Expanded View Figures

Figure EV1. GNL3 prevents DNA resection of stalled replication forks (related to Fig 1).

A Scheme explaining the mini esiRNA screen to determine the best candidates for further characterization. HCT116 cells were transfected in 96-wells plate with each esiRNA from the library (Dataset EV1). After 48 h cells were treated with 1 μM camptothecin for 4 h and subjected to immunofluorescence using an antibody directed against γH2A.X. The level of γH2A.X within nuclei was analyzed using a Celigo high-throughput microscope.

B The γH2A.X level upon depletion and camptothecin treatment in five biological replicates was used to rank the candidates, GNL3 was ranked first and EGFP (negative control) was ranked at the end of the list (21st). The bounds of the box are the 25th and 75th percentiles, the line in the center is the median and the bounds of the whiskers are the maxima and the minima.

C Graphic representation (related to Fig 1B) of the medians of CldU/IdU ratios in three biological replicates, the average is indicated in red.

D Western-blot analysis of HeLa S3 cells treated with 1 μM camptothecin (CPT) during the indicated time.

E Western-blot analysis of HeLa S3 cells treated with 10 μM etoposide (ETP) during the indicated time.

F Graphic representation (related to Fig 1D) of the medians of CldU/IdU ratios in three biological replicates, the average is indicated in red.

G HeLa S3 cells were sequentially labeled for 30 min with IdU and for 30 min with CldU then treated with 1 μM CPT for 240 min. The ratio between CldU and IdU is plotted, and the red line indicates the median. For statistical analysis, Mann–Whitney test was used; ****$P < 0.0001$. At least 100 individual DNA fibers was counted for each condition, the graphic representation of the medians of CldU/IdU ratios in three biological replicates with the average indicated in red is shown.

H HeLa S3 cells were sequentially labeled for 30 min with IdU and for 30 min with CldU then treated with 10 μM ETP for 120 min. The ratio between CldU and IdU is plotted, and the red line indicates the median. For statistical analysis, Mann–Whitney test was used; ****$P < 0.0001$. At least 100 individual DNA fibers was counted for each condition, the graphic representation of the medians of CldU/IdU ratios in three biological replicates with the average indicated in red is shown.

I Western-blot analysis of HeLa S3 cells depleted for GNL3, MRE11, CtIP or EXO1.

J Graphic representation (related to Fig 1E) of the medians of CldU/IdU ratios in three biological replicates, the average is indicated in red.

K Western-blot analysis of Flp-in T-Rex HeLa cells expressing GNL3-WT tagged with FLAG. Cells were first transfected with siControl or siGNL3 for 48 h then expression of GNL3-WT (resistant to the siRNA against GNL3) was induced using 10 μg/ml of doxycycline (DOX) for 16 h.

L Graphic representation (related to Fig 1F) of the medians of CldU/IdU ratios in three biological replicates, the average is indicated in red.

M Western-blot analysis of the indicated proteins upon chromatin fractionation of Hela S3 cells treated 4 h with 5 mM HU.

Source data are available online for this figure.

