Supplemental Information File

Proper CycE-Cdk2 activity in endocycling tissues requires regulation of the Cyclin-dependent kinase inhibitor Dacapo by dE2F1b in Drosophila.

Authors: Minhee Kim, Keemo Delos Santos and Nam-Sung Moon

Supplemental Information:

Figure S1

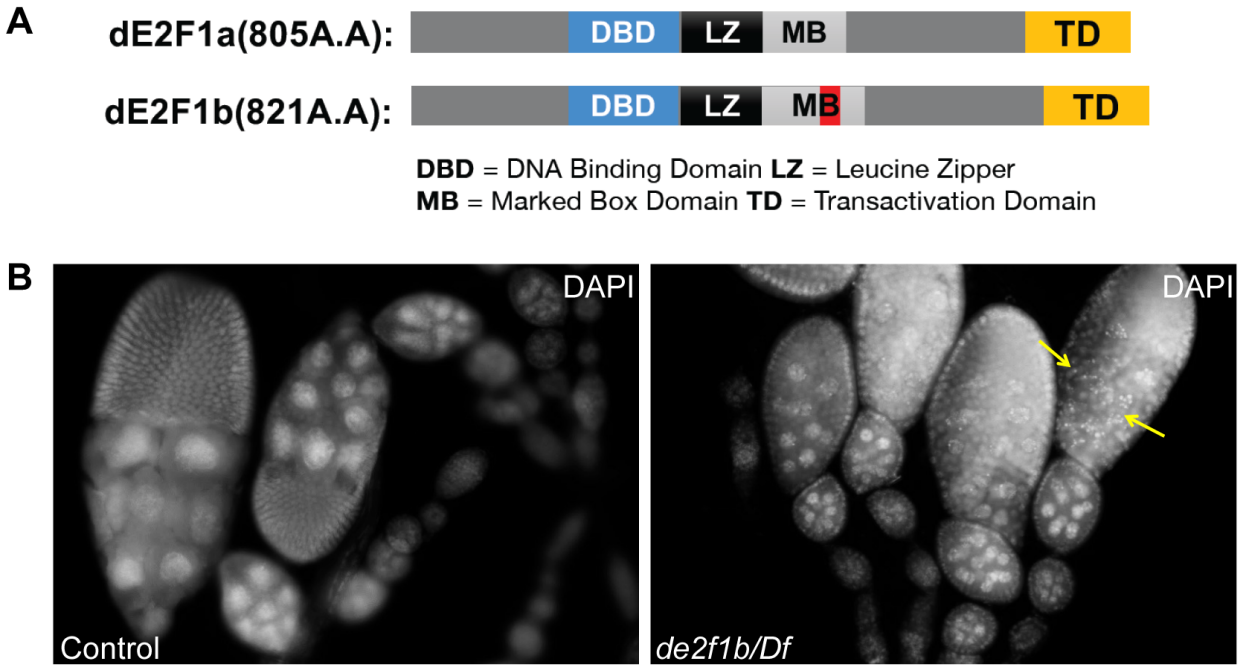

Figure S1. Oogenesis is impaired in *de2f1b* ovaries. (A) dE2F1b only differs from the canonical dE2F1a by a 16 amino acid insertion at the evolutionarily conserved Marked Box domain (red box). (B) Egg chambers stained with DAPI to visualize morphology in control and *de2f1b/Df* backgrounds. Yellow arrows indicate egg chambers with abnormal NC morphology.

