Figure 1 **Antigen Presenting Cell or Tumor Cell**

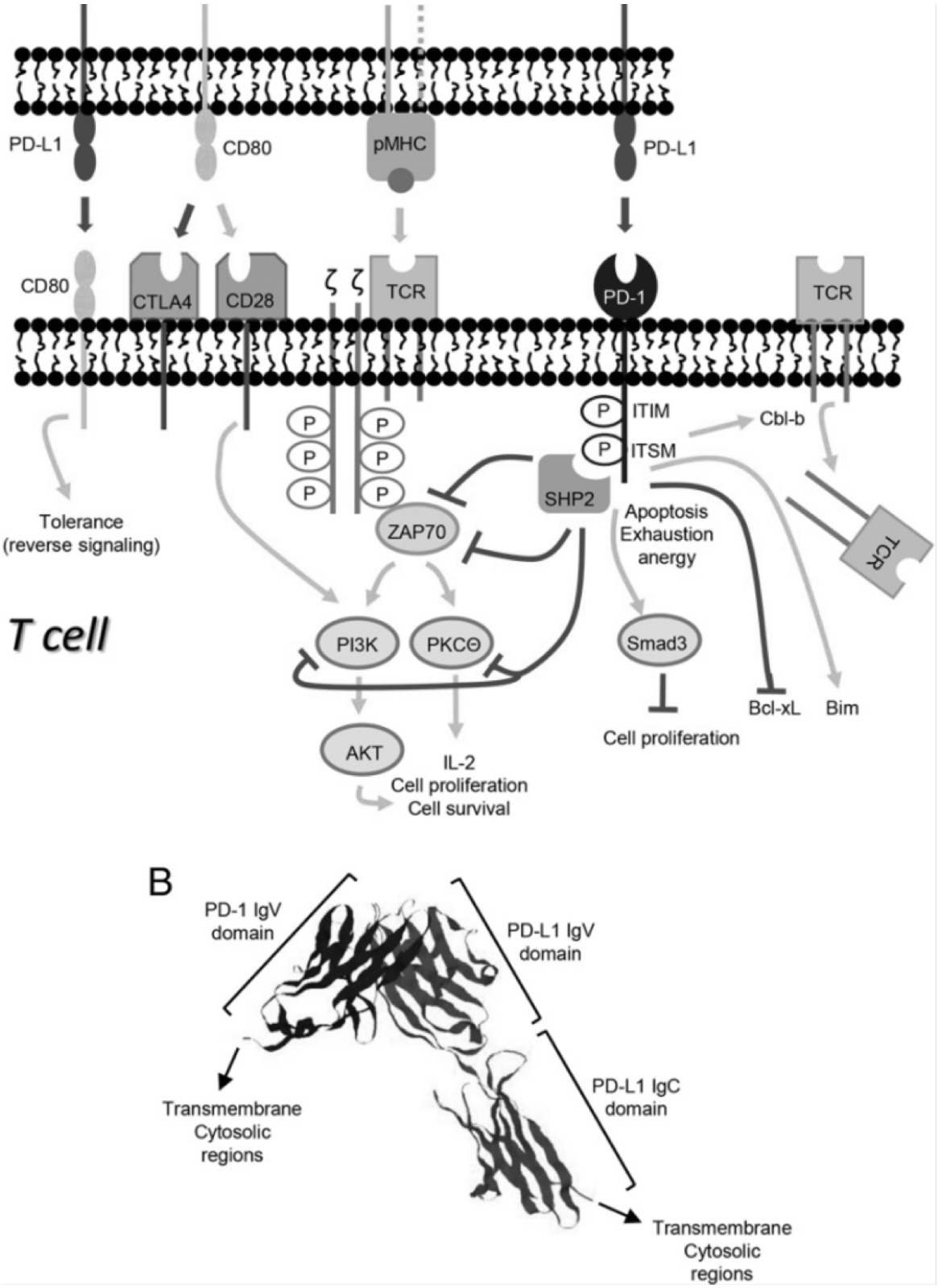


Figure 2

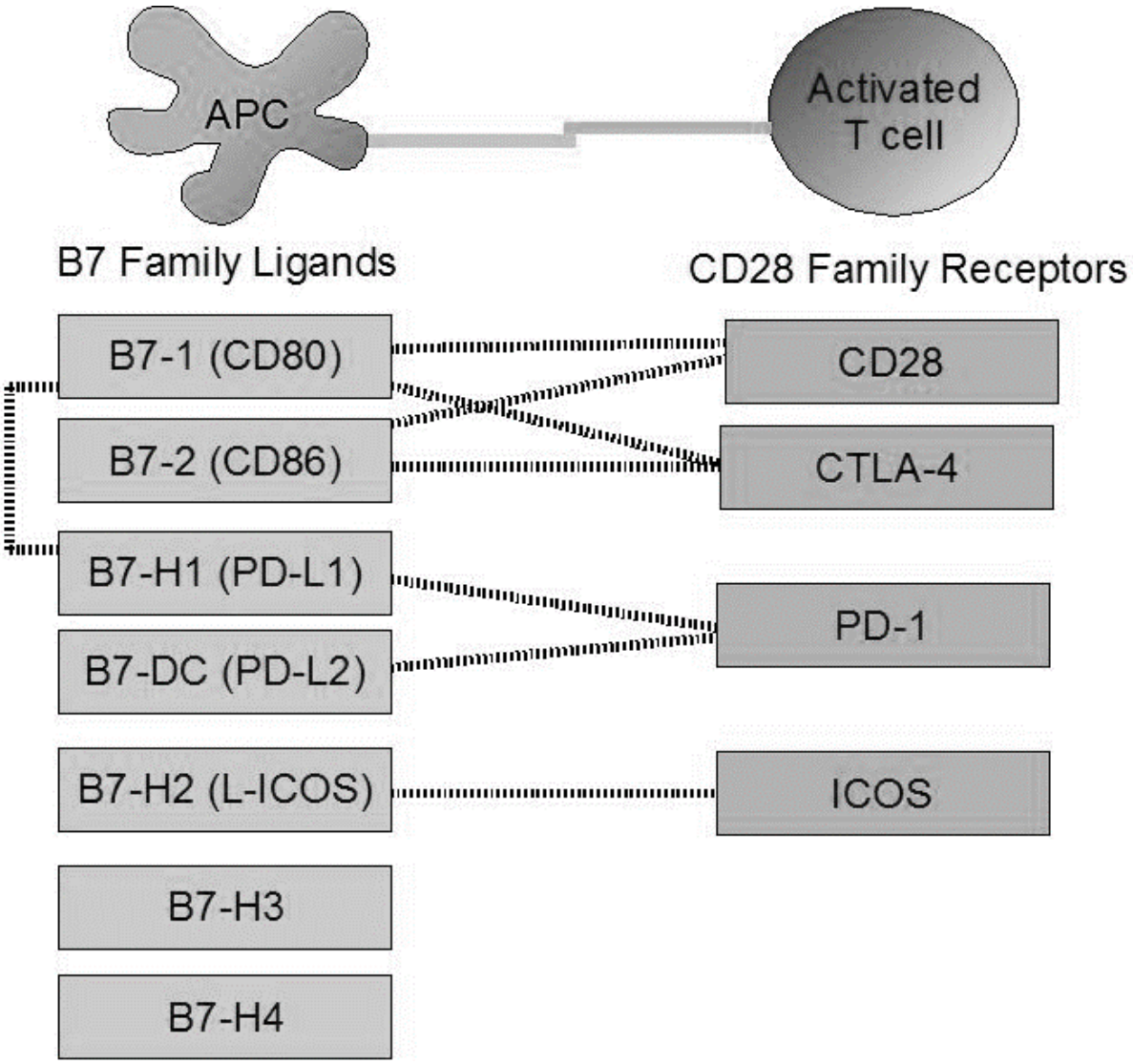

APC
Activated T cell
B7 Family Ligands
CD28 Family Receptors
B7-1 (CD80)
B7-2 (CD86)
B7-H1 (PD-L1)
B7-DC (PD-L2)
B7-H2 (L-ICOS)
B7-H3
B7-H4
CD28
CTLA-4
PD-1
ICOS

Figure 3A

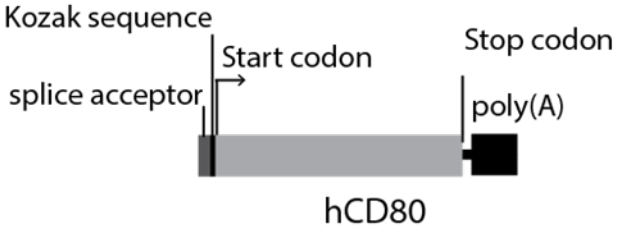


Figure 3B

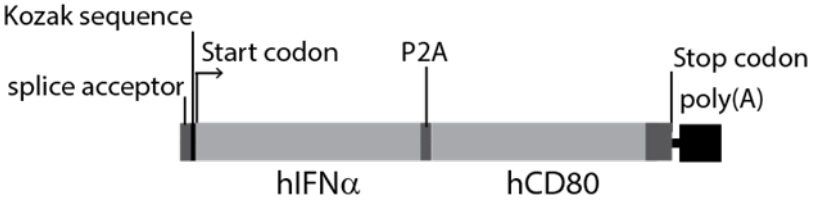


Figure 3C

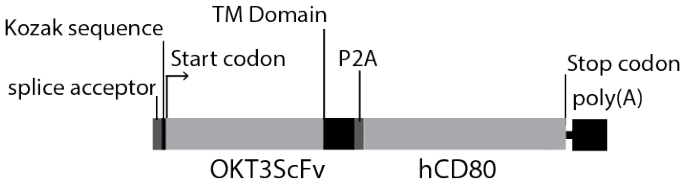


Figure 3D

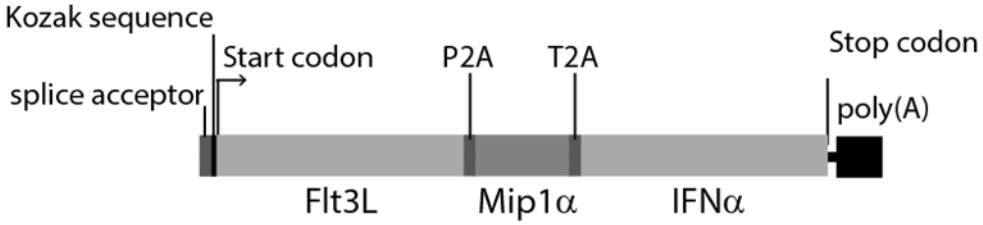


Figure 3E

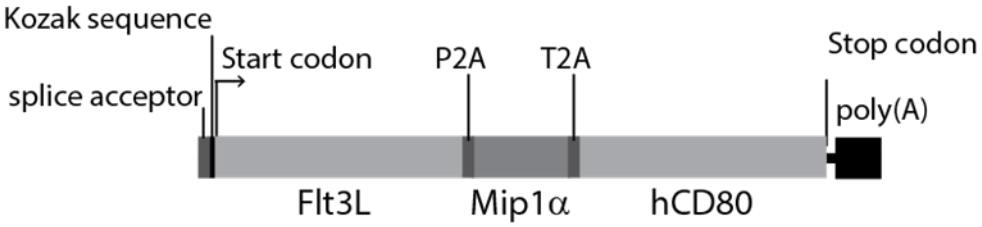


Figure 3F

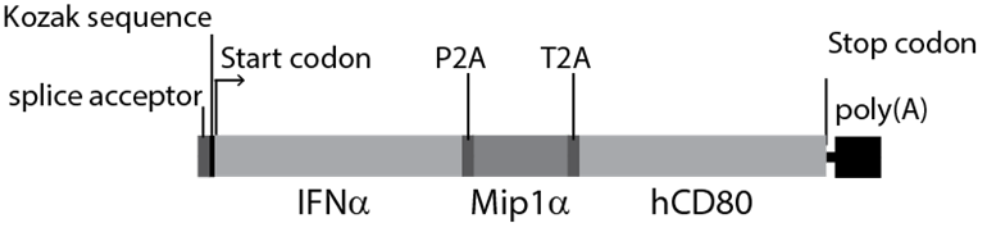


Figure 3G ORF cassette for scFv antibody

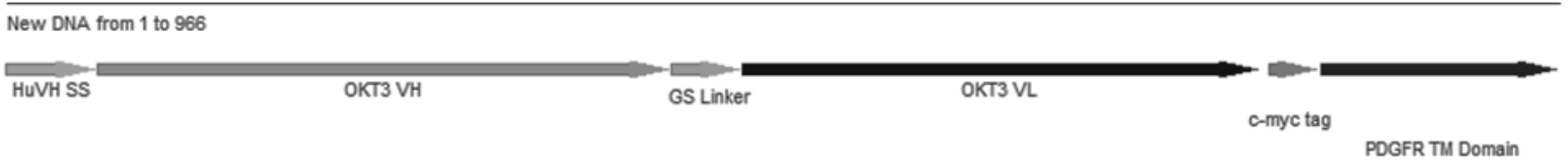


Figure 4A

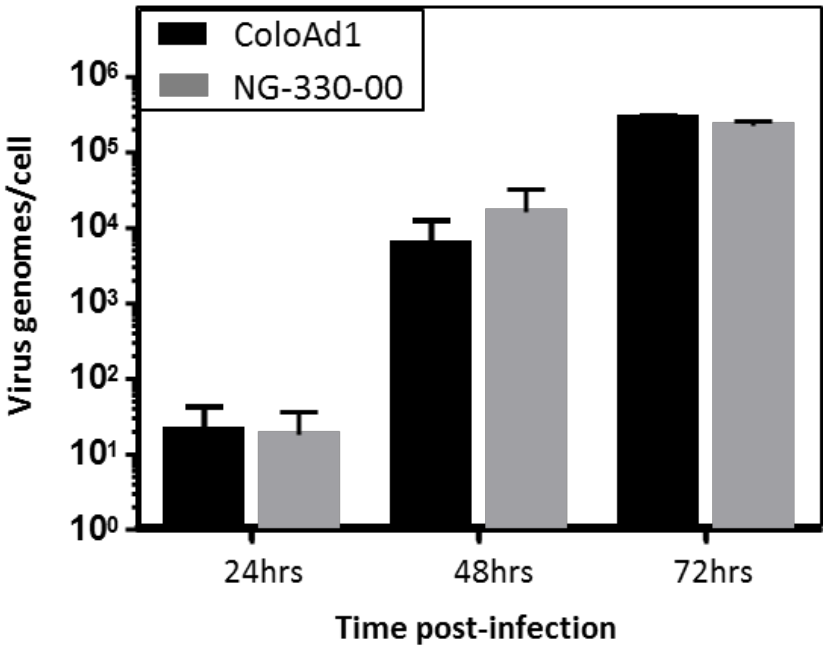


Figure 4B

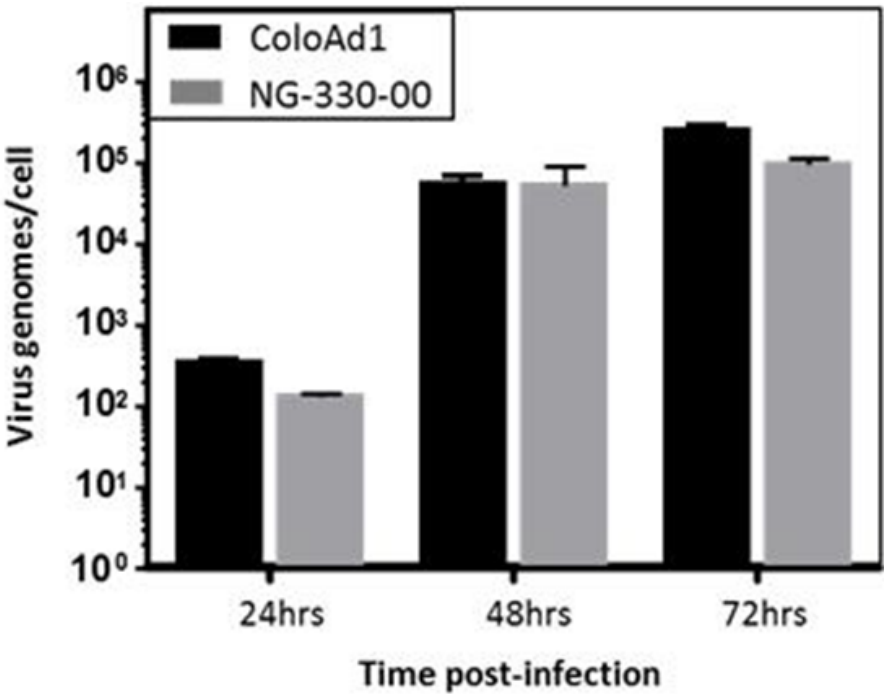


Figure 5

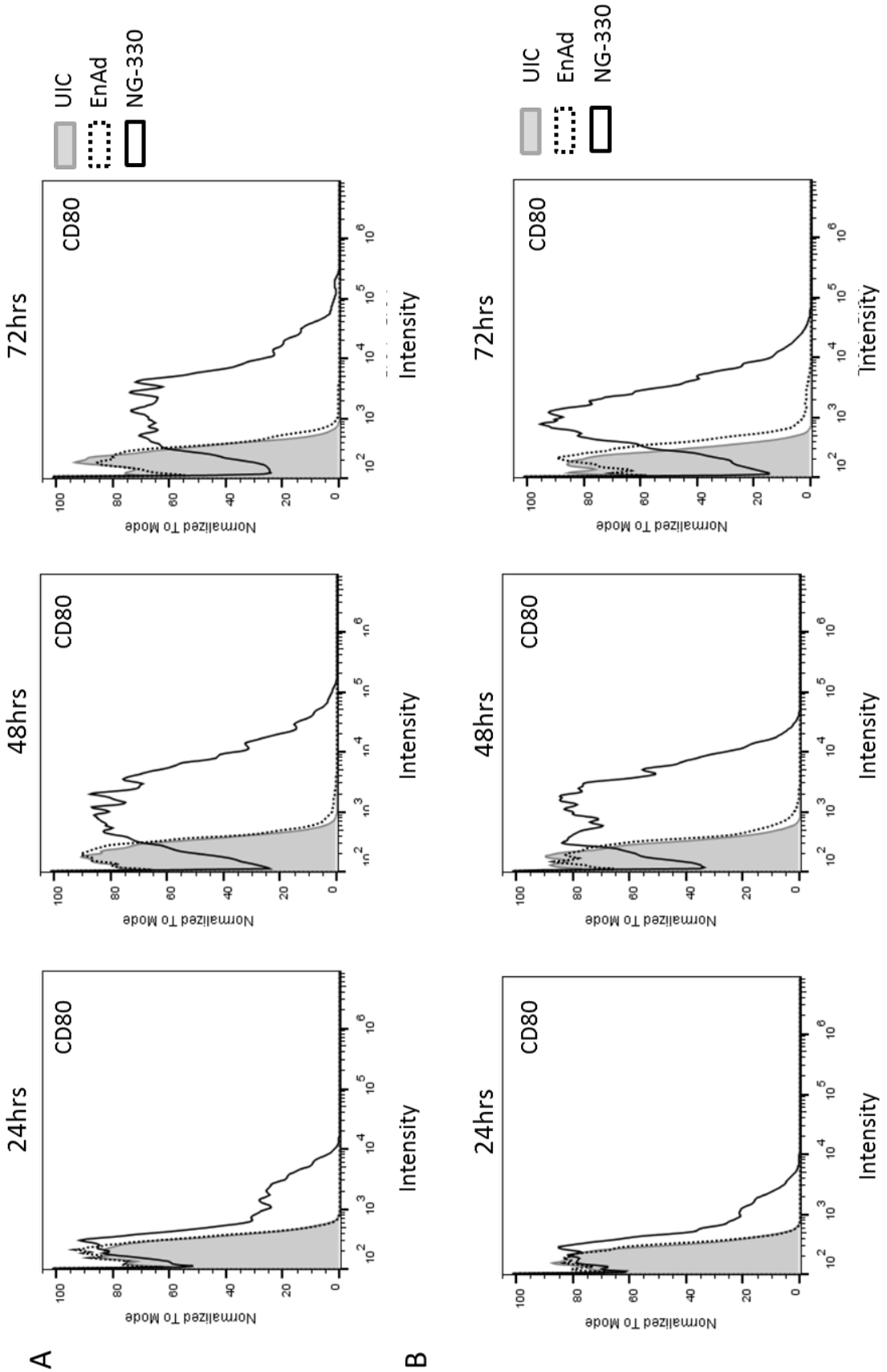
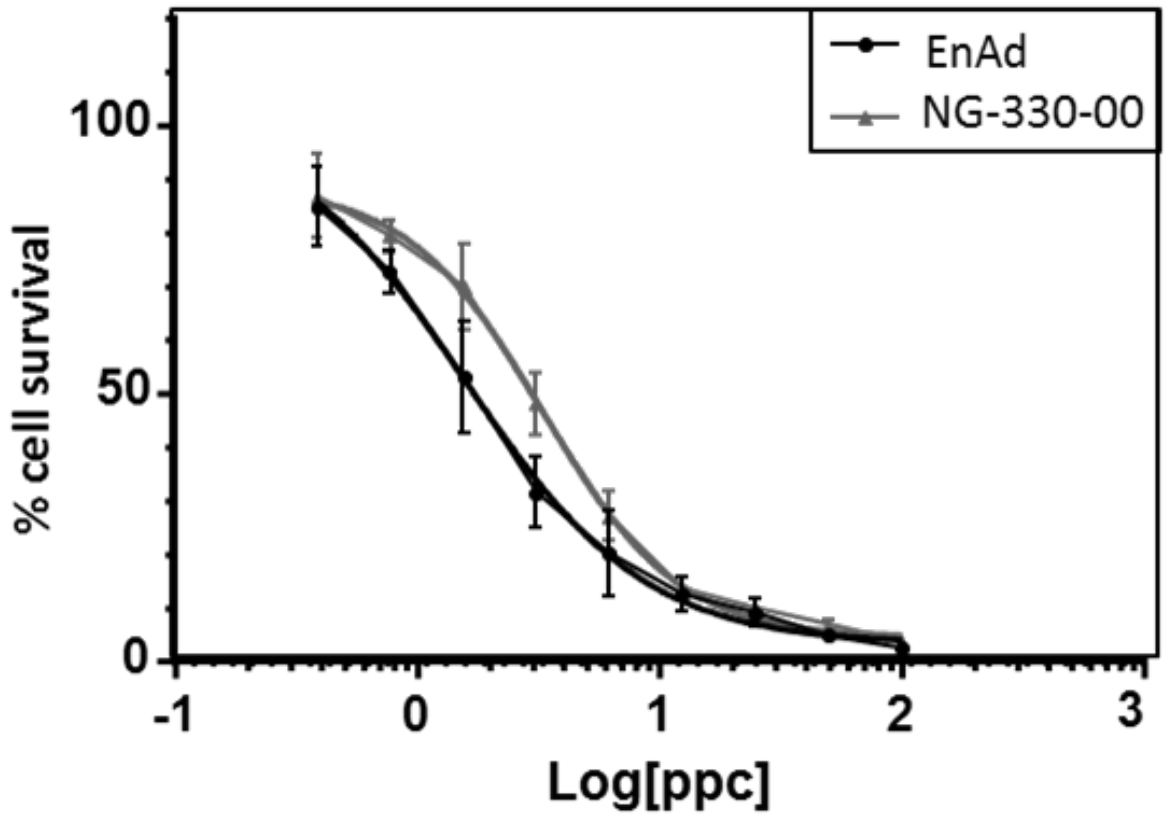

A
B
24hrs
48hrs
72hrs
CD80
UIC
EnAd
NG-330
Normalized To Mode
Intensity

Figure 6



P000145WO

Figure 7

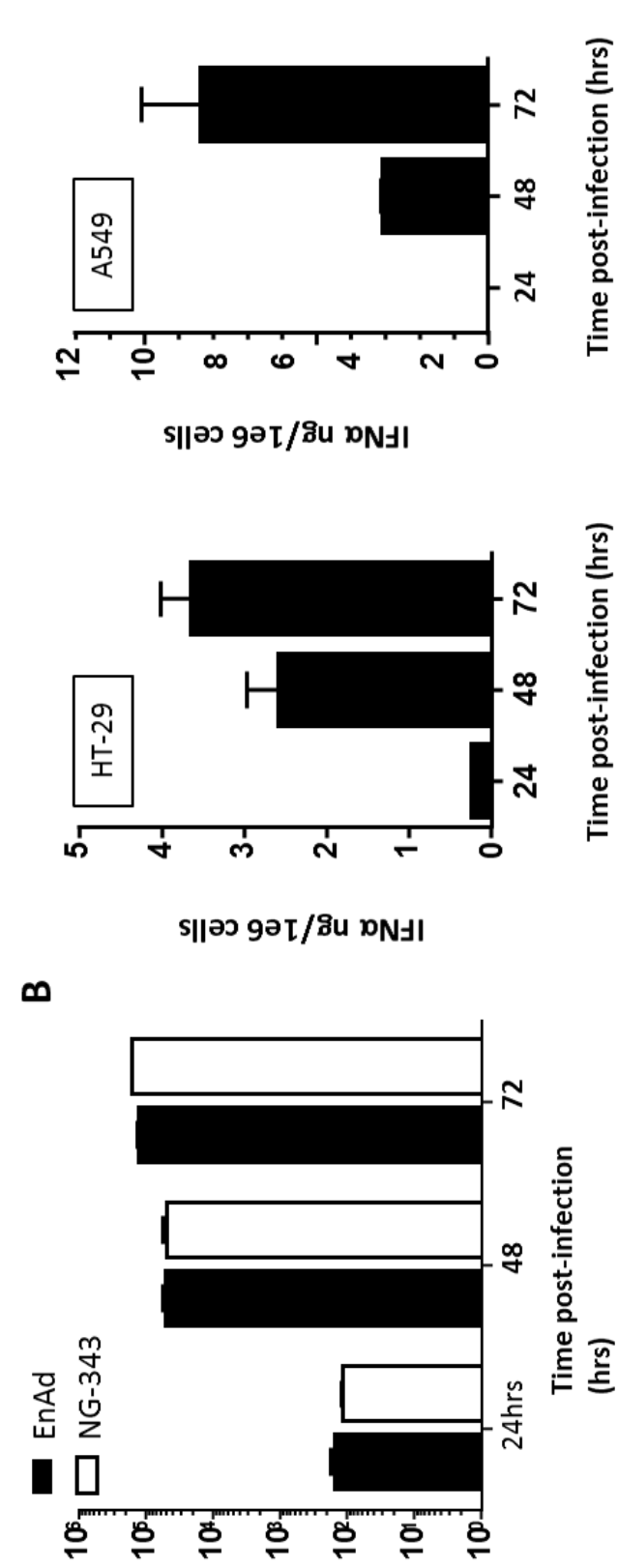


Figure 8

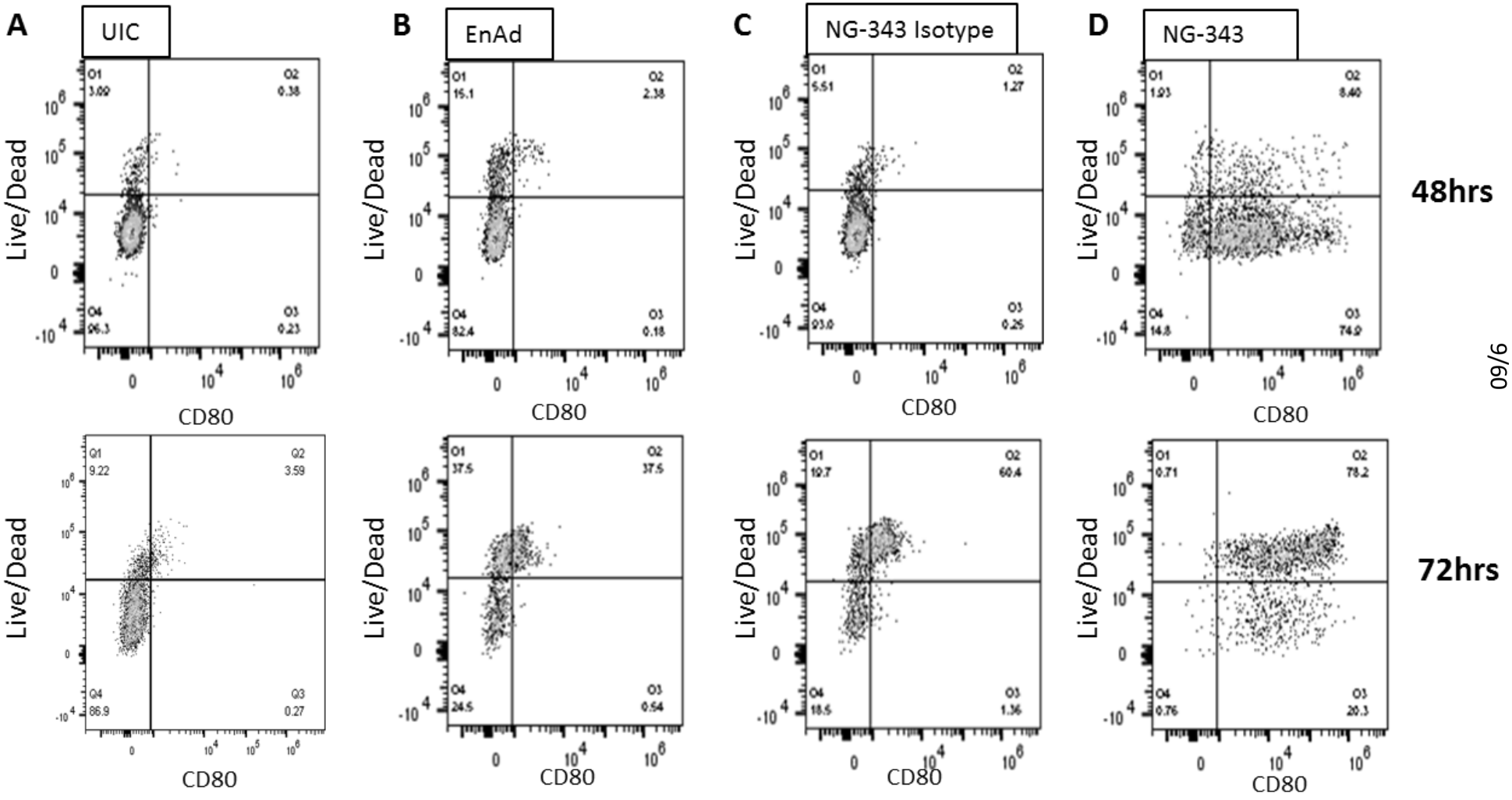

A
UIC
B
EnAd
C
NG-343 Isotype
D
NG-343
48hrs
72hrs
Live/Dead
CD80
Q1 3.69
Q2 0.38
Q4 96.3
Q3 0.23
Q1 15.1
Q2 2.38
Q4 82.4
Q3 0.18
Q1 5.51
Q2 1.27
Q4 93.0
Q3 0.25
Q1 1.93
Q2 8.40
Q4 14.8
Q3 74.9
Q1 9.22
Q2 3.59
Q4 86.9
Q3 0.27
Q1 37.5
Q2 37.5
Q4 24.5
Q3 0.54
Q1 19.7
Q2 60.4
Q4 18.5
Q3 1.36
Q1 0.71
Q2 78.2
Q4 0.76
Q3 20.3

