A) 

B) 

C) 

D)
Schwaiger Figure S2

A) control

HP1 kd

B) control

HP1 kd

S-phase (38%)

S-phase (34%)
Schwaiger Figure S3

A) control vs. HP1 kd

B) DNA vs. % of maximum number of cells

C) Bari1

- % of cells
- control vs. HP1 kd
A) Light

% enrichment

S1  S2  S3  S4

control
HP1 kd

B) Early replication timing

2L chromosomal position [bp]

Kc H3K9me2
Kc transcription
HP1 kd transcription
Kc-HP1 kd transcription

light
hp1 kd
ctrl
H3K9 dimethylation in Kc cells

chromosome: 2 2Het 3 3Het 4 X XHet

Schwaiger Figure S6

A)

B) r = 0.21

C) r = -0.14

D) r = 0.11

E) p < 2.2e-16

F) p < 2.2e-16

G) p < 2.2e-16

H) p < 2.2e-16

I) p = 0.018

J) p = 9.7e-13

RNA Polymerase II binding

RNA levels

replication timing

low  mid  high

H3K27me3

low  mid  high

H3K27me3

low  mid  high

H3K9me2

low  mid  high

H3K9me2

p<2.2e-16
A) H3K27 trimethylation

CTRL: late late early early
HP1 kd: early late early late

p<2.2e-16

B) % repeats

CTRL: early late early late
HP1 kd: early late early late

p=0.47  p=9.8e-16

C) control

replication timing
# initiation zones
early late

D) HP1 knockdown

replication timing
# initiation zones
early late
A)

% of all L:E

% of all E:L

chr4 chr2Het chr3Het chr2 chr3 chrX

0 10 20 30 40
Supplemental Figure Legends

Supplemental Figure 1: Reproducibility of replication timing analysis and specificity of HP1 knockdown. A) Pairwise correlation between two biological replicates of a replication timing analysis of Kc cells on Affymetrix tiling arrays revealing high reproducibility between independent experiments (R=0.98). B) Pairwise correlation between two biological replicates of a replication timing analysis of Kc cells after knockdown of HP1 by RNA interference on Affymetrix tiling arrays revealing high reproducibility between independent experiments (R=0.95). C) Pairwise correlation between replication timing of Kc cells and HP1 depleted cells on Affymetrix tiling arrays. This shows a high degree of similarity between overall replication timing before and after HP1 knockdown (R=0.94). However, there is a significant population of probes replicating later after HP1 RNAi (positive values on x-axis and negative values on y-axis). D) The y-axis shows the relative expression values derived from Affymetrix arrays of control (red bars) and HP1 (blue bars) knockdown cells for the HP1 (HP1A), HP1B, and HP1C genes. Specifically the expression of HP1 (HP1A) is affected by the dsRNA treatment. Error bars represent the standard deviations.

Supplemental Figure 2: S-phase progression is unaffected in HP1 knockdown cells. A) FACS profiles based on DNA content (DAPI) of control (left) and HP1 knockdown (right) cells. B) FACS analysis of cells stained with BrdU (y-axis) and 7-AAD (for DNA, x-axis). Gates around G1, G2 and S-phase cells are marked by circles. Control cells consist of 25% cells in G1, 30% cells in G2, and 38% cells in S-phase. HP1 knockdown cells consist of 27% cells in G1, 33% cells in G2, and 34% cells in S-phase. These data suggest that there is little if any difference in the amount of cells undergoing DNA replication in HP1 RNAi compared to control cells.

Supplemental Figure 3: Replication timing changes of the Bari1 transposable element. A) FACS profiles based on DNA content (DAPI) of control (left) and HP1 knockdown (right) cells. The four gates used for measuring replication in four S-phase fractions are indicated (S1-S4). B) FACS analysis of 1000 sorted cells of each fraction (S1-S4) from Kc cells. Cells were re-stained with DAPI for 10 minutes before FACS analysis. Each line represents the DNA content (x-axis) distribution of one S-phase fraction. The y-axis shows the number of cells of each fraction normalized to the
maximum number of cells in each fraction for ease of comparison. The different S-phase fractions can be nicely separated from each other, which is also reflected by the differential enrichments after BrdU-IP (see Figures 1, 2 and S3 and (Schwaiger et al. 2009)). C) Enrichments of BrdU containing DNA in four FACS sorted fractions (S1-S4) as quantified by real-time PCR. S1 represents the earliest and S4 the latest S phase fraction as measured by DNA content. The Bar1 transposable element is shown. Error bars represent the standard deviations between biological replicates.

