Figure 1

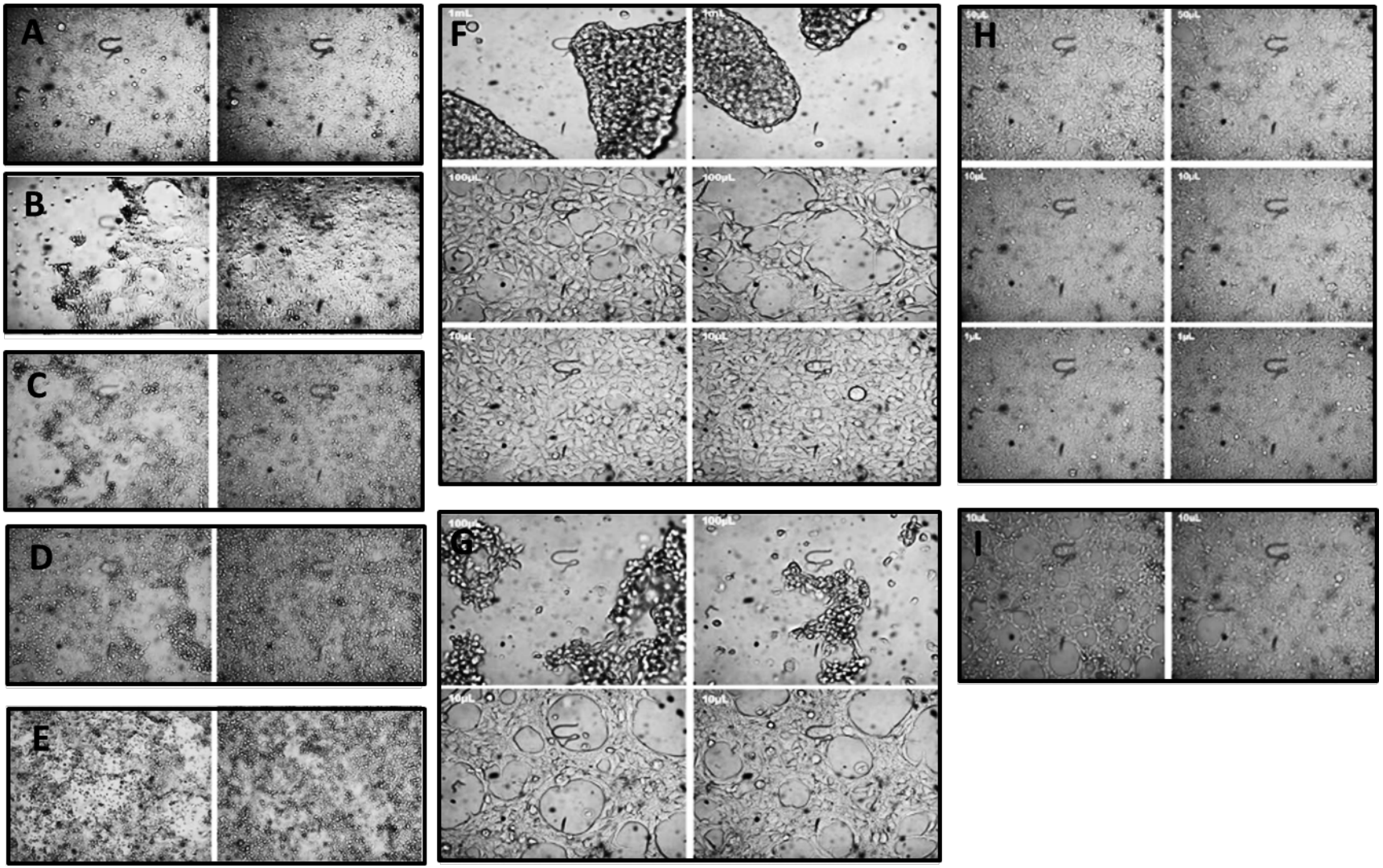


Figure 2

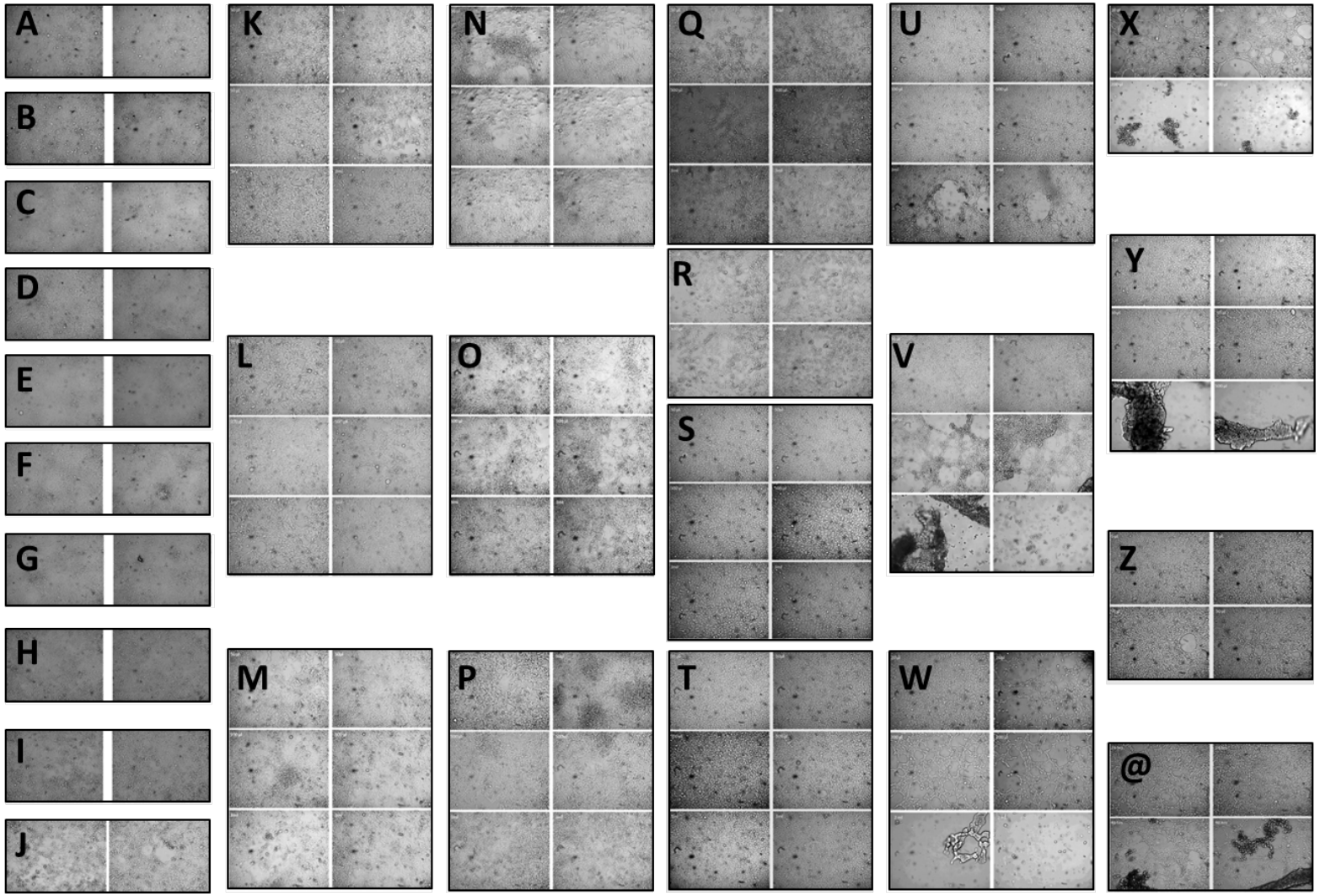


Figure 3

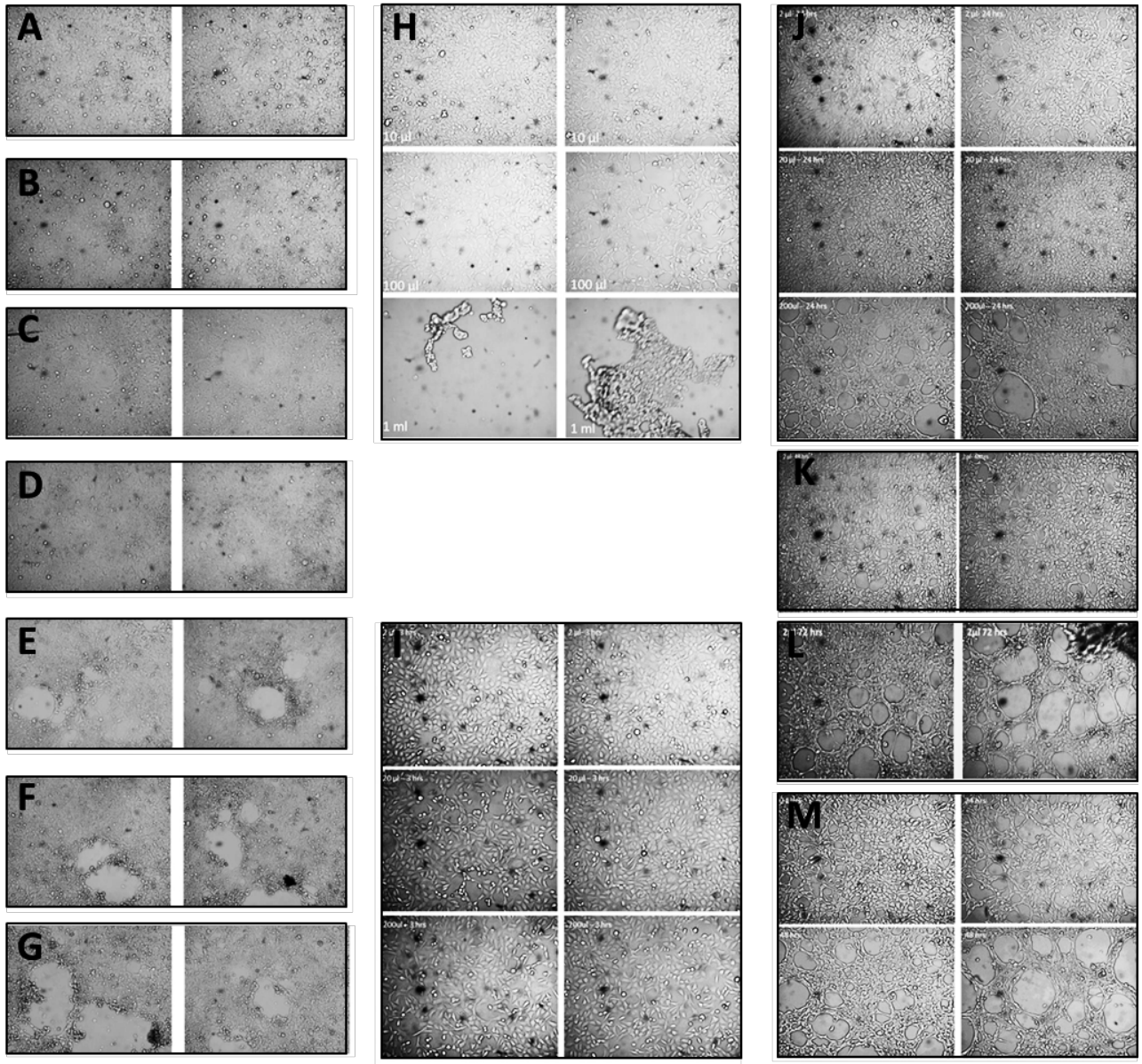


Figure 4A

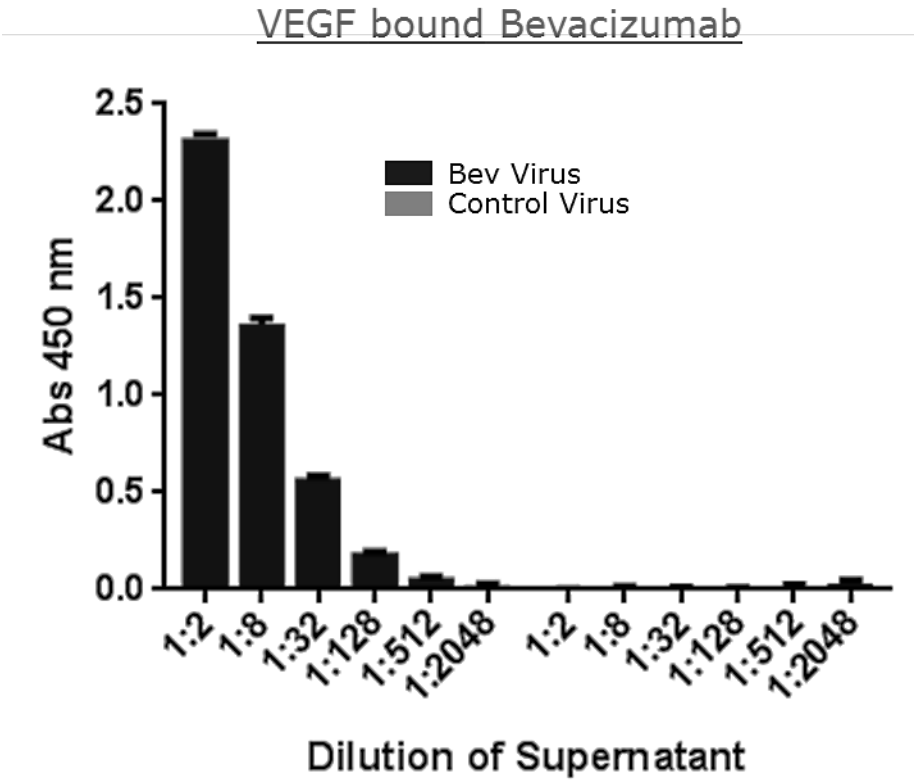


Figure 4B

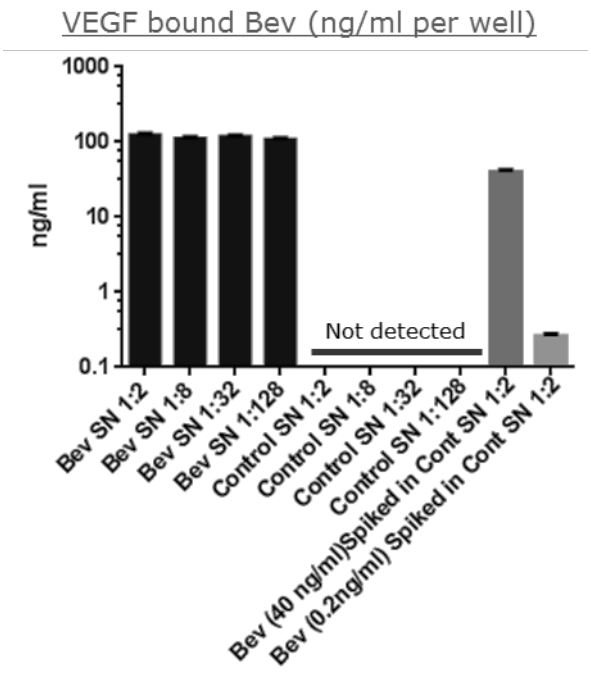


Figure 5

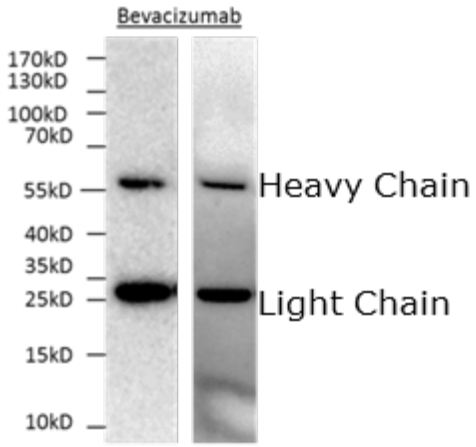


Figure 6

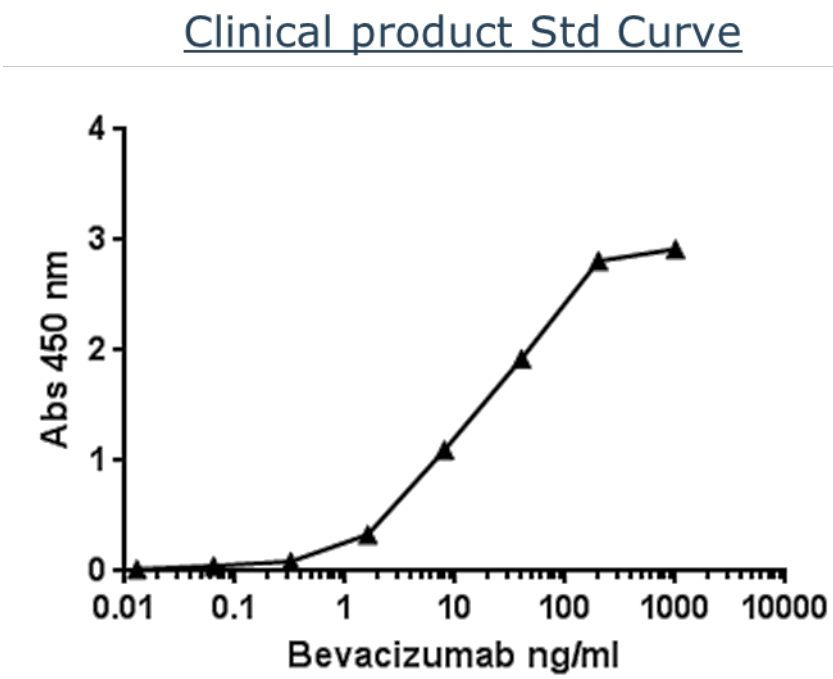


Figure 7

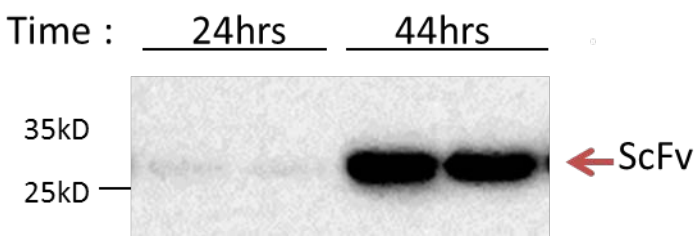


Figure 8

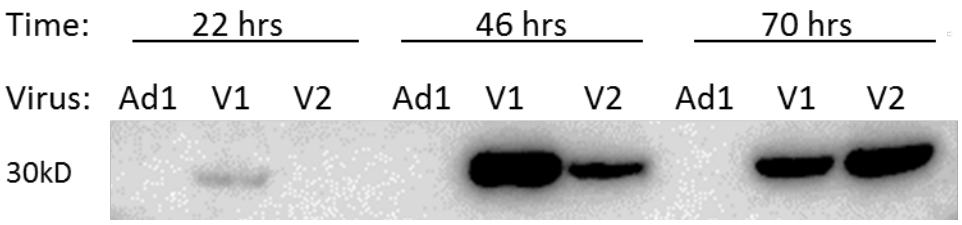


Figure 9

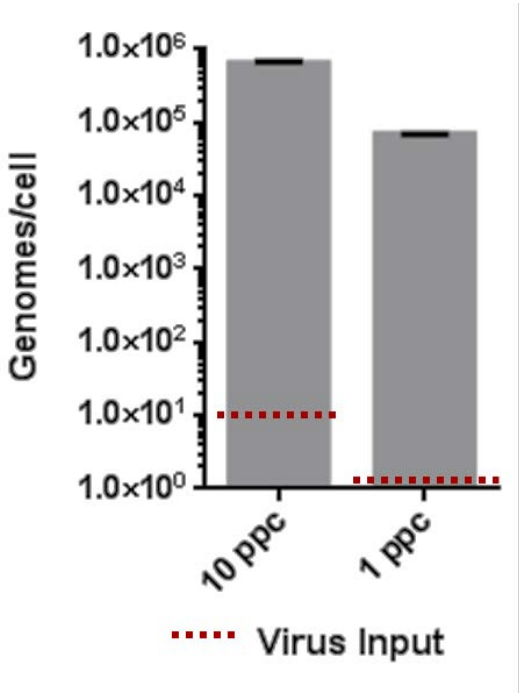
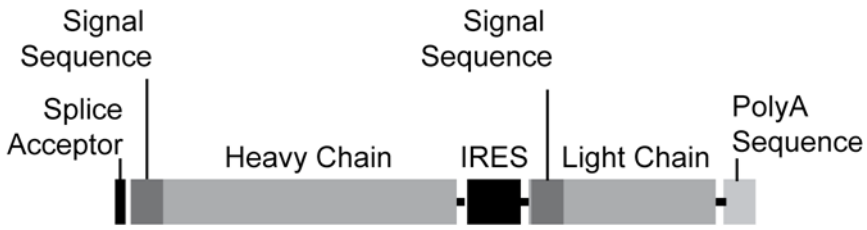
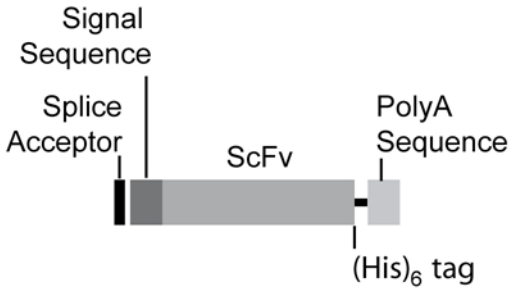


