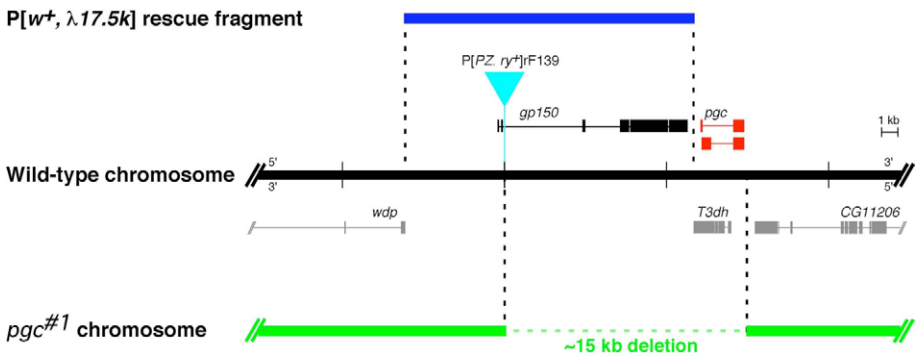
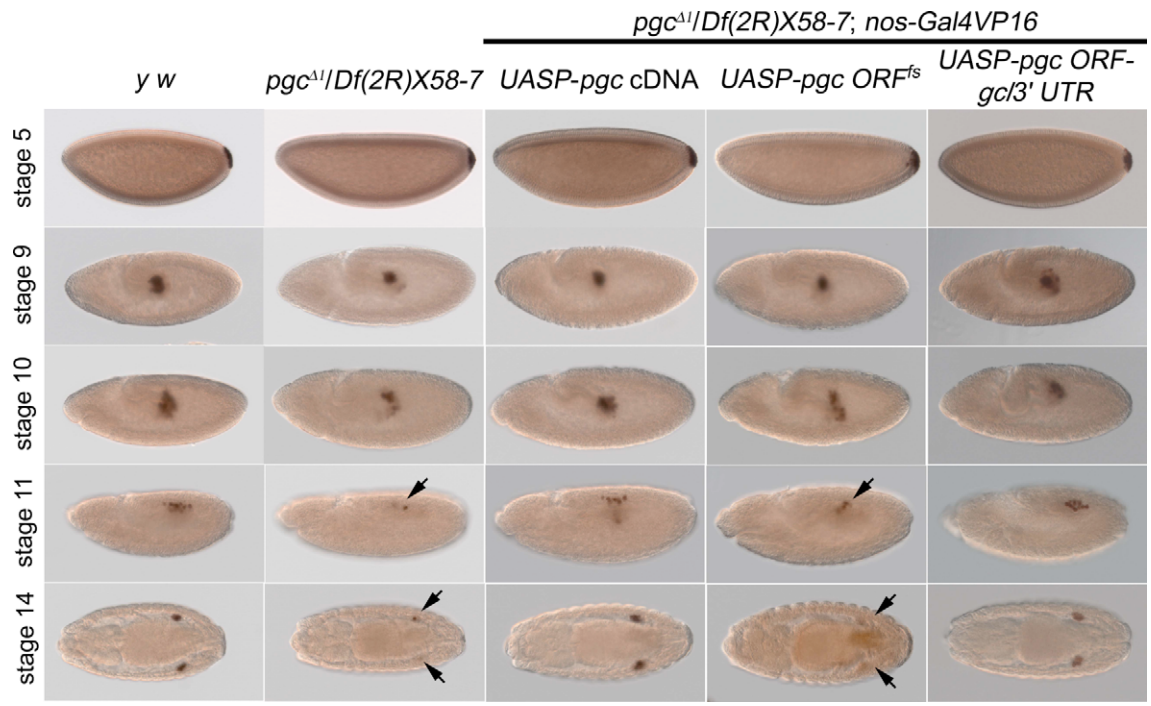
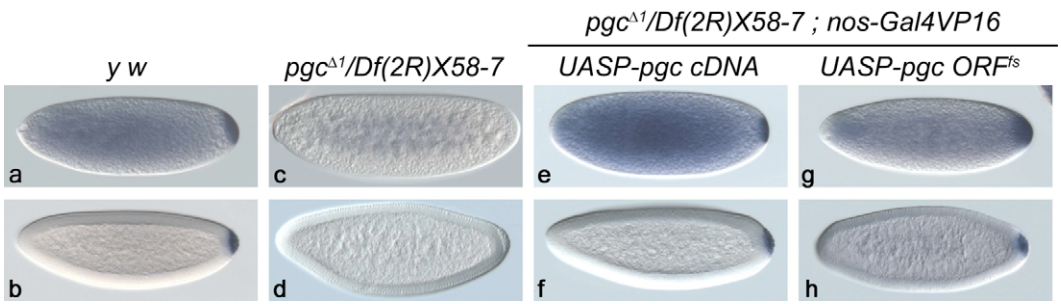


