Science Advances
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Supplementary Materials for

Light-induced control of protein destruction by opto-PROTAC

Jing Liu, He Chen, Leina Ma, Zhixiang He, Dong Wang, Yi Liu, Qian Lin, Tinghu Zhang, Nathanael Gray, H. Ümit Kaniskan, Jian Jin*, Wenyi Wei*

*Corresponding author. Email: wwei2@bidmc.harvard.edu (W.W.); jian.jin@mssm.edu (J.J.)

Published 21 February 2020, *Sci. Adv.* **6**, eaay5154 (2020)
DOI: 10.1126/sciadv.aay5154

This PDF file includes:

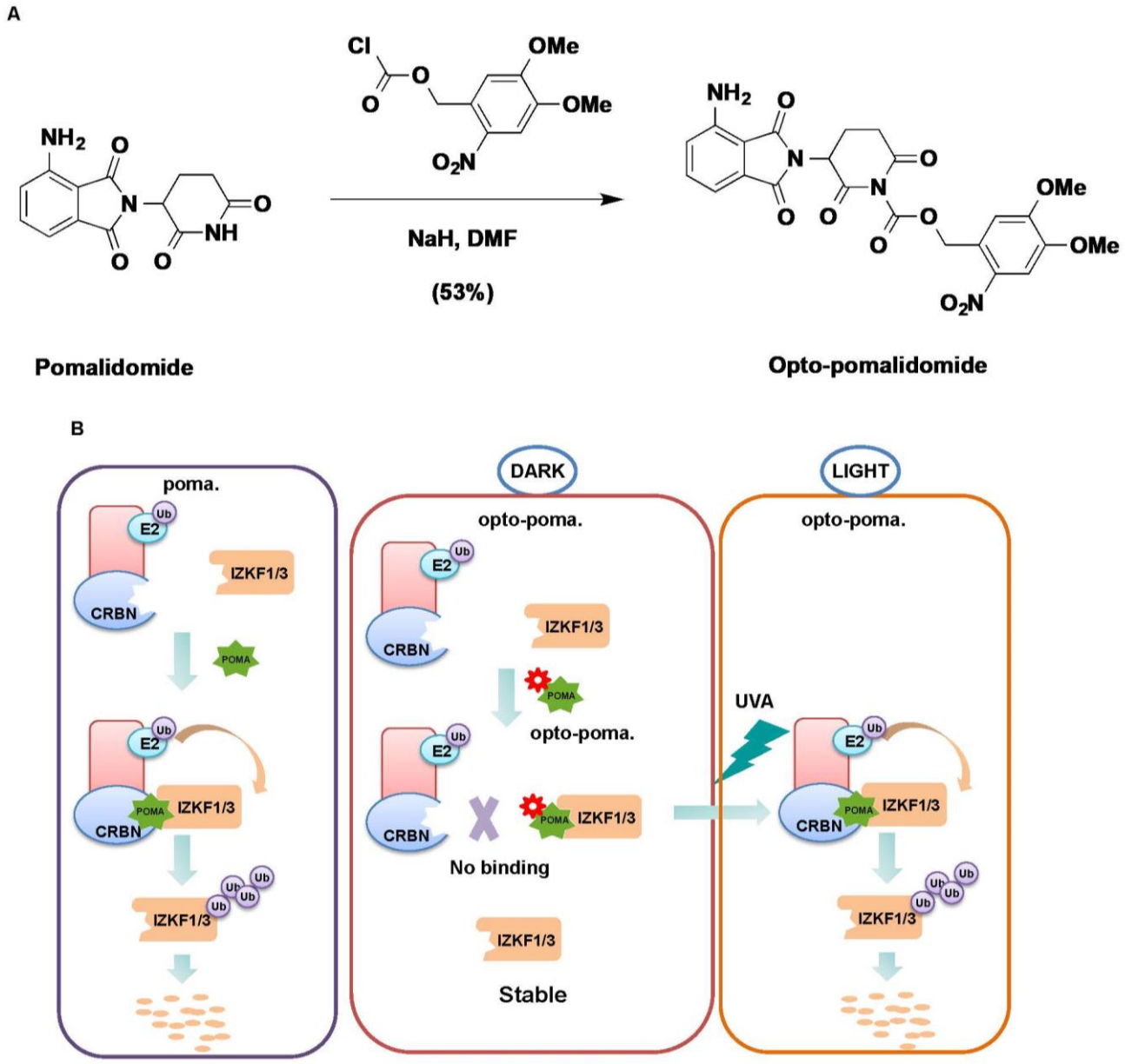

Fig. S1. The schematic illustration of the synthesis of opto-pomalidomide and the working model for opto-pomalidomide to achieve light-induced degradation of IKZF1/3. A) Synthesis of opto-pomalidomide from pomalidomide. B) A schematic illustration for the working model of constitutively active degradation of IKZFs by pomalidomide vs. light inducible degradation of IKZFs by opto-pomalidomide.