**Supplemental Figure 4: Distribution of differential replication timing throughout all chromosomes.** Replication timing profiles of control (red, ctrl) and HP1 knockdown (blue, HP1) Kc cells for each chromosome is shown. X-axis = chromosomal position in basepairs, y axis = log2 (early/late replication). Background coloring denotes regions that replicate earlier in HP1 cells (L:E, blue), regions that replicate earlier in Kc cells (E:L, pink) and regions replicating similarly in both cell types (grey). Regions with small differences over short regions were not included in further analysis (white, see methods for details). These plots illustrate that regions of delayed replication timing after HP1 depletion (E:L, pink) occur preferentially in regions close to the centromeres of chromosome 2 and on chromosome 4. Regions with advanced replication timing (L:E, blue) are scattered throughout the genome and often show only a small difference in replication timing.

**Supplemental Figure 5: Differences in replication timing at heterochromatic genes.** A) Enrichments of BrdU containing DNA in four FACS sorted fractions (S1-S4) at the light gene as quantified by real-time PCR. Error bars represent the standard deviations between three biological replicates. B) Replication timing profiles of control (red, ctrl) and HP1 depleted (blue, HP1 kd) Kc cells for a representative region on chromosome 2L. X-axis = chromosomal position in basepairs, y axis = log2 (early/late replication). Background coloring denotes regions that replicate earlier in HP1 kd cells (L:E, blue), regions that replicate earlier in ctrl cells (E:L, pink) and regions replicating similarly in both cell types (white). Annotated genes are displayed below the profile (boxes=exons, lines=introns, small boxes=UTRs) and colored by their expression status (see methods for details, green= expressed in ctrl and HP1 kd cells, blue= expressed only in HP1 kd cells, red= expressed only in ctrl cells, grey= not expressed in ctrl and HP1 kd cells). H3K9me2 in Kc cells (darkred), transcription levels of ctrl (red) and HP1
kd (blue) cells measured on tiling arrays are displayed on the same scale below, including transcription level differences (black). The direction towards the centromere (cen) is marked by an arrow.

