

Supplemental Materials

Molecular Biology of the Cell

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Figure S1: Related to Figure 1. The mitotic delay observed in *pch-2;ani-2^{RNAi};zyg-1^{RNAi}* small cells is a spindle checkpoint response.

Cartoon of control and *ani-2^{RNAi}* 2-cell embryos (A) and an *ani-2^{RNAi};zyg-1^{RNAi}* 2-cell embryo (B). Mitotic timing in AB cells plotted against cell volume, during unperturbed mitosis (C) and in the presence of monopolar spindles. (D). Lines represent least-squares regression models for each set of data. For (C) equations and p values indicating whether slopes are significantly non-zero for each model are: *ani-2^{RNAi}* (blue): $y = -9.744 \times 10^{-3}x + 3.001$ and $p = 0.6073$; *pch-2;ani-2^{RNAi}* (red): $y = -0.1315x + 3.503$ and $p = 0.0483$; *mad-1;ani-2^{RNAi}* (green): $y = -0.2944x + 4.247$ and $p = 0.0251$. In (D), 95% confidence intervals are indicated by gray shaded areas and equations and p values indicating whether slopes are significantly non-zero for each model are: *san-1;ani-2^{RNAi};zyg-1^{RNAi}* P₁ (blue): $y = -0.4136x + 5.535$ and $p = 0.1885$; *san-1;pch-2;ani-2^{RNAi};zyg-1^{RNAi}* P₁ (red): $y = -0.3541x + 5.647$ and $p = 0.0643$. The regression model of *pch-2;ani-2^{RNAi};zyg-1^{RNAi}* embryos from Figure 1C is indicated by the opaque red line in (D) for comparison.

Figure S2: Related to Figure 2: There is no difference in GFP::MAD-2 fluorescence around mitotic chromosomes in *ani-2^{RNAi};zyg-1^{RNAi}* embryos and *pch-2;ani-2^{RNAi};zyg-1^{RNAi}* AB cells.

(A) Images of MAD-2::GFP in AB cells of control *ani-2^{RNAi};zyg-1^{RNAi}* embryos or *pch-2;ani-2^{RNAi};zyg-1^{RNAi}* embryos after NEBD. Scale bar indicates 5 μm . (B) Quantification of GFP::MAD-2 fluorescence around mitotic chromosomes in AB cells of control and *pch-2* embryos plotted against cell volume.

Figure S3: Related to Figure 3. Nuclear volume does not scale with cell volume in *ani-2^{RNAi}* 2-cell embryos.

(A) Images of a large (top) and small (bottom) AB cell of *ani-2^{RNAi}* embryos. The nuclear area is indicated with a dashed yellow line. Scale bar indicates 5 μ m. (B) Nuclear area plotted against cell volume.

Figure S4: Related to Figure 5. PCH-2 is required for the spindle checkpoint in the germline lineage.

(A) Mitotic timing of control and *pch-2* mutant embryos during unperturbed divisions or in the presence of monopolar spindles. Data for control embryos is the same as Figure 7B. Error bars indicate 95% confidence intervals.

Figure S5: Related to Figure 6. There is no difference in the amount of PCH-2::GFP or GFP::MAD-2 recruited to unattached kinetochores in AB and P₁ cells.

(A) Cartoon and images of PCH-2::GFP recruitment to unattached kinetochores in AB and P₁ cells of 2-cell embryos. Scale bar indicates 1 μ m. (B) Quantification of PCH-2::GFP recruitment at unattached kinetochores in AB and P₁ cells. (C) Cartoon and images of GFP::MAD-2 recruitment to unattached kinetochores in AB and P₁ cells of 2-cell embryos. Scale bar indicates 1 μ m. (D) Quantification of GFP::MAD-2 fluorescence at unattached kinetochores in AB and P₁ cells. All error bars are 95% confidence intervals. NS indicates not significant.

Figure S6: Related to Figure 6. There is no difference in the amount of PCH-2::GFP around mitotic chromosomes in AB cells with bipolar or monopolar spindles. (A) Cartoon