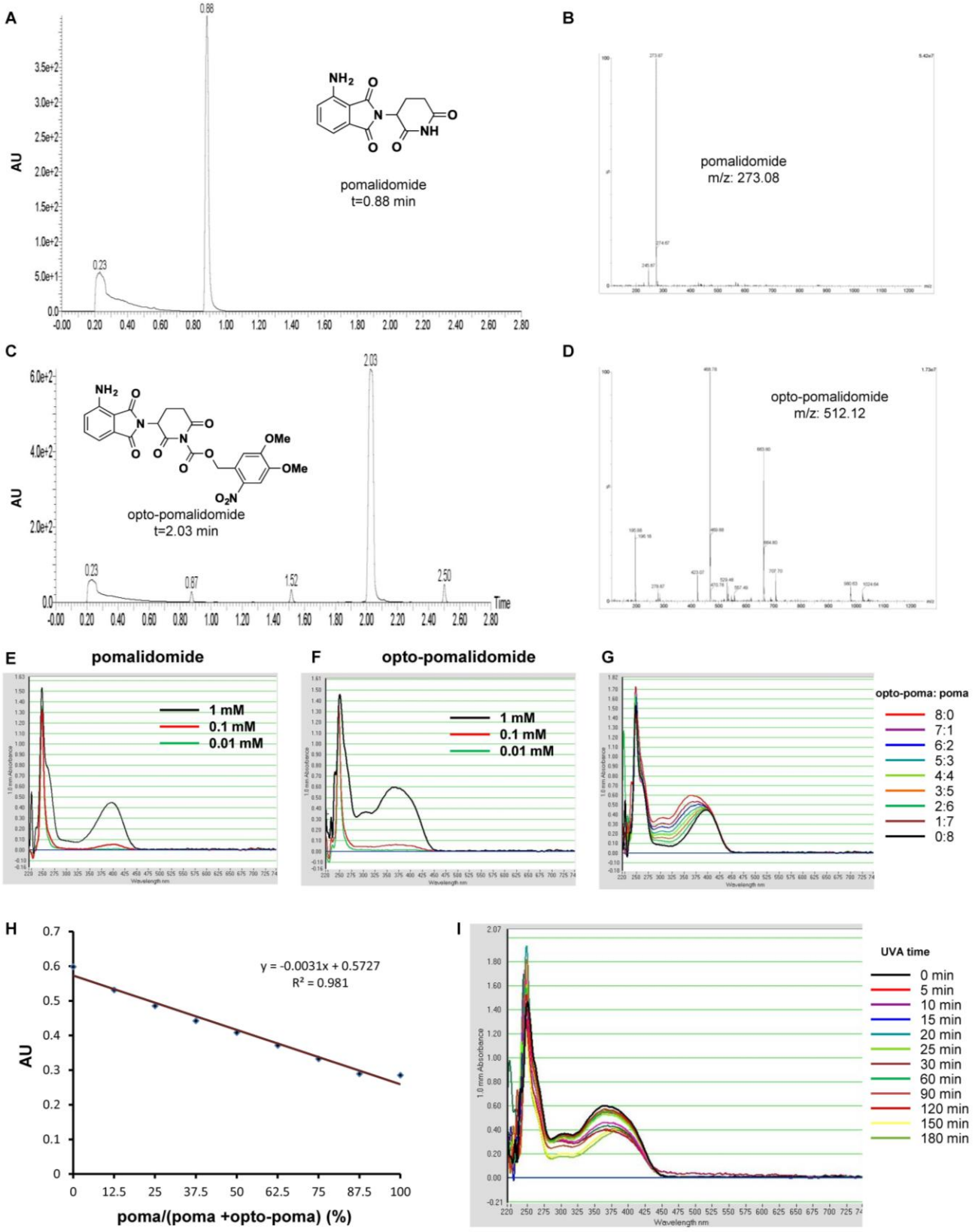
A
B
C
D
E
pomalidomide
F
opto-pomalidomide
G
H
I
AU
pomalidomide
t=0.88 min
pomalidomide
m/z: 273.08
opto-pomalidomide
t=2.03 min
opto-pomalidomide
m/z: 512.12
Time
1 mM
0.1 mM
0.01 mM
opto-poma: poma
8:0
7:1
6:2
5:3
4:4
3:5
2:6
1:7
0:8
y = -0.0031x + 0.5727
R² = 0.981
poma/(poma +opto-poma) (%)
UVA time
0 min
5 min
10 min
15 min
20 min
25 min
30 min
60 min
90 min
120 min
150 min
180 min
Wavelength nm

Fig. S2. UPLC-MS characterization and the time-dependent uncaging of pomalidomide and opto-pomalidomide. A) UPLC analysis of pomalidomide. B) Mass spectrum of pomalidomide by UPLC-MS. C) UPLC analysis of opto-pomalidomide. D) Mass spectrum of opto-pomalidomide by UPLC-MS. E) UV-VIS absorption of pomalidomide. F) UV-VIS absorption of opto-pomalidomide. G) UV-VIS absorption of pomalidomide and opto-pomalidomide mixture. H) Standard curve of pomalidomide and opto-pomalidomide mixed at corresponding ratio. I) UV-VIS absorption of opto-pomalidomide after irradiation with UVA (365nm) for indicated time.

