Supplementary Materials for

ER-mitochondria contacts couple mtDNA synthesis with mitochondrial division in human cells

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This PDF file includes:

Figs. S1 to S7

Figure S1

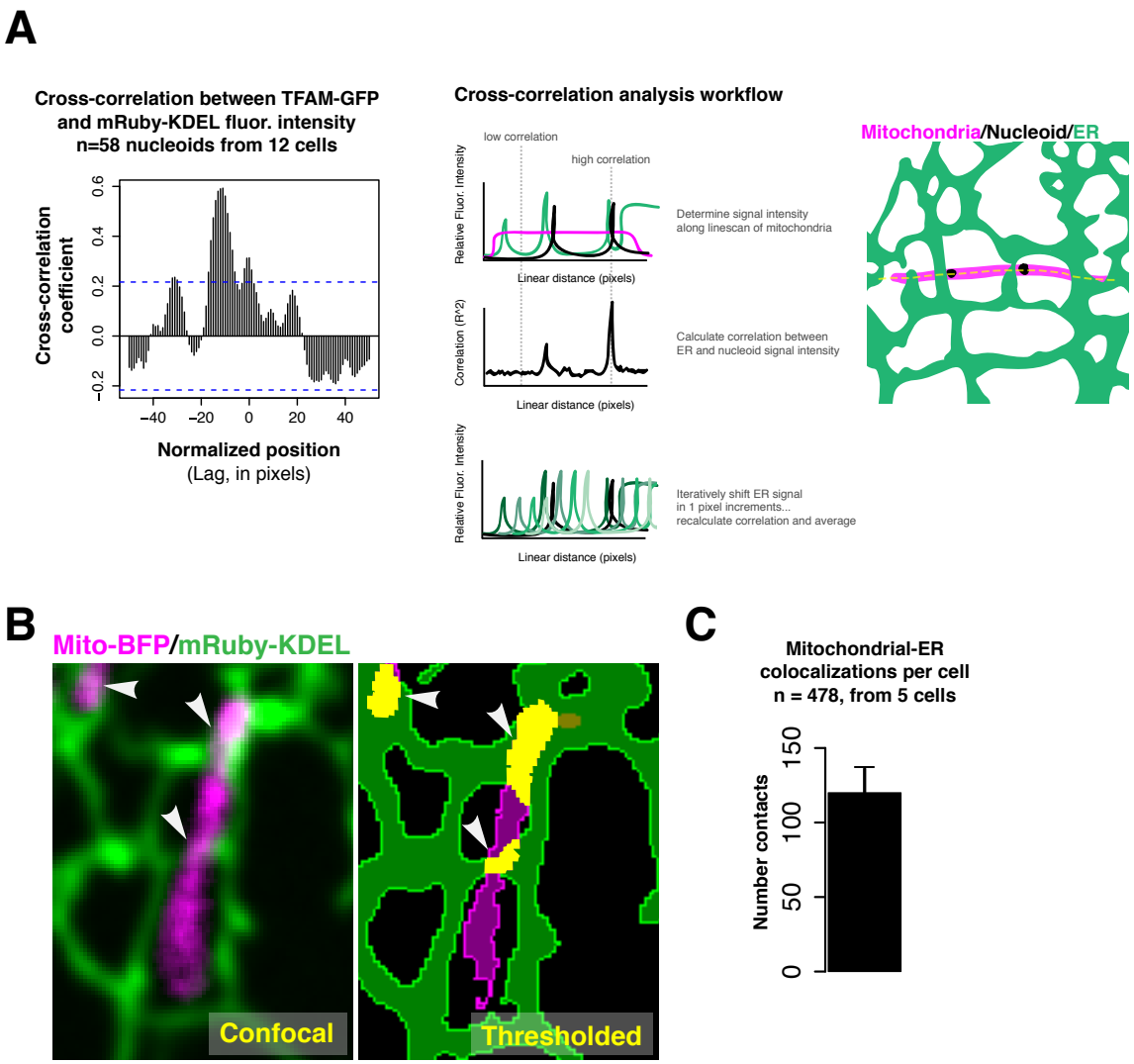

Fig. S1. Dynamics of ER-mitochondria colocalized regions in live mammalian cells

(A) Spatial cross-correlation analysis of nucleoid (TFAM-GFP) and ER (mRuby-KDEL) fluorescence intensity along linescans of mitochondria (n=58) labeled with Mito-BFP in live U2OS cells. Blue dotted lines indicate 95% confidence interval cut-offs. (B) Left, example merged image of a mitochondrion labeled with Mito-BFP (magenta) and ER labeled with mRuby-KDEL (green) from a live U2OS cell. Right, thresholded image from (A), arrows indicate colocalization regions identified for automated tracking (yellow). (C) Quantification of "stable" mitochondria-ER colocalizations (persistent for 5 minutes) per U2OS cell identified via thresholding as in (B).