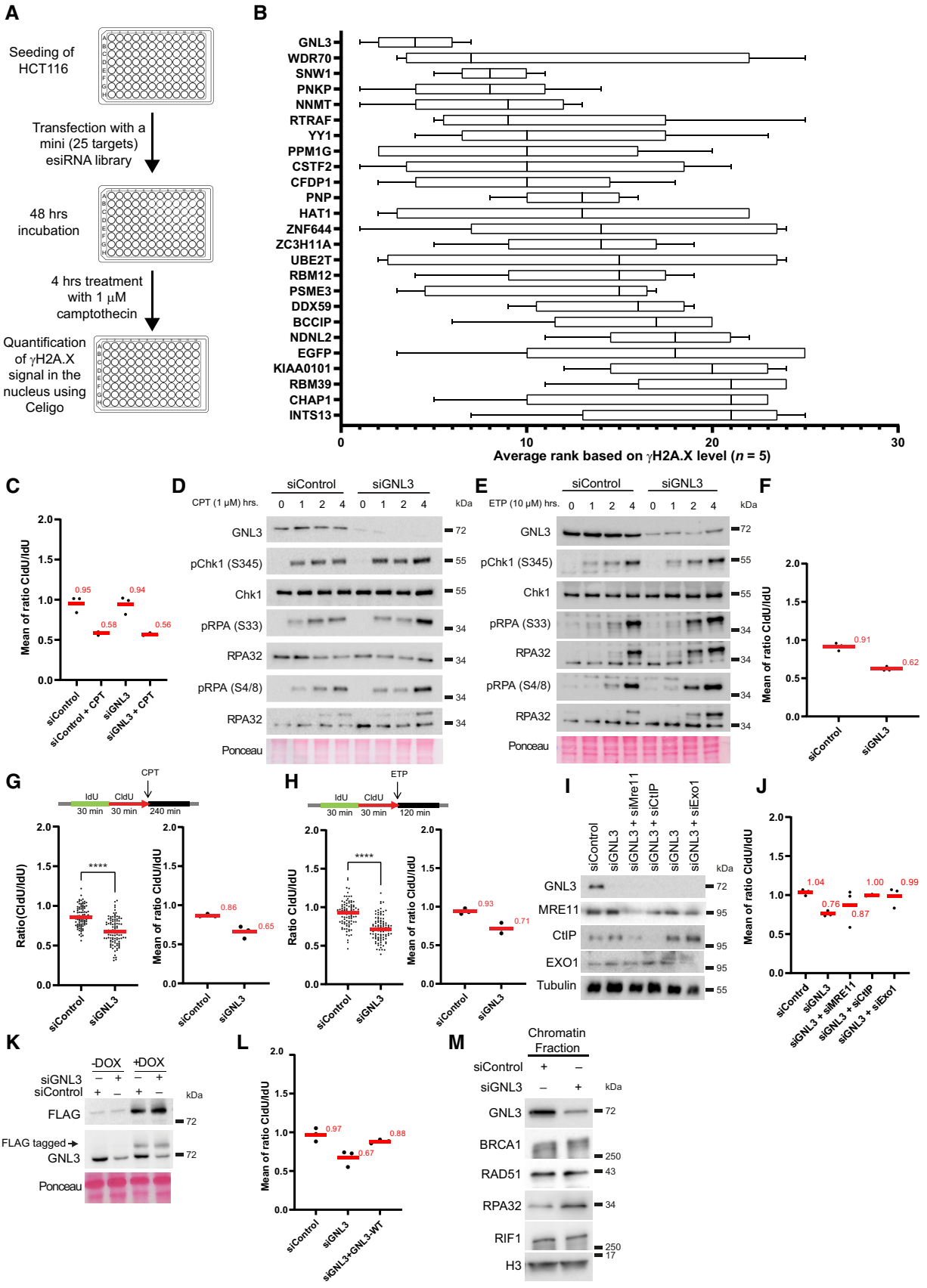

A
Seeding of HCT116
Transfection with a mini (25 targets) esiRNA library
48 hrs incubation
4 hrs treatment with 1 μM camptothecin
Quantification of γH2A.X signal in the nucleus using Celigo
B
GNL3
WDR70
SNW1
PNKP
NNMT
RTRAF
YY1
PPM1G
CSTF2
CFDP1
PNP
HAT1
ZNF644
ZC3H11A
UBE2T
RBM12
PSME3
DDX59
BCCIP
NDNL2
EGFP
KIAA0101
RBM39
CHAP1
INTS13
0
10
20
30
Average rank based on γH2A.X level (n = 5)
C
Mean of ratio CldU/IdU
0.95
0.58
0.94
0.56
siControl
siControl + CPT
siGNL3
siGNL3 + CPT
D
siControl
siGNL3
CPT (1 μM) hrs.
0 1 2 4 0 1 2 4
kDa
GNL3
pChk1 (S345)
Chk1
pRPA (S33)
RPA32
pRPA (S4/8)
RPA32
Ponceau
72
55
55
34
34
34
34
E
siControl
siGNL3
ETP (10 μM) hrs.
0 1 2 4 0 1 2 4
kDa
GNL3
pChk1 (S345)
Chk1
pRPA (S33)
RPA32
pRPA (S4/8)
RPA32
Ponceau
72
55
55
34
34
34
34
F
Mean of ratio CldU/IdU
0.91
0.62
siControl
siGNL3
G
CPT
IdU
CldU
30 min
30 min
240 min
Ratio(CldU/IdU)
****
Mean of ratio CldU/IdU
0.86
0.65
siControl
siGNL3
H
ETP
IdU
CldU
30 min
30 min
120 min
Ratio CldU/IdU
****
Mean of ratio CldU/IdU
0.93
0.71
siControl
siGNL3
I
siControl
siGNL3
siGNL3 + siMre11
siGNL3 + siCtIP
siGNL3
siGNL3 + siExo1
kDa
GNL3
MRE11
CtIP
EXO1
Tubulin
72
95
95
95
55
J
Mean of ratio CldU/IdU
1.04
0.76
0.87
1.00
0.99
siContrd
siGNL3
siGNL3 + siMRE11
siGNL3 + siCtIP
siGNL3 + siExo1
K
-DOX
+DOX
siGNL3
siControl
kDa
FLAG
FLAG tagged
GNL3
Ponceau
72
72
L
Mean of ratio CldU/IdU
0.97
0.67
0.88
siControl
siGNL3
siGNL3+GNL3-WT
M
Chromatin Fraction
siControl
siGNL3
kDa
GNL3
BRCA1
RAD51
RPA32
RIF1
H3
72
250
43
34
250
17

Figure EV1.

