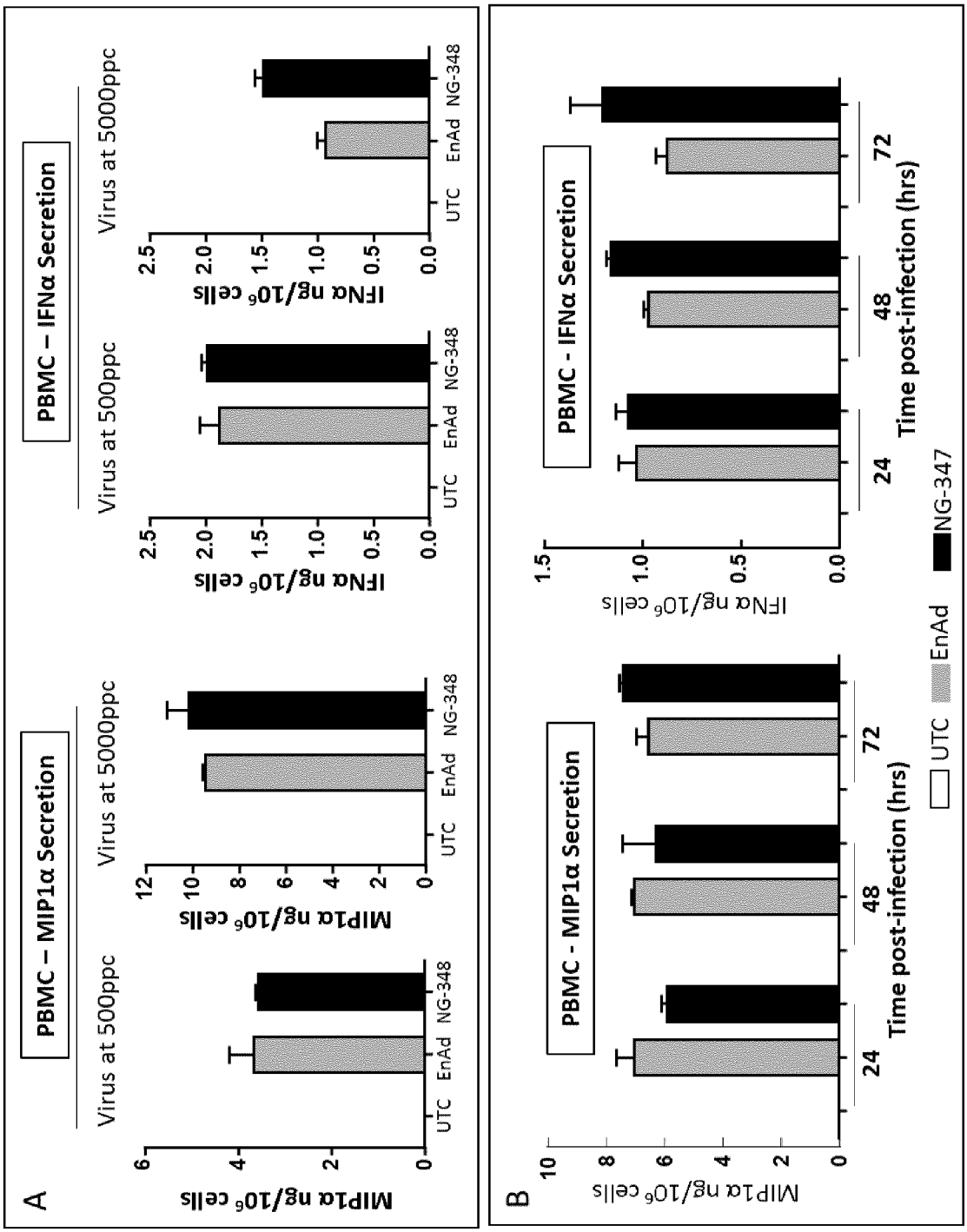

A
PBMC – MIP1α Secretion
Virus at 500ppc
Virus at 5000ppc
MIP1α ng/10^6 cells
UTC EnAd NG-348
PBMC – IFNα Secretion
IFNα ng/10^6 cells
B
PBMC - MIP1α Secretion
PBMC - IFNα Secretion
Time post-infection (hrs)
24
48
72
UTC
EnAd
NG-347

Figure 26

Figure 27

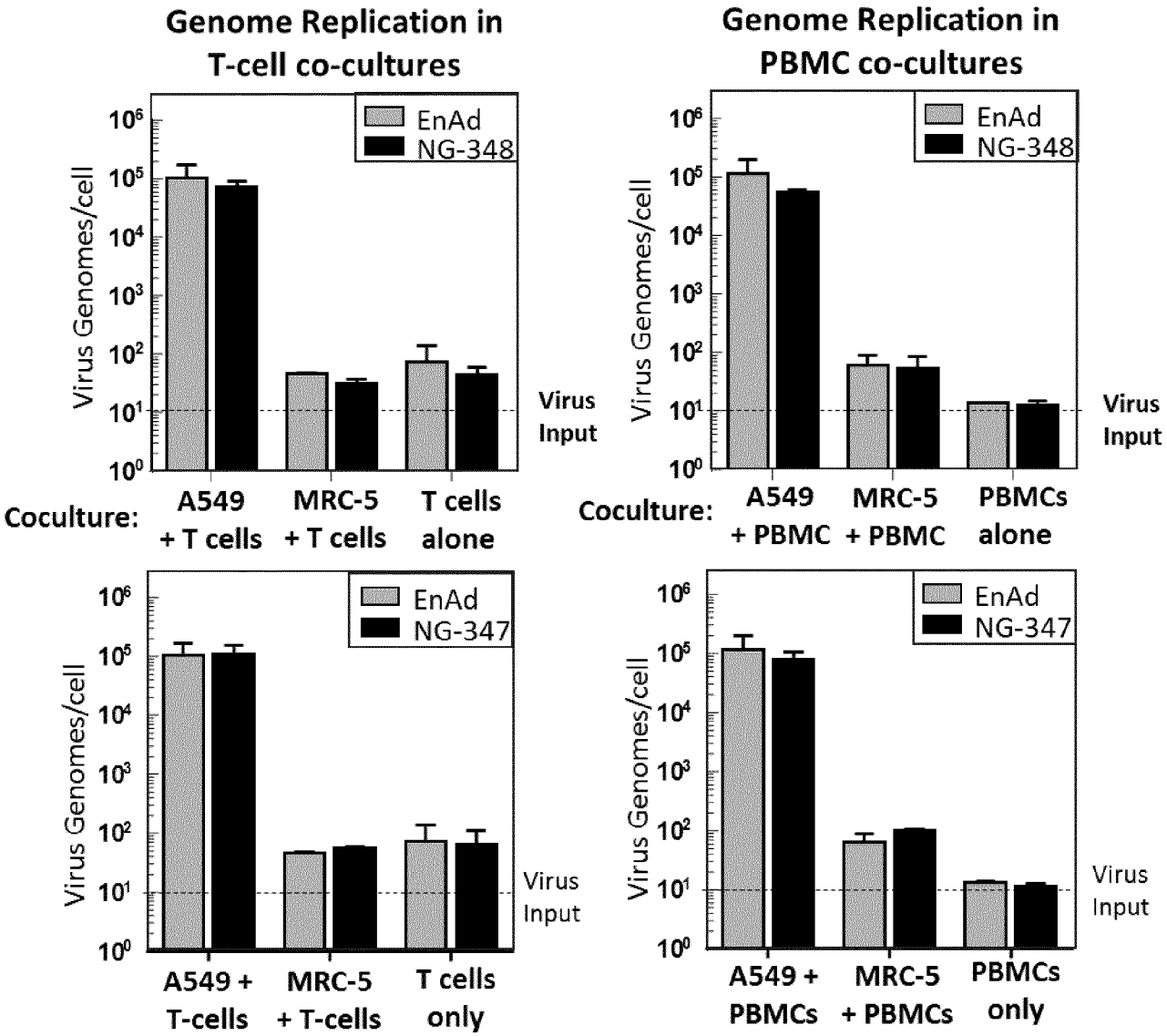

Genome Replication in T-cell co-cultures
Virus Genomes/cell
EnAd
NG-348
Virus Input
Coculture:
A549 + T cells
MRC-5 + T cells
T cells alone
Genome Replication in PBMC co-cultures
Virus Genomes/cell
EnAd
NG-348
Virus Input
Coculture:
A549 + PBMC
MRC-5 + PBMC
PBMCs alone
Virus Genomes/cell
EnAd
NG-347
Virus Input
A549 + T-cells
MRC-5 + T-cells
T cells only
Virus Genomes/cell
EnAd
NG-347
Virus Input
A549 + PBMCs
MRC-5 + PBMCs
PBMCs only

Figure 28

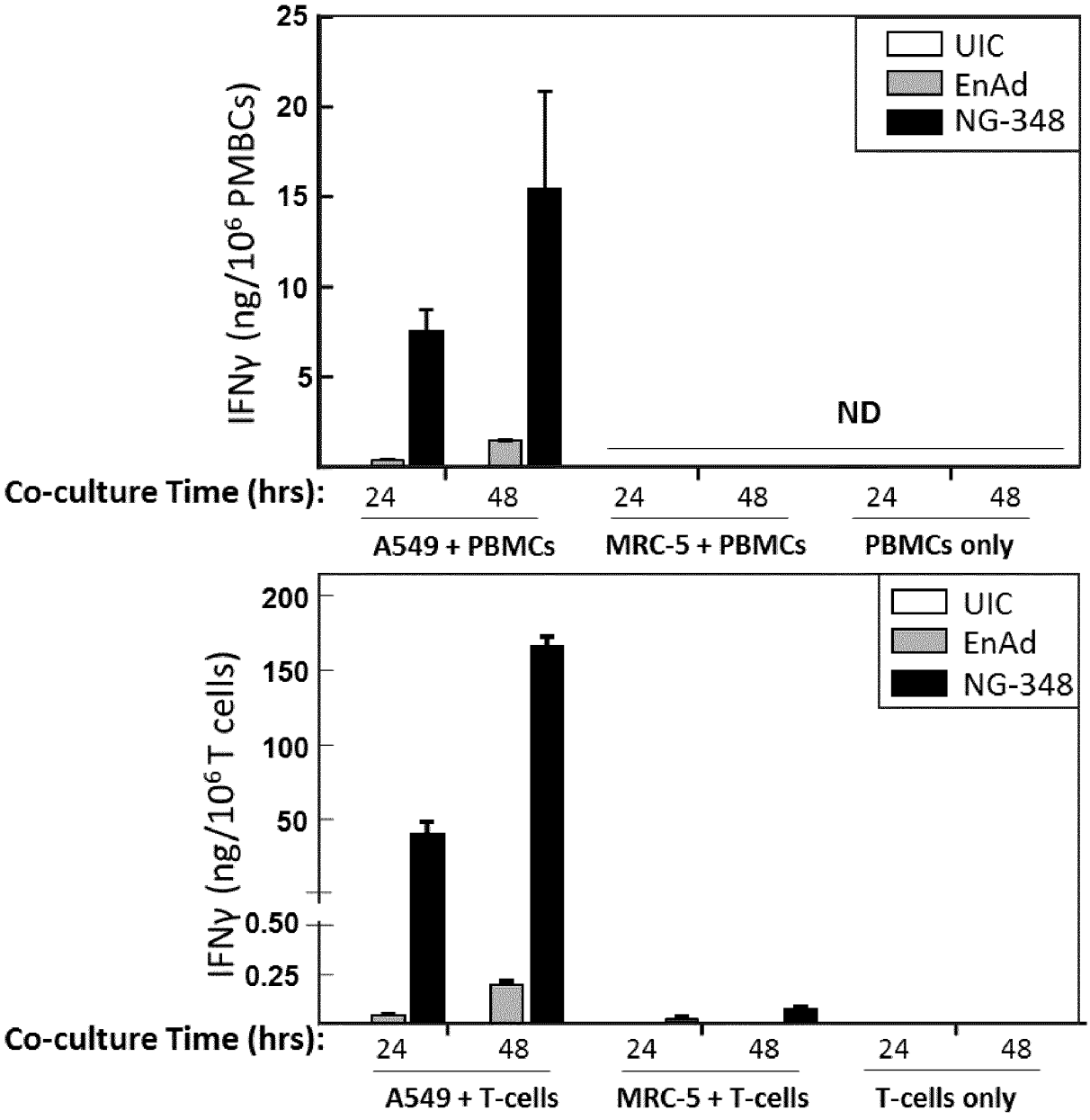

IFNγ (ng/10⁶ PMBCs)
25
20
15
10
5
UIC
EnAd
NG-348
ND
Co-culture Time (hrs):
24
48
24
48
24
48
A549 + PBMCs
MRC-5 + PBMCs
PBMCs only
IFNγ (ng/10⁶ T cells)
200
150
100
50
0.50
0.25
UIC
EnAd
NG-348
Co-culture Time (hrs):
24
48
24
48
24
48
A549 + T-cells
MRC-5 + T-cells
T-cells only