Figure S2

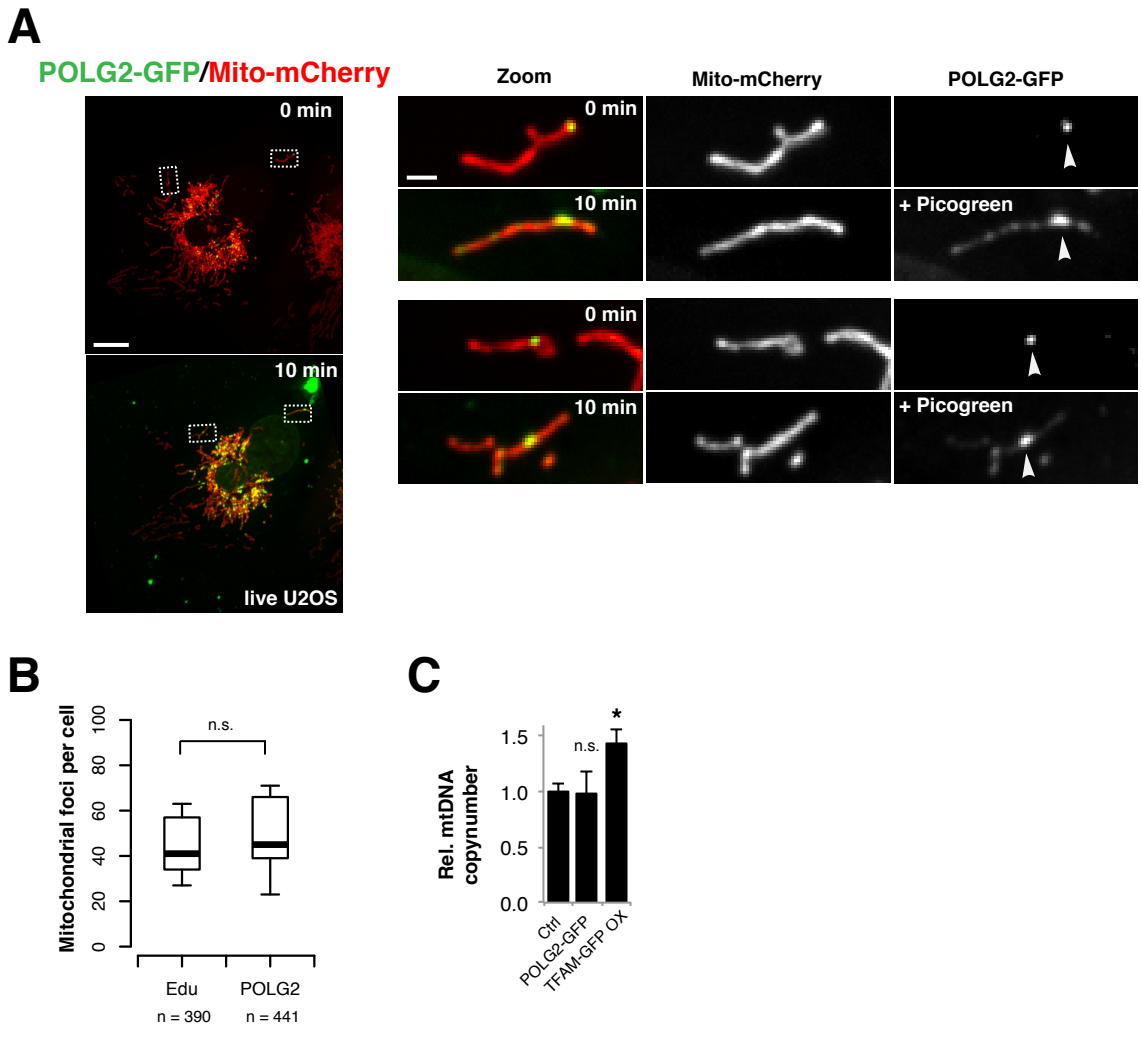

Fig. S2 POLG2-GFP is a faithful reporter for mtDNA replication in cells

(A) Left panels: Additional representative image of a live U2OS cell co-expressing POLG2-GFP and mito-mCherry markers. Right panels: examples of individual mitochondria before (0 min) and after (10 min) Picogreen staining, demonstrating the presence of mtDNA foci which were not labeled by POLG2-GFP. (B) Boxplot demonstrating no significant difference in the number of Edu versus POLG2-GFP foci per U2OS