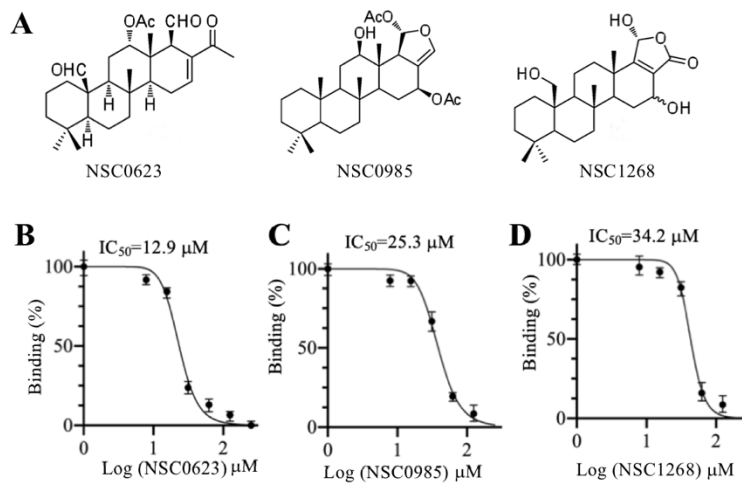


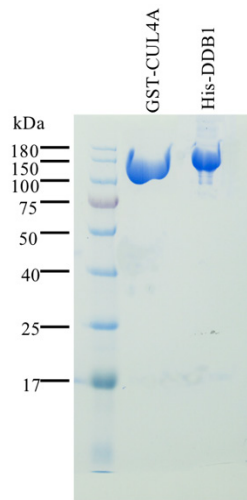
**Supplementary Figure 1. Knockdown of *CUL4A/4B* and *DDB1* significantly inhibited tumor cell proliferation.**

**(A)** The mRNA levels of *CUL4A/4B* and *DDB1* in their corresponding knockdown cells. HCT-116 cells were transfected with pLKO.1, shCUL4A (CUL4A-KD), shCUL4B (CUL4B-KD), and shDDB1 (DDB1-KD), respectively. The obtained knockdown cells were subjected to RNA isolation and qRT-PCR analyses to examine mRNA levels of *CUL4A*, *CUL4B* and *DDB1*. \*\*\* $P < 0.001$ . **(B)** Knockdown of *CUL4A/4B* and *DDB1* decreased cell proliferation. Cells used in (A) were used to examine cell viability at 4 h (0 days), 1, 2, 3, 4, and 5 days by an MTT assay with absorbance measurement at 590 nm. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .



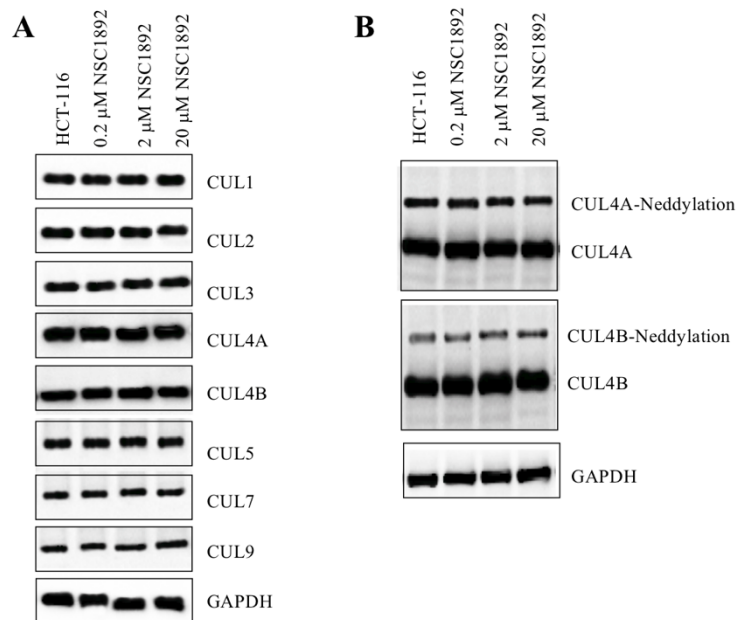
**Supplementary Figure 2. Identified small molecules using AlphaScreen system.**

