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Title: Robust sorting and buffering within condensates control transcriptome stoichiostasis

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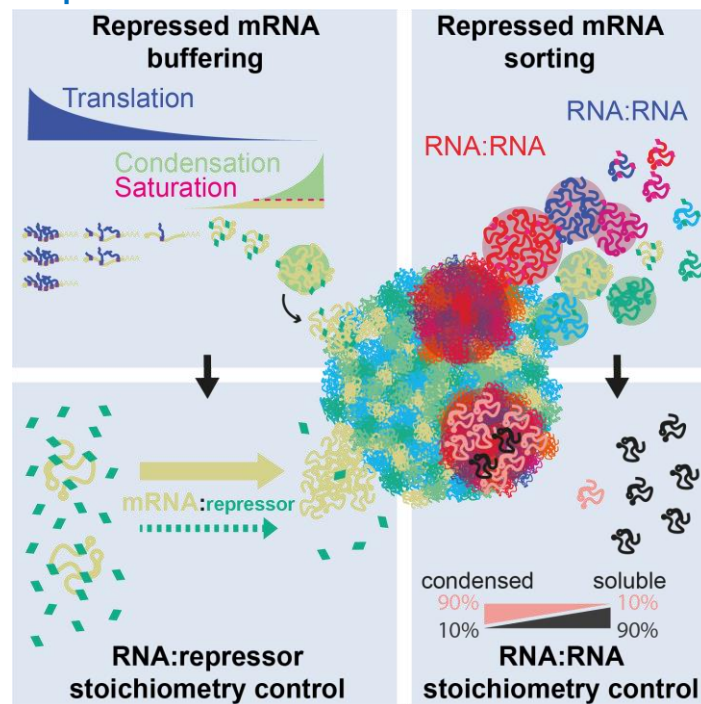
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Graphical abstract



Highlights

mRNA phase separations buffer variation in translational activity

mRNA sorting and buffering into nanoclusters controls transcriptome stoichiometries

Repressed mRNA condensation prevents cytosolic depletion of translation repressors

Transcriptome-wide mRNA compaction is a default but selective state upon quiescence

29 **Abstract**

30
31 Cellular homeostasis requires the robust control of biomolecule concentrations, but how do
32 millions of mRNAs coordinate their stoichiometries in the face of dynamic translational changes?
33 Here, we identified a two-tiered mechanism controlling mRNA:mRNA and mRNA:protein
34 stoichiometries where mRNAs super-assemble into condensates with buffering capacity and
35 sorting selectivity through phase transition mechanisms. Using *C. elegans* oogenesis arrest as a
36 model, we investigated the transcriptome cytosolic reorganization through the sequencing of RNA
37 super-assemblies coupled with single mRNA imaging. Tightly repressed mRNAs self-assembled
38 into same-sequence nanoclusters that further co-assembled into multiphase condensates. mRNA
39 self-sorting was concentration-dependent, providing a self-buffering mechanism that is selective
40 to sequence identity and controls mRNA:mRNA stoichiometries. The cooperative sharing of
41 limiting translation repressors between clustered mRNAs prevented the disruption of
42 mRNA:repressor stoichiometry in the cytosol. The robust control of mRNA:mRNA and
43 mRNA:protein stoichiometries, which we term transcriptome stoichiostasis, emerges from mRNA
44 self-buffering and cooperative super-assembly into multiphase multiscale condensates.

45
46 **Keywords:** phase separations, phase transitions, RNA condensates, homeostasis, RNP
47 stoichiometry, buffering, sorting, translation, multiscale condensates, nanoclusters.

48 **INTRODUCTION**

49
50
51 Cells undergo dramatic activity changes yet must maintain stable internal conditions. Maintaining
52 homeostasis in the face of massive fluctuations in biomolecule concentrations is a challenging
53 task. For example, the stoichiometric information of mRNAs must be robustly controlled
54 transcriptome-wide.^{1,2} Transcription and mRNA decay are two strategies for mRNA copy number
55 control. Translation regulation also uncouples protein production from mRNA expression³ and
56 provides spatiotemporal control of gene expression.⁴ How cells buffer variations in translation to
57 maintain a robust control of transcriptome stoichiometries remains to be addressed.

58
59 To control mRNA translation, regulatory proteins assemble with mRNAs into ribonucleoproteins
60 (RNPs).⁵ In a first layer of coordination, regulators are distributed across mRNA collectives that
61 share the same binding elements, termed RNA regulons.^{1,3} To further ensure robust translation
62 control, mRNAs are co-repressed with redundant repressors or cofactors.⁶⁻⁸ We and others
63 introduced the conceptual framework of phase transitions where coregulated RNAs can further
64 co-assemble into liquid droplets, semi-liquid hydrogels, or solidify into glass or crystal-like forms.⁹⁻
65 ¹¹ Co-repressed mRNAs condense transcriptome-wide^{3,12-14}, and despite the characterization of
66 reconstituted repressive condensates¹⁵⁻¹⁷, whether condensation is a cause or a consequence of
67 translation repression *in vivo* is unclear.⁴ While repression can occur in the absence of
68 condensation, it has been observed that widespread mRNA repression during cellular quiescence
69 and stress triggers condensation¹⁸⁻²⁰. We therefore hypothesized that condensates may buffer
70 the accumulation of repressed mRNAs.¹

71
72 Phase transition theory predictions remain rarely quantitatively tested *in vivo*.²¹⁻²³ An open
73 question is whether endogenous condensates have the storage capacity and selectivity to buffer

74 biomolecules within the crowded and complex cellular environment.¹ In solution, biomolecules
75 remain soluble up to a saturation concentration, above which the fraction in excess co-assembles
76 to concentrate into condensates, limiting the accumulation of biomolecules in the dispersed
77 phase.¹¹ Whether various condensates buffer protein concentrations or reduce protein noise has
78 been debated.^{24,25} The ability of condensates to selectively buffer stoichiometric variations
79 between a vast diversity of mRNAs is unknown. Translation repression has been proposed to be
80 sufficient to target mRNAs to condensates through promiscuous interactions.^{12,13,26} While this may
81 explain how condensates are hijacked to non-specific aggregates in degenerative diseases,^{27,28}
82 this is incompatible with buffering selectivity. In fact, these low selectivity models contradict (i) the
83 diversity of repressed mRNA condensates that coexist within cells,¹ (ii) the coexistence of multiple
84 protein phases within condensates,^{10,29-31} (iii) the absence of mixing between repressed mRNA
85 species that form homotypic nanoclusters³²⁻³⁵. In addition to selectively buffering biomolecules,
86 condensates have been postulated to have storage capacity. Although prevailing models suggest
87 that stress granules and P-bodies have limited storage capacity, large variations in translation
88 nevertheless indicate the existence of subcellular reservoirs. Nanoclusters may represent the
89 invisible stores. Condensate buffering could provide a protective mechanism to maintain
90 homeostasis. However, to date, studies characterizing the adaptive properties of condensates
91 are few.³⁶⁻³⁸

92
93 Using *C. elegans* oogenesis as an in vivo model, we address how phase separations control
94 mRNA stoichiometries. Archetypes of long-lived cells, oocytes maintain fitness through
95 quiescence, and experience dramatic changes in cellular activity upon stimulation. In the absence
96 of transcription within oocytes, maternal mRNA condensation may tend towards equilibrium as
97 metabolic activity drops. Maternal mRNAs can accumulate in viscoelastic *C. elegans* P-
98 bodies,^{10,39} solid *Xenopus* Balbiani-bodies,⁴⁰ or hydrogel-like mammalian MARDOs.⁴¹ We
99 analyzed the supramolecular organization of oocyte transcriptomes and found that oogenesis
100 arrest requires the large-scale, long-term, selective storage of the maternal transcriptome through
101 high compaction into multiscale multiphase condensates that allow the dynamic and sequential
102 retrieval of information as development resumes. We demonstrate that (i) repressed mRNAs
103 saturate in an identity dependent manner providing a selective concentration buffering
104 mechanism, (ii) this saturation-dependent clustering impacts mRNA:mRNA and mRNA:protein
105 stoichiometries transcriptome-wide, (iii) condensation is exponential to the degree of translation
106 repression. Our study provides a quantitative model of how RNA self-organization selectively and
107 robustly buffers mRNA cytosolic stoichiometric variations in a sequence- and translation activity-
108 dependent manner.

109 **RESULTS**

110 **Transcriptome-wide mRNA condensation is a default but selective state upon quiescence**

111
112
113
114 To explore the remodeling of the transcriptome during cellular adaptation to quiescence, we took
115 advantage of *C. elegans* oogenesis rates, which can be reversibly controlled through the depletion
116 of sperm (quiescent oogenesis) or the addition of sperm (active oogenesis). Oogenesis rates
117 differ dramatically between active and quiescent oogenesis with one oocyte produced every ~20
118 minutes to one every ~10 hours.⁴² In both oogenesis states oocytes remarkably maintain their

119 fitness, providing a unique model of cellular adaptation. Quiescence triggers the accumulation of
120 translationally repressed mRNPs into P-body condensates that can grow up to 10 μm in size
121 (Figures 1A, B).^{10,20,39,43–45}

122
123 To address whether P-bodies are quiescence-adaptive reservoirs with storage capacity and
124 selectivity for repressed mRNAs, we first focused on candidate mRNAs that were previously
125 shown to be repressed (*spn-4*, *glp-1*) or translated (*puf-5*, *tbb-2*, *pccb-1*) in oocytes.⁴⁶ The
126 repression cofactor protein GFP:CAR-1 was used to label P-bodies in arrested oocytes,^{10,39,45} and
127 single-molecule fluorescent *in situ* hybridization (smFISH) was used to quantify mRNA
128 clustering.^{47,48} To ensure that RNA condensation did not lead to over- or under-detection of
129 mRNAs we controlled for the linear quantitative scaling between single transcripts and clustered
130 mRNAs after calibrating single mRNA fluorescence intensity (see Figure S1, methods and
131 associated quantitative controls). The two translationally repressed mRNAs, *spn-4* and *glp-1*,
132 were found to be more than 100-fold concentrated in P-bodies compared to the surrounding
133 cytosol (Figures 1B, 1B', S2A-E). In contrast, the two translated *pccb-1* and *tbb-2* mRNAs were
134 concentrated less than 10-fold (Figures 1B, 1B', S2A, S2E). This differential partitioning of mRNAs
135 in P-bodies corresponds to a dynamic range of 90% to 20% of sequestered mRNAs (Figures 1B,
136 1B', S2B, S2E), demonstrating that condensation depletes the cytosol of large but selective
137 mRNA fractions. Although there was a poor correlation between mRNA abundance and mRNA
138 condensation, both translationally repressed mRNAs strongly partitioned to P-bodies while both
139 translated mRNAs partitioned less efficiently (Figure 1B'', S2A-B). For some mRNA species, tens
140 of thousands of copies condensed per cell indicating that condensates could be reservoirs with
141 large storage capacity (Figure 1B''', S2C-D).

142
143 To expand our quantification of condensation transcriptome-wide, we adapted our Fluorescence
144 Activated Particle Sorting and RNA-sequencing (FAPSeq) method to oocytes (Figure 1C).³
145 Oocyte P-bodies were labelled with GFP:CAR-1, formaldehyde fixed, and FAPS sorted by their
146 size and fluorescence using whole animal extracts (Figure 1D), then subjected to RNA-
147 sequencing. Using FAPSeq we computed mRNA enrichment in P-bodies versus whole oocytes
148 (Figures S2F-S2I, Data S1). This strongly correlated ($r^2 > 0.95$) with the mRNA enrichments
149 computed by smFISH imaging for candidate mRNAs, cross-validating the two approaches (Figure
150 S2J). Calibrating RNA-Seq relative levels (Data S2) with imaging absolute numbers (Figures S2K,
151 S2L) allowed us to extrapolate mRNA copy numbers within and outside P-bodies transcriptome-
152 wide (Figure 1E, Data S3). For mRNAs of similar abundance, the degree of enrichment within P-
153 bodies ranged across orders of magnitude, suggesting sorting selectivity (Figure 1E). The
154 dynamic range of condensation was between 10 to 90%. The median across all mRNA species
155 was 65% (Figures 1F, S2M-P). Together, this demonstrated that despite selectivity, large-scale
156 condensation is the rule rather than the exception upon quiescence.

157 158 **Translationally repressed mRNAs condense into oocyte P-body reservoirs**

159
160 To define the features that distinguish condensed RNAs, we computed the degree of P-body
161 enrichment of different RNA types compared to the whole oocyte transcriptome. mRNAs were
162 strongly enriched compared to all ncRNAs we investigated (Figure 2A). Moreover, tRNAs and
163 rRNAs, and components of the translation machineries, were among the most severely depleted

164 (Figures 2A, 2B), suggesting that P-body mRNAs are not translationally active. Ranking candidate
165 mRNAs according to the abundance of their protein output in oocytes⁴⁶ confirmed that mRNA
166 enrichment within P-bodies is inversely correlated with protein production (Figure 2C, Table S1).
167 Accordingly, the mRNA targets of translation repressors PUF-5⁴⁹ and OMA-1/LIN-41⁵⁰ were also
168 strongly enriched in oocyte P-bodies at the developmental stage at which these repressors
169 function, whereas the targets of repressors that function at earlier stages were not (Figure 2D).
170 Translationally repressed P-body mRNAs tended to encode regulatory functions, whereas
171 mRNAs excluded from P-bodies tended to encode for proteins with constitutive, housekeeping,
172 metabolic and structural functions (Figure 2E). By comparing mRNAs enriched in P-bodies to
173 whole oocyte mRNAs we found that P-body mRNAs have longer 3'UTRs, while there was no
174 significant length difference for CDSs or 5'UTRs (Figure S3A). This is in contrast to stress granule
175 and P-granule mRNAs that have been shown to possess longer 3'UTRs, CDSs, and 5'UTRs
176 (Figure S3A).^{12,13} The absence of compositional bias (Figures S3B) and the restriction of length
177 bias to 3'UTRs (Figures S3A) suggests more selective mechanisms for condensate sorting. The
178 targets of some RBPs identified by CLIP are strongly enriched in P-bodies (Figure 2D), yet
179 individually these RBPs do not explain the transcriptome-wide complexity of super-assemblies as
180 these represent a small subset of the P-body transcriptome (Figure 1E).

181

182 **Homotypic mRNA nanoclusters coarsen into multiscale, multiphase condensates**

183

184 Our identification of the coexistence of thousands of mRNAs in condensates raised the question
185 of the mechanism of their super-assembly. Classic phase separation theory predicts that as a
186 system tends toward equilibrium a single large droplet will integrate smaller ones over time in a
187 growth mechanism, termed coarsening, that minimizes the energy of surface tension at the
188 interfaces (Figure 3A). To test whether coarsening applies to the viscoelastic P-bodies that were
189 previously shown to relax under surface tension¹⁰, we took advantage of the spatiotemporal
190 organization of oocytes within gonads to record the sizes of GFP:CAR-1 condensates at various
191 time-points (Figures 3B, 3C). In the oldest quiescent oocytes, 1-2 supersize outliers per cell
192 outgrew the smaller condensates in a time-dependent manner to occupy most of the condensate
193 cumulative volume (Figures 3C, 3C'). Although this coarsening fits the classic phase separation
194 theory, the condensate growth was extremely slow and occurred over a week-long period. In
195 combination with their viscoelastic properties¹⁰, the slow growth of P-bodies is in contrast to the
196 liquid P-granules that grow on a timescale of seconds.⁹

197

198 To determine when condensates arose, we considered two possibilities: (1) mRNA clusters were
199 nucleated and grew during quiescence (2) smaller mRNA clusters existed prior to quiescence but
200 were below detection limits. To distinguish between these two possibilities, we took advantage of
201 the tunable rates of oogenesis.⁴² In the absence of fertilization, oocytes remain arrested on a
202 week-long time scale. In active oogenesis, oogenesis arrest occurred within hours, and macro-
203 condensates were not detected (Figure 3C), as expected given their week-long growth upon
204 quiescence. However, *spn-4* smFISH uncovered nanoclusters that cumulatively accumulated half
205 of the total repressed *spn-4* mRNAs, demonstrating that clustering has been previously largely
206 underestimated due to resolution limitations (Figures 3D, 3D', S4). Although coarsening kinetics
207 were limiting in active cells, nucleation was not, as small mRNA clusters were detected prior to
208 quiescence. Upon quiescence, mRNAs slowly redistributed to supersize outliers. In addition, the

209 slope of the power-law distribution distinguished nanoclusters from macro-condensates,
210 suggesting divergent mechanisms of assembly (Figure 3D, 3D'). Taken together, the ability to
211 segregate large mRNA fractions is not restricted to macro-condensates and includes nanoclusters
212 of active cells.

213
214 The cluster size distribution of *spn-4* mRNAs demonstrated the coexistence of four mRNA
215 populations: single molecules, nanoclusters, macro-condensates, and supersize outliers (Figures
216 3D, 3D', S4D). To test whether compositional differences could explain the size distribution
217 discontinuity between nanoclusters and macro-condensates we simultaneously labeled two
218 translationally repressed mRNAs, *spn-4* and *glp-1* (Figure 3E). Imaging uncovered that smaller
219 clusters were homotypic, they contained only *glp-1* or *spn-4* (Figure 3E-E'). Although homotypic
220 clusters of *spn-4*, *glp-1*, or another repressed mRNA, *mbk-2*, co-assembled into heterotypic
221 macro-condensates (Figure 3E-E', S5), their patterns were anticorrelated within macro-
222 condensates (Figures 3E''-E''', S5I-K), supporting demixing between mRNAs of different
223 identities. In addition to the RNA-protein interactions of single RNP assemblies, new interaction
224 scales emerged from RNP clustering: homotypic RNA-RNA interactions within nanoclusters, and
225 heterotypic interactions between nanoclusters that co-assembled but poorly mixed. Moreover,
226 while RNA-RNA demixing separated mRNA species (Figure 3F), protein-protein demixing defined
227 subcompartments on a larger scale within condensates (Figures 3G).^{10,31} We conclude the
228 multiscale multiphase organization of the transcriptome (Figure 3H).

229 230 **Buffering of repressed mRNA concentration variations by condensates promotes robust** 231 **cytosolic mRNA:protein stoichiometries**

232
233 The observation that condensates grow when repressed mRNAs accumulate upon quiescence
234 (Figure 3) suggested that condensates could provide a buffering mechanism that limits the
235 accumulation of repressed mRNAs in the cytosol. To test whether condensates could buffer
236 mRNAs:repressors stoichiometric variations, we analyzed concentration variations during
237 oogenesis for the repressed mRNA, *spn-4*, and translation repressor CAR-1. Imaging revealed
238 *spn-4* and CAR-1 cytosolic concentrations remained constant across oogenesis, while their
239 relative stoichiometries within condensates dramatically changed (Figures 4A, 4B, S6).
240 Additionally, despite a 4-fold variation between animals in total cellular *spn-4* copy numbers for
241 similarly staged diakinesis oocytes, cytosolic copy numbers remained almost identical (Figure
242 4C). Sequestration to condensates buffered the *spn-4* mRNA variations. To further test the impact
243 of this sequestration on cytosolic concentrations, we depleted the critical P-body component PUF-
244 5 that is essential for RNA condensation into P-bodies (Figures 4D, S6B).¹⁰ *puf-5(RNAi)* induced
245 dissolution led to a more than 8-fold increase in *spn-4* mRNA cytosolic concentrations, consistent
246 with our finding that 90% of *spn-4* mRNAs localized to condensates (Figures 4E, 4F, and Figures
247 S6C-S6D). In contrast, less than 25% of CAR-1 repressors segregated to condensates (Figures
248 4E, S6A-S6A''), and no significant increase upon dissolution was detected (Figures 4F, S6B,
249 S6B'). We conclude that condensates are more potent at sequestering repressed mRNAs than
250 repressors, and thus buffer repressed mRNAs without depleting the cytosol of repressors.

251
252 The buffering capacity of condensates may provide an adaptation mechanism to quiescence
253 during which repressed mRNAs accumulate and repressors may become limiting. Moreover,

254 condensation associated repression systems may become essential upon quiescence. To test
255 this, we first confirmed that condensation associated repression systems are not limiting in active
256 oogenesis, where PUF-5 represses redundantly with PUF-6/7.^{6,7} As previously reported, PUF-5
257 depletion had a very limited impact in active oogenesis: mRNA repression was maintained (Figure
258 4G), and oocytes remained fit enough to produce viable embryos (Figure 4H). However, depletion
259 in quiescent oocytes not only induced a dissolution of mRNAs (Figure 4D, F),¹⁰ but also a dramatic
260 increase in translation for some mRNAs (Figure 4G), and a drop in fitness of quiescent oocytes,
261 as they no longer produced viable embryos (Figure 4H). Of note, mRNAs were differentially
262 sensitive to the disruption of the condensation-repression system that was limiting upon
263 quiescence: *fog-1* translation dramatically increased whereas *spn-4* did not (Figure 4G).
264 Altogether, we provide evidence that a condensation associated repression system is not only
265 critical for buffering the accumulation of repressed mRNAs and maintaining robust translation
266 control as repressors become limiting, but is also essential for oocytes to adapt to repressed
267 mRNA accumulations upon quiescence.

268

269 **Repressed mRNAs saturate autonomously but partition cooperatively**

270

271 Many mRNAs co-exist within oocyte condensates (Figure 1E), raising the question of whether
272 each mRNA species' condensation is individually regulated. In a concentration-dependent phase
273 transition, biomolecules remain soluble at low concentrations, but condense past saturation
274 (Figure 5A).¹¹ In a simple homotypic phase transition model, as molecules in excess of the
275 saturation concentration self-assemble into condensates, the dispersed phase concentration
276 plateaus, providing a buffering mechanism (Figure 5B).²⁴ In a heterotypic model, when molecules
277 co-assemble with binding partners the saturation concentration may become partner-dependent,
278 and consequently can hinder buffering (Figure 5B).²⁵ Hence, each repressed mRNA species
279 could either autonomously self-assemble according to its own concentration, or any repressed
280 mRNA could cooperatively co-assemble as a function of total repressed mRNA concentrations,
281 independent of sequence identity. To distinguish between these two models, we first tested
282 mRNA clustering response to variations in *spn-4* mRNA concentrations. We analyzed arrested
283 oocytes with various levels of *spn-4* depending on *spn-4* (RNAi) efficiency, and computed *spn-4*
284 clustering by smFISH as a function of *spn-4* total cellular concentration (Figure 5C). Strikingly,
285 *spn-4* could accumulate as a soluble, single transcript in the dispersed phase until it plateaued at
286 a saturation concentration and nanoclusters appeared as a threshold effect (Figures 5C, 5D). The
287 dose-response fit a homotypic rather than a heterotypic phase separation model (Figures 5D and
288 5B comparison). Furthermore, macro-condensate imaging showed *spn-4* preferentially clustered
289 with itself (Figures 3E-3F, S5). These two results confirmed that *spn-4* mRNA saturates
290 independently of other mRNAs in a model where homotypic interactions dominate over
291 heterotypic.

292

293 While scaffolding components are essential for condensate assemblies, clients are recruited to
294 pre-existing condensates⁵¹. At very low *spn-4* concentrations, *spn-4* homotypic nanoclusters
295 dissolved, but heterotypic macro-condensates did not (Figure 5C, 5D). We also observed a
296 biphasic partition coefficient of *spn-4* across concentrations confirming the existence of two
297 regimes: (1) recruitment to pre-existing macro-condensates through heterotypic interactions as a
298 client at lower concentrations, and (2) self-assembly into nanoclusters at higher concentrations

299 (Figure 5E). At low concentrations *spn-4* partitioned strongly to condensates (>100-fold), but
300 partitioning quickly dropped as *spn-4* concentrations increased, suggesting that the recruitment
301 as a client relied on specific but limited binding sites that became quickly occupied. Most strikingly,
302 towards higher concentrations, self-assemblies provided a *spn-4* self-buffering mechanism:
303 above saturation concentration repressed *spn-4* mRNAs in excess self-segregated instead of
304 flooding the cytosol where *spn-4* concentrations remained clamped at a fixed concentration.

305
306 The coexistence of diverse homotypic mRNA clusters within macro-condensates (Figure 3E-F,
307 Figure S5I-K) suggested that this self-buffering mechanism is not restricted to *spn-4*, but instead
308 extends transcriptome-wide. We analyzed the distributions of mRNA condensation as a function
309 of mRNA concentrations using FAPSeq data (Figure 5F). Two distinct classes of distribution
310 profiles of mRNA condensation existed depending on mRNA concentrations. Above a
311 concentration threshold, the distributions of mRNAs shifted toward higher condensation, and the
312 dispersion of the distribution narrowed down (Figure 5F). This sharp shift that was reminiscent of
313 a thresholding effect where condensation occurs past a saturation concentration. Despite the
314 general trend of increased condensation above a copy number threshold, a number of poorly
315 expressed mRNAs were strongly enriched, likely through a client mechanism as described for
316 *spn-4*. Conversely some highly expressed mRNAs were poorly enriched in P-bodies, and may
317 represent a population of translated mRNAs that remain soluble at high concentrations. These
318 two independent approaches, (i) the single-molecule imaging of *spn-4* candidate mRNA, and (2)
319 the purification sequencing of condensed mRNAs, independently confirmed that mRNA clustering
320 into condensates buffered the accumulation of approximately half of oocyte mRNAs as their
321 expression exceeded a concentration threshold that was mRNA identity-dependent.

322

323 **Condensates buffer the tightest states of mRNA translation repression**

324

325 Because mRNA translation repression occurred in the absence of macro-condensates in active
326 oogenesis, while condensates buffered the accumulation of repressed mRNAs upon quiescence
327 (Figure 5), we wanted to functionally characterize and quantitatively model the relationship between
328 repression and condensation. Our results, thus far, suggest that ribosome-free repressed mRNAs
329 condense, while translated mRNAs remain soluble at high concentrations (Figure 1B", 2A-C, 5F).
330 In a simple linear model, as the translation output doubles, the number of ribosome-bound
331 mRNAs, and thus soluble mRNAs, also doubles (Figure 6A). In a second model, translation output
332 depends on ribosome density. Only when translation output tends toward zero, does the
333 probability to be ribosome free (condensed) exponentially increases (Figure 6B). To distinguish
334 between these two models, we focused on *spn-4* translation activation during oocyte maturation.
335 Translation rates were estimated from recording changes in SPN-4:GFP levels across oogenesis
336 time-points (Figures 6C-6D'), while mRNA clustering was quantified from *spn-4* smFISH imaging
337 (Figures 6E-6F"). In both active and quiescent oogenesis, SPN-4:GFP protein only accumulated
338 during late oogenesis (Figures 6C-6D'), recapitulating the pattern of *spn-4* translation activation.⁵⁰
339 During the slow translation activation of quiescent oogenesis, macro-condensates dissolved into
340 smaller clusters (Figures 6E-6E"), and upon the robust translation activation of active oogenesis,
341 nanoclusters dissolved into single molecules (Figures 6F-6F").

