

Fig. S1. CENP-C is required for PLK-1 kinetochore localisation. (A) Immunofluorescence analysis using a specific anti-PLK-1 antibody was carried out in wild type and *CENP-C PD^{mut}* embryos at metaphase. Scale bar, 5 μ m. (B) Fluorescently labelled BUB-1 was followed throughout mitosis in wild type and *CENP-C PD^{mut}* embryos. Scale bar, 5 μ m. (C) Chromosomal BUB-1 levels were quantified and normalised to the chromosome (histone) signal. Individual values are shown and the dashed line represents the median. 'n' denotes the number of embryos analysed. Wild type and *CENP-C PD^{mut}* embryos were compared using a non-parametric test (Mann-Whitney) and the P value is shown.

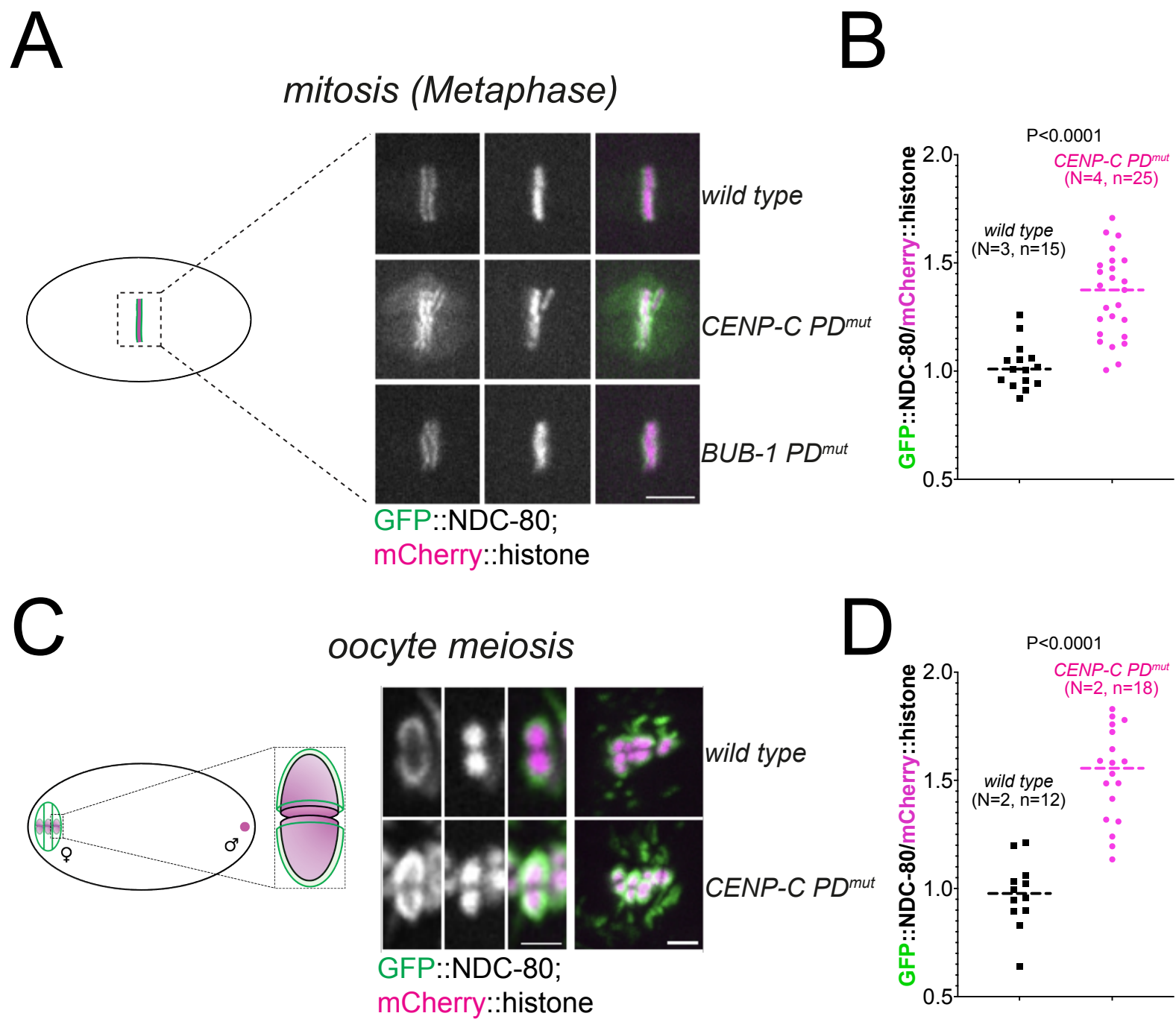


Fig. S2. NDC-80 kinetochore localisation in the *CENP-C PD^{mut}*. (A) GFP-tagged endogenous NDC-80 was followed throughout mitosis in wild type, *CENP-C PD^{mut}*, and *BUB-1 PD^{mut}*. Scale bar, 5 μ m. (B) Chromosomal NDC-80 levels at metaphase were quantified and normalised to the chromosome (histone) signal. Individual values are shown and the dashed line represents the median. ‘N’ denotes the number of experiments and ‘n’ denotes the number of embryos analysed. Wild type and *CENP-C PD^{mut}* embryos were compared using a non-parametric test (Mann-Whitney) and the P value is shown. (C) GFP-tagged endogenous NDC-80 was imaged during oocyte meiosis and a single image corresponding to prometaphase I is shown for wild type and polo docking mutant *CENP-C PD^{mut}*. Scale bars, 2 μ m in the zoomed panel (left) and 3 μ m in right panel. (D) Chromosomal NDC-80 levels at prometaphase of Meiosis I were quantified and normalised to the chromosome (histone) signal. Individual values are shown and the dashed line represents the median. ‘N’ denotes the number of experiments and ‘n’ denotes the number of embryos analysed. Wild type and *CENP-C PD^{mut}* embryos were compared using a non-parametric test (Mann-Whitney) and the P value is shown.