Figure 8 continued

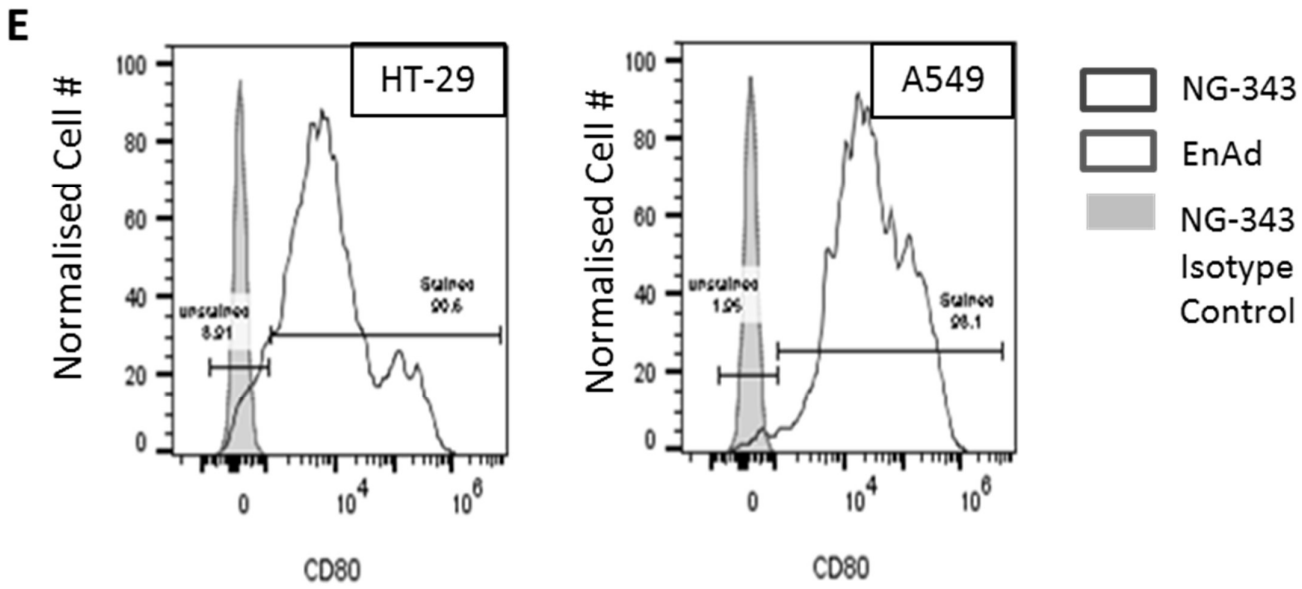


Figure 9A and B

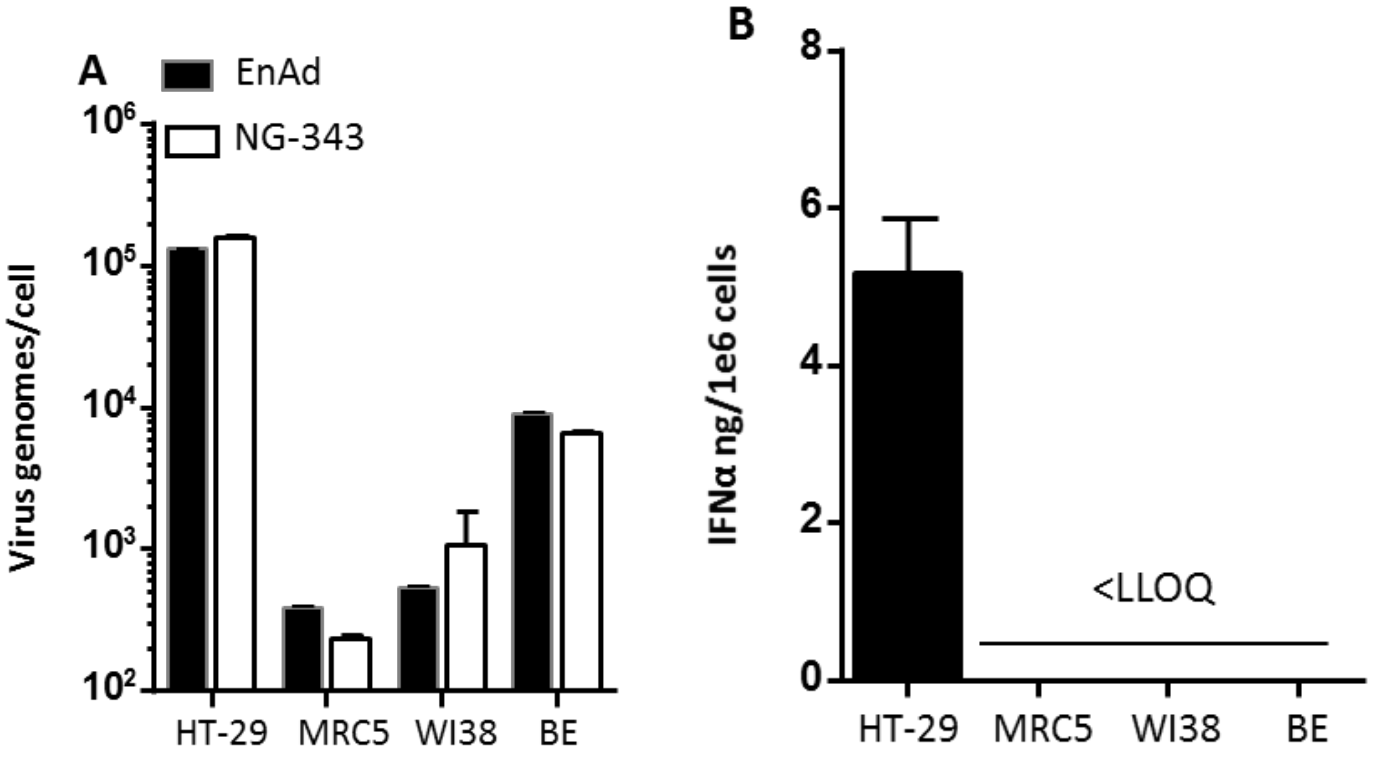


Figure 9C

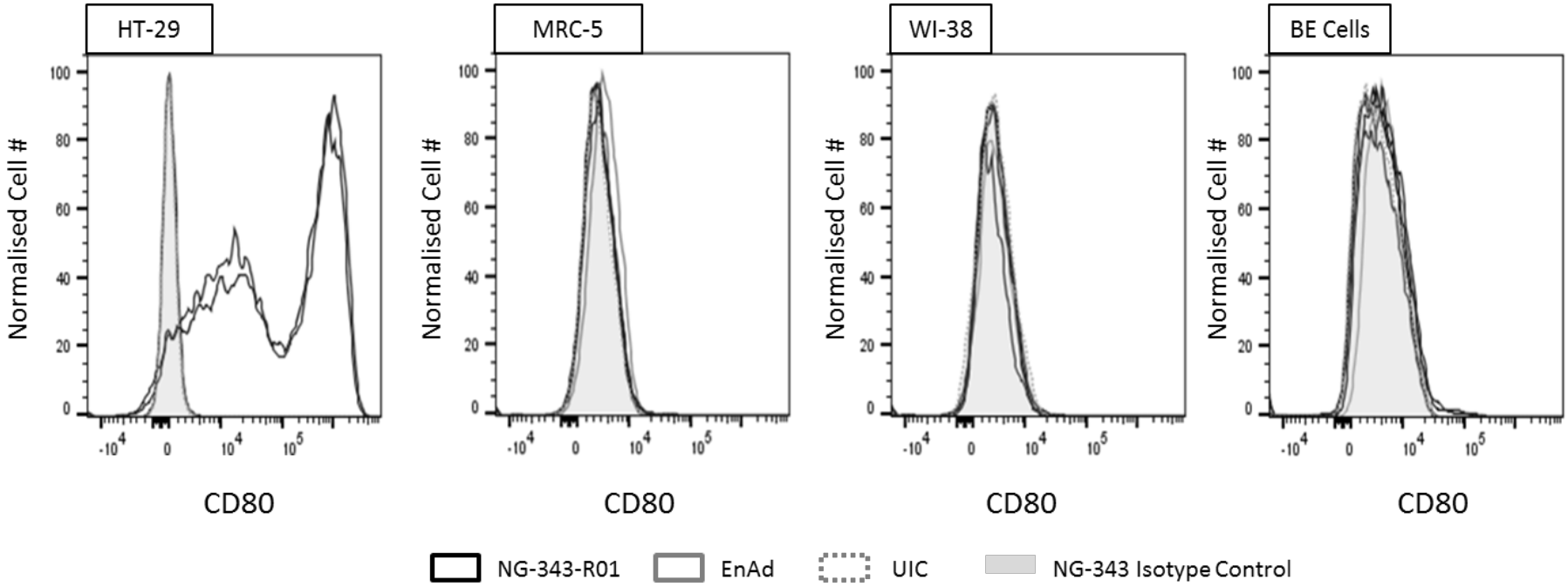

HT-29
MRC-5
WI-38
BE Cells
Normalised Cell #
CD80
NG-343-R01
EnAd
UIC
NG-343 Isotype Control

Figure 10

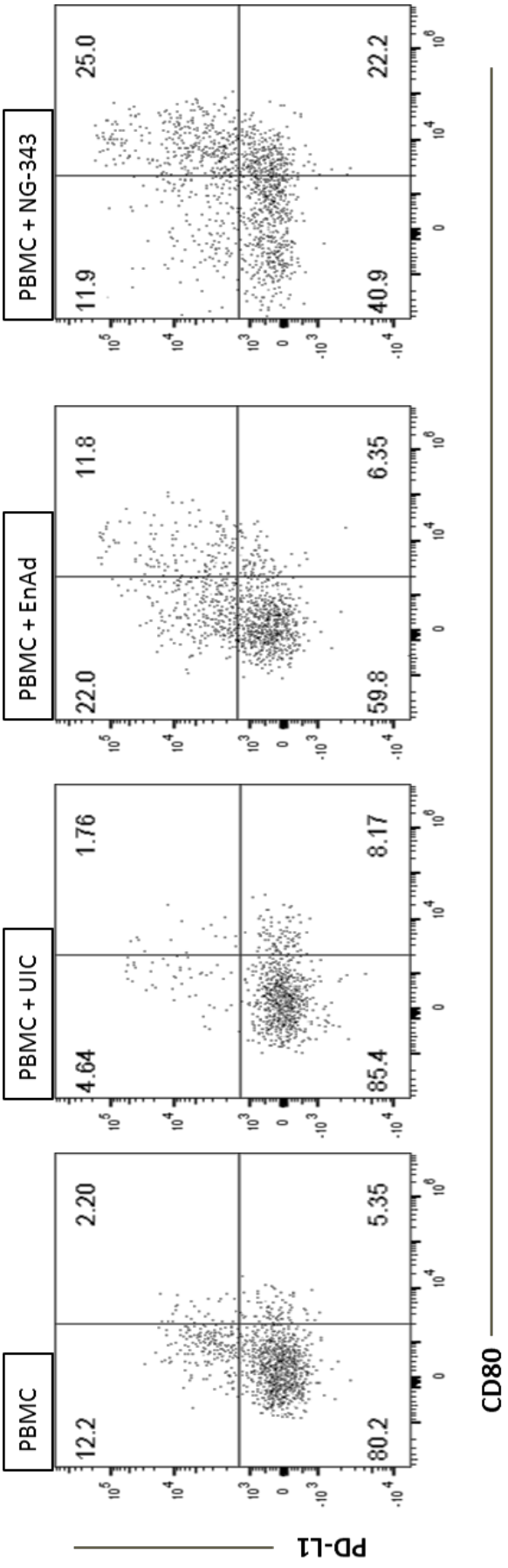

PBMC
12.2
2.20
80.2
5.35
PBMC + UIC
4.64
1.76
85.4
8.17
PBMC + EnAd
22.0
11.8
59.8
6.35
PBMC + NG-343
11.9
25.0
40.9
22.2
PD-L1
CD80

Figure 11A and B

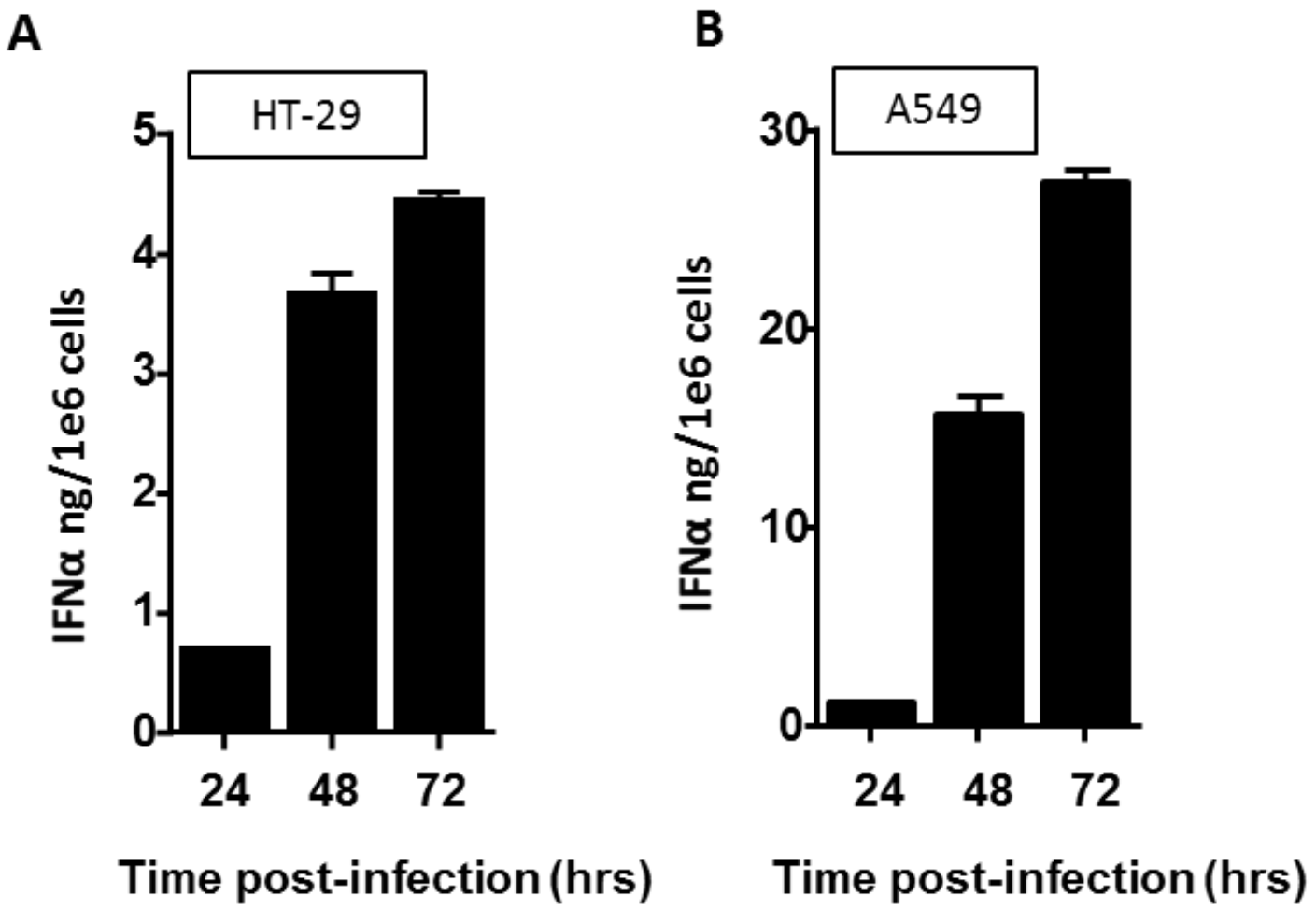


Figure 11C

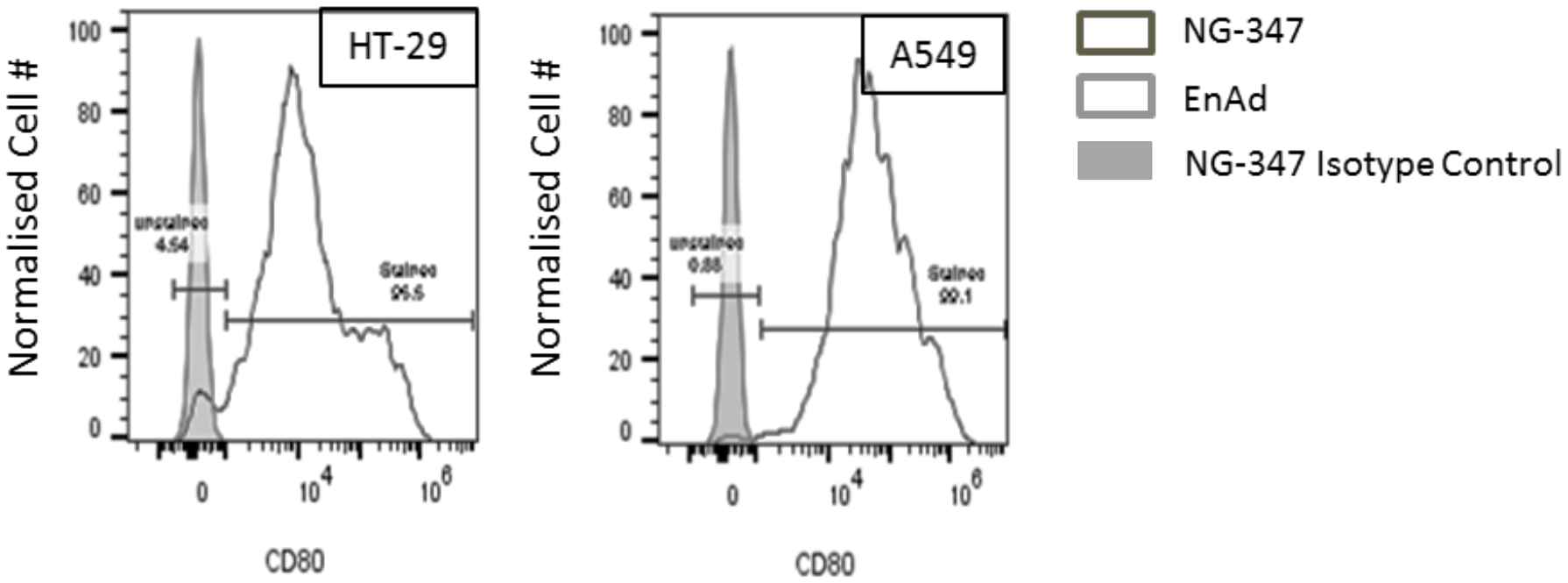

HT-29
A549
Normalised Cell #
CD80
NG-347
EnAd
NG-347 Isotype Control

Figure 12

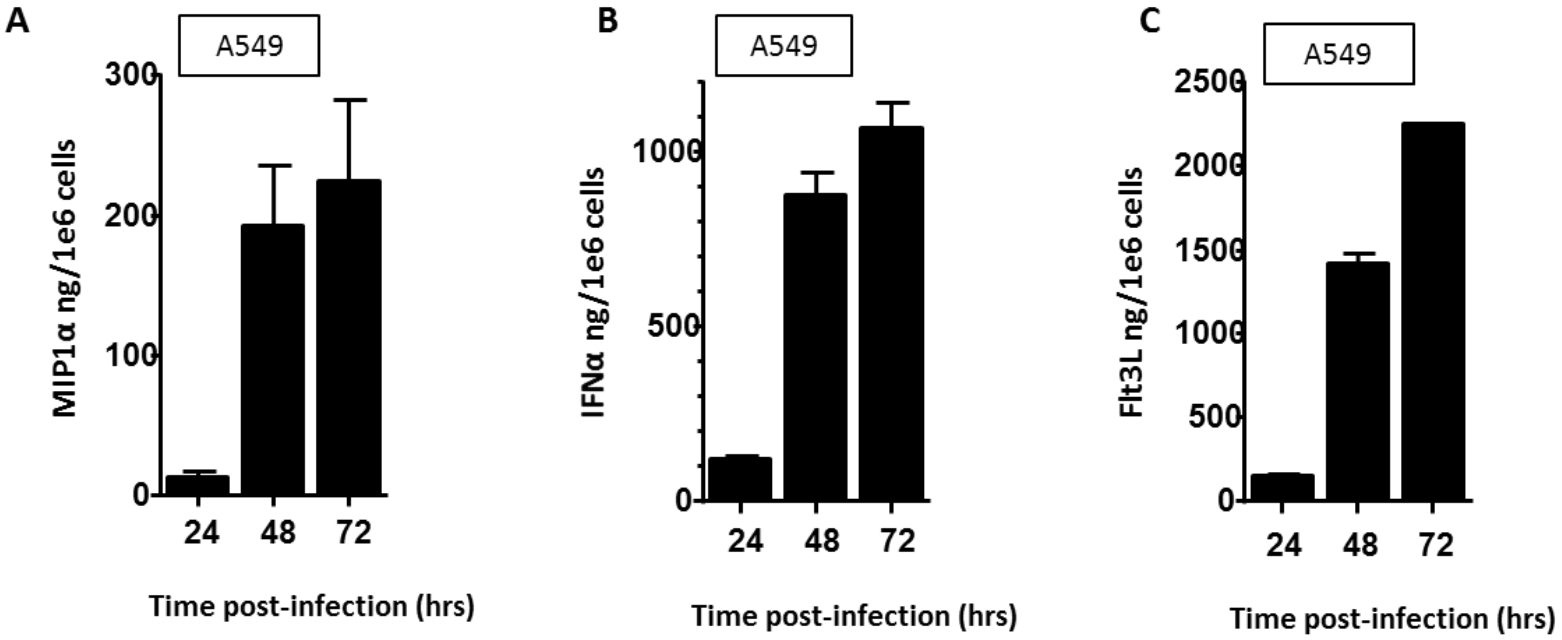
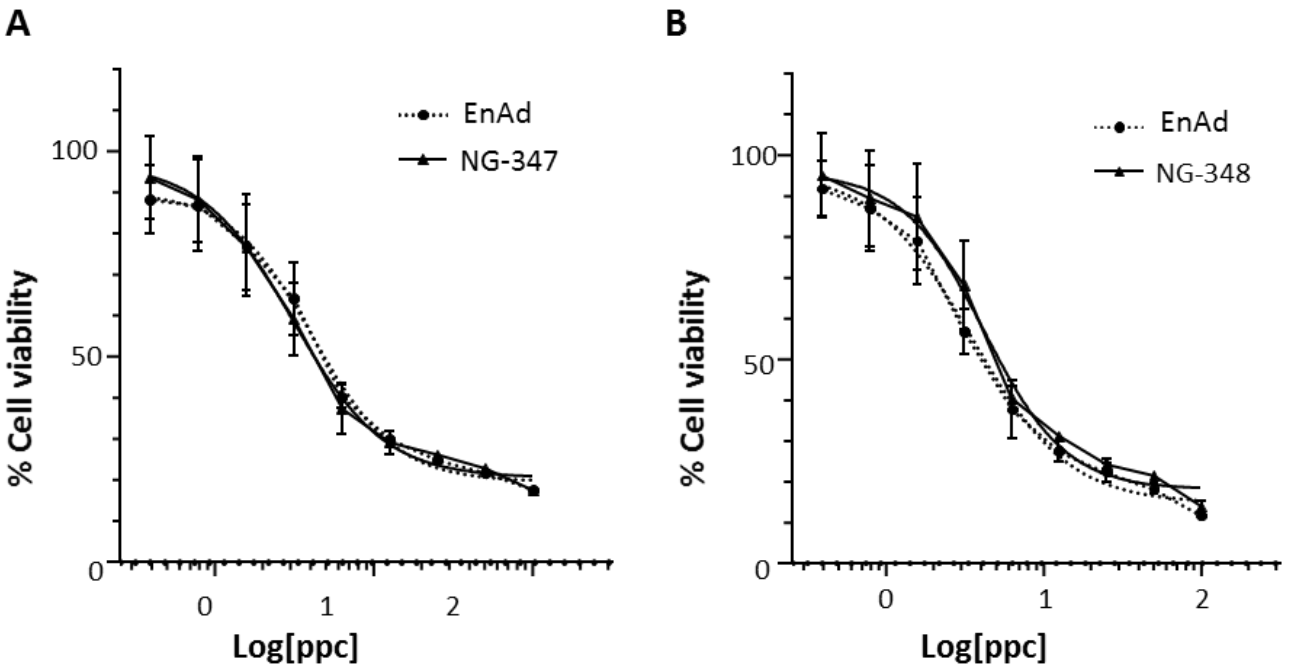

A
A549
MIP1α ng/1e6 cells
300
200
100
0
24
48
72
Time post-infection (hrs)
B
A549
IFNα ng/1e6 cells
1000
500
0
24
48
72
Time post-infection (hrs)
C
A549
Flt3L ng/1e6 cells
2500
2000
1500
1000
500
0
24
48
72
Time post-infection (hrs)

