

Supplemental File including:

- Yeast strains generated and used in this study
- Sequences of fusions for generating yeast strains
- Supplemental Figures 1-5 with legends
- References for Supplemental File

Yeast strains:

YGY663	MATa his3 Δ 1 leu2 Δ 0 LYS2 met15 Δ 0 ura3 Δ 0 HTB2-TEV, HHT1-HA-TEVsite-MYC, <i>bar1</i>
YGY672	MATa his3 Δ 1 leu2 Δ 0 LYS2 met15 Δ 0 ura3 Δ 0 HTB2-TEV, HHT1-HA-TEVsite-MYC, HHT2-HA-TEVsite-MYC, <i>bar1</i>
YGY673	MATa his3 Δ 1 leu2 Δ 0 LYS2 met15 Δ 0 ura3 Δ 0 HTB2-TEV, HHT1-HA-TEVsite-MYC, HHT2-HA-TEVsite-MYC, <i>bar1</i>
YGY674	MATa his3 Δ 1 leu2 Δ 0 LYS2 met15 Δ 0 ura3 Δ 0 HTB2-TEV, HHT1-HA-TEVsite-MYC, HHT2-HA-TEVsite-MYC, <i>bar1</i> , <i>rtt109::NAT</i>
YGY675	MATa his3 Δ 1 leu2 Δ 0 LYS2 met15 Δ 0 ura3 Δ 0 HTB2-TEV, HHT1-HA-TEVsite-MYC, HHT2-HA-TEVsite-MYC, <i>bar1</i> , <i>rtt109::NAT</i>
YGY721	MATa his3 Δ 1 leu2 Δ 0 LYS2 met15 Δ 0 ura3 Δ 0 HTB2-TEV, HHT1-HA-TEVsite-MYC, HHT2-HA-TEVsite-MYC, <i>bar1</i> , <i>vps75::HYG</i>
YGY722	MATa his3 Δ 1 leu2 Δ 0 LYS2 met15 Δ 0 ura3 Δ 0 HTB2-TEV, HHT1-HA-TEVsite-MYC, HHT2-HA-TEVsite-MYC, <i>bar1</i> , <i>vps75::HYG</i>

Sequences of histone exchange tags:

Sequences including 200 bp up and downstream of the modified histone fusions. Silent mutations that introduce a mismatch/es to rescue from the guide RNA target sequence are indicated in blue. TEV carries an S219P mutation to inhibit autoproteolysis (Yi et al. 2013). 2 $\times$ (GGSG) linkers were added between the C-terminus of each modified histone subunit and its fusion. Note that the 5' and 3' UTRs regions in these modified loci remain untouched, such that the fusion is under the control of the native promoter and terminator. All other histone genes remain wild type.

