SUPPLEMENTARY FIGURES

Figure S1 Recombinant PICH and BLM interact in Sf9 cells. (**A**) Lysates of Sf9 cells infected with baculoviruses encoding His_6-PICH and His_6-BLM were fractionated on a Superose 6 column. The fractions were analyzed by SDS-PAGE followed by immunoblotting with α-PICH and α-BLM. The elution positions of the molecular mass standards are indicated. (**B**) Lysates of Sf9 cells infected with the indicated baculoviruses were immunoprecipitated with control or a-PICH beads. Lysates and IPs were blotted with the indicated antibodies.

Figure S2 PICH localizes to kinetochores and anaphase threads. HeLa Tet-On cells at different mitotic stages were stained with DAPI (blue in overlay), CREST (green in overlay), and anti-PICH (red in overlay).

Figure S3 PICH and BLM do not colocalize well in interphase cells. (**A**) Interphase HeLa Tet-On cells transfected with Myc-PICH and GFP-BLM plasmids were stained with anti-Myc and DAPI. In the overlay image, anti-Myc staining is in red. GFP signal is in green. DAPI staining is in blue. Scale bar, 10 μm. (**B**) Total lysates, cytoplasmic fraction, and nuclear fraction of HeLa Tet-On cells were blotted with the indicated antibodies. Tubulin and lamin were exclusively present in cytoplasmic and nuclear fractions, respectively, validating the fractionation protocol.

Figure S4 The PICH–BLM complex prevents micronuclei formation. (**A**) HeLa Tet-On cells mock transfected or transfected with siPICH4 or siBLM were stained with DAPI. Micronuclei

are indicated by arrows and asterisks (which indicate micronuclei possibly formed in two daughter cells from the same cell division event). Scale bar, 10 μm. (**B**) Lysates of cells described in Figure 4B were blotted with anti-PICH and anti-tubulin. (**C**) Lysates of a HeLa Tet-On cell line stably expressing siPICH4-resistant Myc-PICH cultured in the absence or presence of doxycycline and transfected with or without siPICH4 were blotted with anti-PICH and anti-tubulin. (**D**) Quantification of the percentage of micronuclei-containing cells described in (**C**). Mean and standard deviation of three independent experiments are shown. (**E**) Quantification of the percentage of micronuclei-containing HeLa Tet-On cells transfected with the indicated siRNAs. Mean and standard deviation of three independent experiments are shown.

Figure S5 Breakage of anaphase threads produces micronuclei in PICH- and BLM-RNAi cells. Live cell imaging of HeLa cells expressing H2B-mCherry (pseudo-colored green in overlay) and GFP-tubulin (colored red in overlay) mock transfected (**A**) or transfected with siPICH4 (**B**) or siBLM (**C**). The anaphase threads and micronuclei are indicated by arrows. The two micronuclei in the BLM-RNAi cell are magnified and shown in insets. Times indicate the time after NEBD in minutes. The 25 min and 50 min images of the PICH-RNAi cell are shown in Figure 4C. Scale bars, 10 μm.

Figure S6 BLM prevents histone association with PICH threads. (**A**) Mock-RNAi H2B-GFP-expressing HeLa cells in anaphase were stained with DAPI, anti-PICH, and CREST. CREST, PICH and H2B signals in the overlay are colored green, red, and blue, respectively. The PICH threads are indicated by arrows. Scale bars, 5 μm. (**B**) A telophase BLM-RNAi H2B-GFP-expressing HeLa cell stained with DAPI, anti-PICH, and CREST. CREST, PICH and H2B

signals in the overlay are colored green, red, and blue, respectively. PICH threads are magnified and shown in the bottom panel. Scale bars, 5 μm.

Figure S7 The ATPase-dead PICH mutant (K128A) is deficient in thread formation. (**A**) Lysates of HeLa cells transfected with the indicated siRNAs and plasmids were blotted with the indicated antibodies. The positions of Myc-PICH and the endogenous (Endo.) PICH are labeled. The asterisk indicates a weak crossreacting band in the anti-PICH blot. (**B**) Anaphase PICH-RNAi HeLa cells expressing Myc-PICH WT or K128A were stained with anti-Myc and DAPI. Scale bars, 5 μm. (**C**) Quantification of the intensities of the PICH threads in cells expressing Myc-PICH WT or K128A.

