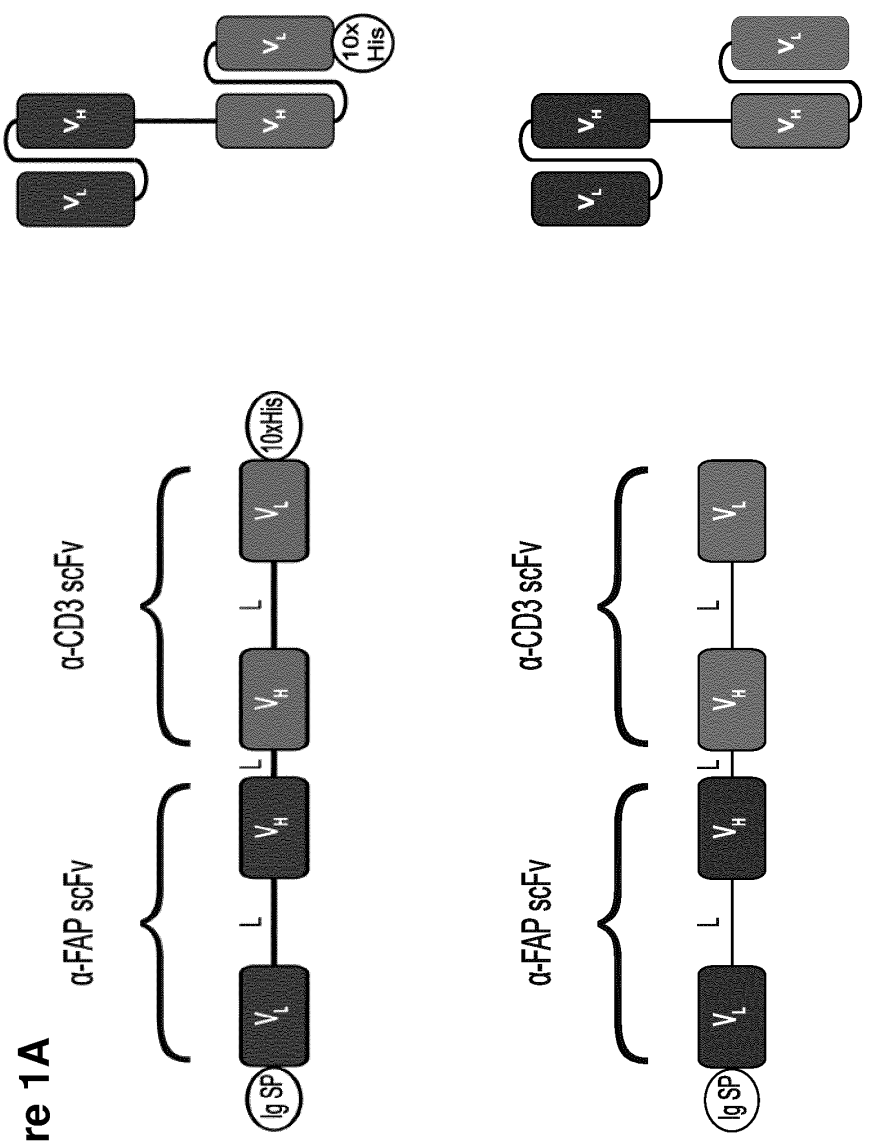
Figure 1A



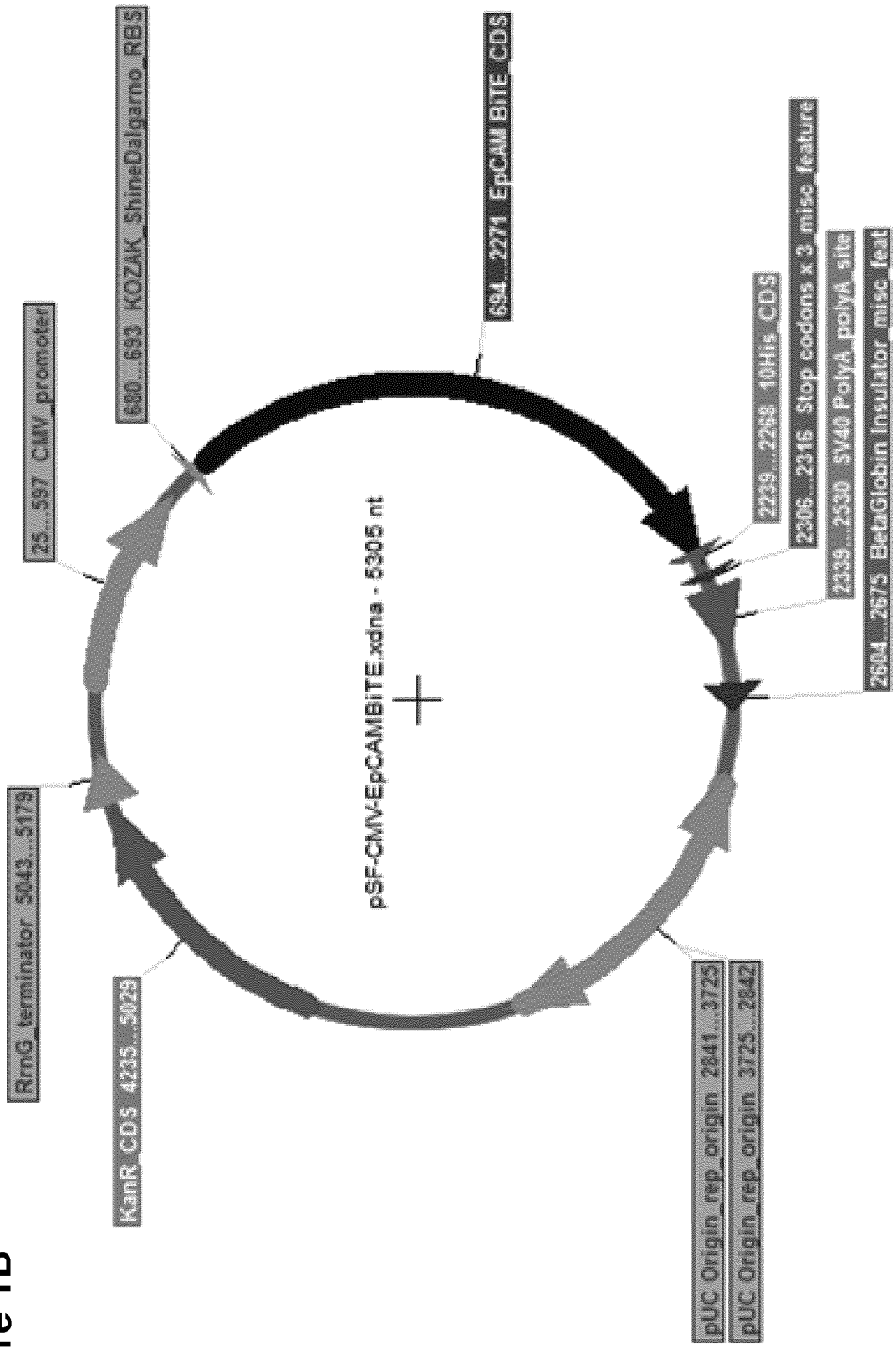

pSF-CMV-EpCAMBiTE.xdna - 5305 nt
25...597 CMV_promoter
680...693 KOZAK_ShineDalgarno_RBS
694...2271 EpCAM BITE_CDS
2239...2268 10His_CDS
2306...2316 Stop codons x 3_misc_feature
2339...2530 SV40 PolyA_polyA_site
2604...2675 BetaGlobin Insulator_misc_feat
pUC Origin_rep_origin 2841...3725
pUC Origin_rep_origin 3725...2842
KanR_CDS 4235...5029
RrnG_terminator 5043...5179

Figure 1B

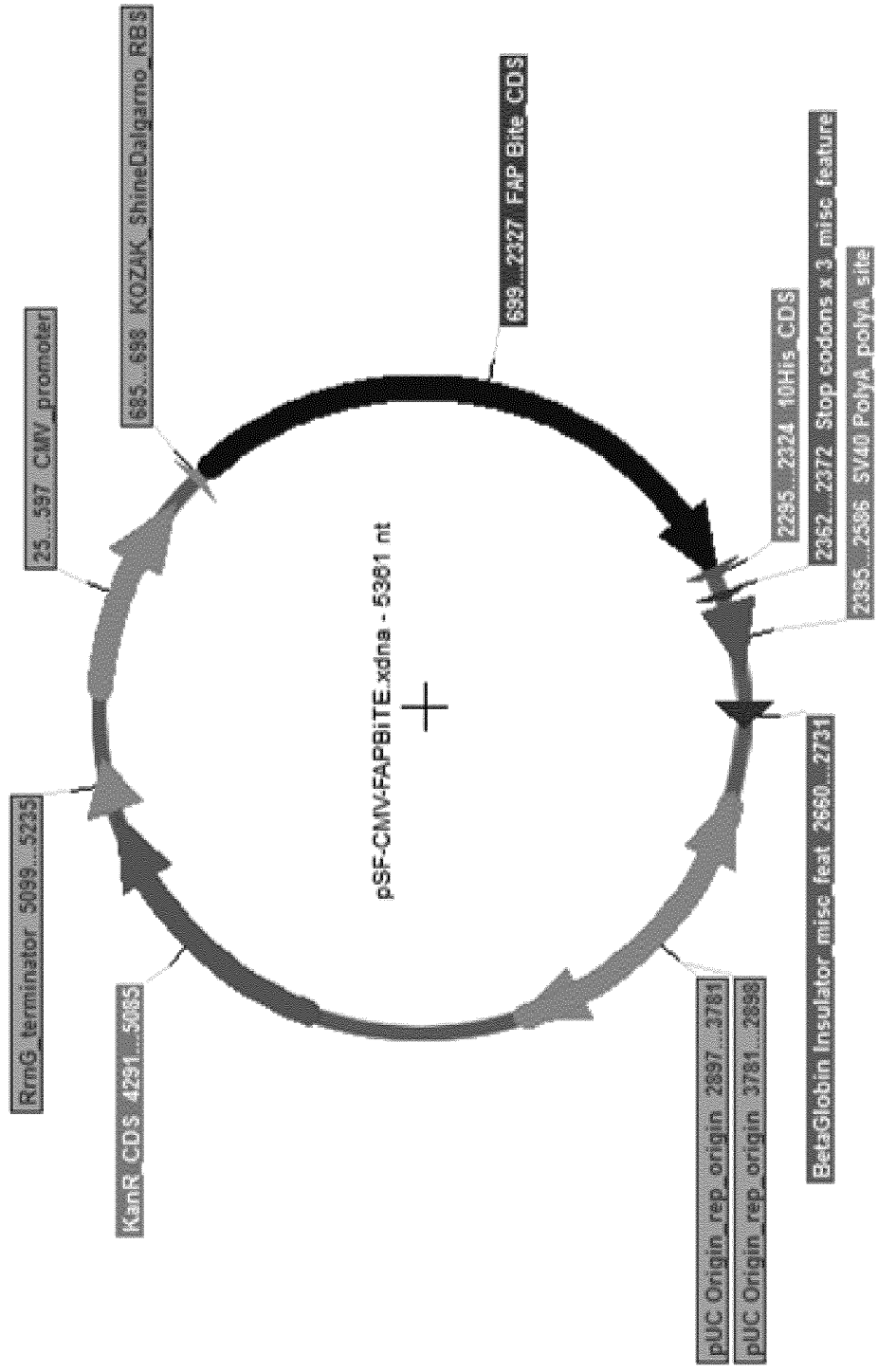

pSF-CMV-FAPBiTE.xdna - 5381 nt
685...698 KOZAK_ShineDalgarno_RBS
699...2327 FAP Bite_CDS
2295...2324 10His_CDS
2362...2372 Stop codons x 3 misc_feature
2395...2586 SV40 PolyA_polyA_site
2660...2731 BetaGlobin Insulator misc_feat
2897...3781 pUC Origin rep_origin
3781...2898 pUC Origin rep_origin
4291...5085 KanR_CDS
5099...5235 RrnG_terminator
25...537 CMV_promoter

Figure 1C

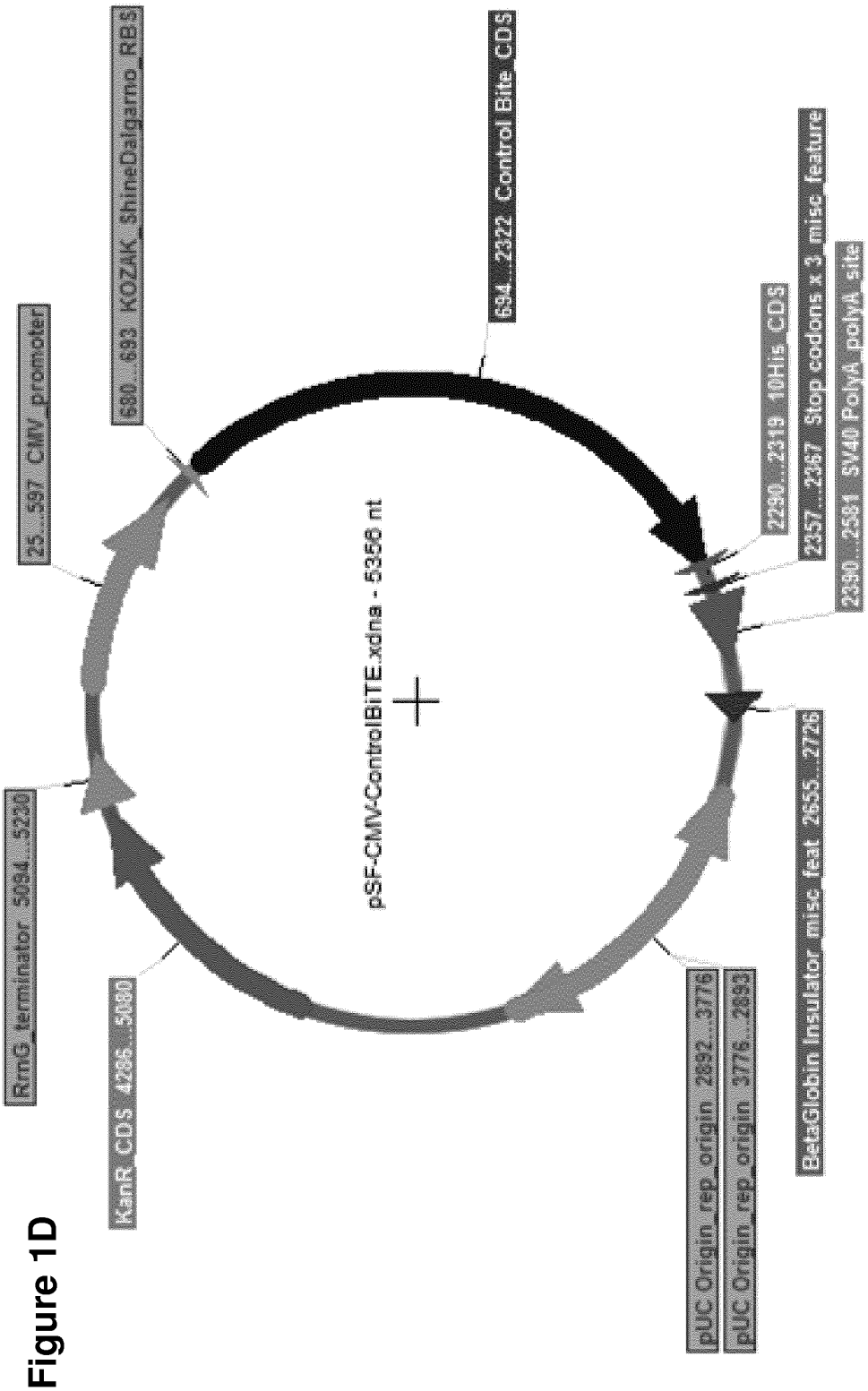
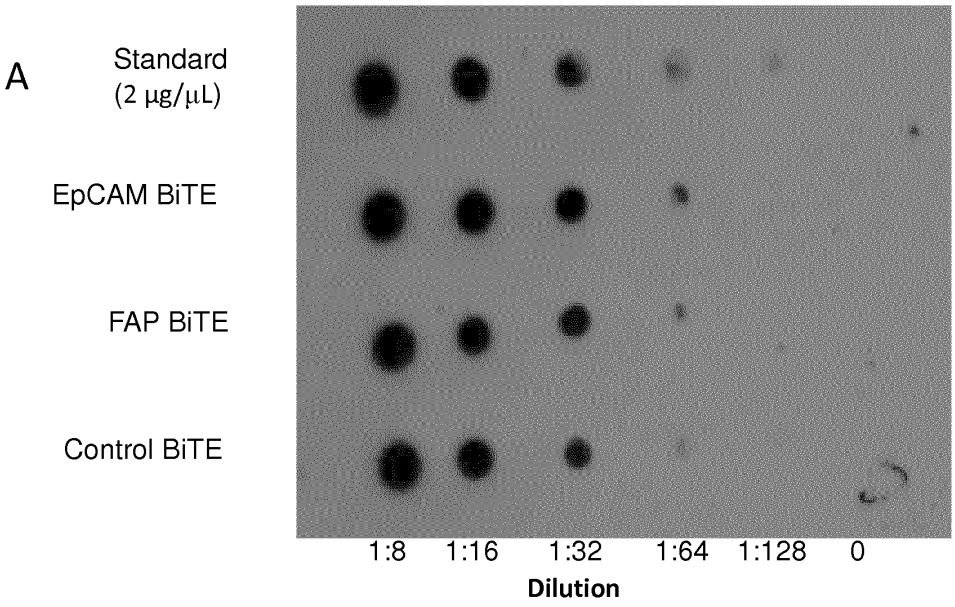

pSF-CMV-ControlBiTE.xdna - 5356 nt
680...693 KOZAK_ShineDalgarno_RBS
694...2322 Control Bite_CDS
2290...2319 10His_CDS
2357...2367 Stop codons x 3_misc_feature
2390...2581 SV40 PolyA_polyA_site
2655...2726 BetaGlobin Insulator_misc_feat
3776...2883 pUC Origin_rep_origin
2892...3776 pUC Origin_rep_origin
4286...5080 KanR_CDS
5084...5230 RrnG_terminator
25...597 CMV_promoter

Figure 1D

Figure 2


A
Standard
(2 µg/µL)
EpCAM BiTE
FAP BiTE
Control BiTE
1:8
1:16
1:32
1:64
1:128
0
Dilution

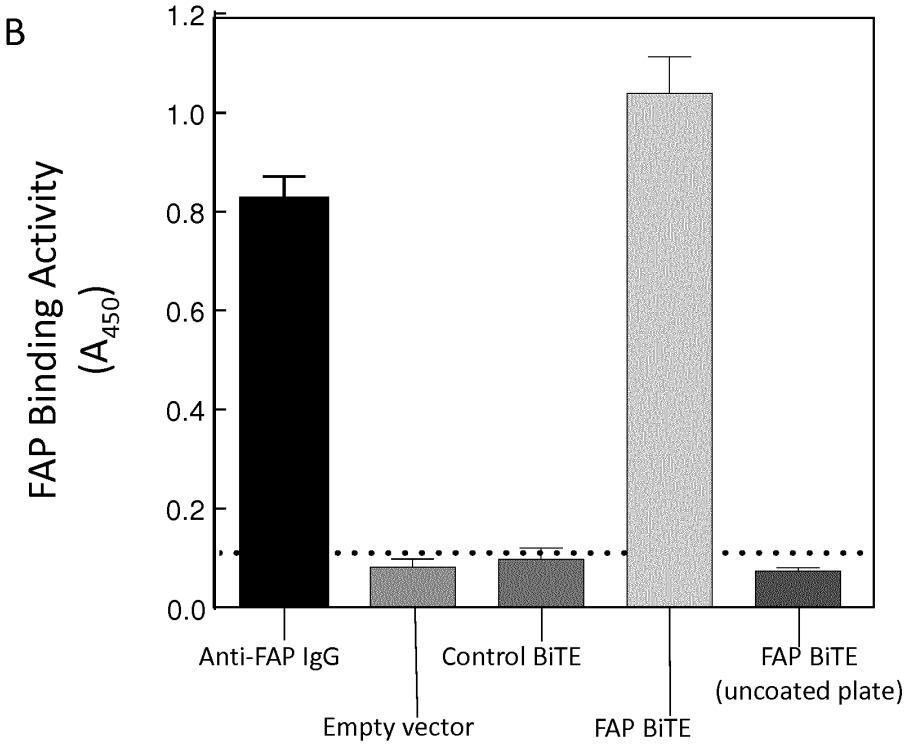

B
FAP Binding Activity
(A_{450})
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Anti-FAP IgG
Empty vector
Control BiTE
FAP BiTE
FAP BiTE
(uncoated plate)

Figure 2C

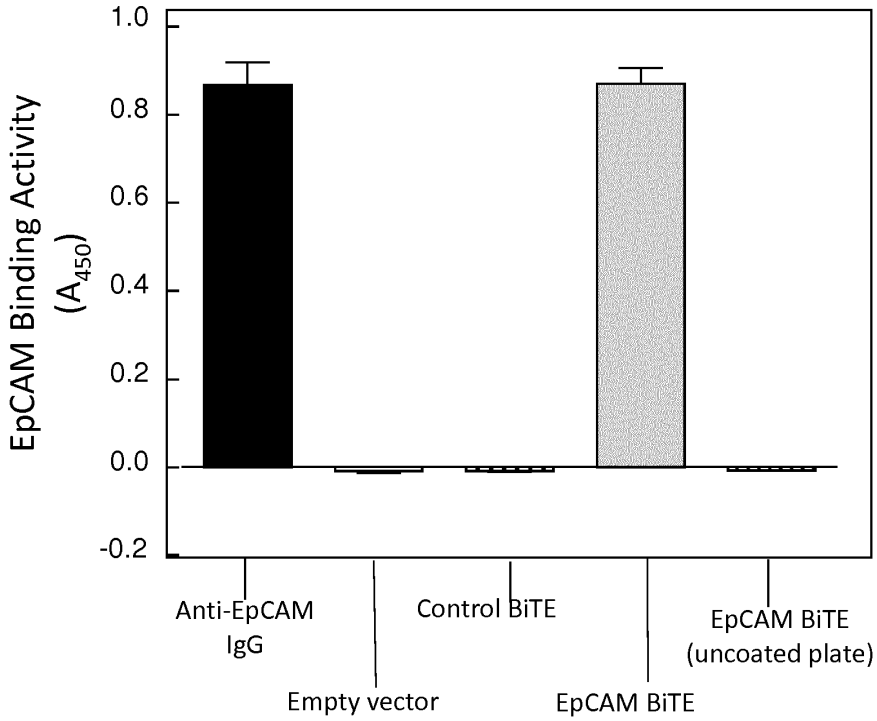
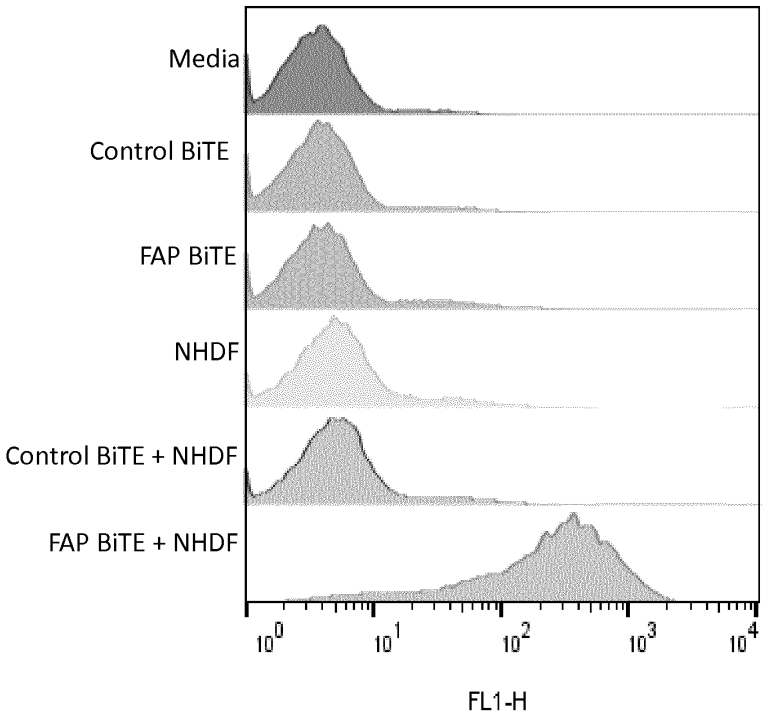

EpCAM Binding Activity
(A_{450})
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
Anti-EpCAM IgG
Empty vector
Control BiTE
EpCAM BiTE
EpCAM BiTE (uncoated plate)

Figure 3A


Media
Control BiTE
FAP BiTE
NHDF
Control BiTE + NHDF
FAP BiTE + NHDF
10^0
10^1
10^2
10^3
10^4
FL1-H

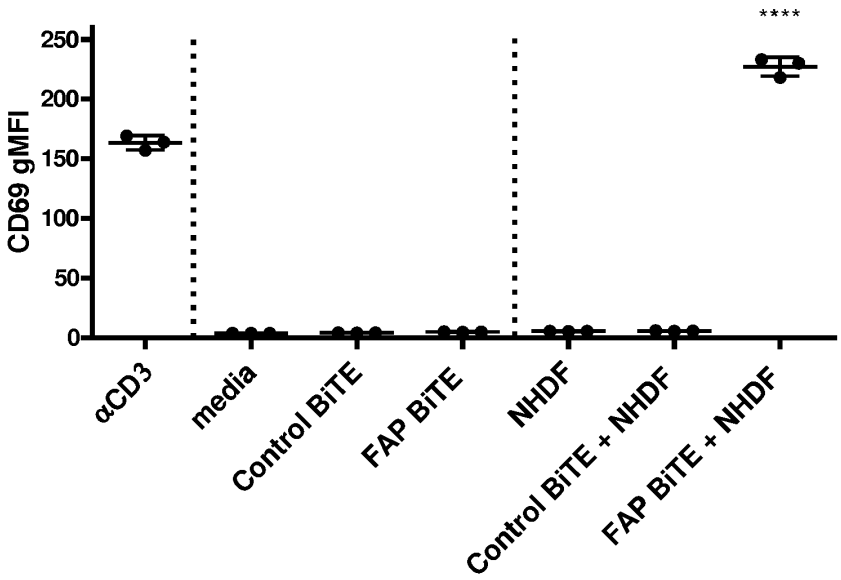
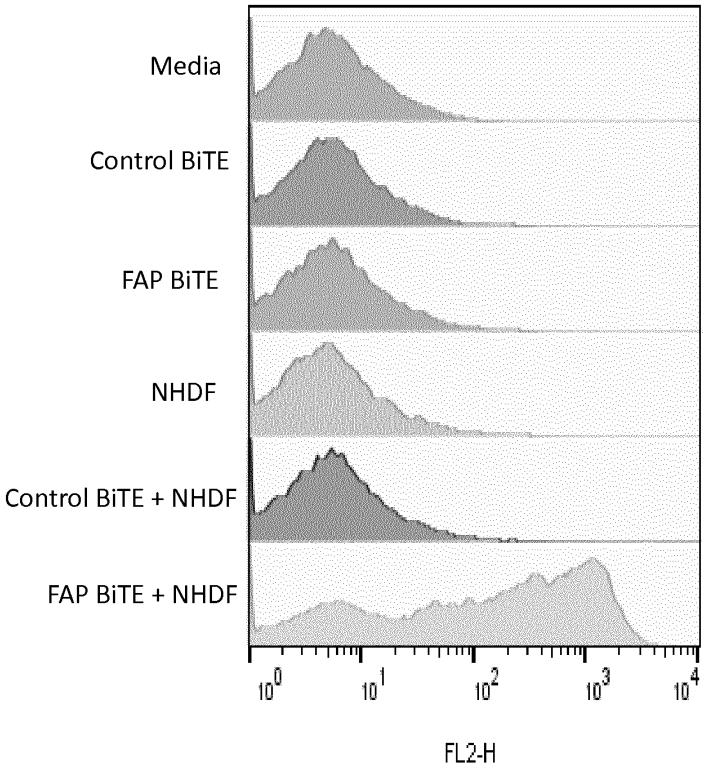

****
250
200
150
100
50
0
CD69 gMFI
αCD3
media
Control BiTE
FAP BiTE
NHDF
Control BiTE + NHDF
FAP BiTE + NHDF

Figure 3B


Media
Control BiTE
FAP BiTE
NHDF
Control BiTE + NHDF
FAP BiTE + NHDF
10^0
10^1
10^2
10^3
10^4
FL2-H

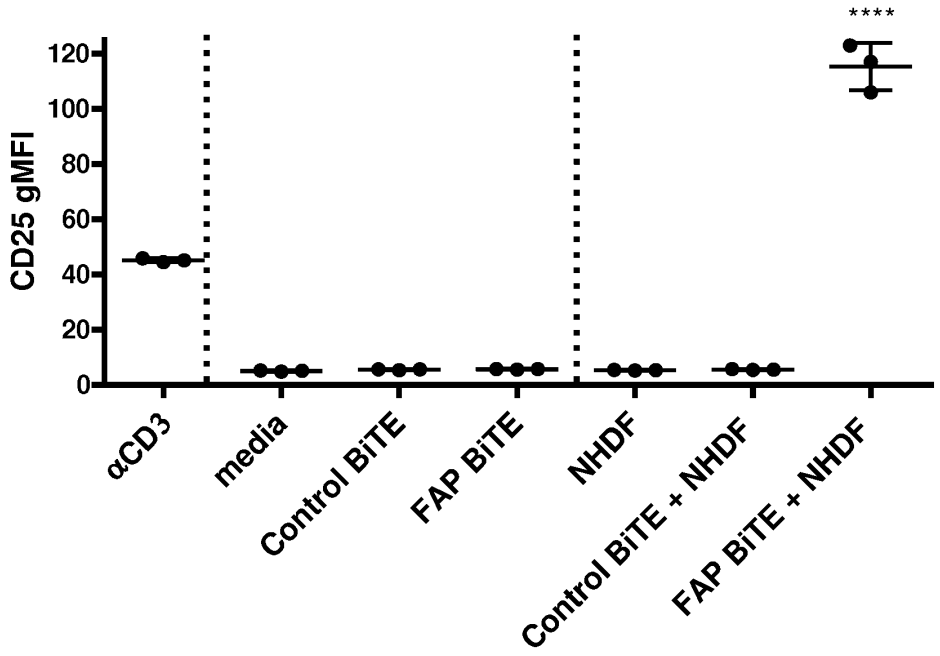

****
CD25 gMFI
120
100
80
60
40
20
0
αCD3
media
Control BiTE
FAP BiTE
NHDF
Control BiTE + NHDF
FAP BiTE + NHDF

Figure 4A

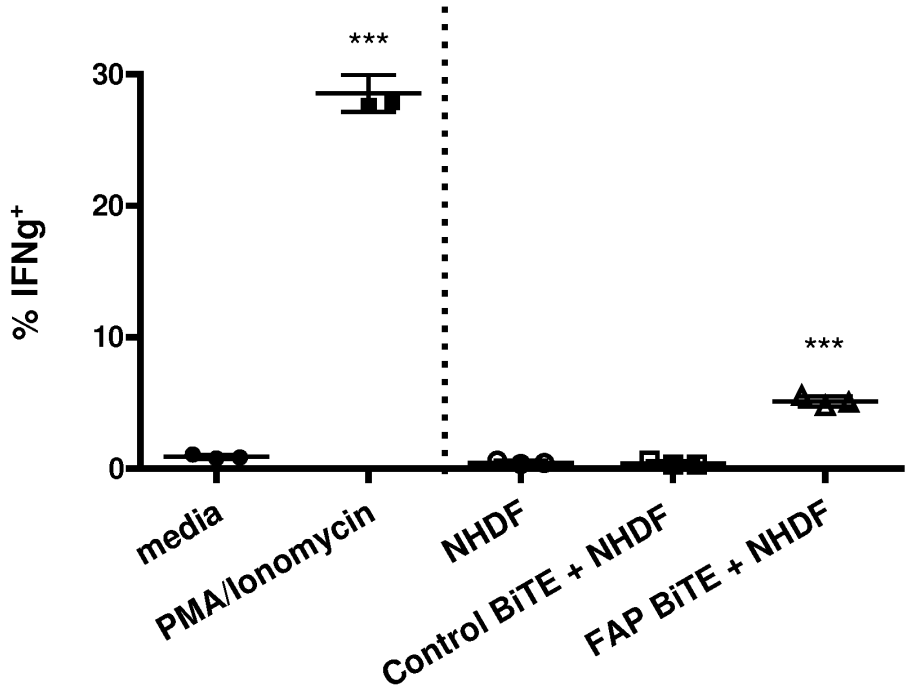


Figure 4B

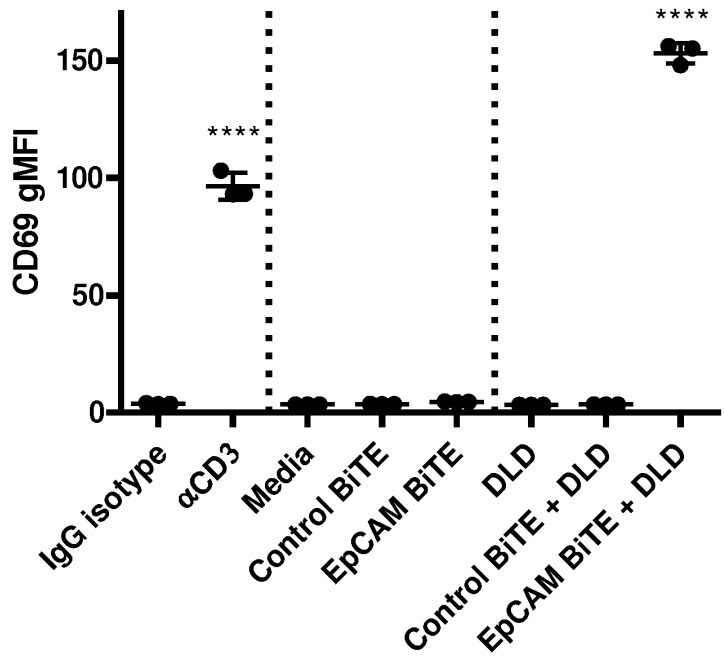


Figure 4C

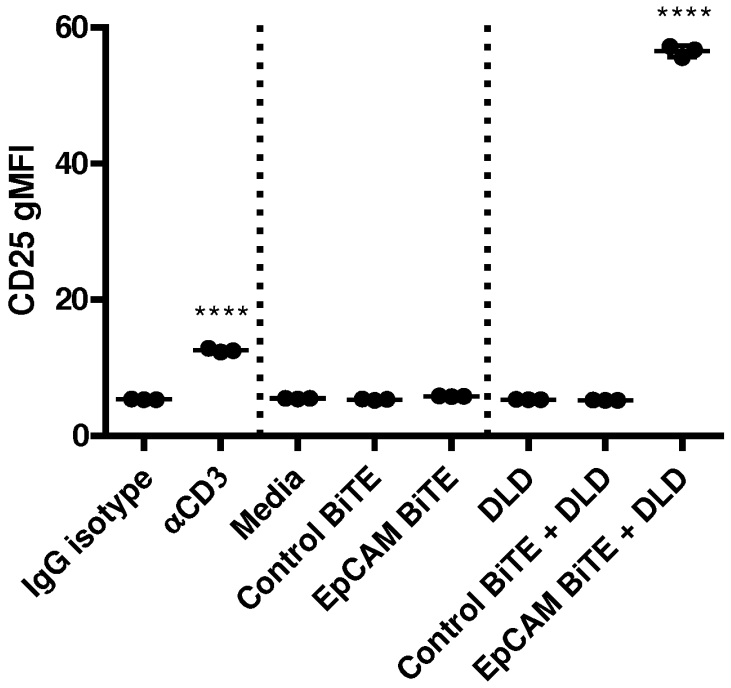


Figure 5A

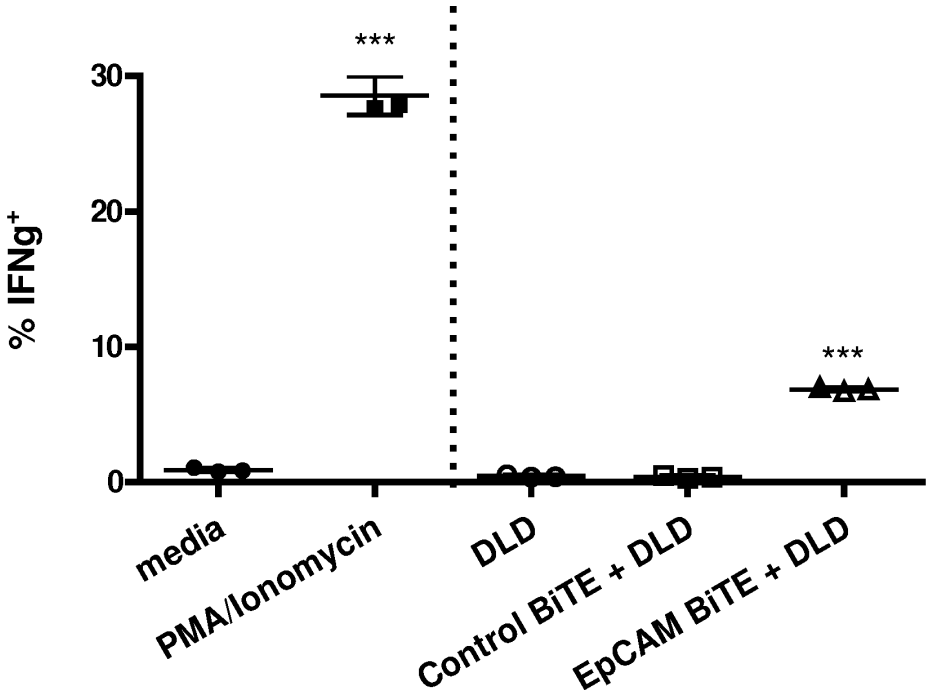
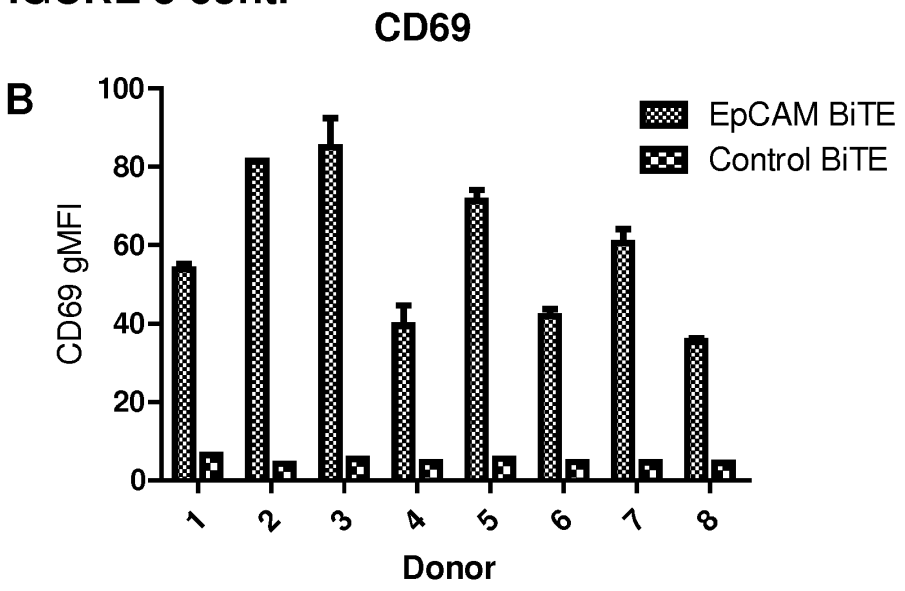


FIGURE 5 cont.



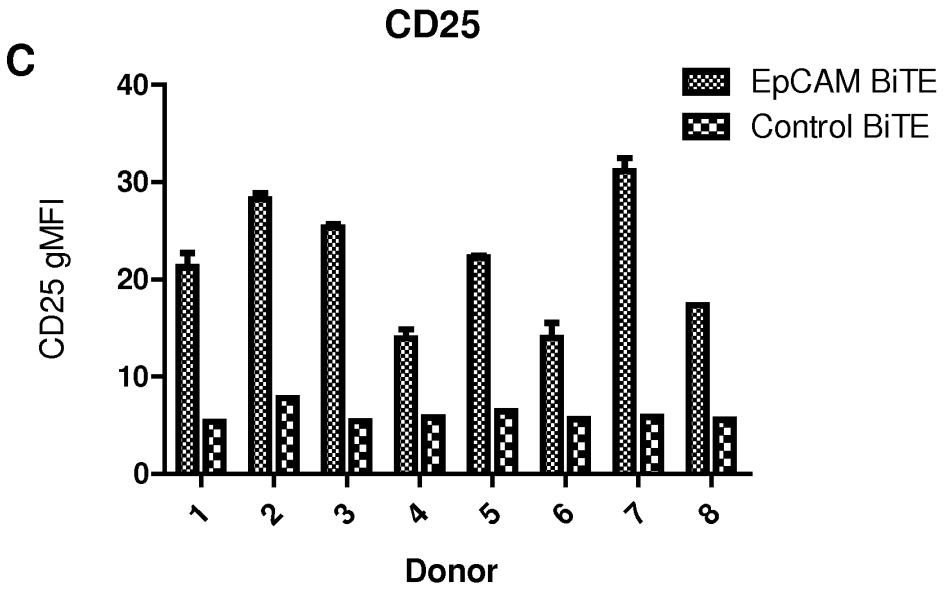


Figure 6

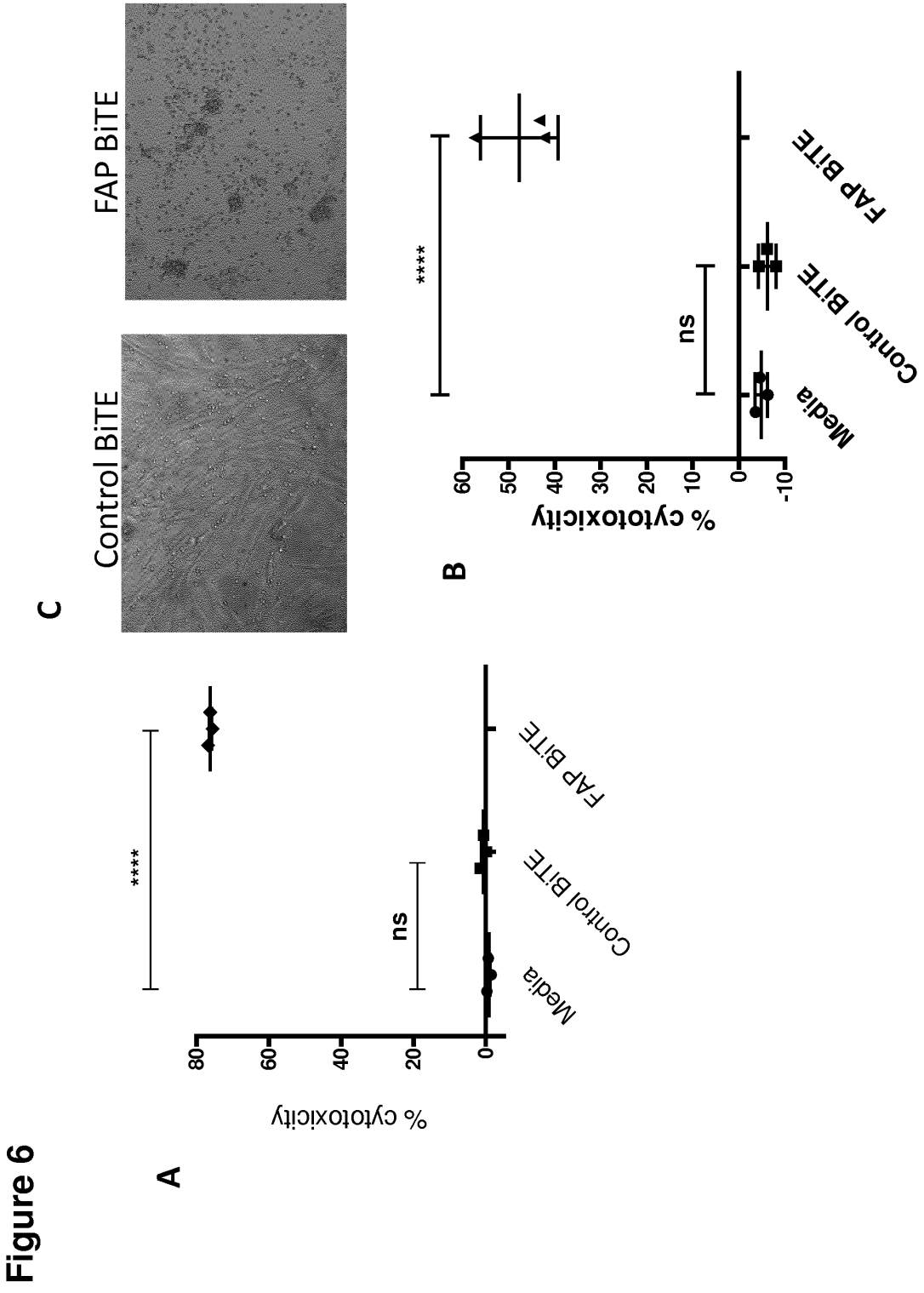
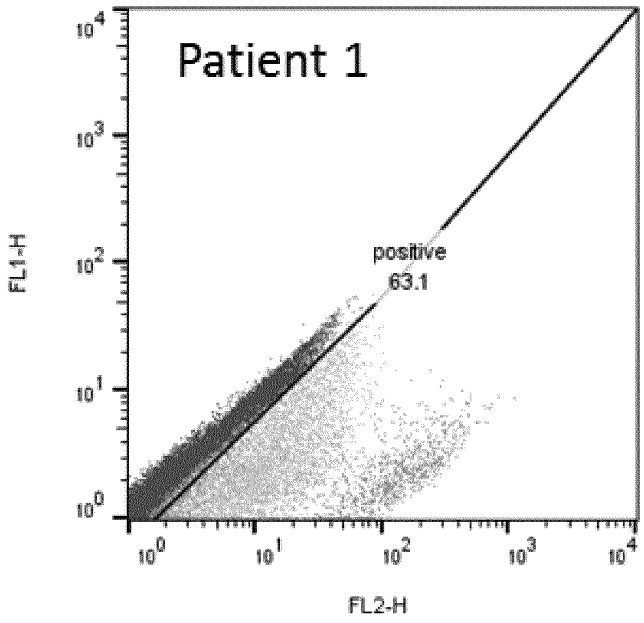
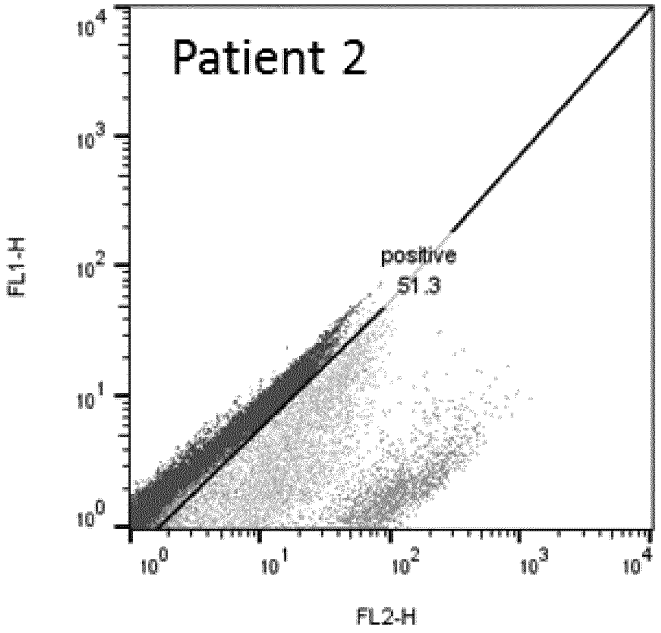

A
% cytotoxicity
80
60
40
20
0
****
ns
Media
Control BiTE
FAP BiTE
B
% cytotoxicity
60
50
40
30
20
10
0
-10
****
ns
Media
Control BiTE
FAP BiTE
C
Control BiTE
FAP BiTE

Figure 7A

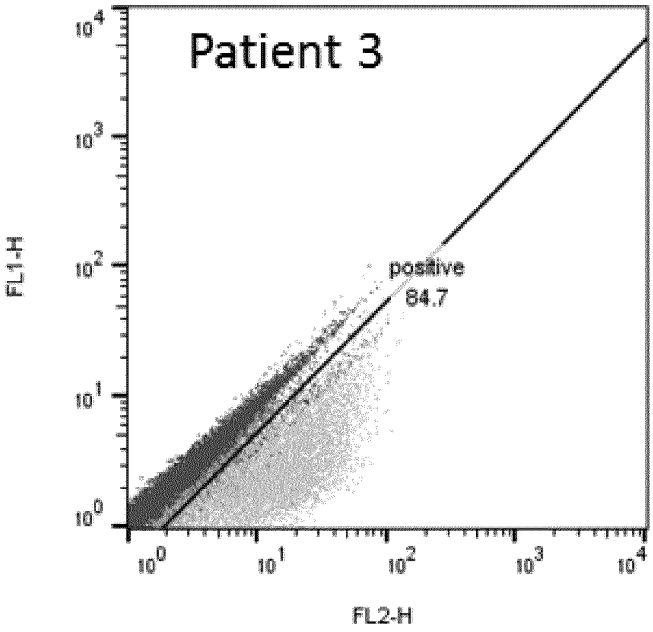


	Sample Name	positive :: Freq. of Paren
	Unstained	0.19
	Isotype C	0.41
	EpCAM BiTE	15.7
	FAP BiTE	63.1

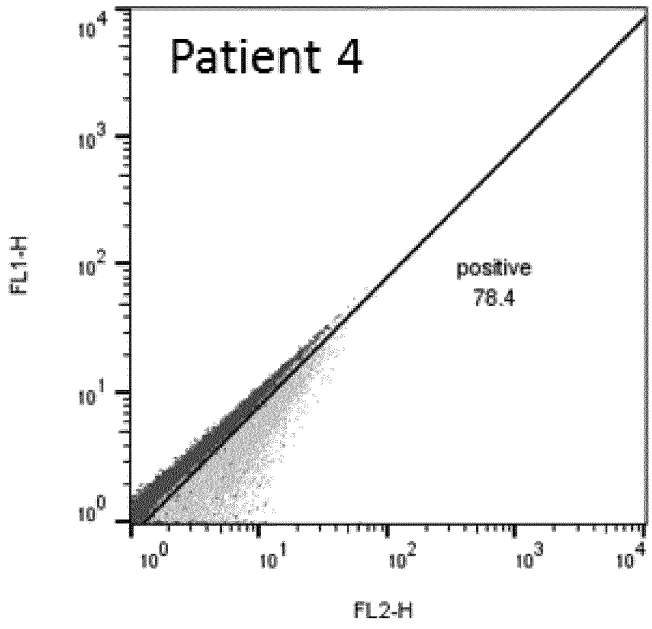


	Sample Name	positive :: Freq. of Paren
	Unstained	0.45
	Isotype C	0.56
	EpCAM BiTE	23.9
	FAP BiTE	51.3

Figure 7A cont.



	Sample Name	positive :: Freq. of Paren
	Unstained	1.06
	Isotype C	1.12
	EpCAM BiTE	4.48
	FAP BiTE	84.7



	Sample Name	positive :: Freq. of Paren
	Unstained	0.78
	Isotype C	0.22
	EpCAM BiTE	0.37
	FAP BiTE	78.4

Figure 7B

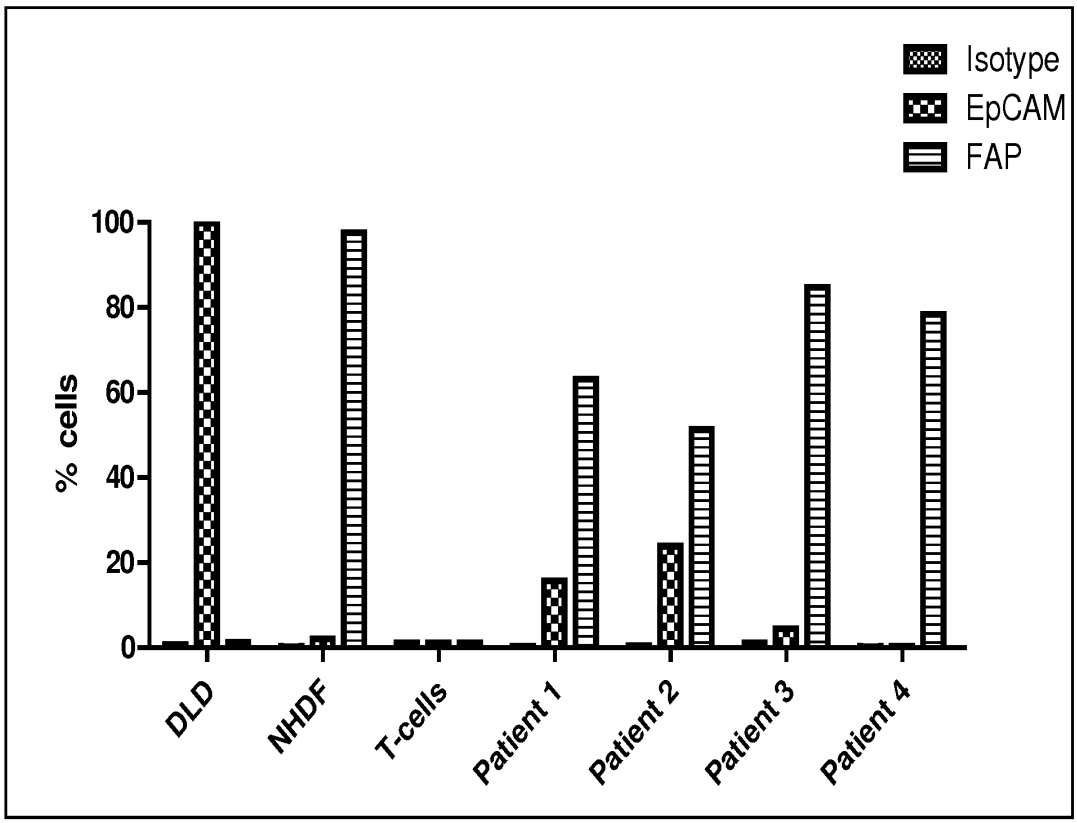


Figure 8A

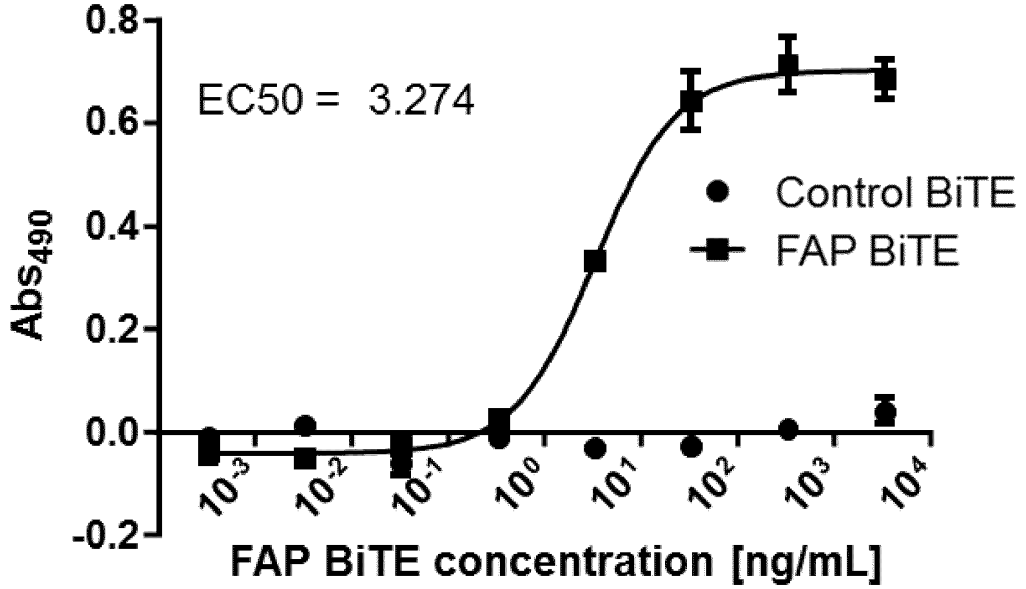


Figure 8B

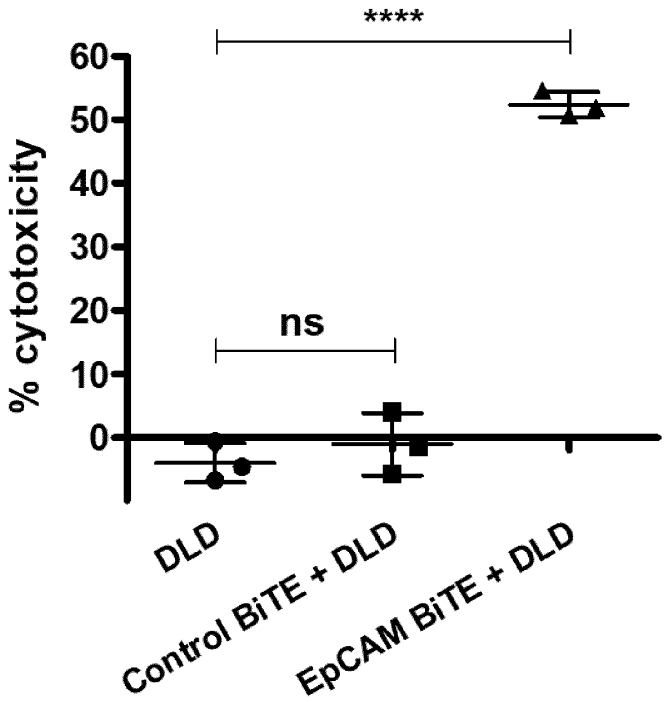
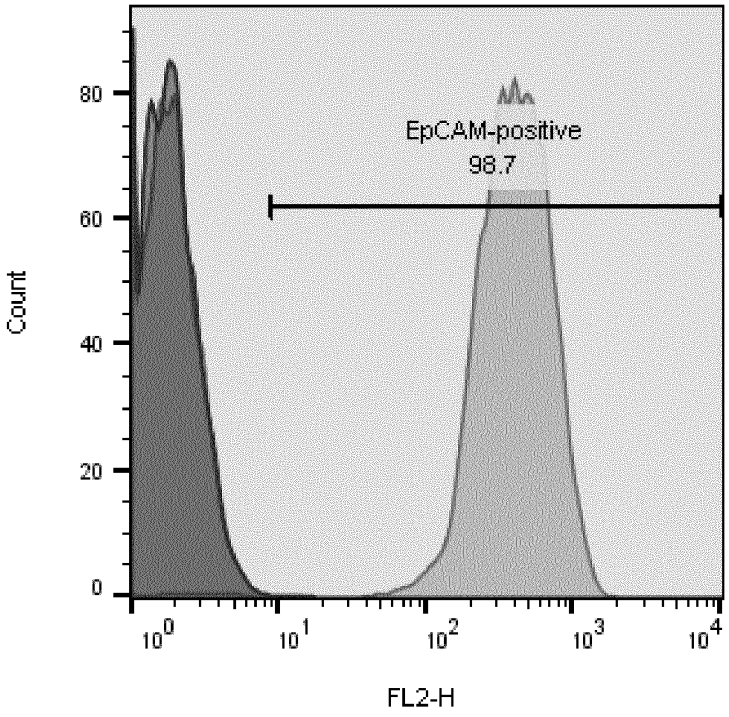
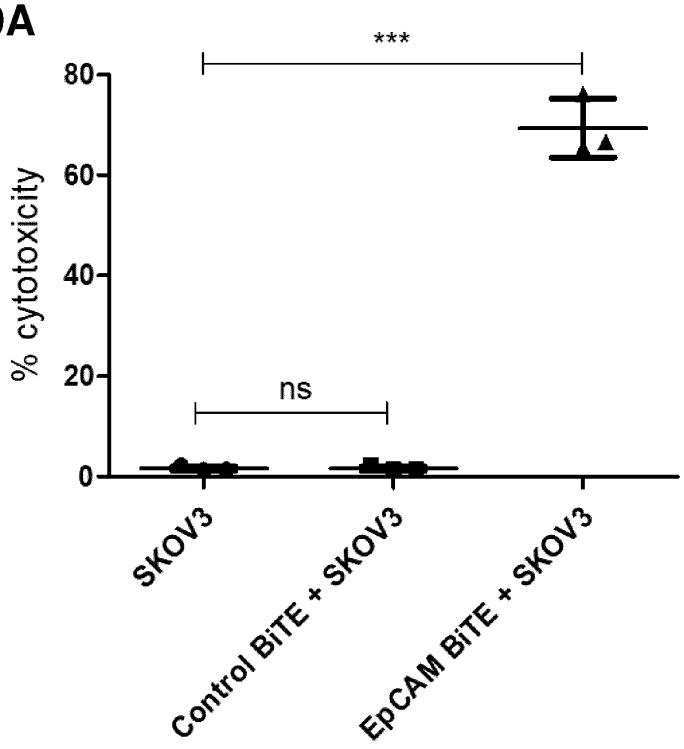