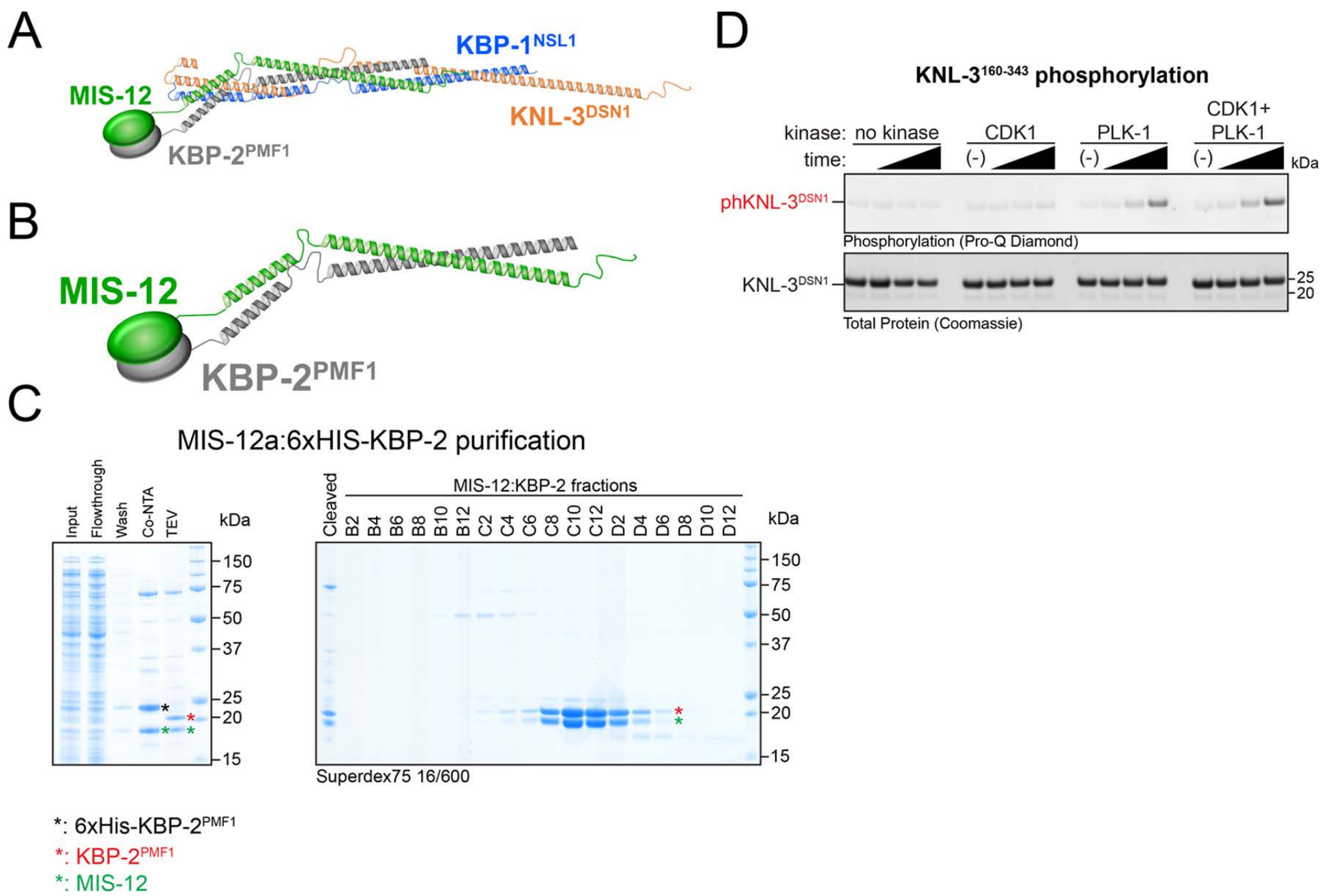


Fig. S3. Purification of the MIS-12:KBP-2^{PMF1} dimer. (A) Schematic of the full MIS-12 complex structure based on an AlphaFold2 prediction. The disordered region of KNL-3^{DSN1} (aa 1-159) is not shown. (B) Enlarged image of the MIS-12:KBP-2^{PMF1} complex from (A). (C) Biochemical purification of the MIS-12:KBP-2^{PMF1} complex from bacteria. (D) Kinase assays were performed with the indicated protein kinases and KNL-3. Time points analysed were 15, 30, and 45 minutes. (D) Kinase assays were performed with the indicated protein kinase and KNL-3^{DSN1} as a substrate. Time points analysed were 0, 15, 30, and 45 minutes.