Figure S2

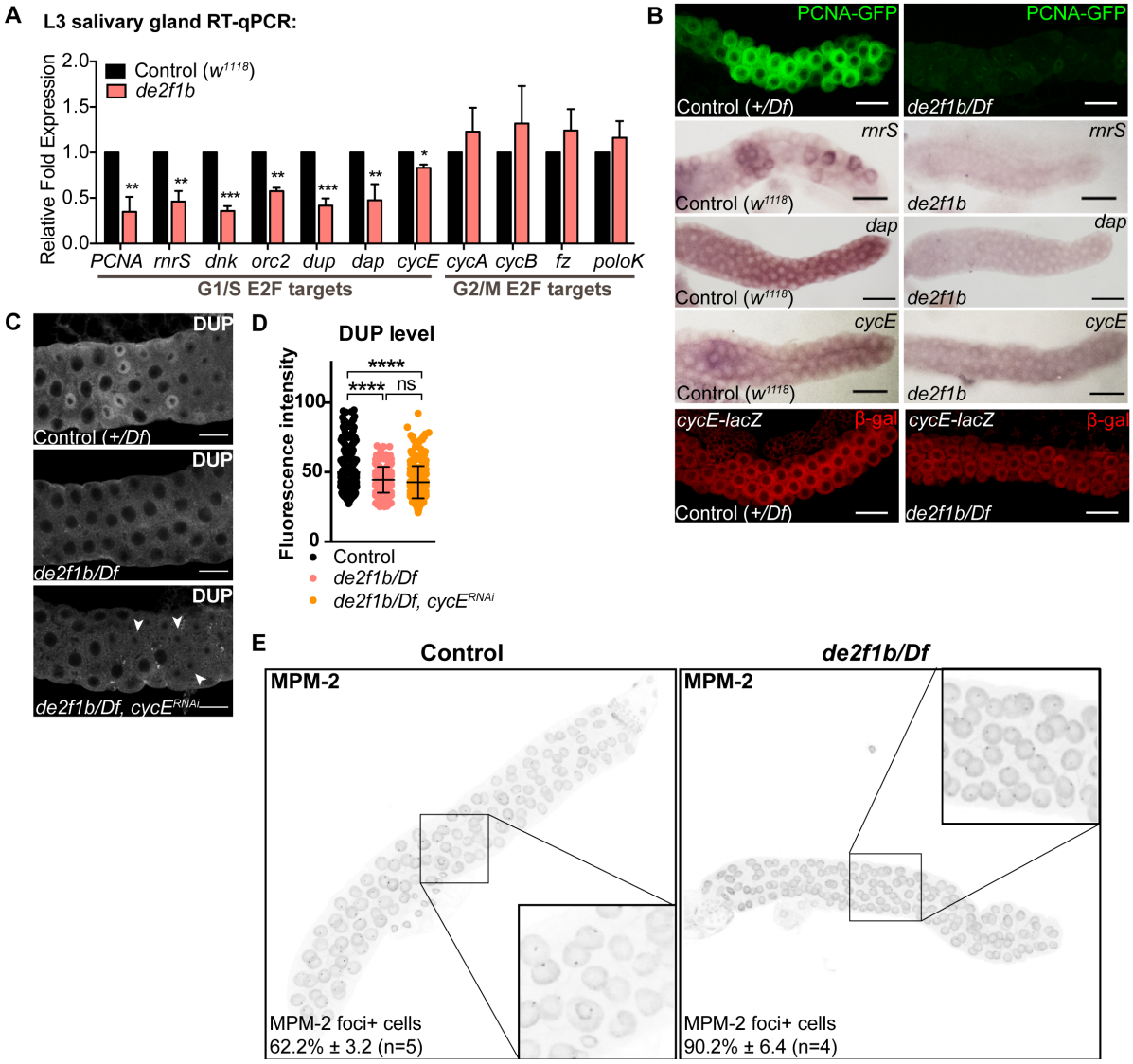

Figure S2. Analysis of *de2f1b* salivary glands. (A) RT-qPCR of E2F targets in control and *de2f1b*. Error bars indicate s.d. from three independent experiments. * = p ≤ 0.05; ** = p ≤ 0.01; *** =p ≤ 0.001. (B) Micrographs of 80-85 hours AEL control and *de2f1b* salivary glands showing expression patterns of PCNA-GFP reporter, *in situ* hybridizations of *rnrS*, *dap*, *cycE* transcripts and β-gal staining to display *cycE-lacZ* reporter expression. Scale bars = 50 μm. (C) Confocal micrographs of 80-85 hr AEL salivary glands from indicated genotypes showing partial restoration of nuclear DUP upon *cycE* depletion in *de2f1b* salivary glands (arrowheads). (D) Quantification of DUP levels from salivary glands shown in (C) (n=5/genotype). ns = p > 0.05; **** = p ≤ 0.0001. All scale bars = 25 μm. (E) Inverted grayscale images of MPM-2 foci. 62% of cells contain MPM-2 foci in control (n=5) while 90% of cells in *de2f1b* salivary glands contain MPM-2 foci (n=4).

