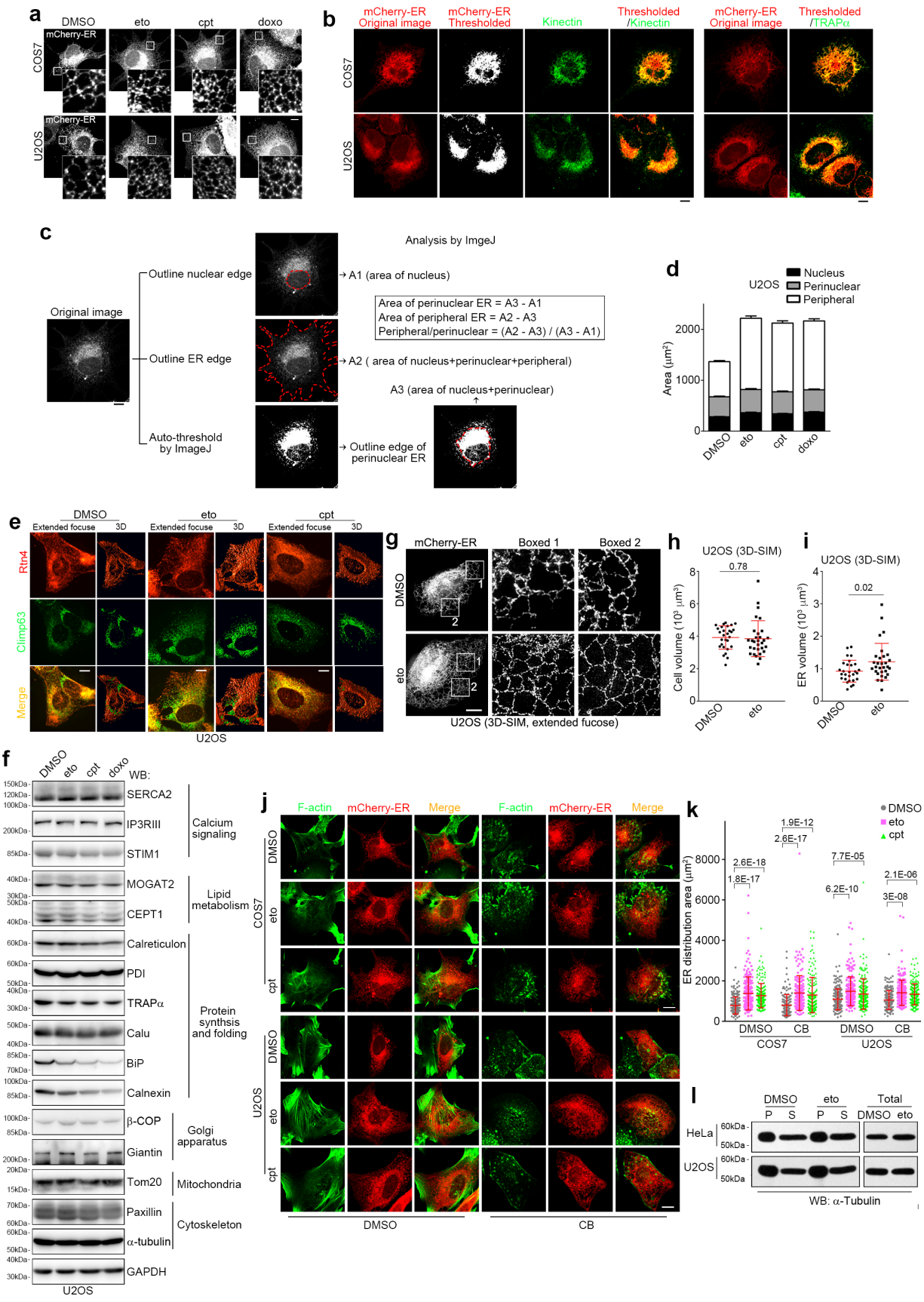
a
DMSO
eto
cpt
doxo
mCherry-ER
COS7
U2OS
b
mCherry-ER Original image
mCherry-ER Thresholded
Kinectin
Thresholded /Kinectin
mCherry-ER Original image
Thresholded /TRAPα
c
Analysis by ImgeJ
Original image
Outline nuclear edge
Outline ER edge
Auto-threshold by ImageJ
→ A1 (area of nucleus)
→ A2 ( area of nucleus+perinuclear+peripheral)
A3 (area of nucleus+perinuclear)
→ Outline edge of perinuclear ER
Area of perinuclear ER = A3 - A1
Area of peripheral ER = A2 - A3
Peripheral/perinuclear = (A2 - A3) / (A3 - A1)
d
U2OS
Nucleus
Perinuclear
Peripheral
Area (μm²)
2000
1000
0
DMSO
eto
cpt
doxo
e
DMSO
eto
cpt
Extended focuse
3D
Rtn4
Climp63
Merge
U2OS
g
mCherry-ER
Boxed 1
Boxed 2
DMSO
eto
U2OS (3D-SIM, extended fucose)
h
U2OS (3D-SIM)
0.78
Cell volume (10³ μm³)
DMSO
eto
i
U2OS (3D-SIM)
0.02
ER volume (10³ μm³)
DMSO
eto
f
DMSO
eto
cpt
doxo
WB:
SERCA2
IP3RIII
STIM1
MOGAT2
CEPT1
Calreticulon
PDI
TRAPα
Calu
BiP
Calnexin
β-COP
Giantin
Tom20
Paxillin
α-tubulin
GAPDH
Calcium signaling
Lipid metabolism
Protein synthsis and folding
Golgi apparatus
Mitochondria
Cytoskeleton
U2OS
j
F-actin
mCherry-ER
Merge
COS7
U2OS
DMSO
eto
cpt
DMSO
CB
k
DMSO
eto
cpt
ER distribution area (μm²)
2.6E-18
1.8E-17
1.9E-12
2.6E-17
7.7E-05
6.2E-10
2.1E-06
3E-08
DMSO
CB
COS7
U2OS
l
DMSO
eto
Total
P
S
HeLa
U2OS
60kDa
50kDa
WB: α-Tubulin