342

343 Contrary to repressed mRNAs, translated mRNAs remained soluble at very high concentrations
344 (Figures 6F"). Although mRNA clusters dissolved upon translation activation, there was no linear
345 relationship between the fraction of soluble molecules and the translation output, supporting the
346 non-linear model (Figure 6G). Only tightly repressed mRNAs were clustered. As translation
347 reached less than 5% of the maximum efficiency, cluster sizes had already dropped by four orders
348 of magnitude (Figures 6C', 6E'). At 15% of maximum translation efficiency, more than 50% of
349 *spn-4* were solubilized (Figure 6G). Conversely, towards high translation output, a less than 2-
350 fold increase in the number of soluble molecules was enough to provide a 10-fold increase in
351 translation (Figure 6G). Translation output was dependent on translation efficiency per soluble
352 mRNA rather than the number of soluble mRNAs. While the soluble mRNA fraction was
353 translationally regulated across wide ranges of efficiency, there was an exponential relationship
354 between mRNA condensation and the degree of repression (Figure 6G-H). Even subtle increases
355 in translation frequency were associated with massive condensate dissolutions (Figure 6G-H), in
356 accordance with the days-long repression requirement for condensate growth. The kinetics of
357 mRNA translation dominated over the kinetics of condensation. We conclude that translation
358 control mainly occurs in the dispersed phase of the cytoplasm where the translation efficiency of
359 single transcripts is adapted to cellular activity. Condensates buffer the exponential accumulation
360 of repressed mRNAs as translation drops, and conversely quickly dissolve upon translation
361 activation.

362
363 The ability of repressed mRNAs to condense autonomously according to their identity through
364 self-assembly into homotypic clusters indicated that mRNAs should similarly dissolve
365 independent of each other according to their respective translation activation profile. To test this,
366 we compared *spn-4* mRNA that is activated upon oocyte maturation to *glp-1*, an mRNA that
367 remains translationally repressed until later embryogenesis (Figure 6I). While, *spn-4* was released
368 from macro-condensates upon translation activation, repressed *glp-1* remained sequestered
369 (Figures 6J-6K). We conclude that multiphase condensates are decentralized systems where
370 distinct mRNA identities coexist within the same macro-condensate while being regulated
371 autonomously.

372 373 **DISCUSSION**

374
375 In the context of gene expression control, whether condensates reduce protein expression noise,
376 or provide a concentration-buffering mechanism has been debated.^{24,25} By focusing on mRNA
377 dynamics rather than those of proteins in our dissection of multi-scale multiphase assemblies, we
378 uncovered how condensates selectively control transcriptome stoichiometries (Figure 7A).
379 mRNAs first assemble with soluble regulators into single mRNPs whose translation is regulated
380 in the dispersed phase. As translation drops, tightly repressed mRNPs do not exceed a fixed
381 saturation concentration, without self-assembling in a self-buffering mechanism that selectively
382 depends on mRNA identity. Diverse nanoclusters further coalesce but poorly mix within macro-
383 condensate subcompartments, that are themselves defined through protein-protein phase
384 separations.^{10,31} Lowly abundant mRNAs below saturation are alternatively recruited as clients to
385 preexisting macro-condensates, diversifying partition mechanisms. Thousands of mRNAs can
386 therefore co-exist within condensates and their concentrations can be independently coordinated
387 to cellular activity (Figure 7A). Their autonomous self-assembly prevents one accumulating

388 repressed mRNA from disrupting others. The better partitioning of mRNAs as compared to
389 regulatory proteins further ensures that mRNA sequestration does not deplete the cytosol of
390 regulators. mRNAs cooperatively share limiting regulators, preventing the disruption of
391 RNA:protein stoichiometry in the dispersed phase. Altogether, condensates allow for a selective
392 and robust buffering of biomolecule stoichiometry variations.

393 Beyond their ability to protect cells from stoichiometry variations, the unique properties of oocyte
394 P-bodies define their reservoir functionality for the long-term and large-scale storage of maternal
395 mRNA information. We have previously shown that the semi-liquid properties of the viscoelastic
396 P-body droplets in arrested oocytes, mechanically “freeze” repressed RNPs that must be
397 preserved for long-term storage until oogenesis resume.¹⁰ Such “solidification” of maternal stores
398 is conserved through evolution.^{40,41} Here, we demonstrate that with a median storage capacity of
399 65% of mRNA copies, oocyte P-bodies provide a transcriptome-wide reservoir for mRNAs
400 controlling oogenesis and early embryogenesis during which transcription is silenced. Disrupting
401 these reservoirs ectopically “floods” the oocytes with mRNA molecules whose cytosolic
402 concentrations can increase up to 9 times. Combined with the solidification of RNPs,^{10,40,41}
403 coarsening into super-size droplets limits the surface/volume ratio, further reducing interface
404 exchange to restrain the diffusion of the repressed mRNA information.

405
406 The ability of condensates to concentrate large cytosolic RNA fractions in small volumes suggests
407 that compaction improves storage capacities. Weighting enrichment by mRNA abundance, we
408 estimate that 50 ± 10 % of the ≈ 18 million mRNAs condense in 5% of the oocyte volume (Figures
409 S7A, B). This concentrates mRNAs to 10^7 nucleotides per μm^3 , which is in the same range as the
410 textbook example of nuclear dsDNA compaction (Figure 7B, Figures S7C, D). Multiplying the
411 volume of a single compacted repressed mRNA⁵² by our computed number of P-body mRNAs
412 gives a cumulative volume slightly larger than P-bodies (Figures 7C, Figure S7D, E), suggesting
413 that P-body mRNAs reach the overlap concentration where mRNAs become entangled by
414 occupying overlapping space. Whether RNA entanglement provides a mechanical mechanism to
415 limit the diffusion of mRNAs remains to be tested. Regardless, segregation through phase
416 separations is a thermodynamically driven process and may have the advantage of being an
417 energy-free storage mechanism for the quiescent or stressed cells.

418
419 Condensates have no fixed internal stoichiometry. This ensures that their buffering functionality
420 is unaltered even when cellular activity variations are encountered. Although these long-term and
421 large-scale reservoirs contain mRNAs in their tightest state of repression, selective condensation
422 and dissolution quickly sense concentration variations to integrate cellular demands, providing a
423 store that can be quickly mobilized through sequential waves of translation activation and
424 dissolution. In some cell types condensates may not reach sufficient volume to serve as
425 reservoirs, in these instances nanoclusters may function as concentration sensors to ensure that
426 robustly measured saturation concentrations of repressed mRNAs remain available to respond to
427 translation demand. Furthermore, we have shown that even in the absence of macro-
428 condensates, the nanocluster pool can be larger than the soluble pool for a given mRNA species.
429 In addition to RNA nanoclusters,^{32–35} protein nanoclusters were also uncovered.^{53,54} This raises
430 the question of the unexplored material properties of this nanoscale of organization and opens
431 intriguing new avenues of investigation. Evolution must have shaped the phase diagram of each
432 mRNA to limit mRNA intermixing and selectively adapt supramolecular organizations to individual

433 molecular functions in a molecular grammar that remains to be deciphered. Beyond stoichiometric
434 control, spatial and biochemical clustering may functionalize every aspect of RNA life,^{55,56}
435 underscoring that there is still much to be discovered about the self-organizing principles of
436 transcriptome supra-molecular polymers.

437

438 **Limitations of the study**

439 The phase transition framework has proven fruitful to uncover key properties of the organization
440 of the transcriptome, however many predictions of the model have not yet been tested. Previously,
441 we characterized mRNP transitions between soluble, semi-liquid and solid states¹⁰, and the
442 condensation of mRNA regulons³. Here, we uncovered an mRNA self-buffering mechanism that
443 is dependent on mRNA concentration, repression, and identity. Further testing of the phase
444 transition model predictions should include comprehensive phase diagrams across transcript
445 diversity. Our results also predict that two types of interactions target mRNAs to condensates. We
446 distinguished mRNA recruitment as a client at low concentrations and as a scaffolding component
447 driving its self-assembly at higher concentrations. However, we did not dissect the molecular
448 grammar driving transcriptome multiphase transitions.

449

450 We concluded that condensates have buffering properties, however we only touched on the
451 protective role of condensate buffering. Condensate dissolution dramatically impacted
452 mRNA:mRNA and mRNA:protein stoichiometries, but what are the direct consequences on
453 biochemical interaction/reactions? Strategies to block buffering without disrupting condensate
454 assembly remain a serious technical challenge but could further discriminate functionalities.
455 Although condensation-repression systems promote fitness in quiescence, the limits up to which
456 condensates can buffer the accumulation of a single transcript without disrupting the others is
457 unanswered. This is critical to tackle pathological aggregations in degenerative diseases^{27,28}.

458

459 Providing an integrated view of mRNA clustering across spatiotemporal scales remains a key
460 challenge. Building on previous work,¹⁰ cluster size distribution across time-points distinguished
461 the rapid self-assembly of homotypic clusters from their slow co-assembly into heterotypic macro-
462 condensates. Although size, composition and kinetics of assembly discriminated these two
463 populations, the underlying interactions and material properties that distinguish them were not
464 addressed. To map cluster size distributions from single transcript to hundreds of thousands
465 mRNA super-assemblies, we relied on single-molecule fluorescence sensitivity without spatially
466 resolving individual mRNAs. In addition, to increase resolution condensate characterization would
467 further benefit from multiphase live-imaging across time scales, from the sub-second diffusion-
468 reaction, to the week-long coarsening. When computing the degree of P-body enrichment of
469 various RNAs we state that mRNAs were strongly enriched compared to ncRNAs, however our
470 RNA-Seq library preparation was size-biased so small RNAs could not be captured.

471

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473

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487

488 **AUTHOR CONTRIBUTIONS**

489

490 AHC, SE and AH conceived the study.

491 AHC collected and analyzed smFISH and fluorescent protein reporter data, developed smFISH
492 image quantification pipelines, nanoclusters fitting, RNA-Seq - smFISH regressions, *In silico*
493 simulations, and feeding RNAi experiments.

494 SE prepared animal extracts, performed FAPS purification, RNA extraction, cDNA libraries
495 libraries, and analyzed RNA-Seq data.

496 MK conducted RNAi microinjection experiments, smiFISH experiments and pre-analysis.

497 ZY analyzed RNA-Seq data under supervision of DA and SE.

498 FV generated *spn-4(RNAi)* feeding clone.

499 ML collected and analyzed production rates from SPN-4:GFP protein reporters, under supervision
500 of AHC.

501 AL, AB, AO and SR participated to the preparation of samples for the FAPS purifications.

502 AL and SR conducted the FAPS purifications.

503 AH collected and analyzed 3'UTR fusion reporters data and embryonic lethality.

504 AHC, SE, and AH made figures; AHC and AH wrote the original draft; DA, AHC, SE and AH
505 reviewed and edited the manuscript.

506

507 **COMPETING INTERESTS**

508

509 Authors declare that they have no competing interests.

510

511

512 **MAIN FIGURE TITLES AND LEGENDS**

513

514 **Figure 1. Quantitative modeling of the selective mRNA condensation within oocyte P-**
515 **bodies.**

516 (A) *C. elegans* oogenesis schematic. The PUF-5 translation repressor and CAR-1 cofactor
517 condense repressed mRNAs in P-bodies of quiescent oocytes.^{10,45}

518 (B-B'') Differential condensation of candidate mRNAs in GFP:CAR-1 P-bodies. (B) Single
519 molecule Fluorescence *in situ* hybridization (smFISH) confocal imaging from pink insets in (A).

520 (B') mRNA selective partitioning. The blue line is the theoretical relationship between the
521 condensed fraction (*CF*) and the partition coefficient (*PC*) (see Methods). (B'') Relationship

522 between mRNA abundance and condensed mRNA fraction. (B'') Relationship between
523 condensed mRNA fraction and the absolute number of condensed mRNAs. n, number of oocytes
524 analyzed for each transcript; errors bars, \pm 95% CI of the mean; r, Pearson correlation coefficient.
525 (C-F) Purification and sequencing of oocyte P-bodies. (C) Schematics of condensate
526 Fluorescence Activated Particle Sorting and RNA-Seq (FAPSeq). (D) FAPSeq separation of
527 condensates according to diffraction and fluorescence. (E) mRNA copy numbers within/outside
528 condensates from the smFISH calibrated FAPSeq (see Figure S2). (F) Distribution of mRNA
529 condensation across transcripts. Median, 65%, range, 10-90%, Q, quartile.

530

531 **Figure 2. Translationally repressed mRNAs condense into oocyte P-bodies.**

532 (A-D) Differential enrichment of RNA classes within P-bodies. RNA-Seq of purified oocyte P-
533 bodies and dissected oocytes were compared. (A) P-body depletion of translation machinery
534 RNAs (rRNA, tRNA), and snRNAs and ncRNAs, as compared to mRNAs. (B) P-body depletion
535 of small and large ribosomal subunit rRNAs. Mean values \pm 95% CI (C) P-body enrichment of
536 mRNAs encoding proteins that are not expressed or poorly expressed. mRNAs were binned
537 according to the abundance of their protein product. (D) P-body enrichment of PUF-5 and OMA-
538 1/LIN-41 translation repressor mRNA targets as compared to targets of repressors that work
539 earlier or later in development. Schematics of repressor expression patterns at the top. n, number
540 of transcripts. (E) P-body depletion of mRNAs encoding housekeeping, structural and metabolic
541 functions, and enrichment of mRNAs encoding regulatory and developmental functions, GO
542 analysis. n, number of transcripts in each category.

543

544 **Figure 3. Homotypic mRNA nanoclusters co-assemble into multiphase multiscale** 545 **condensates.**

546 (A) Condensate coarsening schematic. (B) Spatiotemporal organization of oogenesis. Imaging
547 insets in pink.

548 (C-C') Confocal imaging of P-body coarsening across oogenesis. (C) Comparison of GFP:CAR-
549 1 P-body coarsening in active versus quiescent oogenesis. Estimated time before ovulation in
550 brackets. (C') Size distribution and cumulative volume of P-bodies across quiescent oogenesis
551 time points. n, number of animals.

552 (D-D') mRNA cluster size distribution in staged diakinesis oocytes. (D) smFISH confocal images
553 of single mRNAs (purple arrow), nanoclusters (orange arrow), and macro-condensates (blue
554 arrow). (D') Power law distribution of mRNA clusters and supersize outliers. n, number of animals.
555 Mean values \pm 95% CI.

556 (E-E'') Macro-condensate super-assemblies of homotypic nanoclusters. (E) Simultaneous
557 labeling of *spn-4* and *glp-1* mRNAs in oocytes revealed single molecules (light green and red
558 arrows), homotypic nanoclusters (either *spn-4* or *glp-1* only, green or red arrows), and heterotypic
559 macro-condensates. (E') *glp-1:spn-4* fluorescent intensity ratios within clusters as a function of
560 cluster sizes. Note the discontinuity between the smaller clusters that are homotypic, while macro-
561 condensates are heterotypic. (E'') Example of anticorrelation between *spn-4* and *glp-1* mRNA
562 labeling across a macro-condensate confocal section (dotted line in (E)). FU, fluorescence units.
563 (E''') Pearson correlation coefficients computed as in (E'') for the colocalization of 2 mRNAs
564 across macro-condensate confocal sections (see also Figure S5J). Two-color labeling (Cy3 and
565 Cy5) of the same mRNA provided a positive colocalization control. n, number of sections analyzed
566 (number of animals). Mean values \pm 95% CI.

567 (F-H) Multiscale and multiphase organization of condensates. (F) Confocal imaging of RNA-RNA
568 demixing within condensates. (G) Confocal imaging of protein-protein demixing. (H) Super-
569 assembly schematic.

570

571 **Figure 4. Condensation controls cytosolic mRNA:repressor stoichiometries.**

572 (A) Confocal images of stoichiometric changes between the *spn-4* repressed mRNA and CAR-1
573 repression cofactor across oogenesis. (B) Condensate buffering of *spn-4*:CAR-1 stoichiometry.
574 Note disperse phase constant concentrations. n, number of animals (C) Robustness and noise of
575 *spn-4* mRNA copy number in the dispersed and condensed phases, respectively. Staged
576 diakinesis oocytes of WT animals were compared. n, number of oocytes (D) Confocal images of
577 *spn-4* and *glp-1* mRNAs induced dissolution (*puf-5(RNAi)*) in diakinesis oocytes. (E) Differential
578 condensation of repressors (CAR-1) and repressed mRNAs (*spn-4*, *glp-1*) in diakinesis oocytes.
579 n, number of oocytes. (F) Disruption of mRNA:repressor stoichiometry upon condensate
580 dissolution (*puf-5(RNAi)*). n, number of oocytes. (G) Disruption of translation repression upon
581 PUF-5 depletion is quiescence dependent. GFP translation reporters were under the control of
582 *spn-4* and *fog-1* 3'UTR sequences. n, number of animals. (H) Embryonic lethality upon PUF-5
583 depletion is quiescence dependent. n, number of experiments.

584

585 **Figure 5. Concentration-dependent mRNA clustering buffers repressed mRNA**
586 **accumulations.**

587 (A-B) Models of concentration-dependent phase separations. (A) Biomolecules remain soluble at
588 low concentrations but condense past a saturation concentration. (B) Self-assembly through
589 homotypic interactions leads to fixed saturation concentrations, while co-assembly through
590 heterotypic interactions usually results in non-fixed saturation concentrations.

591 (C-E) *spn-4* mRNA clustering response to *spn4* concentration variations within quiescent oocytes.

592 (C) RNAi manipulated *spn-4* concentrations and smFISH single mRNA quantification (light blue
593 arrow), nanoclusters (dark blue arrow), and GFP:CAR-1 labeled macro-condensates (green
594 arrow). (D) Plateauing of single soluble *spn-4* mRNAs at saturation concentration in the dispersed
595 phase induces *spn-4* nanoclustering. Macro-condensate copy numbers scaled with total
596 abundance. (E) Bimodal response of *spn-4* partition coefficient to total concentration variations.
597 n, number of oocytes.

598 (F) Transcriptome-wide distribution of mRNA enrichment in oocyte P-bodies depending on
599 transcript copy numbers. Enrichment in P-bodies was computed by comparing the P-body and
600 whole oocyte transcriptomes (P-body/whole oocyte $\log_2 FC$), see Figure 1, Figures S2 and S3,
601 Data S1-3). mRNAs were binned according to their total cellular mRNA copy numbers.

602

603 **Figure 6. Low translation dissolves mRNAs whose clustering is exponential to the degree**
604 **of repression.**

605 (A-B) Models of mRNA translation and condensation control. The translation output depends on
606 either the number of translated mRNAs (A) and/or the translation efficiency per mRNA (B).
607 Ribosome free mRNAs (non-translated) condense.

608 (C-H) *spn-4* mRNA dissolution upon translation activation. (C-D) Confocal imaging of SPN-4:GFP
609 protein accumulation across active and quiescent oogenesis (estimated time before ovulation in
610 brackets). (C'-D') SPN-4 accumulation rates computed from SPN-4:GFP intensity variations
611 across oogenesis. (E-F) smFISH confocal imaging of *spn-4* mRNA cluster dissolution. (E'-F') *spn-*

612 4 mRNA cluster size distribution. (E'-F') Cellular concentration of soluble and condensed *spn-4*
613 mRNAs (G-H) *spn-4* clustering is exponential to the degree of translation repression. The fraction
614 of clustered mRNA (G), and the average cluster size distributions (H) are plotted as function of
615 the protein production rate. n , number of oocytes. Mean values \pm 95% CI.
616 (I-J) Sequential dissolutions of mRNA condensed phases across oogenesis. (I) Schematics of
617 SPN-4 and GLP-1 translation temporal waves. (J) smFISH imaging of *spn-4* and *glp-1* mRNAs.
618 (K) Quantification of cluster dissolution.

619
620 **Figure 7. Oocyte P-body multiscale multiphase super-assemblies.** (A) Schematic model of
621 the multiscale multiphase control of mRNA stoichiometry depending on mRNA translational
622 activity and sequence identity. See discussion for details. (B) Compaction of P-body mRNAs and
623 nuclear dsDNA. mRNA compaction was computed for diakinesis P-bodies. DNA compaction was
624 computed for the nuclei of germ stem cells, that were chosen because of their small nuclear
625 volume (C) Model of mRNA compaction within P-bodies. Gyration radius (R_g) of compacted
626 repressed mRNAs estimated elsewhere.⁵² Experimental measures suggest mRNA concentration
627 within P-bodies (c) are near overlap concentration (c^*), suggesting mRNA entanglement. V_p ,
628 volume predicted from multiplying the number of mRNAs within P-bodies by the predicted volume
629 of single repressed mRNA. V_c , cumulative volume of P-bodies experimentally measured.

630

631 SUPPLEMENTAL MAIN FIGURE TITLES AND LEGENDS

632

633 **Figure S1. Single molecule fluorescence in situ hybridization (smFISH) quantifications of**
634 **mRNA clustering within staged oocytes, related to Figures 1, 3, 4, 5, and 6 (see methods**
635 **for detailed descriptions and associated quantification controls).**

636 (A) Confocal imaging section of mRNA condensation in arrested oocytes. The translation
637 repression cofactor GFP:CAR-1 labelled P-bodies, and colocalizing *spn-4* mRNA clusters were
638 detected by smFISH.

639 (B-C) Oocyte segmentation. (B) Lower and upper oocyte limits were identified along the z-stack
640 acquisition using the derivative maxima and minima of the 90th percentile fluorescence intensity.
641 (C) Oocytes were manually segmented along the x-y axis from the maximum intensity projection
642 images. Oocytes were manually staged according to their position along the gonad proximo-distal
643 axis. Oocytes in position -1 are the most differentiated.

644 (D-G) Macro-condensate segmentation using a fluorescence intensity thresholding method. (D)
645 Fluorescent intensity normalization. (E) Background fluorescence removal. (F) Fluorescence
646 intensity thresholding and macro-condensate segmentation and dilatation. (G) Labelling and
647 masking of mRNA macro-condensates.

648 (H) Detection of sub-diffractive mRNA foci using FISH-quant⁵⁷ after masking macro-condensates
649 that were detected in (D-G). White line delineates the masked macro-condensates, white squares
650 mark the detected positions of sub-diffractive mRNA foci (local maxima or brightness pixel within
651 foci). The Image is a maximum projection of 2 z-planes, hence some detected positions are not
652 shown.

653 (I-O) Quality controls and filters for optimal detection of mRNA foci as adapted from⁵⁷. (I)
654 Calibration of the over-detection and under-detection intensity thresholds. The number of
655 detected cytosolic mRNA foci were plotted as a function of the fluorescence intensity detection
656 threshold in a smFISH image of staged oocytes. The curve can be decomposed in 3 segments.

657 In the first segment (red inset 1), the number of detected foci sharply decreases as the intensity
658 detection threshold increases. In this segment, foci are over detected. In the second segment (red
659 insets 2 and 3), the number of detected foci plateaus. This segment provides an objective criterion
660 to define the optimal detection window: changing the intensity detection threshold in that window
661 has no impact on the number of detected foci. The last segment (red inset 4), where the number
662 of detected foci decreases with the intensity threshold, corresponds to the under-detection
663 window. **(J-L)** Examples of different intensity detection thresholds resulting in over-detection **(J)**,
664 optimal detection **(K)**, and under-detection **(L)**. Green arrows indicate instances of over-detection
665 while blue arrows point to examples of low quality spots. **(M)** Quality control filter based on point
666 spread function (PSF) standard deviations⁵⁷. **(N)** Additional filters for mRNA foci detection.
667 Detected spots are fitted to a theoretical PSF. Sigmas, amplitude, and a minimal distance allowed
668 between fitted spots filter out foci that do not fit a sub-diffractive PSF, detections in the
669 background, and double detections, respectively⁵⁷. **(O)** Quantifications of the number of fitted
670 cytosolic mRNA foci in a representative oocyte: detected (upper), after quality control (middle),
671 and after additional filters (lower).
672 **(P-S)** Quantifications of *spn-4* mRNA copy numbers (i) as single molecule transcripts in the
673 dispersed phase, (ii) in sub-diffractive nanocluster foci, and (iii) in macro-condensates. **(P)**
674 Calibration of single mRNA fluorescence intensities. Top panel, confocal images of *spn-4* mRNAs
675 within oocytes. To ensure that mRNAs exist in a single molecule form, *spn-4* mRNA
676 concentrations were diluted in oocytes by increasing concentrations of *RNAi*. Bottom panel,
677 fluorescence intensity distribution of *spn-4* mRNA foci. A right-skewed Gaussian in purple was fit
678 to the experimental fluorescence intensity distribution in grey. The relative fluorescence intensities
679 were calibrated so that the maximum of the distribution corresponded to the normalized
680 fluorescence of 1X in the diluted conditions, which corresponds to the fluorescence of a single
681 mRNA molecule. Note that upon dilution almost no foci were detected with a fluorescence
682 intensity equal or superior to 2X. Such a narrow skewed Gaussian distribution with a fluorescence
683 intensity distributed between 0 and 2 X, and centered around 1X, demonstrated our ability to
684 detect mRNAs with single molecule sensitivity. Inset, confocal image of an average single mRNA
685 imaged at high dilution. **(Q)** Control confirming that the fluorescence intensity of single mRNA
686 molecules does not change within versus outside P-body condensates. Following *spn-4(RNAi)* to
687 dilute *spn-4* mRNAs and ensure single molecule distributions **(P)**, fluorescence intensity
688 distributions were computed either for *spn-4* single molecule foci within CAR-1 condensates
689 (condensed phase), or for *spn-4* single molecule foci within the cytosol (dispersed phase). Of
690 note, other controls confirming that the fluorescence intensity of mRNA molecules does not
691 change between the soluble and clustered states included the physiological or artificial dissolution
692 of macro-condensates and nanoclusters, where the total quantified number of mRNA copies
693 remained unchanged independent of mRNA clustering status (**Figures 6F"**, **6E'-E"**, **Figure S6D**).
694 See also methods **(R)** Quantification of *spn-4* mRNA copy numbers within the macro-condensates
695 segmented in **(F)**. The average fluorescent intensity of a single transcript computed in **(I)** was
696 either integrated or cumulated up to reach the fluorescence intensity of the macro-condensates.
697 Both methods gave similar results (see **Figure S2O**). **(S)** Quantifications of *spn-4* mRNA copy
698 numbers in the dispersed phase (single) and in sub-diffractive nanocluster foci. The fluorescence
699 intensity distribution of sub-diffractive mRNA foci in a staged diakinesis oocytes is shown in grey,
700 a right-skewed Gaussian model fit is shown in blue. The distribution was further decomposed in

701 a single molecule fit in purple as defined in (I) and a nanocluster fit in orange, so as to compute
702 the number of single mRNAs and the number of mRNA in nanoclusters, respectively.

703

704 **Figure S2. Scaling the transcriptome-wide FAPS purification and RNA-sequencing method**
705 **of condensates with single molecule imaging approaches, related to Figure 1.**

706 **(A-C)** Quantifications, in staged diakinesis arrested oocytes, of the differential condensation of
707 candidate mRNAs in GFP:CAR-1 P-bodies from single molecule Fluorescence *in situ*
708 hybridization (smFISH) confocal imaging (See Fig. S1 and methods for imaging pipeline). **(A)**
709 mRNA selective partition. **(B)** Condensed fractions. **(C)** Condensed copy numbers. *n*, number of
710 quantified oocytes. errors bars, \pm 95% CI of the mean.

