Expanded View Figures

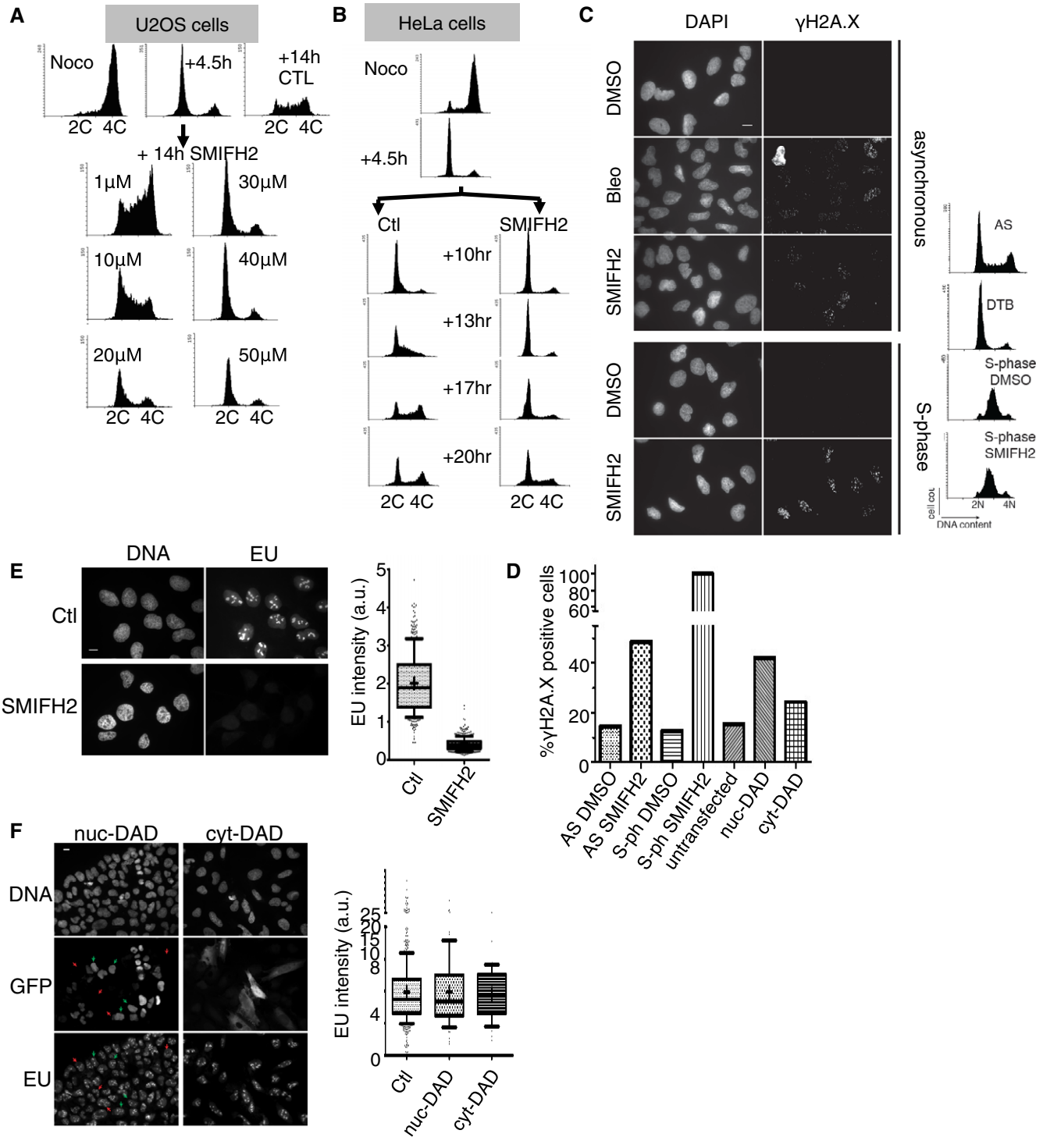


Figure EV1.

Figure EV1. Blocking formins inhibits DNA replication and general transcription and causes replication stress in human cells.

A FACS analysis of M-phase-synchronised U2OS cells, released into G1 in the presence of increasing concentrations of SMIFH2. Cells were collected when control cells were in S-phase (+14 h).

B FACS analysis of HeLa cells, synchronised in M-phase and released into G1 as in Fig 1E, and treated with DMSO (Ctl) or SMIFH2, collected at the time points indicated.

