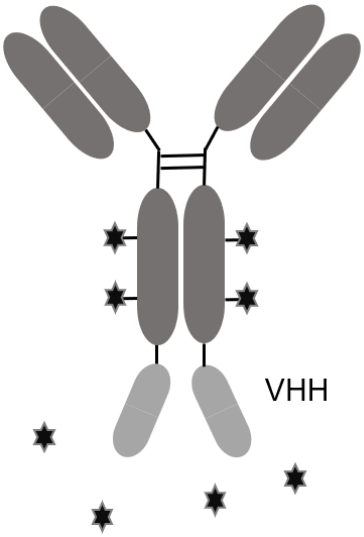



FIG.1

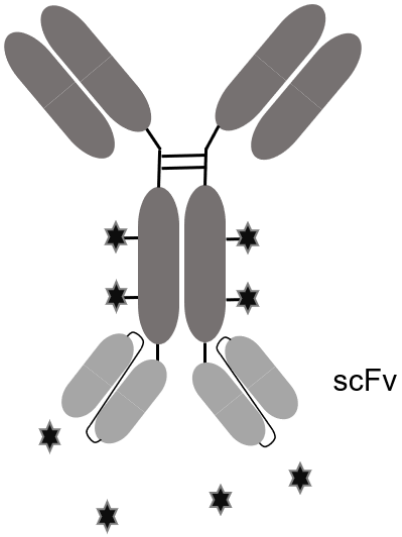



FIG. 2

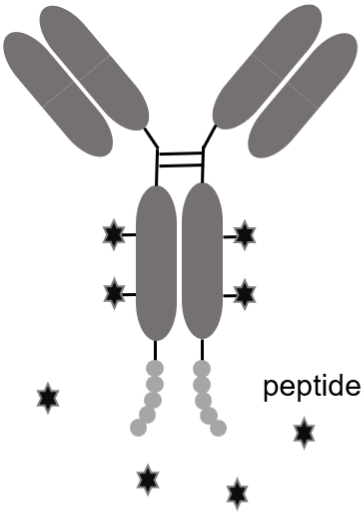



FIG. 3

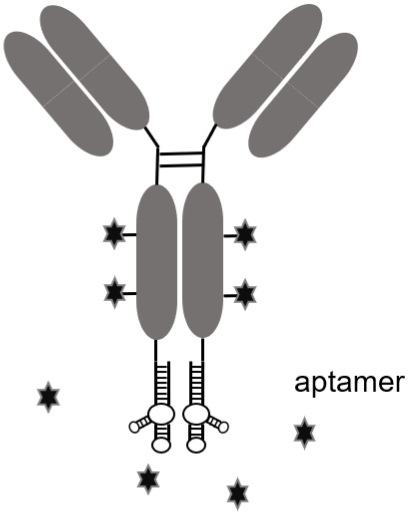



FIG. 4

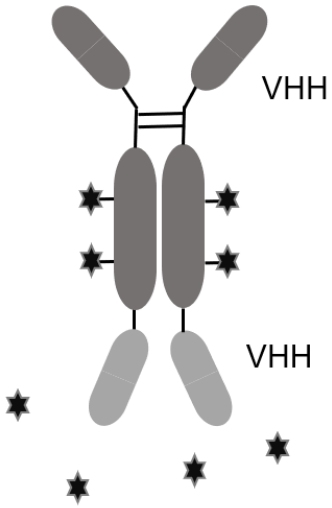



FIG. 5

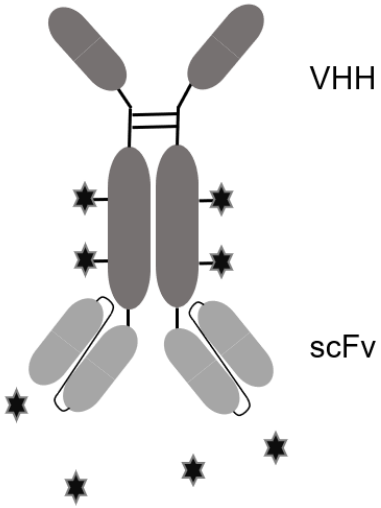



FIG. 6

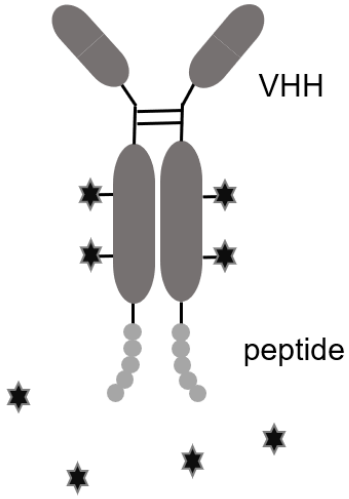



FIG. 7

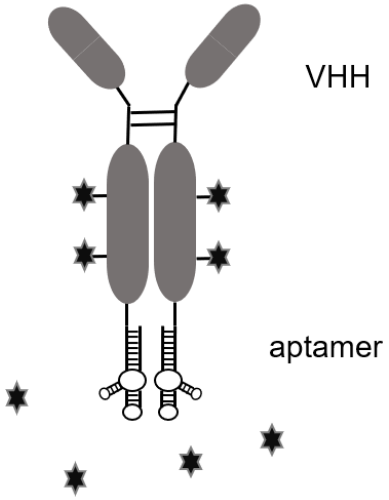



FIG. 8

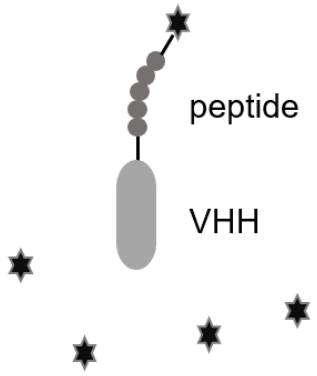



FIG. 9

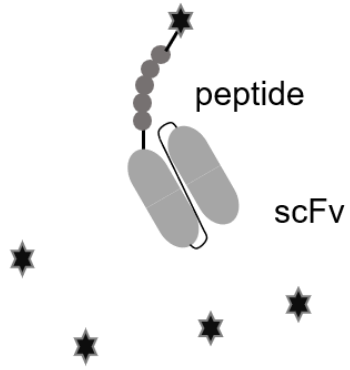



FIG. 10

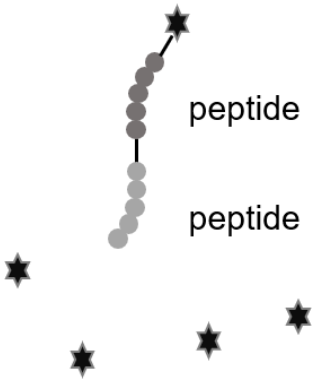



FIG. 11

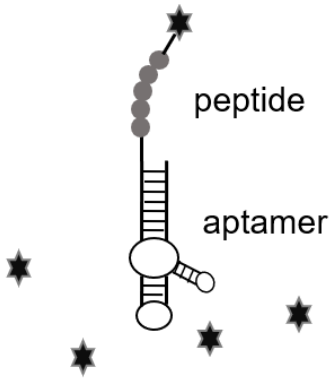



FIG. 12

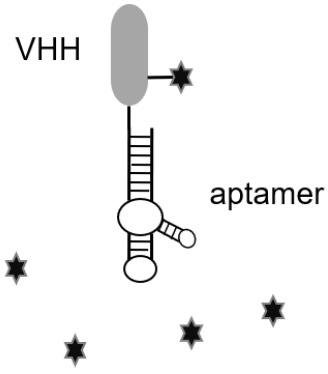



FIG. 13

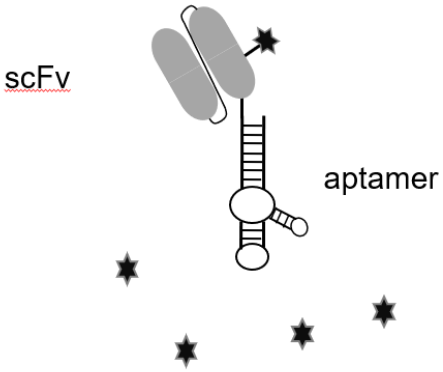



FIG. 14

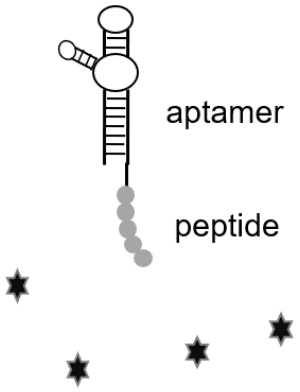



FIG. 15

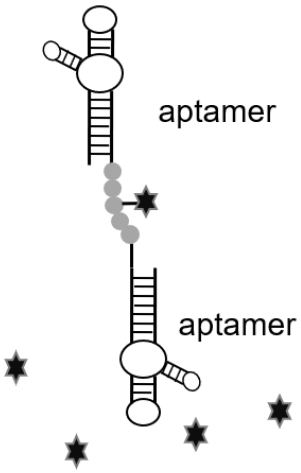



FIG. 16

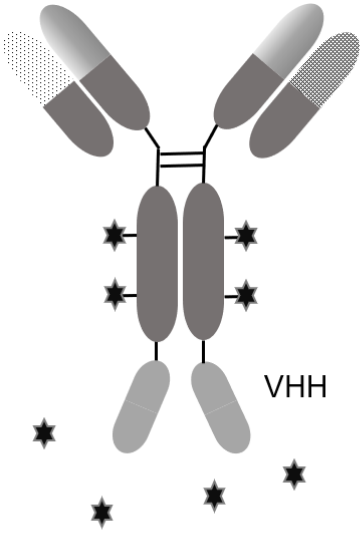



FIG. 17

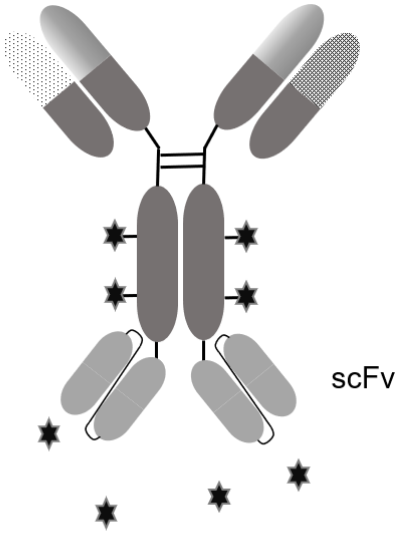



FIG. 18

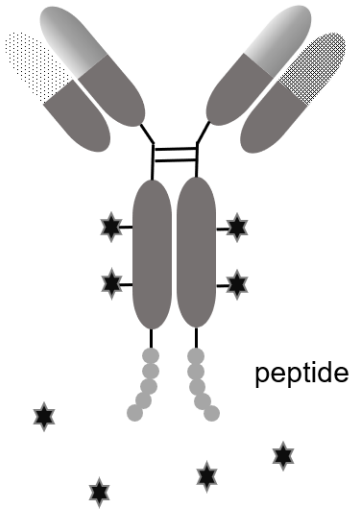



FIG. 19

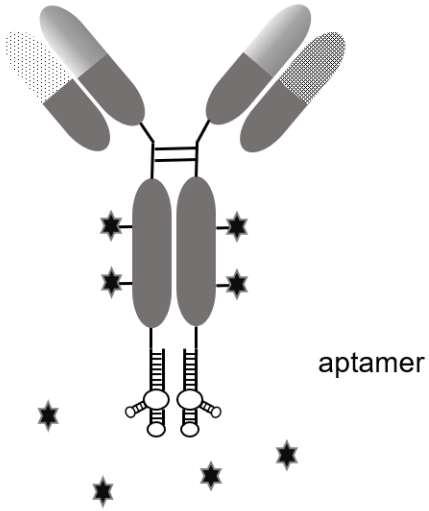



FIG. 20

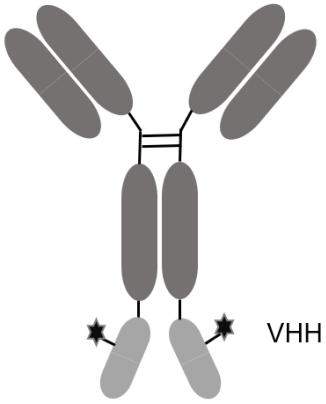



