Supplementary Materials (including Sup. Figures, Movies, Methods, References and Table)

Legends for Supplementary Figures

Sup. Fig. 1: P granule dynamics.

A) Average number of P granules from 3 zygotes filmed from meiotic exit (pronuclear formation, PF), interphase (pronuclear migration, PM), and mitosis (M). Green color in each bar indicates portion of GFP::PGL-1 granules with diameters larger than 0.52μm (numbers show percentage). P granule sizes and numbers are intermediate *in par-1(RNAi);mex-5/6(RNAi)* zygotes compared to *par-1(RNAi)* and *mex-5/6(RNAi)* zygotes, suggesting that PAR-1 promotes P granule assembly both by antagonizing MEX-5 and MEX-6, and by another mechanism.

B) Time-lapse confocal images of the anterior cytoplasm of a zygote expressing PGL-1::GFP and mCherry::MEX-5. Arrows point to two examples of PGL-1::GFP granules that associate with mCherry::MEX-5 before disassembly, leaving mCherry::MEX-5 behind. See movie 4.

Sup Fig 2. Dynamics of P granule components.

In all experiments, GFP or photoconverted Dendra fluorescence was measured in the anterior and posterior halves of the zygote over time. Error bars are standard deviation of mean values obtained from 3 embryos. Blue lines indicate

the first time point at which P granules are restricted to the posterior, and green lines indicate cytokinesis.

A, B, and C) Time zero is pronuclear meeting. Like GFP::PGL-1 (Fig. 1I), GFP::PGL-3 and GFP::GLH-1 levels remain equal in the anterior and posterior during interphase, even though visible granules disappear from the anterior during this time. GFP::PGL-3 and GFP::GLH-1 levels decrease significantly in the anterior only during mitosis (prophase begins shortly before pronuclear meeting). By contrast, GFP::PIE-1 begins to segregate during interphase.

D) Time zero is pronuclear meeting. Dendra::PGL-1 was photoconverted throughout the zygote approximately 10-15 seconds before the first time point. Anterior levels increase and posterior levels decreases during mitosis (with no change in total levels), indicating that GFP::PGL-1 relocalizes from anterior to posterior.

E-H) Dendra::PGL-1 was photoconverted in the region indicated. Time zero is first time point 10-15 seconds after photoconversion. At this time, some photoactivated Dendra::PGL-1 has already diffused into the non-photoconverted half of the embryo. Value in photoconverted half at first time point was set to one and all other values were scaled accordingly. Note that in posterior photoconversion experiments, Dendra::PGL-1 levels remain higher in the posterior throughout the experiment. In anterior photoconversion experiments, Dendra::PGL-1 redistributes from anterior to posterior, and redistribution is faster during mitosis.

Sup. Fig. 3: P granule components are segregated symmetrically in *pptr-1* mutants.

A) Time-lapse confocal images from Movie 5 of 2-to-8 cell wild-type and *pptr-1* embryos expressing GFP::PGL-1 (green) and mCherry:Histone H2B (red). The P blastomere is outlined. GFP::PGL-1 granules are present in interphase/prophase and disperse during metaphase/anaphase. Granules are absent in AB (anterior blastomere in first panel) due to high MEX-5/6 levels, and re-appear in the AB daughters (last two panels) in a short period after sufficient MEX-5/6 has turned over and before the cells enter mitosis.

B) Confocal images (maximum projection of confocal Z-stacks spanning 8µm) of 100-cell embryos expressing GFP::PGL-1. At this stage GFP::PGL-1 is restricted to the primordial germ cells Z2 and Z3 in wild-type embryos, but is present in more cells in *pptr-1* embryos. *lgg-1(RNAi)* blocks PGL-1 degradation in somatic cells. Note that GFP::PGL-1 still accumulates preferentially in Z2 and Z3 in *lgg-1(RNAi)* embryos (arrow), but is evenly distributed in all cells in *pptr-1; lgg-1(RNAi)* embryos.

