Nelson et al., http://www.jcb.org/cgi/content/full/jcb.201505114/DC1

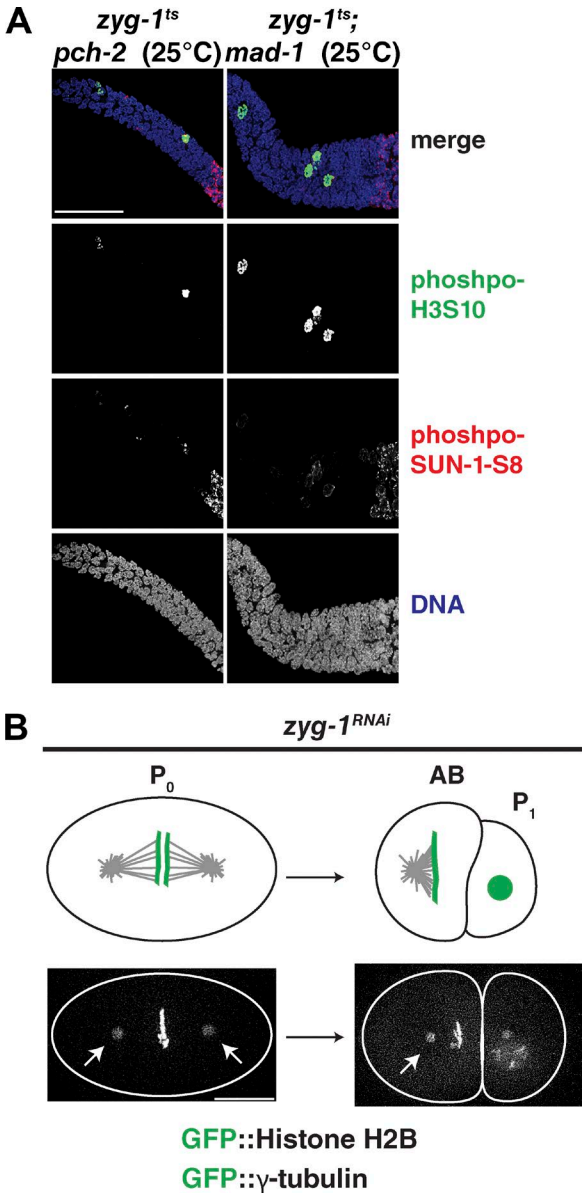


Figure S1. **Mutation of *pch-2* or *mad-1* reduces the mitotic index of *zyg-1*^{ts} germlines.** (A) Representative images of *zyg-1*^{ts}*pch-2* and *zyg-1*^{ts}*;mad-1* mutant germlines stained with DAPI and antibodies recognizing phospho-H3S10 and phospho-SUN-1-S8. Bar, 20 µm. (B, top) Schematic of the first embryonic divisions in *C. elegans* after 24 h of feeding with bacteria expressing *zyg-1* dsRNA. The first division (P_0) proceeds normally with a bipolar spindle, but subsequent divisions take place with monopolar spindles. (B, bottom) Selected frames from the first 2 divisions (P_0 and AB) of embryos at metaphase expressing both GFP::Histone H2B and GFP::γ-tubulin. Arrows denote spindle poles. The white lines surrounding cells were drawn in Adobe Illustrator. Bar, 10 µm.

