Expanded View Figures

Figure EV1. UBAP2L depletion leads to irregular nuclear shape.

A Representative microscopy images from high-content visual validation siRNA screen in HeLa cells for known and predicted human UBD proteins (Krupina *et al*, 2016). ROIs are shown in the corresponding numbered panels. Scale bar, 10 μm.

B, C Validation of CRISPR–Cas9 mediated UBAP2L KO HeLa cell clones by WB analysis (B) and Sanger-sequencing (C). Proteins MW is indicated in kDa. WB is representative of three independent replicates.

D WT and UBAP2L KO HeLa cells were analyzed by FACS and the DNA content is indicated. At least 10,000 events per condition were counted ($n = 3$).

E, F DLD-1 cells were transfected with the indicated siRNAs for 48 h and the presence of micronuclei was assessed by IF microscopy (E) and quantified in (F). Scale bar, 5 μm. Graphs represent the mean of three replicates ± standard deviation (SD) (two sample two-tailed *t*-test $^{**}P < 0.01$).

G, H U2OS cells were transfected with the indicated siRNAs for 48 h and the presence of micronuclei was assessed by IF microscopy (G) and quantified in (H). Scale bar, 5 μm. Graphs represent the mean of three replicates ± standard deviation (SD) (two sample two-tailed *t*-test $^{*}P < 0.05$).

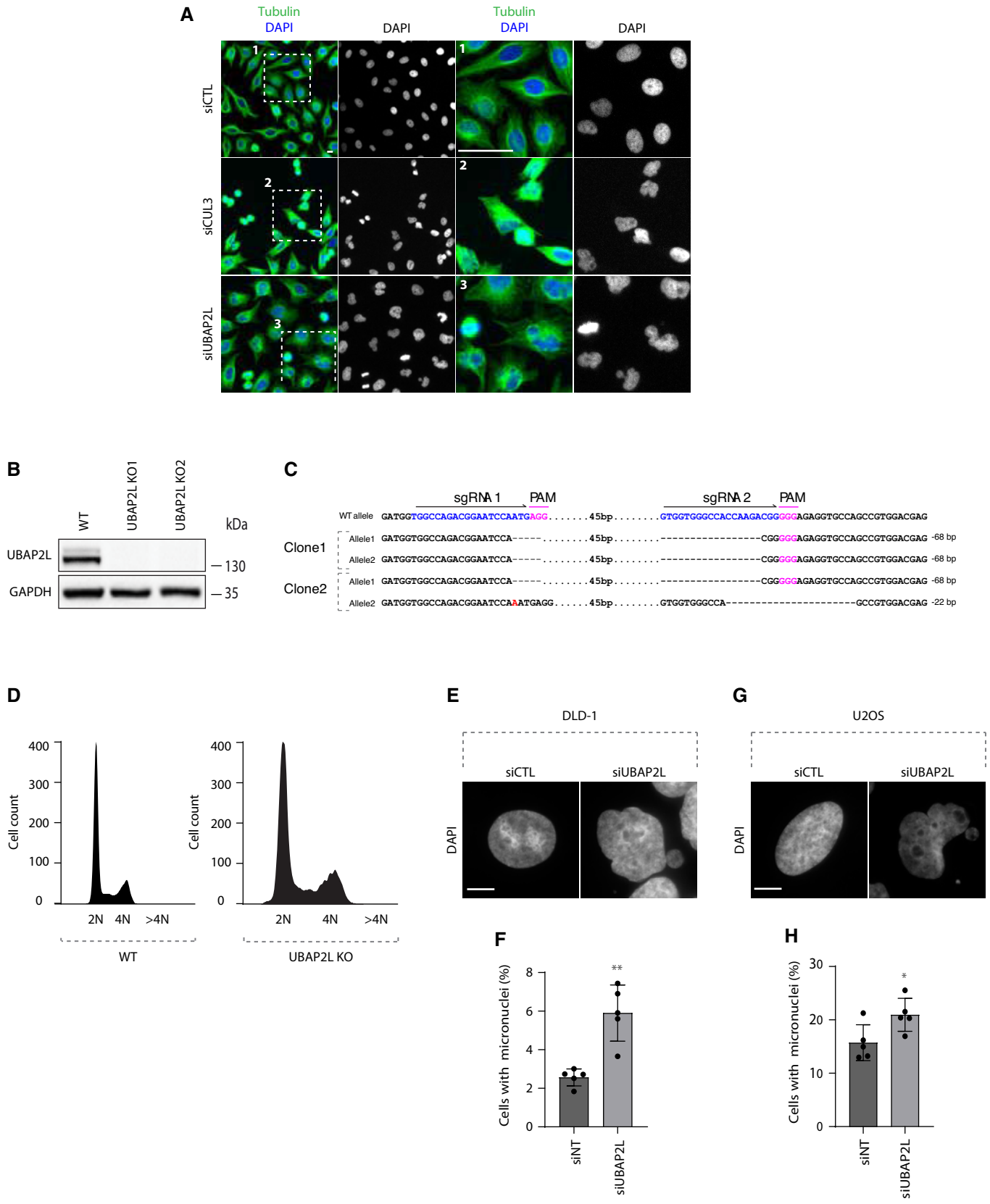
A
Tubulin
DAPI
DAPI
Tubulin
DAPI
DAPI
siCTL
siCUL3
siUBAP2L
1
2
3
B
WT
UBAP2L KO1
UBAP2L KO2
kDa
UBAP2L
GAPDH
130
35
C
sgRNA 1
PAM
sgRNA 2
PAM
WT allele GATGGTGGCCAGACGGAATCCAATGAGG.....45bp.....GTGGTGGGCCACCAAGACGGGGGAGAGGTGCCAGCCGTGGACGAG
Clone1
Allele1 GATGGTGGCCAGACGGAATCCA-----.....45bp.....----------CGGGGGAGAGGTGCCAGCCGTGGACGAG -68 bp
Allele2 GATGGTGGCCAGACGGAATCCA-----.....45bp.....----------CGGGGGAGAGGTGCCAGCCGTGGACGAG -68 bp
Clone2
Allele1 GATGGTGGCCAGACGGAATCCA-----.....45bp.....----------CGGGGGAGAGGTGCCAGCCGTGGACGAG -68 bp
Allele2 GATGGTGGCCAGACGGAATCCAATGAGG.....45bp.....GTGGTGGGCCA----------GCCGTGGACGAG -22 bp
D
Cell count
400
300
200
100
0
2N 4N >4N
WT
UBAP2L KO
E
DLD-1
siCTL
siUBAP2L
DAPI
G
U2OS
siCTL
siUBAP2L
DAPI
F
Cells with micronuclei (%)
8
6
4
2
0
**
siNT
siUBAP2L
H
Cells with micronuclei (%)
30
20
10
0
*
siNT
siUBAP2L

