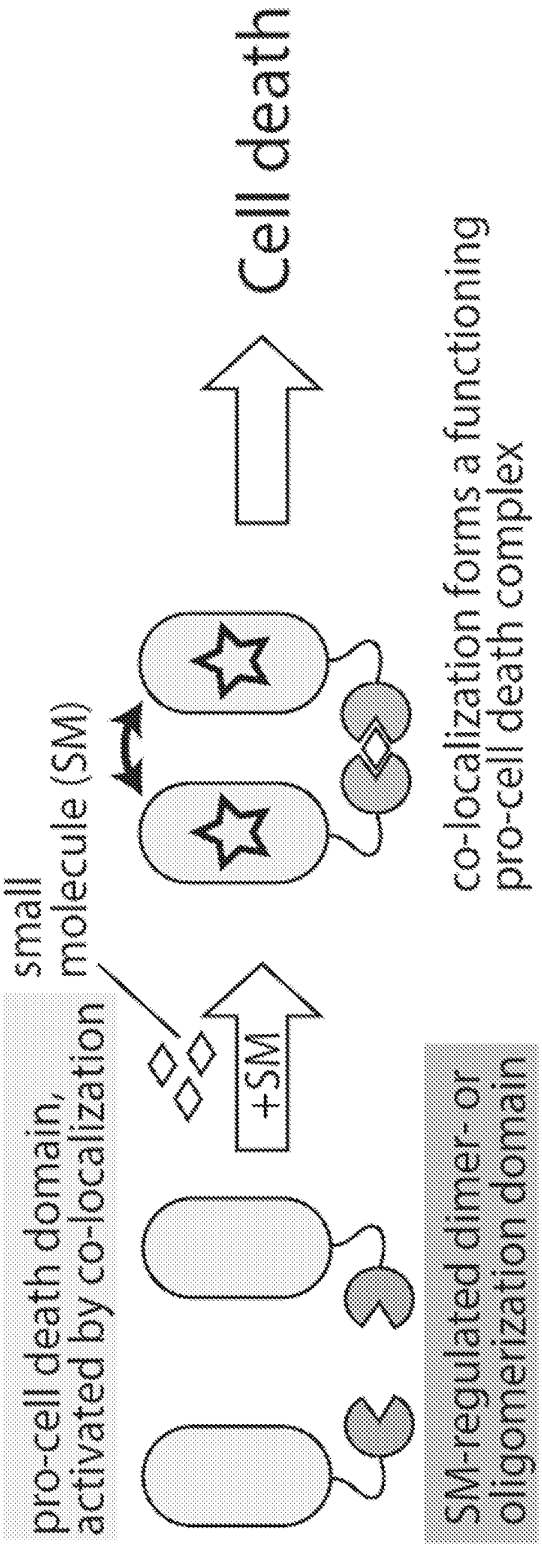

pro-cell death domain, activated by co-localization
small molecule (SM)
+SM
co-localization forms a functioning pro-cell death complex
Cell death
SM-regulated dimer- or oligomerization domain

Figure 1A

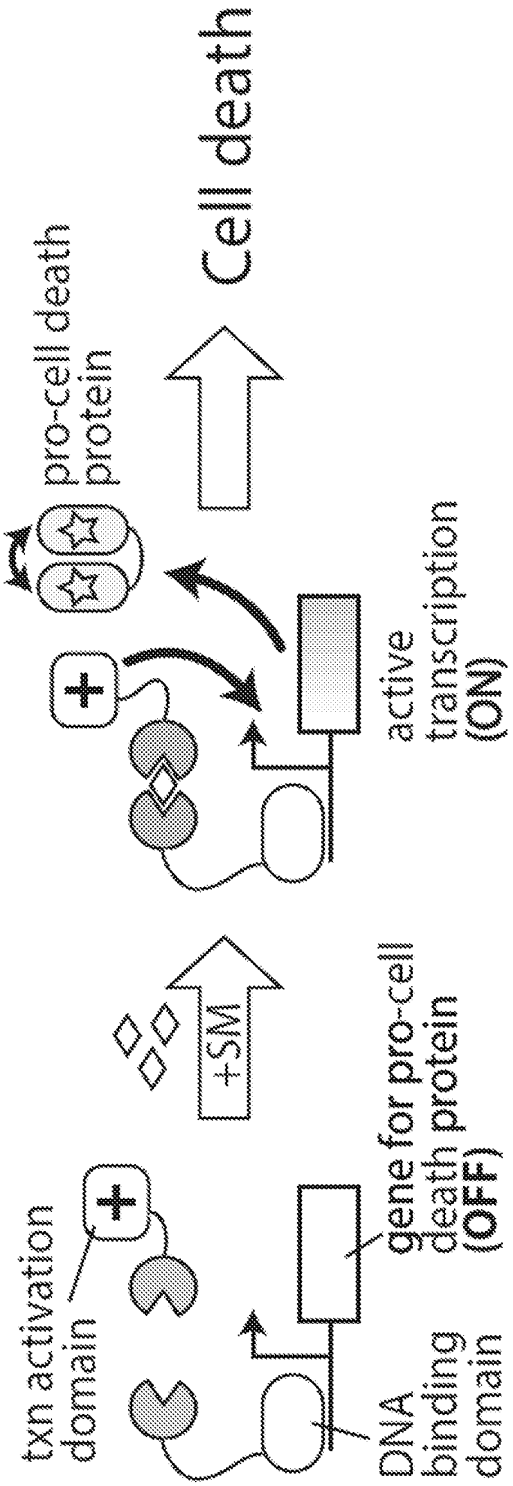

txn activation domain
DNA binding domain
gene for pro-cell death protein (OFF)
+SM
active transcription (ON)
pro-cell death protein
Cell death

Figure 1B

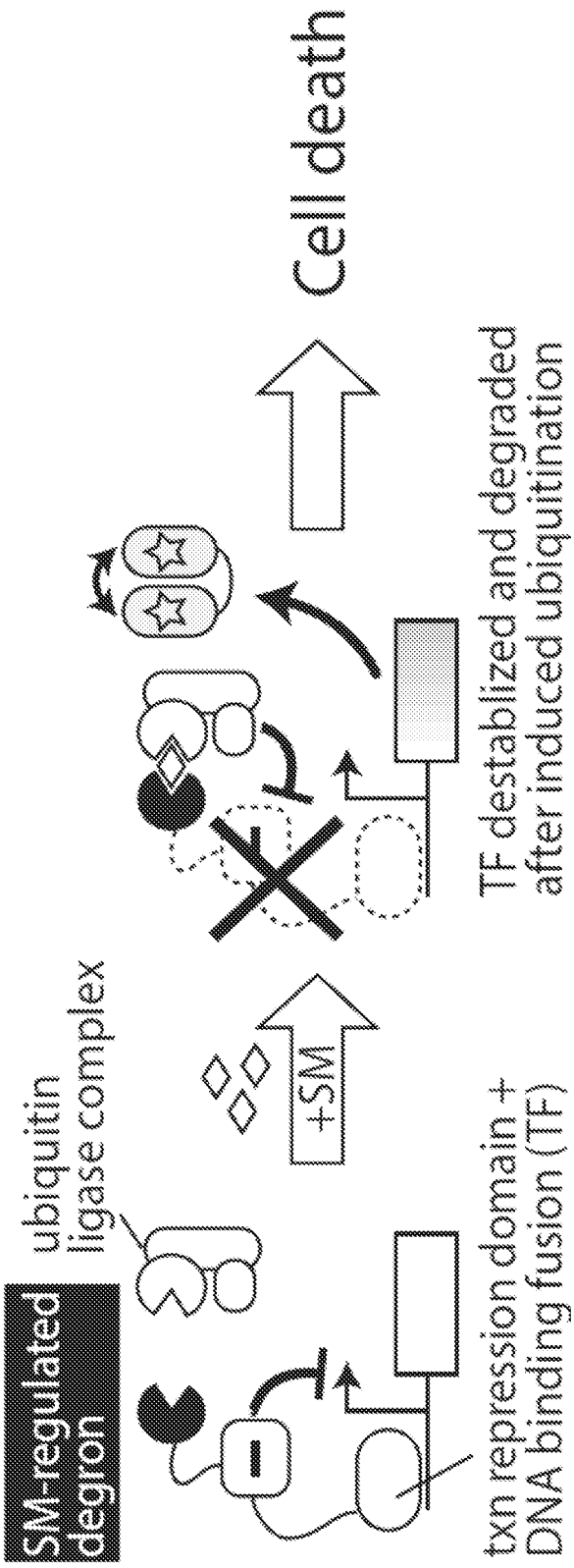

SM-regulated degron
ubiquitin ligase complex
txn repression domain + DNA binding fusion (TF)
+SM
TF destablized and degraded after induced ubiquitination
Cell death

