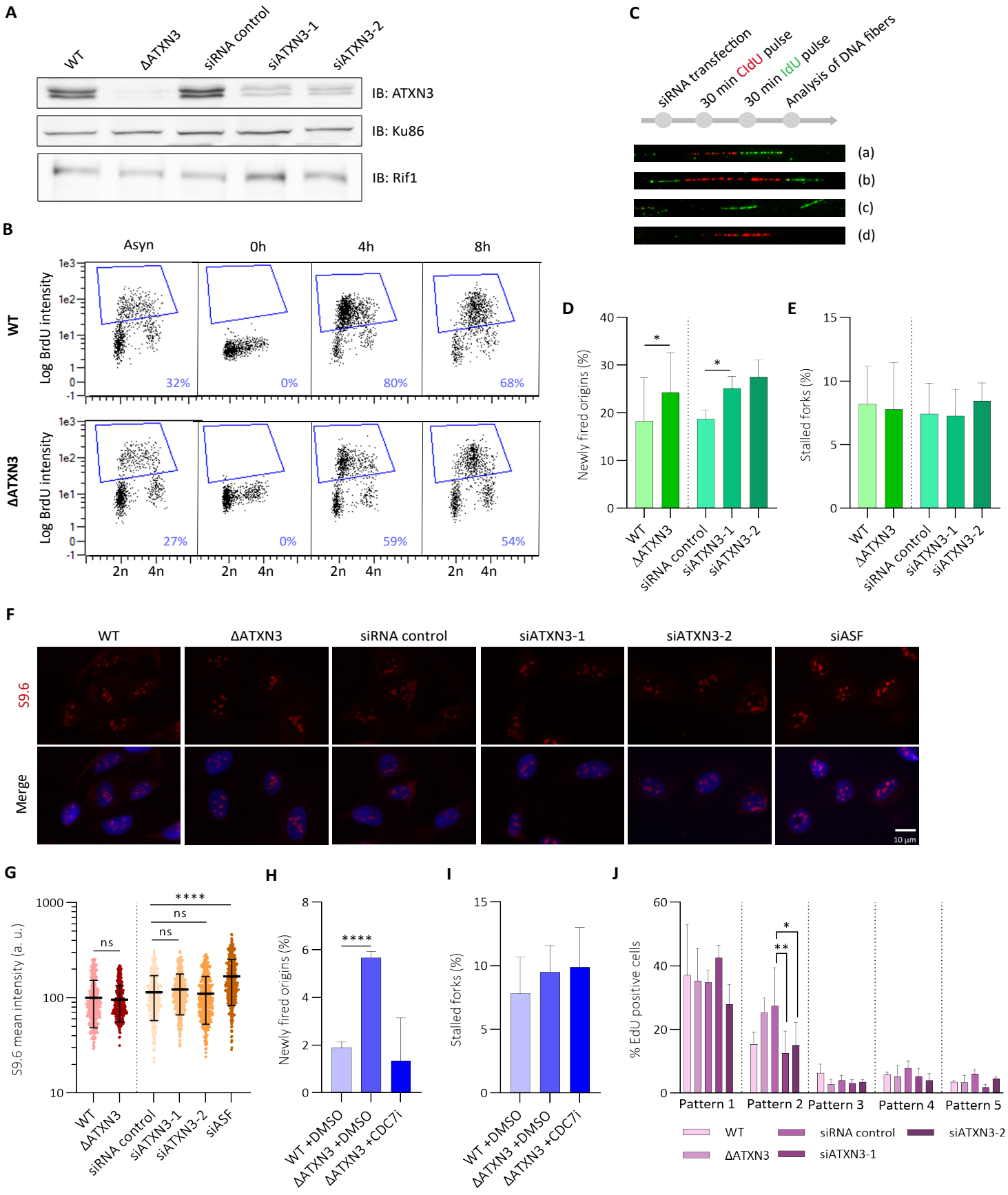
A
WT
ΔATXN3
siRNA control
siATXN3-1
siATXN3-2
IB: ATXN3
IB: Ku86
IB: Rif1
B
Asyn
0h
4h
8h
WT
ΔATXN3
Log BrdU intensity
32%
0%
80%
68%
27%
0%
59%
54%
2n
4n
C
siRNA transfection
30 min CldU pulse
30 min IdU pulse
Analysis of DNA fibers
(a)
(b)
(c)
(d)
D
Newly fired origins (%)
E
Stalled forks (%)
F
WT
ΔATXN3
siRNA control
siATXN3-1
siATXN3-2
siASF
S9.6
Merge
10 µm
G
S9.6 mean intensity (a. u.)
