Supplemental Materials

Molecular Biology of the Cell

Chen et al.

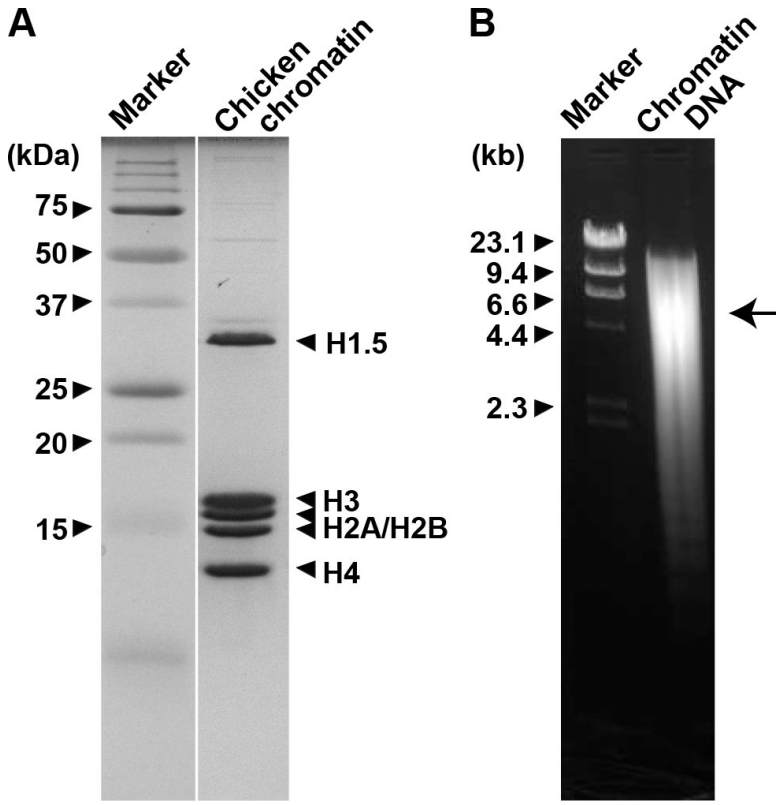

Figure S1. Isolation of chicken erythrocyte chromatin

(A) SDS-PAGE of purified chromatin. Nucleosome core histones H2A/H2B, H3 and H4 as well as linker histone H1.5 are present in the sample. **(B)** Agarose gel of isolated chromatin DNA. Average DNA length is ~5 kb (arrow).