cell, labeled as in Figure 2C. (C) Comparison of mtDNA copynumber in U2OS cells transfected with empty pcDNA3.1 vector (Ctrl), POLG2-GFP, or overexpressing TFAM-GFP (*p<.05, two-tailed t test from three biological replicates). Scale bars: 10 um (left), 2 um (right).

Figure S3

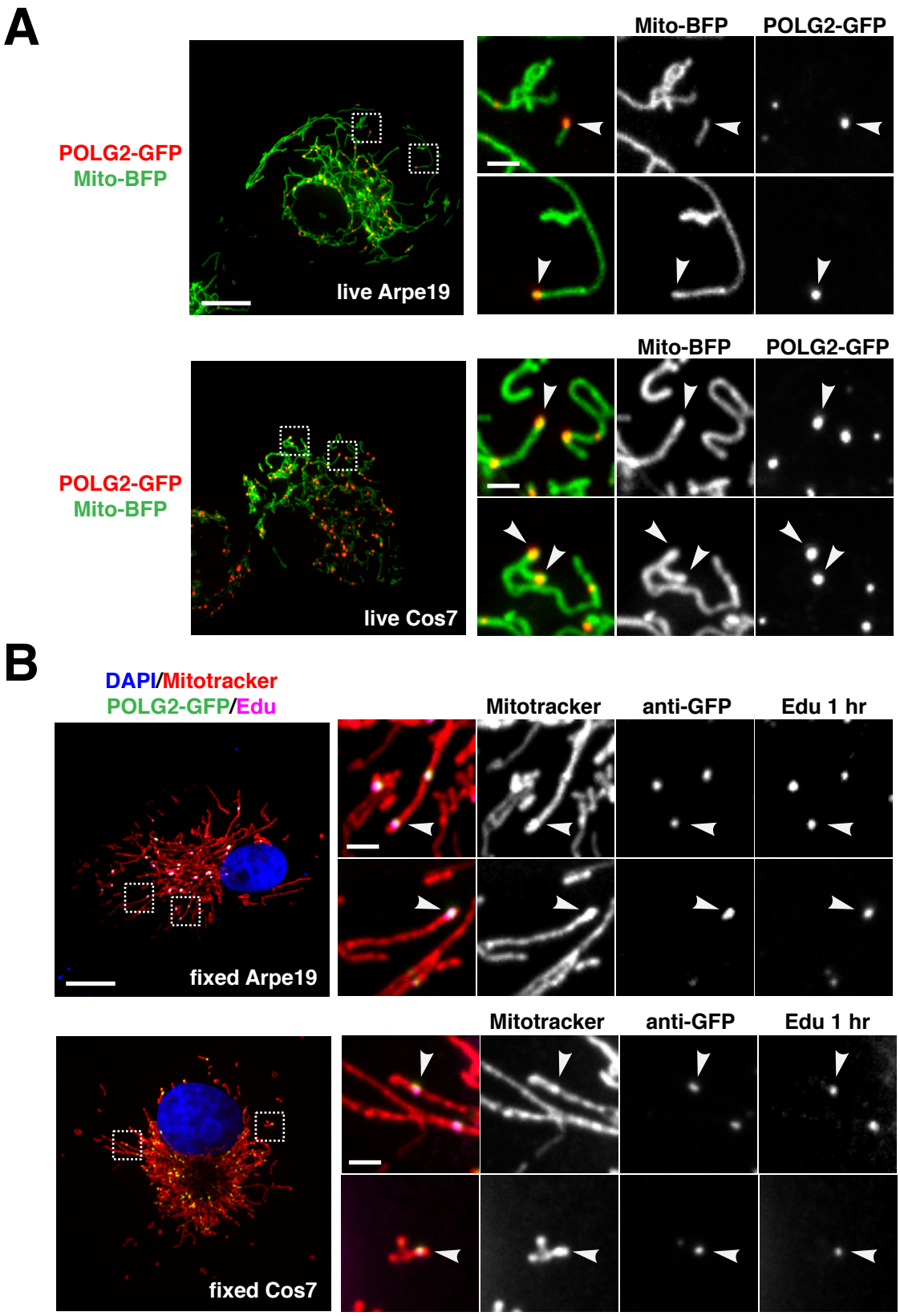
A
Mito-BFP
POLG2-GFP
POLG2-GFP
Mito-BFP
live Arpe19
Mito-BFP
POLG2-GFP
POLG2-GFP
Mito-BFP
live Cos7
B
DAPI/Mitotracker
POLG2-GFP/Edu
Mitotracker
anti-GFP
Edu 1 hr
fixed Arpe19
Mitotracker
anti-GFP
Edu 1 hr
fixed Cos7

