FACS assay on affinity of anti-EGFR VHH-Fc for target cells NCI-H1975

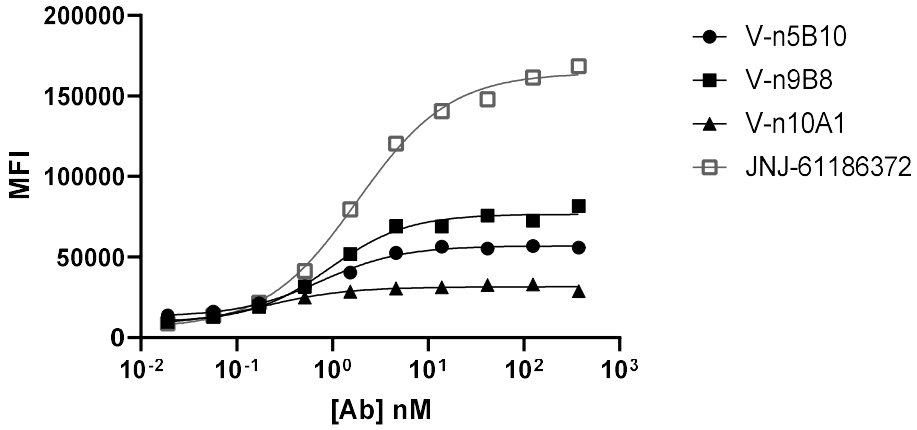


FIG. 1

FACS assay on affinity of anti-cMet VHH-Fc for target cells EBC-1

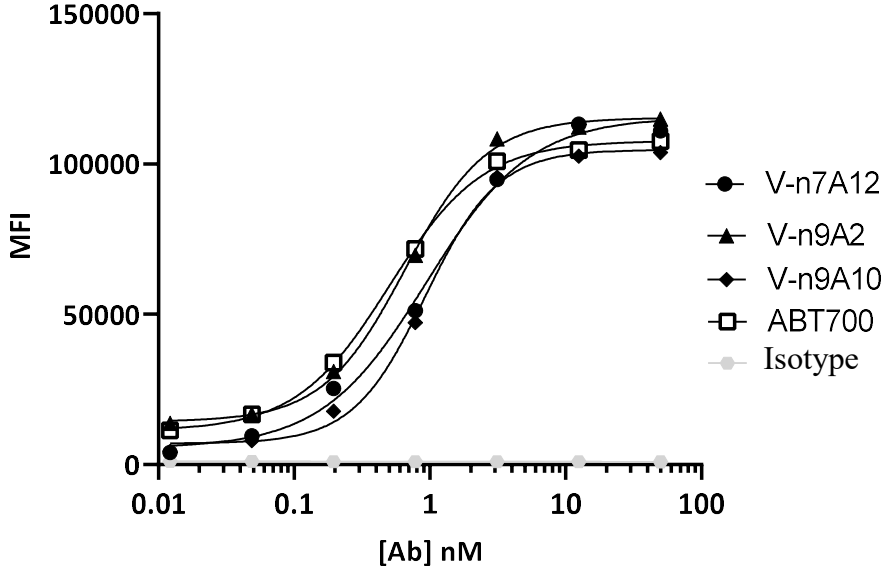


FIG. 2

Blocking effect of anti-cMet VHH-Fc on binding of HGF to EBC-1

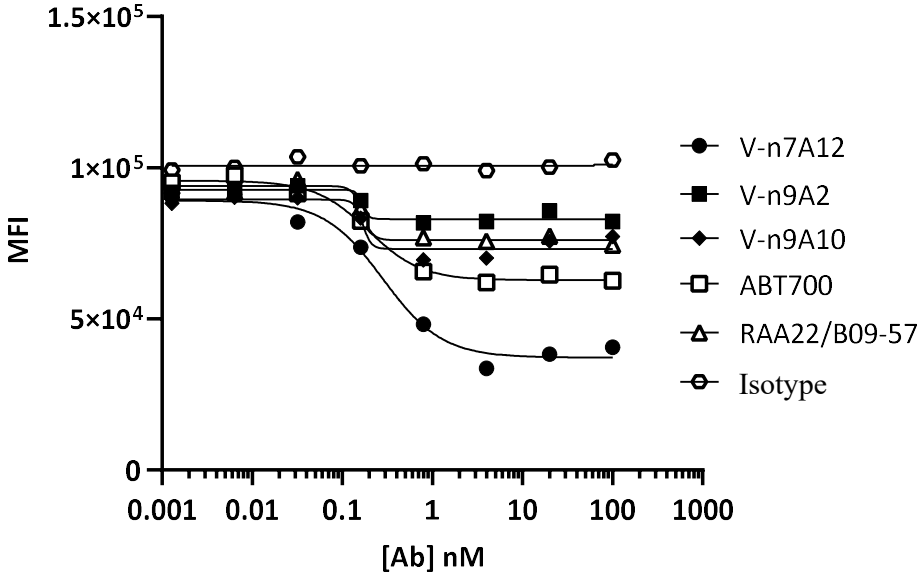


FIG. 3

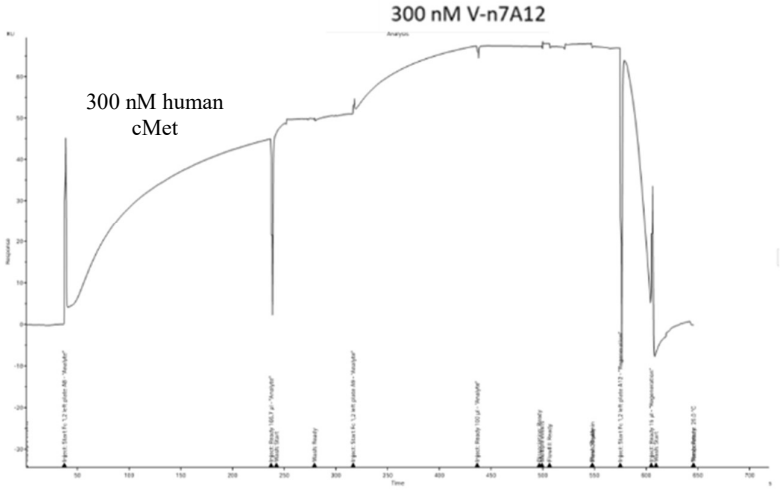
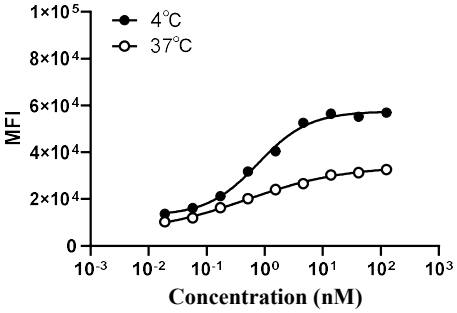
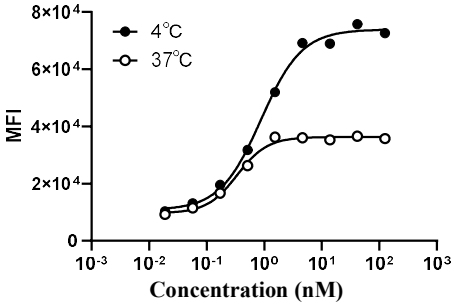