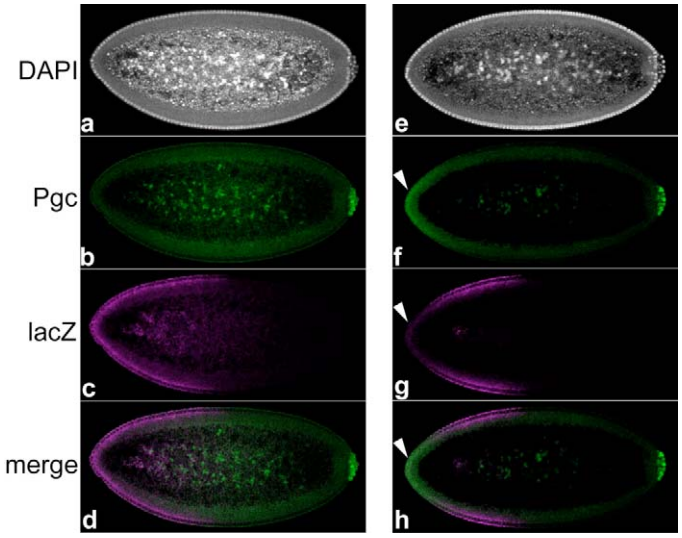
Supplementary Figure 1 | **Generation of *pgc*-null mutant.** Schematic diagram of the *pgc* genomic region. The *Type 3 alcohol dehydrogenase* (*T3dh*) gene is nested in the opposite strand, in the intron and the first exon region of the *pgc* locus. Imprecise mobilization of a P-element, *rF139* (triangle), generated a ~15-kb deletion that completely removed the *pgc* locus ($pgc^{\#1}$; green line). *gp150* was rescued by introducing a ~17.5-kb genomic fragment (blue line) into the $pgc^{\#1}$ chromosome, thereby generating $pgc^{\Delta 1}$, a chromosomal null for *pgc* and *T3dh*. The maternal-effect defects in $pgc^{\Delta 1}$ mutant were fully rescued by *pgc* cDNA expression in oogenesis (Fig. 1c and Supplementary Fig. 2), indicating that the nested gene, *T3dh*, is dispensable for normal development, and that defects observed in pgc^{-} embryos are caused solely by the loss of *pgc* function.