Figure 10

A NG-135



B NG-78



C NG-76

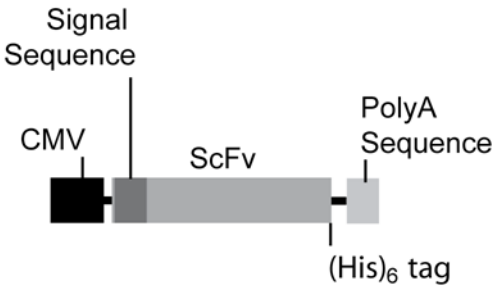


Figure 11

ColoAd1 = EnAd

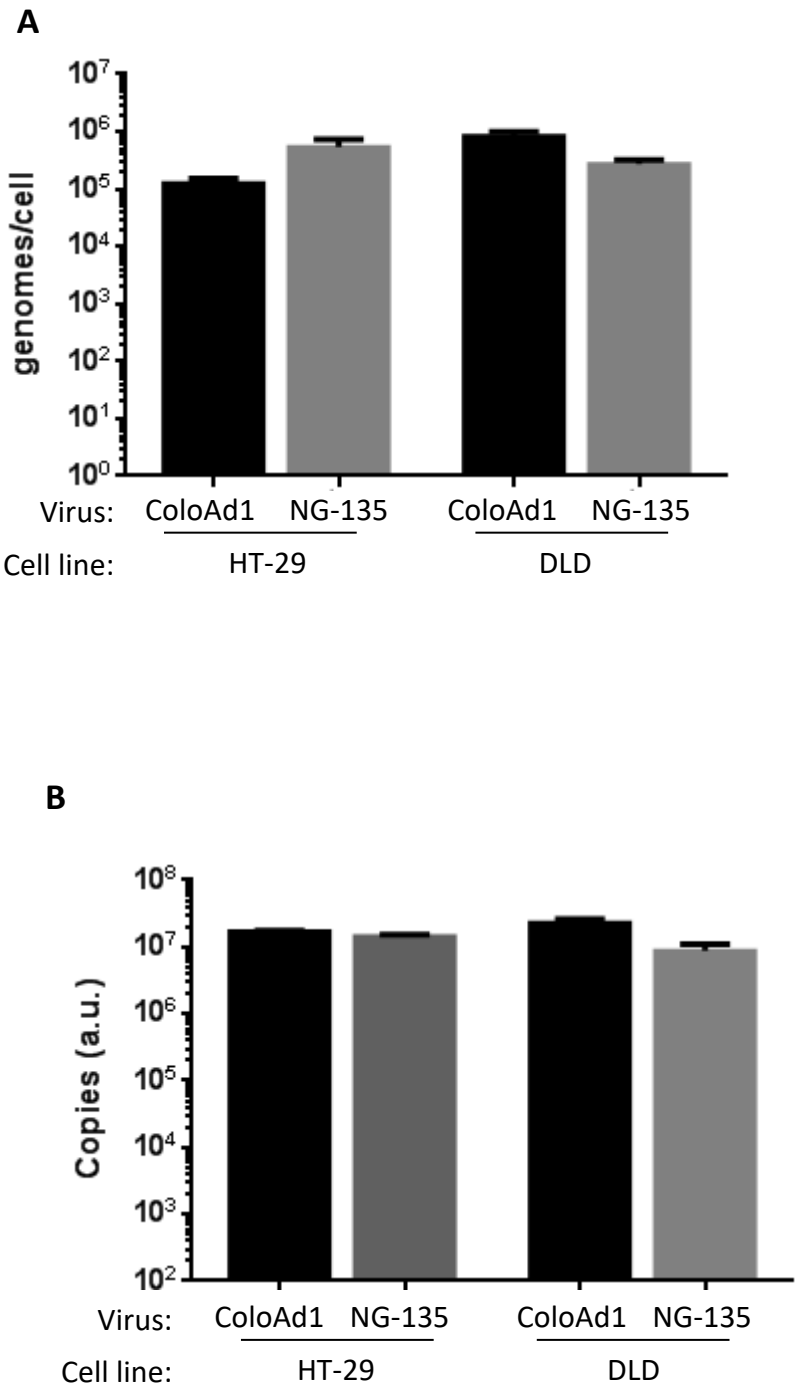


Figure 12 ColoAd1 = EnAd

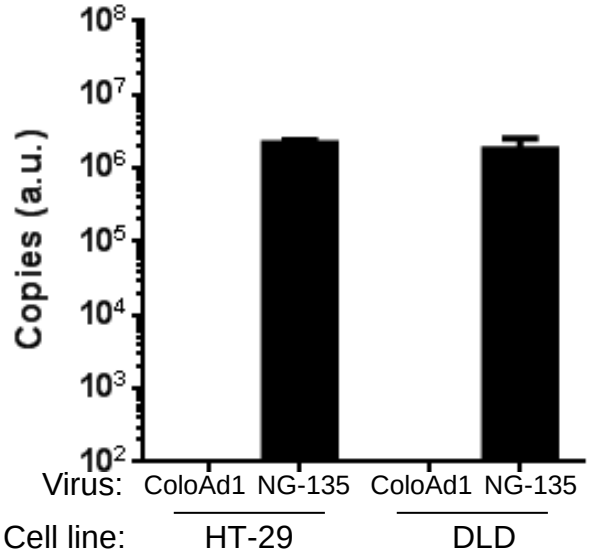


Figure 13

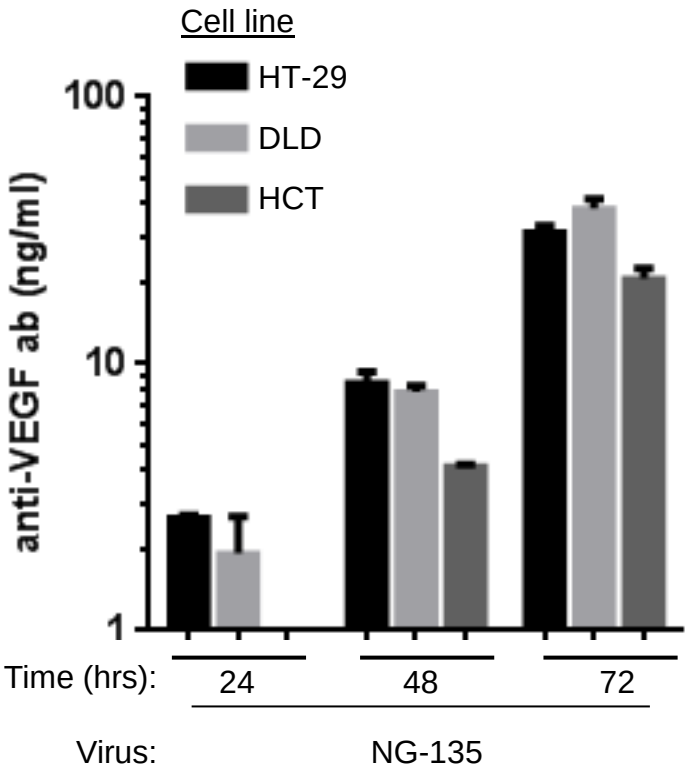


Figure 14

ColoAd1 = EnAd

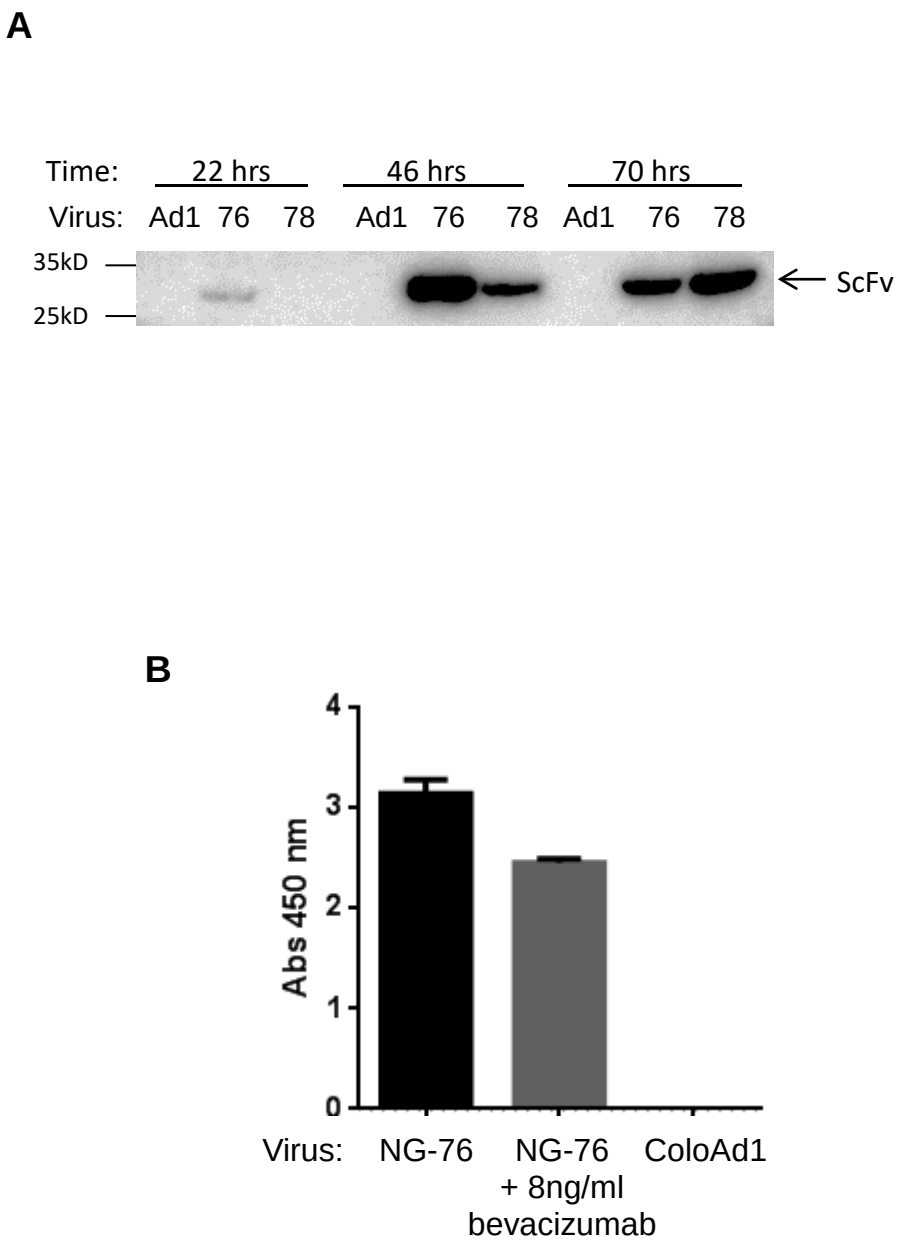


Figure 15 ColoAd1 = EnAd

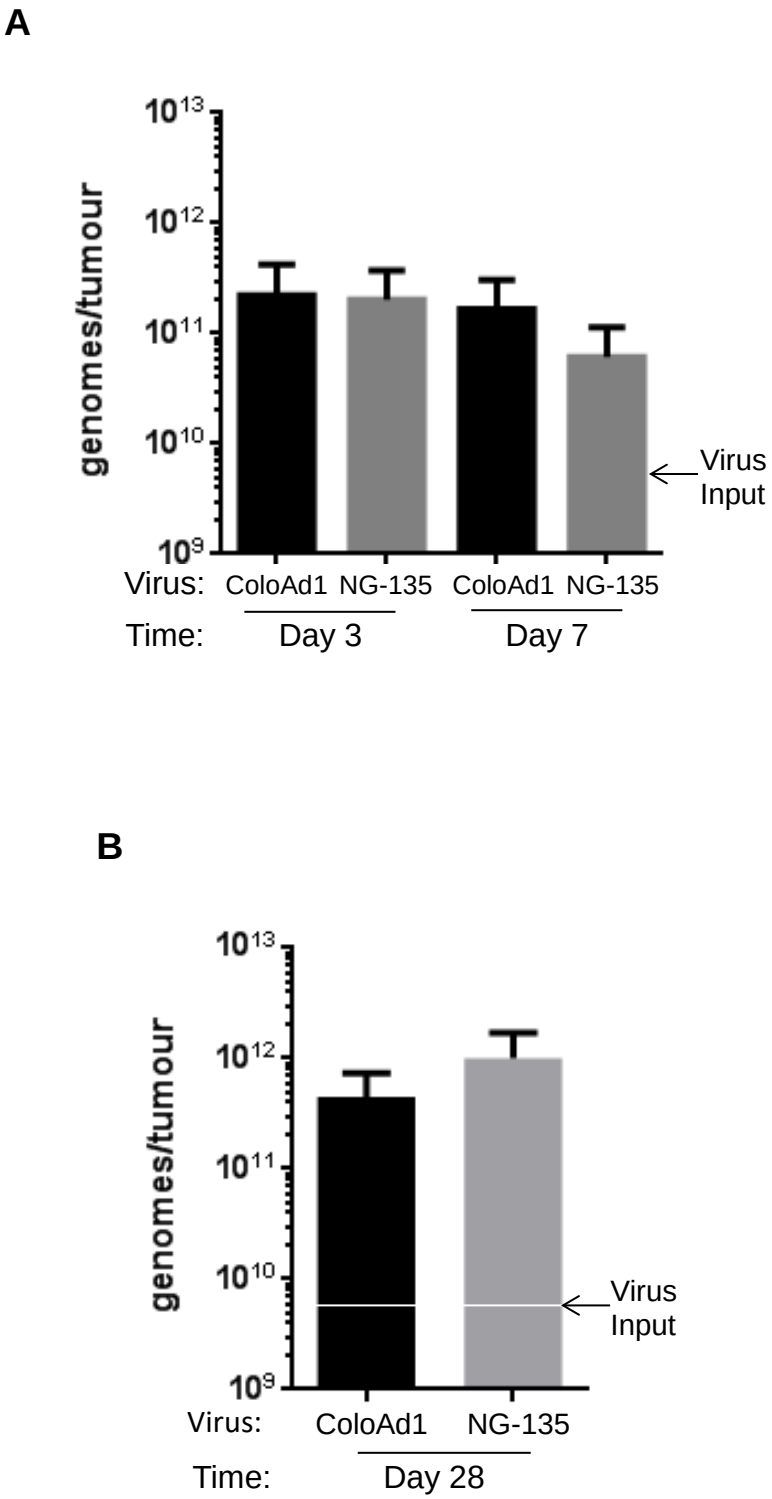


Figure 16 ColoAd1 = EnAd

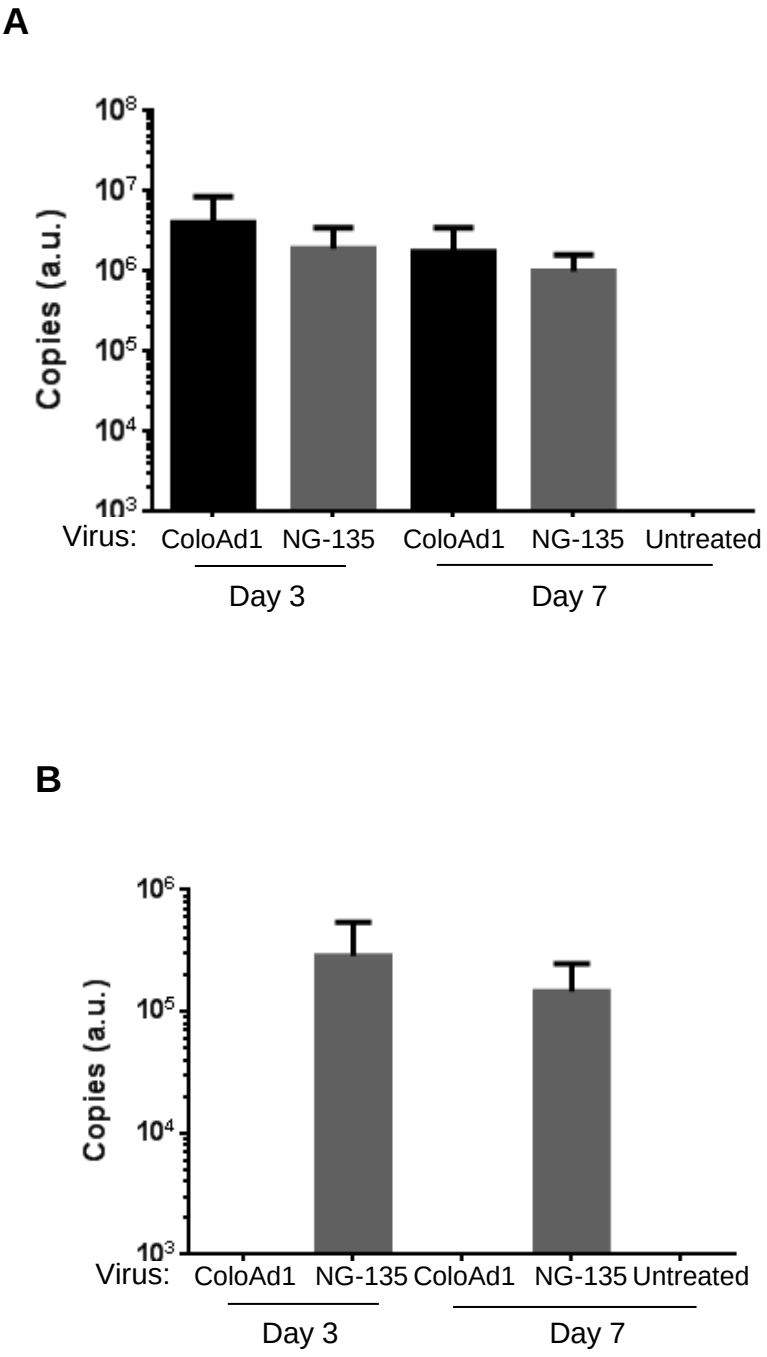


Figure 17 ColoAd1 = EnAd

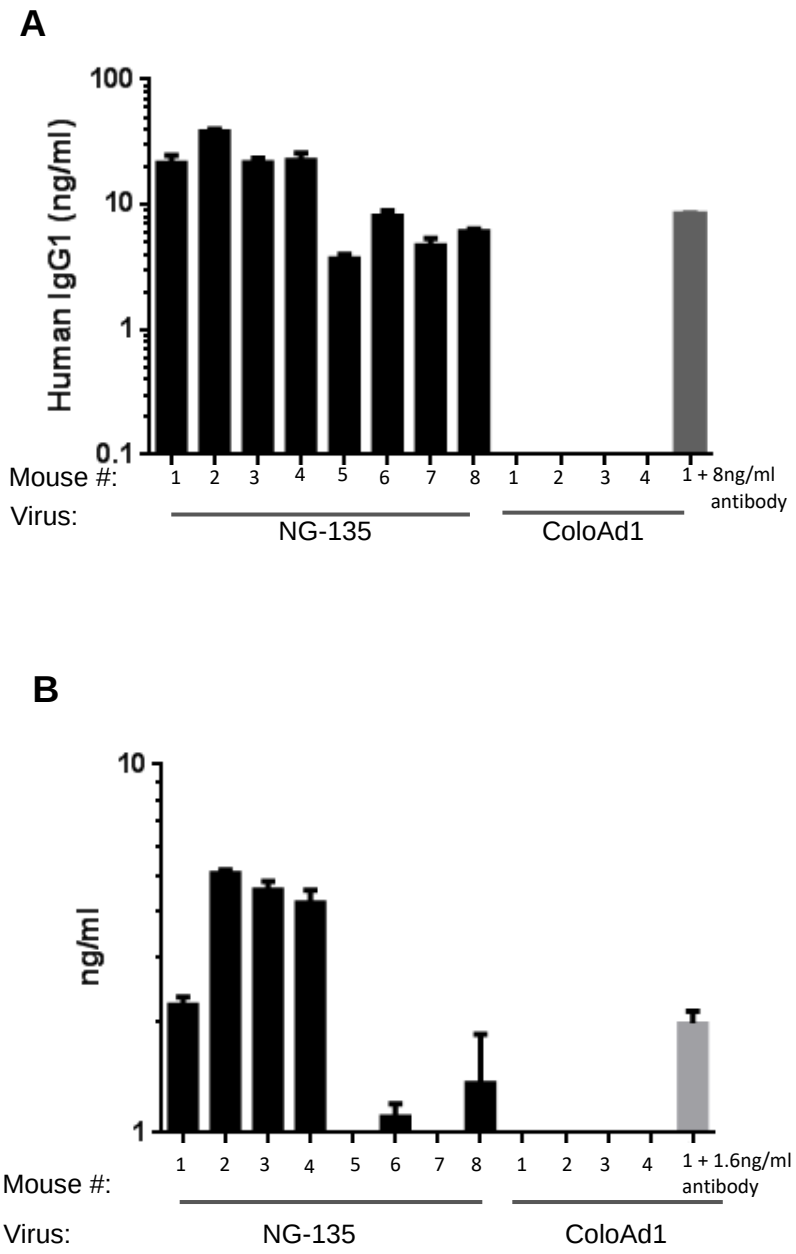


Figure 18 ColoAd1 = EnAd

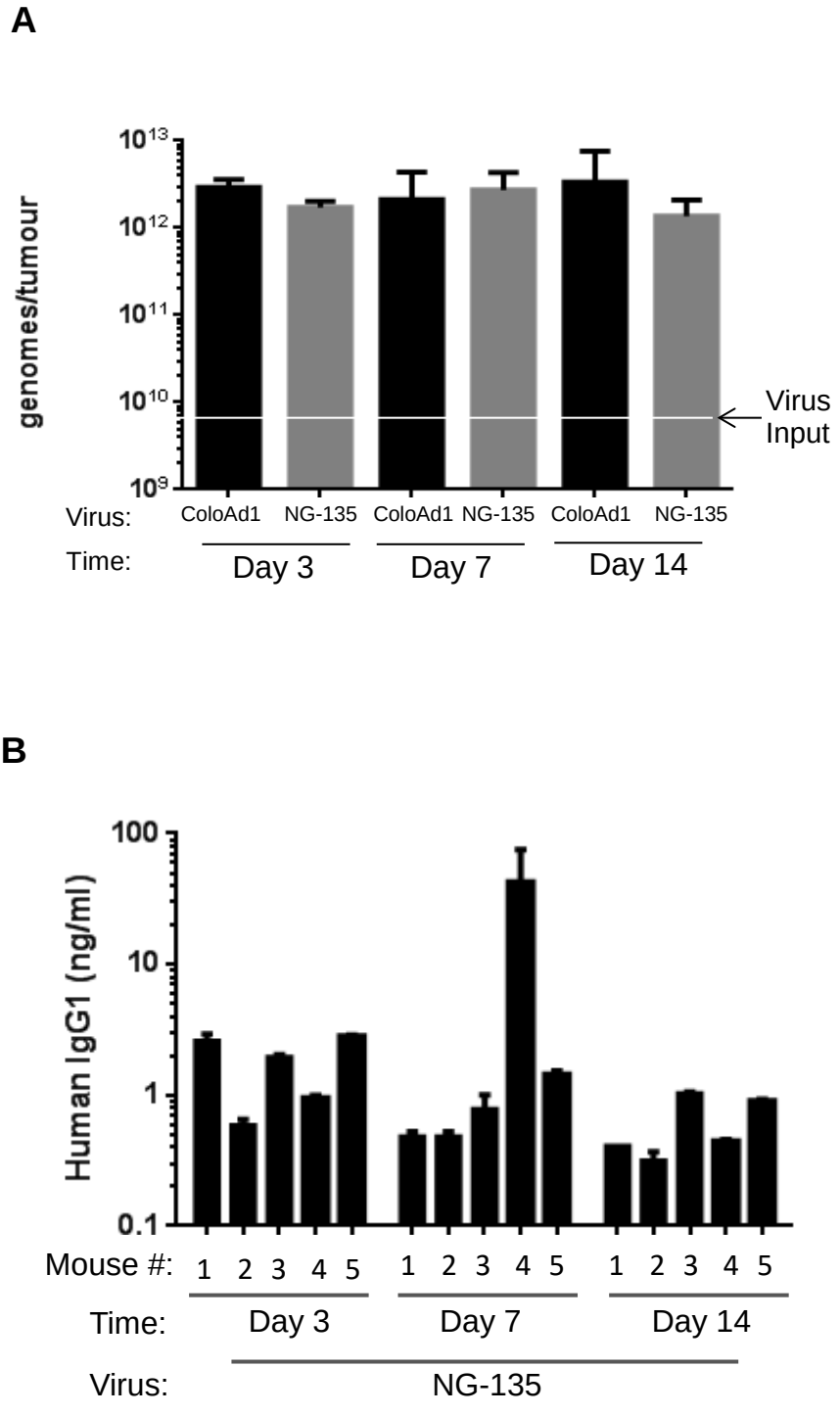


Figure 19 ColoAd1 = EnAd

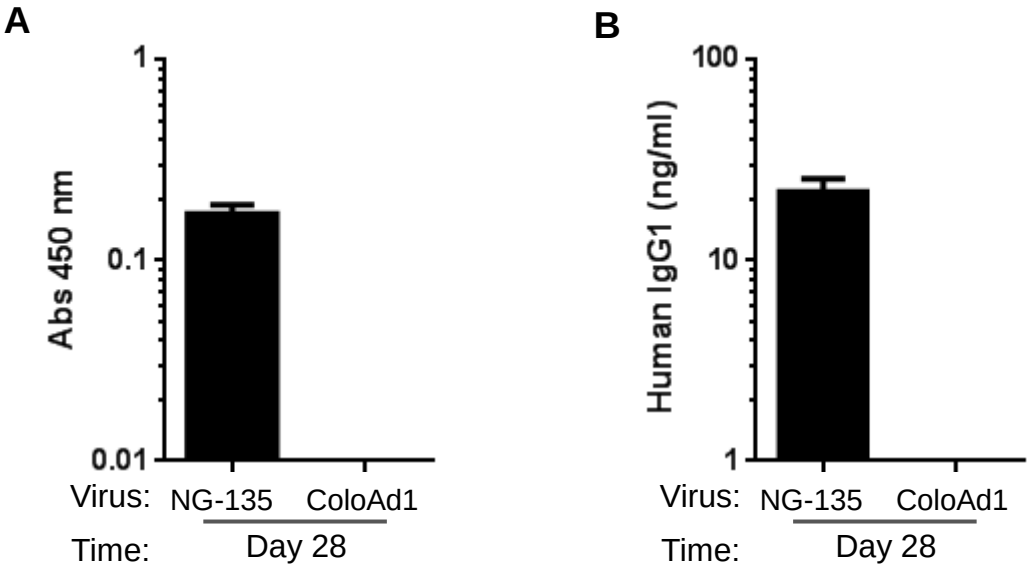


Figure 20

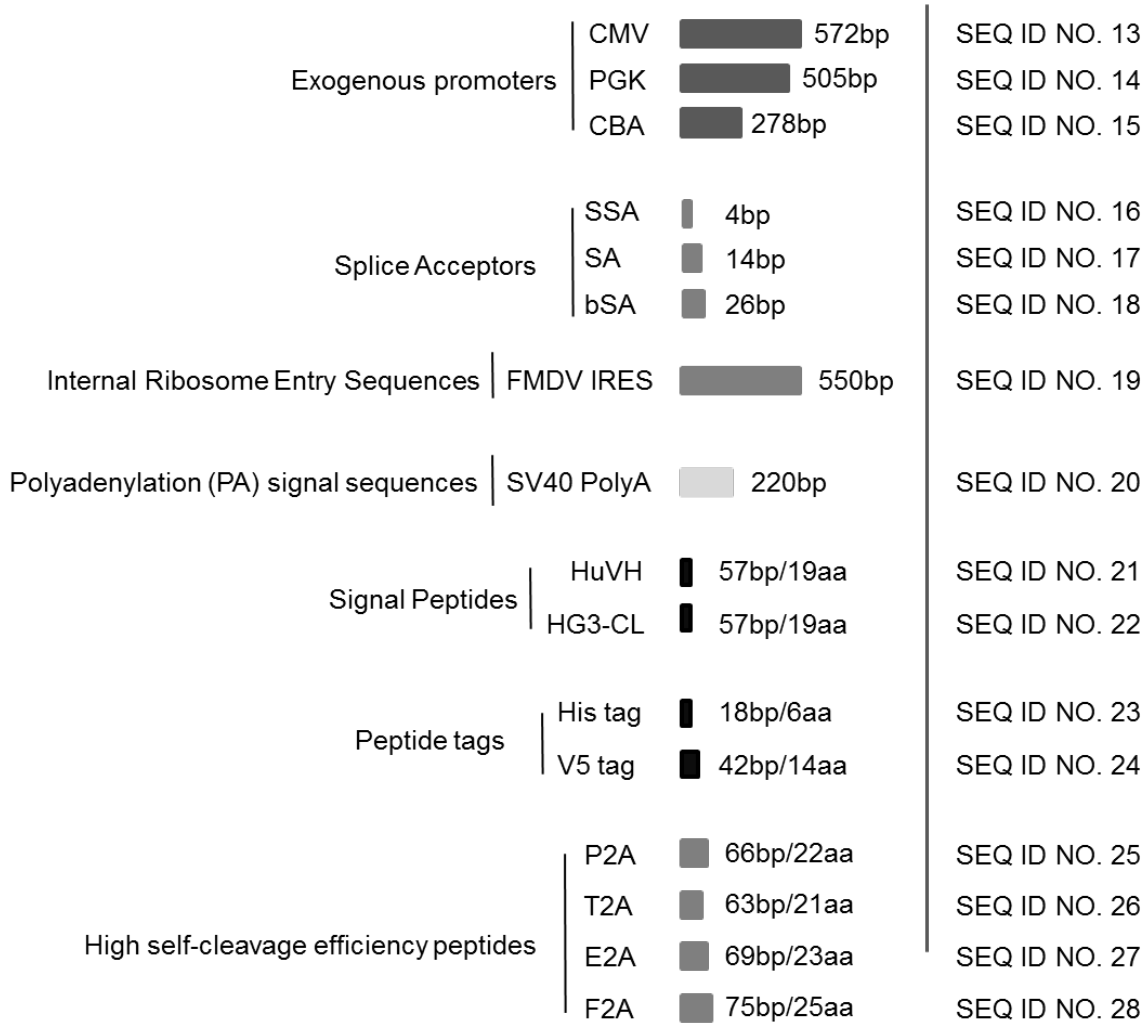

Exogenous promoters
CMV
572bp
SEQ ID NO. 13
PGK
505bp
SEQ ID NO. 14
CBA
278bp
SEQ ID NO. 15
Splice Acceptors
SSA
4bp
SEQ ID NO. 16
SA
14bp
SEQ ID NO. 17
bSA
26bp
SEQ ID NO. 18
Internal Ribosome Entry Sequences
FMDV IRES
550bp
SEQ ID NO. 19
Polyadenylation (PA) signal sequences
SV40 PolyA
220bp
SEQ ID NO. 20
Signal Peptides
HuVH
57bp/19aa
SEQ ID NO. 21
HG3-CL
57bp/19aa
SEQ ID NO. 22
Peptide tags
His tag
18bp/6aa
SEQ ID NO. 23
V5 tag
42bp/14aa
SEQ ID NO. 24
High self-cleavage efficiency peptides
P2A
66bp/22aa
SEQ ID NO. 25
T2A
63bp/21aa
SEQ ID NO. 26
E2A
69bp/23aa
SEQ ID NO. 27
F2A
75bp/25aa
SEQ ID NO. 28

Figure 21

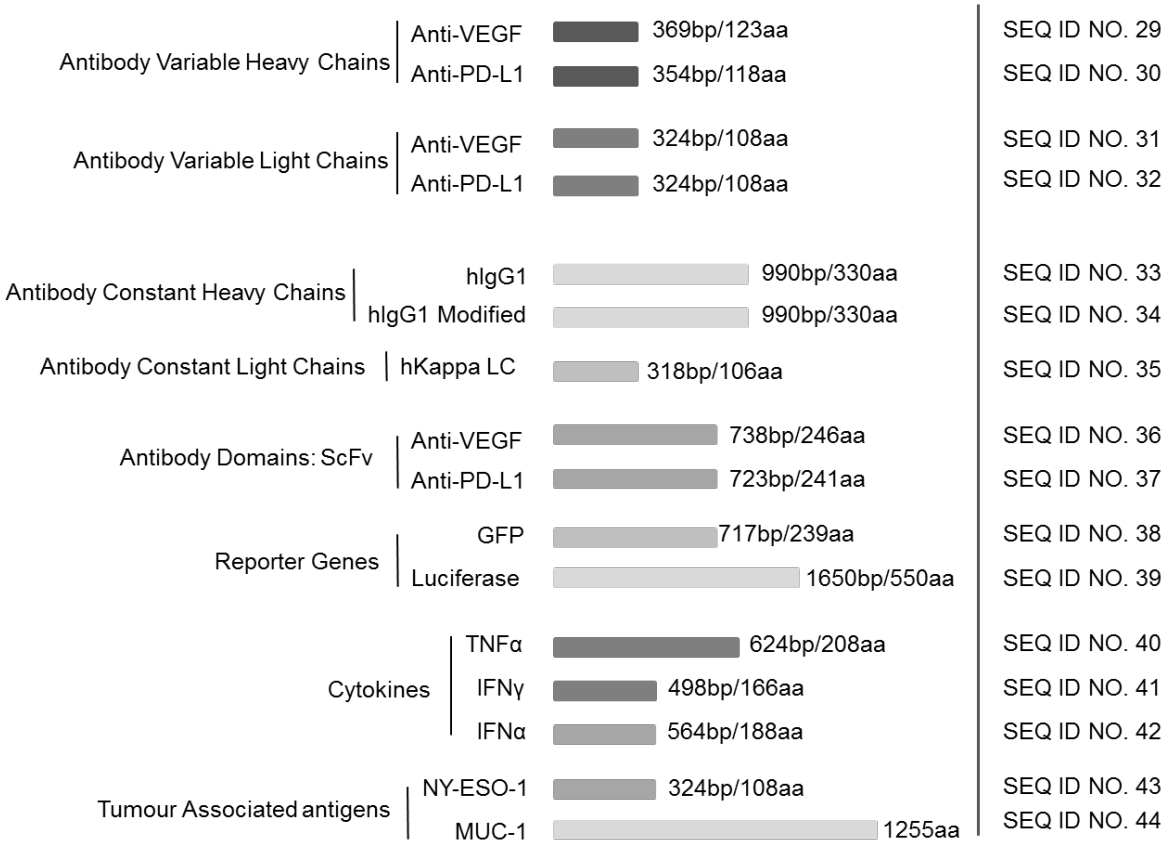

Antibody Variable Heavy Chains
Anti-VEGF
369bp/123aa
SEQ ID NO. 29
Anti-PD-L1
354bp/118aa
SEQ ID NO. 30
Antibody Variable Light Chains
Anti-VEGF
324bp/108aa
SEQ ID NO. 31
