**Depletion levels** 

by Western Blot

32%

Morais da Silva et al., http://www.jcb.org/cgi/content/full/jcb.201210018/DC1

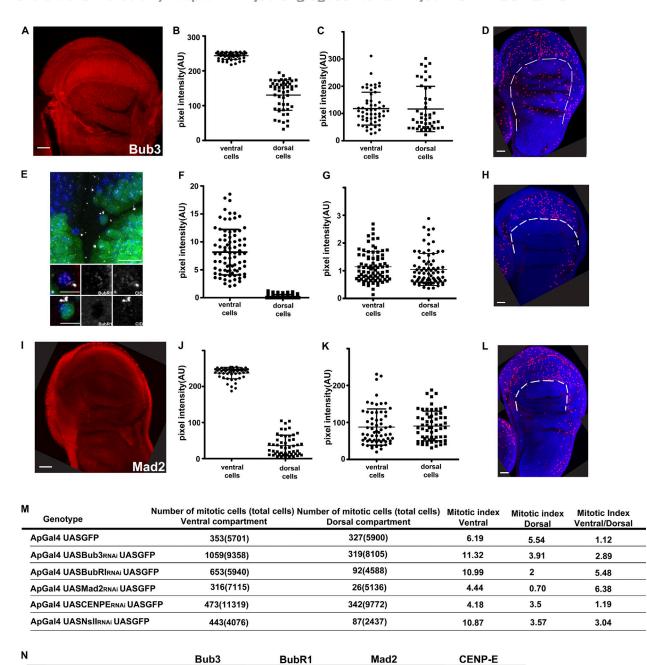


Figure S1. **Protein depletion levels and mitotic index in wing imaginal cells.** (A, E, and I) Immunohistochemistry with a Bub3 (A), BubR1 (E), and Mad2 (I) antibodies revealed the protein depletions in the dorsal domain of wing imaginal discs expressing the respective RNAis. CID, centromere identifier. (B, C, F, G, J, and K) Quantification of the knockdown for Bub3 (B), BubR1 (F), and Mad2 (J) and the respective wt controls for Bub3 (C), BubR1 (G), and Mad2 (K). Immunofluorescence intensity was assessed in cells from the dorsal or ventral side independently and shown in a scatter plot with the mean (large horizontal lines) and SD (small horizontal bars). (D, H, and L) Immunohistochemistry with a phospho–histone-3 antibody revealed mitotic cells on colchicine-treated wing imaginal discs with *Bub3* (D), *BubR1* (H), and *Mad2* (L) knockdown in the dorsal domain. In D, H, and L, the dotted lines separate the dorsal (bottom) from the ventral (top) sides of the wing disc. (M) Quantification of mitotic indexes for wing imaginal discs treated with colchicine expressing GFP and with Bub3, BubR1, and Mad2 knocked down in the dorsal domain. Note the difference in the mitotic index between the dorsal and ventral compartments of the wing discs as a result of an impaired SAC in the dorsal compartment, except for the wt, and CENP-E–knocked down wing imaginal discs. (N) Quantification of the protein depletions by Western blot. Error bars represent SD in B, C, F, G, J, and K. AU, arbitrary unit. Bars: (A, D, H, I, and L) 40 μm; (E, top) 5 μm; (E, bottom) 10 μm.

41%

46%

74%

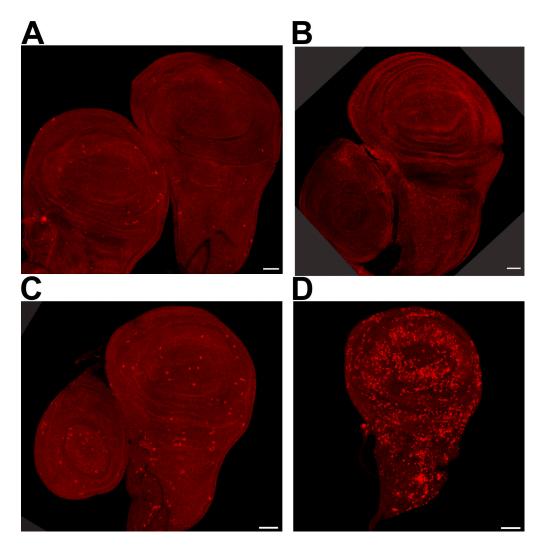


Figure S2. **Apoptosis in wing imaginal discs.** Apoptosis in wing imaginal discs as revealed by a caspase-3 antibody. (A) wt wing imaginal discs. (B) Apoptosis in wing imaginal discs expressing p35 in the dorsal compartment. Note the lack of apoptotic cells. (C and D) Two imaginal discs from a  $Mad2^p$  mutant. Note the variation in the amount of apoptotic cells. Bars, 70  $\mu$ m.