Tudor domain containing protein 5-like (*Tdrd5l*) identifies a novel germline body and regulates maternal RNAs during oogenesis

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Short title: Tdrd5l regulates maternally deposited RNAs

Key words: Tudor-domain, germ cell, maternal RNA, Drosophila, Tdrd5l, Tdrd5, grk, orb

Abstract

Tudor domain-containing proteins are conserved across the animal kingdom for their function in germline development and fertility. Previously, we demonstrated that *Tudor domain-containing protein 5-like (Tdrd5l)* plays an important role in the germline where it promotes male identity. However, Tdrd5l is also expressed in both the ovary and testis during later stages of germline development, suggesting that it plays a role in germline differentiation in both sexes. We found that Tdrd5l localizes to a potentially novel germline body and plays a role in post-transcriptional gene regulation. RNA sequencing of *Tdrd5l*-mutant ovaries compared to wild-type showed that differentially expressed genes were enriched for maternally deposited RNAs. Additionally, embryos laid by *Tdrd5l*-mutant females exhibited reduced viability and displayed dorsal appendage defects suggesting a failure of proper dorsal-ventral (D/V) patterning. As D/V patterning is dependent on *gurken (grk),* we examined Grk expression during oogenesis. We observed premature accumulation of Grk protein in nurse cells indicating that translation is no longer properly repressed during mRNA transport to the oocyte. We also observed increased nurse cell accumulation of the cytoplasmic polyadenylation element binding protein Oo18 RNA-Binding Protein (Orb or CPEB), a translational activator of Grk. Decreasing *orb* function was able to partially rescue the *Tdrd5l*-mutant phenotype, and so defects in Orb are likely a primary cause of the defects in *Tdrd5l* mutants. Our data indicate that *Tdrd5l* is important for translational repression of maternal mRNAs such as *orb*, and possibly others, following their synthesis in the nurse cells and during their transport to the oocyte.

Introduction

50 Tudor domain-containing proteins are conserved across the animal kingdom and one important
51 function of these proteins is germline specification and maintenance (1). Mutations in the
52 genes for Tudor proteins have been shown to cause infertility in flies and mammals alike (2,3).
53 While the molecular function of a tudor domain is to promote protein-protein interaction by
54 binding to dimethylated arginine and lysine residues (4,5), many tudor domain-containing
55 proteins also have additional domains such as RNA recognition motifs (6). Together this allows
56 for tudor domains to scaffold larger protein and ribonucleoprotein complexes (RNPs) such as in
57 RNA granules.

58 RNA granules allow for multiple modes of RNA regulation in locally concentrated and
59 protected membrane-less organelles (7). One germline-specific granule that is enriched for
60 tudor domain-containing proteins is the nuage (8–10), which produces piRNAs that silence
61 transposons (11). In addition to the nuage, germ cells have a number of other types of granules
62 that can also be found in other tissues, the most common of which are the processing bodies
63 (P-bodies) (12). These granules can take on different functions all centered around post-
64 transcriptional regulation of mRNAs such as inducing mRNA degradation via removal of the 5'
65 cap and translational repression via regulation of the poly(A) tail. mRNAs are often targeted to
66 P-bodies through binding of miRNAs which leads to the formation of a protein complex around
67 the RNA (13,14). RNA granules are particularly important in the germline where RNAs are often
68 transcribed at a different time or place from their translation (15). mRNAs can be sent to these
69 granules for repression until they are needed for translation or to form RNPs that are
70 translationally repressed and targeted for transport to another location (16).

71 One special class of germline RNAs are the maternal RNAs generated during oogenesis
72 and deposited into the oocyte for control of early embryonic development, in particular before
73 activation of the zygotic genome (17). During *Drosophila* oogenesis, maternal RNAs are
74 transcribed in nurse cells and then transported into the oocyte where they are later translated.
75 While in the nurse cells, maternally deposited RNAs stay translationally silenced through
76 mechanisms that are not totally understood (18). Some of these RNAs become localized to
77 specific regions of the oocyte and act to establish the body axes of the embryo. One such RNA,
78 *gurken (grk),* is translated first at the posterior of the oocyte to specify posterior fate before
79 localizing to the dorsal-anterior corner of the oocyte to specify dorsal fate (19–21). Other
80 important RNAs include *oskar (osk),* which localizes to the posterior to help specify posterior
81 identity and the germline (22) (23), and *bicoid (bcd),* which specifies the anterior (24).
82 Previous work in our lab identified a novel tudor domain-containing protein, Tdrd5l
83 (Tudor domain-containing protein 5-like), during a screen for genes involved in germline sex
84 determination (25). Tdrd5l is homologous to mouse Tdrd5 as well as to another Drosophila
85 protein, Tejas, both of which are required for fertility (10,26). All of these proteins have a highly
86 related tudor domain, while Tejas and mouse Tdrd5 also have LOTUS domains that interact
87 with the RNA helicase Vasa (27). *Drosophila* Tejas and mouse Tdrd5 both localize with Vasa to
88 the nuage where they act in piRNA synthesis and retrotransposon repression (10,26).
89 While Tdrd5l is male-specific in the early germline, it is expressed in differentiating germ
90 cells in both males and females, suggesting that it plays a role in post-transcriptional gene
91 regulation of both spermatogenesis and oogenesis. In this study we demonstrate that Tdrd5l
92 localizes to a novel germline body that associates with, but is distinct from, the nuage. Further,

93 we demonstrate that, in females, *Tdrd5l* is important for post-transcriptional regulation of
94 maternal RNAs. Embryos from *Tdrd5l*-mutant mothers exhibit defects in dorsal-ventral
95 patterning and both Grk and Orb (Oo-18 RNA-Binding Protein) prematurely accumulate in nurse
96 cells instead of being primarily expressed in the oocyte. Thus, one important role for *Tdrd5l* is in
97 regulation of maternal RNAs as they are being transported from the nurse cells to the oocyte.
98

99 **Results**

100 **Tdrd5l localizes to cytoplasmic bodies in the *Drosophila* germline**

101 Previously, we characterized Tdrd5l expression using an N-terminally tagged exogenous
102 BAC construct (25). Subsequently, we have used CRISPR/CAS9 to generate both N- and C-
103 terminally tagged versions of the endogenous *Tdrd5l* locus. Interestingly, both N- and C-
104 terminally tagged alleles behaved as loss of function alleles, indicating that these regions are
105 important for *Tdrd5l* function. In particular, the N-terminally tagged allele exhibited localization
106 to punctae in the germline when wild-type (wt) Tdrd5l protein was present, but not when the
107 N-terminally tagged allele was the only source of Tdrd5l protein (Fig S1). This indicates that the
108 N-terminus is important for localization of the Tdrd5l protein, but that there is an independent
109 mechanism for Tdrd5l localization to punctae dependent on the wt protein. To visualize
110 localization of functional Tdrd5l protein, we generated internal hemagglutinin (HA) and FLAG
111 epitope-tagged alleles (Fig S1A), which behaved as wt alleles of *Tdrd5l*. We also generated a
112 peptide guinea pig polyclonal antibody using amino acids 65-81 of Tdrd5l. This antiserum
113 yielded a similar staining pattern to the internally tagged *Tdrd5l* alleles and immunostaining
114 was eliminated in *Tdrd5l* mutants (Figure S2).

115 Using the internally FLAG-tagged allele, we observed anti-FLAG immunoreactivity in a
116 mixed population of small and larger cytoplasmic bodies in the pre-meiotic male germline (Fig
117 1A), in agreement with our previous work using the exogenous HA-tagged BAC construct (25).
118 In the germline stem cells (GSCs) and gonioblasts (GBs) (Fig 1A white arrow) there was a trend
119 to have more smaller bodies and the occasional large body. In later spermatogonia and
120 spermatocytes the granules are almost all large bodies 0.9-1 micron in diameter (Fig 1A yellow
121 arrow). Interestingly, in the larger bodies Tdrd5l localized in a hollow, spherical pattern
122 indicating that it formed the periphery of the body (Fig 1G).
123 In the female germline, Tdrd5l immunostaining was greatly reduced compared to the
124 male germline when using identical confocal settings (Fig S3A-B), but still localized to
125 cytoplasmic bodies (Fig 1C). Unlike in the male GSCs (Fig 1B brackets), Tdrd5l expression was
126 absent from the germline stem cells (GSCs) in females (Fig 1D, brackets). Since Sxl represses
127 Tdrd5l expression (25), this likely accounts for the lack of Tdrd5l expression in female GSCs
128 where Sxl is expressed at high levels (28). At later stages in the female germline, we observed
129 Tdrd5l localizing to bodies in the early differentiating germline of the germarium (Fig 1C), as
130 well as in the nurse cells (Fig 1E and 1F, white arrow) and oocyte (Fig 1E, yellow arrow) until
131 stages 6-7 of oogenesis.
132
133 **Tdrd5l localizes to an unknown body**
134 Since many tudor domain-containing proteins, including the Tdrd5l homolog Tejas, are
135 known to localize to RNA granules, we next analyzed the "Tdrd5l Body" to determine whether it
136 corresponds to any previously identified RNA granules.

137 One prominent germline RNA granule is the perinuclear nuage, which acts as the site of
138 piRNA production for transposon repression (29–31) and contains other tudor-domain
139 containing proteins such as the Tdrd5l homolog Tejas in flies, and TDRD5 in mice (8,9,32). One
140 hallmark of the nuage is the presence of Vasa protein (33). We observed that the Tdrd5l-
141 containing bodies often associate with, or are imbedded in regions of Vasa immunoreactivity,
142 but that Vasa staining is not detected within the Tdrd5l bodies themselves (Fig 2A, yellow
143 arrow). Analysis of the distribution of Tdrd5l bodies revealed that there was an equal number
144 that exhibited cytoplasmic vs. perinuclear localization, and bodies in both locations were
145 associated with Vasa immunoreactivity (Fig S4A-B, N= 236 granules). Additionally, we co-
146 stained for Tdrd5l and either Ago3 (Fig 2C, arrows) or Aub (Fig 2B, arrows), two other
147 components of the nuage that are involved in piRNA biogenesis. In both cases we saw similar
148 results as with Vasa, with the proteins enriched next to but not within the Tdrd5l body. These
149 data indicate that either the Tdrd5l body is distinct from the nuage or forms a sub-
150 compartment of nuage not marked by piRNA biogenesis factors. Consistent with this, we
151 observed no changes in transposon expression in *Tdrd5l* mutant gonads of both sexes ((25) and
152 Fig S5), thus suggesting a function for Tdrd5l distinct from its homolog Tejas.
153 Two additional RNA granules that are commonly found in many cell types are the P
154 bodies, sites of post-transcriptional RNA regulation, and U bodies, which are involved in snRNP
155 maturation (34). A common P body component is Decapping protein 1 (Dcp1) (35) and we
156 visualized Dcp1 immunofluorescence in combination with Tdrd5l (Fig 2D). We did not observe
157 co-localization between Dcp1-containing punctae and Tdrd5l, and the Dcp1-containing bodies
158 were also of much smaller size than Tdrd5l bodies. In 63% of testes, we did observe Dcp1

159 labeled P-bodies associated with the periphery of Tdrd5l bodies (e.g. Fig 2D arrow, N= 17
160 testes). This suggests that there may be a relationship between P bodies and Tdrd5l bodies or
161 exchange of materials between these structures, but these data indicate that the two types of
162 bodies are distinct. We visualized U bodies using an endogenously tagged allele of Survival
163 Motor Neuron (SMN) (34) and we failed to observe co-localization between SMN-containing
164 bodies and Tdrd5l (Fig S4C,). Taken together, these data indicate that the Tdrd5l bodies are
165 distinct structures from the Vasa positive nuage, P bodies and U bodies.
166
167 **Tdrd5l genetically interacts with post-transcriptional regulatory factors.**
168 Since cytoplasmic bodies are often important for post-transcriptional gene regulation,
169 we determined whether *Tdrd5l* exhibited genetic interaction with known post-transcriptional
170 regulation pathways. To ask whether Tdrd5l functions in regulating translation and RNA
171 stability by influencing poly(A) tail length, we tested for a genetic interaction between *Tdrd5l*
172 and *twin*, which encodes the CCR4 homolog and major deadenylase in *Drosophila* (36).
173 Expression of a weak RNAi trigger for *twin* in the germline of wild-type (wt) females (*nos-Gal4,*
174 UAS-*twin* RNAi) caused no morphological or fertility defects (Fig 3A). Animals heterozygous for
175 a deletion allele of *Tdrd5l* also exhibit no morphological or fertility defects on their own.
176 However, when the *twin* RNAi trigger was expressed in the germline of females heterozygous
177 for a mutation in *Tdrd5l* (*nos-Gal4,* UAS-*twin* RNAi, *Tdrd5l/+*), we observed severely
178 disorganized ovaries that lacked recognizable egg chambers in 100% of ovaries (Fig 3B, N= 20),
179 and these animals were completely sterile. Additionally, to test whether Tdrd5l functions in
180 regulating mRNA stability through removal of the mRNA 5' cap, we tested for a genetic

181 interaction with *Dcp1,* which encodes one of the major mRNA decapping enzymes in Drosophila
182 (13). As in the case of the *twin* knockdown, there were no morphological or fertility defects
183 when our *Dcp1* RNAi line was expressed in the germline of wt females (*nos-Gal4, UAS-Dcp1*
184 *RNAi,* Fig 3C). However, when *Dcp1* was knocked down in the germline of females heterozygous
185 for a mutation in *Tdrd5l,* the ovaries exhibited a severe morphological defect and lacked
186 germline in 100% of ovaries (Fig 3D, N = 20), and these animals were also completely sterile.
187 These dramatic, dose-sensitive genetic interactions between *Tdrd5l* and *twin* or *Dcp1* indicate
188 that Tdrd5l could function in post-transcriptional repression of mRNAs through both the
189 deadenylation and decapping pathways.
190
191 **Tdrd5l is important for proper egg development**
192 One place where post-transcriptional gene regulation is particularly important is in the
193 developing egg chambers of the female germline. Females trans-heterozygous for predicted
194 null mutant alleles of *Tdrd5l* exhibit a relatively normal ovary morphology but lay reduced
195 numbers of eggs (Fig S6A and Fig 6C). To determine whether the levels of any mRNAs are
196 regulated by *Tdrd5l* we conducted RNAseq in wildtype vs *Tdrd5l*-mutant ovaries. Interestingly,
197 the mRNAs altered in *Tdrd5l* mutants were enriched for genes that are maternally deposited in
198 the embryo, as defined by mRNAs present in 0-2 hr old embryos (37). While maternal genes
199 represent 23.3% of all Drosophila genes, they represent 30% of genes altered in *Tdrd5l* mutants
200 (Fig S7A-B). Further, when the analysis is restricted to genes with a 2-fold or greater change in
201 mRNA level in *Tdrd5l* mutants, the percentage of maternally-expressed genes rises to 94% (Fig
202 S7C). Additionally, we compared our RNAseq data to previously published data identifying RNAs

203 associated with the BicaucaID (BicD)/Egalitarian (Egl) complex involved in transport of RNAs
204 from nurse cells to the oocyte (38). While mRNAs identified in our RNA-seq analysis represent
205 10.4% of all genes in the Drosophila genome, they represent 30% of the top 100 mRNAs
206 associated with the BicD/Egl complex, further suggesting a role for Tdrd5l in regulating mRNAs
207 that are deposited maternally in the oocyte during oogenesis (Fig S7D).