Figure 1C

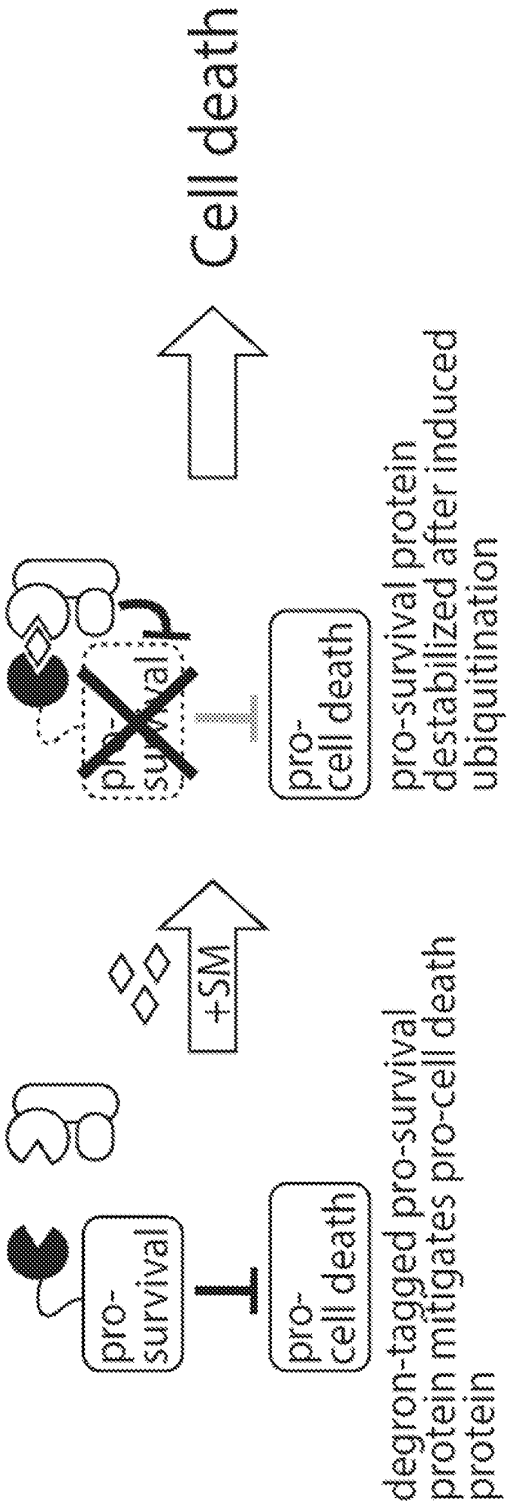

pro-survival
pro-cell death
degron-tagged pro-survival protein mitigates pro-cell death protein
+SM
pro-cell death
pro-survival protein destabilized after induced ubiquitination
Cell death

Figure 1D

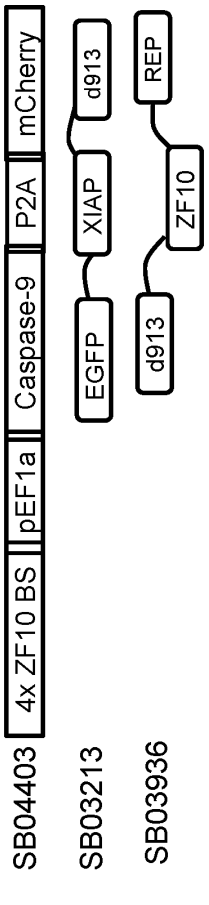

SB04403
4x ZF10 BS
pEF1a
Caspase-9
P2A
mCherry
Toxin
SB03213
EGFP
XIAP
d913
Anti-toxin
SB03936
d913
ZF10
REP
SynTF_Repressor

FIG. 2A

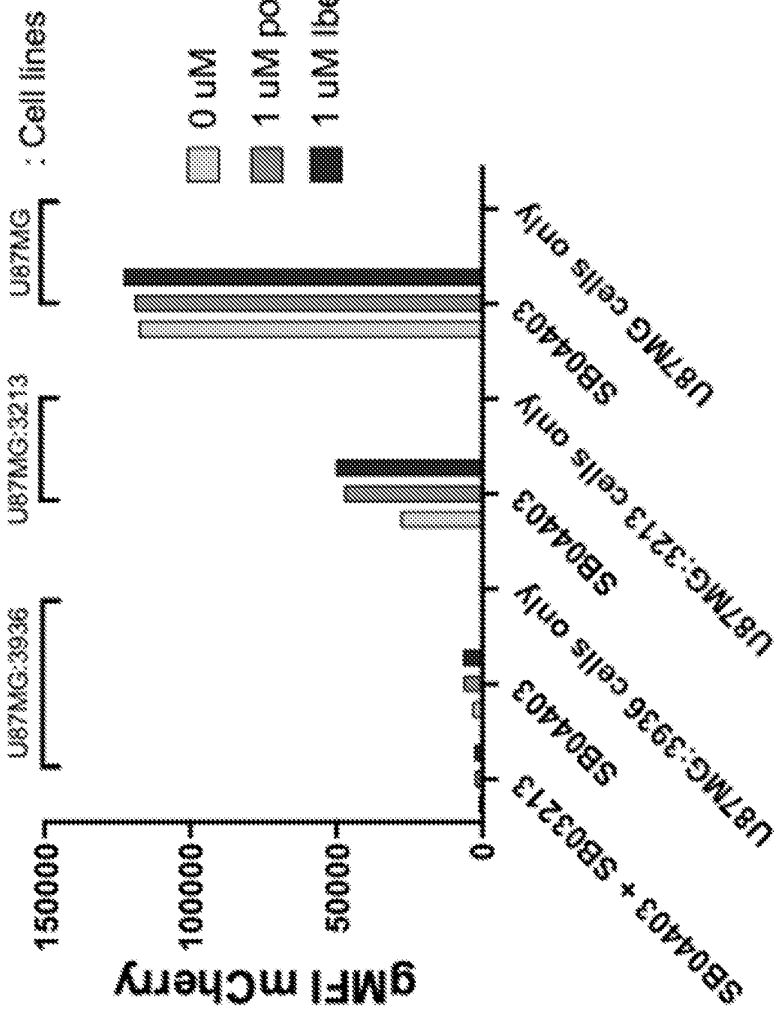

gMFI mCherry
150000
100000
50000
0
U87MG:3936
U87MG:3213
U87MG
: Cell lines
0 uM
1 uM pomalidmide
1 uM lberdomide
SB04403 + SB03213
SB04403
U87MG:3936 cells only
SB04403
U87MG:3213 cells only
SB04403
U87MG cells only

FIG. 2B

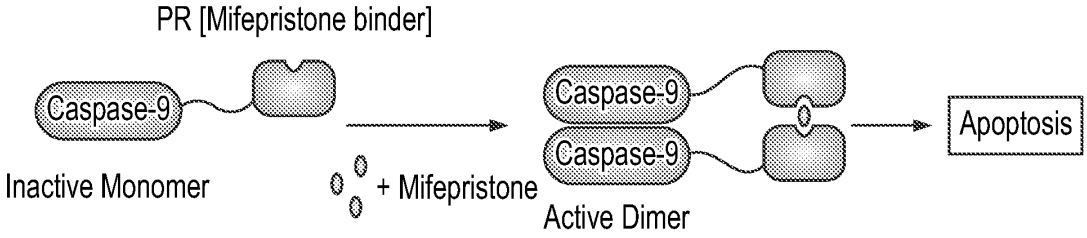

PR [Mifepristone binder]
Caspase-9
Inactive Monomer
+ Mifepristone
Caspase-9
Caspase-9
Active Dimer
Apoptosis