Figure S3

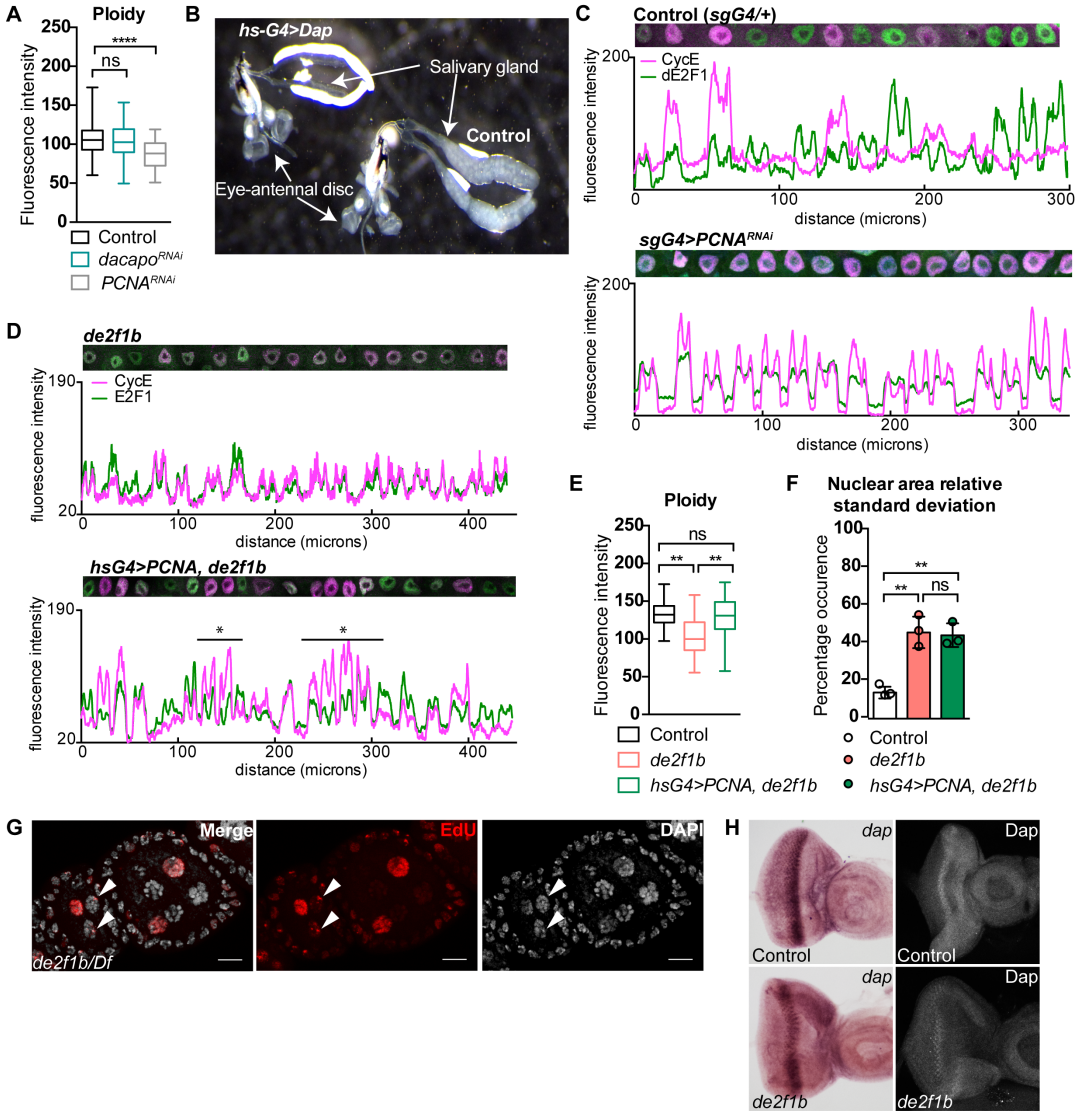

Figure S3. Dap and PCNA are crucial dE2F1b targets during the salivary gland endocycle. (A) Ploidy quantification between control, *sgG4>dacapo*^{RNAi}, and *sgG4>PCNA*^{RNAi} in 80-85 hr AEL salivary glands (n=3/genotype). (B) Control and Dap expressing late-L3 salivary glands attached to eye-antenna discs. Dap expression via the basal promoter activity of *hs-Gal4* severely inhibits the tissue growth. (C) Plot profiles depicting the disruption of oscillation of dE2F1 (green) and CycE (magenta) by *sgG4>PCNA*^{RNAi} from 80-85 hr AEL salivary glands. (D) Plot profiles showing partial restoration of dE2F1 (green) and CycE (magenta) oscillation in *de2f1b* salivary glands through expression of PCNA (*hsG4>PCNA, de2f1b*). (E) A box and whiskers plot showing differences in ploidy of 105-110 hr AEL salivary glands of the indicated genotypes (n=3/genotype). (F) Average of the relative standard deviation of nuclear area of indicated genotypes (n=3/genotype). Error bars indicate s.d. (G) Arrowheads indicate NC nuclei in which EdU incorporation is localized to DAPI dense regions. 