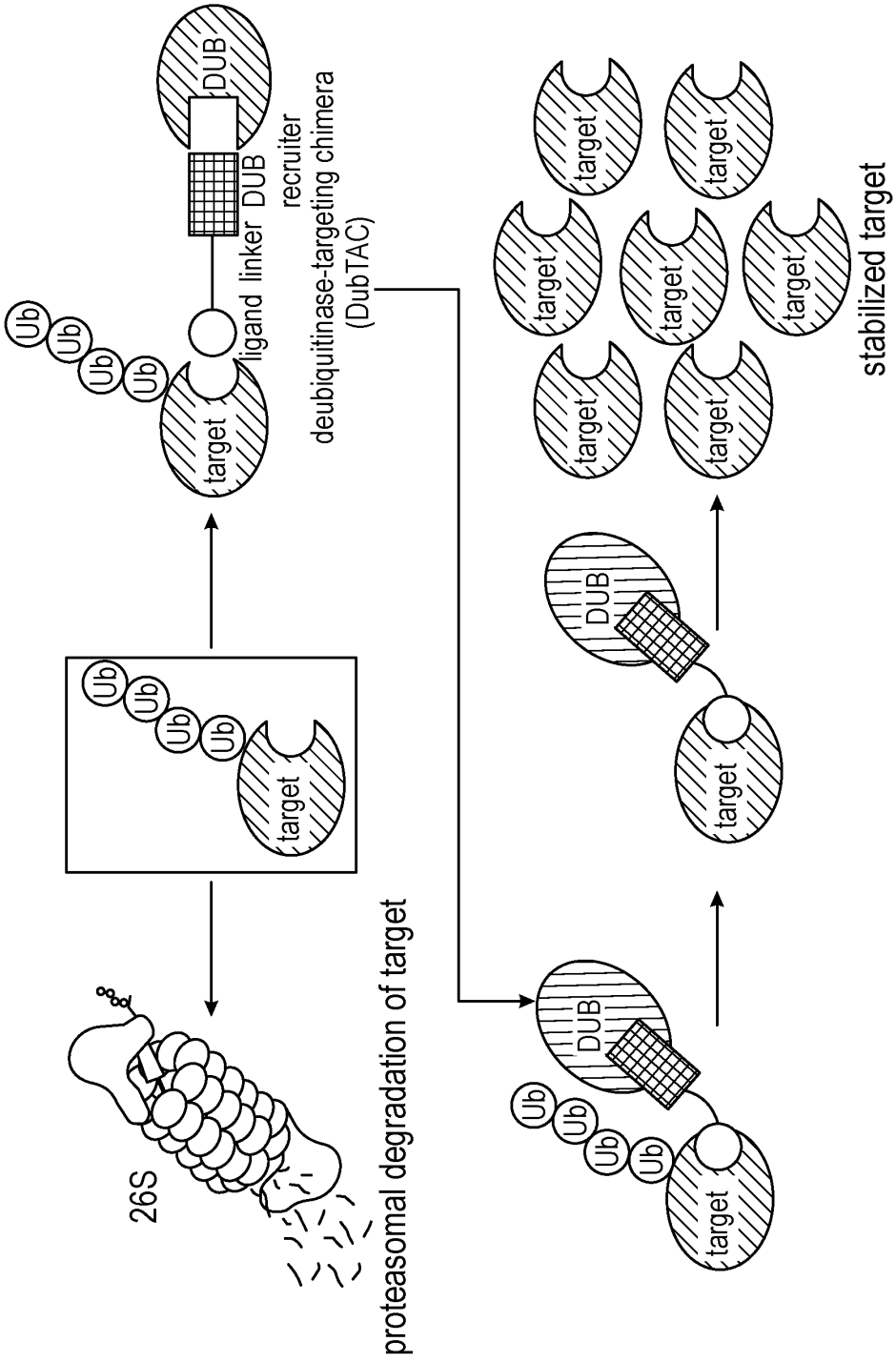

DUB
recruiter
DUB
ligand linker
deubiquitinase-targeting chimera
(DubTAC)
Ub
Ub
Ub
Ub
target
target
target
target
target
target
target
target
target
stabilized target
DUB
DUB
target
target
Ub
Ub
Ub
Ub
target
26S
proteasomal degradation of target

FIG. 1

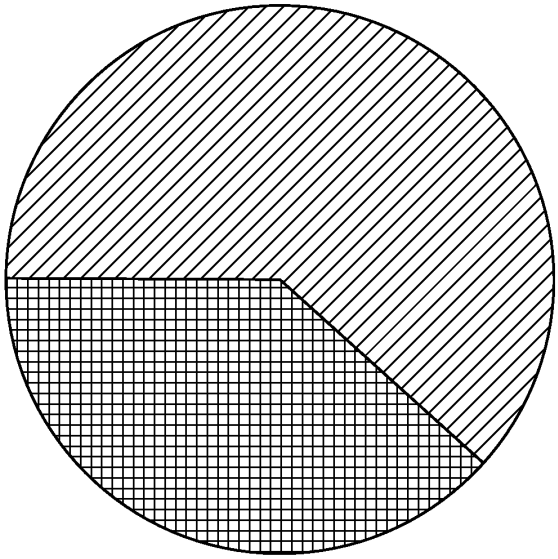

DUBs showing labeling of catalytic cysteines by IA-alkyne
DUBs not showing labeling of catalytic cysteines by IA-alkyne

FIG. 2B

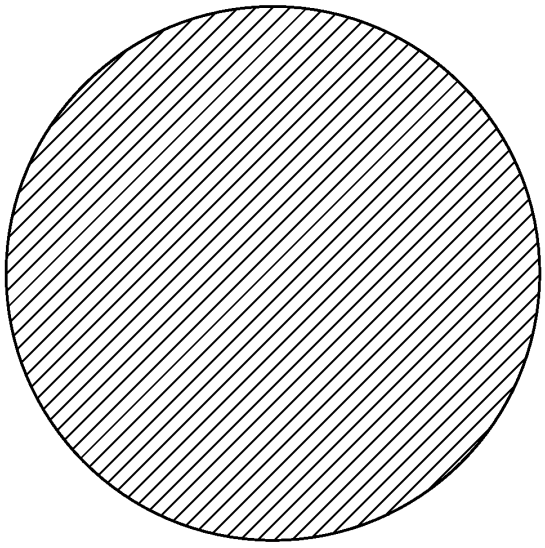

Total=65 DUBs
DUBs with IA-alkyne sites of labeling
DUBs with no IA-alkyne sites of labeling