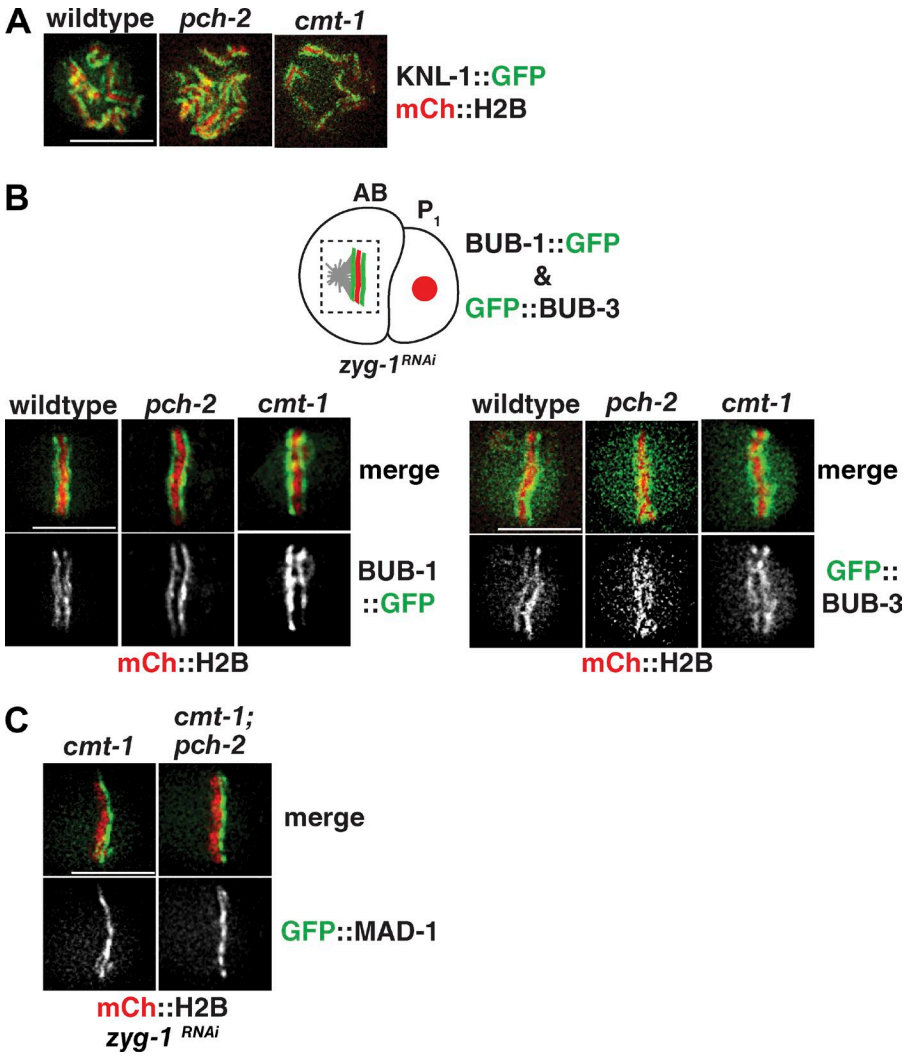


Figure S2. **Mutation of *pch-2* or *cmt-1* has no effect on the localization of KNL-1, BUB-1, BUB-3, and MAD-1 to kinetochores.** (A) No defects in KNL-1::GFP kinetochore signal intensity or loading were apparent in *pch-2* or *cmt-1* mutants. Images of pro-metaphase kinetochores are shown. (B, top) Schematic shows the localization of BUB-1::GFP and GFP::BUB-3 to kinetochores at metaphase after *zyg-1* RNAi. Note that both proteins localize to both sides of pseudo-metaphase plate, indicating their localization is not limited to unattached kinetochores. (B, bottom) BUB-1::GFP and GFP::BUB-3 properly localize to kinetochores in *pch-2* and *cmt-1* mutants. (C) GFP::MAD-1 properly localizes to unattached kinetochores after *zyg-1* RNAi in both *cmt-1* and *cmt-1;pch-2* mutants. Bars, 5 µm.

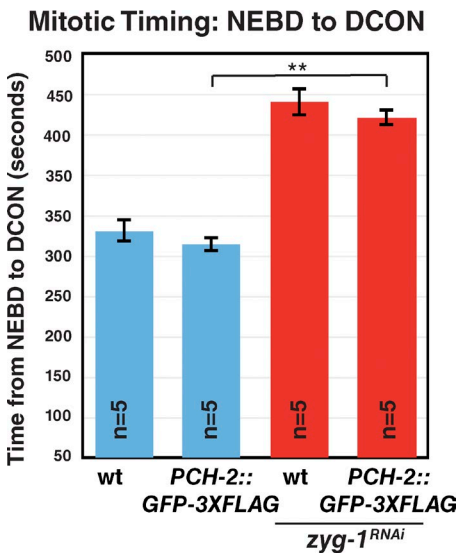


Figure S3. **PCH-2::GFP-3XFLAG embryos are competent for spindle checkpoint activation.** RNAi of *zyg-1* in embryos expressing PCH-2::GFP-3XFLAG induces a statistically significant delay in mitotic timing as in wild-type embryos. DCON was used as a marker of mitotic exit. Error bars represent SEM. **, $P < 0.0001$. Significance was assessed using a paired *t* test.

