Buster et al., http://www.jcb.org/cgi/content/full/jcb.201207183/DC1

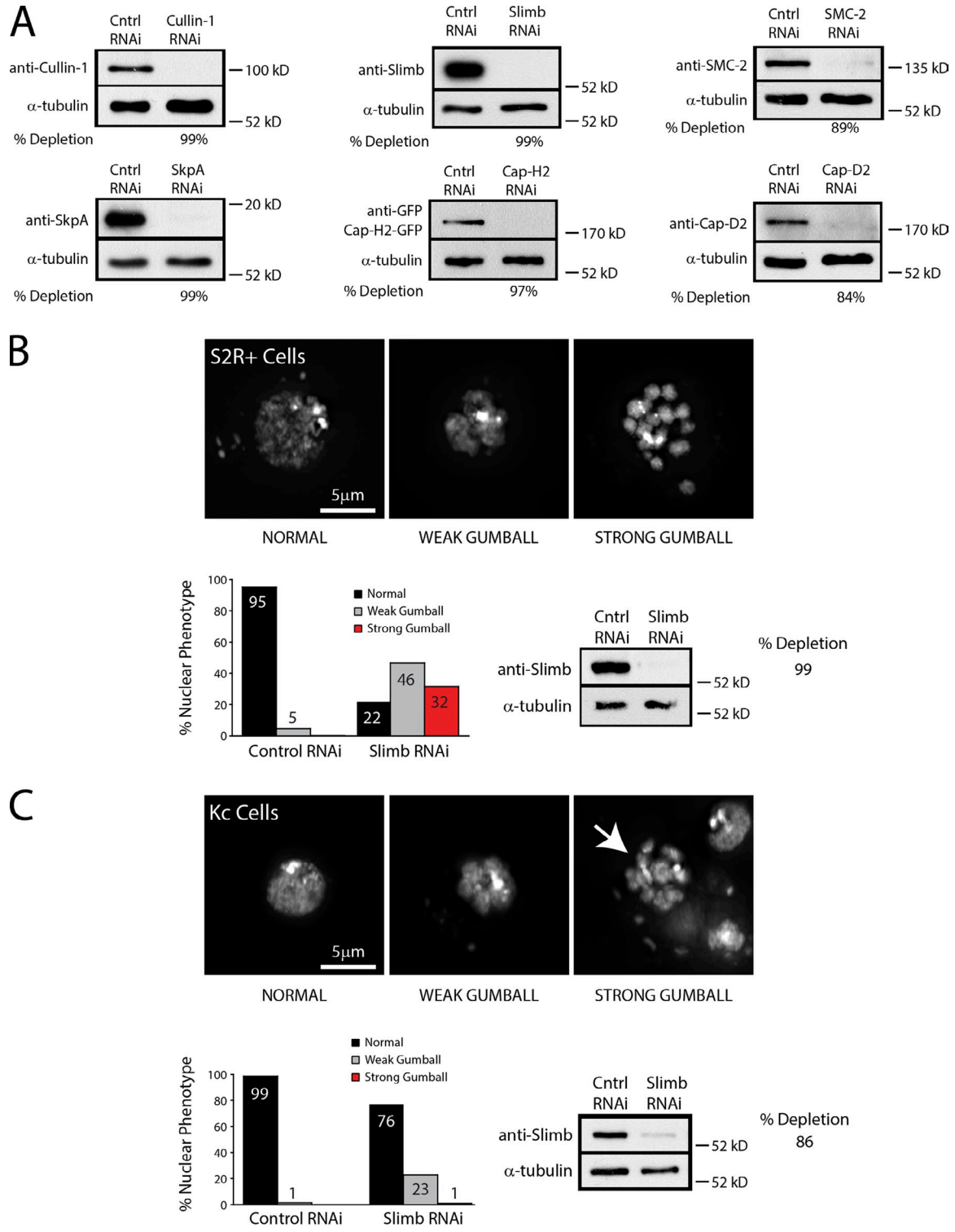

Figure S1. **SCF^{Slimb} is required for interphase chromatin reorganization in cultured *Drosophila* S2R+ and Kc cells.** (A) Immunoblotting demonstrates the efficiency of RNAi-mediated protein depletion from S2 cells. For each blot, lysates from 7-d control and experimental RNAi-treated S2 cells are shown. Equal total protein amounts were loaded. Percent depletion of the target protein was calculated from the ratio of the normalized intensities of the target protein bands in the control and experimental lysates (measured using integrated densitometry [ImageJ], and normalized with similar measurements of the α-tubulin bands, the loading controls). (B, top) Representative images of nuclear phenotypes from 7-d RNAi-treated S2R+ cells stained for DNA. (B, bottom left) Frequency histogram of the nuclear phenotypes in S2R+ cells after 7 d of control and *slimb* RNAi. Numbers in bars are the averages measured for the indicated phenotypes and treatments. (B, bottom right) Immunoblot demonstrates the efficiency of the 7-d RNAi treatment to deplete Slimb. Percent Slimb depletion was calculated as described in A. (C, top) Representative images of nuclear phenotypes from 7-d RNAi-treated Kc cells stained for DNA. The arrow indicates a nucleus displaying the strong chromatin-gumball phenotype. (C, bottom left) Frequency histogram of the nuclear phenotypes in Kc cells after 7 d of control and *slimb* RNAi. (C, bottom right) Immunoblot demonstrates the efficiency of the 7-d RNAi treatment to deplete Slimb. Percent Slimb depletion was calculated as described in A.