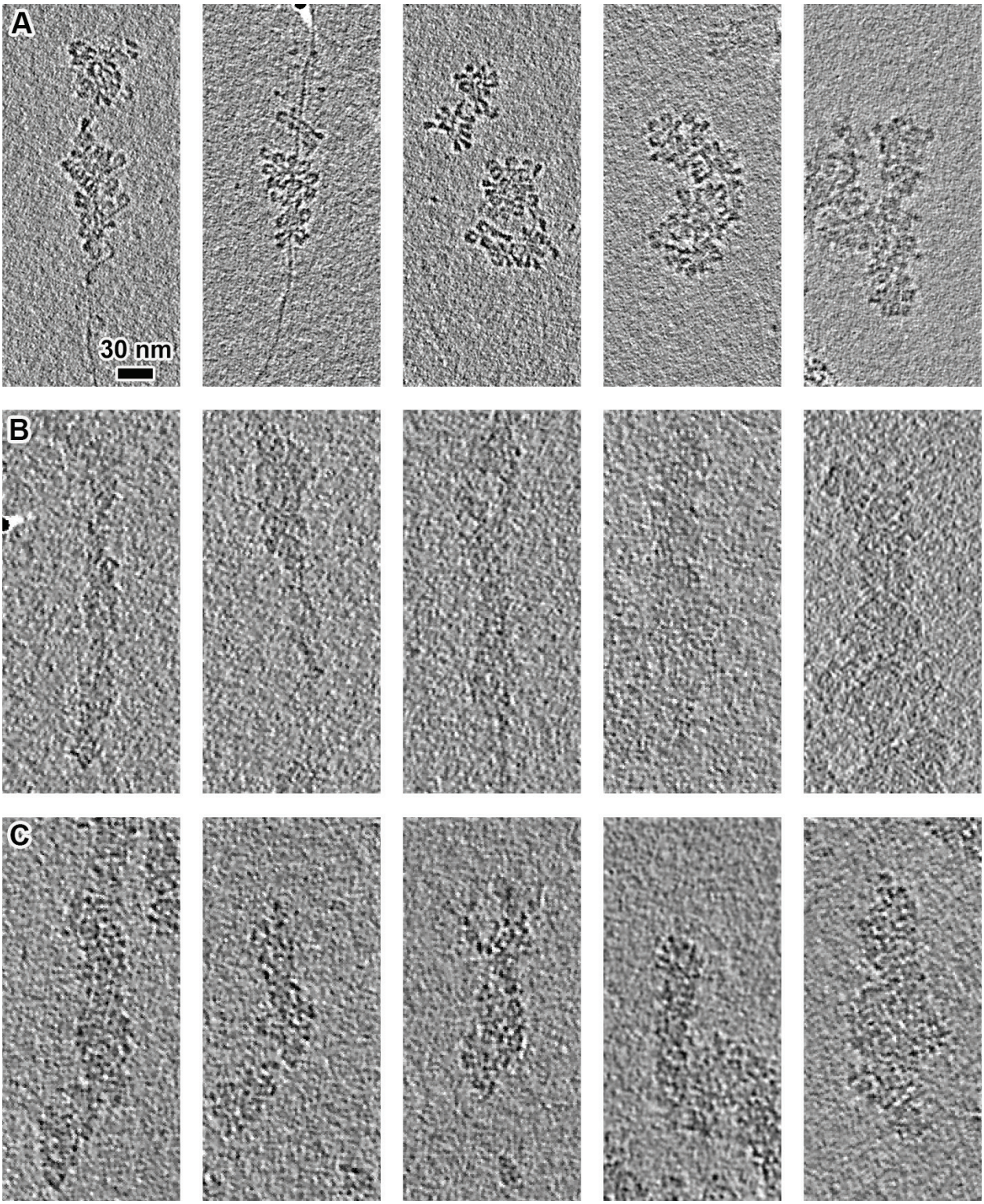

Figure S2. 30-nm fibers are recognizable regardless of cryo-EM sample-preparation method

Row **(A)**: plunge-frozen in isolation buffer; row **(B)**: plunge-frozen in isolation buffer plus 20% dextran; row **(C)**: high-pressure frozen in isolation buffer plus 20% dextran, then cryosectioned. Note that the addition of dextran substantially lowers the image contrast.