Table S1. List of phospho-sites identified from in vitro kinase assays

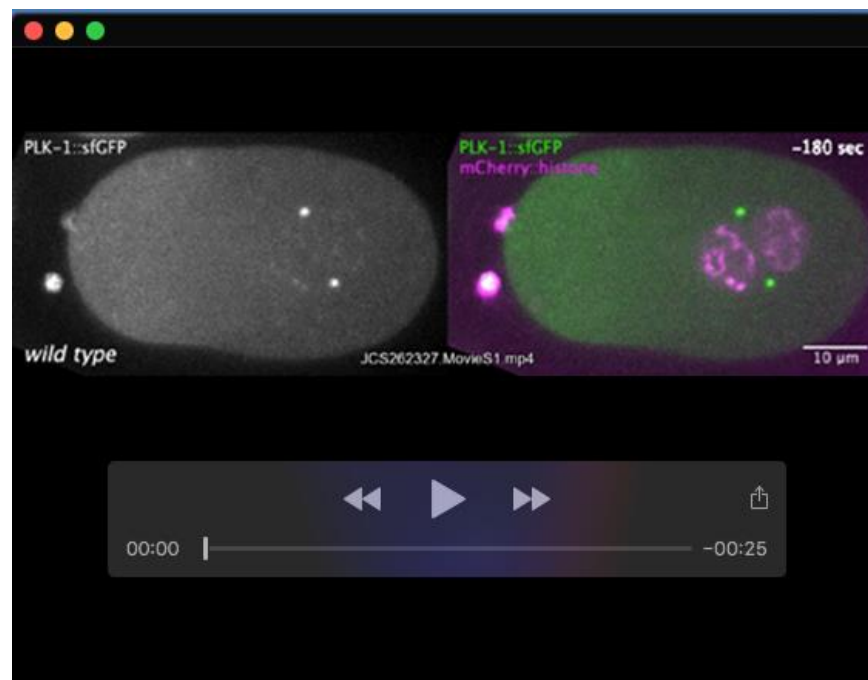
Substrate Protein	PLK-1	Cdk1	Cdk1+PLK-1
CENP-C	Ser 9	Ser 9	Ser 9
	Thr 10	Thr 10	Thr 10
	Thr 19	Thr 19	Thr 19
	Thr 41		Thr 41
	Ser 47		Ser 47
	Ser 52		Ser 52
		Ser 66	Ser 66
			Thr 80
	Thr 98	Thr 98	Thr 98
			Ser 109
			Thr 118
	Ser 125		Ser 125
		Ser 127	Ser 127
	Ser 142	Ser 142	Ser 142
		Ser 146	Ser 146
	Ser 160		Ser 160
	Ser 162		Ser 162
		Thr 163	Thr 163
		Ser 166	Ser 166
	Ser 171		Ser 171
		Ser 174	Ser 174
	Ser 177	Ser 177	
Ser 181		Ser 181	
Ser 200		Ser 200	
Thr 205	Thr 205	Thr 205	
Thr 213		Thr 213	

Substrate Protein	PLK-1
KBP-2	Thr 15
	Ser 22
	Thr 64
	Ser 72
	Thr 84
	Ser 114
	Thr 116
MIS-12	Ser 90
	Thr 92
KNL-3	Ser 177
	Thr 185
	Ser 191
	Ser 225
	Ser 229
	Thr 241
	Ser 258
	Thr 263
Ser 292	

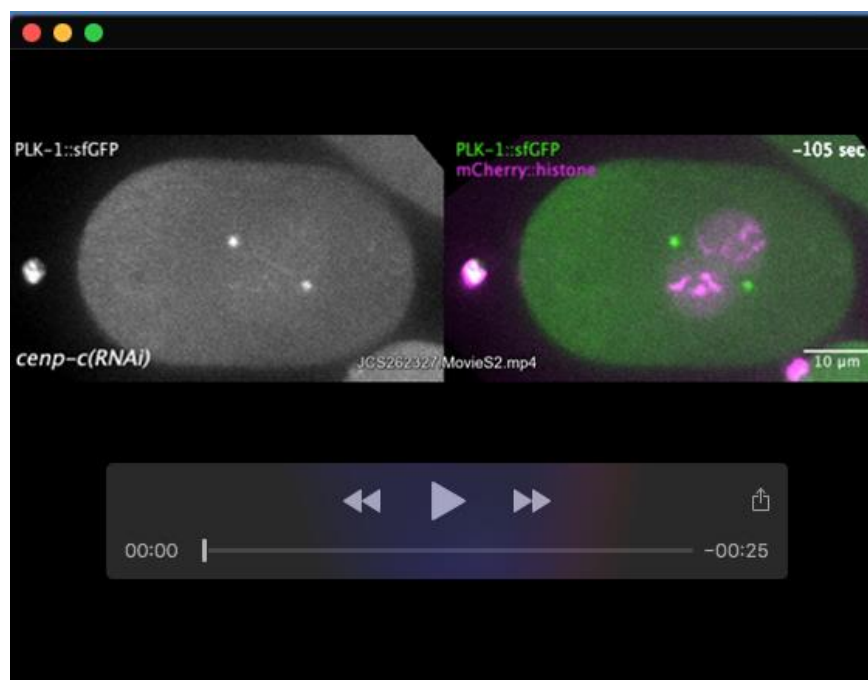
Table S2. *C. elegans* strains used in this study