FIG. 4

FACS assay on endocytosis of V-n5B10 by target cells NCI-H1975



FACS assay on endocytosis of V-n9B8 by target cells NCI-H1975



FACS assay on endocytosis of V-n10A1 by target cells NCI-H1975

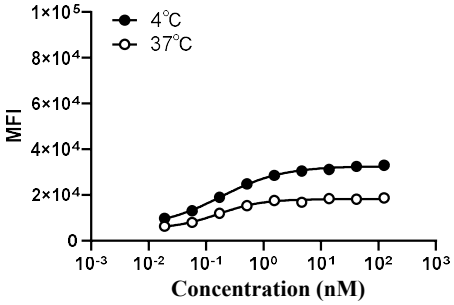


FIG. 5A

FACS assay on endocytosis of anti-cMet VHH-Fc by target cells EBC-1

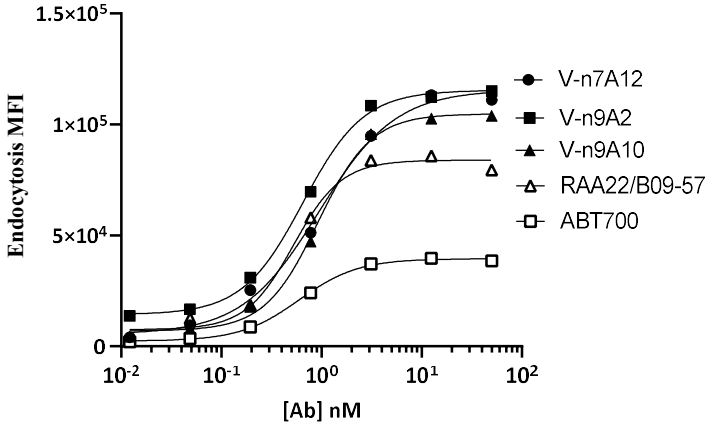


FIG. 5B

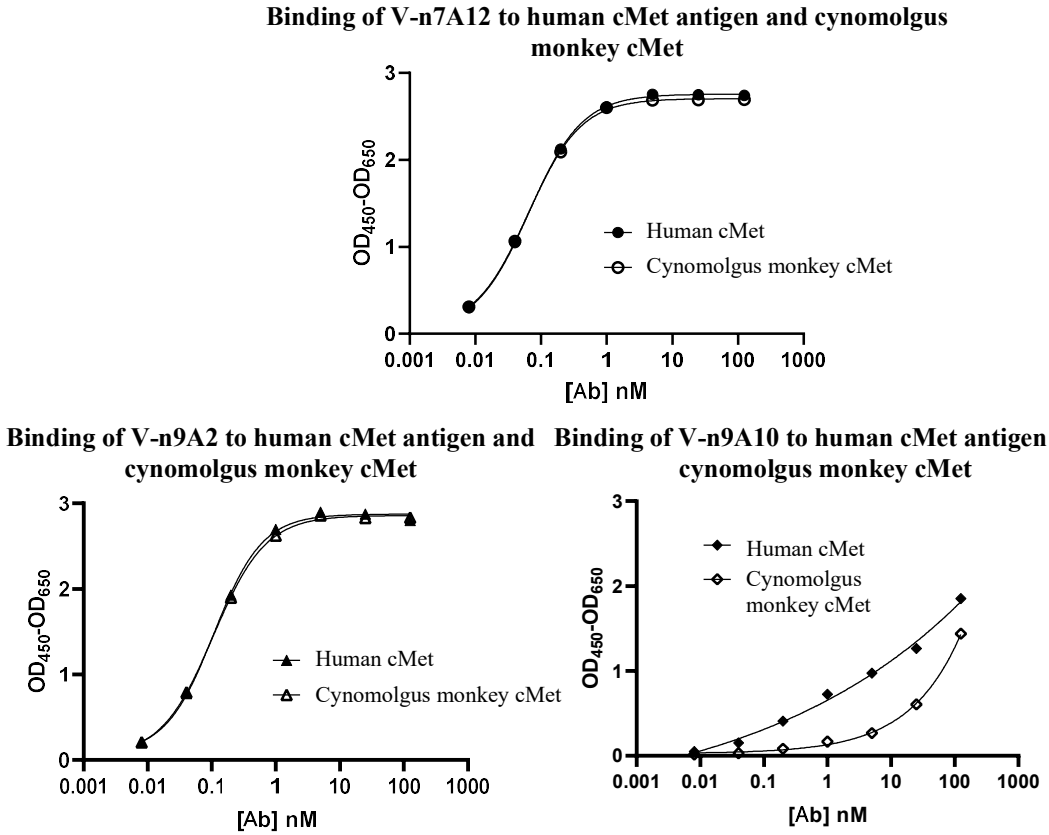

Binding of V-n7A12 to human cMet antigen and cynomolgus monkey cMet
OD450-OD650
[Ab] nM
Human cMet
Cynomolgus monkey cMet
Binding of V-n9A2 to human cMet antigen and cynomolgus monkey cMet
Binding of V-n9A10 to human cMet antigen and cynomolgus monkey cMet

FIG. 6

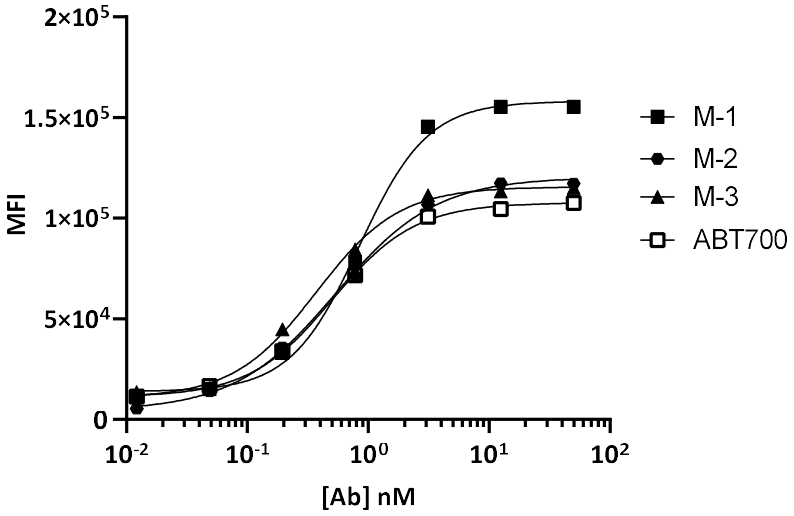

Binding of anti-cMet biepitopic molecule to target cells EBC-1
MFI
[Ab] nM
M-1
M-2
M-3
ABT700