Figure S8 BLM alone is insufficient to stimulate the nucleosome remodeling activity of PICH. (**A**) Coomassie-stained gel of partially purified His_6-PICH, His_6-BLM, His_6-BLM HD (helicase dead mutant), and the PICH–BLM complexes. (**B**) Nucleosome sliding assay of PICH, PICH–BLM, and PICH–BLM HD. All reactions contain ATP.

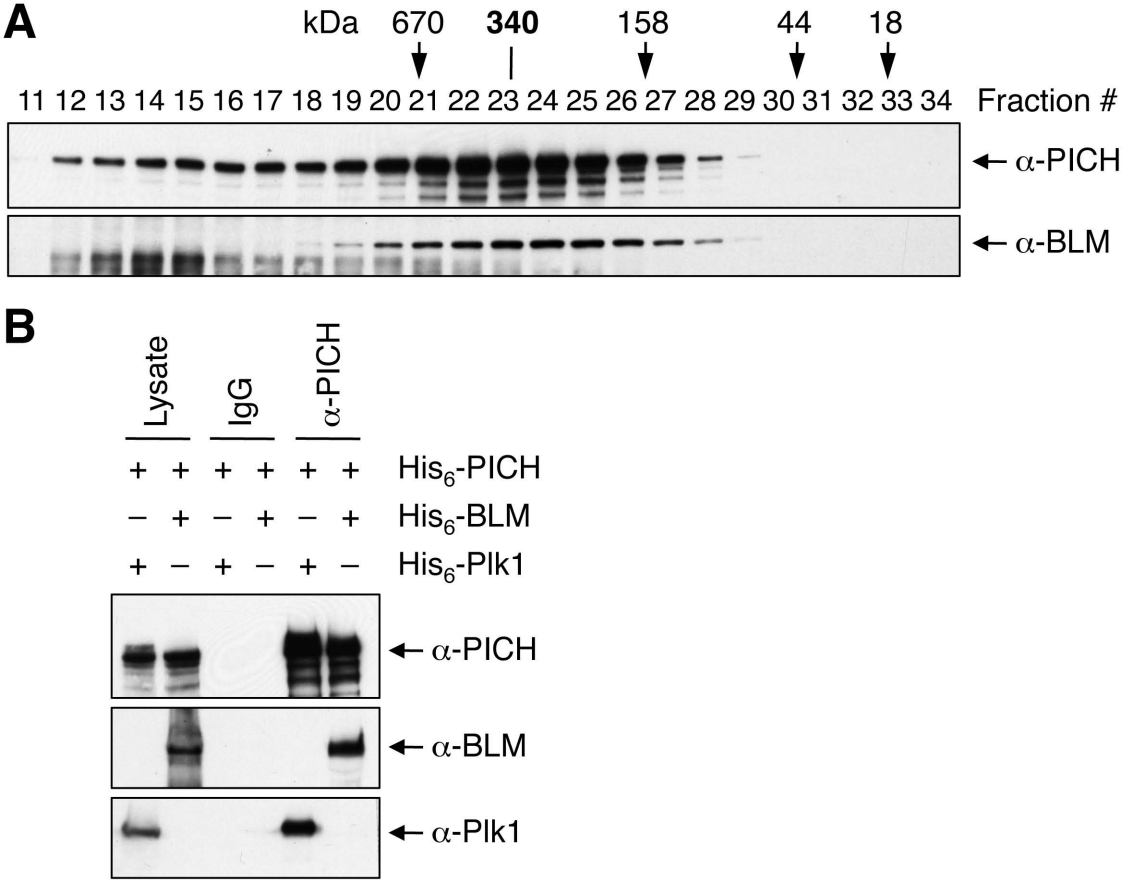
A
kDa
670
340
158
44
18
11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
Fraction #
← α-PICH
← α-BLM
B
Lysate
IgG
α-PICH
+ + + + + + His6-PICH
− + − + − + His6-BLM
+ − + − + − His6-Plk1
← α-PICH
← α-BLM
← α-Plk1