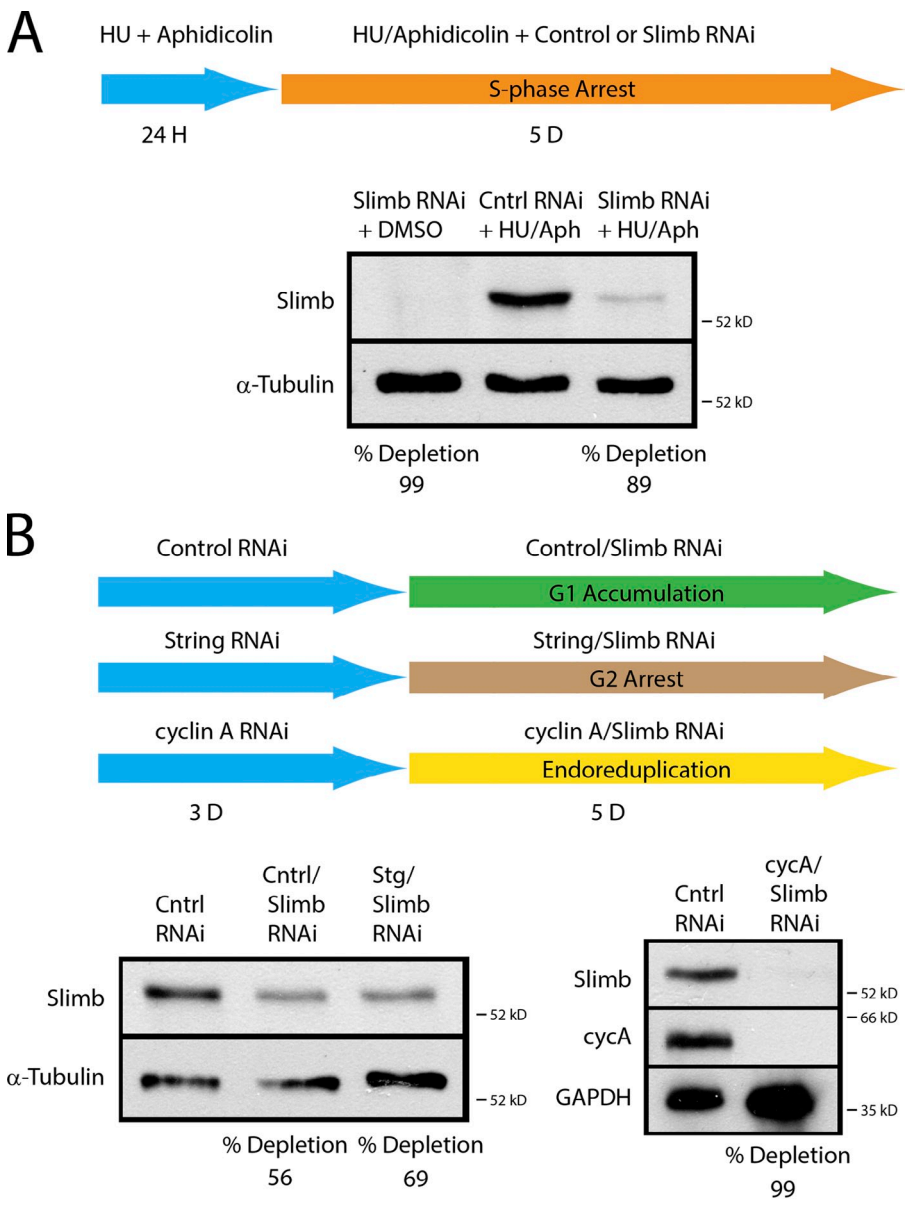

Figure S2. **Outlines of treatment protocols to arrest cells in interphase.** (A, top) Schematic of the drug/RNAi experiment strategy. S2 cells were first treated with a cocktail of hydroxyurea (HU) and aphidicolin for 24 h to cause an S-phase arrest. While maintaining the arrest with drug application, cells were then also *slimb* RNAi-treated every 24 h for the next 5 d. At the end of the treatment regimen, cells were fixed, Hoechst stained, and scored manually for normal (wild type), weak gumball, and strong gumball phenotypes (shown in Fig. 1 E). (A, bottom) Immunoblots demonstrate the efficiency of *slimb* RNAi in drug-arrested cells. The efficiency of *slimb* RNAi in DMSO-treated cells is shown as a control. α-Tubulin was used as a loading control. (B, top) Schematic of the double RNAi experiment strategy. Chromatin compaction was assessed in Slimb-depleted interphase-arrested S2 cells using two different RNAi-based approaches. Cells were treated with String (Stg) or cyclin A (cycA) dsRNA for 3 d to induce interphase arrest and then double RNAi-treated with the addition of Slimb dsRNA for five more days. *Stg* RNAi blocks mitotic entry and arrests cells in G2 phase (Chen et al., 2007), causing a 2.2-fold decrease in the mitotic index (0.3%) after 3 d. *cycA* RNAi also blocks mitotic entry but drives cells into an endoreduplication cycle (promoting repetitive, alternating G- and S-phase cycles; Mihaylov et al., 2002). For comparison, the *slimb*/control double RNAi induces a dramatic accumulation of G1-phase cells, similar to *e2f1* RNAi (Rogers et al., 2009). After 8 d, cells were scored for normal (wild type), weak gumball, and strong gumball phenotypes. (B, bottom left) Immunoblots demonstrate the efficiency of Slimb depletion after the 8-d RNAi treatments. Percent Slimb depletion was calculated as in Fig. S1 A. (B, bottom right) Immunoblot demonstrates the efficiency of Slimb and cycA depletion after the 8-d RNAi treatment. Percent Slimb depletion was calculated as in Fig. S1 A.

