



**Figure S5. REEP1/2 and EI24 are Required for DNA Damage-induced Apoptosis.**

- (a) Western blot (WB) analysis of lysates from U2OS cells transfected with control vector, REEP1 or REEP2, and treated with 1  $\mu$ M doxo for 24 hours.
- (b) Western blot analysis of lysates from U2OS cells stably expressing control (shControl) or REEP1/2 shRNAs (shREEP1/2), transfected with the indicated plasmids, and treated with 1  $\mu$ M doxo for 24 hours. GAPDH and tubulin served as the loading controls.
- (c) Western blot analysis of lysates from HEK293 cells transfected with control or EI24 shRNA (shEI24) and treated with 50 nM cpt or 1  $\mu$ M eto for 12 hours.
- (d) Western blot analysis of HEK293 cells transfected with the indicated shRNA alone or together with either 3 $\times$ Flag-EI24 or 3 $\times$ Flag-EI24 $\Delta$ H1 rescue plasmids and treated with 1  $\mu$ M eto for 12 hours.
- (e) Representative images of flow cytometry analysis for wild-type (WT), REEP1/2 double knockout (DKO), or EI24 knockout (KO) cells positive for propidium iodide (PI) or Annexin V. Cells were treated with 1  $\mu$ M cpt for 14 hours.
- (f) MTT assay of HEK293 cells transfected with REEP1/2 shRNAs and treated with 1  $\mu$ M eto at 48 hours after transfection. n = 8.
- (g) MTT assays of HEK293 cells transfected with the vector, 3 $\times$ Flag-EI24 or 3 $\times$ Flag-EI24 $\Delta$ H1 and treated with eto 48 hours after transfection (n = 8).
- (h) MTT assays of HEK293 cells transfected with control or EI24 shRNA (shEI24) and treated with eto 48 hours after transfection (n = 8).
- (i) MTT assays of wild-type and EI24-knockout (KO) U2OS cells treated with DMSO or cpt (n = 8).
- (j) Western blot analysis of lysates from U2OS cells transfected with control (vector), REEP1/2 or GFP-Rtn4 for 24, 48 or 72 hours.
- (k) Distribution ratios of peripheral/perinuclear ER in U2OS cells overexpressing control (vector), REEP1 or REEP1 $\Delta$ H2.
- (l) Western blot analysis of lysates from HEK293 cells transfected with vector, REEP1 or REEP1 $\Delta$ H2 and treated with 1  $\mu$ M eto for 12 hours.
- (m) Western blot analysis of lysates from U2OS cells transfected with the vector, 3 $\times$ Flag-EI24, 3 $\times$ Flag-EI24 $\Delta$ H1, 3 $\times$ Flag-EI24 $\Delta$ H2 or 3 $\times$ Flag-EI24 $\Delta$ H1+2 and treated with 1  $\mu$ M cpt for 24 hours.
- (n) Western blot analysis of lysates from HEK293 cells transfected with control vector or 3 $\times$ Flag-EI24, and treated with 1  $\mu$ M TM, 100 ng/mL TNF $\alpha$ , 100 nM TG, 500 ng/mL puromycin (puro), 600  $\mu$ g/mL G418, or 20 nM STS for 12 hours.
- (o) Western blot analysis of lysates from wild-type or EI24 knockout U2OS cells treated with 1  $\mu$ M TM, 100 nM TG, 1.5 mM H<sub>2</sub>O<sub>2</sub>, 20 nM STS, 500 ng/mL puromycin, 600  $\mu$ g/mL G418, or 100 ng/mL TNF $\alpha$  for 24 hours, or with 40 J/m<sup>2</sup> UV radiation.
- (p) Western blot analysis of HEK293 cells transfected with 3 $\times$ Flag-EI24 and Bcl2-myc as indicated and treated with 100 nM cpt for 12 hours.