FIG. 21

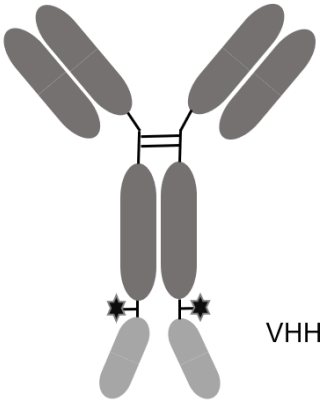



FIG. 22

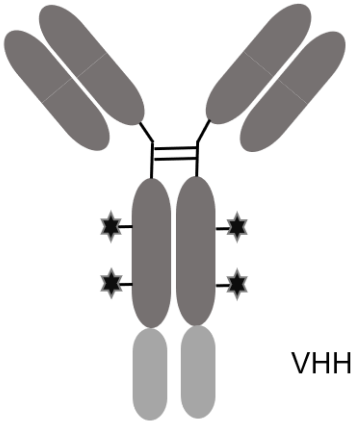



FIG. 23

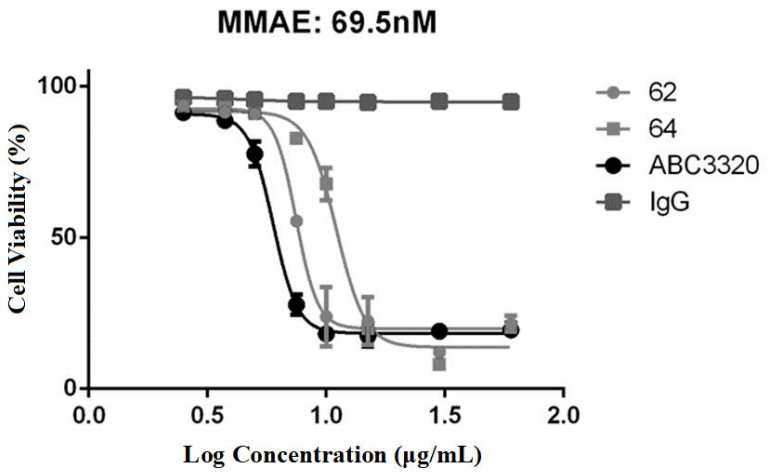

MMAE: 69.5nM
Cell Viability (%)
100
50
0
0.0
0.5
1.0
1.5
2.0
Log Concentration (μg/mL)
62
64
ABC3320
IgG

FIG. 24

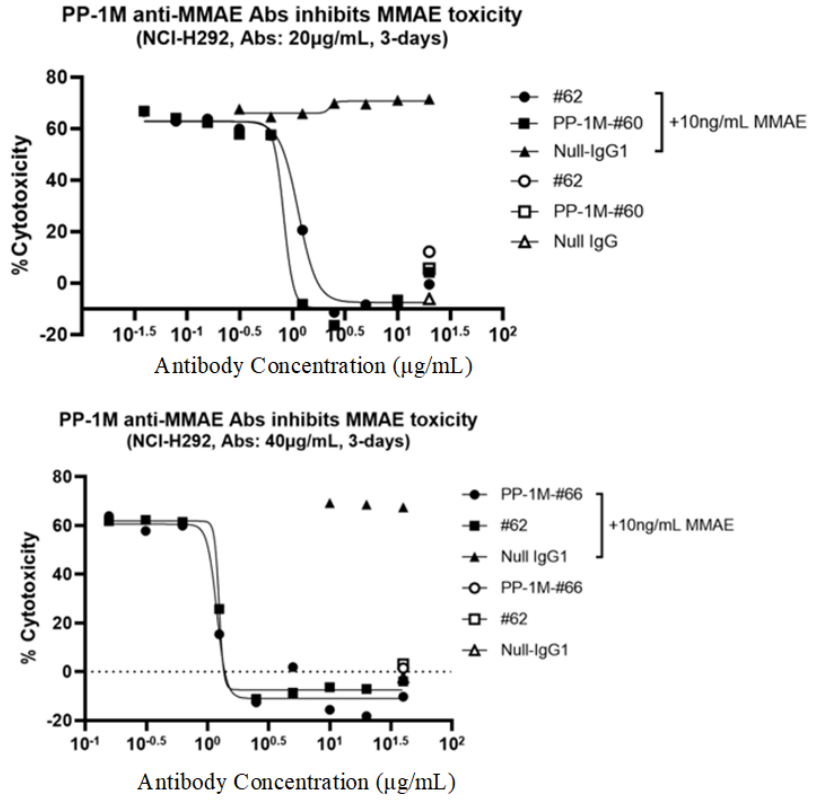

PP-1M anti-MMAE Abs inhibits MMAE toxicity
(NCI-H292, Abs: 20μg/mL, 3-days)
% Cytotoxicity
Antibody Concentration (μg/mL)
#62
PP-1M-#60
Null-IgG1
+10ng/mL MMAE
#62
PP-1M-#60
Null IgG
PP-1M anti-MMAE Abs inhibits MMAE toxicity
(NCI-H292, Abs: 40μg/mL, 3-days)
% Cytotoxicity
Antibody Concentration (μg/mL)
PP-1M-#66
#62
Null IgG1
+10ng/mL MMAE
PP-1M-#66
#62
Null-IgG1

FIG. 25

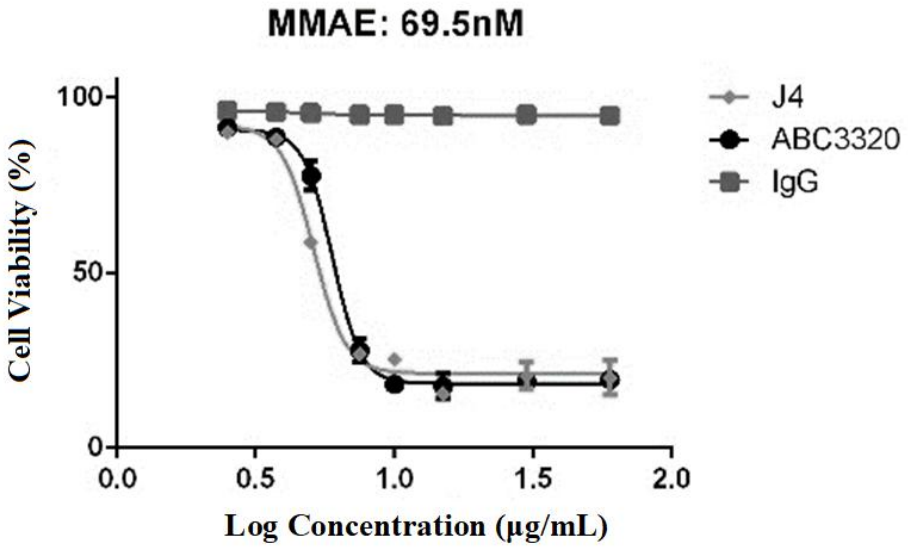

MMAE: 69.5nM
Cell Viability (%)
100
50
0
0.0
0.5
1.0
1.5
2.0
Log Concentration (μg/mL)
J4
ABC3320
IgG

FIG. 26

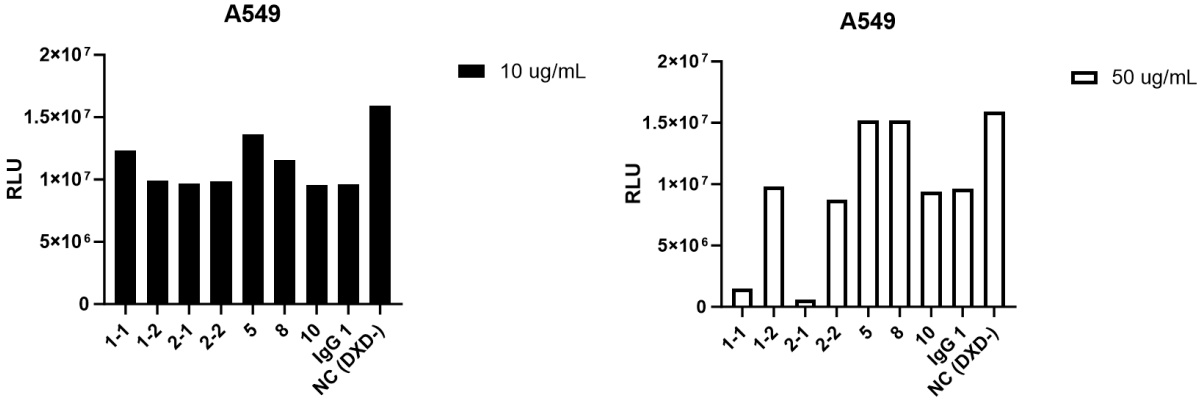

A549
RLU
2×10⁷
1.5×10⁷
1×10⁷
5×10⁶
0
1-1
1-2
2-1
2-2
5
8
10
IgG 1
NC (DXD-)
10 ug/mL
A549
RLU
2×10⁷
1.5×10⁷
1×10⁷
5×10⁶
0
1-1
1-2
2-1
2-2
5
8
10
IgG 1
NC (DXD-)
50 ug/mL