Figure EV2. GNL3 depletion increases the firing of replication origins (related to Fig 2).

A Flow cytometry experiment of HeLa S3 cells. Nascent DNA was labeled with IdU and total DNA stained with propidium iodide.

B Percentage of HeLa S3 cells positive for histone H3 phosphorylated on Histone 10 (pH3S10) upon thymidine block and release. The error bars represent the standard deviation between three biological replicates.

C Graphic representation (related to Fig 2C) of the average fork velocity in three biological replicates, the average is indicated in red.

D Quantification of chromatin fractionation based on three biological replicates (related to Fig 2E), the error bars represent the standard deviation.

E Graphic representation (related to Fig 2F) of the medians of IdU lengths in three biological replicates, the average is indicated in red. Average length of IdU tracts (indicated in red) in three independent experiments. The average value is indicated in red.

F Loss of RIF1 has effect on replication timing in specific genomic loci. Cells were pulse-labeled with BrdU for 90 min and sorted by flow cytometry in two fractions, S1 and S2, corresponding to early and late S-phase. Neo-synthesized DNA was immunoprecipitated with BrdU antibodies. Early and late neo-synthesized DNAs were labeled with Cy3 and Cy5 and hybridized on microarrays. After processing analyzing with the START-R software, replication-timing profiles can be obtained from two replicates. Shown are the zoomed microarray profiles of the timing of replication on chromosome 1 and chromosome 15 as example. Blue lines represent replication timing from siControl cells and red lines represent siRIF1 cells and gray spots represent the log ratio intensity for each probe of the microarray. Significantly disturbed regions are detected by START-R software and advanced regions are indicated with green line and delayed regions by a pink line.