FIG. 2A

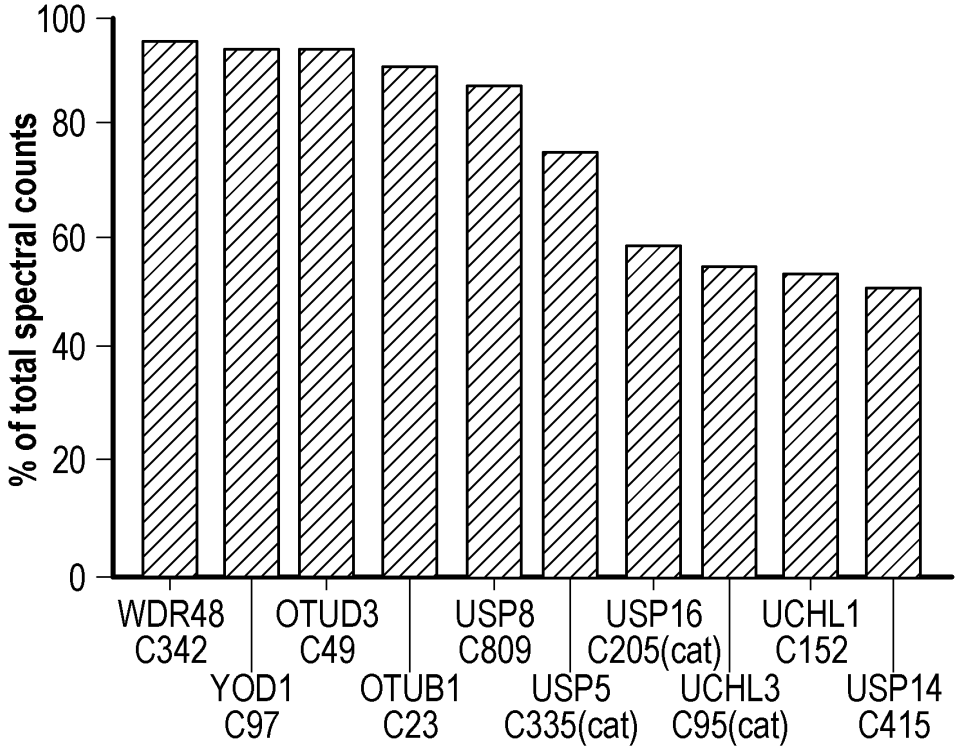

100
80
60
40
20
0
% of total spectral counts
WDR48 C342
YOD1 C97
OTUD3 C49
OTUB1 C23
USP8 C809
USP5 C335(cat)
USP16 C205(cat)
UCHL3 C95(cat)
UCHL1 C152
USP14 C415

FIG. 3A

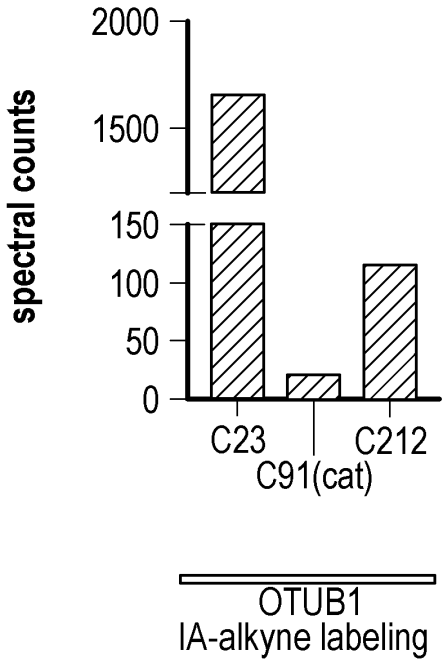

2000
1500
150
100
50
0
spectral counts
C23
C212
C91(cat)
OTUB1
IA-alkyne labeling