711 **(D)** Computed subcellular compartment volumes in staged diakinesis arrested oocytes (see
712 methods). *n*, number of quantified oocytes. errors bars, \pm 95% CI of the mean.

713 **(E)** Relationship between mRNA partition coefficients and mRNA condensed fractions in staged
714 diakinesis arrested oocytes. The blue line is the theoretical relationship between the condensed
715 fraction (*CF*) and the partition coefficient (*PC*): $PC = CF / (1 - CF) * V_d / V_c$ where V_d is the volume
716 of the dilute fraction and V_c is the volume of the condensed fraction as computed in (D). *r*, Pearson
717 correlation coefficient.

718 **(F-I)** Transcriptomics analysis of the FAPS purified diakinesis oocytes P-bodies. **(F)** The principal
719 component analysis (PCA) separates the transcriptomes of FAPS purified diakinesis oocyte P-
720 bodies, from the pre-sorted fractions (whole animal extracts) and the dissected diakinesis
721 oocytes. *n*=3 independent biological replicates **(G)** Differential enrichment of RNAs in the FAPS
722 purified oocyte P-bodies as compared to the pre-sorted whole animal extracts. The dot plot
723 represents all RNAs from the RNA-Seq analysis. Note that somatic enriched transcripts labeled
724 in pink are strongly depleted from oocyte P-bodies, validating the purification quality. **(H)** The
725 distribution of RNA enrichment in oocyte P-bodies was computed from (G). **(I)** Differential
726 enrichment of RNAs in the FAPS purified diakinesis oocyte P-bodies as compared to the whole
727 diakinesis oocyte transcriptome. RNA relative expression levels in oocytes were computed in
728 FPKM.

729 **(J-L)** Correlations between the imaging (smFISH) and FAPS purification-RNA-sequencing
730 (FAPSeq) methods for the quantification of mRNA subcellular localizations. **(J)** Correlation for the
731 mRNA enrichment in P-bodies. **(K)** Correlation for the mRNA abundance in oocyte P-bodies. **(L)**
732 Correlation for the mRNA abundance in oocytes. *n*, number of animals analyzed per mRNA for
733 the smFISH. *n*, number of independent biological replicates for the FAPSeq. errors bars, \pm 95%
734 CI of the mean. r^2 , R-squared of linear regression model (blue line). Shaded areas, 95% CI of
735 linear regressions.

736 **(M-P)** Distributions of mRNA enrichment in P-bodies. **(M)** mRNA enrichment was computed by
737 comparing the oocyte P-body transcriptome to the whole oocyte transcriptome. To avoid bias
738 from the technical dispersion because of low sequencing read counts for poor abundance
739 transcripts, only transcripts in the top 50% of expression levels were plotted **(N)** Comparison of
740 the cumulative distribution of mRNA relative enrichment in P-bodies depending on mRNA
741 expression levels in oocytes. mRNAs were binned according to their expression levels in oocytes
742 (FPKM), demonstrating that mRNA enrichment in P-bodies increases with mRNA abundance.
743 The large dispersion of the lowest mRNA abundance bins is technical, and reflects dispersion
744 noise due to the low sequencing read counts. **(O)** smFISH calibrated distribution of mRNA
745 enrichment in P-bodies. mRNA enrichment was computed using the [S2J](#) linear regression.

746 smFISH integrative and cumulative methods to compute mRNA condensation gave similar
747 results. (P) Distribution of the percentage of condensed mRNAs as computed from the integrated
748 method in (O), including low abundance transcripts.

749

750 **Figure S3. Features of condensed mRNAs, related to Figure 2.**

751 (A) mRNA length comparisons between P-bodies and P-granules. (B) Absence of nucleotide
752 compositional bias for P-body mRNAs. mRNAs were split in 5 bins according to their enrichment
753 in P-bodies, and the % of A, U, C, G nucleotides was computed for 5'UTRs, CDS, and 3'UTRs.

754

755 **Figure S4. *In silico* simulations and corrections of experimental measures for the
756 quantification of *spn-4* mRNA clustering, related to Figures 1, 3, 4, 5, and 6.**

757 (A) Synthetic images of single mRNA foci randomly placed at increasing concentrations within
758 the oocyte volume. Lower panels are one-plane magnifications of the inset. Note that because of
759 the lack of imaging resolution to separate the mRNAs that are randomly placed at high density,
760 some mRNAs artificially appear to cluster in brighter foci towards higher concentrations despite
761 the absence of clustering within the simulation input parameters, demonstrating that mRNA
762 clusters can be overestimated, as further quantified in (B). (B) Comparison of *spn-4* mRNA
763 distribution across cluster sizes in simulated images (*in silico*) versus experimental results.
764 Experimental distributions of cluster sizes were computed at low (*spn-4(RNAi)*) and high (WT
765 animals) mRNA concentrations, either in active oocytes or arrested diakinesis oocytes that were
766 staged in position -4 along the gonad proximo-distal axis. *In silico* distributions as simulated in (A)
767 were computed for concentrations matching the experimental measures. See Figure S1 for the
768 quantification pipeline of smFISH experimental and simulated images, and Figure 5C for the
769 experimental imaging of *spn-4* in RNAi conditions. Note that in the simulation, the nanoclusters
770 detected at high concentrations were artefacts because single mRNAs were not well separated
771 because of imaging resolution limits as shown in (A). n, number of animals analyzed for
772 quantifications. n, number of replicates for the simulation. (C) Comparison of *spn-4* mRNA cluster
773 size distributions at various mRNA concentrations between simulated images (left panel) and
774 experimental results (right panel). *spn-4* mRNA concentrations were experimentally varied using
775 an RNAi approach, for imaging see Figure 5C. Note the concentration threshold effect on mRNA
776 clustering in the experimental data that is not detected in the simulation. (D) Experimental
777 quantifications of *spn-4* mRNA distribution across cluster sizes with a correction from the *in silico*
778 simulation. Corrections subtracted the artificial clusters that were detected in the simulation at
779 similar concentrations, and that were instead accounted as single soluble transcripts. n, number
780 of animals. Mean values \pm 95% CI. (E) smFISH quantifications of single dispersed mRNAs and
781 mRNA nanoclusters within simulated images versus experimental images. (F) Corrected
782 experimental quantifications of single dispersed mRNAs and mRNA nanoclusters. Corrections
783 subtracted the artificial clusters detected in the simulation at similar concentrations that were
784 instead accounted as single soluble transcripts.

785

786 **Figure S5. Controls for the smFISH imaging of mRNA demixing, related to Figure 3.**

787 (A-H) Bleed through controls for the two-color smFISH imaging of mRNAs. (A-C) Bleed through
788 controls for the demixing of *spn-4* and *glp-1* mRNAs within macro-condensates. (A) *spn-4* and
789 *glp-1* mRNAs were fluorescently labelled with Cy3 and Cy5 fluorophores. (B) Bleed through
790 control for Cy3 in the absence of Cy5 labeling. (C) Bleed through control for Cy5 in the absence

791 of Cy3 labeling. **(D-F)** Bleed through controls for *spn-4* and *glp-1* mRNAs in the cytosol. **(D)** *spn-*
792 *4* and *glp-1* mRNAs were fluorescently labelled with Cy3 and Cy5 fluorophores. **(E)** Bleed through
793 control for Cy3 in the absence of Cy5 labeling. **(F)** Bleed through control for Cy5 in the absence
794 of Cy3 labeling. Fluorescence intensity scales are presented above images. Laser intensities
795 were matched. **(G)** Quantifications of Cy3 bleed-through within the Cy5 collection channel. **(H)**
796 Quantifications of the Cy5 bleed-through within the Cy3 collection channel.
797 **(I-K)** smFISH imaging and controls for mRNA-mRNA demixing within quiescent diakinesis
798 oocytes. **(I)** smFISH images of mRNA-mRNA demixing within macro-condensates. *spn-4*, *glp-1*
799 and *mbk-2* mRNAs are shown. Right panels, fluorescent intensity distributions across xy axis as
800 shown as white dashed line on merged panels. **(J)** Controls for mRNA-mRNA demixing within
801 macro-condensates. Top panel imaging of *spn-4* and *glp-1* mRNA demixing. Middle and bottom
802 panels are positive controls for mRNA colocalization, the same mRNA, either *spn-4* or *glp-1*, was
803 labelled with two colors. **(K)** Quantification controls for the demixing between homotypic clusters.
804 n, number of analyzed clusters (number of animals). FU: fluorescence units.

805

806 **Figure S6. Condensation quantifications for the repression cofactor GFP:CAR-1, and the**
807 ***spn-4* and *glp-1* repressed mRNAs, related to Figures 4 and 5.**

808 **(A-B')** Quantifications of the translation repression cofactor GFP:CAR-1 condensation in arrested
809 diakinesis oocytes from confocal z-stack acquisitions. **(A)** Confocal images of GFP:CAR-1
810 depletion (*car-1(RNAi)*) in arrested diakinesis oocytes. **(A')** Control for the GFP:CAR-1
811 fluorescence specificity and quantitative sensitivity. The detected fluorescence in the dispersed
812 phase (outside of condensates) dropped to ≈ 0 upon CAR-1 depletion (*car-1(RNAi)*). **(A'')**
813 Quantifications of the GFP:CAR-1 cumulative fluorescence intensity in the dispersed versus
814 condensed phase. **(B)** Confocal images of GFP:CAR-1 condensates dissolution upon PUF-5
815 depletion (*puf-5(RNAi)*). **(B')** Control for the condensed versus soluble GFP:CAR-1
816 quantifications. The total GFP:CAR-1 measured concentration remained unchanged as
817 GFP:CAR-1 was released to the cytoplasm upon the *puf-5(RNAi)* induced dissolution of
818 condensates, confirming the absence of bias in the quantification of condensed versus soluble
819 molecules.

820 **(C-D)** Quantifications of the repressed mRNAs (*spn-4*, *glp-1*) condensation in oocytes from
821 confocal z-stack acquisitions of smFISH images (see [Figure S1](#) for quantification pipeline details).
822 **(C)** Confocal images of *spn-4* mRNA depletion (*spn-4(RNAi)*). Magnifications are in insets. **(C')**
823 Control for the *spn-4* mRNA detection fluorescence specificity and quantitative sensitivity. The
824 detected cytosolic *spn-4* mRNA concentrations dropped to ≈ 0 upon *spn-4* mRNA depletion (*spn-*
825 *4(RNAi)*). **(C'')** Quantifications of *spn-4* mRNA molecules, in mRNA copy number, within the
826 dispersed and condensed phases. **(D)** Image quantifications of *spn-4* and *glp-1* mRNAs induced
827 dissolution (*puf-5(RNAi)*) in arrested oocytes (see [Figure 4D](#) for corresponding smFISH confocal
828 images). Left panel, control for the condensed versus soluble mRNA quantifications. The total
829 cellular mRNA density remained unchanged upon the *puf-5(RNAi)* induced dissolution of mRNA
830 condensates, confirming the absence of bias in the quantification of condensed versus soluble
831 molecules. Right panel, quantifications of cytosolic mRNA concentration increase upon mRNA
832 release from condensate dissolution.

833

834 **Figure S7. Transcriptome-wide quantification of mRNA enrichment and compaction within**
835 **oocyte P-bodies, related to Figure 7.**

836 **(A-B)** mRNA condensation estimates from the smFISH calibrated FAPSeq (see [method details](#)).
837 **(A)** Condensation of half of mRNAs within 5% of the cellular volume. The number of condensed
838 mRNA (Data S3) was computed from the smFISH calibrated FAPSeq (see [method details](#)).
839 Subcellular volumes were computed in [S2D](#) **(B)** Local mRNA concentrations within and outside
840 condensates. **(C)** mRNA length distributions across transcripts that were used to compute mRNA
841 compactions in (C) and (D). **(D)** Schematic representation of the computing of mRNA compaction
842 within condensates. DNA compaction was computed for germ stem cell nuclei, which were
843 chosen because of their high compaction. RNA compaction was computed for a diakinesis oocyte
844 P-body **(E)** Estimation of the cumulative volume that would be occupied by repressed P-body
845 mRNAs, taking into account the gyration radius of repressed mRNAs as defined by⁵² and the
846 number of P-body mRNAs of the current study.

847 **STAR Methods**

848

849 **Robust sorting and buffering within condensates control transcriptome**
850 **stoichiometries**

851

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863

864 **Contents:**

865

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867 2. Experimental model and study participant details

868 3. Method details

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KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
<i>E. coli</i> OP50	Caenorhabditis Genetics Center	WormBase ID: WBStrain00041969; https://cgc.umn.edu/strain/OP50
<i>E. coli</i> HT115(DE3)	Caenorhabditis Genetics Center	WormBase ID: WBStrain00041080; https://cgc.umn.edu/strain/HT115(DE3)
Chemicals, peptides, and recombinant proteins		
RNaseOUT Recombinant Ribonuclease Inhibitor	Invitrogen (Thermo Fisher Scientific)	Cat#10777019
PBS, pH 7.4 (flow cytometry grade)	ThermoFisher Scientific	Cat#A1286301
Zirconium Oxide Beads 1.0 mm RNase Free	Next Advance	Cat#ZROB10-RNA
SUPERase In RNase Inhibitor (20U/uL)	Invitrogen (Thermo Fisher Scientific)	Cat#AM2696
HEPES	Sigma-Aldrich (Merck)	Cat#H3375; CAS: 7365-45-9
Triton X-100	Sigma-Aldrich (Merck)	Cat#X100; CAS: 9036-19-5
Poly-L-Lysine solution (0.1%)	Sigma-Aldrich (Merck)	Cat#P8920; CAS: 25988-63-0
Gelatine solution (2%)	Sigma-Aldrich (Merck)	Cat#G1393; CAS: 9000-70-8
Chromium (III) Potassium Sulfate	Merck	Cat#1.01036; CAS: 7788-99-0
Sodium phosphate dibasic dodecahydrate	Sigma-Aldrich (Merck)	Cat#71649; CAS: 10039-32-4
Sodium Phosphate, Monobasic	Merck	Cat#567545; CAS: 10049-21-5
Formaldehyde solution	Sigma-Aldrich (Merck)	Cat#F8775; CAS: 50-00-0
Qiagen Proteinase K (>600 mAU/ml)	Qiagen	Cat#19131; CAS: 39450-01-6
SSC buffer (20x)	Jena Bioscience	Cat#BU-118L
Formamide (≥99.5%)	Sigma-Aldrich (Merck)	Cat#F9037; CAS: 75-12-7
Stellaris RNA FISH Hybridization Buffer	LGC Biosearch Technologies	Cat#SMF-HB1-10
ProLong Glass Antifade Mountant	Invitrogen (Thermo Fisher Scientific)	Cat#P36980
Peptone	Thermo Fisher Scientific	Cat#211677
Yeast extract BioChemica	ITW Reagents	Cat#A1552
Sodium chloride	Sigma-Aldrich (Merck)	Cat#S7653; CAS: 7647-14-5
Agar, Bacteriological grade	Thermo Fisher Scientific	Cat#443572500; CAS: 9002-18-0
Magnesium sulfate	Sigma-Aldrich (Merck)	Cat#M7506; CAS: 7487-88-9

Calcium chloride hexahydrate	Sigma-Aldrich (Merck)	Cat#442909; CAS: 7774-34-7
Potassium phosphate monobasic	Sigma-Aldrich (Merck)	Cat#P979; CAS: 7778-77-0
IPTG	Sigma-Aldrich (Merck)	Cat#I6758; CAS: 367-93-1
Cholesterol	Sigma-Aldrich (Merck)	Cat#C8667; CAS: 57-88-5
Carbenicillin	Condalab	Cat#6803
Critical commercial assays		
miRNeasy FFPE kit	Qiagen	Cat#217504
Agilent RNA 6000 Pico Kit	Agilent	Cat#5067-1513
SMARTer Stranded Total RNA-Seq Kit v2 - Pico	Takara	Cat#634411
Agilent High Sensitivity DNA Chips	Agilent	Cat#5067-4626
Qubit RNA HS Assay Kit (Invitrogen)	Invitrogen (Thermo Fisher Scientific)	Cat#Q32852
Qubit dsDNA HS Assay Kit (Invitrogen)	Invitrogen (Thermo Fisher Scientific)	Cat#Q32851
Illumina TruSeq HT assay	Illumina	Cat#FC-121-2003
Deposited data		
RNA-Seq raw data	This paper	GEO: GSE213222
HTSeqCounts raw reads	This paper	GEO: GSE213222
RNA relative enrichment within oocyte P-bodies	This paper	Data S1
RNA-Seq abundances	This paper	Data S2; GEO: GSE213222
Estimated condensed and dissolved mRNA copy numbers in oocytes	This paper	Data S3
GO analysis of P-body enriched and depleted mRNAs	This paper	Data S4
smFISH raw microscopy data	This paper; Mendeley data	https://doi.org/10.17632/ys7pzk9g.1 ; https://doi.org/10.17632/n7sf6f4c22.1 ; https://doi.org/10.17632/qcbvzm5kzg.1
Fluorescent reporters raw microscopy data	This paper; Mendeley data	https://doi.org/10.17632/5jt3m3twsh.1
Protein expression of maternal mRNAs in oocytes	Merritt et al. ⁴⁶	Table S2
mRNA targets of FBF-1 and GLD-1	Hu et al. ⁵⁸	http://POSTAR.ncrn.alab.org
mRNA targets of OMA-1 and LIN-41	Tsukamoto et al. ⁵⁰	https://academic.oup.com/genetics/article/206/4/2007/6072647#supplementary-data
mRNA targets of PUF-5	Stumpf et al. ⁴⁹	https://majournal.cshlp.org/content/14/8/1550/suppl/DC1
Experimental models: Organisms/strains		
<i>C. elegans</i> : fog-2(q71) V	Caenorhabditis Genetics Center	WormBase ID: WBStrain00004538; https://cgc.umn.edu/strain/CB4108

<i>C. elegans</i> wild isolate	Caenorhabditis Genetics Center	WormBase ID: WBStrain00000001; https://cgc.umn.edu/strain/N2
<i>C. elegans</i> : $P_{pie-1}::gfp::car-1::UTR^{pie-1}$	Squirrell et al. ⁵⁹	N/A
<i>C. elegans</i> : $fog-2(q71)$; $P_{pie-1}::gfp::car-1::UTR^{pie-1}$	This paper	N/A
<i>C. elegans</i> (TE73): $fog-2(q71)$; $car-1(oc8[car-1:gfp:FLAG])$	gift of T. Evans	N/A
<i>C. elegans</i> (TE51): $P_{pie-1}::gfp::car-1::UTR^{pie-1}$; $P_{nmy-2}::pgl-1::mrfp$	Hubstenberger et al. ¹⁰	N/A
<i>C. elegans</i> (TE71): $fog-2(q71)$; $P_{pie-1}::gfp::car-1::UTR^{pie-1}$; $P_{nmy-2}::pgl-1::mrfp$	Hubstenberger et al. ¹⁰	N/A
<i>C. elegans</i> (DG4158): $spn-4(tn1699[spn-4::gfp::3xflag])$	Tsukamoto et al. ⁵⁰ ; Caenorhabditis Genetics Center	WormBase ID: WBStrain00005730; https://cgc.umn.edu/strain/DG4158
<i>C. elegans</i> : $fog-2(q71)$; $spn-4(tn1699[spn-4::gfp::3xflag])$	This paper	N/A
<i>C. elegans</i> (DG4215): $puf-5(tn1726[gfp::3xflag::puf-5])$	Tsukamoto et al. ⁵⁰ ; Caenorhabditis Genetics Center	WormBase ID: WBStrain00005742; https://cgc.umn.edu/strain/DG4215
<i>C. elegans</i> : $fog-2(q71)$; $puf-5(tn1726[gfp::3xflag::puf-5])$	This paper	N/A
<i>C. elegans</i> : $P_{pie-1}::GFP::H2B::3'UTR^{fog-1} + unc-119(+)$	Caenorhabditis Genetics Center	WormBase ID: WBStrain00022333; https://cgc.umn.edu/strain/JH2423
<i>C. elegans</i> : $P_{pie-1}::GFP::H2B::3'UTR^{spn-4} + unc-119(+)$	Caenorhabditis Genetics Center	WormBase ID: WBStrain00022302; https://cgc.umn.edu/strain/JH2311
<i>C. elegans</i> : $fog-2(q71)$; $P_{pie-1}::GFP::H2B::3'UTR^{fog-1} + unc-119(+)$	This paper	N/A
<i>C. elegans</i> : $fog-2(q71)$; $P_{pie-1}::GFP::H2B::3'UTR^{spn-4} + unc-119(+)$	This paper	N/A
Oligonucleotides		
<i>spn-4</i> smiFISH probes	This paper (synthesized by IDT)	Table S3
<i>glp-1</i> smiFISH probes	This paper (synthesized by IDT)	Table S4
<i>puf-5</i> smiFISH probes	This paper (synthesized by IDT)	Table S5
<i>tbb-2</i> smiFISH probes	This paper (synthesized by IDT)	Table S6
<i>pccb-1</i> smiFISH probes	This paper (synthesized by IDT)	Table S7
complementary FLAPx probes	This paper (synthesized by IDT)	Table S8
Recombinant DNA		
L4440 vector	Kamath et. al.; Timmons et al. ⁶⁰⁻⁶²	Addgene Plasmid #1654 (pPD129.36)
<i>puf-5</i> (RNAi) in L4440 vector	Hubstenberger et al. ⁶³	N/A
<i>car-1</i> (RNAi) in L4440 vector	Hubstenberger et al. ^{39,63}	N/A
Software and algorithms		

R 4.1.2	R	https://www.r-project.org/
RStudio 2021.09.1+372	RStudio; Posit	https://www.rstudio.com/ ; https://posit.co/downloads/
Fiji 2.9.0/1.53t	Schindelin et al. ⁶⁴	https://imagej.net/software/fiji/
MATLAB R2018b	MathWorks	https://www.mathworks.com/
FISH-quant	Mueller et al.; Tsanov et al. ^{47,48}	https://bitbucket.org/muellerflorian/fish_quant/src/master/
Oligostan	Mueller et al.; Tsanov et al. ^{47,48}	https://bitbucket.org/muellerflorian/fish_quant/src/master/Oligostan/
bcl2fastq	Illumina	https://support.illumina.com/sequencing/sequencing_software/bcl2fastq-conversion-software.html
FastQC	Andrews et al. ⁶⁵	https://qubeshub.org/resources/fastqc
Galaxy server	Goecks et al. ⁶⁶	https://usegalaxy.org/
HISAT2 v2.1.0	Kim et al. ⁶⁷	http://daehwankimlab.github.io/hisat2/ ; https://github.com/DaehwanKimLab/hisat2
StringTie	Pertea et al. ⁶⁸	https://ccb.jhu.edu/software/stringtie/
HTseq count	Anders et al. ⁶⁹	https://htseq.readthedocs.io/en/release_0.11.1/count.html
SamTools	Li et al. ⁷⁰	http://www.htslib.org/
IGV v2.6.1	Robinson et al.; Thorvaldsdóttir et al. ^{71,72}	https://software.broadinstitute.org/software/igv/home
Bioconductor 3.14	Huber et al. ⁷³	https://bioconductor.org/
DESeq2	Love et al. ⁷⁴	https://bioconductor.org/packages/release/bioc/html/DESeq2.html
GenomicRanges	Lawrence et al. ^{75,76}	https://bioconductor.org/packages/release/bioc/html/GenomicRanges.html
rtracklayer	Lawrence et al. ^{75,76}	https://bioconductor.org/packages/release/bioc/html/rtracklayer.html
Panther (release 20191216)	Panther	http://www.pantherdb.org/

Original code: fluorescent reporters quantification, smFISH image quantification, smFISH image simulation	This paper; Mendeley data; GitHub	https://doi.org/10.17632/8jvrnztdvc.1 ; https://github.com/CarдонаEA/code-image-analysis-c-elegans
Other		
MoFlo Astrios EQ	Beckman Coulter	Cat#B25982
Qubit 4.0	Thermo Fisher Scientific	Cat#Q33238
Agilent 2100 BioAnalyser	Agilent	Cat#G2939BA
Zeiss LSM 880	Zeiss	N/A
Zeiss ZEN (black edition) software	Zeiss	N/A

874

875 RESOURCE AVAILABILITY

876

877 Lead contact

878 Further information and requests for resources and reagents should be directed to and will be
879 fulfilled by the lead contact, Arnaud Hubstenberger ([Arnaud.HUBSTENBERGER@univ-](mailto:Arnaud.HUBSTENBERGER@univ-cotedazur.fr)
880 [cotedazur.fr](mailto:Arnaud.HUBSTENBERGER@univ-cotedazur.fr)).

881

882 Materials availability

883 All unique materials and reagents generated in this study are available from the [lead contact](#) with
884 a completed material transfer agreement.

885

886 Data and code availability

- 887
- 888 • RNA-seq raw data have been deposited at GEO and are publicly available as of the date
889 of publication. Accession number is listed in the key resources table. Relative enrichment,
890 abundance, copy number, and GO terms of oocyte mRNAs are provided with this paper
891 as Data S1 to S4. Raw microscopy data from smFISH and fluorescent reporters have
892 been deposited at Mendeley Data and are publicly available as of the date of publication.
893 DOIs are listed in the key resources table. This paper analyzes existing, publicly available
894 data. These sources and identifiers for the datasets are listed in the key resources table.
895 All data reported in this paper will be shared by the lead contact upon request.
 - 896 • All original code has been deposited at Mendeley Data and GitHub and is publicly
897 available as of the date of publication. DOIs and identifiers are listed in the key resources
898 table.
 - 899 • Any additional information required to reanalyze the data reported in this paper is available
900 from the lead contact upon request.

901

902 EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

903

903 C. elegans strains, maintenance and RNAi

904 *C. elegans* strains were maintained by standard methods on feeding plates seeded with OP-50
905 *E. coli*⁷⁷. All transgenic strains were crossed either into hermaphrodites *N2* (Bristol wild-type
906 strain) or feminized CB4108 (*fog-2(q71)*) backgrounds that are depleted of sperm. Strains are
907 listed in [Table S2](#). To deplete the expression of genes of interest, RNAi in *C. elegans* was
908 performed as previously described, either by feeding^{61,63} or injecting (dsRNA)⁷⁸. Briefly, for the
909 feeding method, synchronized L4 larvae were grown at 20°C for 48 h on HT115(DE3) *E. coli*
910 strains expressing the dsRNA of interest from an L4440 plasmid, whose inserted sequence was
911 directly cloned from the corresponding *C. elegans* cDNA^{61,63}. A random dsRNA that is absent
912 from the *C. elegans* genome was expressed from the L4440 plasmid as a *mock(RNAi)* control.
913 For the alternative direct injection of double-stranded RNA (dsRNA) in the gonad⁷⁸, worm gonads
914 were analyzed 48 h post injection.