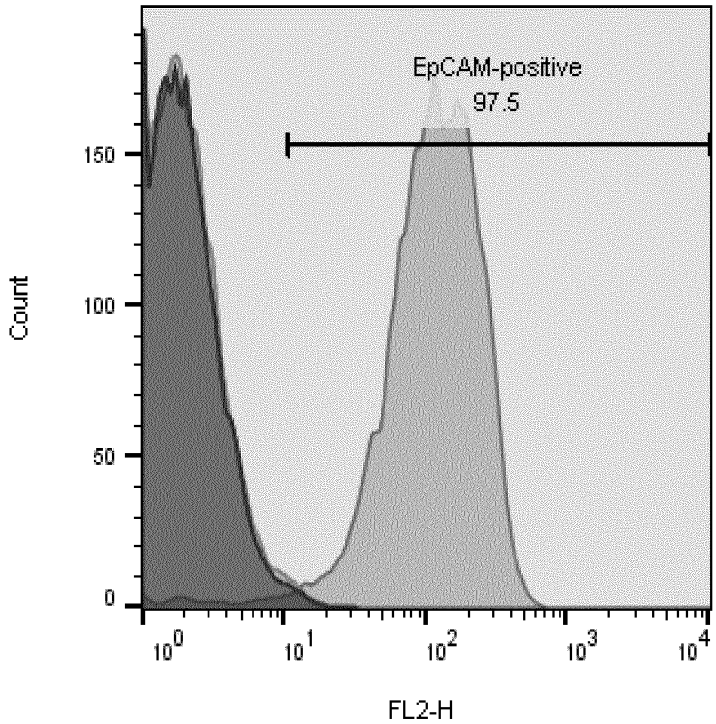
Figure 8C



	Sample Name	EpCAM-positive :: Freq. of Parent	Geometric Mean : FL2-H
	[DLD unstained.021]	0.40	1.77
	[DLD iso.022]	0.36	1.81
	[DLD ep.023]	98.7	361

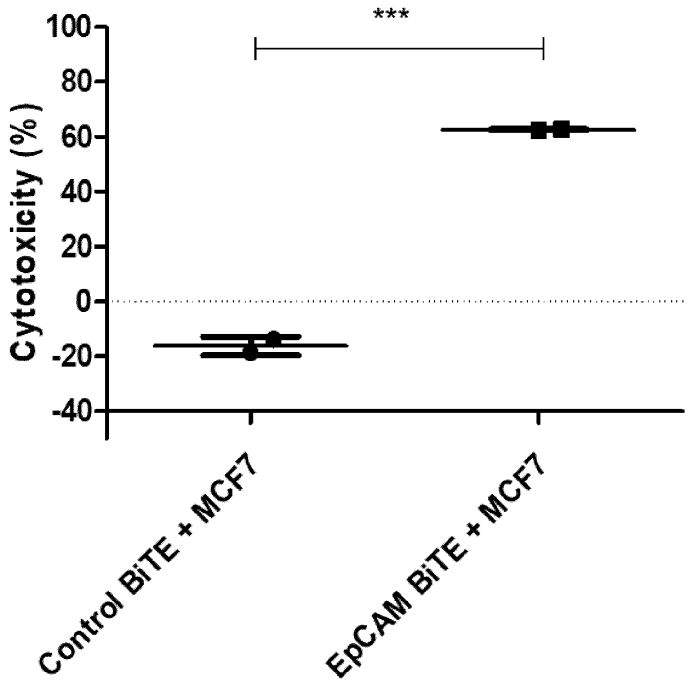
Figure 9A

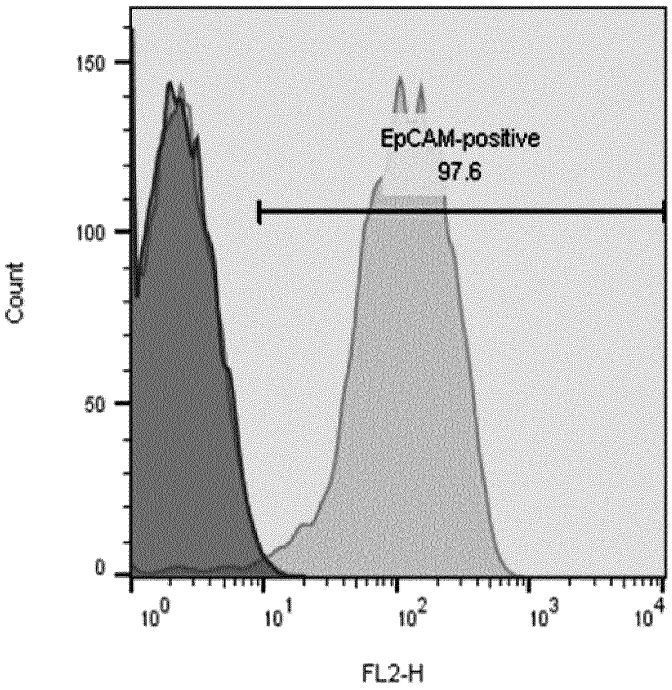




	Sample Name	EpCAM-positive :: Freq. of Parent	Geometric Mean : FL2-H
	[Skov unstained.024]	1.48	1.99
	[Skov iso.025]	1.53	2.00
	[Skov ep.026]	97.5	103

Figure 9B





	Sample Name	EpCAM-positive :: Freq. of Parent	Geometric Mean : FL2-H
	[MCF7 unstained.018]	0.84	2.24
	[MCF7 iso.019]	0.86	2.21
	[MCF7 ep.020]	97.6	101

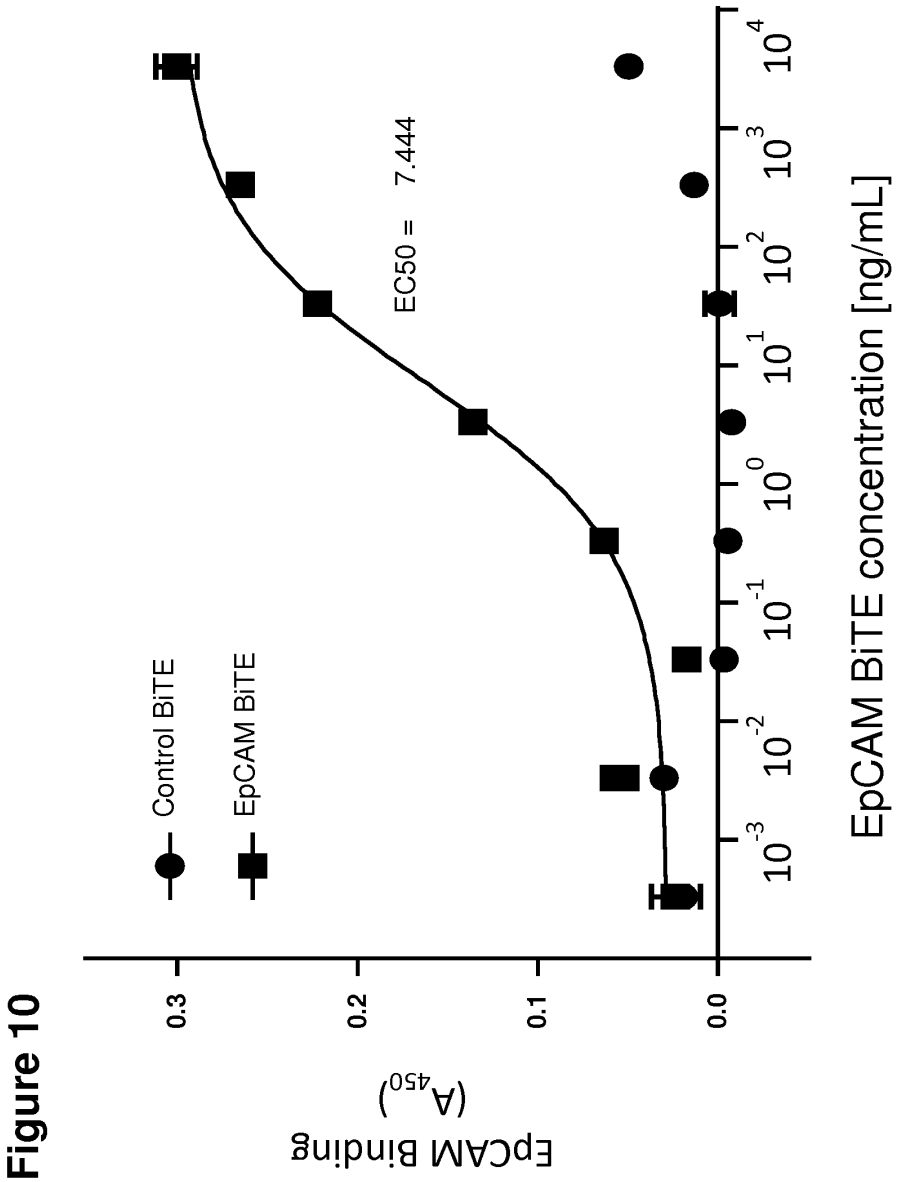

EpCAM Binding
(A_{450})
0.3
0.2
0.1
0.0
Control BiTE
EpCAM BiTE
EC50 = 7.444
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
EpCAM BiTE concentration [ng/mL]

Figure 10

Figure 11A

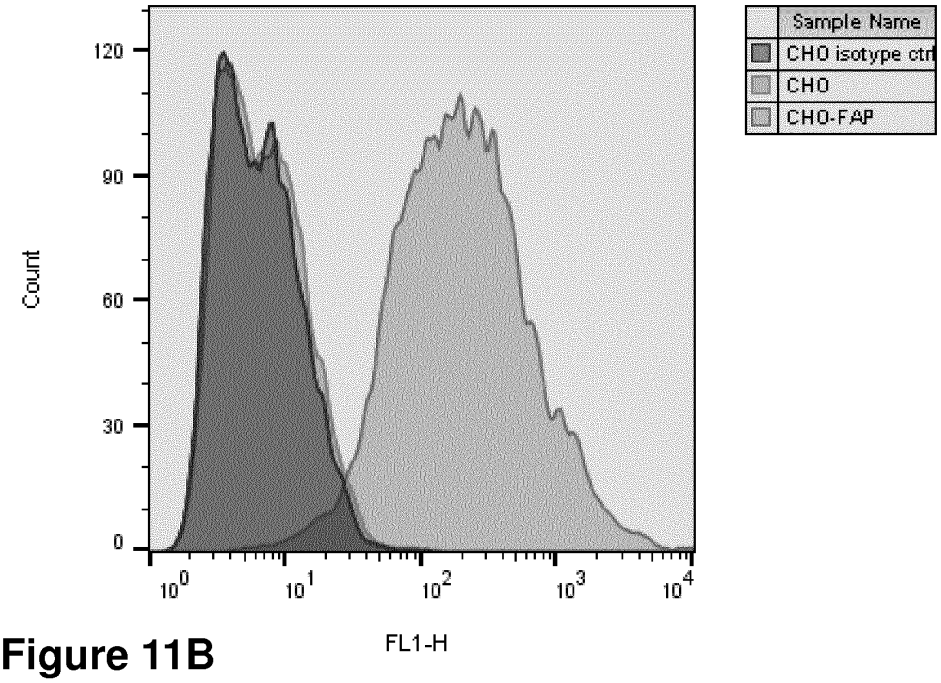


Figure 11B

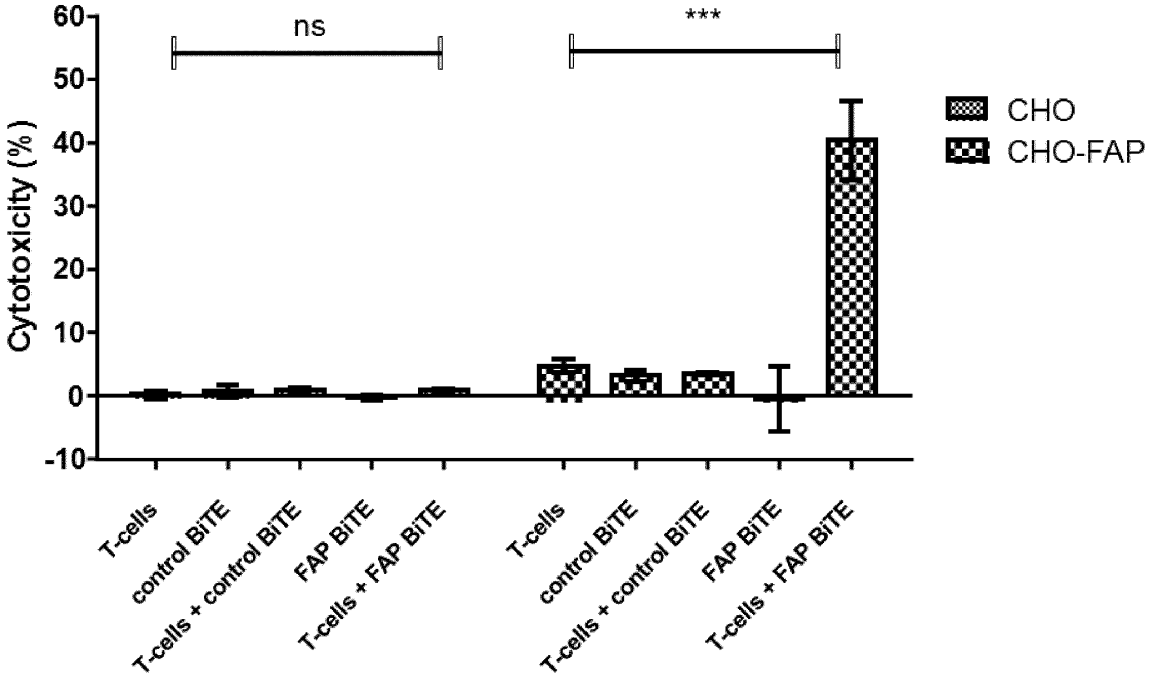


Figure 12

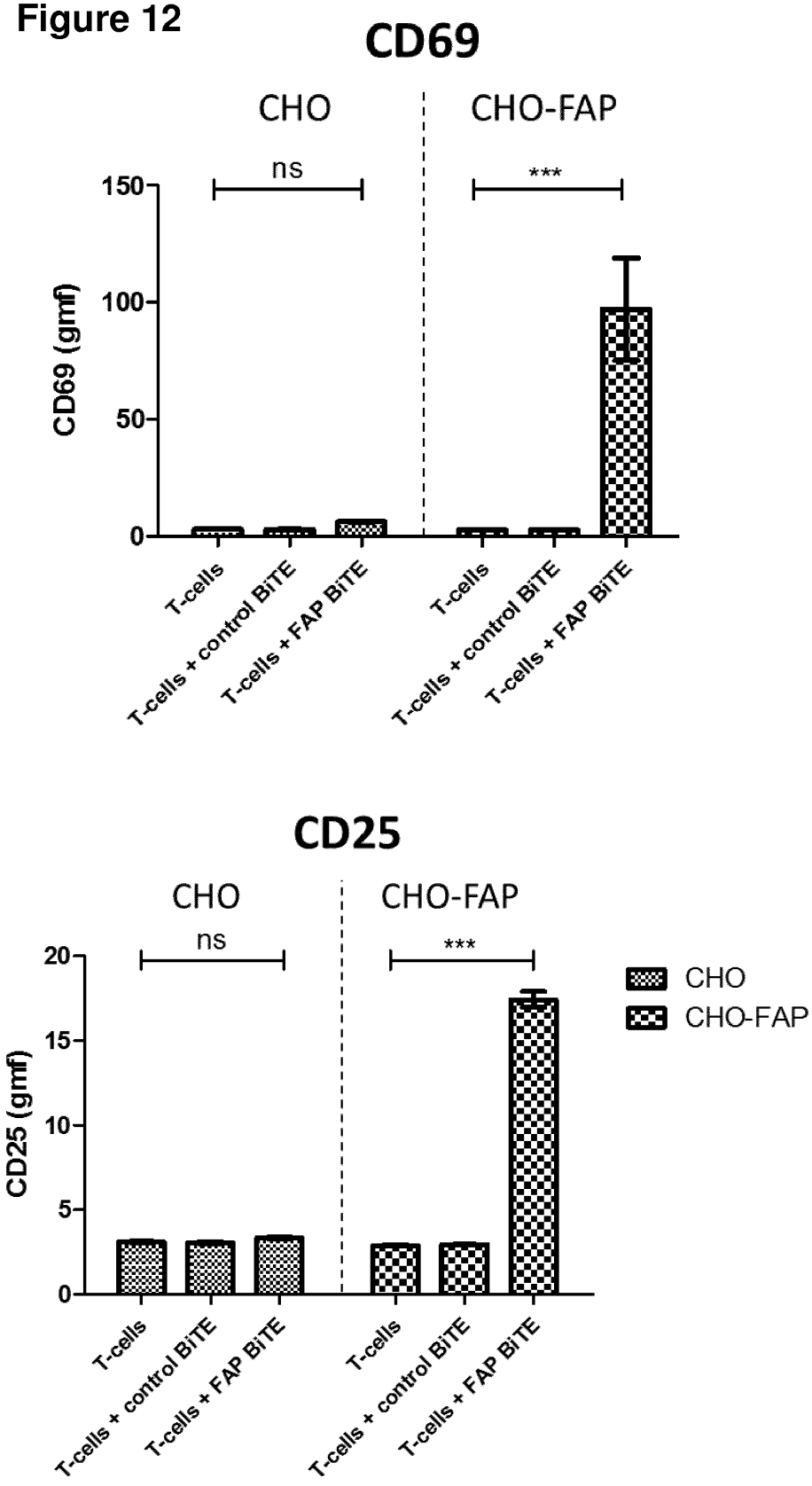
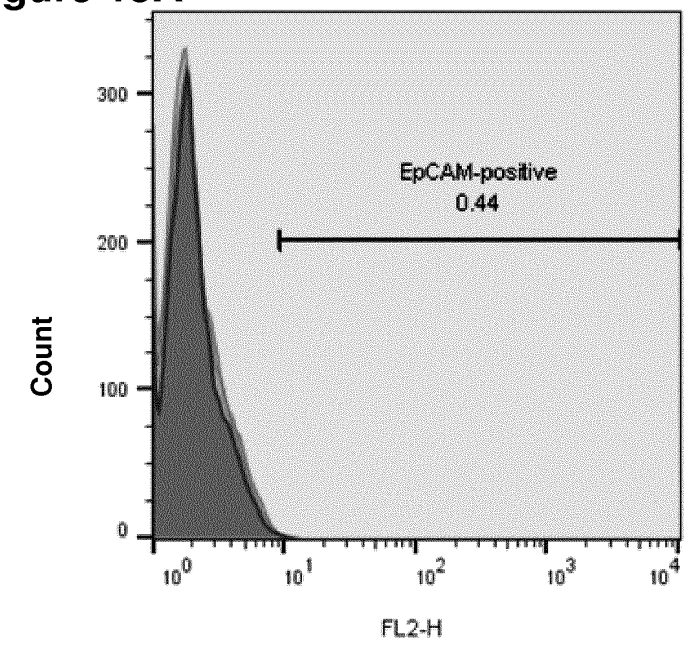
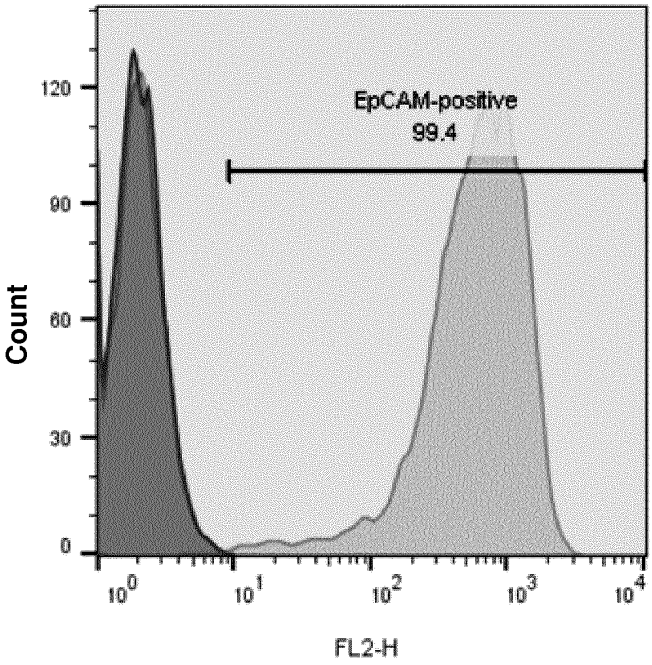

CD69
CHO
CHO-FAP
ns
***
150
100
50
0
CD69 (gmf)
T-cells
T-cells + control BiTE
T-cells + FAP BiTE
T-cells
T-cells + control BiTE
T-cells + FAP BiTE
CD25
CHO
CHO-FAP
ns
***
20
15
10
5
0
CD25 (gmf)
T-cells
T-cells + control BiTE
T-cells + FAP BiTE
T-cells
T-cells + control BiTE
T-cells + FAP BiTE
CHO
CHO-FAP

Figure 13A



	Sample Name	EpCAM-positive :: Freq. of Parent	Geometric Mean : FL2-H
■	[CHO unstained.036]	0.38	2.06
■	[CHO iso.037]	0.42	2.08
■	[CHO ep.038]	0.44	2.02



	Sample Name	EpCAM-positive :: Freq. of Parent	Geometric Mean : FL2-H
■	[CHO-epcam unstained.039]	0.093	2.03
■	[CHO-epcam iso.040]	0.15	2.05
■	[CHO-epcam ep.041]	99.4	513

Figure 13B

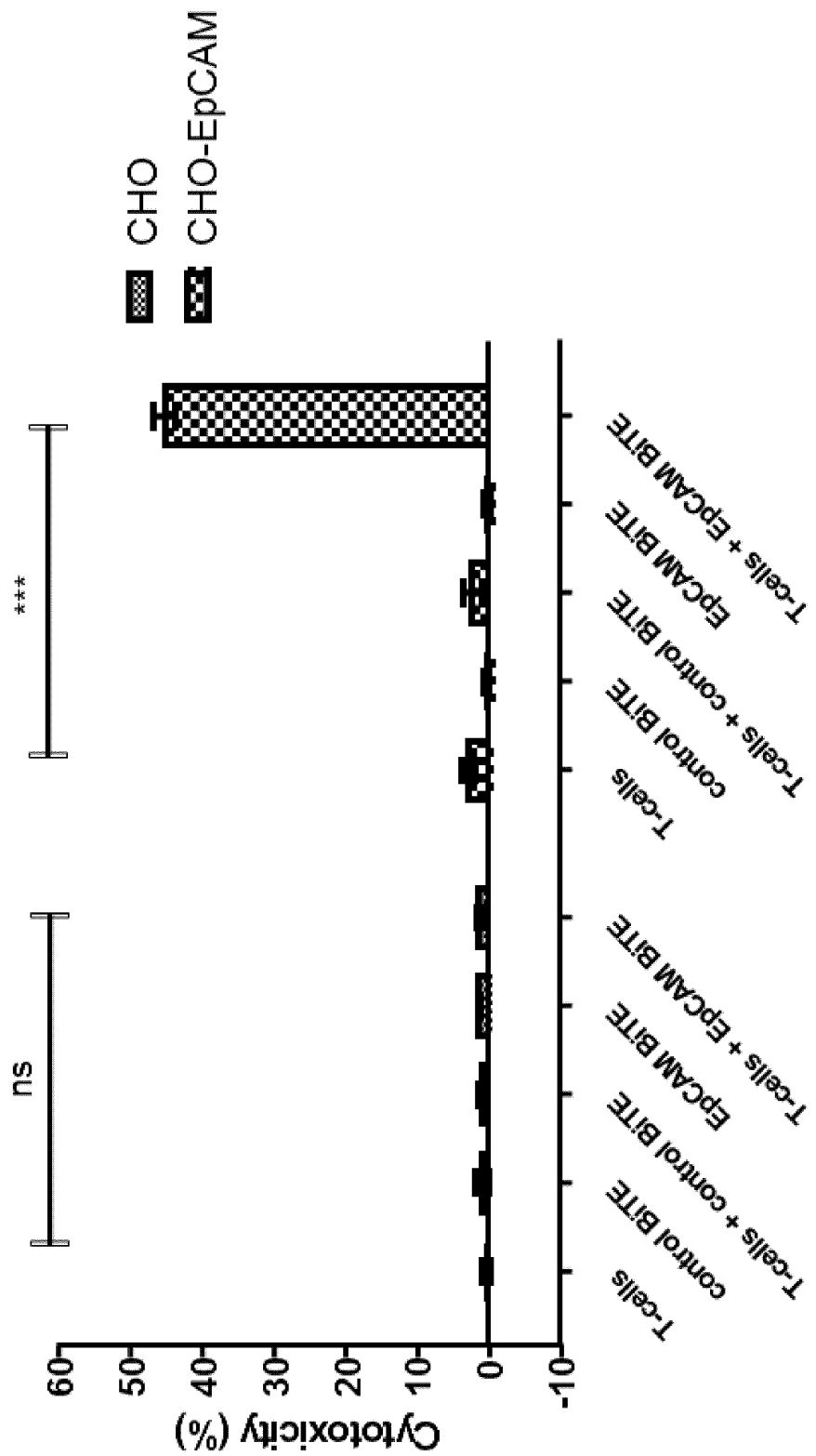

CHO
CHO-EpCAM
Cytotoxicity (%)
60
50
40
30
20
10
0
-10
ns
***
T-cells
control BiTE
T-cells + control BiTE
EpCAM BiTE
T-cells + EpCAM BiTE
T-cells
control BiTE
T-cells + control BiTE
EpCAM BiTE
T-cells + EpCAM BiTE

Figure 14

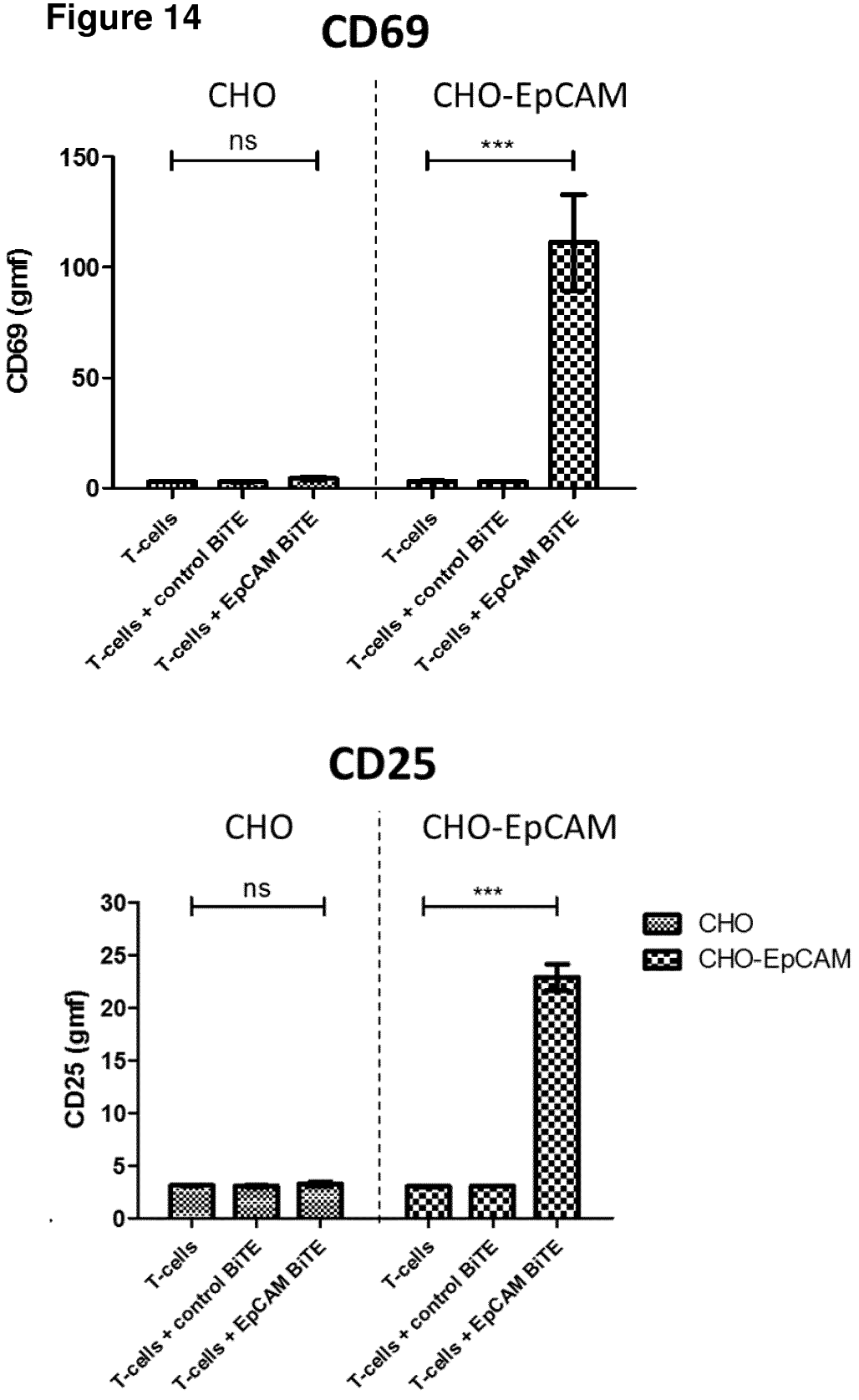

CD69
CHO
CHO-EpCAM
ns
***
CD69 (gmf)
150
100
50
0
T-cells
T-cells + control BiTE
T-cells + EpCAM BiTE
T-cells
T-cells + control BiTE
T-cells + EpCAM BiTE
CD25
CHO
CHO-EpCAM
ns
***
CD25 (gmf)
30
25
20
15
10
5
0
T-cells
T-cells + control BiTE
T-cells + EpCAM BiTE
T-cells
T-cells + control BiTE
T-cells + EpCAM BiTE
CHO
CHO-EpCAM

Figure 15

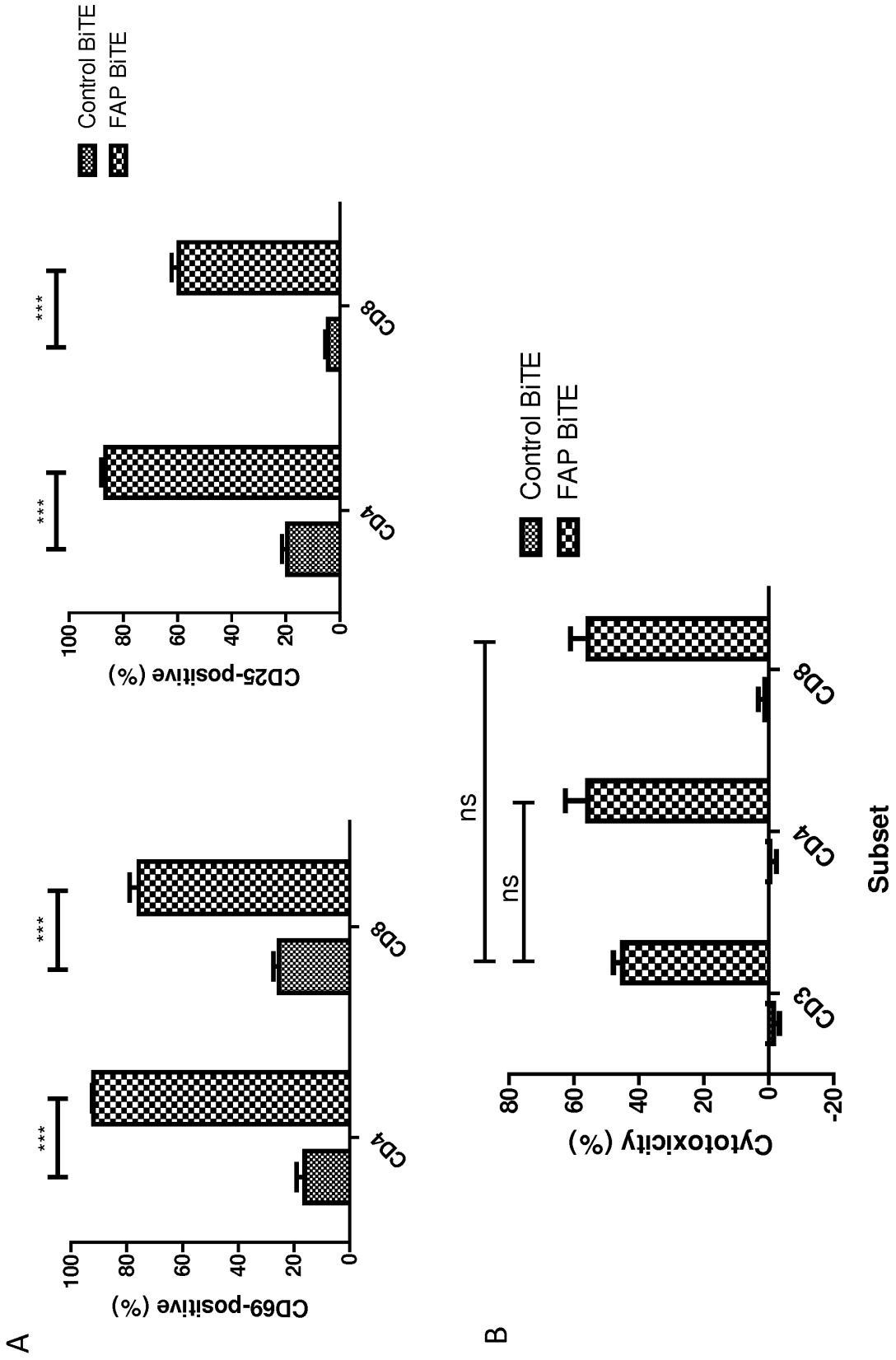

A
CD69-positive (%)
100
80
60
40
20
0
***
***
CD4
CD8
Control BiTE
FAP BiTE
CD25-positive (%)
100
80
60
40
20
0
***
***
CD4
CD8
B
Cytotoxicity (%)
80
60
40
20
0
-20
ns
ns
CD3
CD4
CD8
Subset
Control BiTE
FAP BiTE

Figure 16

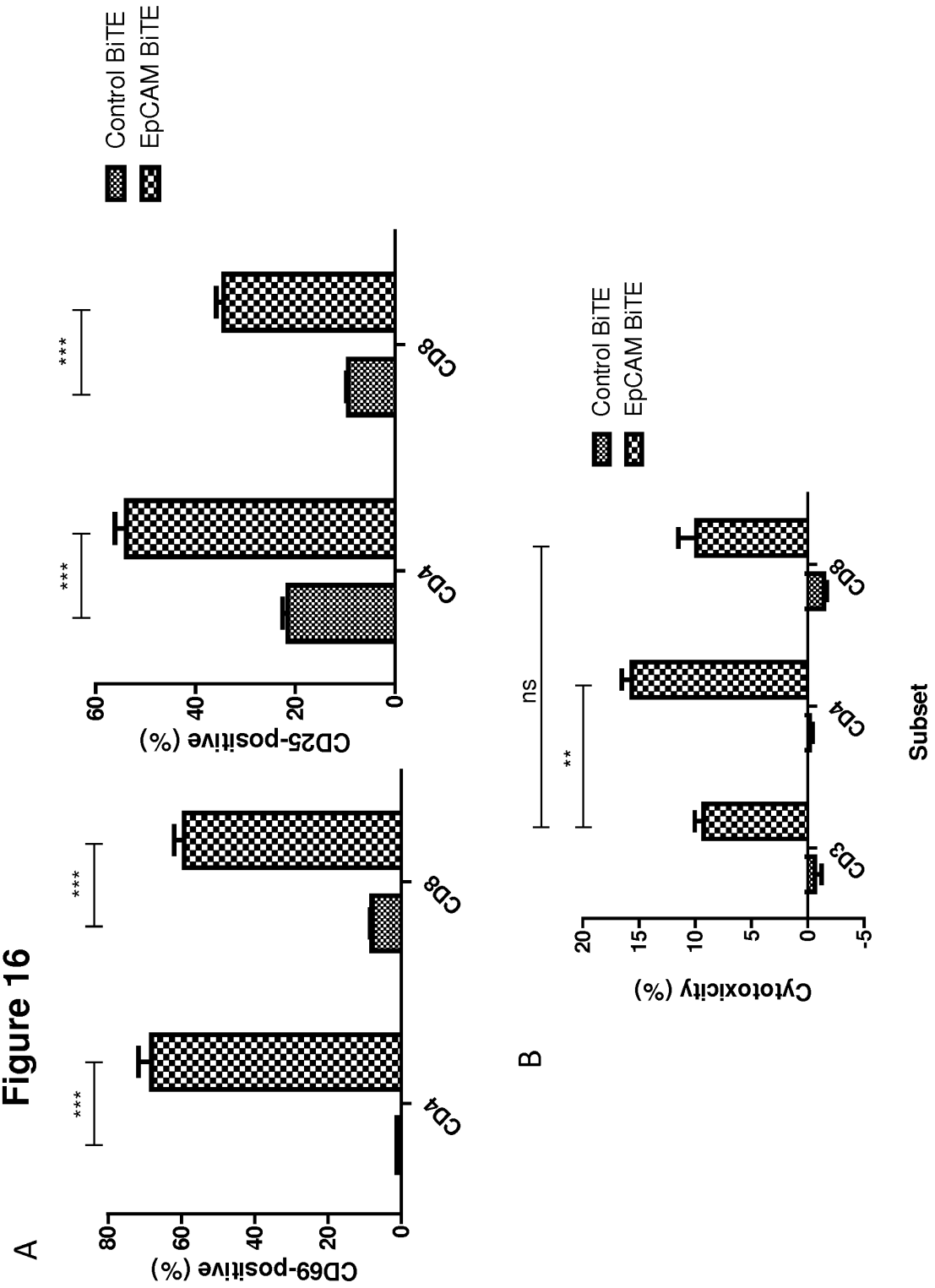

A
Control BiTE
EpCAM BiTE
CD69-positive (%)
0
20
40
60
80
***
***
CD4
CD8
CD25-positive (%)
0
20
40
60
***
***
CD4
CD8
B
Control BiTE
EpCAM BiTE
Cytotoxicity (%)
-5
0
5
10
15
20
ns
**
CD3
CD4
CD8
Subset

Figure 17

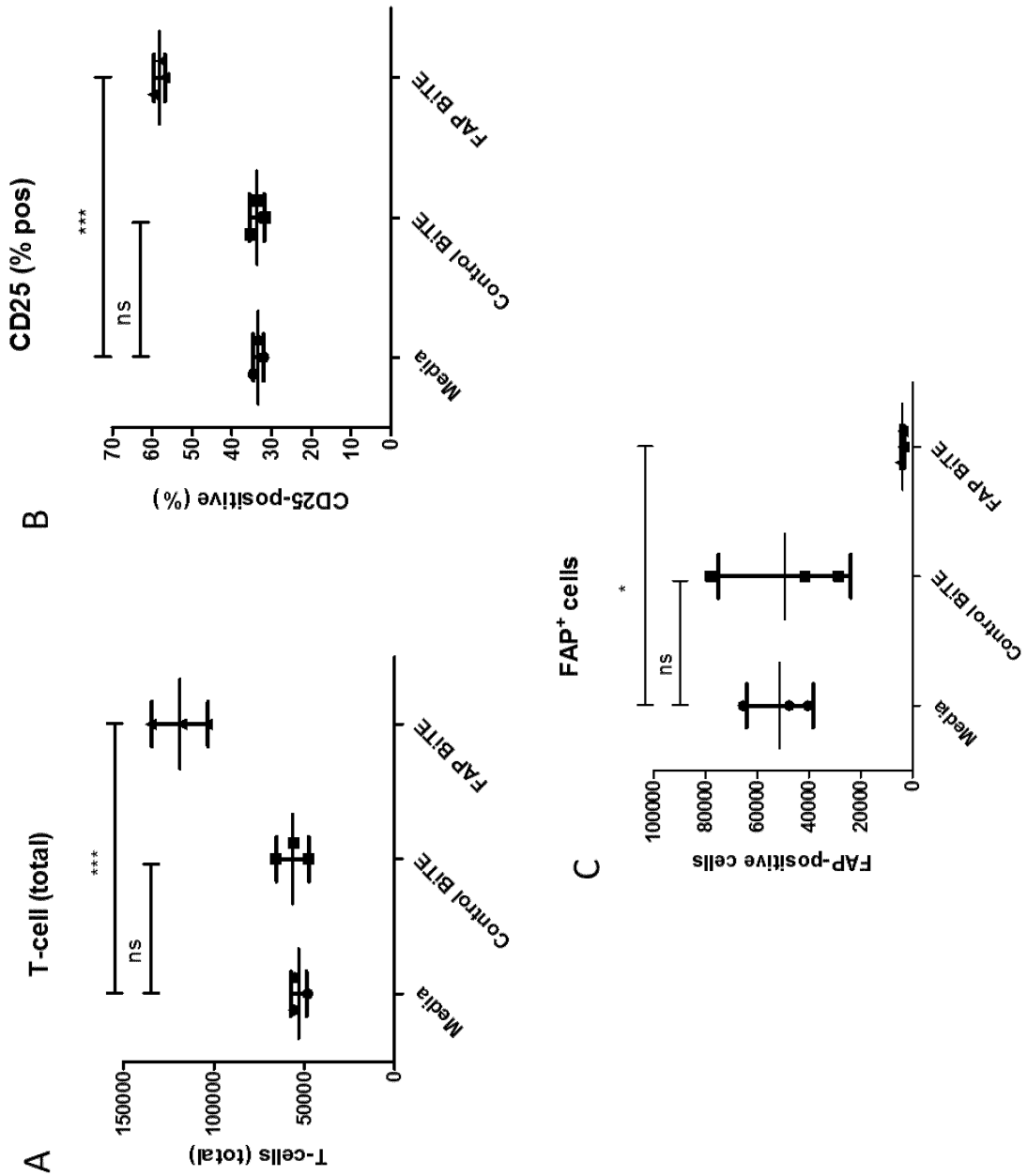

A
T-cell (total)
T-cells (total)
150000
100000
50000
0
***
ns
Media
Control BiTE
FAP BiTE
B
CD25 (% pos)
CD25-positive (%)
70
60
50
40
30
20
10
0
***
ns
Media
Control BiTE
FAP BiTE
C
FAP+ cells
FAP-positive cells
100000
80000
60000
40000
20000
0
*
ns
Media
Control BiTE
FAP BiTE

Figure 18

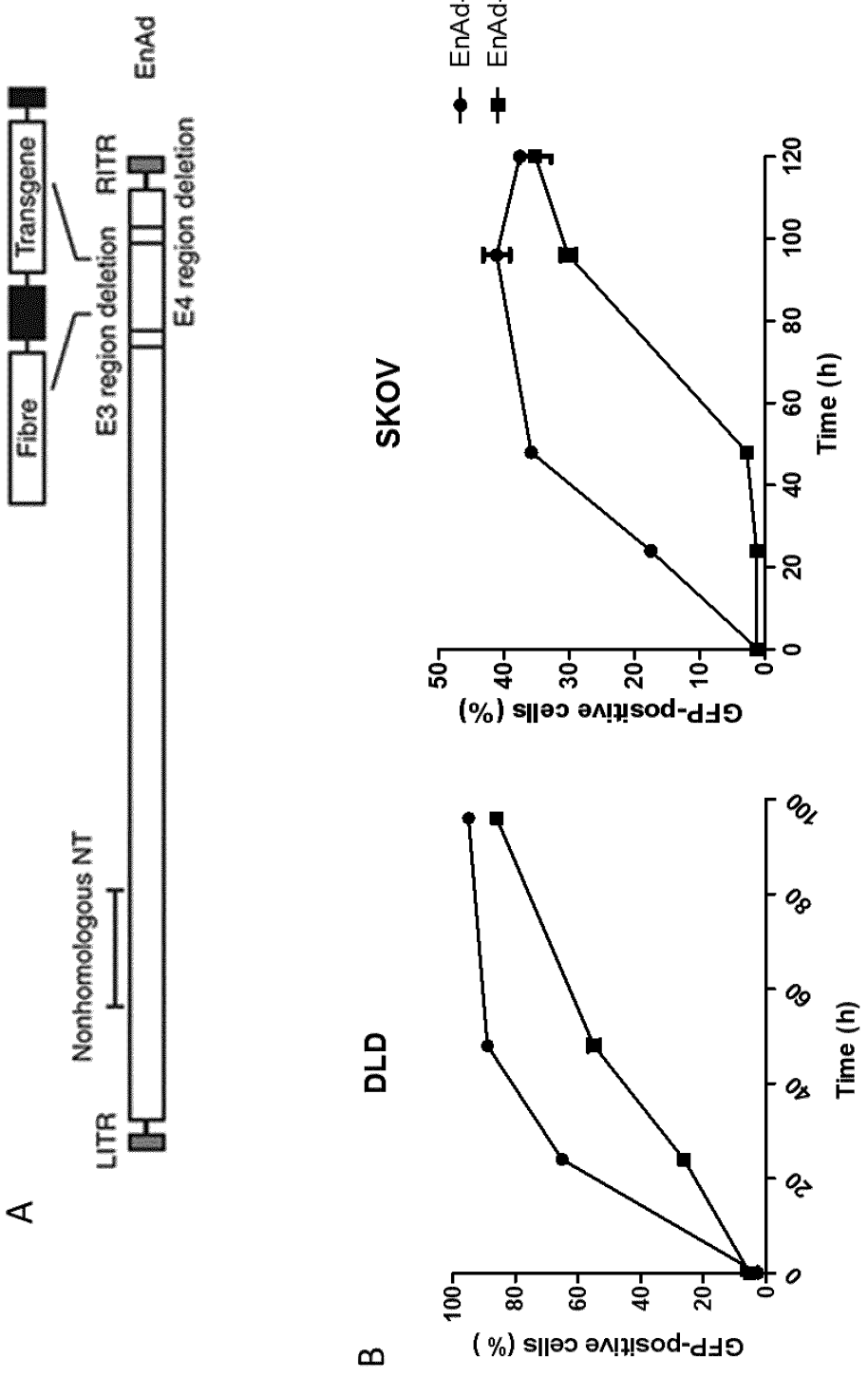

A
pA
Transgene
P
Fibre
E3 region deletion
RITR
EnAd
E4 region deletion
Nonhomologous NT
LITR
B
DLD
GFP-positive cells (%)
Time (h)
SKOV
EnAd-CMV-GFP
EnAd-SA-GFP
GFP-positive cells (%)
Time (h)

Figure 19

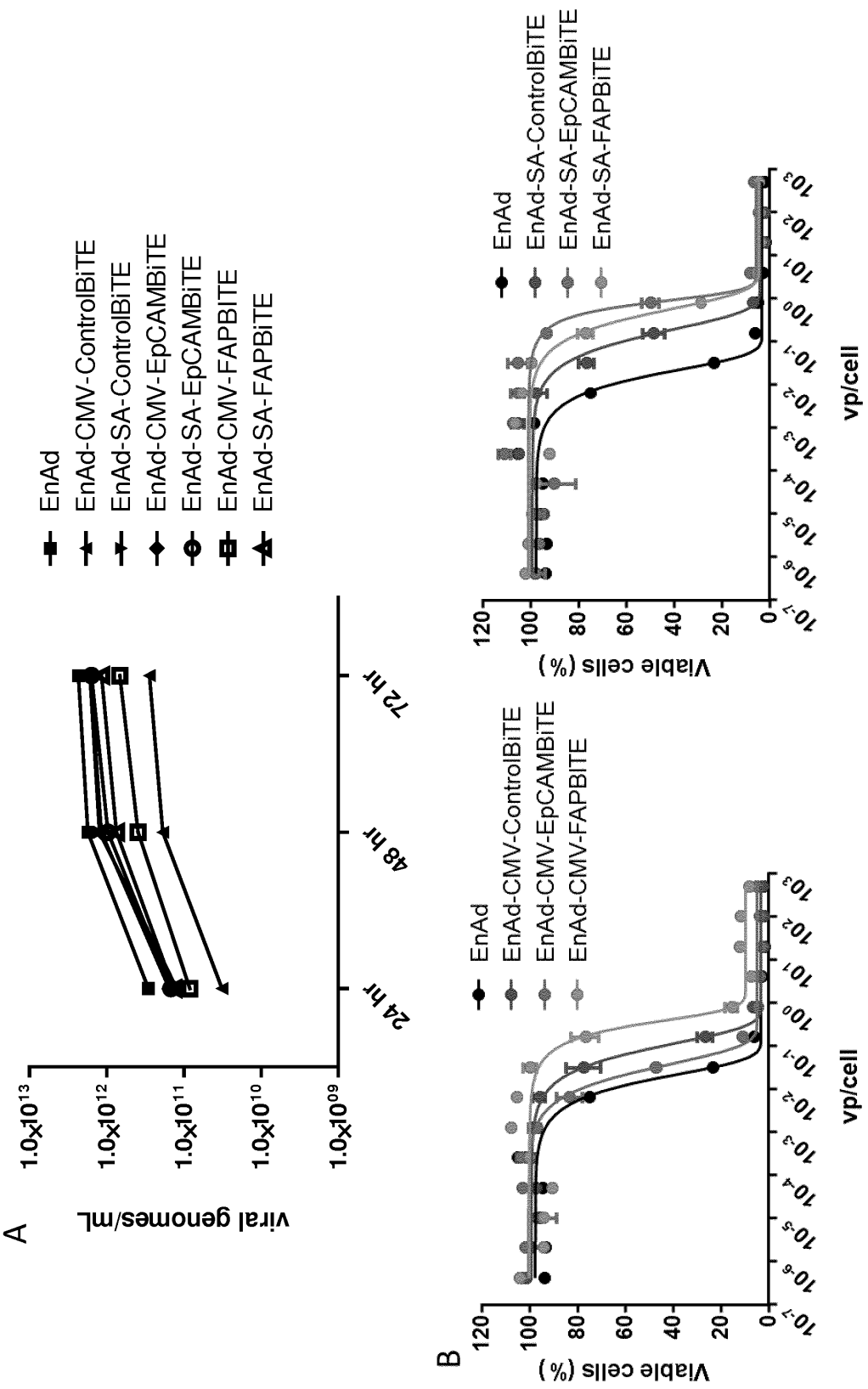

A
viral genomes/mL
1.0x10^13
1.0x10^12
1.0x10^11
1.0x10^10
1.0x10^09
24 hr
48 hr
72 hr
EnAd
EnAd-CMV-ControlBiTE
EnAd-SA-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-EpCAMBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-FAPBiTE
B
Viable cells (%)
vp/cell
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-CMV-FAPBiTE
Viable cells (%)
vp/cell
EnAd
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE
EnAd-SA-FAPBiTE

Figure 20A

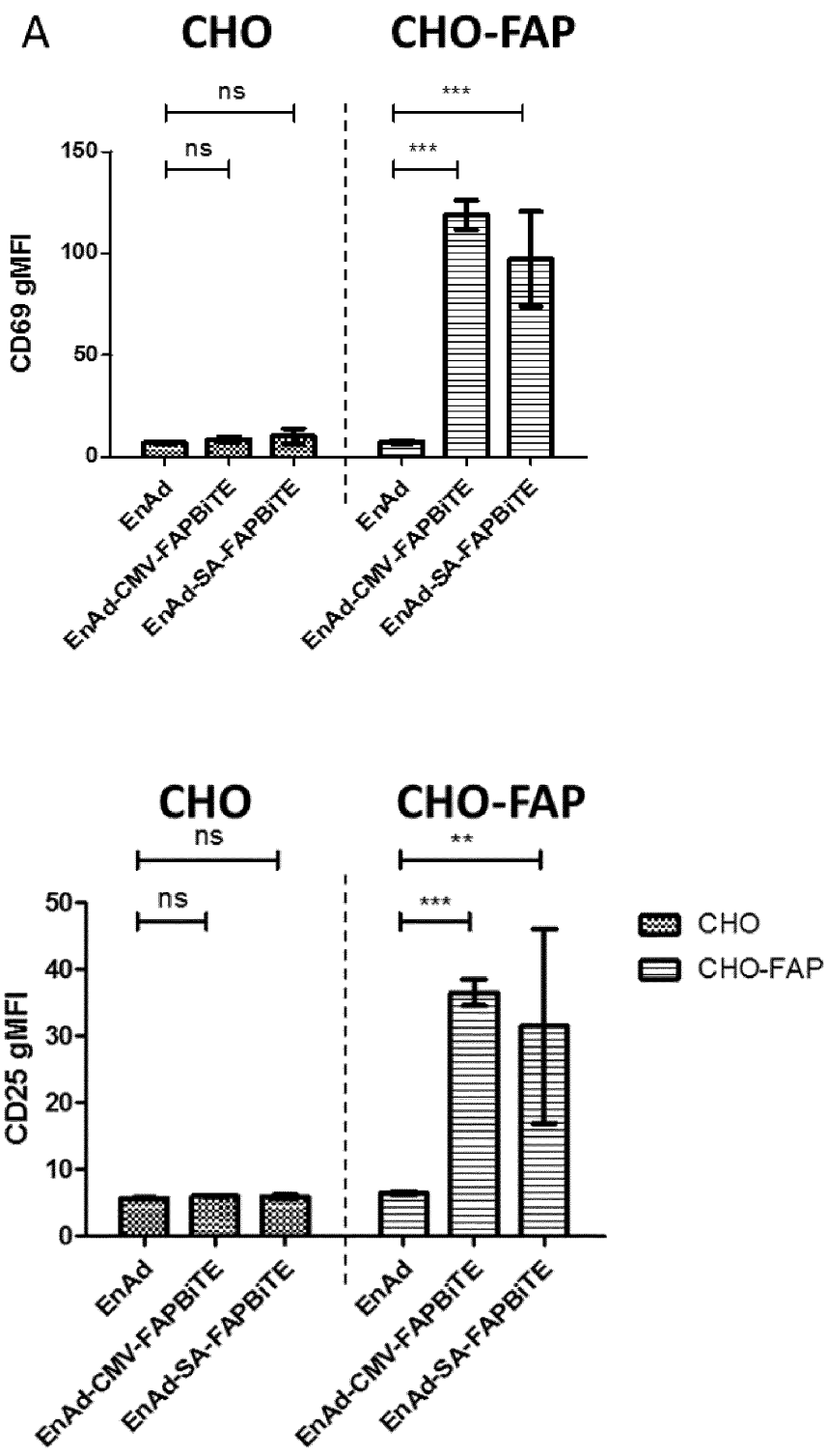

A
CHO
CHO-FAP
ns
ns
***
***
CD69 gMFI
150
100
50
0
EnAd
EnAd-CMV-FAPBiTE
EnAd-SA-FAPBiTE
EnAd
EnAd-CMV-FAPBiTE
EnAd-SA-FAPBiTE
CHO
CHO-FAP
ns
ns
**
***
CD25 gMFI
50
40
30
20
10
0
EnAd
EnAd-CMV-FAPBiTE
EnAd-SA-FAPBiTE
EnAd
EnAd-CMV-FAPBiTE
EnAd-SA-FAPBiTE
CHO
CHO-FAP

Figure 20B

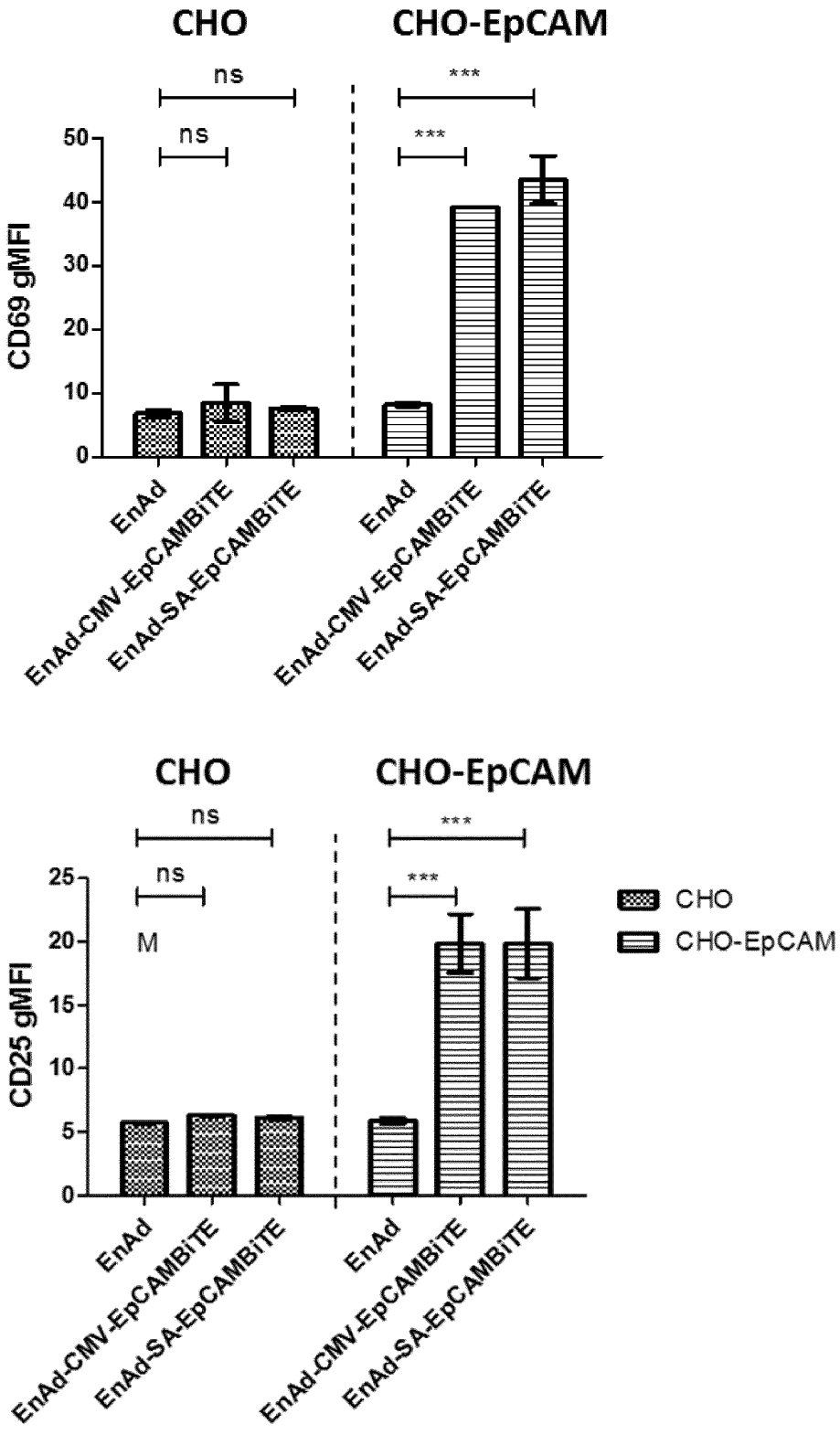
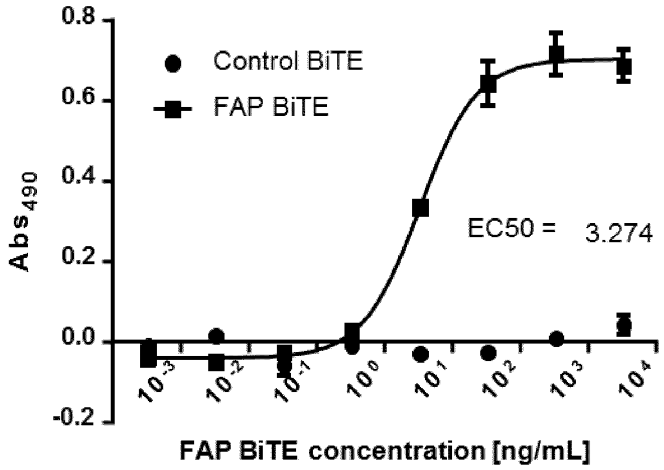
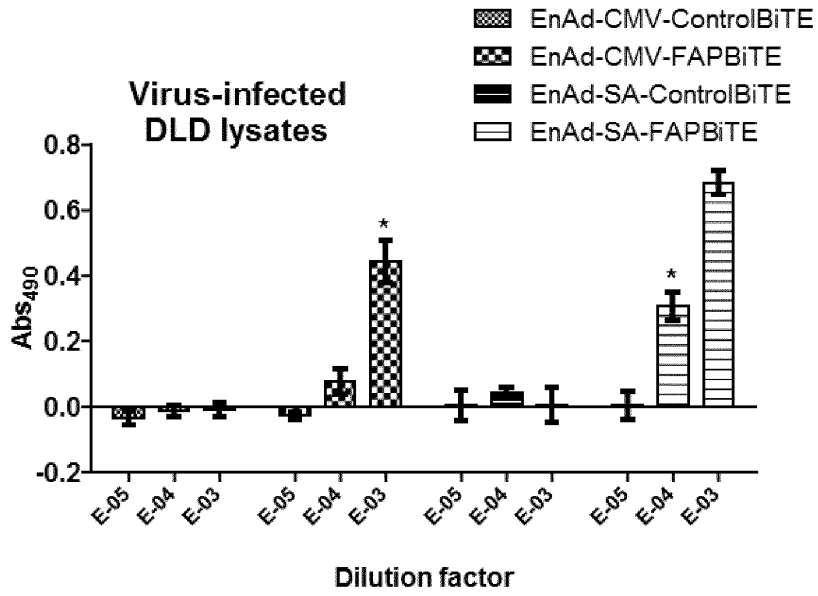

CHO
CHO-EpCAM
ns
ns
***
***
CD69 gMFI
50
40
30
20
10
0
EnAd
EnAd-CMV-EpCAMBiTE
EnAd-SA-EpCAMBiTE
CHO
CHO-EpCAM
ns
ns
***
***
M
CD25 gMFI
25
20
15
10
5
0
CHO
CHO-EpCAM

Figure 21


Control BiTE
FAP BiTE
EC50 = 3.274
Abs490
FAP BiTE concentration [ng/mL]


EnAd-CMV-ControlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ControlBiTE
EnAd-SA-FAPBiTE
Virus-infected DLD lysates
Abs490
Dilution factor

