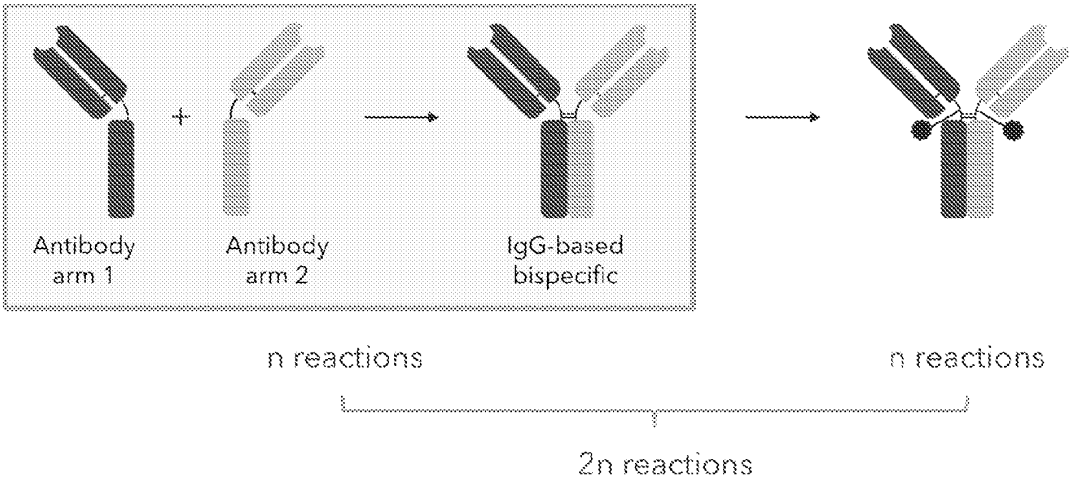
a) State-of-the-art approach with drug conjugation bottleneck



b) Inverse approach avoids drug conjugation bottleneck

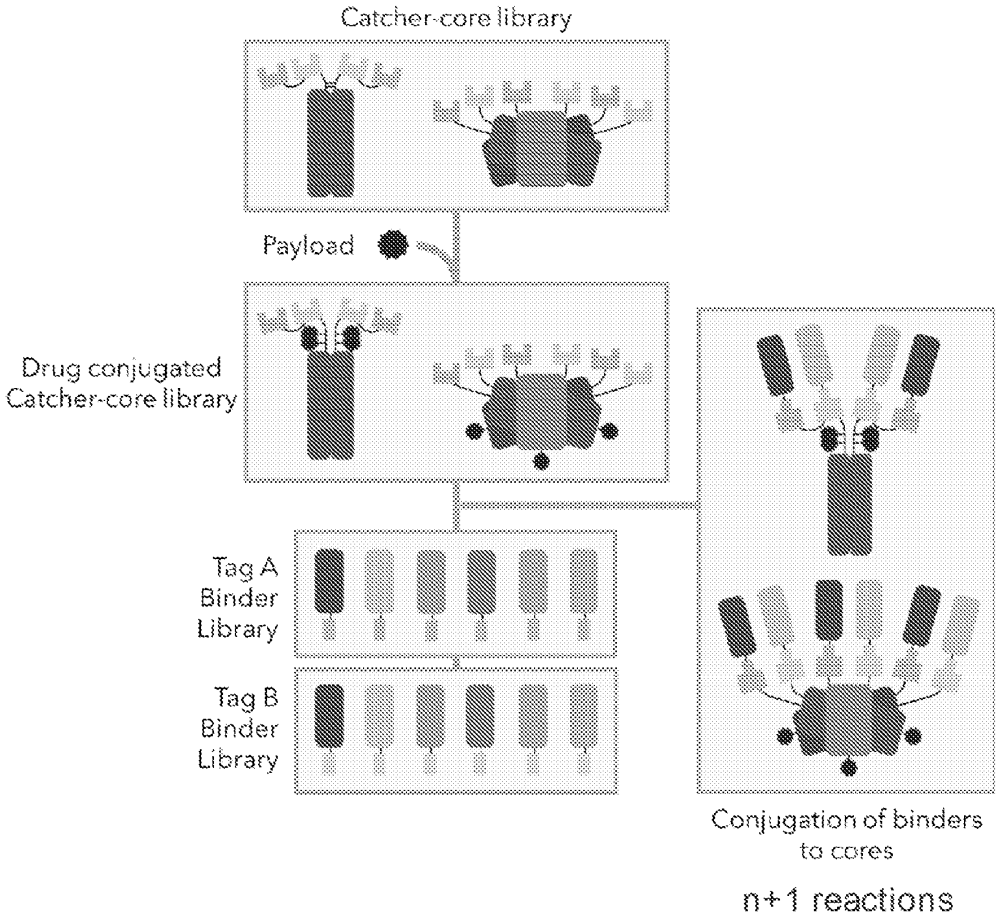


Figure 1

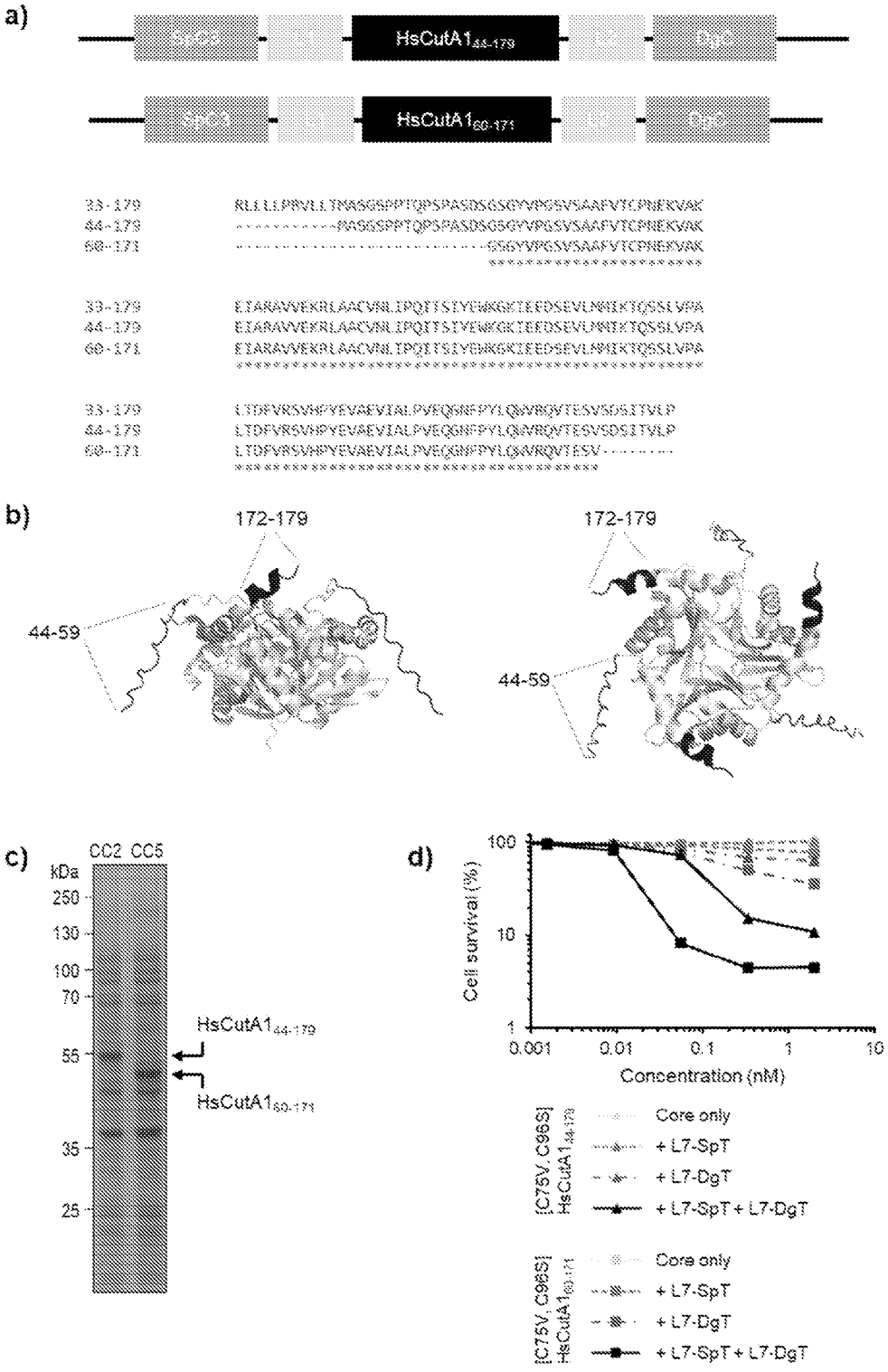
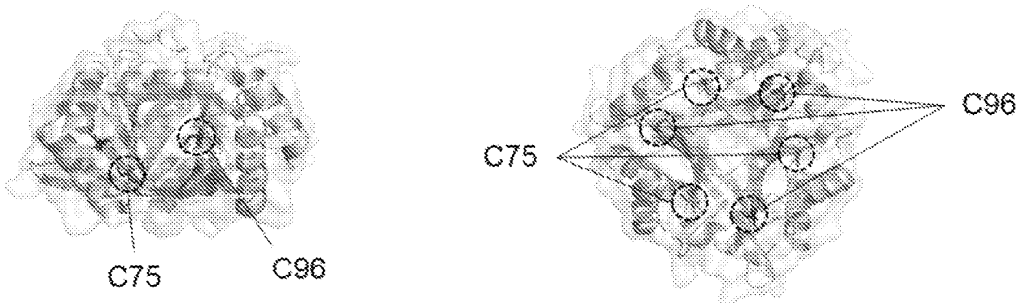

a)
HsCutA1$_{44-179}$
HsCutA1$_{60-171}$
b)
172-179
44-59
c)
CC2 CC5
kDa
250
130
100
70
55
35
25
HsCutA1$_{44-179}$
HsCutA1$_{60-171}$
d)
Cell survival (%)
100
10
1
0.001
0.01
0.1
1
10
Concentration (nM)
[C75V, C96S] HsCutA1$_{44-179}$
[C75V, C96S] HsCutA1$_{60-171}$
Core only
+ L7-SpT
+ L7-DgT
+ L7-SpT + L7-DgT

Figure 2

2 / 25

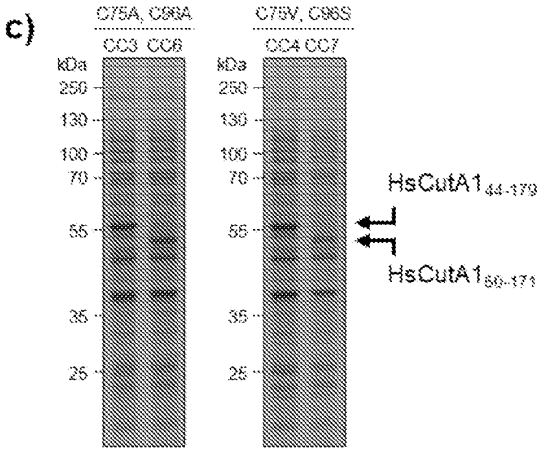
a)



b)

	% frequency of amino acid									
Position	C	A	F	G	I	L	S	T	V	Y
75	27.0	17.4	1.47	0.24	2.94	3.43	0.24	16.0	28.5	0.74
96	94.3			0.73			4.42			0.50

c)



d)

