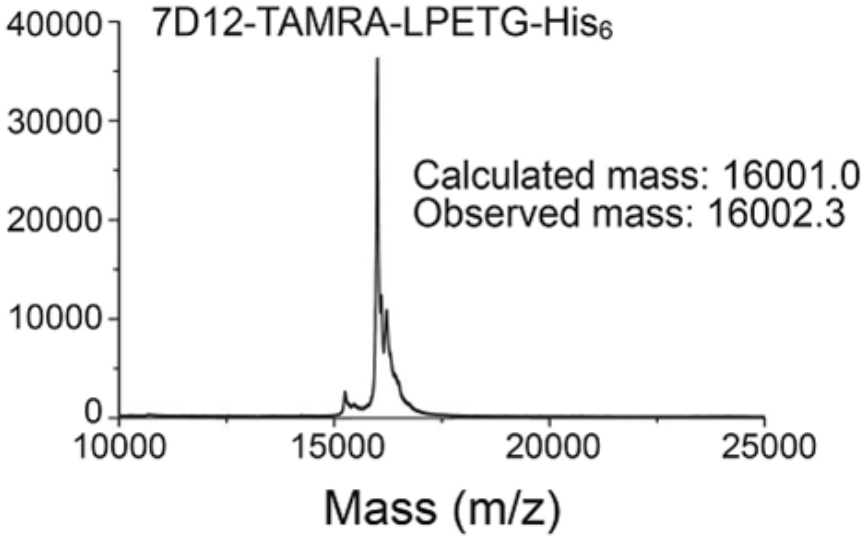

7D12-TAMRA-LPETG-His6
Calculated mass: 16001.0
Observed mass: 16002.3
40000
30000
20000
10000
0
10000
15000
20000
25000
Mass (m/z)

Fig. 1

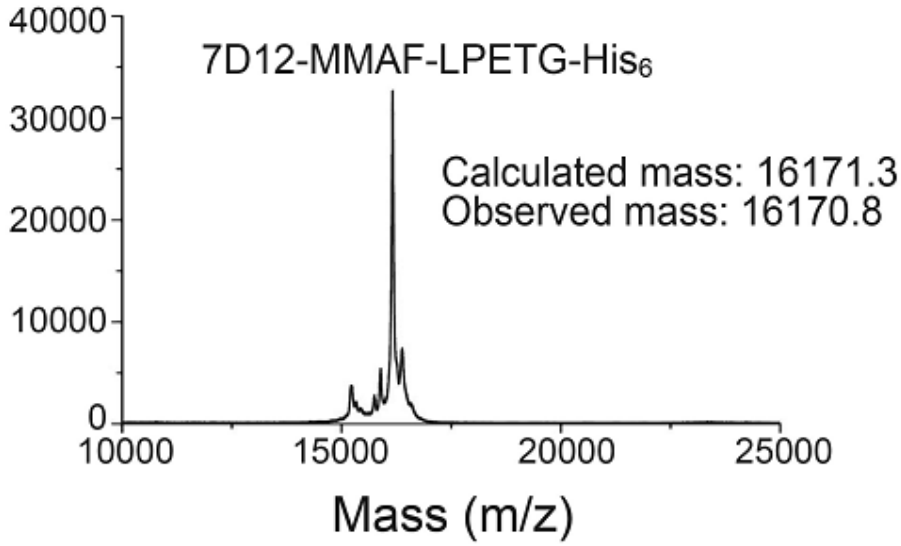

7D12-MMAF-LPETG-His6
Calculated mass: 16171.3
Observed mass: 16170.8
40000
30000
20000
10000
0
10000
15000
20000
25000
Mass (m/z)

Fig. 2

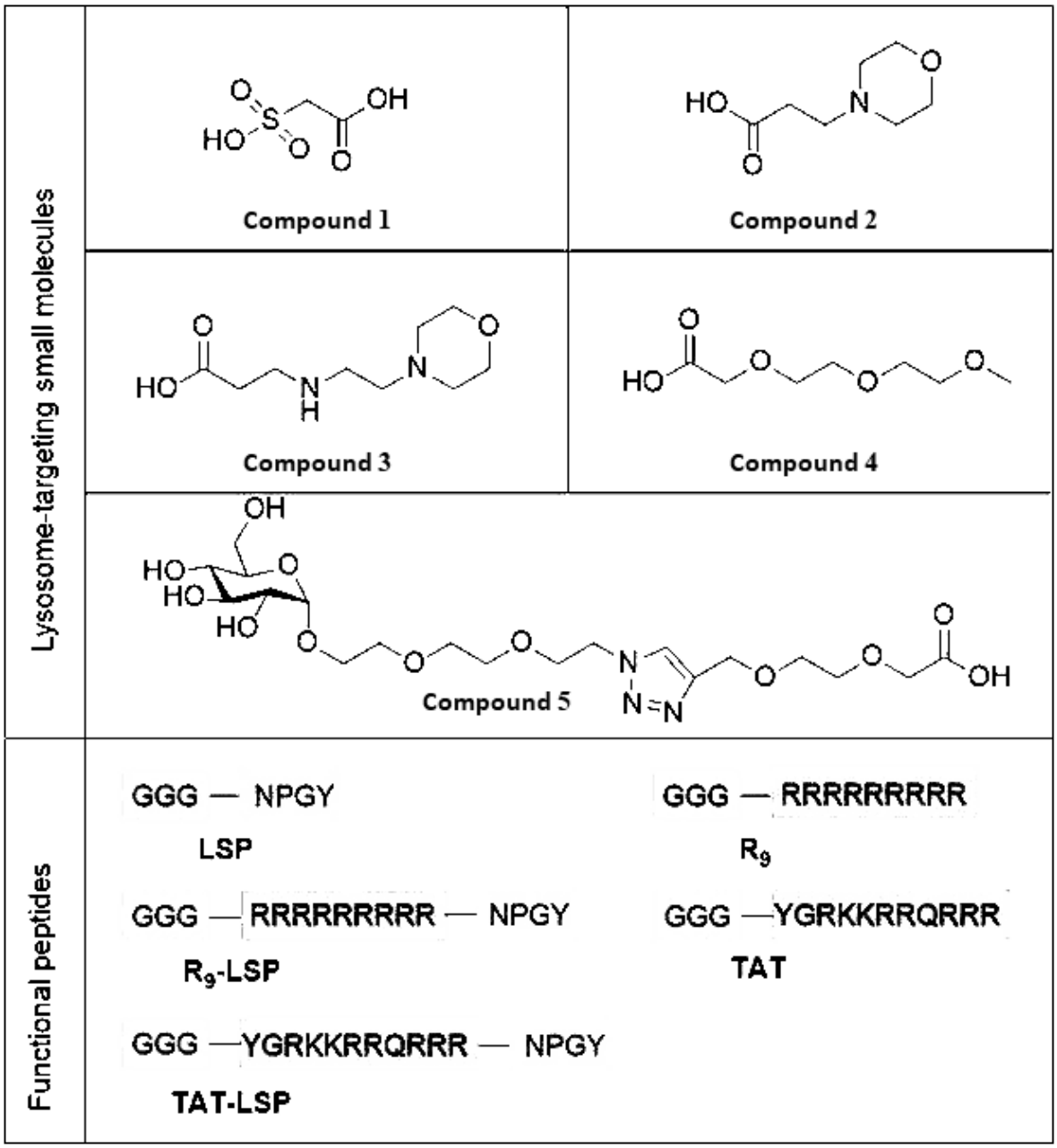

Lysosome-targeting small molecules
Compound 1
Compound 2
Compound 3
Compound 4
Compound 5
Functional peptides
GGG — NPGY
LSP
GGG — RRRRRRRRR
R9
GGG — RRRRRRRRR — NPGY
R9-LSP
GGG —YGRKKRRQRRR
TAT
GGG —YGRKKRRQRRR — NPGY
TAT-LSP

Fig. 3

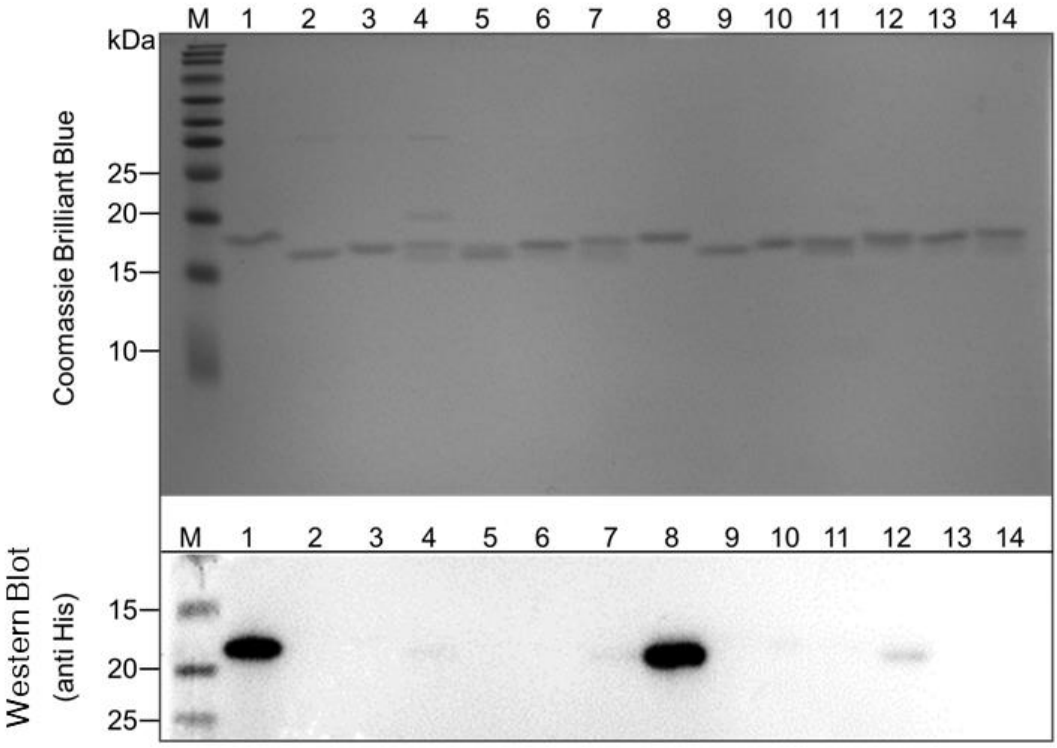

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14
kDa
25
20
15
10
Coomassie Brilliant Blue
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14
15
20
25
Western Blot
(anti His)

