

Supplementary Materials

Molecular Biology of the Cell

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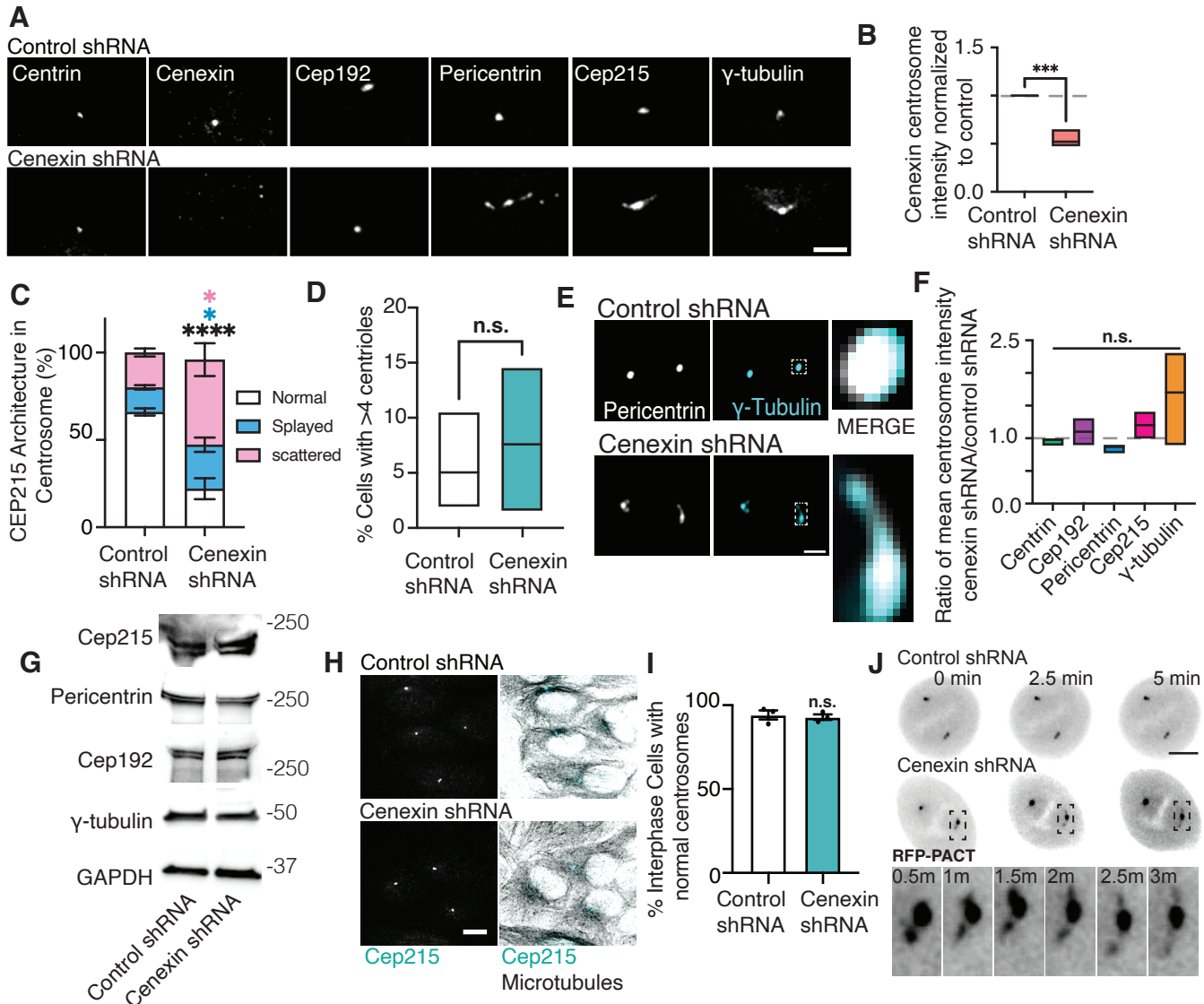