Figure 29

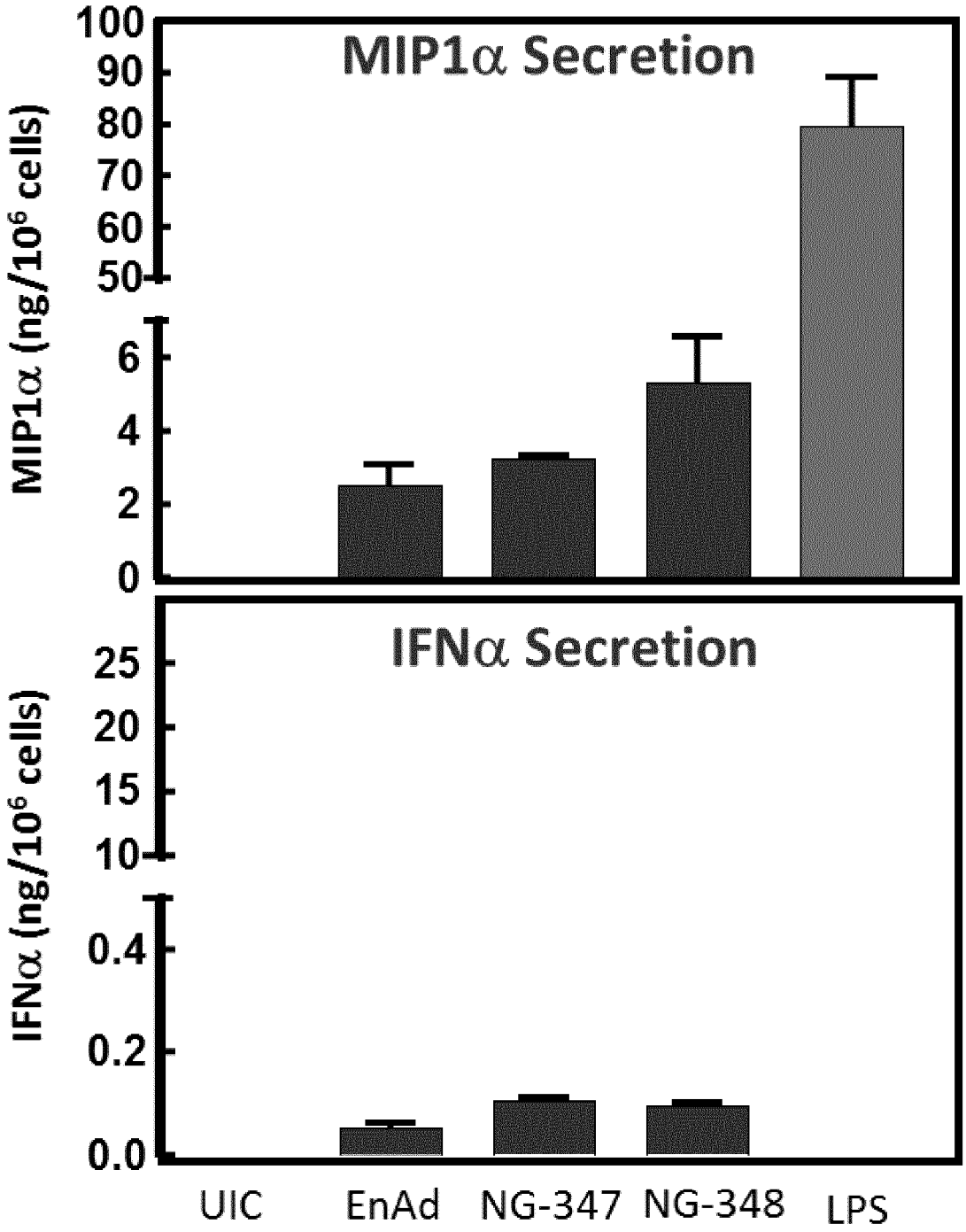
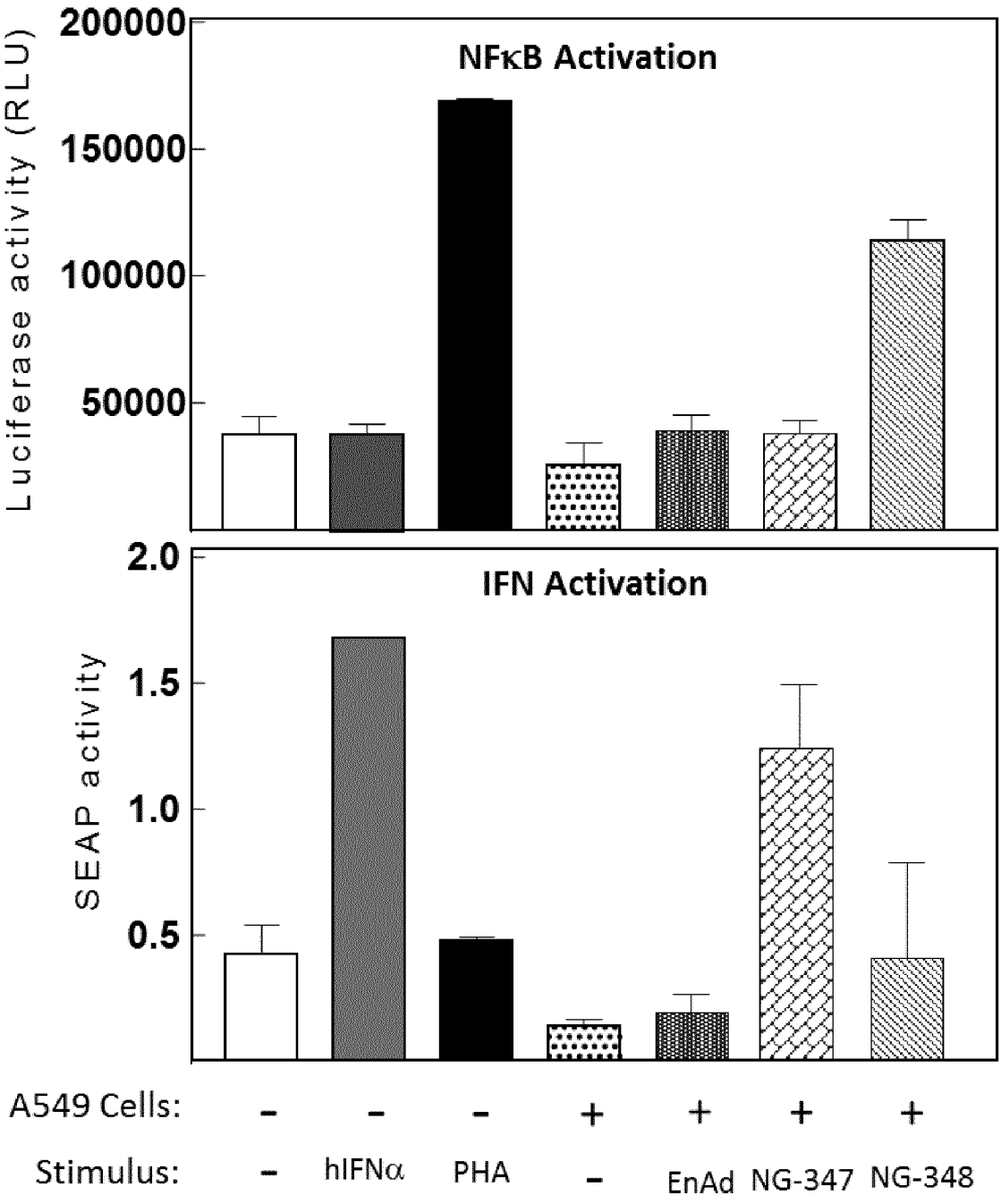

MIP1α Secretion
MIP1α (ng/10^6 cells)
100
90
80
70
60
50
6
4
2
0
IFNα Secretion
IFNα (ng/10^6 cells)
25
20
15
10
0.4
0.2
0.0
UIC
EnAd
NG-347
NG-348
LPS

Figure 30


NFκB Activation
Luciferase activity (RLU)
200000
150000
100000
50000
IFN Activation
SEAP activity
2.0
1.5
1.0
0.5
A549 Cells: - - - + + + +
Stimulus: - hIFNα PHA - EnAd NG-347 NG-348