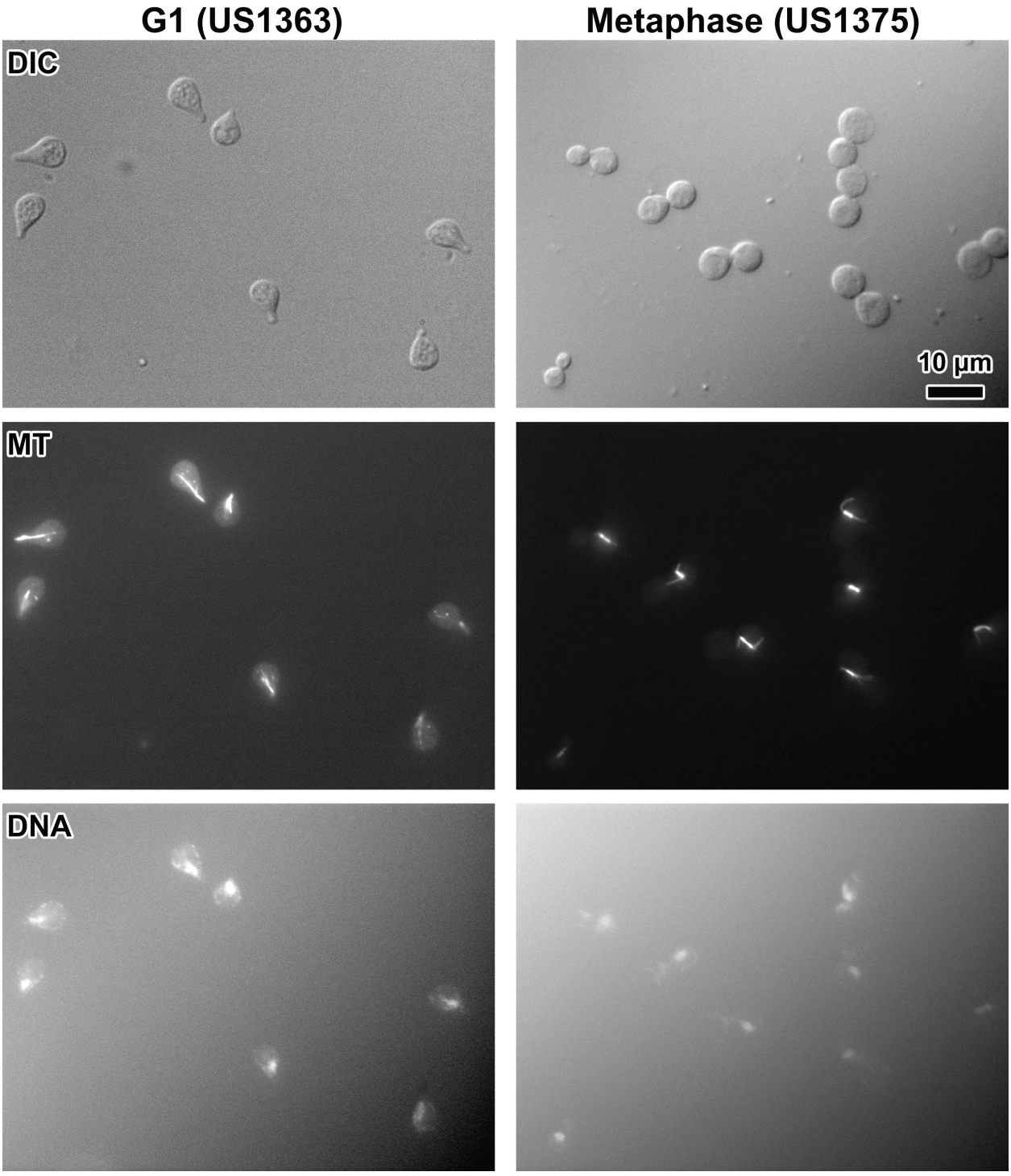

Figure S3. Synchronization of *S. cerevisiae*

US1363 cells are arrested at G1 and show the characteristic “shmoo” morphology (left column) when incubated with α-factor; *cdc20Δ GAL-CDC20* (US1375) mutants are

arrested at metaphase in presence of glucose. These metaphase cells have a characteristic large-bud morphology and a short, bar-shaped spindle (right column).