FIG. 3B

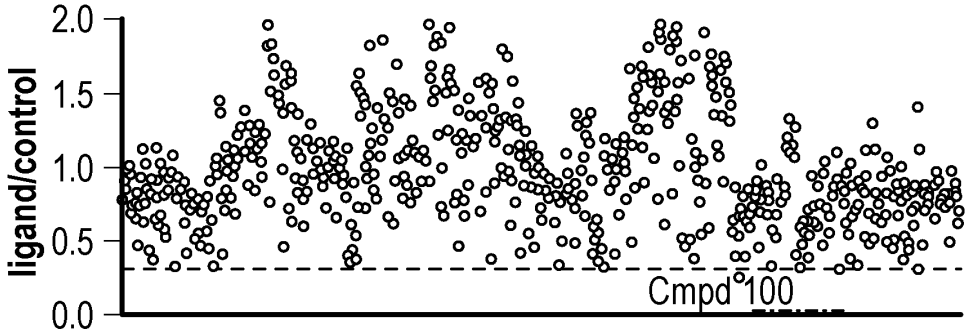

2.0
1.5
1.0
0.5
0.0
ligand/control
Cmpd 100

FIG. 4

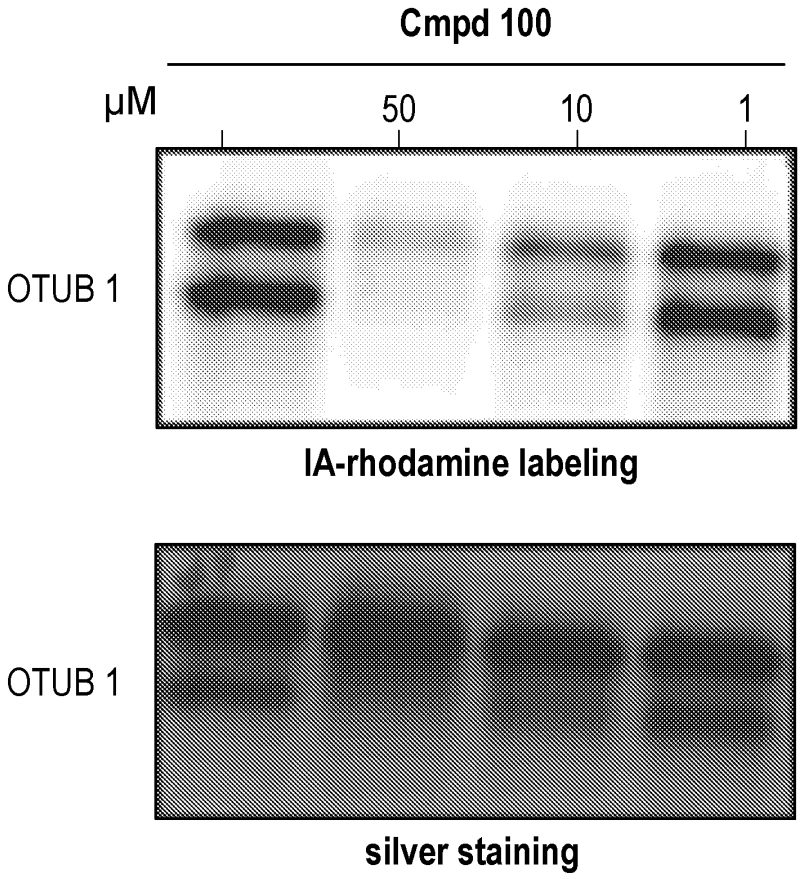

Cmpd 100
μM
50
10
1
OTUB 1
IA-rhodamine labeling
OTUB 1
silver staining

FIG. 5

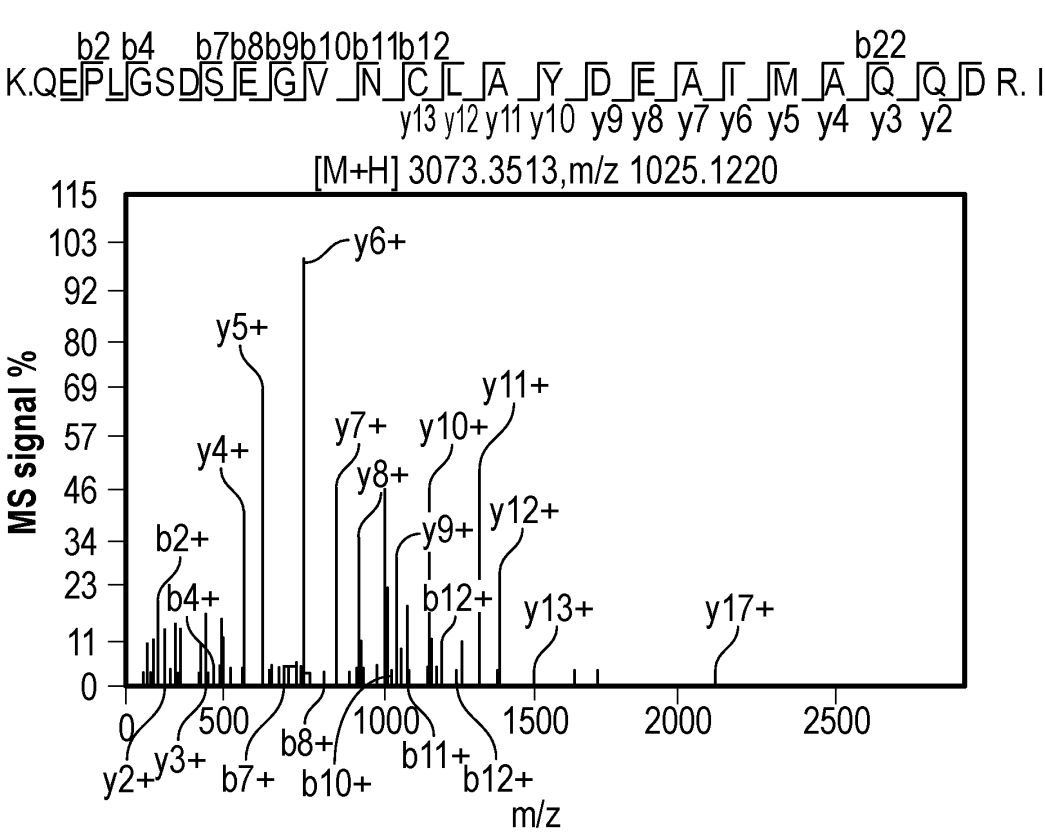

OTUB1 C23
b2 b4 b7 b8 b9 b10 b11 b12 b22
K.QEPLGSDSEGV_N_C_L_A_Y_D_E_A_I_M_A_Q_Q_D R. I
y13 y12 y11 y10 y9 y8 y7 y6 y5 y4 y3 y2
[M+H] 3073.3513,m/z 1025.1220
MS signal %
115
103
92
80
69
57
46
34
23
11
0
y6+
y5+
y11+
y7+
y10+
y4+
y8+
y12+
y9+
b2+
b4+
b12+
y13+
y17+
0
500
1000
1500
2000
2500
y2+
y3+
b7+
b8+
b10+
b11+
b12+
m/z

FIG. 6

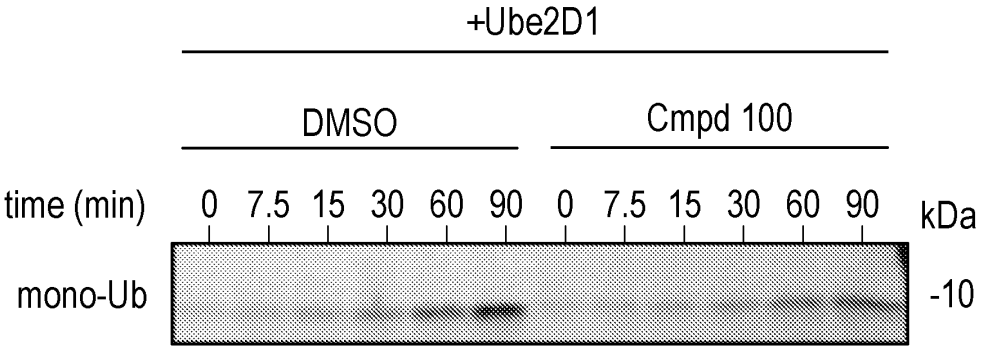

+Ube2D1
DMSO
Cmpd 100
time (min)
0 7.5 15 30 60 90 0 7.5 15 30 60 90
kDa
mono-Ub
-10

FIG. 7

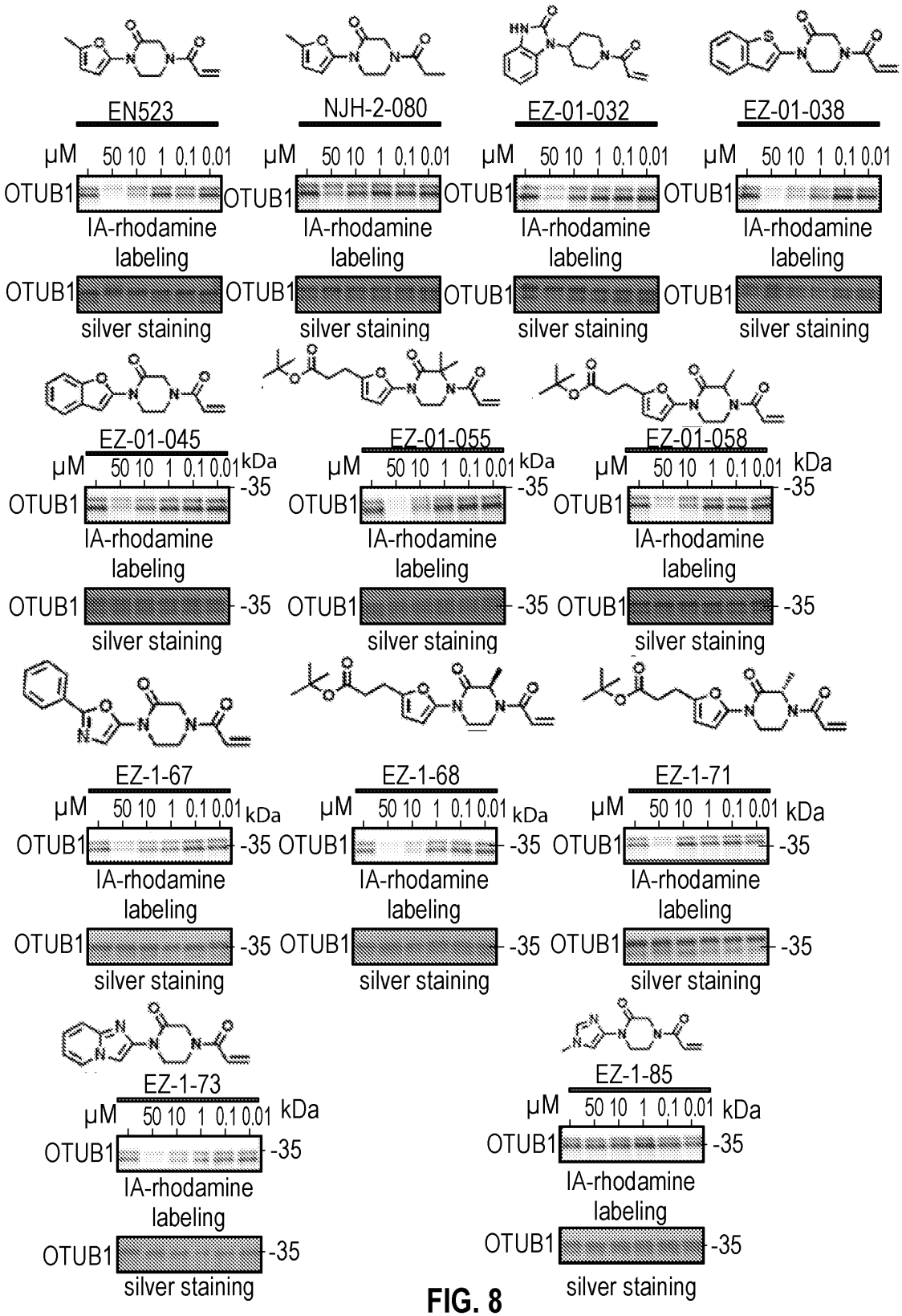

EN523
NJH-2-080
EZ-01-032
EZ-01-038
EZ-01-045
EZ-01-055
EZ-01-058
EZ-1-67
EZ-1-68
EZ-1-71
EZ-1-73
EZ-1-85
μM 50 10 1 0.1 0.01
kDa
-35
OTUB1
IA-rhodamine labeling
silver staining