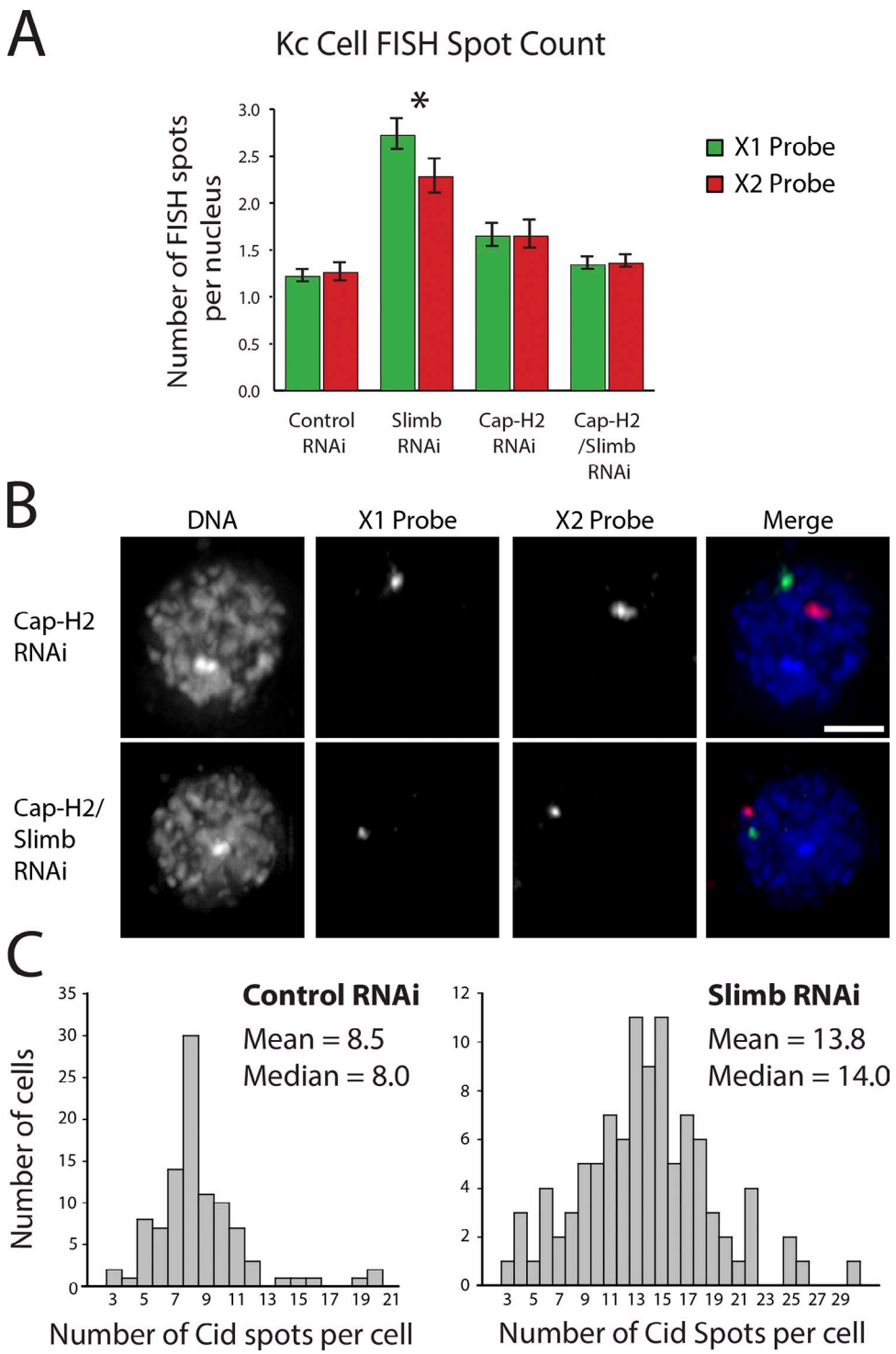

Figure S3. **Depletion of the condensin II subunit cap-H2 rescues *slimb* RNAi-induced chromosome pairing.** (A) Analysis of the number of X chromosome FISH spots after 7 d of RNAi for the indicated gene in Kc cells (n = 27–44 cells per treatment). The asterisk indicates a significant difference when compared with the control ($P < 0.0001$). In this assay, two different X chromosome–specific FISH probes were used (X1 and X2). Error bars indicate SEM. (B) Kc cells were RNAi-treated for the indicated gene for 7 d and labeled with two different euchromatic FISH probes specific for the X chromosome (green and red) and DNA (blue). The presence of only single fluorescent spots for each FISH probe indicates that the X chromosomes are paired in these RNAi treatments at each of the two loci that were probed, and that *cap-H2/slimb* double RNAi rescues the anti-pairing effect observed after *slimb* RNAi (see Fig. 2 C). Bar, 2.5 µm. (C) *slimb* RNAi increases the number of centromeric Cid spots in interphase S2 cells. The number of Cid spots per nucleus was counted from control and Slimb-depleted interphase cells (100 cells per histogram). Values of the mean and median numbers of Cid spots are shown.