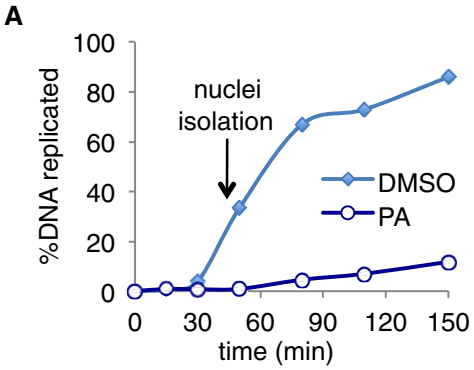
C Asynchronous (AS) or double-thymidine block (DTB) S-phase-synchronised U2OS cells were treated for 2 h with DMSO, bleomycin (Bleo) or SMIFH2, and stained for γH2A.X. Corresponding FACS profiles are shown. Scale bar, 5 μm.

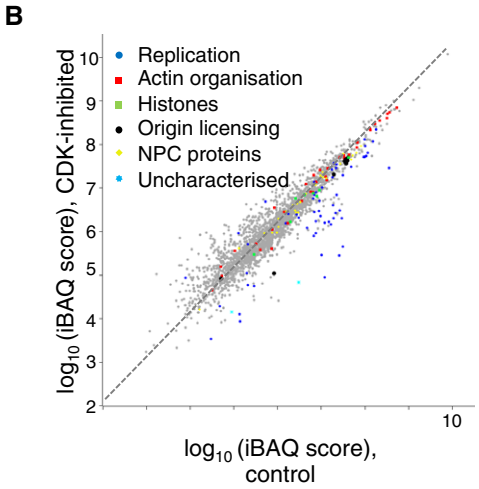
D Quantification of γH2A.X-positive cell number from experiment presented in (C), and in cells transiently transfected with mDia2 nuc-DAD or cyt-DAD constructs. Cells with > 10 foci were considered positive.

E Left: Immunofluorescent images of control or SMIFH2-treated (1 h pre-treatment, 1 h co-incubation) U2OS cells pulsed for 1 h with EU. Scale bar, 5 μm. Right: Quantification of EU signal intensity ($n > 400$). Crosses, mean values; whiskers, 10^{th} and 90^{th} percentiles.

F Left: Immunofluorescent images of U2OS cells transfected with GFP-mDia2 nuc-DAD or cyt-DAD constructs, pulsed for 1 h with EU. Right: Quantification of EU incorporation ($n > 100$, $n = 61$ and $n = 47$, respectively). Scale bar, 5 μm. Red arrows: examples of non-transfected cells; green arrows: examples of GFP-expressing (transfected) cells. Positions of arrows are the same in both panels. Graphs: crosses, mean values; whiskers, 10^{th} and 90^{th} percentiles.

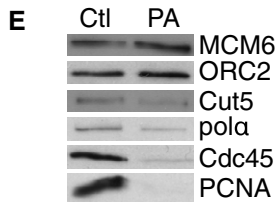
Source data are available online for this figure.





D

GO BP Category	Log10 P-value		
	Entire dataset	Ctrl enriched	CDK1/2-inh. enriched
GO:0006260 I DNA replication	-12.7	-24.1	NE
GO: 0006259 I DNA metabolic process	-21.3	-22.7	NE
GO: 0006297 I DNA gap filling	NE	-10.2	NE
GO:0007049 I Cell cycle	-5.2	-5.4	NE
GO:0000731 I DNA synthesis during DNA repair	NE	-4.0	NE
GO:0006413 I Translation initiation	-4.3	NE	-3.3
GO:0016071 I mRNA metabolic process	-3.6	NE	-2.4
GO:0022402 I Cell cycle process	-2.7	NE	-2.0
GO:0010608 I Posttranscriptional regulation of gene expression	NE	NE	-2.4
GO:0051276 I Chromosome organization	-9.6	NE	NE
GO:0032970 I Regulation of actin filament-based process	-5.9	NE	NE