Figure S1

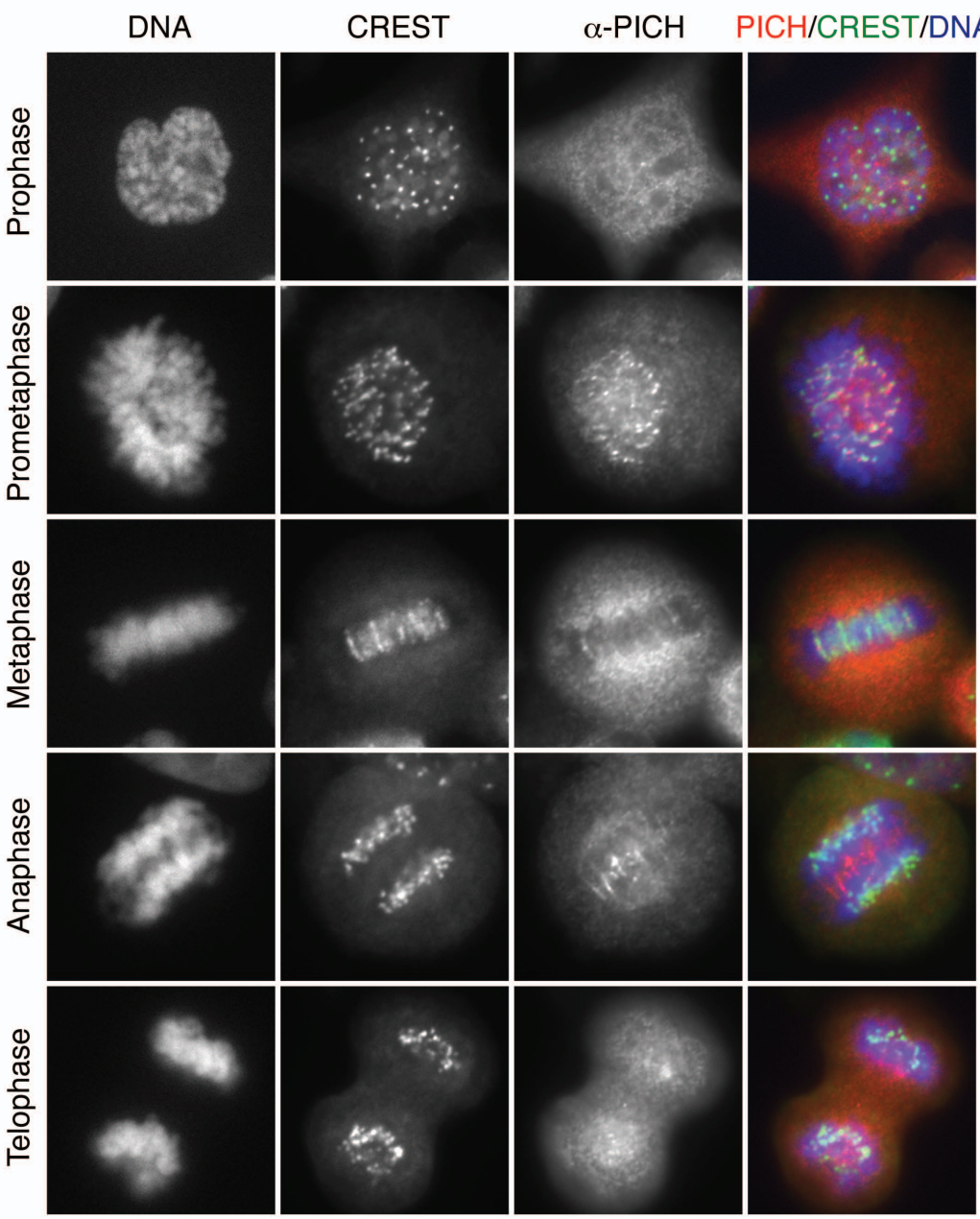
DNA
CREST
α-PICH
PICH/CREST/DNA
Prophase
Prometaphase
Metaphase
Anaphase
Telophase