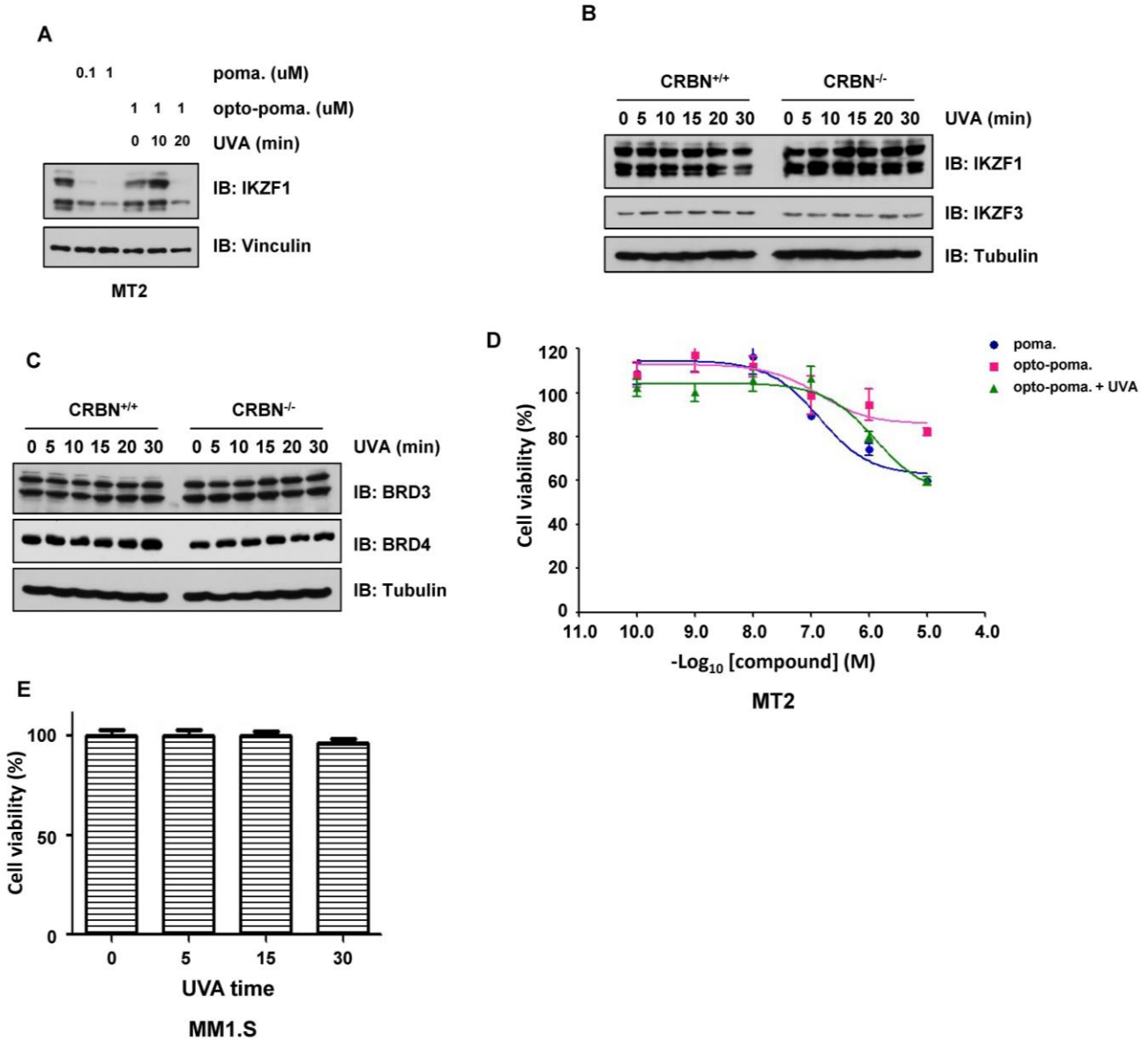
A
0.1 1
poma. (uM)
1 1 1
opto-poma. (uM)
0 10 20
UVA (min)
IB: IKZF1
IB: Vinculin
MT2
B
CRBN+/+
CRBN-/-
0 5 10 15 20 30 0 5 10 15 20 30
UVA (min)
IB: IKZF1
IB: IKZF3
IB: Tubulin
C
CRBN+/+
CRBN-/-
0 5 10 15 20 30 0 5 10 15 20 30
UVA (min)
IB: BRD3
IB: BRD4
IB: Tubulin
D
poma.
opto-poma.
opto-poma. + UVA
Cell viability (%)
120
100
80
60
40
20
0
11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0
-Log10 [compound] (M)
MT2
E
Cell viability (%)
100
50
0
0 5 15 30
UVA time
MM1.S

Fig. S3. Uncaging of opto-pomalidomide by UVA irradiation leads to active degradation of IKZFs in multiple myeloma cancer cell lines. A) UVA irradiation activates opto-pomalidomide to mediate IKZF1 degradation in MT2 cells. B) UVA irradiation (365nm) alone does not lead to degradation of IKZF1/3 in MM1.S. C) UVA irradiation (365nm) alone does not lead to degradation of IKZF1/3 in 293FT cells. D) UVA irradiation-activated opto-pomalidomide inhibits MT2 cell proliferation in a dose-dependent manner. MT2 cells were treated by pomalidomide vs. opto-pomalidomide with or without UVA irradiation (365 nm) for 15 min, and then subjected to CCK-8 cell viability assay. E) UVA irradiation (365 nm) for 5-30 min has minimal effect on the cell viability of MM1.S cells in the absence of opto-pomalidomide. MM1.S cells were illuminated with UVA for indicated time, and then subjected to CCK-8 cell viability assay.