(A) The chemical structure of small molecules. The chemical structures of three other small molecules (NSC0623, NSC0985 and NSC1268) that disrupt the CUL4A-DDB1 interaction. (B-D) Different effects of small molecules on the CUL4A-DDB1 interaction. A secondary AlphaScreen assay was performed to determine the inhibitory effect of NSC0623, NSC0985 and NSC1268 on the CUL4A-DDB1 interaction.



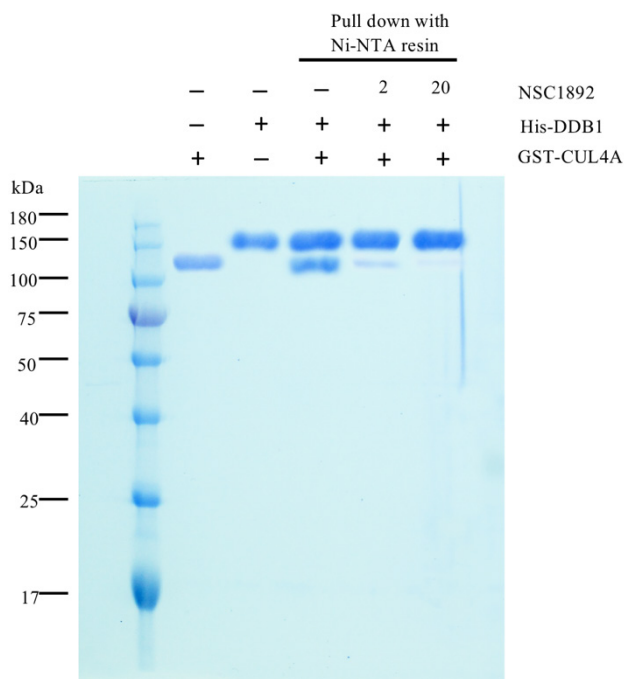
**Supplementary Figure 3. The purified GST-CUL4A and His-DDB1 proteins.**

The pET28a-DDB1 and pGEX-6P-1-CUL4A plasmids were transformed into an *Escherichia coli* strain BL21 (DE3.0), respectively. The positive colonies were grown in liquid lysogeny broth (LB) medium containing antibiotics to logarithmic phase. The GST-CUL4A and His-DDB1 proteins were purified using Glutathione Sepharose 4B resin and Ni-NTA resin, respectively. Equal amounts of GST-CUL4A and His-DDB1 proteins were loaded into a 10% SDS-PAGE gel for separation and then stained with Coomassie blue.



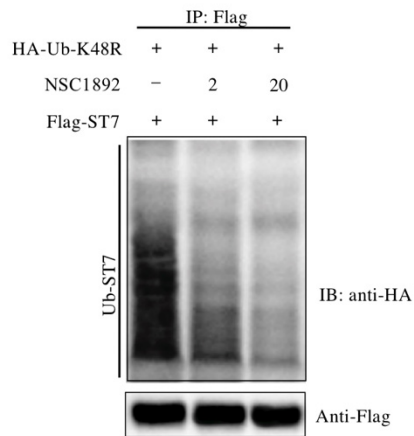
**Supplementary Figure 4. NSC1892 could not change the protein levels of cullin members and the neddylation of ST7.**

(A) NSC1892 treatment could not change the protein levels of cullin members. The HCT-116 cells were treated with different concentrations (0, 0.2, 2, and 20 μM) of NSC1892 for 6 hrs. The resulting cells were subjected to protein isolation. The protein levels of CUL1, CUL2, CUL3, CUL4A, CUL4B, CUL5, CUL7 and CUL9 were determined by western blotting. GAPDH was used as a loading control. (B) NSC1892 treatment could not change the neddylation of ST7. Proteins used in (A) were loaded into a 10% SDS-PAGE for efficient separation (run 2 hrs at 180 V). The neddylation of CUL4A /4B was determined using anti-CUL4A and anti-CUL4B antibodies, respectively.