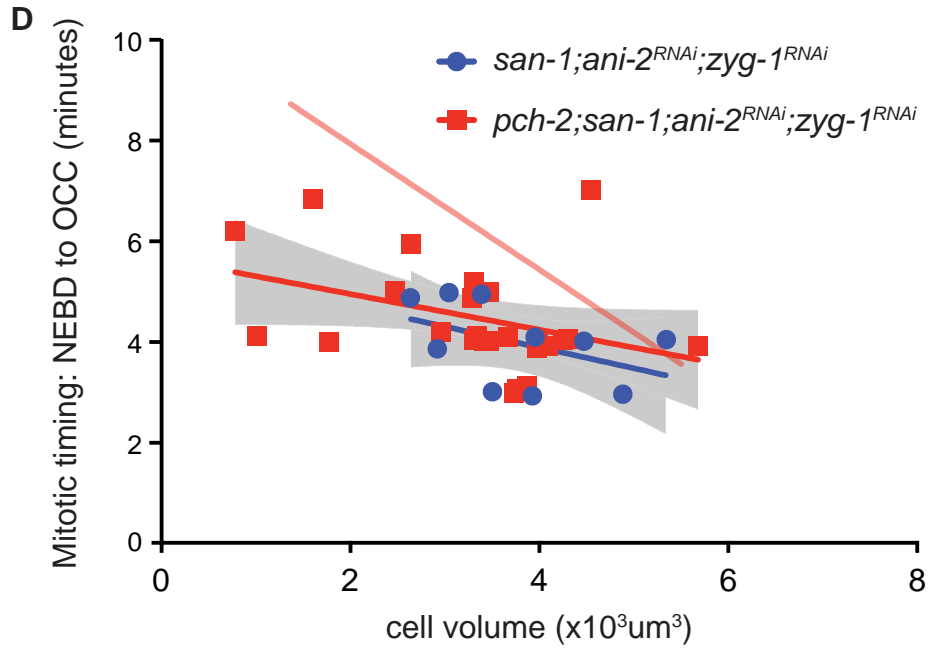
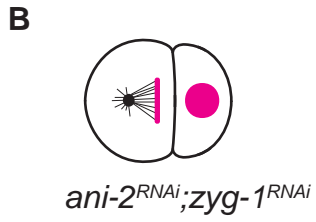
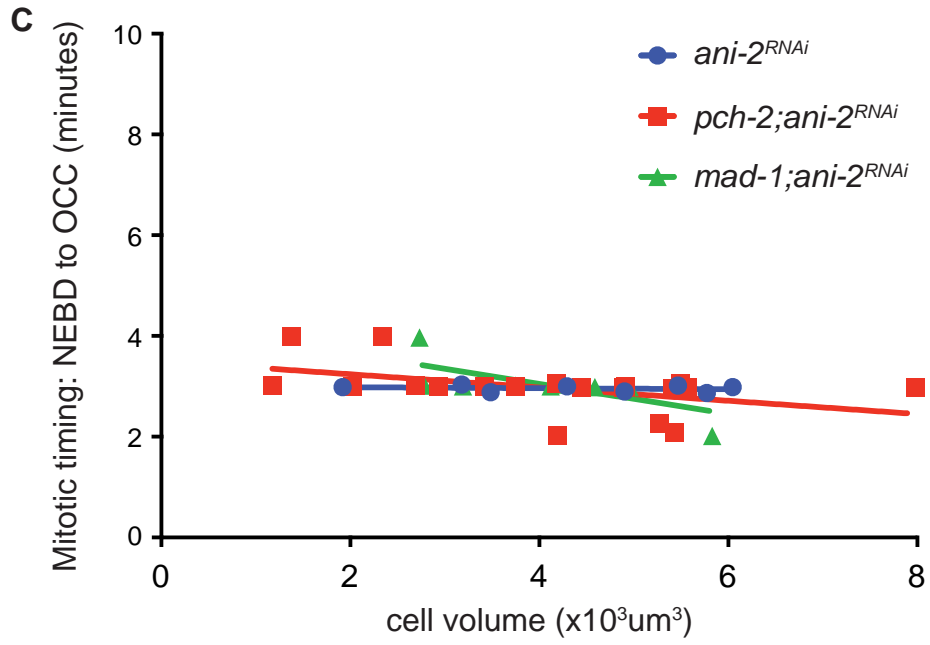
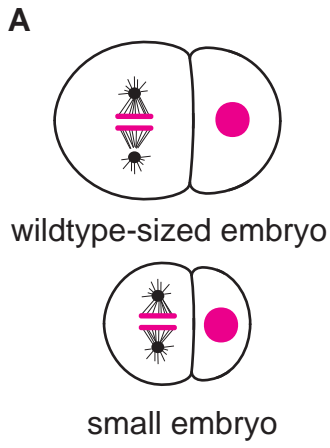
and images of PCH-2::GFP localization around mitotic chromosomes in AB cells of 2-cell embryos. Scale bar indicates 5 μ m. Yellow dashed circle indicates area of PCH-2::GFP fluorescence. (B) Quantification of PCH-2::GFP fluorescence in AB cells with bipolar spindles (control) or monopolar spindles (*zyg-1^{RNAi}*). (C) Quantification of area of PCH-2::GFP fluorescence in AB cells with bipolar spindles or monopolar spindles. (D) Quantification of

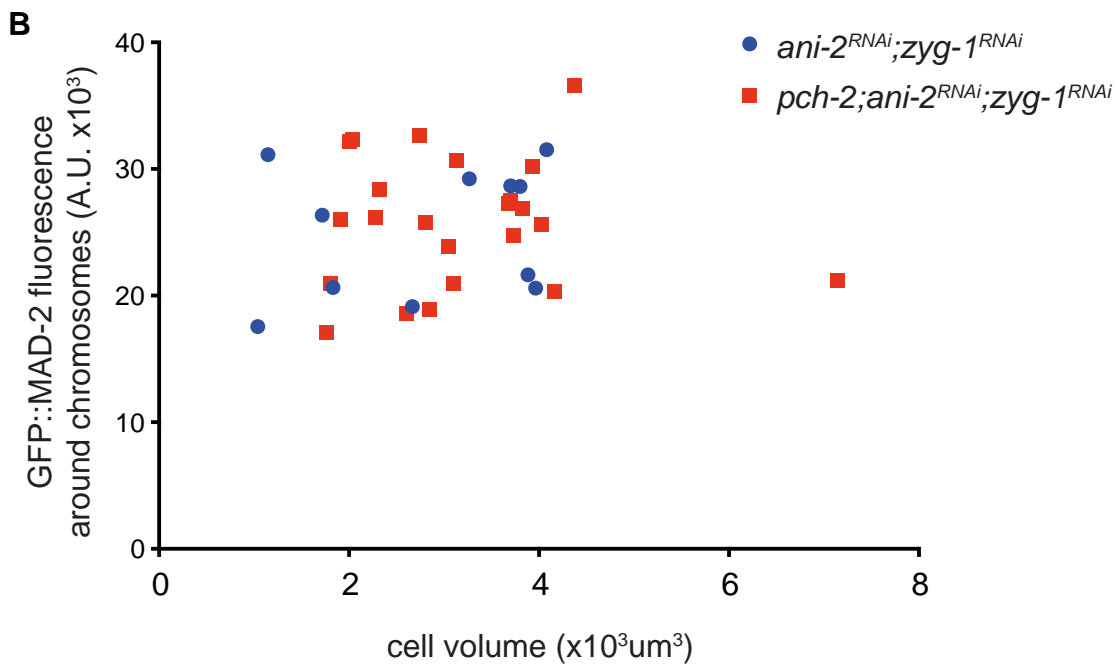
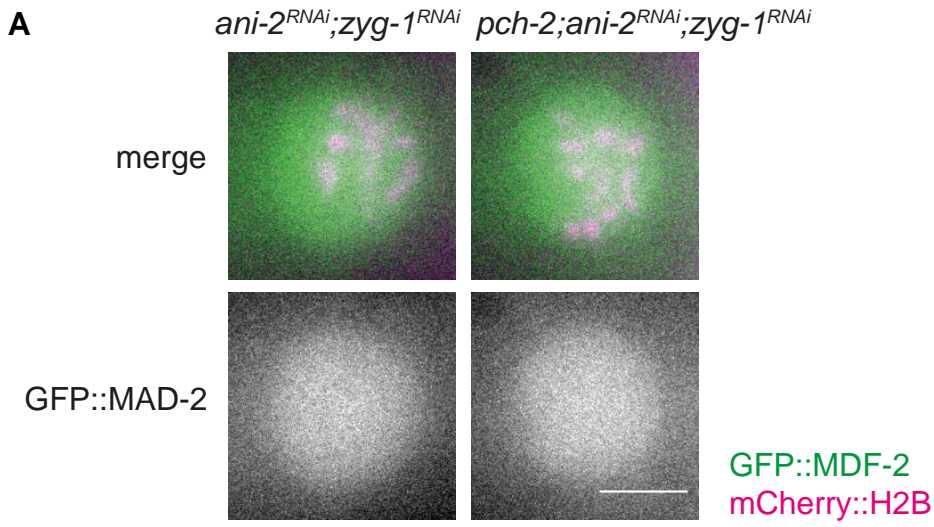
integrated density of PCH-2::GFP fluorescence in AB cells with bipolar spindles or monopolar spindles. All error bars are 95% confidence intervals. NS indicates not significant.