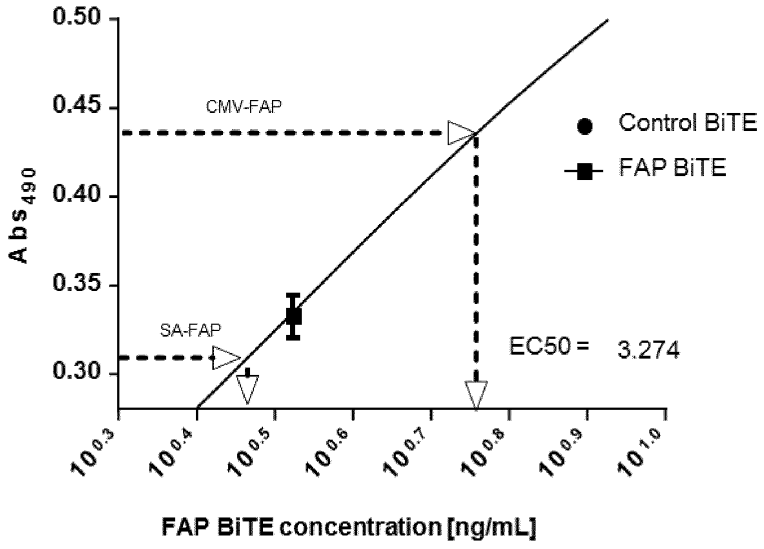
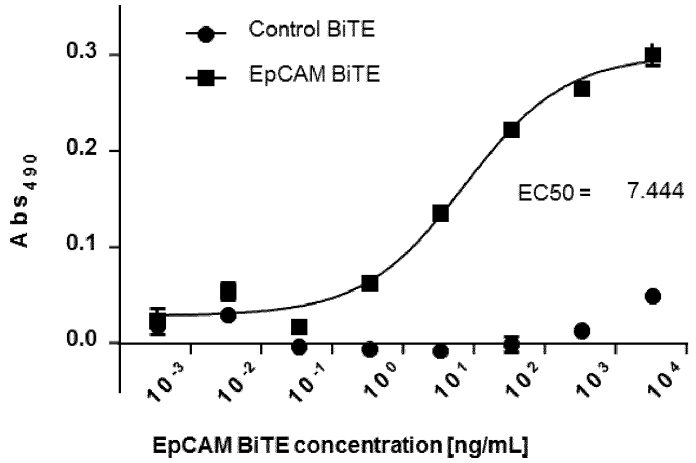
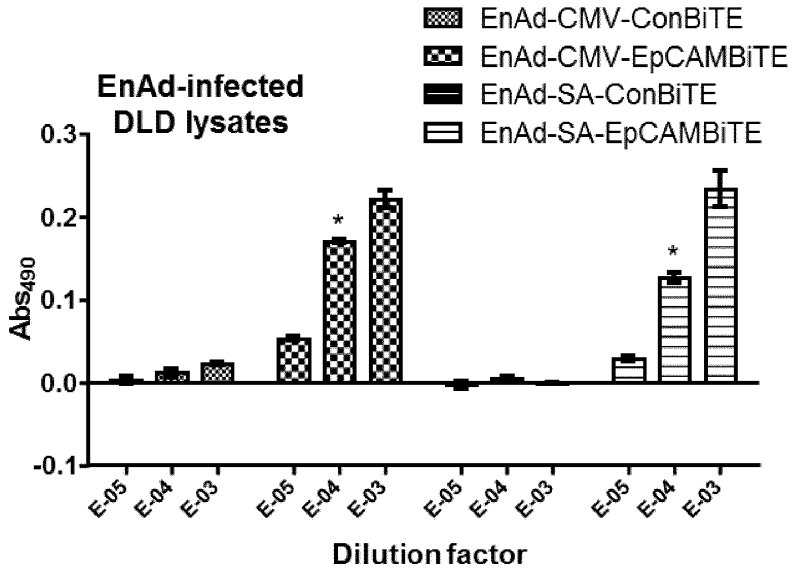
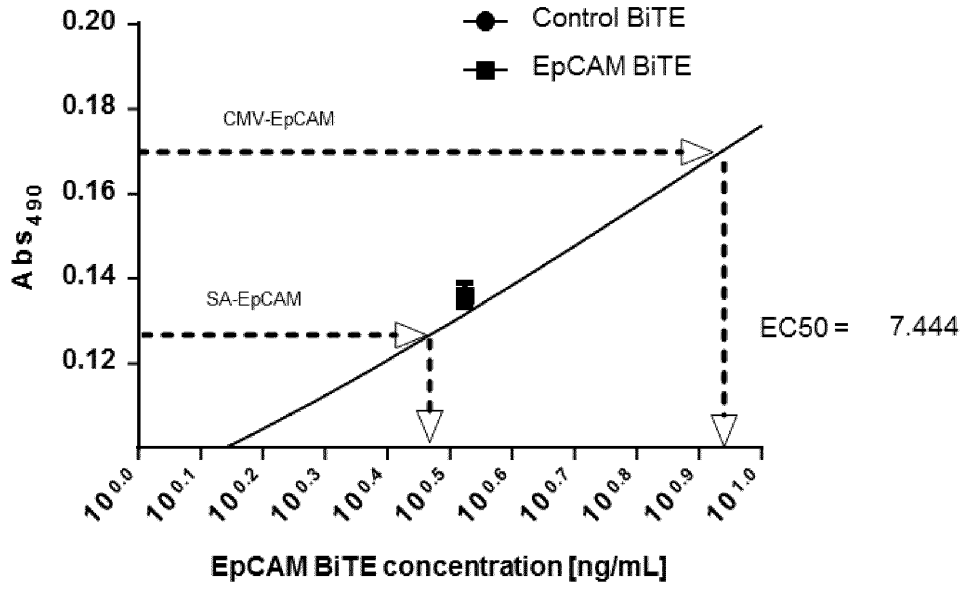

CMV-FAP
SA-FAP
Control BiTE
FAP BiTE
EC50 = 3.274
Abs490
FAP BiTE concentration [ng/mL]

Figure 22


Control BiTE
EpCAM BiTE
EC50 = 7.444
Abs490
0.3
0.2
0.1
0.0
10-3
10-2
10-1
100
101
102
103
104
EpCAM BiTE concentration [ng/mL]


EnAd-CMV-ConBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ConBiTE
EnAd-SA-EpCAMBiTE
EnAd-infected
DLD lysates
Abs490
0.3
0.2
0.1
0.0
-0.1
*
*
E-05
E-04
E-03
Dilution factor


Control BiTE
EpCAM BiTE
CMV-EpCAM
SA-EpCAM
EC50 = 7.444
Abs490
0.20
0.18
0.16
0.14
0.12
100.0
100.1
100.2
100.3
100.4
100.5
100.6
100.7
100.8
100.9
101.0
EpCAM BiTE concentration [ng/mL]

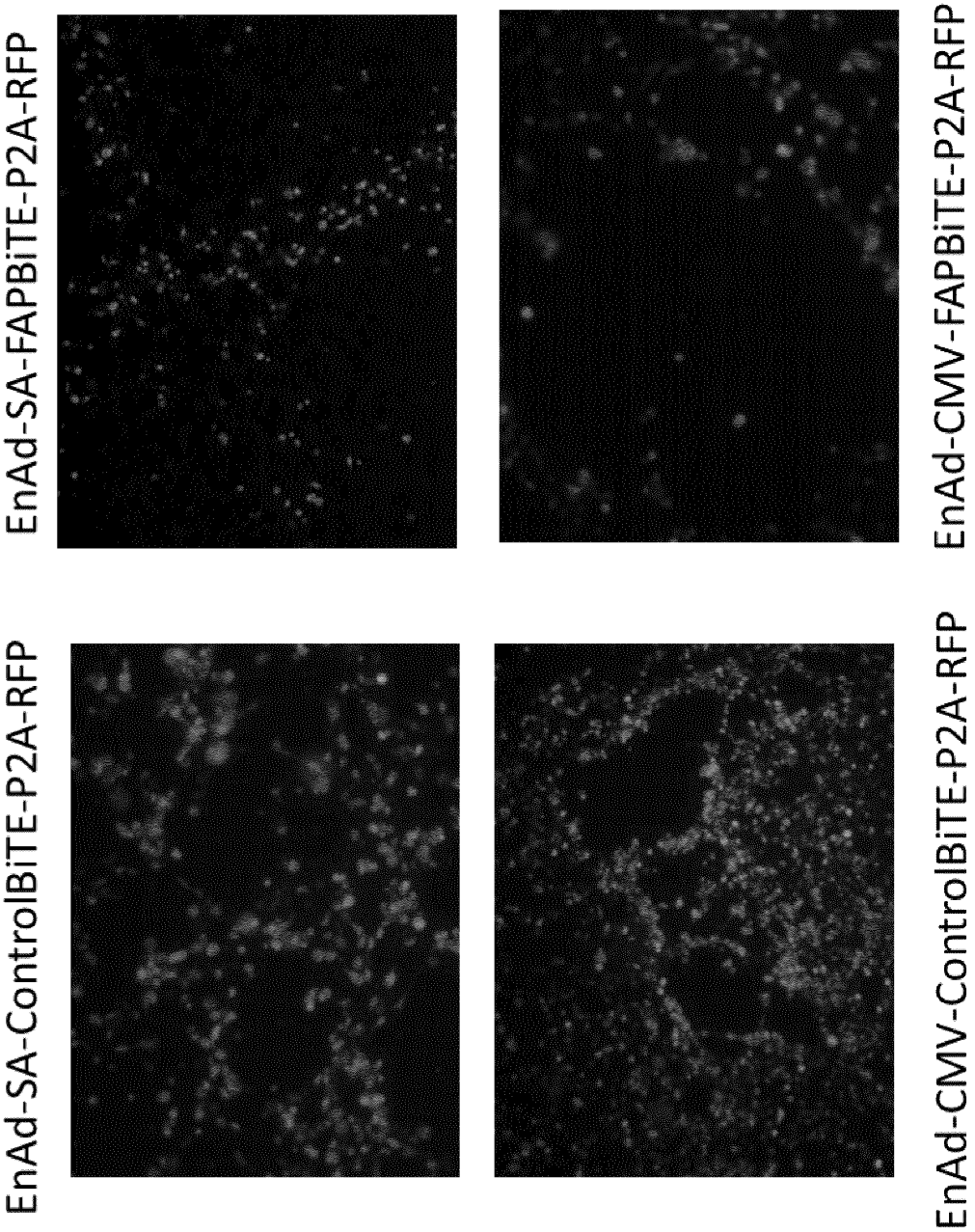

EnAd-SA-FAPBiTE-P2A-RFP
EnAd-CMV-FAPBiTE-P2A-RFP
EnAd-SA-ControlBiTE-P2A-RFP
EnAd-CMV-ControlBiTE-P2A-RFP

Figure 23

Figure 24

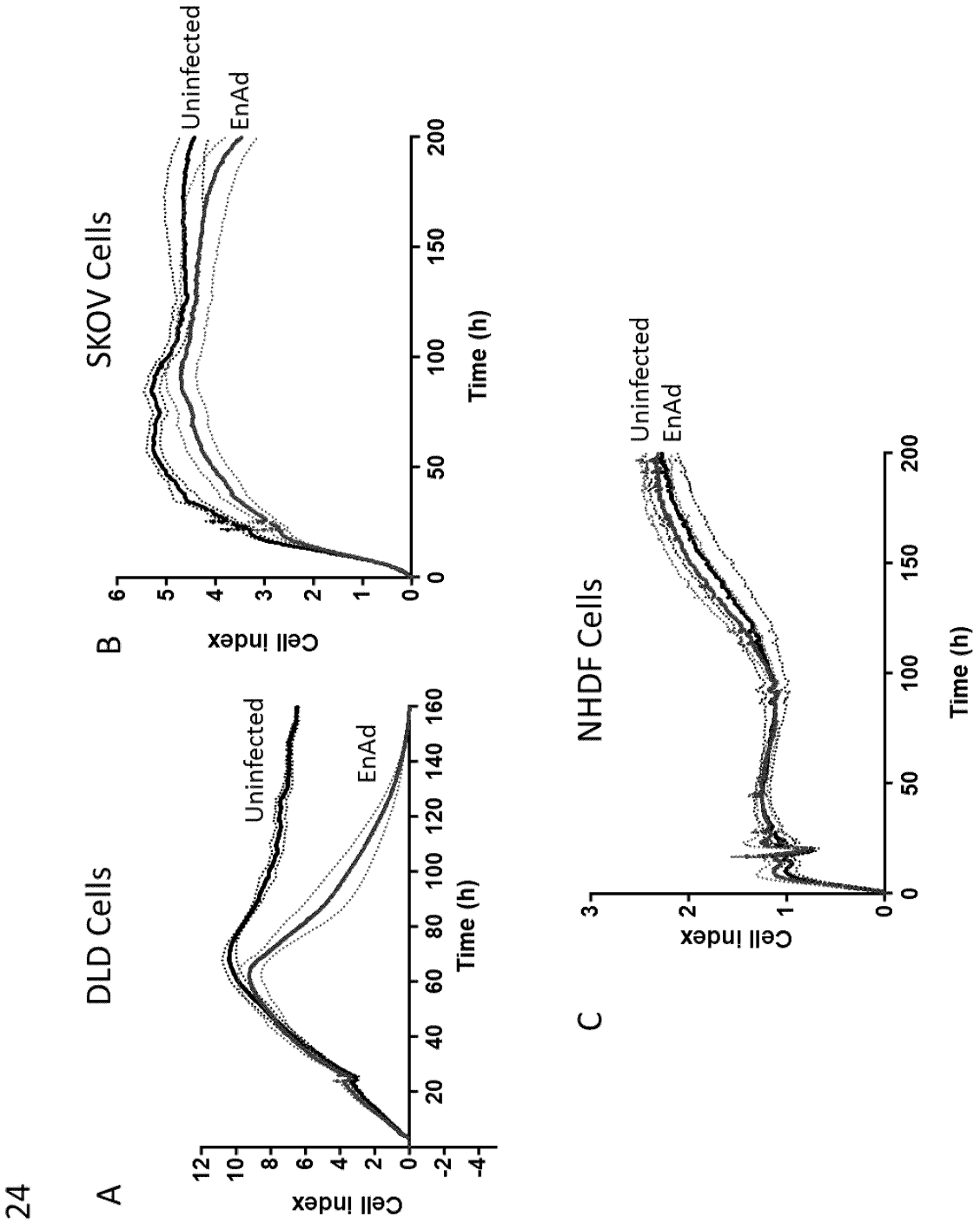

A
DLD Cells
Uninfected
EnAd
Cell Index
Time (h)
B
SKOV Cells
Uninfected
EnAd
Cell Index
Time (h)
C
NHDF Cells
Uninfected
EnAd
Cell Index
Time (h)

Figure 25A

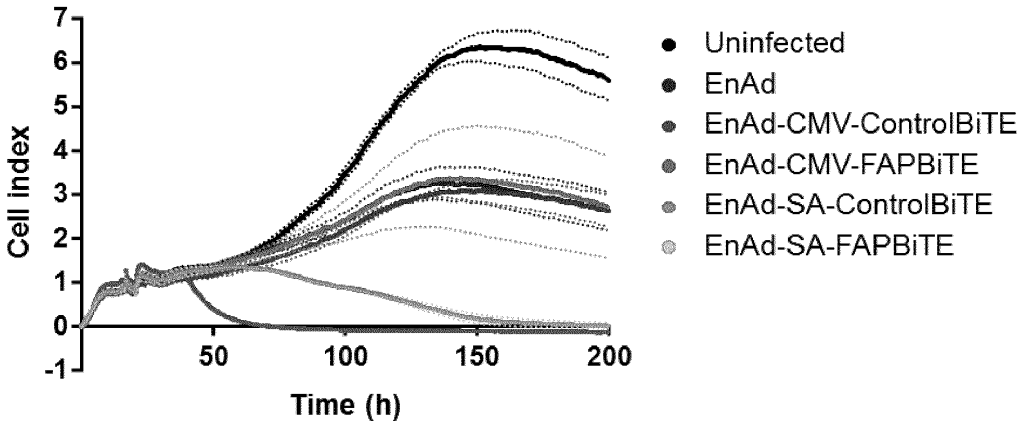


Figure 25B

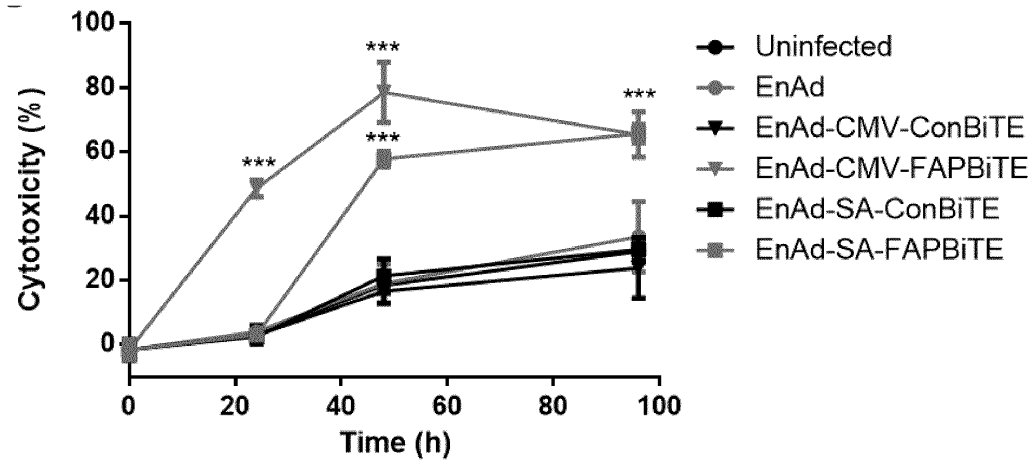


Figure 26

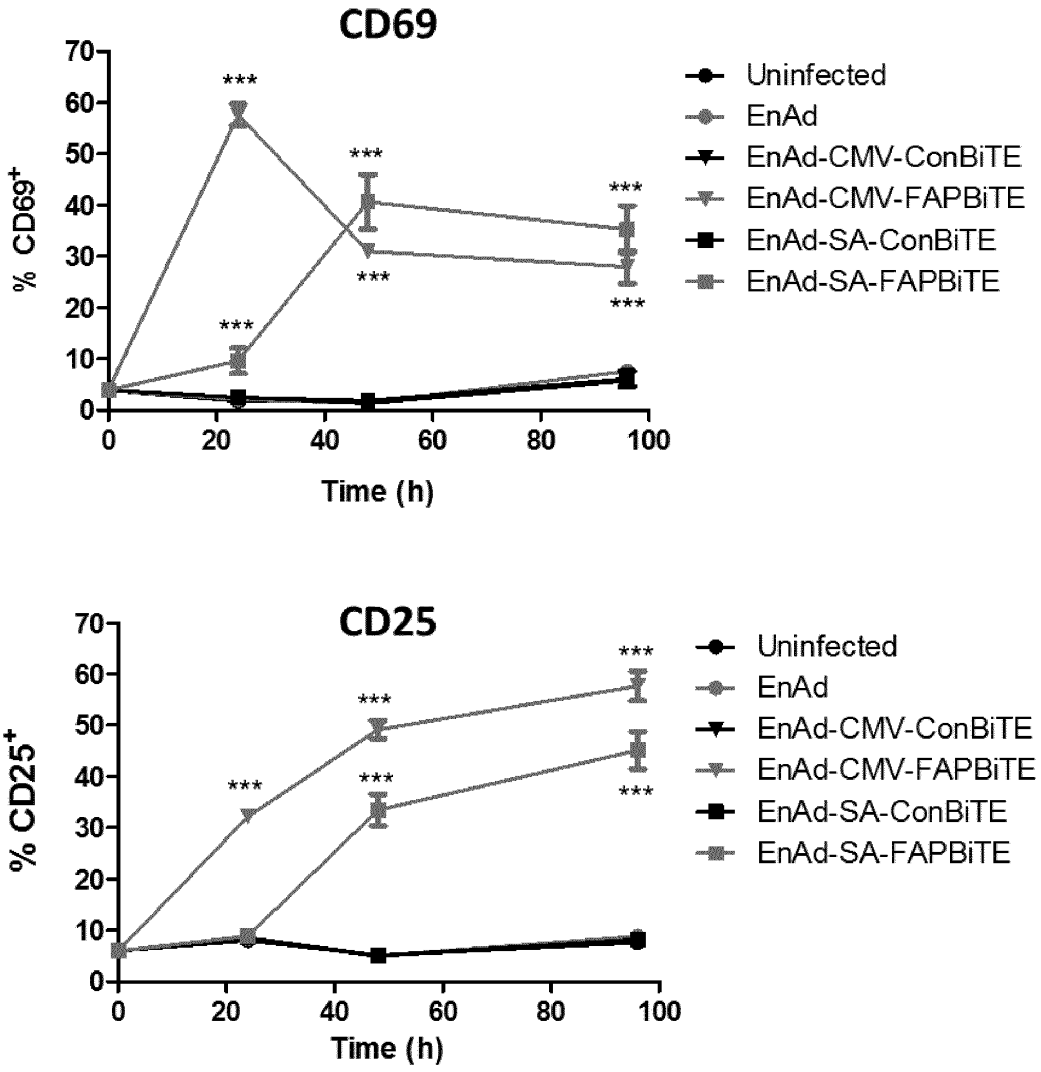

CD69
% CD69+
Time (h)
Uninfected
EnAd
EnAd-CMV-ConBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ConBiTE
EnAd-SA-FAPBiTE
CD25
% CD25+
Time (h)
Uninfected
EnAd
EnAd-CMV-ConBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ConBiTE
EnAd-SA-FAPBiTE

Figure 27

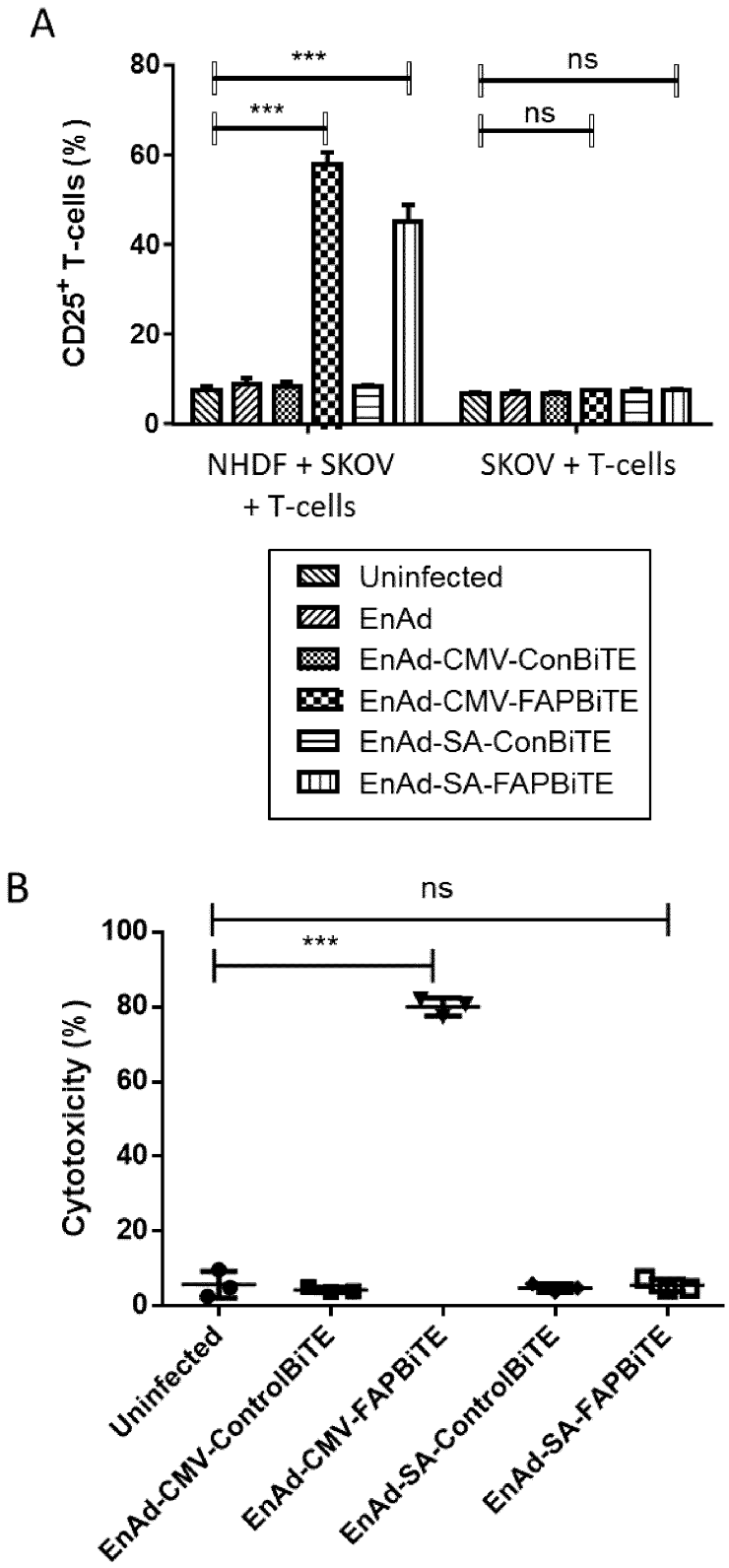
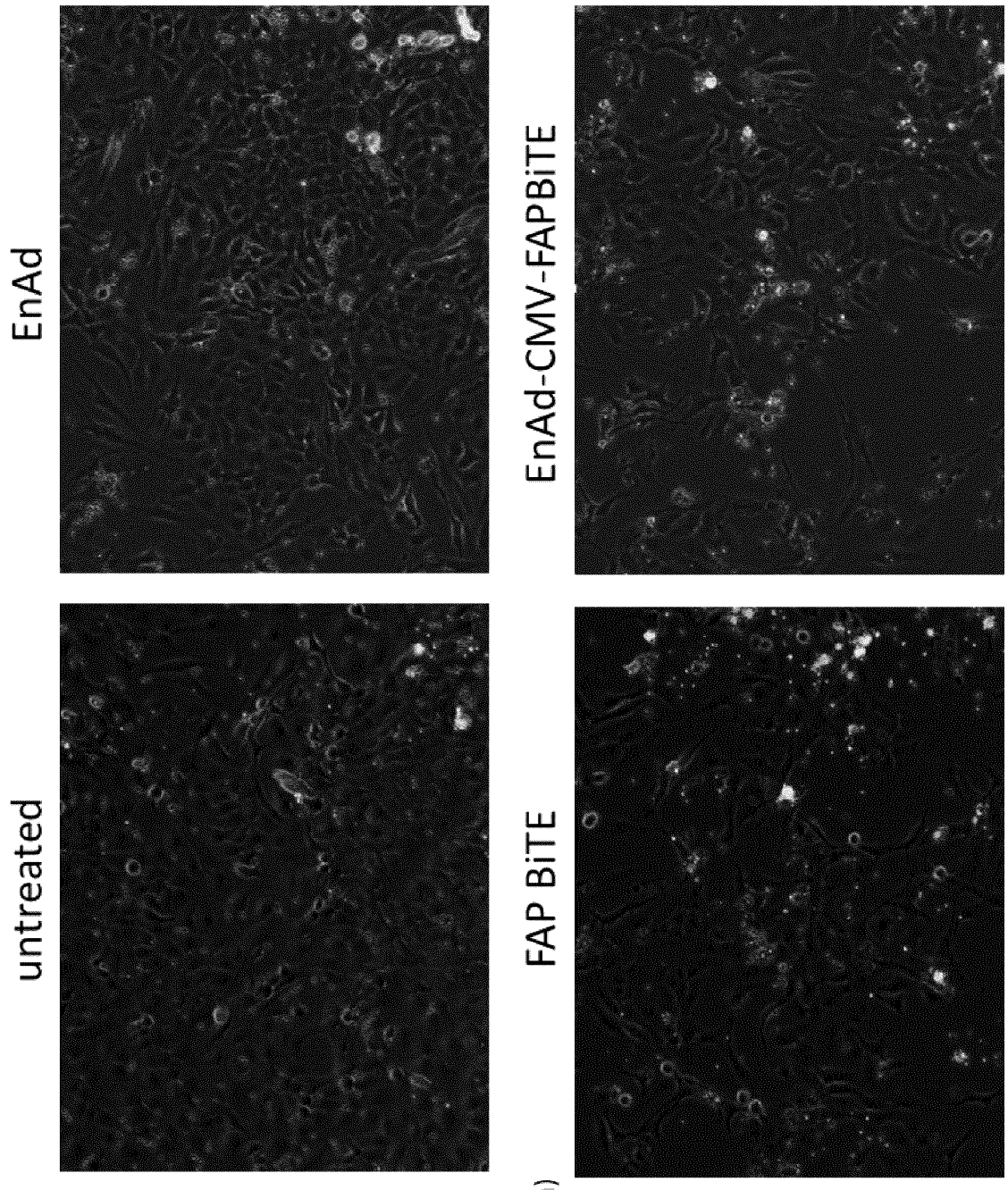

A
CD25+ T-cells (%)
80
60
40
20
0
***
***
ns
ns
NHDF + SKOV + T-cells
SKOV + T-cells
Uninfected
EnAd
EnAd-CMV-ConBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ConBiTE
EnAd-SA-FAPBiTE
B
Cytotoxicity (%)
100
80
60
40
20
0
ns
***
Uninfected
EnAd-CMV-ControlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ControlBiTE
EnAd-SA-FAPBiTE

Figure 28



- SKOV (unstained)
- NHDF (red)
- caspase stain (green)
- Virus – 100 vp/cell

Figure 29

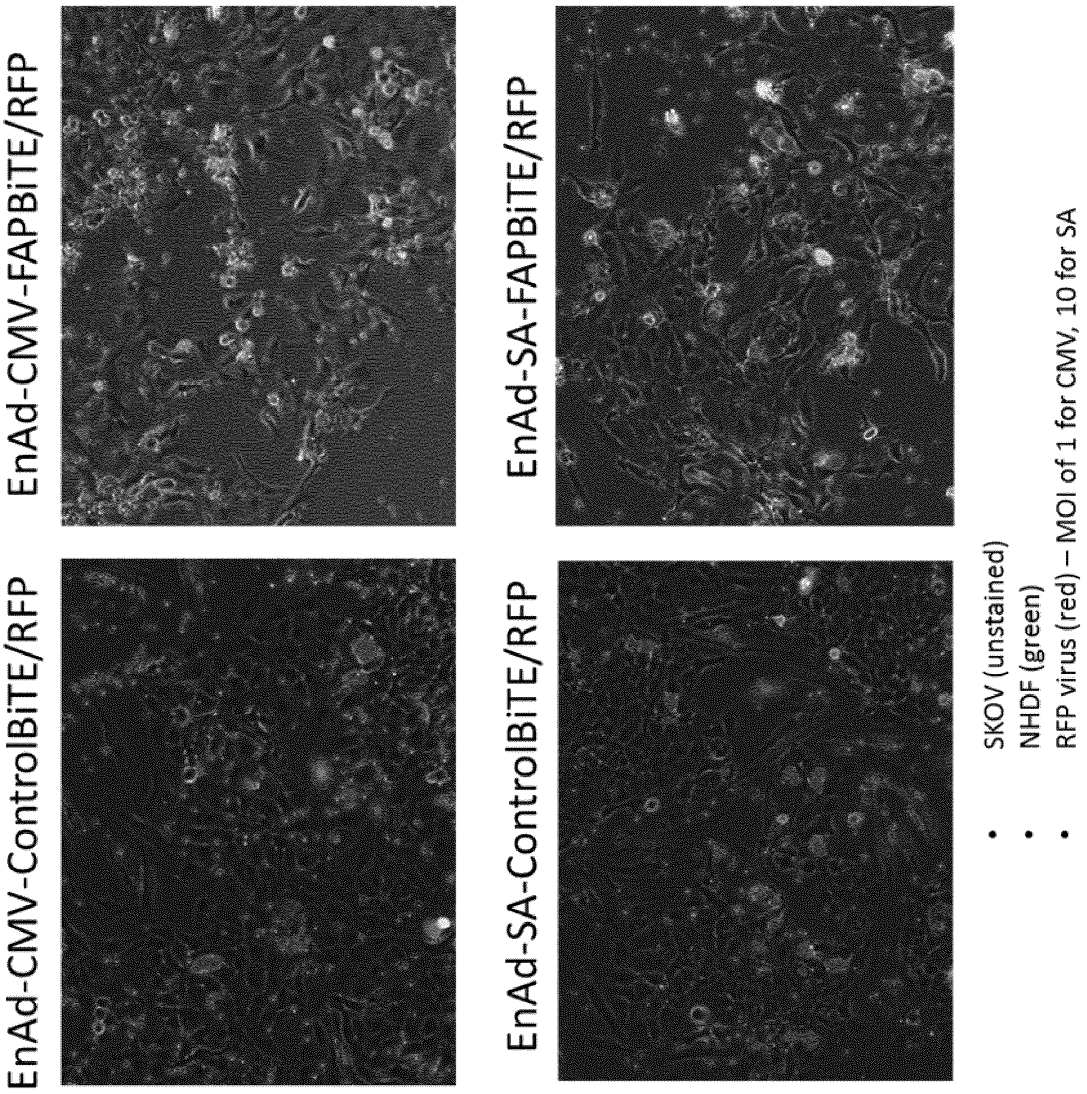

EnAd-CMV-ControlBiTE/RFP
EnAd-CMV-FAPBiTE/RFP
EnAd-SA-ControlBiTE/RFP
EnAd-SA-FAPBiTE/RFP
SKOV (unstained)
NHDF (green)
RFP virus (red) – MOI of 1 for CMV, 10 for SA

Figure 30

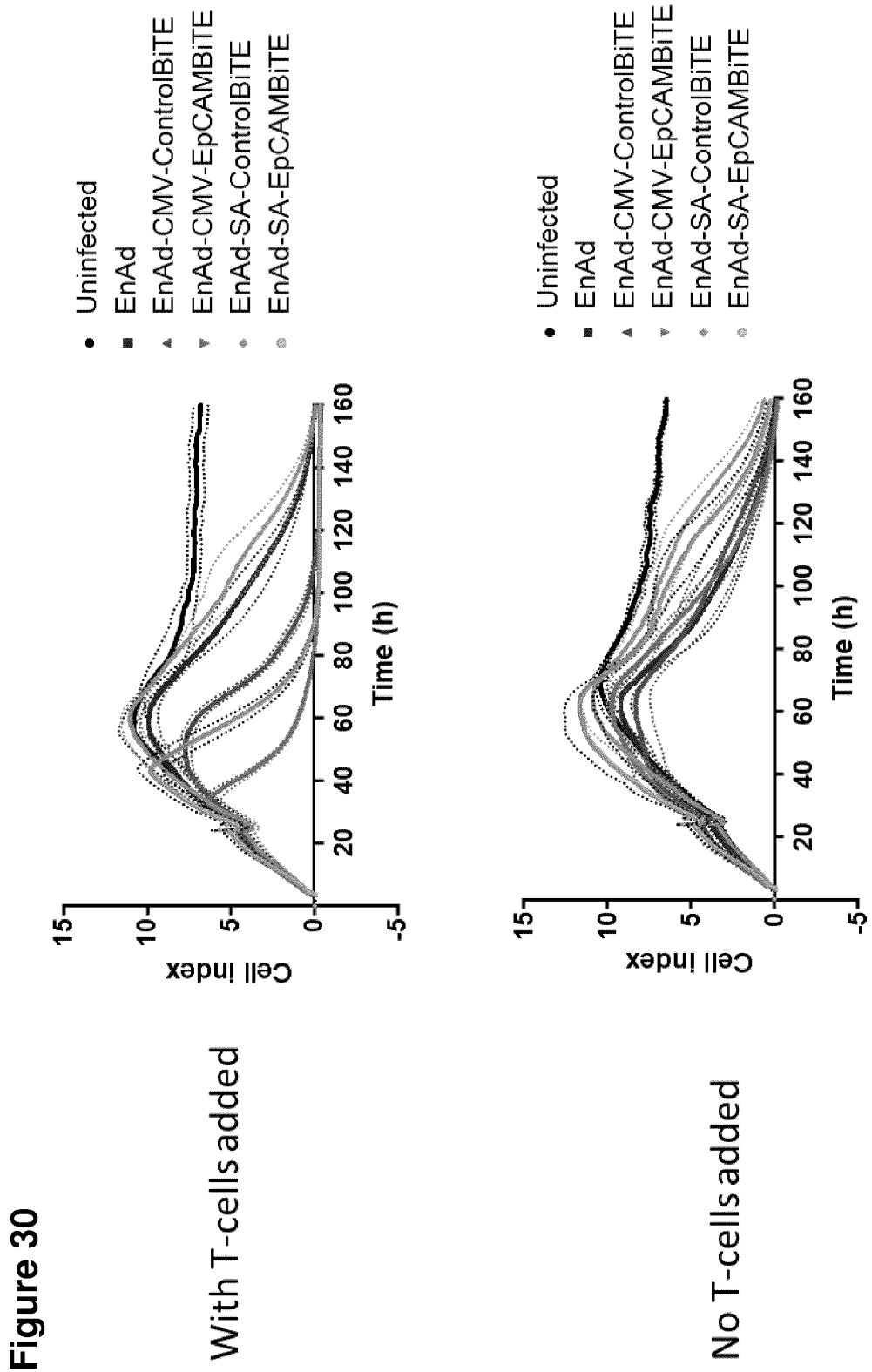
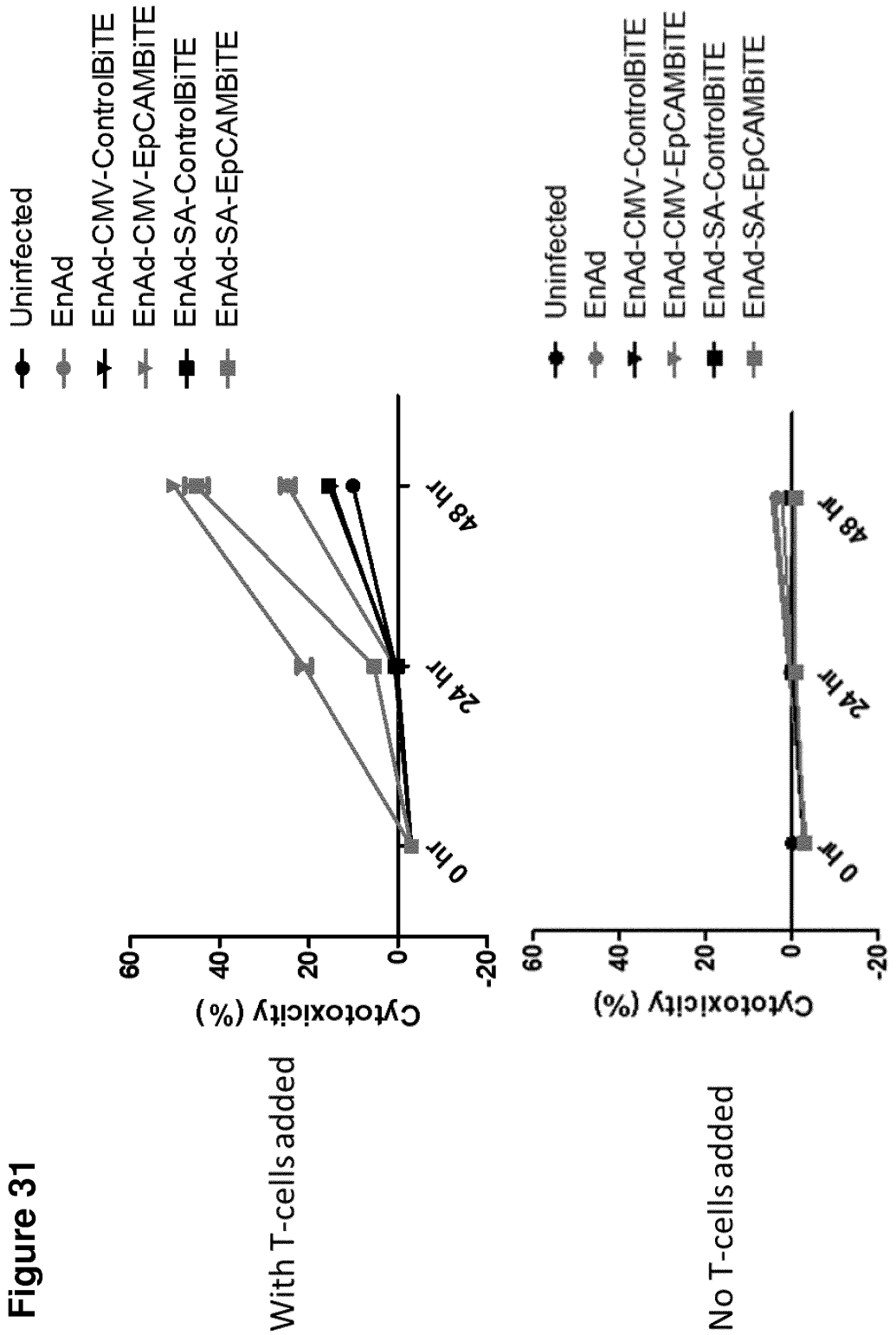

With T-cells added
No T-cells added
Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE
Cell Index
Time (h)
15
10
5
0
-5
20
40
60
80
100
120
140
160

Figure 31


Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE
Cytotoxicity (%)
60
40
20
0
-20
0 hr
24 hr
48 hr
With T-cells added
No T-cells added

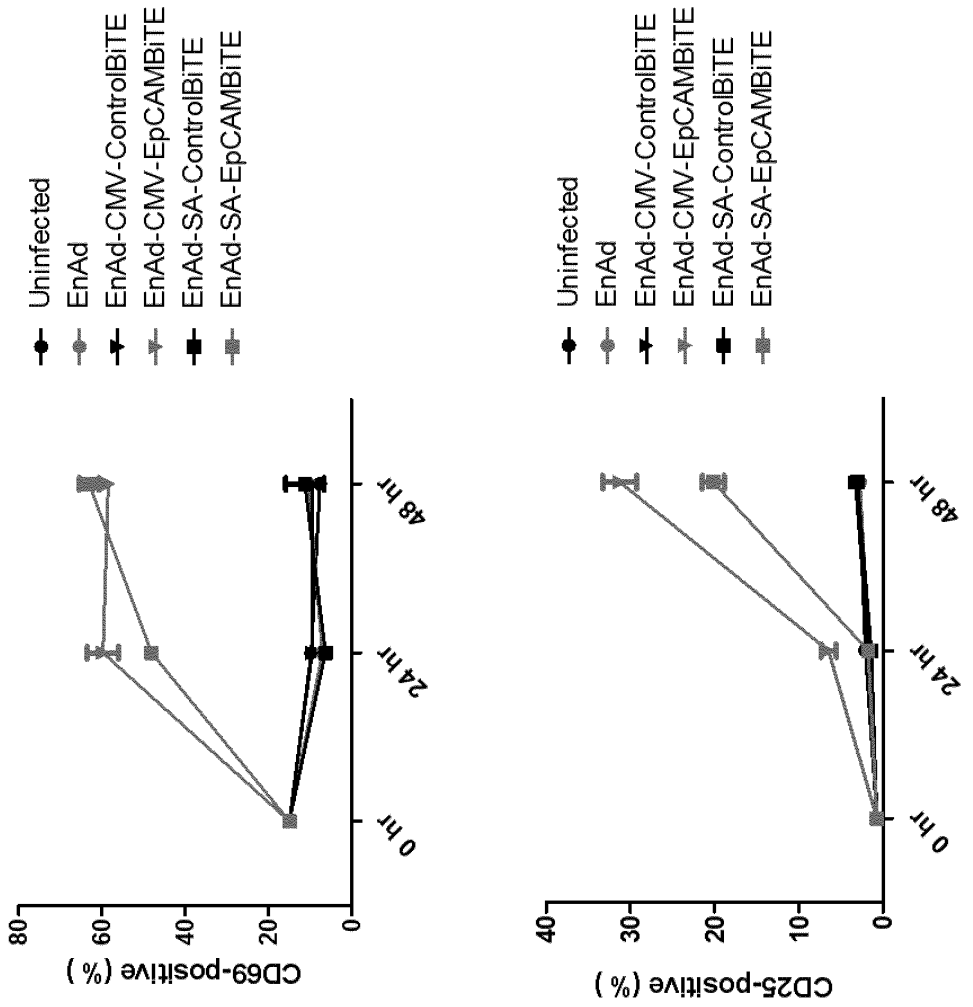
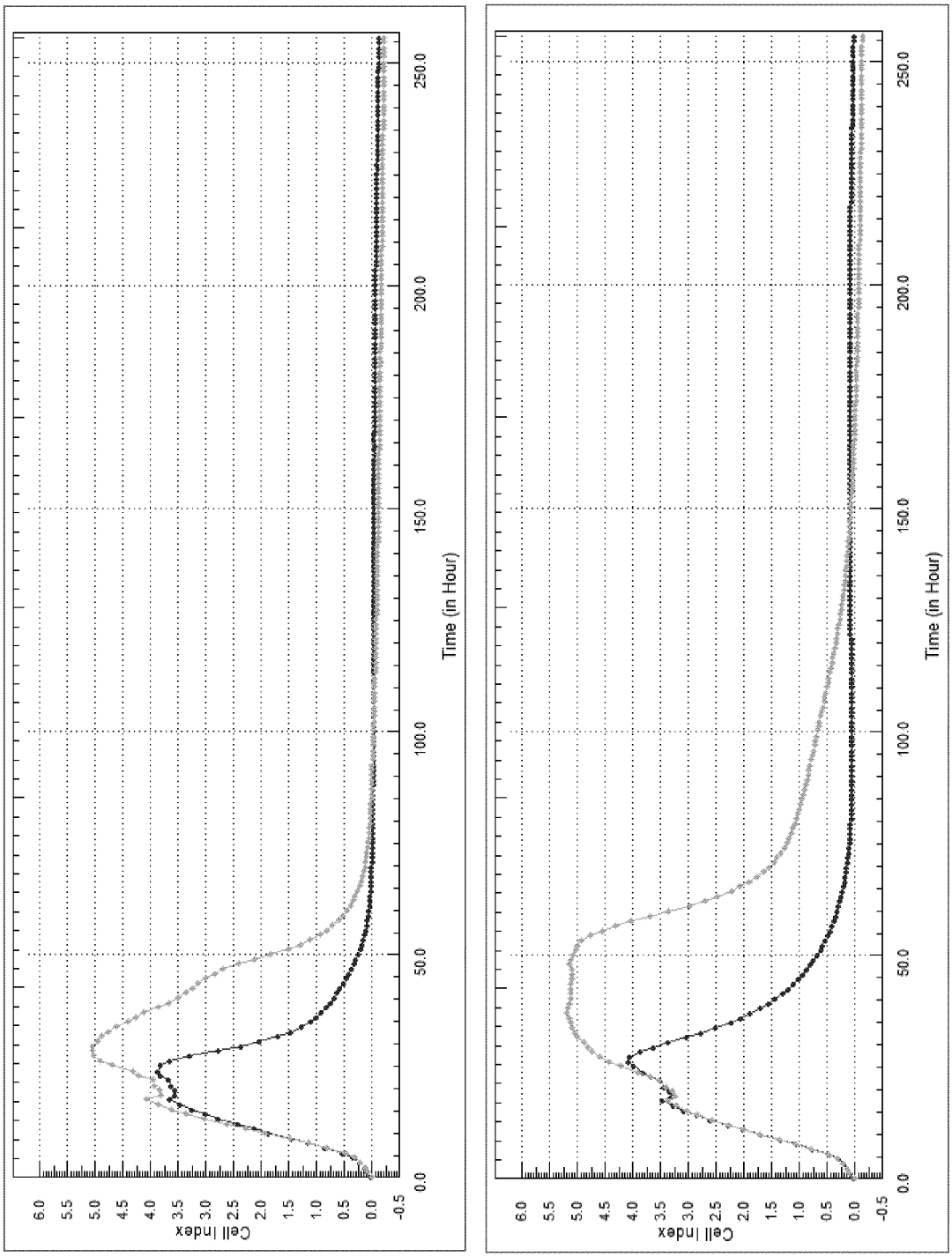

Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE
CD69-positive (%)
0
20
40
60
80
0 hr
24 hr
48 hr
CD25-positive (%)
0
10
20
30
40

Figure 32

Figure 33



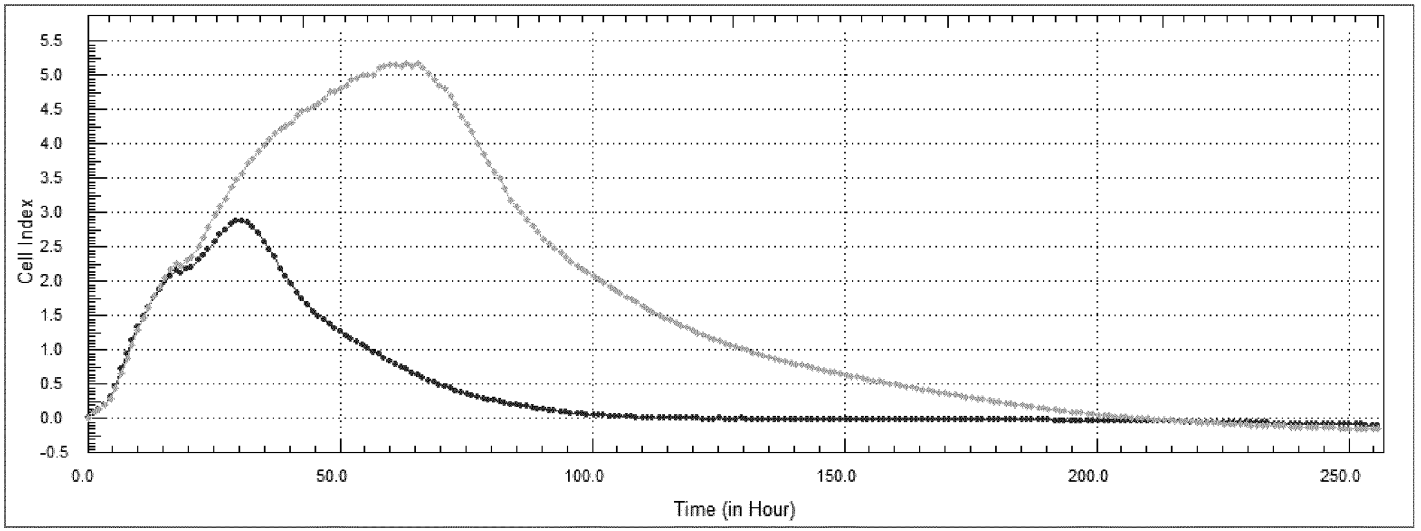
MOI: 10

Light grey – no T-cells added
Dark grey - T-cells added

MOI: 1

Figure 33 cont.

MOI: 0.1



Light grey –
no T-cells added
Dark grey -
T-cells added

MOI: 0.01

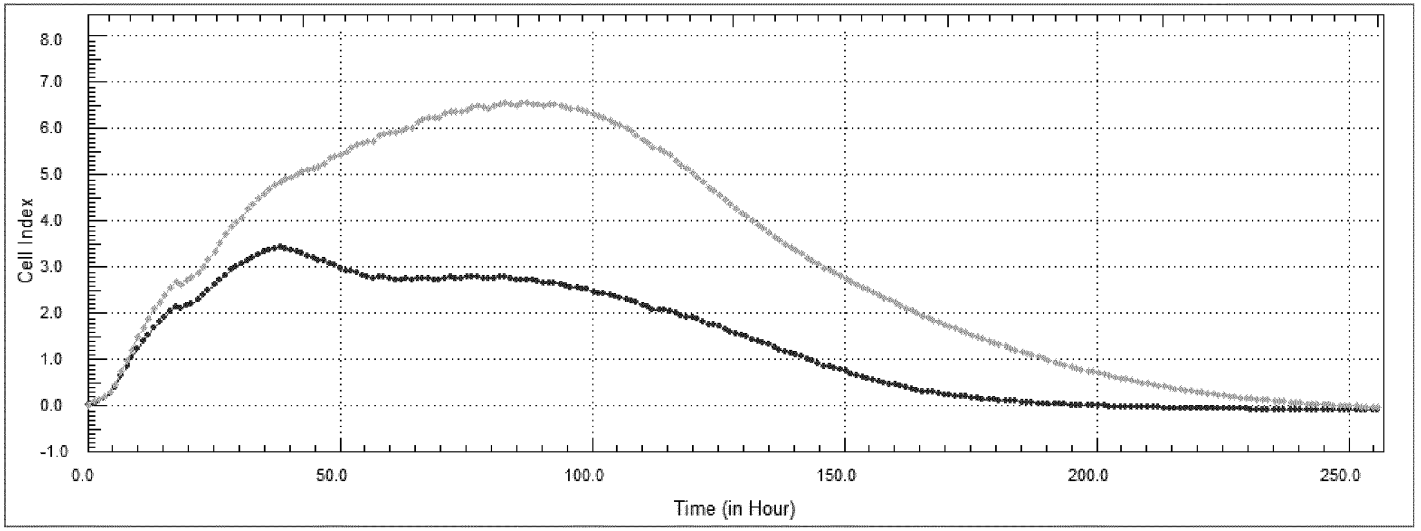
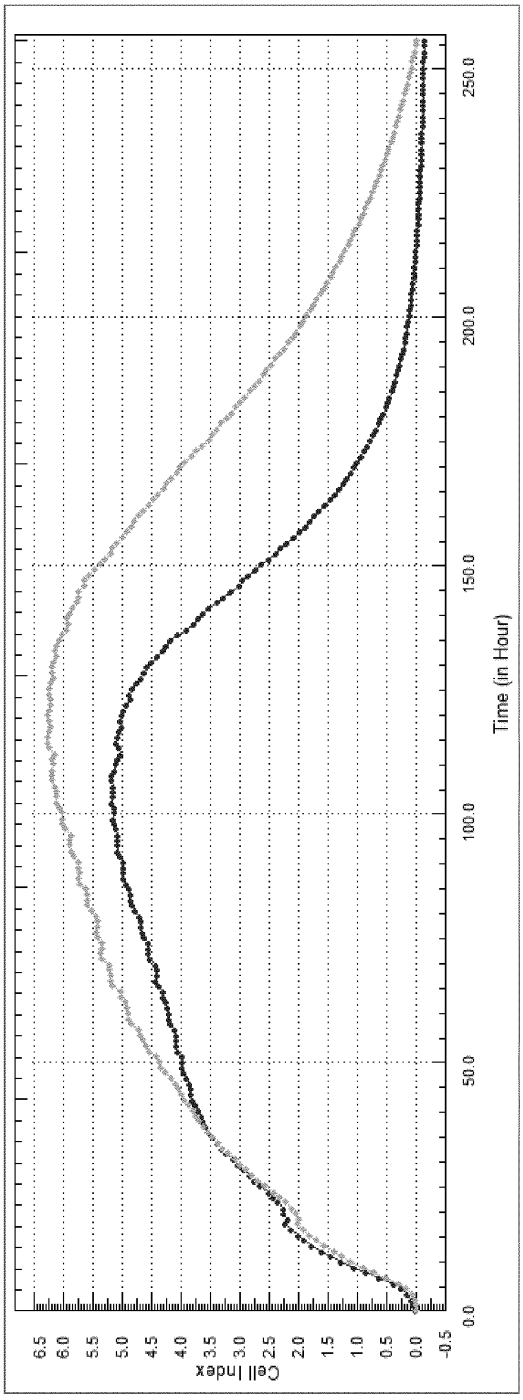
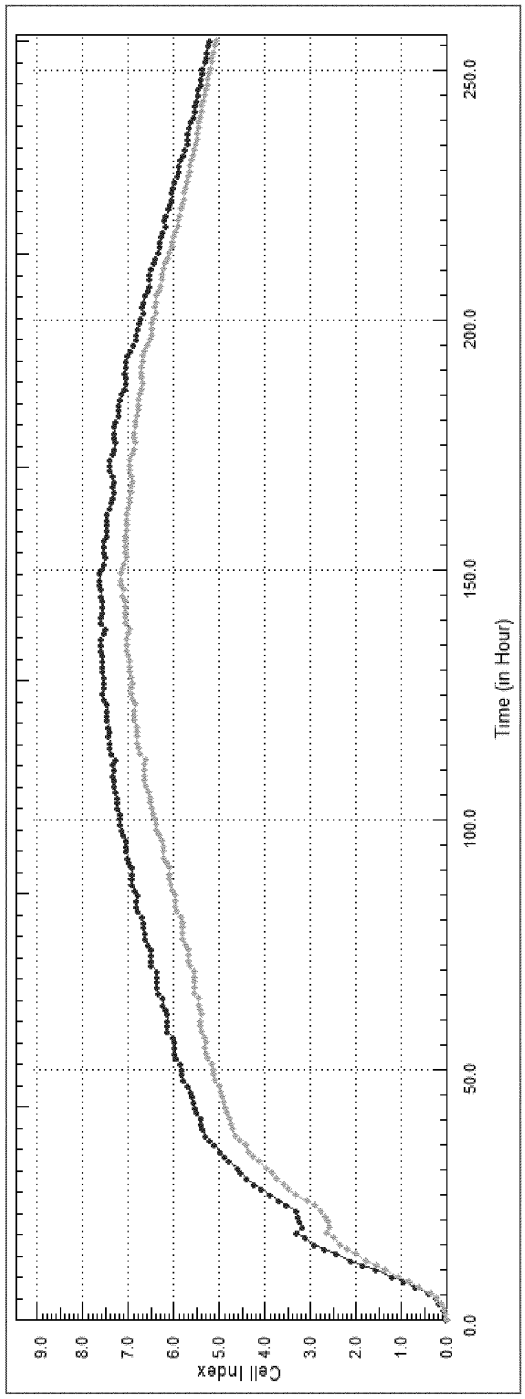


Figure 33 cont.



