

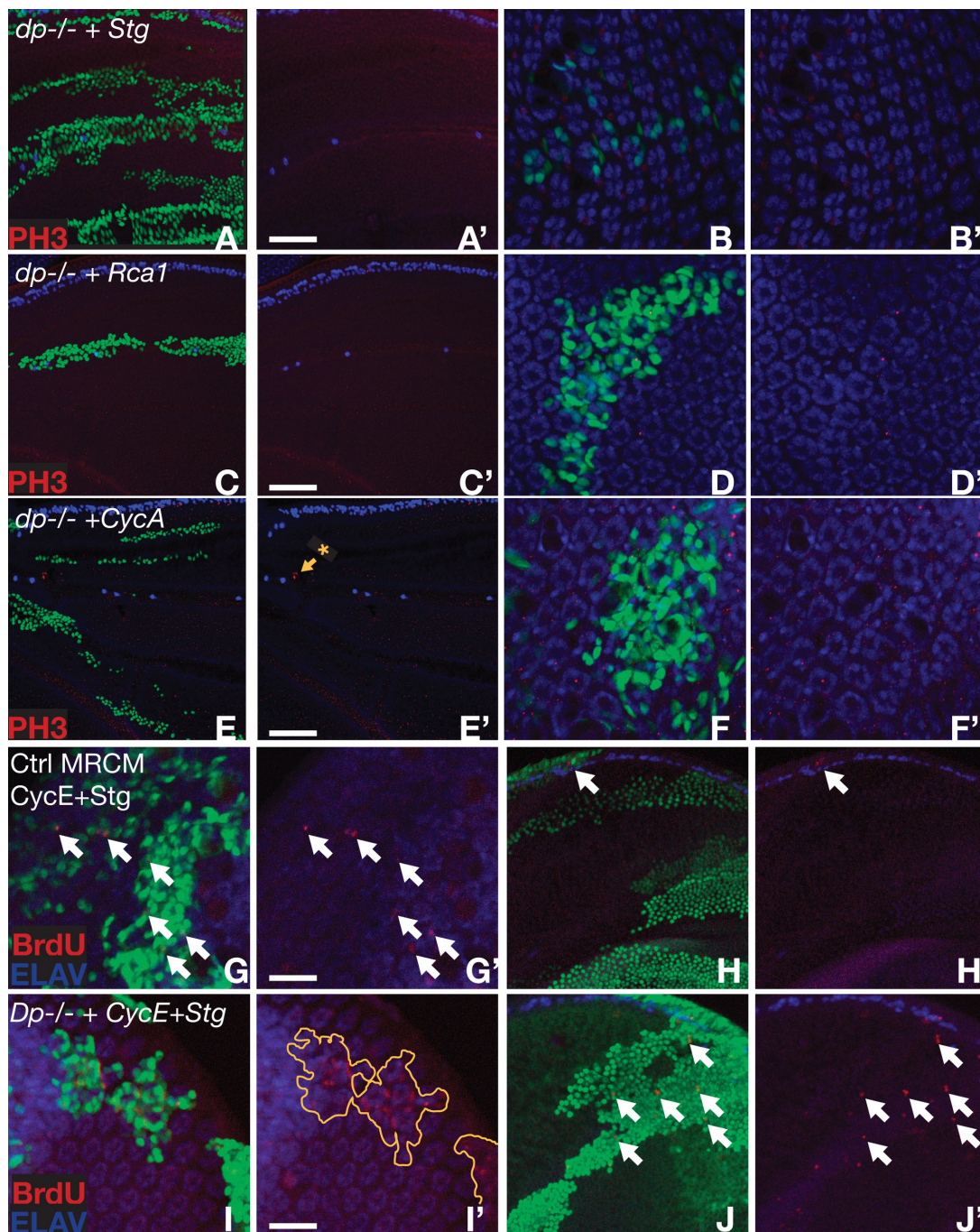
Buttitta et al., <http://www.jcb.org/cgi/content/full/jcb.200910006/DC1>

Figure S1. **Bypass of cell cycle exit is limited, even in the absence of all E2F-DP function.** (A–J) GFP-marked control or *Dp*-null mutant clones expressing the indicated cell cycle regulators were generated using the MARCM system and examined for ectopic mitoses by phospho-histone H3 (PH3; A–F) or S phases by BrdU incorporation (G–J). Neurons are indicated by staining for Elav. *Dp*<sup>-/-</sup> cells expressing *Stg*, *Rca1*, or *CycA* do not bypass exit after 36 h APF (40–44 h APF shown) in the wing (A, C, and E) or eye (B, D, and F). A few nonneural control cells expressing *CycE* + *Stg* are in S phase at 44 h APF in the eye (G, arrows), whereas only cells of the anterior margin are in S phase in the wing at 44 h APF (H, arrows). Some *Dp*<sup>-/-</sup> cells expressing *CycE* + *Stg* enter S phases at 44 h APF in the eye and wing (I and J [arrows]), including cells outside of the wing margin in the blade proper. The arrow with an asterisk in E indicates mitosis of cell in the vein lumen, likely to be a hemocyte, not associated with a clone. (I') Yellow outlines indicate clone boundaries. Bars, 50  $\mu$ m.