Figure S2

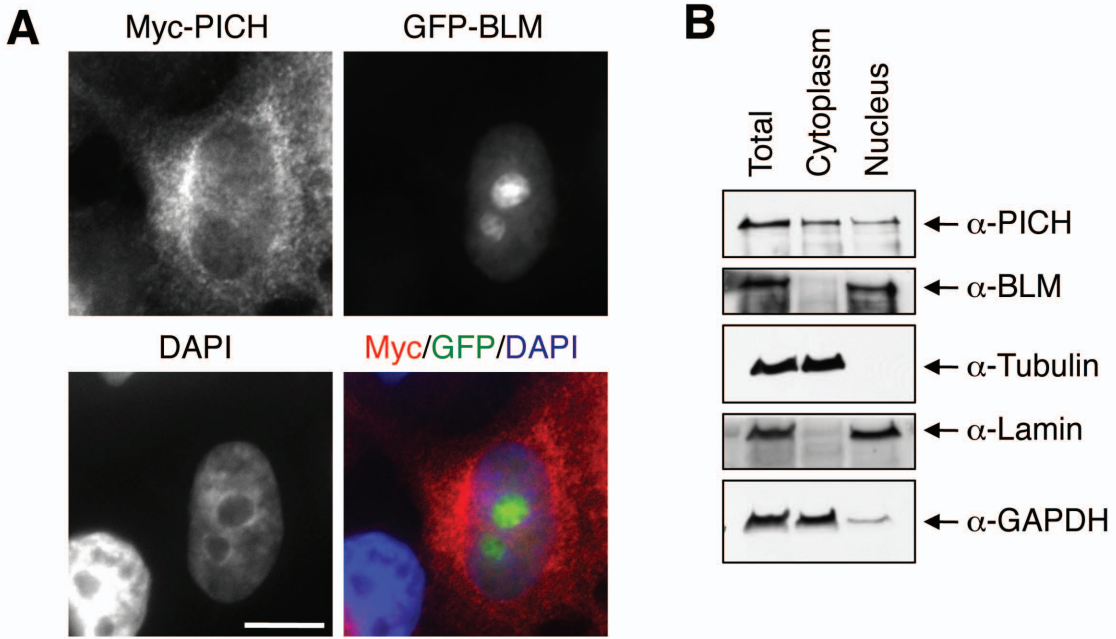
A
Myc-PICH
GFP-BLM
DAPI
Myc/GFP/DAPI
B
Total
Cytoplasm
Nucleus
α-PICH
α-BLM
α-Tubulin
α-Lamin
α-GAPDH

Figure S3

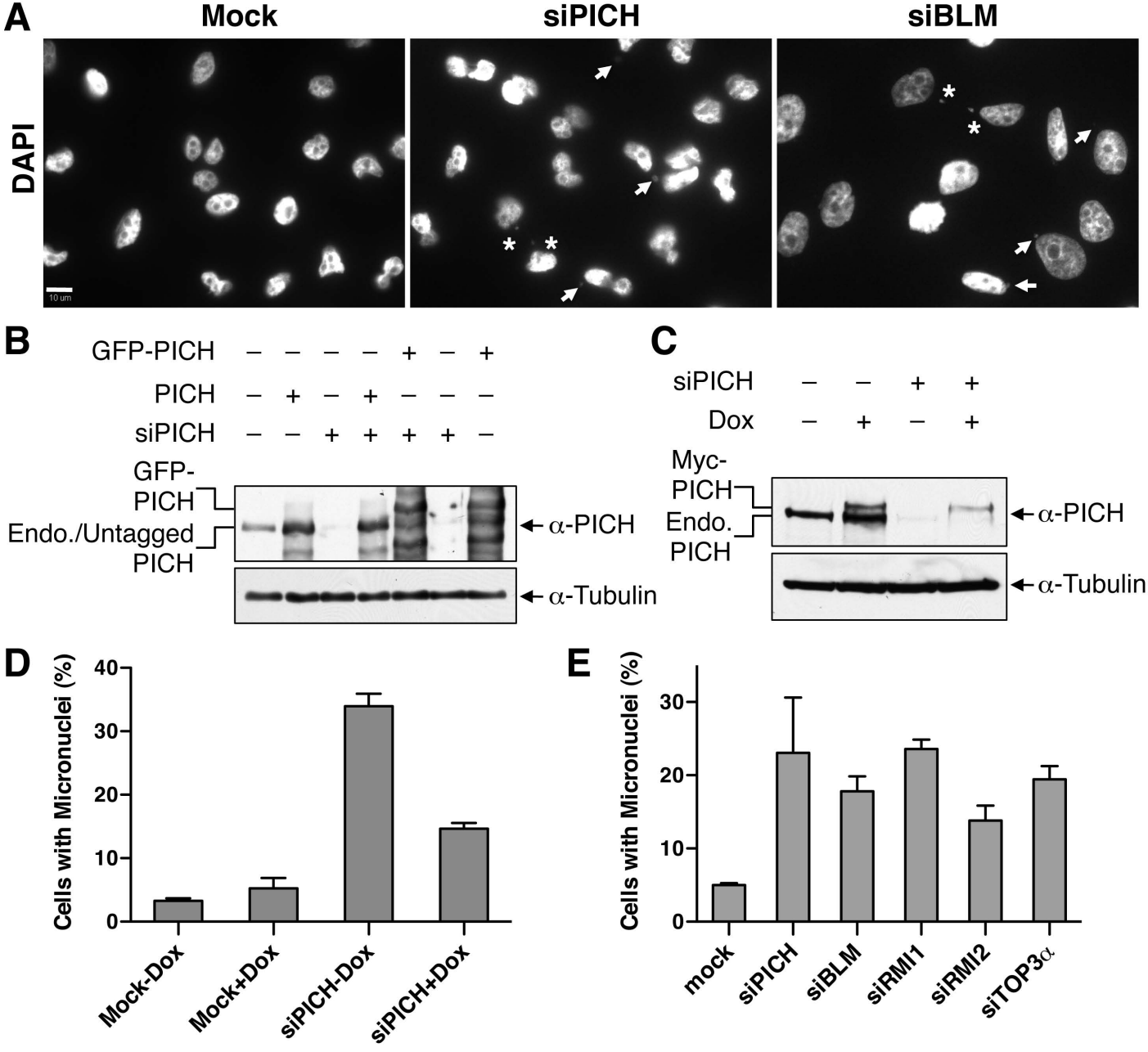
A
Mock
siPICH
siBLM
DAPI
10 um
B
GFP-PICH
PICH
siPICH
GFP-PICH
Endo./Untagged PICH
α-PICH
α-Tubulin
C
siPICH
Dox
Myc-PICH
Endo. PICH
α-PICH
α-Tubulin
D
Cells with Micronuclei (%)
Mock-Dox
Mock+Dox
siPICH-Dox
siPICH+Dox
E
Cells with Micronuclei (%)
mock
siPICH
siBLM
siRMI1
siRMI2
siTOP3α

