SUPPLEMENTAL EXPERIMENTAL PROCEDURES

BAC-mediated recombineering*:*

The *GFP-SuUR* transgenic line was generated through BAC-mediated recombineering (Sharan et al., 2009). First, a *galK* cassette was PCR amplified and used to replace the start codon of *SuUR* in an *attB-P[acman]* clone with a 21-kb genomic region containing the *SuUR* locus (CH322-163L18; pacmanfly.org; bacpac.chori.org). Next the *galK* cassette was replaced with *GFP* lacking a stop codon to create a seamless in-frame fusion between *GFP* and the 5' end of *SuUR* by selecting for loss of *galK*. After fingerprinting and PCR verification, the resulting BAC was injected into a strain harboring the *attP40* landing site (Genetic Services).

Complementation test by copy number analysis:

The function of the GFP-SUUR fusion protein was evaluated by complementation tests of the *SuUR* mutant, measuring copy number of underreplicated regions. Salivary glands were dissected from 10 wandering 3rd instar larvae and genomic DNA was extracted. Quantitative real-time PCR was used to assay copy number at two underreplicated loci as described (Sher et al., 2012).

SUUR antibodies

For immunofluorescence studies of follicle cells anti-SUUR serum was produced in guinea pigs (Covance, Inc). GST was fused to residues 371-583 of SUUR and expressed in *E. coli*. The insoluble fraction was separated by SDS-PAGE and the fusion protein was excised. The resulting protein was used to inject guinea pigs. Full

length 3XFLAG-SUUR-6XHis was produced from Sf9 insect cells and used for affinity purification as described (Moore et al., 1998).

For immunoprecipitations a GST fusion to full-length SUUR was produced in *E. coli* and used to immunize both rabbit and guinea pig. The same protein was used for purification of anti-SUUR antibodies from sera obtained as described (Chalkley and Verrijzer, 2004).

Cytological analysis and microscopy:

Ovaries were fixed in 4% formaldehyde for 15 minutes and extracted in PBS containing 0.1% Triton X-100. Blocking was done in PBS containing 0.1% Triton X-100, 1% BSA and 2% normal goat serum. Affinity-purified guinea pig anti-SUUR antibody was used at a dilution of 1:200. GFP-Booster (Chromotek) was used at a dilution of 1:200. Alexa Fluor-488-conjugated secondary (Life Technologies) was used at a dilution of 1:500. After incubation in secondary antibody, the EdU detection reaction was performed according to the manufactures recommendations using Click-iT EdU Alexa Fluor-555 (Invitrogen). For EdU labeling, ovaries were dissected, pulsed, fixed, permeabilized and detected as above.

Amplification foci quantification: To quantify the fraction of follicle cells incorporating EdU, all egg chambers from the indicated genotypes were prepared and imaged as a Z series in parallel with equal exposure times. After image deconvolution, maximum projection images were used to score for the presence or absence of amplification foci.

Double bar distance meausurements: The distance between double bars has been shown to correlate with replication fork progression (Park et al., 2007). Follicle cells from stage 12 egg chambers were imaged as a Z series and deconvolved. The center-to-center distance between double bars were measured using the NIS-Elements AR 3.2 software from a single plane image. Each data point represents the average of 6-12 measurements per double-bar structure.

CDC45-RNAi Analysis:

For polytene chromosome squashes, flies were raised on standard *Drosophila* cornmeal-yeast-agar medium at 23°C. *Oregon R* and *SuUR* were used as controls for the Western blot analysis. Salivary glands from wandering third-instar larvae were dissected in PBS. Glands were then transferred into a formaldehyde-based fixative (3% lactic acid, 2 mM KCl, 45% acetic acid, 3.7% formaldehyde) for 2 min. The primary antibody dilutions used were as follows: rabbit polyclonal anti-SUUR (E-45) (Makunin et al., 2002), 1:50; mouse monoclonal anti-PCNA (ab29, Abcam) 1:500. The squashes were incubated with secondary goat anti-rabbit and anti-mouse antibodies labeled with Alexa Fluor-488 and Alexa Fluor-568 (Life Technologies). DNA was visualized by DAPI staining.