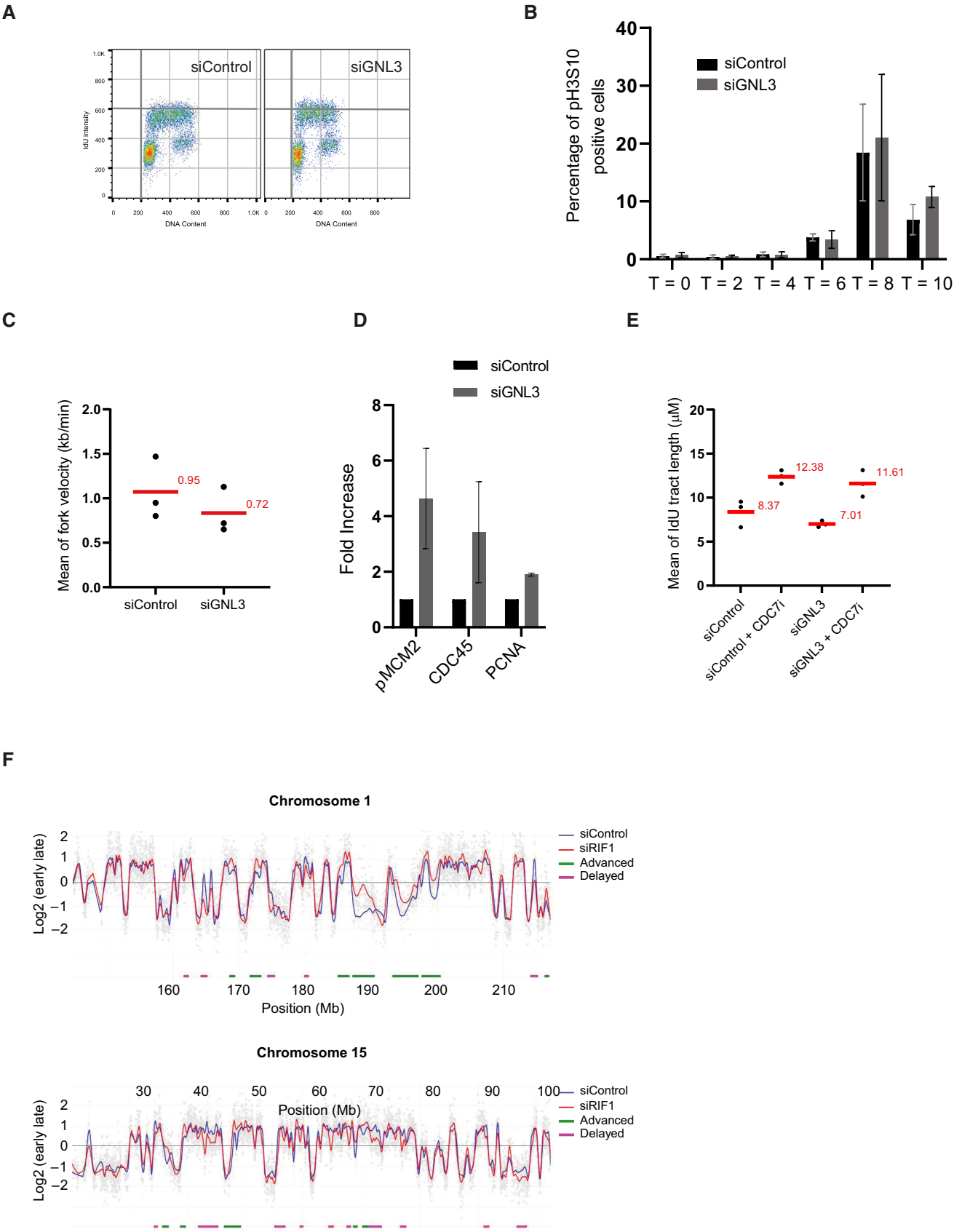

A
siControl
siGNL3
IdU intensity
DNA Content
DNA Content
B
Percentage of pH3S10 positive cells
siControl
siGNL3
T = 0
T = 2
T = 4
T = 6
T = 8
T = 10
C
Mean of fork velocity (kb/min)
0.95
0.72
siControl
siGNL3
D
siControl
siGNL3
Fold Increase
pMCM2
CDC45
PCNA
E
Mean of IdU tract length (μM)
8.37
12.38
7.01
11.61
siControl
siControl + CDC7i
siGNL3
siGNL3 + CDC7i
F
Chromosome 1
Log2 (early late)
siControl
siRIF1
Advanced
Delayed
Position (Mb)
Chromosome 15
Position (Mb)

Figure EV2.

Figure EV3. DNA resection in the absence of GNL3 is a consequence of increased origin firing (related to Fig 3).

A Western-blot analysis upon treatment with HU and inhibition of CDC7, WEE1, or ATR.
B Graphic representation (related to Fig 3B) of the medians of CldU/IdU ratios in three biological replicates, the average is indicated in red.
C Graphic representation (related to Fig 3C) of the medians of CldU/IdU ratios in three biological replicates, the average is indicated in red.
D Graphic representation (related to Fig 3D) of the medians of CldU/IdU ratios in three biological replicates, the average is indicated in red.
E Quantification of RPA phosphorylation (S4/8) upon GNL3 depletion and CDC7 inhibition. The error bars represent the standard deviation between three biological replicates.
F Western-blot analysis of HeLa S3 cells depleted or not for BRCA1 upon treatment with CDC7 inhibitor.
G Graphic representation (related to Fig 3F) of the medians of CldU/IdU ratios in three biological replicates, the average is indicated in red.
H Graphic representation (related to Fig 3G) of the medians of CldU/IdU ratios in three biological replicates, the average is indicated in red.
I Western-blot analysis of HeLa S3 cells.
J Graphic representation (related to Fig 3H) of the medians of CldU/IdU ratios in three biological replicates, the average is indicated in red.
K Western-blot analysis of control U-2 OS cells (U-2 OS) and U-2 OS cells that express the three RPA subunits (SuperRPA).
L Graphic representation (related to Fig 3I) of the medians of CldU/IdU ratios in three biological replicates, the average is indicated in red.