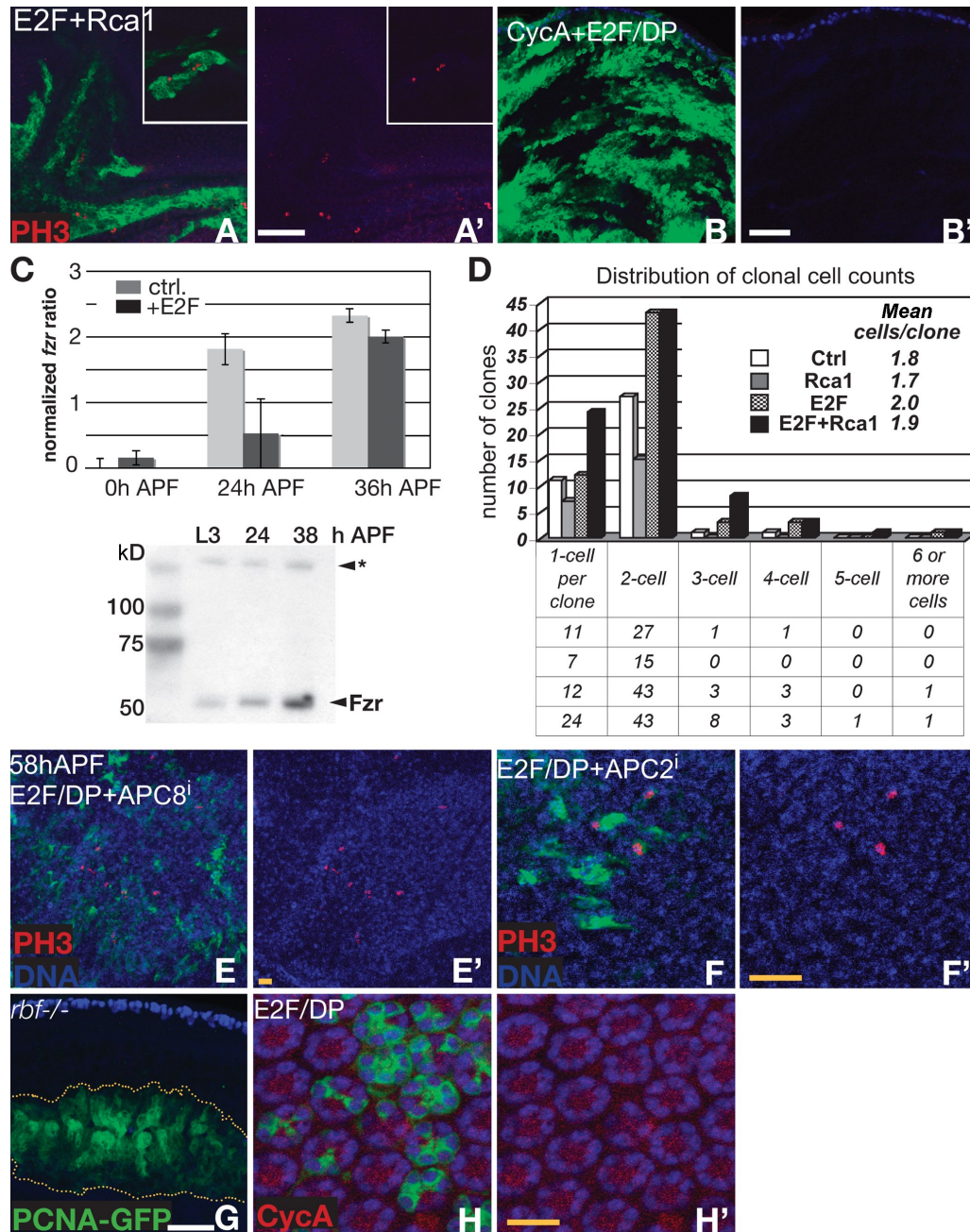


Figure S2. **GFP-labeled clones expressing the indicated cell cycle regulators were generated using *hs-FLP tub>Gal4/UAS, tub-Gal80<sup>TS</sup>*.** (A) Coexpression of E2F and Rca1 in the wing leads to continued mitoses, indicated by PH3, at 44 h APF (insets show an additional example). (B) However, coexpression of CycA with E2F1–DP does not bypass cell cycle exit, as assessed by PH3 staining after 40–44 h APF in wings or eyes (wing shown). (C) Normalized  $\log_2$   $fzr$  ratios (compared with WT L3 wings  $n = 5$ ; y axis) measured by microarray were plotted versus developmental stage (x axis) for control wings and wings expressing E2F. In both cases,  $fzr$  levels increase after exit, although the  $fzr$  increase and cell cycle exit are delayed until 36 h APF in E2F-expressing wings. Error bars indicate the standard deviation. Endogenous Fzr protein levels were assayed by Western blotting in 10 WT wings of the indicated stages. Anti-Fzr antibody reveals a band at  $\sim 57$  kD, which is consistent with the observed Fzr size (Jacobs et al., 2002), as well as an unidentified cross-reacting band  $>150$  kD serving as a loading control (indicated by an asterisk). (D) Sparse clones overexpressing GFP alone (control [ctrl]), Rca1, E2F1–DP (E2F), or E2F + Rca1 were induced at 0 h white prepupae, and cells per clone for at least 30 clones/genotype were quantified at 48–56 h APF. The distributions of clonal cell counts and mean cells/clone are indicated. Although mean clone size is similar, E2F + Rca1 expression leads to an overall increase in  $>2$ -cell clones (compare 13 