ns
****
H
Newly fired origins (%)
WT +DMSO
ΔATXN3 +DMSO
ΔATXN3 +CDC7i
I
Stalled forks (%)
J
% EdU positive cells
Pattern 1
Pattern 2
Pattern 3
Pattern 4
Pattern 5

Figure S1

Figure S1. ATXN3 controls DNA replication timing. (A) Whole cell extracts (WCE) from wild type (WT) and ATXN3 knockout (ΔATXN3) U2OS cells and U2OS cells transfected with the indicated siRNA oligonucleotides were analyzed by western blot with the indicated antibodies. (B) WT and ΔATXN3 cells were synchronized at G1/S phase by a thymidine block, released for the indicated times and pulsed with BrdU during the last 15 min before fixing. Flow cytometry analysis for BrdU and PI was performed. Blue boxes indicate BrdU-positive cells. (C) Schematic representation of DNA replication fork analysis by DNA fiber assay (top) and different outcomes in the analysis (bottom): (a) ongoing replication fork, (b) origin of replication fired during the first (CldU) pulse, (c) origin of replication fired during second (IdU) pulse (newly fired origin) and (d) stalled fork. (D) Quantification of the percentage of newly fired origins (IdU staining only) in WT, ΔATXN3 and ATXN3-depleted U2OS cells. (E) Quantification of the percentage of stalled forks (CldU staining only) in WT, ΔATXN3 and ATXN3-depleted U2OS cells. (F) Representative images of R-loop