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Supplemental Information

Dynamics of Nucleosome Positioning Maturation following Genomic Replication

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Yeast Strains

All experiments (except the experiment in Supplementary Figure S2C) were done with the strain PV1 (MATa ade2-1 trp1-1 can1-1000 leu2-3,112 his3-11,15 GAL psi+ RAD5+ ura3::URA3/GPD-TK(7x) AUR1c::ADH-hENT1 bar1D::KanR) that contains the Thymidine transporter hENT1 and the Thymidine Kinase TK, necessary for EdU processing in yeast. PV1 was derived from strain ES3087 (MATa ade2-1 trp1-1 can1-1000 leu2-3,112 his3-11,15 GAL psi+ RAD5+ ura3::URA3/GPD-TK(7x) AUR1c::ADH-hENT1), provided by Etienne Schwob. The *Bar1* gene was replaced with the Kanamycin resistance cassette *KanR* by homologous recombination (cloning primers:
F-TCATACCAAAATAAAAAGAGTGTCTAGAAGGGTCATATACCAGCTGAAGCTTCGTACGC; R-ATATTTATATGCTATAAAGAAATTGTACTCCAGATTTCTTGGCGCGAGGATCGTAATAAG on plasmid pCM224) and KanR insertion was verified by PCR (outside verification primers: F-GAGAAAGCACGTCGAGCCT; R-TATCAGTAAAACTCCCCTTG
inside verification primers: KanF- TATGGGTATAAATGGGCTCGCG and KanR-AGGAATCGAATGCAACCGGC).
Mutant strains from Figure 4:
*hir1*Δ: strain AC5 (MATa ade2-1 his3-11,15 leu2-3,112 ura3-1 TRP+ can1-100 GAL psi+ RAD5+ URA3::GDP-TK(7x) AUR1c::ADH-hENT1 Δhir1::Nat Δbar1::kanR);
*chd1*Δ: strain RZ12 (MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 GAL psi+ RAD5+ URA3::GDP-TK(7x) AUR1c::ADH-hENT1 Δchd1::LEU2);
*ioc3*Δ: strain RZ15 (MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 GAL psi+ RAD5+ URA3::GDP-TK(7x) AUR1c::ADH-hENT1 Δioc3 ::KanR).
RZ12 and RZ15 were obtained from crosses of ES3086 (MATα ade2-1 trp1-1 can1-1000 leu2-3,112 his3-11,15 GAL psi+ RAD5+ ura3::URA3/GPD-TK(7x) AUR1c::ADH-hENT1; from E. Schwob) with DY10675 (MATa ade2, his3, trp1, ura3, can1, leu2, Δchd1::LEU2; from David Stillman) and Y1244(MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100, Δioc3 ::KanR; from Manfred Schmid), respectively.
The *Hir1* gene in strain AC5 was replaced with the nourseothricin resistance cassette NatMX by homologous recombination (cloning primers: first
Leu2NatF- CCTTGTTCATGTGTGTTCAAGTTAATTAAGGCGCGCCAGA and Leu2NatR-TAATGTTAAAGTGCAATTCTCACTAGTGGATCTGATATCA on the pAG25 plasmid and then
HIR1Dleu2F-AGCATAATAAAATTGCCAGTAACCAAAGGTCTCTGATAACCCTTGTTCATGTGTGTTCAA
HIR1Dleu2R-GGAAAAAACTTGTCCAAAGGAAGGGGTATAAGCTTATTATAATGTTAAAGTGCAATTCT
on the product of the first PCR) and NATMX insertion was verified by PCR (outside verification primers:
HIR1Dleu2VF- TAAAATAATTAAGGCTTACC ; HIR1Dleu2VR – CAATGCGAATACTACAATGA
inside verification primers: NatF- GAGGTGCCGGTGGACCCGCC; NatR- TGGATCGCCGGTGCGTTGAC).

The experiment in Supplementary Figure S2C was done with the CvY61HO strain (MATa ade2-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hisG TRP1::BrdU–Inc (BrdU= HSV-TK +hENT1) pJH132 (Gal::HO URA3)). The CvY61 strain from Oscar Aparicio was transformed with the plasmid pJH132 from Jim Haber to obtain CvY61HO.

Yeast culture

For the synchronization experiment in Figure 1 cells were grown over night at 30°C in Synthetic Complete-URA + Dextrose (SCD-URA) media to OD 0.3. After 3.75hrs at 30°C with α factor (0.25μg/ml), cells were pelleted and transferred into preheated and premixed SCD-URA+ 10μM EdU(Carbosynth), with freshly added 20μg/ml pronase (Sigma). 200ml aliquots were taken right before release and then at regular intervals after release starting at 25min from media change. Cell aliquots were fixed with 1% formaldehyde at indicated time points, incubated for 20min at 30°C and quenched with 125mM Glycine. Cell pellets were then washed with water and flash frozen in liquid nitrogen and kept at -80°C until further processing.

In the EdU-Thymidine pulse chase experiments, cells were grown in SCD-URA over night at 30°C to an OD of 1.0. The next day the culture was diluted to OD=0.25 and cells were allowed to double once. Cell pellets were then transferred to preheated and premixed SCD-URA+ 10μM EdU media. Thymidine (to a final concentration of 5mM) was added after 5 or 20min incubation with EdU at 30°C and incubated for another 5 or 10 min. Cells were then pelleted and transferred into fresh media with 5mM Thymidine (and 3μg/ml thiolutin (Abcam) when indicated). 200ml aliquots were taken at indicated time points and fixed as above. Time points 0-4 min and 8-22 min for the 20min EdU pulse experiment in Figure 2 were done on different days. A 5min EdU pulse and 5min Thymidine chase were used for experiments with deletion mutants from Figure 4.

MNase digestion

700µl 0.5mm glass beads were added to frozen cell pellets, re-suspended in 700µl cell breaking buffer (20% glycerol 100mM Tris-HCl 7.5). Cells were then spheroplasted by bead beating in the Bullet Blender (Next Advance) for 4x3min at strength 8 in the cold room. Spheroplasts were recovered by puncturing the cap of the tube and spinning into 5ml eppendorf at 1000rpm for 3 min. Cells were then centrifuged 5min at maximum speed in a micro centrifuge and the clear top layer was discarded, each pellet was re suspended in 600ul NP buffer (50mM NaCl, 10mM Tris-HCl pH 7.4, 5mM $MgCl_2$, 1mM $CaCl_2$,0.075% NP-40, 0.5mM sperimidine, 1mM βME).The amount of MNase (Worthington Biochemical) was adjusted to the cell density in each tube in order to obtain 80-90% mononucleosomal sized fragments after 20min incubation at 37°C. The reaction was stopped with a 5x stop solution (5%SDS, 50mM EDTA, 1.3 mg/ml proteinase K) and incubated over night at 65°C. DNA was extracted with Phenol- Chlorofom- Iso amyl alcohol (PCI) and precipitated with Sodium acetate and Isopropanol. Purified DNA was treated with RNAse A (Qiagen) and CIP (NEB) and purified once more with PCI. Fragments shorter than 100bp were removed with SPRI select beads (BeckmanCoulter) or homemade MagNA beads (SeraMag Speed beads, Thermo Scientific,(Rohland and Reich, 2012)), and 150bp mononucleosomal sized fragments were subsequently purified from 2% agarose gels.

Biotin conjugation to EdU with the Click reaction

10µl DNA solution was mixed with 10µl biotin azide (quanta biosystems) solution in DMSO/tBuOH(3:1). For each pmole of DNA, we added 1mM biotin azide solution (for example for 20pmoles of DNA in 10µl, 10µl 20mM biotin azide were added). 10µl CuBr solution (10mM CuBr (from freshly made stock), 10mM TBTA (Eurogentec), 10mM Ascorbic acid (from freshly made stock) in DMSO/tBuOH 3:1) were then added to the DNA-biotin azide mix and the reaction was shaken for 2hrs at 37°C. 300µl 10mMTris-HCl pH7.5, 8µl 0.25% linearized acrylamide solution, 33µl 3M Sodium Acetate pH5 and 1ml 100% cold EtOH were then added to the Click reaction and DNA was precipitated at -20°C overnight.

Deep sequencing library construction

Biotinylated DNA pellets were re suspended in 25µl TNE0.2 buffer (200mM NaCl, 10mMTris-HCl 7.5, 1mM EDTA) and mixed with 25µl Streptavidin coated magnetic beads (NEB, pre washed in TNE0.2 and blocked with 100µg/ml salmon sperm DNA). The DNA and bead mixture was shaken for 30min at RT, and beads were washed 2x with 0.25ml TNE0.2 buffer and re suspend in 35µl 10mM Tris-HCl pH8. All the subsequent steps were done with DNA attached to the beads. DNA fragments were blunt ended and phosphorylated with the Epicentre End-it-Repair kit (1X buffer, 0.25mM dNTPs,1mM ATP, 1µl Enzyme mix in a 50µl reaction) for 1hr at RT. Beads were washed 2x with TNE0.2 and re suspended in 43µl 10mM Tris-HCl pH8. Adenosine nucleotide overhangs were added using Epicentre exo- Klenow for 45min at RT (with 0.2mM dATP) followed by two TNE0.2 washes and re suspension in 15µl 10mM Tris-HCl pH8. Illumina Genome sequencing adaptors with in line barcodes (

PE1-NNNNN: PhosNNNNNAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAG

PE2-NNNNN: ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNT

, NNNNN indicates the position of the 5bp barcode, (IDT)) were then ligated over night at 16°C using the Epicentre Fast-Link ligation kit. The ligation reaction was washed 2x with TNE0.2 and beads were re suspended in 20µl water. DNA was then subjected to a primer extension reaction with dUTP to separate the nascent strand from its complement (1X NEB buffer2, 0.1µg/µl 5'phosphorylated random hexamers (IDT), 1.72 µM Illumina PE primer 2.0 (IDT), 0.6 mM dNTPs (dUTP instead of dTTP) and 2U/µl Klenow 5NEB). DNA was denatured and annealed to the primers prior to enzyme addition and the reaction was incubated 1.5 hrs at 37°C. Beads were washed 4x and re suspended in 20µL water. The dUTP containing strand was degraded with USER enzyme (NEB) and beads were re suspended after washing in 20µl 10mM Tris-HCl pH8.