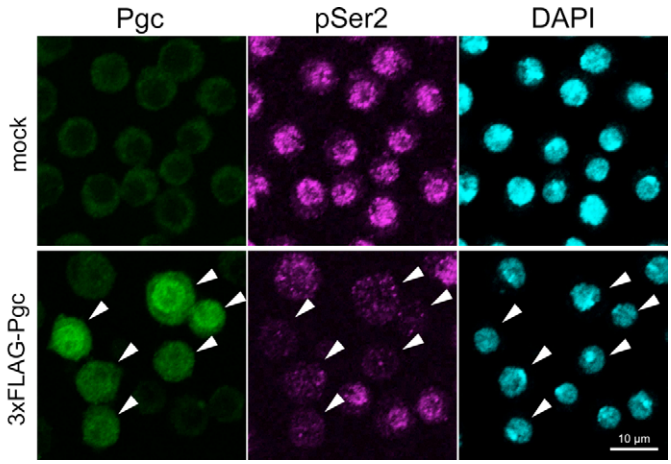
Supplementary Figure 2 | **Pgc is required for pole cell survival.** Embryos were immunostained for Vasa, a marker for germ cells. Maternal genotypes are indicated. In all images, anterior is to the left. Even in the absence of Pgc expression in pole cells (*pgc*$^{\Delta 1}$*/Df(2R)X58-7* and *pgc*$^{\Delta 1}$*/Df(2R)X58-7*; *nos-Gal4VP16/UASP-pgc ORF*^{fs}), normal numbers of pole cells were formed initially (stage 5). However, these *pgc*$^{-}$ pole cells degenerated during mid-embryogenesis (stage 11, arrows), and few or no pole cells coalesced into the embryonic gonads (stage 14, arrows). These defects were rescued by the expression of *pgc* cDNA or the *pgc ORF-gcl 3′ UTR* transgenes.



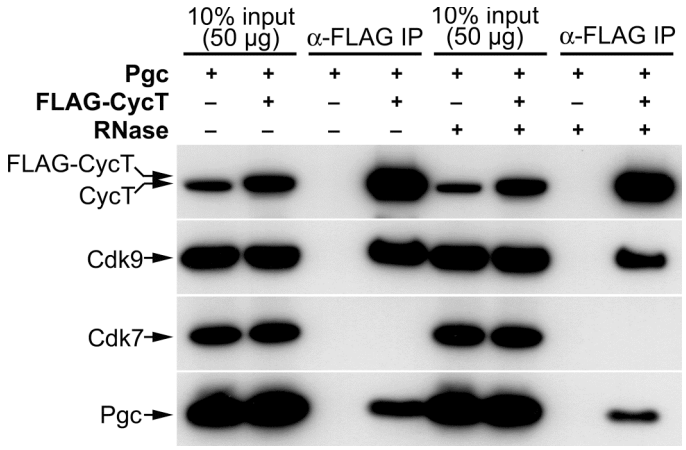
Supplementary Figure 3 | *pgc ORF*^{fs} transcript is concentrated in the germ plasm. Whole-mount *in situ* hybridization with the antisense *pgc* probe was performed in embryos at the syncytial blastoderm (upper panels) and early cellular blastoderm (lower panels) stage. Maternal genotypes are indicated. In all images, anterior is to the left. *pgc* signals were concentrated in the pole plasm (**a, e**) and pole cells (**b, f**) in *pgc*$^{+}$ embryos, but were undetectable in *pgc*$^{-}$ embryos (**c, d**). *pgc ORF*^{fs} transcripts accumulated in the pole plasm (**g**) and were incorporated into the pole cells (**h**).