Fig. 4a

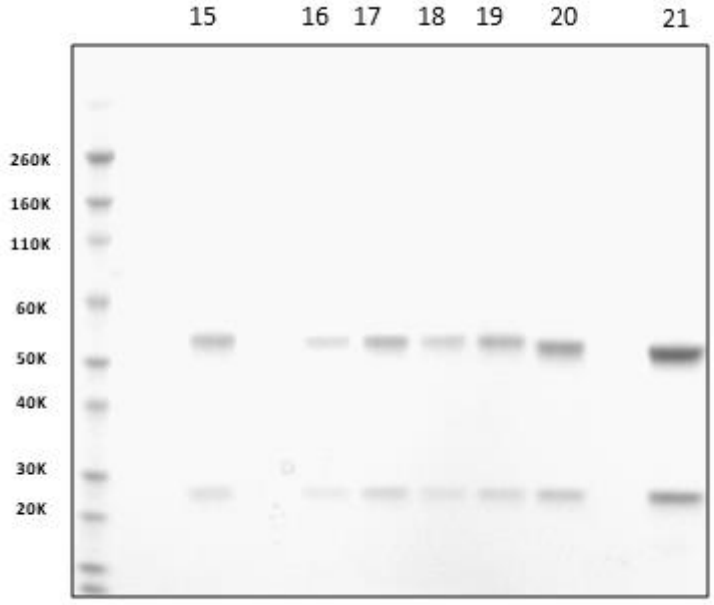

15 16 17 18 19 20 21
260K
160K
110K
60K
50K
40K
30K
20K

Fig. 4b

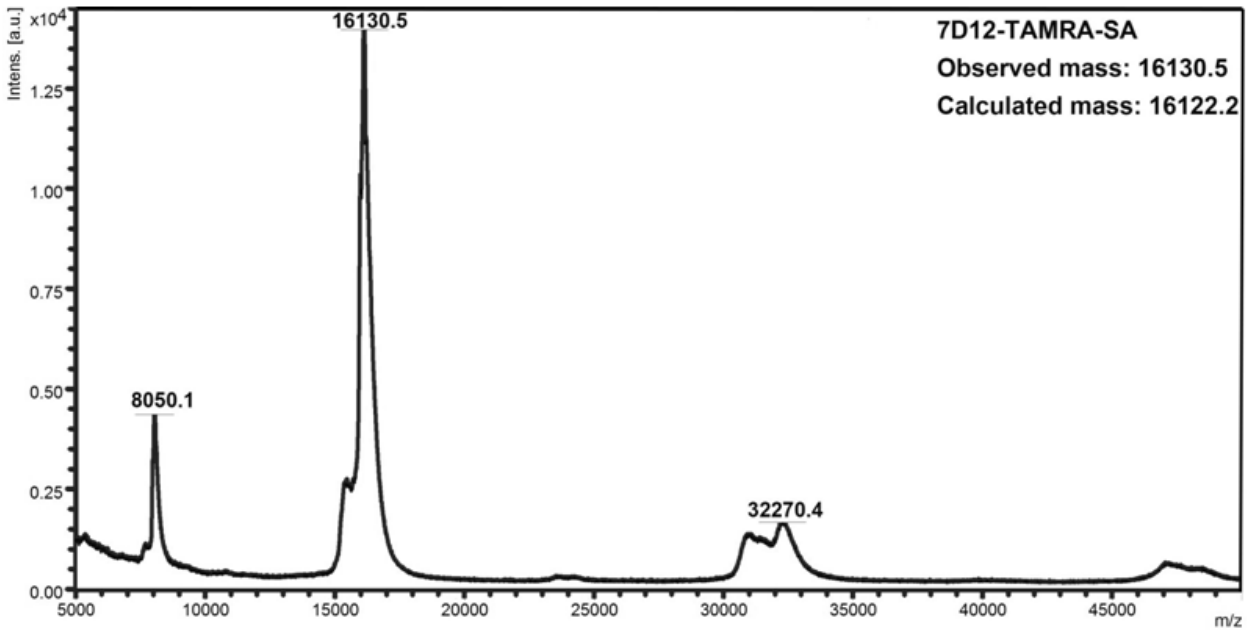

Intens. [a.u.]
x10^4
1.25
1.00
0.75
0.50
0.25
0.00
16130.5
8050.1
32270.4
7D12-TAMRA-SA
Observed mass: 16130.5
Calculated mass: 16122.2
5000
10000
15000
20000
25000
30000
35000
40000
45000
m/z

Fig. 5

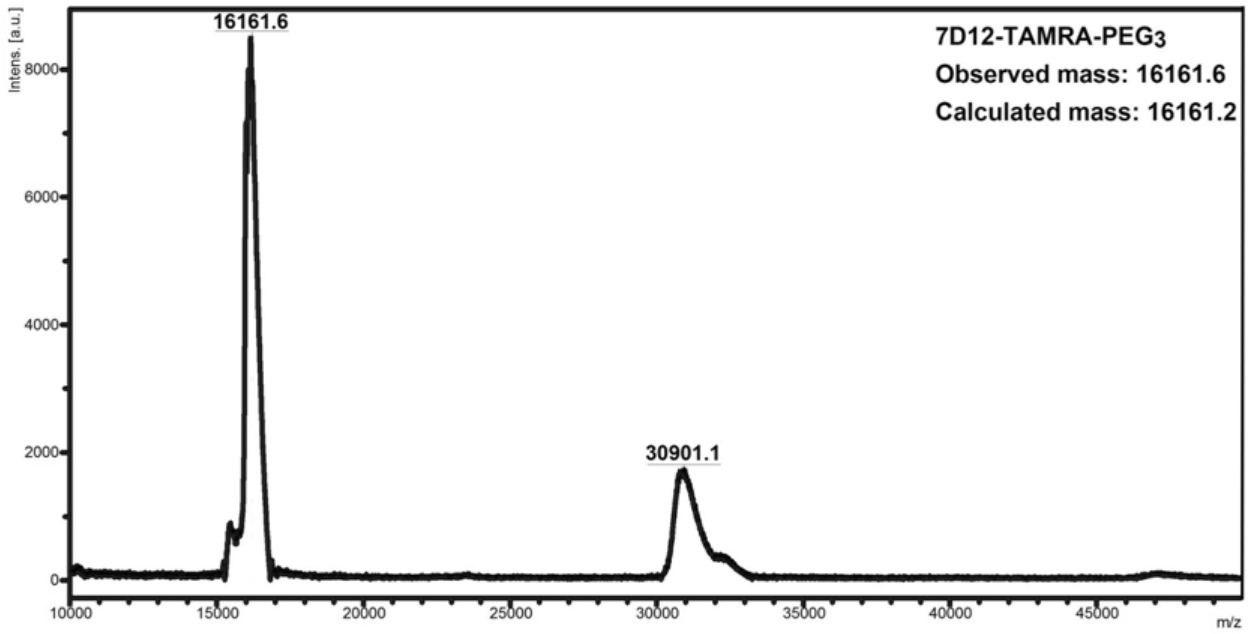

Intens. [a.u.]
8000
6000
4000
2000
0
16161.6
30901.1
7D12-TAMRA-PEG3
Observed mass: 16161.6
Calculated mass: 16161.2
10000
15000
20000
25000
30000
35000
40000
45000
m/z

Fig. 6

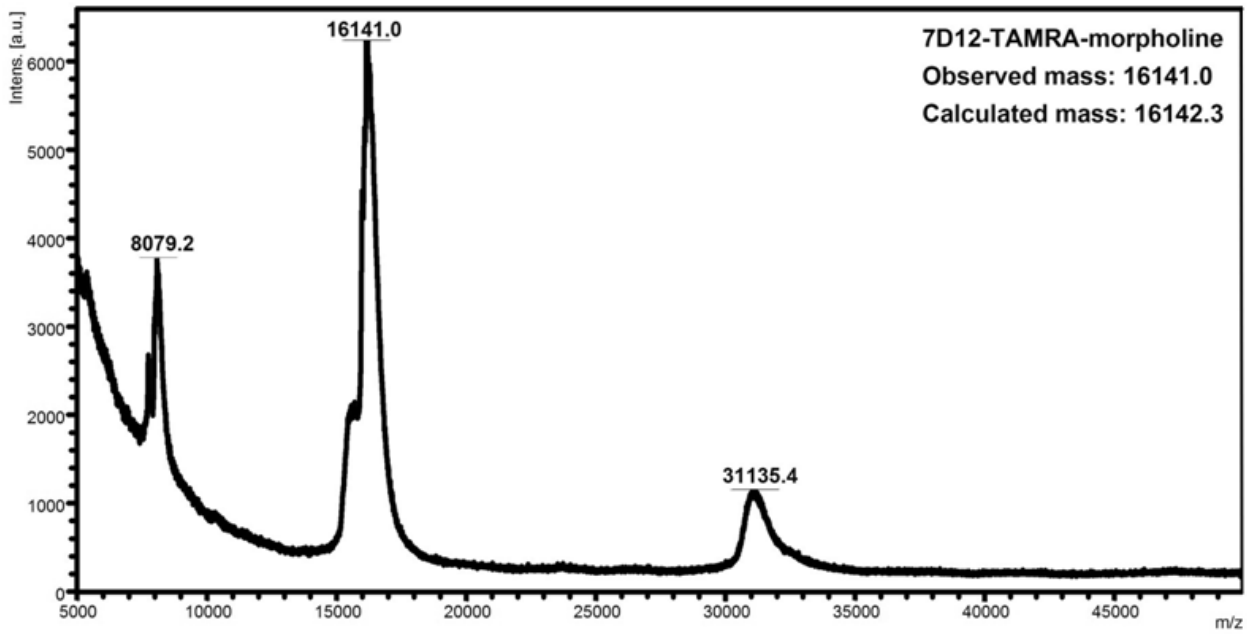

Intens. [a.u.]
6000
5000
4000
3000
2000
1000
0
16141.0
8079.2
31135.4
7D12-TAMRA-morpholine
Observed mass: 16141.0
Calculated mass: 16142.3
5000
10000
15000
20000
25000
30000
35000
40000
45000
m/z

Fig. 7

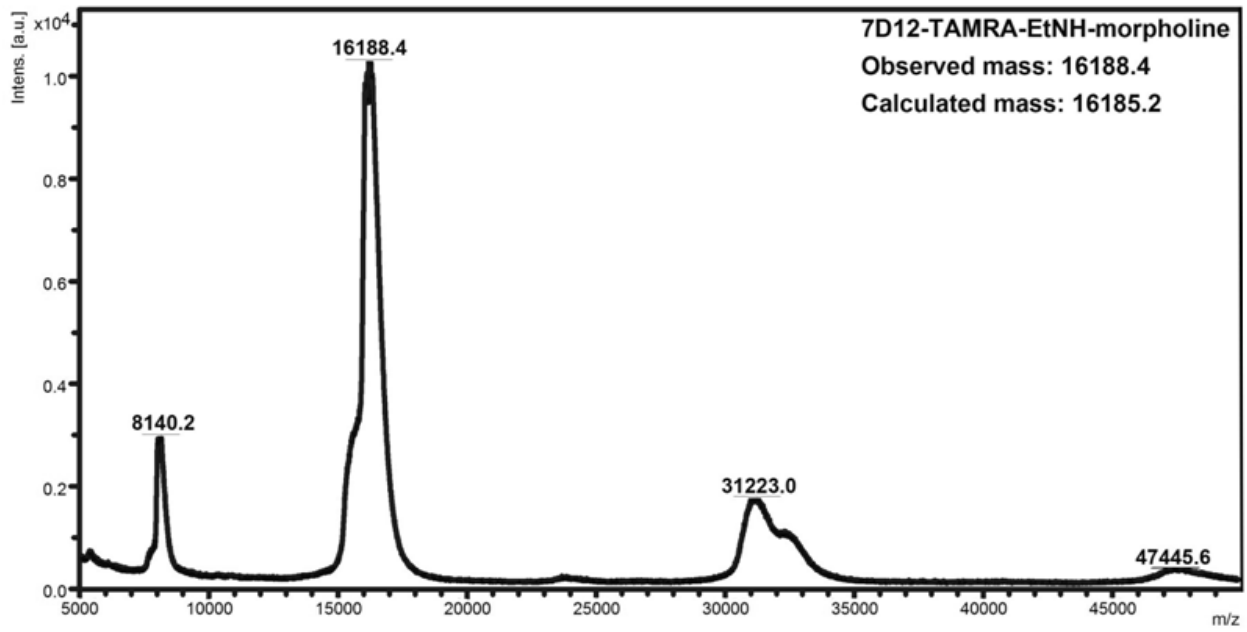

Intens. [a.u.]
x10^4
1.0
0.8
0.6
0.4
0.2
0.0
16188.4
8140.2
31223.0
47445.6
7D12-TAMRA-EtNH-morpholine
Observed mass: 16188.4
Calculated mass: 16185.2
5000
10000
15000
20000
25000
30000
35000
40000
45000
m/z