FIG. 27

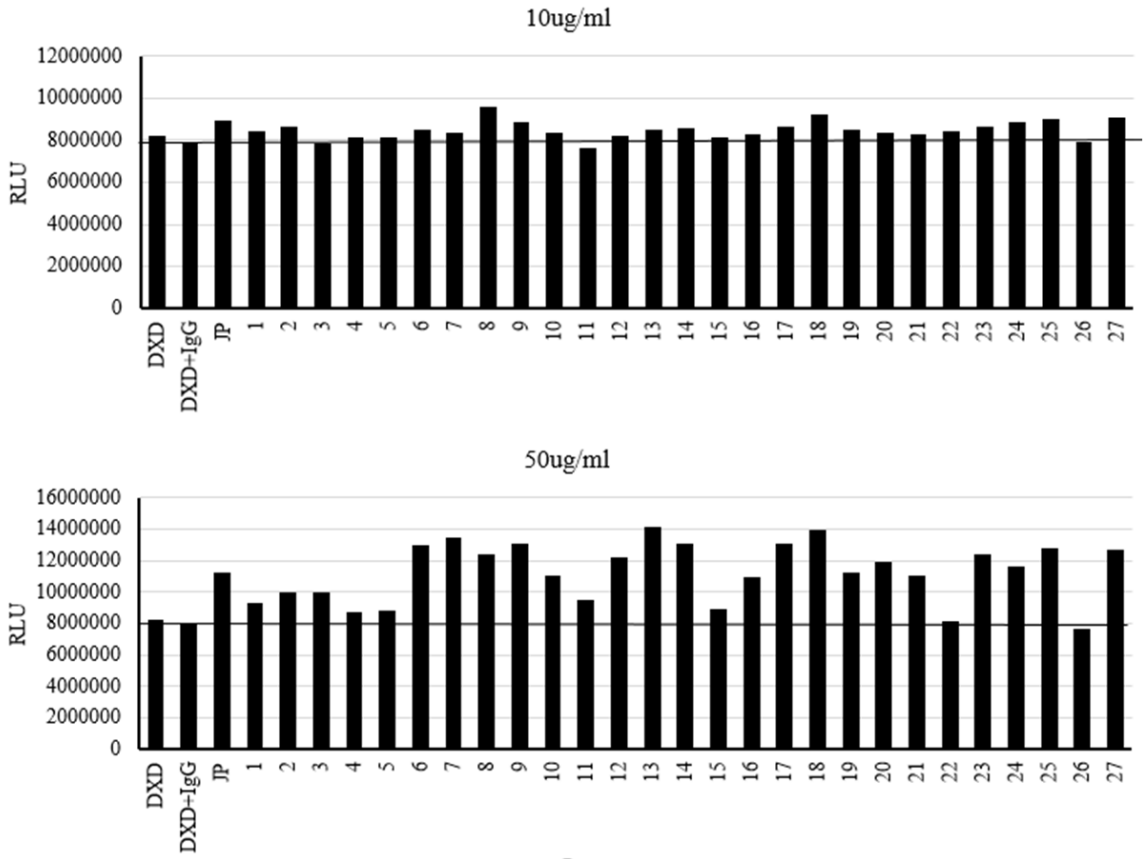

10ug/ml
RLU
12000000
10000000
8000000
6000000
4000000
2000000
0
DXD
DXD+IgG
JP
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
50ug/ml
RLU
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
DXD
DXD+IgG
JP
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27

FIG. 28

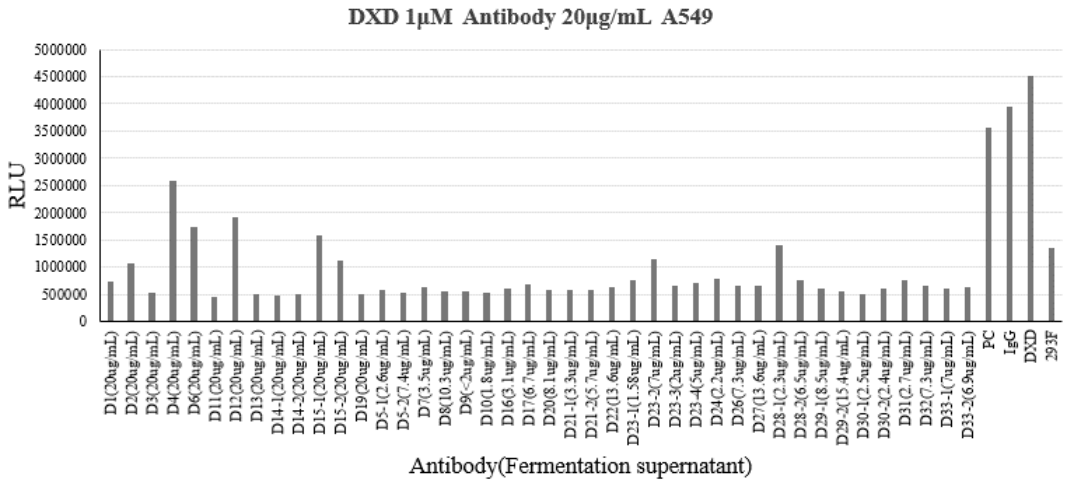

DXD 1μM Antibody 20μg/mL A549
RLU
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
D1(20ug/mL)
D2(20ug/mL)
D3(20ug/mL)
D4(20ug/mL)
D6(20ug/mL)
D11(20ug/mL)
D12(20ug/mL)
D13(20ug/mL)
D14-1(20ug/mL)
D14-2(20ug/mL)
D15-1(20ug/mL)
D15-2(20ug/mL)
D19(20ug/mL)
D5-1(2.6ug/mL)
D5-2(7.4ug/mL)
D7(3.5ug/mL)
D8(10.3ug/mL)
D9(~2ug/mL)
D10(1.8ug/mL)
D16(3.1ug/mL)
D17(6.7ug/mL)
D20(8.1ug/mL)
D21-1(3.3ug/mL)
D21-2(5.7ug/mL)
D22(13.6ug/mL)
D23-1(1.58ug/mL)
D23-2(7ug/mL)
D23-3(2ug/mL)
D23-4(5ug/mL)
D24(2.2ug/mL)
D26(7.3ug/mL)
D27(13.6ug/mL)
D28-1(2.3ug/mL)
D28-2(6.5ug/mL)
D29-1(8.5ug/mL)
D29-2(15.4ug/mL)
D30-1(2.5ug/mL)
D30-2(2.4ug/mL)
D31(2.7ug/mL)
D32(7.3ug/mL)
D33-1(7ug/mL)
D33-2(6.9ug/mL)
PC
IgG
DXD
293F
Antibody(Fermentation supernatant)

FIG. 29

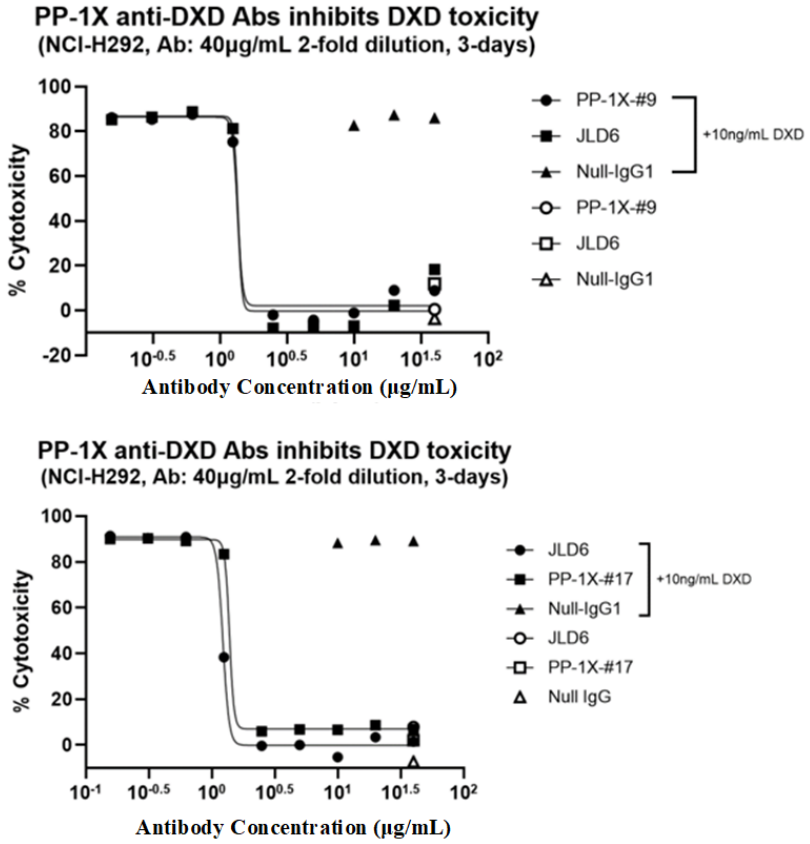

PP-1X anti-DXD Abs inhibits DXD toxicity
(NCI-H292, Ab: 40μg/mL 2-fold dilution, 3-days)
% Cytotoxicity
Antibody Concentration (μg/mL)
PP-1X-#9
JLD6
Null-IgG1
+10ng/mL DXD
PP-1X-#9
JLD6
Null-IgG1
PP-1X anti-DXD Abs inhibits DXD toxicity
(NCI-H292, Ab: 40μg/mL 2-fold dilution, 3-days)
% Cytotoxicity
Antibody Concentration (μg/mL)
JLD6
PP-1X-#17
Null-IgG1
+10ng/mL DXD
JLD6
PP-1X-#17
Null IgG