C) Quantification of *nos-2* and *cey-2 in situ* hybridization data. All embryos were scored in the 8-16 cell stage. At this stage, in wild-type embryos, *cey-2* and *nos-2* RNA are detected in 2 cells (P_3 and C, with stronger signal in P_3, due to asymmetric segregation during division) or in just 1 cell (P_3, due to rapid RNA degradation in C). In contrast, in ~60% of *pptr-1* embryos, *nos-2* and *cey-2* RNAs were detected at equal levels in both P_3 and C (green color in graph), a pattern never observed in wild-type. In the remaining embryos, levels were lower or

undetectable in C due to rapid RNA degradation in that blastomere as in wild-type.

Sup. Fig. 4: P granule components are segregated symmetrically in *pptr-1* mutants.

Fixed wild-type and *pptr-1* embryos stained with DAPI (blue) and antibodies (red or green) against core P granule proteins as indicated. All images are maximum projections of confocal Z-stacks (spanning entire embryo), except for GLH-1 stainings in P_0, which show a single focal plane. Note the absence of bright asymmetric granules during mitosis. For some antibodies, it is difficult to distinguish diffuse cytoplasmic staining from background.

Sup Fig 5: *pptr-1* does not affect other soma-germline asymmetries in embryos.

A**)** Embryos expressing the indicated fusions or stained with the indicated antibodies. Patterns are identical in wild-type and *pptr-1* embryos.

B) GFP::PGL-1 dynamics in *pptr-1;mex-5/6* embryos. MEX-5/6 are required for P granule disassembly in the anterior during interphase in *pptr-1* embryos, but are not required for P granule disassembly during mitosis.

C) Z2 and Z3 descendants in L1 larvae derived from wild-type or *pptr-1* mothers express a paternally-inherited GFP::PGL-1 transgene. L1 was the earliest stage at which we could detect zygotic expression of the GFP::PGL-1 transgene;

zygotic expression of endogenous PGL-1, however, can be detected as early as the comma-stage (see PGL-1 panel in Fig. 3 and (*1*)).

Sup Fig 6: *pptr-1* is required maternally for GFP::PGL-1 enrichment in germline blastomeres.

Wild-type (control) or *pptr-1* hermaphrodites expressing GFP::PGL-1 were crossed with males homozygous for the *pes-10:GFP* transgene, which is transcribed transiently in each somatic lineage (*2*). Cross-progeny embryos (> 100 cell and younger) were identified by *pes-10:GFP* expression (zygotic expression only) and scored for GFP::PGL-1 (maternal expression only at this stage). All cross progeny from wild-type mothers (M2Z2) had normal P granules, whereas all cross progeny from *pptr-1* mothers (M0Z1) showed the *pptr-1* phenotype (no P granule enrichment). The latter were allowed to self-fertilize; all self progeny (M1Z0, M1Z1, M1Z2) had normal P granules demonstrating that the requirement for *pptr-1* with respect to P granule partitioning in embryos is strictly maternal.

Sup Fig 7. *pptr-1* is required zygotically for maximal brood size.

A) Graph comparing average brood size for mothers (n) with different maternal and zygotic *pptr-1* copy numbers (MZ nomenclature as in Fig. 3 and Sup. Fig. 6). Numbers are number of mothers examined for each genotype, aggregated from at least 2 independent experiments. Error bars are standard error of the mean.

At 24°C, *pptr-1* hermaphrodites (M0Z0) have a small brood size compared to wild-type (M2Z2). This defect is partially rescued by introducing a zygotic copy of *pptr-1* (M0Z1), but not by introducing a maternal copy (M1Z0). Brood size increases with increased zygotic copy (compare M1Z0, M1Z1 and M1Z2), suggesting that *pptr-1* is required zygotically for maximal brood size and is haploinsufficient.

B) Graph comparing average brood size for mothers (n) of the indicated genotypes. Error bars are standard error of the mean. p values were obtained from an unpaired T-test. RNAi in wild-type, *ppw-1(pk1425)*, and *rrf-1(pk1417)* hermaphrodites depletes *pptr-1* from both soma and germline, soma only, and germline only, respectively (*3, 4*).