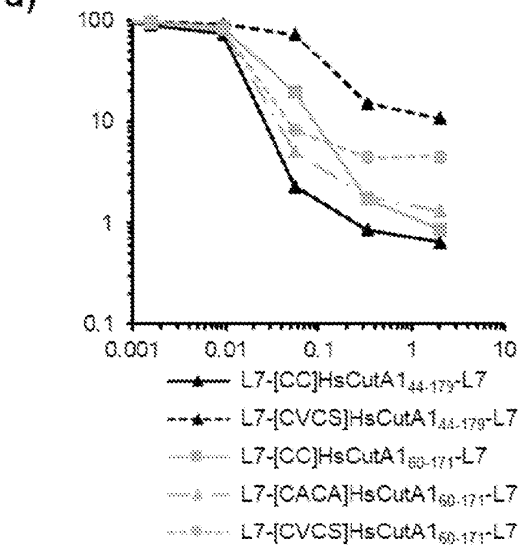


Figure 3

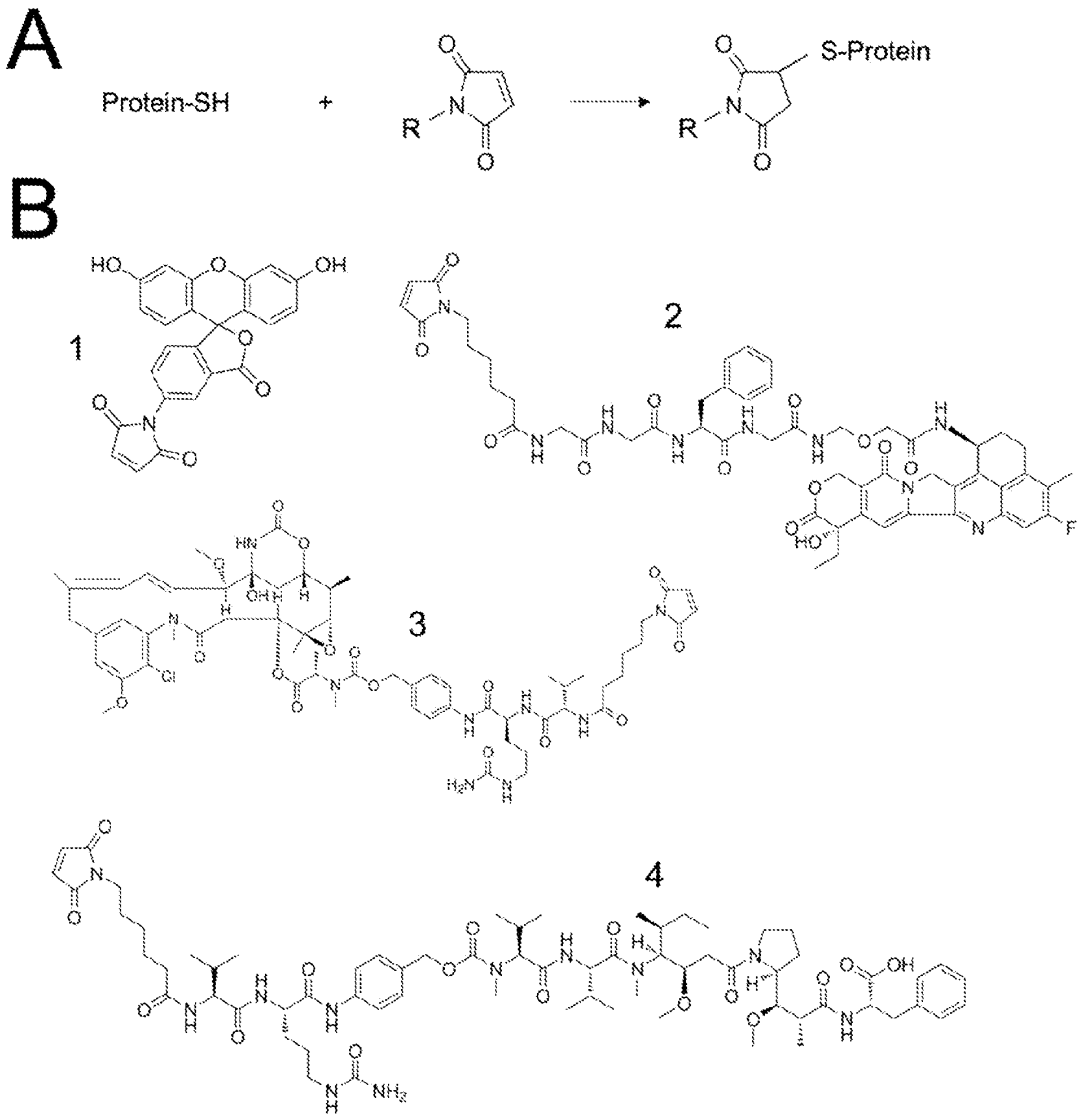


Figure 4

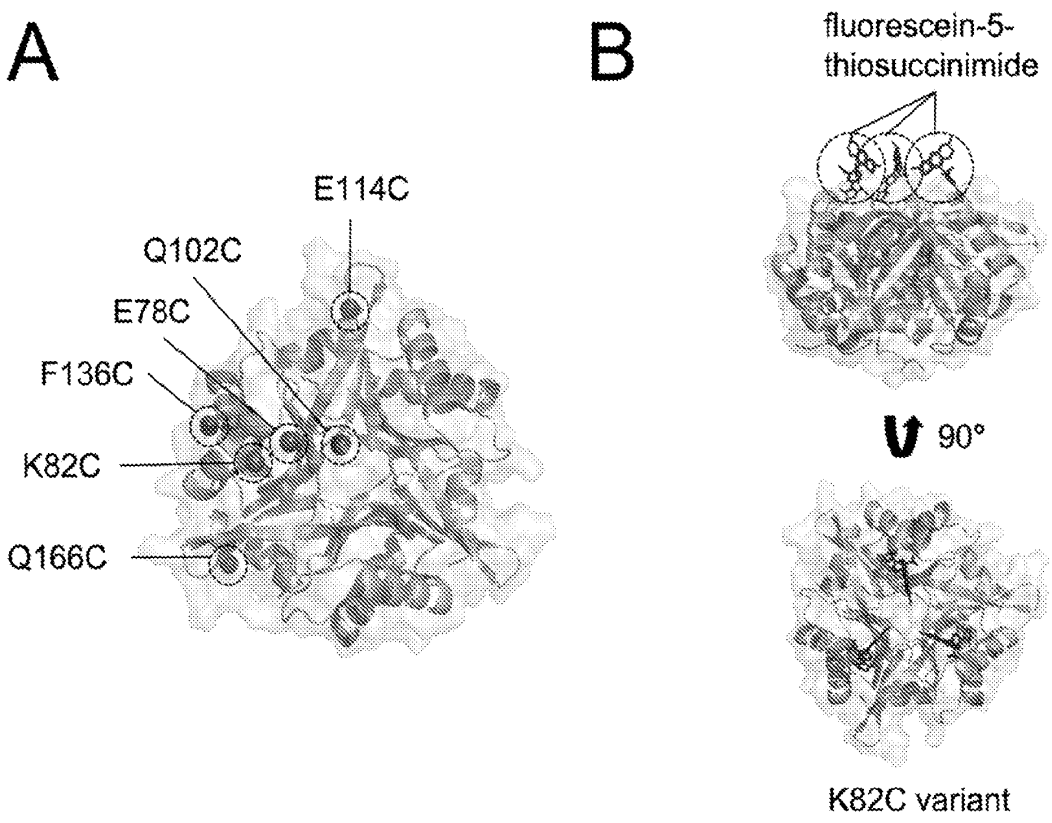

A
E114C
Q102C
E78C
F136C
K82C
Q166C
B
fluorescein-5-
thiosuccinimide
90°
K82C variant

Figure 5

A

Construct	Mutations relative to CC1	SEQ ID NO:	fluorophore/protein ratio
CC1	None	15	0.5
CC041	C75A, C96A	36	0.16
CC038	C75A, C96A, E114C	40	1.38
CC042	C75A, C96A, K82C	38	1.16
CC044	C75A, C96A, F136C	41	0.92
CC058	C75A, C96A, Q166C	42	0.87
CC057	C75A, C96A, Q102C	39	0.69
CC056	C75A, C96A, E78C	37	0.55

B

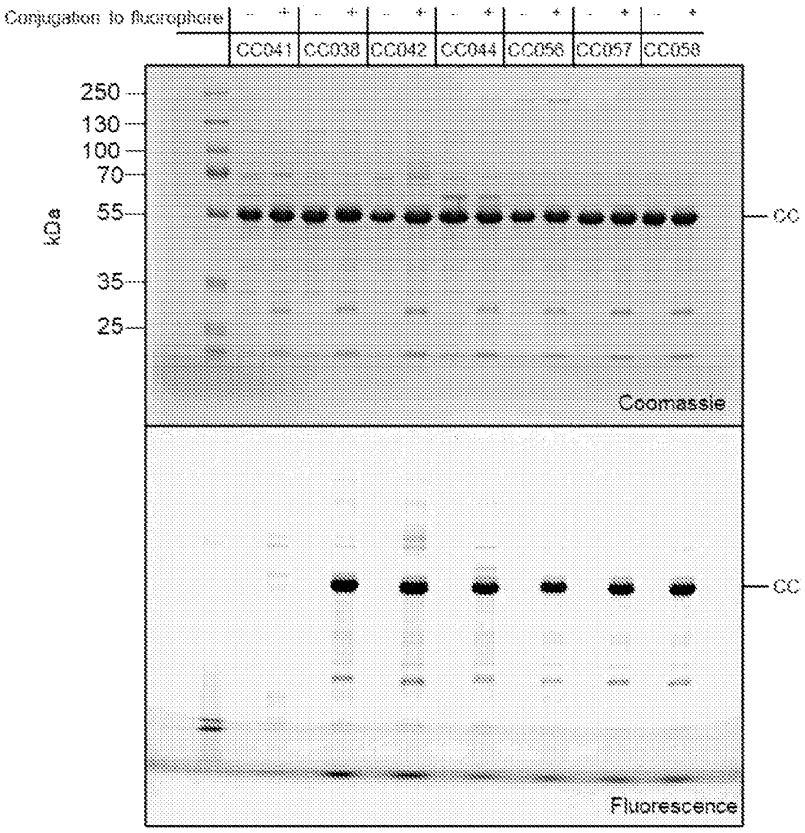


Figure 6

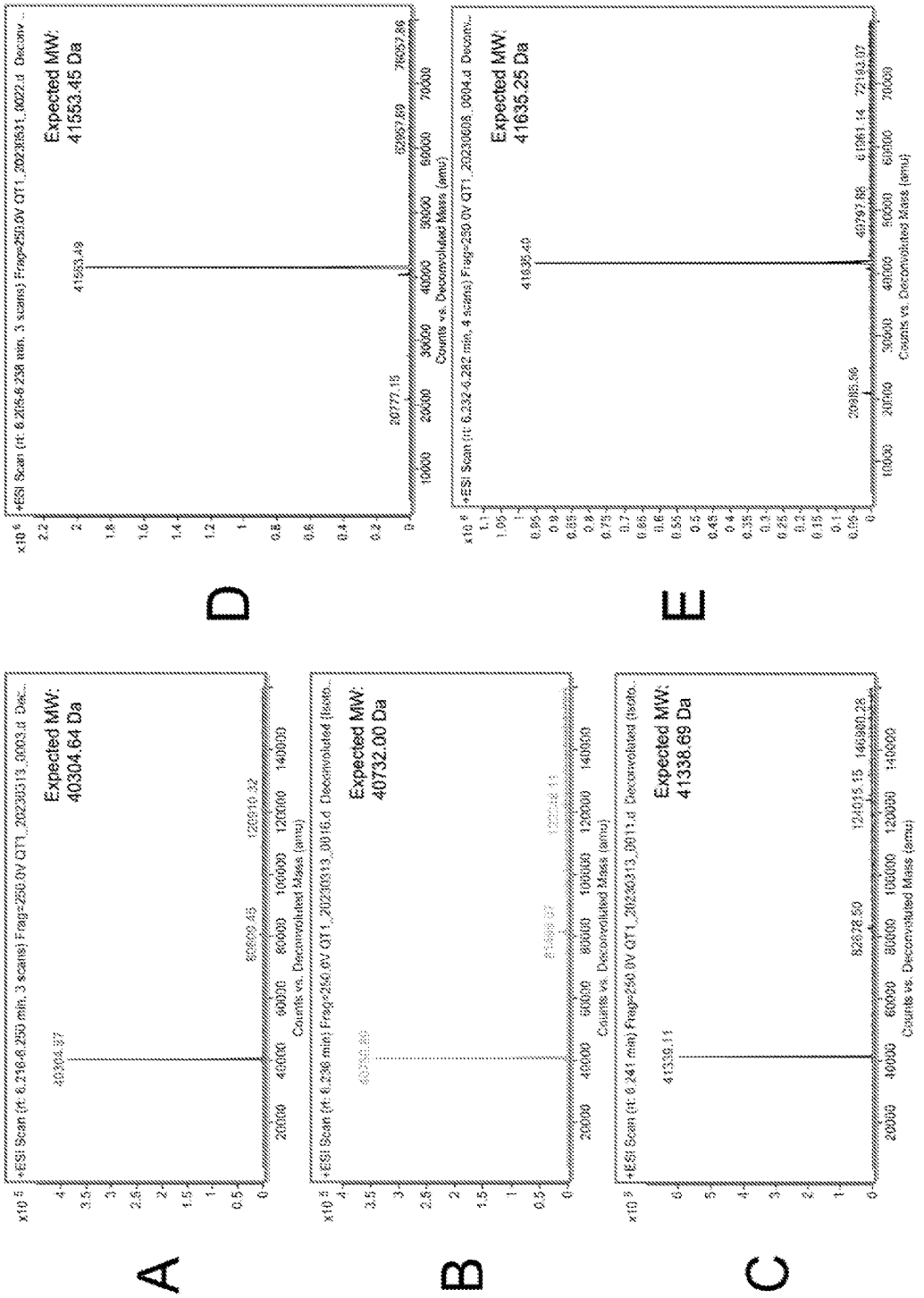

A
Expected MW:
40304.64 Da
B
Expected MW:
40732.00 Da
C
Expected MW:
41338.69 Da
D
Expected MW:
41553.45 Da
E
Expected MW:
41635.25 Da

