Proximity labeling identifies LOTUS domain proteins that promote the formation of perinuclear germ granules in *C. elegans*

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Abstract

The germline produces gametes that transmit genetic and epigenetic information to the next generation. Maintenance of germ cells and development of gametes require germ granules—well-conserved membraneless and RNA-rich organelles. The composition of germ granules is elusive owing to their dynamic nature and their exclusive expression in the germline. Using *C. elegans* germ granule, called P granule, as a model system, we employed a proximity-based labeling method in combination with mass spectrometry to comprehensively define its protein components. This set of experiments identified over 200 proteins, many of which contain intrinsically disordered regions. An RNAi-based screen identified factors that are essential for P granule assembly, notably EGGD-1 and EGGD-2, two previously uncharacterized LOTUS-domain proteins. Loss of *eggd-1* and *eggd-2* results in separation of P granules from nuclear envelope, germline atrophy and reduced fertility. We show that intrinsically disordered regions of EGGD-1 are required to anchor EGGD-1 to the nuclear periphery while its LOTUS domains are required to promote perinuclear localization of P granules. Together, our work expands the repertoire of P granule constituents and provides new insights into the role of LOTUS-domain proteins in germ granule organization.

Background

Germ cells are unique stem cells that give rise to eggs and sperm, and ultimately to an entire organism. Germ cells of diverse species are characterized by the accumulation of electron-dense and membraneless structures referred to as germ granules (Lehtiniemi and Kotaja, 2018; Trcek and Lehmann, 2019; Voronina et al., 2011). Germ granules are widely distributed throughout the animal kingdom. For example, they are known as “P granules” in worms (Strome and Wood, 1982), “nuage and polar granules” in fruit flies (Mahowald, 1968), “intermitochondrial cement” in frogs (Bilinski et al., 2004), and “chromatoid bodies” in mice (Parvinen, 2005).

Genetic and molecular analyses uncovered common classes of proteins that are enriched in germ granules across phyla (Anderson and Kedersha, 2009; Trcek and Lehmann, 2019; Updike and Strome, 2010; Voronina et al., 2011). These include: Piwi proteins and their small RNA cofactors—piRNAs that recognize and silence transposable elements (Aravin et al., 2006; Brennecke et al., 2007; Lau et al., 2006; Vagin et al., 2006); ATP-dependent helicases such as Vasa proteins that are involved in RNA metabolism (Gustafson and Wessel, 2010; Spike et al., 2008), and LOTUS-domain proteins (Limkain /MARF1, Oskar and Tudor domain-containing proteins 5 and 7) (Anantharaman et al., 2010; Callebaut and Mornon, 2010; Kubikova et al., 2020). Metazoan LOTUS domain proteins promote germ cell development. For example, *Drosophila* Oskar interacts with Vasa through its LOTUS domain and serves as scaffold for germ plasm assembly (Jeske et al., 2017; Lehmann, 2016). Mouse TDRD5 and TDRD7 are required for spermatogenesis (Lachke et al., 2011; Smith et al., 2004; Tanaka et al., 2011; Yabuta et al., 2011). The identity and/or role of LOTUS-domain protein(s) in *C. elegans* remain elusive.

C. elegans germ granules—P granules—provide an exceptional *in vivo* model to study granule formation and function (Seydoux, 2018; Strome and Wood, 1982; Updike and Strome, 2010). Biophysical studies combined with high-resolution microscopy describe P granules as phase-transitioned condensates (Brangwynne et al., 2009). P granules are present in germ cells throughout the entire *C. elegans* life cycle. Yet strikingly, they appear in different forms. In the adult gonad where germ cells undergo mitosis and differentiation,

abundant P granules are associated with the cytoplasmic face of nuclear pores (Pitt et al., 2000; Sheth et al., 2010; Updike et al., 2011). During oogenesis, P granules detach from the nuclear membrane and become diffuse in the cytoplasm. Passed on to progeny, P granules partition to the posterior of one-cell embryos. During following cell divisions, P granules are segregated into the germ line blastomeres and begin to coalesce and attach to the nuclear periphery. P granules ultimately become perinuclear in the primordial germ cells which give rise to the adult germline (Updike and Strome, 2010).

More than 40 protein components are enriched in P granules (Updike and Strome, 2010). Genetic analyses have revealed an assembly pathway which involves core proteins including DEPS-1, GLH-1, PGL-1 and IFE-1. DEPS-1 is a nematode specific protein that is required for GLH-1 accumulation (Spike et al., 2008). GLH-1 belongs to a conserved Vasa family that contributes to germ line develop and fertility (Gustafson and Wessel, 2010; Spike et al., 2008). In addition to the conserved DEAD-box helicase domains, GLH-1 and some of its paralogs contain phenylalanine-glycine (FG) repeats which are postulated to promote perinuclear localization of P granules (Chen et al., 2020; Marnik et al., 2019; Updike et al., 2011). PGL-1 contains both RNA binding and dimerization domains and serves as a P granule scaffold protein (Aoki et al., 2016; Kawasaki et al., 1998). Loss of either DEPS-1 or GLH-1 causes dispersal of PGL-1 into the cytoplasm, suggesting DEPS-1 and GLH-1 act upstream of PGL-1 (Kawasaki et al., 2004; Kawasaki et al., 1998). IFE-1 is a *C. elegans* homolog of eIF4E, an mRNA cap-binding protein (Keiper et al., 2000). IFE-1 and PGL-1 interact directly and association of IFE-1 with P granules depends on PGL-1 (Amiri et al., 2001). So far, P granule components are primarily identified by genetic approaches (Updike and Strome, 2010). Due to their perinuclear localization and the nature of membraneless compartments, P granules cannot be easily purified via a conventional fractionation-based approach. Therefore, P granule composition and the molecular rules underlying its assembly and migration remain largely unknown.

Our current study used a proximity-based labeling method in conjunction with mass spectrometry to define the P granule proteome. This uncovered over 200 protein

95 candidates. We show that EGGD-1 and EGGD-2 (embryonic and germline P
 96 granule detached) play a key role in promoting perinuclear localization of P granules.
 97 EGGD-1 associates with the nuclear periphery and its localization depends on its
 98 intrinsically disordered regions. EGGD-1 recruits the Vasa protein GLH-1 possibly
 99 through its LOTUS domains. Loss of *eggd-1* and *eggd-2* causes detachment of P
 100 granules from the nuclear membrane, germline atrophy and infertility. Together, our
 101 findings define the germ granule proteome and shed light on organization principles of
 102 germ granules.

Results

A proximity labeling system to enrich P granule proteins

To probe the composition of P granules, we employed a biotin ligase-based proximity labeling approach to label P granule proteins. TurboID — an engineered promiscuous biotin ligase derived from bacterial BirA — generates reactive biotin derivatives that label proteins in close proximity to the enzyme (Branon et al., 2018). TurboID is active from 20 °C to 25 °C, a range of temperature suitable for *C. elegans* cultivation (Branon et al., 2018). We thus sought to target TurboID to P granules (Figure 1A). To this end, we used CRISPR/Cas9 to introduce TurboID sequences to genomic loci of *deps-1*, *glh-1*, *pgl-1* or *ife-1*, which encode proteins known to reside in P granules (Figures 1A and 1B).

We first assessed if tagged alleles generate functional proteins by examining the fertility of animals expressing individual TurboID-tagged P granule proteins. Loss of *deps-1*, *glh-1*, *pgl-1* or *ife-1* result in reduced fertility (Updike and Strome, 2010). Under normal growth conditions, wild type strains produced ~209 progeny/animal. We found that strains expressing DEPS-1::TurboID and TurboID::IFE-1 yielded ~88 and ~167 progeny/animal respectively (Figure 1B). The brood size of *TurboID::glh-1* animals exhibited a large variation. A small portion of animals became completely sterile and each animal on average produced ~77 progeny (Figure 1B). The strain expressing PGL-1::TurboID was completely sterile, a phenotype that is more severe than *pgl-1* null mutants (Figure 1B) (Kawasaki et al., 1998). This finding suggests that expression of TurboID could cause toxicity. We therefore used two healthiest strains, *deps-1::TurboID* and *TurboID::ife-1*, for the proximity labeling experiments.

We examined biotinylation of proteins to assess the activity of TurboID. Two assays were employed: streptavidin blot analysis of whole-animal lysate and immunofluorescence staining of dissected gonads. For the first assay, we lysed adult animals, prepared protein lysates, and visualized biotinylated proteins using streptavidin–horseradish peroxidase blot analysis. In the lane with the untagged control, we detected a few signals which presumably corresponded to biotinylated endogenous proteins (Figure 1C) (Watts et al.,

2018). In strains expressing DEPS-1::TurboID and TurboID::IFE-1, more proteins were biotinylated (Figure 1C).

For the second assay, we stained dissected gonads with fluorescently labeled streptavidin to examine the subcellular localization of biotinylated proteins. We observed weak cytoplasmic signals in the stained wild type gonad (Figure 1D, upper panel). In the *deps-1::TurboID* and *TurboID::ife-1* gonads, signals of biotinylated proteins were observed in the cytoplasm, but highly enriched in perinuclear structures (Figure 1D, middle and bottom panels). These findings indicate that TurboID can be applied to label proteins in *C. elegans* germline.

Proteomic analysis of P granules

We next carried out streptavidin affinity pulldown to enrich TurboID-biotinylated proteins. In brief, adult animal lysate was prepared under a denaturing condition. After incubating with streptavidin beads, samples were washed under stringent and denaturing conditions to reduce nonbiotinylated protein contaminants and enrich proteins covalently tagged by TurboID (Branon et al., 2018). We found that biotinylated proteins were depleted from the flow-through and efficiently enriched in the pulldown (Figure S1).

Biotinylated proteins from untagged control, *TurboID::ife-1* and *deps-1::TurboID* strains were enriched in three independent biological replicates, and identified by mass spectrometry (Figures 2A and 2B). As compared to untagged control, 155 and 127 proteins were significantly enriched by labeling with TurboID::IFE-1 and DEPS-1::TurboID, respectively (fold-change ≥ 8 , p-value < 0.05) (Figures 2A). Table S1 provides a detailed overview of candidate proteins identified in TurboID strains. Of the combined 204 candidates, 38.2% (78/204) were recovered from both *deps-1::TurboID* and *TurboID::ife-1* strain (Figure 2B and Table S1). We inspected candidates identified in both TurboID experiments and found that some are known P granules proteins (Table S1). For example, the PGL family members PGL-1 and PGL-3, and four Vasa family members GLH-1, GLH-2, GLH-3, and GLH-4 were enriched (Gruidl et al., 1996; Kawasaki et al., 2004; Kawasaki et al., 1998; Spike et al., 2008). We also identified a subset of Argonaute proteins. These

include WAGO-1, WAGO-4, CSR-1 and PRG-1 which localize to P granule as well as PPW-1/WAGO-7 and PPW-2/WAGO-3 whose localization requires further investigation (Batista et al., 2008; Claycomb et al., 2009; Das et al., 2008; Xu et al., 2018; Yigit et al., 2006). Of note, HRDE-1/WAGO-9, a germline nuclear Argonaute protein was not on the list, suggesting TurboID preferentially labeled P granule proteins (Buckley et al., 2012). Consistent with idea that germ granules are the hubs for RNA metabolism (Trcek and Lehmann, 2019), many factors involved in RNA synthesis, processing, and decay were enriched from TurboID. These include the Dicer-related helicase DRH-3, Tudor-domain protein EKL-1, and RNA-dependent RNA polymerase EGO-1, terminal nucleotidyl transferase CDE-1, helicase domain containing proteins ZNFX-1 and RDE-12, decapping related proteins DCAP-1 and EDC-4, and 5'-3' exonuclease XRN-1 (Ishidate et al., 2018; Lall et al., 2005; Shirayama et al., 2014; Smardon et al., 2000; van Wolfswinkel et al., 2009; Wan et al., 2018).

To assess the specificity of proximity labeling, we examined labeled components of nuclear pore complexes. Nuclear pore complexes are among the largest protein structures in cells and comprised of multiple copies of ~30 different proteins known as nucleoporins (Nups) in human or nuclear pore proteins (NPPs) in *C. elegans* (Strambio-De-Castillia et al., 2010; Updike et al., 2011). The structure of the nuclear pore complex contains two main functional regions: the central structure which is embedded within the nuclear envelope, and the peripheral structures which extend to both the nuclear interior and cytoplasm termed the cytoplasmic filaments and the nuclear basket respectively (Figure 2C) (Strambio-De-Castillia et al., 2010). In *C. elegans* germ cells, P granules are associated with the cytoplasmic face of nuclear pore complexes (Pitt et al., 2000; Sheth et al., 2010; Updike et al., 2011). Under stringent purification conditions, we expected to purify specifically cytoplasmic NPPs from DEPS-1::TurboID and TurboID::IFE-1 expressing strains. Indeed, components of cytoplasmic filaments NPP-9 and NPP-14, and cytoplasmic ring NPP-6 were significantly enriched in both TurboID experiments (Figures 2B and 2C) (Strambio-De-Castillia et al., 2010). In summary, we demonstrate that Turbo proximity labeling can be applied to enrich proteins within P granules.