915

916 METHOD DETAILS

917

918 Live-imaging of GFP:protein fusions

919 Live *C. elegans* nematodes were anaesthetized with 50 mM NaN₃ in Phosphate Buffer Saline
920 (PBS), and transferred between slides and coverslips to 4% agarose pads, and imaged on a Zeiss
921 LSM 880 microscope with a Plan-Apochromat 40x/1.4 Oil DIC M27 objective and AiryScan SR
922 module. Imaging was conducted in the first 15 min of anesthesia. To avoid signal interference
923 from the intestine, images of GFP-tagged proteins were acquired from the gonad closer to the
924 objective. 3D image data was obtained using Zeiss ZEN software and a 488 nm laser (BP 420-
925 480 + BP 495-550). Z-stacks were acquired with a step-size of 0.5 μm (2.0 optical zoom, 0.66 μs
926 pixel dwell, master gain of 750, digital offset of 1, 79 μm pinhole) and processed using the in-built
927 Zeiss Airyscan Processing algorithm. Images were acquired across half of the oocyte depth, from
928 cortex to nuclei (~ 6 μm), to prevent differences in signal intensity due to tissue depth.

929

930 **Single molecule FISH (smFISH)**

931 ***smFISH probes preparation***

932 Our smFISH method to visualize mRNA subcellular localization with single-molecule sensitivity in
933 formaldehyde fixed *C. elegans* gonads was adapted from.⁴⁸ For that purpose, we designed 37 to
934 48 smFISH probes per tested transcript using the Oligostan R script.⁴⁸ Primary probes carried
935 FLAPx extensions (Tables S3-S7) that anneal to complementary FLAPx secondary probes
936 labeled with two Cy3 fluorophores (Table S8) to fluorescently label target mRNAs. The theoretical
937 expected amplified fluorescence would correspond to a minimum 37x2 fluorophore per single
938 hybridized transcript. For smFISH probes preparation, primary probes (~60nM/probe) were mixed
939 with labeled FLAPx secondary probes (5μM) in TSE buffer (10 mM Tris; 1 mM EDTA; 100 mM
940 NaCl; pH 7.95), and annealed as previously described⁷⁹.

941

942 ***Sample preparation and hybridization***

943 Briefly, synchronized *C. elegans* adult gonads were dissected on coated coverslips (0.05% Poly-
944 L-lysine; 0.2% gelatin; 0.02% CrK₂O₈S₂), fixed for 5 min in 4% formaldehyde in Phosphate Buffer
945 (77.4mM Na₂HPO₄; 4mM NaH₂PO₄) at RT, and freeze-cracked as previously described³⁹. After
946 freeze-cracking, gonads were secondary fixed in 4% formaldehyde for 20 min at RT, washed in
947 Phosphate Buffer, permeabilized in 70% ethanol overnight at 4°C, and washed again in
948 Phosphate Buffer. Next, to unmask RNAs from bound proteins, and facilitate probe hybridization,
949 samples were treated with proteinase K (QIAGEN, 4 μg/ml in 2xSSC) for 5 min at RT, followed
950 by a secondary fixation in 4% formaldehyde for 15 min to restore crosslinks disrupted by the
951 proteinase K, then washed twice in Phosphate Buffer and once in 15% formamide in 1xSSC. For
952 hybridization, samples were preincubated in 15% formamide in 1xSSC for 15 min at RT, then
953 hybridized for 16 h at 37°C with 2.5 μl of smFISH probes as prepared above and further diluted
954 in 100 μl of Stellaris RNA FISH Hybridization Buffer (LCG Biosearch Technologies) containing
955 10% formamide. For mounting, samples were incubated twice in 25% formamide in 1xSSC for 30
956 min at 37°C, washed twice in Phosphate Buffer, mounted on slides using ProLong Antifade
957 (Invitrogen), and cured for 60 h before imaging.

958

959 ***smFISH imaging***

960 smFISH images of *C. elegans* oocytes were acquired on a Zeiss LSM 880 microscope with a
961 Plan-Apochromat 63x/1.4 Oil DIC M27 objective. Images were processed with the AiryScan
962 super-resolution (SR) module, using Zeiss ZEN software. A 561 nm laser (BP 570-620 + LP 645
963 + SBS SP 615) was used for mRNA detection, and a 488 nm laser (BP 420-480 + BP 495-550)

964 for GFP:CAR-1 signal to assess mRNA localization into P-bodies that are GFP:CAR-1 labelled.
 965 For AiryScan acquisitions, images were taken with a z-spacing of 0.185 μm , 2.0 optical zoom,
 966 0.66 μs pixel dwell, average of 4, master gain of 750, digital offset of 1, and 143 μm pinhole. For
 967 Fast AiryScan acquisitions, z-stacks were obtained with a step-size of 0.250 μm , 1.8 optical zoom,
 968 0.52 μs pixel dwell, average of 4, master gain of 750, digital offset of 1, and 384 μm pinhole.
 969 Multiphase 3D smFISH images were processed using the in-built Zeiss Airyscan Processing
 970 algorithm.

971

972 **smFISH image analysis of mRNA clustering**

973 Image quantifications of condensed and dissolved mRNAs were performed with MATLAB
 974 R2018b. Our image analysis approach is detailed below and in [Figure S1](#).

975

976 **Segmentation of oocyte boundaries and staging**

977 To conduct mRNA clustering analysis in smFISH images of staged diakinesis oocytes, the first
 978 segmentation delineated oocyte boundaries within the dissected gonads ([Figure S1A](#)). A pre-
 979 processing step defined the upper and the lower limits of oocytes within the z-stack acquisition,
 980 the z-upper (Z_{up}) and z-lower (Z_{lo}) oocyte boundaries. For that purpose, the 90th percentile of the
 981 fluorescence intensities was computed for each stack along the z-axis, and the partial derivative
 982 (numerical gradient) of the 90th percentiles was calculated using the MATLAB function *gradient*.
 983 The z-stack positions corresponding to the derivative's maximum and minimum defined the
 984 oocyte lower and upper limits by detecting the transition between background and oocyte signal
 985 ([Figure S1B](#)). Next, to set oocyte limits along x- and y-axis, the oocyte contours were identified
 986 manually in maximum intensity projections of the z-stacks ([Figure S1C](#)). Following oocyte
 987 segmentations, each oocyte was staged according to its position along the proximo-distal axis of
 988 the gonad. The most proximal oocyte, ranked in position -1, is the most mature oocyte paused for
 989 fertilization. Other oocytes were numbered according to their proximo-distal positions, where the
 990 -4 oocyte is more differentiated than the -5 along the gonad spatiotemporal axis ([Figure S1C](#)).

991

992 **Segmentation of mRNA condensates**

993 The second segmentation step was used to identify the mRNA condensates that were larger than
 994 32 pixels in size ($\sim 0.02 \mu\text{m}^3$) in each segmented oocyte ([Figures S1D-S1F](#)). This thresholding
 995 segmentation method was applied in the GFP:CAR-1 and the mRNA channels, which are both
 996 independent and overlapping markers of condensates ([Figure S1A](#)). Briefly, pixels with intensities
 997 lower than the image's 90th quantile were filtered out as background. Then, 2-by-2-by- pixel
 998 regions were normalized (N) between 0 (\approx cytoplasm) and 1 (\approx condensates), according to each
 999 plane maximum intensity ([Figure S1D](#)):

1000

$$N_{i,j} = \begin{cases} \frac{\ln \bar{I}_i}{\ln \max(I_{z_{lo}})}, & j < Z_{lo} \\ \frac{\ln \bar{I}_i}{\ln \max(I_j)}, & Z_{lo} \leq j \leq Z_{up} \\ \frac{\ln \bar{I}_i}{\ln \max(I_{z_{up}})}, & j > Z_{up} \end{cases}$$

1001

1002

1003 where N is the normalized value of a 2-by-2-by-1 region, i denotes x, y coordinates, j is the z -
1004 stack, \bar{I} is the average intensity, and I represents intensities at stack j , z -upper (Z_{up}) or z -lower
1005 (Z_{lo}) oocyte boundaries.

1006
1007 After normalizing the fluorescence intensity (Figure S1D), the local fluorescence background was
1008 removed (Figure S1E). To this end, continuums of weak normalized fluorescent intensities ($N <$
1009 0.65) were identified using the 3D-connected component function. Large 3D-connected regions
1010 (> 5000 pixels) with low N ($N < 0.65$) were defined as local background and were therefore filtered
1011 out (Figure S1E). Next, to segment mRNA condensates, z -stack regions that passed a specific
1012 intensity threshold ($N > 0.6$) were labeled using the 3D-connected component function. 3D-
1013 connected objects larger than $0.02 \mu\text{m}^3$ were considered as macro-condensates. To ensure that
1014 condensate edges were included, each macro-condensate binary mask was dilated using
1015 MATLAB function *imdilate* (Figure S1F).

1016 1017 **Identification of mRNA sub-diffractive foci**

1018 After delineating oocytes and segmenting the largest mRNA condensates (see above), the third
1019 step identified sub-diffractive fluorescent foci, that represent both single mRNAs and mRNA
1020 nanoclusters, using the open-source MATLAB package FISH-quant.^{47,48} Briefly, the raw smFISH
1021 signal was enhanced by a two-step convolution of the image with Gaussian Kernels using the
1022 FISH-quant function *img_filter_Gauss_v5* as detailed.^{47,48} To limit detection to the sub-diffractive
1023 foci, mRNA macro-condensates were masked (Figure S1G). Next, to quantify foci depending on
1024 oocyte differentiation stages, oocyte limits along x - and y -axis as defined above were used to
1025 generate compatible image outlines files readable by the FISH-quant software. Finally, cytosolic
1026 mRNA foci were detected (3D local maximum) and filtered in FISH-quant (Figure S1H). To avoid
1027 single mRNA over- or under- detections, the applied filters included the determination of the
1028 Fluorescence intensity detection threshold (Figure S1I-L), the quality control filter (Figure S1M),
1029 the fit to the theoretical point-spread function (PSF) (Figure S1N), filters whose respective impacts
1030 were quantified (Figure S1O) as previously described.^{47,48}

1031 1032 **Single molecule calibration**

1033 To quantify smFISH images with single molecule sensitivity it was critical to define the
1034 fluorescence intensity distribution of single mRNA molecules. For that purpose, the intensity
1035 distribution of sub-diffractive mRNA foci was quantified at very low RNA concentrations to ensure
1036 that all mRNAs exist as single molecules (Figure S1P). mRNA foci were detected with the open-
1037 source MATLAB package FISH-quant.^{47,48} The point-spread function (PSF) and amplitude of
1038 single molecules were defined in images with mRNA concentrations lower than $0.5 \text{ mRNAs}/\mu\text{m}^3$.
1039 Such low concentrations were obtained either by depleting most RNAs by RNAi (Figure S1P), or
1040 by restricting the analysis at the onset of transcriptional activation in the distal tip regions of the
1041 gonad when transcripts have not yet reach high concentrations (up to 30 to 50 μm from the distal
1042 gonad tip cell). Both methods yielded almost identical distributions of intensities for single mRNAs.
1043 A right-skewed Gaussian was fit to obtain a function that describes the distribution of single mRNA
1044 molecule amplitudes:

$$1045$$
$$1046 \hat{A}(x) = h * \phi(x)\Phi(ax)$$
$$1047$$

1048 where \hat{A} is the kernel density estimation (KDE) for the amplitude of individual mRNA molecules,
 1049 h represents the height of the distribution, and $\phi(x)\Phi(\alpha x)$ is the skewed Gaussian function with
 1050 the shape parameter α :
 1051

$$1052 \quad \phi(x)\Phi(\alpha x) = e^{-\frac{1}{2}\left(\frac{x-\text{centroid}}{sd}\right)^2} * \left[\text{erf}\left(\frac{\alpha\left(\frac{x-\text{centroid}}{sd}\right)}{\sqrt{2}}\right) + 1 \right]$$

1053
 1054 The fitting was performed with the MATLAB function *lsqcurvefit*, thus yielding estimates of h , α ,
 1055 *centroid* and *sd* (standard deviation). After defining the relative 1X fluorescence intensity at which
 1056 the skewed Gaussian distribution of single molecule peaked, we confirmed that we detected
 1057 almost no foci with fluorescence at 2X intensities in our highly diluted conditions (Figure S1P).
 1058 Thus, single mRNAs can be distinguished from clusters containing two mRNAs whose intensity
 1059 is expected at 2X, validating that we can quantify mRNA clustering with single molecule
 1060 sensitivity. In addition we further confirmed that at high dilution, the fluorescence intensity
 1061 distributions of single molecules overlapped whether they were recorded inside GFP:CAR-1 P-
 1062 bodies (condensed phase), or outside in the cytosol (dispersed phase) (Figure S1Q). This, in
 1063 combination with further controls detailed below (Section of Single molecule fluorescence
 1064 intensity controls) confirms that single molecule fluorescent intensities do not significantly change
 1065 between the condensed and soluble phases.
 1066

1067 ***mRNAs copies within sub-diffractive foci***

1068 While in diluted conditions, all mRNA foci have an almost identical fluorescence intensity
 1069 distribution which corresponds to the intensity of single mRNAs, at higher mRNA concentrations
 1070 many brighter foci corresponding to mRNA clusters containing multiple mRNAs were detected
 1071 (Figure S1P). The modeled KDE of single molecule amplitudes (Figure S1P) was then used to
 1072 estimate the number of single mRNA foci detected in oocyte cytosols (Figure S1S). To this end,
 1073 the distribution of fluorescent amplitudes of cytosolic mRNA foci was fit with a right-skewed
 1074 Gaussian model. This fit was further decomposed in two Gaussian distributions. The peak of the
 1075 first Gaussian was imposed to be centered at the single molecule fluorescence amplitude whose
 1076 parameters were calibrated at lower concentration in Figure S1P as described above. The second
 1077 Gaussian centered at higher fluorescence amplitudes, and described foci containing more than
 1078 one mRNA molecule (Figure S1S). This second population of foci were considered as
 1079 nanoclusters. The right-skewed Gaussian function used to describe fluorescence intensity
 1080 distribution was the following:
 1081

$$1082 \quad \hat{A}_f(x) = h_s * \phi(x)\Phi(\alpha x) + h_n * \phi_n(x)\Phi_n(\alpha_n x)$$

1083
 1084 where \hat{A}_f denotes the KDE for the amplitude of cytosolic mRNA foci. The fitting yields estimates
 1085 of the height (h_s) for the single molecule fraction with fixed parameters given by $\phi(x)\Phi(\alpha x)$, as
 1086 well as estimates of h_n , α_n , *centroid_n* and *sd_n*, for the nanocluster fraction. To estimate the
 1087 number of molecules, the functions that describe the KDEs of single molecule ($h_s * \phi(x)\Phi(\alpha x)$)
 1088 and nanoclusters ($h_n * \phi_n(x)\Phi_n(\alpha_n x)$) populations were integrated, multiplied by the number of
 1089 cytosolic foci, and divided by the estimated peak amplitude given by \hat{A} . This integration allowed

1090 us to estimate the proportion of single mRNAs and nanoclusters, and the number of molecules in
1091 each fraction.

1092

1093 **mRNAs copy number quantification within macro-condensates**

1094 To quantify the number of mRNA molecules that were clustered within macro-condensates, we
1095 took advantage of the average PSF of single mRNAs computed at low concentration (see above
1096 and [Figure S1R](#)) and used two complementary and cross-validating methods, an integrative
1097 method and a cumulative method ([Figure S1R](#)). For the integrated intensity approach, we used
1098 the dedicated FISH-quant biocomputing pipeline⁵⁷ that we modified to adapt integration windows
1099 to each condensate volume. For the cumulative method, we computed the number of mRNA
1100 molecules per condensates as follows:

1101

$$1102 \quad N_{(cumulative\ intensity)} = \frac{\sum_{j=1}^V I_{mc_j} - BGD_{sm} \geq 0}{\sum_{i=1}^V I_{sm_i} - BGD_{sm} \geq 0},$$

1103

1104 where V represents the number of voxels, mc a given macro-condensate, sm the average PSF
1105 of single mRNAs, I is the grayscale intensity, and BGD denotes background.

1106

1107 **Quantifications of mRNA phase parameters**

1108 *Dispersed mRNA copy number or dissolved mRNAs ($N_{dis(smFISH)}$):*

1109 Sum of the mRNAs that are detected as single transcripts in the cytosolic phase as described in
1110 our smFISH quantification pipeline detailed above.

1111

1112 *Condensed mRNA copy number or clustered mRNAs ($N_{cond(smFISH)}$):*

1113 Sum of the mRNAs that are detected either as belonging to macro-condensates or nanoclusters
1114 in our smFISH quantification pipeline detailed above.

1115

1116 *Total mRNA copy number ($N_{tot(smFISH)}$):*

1117 Sum of dispersed and condensed mRNA copy numbers.

1118

1119 *Condensed mRNA fraction (% $cond_{smFISH}$):*

1120 Ratio between the condensed mRNA copy number and the total mRNA copy number;

$$1121 \quad \% \text{cond}_{smFISH} = \frac{N_{cond(smFISH)}}{N_{tot(smFISH)}} * 100.$$

1122

1123

1124 *P-body enrichment (FC_{smFISH}):*

1125 Ratio between the condensed mRNA copy number and the dispersed mRNA copy number;

$$1126 \quad FC_{smFISH} = \frac{N_{cond(smFISH)}}{N_{dis(smFISH)}}.$$

1127

1128 *Phase volumes:*

1129 The [segmentation of oocyte boundaries and oocyte staging](#) (see above) was used to obtain 3D
1130 reconstructions of staged oocytes and nuclei, and measure respective volumes. The

1131 [segmentation of mRNA condensates using a thresholding approach](#) (see above) yielded macro-
1132 condensate volumes.

1133

1134 *Total cytoplasmic concentration ($C_{tot(smFISH)}$):*

1135 Total copy number divided by the cytoplasm volume where the cytoplasm volume ($vol_{cytoplasm}$)
1136 was defined as the oocyte volume without the nucleus; $C_{tot(smFISH)} = \frac{N_{tot(smFISH)}}{vol_{cytoplasm}}$.

1137

1138 *Dispersed phase cytosolic concentration ($C_{dis(smFISH)}$):*

1139 Concentration of mRNAs that are present as single transcript (non-clustered) in the dispersed
1140 phase. The dispersed mRNA copy number was divided by the cytosol volume ($vol_{cytosol}$), defined
1141 as the oocyte volume to which the volume of macro-condensates and the nucleus was subtracted;

1142 $C_{dis(smFISH)} = \frac{N_{dis(smFISH)}}{vol_{cytosol}}$.

1143

1144 *Condensed phase concentration ($C_{cond(smFISH)}$):*

1145 Condensed mRNA concentrations were measured in macro-condensates larger than $19 \mu\text{m}^3$ as,
1146 $\frac{\text{mRNAs in macro-condensate}}{vol_{macro-condensate}}$, because volume measurements are more accurate in larger objects.

1147 $C_{cond(smFISH)}$ is the average condensed mRNA concentrations.

1148

1149 *Partition coefficient (PC_{smFISH}):*

1150 The partition coefficient was computed as the ratio between the condensed phase concentration
1151 and the dispersed phase cytosolic concentration; $PC_{smFISH} = \frac{C_{cond(smFISH)}}{C_{dis(smFISH)}}$.

1152

1153 *Condensate cumulative volume ($\sum vol_{cond}$):*

1154 The condensate cumulative volume could not be directly measured because the smallest
1155 condensates are below microscopy resolution. Instead we computed the condensate cumulative

1156 volumes as follow: $\sum vol_{cond} = \frac{N_{cond(smFISH)}}{C_{cond(smFISH)}}$.

1157

1158 *$spn-4$ mRNA distribution across cluster sizes:*

1159 The cluster sizes were defined as the number of condensed mRNA copies per cluster (mRNA
1160 molecules per foci, [see quantification of the number of mRNAs copies within sub-diffractive mRNA
1161 foci and macro-condensates sections](#)). Second to compute $spn-4$ mRNA distributions across
1162 cluster sizes, the number of clusters was multiplied by the number of $spn-4$ molecules per cluster.

1163

1164 *Theoretical relationship between the Partition coefficient (PC) and the Condensed mRNA fraction
1165 (% cond, CF):*

1166 By combining:

1167

1168 (a) $CF = \frac{N_{cond}}{N_{tot}} = \frac{N_{cond}}{N_{dis} + N_{cond}}$

1169 (b) $C_{cond} = \frac{N_{cond}}{V_{cond}}$

1170 (c) $C_{dis} = \frac{N_{dis}}{V_{dis}}$
 1171 (d) $PC = \frac{C_{cond}}{C_{dis}} = \frac{N_{cond}}{N_{dis}} * \frac{V_{dis}}{V_{cond}}$
 1172 (e) $\frac{N_{dis}}{N_{cond}} = \frac{1}{PC} * \frac{V_{dis}}{V_{cond}}$

1173
 1174 We obtained the following theoretical relationship:
 1175

1176
$$PC = \frac{CF}{1 - CF} * \frac{V_{dis}}{V_{cond}}$$

 1177
 1178
$$PC = \frac{\% cond}{100 - \% cond} * \frac{V_{dis}}{V_{cond}}$$

 1179

1180
 1181 where V_{dis} is the cytosol volume (see [phase volumes](#)) and V_{cond} is the [condensate cumulative](#)
 1182 [volume](#) ([Figures S2D](#)).
 1183

1184 ***In silico simulations for smFISH***

1185 We used simulated images to evaluate the accuracy of our smFISH measures of mRNA clustering
 1186 ([Figure S4](#)). In the absence of physical interactions, two independently free diffusing molecules
 1187 may artificially appear as belonging to the same cluster by lack of spatial resolution in microscopy
 1188 images as the molecules randomly come close to each other. Such an occurrence will increase
 1189 at high concentrations, leading to cluster overestimates. To take into account this resolution limit
 1190 at high concentrations, and correct for potential clustering overestimates, we took an *in silico*
 1191 simulation approach ([Figure S4](#)). To do this, we generated synthetic smFISH images where
 1192 individual mRNAs were placed randomly within a 3D space, as expected from free diffusion
 1193 without clustering ([Figure S4A](#)). To simulate our experimental imaging resolution limits,
 1194 experimental PSFs of single mRNAs extracted from RNAi experiments were used. The random
 1195 placement of PSFs in 3D was done in a segmented oocyte without nucleus using the MATLAB
 1196 function *randsample*. A variable of our simulation was the input RNA concentration, that was
 1197 artificially increased by adding randomly placed PSFs ([Figure S4A](#)). For the random placement,
 1198 sampling replacement was allowed, and simulations were repeated 3 times for a given RNA
 1199 concentration. The simulated images were next analyzed using our smFISH quantification
 1200 pipeline ([Figure S1, and see above](#)). In this way, we could compare the measured smFISH mRNA
 1201 quantification results from the simulated images that did not include clustering in the input
 1202 parameters (1) to the actual input values of the simulation, (2) to our experimental measures
 1203 ([Figures S4B-S4F](#)). The experimental nanocluster quantifications were corrected by subtracting
 1204 the cluster artifactual over detection in the simulation, and single soluble mRNA quantifications
 1205 were corrected by adding mRNA artifactually detected as nanoclusters ([Figures S4B-S4F](#)).
 1206

1207 ***Single molecule fluorescence intensity controls***

1208 First, we confirmed that single molecule fluorescence intensities distributions were independent
 1209 of RNA condensation ([Figure S1Q, and above section “Single molecule calibration”](#)). To test
 1210 whether our quantifications of clustered mRNAs in the condensed phase were accurate and
 1211 scaled linearly with the quantification of single mRNAs in the dispersed phase, we induced a

1212 dissolution of mRNA clusters, and tested whether our total mRNA quantification remained
1213 unchanged independent of mRNA condensation status. For that purpose, condensate dissolution
1214 was either artificially induced by RNAi (Figures 4D, S6D), or physiologically induced during oocyte
1215 maturation (Figures 6E-6F"). Both approaches quantified the same total mRNA copy number
1216 whether mRNAs were condensed in macro-condensates, in nanoclusters or dissolved, confirming
1217 our quantifications did not over or underestimate mRNA in the condensed form as compared to
1218 the dispersed form. In addition, the smFISH computed mRNA copy number, as well as mRNA
1219 enrichment in condensates, linearly correlated with the results independently obtained from the
1220 condensate purification sequencing method (Figures S2J-S2L), cross-validating our imaging
1221 quantification by a biochemical purification sequencing approach.

1222

1223 **P-body purification and oocyte dissection**

1224 Oocyte P-bodies were purified from *C. elegans* by adapting our published Fluorescent activating
1225 Particle Sorting (FAPS) method that we previously developed starting from human epithelial cells
1226 in culture,³ the most significant improvement being our ability to start from whole animal fixed
1227 tissues rather than non-fixed cell lines.

1228

1229 **Sample preparation**

1230 *C. elegans* oocyte P-bodies were labelled using a fluorescent GFP:CAR-1 reporter under the
1231 germline promoter *pie-1*.¹⁰ As a secondary marker, PGL-1:RFP was used, which labels
1232 subcompartments of the oocyte P-bodies.^{10,39} To sort oocyte P-bodies, ~ 1000 *C. elegans* animals
1233 were used per replicate. For each sorting experiment, staged L4 females (TE71) and L4
1234 hermaphrodites (TE51) were isolated and grown for 72 h at 20°C in the absence of males. In adult
1235 worms, GFP:CAR-1 labelled P-bodies formed in the arrested oocytes of unmated females (TE71).
1236 By contrast, within active oocytes of self-mated hermaphrodites (TE51) that bypass oogenesis
1237 arrest, GFP:CAR-1 did not condensed into P-bodies macro-condensates, providing negative
1238 controls for the P-body purification.

1239

1240 **Worm lysis and extract preparation for FAPS**

1241 Adult worms were collected and washed 3 times in Phosphate Buffer (77.4mM Na₂HPO₄; 4mM
1242 NaH₂PO₄) equilibrated at 20°C, fixed in ~2.2% formaldehyde in a Phosphate Buffer for 12 min at
1243 20°C, and washed twice in 100mM Tris pH 7.5 to quench the formaldehyde. All subsequent steps
1244 were conducted at 4°C to limit RNA degradation. Fixed worms were washed twice in Lysis buffer
1245 (150 mM NaCl; 50 mM Hepes pH 7.5; 1mM EDTA; 1% Triton; 0,2% SDS), resuspended in 300uL
1246 of fresh lysis buffer complemented with 80U of RNaseOut (Invitrogen). Worm lysates were
1247 generated by mechanical bead beating using 1.0 mm zirconium-oxide beads in a Bullet Blender
1248 Gold homogenizer (Next Advance). The bead beating was conducted at medium speed for 15 s,
1249 which was repeated 8 times for total cellular lysis, with intermediate cooling steps of 30 s on ice
1250 between each bead beating to limit RNA degradation that can be induced by samples over-
1251 heating. The resulting fraction was named the whole animal pre-sorted extract. Three biological
1252 replicates from independent experiments were performed.