13 out of 127 EdU positive NCs from stage 3 to 5 egg chambers in *de2f1b* ovaries showed such pattern while only 1 out of 97 were observed in the control. (H) *In situ* hybridization of *dap* and immunohistochemical staining of Dap in third instar larval eye imaginal discs in Control and *de2f1b* backgrounds. ns = $p > 0.05$; ** = $p \leq 0.01$; **** = $p \leq 0.0001$.

Table S1. Drosophila stocks used in this study

Stock	Source
w^{1118}	Bloomington Drosophila Stock Center (BDSC) 5905
sgs3-GAL4	BDSC 6870
heatshock-GAL4	BDSC 2077
UAS-cycE^{RNAi}	BDSC 36092
UAS-rnrS^{RNAi}	BDSC 32864
UAS-dacapo^{RNAi}	BDSC 64026
UAS-dacapo	BDSC 83334 (Stadler *et al.* 2019)
UAS-PCNA^{RNAi}	Vienna Drosophila Resource Center (VDRC) GD51253
UAS-GFP-E2F1$_{1-230}$*, UAS-mRFP*^{nls}*-CycB*$_{1-266}$	BDSC 55121
cycE-lacZ$^{16.4kb}$	BDSC 30722
Df(3R)Exel6186	BDSC 7665
dap-1gm	Provided by C. Lehner (Meyer *et al.* 2002)
3XPCNA-GFP	Provided by R. Duronio (Thacker *et al.* 2003)
UAS-PCNA	This study, generated using the Drosophila Gateway collection (Drosophila Genomic Resource Center)
de2f1b	Lab collection (Kim *et al.* 2018)
Df(3R)Exel6186, UAS-rnrS^{RNAi}	Lab collection Recombined lines
Df(3R)Exel6186, 3XPCNAGFP	Lab collection Recombined lines
Df(3R)Exel6186, sgG4	Lab collection Recombined lines
Df(3R)Exel6186, cycE-lacZ$^{16.4kb}$	Lab collection Recombined lines

Table S2. Description of full genotypes for each experiment performed in this study.