HTB2-TEV

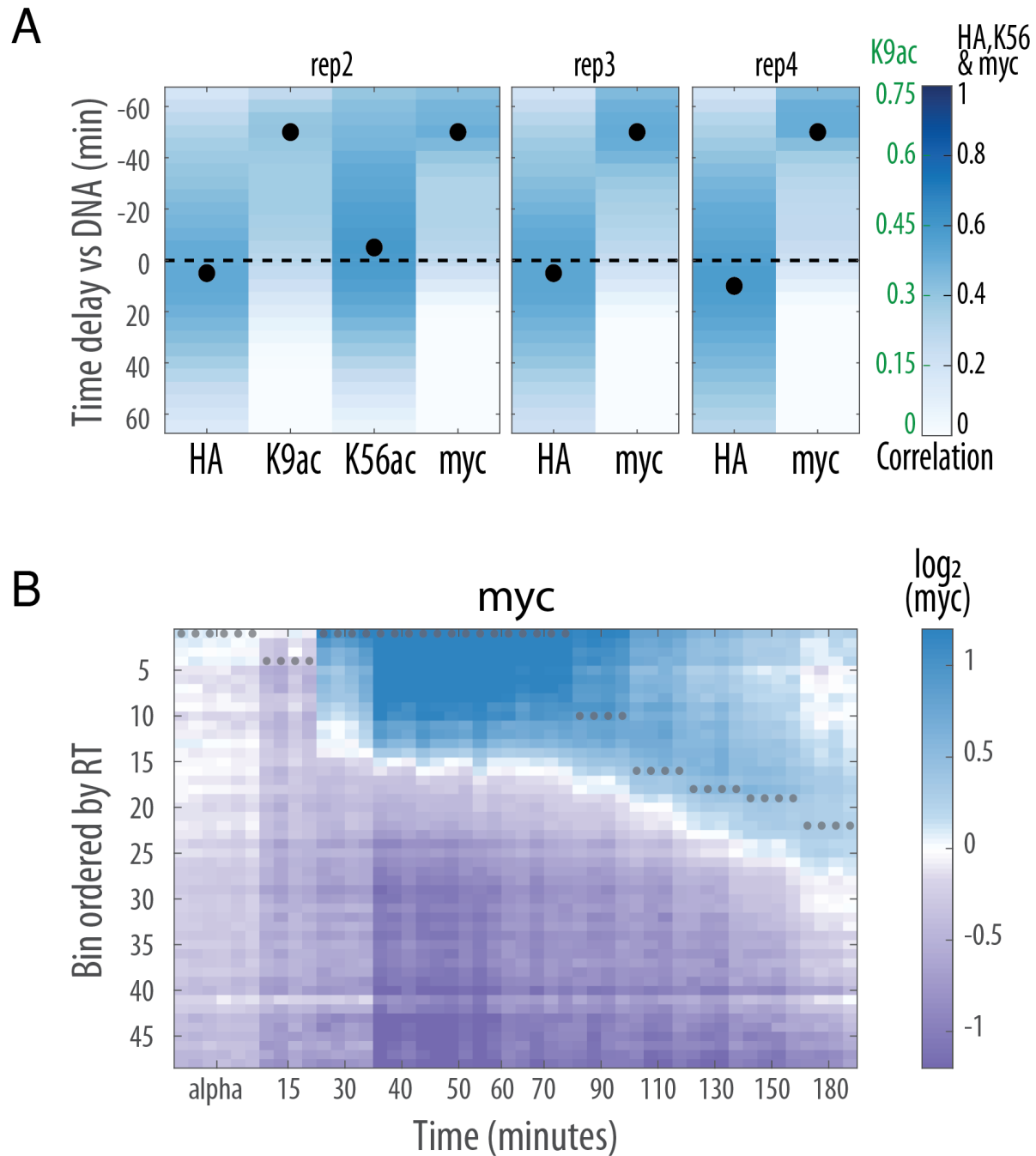
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HHT2-HA-TEVsite-MYC

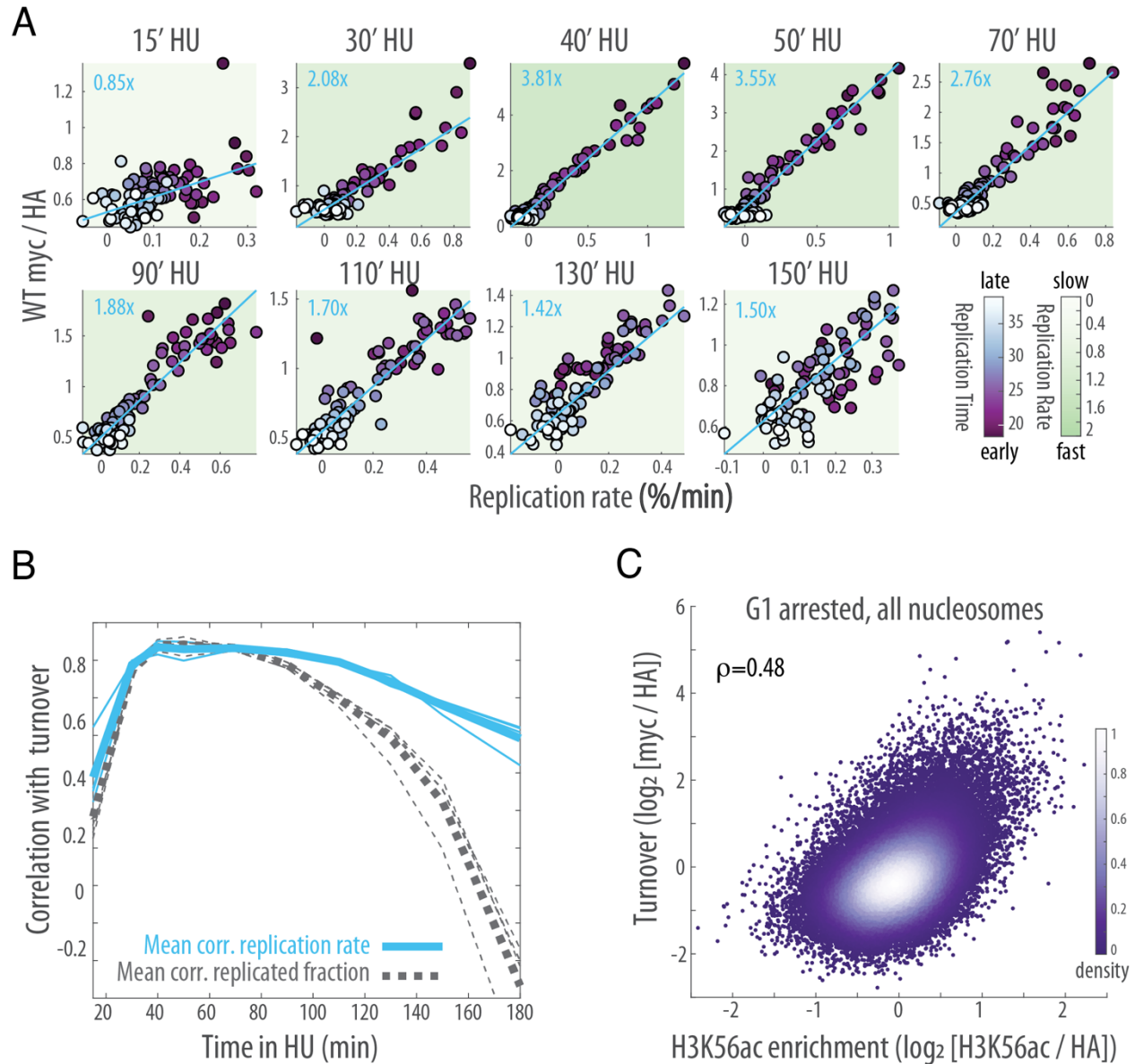
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Supplemental Figure S1) Myc and acetylation dynamics in wild type cells