MOI: 0.001

Light grey – no T-cells added
Dark grey - T-cells added



MOI: 0

Figure 34

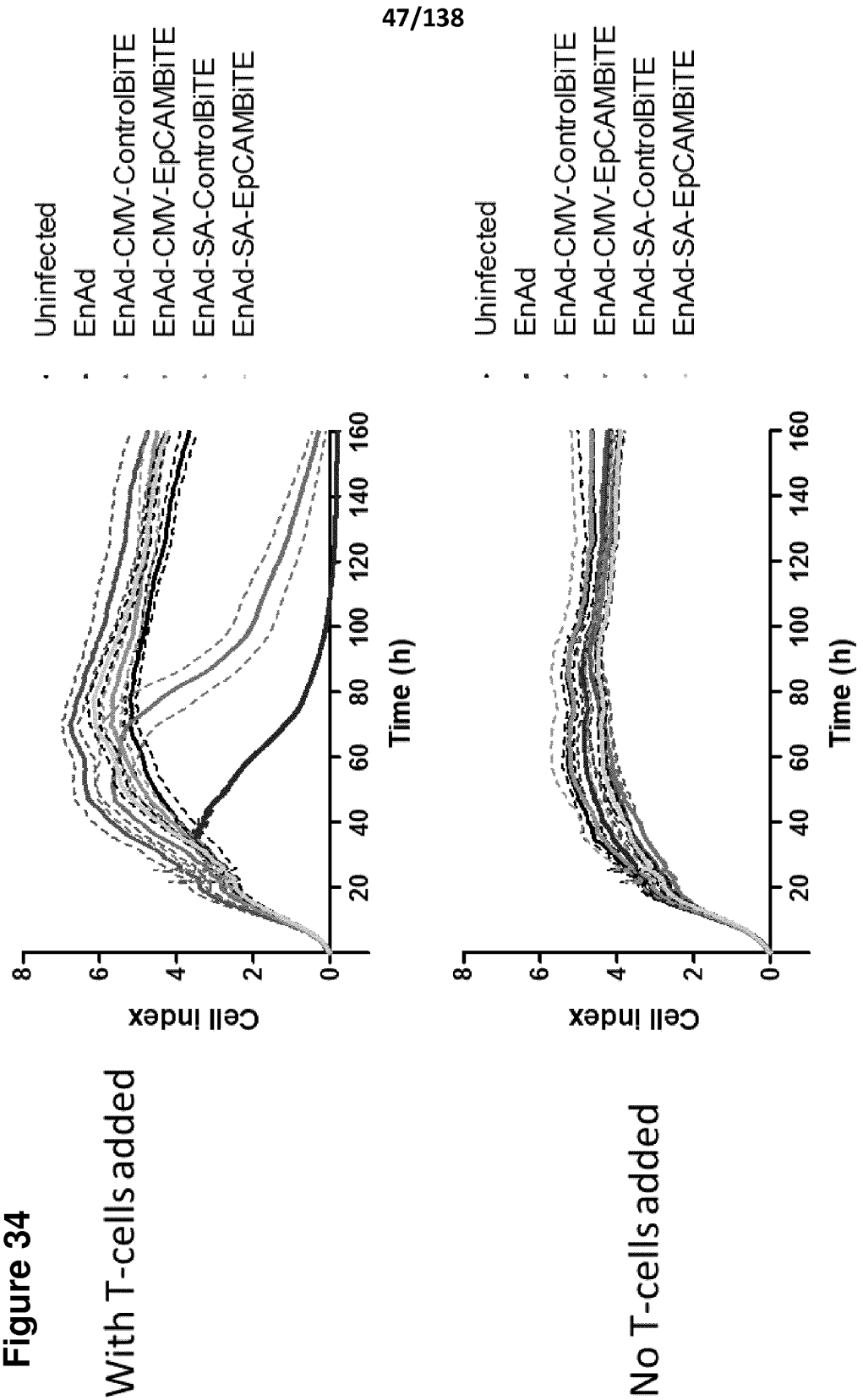

With T-cells added
Cell Index
Time (h)
Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE
No T-cells added
Cell Index
Time (h)
Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE

Figure 35

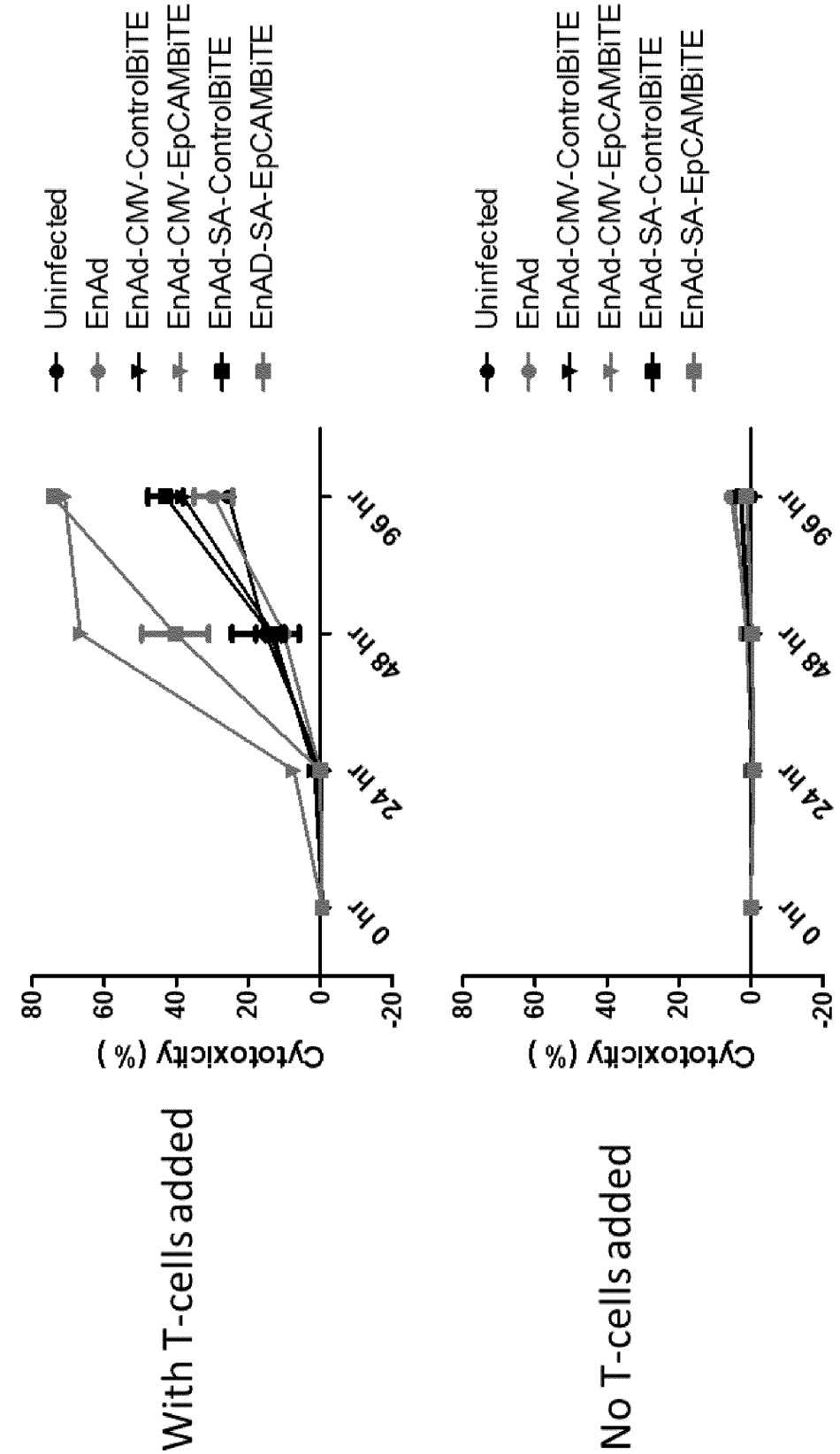
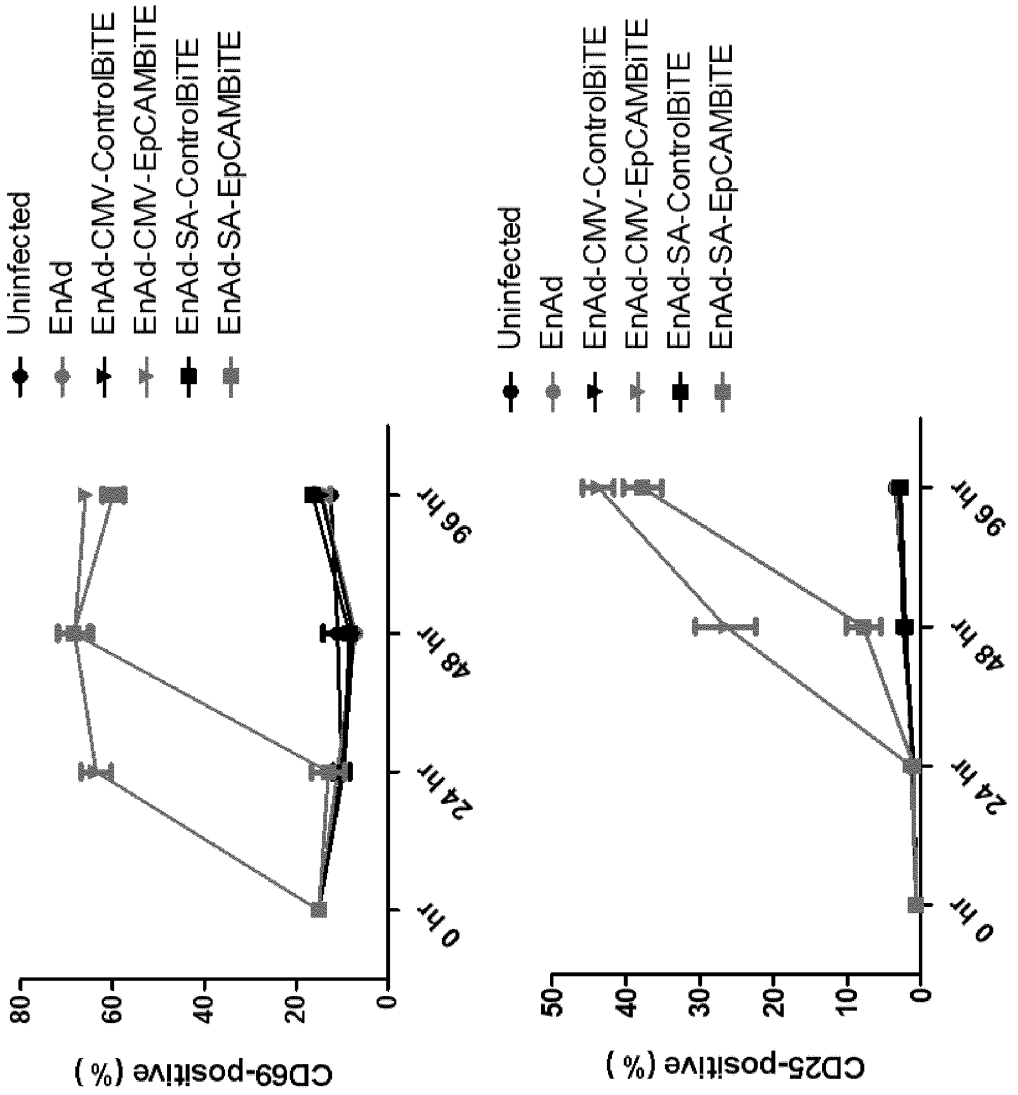

With T-cells added
No T-cells added
Cytotoxicity (%)
80
60
40
20
0
-20
0 hr
24 hr
48 hr
96 hr
Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAD-SA-EpCAMBiTE

Figure 36


Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE
CD69-positive (%)
0
20
40
60
80
0 hr
24 hr
48 hr
96 hr
CD25-positive (%)
0
10
20
30
40
50

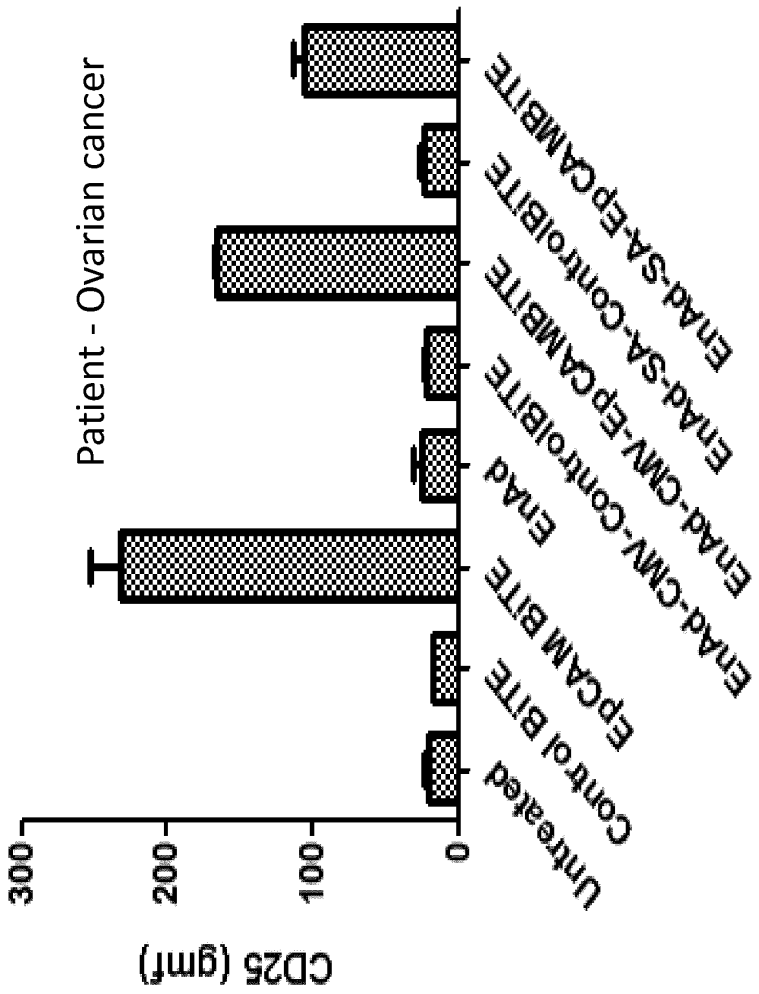
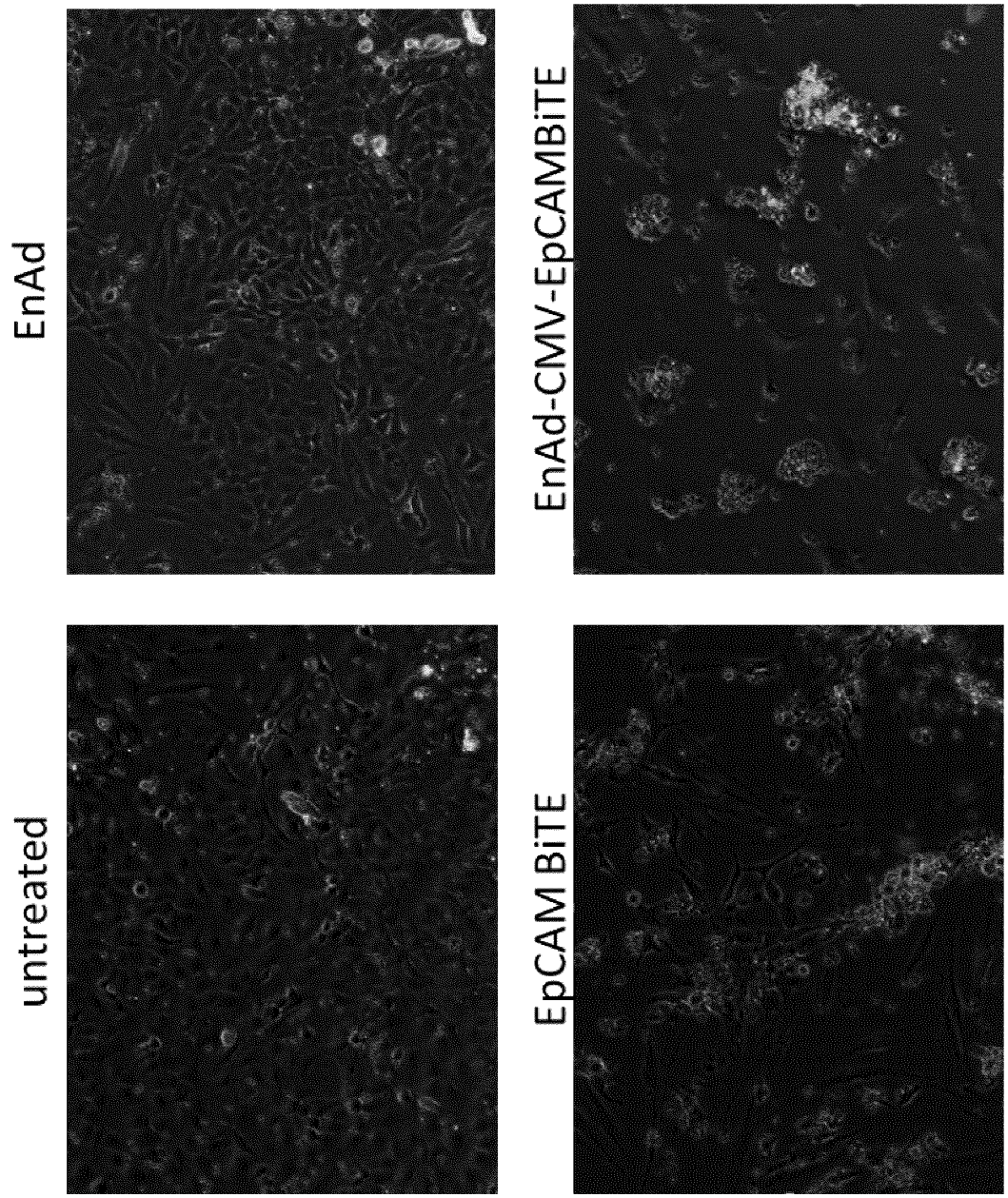

Patient - Ovarian cancer
CD25 (gmf)
300
200
100
0
Untreated
Control BITE
EpCAM BITE
EnAd
EnAd-CMV-ControlBITE
EnAd-CMV-EpCAMBITE
EnAd-SA-ControlBITE
EnAd-SA-EpCAMBITE

Figure 37

Figure 38



- SKOV (unstained)
- NHDF (red)
- caspase stain (green)
- Virus – 100 vp/cell

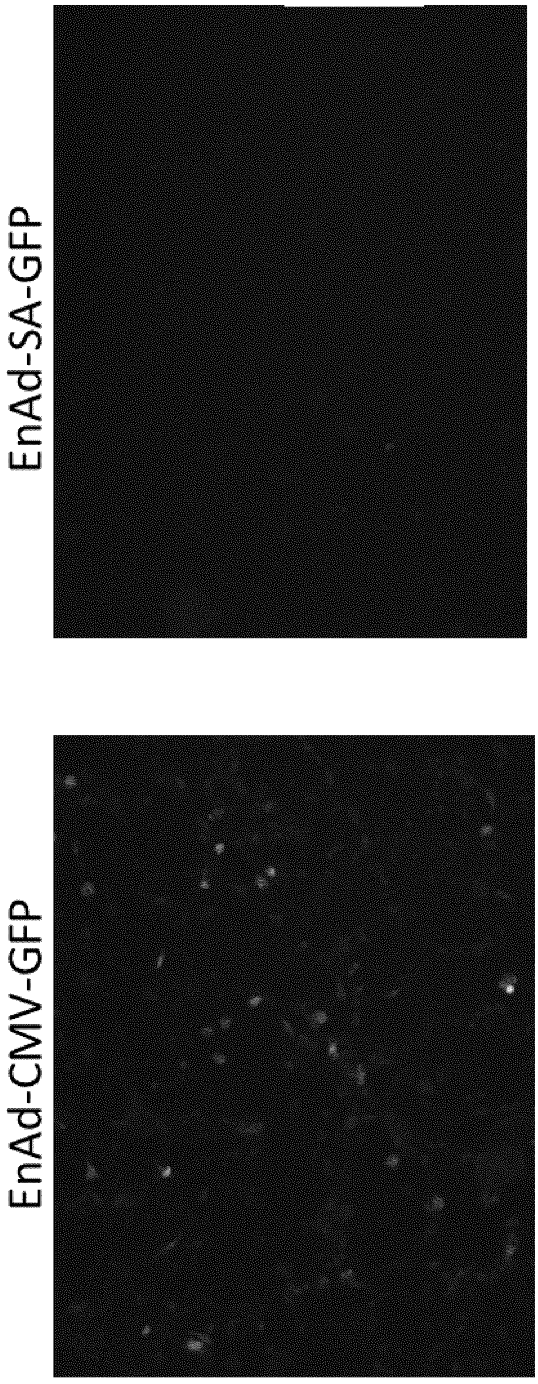
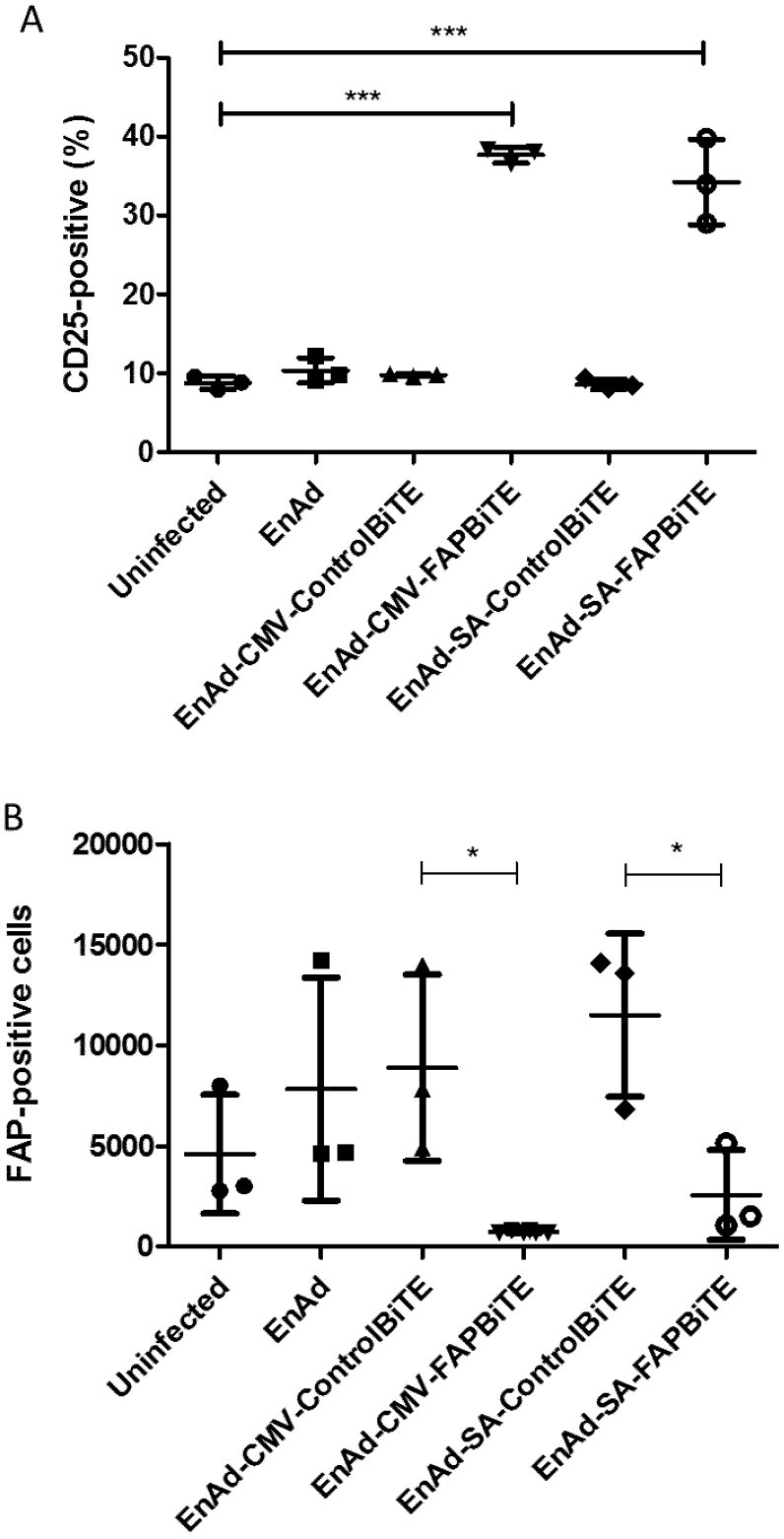


Figure 39

Figure 40


A
CD25-positive (%)
50
40
30
20
10
0
***
***
Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ControlBiTE
EnAd-SA-FAPBiTE
B
FAP-positive cells
20000
15000
10000
5000
0
*
*
Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ControlBiTE
EnAd-SA-FAPBiTE

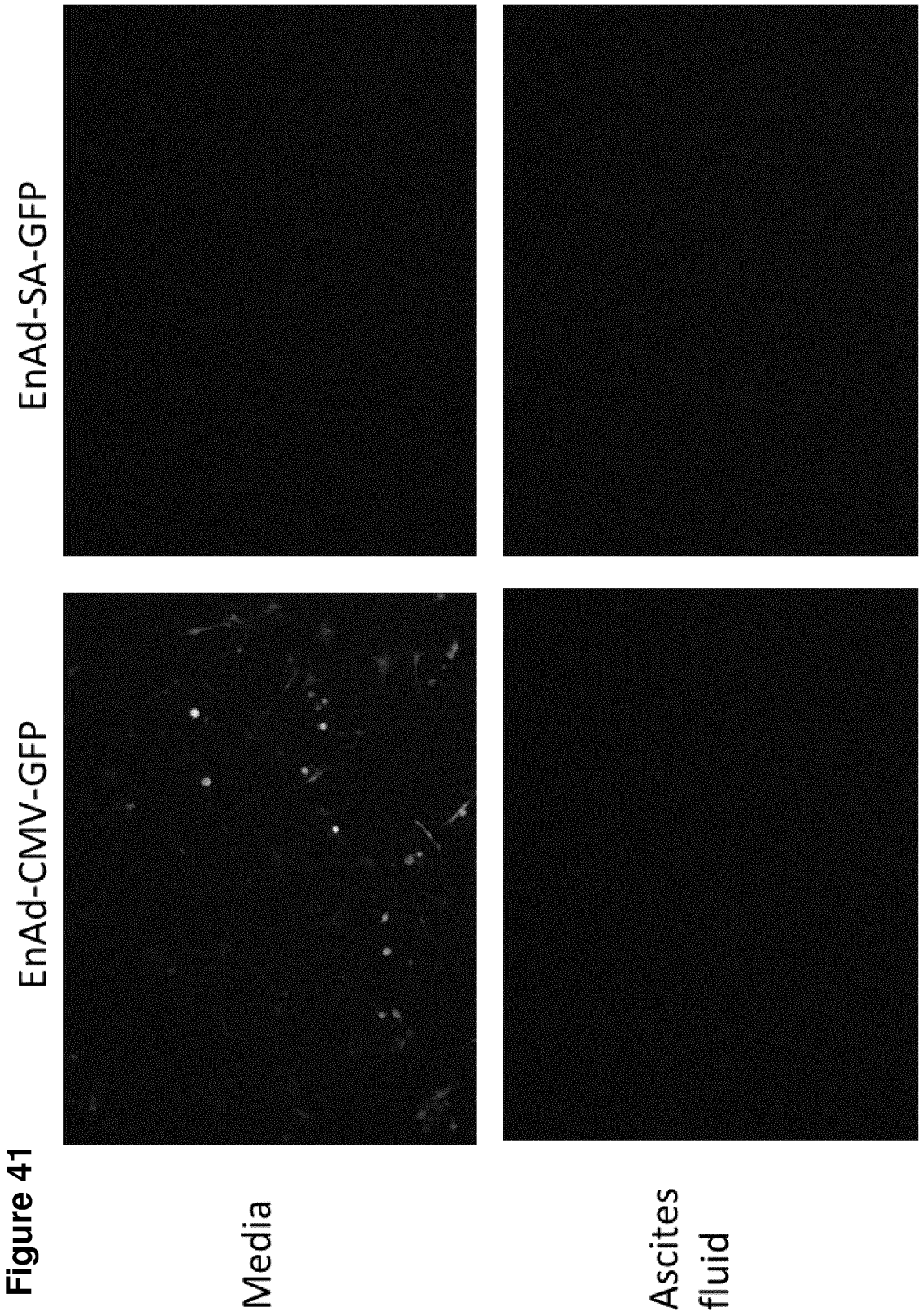


Figure 41

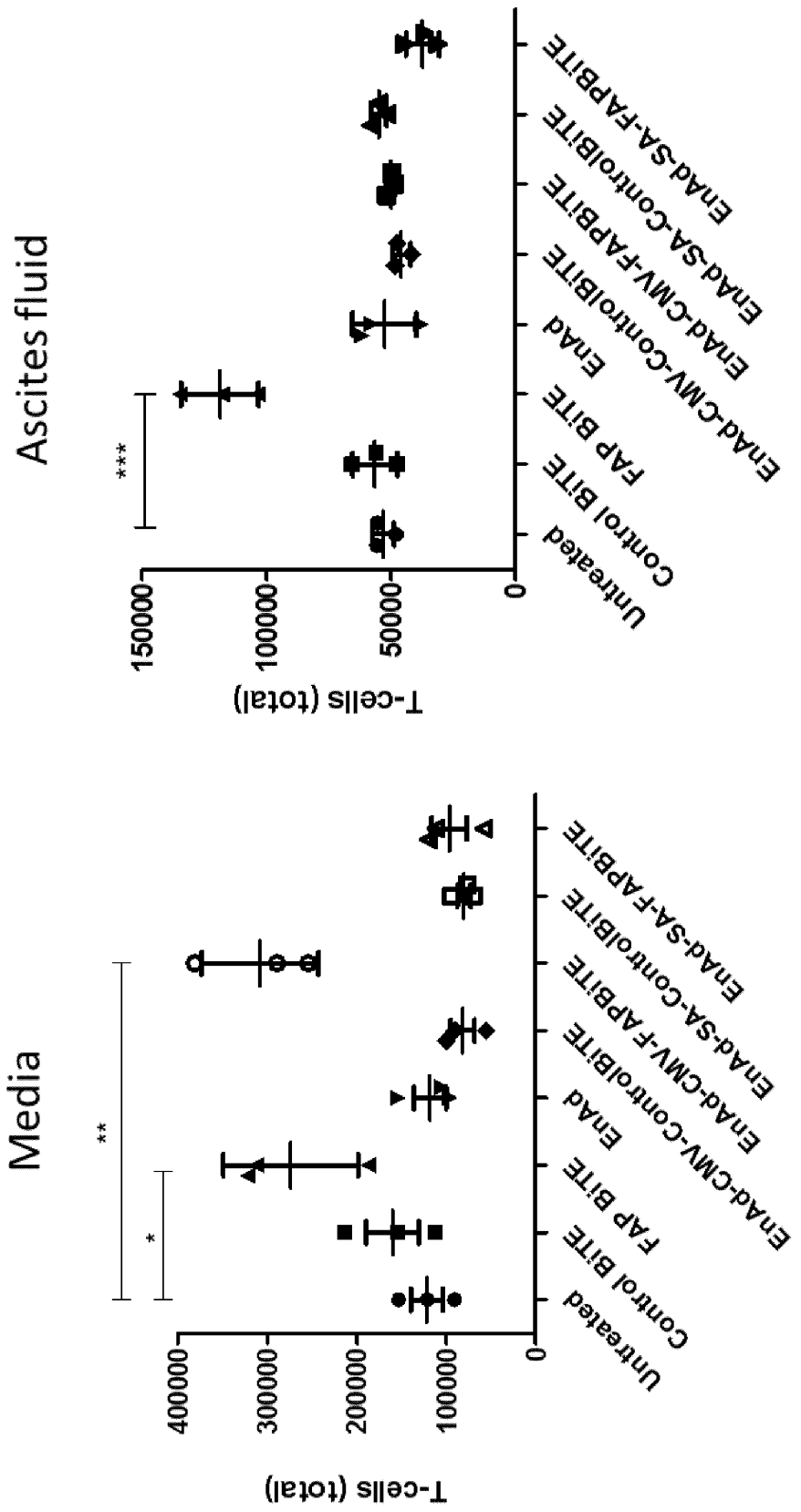

Ascites fluid
T-cells (total)
150000
100000
50000
0
***
Untreated
Control BiTE
FAP BiTE
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ControlBiTE
EnAd-SA-FAPBiTE
Media
T-cells (total)
400000
300000
200000
100000
0
**
*
Untreated
Control BiTE
FAP BiTE
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ControlBiTE
EnAd-SA-FAPBiTE

Figure 42

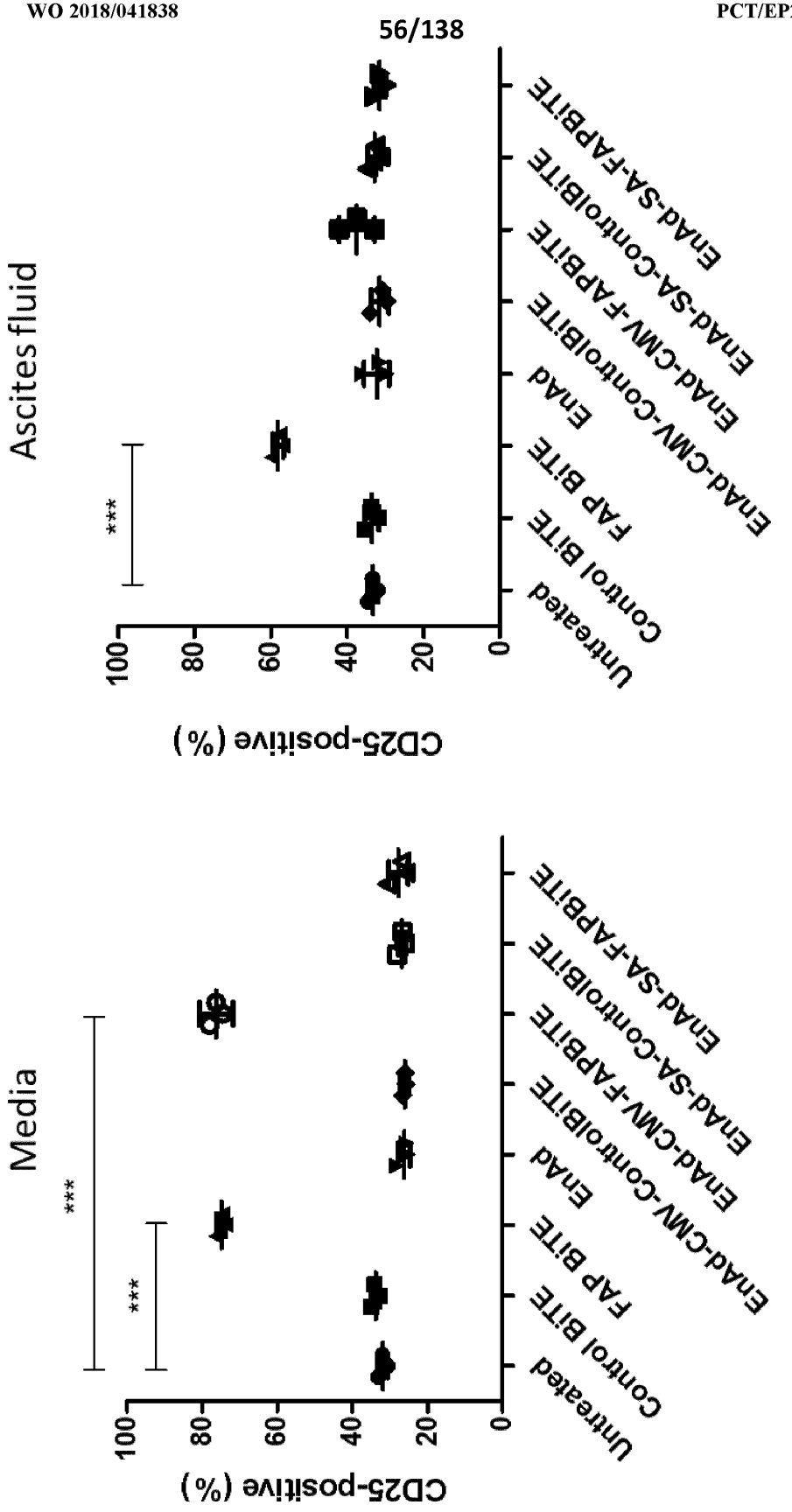

Ascites fluid
CD25-positive (%)
100
80
60
40
20
0
***
Untreated
Control BiTE
FAP BiTE
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ControlBiTE
EnAd-SA-FAPBiTE
Media
CD25-positive (%)
100
80
60
40
20
0
***
***
Untreated
Control BiTE
FAP BiTE
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ControlBiTE
EnAd-SA-FAPBiTE

Figure 43

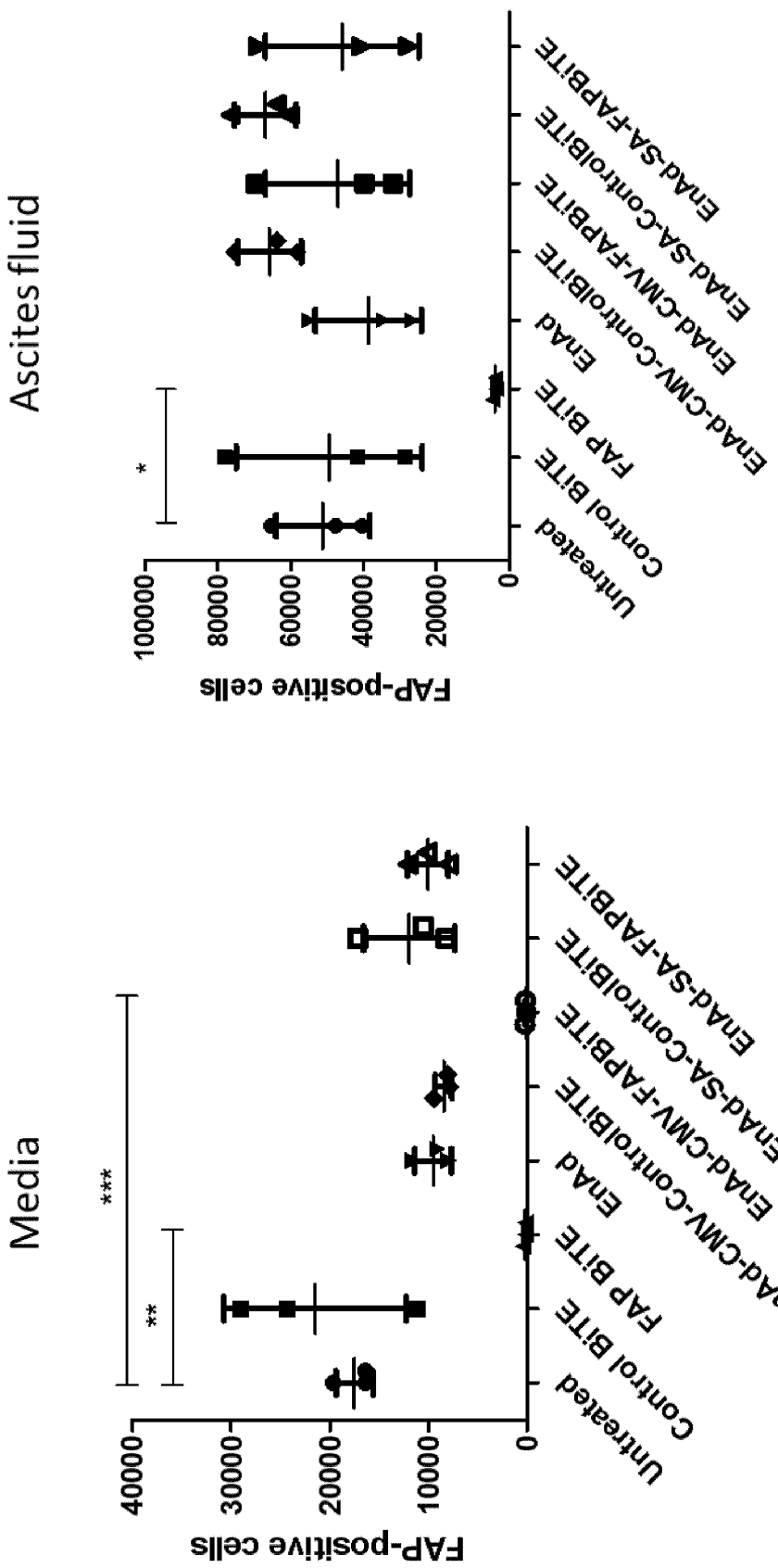
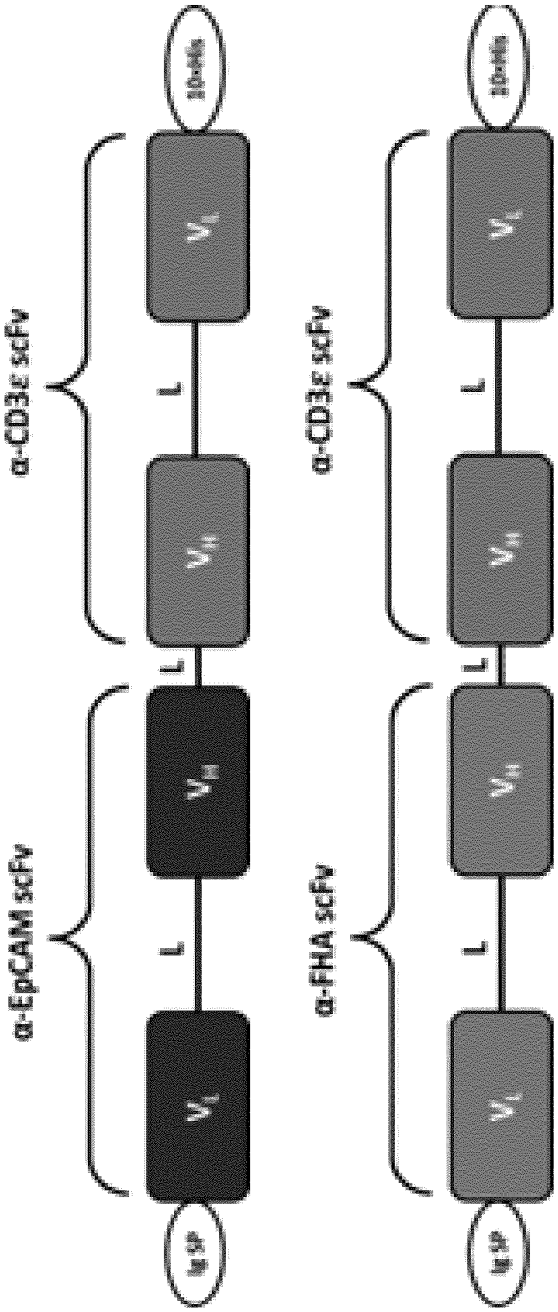

Ascites fluid
FAP-positive cells
100000
80000
60000
40000
20000
0
*
Untreated
Control BiTE
FAP BiTE
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ControlBiTE
EnAd-SA-FAPBiTE
Media
FAP-positive cells
40000
30000
20000
10000
0
***
**
Untreated
Control BiTE
FAP BiTE
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ControlBiTE
EnAd-SA-FAPBiTE

Figure 44

Figure 45A


Ig SP
α-EpCAM scFv
L
L
α-CD3ε scFv
L
L
His6
Ig SP
α-FHA scFv
L
L
α-CD3ε scFv
L
L
His6

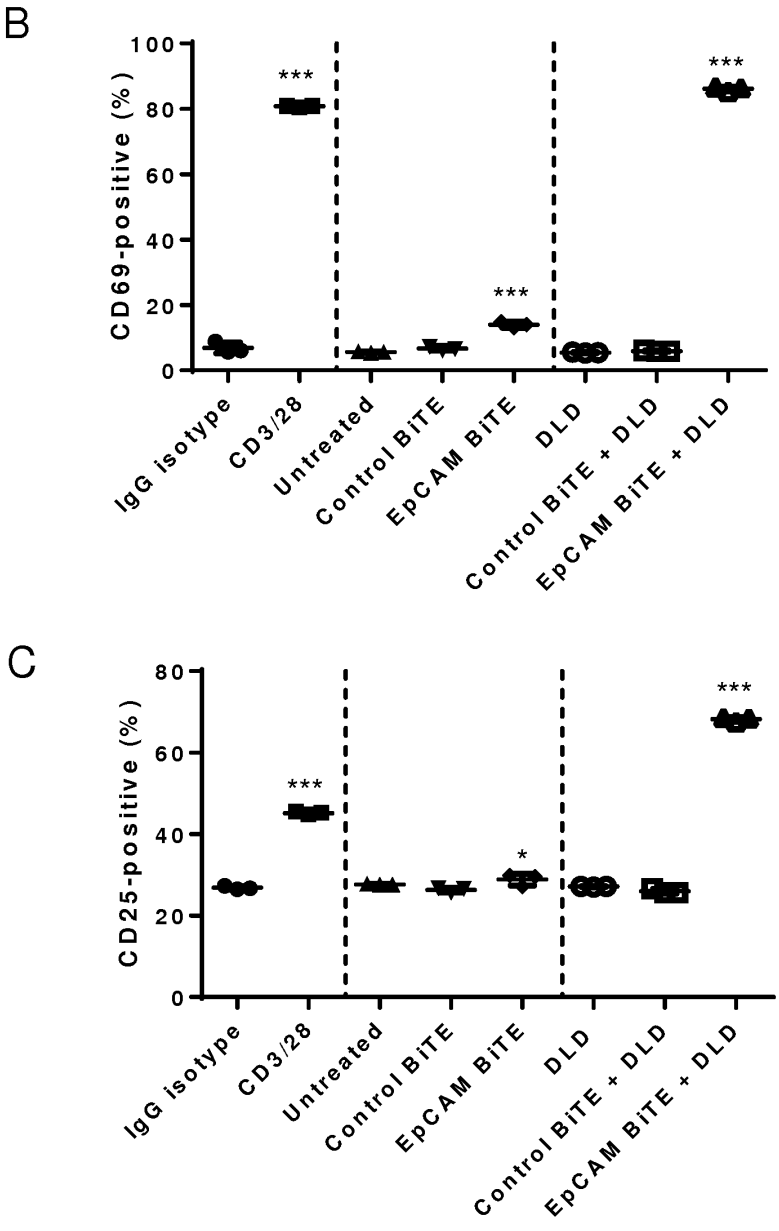


Figure 45 cont.

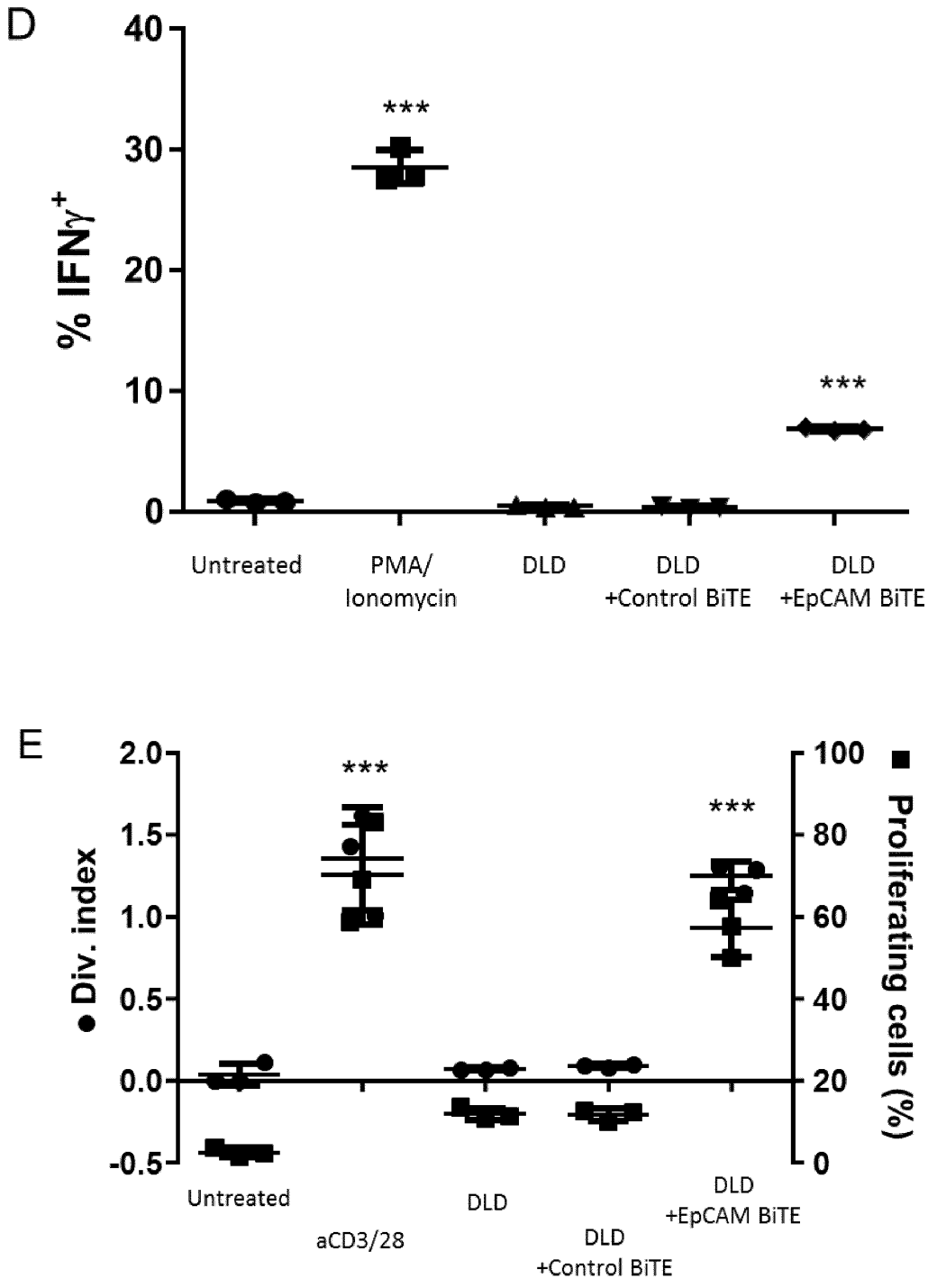
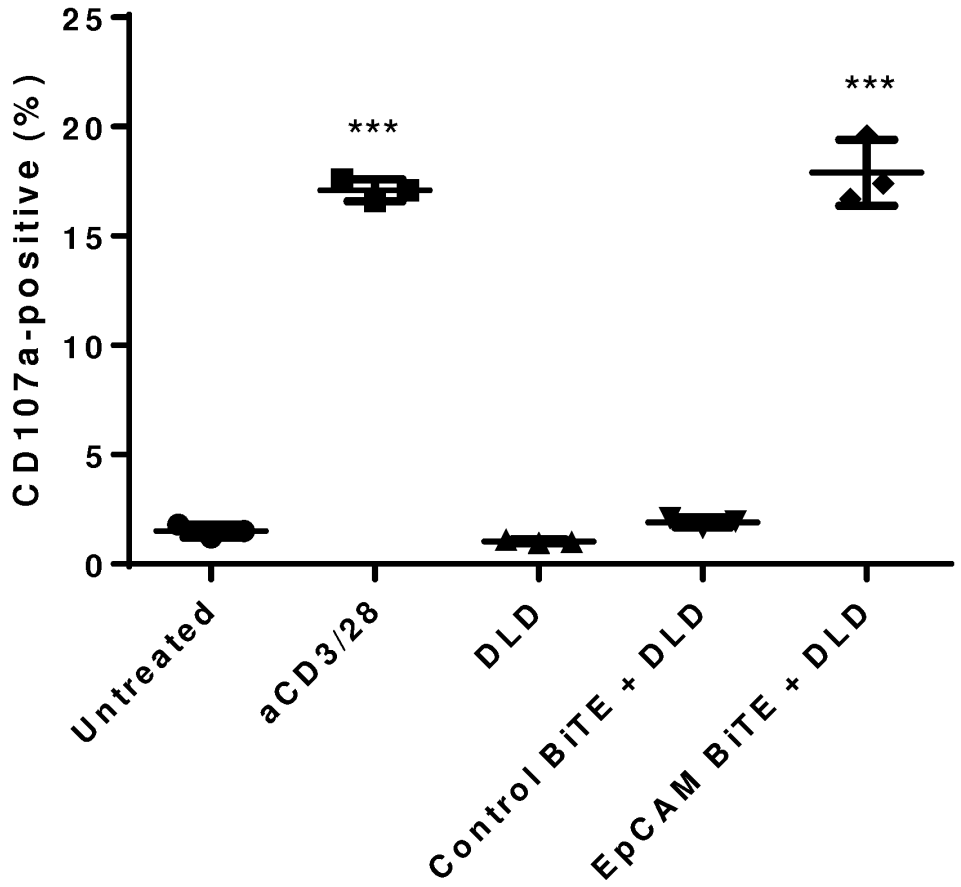


Figure 45 cont.

Figure 45 cont.

F



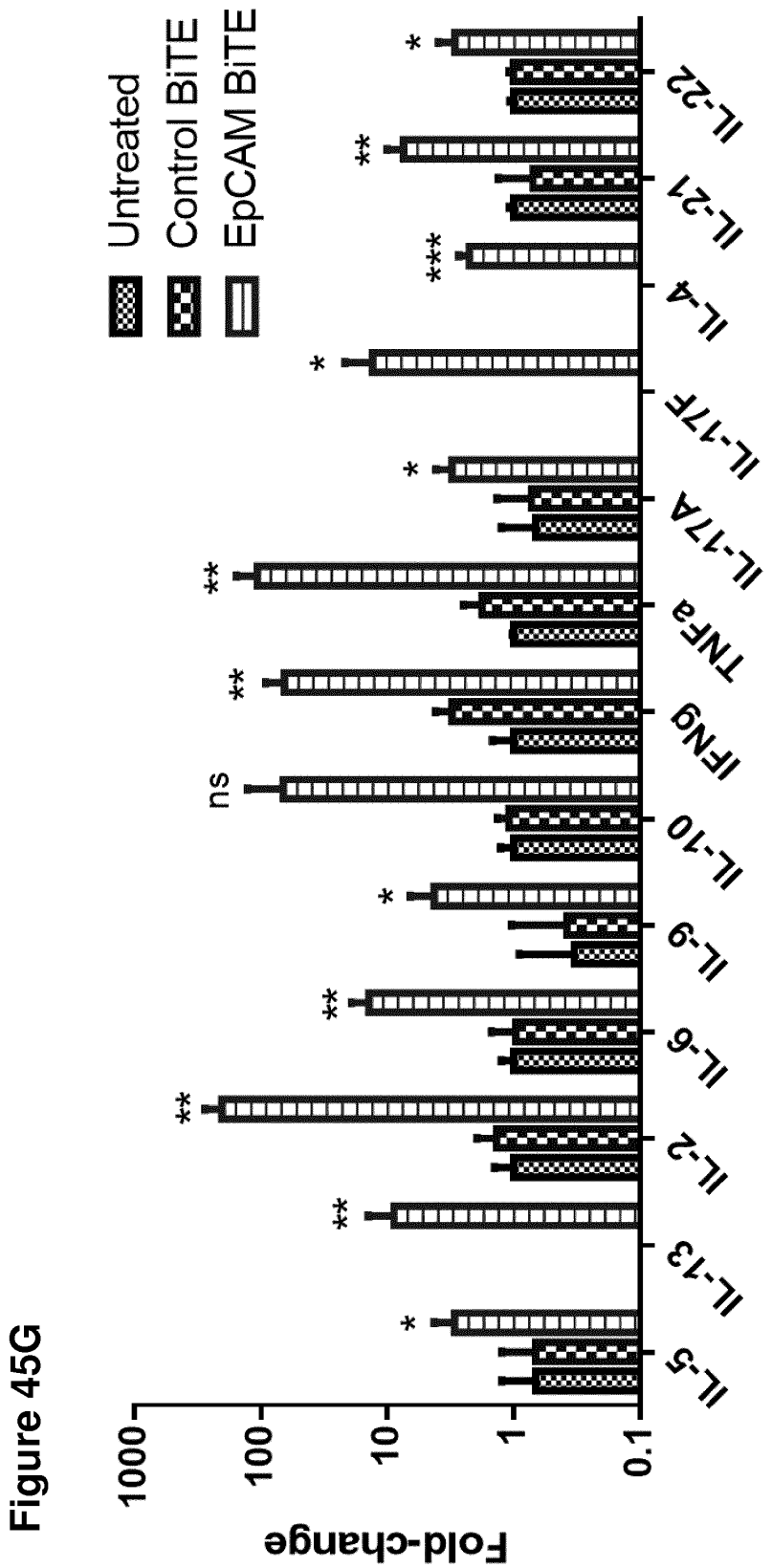

Untreated
Control BiTE
EpCAM BiTE
Fold-change
1000
100
10
1
0.1
IL-5
IL-13
IL-2
IL-6
IL-9
IL-10
IFNg
TNFa
IL-17A
IL-17F
IL-4
IL-21
IL-22
*
**
***
ns

Figure 45G

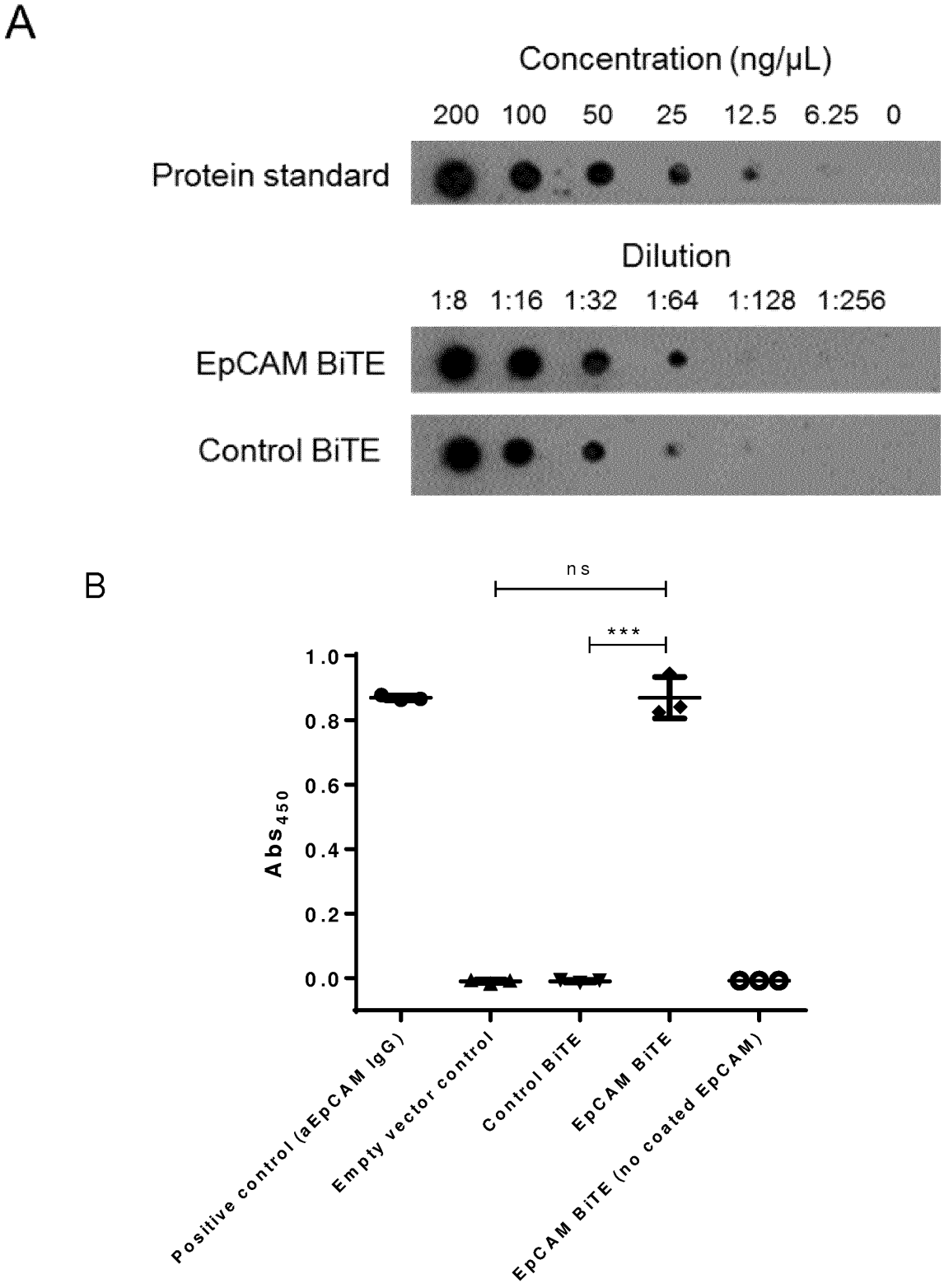

A
Concentration (ng/µL)
200 100 50 25 12.5 6.25 0
Protein standard
Dilution
1:8 1:16 1:32 1:64 1:128 1:256
EpCAM BiTE
Control BiTE
B
ns
***
1.0
0.8
0.6
0.4
0.2
0.0
Abs450
Positive control (aEpCAM IgG)
Empty vector control
Control BiTE
EpCAM BiTE
EpCAM BiTE (no coated EpCAM)

Figure 46

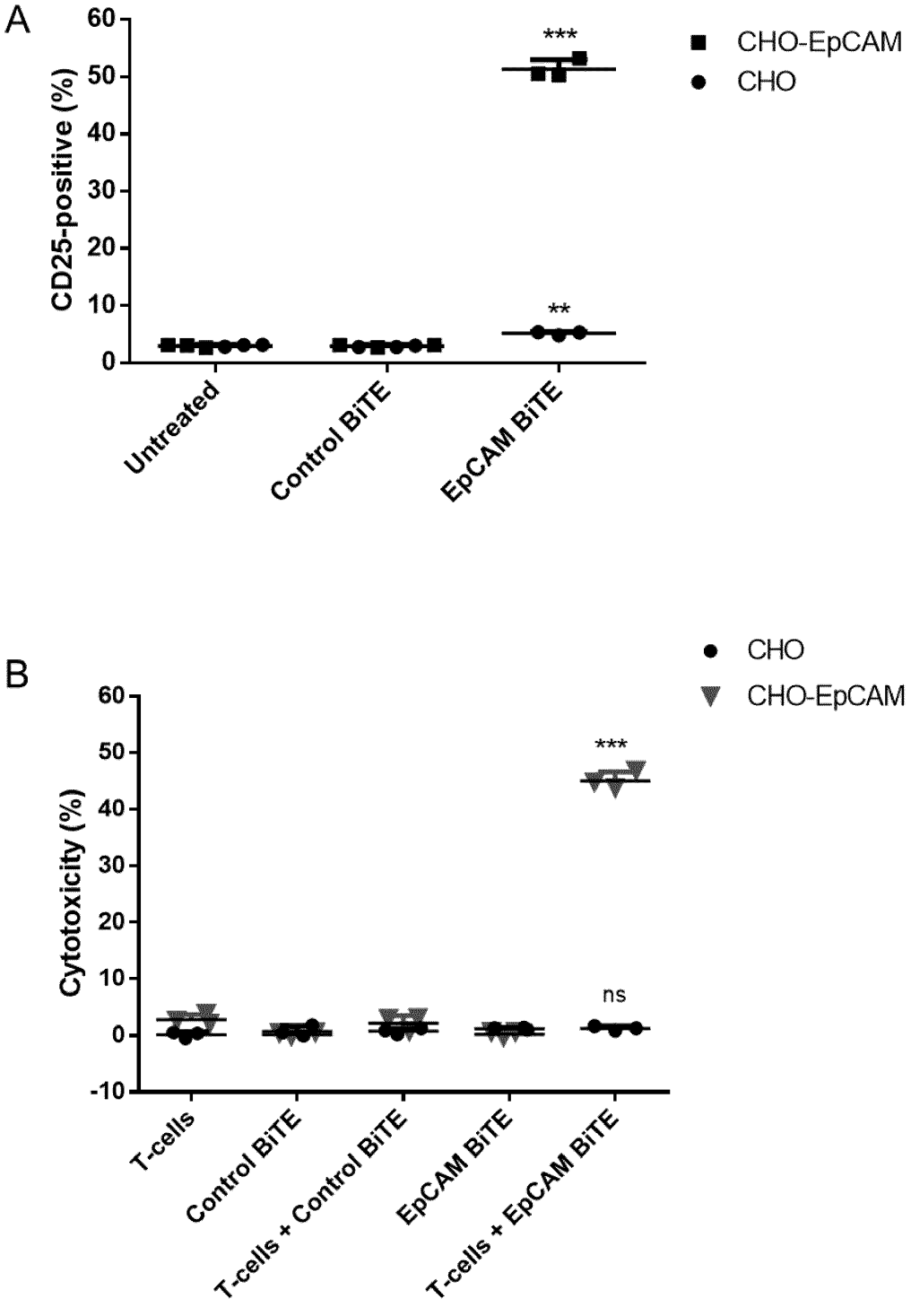

A
CD25-positive (%)
60
50
40
30
20
10
0
***
**
CHO-EpCAM
CHO
Untreated
Control BiTE
EpCAM BiTE
B
Cytotoxicity (%)
60
50
40
30
20
10
0
-10
***
ns
CHO
CHO-EpCAM
T-cells
Control BiTE
T-cells + Control BiTE
EpCAM BiTE
T-cells + EpCAM BiTE

Figure 47

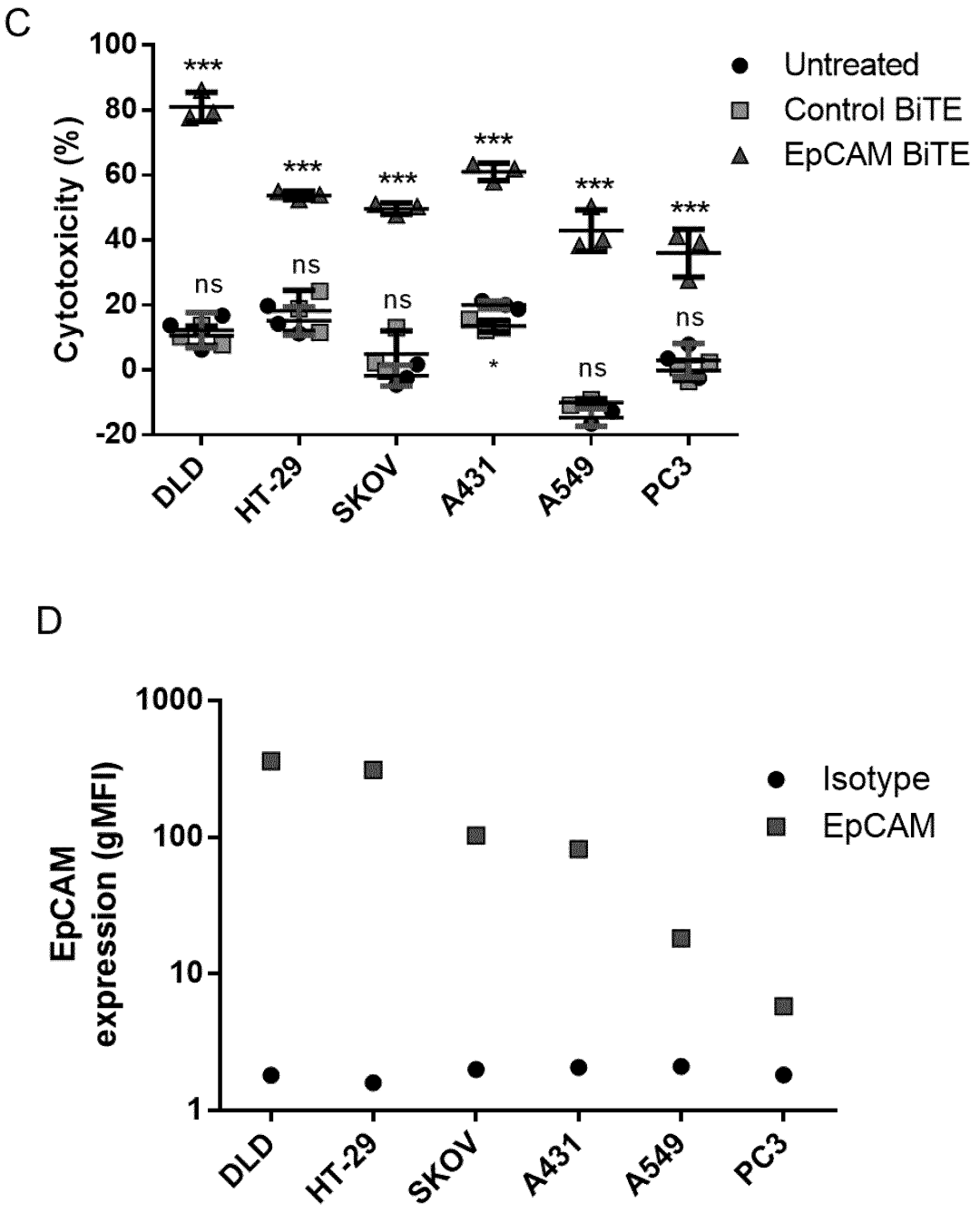


Figure 47 cont.