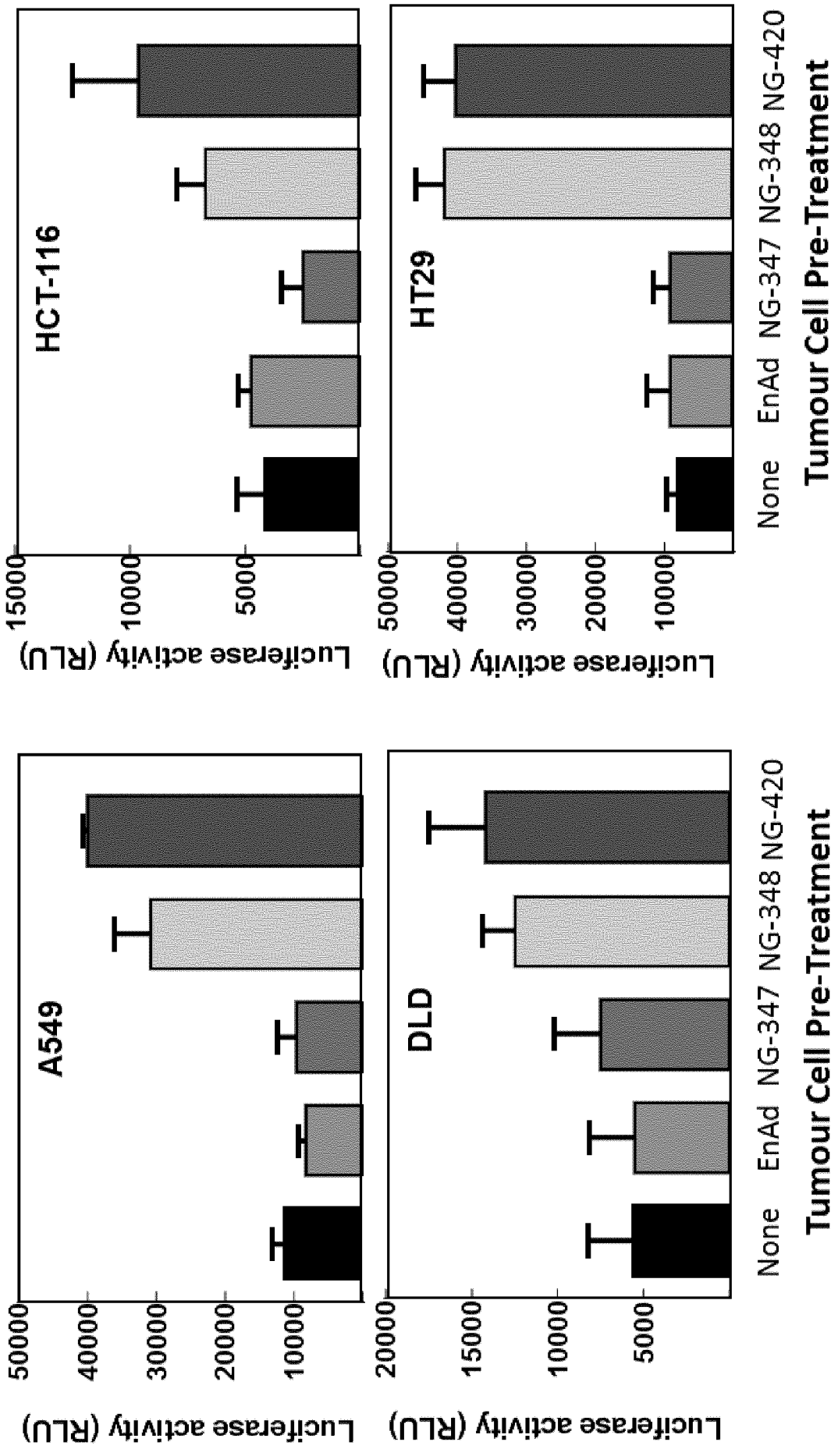

A549
DLD
HCT-116
HT29
Luciferase activity (RLU)
50000
40000
30000
20000
10000
15000
5000
None
EnAd
NG-347
NG-348
NG-420
Tumour Cell Pre-Treatment

Figure 31

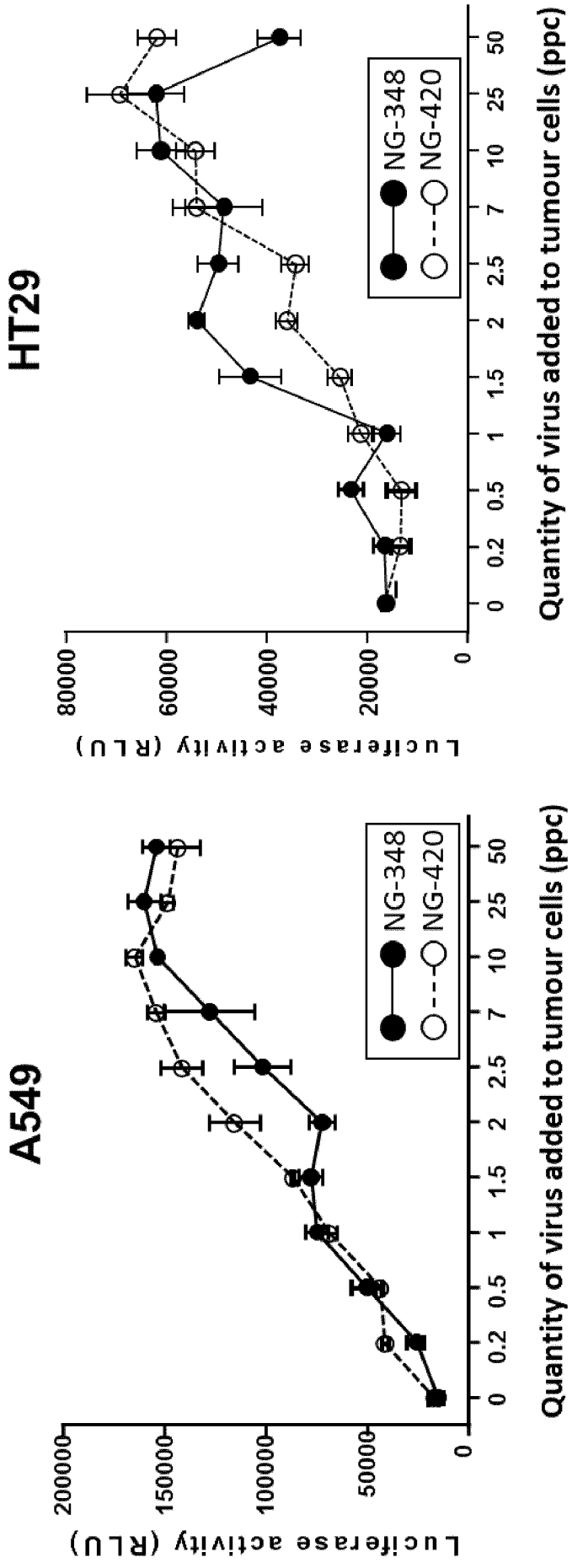

A549
Luciferase activity (RLU)
0
50000
100000
150000
200000
Quantity of virus added to tumour cells (ppc)
0
0.2
0.5
1
1.5
2
2.5
7
10
25
50
NG-348
NG-420
HT29
Luciferase activity (RLU)
0
20000
40000
60000
80000
Quantity of virus added to tumour cells (ppc)
0
0.2
0.5
1
1.5
2
2.5
7
10
25
50
NG-348
NG-420

Figure 32

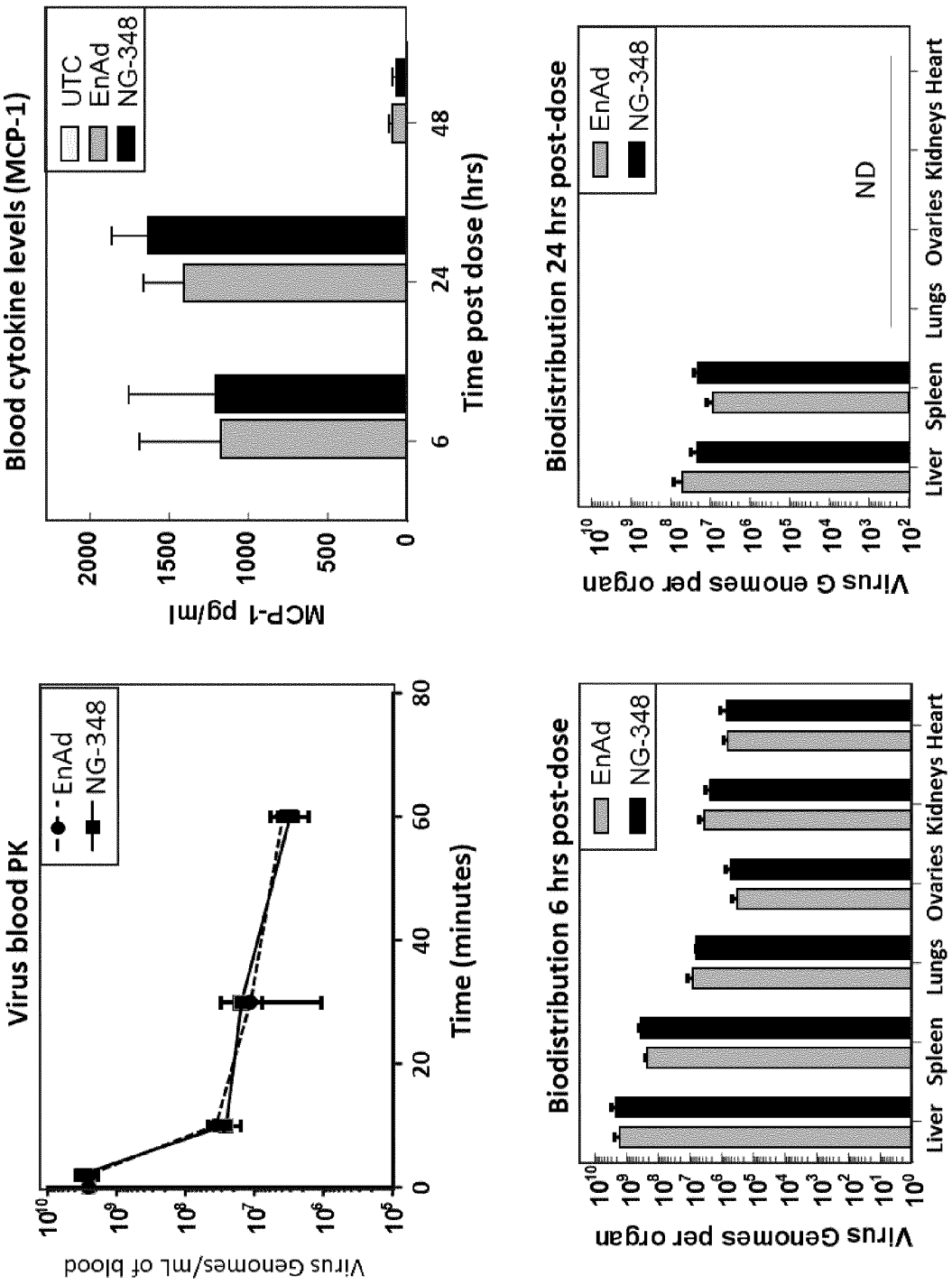

Blood cytokine levels (MCP-1)
UTC
EnAd
NG-348
MCP-1 pg/ml
2000
1500
1000
500
0
6
24
48
Time post dose (hrs)
Biodistribution 24 hrs post-dose
EnAd
NG-348
ND
Virus G enomes per organ
Liver Spleen Lungs Ovaries Kidneys Heart
Virus blood PK
EnAd
NG-348
Virus Genomes/mL of blood
Time (minutes)
Biodistribution 6 hrs post-dose
EnAd
NG-348
Virus Genomes per organ
Liver Spleen Lungs Ovaries Kidneys Heart

Figure 33

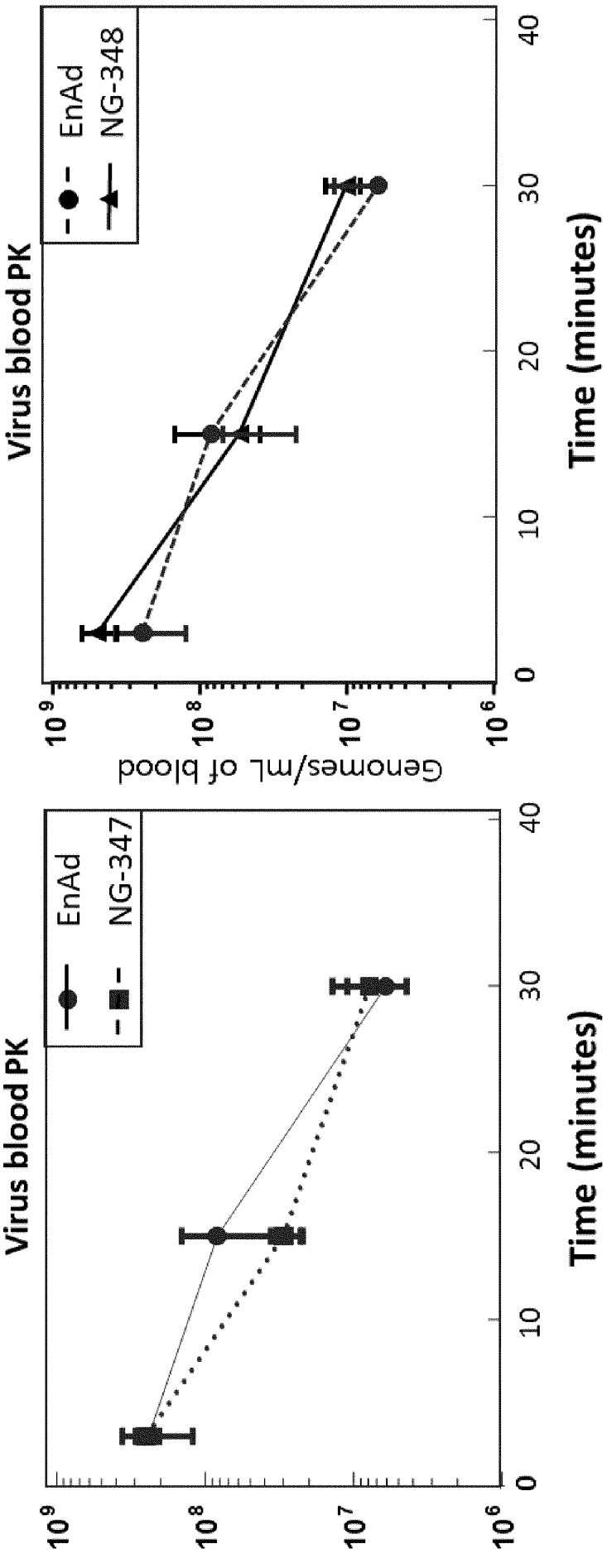

Virus blood PK
EnAd
NG-347
Genomes/mL of blood
Time (minutes)
Virus blood PK
EnAd
NG-348
Genomes/mL of blood
Time (minutes)