FIG. 7

Endocytosis of anti-cMet biepitopic molecule by target cells EBC-1

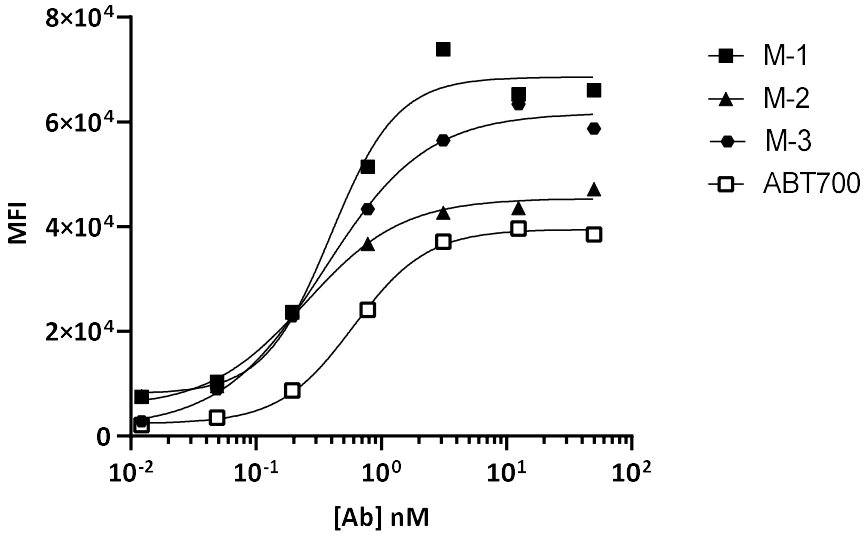


FIG. 8

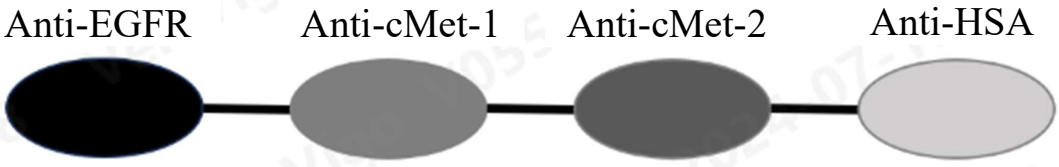


FIG. 9

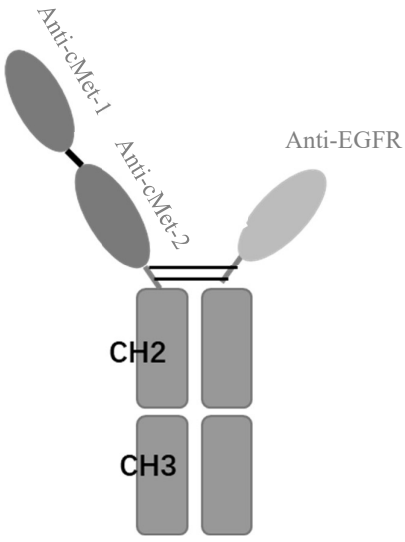


FIG. 10

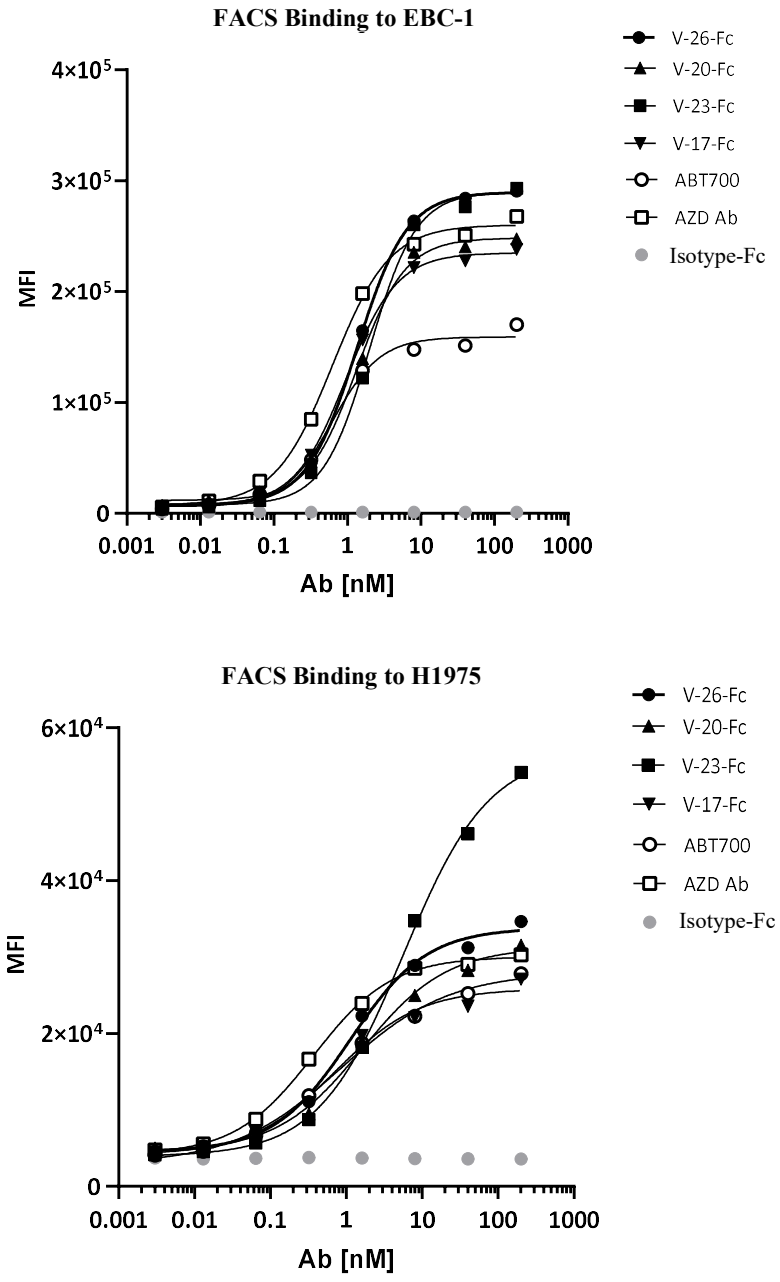

FACS Binding to EBC-1
V-26-Fc
V-20-Fc
V-23-Fc
V-17-Fc
ABT700
AZD Ab
Isotype-Fc
MFI
4×10^5
3×10^5
2×10^5
1×10^5
0
0.001
0.01
0.1
1
10
100
1000
Ab [nM]
FACS Binding to H1975
V-26-Fc
V-20-Fc
V-23-Fc
V-17-Fc
ABT700
AZD Ab
Isotype-Fc
MFI
6×10^4
4×10^4
2×10^4
0
0.001
0.01
0.1
1
10
100
1000
Ab [nM]

FIG. 11

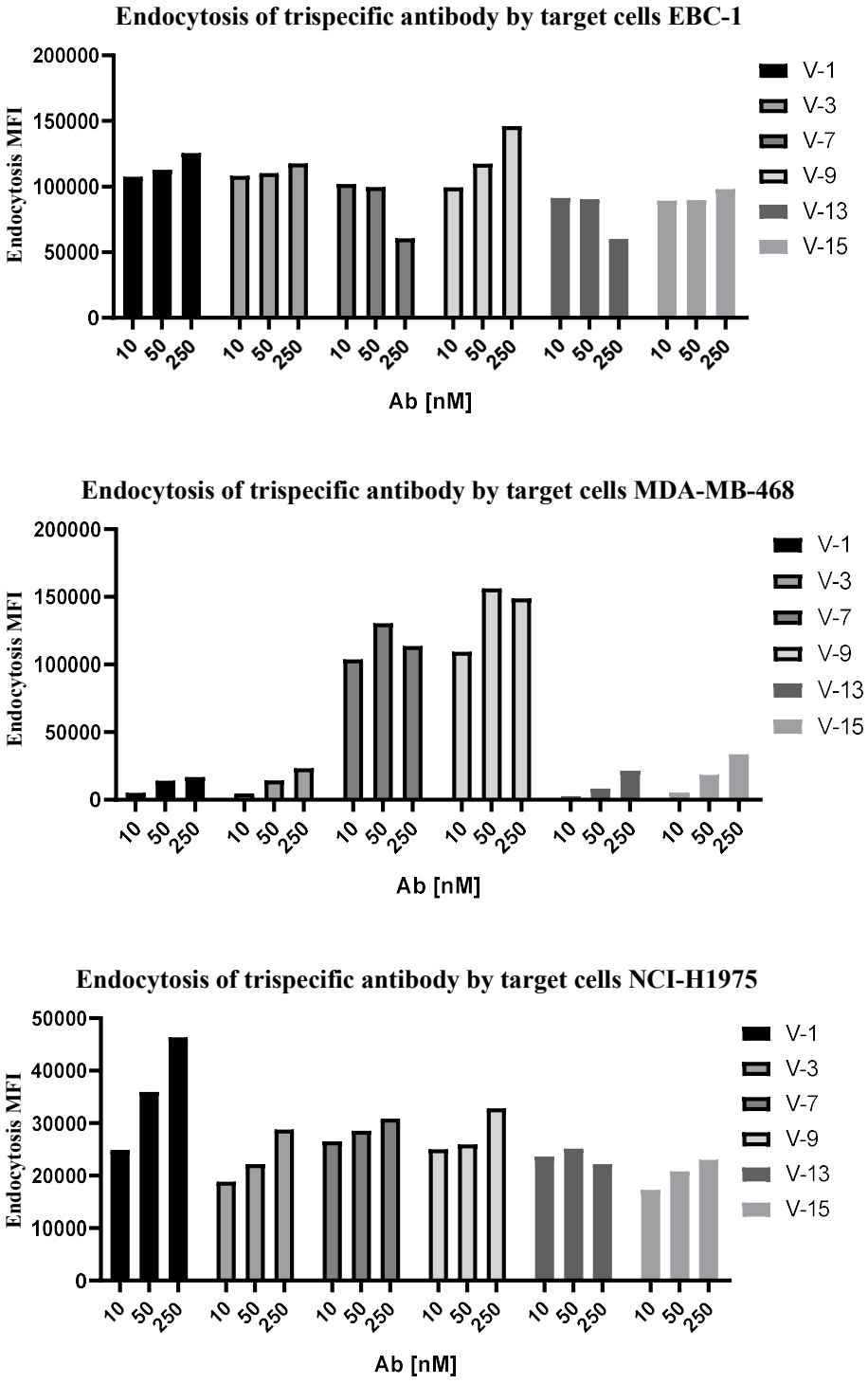

Endocytosis of trispecific antibody by target cells EBC-1
Endocytosis MFI
200000
150000
100000
50000
0
10 50 250 10 50 250 10 50 250 10 50 250 10 50 250 10 50 250
Ab [nM]
V-1
V-3
V-7
V-9
V-13
V-15
Endocytosis of trispecific antibody by target cells MDA-MB-468
Endocytosis MFI
200000
150000
100000
50000
0
10 50 250 10 50 250 10 50 250 10 50 250 10 50 250 10 50 250
Ab [nM]
V-1
V-3
V-7
V-9
V-13
V-15
Endocytosis of trispecific antibody by target cells NCI-H1975
Endocytosis MFI
50000
40000
30000
20000
10000
0
10 50 250 10 50 250 10 50 250 10 50 250 10 50 250 10 50 250
Ab [nM]
V-1
V-3
V-7
V-9
V-13
V-15