Properties of the P granule proteome

We next proceeded to characterize the properties of the P granule proteome. First, we conducted Gene ontology (GO) enrichment analysis (Ashburner et al., 2000; Raudvere et al., 2019). As expected, the top 3 enriched GO terms in the domain of Cellular Component were “cytoplasmic ribonucleoprotein granule”, “ribonucleoprotein granule” and “P granule” (Figure 2D and Table S2). In the domain of Biological Process, the top GO terms are “negative regulation of gene expression”, “gene silencing by RNA”, and “posttranscriptional regulation of gene expression” (Figure 2D and Table S2). Additionally, GO terms including “oocyte construction” and “oocyte anterior/posterior axis specification” were highly enriched (table S2), consistent with the notion that P granules are essential for gametogenesis and germline maintenance. In the domain of Molecular Function, GO terms including “RNA binding”, “nucleic acid binding” and “helicase activity” were significantly enriched, suggesting P granule proteins interact extensively with RNAs (Figure 2D and Table S2).

We constructed the P granule protein interaction network using publicly available protein-protein interaction (PPI) data (Jensen et al., 2009). When examining proteins labeled by both DEPS-1::TurboID and TurboID::IFE-1, we found the resulting network contained 78 nodes and 200 non-redundant edges (Figure S2). The number of edges was significantly higher than expected by chance ($p\text{-value} < 1 \times 10^{-16}$), implying that the proteins are at least partially biologically connected as a group and multiple protein-protein interactions may act in P granule assembly. We found that multiple P granule proteins showed high betweenness centrality within the network (Figure S2). At least three additional clusters were formed (Figure S2). These clusters consist of nuclear pore proteins NPP-6, NPP-9 and NPP-14 (Strambio-De-Castillia et al., 2010), kinetochore components such as HCP-1 (Cheeseman, 2014; Kitagawa, 2009), or proteasome components such as RPN-2 (Marshall and Vierstra, 2019). Together, these findings suggest that known and unknown P granule proteins identified by TurboID form a dense protein interaction network.

Proteins containing intrinsically disordered regions (IDRs) are often found in biomolecular condensates (Markmiller et al., 2018; Molliex et al., 2015; Nott et al., 2015). IDRs

themselves fail to form stable structures. Yet they participate in multivalent protein–protein, protein–DNA, and/or protein–RNA interactions (Oldfield and Dunker, 2014; Uversky, 2017). We next determined if IDR-containing proteins were enriched in the P granule proteome defined by TurboID. We employed the IUPred algorithm which predicts protein disorder by estimating total pairwise inter-residue interaction energy of amino acids (Dosztanyi et al., 2005). A probabilistic score of each residue ranging from 0 (complete order) to 1 (complete disorder) was generated. We calculated the sum of probabilistic scores and further normalized it to the protein length (Table S1). Compared to randomly selected control group, the P granule proteome displayed a higher degree of disorder (Figures 2E and 2F). These data suggest that IDR containing proteins are overrepresented in P granules.

A RNAi-based screen to identify factors required for P granule formation

P granule proteomic data obtained by TurboID provided a unique opportunity to identify factors that directly participate in P granule assembly. To this end, we have begun to conduct a RNA interference (RNAi)-based screen using a reporter strain expressing PGL-1::TagRFP. Different from a previous genome-wide RNAi screen (Updike and Strome, 2009), the reporter in this study had a null allele of *rrf-3*, which renders the strain hypersensitive to RNAi (Simmer et al., 2002). We used fluorescence microscopy to search for changes in PGL-1::TagRFP expression in animals in which candidate genes were depleted. Of 31 genes we screened so far, 11 genes upon depletion caused PGL-1::TagRFP phenotypes in adult animals (Figure 3A). Phenotypes were placed in broad categories including: detached from nuclear envelope, reduced expression, diffuse, large aggregates, and none observed (Figure 3A). This search identified C38D4.4—a previously uncharacterized gene which is required for proper PGL-1::TagRFP localization (Figure 3B). Depletion of C38D4.4 resulted in the formation of large PGL-1-containing aggregates, many of which are separated from nuclear envelope of germ cells. Based on this phenotype, we named C38D4.4 as *eggd-1* for embryonic and germline P granule detached.

F58G11.3 is a predicted paralog of EGGD-1. It is 33.3 % identical to EGGD-1 at the amino acid level. Interestingly, F58G11.3 was also identified from TurboID experiments (Figure 2B and Table S1). We named F58G11.3 as *eggd-2*, although RNAi against *F58G11.3* did not yield noticeable change in PGL-1::TagRFP localization (Figure 3A). Of note, an independent study refers to EGGD-1 and EGGD-2 as MEG-3 interacting proteins MIP-1 and MIP-2 respectively (Cipriani et al., bioRxiv, 2021).

EGGD-1 and EGGD-2 promote perinuclear localization of P granules and fertility

Using CRISPR/CAS9 editing, we generated strains bearing a null allele of *eggd-1* that deletes its open reading frame or a presumptive null allele of *eggd-2* that inserts 17 nucleotide sequences downstream of the start codon and shifts its open reading frame. Consistent with the result from RNAi experiments, PGL-1::TagRFP was dispersed into the cytoplasm in the mutants (Figures 3B, 3C and S3B). In *C. elegans*, germ cell nuclei are situated along the outer surface of the gonadal tube and share a central cytoplasmic core (Amini et al., 2015). We inspected PGL-1::TagRFP fluorescence on the surface and core of germline. In wild type animals, most of fluorescence signal was associated with the nuclear periphery of germ cell nuclei. In *eggd-1* mutants, however, fewer PGL-1 foci were perinuclear, and many diffused into the cytoplasmic core (Figure 3C). Loss of *eggd-2* appeared to have a minor effect on PGL-1 localization (Figure 3C). Deletion of both *eggd-1* and *eggd-2* caused dispersal of perinuclear PGL-1::TagRFP and accumulation of large cytoplasmic PGL-1 aggregates (Figure 3C). We used ImageJ to quantify perinuclear and cytoplasmic fluorescence signals of PGL-1::TagRFP (Figure S3A). Compared to that in wild type, the cytoplasmic/perinuclear ratio was increased in *eggd-1* mutants, and further increased in *eggd-1; eggd-2* double mutants. (Figure 3D).

During the first embryonic cell division, P granules are partitioned to the germ lineage of embryos (Strome and Wood, 1982). During following cell divisions, they are selectively eliminated in somatic cells, and begin to coalesce and attach to the nuclear periphery of germ cells (Seydoux, 2018; Updike and Strome, 2010; Zhang et al., 2009). Consistent with previous findings (Strome and Wood, 1982), PGL-1::TagRFP foci were detected in germ cell as well as in somatic cells around 28-cell stage wild type embryos. By the

comma-stage, PGL-1::TagRFP became predominantly perinuclear (Figure S3B). In contrast, PGL-1 failed to segregate to the germ lineage in *eggd-1*, *eggd-2* and *eggd-1; eggd-2* embryos (Figure S3B). Furthermore, perinuclear P granules were not formed in comma-stage embryos upon loss of *eggd-1* and *eggd-2* (Figure S3B). Together, these findings suggest that EGGD-1 and EGGD-2 promote perinuclear localization of P granules in both adult germline and embryos.

Disruption of germ granule formation causes defects in germline development and infertility in diverse organisms (Anderson and Kedersha, 2009; Trcek and Lehmann, 2019; Voronina et al., 2011). Using PGL-1::TagRFP as a germ cell marker, we examined whether *eggd-1* and *eggd-2* mutants exhibit defects in germline proliferation. Both *eggd-1* and *eggd-2* mutants displayed diminutive germline compared to wild type animals (Figures 3E and S3C). Additive genetic effects were observed in *eggd-1; eggd-2* double mutants (Figures 3E and S3C).

We next evaluated the fertility of *C. elegans* strains upon loss of *eggd-1* and/or *eggd-2*. In brief, we outcrossed mutants with wild type animals and measured the brood sizes of outcrossed strains at approximately generation 12. The wild type strain produced ~263 progeny/animal (Figure 3F). As compared to wild type, *eggd-1* and *eggd-2* animals displayed moderate reduction in brood size, generating ~170 and ~204 progeny/animals, respectively. Strikingly, *eggd-1; eggd-2* double mutants exhibited an additive fertility deficit and produced only ~37 progeny/animal (Figure 3F). These observations suggest that EGGD-1 and EGGD-2 are required for germline maintenance and fertility.

EGGD-1 and EGGD-2 contain two IDRs and two putative LOTUS domains

We next characterized EGGD-1 and EGGD-2 amino acid sequences. First, both EGGD-1 and EGGD-2 were relatively disordered (Table S1). Based on IUPred algorithm, two Intrinsically Disordered Regions (IDRs) were identified in EGGD-1 and EGGD-2, one close to their N-termini and the other located at their C-termini (Figures 4A and 4B) (Dosztanyi et al., 2005).

We next searched for conserved domains within EGGD-1 and EGGD-2 using the HHpred program (Zimmermann et al., 2018). HHpred is one of the most sensitive methods for remote homology detection (Zimmermann et al., 2018). The HHpred search identified two regions in EGGD-1 and EGGD-2 that are homologous to the LOTUS domain of *D. melanogaster* Oskar and *H. sapiens* TDRD5 and TDRD7. LOTUS domains are divided into two subclasses depending on the absence or presence of a C-terminal extension: minimal LOTUS (mLOTUS) and extended LOTUS (eLOTUS) which contains an extra C-terminal alpha-helix (Jeske et al., 2017). Similar to the LOTUS domains in Oskar, TDRD5 or TDRD 7, LOTUS domains in EGGD-1 and EGGD-2 are predicted to contain the C-terminal alpha-helix extension (Figure S4A) (Anantharaman et al., 2010; Callebaut and Mornon, 2010; Kubikova et al., 2020). We conclude that EGGD-1 and EGGD-2 harbor four domains: two eLOTUS domains and two IDRs (Figures 4A and 4B).

Roles of EGGD-1 LOTUS domains and IDRs in perinuclear P granule formation

We next examined EGGD-1 and EGGD-2 protein localization. CRISPR/CAS9 was used to generate fluorescently tagged alleles of *eggd-1* and *eggd-2*. Both proteins are expressed in the adult germline and germ lineage of embryos (Figures S4B and S4C). Consistent with the TurboID data, both EGGD-1::GFP and mCherry::EGGD-2 localize to perinuclear P granules (Figures S4B and S4C). Of note, the fluorescent signal of EGGD-2 was much weaker than EGGD-1::GFP or PGL-1::TagRFP. Considering EGGD-1 has a stronger impact on P granule assembly in the adult germline (Figure 3C), we decided to focus on EGGD-1 for further characterization.

To interrogate the role of IDRs and LOTUS domains, we employed CRISPR/CAS9 to generate a series of *eggd-1* alleles that delete individual IDRs, individual LOTUS domains, both IDRs, or both LOTUS domains (Figure 4C). We next examined the localization of EGGD-1 and PGL-1::tagRFP in live animals using spinning disk confocal microscopy. In the adult germline, full-length EGGD-1::GFP partly co-localized with PGL-1::TagRFP foci (Figure 4C). Super-resolution imaging revealed that EGGD-1::GFP foci surrounds PGL-1::TagRFP foci in some instances, but largely are enriched at the base of P granules (Figures 4D and 4E).

When the first LOTUS domain was deleted (Δ LOTUS 1), EGGD-1 displayed prominent perinuclear localization. In contrast, PGL-1::tagRFP was mis-localized. Upon removal of the second LOTUS domain (Δ LOTUS 2), EGGD-1 localized to the nuclear periphery while perinuclear PGL-1 foci were modestly lost. Deletion of both LOTUS 1 and LOTUS 2 did not appear to affect EGGD-1 localization. However, PGL-1 became predominantly cytoplasmic (Figure 4C). When the first IDR was deleted (Δ IDR 1), perinuclear EGGD-1 foci remained, but some EGGD-1 became dispersed into the cytoplasm. When the second IDR was deleted (Δ IDR 2), both EGGD-1 and PGL-1::tagRFP were largely attached to the nuclear periphery albeit the presence of some large aggregates. When both IDR 1 and IDR 2 were deleted, we found that EGGD-1 was almost completely dispersed in the cytoplasm. And similarly, PGL-1::TagRFP became cytoplasmic (Figure 4C). These findings imply that LOTUS domains and IDRs in EGGD-1 have distinct roles in promoting perinuclear P granules: IDRs are required to anchor EGGD-1 protein to the nuclear periphery, and LOTUS domains likely recruit additional P granule proteins.