bub-1::linker::gfp; mCherry::histone	FGP202	<i>bub-1(syb1134[bub-1::linker::gfp])I; ltIs37 [pAA64; pie-1/mCherry::his-58; unc-119 (+)]IV; unc-119(ed3)III</i>	This study
PLK-1::sfGFP; mCherry::histone	FGP263	<i>plk-1(lt18[plk-1::sGFP]::loxP)III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV</i>	doi.org/10.1016/j.devcel.2017.09.019
NDC-80::GFP; mCherry::histone	FGP372	<i>ndc-80(lt54[ndc-80::GFP::tev::loxP::3xFlag])IV; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV</i>	This study
hcp-4(T163A)	FGP669	<i>hcp-4(fgp58[hcp-4(T163A)])I</i>	doi.org/10.7554/eLife.84057
PLK-1::sGFP; mCherry::histone; hcp-4(T163A)	FGP719	<i>plk-1(lt18[plk-1::sGFP]::loxP)III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV; hcp-4(fgp58[hcp-4(T163A)])I</i>	doi.org/10.7554/eLife.84057
mScarlet::KNL-3; GFP::histone	FGP722	<i>knl-3(dha19 [mScarlet-I³XFLAG::knl-3] V; ruIs32 [pie-1::GFP::histone + unc-119(+)], unc-119 (ed3)</i>	This study
mScarlet::KNL-3; GFP::histone; hcp-4(T163A)	FGP728	<i>knl-3(dha19 [mScarlet-I³XFLAG::knl-3] V; ruIs32 [pie-1::GFP::histone + unc-119(+)], unc-119 (ed3); hcp-4(fgp58[hcp-4(T163A)])I</i>	This study
NDC-80::GFP; mCherry::histone; hcp-4(T163A)	FGP729	<i>ndc-80(lt54[ndc-80::GFP::tev::loxP::3xFlag])IV; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV; hcp-4(fgp58[hcp-4(T163A)])I</i>	This study
PLK-1::sGFP; mCherry::histone; bub-1(wt)	FGP735	<i>plk-1(lt18[plk-1::sGFP]::loxP)III; ; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV; unc-119(ed3)III; ltSi268[pOD/pTK013; Ppub-1::Bub1 reencoded; cb-unc-119(+)]II</i>	This study
PLK-1::sGFP; mCherry::histone; hcp-4(T163A); bub-1(wt)	FGP736	<i>plk-1(lt18[plk-1::sGFP]::loxP)III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV; hcp-4(fgp58[hcp-4(T163A)])I; unc-119(ed3)III; ltSi268[pOD/pTK013; Ppub-1::Bub1 reencoded; cb-unc-119(+)]II</i>	This study
PLK-1::sGFP; mCherry::histone; bub-1(T527A)	FGP737	<i>plk-1(lt18[plk-1::sGFP]::loxP)III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV; unc-119(ed3)III; ltSi1346[pTK070; Ppub-1::BUB-1 T527A reencoded; cb-unc-119(+)]II</i>	This study
PLK-1::sGFP; mCherry::histone; hcp-4(T163A); bub-1(T527A)	FGP738	<i>plk-1(lt18[plk-1::sGFP]::loxP)III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV; hcp-4(fgp58[hcp-4(T163A)])I; unc-119(ed3)III; ltSi1346[pTK070; Ppub-1::BUB-1 T527A reencoded; cb-unc-119(+)]II</i>	This study
mScarlet::KNL-3; GFP::histone; bub-1(wt)	FGP739	<i>knl-3(dha19 [mScarlet-I³XFLAG::knl-3] V; ruIs32 [pie-1::GFP::histone + unc-119(+)], unc-119 (ed3); unc-119(ed3)III; ltSi268[pOD/pTK013; Ppub-1::Bub1 reencoded; cb-unc-119(+)]II</i>	This study
mScarlet::KNL-3; GFP::histone; hcp-4(T163A); bub-1(wt)	FGP740	<i>knl-3(dha19 [mScarlet-I³XFLAG::knl-3] V; ruIs32 [pie-1::GFP::histone + unc-119(+)], unc-119 (ed3); hcp-4(fgp58[hcp-4(T163A)])I; unc-119(ed3)III; ltSi268[pOD/pTK013; Ppub-1::Bub1 reencoded; cb-unc-119(+)]II</i>	This study
mScarlet::KNL-3; GFP::histone; bub-1(T527A)	FGP741	<i>knl-3(dha19 [mScarlet-I³XFLAG::knl-3] V; ruIs32 [pie-1::GFP::histone + unc-119(+)], unc-119 (ed3); unc-119(ed3)III; ltSi1346[pTK070; Ppub-1::BUB-1 T527A reencoded; cb-unc-119(+)]II</i>	This study
mScarlet::KNL-3; GFP::histone; hcp-4(T163A); bub-1(T527A)	FGP742	<i>knl-3(dha19 [mScarlet-I³XFLAG::knl-3] V; ruIs32 [pie-1::GFP::histone + unc-119(+)], unc-119 (ed3); hcp-4(fgp58[hcp-4(T163A)])I; unc-119(ed3)III; ltSi1346[pTK070; Ppub-1::BUB-1 T527A reencoded; cb-unc-119(+)]II</i>	This study

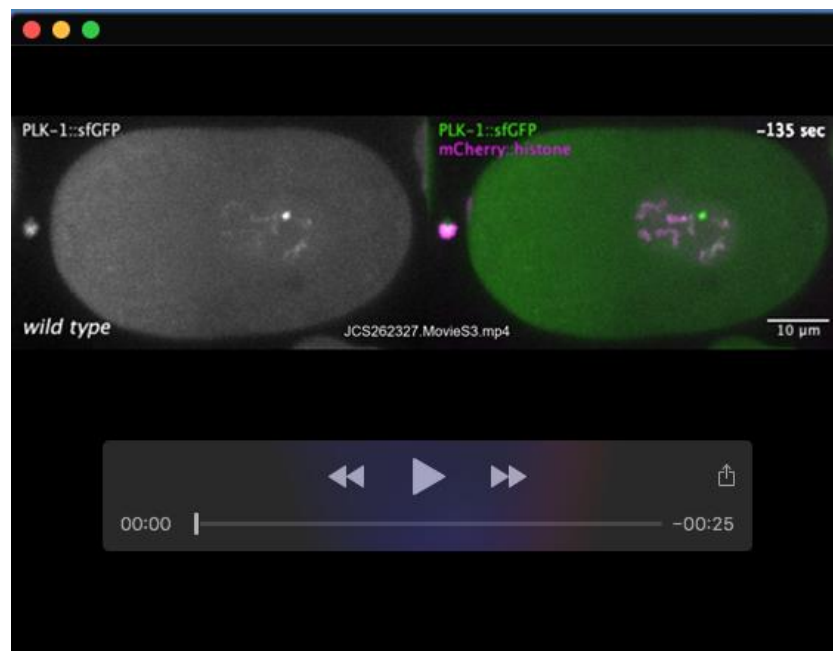
NDC-80::GFP; mCherry::histone; bub-1(wt)	FGP803	<i>ndc-80(lt54[ndc-80::GFP::tev::loxP::3xFlag])IV; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119(+)]IV; Ppub-1::Bub1 reencoded; cb-unc-119(+)]III</i>	This study
NDC-80::GFP; mCherry::histone; hcp-4(T163A); bub-1(wt)	FGP804	<i>ndc-80(lt54[ndc-80::GFP::tev::loxP::3xFlag])IV; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119(+)]IV; hcp-4(fgp58[hcp-4(T163A)])I; unc-119(ed3)III; ltSi268[pOD/pTK013; Ppub-1::Bub1 reencoded; cb-unc-119(+)]III</i>	This study
NDC-80::GFP; mCherry::histone; bub-1(T527A)	FGP805	<i>ndc-80(lt54[ndc-80::GFP::tev::loxP::3xFlag])IV; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119(+)]IV; ltSi1346[pTK070; Ppub-1::BUB-1 T527A reencoded; cb-unc-119(+)]III</i>	This study
NDC-80::GFP; mCherry::histone; hcp-4(T163A); bub-1(T527A)	FGP806	<i>ndc-80(lt54[ndc-80::GFP::tev::loxP::3xFlag])IV; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119(+)]IV; hcp-4(fgp58[hcp-4(T163A)])I; unc-119(ed3)III; ltSi1346[pTK070; Ppub-1::BUB-1 T527A reencoded; cb-unc-119(+)]III</i>	This study
bub-1::linker::gfp; mCherry::histone; hcp4(T163A)	FGP815	<i>bub-1(syb1134[bub-1::linker::gfp])I; ltIs37 [pAA64; pie-1/mCherry::his-58; unc-119(+)]IV; unc-119(ed3)III; hcp-4(fgp58[hcp-4(T163A)])I</i>	This study
GFP::CDC-20; mCherry::histone	OD2591	<i>ltSi814[pPLG047; Pfzy-1::gfp::fzy-1::fzy-1 3'UTR; cb-unc-119(+)]I; unc-119(ed3)III?;; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119(+)] IV</i>	doi.org/10.1101/gad.302067.117
GFP::CDC-20; mCherry::histone; hcp-4(T163A)	FGP829	<i>ltSi814[pPLG047; Pfzy-1::gfp::fzy-1::fzy-1 3'UTR; cb-unc-119(+)]I; unc-119(ed3)III?;; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119(+)] IV; hcp-4(fgp58[hcp-4(T163A)])I</i>	This study