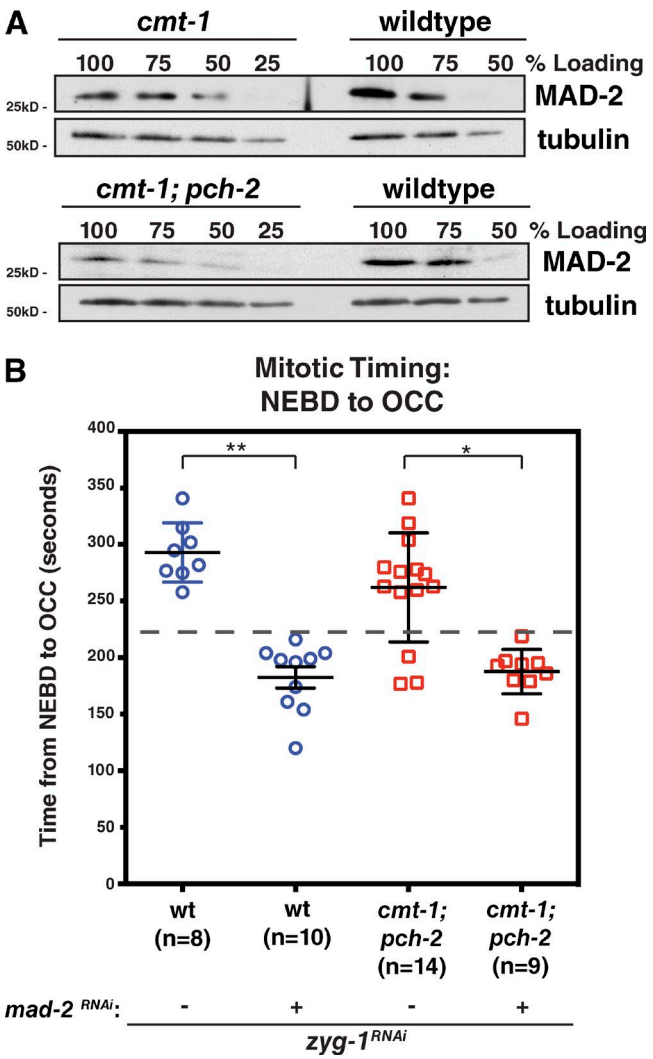


Figure S4. **The mitotic delay produced by *zyg-1* RNAi in *cmt-1;pch-2* mutants is spindle checkpoint dependent.** (A) MAD-2 protein levels are reduced in *cmt-1* and *cmt-1;pch-2* mutants. Whole worm lysates were first normalized for protein concentration and then serial dilutions were analyzed via immunoblot with an anti–MAD-2 antibody and an anti-α-tubulin antibody serving as a loading control. (B) RNAi of *mad-2* restores wild-type mitotic timing in both *zyg-1*^{RNAi} and *cmt-1;pch-2;zyg-1*^{RNAi} embryos. Error bars represent SEM. *, $P < 0.01$; **, $P < 0.0001$. Significance was assessed using a paired *t* test.

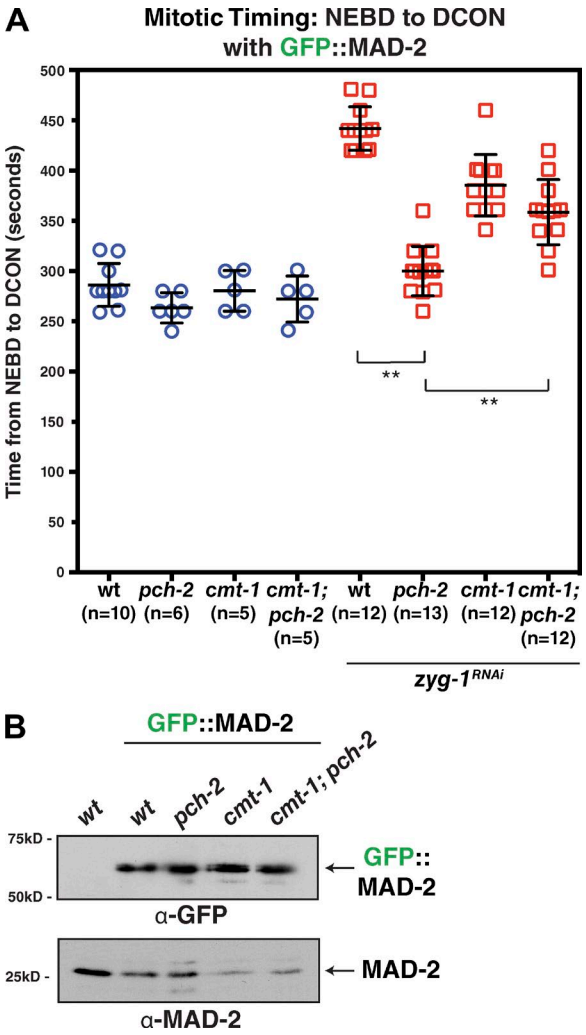
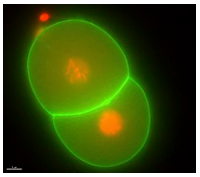
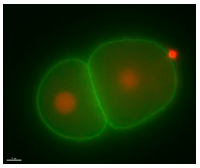