Figure 34

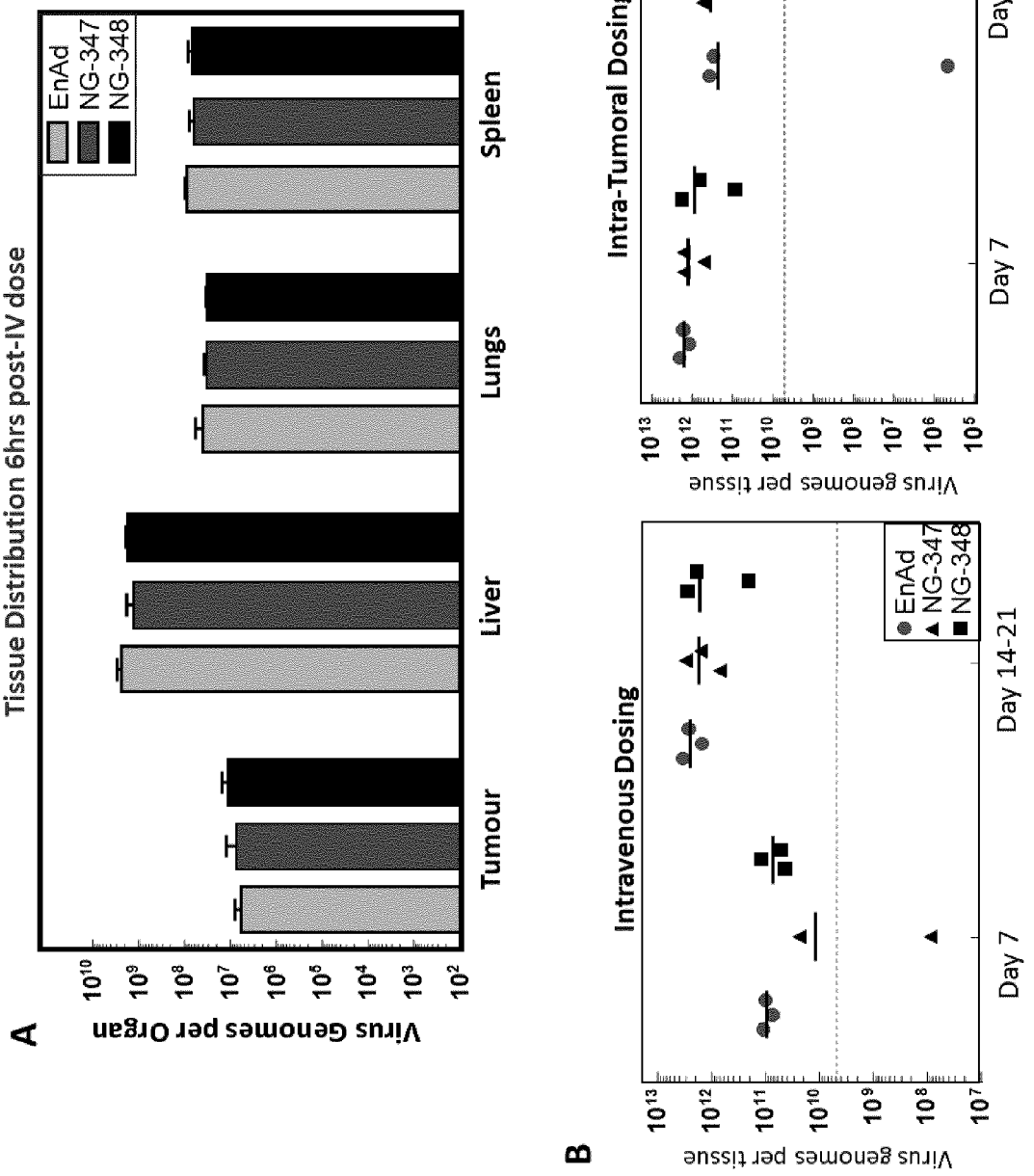

A
Tissue Distribution 6hrs post-IV dose
Virus Genomes per Organ
EnAd
NG-347
NG-348
Tumour
Liver
Lungs
Spleen
B
Intravenous Dosing
Intra-Tumoral Dosing
Virus genomes per tissue
Day 7
Day 14-21
Virus Input

Figure 35

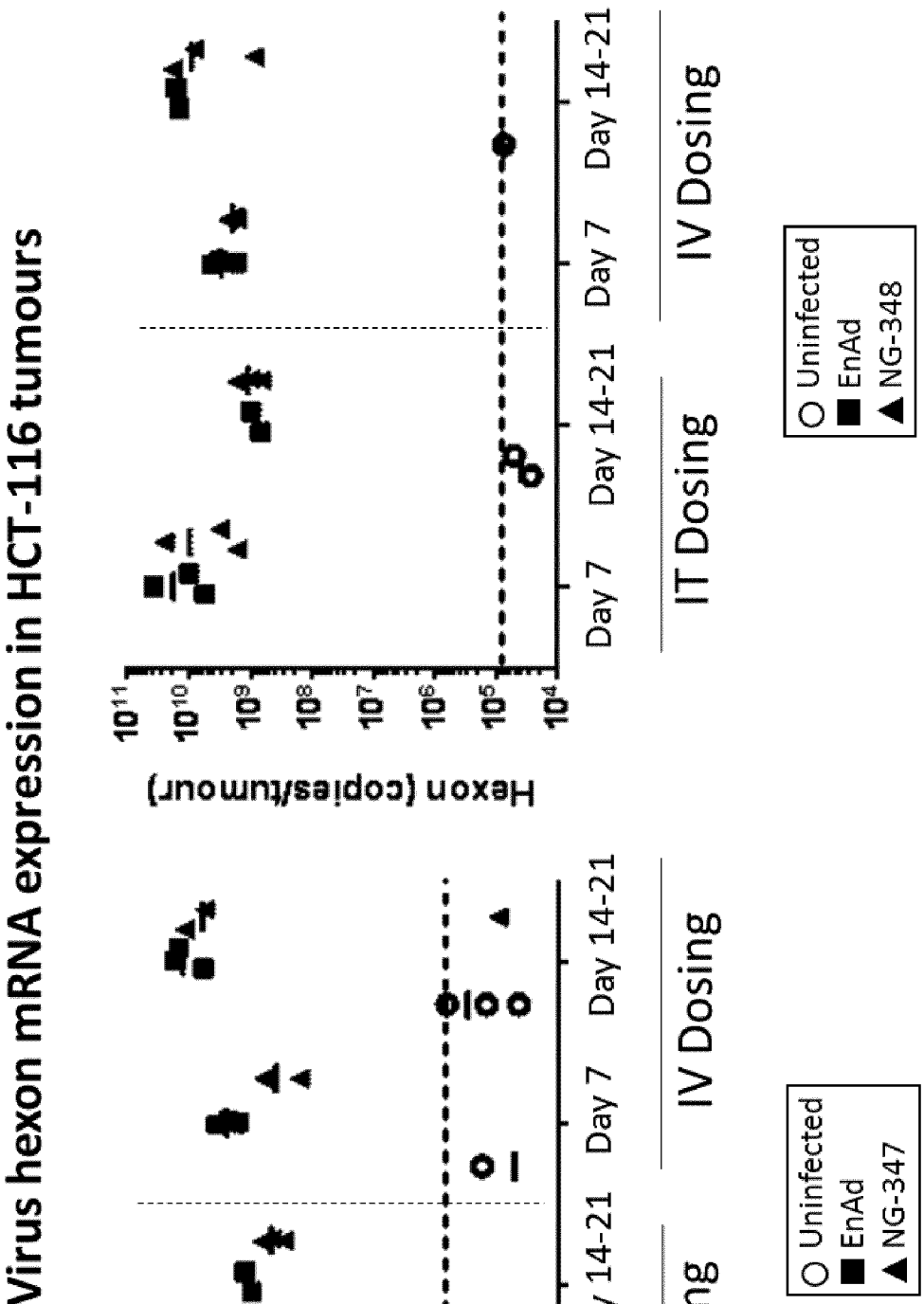

Virus hexon mRNA expression in HCT-116 tumours
Hexon (copies/tumour)
Day 7
Day 14-21
Day 7
Day 14-21
IT Dosing
IV Dosing
Uninfected
EnAd
NG-347
NG-348

Figure 36

Figure 37

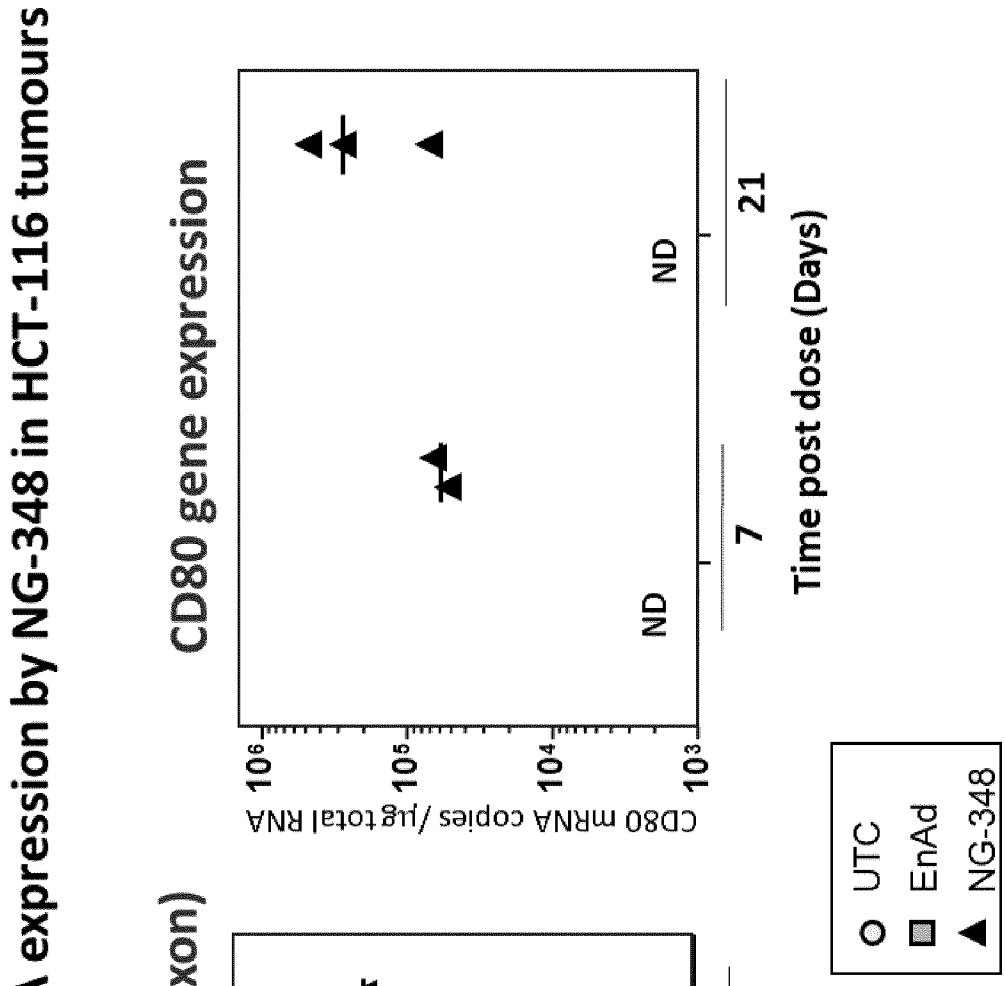

Hexon and CD80 transgene mRNA expression by NG-348 in HCT-116 tumours
Viral Gene expression (Hexon)
Hexon mRNA Copies/µg total RNA
10^7
10^6
10^5
10^4
10^3
ND
ND
7
21
Time post dose (Days)
CD80 gene expression
CD80 mRNA copies /µg total RNA
10^6
10^5
10^4
10^3
ND
ND
7
21
Time post dose (Days)
UTC
EnAd
NG-348