Figure 7

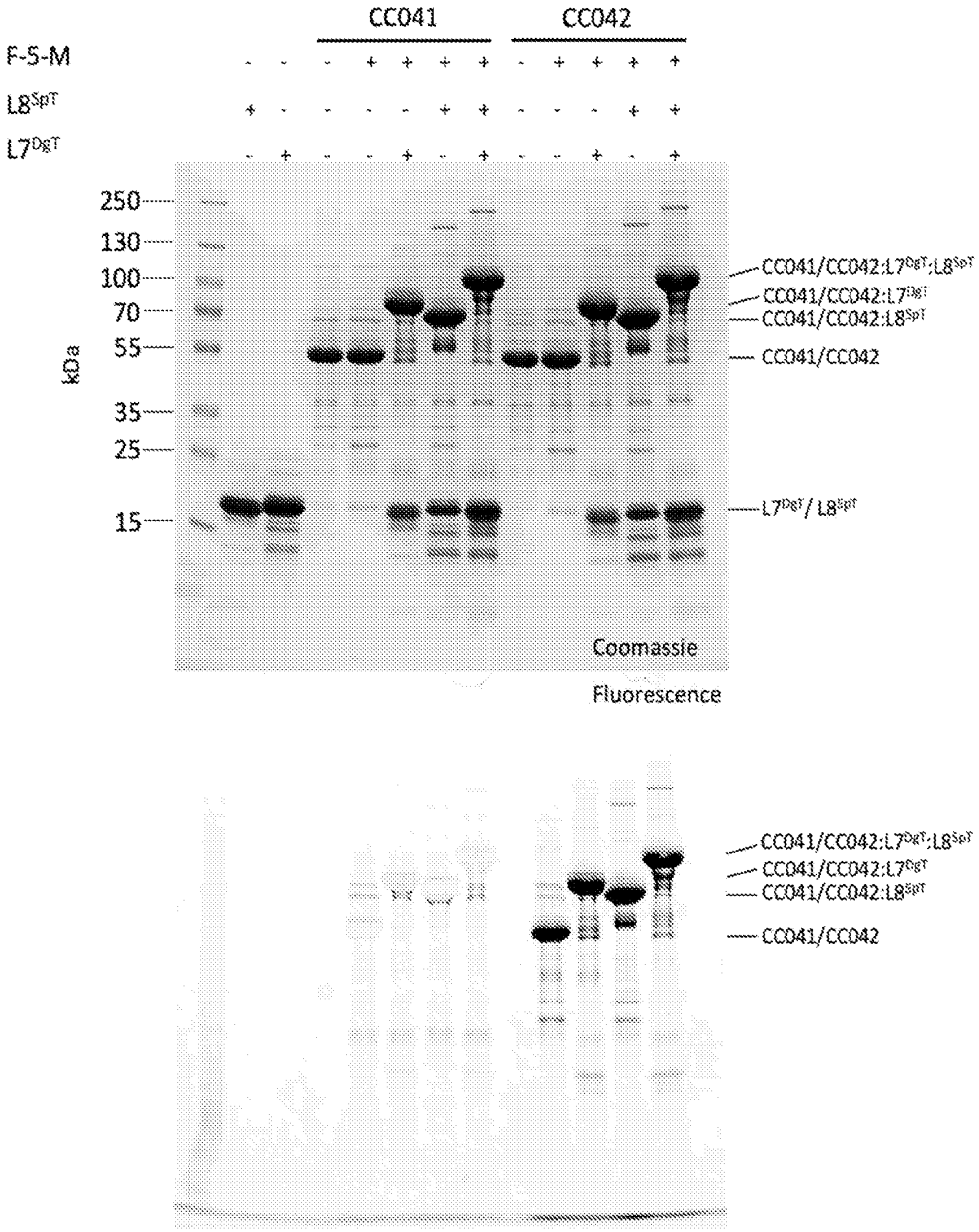

CC041
CC042
F-S-M
L8SpT
L7DgT
250
130
100
70
55
35
25
15
kDa
CC041/CC042:L7DgT:L8SpT
CC041/CC042:L7DgT
CC041/CC042:L8SpT
CC041/CC042
L7DgT / L8SpT
Coomassie
Fluorescence

Figure 8

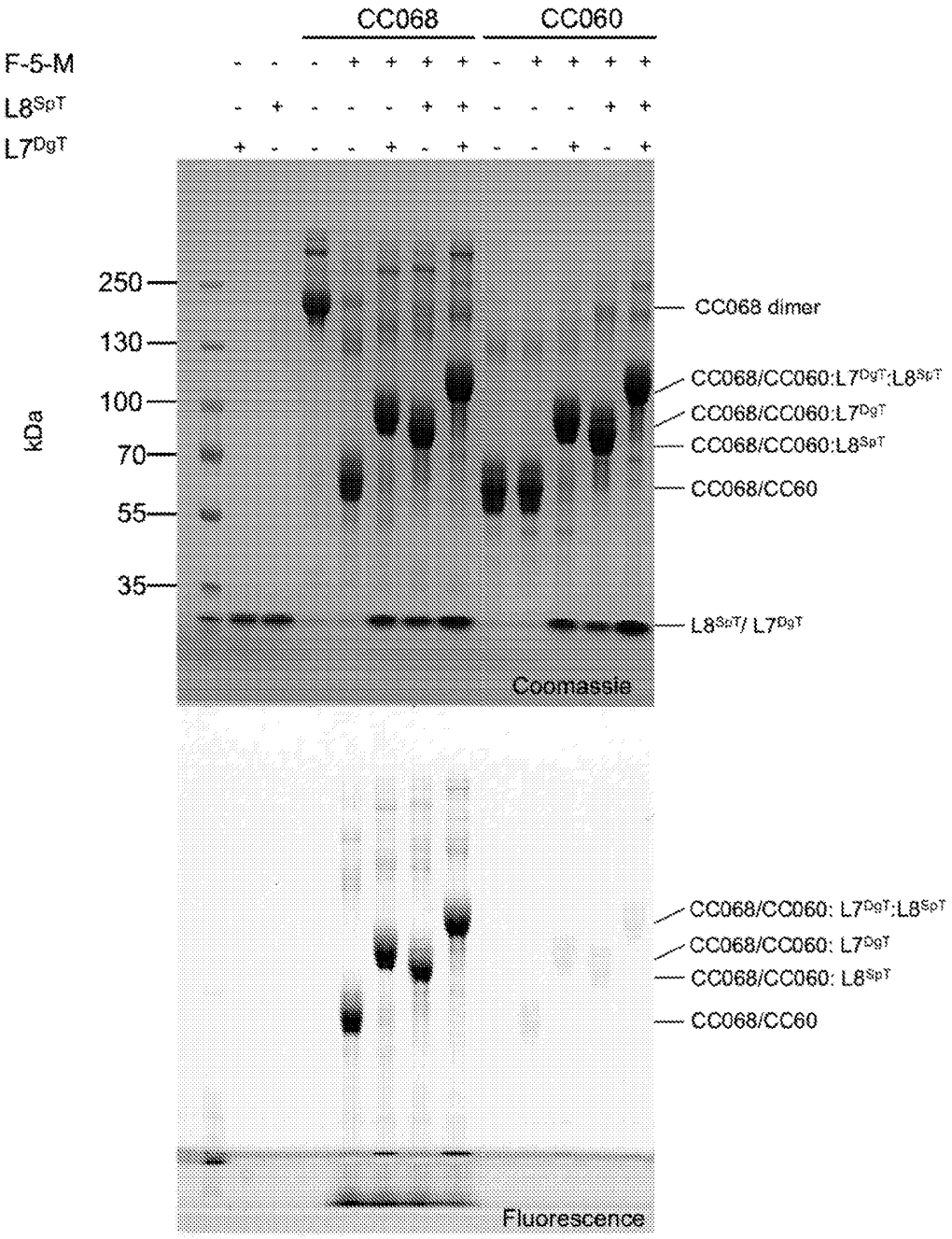

CC068
CC060
F-5-M
L8SpT
L7DgT
kDa
250
130
100
70
55
35
CC068 dimer
CC068/CC060:L7DgT:L8SpT
CC068/CC060:L7DgT
CC068/CC060:L8SpT
CC068/CC60
L8SpT/ L7DgT
Coomassie
CC068/CC060: L7DgT:L8SpT
CC068/CC060: L7DgT
CC068/CC060: L8SpT
CC068/CC60
Fluorescence