Figure S4

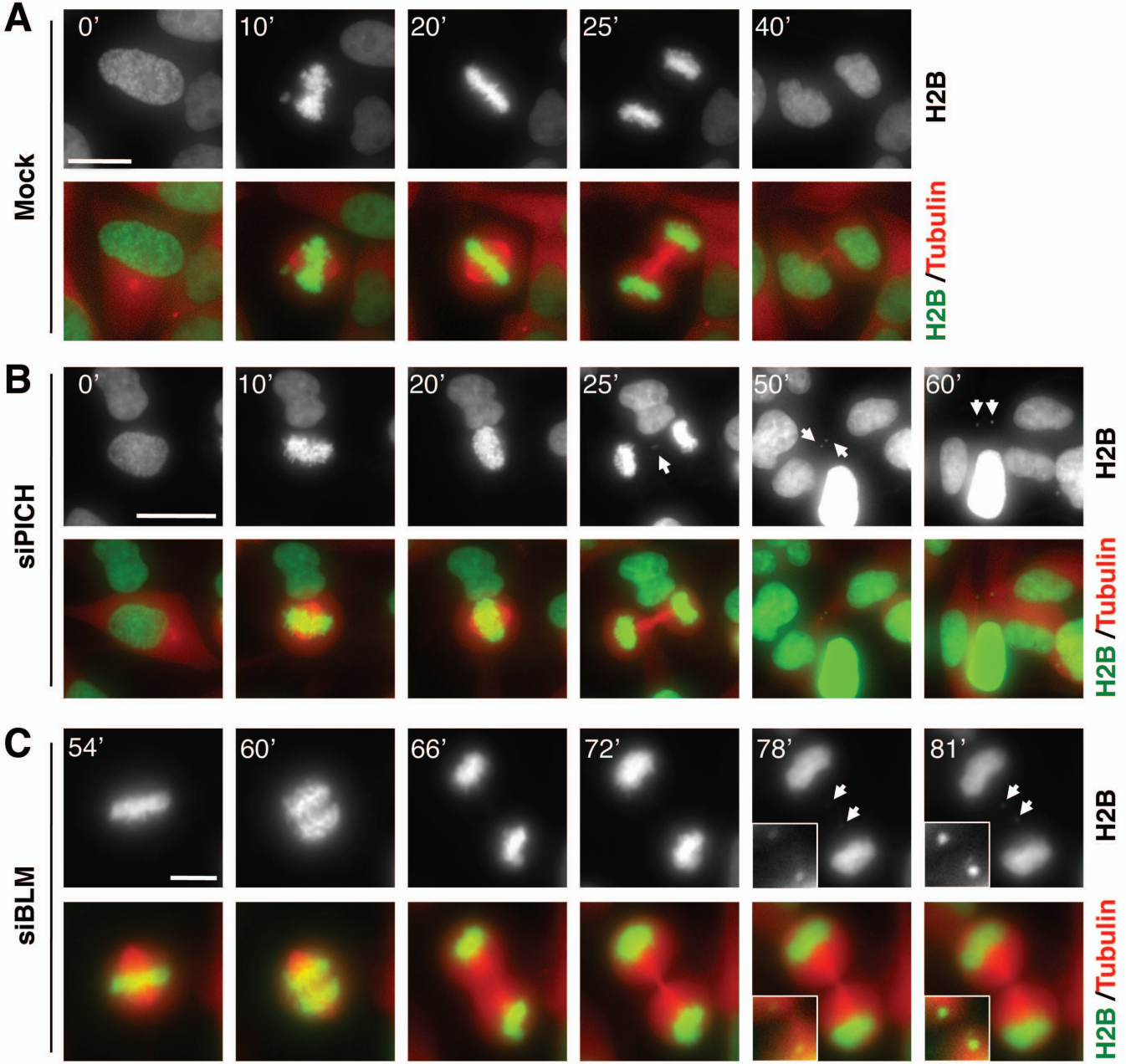
A
Mock
0'
10'
20'
25'
40'
H2B
H2B /Tubulin
B
siPICH
0'
10'
20'
25'
50'
60'
H2B
H2B /Tubulin
C
siBLM
54'
60'
66'
72'
78'
81'
H2B
H2B /Tubulin