The remaining nascent DNA strand was amplified with the Phusion enzyme (NEB) for 18 PCR cycles with Illumina PE1 (AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT) and PE2 (CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT) primers (IDT). Only 2µl of the bead suspension was added to the 50µl PCR mix. Amplified libraries were purified from a 2% agarose gel and fragment size and library concentration were determined from a Bioanalyzer (Agilent) scan and Qubit fluorimetry measurements, respectively. Sample libraries were mixed in equimolar amounts and the final concentration was confirmed by qPCR. Mixed library samples were sequenced on a HiSeq 2000 (2x75bp) (Illumina) at the CNAG, Barcelona, Spain or on a Next Seq sequencer (2x50bp) (Illumina) in O. Rando's laboratory.

***In vitro* and *in vivo* testing of library strand specificity**

The 240bp fragment from the Hygromycin resistance gene was PCR amplified with primers F (ATCGCGCATATGAAATCACGCCATG)

and R (GACATTGGGGAATTCAGCGAGAGCCTGACC) EdUTP (Jena Bioscience) was incorporated with F or R primer extension of the PCR fragment (1xNEB buffer 2, 1.2mM dNTPs (EdUTP instead of dTTP), 0.5µM primer). The primer was annealed to the template prior to the addition of Klenow DNA polymerase (2U/µl, NEB) and the reaction was incubated 1.5 hrs at 37°C. Biotin conjugation to EdU and strand specific deep sequencing library construction were performed as described above. Strand specificity of the library was checked by qPCR (SYBR Fast qPCR, KAPA Biosystems) with primer pairs: Fsense (TCCGCGACCGGCTGCA) or Rsense (TGCAGCCGGTCGCGGA) with Illumina primer PE1 or PE2 as indicated in Figure S2A ad B.

For *in vivo* incorporation of EdU into one strand of the MATalpha1 locus, mid log cells grown in rich dextrose media were treated with nocodazole (15µg/ml) for 3.5 hrs at 30°C. An aliquot was fixed with formaldehyde for the dextrose negative control library and the rest of the G2/M arrested cells were switched to galactose media supplemented with 10µM EdU and nocodazole and incubated for 2hrs at 30°C. Cells were then fixed with formaldehyde, and treated with MNase as above. Isolated DNA fragments were used for strand specific library construction as described above. Strand specificity of the MATalpha1 locus in the library was checked with primers EdU (GGTTAAGATAAGAACAAAGAATGATGCT) or dU (AGCATCATTCTTTGTTCTTATCTTAACC) with the Illumina PE1 primer. The average Ct from reactions with primers specific for the SSL2 gene(SSL2 F (TTTGGATCTCGCCAAGTGACGGTA) and SSL2 R (TAGGCTCTGCAATGGTGACCAGAA)) in combination with PE1 was used as the normalization control, as SSL2 fragments in both orientations relative to the PE1 sequence should be equally represented in the library.

Flow Cytometry profiling

Cell culture aliquots were fixed with 70% EtOH. Cells were washed 2X with PBS+10%EtOH and resuspended in 250µl PBS and treated with RNAse A (0.8µg/µl) and proteinase K (0.2 µg/µl) for 2hrs at 37°C. Cells were then pelleted and re suspended in 250µl PBS. 5µl was used for cell counting. 2.5 million cells were taken for fluorescent labeling of incorporated EdU and re-suspended in 50µl Click reaction solution (16mM $CuSO_4$, 40µM FAM-azide (Lumiprobe), and 80mM Ascorbic Acid in PBS) and incubated for 20min at RT in the dark. Excess dye was washed 3x with PBS+10%EtOH and labeled cells were re suspended in 200µl PBS. Another batch of 2.5 million cells was re-suspended in 2µM Sytox Green in PBS for monitoring DNA content. Labeled cells were sonicated in a cup sonicator 3x2sec at 45% strength immediately before FACS measurements (FACSCalibur (BD Biosciences), FL-1 filter, FSC size cutoff: 70).

We measured the distribution of the FL1-W or FL1-A parameters for FAM-EdU or Sytox fluorescence, respectively. The analysis was done with in house Perl and R scripts.

Gene Expression Microarray hybridization

PV1 cells were arrested in G1 with α factor as above. Genomic DNA was isolated from G1 arrested flash frozen cell pellets with Phenol/Chloroform, and sonicated with the Bioruptor Pico cup sonicator (200µl at 200ng/ µl, 30"ON 30"OFF at 4°C). Cells were released into S phase in media with or without 10µM EdU, as above. 50ml aliquots were flash frozen in liquid nitrogen 32 and 40min after release, for RNA isolation.

Total RNA was isolated from frozen cell pellets with Trizol. Frozen cell pellets were re-suspended directly in Trizol and bead beated in the Bullet Blender (Next Advance) as above. RNA was then purified and DNAseI treated with the RNAeasy Column purification kit (Qiagen).

We used ~30 µg of total RNA for each expression array. RNA was reverse transcribed using oligodTs(0.15µg/µl final) as primers. Reactions (0.5mM dNTP (N=A,G,C),0.2mMdTTP and 0.3mM amino-allyl dUTP (SIGMA),6µg/ml Actinomycine D (SIGMA), 10mMDTT, 1XFS buffer and 10U/µl Superscript III (Life technologies)) were incubated at 50°C for 2hrs. RNA was then degraded with NaOH at 65°C (10µl 1N NaOH and 10µl 0.5M EDTA into 30µl reactions), the solution was neutralized with HEPES pH=7.5 (25µl 1M stock) and the buffer was exchanged for water in Amicon30 centricon spin columns. The resulting cDNA was dye-coupled with Cy5 or Cy3 NHS-esters and purified as described previously (Liu et al., 2005).

The Cy5 or Cy3 labeled cDNA was mixed with Cy3 or Cy5 labeled genomic DNA, respectively (genomic DNA labeling: 2µg (quantified in the Qubit fluorimeter) PV1 genomic DNA from the G1 cell cycle phase in Klenow NEB buffer, 0.3 µg/µl random hexamers, 0.12 mM dNTP (N=A,G,T),0.06mM dCTP and 0.06mM Cy5 or Cy3 conjugated dCTP (GE healthcare), and 1U/µl Klenow enzyme (NEB); incubated 2hrs at 37°C and cleaned up in Amicon-30 centricon spin columns). Labelling efficiency of cDNA and genomic DNA was verified in the Nanodrop spectrophotometer. The labeled mixture was combined with hybridization buffer, following the Agilent microarray hybridization protocol and hybridized to Agilent 8x15K yeast Gene Expression arrays at 65°C for 16hrs. Images were scanned at 5µm with the InnoScan 710 MicroArray scanner (Innopsys) and processed with the Mapix software. Data was normalized by dividing the Cy5/Cy3 ratio for each probe with the average Cy5/Cy3 ratio for the whole array. The GEO accession number for the microarray data is GSE79384.

Data analysis

Sequences were aligned to *S.cerevisiae* genome using BLAT. We kept reads that had at least one uniquely aligned 100% match in the paired end pair. Read count distribution was determined in 1bp windows and then normalized to 1 by dividing each base pair count with the genome-wide average base-pair count. Forward and reverse reads were treated separately.

The repetitive regions map was constructed by "blating" all the possible 70 or 45bp sequences of the yeast genome and parsing all the unique 70 (45)bp sequences. All the base coordinates that were not in those unique sequences were considered repetitive.

Since we noticed a linear proportionality between average correlations to the standard per time point and sequencing read file sizes, all correlation values were corrected for variability in sequencing read number between time points within each dataset. Pearson correlations of nascent chromatin gene profiles with a mid-log standard were corrected for differences in sequencing read numbers as follows: The slope *a* of the regression line: $avgcorrel_t = a * fs_t + b$; (where $avgcorrel_t$ is the average correlation for time point *t* within a dataset (libraries for each time point within one time course experiment, i.e. dataset ,were multiplexed and sequenced in one lane) and fs_t is the sequencing read file size in Gb of time point *t*), was determined using the least square method linear fit. The differences in sequencing read file sizes in different time points were then normalized to the average file size of the dataset by modifying the correlation value for each gene *i* within each time point *t* using the following formula:

$Corrected\ Correlation_i = Correlation_i - (fs_t - avgfs_t) * a$, where $avgfs_t$ is the average file size of all time points in the same dataset.

We used the following criteria for filtering genes that are replicated from efficient origins: 1. Efficient origins were defined as origins whose read density peak heights were bigger or equal to 0.6 at the 25min time point in the NChAP experiment with synchronized cultures (**Figure 1**, read densities were normalized to the maximum peak height per chromosome, the highest peak on every chromosome has therefore an efficiency of 1 or 100%). 2. Genes that were within the boundaries of the read density area around efficient origins at the 25min time point were considered as being replicated from that particular origin in most cells. The resulting dataset matches highly efficient origins from a previously published study in which origins were identified using Okazaki fragment mapping (Figure S10A) (McGuffee et al., 2013).