FIG. 3A

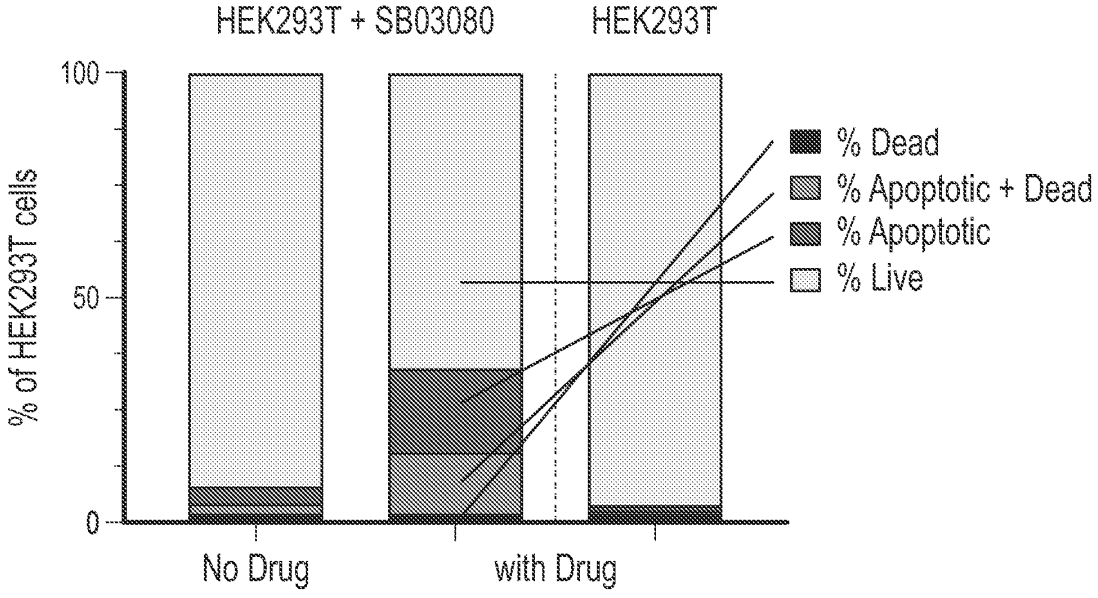

HEK293T + SB03080
HEK293T
100
50
0
% of HEK293T cells
No Drug
with Drug
% Dead
% Apoptotic + Dead
% Apoptotic
% Live

FIG. 3B

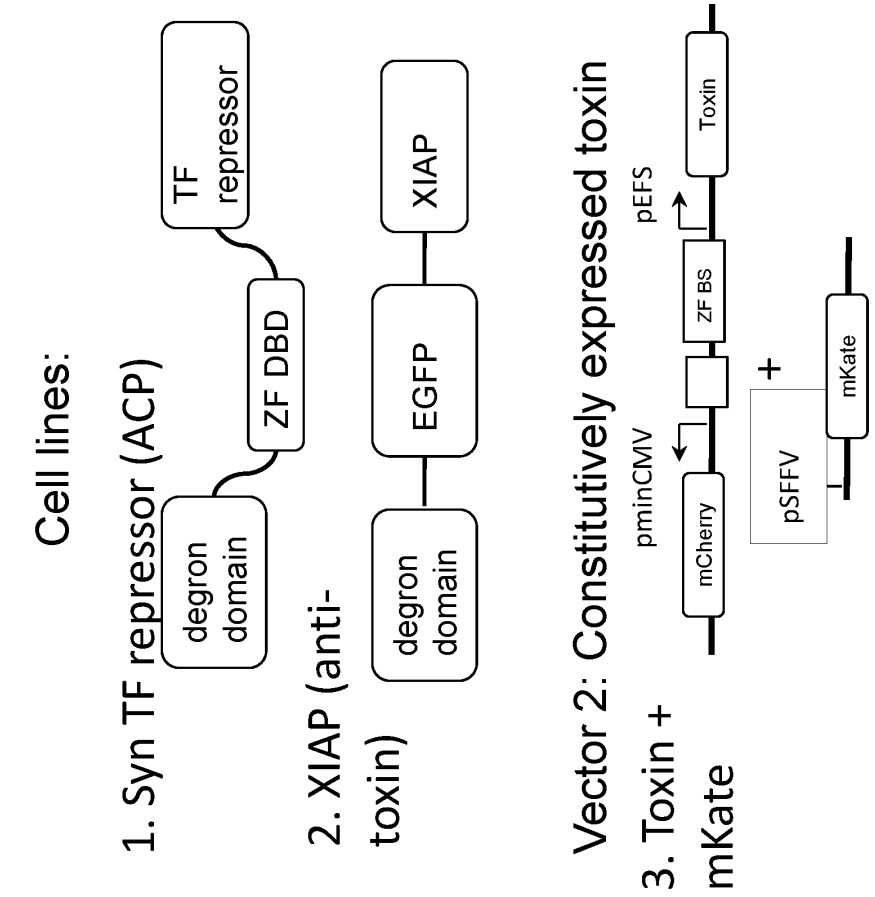
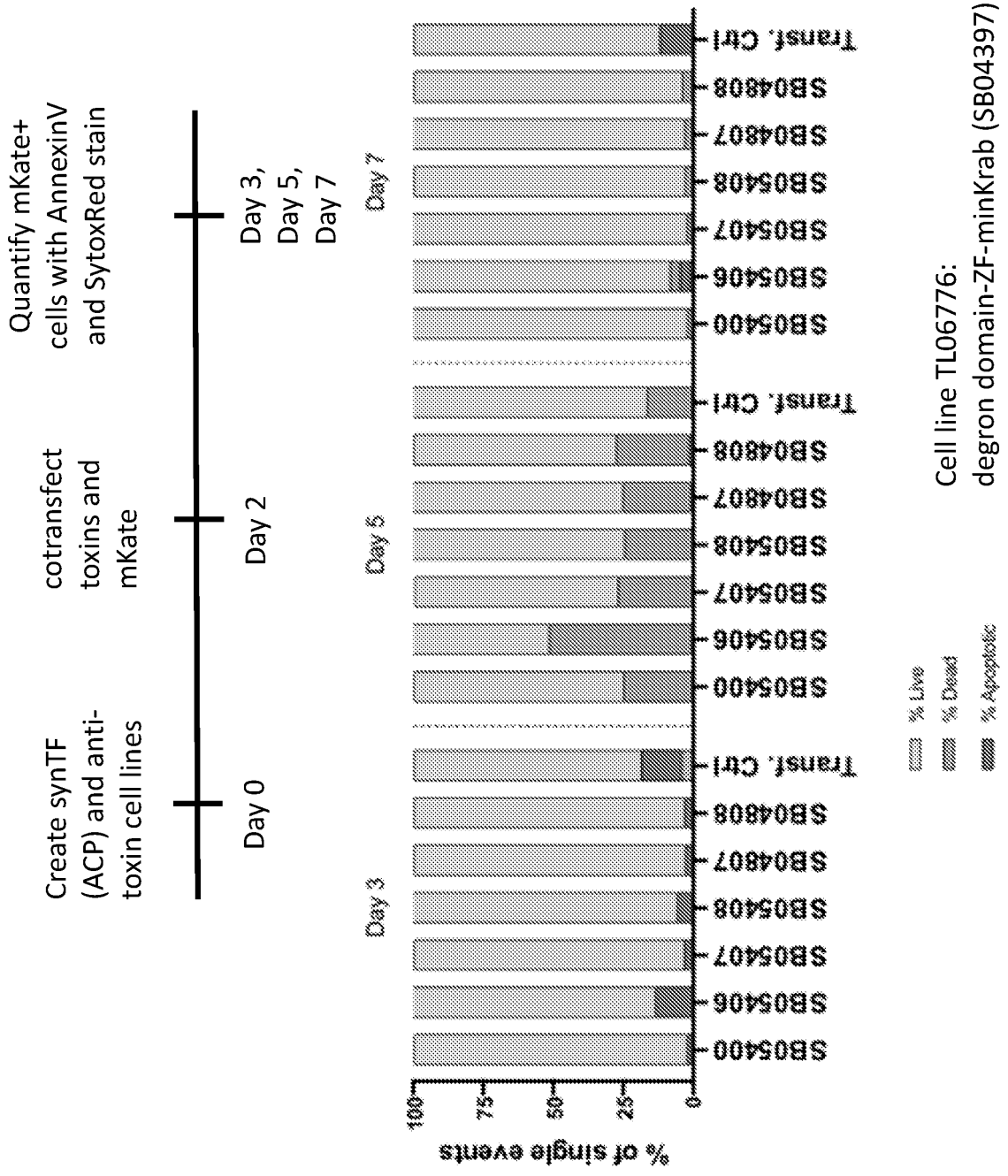