clones for E2F + Rca1 vs. 7 clones for E2F alone). (E and F) GFP-marked clones coexpressing E2F1–DP and RNAis to the indicated APC/C components were generated using *hs-FLP tub>Gal4/UAS, tub-Gal80<sup>TS</sup>* in eyes and examined for mitoses at 50–60 h APF (58 h APF shown). Mitoses are evident in cells coexpressing E2F + APC8<sup>RNAi</sup> or APC2<sup>RNAi</sup> in the eye. (G) *Rbf* null mutant cells in the wing (clone indicated by dotted outline) activate E2F target gene expression, as indicated by the E2F-responsive PCNA-GFP reporter. (H) GFP-labeled cells overexpressing E2F1–DP fail to accumulate CycA protein in the eye. Elav (blue) marks neurons in G and H. Bars: (A, B, and G) 50  $\mu$ m; (E, F, and H) 20  $\mu$ m.

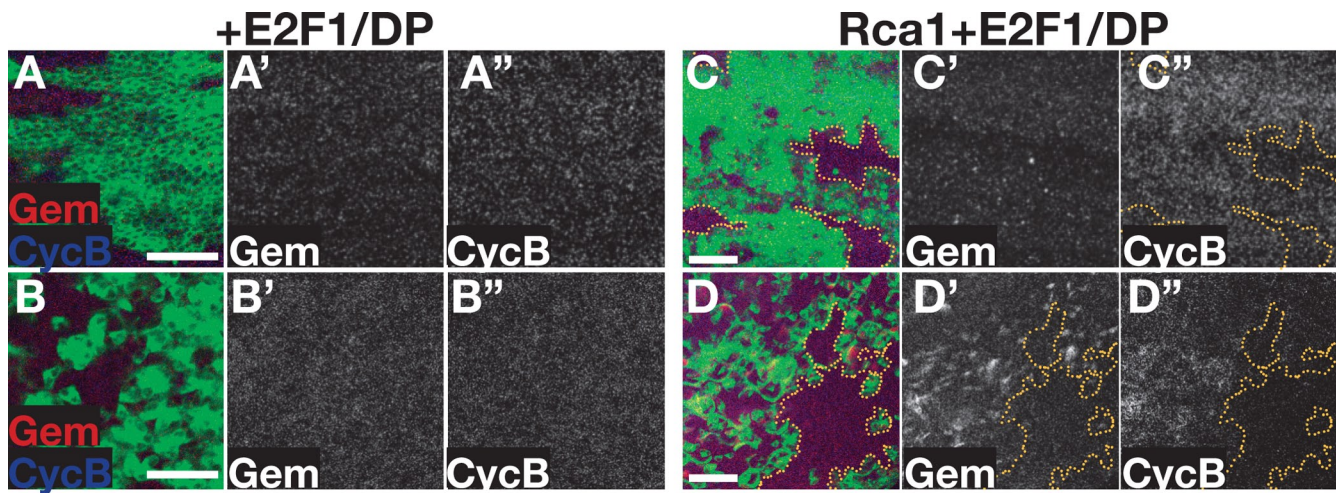


Figure S3. GFP-marked clones expressing the indicated cell cycle regulators were generated using *hs-FLP tub>Gal4/UAS, tub-Gal80<sup>15</sup>* and examined for **CycB or Gem**. (A and B) Cells expressing E2F1–DP in the wing (A) and eye (B) fail to accumulate Gem and CycB at 40–48 h APF. In contrast, cells coexpressing E2F1–DP + Rca1 accumulate very low levels of CycB in the wing (C) and significant levels of Gem in the eye (D). (C and D) Yellow outlines indicate clone boundaries. Bars, 50  $\mu$ m.

The complete E2F and *Dp*<sup>-/-</sup> array dataset (provided as tab-delimited text) is included as a txt file.

## Reference

Jacobs, H., D. Richter, T. Venkatesh, and C. Lehner. 2002. Completion of mitosis requires neither *fzr/trap* nor *fzr2*, a male germline-specific *Drosophila* Cdh1 homolog. *Curr. Biol.* 12:1435–1441. doi:10.1016/S0960-9822(02)01074-6