FIG. 12

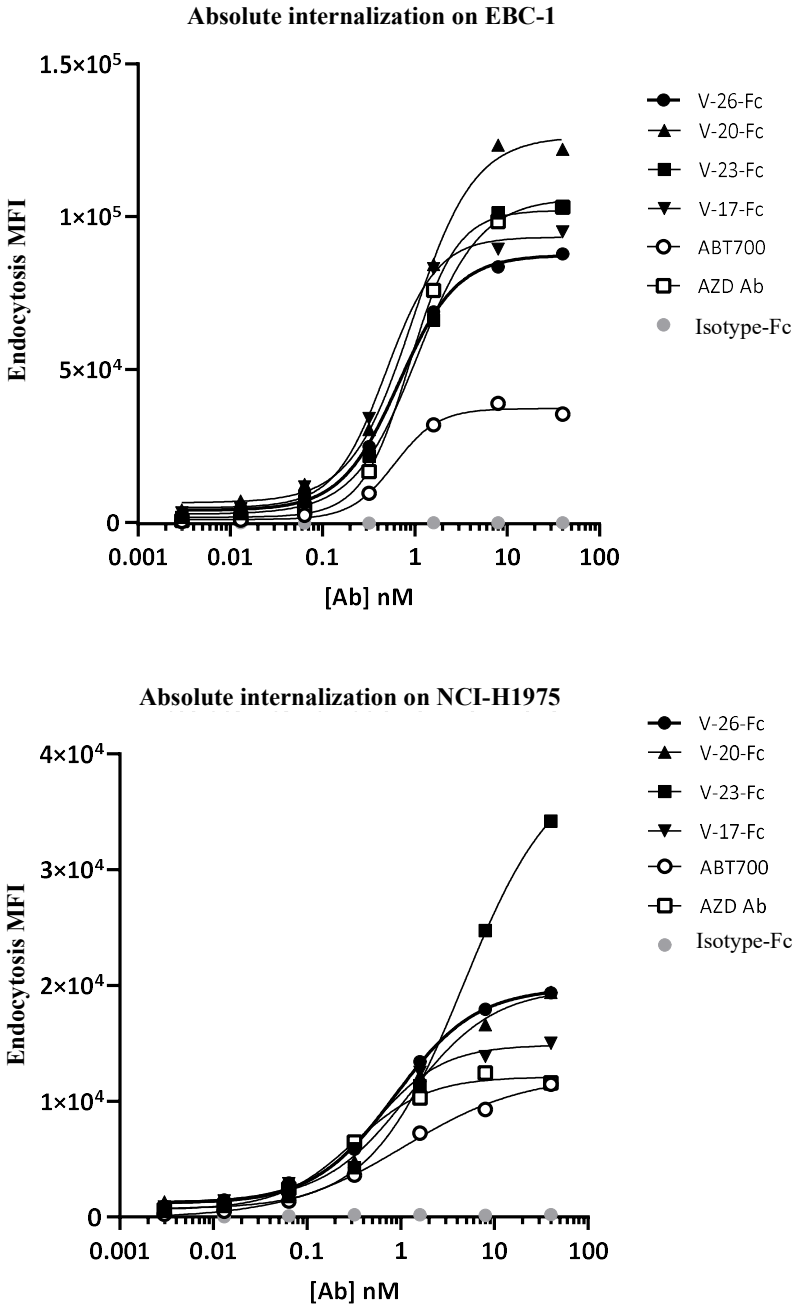

Absolute internalization on EBC-1
Endocytosis MFI
1.5×10^5
1×10^5
5×10^4
0
0.001
0.01
0.1
1
10
100
[Ab] nM
V-26-Fc
V-20-Fc
V-23-Fc
V-17-Fc
ABT700
AZD Ab
Isotype-Fc
Absolute internalization on NCI-H1975
Endocytosis MFI
4×10^4
3×10^4
2×10^4
1×10^4
0
0.001
0.01
0.1
1
10
100
[Ab] nM
V-26-Fc
V-20-Fc
V-23-Fc
V-17-Fc
ABT700
AZD Ab
Isotype-Fc

FIG. 13

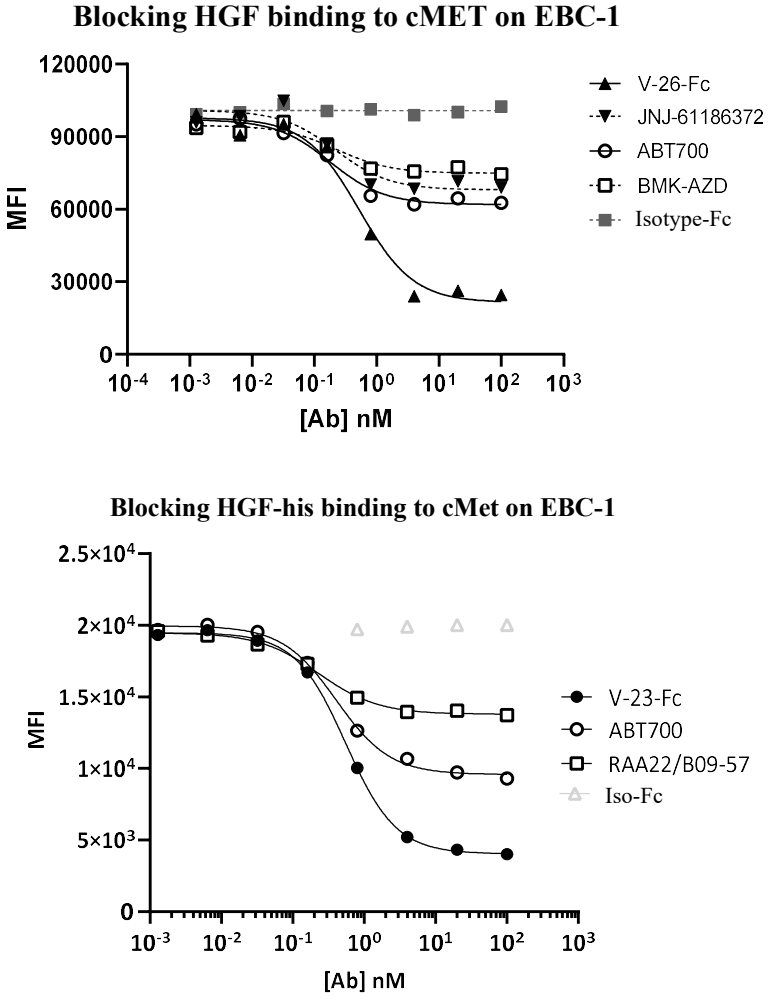

Blocking HGF binding to cMET on EBC-1
120000
90000
60000
30000
0
MFI
10-4 10-3 10-2 10-1 100 101 102 103
[Ab] nM
V-26-Fc
JNJ-61186372
ABT700
BMK-AZD
Isotype-Fc
Blocking HGF-his binding to cMet on EBC-1
2.5×10⁴
2×10⁴
1.5×10⁴
1×10⁴
5×10³
0
MFI
10-3 10-2 10-1 100 101 102 103
[Ab] nM
V-23-Fc
ABT700
RAA22/B09-57
Iso-Fc

FIG. 14

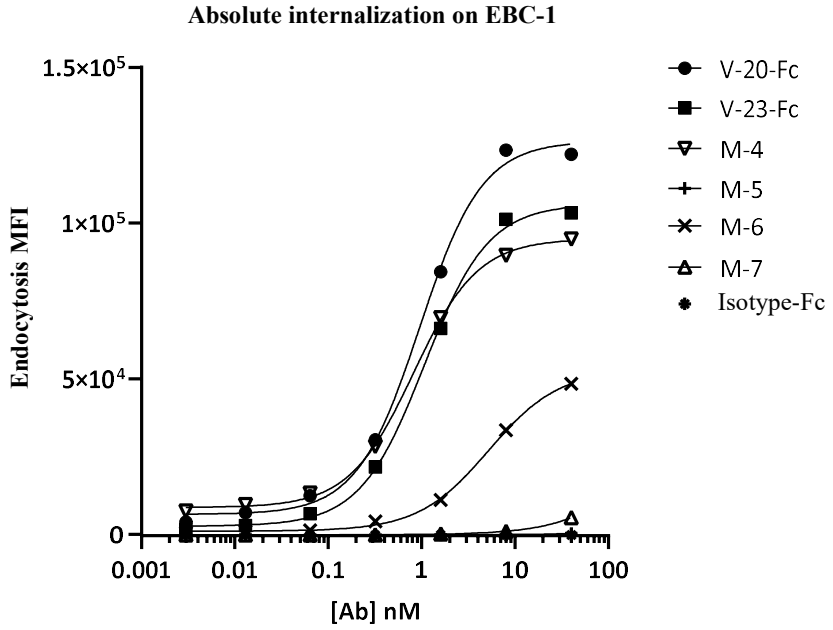

Absolute internalization on EBC-1
Endocytosis MFI
1.5×10^5
1×10^5
5×10^4
0
0.001
0.01
0.1
1
10
100
[Ab] nM
V-20-Fc
V-23-Fc
M-4
M-5
M-6
M-7
Isotype-Fc

