Supporting Information

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SI Materials and Methods

RNA in Situ Hybridization. RNA in situ hybridization with colorimetric signal output was performed as described previously (1). Here 200- to 800-bp exonic fragments were PCR-amplified from *CG7110*, *CG16848*, *CG16956*, *Vm34Ca*, *Tehao*, *CG6866*/*loqs*, *CG9293*, *CG7099*, *CG10859*, and *beta'Cop*. PCR products were cloned into the pCRII-TOPO dual promoter vector (Invitrogen). Sense and antisense probes were in vitro transcribed and digoxygenin-labeled using either T7 or SP6 polymerase depending on the orientation of the insert, according to the manufacturer's instructions (Roche). Ovaries from WT *OrR* fattened females were dissected in Grace's medium and hybridized at 55 °C. Fluorescent hybridization for *CG16956* was performed using the same DIG-labeled probes and hybridized as described previously (2). Colocalization with the *slbo* marker was assessed using α-GFP (a gift from Mary-Lou Pardue, Massachusetts Institute of Technology, Cambridge, MA) immunofluorescence immediately after RNA FISH, as described previously for visualizing other proteins in follicle cells (3).

Isolation of Follicle Cell Populations. For cell population experiments, follicle cells were isolated using a protocol modified from Bryant et al. (4). Approximately 150 whole ovaries were dissected in ice-cold Schneider's medium supplemented with 10% FBS. Tissue was digested with 0.9 mL of 0.25% Trypsin/EDTA and 0.1 mL of 50-mg/mL collagenase for 15 min at room temperature. The supernatant was strained through a 40-μm mesh and spun at 1,000 × *g* for 7 min in the cold medium and washed once with nonsupplemented Grace's medium. GFP sorting was performed on a MoFlo2 (Beckman Coulter) at the MIT Koch Institute's Flow Cytometry Core Facility. Cells were pelleted at 1,000 × *g* for 7 min and processed for genomic DNA isolation as described previously (3).

Transgenic Fly Construction. To test the *cis* requirements for amplification at *DAFC-34B*, we constructed transposons with various sequences from the most-amplified region of *DAFC-34B* flanked by suppressor of Hairy wing binding sites to control for genomic position-specific integration effects. The 10-kb central amplified regions was PCR-amplified from BACR06A03 using exTaq DNA polymerase (Takara) and primers with AscI and AvrII sites on the forward and reverse sequences, respectively, and cloned into a modified PCRA vector with AscI and AvrII sequences engineered into the multiple cloning site (5). This plasmid, PCRA_34B_10kb, was sequence-verified, digested with NotI, and subjected to a partial XhoI digest to transfer the 10-kb insert to the NotI and XhoI sites of Big Parent to generate BP_34B_10kb (5). BP_34B_6kb was generated from the partial digest of PCRA_34B_10kb. The 2.1-kb NheI/XhoI fragment and 1.8-kb XhoI/NheI fragment of PCRA_34B_10kb were cloned into the PCRA vector and subsequently transferred to the NotI and XhoI sites Big Parent to generate BP_34B_2.1kb and BP_34B_1.8kb.

The 1-kb origin mapped by nascent strand analysis was PCR-amplified from BACR06A03 using exTaq DNA polymerase (Takara) and primers with NheI sites. The product was cloned into the original PCRA vector, generating PCRA_34B_1kb, which was subsequently cloned into the BP vector at the NotI and XhoI sites to generate BP_34B_1kb.

1. Ivanovska I, Khandan T, Ito T, Orr-Weaver TL (2005) A histone code in meiosis: The histone kinase, NHK-1, is required for proper chromosomal architecture in *Drosophila* oocytes. *Genes Dev* 19:2571–2582.
2. Xie F, Orr-Weaver TL (2008) Isolation of a *Drosophila* amplification origin developmentally activated by transcription. *Proc Natl Acad Sci USA* 105:9651–9656.
3. Claycomb JM, MacAlpine DM, Evans JG, Bell SP, Orr-Weaver TL (2002) Visualization of replication initiation and elongation in *Drosophila*. *J Cell Biol* 159:225–236.
4. Bryant Z, et al. (1999) Characterization of differentially expressed genes in purified *Drosophila* follicle cells: Toward a general strategy for cell type-specific developmental analysis. *Proc Natl Acad Sci USA* 96:5559–5564.
5. Lu L, Zhang H, Tower J (2001) Functionally distinct, sequence-specific replicator and origin elements are required for *Drosophila* chorion gene amplification. *Genes Dev* 15: 134–146.