Figure EV1.

Figure EV2. UBAP2L localization during mitosis.

A–E Representative IF pictures of HeLa cells synchronized in mitosis using DTBR after chemical pre-extraction of the cytoplasm using 0.01% of Triton X-100 for 1m30. UBAP2L localization was assessed by co-staining with indicated mitotic structures markers. ROIs are shown in the corresponding numbered panels. Scale bar, 5 μm.

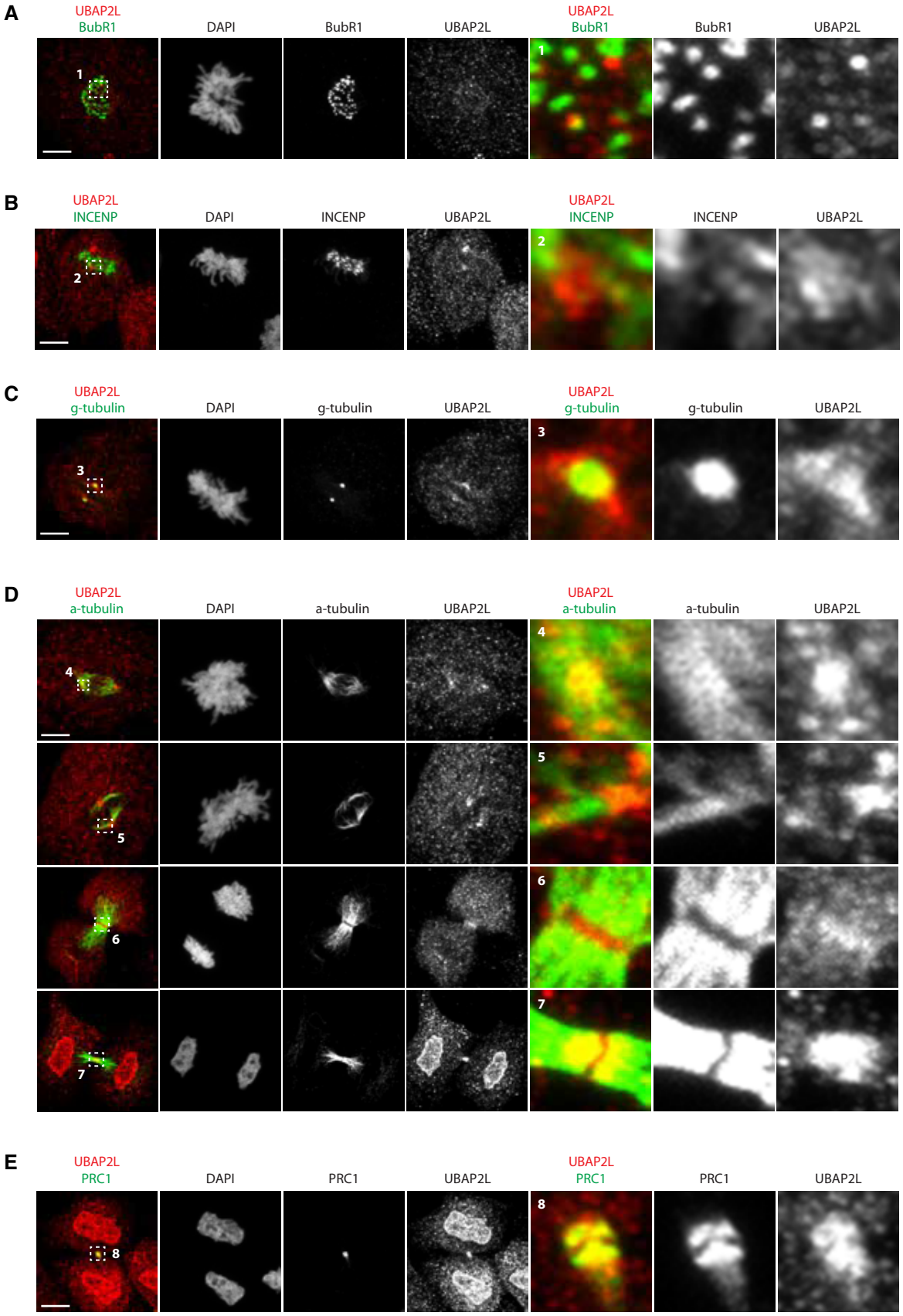

Figure EV2.

Figure EV3. UBAP2L regulates PLK1 expression.

A Quantification of the percentage of cells expressing PLK1 in G1/S synchronized HeLa cells treated with the indicated siRNAs. Graphs represent the mean of three replicates ± SD (two sample two-tailed *t*-test $^{**}P < 0.01$). At least 250 cells were quantified per condition for each replicate.

B Quantification of the percentage of cells expressing PLK1 in G1/S synchronized WT or UBAP2L KO HeLa cells using DTB. Graphs represent the mean of four replicates ± SD (one-way ANOVA with Dunnett's correction $^{***}P < 0.001$, $^{****}P < 0.0001$). At least 250 cells were quantified per condition for each replicate.

C Representative IF images of control or UBAP2L-downregulated HeLa cells synchronized in mitosis using DTBR ($n = 3$). Scale bar, 4 μm.

D–G Representative IF images of WT or UBAP2L KO HeLa cells synchronized in G1/S using DTB. Scale bar, 5 μm. The percentage of cells expressing AurA, Cyclin B1 or AurB was quantified in (E), (F), and (G), respectively. At least 100 cells per condition was analyzed for each experiment.

Source data are available online for this figure.