EGGD-1 acts upstream of GLH-1 in P granule assembly

Previous studies showed one conserved function of eLOTUS domains is to interact with Vasa (Jeske et al., 2015; Jeske et al., 2017). *C. elegans* Vasa protein GLH-1 is known to localize to P granules and promote their assembly (Chen et al., 2020; Marnik et al., 2019; Updike et al., 2011). We thus tested the interaction between EGGD-1/2 and GLH-1. In particular, we performed epistasis analyses to order EGGD-1/2 and GLH-1 in the P granule assembly pathway. As expected, GFP::GLH-1 localized to P granules in wild type animals. However, when *eggd-1* was deleted, GFP::GLH-1 foci became diffuse and many no longer associated with the nuclear membrane. Deletion of *eggd-2* did not appear to affect GFP::GLH-1 localization. However, there was a striking change in GFP:GLH-1 distribution in *eggd-1; eggd-2* double mutant animals, with the majority of fluorescence signals appearing throughout the cytoplasm (Figure 5A). These findings suggest perinuclear GFP::GLH-1 primarily depends on EGGD-1.

We next used RNAi to deplete GLH-1 from EGGD-1::GFP; PGL-1::TagRFP expressing worms. Of note, *glh-1* RNAi likely depleted other GLH family members such as GLH-2 due to the sequence similarity. Consistent with previous studies (Spike et al., 2008), PGL-1::TagRFP became diffused into the cytoplasm upon depletion of GLH protein(s). In contrast, EGGD-1::GFP remained at the nuclear periphery (Figure 5B). These observations suggest that unlike other P granule proteins, perinuclear localization of EGGD-1 does not rely on the GLH family.

A subset of nuclear pore proteins, such as NPP-10, localize to P granules and are indispensable for their integrity (Updike and Strome, 2009; Voronina and Seydoux, 2010). We next sought to determine if EGGD-1 localization depends on nuclear pore proteins. Indeed, depletion of *npp-10* caused diffuse PGL-1::TagRFP (Updike and Strome, 2009; Voronina and Seydoux, 2010). Similarly, EGGD-1::GFP became detached from the nuclear membrane and formed aggregates in the cytoplasm upon depletion of *npp-10* (Figure 5B). Together, the epistasis analysis demonstrates perinuclear EGGD-1 requires intact nuclear pores and recruitment of GLH-1 to the nuclear periphery depends on EGGD-1.

Ectopic expression of EGGD-1 drives formation of perinuclear granules

So far, our data suggest that EGGD-1 is necessary for the perinuclear location of P granules. We wondered if EGGD-1 alone is sufficient to recruit GLH-1 to form perinuclear granules. To test this idea, an ectopic expression system was employed. EGGD-1 and/or GLH-1 were expressed under the muscle-specific *myo-3* promoter so that these proteins were expressed in somatic cells. This enabled us to examine the relationship between these proteins and granule assembly independently of other P granule or germline proteins.

Consistent with previous findings that wild type GLH-1 cannot form granules by itself (Updike et al., 2011), mCherry::GLH-1 was diffuse throughout the cytoplasm in muscle cells (Figure 6A). In contrast, EGGD-1::GFP self-aggregated into granules when it was expressed ectopically. While a few cytoplasmic granules were observed, the majority of

410 EGGD-1::GFP granules appeared to associate with the nuclear periphery (Figure 6B).
 411 We next drove the ectopic expression of EGGD-1::GFP and mCherry::GLH-1
 412 simultaneously. Strikingly, both proteins co-localized and formed perinuclear foci (Figure
 413 6C). Together, these findings indicate that EGGD-1 is intrinsically capable of forming
 414 perinuclear granules, and drives the formation of perinuclear granules by recruiting GLH-
 415 1.

Discussion

Since the discovery of perinuclear P granules, the molecular mechanisms for their assembly has been under intensive investigation (Strome and Wood, 1982; Updike and Strome, 2010). Previous studies showed that P granule formation requires core components including PGL family members PGL-1 and PGL-3, and Vasa family members GLH-1 and GLH-4 (Gruidl et al., 1996; Kawasaki et al., 2004; Kawasaki et al., 1998; Spike et al., 2008; Updike et al., 2011). Zygotic P granule assembly further requires intrinsically disordered proteins MEG-3 and MEG-4 (Smith et al., 2016; Wang et al., 2014). However, it remains unclear how P granules are formed and retained at the nuclear periphery.

Using proximity labeling and genetic approaches, we discovered and characterized EGGD-1 and EGGD-2—LOTUS-domain and IDR-containing proteins. Our work provides insight into the molecular function of EGGD proteins in promoting formation of perinuclear P granules. We show that IDRs are required to anchor EGGD-1 protein to the nuclear periphery, and that the LOTUS domains are essential for perinuclear localization of P granules, likely by recruiting the Vasa protein GLH-1. Remarkably, EGGD-1 is capable of self-assembling into perinuclear granules. Thus, EGGD-1 is not only necessary but also sufficient for recruiting GLH-1 to the nuclear membrane.

Based on these findings, we propose a model for the molecular function of EGGD proteins (Figure 6D). IDRs of EGGD proteins may form multivalent interactions with cytoplasmic filaments of the nuclear pore complex. It is possible that IDRs preferentially associate with phenylalanine/glycine-rich NPPs that are intrinsically disordered. Dimerization of LOTUS domains serves as an interface for the binding of Vasa protein GLH-1 (Jeske et al., 2015; Jeske et al., 2017). Association of GLH-1 with the nuclear membrane initiates the recruitment of PGL-1 and additional P granule proteins. More experiments, both in vitro and in vivo, will be required to dissect this complex protein-protein interaction network.

Interplay between EGGD-1 and EGGD-2

The interplay between EGGD-1 and EGGD-2 seems complex. In the adult germline, EGGD-1 and EGGD-2 function partially redundantly. EGGD-1 plays a more dominant role in promoting perinuclear localization of P granules (Figure 3C). In the embryos, however, both proteins are required for P granule partitioning and attachment to the nuclear periphery (Figure S3B). One speculative explanation is that EGGD-1 and EGGD-2 interact with different Vasa proteins and thus make distinct contributions to P granule assembly in the germline and zygotes.

Most LOTUS domain proteins, including Oskar, TDRD5 and TDRD7, harbor a single eLOTUS domain. In contrast, EGGD-1 and EGGD-2 are predicted to contain two eLOTUS domains (Figure S4A). The eLOTUS domain of *Drosophilla* Oskar is capable of forming dimers (Jeske et al., 2015; Jeske et al., 2017). We speculate that through their eLOTUS domains, EGGD-1 and EGGD-2 may form homodimers, heterodimers, or perhaps even oligomers. Of note, an independent study identified a third LOTUS domain protein LOTR-1 which is homologous to mammalian TDRD5/7 (Marnik et al., bioRxiv, 2021). Interestingly, similar to EGGD-1 and EGGD-2, LOTR-1 was also enriched from our TurboID experiments (Figure 2B and Table S1). Future genetic experiments will be required to determine the interplay of these three LOTUS domain proteins in regulating perinuclear P granule formation.

LOTUS domain proteins as scaffolds for germ granule assembly

LOTUS domains are found in bacteria, plants and animals (Anantharaman et al., 2010; Callebaut and Mornon, 2010). LOTUS domains exhibit minimum sequence homology (Figure S4A) (Anantharaman et al., 2010; Callebaut and Mornon, 2010). Yet they adopt a common helix-turn-helix conformation (Anantharaman et al., 2010; Callebaut and Mornon, 2010; Jeske et al., 2015; Jeske et al., 2017). The fact that diverse sequences yield a similar structure implies that the LOTUS domains acts as structural scaffolds. Indeed, the conserved function of eLOTUS domains is to bind to Vasa (Jeske et al., 2017).

Consistent with the idea the structure of a protein largely determines its functional properties, LOTUS proteins are essential for the development of metazoan germ cells.

In *Drosophila*, Oskar is required for germ plasm assembly and germ cell formation (Jeske et al., 2017; Lehmann, 2016). In mice, TDRD7 localizes to chromatoid bodies (P granule counterpart), and is required for spermatogenesis (Lachke et al., 2011; Smith et al., 2004; Tanaka et al., 2011; Yabuta et al., 2011). In this study, we show that *C. elegans* EGGD-1 and EGGD-2 are key components for germ granules assembly on the nuclear periphery. Together, these findings suggest that LOTUS domain proteins belong to a unique family that has low sequence identity, but high structure homology and functional similarity. It will be important to use a structure-based, but not the sequence-based, search program to identify more LOTUS domain proteins across phyla.

Advantage and limitation of proximity labeling in this study

Proximity labeling is a powerful approach to map the proteome composition of organelles in living cells. Compared to conventional affinity purification, the key advantage lies in its ability to capture weak and transient interaction. In addition, the strong binding of biotin to streptavidin permits stringent protein extraction which reduces background contaminants. It is therefore an ideal tool to define the composition of phase-separated membraneless organelles that are formed by weak multivalent interactions (Bracha et al., 2019). Proximity labeling uses a promiscuous enzyme such as BioID, APEX, or TurboID (Branon et al., 2018; Rhee et al., 2013; Roux et al., 2012). TurboID appears to be the best choice for *C. elegans* labeling for several reasons: 1). TurboID is active from 20 °C to 25 °C, a range of temperature suitable for *C. elegans* cultivation (Branon et al., 2018). 2). TurboID uses ATP and biotin as substrates which are readily available in cells, while APEX requires exogenous cofactors which may not be easily transported into worms (Branon et al., 2018; Rhee et al., 2013); 3). TurboID catalyzes biotinylation with much greater efficiency than BioID (Branon et al., 2018);

In this study, we employed TurboID in combination with mass spectrometry analysis to define the constituents of P granules. While this approach identified many known and unknown P granule proteins, it has some limitations. First, TurboID fusion proteins can be toxic. We found strains expressing TurboID::GLH-1 and PGL-1::TurboID proteins either unhealthy or infertile (Figure 1B). Second, TurboID labeled cytoplasmic proteins as

revealed by streptavidin staining (Figure 1D). It is possible that some proteins are biotinylated by TurboID when passing through P granules and shuttling between the nucleus and cytoplasm. Consistent with this idea, TurboID enriched IMA-2, a member of importin α family of nuclear-cytoplasmic transport factors (Table S1). Alternatively, because P granules are membraneless and highly dynamic, the bait proteins (IFE-1 and DEPS-1) may constantly mix and de-mix with surrounding cytoplasmic components.

While we continue to optimize the protocol for TurboID labeling, several approaches can be used to overcome these limitations. For example, an auxin-inducible degradation system can be applied to deplete the toxic TurboID fusion proteins (Zhang et al., 2015). Removal worms from auxin-containing plates enables transient expression of TurboID proteins and thus proximity labeling. More recently, a split-TurboID method was developed, in which TurboID is split into two inactive fragments, but can be reconstituted in vivo (Cho et al., 2020). It is conceivable to fuse individual fragments of TurboID to two P granule proteins. In this case, TurboID will become active only when two bait proteins interact within the compartment. We envision this approach will greatly improve the specificity in labeling P granule proteins.

In addition to P granules, *C. elegans* germ cells possess other membraneless organelles such as Z granules and Mutator foci (Phillips et al., 2012; Wan et al., 2018). These granules are adjacent to one another, but each has distinct functions in RNA interference and transgenerational epigenetic inheritance (Phillips et al., 2012; Wan et al., 2018). We speculate that they share some common proteins, but also contain unique components. Proximity labeling described in this study can be applied to unravel the proteome of Z granules and Mutator foci, and thus will provide new insight into the organization and function of germ granules.

Materials and Methods

Strains

Worms were cultured according to standard methods at 20°C unless otherwise indicated (Brenner, 1974). N2 strain serves as wild type or untagged control. Mutant animals were generated using CRISPR editing or obtained from the CGC. All strains used in this study are listed in the Key Resources table.

CRISPR genome editing

TurboID strains, FLAG::AID::GFP::TEV tagged *eggd-1*, and mCherry tagged *eggd-2* were generated by the co-CRISPR strategy (Kim et al., 2014). A vector containing a dominant allele of *rol-6* was used as a co-injection marker. Repair templates for insertion were made by PCR and purified by agarose gel purification. Prior to injection, repair templates were melted to improve HDR efficiency (Ghanta and Mello, 2020). Roller F1 were picked and presence of insertions at edited loci were screened for by PCR. Domain deletion alleles of *eggd-1* were generated by co-injecting guide RNAs targeting the region flanking the deletion. Single stranded DNA donors served as a repair template for precise mutations as described (Paix et al., 2017). Guide RNA sequences, repair oligo sequences, and primer sequences for repair template amplification can be found in the Key Resources table.

Molecular cloning and generation of ectopic expression strains

Endogenously tagged loci of *eggd-1::GFP*, and *mCherry::glh-1* were amplified by PCR. pCFJ104 (Pmyo-3::mCherry::unc-54) was digested with XbaI and SacI, the plasmid backbone was separated by gel purification. PCR products were digested with XbaI or AvrII and SacI and ligated into the pCFJ104 backbone. Plasmids were isolated by miniprep and sequence-verified by sanger sequencing. *unc-119(ed9)* animals were injected with a plasmid mixture including the genes of interest, and pCFJ151. non-unc F1 progeny were single-picked. non-unc F2 progeny were used to establish at least 3 independent transmission lines.