For Western blotting, we used the following antibody dilutions: rabbit anti-CDC45 (kindly provided by N. Vorobyeva (Vorobyeva et al., 2013), 1:300; mouse anti-PCNA (ab29, Abcam), 1:1000; rabbit anti-SUUR (E-45), 1:250; mouse anti-tubulin, 1:500 (Bx69, kindly provided by H. Saumweber (Tavares et al., 1996); goat anti-rabbit and anti-mouse HRP-IgG conjugates (G21234, G21040, Life Technologies), 1:3000.

ChIP-seq:

Antibodies: Full length 6His-CDC45-Strep-tagII was produced from Sf9 insect cells and used for affinity purification as described (Moore et al., 1998). Anti-Cdc45 serum was kindly provided by M. Botchan, UC Berkeley (Moyer et al., 2006). The affinity purified guinea pig anti-SUUR antibody used for immunofluorescence also was used in ChIP reactions.

Library preparation, sequencing information and bioinformatics analysis: Libraries were prepared using NEBNext ChIP-Seq for Illumina according the manufactures protocol (New England Biolabs). Libraries were sequenced on Illumina's HiSeq platform and reads were mapped to the Apr. 2006 (BDGP Release 5) assembly of the Drosophila genome using BWA (Li and Durbin, 2009).

For salivary gland samples, ChIP peaks were called using MACS (v1.4) with a p-value of 1e-5, using input samples as controls (Zhang et al., 2008). To further increase stringency, peaks with a false discovery rate of <5% were used in the analysis.

For egg chamber samples, MACS2 was used to call peaks with a p-value of 1e-3. Peaks called in amplified regions tended to be broad, likely due to the changes in copy number. Therefore, we quantified SUUR and CDC45 enrichment in 1kb intervals across *DAFC-66D.* To this end, read counts were summed in non-overlapping 1kb windows across *DAFC-66D* and for both ChIP and input samples. Reads in each sample were then normalized to reads per million (RPM). The ratio of normalized ChIP reads relative to input reads were calculated for each window.

IP-mass spectrometry:

The immunoprecipitates were subjected to mass spectrometry as detailed in (Moshkin et al., 2009; Wilm et al., 1996). The criteria for defining a protein as associated with SUUR was that it was present in IP samples using both antibodies and at least five-fold enriched over background mock-IP samples.

CGH copy number analysis:

Tiling arrays containing genomic regions specifically amplified in follicle cells in combination with control regions at 125bp resolution as recommended by Agilent (AMAID# 027763). The resulting data was LOESS normalized and smoothed using the software package Ringo in R.

To quantify the effect loss of SUUR function or SUUR overexpression has on replication profiles at each site of amplification we first determined the highest point of amplification by quantifying the point in the gradient with the maximum copy number. Next, we determined the location on each arm of the amplicon corresponding to half the maximum copy number, allowing quantification of the distance between the half maximum point of each arm of the replication gradient. This provides a quantitative measure of fork progression at all sites of amplification.

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. SUUR is localized to sites of gene amplification, Related to Figure 1.

(A) Specificity of affinity purified anti-SUUR antibody. Representative image of wild-type and *SuUR* mutant stage 11 follicle cells. Replication forks are marked by EdU incorporation (red), SUUR by immunostaining (green) and DNA by DAPI staining (blue). Individual channels are shown as labeled. Samples were imaged with equal exposure times and brightness was linearly adjusted for presentation. Scale bar = 2μm.

(B) Localization of a functional GFP-SUUR fusion protein in a stage 13 follicle cell. The arrowhead marks SUUR constitutively localized to heterochromatin. Replication forks are marked by EdU incorporation (red), GFP immunostaining (green) and DNA by DAPI staining (blue). A single representative follicle cell nucleus is shown. Individual channels are shown as labeled. Scale bar = 2μm

(C) *GFP-SuUR* complementation assay. Quantitative PCR measurements of copy number at *36C* and *36D* from wandering third-instar salivary glands. Copy number changes are relative to fully replicated diploid embryonic DNA. The graph represents the average of two biological replicates. Error bars represent the SEM. GFP-SUUR is functional, because it leads to reduced copy number at the two normally underreplicated domains.