Anti-PD-L1
324bp/108aa
SEQ ID NO. 32
Antibody Constant Heavy Chains
hIgG1
990bp/330aa
SEQ ID NO. 33
hIgG1 Modified
990bp/330aa
SEQ ID NO. 34
Antibody Constant Light Chains
hKappa LC
318bp/106aa
SEQ ID NO. 35
Antibody Domains: ScFv
Anti-VEGF
738bp/246aa
SEQ ID NO. 36
Anti-PD-L1
723bp/241aa
SEQ ID NO. 37
Reporter Genes
GFP
717bp/239aa
SEQ ID NO. 38
Luciferase
1650bp/550aa
SEQ ID NO. 39
Cytokines
TNFα
624bp/208aa
SEQ ID NO. 40
IFNγ
498bp/166aa
SEQ ID NO. 41
IFNα
564bp/188aa
SEQ ID NO. 42
Tumour Associated antigens
NY-ESO-1
324bp/108aa
SEQ ID NO. 43
MUC-1
1255aa
SEQ ID NO. 44

Figure 22

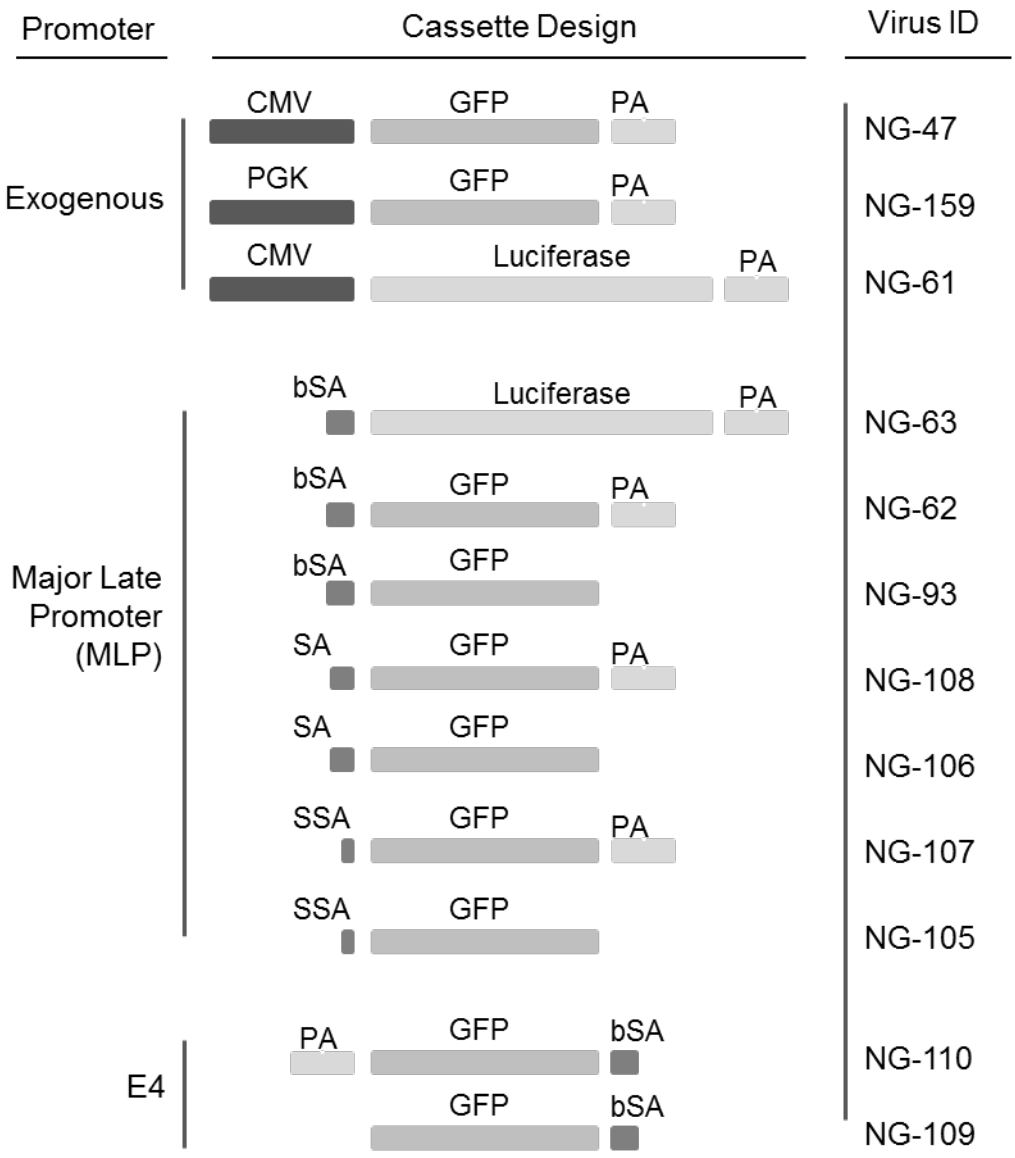

Promoter
Cassette Design
Virus ID
Exogenous
CMV GFP PA
NG-47
PGK GFP PA
NG-159
CMV Luciferase PA
NG-61
Major Late Promoter (MLP)
bSA Luciferase PA
NG-63
bSA GFP PA
NG-62
bSA GFP
NG-93
SA GFP PA
NG-108
SA GFP
NG-106
SSA GFP PA
NG-107
SSA GFP
NG-105
E4
PA GFP bSA
NG-110
GFP bSA
NG-109

Figure 23

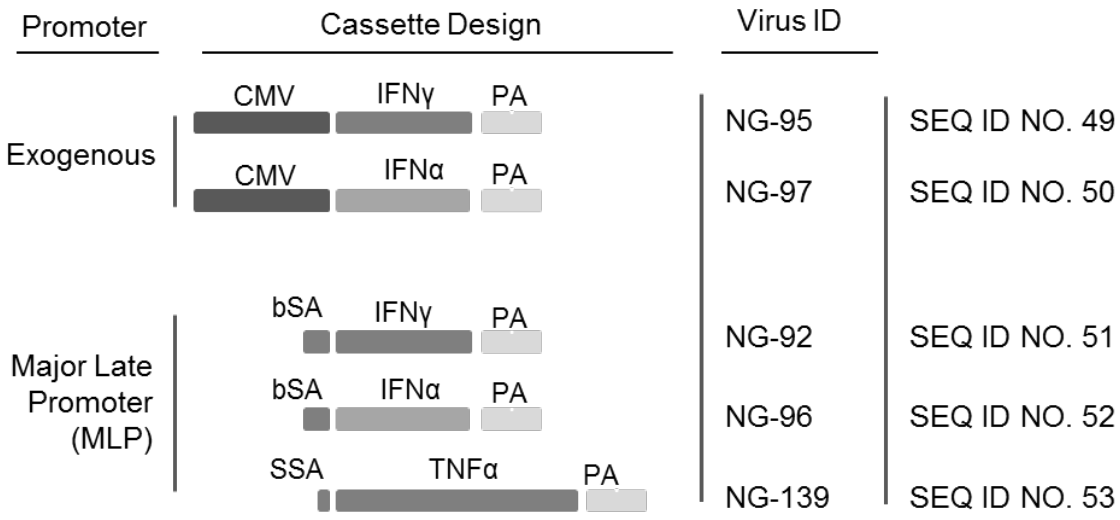

Promoter
Cassette Design
Virus ID
Exogenous
CMV
IFNγ
PA
NG-95
SEQ ID NO. 49
CMV
IFNα
PA
NG-97
SEQ ID NO. 50
Major Late Promoter (MLP)
bSA
IFNγ
PA
NG-92
SEQ ID NO. 51
bSA
IFNα
PA
NG-96
SEQ ID NO. 52
SSA
TNFα
PA
NG-139
SEQ ID NO. 53

Figure 24

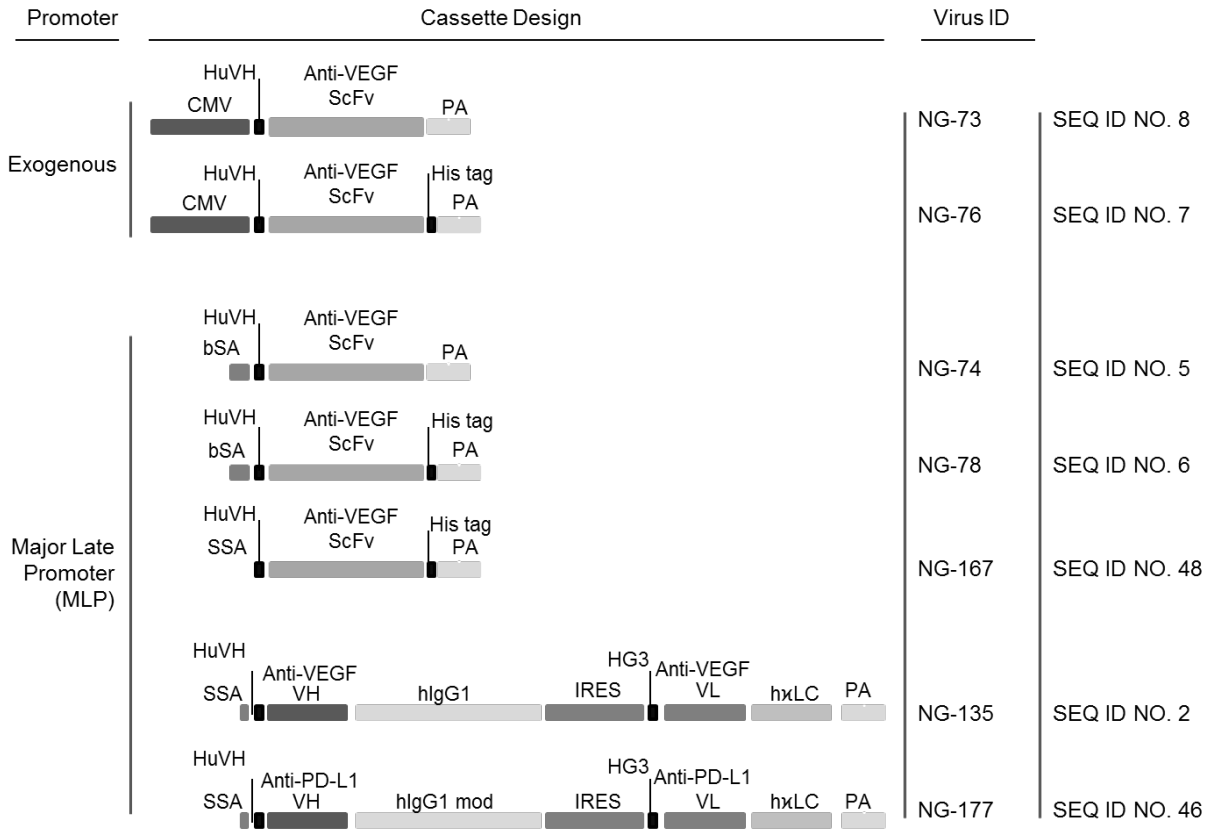

Promoter
Cassette Design
Virus ID
Exogenous
CMV
HuVH
Anti-VEGF ScFv
PA
His tag
NG-73
SEQ ID NO. 8
NG-76
SEQ ID NO. 7
Major Late Promoter (MLP)
bSA
SSA
NG-74
SEQ ID NO. 5
NG-78
SEQ ID NO. 6
NG-167
SEQ ID NO. 48
Anti-VEGF VH
hIgG1
HG3
IRES
Anti-VEGF VL
hκLC
NG-135
SEQ ID NO. 2
Anti-PD-L1 VH
hIgG1 mod
Anti-PD-L1 VL
NG-177
SEQ ID NO. 46