208 In *Drosophila,* maternally deposited RNAs are transcribed in the nurse cells where they
209 are silenced until they get to their proper location in the oocyte and are then translationally
210 activated at specific times (39). To investigate whether Tdrd5l plays a role in this process we
211 tested whether the eggs laid by *Tdrd5l*-mutant females exhibited defects in development when
212 crossed to wildtype males. Indeed, we observed that these eggs were greatly reduced in their
213 ability to hatch and give rise to viable larvae (Fig 4A). To determine if the decrease in hatch rate
214 was due to patterning defects, we examined the dorsal appendages (DA) on the offspring, an
215 indicator of Dorsal/Ventral patterning (40). Offspring from trans-heterozygous *Tdrd5l*-mutant
216 females exhibited a greatly increased frequency of DA defects (Fig 4B), which ranged from
217 alterations of DA length (Fig 4D) to a fusion of DA material into a ring (Fig 4E) or a single fused
218 appendage (Fig 4F). If the defects in dorsal-ventral patterning are the major reason behind the
219 embryonic defects, then we would expect that the majority of unhatched embryos would
220 exhibit DA defects. However, when we examined the DA defects specifically of those embryos
221 that failed to hatch, we did not observe a greater percentage of DA defects than we observed
222 when examining all embryos. This indicates that, while DA and D/V defects are the probable
223 cause of death for many of the embryos, other embryos are likely to die for other reasons.

224 When we attempted to examine the cuticle pattern of unhatched embryos we observed no
225 discernable cuticle pattern, indicating that these embryos die prior to cuticle deposition.
226
227 ***Tdrd5l* is required for repression of Grk expression in nurse cells**
228 The D/V and anterior/posterior (A/P) axes in the developing oocytes are set up by
229 localized translation of maternally deposited RNAs. These RNAs are transcribed in the nurse
230 cells and post-transcriptionally silenced during transport to the oocyte (39). Three of the classic
231 maternally deposited RNAs are *gurken (grk)*, which regulates D/V patterning (20,21), *bicoid*
232 *(bcd)* which determines anterior fate (24), and *oskar (osk)* which specifies posterior identity and
233 the germ plasm (22). To determine if *Tdrd5l* regulates maternally deposited RNAs we
234 immunostained for the protein products of these three mRNAs in wildtype and *Tdrd5l*-mutant
235 ovaries. Both Grk and Osk protein staining were altered in *Tdrd5l*-mutant ovaries (Fig 5A-H),
236 whereas no Bcd staining was observed in the mutant ovaries, similar to wt (data not shown). In
237 wt ovaries, Grk immunoreactivity is normally observed in the dorsal-anterior corner of the
238 oocyte (Fig 5A, arrowhead) and is absent from the nurse cells of developing egg chambers (Fig
239 5C). However, in *Tdrd5l* mutant ovaries we observed Grk staining in the nurse cells of mid-stage
240 egg chambers of 80% of ovaries tested (Fig 5D, outlined and inset, N = 25 ovaries), suggesting
241 the *grk* mRNA is no longer translationally repressed in the nurse cells. In addition, we still
242 observed Grk immunoreactivity at the dorsal-anterior corner of developing oocytes (Fig 5B) in
243 *Tdrd5l* mutants.
244 In wt ovaries, Osk immunostaining is normally restricted to the posterior pole of
245 developing oocytes beginning at stage 10 (Fig 5E, arrowhead) (22). In stage 10 *Tdrd5l*-mutant

246 egg chambers we still observed Osk protein correctly localized to the posterior pole (Fig 5F).
247 However, in 77% of stage 9 *Tdrd5l*-mutant egg chambers we observed premature staining for
248 Osk, and in these cases Osk protein was localized to the middle of the oocyte rather than the
249 posterior end (Fig 5H, N = 25 ovaries). However, unlike with Grk, we did not observe Osk
250 protein expressed in the nurse cells of *Tdrd5l* mutants (Fig 5H).

251 To determine if the ectopic expression of Grk and Osk proteins was due to defects in
252 RNA transport from the nurse cells or RNA localization in the oocyte, we conducted fluorescent
253 *in situ* hybridization (FISH) to visualize the *grk* and *osk* mRNAs (Fig 5I-L). We observed no
254 changes in *grk* mRNA localization between wildtype and *Tdrd5*-mutant ovaries (Fig 5I-J).
255 Contrary to what we observed with the *grk* RNA, we observed altered *osk* mRNA localization in
256 *Tdrd5l*-mutant oocytes (Fig 5L). In *Tdrd5l* mutant oocytes, *osk* mRNA was not tightly localized to
257 the posterior and was instead observed throughout the oocyte in a 31% of ovaries (Fig 5L, N=
258 15 ovaries).

259 Grk is first required at the posterior of the oocyte to specify the posterior follicle cells,
260 which are then required for proper microtubule orientation and localization of *osk* RNA and
261 protein to the posterior pole (41). Thus, it is possible that the mis-localization of *osk* we
262 observed could be secondary to defects in *grk* function. To test whether the microtubule
263 network is properly oriented in *Tdrd5l* mutants, we used Kinesin-LacZ (Kin-LacZ) to mark the
264 plus ends of the microtubules (21). In wt ovaries the microtubule network in the oocyte is
265 polarized with plus ends at the posterior, therefore Kin-LacZ should be localized tightly to the
266 posterior by stages 9 and 10 of egg chamber development (Fig S8A white arrow, and Fig S8B
267 yellow arrow, quantified in Fig S8E). In *Tdrd5l*-mutant ovaries we detected a range of Kin-LacZ

268 localization: some oocytes had tight posterior localization, while others had some posterior
269 localization of Kin-LacZ with plumes of staining extending away from the posterior pole. In the
270 most extreme cases we observed diffuse Kin-LacZ localization in the center of the oocyte (Fig
271 S8C white arrow and Fig S8D yellow arrow, quantified in Fig S8E). These data indicate that the
272 defects in *osk* posterior localization may be caused by loss of *grk* function rather than defects in
273 the direct regulation of *osk* itself.
274
275 ***Tdrd5l* represses Orb protein expression in nurse cells**
276 Work by other labs has shown that Grk translation in the oocyte is activated by Oo18
277 RNA binding protein (Orb) (42–44). Orb is a cytoplasmic polyadenylation element binding
278 protein (CPEB) that is highly expressed in the oocyte where it recruits Wispy to lengthen the *grk*
279 poly(A) tail to activate its translation (43). It has also been shown that ectopic expression of Orb
280 in the nurse cells is sufficient to prematurely activate Grk translation (44). To test whether
281 ectopic expression of Orb in the nurse cells could be the cause of Grk translation in *Tdrd5l*-
282 mutant nurse cells, we stained for Orb protein in *Tdrd5l*-mutant ovaries. As previously
283 described, in all wt ovaries we observed Orb immunofluorescence primarily in the oocyte and it
284 was largely absent from the nurse cells of later egg chambers, except the nurse cell closest to
285 the oocyte (Fig 6A, squared region). In 100 % of *Tdrd5l*-mutant ovaries we saw an expansion of
286 Orb protein expression into almost all the nurse cells suggesting Orb translation had been de-
287 repressed in *Tdrd5l* mutants (Fig 6B, squared region, N= 26).
288 To test whether the phenotype observed in *Tdrd5l* mutants is due to increased Orb
289 expression, we determined whether reduction of *orb* function could suppress the *Tdrd5l*-

290 mutant phenotype. *Tdrd5l*-mutant females that were also heterozygous for an *orb* loss of
291 function allele (*Tdrd5l-/Tdrd5l-; orbF343/+* females) laid significantly more eggs than *Tdrd5l*-
292 mutant females, and a similar number of eggs to wt control females (Fig 6C). However, being
293 heterozygous for an *orb* mutant allele did not rescue the egg hatching defect observed in
294 *Tdrd5l*-mutants (not shown). This suggests that the defects observed in *Tdrd5l* mutants are
295 partially due to the increased Orb expression observed in the nurse cells, but other defects may
296 be due to misregulation of other RNAs or other functions of Tdrd5l.
297

298 **Discussion**

299 Work from a number of labs has demonstrated the importance of RNA granules for
300 proper germ cell identity and function. Here we have extended our study of Tdrd5l and shown
301 that it localizes to the periphery of a novel germline structure, the "Tdrd5l body", that does not
302 exhibit the characteristics of any previously identified germline granule. While we previously
303 demonstrated that Tdrd5l expression is highly male-biased in the undifferentiated germline, we
304 show here that Tdrd5l bodies are also present during germline differentiation in females. *Tdrd5l*
305 does not regulate transposon expression, like other Tdrd5 family members, but instead
306 regulates the expression of maternal RNAs that need to be silenced in nurse cells as they are
307 transported to the oocyte.
308

309 **Analysis of the "Tdrd5l body"**

310 Previous work studying expression of the Tdrd5l protein used a genomic BAC with an N-
311 terminal epitope tag that we have subsequently found interferes with *Tdrd5l* function.

312 Therefore, we used an internal epitope-tag placed into the endogenous *Tdrd5l* locus that
313 retains full *Tdrd5l* function, as well as a peptide polyclonal antibody generated against native
314 Tdrd5l, to characterize the localization of Tdrd5l. We were able to verify that Tdrd5l is more
315 highly expressed overall in the male germline than the female germline, and is expressed in
316 male GSCs but is not observed in female GSCs (Fig 1). This is consistent with *Sex lethal* being a
317 repressor of Tdrd5l expression as we previously found (25). However, we also describe the
318 expression of Tdrd5l in the female germline, where it is found in germ cells that have begun to
319 differentiate in the germarium (Fig 1C-D), and in nurse cells and oocytes in the developing egg
320 chambers (Fig 1E-F). Interestingly, in both male and female germ cells, the punctae of Tdrd5l
321 expression appear "hollow" indicating that Tdrd5l resides on the surface of the structures it
322 identifies.

323 Our analysis also indicates that the Tdrd5l bodies are not representative of any type of
324 previously described germline granule in *Drosophila*. The most prominent germline granule is
325 the perinuclear nuage which contains the helicase Vasa and other factors important for piRNA
326 biogenesis, like Aub and Ago3. While the Tdrd5l bodies are often observed in a perinuclear
327 location, they notably lack Vasa and are not enriched for Aub or Ago3 (Fig 2). Thus, while Tdrd5l
328 bodies may interact with the nuage, they appear to be distinct entities. A large granule, the
329 piRNA nuage giant body, has been described that is associated with the nuage (45). However,
330 unlike the Tdrd5l body, this body contains Vasa and appears only in primary spermatocytes, and
331 so is distinct from the Tdrd5l body. The localization of the Tdrd5l body is also not consistent
332 with it being part of the germ plasm or sponge bodies found in the oocyte (46–49). The Tdrd5l
333 body appears to be distinct from RNA granules found more commonly in different cell types,

334 such as P bodies, U bodies and stress granules. P bodies characteristically contain Dcp1 and are
335 much smaller in size than Tdrd5l bodies. Our previous work suggested some overlap in
336 localization between Tdrd5l and Dcp1 (25) but the more detailed analysis described here
337 reveals that smaller, Dcp1-positive bodies can be seen associating with Tdrd5l bodies, but that
338 Dcp1 is not present in the Tdrd5l bodies themselves (Fig 2). We also do not see overlap
339 between Tdrd5l and the U body protein Smn (Fig S4). Lastly, the Tdrd5l bodies are observed
340 constitutively in the germline and are not affected by stresses such as starvation or changes in
341 temperature (C.P. unpublished data) indicating that they are not stress granules.

342 Germline granules have been most well-studied in C. elegans, and a number of distinct
343 regions of the nuage have been described in this species (50,51). At many stages of germline
344 development, P granules are associated with the nuclear periphery and, like Drosophila nuage,
345 contain Vasa-class helicases (the GLH's) as well as Ago proteins (52–54). However, associated
346 with the P granules are other perinuclear regions known as the Mutator foci, SIMR foci and the
347 Z granules, that have distinct protein components (51). One possibility is that the Tdrd5l body
348 represents a similar sub-structure associated with the nuage in *Drosophila*. In addition to
349 studying the function of *Tdrd5l*, identifying other proteins and possibly RNAs that are present in
350 the Tdrd5l body will be an important next step.

351

352 **Function of the Tdrd5 proteins**

353 There are many types of Tudor domain containing proteins and they can be classified
354 into sub-families based on the homology of their Tudor domains. In diverse animal species,
355 including mouse and humans, there is a single Tdrd5 protein. Interestingly, flies have two such

356 proteins, Tdrd5l and Tejas (10,25). Many Tdrd5 proteins, like mouse TDRD5 and *Drosophila*
357 Tejas, also contain an N-terminal LOTUS domain which is known to bind Vasa-type helicases
358 (27), but Drosophila Tdrd5l lacks this domain. However, the N-terminus of Tdrd5l must be
359 important for its function since placing even a small epitope tag in this position compromises
360 Tdrd5l function. Consistent with its Vasa-binding LOTUS domain, Tejas associates with the Vasa-
361 positive nuage where it acts in the piRNA pathway to repress germline transposon expression
362 (10). In contrast, we did not observe Tdrd5l co-localizing with the nuage (Fig 2A), and we have
363 observed no change in transposon expression in *Tdrd5l* mutants (Fig S5). Instead, we find that
364 *Tdrd5l* exhibits potent genetic interaction with pathways that repress mRNAs at the post-
365 transcriptional level, including the mRNA decapping and deadenylation pathways (Fig 3). This is
366 also consistent with a role for Tdrd5l in regulating mRNA repression in nurse cells (see below).
367 Thus, it may be that Tejas is more specific for transposon regulation while Tdrd5l is involved in
368 post-transcriptional regulation of germline mRNAs.
369 Mouse TDRD5 associates with the Chromatoid Body, a nuage-related structure. In
370 addition, mice mutant for *Tdrd5* exhibit defects in transposon regulation and spermatogenesis
371 (26), similar to Drosophila *tejas* (10). Interestingly, *Tdrd5*-mutant mice also exhibit defects in
372 regulation of pachytene piRNAs (55,56), which are proposed to regulate mRNAs important for
373 spermatogenesis rather than to repress transposons. One intriguing hypothesis is that the role
374 of the single TDRD5 protein in mice in regulating both transposon repression and mRNA
375 expression has been divided between the two Tdrd5 proteins in Drosophila, Tejas and Tdrd5l. In
376 addition, both Drosophila proteins have functions in oogenesis in addition to spermatogenesis
377 (10,25, this work). Similarly, knock down of a Tdrd5 homolog in Locust also affects both

378 oogenesis and spermatogenesis (57). While a role for mouse TDRD5 in the female germline has
379 not been described, consortium data indicate that *Tdrd5* is expressed in the mouse ovary (58),
380 making this an interesting place to look for additional functions of TDRD5 in the mouse.
381
382 **Regulation of Maternally deposited RNAs**
383 Prior work from our lab demonstrated that *Tdrd5l* promotes male identity in the
384 germline. Consistent with this, Tdrd5l is expressed in male GSCs but is repressed in female GSCs
385 by the action of *Sxl* (25). However, Tdrd5l is also expressed in differentiating germ cells in both
386 males and females ((25), and Fig 1). This suggests that Tdrd5l might promote male identity in
387 early germ cells and germline stem cells but also regulate aspects of germline differentiation in
388 both sexes. One process in the female germline that relies heavily on post-transcriptional
389 regulation of RNAs is the production and transport of maternal RNAs from nurse cells into the
390 oocyte.
391 Maternal contribution of RNAs, proteins, and organelles is conserved from flies through
392 vertebrates (59,60), and supports embryonic patterning and embryogenesis prior to activation
393 of the zygotic genome and beyond. In *Drosophila*, maternal RNAs are transcribed in the nurse
394 cells, and are post-transcriptionally silenced to prevent their translation during transport to the
395 oocyte and prior to the specific time they should be activated. The cytoplasmic polyadenylation
396 element binding protein (CPEB) Orb is known to activate translation of maternal RNAs such as
397 *osk* and *grk* in the oocyte (43,44,61). Like other maternal RNAs, *orb* is transcribed in nurse cells
398 and transported to the oocyte where it is translated. Interestingly, Orb/CPEB homologs are
399 critical for regulating translation of maternal RNAs in vertebrate systems as well (62).