Figure S5

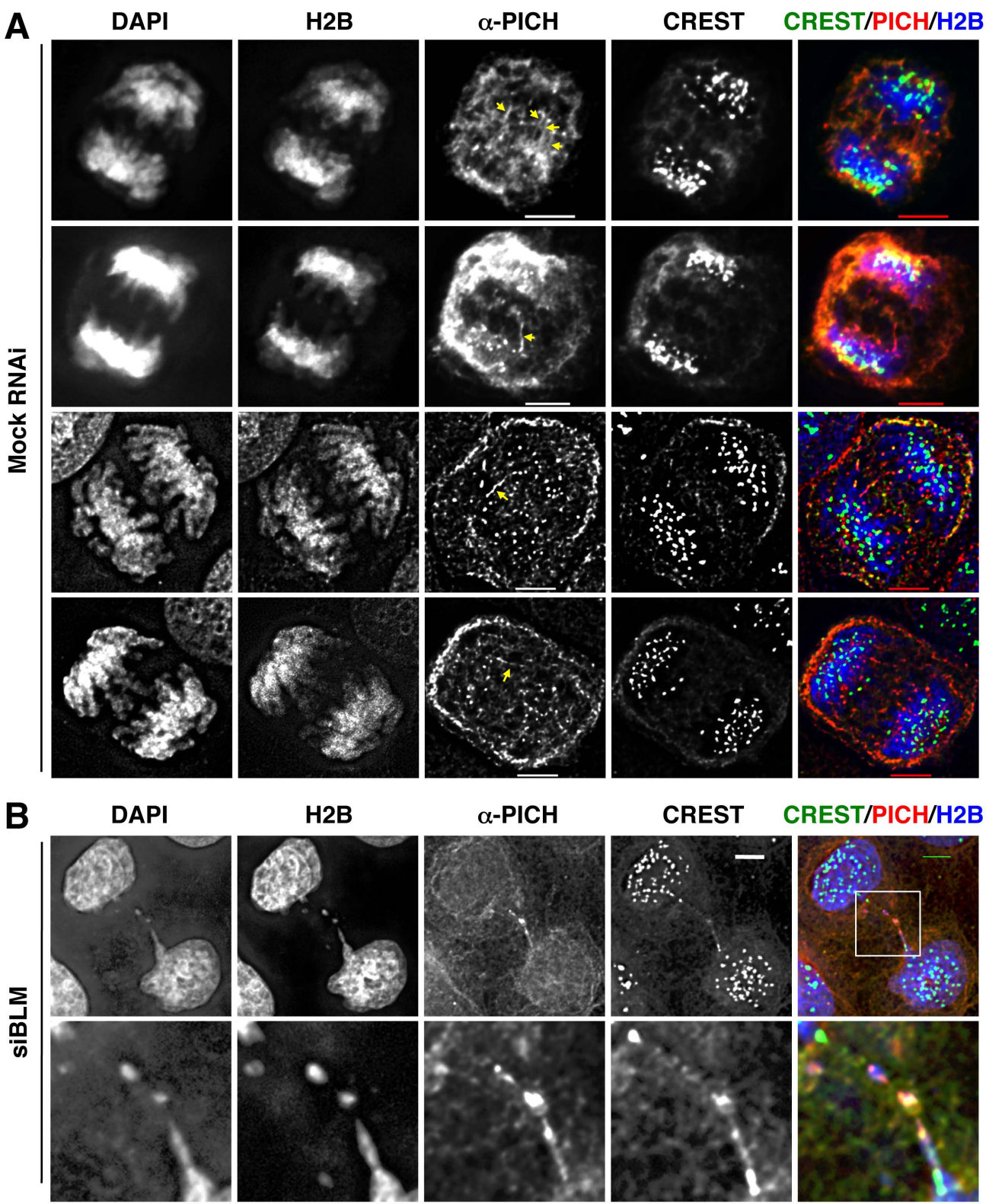
A
DAPI
H2B
α-PICH
CREST
CREST/PICH/H2B
Mock RNAi
B
DAPI
H2B
α-PICH
CREST
CREST/PICH/H2B
siBLM