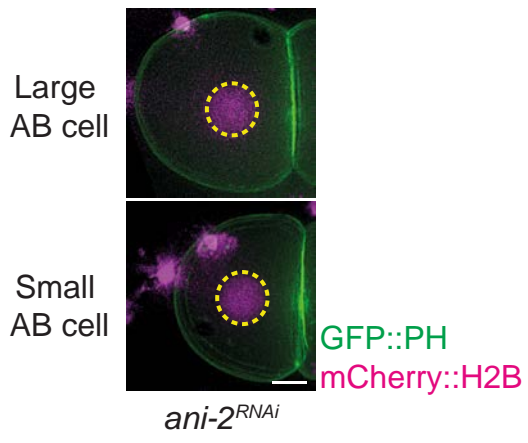
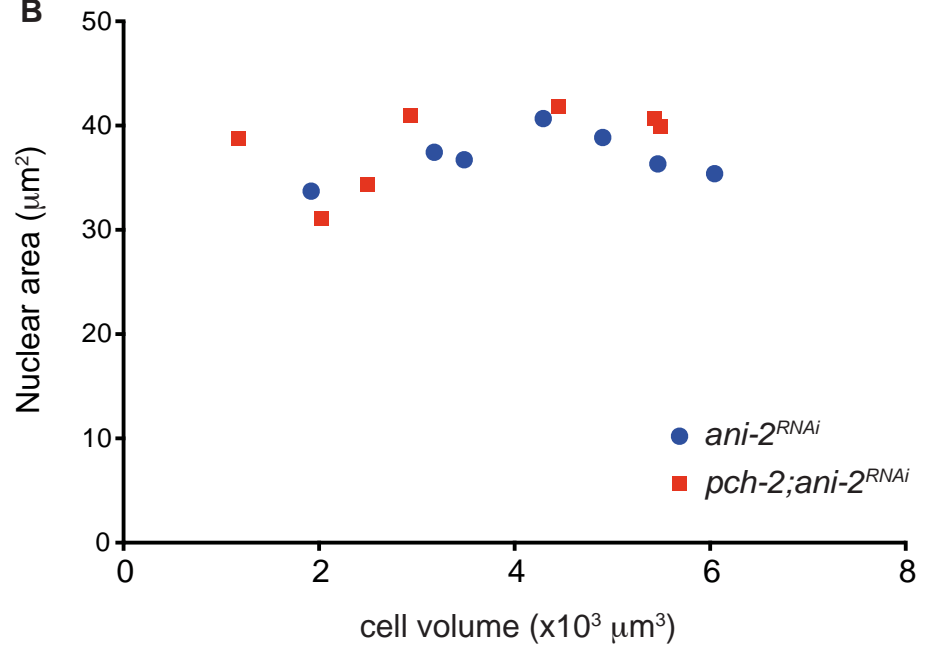
Figure S7: Related to Figure 6. PCH-2's enrichment in P₁ cells is around chromosomes and does not depend on GPR-1/2.