**Supplemental Figure 6: Distribution of H3K9 dimethylation and H3K27 trimethylation.** A) Enrichment of H3K9me2 on different chromosomes. The boxplots illustrate that pericentric regions on chromosome 2 (2Het, p-value < 2.2e-16) and to a lesser extend on chromosome 3, X (3Het, XHet, p-value < 2.2e-16) and 4 (4, p-value < 2.2e-16) show elevated levels of H3K9me2 compared to euchromatin on chromosome 2, 3 and X (2, 3, X). This distribution of H3K9me2 is similar to the distribution of this mark in *Drosophila* embryos (Yasuhara et al. 2008), and overlaps with binding sites of HP1 (de Wit et al. 2007). B) Comparison of the enrichment for H3K9me2 (y-axis) and HP1 binding (x-axis, de Wit et al. 2007)) in Kc cells shows a weak yet significant correlation (R=0.21). C) HP1 binding (x-axis, de Wit et al. 2007) and H3K27m3 (y-axis) almost never occur at the same sites (R=-0.14) in Kc cells. D) Similarly, the enrichments of H3K9me2 (x-axis) and H3K27m3 (y-axis) do not correlate (R=0.11). Pair-wise correlations were measured in 200bp sliding windows across regions covered by all three datasets (H3K9me2, H3K27me3, and HP1). The intensity of the blue color reflects the amount of data points with similar values in the xy scatterplot (dark blue= high number of data points, outliers are drawn as black points). E) Enrichments of RNA Polymerase II in Kc cells in regions of low, mid or high H3K27me3. Regions of high H3H27me3 levels are depleted in RNA Polymerase II. F) RNA levels in Kc cells in regions of low, mid or high H3K27me3. Transcription occurs mostly in regions without H3K27me3 (green boxplot). G) Replication timing of Kc cells in regions of low, mid or high H3K27me3. Regions marked by H3K27me3 tend to replicate late. H) Enrichments of RNA Polymerase II in Kc cells in regions of low, mid or high H3K9me2. Regions of high H3H9me3 levels are depleted in RNA Polymerase II. I) RNA levels in Kc cells in regions of low, mid or high H3K9me2. Regions of high H3K9me2 enrichment are often transcribed. J) Replication timing of Kc cells in regions of low, mid or high H3K9me2. Late replication is not as prominent in regions of high H3K9me2 as in regions of high H3K27me3.
Supplemental Figure 7: H3K27me3 and repeat density in differentially replicating regions. A) Distribution of H3K27me3 for regions with differential replication timing. The boxplots illustrate that regions which change replication timing after HP1 knockdown (red boxplots) are not enriched in H3K27me3. Instead, H3K27me3 is mostly enriched in constitutively late replicating regions. This is in agreement with the observation that HP1/H3K9me2 and Polycomb/H3K27me3 hardly ever occur at the same sites (Fig. X and (de Wit et al. 2007)). L:E=regions replicating earlier in HP1 kd cells, L:L=regions replicating late in both, E:E=regions replicating early in both, E:L=regions replicating earlier in ctrl cells. B) Distribution of repeat density for regions with differential replication timing. The boxplots illustrate that regions replication later after HP1 knockdown (E:L) show a high number of repetitive sequences. L:E=regions replicating earlier in HP1 kd cells, L:L=regions replicating late in both, E:E=regions replicating early in both, E:L=regions replicating earlier in ctrl cells. y-axis= distribution of the percentage of repetitive sequences in each HMM derived region. C) Histogram displaying the number of initiation zones throughout S-phase in control cells (Schwaiger et al. 2009). D) Histogram displaying the number of initiation zones throughout S-phase in HP1 knockdown cells. Initiation zones were defined in the same way as initiation zones in Kc cells (Figure S7C) as described in detail in (Schwaiger et al. 2009). The distribution of initiation zones does not appear to change after HP1 knockdown (p-value=0.29). p-values were calculated using the Wilcoxon rank sum test.

Supplemental Figure 8: Delayed replication timing of chromosome 4 and pericentric heterochromatin (regions containing many dispersed repeats). Regions of differential replication timing were determined using a HMM approach (see methods for details). The percentage of the genomic coverage of all regions with delayed replication timing after HP1 knockdown (E:L) on each chromosome is displayed as blue bars. For example, 2.4% of all basepairs in the genome are covered by E:L regions, 10% of those reside on the 4th chromosome. The percentage of the genomic coverage of all regions with advanced replication timing after HP1 knockdown (L:E) on each chromosome is displayed as red bars. Chromosomes were separated into chromosome 4, heterochromatin on chromosome 2 (including chr2LHet, chr2RHet and the most centromere proximal ~1MB of chromosome 2L and 2R), heterochromatin on chromosome 3 (including chr3LHet,chr3RHet and the most centromere proximal ~1MB
of chromosome 3L and 3R), chromosome 2 and 3 (excluding the centromere proximal regions), and chromosome X. The barplot shows that E:L regions are most abundant in the heterochromatin of chromosome 2 and also on the small fourth chromosome.
Supplementary Materials and Methods

Target sequence amplification and microarray processing

To obtain sufficient target DNA for microarray hybridization, we amplified the denatured and immunoprecipitated DNA as described (Schubeler et al. 2002). The enrichments of several control genes were verified to ensure correct amplification before labeling and hybridization to arrays. For use with Affymetrix tiling arrays, dUTP was incorporated into the amplification reaction at 2 mM, the amplified DNA was fragmented and end-labeled using the GeneChip WT Double-Stranded DNA Terminal labeling Kit (Affymetrix) and hybridized to GeneChip Drosophila Tiling 1.0R arrays (Affymetrix) according to manufacturer’s instructions. All microarray experiments were carried out in at least two biological replicates.