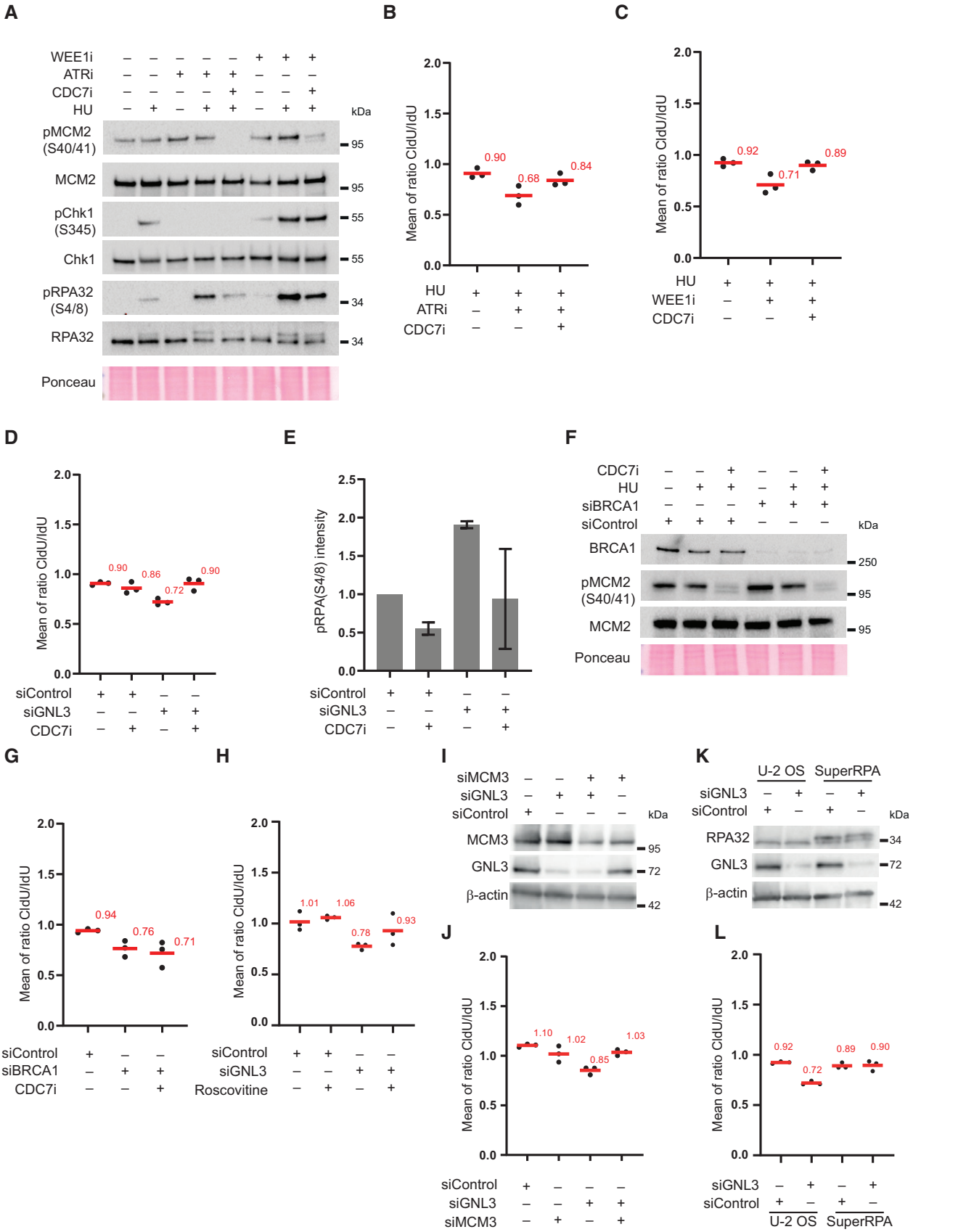

A
WEE1i
ATRi
CDC7i
HU
kDa
pMCM2 (S40/41)
MCM2
pChk1 (S345)
Chk1
pRPA32 (S4/8)
RPA32
Ponceau
95
95
55
55
34
34
B
Mean of ratio CldU/IdU
0.90
0.68
0.84
HU
ATRi
CDC7i
C
Mean of ratio CldU/IdU
0.92
0.71
0.89
HU
WEE1i
CDC7i
D
Mean of ratio CldU/IdU
0.90
0.86
0.72
0.90
siControl
siGNL3
CDC7i
E
pRPA(S4/8) intensity
siControl
siGNL3
CDC7i
F
CDC7i
HU
siBRCA1
siControl
kDa
BRCA1
pMCM2 (S40/41)
MCM2
Ponceau
250
95
95
G
Mean of ratio CldU/IdU
0.94
0.76
0.71
siControl
siBRCA1
CDC7i
H
Mean of ratio CldU/IdU
1.01
1.06
0.78
0.93
siControl
siGNL3
Roscovitine
I
siMCM3
siGNL3
siControl
kDa
MCM3
GNL3
β-actin
95
72
42
J
Mean of ratio CldU/IdU
1.10
1.02
0.85
1.03
siControl
siGNL3
siMCM3
K
U-2 OS
SuperRPA
siGNL3
siControl
kDa
RPA32
GNL3
β-actin
34
72
42
L
Mean of ratio CldU/IdU
0.92
0.72
0.89
0.90
siGNL3
siControl
U-2 OS
SuperRPA

Figure EV3.