Figure	Genotype
1A-D 2A,C 3A-C 4A-E 7A,B S1B S2 S3G,H	**Control:** *w;; +/Df(3R)Exel6186* ***de2f1b/Df:*** *w;; de2f1b/Df(3R)Exel6186*
2B	**Control:** *w;; +/Df(3R)Exel6186, 3XPCNAGFP* ***de2f1b/Df*:** *w;; de2f1b/Df(3R)Exel6186, 3XPCNAGFP*
3A-C,	***de2f1b/Df, cycE^{KD}*:** *w; hsG4/UAS-cycE^{RNAi}; de2f1b/Df(3R)Exel6186* ***de2f1b/Df, UAS-Dap*:** *w; +/hsG4; de2f1b/UAS-dap, Df(3R)Exel6186*
5C-F	**Control:** *w; +/UAS-GFP-E2F1$_{1-230}$, UAS-mRFP^{nls}-CycB$_{1-266}$; +/Df(3R)Exel6186, sgG4* ***de2f1b/Df*:** *w; +/UAS-GFP-E2F1$_{1-230}$, UAS-mRFP^{nls}-CycB$_{1-266}$;* *de2f1b/Df(3R)Exel6186, sgG4*
5G S2C,D	**Control:** *w; +/UAS-GFP-E2F1$_{1-230}$, UAS-mRFP^{nls}-CycB$_{1-266}$; +/Df(3R)Exel6186, sgG4* ***de2f1b/Df*:** *w; +/UAS-GFP-E2F1$_{1-230}$, UAS-mRFP^{nls}-CycB$_{1-266}$;* *de2f1b/Df(3R)Exel6186, sgG4* ***de2f1b/Df, cycE^{RNAi}*:** *w; UAS-cycE^{RNAi}/UAS-GFP-E2F1$_{1-230}$, UAS-mRFP^{nls}-CycB$_{1-266}$;* *de2f1b/Df(3R)Exel6186, sgG4*
6A,B	**Control (*dap-1gm, +/Df*):** *w; dap-1gm/UAS-GFP-E2F1$_{1-230}$, UAS-mRFP^{nls}-CycB$_{1-266}$;* *+/Df(3R)Exel6186, sgG4* ***dap-1gm, de2f1b/Df:*** *w; dap-1gm/UAS-GFP-E2F1$_{1-230}$, UAS-mRFP^{nls}-CycB$_{1-266}$;* *de2f1b/Df(3R)Exel6186, sgG4*
6C-F S3A	**Control:** *w;; +/sgG4* ***sgG4>dap^{RNAi}*:** *w; +/UAS-dacapo^{RNAi}; +/sgG4*
7C,D	***hsG4>PCNA; de2f1b*:**

S3D,E,F	*w; hsG4/UAS-PCNA; de2f1b*
7E	**Control:** *w;; +/Df(3R)Exel6186*) ***de2f1b/Df*:** *w;; de2f1b/Df (3R)Exel6186* ***de2f1b/Df, cycE^{RNAi}:*** *w; hsG4/UAS-cycE^{RNAi}; de2f1b/Df(3R)Exel6186* ***de2f1b/Df, rnrS^{RNAi}:*** *w; hsG4/+; de2f1b/Df(3R)Exel6186, UAS-rnrS^{RNAi}*.
S2B	First panel **Control (*+/Df*):** *w;; +/Df(3R)Exel6186, 3XPCNAGFP* ***de2f1b/Df:*** *w;; de2f1b/Df(3R)Exel6186, 3XPCNAGFP* Fourth panel **Control (*+/Df*):** *w;; +/Df(3R)Exel6186, cycE-lacZ$^{16.4kb}$* ***de2f1b/Df:*** *w;; de2f1b/Df(3R)Exel6186, cycE-lacZ$^{16.4kb}$*
S3B	**Control :** *w;; +/hsG4* ***hs-G4>Dap***: *w; +/hsG4; +/UAS-dacapo*
S3A,C	**Control :** *w;+/UAS-GFP-E2F1$_{1-230}$, UAS-mRFP^{nls}-CycB$_{1-266}$; +/sgG4* ***sgG4>PCNA^{RNAi}***: *w; UAS-GFP-E2F1$_{1-230}$, UAS-mRFP^{nls}-CycB$_{1-266}$/UAS-PCNA^{RNAi}; +/sgG4*

