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Supplementary Information for

Erk1/2 Inactivation Promotes a Rapid Redistribution of COP1 and Degradation of COP1 Substrates

Weiming Ouyang[a,1,2], Pengfei Guo[a,1], Kazuyo Takeda[b], Qiong Fu[a], Hui Fang[a], David M. Frucht[a,2]

[a] Division of Biotechnology Review and Research II, Office of Biotechnology Products, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, [b] Microscopy and Imaging Core Facility, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration

[1] W.O. and P.G. contributed equally to this work.

[2] To whom correspondence should be addressed.

Weiming Ouyang, Ph.D.
E-mail: Weiming.ouyang@fda.hhs.gov

David Frucht, M.D.
E-mail: david.frucht@fda.hhs.gov

This PDF file includes:

Supplementary Figures S1 to S12

Supplementary Figure 1

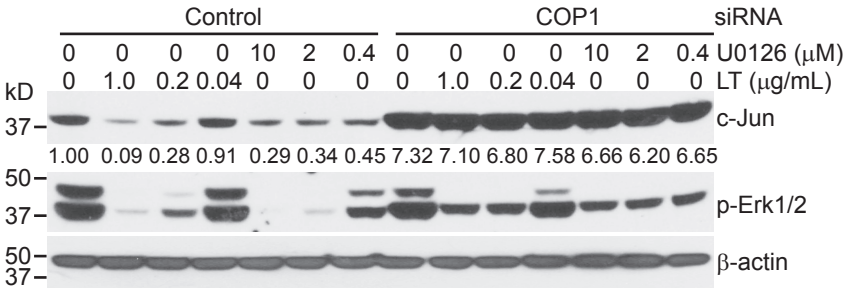

Fig. S1. Knockdown of COP1 prevents c-Jun reduction in cells treated with a range of LT and U0126 concentrations.

Hepa1c1c7 cells were transfected with control or COP1 siRNA. Two days following the transfection, the cells were cultured with medium, LT for 2 hours or U0126 for one hour at the indicated doses and then extracted with NuPAGE LDS sample buffer. The expression levels of c-Jun, p-Erk1/2 and β-actin proteins in the cell lysates were assessed by Western blotting. Band intensities were quantified and normalized to β-actin levels. The relative protein levels are shown under each band. Data shown are representative of two independent experiments.

Supplementary Figure 2

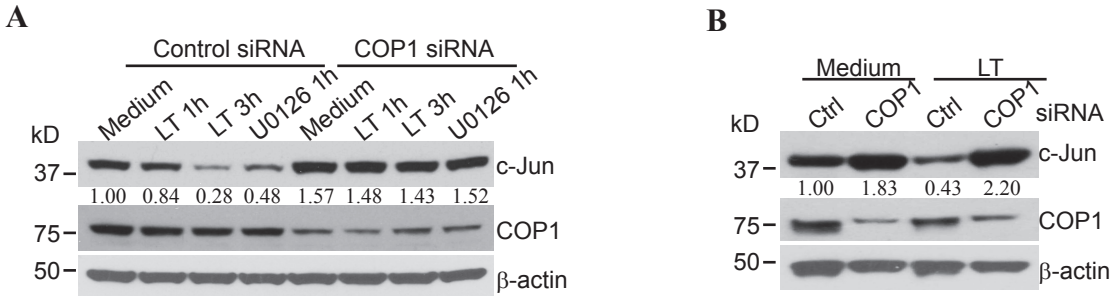

Fig. S2. Knockdown of COP1 prevents c-Jun reduction following treatment with LT or U0126.

HepG2 and primary MEF cells were transfected with control siRNA or siRNA targeting human COP1 (A) or murine Cop1 (B). Two days following the transfection, the cells were cultured with or without LT or U0126, and then extracted with NuPAGE LDS sample buffer. The levels of c-Jun protein were measured by Western blotting. β-actin levels were used as loading controls. Intensities of c-Jun bands were quantified and normalized to β-actin levels. The relative protein levels are shown under each band. Data shown are representative of two independent experiments.