Replication timing in Figures 6 and S10 was determined as described in (Yang et al., 2010). Nascent read distribution densities normalized to 1 per chromosome from each time point in Figure1 were binned in 400bp windows and fitted to the Hill equation: $\%replicated = \frac{1}{1+\left(\frac{t_{50}}{t}\right)^r}$, where *t* is the time since release from arrest and the t_{50} is replication timing, i.e. the time since release at which that 400bp segment has been replicated in 50% of the population. Our replication timing values also correlate with published data (Figure S10B, (Raghuraman et al., 2001)

Analysis was done using in-house Perl and R scripts (available upon request). Sequencing Data are deposited at GEO Database under the number GSE74090.

Statistical Analysis

Pairwise Pearson correlations were calculated for all time points (data points every 30bp) in all datasets presented here (Supplementary Figure S6A, Table S2). Pairwise t-tests from Figures S6B-C and S11 were done using the Perl module Statistics::Ttest. The input data for S6B-C and S11 are in Tables S1 (degrees of freedom:2492; alpha level=0.05) and S3 (degrees of freedom:210; alpha level=0.05), respectively.

Box plots from Figures S6B, S7 and S11 were generated using the R boxplot function; input data for S6B and S7 are from Table S2 and for S11 from Table S3.

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List of Supplemental Tables

Table S1: Pair-wise correlation between time points, related to Figure S6A

Table S2: Nascent nucleosome profile correlations to the total chromatin standard for each gene, related to Figure 2A

Table S3: Leading and Lagging gene copy correlations to the 22min time-point from the 20min EdU pulse experiment (replicate 1), related to Figure 5A

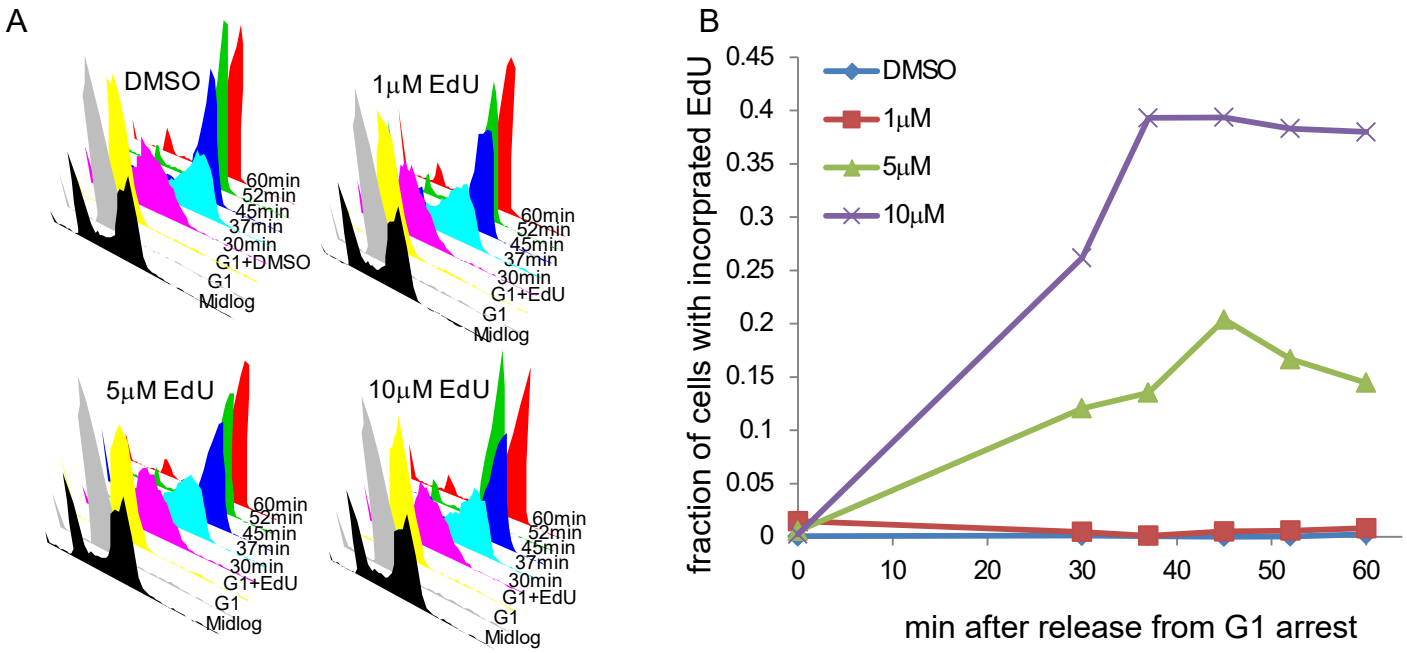


Figure S1 (related to Figure 1): EdU incorporation does not perturb S-phase progression. A. FACS profiles of DNA content in synchronized cell populations after release from G1 arrest in the presence of indicated amount of EdU (DMSO is the mock treatment). **B.** Fraction of cells that incorporated EdU after release from arrest measured by FACS of FAM conjugated to EdU.

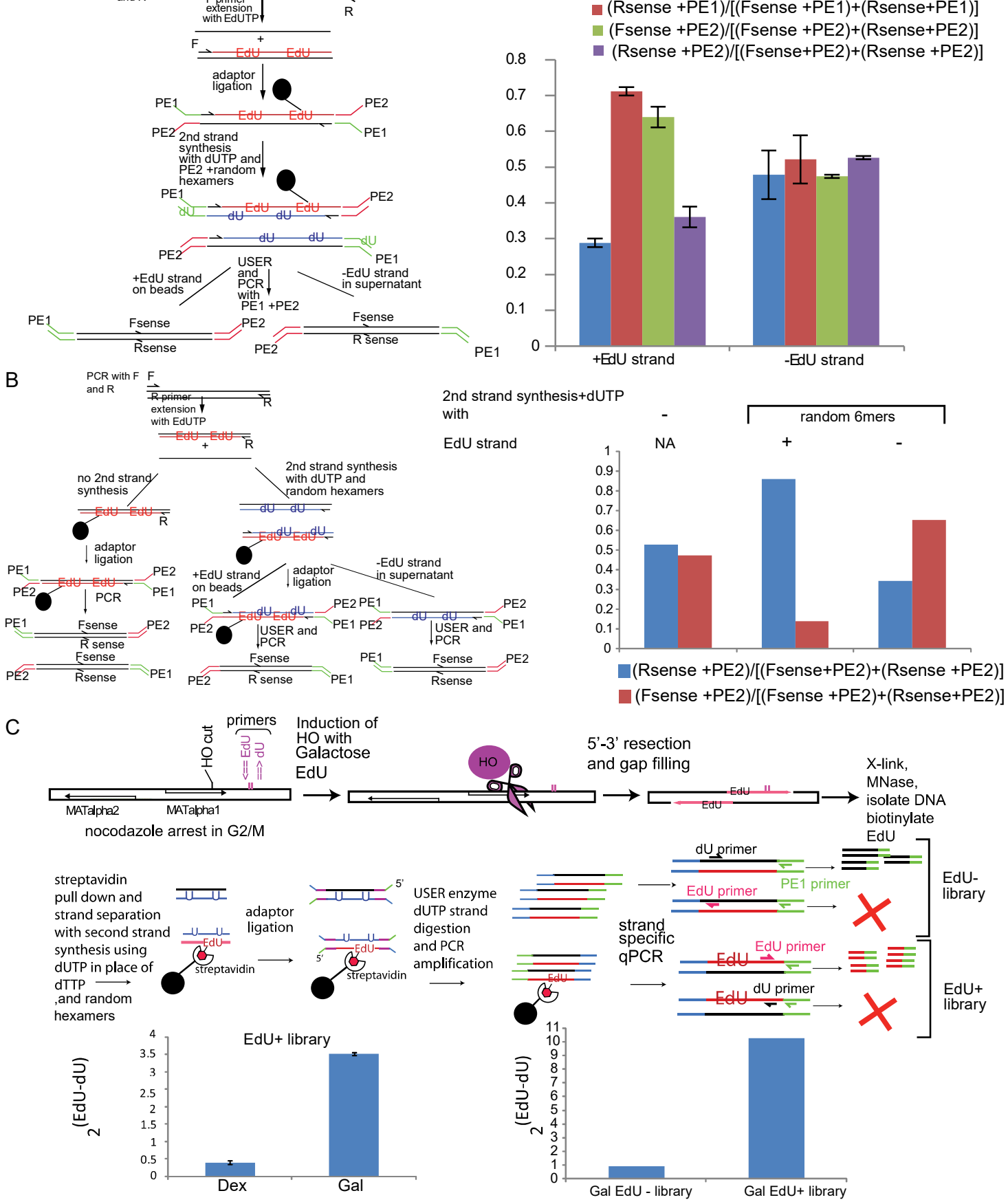


Figure S2 (related to Figure 1): Test for strand specificity of NChAP libraries. A. EdU was incorporated using primer extension with the F primer of a PCR template as shown in the diagram on the left. qPCR results of resulting libraries (bottom row of the diagram) are shown on the right. The height of the bar represents the ratio $2^{-Ct1or2}/(2^{-Ct1}+2^{-Ct2})$ of reactions with indicated combinations of primers illustrated in the diagram, error bars: standard deviation of 2 biological replicates. The -EdU fraction was not used in further NChAP experiments since it shows no strand specificity. We suspect that a fraction of EdU containing strands has detached themselves from streptavidin beads during the primer extension reac-tion and the resulting supernatant contains both the nascent strand and its complement. **B.** As in A but EdU was incorporated in the other strand using the R primer and strand separation through primer extension with random hexamers was performed prior to deep-sequencing adaptor ligation. **C.** In vivo incorporation of EdU into one strand at the MATalph1 locus (top row). The resulting libraries were used as templates in qPCR with the PE primer in combination with the EdU or dU primers as depicted in the diagram. Ct values from a reaction using primers complementary to the W and C strands of the SSL2 gene were used as the internal normalization control. Fragments containing the strand that incorporated EdU are enriched