Table S3: List of primary antibodies used in this study

Antibody	Dilution	Source
Mouse anti-GFP-FITC	1:100	Abcam 6662
Mouse anti-HP1	1:200	Developmental Studies Hybridoma Bank (DSHB) C1A9
Rabbit anti-H3K9me3	1:200	Abcam 8898
Rabbit anti-dE2F1	1:100	Gift from N. Dyson, Massachusetts General Hospital
Mouse anti-Myc	1:200	DSHB 9E10
Guinea pig anti-DUP	1:1000	Gift from T. Orr-Weaver and J. Nordman
Goat anti-CycE	1:200	Santa Cruz sc15903
Rabbit anti-CycE	1:100	Santa Cruz sc33748
Mouse anti-PCNA	1:200	Cell Signaling PC10 2586
Mouse anti-Dap (NP1)	1:500	Gift from I. Hariharan

Table S4. List of primers used in this study

Target	Forward	Reverse
rp49	TACAGGCCCAAGATCGTGAAG	GACGCACTCTGTTGTCGATACC
β-*tub*	ACATCCCGCCCCGTGGTC	AGAAAGCCTTGCGCCTGAACATAG
PCNA	AAGCCACCATCCTGAAGAAG	CGACACATGGGAGTTGTCC
rnrS	AATGGCGTCCAAGGAAAAC	ACATCTTGCGAACGTTGTTG
dnk	ATGCCCTTTCAGAGTTATGTCAC	GTTCTCCACGAAGCAATAGCG
orc2	CAACAAAGGCGGTTACAAGACG	GCATTCCCAACCATTGCCG
dup	ATCAGTATCAAGAACAGGCGTTT	GGCGGTGACAATTAGTCGG
dap	TCAGTGAGTTCTGCAAGATGAGC	GGTCACGCTTTATGCGATTCAA
cycE	GTTTGTGCAAACCTCACAGC	AACAGCGTAAAGCCATCTCC
cycA	CCAATTTCGCCGTGCTCAAT	CTTGAATTGCTCCACCACGG
cycB	GCTGCCGATTCACTTCCTTC	CAGCTGCAATCTCCGATGG
fz	TCCGTCTCGTACAACACCAG	CTGACGGGTGACAACGAGTA
poloK	TCTGGAGTCGACCTTCCTCA	CTTGCAGAATTCGCGGCTTT

Table S5. List of genes whose mutations give rise to nurse cell chromosome dispersal defect