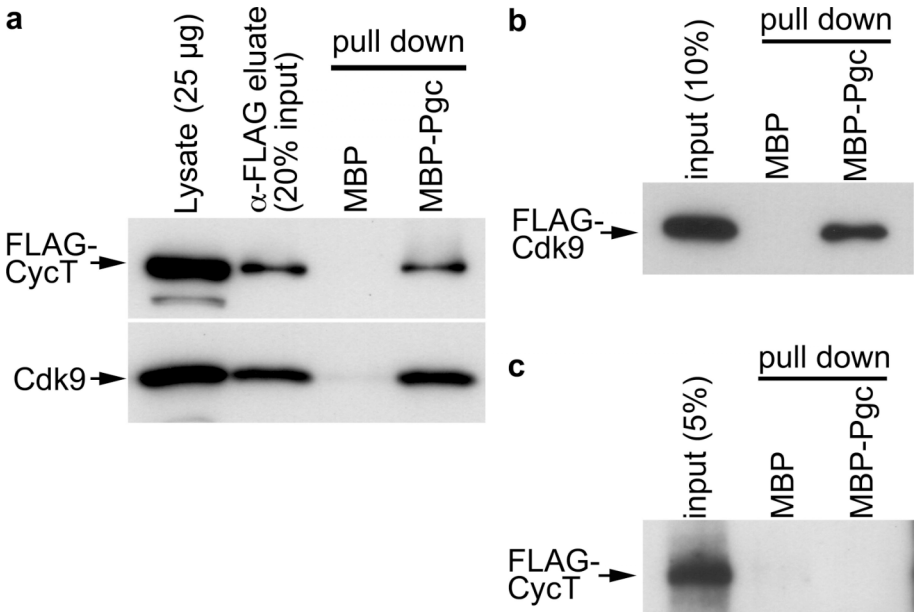
Supplementary Figure 4 | Anterior misexpression of Pgc causes repression of zygotic *hb* expression. a-d, A control embryo from *y w* females mated with *hb-lacZ* males. **e-h,** An embryo from *UASP-pgc ORF-bcd 3′ UTR*; *nos-Gal4VP16* females mated with *hb-lacZ* males. Anterior is to the left. Fixed embryos were stained for Pgc (green; **b**, **f**) and for β-galactosidase (magenta; **c**, **g**). Merged images are shown in **d** and **h**. *The* embryos were counterstained with DAPI to visualize the nuclei (**a**, **e**). Zygotic expression of the *hb-lacZ* reporter was repressed at the anterior region, where ectopic Pgc was detected (arrowheads in **f-h**).



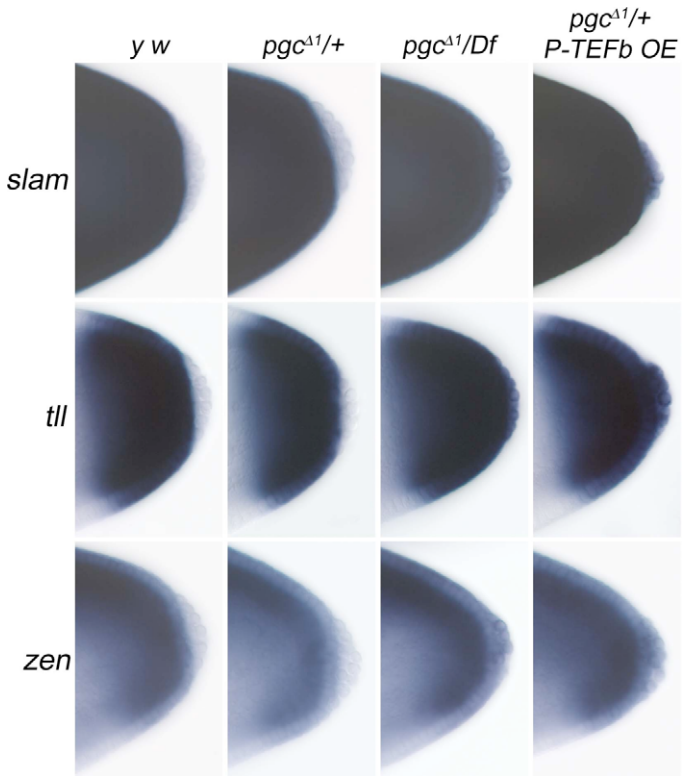
Supplementary Figure 5 | Pgc expression in *Drosophila* S2 cells represses CTD Ser2 phosphorylation. Mock- or pMT-3xFLAG-Pgc-transfected S2 cells werecultured in the presence of 0.5 mM $CuSO_4$ for 8 hours and immunostained for Pgc (green) and pSer2 (magenta). Nuclei were counterstained with DAPI (cyan). Expression of Pgc repressed CTD Ser2 phosphorylation in S2 cells (arrowheads).