**Figure S1. DNA Damage Triggers Tubular ER Extension and Methodology for ER Morphology Analysis.**

(a) Representative images of the ER in COS7 and U2OS cells treated with DMSO, 50 μM eto, 1 μM cpt, or 1 μM doxo for 16 hours. Scale bar, 10 μm. The outlined area is magnified at the lower right of each panel.

(b) Representative images of U2OS and COS7 cells transfected with mCherry-ER, and immuno-stained with anti-Kinectin or anti-TRAPα antibodies. Scale bars, 10 μm.

(c) Schematic figure illustrating the analysis of the distribution area for ER structures.

(d) Areas of different ER structures in U2OS cells treated with DMSO, 50 μM eto, 1 μM cpt or 1 μM doxo for 16 hours. $n \geq 120$ cells.

(e) Representative images of extended focus (left panel) or 3D reconstruction (right panel) results of fluorescently co-immunolabeled Rtn4 (red) and Climp63 (green) in U2OS cells treated with DMSO, 50 μM eto or 1 μM cpt for 16 hours. Data are captured by spinning disc microscopy and deconvolved by Volocity. Scale bars, 10 μm.

(f) Western blot analysis of lysates from U2OS cells treated with DMSO, 50 μM eto, 1 μM cpt or 1 μM doxo for 16 hours. GAPDH served as a loading control. SERCA2, sarco/endoplasmic reticulum $Ca^{2+}$-ATPase; IP3RIII, inositol 1,4,5-trisphosphate (IP3) receptor III; STIM1, stromal interaction molecule 1; MOGAT2, monoacylglycerol *O*-acyltransferase 2; CEPT1, choline/ethanolamine phosphotransferase 1; PDI, protein disulfide isomerase; TRAPα, translocon-associated protein α; BiP, Ig binding protein.

(g) Representative 3D-SIM images of mCherry-ER in U2OS cells treated with DMSO or 50 μM eto for 16 hours. Images are shown in the extended focus manner. Boxed regions are magnified. Scale bar, 10 μm.

(h, i) Statistical analyses of the volumes of total cell (h) and ER (i) from 3D-SIM images of GFP and mCherry-ER in U2OS cells treated with DMSO or 50 μM eto for 16 hours. $n \geq 27$ cells.

(j) COS7 (upper panel) or U2OS (lower panels) cells transfected with mCherry-ER were treated with DMSO, 50 μM eto or 1 μM cpt in the absence or presence of 5 μM cytochalasin B (CB) for 16 hours, and labeled with phalloidin (green) to show actin filaments (F-actin). Scale bar, 10 μm.

(k) Corresponding analyses of the ER distribution area shown in (j).

(l) Assays to determine the status of microtubule polymerization in HeLa and U2OS cells treated with 50 μM eto for 16 hours. After centrifugation, pellet (P) and supernatant (S) fractions were subjected to western blot (WB) analysis.