Supplementary Figure 3

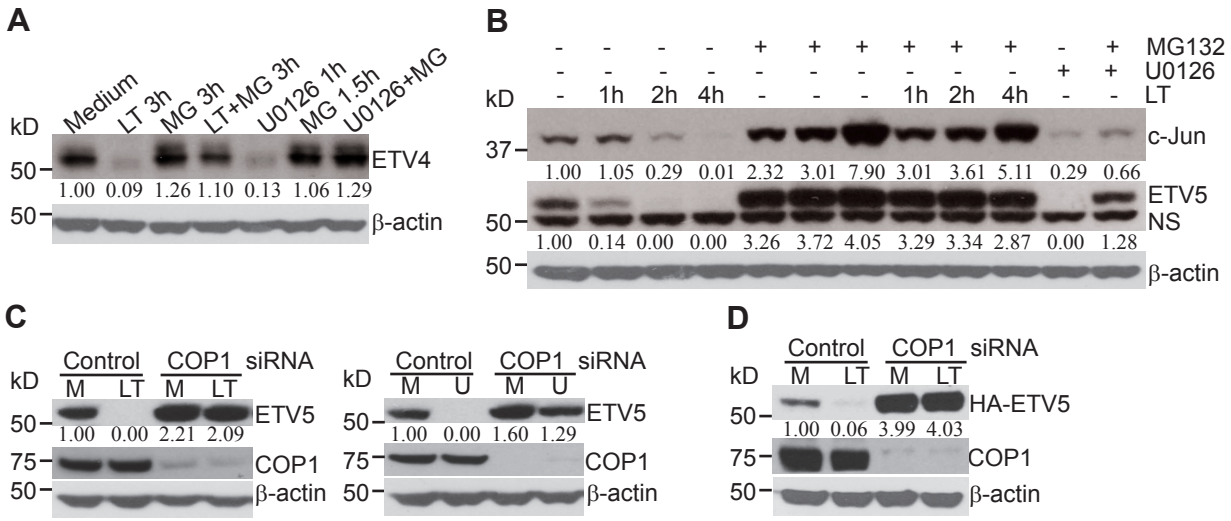

Fig. S3. Treatment with LT or U0126 induces COP1-dependent ETV4 and ETV5 protein degradation

(A, B) HepG2 cells were treated with LT, U0126 and/or MG132 as indicated. MG132 was added to the culture at the same time as LT, but 30 minutes prior to the addition of U0126. (C, D) HepG2 (C) or HepG2-HA-ETV5 (D) cells were transfected with COP1 siRNA. Two days after the transfection, the cells were cultured with or without LT for 2 hours or U0126 (U) for 1 hour, and then extracted with NuPAGE LDS sample buffer. The levels of endogenous ETV4 (A), ETV5 (B, C) and HA-ETV5 (D) proteins were measured by Western blotting. β-actin levels were used as loading controls. Band intensities were quantified and normalized to β-actin. The relative protein levels are shown under each band. Data shown are representative of at least two independent experiments. NS depicts nonspecific bands.