Fig. 8

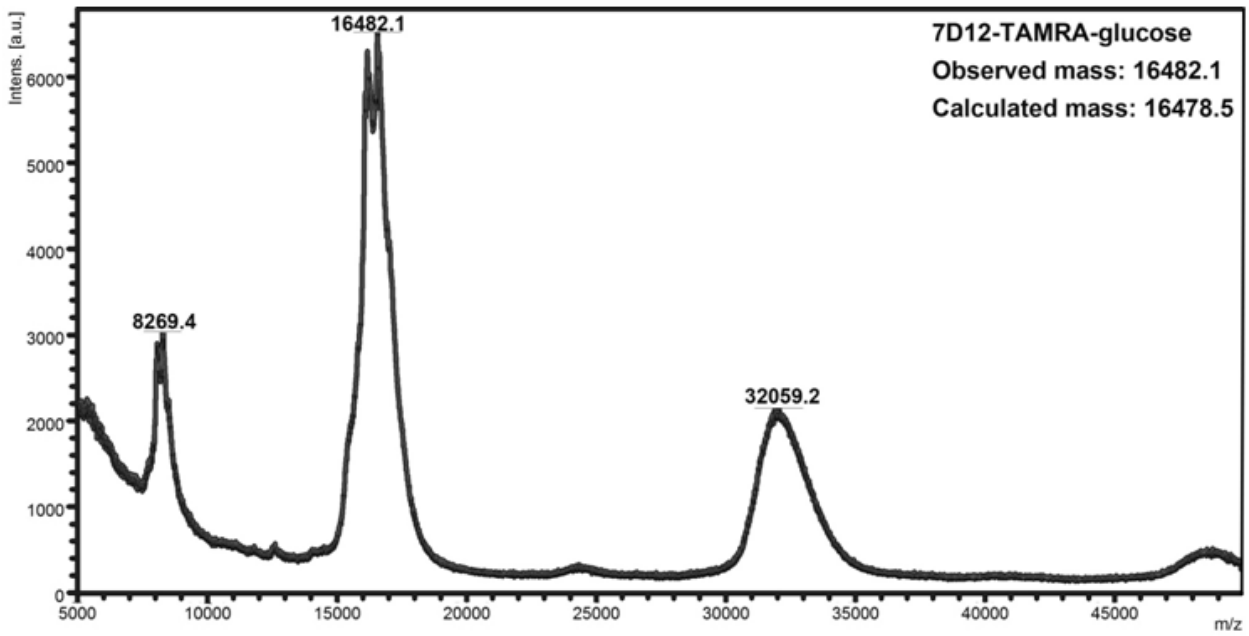

Intens. [a.u.]
16482.1
7D12-TAMRA-glucose
Observed mass: 16482.1
Calculated mass: 16478.5
8269.4
32059.2
m/z

Fig. 9

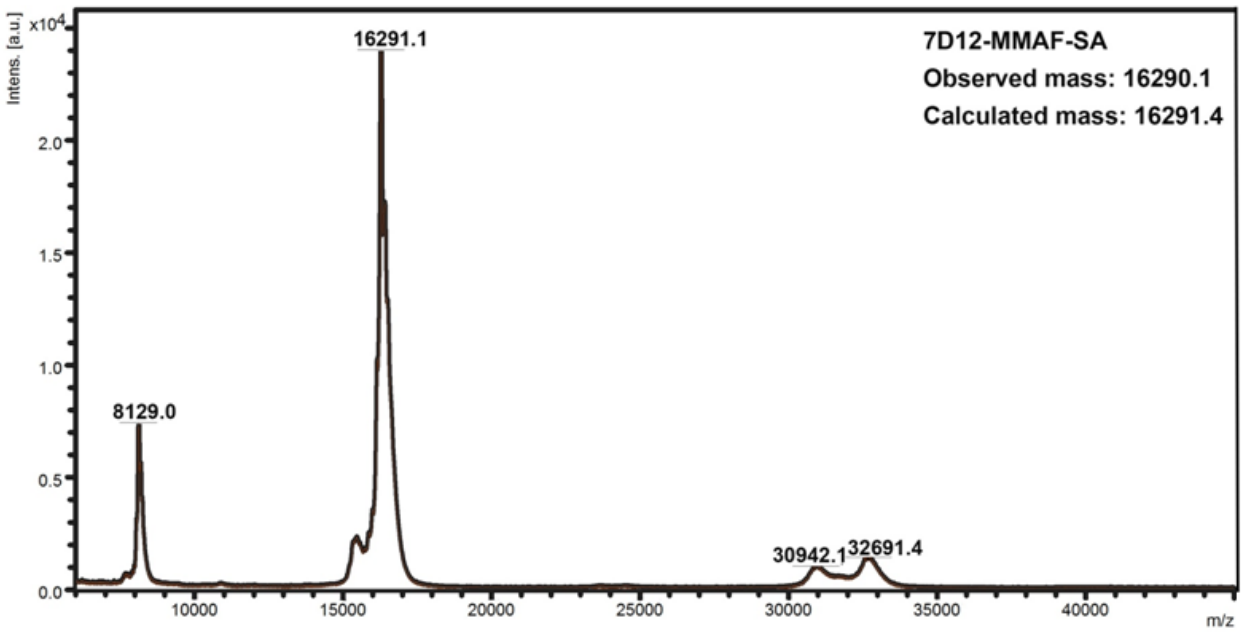

Intens. [a.u.]
x10^4
16291.1
7D12-MMAF-SA
Observed mass: 16290.1
Calculated mass: 16291.4
8129.0
30942.1
32691.4
m/z

Fig. 10

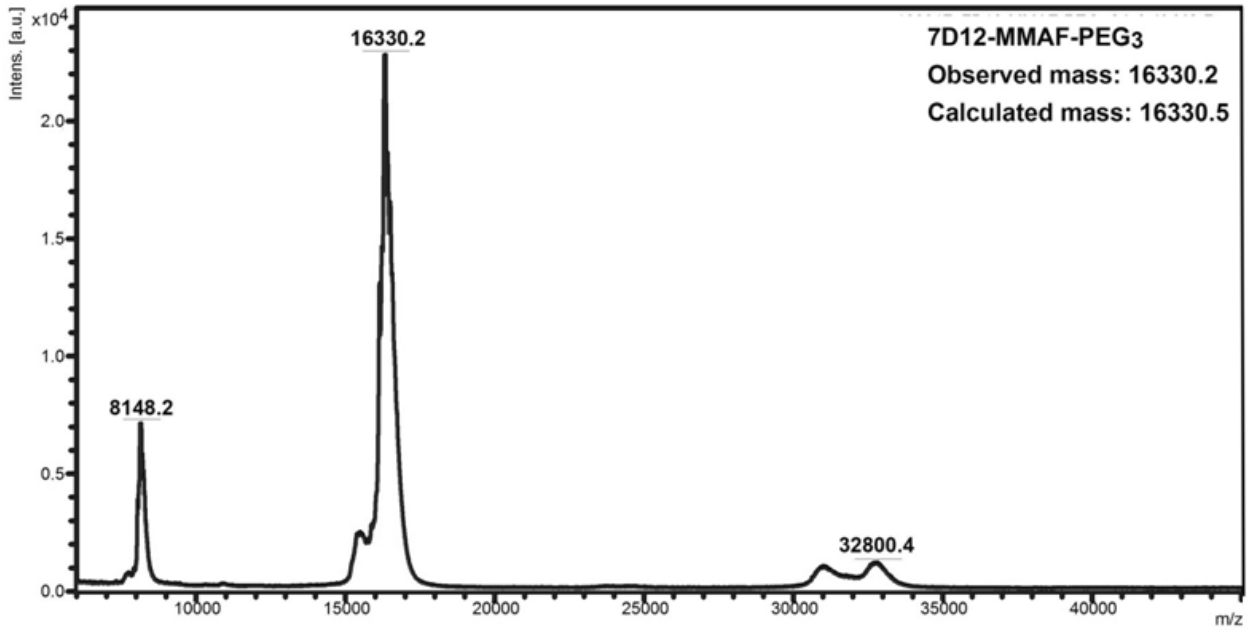

Intens. [a.u.]
x10^4
2.0
1.5
1.0
0.5
0.0
16330.2
8148.2
32800.4
7D12-MMAF-PEG3
Observed mass: 16330.2
Calculated mass: 16330.5
10000
15000
20000
25000
30000
35000
40000
m/z

Fig. 11

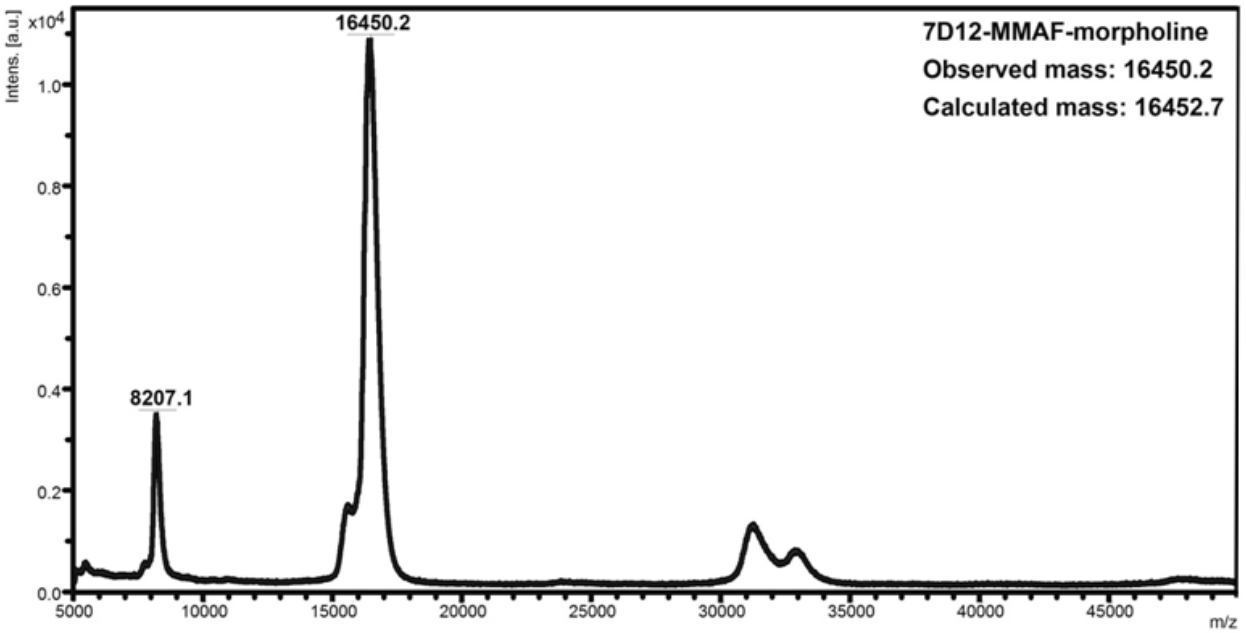

Intens. [a.u.]
x10^4
1.0
0.8
0.6
0.4
0.2
0.0
16450.2
8207.1
7D12-MMAF-morpholine
Observed mass: 16450.2
Calculated mass: 16452.7
5000
10000
15000
20000
25000
30000
35000
40000
45000
m/z

Fig. 12

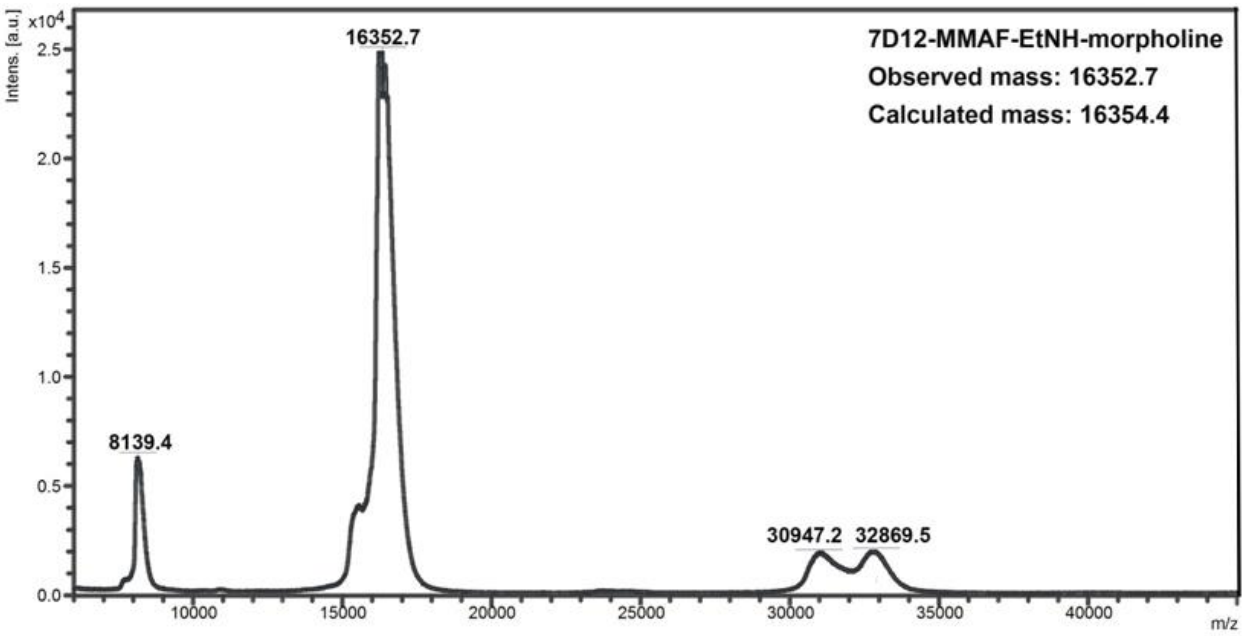

Intens. [a.u.]
x10^4
2.5
2.0
1.5
1.0
0.5
0.0
16352.7
8139.4
30947.2
32869.5
7D12-MMAF-EtNH-morpholine
Observed mass: 16352.7
Calculated mass: 16354.4
10000
15000
20000
25000
30000
35000
40000
m/z