Figure S2. SUUR affects replication fork progression, Related to Figure 3

Array based comparative genomic hybridization (aCGH) of all follicle cell amplicons, with copy number shown in $\log_2$. DNA extracted from stage 13 egg chambers of the

indicated genotypes is compared to diploid 0-2h embryonic DNA. Quantification of replication profiles is listed in Table S1.

Figure S3. Loss of SUUR function does not affect fork rate but results in prolonged EdU incorporation during gene amplification, Related to Figure 3

(A) Quantification of the double-bar distance from stage 12 follicle cells. Each data point represents the average of 6-12 measurements from the center-to-center distance of an individual double bar. Wild-type mean = 0.64 μm (±0.1SD); *SuUR* mean 0.69 (±0.06SD). A Mann-Whitney test determined the mean values are not statistically significant (P = 0.4283).

(B) The fraction of amplifying (EdU positive) follicle cells during each stage of gene amplification quantified from two biological replicates. n > 200 (mean +/- SEM).

Figure S4. DNA damage profiles for the major chromosome arms, Related to Figure 4

aCGH and γH2Av ChIP-seq profiles from wild-type and *SuUR* mutant wandering third-instar larval salivary glands. Each chromosome arm is shown as indicated, except the fourth chromosome. IgG serves as a control for nonspecific binding. ChIP-seq peaks were called by MACS relative to input DNA. aCGH data are from (Sher et al., 2012).

Table S1. Quantitative analysis of CGH profiles at all sites of gene amplification, Related to Figure 3.					
OrR	log2 copy number at peak	Left arm (bp)	Right arm (bp)	half max to half max (bp)	difference relative to *OrR* (bp)
22B	1.2852	17840	27810	45650	0
30B	1.2897	31090	47620	78710	0
34B	1.8756	35680	25660	61340	0
62D	1.2393	44210	28980	73190	0
66D	4.2808	36300	39340	75640	0
7F	3.0303	41490	26260	67750	0
SuUR					
*22B**	0.0161	NA	NA	NA	NA
30B	1.3443	34700	55820	90520	11810
34B	2.0035	51190	48710	99900	38560
62D	1.3325	52580	47180	99760	26570
66D	4.4111	53660	46100	99760	24120
7F	3.0241	46360	53470	99830	32080
4X-SuUR					
*22B**	0.1379	NA	NA	NA	NA
30B	1.4703	20690	26640	47330	-31380
34B	2.246	20140	15990	36130	-25210
62D	1.3557	24250	15800	40050	-33140
66D	5.0383	17630	21410	39040	-36600
7F	3.5175	20980	19420	40400	-27350
6X-SuUR					
*22B**	0.0361	NA	NA	NA	NA
30B	1.4724	18760	22340	41100	-37610
34B	2.2483	23180	16310	39490	-21850
62D	1.2927	28810	16140	44950	-28240
66D	4.9185	20880	23660	44540	-31100
7F	3.3831	22400	21180	43580	-24170

**DAFC-22B* does not amplify in the *SuUR* mutant and overexpression strains. The amplification of this region has been shown previously to be strain specific (Kim et al., 2011)