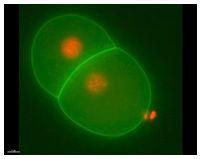
Figure S5. **Overexpression of GFP::MAD-2 does not rescue checkpoint function in *pch-2* or *cmt-1* mutants.** (A) *pch-2;zyg-1*^{RNAi} mutants exhibit wild type mitotic timing and *cmt-1; zyg-1*^{RNAi} and *cmt-1;pch-2; zyg-1*^{RNAi} mutants have a less robust checkpoint response despite overexpressing GFP::MAD-2. (B) Immunoblot showing GFP::MAD-2 levels are unperturbed in *pch-2* and *cmt-1* mutants. The strains expressing GFP::MAD-2 also express endogenous MAD-2 protein (lower immunoblot). Error bars represent SEM. **, $P < 0.0001$. Significance was assessed using a paired *t* test.



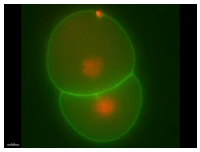
Video 1. **Mitosis in a wild-type two-cell embryo.** A wild-type embryo expressing GFP::PH and mCh::H2B for visualization of the plasma membrane and chromatin, respectively (strain OD95). The AB cell is toward the top. Images were generated by time-lapse microscopy on a DeltaVision Personal DV deconvolution microscope and processed by constrained, iterative deconvolution using the softWoRx software package. Each frame is 20 s.



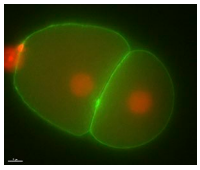
Video 2. **Mitosis in a *pch-2(tm1458)* mutant two-cell embryo.** A *pch-2(tm1458)* mutant embryo expressing GFP::PH and mCh::H2B for visualization of the plasma membrane and chromatin, respectively (strain BHL575). The AB cell is on the right. Images were generated by time-lapse microscopy on a DeltaVision Personal DV deconvolution microscope and processed by constrained, iterative deconvolution using the softWoRx software package. Each frame is 20 s.



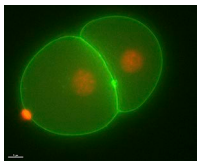
Video 3. **Mitosis in a two-cell embryo with monopolar spindles.** A *zyg-1*^{RNAi} embryo expressing GFP::PH and mCh::H2B for visualization of the plasma membrane and chromatin, respectively (strain OD95). The AB cell is on the bottom right. Note the delay in mitosis due to the spindle checkpoint response. Images were generated by time-lapse microscopy on a DeltaVision Personal DV deconvolution microscope (Applied Precision) and processed by constrained, iterative deconvolution using the softWoRx software package. Each frame is 20 s.



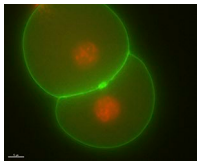
Video 4. **Mitosis in a *pch-2(tm1458)* mutant two-cell embryo with monopolar spindles.** A *pch-2(tm1458);zyg-1*^{RNAi} mutant embryo expressing GFP::PH and mCh::H2B for visualization of the plasma membrane and chromatin, respectively (strain BHL575). The AB cell is on the top. Note the absence of a mitotic delay as compared with Video 3. Images were generated by time-lapse microscopy on a DeltaVision Personal DV deconvolution microscope and processed by constrained, iterative deconvolution using the softWoRx software package. Each frame is 20 s.



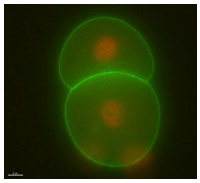
Video 5. **Mitosis in a *cmt-1(ok2879)* mutant two-cell embryo.** A *cmt-1(ok2879)* mutant embryo expressing GFP::PH and mCh::H2B for visualization of the plasma membrane and chromatin, respectively (strain BHL608). The AB cell is on the left. Images were generated by time-lapse microscopy on a DeltaVision Personal DV deconvolution microscope and processed by constrained, iterative deconvolution using the softWoRx software package. Each frame is 20 s.