400 We observed defects in expression of Orb, Grk and Osk proteins in *Tdrd5l* mutants (Fig 5
401 and Fig 6). We saw ectopic accumulation of both Orb and Grk in nurse cells (Fig 5D and Fig 6B),
402 while Osk protein was sometimes observed in the middle of the oocyte instead of its normal
403 location at the posterior pole (Fig 5H). We also observed maternal-effect lethality of embryos
404 from *Tdrd5l*-mutant mothers that included dorsal appendage defects consistent with Grk's role
405 in dorsal-ventral patterning. One possible explanation for all of these phenotypes is a failure of
406 Orb to be properly repressed in nurse cells. Ectopic Orb translation in nurse cells can cause
407 improper activation of Grk translation in these cells (44), and this might also lead to insufficient
408 translation of Grk in the oocyte. Early Grk expression at the posterior pole of the oocyte is
409 necessary for proper posterior determination and defects in this process can lead to disruption
410 of *osk* mRNA and protein localization to the posterior pole (19,21) as observed in *Tdrd5l*
411 mutants (Fig 5L). Further, impaired Grk expression in the dorsal-anterior corner of the oocyte
412 can cause problems in specification of dorsal follicle cells, and defects in dorsal appendage
413 formation and dorsal-ventral patterning of the embryo. Thus, one primary defect in *Tdrd5l*
414 mutants could be a failure to properly repress *orb* translation in the nurse cells. In agreement
415 with this possibility we see a partial rescue of the *Tdrd5l* egg-laying defect when *orb* function is
416 compromised (Fig 6).
417 Other factors have been implicated in repressing *orb* translation in nurse cells, including
418 FMR1, the Drosophila homolog of Fragile X mental retardation protein (63), and Cup, a
419 translational repressor (64). Mutations in both *FMR1* and *cup* cause Orb accumulation in nurse
420 cells similar to what we observe in *Tdrd5l* mutants. One intriguing hypothesis is that the Tdrd5l
421 body in nurse cells is a site where mRNAs such as *orb* get marked for translational repression by

422 factors such as FMR and Cup. Since the nuage and nuage-related material in flies, worms and
423 mice all function in small RNA regulatory pathways, the marking of mRNAs like *orb* in the Tdrd5l
424 granule may also involve such regulatory RNAs.
425
426 **Materials and Methods**
427 **Fly stocks and CRISPR tagging**
428 Fly stocks used in this paper were obtained from the Bloomington stock center unless
429 otherwise noted. *Nos*-gal4 (65), *twin* RNAi(BDSC# 32901), *Dcp1* RNAi(BDSC# 67874), *mcherry*
430 RNAi(BDSC# 35785), *orb* RNAi(BDSC# 43143), Vasa-Cas9(BDSC# 56552), piggyBac (BDSC#
431 32070), Kin-LacZ (T. Schupbach) . *Tdrd5l* mutant alleles were generated previously by our lab
432 (25). *Tdrd5l*:GFP and *Tdrd5l*:FLAG alleles were generated using CRISPR as described by the fly
433 CRISPR group (66). The GFP sequence followed by a linker sequence was inserted in place of the
434 Tdrd5l start codon. The internal flag construct contains a 3x FLAG sequence flanked by linkers in
435 the middle of exon 3 following K225. The GFP and FLAG donor plasmids and pU6 gRNA plasmid
436 were obtained from the *Drosophila* genetics resources center and were stock numbers 1365,
437 1367, 1363 respectively.
438
439 **Immunofluorescence and antibody generation**
440 All gonads were dissected, and fixed, and stained as previously published (67). All images were
441 taken on a Zeiss LSM 700, LSM 800 with airyscan detector (when noted), or LSM 980 with
442 airyscan detector (when noted). Primary antibodies used were Guinea pig anti-Tdrd5l 1:10,000
443 (this paper), rat anti-HA 1:100 (Roche Cat # 3F10), rabbit anti- Vasa 1:10,000 (R, Lehman),

444 Guinea pig anti-TJ 1:1000 (M. Van Doren), mouse anti Armadillo 1:100 (DSHB, N2 7A1), Mouse
445 anti Orb 1:40 (DSHB, 6H4), mouse anti- Gurken 1:20 (DSHB, 1D12), rabbit anti-oskar 1:1000 (A.
446 Ephrussi), rat anti-Ago3 1:500 (T. Kai), mouse anti-Aub 1:1000 (MC. Siomi). Secondary
447 antibodies were used at 1:500 (Goat anti-rabbit alexa-fluor 546 Cat # A11011, Goat anti-rabbit
448 alexa-fluor 488 Cat # A11034, Goat anti-mouse alexa-fluor 488 Cat # A11029, Goat anti-mouse
449 alexa-fluor 546 Cat # A11004, Goat anti-mouse alexa-fluor 633 Cat # A21050, Goat anti-rat
450 alexa-fluor 488 Cat # A11006. Goat anti-rat alexa-fluor 633 Cat # A21094, Goat anti-guinea pig
451 alexa-fluor 488 Cat # A11073, Goat anti-guinea pig alexa-fluor 633 Cat # A21105). Samples were
452 stained in DAPI solution and mounted in DABCO.
453
454 **Quantification of nuage association**
455 To quantify the association of Tdrd5l granules with Vasa, we calculated the number of Tdrd5l
456 granules in the 8 and 16 cell cysts that localized either to the nuclear periphery (perinuclear) or
457 to the cytoplasm. For each group we then calculated the number of Tdrd5l granules that are
458 directly adjacent to Vasa granules, co-localize with Vasa, or do not associate with Vasa at all
459 **Egg lay assays**
460 Trans-heterozygous *Tdrd5l* mutant females and control females were aged 7days with *OregonR*
461 males. Individual females and males were placed in condos on grape juice plates with wet yeast
462 paste for 24hrs, and then taken off the plate. The number of eggs laid and those with dorsal
463 appendage defects were counted for each female. At 48hrs these counts were done again to
464 count how many had hatched.
465 **Genetic interaction assays**

466 UAS-RNAi lines were crossed to Nos-gal4 for controls or *Tdrd5l*^{Q5}*;;nos-gal4* for mutants. *twin*
467 and *Dcp1* were knocked down using a UASp-shRNAi driven in the germline by nos-gal4 in a
468 wildtype background and in a *Tdrd5l-* background. female progeny were heterozygous (*Tdrd5l/*
469 *+*), Progeny were aged 5-10days then dissected immunostained as described above using anti-
470 vasa, anti-TJ, anti-Arm and DAPI.

471 **Fluorescence in situ hybridization**

472 FISH for *gurken* and *oskar* were conducted as published by the Berg lab (68). cDNA clones 2169
473 and 7305 for probes were obtained from the Drosophila Genetics Resource Center.

474 **RNA sequencing**

475 For testis RNA sequencing, Testes were dissected from 5d old males, RNA was prepped using
476 RNA-Bee(Tel-test) 3 biological replicated were used for each genotype. Libraries were prepped
477 using a previously established protocol (69). 100bp paired end sequencing was conducted by
478 the Johns Hopkins Genetics Resources Core facility
479 For ovary RNA sequencing, ovaries were dissected from virgin females, and 3 biological
480 replicates were dissected per genotype. RNA was prepped using The Direct-zol RNA micro prep
481 kit (Zymogen). Library construction and 100bp paired end sequencing was conducted by the
482 Johns Hopkins Genetics Resources Core facility.
483 Read quality for both the ovary and testis RNAseq data was determined using the fastQC kit.
484 Reads were mapped using Tophat and HTSeq to the *Drosophila* genome using Ensemble release
485 BDGP6. Differential gene expression analysis was conducted using DESeq.
486 Differential transposon analysis was carried out as previously (70). Briefly, RNAseq reads were
487 mapped to the FlyBase r6.46 genome using Hisat2 (-k 1 --rna-strandness RF --dta) (PMID:

488 35266522, PMID: 25751142). Read counts for transposons were determined based on their
489 genomic locations according to the UCSC RepeatMasker track and read counts were analyzed
490 with HTSeq-count (PMID: 29106570, PMID: 25260700). Differential expression analysis of
491 transposons was determined with DESeq2 (pAdjustMethod = 'fdr')(PMID: 25516281).
492
493 Strains and plasmids are available upon request. The authors affirm that all data necessary for
494 confirming the conclusions of the article are present within the article, figures, and tables.
495
496

497 **Acknowledgements**
498

499 We thank the *Drosophila* community, the Bloomington stock center, DRGC, and Developmental
500 Studies Hybridoma Bank for stocks and reagents. We would also like to thank the Chou lab for
501 Dcp1-YFP flies, as well as Trudi Schupbach and Liz Gavis for reagents and helpful discussion on
502 this work. We thank members of the Johns Hopkins community; the Johnston lab, and John Kim
503 for helpful discussion as well as Fred Tan for help with bioinformatics. This work was supported
504 by NIH grant R21HD101781 to MVD.
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507

508 **Figure legend**

509 **Figure 1: Tdrd5l localizes to cytoplasmic bodies in the *Drosophila* germline**
510 (A-G) immunofluorescence of adult gonads using an endogenously FLAG-tagged allele of *Tdrd5l*.
511 Panels B, D and F are higher magnification images of panels A, C and E, respectively. Antibodies
512 used are as indicated in the figure. Scale bars = 5 microns. A,B) Adult testes. Note that Tdrd5l

513 localizes to punctae or "bodies" in germ cells. Examples of small bodies are marked by white
514 arrow heads and examples of large bodies are marked by yellow arrowheads. GSCs are
515 indicated by brackets. C,D) Adult ovaries. A germarium is indicated by the dashed line in C.
516 Tdrd5l localizes to bodies in the germline similar to those observed in testes but are not
517 observed in female GSCs (brackets). E,F) Stage 7 egg chamber. Tdrd5l localizes to cytoplasmic
518 bodies in both nurse cells (white arrow) and oocyte (yellow arrow). G) Immunofluorescence of
519 an adult testis using an LSM 980 with an airyscan detector. Note the localization of FLAG-Tdrd5l
520 to the periphery of the body.
521
522 **Figure 2: Tdrd5l localizes to a novel germline body**
523 (A-D) Immunofluorescence of adult testes using endogenously FLAG-tagged *Tdrd5l* compared to
524 markers for known RNA granules as indicated in figure. Examples of Tdrd5l bodies are marked
525 with yellow arrows. A) Immunostaining of gonads for Vasa-positive nuage shows that Vasa
526 staining is observed adjacent to, but not within, Tdrd5l bodies. Scale bar = 0.5 micron. (B,C)
527 Immunostaining for Aub (B) or Ago3 (C) and Tdrd5l shows a similar relationship as Tdrd5l and
528 Vasa, with Nuage factors being adjacent to, but not within, Tdrd5l bodies. D) Immunostaining of
529 endogenously YFP-tagged Dcp1 and Tdrd5l bodies similarly shows punctae of Dcp1-YFP
530 adjacent to, but not within, Tdrd5l bodies. Scale bars in B-D equal 1 micron.
531
532 **Figure 3: *Tdrd5l* genetically interacts with post-transcriptional gene regulatory factors**
533 (A-D) Immunofluorescence of adult ovaries with genotypes and antibodies as indicated in
534 figure. Fertility status of flies is marked in the top right corner of each panel. (A,B) *nos>twin*
535 RNAi in the germline of wildtype (A) or *Tdrd5l*$^{\Delta Q5/+}$ heterozygous (B) females. *nos>twin* females
536 (A) have normal ovaries and are fertile while germline development and egg chamber
537 production is severely compromised in *nos>twin, Tdrd5l*$^{\Delta Q5/+}$ females (B) and these animals are
538 sterile. (C,D) *nos>Dcp1* RNAi in the germline of wildtype (C) or *Tdrd5l*$^{\Delta Q5/+}$ heterozygous (D)
539 females. *nos>dcp1* RNAi females have normal ovaries and are fertile while germ cells are almost
540 completely absent from *nos>Dcp1*, *Tdrd5l*$^{\Delta Q5/+}$ females (D) and these animals are sterile.
541
542 **Figure 4: *Tdrd5l* is important for proper egg development**
543 (A) Quantification of hatch rate of eggs laid during single female fecundity assays. Eggs laid by
544 control females (Cas9 ^{iso}) are represented by blue dots and eggs laid by *Tdrd5l*-mutant females
545 (Tdrd5l$^{\Delta M4/\Delta Q5}$) are represented by orange dots. B) Quantification of dorsal appendage defects
546 observed in eggs laid during single female fecundity assays. Eggs laid by control females
547 (Cas9^{iso}) are represented by blue dots, and eggs laid by *Tdrd5l*-mutant (*Tdrd5l*$^{\Delta M4/\Delta Q5}$) females
548 are indicated by orange dots. (C-F) Examples of dorsal appendages quantified in B. (C) Example
549 of an egg laid by a *Tdrd5l* mutant female with normal dorsal appendages. (D-F) Examples of
550 eggs laid by *Tdrd5l*- females with dorsal appendage defects.
551
552
553 **Figure 5: *Tdrd5l* mutants exhibit premature accumulation of maternal proteins.**
554 (A-H) Immunofluorescence of proteins produced by maternally deposited RNAs using
555 antibodies as indicated in wildtype (Cas9^{iso}) and *Tdrd5l*- (*Tdrd5l*$^{\Delta M4/\Delta Q5}$) ovaries. (A-D)