Fig. 13

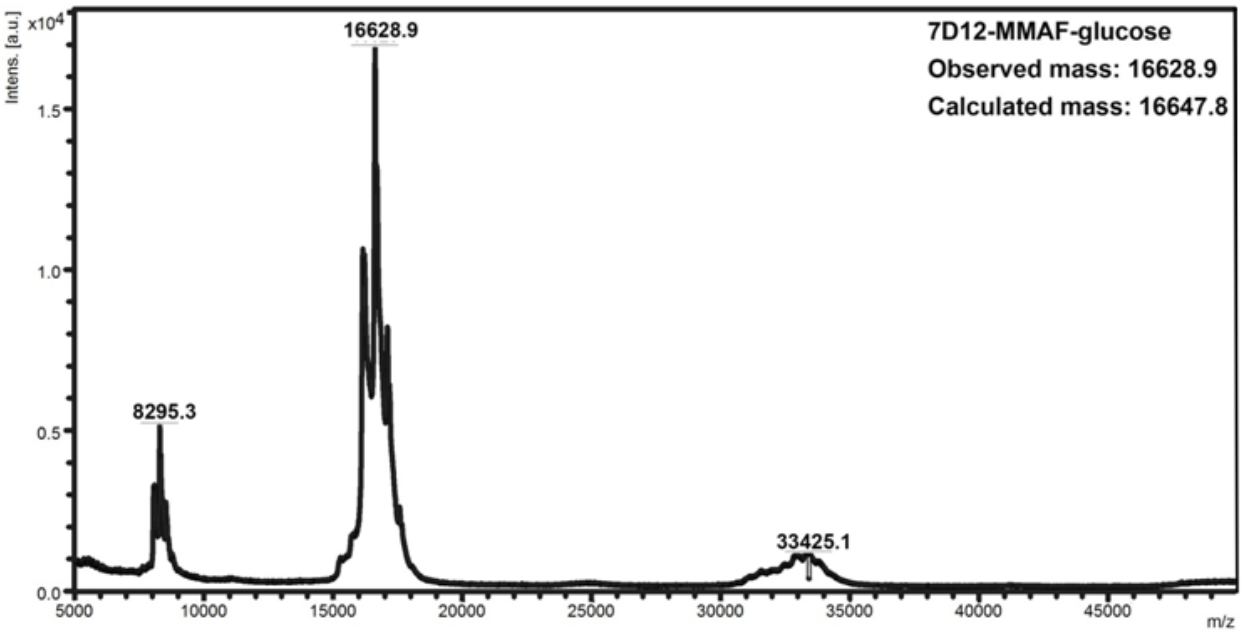

Intens. [a.u.]
x10^4
1.5
1.0
0.5
0.0
16628.9
8295.3
33425.1
7D12-MMAF-glucose
Observed mass: 16628.9
Calculated mass: 16647.8
5000
10000
15000
20000
25000
30000
35000
40000
45000
m/z

Fig. 14

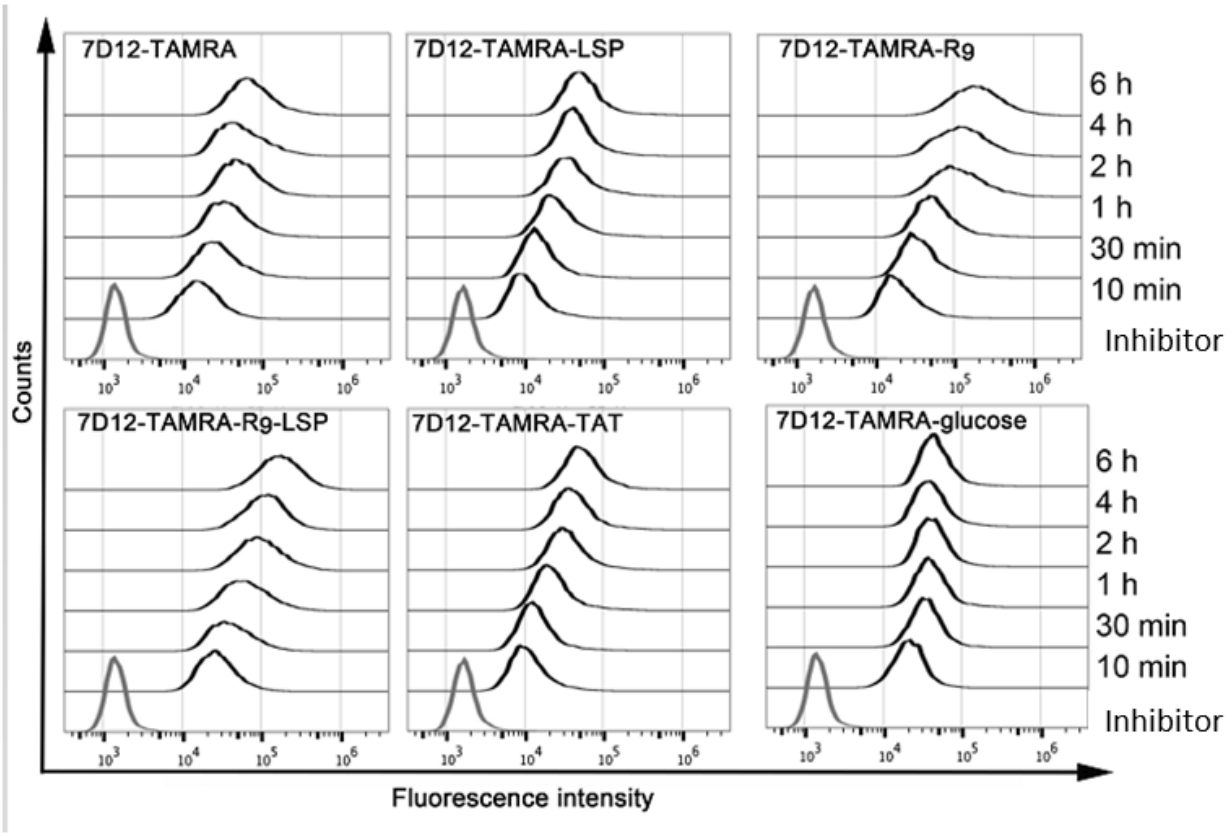

7D12-TAMRA
7D12-TAMRA-LSP
7D12-TAMRA-R9
6 h
4 h
2 h
1 h
30 min
10 min
Inhibitor
7D12-TAMRA-R9-LSP
7D12-TAMRA-TAT
7D12-TAMRA-glucose
6 h
4 h
2 h
1 h
30 min
10 min
Inhibitor
Counts
Fluorescence intensity

Fig. 15

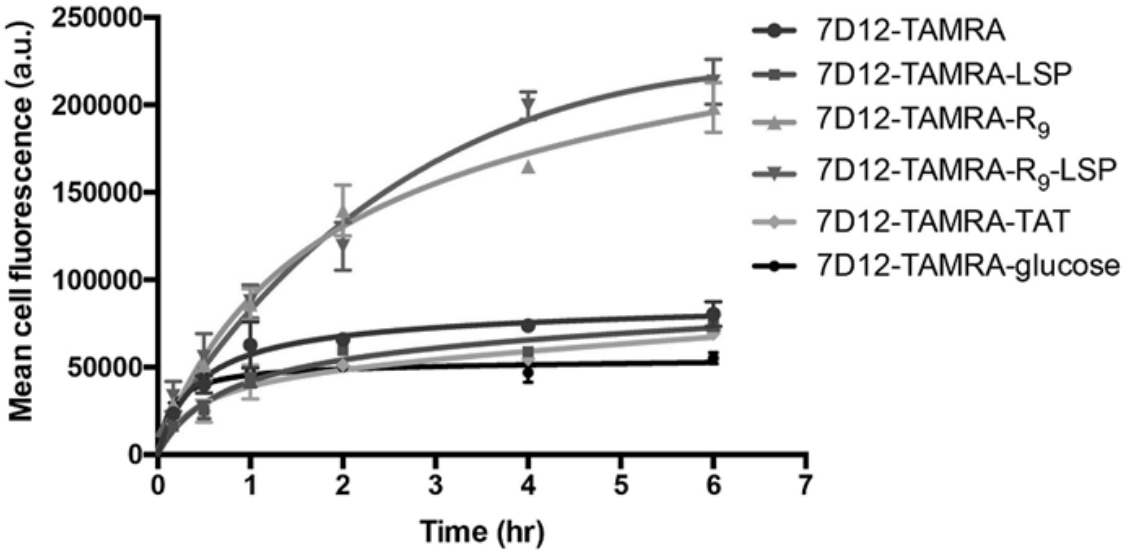

Mean cell fluorescence (a.u.)
250000
200000
150000
100000
50000
0
0
1
2
3
4
5
6
7
Time (hr)
7D12-TAMRA
7D12-TAMRA-LSP
7D12-TAMRA-R9
7D12-TAMRA-R9-LSP
7D12-TAMRA-TAT
7D12-TAMRA-glucose