Figure 13



C

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-347	3.78e12	1.67e11	22.7
NG-348	6.68e12	3.31e11	20.2

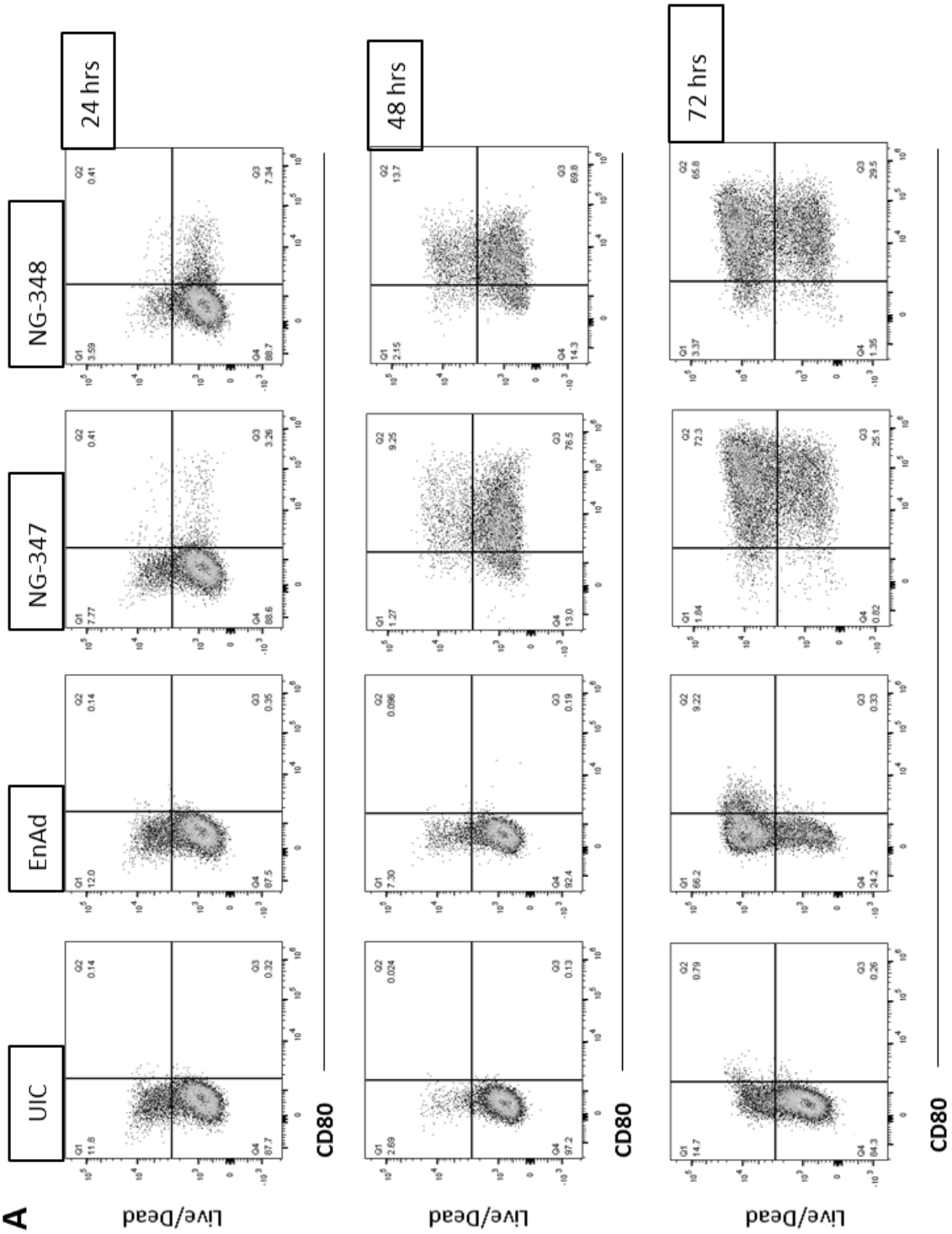


Figure 14A

P000145WO

Figure 14B

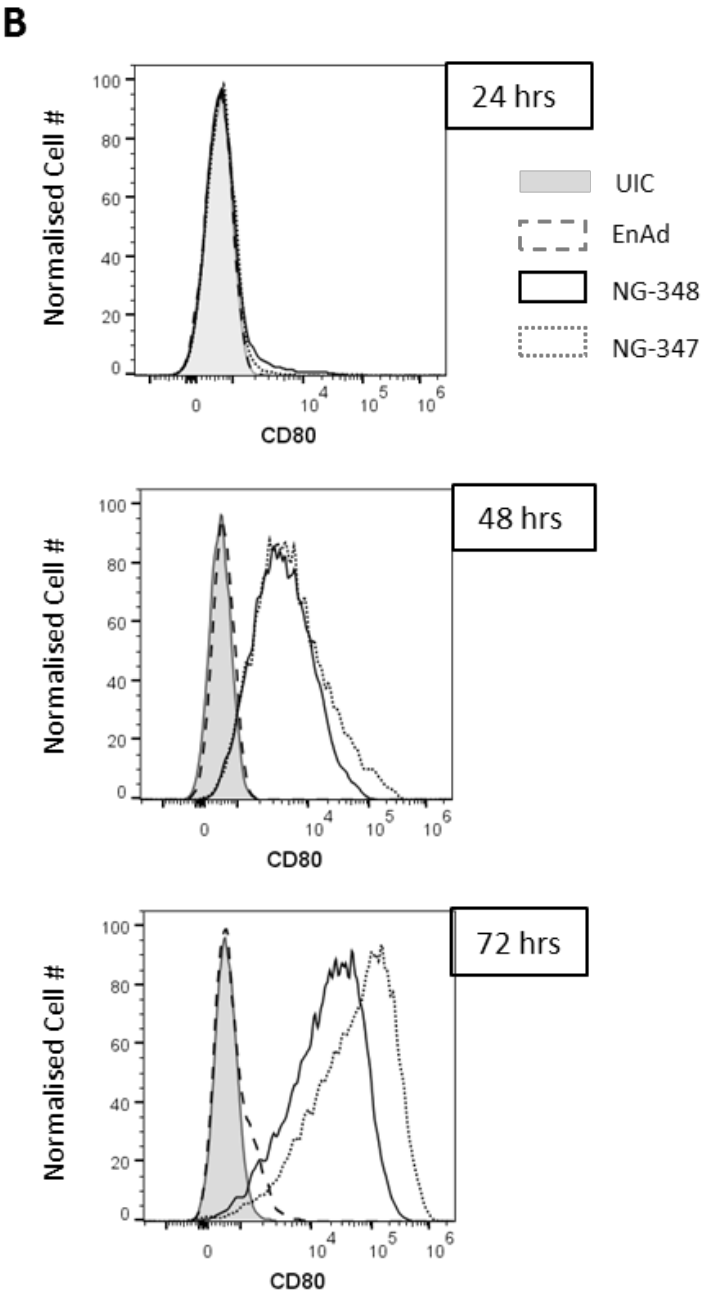

B
24 hrs
48 hrs
72 hrs
UIC
EnAd
NG-348
NG-347
Normalised Cell #
CD80
0
20
40
60
80
100
10^4
10^5
10^6

Figure 15A

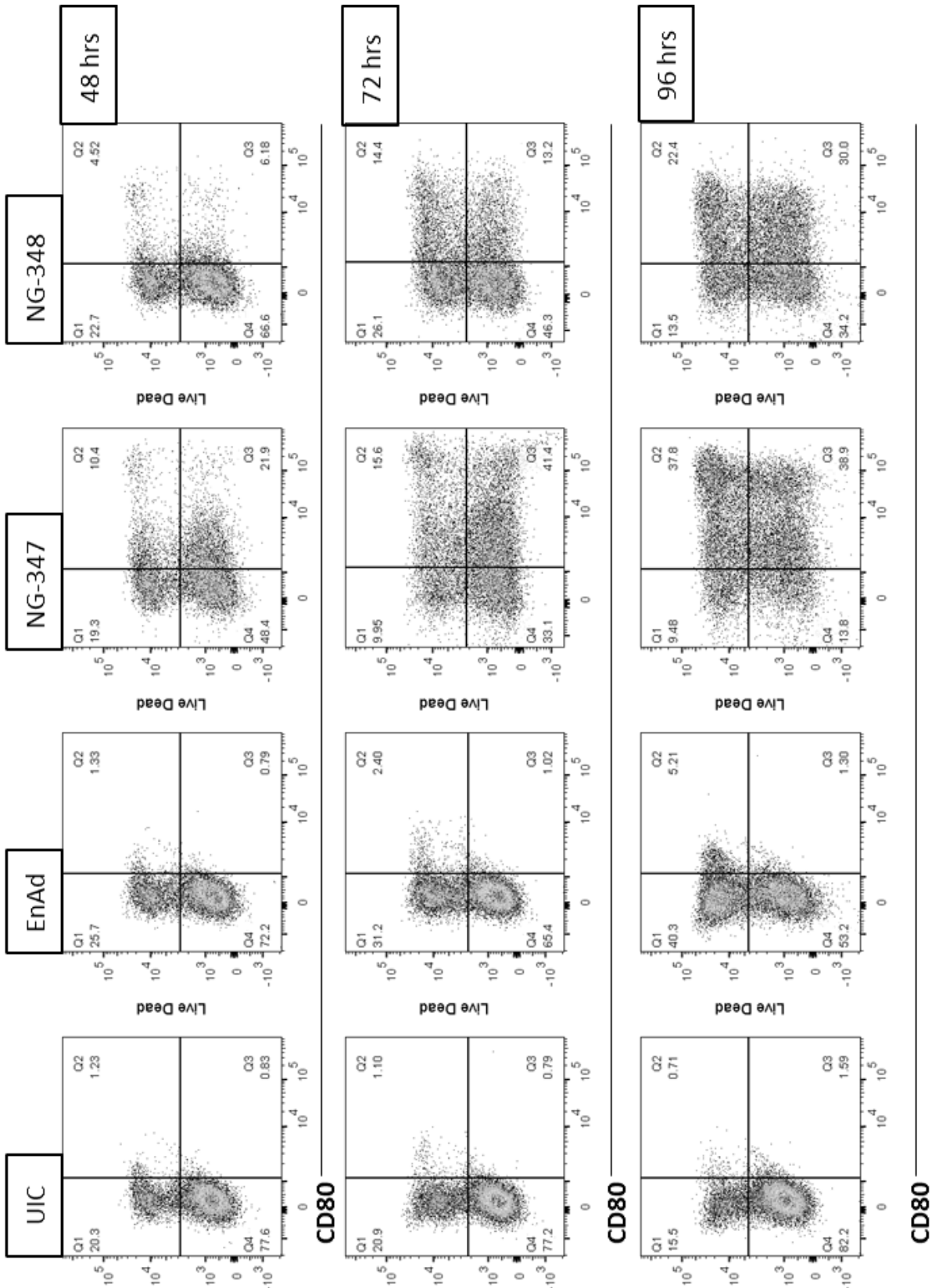

A
UIC
EnAd
NG-347
NG-348
48 hrs
72 hrs
96 hrs
CD80
Live Dead
Q1 20.3
Q2 1.23
Q3 0.83
Q4 77.6
Q1 25.7
Q2 1.33
Q3 0.79
Q4 72.2
Q1 19.3
Q2 10.4
Q3 21.9
Q4 48.4
Q1 22.7
Q2 4.52
Q3 6.18
Q4 66.6
Q1 20.9
Q2 1.10
Q3 0.79
Q4 77.2
Q1 31.2
Q2 2.40
Q3 1.02
Q4 65.4
Q1 9.95
Q2 15.6
Q3 41.4
Q4 33.1
Q1 26.1
Q2 14.4
Q3 13.2
Q4 46.3
Q1 15.5
Q2 0.71
Q3 1.59
Q4 82.2
Q1 40.3
Q2 5.21
Q3 1.30
Q4 53.2
Q1 9.48
Q2 37.8
Q3 38.9
Q4 13.8
Q1 13.5
Q2 22.4
Q3 30.0
Q4 34.2

Figure 15B

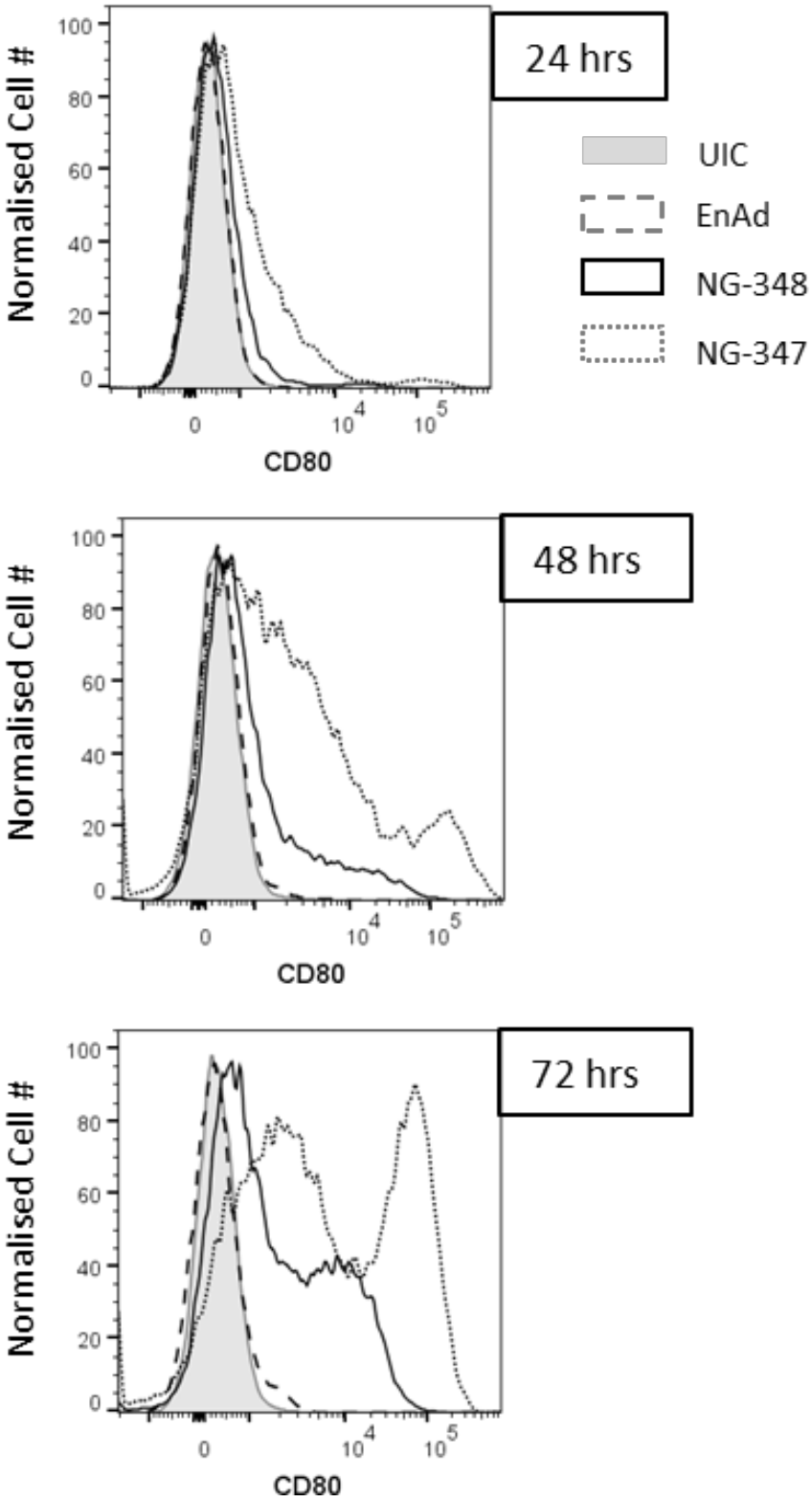

24 hrs
48 hrs
72 hrs
UIC
EnAd
NG-348
NG-347
Normalised Cell #
CD80
0
20
40
60
80
100
10^4
10^5

Figure 16

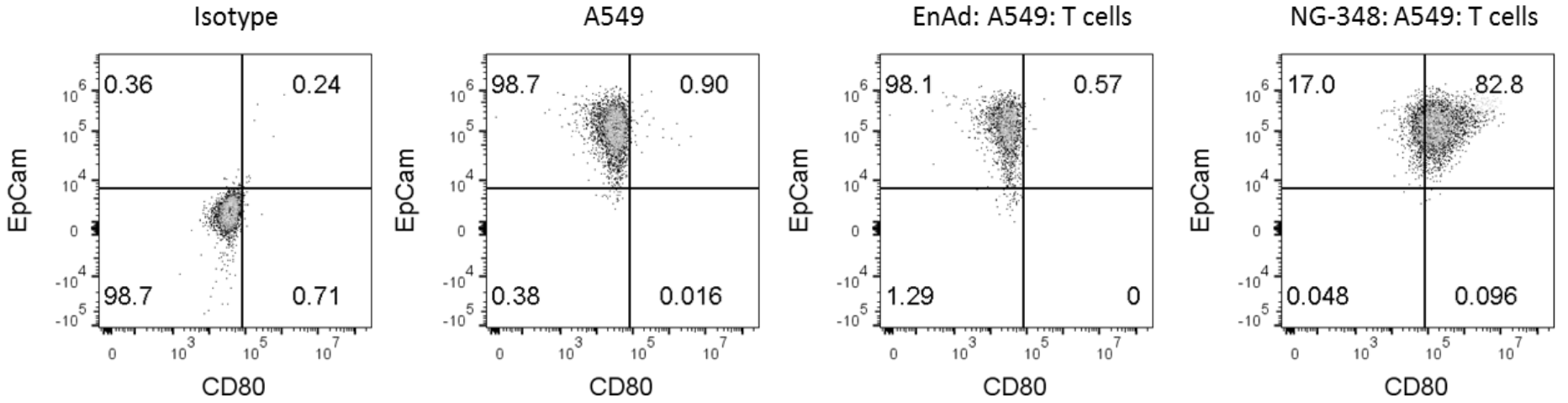


Figure 17A

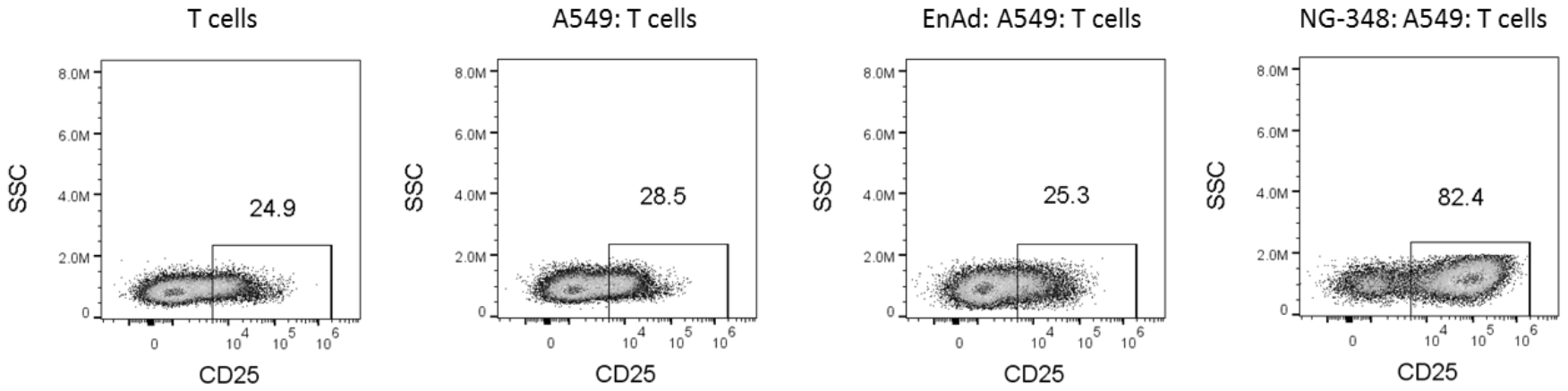


Figure 17B & C

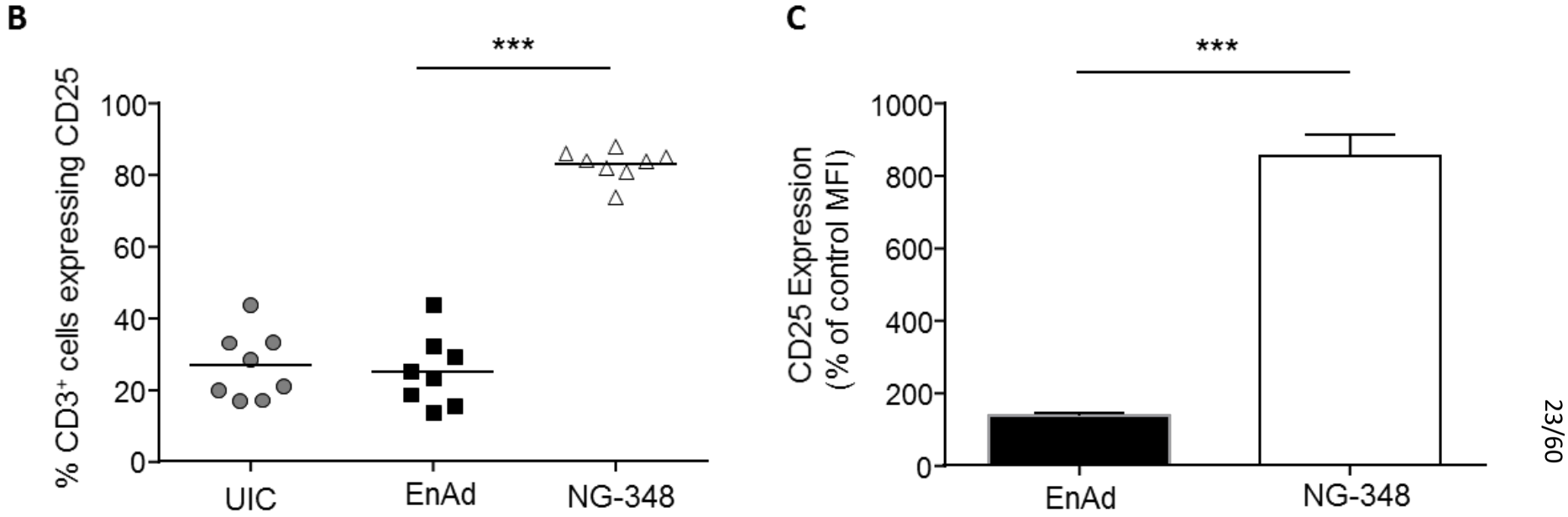

B
***
% CD3+ cells expressing CD25
100
80
60
40
20
0
UIC
EnAd
NG-348
C
***
CD25 Expression
(% of control MFI)
1000
800
600
400
200
0
EnAd
NG-348

Figure 18 A & B

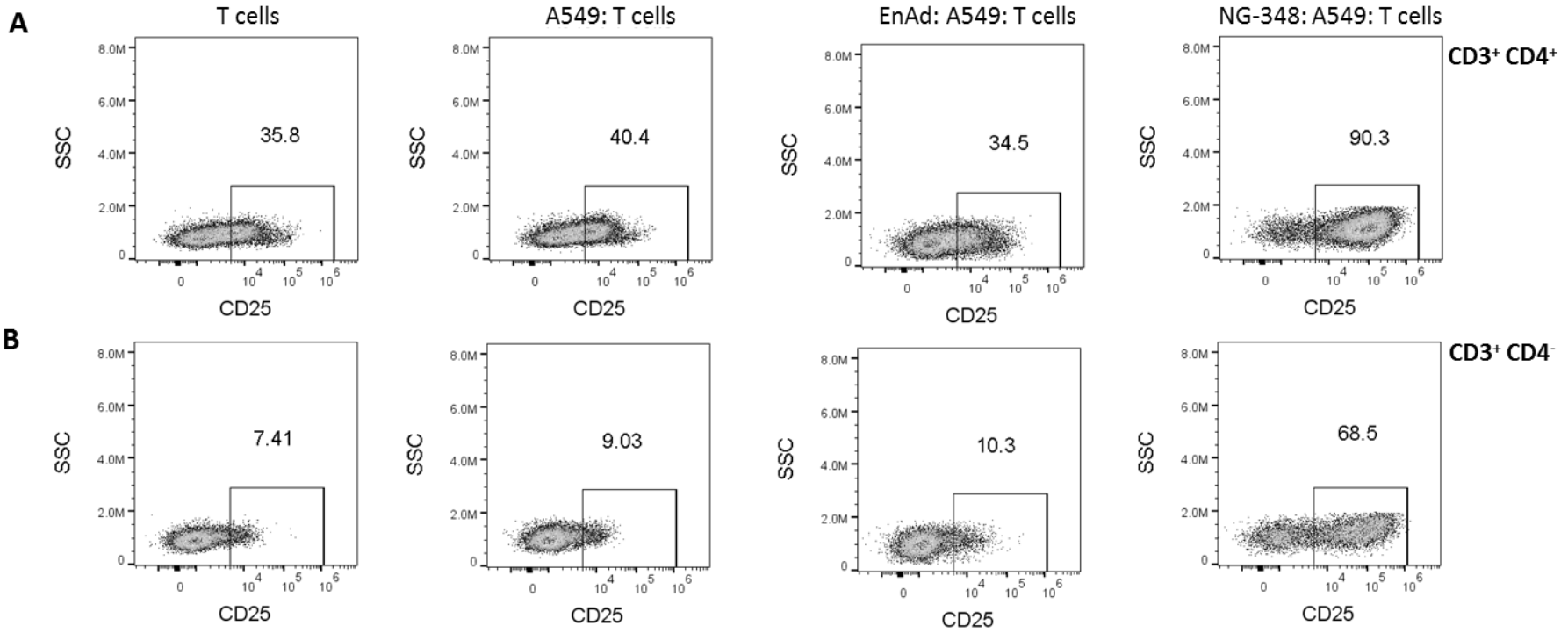

A
T cells
A549: T cells
EnAd: A549: T cells
NG-348: A549: T cells
CD3+ CD4+
35.8
40.4
34.5
90.3
B
CD3+ CD4-
7.41
9.03
10.3
68.5
SSC
CD25
8.0M
6.0M
4.0M
2.0M
0

Figure 18 C & D

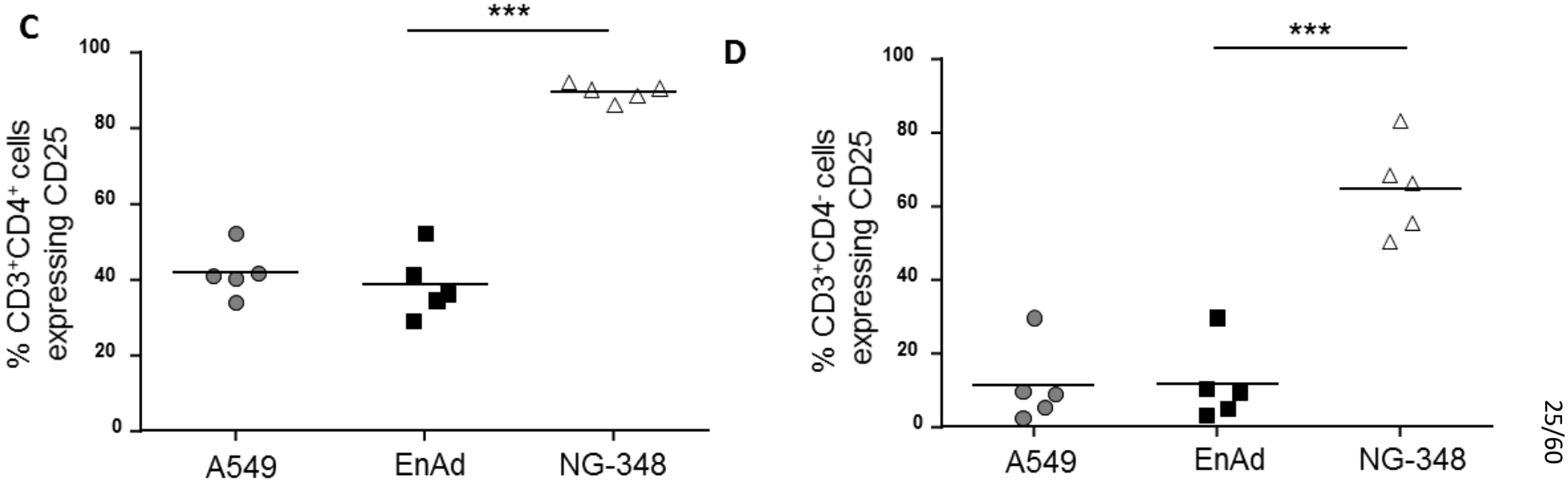

C
***
% CD3+CD4+ cells expressing CD25
100
80
60
40
20
0
A549
EnAd
NG-348
D
***
% CD3+CD4- cells expressing CD25
100
80
60
40
20
0
A549
EnAd
NG-348