FIG. 8

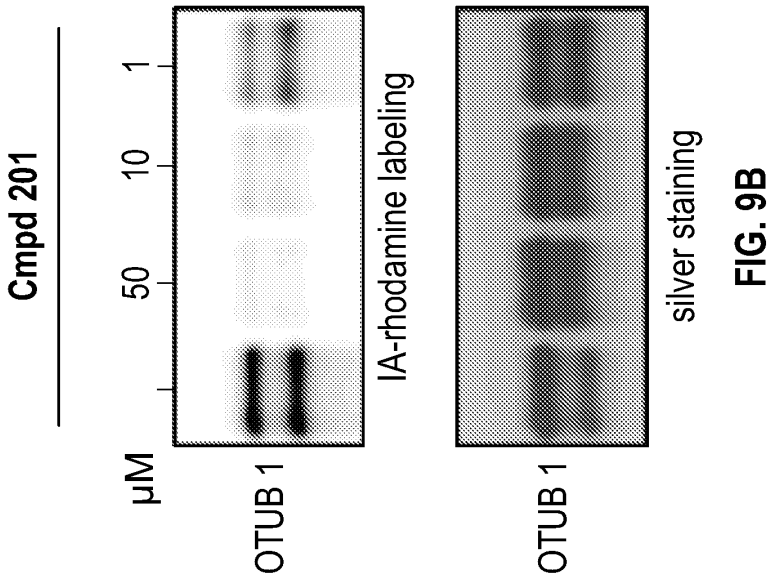

Cmpd 201
μM
1
10
50
OTUB 1
IA-rhodamine labeling
OTUB 1
silver staining

FIG. 9B

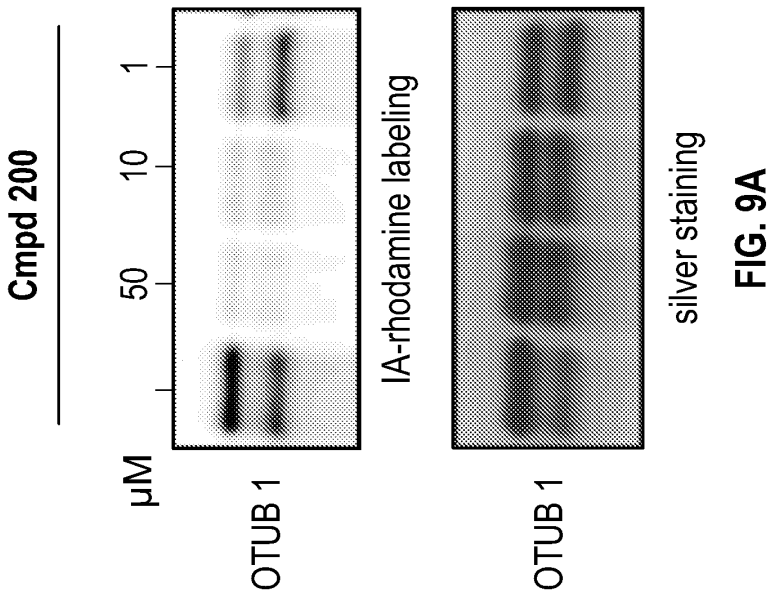

Cmpd 200
μM
1
10
50
OTUB 1
IA-rhodamine labeling
OTUB 1
silver staining

FIG. 9A

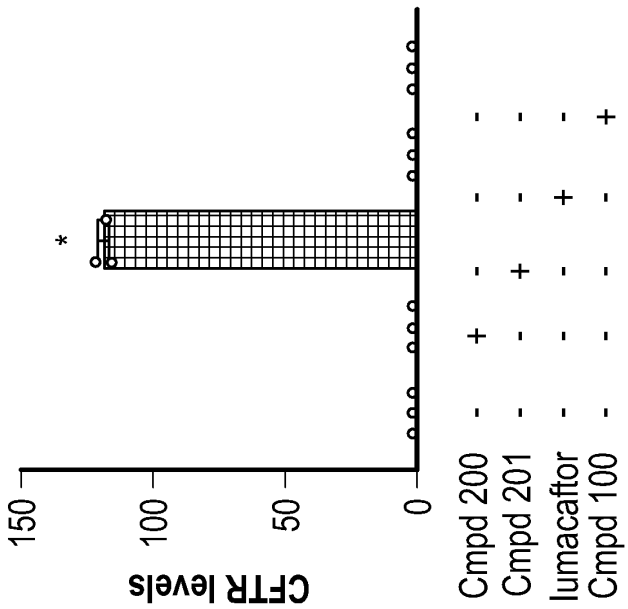



FIG. 10B