staining (using S9.6 antibody) in control, ΔATXN3 and siATXN3 cells by immunofluorescence (IF). A mix of siASF-1 and -2 was used as positive control for R-loops formation (Li & Manley (2005). *Cell*, 122, 365–378). (G) Quantification of R-loop IF in WT, ΔATXN3 and ATXN3-depleted U2OS cells. All samples were analyzed with the same exposure time. Depicted is the mean fluorescence intensity within the nuclear area (identified by DAPI signal) (n=350). (H) Quantification of the percentage of newly fired origins in WT and ΔATXN3 U2OS treated or not with CDC7i (60 μM) for 4 h. (I) Quantification of the percentage of stalled forks in WT and ΔATXN3 U2OS, treated as in (H). (J) Percentage of the described replication patterns shown in Figure 1F in the indicated samples.

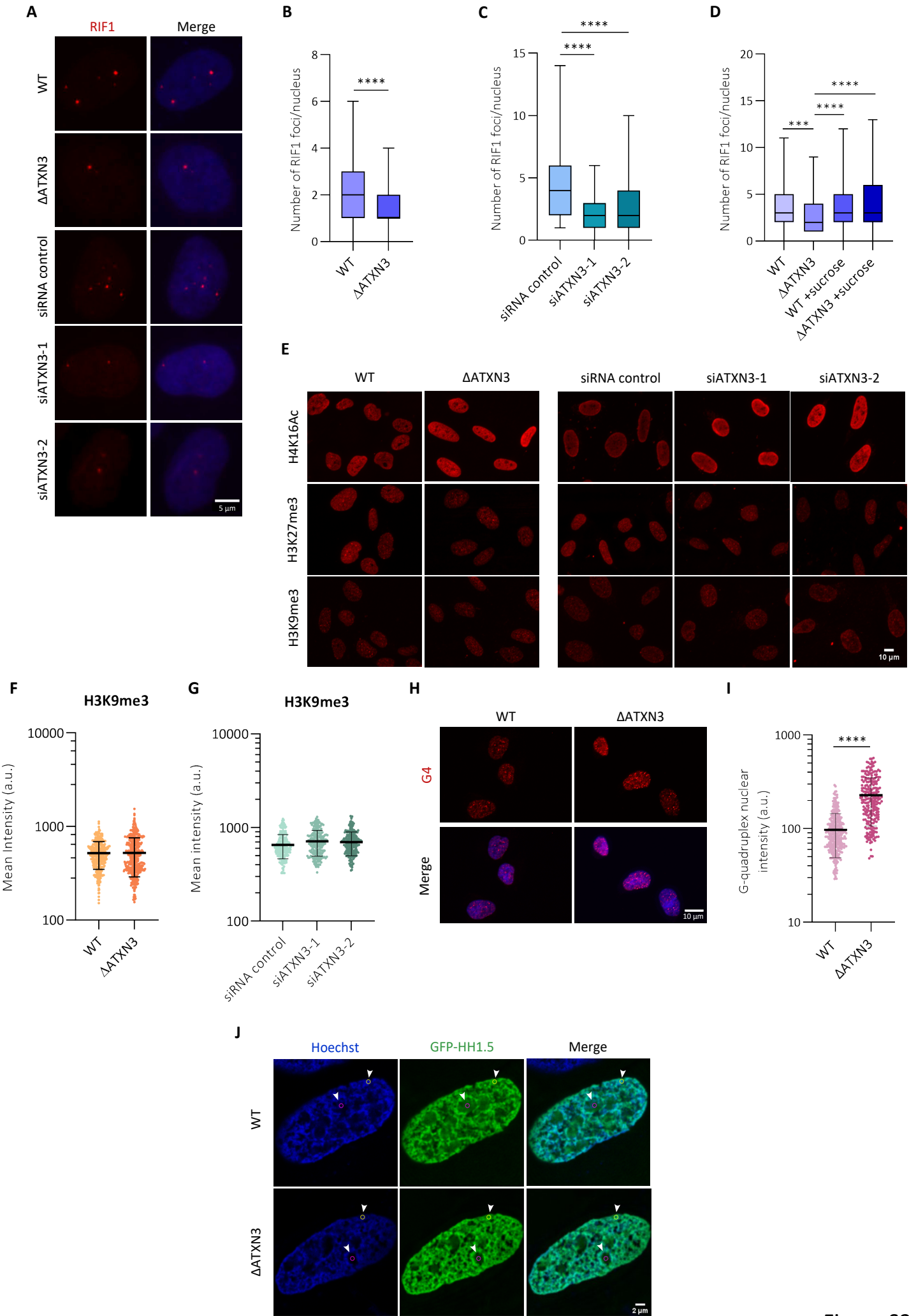
A
RIF1
Merge
WT
ΔATXN3
siRNA control
siATXN3-1
siATXN3-2
5 µm
B
Number of RIF1 foci/nucleus
****
WT
ΔATXN3
C
Number of RIF1 foci/nucleus
****
****
siRNA control
siATXN3-1
siATXN3-2
D
Number of RIF1 foci/nucleus
***
****
****
WT
ΔATXN3
WT +sucrose
ΔATXN3 +sucrose
E
WT
ΔATXN3
siRNA control
siATXN3-1
siATXN3-2
H4K16Ac
H3K27me3
H3K9me3
10 µm
F
H3K9me3
Mean Intensity (a.u.)
WT
ΔATXN3
G
H3K9me3
Mean intensity (a.u.)
siRNA control
siATXN3-1
siATXN3-2
H
WT
ΔATXN3
G4
Merge
10 µm
I
G-quadruplex nuclear intensity (a.u.)