Fig. 16

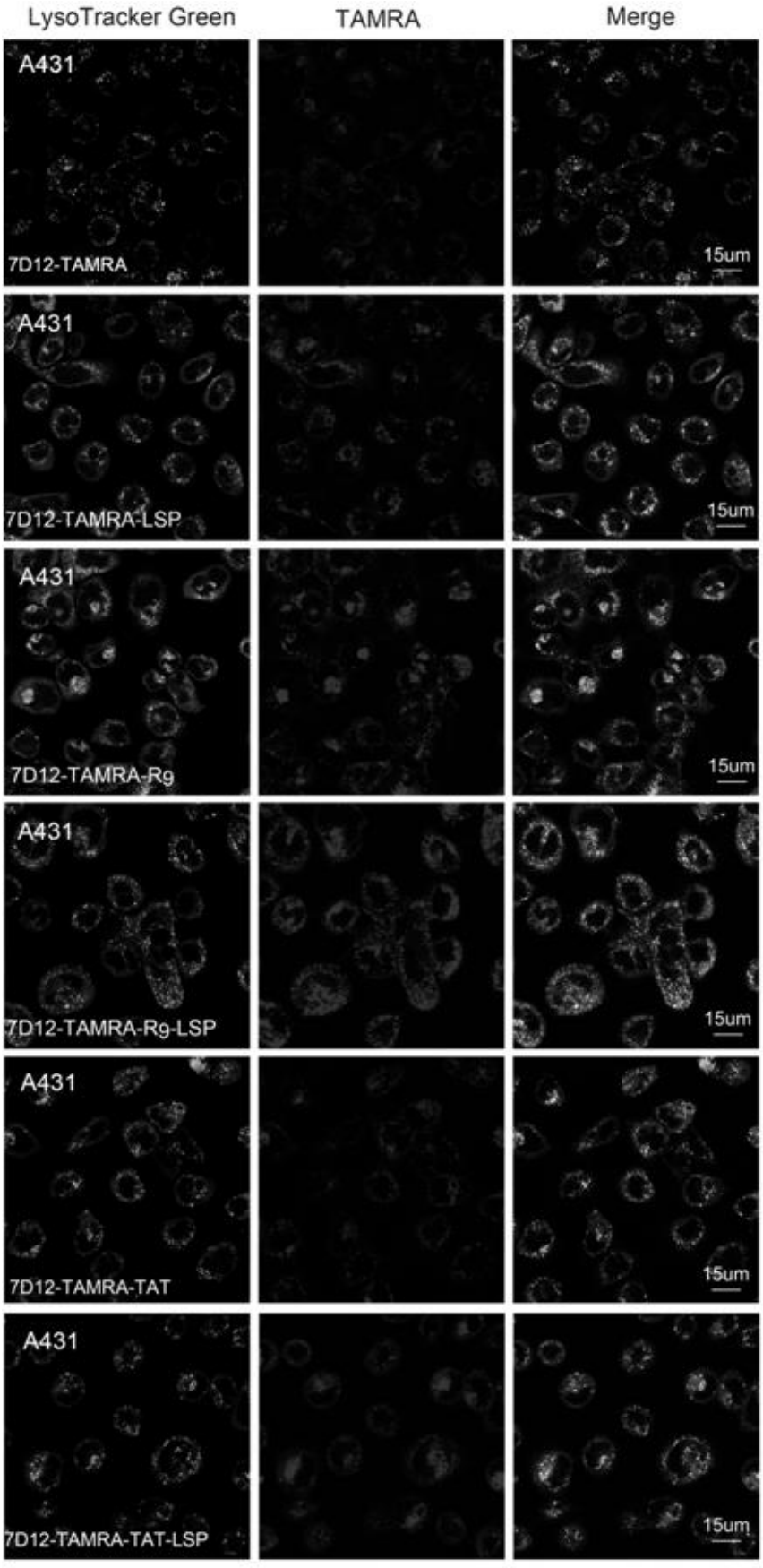

LysoTracker Green
TAMRA
Merge
A431
7D12-TAMRA
15um
A431
7D12-TAMRA-LSP
15um
A431
7D12-TAMRA-R9
15um
A431
7D12-TAMRA-R9-LSP
15um
A431
7D12-TAMRA-TAT
15um
A431
7D12-TAMRA-TAT-LSP
15um

Fig. 17a

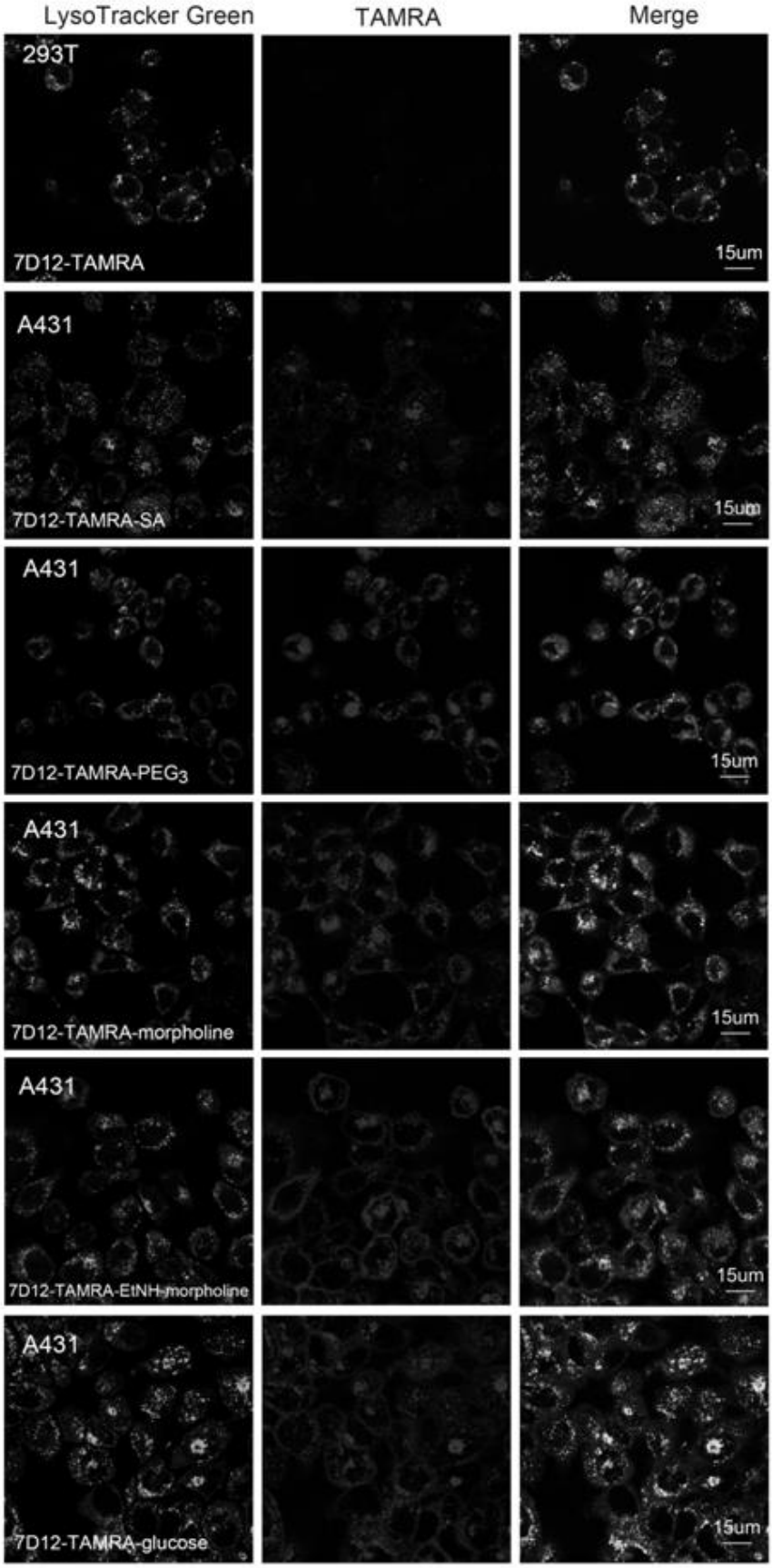

LysoTracker Green
TAMRA
Merge
293T
7D12-TAMRA
15um
A431
7D12-TAMRA-SA
15um
A431
7D12-TAMRA-PEG3
15um
A431
7D12-TAMRA-morpholine
15um
A431
7D12-TAMRA-EtNH-morpholine
15um
A431
7D12-TAMRA-glucose
15um

Fig. 17b

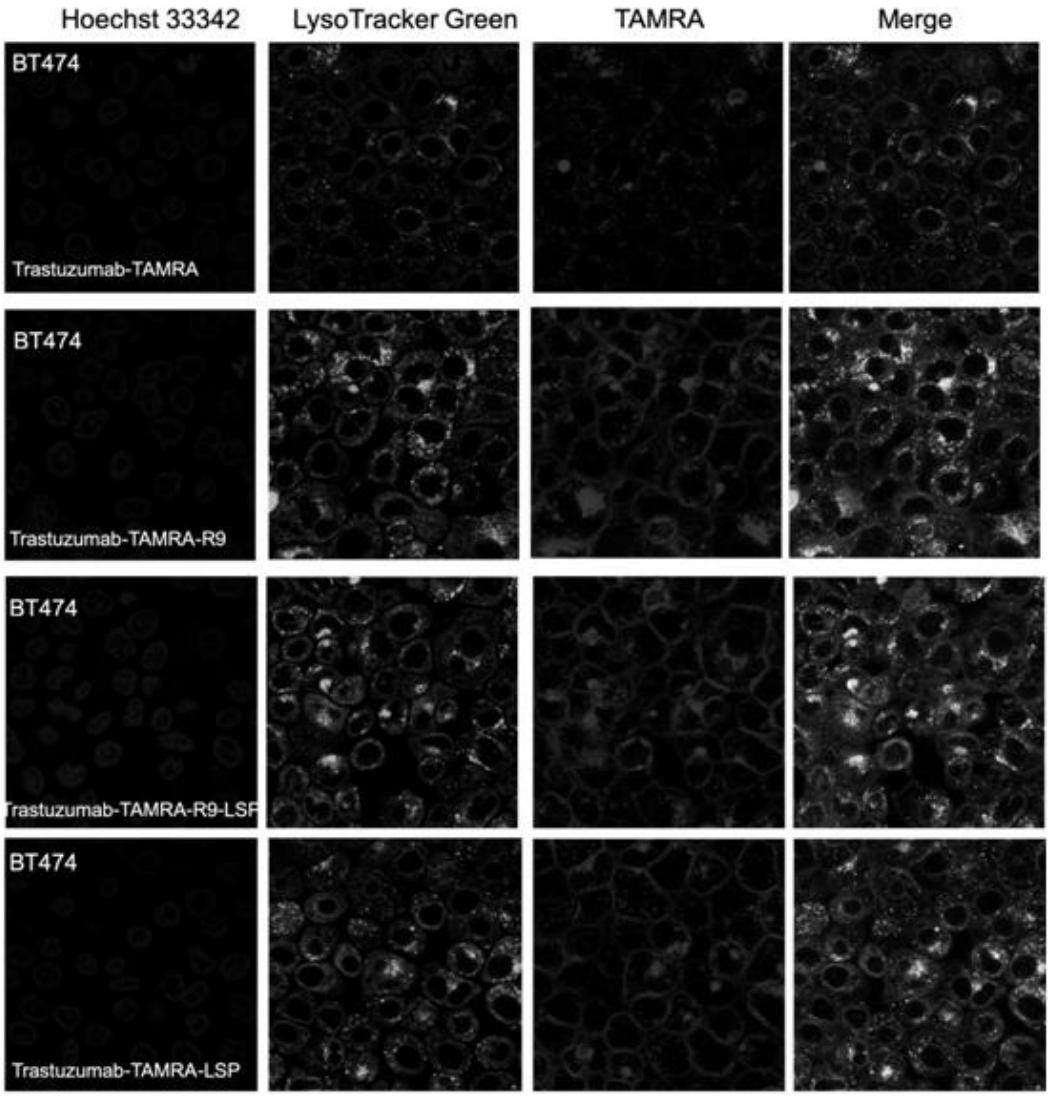

Hoechst 33342
LysoTracker Green
TAMRA
Merge
BT474
Trastuzumab-TAMRA
BT474
Trastuzumab-TAMRA-R9
BT474
Trastuzumab-TAMRA-R9-LSP
BT474
Trastuzumab-TAMRA-LSP

