**MULTIMEDIA FILES**

Movie S1. Related to Figure 2. HP1a is recruited to main nuclei but not to late-segregating acentrics.

Time-lapse movie of a larval neuroblast expressing H2Av-RFP, GFP-HP1a ,and I-CreI treated with DMSO.

Movie S2. Related to Figure 2. Aurora B activity mediates preferential HP1a exclusion from acentrics.

Time-lapse movie of a larval neuroblast expressing H2Av-RFP, GFP-HP1a, and I-CreI treated with DMSO + Binucleine-2.

Movie S3. Related to Figure 3. Late-segregating acentrics enter telophase daughter nuclei through channels.

Time-lapse movie of a larval neuroblast expressing H2Av-RFP, Lamin-GFP, and I-CreI treated with DMSO.

Movie S4. Related to Figure 3. Aurora B activity promotes channel formation.

Time-lapse movie of a larval neuroblast expressing H2Av-RFP, Lamin-GFP, and I-CreI treated with DMSO + Binucleine-2.

Movie S5. Related to Figure 3. HP1a depletion does not affect channel formation.

Time-lapse movie of a larval neuroblast expressing H2Av-RFP, Lamin-GFP, RNAi against HP1a, and I-CreI treated with DMSO.

Movie S6. Related to Figure 3. Depletion of HP1a rescues channel formation upon Aurora B inhibition.

Time-lapse movie of a larval neuroblast expressing H2Av-RFP, Lamin-GFP, RNAi against HP1a, and I-CreI treated with DMSO + Binucleine-2.

Movie S7. Related to Figure 5. Nuclear envelope reassembly initiates on the leading edge of chromosomes segregating to daughter neuroblasts.

Time-lapse movie of a larval neuroblast expressing H2Av-RFP and Lamin-GFP.

Movie S8. Related to Figure 5. HP1a biases the position of initiation of nuclear envelope reassembly.

Time-lapse movie of a larval neuroblast expressing H2Av-RFP, Lamin-GFP, and RNAi against HP1a.

**SUPPLEMENTAL FIGURES**



Figure S1. Aurora B kinase preferentially phosphorylates H3(S10) on acentrics and tethers (supplement to Figure 1)

Images of acentrics in (A) wild-type and (B) Aurora B-depleted anaphase neuroblasts stained for DNA (red) and phospho-H3(S10) (cyan). (C) Graph comparing fold increase in the average phopsho-H3(S10)/DNA pixel intensity ratio for the areas of the acentrics vs. the areas of the main nuclei for I-CreI-expressing neuroblasts (left) and I-CreI- and Aurora B RNAi-expressing neuroblast. Each circle represents one anaphase/telophase cell. Scale bars are 2 µm.



Figure S2. Schematic illustrating logic of HP1a co-depletion with Aurora B inhibition (supplement to Figure 3)

(A) In wild-type conditions, highly localized Aurora B on the acentric and the tether inhibits recruitment of both HP1a and nuclear envelope components specifically to the acentric and tether, leading to the formation of nuclear envelope channels and the reincorporation of acentrics into daughter nuclei. (B) When Aurora B is inhibited, HP1a and nuclear envelope components can now be recruited to acentric and tether and channels do not form. Therefore, acentrics are “locked out” as micronuclei. (C) If Aurora B activity mediates nuclear envelope channel formation through the preferential exclusion of HP1a from the acentric and the tether, RNAi depletion of HP1a should rescue nuclear envelope channel formation in conditions in which Aurora B is inhibited, allowing acentrics to reintegrate into daughter nuclei. NER = nuclear envelope reassembly.



Figure S3. Growth temperature influences strength of HP1a depletion (Supplement to Figures 3 and 5)

(A) Stills from time-lapse movies of a wild-type neuroblast expressing H2Av-RFP (top panel) and a neuroblast expressing H2Av-RFP and HP1a RNAi (bottom panel) from larvae grown at 29ºC. In the wild-type neuroblast, chromosomes separate normally. In the HP1a RNAi-expressing neuroblast, there are lagging chromosomes (arrow) and a chromosome bridge (arrowhead). (A’) Comparison of the percentage of chromosome segregation errors in wild-type neuroblasts (dark blue bars) and HP1a RNAi-expressing neuroblasts (light blue bars) from larvae grown at 22ºC (left bars) and 29ºC (right bars). 14% (N=7) and 13% (N=8) of wild-type neuroblast divisions had chromosome segregation errors when larvae were grown at 22ºC and 29ºC respectively. 29% (N=7) and 45% (N=20) of HP1a RNAi-expressing neuroblast divisions had chromosome segregation errors when larvae were grown at 22ºC and 29ºC respectively. (B) Comparison of the mean survival of wild-type larvae (dark blue bars) and larvae expressing HP1a RNAi (light blue bars) when grown at 22ºC (left bars) and 29ºC (right bars). Error bars represent standard deviation. On average, wild-type larvae developed into adults 85% (SD = 30%, N=13) and 78% (SD = 21%, N=13) when grown at 22ºC and 29ºC respectively. On average, HP1a RNAi-expressing larvae developed into adults 73% (SD = 23%, N=13) and 29% (SD= 31%, N=13) when grown at 22ºC and 29ºC respectively. Asterisks indicate statistical significance by unpaired one-sided t-tests (HP1a RNAi-expressing larvae at 22ºC and 29ºC: p=0.0002; and wild-type and HP1a RNAi-expressing larvae at 29ºC: p=5.5 x 10-5). Time is written as min:sec after anaphase onset. Scale bars are 2 µm.



Figure S4. HP1a is recruited to the leading edge of segregating chromosomes prior to nuclear envelope reassembly (Supplement to Figure 5)

(A) Stills from a time-lapse movie of a wild-type neuroblast expressing H2Av-RFP (red) and GFP-HP1a (green). Also shown is DIC (grey). GFP-HP1a recruited to segregating chromatin is indicated by green arrows. Nuclear envelope reformation is indicated by a yellow arrow. (A’) Graph of the time (sec) from anaphase onset to HP1a recruitment (left circles), anaphase onset to nuclear envelope reassembly (middle circles), and HP1a recruitment to nuclear envelope reassembly (right circles (B) Graphical representation of the average timeline from anaphase to HP1a recruitment to nuclear envelope reassembly. (C) Still image of a wild-type neuroblast expressing H2Av-RFP (red) and GFP-HP1a (green) (left panel) and a graph of the pixel intensity of both H2Av-RFP (red) and GFP-HP1a (green) of the yellow dashed line. Peaks of GFP-HP1a intensity are on the outside shoulders of the peaks of H2Av-RFP intensity, showing GFP-HP1a is recruited to the leading edge of segregating chromosomes. Time is written as min:sec after anaphase onset. Scale bars are 2 µm.