Figure 25 ColoAd1 = EnAd

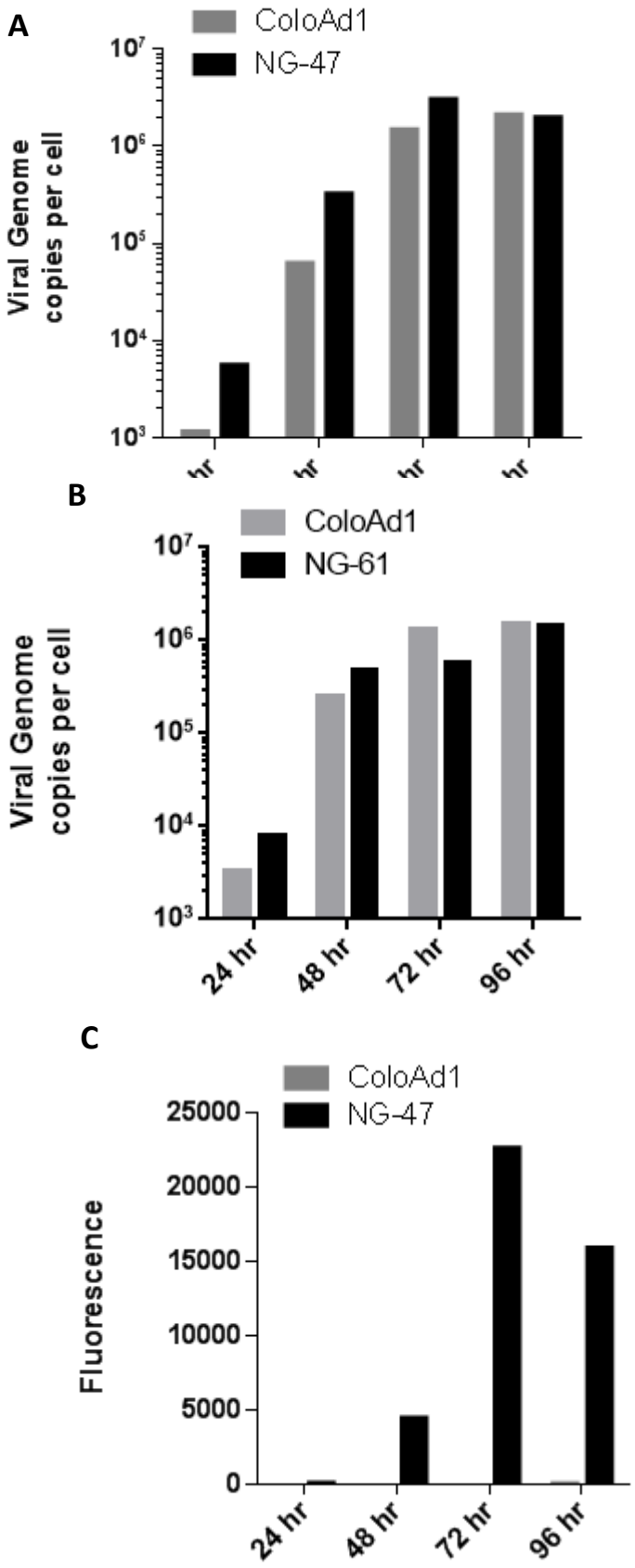


Figure 26 ColoAD1 = EnAd

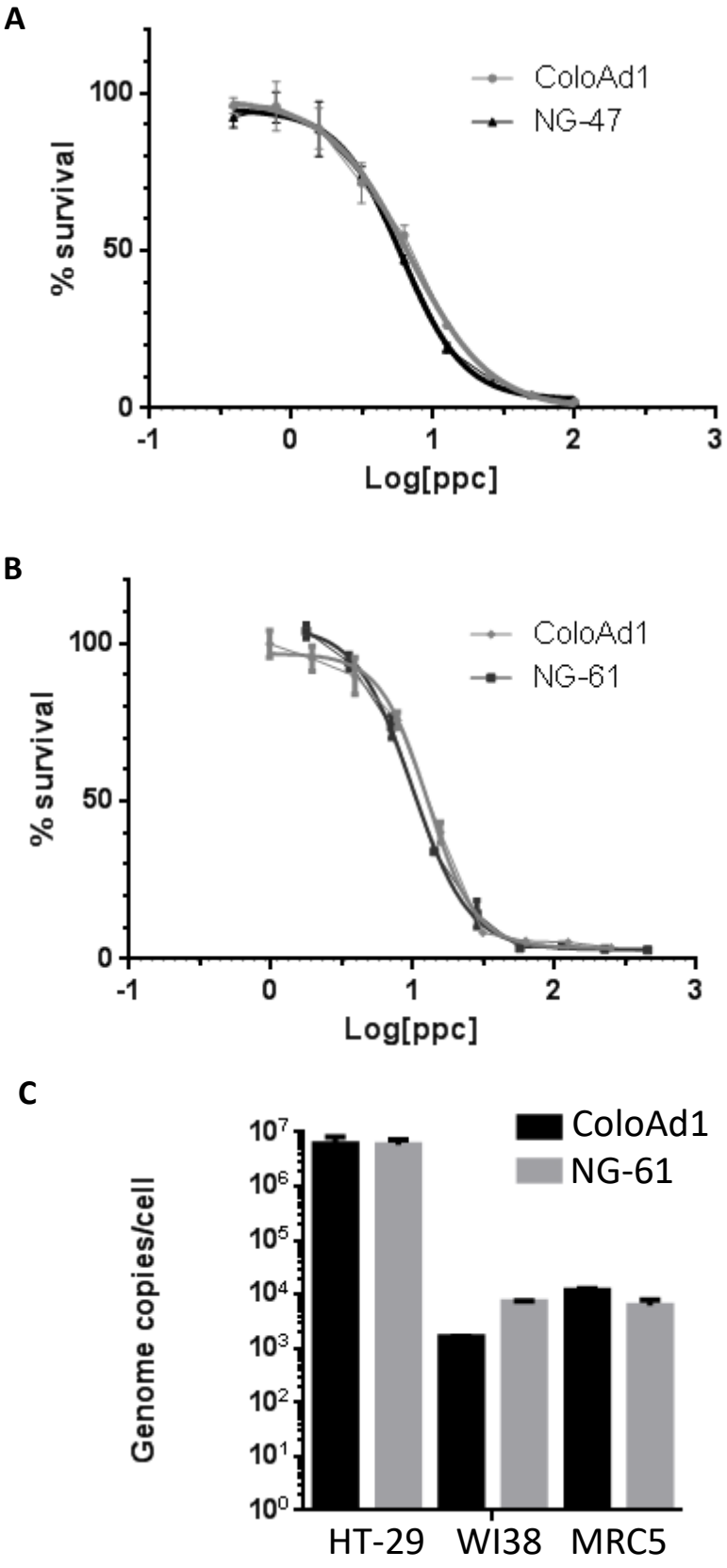
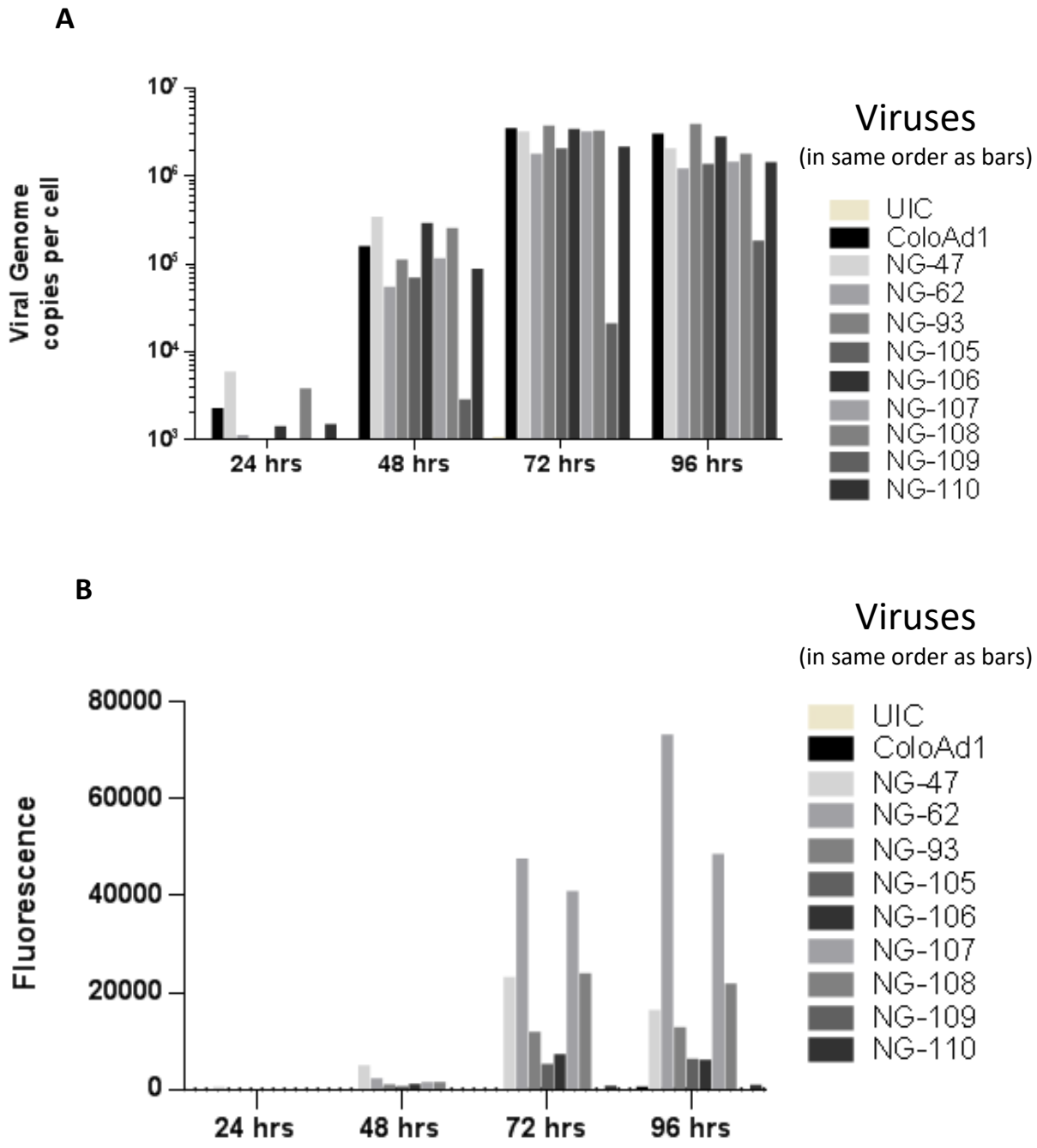


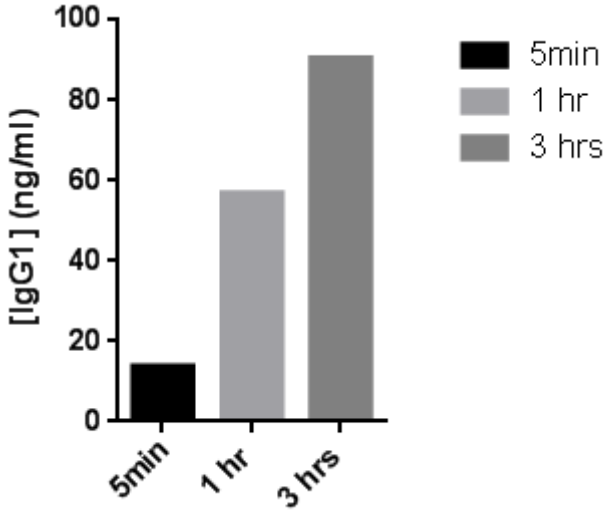
Figure 27



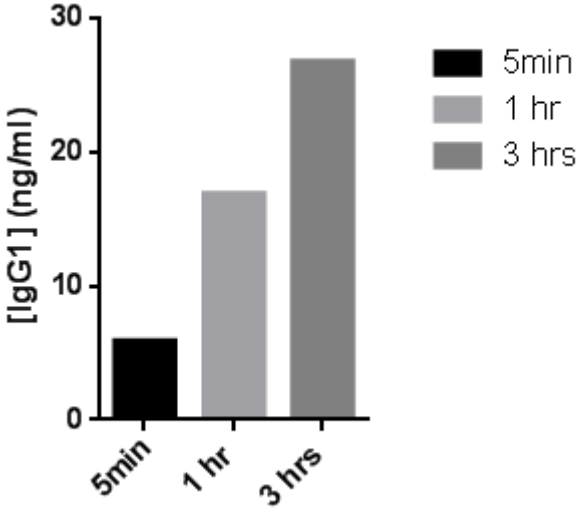
ColoAd1 = EnAd; UIC = uninfected control

Figure 28

A



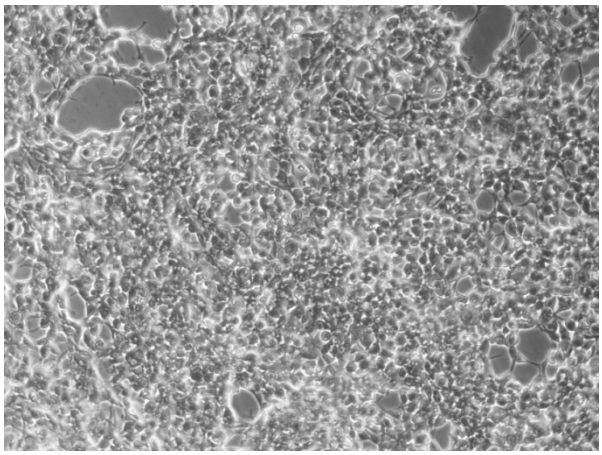
B



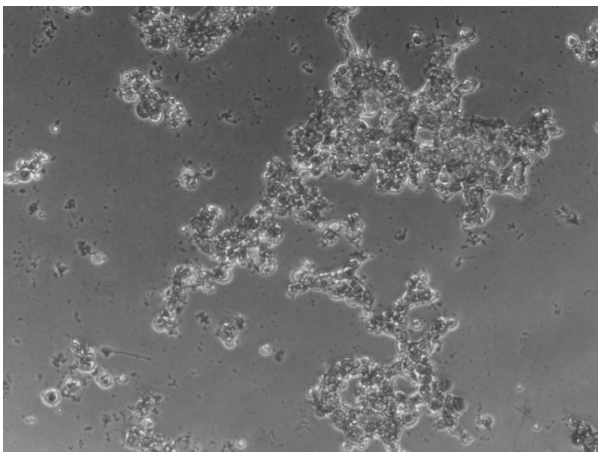
C

	Total Ab production (ng/ml)/1e6 cells	
	HT-29 cells	A549 cells
24hrs	62.4	53.6
48hrs	724	287.2
72hrs	915.2	222.4

Figure 29 ColoAd1 = EnAd



A



B

C

	TNF production (ng/ml)
	HT-29 cells
24hrs	0.233
36hrs	15.7

D

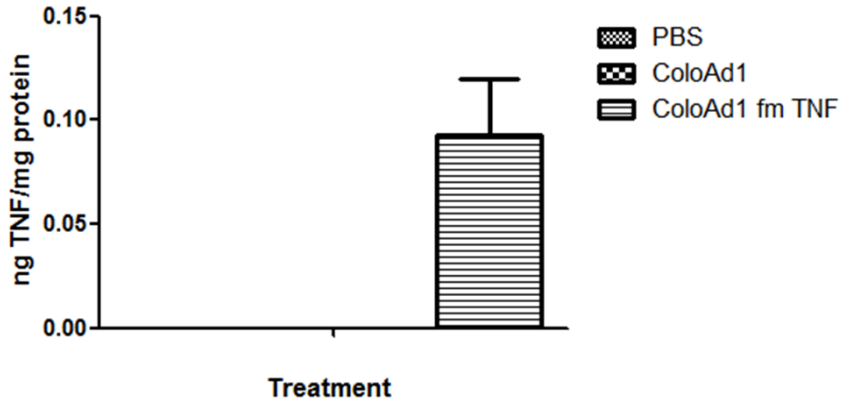


Figure 30

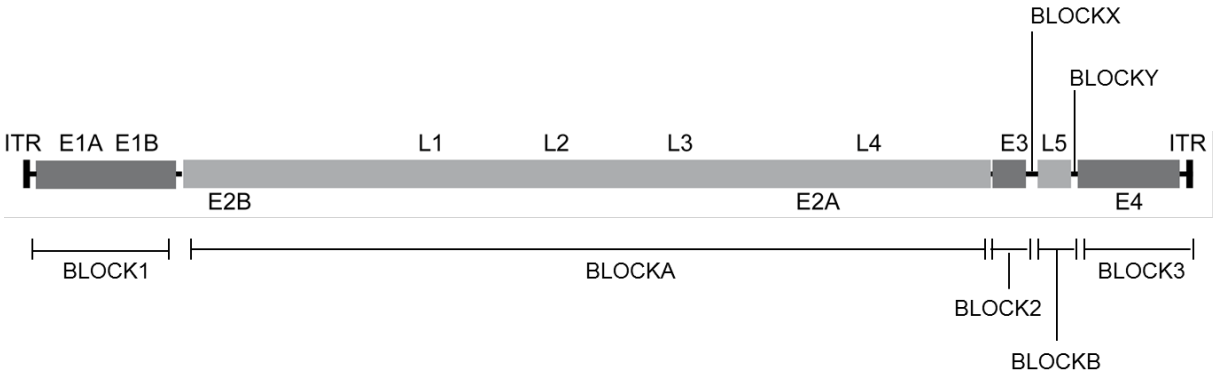


Figure 31

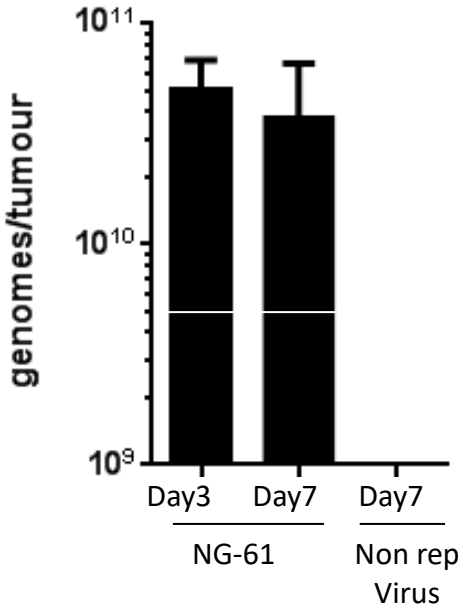


Figure 32

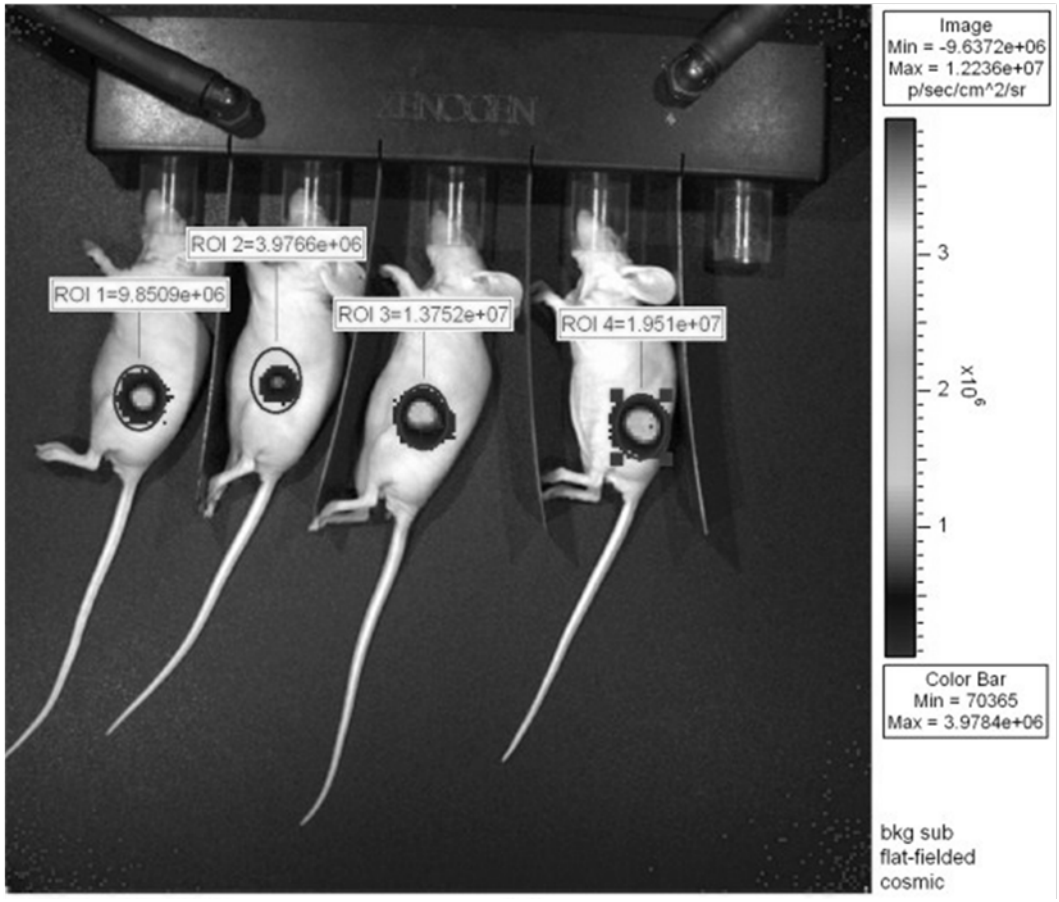
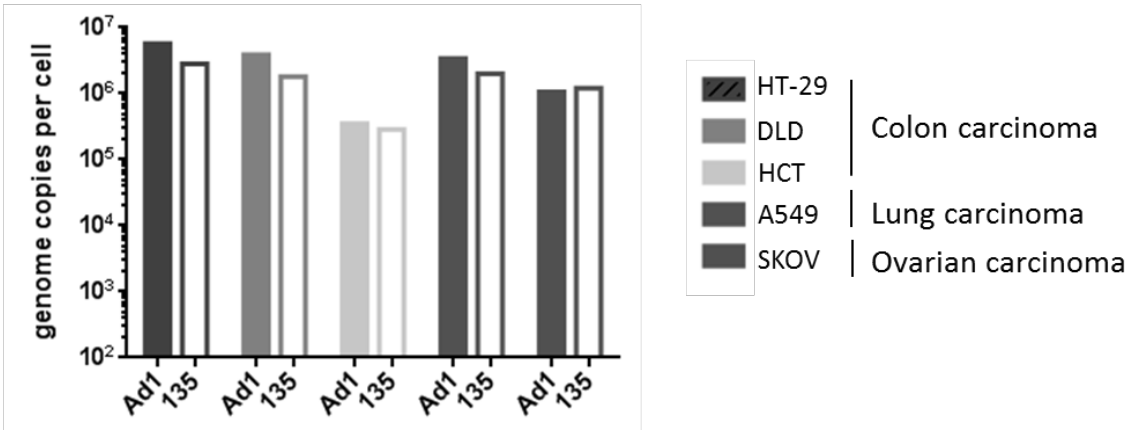