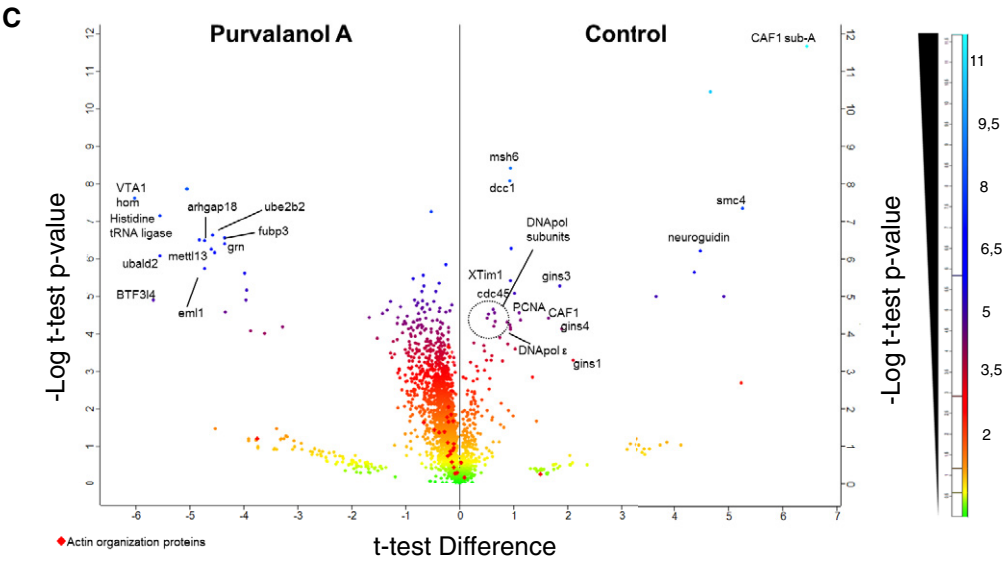


Figure EV2. The proteome of replicating nuclei in XEE.

A Replication time course of sperm chromatin in control and purvalanol A (PA)-treated egg extracts, with nuclei isolated for MS analysis at 50 min.
B Graphical representation of the identified proteome with relative quantitation data (mean values from three replicates). Full dataset, Dataset EV1.
C Volcano plot combining the fold change between control and CDK-inhibited conditions with their log10 *P*-values (Student's *t*-test). The most significantly differentially abundant proteins are highlighted.
D GO analysis using DAVID, showing the most highly enriched GO biological processes in each condition (full GO analysis, Dataset EV2). NE, not enriched.
E Western blots of chromatin fractions from control and PA-treated nuclei used for MS analysis.