Figure S1

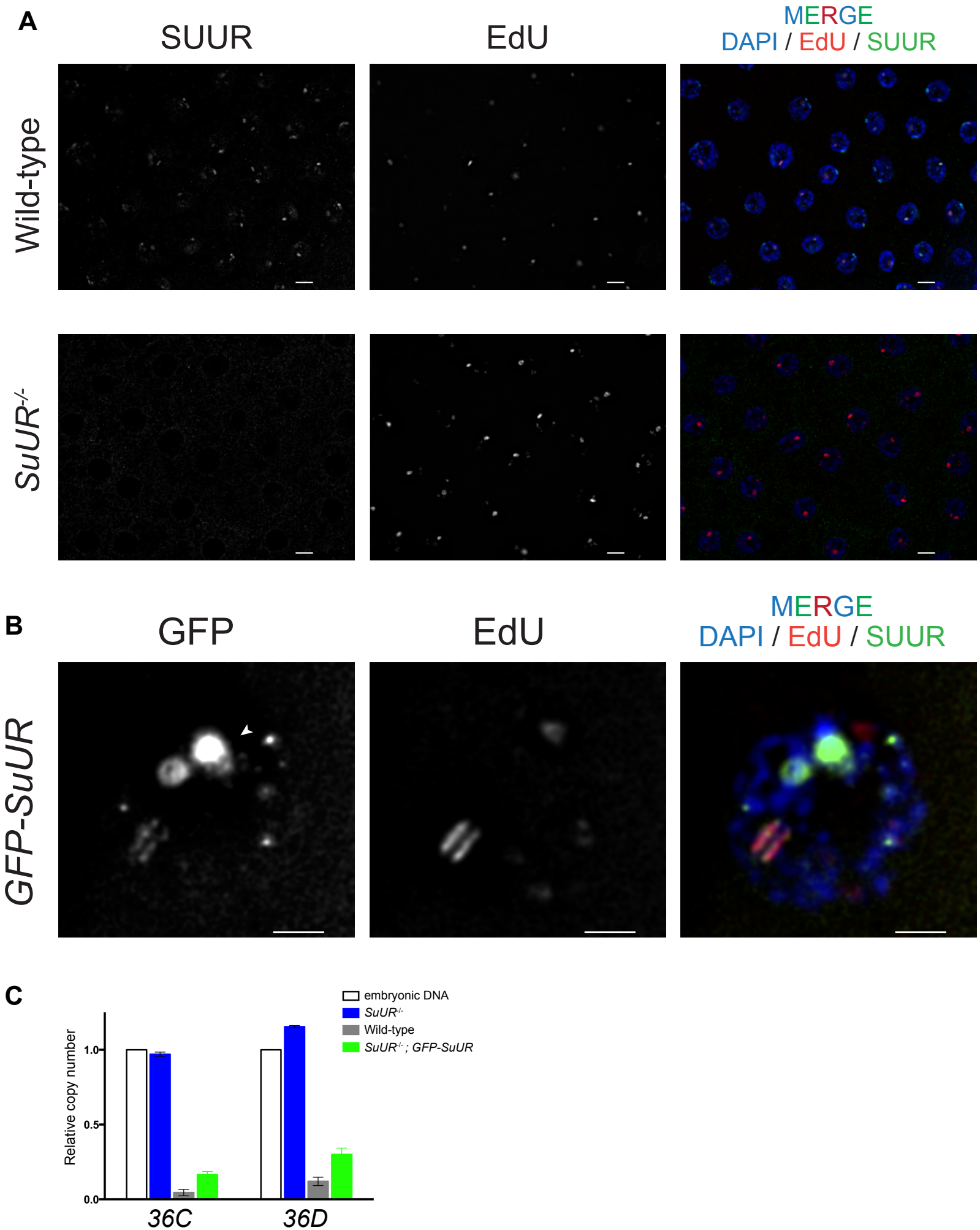

A
SUUR
EdU
MERGE
DAPI / EdU / SUUR
Wild-type
SuUR-/-
B
GFP
EdU
MERGE
DAPI / EdU / SUUR
GFP-SuUR
C
embryonic DNA
SuUR-/-
Wild-type
SuUR-/- ; GFP-SuUR
Relative copy number
1.0
0.5
0.0
36C
36D