Figure 9

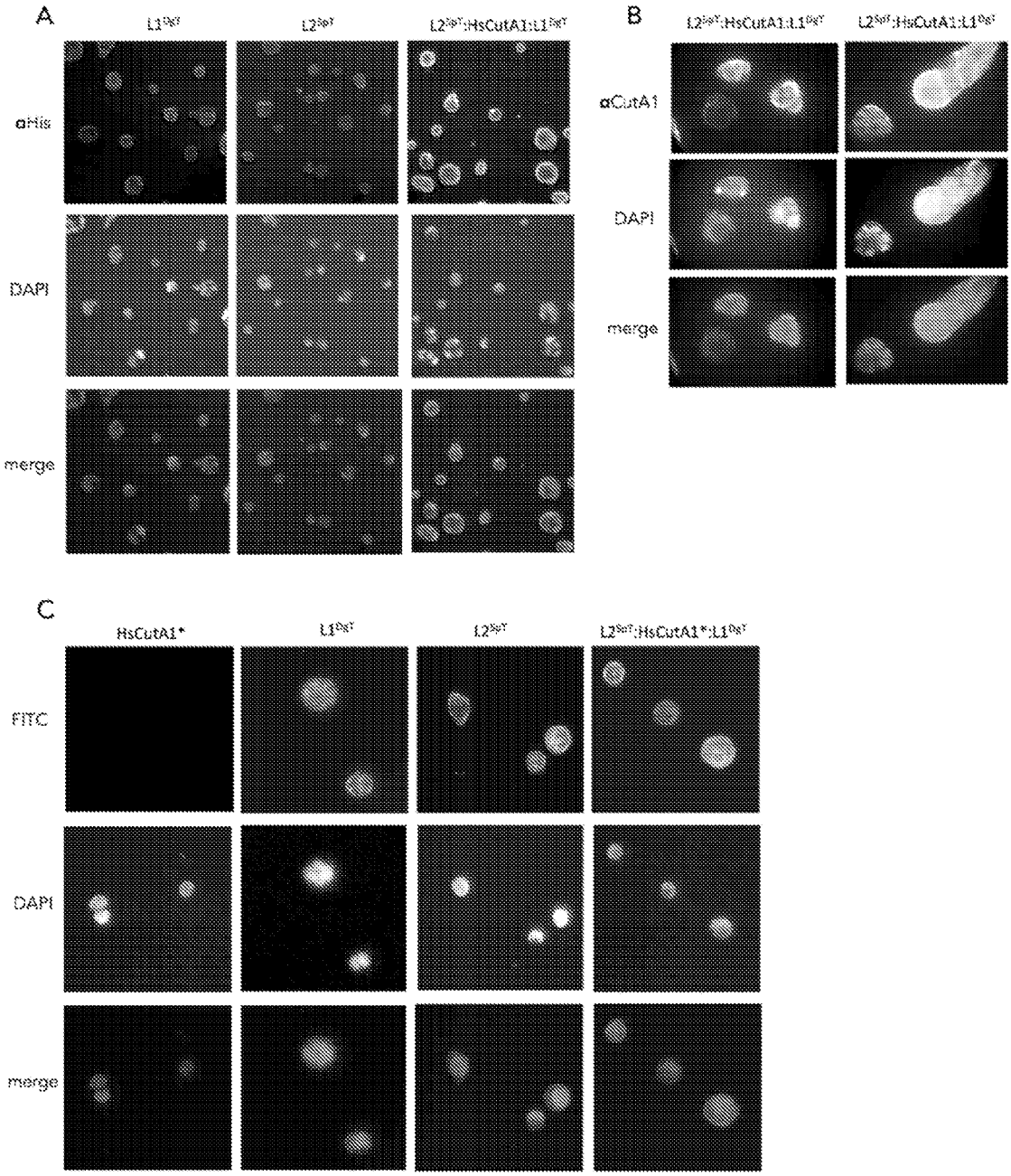

A
αHis
DAPI
merge
B
αCutA1
DAPI
merge
C
HsCutA1*
FITC
DAPI
merge

Figure 10

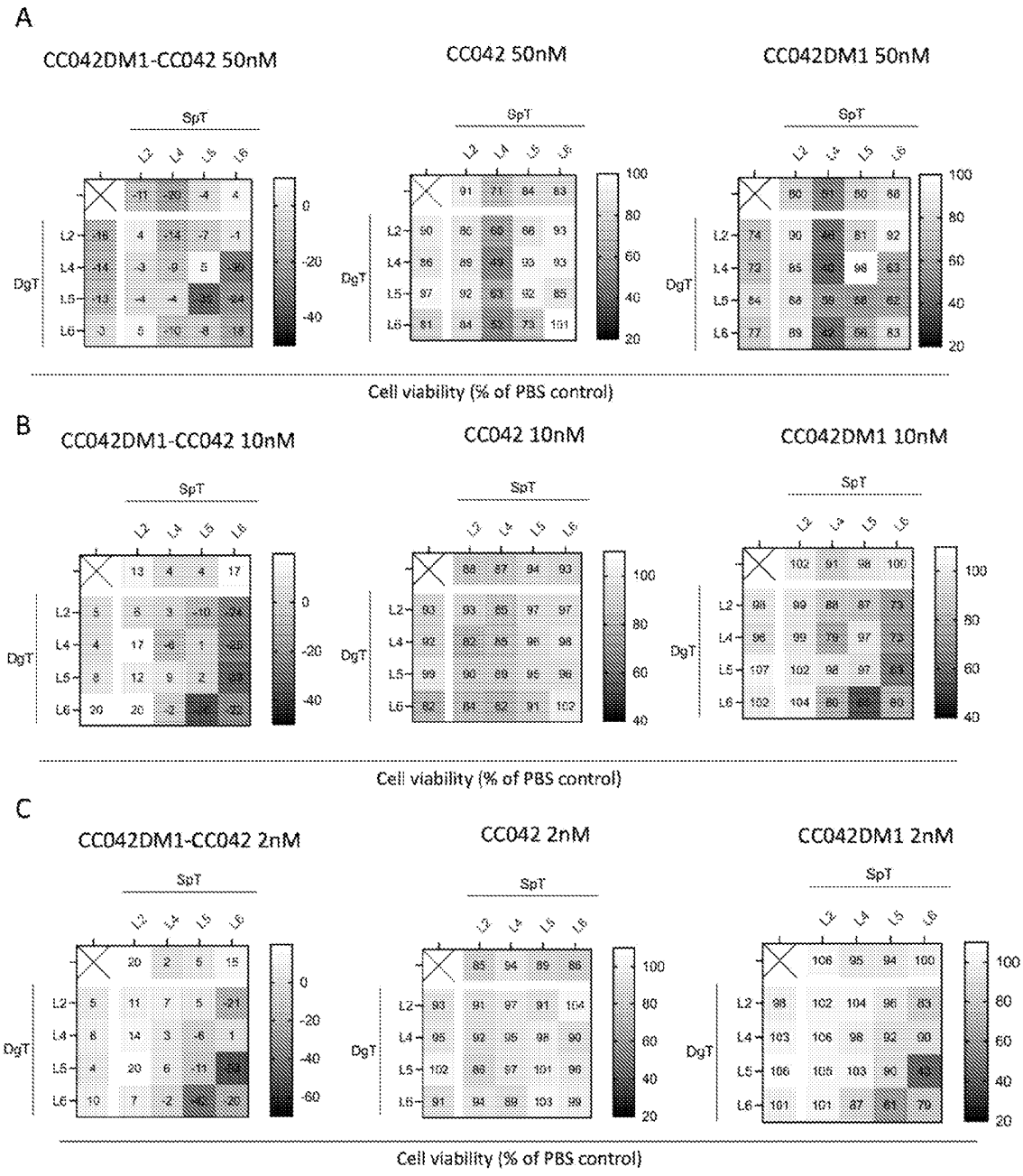

A
CC042DM1-CC042 50nM
CC042 50nM
CC042DM1 50nM
SpT
DgT
L2
L4
L5
L6
Cell viability (% of PBS control)
B
CC042DM1-CC042 10nM
CC042 10nM
CC042DM1 10nM
Cell viability (% of PBS control)
C
CC042DM1-CC042 2nM
CC042 2nM
CC042DM1 2nM
Cell viability (% of PBS control)

Figure 11

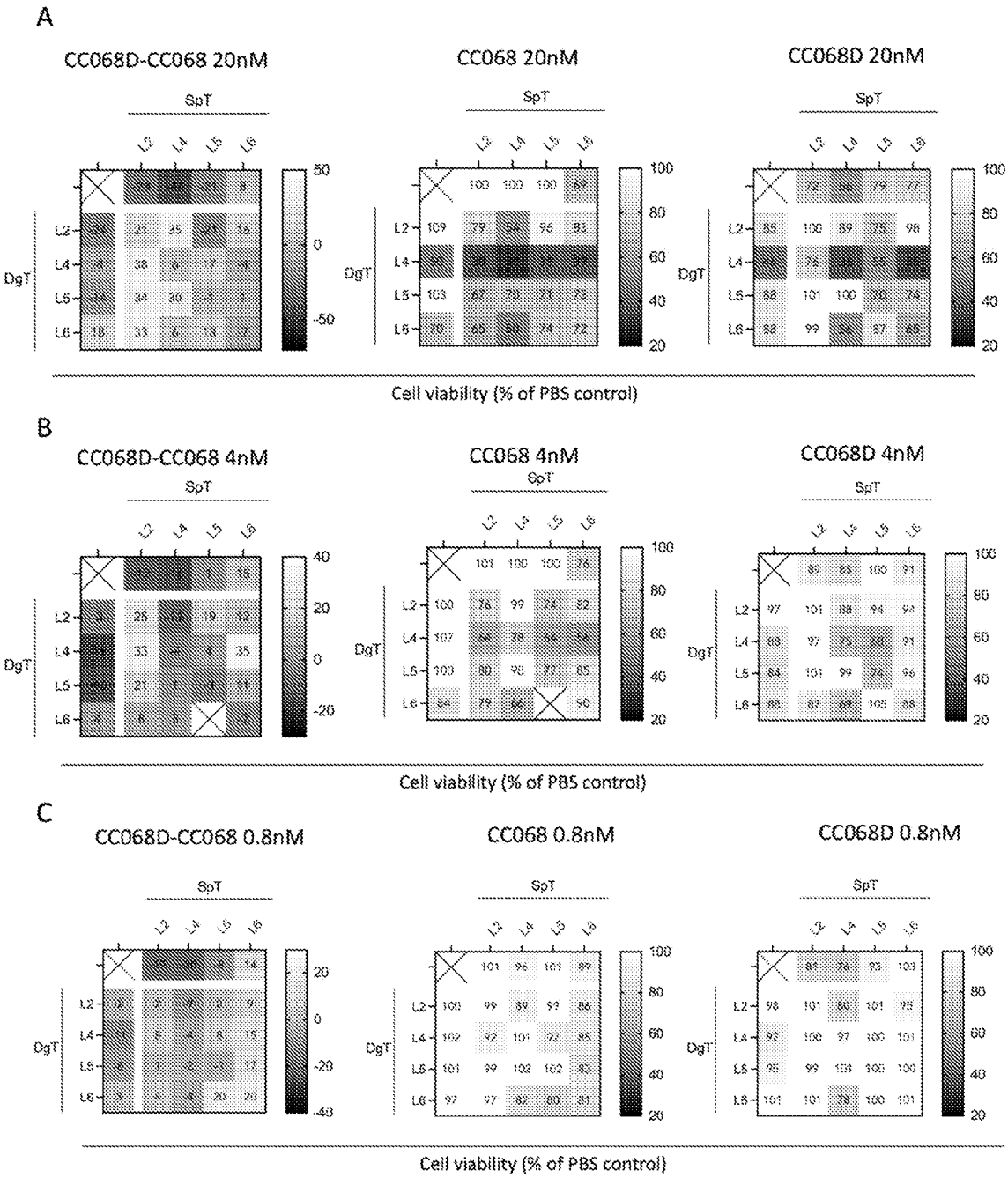

A
CC068D-CC068 20nM
CC068 20nM
CC068D 20nM
SpT
DgT
L2
L4
L5
L6
Cell viability (% of PBS control)
B
CC068D-CC068 4nM
CC068 4nM
CC068D 4nM
Cell viability (% of PBS control)
C
CC068D-CC068 0.8nM
CC068 0.8nM
CC068D 0.8nM
Cell viability (% of PBS control)

Figure 12

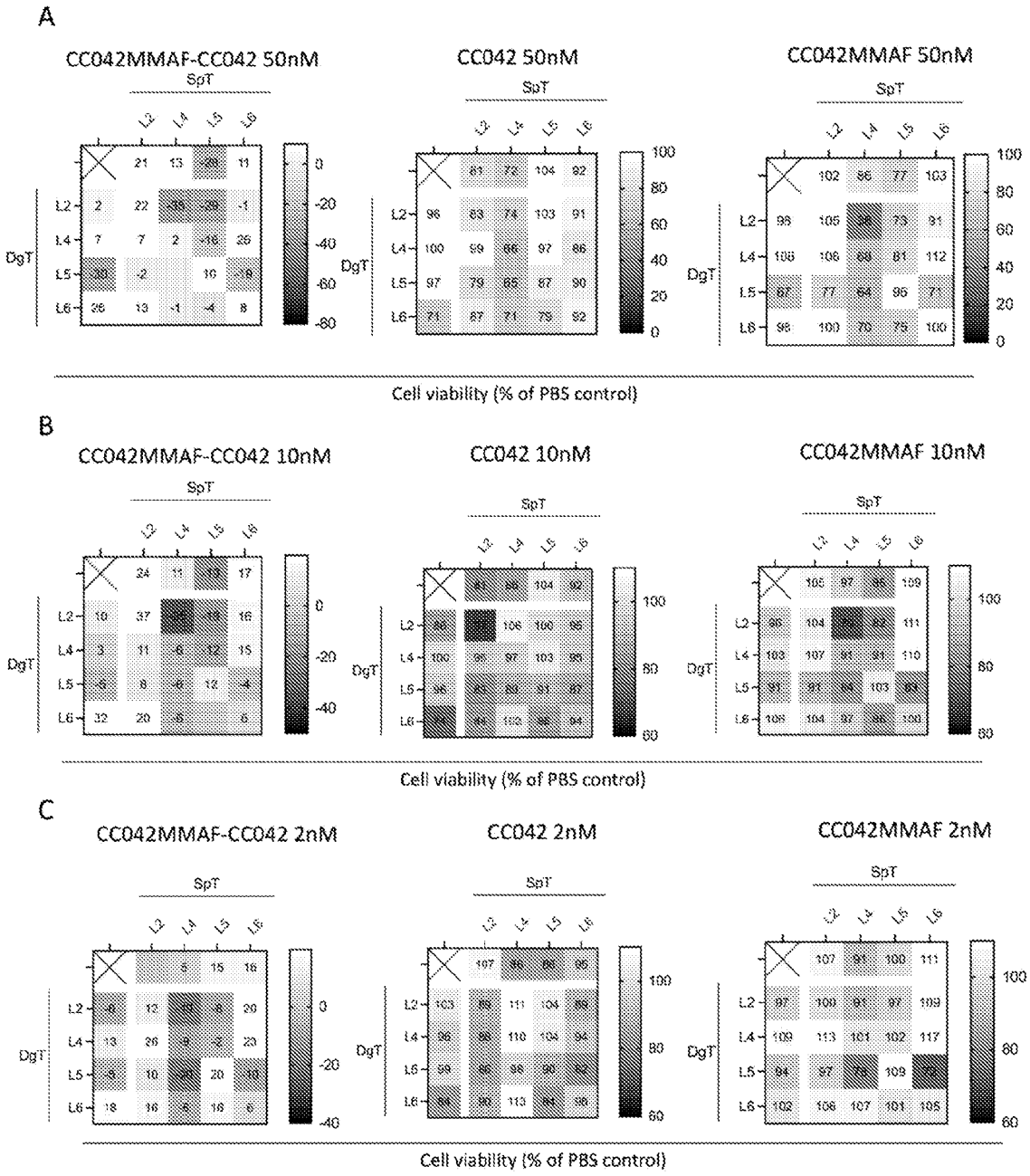

A
CC042MMAF-CC042 50nM
CC042 50nM
CC042MMAF 50nM
SpT
DgT
L2
L4
L5
L6
Cell viability (% of PBS control)
B
CC042MMAF-CC042 10nM
CC042 10nM
CC042MMAF 10nM
Cell viability (% of PBS control)
C
CC042MMAF-CC042 2nM
CC042 2nM
CC042MMAF 2nM
Cell viability (% of PBS control)