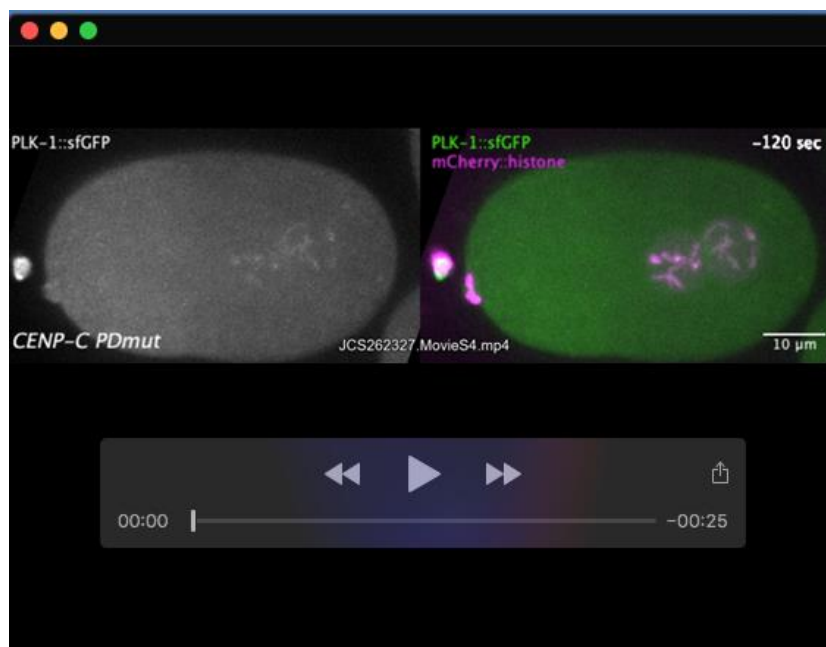
Movie 1. Embryo expressing PLK-1::sfGFP and mCherry::histone was imaged throughout mitosis. Movie corresponds to 'wild type' panels in Fig. 1B.



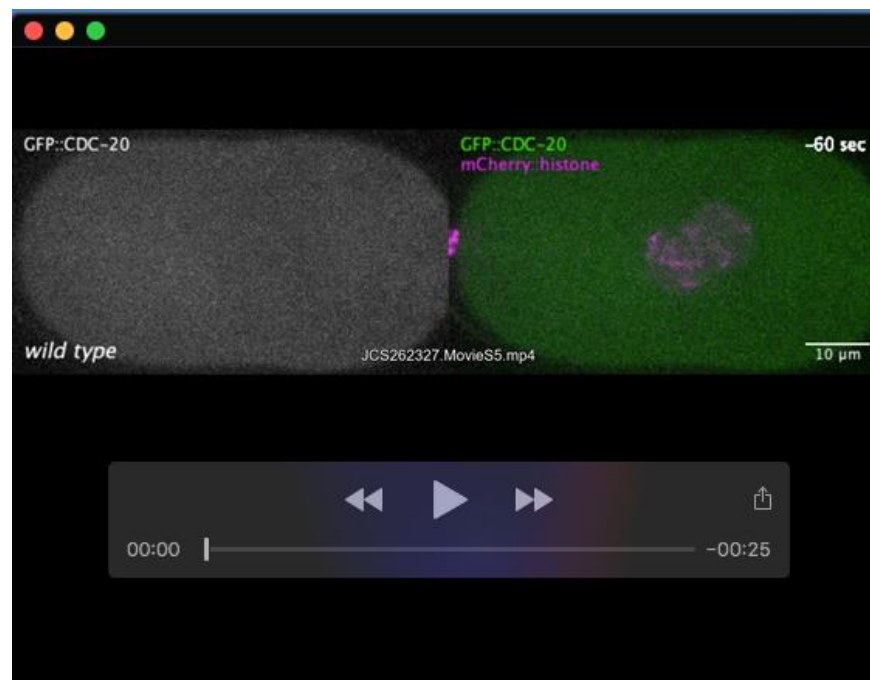
Movie 2. Embryo expressing PLK-1::sfGFP and mCherry::histone was imaged throughout mitosis. Movie corresponds to '*cenp-c(RNAi)*' panels in Fig. 1B.