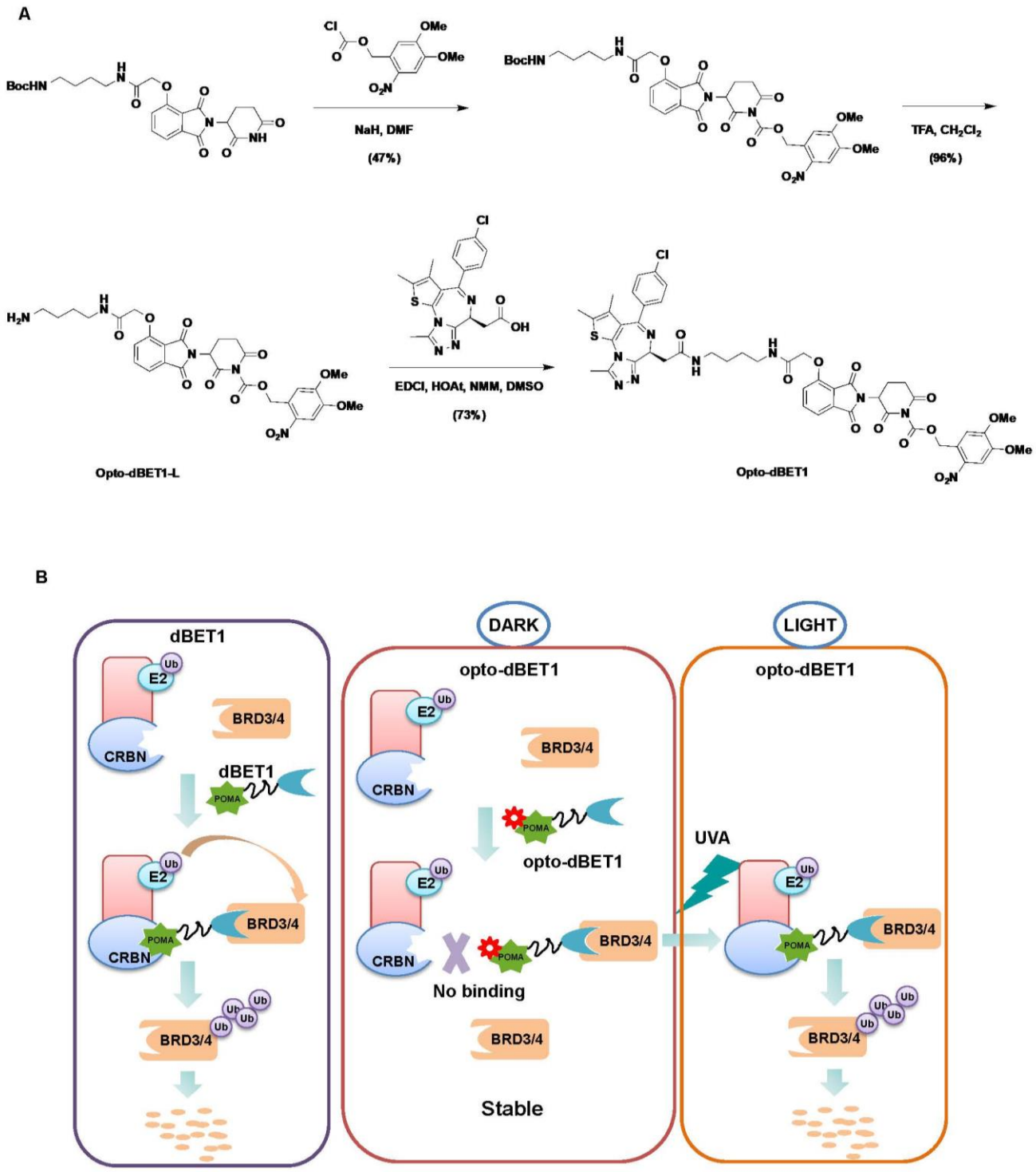

Fig. S4. The schematic illustration of the synthesis of opto-dBET1 and the working model for opto-dBET1 to achieve light-induced degradation of BRDs. A) Synthesis of opto-dBET1. B) A schematic illustration for the working model of dBET1 vs. opto-dBET1 vs. opto-dBET1 + UVA irradiation on promoting degradation of BRDs.

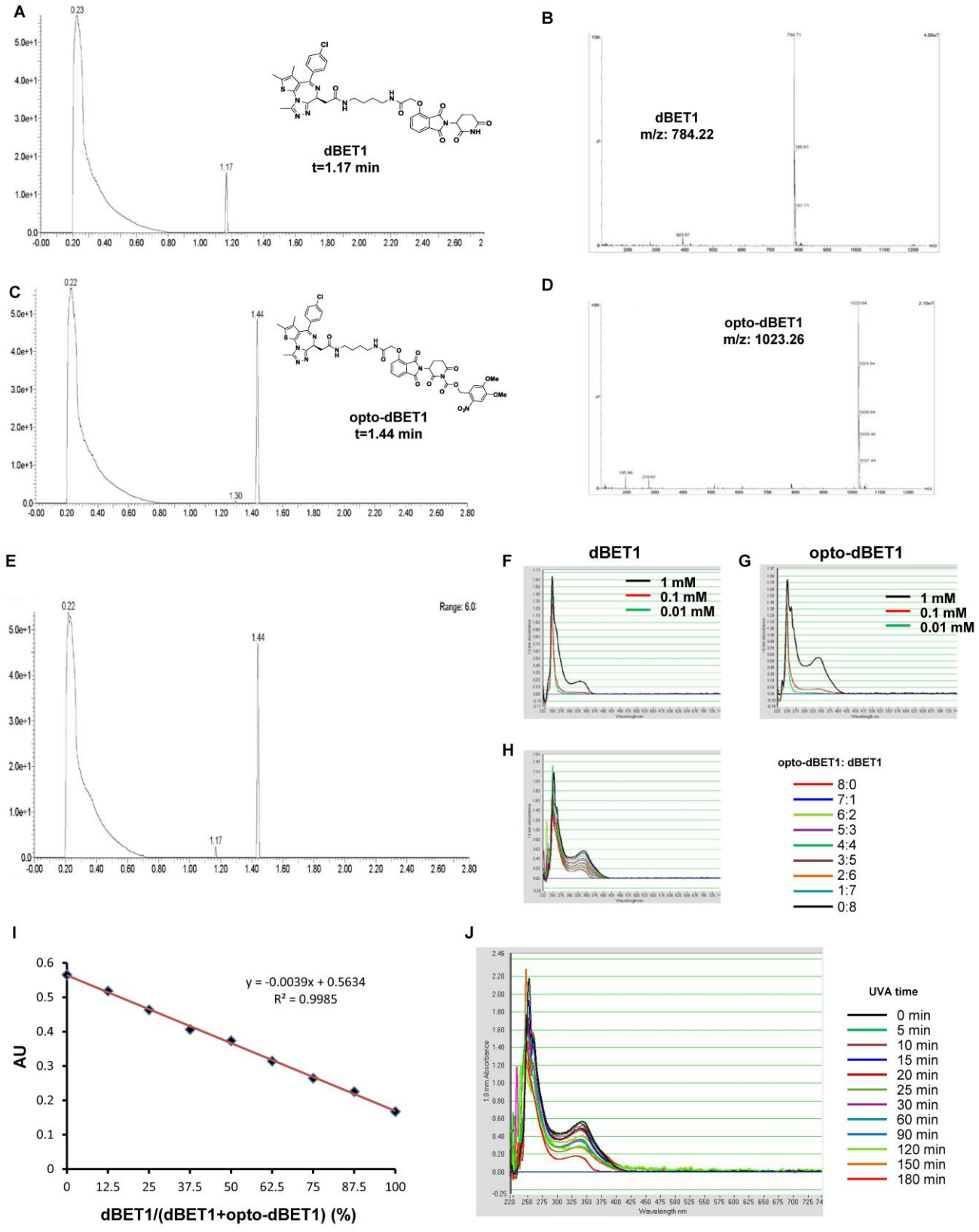
A
dBET1
t=1.17 min
B
dBET1
m/z: 784.22
C
opto-dBET1
t=1.44 min
D
opto-dBET1
m/z: 1023.26
E
F
dBET1
1 mM
0.1 mM
0.01 mM
G
opto-dBET1
1 mM
0.1 mM
0.01 mM
H
opto-dBET1: dBET1
8:0
7:1
6:2
5:3
4:4
3:5
2:6
1:7
0:8
I
y = -0.0039x + 0.5634
R² = 0.9985
AU
dBET1/(dBET1+opto-dBET1) (%)
J
UVA time
0 min
5 min
10 min
15 min
20 min
25 min
30 min
60 min
90 min
120 min
150 min
180 min