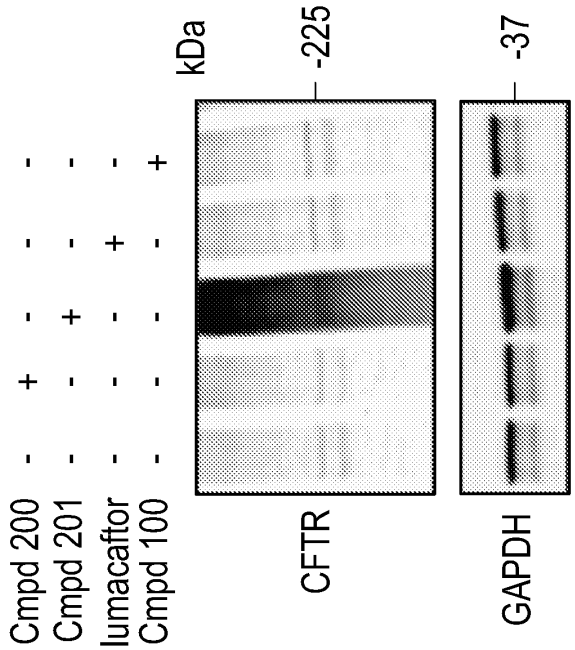



FIG. 10A

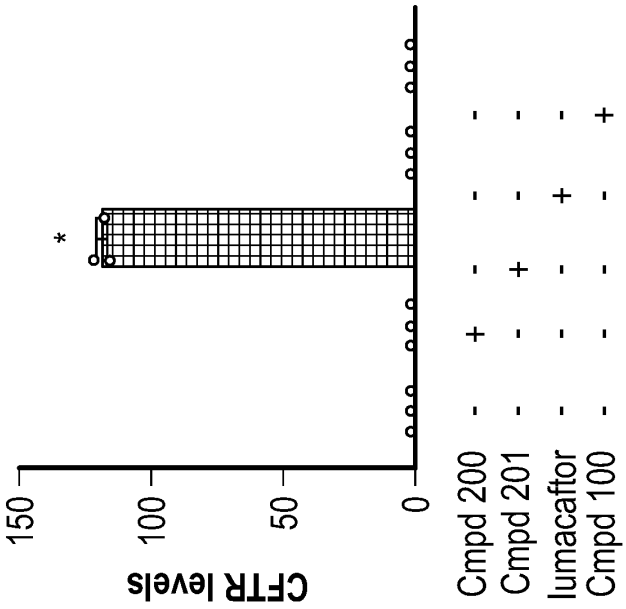



FIG. 11B

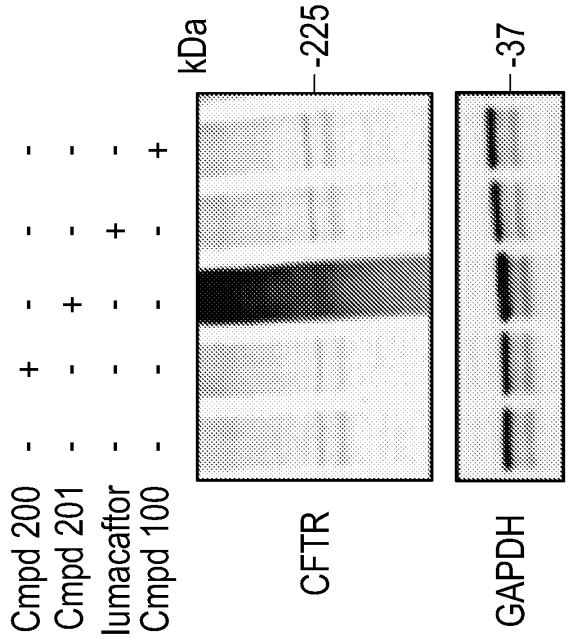



FIG. 11A

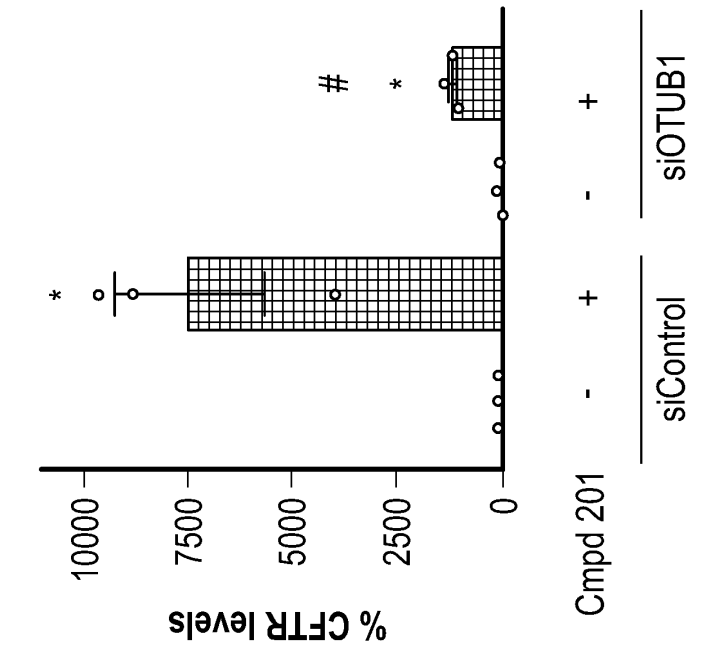



FIG. 12B

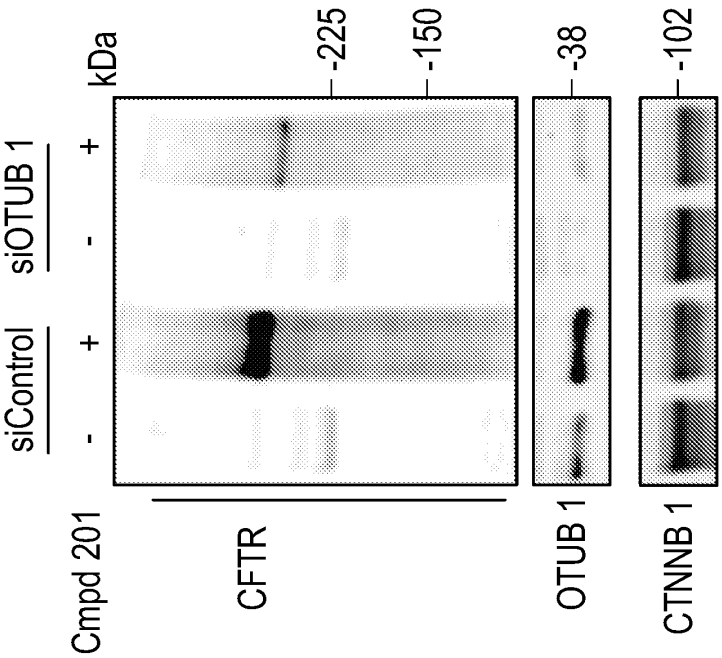



FIG. 12A

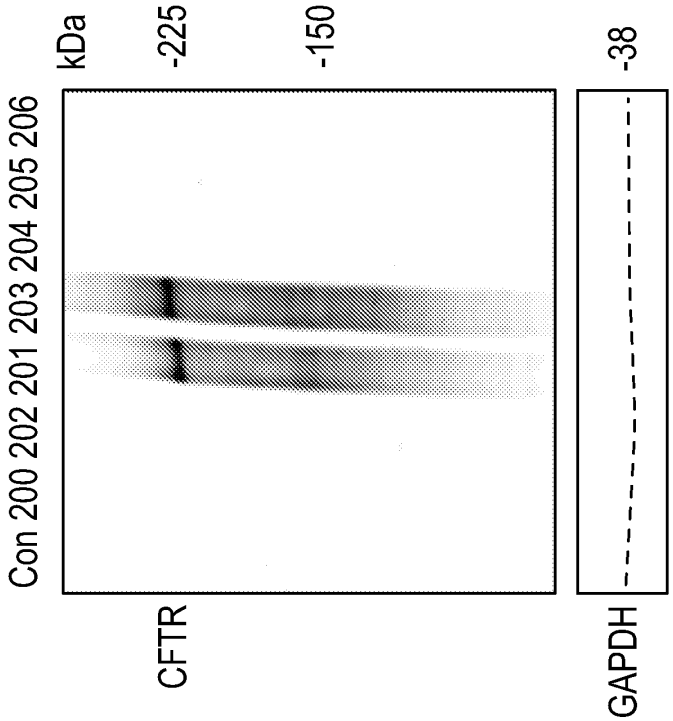