Figure 19

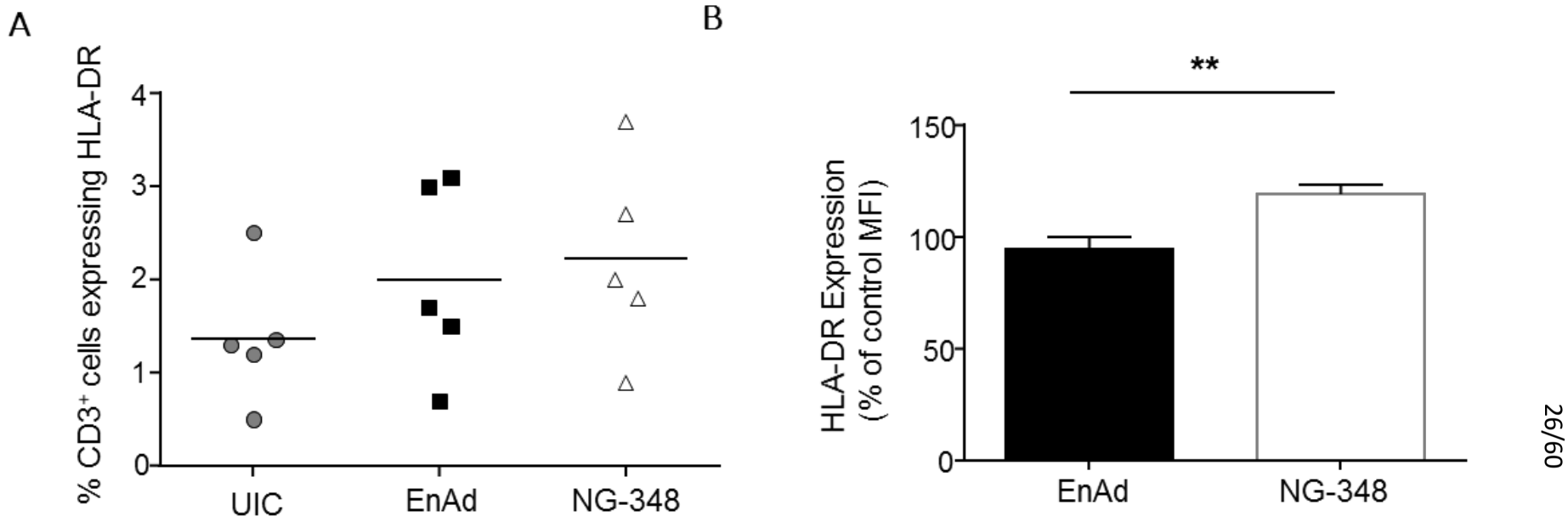

A
% CD3+ cells expressing HLA-DR
0
1
2
3
4
UIC
EnAd
NG-348
B
**
HLA-DR Expression
(% of control MFI)
0
50
100
150
EnAd
NG-348

Figure 20A & B

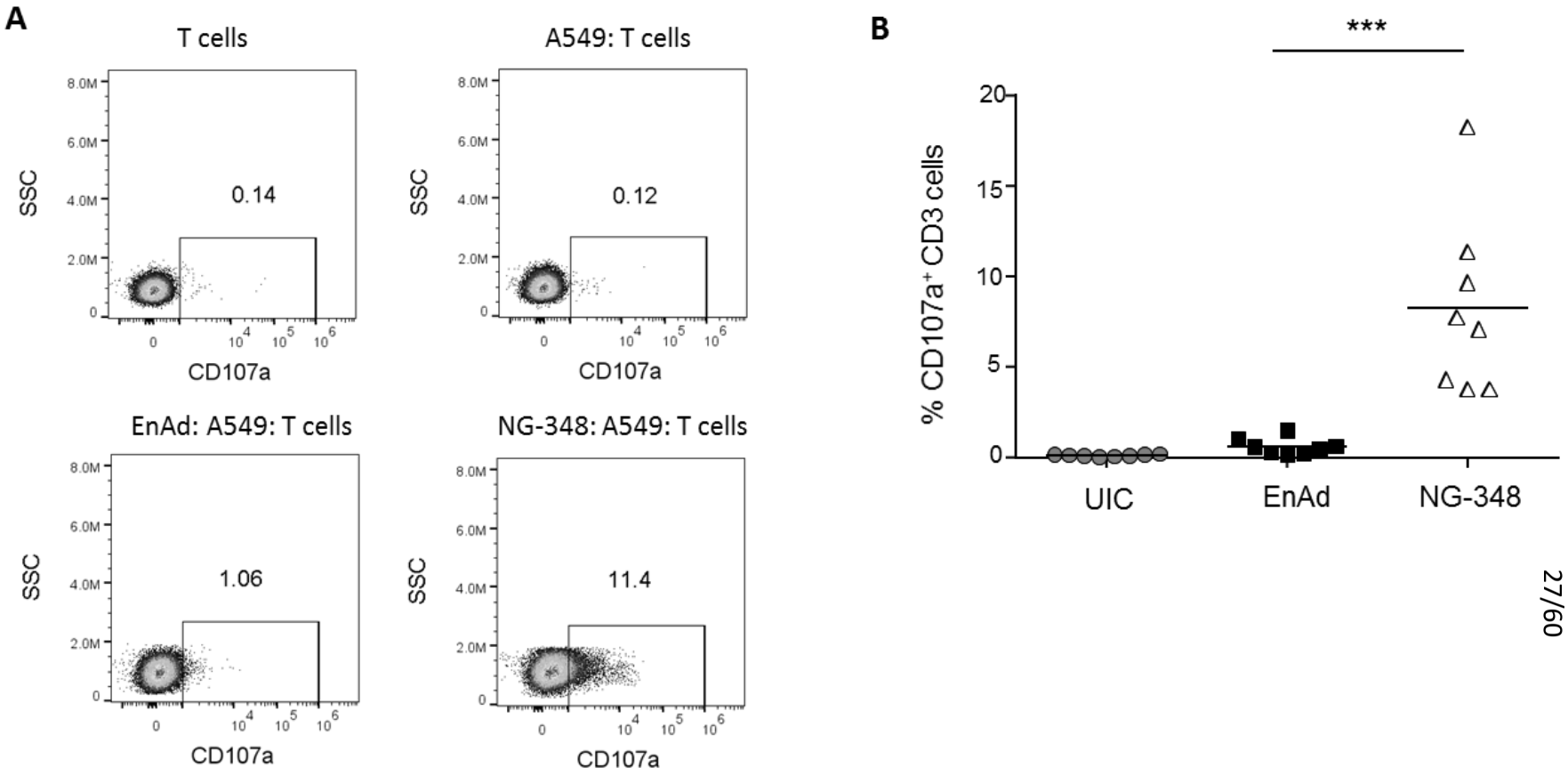

A
T cells
A549: T cells
EnAd: A549: T cells
NG-348: A549: T cells
SSC
CD107a
0.14
0.12
1.06
11.4
B
***
% CD107a+ CD3 cells
UIC
EnAd
NG-348

Figure 21A & B

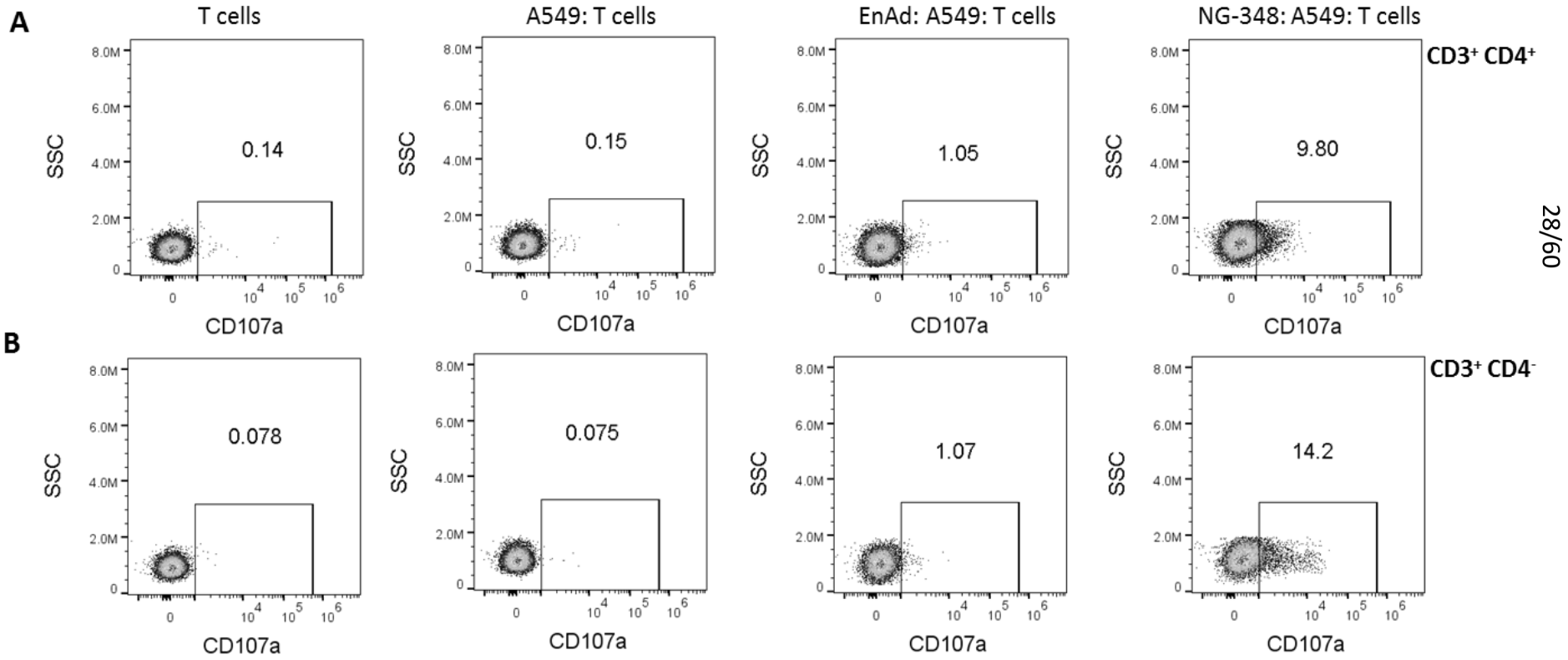

A
T cells
A549: T cells
EnAd: A549: T cells
NG-348: A549: T cells
CD3+ CD4+
0.14
0.15
1.05
9.80
B
CD3+ CD4-
0.078
0.075
1.07
14.2
SSC
CD107a
8.0M
6.0M
4.0M
2.0M
0

Figure 21 C & D

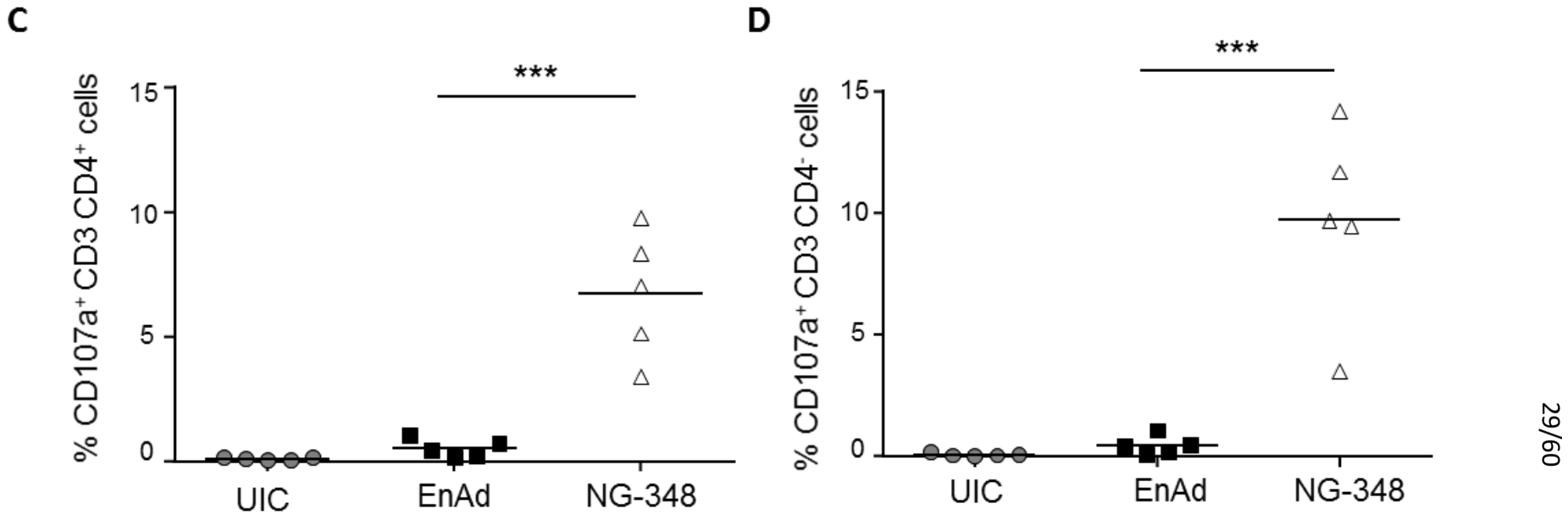

C
D
***
***
% CD107a+ CD3 CD4+ cells
% CD107a+ CD3 CD4- cells
15
10
5
0
UIC
EnAd
NG-348

Figure 22

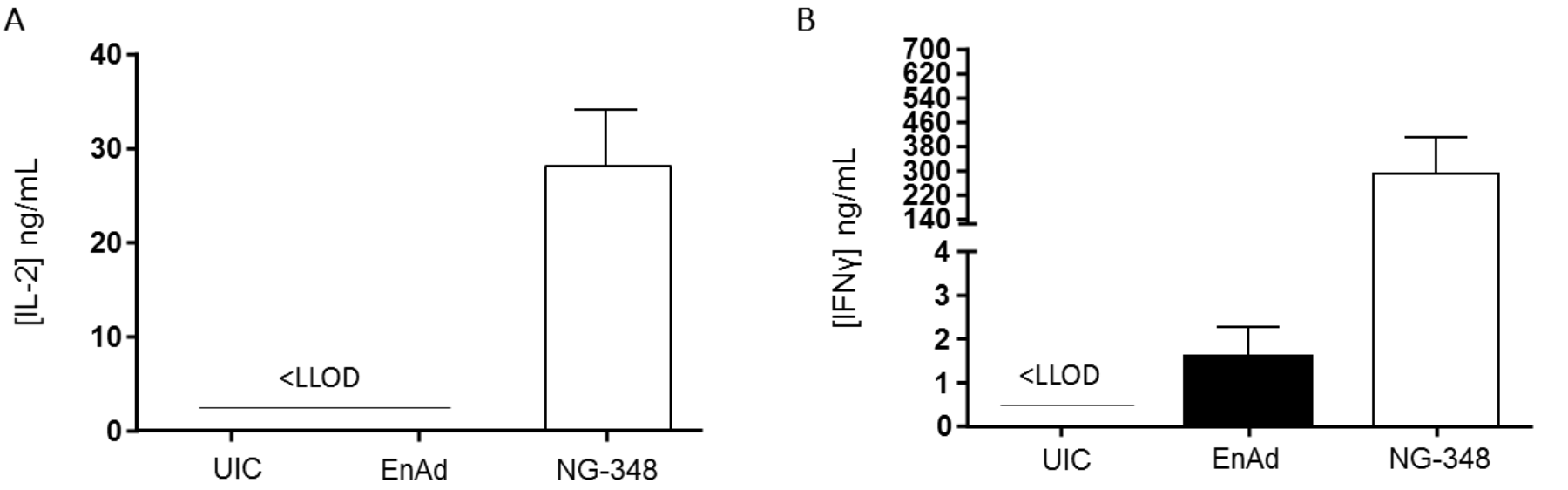

A
[IL-2] ng/mL
40
30
20
10
0
<LLOD
UIC
EnAd
NG-348
B
[IFNγ] ng/mL
700
620
540
460
380
300
220
140
4
3
2
1
0
<LLOD
UIC
EnAd
NG-348

Figure 23

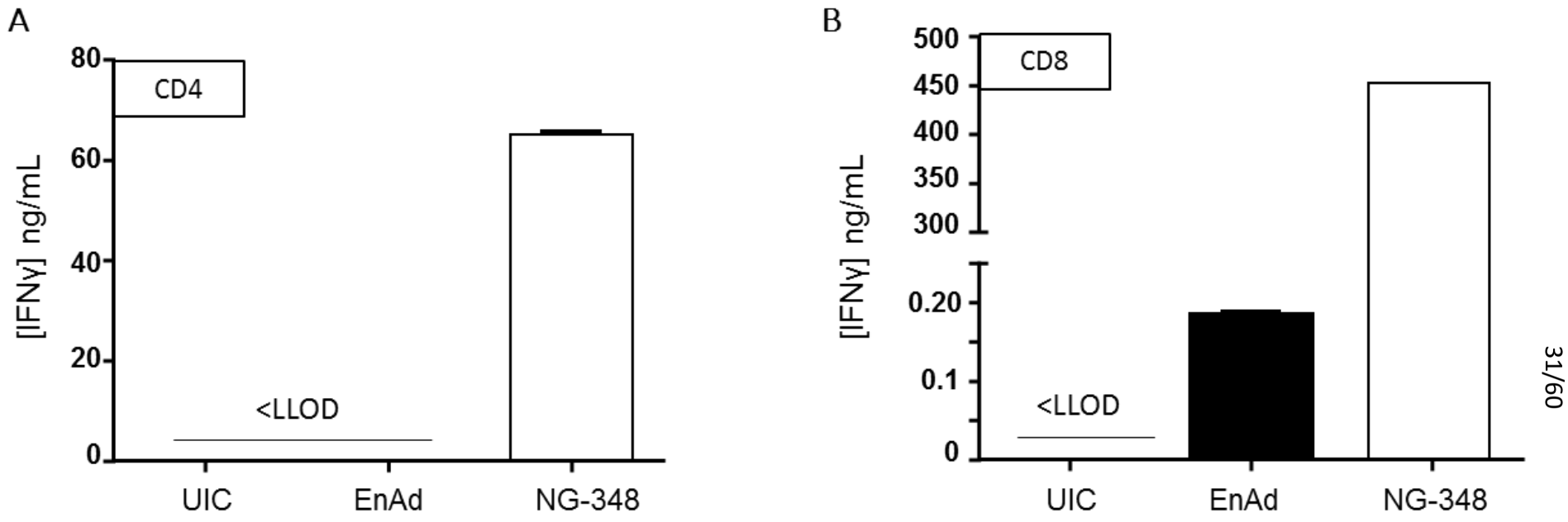

A
CD4
[IFNγ] ng/mL
80
60
40
20
0
<LLOD
UIC
EnAd
NG-348
B
CD8
[IFNγ] ng/mL
500
450
400
350
300
0.20
0.1
0
<LLOD
UIC
EnAd
NG-348

Figure 24

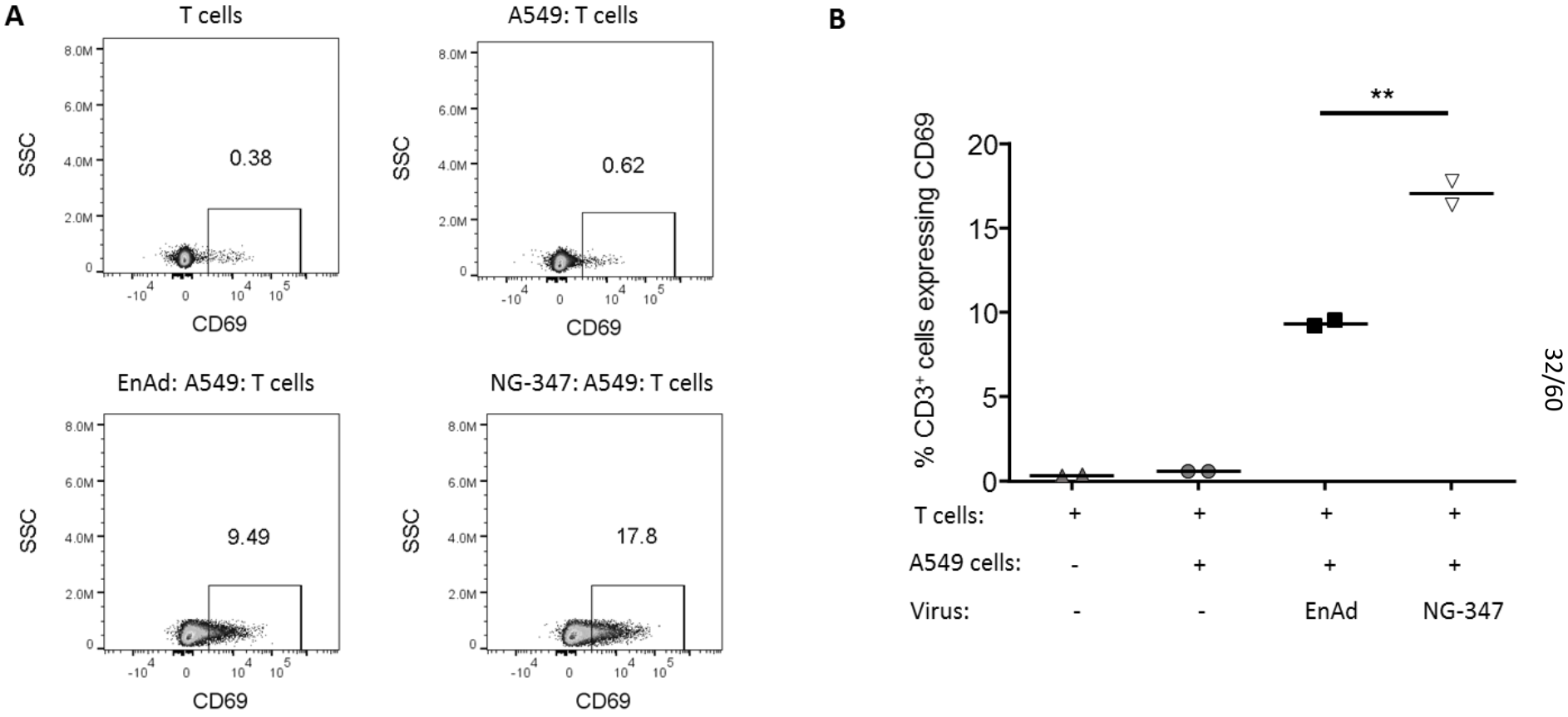

A
T cells
A549: T cells
EnAd: A549: T cells
NG-347: A549: T cells
SSC
CD69
0.38
0.62
9.49
17.8
8.0M
6.0M
4.0M
2.0M
0
B
% CD3+ cells expressing CD69
20
15
10
5
0
**
T cells: + + + +
A549 cells: - + + +
Virus: - - EnAd NG-347
32/60