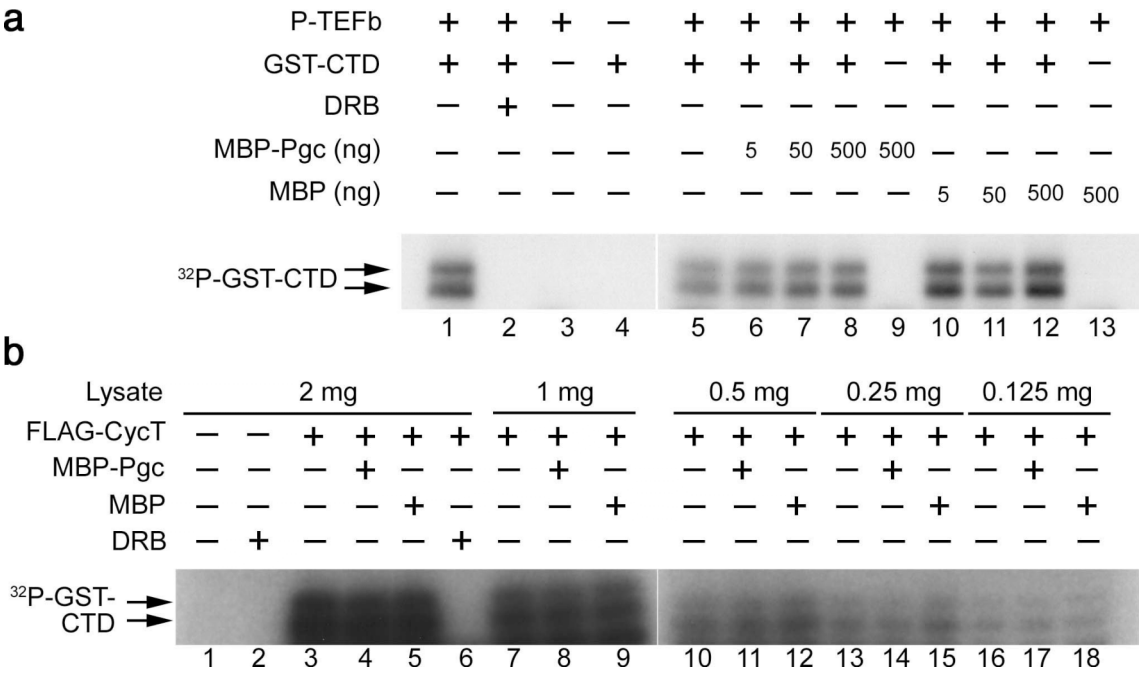
Supplementary Figure 6 | **Pgc forms a complex with P-TEFb.** Lysates of S2 cells expressing FLAG-tagged CycT and non-tagged Pgc were immunoprecipitated with anti-FLAG antibody, and bound proteins were analyzed by western blotting. Pgc and Cdk9, but not Cdk7, were co-immunoprecipitated with FLAG-CycT.