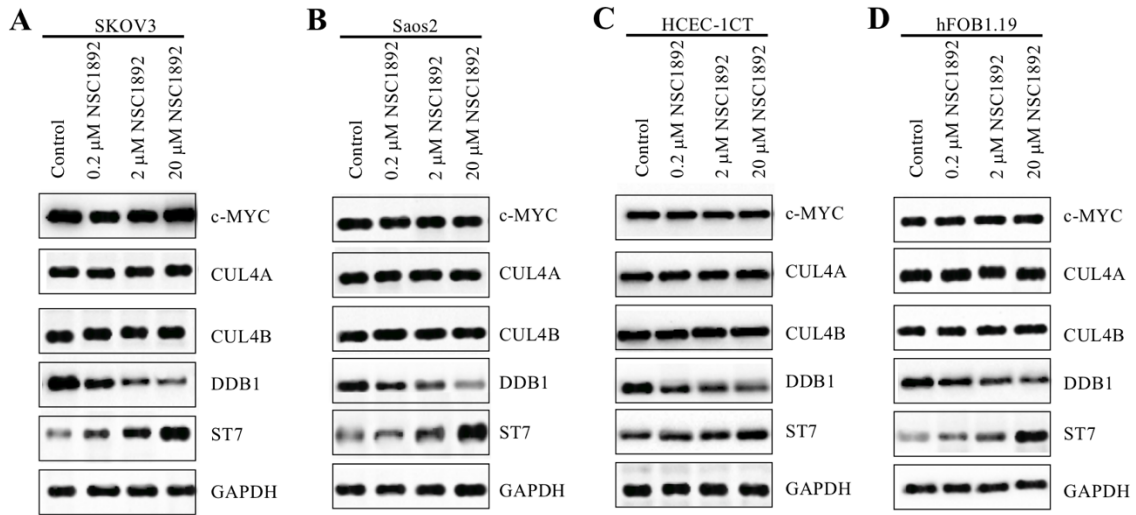
**Supplementary Figure 5. NSC1892 decreased the interaction of CUL4A and DDB1 *in vitro*.**

Equal amounts of GST-CUL4A and His-DDB1 proteins were incubated to assemble a complex at 4°C for 30 min. The CUL4A-DDB1 complex was then treated with DMSO, 2.0 μM or 20 μM NSC1892 at 4°C for 6 hrs, followed by incubating with the Ni-NTA resin at 4°C for 4 hrs. After washing five times, the pulldown proteins were determined by loading into a 10% SDS-PAGE gel and then stained with Coomassie blue.



**Supplementary Figure 6. NSC1892 inhibited the K48-linked poly-ubiquitination of ST7.**

HCT-116 cells under 80% confluence were cotransfected with pCDNA3-3×HA-ubiquitin-K48R and pCDNA3-2×Flag-ST7. After incubation for another 48 hrs, cells were treated with different concentrations (0, 0.2, 2, and 20  $\mu$ M) of NSC1892 for 6 hrs. Cells were immunoprecipitated with an anti-Flag antibody and the ubiquitination of ST7 was detected using an anti-HA antibody. The loading level of ST7 was examined using an anti-Flag antibody.



**Supplementary Figure 7. NSC1892 degraded DDB1 protein in cancerous and noncancerous cells**

SKOV3 (A), Saos2 (B), HCEC-1CT (C), and hFOB1.19 (D) cells were treated with different concentrations (0, 0.2, 2, and 20 μM) of NSC1892 for 6 hrs, respectively. The protein levels of c-MYC, CUL4A, CUL4B, DDB1, and ST7 were determined by western blotting. GAPDH was used as a loading control.