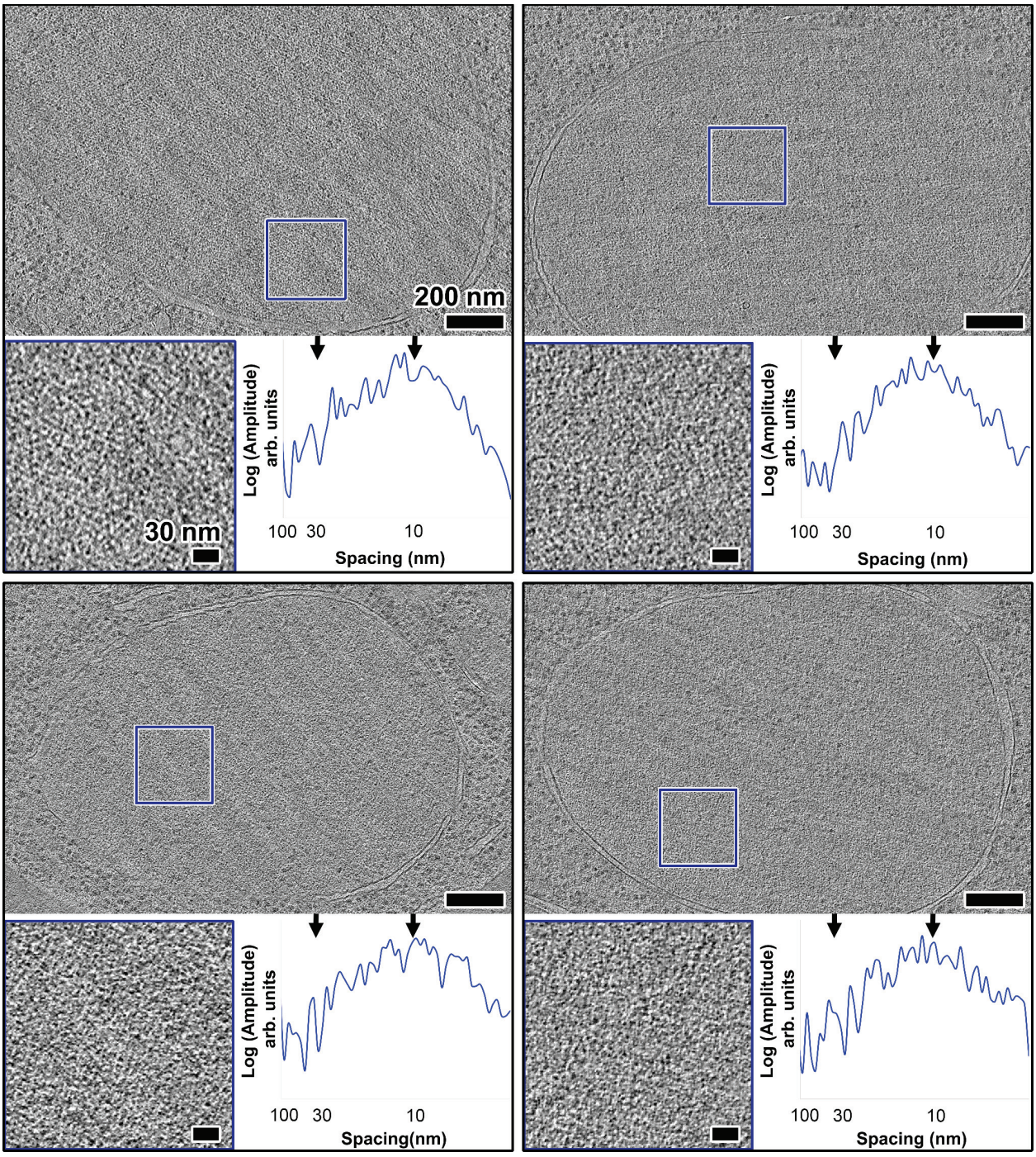

Figure S4A. Additional examples of G1-arrested cells

Tomographic slices (30-nm thick) of four more G1 cells. The lower left subpanel is a 3-fold enlargement of the area boxed in blue. The lower right subpanel shows a rotationally averaged power spectrum of this area. Arrows indicate 30- and 10-nm

spacings.