Fig. S5. UPLC-MS characterization and the time-dependent uncaging of opto-dBET1 by UVA irradiation in vitro. A) UPLC analysis of dBET1. B) Mass spectrum of dBET1 by UPLC-MS. C) UPLC analysis of opto-dBET1. D) Mass spectrum of opto-dBET1 by UPLC-MS. E) UPLC analysis of opto-dBET1 after irradiation with UVA (365nm) for 30 minutes. F) UV-VIS absorption of dBET1. G) UV-VIS absorption of opto-dBET1. H) UV-VIS absorption of dBET1 and opto-dBET1 mixture at the corresponding ratio. D. Standard curve of dBET1 and opto-dBET1 at the corresponding ratio. I) UV-VIS absorption of opto-dBET1 after irradiation with UVA (365nm) for indicated time.

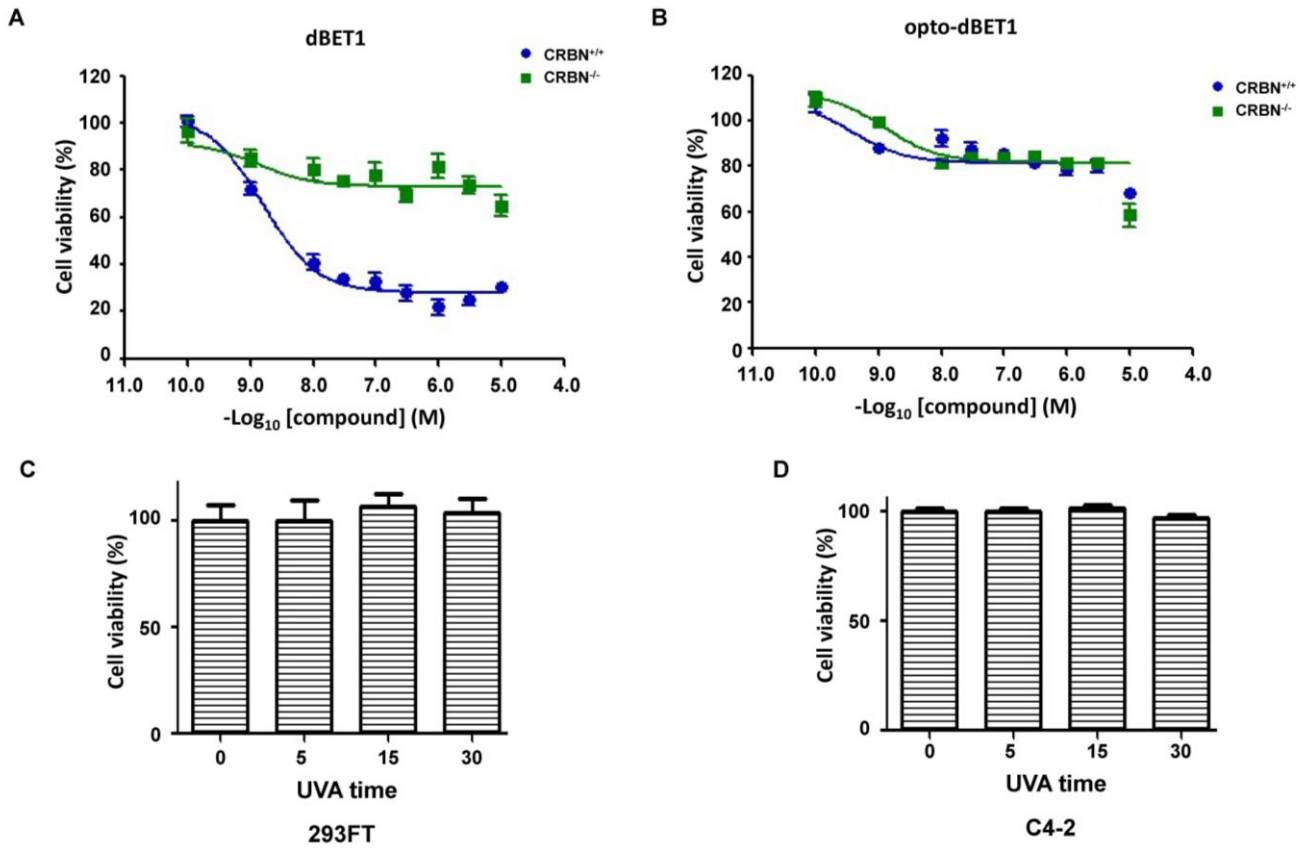

Fig. S6. dBET1 inhibits cell proliferation in a CRBN-dependent manner. A) dBET1 inhibits cell proliferation in 293FT-*CRBN*$^{+/+}$ but not 293FT-*CRBN*$^{-/-}$ cells. B) opto-dBET1 did not inhibit cell proliferation in 293FT-*CRBN*$^{+/+}$ and 293FT-*CRBN*$^{-/-}$ cells. 