A) Temporal sequence of *Rtt109*-dependent histone marks and H3 integration during DNA replication. Cross-correlation analysis as in Figure 1E. Additional repeats of the time course experiment consistently measure time delays between the various epitopes, note the different color scales for the epitopes.

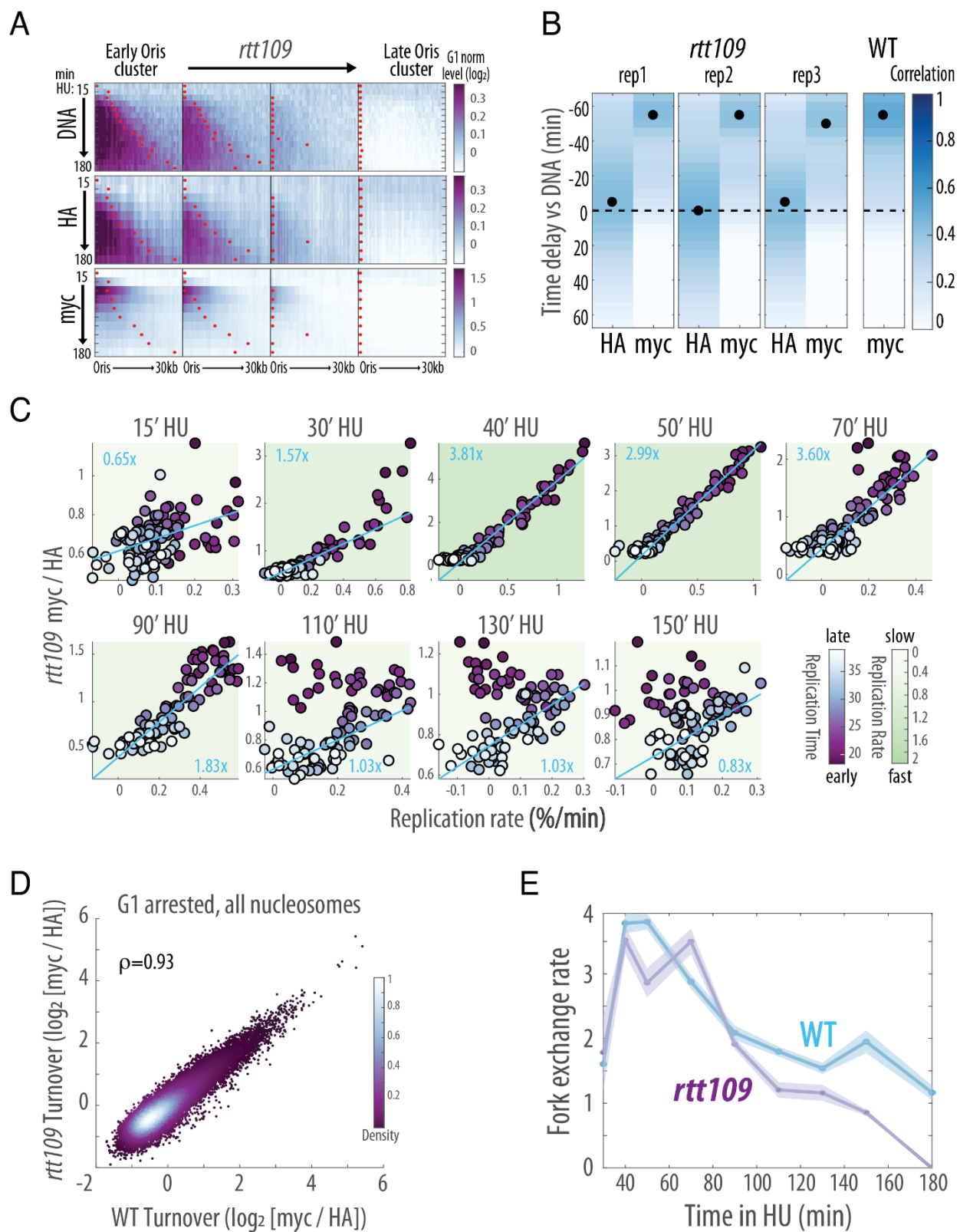
B) *Myc dynamics in replicated versus non-replicated regions*. Median myc coverage in RT clusters (Y-axis) as a function of experiment time point in all time courses (X-axis, each column is a time course centered around the time mark, n=4). Grey dots indicate DNA-derived position of the replication fork.