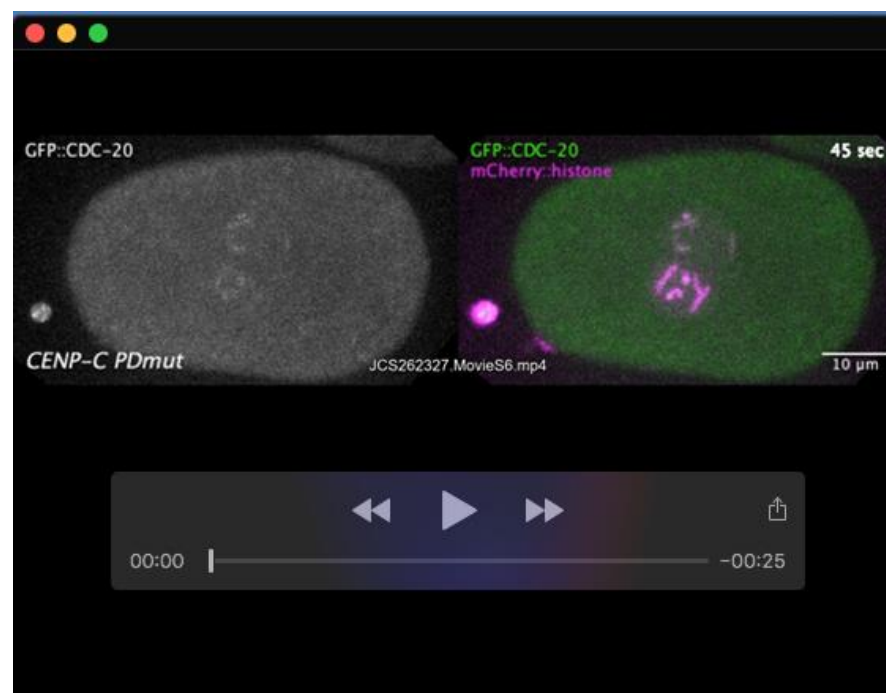
Movie 3. Embryo expressing PLK-1::sfGFP and mCherry::histone was imaged throughout mitosis. Movie corresponds to 'wild type' panels in Fig. 1F.



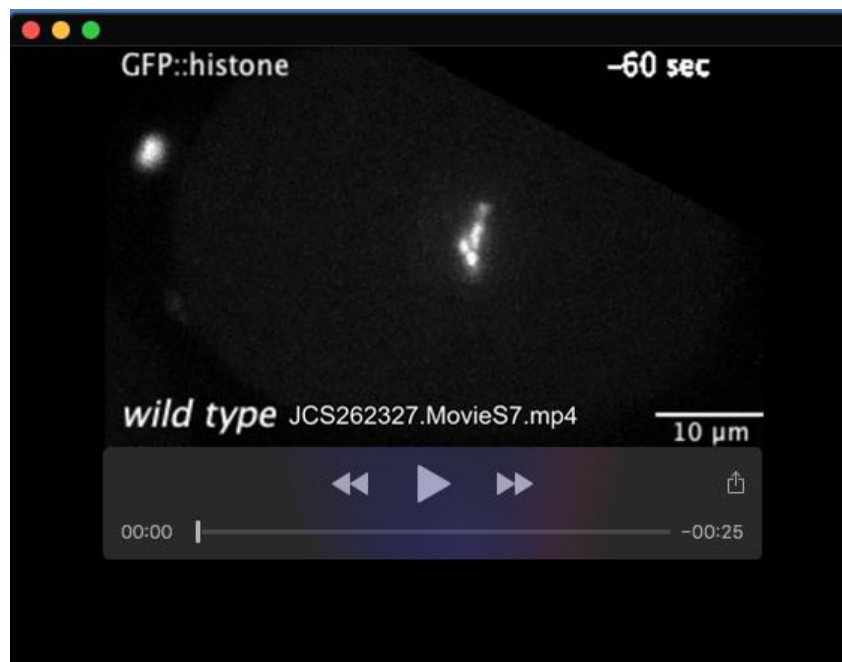
Movie 4. Embryo expressing PLK-1::sfGFP and mCherry::histone was imaged throughout mitosis. Movie corresponds to '*CENP-C PD^{mut}*' panels in Fig. 1F.



Movie 5. Embryo expressing GFP::CDC-20 and mCherry::histone was imaged throughout mitosis. Movie corresponds to 'wild type' panels in Fig. 2A.

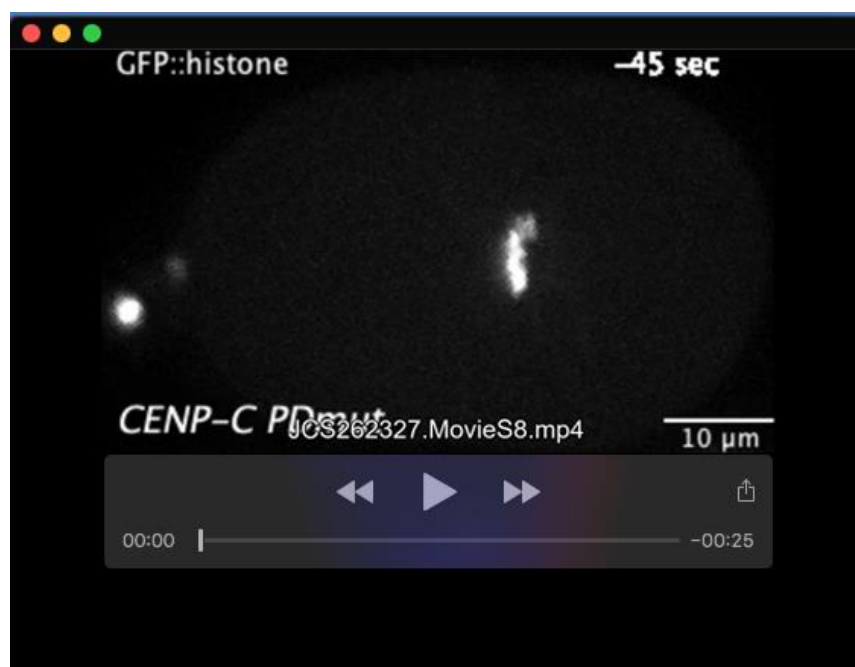


Movie 6. Embryo expressing GFP::CDC-20 and mCherry::histone was imaged throughout mitosis. Movie corresponds to 'CENP-C PD^{mut}' panels in Fig. 2A.