293FT-*CRBN*$^{+/+}$ and 293FT-*CRBN*$^{-/-}$ cells were treated by dBET1 vs. opto-dBET1 for 72 hours, and then subjected to CCK-8 cell viability assay. C-D) UVA irradiation (365 nm) for 5-30 min has minimal effect on the cell viability of 293FT (C) and C4-2 (D) cells in the absence of opto-dBET1. 293FT and C4-2 cells were illuminated with UVA for indicated time, and then subjected to CCK-8 cell viability assay.

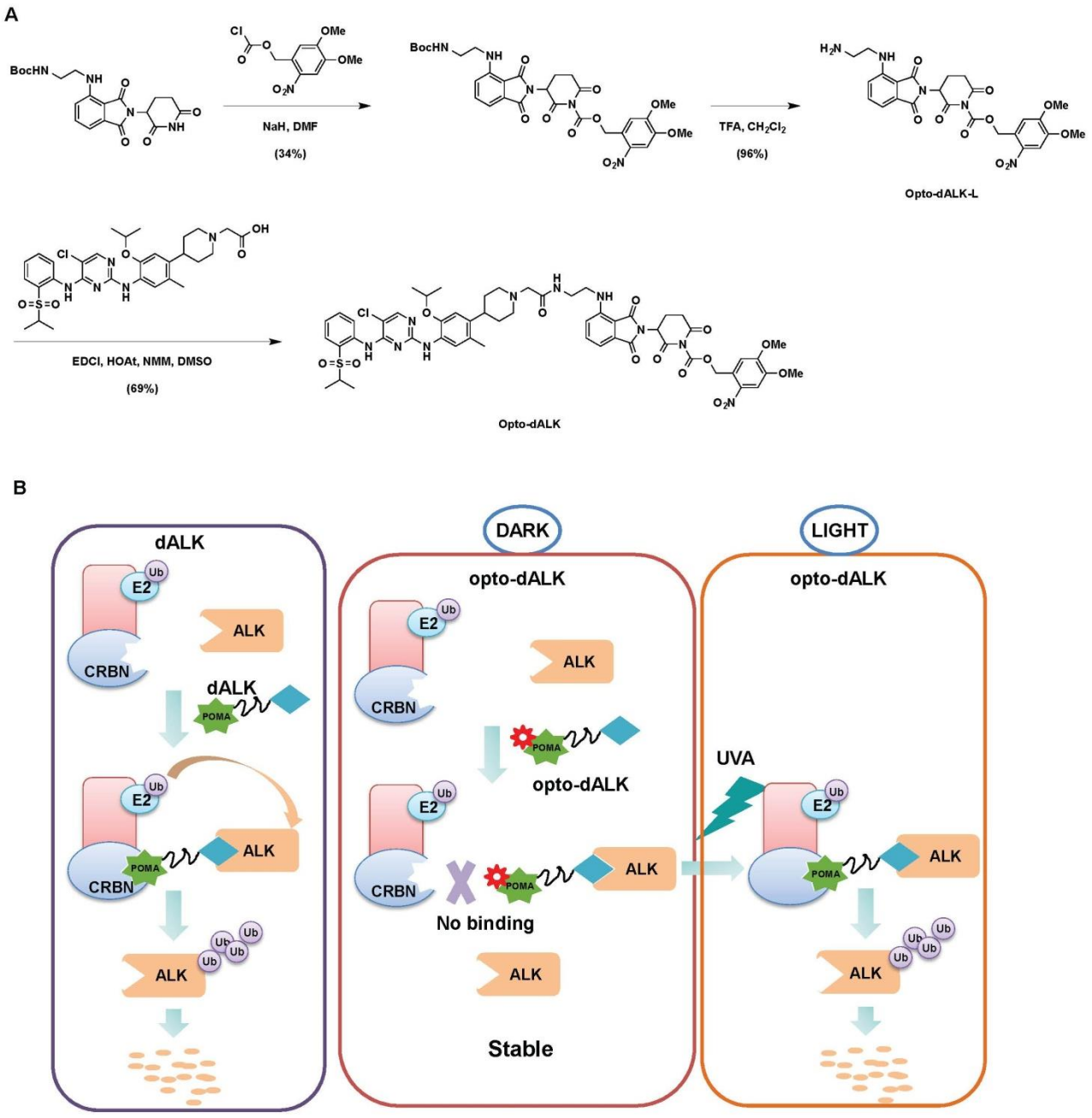

Fig. S7. The schematic illustration of the synthesis of opto-dALK and the working model for opto-dALK to achieve light-induced degradation of ALK fusion proteins. A) Synthesis of opto-dALK. B) A schematic illustration for the working model of dALK vs. opto-dALK vs. opto-dALK + UVA irradiation (365nm) on promoting the degradation of ALK fusion proteins.