Figure S1. Cenexin loss results in PCM specific fragmentation. Related to Figure 1. (A) Metaphase HeLa cells mitotic centrosomes corresponding with Figure 1A labeled for centrosome markers (grey). Control shRNA (top) and cenexin shRNA (bottom). Scale bar, 5 μ m. (B) Box plot depicting cenexin mitotic centrosome intensity, cenexin shRNA normalized to control. Box boundaries denote the 25th and 75th percentiles. Unpaired, two-tailed Student's t-tests, *** $p < 0.001$. (C) Stacked Bar graph depicting percentages of CEP215 architecture in control shRNA and cenexin shRNA treated cells. Means with SEM shown. Unpaired, two-tailed Student's t-test, * $p < 0.05$, **** $p < 0.0001$. (D) Floating bars depicting percentage of mitotic cells with >4 centrioles. Min, max and median are displayed. Unpaired, two-tailed Student's t-tests, n.s. not significant. (E) Projections of control shRNA and cenexin shRNA immunolabeled for pericentrin (grey) and γ -tubulin (cyan). Insets at 3-4X magnification (merge). Scale bar, 5 μ m. (F) Floating bars of mean centrosome intensity ratio cenexin shRNA/control shRNA. Min, max and median displayed. Grey dashed line, ratio of 1. One-way ANOVA, n.s. not significant. (G) Western Blot of cell lysates for centrosome proteins in (F). (H) Interphase cells labeled for Cep215 (cyan in merge) and microtubules (inverted LUT). Scale bar, 5 μ m. (I) Bar graph depicting percentages of interphase cells with normal Cep215 centrosome morphology. Unpaired, two-tailed Student's t-tests, n.s. not significant. (J) RFP-PACT expressing metaphase cells imaged over time. 3x Magnified inset of splayed mitotic centrosome from cenexin shRNA treated cell. Scale bar, 5 μ m. For all graphs: detailed statistical analysis in Table S1.