****
WT
ΔATXN3
J
Hoechst
GFP-HH1.5
Merge
WT
ΔATXN3
2 µm

Figure S2

Figure S2. Lack of ATXN3 influences chromatin status. (A) Representative images of RIF1 foci under physiological conditions in control, ΔATXN3 and the indicated siRNA treated cells by IF. (B) Quantification of the number of RIF1 foci per nucleus in WT and ΔATXN3 U2OS cells (n=350). (C) As (B), but for control and siATXN3 cells (n=300). (D) RIF1 IF analysis in the indicated cells, treated or not with sucrose (80 mM) for 30 min (n=300). (E) Representative images of the IF staining for H4K16Ac, H3K27me3 and H3K9me3 in WT, ΔATXN3 and cells transfected with the indicated siRNAs. (F) WT and ΔATXN3 cells stained for H3K9me3 were imaged by fluorescence microscopy using the same exposure time. The mean fluorescence intensity within the nuclear area (identified by DAPI signal) was quantified and analyzed (n=300). (G) As in (F), but for cells transfected with the indicated siRNAs (n=250). (H) Representative images of G-quadruplex (G4) IF in WT and ΔATXN3 U2OS cells after pre-extraction. (I) Quantification of (D). All samples were analyzed with the same exposure time. The mean fluorescence intensity within the nuclear area (identified by DAPI signal) was quantified and analyzed (n=300). (J) Representative images of WT and U2OS cells transiently transfected with GFP-Histone H1.5 and stained with Hoechst 33342. Yellow and magenta circles encompass examples of heterochromatin and euchromatin areas, respectively, where bleaching was performed in the FRAP experiments.

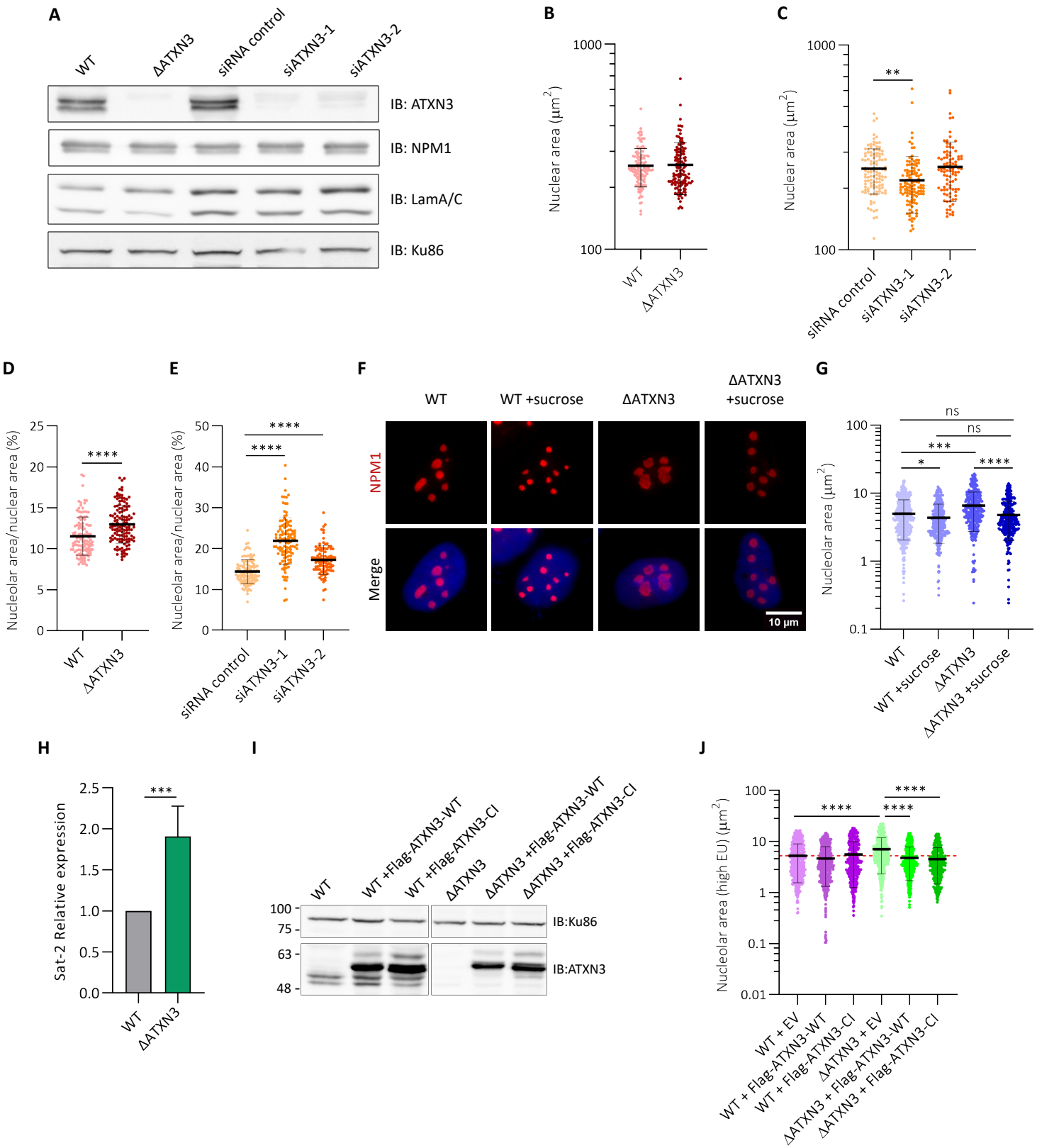
A
WT
ΔATXN3
siRNA control
siATXN3-1
siATXN3-2
IB: ATXN3
IB: NPM1
IB: LamA/C
IB: Ku86
B
Nuclear area (μm²)
1000
100
WT
ΔATXN3
C
Nuclear area (μm²)
1000
100
**
siRNA control
siATXN3-1