FIG. 15

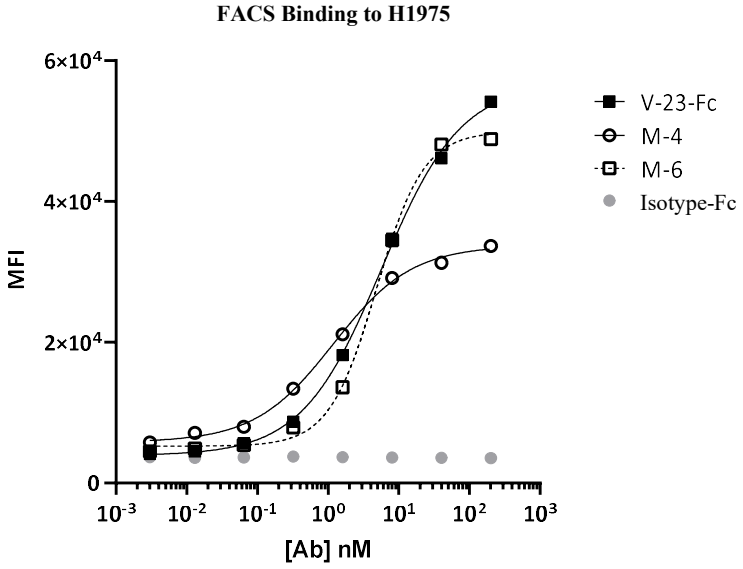
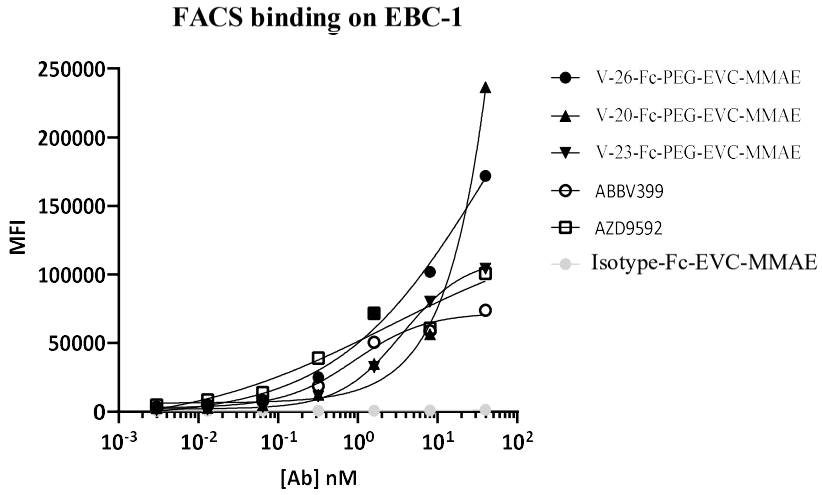
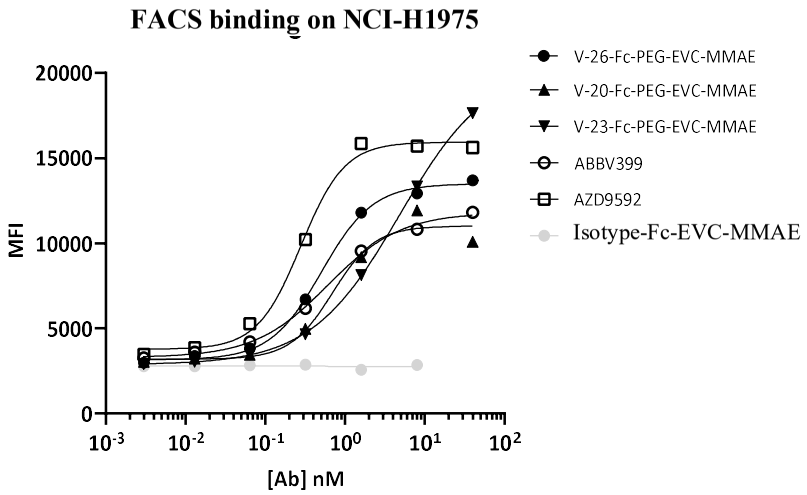
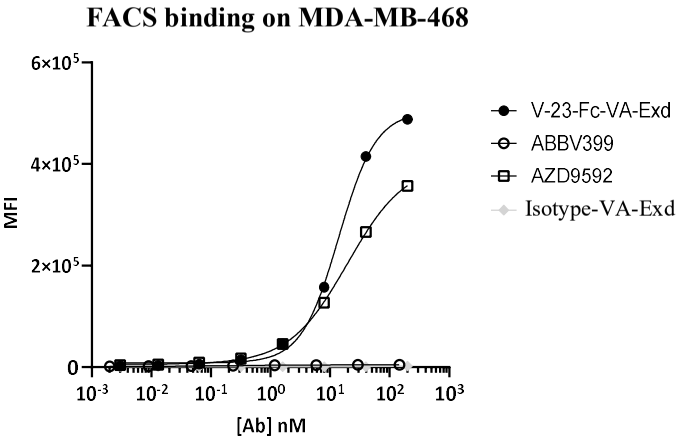

FACS Binding to H1975
MFI
6×10^4
4×10^4
2×10^4
0
10^-3
10^-2
10^-1
10^0
10^1
10^2
10^3
[Ab] nM
V-23-Fc
M-4
M-6
Isotype-Fc

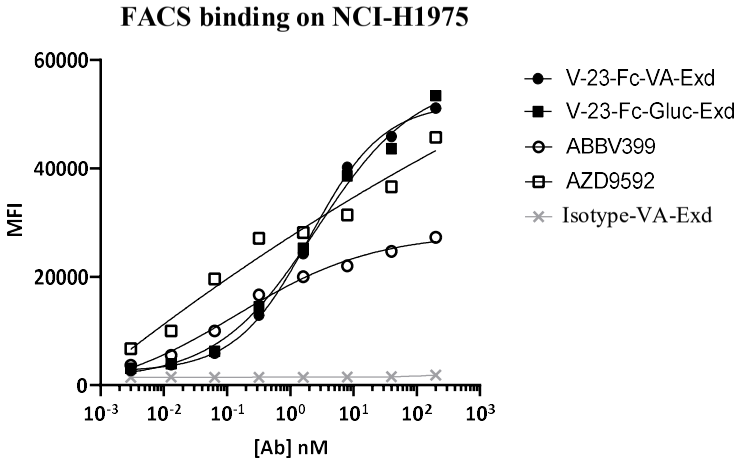
FIG. 16

FIG. 17


FACS binding on EBC-1
MFI
250000
200000
150000
100000
50000
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2}
[Ab] nM
V-26-Fc-PEG-EVC-MMAE
V-20-Fc-PEG-EVC-MMAE
V-23-Fc-PEG-EVC-MMAE
ABBV399
AZD9592
Isotype-Fc-EVC-MMAE


FACS binding on NCI-H1975
MFI
20000
15000
10000
5000
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2}
[Ab] nM
V-26-Fc-PEG-EVC-MMAE
V-20-Fc-PEG-EVC-MMAE
V-23-Fc-PEG-EVC-MMAE
ABBV399
AZD9592
Isotype-Fc-EVC-MMAE


FACS binding on MDA-MB-468
MFI
6×10^5
4×10^5
2×10^5
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3}
[Ab] nM
V-23-Fc-VA-Exd
ABBV399
AZD9592
Isotype-VA-Exd


FACS binding on NCI-H1975
MFI
60000
40000
20000
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3}
[Ab] nM
V-23-Fc-VA-Exd
V-23-Fc-Gluc-Exd
ABBV399
AZD9592
Isotype-VA-Exd