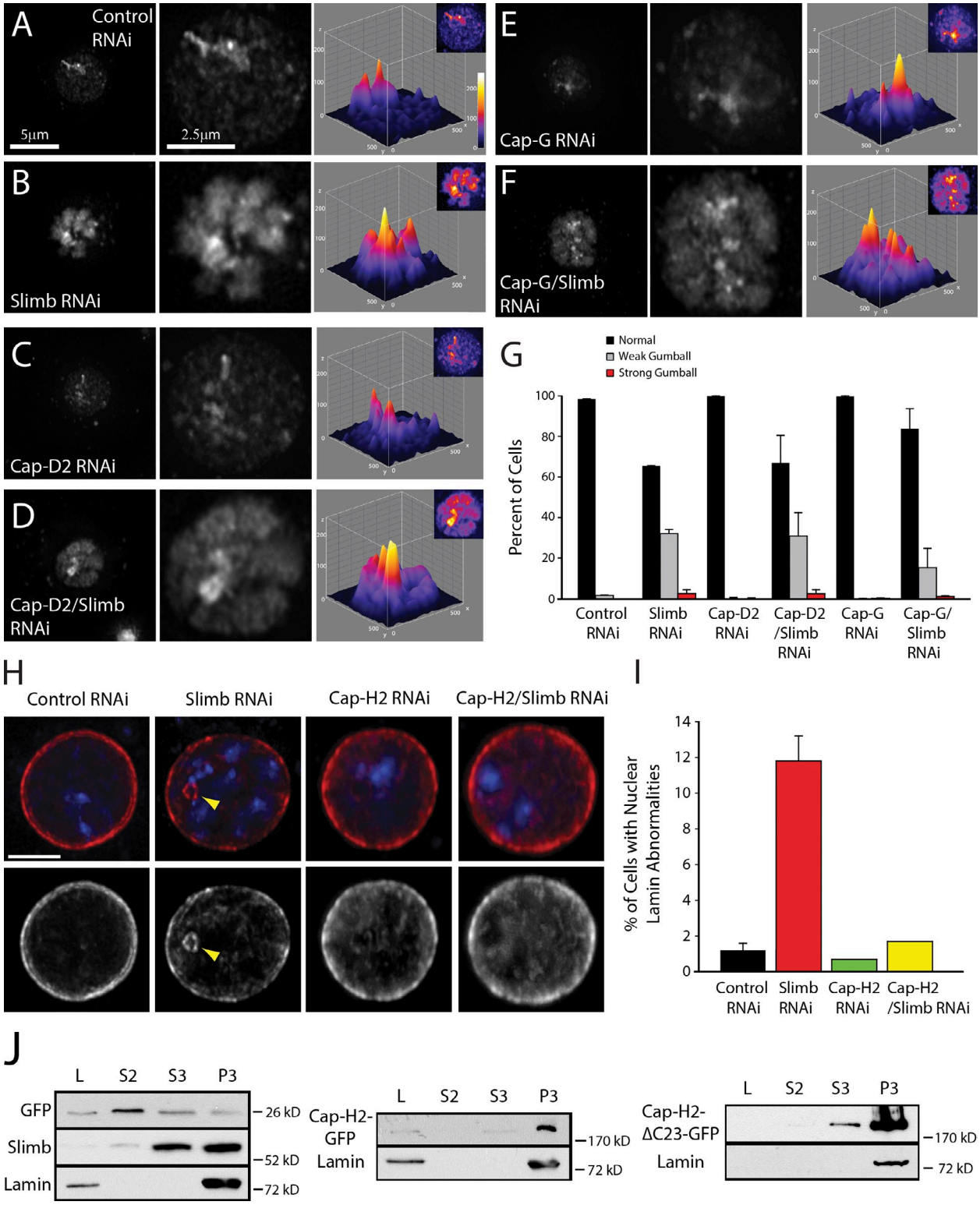

Figure S4. **Depletion of condensin I subunits does not rescue *slimb* RNAi-induced chromatin reorganization, but Cap-H2 depletion rescues *slimb* RNAi-induced nuclear envelope defects.** (A–F) 4-d RNAi-treated S2 cells Hoechst-stained to visualize DNA. Condensin I depletion in S2 cells causes significant cell death after 4 d of RNAi due to mitotic defects in resolving sister chromatids (Somma et al., 2003; Savvidou et al., 2005). Thus, RNAi to Slimb and the condensin I subunits, Cap-D2 and Cap-G, was limited to 4 d. Slimb depletion (B) promotes an increase in weak chromatin-gumball formation that is not rescued with *cap-D2* (D) or *cap-G* (F) double RNAi. (Note that stronger chromatin-gumball phenotypes are observed after a longer period of Slimb depletion; Fig. 1, D and F; and Fig. 3, B and I). Control (A), *cap-D2* (C), and *cap-G* (E) RNAi-treated cells are also shown. Cells are shown at low and high magnifications (left and middle). Shown on the right are 3D surface plots of the fluorescence intensities of the DNA (insets). (G) Frequency histogram of the nuclear phenotypes in S2 cells after 4 d of RNAi (n = 1,000–1,800 cells per treatment). (H) S2 cells were RNAi-treated for the indicated gene for 7 d and then immunostained for nuclear lamin (red; grayscale in bottom panels). Whereas the morphology of the nuclear envelope remains intact in control, *cap-H2*, and *cap-H2/slimb* RNAi-treated cells, Slimb-depleted cells display an increase in nuclear envelope defects. Arrowheads mark an abnormal internalized nuclear microsphere present in the Slimb-depleted cell. DNA, blue. Bar, 2.5 µm. (I) Frequency histogram of nuclear lamin abnormalities (similar to those shown in H and Fig. 2, G–I) after RNAi treatment (n = 500–1,000 cells per treatment). (J) Slimb associates with chromatin and prevents accumulation of Cap-H2. S2 cells transiently expressing EGFP, Cap-H2-EGFP, or Cap-H2-ΔC23-EGFP were lysed and fractionated to isolate chromatin. Whole cell lysate (L), cytoplasmic (S2), nuclear-soluble (S3), and chromatin (P3) fractions are shown and immunoblotted for the indicated proteins. Lamin is a marker for the insoluble chromatin-containing fraction. Error bars indicate SEM.