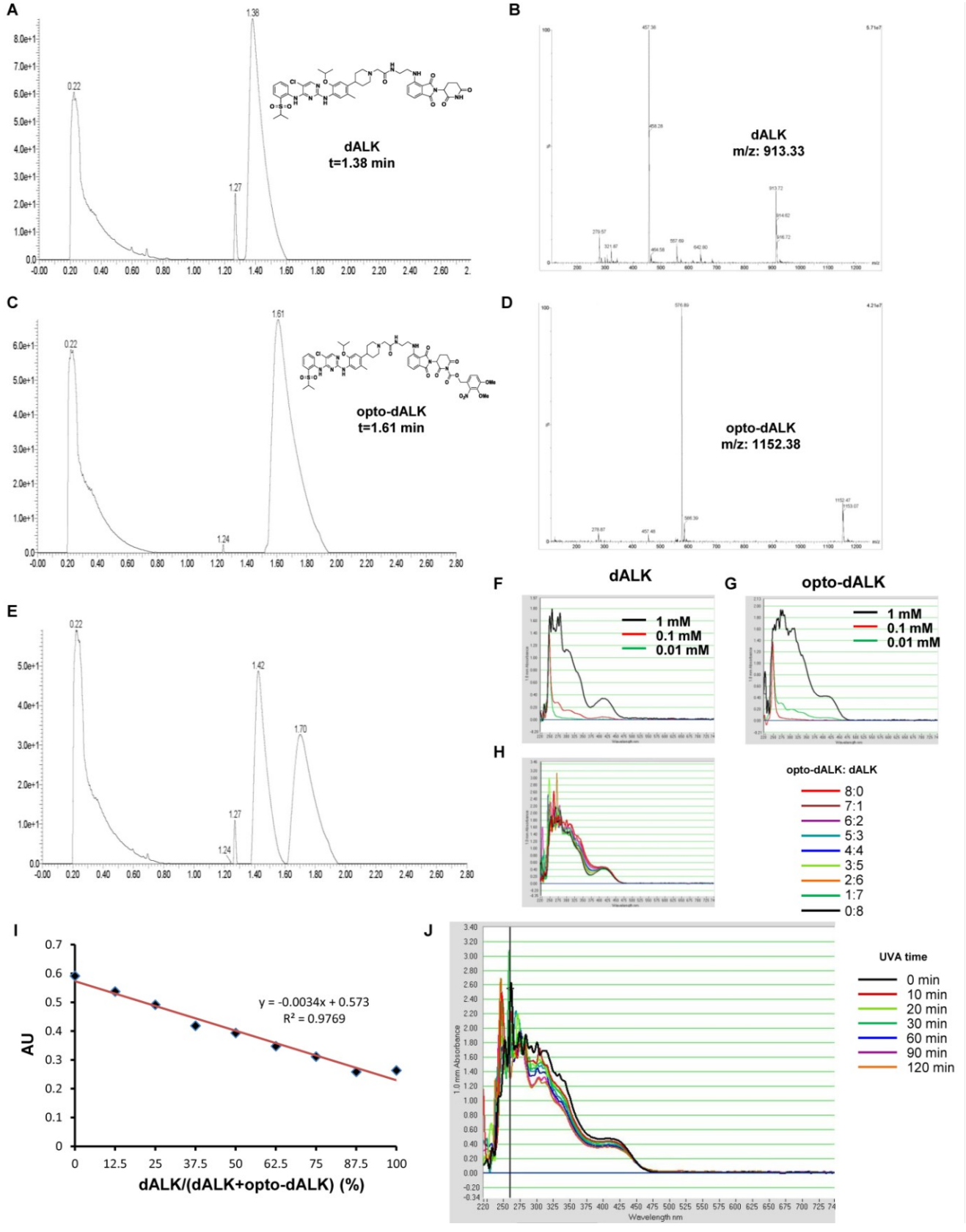
A
dALK
t=1.38 min
B
dALK
m/z: 913.33
C
opto-dALK
t=1.61 min
D
opto-dALK
m/z: 1152.38
E
F
dALK
1 mM
0.1 mM
0.01 mM
G
opto-dALK
1 mM
0.1 mM
0.01 mM
H
opto-dALK: dALK
8:0
7:1
6:2
5:3
4:4
3:5
2:6
1:7
0:8
I
y = -0.0034x + 0.573
R² = 0.9769
AU
dALK/(dALK+opto-dALK) (%)
J
UVA time
0 min
10 min
20 min
30 min
60 min
90 min
120 min
1.0 mm Absorbance
Wavelength nm

Fig. S8. UPLC-MS characterization and the time-dependent uncaging process of opto-dALK. A) UPLC analysis of dALK. B) Mass spectrum of dALK by UPLC-MS. C) UPLC analysis of opto-dALK. D) Mass spectrum of opto-dALK by UPLC-MS. E). UPLC analysis of opto-dALK after irradiation with UVA (365nm) for 30 minutes. F) UV-VIS absorption of dALK. G) UV-VIS absorption of opto-dALK. H) UV-VIS absorption of dALK and opto-dALK mixture at corresponding ratio. I) Standard curve of dALK and opto-dALK mixed at corresponding ratio. J) UV-VIS absorption of opto-dALK after irradiation with UVA (365nm) for indicated time.