Figure 48

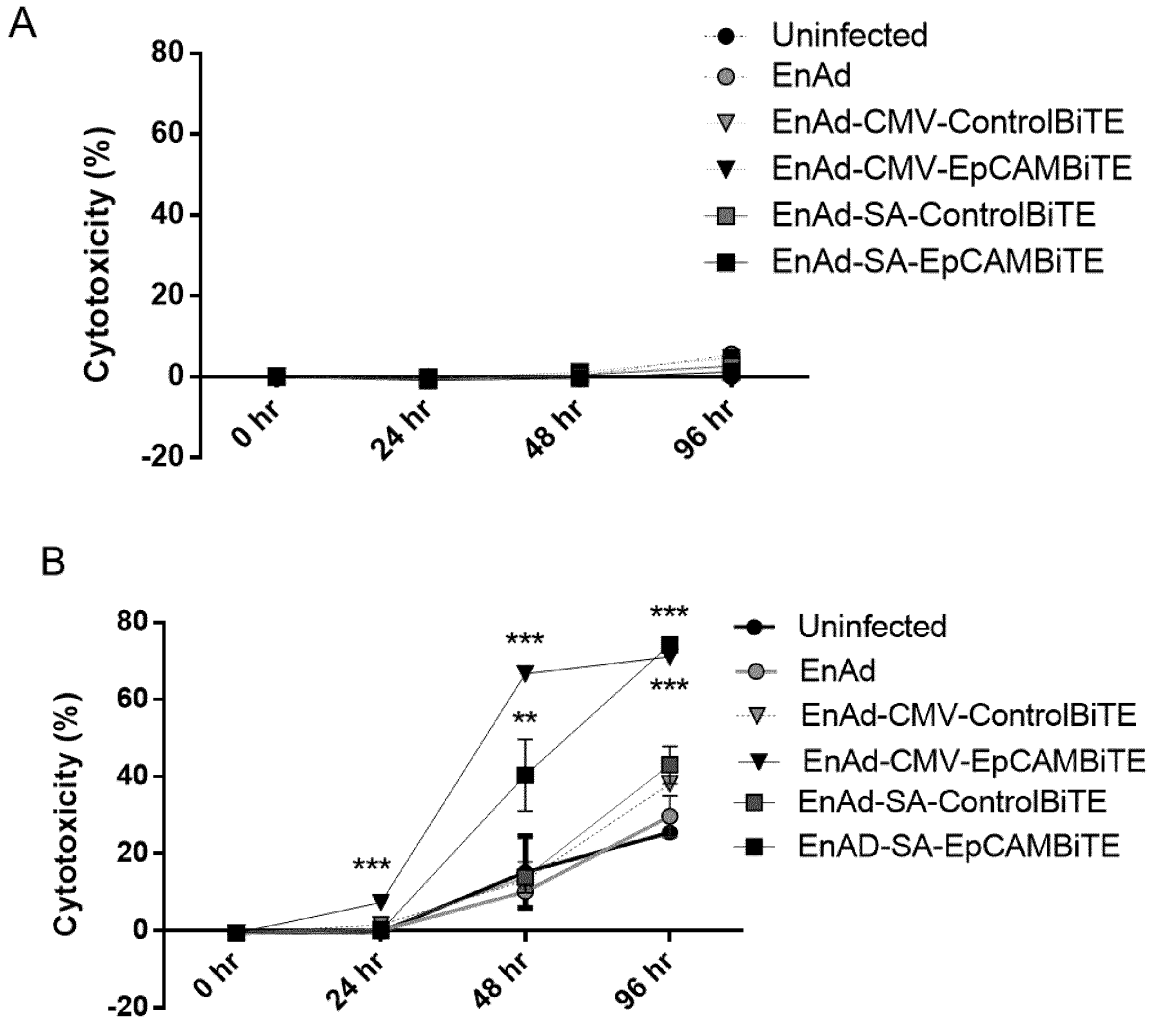
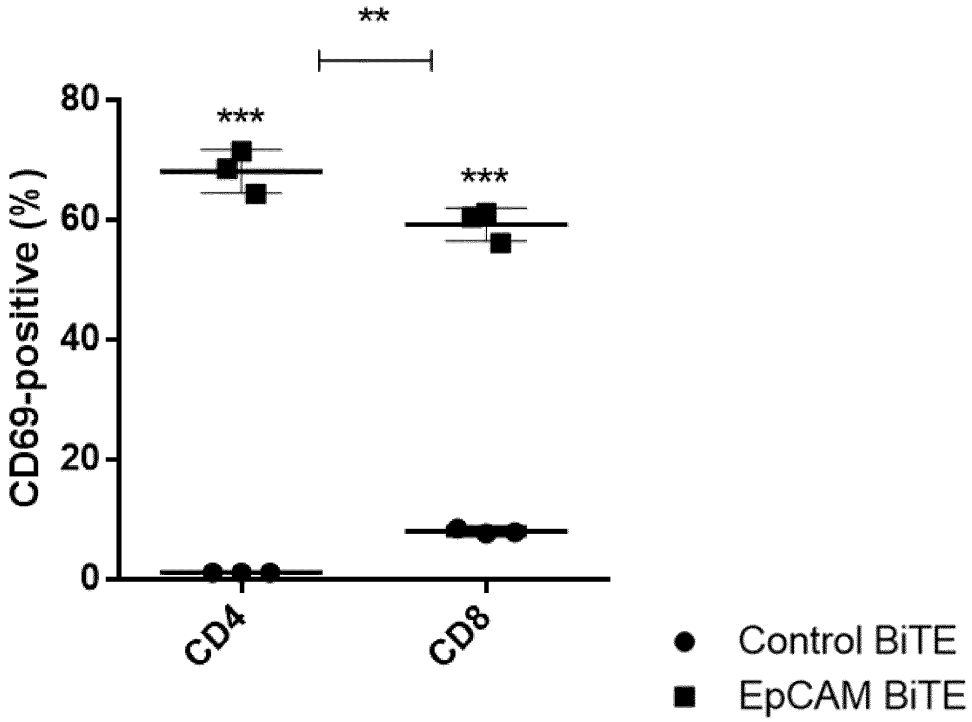
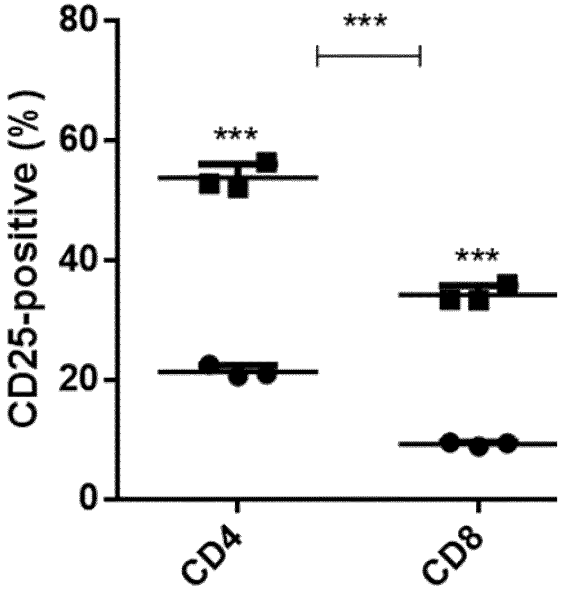

A
Cytotoxicity (%)
80
60
40
20
0
-20
0 hr
24 hr
48 hr
96 hr
Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE
B
Cytotoxicity (%)
***
**
***
***
***
Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAD-SA-EpCAMBiTE

Figure 49A


**
***
***
80
60
40
20
0
CD69-positive (%)
CD4
CD8
Control BiTE
EpCAM BiTE


***
***
***
80
60
40
20
0
CD25-positive (%)
CD4
CD8

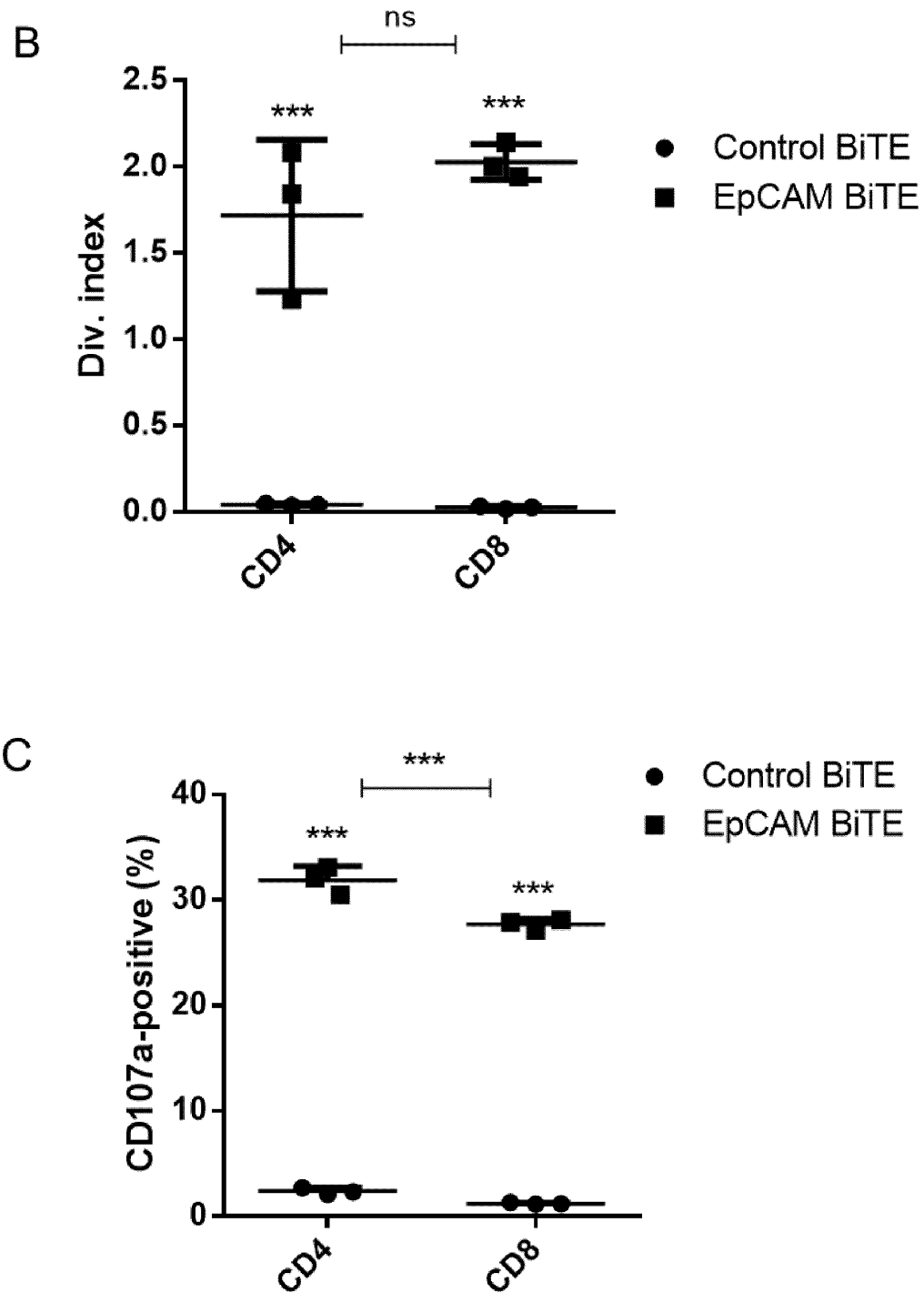


Figure 49 cont.

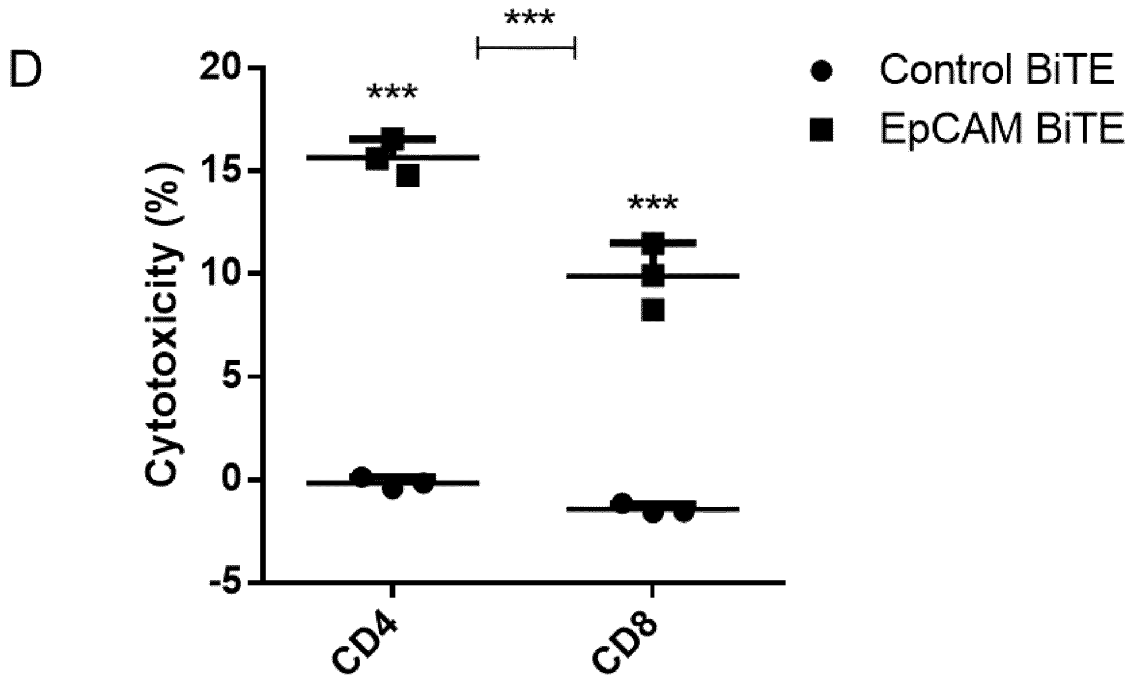


Figure 49 cont.

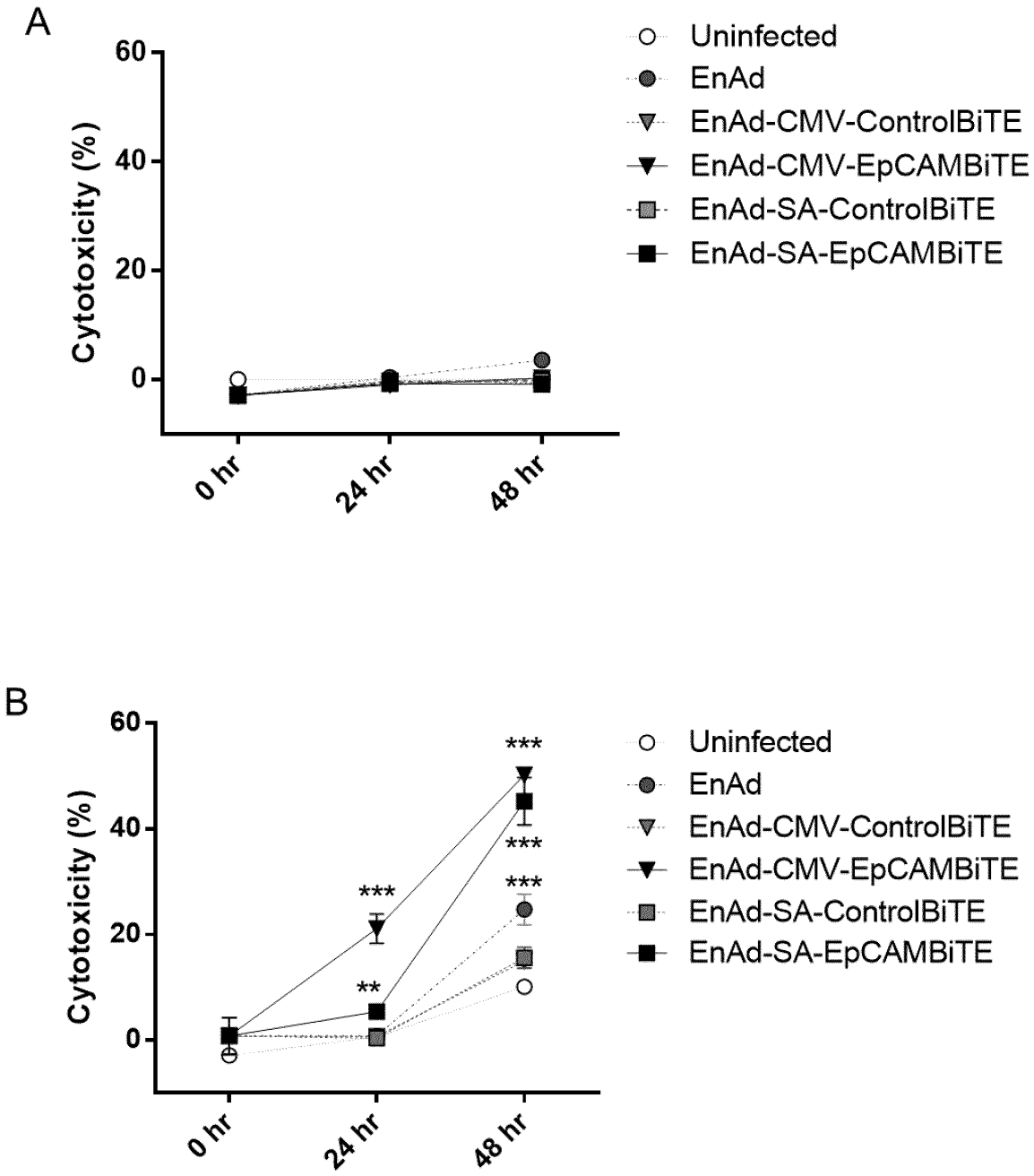

A
Cytotoxicity (%)
60
40
20
0
0 hr
24 hr
48 hr
Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE
B
Cytotoxicity (%)
60
40
20
0
***
***
***
**
0 hr
24 hr
48 hr
Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE

Figure 50

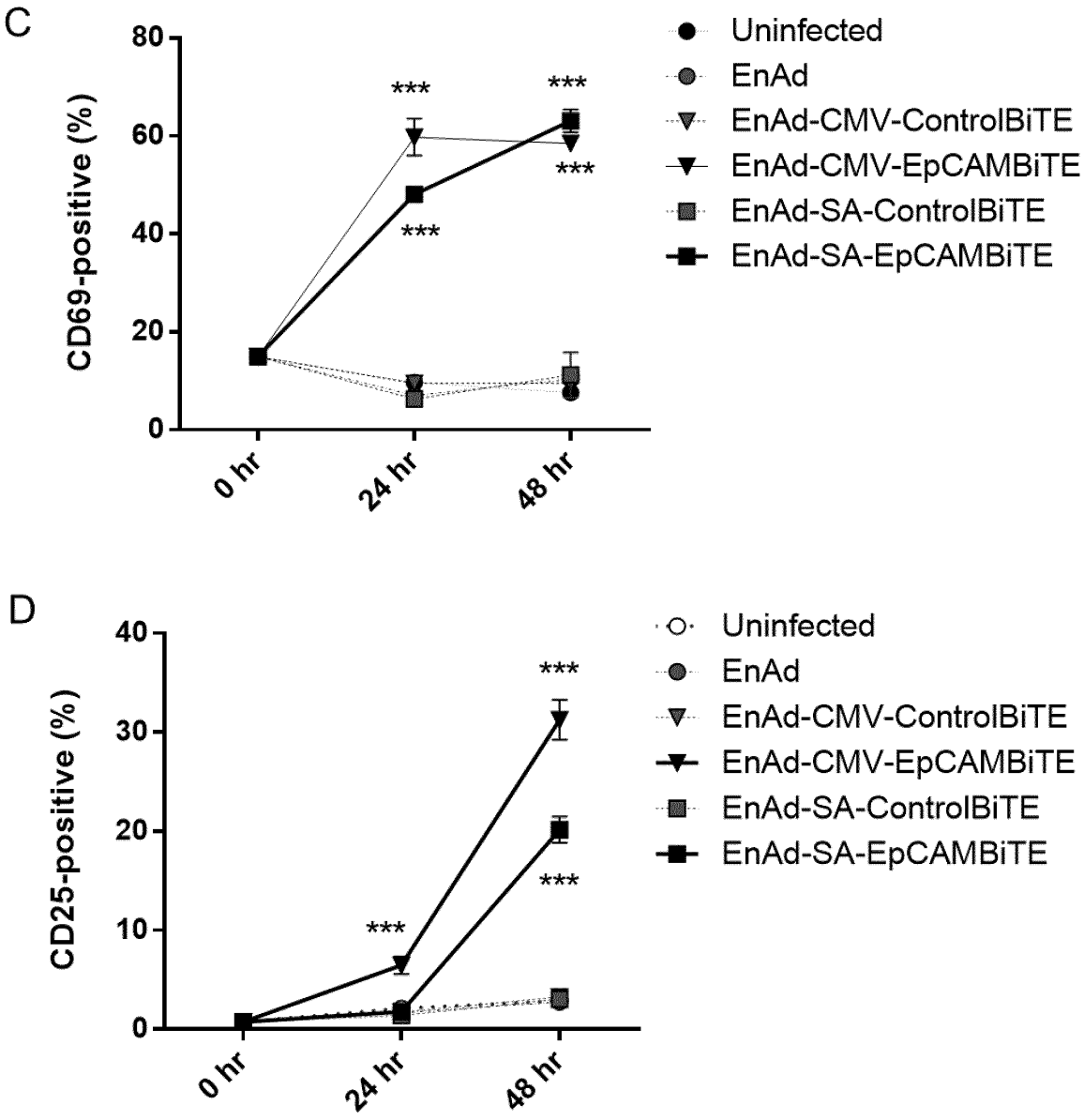
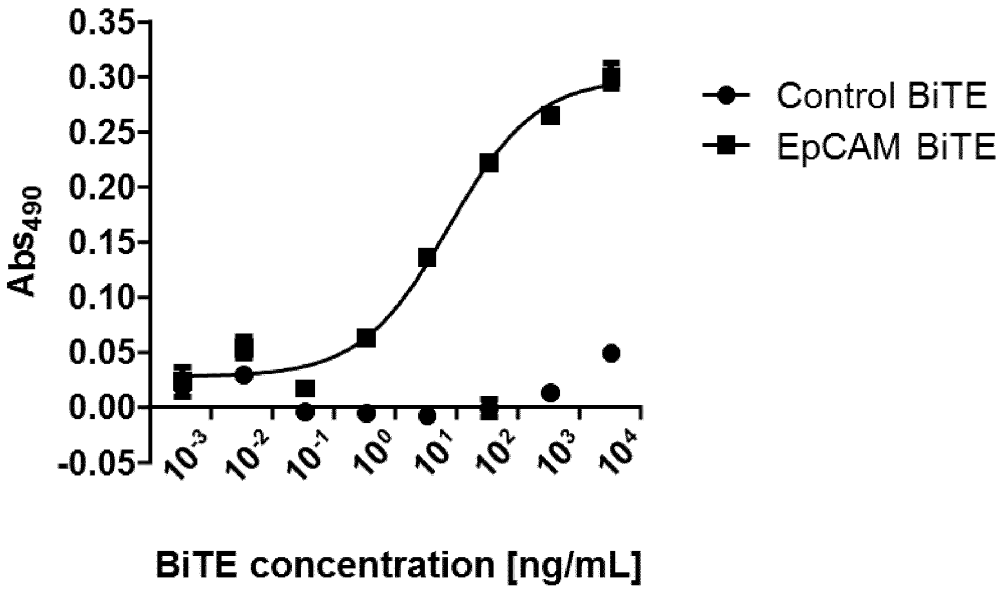


Figure 50 cont.

E



EC50 7.444

F

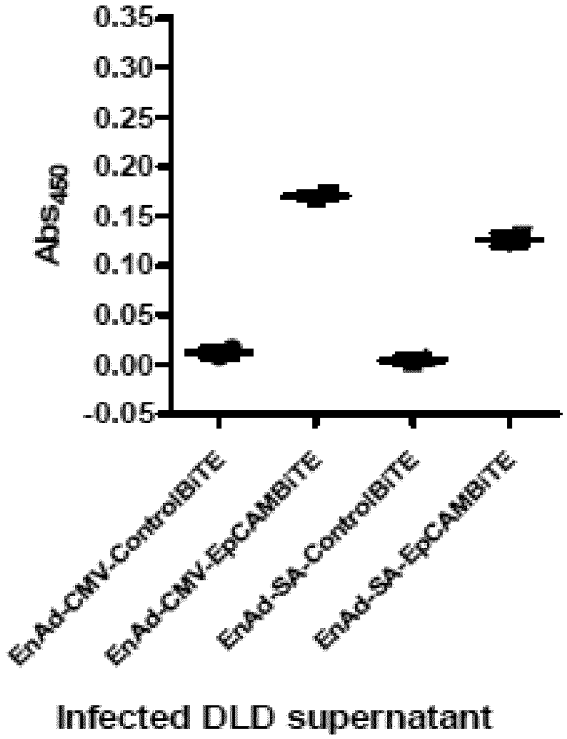


Figure 50 cont.

G

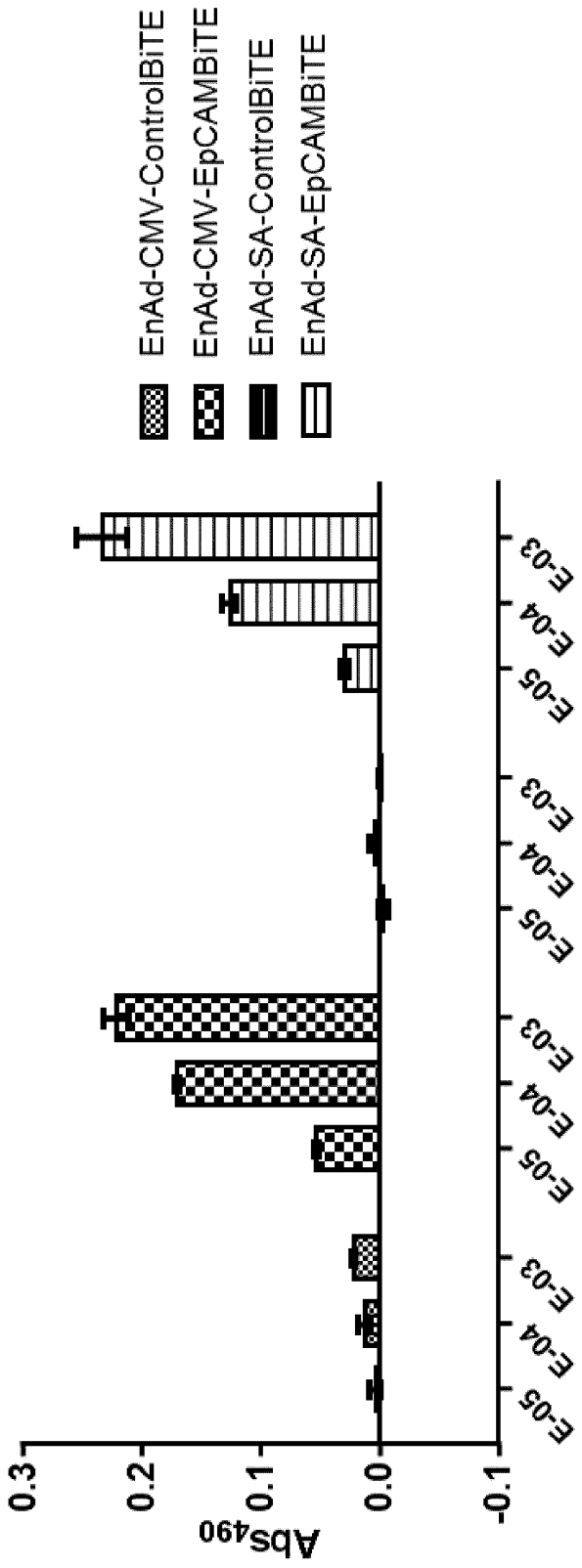


Figure 50 cont.

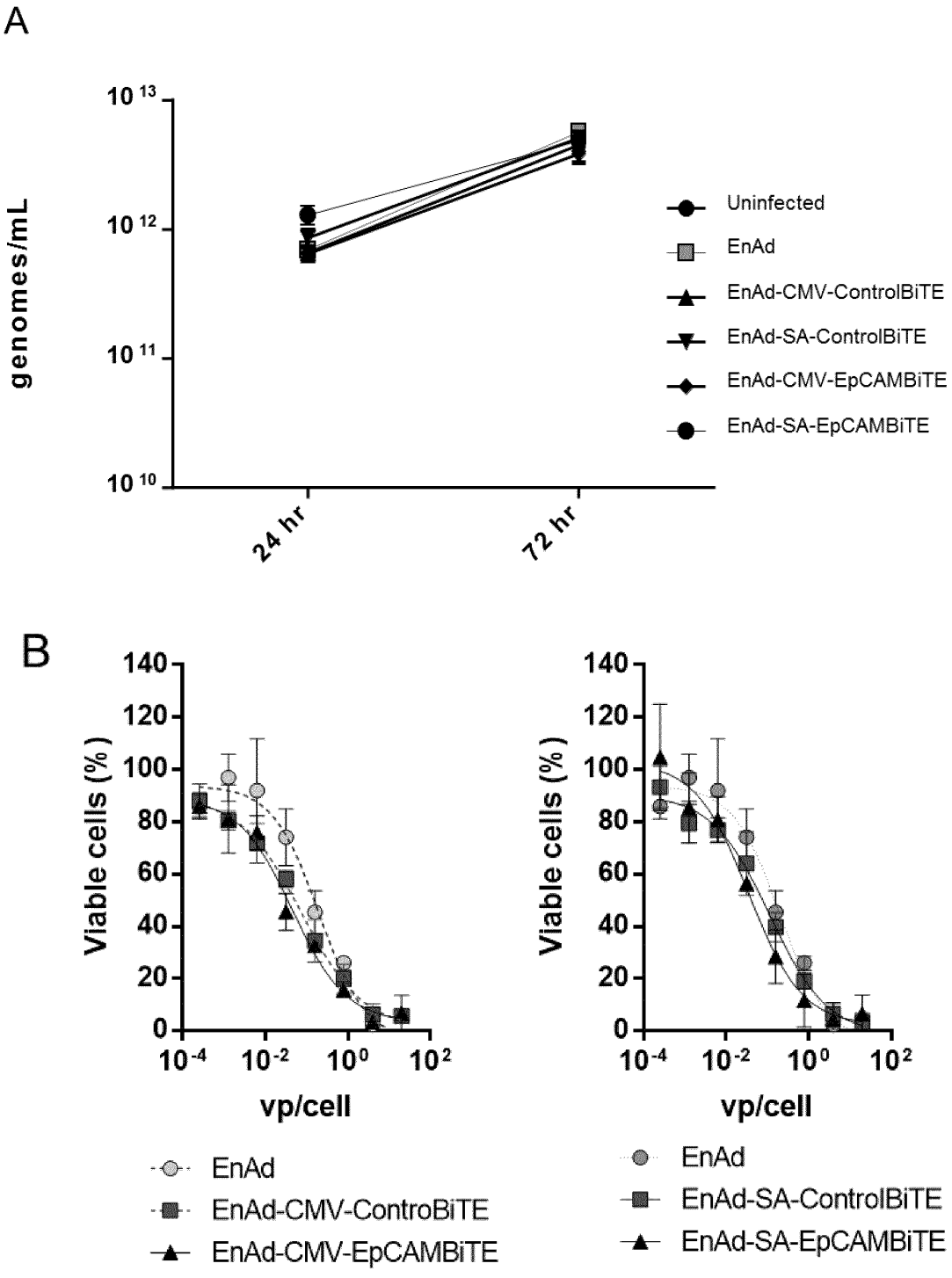

A
genomes/mL
10 13
10 12
10 11
10 10
24 hr
72 hr
Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-SA-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-EpCAMBiTE
B
Viable cells (%)
140
120
100
80
60
40
20
0
10-4
10-2
100
102
vp/cell
EnAd
EnAd-CMV-ControBiTE
EnAd-CMV-EpCAMBiTE
Viable cells (%)
vp/cell
EnAd
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE

Figure 51

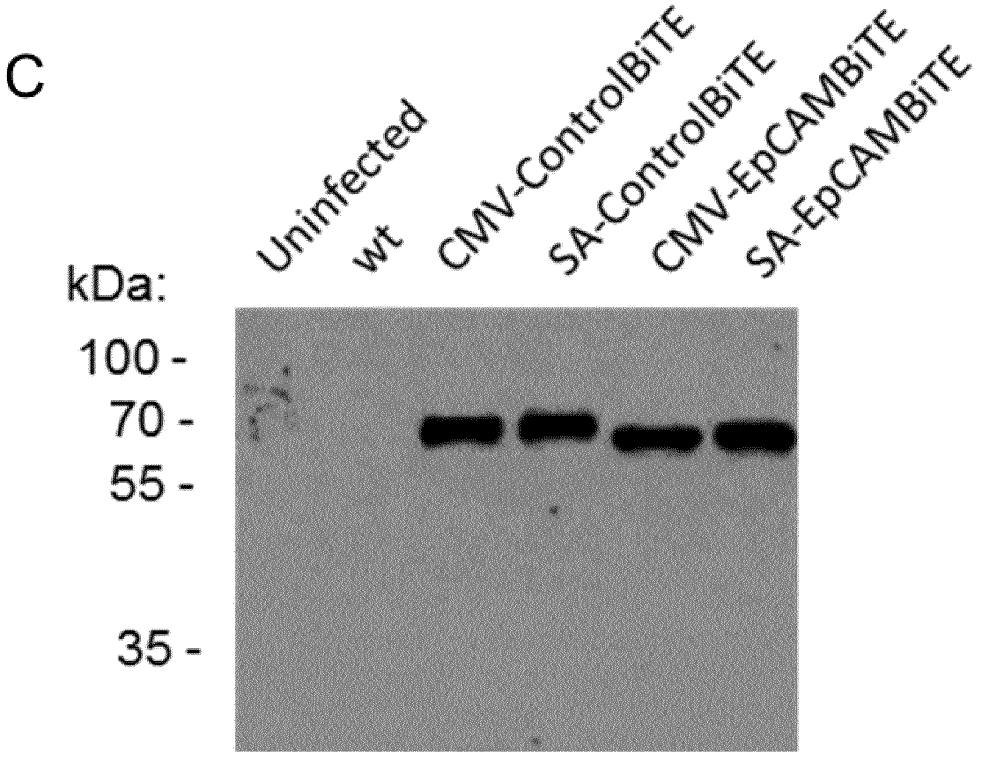


Figure 51 cont.

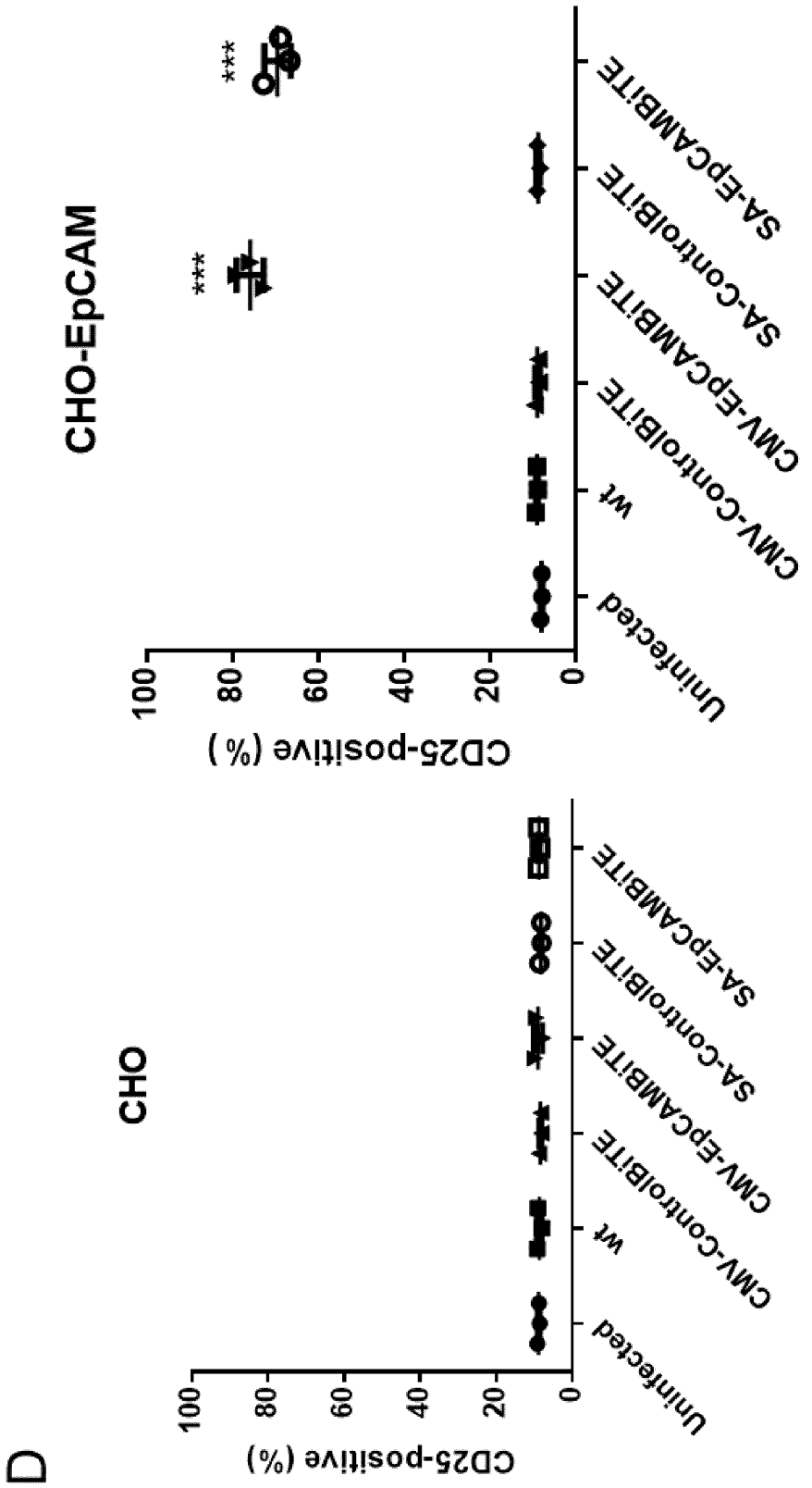


Figure 51 cont.

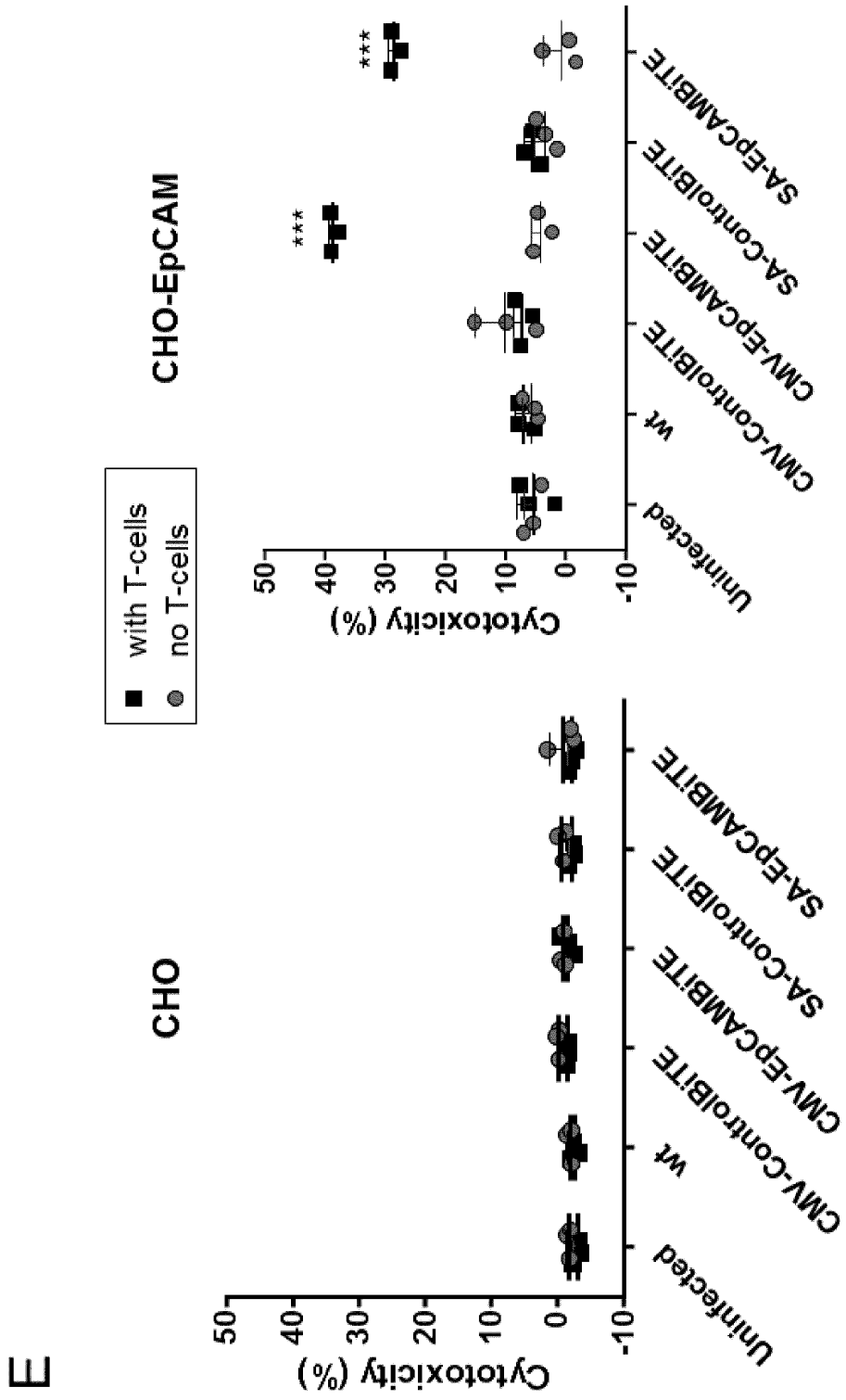


Figure 51 cont.

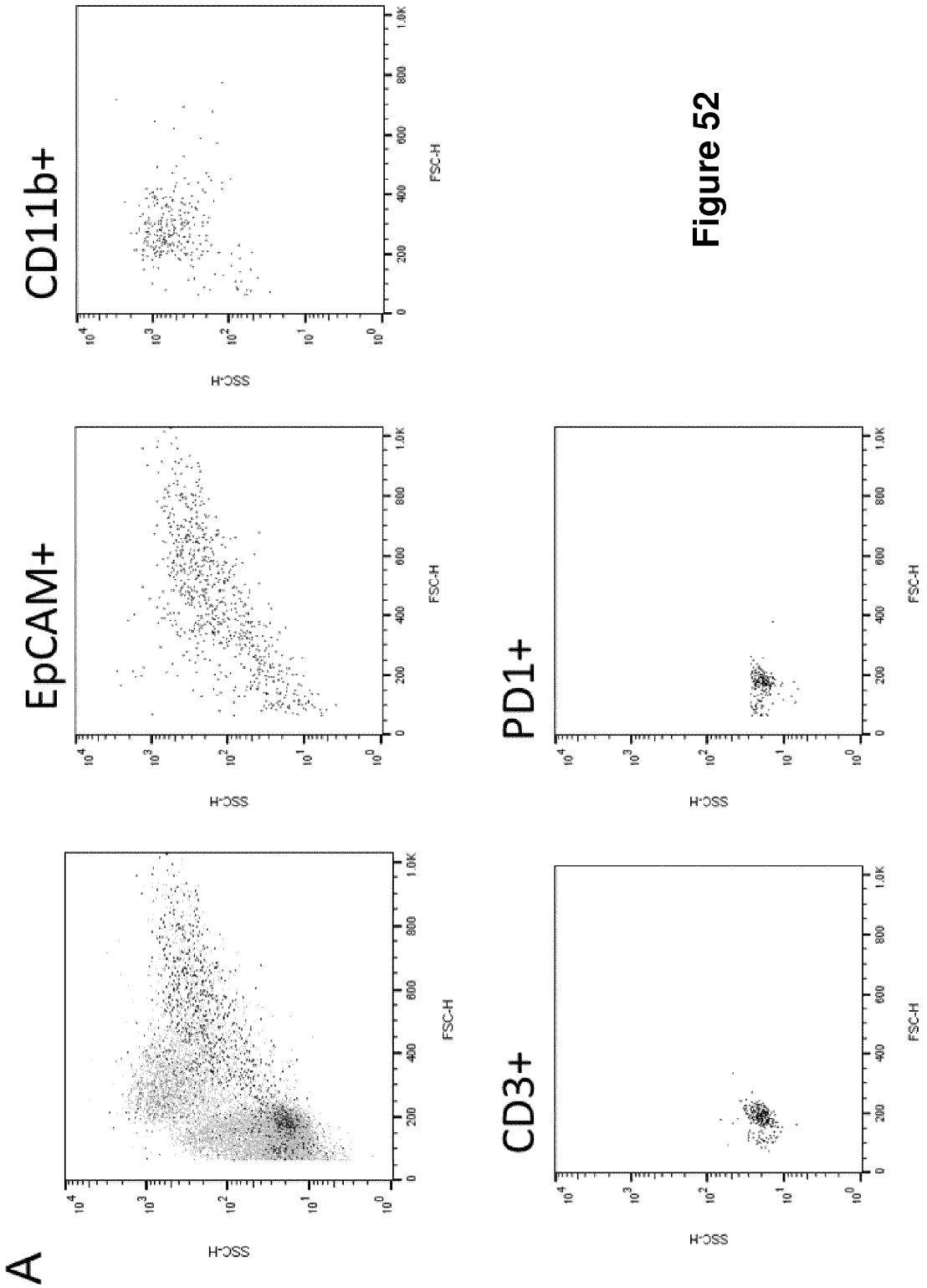


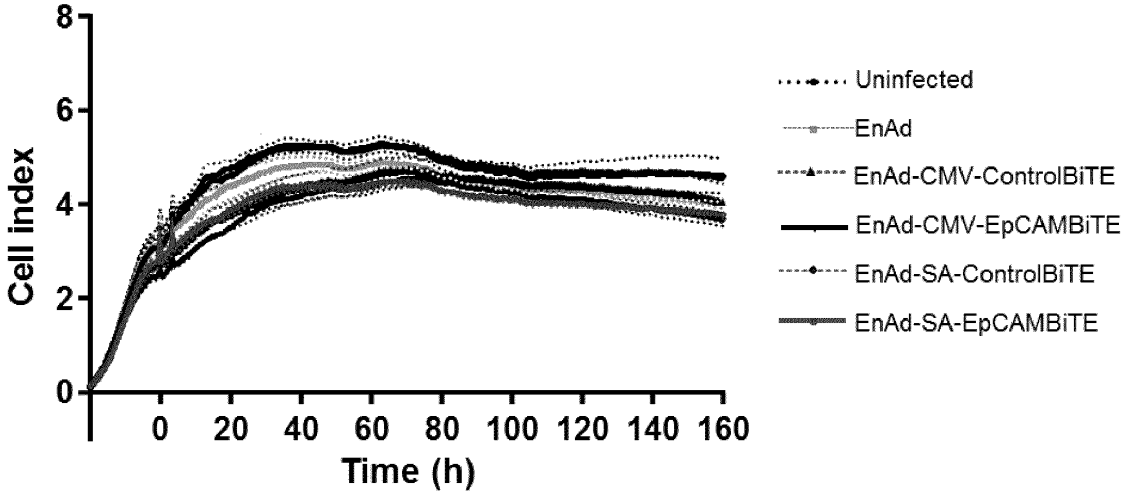
Figure 52

B

Subset	Count
EpCAM	749
CD3	289
PD1	206
CD11b	277
Total	10000

Figure 52

A


Cell index
Time (h)
0
2
4
6
8
0 20 40 60 80 100 120 140 160
Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE

B

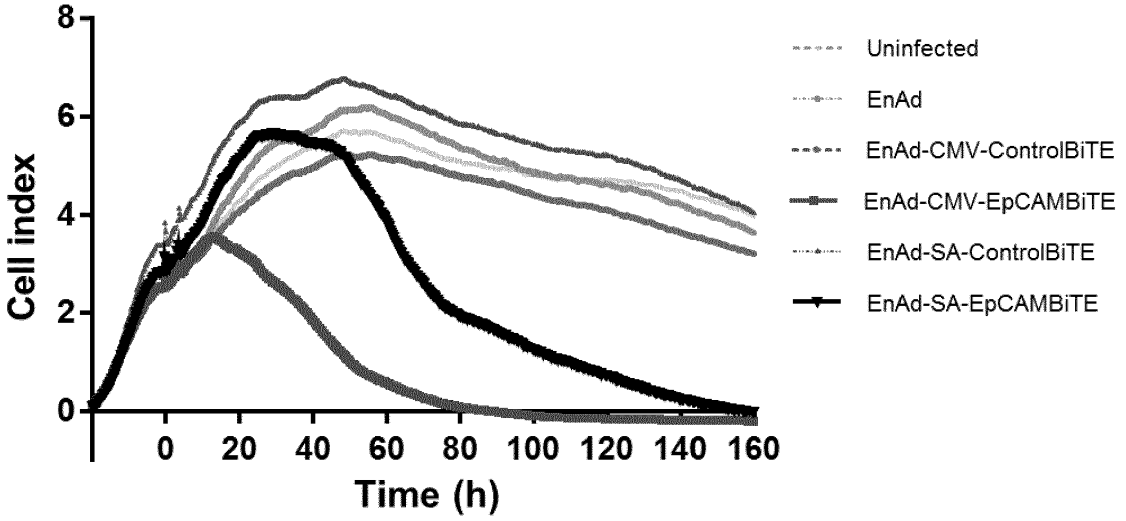

Cell index
Time (h)
0
2
4
6
8
0 20 40 60 80 100 120 140 160
Uninfected
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE

Figure 53

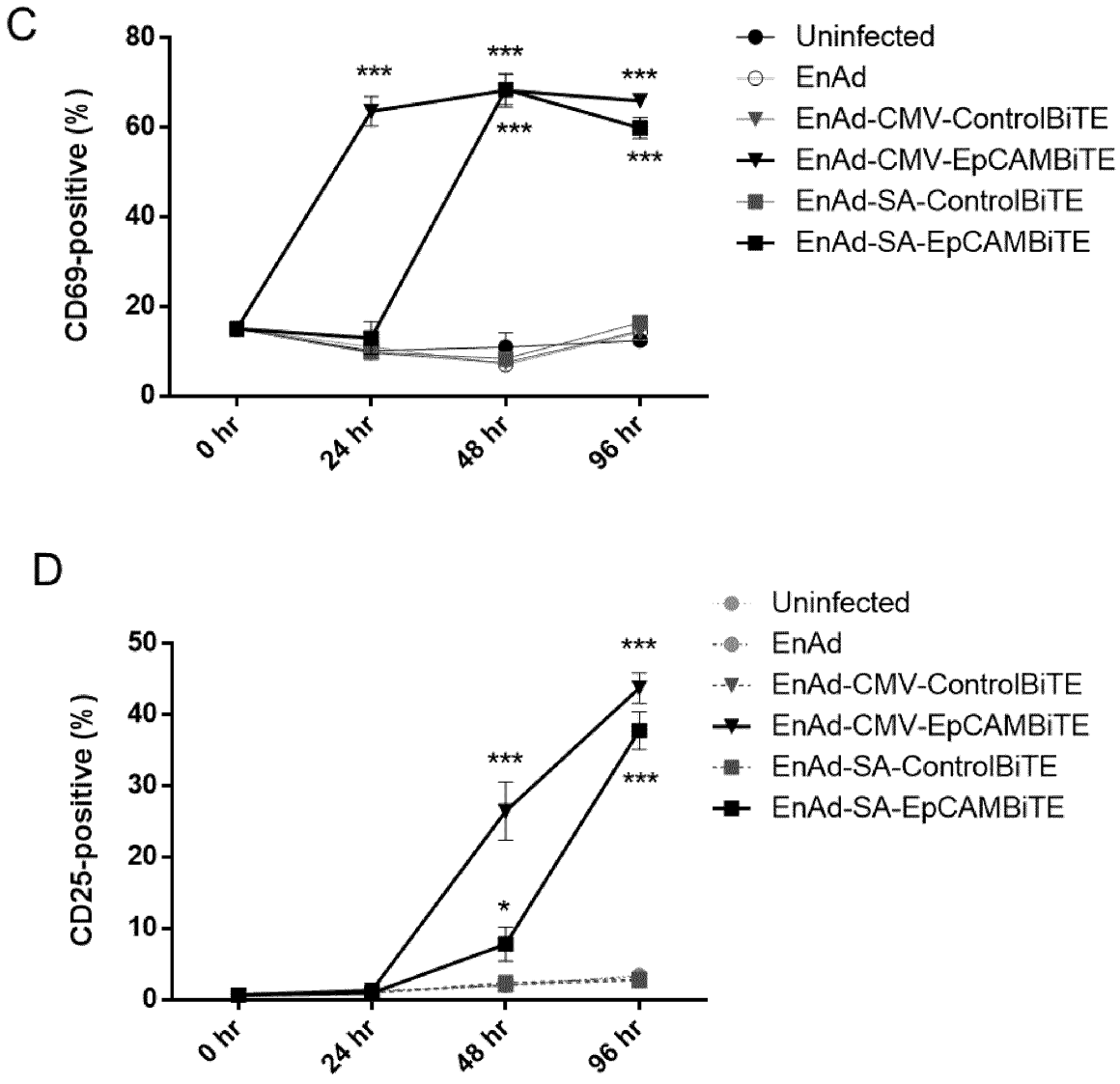


Figure 53 cont.

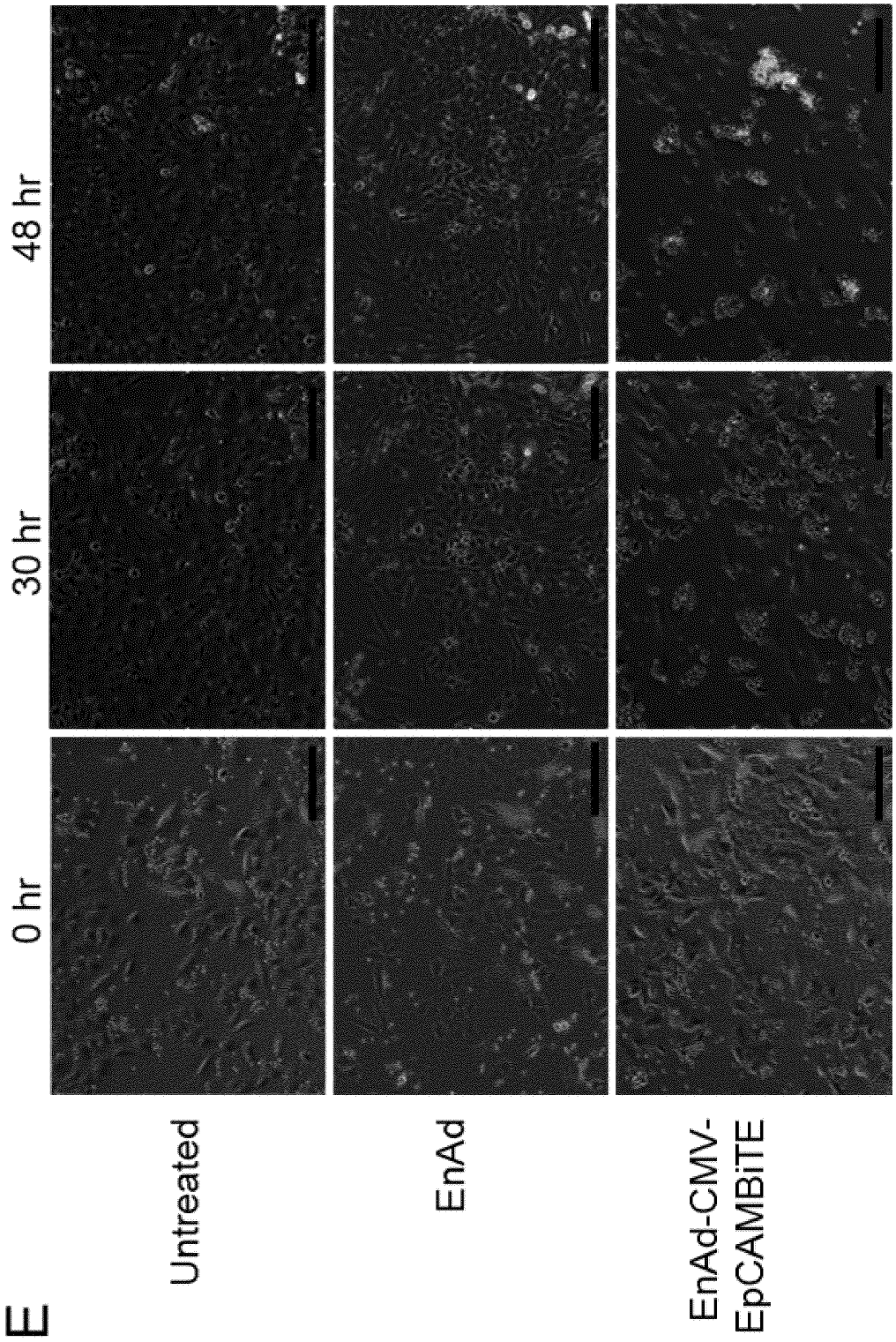


Figure 53 cont.