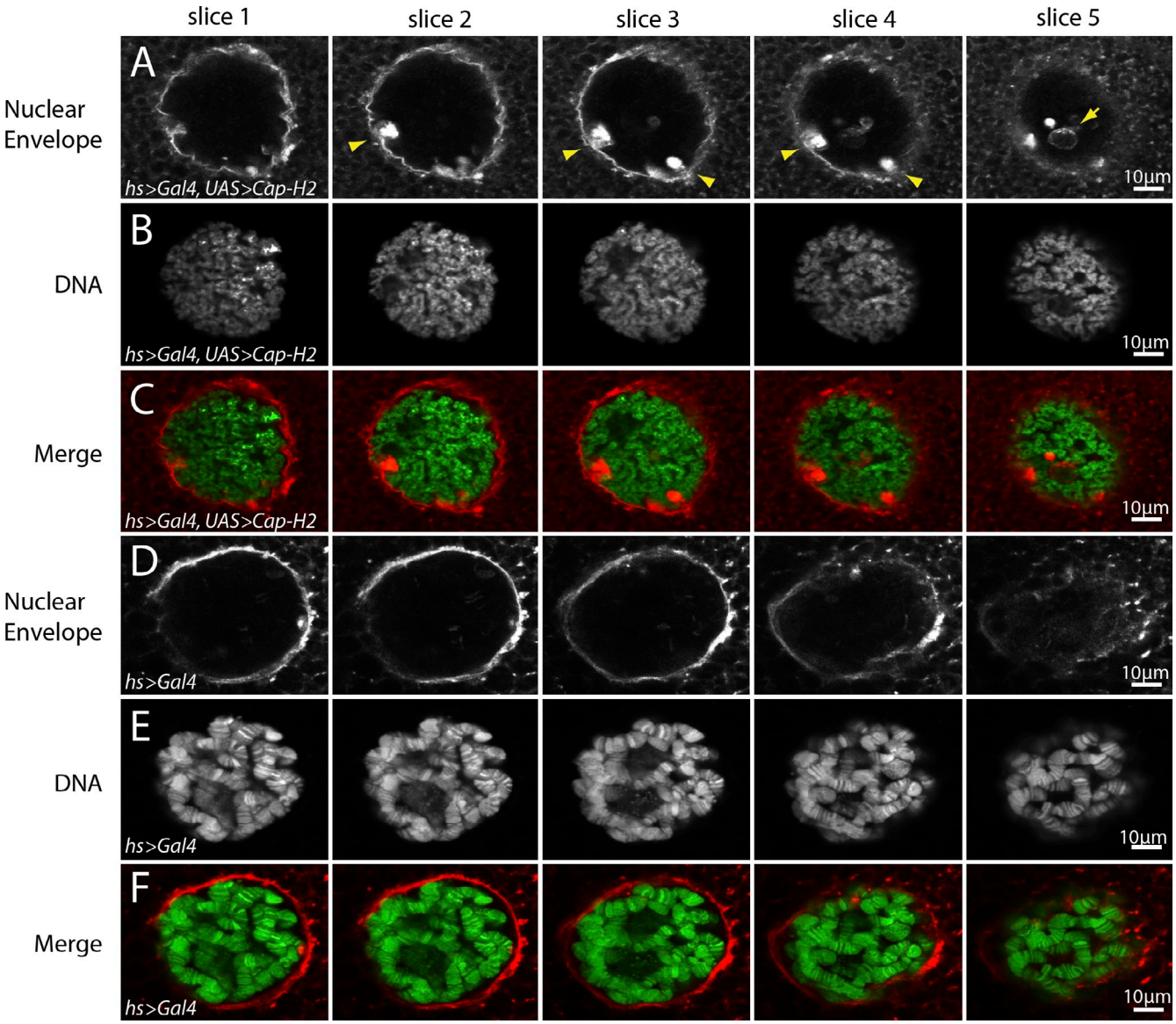

Figure S5. **Cap-H2 overexpression induces nuclear envelope defects in larval salivary gland cells.** Cells isolated from the salivary glands of third-instar larvae were DAPI-stained to visualize DNA and stained with wheat germ agglutinin conjugated to Alexa Fluor 488 to visualize nuclear envelopes. (A–C) Optical sections of a single nucleus from a salivary gland cell of a heat-shocked (*hs*) larva carrying the Gal4 (*hs>Gal4*) and *UAS>Cap-H2*^{EY09979} transgenes. Sequential 1-µm z-stack sections are shown. In this cell, which has been genetically manipulated to overexpress wild-type Cap-H2, note the wrinkled envelope in slice 1, the envelope invaginations in slices 2–4 (arrowheads), and the microspheres in slices 4 and 5 (arrow). (A) Fluorescently labeled nuclear envelope. (B) DAPI-stained DNA; note the nonpolytene appearance. (C) Merged images showing the nuclear envelope (red) and DNA (green). (D–F) Optical sections of a single nucleus from a salivary gland cell of a heat-shocked control larva carrying only the Gal4 (*hs>Gal4*) transgene. Sequential z-stack sections are shown as in A–C. Note the smoothness of the envelope interior and lack of invaginations (D), and the distinctive polytene banding pattern (E).

Table S1. **Primer sequences used to generate dsRNA for RNAi**