Figure 38

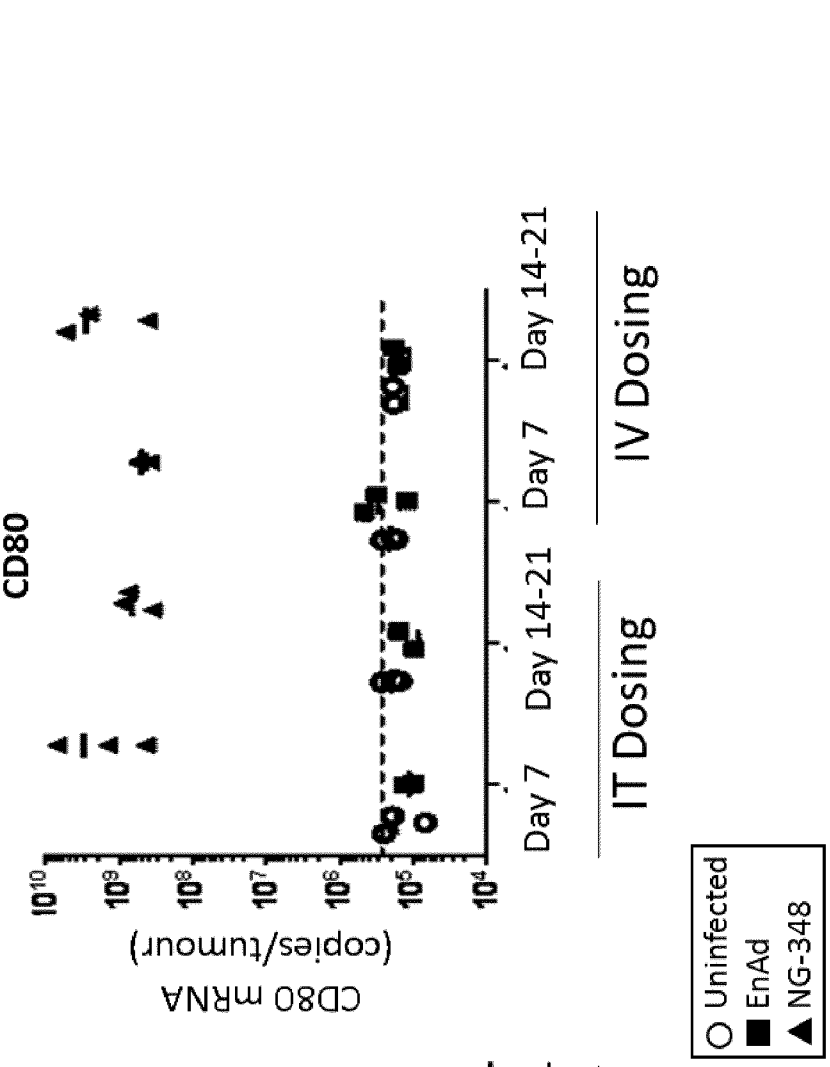

Anti-CD3-ScFv and CD80 transgene mRNA expression by NG-348 in HCT-116 tumours
Anti-CD3-ScFv mRNA (copies/tumour)
Anti-CD3-ScFv
Tissue Assay Background
CD80 mRNA (copies/tumour)
CD80
Day 7
Day 14-21
Day 7
Day 14-21
IT Dosing
IV Dosing
Uninfected
EnAd
NG-348

Figure 39

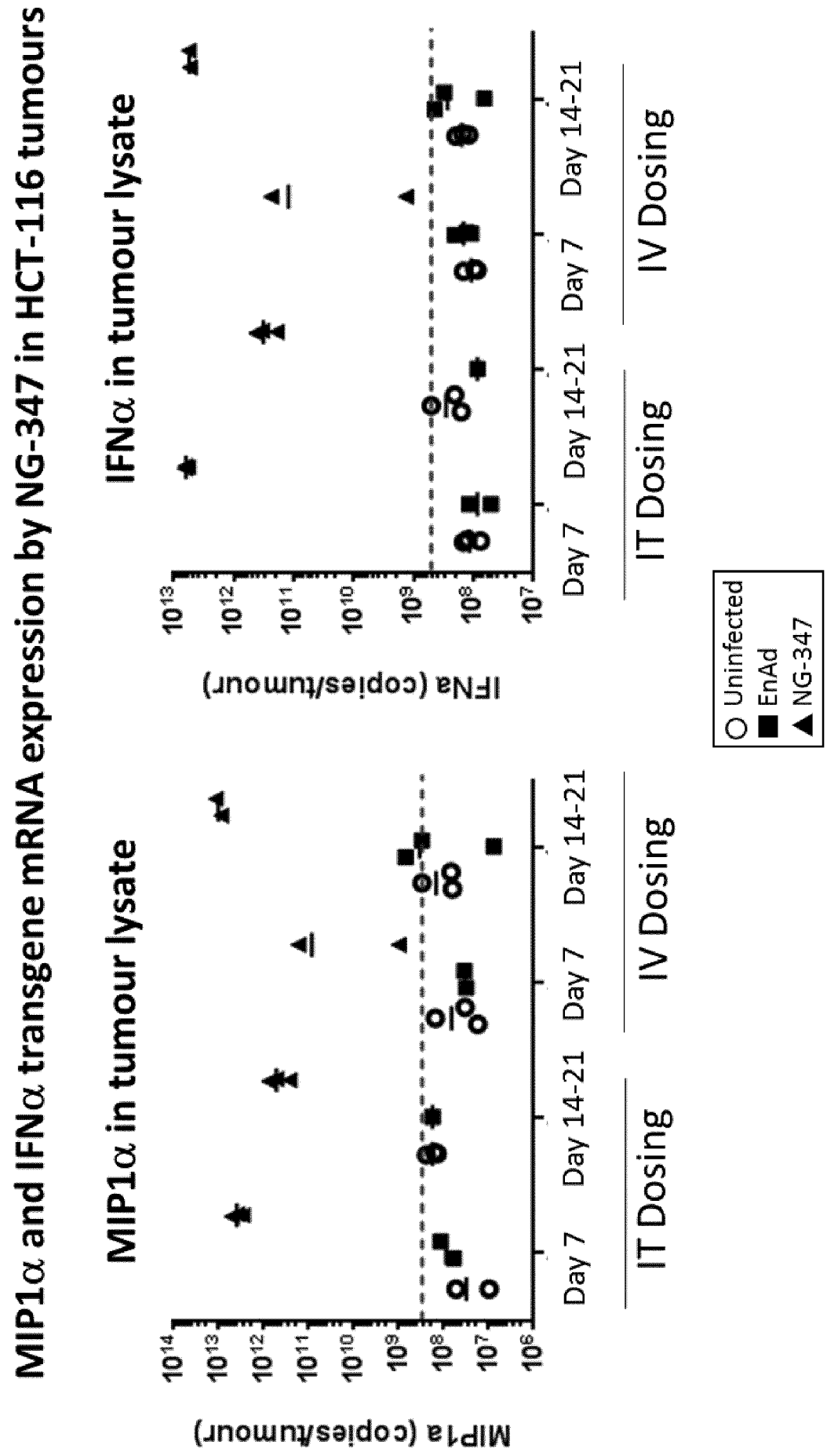

MIP1α and IFNα transgene mRNA expression by NG-347 in HCT-116 tumours
MIP1α in tumour lysate
MIP1a (copies/tumour)
IFNα in tumour lysate
IFNa (copies/tumour)
Day 7
Day 14-21
Day 7
Day 14-21
IT Dosing
IV Dosing
Uninfected
EnAd
NG-347