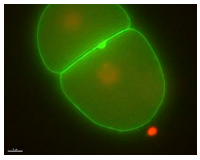
Video 6. **Delayed mitotic timing in a *cmt-1(ok2879)* mutant two-cell embryo with monopolar spindles.** A *cmt-1(ok2879);zyg-1*^{RNAi} mutant embryo expressing GFP::PH and mCh::H2B for visualization of the plasma membrane and chromatin, respectively (strain BHL608). The AB cell is on the left. Note the delay in mitosis due to the spindle checkpoint response. Images were generated by time-lapse microscopy on a DeltaVision Personal DV deconvolution microscope and processed by constrained, iterative deconvolution using the softWoRx software package. Each frame is 20 s.



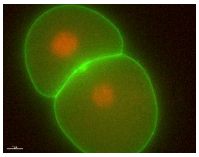
Video 7. **Normal mitotic timing in a *cmt-1(ok2879)* mutant two-cell embryo with monopolar spindles.** A *cmt-1(ok2879);zyg-1*^{RNAi} mutant embryo expressing GFP::PH and mCh::H2B for visualization of the plasma membrane and chromatin, respectively (strain BHL608). The AB cell is on the top left. Note the absence of a mitotic delay as compared with Video 6. Images were generated by time-lapse microscopy on a DeltaVision Personal DV deconvolution microscope and processed by constrained, iterative deconvolution using the softWoRx software package. Each frame is 20 s.



Video 8. **Mitosis in a *cmt-1(ok2879);pch-2(tm1458)* mutant two-cell embryo.** A *cmt-1(ok2879);pch-2(tm1458)* mutant embryo expressing GFP::PH and mCh::H2B for visualization of the plasma membrane and chromatin, respectively (strain BHL607). The AB cell is on the bottom. Images were generated by time-lapse microscopy on a DeltaVision Personal DV deconvolution microscope and processed by constrained, iterative deconvolution using the softWoRx software package. Each frame is 20 s.



Video 9. **Delayed mitotic timing in a *cmt-1(ok2879);pch-2(tm1458)* mutant two-cell embryo with monopolar spindles.** A *cmt-1(ok2879);pch-2(tm1458);zyg-1*^{RNAi} mutant embryo expressing GFP::PH and mCh::H2B for visualization of the plasma membrane and chromatin, respectively (strain BHL607). The AB cell is on the bottom right. Note the delay in mitosis due to the spindle checkpoint response. Images were generated by time-lapse microscopy on a DeltaVision Personal DV deconvolution microscope and processed by constrained, iterative deconvolution using the softWoRx software package. Each frame is 20 s.



Video 10. **Normal mitotic timing in a *cmt-1(ok2879);pch-2(tm1458)* mutant two-cell embryo with monopolar spindles.** A *cmt-1(ok2879);pch-2(tm1458);zyg-1*^{RNAi} mutant embryo expressing GFP::PH and mCh::H2B for visualization of the plasma membrane and chromatin, respectively (strain BHL607). The AB cell is on the bottom right. Note the absence of a mitotic delay compared with Video 9. Images were generated by time-lapse microscopy on a DeltaVision Personal DV deconvolution microscope and processed by constrained, iterative deconvolution using the softWoRx software package. Each frame is 20 s.