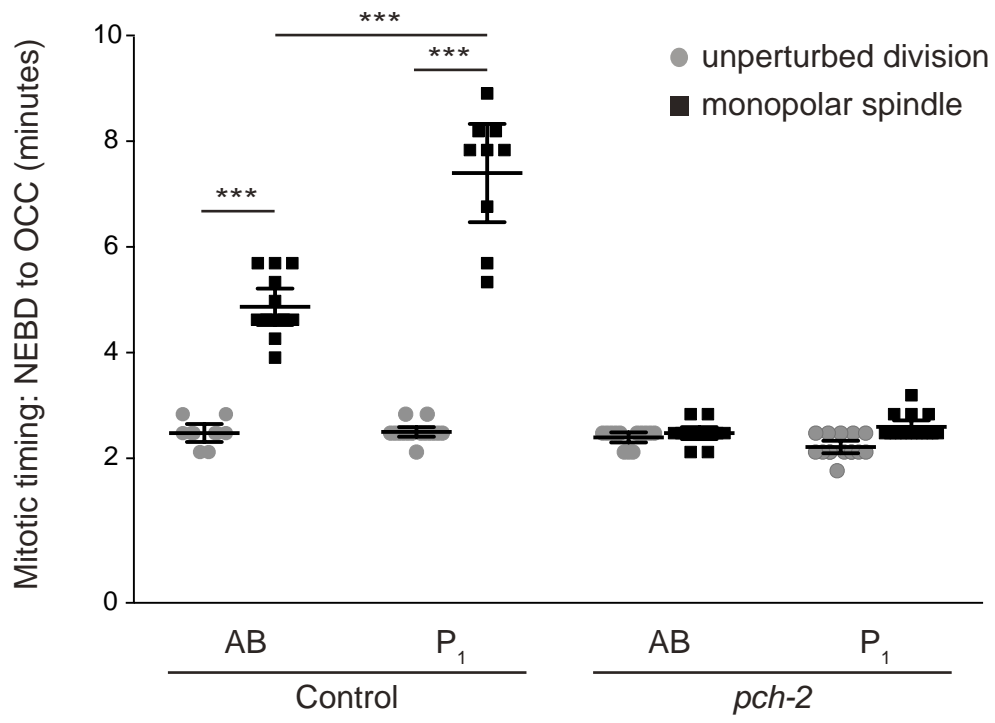
(A) Quantification of PCH-2::GFP fluorescence in the cytoplasm of AB and P₁ cells. (B) Images of AB (left) and P₁ (right) cells after NEBD. Scale bar indicate 5 μm. (C) Quantification of cell area in AB and P₁ cells of control RNAi and *gpr-1/2*^{RNAi} 2-cell embryos. Red symbols indicate cells in which PCH-2::GFP fluorescence was quantified in (D). (D) Quantification of PCH-2::GFP fluorescence in AB and P₁ cells of control RNAi and *gpr-1/2*^{RNAi} embryos. (E) Cartoon and images of PCH-2::GFP localization around mitotic chromosomes in AB and P₁ cells of control RNAi and *gpr-1/2*^{RNAi} 2-cell embryos. Scale bars indicate 5 μm. Error bars are 95% confidence intervals. NS indicates not significant.

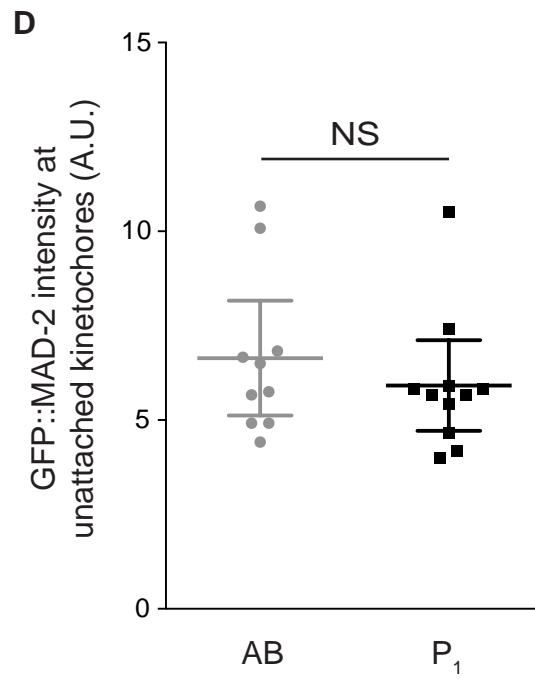
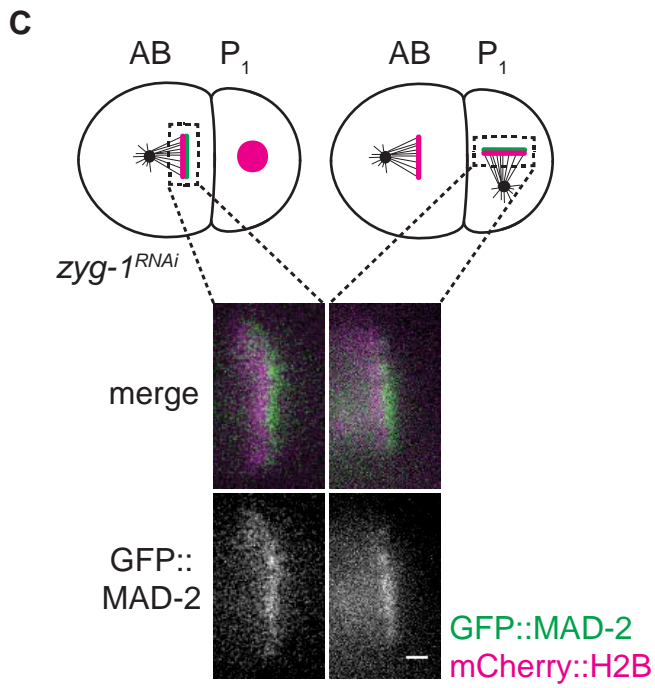
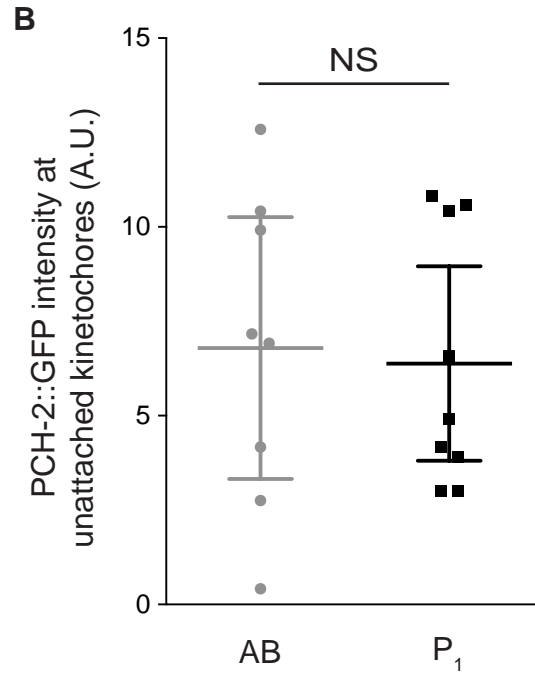
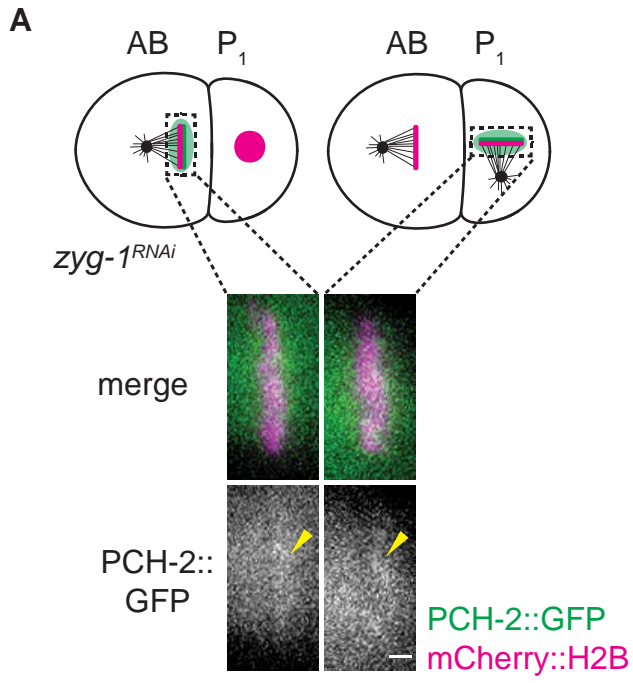
Figure S8: Related to Figure 6. PCH-2's enrichment around mitotic chromosomes in P₁ cells depends on PAR-6. (A and B) Quantification of cell area in AB and P₁ cells of control RNAi, *par-1*^{RNAi} and *par-6*^{RNAi} 2-cell embryos. (C) Cartoon and images of PCH-2::GFP localization around mitotic chromosomes in AB and P₁ cells of control RNAi and *par-6*^{RNAi} 2-cell embryos. Scale bars indicate 5 μm. (D) Quantification of PCH-2::GFP fluorescence in AB and P₁ cells of control RNAi and *par-6*^{RNAi} embryos. Error bars are 95% confidence intervals. NS indicates not significant.