Figure 40

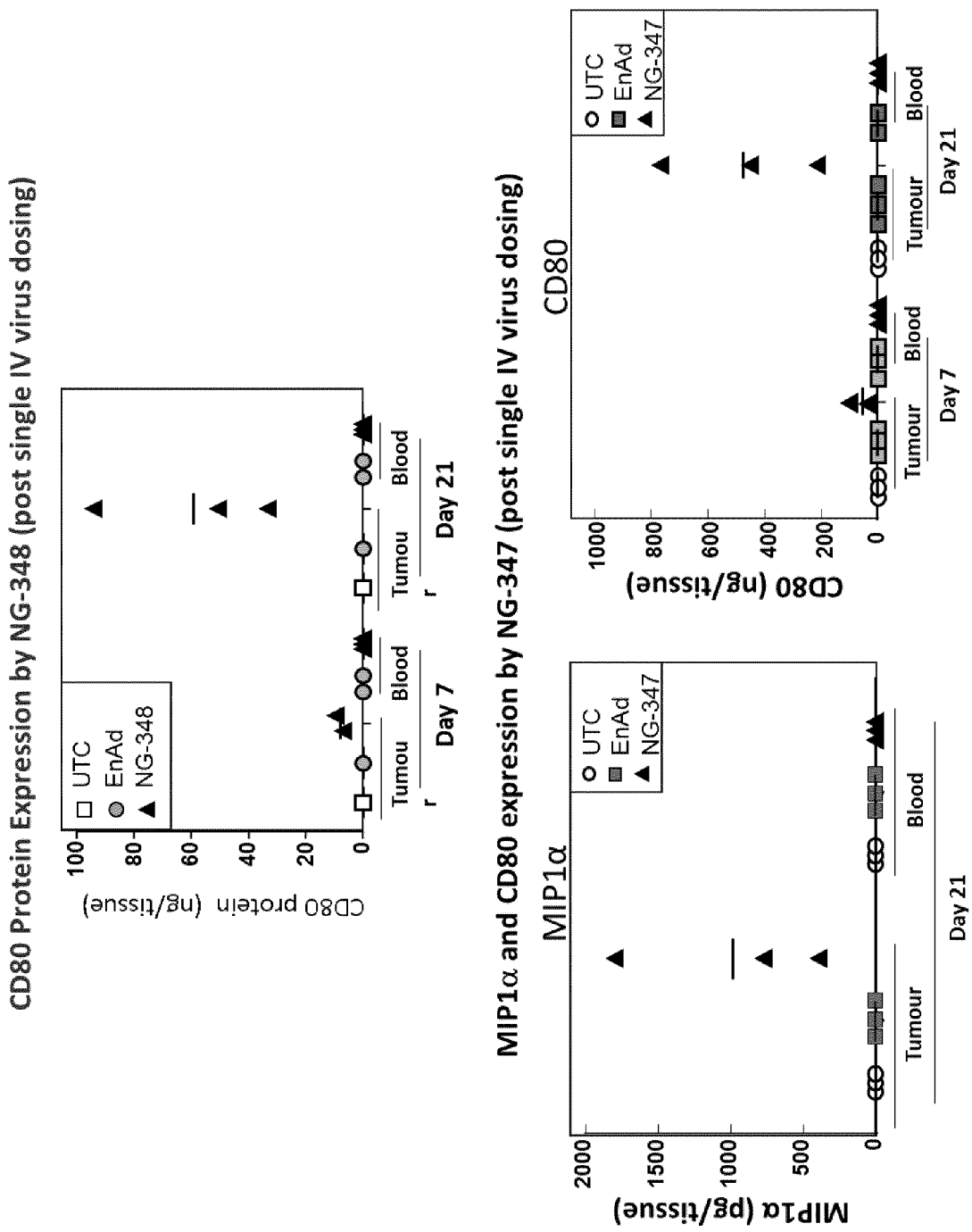

CD80 Protein Expression by NG-348 (post single IV virus dosing)
UTC
EnAd
NG-348
CD80 protein (ng/tissue)
100
80
60
40
20
0
Tumou
Blood
Tumou
Blood
Day 7
Day 21
MIP1α and CD80 expression by NG-347 (post single IV virus dosing)
MIP1α
UTC
EnAd
NG-347
MIP1α (pg/tissue)
2000
1500
1000
500
0
Tumour
Blood
Day 21
CD80
UTC
EnAd
NG-347
CD80 (ng/tissue)
1000
800
600
400
200
0
Tumour
Blood
Tumour
Blood
Day 7
Day 21

Figure 41

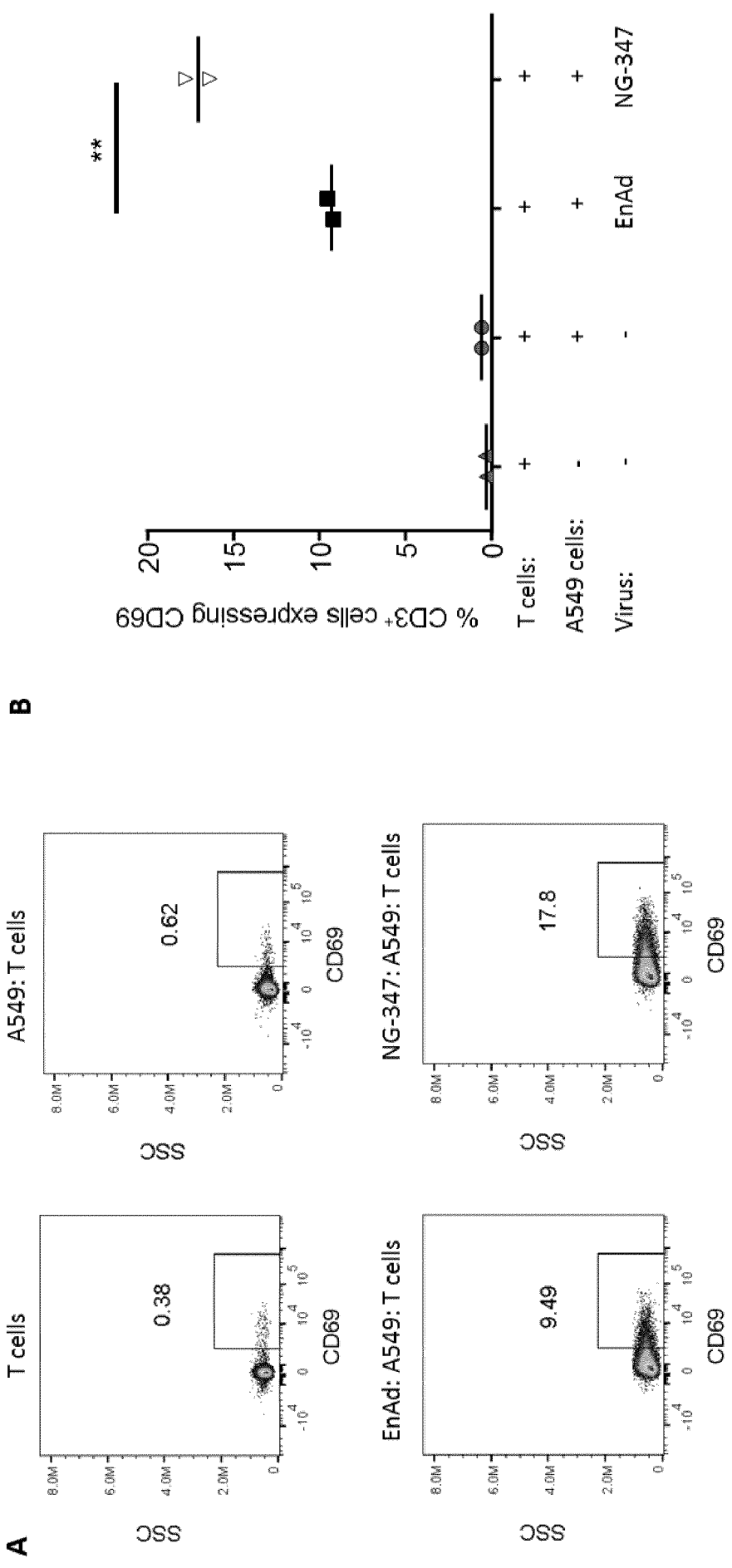
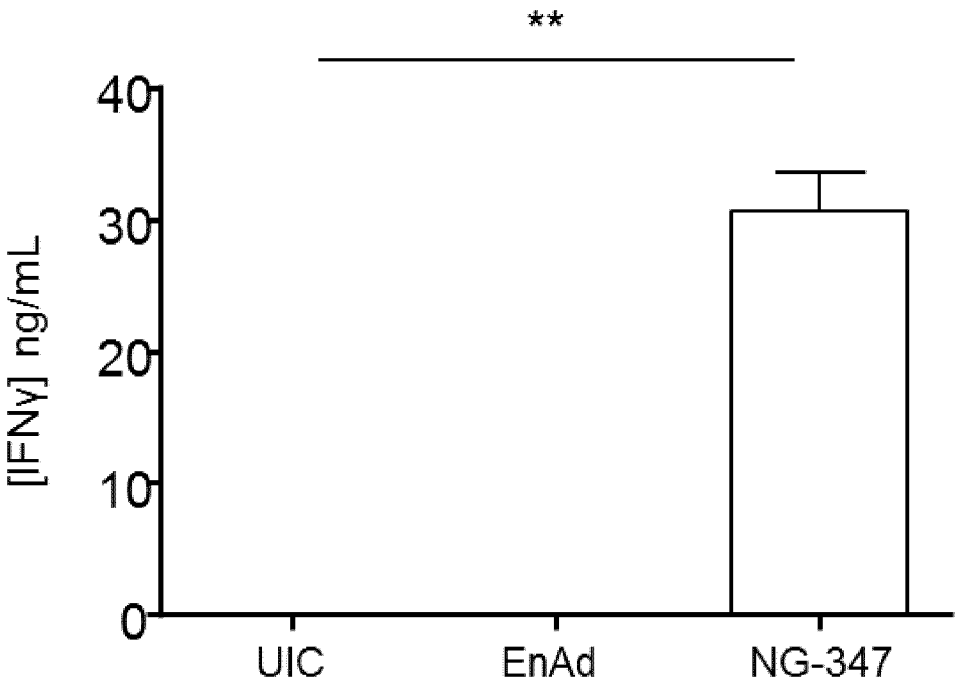

A
T cells
0.38
A549: T cells
0.62
EnAd: A549: T cells
9.49
NG-347: A549: T cells
17.8
SSC
CD69
B
% CD3+ cells expressing CD69
**
T cells:
A549 cells:
Virus:
EnAd
NG-347

Figure 42



VIRUS	TITRE (vp/mL)	INFECTIOUS TITRE (IFU/mL)	Infectivity Ratio (P:I)
EnAd (Ref 1)	3e12	7.83e10	38.3
EnAd (Ref 2)	3e12	9.37e10	32.0
NG-348	6.68e12	3.31e11	20.2

