

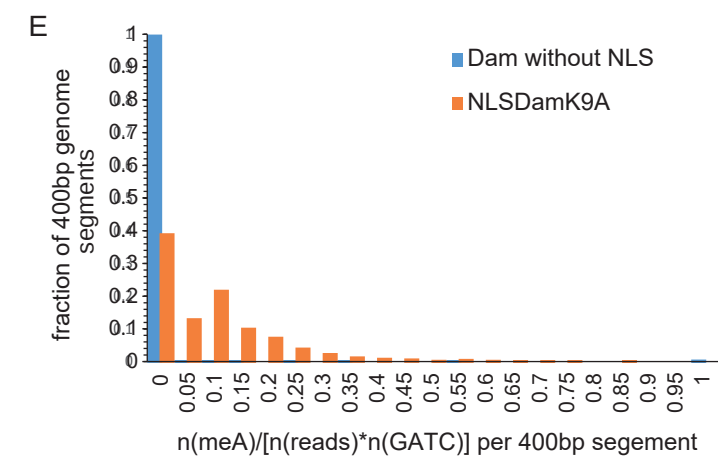
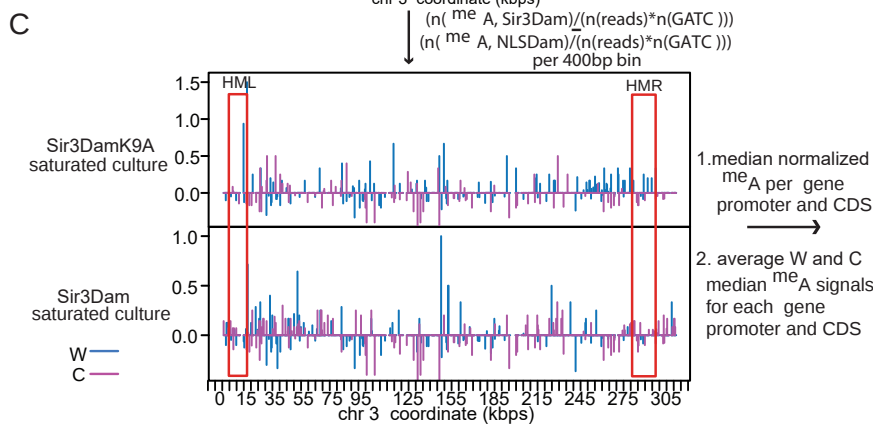
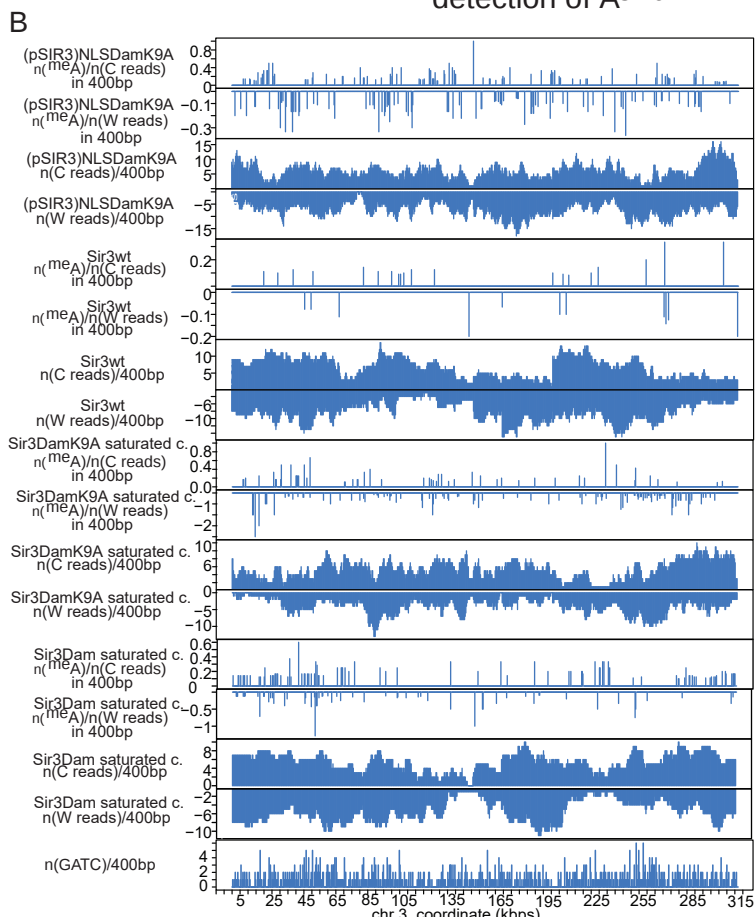
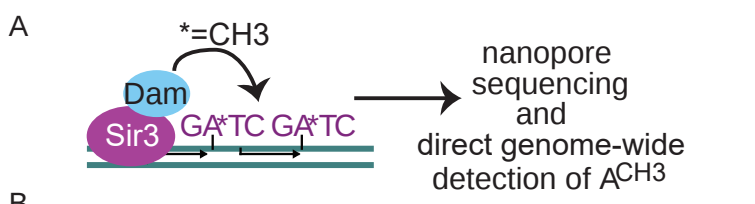
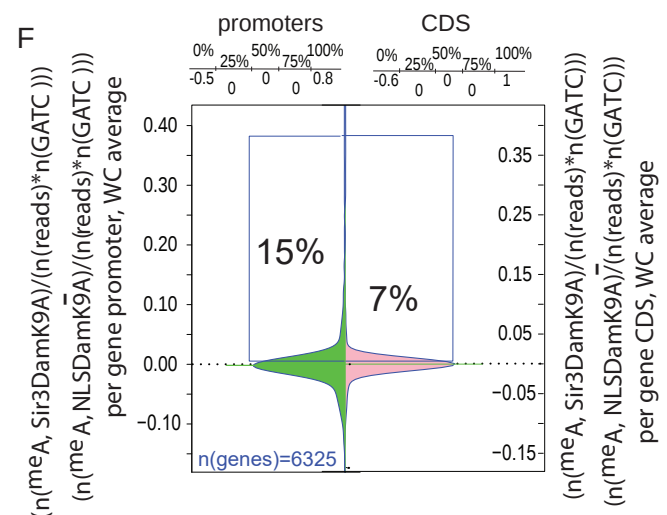
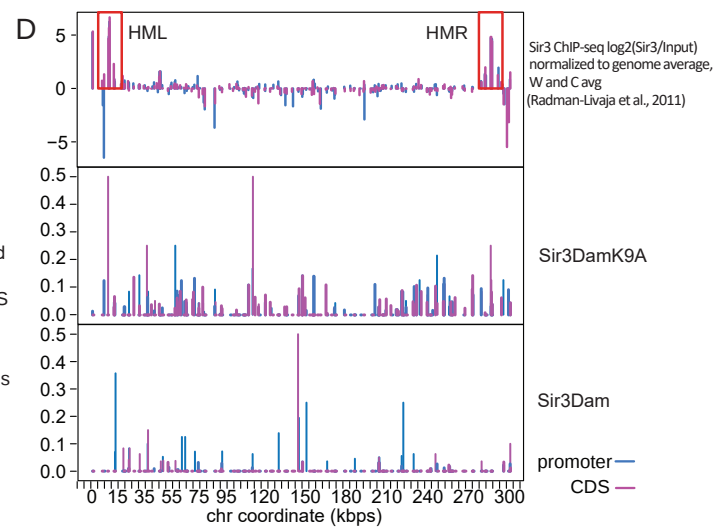
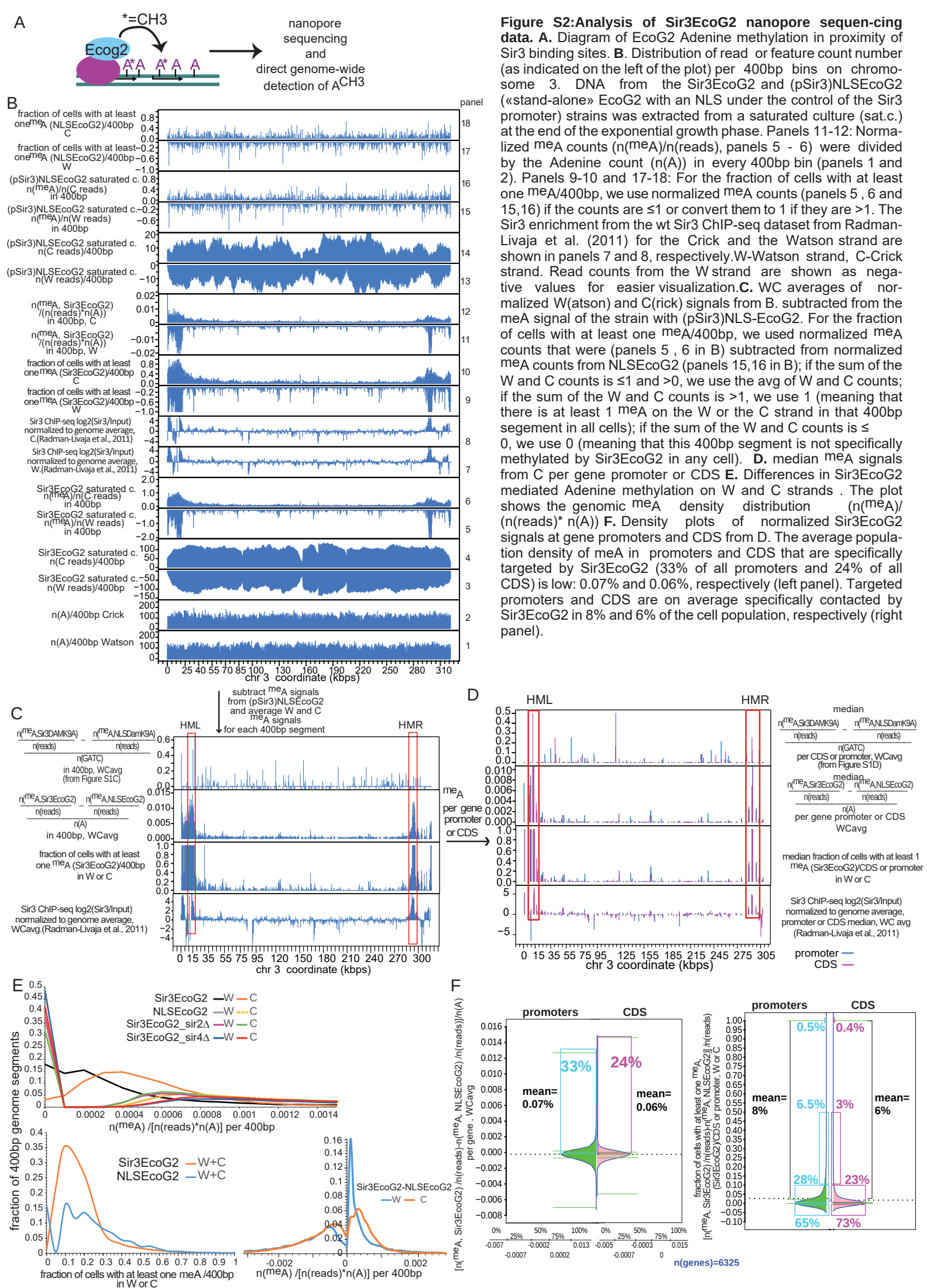


Figure S1: Analysis of Sir3Dam nanopore sequencing data. **A.** Diagram of Dam Adenine methylation within GATC sequences in proximity of Sir3 contact sites. **B.** Distribution of read or feature count number (as indicated on the left of the plot) per 400bp bins on chromosome 3. DNA from Sir3Dam, Sir3DamK9A, Sir3wt (untagged Sir3) or (pSIR3)NLSDamK9A («standalone» DamK9A with an NLS sequence