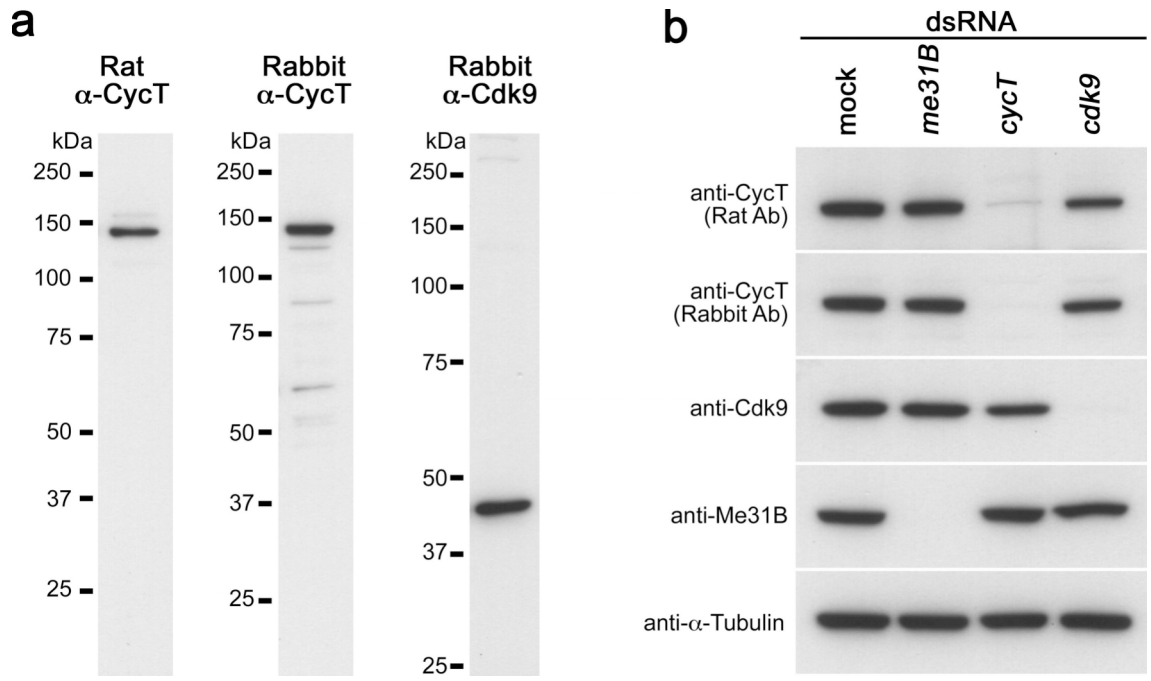
Supplementary Figure 7 | **Pgc interacts with P-TEFb *in vitro*. a,** MBP-Pgc pulled down P-TEFb complex that was partially purified from S2 cells expressing FLAG-CycT. **b,c,** MBP-Pgc pulled down *in vitro*-synthesized FLAG-Cdk9 (**b**), but not FLAG-CycT (**c**).



Supplementary Figure 8 | **Overexpression of P-TEFb promotes misexpression of somatic genes in pole cells.** Overexpression of P-TEFb in pole cells (*P-TEFb OE*) induced ectopic expression of *slam, tll* and *zen*, as in *pgc*$^{-}$ pole cells.



Supplementary Figure 9 | **Pgc alone fails to inhibit the kinase activity of P-TEFb *in vitro*. a,** A fixed amount of P-TEFb immobilized on anti-FLAG beads was incubated with GST-CTD in the presence of various amounts of MBP-Pgc or MBP protein. The immobilized P-TEFb phosphorylated GST-CTD (lanes 1 and 4). This phosphorylation was completely inhibited by 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) (lane 2), indicating that the kinase activity corresponded to that of P-TEFb. The ability of immobilized P-TEFb to phosphorylate GST-CTD was unaffected by MBP-Pgc (lanes 5-9), which binds Cdk9 *in vitro* (Fig. 4d). The addition of MBP to the reaction had no effect on the kinase activity (lanes 10-13). **b,** Various amounts of P-TEFb were immobilized on anti-FLAG M2 beads, and incubated with GST-CTD in the absence (lanes 3, 7, 10, 13, and 16) or presence of a fixed amount (1 μg) of MBP-Pgc (lanes 4, 8, 11, 14, and 17) or MBP (lanes 5, 9, 12, 15, 18). GST-CTD phosphorylation was completely inhibited by DRB (lane 6), indicating that the kinase activity corresponded to that of P-TEFb. Given the binding capacity of anti-FLAG M2 beads [6 μg (~120 pmol) bacterial alkaline phosphatase (50 kDa) per 10 μl beads, according to the manufacturer's data sheet], ~7.5 pmol P-TEFb, at a maximum, would have been pulled down in the samples in lanes 16-18 (we suspect that very much less than this amount of P-TEFb would have been immobilized). Since 1 μg MBP-Pgc (~50 kDa) is calculated to be 20 pmol, at least a 2.5 times molar excess of MBP-Pgc over P-TEFb was estimated to be present in the reactions in lane 17.