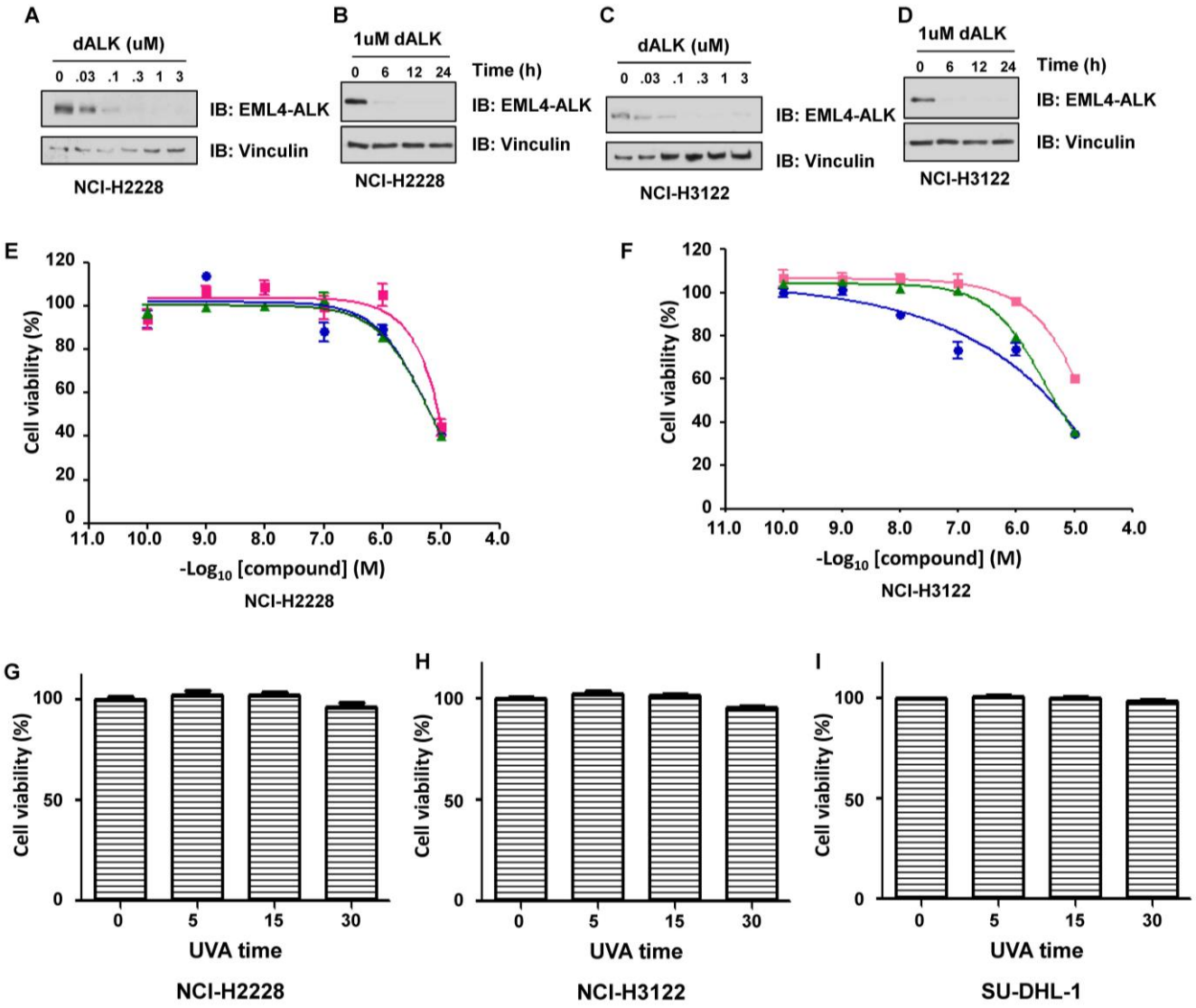
A
dALK (uM)
0 .03 .1 .3 1 3
IB: EML4-ALK
IB: Vinculin
NCI-H2228
B
1uM dALK
0 6 12 24
Time (h)
IB: EML4-ALK
IB: Vinculin
NCI-H2228
C
dALK (uM)
0 .03 .1 .3 1 3
IB: EML4-ALK
IB: Vinculin
NCI-H3122
D
1uM dALK
0 6 12 24
Time (h)
IB: EML4-ALK
IB: Vinculin
NCI-H3122
E
Cell viability (%)
-Log10 [compound] (M)
NCI-H2228
F
Cell viability (%)
-Log10 [compound] (M)
NCI-H3122
G
Cell viability (%)
UVA time
NCI-H2228
H
Cell viability (%)
UVA time
NCI-H3122
I
Cell viability (%)
UVA time
SU-DHL-1

Fig. S9. Dose-dependent degradation of ALK fusion proteins by dALK. A-D) dALK promotes the degradation of EML4-ALK fusion proteins in NCI-H2228 (A, B) and NCI-H3122 (C, D) NSCLC cell lines. NCI-H2228 and NCI-H3122 were treated with dALK at indicated dose or for indicated time, and then subjected to analysis of the level of EML-ALK fusion protein. E-F) The cell proliferation of NCI-H2228 (E) and NCI-H3122 (F) NSCLC cell lines was relative resistant to ALK degrader. NCI-H2228 and NCI-H3122 NSCLC cell lines were treated by dALK vs. opto-dALK with or without UVA irradiation, and then subjected to CCK-8 cell viability assay. G-I) UVA irradiation alone (365 nm) for 5-30 min has minimal effect on the cell viability of NCI-H2228 (G), NCI-H3122 (H) and SU-DHL-1 (I) cells in the absence of opto-ALK. NCI-H2228, NCI-H3122 and SU-DHL-1 cells were illuminated with UVA for indicated time, and then subjected to CCK-8 cell viability assay.