N2 (wild-type), *rrf-1(pk1417)*, or *ppw-1(pk1425)* L4 hermaphrodites ("P0" generation) were soaked in *pptr-1* dsRNA (Methods), recovered on *pptr-1(RNAi)* plates for 24 hours, and allowed to lay eggs (F1) for 2 hours onto fresh *pptr-1(RNAi)* plates. Gravid P0 mothers were squashed and stained to visualize P granules in F1 embryos (photomicropgraphs below graph). F1 L4 stage hermaphrodites were transferred to fresh *pptr-1(RNAi)* plates for 30 hours and passaged to a second plate to lay remainder of their brood. Total broods were determined by counting number of L4 larvae on both plates (F2s). Note that depletion of *pptr-1* in the soma (*ppw-1* background) does not affect P granule partitioning during embryogenesis but these embryos grow up to have reduced brood size. In contrast, depletion of *pptr-1* in the germline (*rrf-1* background) disrupts embryonic partitioning of P granules, but these embryos grow up to have

a normal brood size. *rrf-1* mutants are sterile are high temperatures (*5*) so we could not determine whether depletion of *pptr-1* in the germline also leads to low penetrance sterility at high temperatures as expected.

Legends for Supplementary Movies

All movies oriented anterior to the left and posterior to the right. Movies were acquired with a Zeiss Axio Imager fitted with a Yokogawa spinning-disc confocal scanner (63x 1.4NA plan apochromat lens). Slices were taken every 1μm for a total of 8μm for time-lapse images. For Movies 1, 4, and 5, images were captured every 8s and movies are shown at 15 frames/sec. For Movies 2 and 3, images were captured every 2 min and movies are shown at 6 frames/sec.

Movie 1. Wild-type Embryo expressing PGL-1::GFP (JH2330, Figure 2C)

Movie 2. Wild-type embryo expressing GFP::PGL-3 (JH2017)

Movie 3. Wild-type embryo expressing GFP::GLH-1 (JH2172)

Movie 4. Wild-type embryo expressing PGL-1::GFP and mCherry::MEX-5 (JH2840, Sup. Figure 1)

Movie 5. Combined movie of wild-type and *pptr-1* embryos expressing GFP::PGL-1 and mCherry::H2B (JH2842 and JH2843, respectively; Sup. Figure 3A)

Methods

Nematode Strains

C. elegans strains (Table S1) were derived from the wild-type Bristol N2 and reared with standard procedures (*6*). All experiments, including movies, were performed at 24^{o}C, unless otherwise indicated. The P granule partitioning defect of *pptr-1* mutants is fully penetrant at 20^{o}C and 24^{o}C, but the low penetrance sterility phenotype is only seen at 24^{o}C and 26^{o}C. *pptr-1* was identified as follows: 231 candidate miRNA pathway genes (*7*) were screened by RNAi for disruption of GFP::PGL-1 expression in embryos. This primary screen identified the PP2A catalytic subunit *let-92.* We then screened by RNAi the 14 PP2A regulatory subunits predicted by WormBase; only *pptr-1(RNAi)* disrupted GFP::PGL-1 distribution. We obtained a *pptr-1* deletion allele (*tm3103*) from the National BioResource Project for the Experimental Animal, Japan. *tm3103* was outcrossed 6 times before use in all the experiments described here. JH2842 and JH2483 were made by crossing JH2108 and JH2841, respectively, with OD57 (*8*).

Transgenics

Gateway cloning (Invitrogen) (*9*) was used to generate all constructs (Table S1). Transgenes were introduced into worms by microparticle bombardment (*10*) . Dendra DNA sequence (*11*) was recoded to conform to *C. elegans* codon usage

(*12*) and to include three synthetic introns (*13*) (Genscript, NJ, USA; Sequence is available upon request).