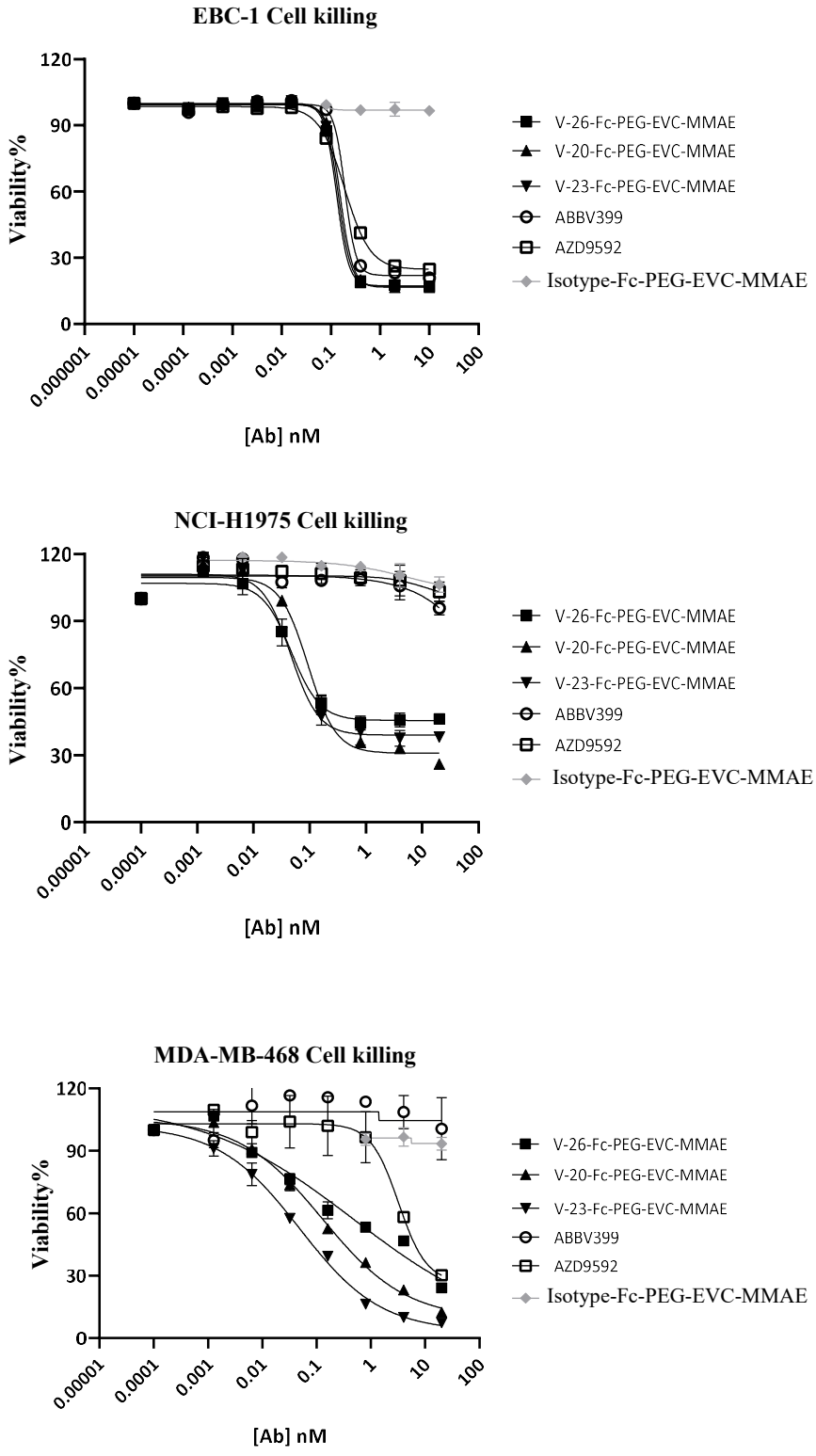

EBC-1 Cell killing
Viability%
120
90
60
30
0
0.000001
0.00001
0.0001
0.001
0.01
0.1
1
10
100
[Ab] nM
V-26-Fc-PEG-EVC-MMAE
V-20-Fc-PEG-EVC-MMAE
V-23-Fc-PEG-EVC-MMAE
ABBV399
AZD9592
Isotype-Fc-PEG-EVC-MMAE
NCI-H1975 Cell killing
Viability%
120
90
60
30
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
[Ab] nM
V-26-Fc-PEG-EVC-MMAE
V-20-Fc-PEG-EVC-MMAE
V-23-Fc-PEG-EVC-MMAE
ABBV399
AZD9592
Isotype-Fc-PEG-EVC-MMAE
MDA-MB-468 Cell killing
Viability%
120
90
60
30
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
[Ab] nM
V-26-Fc-PEG-EVC-MMAE
V-20-Fc-PEG-EVC-MMAE
V-23-Fc-PEG-EVC-MMAE
ABBV399
AZD9592
Isotype-Fc-PEG-EVC-MMAE

FIG. 18A

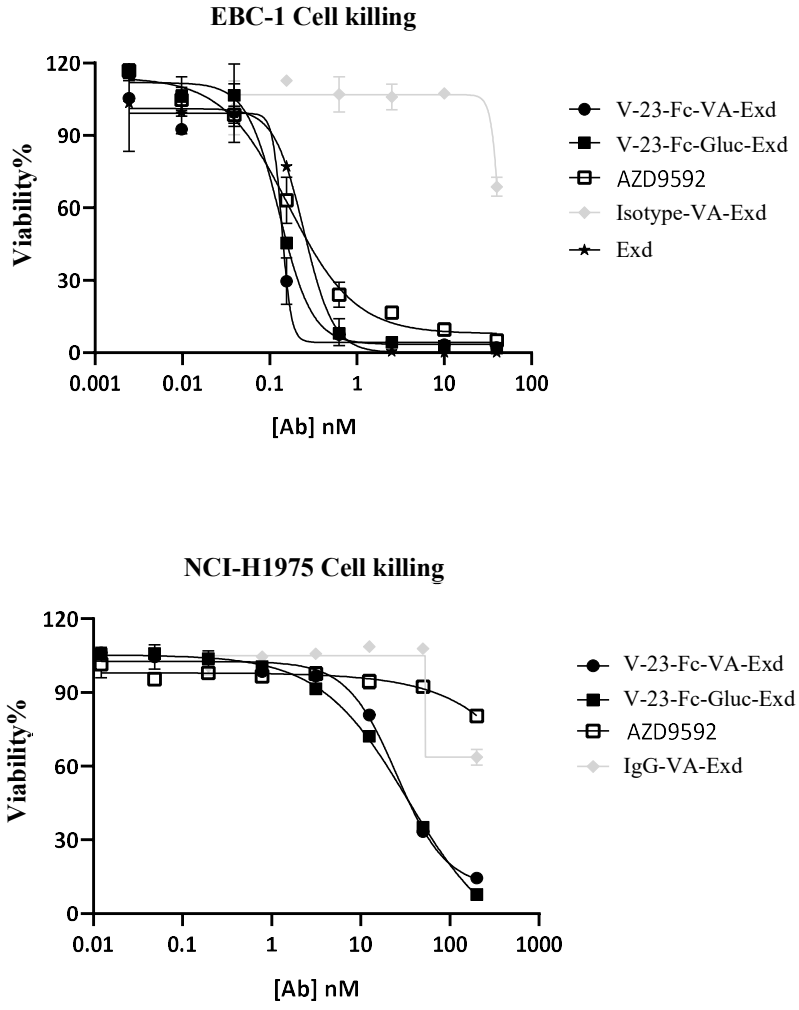
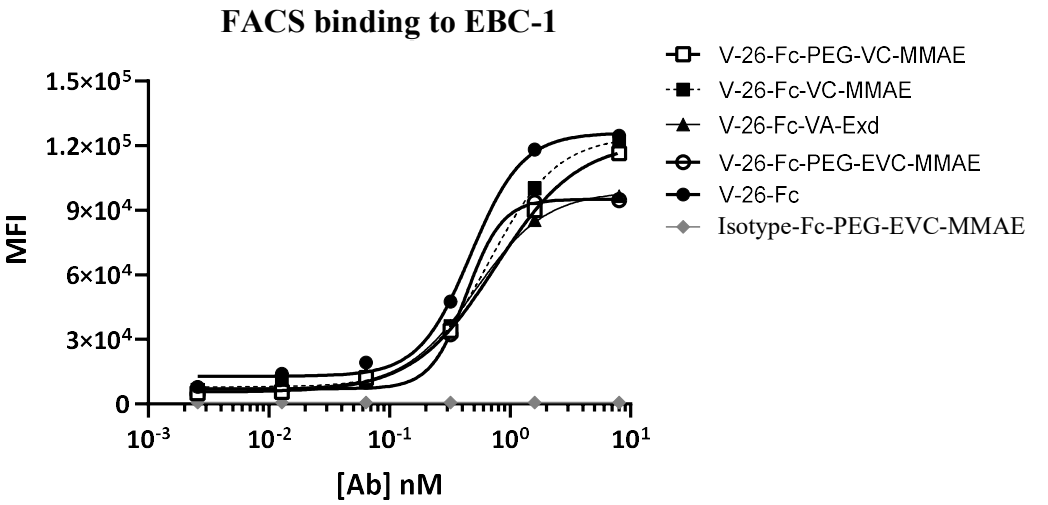

EBC-1 Cell killing
Viability%
120
90
60
30
0
0.001
0.01
0.1
1
10
100
[Ab] nM
V-23-Fc-VA-Exd
V-23-Fc-Gluc-Exd
AZD9592
Isotype-VA-Exd
Exd
NCI-H1975 Cell killing
Viability%
120
90
60
30
0
0.01
0.1
1
10
100
1000
[Ab] nM
V-23-Fc-VA-Exd
V-23-Fc-Gluc-Exd
AZD9592
IgG-VA-Exd

FIG. 18B


FACS binding to EBC-1
MFI
1.5×10^5
1.2×10^5
9×10^4
6×10^4
3×10^4
0
10^-3
10^-2
10^-1
10^0
10^1
[Ab] nM
V-26-Fc-PEG-VC-MMAE
V-26-Fc-VC-MMAE
V-26-Fc-VA-Exd
V-26-Fc-PEG-EVC-MMAE
V-26-Fc
Isotype-Fc-PEG-EVC-MMAE