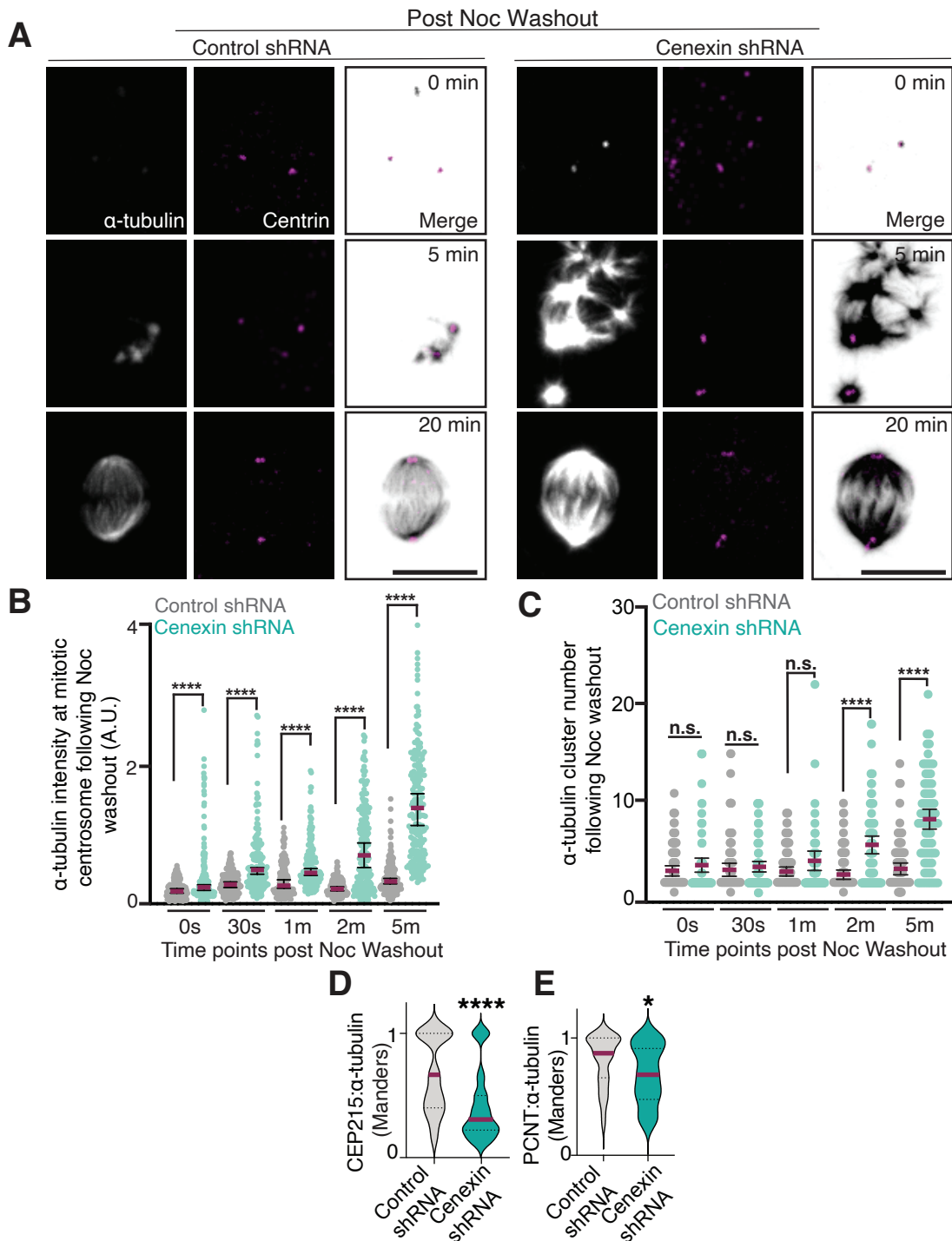


Figure S2. Cenexin tempers microtubule nucleation by mediating pericentrin associated acentrosomal nucleation sites. Related to Figure 2. (A) Control and cenexin shRNA treated HeLa cells are from Figure 1B at 0 min, 5 min and 20 min post Nocodazole washout. α -tubulin (grey), centrin (magenta). Scale bar, 10 μ m. (B-C) Scatter plot depicting α -tubulin intensity at mitotic centrosomes (B) and α -tubulin cluster number (C) 0s, 30s, 1m, 2m and 5m following nocodazole washout. Mean (magenta) with 95% confidence intervals shown. Unpaired, two-tailed Student's t-tests performed between control and cenexin shRNA cells, n.s. not significant, **** $p < 0.0001$. (D-E) Violin plot demonstrating colocalization of CEP215 (D) or pericentrin (E) with α -tubulin clusters measured using Mander's overlap coefficient. Magenta line denotes the median, dashed lines denote the 25th and 75th percentiles. Unpaired, two-tailed Student's t-tests, * $p < 0.05$, **** $p < 0.0001$. For all graphs: detailed statistical analysis in Table S1.