RNAi-mediated Knockdown

RNAi was performed by feeding (except in Fig. S7 see below). HT115 bacteria transformed with feeding vectors were grown at 37º C in LB + ampicillin (100µg/mL), plated on NNGM (nematode nutritional growth media) + ampicillin (100µg/mL) + IPTG (1mM), and grown overnight at room temperature before adding L4 worms at 25º C for 22-28 hours. For *mex-5/6(RNAi)*, the second exon of *mex-5* and *mex-6* were cloned separately into the gateway feeding vector pCD1.01, and bacteria expressing each were mixed before plating. The *pptr-1(RNAi)* and *par-1(RNAi)* constructs were obtained from the Ahringer RNAi library (*14*). To make *pptr-1* dsRNA, 465 bp of *pptr-1* cDNA was amplified using primers containing T7 promoter sequences and corresponding to exons 1, 2, and 3 of pptr-1. dsRNA was then created using AmpliScribe T7-Flash Transcription Kit (Epicentre) and purified using RNeasy kit (Qiagen).

For Fig. S7, P0 generation mothers were soaked in 0.4 µg/µl of *pptr-1* dsRNA and 5x soaking buffer (*15*) for 22 hours before plating on feeding *pptr-1(RNAi)* prepared as described above.

Live Imaging and Spinning-Disc Confocal Microscopy

Embryos were dissected from gravid adults into egg salts (118 mM NaCl, 10mM Hepes pH 7.5, 2mM $CaCl_2$, 48mM KCl, 2mM $MgCl_2$) and placed on a 3%

agarose pad. Time-lapse movies were acquired using a Zeiss Axio Imager fitted with a Yokogawa spinning-disc confocal scanner (63x 1.4NA plan apochromat lens). Slices were taken every 1µm for a total of 8µm for time-lapse images. Dendra photoconversions were performed using a 120W mercury vapor short arc lamp and an exposure of 2.4 seconds with an excitation of 357nm. Local photoconversions were performed by closing down the field diaphragm of the Zeiss Axio Imager and an exposure time of 4.8 seconds with an excitation of 357nm. All images were acquired with Slidebook software (Intelligent Imaging Innovations). For *par-1* experiments, *par-1(zu310ts)* L4s were shifted from 15 ºC to 25.5 ºC and grown overnight. Embryos were then dissected into 25.5 ºC egg salts and imaged at 26 ºC.

In situ Hybridization

In situ hybridization of *nos-2* and *cey-2* mRNA was performed as described previously (*16*), except that probe hybridization was performed at 46º C.

Immunostaining

Gravid adult hermaphrodites were laid on a slide coated with 0.01% poly-L-lysine and embryos, extruded by squashing with a coverslip, and frozen on pre-chilled aluminum blocks. Cover slips were removed and slides were incubated in -20 °C methanol for 15 minutes, followed by -20 °C acetone for 10 minutes. Slides were preblocked in PBS/0.1% Tween/0.1% BSA (PBT) for 30 minutes, and incubated with primary antibody overnight at 4 °C. Primary antibodies were diluted in PBT

as follows: OIC1D4 (1:10, Developmental Studies Hybridoma Bank), K76 (1:10, DSHB), KT3 (1:10, DSHB), chicken anti-GLH-1 (1:100, gift from K. Bennett), chicken anti-GLH-2 (1:200, gift from K. Bennett), rabbit anti-GLH-4 (1:10, gift from K. Bennett), rabbit anti-PAR-1 (1:200, gift from K. Kemphues), and affinity purified rat anti-NOS-1 (1:15). Secondary antibodies were applied for 2 hours at room temperature in the following dilutions: Cy3-conjugated goat anti-mouse IgG Fc fragment specific (1:100, Jackson Immunoresearch), Cy3-conjugated goat anti-mouse IgM (1:100, Jackson Immunoresearch), Cy3-conjugated goat anti-mouse IgG H+L (1:100, Jackson Immunoresearch), Alexa 568-conjugated goat anti-chicken IgY (1:200, Molecular Probes), Alexa 488-conjugated goat anti-rabbit IgG (1:100, Molecular Probes), Alexa 568-conjugated goat anti-rat IgG (1:100, Molecular Probes), and FITC-conjugated goat anti-GFP (1:200, Abcam).

Images were acquired using a Zeiss Axio Imager fitted with a Yokogawa spinning-disc confocal scanner (63x 1.4NA plan apochromat lens). All fluorescence intensities were below saturation during capture. Z-stack images (0.5um intervals spanning entire embryo) were collapsed (maximum projection) using Slidebook software. Collapsed images were normalized based on minimal and maximal values in *pptr-1* embryos. Consequently, maximal wild-type values are saturated in pictures shown.