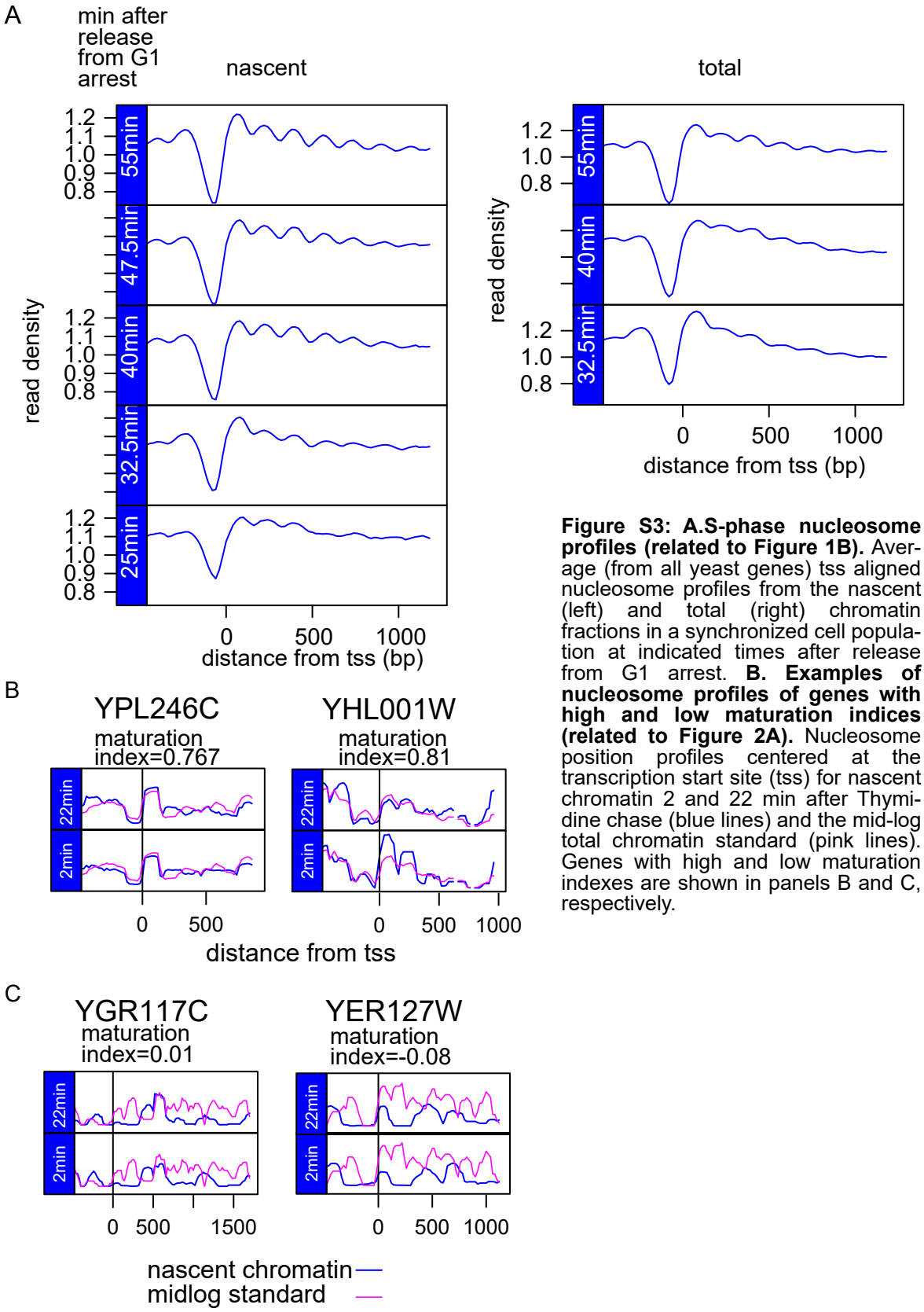


Figure S3: A.S-phase nucleosome profiles (related to Figure 1B). Average (from all yeast genes) tss aligned nucleosome profiles from the nascent (left) and total (right) chromatin fractions in a synchronized cell population at indicated times after release from G1 arrest. **B. Examples of nucleosome profiles of genes with high and low maturation indices (related to Figure 2A).** Nucleosome position profiles centered at the transcription start site (tss) for nascent chromatin 2 and 22 min after Thymidine chase (blue lines) and the mid-log total chromatin standard (pink lines). Genes with high and low maturation indexes are shown in panels B and C, respectively.