Affymetrix tiling arrays were analyzed using MAT (Model-based Analysis of Tiling-array) software (Johnson et al. 2006). The bandwidth was set to 1000bp for replication timing and 200bp for ChIP and transcription data. MAT scores were extracted from the BAR files generated using the Python script ‘Bar2Wig.py’ kindly provided by Wei Li (Harvard University). Data from the Wiggle files were reformatted using Perl for subsequent analysis in R and the ratio of early/late replicating DNA, bound/input DNA and nascent/genomic DNA was calculated. Signal values for Affymetrix expression arrays were estimated using the GC-RMA module from Genedata’s Refiner 4.5 package (Genedata, Basel, Switzerland). Expression data analysis was performed in Genedata’s Analyst 4.5 package. Only those genes which had an Affymetrix detection p-value < 0.05 and signal values > 4 were assumed to be expressed. All analytical procedures were based on Flybase release 5.11 of the Drosophila genome. Data based on arrays of the release 4.3 (dm2 in UCSC) were lifted to release 5.11 (dm3 in UCSC) using the UCSC LiftOver tool. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (Edgar et al. 2002) and are accessible through GEO Series accession numbers GSE13328 (Schwaiger et al. 2009) and GSE18092. Furthermore, genome-wide replication timing profiles can be viewed at replicationdomain.org (Weddington et al. 2008).

Segmentation of replication timing profiles by Hidden Markov Models (HMMs)
All analytical procedures were done using R (R_Development_Core_Team 2006) and all custom scripts are available upon request. To define differentially replicating regions between Kc and HP1 knockdown cells, we segmented Kc minus HP1 replication timing data (in 500bp sliding windows) using HMMs, as described (Birney et al. 2007; Schwaiger et al. 2009). The basic premise of HMMs is that observed data are generated stochastically from a pre-determined number of hidden background probability distributions, or states. We used three states, to distinguish early, mid and late replication. The parameters of the HMMs (emission probabilities, here modeled as normal distributions, and the transition probabilities between states), are estimated via unsupervised learning (Baum-Welch algorithm) from the replication timing profile. In our case, model parameters describe the range of replication timing values that are typical for L:E (advanced replication timing after HP1 knockdown), no difference and E:L (delayed replication timing after HP1 knockdown) differential replication states, and the patterns of changing state along chromosomal positions. In order to rule out the possibility that trained models correspond to suboptimal local minima in parameter space, the training procedure was repeated several times using varying initial parameters, all resulting in highly similar trained models. "L:E", “no difference” and "E:L" replication states were assigned to genomic positions according to the most probable path through the trained model states given the observed data (Viterbi algorithm). Consecutive genomic positions with identical replication states were merged, and to eliminate likely outlier values in the replication timing data, we further merged regions of identical states that were separated by less than 5kb. The segmentation algorithm was implemented in Python using the GHMM library (Schliep et al. 2004). Many regions showed timing differences of various degrees, and about half of the genome showed no replication timing differences. For further analysis of the most prominent differences in replication timing, regions larger than 20kb with an average timing difference higher than the median of timing difference on the raw Kc-HP1 replication timing values plus (E:L) or minus (L:E) 1/12th of the range of the raw Kc-HP1 replication timing values were selected. However, all observed correlations of replication timing with transcription are also observed using different cutoffs and even with all differentially replicating domains as predicted by the Hidden Markov Model. This analysis was also performed on replication timing differences between two control samples. Thereby we found that ~0.5% of the genome showed differences in replication
timing, compared to ~8% between control and HP1 knockdown cells. This suggests that most of the replication timing changes we detect after HP1 RNAi are significant.

**Analysis of repeat density**

Repeat-masked and annotated regions of the genome were downloaded from the UCSC genome browser. The percentage of base-pairs overlapping with such repetitive regions was calculated within each HMM derived region or within 2kb windows.

**Supplementary References**