Gene	FlyBase ID	Function	References
de2f1	FBgn0011766	Cell cycle; transcription factor	(Royzman *et al.* 2002)
dap	FBgn0010316	Cell cycle; $p21^{CIP}/p27^{Kip1}/p57^{Kip2}$-like cyclin-dependent kinase inhibitor	(Hong *et al.* 2003)
mr	FBgn0002791	Cell cycle; APC2 subunit of APC/C; Ubiquitin protein ligase binding and activity	(Reed and Orr-Weaver 1997; Kashevsky *et al.* 2002)
poly	FBgn0086371	Insulin receptor binding; mediator of InR/TOR signaling pathway	(Klusza and Deng 2010)
su(Hw)	FBgn0003567	Chromatin insulator sequence binding activity; Transcription factor; euchromatin binding activity	(Baxley *et al.* 2011)
rhi	FBgn0004400	Female germline-specific; Heterochromatin protein 1 (HP1) family; chromatin binding; piRNA dual-strand cluster binding activity	(Volpe *et al.* 2001)
vnc	FBgn0263251	Histone acetylation; Catalytic subunit of N-terminal acetyltranferase complex; oogenesis	(Wang *et al.* 2010)
spoon	FBgn0263987	RNA binding; Splicing regulation; Protein kinase anchor	(Motola and Silberberg 2004)
hfp	FBgn0028577	RNA binding; RNA splicing; transcription	(Van Buskirk and Schüpbach 2002)
smn	FBgn0036641	RNA binding; alpha-actinin binding; RNA splicing via transesterification reactions; RNP complex assembly	(Lee *et al.* 2009)
sqd	FBgn0263396	RNA binding; heterogeneous ribonucleoprotein (hnRNP) complex member; pre-mRNA splicing regulation	(Goodrich *et al.* 2004)
Rm62	FBgn0003261	RNA binding; DEAD box RNA helicase; alternative splicing regulation; RNA interference	(Buszczak and Spradling 2006)
Rbp9	FBgn0010263	RNA binding; germ cell proliferation; oogenesis	(King *et al.* 1981)
cup	FBgn0000392	RNA binding; translation regulation – repressor; eIF4E binding protein	(Keyes and Spradling 1997)
RpS2	FBgn0004867	RNA binding; structural component of 40S ribosomal complex	(Cramton and Laski 1994)
Hrb27C	FBgn0004838	RNA binding; oogenesis	(Goodrich *et al.* 2004)
otu	FBgn0003023	Subfamily of deubiquitinases; thiol-dependent ubiquitin-specific protease activity; oogenesis	(Storto and King 1988)
Cap-H2	FBgn0037831	Chromatin organization; chromosome separation; chromatin binding; Condensin II subunit	(Hartl *et al.* 2008)
Cap-D3	FBgn0051989	Chromatin organization; chromosome separation; chromatin binding; Condensin II subunit	(Hartl *et al.* 2008)
SMC4	FBgn0015391	Chromosome condensation; ATPase activity; chromatin binding	(Hartl *et al.* 2008)

Supplemental Methods

dacapo RNA probe generation

dap probe was generated using standard PCR-based probe generation methods as described previously (Legendre *et al.* 2013). In brief, the *dap* coding sequence was flanked by T7 and T3 polymerase sequences in the forward and reverse primers, respectively, using the following primers:

Forward primer with T7:

5'-TAATACGACTCACTATAGGGAGAATGGTCAGTGCCCGAGTCCTGAATCC-3'

Reverse primer with T3:

5'-AATTAACCCTCACTAAAGGGAGATTAGTTGTGGCGCGGCCGCTTCAAC-3'

PCR was performed using standard procedures for the Phusion High Fidelity DNA polymerase (NEB) using the *dap* cDNA clone obtained from the Drosophila Genomics Resource Center (#RE12958). PCR product was purified then subjected to DIG-labeled transcription by T7 or T3 polymerase to generate the sense and anti-sense probes.

Supplemental References

Baxley R. M., Soshnev A. A., Koryakov D. E., Zhimulev I. F., Geyer P. K., 2011 The role of the Suppressor of Hairy-wing insulator protein in Drosophila oogenesis. Developmental Biology **356**: 398–410.

Buszczak M., Spradling A. C., 2006 The Drosophila P68 RNA helicase regulates transcriptional deactivation by promoting RNA release from chromatin. Genes Dev. **20**: 977–989.

Cramton S. E., Laski F. A., 1994 string of pearls encodes Drosophila ribosomal protein S2, has Minute-like characteristics, and is required during oogenesis. Genetics **137**: 1039–1048.

Goodrich J. S., Clouse K. N., Schüpbach T., 2004 Hrb27C, Sqd and Otu cooperatively regulate gurken RNA localization and mediate nurse cell chromosome dispersion in Drosophila oogenesis. Development **131**: 1949–1958.

Hartl T. A., Smith H. F., Bosco G., 2008 Chromosome alignment and transvection are antagonized by condensin II. Science **322**: 1384–1387.

Hong A., Lee-Kong S., Iida T., Sugimura I., Lilly M. A., 2003 The p27cip/kip ortholog dacapo maintains the Drosophila oocyte in prophase of meiosis I. Development **130**: 1235–1242.