ROI 1=9.8509e+06
ROI 2=3.9766e+06
ROI 3=1.3752e+07
ROI 4=1.951e+07
Image
Min = -9.6372e+06
Max = 1.2236e+07
p/sec/cm^2/sr
3
2
1
x10^6
Color Bar
Min = 70365
Max = 3.9784e+06
bkg sub
flat-fielded
cosmic

Figure 33 Ad1 = EnAD; 135 = NG-135

A



B

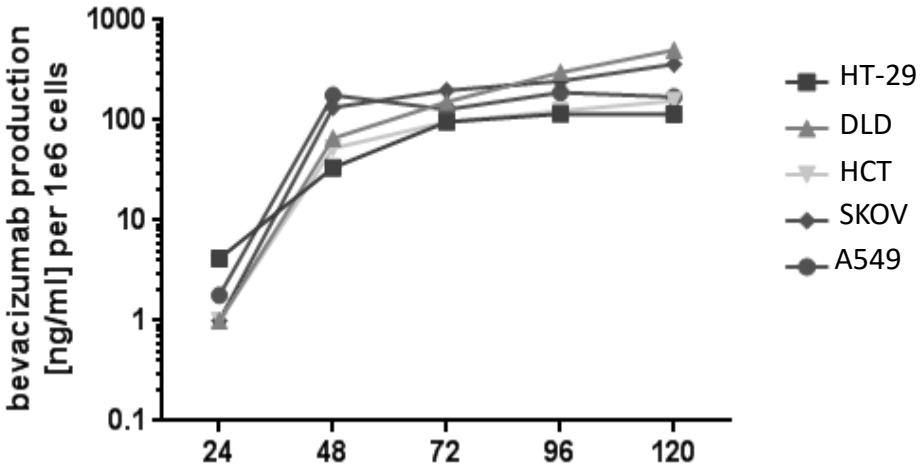


Figure 34

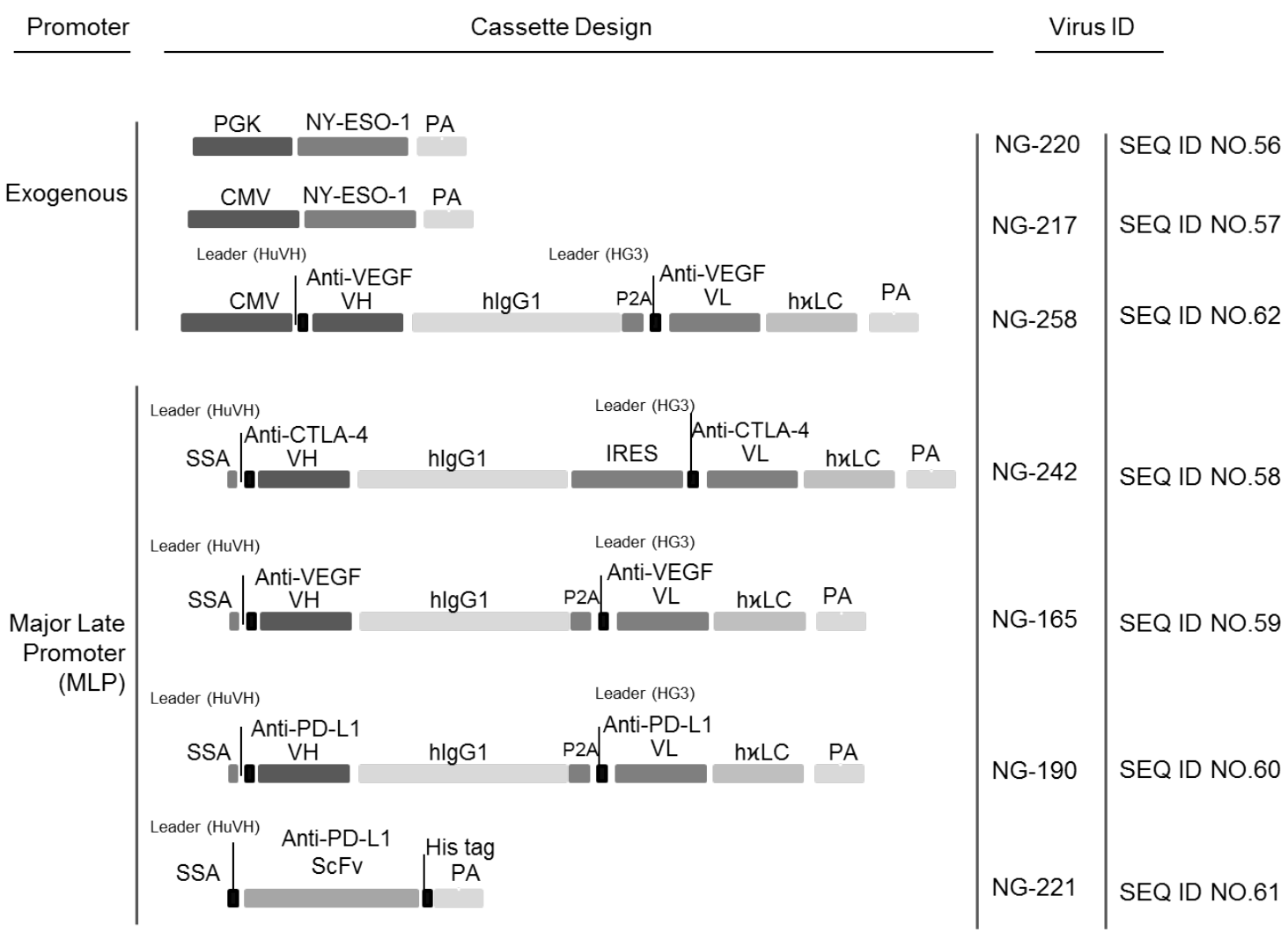

Promoter
Cassette Design
Virus ID
Exogenous
PGK
NY-ESO-1
PA
NG-220
SEQ ID NO.56
CMV
NY-ESO-1
PA
NG-217
SEQ ID NO.57
Leader (HuVH)
Leader (HG3)
CMV
Anti-VEGF VH
hIgG1
P2A
Anti-VEGF VL
hκLC
PA
NG-258
SEQ ID NO.62
Major Late Promoter (MLP)
Leader (HuVH)
Leader (HG3)
SSA
Anti-CTLA-4 VH
hIgG1
IRES
Anti-CTLA-4 VL
hκLC
PA
NG-242
SEQ ID NO.58
Leader (HuVH)
Leader (HG3)
SSA
Anti-VEGF VH
hIgG1
P2A
Anti-VEGF VL
hκLC
PA
NG-165
SEQ ID NO.59
Leader (HuVH)
Leader (HG3)
SSA
Anti-PD-L1 VH
hIgG1
P2A
Anti-PD-L1 VL
hκLC
PA
NG-190
SEQ ID NO.60
Leader (HuVH)
SSA
Anti-PD-L1 ScFv
His tag
PA
NG-221
SEQ ID NO.61

Figure 35

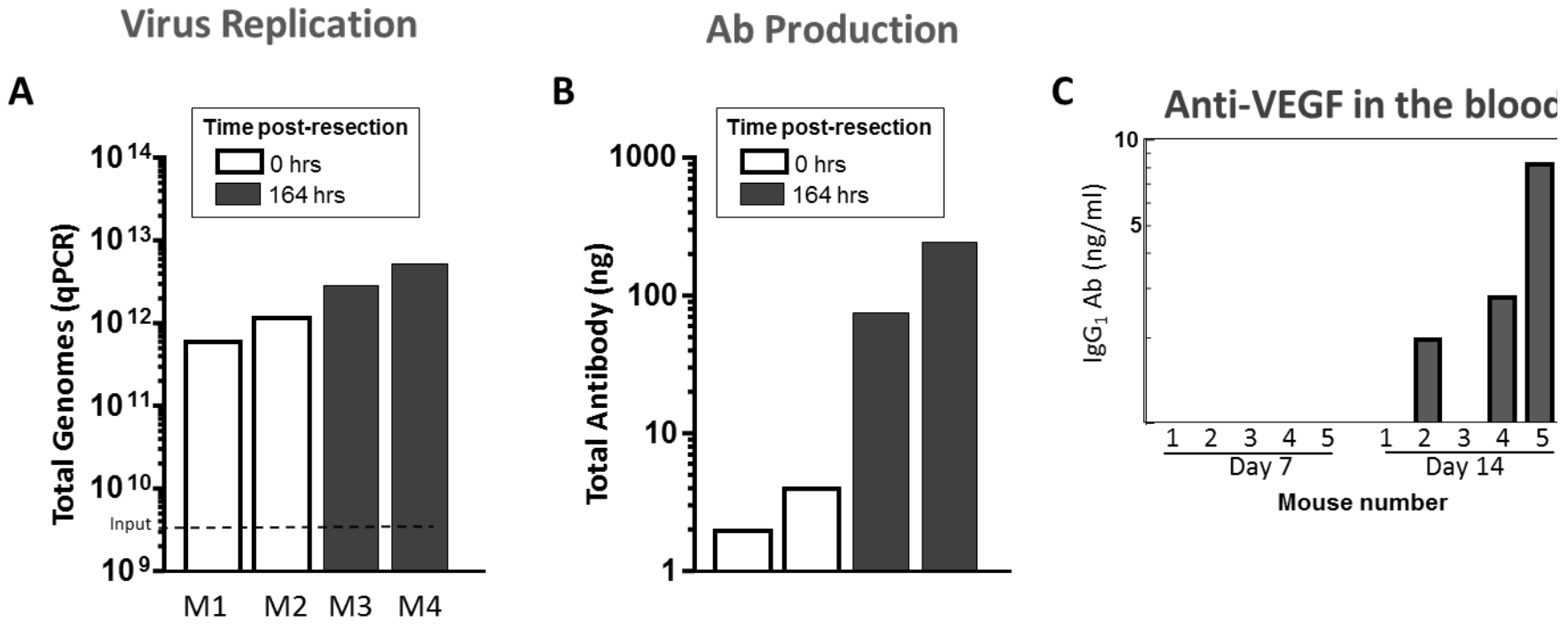

Virus Replication
A
Time post-resection
0 hrs
164 hrs
Total Genomes (qPCR)
10 14
10 13
10 12
10 11
10 10
Input
10 9
M1
M2
M3
M4
Ab Production
B
Time post-resection
0 hrs
164 hrs
Total Antibody (ng)
1000
100
10
1
C
Anti-VEGF in the blood
IgG1 Ab (ng/ml)
10
5
1 2 3 4 5
Day 7
1 2 3 4 5
Day 14
Mouse number

Figure 36

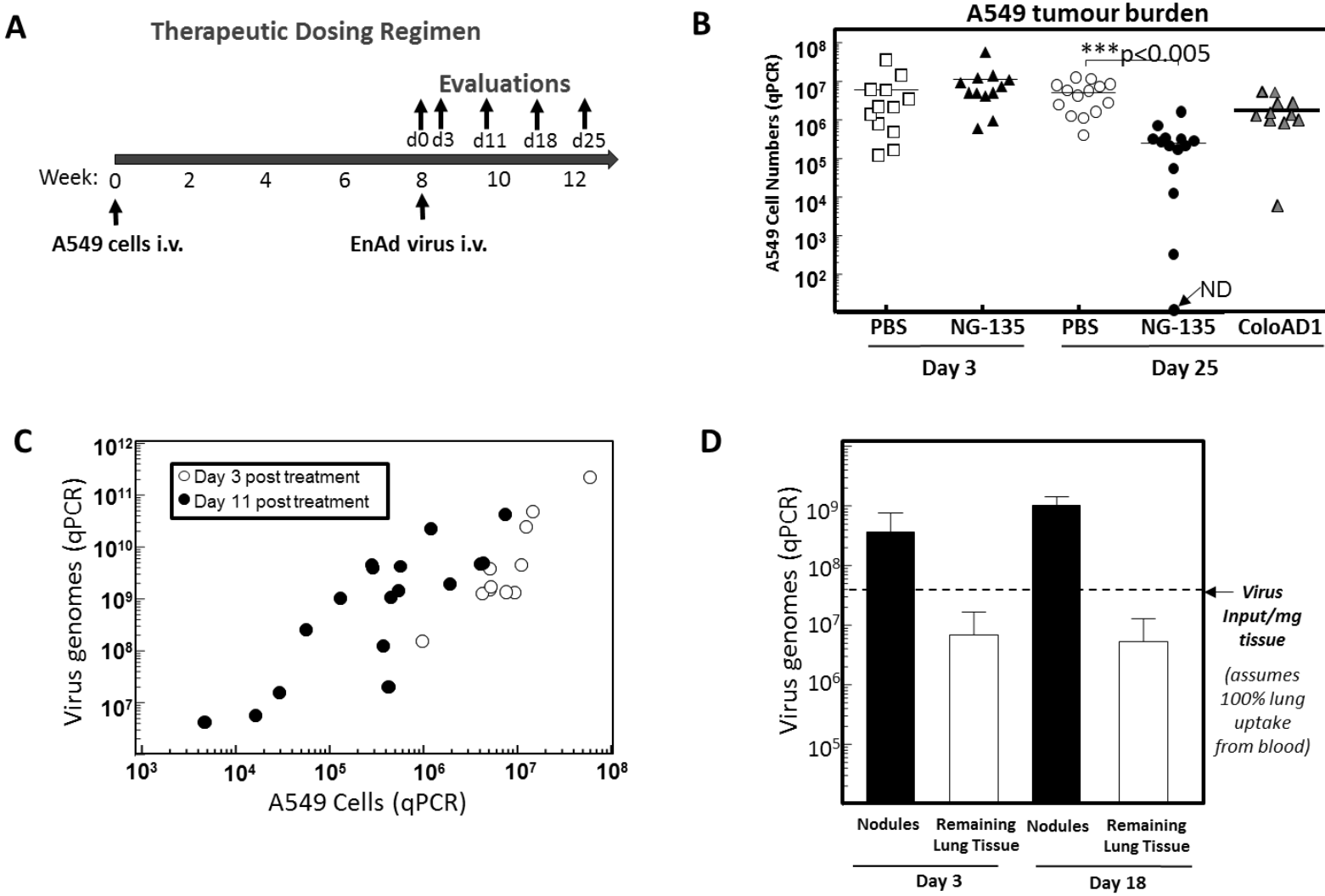

A
Therapeutic Dosing Regimen
Evaluations
d0 d3 d11 d18 d25
Week: 0 2 4 6 8 10 12
A549 cells i.v.
EnAd virus i.v.
B
A549 tumour burden
A549 Cell Numbers (qPCR)
***p≤0.005
ND
PBS NG-135 PBS NG-135 ColoAD1
Day 3
Day 25
C
Virus genomes (qPCR)
Day 3 post treatment
Day 11 post treatment
A549 Cells (qPCR)
D
Virus genomes (qPCR)
Virus Input/mg tissue
(assumes 100% lung uptake from blood)
Nodules
Remaining Lung Tissue
Nodules
Remaining Lung Tissue
Day 3
Day 18

Figure 37

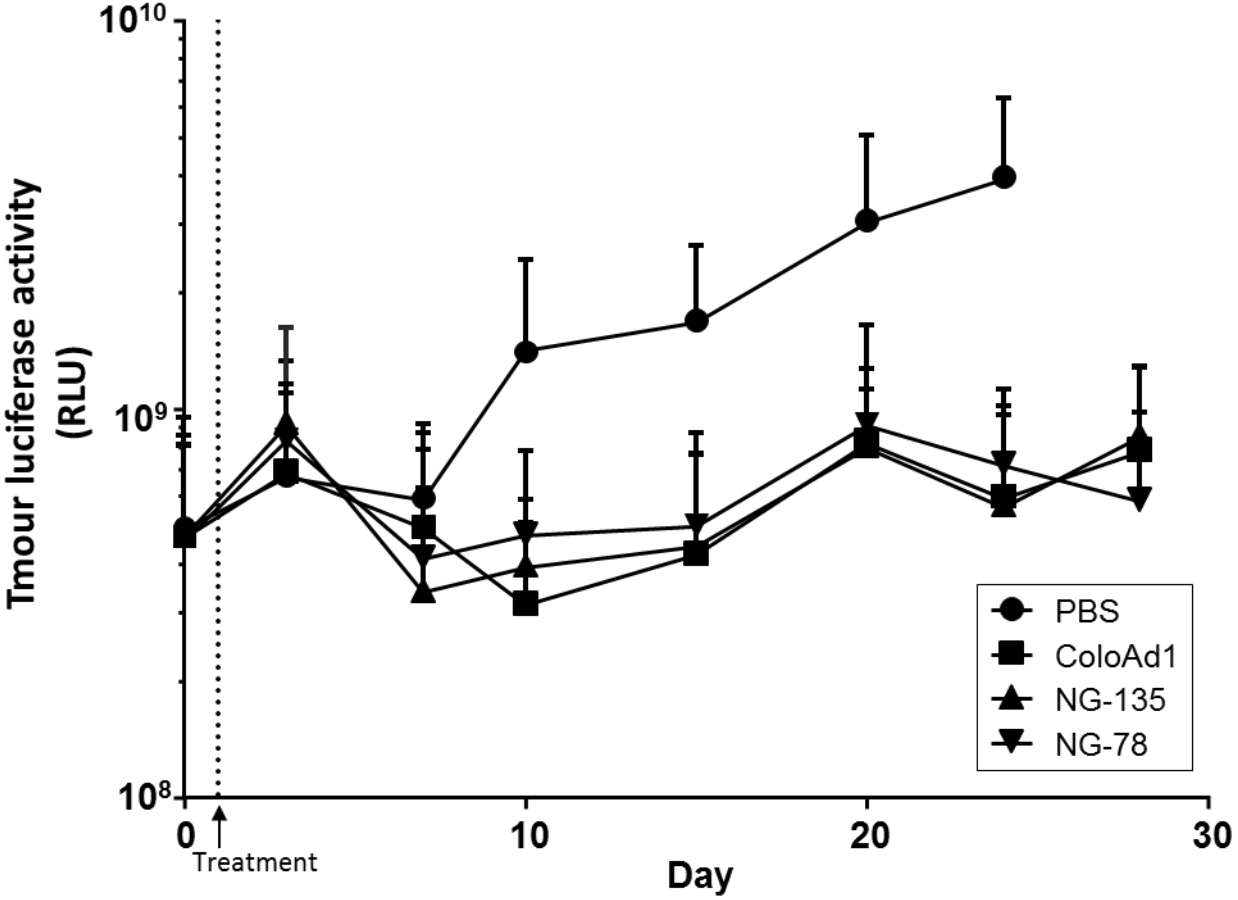
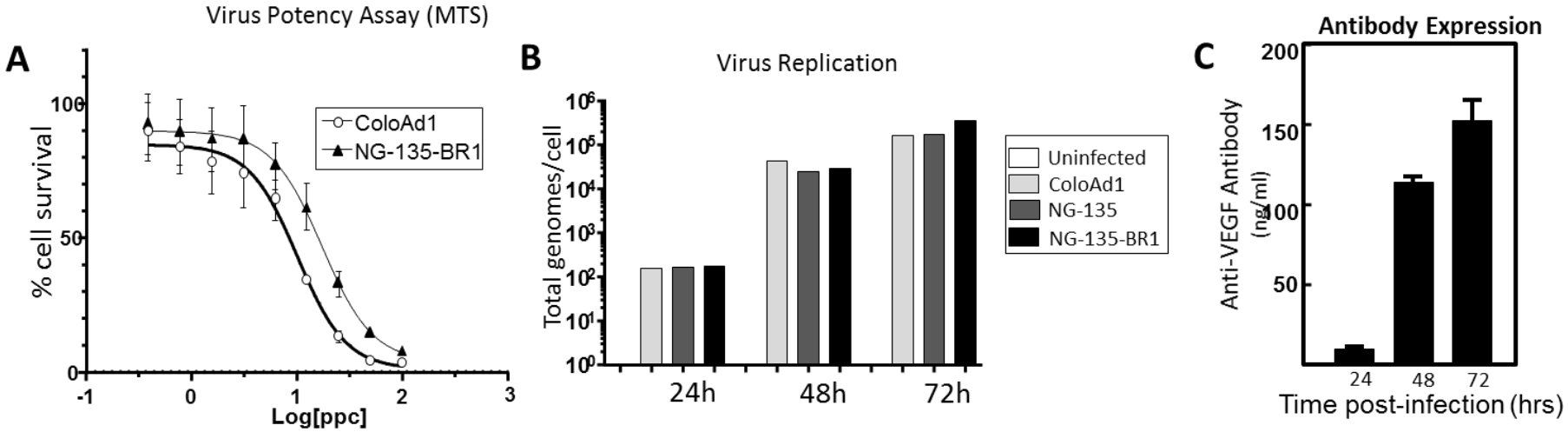
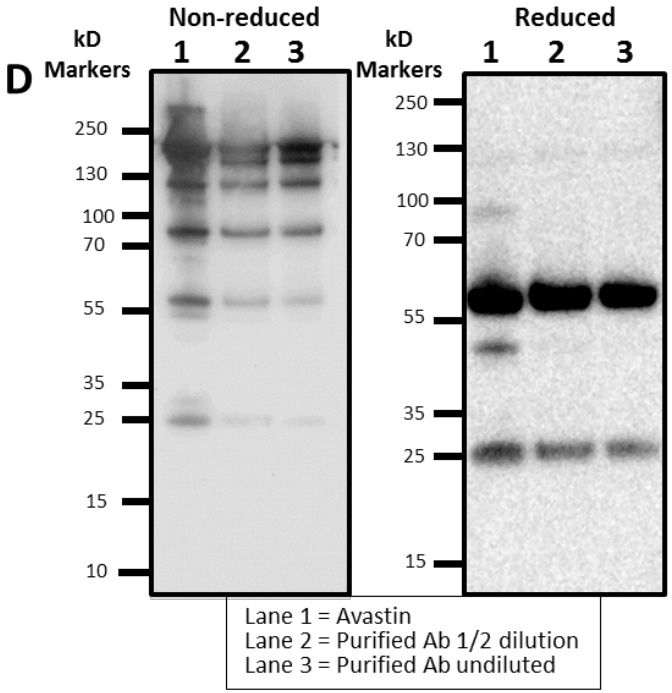