FIG. 13

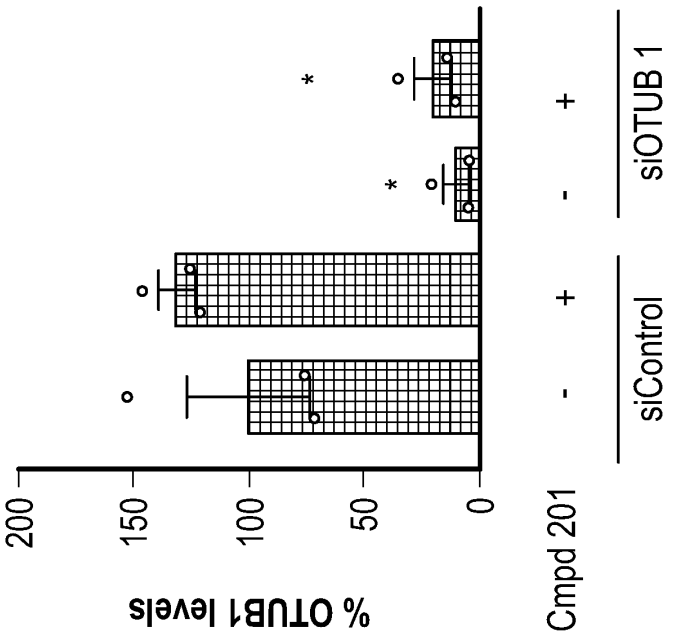



FIG. 12C

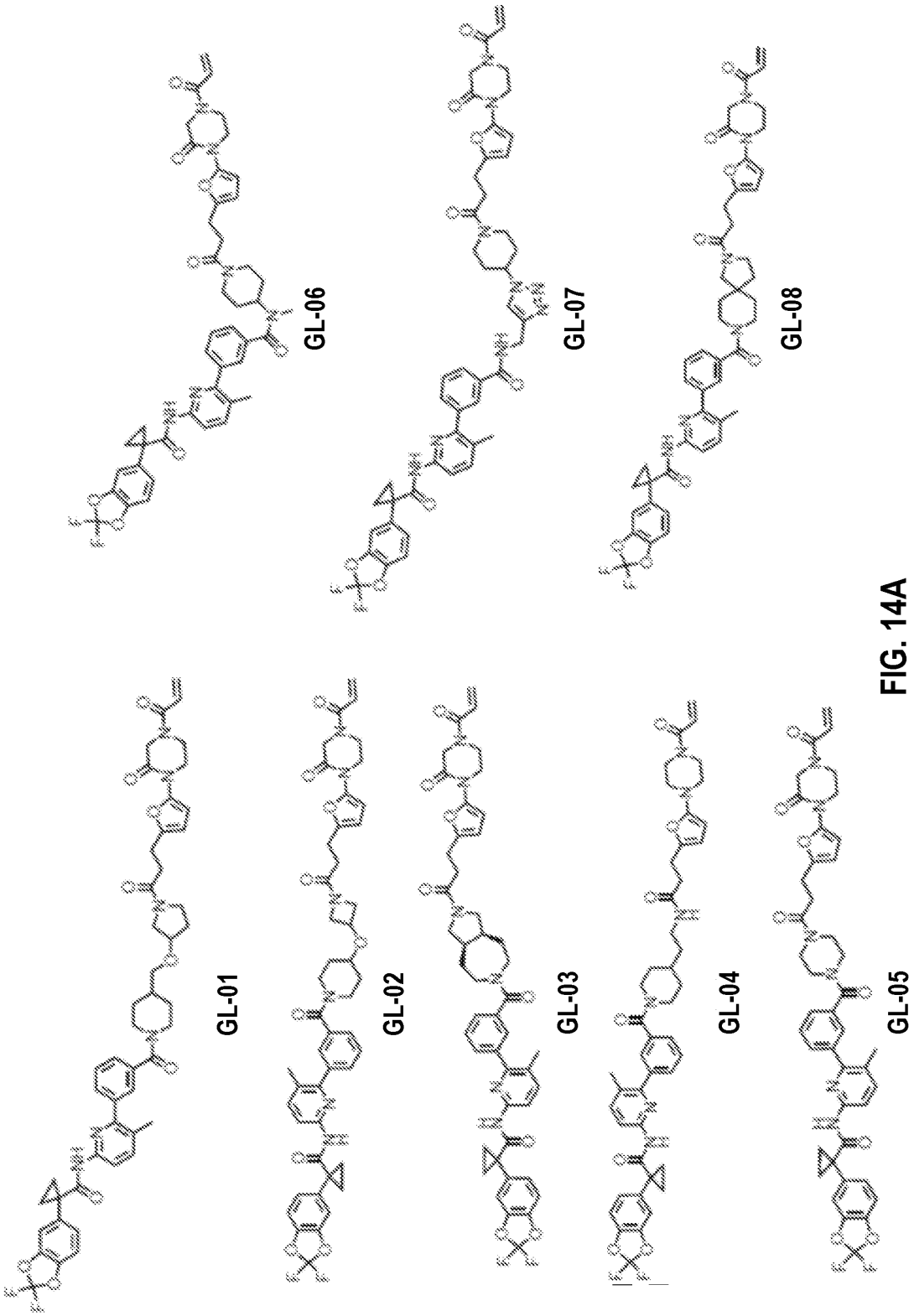



FIG. 14A

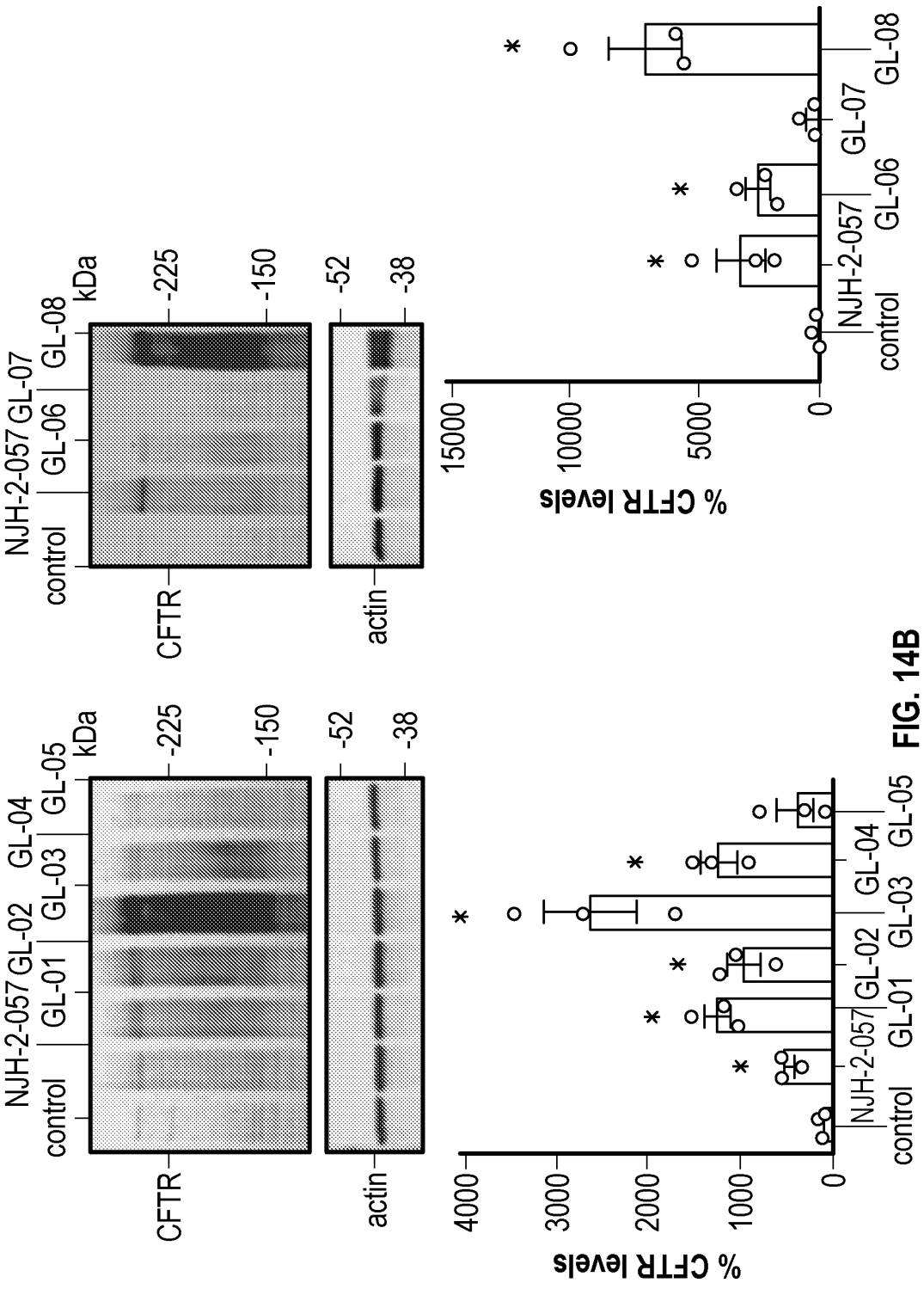

control
NJH-2-057
GL-01
GL-02
GL-03
GL-04
GL-05
GL-06
GL-07
GL-08
kDa
-225
-150
-52
-38
CFTR
actin
% CFTR levels
0
1000
2000
3000
4000
5000
10000
15000