Fig. 17c

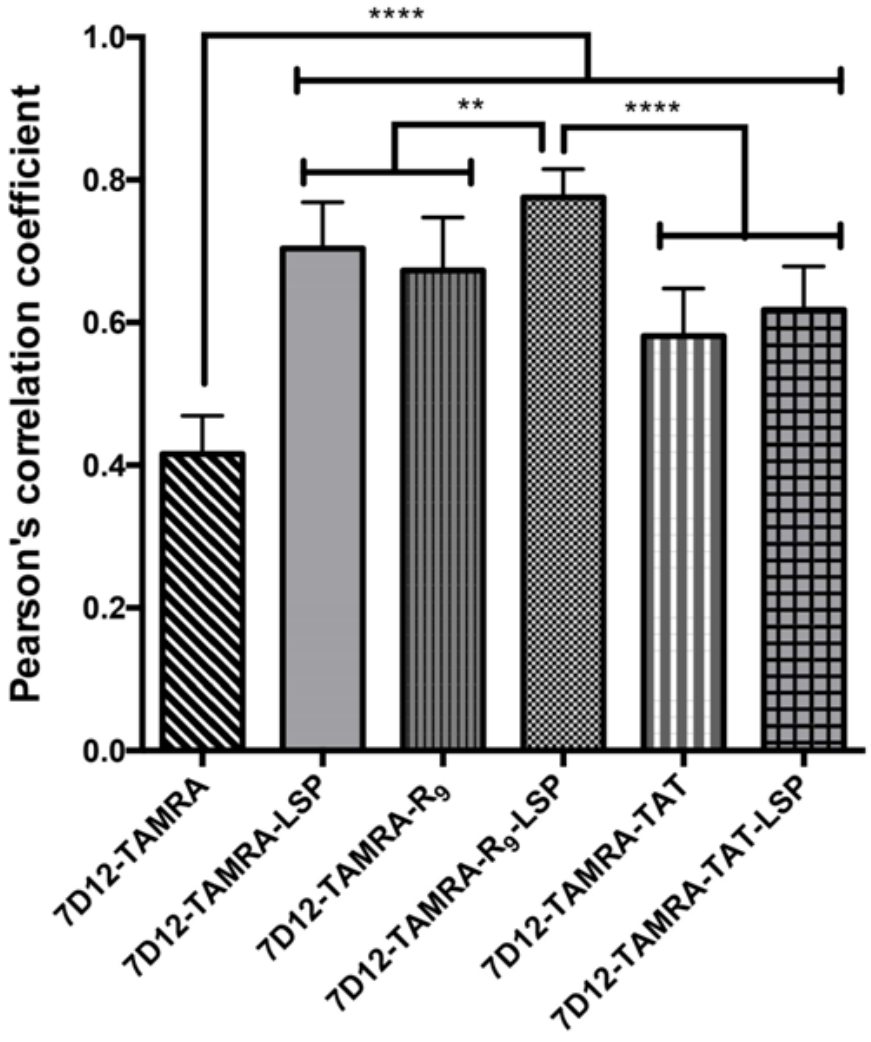

****
**
****
1.0
0.8
0.6
0.4
0.2
0.0
Pearson's correlation coefficient
7D12-TAMRA
7D12-TAMRA-LSP
7D12-TAMRA-R9
7D12-TAMRA-R9-LSP
7D12-TAMRA-TAT
7D12-TAMRA-TAT-LSP

Fig. 18a

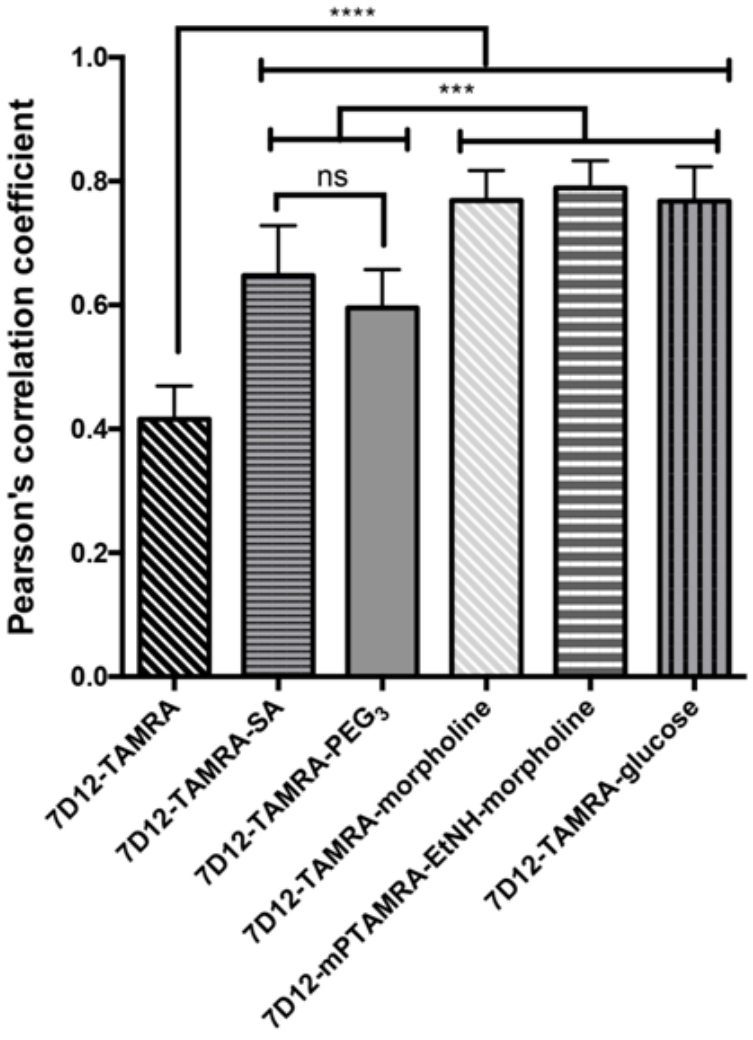

****
***
ns
1.0
0.8
0.6
0.4
0.2
0.0
Pearson's correlation coefficient
7D12-TAMRA
7D12-TAMRA-SA
7D12-TAMRA-PEG3
7D12-TAMRA-morpholine
7D12-mPTAMRA-EtNH-morpholine
7D12-TAMRA-glucose

Fig. 18b

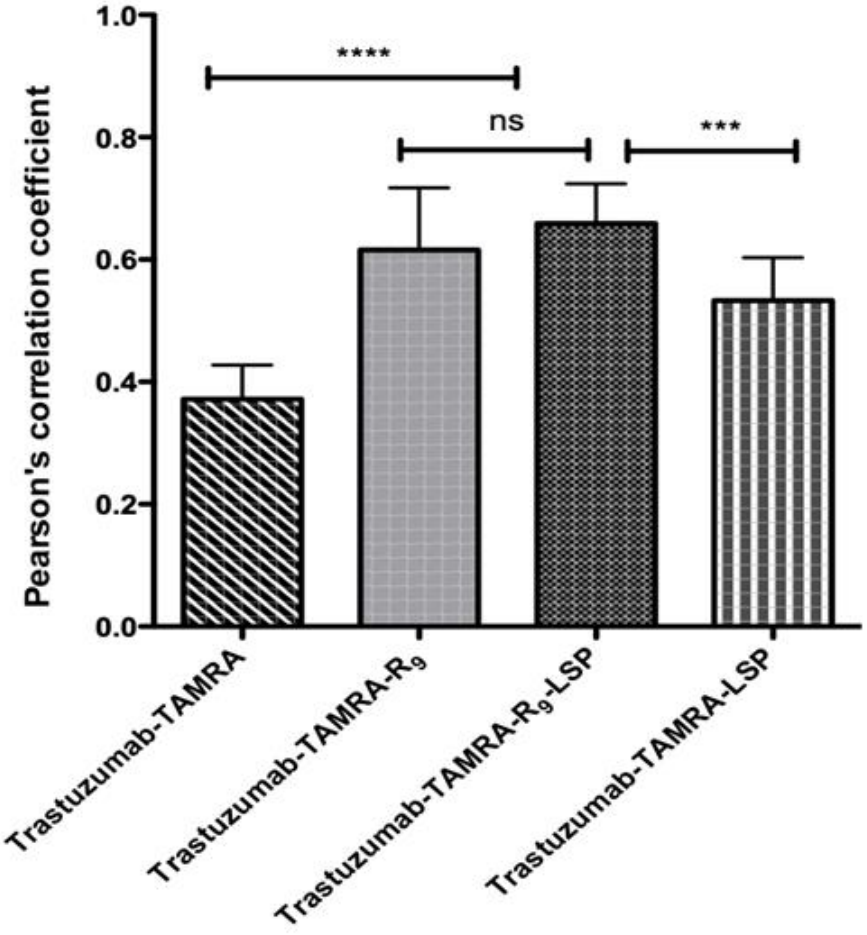

Pearson's correlation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
****
ns
***
Trastuzumab-TAMRA
Trastuzumab-TAMRA-Rg
Trastuzumab-TAMRA-Rg-LSP
Trastuzumab-TAMRA-LSP