Figure 13

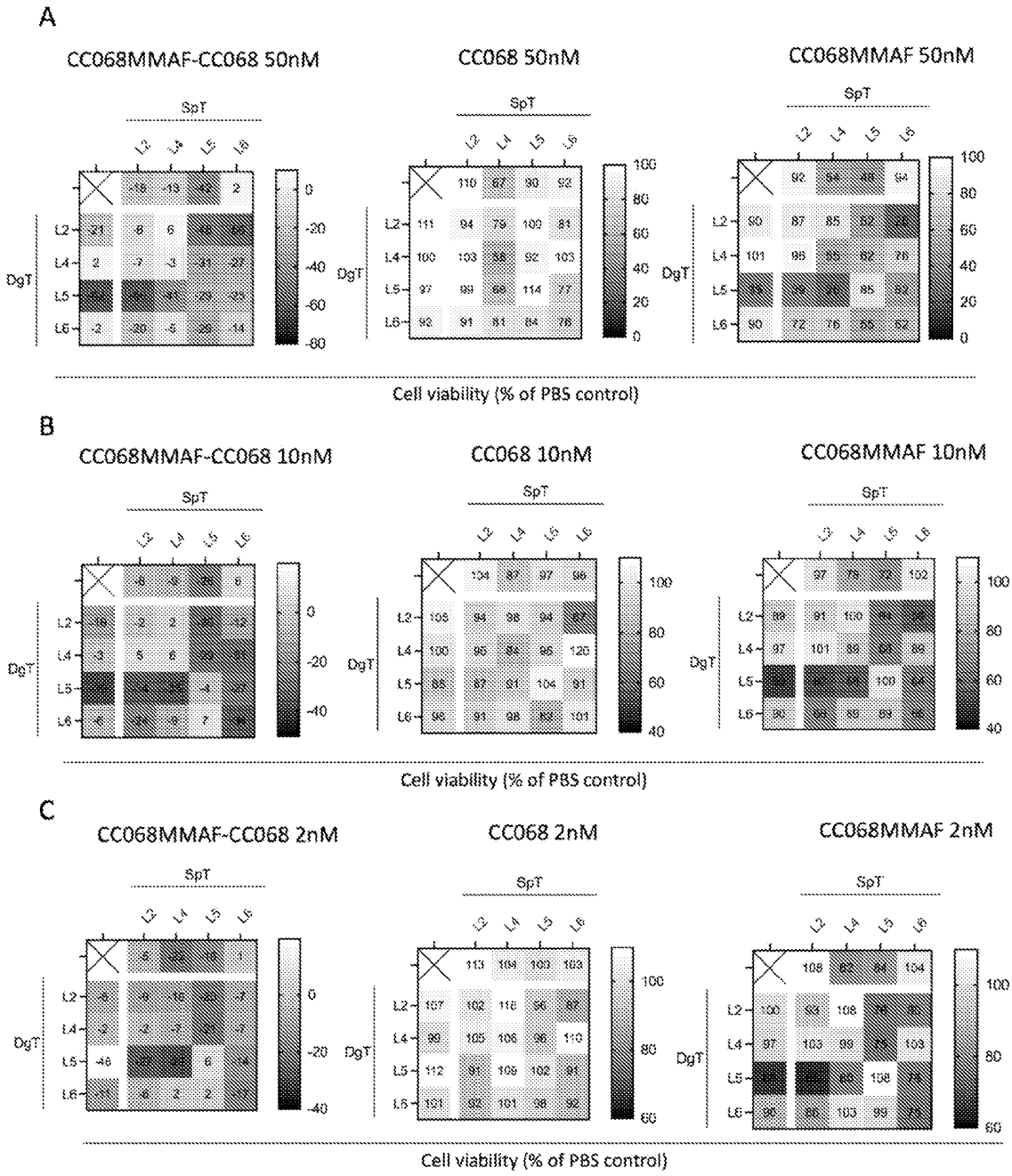

A
CC068MMAF-CC068 50nM
CC068 50nM
CC068MMAF 50nM
SpT
DgT
L2
L4
L5
L6
Cell viability (% of PBS control)
B
CC068MMAF-CC068 10nM
CC068 10nM
CC068MMAF 10nM
Cell viability (% of PBS control)
C
CC068MMAF-CC068 2nM
CC068 2nM
CC068MMAF 2nM
Cell viability (% of PBS control)