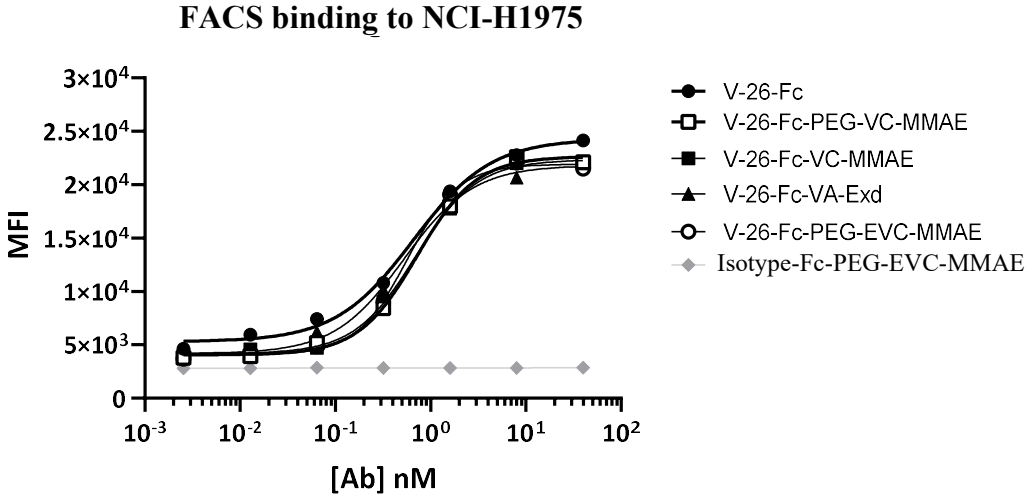

FACS binding to NCI-H1975
MFI
3×10^4
2.5×10^4
2×10^4
1.5×10^4
1×10^4
5×10^3
0
10^-3
10^-2
10^-1
10^0
10^1
10^2
[Ab] nM
V-26-Fc
V-26-Fc-PEG-VC-MMAE
V-26-Fc-VC-MMAE
V-26-Fc-VA-Exd
V-26-Fc-PEG-EVC-MMAE
Isotype-Fc-PEG-EVC-MMAE

FIG. 19

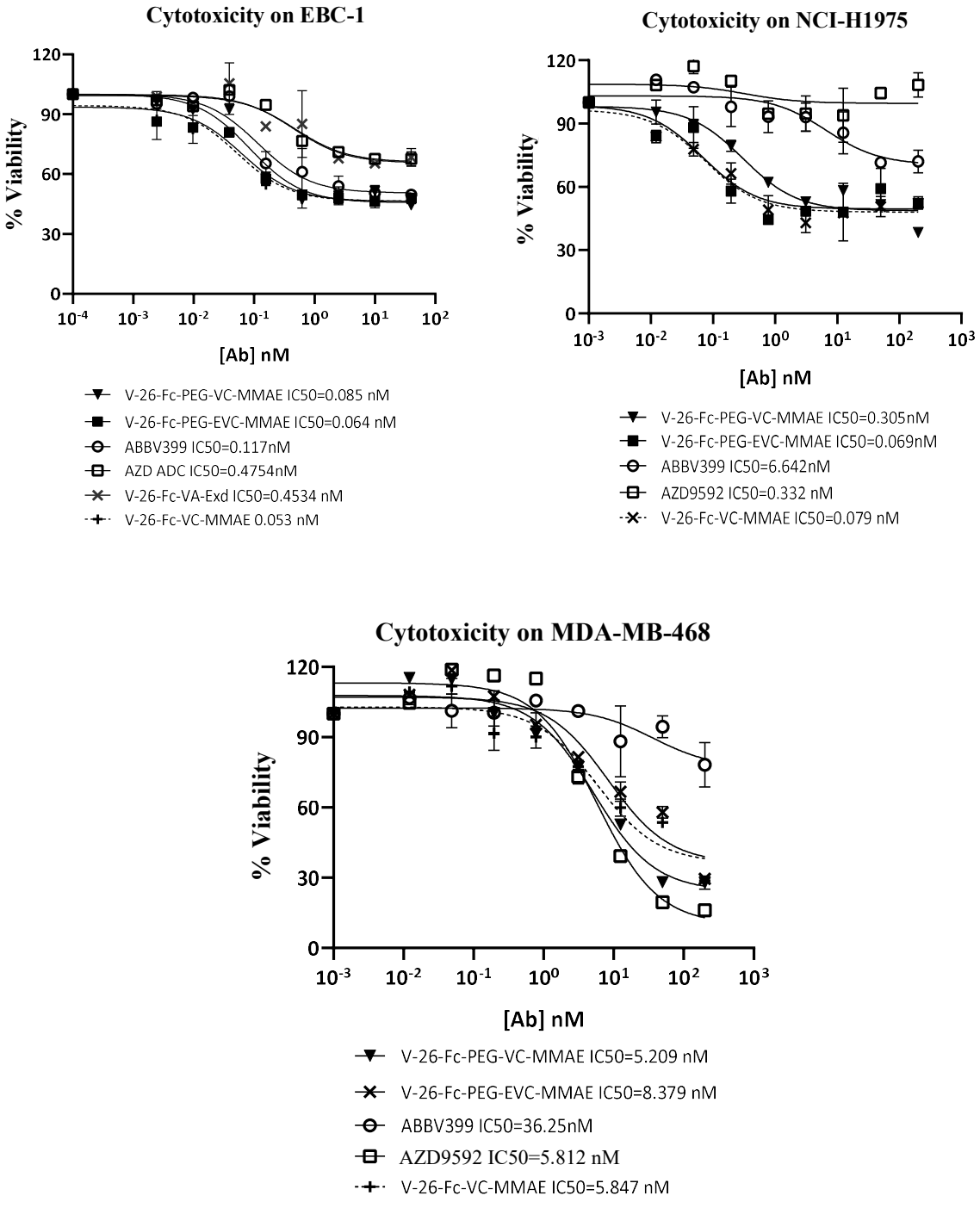

Cytotoxicity on EBC-1
% Viability
[Ab] nM
V-26-Fc-PEG-VC-MMAE IC50=0.085 nM
V-26-Fc-PEG-EVC-MMAE IC50=0.064 nM
ABBV399 IC50=0.117nM
AZD ADC IC50=0.4754nM
V-26-Fc-VA-Exd IC50=0.4534 nM
V-26-Fc-VC-MMAE 0.053 nM
Cytotoxicity on NCI-H1975
% Viability
[Ab] nM
V-26-Fc-PEG-VC-MMAE IC50=0.305nM
V-26-Fc-PEG-EVC-MMAE IC50=0.069nM
ABBV399 IC50=6.642nM
AZD9592 IC50=0.332 nM
V-26-Fc-VC-MMAE IC50=0.079 nM
Cytotoxicity on MDA-MB-468
% Viability
[Ab] nM
V-26-Fc-PEG-VC-MMAE IC50=5.209 nM
V-26-Fc-PEG-EVC-MMAE IC50=8.379 nM
ABBV399 IC50=36.25nM
AZD9592 IC50=5.812 nM
V-26-Fc-VC-MMAE IC50=5.847 nM

FIG. 20

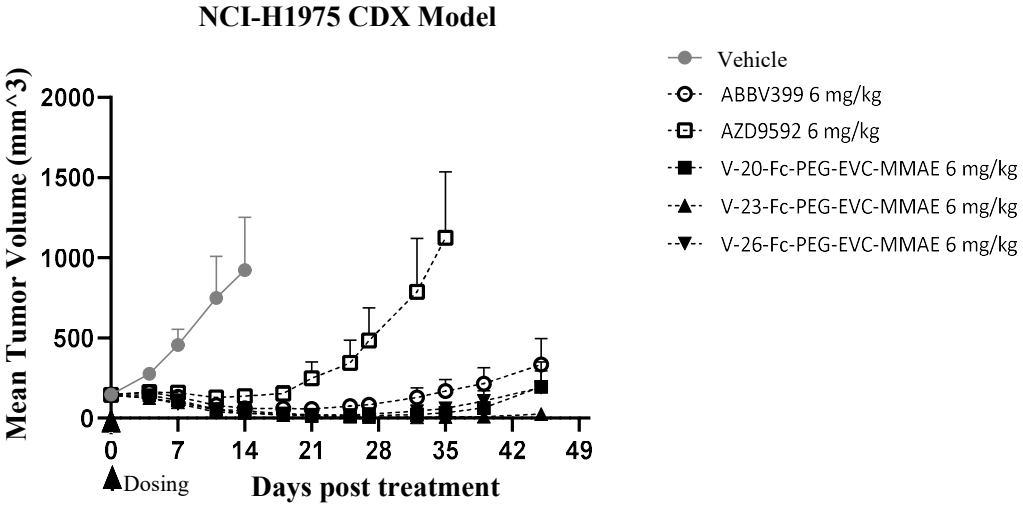

NCI-H1975 CDX Model
Mean Tumor Volume (mm^3)
2000
1500
1000
500
0
0 7 14 21 28 35 42 49
Dosing
Days post treatment
Vehicle
ABBV399 6 mg/kg
AZD9592 6 mg/kg
V-20-Fc-PEG-EVC-MMAE 6 mg/kg
V-23-Fc-PEG-EVC-MMAE 6 mg/kg
V-26-Fc-PEG-EVC-MMAE 6 mg/kg