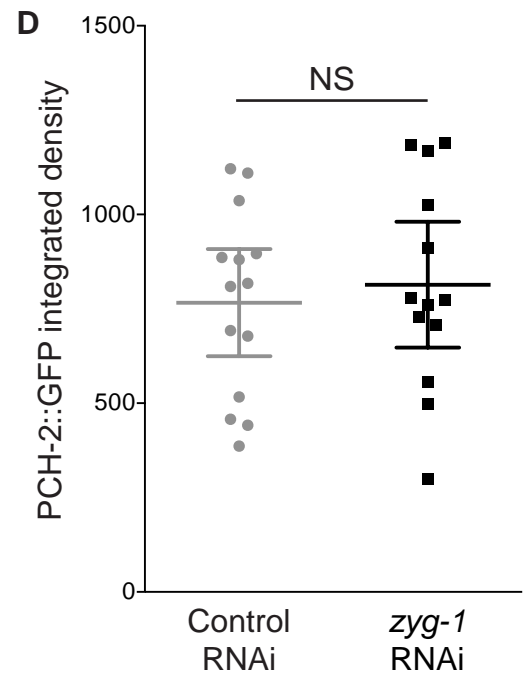
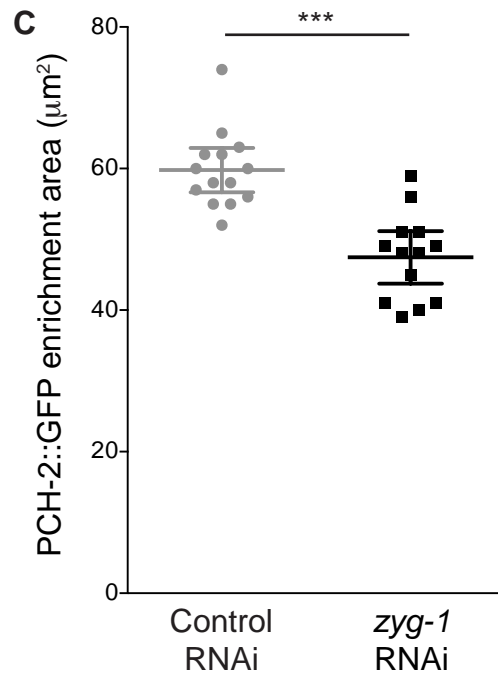
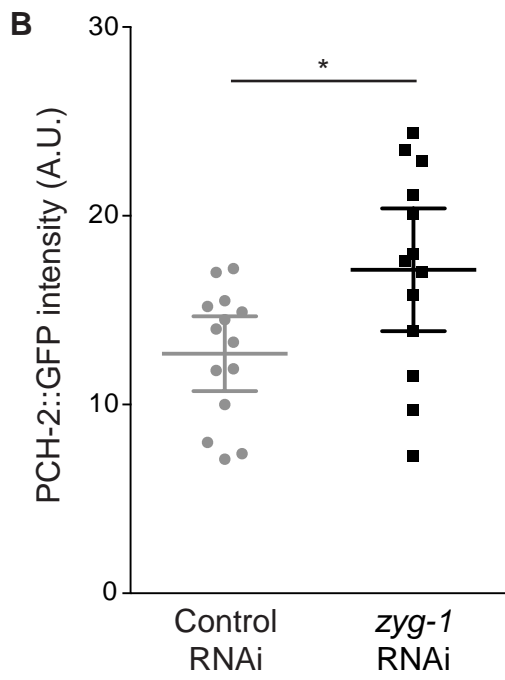
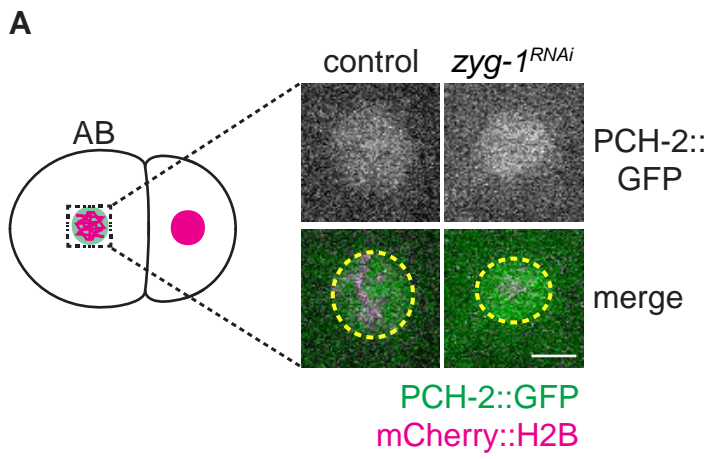