1253

1254 **P-body sorting by FAPS**

1255 From the whole animal pre-sorted extracts, oocyte P-bodies were sorted on a cell sorter (MoFlo
1256 Astrios EQ, Beckman Coulter) equipped with a nozzle size of 70 μm, and working at 66 000 Hz

1257 with a 60 psi pressure, using the 488 nm excitation laser and the 526/26 band pass filter to detect
1258 the GFP labelled P-bodies, and the 561 nm excitation laser and the 579/8 band pass filter to
1259 detect the RFP. Particles were detected according to their intensities (either GFP or RFP) and
1260 sizes (Forward Scattered Light). The sorting window was defined to keep GFP:CAR-1 labeled
1261 oocyte P-bodies, by comparing TE71 extracts that contained P-bodies to TE51 extracts that did
1262 not contain enlarged P-bodies (Figure 1D). The purity mode (1-2 envelopes) and a differential
1263 pressure of 0.5 were applied for sorting. Note that the starting dilution of the pre-sorted extract
1264 was critical for the purity of sorted P-bodies. A 1/2000 dilution of the pre-sorted extract was
1265 therefore adjusted to detect maximum 10000 Events Per Second (EPS). After 12 h of sorting,
1266 1×10^6 P-bodies were collected. The pre-sorted and sorted fractions were stored at 80 °C. The
1267 presence of sorted P-bodies was confirmed by microcopy, and their stability was verified by re-
1268 sorting collected P-bodies³.

1269

1270 **Oocyte dissections**

1271 For transcriptomic analyses, an additional control of dissected oocytes was included. Briefly, ~100
1272 adult *C. elegans* females (TE71), that were matched to the synchronized *C. elegans* used for P-
1273 body purification, were washed in Phosphate Buffer and dissected on coverslips. Dissected
1274 oocytes were transferred into 1ml Phosphate Buffer, centrifuged at 1000G for 10 min, and
1275 resuspended in ~150 μ l Phosphate Buffer. Lysis and FAPS steps were skipped. Three biological
1276 replicates from independent experiments were performed.

1277

1278 **Transcriptome analysis of oocyte P-bodies**

1279 Sorted oocyte P-bodies, whole animals extracts (pre-sorted extracts containing somatic and
1280 germline RNAs), and dissected oocytes fractions (oocyte RNAs) were processed in parallel for
1281 RNA-sequencing (RNA-Seq).

1282

1283 **RNA sequencing**

1284 In order to obtain high-quality RNAs from fixed samples (sorted oocyte P-bodies and animal
1285 extracts) and oocyte dissections, total RNA was extracted using a miRNeasy FFPE kit (Qiagen)
1286 with adaptations: i) the deparaffinization step was skipped, and ii) since sorted oocyte P-bodies
1287 were subcellular fractions, the separation of cell debris by centrifugation was omitted for these
1288 samples. The subsequent steps followed the recommendations of the supplier, and notably
1289 included DNase-I treatment and reverse crosslink of fixed fractions. After extraction, RNA integrity
1290 was confirmed using Agilent RNA Pico Chips (Agilent 2100 BioAnalyser), and RNA concentration
1291 was quantified with RNA HS Assay Kit (Invitrogen) using a Qubit 4.0. For cDNA libraries
1292 preparation, 10 ng of total RNA were used. Briefly, paired-end RNA-Seq libraries with barcodes
1293 were generated using SMARTer Stranded Total RNA-Seq Kit v2 - Pico (Takara). The quality and
1294 average size of the libraries were confirmed with Agilent High Sensitivity DNA Chips (Agilent 2100
1295 BioAnalyser), and the concentrations were measured with dsDNA HS Assay Kit (Invitrogen) using
1296 a Qubit 4.0. For RNA-Seq, libraries were pooled in equimolar concentrations of 4 nM and paired-
1297 end sequencing (Illumina TruSeq HT assay) was performed at UCAGenomix - Functional
1298 Genomics platform (Université Cote d'Azur, Nice, France).

1299

1300 **RNA-Seq analysis**

1301 RNA-Seq libraries were built from 3 biological replicates of the sorted P-bodies, whole animal pre-
1302 sorted extracts, and dissected oocytes. After a 75 base pair long paired-end sequencing of pooled
1303 samples, demultiplexing (*bcl2fastq application*) was performed. Sequencing reads were checked
1304 for quality by FastQC (FastQC: A Quality Control Tool for High Throughput Sequence Data,
1305 <https://qubeshub.org/resources/fastqc>) and 4 bases of the 5'-end, and 1 base of the 3'-end were
1306 trimmed. Read mapping was then performed on a local Galaxy server.⁶⁶ Briefly, reads were
1307 aligned to the *C. elegans* genome (WBcel235.96) using *HISAT2 v2.1.0*.⁶⁷ For the alignment, the
1308 following stranded reverse-forward design parameters were used: function governing the
1309 maximum number of ambiguous characters was allowed, maximum mismatch penalty = 6,
1310 minimum mismatch penalty = 2, maximum soft clipping penalty = 2, minimum soft clipping penalty
1311 = 1, ambiguous read penalty = 1, read gap open penalty = 5, read gap extend penalty = 3,
1312 reference gap open penalty = 5, reference gap extend penalty = 3, penalty for canonical splice
1313 sites = 0, penalty for non-canonical splice sites = 12, minimum intron length = 20, maximum intron
1314 length = 140000. To estimate relative RNA abundance for each transcript species, FPKM
1315 (Fragments Per Kilobase Million) and TPM (Transcripts Per Kilobase Million) were computed with
1316 *StringTie* using default parameters.⁶⁸

1317

1318 **RNA enrichment in oocyte P-bodies**

1319 To compute the relative RNA enrichment between FAPS purified P-bodies, whole animal pre-
1320 sorting extracts, and whole oocytes, read raw counts were determined for each annotated gene
1321 by *HTseq-count* using default stranded parameters.⁶⁹ Next, the *DESeq2* Bioconductor package
1322 was used to detect fold-change ($FC_{RNA-Seq}$) differences between groups taking into account
1323 internal normalization with correction for library size and RNA composition bias.⁷⁴ The geometric
1324 mean was calculated for each gene across samples. Then, the counts per gene were divided by
1325 the geometric mean. The ratios generated by this procedure were the size factors for each
1326 sample. Shrinkage estimation was applied for dispersions using the samples from the different
1327 conditions as replicates. To determine gene expression differences between conditions, negative
1328 binomial generalized linear models were fit to each gene and the Wald test was applied for
1329 significance testing. Count outliers and genes with mean of normalized counts below threshold
1330 were removed by Cook's distance. For statistical significance, *p*-value < 0.05 was considered the
1331 threshold of FDR filtering for Type II error. All the statistical analyses were conducted in R version
1332 4.1.2.

1333

1334 **Feature analysis of oocyte P-body mRNAs**

1335 *Differential enrichment of RNA biotypes in oocyte P-bodies, including rRNAs.*

1336 GenomicRanges and rtracklayer Bioconductor packages were used to retrieve biotype
1337 information from the Gene Transfer Format file corresponding to WBcel235.96.^{75,76} Transcripts
1338 were classified into the following biotypes: mRNA, pseudogenes, antisense, lincRNA, snRNA,
1339 ncRNA, rRNA, snoRNA, tRNA, and the $FC_{RNA-Seq}$ were computed for each biotype by comparing
1340 the oocyte P-body transcriptome to the whole oocyte transcriptome ([Data S1](#)).

1341

1342 *Differential enrichment of mRNAs in oocyte P-bodies depending on the abundance of their protein
1343 product.*

1344 mRNAs were binned in 3 classes (no, poor and high protein product) according to the abundance
1345 of the encoded proteins in diakinesis oocytes as estimated using GFP fluorescent reporters.⁴⁶

1346
1347 *Differential P-body enrichment for the RNA targets of various RNA binding proteins.*
1348 The mRNA targets of various RNA binding proteins were predicted as follow. The top 100 mRNA
1349 targets of FBF-1 and GLD-1 were ranked according to their binding site records from CLIP data
1350 sets as computed in.⁵⁸ The targets of OMA-1 and LIN-41 were identified in ⁵⁰ and selected with a
1351 corrected p-value bellow 0.05. mRNA targets of PUF-5 were predicted from.⁴⁹

1352
1353 *Gene Ontology (GO) analysis.*

1354 To determine the GO terms of our P-body enriched and depleted mRNAs, a Panther enrichment
1355 test (release 20191216) was conducted at <http://www.pantherdb.org/>, using the GO Ontology
1356 database DOI: 10.5281/zenodo.3727280 Released 2020-03-23. Representative categories are
1357 shown in [Figure 2E](#), and complete analysis can be found in [Data S4](#).

1358
1359 *Nucleotide length for mRNAs enriched in oocyte P-bodies and embryonic P-granules.*

1360 For P-body and P-granule enriched mRNAs (corrected p-value $p < 0.05$), we computed for the
1361 longest isoform of each coding transcript the 5'UTR, 3'UTR, and CDS nucleotide length using the
1362 *C. elegans* genome annotation (WBcel235.96). P-granule enriched mRNAs were identified in ¹³.

1363
1364 *Nucleotide compositional bias of mRNAs enriched in oocyte P-bodies.*

1365 mRNAs were ranked from low to high enrichment in P-bodies ($FC_{RNA-Seq}$) and distributed in 5
1366 equal sizes bins. For each bin, the nucleotide composition (% of A,U,C,G) was computed in the
1367 5'UTR, 3'UTR, and CDS.

1368
1369 ***Transcriptome-wide scaling of condensation***

1370 For each transcript of the transcriptome, the condensed mRNA copy number in the cytosolic P-
1371 bodies ($N_{cond(p)}$), the dissolved mRNA copy number in the dispersed cytosolic phase ($N_{dis(e)}$),
1372 and their P-body enrichment as compared to the dispersed cytosolic phase (FC_p) were
1373 extrapolated by calibrating the RNA condensate purification and sequencing results with smFISH
1374 imaging quantifications of RNA condensation ([Figure S2A-L](#)). The RNA-Seq provided information
1375 about mRNA abundance (FPKM units) and enrichment ($FC_{RNA-Seq}$) on a transcriptome-wide
1376 scale, but values were relative ([Figures S2F-S2I](#)). In a complementary approach, the smFISH
1377 quantified mRNA absolute copy number in the condensed ($N_{cond(smFISH)}$) and dispersed phases
1378 ($N_{dis(smFISH)}$) and their exact enrichment in P-bodies (FC_{smFISH}), but for a limited number of
1379 candidate mRNAs ([Figure S1, S2A-E](#)). To combine the advantages of these two approaches, we
1380 scaled the transcriptome-wide RNA-Seq with the absolute mRNA measurements of the smFISH
1381 imaging using linear regressions ([Figures S2J-S2L](#)).

1382
1383 Linear regressions demonstrated the strong correlation and therefore cross-validation between
1384 the two approaches when comparing:

1385
1386 (i) mRNA enrichment in P-bodies measured by smFISH ($\log_2 FC_{smFISH}$) vs. mRNA enrichment in
1387 P-bodies measured by RNA-Seq ($\log_2 FC_{RNA-Seq}$) ([Figure S2J](#)).

1388 (ii.a) smFISH condensed mRNA copy number ($N_{cond(smFISH)}$) vs. RNA-Seq abundance in
1389 condensates (FPKM units) ([Figure S2K](#)).

1390 (ii.b) smFISH total mRNA copy number in oocytes ($N_{tot(smFISH)}$) vs. RNA-Seq abundance in
1391 oocytes (FPKM units) (Figure S2L).

1392 Note that $N_{tot(smFISH)} = N_{dis(smFISH)} + N_{cond(smFISH)}$, and the linear regressions of absolute
1393 counts (ii.a and ii.b) were constrained to intercept the origin (Figures S2K, S2L).

1394
1395 We took advantage of these linear regressions whose confidence interval were narrow to predict
1396 (p) the $\log_2 FC_p$ (regression i) and the condensed mRNA counts ($N_{cond(p)}$) (regression ii.a) for
1397 ~11140 mRNA species (Data S3), as follows.

1398
1399 The linear regression (i) of the mRNA enrichment in P-bodies yielded smFISH calibrated
1400 predictions (p) for the FC_p :

1401
1402 $\log_2 FC_p \approx \log_2 \frac{condensed}{dissolved}$, and therefore $FC_p \approx 2^{\log_2 FC_p} \approx \frac{condensed}{dissolved}$

1403
1404 $FC_p \approx \frac{condensed}{dissolved}$ yielded estimates (e) for the percentage of condensed molecules (% $cond_e$) and
1405 the percentage of dissolved molecules (% dis_e) as follows:

1406
1407 $\% cond_e = \frac{FC_p}{FC_p+1} * 100$, $\% dis_e = \frac{1}{FC_p+1} * 100$

1408
1409 The linear regression (ii.a) of P-body mRNA abundances predicted condensed mRNA counts
1410 ($N_{cond(p)}$), which combined with FC_p returned estimates for the dissolved counts as $N_{dis(e)} =$
1411 $\frac{N_{cond(p)}}{FC_p}$, and total counts as $N_{tot(e)} = N_{cond(p)} * \left(\frac{FC_p+1}{FC_p}\right)$.

1412
1413 Linear regressions, model predictions, and statistical analyses were conducted using R version
1414 4.1.2.

1415
1416 **Transcriptome-wide percentage of condensation**

1417 To obtain an estimate of the condensation percentage for all mRNAs combined together while
1418 minimizing the fitting dependence, we computed a weighted average of the relative $\log_2 FC_{RNA-Seq}$
1419 enrichments ($\overline{\log_2 FC_w}$) calculated using the RNA-Seq of dissected oocytes, and in which the
1420 weight of each transcript depends on its relative copy number (FPKM) within the oocyte as follows:

1421
1422
$$\overline{\log_2 FC_w} = \frac{\sum_{n=1}^N \log_2 FC_n * oocyte\ FPKM_n}{\sum_{n=1}^N oocyte\ FPKM_n}$$

1423 Where n represents a transcript and N is the total number of transcripts in dissected oocytes.
1424 Next, we used the model (i) to obtain the $\log_2 FC_p$ enrichment calibrated by smFISH, and
1425 calculated the estimated percentage of condensation (% $cond_e$) and the confidence interval for
1426 all oocyte mRNA transcripts (Figure S7A).

1427
1428 **Transcriptome-wide compaction in condensates**

1429 To estimate the mRNA compaction in P-body condensates, we computed the transcriptome-wide
 1430 condensed mRNA local concentration within P-bodies as follow:
 1431

$$1432 \quad \text{transcriptome-wide } C_{cond} = \frac{\sum N_{cond(p)}}{\sum vol_{cond}}$$

1433
 1434 Confidence interval was computed as follows:
 1435

$$1436 \quad \min(\text{transcriptome-wide } C_{cond}) = \frac{\sum N_{cond(p)} \text{ lower limit (95\% CI)}}{\sum vol_{cond} \text{ upper limit (95\% CI)}}$$

$$1437 \quad \max(\text{transcriptome-wide } C_{cond}) = \frac{\sum N_{cond(p)} \text{ upper limit (95\% CI)}}{\sum vol_{cond} \text{ lower limit (95\% CI)}}$$

1438
 1439 The P-body mRNA condensed copy number ($N_{cond(p)}$) and its minimum and maximum 95 %
 1440 confidence interval (95% CI) were computed for each transcript transcriptome-wide using the
 1441 linear regression (ii.a) (Data S3), from which the cumulative condensed copy number was
 1442 computed for all oocyte mRNAs ($\sum N_{cond(p)}$) and its upper and lower limits. The corresponding
 1443 cumulative volume of P-bodies ($\sum vol_{cond}$) was computed as described in the smFISH method
 1444 section.

1445
 1446 We further calculated the mRNA compaction in condensates in nucleotide per μm^3
 1447 (mRNA nt concentration) (Figures 7B-C, S7A-E), as follows, where nt is the annotated length in
 1448 nucleotides of each corresponding full-length transcript (Data S3):
 1449

$$1450 \quad \text{mRNA nt concentration} = \frac{\sum N_{cond(p)} * nt}{\sum vol_{cond}}$$

1451 **Quantification of coarsening across oogenesis**

1452 The proximo-distal axis in *C. elegans* gonads, along which oocytes are aligned in a single row, is
 1453 a convenient spatiotemporal axis, to study condensate growth across time. For each oocyte the
 1454 time before ovulation can be estimated knowing the oocyte position in the row and the ovulation
 1455 rate (the frequency at which one oocyte move from one position to the next) (Figure 3A-C). To
 1456 quantify P-body coarsening, we recorded the sizes of fluorescently labelled GFP:CAR-1 P-bodies
 1457 depending on oocyte position along the gonad axis. To image the entire gonads, up to 4 z-stack
 1458 acquisitions were analyzed per animal, using the parameters described in the [live-imaging](#)
 1459 [methods section](#). Next, condensates were segmented within staged oocytes using the script
 1460 detailed in the smFISH quantification methods (Figure S1). We computed the condensate volume
 1461 distribution and the cumulative volume distribution for each time-point before ovulation (oocyte
 1462 position) (Figure 3C').
 1463

1464 **mRNA compositional bias across cluster sizes**

1465 The repressed mRNAs *spn-4* and *glp-1* were simultaneously detected by two-color smFISH using
 1466 Cy3 and Cy5 labeled probes. Images were acquired as described in the [smFISH imaging section](#)
 1467 with an additional channel for Cy5 (633 nm laser, BP 570-620 + LP 645). Laser power used for
 1468

1469 both channels was 5% (Fast Airyscan). Single-labeled controls showed no bleed-through between
1470 Cy3 and Cy5 channels (Figure S5A-H).

1471
1472 To assess mRNA compositional differences across cluster sizes, the ratio between *glp-1* and
1473 *spn4* mRNA cumulative fluorescence intensities was computed for each mRNA cluster and
1474 plotted as a function of the cluster size. Clusters were segmented using the thresholding approach
1475 described in Segmentation of mRNA condensates. The stringency of the thresholding was slightly
1476 reduced to include small clusters.

1477 1478 **mRNA demixing within macro-condensates**

1479 The degree of *spn-4* and *glp-1* demixing within macro-condensates was evaluated by computing
1480 the Pearson correlation coefficient (r) along x-y transversal sections of macro-condensates
1481 (Figure 3E'''). As positive control of mixing, *spn-4* or *glp-1* simultaneously with Cy3 and Cy5
1482 fluorescent probes (see Figures 3E''', S5J). In addition, the ratio between Cy3 and Cy5 cumulative
1483 fluorescence intensities was plotted as a function of cluster size, which confirmed the homotypic
1484 feature of small clusters as compared to large macro-condensates (Figures S5J, K).

1485 1486 **Analysis of relative protein concentrations**

1487 **Protein concentrations across differentiation**

1488 The relative GFP:CAR-1 and PUF-5:GFP protein concentrations were calculated along oocyte
1489 differentiation using z-stack confocal images of live *C. elegans* gonads (see Live imaging section).
1490 Oocytes were manually delimited and staged according to their differentiation along the gonad
1491 proximo-distal axis, and GFP condensates were segmented (see Quantification of P-body
1492 coarsening across oogenesis). The relative concentration in oocytes (rC_{oocyte}), dissolved (rC_{dis}),
1493 condensed (rC_{cond}), and in each macro-condensate (rC_{MC}) was computed per volume unit as
1494 follows:

$$1495$$
$$1496 \quad rC_{phase} = \frac{\sum FU}{phase\ volume}$$

1497
1498 Where FU is the fluorescence intensity units and $phase\ volume$ is the volume given in either
1499 voxels or μm^3 . RNAi controls depleting the fluorescent protein of interest were used to confirm the
1500 specificity and sensitivity of our measures (Figures S6A-S6B').

1501
1502 To measure GFP:CAR-1 local concentrations (Figure 4B) in condensates across oocyte
1503 differentiation, values were obtained from the eight larger condensates segmented in each
1504 oocyte; in the same fashion as the condensed phase concentration (see $C_{cond(smFISH)}$) of mRNAs
1505 was computed.

1506 1507 ***fog-1* and *spn-4* translation reporters**

1508 The $P_{pie-1}::GFP::H2B::3'UTR^{fog-1/spn-4}$ fusions reporters were reported to recapitulate endogenous
1509 expression patterns.⁴⁶ The accumulation of the GFP:H2B reporter in nuclei was quantified in one
1510 focal plane by computing the maximum intensity of a selected nucleus minus the background of
1511 the surrounding cytosol, as previously reported.^{6,39}

1512

1513 **SPN-4:GFP protein production rates**

1514 SPN-4:GFP protein production rates, computed in Fluorescence units per μm^3 per min, were
1515 derived from SPN-4:GFP concentrations (in Fluorescence units per μm^3) across oogenesis time
1516 points (in min). Briefly, synchronized unmated L4 larvae females were either isolated or kept in
1517 culture with males, to compare protein production rates in quiescent (unfertilized) and active
1518 oogenesis (fertilized). Time points between N and N+1 oocytes in the gonad correspond to the
1519 time between 2 ovulation events, and are 0,33 h and 10 h, in active and quiescent oogenesis,
1520 respectively. *C. elegans* gonads from adults that were 48 h post L4 larvae stage were imaged
1521 and the relative SPN-4:GFP concentration (rC_{SPN4}) was calculated at each oogenesis time point
1522 along the gonad using Fiji. For image background subtraction, a representative region (~250 x
1523 250 pixels) was defined in the distal gonad that does not express SPN-4:GFP. The rC_{SPN4} per
1524 segmented and staged oocyte was computed from cumulative z-projections (sum of slices) of 3D
1525 z-stacks as follows:

1526

$$1527 \quad rC_{SPN4} = \frac{\bar{I}_{outline} * px_{outline} - BGD * vx_{oocyte}}{V_{vx} * vx_{oocyte}} = \frac{\sum I_{oocyte-BGD}}{V_{oocyte}}$$

1528

1529 $\bar{I}_{outline}$ represents the mean fluorescence intensity of the z-projected oocyte (*outline*), $px_{outline}$
1530 is the number of pixels in the *outline*, vx_{oocyte} is the number of voxels in the oocyte given by
1531 $N_{stacks} * px_{outline}$ where N_{stacks} is the number of slices. V_{vx} represents the voxel volume in μm^3 .

1532 The estimated mean background was calculated as $BGD = \frac{\bar{I}_{BGD} * px_{BGD}}{vx_{BGD}} = \frac{\bar{I}_{BGD}}{N_{stacks}}$, where \bar{I}_{BGD} is the
1533 mean fluorescence intensity of the z-projected *BGD* representative region (*outline_{BGD}*), px_{BGD} is
1534 the number of pixels in the *outline_{BGD}*, and vx_{BGD} is the number of voxels given by $N_{stacks} * px_{BGD}$.

1535

1537 From the difference in relative SPN-4:GFP concentration (ΔrC_{SPN4}) between the N and N+1
1538 oocyte separated by the ovulation time, we computed the relative SPN-4 protein production rates
1539 ($rPR_{SPN4} = \frac{\Delta rC_{SPN4}}{ovulation\ time}$).

1540

1541 **QUANTIFICATION AND STATISTICAL ANALYSIS**

1542

1543 **Quantification**

1544 The details of the quantification are included in the sections of [method details](#).

1545

1546 **Statistical analysis**

1547 Statistical analyses were performed in R v. 4.1.2. When comparing two datasets, normality
1548 distribution was assessed using a Shapiro-Wilk test. Two-sided Mann-Whitney U-test was used
1549 for non-normally distributed data, and two-sided t-test for normally distributed (parametric) data.
1550 For parametric hypothesis testing, equality of variances was determined (F test; *var.test* function).
1551 When comparing more than two datasets, regression models were fitted and model assumptions
1552 checked including the normality of residuals (Shapiro-Wilk test). One-way ANOVA was used if a
1553 model met assumptions, otherwise, Kruskal-Wallis tests were implemented. Parametric or non-

1554 parametric pairwise comparisons were conducted with a false discovery rate (FDR) adjustment
1555 method. p -value less than 0.05 was considered statistically significant.

1556

1557 Plotted data are presented as mean values with 95% confidence intervals, violin plots with the
1558 probability density distribution of the data, or boxplots with median (center line), first and third
1559 quartiles (box bounds), whiskers (1.5 times the interquartile range), and outliers. Sample sizes
1560 (n) are indicated in figure captions or the relevant sections of [method details](#).

1561

1562 The details of RNA-Seq statistical analyses are included in the corresponding sections of [method](#)
1563 [details](#).

1564
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1566

SUPPLEMENTARY TABLES

Protein abundance	Gene id (wormbase)	mRNA	Enrichment in P-bodies
High	WBGene00001482	<i>fog-2</i>	-1.580621583
High	WBGene00003220	<i>mes-2</i>	-0.3786363
High	WBGene00003920	<i>par-5</i>	-1.16238
High	WBGene00006537	<i>tbb-2</i>	-1.272879
High	WBGene00003994	<i>pgl-3</i>	-0.7272095
High	WBGene00003043	<i>lip-1</i>	1.009881
High	WBGene00004374	<i>rme-2</i>	-0.799679
High	WBGene00003230	<i>mex-5</i>	1.076572
High	WBGene00004241	<i>puf-5</i>	0.1067736
High	WBGene00003912	<i>pal-1</i>	1.182124
High	WBGene00000871	<i>cye-1</i>	-0.436435661
High	WBGene00003221	<i>mes-3</i>	0.3024479
High	WBGene00000467	<i>cep-1</i>	0.431863468
High	WBGene00004976	<i>spe-41</i>	-0.02561671
High	WBGene00004027	<i>pie-1</i>	-0.3722637
Poor	WBGene00003992	<i>pgl-1</i>	0.1226306
Poor	WBGene00003785	<i>nos-3</i>	0.956317
Poor	WBGene00000935	<i>daz-1</i>	1.093645633
Poor	WBGene00001862	<i>him-3</i>	1.293189
Poor	WBGene00003229	<i>mex-3</i>	0.5136953
Poor	WBGene00001481	<i>fog-1</i>	1.471059837
No	WBGene00001609	<i>glp-1</i>	1.270785647
No	WBGene00001401	<i>fbf-1</i>	1.05501891
No	WBGene00001402	<i>fbf-2</i>	0.941915491
No	WBGene00001595	<i>gld-1</i>	0.98989342
No	WBGene00004984	<i>spn-4</i>	1.849331
No	WBGene00004078	<i>pos-1</i>	1.533989

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1570

Table S1. mRNA enrichment within oocyte P-bodies according to the abundance of their protein product in oocytes, related to [Figure 2](#).