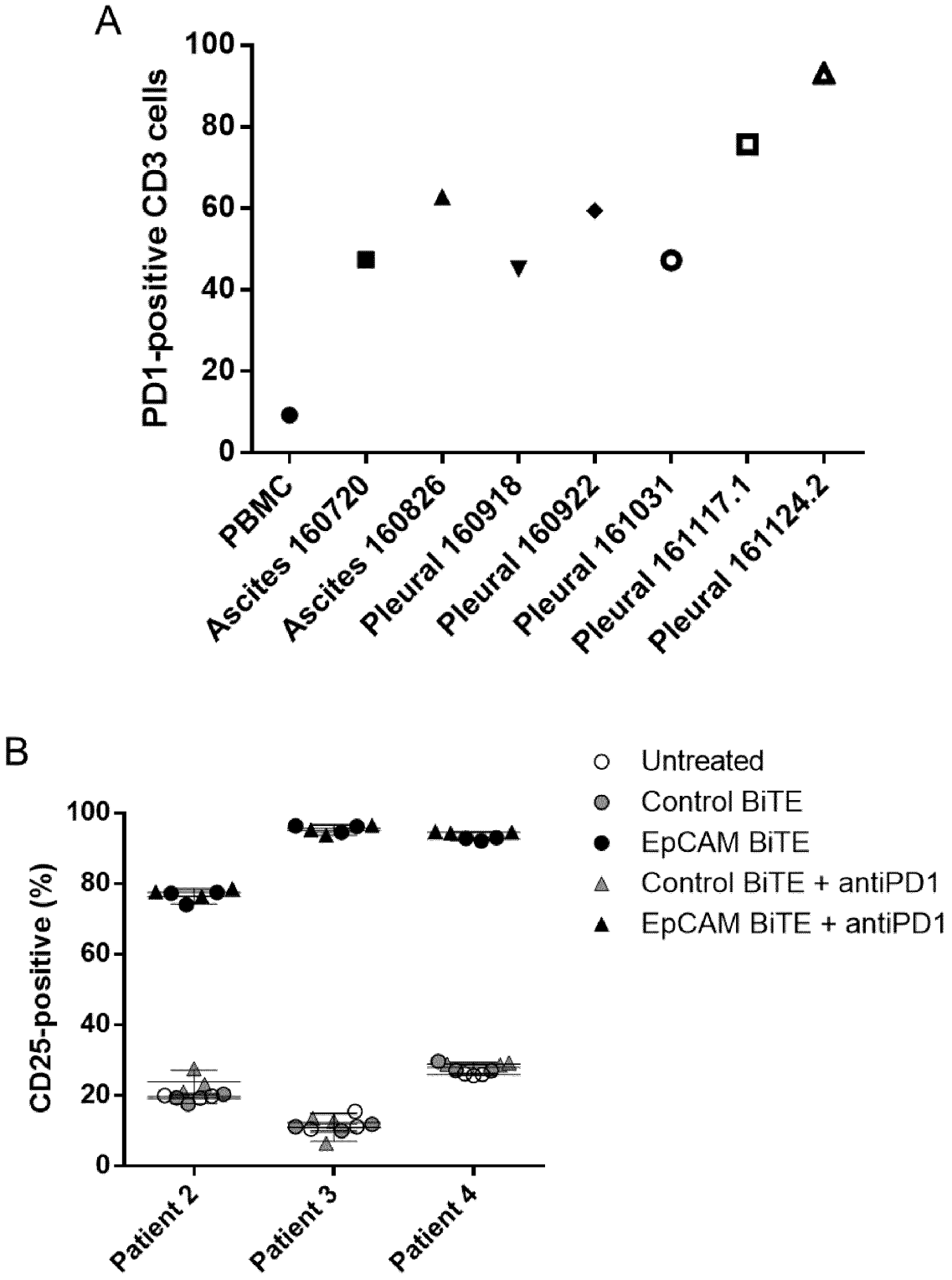

A
PD1-positive CD3 cells
100
80
60
40
20
0
PBMC
Ascites 160720
Ascites 160826
Pleural 160918
Pleural 160922
Pleural 161031
Pleural 161117.1
Pleural 161124.2
B
CD25-positive (%)
100
80
60
40
20
0
Patient 2
Patient 3
Patient 4
Untreated
Control BiTE
EpCAM BiTE
Control BiTE + antiPD1
EpCAM BiTE + antiPD1

Figure 54

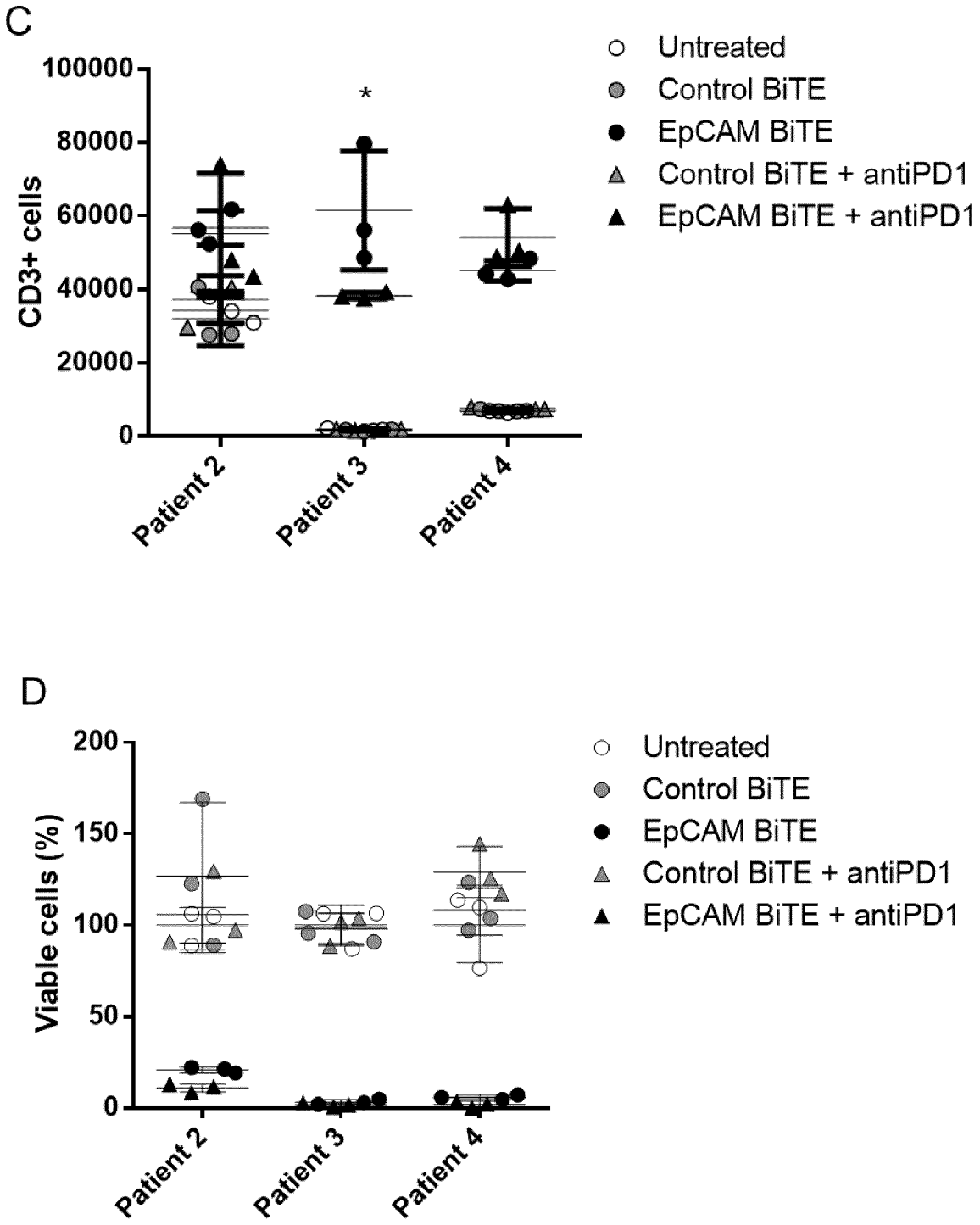


Figure 54 cont.

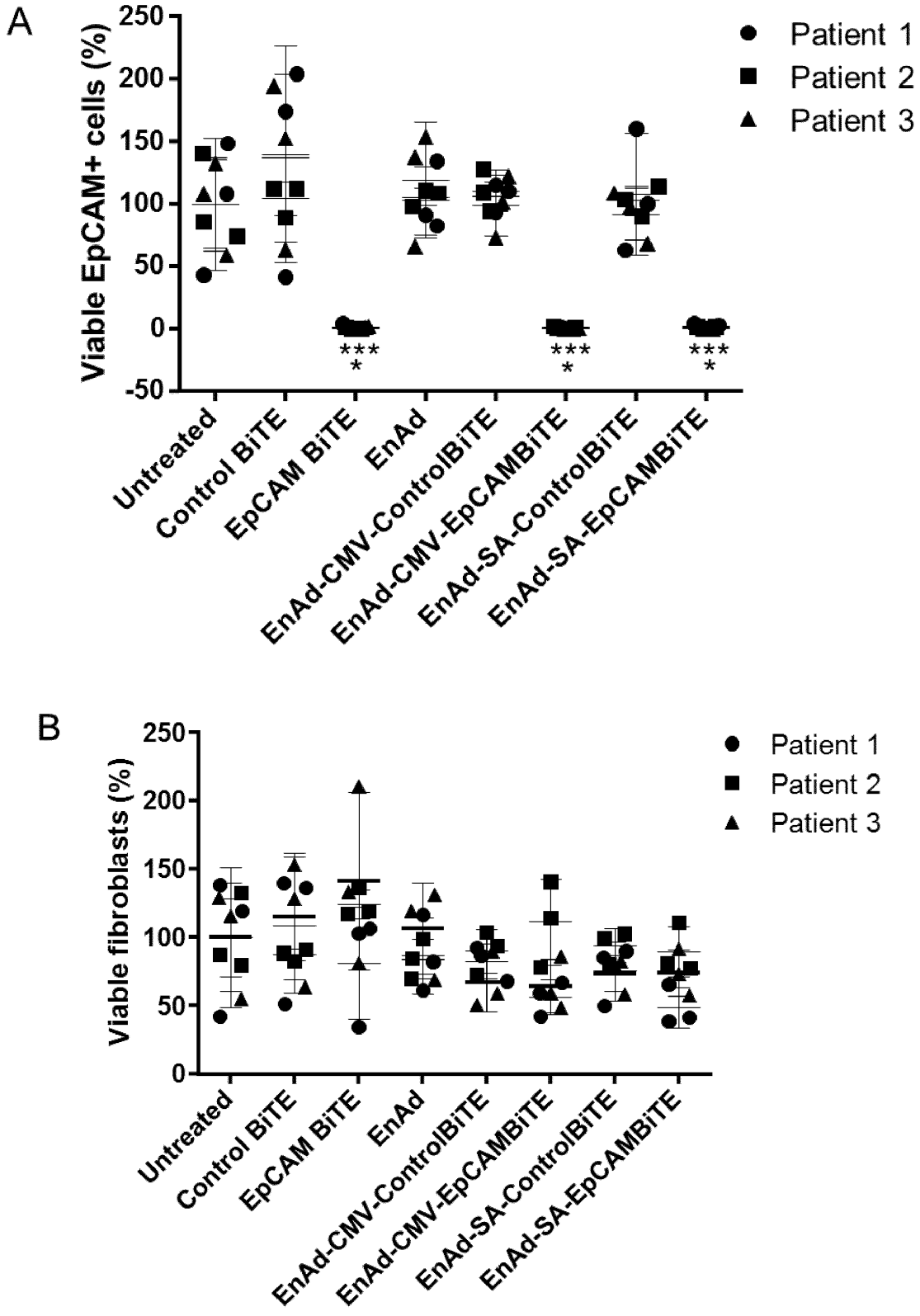

A
Viable EpCAM+ cells (%)
250
200
150
100
50
0
-50
***
*
Patient 1
Patient 2
Patient 3
Untreated
Control BiTE
EpCAM BiTE
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE
B
Viable fibroblasts (%)
250
200
150
100
50
0
Patient 1
Patient 2
Patient 3
Untreated
Control BiTE
EpCAM BiTE
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE

Figure 55

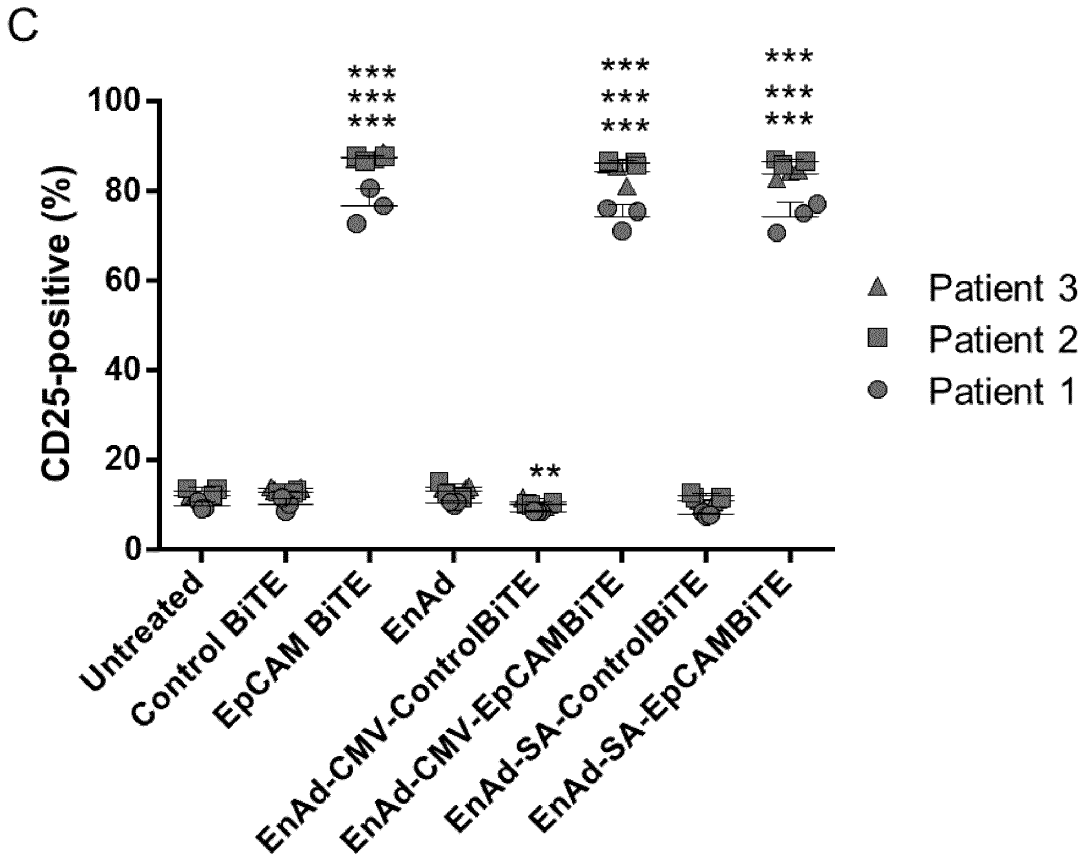


Figure 55 cont.

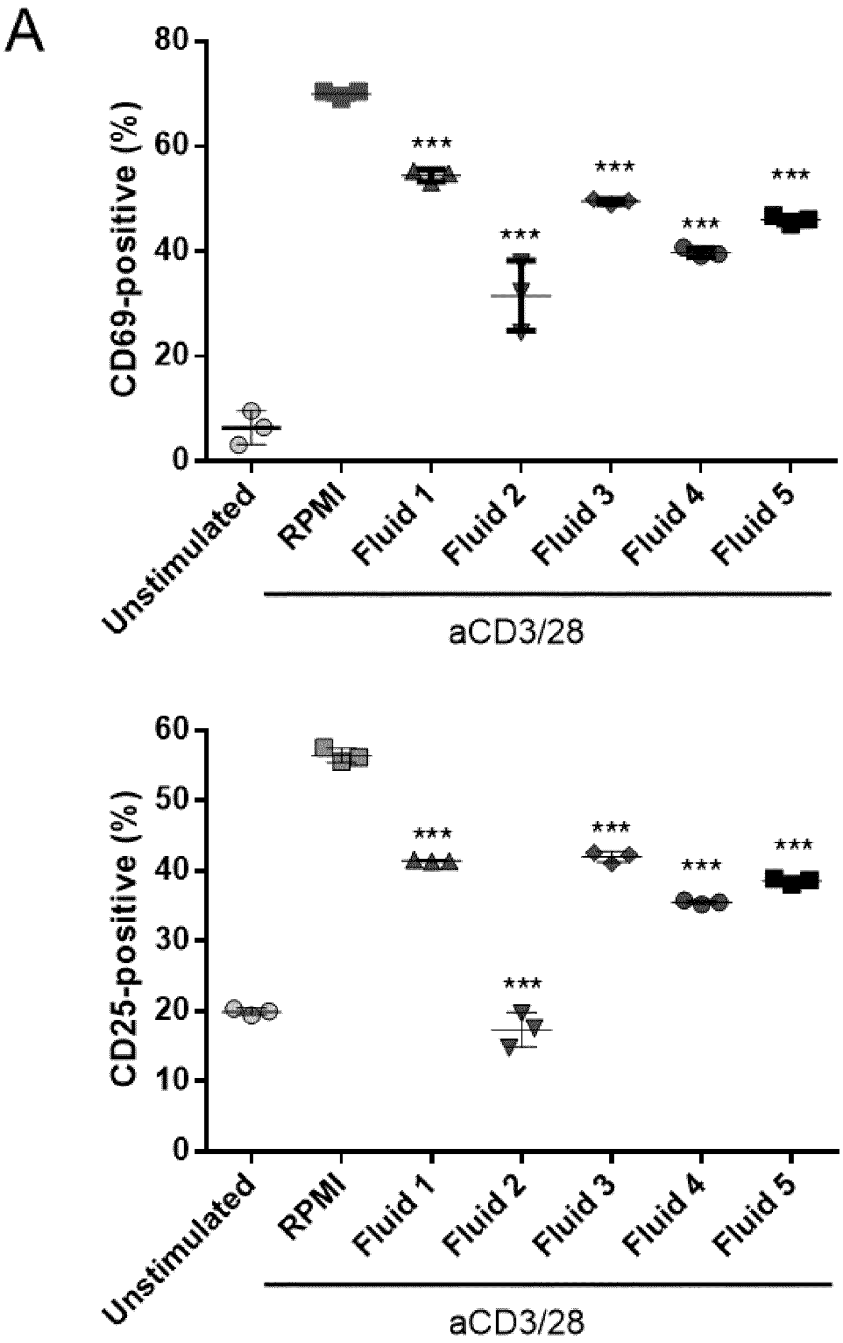

A
CD69-positive (%)
80
60
40
20
0
***
Unstimulated
RPMI
Fluid 1
Fluid 2
Fluid 3
Fluid 4
Fluid 5
aCD3/28
CD25-positive (%)
60
50
40
30
20
10
0

Figure 56

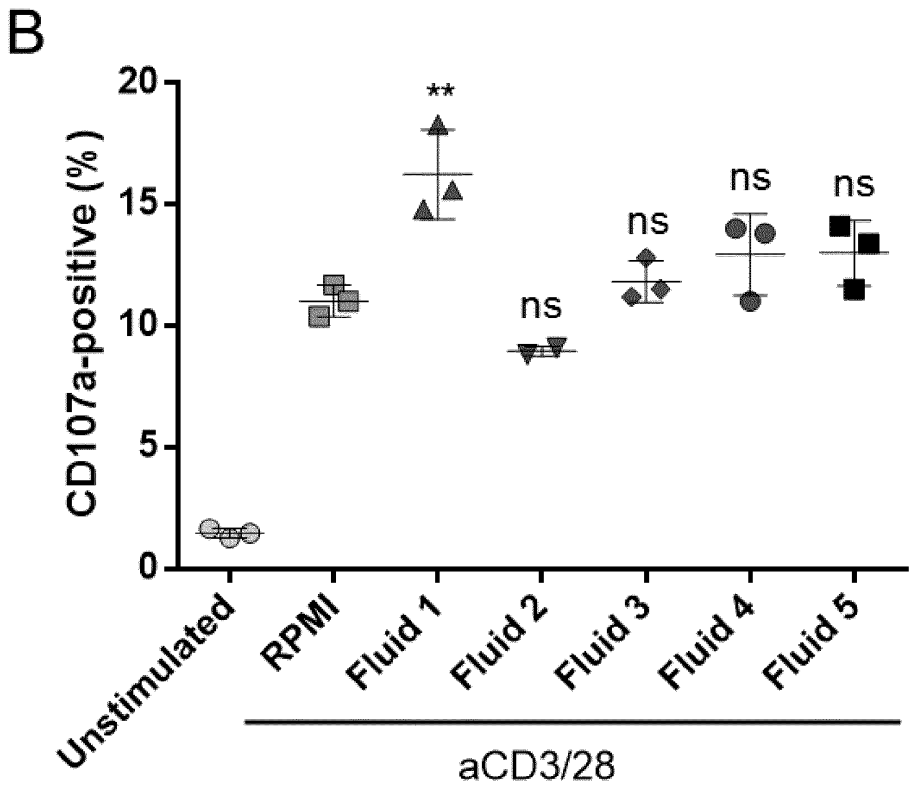


Figure 56 cont.

C

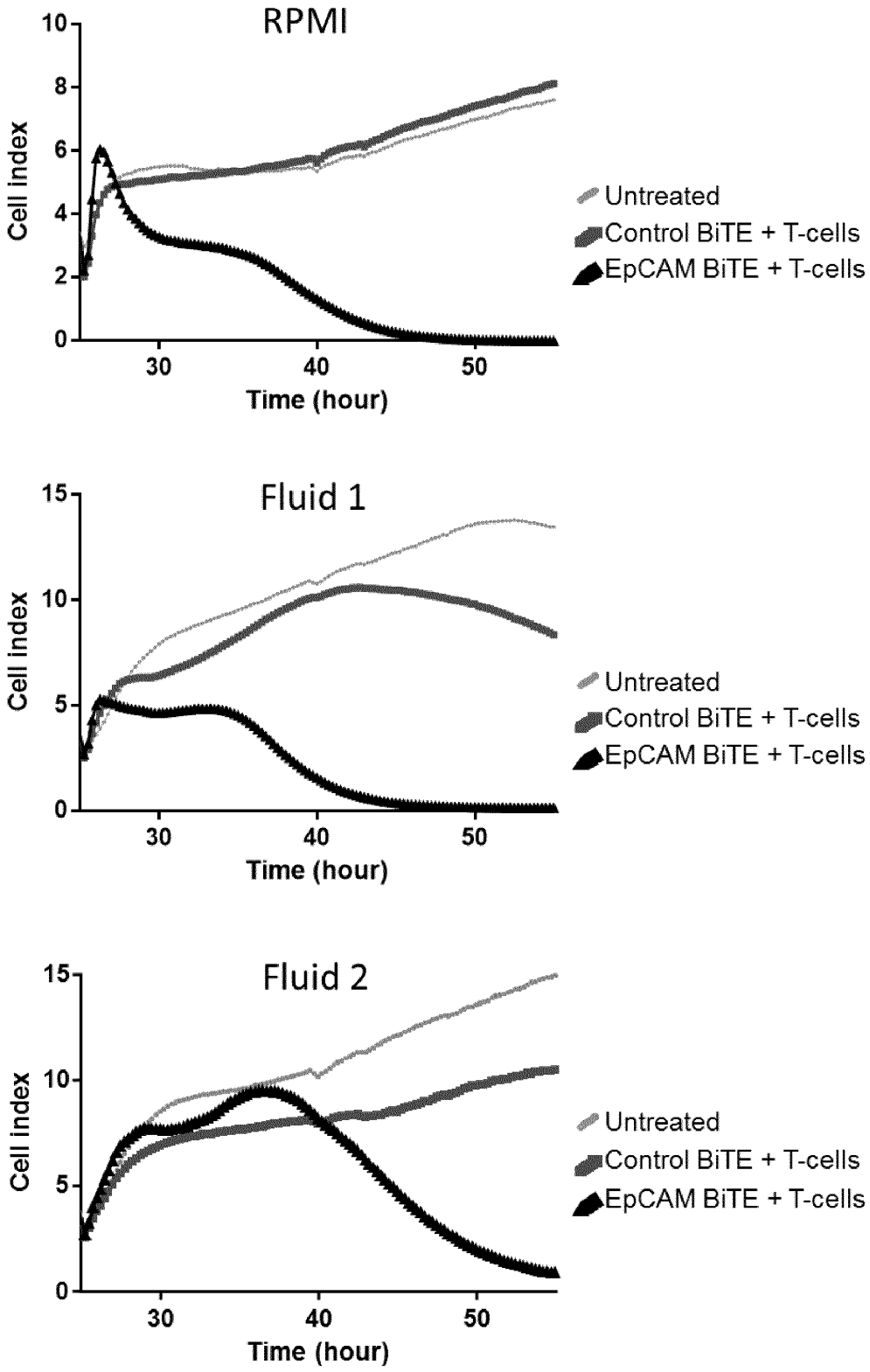


Figure 56 cont.

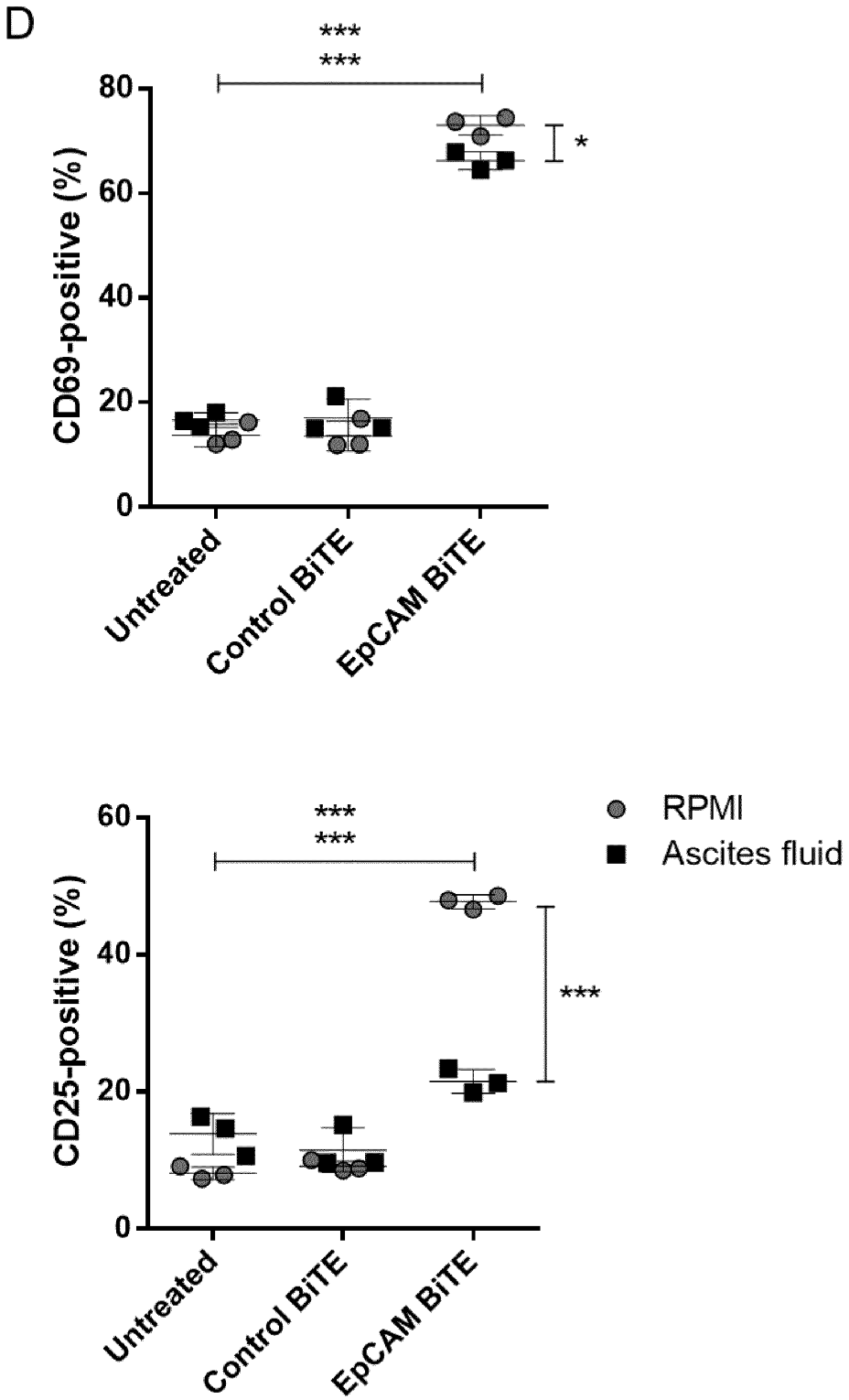


Figure 56 cont.

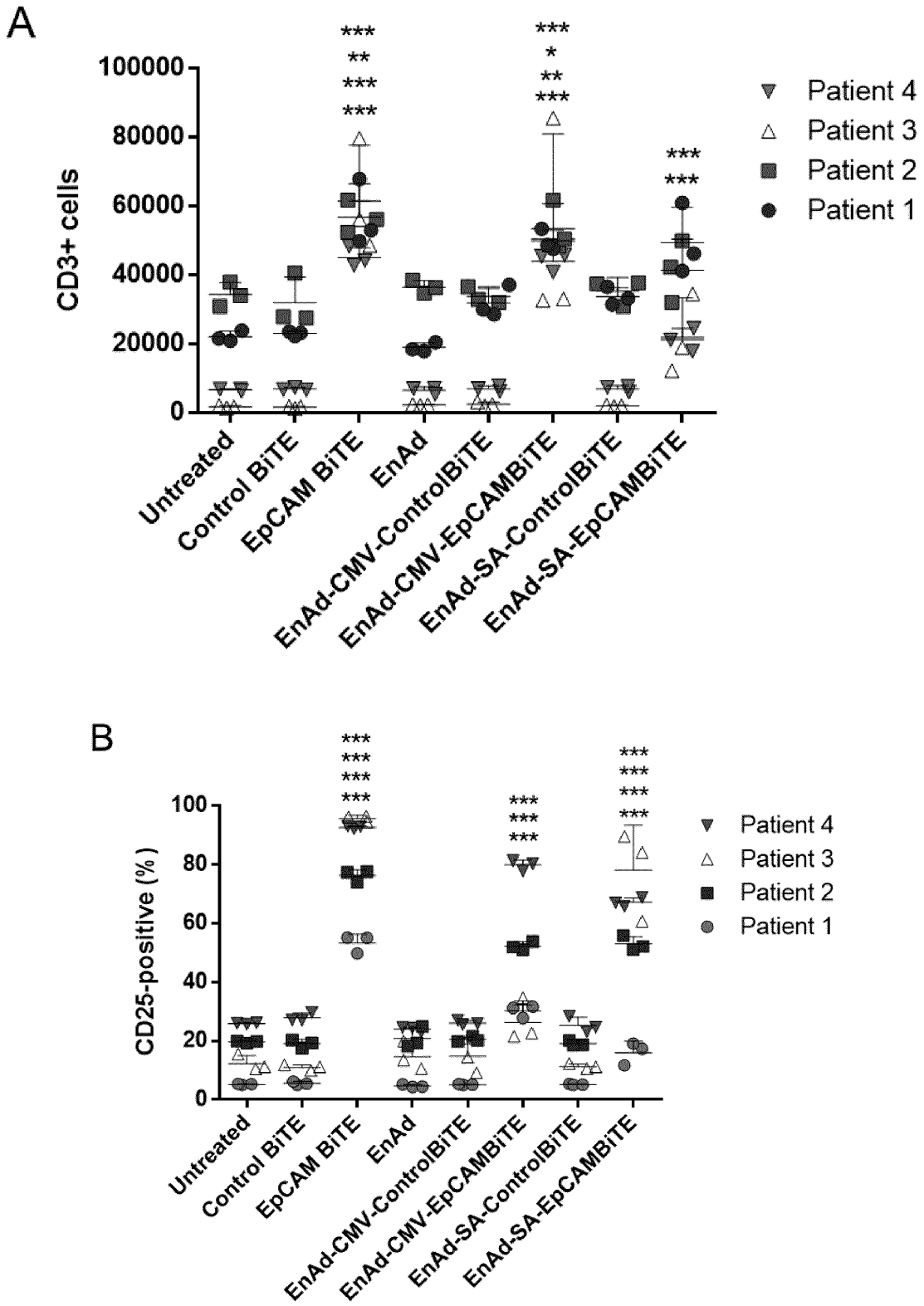

A
CD3+ cells
100000
80000
60000
40000
20000
0
Patient 4
Patient 3
Patient 2
Patient 1
Untreated
Control BiTE
EpCAM BiTE
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE
B
CD25-positive (%)
100
80
60
40
20
0

Figure 57

C

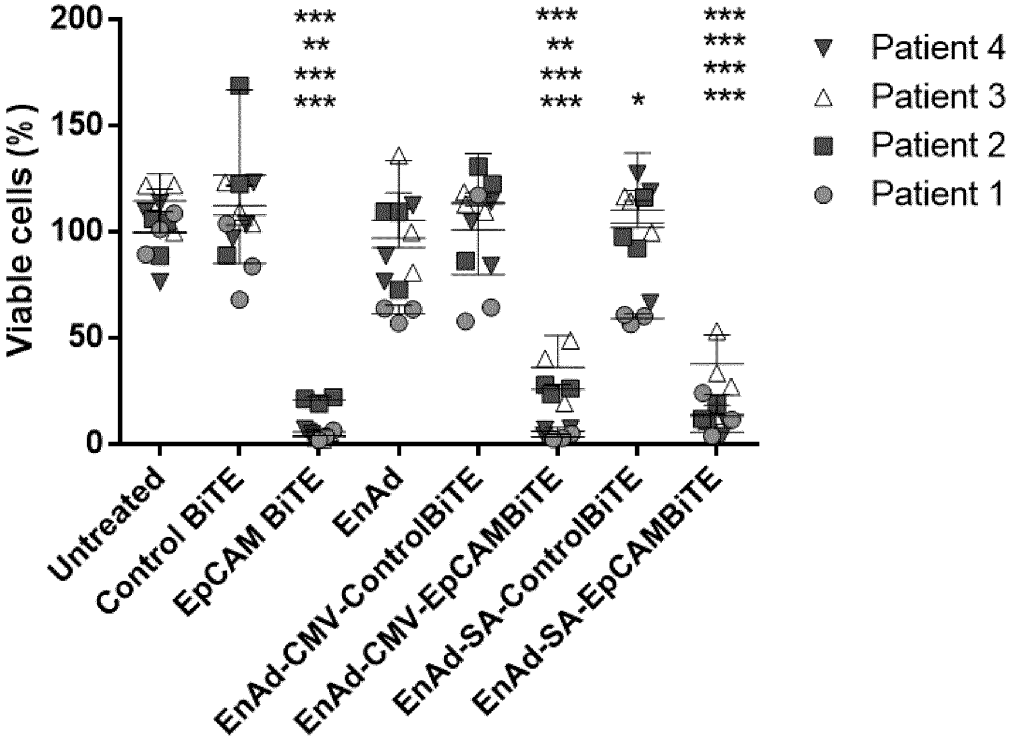
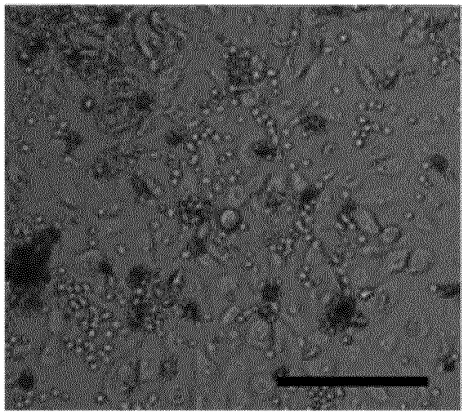
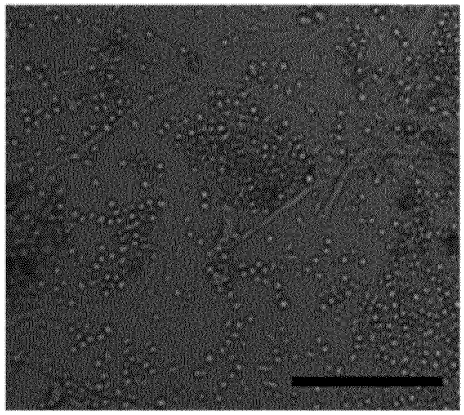


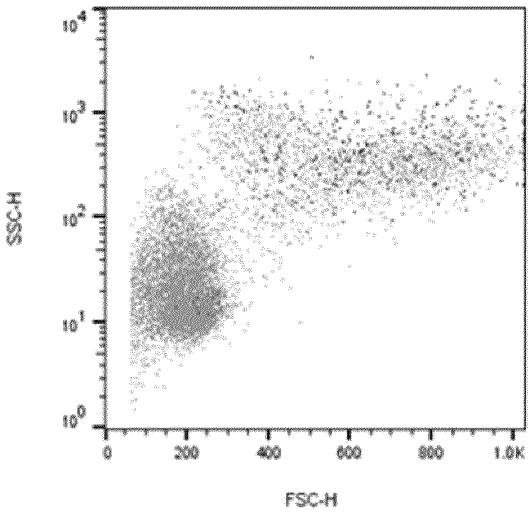
Figure 57 cont.

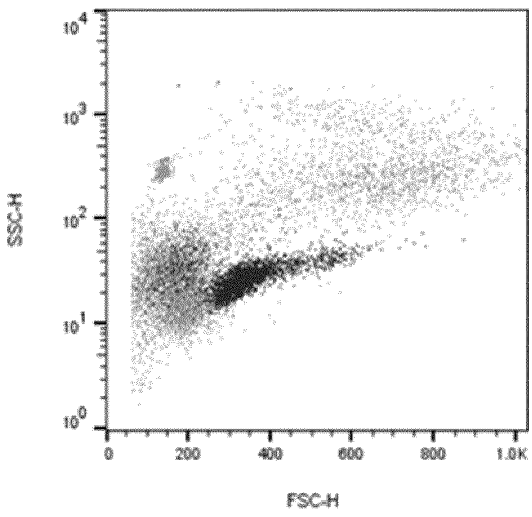
D EnAd

EnAd-CMV-EpCAMBiTE









	Sample Name	Subset Name	Count
■	[conBiTE 1.063]	EpCAM-positive	310
■	[conBiTE 1.063]	CD25-positive	16.0
■	[conBiTE 1.063]	CD3	160
■	[conBiTE 1.063]	Cells only	9408

	Sample Name	Subset Name	Count
■	[epBiTE 1.066]	EpCAM-positive	6.00
■	[epBiTE 1.066]	CD25-positive	3214
■	[epBiTE 1.066]	CD3	3400
■	[epBiTE 1.066]	Ungated	10000

Figure 57 cont.

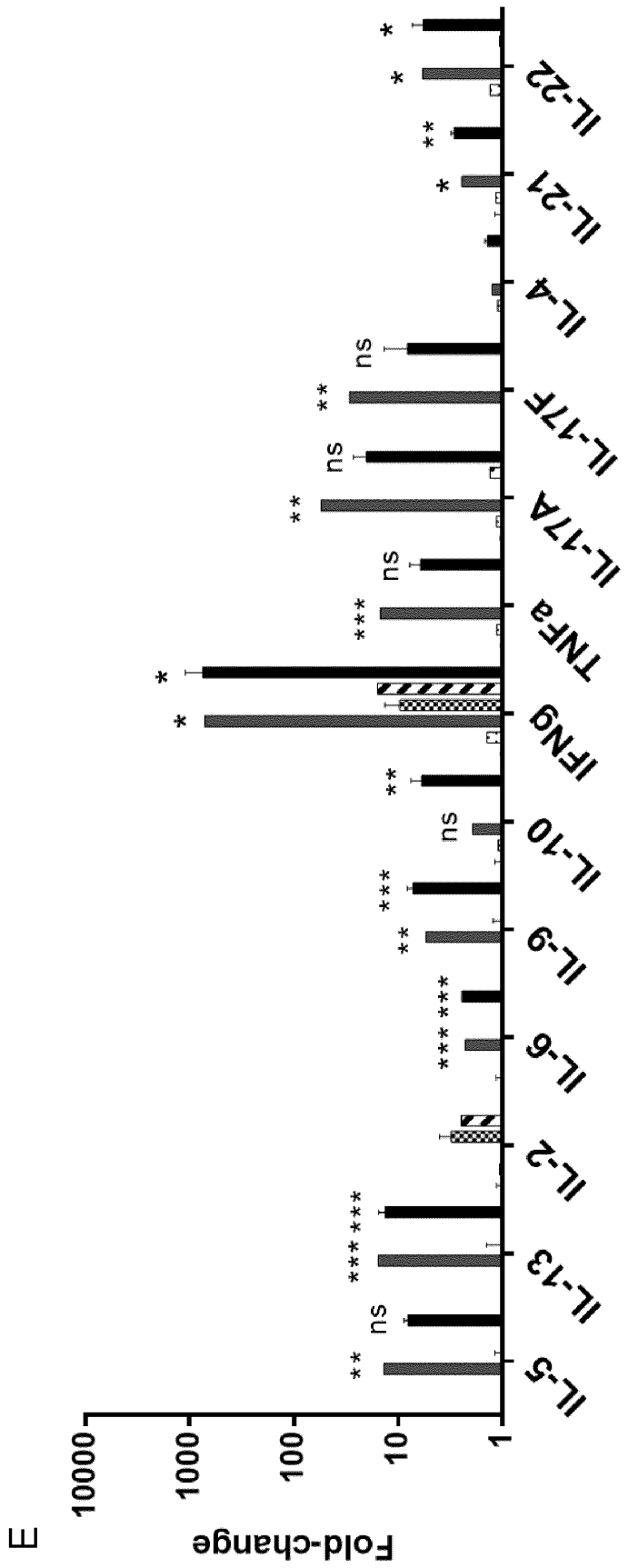


Figure 57 cont.

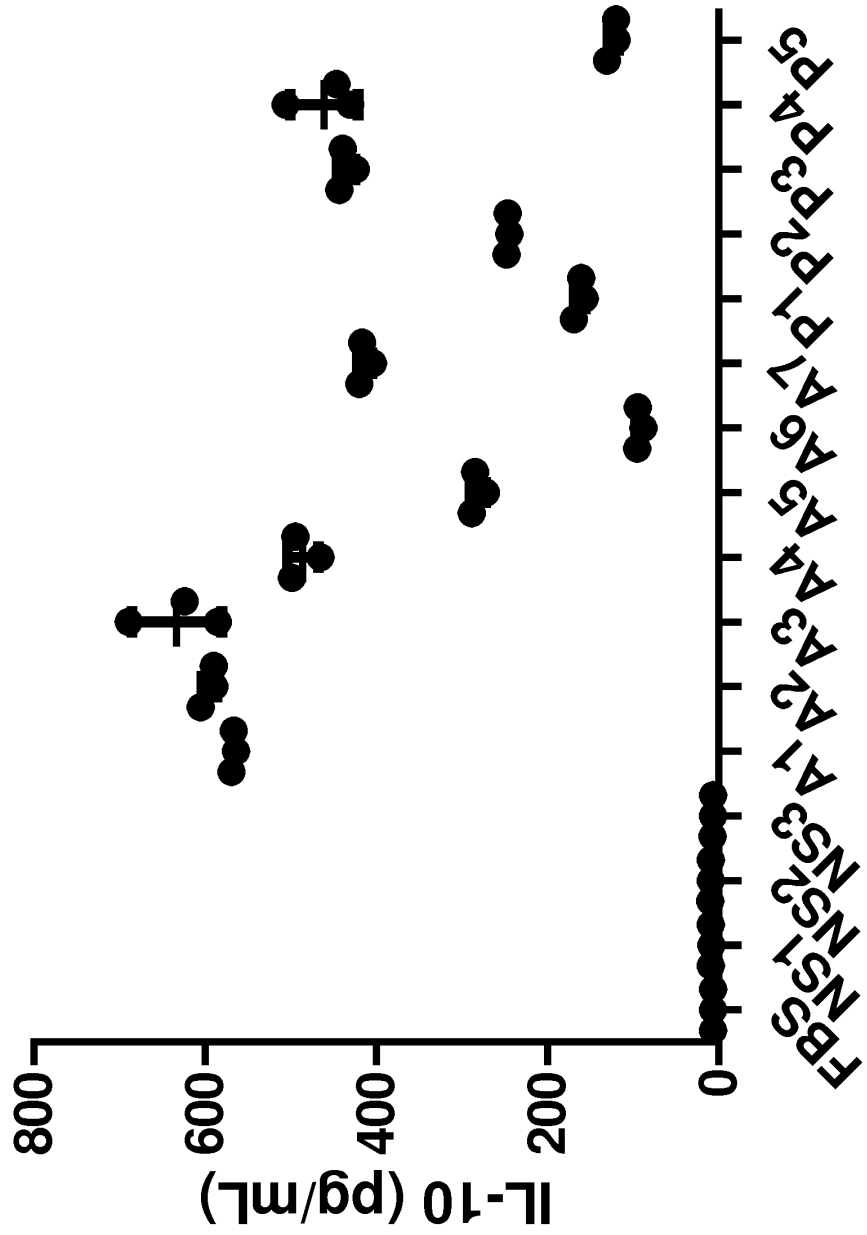

IL-10 (pg/mL)
800
600
400
200
0
FBS1
FNS1
FNS2
FNS3
A1
A2
A3
A4
A5
A6
A7
P1
P2
P3
P4
P5

FIGURE 58

Untreated
CD3/CD28
CD69+CD25+ (%)
0
20
40
60
80
normal serum
A1
A2
A3
A4
A5
A6
A7
P1
P2
P3
P4
P5
*
***
***
***
***
***

FIGURE 59

Untreated
CD3/CD28
CD107a-positive (%)
30
20
10
0
normal serum
A1
A2
A3
A4
A5
A6
A7
P1
P2
P3
P4
P5
***

FIGURE 60

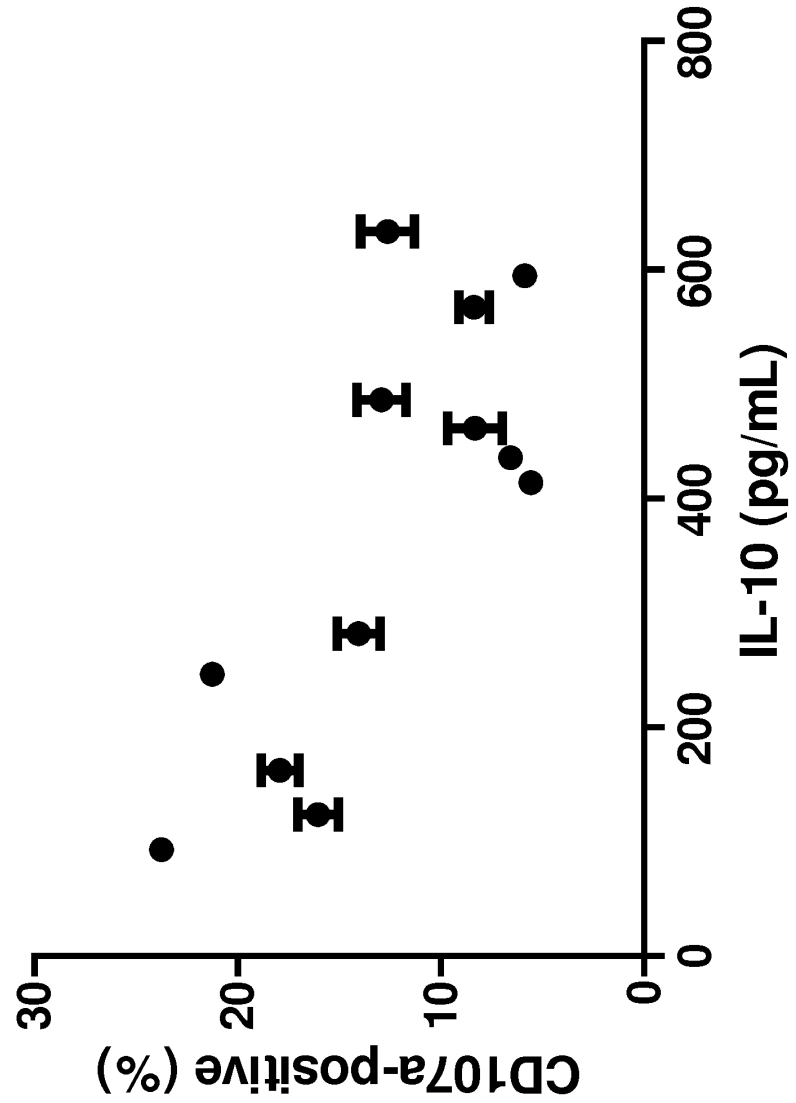

IL-10 vs CD107a
CD107a-positive (%)
IL-10 (pg/mL)
0
10
20
30
0
200
400
600
800

FIGURE 61

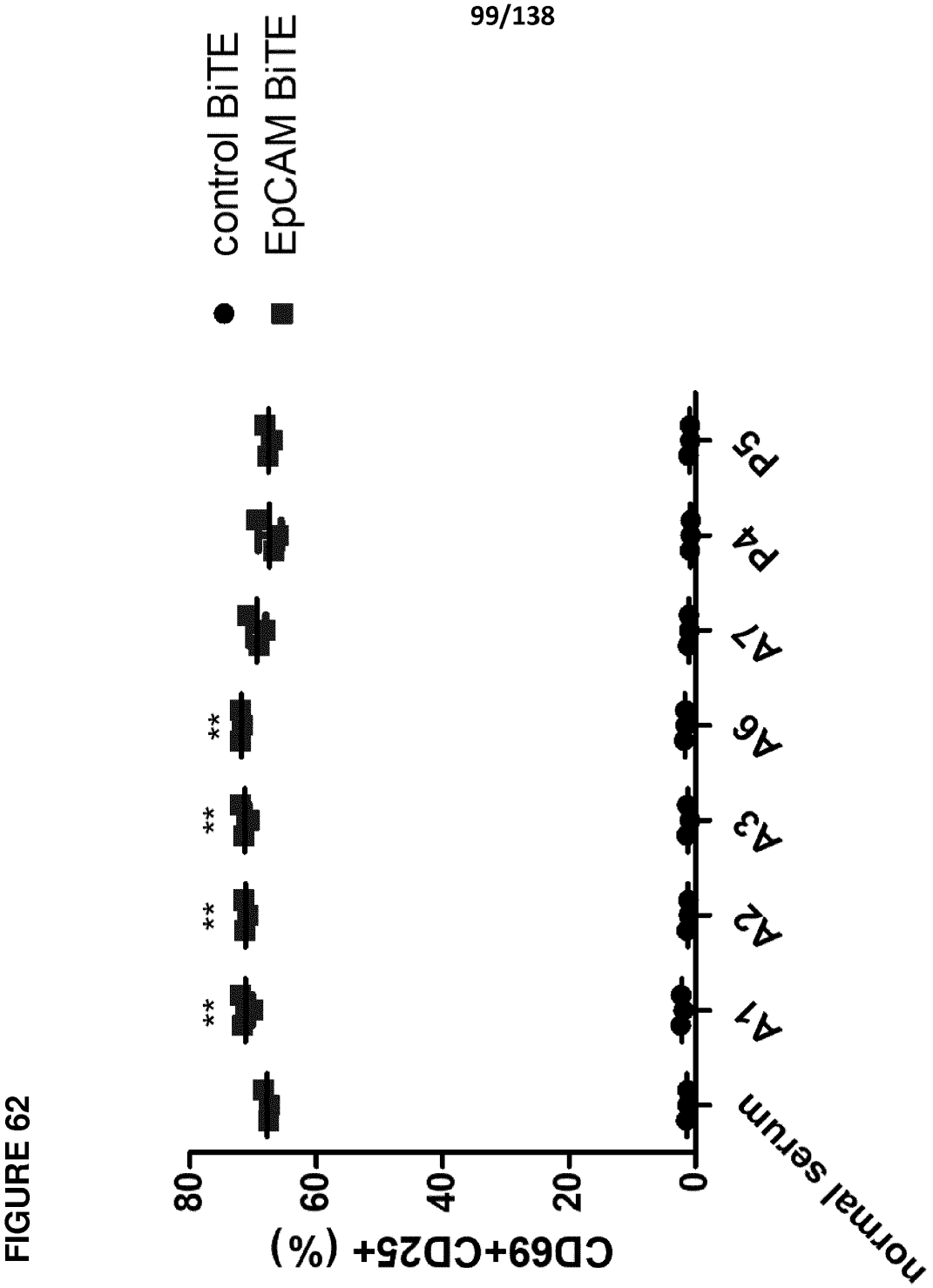

control BiTE
EpCAM BiTE
CD69+CD25+ (%)
0
20
40
60
80
**
**
**
**
normal serum
A1
A2
A3
A6
A7
P4
P5

FIGURE 62

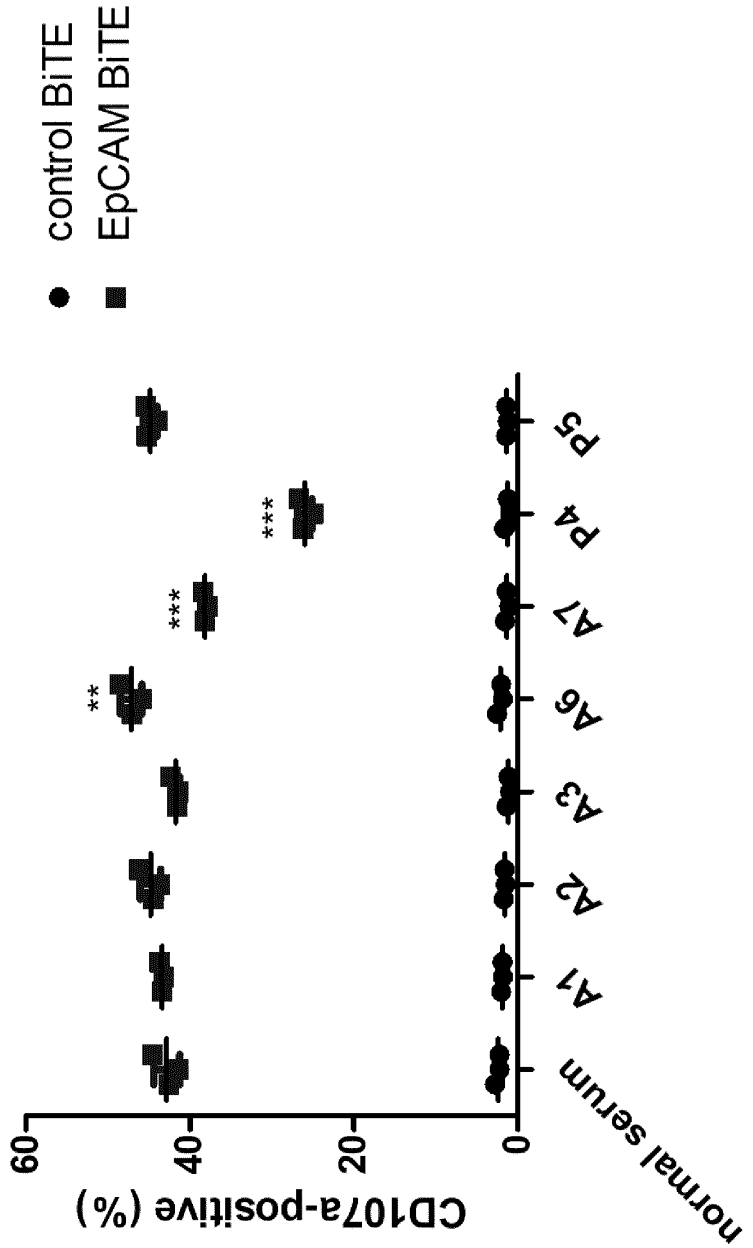
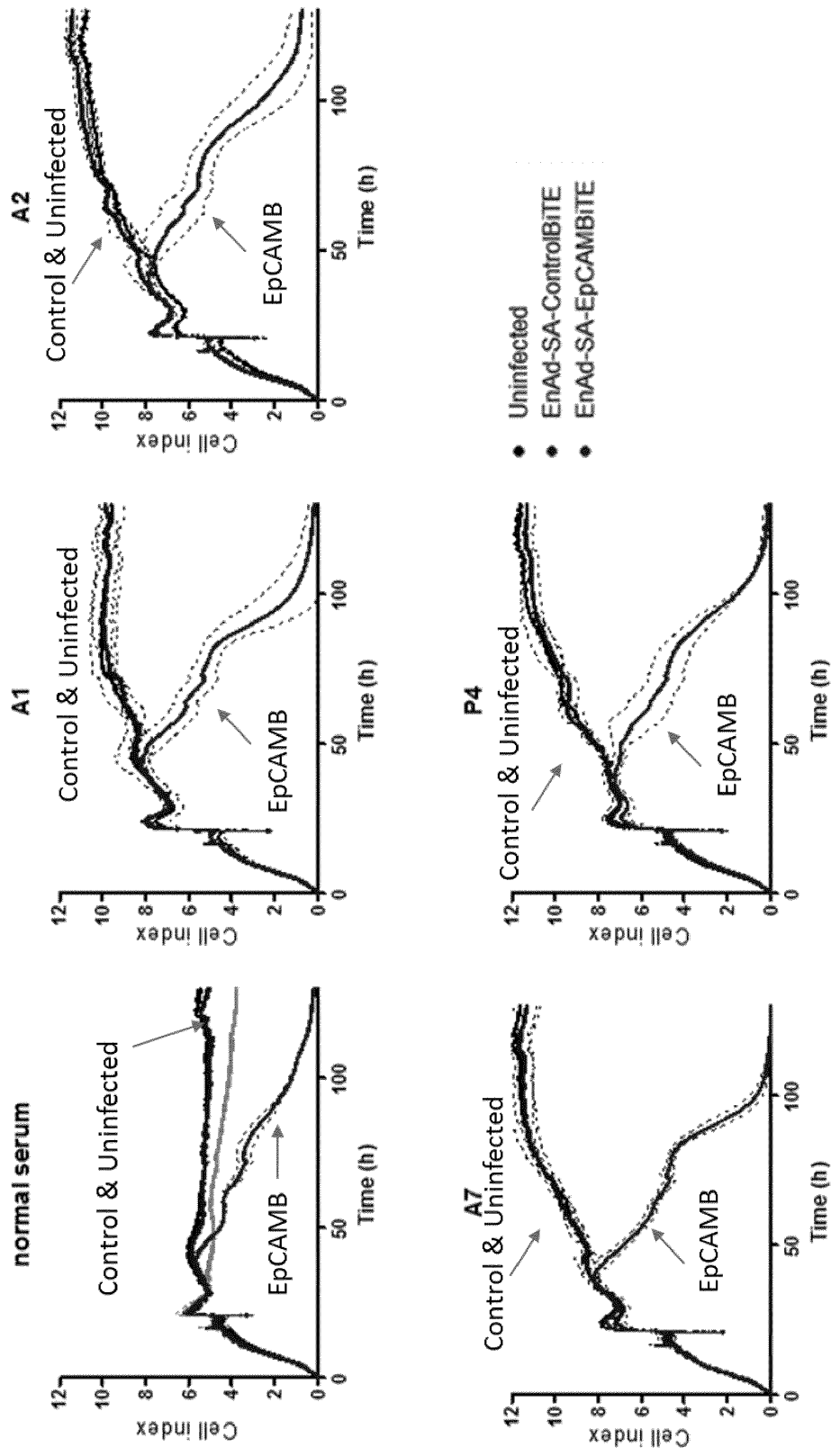

control BiTE
EpCAM BiTE
CD107a-positive (%)
0
20
40
60
normal serum
A1
A2
A3
A6
A7
P4
P5
**
***
***

FIGURE 63

FIGURE 64


normal serum
A1
A2
P4
A7
Control & Uninfected
EpCAMB
Cell index
Time (h)
Uninfected
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE

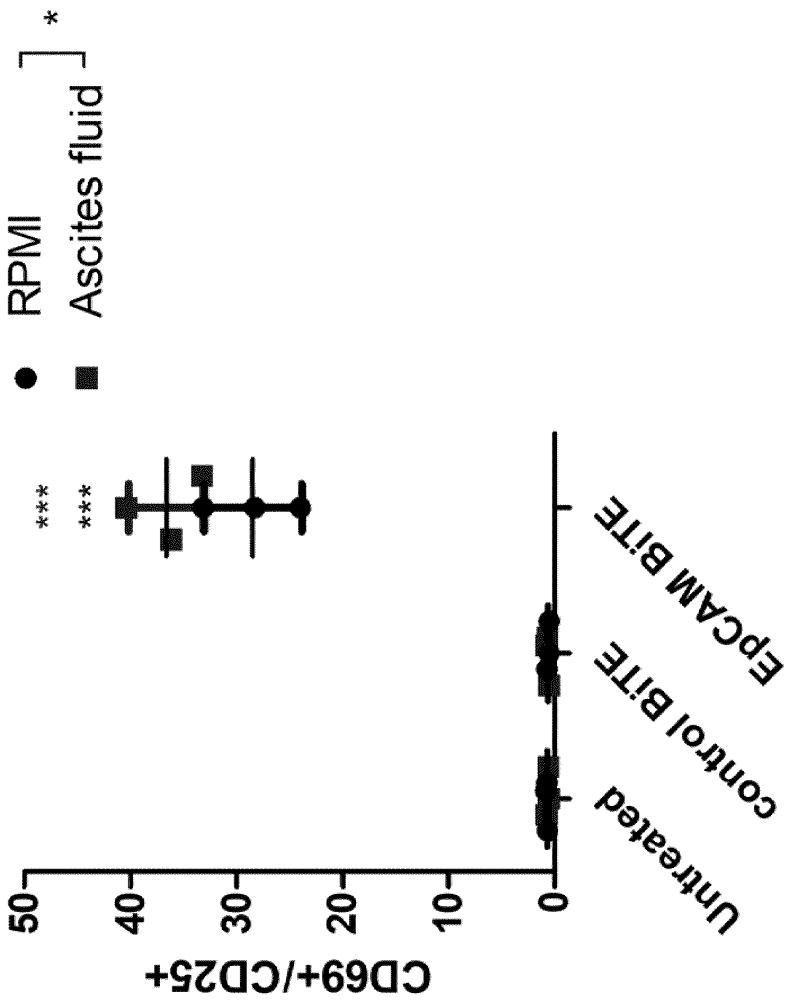

RPMI
Ascites fluid
*
***
***
CD69+/CD25+
50
40
30
20
10
0
Untreated
control BiTE
EpCAM BiTE