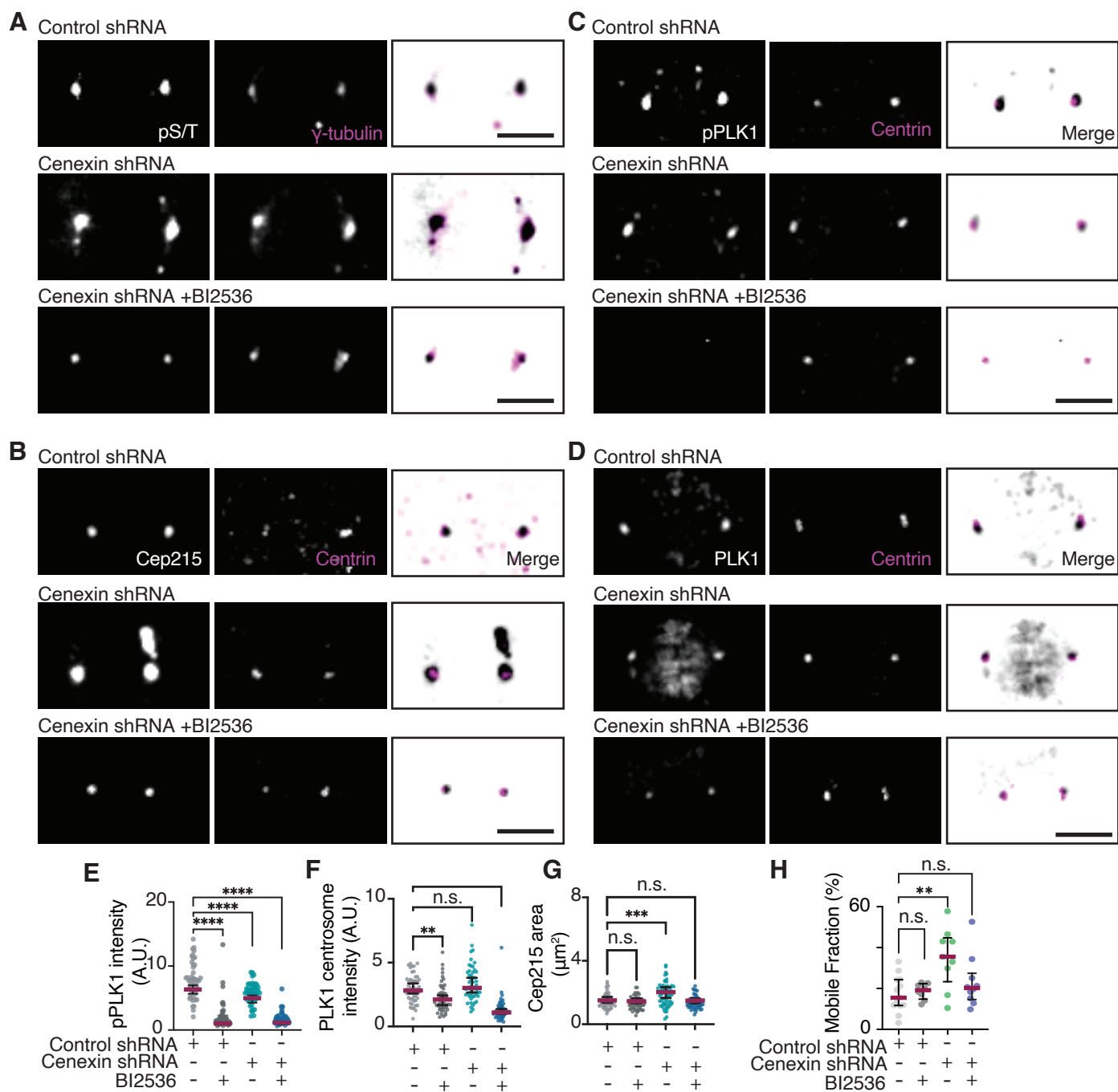


Figure S3. Cenexin is required for PCM cohesion and PLK1 is required for PCM dispersion. Related to Figure 3. (A-D) Projections of mitotic control shRNA, cenexin shRNA and cenexin shRNA treated with BI2536 cells labeled for pS/T (A), γ -tubulin (A), centrin (B, C, D), Cep215 (B), pPLK1 (C), and PLK1 (D). Scale bar, 5 μm . (E-G) Scatter plots depicting pPLK1 (A.U., E) and PLK1 (A.U., F) mean centrosome intensity, and Cep215 area (μm^2 , G) under control shRNA and cenexin shRNA with and without BI2536. Mean (magenta) and 95% confidence intervals are shown. One-way ANOVA with multiple comparisons to control cells, n.s. not significant, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. (H) Scatter plots of mobile fraction of RFP-PACT at metaphase spindle poles in control shRNA, cenexin shRNA and cenexin shRNA +BI2536. Box boundaries denote the 25th and 75th percentiles. One-way ANOVA with multiple comparisons to control cell, n.s. not significant, * $p < 0.05$. For all graphs: detailed statistical analysis in Table S1.