Figure 25

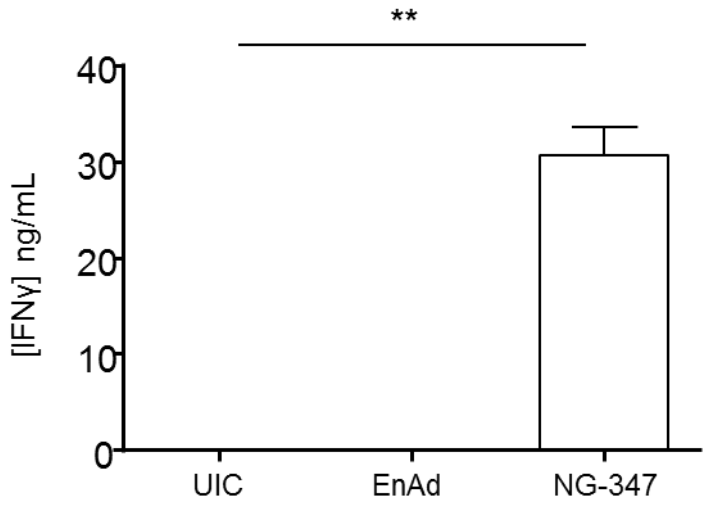


Figure 26

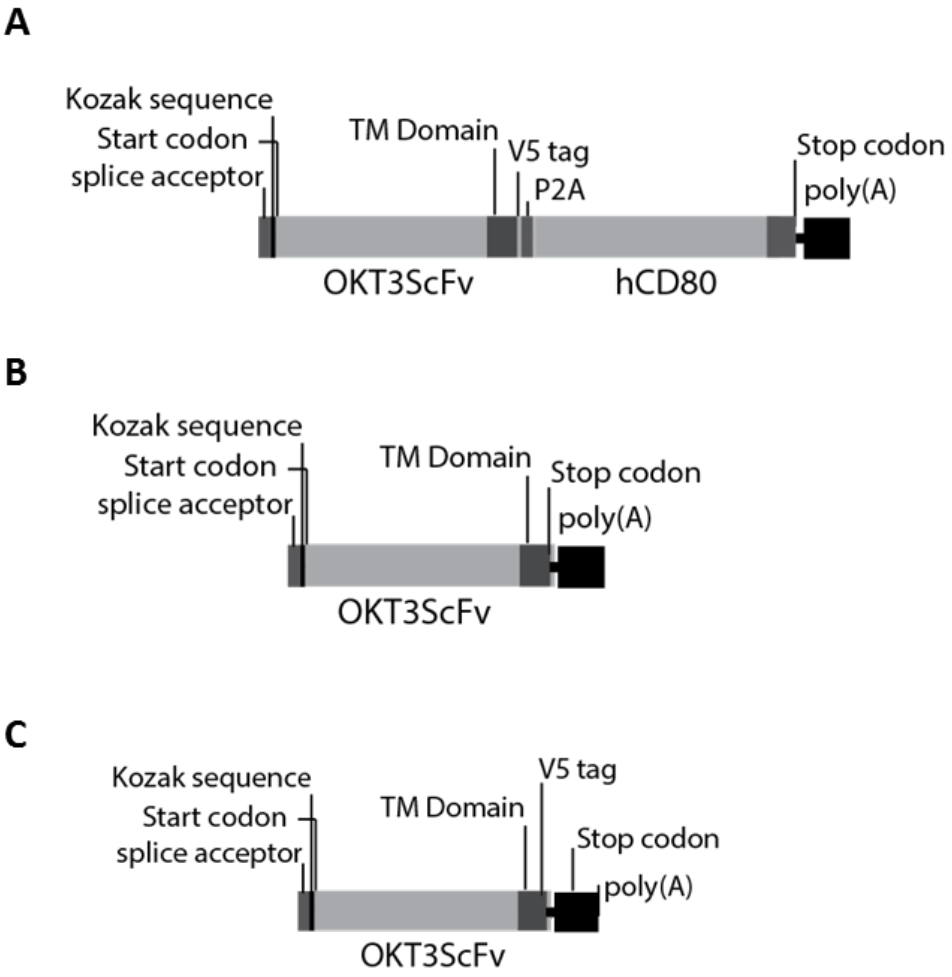


Figure 27

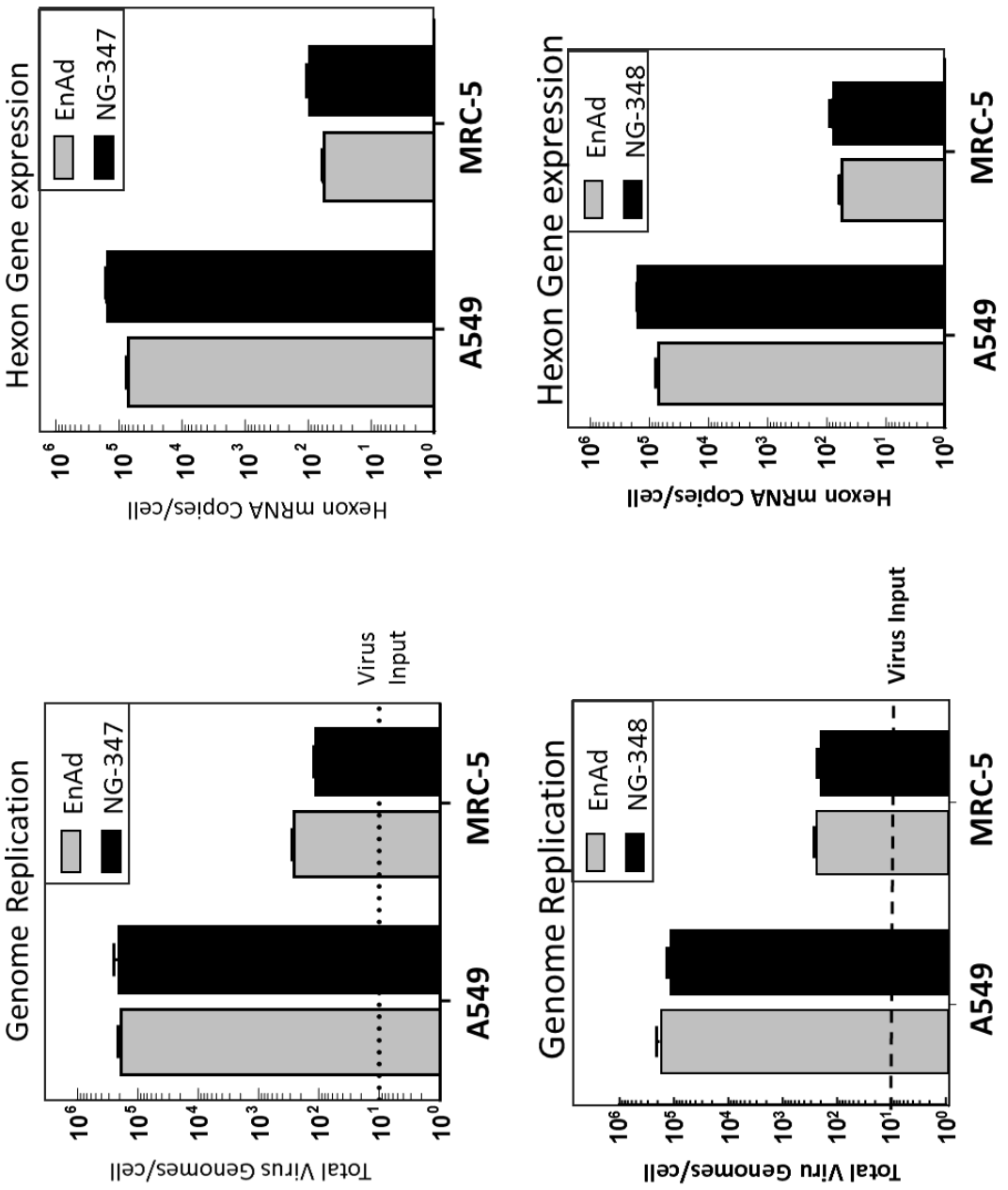
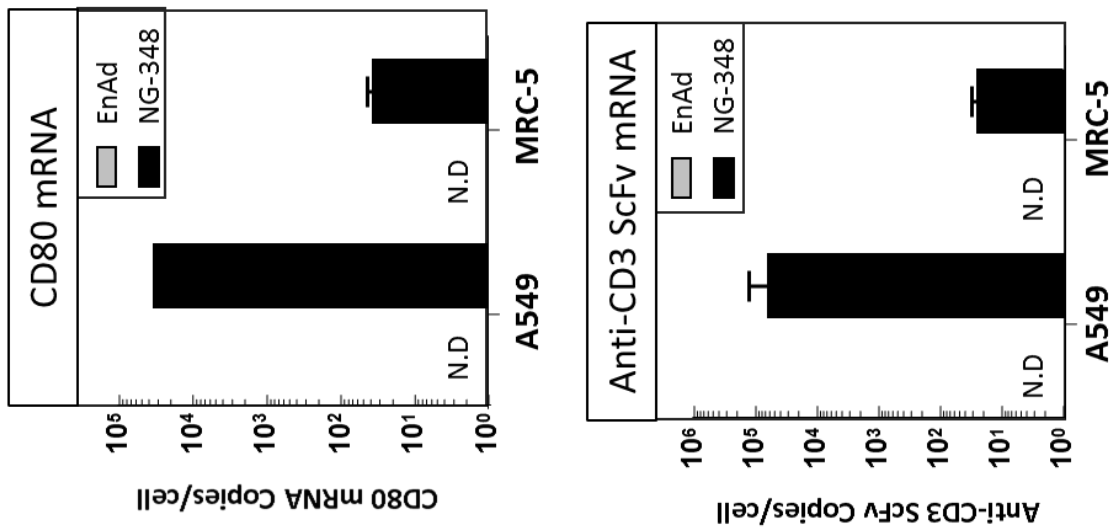

Hexon Gene expression
EnAd
NG-347
Hexon mRNA Copies/cell
A549
MRC-5
Hexon Gene expression
EnAd
NG-348
Hexon mRNA Copies/cell
A549
MRC-5
Genome Replication
EnAd
NG-347
Total Virus Genomes/cell
Virus Input
A549
MRC-5
Genome Replication
EnAd
NG-348
Total Viru Genomes/cell
Virus Input
A549
MRC-5

Figure 28


CD80 mRNA
EnAd
NG-348
CD80 mRNA Copies/cell
N.D
N.D
A549
MRC-5
Anti-CD3 ScFv mRNA
EnAd
NG-348
Anti-CD3 ScFv Copies/cell
N.D
N.D
A549
MRC-5

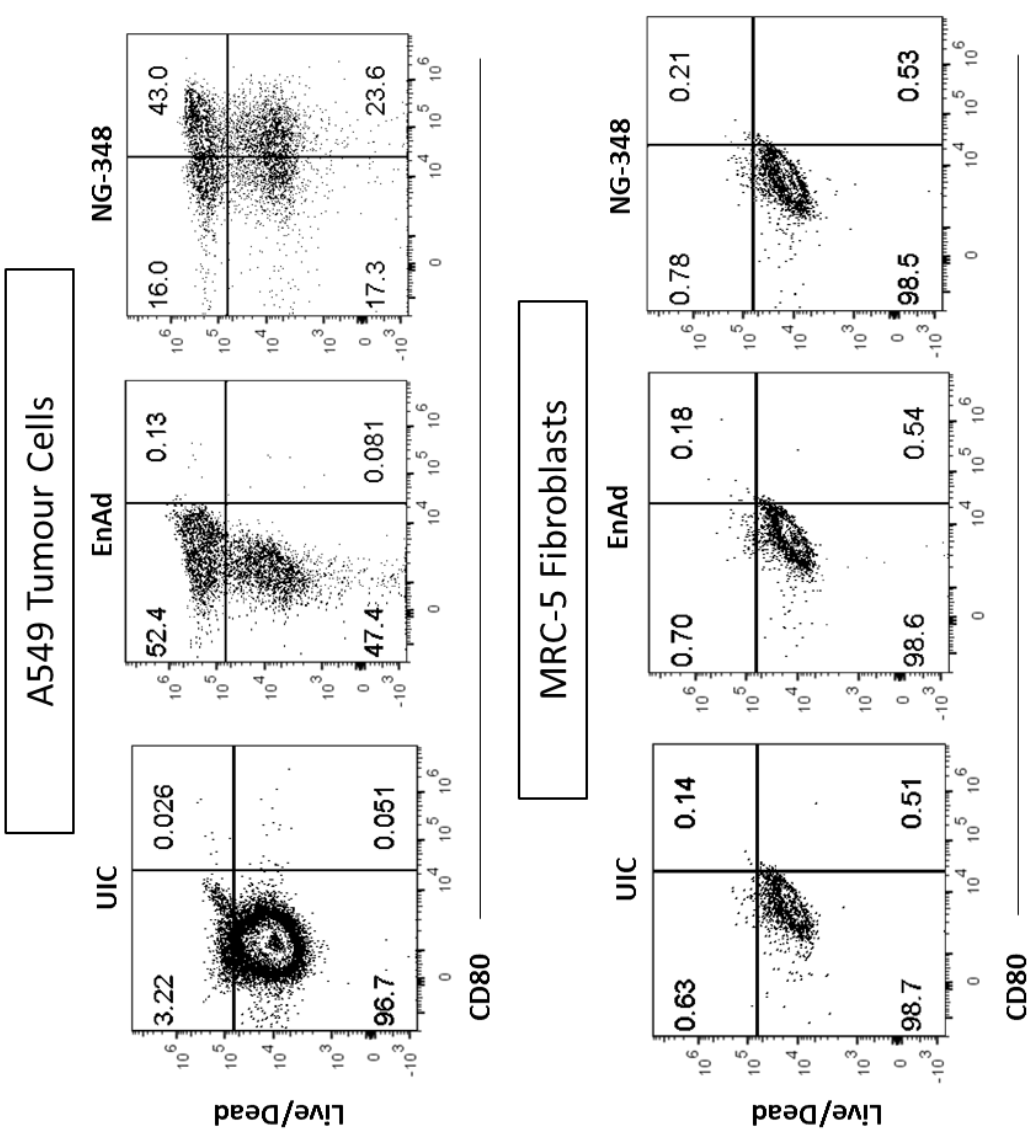

A549 Tumour Cells
UIC
EnAd
NG-348
3.22
0.026
96.7
0.051
52.4
0.13
47.4
0.081
16.0
43.0
17.3
23.6
Live/Dead
CD80
MRC-5 Fibroblasts
UIC
EnAd
NG-348
0.63
0.14
98.7
0.51
0.70
0.18
98.6
0.54
0.78
0.21
98.5
0.53
Live/Dead
CD80

Figure 29

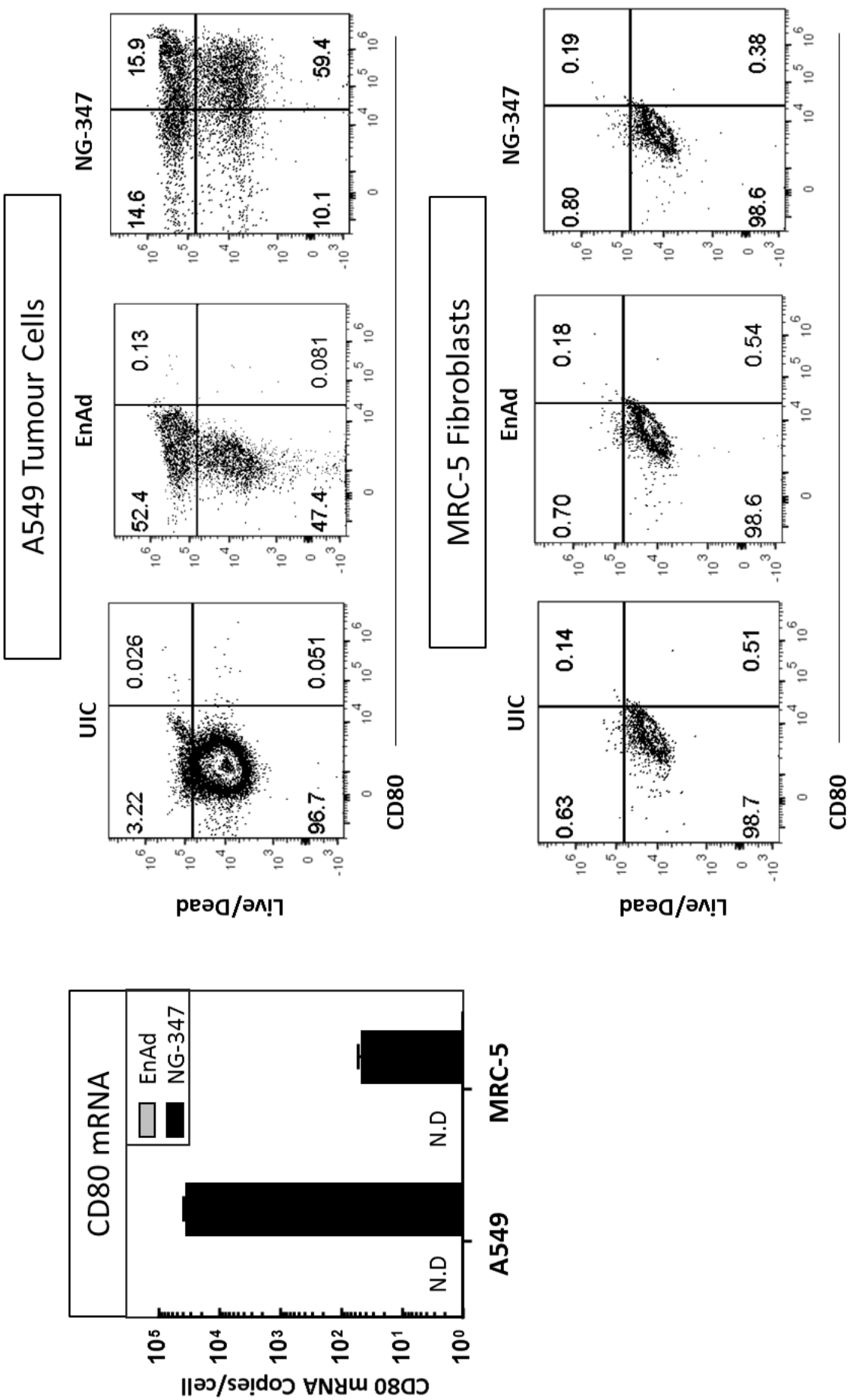
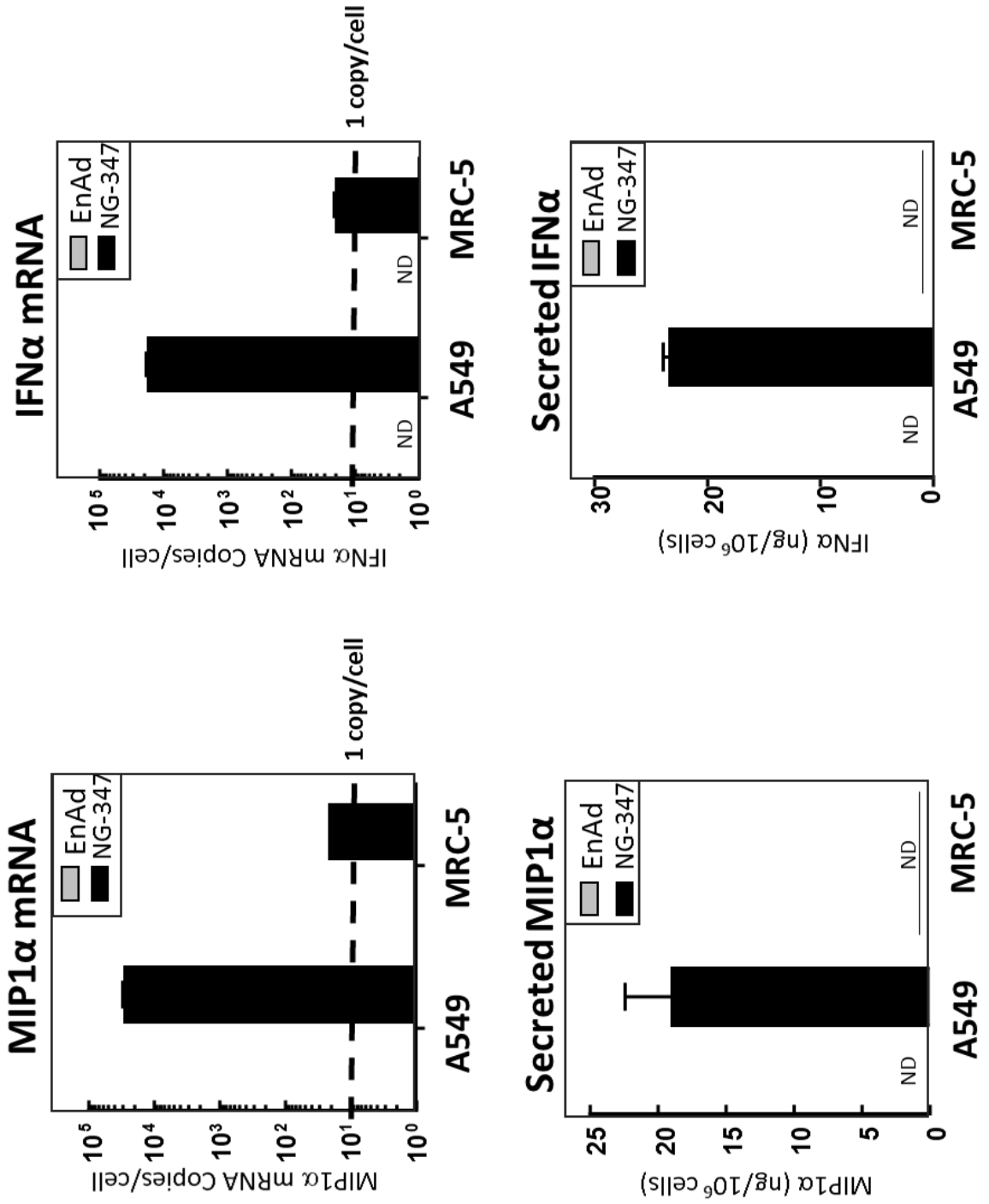

A549 Tumour Cells
MRC-5 Fibroblasts
UIC
EnAd
NG-347
Live/Dead
CD80
3.22
0.026
96.7
0.051
52.4
0.13
47.4
0.081
14.6
15.9
10.1
59.4
0.63
0.14
98.7
0.51
0.70
0.18
98.6
0.54
0.80
0.19
98.6
0.38
CD80 mRNA
EnAd
NG-347
CD80 mRNA Copies/cell
N.D
N.D
A549
MRC-5

Figure 30


MIP1α mRNA
EnAd
NG-347
MIP1α mRNA Copies/cell
1 copy/cell
A549
MRC-5
IFNα mRNA
IFNα mRNA Copies/cell
ND
Secreted MIP1α
MIP1α (ng/10^6 cells)
Secreted IFNα
IFNα (ng/10^6 cells)

P000145WO

Figure 31

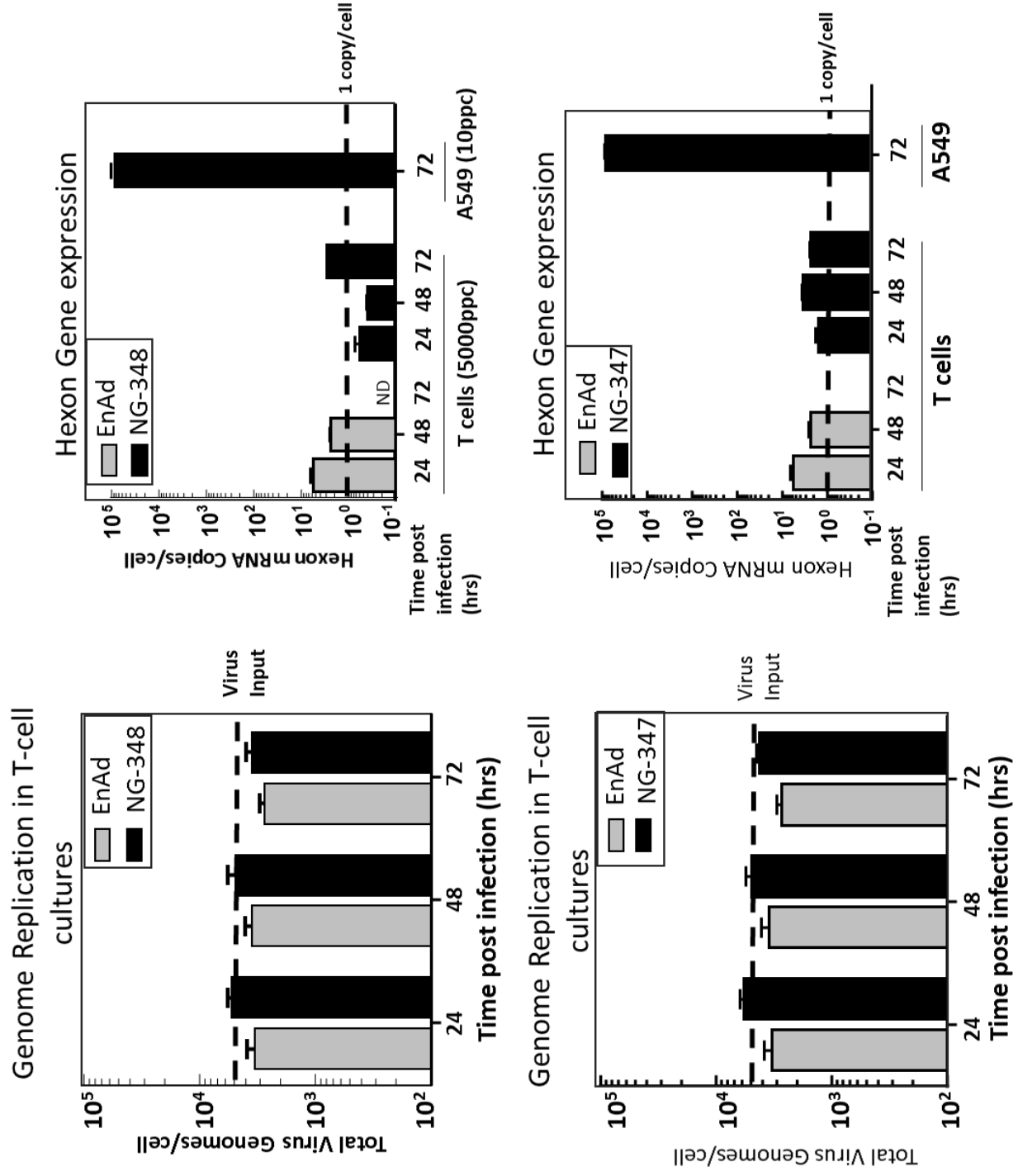


Figure 32

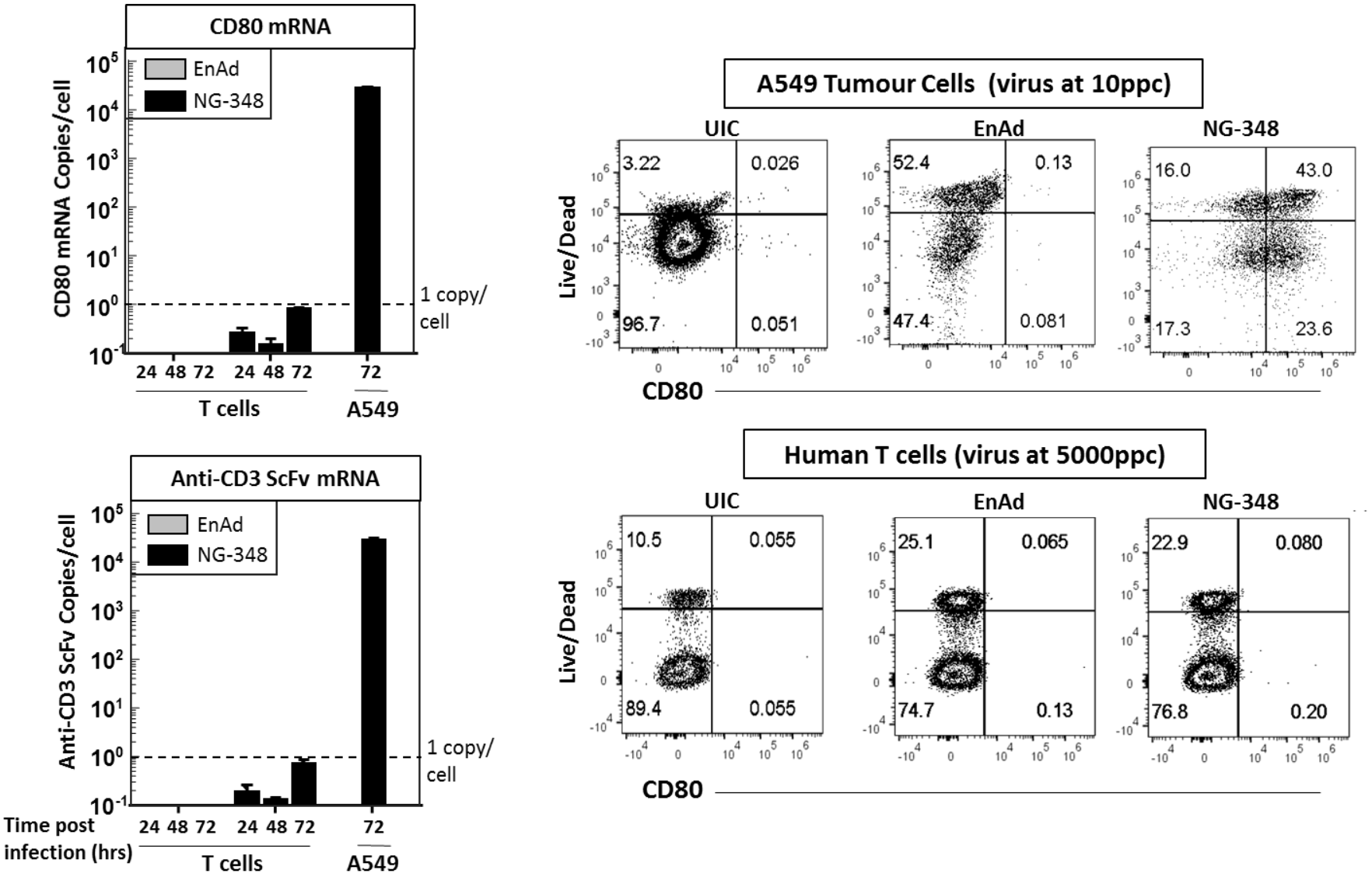

CD80 mRNA
EnAd
NG-348
CD80 mRNA Copies/cell
1 copy/ cell
24 48 72 24 48 72 72
T cells
A549
Anti-CD3 ScFv mRNA
Anti-CD3 ScFv Copies/cell
Time post infection (hrs)
A549 Tumour Cells (virus at 10ppc)
UIC
EnAd
NG-348
Live/Dead
CD80
3.22
0.026
96.7
0.051
52.4
0.13
47.4
0.081
16.0
43.0
17.3
23.6
Human T cells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