Tmour luciferase activity (RLU)
10^{10}
10^{9}
10^{8}
0
Treatment
10
20
30
Day
PBS
ColoAd1
NG-135
NG-78

Figure 38 ColoAd1 = EnAD





E

BiaCore VEGF Binding Data

Sample	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU²)	U-value
Ref Std Run 1	227681.3	5.66E-05	**2.49E-10**	130.1	4.543	6.893
Purified NG-135-BR1 Sample	302655.0	5.24E-05	**1.73E-10**	135.3	11.560	11.649
Ref Std Run 2	217793.5	4.46E-05	**2.05E-10**	126.2	4.260	8.961

Figure 39

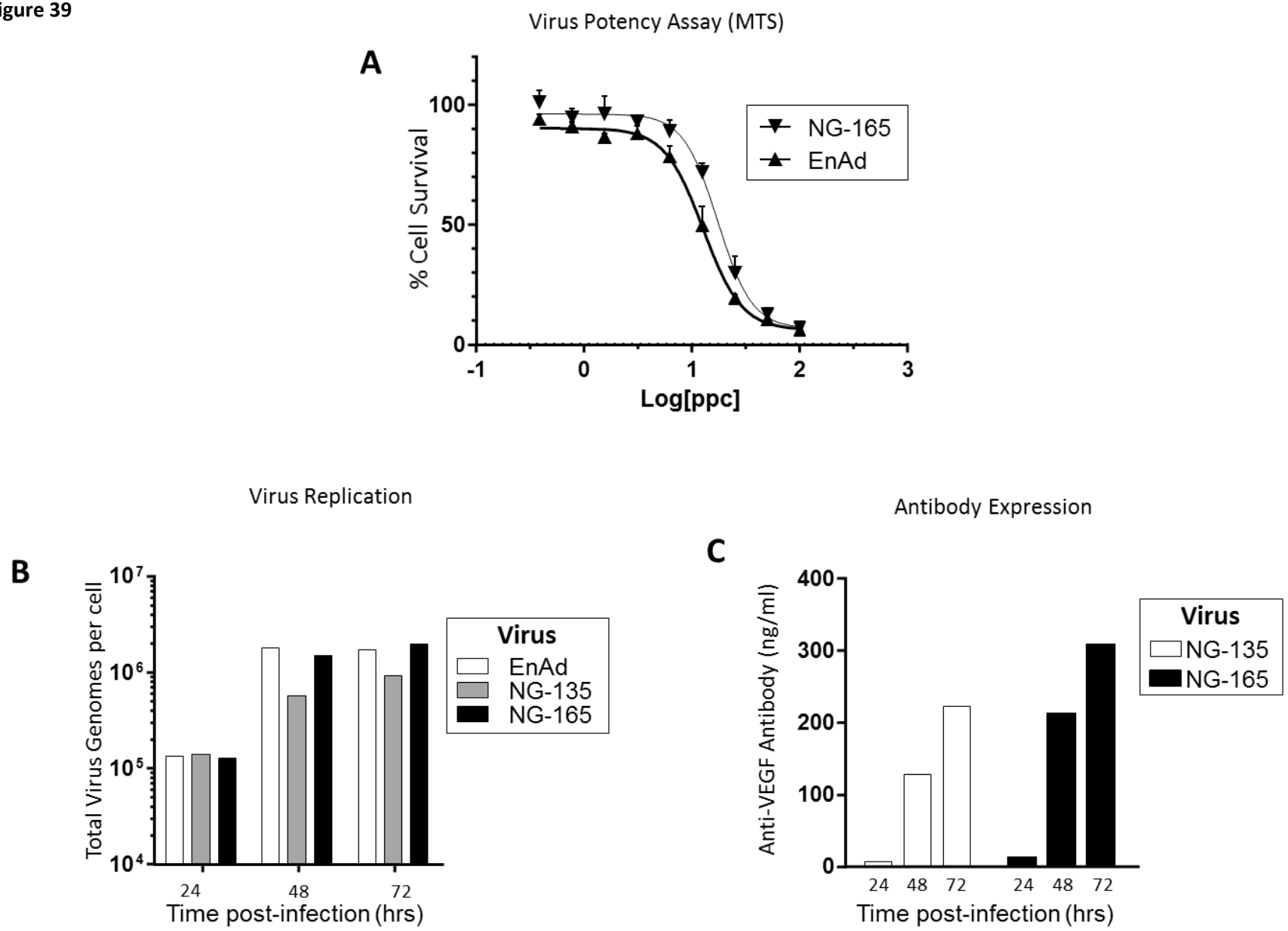

Virus Potency Assay (MTS)
A
% Cell Survival
100
50
0
-1
0
1
2
3
Log[ppc]
NG-165
EnAd
Virus Replication
B
Total Virus Genomes per cell
10^7
10^6
10^5
10^4
24
48
72
Time post-infection (hrs)
Virus
EnAd
NG-135
NG-165
Antibody Expression
C
Anti-VEGF Antibody (ng/ml)
400
300
200
100
0
24
48
72
24
48
72
Time post-infection (hrs)
Virus
NG-135
NG-165

Figure 40 ColoAD1 = EnAd

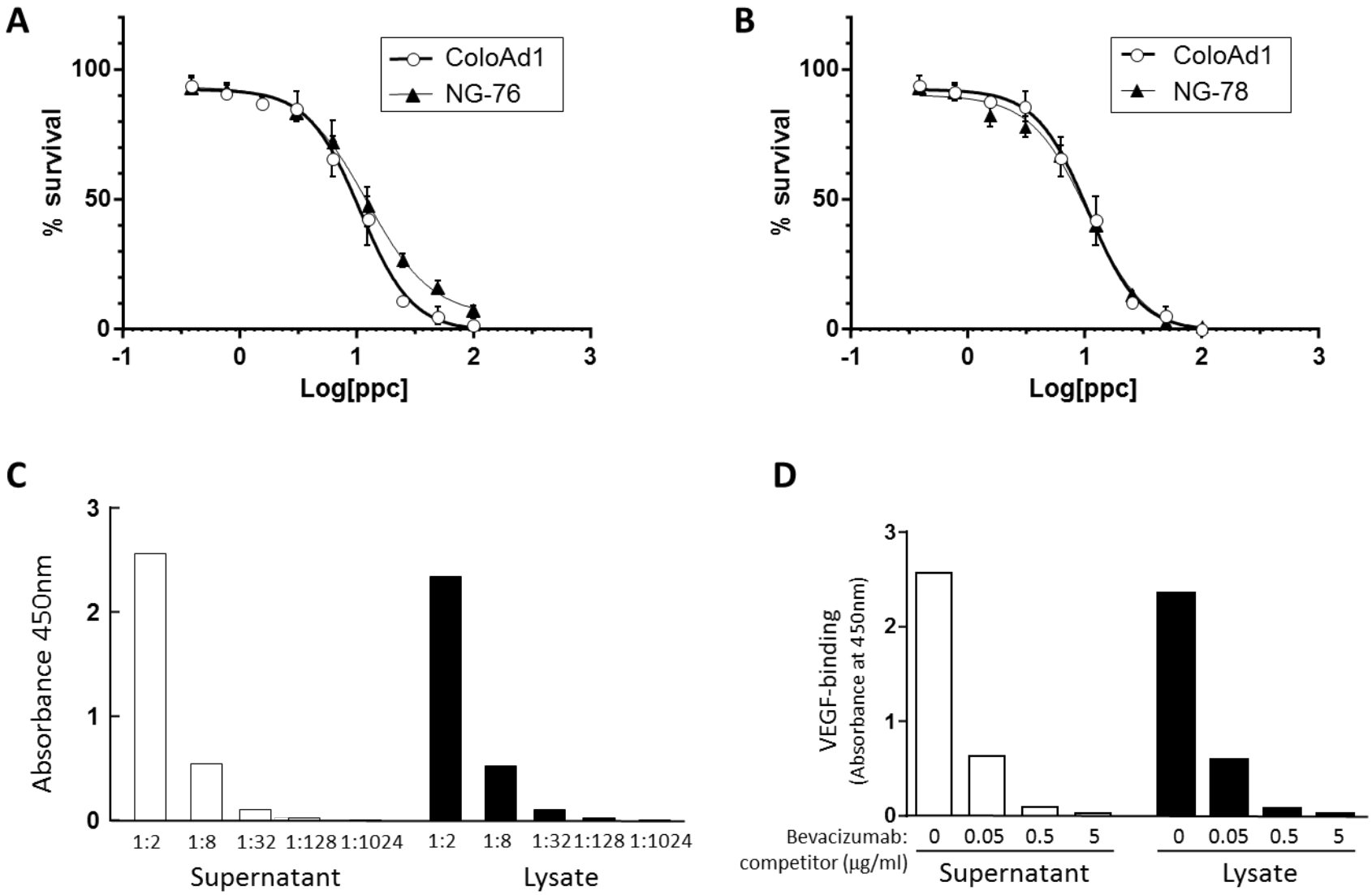


Figure 41

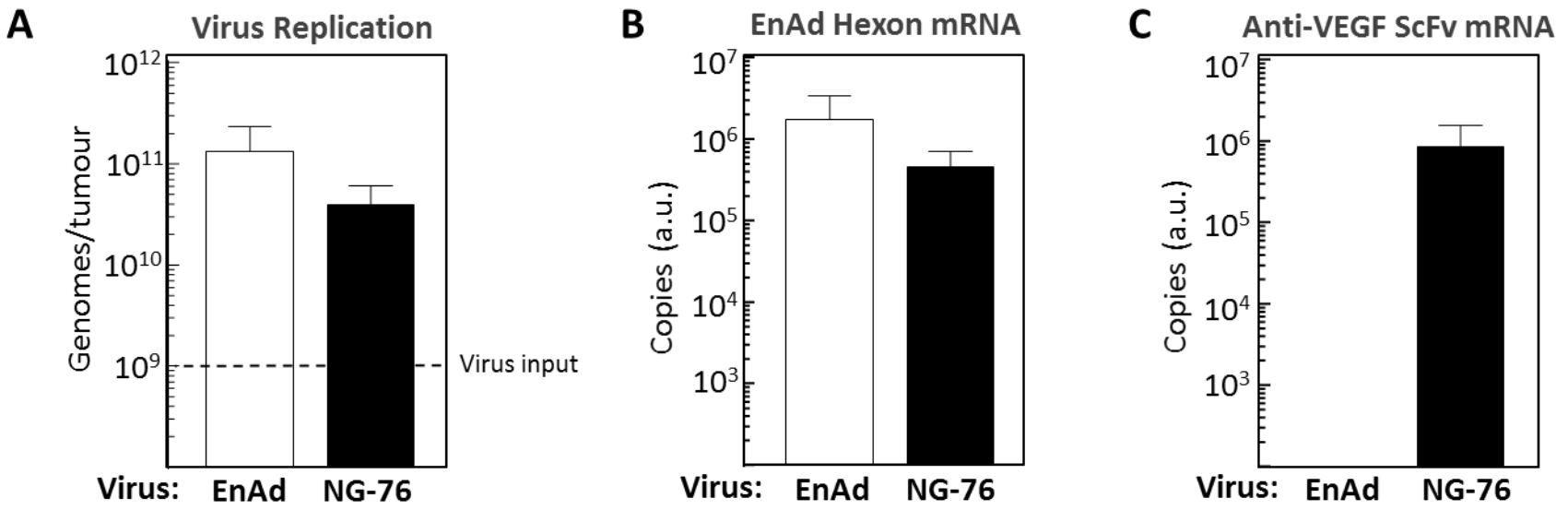

A
Virus Replication
Genomes/tumour
10^{12}
10^{11}
10^{10}
10^{9}
Virus input
Virus: EnAd NG-76
B
EnAd Hexon mRNA
Copies (a.u.)
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
Virus: EnAd NG-76
C
Anti-VEGF ScFv mRNA
Copies (a.u.)
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
Virus: EnAd NG-76

Figure 42

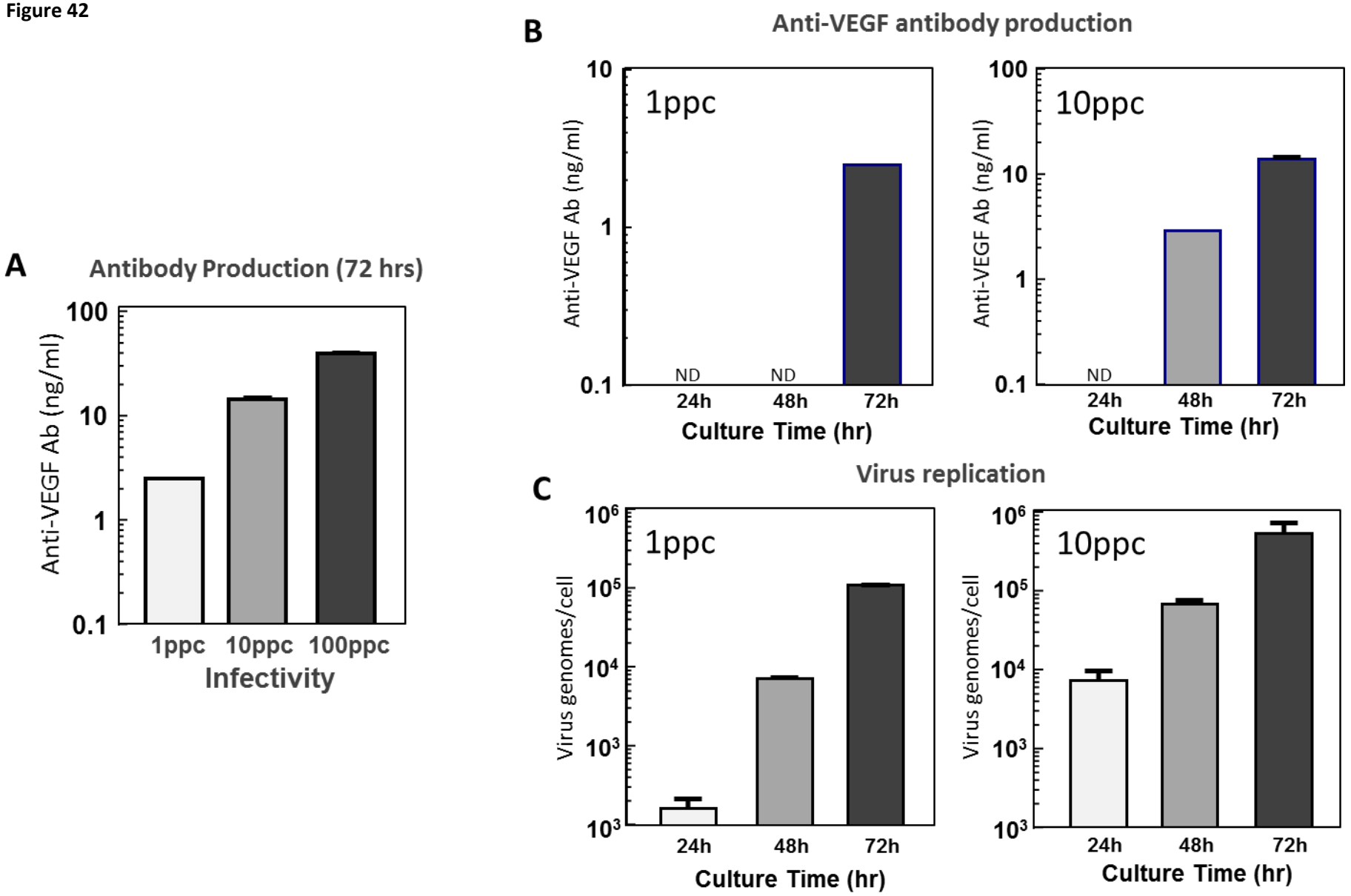

A
Antibody Production (72 hrs)
Anti-VEGF Ab (ng/ml)
100
10
1
0.1
1ppc
10ppc
100ppc
Infectivity
B
Anti-VEGF antibody production
1ppc
10ppc
Anti-VEGF Ab (ng/ml)
ND
ND
24h
48h
72h
Culture Time (hr)
C
Virus replication
Virus genomes/cell
10^6
10^5
10^4
10^3

Figure 43

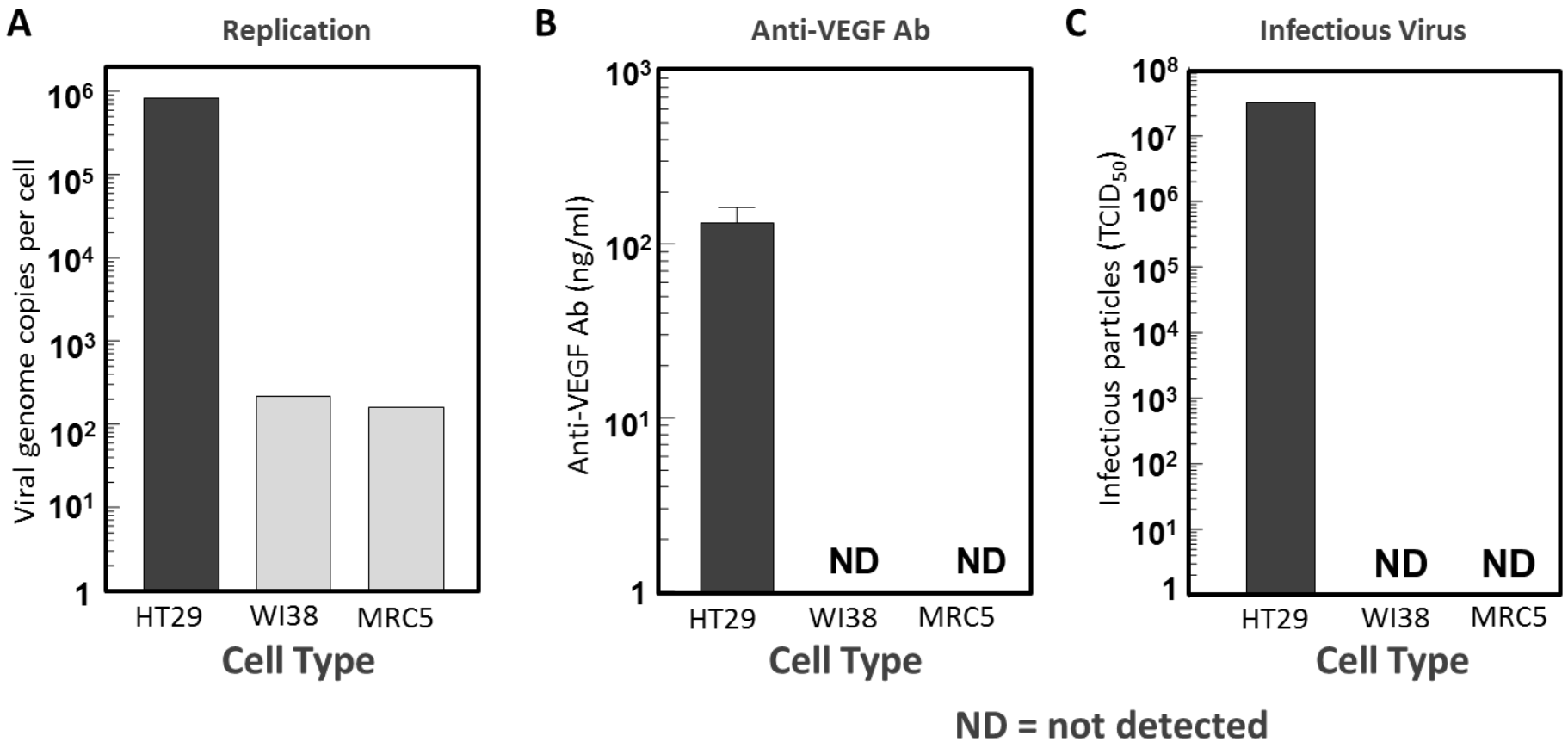

A
Replication
Viral genome copies per cell
10^6
10^5
10^4
10^3
10^2
10^1
1
HT29
WI38
MRC5
Cell Type
B
Anti-VEGF Ab
Anti-VEGF Ab (ng/ml)
10^3
10^2
10^1
1
ND
ND
HT29
WI38
MRC5
Cell Type
C
Infectious Virus
Infectious particles (TCID50)
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
1
ND
ND
HT29
WI38
MRC5
Cell Type
ND = not detected

