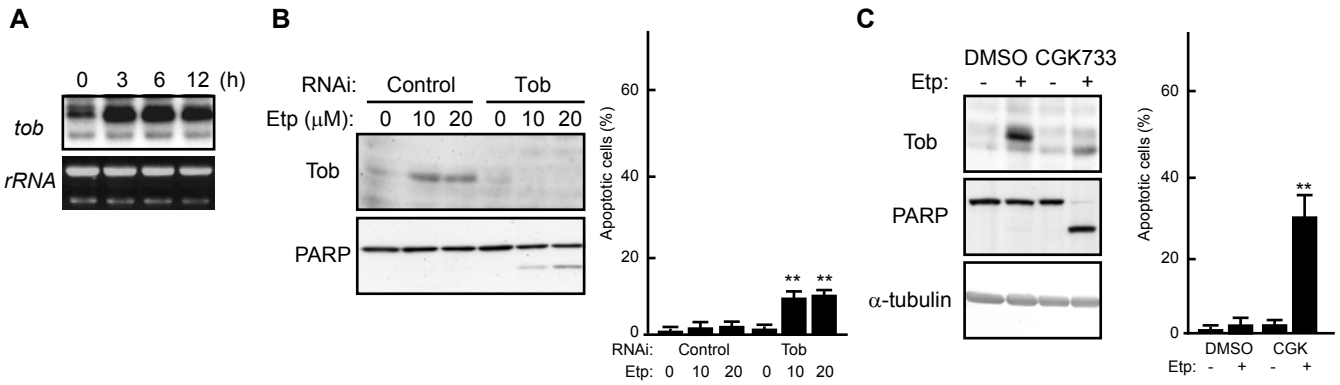
Supplementary materials and methods

SiRNAs. The sequences of siRNAs are as follows:
Tob 5'-CAUUUUGGUAGAGCCGAACTT-3'; DDB1
5'-GUUUCAUCGACGGUGACUUGA-3'; b-TrCP1
5'-CGUUGUAUUCGAUUUGAUAAC-3'; Cdc4 5'-CCGAAUAGUUAGUGGUUCUGA-3';
Skp2 5'-GCAUGUACAGGUGGCUGUUTT-3'; Cdc20
5'-GACCUGCCGUUACAUUCCUUC-3'; Cdh1 5'-CGCACGGCUACUCACAGAACC-3';
Cdt2 5'-CUUCAAAUACUCCUACGUUCU-3'; DDB2
5'-CACAGCCUCCGUAGAUCAAAC-3'; ATM 5'-GGAUCGUAAACGCUUCUUAUG-3';
ATR 5'-CCCGAGAGAUAUCAUAUCACC-3'; Chk1
5'-GAAGCGUGCCGUAGACUGUCC-3'; Chk2 5'-CAGAUAAAUACCGAACAUACA-3';
Cdc7 5'-GCCCUGCAGUGAAACUCAUGA-3'; Dbf4
5'-CACAAACCUUGGGUCGAAUUU-3'; Drf1 5'-GUUGACUCGGUGCCUCUAAGC-3';
HMGB1 5'-GAAGAGUAAUCAAUCUACUCA-3'; Control
5'-UUCUCCGAACGUGUCACGUTT-3'.

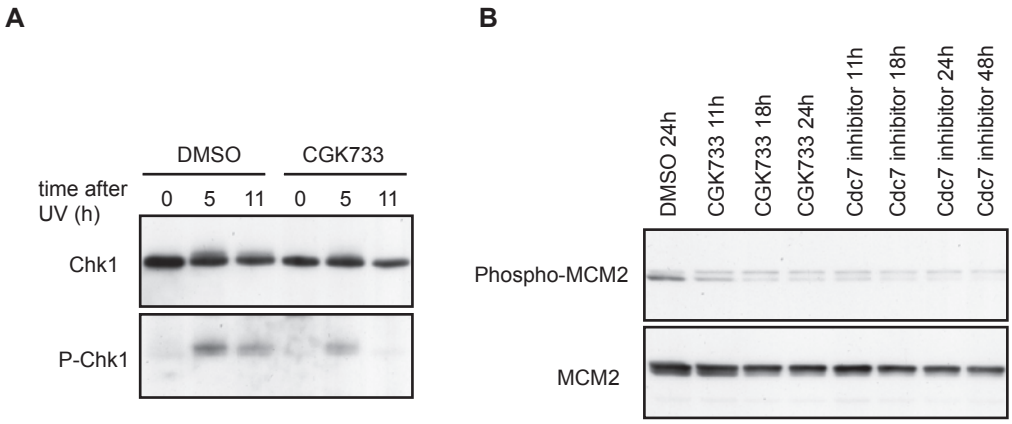
2D-DIGE. U2OS cells were pretreated with 20 μM CGK733 or DMSO for 40 min and were subsequently irradiated with UV (30 J/m^2). Immobilized metal ion–affinity chromatography, using the PhosphoProtein Purification Kit (Qiagen) was performed 30 min after irradiation. After removal of non-protein material (2-D Clean-Up Kit; GE Healthcare), we minimally labeled 25 μg of the phosphoprotein-enriched fractions with 400 pmol Cy3 or Cy5 fluorescent dye (CyDye DIGE Fluors, GE Healthcare) for 30 min on ice in buffer (30 mM Tris-HCl, pH 8.5, 7 M urea, 2 M thiourea and 4% (w/v) CHAPS). We quenched the labeling reaction with the addition of 200 μM lysine. We mixed the paired samples and performed first-dimension isoelectric focusing on immobilized pH gradient strips (24 cm; pH 3–10 nonlinear) using an Ettan IPGphor II system. We performed second-dimension separation on 10% SDS-PAGE gels (20 x 24 cm). We acquired fluorescence images of the gels at appropriate wavelengths for Cy3 and Cy5 dyes on a Typhoon 8600 scanner (GE Healthcare).