Supplementary Figure 10 | Specificities of newly raised anti-Cdk9 and anti-CycT antibodies. a, Rabbit anti-Cdk9, and rat and rabbit anti-CycT antibodies were used for western blot analyses of S2 nuclear lysates (10 μg). **b,** S2 cells were cultured in the absence or presence of the indicated dsRNA for 4 days, and the total cell lysates (10 μg) were analyzed by western blot.

Supplementary Table 1 | Effects of P-TEFb overexpression on pole cell development

Fly strain	Maternal genotype			Distribution of pole cell number per gonad									Agametic ovaries in progeny (n)
	pgc	*UAS-cdk9*	*UAS-cycT*	0	1	2	3	4	5	>5	Mean ± s.d.	(n)	
1	**+/+**	**-**	**-**	0	0	0	0	0	1	59	11.0 ± 2.3	(60)	0 (200)
2	**+/+**	**+**	**+**	0	0	3	6	4	2	42	7.2 ± 3.0	(57)	4.0% (400)
3	**+/-**	**-**	**-**	0	0	0	0	0	0	60	9.9 ± 1.6	(60)	0 (800)
4	**+/-**	**+**	**-**	0	0	0	0	0	1	39	9.7 ± 1.9	(40)	0 (800)
5	**+/-**	**+**	**-**	0	0	0	0	0	0	40	10.1 ± 2.3	(40)	0 (800)
6	**+/-**	**-**	**+**	0	0	1	1	1	1	36	9.3 ± 3.0	(40)	0.8% (800)
7	**+/-**	**-**	**+**	0	0	2	0	1	3	33	9.2 ± 3.2	(39)	1.6% (800)
8	**+/-**	**+**	**+**	12	7	7	6	10	9	18	3.9 ± 3.1	(69)	28% (800)
9	**+/-**	**+**	**+**	10	10	6	6	5	4	17	3.9 ± 3.7	(58)	27% (800)
10	**-/-**	**-**	**-**	33	21	11	6	5	4	0	1.3 ± 1.5	(80)	85% (480)

Females of the indicated genotypes were mated with *y w* males. Pole cell number in the gonads of stages 14-16 embryos was counted by anti-Vasa staining.

Maternal genotype of each strain is as follows:

Strain 1: *nos-Gal4VP16*/+

Strain 2: *UASP-cdk9-nos 3′ UTR*^{f5}/+; *nos-Gal4VP16*/*UASP-cycT-nos 3′ UTR*^{m6}

Strain 3: *pgc*$^{\Delta 1}$/+; *nos-Gal4VP16*/+

Strain 4: *pgc*$^{\Delta 1}$/*UASP-cdk9-nos 3′ UTR*^{f5}; *nos-Gal4VP16*/+

Strain 5: *pgc*$^{\Delta 1}$/*UASP-cdk9-nos 3′ UTR*^{f6}; *nos-Gal4VP16*/+

Strain 6: *pgc*$^{\Delta 1}$/+; *nos-Gal4VP16*/*UASP-cycT-nos 3′ UTR*^{m6}

Strain 7: *pgc*$^{\Delta 1}$/+; *nos-Gal4VP16*/*UASP-cycT-nos 3′ UTR*^{m7}

Strain 8: *pgc*$^{\Delta 1}$/*UASP-cdk9-nos 3′ UTR*^{f5}; *nos-Gal4VP16*/*UASP-cycT-nos 3′ UTR*^{m6}