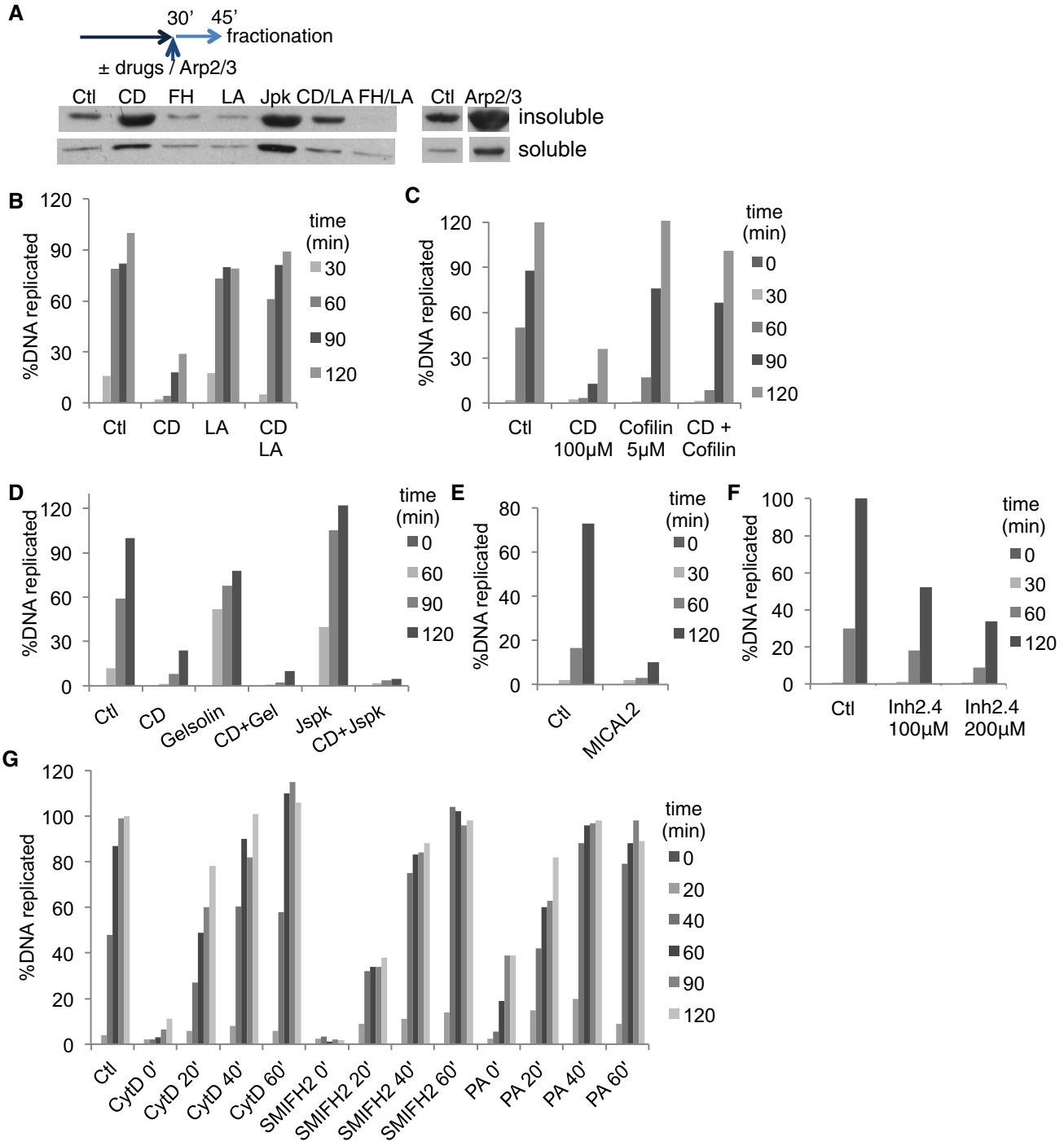


Figure EV3. Actin dynamics is required for DNA replication.

A Nuclei were allowed to form for 30 min before drugs (CytD, CD; SMIFH2, FH; latrunculin A, LA; jasplakinolide, Jpk; CytD and latrunculin A, CD/LA; SMIFH2 and latrunculin A, FH/LA) or Arp2/3 recombinant protein (in combination with VCA domain of WASP) was added, then purified at 45 min. Soluble and insoluble nuclear fractions were blotted for actin. Equal number of nuclei was used in each condition.

B–F DNA replication assessed in control extract, or extracts supplemented with CytD (CD), with or without latrunculin A (LA) (B); CytD (CD) with or without cofilin (C); CytD (CD), gelsolin, CytD and gelsolin (CD + Gel), jasplakinolide (Jspk), or CytD and jasplakinolide (CD + Jspk) (D); recombinant MICAL2 protein (E), or formin inhibitor 2.4 (F). Each panel is representative of multiple experiments: (B) 5 experiments; (C–F) 2 experiments.

G DNA replication analysed in control extract, or extracts supplemented with CytD, SMIFH2 or PA that was added at the time points indicated. Representative of two independent experiments.

Source data are available online for this figure.

Figure EV4. Active nuclear transport requires actin dynamics.

A Immunofluorescence images of nuclei formed in control extracts, or extracts supplemented with the indicated drug or MICAL2 recombinant protein. DNA was stained with Hoechst and the number of normally assembled nuclei was determined. Scale bar, 50 μm.

B Immunofluorescence images of nuclei formed in control or CytD (CD)- or SMIFH2-treated extracts as in Fig 5B. Nuclear membranes were visualised with the lipid dye DHCC. Nuclei were analysed at 60 min. Scale bar, 10 μm.

C Scheme: Nuclei were formed in control extract or in the presence of CytD (CD), SMIFH2, WGA or Triton; at 35 min Dextran10-Alexa488 (Dex10-A488) or Dextran70-rhodamine (Dex70-rhod) were added; nuclei were imaged directly at 50 min. Scale bar, 10 μm.

D Scheme: Nuclei were formed in control extract; at 45 min, CytD (CD), SMIFH2, WGA or Triton was added, followed by the addition of Dextran10-Alexa488 (Dex10-A488) or Dextran70-rhodamine (Dex70-rhod); nuclei were imaged directly at 75 min. Scale bar, 10 μm.

E Western blots of total nuclear fractions in control and formin-inhibited (SMIFH2) extracts, probed with antibodies to the NUPs indicated; the same number of nuclei was analysed for each condition.

F Immunofluorescence images of nuclei formed for 5 and 15 min in control or cytochalasin D-treated extracts, stained for DNA, Elys, RCC1, importin-β and FG-NUPs (mAb414). Scale bar, 10 μm.

Source data are available online for this figure.