556 immunostaining for Grk protein. Grk protein localizes to the dorsal anterior corner of both
557 wildtype (A) and *Tdrd5l-* (B) oocytes in stage 9 egg chambers as marked by the white
558 arrowheads. Grk protein is absent from wildtype nurse cells (C) but is present in *Tdrd5l*-mutant
559 nurse cells (D). Examples of individual Stage 7-8 egg chambers are outlined and enlarged in
560 insets. (E-H) Immunostaining for Osk protein. Osk protein is localized to the posterior of both
561 wild type (E) and *Tdrd5l-* (F) Stage 9-10 oocytes as marked by yellow arrowheads. (G) Osk
562 protein is absent from stage 7 wildtype nurse cells and oocytes. However, Osk protein is
563 observed in the center of 77% stage 7 *Tdrd5l-* oocytes. Oocytes are outlined by dashed yellow
564 line in F and G. (I-L) FISH for maternally deposited RNAs as indicated in wildtype (Cas9^{iso}) and
565 *Tdrd5l* mutant (*Tdrd5l*$^{\Delta M4/\Delta Q5}$) ovaries. (I,J) FISH for *grk* mRNA. *grk* mRNA is localized to the
566 dorsal anterior corner of oocytes in stage 9 egg chambers in both wildtype (I) and *Tdrd5l*
567 mutants (J). The dorsal anterior corner is marked by an asterix. (K,L) FISH for *osk* mRNA. *osk*
568 mRNA is localized to the posterior of wildtype stage 7-8 oocytes (K) but is localized throughout
569 the oocyte in 31% of *Tdrd5l* mutants. Oocytes are marked by white arrowheads
570
571
572 **Figure 6: Tdrd5l represses Orb translation**
573 (A-B) Immunofluorescence of Orb in wildtype (Cas9^{iso}) and *Tdrd5l* mutant (*Tdrd5l*$^{\Delta M4/\Delta Q5}$)
574 ovaries. Scale bars = 5 microns. A) Orb protein expression is detected at high levels in wildtype
575 oocytes. (A') boxed area is enlarged in (A''). Orb protein expression is detected at high levels in
576 both oocytes and nurse cells of *Tdrd5l-* ovaries(B). B') boxed area is enlarged in (B''). C)
577 Quantification of egg laid by females from an *Orb/Tdrd5l* genetic interaction assay. Fewer eggs
578 are laid by *Tdrd5l-* (*Tdrd5l*$^{\Delta M4/\Delta Q5}$) females than by *Tdrd5l-* mutant females transheterozygous
579 for an *orb*^{F343} allele (*Tdrd5l*$^{\Delta M4/\Delta Q5}$*;; Orb*$^{F343/+}$) . Note that eggs laid by transheterozygous flies are
580 at a similar level to eggs laid by wildtype (OregonR) flies. P-value $\leq$ 0.01 is denoted by **. P-
581 value $\leq$ 0.001 is denoted by ***.
582
583 **Supplemental Figure 1: Tdrd5l localization to cytoplasmic bodies in the *Drosophila* germline is**
584 **dependent on the N-terminus.**
585 (A) Schematic of the *Tdrd5l* gene locus, insertion sites for endogenous tags are marked as N-
586 Term tag, Internal tag, and C-Term tag. Antibody was raised against the epitope marked by the
587 purple line. (B-D) Immunofluorescence of adult gonads expressing endogenous Tdrd5l N-
588 terminally tagged by GFP. Antibodies used as indicated. (B) Tdrd5l-GFP expression in ovaries
589 heterozygous for *GFP-Tdrd5l/untagged-Tdrd5l*. Note the localization of GFP-Tdrd5l to
590 cytoplasmic bodies. (C) GFP expression in ovaries homozygous for GFP-Tdrd5l. Note the lack of
591 GFP signal. (D) GFP expression in males hemizygous for GFP-Tdrd5l (*GFP-Tdrd5l/Y*). Note the
592 lack of GFP signal. The testis is outlined by a white dotted line in (D').
593
594 **Supplemental Figure 2: Validation of Tdrd5l antibody**
595 (A-B) Immunofluorescence of the anti-Tdrd5l antibody in adult testes using antibodies as
596 indicated. Tdrd5l expression is detected in wildtype testes (Oregon R) where the antibody
597 recognizes Tdrd5l bodies (A). Tdrd5l expression was not detected in *Tdrd5l-* (*Tdrd5l*$^{\Delta M4}$) testes
598 (B).
599

600 **Supplemental Figure 3: Tdrd5l expression is higher in males than females**
601 A-B) Immunofluorescence of internal FLAG-Tdrd5l using the same confocal settings to image
602 male and female gonads. FLAG-Tdrd5l is expressed at higher levels in testes (A) compared to
603 Tdrd5l-FLAG staining in ovaries (B) when imaged with the same confocal settings. Note the
604 localization of Tdrd5l to cytoplasmic bodies in both ovaries and testes.
605
606 **Supplemental Figure 4: Tdrd5l localizes to an unknown body**
607 (A) Quantification of Tdrd5l body association with Vasa stained nuage. Red bars represent
608 cytoplasmic Tdrd5l bodies while blue bars represent perinuclear Tdrd5l bodies. Bodies were
609 classified as localizing adjacent to Vasa as in (B), overlapping with Vasa, or having no association
610 with Vasa. (B) Example of a cytoplasmic Tdrd5l body visualized using an internal FLAG tagged
611 allele of Tdrd5l localized next to Vasa-positive granules. (C) Immunofluorescence of FLAG-
612 Tdrd5l and HA-SMN. Tdrd5l bodies shown in green and marked by a white arrowhead. SMN
613 positive U-bodies are shown in magenta and marked by a yellow arrowhead. Note Tdrd5l
614 bodies do not co-localize with SMN positive U-bodies.
615
616 **Supplemental Figure 5: Tdrd5l does not repress transposon expression**
617 (A-B) Differential expression analysis of transposons in *Tdrd5l*- gonads (Females = $Tdrd5l^{\Delta M4/\Delta Q5}$,
618 Males = $Tdrd5l^{\Delta M4}/Y$) compared to wt. Each dot signifies a single transposon gene. Blue dotes
619 are down-regulated genes, red dotes are upregulated-genes, and black dots are transposons
620 with no change in expression levels. A) RNA sequencing of *Tdrd5l* mutant ovaries compared to
621 wildtype ($Cas9^{iso}$) ovaries showed no de-repression of transposon expression. B) RNA
622 sequencing of *Tdrd5l* mutant testes compared to wildtype ($Cas9^{iso}$) testes showed no global
623 changes in transposon expression.
624
625 **Supplemental Figure 6: Tdrd5l is important for proper egg development**
626 (A-B) Quantification of fecundity assays performed using single female egg lay assays. (A) Eggs
627 laid per *Tdrd5l*- ($Tdrd5l^{\Delta M4/\Delta Q5}$) female (orange dots) compared to control ($Cas9^{iso}$) females (blue
628 dots). Note the decreased number of eggs laid by *Tdrd5l*-mutants. (B) Number of unhatched
629 embryos with dorsal appendage defects per *Tdrd5l*- ($Tdrd5l^{\Delta M4/\Delta Q5}$) female (orange dots)
630 compared to wt control ($Cas9^{iso}$, blue dots). Note that the % of unhatched embryos with Dorsal
631 Appendage defects is similar to the % of ALL embryos with Dorsal Appendage defects (Fig 4B),
632 indicating that other defects in addition to D/V patterning defects affect viability of embryos
633 from *Tdrd5l*-mutant mothers.
634
635
636 **Supplemental figure 7: Tdrd5l regulates maternally deposited RNAs**
637 (A-C) Quantification of changes in maternal RNA expression in *Tdrd5l*- ($Tdrd5l^{\Delta M4/\Delta Q5}$) ovaries
638 compared to control ($Cas9^{iso}$) ovaries. Maternally deposited RNAs are defined as RNAs found in
639 0-2hr embryos from modENCODE. In each pie chart the share of maternal RNAs is shown in
640 magenta, while the share of non-maternal RNAs is shown in yellow. (A) 23% of all genes in the
641 genome are maternally deposited. (B) 30% of genes differentially expressed in *Tdrd5l*-mutant
642 ($Tdrd5l^{\Delta M4/\Delta Q5}$) ovaries are maternally deposited. (C) 90% of genes with a greater than 2-fold

643 change in gene expression in *Tdrd5l*-mutant (*Tdrd5l*$^{\Delta M4/\Delta Q5}$) ovaries are maternally deposited.
644 (D) 25% of RNAs pulled down in a previously published BicD/Egl RIP-seq data set are
645 upregulated in *Tdrd5l*-mutant (*Tdrd5l*$^{\Delta M4/\Delta Q5}$) ovaries.
646
647
648 **Supplemental figure 8: Kinesin mis-localizes in *Tdrd5l*-mutant oocytes**
649 (A-D) Immunofluorescence of a Kin-LacZ reporter in developing ooctyes. Kin-LacZ expression is
650 localized to the poster of the oocyte in wildtype (Kin-LacZ) stage 9 egg chambers (A) as marked
651 by a white arrowhead and in wildtype (Kin-LacZ) stage 10 egg chambers (B) as marked by a
652 yellow arrowhead. Kin-LacZ expression is not tightly localized to the posterior of the oocyte in
653 *Tdrd5l*-mutant (*Tdrd5l*$^{\Delta M4/\Delta Q5}$ *;; Kin-LacZ*) stage 9 egg chambers (C) as marked by a white
654 arrowhead, or in *Tdrd5l*-mutant (*Tdrd5l*$^{\Delta M4/\Delta Q5}$ *;; Kin-LacZ*) stage 10 egg chambers (D) as marked
655 by a yellow arrowhead. (E) Quantification of observations in A-D. Quantifications of stage 9 egg
656 chambers are in the yellow portion of the graph. Quantifications of stage 10 egg chambers are
657 in the pink portion of the graph. *Tdrd5l* mutants are represented by orange bars, and controls
658 are represented by blue bars. Each bar represents the percentage of egg chambers analyzed for
659 that stage. N is noted inside each bar.
660
661
662 **Supplemental table 1: Male RNAseq**
663
664 **Supplemental table 2: Female RNAseq**
665
666

667 **References**

668 1. Pek JW, Anand A, Kai T. Tudor domain proteins in development. Development. 2012 Jul
669 1;139(13):2255–66.

670 2. Boswell RE, Mahowald AP. tudor, a gene required for assembly of the germ plasm in
671 Drosophila melanogaster. Cell. 1985 Nov 1;43(1):97–104.

672 3. Chuma S, Hosokawa M, Kitamura K, Kasai S, Fujioka M, Hiyoshi M, et al. Tdrd1/Mtr-1, a
673 tudor-related gene, is essential for male germ-cell differentiation and nuage/germinal
674 granule formation in mice. Proc Natl Acad Sci. 2006 Oct 24;103(43):15894–9.

675 4. Brahms H, Meheus L, De Brabandere V, Fischer U, Lührmann R. Symmetrical dimethylation
676 of arginine residues in spliceosomal Sm protein B/B′ and the Sm-like protein LSm4, and
677 their interaction with the SMN protein. RNA. 2001 Nov;7(11):1531–42.

678 5. Friesen WJ, Massenet S, Paushkin S, Wyce A, Dreyfuss G. SMN, the Product of the Spinal
679 Muscular Atrophy Gene, Binds Preferentially to Dimethylarginine-Containing Protein
680 Targets. Mol Cell. 2001 May 25;7(5):1111–7.

681 6. Handler D, Olivieri D, Novatchkova M, Gruber FS, Meixner K, Mechtler K, et al. A
682 systematic analysis of Drosophila TUDOR domain-containing proteins identifies Vreteno
683 and the Tdrd12 family as essential primary piRNA pathway factors. EMBO J. 2011 Oct
684 5;30(19):3977–93.

685 7. Tauber D, Tauber G, Parker R. Mechanisms and Regulation of RNA Condensation in RNP
686 Granule Formation. Trends Biochem Sci. 2020 Sep;45(9):764–78.

687 8. Bardsley A, McDonald K, Boswell RE. Distribution of tudor protein in the Drosophila
688 embryo suggests separation of functions based on site of localization. Dev Camb Engl.
689 1993 Sep;119(1):207–19.

690 9. Chuma S, Hiyoshi M, Yamamoto A, Hosokawa M, Takamune K, Nakatsuji N. Mouse Tudor
691 Repeat-1 (MTR-1) is a novel component of chromatoid bodies/nuages in male germ cells
692 and forms a complex with snRNPs. Mech Dev. 2003 Sep 1;120(9):979–90.

693 10. Patil VS, Kai T. Repression of Retroelements in Drosophila Germline via piRNA Pathway by
694 the Tudor Domain Protein Tejas. Curr Biol. 2010 Apr 27;20(8):724–30.

695 11. Brennecke J, Aravin AA, Stark A, Dus M, Kellis M, Sachidanandam R, et al. Discrete Small
696 RNA-Generating Loci as Master Regulators of Transposon Activity in Drosophila. Cell. 2007
697 Mar 23;128(6):1089–103.

698 12. Balagopal V, Parker R. Polysomes, P bodies and stress granules: states and fates of
699 eukaryotic mRNAs. Curr Opin Cell Biol. 2009 Jun 1;21(3):403–8.

700 13. Behm-Ansmant I, Rehwinkel J, Doerks T, Stark A, Bork P, Izaurralde E. mRNA degradation
701 by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping
702 complexes. Genes Dev. 2006 Jul 15;20(14):1885–98.

703 14. Liu J, Valencia-Sanchez MA, Hannon GJ, Parker R. MicroRNA-dependent localization of
704 targeted mRNAs to mammalian P-bodies. Nat Cell Biol. 2005 Jul;7(7):719–23.

705 15. Dodson AE, Kennedy S. Phase Separation in Germ Cells and Development. Dev Cell. 2020
706 Oct 12;55(1):4–17.

707 16. Nakamura A, Amikura R, Hanyu K, Kobayashi S. Me31B silences translation of oocyte-
708 localizing RNAs through the formation of cytoplasmic RNP complex during Drosophila
709 oogenesis. Development. 2001 Sep 1;128(17):3233–42.

710 17. Johnston DS, Nüsslein-Volhard C. The origin of pattern and polarity in the Drosophila
711 embryo. Cell. 1992 Jan 24;68(2):201–19.