Fig. 18c

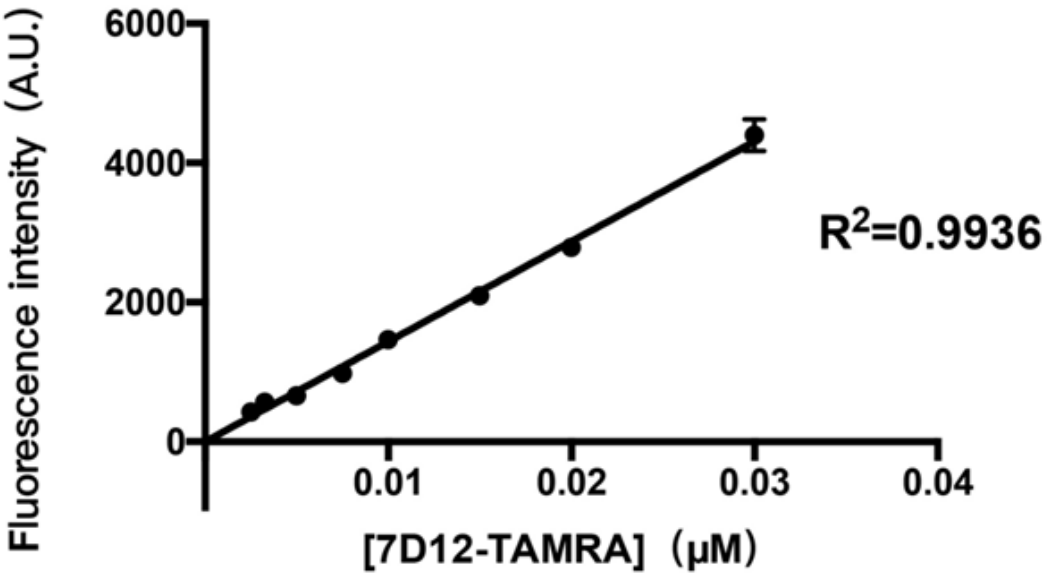


Fig. 19a

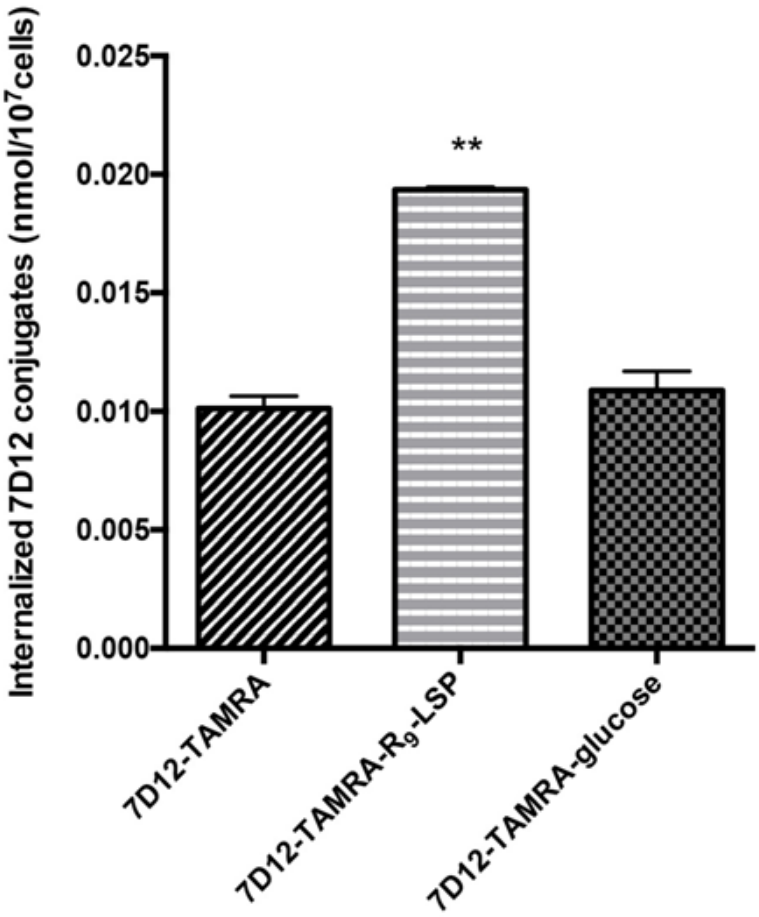


Fig. 19b

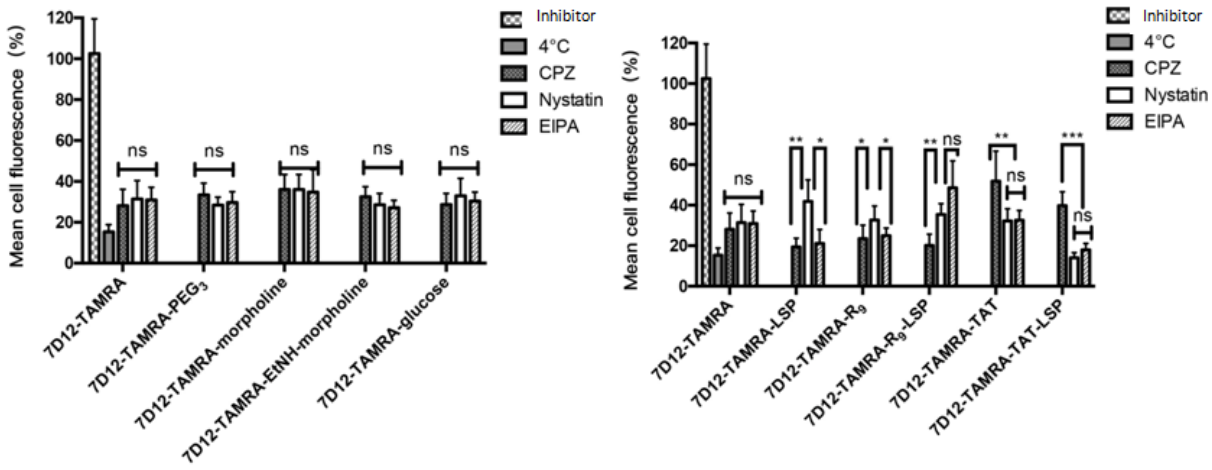

Mean cell fluorescence (%)
120
100
80
60
40
20
0
Inhibitor
4°C
CPZ
Nystatin
EIPA
ns
7D12-TAMRA
7D12-TAMRA-PEG3
7D12-TAMRA-morpholine
7D12-TAMRA-EtNH-morpholine
7D12-TAMRA-glucose
Mean cell fluorescence (%)
**
*
ns
***
7D12-TAMRA
7D12-TAMRA-LSP
7D12-TAMRA-R9
7D12-TAMRA-R9-LSP
7D12-TAMRA-TAT
7D12-TAMRA-TAT-LSP

Fig. 20

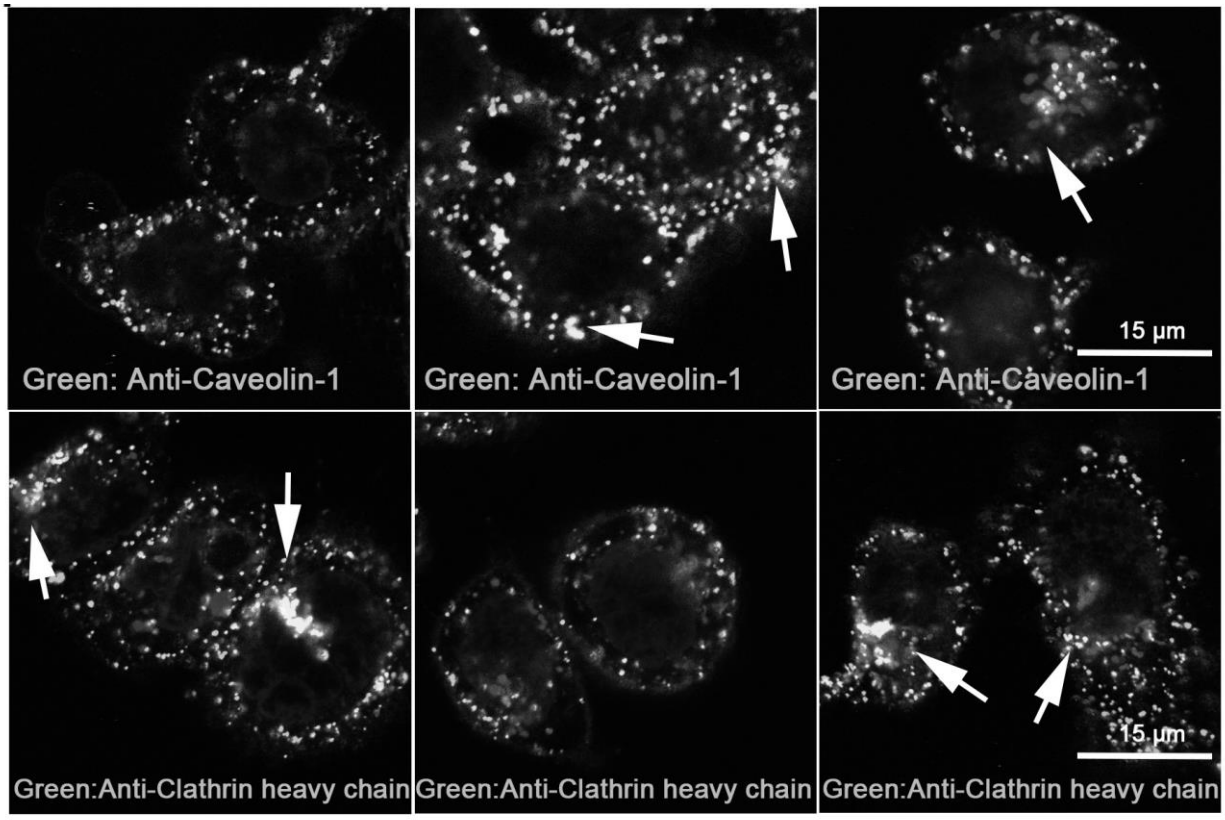

Green: Anti-Caveolin-1
Green: Anti-Caveolin-1
Green: Anti-Caveolin-1
15 μm
Green:Anti-Clathrin heavy chain
Green:Anti-Clathrin heavy chain
Green:Anti-Clathrin heavy chain
15 μm

Fig. 21

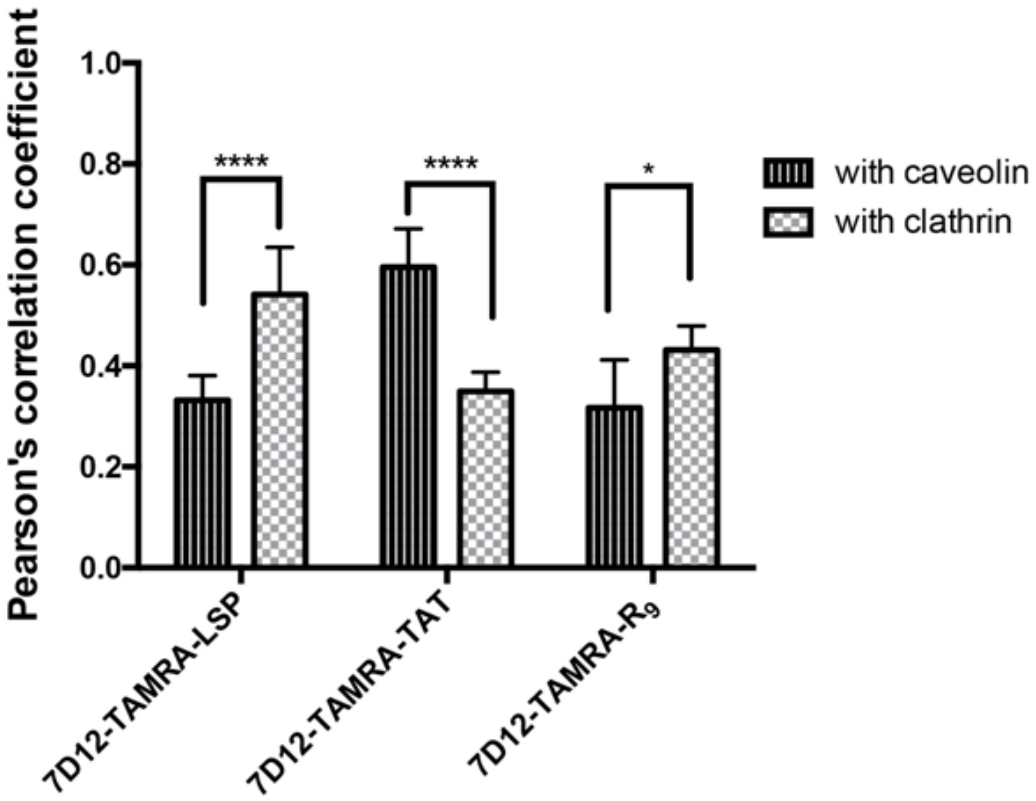

Pearson's correlation coefficient
1.0
0.8
0.6
0.4
0.2
0.0
****
****
*
with caveolin
with clathrin
7D12-TAMRA-LSP
7D12-TAMRA-TAT
7D12-TAMRA-R9

Fig. 22

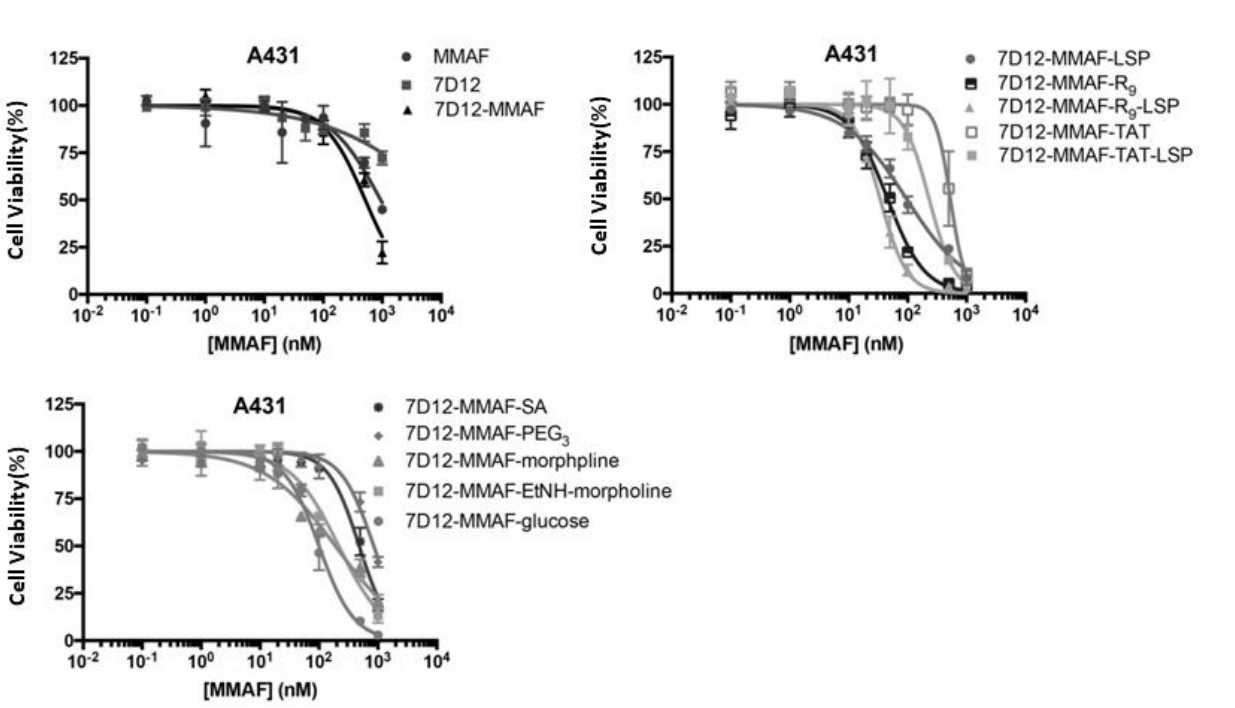

A431
MMAF
7D12
7D12-MMAF
Cell Viability(%)
[MMAF] (nM)
A431
7D12-MMAF-LSP
7D12-MMAF-R9
7D12-MMAF-R9-LSP
7D12-MMAF-TAT
7D12-MMAF-TAT-LSP
Cell Viability(%)
[MMAF] (nM)
A431
7D12-MMAF-SA
7D12-MMAF-PEG3
7D12-MMAF-morphpline
7D12-MMAF-EtNH-morpholine
7D12-MMAF-glucose
Cell Viability(%)
[MMAF] (nM)