Fig. S3 Nascent mtDNA is distributed to mitochondrial tips in live and fixed cells

(A) Representative images of live Arpe19 (top panel) and Cos7 cells (bottom panel) co-expressing mito-BFP and POLG2-GFP. (B) Representative images of fixed Arpe19 (top panel) or Cos7 cells (bottom panel) transfected with POLG2-GFP and labeled with DAPI (DNA, blue), Mitotracker Red (mitochondria, red), anti-GFP AlexaFluor488 (POLG2-GFP, green) and Edu-AlexaFluor647 (replicating nucleoids, magenta). Scale bars: 10 um (full field view), 2 um (zoom).

Figure S4

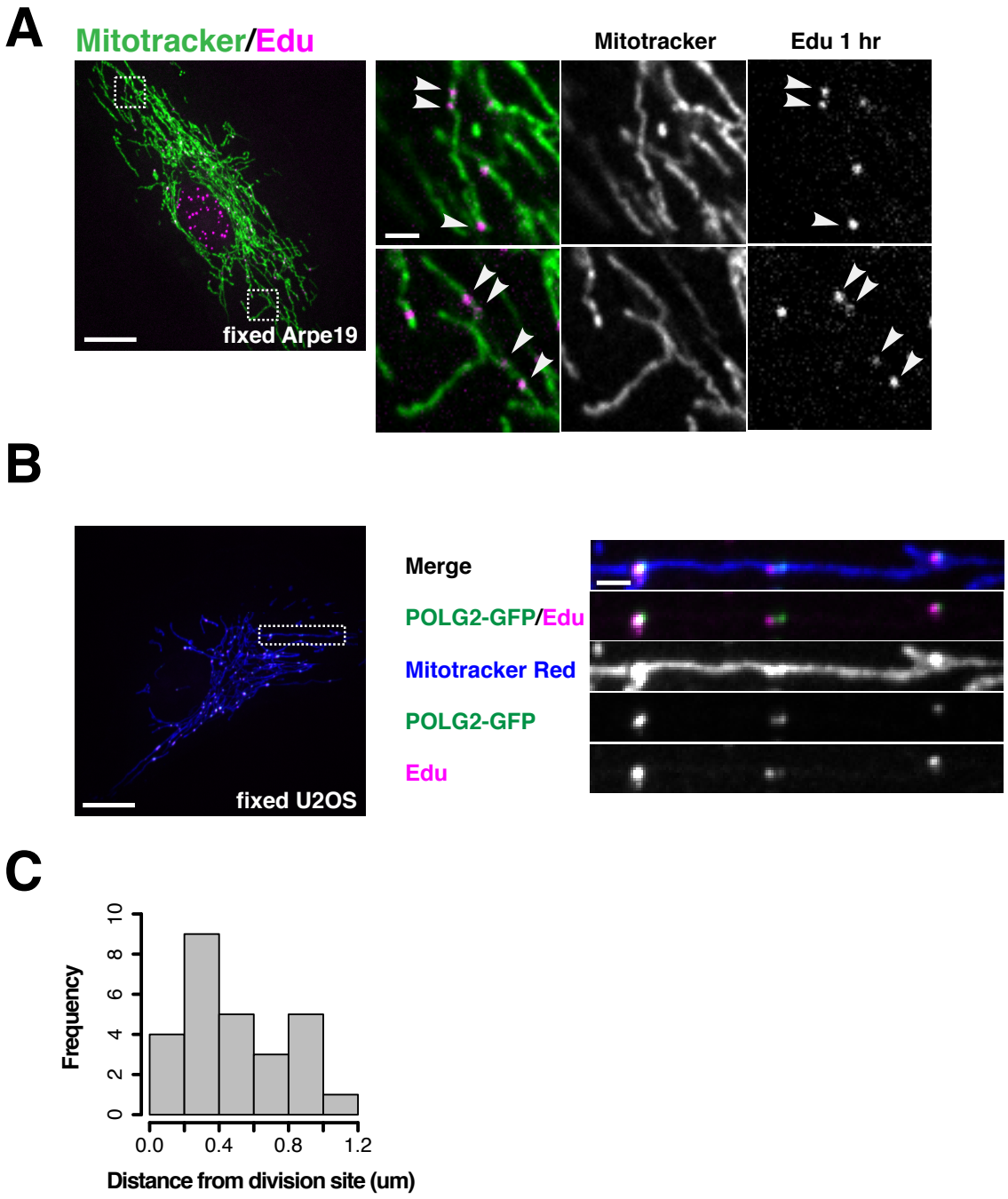

Fig. S4 Segregating genomes are labeled by both Edu and POLG2-GFP

Representative images of fixed Arpe19 cells labeled with Mitotracker Red (mitochondria, blue) and Edu-AlexaFluor647 (replicating nucleoids, magenta) demonstrating Edu focal pairs in unbranched organelle (top panels), or an branched organelle (bottom panels). (B) Representative image of a fixed Arpe19 cell previously transfected with POLG2-GFP

and labeled with Mitotracker Red (mitochondria, blue), anti-GFP AlexaFluor488 (POLG2-GFP, green) and Edu-AlexaFluor647 (replicating nucleoids, magenta), demonstrating an Edu focal pair. (C) Histogram indicating the proximity of division sites to the center of POLG2-GFP foci in live U2OS cells, in microns. Scale bars: 10 um (full field view), 2 um (zoom).