Figure EV4. GNL3 interacts with ORC2 in the nucleolus (related to Fig 4).

A Immunofluorescence experiment in Flp-In T-Rex HEK293 cells showing the expression of GNL3-BirA-FLAG and the biotinylation by BirA (revealed by streptavidin coupled with Alexa-488) upon addition of biotin.

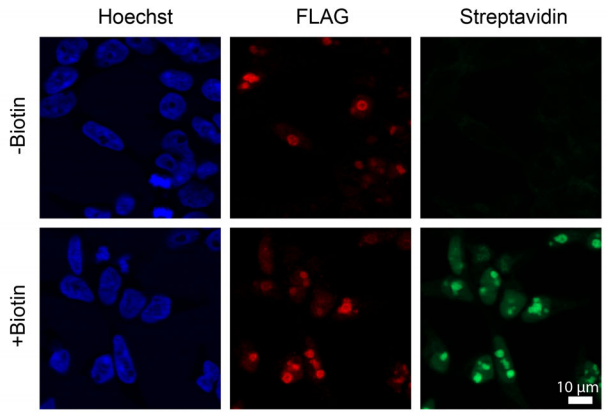
B Common hits found by mass spectrometry between ORC2 immunoprecipitation and GNL3 BioID. The localization was determined using The Human Protein Atlas database (https://www.proteinatlas.org/).

C Examples of GNL3 peaks on chromosome 19 obtained by ChIP-seq of GNL3, INPUT is shown as negative control. The ORC2 ChIP-seq data are obtained from (Miotto *et al*, 2016).

D PLA (proximity ligation assay) analyzing the proximity between ORC2 and GNL3 in HeLa S3 cells.

E PLA (proximity ligation assay) analyzing the proximity between GNL3 and CENP-A in HeLa S3 cells.

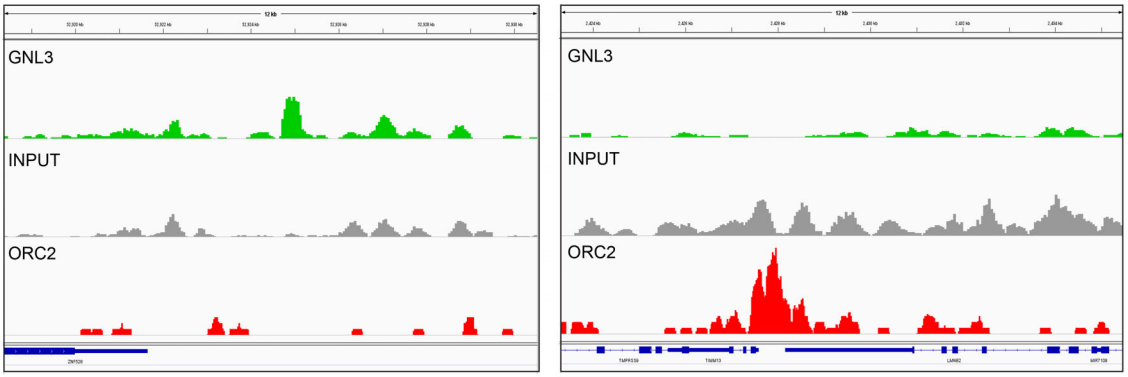
A



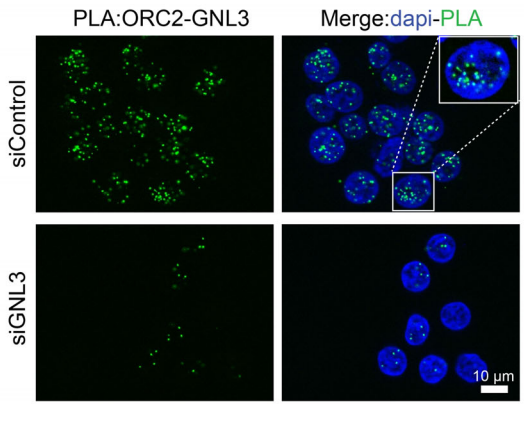
B

Commons hits between ORC2-IP and GNL3 BioID	
Present in the nucleolus	**Not detected in the nucleolus**
COIL	DDX49
DDX10	JMJD1C
DDX18	MRE11
DDX21	PRPF3
EBNA1BP2	SF3B1
ESF1	SNRPA1
EXOSC10	THRAP3
FTSJ3	TMPO
GNL2	TRAP1
MKI67	XRCC6
MYBBP1A	ZC3H11A
NAT10	
NCL	
NKRF	
NOP53	
NPM1	
PES1	
RPF2	
RPL5	
TCOF1	
TRMT1L	
UTP14A	
WDR36	
XRN2	