Kashevsky H., Wallace J. A., Reed B. H., Lai C., Hayashi-Hagihara A., Orr-Weaver T. L., 2002 The anaphase promoting complex/cyclosome is required during development for modified cell cycles. PNAS **99**: 11217–11222.

Keyes L. N., Spradling A. C., 1997 The Drosophila gene fs(2)cup interacts with otu to define a cytoplasmic pathway required for the structure and function of germ-line chromosomes. Development **124**: 1419–1431.

Kim M., Tang J. P., Moon N. S., 2018 An alternatively spliced form affecting the Marked Box domain of Drosophila E2F1 is required for proper cell cycle regulation. PLoS Genet. **14**: e1007204.

King R. C., Riley S. F., Cassidy J. D., White P. E., Paik Y. K., 1981 Giant polytene chromosomes from the ovaries of a Drosophila mutant. Science **212**: 441–443.

Klusza S., Deng W.-M., 2010 poly is required for nurse cell chromosome dispersal and oocyte polarity in Drosophila. Fly (Austin).

Lee L., Davies S. E., Liu J.-L., 2009 The spinal muscular atrophy protein SMN affects Drosophila germline nuclear organization through the U body–P body pathway. Developmental Biology **332**: 142–155.

Legendre F., Cody N., Iampietro C., Bergalet J., Lefebvre F. A., Moquin-Beaudry G., Zhang O., Wang X., Lécuyer E., 2013 Whole mount RNA fluorescent in situ hybridization of Drosophila embryos. J Vis Exp: e50057.

Meyer C. A., Kramer I., Dittrich R., Marzodko S., Emmerich J., Lehner C. F., 2002 Drosophila p27Dacapo expression during embryogenesis is controlled by a complex regulatory region independent of cell cycle progression. Development **129**: 319–328.

Motola S., Silberberg F. S. N., 2004 spoonbill, a new Drosophila female-sterile mutation, interferes with chromosome organization and dorsal–ventral patterning of the egg. Dev. Dyn. **230**: 535–545.

Reed B. H., Orr-Weaver T. L., 1997 The Drosophila gene morula inhibits mitotic functions in the endo cell cycle and the mitotic cell cycle. Development **124**: 3543–3553.

Royzman I., Hayashi-Hagihara A., Dej K. J., Bosco G., Lee J. Y., Orr-Weaver T. L., 2002 The E2F cell cycle regulator is required for Drosophila nurse cell DNA replication and apoptosis. Mech. Dev. **119**: 225–237.

Stadler C. B., Arefin B., Ekman H., Thor S., 2019 PIP degron-stabilized Dacapo/p21Cip1 and mutations in ago act in an anti- versus pro-proliferative manner, yet both trigger an increase in Cyclin E levels. Development **146**: dev175927.

Storto P. D., King R. C., 1988 Multiplicity of functions for the otu gene products during Drosophila oogenesis. Developmental Genetics **9**: 91–120.

Thacker S. A., Bonnette P. C., Duronio R. J., 2003 The contribution of E2F-regulated transcription to Drosophila PCNA gene function. Curr. Biol. **13**: 53–58.

Van Buskirk C., Schüpbach T., 2002 half pint Regulates Alternative Splice Site Selection in Drosophila. Developmental Cell **2**: 343–353.

Volpe A. M., Horowitz H., Grafer C. M., Jackson S. M., Berg C. A., 2001 Drosophila rhino Encodes a Female-Specific Chromo-domain Protein That Affects Chromosome Structure and Egg Polarity. Genetics **159**: 1117–1134.

Wang Y., Mijares M., Gall M. D., Turan T., Javier A., Bornemann D. J., Manage K., Warrior R., 2010 Drosophila variable nurse cells encodes arrest defective 1 (ARD1), the catalytic subunit of the major N-terminal acetyltransferase complex. Dev. Dyn. **239**: 2813–2827.