### The Ser/Thr sites of DDB1

M S Y N Y V V T A Q K P T A V N G C V T G H F T S A E D L N L L I A K N T R L E I Y V V T A E G L R P V K E V G M Y G K  
I A V M E L F R P K G E S K D L L F I L T A K Y N A C I L E Y K O S G E S I D I I T R A H G N V Q D R I G R P S E T G I  
I G I I D P E C R M I G L R L Y D G L F K V I P L D R D N K E L K A F N I R L E E L H V I D V K F L Y G C Q A P T I C F  
V Y Q D P Q G R H V K T Y E V S L R E K E F N K G P W K Q E N V E A E A S M V I A V P E P F G G A I I G Q E S I T V H  
N G D K Y L A I A P P I I K O S T I V C H N R V D P N G S R Y L L G D M E G R L F M L L L E K E E Q M D G T V T L K D L  
R V E L L G E T S I A E C L T Y L D N G V V F V G S R L G D S Q L V K L N V D S N E Q G S Y V V A M E T F T N L G P I V  
D M C V V D L E R Q G Q G Q L V T C S G A F K E G S L R I I R N G I G I H E H A S I D L P G I K G L W P L R S D P N R E  
T D D T L V L S F V G Q T R V L M L N G E E V E E T E L M G F V D D Q Q T F F C G N V A H Q Q L I Q I T S A S V R L V S  
Q E P K A L V S E W K E P Q A K N I S V A S C N S S Q V V V A V G R A L Y Y L Q I H P Q E L R Q I S H T E M E H E V A C  
L D I T P L G D S N G L S P L C A I G L W T D I S A R I L K L P S F E L L H K E M L G G E I I P R S I L M T T F E S S H  
Y L L C A L G D G A L F Y F G L N I E T G L L S D R K K V T L G T Q P T V L R T F R S L S T T N V F A C S D R P T V I Y  
S S N H K L V F S N V N L K E V N Y M C P L N S D G Y P D S L A L A N N S T L T I G T I D E I Q K L H I R T V P L Y E S  
P R K I C Y Q E V S Q C F G V L S S R I E V Q D T S G G T T A L R P S A S T Q A L S S S V S S S K L F S S S T A P H E T  
S F G E E V E V H N L L I D Q H T F E V L H A H Q F L Q N E Y A L S L V S C K L G K D P N T Y F I V G T A M V Y P E E  
A E P K Q G R I V V F Q Y S D G K L O T V A E K E V K G A V Y S M V E F N G K L L A S I N S T V R L Y E W T T E K E L R  
T E C N H Y N N I M A L Y L K T K G D F I L V G D L M R S V L L L A Y K P M E G N F E E I A R D F N P N W M S A V E I L  
D D D N F L G A E N A F N L F V C Q K D S A A T T D E E R Q H L Q E V G L F H L G E F V N V F C H G S L V M Q N L G E T  
S T P T Q G S V L F G T V N G M I G L V T S L S E S W Y N L L L D M Q N R L N K V I K S V G K I E H S F W R S F H T E R  
K T E P A T G F I D G D L I E S F L D I S R P K M Q E V V A N L Q Y D D G S G M K R E A T A D D L I K V V E E L T R I H

### Supplementary Figure 8. The Ser/Thr sites in the protein sequence of DDB1.

The Ser/Thr sites in the protein sequence of DDB1 were labelled with green and purple colors, respectively.