siATXN3-2
D
Nucleolar area/nuclear area (%)
25
20
15
10
5
0
****
WT
ΔATXN3
E
Nucleolar area/nuclear area (%)
50
40
30
20
10
0
****
****
siRNA control
siATXN3-1
siATXN3-2
F
WT
WT +sucrose
ΔATXN3
ΔATXN3 +sucrose
NPM1
Merge
10 μm
G
Nucleolar area (μm²)
100
10
1
0.1
ns
ns
***
*
****
WT
WT +sucrose
ΔATXN3
ΔATXN3 +sucrose
H
Sat-2 Relative expression
2.5
2.0
1.5
1.0
0.5
0.0
***
WT
ΔATXN3
I
WT
WT +Flag-ATXN3-WT
WT +Flag-ATXN3-CI
ΔATXN3
ΔATXN3 +Flag-ATXN3-WT
ΔATXN3 +Flag-ATXN3-CI
100
75
63
48
IB:Ku86
IB:ATXN3
J
Nucleolar area (high EU) (μm²)
100
10
1
0.1
0.01
****
****
****
WT + EV
WT + Flag-ATXN3-WT
WT + Flag-ATXN3-CI
ΔATXN3 + EV
ΔATXN3 + Flag-ATXN3-WT
ΔATXN3 + Flag-ATXN3-CI

Figure S3

Figure S3. ATXN3 deficiency disturbs the nucleolar area. (A) WCE of WT, ΔATXN3 and cells depleted for ATXN3 were analyzed by western blot with the indicated antibodies. (B) Quantification of the nuclear area based on DAPI staining in WT and ΔATXN3 cells from Figure 3E. (C) As in (B), but for U2OS cells transfected with control or ATXN3 siRNAs. (D) Ratio of the quantified nucleolar to nuclear areas from Figure 3E in WT and ΔATXN3 cells. (E) As in (D), but for control and U2OS cells depleted for ATXN3. In all panels, n=100. (F) NPM1 IF after pre-extraction in WT and ΔATXN3 cells treated or not with sucrose (80 mM) for 30 min. (G) Quantification of nucleolar area, as determined by NMP1 signal, of (F) (n=300). (H) *Sat-2* relative expression was measured by real-time PCR in WT or ΔATXN3 cells (N=5). (I) WCE from cells in Figure 5A were analyzed by with the indicated antibodies. (J) Quantification of nucleolar area (areas with higher EU intensity) from Figure 5A. Dash red line indicates the mean of the reference condition (WT cells transfected with EV) (n=400).