712 18. Becalska AN, Gavis ER. Lighting up mRNA localization in Drosophila oogenesis. Dev Camb
713 Engl. 2009 Aug;136(15):2493–503.

714 19. González-Reyes A, Elliott H, St Johnston D. Polarization of both major body axes in
715 Drosophila by gurken-torpedo signalling. Nature. 1995 Jun;375(6533):654–8.

716 20. Neuman-Silberberg FS, Sch T. The Drosophila Dorsoventral Patterning Gene gurken
717 Produces a Dorsally Localized RNA and Encodes a TGF(-like Protein. 1993;10.

718 21. Roth S, Neuman-Silberberg FS, Barcelo G, Schüpbach T. cornichon and the EGF receptor
719 signaling process are necessary for both anterior-posterior and dorsal-ventral pattern
720 formation in Drosophila. Cell. 1995 Jun 16;81(6):967–78.

721 22. Ephrussi A, Dickinson LK, Lehmann R. oskar organizes the germ plasm and directs
722 localization of the posterior determinant nanos. Cell. 1991 Jul 12;66(1):37–50.

723 23. Wang C, Lehmann R. Nanos is the localized posterior determinant in Drosophila. Cell. 1991
724 Aug;66(4):637–47.

725 24. Driever W, Nüsslein-Volhard C. The bicoid protein determines position in the Drosophila
726 embryo in a concentration-dependent manner. Cell. 1988 Jul 1;54(1):95–104.

727 25. Primus S, Pozmanter C, Baxter K, Doren MV. Tudor-domain containing protein 5-like
728 promotes male sexual identity in the Drosophila germline and is repressed in females by
729 Sex lethal. PLOS Genet. 2019 Jul 22;15(7):e1007617.

730 26. Yabuta Y, Ohta H, Abe T, Kurimoto K, Chuma S, Saitou M. TDRD5 is required for
731 retrotransposon silencing, chromatoid body assembly, and spermiogenesis in mice. J Cell
732 Biol. 2011 Mar 7;192(5):781–95.

733 27. Jeske M, Müller CW, Ephrussi A. The LOTUS domain is a conserved DEAD-box RNA helicase
734 regulator essential for the recruitment of Vasa to the germ plasm and nuage. Genes Dev.
735 2017 May 1;31(9):939–52.

736 28. Bopp D, Horabin JI, Lersch RA, Cline TW, Schedl P. Expression of the Sex-lethal gene is
737 controlled at multiple levels during Drosophila oogenesis. :16.

738 29. Lim AK, Kai T. Unique germ-line organelle, nuage, functions to repress selfish genetic
739 elements in Drosophila melanogaster. Proc Natl Acad Sci. 2007 Apr 17;104(16):6714–9.

740 30. Pane A, Wehr K, Schüpbach T. zucchini and squash encode two putative nucleases
741 required for rasiRNA production in the Drosophila germline. Dev Cell. 2007 Jun;12(6):851–
742 62.

743 31. Soper SFC, van der Heijden GW, Hardiman TC, Goodheart M, Martin SL, de Boer P, et al.
744 Mouse maelstrom, a component of nuage, is essential for spermatogenesis and
745 transposon repression in meiosis. Dev Cell. 2008 Aug;15(2):285–97.

746 32. Pan J, Goodheart M, Chuma S, Nakatsuji N, Page DC, Wang PJ. RNF17, a component of the
747 mammalian germ cell nuage, is essential for spermiogenesis. Development. 2005 Sep
748 15;132(18):4029–39.

749 33. Liang L, Diehl-Jones W, Lasko P. Localization of vasa protein to the Drosophila pole plasm is
750 independent of its RNA-binding and helicase activities. Development. 1994 May
751 1;120(5):1201–11.

752 34. Liu JL, Gall JG. U bodies are cytoplasmic structures that contain uridine-rich small nuclear
753 ribonucleoproteins and associate with P bodies. Proc Natl Acad Sci. 2007 Jul
754 10;104(28):11655–9.

755 35. Parker R, Sheth U. P Bodies and the Control of mRNA Translation and Degradation. Mol
756 Cell. 2007 Mar 9;25(5):635–46.

757 36. Morris JZ, Hong A, Lilly MA, Lehmann R. twin, a CCR4 homolog, regulates cyclin poly(A) tail
758 length to permit Drosophila oogenesis. Development. 2005 Mar 15;132(6):1165–74.

759 37. Graveley BR, Brooks AN, Carlson JW, Duff MO, Landolin JM, Yang L, et al. The
760 Developmental Transcriptome of Drosophila melanogaster. Nature. 2011 Mar
761 24;471(7339):473–9.

762 38. Vazquez-Pianzola P, Schaller B, Colombo M, Beuchle D, Neuenschwander S, Marcil A, et al.
763 The mRNA transportome of the BicD/Egl transport machinery. RNA Biol. 2017 Jan
764 2;14(1):73–89.

765 39. Johnstone O, Lasko P. Translational regulation and RNA localization in Drosophila oocytes
766 and embryos. Annu Rev Genet. 2001;35:365–406.

767 40. Berg CA. The Drosophila shell game: patterning genes and morphological change. Trends
768 Genet. 2005 Jun 1;21(6):346–55.

769 41. Zimyanin V, Lowe N, St Johnston D. An Oskar-Dependent Positive Feedback Loop
770 Maintains the Polarity of the Drosophila Oocyte. Curr Biol. 2007 Feb 20;17(4):353–9.

771 42. Chang JS, Tan L, Wolf MR, Schedl P. Functioning of the Drosophila orb gene in gurken
772 mRNA localization and translation. Development. 2001 Aug 15;128(16):3169–77.

773 43. Norvell A, Wong J, Randolph K, Thompson L. Wispy and Orb cooperate in the cytoplasmic
774 polyadenylation of localized gurken mRNA. Dev Dyn. 2015;244(10):1276–85.

775 44. Davidson A, Parton RM, Rabouille C, Weil TT, Davis I. Localized Translation of gurken/TGF-
776 α mRNA during Axis Specification Is Controlled by Access to Orb/CPEB on Processing
777 Bodies. Cell Rep. 2016 Mar 15;14(10):2451–62.

778 45. Kibanov MV, Egorova KS, Ryazansky SS, Sokolova OA, Kotov AA, Olenkina OM, et al. A
779 novel organelle, the piNG-body, in the nuage of Drosophila male germ cells is associated
780 with piRNA-mediated gene silencing. Mol Biol Cell. 2011 Jul 20;22(18):3410–9.

781 46. Hay B, Ackerman L, Barbel S, Jan LY, Jan YN. Identification of a component of Drosophila
782 polar granules. :16.

783 47. Wilsch-Bräuninger M, Schwarz H, Nüsslein-Volhard C. A Sponge-like Structure Involved in
784 the Association and Transport of Maternal Products during Drosophila Oogenesis. J Cell
785 Biol. 1997 Nov 3;139(3):817–29.

786 48. Mahowald AP. Fine structure of pole cells and polar granules in Drosophila melanogaster. J
787 Exp Zool. 1962;151(3):201–15.

788 49. Lasko PF, Ashburner M. Posterior localization of vasa protein correlates with, but is not
789 sufficient for, pole cell development. Genes Dev. 1990 Jun;4(6):905–21.

790 50. Ouyang JPT, Seydoux G. Nuage condensates: accelerators or circuit breakers for sRNA
791 silencing pathways? RNA N Y N. 2022 Jan;28(1):58–66.

792 51. Sundby AE, Molnar RI, Claycomb JM. Connecting the Dots: Linking Caenorhabditis elegans
793 Small RNA Pathways and Germ Granules. Trends Cell Biol. 2021 May;31(5):387–401.

794 52. Gruidl ME, Smith PA, Kuznicki KA, McCrone JS, Kirchner J, Roussell DL, et al. Multiple
795 potential germ-line helicases are components of the germ-line-specific P granules of
796 Caenorhabditis elegans. Proc Natl Acad Sci U S A. 1996 Nov 26;93(24):13837–42.

797 53. Batista PJ, Ruby JG, Claycomb JM, Chiang R, Fahlgren N, Kasschau KD, et al. PRG-1 and
798 21U-RNAs interact to form the piRNA complex required for fertility in C. elegans. Mol Cell.
799 2008 Jul 11;31(1):67–78.

800 54. Wang G, Reinke V. A C. elegans Piwi, PRG-1, regulates 21U-RNAs during spermatogenesis.
801 Curr Biol CB. 2008 Jun 24;18(12):861–7.

802 55. Ding D, Liu J, Midic U, Wu Y, Dong K, Melnick A, et al. TDRD5 binds piRNA precursors and
803 selectively enhances pachytene piRNA processing in mice. Nat Commun. 2018 Jan
804 9;9(1):127.

805 56. Sun YH, Jiang F, Li XZ. Disruption of Tdrd5 decouples the stepwise processing of long
806 precursor transcripts during pachytene PIWI-interacting RNA biogenesis. Biol Reprod. 2018
807 Oct 1;99(4):684–5.

808 57. Deng S, Wang J, Ma E, Zhang J, Xing S. TDRD5 Is Required for Spermatogenesis and
809 Oogenesis in Locusta migratoria. Insects. 2022 Feb 24;13(3):227.

810 58. Bult CJ, Blake JA, Smith CL, Kadin JA, Richardson JE, Mouse Genome Database Group.
811 Mouse Genome Database (MGD) 2019. Nucleic Acids Res. 2019 Jan 8;47(D1):D801–6.

812 59. Hutchison CA, Newbold JE, Potter SS, Edgell MH. Maternal inheritance of mammalian
813 mitochondrial DNA. Nature. 1974 Oct;251(5475):536–8.

814 60. Wang QT, Piotrowska K, Ciemerych MA, Milenkovic L, Scott MP, Davis RW, et al. A
815 Genome-Wide Study of Gene Activity Reveals Developmental Signaling Pathways in the
816 Preimplantation Mouse Embryo. Dev Cell. 2004 Jan 1;6(1):133–44.

817 61. Castagnetti S, Ephrussi A. Orb and a long poly(A) tail are required for efficient oskar
818 translation at the posterior pole of the Drosophila oocyte. Development. 2003 Mar
819 1;130(5):835–43.

820 62. Richter JD. CPEB: a life in translation. Trends Biochem Sci. 2007 Jun;32(6):279–85.

821 63. Costa A, Wang Y, Dockendorff TC, Erdjument-Bromage H, Tempst P, Schedl P, et al. The
822 Drosophila Fragile X Protein Functions as a Negative Regulator in the orb Autoregulatory
823 Pathway. Dev Cell. 2005 Mar 1;8(3):331–42.

824 64. Wong LC, Schedl P. Cup Blocks the Precocious Activation of the Orb Autoregulatory Loop.
825 PLOS ONE. 2011 Dec 2;6(12):e28261.

826 65. Doren MV, Williamson AL, Lehmann R. Regulation of zygotic gene expression in Drosophila
827 primordial germ cells. Curr Biol. 1998 Feb 12;8(4):243–6.

828 66. Gratz SJ, Ukken FP, Rubinstein CD, Thiede G, Donohue LK, Cummings AM, et al. Highly
829 specific and efficient CRISPR/Cas9-catalyzed homology-directed repair in Drosophila.
830 Genetics. 2014 Apr;196(4):961–71.

831 67. Gonczy P, Matunis E, DiNardo S. bag-of-marbles and benign gonial cell neoplasm act in the
832 germline to restrict proliferation during Drosophila spermatogenesis. Development. 1997
833 Nov 1;124(21):4361–71.

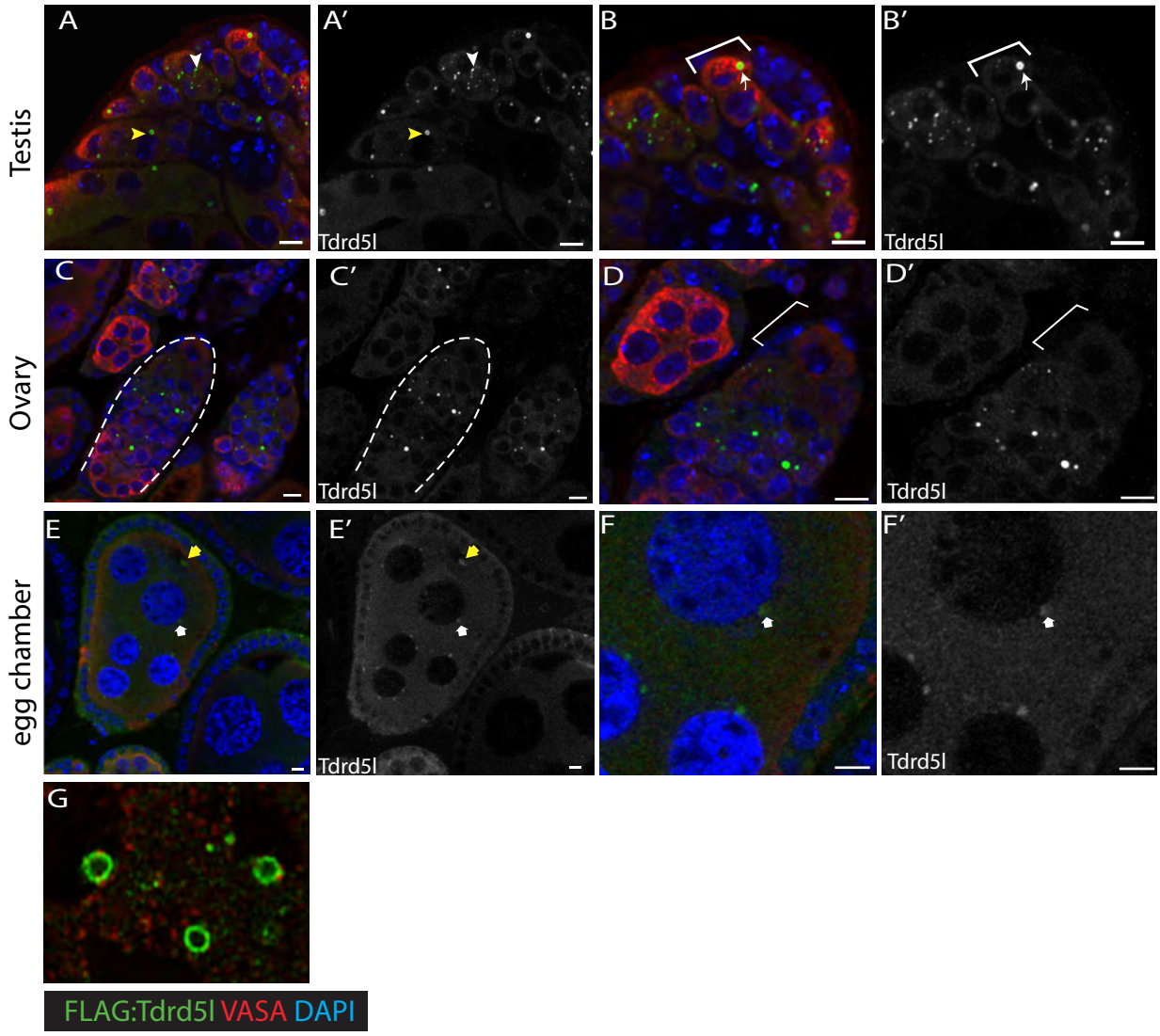
834 68. Zimmerman SG, Peters NC, Altaras AE, Berg CA. Optimized RNA ISH, RNA FISH and protein-
835 RNA double labeling (IF/FISH) in Drosophila ovaries. Nat Protoc. 2013 Nov;8(11):2158–79.