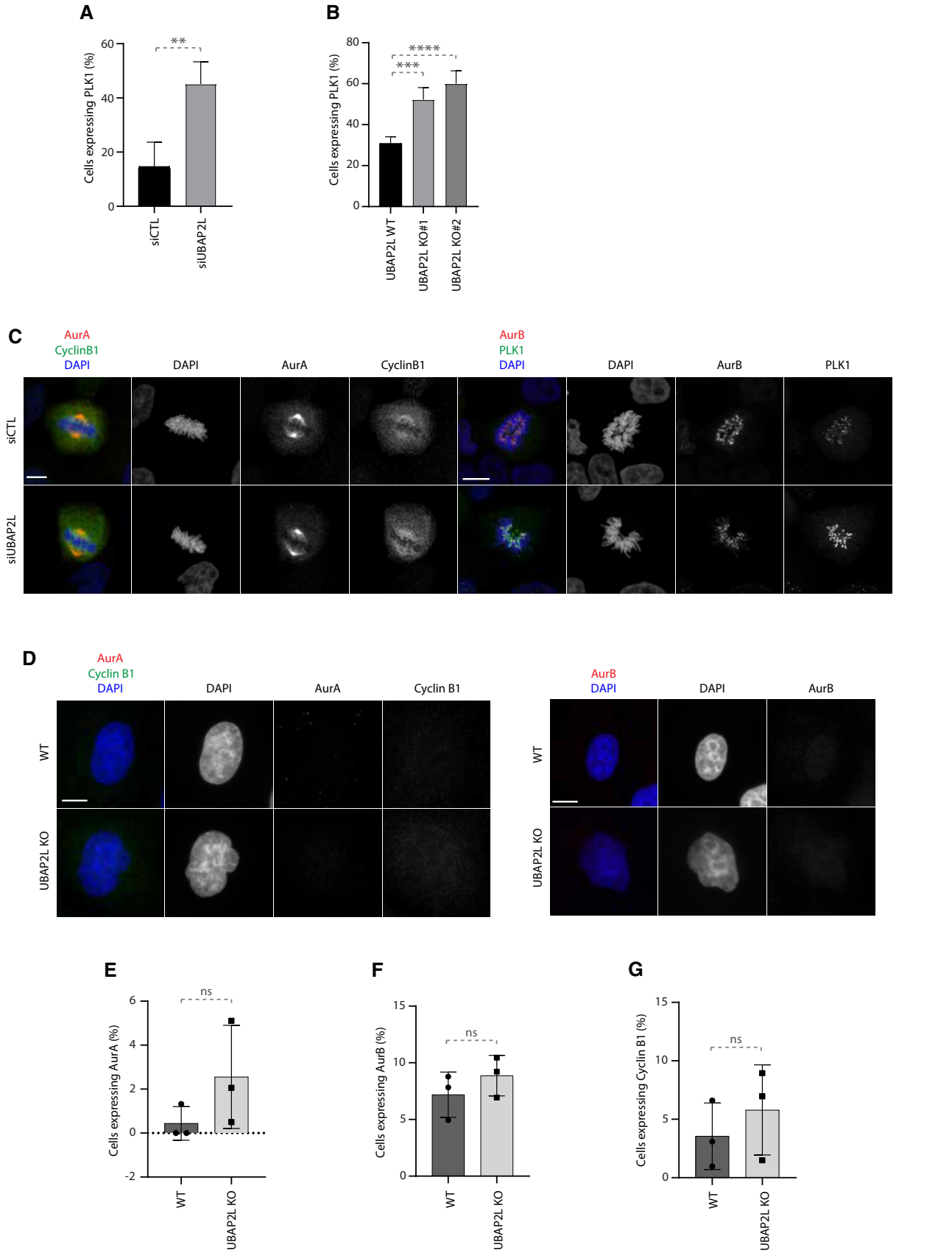
A
Cells expressing PLK1 (%)
60
40
20
0
**
siCTL
siUBAP2L
B
Cells expressing PLK1 (%)
80
60
40
20
0
****
***
UBAP2L WT
UBAP2L KO#1
UBAP2L KO#2
C
AurA
CyclinB1
DAPI
DAPI
AurA
CyclinB1
AurB
PLK1
DAPI
DAPI
AurB
PLK1
siCTL
siUBAP2L
D
AurA
Cyclin B1
DAPI
DAPI
AurA
Cyclin B1
AurB
DAPI
DAPI
AurB
WT
UBAP2L KO
E
Cells expressing AurA (%)
6
4
2
0
-2
ns
WT
UBAP2L KO
F
Cells expressing AurB (%)
15
10
5
0
ns
WT
UBAP2L KO
G
Cells expressing Cyclin B1 (%)
15
10
5
0
ns
WT
UBAP2L KO

Figure EV3.