FIG. 30

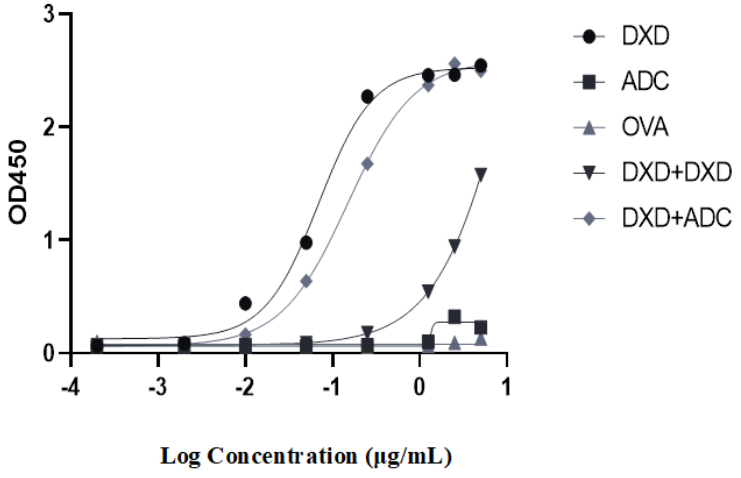

OD450
Log Concentration (μg/mL)
DXD
ADC
OVA
DXD+DXD
DXD+ADC

FIG. 31

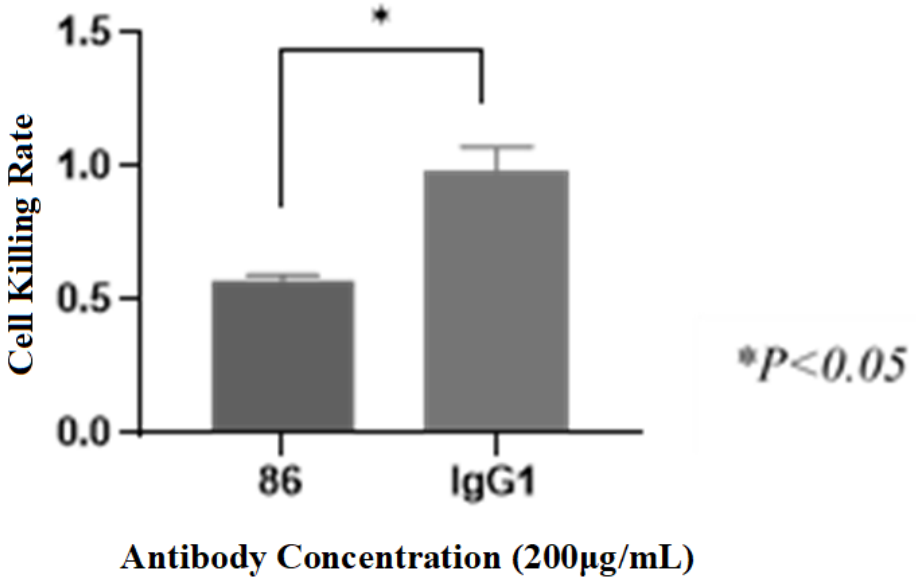

1.5
1.0
0.5
0.0
Cell Killing Rate
*
86
IgG1
Antibody Concentration (200μg/mL)
*P<0.05

FIG. 32

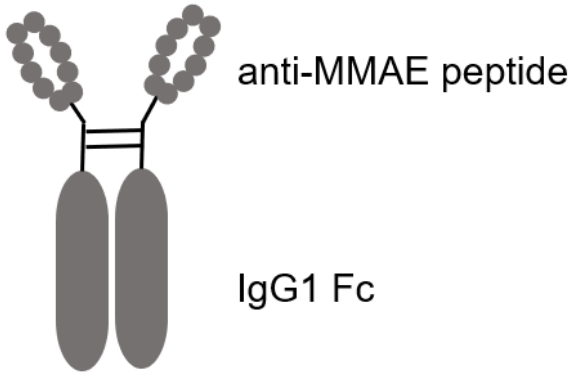

anti-MMAE peptide
IgG1 Fc

FIG. 33

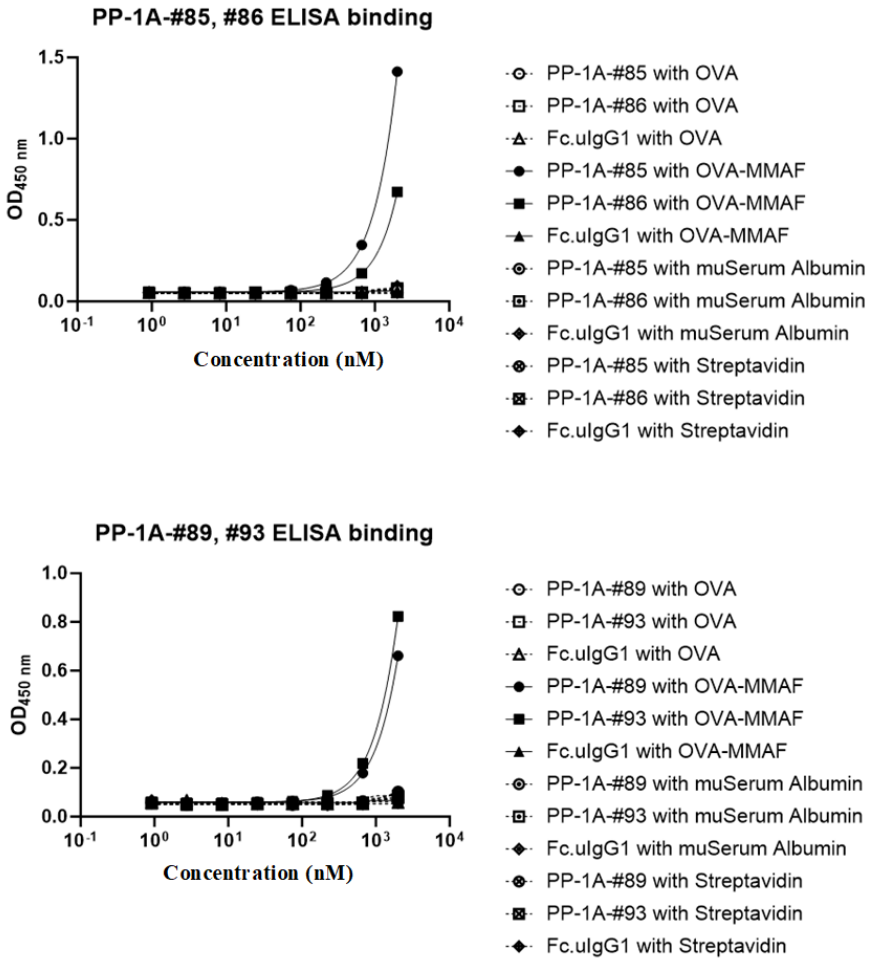

PP-1A-#85, #86 ELISA binding
OD450 nm
Concentration (nM)
PP-1A-#85 with OVA
PP-1A-#86 with OVA
Fc.uIgG1 with OVA
PP-1A-#85 with OVA-MMAF
PP-1A-#86 with OVA-MMAF
Fc.uIgG1 with OVA-MMAF
PP-1A-#85 with muSerum Albumin
PP-1A-#86 with muSerum Albumin
Fc.uIgG1 with muSerum Albumin
PP-1A-#85 with Streptavidin
PP-1A-#86 with Streptavidin
Fc.uIgG1 with Streptavidin
PP-1A-#89, #93 ELISA binding
OD450 nm
Concentration (nM)
PP-1A-#89 with OVA
PP-1A-#93 with OVA
Fc.uIgG1 with OVA
PP-1A-#89 with OVA-MMAF
PP-1A-#93 with OVA-MMAF
Fc.uIgG1 with OVA-MMAF
PP-1A-#89 with muSerum Albumin
PP-1A-#93 with muSerum Albumin
Fc.uIgG1 with muSerum Albumin
PP-1A-#89 with Streptavidin
PP-1A-#93 with Streptavidin
Fc.uIgG1 with Streptavidin
PP-1A ELISA binding on OVA-MMAF and OVA
OD450 nm
PP-1A-#84 with OVA
PP-1A-#84 with OVA-MMAF
PP-1A-#87 with OVA
PP-1A-#87 with OVA-MMAF
PP-1A-#88 with OVA
PP-1A-#88 with OVA-MMAF
PP-1A-#90 with OVA
PP-1A-#90 with OVA-MMAF
PP-1A-#91 with OVA
PP-1A-#91 with OVA-MMAF
PP-1A-#92 with OVA
PP-1A-#92 with OVA-MMAF
PP-1A-#94 with OVA
PP-1A-#94 with OVA-MMAF

FIG. 34

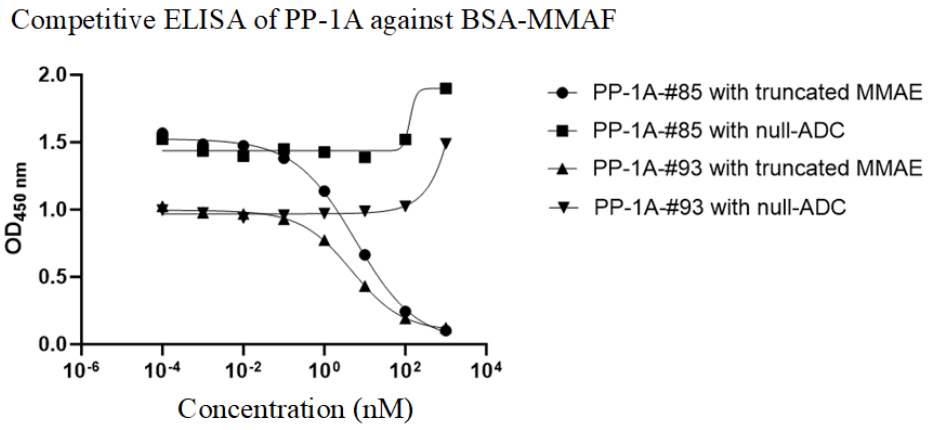

Competitive ELISA of PP-1A against BSA-MMAF
2.0
1.5
1.0
0.5
0.0
OD450 nm
10-6
10-4
10-2
100
102
104
Concentration (nM)
PP-1A-#85 with truncated MMAE
PP-1A-#85 with null-ADC
PP-1A-#93 with truncated MMAE
PP-1A-#93 with null-ADC