836 69. Zhang Z, Theurkauf WE, Weng Z, Zamore PD. Strand-specific libraries for high throughput
837 RNA sequencing (RNA-Seq) prepared without poly(A) selection. Silence. 2012 Dec
838 28;3(1):9.

839 70. Benner L, Castro EA, Whitworth C, Venken KJT, Yang H, Fang J, et al. Drosophila
840 Heterochromatin Stabilization Requires the Zinc-Finger Protein Small Ovary. Genetics.
841 2019 Nov;213(3):877–95.

842

Figure 1


Testis
Ovary
egg chamber
A
A′
B
B′
C
C′
D
D′
E
E′
F
F′
G
Tdrd5l
FLAG:Tdrd5l VASA DAPI

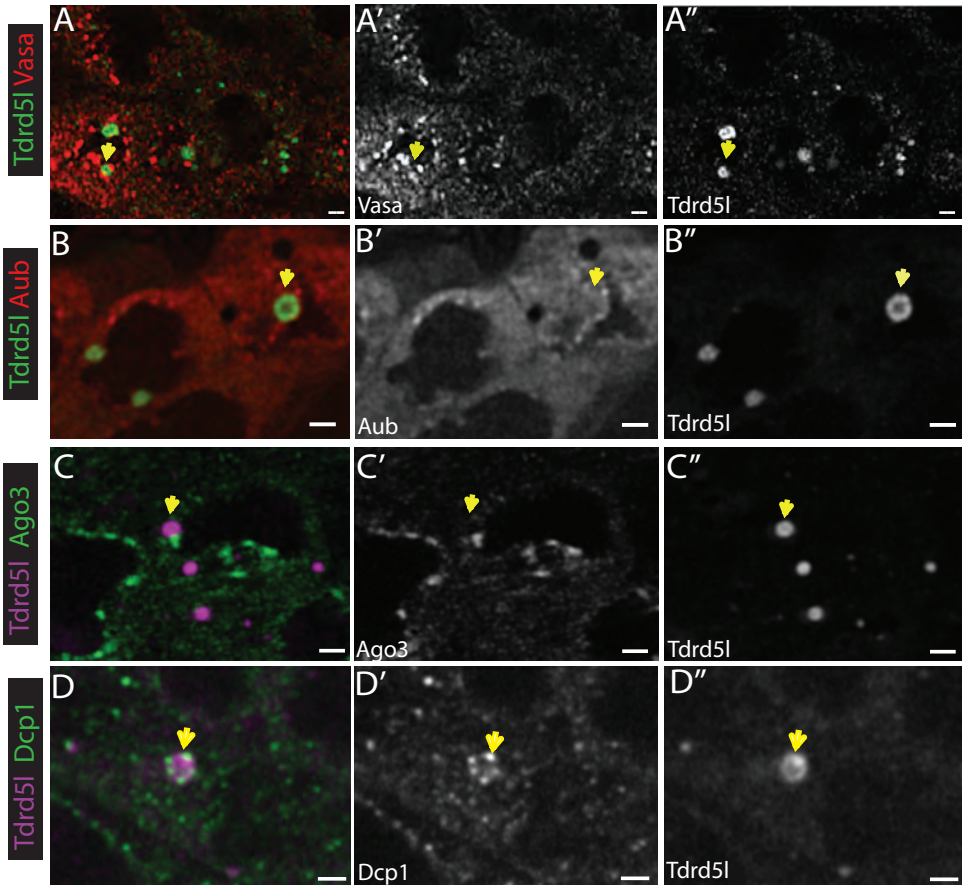

Tdrd5l Vasa
A
A′
Vasa
A″
Tdrd5l
Tdrd5l Aub
B
B′
Aub
B″
Tdrd5l
Tdrd5l Ago3
C
C′
Ago3
C″
Tdrd5l
Tdrd5l Dcp1
D
D′
Dcp1
D″
Tdrd5l

Figure 3

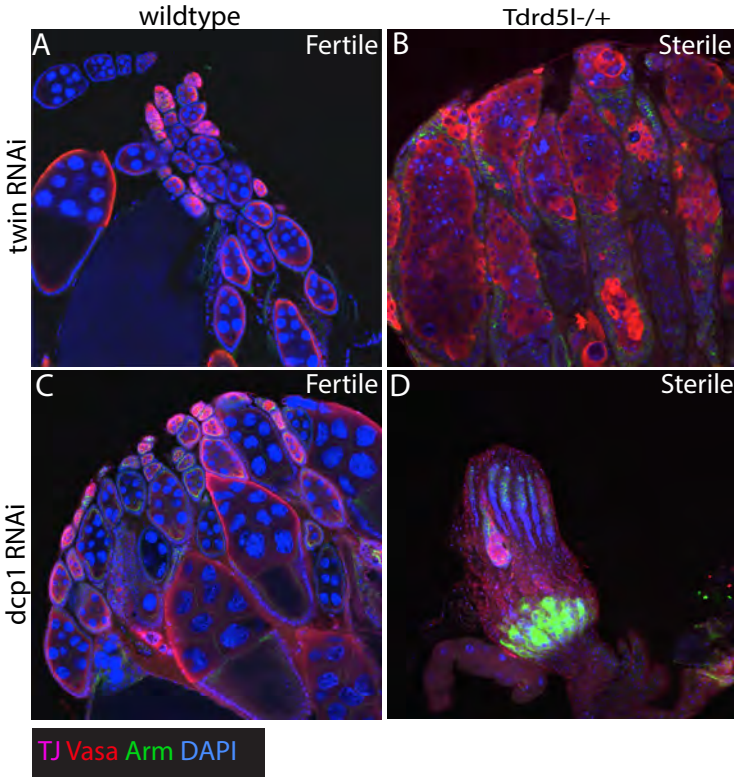

wildtype
Tdrd5l-/+
twin RNAi
dcp1 RNAi
A
Fertile
B
Sterile
C
Fertile
D
Sterile
TJ Vasa Arm DAPI

Figure 4

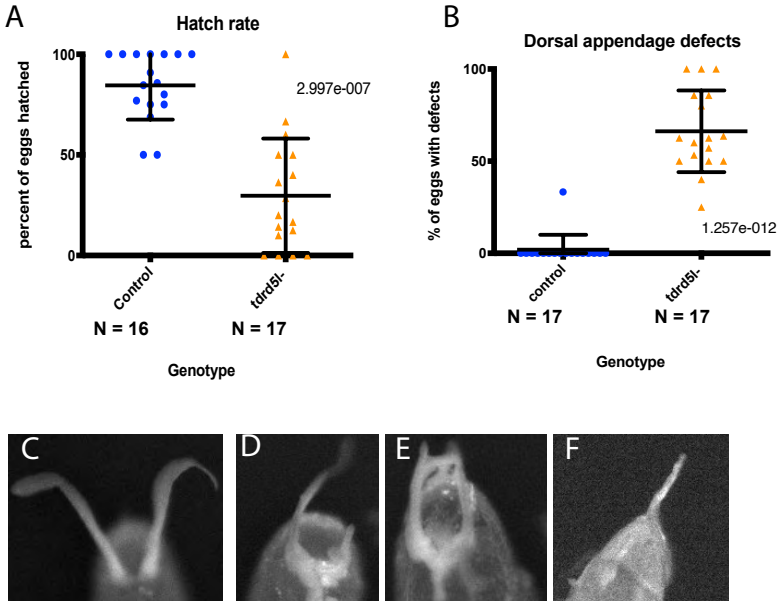

A
Hatch rate
percent of eggs hatched
100
50
0
2.997e-007
Control
tdrd5l-
N = 16
N = 17
Genotype
B
Dorsal appendage defects
% of eggs with defects
100
50
0
1.257e-012
control
tdrd5l-
N = 17
N = 17
Genotype
C
D
E
F

Figure 5

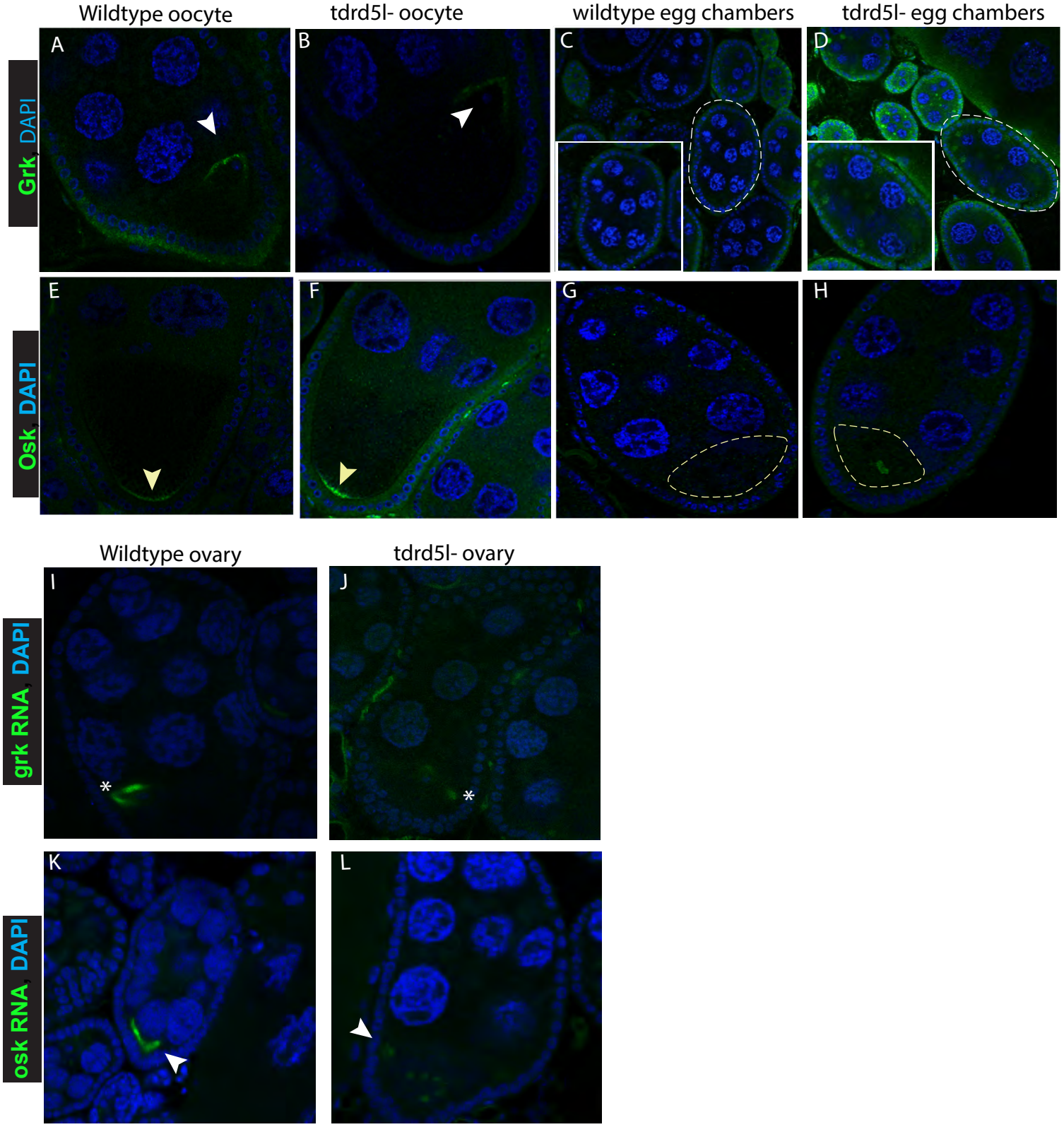
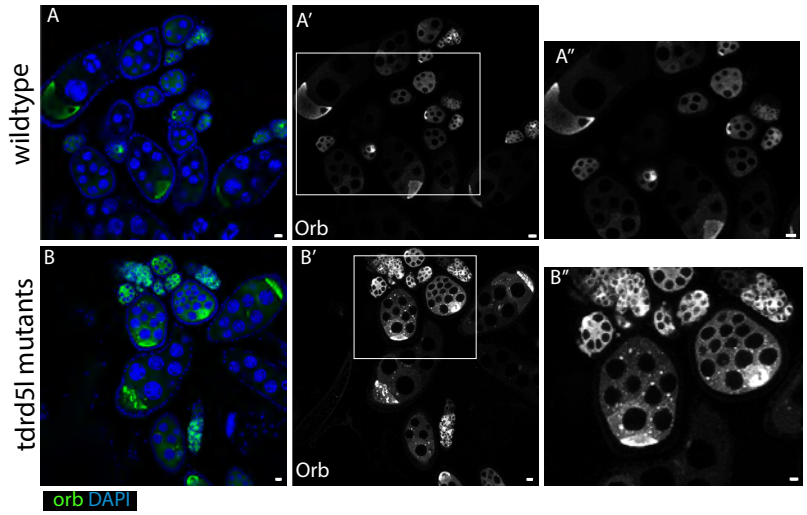
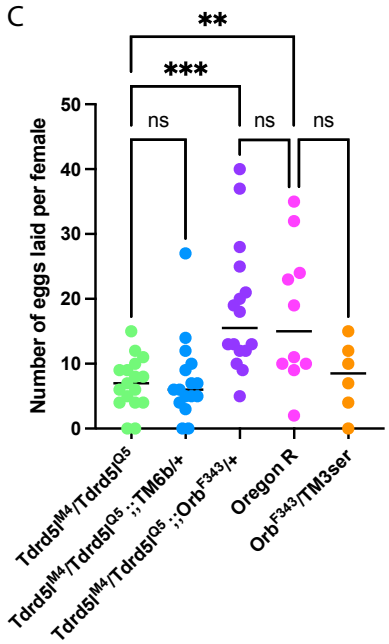
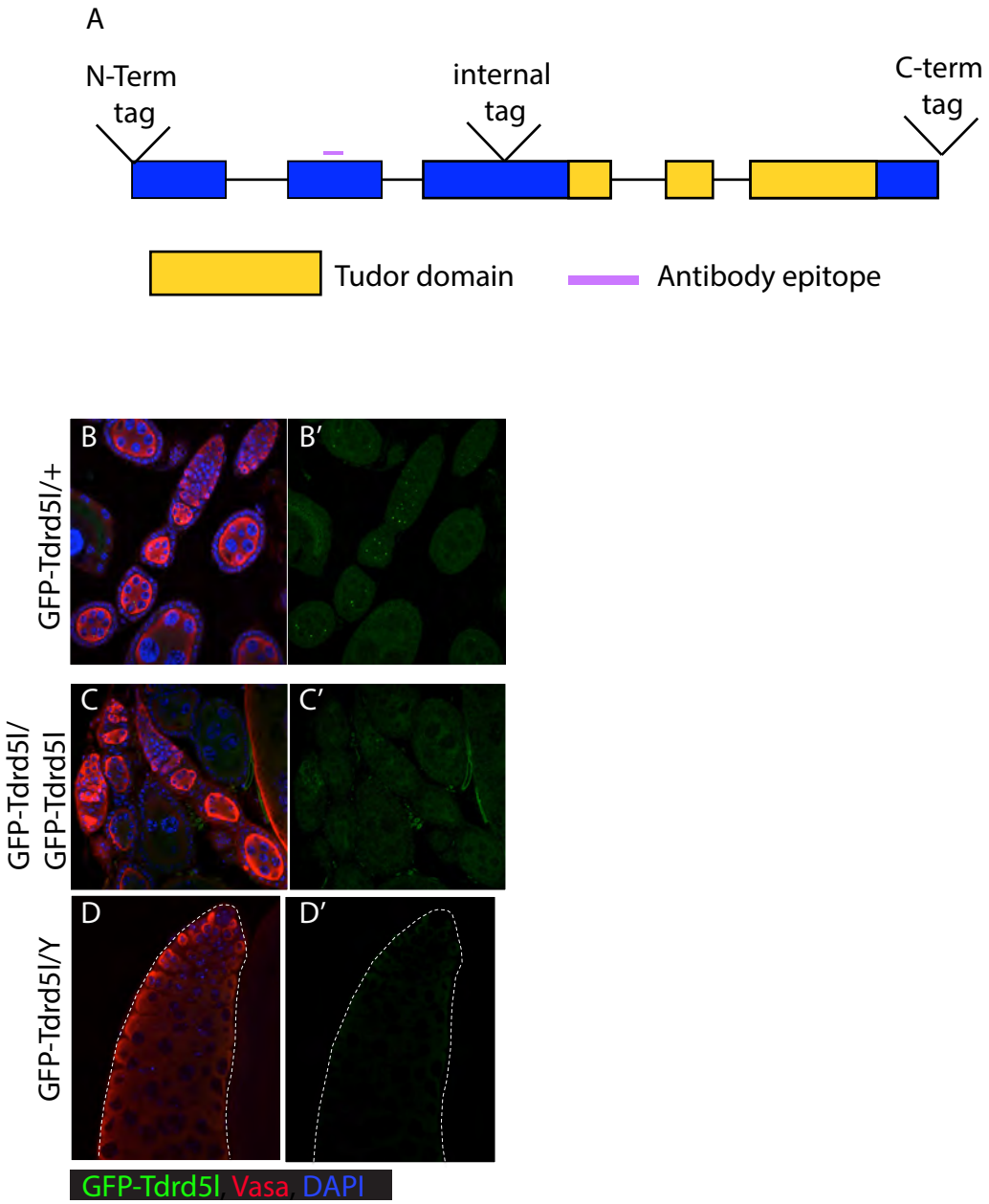