Figure S2

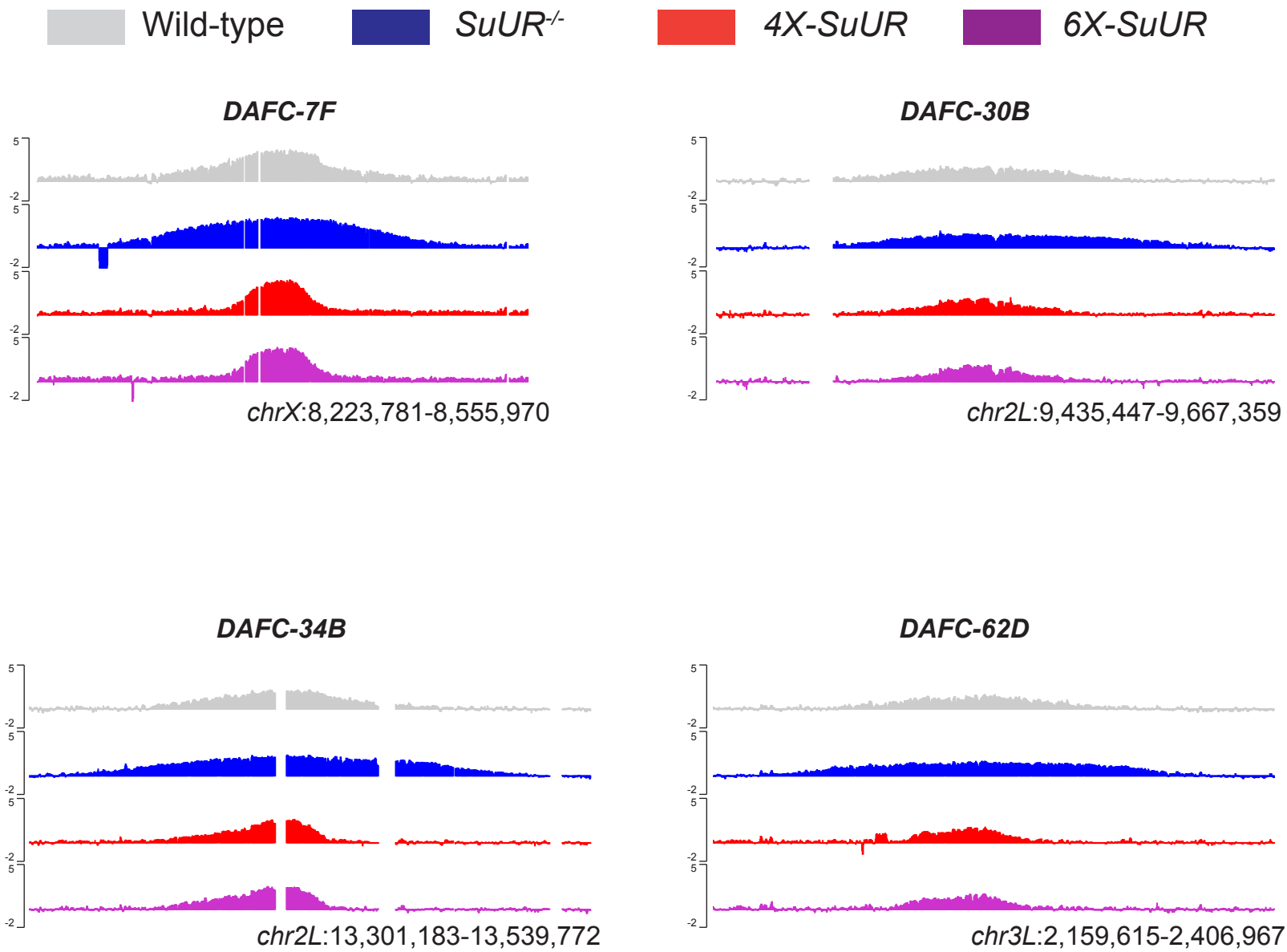

Wild-type
SuUR-/-
4X-SuUR
6X-SuUR
DAFC-7F
chrX:8,223,781-8,555,970
DAFC-30B
chr2L:9,435,447-9,667,359
DAFC-34B
chr2L:13,301,183-13,539,772
DAFC-62D
chr3L:2,159,615-2,406,967