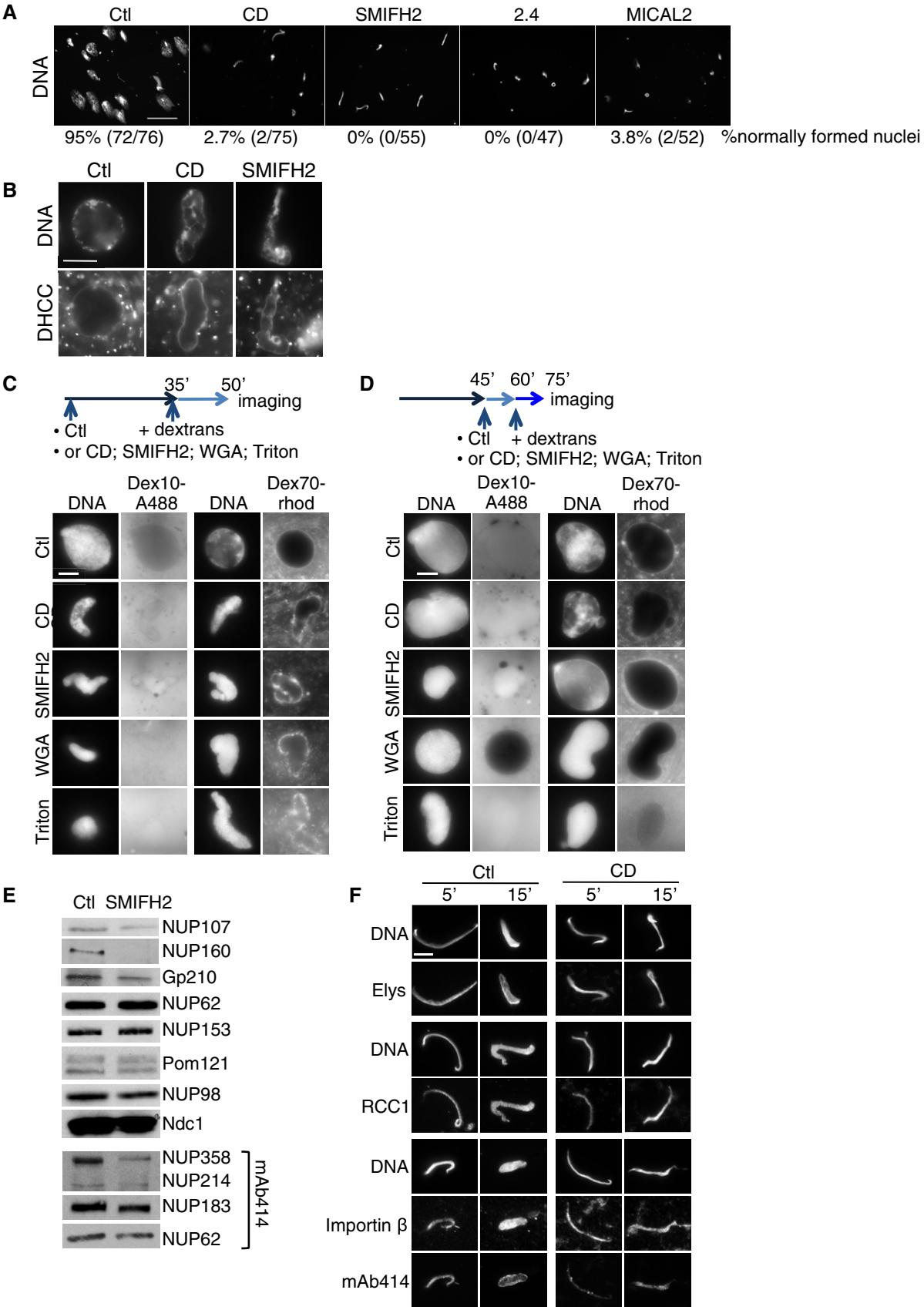


Figure EV4.

Figure EV5. Ongoing nuclear transport is required to promote DNA replication.

A Replication assay in control extract and in extract supplemented with WGA at the concentrations indicated (mg/ml).

B, C XEE was control (Mock) or WGA-depleted (WGA-bpΔ), and assayed for depletion efficiency (after one (1x) and two (2x) rounds of depletion) by Western blot with mAb414 (B) and replication (C).

D Scheme: Nuclei were formed in extract containing aphidicolin to block initiation of DNA replication, then transferred to a second extract, control or treated with 0.1% Triton X-100, and supplemented with Dextran70-rhodamine (Dex70-rhod); nuclei were imaged directly 15 min later. Scale bar, 10 μm.