Cell lines:
1. Syn TF repressor (ACP)
degron domain
ZF DBD
TF repressor
2. XIAP (anti-toxin)
degron domain
EGFP
XIAP
Vector 2: Constitutively expressed toxin
3. Toxin + mKate
pminCMV
mCherry
ZF BS
pEFS
Toxin
pSFFV
+
mKate

FIG. 4A

FIG. 4B


Create synTF (ACP) and anti-toxin cell lines
Day 0
cotransfect toxins and mKate
Day 2
Quantify mKate+ cells with AnnexinV and SytoxRed stain
Day 3, Day 5, Day 7
Day 3
Day 5
Day 7
% of single events
100
75
50
25
0
Transf. Ctrl
SB05400
SB05406
SB05407
SB05408
SB04807
SB04808
% Live
% Dead
% Apoptotic
% Apoptotic and Dead
Cell line TL06776:
degron domain-ZF-minKrab (SB04397)

Cell line TL06776: degron domain-ZF-minKrab (SB04397)

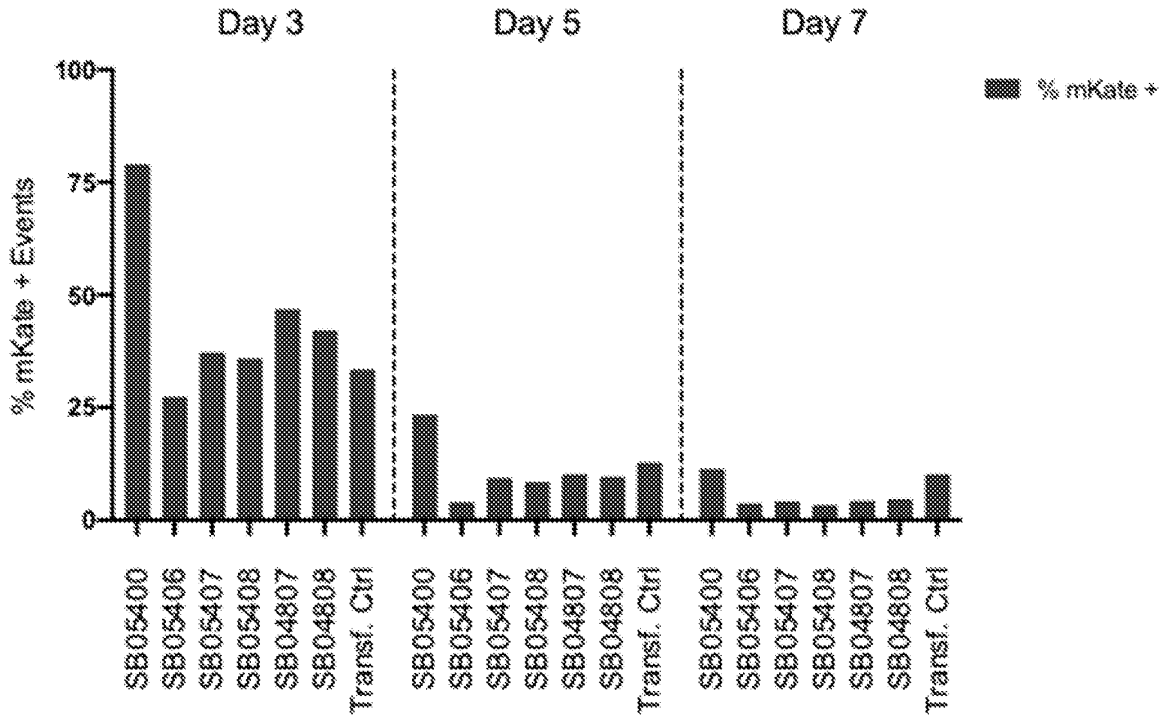


FIG. 4C

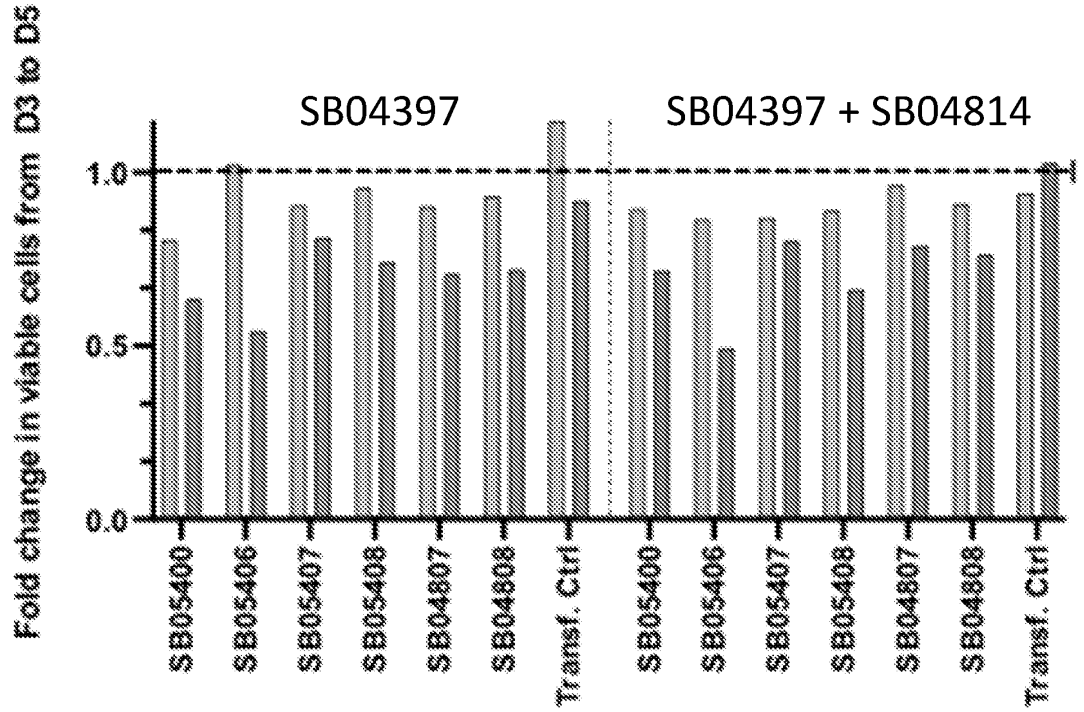


FIG. 4D

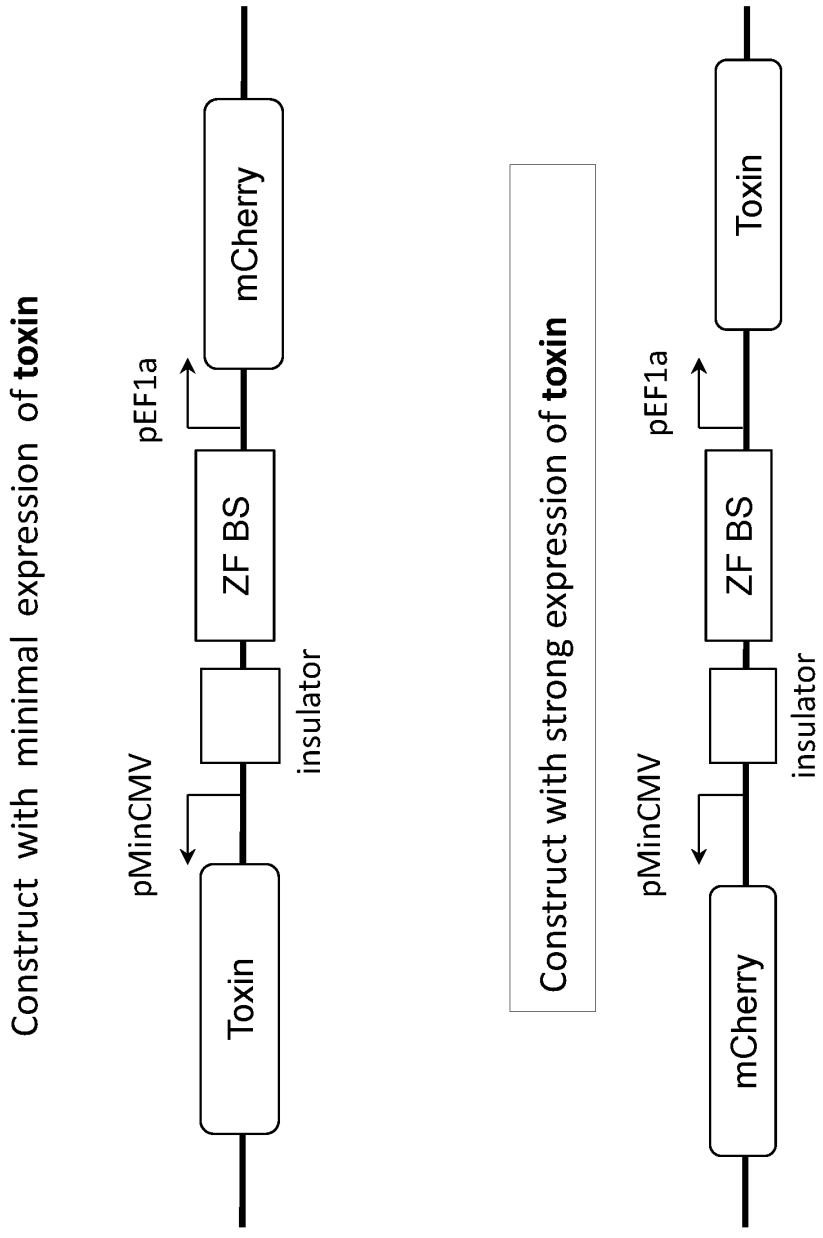
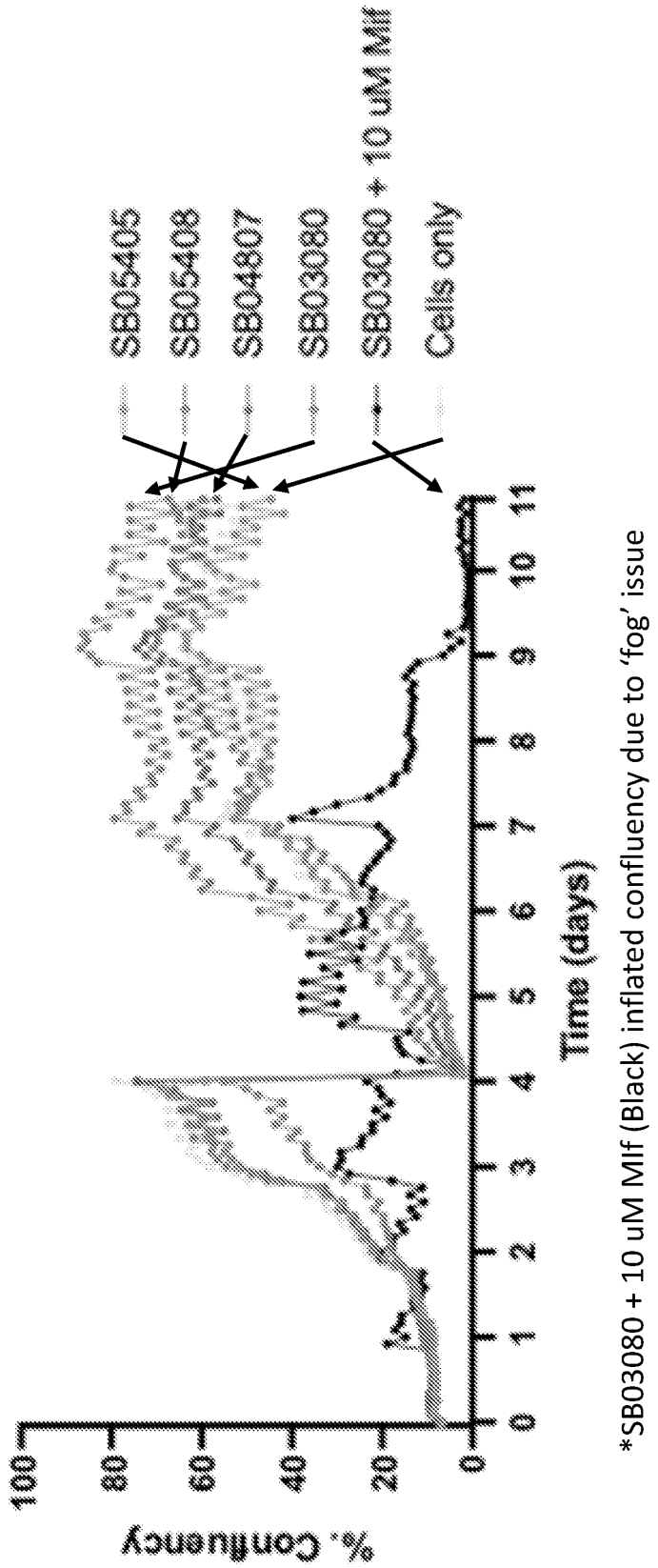
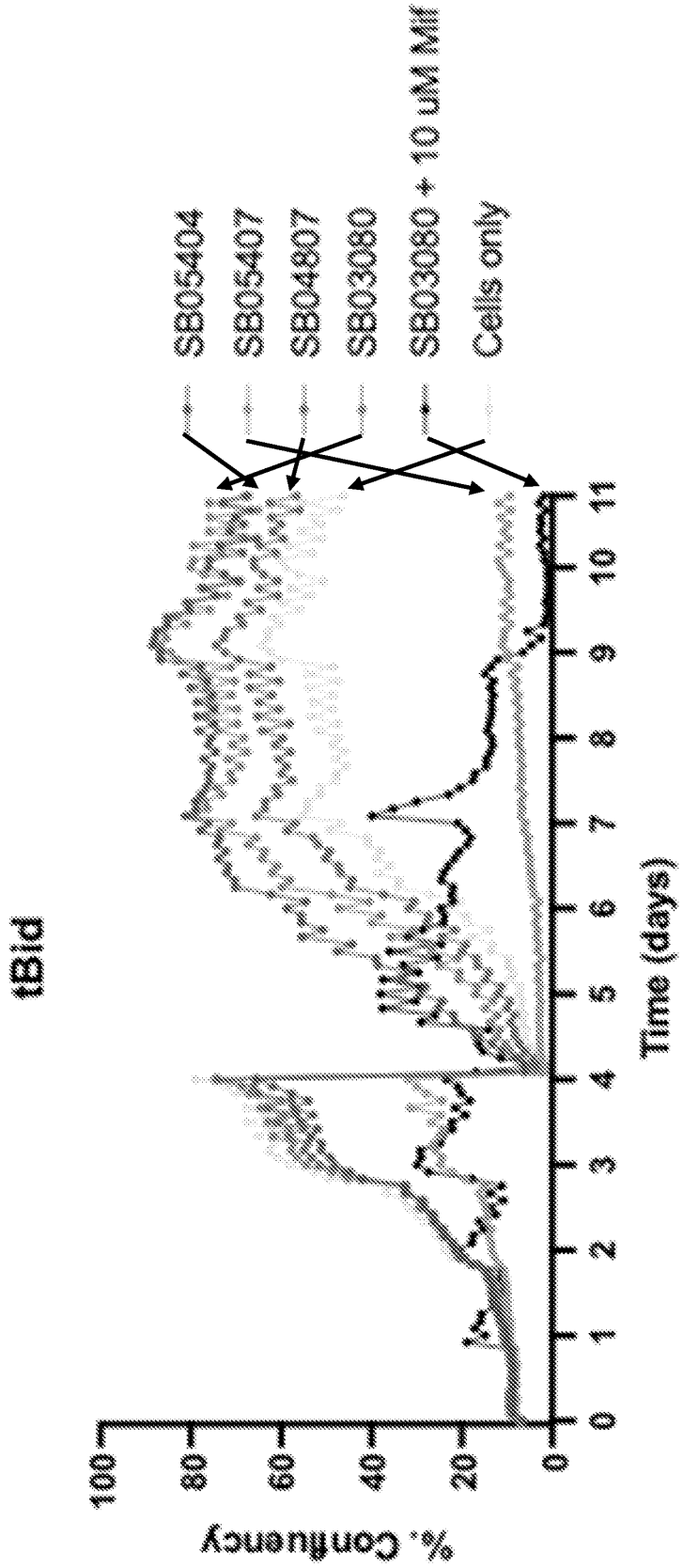