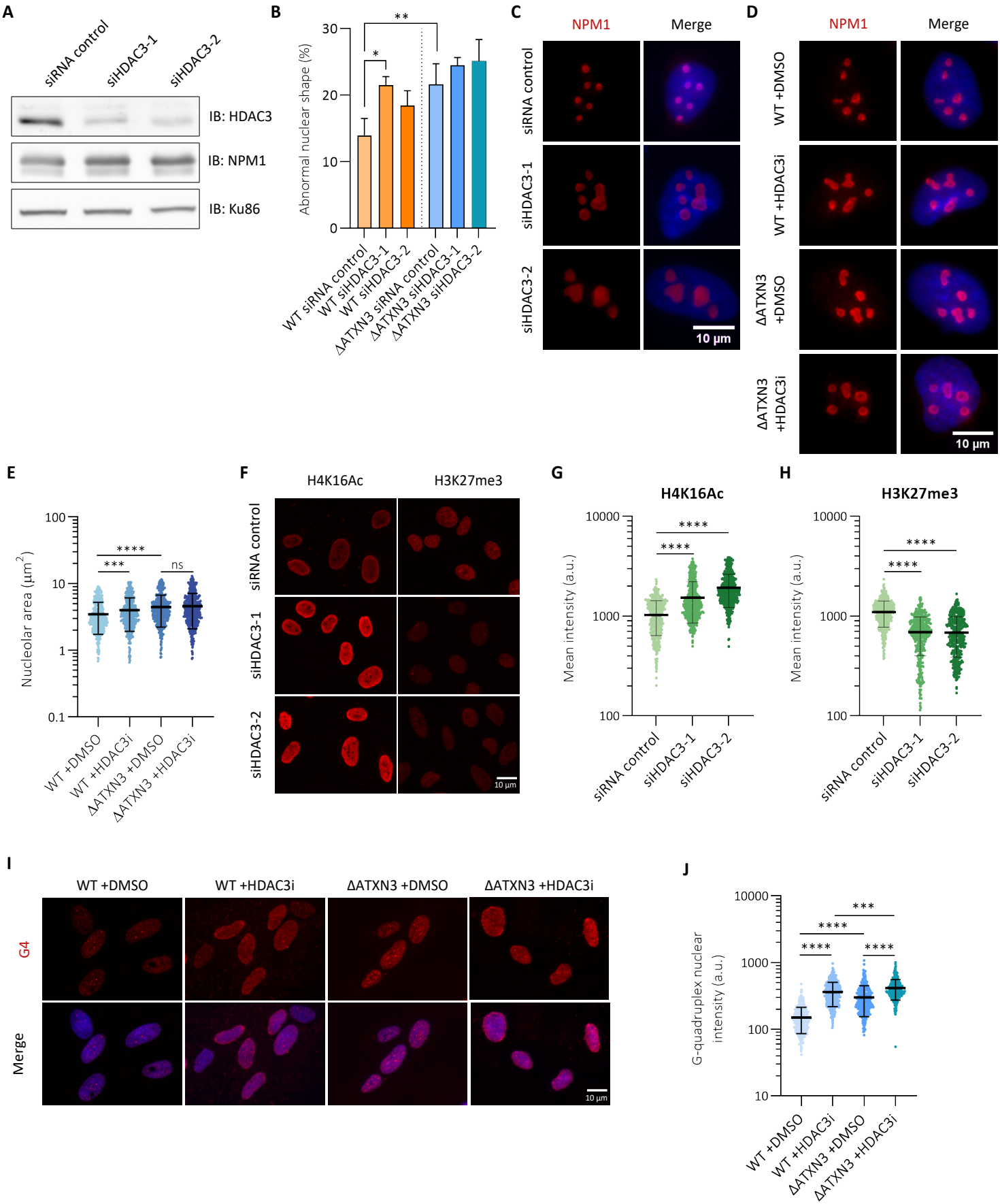
A
siRNA control
siHDAC3-1
siHDAC3-2
IB: HDAC3
IB: NPM1
IB: Ku86
B
Abnormal nuclear shape (%)
WT siRNA control
WT siHDAC3-1
WT siHDAC3-2
ΔATXN3 siRNA control
ΔATXN3 siHDAC3-1
ΔATXN3 siHDAC3-2
C
NPM1
Merge
siRNA control
siHDAC3-1
siHDAC3-2
10 µm
D
NPM1
Merge
WT +DMSO
WT +HDAC3i
ΔATXN3 +DMSO
ΔATXN3 +HDAC3i
10 µm
E
Nucleolar area (µm²)
ns
WT +DMSO
WT +HDAC3i
ΔATXN3 +DMSO
ΔATXN3 +HDAC3i
F
H4K16Ac
H3K27me3
siRNA control
siHDAC3-1
siHDAC3-2
10 µm
G
H4K16Ac
Mean intensity (a.u.)