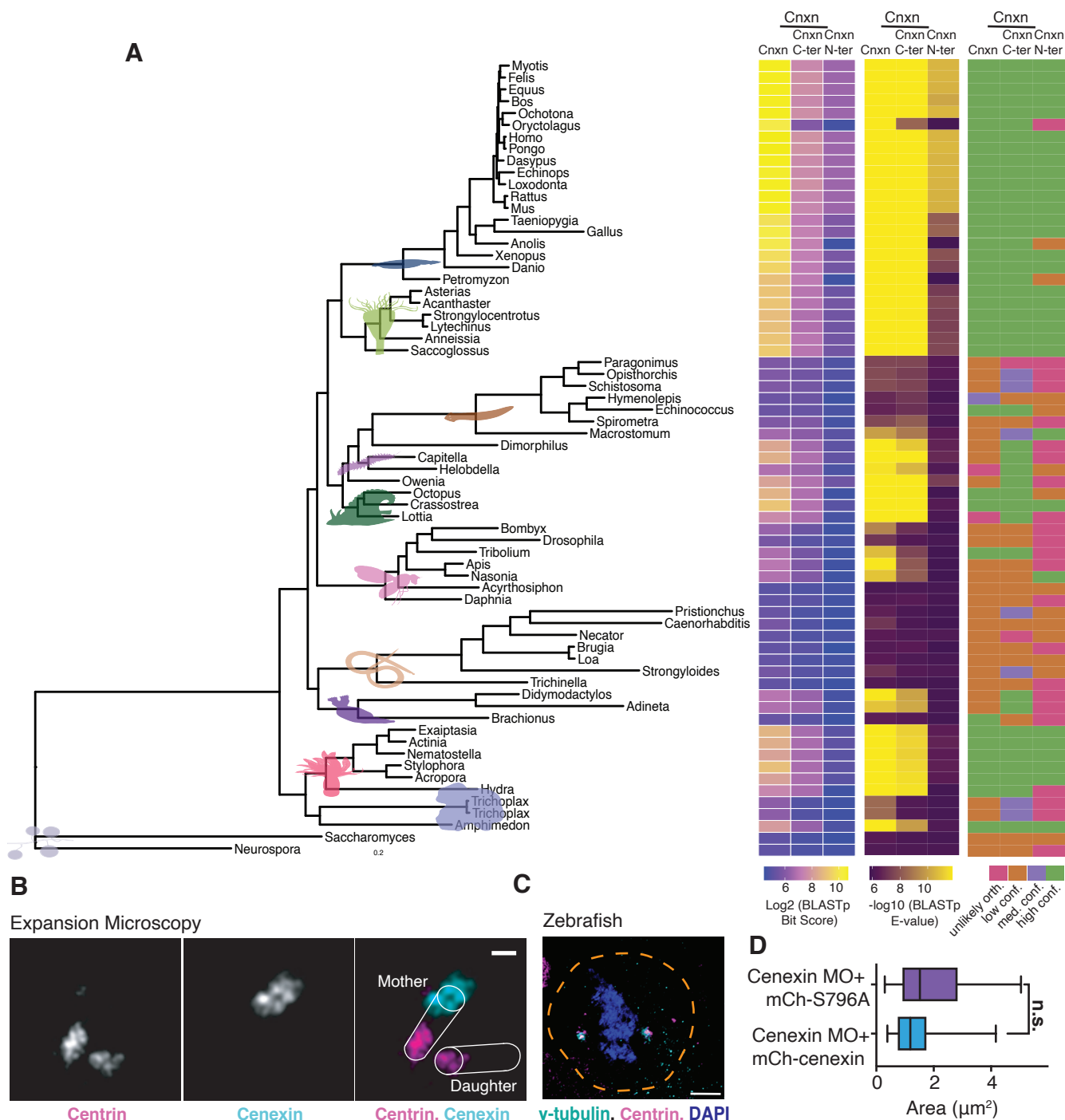


Figure S4. Cenexin phosphorylation at its conserved C-terminal PLK1 binding site is required for maintenance of PCM in vivo. Related to Figure 4. (A) BlastP searches using local BLAST installation where hit confidence as potential orthologs were determined by hit length, percent identity, and NCBI annotation. High confidence orthologs were noted if NCBI annotated as a Cnxn ortholog or had a matched alignment length >100 a.a (Cnxn), >80 a.a (C-term), or >30 a.a. (N-term) and a percent identity >60% (Cnxn) or >40% (C-term). Additional considerations for unlikely, low confidence, and medium confidence discussed in methods. See Supplemental Files S1-S5. (B) Projections of expansion confocal images of an interphase Hela cell labeled for centrin (grey, magenta in merge) and cenexin (grey, cyan in merge). Centrioles are traced to show mother and daughter. Scale bar 1 μ m. (C) Representative cell from a 512-cell zebrafish control embryo. Centrin-GFP (magenta) and γ -tubulin (cyan) are shown. Scale bar, 5 μ m. (D) Box-and-whisker plots of mCh-cenexin and mCh-cenexin-S796A area (μ m²) at mitotic centrosomes in cenexin depleted embryos (MO). Box boundaries denote the 25th and 75th percentiles. Unpaired, two-tailed Student's t-tests, n.s. not significant. For all graphs: detailed statistical analysis in Table S1.