Construct with minimal expression of **toxin**
pMinCMV
Toxin
insulator
ZF BS
pEF1a
mCherry
Construct with strong expression of **toxin**
pMinCMV
mCherry
insulator
ZF BS
pEF1a
Toxin

FIG. 5A



*SB03080 + 10 uM Mif (Black) inflated confluency due to 'fog' issue

FIG. 5B



*SB03080 + 10 uM Mif (Black) inflated confluency due to 'fog' issue

FIG. 5C

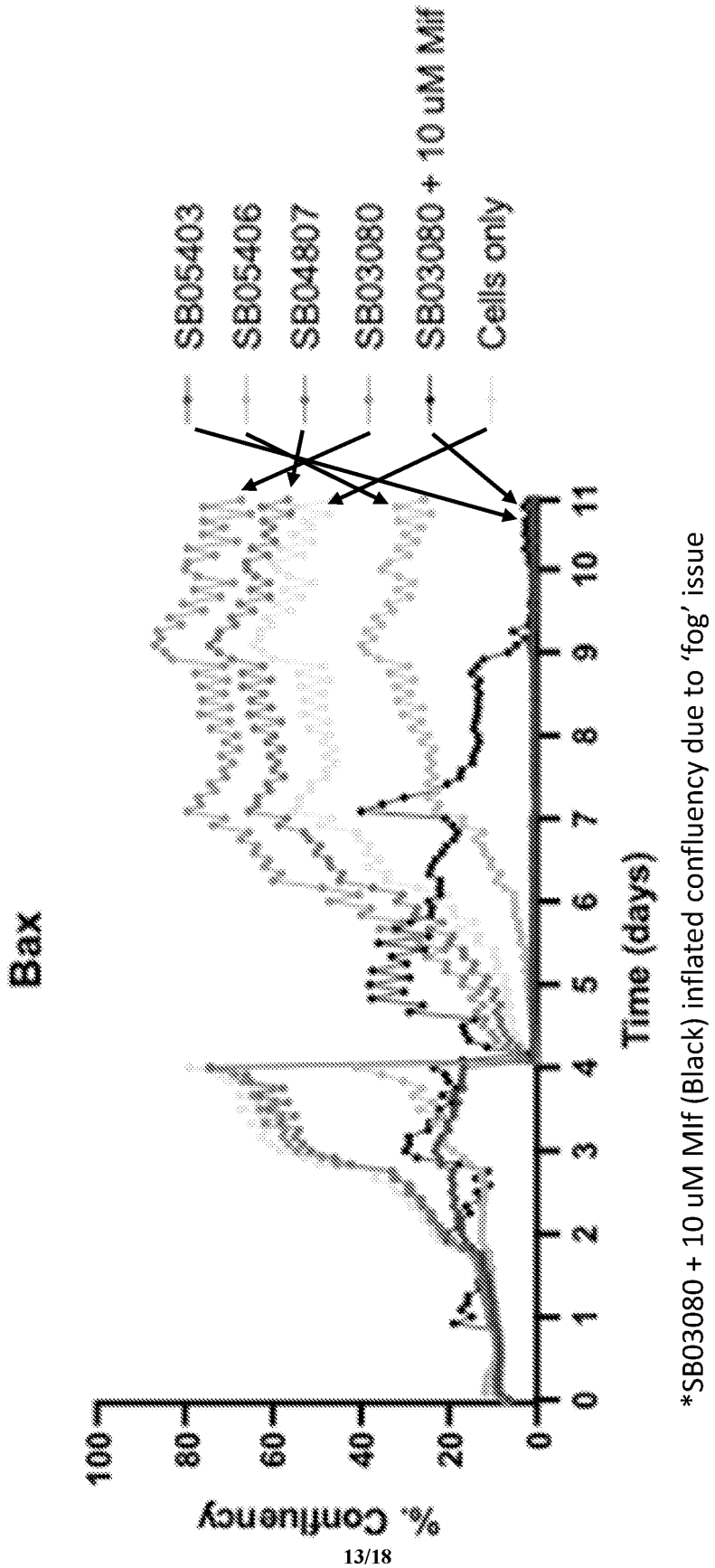

Bax
SB05403
SB05406
SB04807
SB03080
SB03080 + 10 uM Mif
Cells only
% Confluency
0
20
40
60
80
100
0 1 2 3 4 5 6 7 8 9 10 11
Time (days)
*SB03080 + 10 uM Mif (Black) inflated confluency due to 'fog' issue