Table S1. ***C. elegans* strains used in this study**

Strain number	Genotype
N2 (ancestral)	
TH32	*unc-119(ed3) III; ruIs32* [*pAZ132; Ppie-1::GFP::histone H2B*] *III; ddIs6* [*Ppie-1::GFP::tbg-1; unc-119(+)]*
OD95	*unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV; ltIs38* [*pAA1; Ppie-1::GFP::PH(PLC1delta1); unc-119(+)*]
BHL16	*pch-2(tm1458)* II
BHL575	*pch-2(tm1458)* II; *unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV; ltIs38* [*pAA1; Ppie-1::GFP::PH(PLC1delta1); unc-119(+)*]
BHL596	*unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV; ltIs38* [*pAA1; Ppie-1::GFP::PH(PLC1delta1); unc-119(+)*]; *mdf-1(av19)* V
BHL600	*unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV; ltIs52* [*pOD379; Ppie-1::GFP::MDF-2; unc-119 (+)*]
BHL601	*cmt-1(ok2879)* I
BHL604	*pch-2(tm1458)* II*; unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV; ltIs52* [*pOD379; Ppie-1::GFP::MDF-2; unc-119 (+)*]
BHL607	*cmt-1(ok2879)* I*; pch-2(tm1458)* II; *unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV; ltIs38* [*pAA1; Ppie-1::GFP::PH(PLC1delta1); unc-119(+)*]
BHL608	*cmt-1(ok2879)* I*; unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV; ltIs38* [*pAA1; Ppie-1::GFP::PH(PLC1delta1); unc-119(+)*]
BHL622	*pch-2(tm1458)* II*; unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV; mdf-1(gk2) V; jzIs1* [*pRK139; PPpie-1::GFP::mdf-1; unc-119*(+)];
BHL623	*cmt-1(ok2879)* I; *pch-2(tm1458)* II
BHL626	*unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV; mdf-1(gk2) V; jzIs1* [*pRK139; Ppie-1::GFP::mdf-1; unc-119*(+)];
BHL629	*pch-2(tm1458) unc-4(e120) II*
BHL630	*zyg-1(b1) unc-4(e120) II*
BHL632	*cmt-1(ok2879)* I*; pch-2(tm1458)* II; *unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV; ltIs52* [*pOD379; Ppie-1::GFP::MDF-2; unc-119 (+)*]
BHL633	*cmt-1(ok2879)* I; *unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV; ltIs52* [*pOD379; Ppie-1::GFP::MDF-2; unc-119 (+)*]
BHL635	*cmt-1(ok2879)* I; *pch-2(tm1458)* II*; unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV; mdf-1(gk2) V; jzIs1* [*pRK139; Ppie-1::GFP::mdf-1; unc-119*(+)];
BHL636	*cmt-1(ok2879)* I*; unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV; mdf-1(gk2) V; jzIs1* [*pRK139; Ppie-1::GFP::mdf-1; unc-119*(+)];
BHL642	*unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV; mdf-1(av19)* V*; ltIs52* [*pOD379; Ppie-1::GFP::mdf-2; unc-119 (+)*]
BHL650	*unc-4(e120) II*
BHL658	*unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV;* ddIs68 [*bub-1::TY1::EGFP::3xFLAG*(92C12); *unc-119*(+)]
BHL660	*unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV;* ddIs153 [*knl-1::TY1::EGFP::3xFLAG*(92C12); *unc-119*(+)]
BHL663	*unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV;* ltIs73 [pOD377; *Ppie-1::GFP::bub-3; unc-119* (+)]
BHL664	*Ppch-2::pch-2::GFP-3FLAG(blt04, pCN94) II; unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV*
BHL667	*unc-4(e120) II; mdf-1(av19) V*
BHL669	*zyg-1(b1) unc-4(e120) II; mdf-1(av19) V*
BHL673	*cmt-1(ok2879) I; unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV;* ddIs153 [*knl-1::TY1::EGFP::3xFLAG*(92C12); *unc-119*(+)]
BHL674	*zyg-1(b1) pch-2(tm1458) unc-4(e120) II*
BHL675	*cmt-1(ok2879) I; unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV;* ltIs73 [pOD377; *Ppie-1::GFP::bub-3; unc-119* (+)]
BHL676	*pch-2(tm1458) II; unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV;* ddIs68 [*bub-1::TY1::EGFP::3xFLAG*(92C12); *unc-119*(+)]
BHL677	*cmt-1(ok2879) I; unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV;* ddIs68 [*bub-1::TY1::EGFP::3xFLAG*(92C12); *unc-119*(+)]
BHL679	*pch-2(tm1458) II; unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV;* ltIs73 [pOD377; *Ppie-1::GFP::bub-3; unc-119* (+)]
BHL682	*pch-2(tm1458) II; unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV;* ddIs153 [*knl-1::TY1::EGFP::3xFLAG*(92C12); *unc-119*(+)]
BHL685	*cmt-1(ok2879) I; Ppch-2::pch-2::GFP-3FLAG(blt04, pCN94) II; unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV*
BHL697	*Ppch-2::pch-2::GFP-3FLAG(blt04, pCN94) II; unc-119(ed3) III; ltIs37* [*pAA64; Ppie-1::mCherry::his-58; unc-119 (+)*] *IV; mdf-1(av19)* V