Figure S5

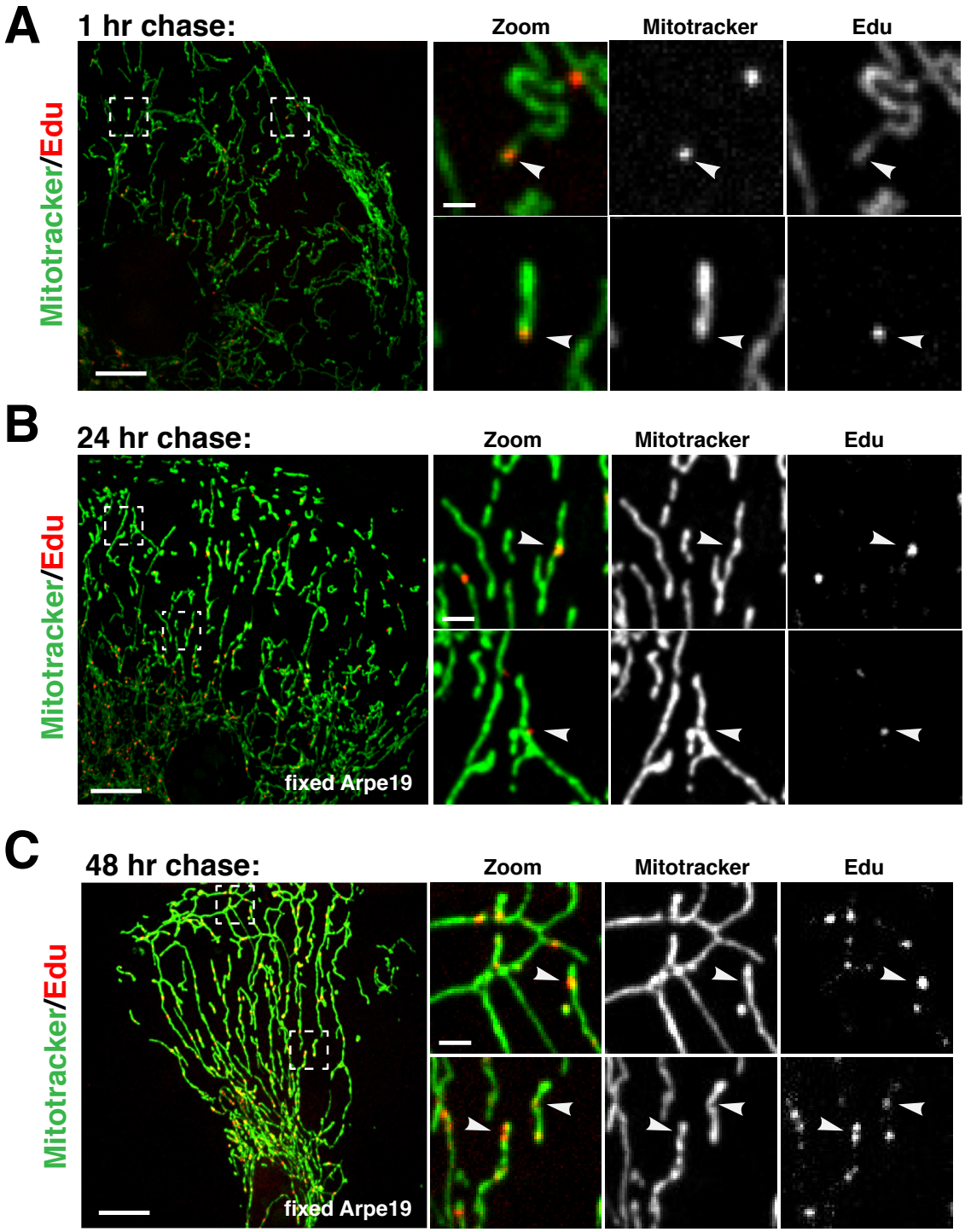
A
1 hr chase:
Zoom
Mitotracker
Edu
Mitotracker/Edu
B
24 hr chase:
Zoom
Mitotracker
Edu
Mitotracker/Edu
fixed Arpe19
C
48 hr chase:
Zoom
Mitotracker
Edu
Mitotracker/Edu
fixed Arpe19

Fig. S5 Edu pulse-chase of mtDNA in Arpe19 cells

Representative images of Arpe19 cells labeled with Mitotracker Red (mitochondria, in green) and pulse-labeled with Edu (replicating nucleoids, red) for 1 hour as described in the text, then chased in Edu-free media for 1 hour (A), 24 hours (B), or 48 hours (C). Arrows indicate nascent mtDNA. Scale bars: (A-C) 10 um (full field view), 2 um (zoom).