under the control of the SIR3 promoter) strains was extracted from saturated cultures at the end of the exponential growth phase. W-Watson strand, C-Crick strand. Read counts from the W strand are shown as negative values for easier visualization. **C.** Normalized meA counts ($n(\text{meA})/n(\text{reads})$) from Sir3Dam, Sir3DamK9A and (pSIR3)NLSDamK9A from B were divided by the GATC count ($n(\text{GATC})$) in every 400bp bin (if there are no GATCs in the bin the signal was considered as NA) and the normalized meA signal from the (pSIR3)NLSDamK9A strain was subtracted from the meA Sir3Dam and Sir3DamK9A signals. Signals from the W(atson) (blue) and the C(rick) (magenta) strands are shown in the same panel. **D.** The median meA signal of signals from C were first determined for each gene promoter and each gene CDS and then these signals were averaged between the Watson and the Crick strands if neither was marked as NA, otherwise only the non NA value was kept. The WC average Sir3 enrichment per gene promoter and CDS from a wt Sir3 ChIP-seq dataset from Radman-Livaja et al. (2011) is shown in the top panel for comparison. **E.** Comparison of GATC methylation probabilities between a strain that contains a stand alone Dam gene without the NLS sequence and the NLSDamK9A strain. Both constructs are under the control of the Sir3 promoter. We first selected 