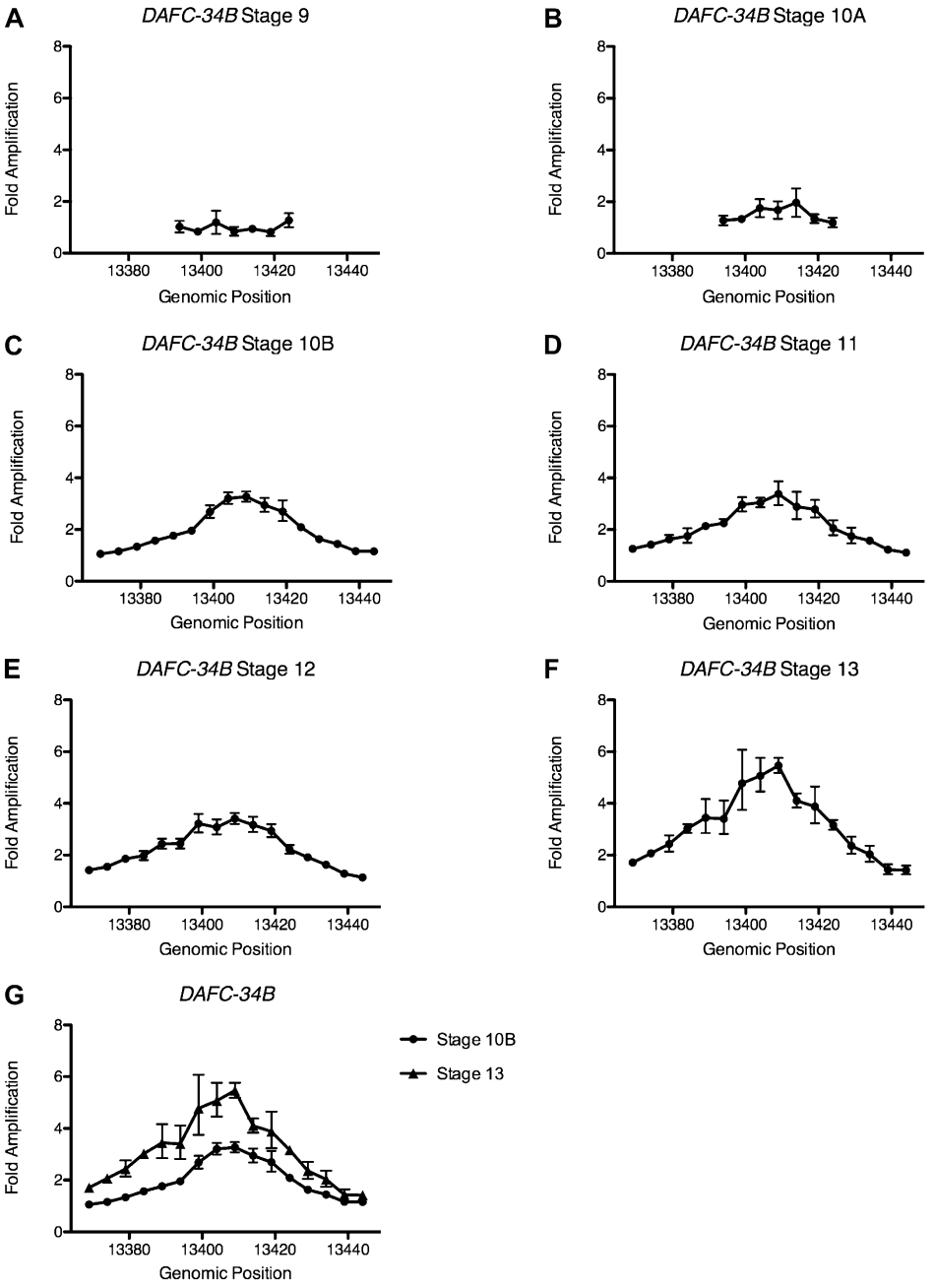

Fig. S1. *DAFC-34B* exhibits two stages of replication initiation. (*A*–*G*) Genomic DNA was isolated from hand-sorted staged egg chambers, and DNA copy levels were quantified by qPCR compared with a nonamplified locus at *62C5*. Error bars show SD for triplicate reactions. Genomic position is shown on the *x*-axis (13380 is Chr2L:13,380,000). Although *Vm34Ca* expression starts at stage 8, gene amplification is not observed until stage 10A, when there is a twofold increase in DNA copy number (*B*). Replication initiation occurs in stage 10B to reach approximately threefold amplification (*C*). Another period of replication initiation at stage 13 results in a doubling of copy number at *DAFC-34B* (*F*). (*G*) Overlay of the stage 10B and stage 13 replication profiles.