C



D



E

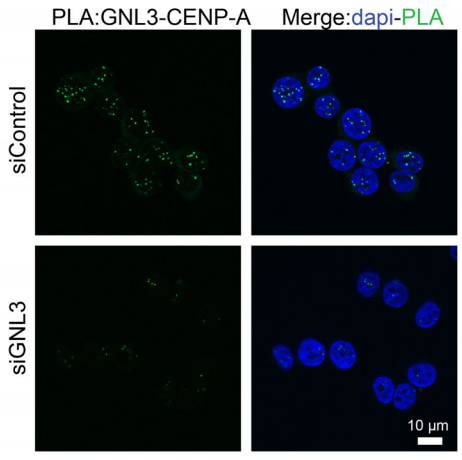


Figure EV4.

Figure EV5. Accumulation of GNL3 into the nucleolus limits origin firing (related to Figs 5 and 6).

A PLA (proximity ligation assay) analyzing the proximity between ORC2 and GNL3-WT-FLAG or GNL3-dB-FLAG in HeLa Flp-In cells upon doxycycline induction using the indicated antibodies.
B PLA (proximity ligation assay) analyzing the proximity between ORC2 and GNL3-dB-FLAG in HeLa Flp-In cells upon doxycycline induction using the indicated antibodies.
C Biological replicate of the GIFD (Global Instant Fork Density) analysis performed in Fig 5G. GIFD value is indicated in red.
D Graphic representation (related to Fig 6A) of the medians of CldU/IdU ratios in three biological replicates, the average is indicated in red.
E Graphic representation (related to Fig 6B) of the medians of CldU/IdU ratios in three biological replicates, the average is indicated in red.
F Western-blot analysis of Flp-In T-Rex HeLa cells expressing exogenous GNL3-WT or GNL3-dB mutants. Cells were transfected with siControl or siGNL3 for 48 h then the expression of GNL3-WT and GNL3-dB (resistant to the siRNA against GNL3) was induced using the indicated doses of doxycycline for 16 h.
G Immunofluorescence analysis of Flp-in T-Rex HeLa cells expressing GNL3-dB at the indicated doses of doxycycline.
H Graphic representation (related to Fig 6C) of the medians of CldU/IdU ratios in three biological replicates, the average is indicated in red.
I Immunofluorescence experiment using the indicated antibodies of Flp-in T-Rex HeLa cells expressing or not exogenous FLAG-ORC2.
J Graphic representation (related to Fig 6E) of the medians of CldU/IdU ratios in three biological replicates, the average is indicated in red.