400bp segments from the genome whose sequences were present in both datasets, then we determined the distribution of GATC methylation probabilities in the set of selected 400bp segments from the Dam without NLS (blue) and NLSDamK9A (orange) strains. **F.** Density plots of normalized Sir3DamK9A signals at gene promoters and CDS from saturated cultures from D. 15% and 7% of gene promoters and CDS, respectively, have a normalized Sir3DamK9A signal >0





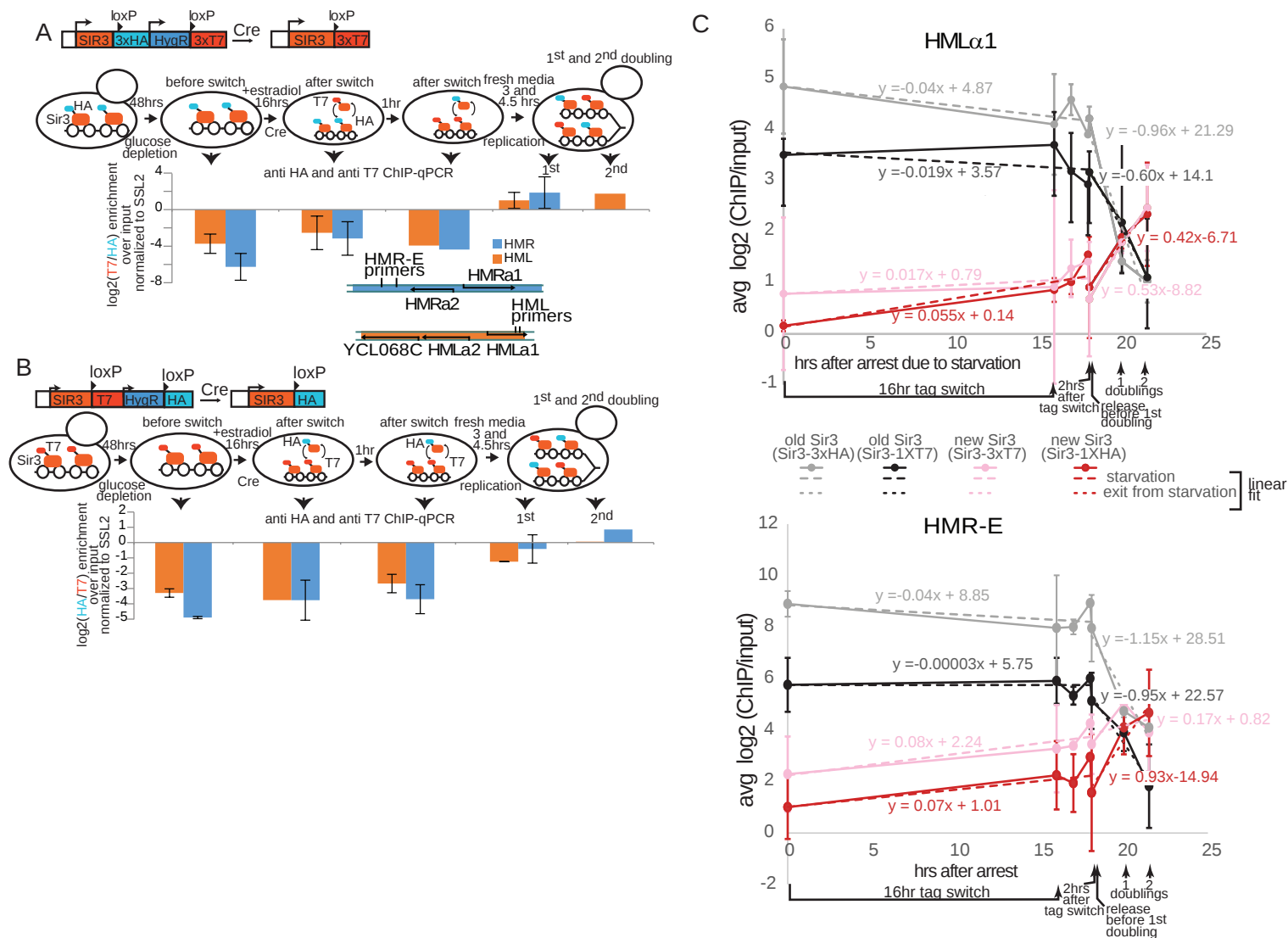
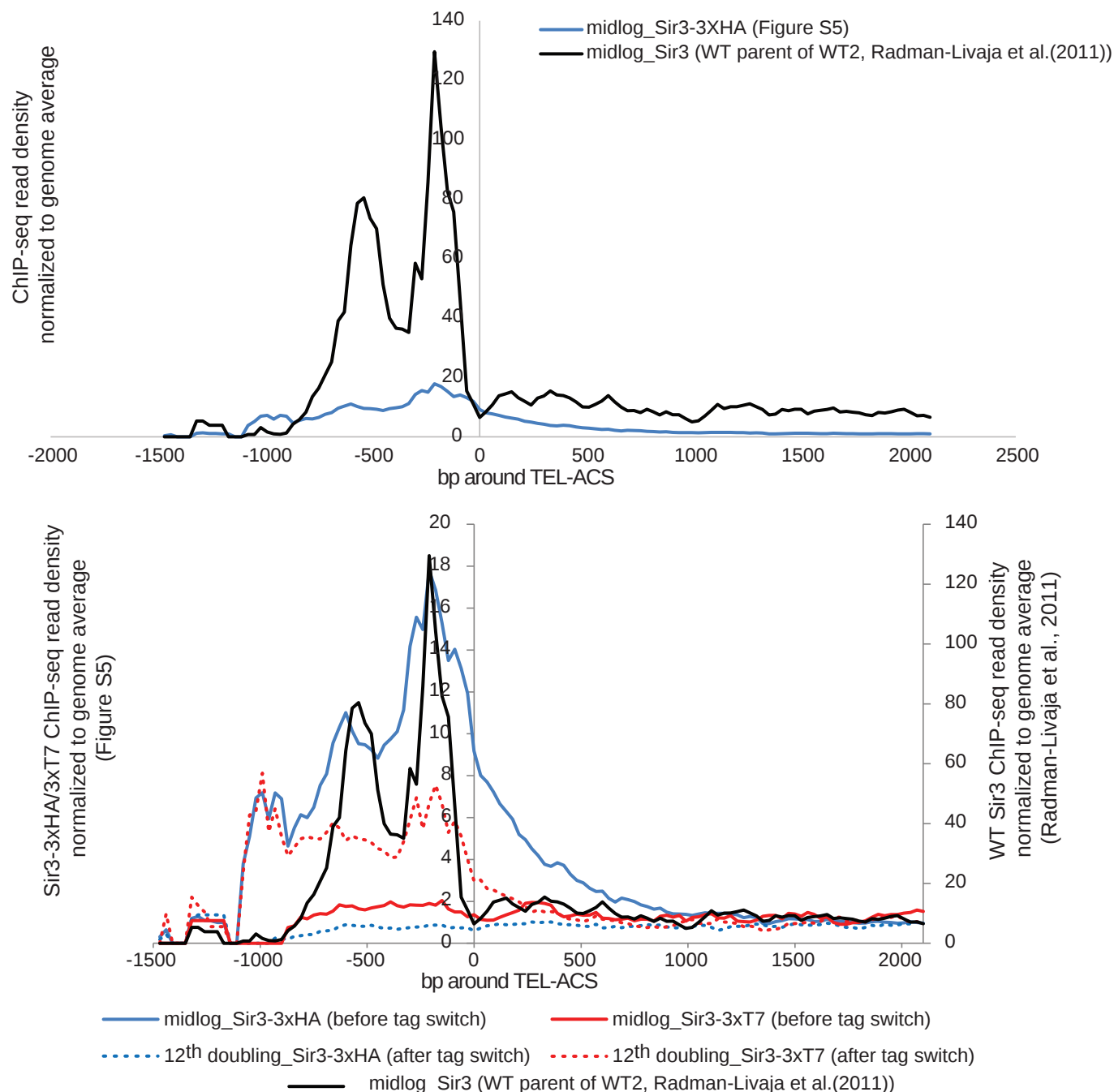


Figure S3: Cell growth dependent and independent Sir3 turnover at silent mating type loci. A.