Figure S3

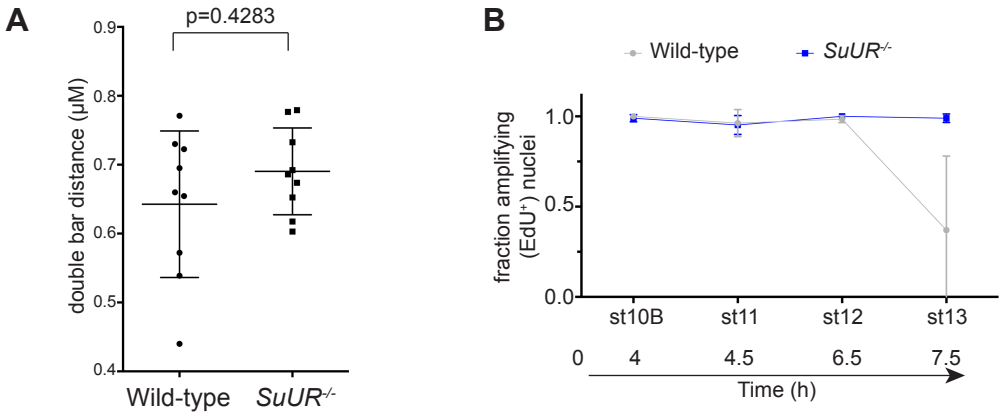
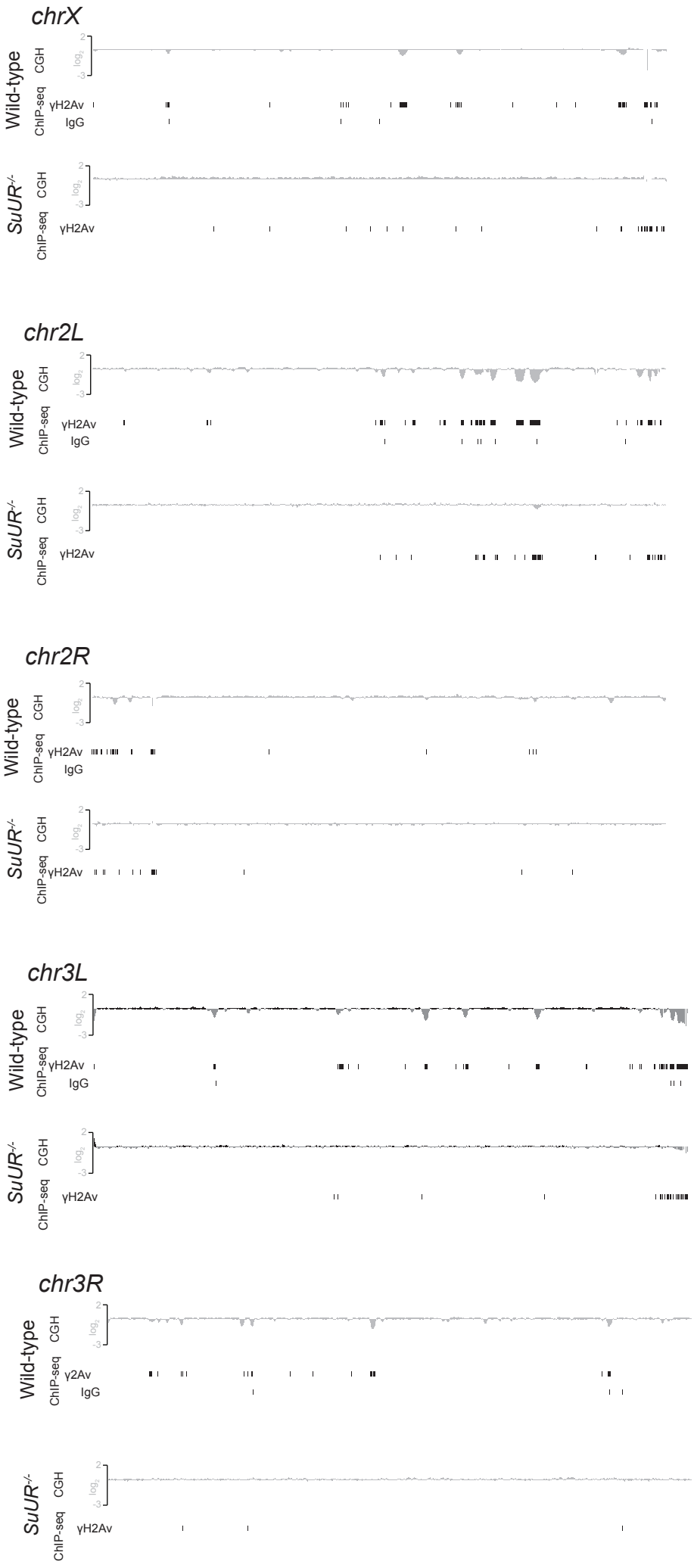

A
p=0.4283
double bar distance (µM)
0.9
0.8
0.7
0.6
0.5
0.4
Wild-type
SuUR-/-
B
Wild-type
SuUR-/-
fraction amplifying (EdU+) nuclei
1.0
0.5
0.0
st10B
st11
st12
st13
0
4
4.5
6.5
7.5
Time (h)

Figure S4


chrX
Wild-type
CGH
ChIP-seq
γH2Av
IgG
SuUR-/-
chr2L
chr2R
chr3L
chr3R
γ2Av