FIG. 14B

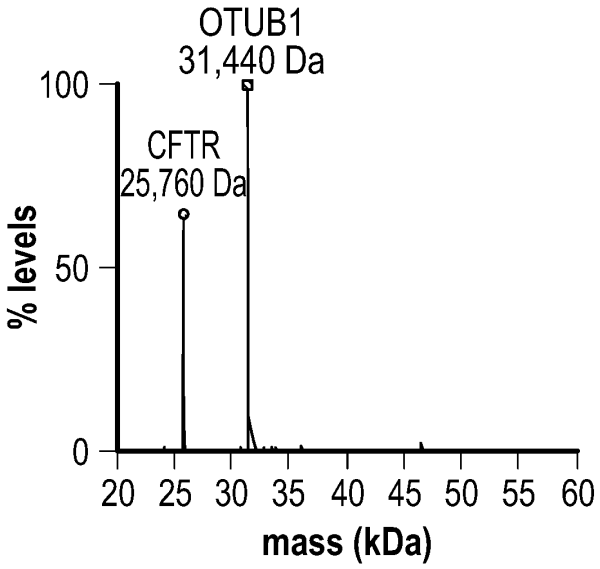



FIG. 15A

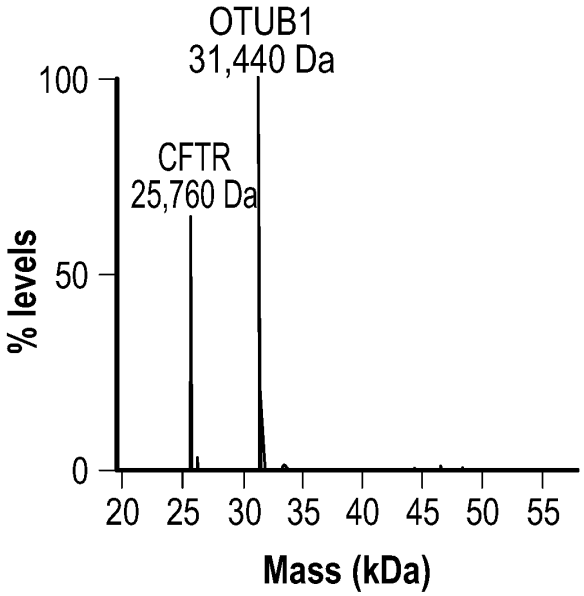



FIG. 15B

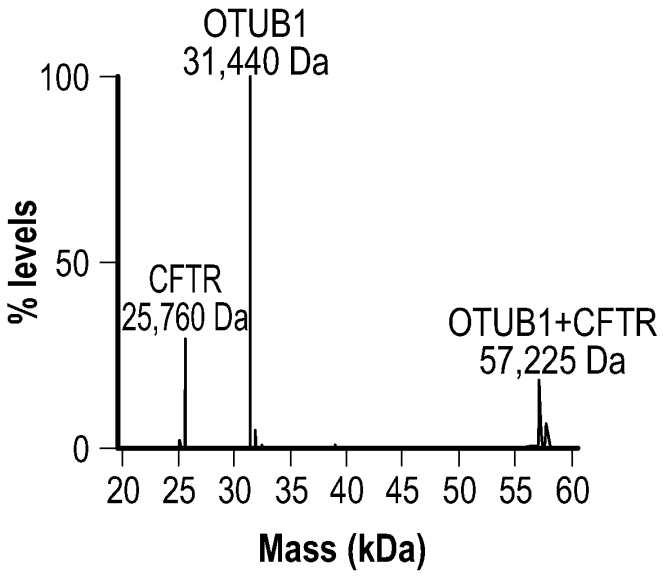

OTUB1
31,440 Da
100
50
0
% levels
CFTR
25,760 Da
OTUB1+CFTR
57,225 Da
20 25 30 35 40 45 50 55 60
Mass (kDa)

FIG. 15C

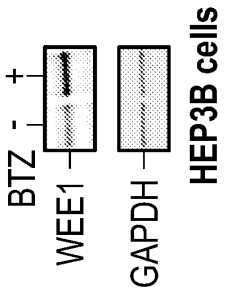
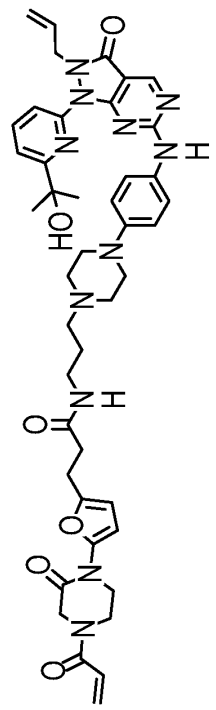
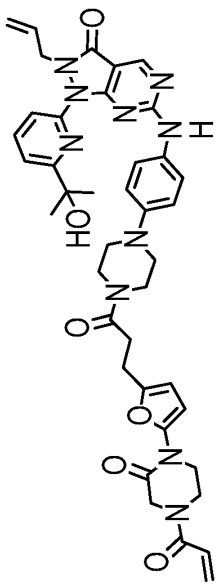