Fluorescence Quantification

At least 3 embryos were analyzed for all quantifications. Image analysis was performed using Slidebook software.

For all graphs in Fig. 1 and Sup. Fig, 2, average fluorescence levels from time-lapse movies were measured from average projections of an 8μm stack (spanning approximately half the embryo). For the GFP fusions and whole embryo photoconversions of Dendra::PGL-1, movies were started at pronuclear formation (meiotic exit) and the first recorded anterior and posterior values were set to 1 and all other values scaled accordingly (Average variance between anterior and posterior values at meiotic exit was less than 3% with no preference for anterior or posterior). For local photoconversions of Dendra::PGL-1, the value of the converted half of the embryo was set to 1.

For Sup. Fig. 1A, granules were manually counted. To measure anterior rates of granule disappearance versus movement into the posterior, granules were followed manually through time and Z-stacks (slices 1-8). Only granules that disappeared while in slices 2-7 were counted to rule out movement into a focal plane that was not acquired.

Values in Figure 2 were measured by imaging three different focal planes in 3 embryos for each genotype and stage. Sum fluorescence intensities were measured in the P blastomere and in an identically-sized region positioned over the somatic sister. Fold enrichment was calculated as the average ratio of P cell:soma. Error bars are the standard error of the mean.

Sterility and brood size counts

Sterile animals were identified under the dissecting microscope among synchronized broods (12-24 hour lay) as adult hermaphrodites with empty (clear)

uteri. When examined at higher magnification, 10/10 hermaphrodites with clear uteri had an underproliferated gonad as shown in Fig. 4C. M0Z0 and M0Z1 animals were analyzed on the same plates among the brood of *pptr-1/pptr-1* mothers mated to +/+ males carrying the *myo-2::GFP transgene* (PD4790): M0Z1 (cross progeny) were GFP+, whereas M0Z0 (self-progeny) were GFP-. Fertile M0Z1 mothers were allowed to self to generate M1Z0, M1Z1, M1Z2 progeny. All sterile animals were genotyped; totals in each category were estimated from expected Mendelian ratios.

To obtain total brood sizes, mothers were cloned as L4s onto individual plates and passaged to a new plate after 30 hours (1 mother → 2 plates). Progeny from both plates were counted at the L4 stage. *pptr-1/pptr-1* hermaphrodites were crossed with +/+ (N2) males to generate M0Z1 hermaphrodites, and these were selfed to generate M1Z0, M1Z1, M1Z2 hermaphrodites. The genotype of each mother was determined by whole-worm PCR after the mother had laid her complete brood.

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Table S1. Strains used in this study