Supplemental Figure S2) H3 exchange during DNA replication

A-B) *H3 exchange is strongly correlated with replication rate throughout the time course.* (A) as Figure 2A for all time points, with the background color indicating the maximal replication rate at a given timepoint. The linear fit and its slope measuring fork exchange is shown in blue. (B) Correlation between H3 turnover ($\log_2(\text{myc}/\text{HA})$) and replication rate or replicated fraction across all clusters. The thick line corresponds to the mean of 4 experiments indicated in thin lines. Note the drop in correlation at the later times.

C) *Turnover correlates with H3K56ac in G1-arrested cells.* Histone turnover ($\log_2(\text{myc}/\text{HA})$) and acetylation enrichment ($\log_2(\text{K56ac}/\text{HA})$) of wild type cells in non-replicating, G1-arrested cells.



Supplemental Figure S3) Replication-associated histone dynamics in *RTT109*-deleted cells

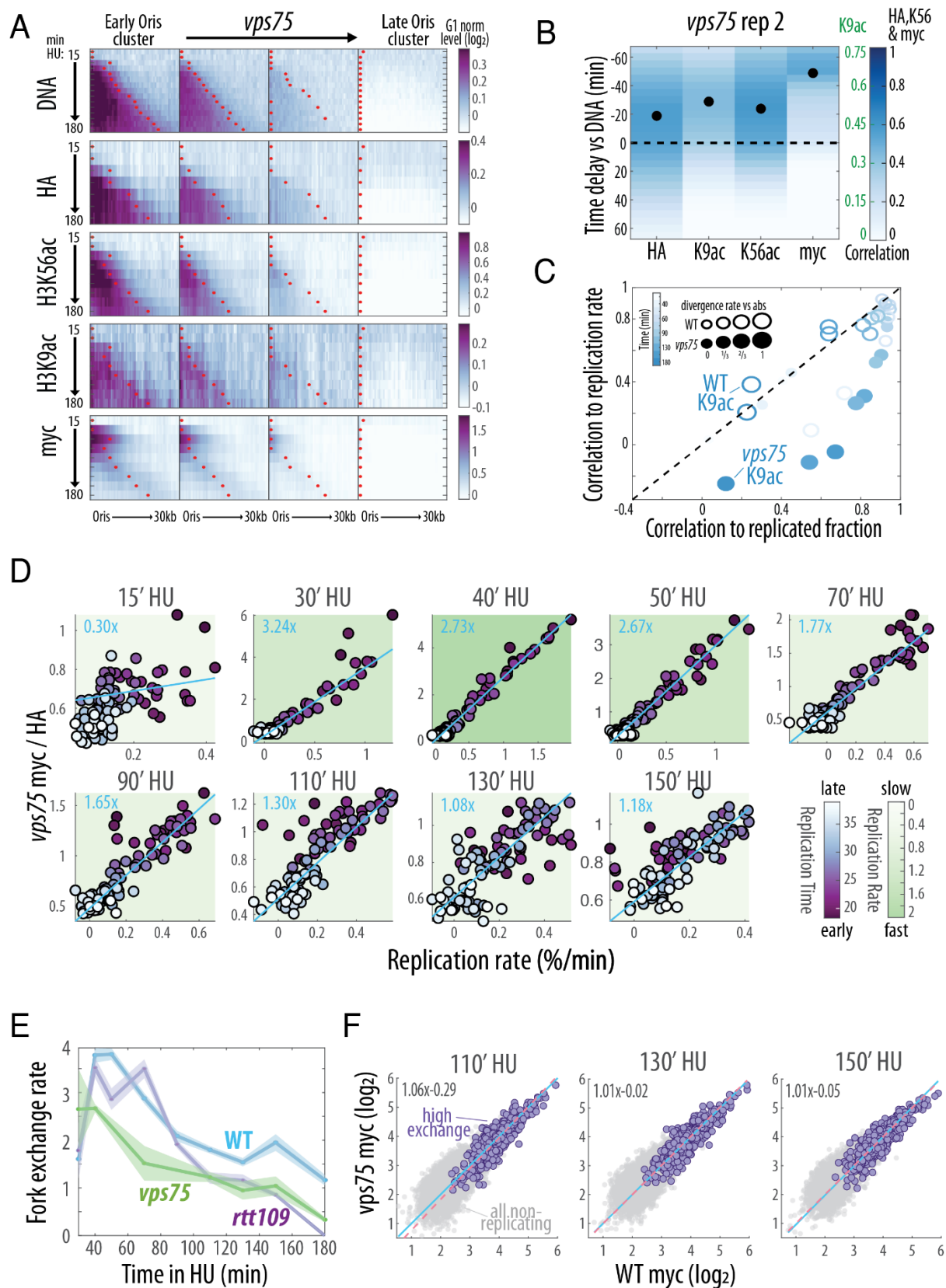
A) *H3 sensor levels around ORIs of RTT109-deleted cells.* As Figure 1C for *RTT109*-deleted cells with same scale intensities.

B) *Temporal relation between myc and fork progression in RTT109-deleted cells.* As Supplemental Fig. S1A for three repeats of *RTT109*-deleted cells. WT myc correlation is shown for comparison.

C) *Loss of correlation between H3 exchange and replication rate at mostly replicated regions after RTT109 deletion.* As Supplemental Fig. S2A for *RTT109*-deleted cells. Note the divergence of early replicating clusters from the line in later time points when they are mostly (>50%) replicated.

D) *Replication-independent histone dynamics are invariant to RTT109 deletion.* Mean turnover ($\log_2$ myc/HA) of *rtt109* versus WT cells showing all nucleosomes (n=5 for each strain).

E) *Reduced fork-associated histone exchange in RTT109-deleted cells.* As Figure 2D for the indicated strains (n=4 for WT, n=3 for *rtt109*). Shading is standard error.



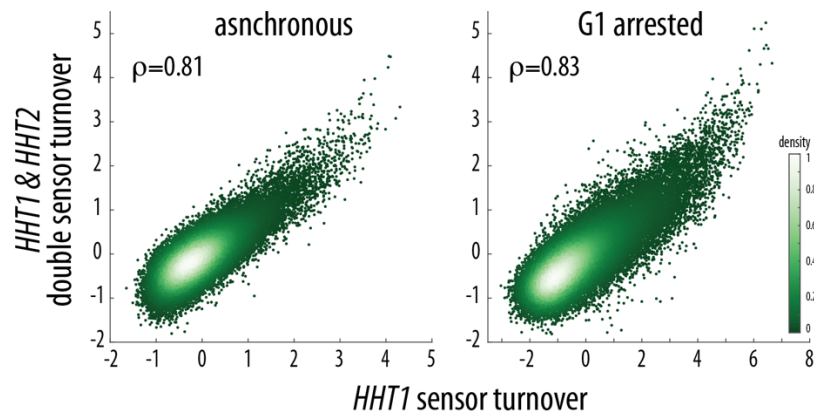
Supplemental Figure S4) Replication-associated histone dynamics in *VPS75*-deleted cells

A-C) *VPS75 deletion removes fork associated K9 acetylation.* (A-C) as Figures 1C, 1E and 1D respectively for the indicated epitopes in the *vps75* mutant. (B) is a repeat for Figure 4B. Of note, the parallel shift of all epitopes relative to the DNA standard supports temporal differences between this repeat and the external DNA time course. Most importantly, this does not affect the relation between K9ac and the two internal measurements for the replication fork, HA and K56ac. (C) As in Figure 1D, both *vps75* repeats are included.