Figure 14

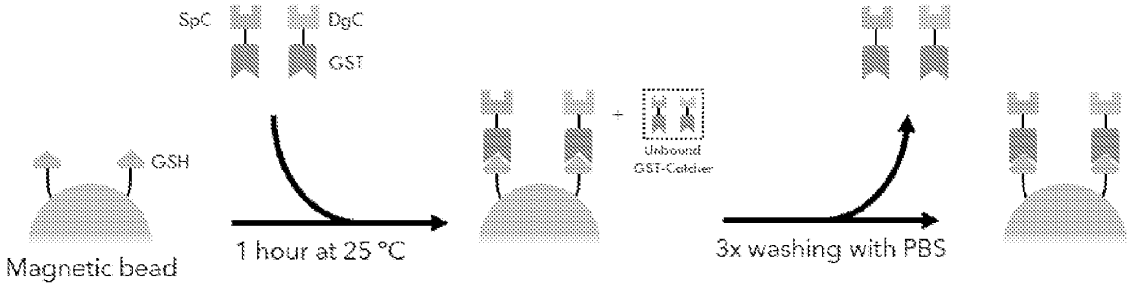


Figure 15

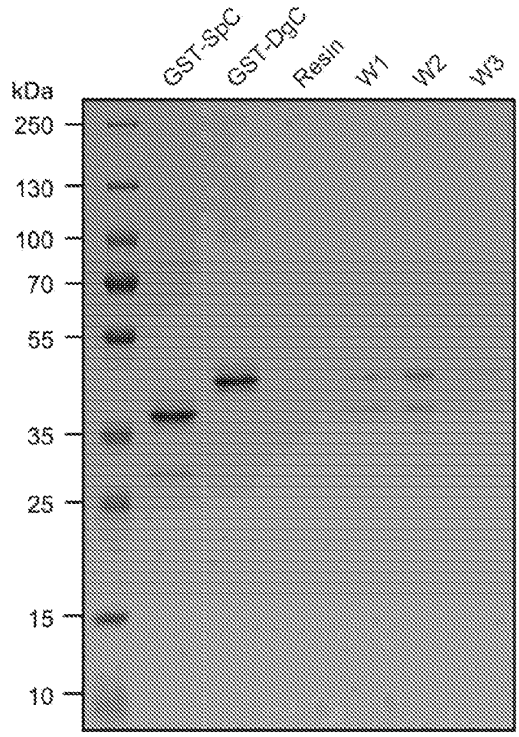


Figure 16

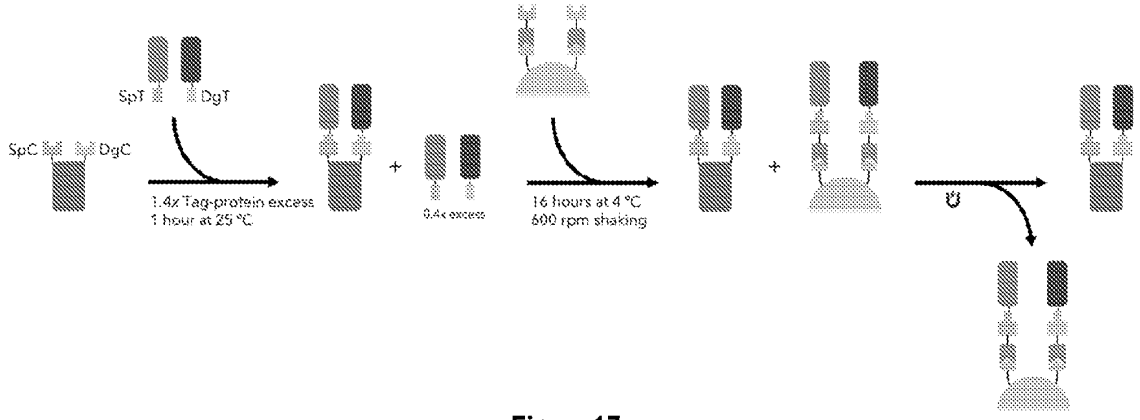


Figure 17

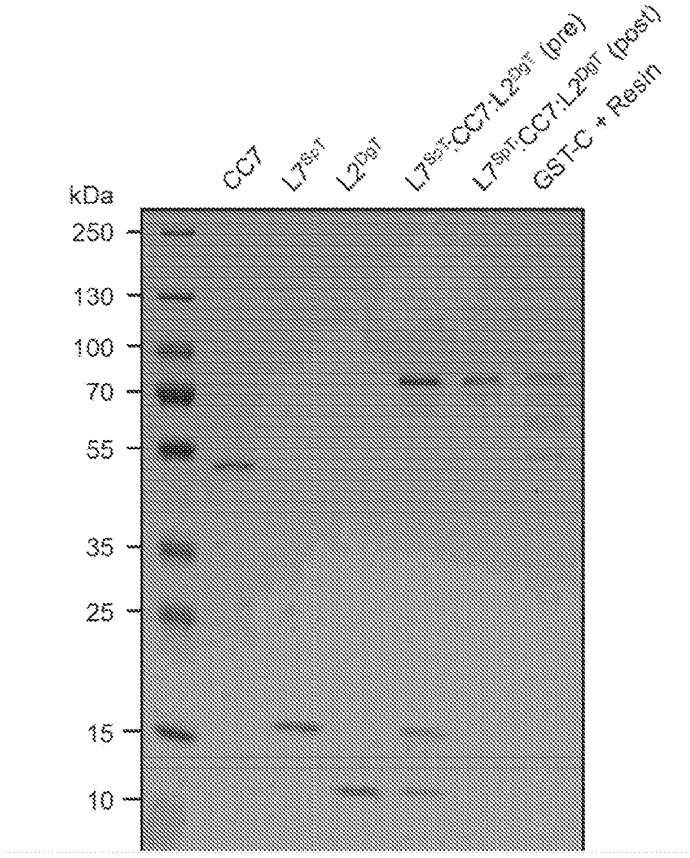


Figure 18

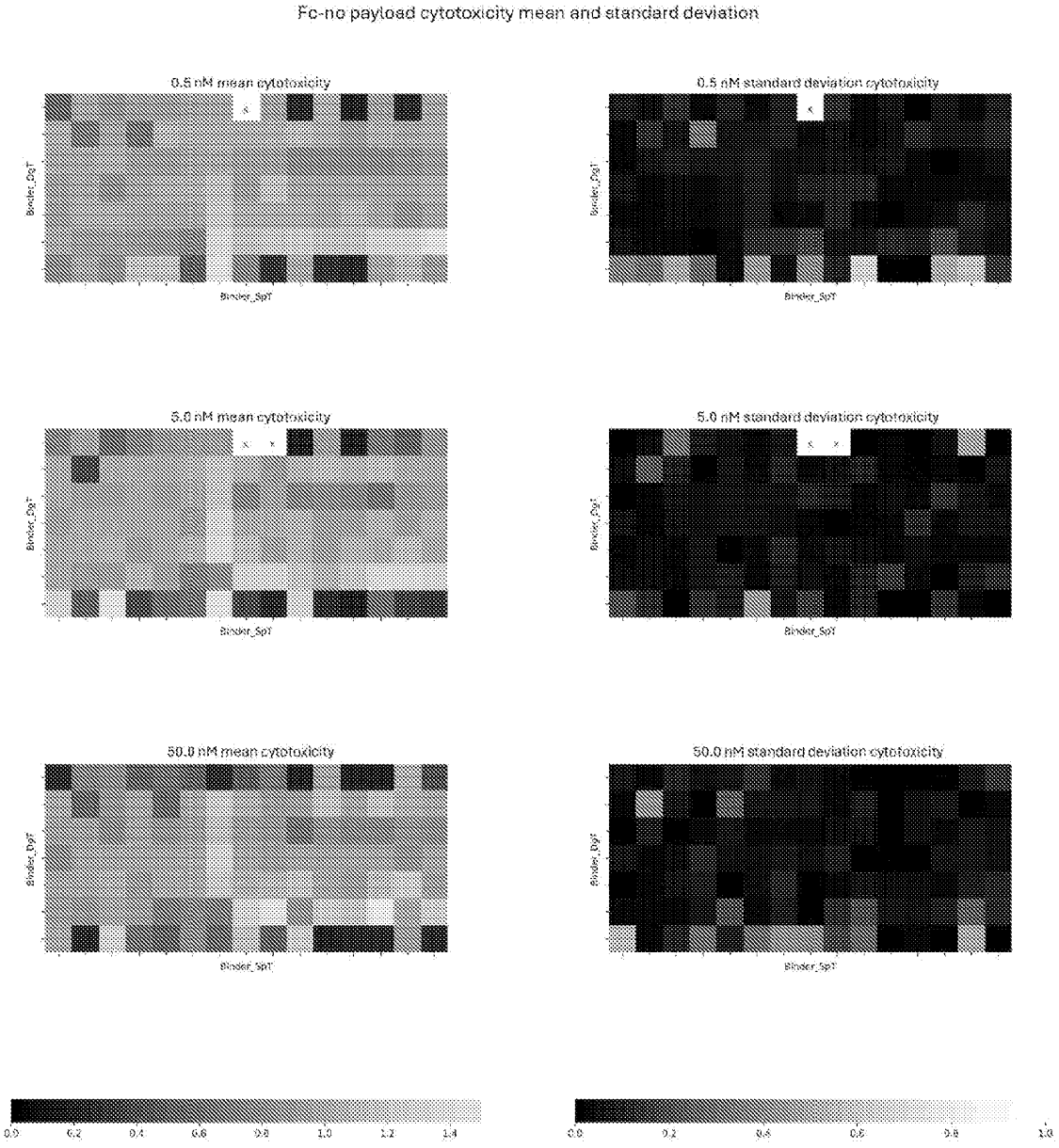

Fc-no payload cytotoxicity mean and standard deviation
0.5 nM mean cytotoxicity
0.5 nM standard deviation cytotoxicity
5.0 nM mean cytotoxicity
5.0 nM standard deviation cytotoxicity
50.0 nM mean cytotoxicity
50.0 nM standard deviation cytotoxicity
Binder_DpT
Binder_SpT
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4

Figure 19

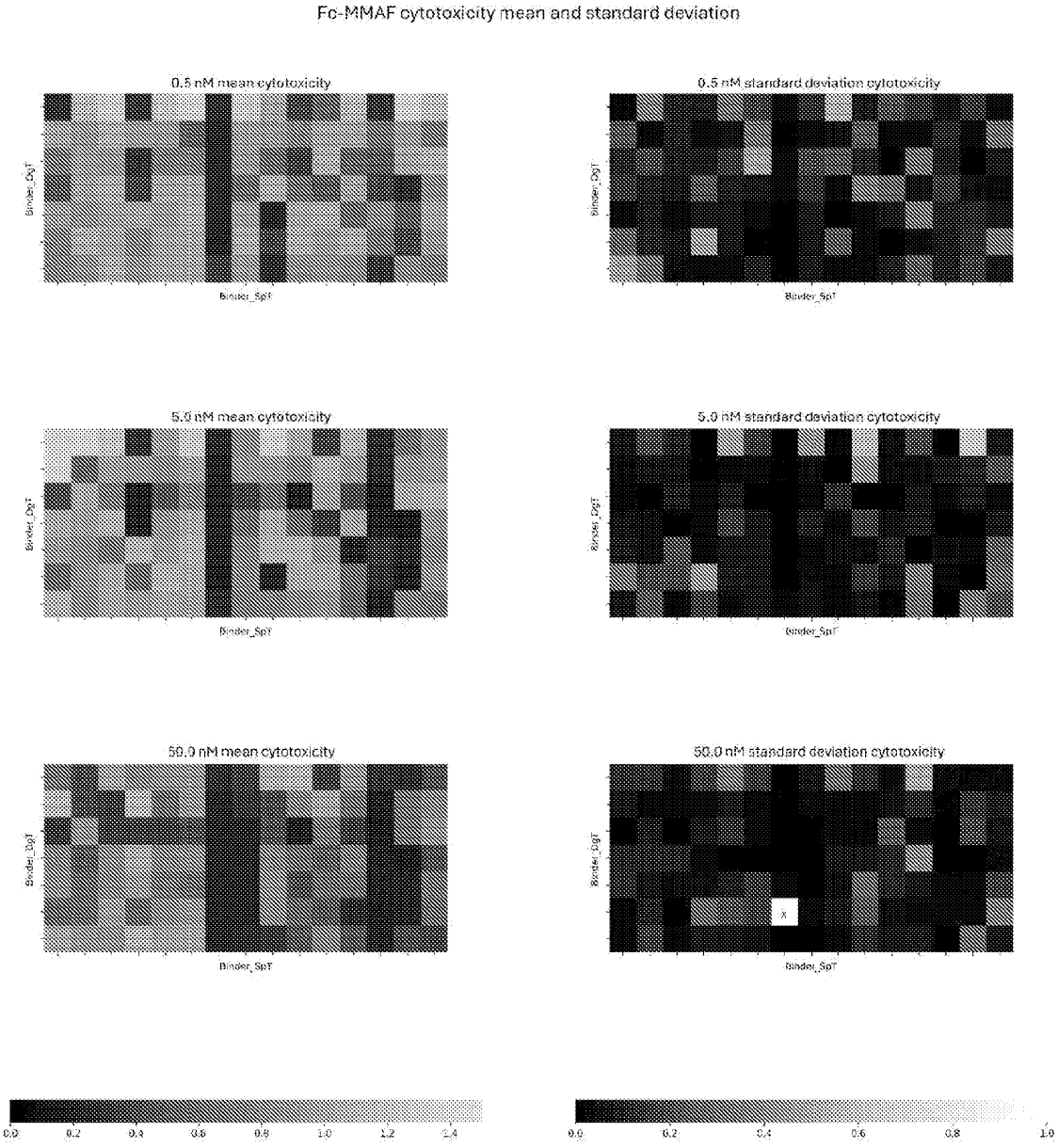

Fc-MMAF cytotoxicity mean and standard deviation
0.5 nM mean cytotoxicity
0.5 nM standard deviation cytotoxicity
5.0 nM mean cytotoxicity
5.0 nM standard deviation cytotoxicity
50.0 nM mean cytotoxicity
50.0 nM standard deviation cytotoxicity
Binder_DpT
Binder_SpT
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4

Figure 20

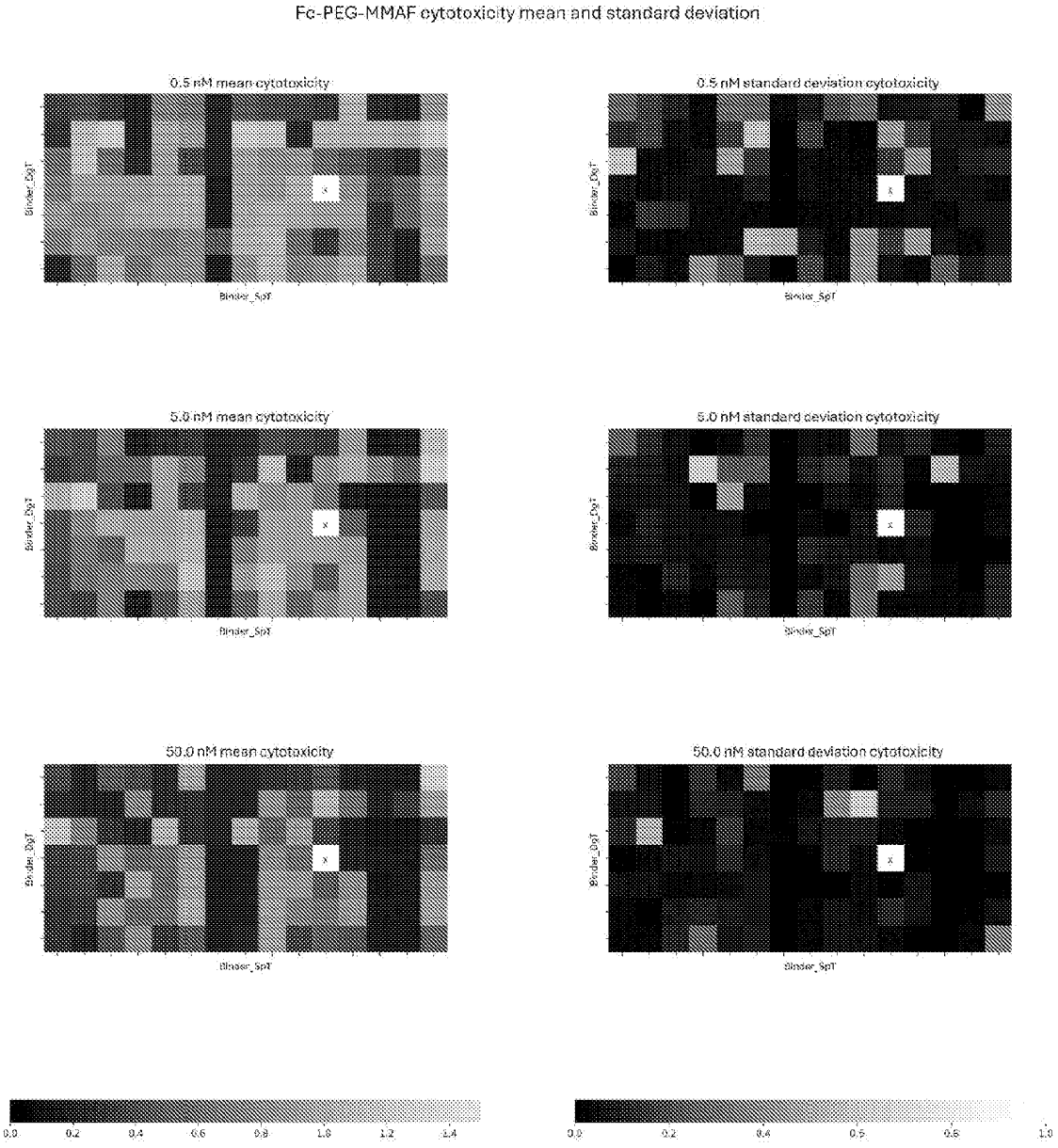

Fc-PEG-MMAF cytotoxicity mean and standard deviation
0.5 nM mean cytotoxicity
0.5 nM standard deviation cytotoxicity
5.0 nM mean cytotoxicity
5.0 nM standard deviation cytotoxicity
50.0 nM mean cytotoxicity
50.0 nM standard deviation cytotoxicity
Binder_DgT
Binder_SpT
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0.0
0.2
0.4
0.5
0.6
1.0