Figure 44

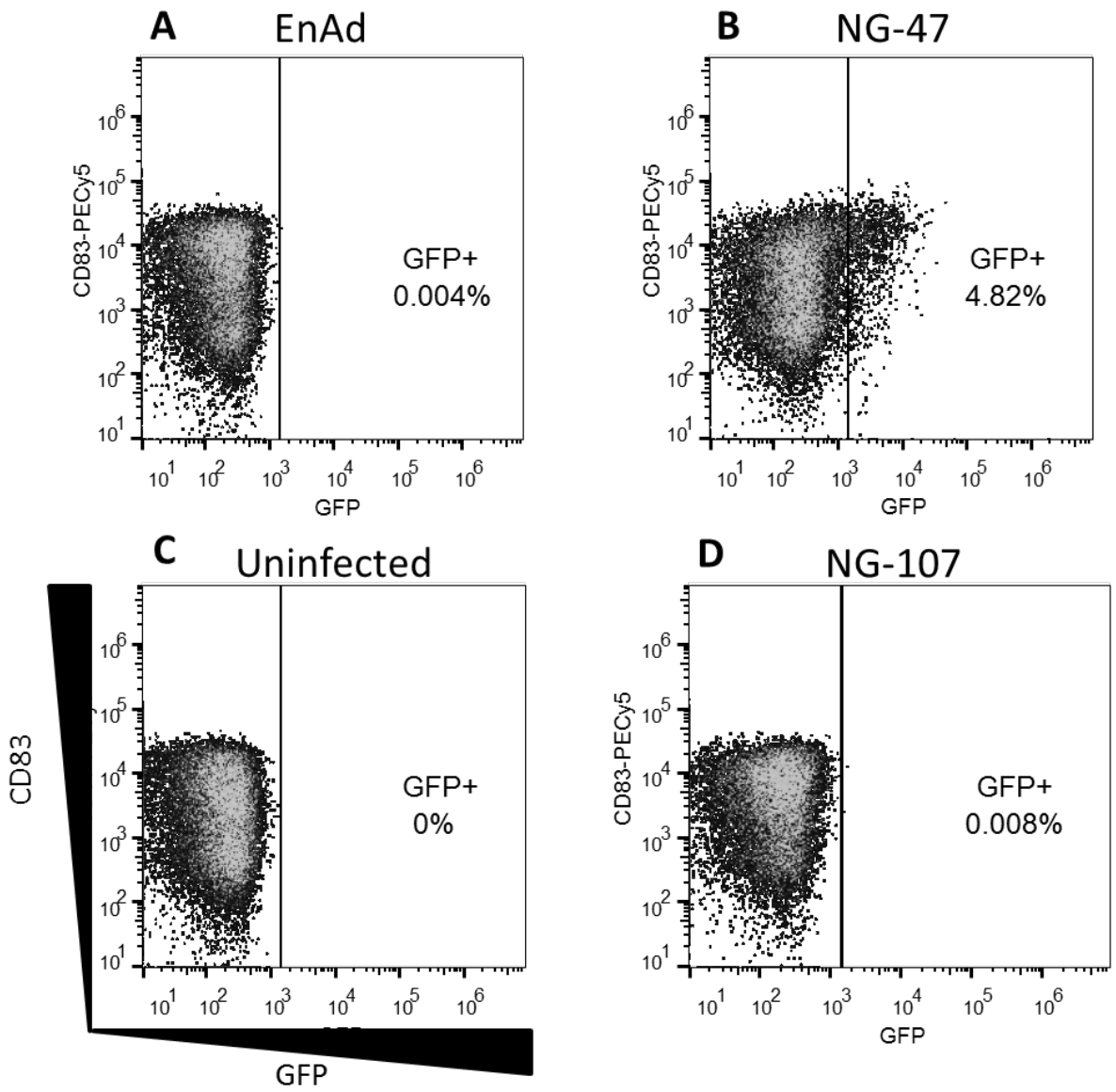

A
EnAd
GFP+
0.004%
B
NG-47
GFP+
4.82%
C
Uninfected
GFP+
0%
D
NG-107
GFP+
0.008%
CD83-PECy5
CD83
GFP

Figure 45

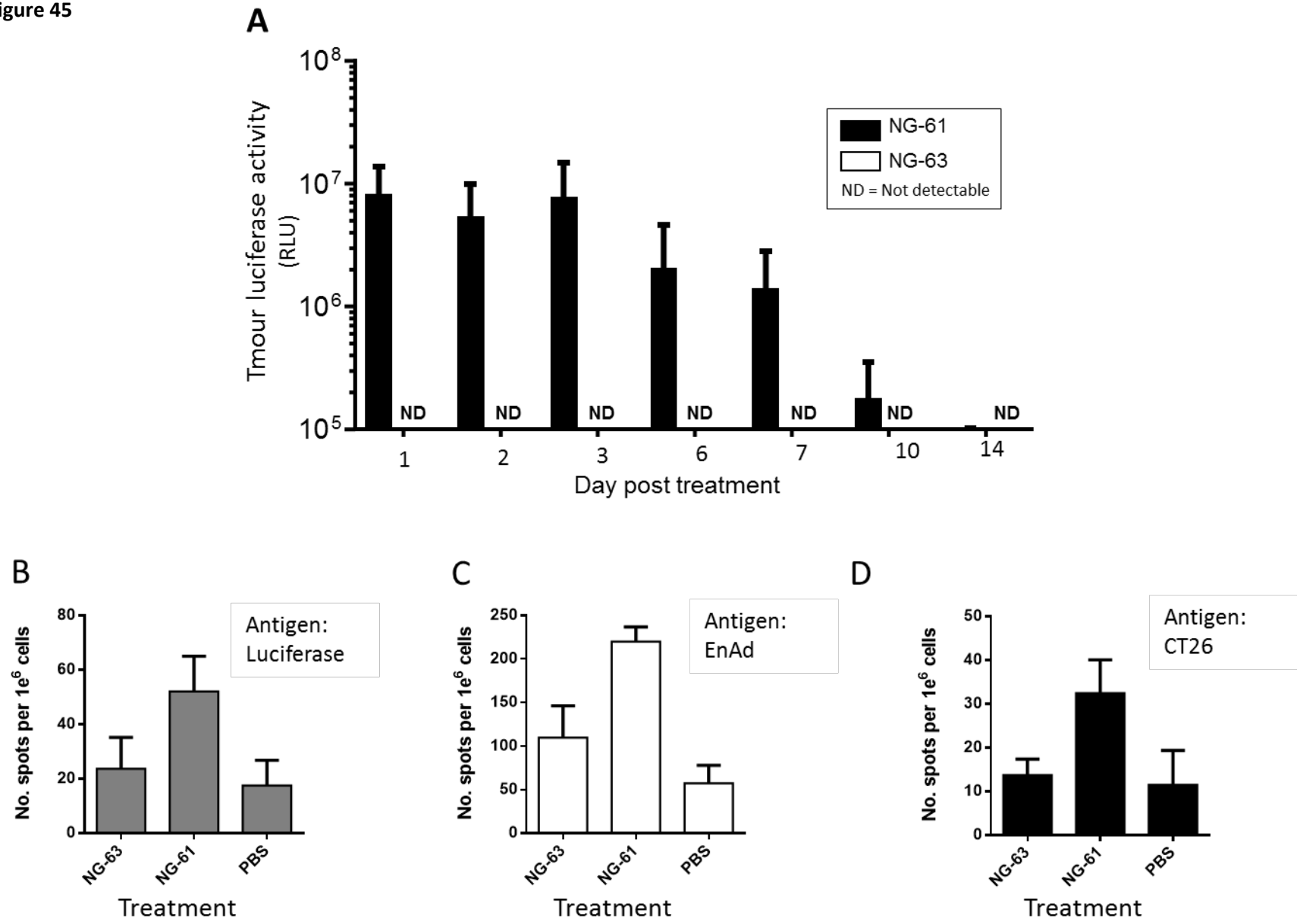

A
Tmour luciferase activity (RLU)
10^8
10^7
10^6
10^5
NG-61
NG-63
ND = Not detectable
ND
1
2
3
6
7
10
14
Day post treatment
B
No. spots per 1e6 cells
Antigen: Luciferase
NG-63
NG-61
PBS
Treatment
C
No. spots per 1e6 cells
Antigen: EnAd
NG-63
NG-61
PBS
Treatment
D
No. spots per 1e6 cells
Antigen: CT26
NG-63
NG-61
PBS
Treatment

Figure 46

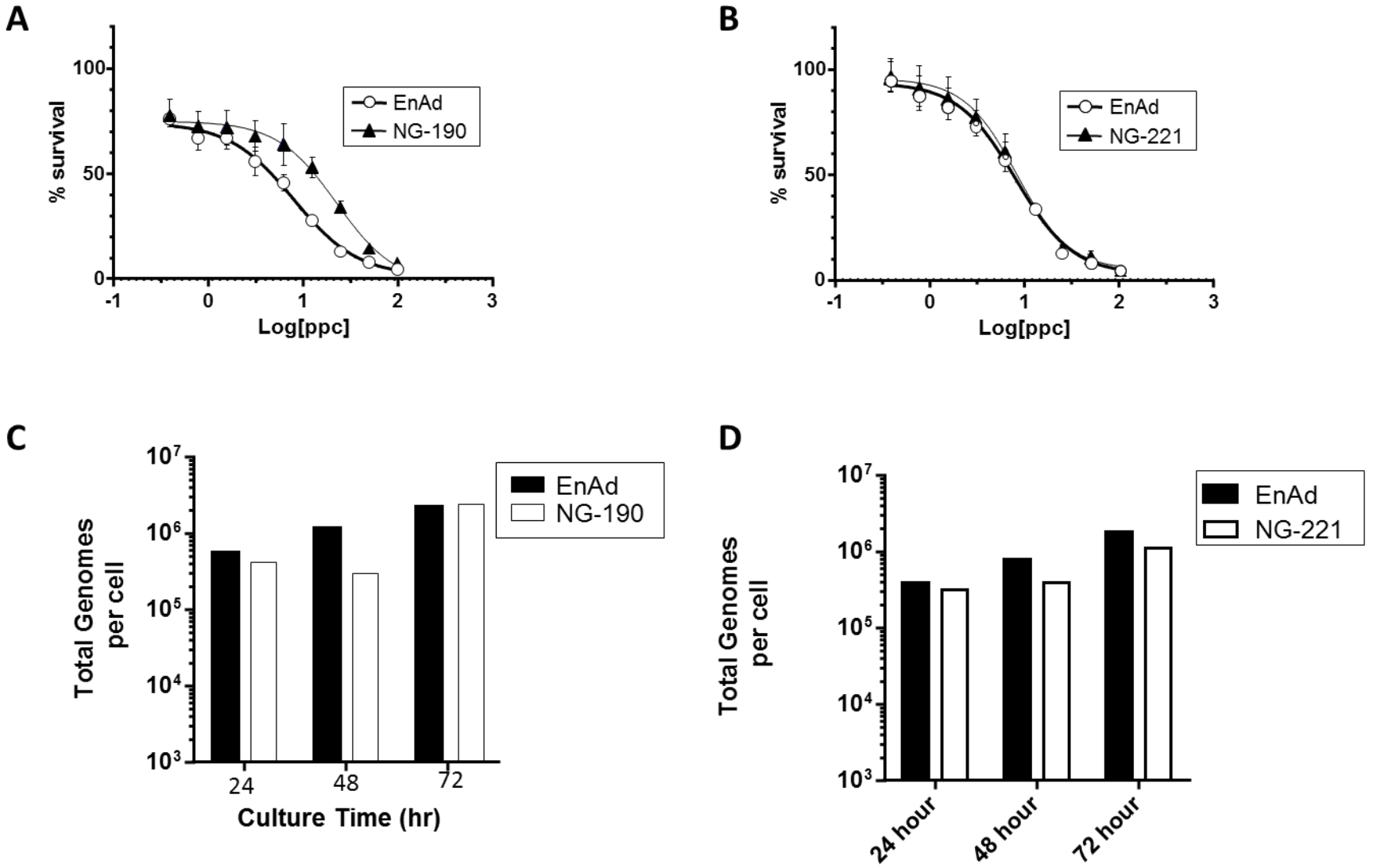
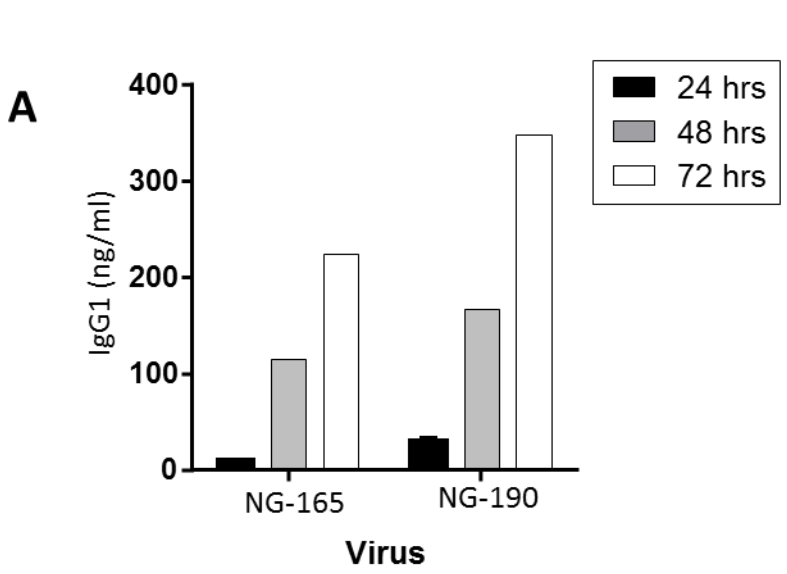
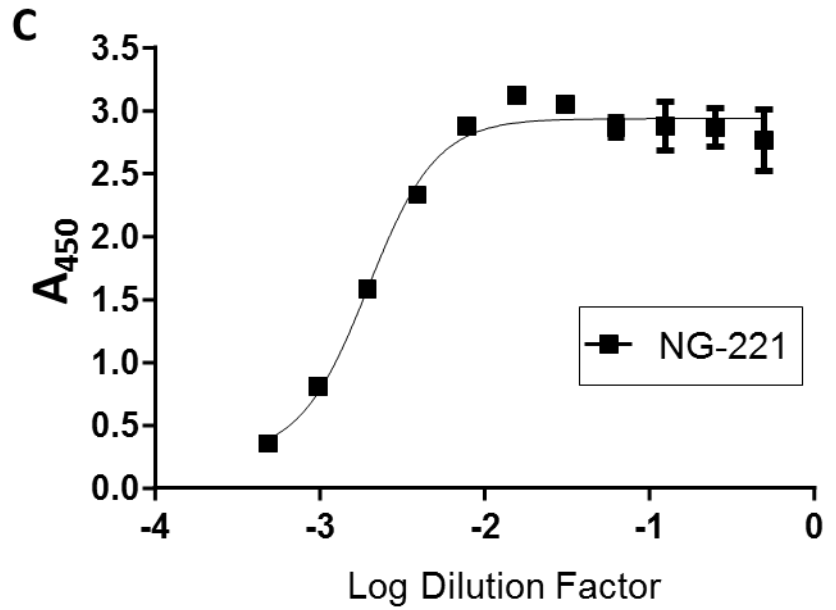
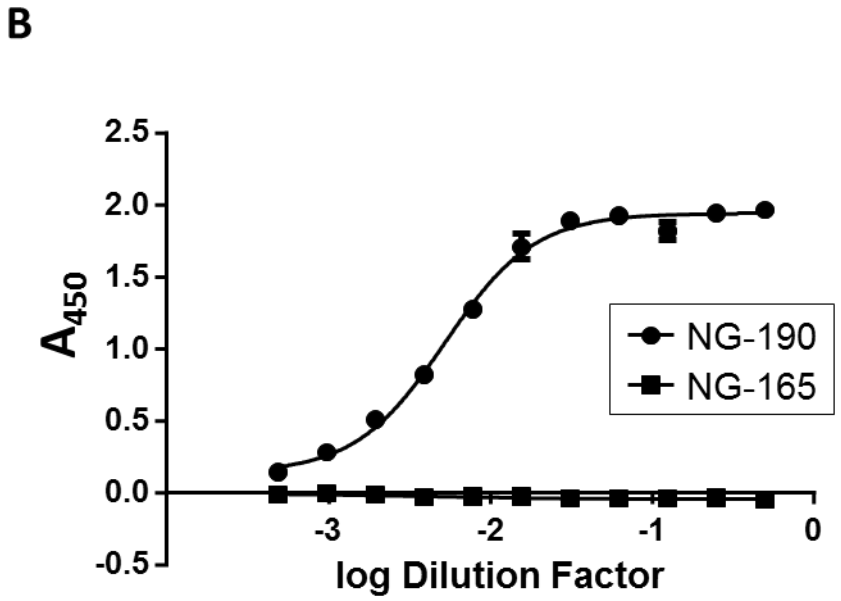

A
100
50
0
% survival
-1
0
1
2
3
Log[ppc]
EnAd
NG-190
B
100
50
0
% survival
-1
0
1
2
3
Log[ppc]
EnAd
NG-221
C
10^7
10^6
10^5
10^4
10^3
Total Genomes per cell
24
48
72
Culture Time (hr)
EnAd
NG-190
D
10^7
10^6
10^5
10^4
10^3
Total Genomes per cell
24 hour
48 hour
72 hour
EnAd
NG-221

Figure 47


A
400
300
200
100
0
IgG1 (ng/ml)
24 hrs
48 hrs
72 hrs
NG-165
NG-190
Virus


C
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
A450
NG-221
-4
-3
-2
-1
0
Log Dilution Factor


B
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
A450
NG-190
NG-165
-3
-2
-1
0
log Dilution Factor

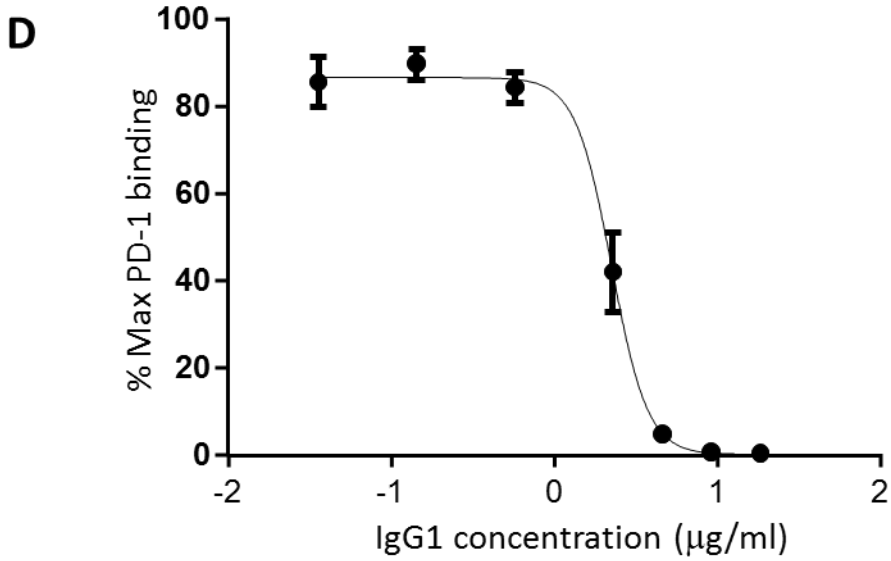

D
100
80
60
40
20
0
% Max PD-1 binding
-2
-1
0
1
2
IgG1 concentration (µg/ml)

Figure 48

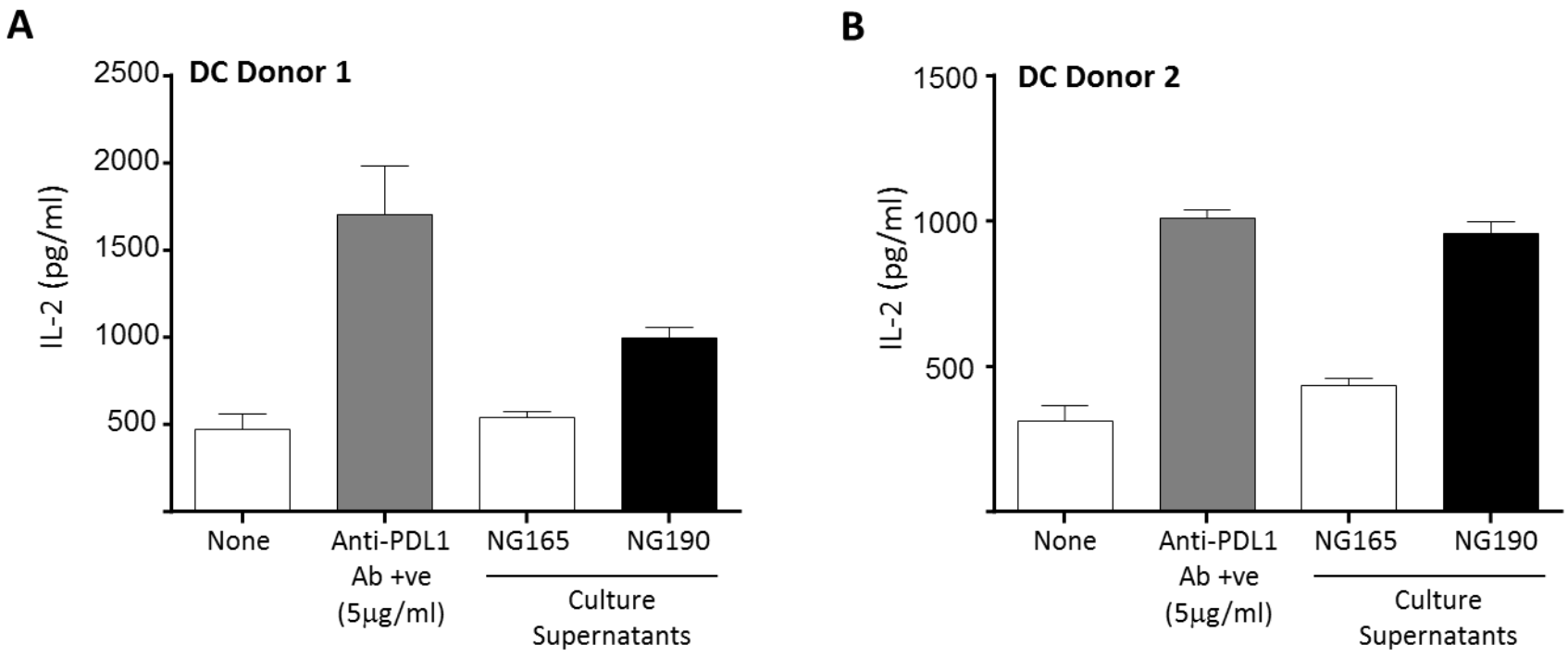

A
DC Donor 1
IL-2 (pg/ml)
2500
2000
1500
1000
500
None
Anti-PDL1 Ab +ve (5µg/ml)
NG165
NG190
Culture Supernatants
B
DC Donor 2
IL-2 (pg/ml)
1500
1000
500
None
Anti-PDL1 Ab +ve (5µg/ml)
NG165
NG190
Culture Supernatants