Figure EV4. The C-terminal domain of UBAP2L mediates its function on PLK1 and on mitosis.

A Schematic representation of UBAP2L protein fragments. Indicated numbers stand for aminoacids (aa).

B WB analysis of G1/S synchronized (DTB) WT or UBAP2L KO HeLa cells transiently transfected with the indicated flag-tagged UBAP2L protein fragments. Proteins MW is indicated in kDa. Arrows point to the migration of each fragment. WB is representative of three independent replicates.

C IF representative pictures of WT or UBAP2L KO HeLa cells transfected with the indicated flag-tagged UBAP2L protein fragments for 48 h and synchronized in mitosis using MR. Scale bar, 8 μm.

D WB analysis of WT or UBAP2L KO HeLa cells synchronized in G1/S with DTB and transfected with the indicated flag-tagged UBAP2L constructs and control or G3BP1/2 siRNAs for 48 h. Proteins MW is indicated in kDa. WB is representative of three independent replicates.

E Quantification of PLK1 nuclear intensity from the experiment described in (D). At least 150 cells were quantified per condition for each replicate. Each dot of the graph represents PLK1 nuclear intensity in a single nucleus. The measurements of three biological replicates are combined, red bars represent the mean (Kruskal-Wallis test with Dunn's correction $*P < 0.05$, $****P < 0.0001$, ns, non-significant).

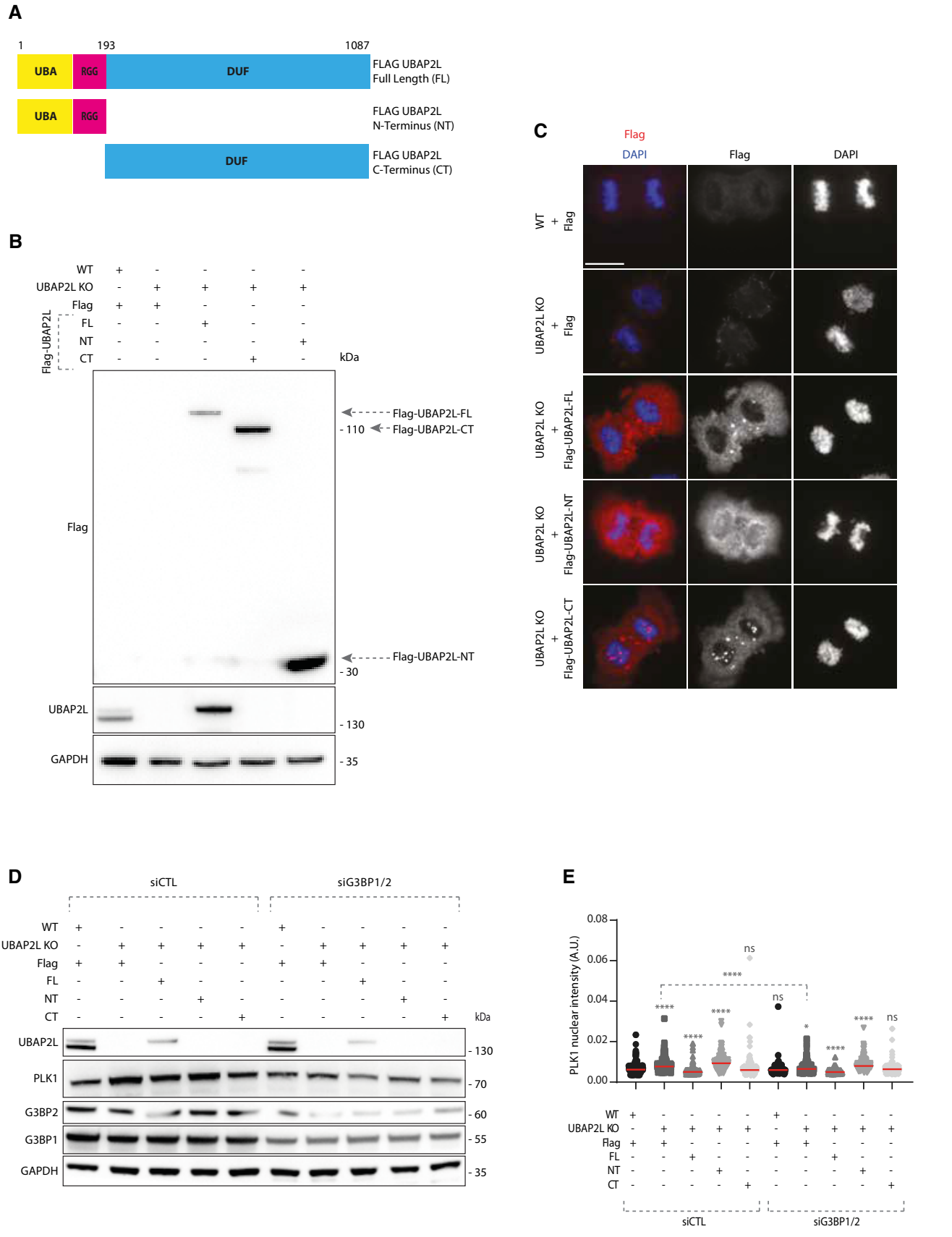
A
1
193
1087
UBA
RGG
DUF
FLAG UBAP2L
Full Length (FL)
FLAG UBAP2L
N-Terminus (NT)
FLAG UBAP2L
C-Terminus (CT)
B
WT
UBAP2L KO
Flag
FL
NT
CT
Flag-UBAP2L
kDa
Flag-UBAP2L-FL
110
Flag-UBAP2L-CT
Flag
Flag-UBAP2L-NT
30
UBAP2L
130
GAPDH
35
C
Flag
DAPI
Flag
DAPI
WT + Flag
UBAP2L KO + Flag
UBAP2L KO + Flag-UBAP2L-FL
UBAP2L KO + Flag-UBAP2L-NT
UBAP2L KO + Flag-UBAP2L-CT
D
siCTL
siG3BP1/2
WT
UBAP2L KO
Flag
FL
NT
CT
kDa
UBAP2L
130
PLK1
70
G3BP2
60
G3BP1
55
GAPDH
35
E
PLK1 nuclear intensity (A.U.)
0.08
0.06
0.04
0.02
0.00
ns
****
ns
****
****
****
*
****
****
ns
WT
UBAP2L KO
Flag
FL
NT
CT
siCTL
siG3BP1/2