Name	Description	Genotype	Reference
JH1999	pie-1 prom::GFP-H2B-nos-2 3'UTR	*unc-119(ed3); axIs1448[pCM1.01]*	D'Agostino et al., 2006
JH2015	pie-1 prom::GFP::PIE-1-pie-1 3'UTR	*unc-119(ed3); axIs1462[pCM4.08]*	Merritt et al., 2008
JH2017	pie-1 prom::GFP::PGL-3-pgl-3 3'UTR	*unc-119(ed3); axIs1464[pCM4.10]*	Merritt et al., 2008
JH2078	pie-1 prom-LAP::MEX-5::pie-1 3'UTR	*unc-119(ed3); axIs1504[pCG2]*	This study
JH2108	pie-1 prom::GFP::PGL-1-pgl-1 3'UTR	*unc-119(ed3); axIs1522[pCM4.11]*	Merritt et al., 2008
JH2172	pie-1 prom::LAP-GLH-1-nos-2 3'UTR	*unc-119(ed3); axIs1485[pEV1.02]*	Gift from E. Voronina
JH2330	nmy-2 prom-PGL-1-GFP-nmy-2 3'UTR; pie-1 prom::mCherry-PATR-1::pie-1 3'UTR	*unc-119(ed3); axIs1488[pCG176]; NA*	Gallo et al., 2008
JH2758	pie-1 prom::mCherry-PAR-2::pie-1 3'UTR	*unc-119(ed3); axIs1929[pFM033]*	Zonies et al., 2010
JH2773	pie-1 prom::Dendra::PGL-1-pgl-1 3'UTR	*unc-119(ed3); axIs1946[pCG391]*	This study
JH2787	*pptr-1* mutant	*pptr-1(tm3103)*	This study
JH2791	*pptr-1* mutant; pie-1 prom::GFP-H2B-nos-2 3'UTR	*pptr-1(tm3103); axIs1448[pCM1.01]*	This study
JH2794	*pptr-1* mutant; pie-1 prom::GFP::PIE-1-pie-1 3'UTR	*pptr-1(tm3103); axIs1462 [pCM4.08]*	This study
JH2835	*pptr-1* mutant; pie-1 prom-LAP::MEX-5::pie-1 3'UTR	*pptr-1(tm3103); axIs1504[pCG2]*	This study
JH2836	nmy-2 prom-PGL-1-GFP-nmy-2 3'UTR	*unc-119(ed3); NA*	Wolke et al., 2007
JH2838	*par-1* ts; nmy-2 prom-PGL-1-GFP-nmy-2 3'UTR	*par-1(zu310ts); NA*	This study
JH2839	*pptr-1* mutant; pie-1 prom::GFP::PGL-1-pgl-1 3'UTR; pie-1 prom::mCherry-PAR-2::pie-1 3'UTR	*pptr-1(tm3103); axIs1522[pCM4.11]; axIs1929[pFM033]*	This study
JH2840	nmy-2 prom-PGL-1-GFP-nmy-2 3'UTR; pie-1 prom::mCherry-MEX-5::pie-1 3'UTR	*unc-119(ed3); NA; axIs1731[pEG56]*	This study
JH2841	*pptr-1* mutant; pie-1 prom::GFP::PGL-1-pgl-1 3'UTR	*pptr-1(tm3103); axIs1522[pCM4.11]*	This study
JH2842	pie-1 prom::GFP::PGL-1-pgl-1 3'UTR; pie-1 prom::mCherry-H2B::pie-1 3'UTR	*unc-119(ed3); axIs1522[pCM4.11]; NA*	This study
JH2843	*pptr-1* mutant; pie-1 prom::GFP::PGL-1-pgl-1 3'UTR; pie-1 prom::mCherry-H2B::pie-1 3'UTR	*pptr-1(tm3103); axIs1522[pCM4.11]; NA*	This study
KK289	*par-1* kinase dead	*par-1(it51) rol-4(sc8)/DnT1*	Gift from K. Kemphues
KK822	*par-1* ts	*par-1(zu310ts)*	Gift from K. Kemphues
NL2098	*rrf-1* mutant	*rrf-1(pk1417)*	Sijen et al., 2001
NL3511	*ppw-1* mutant	*ppw-1(pk1425)*	Tijsterman et al., 2002

PD4790	myo-2 prom::GFP; pes-10 prom::GFP	*mIs12*	Edgley et al., 1999
TH202[#]	glh-1 prom::GLH-1::GFP::glh-1 3'UTR	Recombineered fosmid integrated by bombardment	Brangwynne et al., 2009
TH206*	pgl-1 prom::PGL-1::GFP::pgl-1 3'UTR	Recombineered fosmid integrated by bombardment	Brangwynne et al., 2009

Abbreviations:

Prom – Promoter
3' UTR - 3' untranslated region
All CAPs – coding regions of indicated genes
:: - GATEWAY recombination sequence used for cloning (for JH strains only).

All transgenes (except for *myo-2* and *pes-10*) also contain a wild-type copy of *unc-119* (transformation marker). Note that we used four different GFP::PGL-1 lines: each with different promoter and 3' UTR combinations. All lines behaved identically in movies. Nevertheless for quantification of PGL-1 levels as shown in Fig. 2F,G and Sup. Fig. 2, we specifically used lines with the *pgl-1* 3' UTR (JH2108, JH2773, and JH2841).

TH202 was used for experiments shown in Fig. 2G.
* TH206 was used for experiments shown in Supp. Fig. 5C.