Figures	Category	n cells	n experiments	Statistical Test	Parameters	Result	p-value
1B	Human cell (HeLa) control shRNA	25	n=3, representing 1	Two-tailed Student's t-test	t=0.7746, df=98	n.s.	0.4404
	Human cell (HeLa) cenexin shRNA	25	n=3, representing 1				
1C	Human cell (HeLa) control shRNA	25	n=3, representing 1	Two-tailed Student's t-test	t=0.3695, df=98	n.s.	0.7125
	Human cell (HeLa) cenexin shRNA	25	n=3, representing 1				
1D	Human cell (HeLa) control shRNA	25	n=3, representing 1	Two-tailed Student's t-test	t=5.055, df=98	****	<0.0001
	Human cell (HeLa) cenexin shRNA	25	n=3, representing 1				
1E	Human cell (HeLa) control shRNA	25	n=3, representing 1	Two-tailed Student's t-test	t=6.469, df=106	****	<0.0001
	Human cell (HeLa) cenexin shRNA	25	n=3, representing 1				
1F	Human cell (HeLa) control shRNA	27	n=3, representing 1	Two-tailed Student's t-test	t=3.885, df=98	***	0.0002
	Human cell (HeLa) cenexin shRNA	25	n=3, representing 1				
S1B	Human cell (HeLa) control shRNA	75	3	Two-tailed Student's t-test	t=8.953, df=4	***	0.0009
	Human cell (HeLa) cenexin shRNA	75	3				
S1C	Human cell (HeLa) control shRNA	>500	6	Two-tailed Student's t-test	t=6.944, df=10	Normal: ****	Normal: <0.0001
					t=2.581, df=10	Splayed: *	Splayed: 0.0273
		>500	6				

	Human cell (HeLa) cenexin shRNA				t=2.988, df=10	Scattered: *	Scattered: 0.0136
S1D	Human cell (HeLa) control shRNA		6	Two-tailed Student's t-test	t=1.136, df=9	n.s.	0.2854
	Human cell (HeLa) cenexin shRNA		5				
S1F	CEP192	>75	3	One Way ANOVA	F (5, 12) = 2.399	n.s.	P=0.0995
	Pericentrin		3				
	CEP215		3				
	γ-tubulin		3				
S1I	Human cell (HeLa) control shRNA	>100	3	Two-tailed Student's t-test	t=0.4201, df=4	n.s.	0.690
	Human cell (HeLa) cenexin shRNA						
2C	Human cell (HeLa) control shRNA	>174	3	Two-tailed Student's t-test	t=17.30, df=359	****	<0.0001
	Human cell (HeLa) cenexin shRNA		3				
2D	Human cell (HeLa) control shRNA	>79	3	Two-tailed Student's t-test	t=6.816, df=157	****	<0.0001
	Human cell (HeLa) cenexin shRNA		3				
2F	Human cell (HeLa) cenexin shRNA	>52	n=3, representing 1	Two-tailed Student's t-test	t=7.391, df=133	****	<0.0001
	Human cell (HeLa) cenexin shRNA +BI2536		n=3, representing 1				
2G	Human cell (HeLa)	>56	n=3, representing 1		t=1.046, df=127	n.s.	0.2976

	cenexin shRNA						
	Human cell (HeLa) cenexin shRNA +BI2536		n=3, representing 1	Two-tailed Student's t-test			
2I	Human cell (HeLa) control shRNA	>76	3	Two-tailed Student's t-test	t=4.281, df=162	****	<0.0001
	Human cell (HeLa) cenexin shRNA		3				
2J	Human cell (HeLa) control shRNA	>78	3	Two-tailed Student's t-test	t=0.3306, df=164	n.s.	0.7414
	Human cell (HeLa) cenexin shRNA		3				
S2B	Human cell (HeLa) control shRNA	>158	3	Two-tailed Student's t-test	t=5.107, df=321	****	<0.0001
			3		t=7.961, df=337	****	
			3		t=6.443, df=325	****	
	Human cell (HeLa) cenexin shRNA		3		t=11.73, df=347	****	
			3		t=12.97, df=373	****	
S2C	Human cell (HeLa) control shRNA	>75	3	Two-tailed Student's t-test	t=0.5556, df=153	n.s.	0.5793
			3		t=1.214, df=161	n.s.	0.2265
			3		t=0.5107, df=154	n.s.	0.6103
	Human cell (HeLa) cenexin shRNA		3		t=4.333, df=157	****	<0.0001
			3		t=7.369, df=151	****	<0.0001
S2D	Human cell (HeLa) control shRNA	>76	n=3	Two-tailed Student's t-test	t=5.516, df=152	****	<0.0001
	Human cell (HeLa) cenexin shRNA		n=3				
S2E	Human cell (HeLa) control shRNA	>27	n=3, representing 1	Two-tailed Student's t-test		*	0.0434
	Human cell (HeLa)		n=3, representing 1				