FIGURE 65

FIGURE 66A

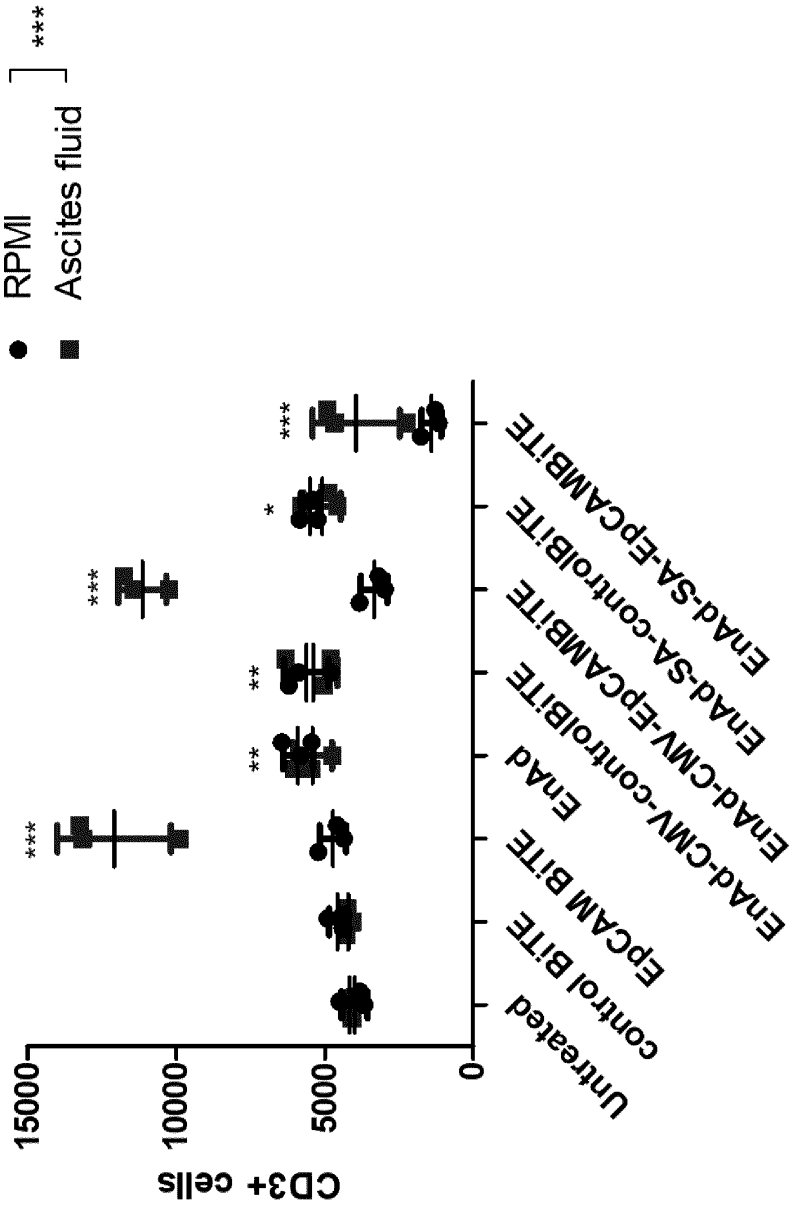

RPMI
Ascites fluid
***
CD3+ cells
15000
10000
5000
0
Untreated
control BiTE
EpCAM BiTE
EnAd
EnAd-CMV-controlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-controlBiTE
EnAd-SA-EpCAMBiTE
***
**
**
***
*
***

FIGURE 66B

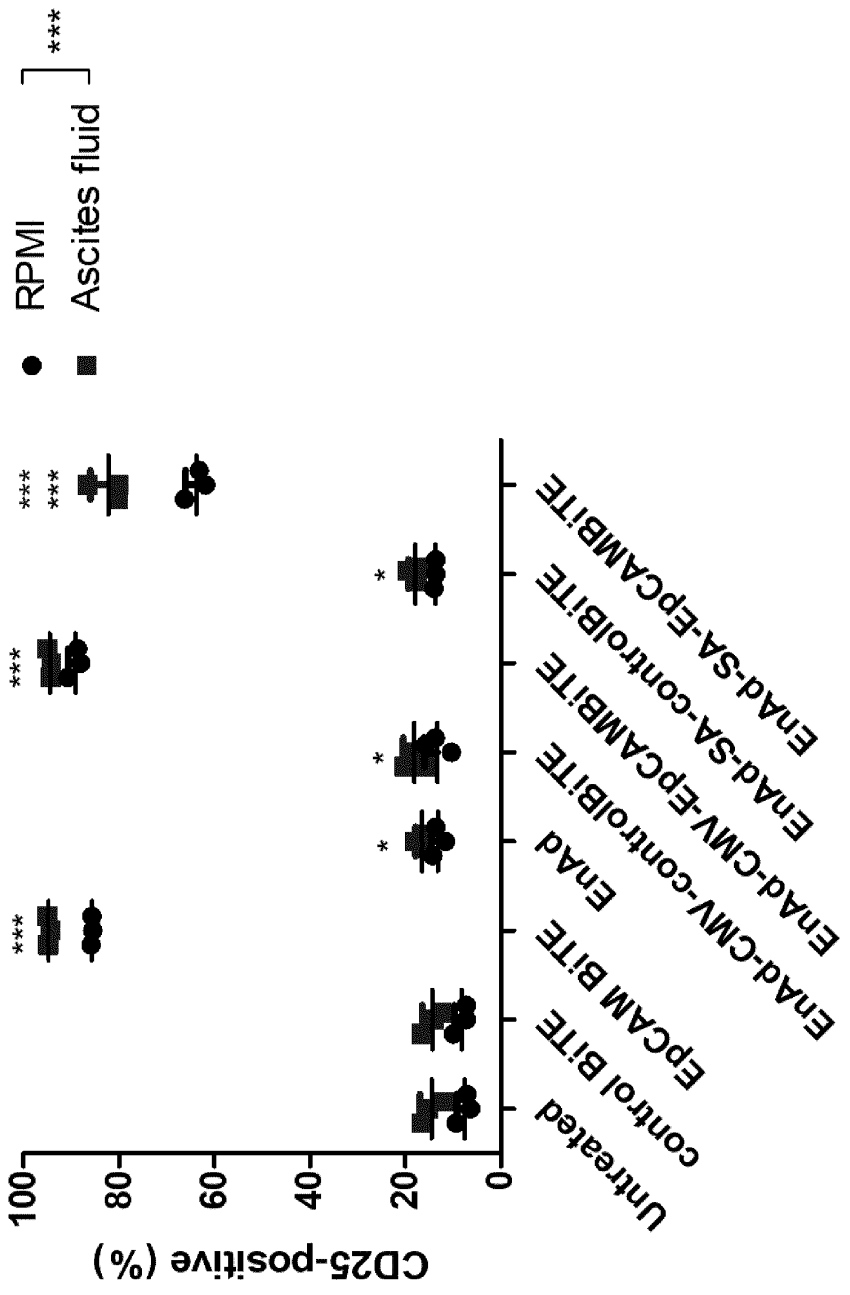
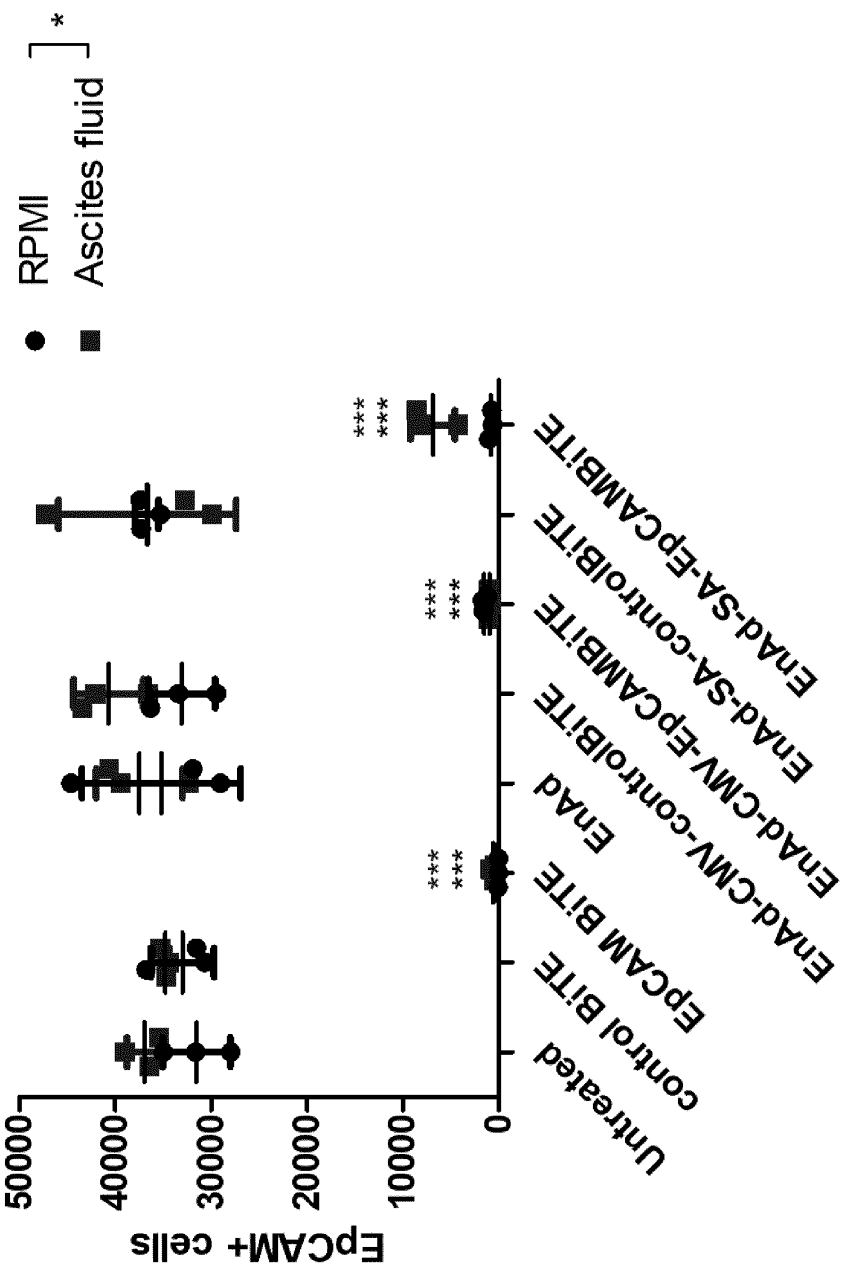

RPMI
Ascites fluid
***
CD25-positive (%)
100
80
60
40
20
0
Untreated
control BiTE
EpCAM BiTE
EnAd
EnAd-CMV-controlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-controlBiTE
EnAd-SA-EpCAMBiTE

FIGURE 66C


RPMI
Ascites fluid
*
EpCAM+ cells
50000
40000
30000
20000
10000
0
***
***
***
***
***
***
Untreated
control BiTE
EpCAM BiTE
EnAd
EnAd-CMV-controlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-controlBiTE
EnAd-SA-EpCAMBiTE

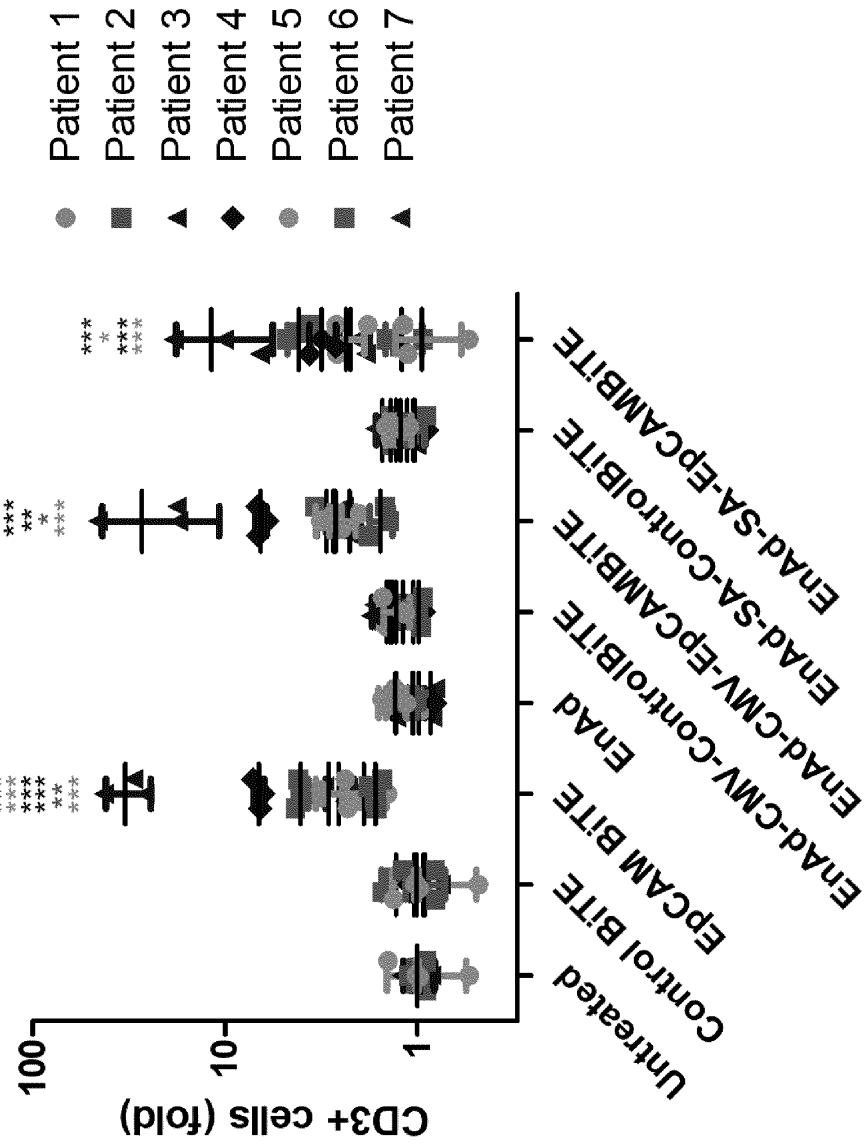

Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
CD3+ cells (fold)
100
10
1
Untreated
Control BiTE
EpCAM BiTE
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE

FIGURE 67A

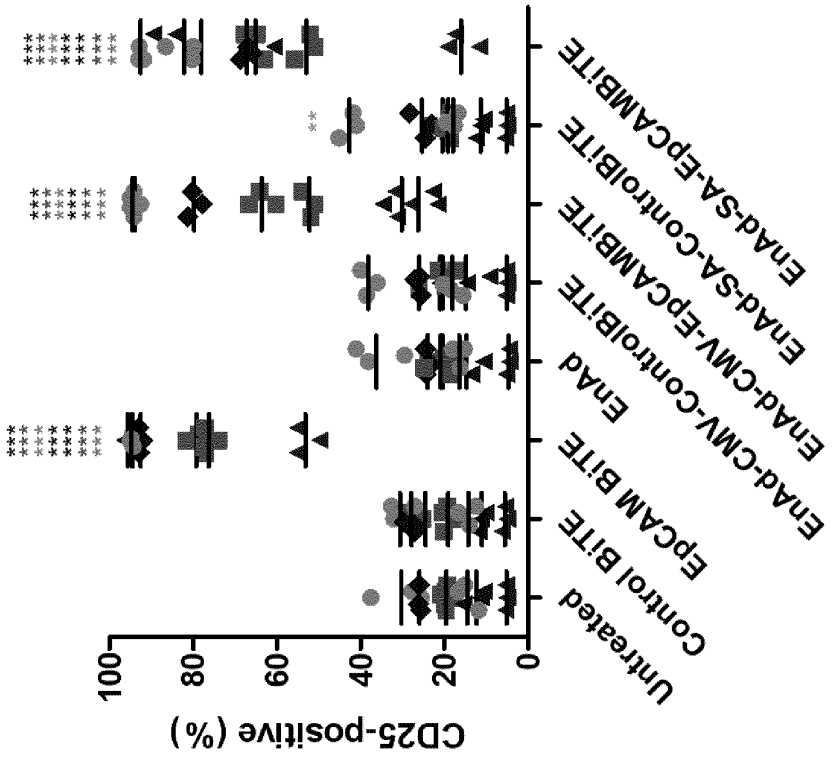

CD25-positive (%)
0
20
40
60
80
100
Untreated
Control BiTE
EpCAM BiTE
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE

FIGURE 67B

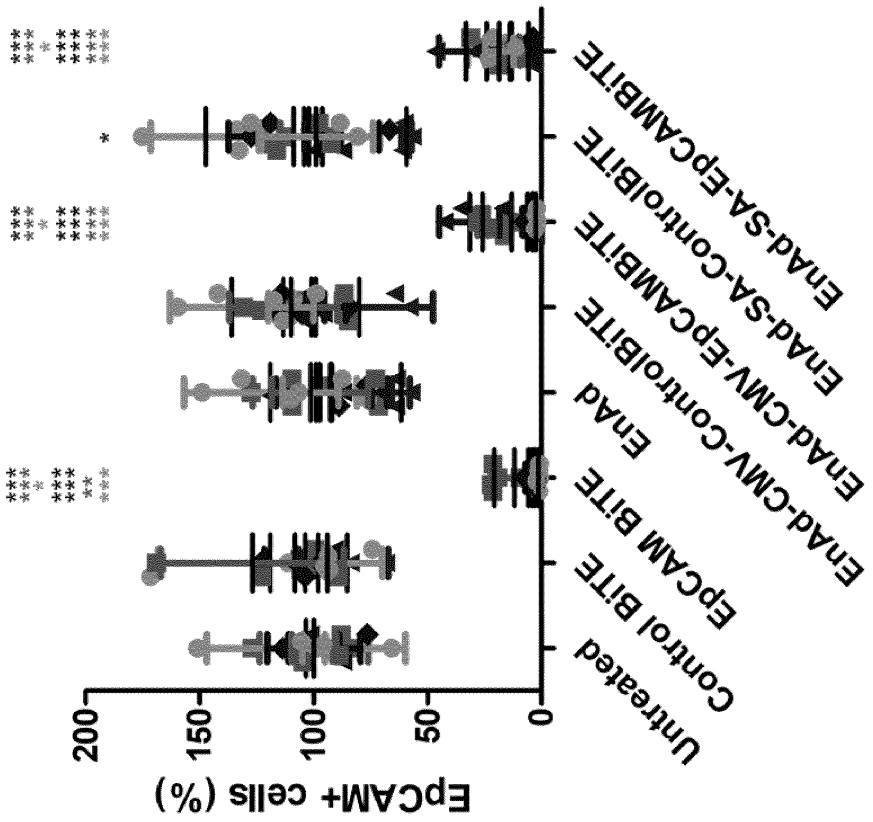
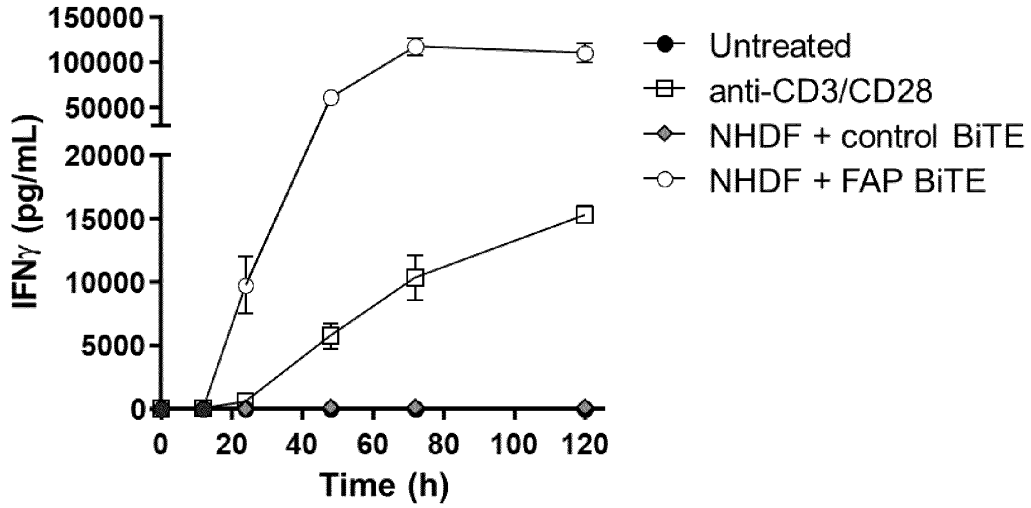

EpCAM+ cells (%)
200
150
100
50
0
Untreated
Control BiTE
EpCAM BiTE
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE

FIGURE 67C

Figure 68

A


150000
100000
50000
20000
15000
10000
5000
0
IFNγ (pg/mL)
0
20
40
60
80
100
120
Time (h)
Untreated
anti-CD3/CD28
NHDF + control BiTE
NHDF + FAP BiTE

B

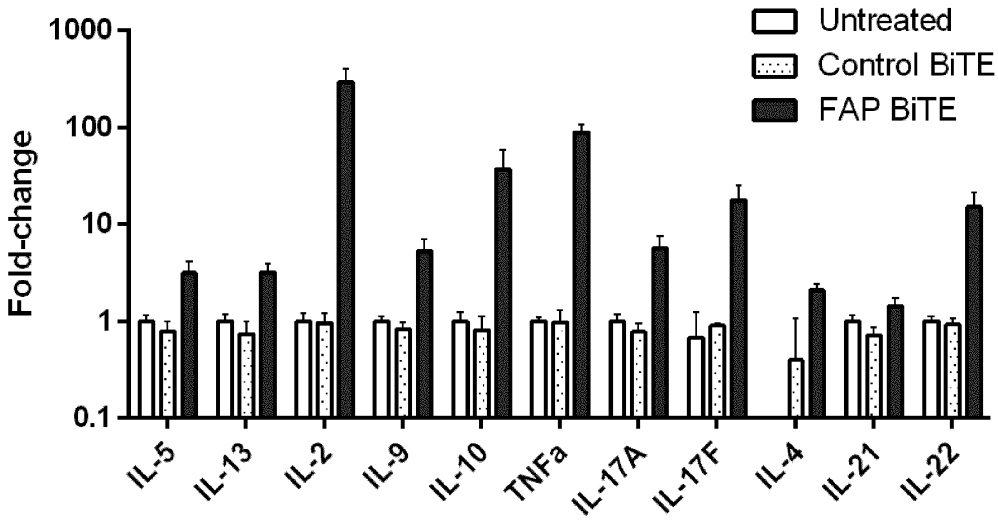

1000
100
10
1
0.1
Fold-change
Untreated
Control BiTE
FAP BiTE
IL-5
IL-13
IL-2
IL-9
IL-10
TNFa
IL-17A
IL-17F
IL-4
IL-21
IL-22

Figure 69

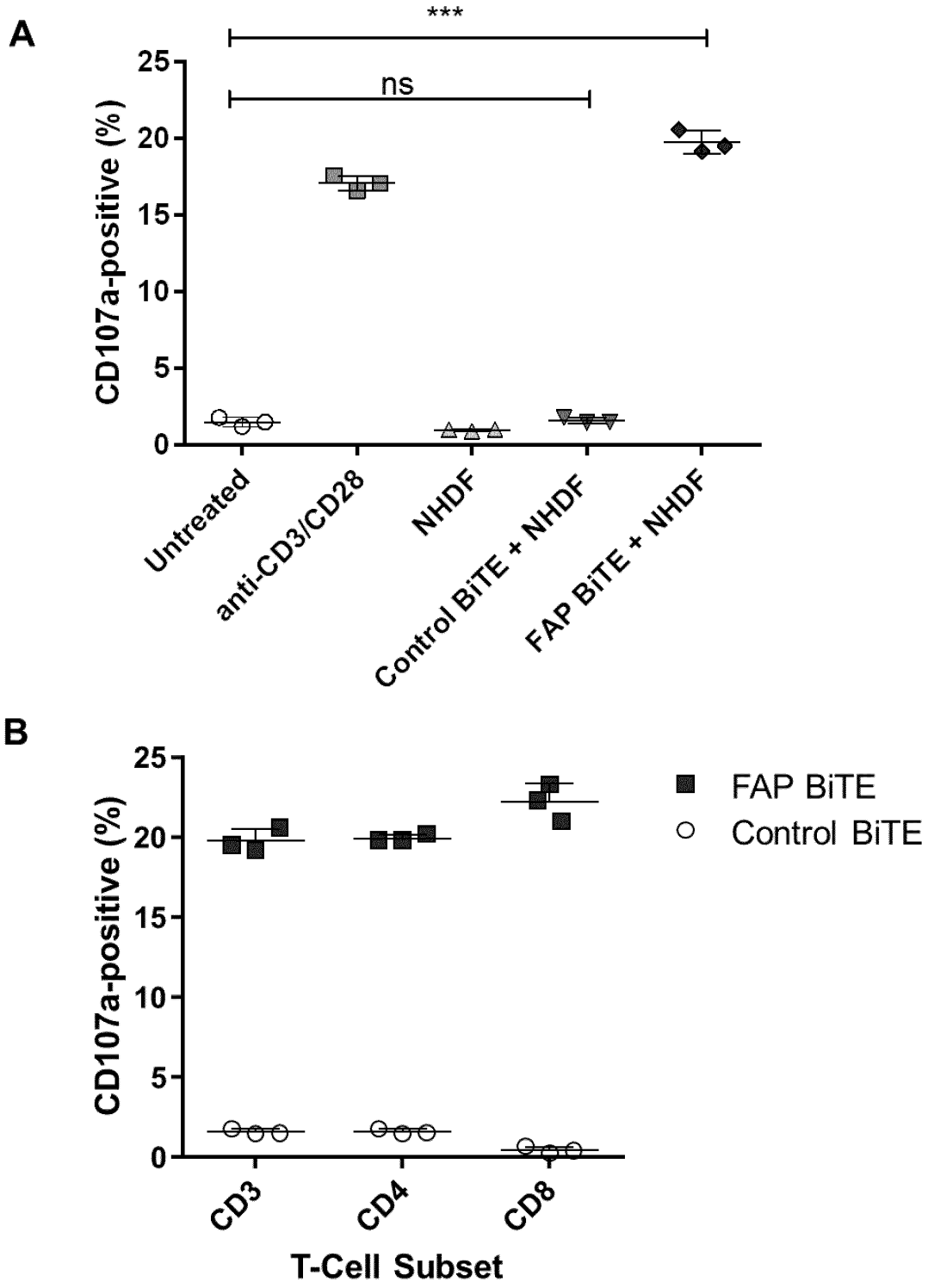

A
***
ns
CD107a-positive (%)
25
20
15
10
5
0
Untreated
anti-CD3/CD28
NHDF
Control BiTE + NHDF
FAP BiTE + NHDF
B
CD107a-positive (%)
25
20
15
10
5
0
FAP BiTE
Control BiTE
CD3
CD4
CD8
T-Cell Subset

Figure 69

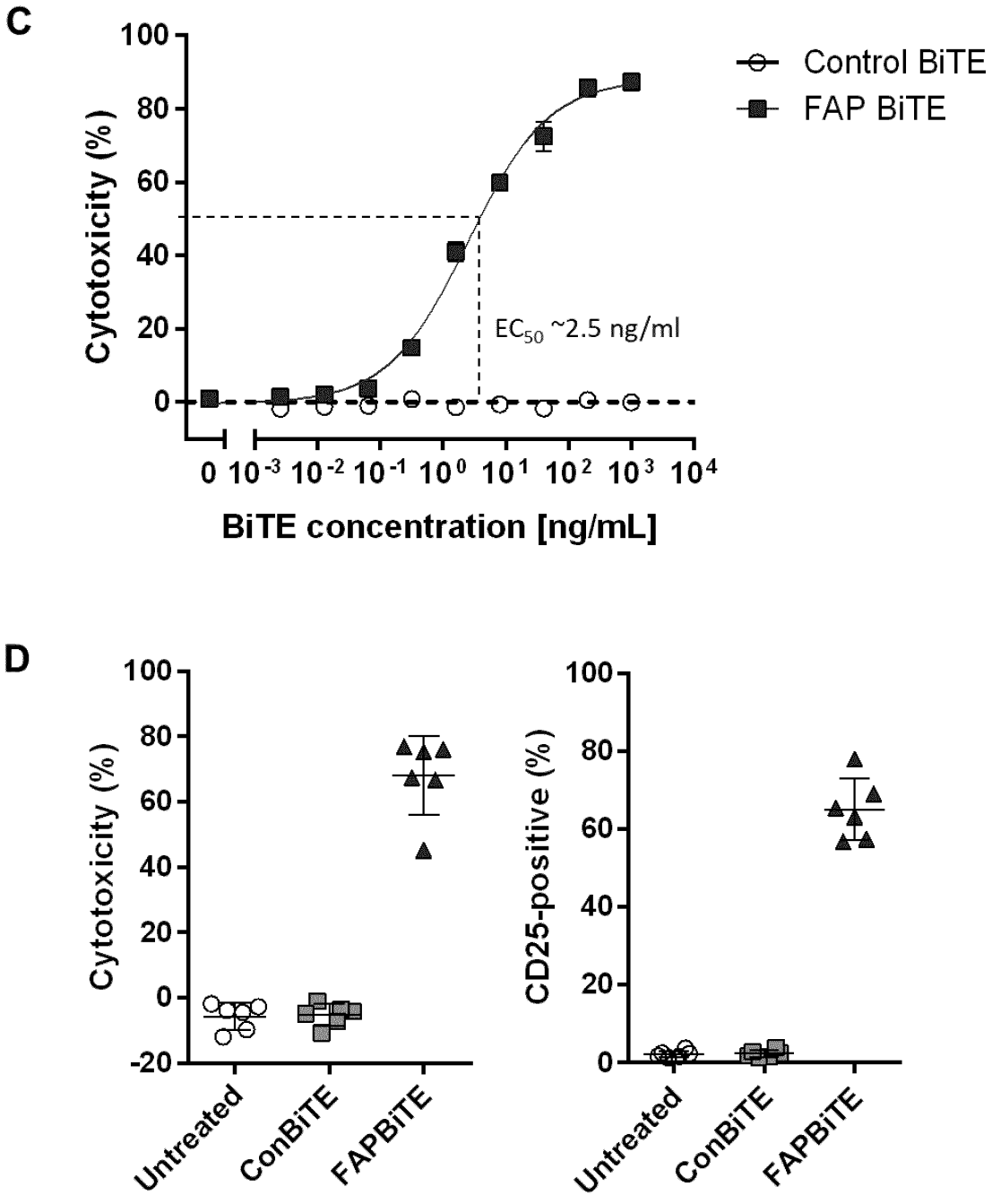

C
Cytotoxicity (%)
100
80
60
40
20
0
Control BiTE
FAP BiTE
EC50 ~2.5 ng/ml
0 10-3 10-2 10-1 100 101 102 103 104
BiTE concentration [ng/mL]
D
Cytotoxicity (%)
CD25-positive (%)
Untreated
ConBiTE
FAPBiTE

Figure 70

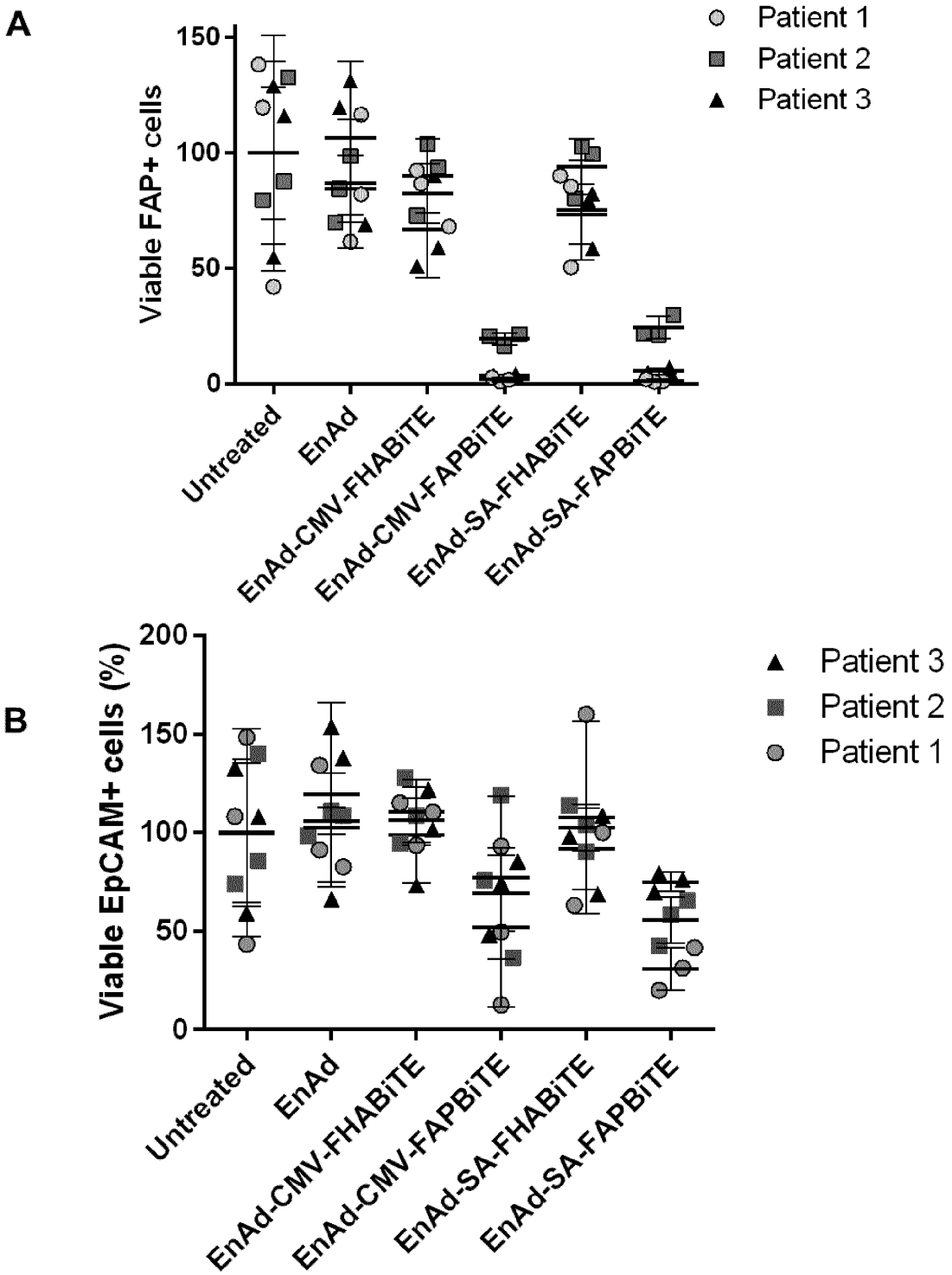

A
Patient 1
Patient 2
Patient 3
Viable FAP+ cells
150
100
50
0
Untreated
EnAd
EnAd-CMV-FHABiTE
EnAd-CMV-FAPBiTE
EnAd-SA-FHABiTE
EnAd-SA-FAPBiTE
B
Patient 3
Patient 2
Patient 1
Viable EpCAM+ cells (%)
200
150
100
50
0
Untreated
EnAd
EnAd-CMV-FHABiTE
EnAd-CMV-FAPBiTE
EnAd-SA-FHABiTE
EnAd-SA-FAPBiTE

Figure 70

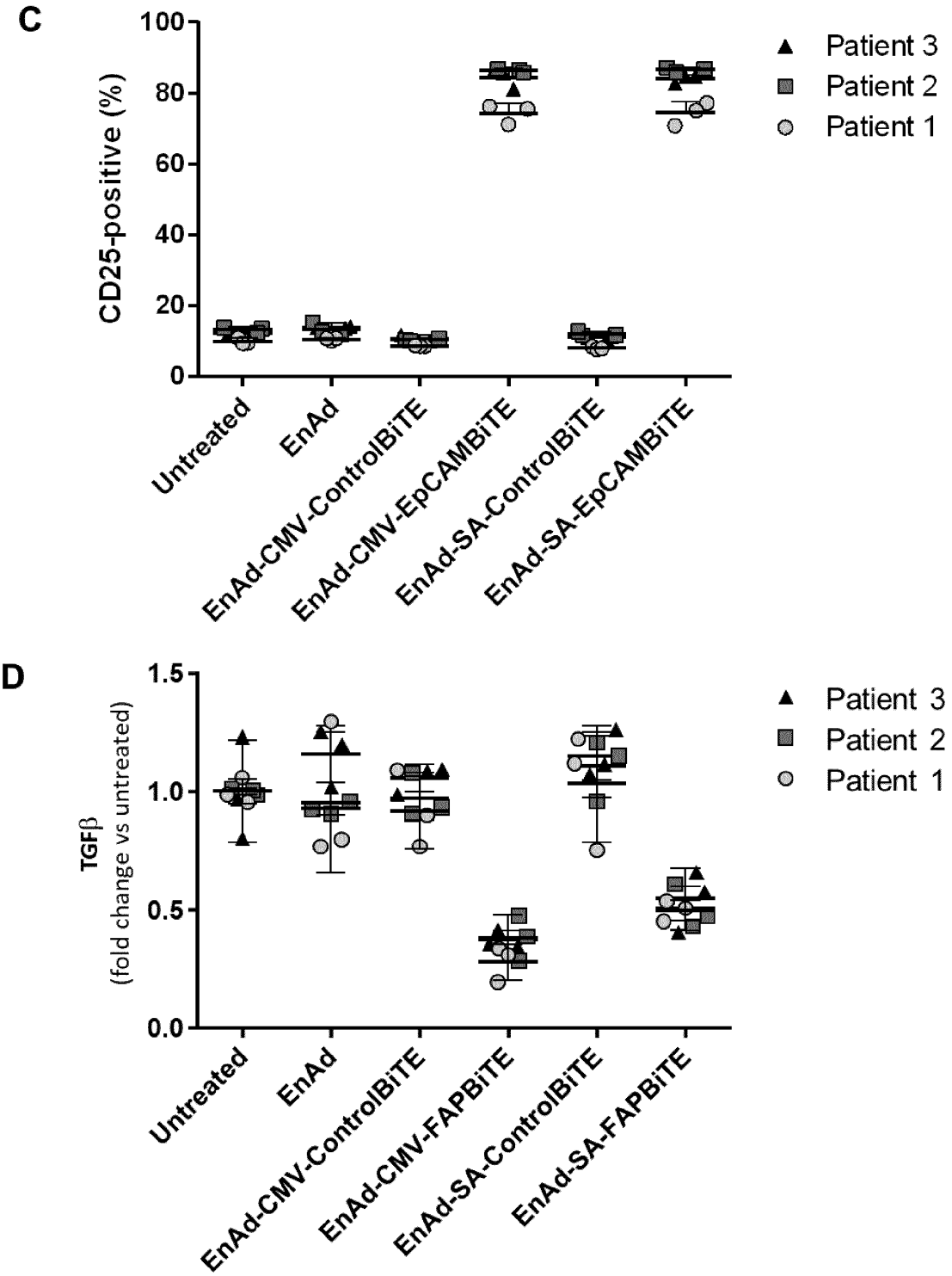

C
CD25-positive (%)
100
80
60
40
20
0
Patient 3
Patient 2
Patient 1
Untreated
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-EpCAMBiTE
EnAd-SA-ControlBiTE
EnAd-SA-EpCAMBiTE
D
TGFβ
(fold change vs untreated)
1.5
1.0
0.5
0.0
Patient 3
Patient 2
Patient 1
Untreated
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ControlBiTE
EnAd-SA-FAPBiTE

Figure 71

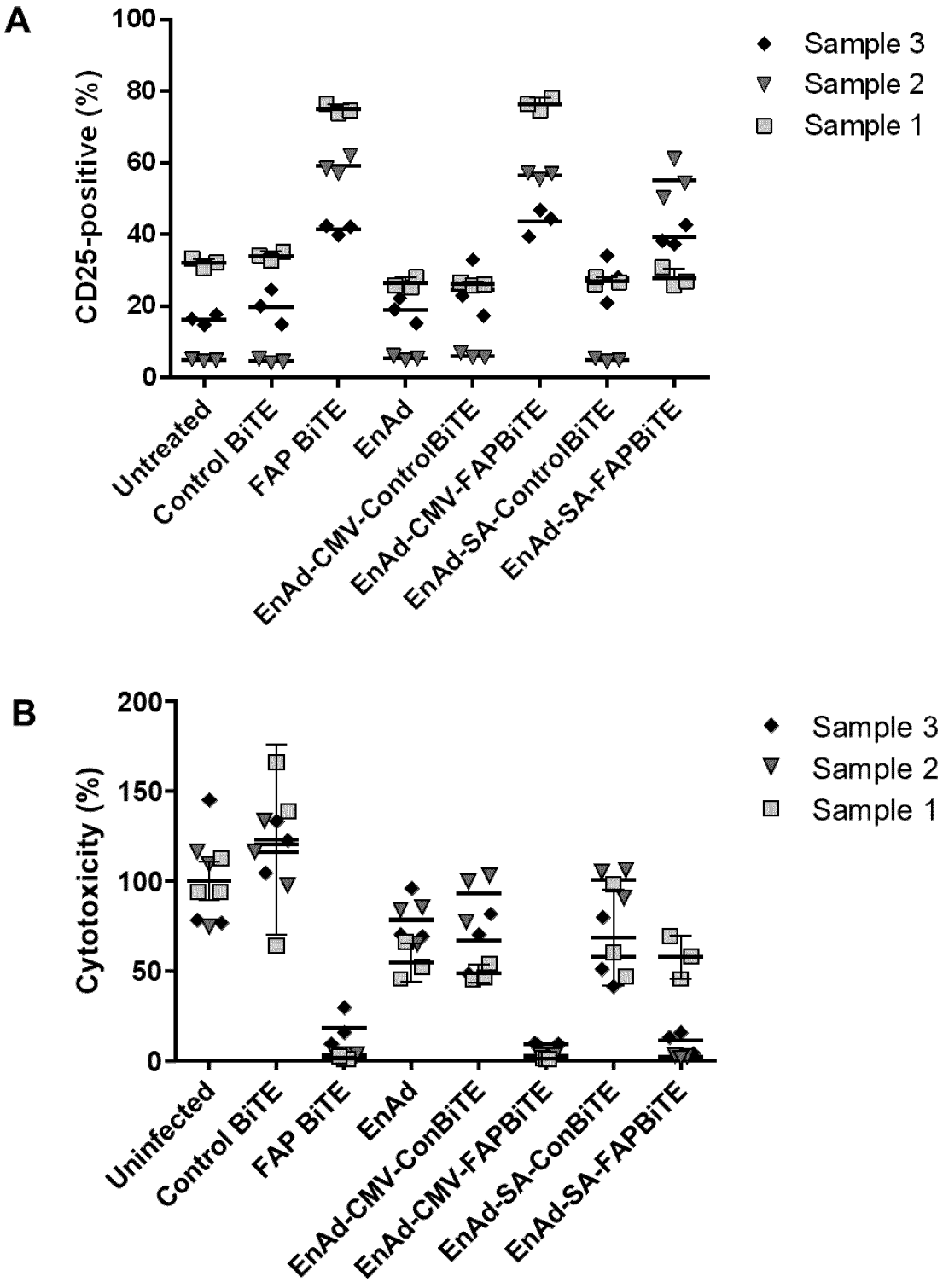

A
CD25-positive (%)
100
80
60
40
20
0
Sample 3
Sample 2
Sample 1
Untreated
Control BiTE
FAP BiTE
EnAd
EnAd-CMV-ControlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ControlBiTE
EnAd-SA-FAPBiTE
B
Cytotoxicity (%)
200
150
100
50
0
Sample 3
Sample 2
Sample 1
Uninfected
Control BiTE
FAP BiTE
EnAd
EnAd-CMV-ConBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ConBiTE
EnAd-SA-FAPBiTE

Figure 72

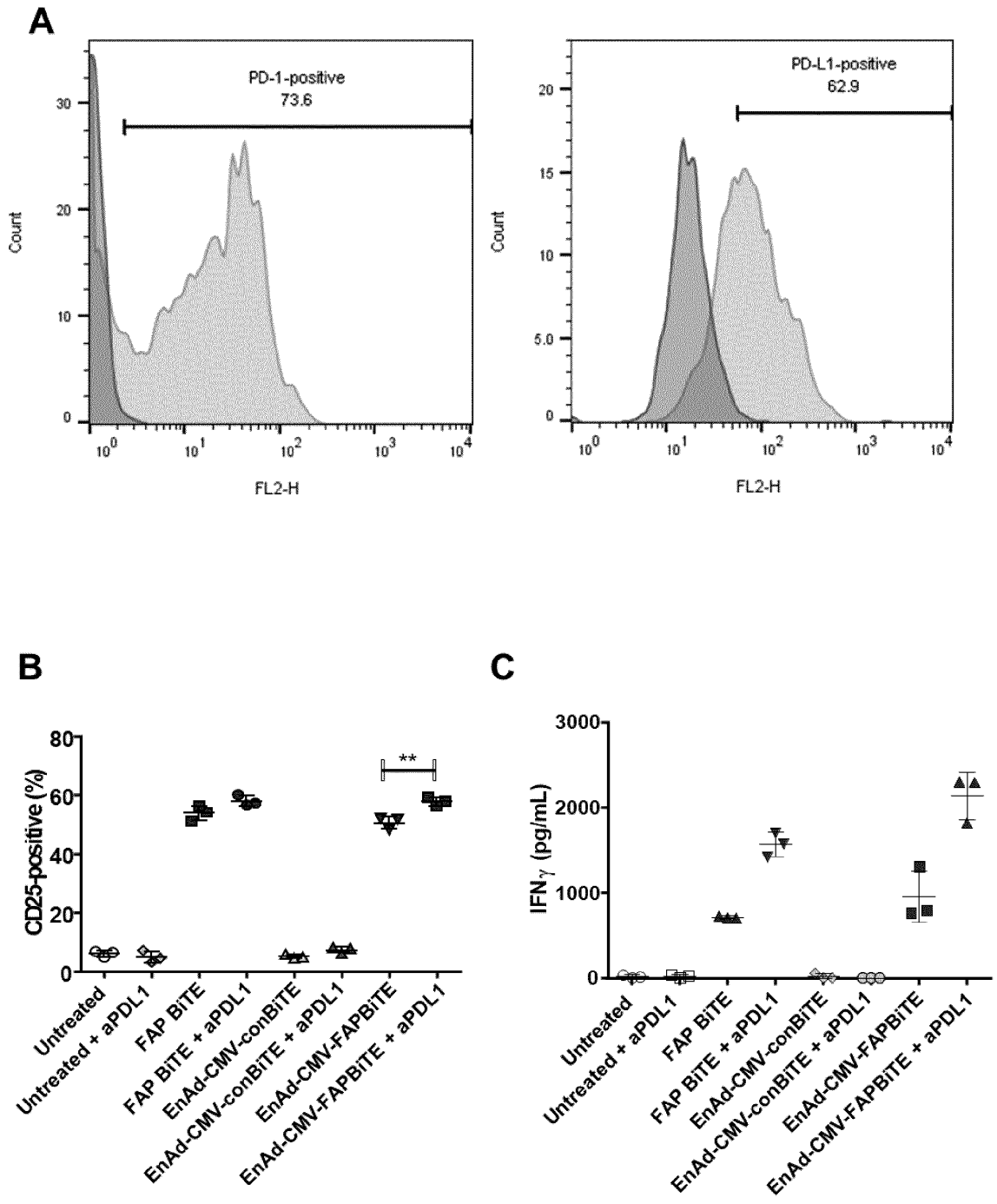

A
PD-1-positive
73.6
Count
FL2-H
PD-L1-positive
62.9
Count
FL2-H
B
CD25-positive (%)
**
Untreated
Untreated + aPDL1
FAP BiTE
FAP BiTE + aPDL1
EnAd-CMV-conBiTE
EnAd-CMV-conBiTE + aPDL1
EnAd-CMV-FAPBiTE
EnAd-CMV-FAPBiTE + aPDL1
C
IFNγ (pg/mL)
Untreated
Untreated + aPDL1
FAP BiTE
FAP BiTE + aPDL1
EnAd-CMV-conBiTE
EnAd-CMV-conBiTE + aPDL1
EnAd-CMV-FAPBiTE
EnAd-CMV-FAPBiTE + aPDL1

Figure 72

D

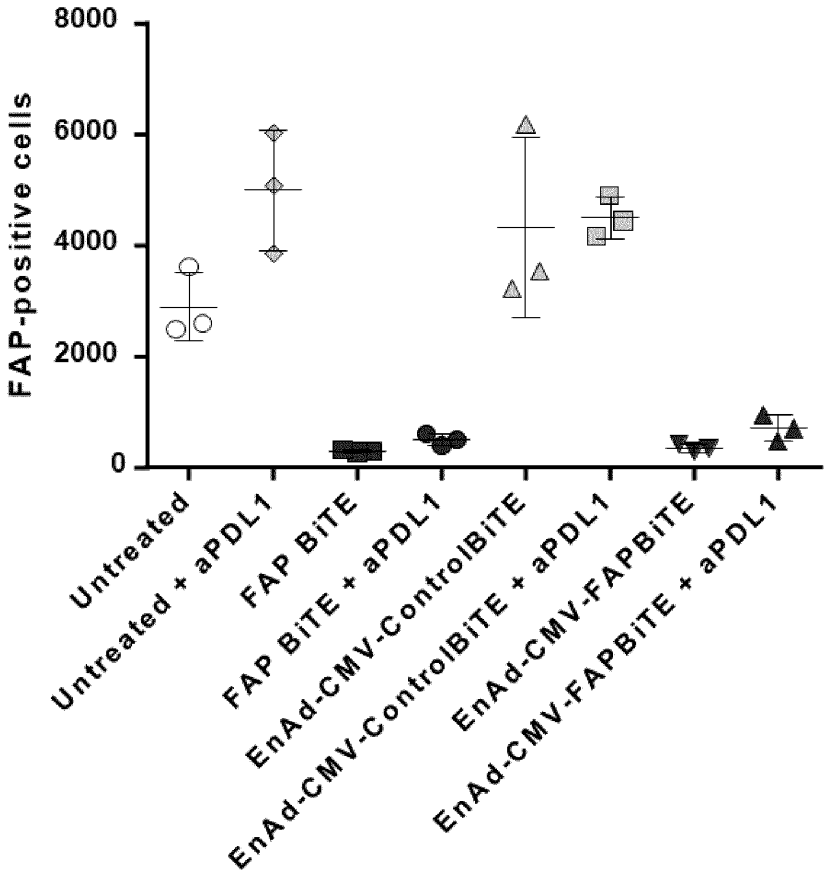

FAP-positive cells
8000
6000
4000
2000
0
Untreated
Untreated + aPDL1
FAP BiTE
FAP BiTE + aPDL1
EnAd-CMV-ControlBiTE
EnAd-CMV-ControlBiTE + aPDL1
EnAd-CMV-FAPBiTE
EnAd-CMV-FAPBiTE + aPDL1

Figure 73

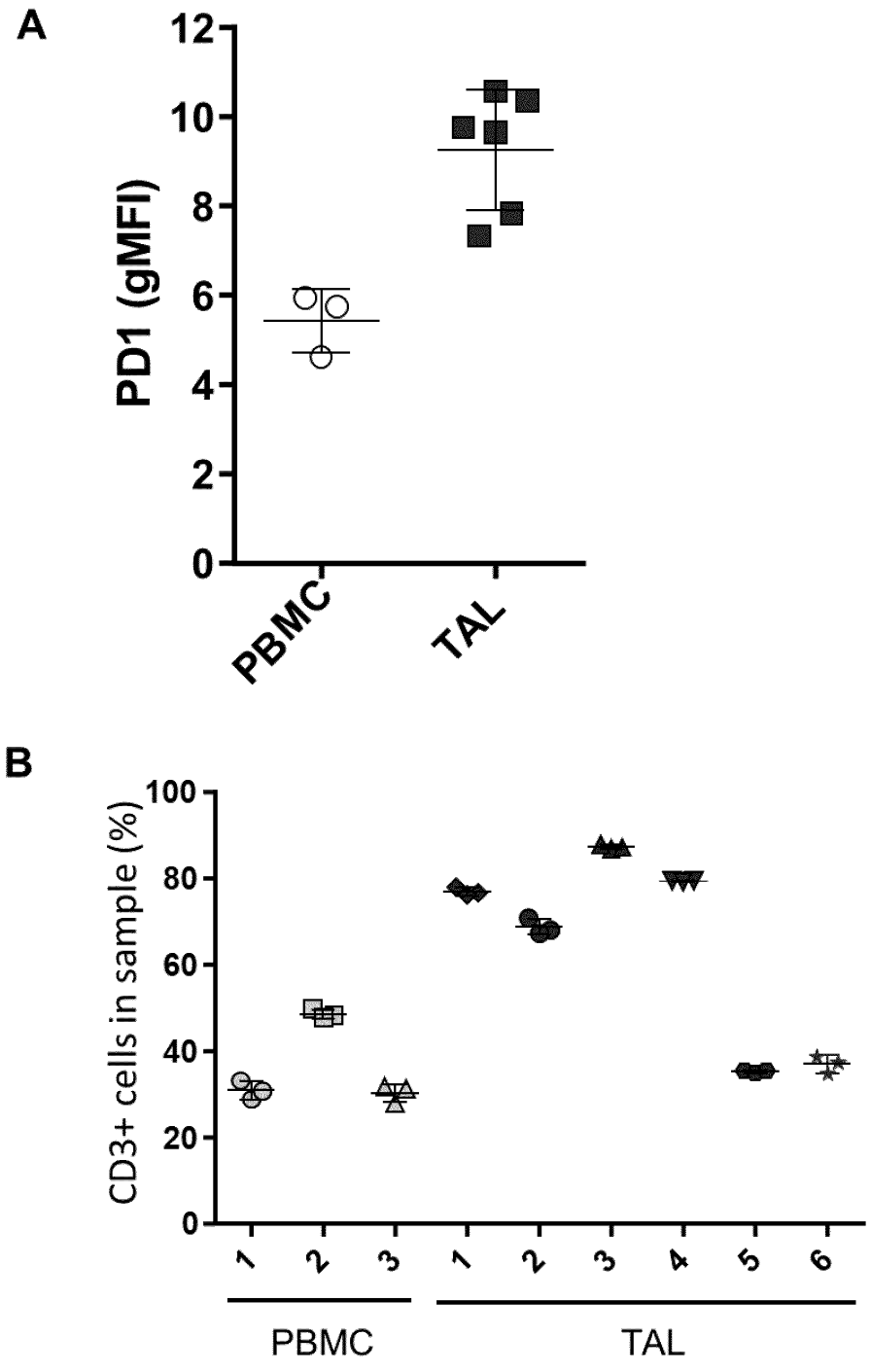

A
PD1 (gMFI)
0
2
4
6
8
10
12
PBMC
TAL
B
CD3+ cells in sample (%)
0
20
40
60
80
100
1
2
3
1
2
3
4
5
6
PBMC
TAL

Figure 73

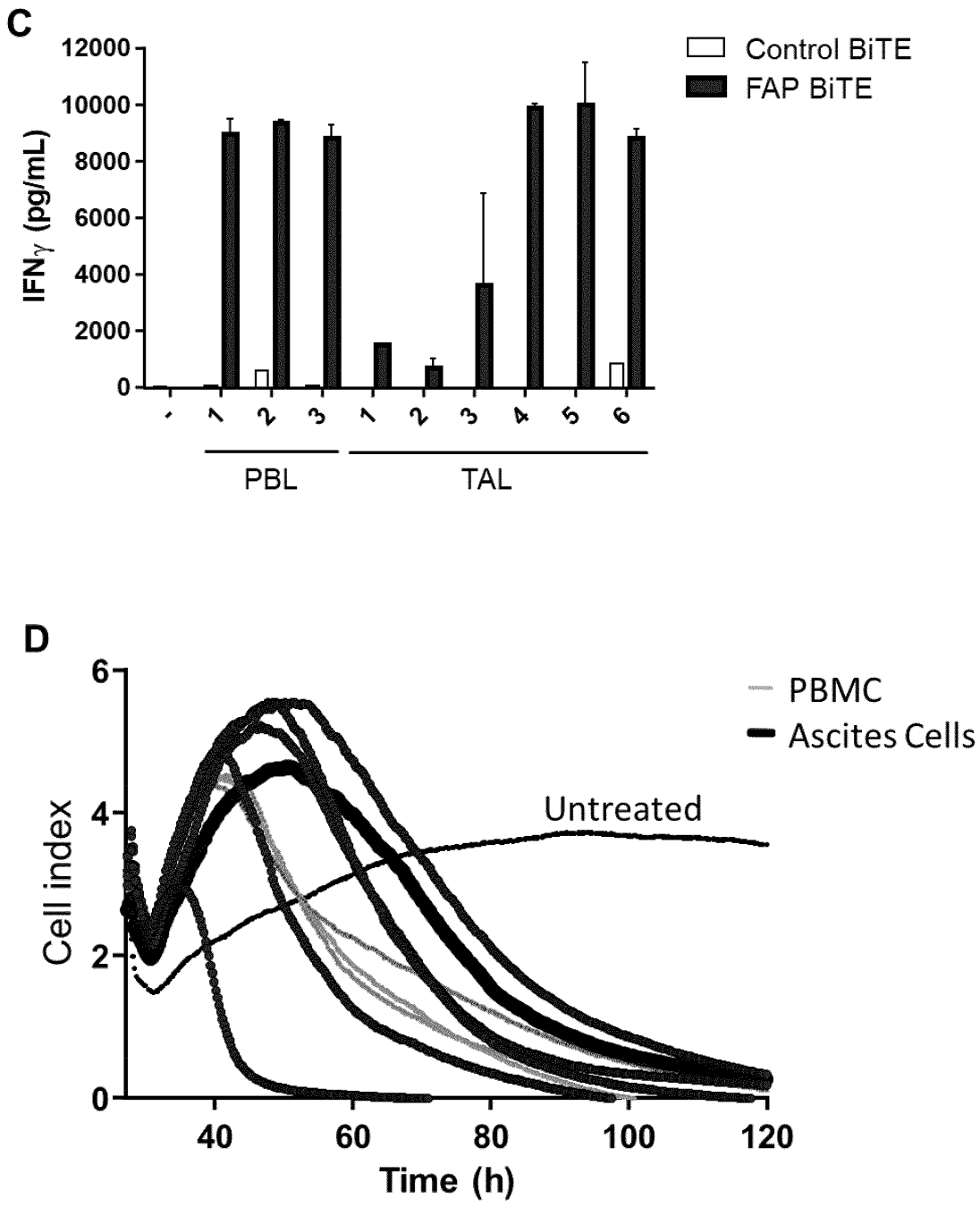

C
12000
10000
8000
6000
4000
2000
0
IFNγ (pg/mL)
Control BiTE
FAP BiTE
-
1
2
3
1
2
3
4
5
6
PBL
TAL
D
6
4
2
0
Cell index
PBMC
Ascites Cells
Untreated
40
60
80
100
120
Time (h)

Figure 74

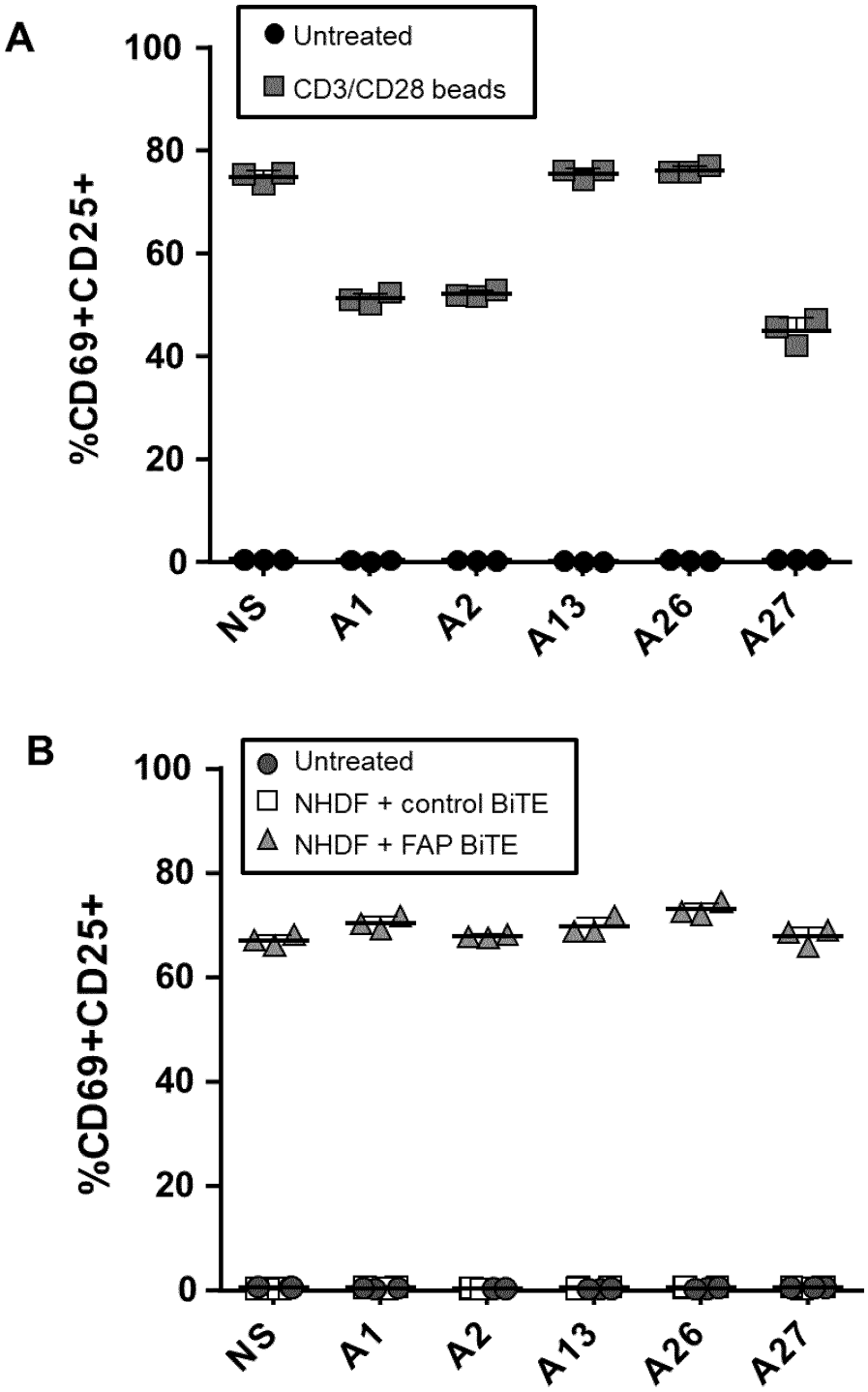

A
Untreated
CD3/CD28 beads
%CD69+CD25+
100
80
60
40
20
0
NS
A1
A2
A13
A26
A27
B
Untreated
NHDF + control BiTE
NHDF + FAP BiTE
%CD69+CD25+
100
80
60
40
20
0
NS
A1
A2
A13
A26
A27