Figure 21

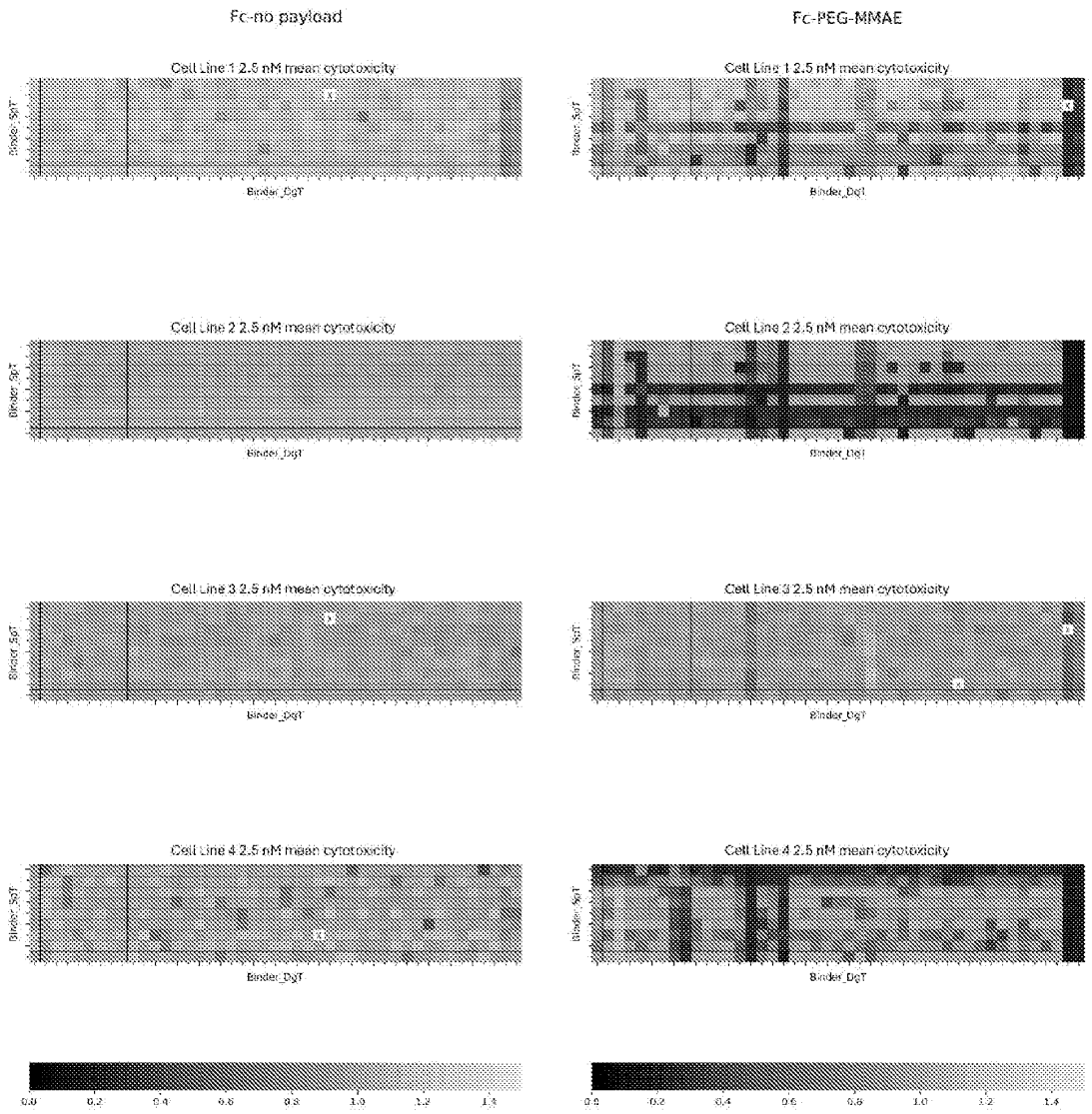

Fc-no payload
Fc-PEG-MMAE
Cell Line 1 2.5 nM mean cytotoxicity
Cell Line 2 2.5 nM mean cytotoxicity
Cell Line 3 2.5 nM mean cytotoxicity
Cell Line 4 2.5 nM mean cytotoxicity
Binder_SpT
Binder_DgT
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4

Figure 22A

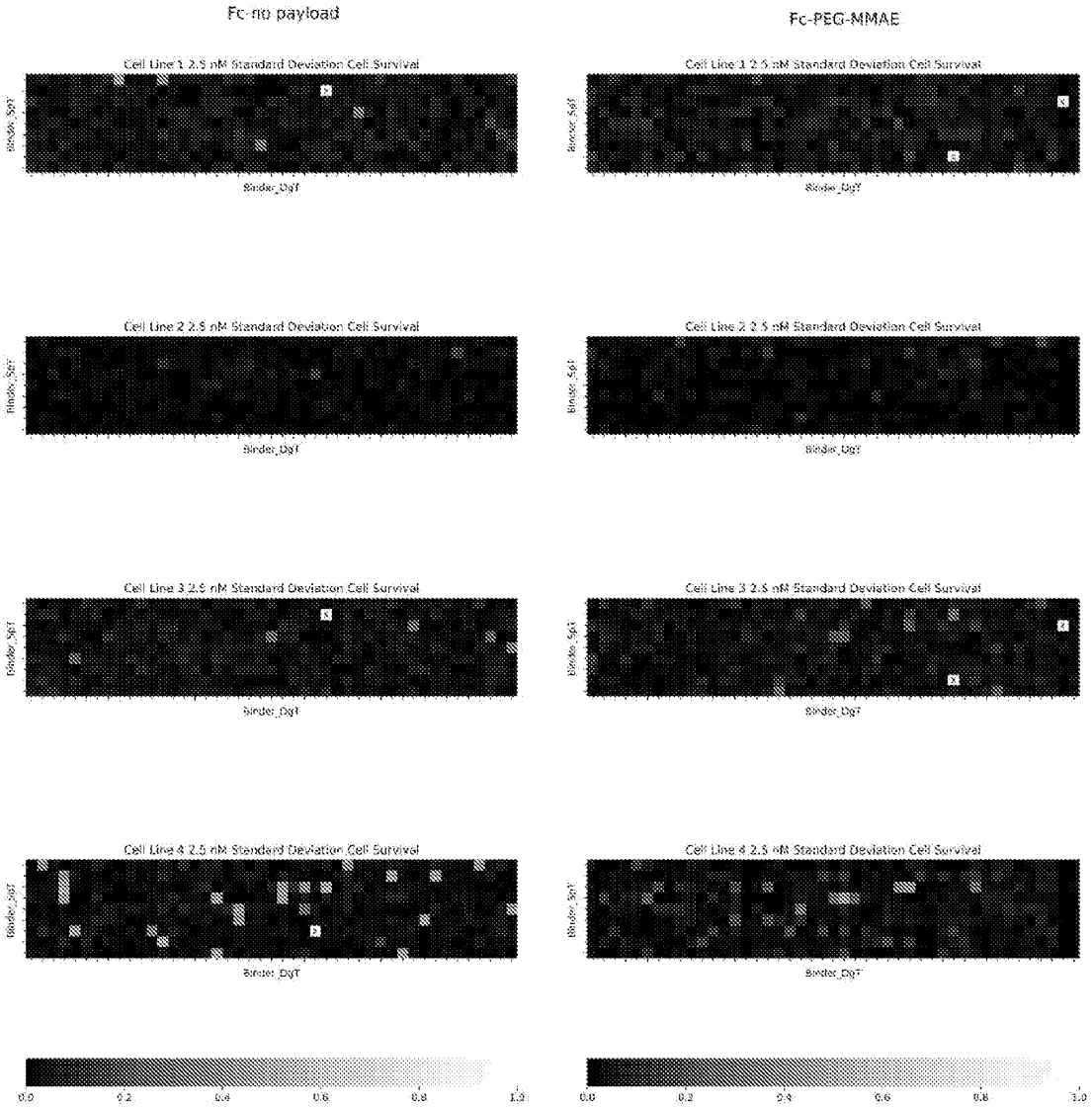

Fc-no payload
Fc-PEG-MMAE
Cell Line 1 2.5 nM Standard Deviation Cell Survival
Cell Line 2 2.5 nM Standard Deviation Cell Survival
Cell Line 3 2.5 nM Standard Deviation Cell Survival
Cell Line 4 2.5 nM Standard Deviation Cell Survival
Binder_Spf
Binder_DgT
0.0
0.2
0.4
0.6
0.8
1.0

Figure 22B

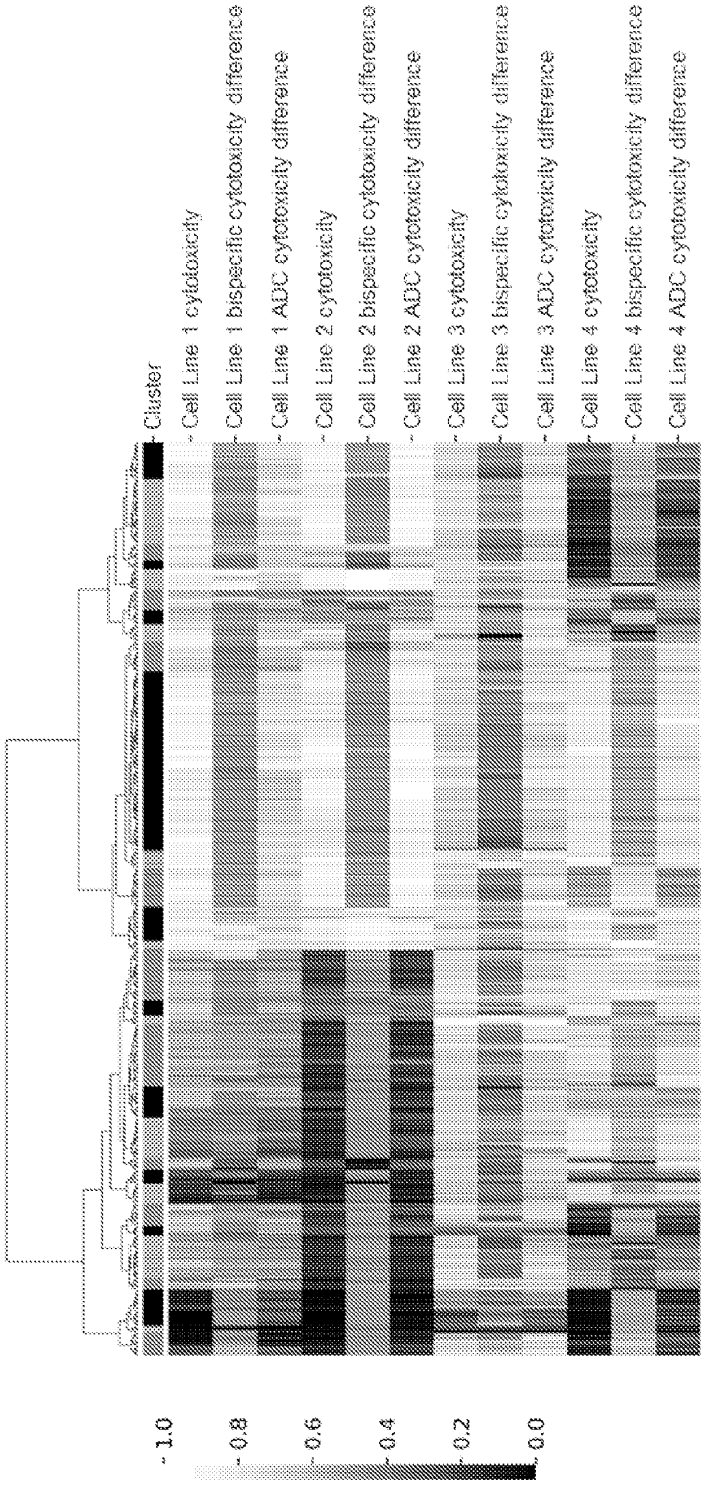

Cluster
Cell Line 1 cytotoxicity
Cell Line 1 bispecific cytotoxicity difference
Cell Line 1 ADC cytotoxicity difference
Cell Line 2 cytotoxicity
Cell Line 2 bispecific cytotoxicity difference
Cell Line 2 ADC cytotoxicity difference
Cell Line 3 cytotoxicity
Cell Line 3 bispecific cytotoxicity difference
Cell Line 3 ADC cytotoxicity difference
Cell Line 4 cytotoxicity
Cell Line 4 bispecific cytotoxicity difference
Cell Line 4 ADC cytotoxicity difference
1.0
0.8
0.6
0.4
0.2
0.0