Strain 9: *pgc*$^{\Delta 1}$/*UASP-cdk9-nos 3′ UTR*^{f6}; *nos-Gal4VP16*/*UASP-cycT-nos 3′ UTR*^{m7}

Strain 10: *pgc*$^{\Delta 1}$/*Df(2R)X58-7*

Supplementary Discussion

Two other maternal germ plasm proteins, Gcl and Nos, have been implicated in repressing CTD Ser2 phosphorylation during germ cell specification[34,35]. Gcl, in particular, was reported to be involved in the repression of CTD Ser2 phosphorylation in pole bud nuclei[34]. However, we noticed that the reactivity of the widely used anti-pSer2 antibody (H5) is highly sensitive to methanol, which is generally used for the devitellinization of fixed embryos. When we stained hand-devitellinized embryos that were not exposed to methanol with the H5 antibody, we consistently detected pSer2 signals in pole-bud interphase nuclei (Fig. 1b). We also confirmed the presence of pSer2 in pole-bud nuclei using another anti-pSer2 antibody (data not shown), indicating that the repression of CTD phosphorylation occurs only after the pole-bud stage in wild-type embryos. Furthermore, pSer2 is properly repressed in the few pole cells present in embryos lacking *gcl*[34]. Thus, we consider it unlikely that Gcl directly regulates CTD Ser2 phosphorylation in pole cells during normal development.

Although Nos was thought not to be involved in the repression of CTD Ser2 phosphorylation[2], a recent report argues that the loss of Nos in pole cells causes modest CTD Ser2 phosphorylation in stage-4 pole cells[35]. Notably, we found that in some pole cells in early stage-4 wild type embryos (nuclear cycle 10), CTD Ser2 phosphorylation was not fully repressed (Fig. 1b). We also found that Nos protein expression in stage 4-5 pole cells was not affected by loss of Pgc and *vice versa* (data not shown). Furthermore, Nos is a cytoplasmic protein[1], while Pgc concentrates in the nucleus (Fig. 1b and Supplementary Fig. 5). Thus, the contribution of Nos to the repression of CTD Ser2 phosphorylation, if any, must be independent of *pgc*.

Pgc fails to inhibit the kinase activity of P-TEFb *in vitro* (Supplementary Fig. 9), but instead prevents its recruitment to transcriptional sites (Fig. 4). We therefore

consider that transcription factors could compete with Pgc for P-TEFb recruitment, and that Pgc-mediated repression is not absolute. If sufficient amounts of such transcription factors were present in pole cell nuclei, Pgc would fail to sequester P-TEFb efficiently, resulted in the misexpression of specific sets of genes. Given that Nos is a translational repressor[1] and Gcl is a nuclear-pore associated protein[36], they might inhibit the production and/or nuclear import of specific transcription factors that promote mRNA transcription in a gene specific manner. According to this model, specific transcriptional factors are ectopically accumulated in pole cell nuclei in the absence of Nos or Gcl, and they outcompete Pgc for P-TEFb recruitment, promoting the misexpression of specific sets of somatic genes, such as *eve* and *ftz*.

References

34. Leatherman, J. L., Levin, L., Boero, J. & Jongens, T. A. *germ cell-less* acts to repress transcription during the establishment of the *Drosophila* germ cell lineage. *Curr. Biol.* **12**, 1681-1685 (2002).

35. Deshpande, G., Calhoun, G., Jinks, T. M., Polydorides, A. D. & Schedl, P. Nanos downregulates transcription and modulates CTD phosphorylation in the soma of early *Drosophila* embryos. *Mech. Dev.* **122**, 645-657 (2005).

36. Jongens, T. A., Ackerman, L. D., Swedlow, J.R., Jan, L. Y. & Jan, Y. N. *Germ cell-less* encodes a cell-type specific nuclear pore-associated protein and functions early in the germ-cell specification pathway of *Drosophila*. *Genes Dev.* **8**, 2123-2136 (1994).