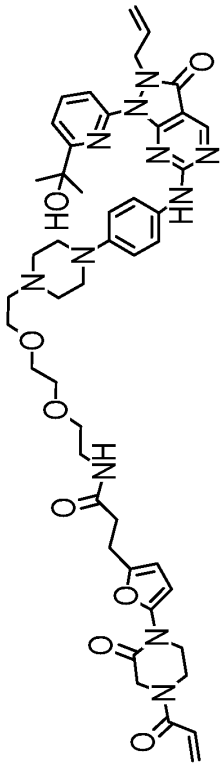

FIG. 16A



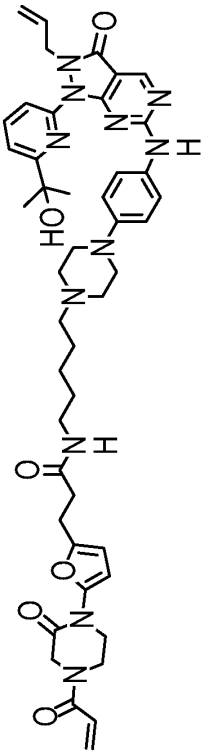
LEB-03-144



LEB-03-153



LEB-03-146



LEB-03-145

FIG. 16B

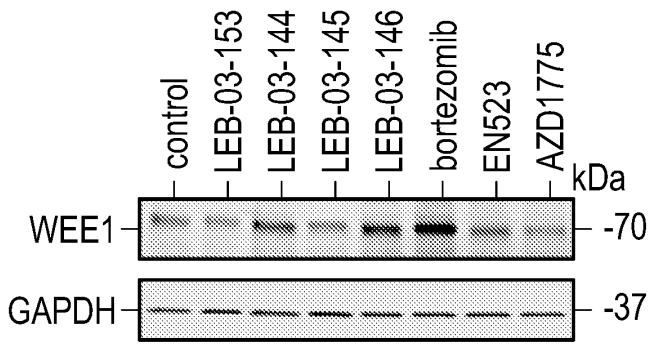



FIG. 16C

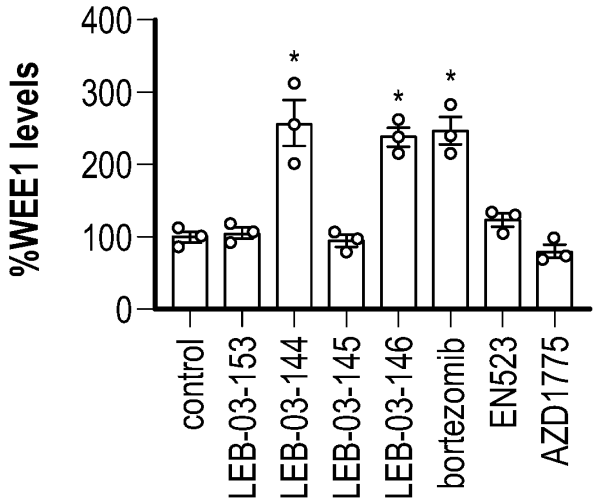



FIG. 16D

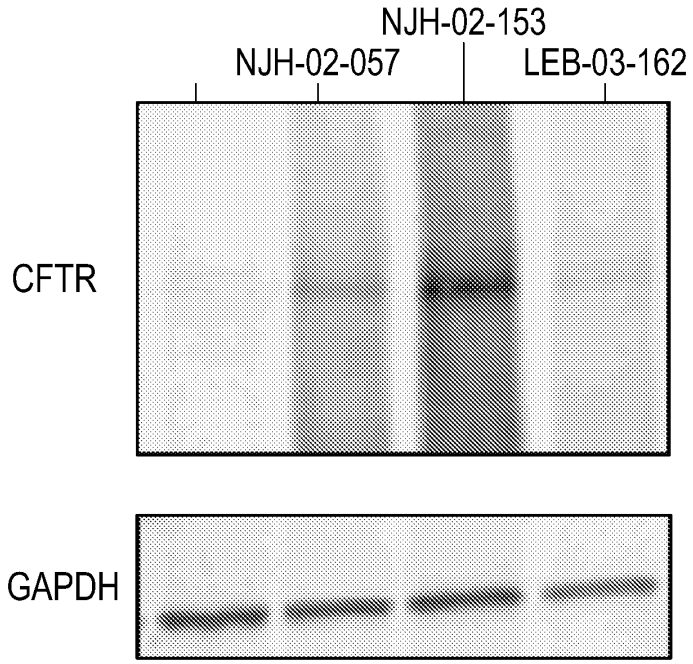



FIG. 17

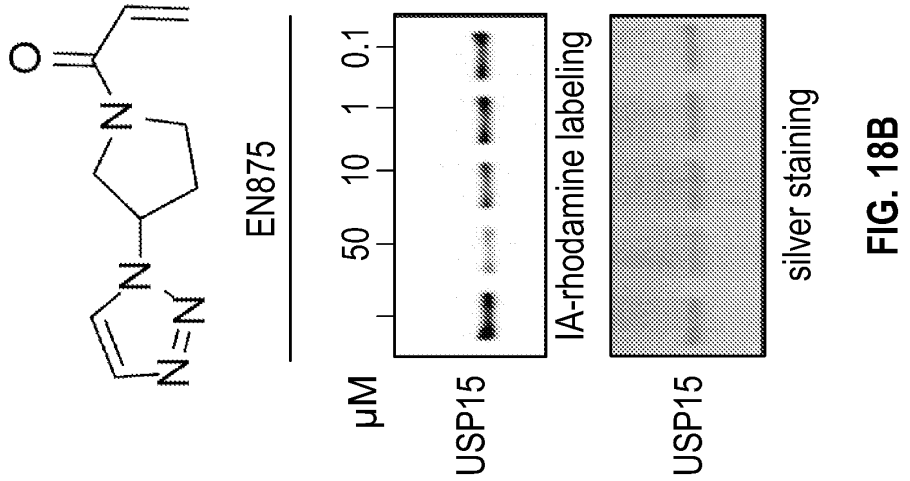



FIG. 18B

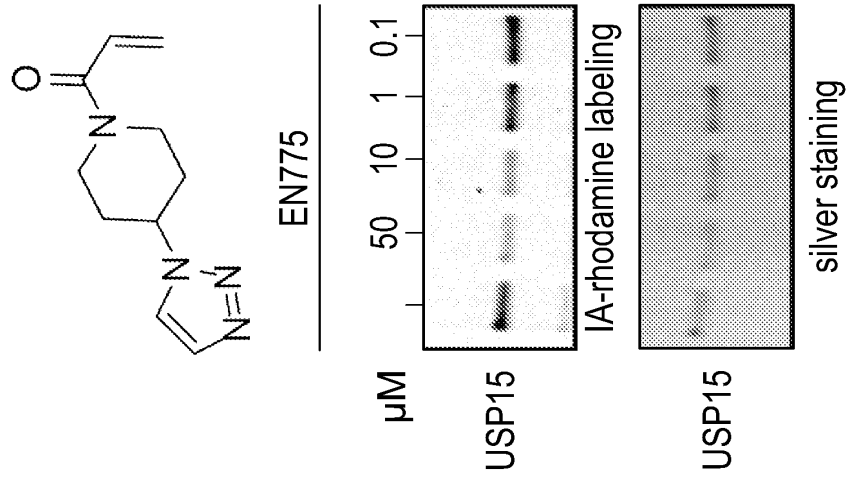



FIG. 18A

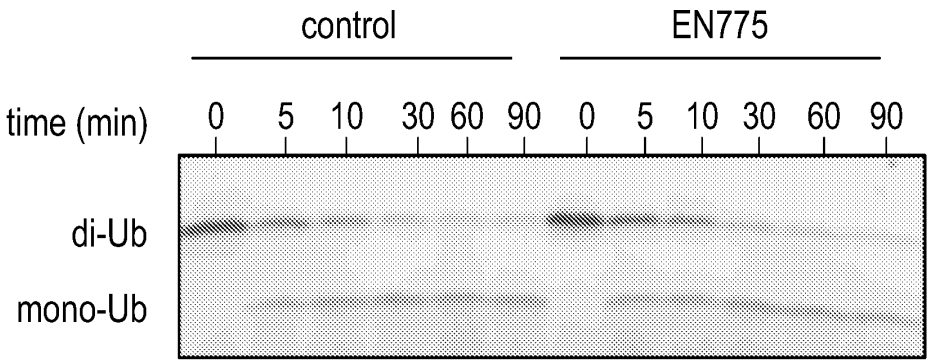



FIG. 18C

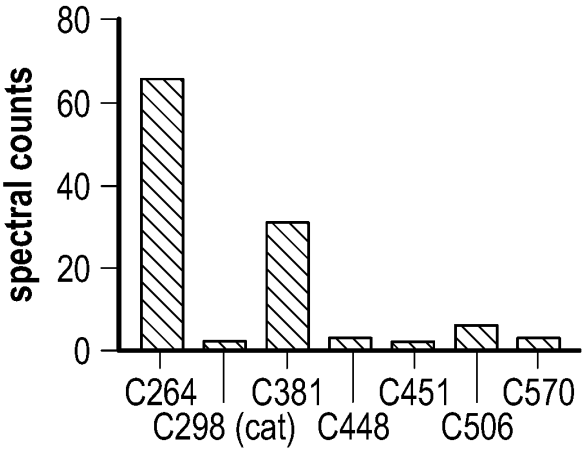



FIG. 18D

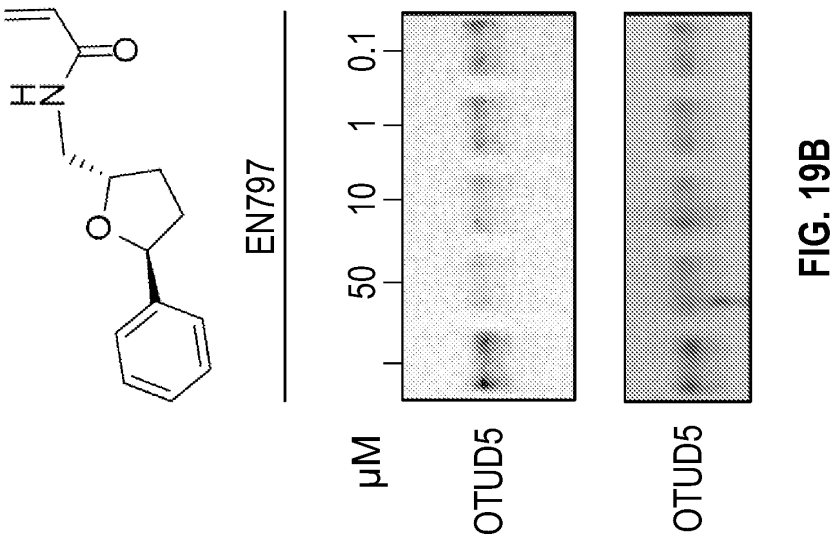



FIG. 19B

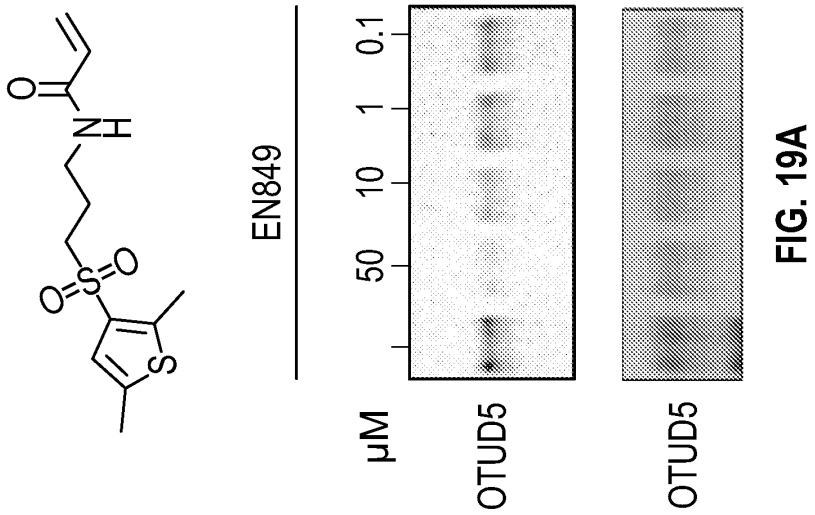



FIG. 19A