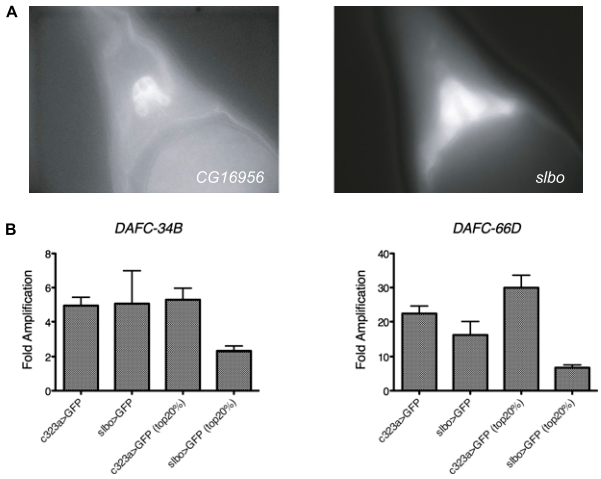

Fig. S2. Follicle cells expressing *CG16956* do not selectively or more greatly amplify *DAFC-34B*. (*A*) RNA FISH was performed along with α-GFP immunofluorescence on *slbo-GAL4;UAS-GFP* ovaries to determine colocalization of *CG16956*. *CG16956* is expressed in *slbo*-positive cells, a border cell-specific marker. (*B*) GFP-positive *slbo*-expressing cells were recovered by FACS, and genomic DNA from these samples was compared with GFP-positive cells driven by the ubiquitous follicle cell driver, *c323a*. Results for the total GFP-positive cells and the top 20% GFP-positive cells are shown; *DAFC-34B* (*Left*) and the control *DAFC-66D* (*Right*).

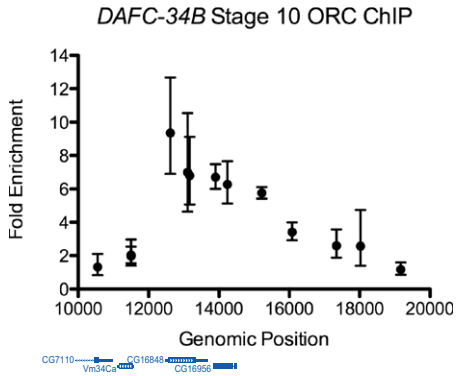

Fig. S3. ORC localizes in a 10-kb zone at *DAFC-34B*. ChIP-qPCR experiments using antibodies recognizing ORC2 performed on stage 10 egg chambers revealed the same pattern of localization as with ChIP-chip.

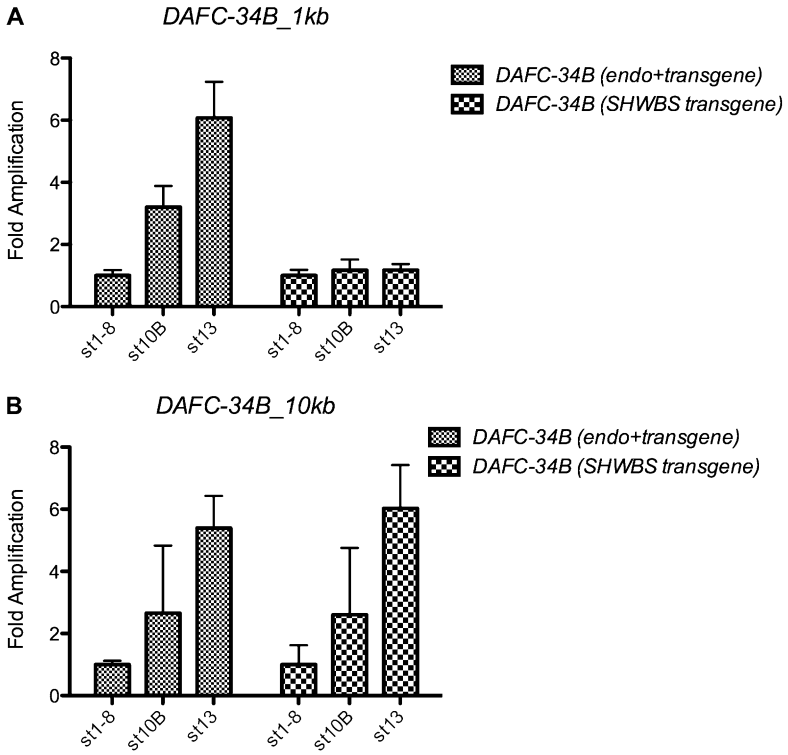

Fig. S4. Testing *cis* requirements for amplification at *DAFC-34B*. (*A*) The 1-kb transposon containing the *DAFC-34B* replication origin does not show ectopic amplification. Primers specific for the transgene (including Suppressor of Hairy Wing binding sequences) can distinguish it from the endogenous *DAFC-34B* locus. (*B*) The 10-kb transposon containing the *DAFC-34B* replication origin and the ORC binding zone shows ectopic amplification.