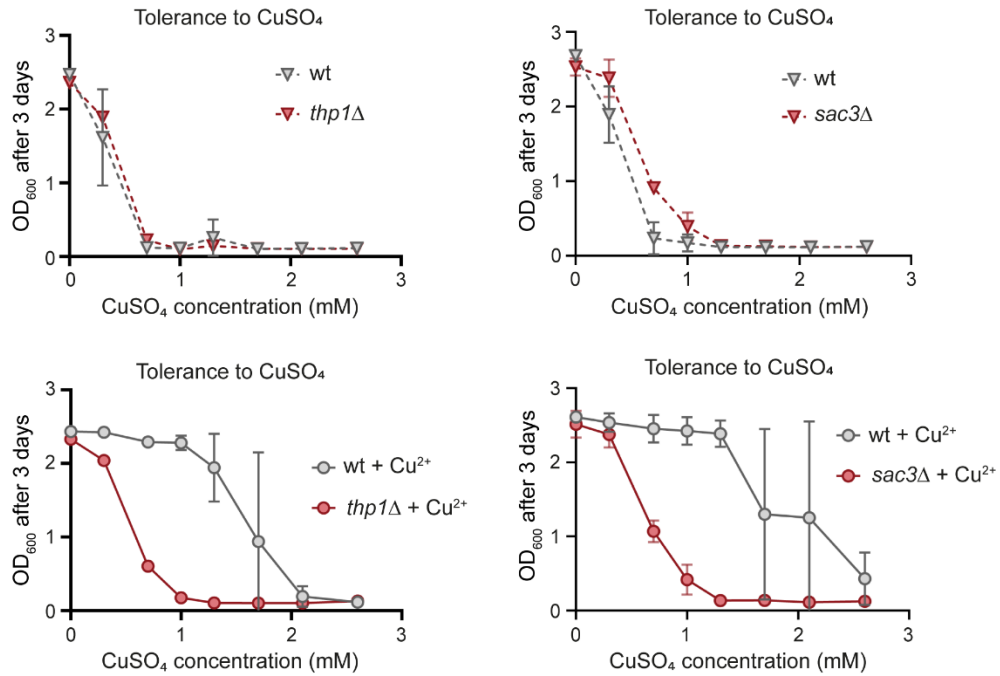


Supplementary Material

Supplementary Figures

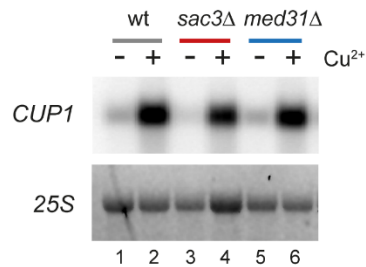
Supplementary figures are numbered 2-6 as each forms a supplement to one of the main figures 2-6.

A

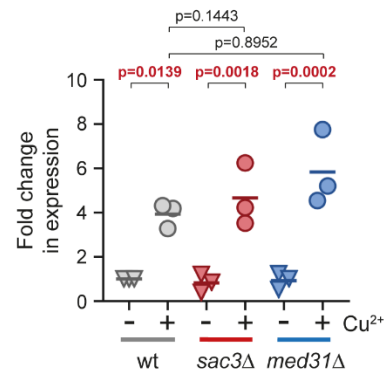


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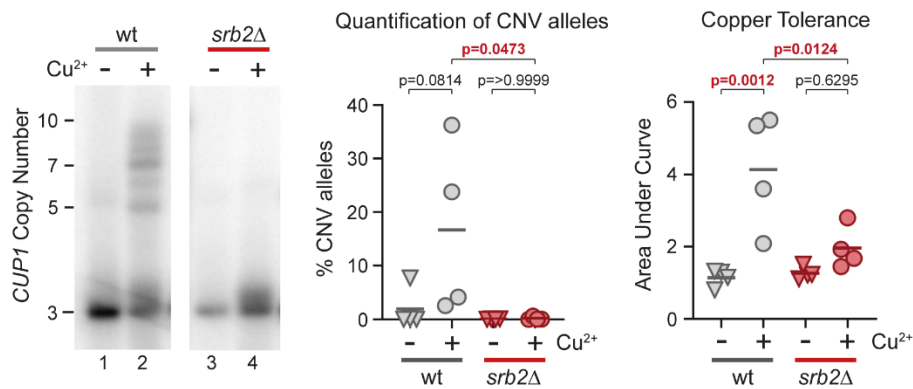
Northern analysis of *CUP1* mRNA induction