Wildtype oocyte
tdrd5l- oocyte
wildtype egg chambers
tdrd5l- egg chambers
Grk, DAPI
Osk, DAPI
A
B
C
D
E
F
G
H
Wildtype ovary
tdrd5l- ovary
grk RNA, DAPI
osk RNA, DAPI
I
J
K
L

Figure 6

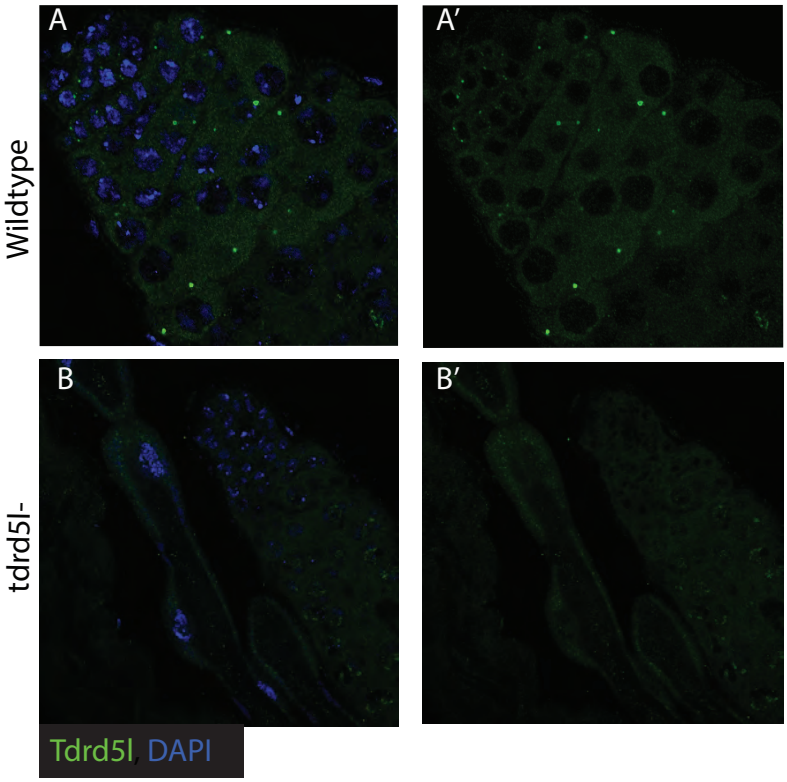

wildtype
tdrd5l mutants
A
A′
A″
B
B′
B″
Orb
Orb
orb DAPI

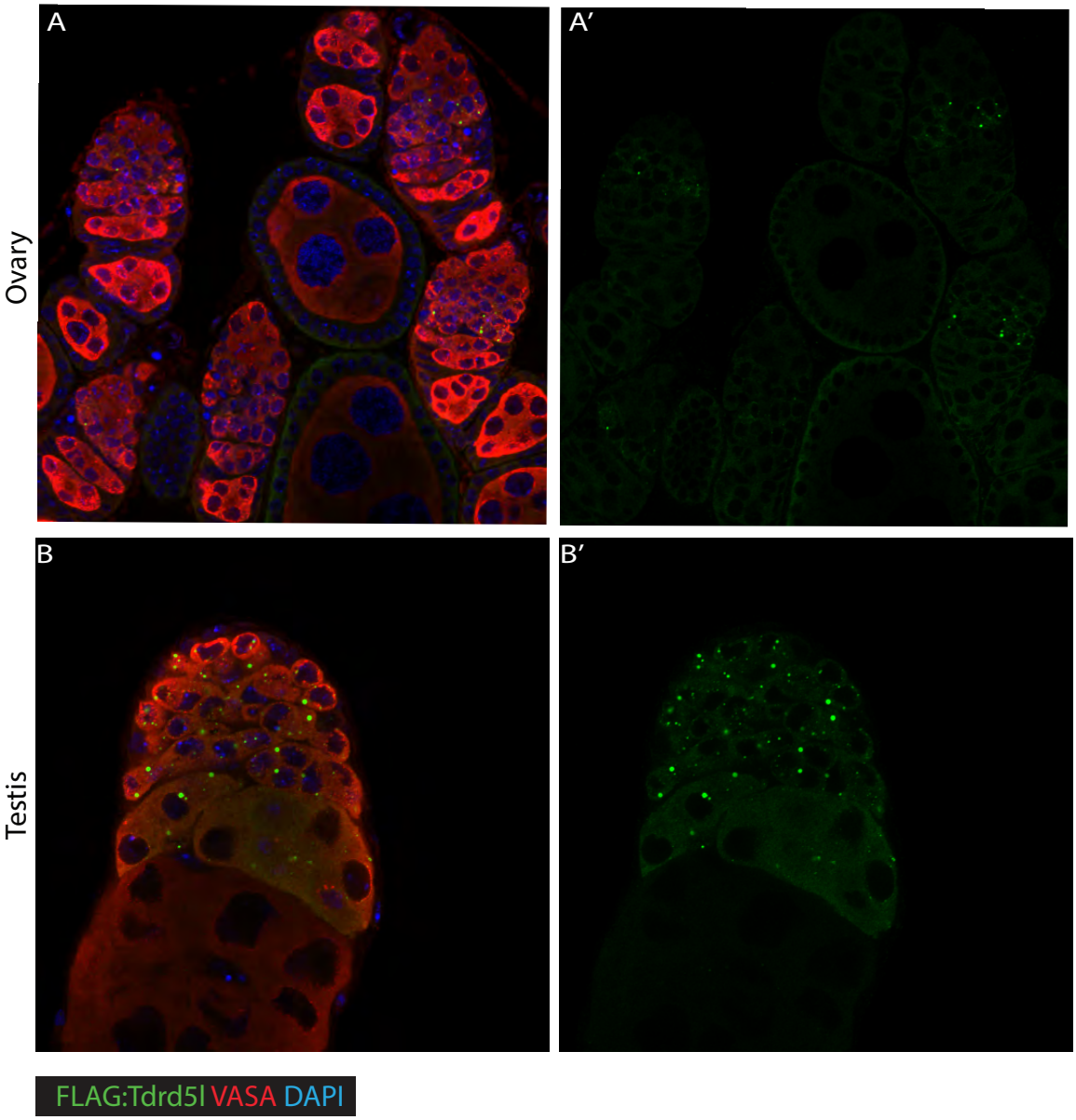

C
**
***
ns
ns
ns
Number of eggs laid per female
50
40
30
20
10
0
Tdrd5l^M4/Tdrd5l^Q5
Tdrd5l^M4/Tdrd5l^Q5 ;;TM6b/+
Tdrd5l^M4/Tdrd5l^Q5 ;;Orb^F343/+
Oregon R
Orb^F343/TM3ser