RNAi by feeding

The HT115 RNAi feeding strains were picked from the *C. elegans* RNAi Collections (Key Resources table) (Kamath and Ahringer, 2003; Lamesch et al., 2004). All RNAi experiments were performed on NGM plates supplemented with 50 ug/ml ampicillin and 5mM IPTG. For the genetic analysis, L4 larvae were transferred to plates seeded with HT115 bacteria expressing dsRNAs against the gene of interest. After 4-5 days their progeny was imaged and scored.

Brood size counting

TurbID animals were assayed at 15°C. Wild type, *eggd-1*, *eggd-2*, and *eggd-1; eggd-2* animals were assayed at 20°C at approximately generation 12 after outcross. Newly hatched L1 larvae were placed singly on plates. Halfway through egg-laying, animals were transferred to fresh plates until egg-laying stopped. The brood size for each animal was calculated by adding the progeny on the original and transferred plates.

Streptavidin staining

Animals were synchronized by hypochlorite lysis. Synchronized L1 were transferred to NGM seeded with OP50 and incubated at 15 °C until L4 stage. Animals were then transferred to 25 °C overnight for biotin labeling. Worms were resuspended in M9 and washed three times. Animals were then transferred to M9 supplemented with 0.25mM levamisole and dissected on slides coated with poly-L-Lysine. Slides were submerged in -20 °C methanol for 10 minutes, followed by 15 minutes in 3% paraformaldehyde solution in PBS at room temperature. Paraformaldehyde solution was removed, and slides were washed 3x in PBS and then 1:2000 streptavidin-Alexa Fluor 488 in PBS + 0.1% Triton X-100 was added to the slides. Slides were kept in a humidified chamber overnight at 4 °C. The slides were then washed 4 times for 30 minutes with PBS + 0.1% Triton X-100 and twice with PBS. 5 ul of antifade media with DAPI was added to the slides and covered with a coverslip. The slides were sealed with nail polish and kept at 4°C until imaging.

Whole worm fixation

Animals expressing *myo-3p::glh-1* and/or *myo-3p::eggd-1* constructs were resuspended in M9 and washed twice to remove bacteria. M9 was replaced with -20 °C methanol.

Animals were incubated at -20°C for 15 minutes and then briefly centrifuged at 100 x g. methanol was removed and replaced with -20 °C acetone. Tubes were kept at -20°C for 20 minutes, and animal were centrifuged as before. Acetone was replaced with a solution of 50% acetone, 50% ultrapure water and animals were incubated at -20 °C for 15 minutes. This process was repeated with 25% acetone at 4°C. Fixed animals were centrifuged and supernatant was removed. 30 ul of antifade medium + DAPI was added to the tube. Fixed animals were transferred to slides using a glass Pasteur pipette, covered with a coverslip, and sealed with nail polish.

Microscopy

Live animals were suspended in M9 buffer, immobilized using 0.5mM levamisole and mounted on fresh 5% agar pads. Live embryos were dissected from gravid adult worms in M9 buffer and transferred to agar pads for imaging. Spinning disc confocal images were acquired using a Nikon TiE inverted microscope equipped with an Andor Revolution WD spinning disc system. Images were taken using a CFI Plan Apo VC 60x/1.2NA water immersion objective, or a CFI Plan Apo VC 100X/1.4NA oil immersion objective with an Andor Neo sCMOS detector. Airyscan images were acquired using a Zeiss Axio Observer microscope equipped with an Airyscan 2 detector and a Plan Apo 63x/1.4NA objective. Image processing was performed using standard 3D Airyscan processing.

Quantification of cytoplasmic PGL-1::TagRFP over perinuclear PGL-1::TagRFP

Single-plane 54 x 37.8 micron rectangular optical cross sections images of adult germlines in the pachytene region were obtained for 4 separate animals. A ROI (region of interest) was drawn about each edge of the germline, and the cytoplasmic space (see Figure S3A). The background fluorescence of the image was determined by drawing a ROI outside of where the animal was located. The mean intensity of each ROI was determined using the measure function in imageJ and the ratio of cytoplasmic and perinuclear PGL-1::TagRFP was calculated as follows:

$$\frac{2 * (cytoplasm - background)}{((edge1 + edge2) - (2 * background))}$$

Germline atrophy characterization

Wild type animals and *eggd* mutants expressing PGL-1::TagRFP were synchronized by hypochlorite lysis and plated to NGM on day 1. Animals were maintained at 20°C until day 4 and imaged using a Leica DMIRE2 inverted microscope equipped with a Zeiss Axiocam 305 mono camera using a dry 40x objective. Germline defects were broadly sorted into 4 categories: normal, small, atrophy, and absent/few cells.

Streptavidin-HRP blotting

100 synchronized L1 animals were plated to NGM. Animals were grown at 15 °C until they reached the L4 stage. Animals were then incubated overnight at 25 °C and harvested. Cell lysates were prepared by boiling animals at 100 °C, and then were separated on precast denaturing polyacrylamide gels, transferred onto PVDF membrane (Bio-Rad), and probed with HRP-Conjugated Streptavidin 1:4000 (Thermo Fisher) for detection using Clarity ECL Western Blotting Substrate (Bio-Rad). The blot then was stained by Coomassie blue (Thermo Fisher).

TurboID proximity-based labeling

TurboID strains and N2 animals were synchronized by hypochlorite lysis. 40,000 synchronized L1 animals were plated to NGM seeded with concentrated OP50 food. Animals were grown at 15 °C until they reached the L4 stage. Animals were then incubated overnight at 25 °C and collected in M9 and washed 2x in M9, once in ddH₂O and once in RIPA buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.125% SDS, 0.125% sodium deoxycholate, 1% Triton X-100 in ddH₂O). Animals were then resuspended in RIPA buffer supplemented with cOmplete mini EDTA-free Protease Inhibitor Cocktail tablets (Sigma). Resuspended pellets of animals were flash-frozen in liquid N₂ until further use. Worm pellets were lysed using a bead mill homogenizer. Lysate were centrifuged at 14000x RPM. The supernatant was mixed with 80 µl Streptavidin magnetic beads (Thermo Fisher) and incubated overnight at 4 °C with constant rotation. Beads were then washed for 5 minutes, twice with RIPA buffer, once with 1M KCl, once with 0.1

M Na₂CO₃ and once with 2 M urea in 10 mM Tris-HCl (pH 8.0). Beads were resuspended in PBS and subjected to on-beads trypsin digestion.

On Beads Digestion and Mass spectrometry analysis

Streptavidin magnetic beads were washed with 50 mM ammonium bicarbonate three times. After the third wash, DTT (Thermo Fisher) was added and the sample is incubated at 4 °C for 15 min. After the incubation, Iodoacetamide was added and the sample is kept in dark at room temperature for 30 min. 250 ng of sequencing grade-modified trypsin (Promega) prepared in 50 mM ammonium bicarbonate was mixed with sample at 37 °C overnight. The reaction was quenched by adding acetic acid for acidification. Supernatant was taken out, concentrated for LC/MSMS analysis.

Capillary-liquid chromatography-nanospray tandem mass spectrometry of protein identification was performed on an orbitrap Fusion mass spectrometer equipped with an EASY-Spray sources (Thermo Fisher). Sequence information from the MS/MS data was processed by converting the .raw files into a merged file (.mgf) using MSConvert (ProteoWizard). Isotope distributions for the precursor ions of the MS/MS spectra were deconvoluted to obtain the charge states and monoisotopic *m/z* values of the precursor ions during the data conversion. The resulting mgf files were searched using Mascot Daemon by Matrix Science version 2.5.1 and the database searched against *C. elegans* Uniprot database.

TurboID protein enrichment analysis

Following pre-processing the data were further analyzed using custom R scripts. Briefly, the mean spectral count (n = 3) was compared between turboID tagged strains and wild type using a pseudo-count of 0.01 to account for zeros in the data; p values were derived using a one tailed t-test. Volcano plots were generated using ggplot2 and venn diagrams of enriched proteins were generated using BioVenn.

Protein domain identification

Putative LOTUS domains of EGGD-1 and EGGD-2 were first identified using HHpred (Zimmermann et al., 2018). To confirm the presence of LOTUS domains in EGGD-1 and EGGD-2, secondary structure prediction was performed using 103 amino acid long windows about the putative LOTUS domains along with the known extended LOTUS domains from *Drosophila melanogaster* Oskar, and *Mus musculus* TDRD5 and TDRD7 using PROMALS3D (Pei et al., 2008). EGGD-1 and EGGD-2 LOTUS domains were determined to be extended LOTUS domains by the presence of an alpha-helical C-terminal extension ($\alpha 5$) (Jeske et al., 2017). Intrinsically disordered regions were defined using the IUPRED server (Dosztanyi et al., 2005). Regions with sustained IUPRED scores over 0.5 were classified as disordered regions.

Protein-protein interaction network

Proteins enriched in both DEPS-1 and IFE-1 TurboID pull-down experiments were submitted for protein-protein interaction network analysis to STRING (string-db.org) using a high confidence and full interaction method with all active interaction sources selected (Jensen et al., 2009). The resulting network was exported to Cytoscape for visualization (Shannon et al., 2003).

Protein disorder analysis

Using a custom shell script, the longest protein isoform for each protein in the *C. elegans* proteome was parsed from a protein annotation fasta file (*C. elegans* release WS230). The per-residue disorder for each protein was then calculated using IUPRED (Dosztanyi et al., 2005). A custom python script was then used to calculate the mean disorder for each protein by dividing total IUPRED score by protein length. A custom R script was used to compare the mean disorder of proteins between proteins enriched in either/both turboID tagged strain with that in a random control. The random sample of proteins used for comparison was generated using the sample_n() R function from the dplyr R package. A Wilcoxon rank-sum test was used to statistically compare the disorder of tagged proteins with that of the randomized control group.

Gene Ontology (GO) Analysis

718 Enriched proteins present in both turboID tagged strains were used in GO analysis using
 719 g:Profiler (Raudvere et al., 2019). Results from g:Profiler GO analysis were plotted using
 720 a custom R script.

721

722 **Data availability**

723 All scripts used in this report are available at <https://github.com/benpastore/TurboID>.

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Competing interests: No competing interests declared

Figure Legend

Figure 1. A proximity labeling system for specific biotin labeling of P granule proteins

(A) Schematic of proximity-based labeling. Known P granule bait proteins are tagged with the promiscuous biotin ligase—TurboID—to label proteins present in P granules.

(B) Brood sizes of strains endogenously tagged at the loci encoding *deps-1*, *ife-1*, *glh-1*, and *pgl-1* and N2 control. 5 independent lines of *pgl-1::TurboID* heterozygotes are sterile. ns: not significant, *** p-value < 0.0005, two tailed Student's t-test.

(C) Streptavidin-horseradish peroxidase blotting. The left panel shows whole animal lysates prepared from N2 (untagged control), and strains expressing DEPS-1::TurboID or TurboID::IFE-1 blotted with streptavidin-HRP to visualize biotinylated proteins. The right panel shows Coomassie blue stain of the same membrane. An asterisk marks endogenously biotinylated protein (Based on its size, the protein likely is PCCA-1).

(D) Streptavidin-Alexa Fluor 488 staining of gonad dissected from N2 (untagged control), and animals expressing TurboID::IFE-1, and DEPS-1::TurboID. Scale bar = 10 microns.

Figure 2. Proteomic analysis of P granules and properties of P granule proteome

(A) Volcano plots showing statistically significant enriched proteins from strains expressing DEPS-1::turboID and turboID::IFE-1 in green. One tailed Student's t-test, p-value < 0.05, $\log_2(\text{Fold change}) \geq 3$. DEPS-1 and IFE-1 proteins are shown in red on the respective plots.

(B) Venn diagram showing overlapping proteins recovered from strains expressing TurboID::IFE-1 and DEPS-1::TurboID. The list shows proteins enriched by both pull-down experiments. Argonaute proteins (purple), nuclear pore proteins (green), and core P granule components GLH and PGL-1 family proteins (red).

(C) Schematic of nuclear pores and P granules. Pull-down experiments enrich nuclear pore proteins (NPP-6, NPP-9, NPP-14) composing either the cytoplasmic filaments or outer ring (purple). NPPs and their vertebrate homologs are shown.

(D) Functional gene set enrichment analysis of proteins significantly enriched in both pull-down experiments organized by molecular function, biological processes, and cellular compartments. Top three enriched categories (based on p-value) are labeled.

(E) Boxplot showing the average disorder of proteins enriched in both TurboID::IFE-1 and DEPS-1::TurboID ($n = 78$) as predicted using IUPRED compared to a random control set ($n = 78$). The average disorder of proteins was derived by comparing the total IUPRED score of each protein to its length. Wilcoxon rank-sum test ($p < 0.01$ **).

(F) The same analysis as in E, but with proteins labeled in either TurboID::IFE-1 or DEPS-1::TurboID pull-down experiments ($n = 204$). Wilcoxon rank-sum test ($p < 0.0001$ ****).