-B. Sir3 tag switch ChIP qPCR experiments for 3xHA to 3xT7 (A) and 1xT7 to 1xHA tag switches (B). Cells are arrested by glucose depletion before the tag switch. The switch is induced with estradiol addition to arrested cells (98% of cells have switched after a 16hr incubation). Cells are then released from arrest with addition of fresh media and allowed to grow for one or two doublings. Cell aliquots were fixed with 1% formaldehyde for 20min at times indicated in the diagrams and anti-HA and anti-T7 ChIPs were performed on sonicated chromatin. Bar graphs show the ratios of new (T7 in A or HA in B) over old (HA in A or T7 in B) Sir3 enrichment at HMR (blue) and HML (orange) silent mating type loci measured by qPCR (Cts were normalized to the SSL2 locus (where Sir3 does not bind) for ChIPs and inputs, separately, and the ChIP Δ Cts were then normalized to input Δ Cts). The location of qPCR primers are shown in the diagram below the bar graph in A. The bar graphs show the average of two biological replicates (error bars are deviations from the average). **C.** The scatter plots show the apparent ON (change in new Sir3p (ChIP/Input) log₂ ratio in hr⁻¹) and OFF (change in old Sir3p (ChIP/Input) log₂ ratio in hr⁻¹) Sir3p rates at HML (top) and HMR (bottom), calculated from the slopes of linear fits before and after release, as shown. Error bars are standard deviations from the average of two biological replicates.