Supplementary Figure 4

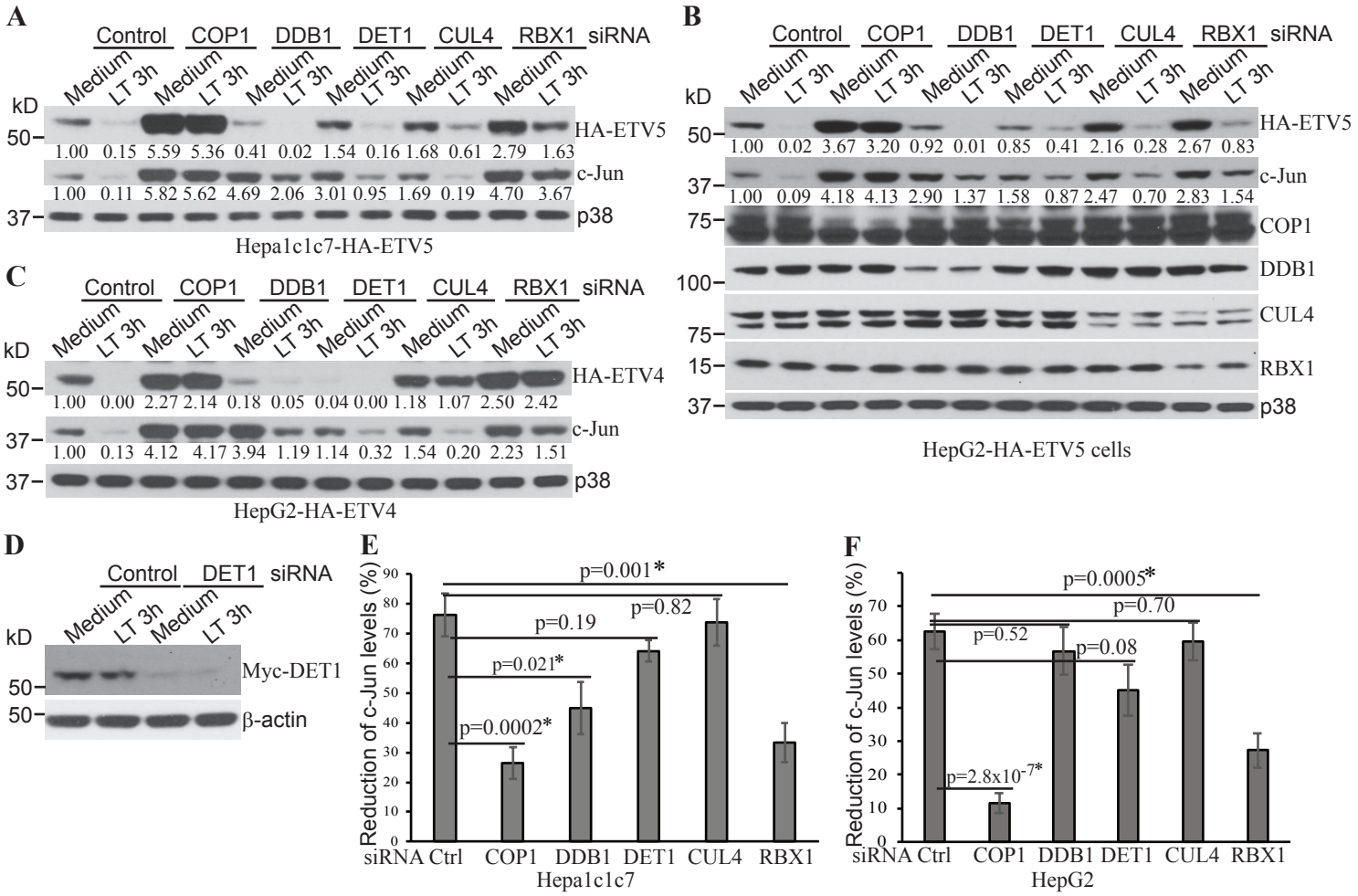

Fig. S4. Effects of knockdown of CRL4$^{COP1-DET1}$ complex components on the levels on COP1 substrates in LT-treated cells.

Hepa1c1c7-HA-ETV5 (A), HepG2-HA-ETV5 (B) and HepG2-HA-ETV4 (C) cells were transfected with siRNA targeting components of CRL4$^{COP1-DET1}$ complex as indicated. Two days following the transfection, the cells were cultured with or without LT for 2 (A) or 3 hours (B, C), and then extracted with NuPAGE LDS sample buffer. The levels of endogenous c-Jun (A-C), HA-ETV5 (A, B) and HA-ETV4 (C) proteins were measured by Western blotting. siRNA-mediated suppression of targets was confirmed by Western blotting. p38 levels were used as loading controls. Band intensities were quantified and normalized to p38 levels. The relative protein levels are shown under each band. Data shown are representative of two independent experiments. (D) HepG2-Myc-Det1 cells were transfected with control or DET1 siRNA, treated with LT for 3 hour and then extracted with NuPAGE LDS sample buffer. The levels of Myc-Det1 were determined by Western blotting. β-actin levels were used as loading controls. (E, F) Reductions in the levels of c-Jun protein in Hepa1c1c7 (E) and HepG2 (F) cells were analyzed using data from five independent experiments and presented as average ± SE (bottom). Asterisks depict statistically significant differences between the two groups ($p<0.05$).