Figure 3. EGGD-1 and EGGD-2 promote perinuclear localization of P granules

(A) PGL-1::TagRFP phenotypes. Summarized PGL-1::TagRFP from a RNAi based screen. Genes are grouped by the observed PGL-1::TagRFP phenotypes in *rrf-3*; *pgl-1::TagRFP* adult germline.

(B) Fluorescence micrographs of dissected gonads from PGL-1::TagRFP reporter animals after 2 successive generations of control RNAi or C38D4.4/*eggd-1* RNAi. Scale bar = 10 microns.

(C) Single confocal slices of the surface, and cytoplasmic core of the germline in live adult animals expressing PGL-1::TagRFP. Wild type animals, and animals bearing mutations in *eggd-1*, *eggd-2*, and *eggd-1;eggd-2* are shown. Scale bar = 10 microns. Images are representative of at least 4 animals.

(D) Boxplot of quantified cytoplasmic vs. perinuclear PGL-1::TagRFP signal intensity in *eggd-1*, *eggd-2* and *eggd-1; eggd-2* mutant backgrounds. ns: not significant, * p-value < 0.05, ** p-value < 0.005, two tailed Student's t-test ($n = 4$).

(E) Germline morphology in adult *eggd-1*, *eggd-2*, and *eggd-1; eggd-2* mutants compared to wild type animals. Representative images of absent, atrophy, small, and normal classifications are shown in Figure S3C.

(F) Brood counts of wild type, *eggd-1*, *eggd-2*, and *eggd-1; eggd-2*, mutant animals. ** p-value < 0.005, *** p-value < 0.0005, two tailed Student's t-test.

Figure 4. EGGD-1 and EGGD-2 contain IDRs and LOTUS domains.

(A-B). Domain architecture and IUPRED score for EGGD-1 (A) and EGGD-2 (B). Regions of proteins with an IUPRED score above 0.5 are predicted to be disordered, while regions below 0.5 are predicted to be ordered. The intrinsically disordered region is shown in blue and abbreviated as 'IDR'. LOTUS domains are shown in green.

(C) Spinning disc confocal images (100x objective) showing the localization of EGGD-1::GFP protein, and a series of EGGD-1::GFP domain deletion mutants in pachytene germ cells of live animals expressing PGL-1::TagRFP. Images are representative of over five animals. Scale bar = 10 microns.

(D) Super-resolution Zeiss Airyscan image of a single pachytene nucleus in animals co-expressing EGGD-1::GFP and PGL-1::TagRFP.

(E) Intensity profile of EGGD-1::GFP and PGL-1::TagRFP signals along the dotted arrow in panel (D). AU: arbitrary unit.

Figure 5. EGGD-1 acts upstream of GLH-1 in P granule assembly and localization.

(A) Micrographs showing pachytene nuclei of wild type and mutant animals expressing GFP::GLH-1 (60x objective). The contrast of images in *eggd-1*, and *eggd-1; eggd-2* mutants is manually adjusted to visualize localization of GFP::GLH-1. Images are representative of at least 4 animals. Scale bar = 10 microns.

(B) Fluorescence micrographs of pachytene nuclei from live animals expressing EGGD-1::GFP and PGL-1::TagRFP under the indicated RNAi conditions (60x objective). The contrast in images upon *glh-1* RNAi and *npp-10* RNAi is manually adjusted to visualize EGGD-1::GFP. Scale bar = 10 microns.

Figure 6. EGGD-1 intrinsically localizes to the nuclear envelope and is sufficient to recruit GLH-1 to the nuclear periphery.

(A-C) Maximum intensity projection of a z stack spanning the pharynx of fixed adult animals ectopically expressing mCherry::GLH-1 (A), EGGD-1::GFP (B), or mCherry::GLH-1 and EGGD-1::GFP (C) under the muscle-specific *myo-3* promoter. Top panel shows the entire pharynx. Bottom panel shows an individual nucleus outlined by a dashed box in the top panel. Images are representative of at least 6 animals (60x objective).

(D) Model illustrating the proposed role of EGGD-1 in P granule assembly.

Supplementary Figure 1. Streptavidin pull-down of biotinylated proteins. Related to Figure 1.

Streptavidin-horseradish peroxidase blotting showing pulldown efficiency. Biotinylated proteins are present in the input, depleted in the flow-through and enriched in the pulldown fraction.

Supplementary Figure 2. Network analysis of proteins enriched from TurboID. Related to Figure 2.

STRING network analysis of proteins enriched in both pull-down experiments from strains expressing DEPS-1::TurboID and TurboID::IFE-1 (n=78). Known P granule proteins are shown in red, nuclear pore proteins are shown in turquoise, EGGD-1 is shown in green, and EGGD-2 is shown in orange. Other proteins are shown in gray. Network was visualized in Cytoscape.

Supplementary Figure 3. Embryonic PGL-1::TagRFP localization and images of diminutive germline. Related to Figure 3.

(A) One example of regions of interest drawn to quantify PGL-1::TagRFP signal intensity plotted in Figure 3D. The equation used to quantify cytoplasmic/perinuclear signal intensity is located in the Materials and Methods section.

(B) Maximum intensity projections of confocal z stacks spanning the depth of embryos expressing PGL-1::TagRFP at the indicated stages in *eggd-1*, *eggd-2* and *eggd-1; eggd-2* mutants. Dashed line outlines the embryos. Images are representative of at least 4 embryos.

(C) Brightfield and fluorescence images of adult animals with normal, small, atrophied, and absent germlines. The gonad is outlined by a yellow dashed line in the brightfield images. The genotype of imaged animals is indicated at the bottom left of each brightfield image.

Supplementary Figure 4. Localization of EGGD-1 and EGGD-2 proteins. Related to Figure 4.

(A) Alignment between the extended LOTUS domains of *Drosophila melanogaster* Oskar mouse TDRD7, TDRD5, and the putative LOTUS domains present in EGGD-1 and EGGD-2. In the “secondary structure” row, “h” indicates a consensus prediction for an alpha-helical secondary structure, while “e” indicates a prediction for a beta sheet.

(B) Single confocal slices of EGGD-1::GFP and mCherry::EGGD-2 protein in the adult germline.

(C) Maximum intensity projections made from confocal images spanning the depth of embryos, visualizing EGGD-1::GFP, and mCherry::EGGD-2 during embryonic development.

Supplementary Table 1: P granule proteins revealed by TurboID and their IUPRED score

Supplementary Table 2: Significantly enriched Gene Ontology terms for TurboID hits

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1124

Figure 1

Figure 2

A

PGL-1::TagRFP phenotype	Gene name
detached from nuclear envelope	C38D4.4/ <i>eggd-1</i>
reduced expression	<i>wago-1</i> , <i>hsp-110</i> , <i>cpf-2</i>
diffuse	<i>puf-5</i> , <i>ran-2</i>
large aggregates	<i>cey-2</i> , <i>cey-3</i> , <i>ubh-4</i> , <i>ani-1</i> , <i>ifg-1</i>
none observed	<i>maph-1.2</i> , F56C9.6, <i>ppw-2</i> , M01H9.3, Y37E11B.10, F01G4.4, <i>hmg-12</i> , <i>edc-4</i> , <i>ppw-1</i> , <i>frm-4</i> , <i>haf-9</i> , <i>prg-1</i> , F52B5.3, <i>hip-1</i> , F58G11.3/ <i>eggd-2</i> , <i>lotr-1</i> , D2005.4, <i>pqn-59</i> , <i>lea-1</i> , <i>dnj-11</i>

B

C

D

E

F

Figure 3

Figure 4

Figure 5

Figure 6

Figure S1

Number of nodes: 78
 Number of edges: 200
 Expected number of edges: 31
 Average local clustering coefficient: 0.444
 PPI enrichment p-value: $< 1 \times 10^{-16}$

Figure S2

B

PGL-1::TagRFP

C

Figure S3

A

```

OSKAR LOTUS1: 1 --MTII--ESNYISVREEYPDIDSEVRAT-LLSHAQNGTITISSIKSEYRKLTGNFFP--L-----HDNVTFDLITIPNVT-AECSESGKRIFNLKASLKNHLLDMVLNQKERTSD----- 103
TDRD7 LOTUS1: 1 --MLEA-----DLVSKMLRAV-LQSH-KNGIVLPRLQGEYRSLTGDWIP--FKQLG-----YPTLEAYLRSPAVVRIEASRSGEIVCYAVACTETARIAQLVARQRTSKRKIGRQINC----- 103
TDRD5 LOTUS1: 1 --MSEQ-----ERIQDCLRKEIRSL-LIST-KDGLTPQOLEKEYLLMVGNHLP--LRILG-----YRSTMELVLDMPDVVSVCPPGGDGTIVLKAIPDESTKGIASLVAKQRRSHKVRNS----- 103
EGGD-1 LOTUS1: 1 --MTEA--DDPK---KKIRARIERDVYSV-LLSF-QEGATPNQVERRMDDTLGYTYVGKYVQAG-----VRDMAQLLREHKDVRQN--LSG--VFSVVANEDNQDLINLISKQKKKKGGK----- 103
EGGD-2 LOTUS1: 1 --MSEEEGKEKM---NKMRKRIERDAYSV-LLSF-KDGASPKQVEQRMDDTLGYDYCSKYHLAG-----VYDMAQILIEHPDVRQT--YEG--KFKI IANEDNQDLVDLISKQSDGPRN----- 103
EGGD-1 LOTUS2: 1 KCMPSIV-----DFSNNVKRMMLLEY-PADVHLSELTEIYDKHFDVSIID-PLQLFSKSWMMFVKTSFKEWLQLG-----TDG--RVEV---KEPWFNTNFGAALKKSMPPAPEKKKPTTLH- 103
EGGD-2 LOTUS2: 1 SHMPTIT-----EFMNNTRKMMVKEL-PASVHLSEMTELYEREFGAPVD-PLLLFSKSWMLMAKTTTFKEFLQV-----IDG--EVSL---REKWLADQGTTSKSVLELPSRKVEATTVAQ 103
secondary structure:      hh      hhh hhhhhhhhhh hhh      hhhhhhhhhh      hhhhhh      hhhhhhhhhh      eeeee     hhhhhhhhhhhh
                        [red box] [red box] [red box] [red box] [red box] [red box] [red box] [red box]
                        α1      α2      α3 and α4      α5

```

B

C

Figure S4

Table S1. Proteins enriched by DEPS-1::TurboID and TurboID::IFE-1

Protein ID	Stable gene ID	Disorder (%)	Bait
AAGR-4	WBGene00018682	22.435	IFE-1
AARS-2	WBGene00000197	27.589	IFE-1
ABCE-1	WBGene00012714	22.922	DEPS-1
ABCF-3	WBGene00018339	30.594	DEPS-1
ACDH-13	WBGene00001566	17.785	DEPS-1
ACLY-2	WBGene00007150	23.134	DEPS-1
ALDO-2	WBGene00017166	25.604	IFE-1/DEPS-1
ALFA-1	WBGene00017547	24.405	IFE-1
ANI-1	WBGene00013038	54.068	IFE-1
ARX-3	WBGene00000201	15.878	IFE-1
ATX-2	WBGene00000231	71.787	IFE-1
BMK-1	WBGene00000257	42.078	IFE-1/DEPS-1
BUB-1	WBGene00000275	34.895	IFE-1
C27B7.5	WBGene00007762	55.849	IFE-1
C38D4.4/EGGD-1	WBGene00008005	48.513	IFE-1/DEPS-1
CASH-1	WBGene00010631	44.708	IFE-1/DEPS-1
CCT-8	WBGene00021934	26.353	DEPS-1
CDC-48.2	WBGene00008053	32.159	IFE-1
CDE-1	WBGene00019629	30.288	IFE-1
CEY-2	WBGene00000473	59.354	IFE-1/DEPS-1
CEY-3	WBGene00000474	47.647	IFE-1/DEPS-1
CLS-2	WBGene00000549	33.205	IFE-1
CPB-3	WBGene00000772	45.670	IFE-1/DEPS-1
CPF-2	WBGene00000774	62.558	IFE-1
CSN-1	WBGene00000813	32.717	DEPS-1
CSR-1	WBGene00017641	33.531	IFE-1/DEPS-1
CZW-1	WBGene00017643	14.885	IFE-1/DEPS-1
D2005.4	WBGene00008399	31.553	IFE-1/DEPS-1
DAF-18	WBGene00000913	40.114	DEPS-1
DCAP-1	WBGene00021929	36.774	IFE-1/DEPS-1
DCAP-2	WBGene00003582	49.940	IFE-1
DCR-1	WBGene00000939	28.061	DEPS-1
DDX-19	WBGene00011580	44.541	IFE-1
DEPS-1	WBGene00022034	26.478	DEPS-1
DNJ-11	WBGene00001029	43.988	IFE-1/DEPS-1
DRH-1	WBGene00001090	22.063	DEPS-1
DRH-3	WBGene00008400	25.466	IFE-1/DEPS-1
ECH-5	WBGene00001154	19.163	DEPS-1
EDC-3	WBGene00011036	39.163	IFE-1
EDC-4	WBGene00021551	31.835	IFE-1/DEPS-1
EGO-1	WBGene00001214	22.238	IFE-1/DEPS-1
EIF-2A	WBGene00008480	33.226	IFE-1/DEPS-1