Movie 7. Embryo expressing GFP::histone was imaged throughout mitosis.

Movie corresponds to 'wild type' panels in Fig. 2D. The strain used here was FGP722.



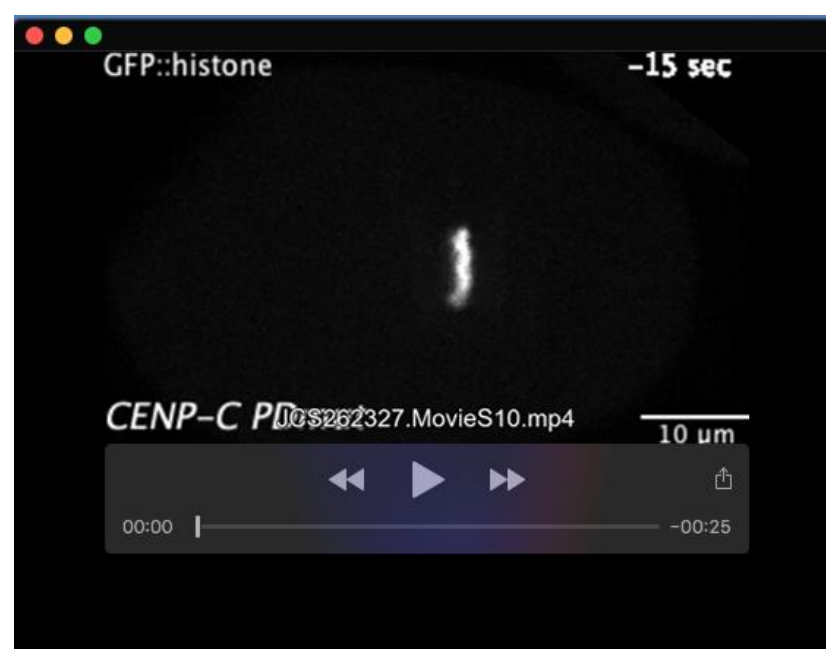
Movie 8. Embryo expressing GFP::histone was imaged throughout mitosis.

Movie corresponds to 'CENP-C PD^{mut}' panels in Fig. 2D. The strain used here was FGP728.



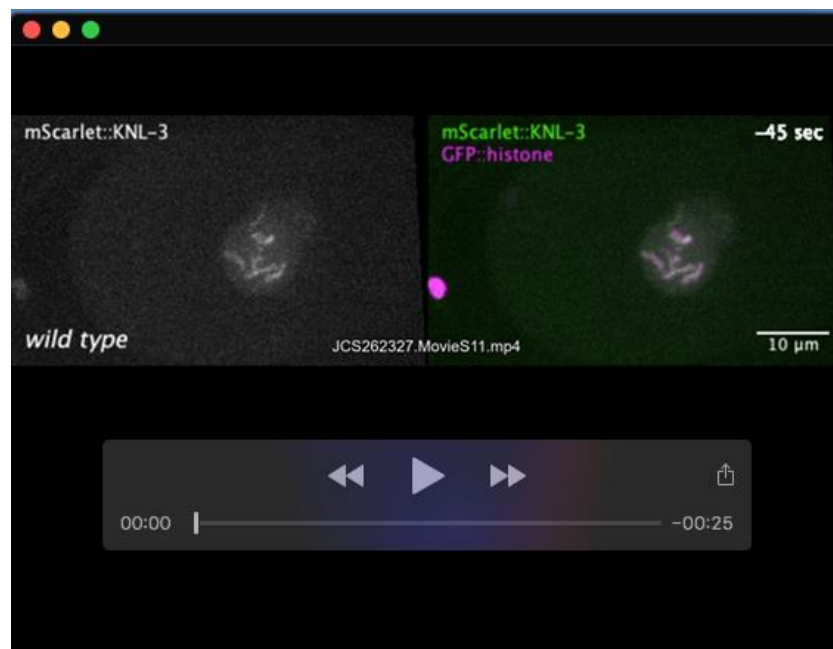
Movie 9. Embryo expressing GFP::histone was imaged throughout mitosis.

Movie corresponds to 'wild type' panels in Fig. 2E. The strain used here was FGP722.

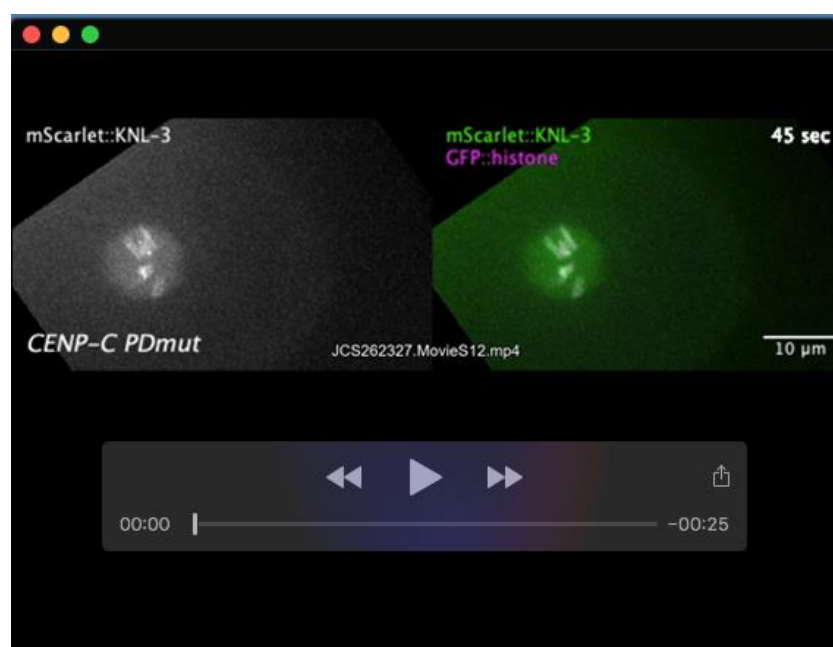


Movie 10. Embryo expressing GFP::histone was imaged throughout mitosis.