Figure S6

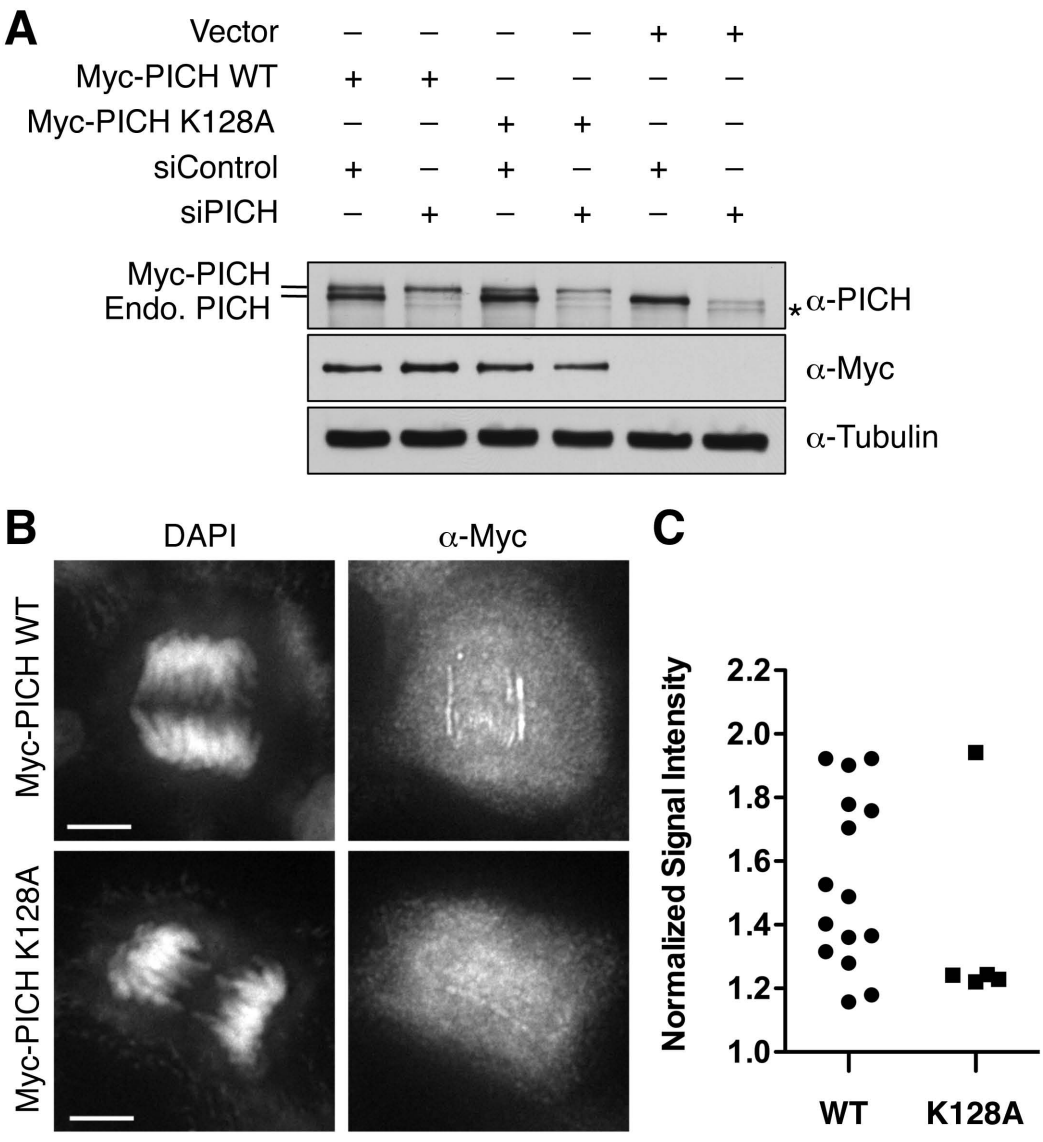
A
Vector
Myc-PICH WT
Myc-PICH K128A
siControl
siPICH
Myc-PICH
Endo. PICH
α-PICH
α-Myc
α-Tubulin
B
DAPI
α-Myc
Myc-PICH WT
Myc-PICH K128A
C
Normalized Signal Intensity
2.2
2.0
1.8
1.6
1.4
1.2
1.0
WT
K128A