EIF-3.H	WBGene00001231	29.227	DEPS-1
EKL-1	WBGene00009052	25.103	IFE-1/DEPS-1
EKL-6	WBGene00011806	14.842	IFE-1
EMB-5	WBGene00001259	36.002	DEPS-1
ERFA-3	WBGene00010405	33.883	IFE-1/DEPS-1
F01G4.4	WBGene00008503	62.340	IFE-1
F07B7.2	WBGene00017186	41.465	DEPS-1
F10G7.10	WBGene00017373	22.424	DEPS-1
F13D12.5	WBGene00008740	22.746	IFE-1/DEPS-1
F42A10.5	WBGene00018341	71.174	IFE-1
F42G9.1	WBGene00018362	50.389	IFE-1/DEPS-1
F46F11.1	WBGene00018508	37.958	IFE-1/DEPS-1
F52B5.3	WBGene00009922	37.892	IFE-1/DEPS-1
F52C6.2	WBGene00018659	46.107	IFE-1
F55A12.5	WBGene00018864	35.570	IFE-1/DEPS-1
F55F8.2	WBGene00018890	39.037	DEPS-1
F56B3.4	WBGene00004824	31.070	DEPS-1
F56C9.6	WBGene00018950	60.826	IFE-1
F58G11.3/EGGD-2	WBGene00010281	46.534	IFE-1/DEPS-1
FARD-1	WBGene00022200	12.998	IFE-1
FKB-6	WBGene00001431	37.393	DEPS-1
FRM-4	WBGene00001491	36.098	IFE-1
FUBL-2	WBGene00022738	54.544	IFE-1
GCN-1	WBGene00021697	19.249	DEPS-1
GLD-1	WBGene00001595	45.039	IFE-1/DEPS-1
GLD-2	WBGene00001596	60.607	IFE-1/DEPS-1
GLD-3	WBGene00001597	48.070	IFE-1
GLH-1	WBGene00001598	39.167	IFE-1/DEPS-1
GLH-2	WBGene00001599	45.086	IFE-1/DEPS-1
GLH-3	WBGene00001600	32.808	IFE-1/DEPS-1
GLH-4	WBGene00001601	48.884	IFE-1/DEPS-1
GLS-1	WBGene00007975	57.054	IFE-1
GPX-2	WBGene00011045	17.744	DEPS-1
GYF-1	WBGene00016002	62.005	IFE-1/DEPS-1
H05L14.2	WBGene00010367	36.498	IFE-1
H34C03.2	WBGene00019259	31.572	IFE-1/DEPS-1
HAF-9	WBGene00001819	17.316	IFE-1/DEPS-1
HCP-1	WBGene00001829	52.862	IFE-1/DEPS-1
HIP-1	WBGene00011735	58.625	IFE-1/DEPS-1
HPO-32	WBGene00015313	61.060	IFE-1
HPO-40	WBGene00014113	45.143	IFE-1/DEPS-1
HSP-110	WBGene00016250	34.442	DEPS-1
HSP-16.2	WBGene00002016	34.314	DEPS-1
IFE-1	WBGene00002059	24.172	IFE-1/DEPS-1
IFG-1	WBGene00002066	56.293	IFE-1

IMA-2	WBGene00002073	32.587	IFE-1/DEPS-1
INTS-5	WBGene00013075	10.263	DEPS-1
INTS-8	WBGene00013021	18.017	DEPS-1
ITSN-1	WBGene00006405	45.887	IFE-1/DEPS-1
K07H8.10	WBGene00019510	73.161	DEPS-1
K12D12.5	WBGene00010787	24.896	IFE-1
KLP-7	WBGene00002219	48.719	IFE-1
LEA-1	WBGene00002263	63.949	IFE-1/DEPS-1
LET-418	WBGene00002637	43.883	DEPS-1
LET-502	WBGene00002694	37.066	IFE-1
LET-711	WBGene00002845	27.144	IFE-1/DEPS-1
LOTR-1	WBGene00008385	33.454	IFE-1/DEPS-1
LPD-7	WBGene00003063	27.950	IFE-1
M01H9.3	WBGene00019719	59.714	IFE-1
MAP-2	WBGene00003130	34.482	IFE-1
MAPH-1.2	WBGene00009113	54.288	IFE-1/DEPS-1
MASK-1	WBGene00011240	47.096	IFE-1
MCT-3	WBGene00010834	15.594	DEPS-1
MDT-4	WBGene00007012	60.518	IFE-1/DEPS-1
MDT-6	WBGene00003164	42.333	DEPS-1
MEG-3	WBGene00001569	54.300	IFE-1
MEG-4	WBGene00016485	56.138	IFE-1
MEL-46	WBGene00020284	40.659	IFE-1
METR-1	WBGene00010988	21.967	IFE-1/DEPS-1
MEX-3	WBGene00003229	45.903	IFE-1/DEPS-1
MIX-1	WBGene00003367	36.995	IFE-1
MOG-4	WBGene00003392	33.947	IFE-1
MOG-5	WBGene00003393	39.478	IFE-1
NAP-1	WBGene00017075	32.757	DEPS-1
NASP-2	WBGene00008218	61.975	IFE-1/DEPS-1
NATC-1	WBGene00020719	22.441	IFE-1/DEPS-1
NHL-2	WBGene00003598	41.222	IFE-1
NPP-10	WBGene00003796	42.909	IFE-1
NPP-13	WBGene00003799	26.928	IFE-1
NPP-14	WBGene00003800	48.520	IFE-1/DEPS-1
NPP-21	WBGene00019940	60.111	IFE-1
NPP-24	WBGene00022672	22.465	DEPS-1
NPP-4	WBGene00003790	43.775	IFE-1
NPP-6	WBGene00003792	22.285	IFE-1/DEPS-1
NPP-7	WBGene00003793	57.434	IFE-1
NPP-9	WBGene00003795	48.118	IFE-1/DEPS-1
NUD-1	WBGene00003829	45.597	DEPS-1
OMA-1	WBGene00003864	45.403	IFE-1
PAT-4	WBGene00003931	21.340	IFE-1
PERM-4	WBGene00016638	45.917	DEPS-1

PFD-2	WBGene00019220	40.912	DEPS-1
PGL-1	WBGene00003992	34.188	IFE-1/DEPS-1
PGL-2	WBGene00003993	18.808	IFE-1
PGL-3	WBGene00003994	34.577	IFE-1/DEPS-1
PID-3	WBGene00021270	21.150	IFE-1
PID-5	WBGene00021555	22.426	IFE-1/DEPS-1
PLK-1	WBGene00004042	32.578	IFE-1
PPFR-4	WBGene00022193	40.587	IFE-1/DEPS-1
PPW-1	WBGene00004093	25.101	IFE-1/DEPS-1
PPW-2	WBGene00004094	29.475	IFE-1/DEPS-1
PQN-59	WBGene00004143	72.500	IFE-1/DEPS-1
PQN-87	WBGene00004167	58.976	IFE-1
PRG-1	WBGene00004178	27.052	IFE-1/DEPS-1
PUF-3	WBGene00004239	21.936	IFE-1
PUF-5	WBGene00004241	16.963	IFE-1/DEPS-1
R05F9.6	WBGene00019890	20.729	DEPS-1
R09E10.6	WBGene00011176	32.845	DEPS-1
R74.8	WBGene00011282	30.281	IFE-1
RAN-2	WBGene00004303	24.664	IFE-1
RBM-39	WBGene00021921	52.407	IFE-1
RDE-12	WBGene00010280	59.068	IFE-1/DEPS-1
RHA-1	WBGene00004355	33.150	IFE-1
RPAC-40	WBGene00019275	27.143	DEPS-1
RPB-2	WBGene00016140	27.990	IFE-1
RPL-32	WBGene00004446	43.596	IFE-1
RPN-11	WBGene00004467	28.425	IFE-1/DEPS-1
RPN-2	WBGene00004459	25.854	IFE-1/DEPS-1
RPN-9	WBGene00004465	15.222	IFE-1/DEPS-1
SAO-1	WBGene00011195	55.711	DEPS-1
SART-3	WBGene00007111	32.965	IFE-1
SEC-6	WBGene00017284	24.931	IFE-1/DEPS-1
SKR-7	WBGene00004813	36.492	IFE-1/DEPS-1
SMC-4	WBGene00004874	46.578	IFE-1/DEPS-1
SMG-2	WBGene00004880	34.254	IFE-1
SNA-2	WBGene00011747	43.893	IFE-1
SNAP-29	WBGene00019305	67.567	IFE-1
SPE-15	WBGene00004969	27.017	DEPS-1
SPTL-3	WBGene00011932	19.441	IFE-1
SQV-4	WBGene00005022	17.047	IFE-1/DEPS-1
SYF-1	WBGene00016837	24.305	IFE-1
T02G5.7	WBGene00020166	20.461	DEPS-1
T04C9.1	WBGene00020209	32.314	IFE-1
T05F1.2	WBGene00011489	31.788	DEPS-1
T05H10.1	WBGene00011507	33.117	DEPS-1
T20B12.7	WBGene00020604	28.978	DEPS-1

T22B7.7	WBGene00020674	27.879	DEPS-1
T23H2.3	WBGene00020742	45.670	DEPS-1
T26A5.2	WBGene00020819	30.578	IFE-1
TANK-1	WBGene00004053	29.619	DEPS-1
TCC-1	WBGene00013340	53.895	IFE-1
TIAR-1	WBGene00015943	46.127	IFE-1/DEPS-1
TTC-1	WBGene00016390	43.366	DEPS-1
TXL-1	WBGene00021826	22.706	DEPS-1
UBH-4	WBGene00006724	28.172	IFE-1
UBXN-6	WBGene00019163	36.318	IFE-1
ULE-5	WBGene00013394	39.522	DEPS-1
ULP-1	WBGene00006736	40.548	IFE-1
USO-1	WBGene00010713	25.882	IFE-1/DEPS-1
W07E11.1	WBGene00012326	25.882	IFE-1/DEPS-1
W09D10.1	WBGene00012359	48.492	IFE-1
WAGO-1	WBGene00011061	31.447	IFE-1/DEPS-1
WAGO-4	WBGene00010263	26.335	IFE-1/DEPS-1
XRN-1	WBGene00012730	41.068	IFE-1/DEPS-1
Y110A7A.15	WBGene00022462	23.218	IFE-1/DEPS-1
Y111B2A.3	WBGene00013729	25.825	IFE-1/DEPS-1
Y38E10A.17	WBGene00012595	45.381	IFE-1
Y39A3CL.4	WBGene00021442	31.311	IFE-1/DEPS-1
Y66D12A.19	WBGene00013445	56.269	IFE-1
Y71F9AL.12	WBGene00022117	38.997	DEPS-1
Y75B8A.25	WBGene00013558	45.241	IFE-1
ZNFX-1	WBGene00014208	24.829	IFE-1/DEPS-1
ZWL-1	WBGene00021460	23.625	IFE-1