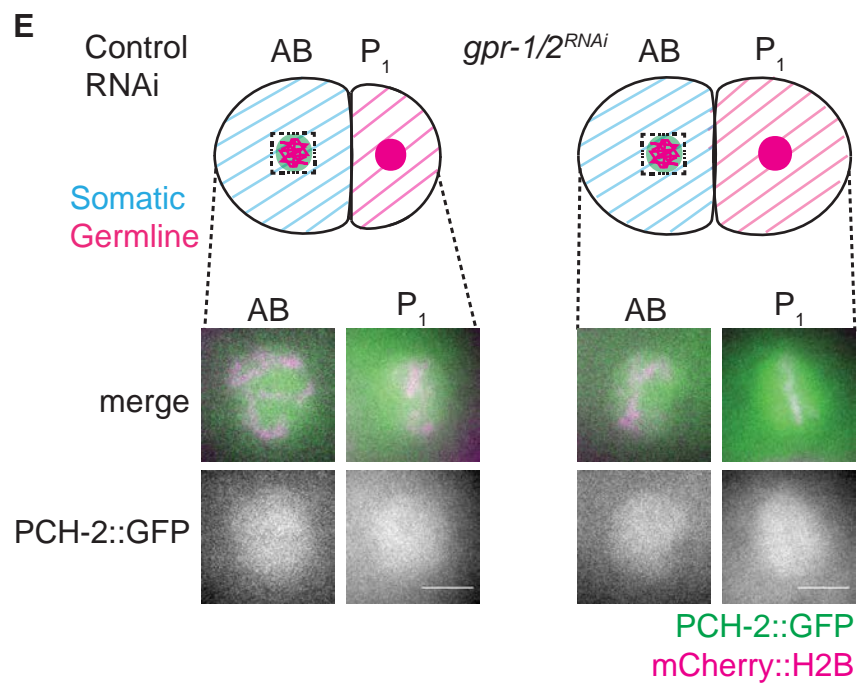
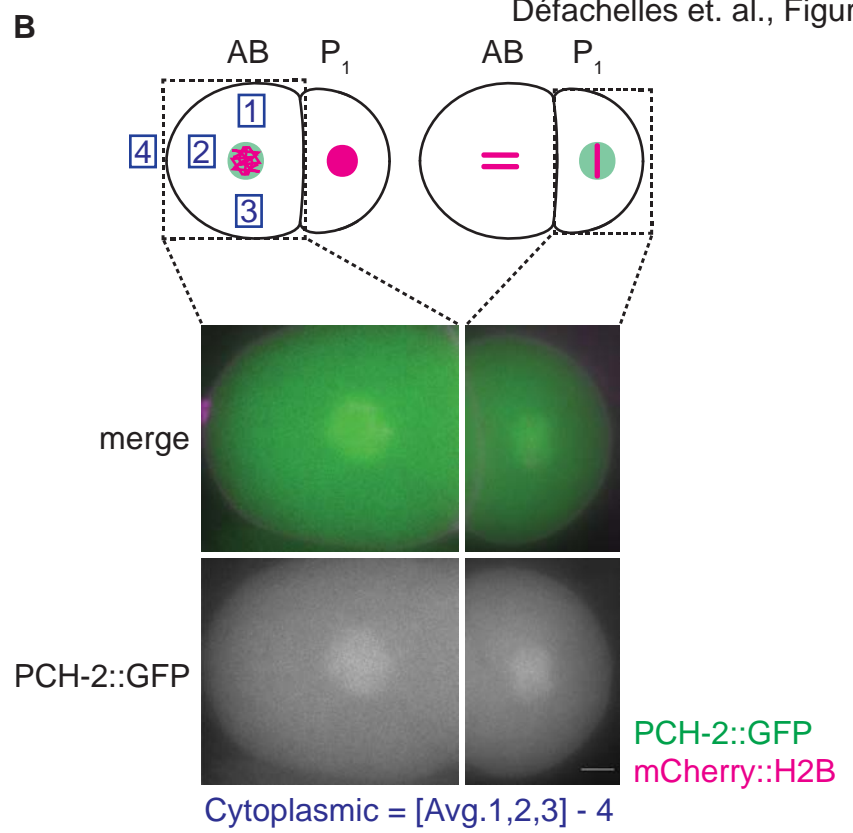
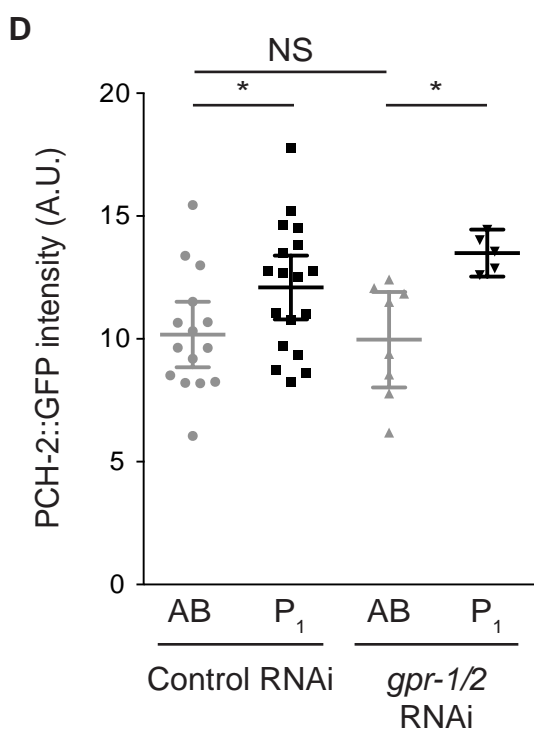
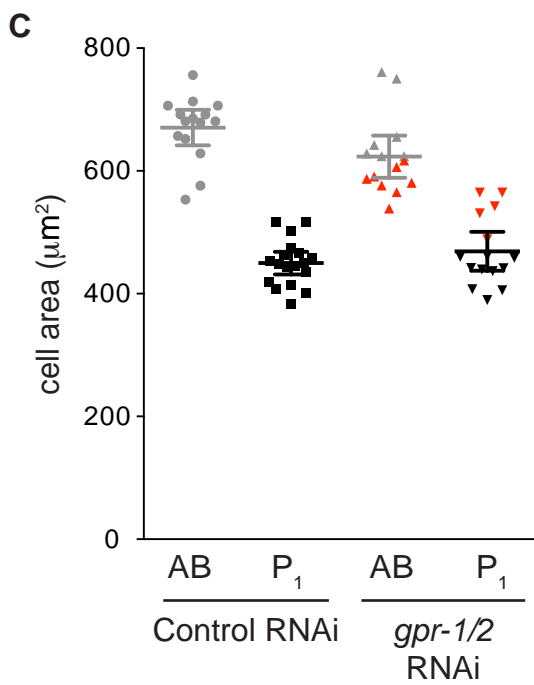
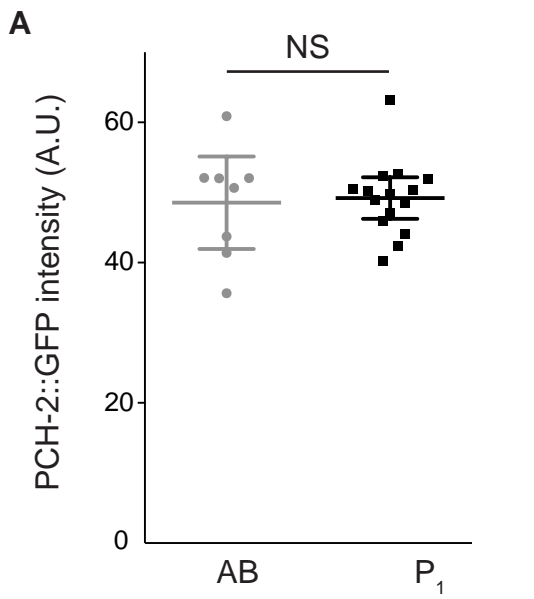


