

Supplemental Figure legends

Supplementary Figure 1. The following data are shown for the entire chromosome arms *X*, *2L*, *2R*, *3L*, and *3R*. *OrR* CGH, *OrR* RNA Sequencing, *OrR* RNA Pol II ChIP-chip, *OrR* H3K27me3 ChIP-chip, and *OrR* ORC ChIP-Seq. Locations of euchromatic under-replicated regions are marked by vertical light green bars, and heterochromatic under-replicated regions (5 additional loci) are marked with vertical light blue bars. Data are normalized as explained in Fig. 1 in main text.

Supplementary Figure 2. Quantitative PCR of eleven most under-replicated genomic loci confirms aCGH data. qPCR was performed with primers against 11 under-replicated loci, normalized to a control fully replicated locus, corroborating levels of under-replication as indicated by probe closest to the qPCR primer present on the aCGH array.

Supplementary Figure 3. Comparison of the transcription properties of the regions that are under-replicated in salivary glands to the corresponding regions in dividing cell culture lines. **A**, Each under-replicated region was divided into 100 windows and each flanking region (the length of half the corresponding under-replicated region) was divided into 50 windows. The mean expression value of each gene (RPKM) is shown across each window. The KC RNA-Seq data are those from the modENCODE consortium, which is modENCODE_2593 from the web site <http://intermine.modencode.org/release-22/objectDetails.do?id=1166000188>. In the bottom panel the exon densities within the windows are shown. The 34 under-replicated domains are shown in the same order vertically in each panel. **B**, GO enrichment of genes in the under-replicated regions, number of genes and p values for each category.

Supplemental Figure 4. Comparison of H3K27me3 ChIP-chip normalized to either input DNA or to control IgG pulldown. The identical results indicate that either can be used for normalization of the data with similar results.

Supplementary Figure 5. Comparison between ChIP-chip and ChIP-Seq results for *OrR* ORC2 binding sites. Shown is the proximal region of 2L. In both experiments, the under-replicated regions are sparse in ORC2 binding sites. Plotted is the mean probe intensity.

Supplementary Figure 6. A, Ploidy levels of *orc1* mutants and heterozygous sibling controls were quantified using DAPI microdensitometry. The intensity of DAPI staining was measured relative to diploid cells to calculate their ploidy. **B,** The following data are shown for chromosome arms *X*, 2L, 2R, 3L, and 3R. *OrR* aCGH, *orc2^{k43}/Df(3R)Exel6288* aCGH, *orc2^{k43}/Df(3R)Exel6171* aCGH, *orc1* aCGH. Plotted is the mean probe intensity.

Supplementary Figure 7. Quantitative PCR of four under-replicated genomic loci confirms *SuUR* mutant aCGH data. qPCR was performed with primers against four under-replicated loci, normalized to a control fully replicated locus, corroborating recovery of replication in the *SuUR* mutant, as indicated by aCGH.

Supplementary Figure 8. The following data are shown for chromosome arms *X*, 2L, 2R, 3L, and 3R. *OrR* aCGH, *SuUR* mutant aCGH, *SuUR* mutant ORC ChIP-Seq, *SuUR* mutant RNA Sequencing and *SuUR* mutant H3K27me3 ChIP-chip. Locations of euchromatic under-replicated regions are marked by vertical light green bars, and heterochromatic under-replicated regions (5 additional loci) are marked with vertical light blue bars. Data are normalized as described in figures in main text.

Supplementary Figure 9. Comparison between ChIP-chip and ChIP-Seq results for *SuUR* mutant ORC2 binding sites. Shown is the proximal region of 2L. Plotted is the mean probe intensity.

Supplementary Figure 10. Increased replication fork progression in *SuUR* mutants is not due to developmental delays in oogenesis. Egg chambers stages 8-14 were quantified in *OrR*, *SuUR* mutants, or the heterozygote *SuUR/TM3*, with frequency of occurrence plotted on y axis. At least 250 egg chambers were counted from at least four different ovaries.