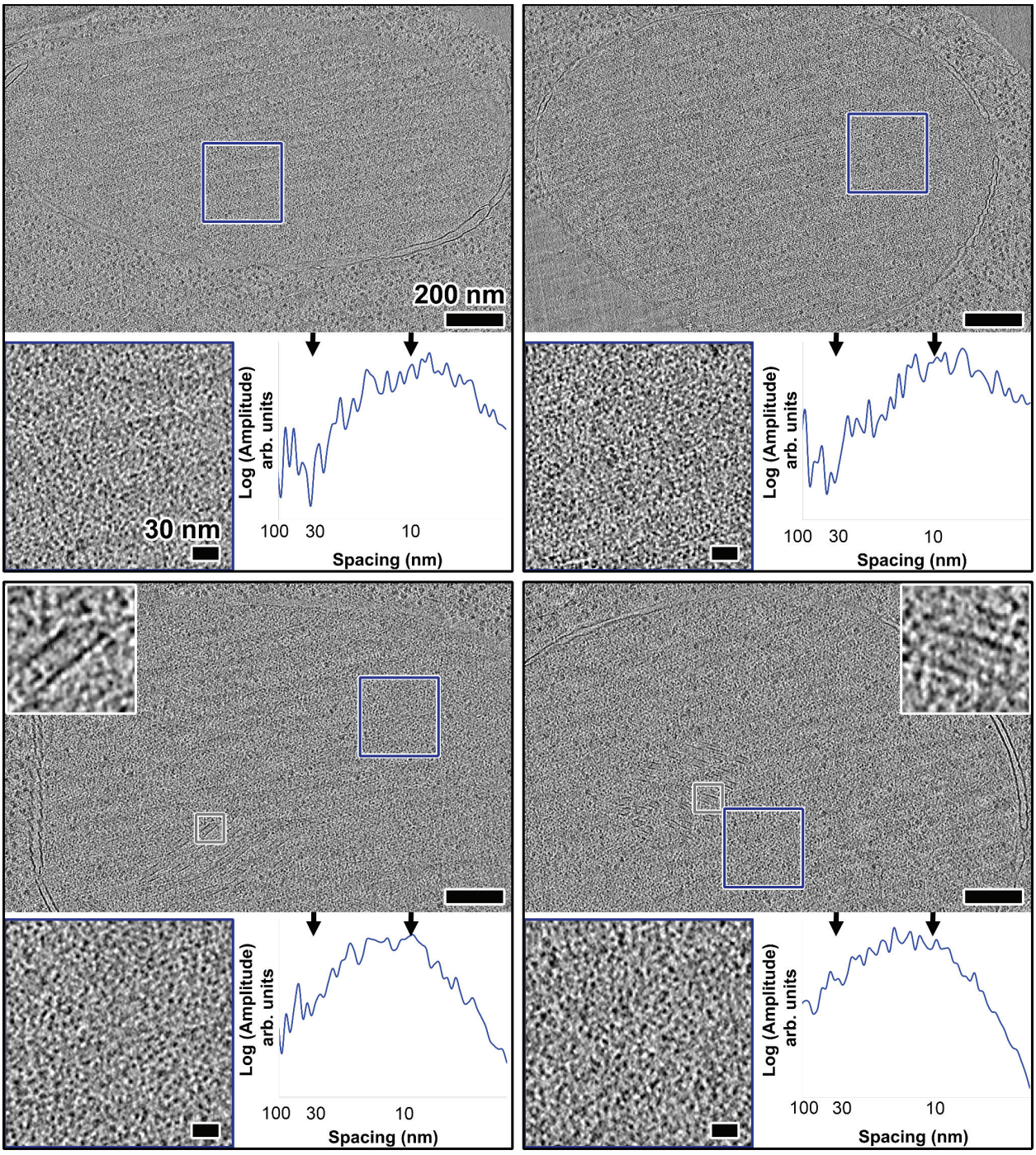

Figure S4B. Additional examples of metaphase-arrested cells

Tomographic slices (30-nm thick) of four more metaphase cells. The lower left subpanel is a 3-fold enlargement of the area boxed in blue. The lower right subpanel shows a rotationally averaged power spectrum of this area. Arrows indicate 30- and 10-nm

35 spacings. For the two cells in the lower panels, the upper subpanels show spindle
36 microtubules, boxed in grey and enlarged 5-fold.
37