Figure 33

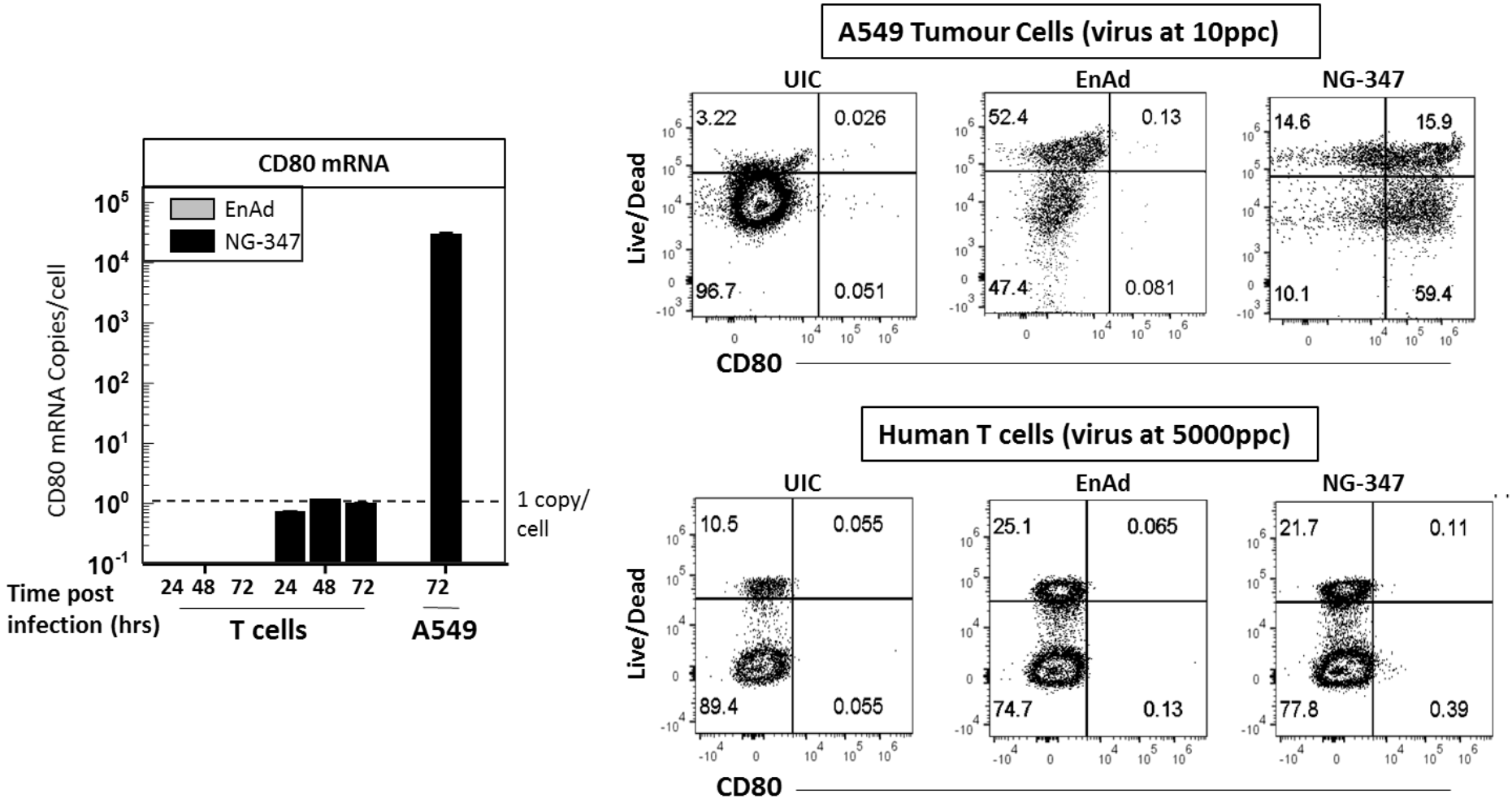
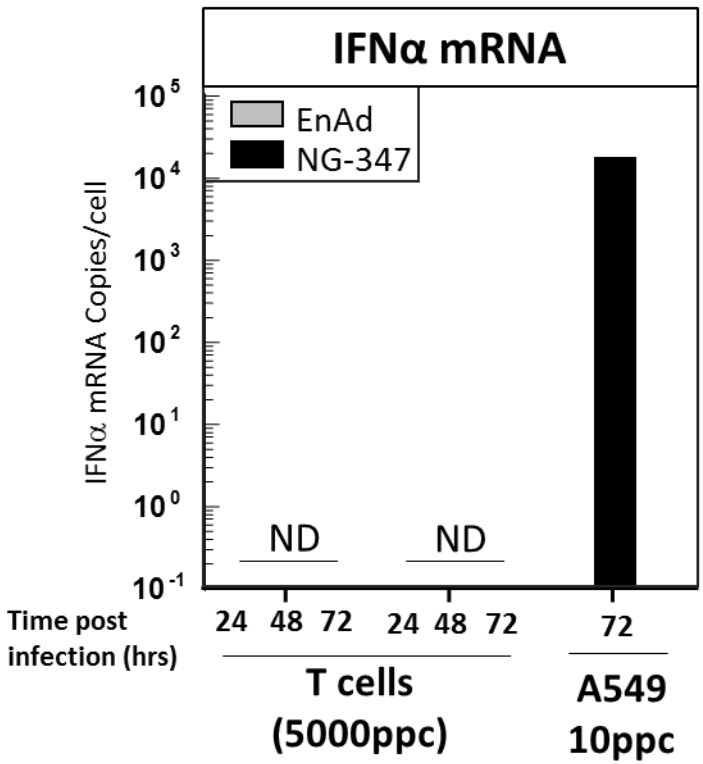

CD80 mRNA
EnAd
NG-347
CD80 mRNA Copies/cell
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
1 copy/ cell
Time post infection (hrs)
24 48 72 24 48 72 72
T cells
A549
A549 Tumour Cells (virus at 10ppc)
UIC
EnAd
NG-347
Live/Dead
3.22
0.026
96.7
0.051
52.4
0.13
47.4
0.081
14.6
15.9
10.1
59.4
CD80
Human T cells (virus at 5000ppc)
UIC
EnAd
NG-347
Live/Dead
10.5
0.055
89.4
0.055
25.1
0.065
74.7
0.13
21.7
0.11
77.8
0.39
CD80

Figure 34


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
Time post infection (hrs)
24 48 72 24 48 72 72
T cells (5000ppc)
A549 10ppc

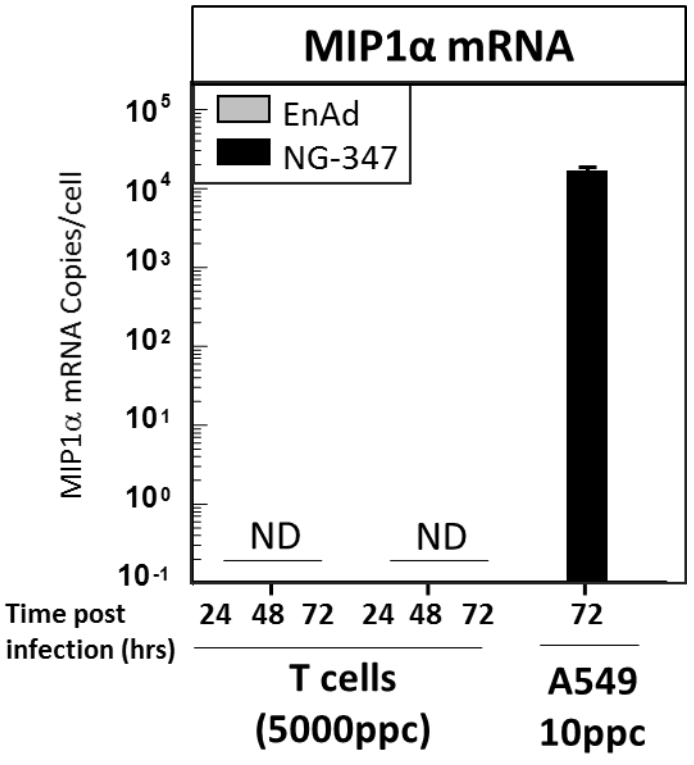

MIP1α mRNA
EnAd
NG-347
MIP1α mRNA Copies/cell
10^5
10^4
10^3
10^2
10^1
10^0
10^-1
ND
ND
Time post infection (hrs)
24 48 72 24 48 72 72
T cells (5000ppc)
A549 10ppc

Figure 35

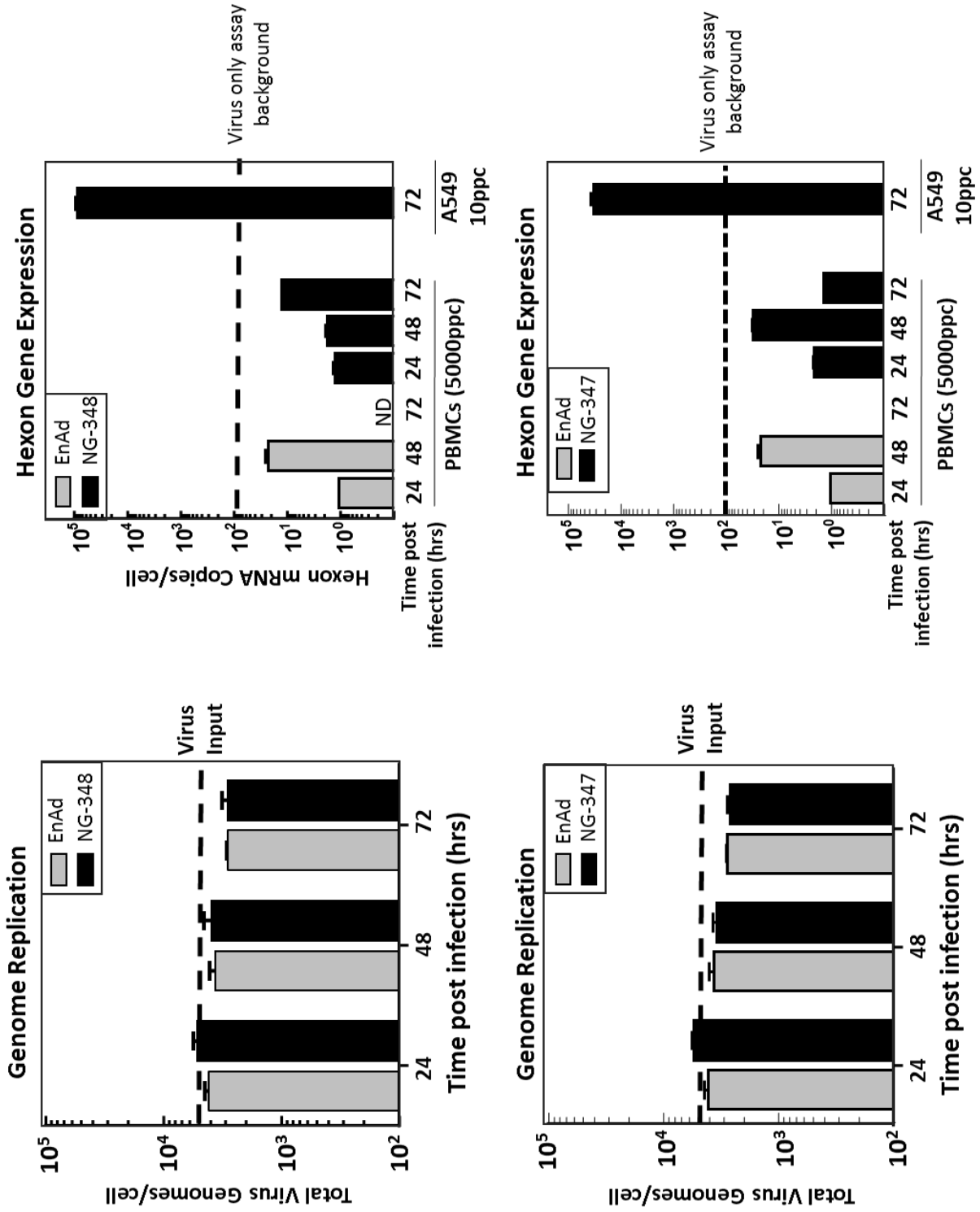

Hexon Gene Expression
EnAd
NG-348
Virus only assay background
Hexon mRNA Copies/cell
ND
Time post infection (hrs)
24 48 72 24 48 72
PBMCs (5000ppc)
A549 10ppc
Genome Replication
Virus Input
Total Virus Genomes/cell
Time post infection (hrs)
Hexon Gene Expression
EnAd
NG-347
Virus only assay background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

Figure 36

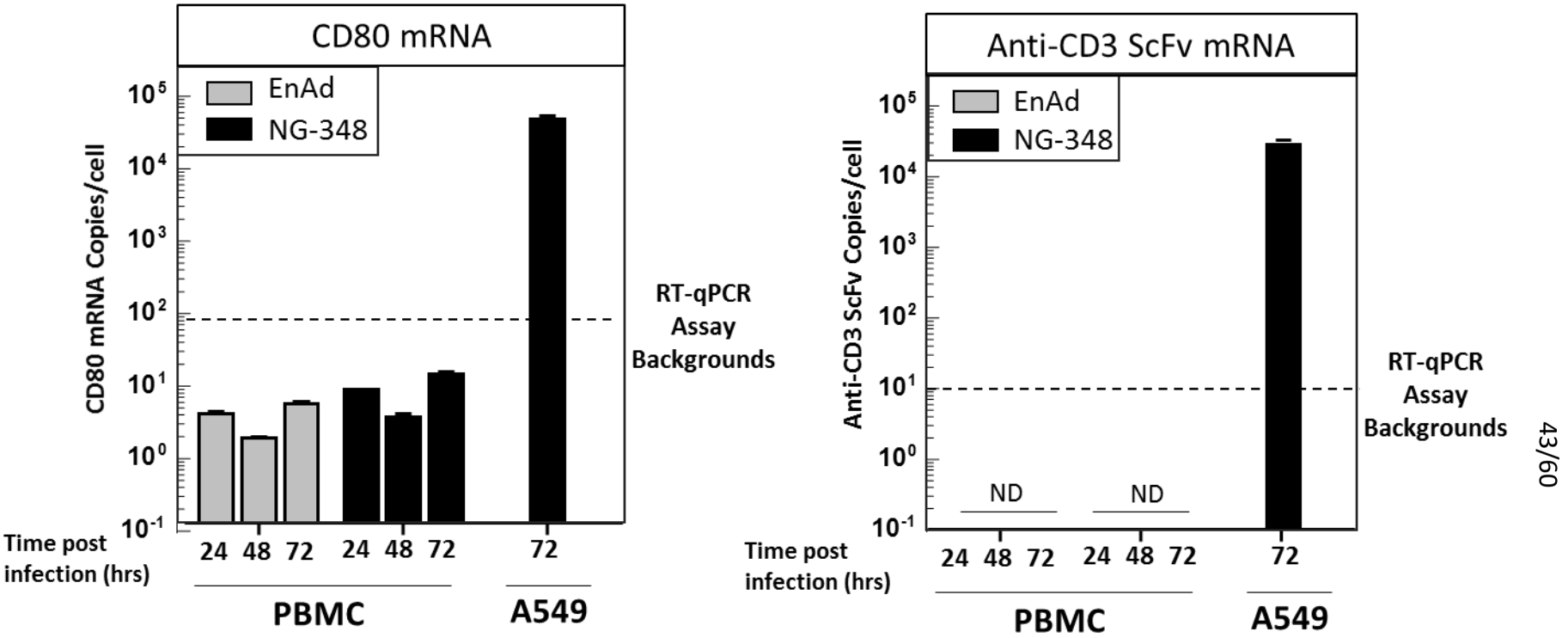

CD80 mRNA
EnAd
NG-348
CD80 mRNA Copies/cell
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
RT-qPCR Assay Backgrounds
Time post infection (hrs)
24 48 72 24 48 72 72
PBMC
A549
Anti-CD3 ScFv mRNA
EnAd
NG-348
Anti-CD3 ScFv Copies/cell
ND
ND
RT-qPCR Assay Backgrounds
Time post infection (hrs)
24 48 72 24 48 72 72
PBMC
A549

Figure 37

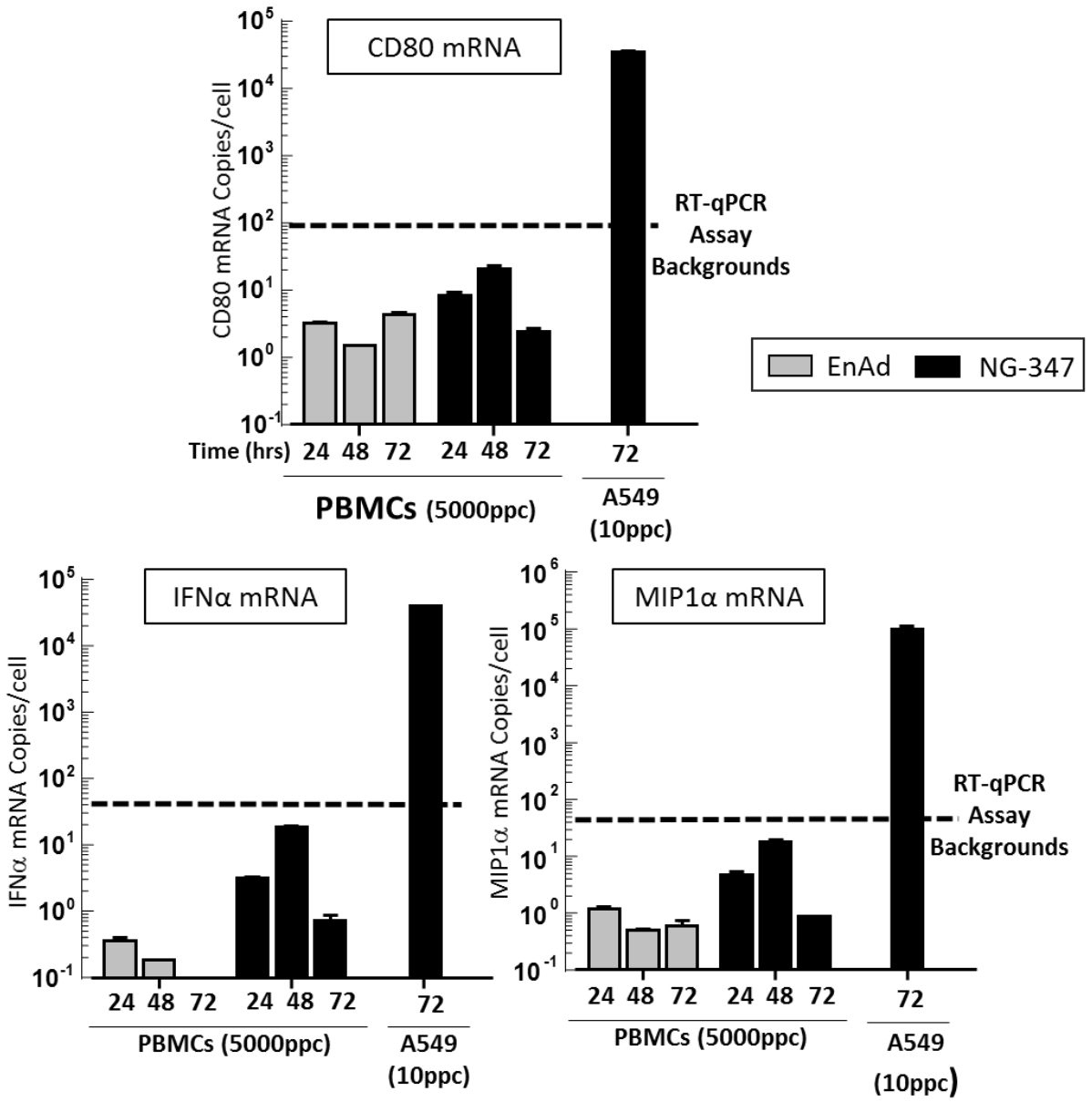

CD80 mRNA
CD80 mRNA Copies/cell
RT-qPCR Assay Backgrounds
EnAd
NG-347
Time (hrs) 24 48 72 24 48 72 72
PBMCs (5000ppc)
A549 (10ppc)
IFNα mRNA
IFNα mRNA Copies/cell
24 48 72 24 48 72 72
PBMCs (5000ppc)
A549 (10ppc)
MIP1α mRNA
MIP1α mRNA Copies/cell
RT-qPCR Assay Backgrounds
24 48 72 24 48 72 72
PBMCs (5000ppc)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

Figure 38

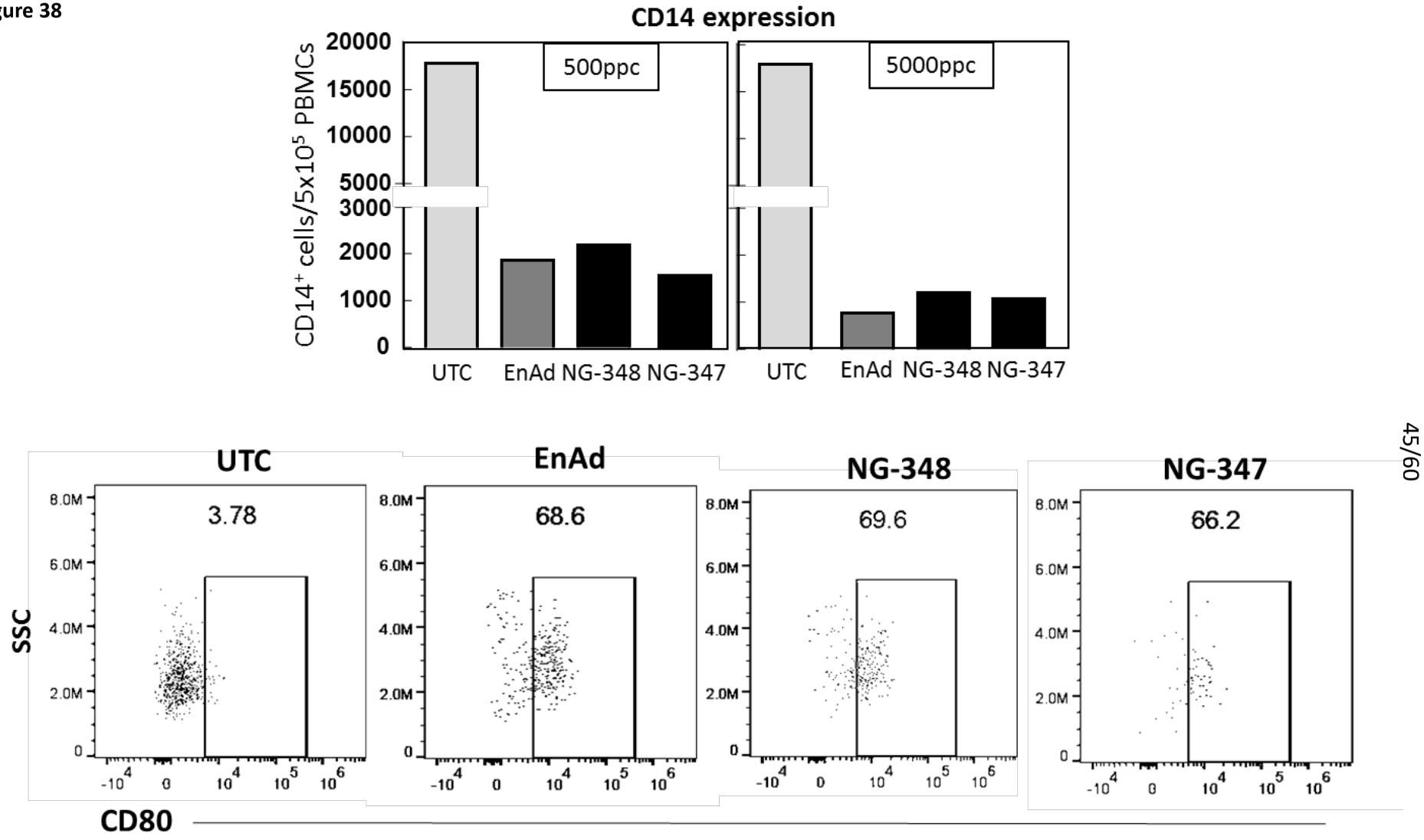

CD14 expression
CD14+ cells/5x10^5 PBMCs
20000
15000
10000
5000
3000
2000
1000
0
500ppc
5000ppc
UTC
EnAd
NG-348
NG-347
UTC
EnAd
NG-348
NG-347
UTC
EnAd
NG-348
NG-347
3.78
68.6
69.6
66.2
SSC
CD80
8.0M
6.0M
4.0M
2.0M
0
-10^4
0
10^4
10^5
10^6

Figure 39

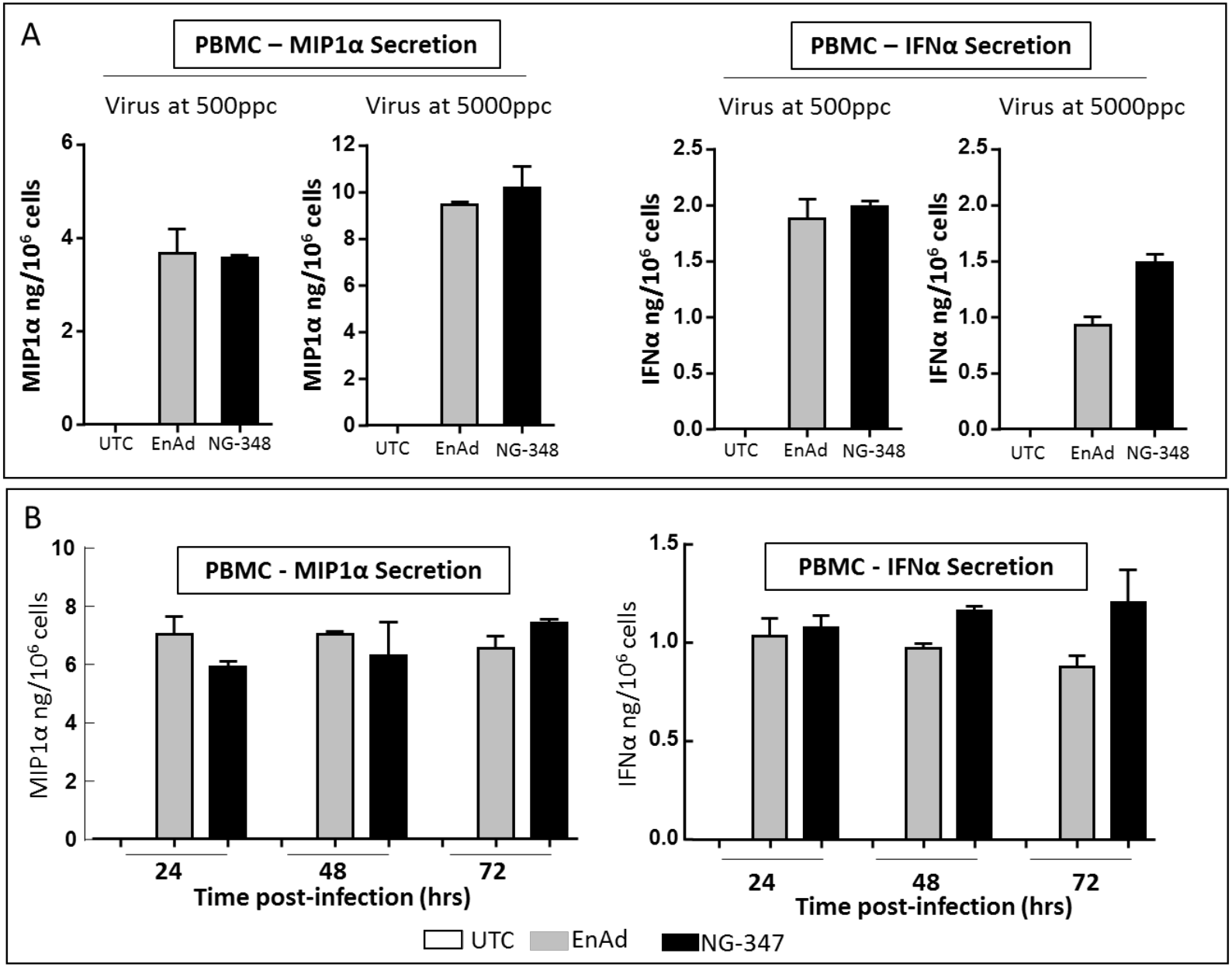

A
PBMC – MIP1α Secretion
Virus at 500ppc
Virus at 5000ppc
MIP1α ng/10^6 cells
UTC
EnAd
NG-348
PBMC – IFNα Secretion
IFNα ng/10^6 cells
B
PBMC - MIP1α Secretion
PBMC - IFNα Secretion
Time post-infection (hrs)
24
48
72
UTC
EnAd
NG-347