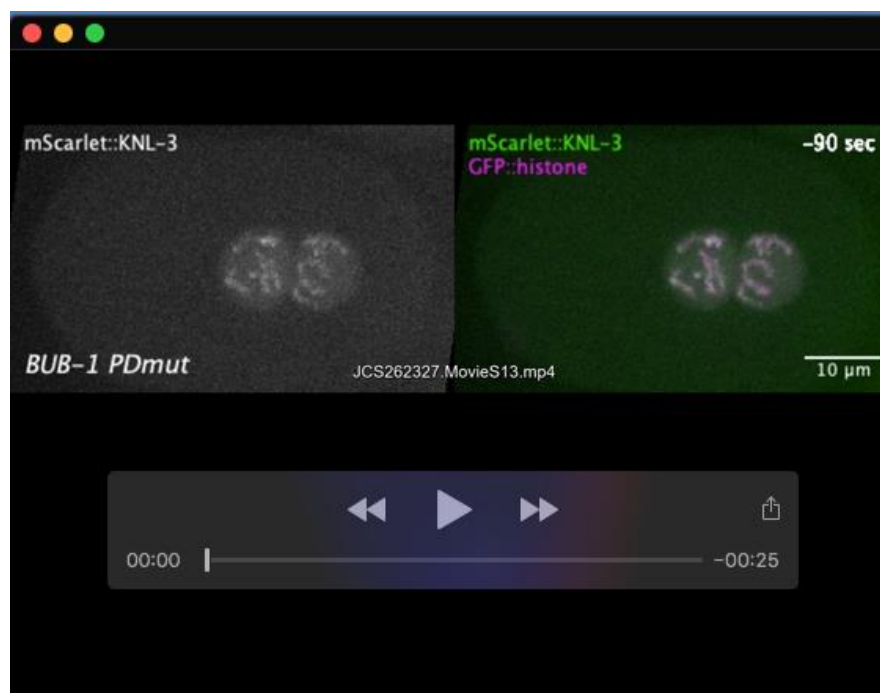
Movie corresponds to 'CENP-C PD^{mut}' panels in Fig. 2E. The strain used here was FGP728.



Movie 11. Embryo expressing Scarlet::KNL-3 and GFP::histone was imaged throughout mitosis. Movie corresponds to 'wild type' panels in Fig. 3B.



Movie 12. Embryo expressing Scarlet::KNL-3 and GFP::histone was imaged throughout mitosis. Movie corresponds to 'CENP-C PD^{mut}' panels in Fig. 3B.



Movie 13. Embryo expressing Scarlet::KNL-3 and GFP::histone was imaged throughout mitosis. Movie corresponds to '*BUB-1 PD^{mut}*' panels in Fig. 3B.

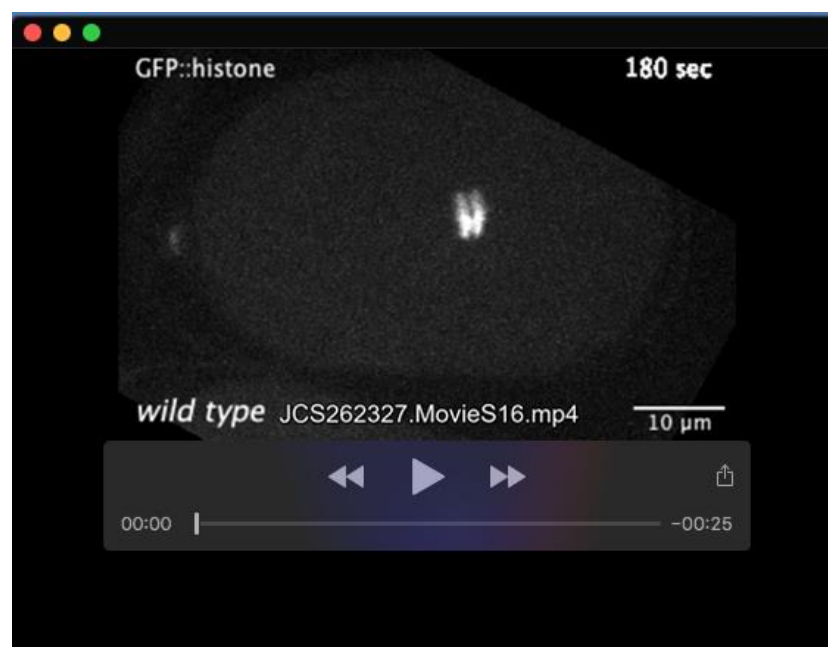


Movie 14. Embryo expressing GFP::histone was imaged throughout mitosis. Movie corresponds to '*wild type*' panels in Fig. 4A.



Movie 15. Embryo expressing GFP::histone was imaged throughout mitosis.

Movie corresponds to '*CENP-C PD^{mut} + BUB-1 PD^{mut}*' panels in Fig. 4A.



Movie 16. Embryo expressing Scarlet::KNL-3 and GFP::histone was imaged throughout mitosis. Movie corresponds to '*wild type*' panels in Fig. 4B.



Movie 17. Embryo expressing Scarlet::KNL-3 and GFP::histone was imaged throughout mitosis. Movie corresponds to '*CENP-C PD^{mut}*' panels in Fig. 4B.



Movie 18. Embryo expressing GFP::histone was imaged throughout mitosis. Movie corresponds to '*BUB-1 PD^{mut}*' panels in Fig. 4B.



Movie 19. Embryo expressing GFP::histone was imaged throughout mitosis.
Movie corresponds to 'CENP-C PD^{mut} + BUB-1 PD^{mut}' panels in Fig. 4B.