Supplementary Figure 5

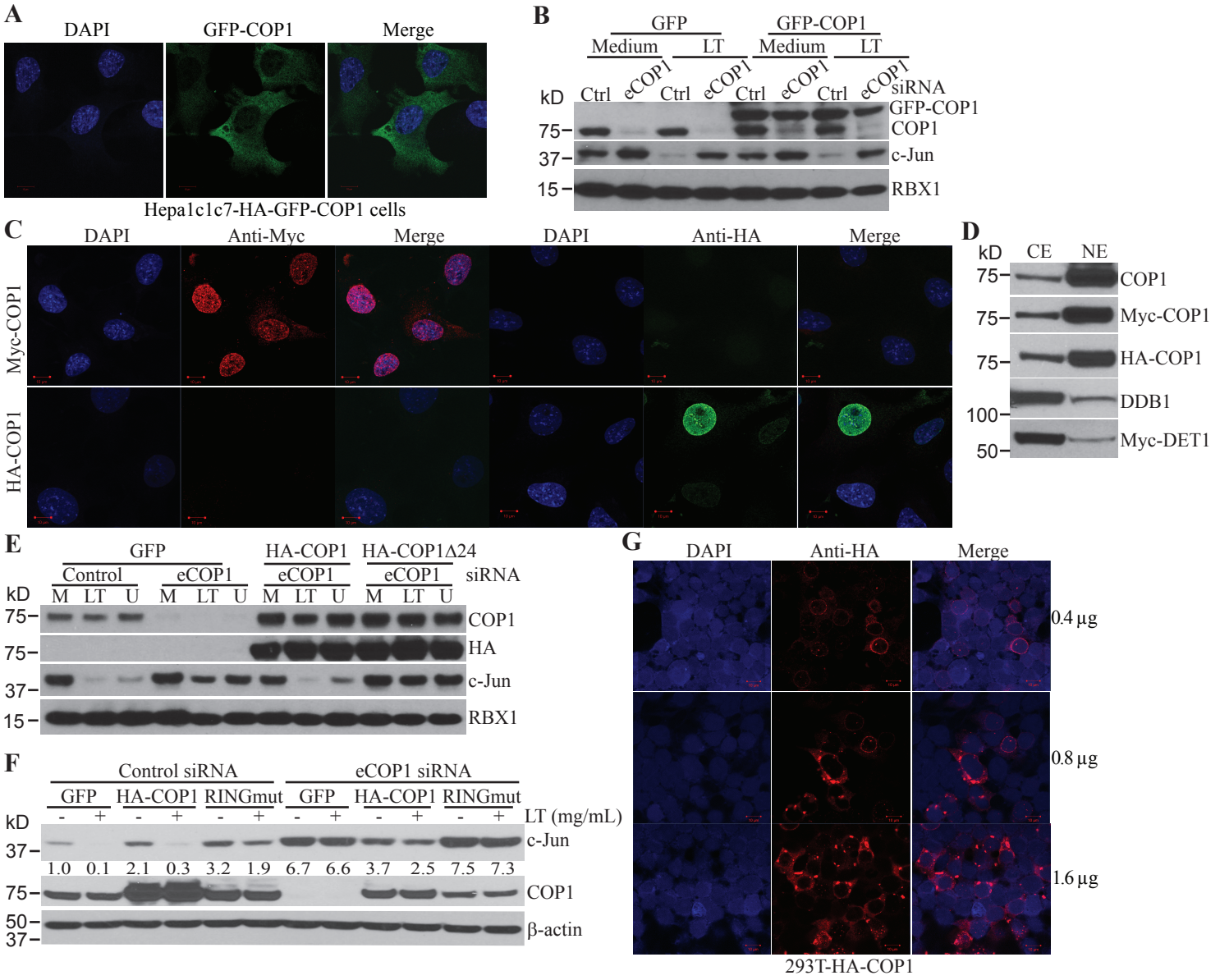

Fig. S5. Effect of tagging on the location and function of COP1.

(A) Hepa1c1c7 cells were transduced with pseudovirus expressing a GFP-COP1 fusion protein. The location of the fusion protein was assessed by LSM 880 confocal microscope. (B) Hep1c1c7 cells stably expressing GFP or GFP-COP1 were transfected with control siRNA or siRNA targeting endogenous murine COP1 (eCOP1). Two days following transfection, the cells were cultured with or without LT for 2 hours and then extracted with NuPAGE LDS sample buffer. The levels of c-Jun and COP1 proteins were measured by Western blotting. Rbx1 levels were used as loading controls. (C) Hepa1c1c7 cells were transduced with pseudovirus expressing Myc-COP1 or HA-COP1. The locations of Myc-COP1 and HA-COP1 were analyzed by immunofluorescent staining with rabbit anti-HA tag (C29F4, Cell Signaling #3724, 1:1500) and mouse anti-Myc tag (9B11, Cell Signaling #2276, 1:3000) followed with goat-anti-rabbit IgG-Alex Fluor568 and goat-anti-mouse IgG-Alex Fluor633 (ThermoFisher, 1:200). The stained cells were analyzed by LSM 880 confocal microscope. (D) Hepa1c1c7 cells were transduced with pseudo virus expressing Myc-COP1, HA-COP1 and Myc-DET1. Cytoplasmic (CE) and nuclear extracts (NE) from parental and transduced cells were prepared using a kit from Active Motif. The levels of COP1, DDB1, DET1 proteins were assessed by Western blotting. (E) Hep1c1c7 cells stably expressing GFP, HA-COP1 or HA-COP1Δ24 were transfected with control siRNA or siRNA specifically targeting endogenous murine COP1, treated with LT and U0126, and analyzed for the levels of c-Jun by Western blotting as described in Fig. S5B. (F) Hep1c1c7 cells stably expressing GFP, HA-COP1 or RING domain mutated HA-COP1 (RINGmut) were transfected with control siRNA or siRNA specifically targeting endogenous murine COP1, treated with LT for 2 hours, and analyzed for the levels of c-Jun by Western blotting as described above. Intensities of c-Jun bands were quantified and normalized to β-actin levels. The relative amounts are shown under each band. Data shown are representative of two independent experiments. (G) 293T cells were transiently transfected with 0.4, 0.8 or 1.6 μg plasmid expressing HA-COP1, immuno-stained, and analyzed by confocal microscopy as described above.