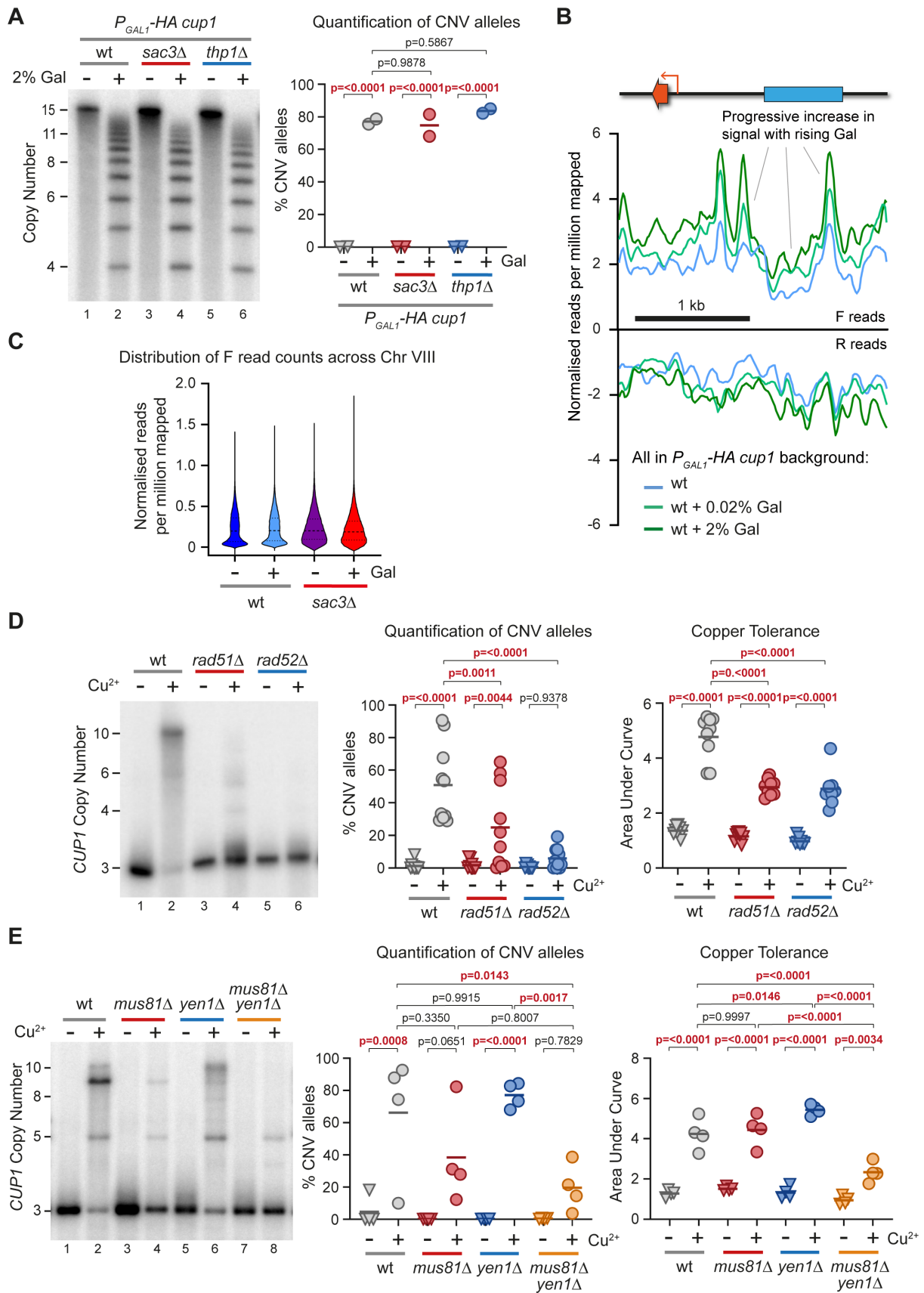
Quantification of *CUP1* mRNA



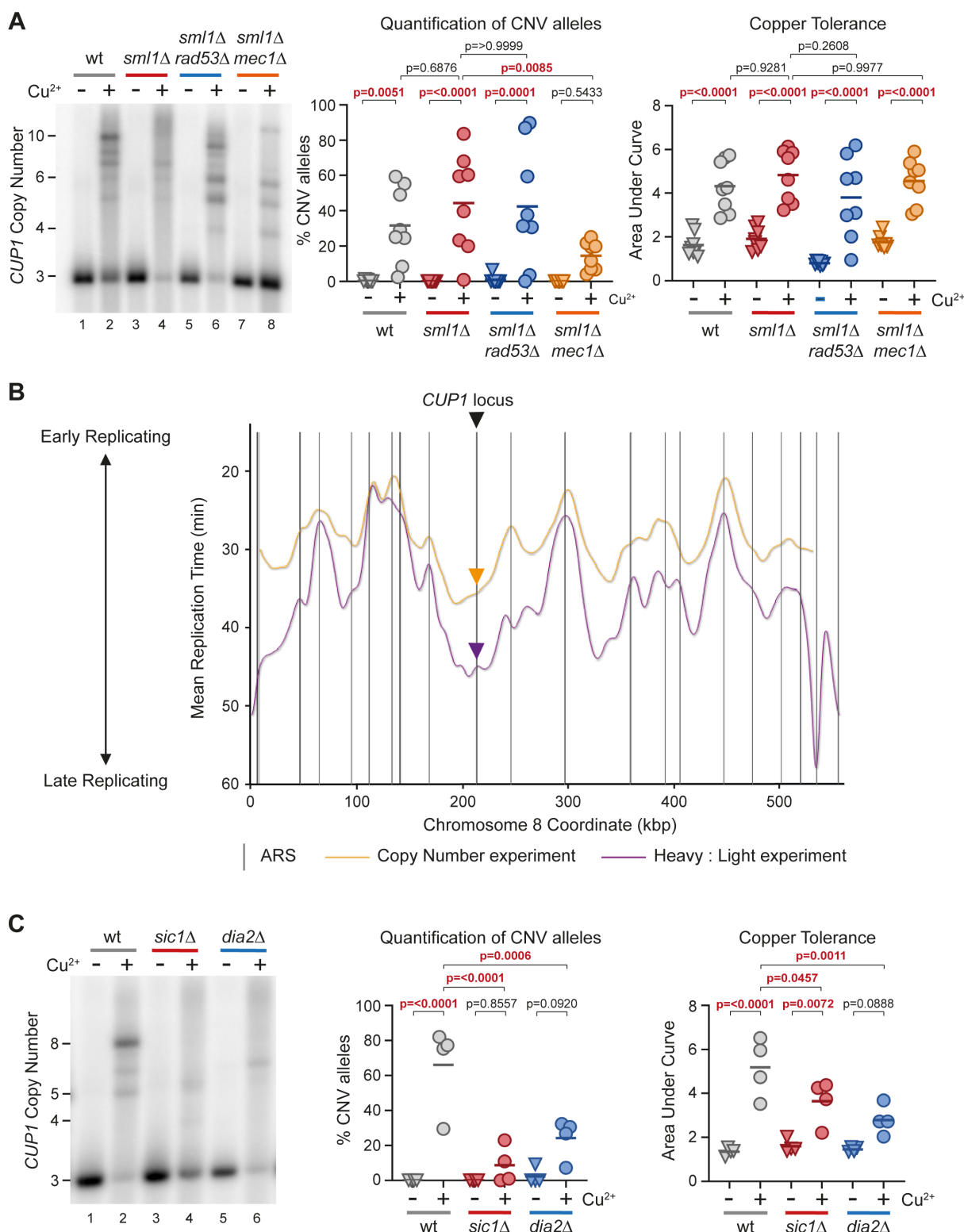
C



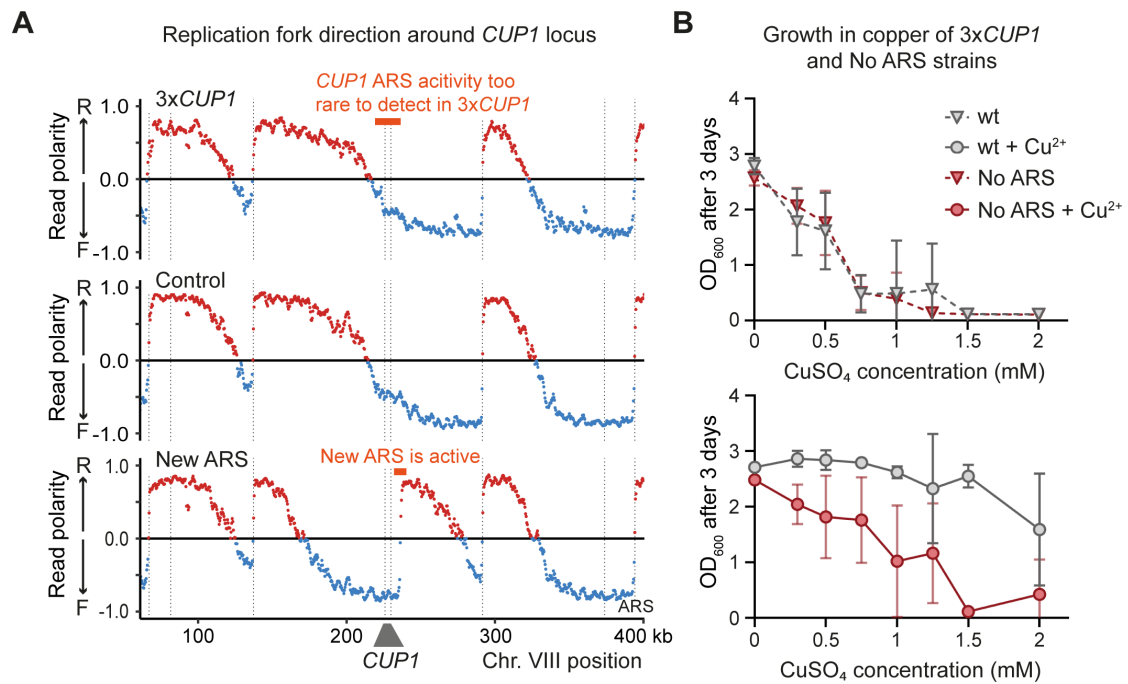
Supplementary Figure 2. Supplement to TREX-2 and Mediator are required for transcription stimulated *CUP1* CNV. **(A)** Plots of final OD₆₆₀ versus [CuSO₄] for wild type, *thp1Δ* and *sac3Δ*. Upper plots show naïve cells that have not been pre-exposed to CuSO₄, lower plots show cells that have been pre-cultured in 0.3mM CuSO₄. Tolerance of mutants that have not been pre-cultured in CuSO₄ is similar or higher than wild type, showing that loss of Thp1 and Sac3 does not impair the normal response to environmental copper. This is the source data underlying the plots in Figures 2B, 2C. **(B)** Northern blot analysis of *CUP1* mRNA, in wild-type, *sac3Δ* and *med31Δ* cells. Mid-log cells were treated with 0.3mM CuSO₄ for 6 hours and fold change in *CUP1* expression was quantified relative to the 25S rRNA highlighted by ethidium bromide staining, p-values calculated by 1-way ANOVA; n = 3. **(C)** Southern blot analysis of *CUP1* copy number and copper tolerance analysis of 3x*CUP1* wild-type (wt) and *srb2Δ* cells, performed as in Figure 2A, n = 3.



