Supplemental Material for Seller et al.,

A) Diagram of the endogenously tagged *Drosophila melanogaster* stocks generated in this study. Fluorescent tags were fused to the N-terminus of the indicated proteins. Successful modification was confirmed by PCR followed by Sanger sequencing and by Western Blotting. Founding lines were backcrossed for at least five generation to our lab’s wild-type stock (Canton-S). All lines were viable and fertile. B) Schematic showing the CRISPR-Cas9 strategies used to generate the tagged lines indicated in panel A. CRISPR gRNA targets near the N-terminus of the gene of interest were identified using the CRISPR Target Finder tool (Gratz et al. 2014). Vasa-Cas9 embryos were co-injected with gRNA and Homology Donor plasmids by Rainbow Transgenic Flies Inc (Camarillo, CA). (Bi) Shows N-terminal tagging using the Scarless-DsRed method. The inserted fluorescent protein tag is accompanied by a DsRed marker under the control of the eye specific 3xP3 regulatory sequence. The 3xP3-DsRed cassette is flanked by recognition sequences for the PiggyBac Transposase (yellow boxes on schematic). Flies carrying successful integration of the donor DNA sequence were identified by DsRed expression in their eyes. DsRed positive flies were then crossed to a stock expressing the PiggyBac Transposase which catalyzes the excision of the 3xP3-DsRed cassette. (Bii) Tagging *eggless* with the Halotag sequence was performed without a screenable DsRed marker. Insertion of the halotag was assessed directly by single fly pcr.

Supplemental Figure S1
Supplemental Figure S2

A) Selected live stills from live imaging during cycle 11 of a GFP-egg embryo after maternal expression of the JabbaTrap. Note that Egg localizes to small puncta in the cytoplasm, and not to foci in the nucleus as it does in embryos that do not express the JabbaTrap (compare to Figure 3). Scale bars are 2 µm and the beginning of interphase 11 is set as 0:00. B) Maternally expressed JabbaTrap prevents the nuclear accumulation of GFP-Egg through the MBT. Selected live stills of late cycle 14 embryos from mothers of the indicated genotypes injected with mCherry-HP1a. In control embryos (no Gal4) GFP-Egg co-localizes with HP1a at heterochromatic foci. In contrast, GFP-Egg does not localize to the nucleus in embryos expressing the JabbaTrap.
Supplemental Figure S3

A) Confocal micrograph showing the representative output of the Volocity protocol used to identify the 359 bp repeat using signal from the 359-TALE-HaloJF646 protein. Briefly the software identified objects with an upper intensity threshold of 2 standard deviations and a size threshold of 0.21 µm³. A representative output is show where the objects identified by the protocol are outlined in yellow, in green is the signal from mCherry-HP1a. Scale bar is 4 µm. B) Representative stills from live imaging following mCherry-HP1a and 359-TALE-HaloJF646 during interphase 14 in either JabbaTrap control or JabbaTrap GFP-Egg embryos. Two representative 359-TALE signals are outlined in yellow on the mCherry-HP1a channel. Note that HP1a colocalizes with 359-TALE signal in the JabbaTrap control embryo (27:00 and on), but not in the JabbaTrap GFP-Egg embryo. Scale bars are 2 µm, and the beginning of interphase 14 was set as 0:00. C) JabbaTrap mislocalization of Egg blocks establishment of H3K9me3-HP1a. Fixed gastrulation stage embryos of the indicated genotype immunostained for H3K9me3 and HP1a. D) The 1.686 satellite does not recruit mCherry-HP1a during interphase 14. Compare to Fig. 2F. Plot derived using live imaging data from a wild-type embryo microinjected with mCherry-HP1a and 1.686-TALE-GFP purified proteins. The Volocity protocol outline in A) was used to identify the region of the nucleus occupied by the 1.686 satellite. The mean intensity of mCherry-HP1a signal in this region was calculated for each timepoint. After setting the intensity at the beginning of interphase 14 as zero, the mean intensity of mCherry-HP1a at the 1.686 repeat is graphed over time (in min.) during interphase 14. Error bars represent standard deviation.
Supplemental Figure S4

A) Confocal micrographs of a live cycle 11 embryo from a *gfp-egg/+* female expressing the JabbaTrap. The JabbaTrap mislocalizes GFP-Egg to cytoplasmic puncta and prevents its recruitment to the nucleus. In the merged image signal from mCherry-HP1a is shown in magenta and signal from GFP-Egg is shown in green. When the GFP-Egg channel is shown alone, the position of two nuclei is outlined with a white dashed line. Scale bar is 2 µm.

B) JabbaTrap mislocalization of GFP-Egg does not block the recruitment of HP1a to the 359 bp repeat in a *gfp-egg/+* (heterozygous) maternal background. Live confocal micrographs of a late cycle 14 embryo from a *gfp-egg/+* female expressing the JabbaTrap. Embryos were co-injected with purified proteins of mCherry-HP1a and TALE-359-HaloJF646 prior to filming. The position of several 359 satellites is outlined in yellow on the image of the HP1a channel. The scale bar is 2 µm.