Supplementary Figure 6

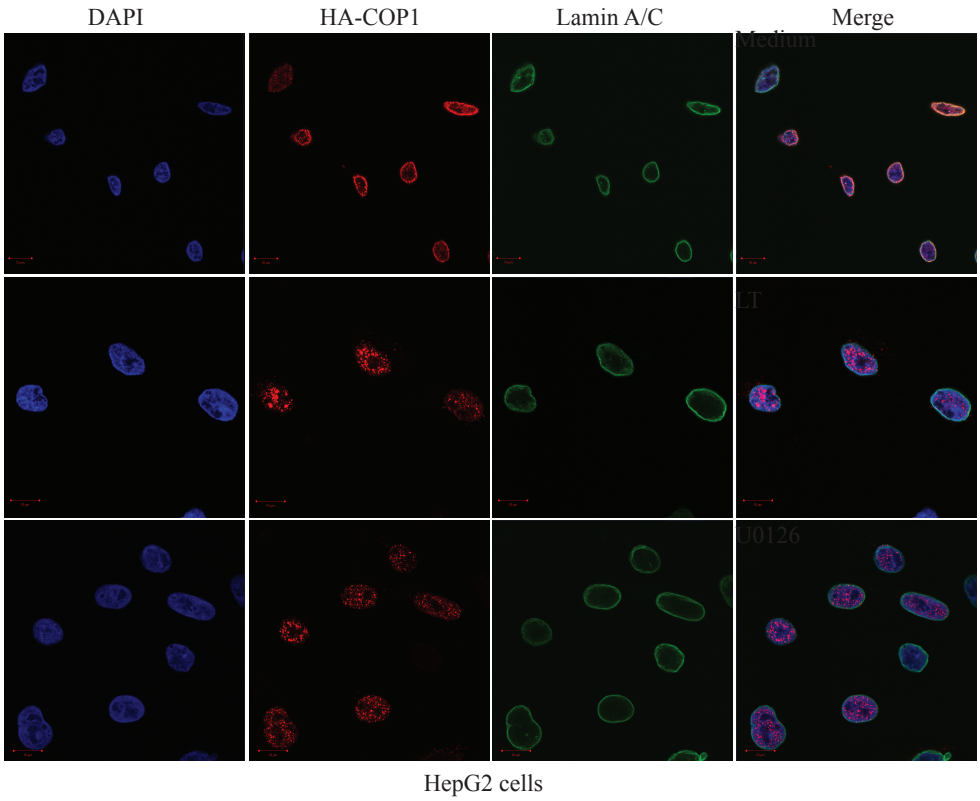

Fig. S6. LT or U0126 treatment promotes COP1 translocation from the nuclear envelope to the nucleoplasm.

HepG2 cells stably expressing HA-COP1 were cultured with or without LT for 2 hours or U0126 for 1 hour. The cells were then analyzed by immunofluorescence staining with mouse anti-Lamin A/C (4C11, Cell Signaling #4777, 1:100) and rabbit anti-HA primary antibodies followed with goat-anti-mouse IgG-Alexa Fluor 633 and goat-anti-rabbit IgG-Alexa Fluor 568 secondary antibodies. The locations of Lamin A/C, HA-COP1 and the nucleus were assessed by LSM 880 confocal microscope.