**The K sites of DDB1**

MSYNYVVTAQKPTAVNGCVTGHFTSAEDLNLLIAKNTREIYVVTAEGLRPVKEVGMYGK  
IAVMELFRPKGESKDLLFILTAKYNACILEYKQSGESIDIIITRAHGNVQDRIGRPSETGI  
IGIIDPECRMIGLRLYDGLFKVIPLDRDNKELKAFNIRLEELHVIDVKFLYGCQAPTICF  
VYQDPQGRHVKTYEVSLEKRFNKGPWKQENVEAEASMVIAVPEPFGGAIIGQESITYH  
NGDKYLAIAPIIIKQSTIVCHNRVDPNGSRVLLGDMEGRFLMLLEKKEEQMDGTVTLKDL  
RVELLGETSIAECLTYLDNGVVFVGSRLGDSQLVKLNVDSENEQGSYVVAMETFTNLGPIV  
DMCVVDLERQGGQLVTCGSAFKEGSLRIIRNGIGIHEHASIDLPGIKGLWPLRSDPNRE  
TDDTLVLSFVGQTRVLMNGEVEETEELMGFVDDQQTFFCGNVAHQQLIQITSASVRLVS  
QEPKALVSEWKEPQAKNISVASCNSQVVAVGRALYYLQIHPQELRQISHTEMEHEVAC  
LDITPLGDSNGLSPLCAIGLWTDISARILKLPSEFELHKEMLGGEIIPRSILMTTFESSH  
YLLCALGDGALFYFGLNIETGLLSDRKKVTLGTQPTVLRFRSLSTNVFACSDRPTVIY  
SSNHKLVFSNVNLKEVNYMCPNLSGYPDSLALANNSTLTIGTIDEIQKLHIRTVPPLYES  
PRKICYQEVSQCFVLSRRIEVQDTSGGTTALRPSASTQALSSSVSSSKLFSSTAPHET  
SPGEEVEVHLLIIDQHTFEVLHAHQFLQNEYALSLVSCKLGKDPNTYFIVGTAMVYPEE  
AEPKQGRIVVFQYSDGKLQTVAEKEVKGAVYSMVEFNGKLLASINSTVRLYEWTTEKELR  
TECNHYNNIMALYLKTKGDFILVGDLMRSVLLLAYKPMEGNFEEIARDFNPWNMSAVEIL  
DDDNFLGAENAFNLFVCQKDSAATTDEERQHLQEVGLFHLGFEVNVFCHGSLVMQNLET  
STPTQGSVLFGTVNGMIGLVTSLSESWYNLLDMQNRLNKVIKSVGKIEHSFWRSPHTER  
KTEPATGFIDGDLIESFLDISRPKMQEVVANLQYDDGSGMKREATADDLIKVVVEELTRIH

**Supplementary Figure 9. The Lys sites in the protein sequence of DDB1.**

The Lys (K) sites in the protein sequence of DDB1 were labelled with light blue color.

**Supplementary Table-1. Primers used for qRT-PCR analyzes**

<b>Gene</b>	<b>Forward Primers</b>	<b>Reverse primers</b>
CUL4A	5'- ACCATTGATGGAATCCTACTGC-3'	5'- ACCTTTGGCCTTCGGCAGCATA-3'
CUL4B	5'-ACTTCGTGCAGGCAACAAAGA -3'	5'- CAGCATCTACAGATGCACTCT-3'
c-Myc	5'-ACCACCAGCAGCGACTCT-3'	5'- GCGTAGTTGTGCTGATGTGT-3'
DDB1	5'- TAGAGATCTATGTGGTCAC-3'	5'- ATGCAGGCATTGTACTIONTCGCTG-3'
ST7	5'-GTCTCAGTAAGCCACTTGC-3'	5'- GTCATAGTAAGTAAGAGGCT-3'
p21	5'-CCTCAGGCAGCTCAAGCAGCG-3'	5'-AGGGGCCAGTGTCTCCCTCCT-3'
p27	5'-AACTGACGTGGAGCGGGGTAT-3'	5'-AATGAAGTATCAGCTGTCTCT-3'
$\beta$ -Actin	5'- AGAGCTACGAGCTGCCTGAC-3'	5'- AGCACTGTGTTGGCGTACAG -3'