FIG. 5D

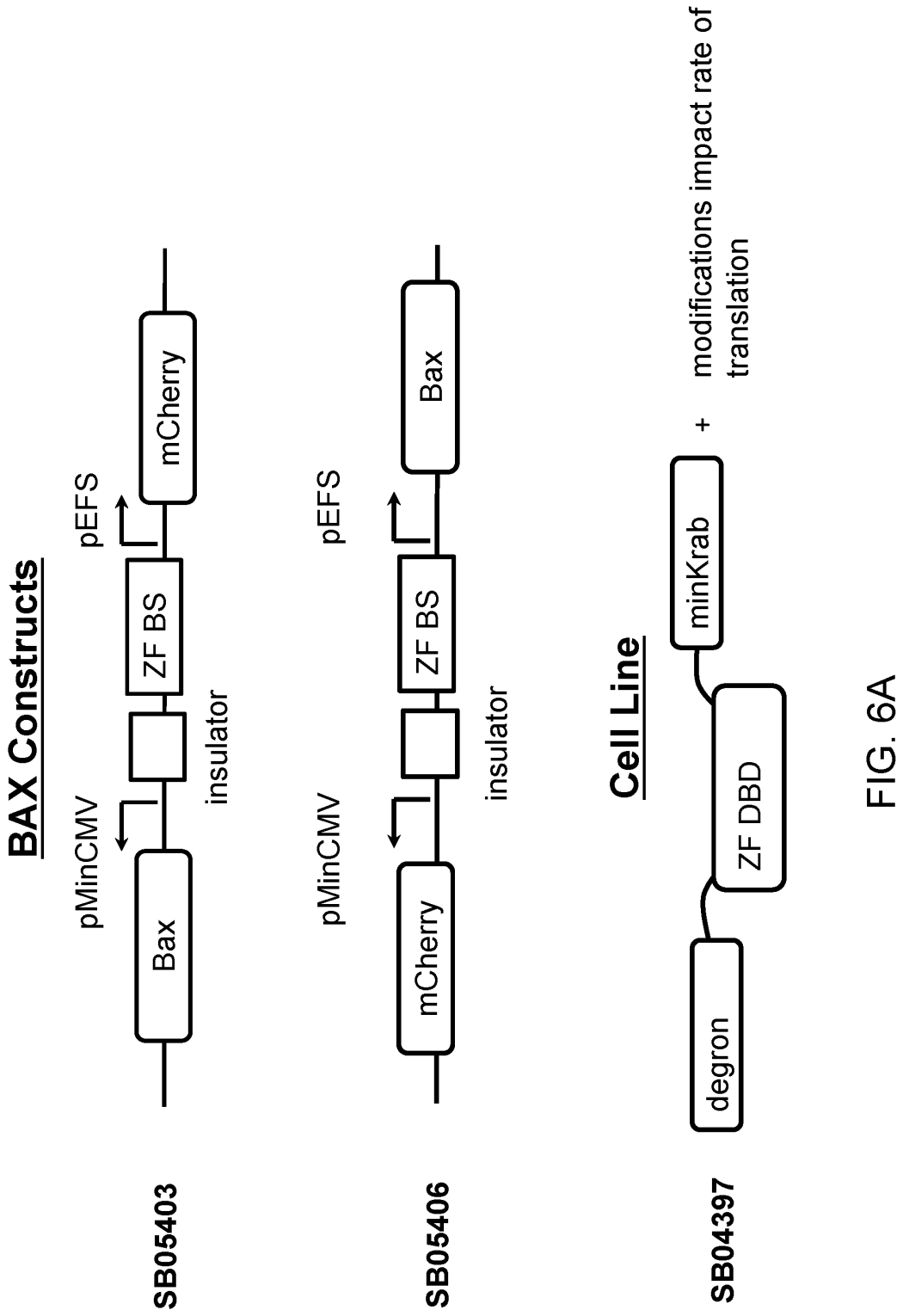

BAX Constructs
pMinCMV
pEFS
Bax
ZF BS
mCherry
insulator
SB05403
pMinCMV
pEFS
mCherry
ZF BS
Bax
insulator
SB05406
Cell Line
degron
ZF DBD
minKrab
+
modifications impact rate of translation
SB04397

FIG. 6A

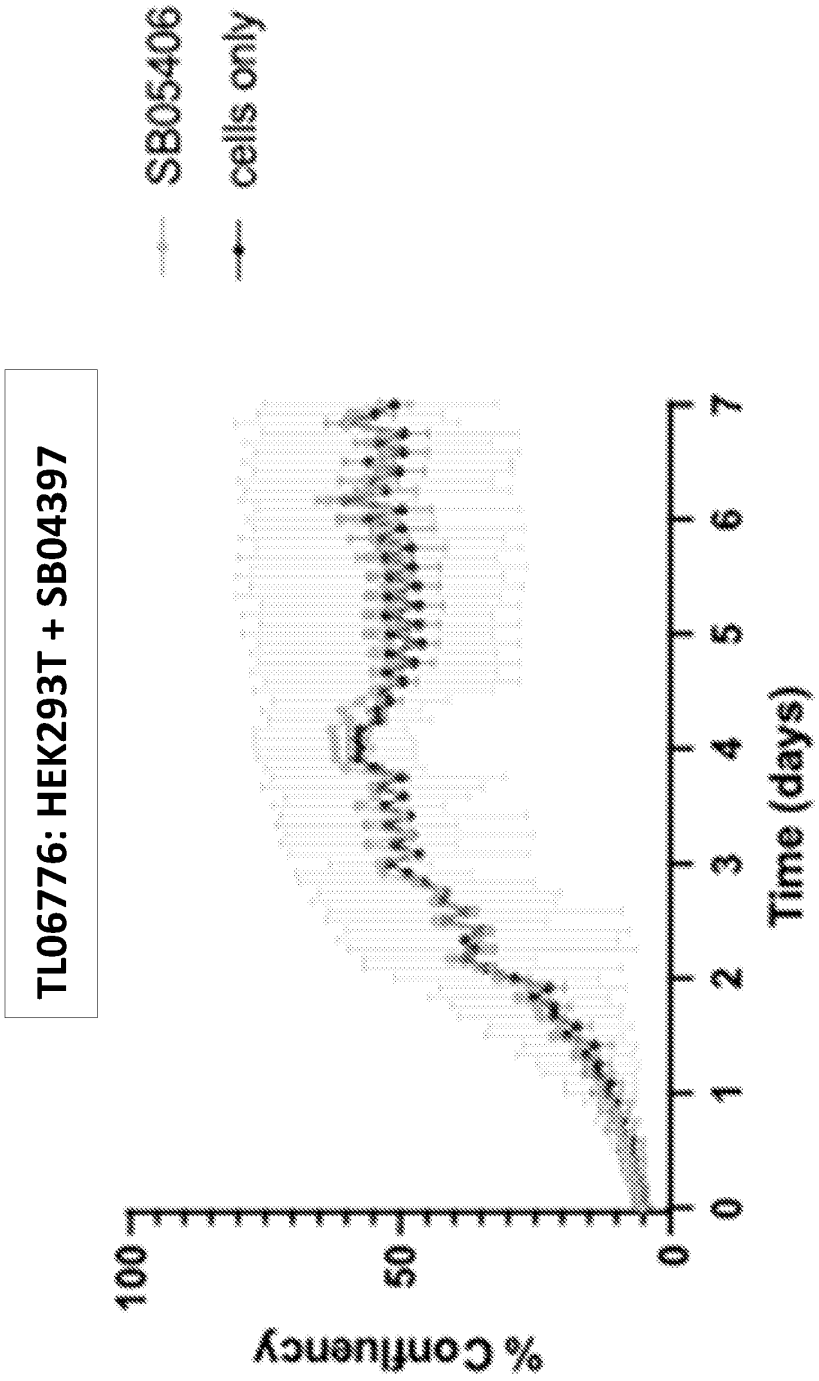

TL06776: HEK293T + SB04397
SB05406
cells only
% Confluency
100
50
0
0
1
2
3
4
5
6
7
Time (days)