Fig. 23

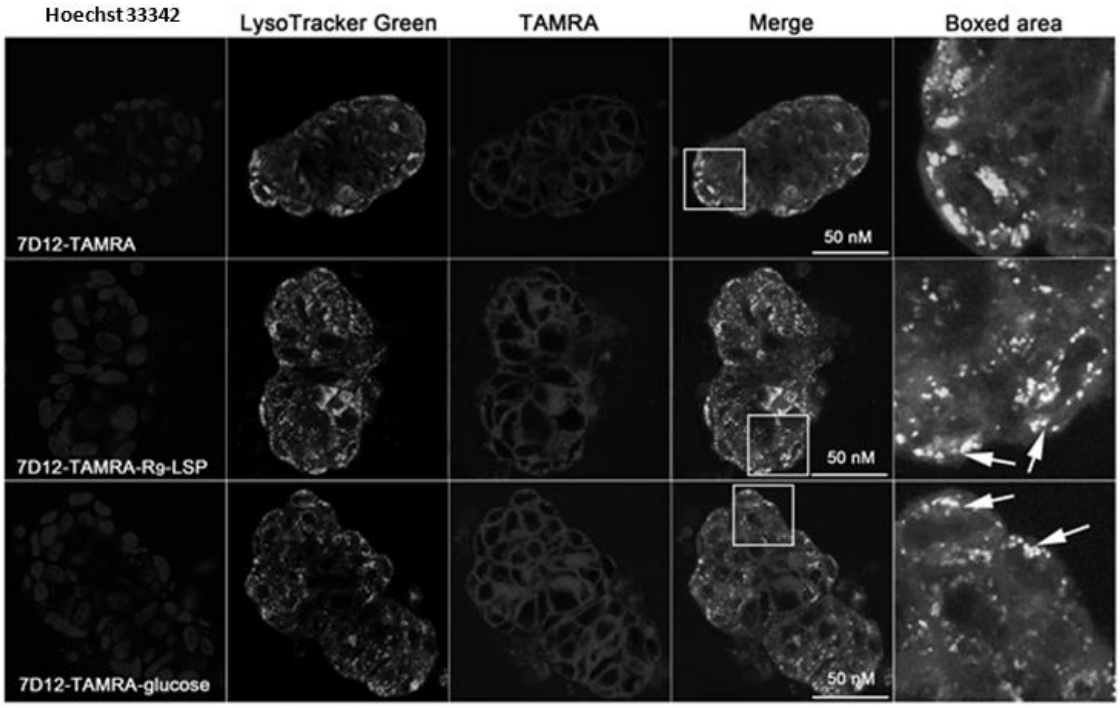

Hoechst 33342
LysoTracker Green
TAMRA
Merge
Boxed area
7D12-TAMRA
7D12-TAMRA-R9-LSP
7D12-TAMRA-glucose
50 nM
50 nM
50 nM

Fig. 24

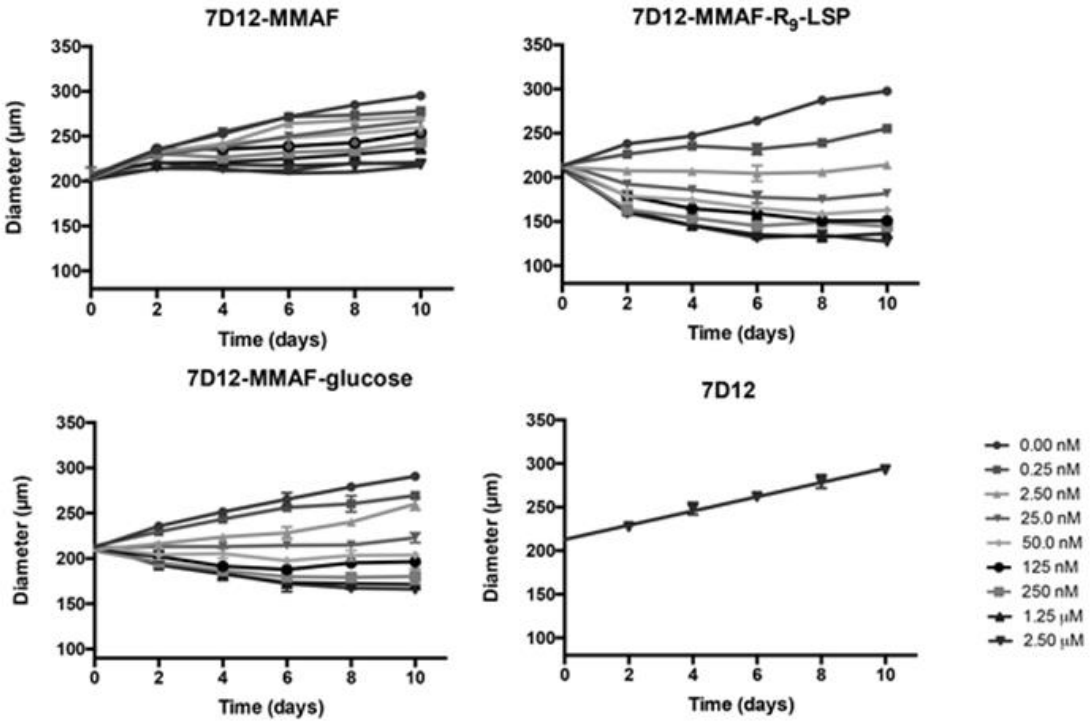

7D12-MMAF
7D12-MMAF-R9-LSP
7D12-MMAF-glucose
7D12
Diameter (μm)
Time (days)
0.00 nM
0.25 nM
2.50 nM
25.0 nM
50.0 nM
125 nM
250 nM
1.25 μM
2.50 μM

Fig. 25

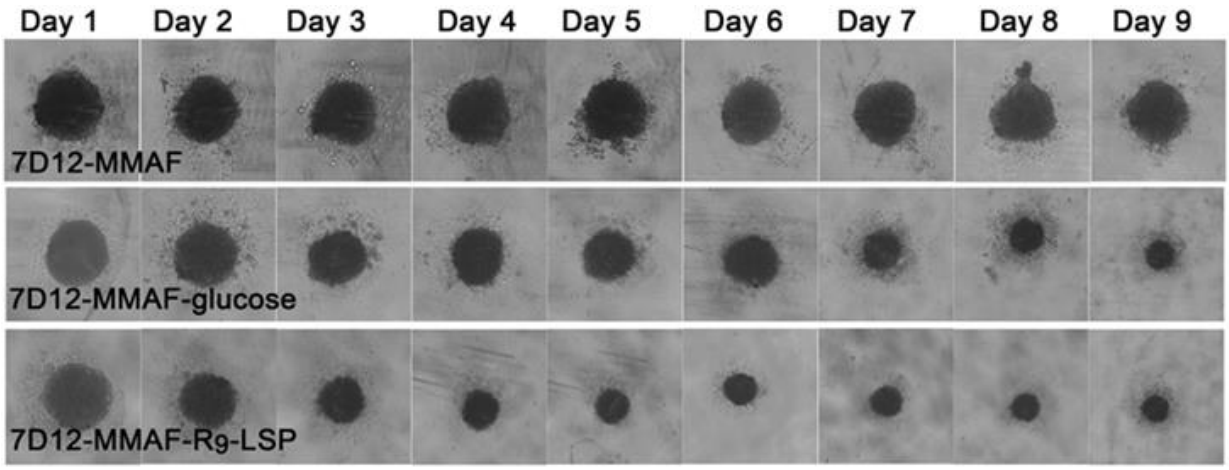

Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
7D12-MMAF
7D12-MMAF-glucose
7D12-MMAF-Rg-LSP

Fig. 26

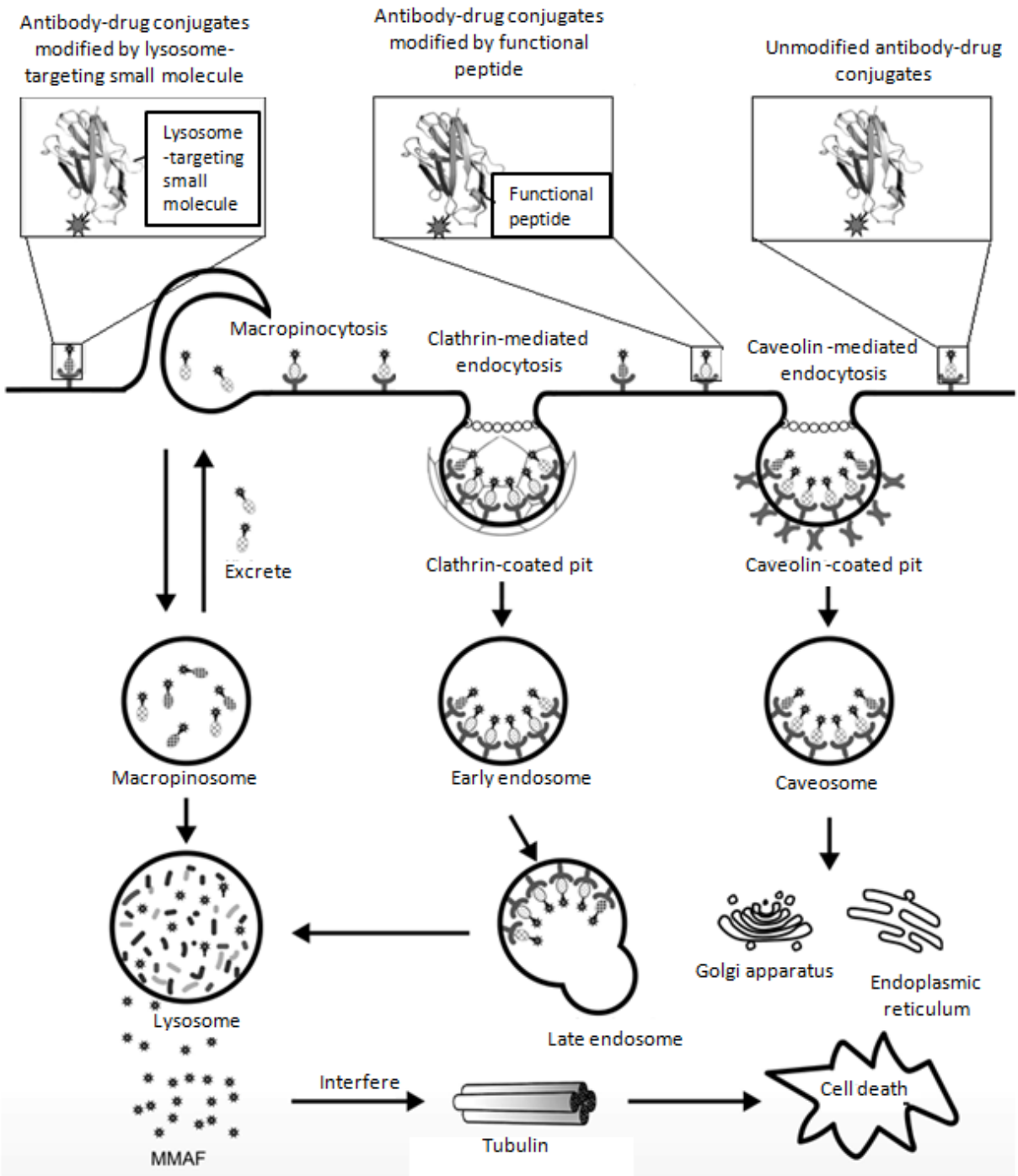

Antibody-drug conjugates modified by lysosome-targeting small molecule
Lysosome -targeting small molecule
Antibody-drug conjugates modified by functional peptide
Functional peptide
Unmodified antibody-drug conjugates
Macropinocytosis
Clathrin-mediated endocytosis
Caveolin -mediated endocytosis
Excrete
Clathrin-coated pit
Caveolin -coated pit
Macropinosome
Early endosome
Caveosome
Lysosome
Late endosome
Golgi apparatus
Endoplasmic reticulum
Interfere
Tubulin
Cell death
MMAF

Fig. 27