siRNA control
siHDAC3-1
siHDAC3-2
H
H3K27me3
Mean intensity (a.u.)
siRNA control
siHDAC3-1
siHDAC3-2
I
WT +DMSO
WT +HDAC3i
ΔATXN3 +DMSO
ΔATXN3 +HDAC3i
G4
Merge
10 µm
J
G-quadruplex nuclear intensity (a.u.)
WT +DMSO
WT +HDAC3i
ΔATXN3 +DMSO
ΔATXN3 +HDAC3i

Figure S4

Figure S4. HDAC3 and ATXN3 cooperate to control chromatin structure. (A) WCE of U2OS cells depleted for HDAC3 were analyzed by western blot with the indicated antibodies. (B) Quantification of abnormal nuclear shape based on lamin A/C staining in WT or ΔATXN3 cells transfected with the indicated siRNAs. (C) U2OS cells transfected with the indicated siRNA were pre-extracted, fixed, and stained for NPM1. (D) WT and ΔATXN3 cells were incubated with DMSO or a HDAC3 inhibitor (HDAC3i, 2 μM) for 24 h and stained for NPM1 as in (C). (E) Quantification of nucleolar area (identified by NPM1 signal) from (D) (n=500). (F) U2OS cells transfected with the indicated siRNAs were stained using H4K16Ac and H3K27me3 antibodies. Representative images are shown. (G) Quantification of the mean intensity of H4K16Ac in the nuclear area (identified by DAPI staining) from (F) (n=400). (H) As (G), but for H3K27me3. (I) WT and ΔATXN3 cells were incubated with DMSO or HDAC3i (2 μM) for 24 h and stained for G4 after pre-extraction. (J) Quantification of G4 nuclear mean intensity from (I). All samples were analyzed with the same exposure time. The mean fluorescence intensity within the nuclear area (identified by DAPI signal) was quantified and analyzed (n=350).