1571

Strain	Genotype	Mutagen	Reference
-	$P_{pie-1}::gfp::car-1::UTR^{pie-1}$	Bombardment	59
-	$fog-2(q71); P_{pie-1}::gfp::car-1::UTR^{pie-1}$	Bombardment	This study
TE73	$fog-2(q71); car-1(oc8[car-1:gfp:FLAG])$	CRISPR-Cas9	gift of T. Evans
TE51	$P_{pie-1}::gfp::car-1::UTR^{pie-1}; P_{nmy-2}::pgl-1::mrfp$	Bombardment	10
TE71	$fog-2(q71); P_{pie-1}::gfp::car-1::UTR^{pie-1}; P_{nmy-2}::pgl-1::mrfp$	Bombardment	10
DG4158	$spn-4(tn1699[spn-4::gfp::3xflag])$	CRISPR-Cas9	50
-	$fog-2(q71); spn-4(tn1699[spn-4::gfp::3xflag])$	CRISPR-Cas9	This study
DG4215	$puf-5(tn1726[gfp::3xflag::puf-5])$	CRISPR-Cas9	50
-	$fog-2(q71); puf-5(tn1726[gfp::3xflag::puf-5])$	CRISPR-Cas9	This study

P: promoter. *UTR*: 3' untranslated region.

Table S2. *C. elegans* strains.

1572
1573
1574
1575

mRNA	Probe	5' - probe - FLAPx - 3'
<i>spn-4</i>	1	ATGGCGAAGCACTTCATTTGACTTCGAGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	2	CATTGCTACGGTATGGTGCCATCATGAATCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	3	TTGAACAACCTGATGCATTGGCTCAGCTCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	4	GGGCTGTTATGTGGGGACCAATGAATCTCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	5	TGCTCTCTTTCTTTGTTAGCACTATTATTTCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	6	TGCTGCATGGTGAGTTTTGTCTGTGCATGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	7	CATCAGAATACTTGTTCCTCGTAGGCTCGCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	8	CTCGCTGTGCTTGGTGCATCGAGGTGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	9	GTTGATAAGCTTTCTGCGGTTGGTGTGCGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	10	TTCTCACTTCTATAATTTCTTGAACATGTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	11	TTCGTGAAGCGCAGCAGCAGCTTTCTCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	12	ACCCCTTTGGATCCACGATTTTGTGACGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	13	AGAAGCCGGCTCACGAGTTTTCGGTTTGACCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	14	AATTGGATCTTTGTTGGAAGGATTCGCACTGTCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	15	TGTTAGCAAGCGAGGTAGGTTAAACGGACTGGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	16	ACGAATCATTTGACAAGGACAACGAGTGTGCGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	17	AAATGGGCGTATCAGTTGAACTGATTACTGGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	18	TTGGTGGAGAAACAGACTTTCCGTGGAACGATCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	19	GGAACCTTCGTAGAGAGCAGCGAGAAGATAATCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	20	CTGATGATCTCAATCCAGAAGATGTCACAGATCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	21	GAAGTGTGAGAAAGAGCAGTAATCTGGTACGCGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	22	ATGATGTGGGAATATAGAGTTCAACTCCACATCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	23	AACGGTAATCCATCATGTGCTCTGTGAGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	24	TTCCACACTCAGGACTTTCATATGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	25	GACAGCTCGTGATTTCGAAGAATCAGAACACTTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	26	TTTGCAGAAGTATTCACCAGCTGAAGAAAGCTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	27	AGCTTCGGGAAAACATGTTGTGTTCTCTTTTCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	28	CATAAATTATGAGGGGCTCGAAGATCGACACACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	29	GCTTTGGTGGTGGCAAAACTTCATTTTGTGGACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	30	AGGATTTCGATGGAATCGAGTGTGACAAAACCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	31	AGAACATTGCCGCCAAATCTTGTTCACGAAAACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	32	AGGGAATATTGCTGACATATATCCTGTTATCTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	33	ACAGGAATGCAAAAATAACTGGCGAACACAGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	34	AAGATGCTCAGAAAATACAATACGTGGGCACACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	35	GAGATGGCAAAATAGGGAAGATGCTGAAACGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	36	TCCTACAAAATAACACCGGGGGGTCGATCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>spn-4</i>	37	TTGGTCTGCACAAGACTACGCGTGTGAAAATCCTCCTAAGTTTCGAGCTGGACTCAGTG

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Table S3. Sequence of probes against *spn-4*. Primary probes carried the FLAPx extension shown in blue.

mRNA	Probe	5' - probe - FLAPx - 3'
<i>glp-1</i>	1	TGACCGGTGGTGCCATTTTCATGGTTAAACA CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	2	CCCAGACTTAGCACCATGACCTGACT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	3	AAGTTCATGATGGTAGCTGGCTTCAGCAAC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	4	GAGCAGCAGTCATGTCCATTGAATCGACT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	5	CTCCTTTTGAAGAAGAAGCTCTGCCATCT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	6	ATCAATGCAGTCATACCATTCCGGTCC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	7	CATTCACATCGGCTCCAGCCCTTCAAG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	8	TTTTCTCTGACATTGGCAGCAAGCCAC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	9	GCAGAACTCGTCGTCTATACTTTGAATCCA CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	10	AATGATTTGACTGATTCCGACGACCTTTCTC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	11	ACAGTACCATTGCTTCCGTAATTGGGAG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	12	ATACGTACGGTTGCTCGAAGATTGCACTG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	13	CTCCATTTGCGAATTGATCAGCACATCTTG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	14	GAAGCAAGATCCATACACTTTCGTTGCTCG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	15	CCATATTGGCATTTCGCAATATCCAGTATT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	16	GGAGCATAACTGTCTATCCTTTATTTTCGCAG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	17	ATCTCCAGAGAATCCGTTGGGGCAGTTAC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	18	GCAGTGTGGTCTCTGTAGTGGAAGATCACAA CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	19	ACATGTCGATAGCCTCCTCGCAATACT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	20	AGTTGTTAACACACGTTTCTTCGATGAGGCAT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	21	CCCGAGTCGCATACACATCTGAAGTCTT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	22	GTTCACAATATCGACCGGATTTTCCAATTGGG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	23	TAGCAGAGTCCGTTGTTGCAAGGATCCGAAC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	24	TCTTGACAGATACACGTCGGATTTTACCAT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	25	TACTCCGGACTTGTTCATTTCGATTCGGT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	26	GACGGTGGTGGTGTGATGATTTGTTTCGCC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	27	GACGATGCTGCACCTTGTGGCGTTTTTCGC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	28	ACCGTCTTTTACATCCTTCCTTAGCG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	29	AAGCATTATCGGTGTTCTTCCGTCCTCGT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	30	GTTTGTCTTATTAGAGCTTCGTCGGACCAG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	31	TATTACCATTTCTCGTTATCGTGCATCG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	32	ATGAAGAGCTGTTGTCCTTTTACTTGTGG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	33	ACTTGATGTTTTCCGAGTTCACGTGCTACT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	34	TCGAATTGCCTCTGTTGTAATGACATCTTCC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	35	TGATTGACTGACTCATTGTGATTGGAGCTGT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	36	TCGCATCTGGTCCCTGCAGCTTGAACA CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	37	GAGAGGGATTTTCGTCGGCTGGTAGATCAG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	38	ACTATAATCGCTACAGTGTCTCTTGGTATTGG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	39	TCGTTGATTCCATCGGAGGCATCCAGAC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	40	AACTCTTTCCATTTTCATGTTTACCATCCCC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	41	TGCATTAGTGTCTCTCCTCCGGTAGCTTG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	42	GGTATTGACATTTGGAGAATGGTCTTTGCC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	43	GATCACGAATGACAGTCTCTTTGATTTCTCG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	44	CTCCGTTAGCACATGTAGTTGGAGTGCAC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	45	CATCGTTGTAACACGTTCCATTGCTCCGAC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	46	TCTCACATCGTTGTCCAGTGAATCCTTGT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	47	CTTCATCTCTGTTCCGAATACATCTTCCAT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>glp-1</i>	48	CTCCATAGCCTTCTTACAGCCTTTTTG CCTCCTAAGTTTCGAGCTGGACTCAGTG

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Table S4. Sequence of probes against *glp-1*. Primary probes carried the FLAPx extension shown in blue.

mRNA	Probe	5' - probe - FLAPx - 3'
<i>puf-5</i>	1	TTATCACTAGATGGCGTTTCTAGTAGAGGCAC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	2	ACGGTTGACACGATTCTTGATCCGATCGAAC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	3	TGTTGGACGACATAGTTCCCGTATTGATGGAAC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	4	ACAAGATGTCCAGAGCATCACGGTTTCGT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	5	CTGATCCTTCACATATCCATCGAAGATTTTCG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	6	ACTTGTCTTGGACATCGATAGAATGTTACGG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	7	ACGAGACGGCAACCATACTTGTCTTGGC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	8	CAGTTTGACAACCTTCTGCGAAGCTGGATGG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	9	GATCTTCTGGGAAATGGCGCTCCAGGAAC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	10	GATCGAACATTGCTCTGGACGTTTCCAACG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	11	TGACGTATTGTTGGCGTGAATCGGCAACG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	12	AGTCAGCAGGAGTCAAGTTCAATGGGCT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	13	TTCAGTTTGTTCATGAAATGGGCACTGATG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	14	TGAGTGGAGTTGGCATAGCCCACTG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	15	TTGGCTCATTTCGTCATCGTCATTGGCAC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	16	TGAGCTTCTGGAGCGACTCGATTATTTTCT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	17	TTTGGCATAGAGCGCATTTCGGATGC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	18	GCGTGCCACTGATGTCATGAGTCCAT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	19	GGATTATCAGACAGCTTGTTCGATGGTTT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	20	TGATCTTTTCGACGAGCTTCATCGCGAT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	21	TTTCGATCAATCCGTTGTCACACATCTT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	22	GGCGAGTATTGGAAGCGCTGTTCCAC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	23	TTTGGTGATCGCTGATCGACGATGAATCC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	24	GCTTTTCGAGTTGATTCTTCCAGTACTG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	25	GAGGTCATCCCGAATGTTTCCGCTGTT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	26	TTTCGATGATTGATCACGGTACATTTCCAT CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	27	GACATCGAATTTCTGGATCGACATCTGC CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	28	CGGTGTAGACGGTGTCAAGGCAGCCAG CCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	29	CCATCTTACGTTTCTCTTTTCCGAGAA GTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	30	AGGCGTTCCTGTTTCGTCGAAGGGTGC ATCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	31	CCAGAGGACTTGATGACGTATTGAACG ACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	32	TAGCGAACTCGTTTCGATGAAAGTCG GAAGCAGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	33	AGGAGCTGAAGACGTGTGTTGAAGCA ATGGAGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	34	AACAGTCATCAGGTTGTCATCGCGAC ACACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	35	ATTTGACGAAGAACTCCACGCACTA ATTGGTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	36	TCAACAACTTCTGGATGATGAAATTT CCGGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	37	CACTGCGGCAAAGACTGGTGAAGAC AGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	38	TTGTCAAAGAGCACAAAGTGCATCTC GTTATCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	39	TGACTCCAGTTCTATCCATAGCGAA ATCGACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	40	AGCGATCCGTTGCTAACAACATCTT GGAGACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	41	ACACGGATCACTCGACTTGGCAA ATGGATCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	42	TCCCATGAAGTTGAACAGAGGA ATGTTGGACGCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	43	GAGAATGCACCTCCATCGAAGG ATCCGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	44	CAGAGGAAAAACGCTCAAGGCGG CCGGAACTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	45	AGCAAGAGAGGTGGAGCAAAGAG AAAAGCTCCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	46	TGATCGGTGCAAAGTGGCAGAA ATCAAGACTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	47	TCGTTTCCAACGGAAAAATCGG CAAGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>puf-5</i>	48	GACAGCCCAGGCAGGCAAAAGACGG GCCTCCTAAGTTTCGAGCTGGACTCAGTG

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Table S5. Sequence of probes against *puf-5*. Primary probes carried the FLAPx extension shown in blue.

mRNA	Probe	5' - probe - FLAPx - 3'
<i>tbb-2</i>	1	AGTGTACCAATGGAGGAAAGCCTTGCGGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	2	GTTCCGGGATCCATTCGACGAAGTAGGACGAGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	3	TGGGAATCGGAGACAGGTGGTAACTCCGGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	4	ACCGAGAATGAGCTCATGATTCTGTCTGGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	5	CACATTGTCGACAAGCTCAGCTCCTTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	6	GACAGAATCCATGGTTCCTGGTTCGAGATCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	7	CCAACACGGCGCGTGGCACATACTTTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	8	GTCGGAGATGACCTCCAGAATTTGGATCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	9	TTATTGCTCCGATTTCGTAAGTCTCTCCAGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	10	GGCAGTTGCTTCTTGGTATTGTTGGTATTCTGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	11	ATGAGATCGTTCATGTTGCTCTCGGCTTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	12	GTGAACTCCATCTCGTCCATCTCTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	13	AAGGTGGCAGCCATCTTGAGTCTCTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	14	GTGGGATGTCACAAACGGCGGTCTTGACGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	15	CTTGTTCTGAACGTTGAGCATTGCTCGTCACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	16	CTCTCTCATCGACATGCGTCCACGGACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	17	GTCTTGGGTCACAGGCAGCCATCATGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	18	CTTGGCATCGAACATTTGTTGGGTGAGCTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	19	CCCTTGGCGGAAAGTGGTGCGAATCCTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	20	AAGCTTGCAGGATCGGCATTGAGCTGTCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	21	GGTTGGGTTGGTGTAGTTTGGGGTACGGTAGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	22	ATGTCGTAGAGGGCCTCGTTGTCAATGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	23	TAGGTCTCATCGGTGTTCTCAACAAGCTGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	24	GGACGGAGAGGGTGGCATTGTATGGTCTACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	25	ACTGTGTCGGACACCTTTGGCGATGGTACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	26	TCTTCGCGGATTTTGGAGATGAGAAGTGTCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	27	TTCCAGATCCGGTTCCTCCTCCGAGACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	28	CTTCGGCTTCCTTGCAGATCACGTCACTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	29	TGTAGTGACCTTGGCCCAGTTGTTCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	30	CTCCGCTTTGTCCGAACACAAAGTTGTCAGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	31	GTTGTTGGCTTCGTTGTAGTAGACGTCAATGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	32	TCAAGCTGGAGATCCGTCTCTCCCTTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	33	ATTTGGTTTCCGCATTGTCCGGCTTGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	34	ATAGCGGCCACGGTCAGGTAACGTCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	35	CGACGGTAAGGGCACGGTAAGCCTGGGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	36	CGGAACAGCTGGCCGAATGGTCCAGAGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	37	CCTCAGCGTATCCGTGACGTGCTCTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	38	AGGTTCCGTCTGGCTGGATTCCGTGCTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	39	AACATAGCAGTGAAGTCTGAGATACGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	40	AGTGAAGACGTGGGAATGGAACCATGTTGACGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	41	ATGGTGAGGGATACAAGATGGTTCAAATCACCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	42	GTGAGTGAGTTGGAATCCTTGAAGACAATCGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>tbb-2</i>	43	GAAAAGCTCTTGATAGCGGTGAGTTTCCAACTCCTAAGTTTCGAGCTGGACTCAGTG

Table S6. Sequence of probes against *tbb-2*. Primary probes carried the FLAPx extension shown in blue.

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mRNA	Probe	5' - probe - FLAPx - 3'
<i>pccb-1</i>	1	CCAGGCGTAGTTGATGTCTCCACGAAGATCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	2	TTTGATGACATGACATCGTAAGCTCCTCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	3	GAGTAATGATGGTAATCTTTGGAACGGTGGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	4	GCATCCAGCAGCAAACCTTTGGATTGTTCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	5	GAATCCAATGACCAAGTTCTTGGCGTAGTCTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	6	AGCGTGAACAACGTCCTTCATGTTGTAGGCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	7	GCAGCACGGATTGGAGCAGCATCAGTGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	8	TGAGCGACTCCAGAGGTGACAGTGTAGTCTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	9	GCTTTGACAACATCTGGGCCAGTGTAACTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	10	CGAGTAGACGGCTCCTCCAGCACATGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	11	AAGCCATCACGTTCTCTTGAAGATCGGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	12	CCGAGACTGGGCTCCGACAAGCATCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	13	CAGGATATTTCTCTTTCTGCATACCGAAGTCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	14	TGCAGTGTGCTCGGCAAACATGTCATACTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	15	GCAACCTTGATGGTATGAGCGATCGAAGTGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	16	TTAGAGTGGAAATGTTCCGTGCTTCTCCATCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	17	GTTCTTCAACTTCTTCTTCAAGCATGTTACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	18	GCGGCTGGGAACGGGTTGCTGAACAGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	19	CAGTGTATTCCTCCTCGTGTGAACGGCGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	20	CTCCCATCACAGCAACCTCAGCAGTTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	21	GATGATACCTCCGACTCTTGGCAGTTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	22	CAATTCGACAGTGCCTCCGTTTATTCTGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	23	TGGAACAGCTCTGTCCCATGGATCCTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	24	CTCCTGGGTGACCTCCTCGTTGGTAACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	25	TACCCAGCAAGCGACTCAACTCCTTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	26	GGATACGGGCACCTCCGGAGTCATTGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	27	ATTTCTCCACGTCCAGTGACAACCGAATCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	28	TGTTAGCTTTCCGCGAGCATGTTGAGCGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	29	GACGCGCTTCTTTCCACCTCCGAGAACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	30	GCTTTCTCTCTGGTCTCATCGATCTTATCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	31	CTCGCACACCTTCTTTCTGGTCTCTGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	32	GGAATGATAATGTCGTGACGAATCCTCTGACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	33	TGGCAAGAAACCGGGAACGTCAACCAAAGTGACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	34	ATTGGGATGTTGAAAGCATCGCAGAATCGGACCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	35	AACGAGCTCCTTTCACGGAAGAGTTGATATCACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	36	CATAATCTCGAAGAAATCTCCCTCGTCGACCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	37	CAGTGCTTTCCAATGGAACAACAGTATCCAAAACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	38	TGAGCGCATCAACATCGTTGTGCAAAGCACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	39	GGAGGTATCACGAACCATGAAAGTGAAAGTCGGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	40	CCCATAATCATAGAGATTTGAGGAACGACACCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	41	CCTCTCTCATGATCTTGACGATCTTTTTGGCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	42	TATCATTACGGAAGAAAGATGGAGACTGCACCCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	43	GGCAAAAGCGTAGAGAAGTTTGGCTCCGTGACCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	44	AGTGGAAAGATAGTTGAAAAGCTCTCTGAGGCTCCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	45	TGAATGGATGACAGAGATCCTCCGAAAACAGTCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	46	GTCTTGGGAGAAAACAAAGACGGTACGTCCGTCTCCTAAGTTTCGAGCTGGACTCAGTG
<i>pccb-1</i>	47	TTCTATCCAAAAGAAGATCAATGCGTTCGCGACCTCCTAAGTTTCGAGCTGGACTCAGTG

Table S7. Sequence of probes against *pccb-1*. Primary probes carried the FLAPx extension shown in blue.

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Fluorophores	Complementary FLAPx, 5' -> 3'
Cy3	/5Cy3/CACTGAGTCCAGCTCGAAACTTAGGAGG/3Cy3Sp/
Cy5	/5Cy5/CACTGAGTCCAGCTCGAAACTTAGGAGG/3Cy5Sp/

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1601 **Table S8.** Sequence of complementary FLAPx probes. The probes are modified with two Cy3 or
1602 Cy5 moieties at 5' and 3'. Modification symbols are shown.

1603

1604 SUPPLEMENTARY DATA

1605

1606 **Data S1. RNA relative enrichment within oocyte P-bodies, related to Figures 1 and 2.**
1607 (separate file)

1608 RNA relative enrichments within oocytes P-bodies.

1609

1610 **Data S2. RNA-Seq abundances, related to Figures 1 and 2.** (separate file)

1611 RNA-Seq abundances of sorted P-bodies, whole animal pre-sorted extracts, and dissected
1612 oocytes.

1613

1614 **Data S3. Estimated condensed and dissolved mRNA copy numbers in oocytes, related to**
1615 **Figure 1.** (separate file)

1616 Transcriptome-wide extrapolation of the mRNA copy numbers condensed in cytosolic P-bodies
1617 and dispersed in the cytosolic phase.

1618

1619 **Data S4. GO analysis of P-body enriched and depleted mRNAs, related to Figure 2.**
1620 (separate file)