FIG. 21

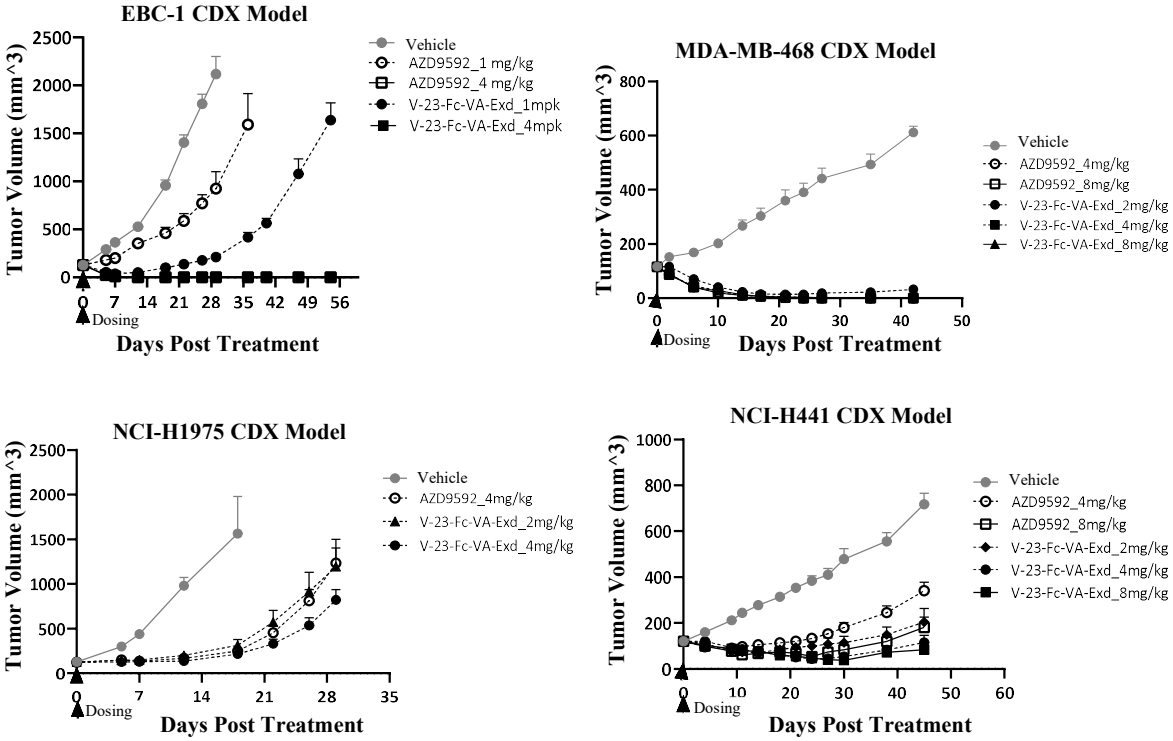

EBC-1 CDX Model
Tumor Volume (mm^3)
2500
2000
1500
1000
500
0
0 7 14 21 28 35 42 49 56
Dosing
Days Post Treatment
Vehicle
AZD9592_1 mg/kg
AZD9592_4 mg/kg
V-23-Fc-VA-Exd_1mpk
V-23-Fc-VA-Exd_4mpk
MDA-MB-468 CDX Model
Tumor Volume (mm^3)
800
600
400
200
0
0 10 20 30 40 50
Dosing
Days Post Treatment
Vehicle
AZD9592_4mg/kg
AZD9592_8mg/kg
V-23-Fc-VA-Exd_2mg/kg
V-23-Fc-VA-Exd_4mg/kg
V-23-Fc-VA-Exd_8mg/kg
NCI-H1975 CDX Model
Tumor Volume (mm^3)
2500
2000
1500
1000
500
0
0 7 14 21 28 35
Dosing
Days Post Treatment
Vehicle
AZD9592_4mg/kg
V-23-Fc-VA-Exd_2mg/kg
V-23-Fc-VA-Exd_4mg/kg
NCI-H441 CDX Model
Tumor Volume (mm^3)
1000
800
600
400
200
0
0 10 20 30 40 50 60
Dosing
Days Post Treatment
Vehicle
AZD9592_4mg/kg
AZD9592_8mg/kg
V-23-Fc-VA-Exd_2mg/kg
V-23-Fc-VA-Exd_4mg/kg
V-23-Fc-VA-Exd_8mg/kg

FIG. 22

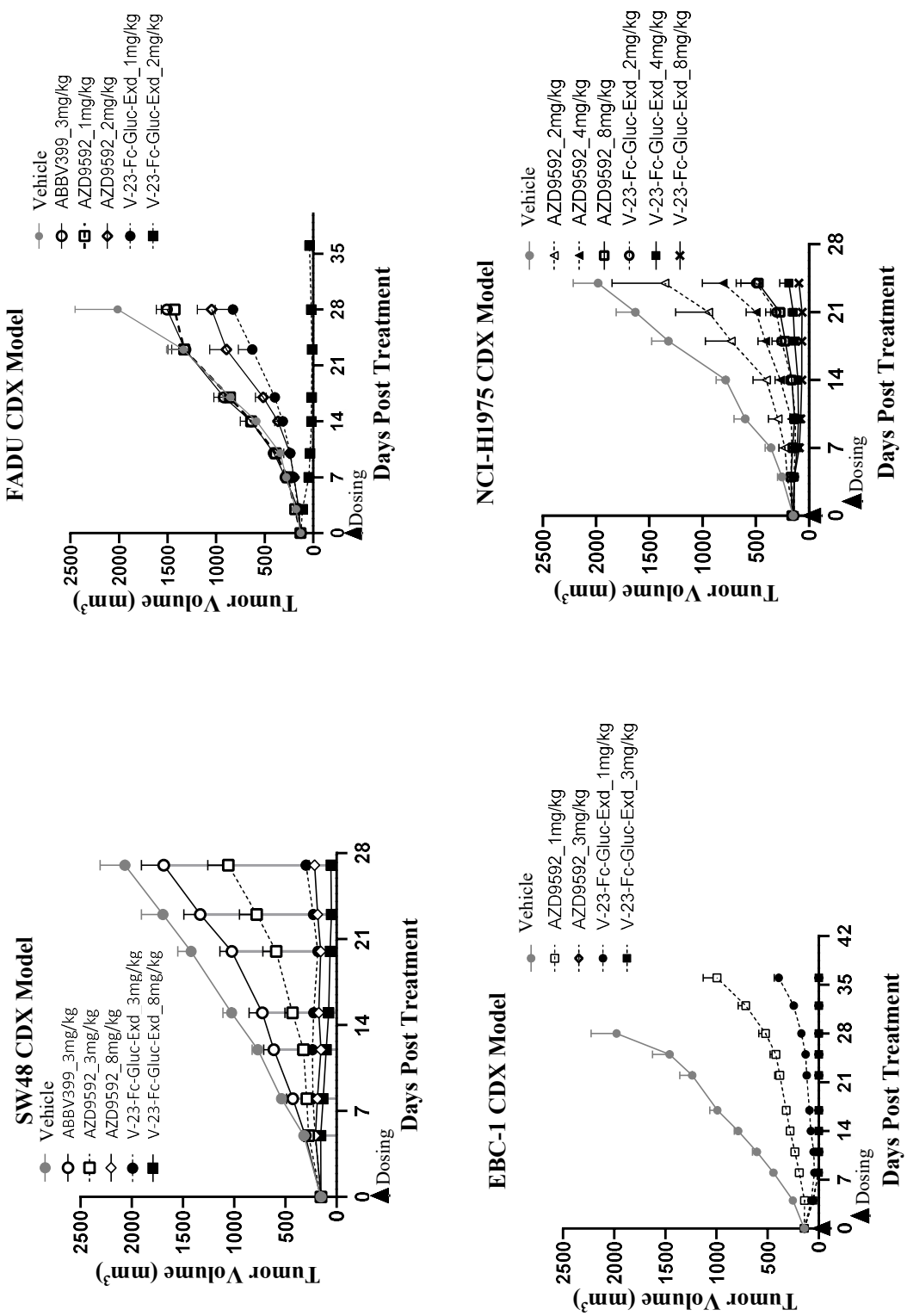

FADU CDX Model
Vehicle
ABBV399_3mg/kg
AZD9592_1mg/kg
AZD9592_2mg/kg
V-23-Fc-Gluc-Exd_1mg/kg
V-23-Fc-Gluc-Exd_2mg/kg
Tumor Volume (mm³)
Days Post Treatment
Dosing
NCI-H1975 CDX Model
Vehicle
AZD9592_2mg/kg
AZD9592_4mg/kg
AZD9592_8mg/kg
V-23-Fc-Gluc-Exd_2mg/kg
V-23-Fc-Gluc-Exd_4mg/kg
V-23-Fc-Gluc-Exd_8mg/kg
Tumor Volume (mm³)
Days Post Treatment
Dosing
SW48 CDX Model
Vehicle
ABBV399_3mg/kg
AZD9592_3mg/kg
AZD9592_8mg/kg
V-23-Fc-Gluc-Exd_3mg/kg
V-23-Fc-Gluc-Exd_8mg/kg
Tumor Volume (mm³)
Days Post Treatment
Dosing
EBC-1 CDX Model
Vehicle
AZD9592_1mg/kg
AZD9592_3mg/kg
V-23-Fc-Gluc-Exd_1mg/kg
V-23-Fc-Gluc-Exd_3mg/kg
Tumor Volume (mm³)
Days Post Treatment
Dosing

FIG. 23