Protein identification by mass spectrometry. We performed 2D-DIGE in the presence of 400 μg unlabeled phosphoprotein fractions. We transferred proteins from the gel onto a PVDF membrane (ProBlott; Applied Biosystems) and used the Typhoon 8600 fluorescence scanner to detect the spot pattern. After staining with CBB R-350 (GE Healthcare), we excised the appropriate spots and digested them with lysyl endopeptidase (Acromobacter protease I; Wako). We desalted and concentrated recovered peptides using ZipTip HPL (Millipore), eluted them

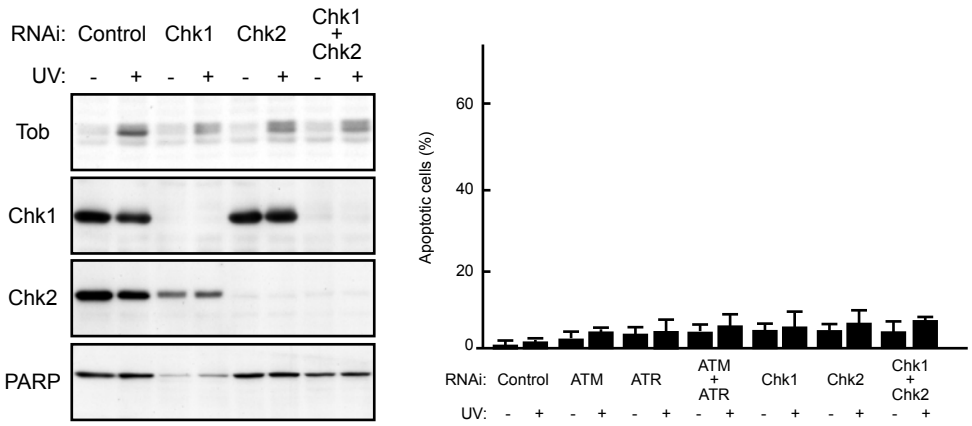
with 50% (v/v) acetonitrile and 0.1% (v/v) acetic acid containing α-cyano-4-hydroxycinnamic acid (Laser BioLabs) as the matrix, and applied them directly onto the metal target. We acquired all MALDI-TOF mass spectra in the positive ion reflector mode on a Voyager-DEPRO instrument (Applied Biosystems) with an *m*/*z* range of 800–4000 Da. We searched data sets against the NCBI non-redundant protein database restricted to *Homo sapiens* using the Mascot search engine (Matrix Science) with a peptide mass tolerance of 0.15 Da. We allowed one missed cleavage and partial oxidation of methionine. We evaluated the search results by considering the Mascot score, probability value, peptide coverage, pattern of coverage and correspondence between the predicted and experimental pI and molecular mass.