Figure EV4.

Figure EV5. Generation of PLK1-eGFP Knock-In (KI) HeLa cells.

A Schematic representation of the screening strategy used to identify PLK1–eGFP positive clones. Forward (Fw) and Reverse (Rv) primers used are annotated. Bp stands for base pair.

B, C Agarose gel electrophoresis (B) and WB analysis (C) of PLK1 WT and PLK1–eGFP HeLa cells lysates. DNA fragments length is indicated in bp. Proteins MW is indicated in kDa.

D Scatterplot representing the time from prophase to anaphase (seconds) in HeLa PLK1 WT and PLK1–eGFP cell lines. Error bars indicate Standard Error of the Mean. The number of analyzed cells is indicated in the graph. Statistical significance was determined using Mann–Whitney test (ns, non-significant).

E Representative time frames of a 12 h movie of HeLa PLK1–eGFP. Scale bar, 10 μm. Time is indicated as hh:mm.

F, G Representative IF pictures of WT or UBAP2L KO HeLa cells synchronized in mitosis with DTBR (G) and quantification of the relative PLK1 intensity at the outer kinetochore (A.U.) (G). ROIs are shown in the corresponding numbered panels. Scale bar, 5 μm. At least 50 cells were quantified per condition for each experiment and a minimum of 10 pairs of kinetochores per cell was analyzed. Each dot represents PLK1/BubR1 intensity ratio at a single pair of kinetochores. The measurements of three biological replicates are combined, red bars represent the mean (Mann–Whitney test ****$P < 0.0001$).