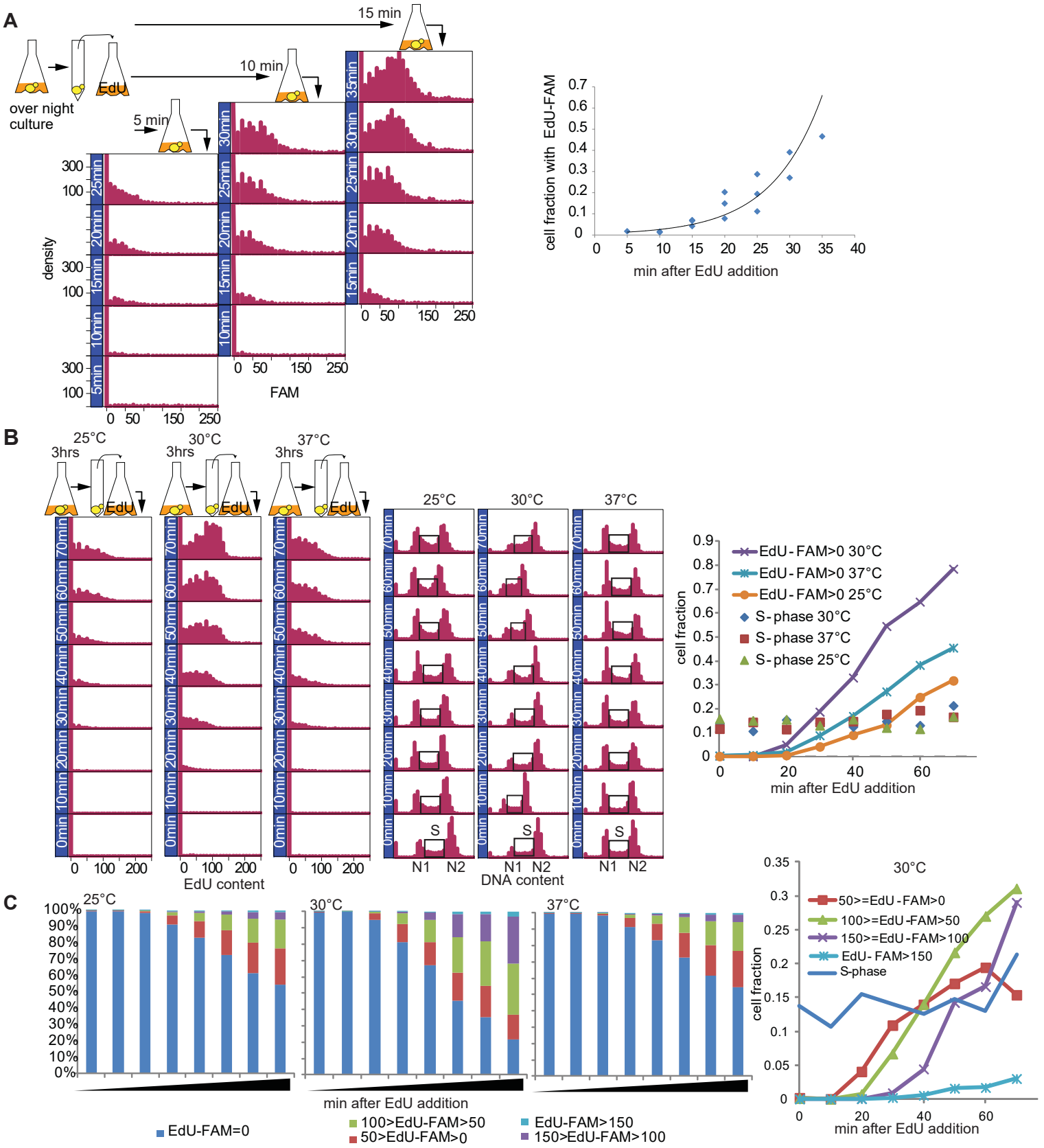


Figure S4 (related to Figure 2): Kinetics of EdU incorporation in asynchronous cultures. A. Density distribution of Fluorescein (FAM) labeled cells after incubation of asynchronous cell populations with EdU (10μM) for indicated times (5,10 and 15 min). Aliquots were taken in 5min intervals after the initial incubation. EdU stayed in the culture throughout the time course. FAM was conjugated to EdU in ethanol fixed cells using Click chemistry. The plot shows the increase in the fraction of FAM positive cells over time, with a 15 min lag between EdU addition and the start of EdU incorporation into cells. **B.** EdU incorporation at different temperatures. Cells were grown over night at 30ºC and then shifted to 25ºC or 37ºC or kept at 30ºC and incubated for 3 hrs. 10μM EdU was subsequently added and FACS aliquots were taken every 10min after EdU addition. EdU-FAM density distribution is shown on the left and corresponding DNA content (detected with Sytox Green labeling) is shown on the right. The black rectangles show the fraction of cells in S-phase (the quantification is shown in the plot on the right). The S-phase fraction is the same at all three temperatures, but the G1 fraction (indicated by the N1 peak in the DNA content density distribution plots) is higher at 25ºC and 37ºC. This suggests that both G1 and S-phase are longer at suboptimal temperatures. There is a lag time of 20 min before EdU could be detected in all three cultures. The lag time is probably due to the length of time it takes for cells to import EdU and process it into EdUTP for incorporation into nascent DNA. The fraction of EdU-FAM positive cells reaches the levels of S-phase cells 25, 40 and 50 min after EdU addition at 30ºC, 37ºC and 25ºC, respectively (plot on the right), reflecting slower EdU import at 25ºC and 37ºC.**C.** EdU-FAM intensity distribution at different temperatures. The bar graph shows a progressive shift to higher EdU-FAM intensities as DNA replication progresses in cells that have incorporated EdU the earliest. The low intensity fraction reaches equilibrium 40 min after EdU addition at 30ºC as equal numbers of cells enter and progress through S-phase and shift to higher EdU-FAM intensities (plot on the right). A delayed intensity shift at 25ºC and 37ºC compared to 30ºC is consistent with slower S-phase progression at 25ºC and 37ºC.

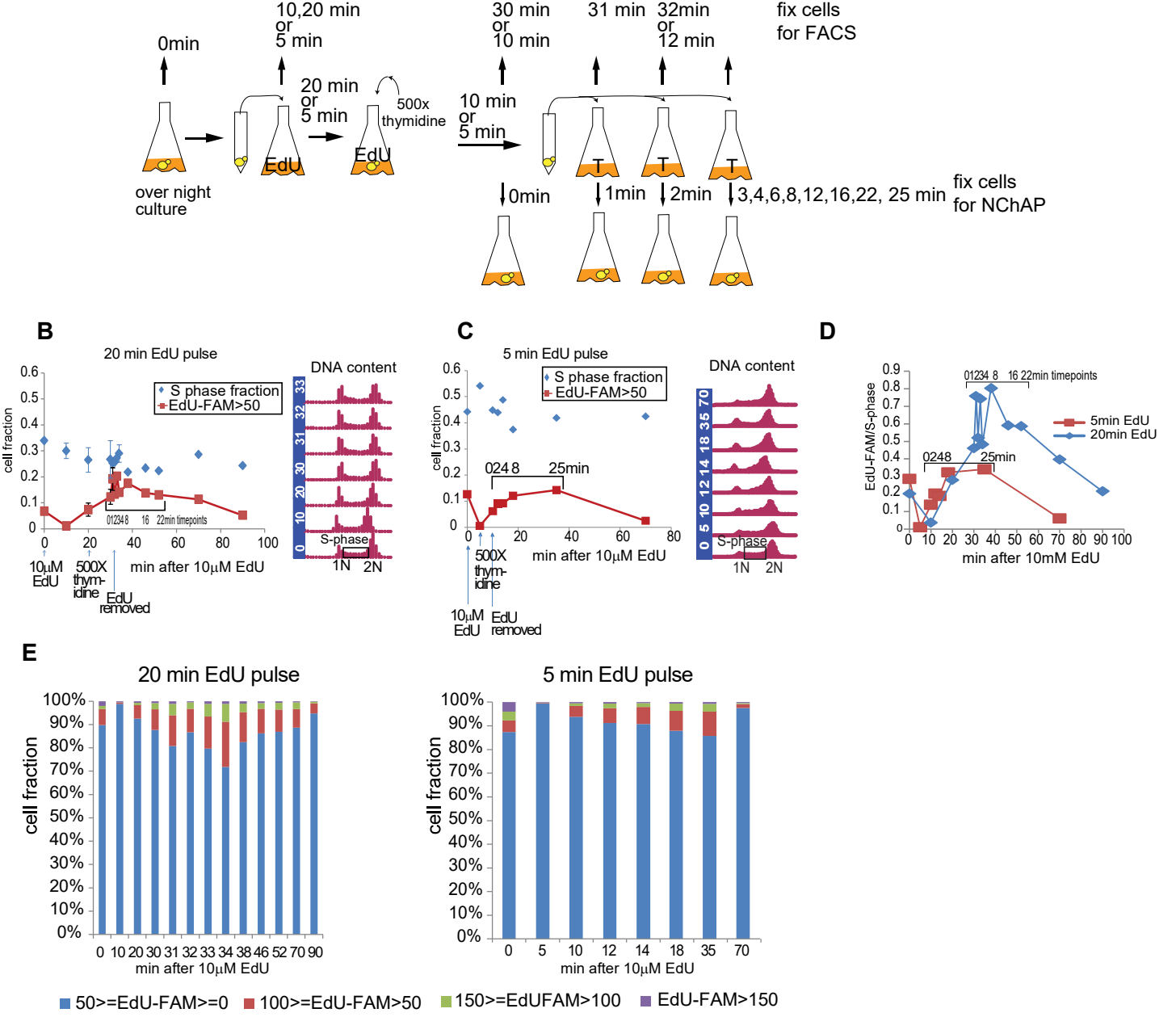


Figure S5 (related to Figure 2): EdU incorporation kinetics in asynchronous cells. A. Diagram of the pulse chase experiment. Exponentially growing cells were transferred to media containing EdU. Thymidine was added after a 20 or 5 min incubation with EdU and the culture was incubated another 10 or 5 min, respectively. Cells were then transferred to media with excess Thymidine and without EdU. Samples for flow cytometry and NChAP were fixed at indicated times. **B.** (20 min EdU pulse),**C.** (5 min EdU pulse).Flow cytometry measurement of EdU incorporation measured from the fluorescence of FAM conjugated to EdU (EdU-FAM). The graph on the left shows the fraction of cells with a positive FAM signal (higher than background (A.U.)) at indicated times after the beginning of the EdU pulse; error bars: standard deviation of two biological replicates. The S-phase fraction was estimated from flow cytometry profiles of DNA content (DNA labeled with Sytox green) as shown in the right panel. 10, 20, 30 and 31 min points in B are an average of two replicates. FAM Fluorescence is sometimes detected prior to EdU addition (0 min point) due to accumulation of hydrophobic FAM-azide in vacuoles after the click reaction in the absence of EdU (as seen by fluorescence microscopy, data not shown). We probably don't detected this non-specific background fluorescence after EdU addition in subsequent time points because FAM-azide can now react with both free EdU and EdU incorporated into DNA instead of ending up in vacuoles. The FAM-azide conjugated with the free EdU can then be better washed away, resulting in lower background fluorescence. **D.** The plot shows the proportion of S-phase cells that have incorporated EdU (measured as described in B and C) after a 5 min EdU pulse (red squares) or a 20 min EdU pulse (blue diamonds). **E.** Fluorescence inten-sity distribution for the three experiments from B and C. EdU-FAM intensity distributions are similar after a 5 or a 20 min EdU pulse, but the fraction of EdU positive S-phase cells is 2 to 3 fold lower after a 5 min pulse than after a 20 min pulse as shown in D. We therefore suspect that we are sampling cells that process EdU more or less rapidly when doing a 5 min or a 20 min EdU pulse, respectively. Consequently, the majority of cells in either experiment will have replicated similar fractions of their genomes, as evidenced by similar EdU-FAM intensity distributions in both experiments.