	cenexin shRNA						
3B	Human cell (HeLa) control shRNA	25	n=3, representing 1	One Way ANOVA	F (3, 194) = 199.8	Control	Control
	Human cell (HeLa) control shRNA+ BI2536	25	n=3, representing 1			****	<0.0001
	Human cell (HeLa) cenexin shRNA	25	n=3, representing 1			****	<0.0001
	Human cell (HeLa) cenexin shRNA+ BI2536	25	n=3, representing 1			***	<0.0002
3C	Human cell (HeLa) control shRNA	7	n=3, representing 1	One Way ANOVA	F (3, 65) = 15.09	Control	Control
	Human cell (HeLa) control shRNA+ BI2536	6	n=3, representing 1			n.s.	>0.9999
	Human cell (HeLa) cenexin shRNA	12	n=3, representing 1			****	<0.0001
	Human cell (HeLa) cenexin shRNA+ BI2536	9	n=3, representing 1			n.s.	0.8196
3D	Human cell (HeLa) control shRNA	13	n=3, representing 1	One Way ANOVA	F (3, 79) = 95.24	Control	Control
	Human cell (HeLa) control shRNA+ BI2536	12	n=3, representing 1			*	0.0163
	Human cell (HeLa) cenexin shRNA	8	n=3, representing 1			****	<0.0001
	Human cell (HeLa) cenexin shRNA+ BI2536	9	n=3, representing 1			n.s.	0.2868
3F	Human cell (HeLa)	13	>3				

	control shRNA						
	Human cell (HeLa) control shRNA + BI2536	8					
	Human cell (HeLa) cenexin shRNA	9					
	Human cell (HeLa) cenexin shRNA+ BI2536	10					
S3E	Human cell (HeLa) control shRNA	25	n=3, representing 1	One Way ANOVA	F (3, 200) = 108.6	Control	Control
	Human cell (HeLa) control shRNA+ BI2536	25	n=3, representing 1			****	<0.0001
	Human cell (HeLa) cenexin shRNA	25	n=3, representing 1			****	<0.0001
	Human cell (HeLa) cenexin shRNA+ BI2536	25	n=3, representing 1			****	<0.0001
S3F	Human cell (HeLa) control shRNA	26	n=3, representing 1	One Way ANOVA	F (3, 195) = 30.64	Control	Control
	Human cell (HeLa) control shRNA+ BI2536	26	n=3, representing 1			**	0.0083
	Human cell (HeLa) cenexin shRNA	25	n=3, representing 1			n.s.	0.1330
	Human cell (HeLa) cenexin shRNA+ BI2536	25	n=3, representing 1			****	<0.0001
S3G	Human cell (HeLa) control shRNA	25	n=3, representing 1	One Way ANOVA	F (3, 215) = 13.32	Control	Control
	Human cell (HeLa) control	27	n=3, representing 1			n.s.	0.4275

	shRNA+ BI2536						
	Human cell (HeLa) cenexin shRNA	29	n=3, representing 1			***	0.0001
	Human cell (HeLa) cenexin shRNA+ BI2536	27	n=3, representing 1			n.s.	0.7262
S3H	Human cell (HeLa) control shRNA	13	>3	One Way ANOVA	F (3,36) = 1.788	Control	Control
	Human cell (HeLa) control shRNA + BI2536	8				n.s.	0.9877
	Human cell (HeLa) cenexin shRNA	9				**	0.0015
	Human cell (HeLa) cenexin shRNA+ BI2536	10				n.s.	0.4739
4G	Control injection	12, 3 embryos	1	One Way ANOVA	F (3, 82) = 28.98	Control	Control
	Cenexin MO injection	13, 3 embryos	1			**	0.0092
	Cenexin MO injection +mCherry cenexin-WT	15, 4 embryos	1			n.s.	0.3406
	Cenexin MO injection +mCherry cenexin-S796A	12, 4 embryos	1			****	<0.0001
4H	Cenexin MO injection +mCherry cenexin-WT	14, 3 embryos	1	Two-tailed Student's t-test	t=4.348, df=50	****	<0.0001
	Cenexin MO injection +mCherry cenexin-S796A	13, 3 embryos	1				

S4D	Cenexin area (μm^2)	10, 3 embryos	1	Two-tailed Student's t-test	t=1.715, df=34	n.s.	0.0955
		8, 3 embryos	1				

Table S1. Detailed statistical analysis of results reported in this study.