1621 Full list of GO terms found for P-body enriched and depleted mRNAs.

1622

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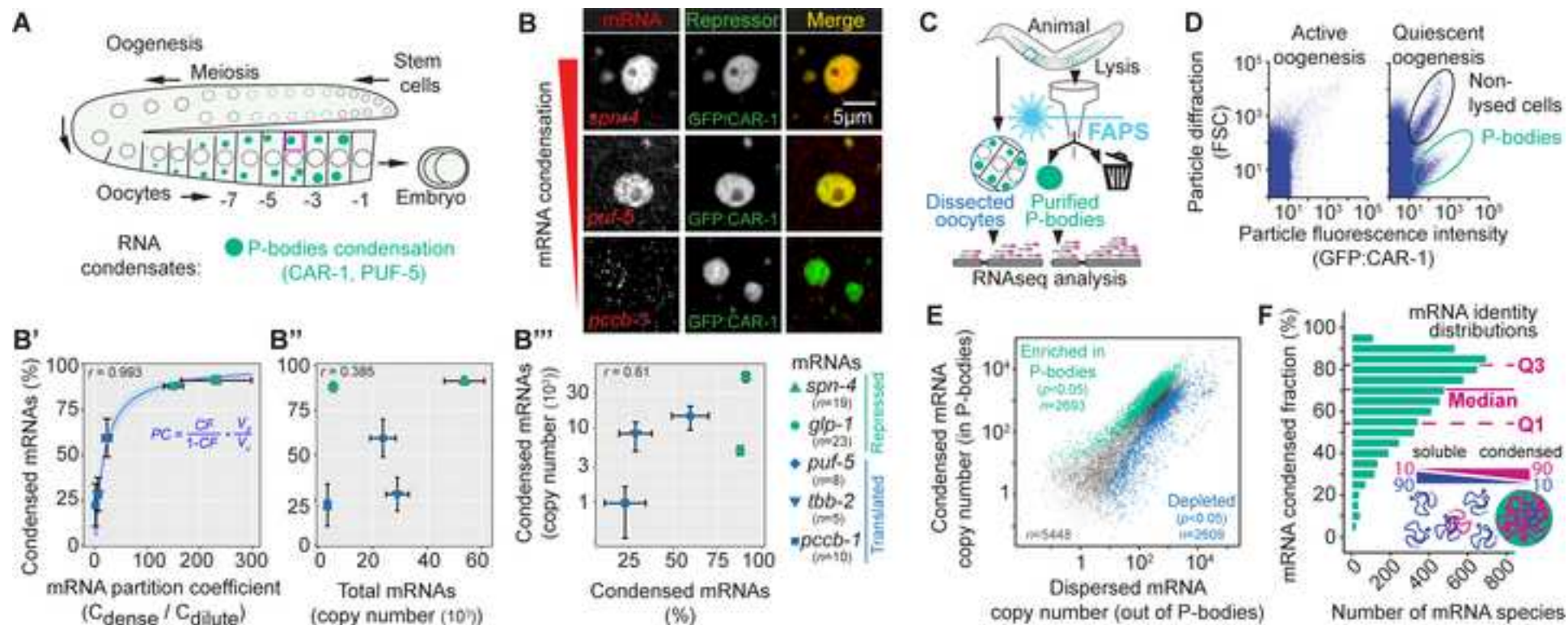
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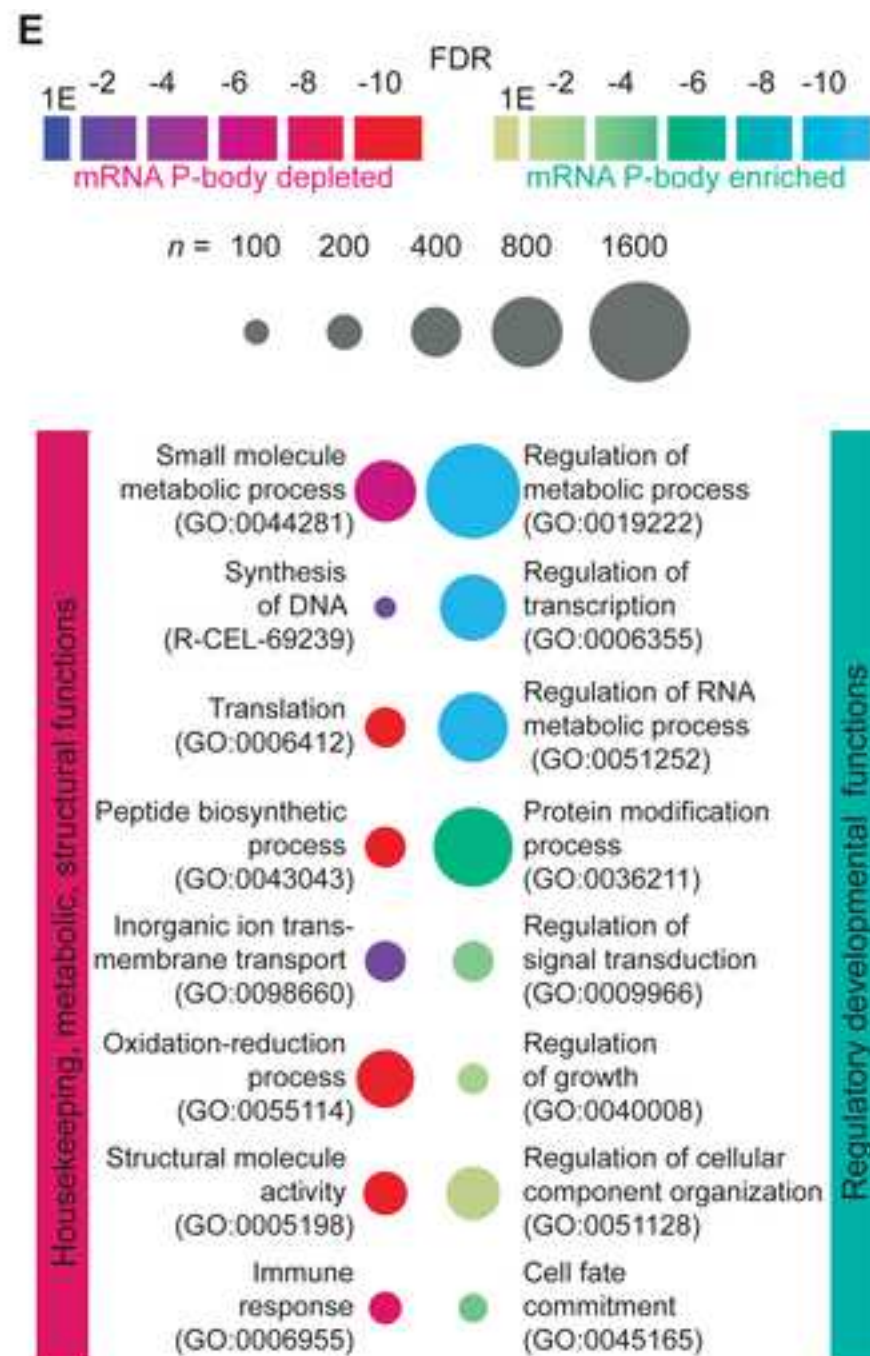
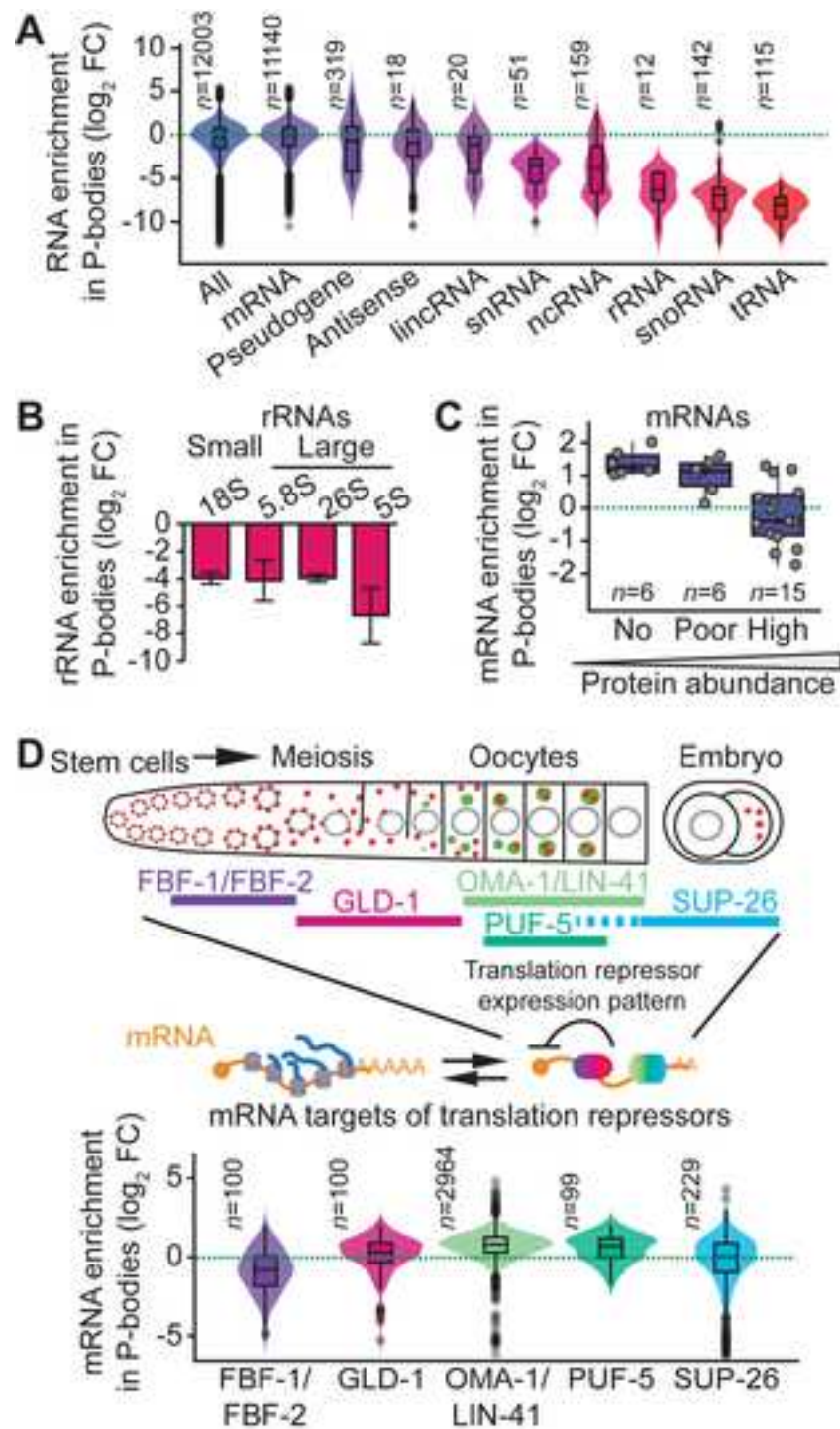
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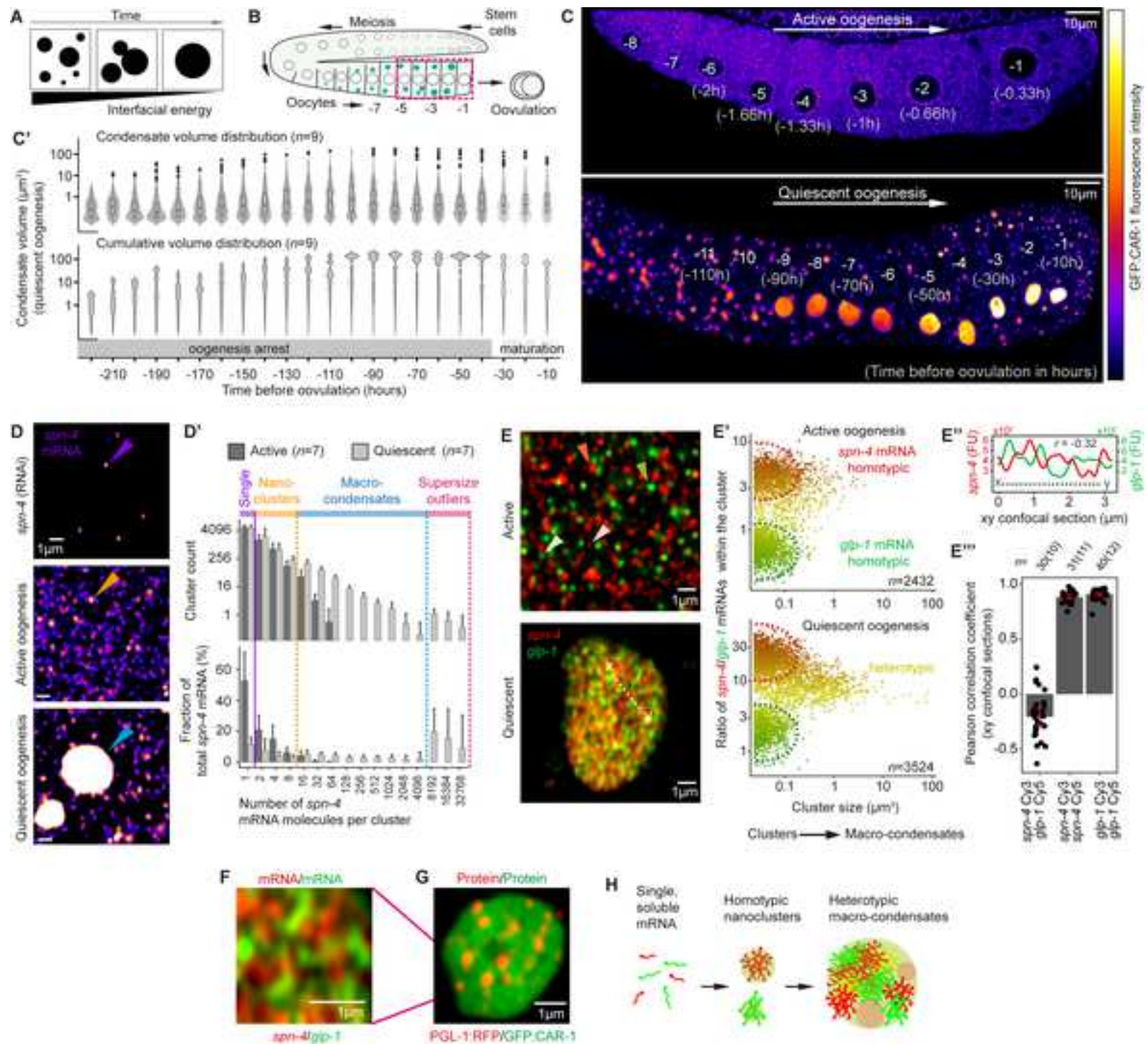
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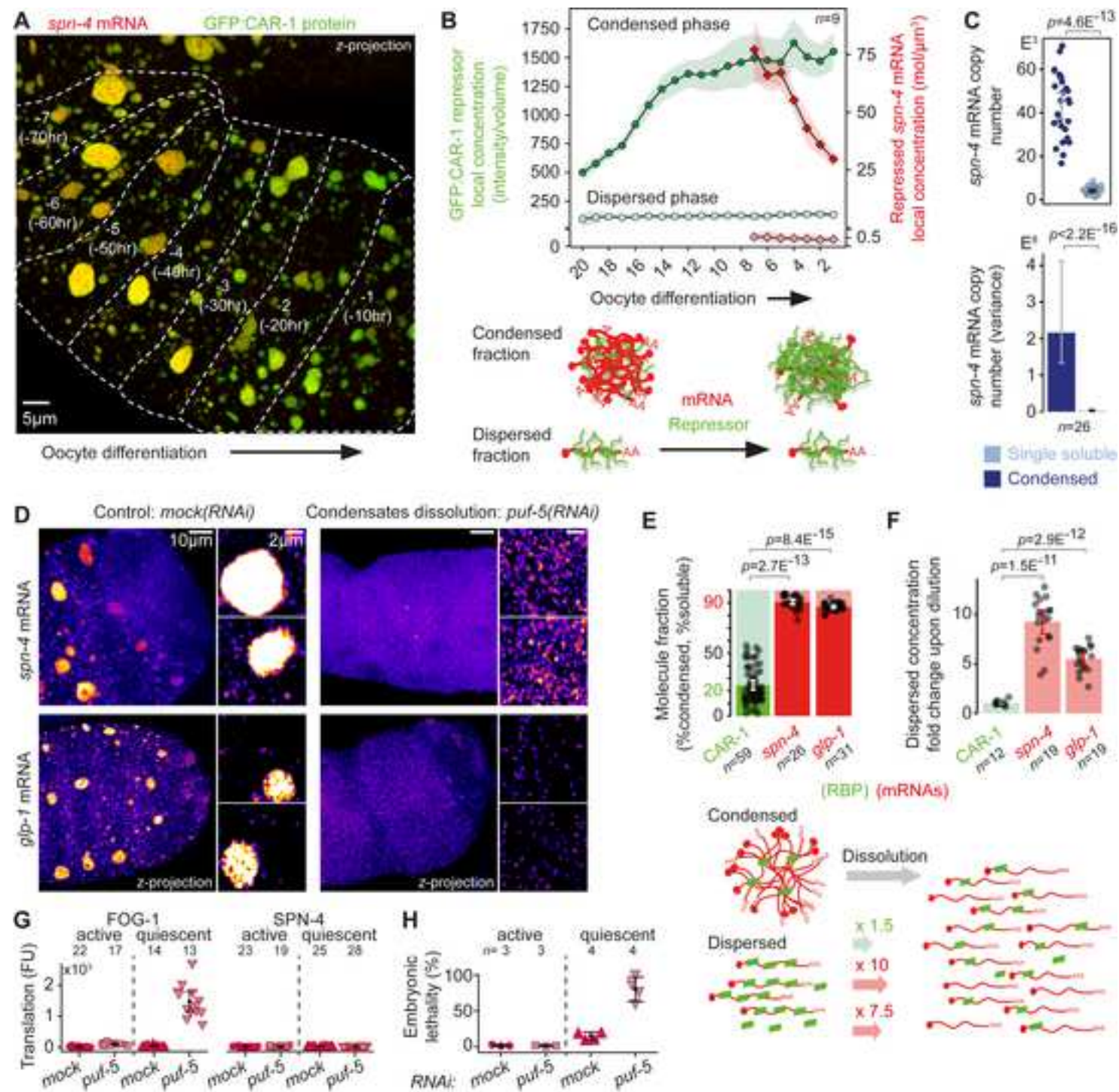
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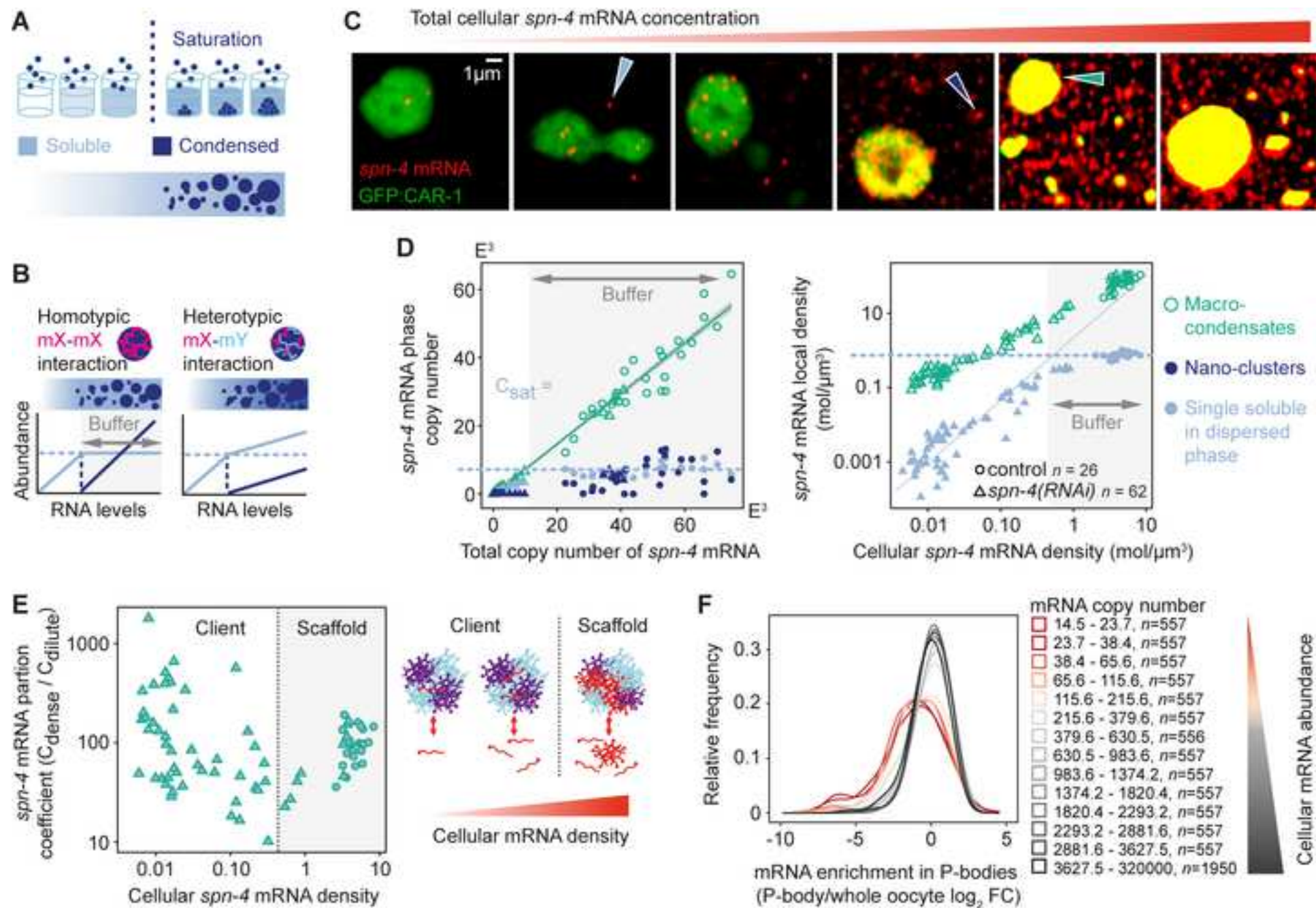
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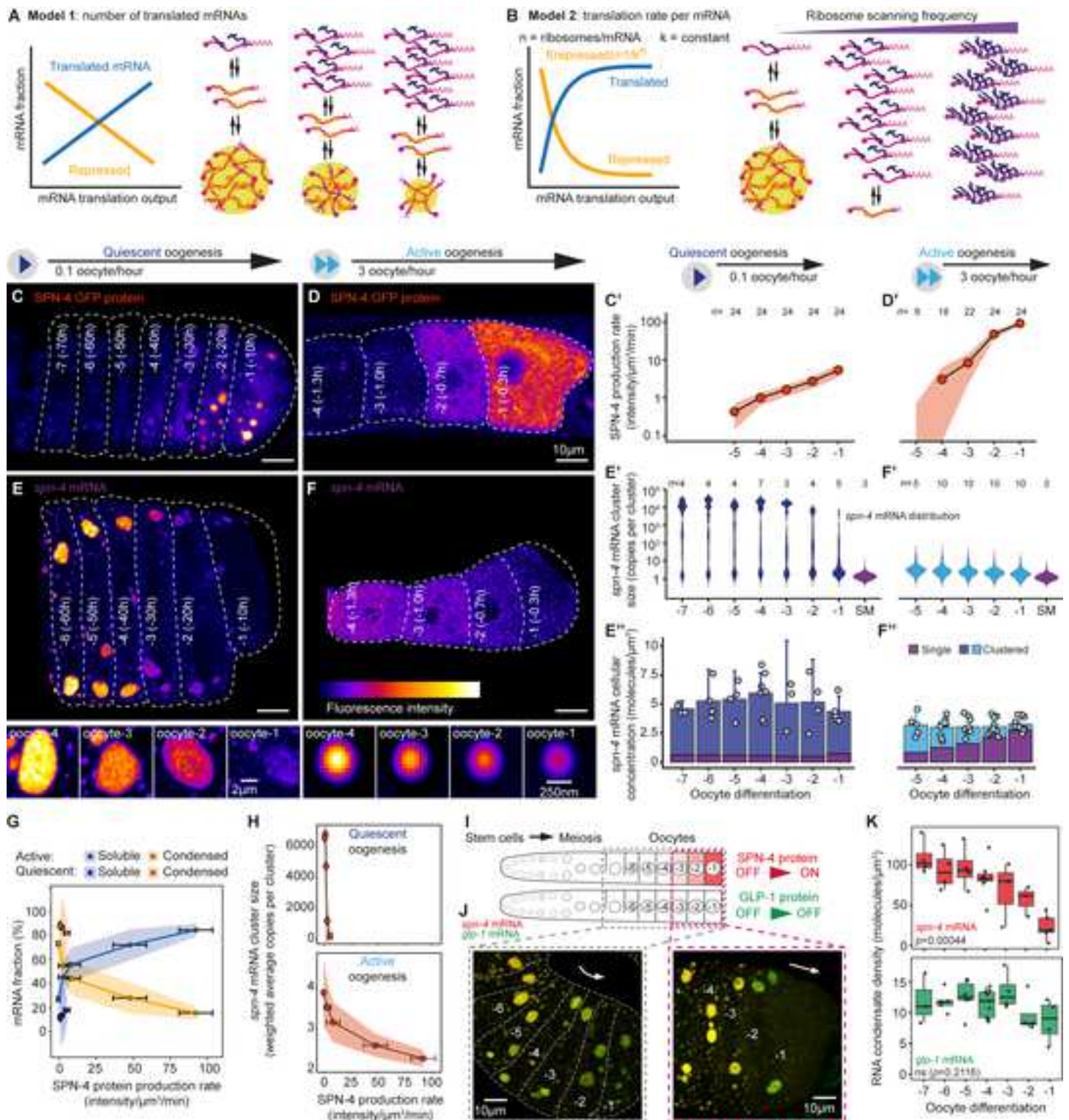


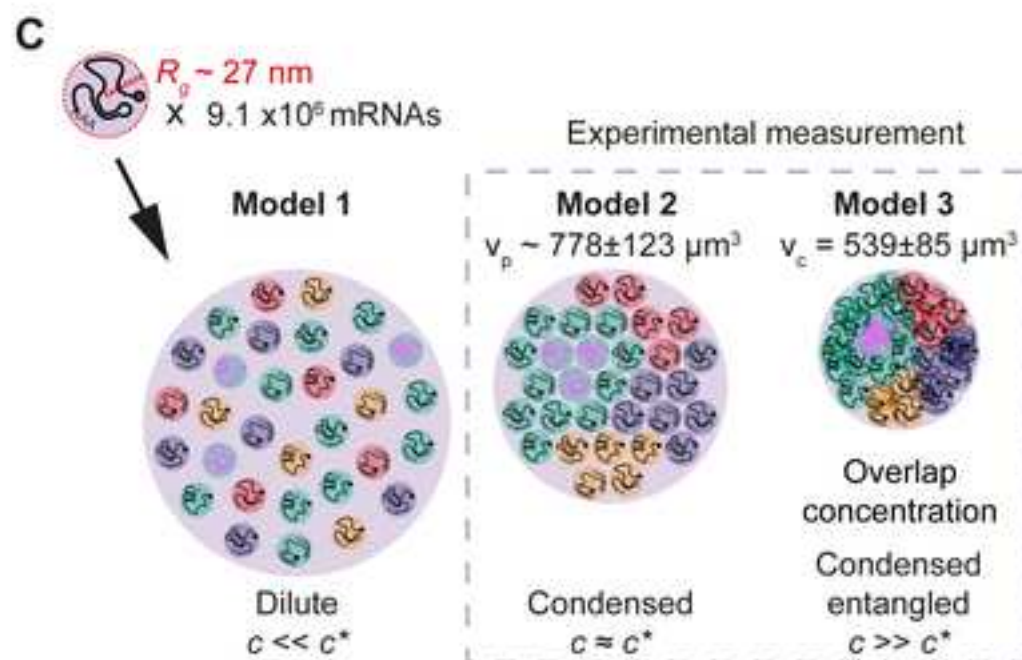
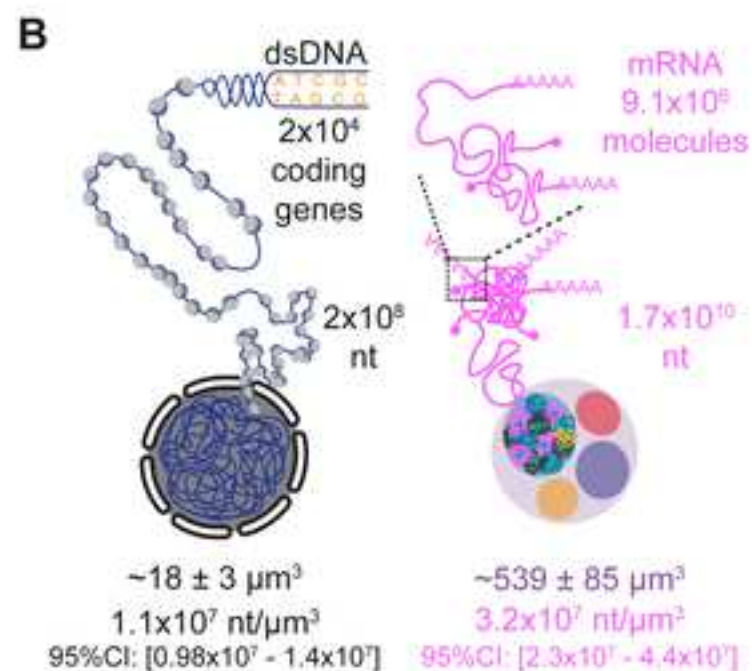
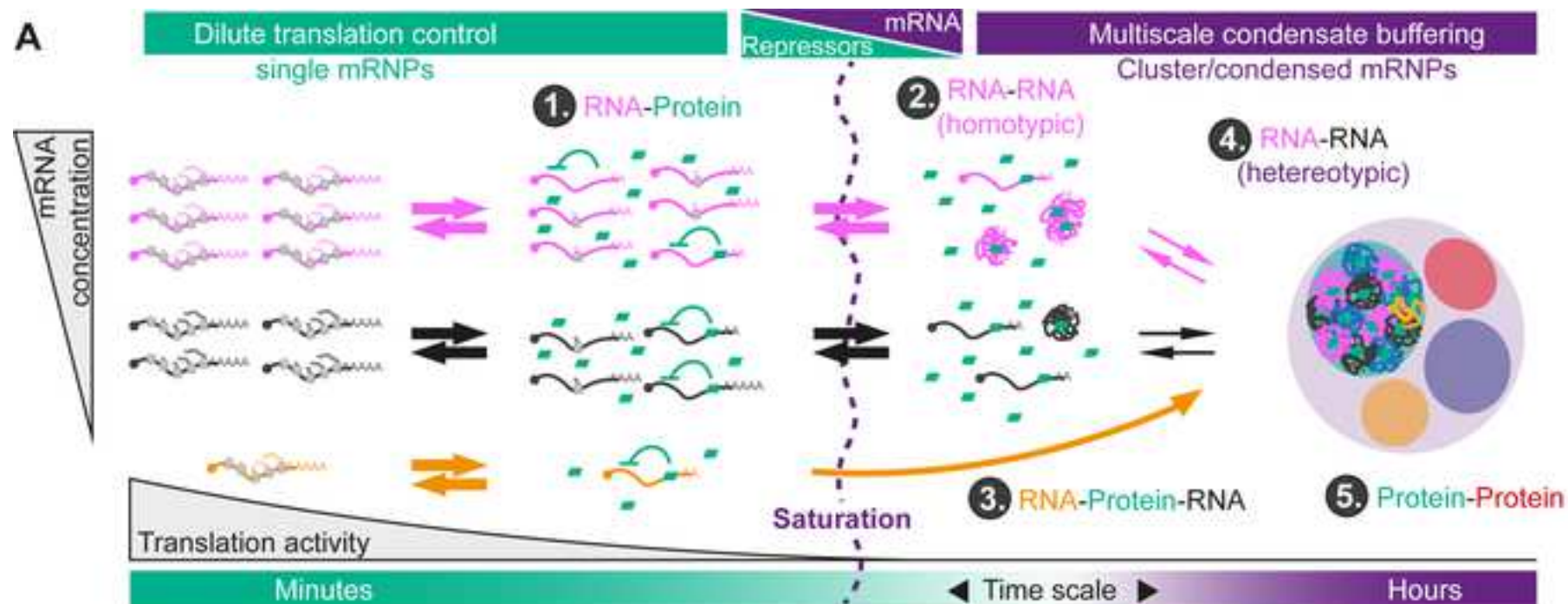