Table S2: Significantly enriched Gene Ontology terms for TurboID hits

Domain	term_name	term_id	Adjusted_p_value	intersections
Molecular Function	RNA binding	GO:0003723	2.07192E-06	WBGENE00000772, WBGENE00021929, WBGENE00008400, WBGENE00001214, WBGENE00008480, WBGENE0010405, WBGENE00009922, WBGENE00001595, WBGENE00002059, WBGENE00003229, WBGENE00003992, WBGENE00004178, WBGENE00004241, WBGENE00001280, WBGENE00015943, WBGENE0001263, WBGENE00012730, WBGENE00014208
Molecular Function	nucleic acid binding	GO:0003676	5.29683E-05	WBGENE00000473, WBGENE00000474, WBGENE00000772, WBGENE00017641, WBGENE00021929, WBGENE00008400, WBGENE00001214, WBGENE00008480, WBGENE00010405, WBGENE00009922, WBGENE00001595, WBGENE00001598, WBGENE00001599, WBGENE00001600, WBGENE00001601, WBGENE00002059, WBGENE00003229, WBGENE00003992, WBGENE00004093, WBGENE00004094, WBGENE00004178, WBGENE00004241, WBGENE00001280, WBGENE00015943, WBGENE00011061, WBGENE00010263, WBGENE00012730, WBGENE00014208
Molecular Function	helicase activity	GO:0004386	6.00079E-05	WBGENE00008400, WBGENE00009922, WBGENE00001598, WBGENE00001599, WBGENE00001600, WBGENE0001601, WBGENE0001280, WBGENE00014208
Molecular Function	ATPase, acting on DNA	GO:0008094	0.000202981	WBGENE00008400, WBGENE00009922, WBGENE00001598, WBGENE00001599, WBGENE00001600, WBGENE0001601, WBGENE0001280, WBGENE00014208
Molecular Function	catalytic activity, acting on DNA	GO:0140097	0.002195264	WBGENE00008400, WBGENE00009922, WBGENE00001598, WBGENE00001599, WBGENE00001600, WBGENE0001601, WBGENE0001280, WBGENE00014208
Molecular Function	ATPase	GO:0016887	0.002834403	WBGENE00000257, WBGENE00008400, WBGENE00009922, WBGENE00001598, WBGENE00001599, WBGENE00001600, WBGENE00001601, WBGENE00001819, WBGENE0001280, WBGENE00004874, WBGENE00014208
Molecular Function	binding	GO:0005488	0.003305502	WBGENE00000257, WBGENE00000473, WBGENE00000474, WBGENE00000772, WBGENE000017641, WBGENE00017643, WBGENE00021929, WBGENE00001029, WBGENE00008400, WBGENE000021551, WBGENE00001214, WBGENE00008480, WBGENE00009052, WBGENE00010405, WBGENE00018362, WBGENE00018508, WBGENE00009922, WBGENE00001595, WBGENE00001598, WBGENE00001599, WBGENE00001600, WBGENE0001601, WBGENE00001819, WBGENE00002059, WBGENE00001829, WBGENE00001735, WBGENE00002059, WBGENE00002073, WBGENE00006045, WBGENE00002263, WBGENE000090913, WBGENE00010988, WBGENE00003229, WBGENE00008218, WBGENE00003800, WBGENE00003795, WBGENE00003992, WBGENE00004093, WBGENE00004094, WBGENE00004143, WBGENE00004178, WBGENE00004241, WBGENE00010280, WBGENE00004467, WBGENE00004465, WBGENE00004813, WBGENE00004874, WBGENE00005022, WBGENE00015943, WBGENE00010713, WBGENE00012326, WBGENE00011061, WBGENE00010263, WBGENE00012730, WBGENE00014208
Molecular Function	heterocyclic compound binding	GO:1901363	0.00472517	WBGENE00000257, WBGENE00000473, WBGENE00000474, WBGENE00000772, WBGENE000017641, WBGENE00021929, WBGENE00008400, WBGENE00001214, WBGENE00008480, WBGENE00018508, WBGENE00009922, WBGENE00001595, WBGENE00001598, WBGENE00001599, WBGENE00001600, WBGENE0001601, WBGENE00001819, WBGENE00002059, WBGENE00010988, WBGENE00003229, WBGENE00003992, WBGENE00004093, WBGENE00004094, WBGENE00004178, WBGENE00004241, WBGENE00010280, WBGENE00004874, WBGENE00005022, WBGENE00015943, WBGENE00012326, WBGENE00011061, WBGENE00010263, WBGENE00012730, WBGENE00014208
Molecular Function	organic cyclic compound binding	GO:0097159	0.005071418	WBGENE00000257, WBGENE00000473, WBGENE00000474, WBGENE00000772, WBGENE000017641, WBGENE00021929, WBGENE00008400, WBGENE00001214, WBGENE00008480, WBGENE00018508, WBGENE00009922, WBGENE00001595, WBGENE00001598, WBGENE00001599, WBGENE00001600, WBGENE0001601, WBGENE00001819, WBGENE00002059, WBGENE00010988, WBGENE00003229, WBGENE00003992, WBGENE00004093, WBGENE00004094, WBGENE00004178, WBGENE00004241, WBGENE00010280, WBGENE00004874, WBGENE00005022, WBGENE00015943, WBGENE00012326, WBGENE00011061, WBGENE00010263, WBGENE00012730, WBGENE00014208
Biological Process	negative regulation of gene expression	GO:0010629	2.05E-10	WBGENE00017641, WBGENE00021929, WBGENE00008400, WBGENE00021551, WBGENE00001214, WBGENE00009052, WBGENE00001819, WBGENE00002845, WBGENE00003229, WBGENE000010280, WBGENE00015943, WBGENE00011061, WBGENE00010263, WBGENE00012730, WBGENE00014208
Biological Process	gene silencing by RNA	GO:0031047	1.46E-07	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00001819, WBGENE00010280, WBGENE00011061, WBGENE00010263, WBGENE00014208
Biological Process	posttranscriptional regulation of gene expression	GO:0010608	4.98E-07	WBGENE00000772, WBGENE00017641, WBGENE00021929, WBGENE00008400, WBGENE00001214, WBGENE00001595, WBGENE00001819, WBGENE00002845, WBGENE00003229, WBGENE00010280, WBGENE00015943, WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00001819, WBGENE00010280, WBGENE00011061, WBGENE00010263, WBGENE00014208
Biological Process	gene silencing	GO:0016458	1.09471E-06	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00001819, WBGENE00010280, WBGENE00011061, WBGENE00010263, WBGENE00014208
Biological Process	oocyte construction	GO:0007308	3.33148E-06	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00003992
Biological Process	oocyte anterior/posterior axis specification	GO:0007314	3.33148E-06	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00003992
Biological Process	maternal determination of anterior/posterior axis	GO:0008358	3.33148E-06	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00003992
Biological Process	pole plasm assembly	GO:0007315	3.33148E-06	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00003992
Biological Process	oocyte axis specification	GO:0007309	3.33148E-06	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00003992
Biological Process	P granule organization	GO:0030719	3.33148E-06	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00003992
Biological Process	anatomical structure maturation	GO:0071695	8.05529E-06	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00002059, WBGENE00003992
Biological Process	cell maturation	GO:0048469	8.05529E-06	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00003992
Biological Process	cytoplasm organization	GO:0007028	8.34498E-06	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00003992
Biological Process	embryonic pattern specification	GO:0009880	1.01102E-05	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00003229, WBGENE00003992
Biological Process	negative regulation of macromolecule metabolism	GO:0010605	1.05027E-05	WBGENE00017641, WBGENE00021929, WBGENE00008400, WBGENE00021551, WBGENE00001214, WBGENE00009052, WBGENE00001819, WBGENE00002845, WBGENE00003229, WBGENE00010280, WBGENE00015943, WBGENE00011061, WBGENE00010263, WBGENE00012730, WBGENE00014208
Biological Process	negative regulation of metabolic process	GO:0009892	1.43659E-05	WBGENE00017641, WBGENE00021929, WBGENE00008400, WBGENE00021551, WBGENE00001214, WBGENE00009052, WBGENE00001819, WBGENE00002845, WBGENE00003229, WBGENE00010280, WBGENE00015943, WBGENE00011061, WBGENE00010263, WBGENE00012730, WBGENE00014208
Biological Process	oogenesis	GO:0048477	1.57527E-05	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00002059, WBGENE00002845, WBGENE00003992, WBGENE00004178
Biological Process	germ cell development	GO:0007281	2.68658E-05	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00002059, WBGENE00002845, WBGENE00003992, WBGENE00004178
Biological Process	negative regulation of biological process	GO:0048519	2.70853E-05	WBGENE00000257, WBGENE00017641, WBGENE00017643, WBGENE00021929, WBGENE00008400, WBGENE00021551, WBGENE00001214, WBGENE00009052, WBGENE00001819, WBGENE00002845, WBGENE00003229, WBGENE00010280, WBGENE00004813, WBGENE000015943, WBGENE00011061, WBGENE00010263, WBGENE00012730, WBGENE00014208
Biological Process	developmental maturation	GO:0021700	2.7976E-05	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00002059, WBGENE00003992
Biological Process	organelle organization	GO:0006996	3.8901E-05	WBGENE00000257, WBGENE00010631, WBGENE00017641, WBGENE00017643, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE0001829, WBGENE00002845, WBGENE00009113, WBGENE00003229, WBGENE0000218, WBGENE00001792, WBGENE00003992, WBGENE00004874, WBGENE00015943, WBGENE00010713, WBGENE00010263, WBGENE00012730, WBGENE00014208
Biological Process	oocyte differentiation	GO:0009994	4.01473E-05	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00002059, WBGENE00003992
Biological Process	oocyte development	GO:0048599	4.01473E-05	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00002059, WBGENE00003992
Biological Process	gamete generation	GO:0007276	5.58514E-05	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00002059, WBGENE00002845, WBGENE00003992, WBGENE00004178, WBGENE00010280
Biological Process	cellular component organization	GO:0016043	7.01758E-05	WBGENE00000257, WBGENE00010631, WBGENE00017641, WBGENE00017643, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00010405, WBGENE00001829, WBGENE00011735, WBGENE00006045, WBGENE00002845, WBGENE00009113, WBGENE00003229, WBGENE00008218, WBGENE00003792, WBGENE00003795, WBGENE00003992, WBGENE00004178, WBGENE00004465, WBGENE00004874, WBGENE00015943, WBGENE00010713, WBGENE00010263, WBGENE00012730, WBGENE00014208
Biological Process	anterior/posterior axis specification, embryonic	GO:0008595	8.2164E-05	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00003992
Biological Process	tripartite regional subdivision	GO:0007351	8.2164E-05	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00003992
Biological Process	embryonic axis specification	GO:0000578	8.2164E-05	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00003992
Biological Process	cellular process involved in reproduction in P granule assembly	GO:0022412	9.41411E-05	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE00002059, WBGENE00002845, WBGENE00003992, WBGENE00004178
Biological Process	P granule assembly	GO:1903863	9.6297E-05	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052