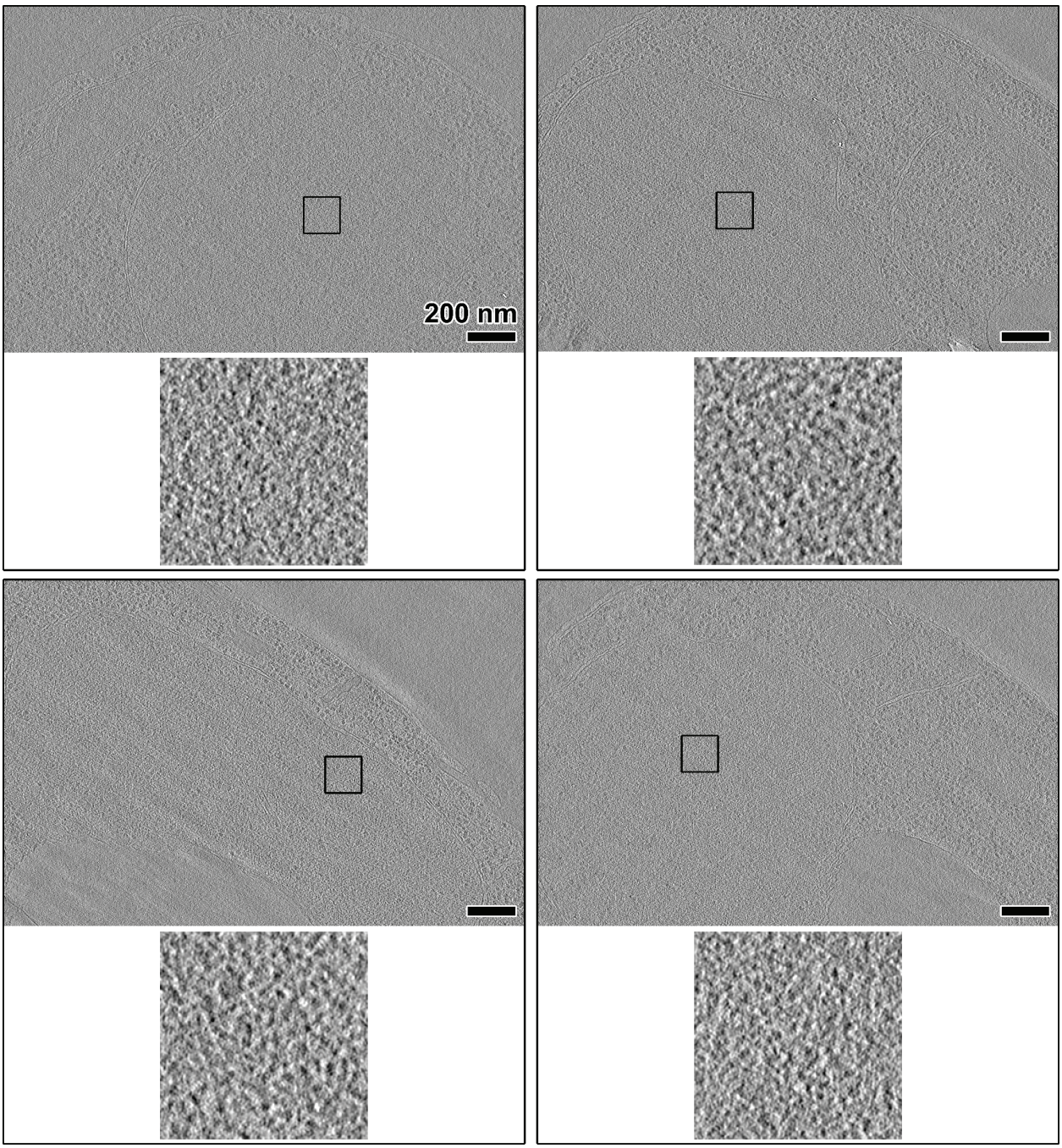

Figure S4C. Additional examples of formaldehyde-fixed cells

Tomographic slices (10-nm thick) of four more wild-type cells, fixed in formaldehyde. A region inside each nucleus is boxed out and enlarged 6-fold in the lower subpanel.