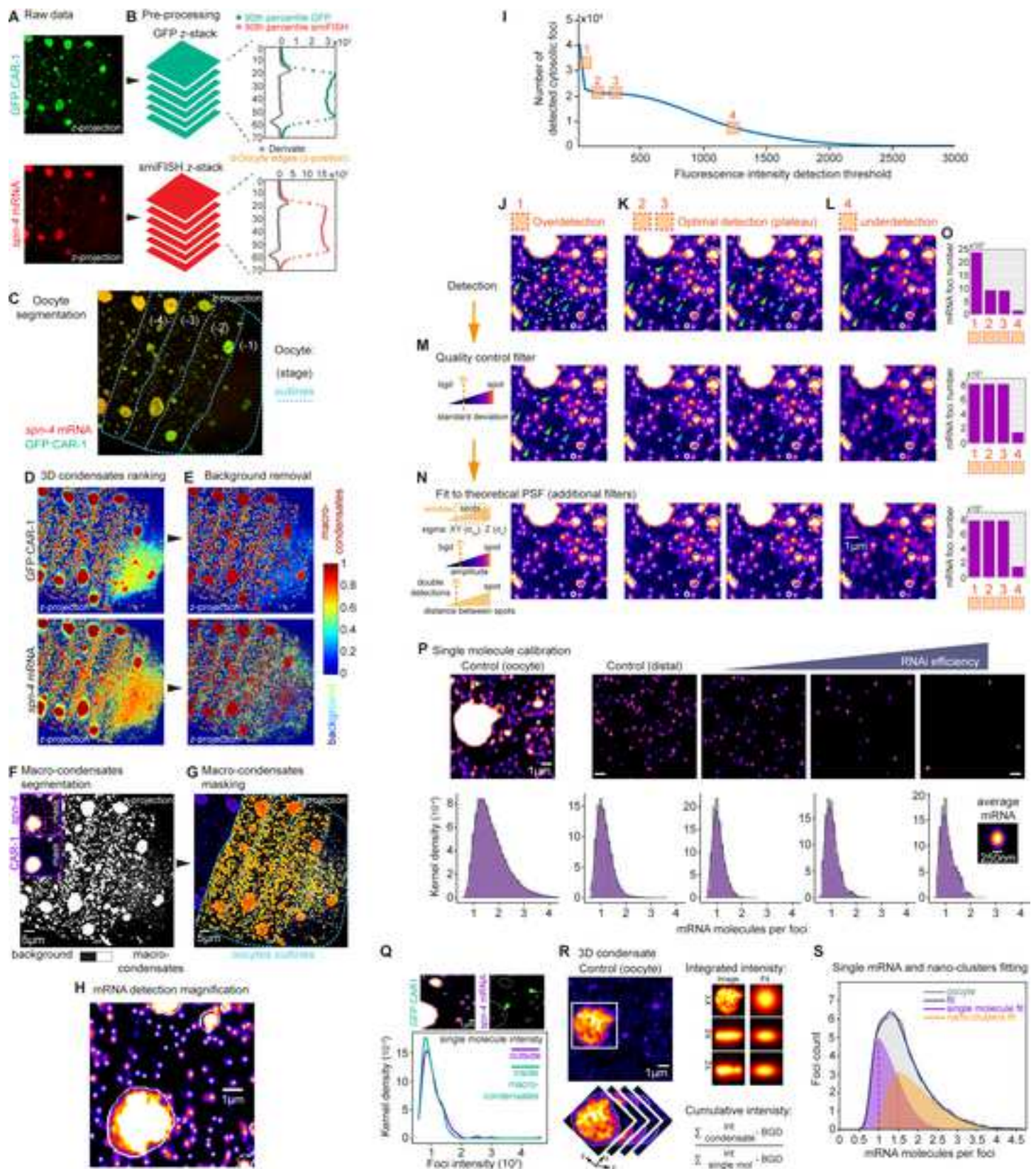


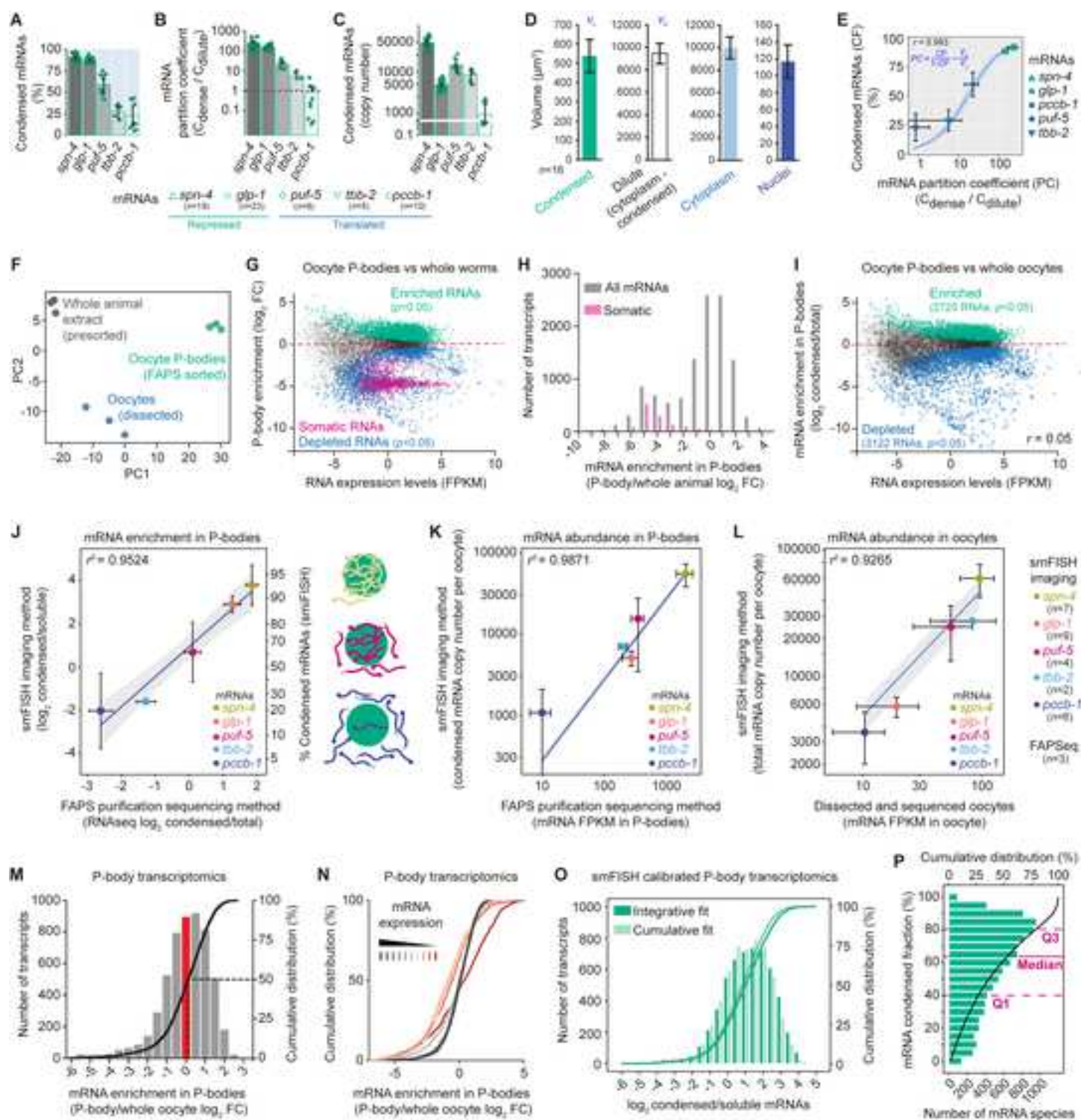


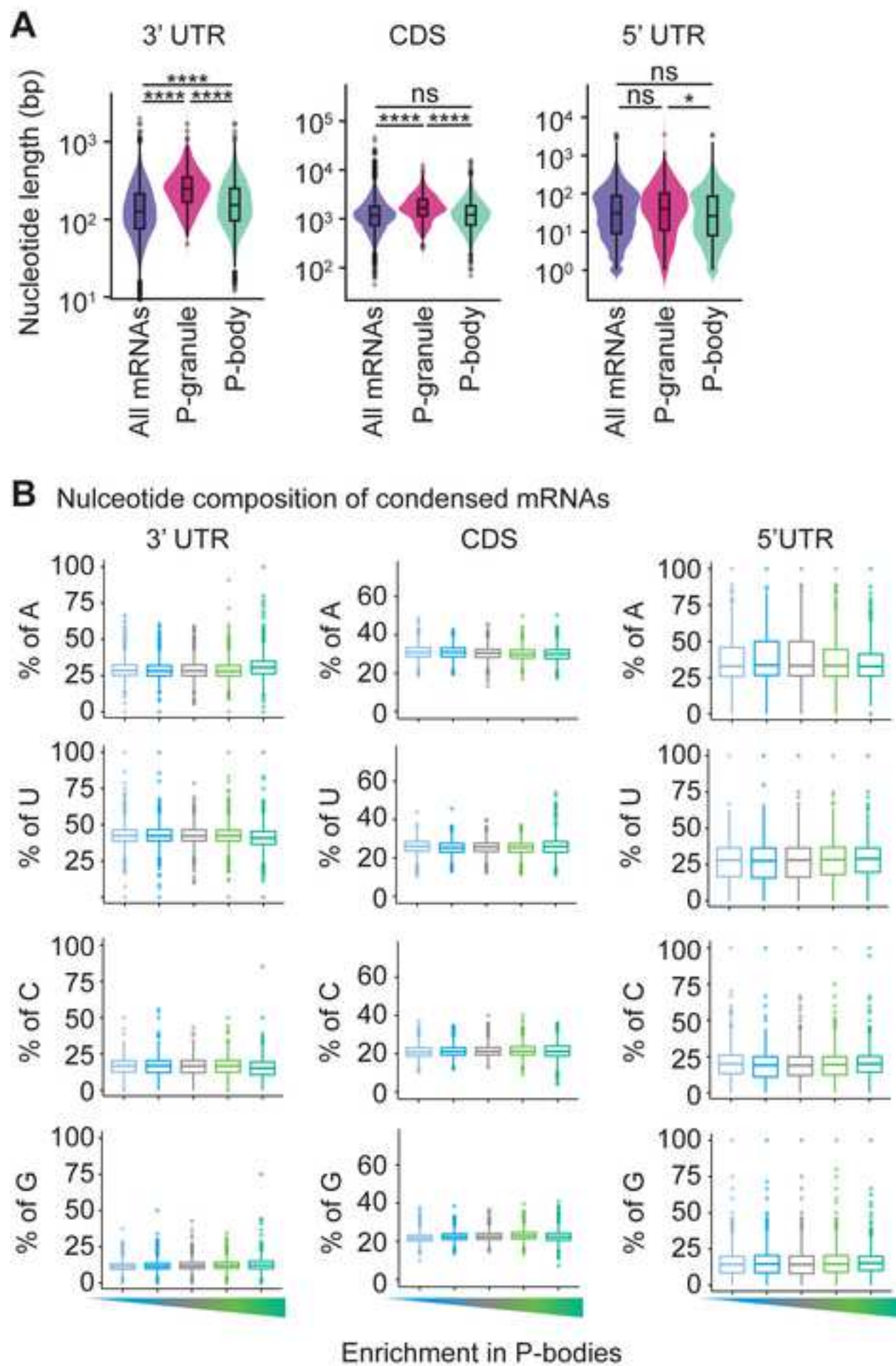


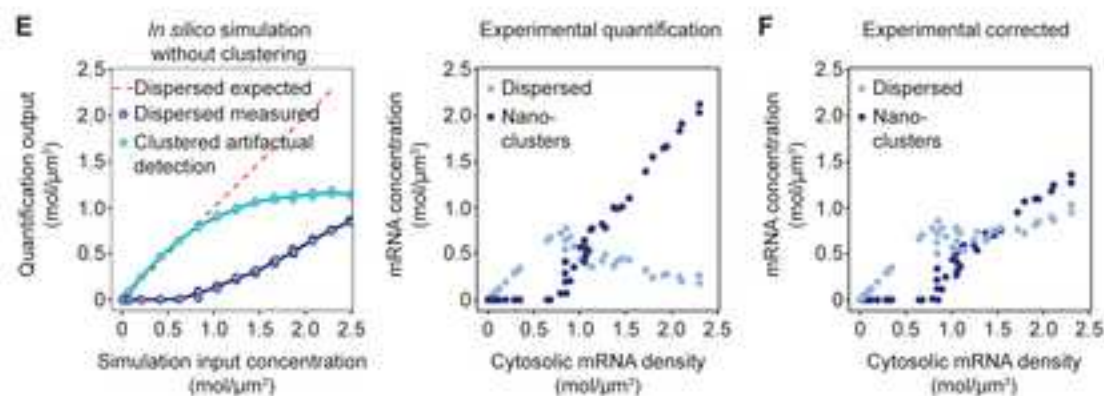
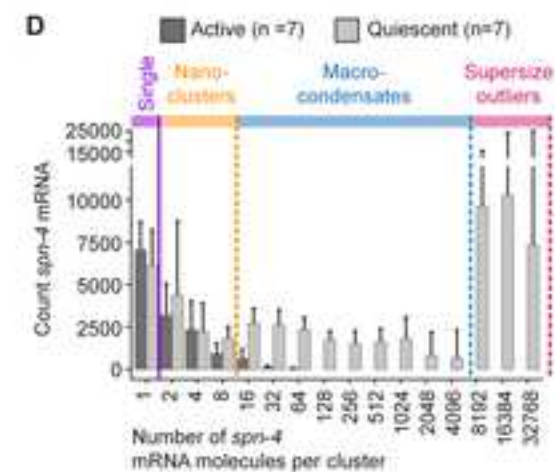
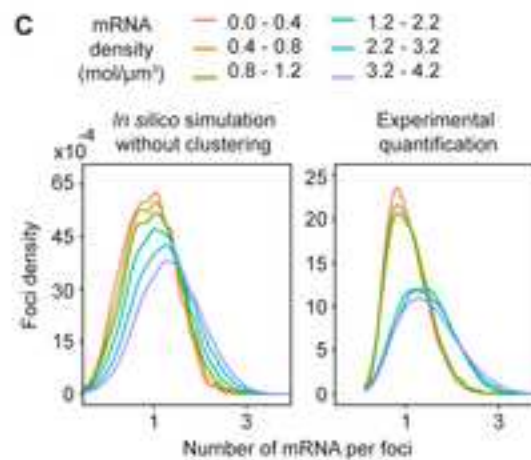
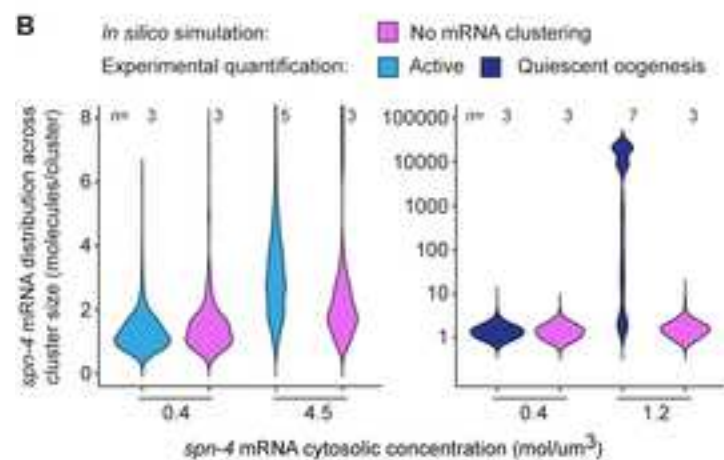
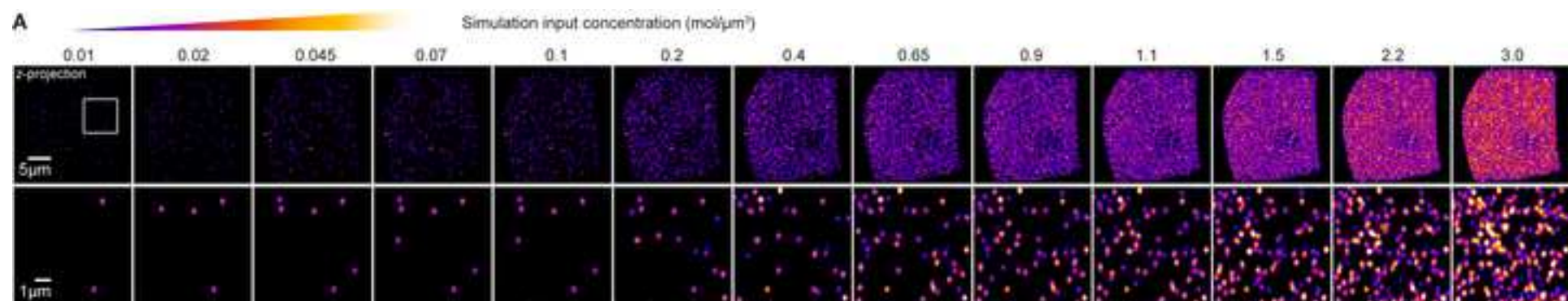


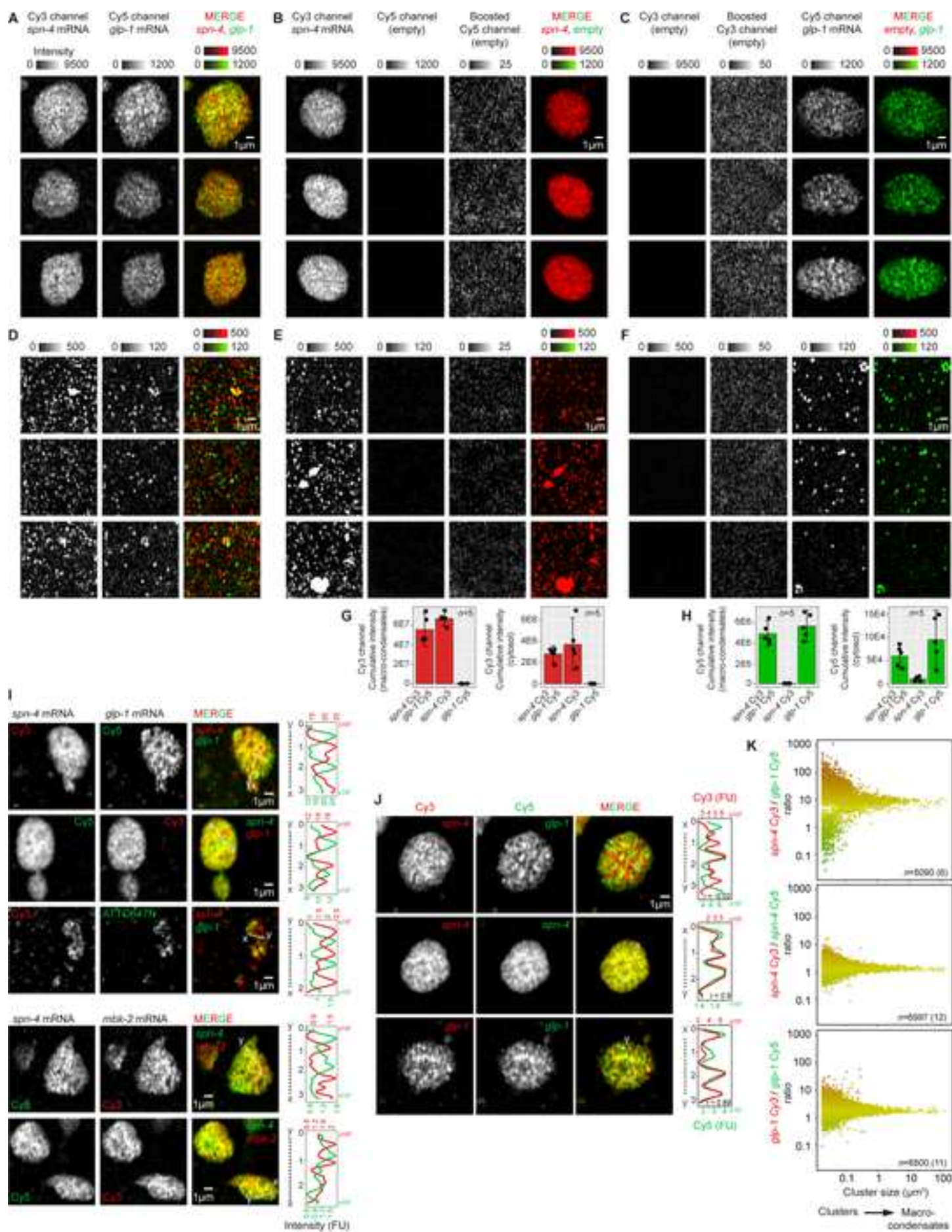


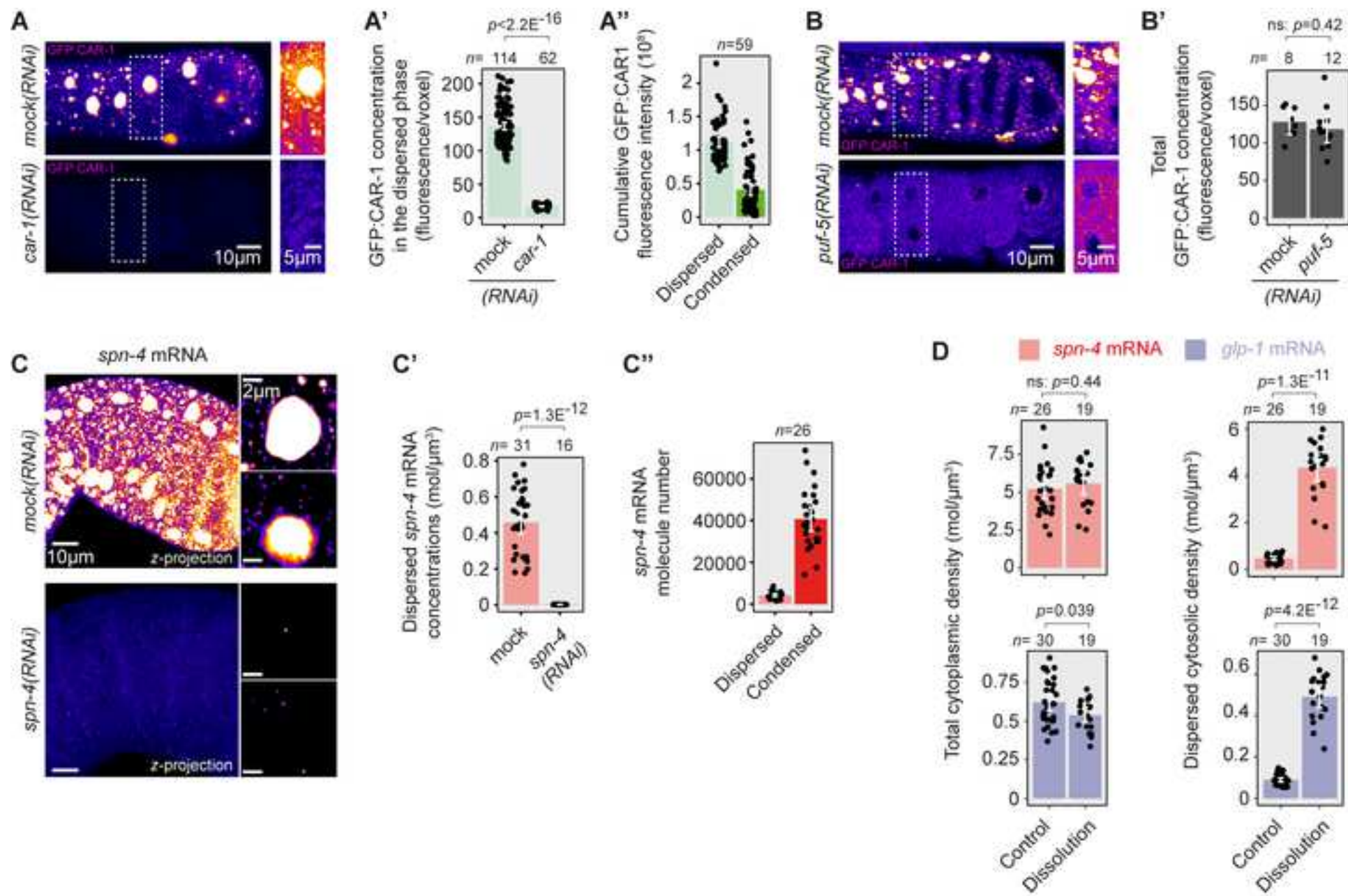


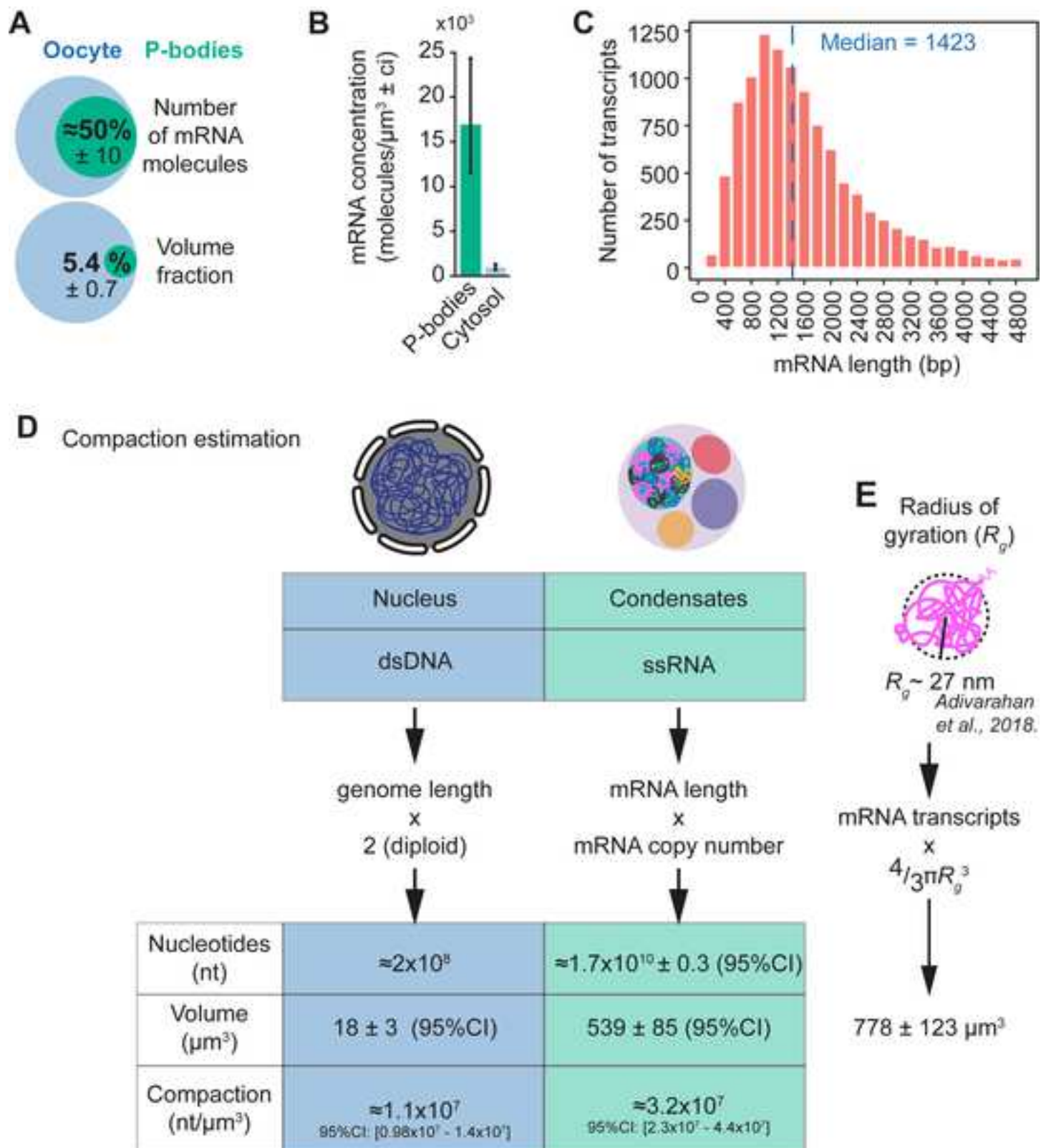














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