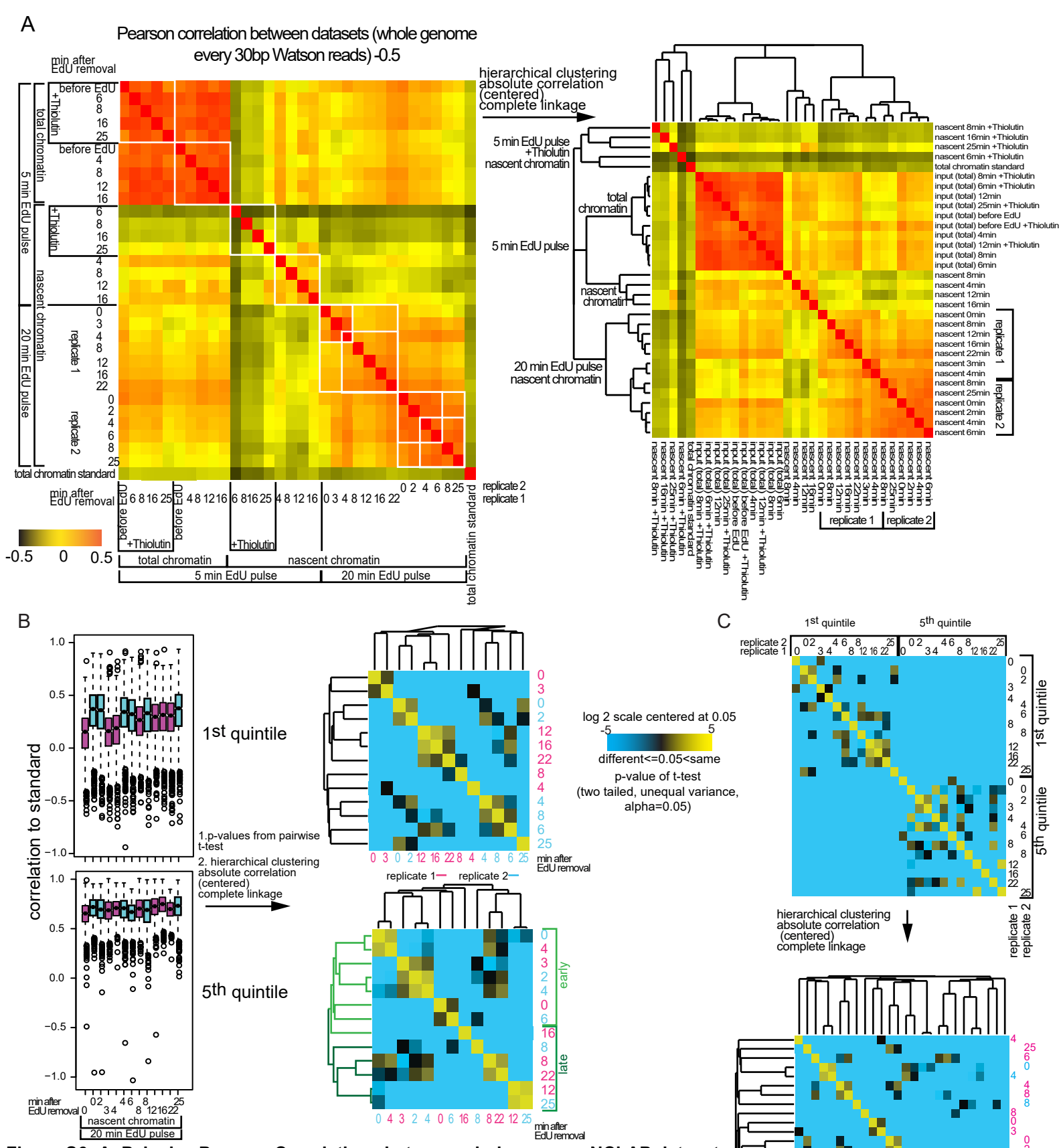


Figure S6: A. Pairwise Pearson Correlations between whole genome NChAP datasets (related to Figure 2). Correlations were calculated for indicated Watson strand datasets with data points every 30bp covering the whole genome (Crick strand analysis yields similar results). Values in the heat map show correlation values decreased by 0.5 to increase color contrast. The heat map on the right shows the result of hierarchical clustering of the matrix on the left. Proximal time points are more similar to each other than to distant time points from the same time course in the 5min and the two 20min EdU pulse experiments, consistent with a gradual chromatin maturation process. The two 20min EdU pulse replicates are more similar to each other than either is to the 5min EdU pulse experiment suggesting that we are detecting different stages of chromatin maturation with a 5 and a 20 min EdU pulse. The total chromatin datasets are all highly correlated to each other irrespective of time after EdU removal and nascent chromatin datasets cluster separately from the total chromatin datasets, indicating that we are indeed measuring nucleosome positioning dynamics specific to newly replicated chromatin. **B.** P-values from pairwise t-tests for gene correlations to the standard from two 20min EdU pulse experiments. Left: Box plot distributions of gene profile correlation to the standard gene profile for slow maturing 1st (top) and fast maturing 5th (bottom) gene quintiles, as defined in Figure 2. Right: Hierarchical clustering of p-values from pairwise t-tests of distributions shown in the box plots. Early (0 to 6min) and late (8 to 25min) time points are for the most part significantly different between each other (blue and black tiles, p-value<=0.05) with some similarities between transitional time points (4 to 8min). Early and late points tend to cluster together in both quintiles, although in addition to the early and late clusters experimental variability between replicates also produces clustering by replicate in the 1st quintile. **C.** P-values from pairwise t-tests between 1st and 5th quintile correlation distributions shown in box plots in B. All time points from the fifth quintile are significantly different from all the time points from the first quintile.

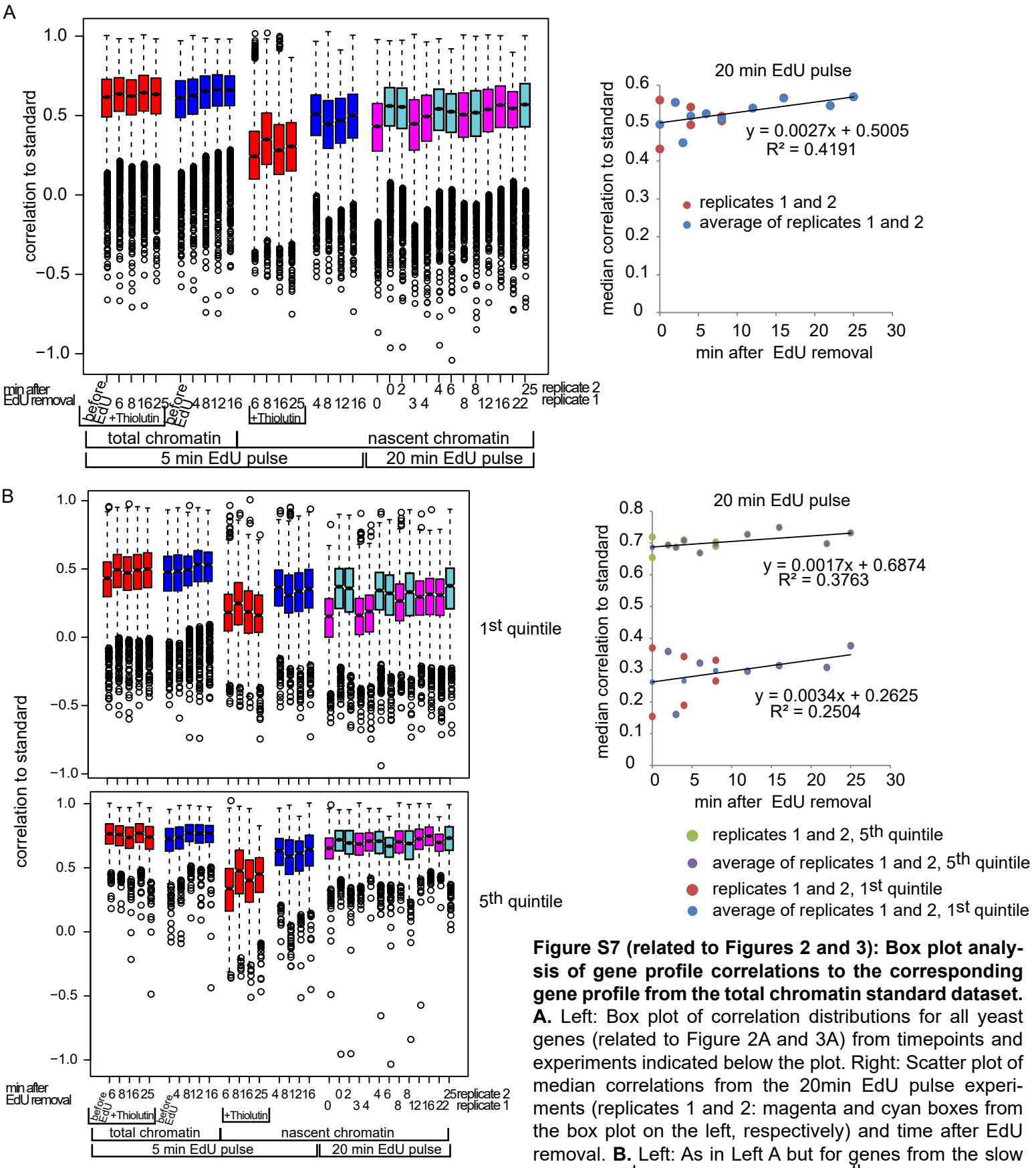
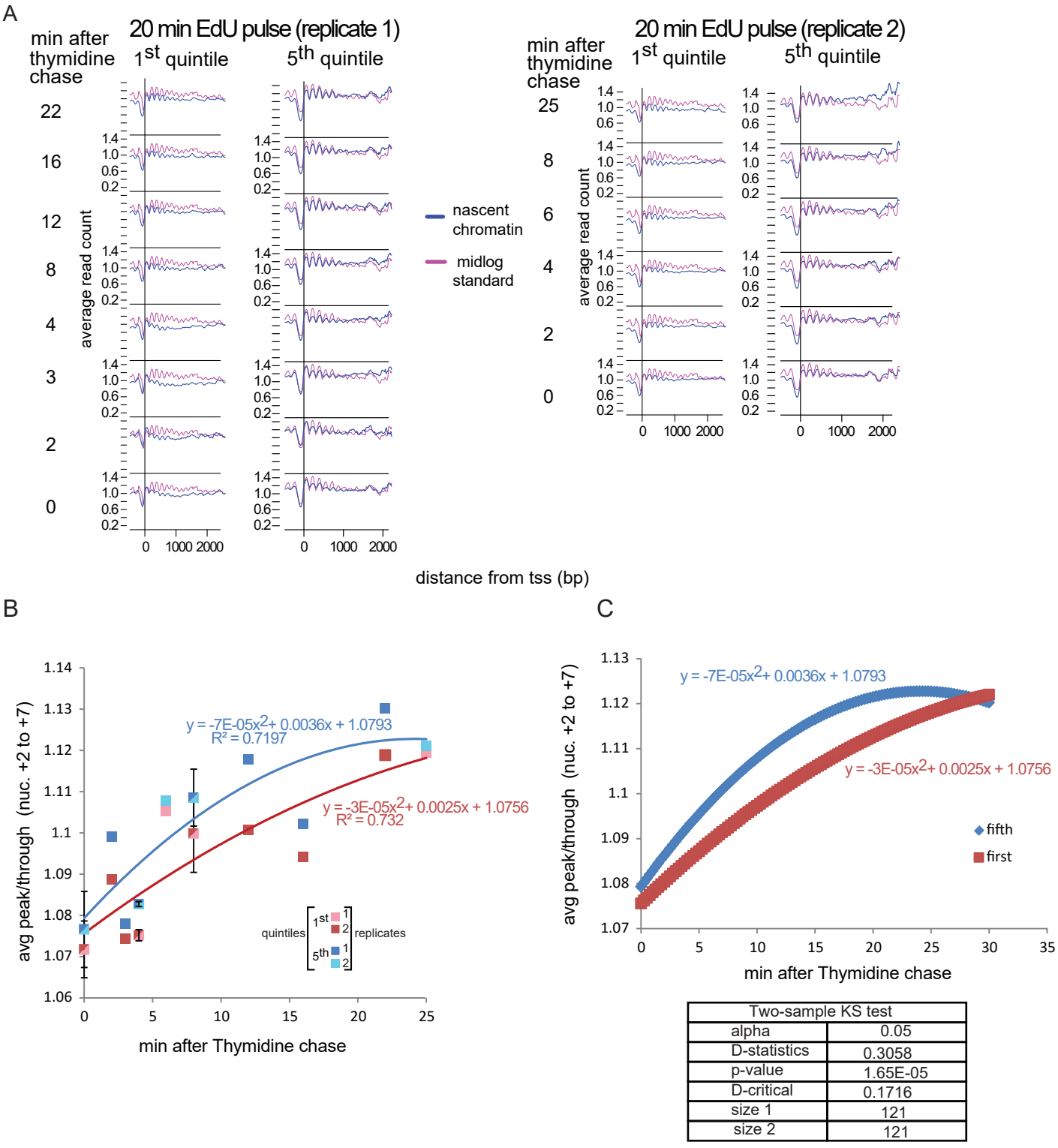