FIG. 35

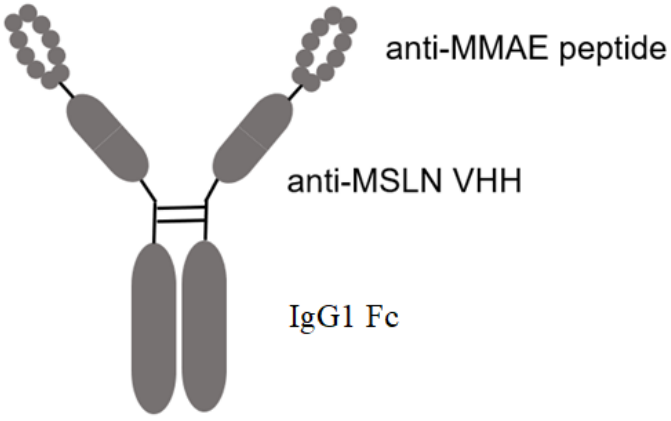

anti-MMAE peptide
anti-MSLN VHH
IgG1 Fc

FIG. 36

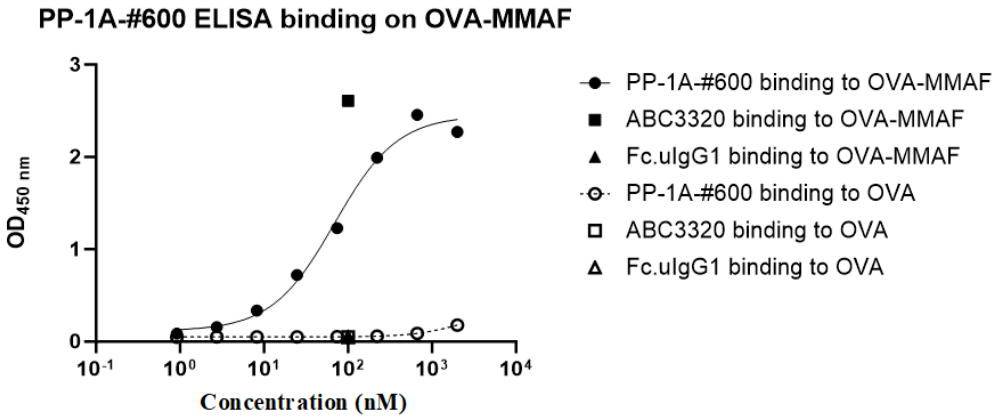

PP-1A-#600 ELISA binding on OVA-MMAF
OD450 nm
Concentration (nM)
PP-1A-#600 binding to OVA-MMAF
ABC3320 binding to OVA-MMAF
Fc.uIgG1 binding to OVA-MMAF
PP-1A-#600 binding to OVA
ABC3320 binding to OVA
Fc.uIgG1 binding to OVA

FIG. 37

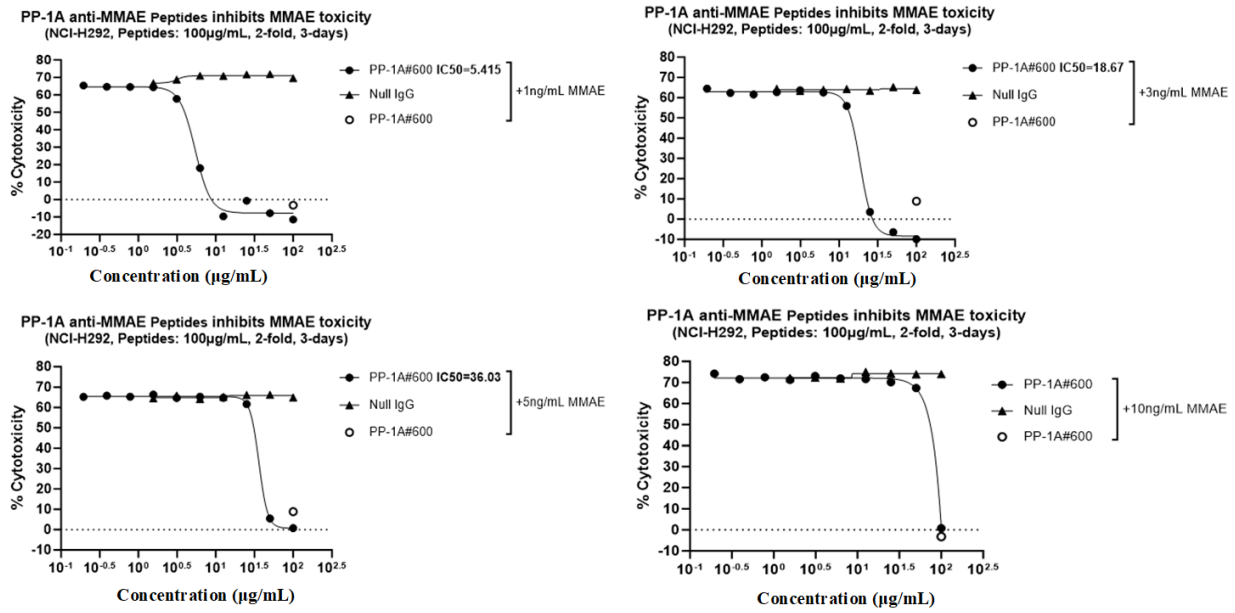

PP-1A anti-MMAE Peptides inhibits MMAE toxicity
(NCI-H292, Peptides: 100μg/mL, 2-fold, 3-days)
% Cytotoxicity
Concentration (μg/mL)
PP-1A#600 IC50=5.415
Null IgG
PP-1A#600
+1ng/mL MMAE
PP-1A anti-MMAE Peptides inhibits MMAE toxicity
(NCI-H292, Peptides: 100μg/mL, 2-fold, 3-days)
% Cytotoxicity
Concentration (μg/mL)
PP-1A#600 IC50=18.67
Null IgG
PP-1A#600
+3ng/mL MMAE
PP-1A anti-MMAE Peptides inhibits MMAE toxicity
(NCI-H292, Peptides: 100μg/mL, 2-fold, 3-days)
% Cytotoxicity
Concentration (μg/mL)
PP-1A#600 IC50=36.03
Null IgG
PP-1A#600
+5ng/mL MMAE
PP-1A anti-MMAE Peptides inhibits MMAE toxicity
(NCI-H292, Peptides: 100μg/mL, 2-fold, 3-days)
% Cytotoxicity
Concentration (μg/mL)
PP-1A#600
Null IgG
PP-1A#600
+10ng/mL MMAE

FIG. 38

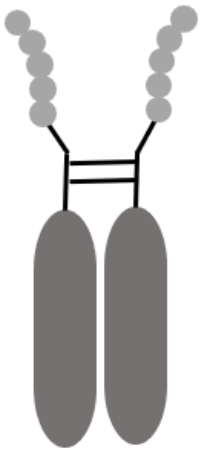
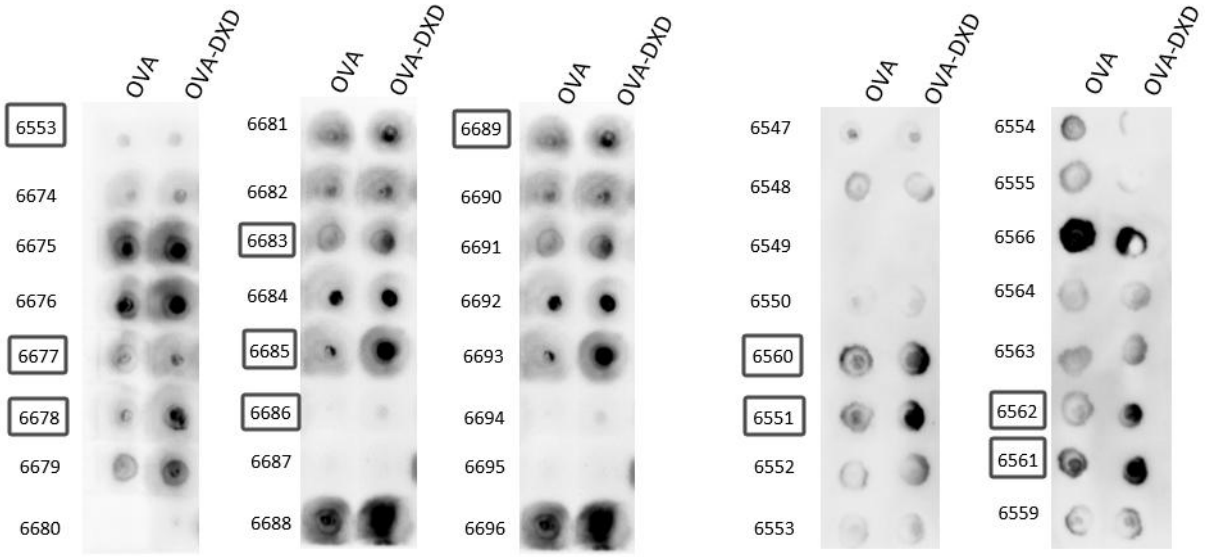


FIG. 39


OVA
OVA-DXD
6553
6674
6675
6676
6677
6678
6679
6680
6681
6682
6683
6684
6685
6686
6687
6688
6689
6690
6691
6692
6693
6694
6695
6696
6547
6548
6549
6550
6560
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6552
6553
6554
6555
6566
6564
6563
6562
6561
6559