Biological Process	female gamete generation	GO:0007292	0.000137976	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00002059, WBGene00002845, WBGene00003992
Biological Process	segmentation	GO:0035282	0.000169295	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00003992
Biological Process	blastoderm segmentation	GO:0007350	0.000169295	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00003992
Biological Process	reproduction	GO:0000003	0.000379609	WBGene00017641, WBGene000021929, WBGene000008400, WBGene00001214, WBGene000009052, WBGene0001829, WBGene00002059, WBGene00002845, WBGene00003792, WBGene00003992, WBGene00004178, WBGene00010280, WBGene00004874, WBGene00005022, WBGene00015943
Biological Process	cellular component organization or biogenesis	GO:0071840	0.000395528	WBGene00000257, WBGene00010631, WBGene00017641, WBGene00017643, WBGene000008400, WBGene0001214, WBGene000009052, WBGene00010405, WBGene0001829, WBGene00011735, WBGene000006405, WBGene00002845, WBGene00009113, WBGene00003229, WBGene000008218, WBGene00003792, WBGene00003795, WBGene00003992, WBGene00004178, WBGene00004465, WBGene00004874, WBGene00015943, WBGene00010713, WBGene00010263, WBGene00012730, WBGene00014208
Biological Process	anterior/posterior axis specification	GO:0009948	0.000462378	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00003992
Biological Process	sexual reproduction	GO:0019953	0.000521071	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00002059, WBGene00002845, WBGene00003992, WBGene00004178, WBGene00010280
Biological Process	developmental process	GO:0032502	0.000639192	WBGene00010631, WBGene00017641, WBGene000021929, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00001819, WBGene00002059, WBGene00002845, WBGene00009113, WBGene00003229, WBGene000020719, WBGene00003792, WBGene00003795, WBGene00003992, WBGene00004178, WBGene00010280, WBGene00004874, WBGene00005022, WBGene00015943, WBGene00011061, WBGene00012730
Biological Process	multi-organism reproductive process	GO:0044703	0.00083288	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00002059, WBGene00002845, WBGene00003992, WBGene00004178, WBGene00010280
Biological Process	multi-organism process	GO:0051704	0.00083288	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00002059, WBGene00002845, WBGene00003992, WBGene00004178, WBGene00010280
Biological Process	multicellular organismal reproductive process	GO:0048609	0.000835615	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00002059, WBGene00002845, WBGene00003992, WBGene00004178, WBGene00010280, WBGene00005022
Biological Process	axis specification	GO:0009798	0.001229972	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00003992
Biological Process	multicellular organism reproduction	GO:0032504	0.001613892	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00002059, WBGene00002845, WBGene00003992, WBGene00004178, WBGene00010280, WBGene00005022
Biological Process	pattern specification process	GO:0007389	0.00178449	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00003229, WBGene00003992
Biological Process	anatomical structure development	GO:0048856	0.002155765	WBGene00010631, WBGene00017641, WBGene000021929, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00002059, WBGene00002845, WBGene00009113, WBGene00003229, WBGene000020719, WBGene00003792, WBGene00003795, WBGene00003992, WBGene00004178, WBGene00004874, WBGene00005022, WBGene00015943, WBGene00011061, WBGene00012730
Biological Process	anterior/posterior pattern specification	GO:0009952	0.002437077	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00003992
Biological Process	mitotic nuclear division	GO:0140014	0.002961395	WBGene00000257, WBGene00017641, WBGene00017643, WBGene00001829, WBGene00004178, WBGene00004874
Biological Process	RNA interference	GO:0016246	0.00312214	WBGene00017641, WBGene000008400, WBGene00001214, WBGene00001819, WBGene00010280
Biological Process	reproductive process	GO:0022414	0.004562864	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00002059, WBGene00002845, WBGene00003992, WBGene00004178, WBGene00010280, WBGene00004874, WBGene00005022
Biological Process	developmental process involved in reproduction	GO:0003006	0.004894145	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00002059, WBGene00002845, WBGene00003992, WBGene00004178, WBGene00004874
Biological Process	DNA packaging	GO:0006323	0.00550348	WBGene00001214, WBGene000008218, WBGene00004874, WBGene00010263, WBGene00014208
Biological Process	cellular component assembly	GO:0022607	0.005535603	WBGene00000257, WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene0001829, WBGene00011735, WBGene000008218, WBGene00003992, WBGene00004465, WBGene00015943, WBGene00010263, WBGene00012730, WBGene00014208
Biological Process	regulation of cellular amide metabolic process	GO:0034248	0.006272073	WBGene00000772, WBGene000021929, WBGene00001595, WBGene00002845, WBGene00003229, WBGene00010280
Biological Process	regulation of translation	GO:0006417	0.006272073	WBGene00000772, WBGene000021929, WBGene00001595, WBGene00002845, WBGene00003229, WBGene00010280
Biological Process	regulation of gene expression	GO:0010468	0.006696267	WBGene00000473, WBGene00000474, WBGene00000772, WBGene00017641, WBGene000021929, WBGene000008400, WBGene000021551, WBGene00001214, WBGene000009052, WBGene00001595, WBGene00001819, WBGene00002845, WBGene00007012, WBGene00003229, WBGene00010280, WBGene00015943, WBGene00011061, WBGene00010263, WBGene00012730, WBGene00014208
Biological Process	multicellular organism development	GO:0007275	0.007035631	WBGene00010631, WBGene00017641, WBGene000021929, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00002059, WBGene00002845, WBGene00009113, WBGene00003229, WBGene00003792, WBGene00003795, WBGene00003992, WBGene00004874, WBGene00005022, WBGene00015943, WBGene00011061, WBGene00012730
Biological Process	heterochromatin assembly by small RNA	GO:0031048	0.007559293	WBGene00001214, WBGene00010263, WBGene00014208
Biological Process	regionalization	GO:0003002	0.008276267	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00003992
Biological Process	dsRNA processing	GO:0031050	0.010593275	WBGene000008400, WBGene00001214, WBGene000009052, WBGene00010280
Biological Process	production of small RNA involved in gene silencing	GO:0070918	0.010593275	WBGene000008400, WBGene00001214, WBGene000009052, WBGene00010280
Biological Process	post-transcriptional gene silencing by RNA	GO:0035194	0.013155992	WBGene00017641, WBGene000008400, WBGene00001214, WBGene00001819, WBGene00010280
Biological Process	posttranscriptional gene silencing	GO:0016441	0.013155992	WBGene00017641, WBGene000008400, WBGene00001214, WBGene00001819, WBGene00010280
Biological Process	organelle assembly	GO:0070925	0.016809643	WBGene00000257, WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene0001829, WBGene00015943, WBGene00012730
Biological Process	mitotic sister chromatid segregation	GO:0000070	0.017009478	WBGene00000257, WBGene00017641, WBGene00017643, WBGene00001829, WBGene00004874
Biological Process	heterochromatin assembly	GO:0031507	0.021990444	WBGene00001214, WBGene00010263, WBGene00014208
Biological Process	chromatin assembly	GO:0031497	0.024809995	WBGene00001214, WBGene000008218, WBGene00010263, WBGene00014208
Biological Process	mitotic cell cycle process	GO:1903047	0.025821342	WBGene00000257, WBGene00017641, WBGene00017643, WBGene00001829, WBGene00002845, WBGene00004874
Biological Process	heterochromatin organization	GO:0070828	0.029196162	WBGene00001214, WBGene00010263, WBGene00014208
Biological Process	nuclear-transcribed mRNA catabolic process	GO:0000956	0.029414231	WBGene000021929, WBGene000021551, WBGene00002845, WBGene00015943, WBGene00012730
Biological Process	cell development	GO:0048468	0.033149687	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00002059, WBGene00002845, WBGene00009113, WBGene00003992, WBGene00004178
Biological Process	regulation of macromolecule metabolic process	GO:0060255	0.038735749	WBGene00000473, WBGene00000474, WBGene00000772, WBGene00017641, WBGene000021929, WBGene000008400, WBGene000021551, WBGene00001214, WBGene000009052, WBGene00001595, WBGene00001819, WBGene00002845, WBGene00007012, WBGene00003229, WBGene00010280, WBGene00004459, WBGene00015943, WBGene00011061, WBGene00010263, WBGene00012730, WBGene00014208
Biological Process	chromatin assembly or disassembly	GO:0006333	0.0399247	WBGene00001214, WBGene000008218, WBGene00010263, WBGene00014208
Biological Process	sister chromatid segregation	GO:0000819	0.041891373	WBGene00000257, WBGene00017641, WBGene00017643, WBGene00001829, WBGene00004874
Biological Process	chromatin remodeling	GO:0006338	0.044565682	WBGene00001214, WBGene000008218, WBGene00010263, WBGene00014208
Biological Process	embryo development	GO:0009790	0.045424236	WBGene00017641, WBGene000008400, WBGene00001214, WBGene000009052, WBGene00003229, WBGene00003792, WBGene00003795, WBGene00003992, WBGene00005022
Cellular Compartment	cytoplasmic ribonucleoprotein granule	GO:0036464	3.02E-18	WBGene00017641, WBGene000021929, WBGene000008400, WBGene00001214, WBGene000009052, WBGene0001829, WBGene00002059, WBGene00002845, WBGene00003229, WBGene00003992, WBGene00004178, WBGene00010280, WBGene00015943, WBGene00011061
Cellular Compartment	ribonucleoprotein granule	GO:0035770	3.72E-18	WBGene00017641, WBGene000021929, WBGene000008400, WBGene000021551, WBGene00001214, WBGene00001819, WBGene00002059, WBGene00002845, WBGene00003229, WBGene00003992, WBGene00004178, WBGene00010280, WBGene00015943, WBGene00011061
Cellular Compartment	P granule	GO:0043186	5.52E-16	WBGene00017641, WBGene000021929, WBGene000008400, WBGene00001214, WBGene00002059, WBGene00003229, WBGene00003992, WBGene00004178, WBGene00010280, WBGene00015943, WBGene00011061
Cellular Compartment	germ plasm	GO:0060293	5.52E-16	WBGene00017641, WBGene000021929, WBGene000008400, WBGene00001214, WBGene00002059, WBGene00003229, WBGene00003992, WBGene00004178, WBGene00010280, WBGene00015943, WBGene00011061
Cellular Compartment	pole plasm	GO:0045495	5.52E-16	WBGene00017641, WBGene000021929, WBGene000008400, WBGene00001214, WBGene00002059, WBGene00003229, WBGene00003992, WBGene00004178, WBGene00010280, WBGene00015943, WBGene00011061
Cellular Compartment	supramolecular complex	GO:0099080	1.24E-15	WBGene00000257, WBGene00017641, WBGene00017643, WBGene000021929, WBGene000008400, WBGene000021551, WBGene00001214, WBGene00001819, WBGene00002059, WBGene00002845, WBGene00009113, WBGene00003229, WBGene00003992, WBGene00004178, WBGene00010280, WBGene00015943, WBGene00011061

Cellular Compartment	intracellular anatomical structure	GO:0005622	5.69E-08	WBGENE00000257, WBGENE00000473, WBGENE00000474, WBGENE00017641, WBGENE00017643, WBGENE0001929, WBGENE0001029, WBGENE00008400, WBGENE00021551, WBGENE0001214, WBGENE00009052, WBGENE00010405, WBGENE00018508, WBGENE0001819, WBGENE0001829, WBGENE00011735, WBGENE00014113, WBGENE00002059, WBGENE00002073, WBGENE00006405, WBGENE00002845, WBGENE00009113, WBGENE00007012, WBGENE00003229, WBGENE00008218, WBGENE000020719, WBGENE00003792, WBGENE00003795, WBGENE00003992, WBGENE00004143, WBGENE00004178, WBGENE00010280, WBGENE00004465, WBGENE00017284, WBGENE00004813, WBGENE00004874, WBGENE00005022, WBGENE00015943, WBGENE00010713, WBGENE00011061, WBGENE00010263, WBGENE00012730, WBGENE00014208
Cellular Compartment	metaphase plate	GO:0070090	2.00E-07	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052
Cellular Compartment	cytoplasm	GO:0005737	2.40E-07	WBGENE00017641, WBGENE00017643, WBGENE00021929, WBGENE00001029, WBGENE00008400, WBGENE00021551, WBGENE00001214, WBGENE00009052, WBGENE00010405, WBGENE00018508, WBGENE0001819, WBGENE00011735, WBGENE00014113, WBGENE00002059, WBGENE00002073, WBGENE00006405, WBGENE00002845, WBGENE00009113, WBGENE00003229, WBGENE000020719, WBGENE00003795, WBGENE00003992, WBGENE00004143, WBGENE00004178, WBGENE00010280, WBGENE00004465, WBGENE00017284, WBGENE00004813, WBGENE00005022, WBGENE00015943, WBGENE00010713, WBGENE00011061, WBGENE00010263, WBGENE00012730, WBGENE00014208
Cellular Compartment	intracellular non-membrane-bounded orga	GO:0043232	3.56E-07	WBGENE00000257, WBGENE00017641, WBGENE00017643, WBGENE00021929, WBGENE00008400, WBGENE00021551, WBGENE00001214, WBGENE00009052, WBGENE0001829, WBGENE00011735, WBGENE00014113, WBGENE00002059, WBGENE00002845, WBGENE00009113, WBGENE00003229, WBGENE00004874, WBGENE00015943, WBGENE00011061
Cellular Compartment	non-membrane-bounded organelle	GO:0043228	3.56E-07	WBGENE00000257, WBGENE00017641, WBGENE00017643, WBGENE00021929, WBGENE00008400, WBGENE00021551, WBGENE00001214, WBGENE00009052, WBGENE0001829, WBGENE00011735, WBGENE00014113, WBGENE00002059, WBGENE00002845, WBGENE00009113, WBGENE00003229, WBGENE00004874, WBGENE00015943, WBGENE00011061
Cellular Compartment	condensed chromosome	GO:0000793	2.21025E-05	WBGENE00017641, WBGENE00017643, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE0001829, WBGENE00004874
Cellular Compartment	intracellular organelle	GO:0043229	9.69046E-05	WBGENE00000257, WBGENE00000473, WBGENE00000474, WBGENE00017641, WBGENE00017643, WBGENE00021929, WBGENE00008400, WBGENE00021551, WBGENE00001214, WBGENE00009052, WBGENE0001829, WBGENE00011735, WBGENE00014113, WBGENE00002059, WBGENE00006405, WBGENE00002845, WBGENE00009113, WBGENE00007012, WBGENE00003229, WBGENE00008218, WBGENE00003792, WBGENE00003795, WBGENE00003992, WBGENE00004143, WBGENE00004178, WBGENE00010280, WBGENE00004465, WBGENE00004813, WBGENE00004874, WBGENE00005022, WBGENE00015943, WBGENE00010713, WBGENE00011061, WBGENE00012730, WBGENE00014208
Cellular Compartment	organelle	GO:0043226	0.000156429	WBGENE00000257, WBGENE00000473, WBGENE00000474, WBGENE00017641, WBGENE00017643, WBGENE00021929, WBGENE00008400, WBGENE00021551, WBGENE00001214, WBGENE00009052, WBGENE0001829, WBGENE00011735, WBGENE00014113, WBGENE00002059, WBGENE00006405, WBGENE00002845, WBGENE00009113, WBGENE00007012, WBGENE00003229, WBGENE00008218, WBGENE00003792, WBGENE00003795, WBGENE00003992, WBGENE00004143, WBGENE00004178, WBGENE00010280, WBGENE00004465, WBGENE00004813, WBGENE00004874, WBGENE00005022, WBGENE00015943, WBGENE00010713, WBGENE00011061, WBGENE00012730, WBGENE00014208
Cellular Compartment	P-body	GO:0000932	0.00033481	WBGENE00021929, WBGENE00021551, WBGENE00002845, WBGENE00010280
Cellular Compartment	nucleus	GO:0005634	0.000868445	WBGENE00000257, WBGENE00000473, WBGENE00000474, WBGENE00017641, WBGENE00017643, WBGENE00008400, WBGENE00001214, WBGENE00009052, WBGENE0001829, WBGENE00002845, WBGENE00007012, WBGENE00008218, WBGENE00003792, WBGENE00003795, WBGENE00004143, WBGENE00010280, WBGENE00004465, WBGENE00004813, WBGENE00004874, WBGENE00005022, WBGENE00015943, WBGENE00012730, WBGENE00014208
Cellular Compartment	condensed nuclear chromosome	GO:0000794	0.002137633	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052
Cellular Compartment	nuclear RNA-directed RNA polymerase co	GO:0031380	0.02387864	WBGENE00001214, WBGENE00014208
Cellular Compartment	RNA-directed RNA polymerase complex	GO:0031379	0.02387864	WBGENE00001214, WBGENE00014208
Cellular Compartment	perinuclear region of cytoplasm	GO:0048471	0.035149172	WBGENE00014113, WBGENE00010280, WBGENE00010263, WBGENE00014208
Cellular Compartment	nuclear chromosome	GO:0000228	0.03704086	WBGENE00017641, WBGENE00008400, WBGENE00001214, WBGENE00009052