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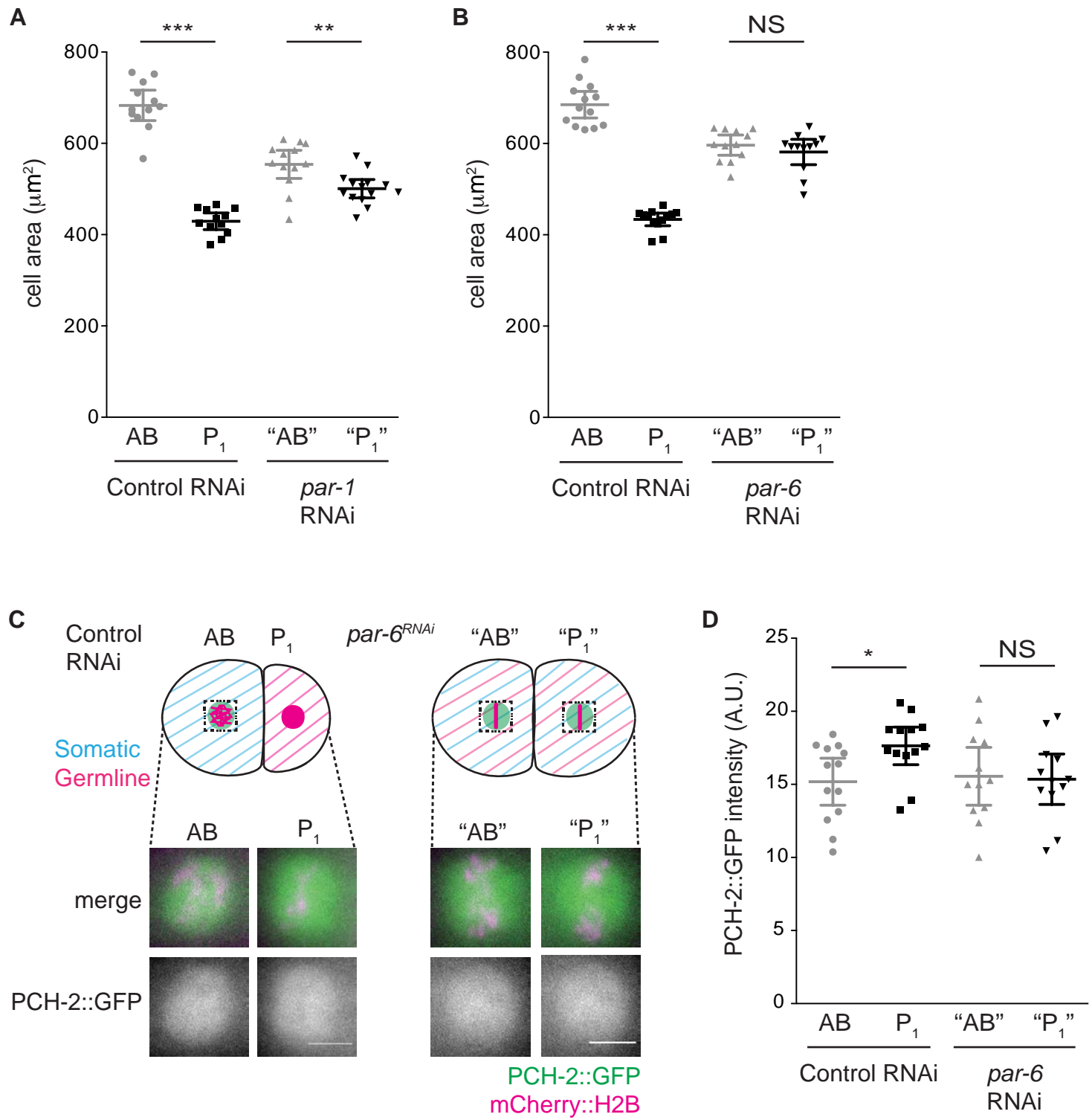