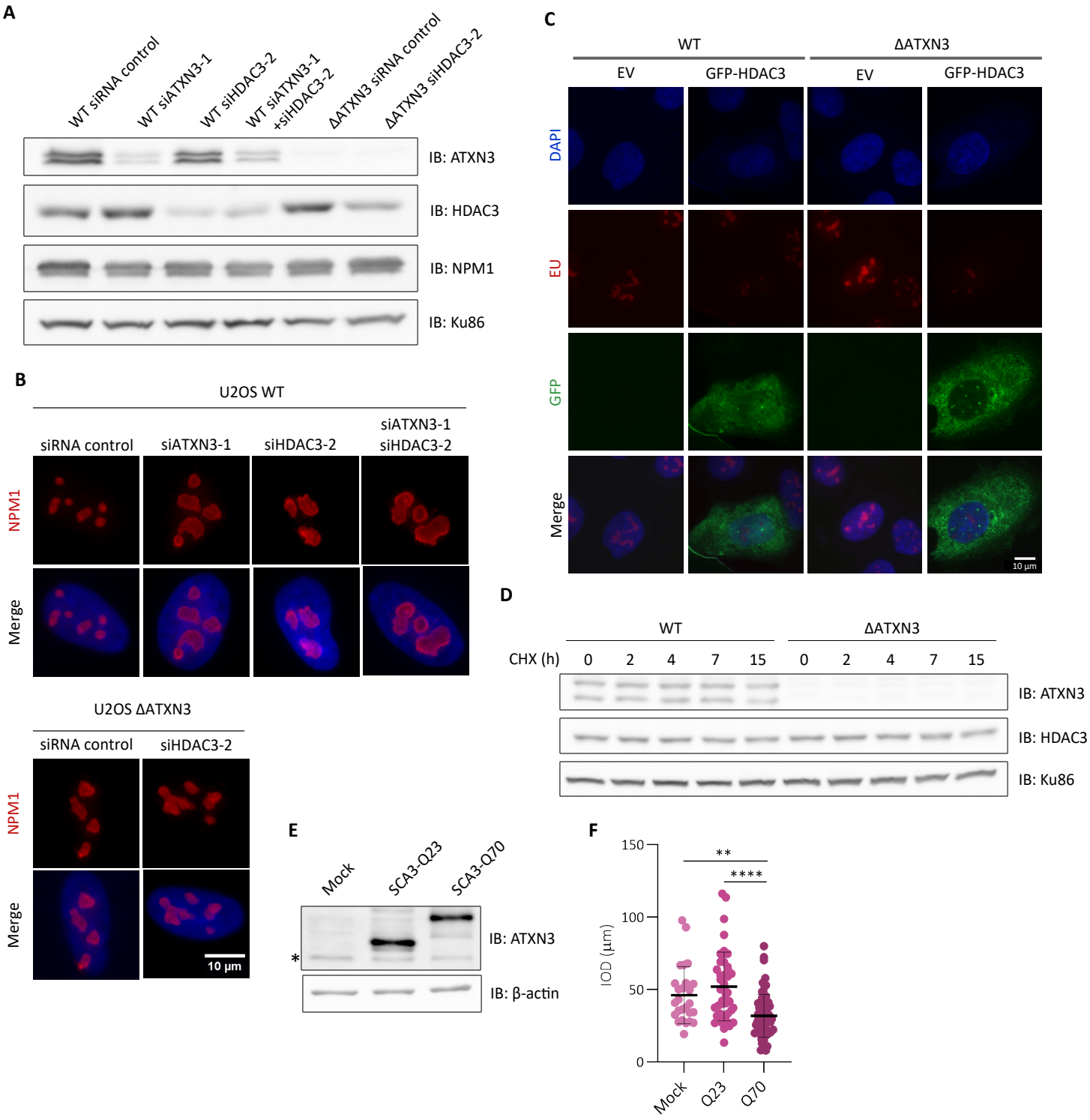
A
WT siRNA control
WT siATXN3-1
WT siHDAC3-2
WT siATXN3-1 +siHDAC3-2
ΔATXN3 siRNA control
ΔATXN3 siHDAC3-2
IB: ATXN3
IB: HDAC3
IB: NPM1
IB: Ku86
B
U2OS WT
siRNA control
siATXN3-1
siHDAC3-2
siATXN3-1 siHDAC3-2
NPM1
Merge
U2OS ΔATXN3
siRNA control
siHDAC3-2
NPM1
Merge
10 μm
C
WT
ΔATXN3
EV
GFP-HDAC3
EV
GFP-HDAC3
DAPI
EU
GFP
Merge
10 μm
D
WT
ΔATXN3
CHX (h)
0 2 4 7 15 0 2 4 7 15
IB: ATXN3
IB: HDAC3
IB: Ku86
E
Mock
SCA3-Q23
SCA3-Q70
*
IB: ATXN3
IB: β-actin
F
IOD (μm)
150
100
50
0
**
****
Mock
Q23
Q70

Figure S5

Figure S5. HDAC3-depletion phenocopies ATXN3-deficiency. (A) WT, ΔATXN3 and cells depleted for ATXN3 or HDAC3 were lysed, and extracts were analyzed by western blot with the indicated antibodies. (B) Representative NPM1 IF images in the indicated cells. (C) WT or ΔATXN3 cells were transfected with an empty vector (EV) or a plasmid expressing GFP-HDAC3. 36 h after transfection cells were incubated with and stained for EU. (D) WT and ΔATXN3 U2OS cells were incubated with cycloheximide (CHX) for the indicated times and then WCE were analyzed by western blot using the indicated antibodies. (E) WCE of CSM14.1 cells expressing normal (SCA3-Q23) or expanded (SCA3-Q70) human ATXN3 were analyzed by western blot with the indicated antibodies. Asterisk indicates endogenous ATXN3. (F) Quantification of the inter origin distance (IOD) in CSM14.1 cells (n=40).