Figure S7 (related to Figures 2 and 3): Box plot analysis of gene profile correlations to the corresponding gene profile from the total chromatin standard dataset. **A.** Left: Box plot of correlation distributions for all yeast genes (related to Figure 2A and 3A) from timepoints and experiments indicated below the plot. Right: Scatter plot of median correlations from the 20min EdU pulse experiments (replicates 1 and 2: magenta and cyan boxes from the box plot on the left, respectively) and time after EdU removal. **B.** Left: As in Left A but for genes from the slow maturing 1st (top) and fast maturing 5th (bottom) quintiles, defined in Figure 2. Right: as in Right A but for 1st and 5th quintiles. Early timepoints in the first quintile contribute to most of the variability between replicates.The trend towards increasing correlation is detectable in both replicates in later time points (from 8 to 25min after EdU removal).



Two-sample KS test	
alpha	0.05
D-statistics	0.3058
p-value	1.65E-05
D-critical	0.1716
size 1	121
size 2	121

Figure S8 (related to Figure 2): Replicate of the 20 min EdU, 10 min Thymidine chase experiment. A. Average nucleosome profiles at indicated time points after Thymidine incubation from the nascent chromatin fraction (blue line) from the slow and fast maturing first and fifth quintiles (1245 genes each), respectively (as defined in Figure 2A). left: 20 min EdU pulse dataset from Figure 2 (replicate 1); right: 20 min EdU pulse replicate dataset (replicate 2). The pink line shows the average profile of genes from the mid-log standard in the corresponding quintiles. Note lower peak/through ratios in the firts kb of the CDS on nascent profiles (blue) in early time points compared to late ones. **B.** Change in average peak/through ratios for nucleosomes +2 to +7 in quintiles 1 and 5. Points are combined from replicates 1 and 2.The average values between replicates 1 and 2 (where time points were the same in both replicates) are shown as two-color squares and the error bars represent the standard deviation from the mean. **C.** Kolmogorov-Smirnov test for a significant difference between quadratic fit curves from B. Quadratic curves were drawn with 121 time points using the equations determined in B for quintiles 1 (red) and 5 (blue). The null hypothesis (i.e. that the two curves are identical) was tested with a two-sample KS test with alpha=0.05. Since 0.3058>0.1716 and p-value<alpha, the null hypothesis is rejected and the two curves are significantly different.

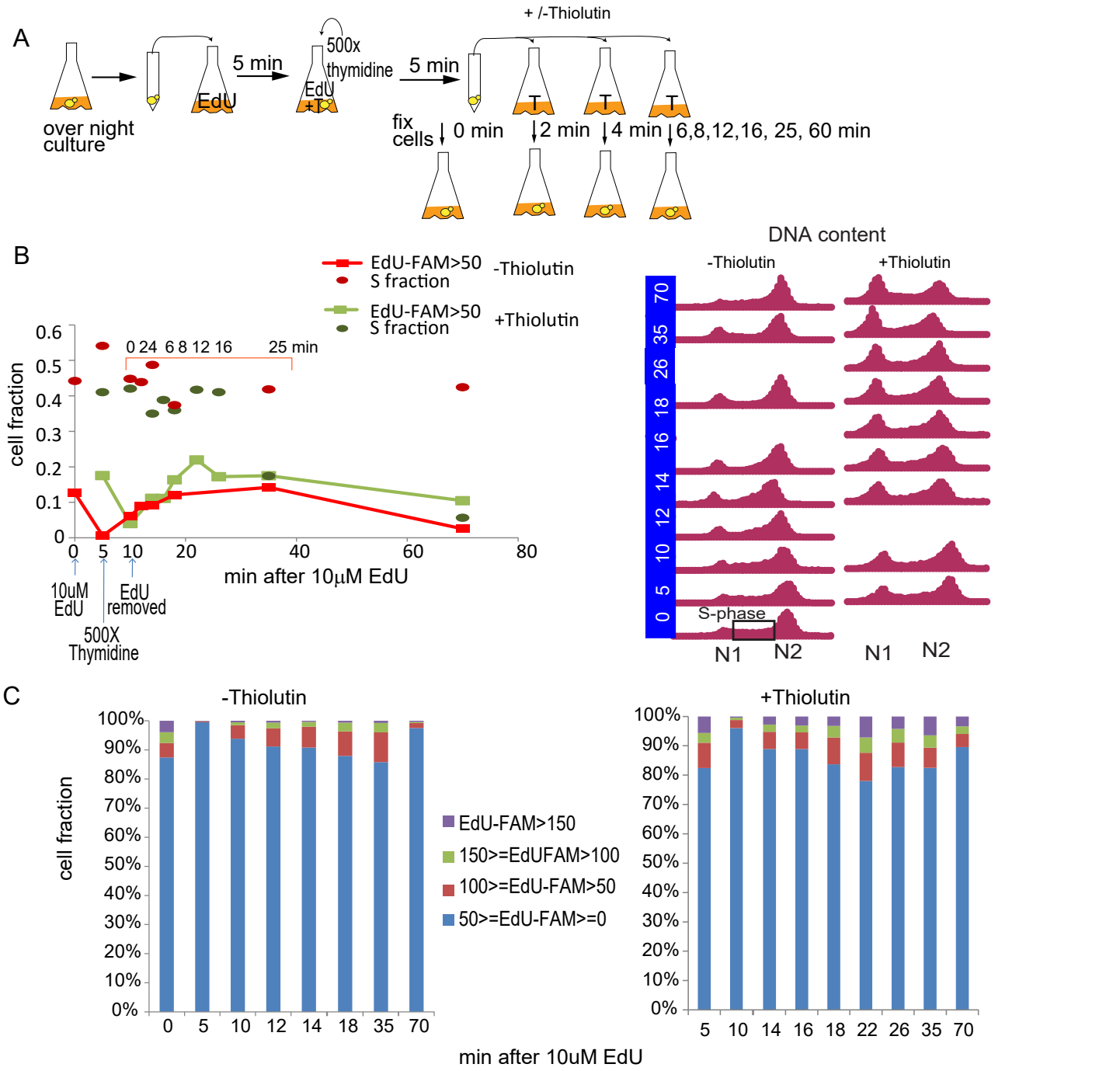


Figure S9 (related to Figure 3): 5min EdU-Thymidine pulse chase in an asynchronous culture with thiolutin treatment. A. Diagram of the pulse chase experiment. **B.** Flow cytometry measurement of EdU incorporation measured from the fluorescence of FAM conjugated to EdU (EdU-FAM). The graph on the left shows the fraction of cells with a FAM signal above background (higher than 50 (A.U.)) at indicated times after the beginning of the EdU pulse. The fractions of cells in S-phase are indicated as red and green circles for non-treated and thiolutin treated cells, respectively. The S-phase fraction was estimated from flow cytometry profiles of DNA content (DNA labeled with Sytox Green) as shown in the right panel. The effectiveness of the Thiolutin treatment is supported by cytometry profiles of DNA content, as thiolutin treated cells in contrast to non-treated cells accumulate in G1 in later time points due to an arrest in cell cycle progression in the absence of transcription. **C.** Bar plot of EdU-FAM fluorescence intensity distribution.