FIG. 6B

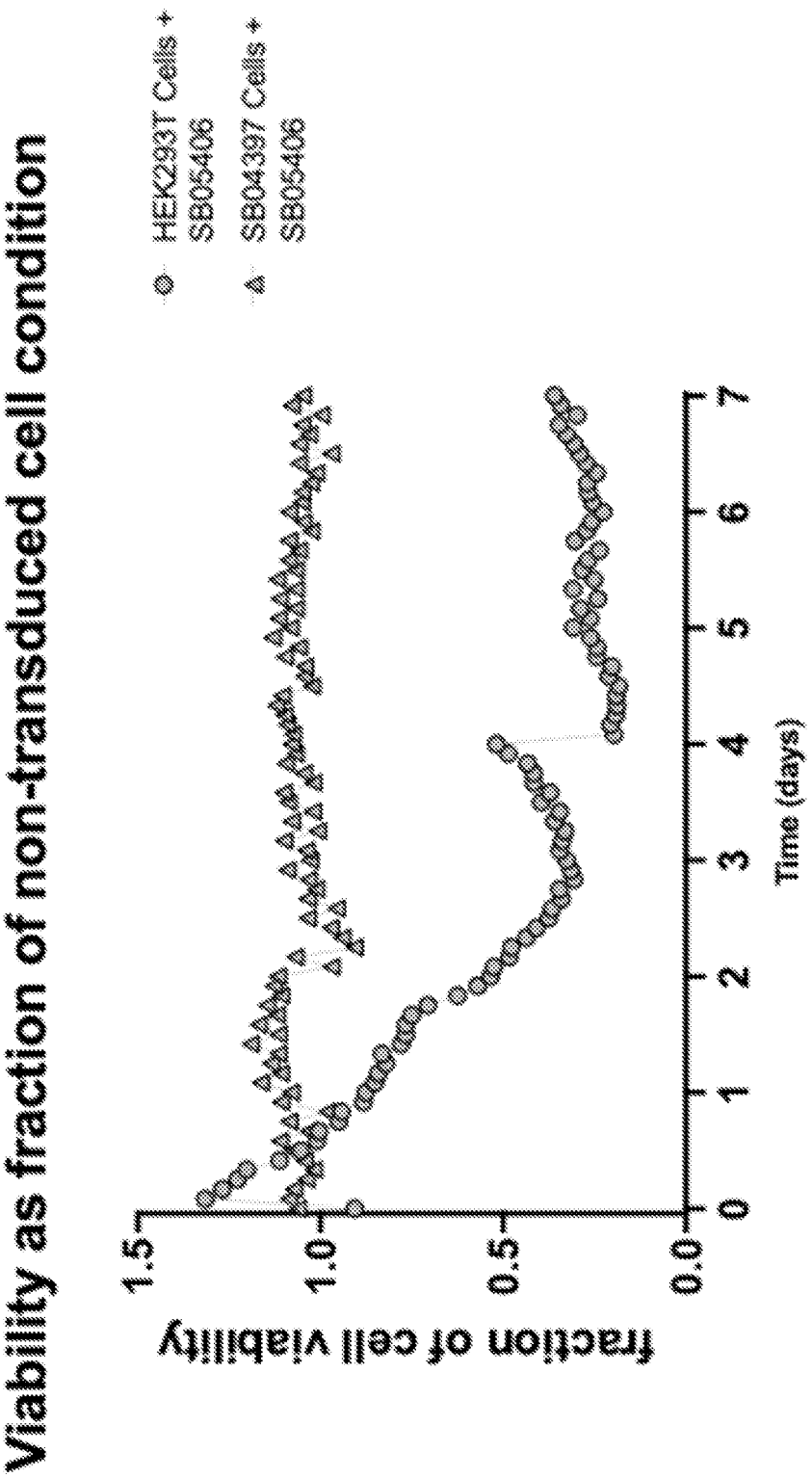

Viability as fraction of non-transduced cell condition
HEK293T Cells + SB05406
SB04397 Cells + SB05406
fraction of cell viability
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7
Time (days)

FIG. 6C

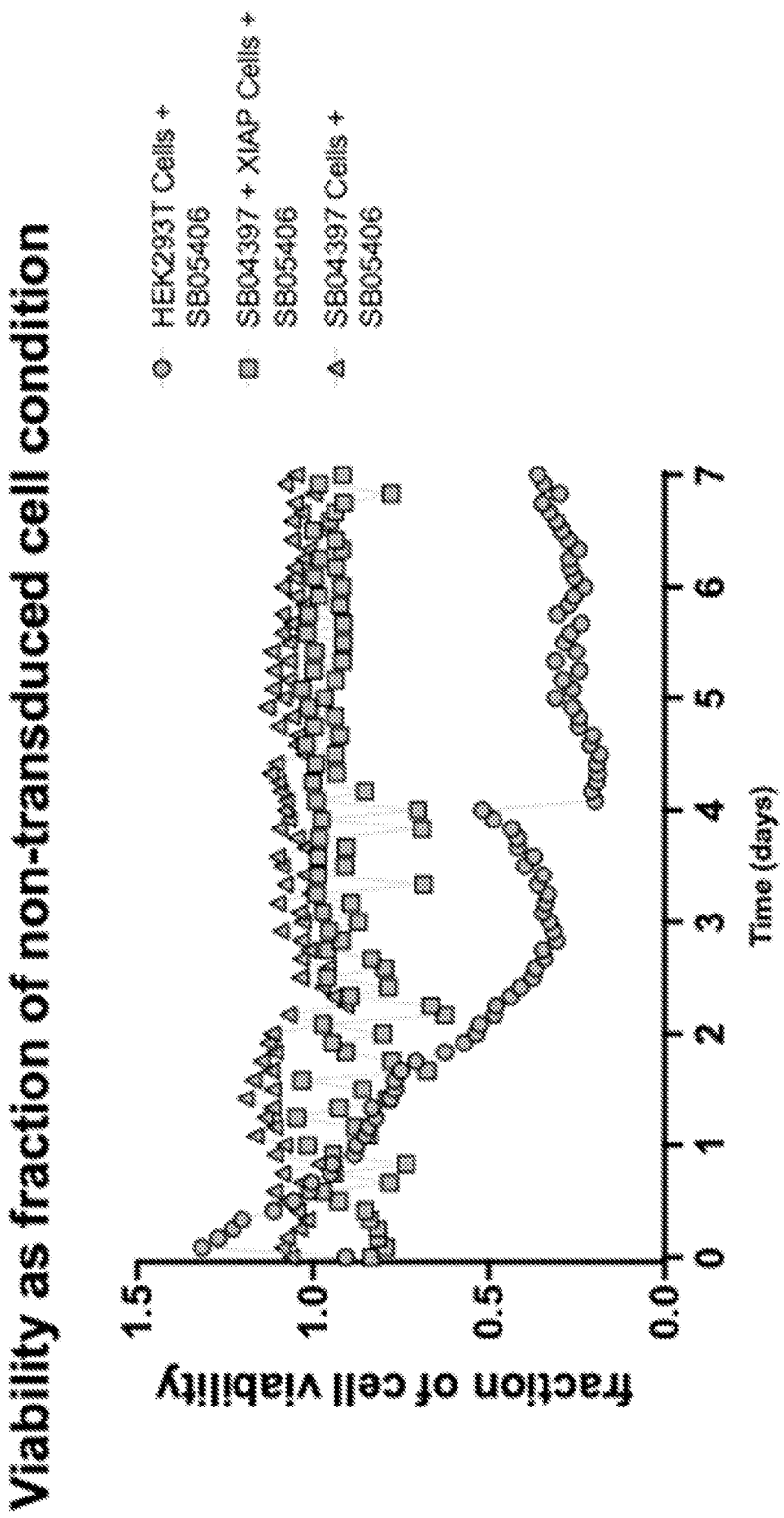

Viability as fraction of non-transduced cell condition
HEK293T Cells + SB05406
SB04397 + XIAP Cells + SB05406
SB04397 Cells + SB05406
fraction of cell viability
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7
Time (days)

FIG. 6D

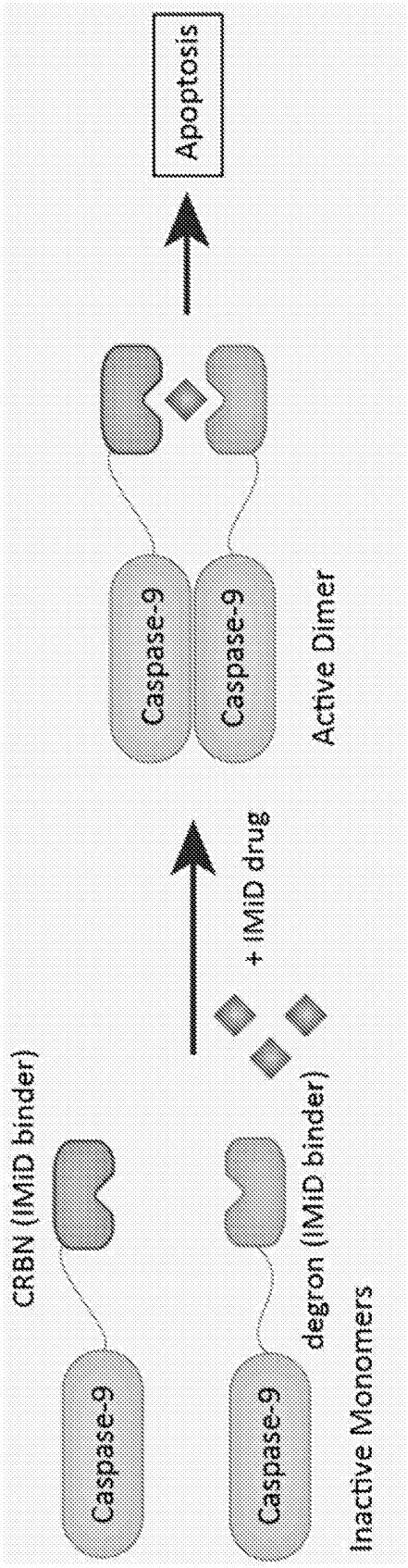

Apoptosis
Caspase-9
Caspase-9
Active Dimer
+ IMiD drug
CRBN (IMiD binder)
degron (IMiD binder)
Caspase-9
Caspase-9
Inactive Monomers

FIG. 7