Supplementary Figure 7

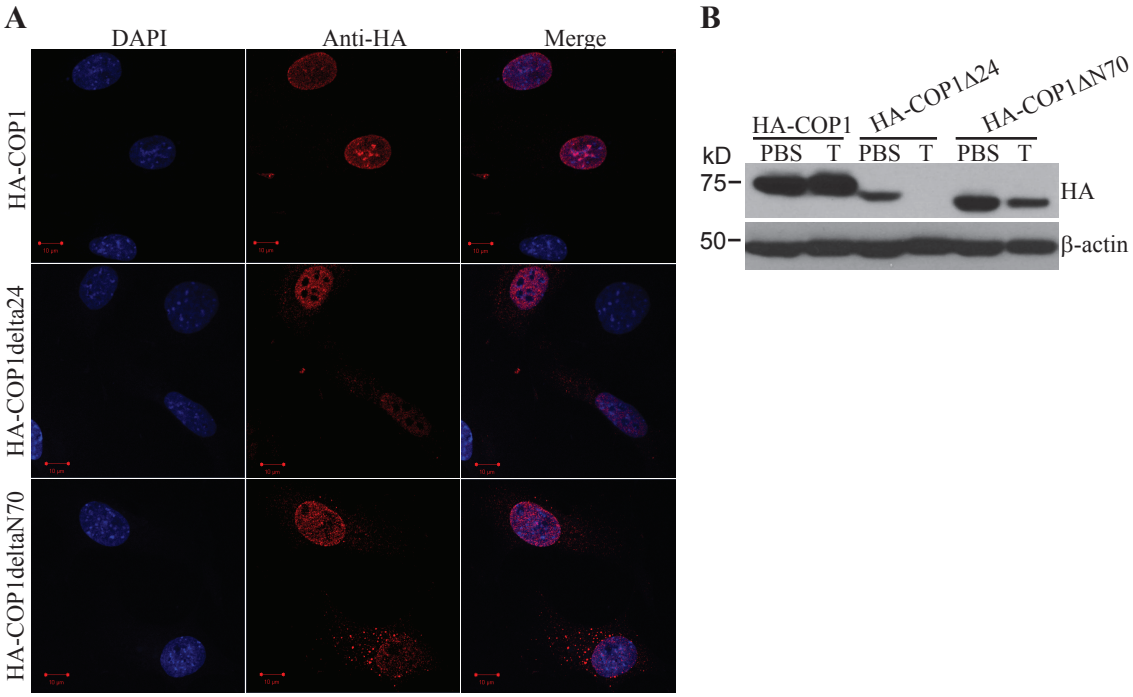

Fig. S7. Localization of tagged-COP1 and COP1 mutants.

(A) Hepa1c1c7 cells were transduced with pseudovirus expressing HA-COP1, HA-COP1Δ24 or HA-COP1ΔN70. The locations of COP1 and COP1 mutants were analyzed by immunofluorescent staining with rabbit anti-HA tag followed with goat-anti-rabbit IgG-Alex Fluor 568 and LSM 880 confocal microscope. (B) Hepa1c1c7 cells stably expressing HA-tagged full length COP1, COP1Δ24 or COP1ΔN70 were cultured with or without 1% Triton X-100 (T) for 1 min, washed with cold PBS twice (3 min/wash) and extracted with NuPAGE LDS sample buffer. The levels of HA-tagged proteins and β-actin were assessed by Western blotting.

Supplementary Figure 8

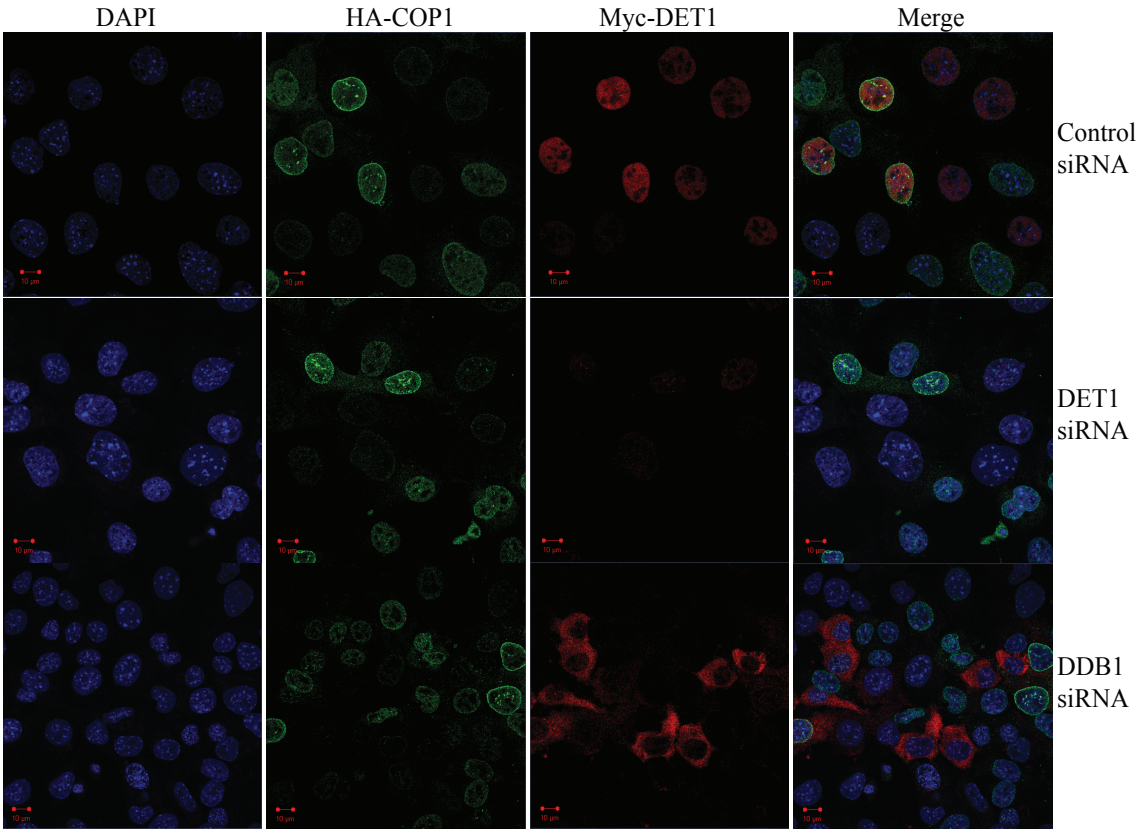

Fig. S8. Knockdown of DDB1 and DET1 does not affect the location of COP1.