FIG. 40

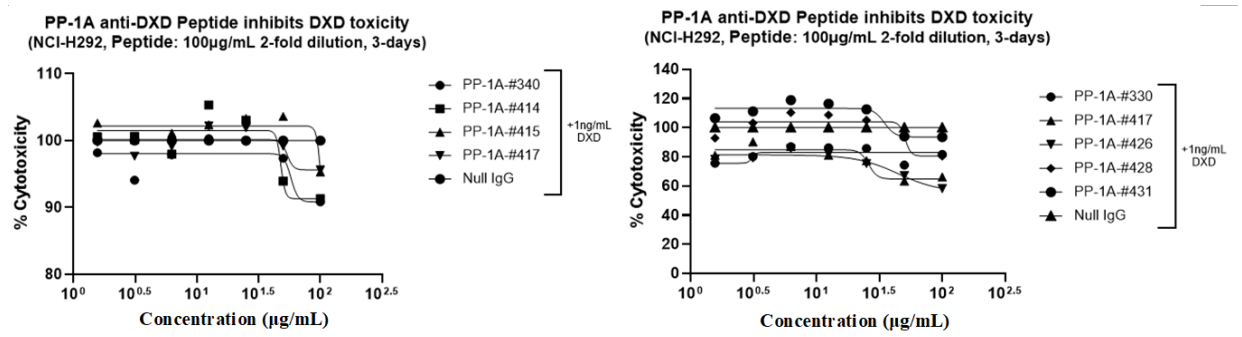


FIG. 41

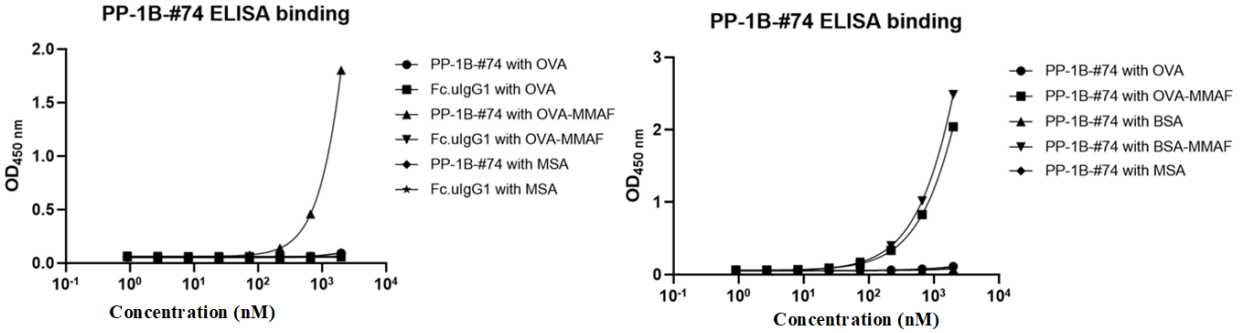


FIG. 42

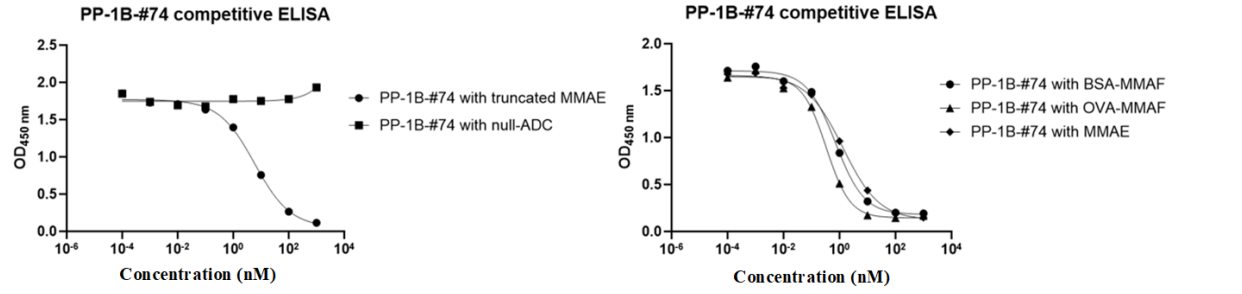


FIG. 43

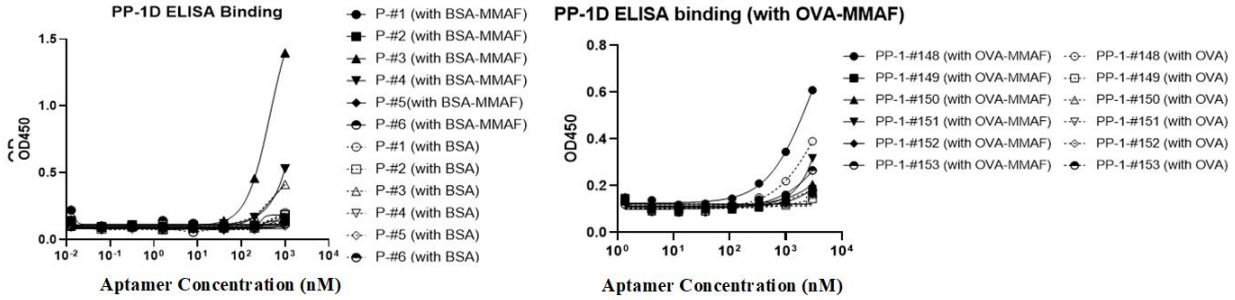
PP-1D ELISA Binding
OD450
Aptamer Concentration (nM)
P-#1 (with BSA-MMAF)
P-#2 (with BSA-MMAF)
P-#3 (with BSA-MMAF)
P-#4 (with BSA-MMAF)
P-#5(with BSA-MMAF)
P-#6 (with BSA-MMAF)
P-#1 (with BSA)
P-#2 (with BSA)
P-#3 (with BSA)
P-#4 (with BSA)
P-#5 (with BSA)
P-#6 (with BSA)
PP-1D ELISA binding (with OVA-MMAF)
OD450
Aptamer Concentration (nM)
PP-1-#148 (with OVA-MMAF)
PP-1-#149 (with OVA-MMAF)
PP-1-#150 (with OVA-MMAF)
PP-1-#151 (with OVA-MMAF)
PP-1-#152 (with OVA-MMAF)
PP-1-#153 (with OVA-MMAF)
PP-1-#148 (with OVA)
PP-1-#149 (with OVA)
PP-1-#150 (with OVA)
PP-1-#151 (with OVA)
PP-1-#152 (with OVA)
PP-1-#153 (with OVA)

FIG. 44

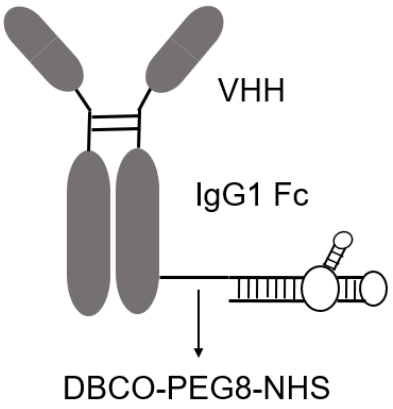
VHH
IgG1 Fc
DBCO-PEG8-NHS

FIG. 45

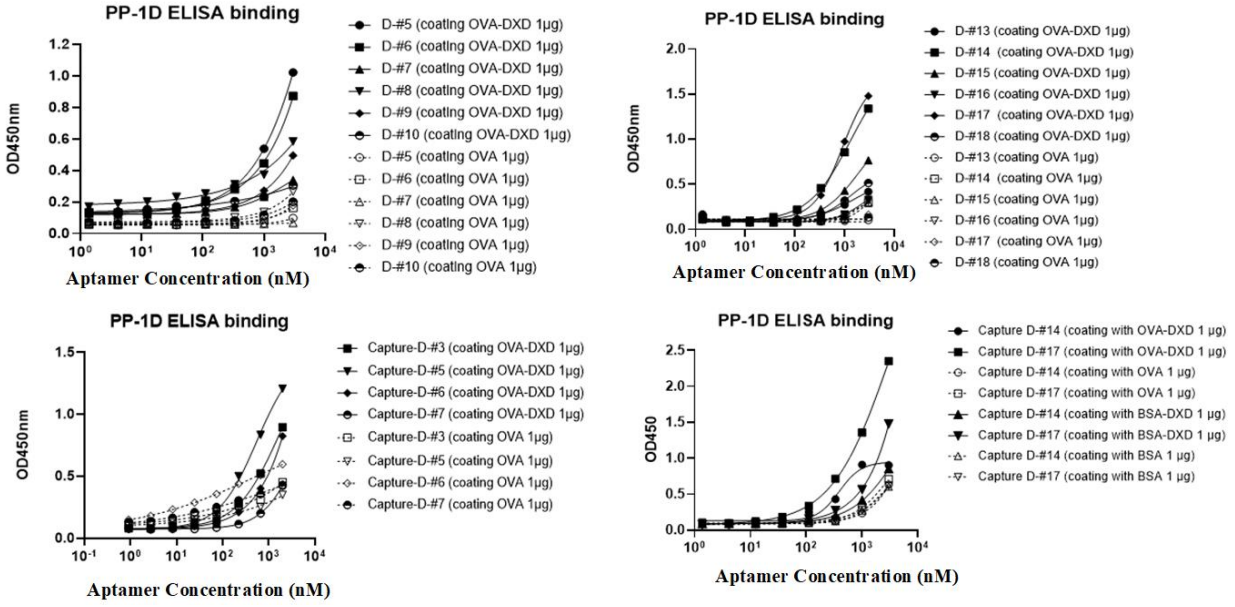
PP-1D ELISA binding
OD450nm
Aptamer Concentration (nM)
D-#5 (coating OVA-DXD 1μg)
D-#6 (coating OVA-DXD 1μg)
D-#7 (coating OVA-DXD 1μg)
D-#8 (coating OVA-DXD 1μg)
D-#9 (coating OVA-DXD 1μg)
D-#10 (coating OVA-DXD 1μg)
D-#5 (coating OVA 1μg)
D-#6 (coating OVA 1μg)
D-#7 (coating OVA 1μg)
D-#8 (coating OVA 1μg)
D-#9 (coating OVA 1μg)
D-#10 (coating OVA 1μg)
PP-1D ELISA binding
OD450nm
Aptamer Concentration (nM)
D-#13 (coating OVA-DXD 1μg)
D-#14 (coating OVA-DXD 1μg)
D-#15 (coating OVA-DXD 1μg)
D-#16 (coating OVA-DXD 1μg)
D-#17 (coating OVA-DXD 1μg)
D-#18 (coating OVA-DXD 1μg)
D-#13 (coating OVA 1μg)
D-#14 (coating OVA 1μg)
D-#15 (coating OVA 1μg)
D-#16 (coating OVA 1μg)
D-#17 (coating OVA 1μg)
D-#18 (coating OVA 1μg)
PP-1D ELISA binding
OD450nm
Aptamer Concentration (nM)
Capture-D-#3 (coating OVA-DXD 1μg)
Capture-D-#5 (coating OVA-DXD 1μg)
Capture-D-#6 (coating OVA-DXD 1μg)
Capture-D-#7 (coating OVA-DXD 1μg)
Capture-D-#3 (coating OVA 1μg)
Capture-D-#5 (coating OVA 1μg)
Capture-D-#6 (coating OVA 1μg)
Capture-D-#7 (coating OVA 1μg)
PP-1D ELISA binding
OD450
Aptamer Concentration (nM)
Capture D-#14 (coating with OVA-DXD 1 μg)
Capture D-#17 (coating with OVA-DXD 1 μg)
Capture D-#14 (coating with OVA 1 μg)
Capture D-#17 (coating with OVA 1 μg)
Capture D-#14 (coating with BSA-DXD 1 μg)
Capture D-#17 (coating with BSA-DXD 1 μg)
Capture D-#14 (coating with BSA 1 μg)
Capture D-#17 (coating with BSA 1 μg)