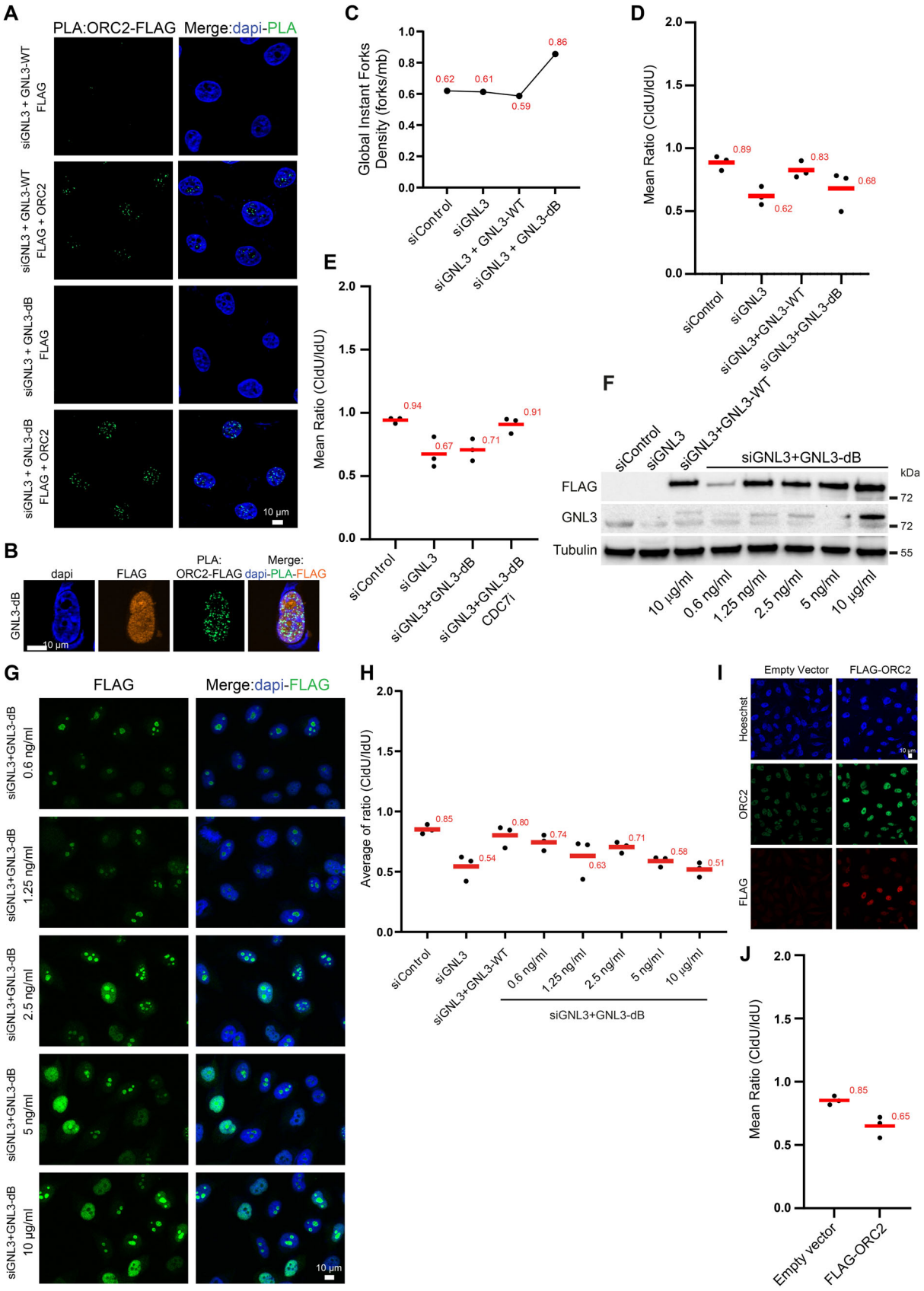

A
PLA:ORC2-FLAG
Merge:dapi-PLA
siGNL3 + GNL3-WT FLAG
siGNL3 + GNL3-WT FLAG + ORC2
siGNL3 + GNL3-dB FLAG
siGNL3 + GNL3-dB FLAG + ORC2
10 µm
B
dapi
FLAG
PLA: ORC2-FLAG
Merge: dapi-PLA-FLAG
GNL3-dB
10 µm
C
Global Instant Forks Density (forks/mb)
0.62
0.61
0.59
0.86
siControl
siGNL3
siGNL3 + GNL3-WT
siGNL3 + GNL3-dB
D
Mean Ratio (CldU/IdU)
0.89
0.62
0.83
0.68
siControl
siGNL3
siGNL3+GNL3-WT
siGNL3+GNL3-dB
E
Mean Ratio (CldU/IdU)
0.94
0.67
0.71
0.91
siControl
siGNL3
siGNL3+GNL3-dB
siGNL3+GNL3-dB CDC7i
F
siControl
siGNL3
siGNL3+GNL3-WT
siGNL3+GNL3-dB
kDa
FLAG
GNL3
Tubulin
72
72
55
10 µg/ml
0.6 ng/ml
1.25 ng/ml
2.5 ng/ml
5 ng/ml
10 µg/ml
G
FLAG
Merge:dapi-FLAG
siGNL3+GNL3-dB 0.6 ng/ml
siGNL3+GNL3-dB 1.25 ng/ml
siGNL3+GNL3-dB 2.5 ng/ml
siGNL3+GNL3-dB 5 ng/ml
siGNL3+GNL3-dB 10 µg/ml
10 µm
H
Average of ratio (CldU/IdU)
0.85
0.54
0.80
0.74
0.63
0.71
0.58
0.51
siControl
siGNL3
siGNL3+GNL3-WT
0.6 ng/ml
1.25 ng/ml
2.5 ng/ml
5 ng/ml
10 µg/ml
siGNL3+GNL3-dB
I
Empty Vector
FLAG-ORC2
Hoeschst
ORC2
FLAG
10 µm
J
Mean Ratio (CldU/IdU)
0.85
0.65
Empty vector
FLAG-ORC2

Figure EV5.