Hepa1c1c7 cells stably expressing HA-COP1 and Myc-DET1 were transfected with control siRNA or siRNA targeting DET1 and DDB1. Two days following the transfection, the cells were stained with rabbit anti-HA tag and mouse anti-Myc followed with goat-anti-rabbit IgG-Alex Fluor568 and goat-anti-mouse IgG-Alex Fluor633. The locations of HA-COP1, Myc-DET1 and the nucleus were assessed by LSM 880 confocal microscope.

Supplementary Figure 9

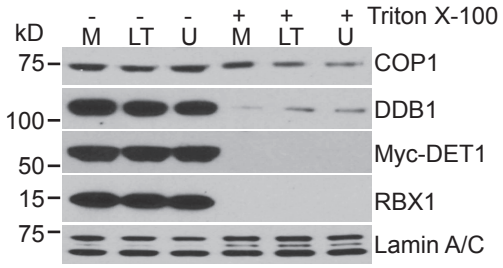

Fig. S9. Effect of Triton X-100 treatment on the levels of CRL4$^{COP1-DET1}$ complex components.

Hepa1c1c7 cells stably expressing Myc-DET1 were cultured with (LT) or without (M) LT for 2 hours or U0126 (U) for 1 hour. The cells were then treated with 1% Triton X-100 for 1 min, washed with cold PBS twice (3 min/wash) and extracted with NuPAGE LDS sample buffer. The levels of COP1, DDB1, Myc-DET1, RBX1 and Lamin A/C were assessed by Western blotting.

Supplementary Figure 10

TPR peptides

	MG132	LT/MG132		Reduction
	0.41071	0.06571		84.00
	0.12732	0.06123		51.91
	0.14375	0.04443		69.09
	0.184	0.0368		80.00
	0.275	0.09167		66.67
	0.12283	0.02444		80.10
Average				71.96
SD				11.95

Fig. S10. LT treatment disrupts the interaction of COP1 with TPR.

Hepa1c1c7 cells stably expressing HA-COP1 were cultured with MG132 or MG132 with LT for 2 hours. Nuclear extracts were prepared from the treated cells and incubated with anti-HA magnetic beads. The relative contents of TPR peptides identified by mass spectrometry in the immunoprecipitated (IP) complexes from two independent experiments are shown. Relative reductions in TPR content by LT treatment were calculated using the formula: (Relative content in MG132 treated group - Relative content in MG132/LT treated group)/Relative content in MG132 treated group × 100%.

Supplementary Figure 11

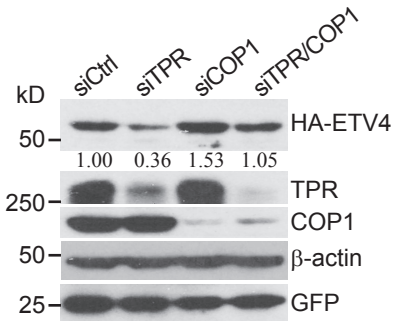

Fig. S11. TPR knockdown reduces the levels of HA-ETV4 protein in a COP1-dependent manner.

Hepa1c1c7 cells stably expressing HA-ETV4 were transfected with control, TPR or COP1 siRNA. Two days following the transfection, the cells were extracted with NuPAGE LDS sample buffer and analyzed for the levels of indicated proteins by Western blotting. β-actin and GFP levels were used as loading controls. Intensities of HA-ETV4 bands were quantified and normalized to β-actin levels. The relative protein levels are shown under each band. Data shown are representative of two independent experiments.