FIG. 46

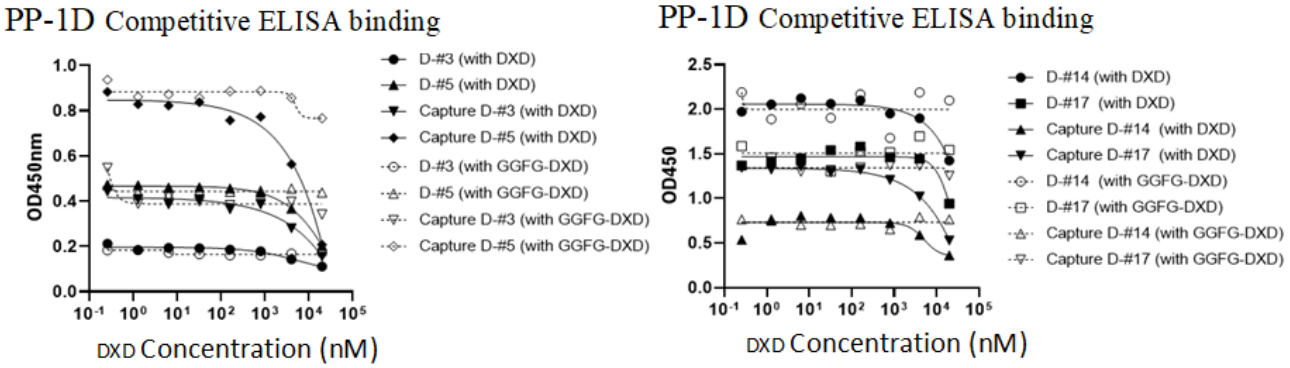
PP-1D Competitive ELISA binding
OD450nm
DXD Concentration (nM)
D-#3 (with DXD)
D-#5 (with DXD)
Capture D-#3 (with DXD)
Capture D-#5 (with DXD)
D-#3 (with GGFG-DXD)
D-#5 (with GGFG-DXD)
Capture D-#3 (with GGFG-DXD)
Capture D-#5 (with GGFG-DXD)
PP-1D Competitive ELISA binding
OD450
DXD Concentration (nM)
D-#14 (with DXD)
D-#17 (with DXD)
Capture D-#14 (with DXD)
Capture D-#17 (with DXD)
D-#14 (with GGFG-DXD)
D-#17 (with GGFG-DXD)
Capture D-#14 (with GGFG-DXD)
Capture D-#17 (with GGFG-DXD)

FIG. 47

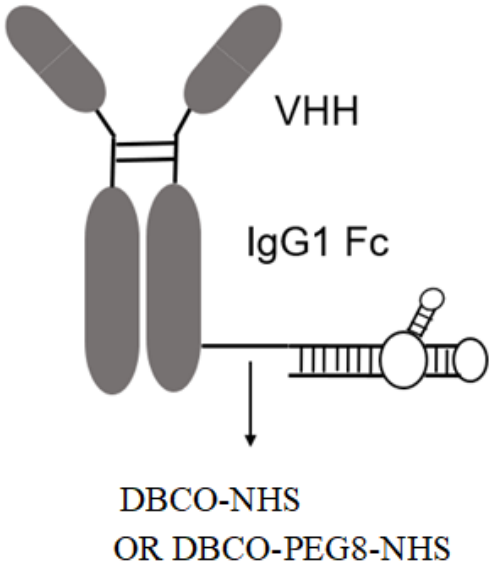
VHH
IgG1 Fc
DBCO-NHS
OR DBCO-PEG8-NHS

FIG. 48

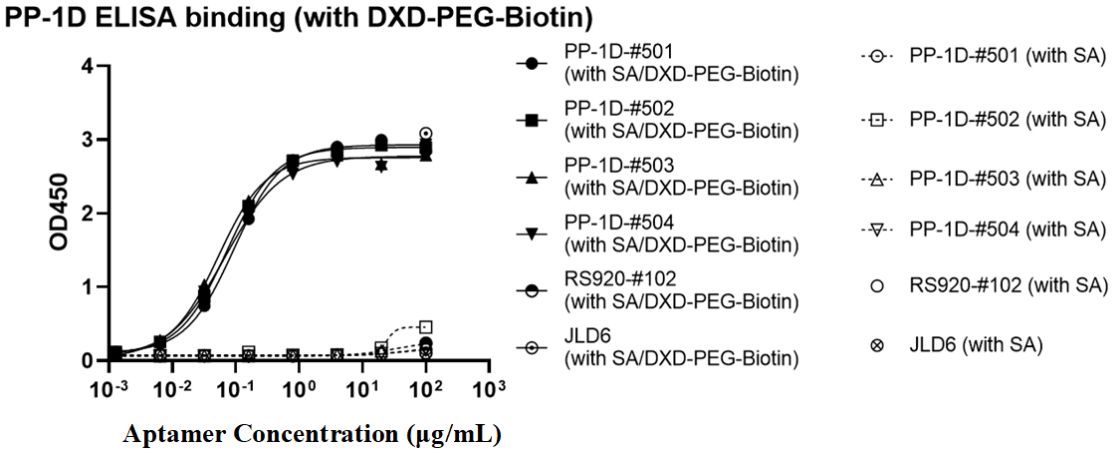
PP-1D ELISA binding (with DXD-PEG-Biotin)
OD450
Aptamer Concentration (μg/mL)
PP-1D-#501 (with SA/DXD-PEG-Biotin)
PP-1D-#502 (with SA/DXD-PEG-Biotin)
PP-1D-#503 (with SA/DXD-PEG-Biotin)
PP-1D-#504 (with SA/DXD-PEG-Biotin)
RS920-#102 (with SA/DXD-PEG-Biotin)
JLD6 (with SA/DXD-PEG-Biotin)
PP-1D-#501 (with SA)
PP-1D-#502 (with SA)
PP-1D-#503 (with SA)
PP-1D-#504 (with SA)
RS920-#102 (with SA)
JLD6 (with SA)

FIG. 49

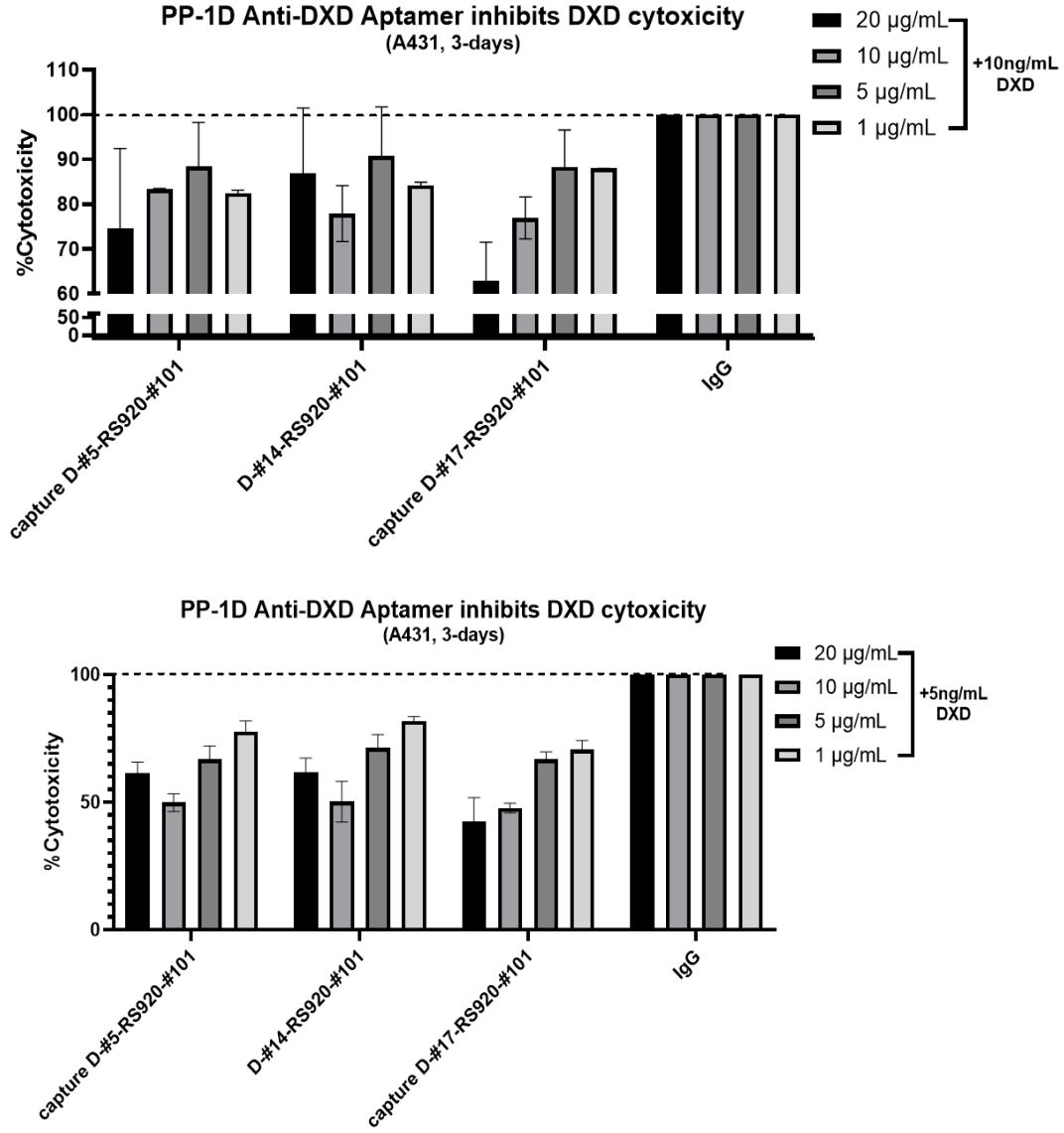
PP-1D Anti-DXD Aptamer inhibits DXD cytoxicity
(A431, 3-days)
%Cytotoxicity
110
100
90
80
70
60
50
0
20 μg/mL
10 μg/mL
5 μg/mL
1 μg/mL
+10ng/mL DXD
capture D-#5-RS920-#101
D-#14-RS920-#101
capture D-#17-RS920-#101
IgG
PP-1D Anti-DXD Aptamer inhibits DXD cytoxicity
(A431, 3-days)
% Cytotoxicity
100
50
0
20 μg/mL
10 μg/mL
5 μg/mL
1 μg/mL
+5ng/mL DXD
capture D-#5-RS920-#101
D-#14-RS920-#101
capture D-#17-RS920-#101
IgG