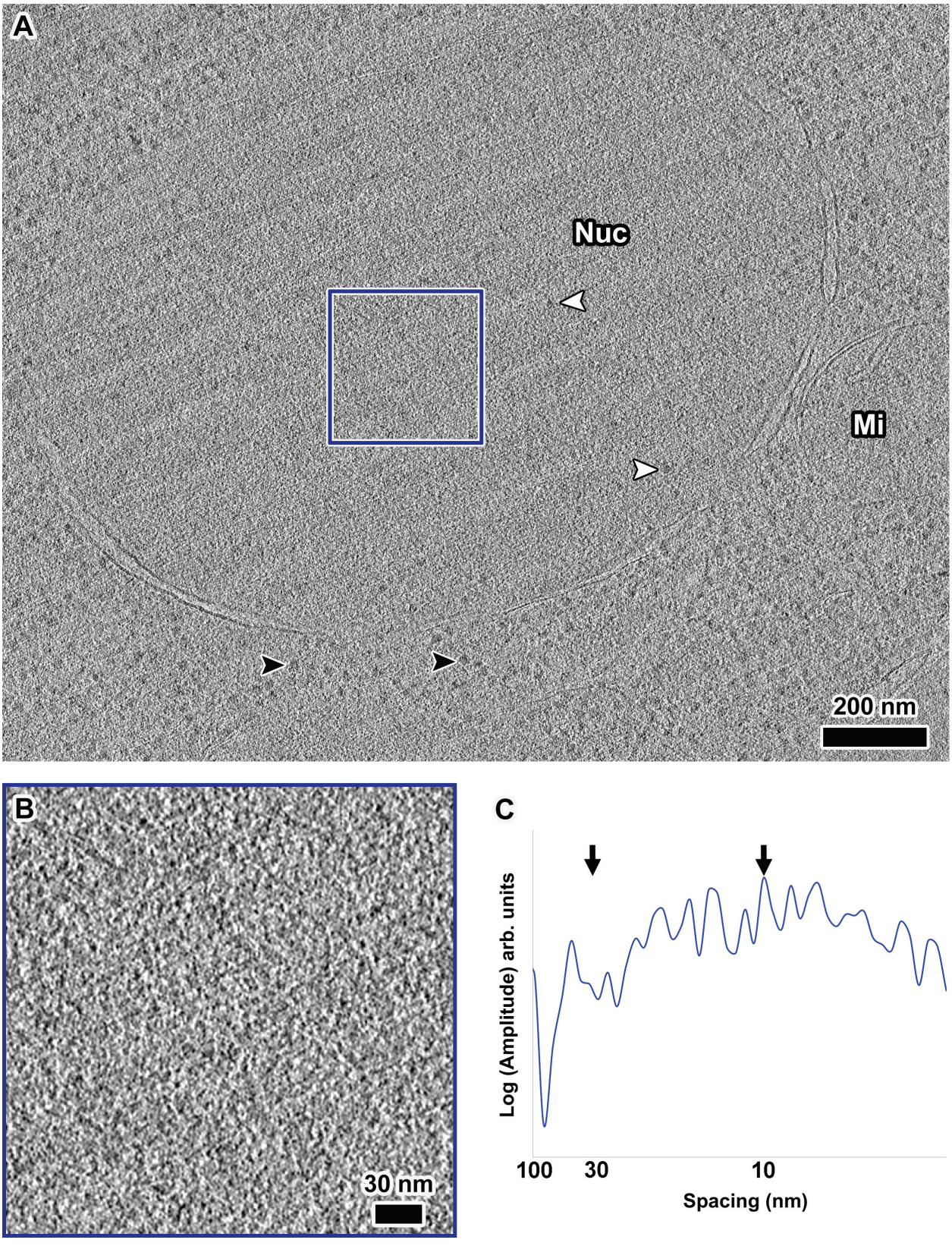

Figure S5. 30-nm fiber signals are absent regardless of imaging conditions

(A) Tomographic slice (30-nm thick) of a nucleus in a G1-arrested cell that was imaged much closer to focus (-3 μm) than the other cells presented in this paper. The nucleus (Nuc) and a mitochondrion (Mi) are labeled. White arrows: intranuclear ribosome-sized densities; black arrows: cytoplasmic ribosome densities. **(B)** A 3-fold enlargement of the intranuclear position boxed in panel (A). **(C)** Rotationally averaged power spectrum of (B). Arrows point to 30- and 10-nm spacings.

Table S1. Tomogram details

ID	Figure	Sample	Method	Dose	Defocus
15apr11_chrom_01	1A,B	F	PF	100	-6
15may29_chrom_19	1C,D	F	PF	100	-6
15apr29_chrom_15	1E,F	F	VS	100	-10
15mar02a__070	2A,C,D	G1	VS	100	-8
14oct01a__027	2B,E,F + Movie S1	M	VS	100	-10
15mar02a__074	3A,B,C,D	G1	VS	100	-8
14oct01a__032	3E,F,G,H	M	VS	100	-8
15oct12_yeast_12	4A,B	G1	VS	100	-8
16jan29_yeast_10	4C,D	UF	VS	100	-12
15mar02a_065	5A,B	G1	VS	100	-11
15apr11_chrom_01	S2A	F	PF	100	-6
15apr11_chrom_02	S2A	F	PF	100	-6
15may29_chrom_11	S2B	F	PF	100	-8
15may29_chrom_15	S2B	F	PF	100	-6

15may29_chrom_21	S2B	F	PF	100	-6
15apr29_chrom_32	S2C	F	VS	100	-10
15may05_chrom_07	S2C	F	VS	100	-10
15mar02a__046	S4A	G1	VS	100	-8
15oct12_yeast_02	S4A	G1	VS	100	-6