Figure 40

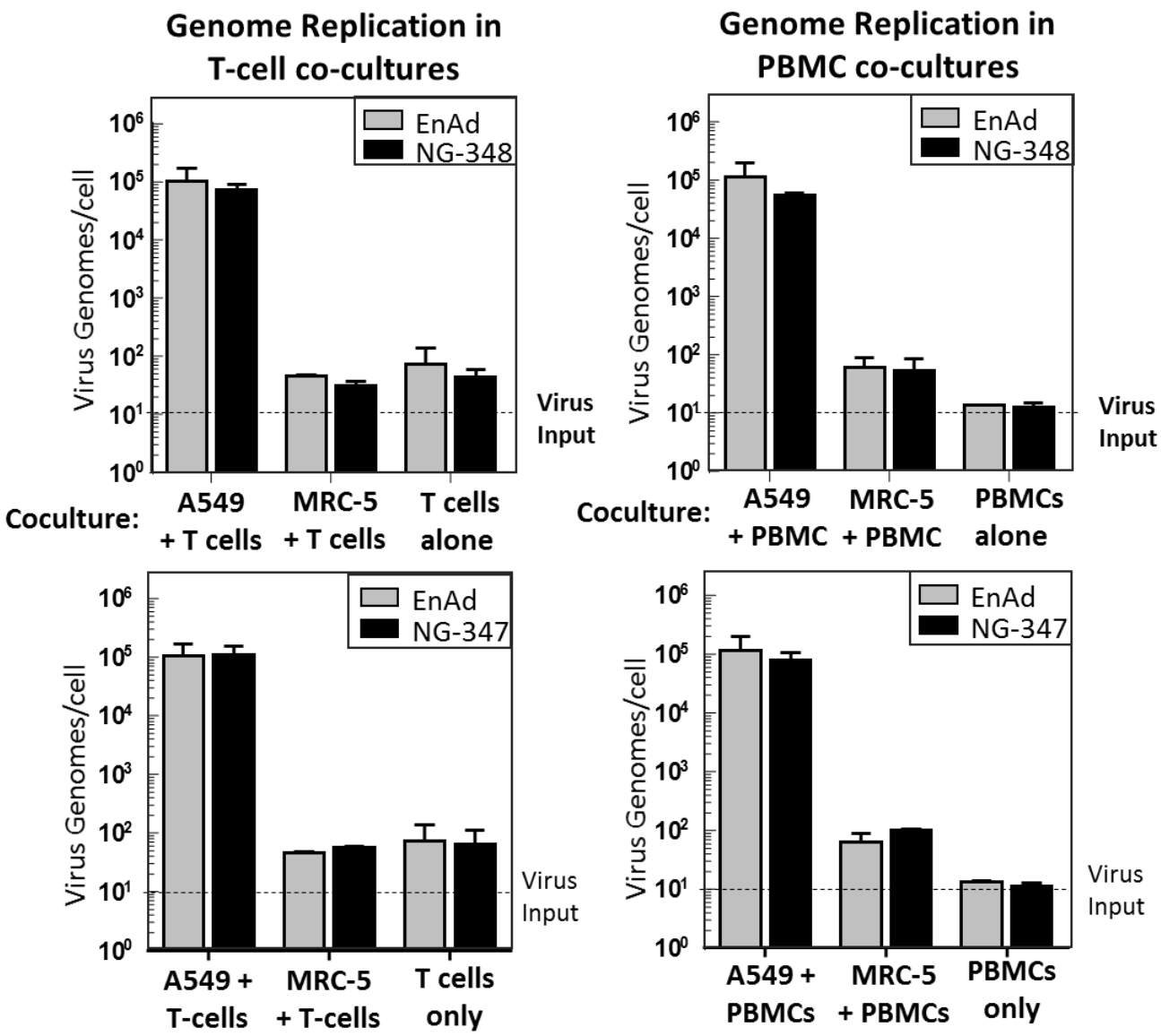

Genome Replication in T-cell co-cultures
Virus Genomes/cell
EnAd
NG-348
Virus Input
Coculture:
A549 + T cells
MRC-5 + T cells
T cells alone
Genome Replication in PBMC co-cultures
Virus Genomes/cell
EnAd
NG-348
Virus Input
Coculture:
A549 + PBMC
MRC-5 + PBMC
PBMCs alone
Virus Genomes/cell
EnAd
NG-347
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 41

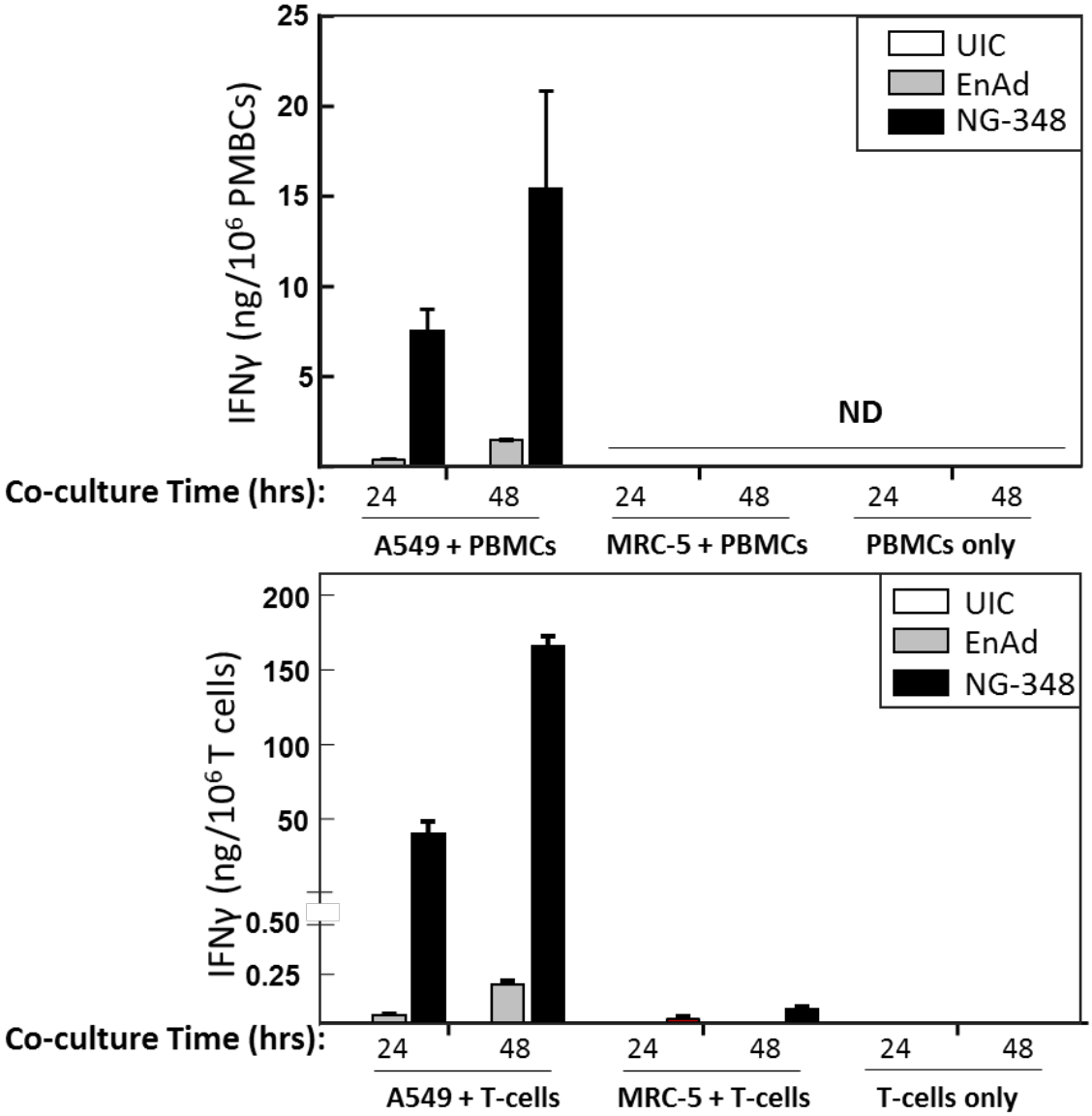

IFNγ (ng/10⁶ PMBCs)
25
20
15
10
5
UIC
EnAd
NG-348
ND
Co-culture Time (hrs):
24
48
24
48
24
48
A549 + PBMCs
MRC-5 + PBMCs
PBMCs only
IFNγ (ng/10⁶ T cells)
200
150
100
50
0.50
0.25
UIC
EnAd
NG-348
Co-culture Time (hrs):
24
48
24
48
24
48
A549 + T-cells
MRC-5 + T-cells
T-cells only

Figure 42

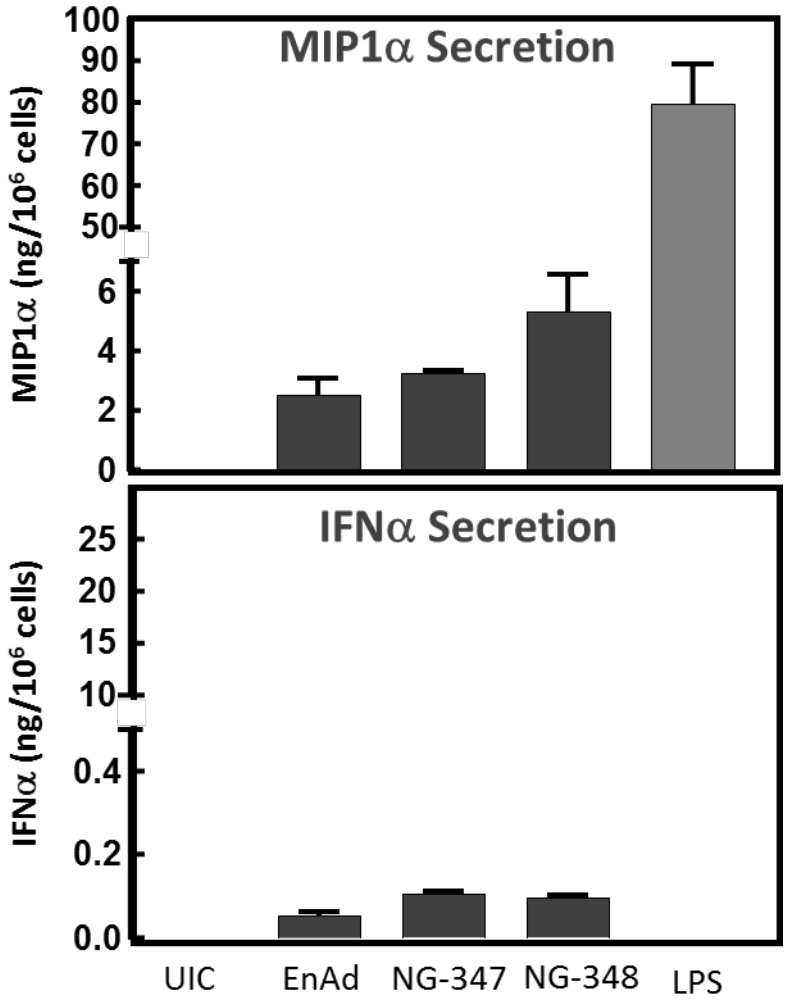

MIP1α Secretion
MIP1α (ng/10^6 cells)
100
90
80
70
60
50
6
4
2
0
IFNα Secretion
IFNα (ng/10^6 cells)
25
20
15
10
0.4
0.2
0.0
UIC
EnAd
NG-347
NG-348
LPS

Figure 43

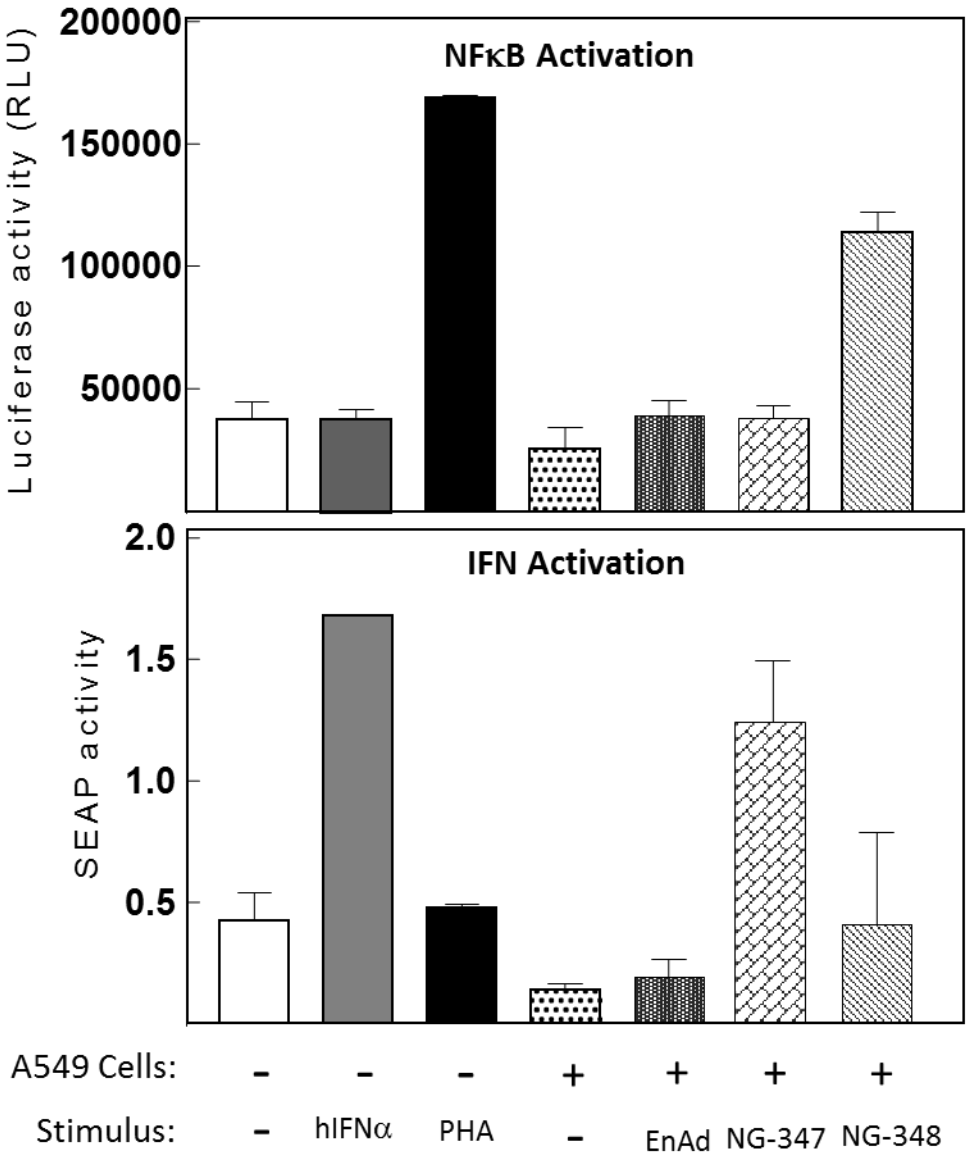

NFκB Activation
Luciferase activity (RLU)
200000
150000
100000
50000
IFN Activation
SEAP activity
2.0
1.5
1.0
0.5
A549 Cells: - - - + + + +
Stimulus: - hIFNα PHA - EnAd NG-347 NG-348

Figure 44

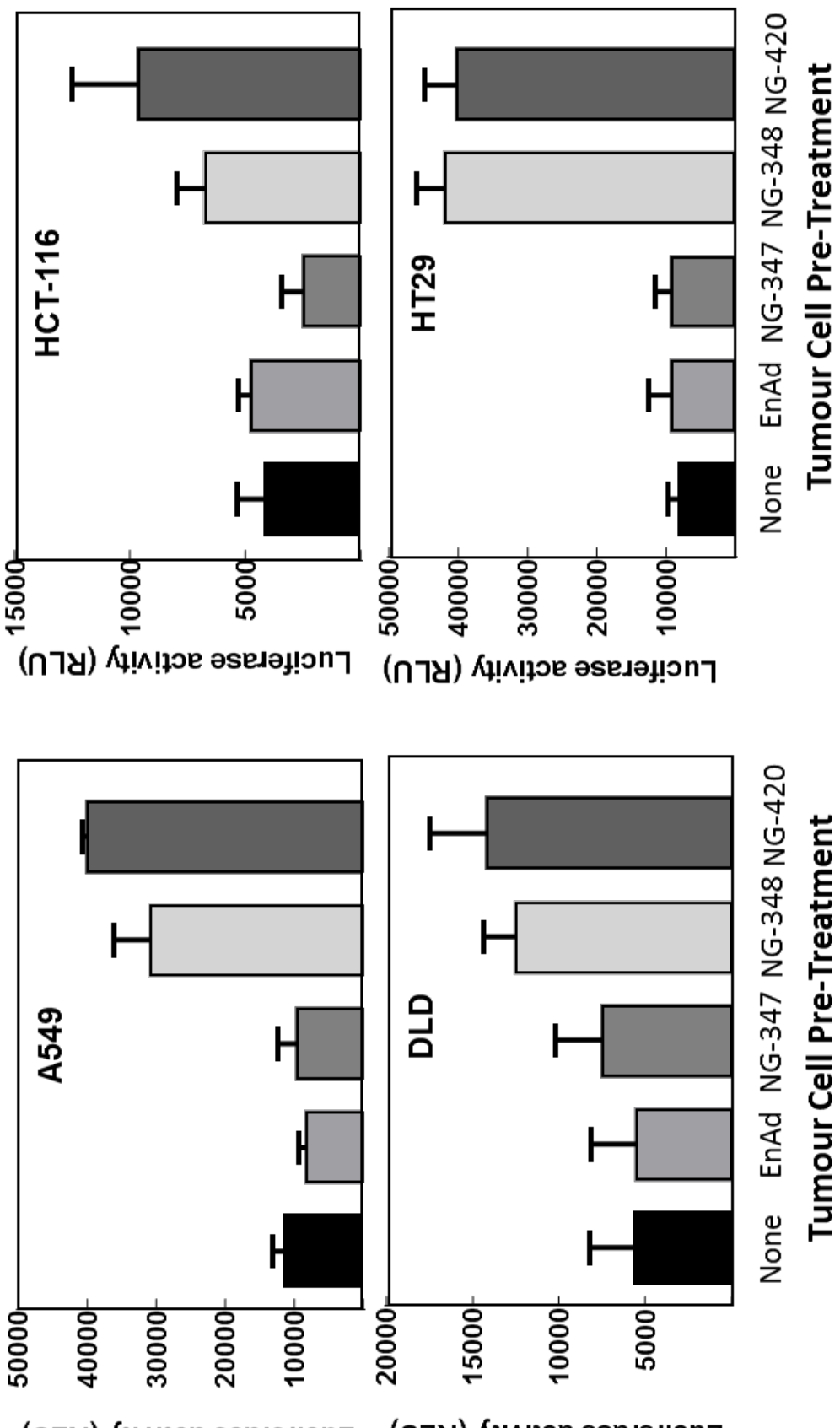

A549
DLD
HCT-116
HT29
Luciferase activity (RLU)
50000
40000
30000
20000
10000
15000
5000
None
EnAd
NG-347
NG-348
NG-420
Tumour Cell Pre-Treatment

Figure 45

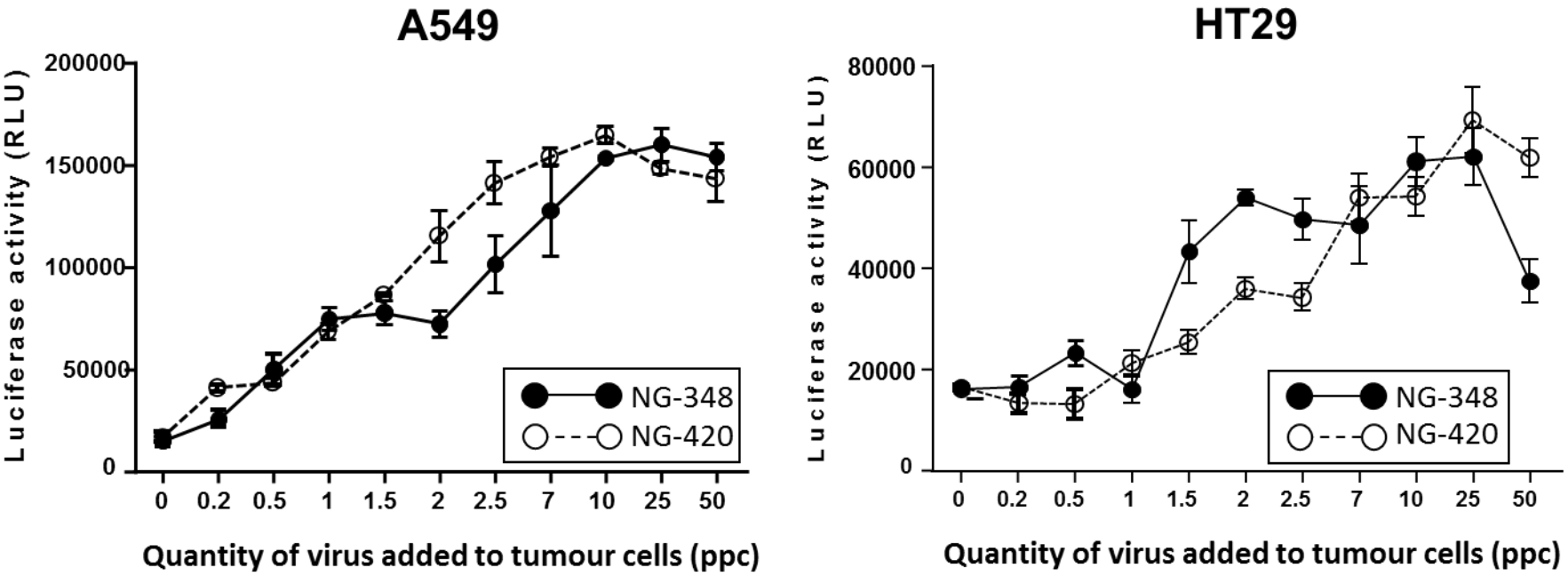
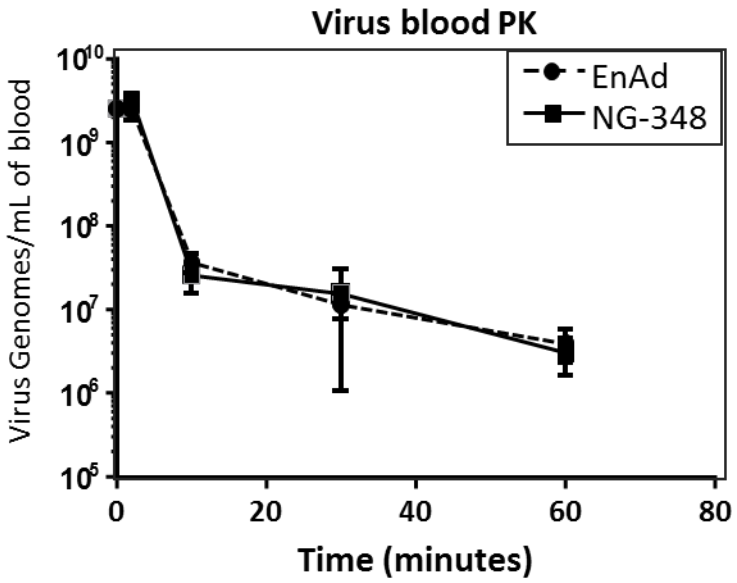
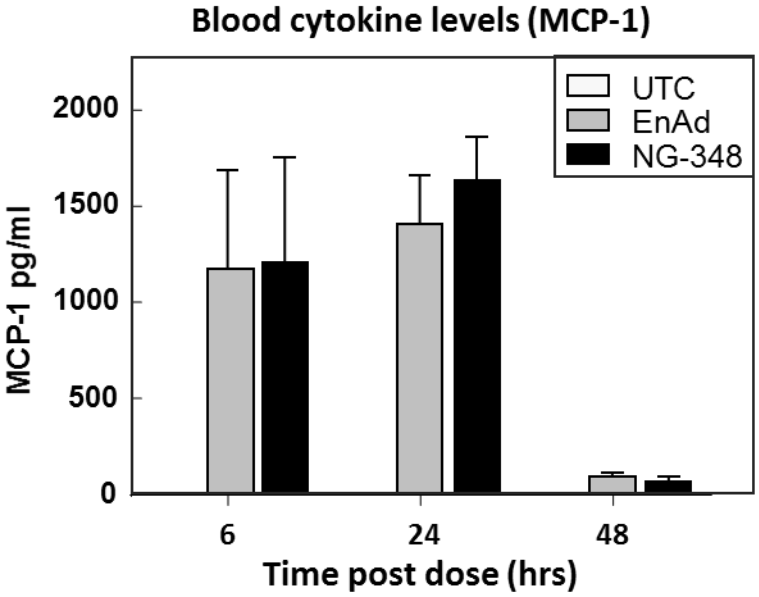
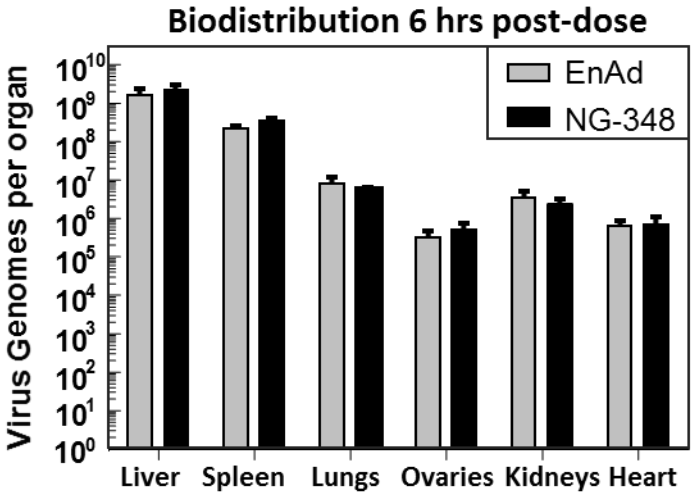

A549
Luciferase activity (RLU)
200000
150000
100000
50000
0
0 0.2 0.5 1 1.5 2 2.5 7 10 25 50
Quantity of virus added to tumour cells (ppc)
NG-348
NG-420
HT29
Luciferase activity (RLU)
80000
60000
40000
20000
0
0 0.2 0.5 1 1.5 2 2.5 7 10 25 50
Quantity of virus added to tumour cells (ppc)
NG-348
NG-420

Figure 46


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


Blood cytokine levels (MCP-1)
MCP-1 pg/ml
UTC
EnAd
NG-348
Time post dose (hrs)


Biodistribution 6 hrs post-dose
Virus Genomes per organ
EnAd
NG-348
Liver
Spleen
Lungs
Ovaries
Kidneys
Heart

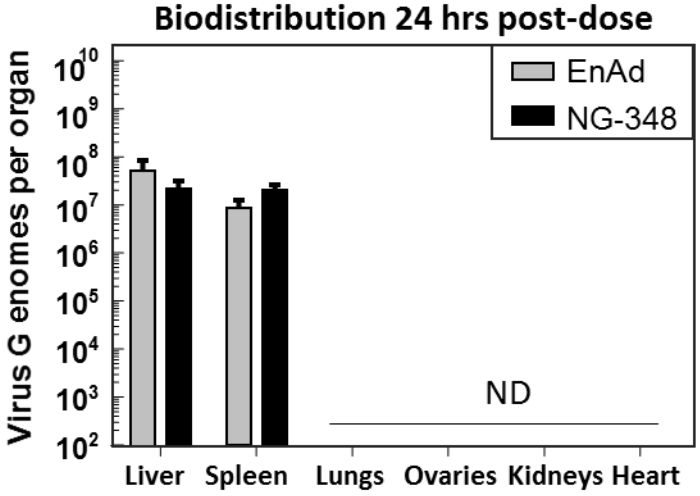
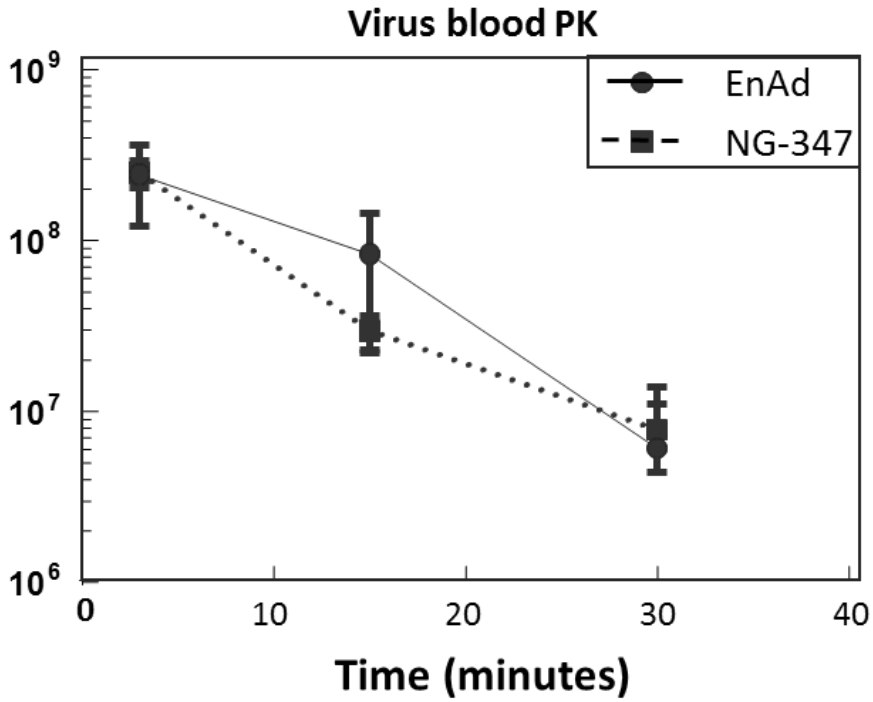