Figure S6

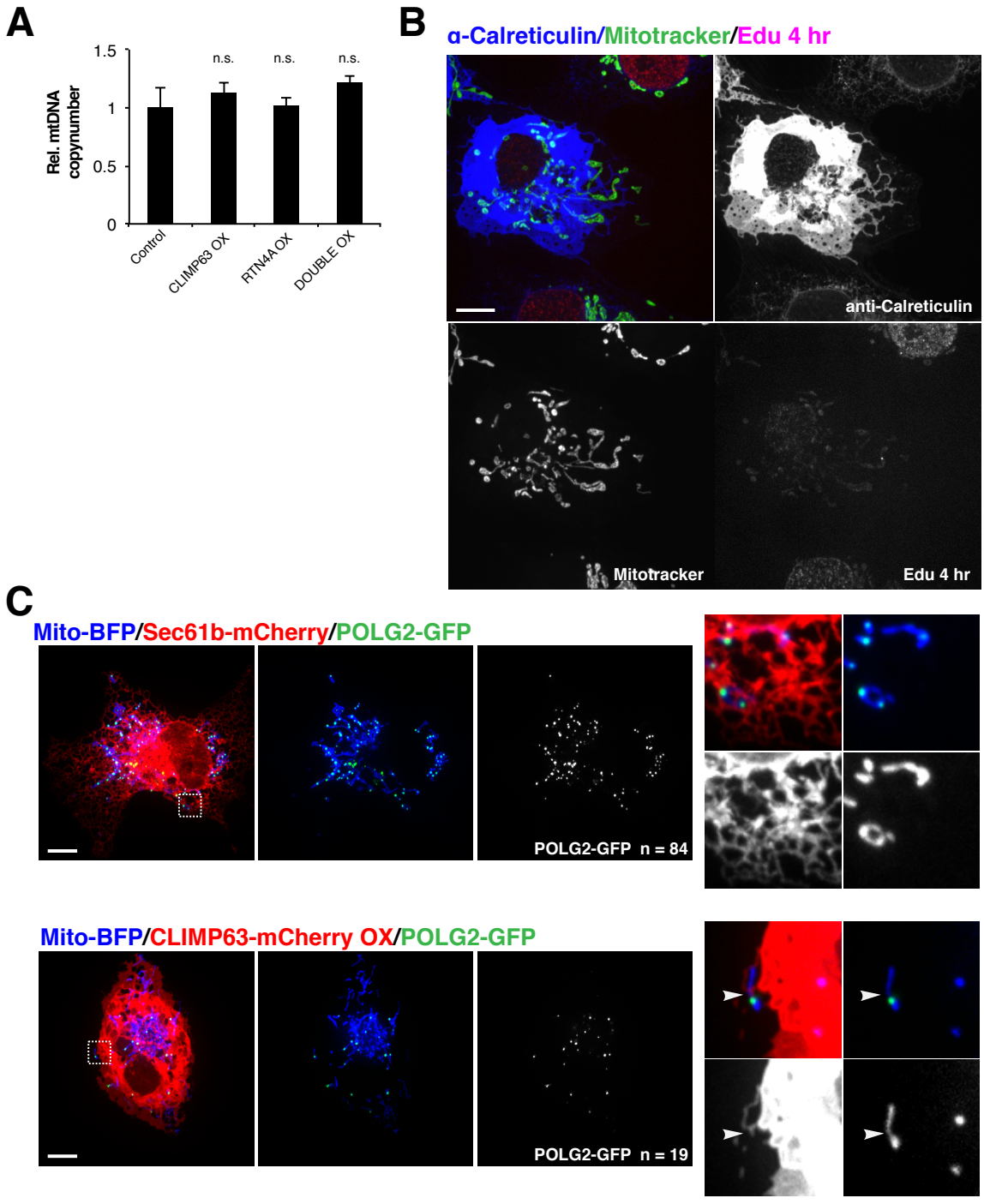

Fig. S6 Replication initiation, but not mtDNA copy number, is linked to ER morphology

(A) Comparison of mtDNA copy number in U2OS cells transfected with empty pcDNA3.1 vector (Control), CLIMP63-GFP, RTN4A-GFP, or both CLIMP63-GFP and

RTN4A-GFP (n.s. $p>0.05$, two-tailed t test from three biological replicates). (B) Full field views of Cos7 cell overexpressing CLIMP63-mChery from Figure 4D showing anti-Calreticulin, Mitotracker Red and Edu signal. (C) Top, representative image of Cos7 cell co-expressing mito-BFP (mitochondria, blue), Sec61-mCherry (ER, red) and POLG2-GFP. Bottom, representative image of Cos7 cell co-expressing mito-BFP and POLG2-GFP and overexpressing CLIMP63-mCherry. Magnified regions shown at right; arrow indicates a residual tubular mitochondria-ER contact marked by a POLG2-GFP focus. Scale bars: (B-C) 10 um (full field view).