Figure S7

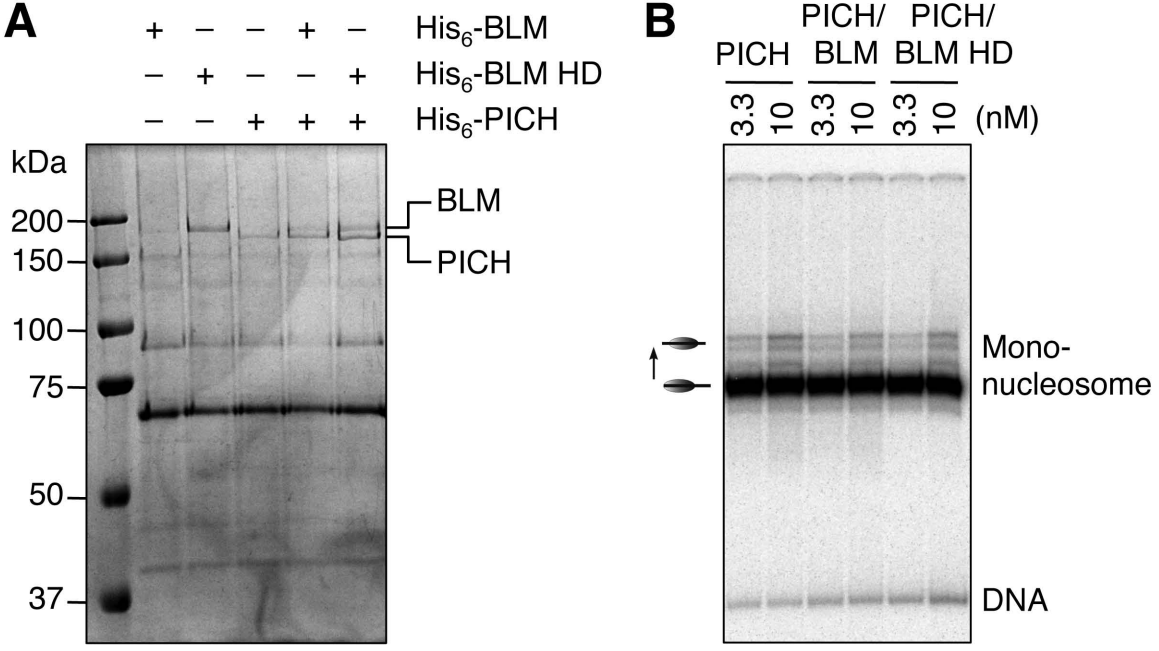
A
+ − − + − His6-BLM
− + − − + His6-BLM HD
− − + + + His6-PICH
kDa
200
150
100
75
50
37
BLM
PICH
B
PICH
PICH/ BLM
PICH/ BLM HD
3.3
10
3.3
10
3.3
10
(nM)
Mono-nucleosome
DNA

Figure S8