Table S1: Strains used in this study

Strain	Genotype
OD56	<i>unc-119(ed3) III; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV</i>
OD95	<i>unc-119(ed3) III; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV; Itls38 [pAA1; Ppie-1::GFP::PH(PLC1delta1); unc-119(+)]</i>
BHL575	<i>pch-2(tm1458) II; unc-119(ed3) III; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV; Itls38 [pAA1; Ppie-1::GFP::PH(PLC1delta1); unc-119(+)]</i>
BHL596	<i>unc-119(ed3) III; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV; Itls38 [pAA1; Ppie-1::GFP::PH(PLC1delta1); unc-119(+)]; mdf-1(av19) V</i>
BHL600	<i>unc-119(ed3) III; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV; Itls52 [pOD379; Ppie-1::GFP::MDF-2; unc-119 (+)]</i>
BHL604	<i>pch-2(tm1458) II; unc-119(ed3) III; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV; Itls52 [pOD379; Ppie-1::GFP::MDF-2; unc-119 (+)]</i>
BHL608	<i>cmt-1(ok2879) I; unc-119(ed3) III; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV; Itls38 [pAA1; Ppie-1::GFP::PH(PLC1delta1); unc-119(+)]</i>
BHL664	<i>Ppch-2::pch-2::GFP-3XFLAG (blt4 [pCN94]) II; unc-119(ed3) III; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV</i>
BHL666	<i>unc-119(ed3) III; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)]; mdf-2(tm2190) IV/nT1[qIs51](IV;V) Itls38 [pAA1; Ppie-1::GFP::PH(PLC1delta1); unc-119(+)]</i>
BHL883	<i>san-1/mdf-3 (ok1580) I; pch-2(tm1458) II; unc-119(ed3) III; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV; Itls38 [pAA1; Ppie-1::GFP::PH(PLC1delta1); unc-119(+)]</i>
BHL887	<i>cmt-1(ok2879) I; pch-2::GFP-3XFLAG (blt4 [pCN94]) II; unc-119(ed3) III; Itls44[pAA173; pPie-1::mCherry::PH(PLC1delta1); unc-119(+)]; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV</i>
BHL888	<i>unc-119(ed3) III; Itls44 [pAA173; pPie-1::mCherry::PH(PLC1delta1); unc-119(+)]; Itls52 [pOD379; Ppie-1::GFP::mdf-2; unc-119 (+)]; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV</i>
BHL889	<i>pch-2(tm1458) II; unc-119(ed3) III; Itls44 [pAA173; pPie-1::mCherry::PH(PLC1delta1); unc-119(+)]; Itls52 [pOD379; Ppie-1::GFP::mdf-2; unc-119 (+)]; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV</i>
BHL891	<i>unc-119(ed3) III; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV; Itls24[pAZ132; pPie-1::GFP::tba-2; unc-119(+)]</i>
BHL892	<i>pch-2 (tm1458) II; unc-119(ed3) III; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV; Itls24 [pAZ132; pPie-1::GFP::tba-2; unc-119(+)]</i>
BHL893	<i>san-1/mdf-3 (ok1580) I; unc-119(ed3) III; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV; Itls38 [pAA1; Ppie-1::GFP::PH(PLC1delta1); unc-119(+)]</i>
BHL904	<i>Ppch-2::pch-2::GFP-3XFLAG (blt4 [pCN94]) II; unc-119(ed3) III; Itls44[pAA173; pPie-1::mCherry::PH(PLC1delta1); unc-119(+)]; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV</i>
BHL950	<i>pch-2(tm1458) II; unc-119(ed3) III; Itls37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)]; mdf-2(tm2190) IV/nT1[qIs51](IV;V); Itls38 [pAA1; Ppie-1::GFP::PH(PLC1delta1); unc-119(+)]</i>

BHL958	<i>unc-119(ed3) III; ltIs37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV/nT1[qIs51](IV;V); ltIs38 [pAA1; Ppie-1::GFP::PH(PLC1delta1); unc-119(+)]</i>
BHL964	<i>zyg-1(or297) unc-4(e120) II unc-119(ed3) III; ltIs37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV; ltIs24[pAZ132; pPie-1::GFP::tba-2; unc-119(+)]</i>
BHL965	<i>zyg-1(or297) pch-2(tm1458) unc-4(e120) II; unc-119(ed3) III; ltIs37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV; ltIs24[pAZ132; pPie-1::GFP::tba-2; unc-119(+)]</i>
GAL5	<i>san-1/mdf-3 (mat5) I; unc-119(ed3) III; ruls57[Ppie-1::GFP::tubulin + unc-119(+)]; ltIs37[pie-1p::mCherry::his-58 (pAA64) + unc-119(+)] IV</i>