FIG. 50

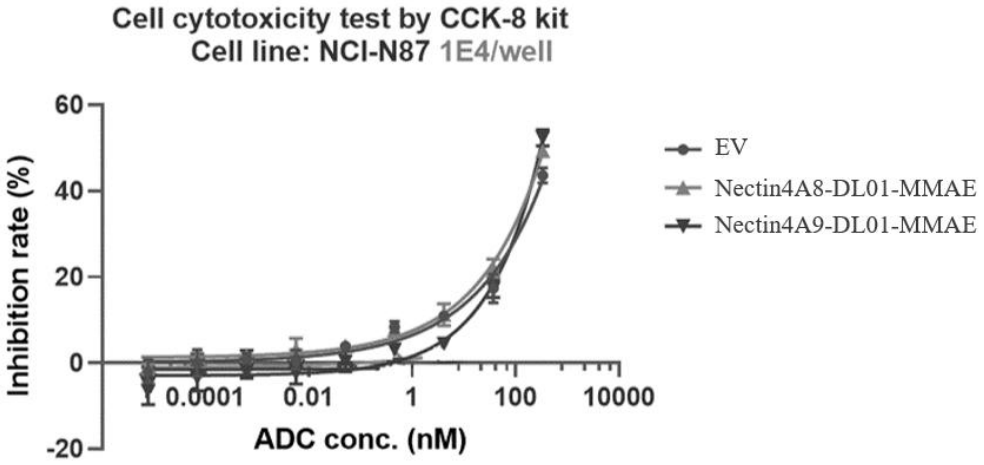
Cell cytotoxicity test by CCK-8 kit
Cell line: NCI-N87 1E4/well
Inhibition rate (%)
60
40
20
0
-20
0.0001
0.01
1
100
10000
ADC conc. (nM)
EV
Nectin4A8-DL01-MMAE
Nectin4A9-DL01-MMAE

FIG. 51

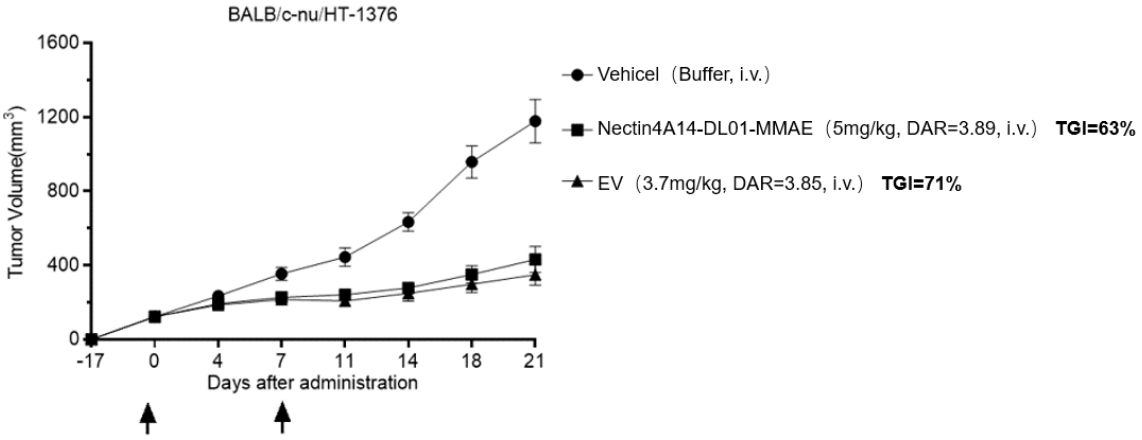
BALB/c-nu/HT-1376
Tumor Volume(mm3)
1600
1200
800
400
0
-17
0
4
7
11
14
18
21
Days after administration
Vehicel（Buffer, i.v.）
Nectin4A14-DL01-MMAE（5mg/kg, DAR=3.89, i.v.） TGI=63%
EV（3.7mg/kg, DAR=3.85, i.v.） TGI=71%

FIG. 52

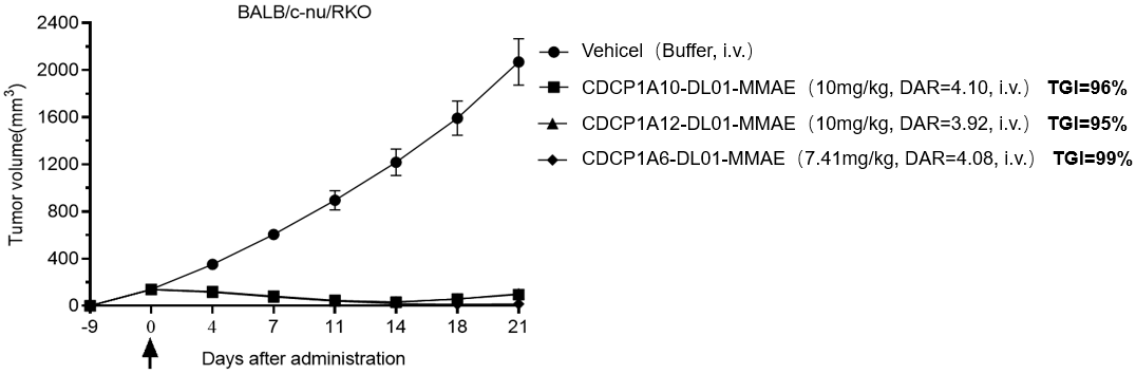


FIG. 53

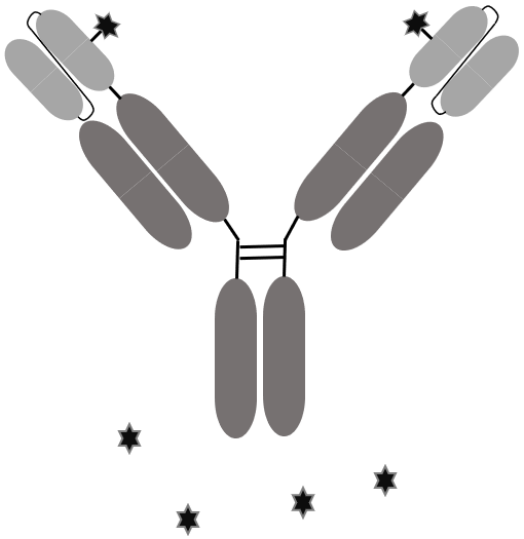

FIG. 54

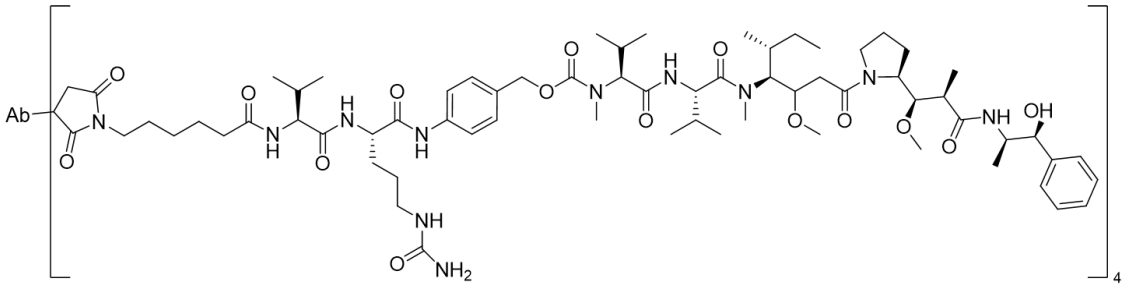


FIG.55

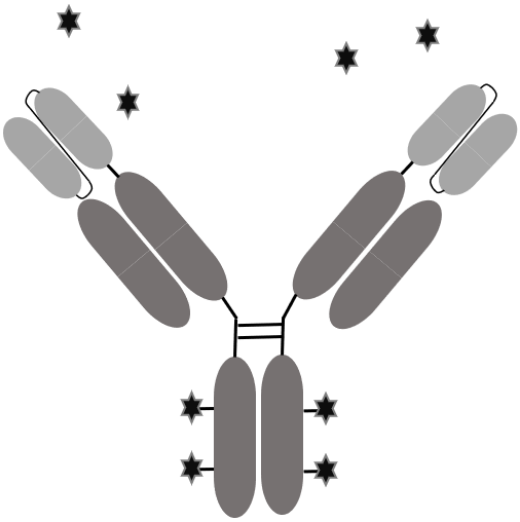

FIG. 56

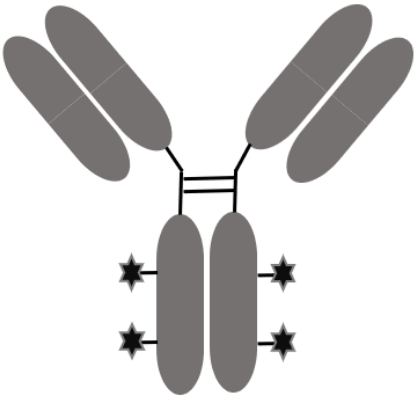

FIG. 57

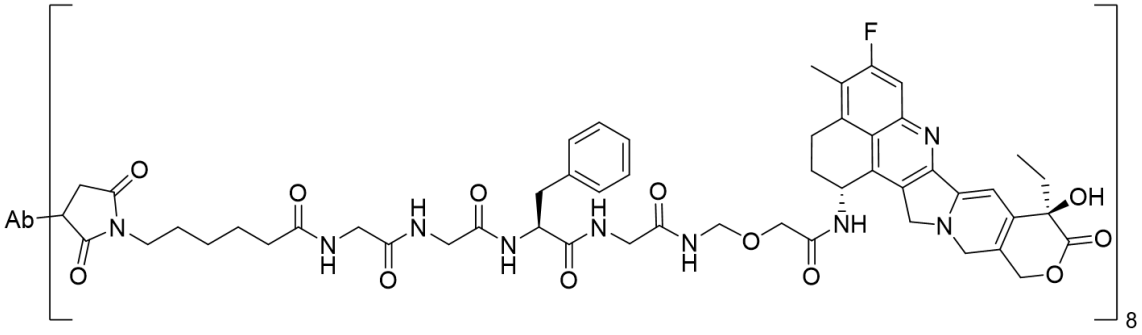


FIG. 58