Figure 43

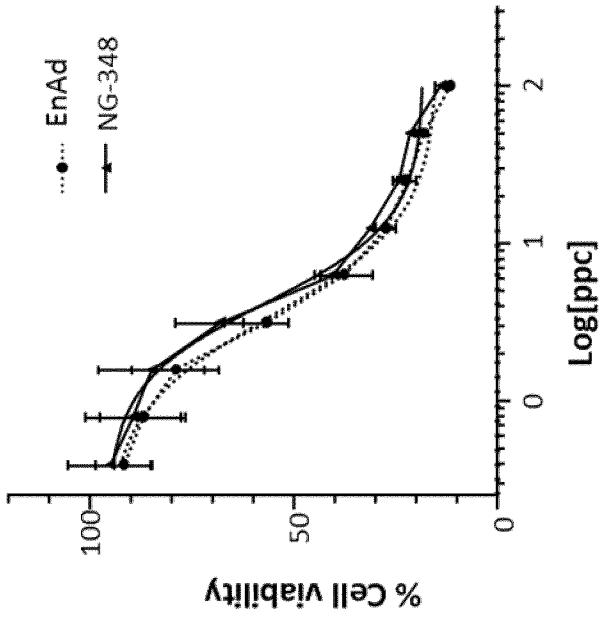


Figure 44

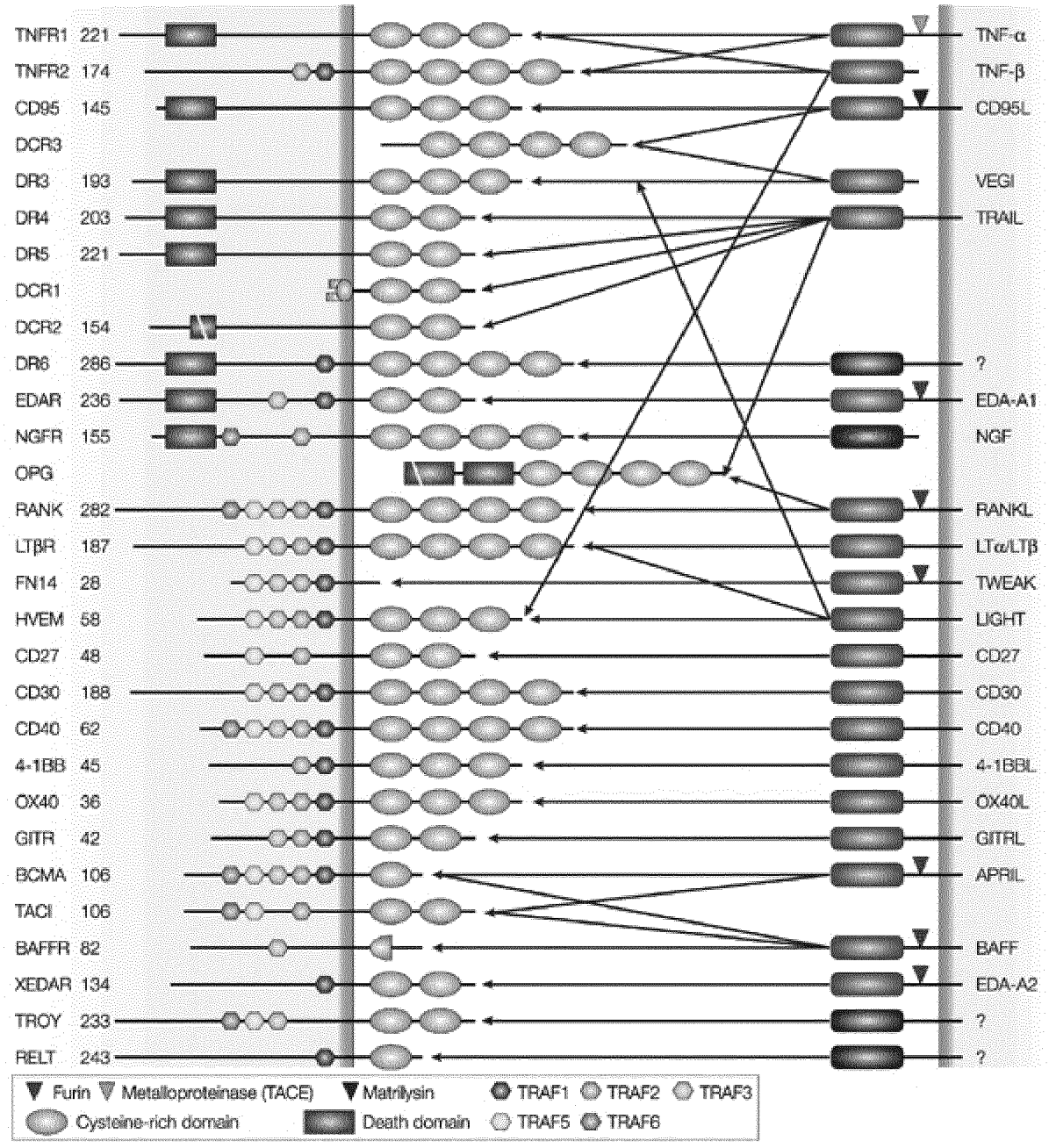


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Figure 45

SEQ ID NO: 1 Muromonab-CD3 (OKT3) VH

QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKAT
LTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS

SEQ ID NO: 2 Muromonab-CD3 (OKT3) VL

DIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLASGVPAHFRGSGSGTS
YSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINR

SEQ ID NO: 3 Muromonab –CD3 (OKT3) single chain Fv

QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKAT
LTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSGGGGSGGGGSGGGGSDIVL
TQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLASGVPAHFRGSGSGTSYSLT
ISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINR

SEQ ID NO: 4 Membrane anchored form of the anti-human CD3 single chain Fv

MGWSCIILFLVATATGVHSQVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGY
INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS
GGGGSGGGGSGGGGSDIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLA
SGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINRGSEQKLISEEDLNAVG
QDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

SEQ ID NO: 5 Membrane anchored form of anti-human CD3 single chain Fv with C-terminal V5 tag

MGWSCIILFLVATATGVHSQVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGY
INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS
GGGGSGGGGSGGGGSDIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLA
SGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINRGSEQKLISEEDLNAVG
QDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPRGS**IPNPLLGLD** **Tag in bold**

SEQ ID NO: 6 Teplizumab VH sequence

QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQKVKDRFT
ISRDNSKNTAFLQMDSLRPEDTGVYFCARYYDDHYCLDYWGQGTPVTVSS

Figure 46

SEQ ID NO: 7 Teplizumab VL sequence

DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTD
YTFTISSLQPEDIATYYCQQWSSNPFTFGQGTKLQITR

SEQ ID NO: 8 Teplizumab Heavy chain sequence

QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQKVKDRFT
ISRDNSKNTAFLQMDSLRPEDTGVYFCARYYDDHYCLDYWGQGTPVTVSSASTKGPSVFPLAPSSKSTS
GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK
PSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP
REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 9 Teplizumab Light Chain Sequence

DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTD
YTFTISSLQPEDIATYYCQQWSSNPFTFGQGTKLQITRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF
YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC

SEQ ID NO: 15 PDGFR TM Domain

AVGQDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

SEQ ID NO: 18 PDGFR TM Domain with N-terminal c-myc tag

gsEQKLISEEDLnAVGQDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

Figure 47

SEQ ID NO: 19 HuVH human VH leader sequence

MGWSCIILFLVATATGVHS

SEQ ID NO. 21 EnAd Genome

TCTATCTATATAATATACCTTATAGATGGAATGGTGCCAATATGTAAATGAGGTGATTTTAAAAAGTGT
GGATCGTGTGGTGATTGGCTGTGGGGTTAACGGCTAAAAGGGGCGGTGCGACCGTGGGAAAATGACGTT
TTGTGGGGGTGGAGTTTTTTTGCAAGTTGTCGCGGGAAATGTGACGCATAAAAAGGCTTTTTTCTCACG
GAACTACTTAGTTTTCCCACGGTATTTAACAGGAAATGAGGTAGTTTTGACCGGATGCAAGTGAAAATT
GTTGATTTTCGCGCGAAAACTGAATGAGGAAGTGTTTTTCTGAATAATGTGGTATTTATGGCAGGGTGG
AGTATTTGTTCAGGGCCAGGTAGACTTTGACCCATTACGTGGAGGTTTCGATTACCGTGTTTTTTACCT
GAATTTCCGCGTACCGTGTCAAAGTCTTCTGTTTTTACGTAGGTGTCAGCTGATCGCTAGGGTATTTAT
ACCTCAGGGTTTGTGTCAAGAGGCCACTCTTGAGTGCCAGCGAGAAGAGTTTTCTCCTCTGCGCCGGCA
GTTTAATAATAAAAAAATGAGAGATTTGCGATTTCTGCCTCAGGAAATAATCTCTGCTGAGACTGGAAA
TGAAATATTGGAGCTTGTGGTGCACGCCCTGATGGGAGACGATCCGGAGCCACCTGTGCAGCTTTTTGA
GCCTCCTACGCTTCAGGAACTGTATGATTTAGAGGTAGAGGGATCGGAGGATTCTAATGAGGAAGCTGT
AAATGGCTTTTTTACCGATTCTATGCTTTTAGCTGCTAATGAAGGGTTAGAATTAGATCCGCCTTTGGA
CACTTTTGATACTCCAGGGGTAATTGTGGAAAGCGGTACAGGTGTAAGAAAATTACCTGATTTGAGTTC
CGTGGACTGTGATTTGCACTGCTATGAAGACGGGTTTCCTCCGAGTGATGAGGAGGACCATGAAAAGGA
GCAGTCCATGCAGACTGCAGCGGGTGAGGGAGTGAAGGCTGCCAATGTTGGTTTTCAGTTGGATTGCCC
GGAGCTTCCTGGACATGGCTGTAAGTCTTGTGAATTTCACAGGAAAAATACTGGAGTAAAGGAACTGTT
ATGTTCGCTTTGTTATATGAGAACGCACTGCCACTTTATTTACAGTAAGTGTGTTTAAGTTAAAATTTA
AAGGAATATGCTGTTTTTCACATGTATATTGAGTGTGAGTTTTGTGCTTCTTATTATAGGTCCTGTGTC
TGATGCTGATGAATCACCATCTCCTGATTCTACTACCTCACCTCCTGAGATTCAAGCACCTGTTCCTGT
GGACGTGCGCAAGCCCATTCCTGTGAAGCTTAAGCCTGGGAAACGTCCAGCAGTGGAAAAACTTGAGGA
CTTGTTACAGGGTGGGGACGGACCTTTGGACTTGAGTACACGGAAACGTCCAAGACAATAAGTGTTCCA
TATCCGTGTTTACTTAAGGTGACGTCAATATTTGTGTGACAGTGCAATGTAATAAAAATATGTTAACTG
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Figure 48

SEQ ID NO: 87 DNA CORRESPONDING TO E2B REGION OF THE ENAD GENOME (BP 10355-5068)

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GAGCCTTTCTGTGCGAGGATGAGAGCCAATCGGGAAGAACTGGATCTCCTGCCACCAGTTGGAGGAATG
GCTGTTGATGTGATGGAAGTAGAACTCCCTGCGACGCGCCGAGCATTCATGCTTGTGCTTGTACAGACG
GCCGCAGTAGTCGCAGCGTTGCACGGGTTGTATCTCGTGAATGAGTTGTACCTGGCTTCCCTTGACGAG
AAATTTCAGTGGGAAGCCGAGGCCTGGCGATTGTATCTCGTGCTTTACTATGTTGTCTGCATCGGCCTG
TTCATCTTCTGTCTCGATGGTGGTCATGCTGACGAGCCCTCGCGGGAGGCAAGTCCAGACCTCGGCGCG
GCAGGGGCGGAGCTCGAGGACGAGAGCGCGCAGGCTGGAGCTGTCCAGGGTCCTGAGACGCTGCGGACT
CAGGTTAGTAGGCAGTGTCAGGAGATTAACTTGCATGATCTTTTGGAGGGCGTGCGGGAGGTTCAGATA
GTACTTGATCTCAACGGGTCCGTTGGTGGAGATGTCGATGGCTTGCAGGGTTCCGTGTCCCTTGGGCGC
TACCACCGTGCCCTTGTTTTTCATTTTGGACGGCGGTGGCTCTGTTGCTTCTTGCATGTTTAGAAGCGG
TGTCGAGGGCGCGCACCGGGCGGCAGGGGCGGCTCGGGACCCGGCGGCATGGCTGGCAGTGGTACGTCG
GCGCCGCGCGCGGGTAGGTTCTGGTACTGCGCCCTGAGAAGACTCGCATGCGCGACGACGCGGCGGTTG
ACATCCTGGATCTGACGCCTCTGGGTGAAAGCTACCGGCCCCGTGAGCTTGAACCTGAAAGAGAGTTCA
ACAGAATCAATCTCGGTATCGTTGACGGCGGCTTGCCTAAGGATTTCTTGCACGTCACCAGAGTTGTCC
TGGTAGGCGATCTCCGCCATGAACTGCTCGATCTCTTCCTCTTGAAGATCTCCGCGGCCCGCTCTCTCG
ACGGTGGCCGCGAGGTCGTTGGAGATGCGCCCAATGAGTTGAGAGAATGCATTCATGCCCGCCTCGTTC
CAGACGCGGCTGTAGACCACGGCCCCCACGGGATCTCTCGCGCGCATGACCACCTGGGCGAGGTTGAGC
TCCACGTGGCGGGTGAAGACCGCATAGTTGCATAGGCGCTGGAAAAGGTAGTTGAGTGTGGTGGCGATG
TGCTCGGTGACGAAGAAATACATGATCCATCGTCTCAGCGGCATCTCGCTGACATCGCCCAGAGCTTCC
AAGCGCTCCATGGCCTCGTAGAAGTCCACGGCAAAATTAAAAAACTGGGAGTTTCGCGCGGACACGGTC
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GGAACGCGGCGACGCCGGCGGCGCACGGGCAGACGGTCGATGAATCTTTCAATGACCTCTCCGCGGCGG
CGGCGCATGGTTTCAGTGACGGCGCGGCCGTTCTCGCGCGGTCGCAGAGTAAAAACACCGCCGCGCATC
TCCTTAAAGTGGTGACTGGGAGGTTCTCCGTTTGGGAGGGAGAGGGCGCTGATTATACATTTTATTAAT
TGGCCCGTAGGGACTGCACGCAGAGATCTGATCGTGTCAAGATCCACGGGATCTGAAAACCTTTCGACG
AAAGCGTCTAACCAGTCACAGTCACAAGGTAGGCTGAGTACGGCTTCTTGTGGGCGGGGGTGGTTATGT
GTTCGGTCTGGGTCTTCTGTTTCTTCTTCATCTCGGGAAGGTGAGACGATGCTGCTGGTGATGAAATTA
AAGTAGGCAGTTCTAAGACGGCGGATGGTGGCGAGGAGCACCAGGTCTTTGGGTCCGGCTTGCTGGATA
CGCAGGCGATTGGCCATTCCCCAAGCATTATCCTGACATCTAGCAAGATCTTTGTAGTAGTCTTGCATG
AGCCGTTCTACGGGCACTTCTTCCTCACCCGTTCTGCCATGCATACGTGTGAGTCCAAATCCGCGCATT
GGTTGTACCAGTGCCAAGTCAGCTACGACTCTTTCGGCGAGGATGGCTTGCTGTACTTGGGTAAGGGTG
GCTTGAAAGTCATCAAAATCCACAAAGCGGTGGTAAGCTCCTGTATTAATGGTGTAAGCACAGTTGGCC
ATGACTGACCAGTTAACTGTCTGGTGACCAGGGCGCACGAGCTCGGTGTATTTAAGGCGCGAATAGGCG
CGGGTGTCAAAGATGTAATCGTTGCAGGTGCGCACCAGATACTGGTACCCTATAAGAAAATGCGGCGGT
GGTTGGCGGTAGAGAGGCCATCGTTCTGTAGCTGGAGCGCCAGGGGCGAGGTCTTCCAACATAAGGCGG
TGATAGCCGTAGATGTACCTGGACATCCAGGTGATTCCTGCGGCGGTAGTAGAAGCCCGAGGAAACTCG
CGTACGCGGTTCCAAATGTTGCGTAGCGGCATGAAGTAGTTCAT