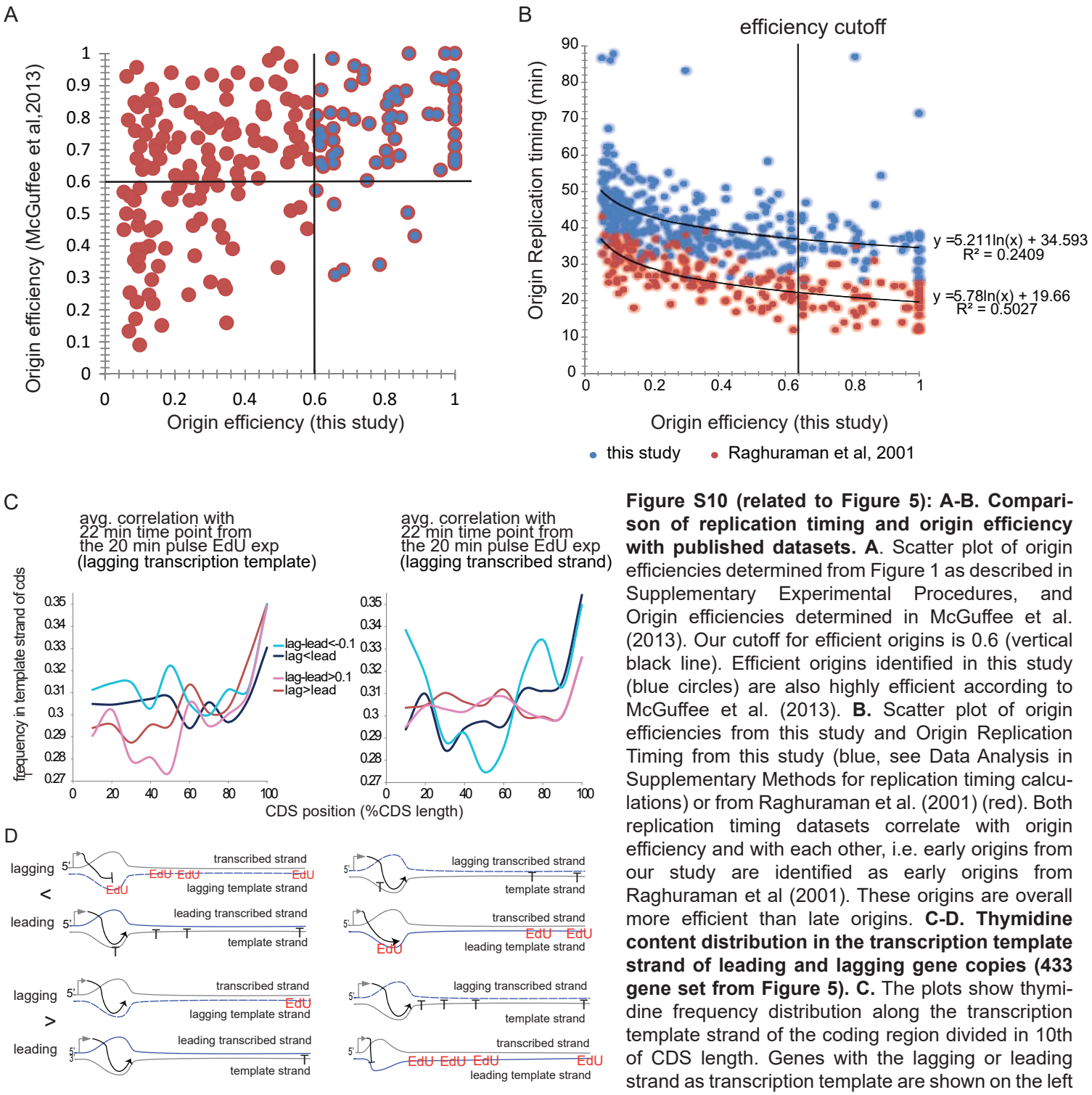


Figure S10 (related to Figure 5): A-B. Comparison of replication timing and origin efficiency with published datasets. A. Scatter plot of origin efficiencies determined from Figure 1 as described in Supplementary Experimental Procedures, and Origin efficiencies determined in McGuffee et al. (2013). Our cutoff for efficient origins is 0.6 (vertical black line). Efficient origins identified in this study (blue circles) are also highly efficient according to McGuffee et al. (2013). **B.** Scatter plot of origin efficiencies from this study and Origin Replication Timing from this study (blue, see Data Analysis in Supplementary Methods for replication timing calculations) or from Raghuraman et al. (2001) (red). Both replication timing datasets correlate with origin efficiency and with each other, i.e. early origins from our study are identified as early origins from Raghuraman et al (2001). These origins are overall more efficient than late origins. **C-D. Thymidine content distribution in the transcription template strand of leading and lagging gene copies (433 gene set from Figure 5). C.** The plots show thymidine frequency distribution along the transcription template strand of the coding region divided in 10th of CDS length. Genes with the lagging or leading strand as transcription template are shown on the left and right, respectively. **D**. Diagrams illustrating the position of thymidine (T) enrichment along the CDS (the grey arrow marks the location of the tss); top panel for average lagging correlations smaller than leading and the opposite in the bottom panel. The nascent strands are shown in blue (dashed lines-lagging, solid line -leading). EdU replaced T in nascent strands. Nascent RNA is shown as a black line. Thymidine is slightly enriched (~2% to 5%) in the lagging template strand in the gene set with the slower maturing lagging copy, from the 30th to the 50th percentile of CDS length. It is not clear whether this slight increase is sufficient to slow down RNA pol2 progression. The pattern is reversed when the leading strand is the transcription template, as expected if the observed differences between maturation rates of the leading and lagging transcription template strands are due to EdU interference with RNA pol2 progression, when EdU is in the template strand.

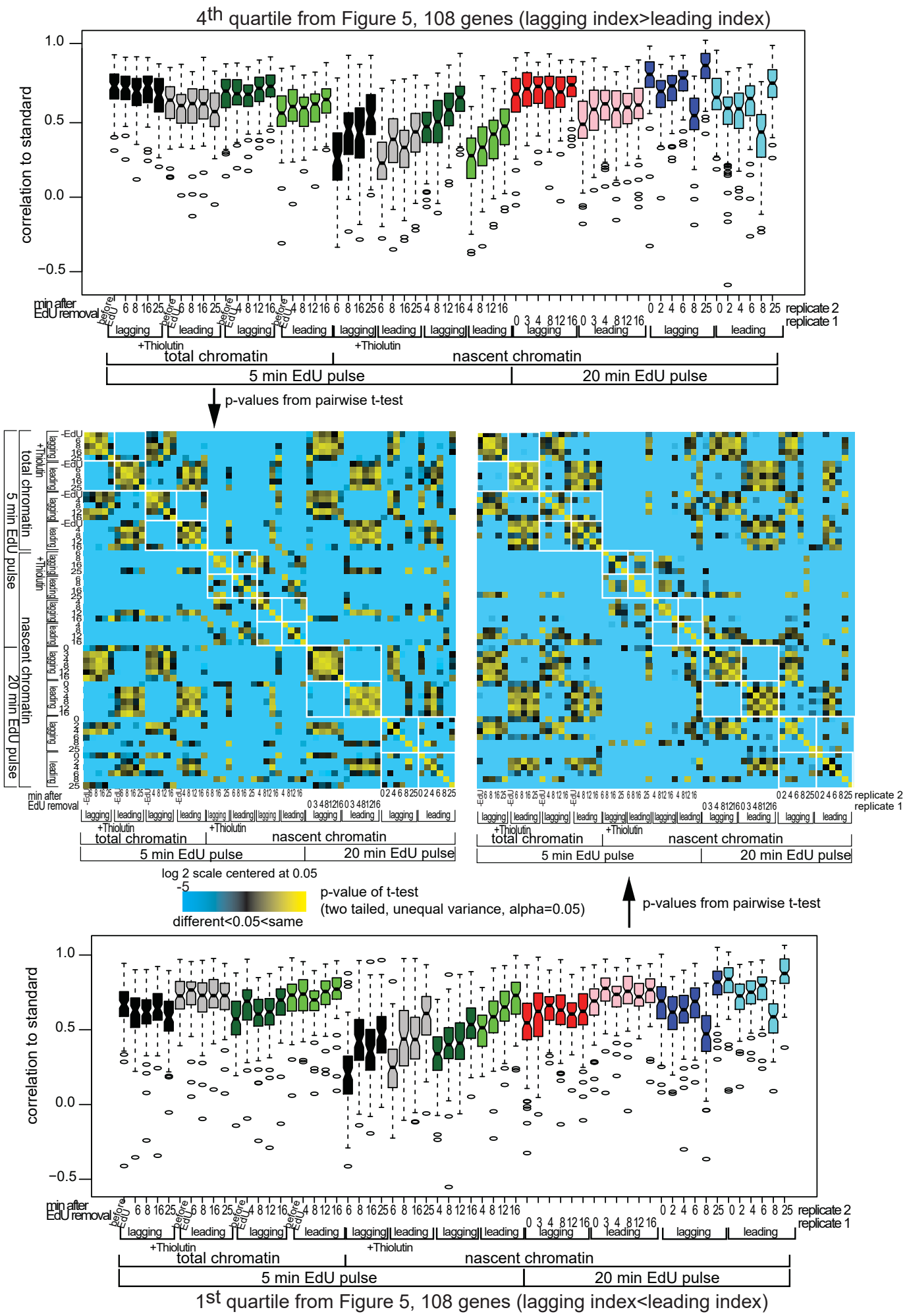


Figure S11 (related to Figure 5):Box plot and t-test for correlations to the standard of lagging and leading gene copies (related to Figure 5). Top: Box plot of correlation distributions for genes from the quartile 4 of Figure 5C from timepoints and experiments indicated below the plot. P-values of pairwise t-tests of distributions in the box plot are shown in the heat map in the middle left. Note the checkered pattern of the heat map, indicating a significant difference between lading and lagging correlation distributions from the same timepoints within one time course. Bottom: Same as Top but for quartile 1 from Figure 5C. The corresponding t-test p-values are on the heat map in the middle right.