Gene	Flybase CG number	Sense primer[a]	Antisense primer[a]	Expected size[b]
				bp
Control[c]	NA	5′-CGCTTTTCTGGATTCATCGAC-3′	5′-TGAGTAACCTGAGGCTATGG-3′	600
cullin-1	1877	5′-CTGCTCAACGCAGACCG-3′	5′-TGTCCTGCAGTTGCTGG-3′	967
FBXW1/*slimb*	3412	5′-GGCCGCCACATGCTGCG-3′	5′-CGGTCTTGTTCTCATTGGG-3′	912
skpA	16983	5′-CAGTTGGAGCCTGCGTGG-3′	5′-TGCAGGTTATGCCTGTGTGG-3′	484
Cap-D3	31989	5′-AGCGCATGACCAACTGG-3′	5′-GGCCATTTGTGAACCCTTCAGAGG-3′	515
Cap-D2	1911	5′-CCAGATATCATCTCCCG-3′	5′-TCTGAGGTGGAATCCCG-3′	551
Cap-G	34438	5′-ACTGCTTGCGCTGGCGG-3′	5′-CCTCCTCAGTGGAGGGC-3′	566
SMC-2	10212	5′-ATAGCTCCAGTGCCGCG-3′	5′-GCCTGTCTGCTGACCTGCC-3′	949
Cap-H2	14685	5′-ACCGGAGAAAAACGAGCGCAGGCC-3′	5′-GGGATCCACTCTCGTGC-3′	838
string (stg)	1395	5′-GCTAATCGGCGACTTCAGC-3′	5′-GCATCAGTCGCGAGCGC-3′	608
cyclin A (cycA)	5940	5′-GAGAAGCTGAAGTACATGACGC-3′	5′-TATTAATATCCGGCTGCTGG-3′	391

NA, not applicable.
[a]All primers began with the T7 promoter sequence 5′-TAATACGACTCACTATAGGG-3′ immediately followed by a gene-specific sequence.
[b]In most cases, a large single exon was PCR amplified from a cDNA template to generate dsRNA, otherwise genomic DNA was used.
[c]Control template was generated from a region of the plasmid pEGFP-N1 that is dissimilar to any *Drosophila* coding sequence.

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