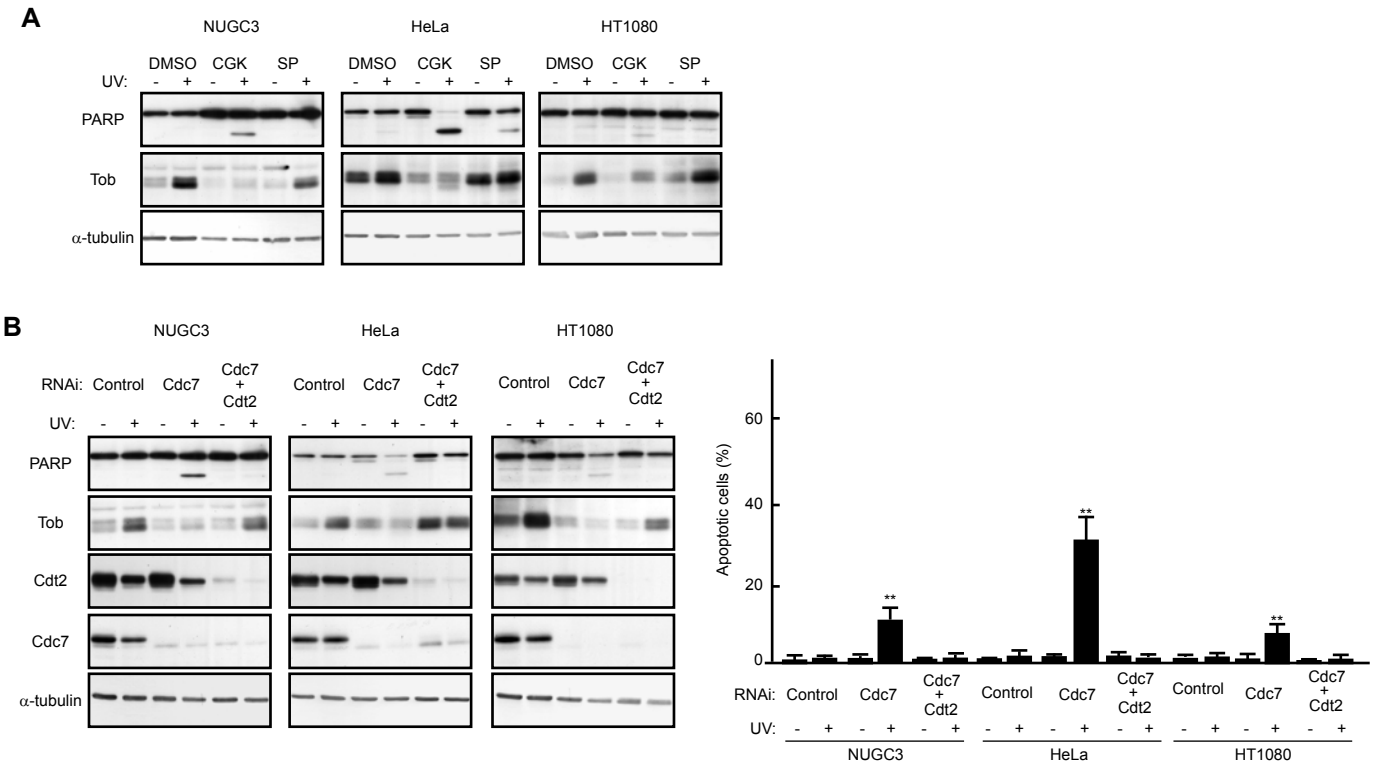
Supplemental Figure S1. Etp-induced Tob is protected from degradation to suppress apoptosis.
(A) Total RNA was prepared from U2OS cells at the indicated times after Etp treatment and subjected to Northern blotting using *tob* cDNA as a probe. Ethidium bromide-stained agarose gel is shown as a loading control (*rRNA*).
(B) U2OS cells transfected with the indicated siRNAs were treated with Etp. The cell lysates were analyzed using immunoblotting.
(C) U2OS cells pretreated with the indicated chemicals were treated with Etp (10 μM). The cell lysates were analyzed using immunoblotting.
The graphs show the percentages of apoptotic cells with condensed nuclei in the indicated siRNA-transfected or chemical-treated cells. At least 100 cells are scored for each experiment. Values are shown as the mean + S.D. from triplicate experiments. (**p<0.01)



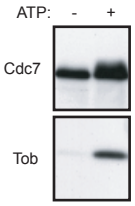
Supplemental Figure S2. CGK733 inhibits both ATR and Cdc7 activity
(A) U2OS cells pretreated with DMSO or CGK733 (20 μM) were irradiated with UV (30 J/m^2). The cell lysates were prepared at the indicated times and analyzed using immunoblotting.
(B) U2OS cells were treated with the indicated chemicals for the indicated periods. The cell lysates were analyzed using immunoblotting.



Supplemental Figure S3. Chk1 and Chk2 are not invovled in the UV-induced Tob increase.
U2OS cells transfected with the indicated siRNAs were irradiated with UV (30 J/m^2). Cell lysates were analyzed using immunoblotting.
The graph shows the percentages of apoptotic cells with condensed nuclei in the indicated siRNA-transfected cells.
At least 100 cells are scored for each experiment. Values are shown as the mean + S.D. from triplicate experiments.



Supplemental Figure S4. The Cdc7-Tob axis-mediated cell survival against mild DNA damage functions in several types of cells.
(A) Cells treated with the indicated chemicals were irradiated with UV (30 J/m^2). Cell lysates were analyzed using immunoblotting.
(B) Cells transfected with the indicated siRNAs were irradiated with UV (30 J/m^2). Cell lysates were analyzed using immunoblotting. The graph shows the percentages of apoptotic cells with condensed nuclei in the indicated siRNA-transfected or chemical-treated cells. At least 100 cells are scored for each experiment. Values are shown as the mean + S.D. from triplicate experiments. (**p<0.01)



Supplemental Figure S5. Cdc7-dependent phosphorylation promotes Tob-Cdc7 interaction *in vitro*.
GST-Cdc7 was purified after an *in vitro* phosphorylation reaction containing Tob as a substrate in the presence (+) or the absence (-) of ATP. The purified products were analyzed using immunoblotting