Figure 23

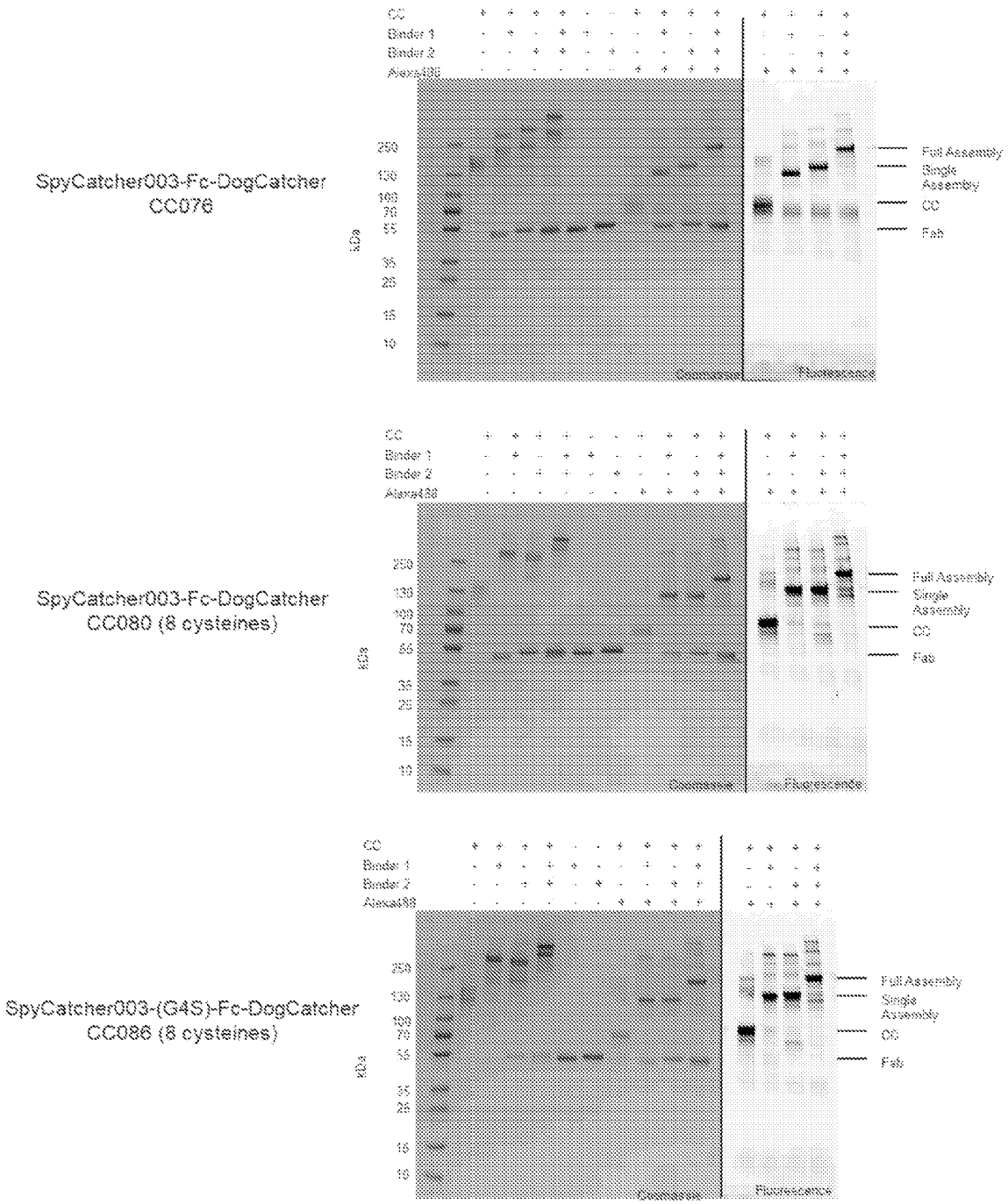

SpyCatcher003-Fc-DogCatcher CC076
SpyCatcher003-Fc-DogCatcher CC080 (8 cysteines)
SpyCatcher003-(G4S)-Fc-DogCatcher CC086 (8 cysteines)
CC
Binder 1
Binder 2
Alexa488
kDa
250
130
100
70
55
35
25
15
10
Coomassie
Fluorescence
Full Assembly
Single Assembly
CC
Fab

Figure 24

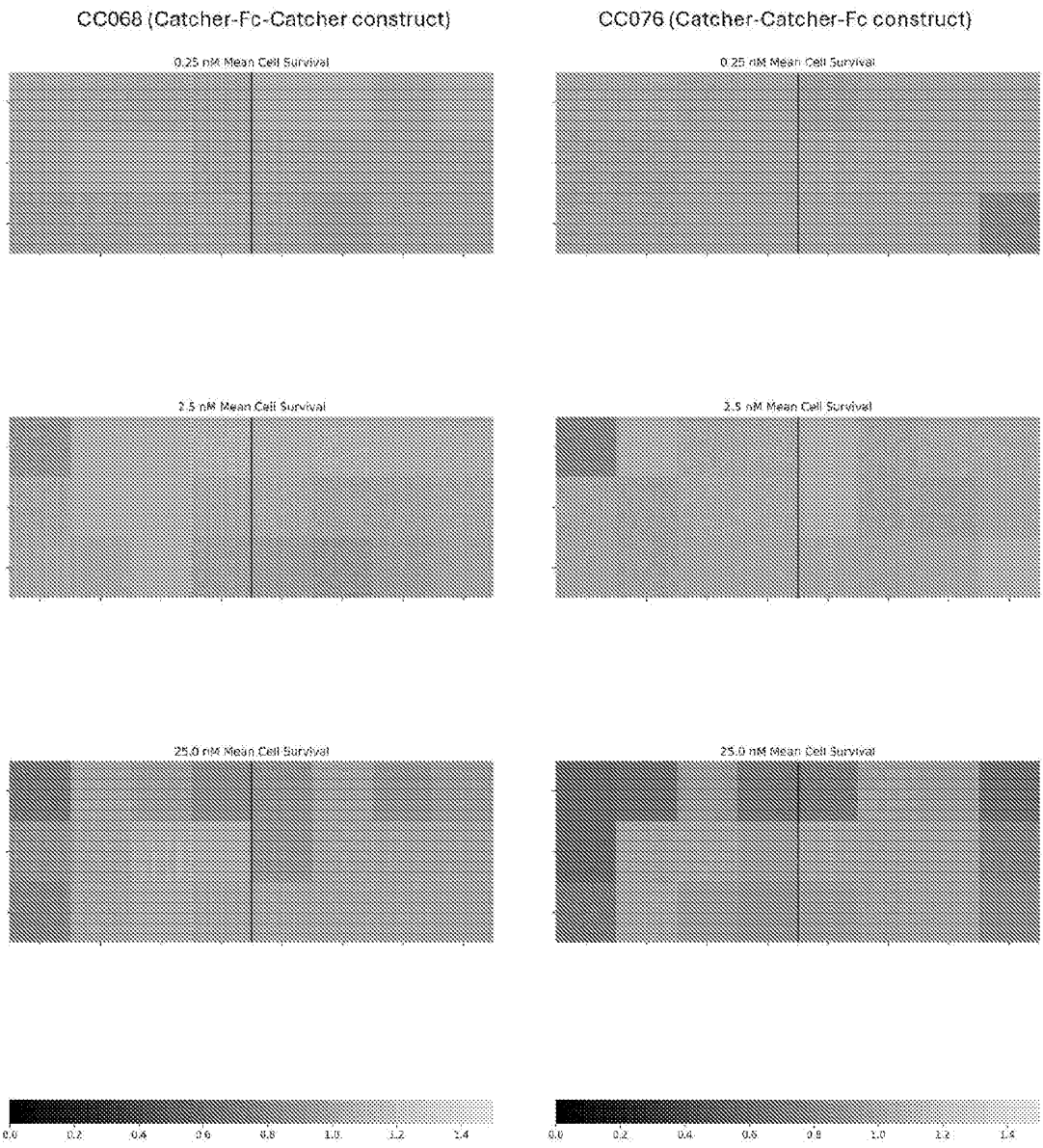

CC068 (Catcher-Fc-Catcher construct)
CC076 (Catcher-Catcher-Fc construct)
0.25 nM Mean Cell Survival
0.25 nM Mean Cell Survival
2.5 nM Mean Cell Survival
2.5 nM Mean Cell Survival
25.0 nM Mean Cell Survival
25.0 nM Mean Cell Survival
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4

Figure 25

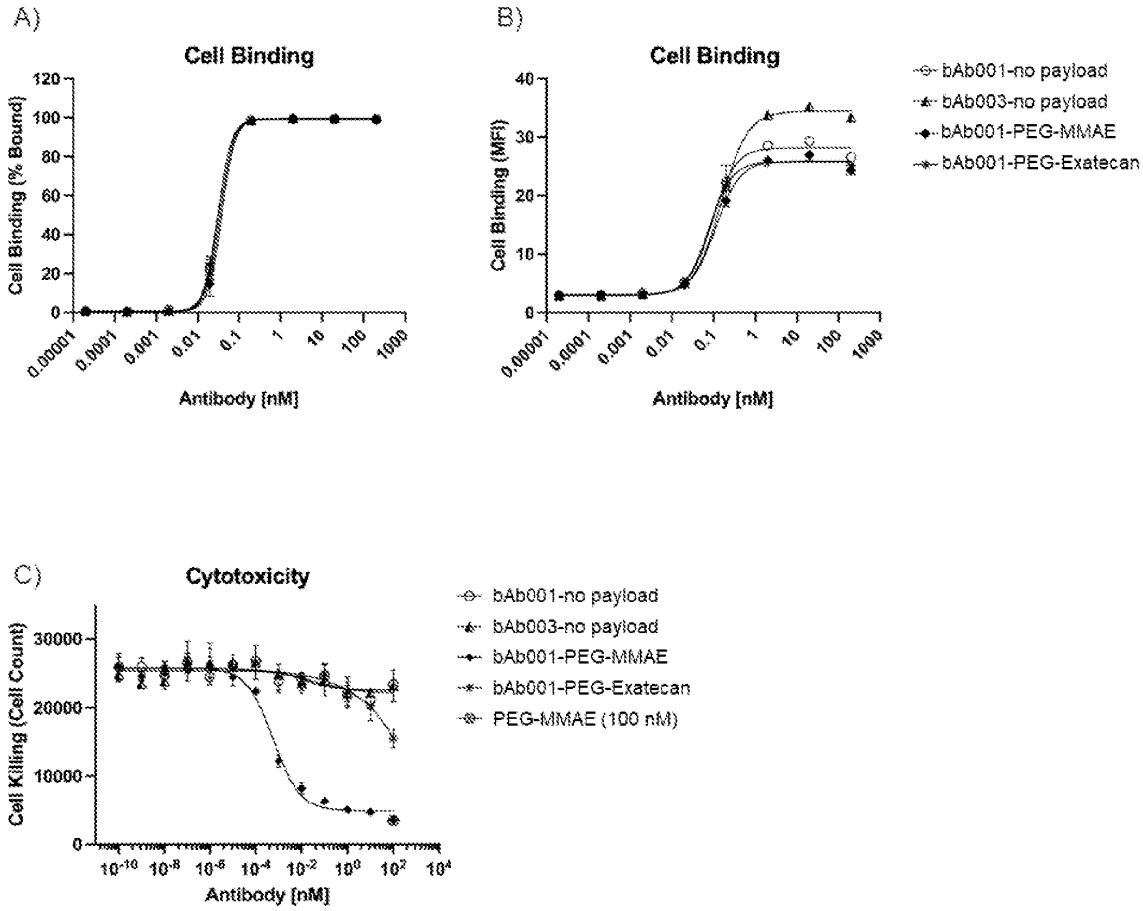

A)
Cell Binding
Cell Binding (% Bound)
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000
Antibody [nM]
B)
Cell Binding
Cell Binding (MFI)
40
30
20
10
0
Antibody [nM]
bAb001-no payload
bAb003-no payload
bAb001-PEG-MMAE
bAb001-PEG-Exatecan
C)
Cytotoxicity
Cell Killing (Cell Count)
30000
20000
10000
0
10^{-10} 10^{-8} 10^{-6} 10^{-4} 10^{-2} 10^{0} 10^{2} 10^{4}
Antibody [nM]
bAb001-no payload
bAb003-no payload
bAb001-PEG-MMAE
bAb001-PEG-Exatecan
PEG-MMAE (100 nM)

Figure 26