E Immunofluorescence images of nuclei formed for 60 min in control and geminin (40 nM)-containing extracts, stained for DNA, NUPs (mAb414) and lamin B3. Scale bar, 10 μm.

F Replication time course of control and geminin-treated extract.

G Replication time course of sperm chromatin in NPE extract treated with SMIFH2 or 2.4 formin inhibitor (mean values of two replicates), both used at 200 μM.

H Replication time course of ssDNA in high-speed extract, control (Ctl) or in the presence of SMIFH2 (mean ± SEM; n = 3 and 4, respectively).

Source data are available online for this figure.

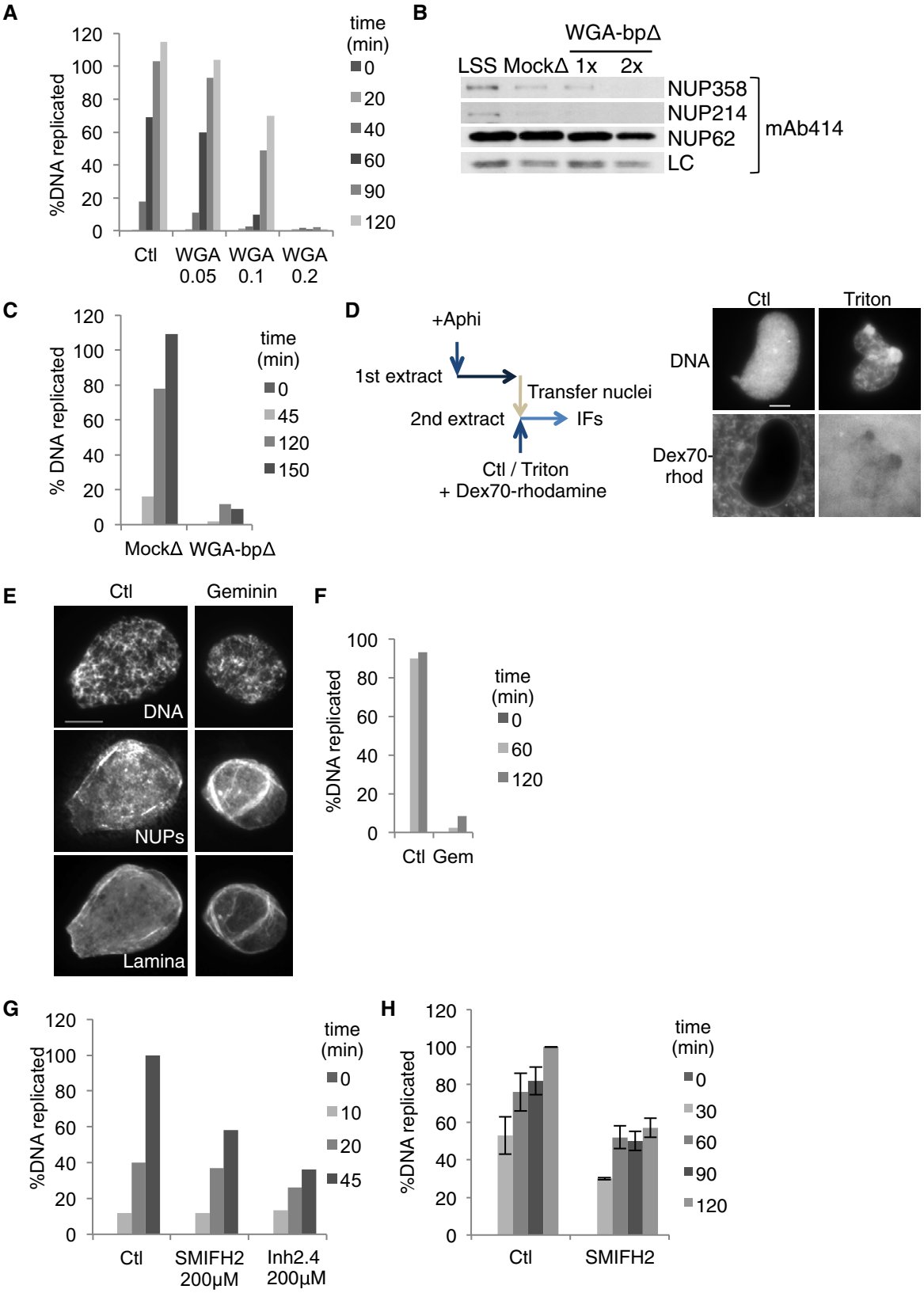


Figure EV5.