Biodistribution 24 hrs post-dose
Virus G enomes per organ
EnAd
NG-348
ND
Liver
Spleen
Lungs
Ovaries
Kidneys
Heart

Figure 47


Virus blood PK
EnAd
NG-347
10^9
10^8
10^7
10^6
0
10
20
30
40
Time (minutes)

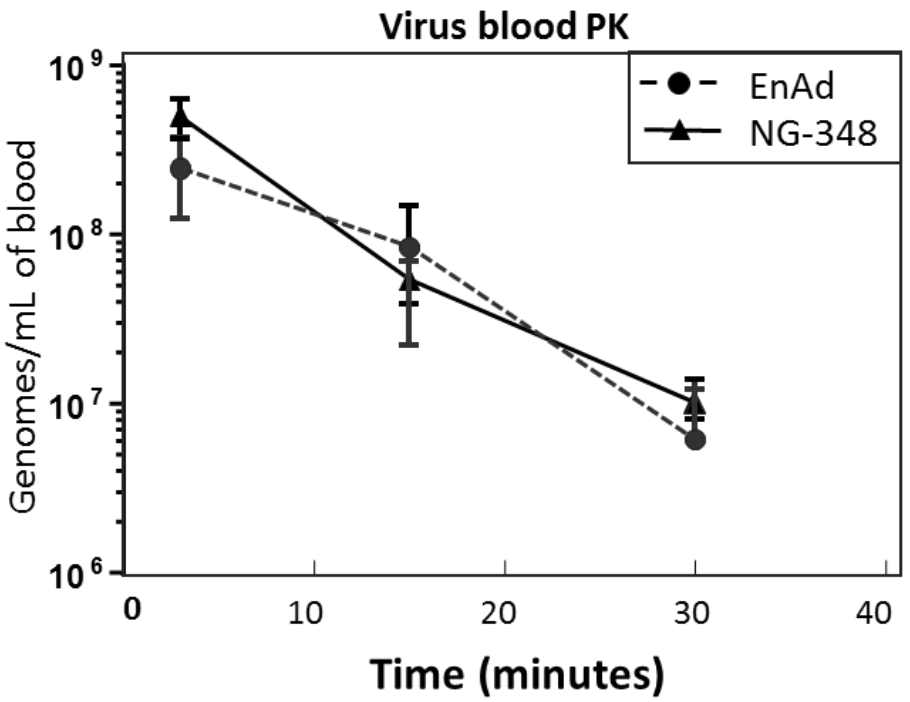

Virus blood PK
EnAd
NG-348
Genomes/mL of blood
10^9
10^8
10^7
10^6
0
10
20
30
40
Time (minutes)

Figure 48

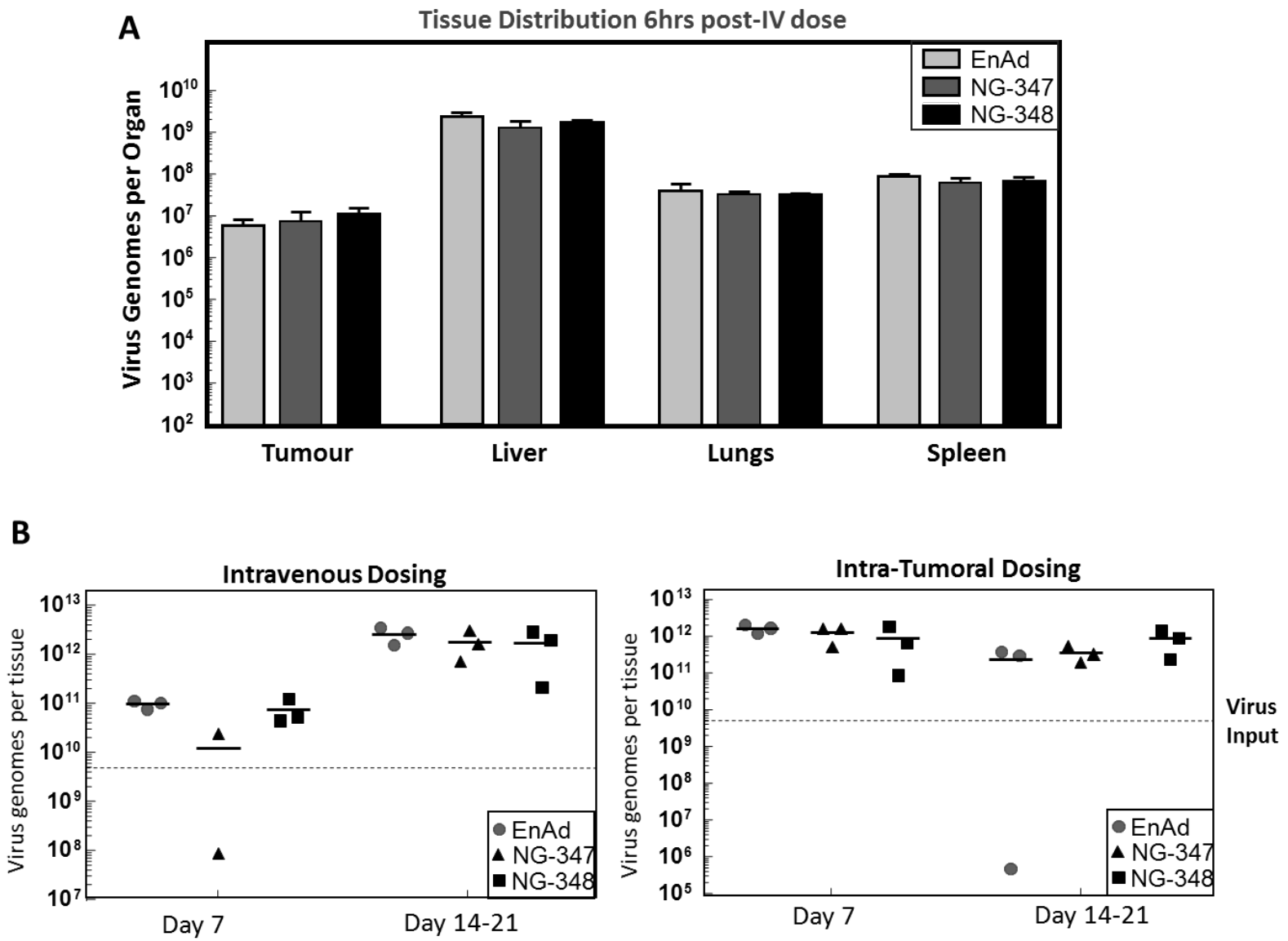

A
Tissue Distribution 6hrs post-IV dose
Virus Genomes per Organ
EnAd
NG-347
NG-348
Tumour
Liver
Lungs
Spleen
B
Intravenous Dosing
Intra-Tumoral Dosing
Virus genomes per tissue
Virus Input
Day 7
Day 14-21

Figure 49

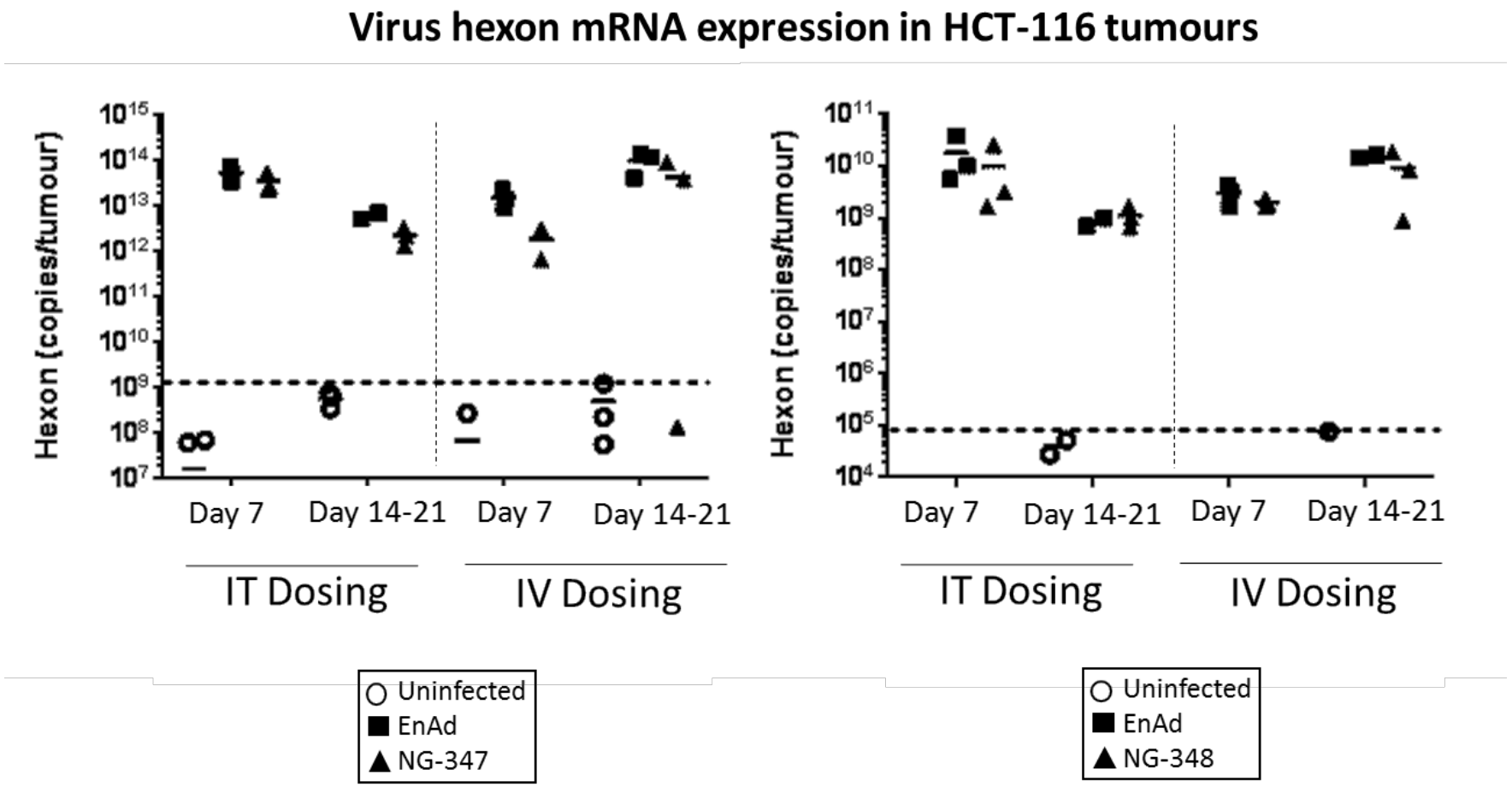

Virus hexon mRNA expression in HCT-116 tumours
Hexon (copies/tumour)
Day 7
Day 14-21
Day 7
Day 14-21
IT Dosing
IV Dosing
Uninfected
EnAd
NG-347
Hexon (copies/tumour)
Day 7
Day 14-21
Day 7
Day 14-21
IT Dosing
IV Dosing
Uninfected
EnAd
NG-348

Figure 50

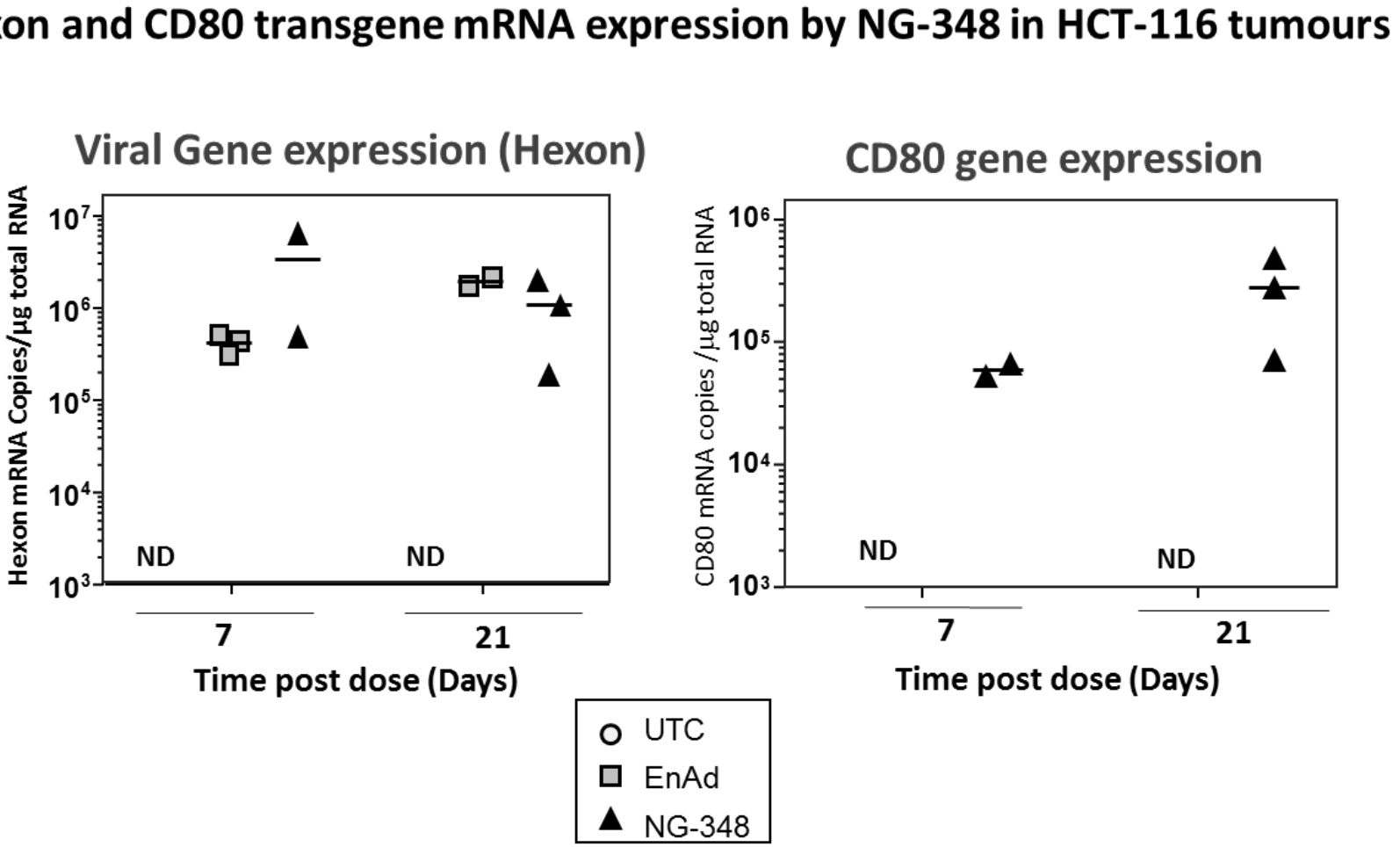

Hexon and CD80 transgene mRNA expression by NG-348 in HCT-116 tumours
Viral Gene expression (Hexon)
Hexon mRNA Copies/µg total RNA
10^7
10^6
10^5
10^4
10^3
ND
ND
7
21
Time post dose (Days)
CD80 gene expression
CD80 mRNA copies /µg total RNA
10^6
10^5
10^4
10^3
ND
ND
7
21
Time post dose (Days)
UTC
EnAd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

Figure 51

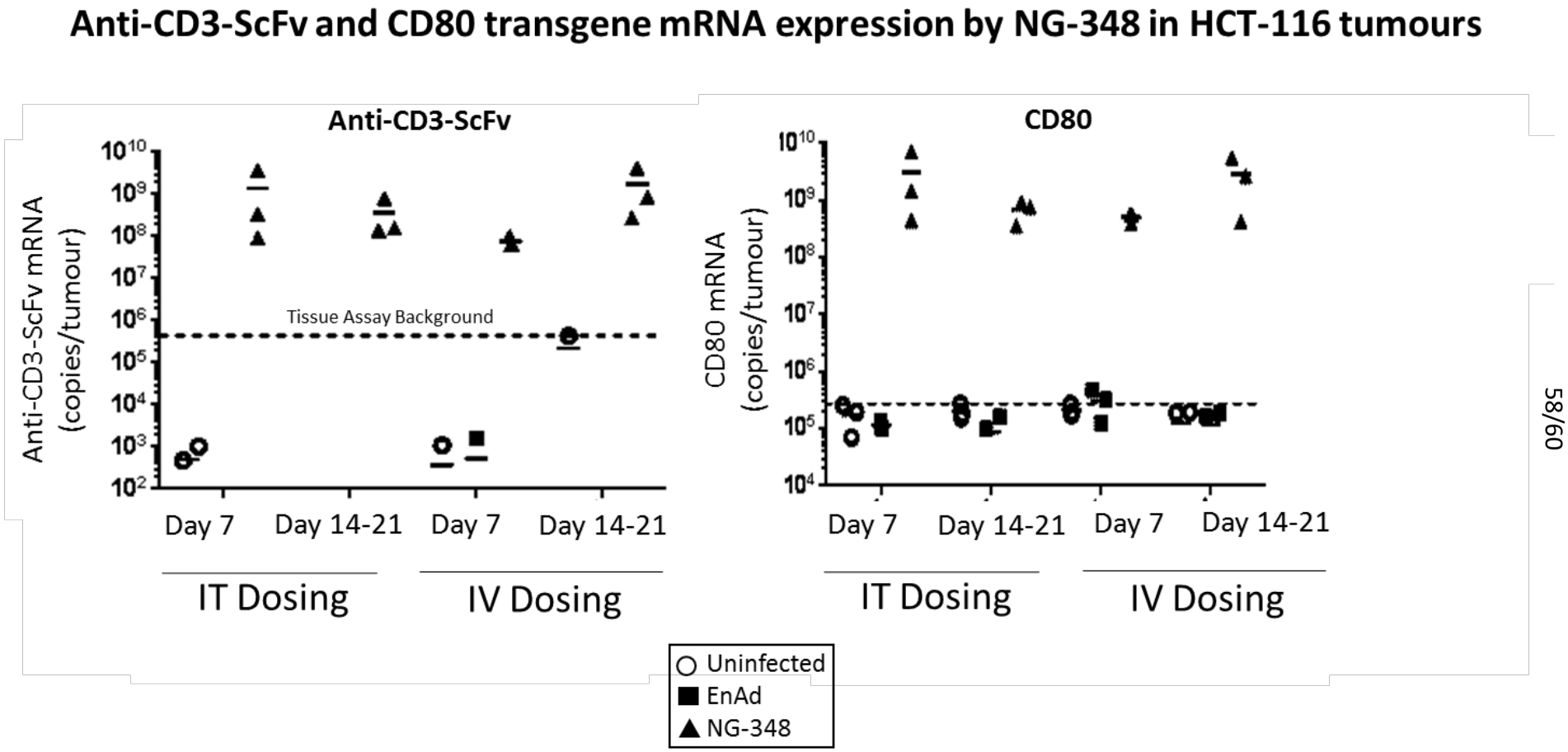

Anti-CD3-ScFv and CD80 transgene mRNA expression by NG-348 in HCT-116 tumours
Anti-CD3-ScFv
Anti-CD3-ScFv mRNA (copies/tumour)
Tissue Assay Background
Day 7
Day 14-21
Day 7
Day 14-21
IT Dosing
IV Dosing
CD80
CD80 mRNA (copies/tumour)
Day 7
Day 14-21
Day 7
Day 14-21
IT Dosing
IV Dosing
Uninfected
EnAd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

Figure 52

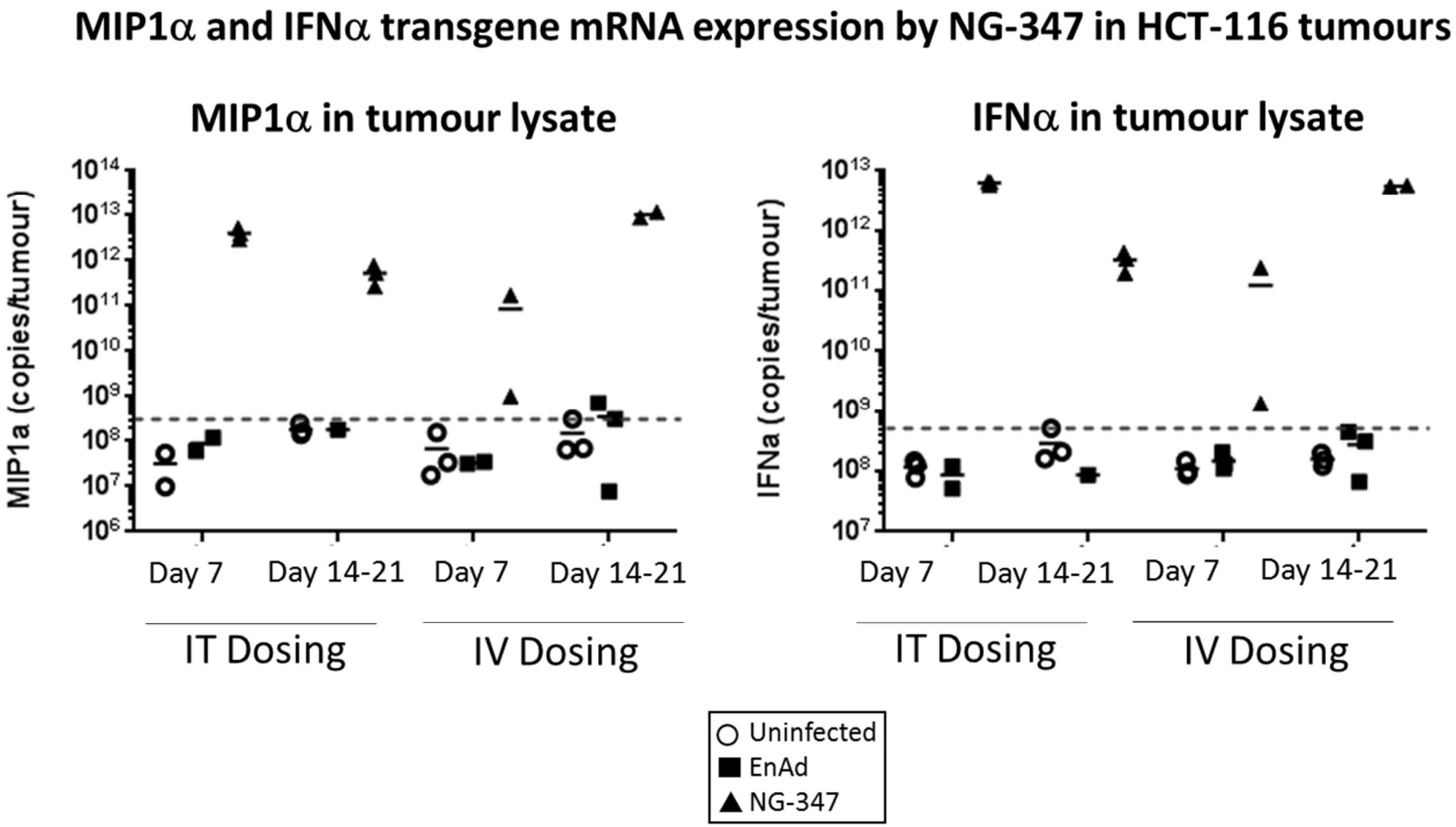
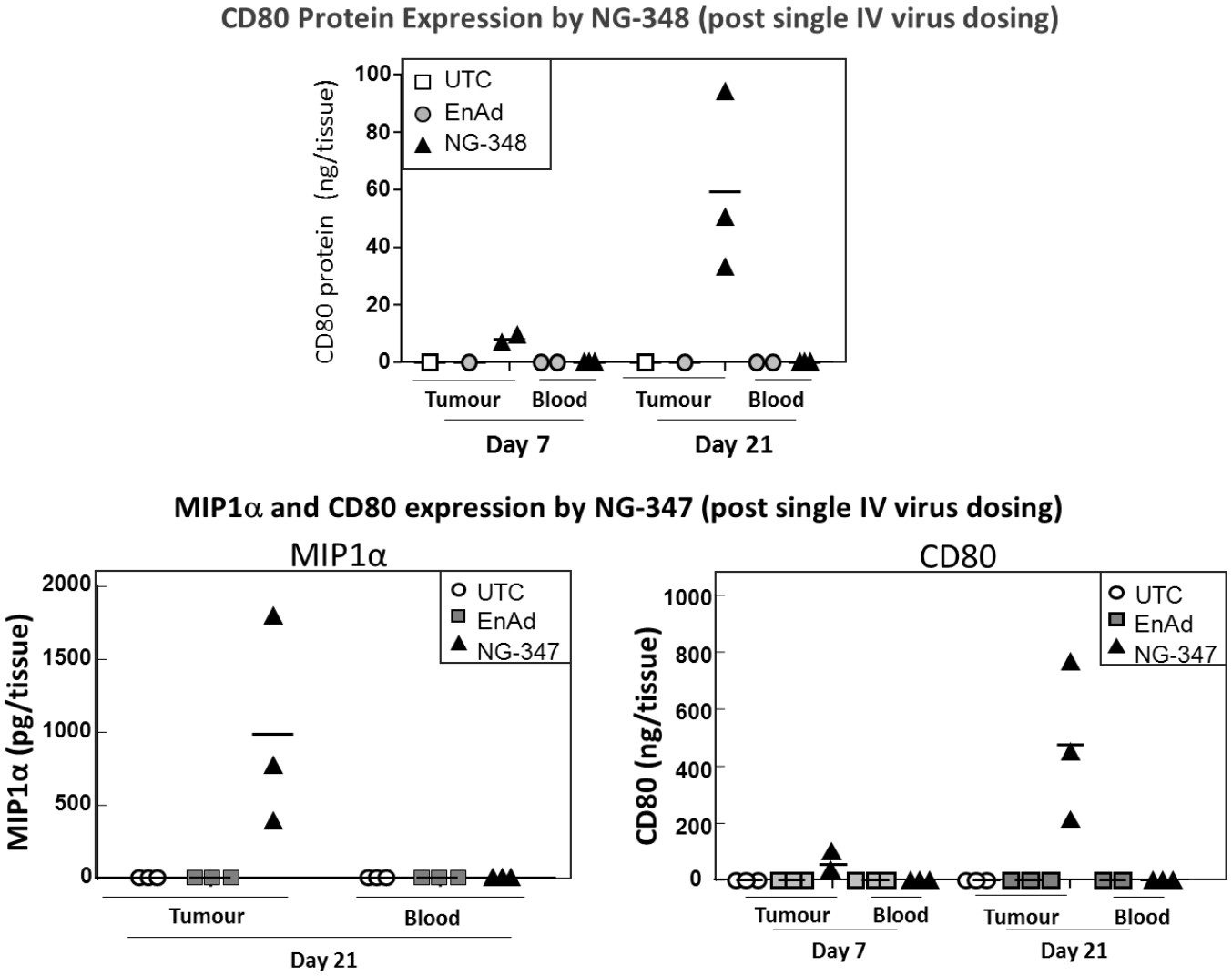

MIP1α and IFNα transgene mRNA expression by NG-347 in HCT-116 tumours
MIP1α in tumour lysate
MIP1a (copies/tumour)
Day 7
Day 14-21
Day 7
Day 14-21
IT Dosing
IV Dosing
IFNα in tumour lysate
IFNa (copies/tumour)
Day 7
Day 14-21
Day 7
Day 14-21
IT Dosing
IV Dosing
Uninfected
EnAd
NG-347

Figure 53


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