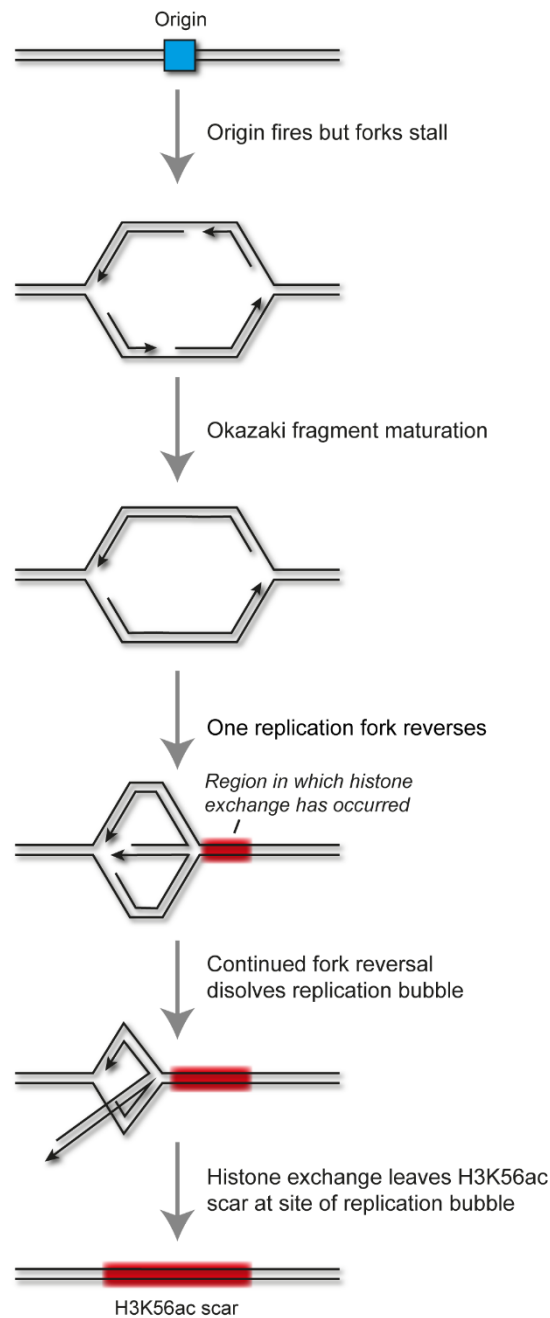
Supplementary Figure 3. Supplement to replication fork stalling and cleavage at *CUP1* locus. **(A)** Cells with every *CUP1* ORF and promoter in each *CUP1* copy replaced by *P_{GAL1}-HA*, grown for 10 generations in raffinose \pm 2% galactose. Analysis by Southern blot comparing *sac3* Δ and *thp1* Δ cells to wild type. Quantification shows the percentage of alleles deviating from the parental copy number of 17 copies; n = 2. **(B)** Plots of TrAEL-seq read density on forward and reverse strands across a *P_{GAL1}-HA cup1* repeat for wild-type cells induced for 6 hours with 0%, 0.02% or 2% galactose. TrAEL-seq profiles are an average of two biological replicates, 0% and 0.02% conditions are the same data as Figure 3C, data is processed as in Figure 3C. **(C)** Violin plots showing the distribution of read counts for all 50 bp windows spaced at 10 bp intervals across chromosome VIII that do not overlap multi-copy elements, data was processed as in Figure 3C. **(D)** and **(E)** Southern blot analysis of *CUP1* copy number and copper adaptation analysis of 3x*CUP1* wild-type (wt) and indicated mutant cells, performed as Figure 3E, n = 10 for (D), n = 4 for (E).



Supplementary Figure 4. Supplement to replication timing and fork progression control *CUP1* CNV. **(A)** Southern blot analysis of *CUP1* copy number and copper adaptation analysis of $3\times CUP1$ wild-type (wt), *sml1* Δ , *rad53* Δ *sml1* Δ and *mec1* Δ *sml1* Δ cells, performed as in Figure 4A, $n = 8$. **(B)** Replication timing on Chromosome 8 from OriDB (1), with data collected by two independent studies measuring change in copy number (2) (shown in yellow) or Heavy : Light isotope transfer (3) (shown in purple). Grey lines correspond to Replication origins and arrows indicate the late replication timing of the *CUP1* locus from the respective studies. **(C)** Southern and growth curve analysis of $3\times CUP1$ wildtype (wt), and indicated mutant grown for 10 generations ± 0.3 mM $CuSO_4$ as in Figure 4A; $n = 4$.



Supplementary Figure 5. Supplement to local replication origin firing regulates CNV at *CUP1* locus. **(A)** TrAEL-seq read polarity plots for *3xCUP1* strains wild type, control or New ARS (defined in Figure 5B), showing region surrounding *CUP1* on chromosome VIII, processed as Figure 5A, n=1. **(B)** Plots of final OD_{600} versus $[CuSO_4]$ for wild type and No ARS cells that are naïve (above) or pre-cultured in 0.3 mM $CuSO_4$ (below). Copper resistance of No ARS cells that have not been pre-cultured in $CuSO_4$ is similar to wild type, showing that deletion of this large region of the *CUP1* repeat does not impair the normal response to environmental copper. This is the source data underlying the plots in Figure 5D.



Supplementary Figure 6. Proposed mechanism for H3K56ac scar incorporation during unsuccessful replication origin firing. DNA strands are shown in black, the replication origin is shown in blue and chromatin containing H3K56ac shown in red. In the presence of topological stress, replication origins may fire but stall soon after. Positive supercoiling may promote reversal of such stalled fork structures to the point of dissolution, leaving behind a “H3K56ac scar” from initial chromatin assembly

Supplementary Tables

Tables are provided as Excel files.

S1 Table *S. cerevisiae* yeast strains used in this study

S2 Table Oligonucleotide pairs used in this study.

S3 Table Quantification of CNV derived from Mutant Screen. % CNV alleles were calculated as intensity of bands by southern blot analysis: $\text{CNV alleles} / (\text{CNV alleles} + \text{Parental allele}) \times 100$. Fold-change in CNV was calculated as $\% \text{ CNV alleles for } gene\Delta / \% \text{ CNV alleles of Wild Type}$.

Supplementary References

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