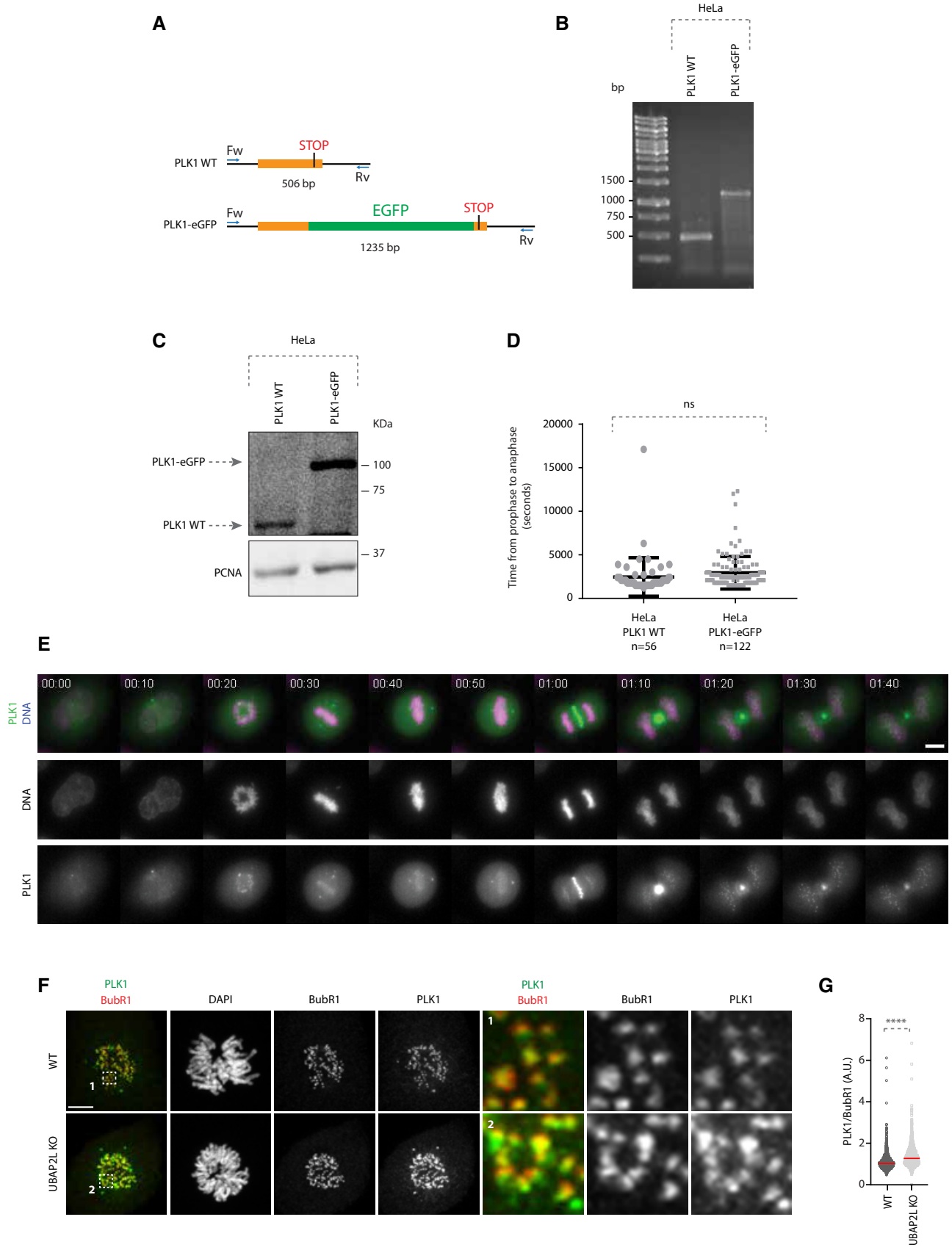
A
PLK1 WT
Fw
STOP
Rv
506 bp
PLK1-eGFP
Fw
EGFP
STOP
Rv
1235 bp
B
HeLa
PLK1 WT
PLK1-eGFP
bp
1500
1000
750
500
C
HeLa
PLK1 WT
PLK1-eGFP
KDa
PLK1-eGFP
PLK1 WT
PCNA
100
75
37
D
ns
Time from prophase to anaphase (seconds)
20000
15000
10000
5000
0
HeLa PLK1 WT n=56
HeLa PLK1-eGFP n=122
E
PLK1 DNA
DNA
PLK1
00:00
00:10
00:20
00:30
00:40
00:50
01:00
01:10
01:20
01:30
01:40
F
PLK1 BubR1
DAPI
BubR1
PLK1
PLK1 BubR1
BubR1
PLK1
WT
UBAP2L KO
1
2
G
****
PLK1/BubR1 (A.U.)
8
6
4
2
0
WT
UBAP2L KO

Figure EV5.