Figure 75

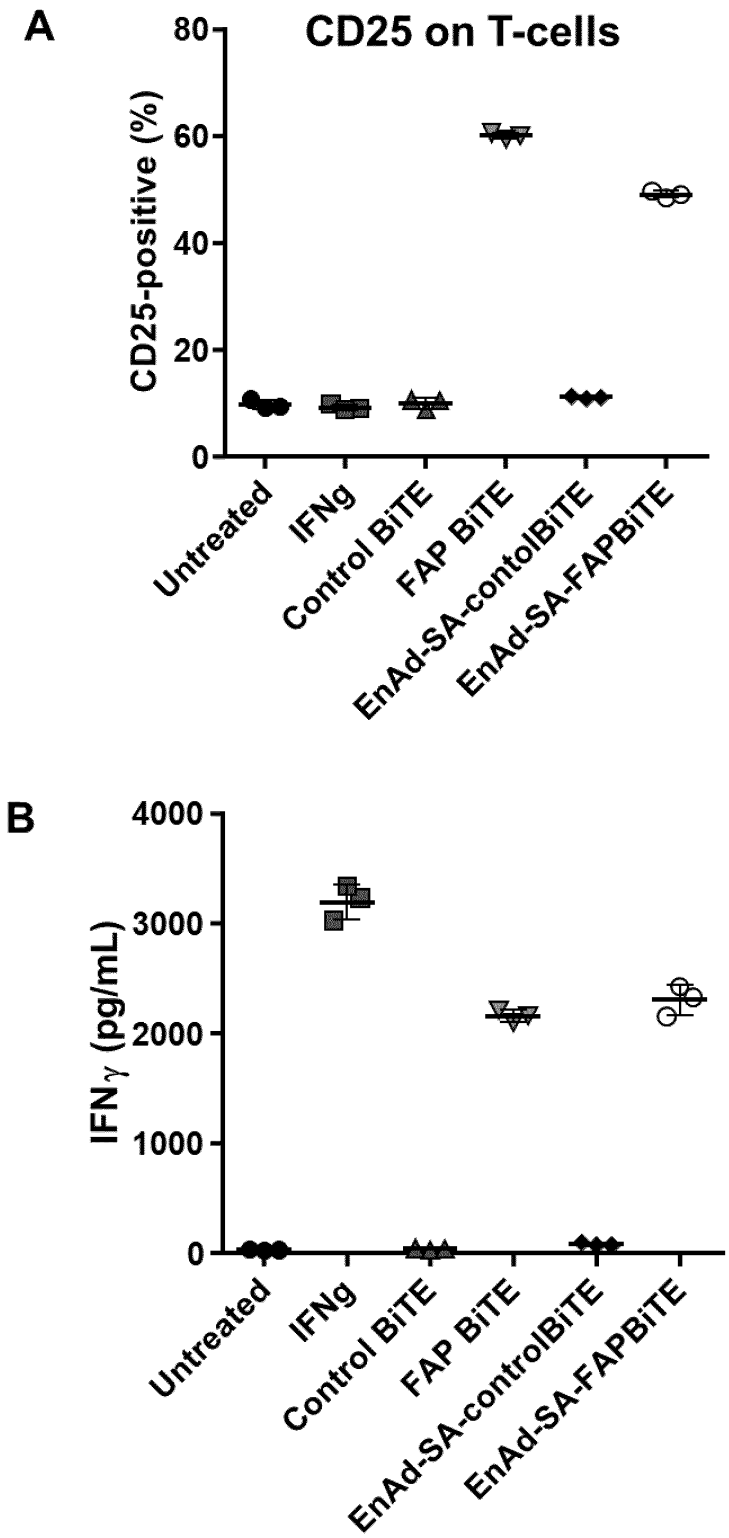

A
CD25 on T-cells
CD25-positive (%)
80
60
40
20
0
Untreated
IFNg
Control BiTE
FAP BiTE
EnAd-SA-contolBiTE
EnAd-SA-FAPBiTE
B
IFNγ (pg/mL)
4000
3000
2000
1000
0
Untreated
IFNg
Control BiTE
FAP BiTE
EnAd-SA-controlBiTE
EnAd-SA-FAPBiTE

Figure 75

C

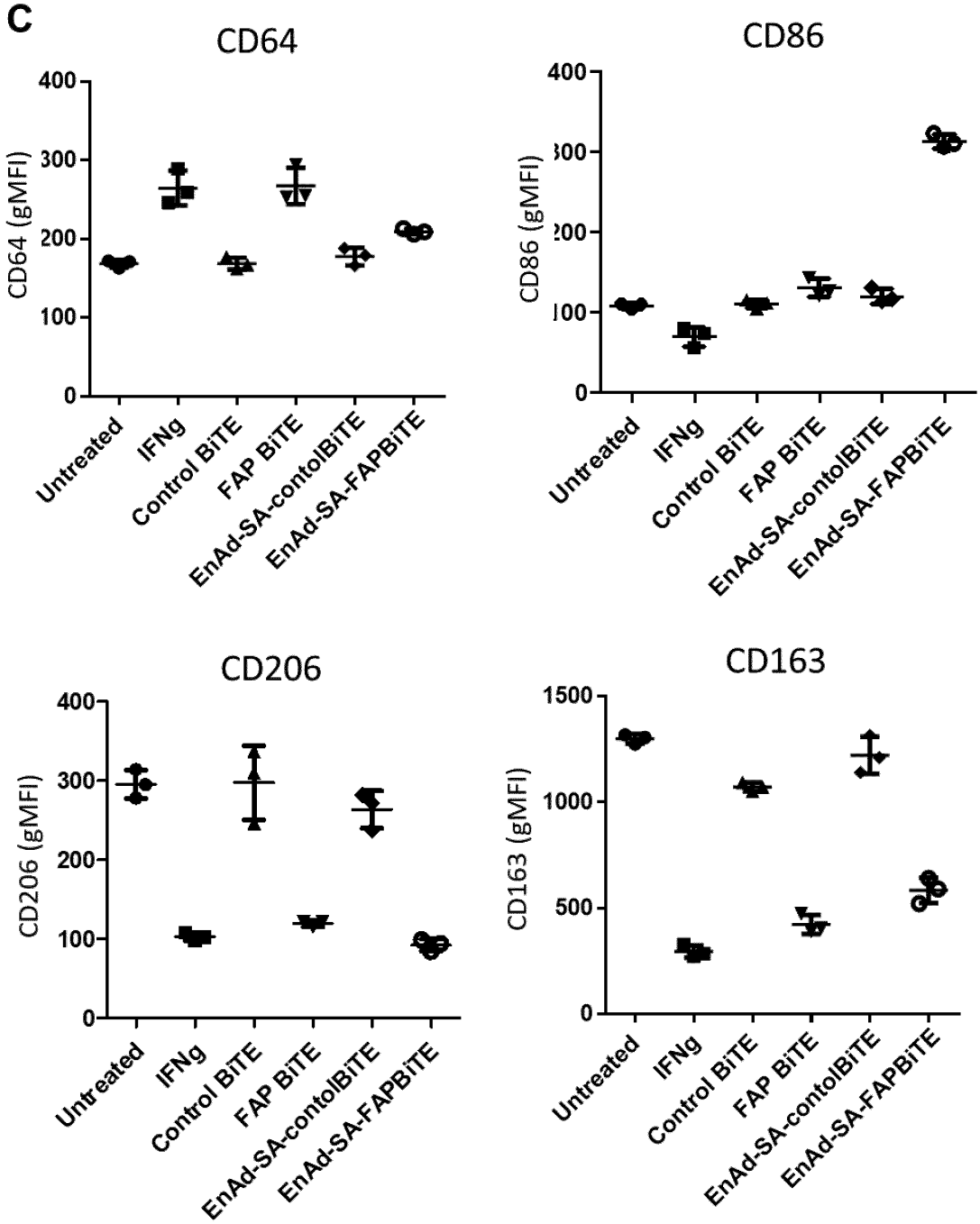
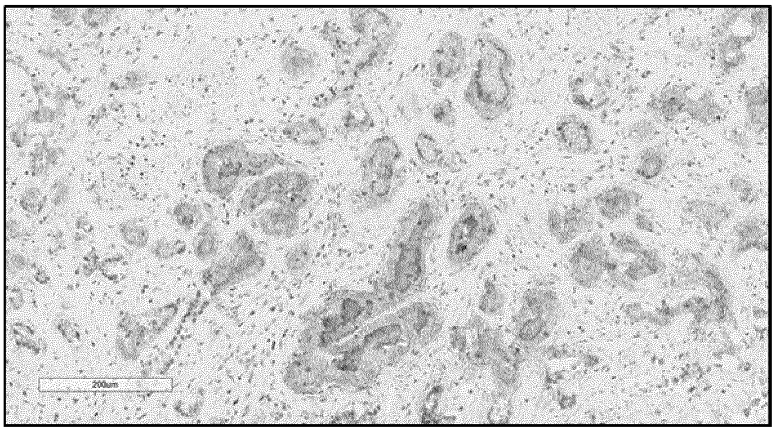

CD64
CD64 (gMFI)
400
300
200
100
0
Untreated
IFNg
Control BiTE
FAP BiTE
EnAd-SA-contolBiTE
EnAd-SA-FAPBiTE
CD86
CD86 (gMFI)
CD206
CD206 (gMFI)
CD163
CD163 (gMFI)
1500
1000
500

Figure 76

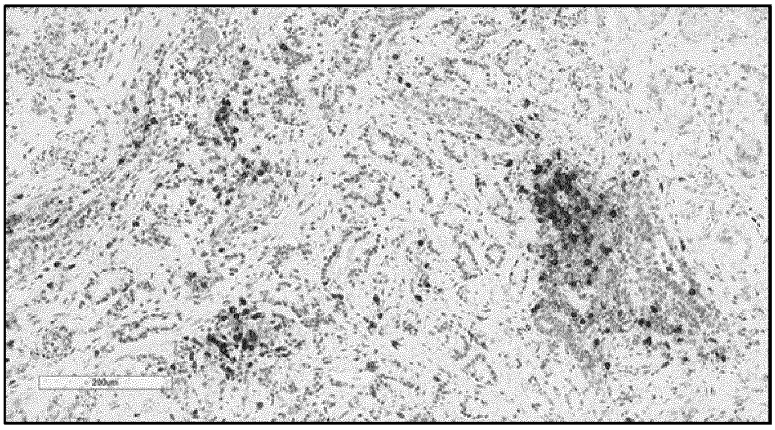
A

EpCAM Staining



B

CD8 Staining



C

FAP Staining

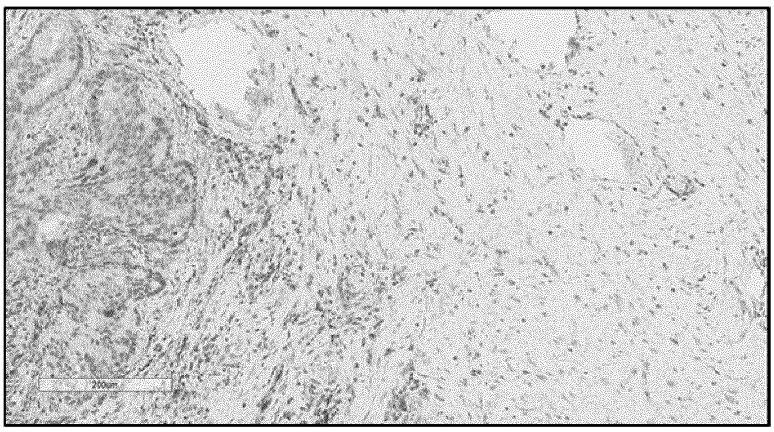


Figure 76

D

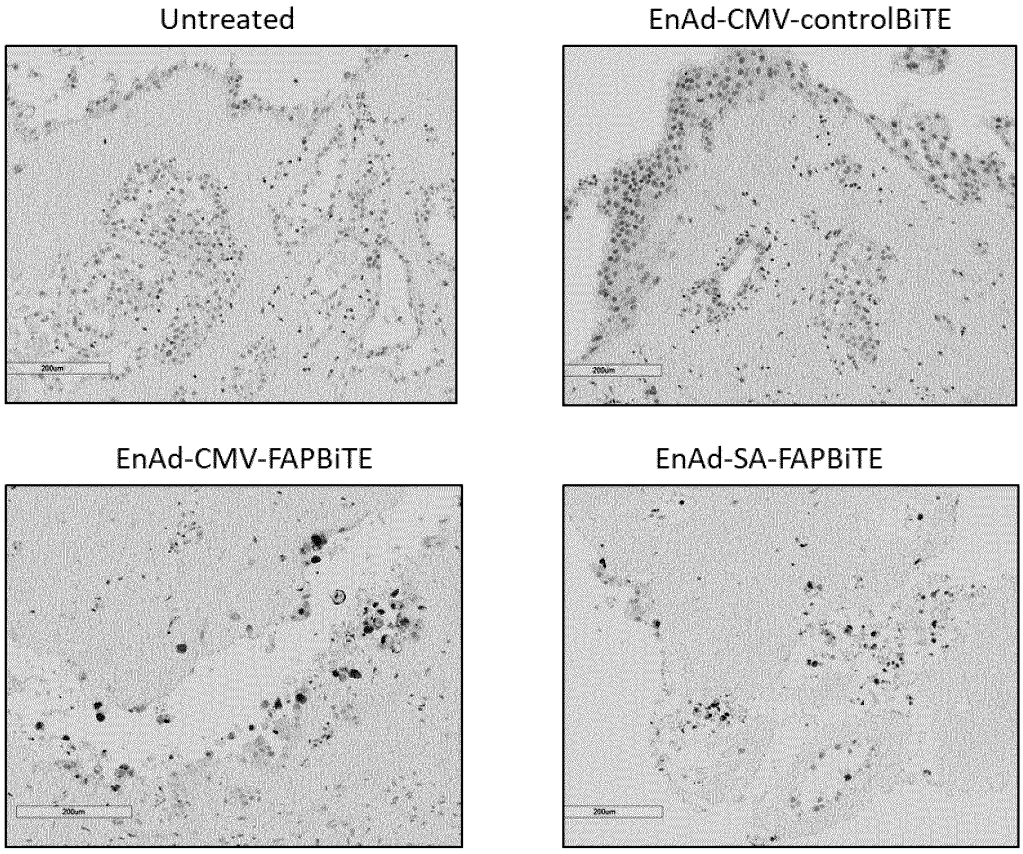

Untreated
EnAd-CMV-controlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-FAPBiTE

Figure 76

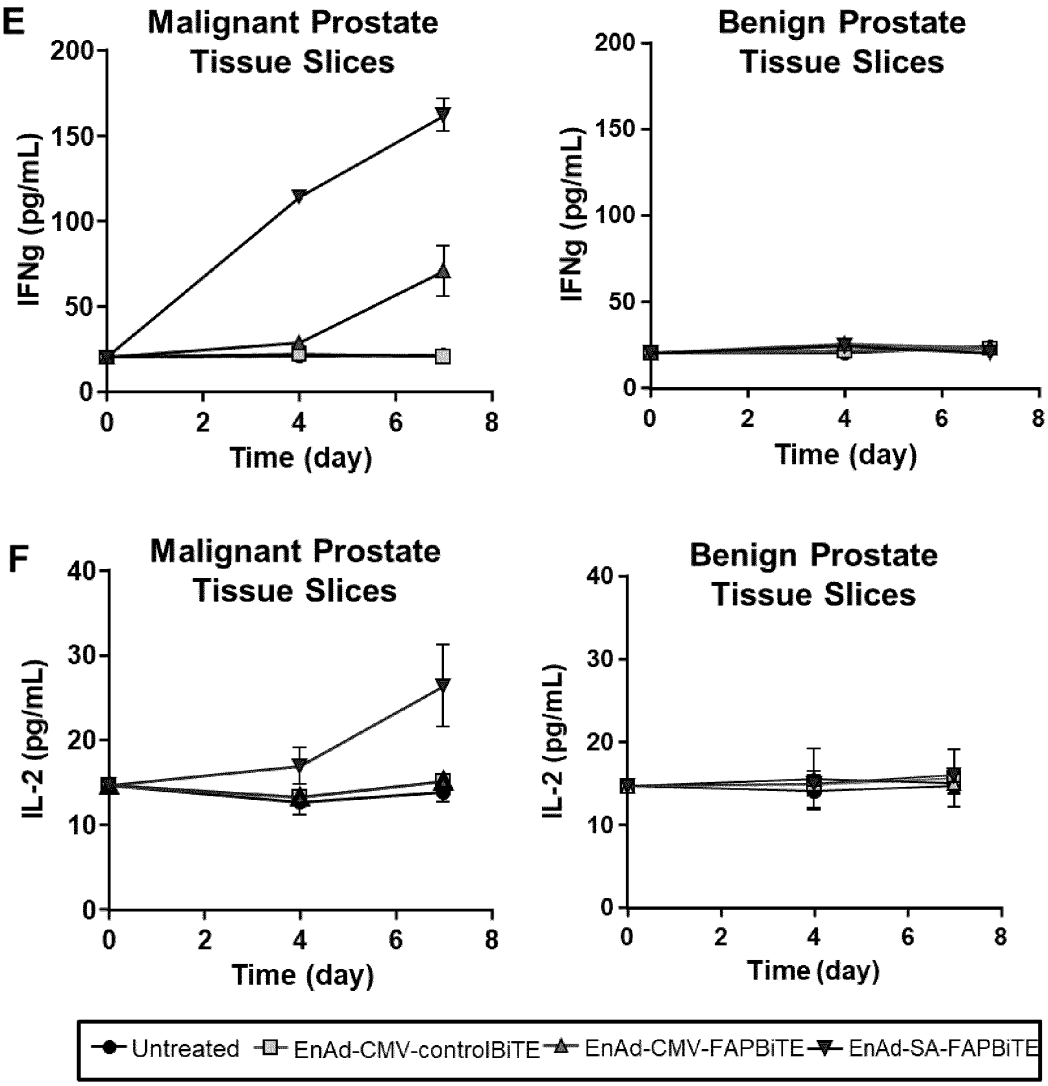

E
Malignant Prostate Tissue Slices
IFNg (pg/mL)
Time (day)
Benign Prostate Tissue Slices
IFNg (pg/mL)
Time (day)
F
Malignant Prostate Tissue Slices
IL-2 (pg/mL)
Time (day)
Benign Prostate Tissue Slices
IL-2 (pg/mL)
Time (day)
Untreated
EnAd-CMV-controlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-FAPBiTE

Figure 77

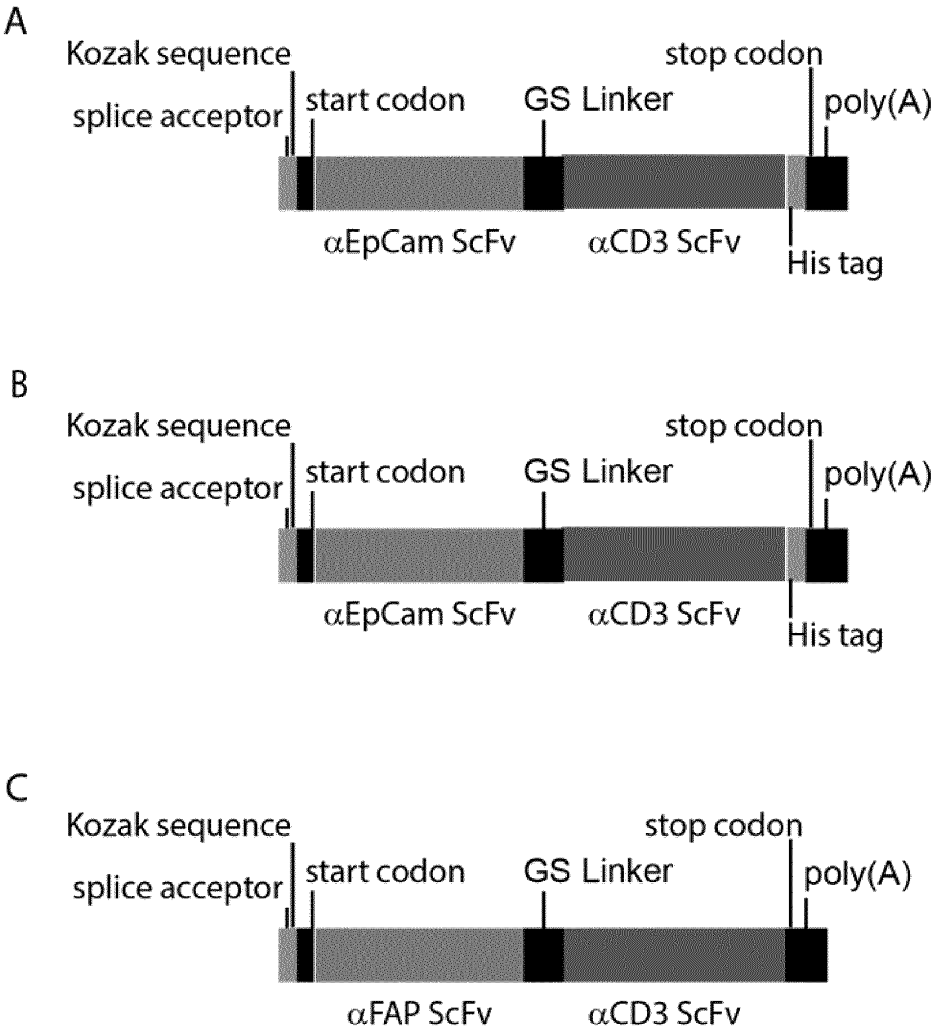

A
Kozak sequence
splice acceptor
start codon
GS Linker
stop codon
poly(A)
αEpCam ScFv
αCD3 ScFv
His tag
B
Kozak sequence
splice acceptor
start codon
GS Linker
stop codon
poly(A)
αEpCam ScFv
αCD3 ScFv
His tag
C
Kozak sequence
splice acceptor
start codon
GS Linker
stop codon
poly(A)
αFAP ScFv
αCD3 ScFv

Figure 77D

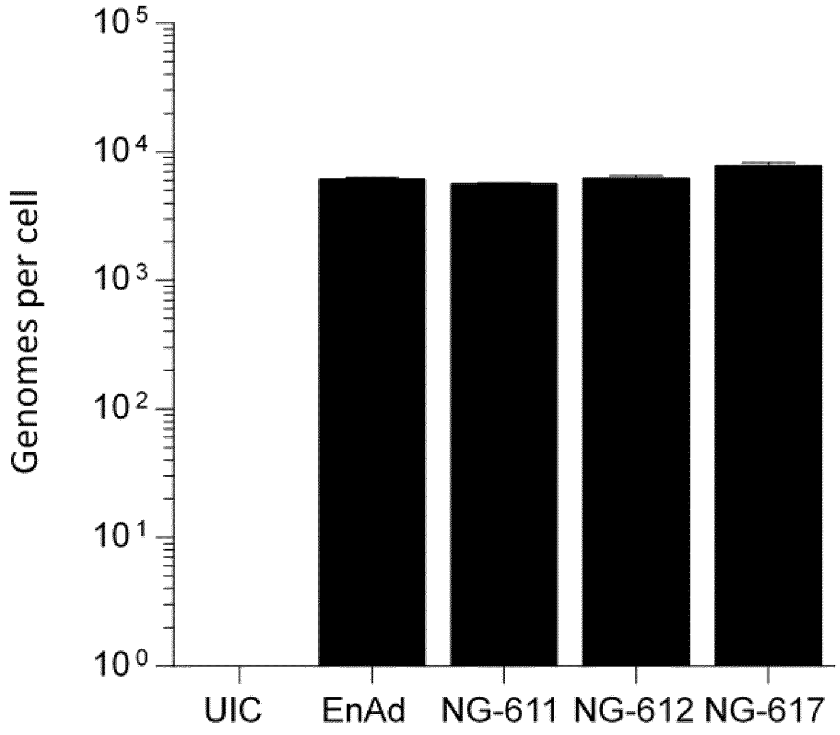

Genomes per cell
10^5
10^4
10^3
10^2
10^1
10^0
UIC
EnAd
NG-611
NG-612
NG-617

Figure 78

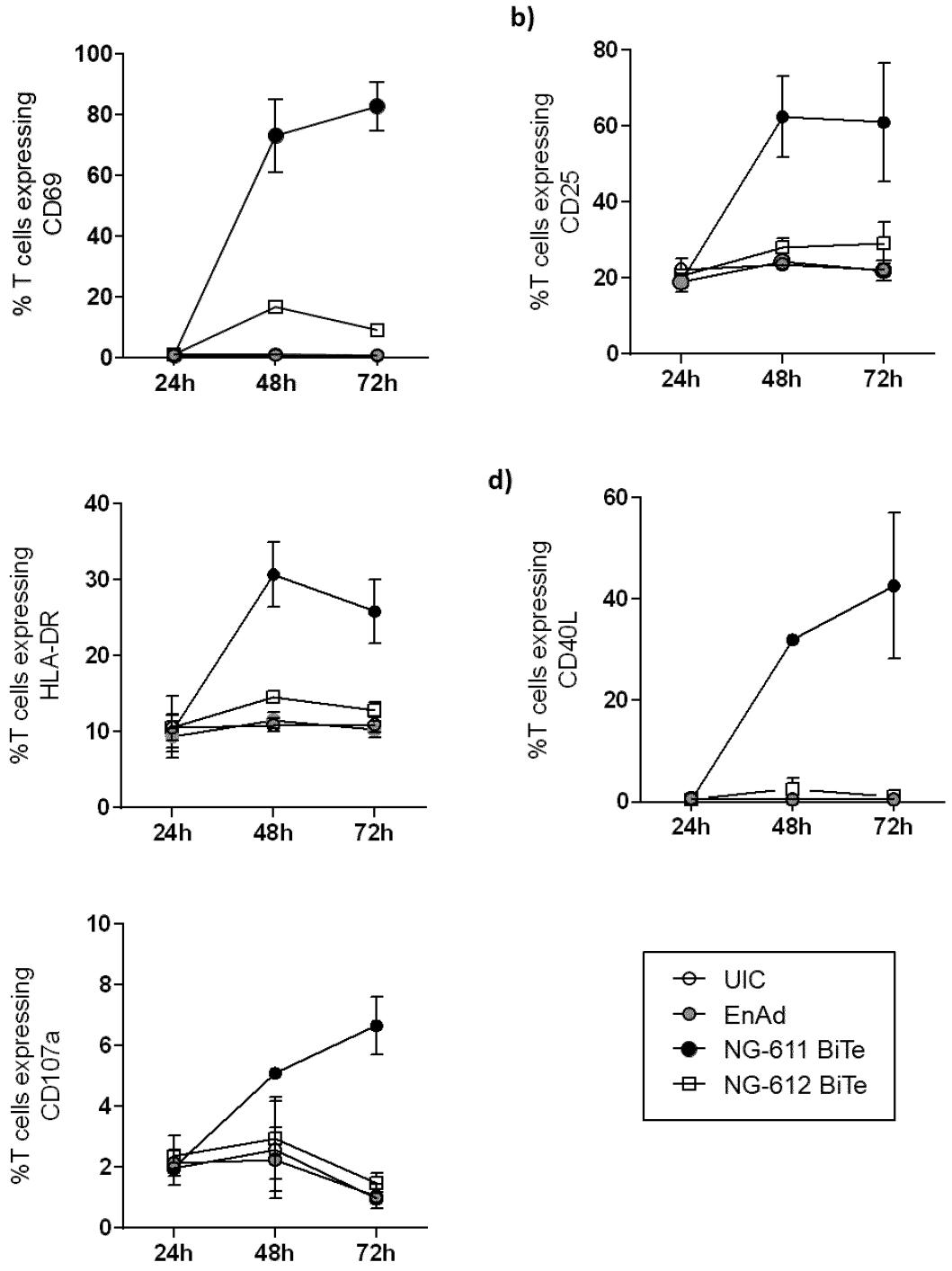

b)
d)
% T cells expressing CD69
%T cells expressing CD25
%T cells expressing HLA-DR
%T cells expressing CD40L
%T cells expressing CD107a
24h
48h
72h
UIC
EnAd
NG-611 BiTe
NG-612 BiTe

Figure 79

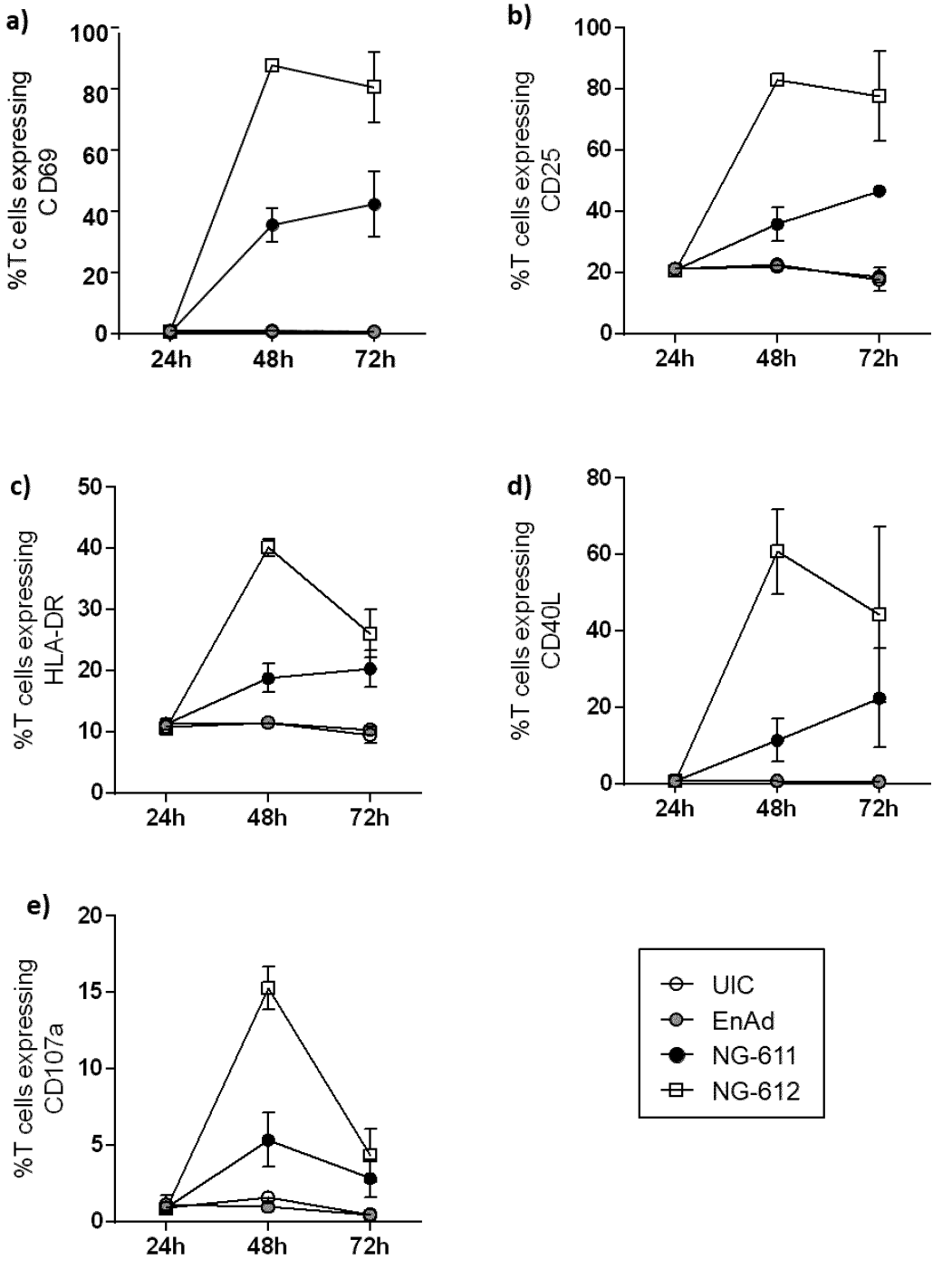

a)
%T cells expressing CD69
100
80
60
40
20
0
24h
48h
72h
b)
%T cells expressing CD25
c)
%T cells expressing HLA-DR
50
40
30
20
10
d)
%T cells expressing CD40L
e)
%T cells expressing CD107a
15
5
UIC
EnAd
NG-611
NG-612

Figure 80

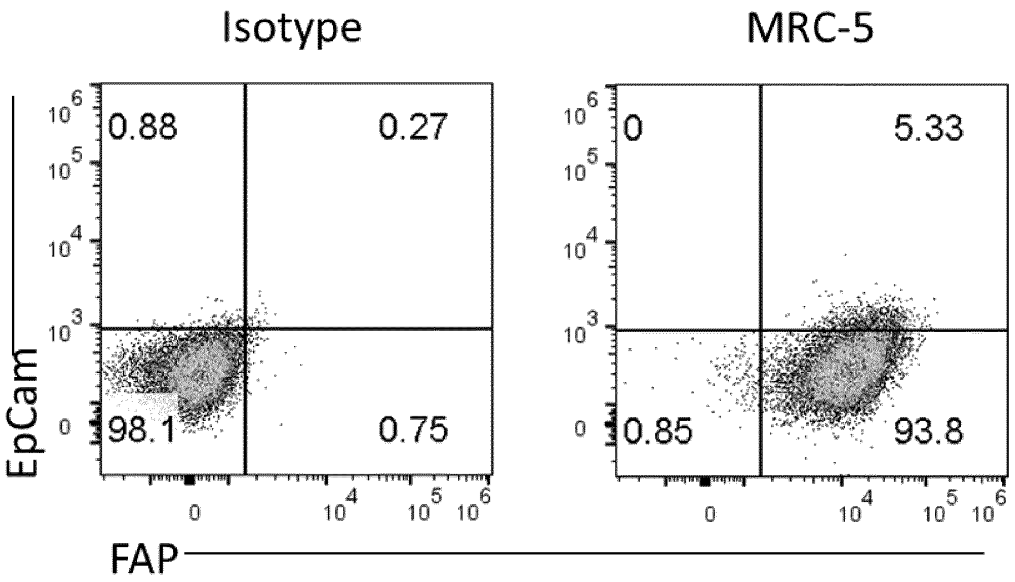


Figure 81A

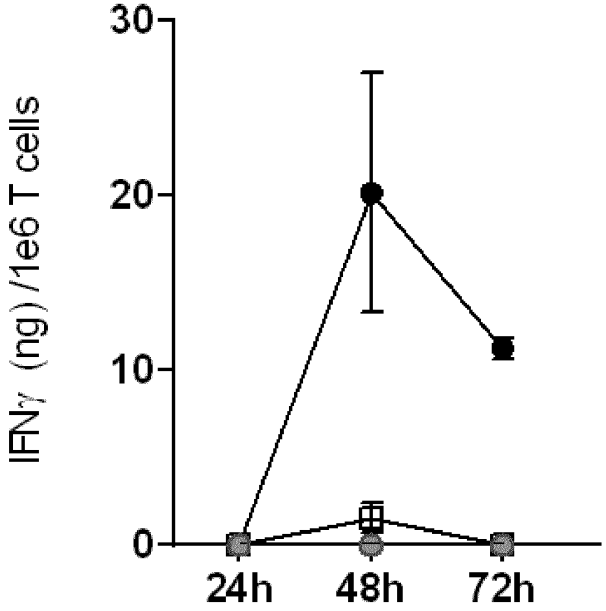


Figure 81B

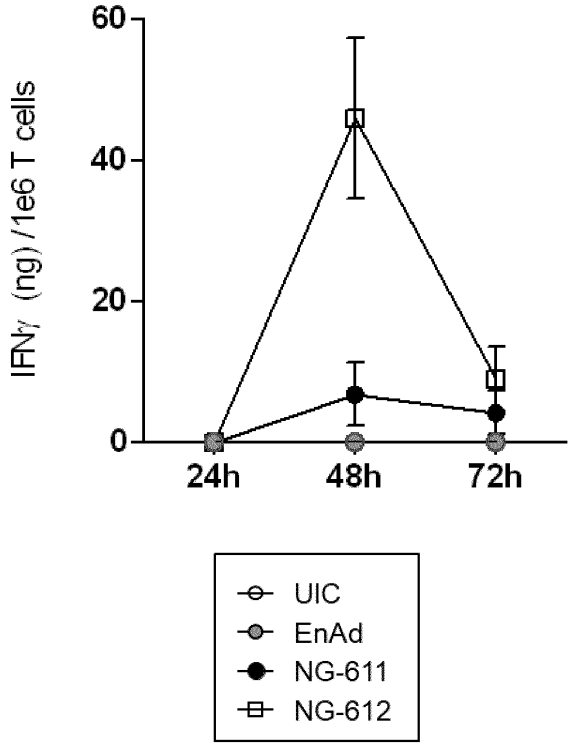


Figure 82

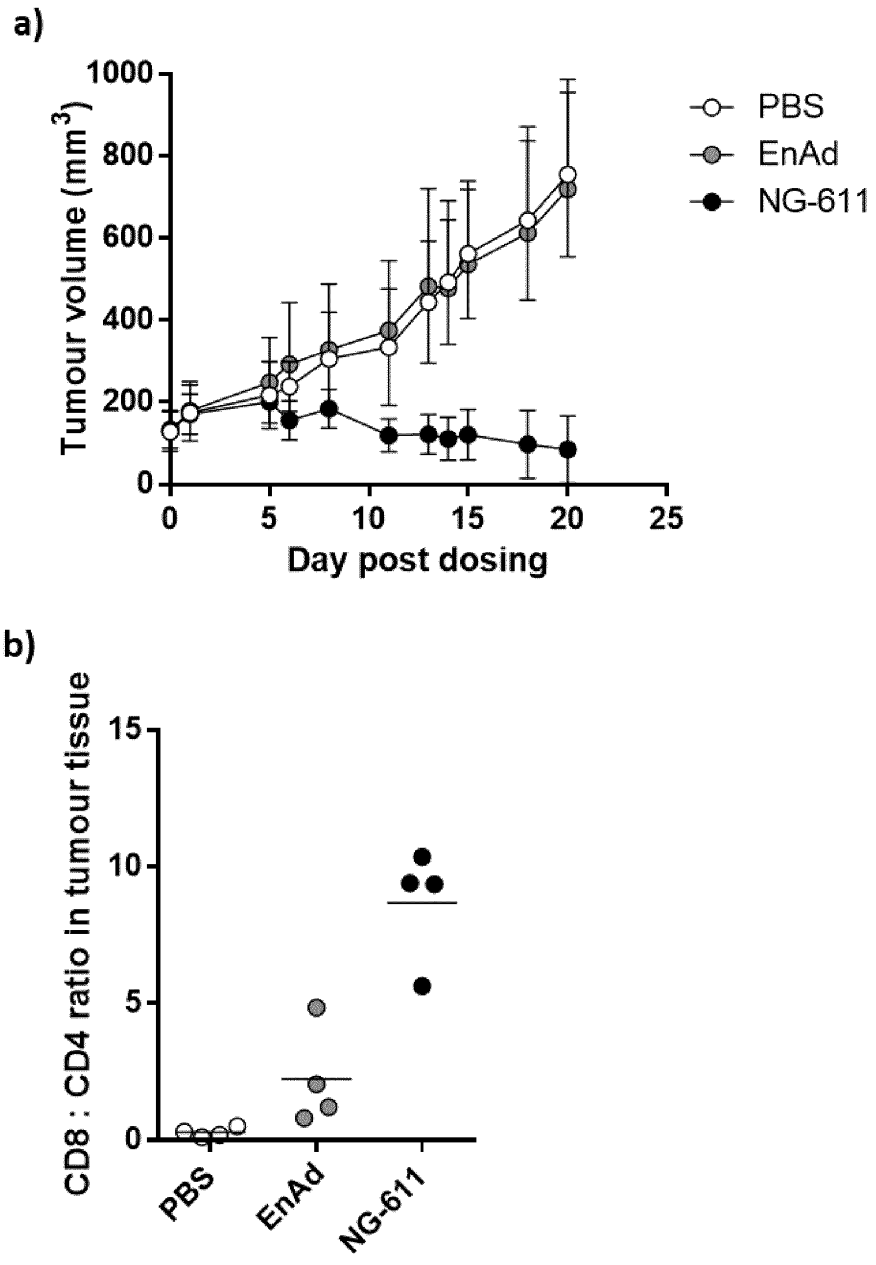

a)
Tumour volume (mm3)
1000
800
600
400
200
0
0
5
10
15
20
25
Day post dosing
PBS
EnAd
NG-611
b)
CD8 : CD4 ratio in tumour tissue
15
10
5
0
PBS
EnAd
NG-611

Figure 83

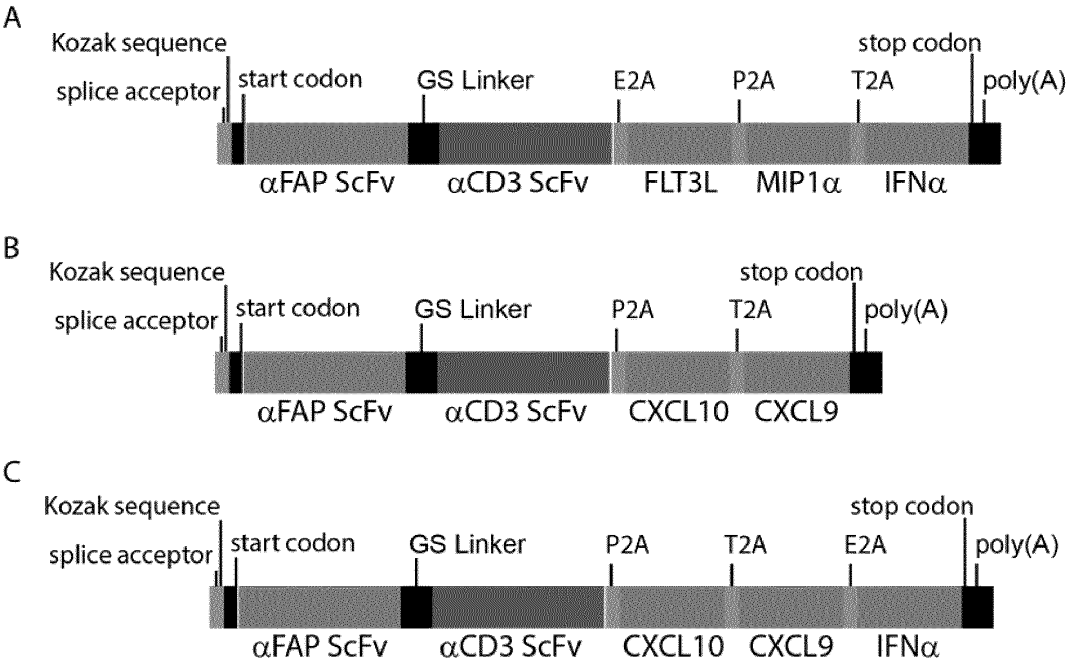


Figure 84

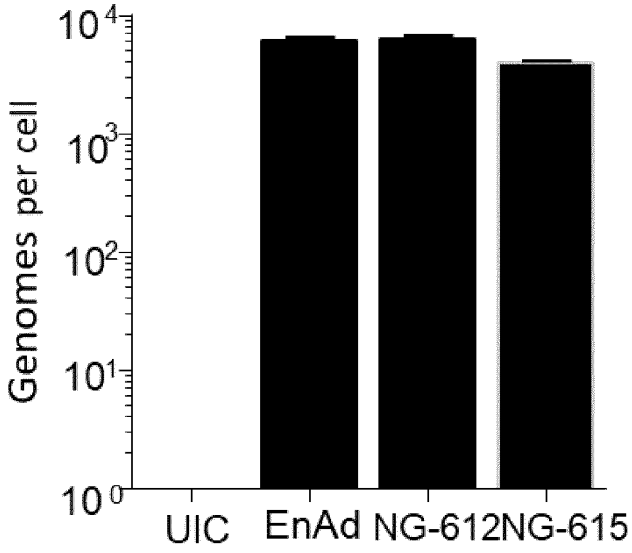


Figure 85

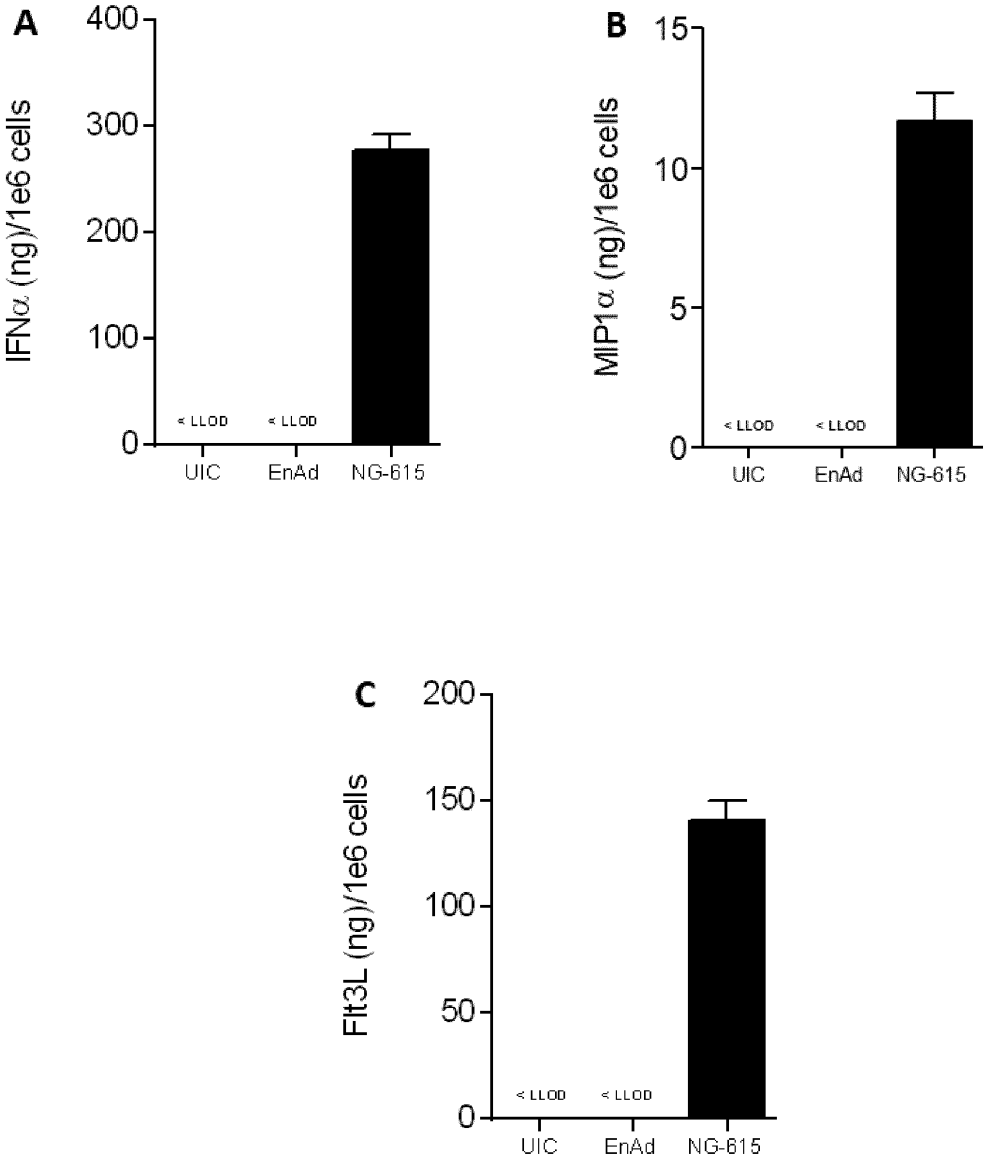

A
IFNα (ng)/1e6 cells
400
300
200
100
0
< LLOD
< LLOD
UIC
EnAd
NG-615
B
MIP1α (ng)/1e6 cells
15
10
5
0
< LLOD
< LLOD
UIC
EnAd
NG-615
C
Flt3L (ng)/1e6 cells
200
150
100
50
0
< LLOD
< LLOD
UIC
EnAd
NG-615

Figure 86

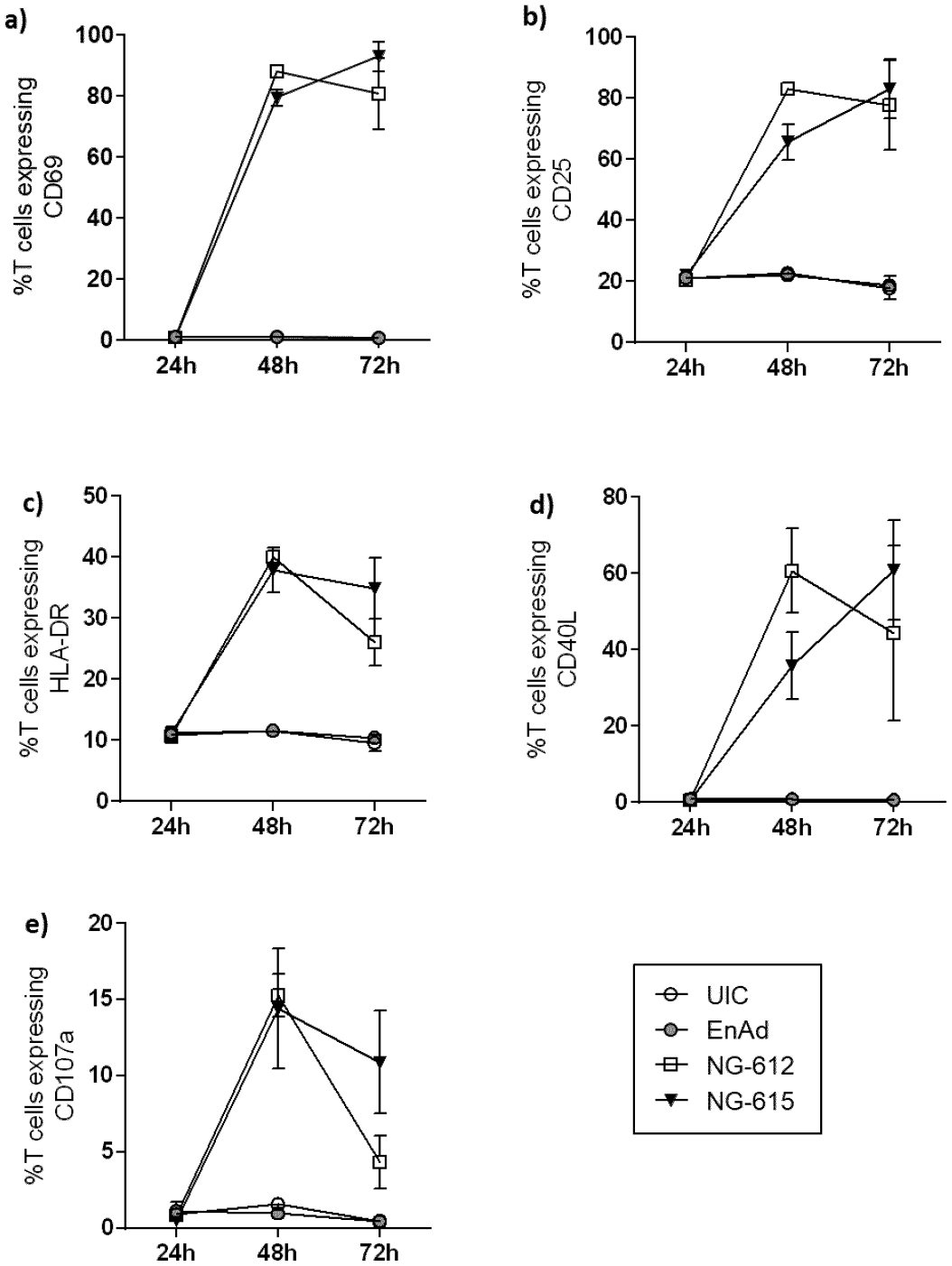

a)
%T cells expressing CD69
100
80
60
40
20
0
24h
48h
72h
b)
%T cells expressing CD25
c)
%T cells expressing HLA-DR
50
40
30
20
10
d)
%T cells expressing CD40L
e)
%T cells expressing CD107a
15
5
UIC
EnAd
NG-612
NG-615

Figure 87

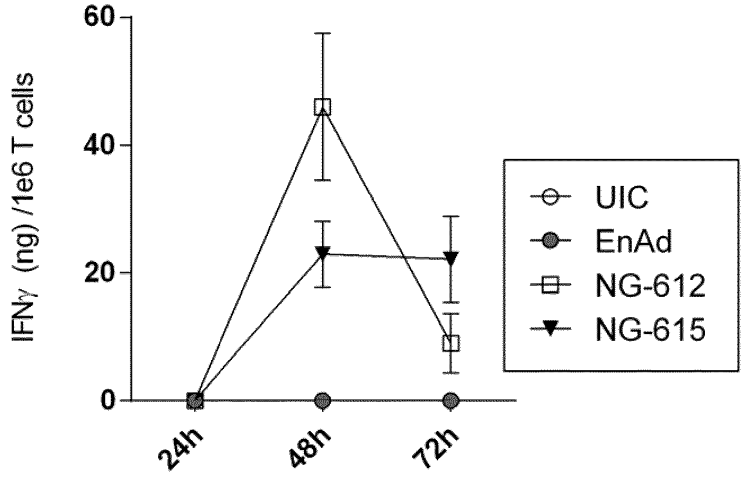


Figure 88

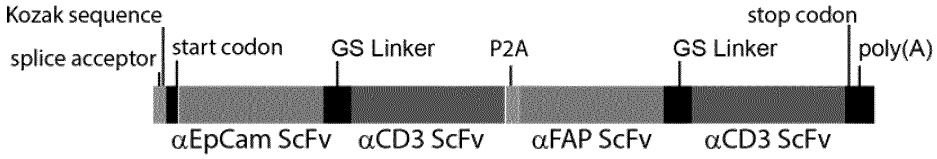


Figure 89

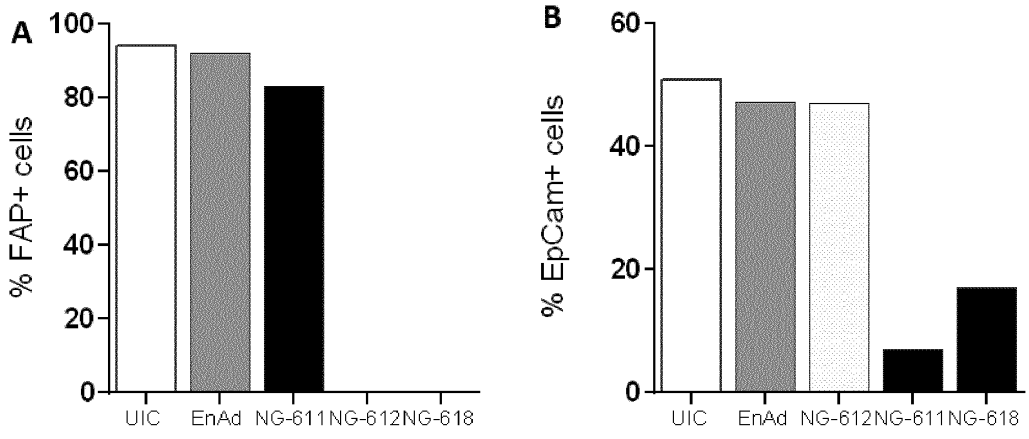
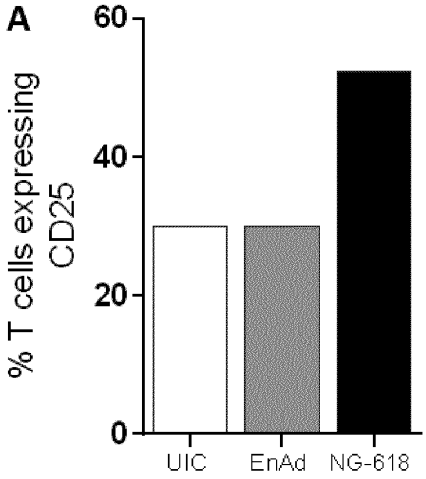
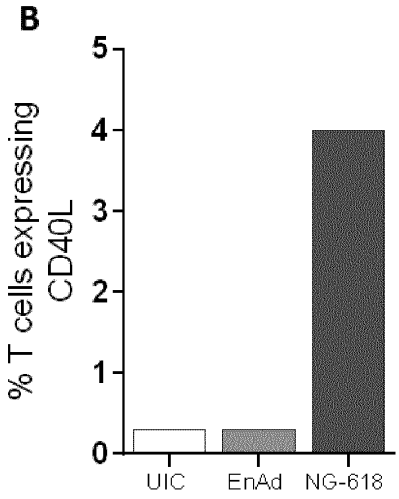


Figure 90


A
% T cells expressing CD25
60
40
20
0
UIC
EnAd
NG-618


B
% T cells expressing CD40L
5
4
3
2
1
0
UIC
EnAd
NG-618

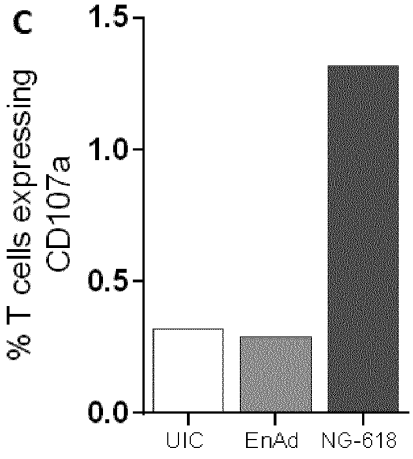

C
% T cells expressing CD107a
1.5
1.0
0.5
0.0
UIC
EnAd
NG-618

Figure 91

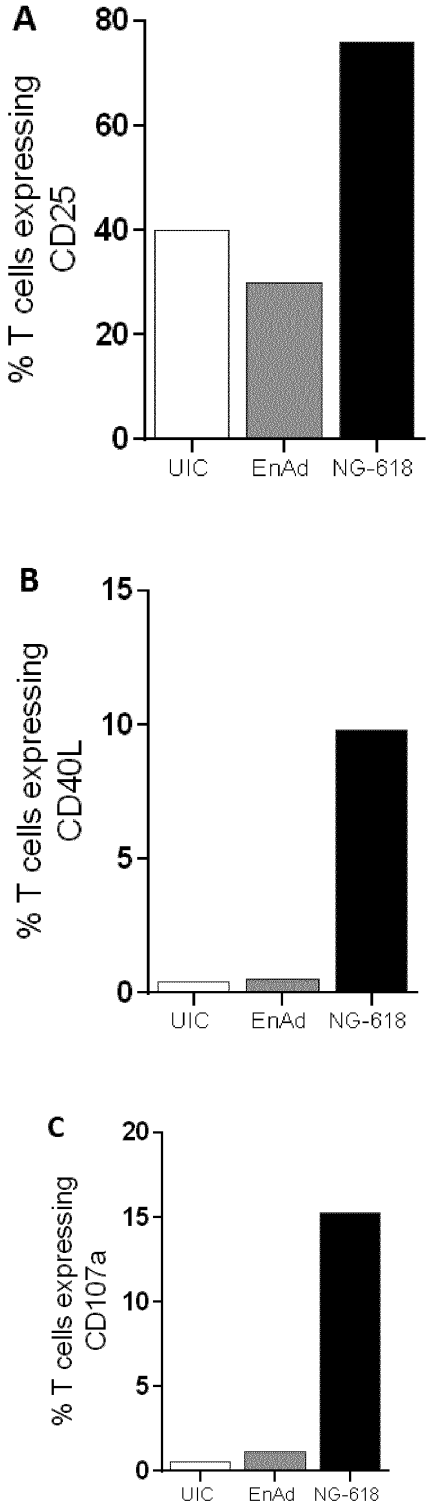
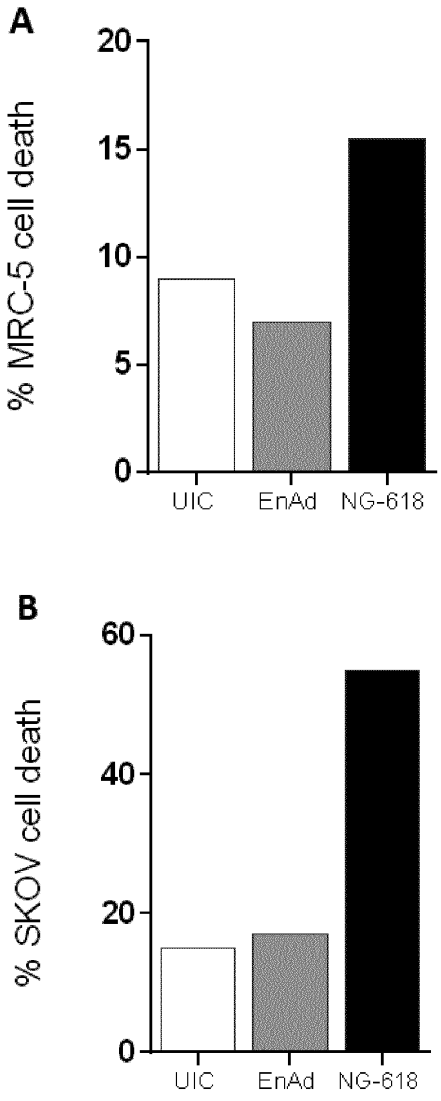

A
% T cells expressing CD25
80
60
40
20
0
UIC
EnAd
NG-618
B
% T cells expressing CD40L
15
10
5
0
UIC
EnAd
NG-618
C
% T cells expressing CD107a
20
15
10
5
0
UIC
EnAd
NG-618

Figure 92


A
% MRC-5 cell death
20
15
10
5
0
UIC
EnAd
NG-618
B
% SKOV cell death
60
40
20
0
UIC
EnAd
NG-618