15oct12_yeast_03	S4A	G1	VS	100	-4
15oct12_yeast_04	S4A	G1	VS	100	-4
14oct01a__014	S4B	M	VS	100	-10
14oct01a__021	S4B	M	VS	100	-10
14oct01a__052	S4B	M	VS	100	-8
14oct01a__067	S4B	M	VS	100	-8
16jan29_yeast_01	S4C	UF	VS	100	-8
16jan29_yeast_05	S4C	UF	VS	100	-12
16jan29_yeast_09	S4C	UF	VS	100	-12
16jan29_yeast_17	S4C	UF	VS	100	-8
15oct12_yeast_15	S5	G1	VS	100	-3

ID: Legion data ID for data collected by Leginon or manually assigned ID for data collected by FEI Tomo.

Sample: (M) metaphase-arrested yeast; (G1) G1-arrested yeast; (UF) unsynchronized and formaldehyde-fixed yeast; (F) purified chicken erythrocyte chromatin.

Method: (VS) vitreous sections, (PF) plunge-freezing.

Dose: Electrons / $Å^2$.

Defocus: Nominal values in μm; fitted values are reported in Table S2.

Table S2. Cryotomography imaging conditions

Sample	Purified chromatin	*S. cerevisiae* cells
EM grid	CF-22-2C-T (Protochips)	CF-42-2C-T (Protochips) / continuous carbon (EMS)
Microscope	Titan Krios	
Energy	300 keV	
Gun type	FEG	
Camera	Falcon II Direct Detector	Falcon I or II Direct Detector
Tomography software	FEI TOMO 3 and 4	Leginon
Calibrated magnification	23,986	15,678 / 19,167
Calibrated pixel size	5.84 Å	8.93 / 7.47 Å
Defocus (fitted)	-6 to -8 µm	-3 to -15 µm
Cumulative dose	80 to 100 electrons / $Å^2$	
Dose fractionation	1 / cosine	
Tilt range	±60°	
Tilt increment	2°	