C) JabbaTrap mislocalization of GFP-Egg does not block the recruitment of HP1a to heterochromatic foci in a *gfp-egg/+* maternal background. Embryos were injected with purified mCherry-HP1a protein and filmed during cycle 14 by confocal microscopy, selected stills of an embryo are shown from the indicated times (in minutes) during interphase 14. The scale bar is 2 µm.
Supplemental Figure S5

A) Domain structure of the Eggless protein (adapted from Clough et al. 2014). B) Selected live images of cycle 12 nuclei showing the localization of ectopically expressed GFP-Egg-tud and microinjected Cy5-Histones. A GFP tagged version of Egg lacking the tandem tudor domain was expressed maternally using Maternal-tubulin-Gal4. Images show a single confocal section and the scale bar is 3 µm. C) The Egg protein present at the MBT is maternally provided. Embryos from the indicated crosses were collected and imaged by confocal microscopy. Scale bars are 5 µm. D) Selected stills from a live cycle 14 embryo showing the coincidence of GFP-Egg and Halo549-Egg. The maternal genotype is indicated on the left. Scale bars are 3 µm. E) Selected stills from live cycle 14 embryos showing the specificity of the in vivo Halo labeling. Embryos of the indicated genotypes were collected, permeabilized with Citrasolv, and labeled with the JF549 Halo ligand. Scale bars are 2 µm.
Supplemental Movie legends.

Supplemental Movie S1

Transient expression of the JabbaTrap mislocalizes Orc2-GFP and blocks S phase. This movie accompanies Fig. 1B. Live imaging of Orc2-EGFP (in green) and His2Av-RFP (in magenta). An orc2-EGFP embryo was injected with JabbaTrap mRNA during cycle 11 and then live records were taken by confocal microscopy. The JabbaTrap mislocalizes Orc2 from the nucleus to cytoplasmic puncta and to membranous structures. Orc2 failed to bind to the anaphase chromosomes during the time when origin licensing normally occurs (00:09:00). Nuclei entered mitosis after a delay and underwent catastrophic anaphase bridging (00:29:00). Records were acquired every minute using a 100x oil objective.

Supplemental Movie S2

Imaging the progressive establishment of H3K9me2 in the early embryo. This movie accompanies Fig. 2A. Live imaging over cycles 12-14 of an embryo microinjected with Cy5 labeled H3K9me2-Fab fragments during cell cycle 10. The pool of injected K9me2 Fab is recruited to heterochromatic foci as the modification accumulates during embryogenesis. Note that the Fab fragment does not bind to chromatin during mitosis, but recruitment is fast once the nuclei exit from mitosis. The Fab signal at heterochromatin increases over the course of interphase, but also as the embryo develops through the MBT. Records were acquired every 2 minutes using a 100x oil objective.

Supplemental Movie S3

Recruitment of HP1a to heterochromatin in JabbaTrap control embryo during cycle 14. This movie accompanies Fig. 2D. Live imaging of the accumulation of mCherry-HP1a in a control embryo (No GFP tag) following maternal expression of the JabbaTrap. Movie begins at the start of cycle 14 and ends in interphase of cycle 15. HP1a is recruited to multiple bright foci that grow in number and size over the course of interphase 14. These foci ultimately coalesce into a bright chromocenter that is inherited into cycle 15 (01:54:00 and on). Compare with Movie 4. Records were acquired every 3 minutes using a 100x oil objective.

Supplemental Movie S4

JabbaTrapping GFP-Egg blocks the MBT recruitment of HP1a to heterochromatic foci. This movie accompanies Fig. 2D. Real time records following the accumulation of mCherry-HP1a in a GFP-egg embryo following maternal expression of the JabbaTrap. Movie begins in mitosis 13 and follows HP1a during cycle 14 and into cycle 15. Note that the recruitment of HP1a to heterochromatic foci is substantially reduced compared to control (Movie 3). The prominent HP1a rich chromocenter that normally forms at the end of cycle 14 is not evident, and the HP1a foci that do form remain separated into cycle 15. Records were acquired every 3 minutes using a 100x oil objective.

Supplemental Movie S5

Gradual recruitment of Egg to chromatin foci during interphase 12. This movie accompanies Fig. 3D. Live imaging of Halo-Egg (in magenta) and GFP-HP1a (in green), note that in this color scheme coincident signal will appear as
white. This movie begins at the start of cycle 12 and ends in mitosis 12. After a brief delay at the start of the cycle, Egg begins to accumulate to bright foci on chromatin. The recruitment of Egg to foci precedes that of HP1a. Both Egg and HP1a dissociate from chromatin during mitosis. Records were acquired every 30 seconds using a 100x oil objective.

**Supplemental Movie S6**

Egg dissociates from chromatin when chromosomes condense in preparation for mitosis. This movie accompanies Fig. 5A. Live imaging of Halo-Egg (in green) and Cy5 labeled Histones (in purple). The movie begins in mitosis 11 and ends in mitosis 12. Records from a Halo-egg embryo labeled with JF549 and then injected with Cy5-labeled Histone proteins to mark the chromosomes. Egg is recruited to chromatin foci during interphase after a brief delay following the exit from mitosis. Accumulation of Egg to chromatin continues for most of interphase until mitotic chromatin condensation leads to its dissociation (00:12:00-00:14:00). Records were acquired every 30 seconds using a 100x oil objective.

**Supplemental Movie S7**

Arresting the embryonic cell cycle in interphase permits continued accumulation of Egg and HP1a at heterochromatin. This movie accompanies Fig. 5B. Live imaging of Halo-Egg (in magenta) and GFP-HP1a (in green). Note that coincident signal appears in white in this color scheme. This movie begins in Mitosis 12 and then continues through the arrested interphase 13. Arresting the cell cycle in interphase allows increased Egg recruitment to chromatin foci, which then persist for the duration of our record (over one hour). This leads to the accumulation of HP1a to large heterochromatic foci that coincide with Egg (00:26:00 – 01:08:00). Records were acquired every 2 minutes using a 100x oil objective.