Supplementary Figure 12

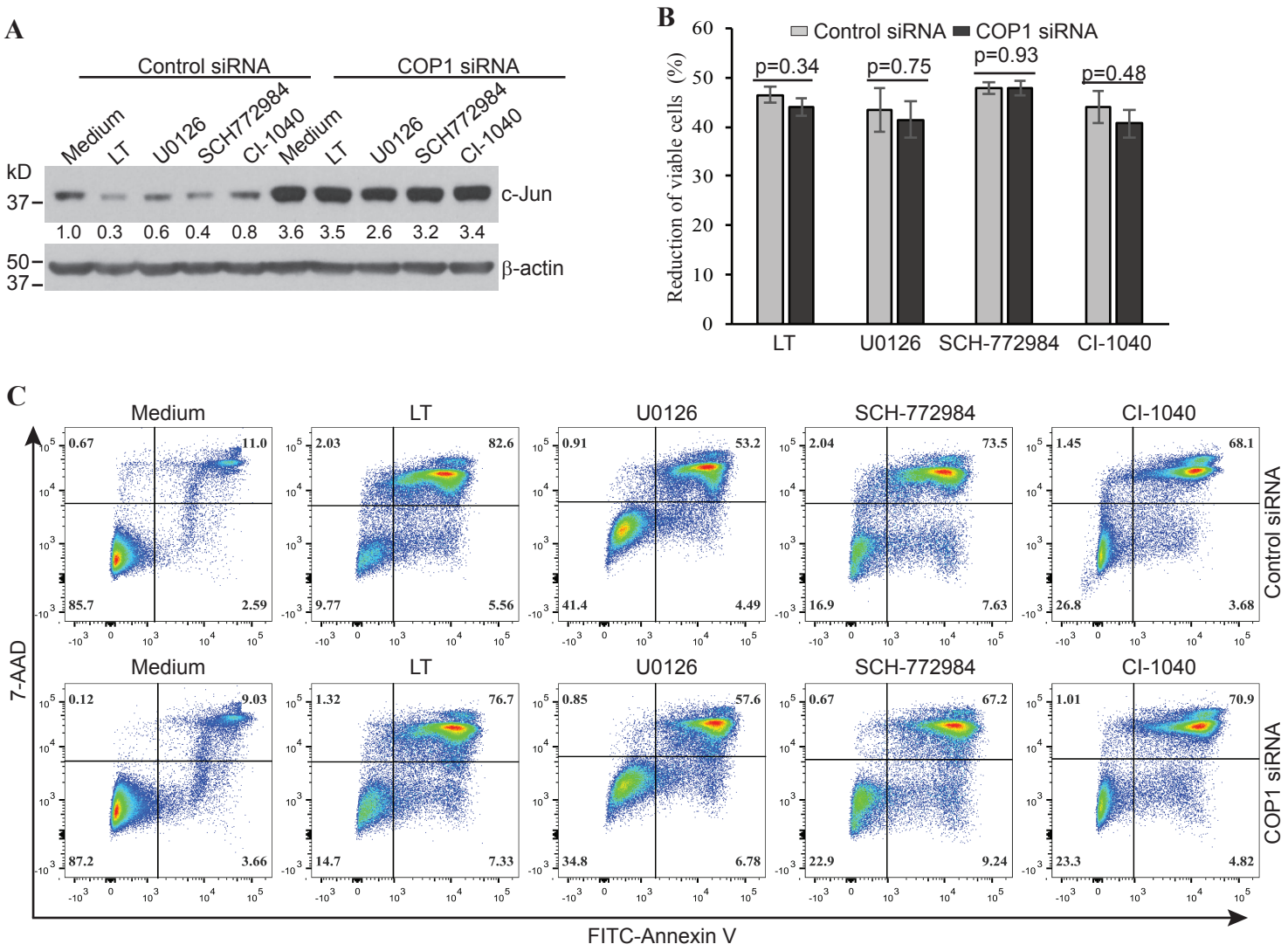

Fig. S12. COP1 knockdown fails to restore the viability of A375 cells treated with LT or MEK1/2 inhibitors.

(A) A375 cells were treated with LT or MEK1/2 inhibitors for 6 hours and then extracted with LDS sample buffer. The levels of c-Jun and β-actin were measured by Western blotting. Band intensities were quantified and normalized to β-actin levels. Relative c-Jun protein levels are shown under each band. Data shown are representative of two independent experiments. (B) Three thousand A375 cells transfected with control or COP1 siRNA were treated with LT or MEK1/2 inhibitors for 48 hours. Cellular proliferation and viability were measured using an MTS proliferation kit. Relative reductions in cellular proliferation and viability were calculated using the following formula: Reduction of viable cells (%) = (O.D.$_{490}$ of untreated group - O.D.$_{490}$ of LT-treated group)/O.D.$_{490}$ of untreated group × 100%. Data from three independent experiments were statistically analyzed using a two-tailed Student's t test and presented as mean ± SE. (C) A375 cell transfected with control or COP1 siRNA were treated with LT or MEK1/2 inhibitors for 72 hours. Cell apoptosis and death were measured using FACS staining with FITC-Annexin V and 7-AAD and analyzed by the FlowJo software. Data shown are representative of two independent experiments.