Supplemental Figure 1

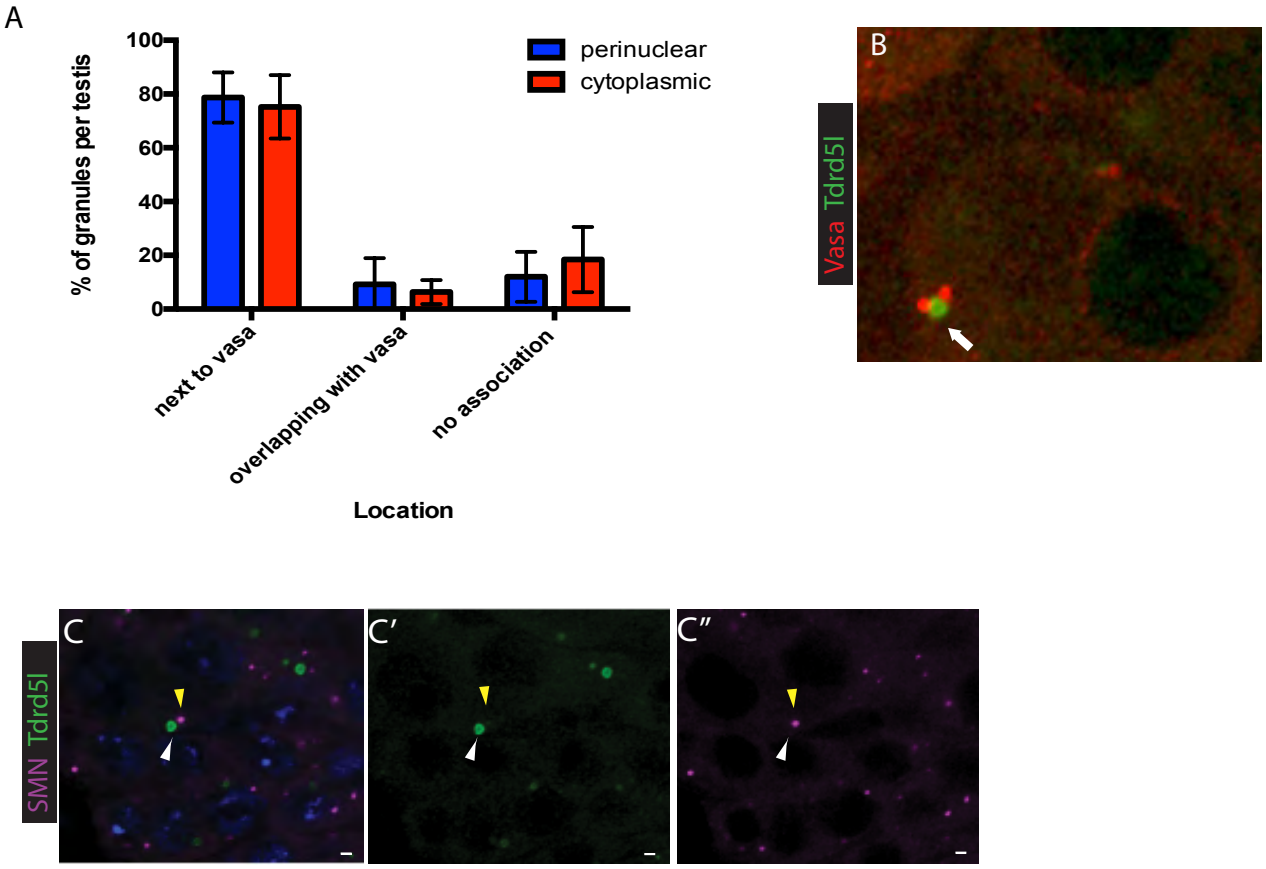


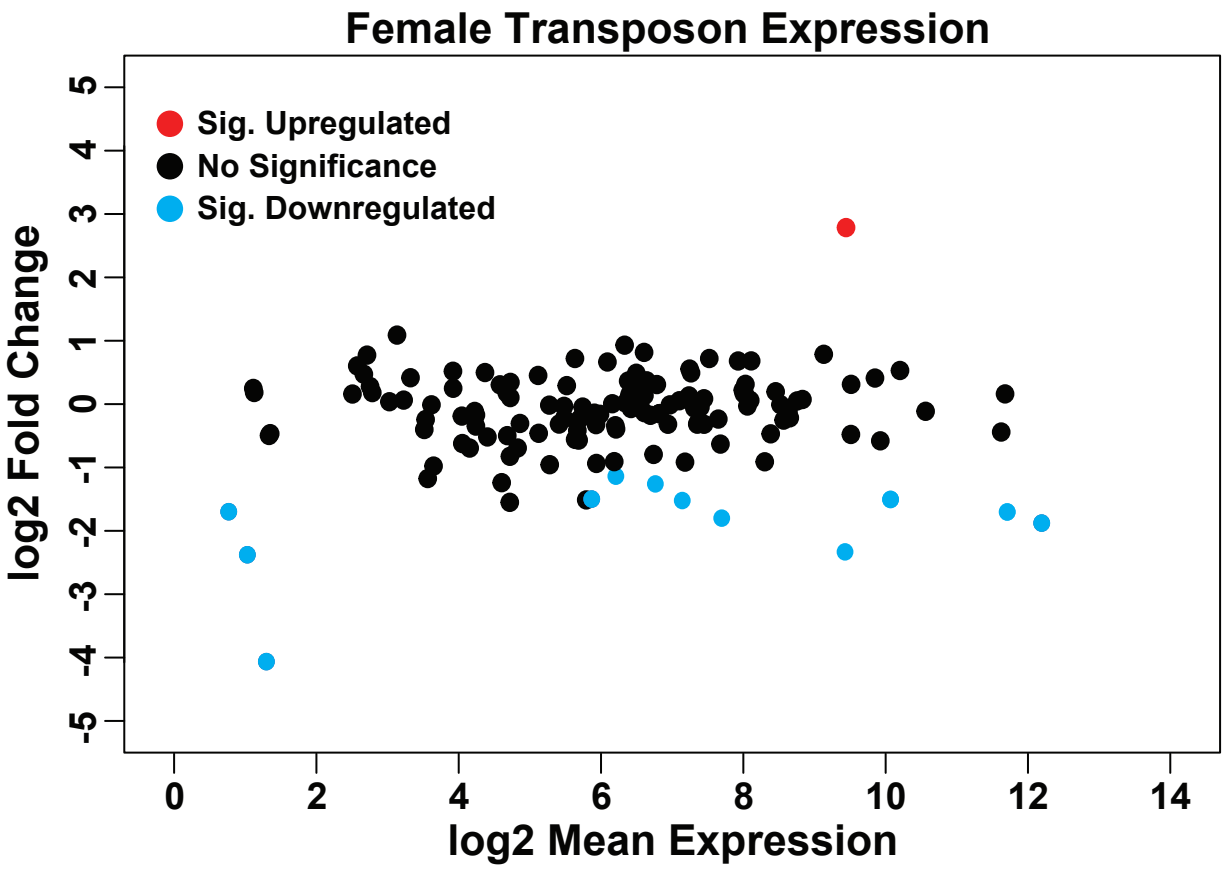
Supplementary Figure 2

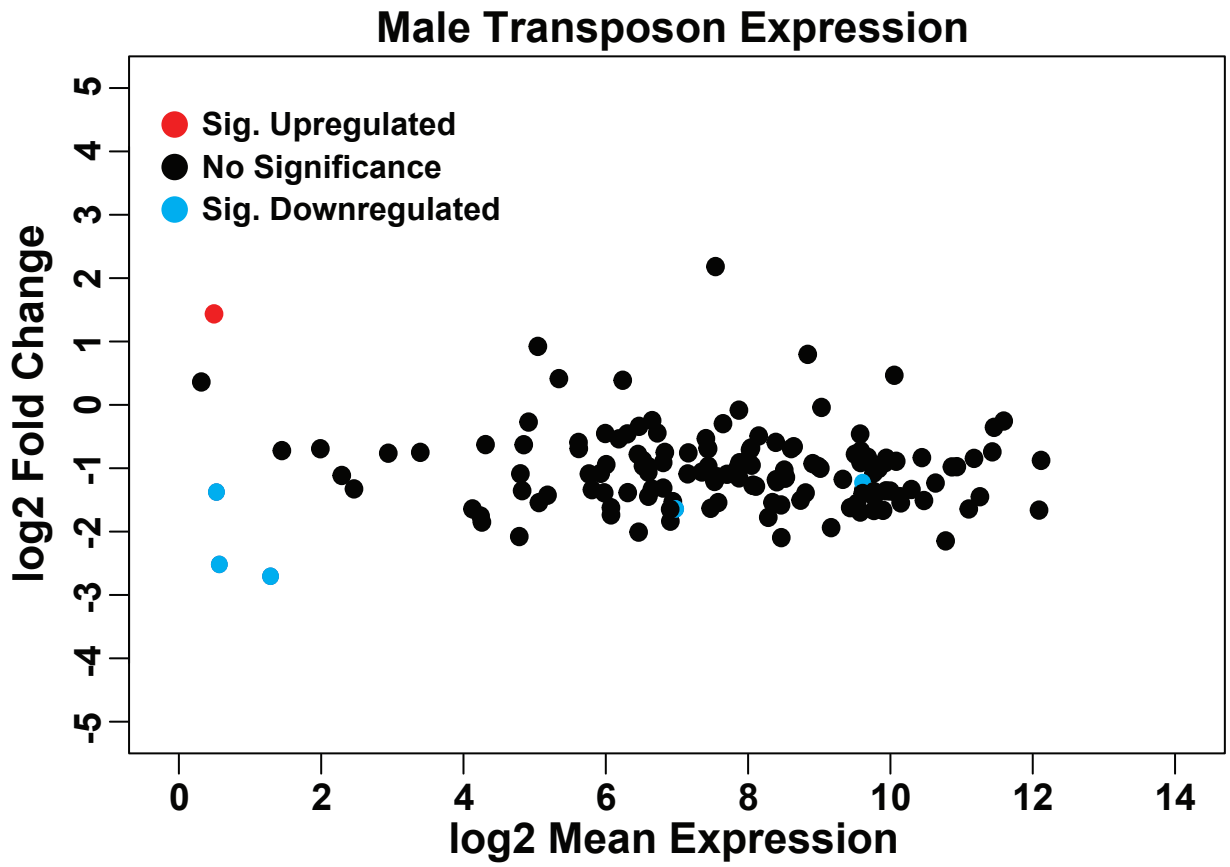



A
A′
Ovary
B
B′
Testis
FLAG:Tdrd5l VASA DAPI

Supplementary figure 4




Female Transposon Expression
Sig. Upregulated
No Significance
Sig. Downregulated
log2 Fold Change
log2 Mean Expression
5
4
3
2
1
0
-1
-2
-3
-4
-5
0
2
4
6
8
10
12
14


Male Transposon Expression
Sig. Upregulated
No Significance
Sig. Downregulated
log2 Fold Change
log2 Mean Expression
5
4
3
2
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-2
-3
-4
-5
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6
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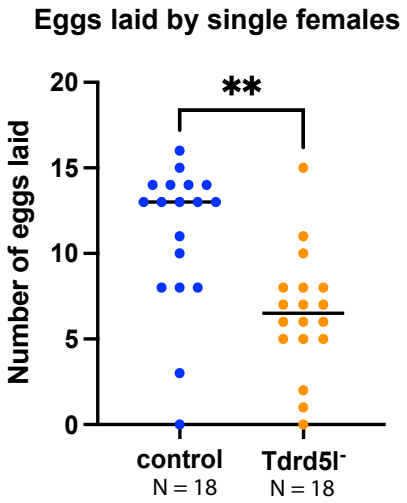
12
14

Supplementary Figure 6

A



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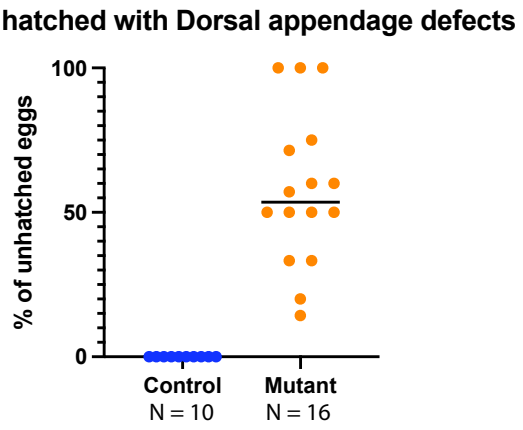
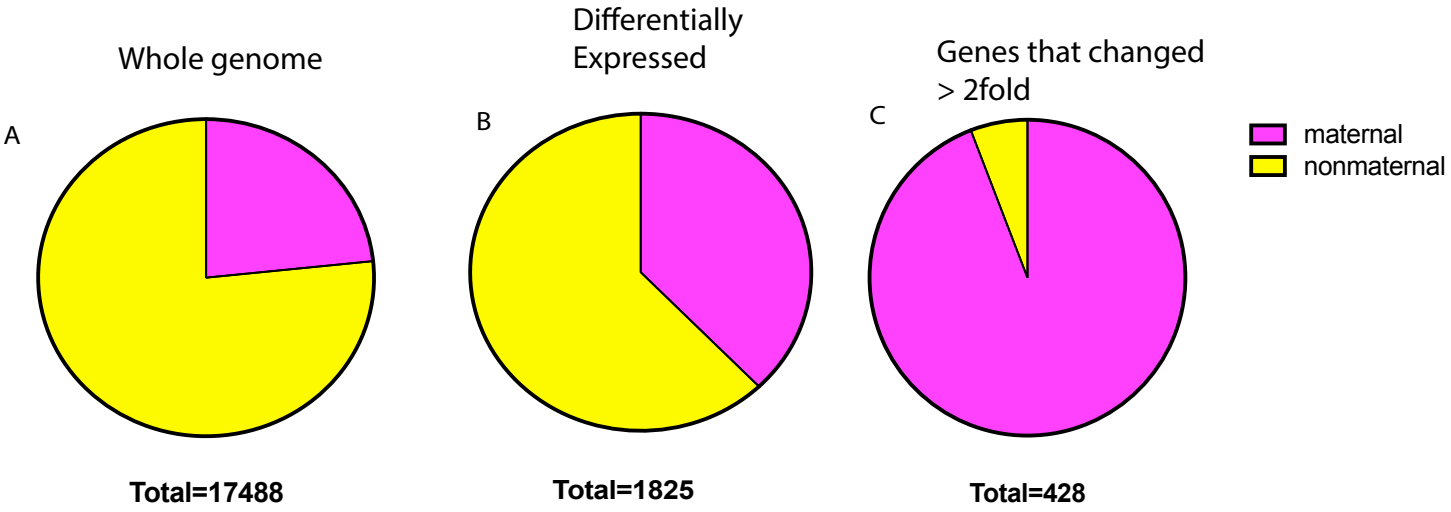
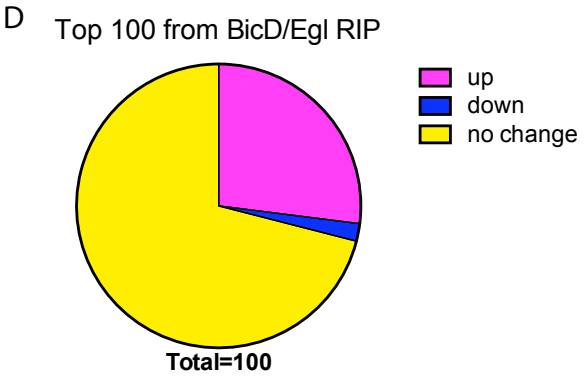
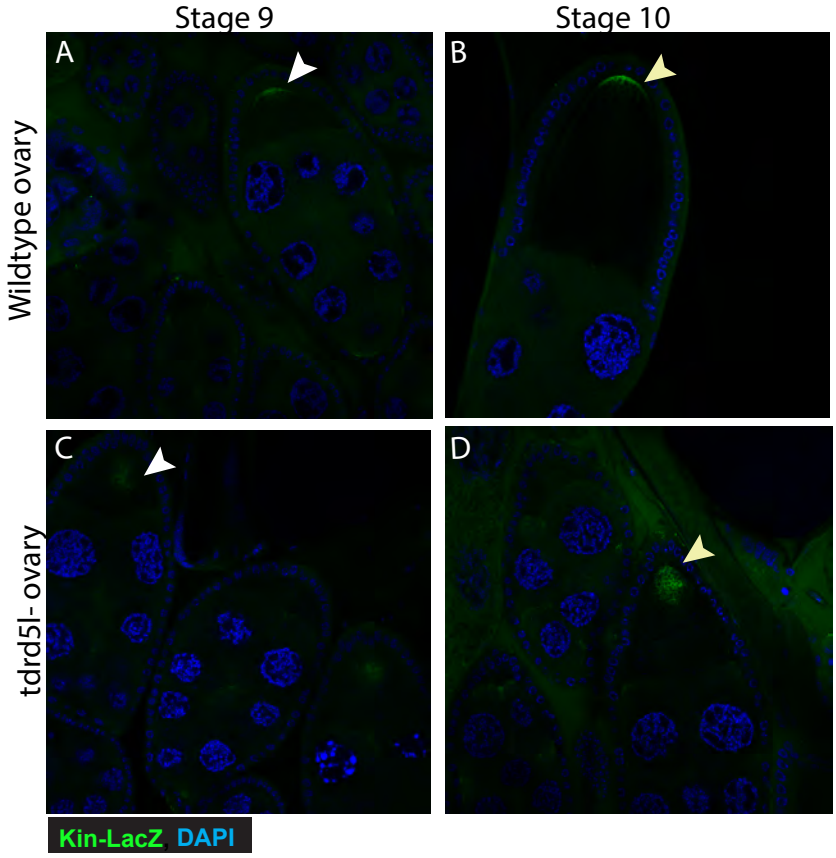
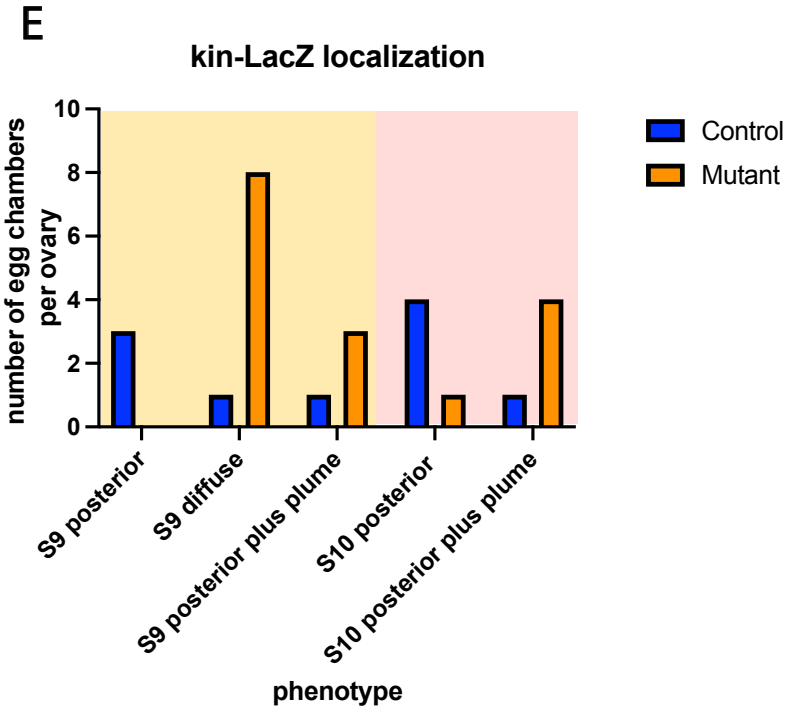


Supplementary Figure 7





Supplementary 8