SEQ ID NO: 88 A NON-CODING SEQUENCE SUITABLE FOR INCLUSION INTO BX

AAAATGATTAATAAAAAATCACTTACTTGAAATCAGCAATAAGGTCTCTGTTGAAATTTTCTCCCAGCA
GCACCTCACTTCCCTCTTCCCAACTCTGGTATTCTAAACCCCGTTCAGCGGCATACTTTCTCCATACTT
TAAAGGGGATGTCAAATTTTAGCTCCTCTCCTGTACCCACAATCTTCATGTCTTTCTTCCCAG

SEQ ID NO: 89 A NON-CODING SEQUENCE SUITABLE FOR INCLUSION INTO BY

CAAATAAAGTTTAACTTGTTTATTTGAAAATCAA

SEQ ID NO. 90 SPLICE ACCEPTOR SEQUENCE

TTTCTCTCTT CAGG

SEQ ID NO. 91 SPLICE ACCEPTOR SEQUENCE

TGCTAATCTT CCTTTCTCTC TTCAGG

Figure 49

SEQ ID NO: 93 Internal Ribosome Entry Sequence (IRES)

CGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATT
GCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGTCT
TTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTC
TTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCT
CTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAG
TTGGATAGTTGTGGAAAGAGTCAAATGGCTCCCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCA
GAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTTCATGTGTTTAGTCGAG
GTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATGATAATA

SEQ ID NO: 94 High efficiency self-cleavable P2A peptide sequence

GSGATNFSLLKQAGDVEENPGP

SEQ ID NO: 95 High efficiency self-cleavable F2A peptide sequence

GSGEGRGSLLTCGDVEENPGP

SEQ ID NO: 96 High efficiency self-cleavable E2A peptide sequence

GSGQCTNYALLKLAGDVESNPGP

SEQ ID NO: 97 High efficiency self-cleavable T2A peptide sequence

GSGVKQTLNFDLLKLAGDVESNPGP

SEQ ID NO: 98 Human CD80 amino acid sequence

MGHTRRQGTSPSKCPYLNFFQLLVLAGLSHFCSGVIHVTKEVKEVATLSCGHNVSVEELAQTRIYWQKE
KKMVLTMMSGDMNIWPEYKNRTIFDITNNLSIVILALRPSDEGTYECVVLKYEKDAFKREHLAEVTLSV
KADFPTPSISDFEIPTSNIRRIICSTSGGFPEPHLSWLENGEELNAINTTVSQDPETELYAVSSKLDFN
MTTNHSFMCLIKYGHLRVNQTFNWNTTKQEHFPDNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRR
NERLRRESVRPV

SEQ ID NO. 99 poly adenylation sequence (SV40 late polyA sequence)

CAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTA
TTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACA
ACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTT

SEQ ID NO. 100

Figure 50

NG-348 virus genome sequence comprising the EnAd genome with a transgene cassette that encodes a membrane-anchored chimeric form of the single chain Fv anti-human CD3e and the T lymphocyte activation antigen, CD80 inserted in the region B_γ. The transgene cassette contains a 5' SSA, membrane-anchored anti-$CD3_\varepsilon$ cDNA sequence, P2A peptide, human CD80 cDNA sequence and a 3' poly(A) sequence

TCTATCTATATAATATACCTTATAGATGGAATGGTGCCAATATGTAAATGAGGTGATTTTAAAAAGTGT
GGATCGTGTGGTGATTGGCTGTGGGGTTAACGGCTAAAAGGGGCGGTGCGACCGTGGGAAAATGACGTT
TTGTGGGGGTGGAGTTTTTTTGCAAGTTGTCGCGGGAAATGTGACGCATAAAAAGGCTTTTTTCTCACG
GAACTACTTAGTTTTCCCACGGTATTTAACAGGAAATGAGGTAGTTTTGACCGGATGCAAGTGAAAATT
GTTGATTTTCGCGCGAAAACTGAATGAGGAAGTGTTTTTCTGAATAATGTGGTATTTATGGCAGGGTGG
AGTATTTGTTCAGGGCCAGGTAGACTTTGACCCATTACGTGGAGGTTTCGATTACCGTGTTTTTTACCT
GAATTTCCGCGTACCGTGTCAAAGTCTTCTGTTTTTACGTAGGTGTCAGCTGATCGCTAGGGTATTTAT
ACCTCAGGGTTTGTGTCAAGAGGCCACTCTTGAGTGCCAGCGAGAAGAGTTTTCTCCTCTGCGCCGGCA
GTTTAATAATAAAAAAATGAGAGATTTGCGATTTCTGCCTCAGGAAATAATCTCTGCTGAGACTGGAAA
TGAAATATTGGAGCTTGTGGTGCACGCCCTGATGGGAGACGATCCGGAGCCACCTGTGCAGCTTTTTGA
GCCTCCTACGCTTCAGGAACTGTATGATTTAGAGGTAGAGGGATCGGAGGATTCTAATGAGGAAGCTGT
AAATGGCTTTTTTACCGATTCTATGCTTTTAGCTGCTAATGAAGGGTTAGAATTAGATCCGCCTTTGGA

CACTTTTGATACTCCAGGGGTAATTGTGGAAAGCGGTACAGGTGTAAGAAAATTACCTGATTTGAGTTC
CGTGGACTGTGATTTGCACTGCTATGAAGACGGGTTTCCTCCGAGTGATGAGGAGGACCATGAAAAGGA
GCAGTCCATGCAGACTGCAGCGGGTGAGGGAGTGAAGGCTGCCAATGTTGGTTTTCAGTTGGATTGCCC
GGAGCTTCCTGGACATGGCTGTAAGTCTTGTGAATTTCACAGGAAAAATACTGGAGTAAAGGAACTGTT
ATGTTCGCTTTGTTATATGAGAACGCACTGCCACTTTATTTACAGTAAGTGTGTTTAAGTTAAAATTTA
AAGGAATATGCTGTTTTTCACATGTATATTGAGTGTGAGTTTTGTGCTTCTTATTATAGGTCCTGTGTC
TGATGCTGATGAATCACCATCTCCTGATTCTACTACCTCACCTCCTGAGATTCAAGCACCTGTTCCTGT
GGACGTGCGCAAGCCCATTCCTGTGAAGCTTAAGCCTGGGAAACGTCCAGCAGTGGAAAAACTTGAGGA
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TTCACTGGTTTTTATTGCTTTTTGGGCGGGGACTCAGGTATATAAGTAGAAGCAGACCTGTGTGGTTAG
CTCATAGGAGCTGGCTTTCATCCATGGAGGTTTGGGCCATTTTGGAAGACCTTAGGAAGACTAGGCAAC
TGTTAGAGAACGCTTCGGACGGAGTCTCCGGTTTTTGGAGATTCTGGTTCGCTAGTGAATTAGCTAGGG
TAGTTTTTAGGATAAAACAGGACTATAAACAAGAATTTGAAAAGTTGTTGGTAGATTGCCCAGGACTTT
TTGAAGCTCTTAATTTGGGCCATCAGGTTCACTTTAAAGAAAAAGTTTTATCAGTTTTAGACTTTTCAA
CCCCAGGTAGAACTGCTGCTGCTGTGGCTTTTCTTACTTTTATATTAGATAAATGGATCCCGCAGACTC
ATTTCAGCAGGGGATACGTTTTGGATTTCATAGCCACAGCATTGTGGAGAACATGGAAGGTTCGCAAGA
TGAGGACAATCTTAGGTTACTGGCCAGTGCAGCCTTTGGGTGTAGCGGGAATCCTGAGGCATCCACCGG
TCATGCCAGCGGTTCTGGAGGAGGAACAGCAAGAGGACAACCCGAGAGCCGGCCTGGACCCTCCAGTGG
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AATGAGTCGCAGACGTCCTGAAACCATTTGGTGGCATGAGGTTCAGAAAGAGGGAAGGGATGAAGTTTC
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CTTCGGGCCCCGGAGCATAGTTCCCCTCACAGATTTGCATTTCCCAAGCTTTCAGTTCCGAGGGTGGAA
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CGGTTCATTGGGGTCAAAAACAAGTTTTCCGCCATATTTTTTGATGCGTTTCTTACCTTTGGTCTCCAT
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GGCTTGCAGGGTTCCGTGTCCCTTGGGCGCTACCACCGTGCCCTTGTTTTTCATTTTGGACGGCGGTGG
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AAAAACTGGGAGTTTCGCGCGGACACGGTCAACTCCTCTTCCAGAAGACGGATAAGTTCGGCGATGGTG
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ACAGCCAATCACCACACGATCCACACTTTTTAAAATCACCTCATTTACATATTGGCACCATTCCATCTA
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SEQ ID NO: 101 V5 TAG

IPNPLLGLD

Figure 51

SEQUENCE ID NO. 102 NG-348A virus genome sequence comprising the EnAd genome with a transgene cassette that encodes a membrane-anchored chimeric form of the single chain Fv anti-human CD3e with C-terminal V5 tag and the T lymphocyte activation antigen, CD80 inserted in the region B_γ. The transgene cassette contains a 5' SSA, membrane-anchored anti-CD3Ɛ cDNA sequence, V5 tag, P2A peptide, human CD80 cDNA sequence and a 3' poly(A) sequence

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CACCTCATTTACATATTGGCACCATTCCATCTATAAGGTATATTATATAGATAGA

Figure 52

SEQ ID NO. 103 NG-420 virus genome sequence comprising the EnAd genome with a transgene cassette that encodes a membrane-anchored chimeric form of the single chain Fv anti-human CD3e inserted in the region B_γ. The transgene cassette contains a 5' SSA, membrane-anchored anti-CD3Ɛ cDNA sequence and a 3' poly(A) sequence

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Figure 53

SEQ ID NO. 104 NG-420A virus genome sequence comprising the EnAd genome with a transgene cassette that encodes a membrane-anchored chimeric form of the single chain Fv anti-human CD3e and a C-terminal V5 tag, inserted in the region B_{γ}. The transgene cassette contains a 5' SSA, membrane-anchored anti-CD3Ɛ cDNA sequence, V5 tag sequence and a 3' poly(A) sequence.

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