Figure 49

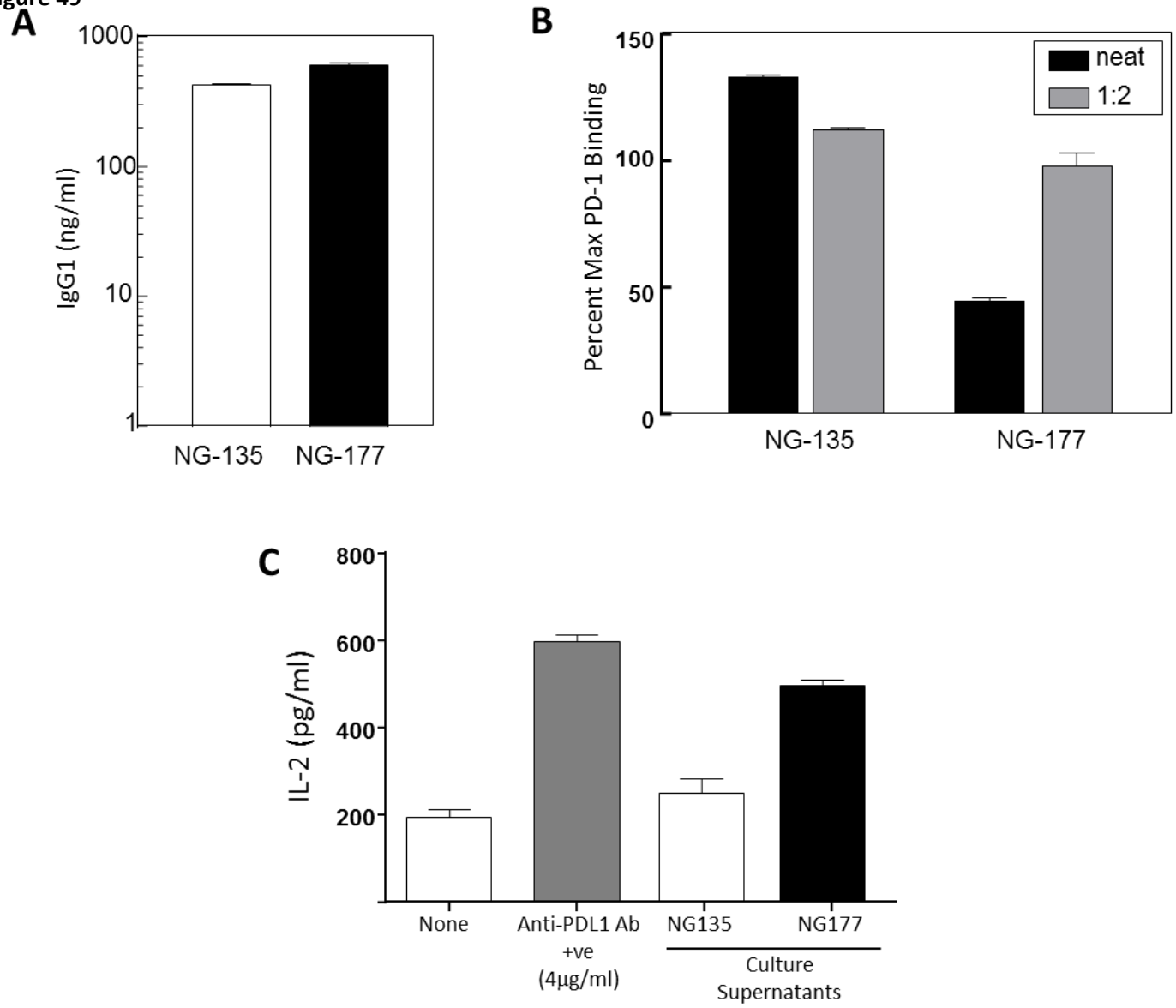

A
1000
100
10
1
IgG1 (ng/ml)
NG-135
NG-177
B
150
100
50
0
Percent Max PD-1 Binding
neat
1:2
NG-135
NG-177
C
800
600
400
200
IL-2 (pg/ml)
None
Anti-PDL1 Ab
+ve
(4µg/ml)
NG135
NG177
Culture
Supernatants

Figure 50

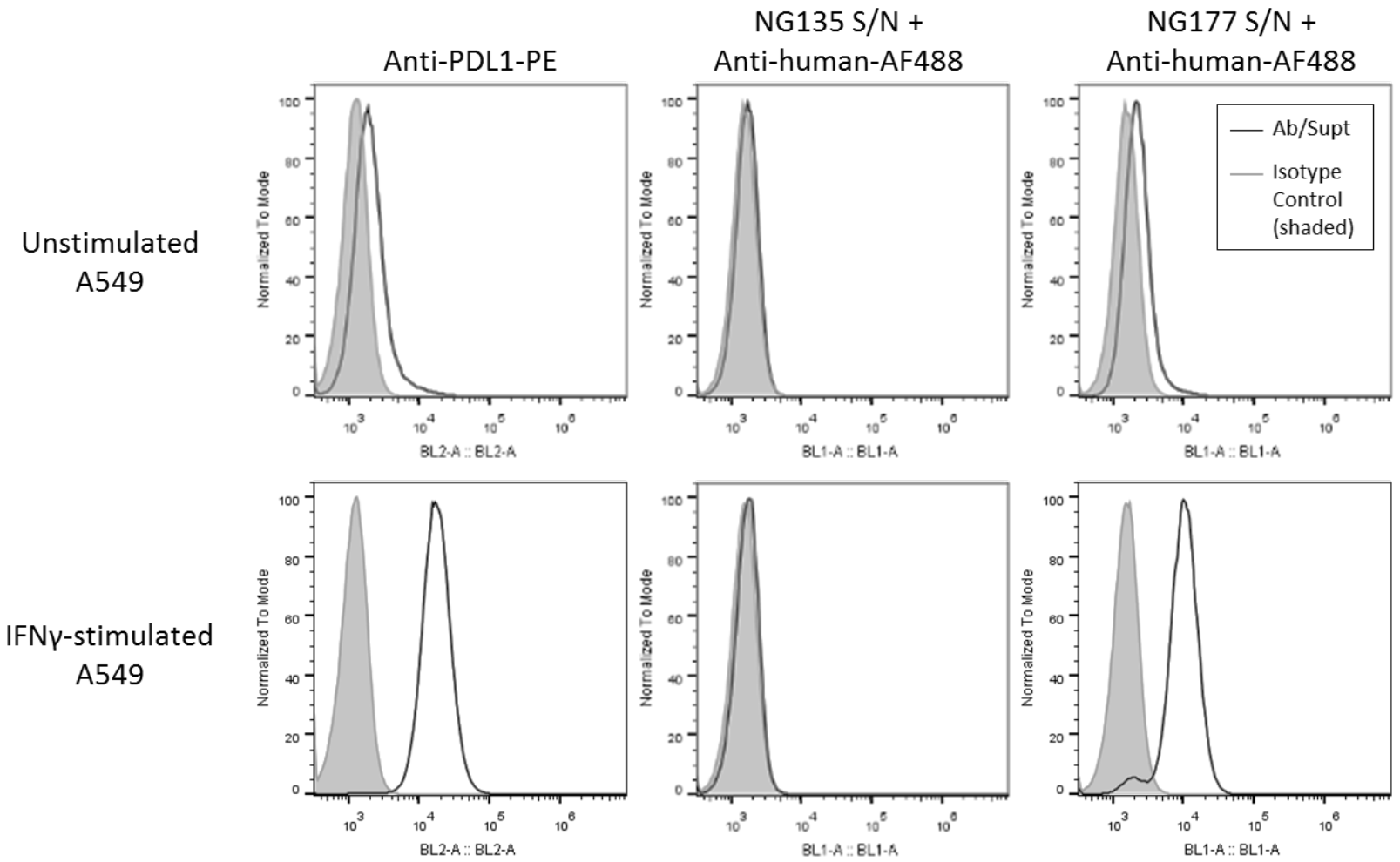

Anti-PDL1-PE
NG135 S/N + Anti-human-AF488
NG177 S/N + Anti-human-AF488
Unstimulated A549
IFNγ-stimulated A549
Normalized To Mode
BL2-A :: BL2-A
BL1-A :: BL1-A
Ab/Supt
Isotype Control (shaded)

Figure 51 ColoAd1 = EnAd

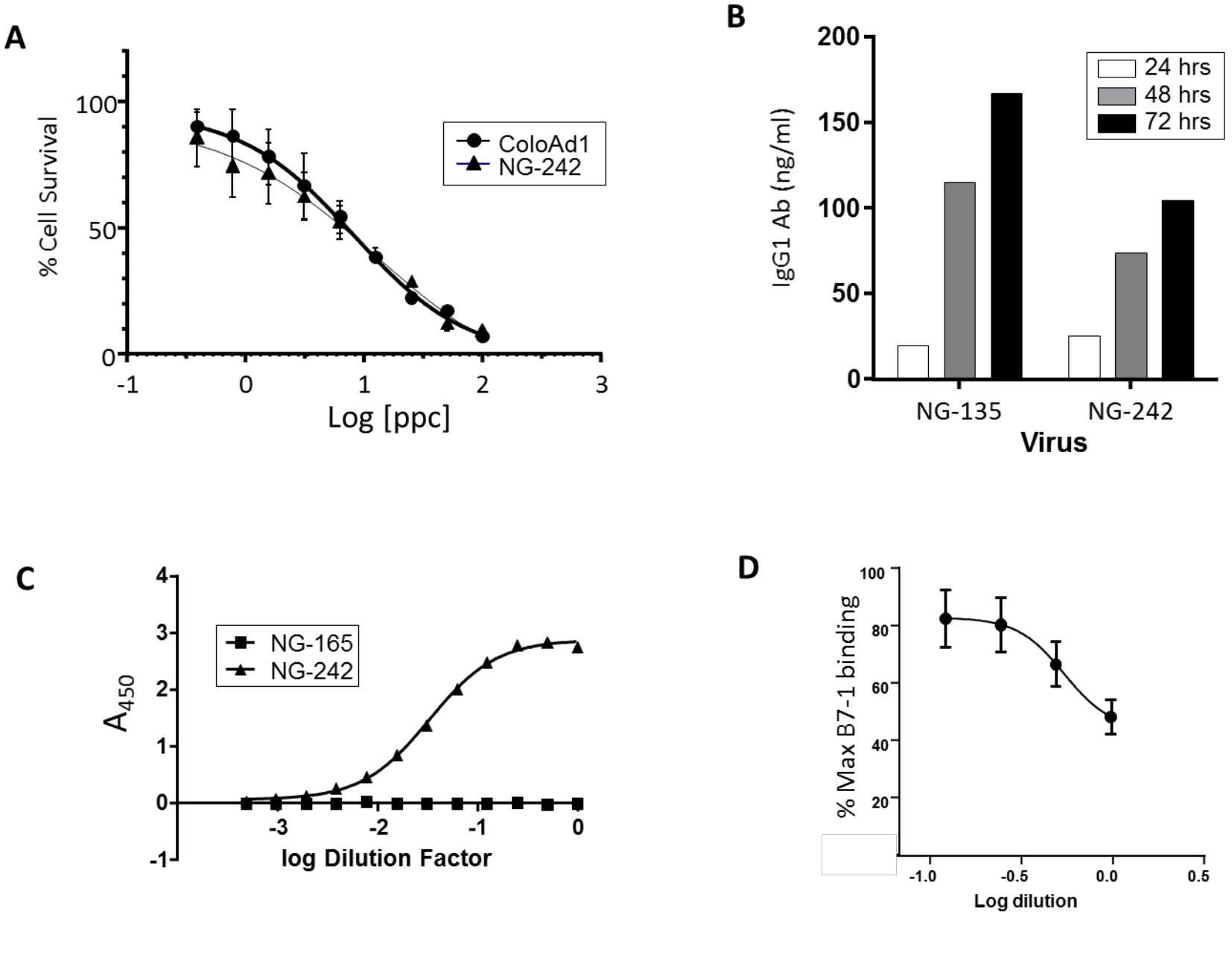


Figure 52 ColoAd1 = EnAd

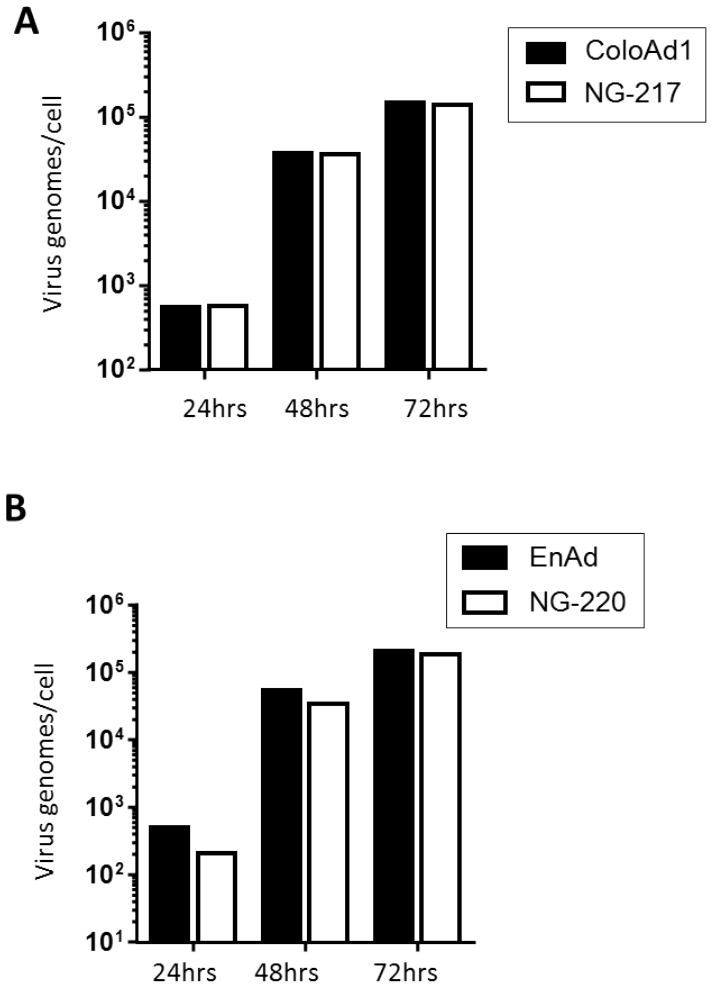


Figure 53 ColoAd1 = EnAd

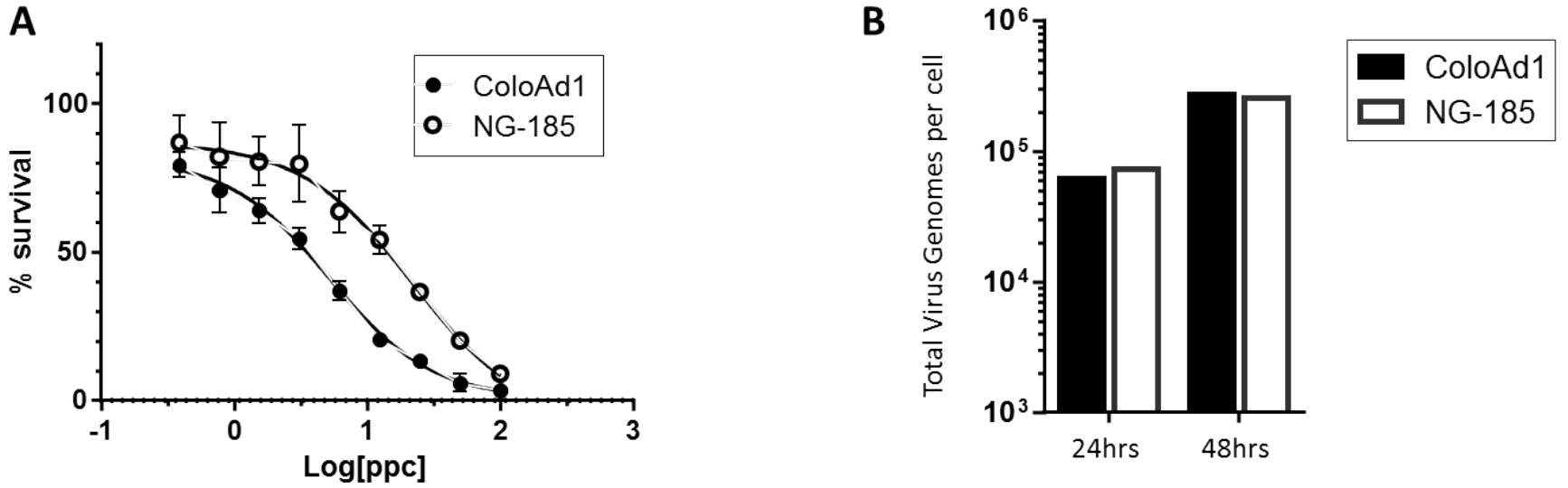
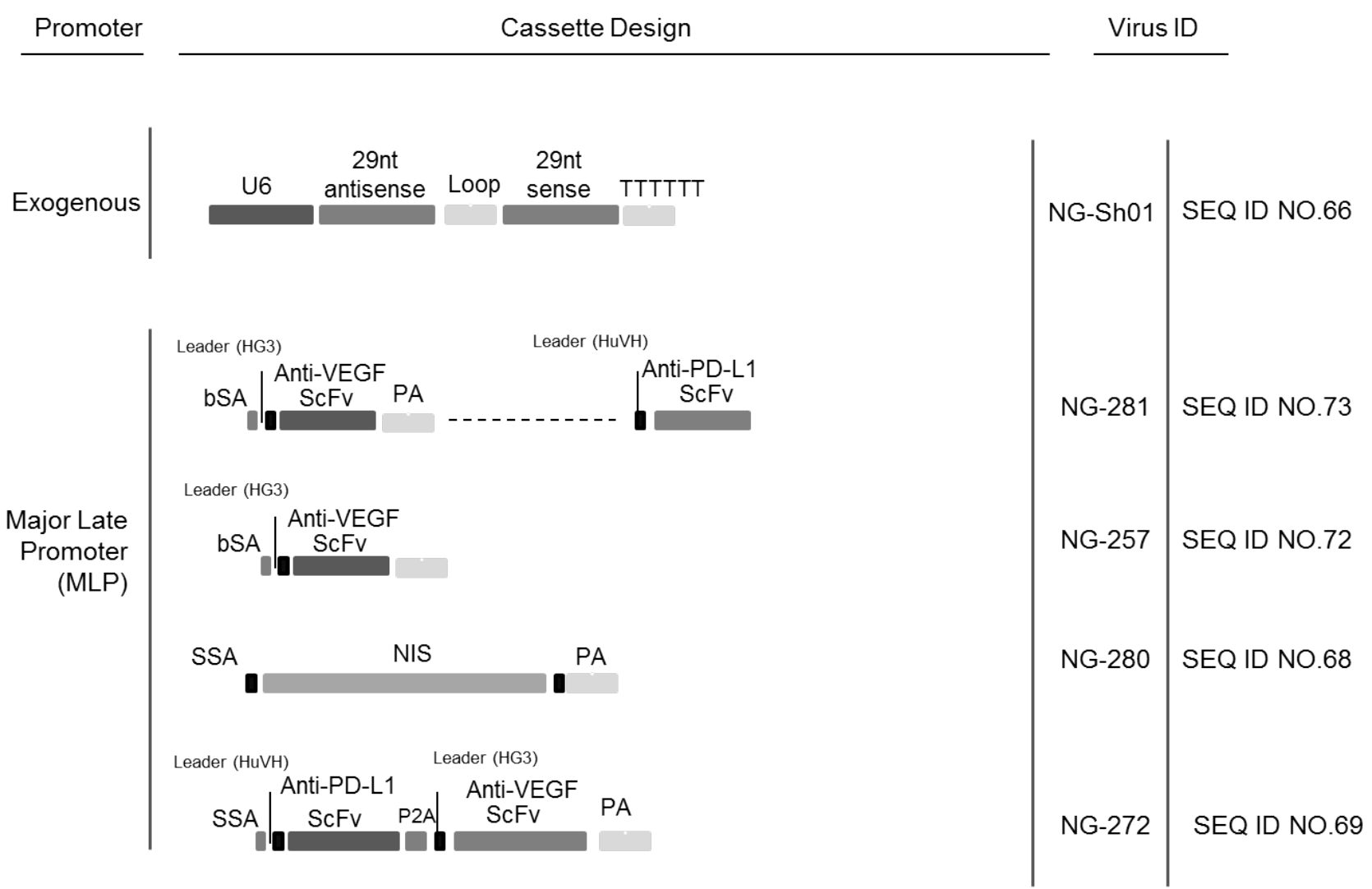


Figure 54


Promoter
Cassette Design
Virus ID
Exogenous
U6
29nt antisense
Loop
29nt sense
TTTTTT
NG-Sh01
SEQ ID NO.66
Major Late Promoter (MLP)
Leader (HG3)
bSA
Anti-VEGF ScFv
PA
Leader (HuVH)
Anti-PD-L1 ScFv
NG-281
SEQ ID NO.73
Leader (HG3)
bSA
Anti-VEGF ScFv
NG-257
SEQ ID NO.72
SSA
NIS
PA
NG-280
SEQ ID NO.68
Leader (HuVH)
Anti-PD-L1 ScFv
SSA
P2A
Leader (HG3)
Anti-VEGF ScFv
PA
NG-272
SEQ ID NO.69