Figure S7

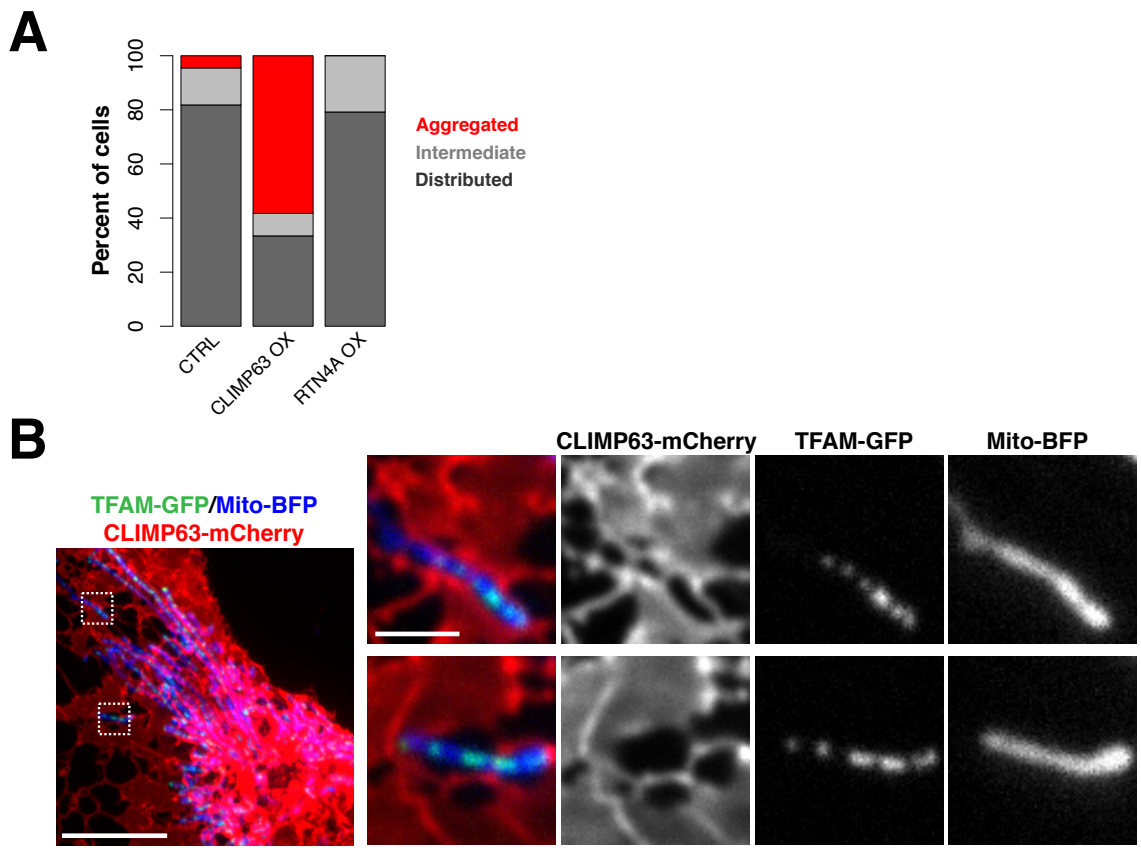

Fig. S7 ER tubules are correlated with nucleoid distribution within mitochondria

(A) Barplot indicating the extent of population-level nucleoid aggregation in live Cos7 cells overexpressing CLIMP63-mCherry, RTN4A-GFP, or both (CTRL). (B) Representative image of a live Cos7 cell overexpressing CLIMP63-mCherry (ER, red) and co-expressing TFAM-GFP (nucleoids, green) and mito-BFP (mitochondria, blue), showing residual tubular mitochondria-ER contacts adjacent to nucleoid foci. Scale bars: (B) 10 um (full field view), 2 um (zoom).