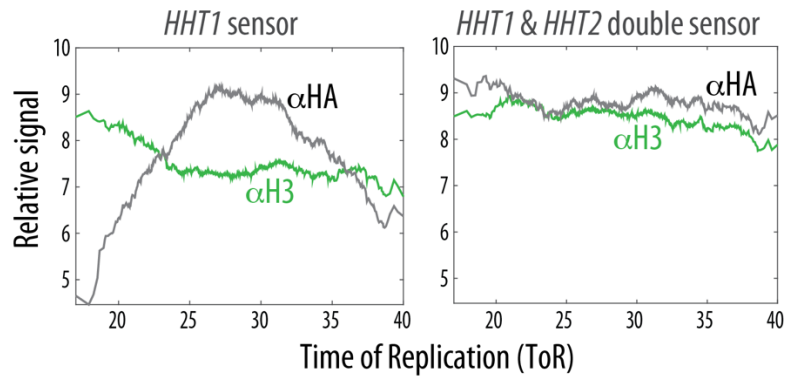
D-E) *Reduced fork exchange rate in VPS75-deleted cells.* (D) as Supplemental Fig. S2A for *VPS75*-deleted cells. HA data for times 50 & 90 was interpolated, see Methods. (E) as Supplemental Fig. S3E with the addition of the *vps75* strain (n=2). Shaded line is standard error.

F) *Replication-independent histone dynamics remain unaffected by VPS75 deletion.* As Figure 3D for *VPS75*-deleted cells (mean of n=2).

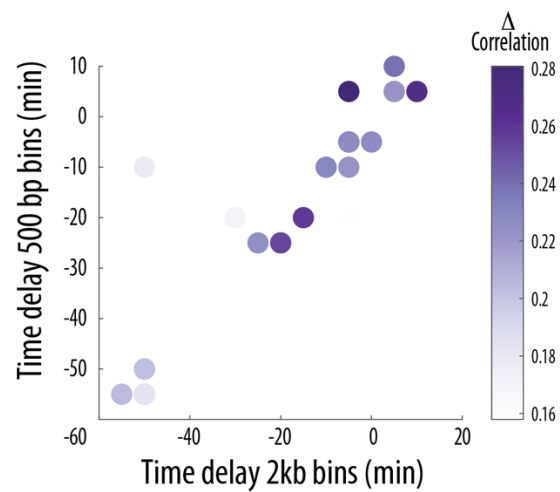
A



B



C



Supplemental Figure S5) An H3 sensor to measure nucleosome exchange during DNA replication

A-B) *Strains with both H3 alleles tagged with the exchange sensor accurately measure replication-independent histone turnover, and ameliorate replication-associated allele imbalances.* (A) Turnover ($\log_2(\text{myc}/\text{HA})$) of all nucleosomes in asynchronously growing (left) or non-replicating G1-arrested (right) cells with the single-tagged (*HHT1*) versus double-tagged (*HHT1* and *HHT2*) H3 sensors. (B) Mean nucleosome occupancy of total histone H3 (using an anti-H3 antibody) versus sensor-tagged histone H3 (anti-HA antibody) across all RTs. Note the replication-associated imbalance of the *HHT1*-tagged single-allele sensor that is abolished in the double-tagged sensor.

C) *Time delays between epitopes are robust to variations in bin size.* Compared are all calculated time delays (all epitopes, genotypes and repeats) when using 500 or 2000 base pair bins. Color indicates the difference in cross-correlation score between the two measures.

References for Supplemental Material

Yi L, Gebhard MC, Li Q, Taft JM, Georgiou G, Iverson BL. 2013. Engineering of TEV protease variants by yeast ER sequestration screening (YESS) of combinatorial libraries. *Proc Natl Acad Sci U S A* **110**: 7229–7234. www.pnas.org/cgi/doi/10.1073/pnas.1215994110 (Accessed July 30, 2020).