A



B

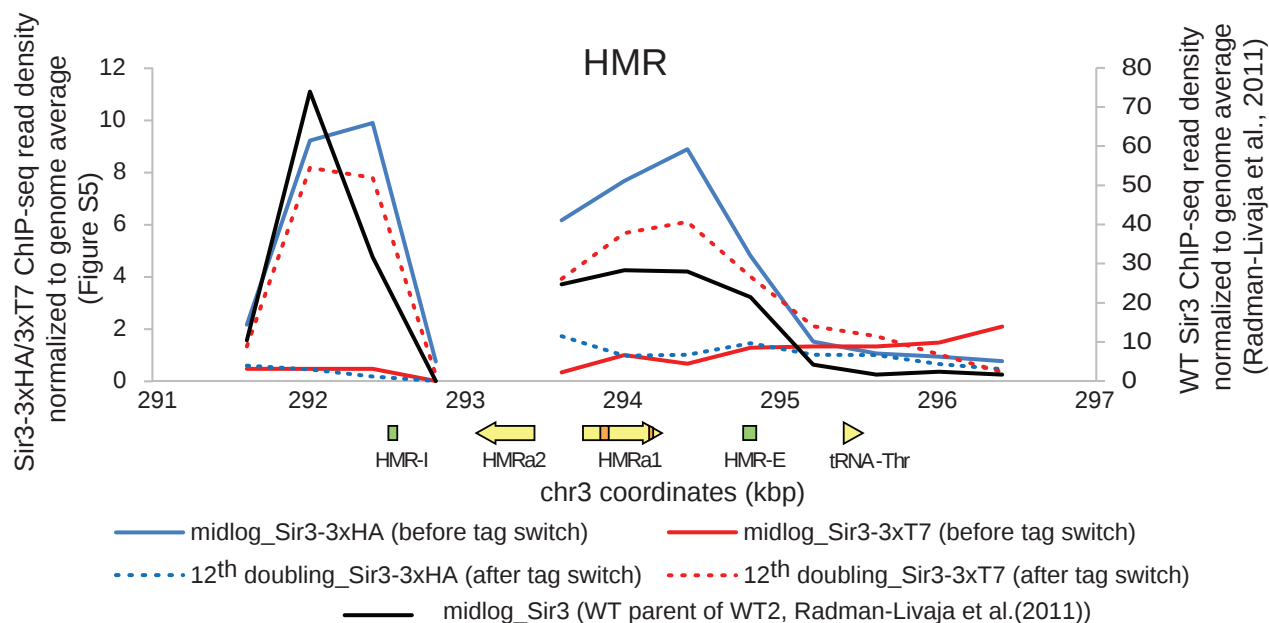
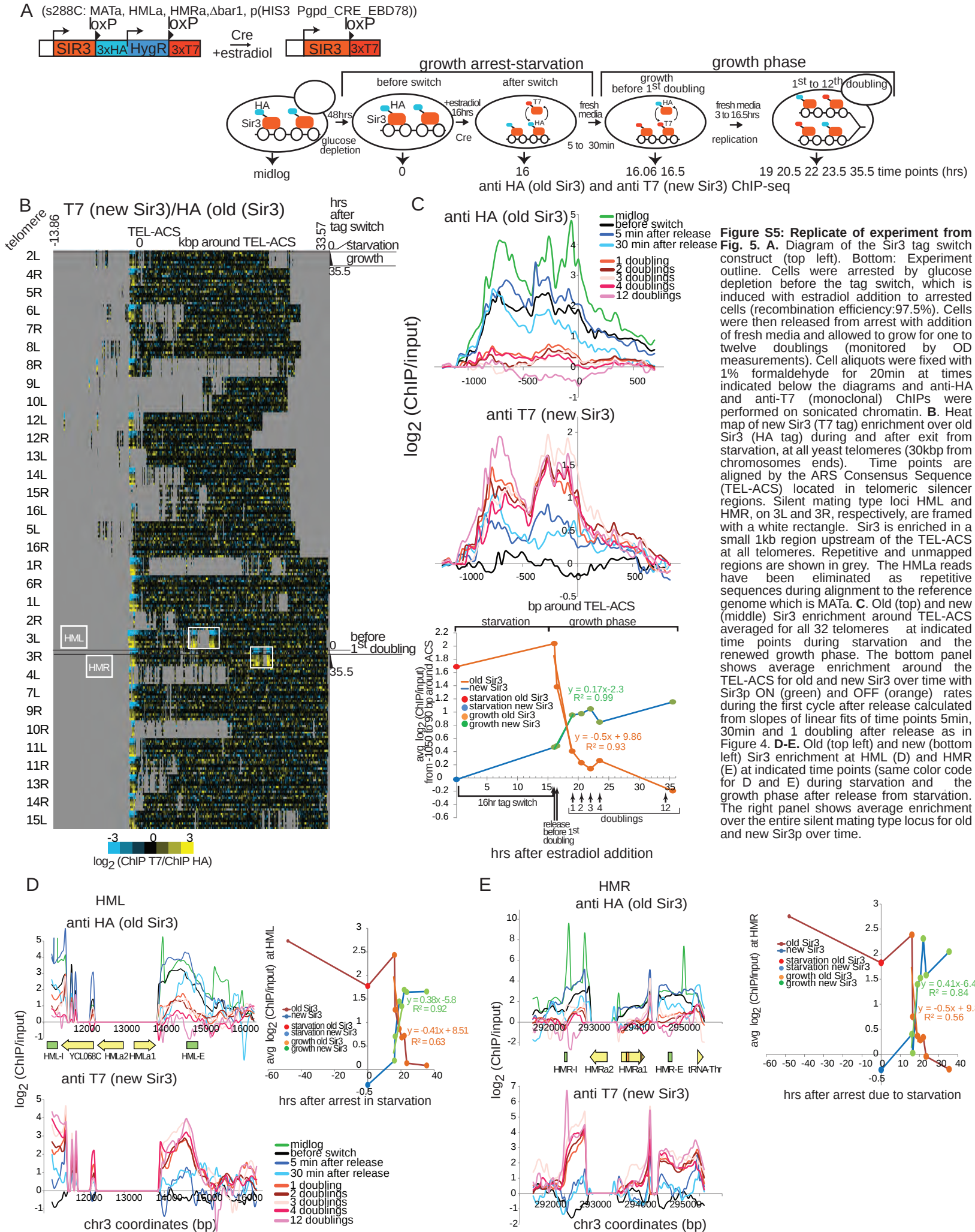
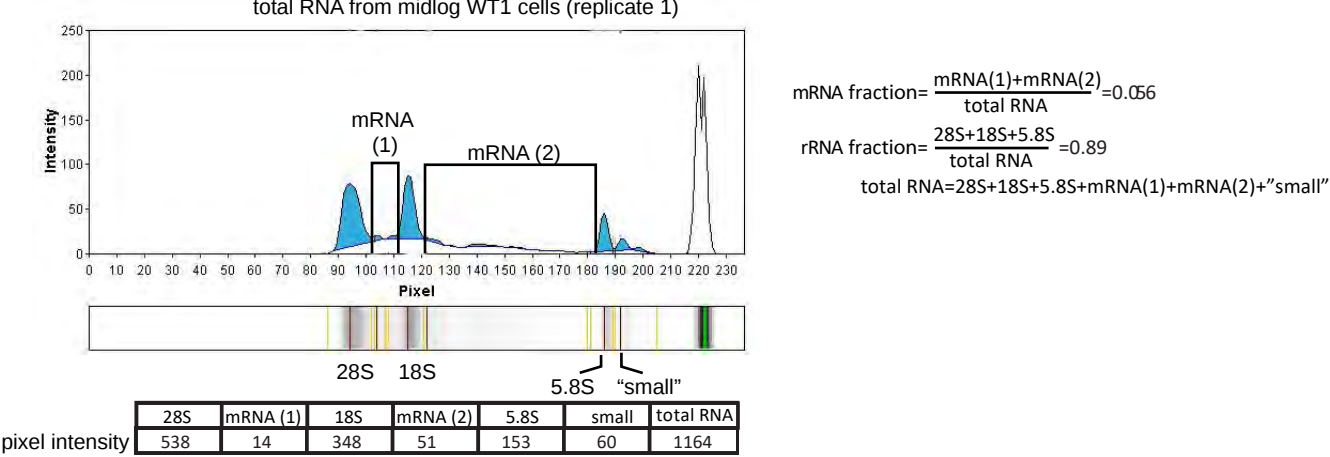


Figure S4: Comparison of untagged and 3xHA/3xT7 tagged Sir3 ChIP-seq in subtelomeric regions (A) and at the HMR silent mating type locus (B)



A



B

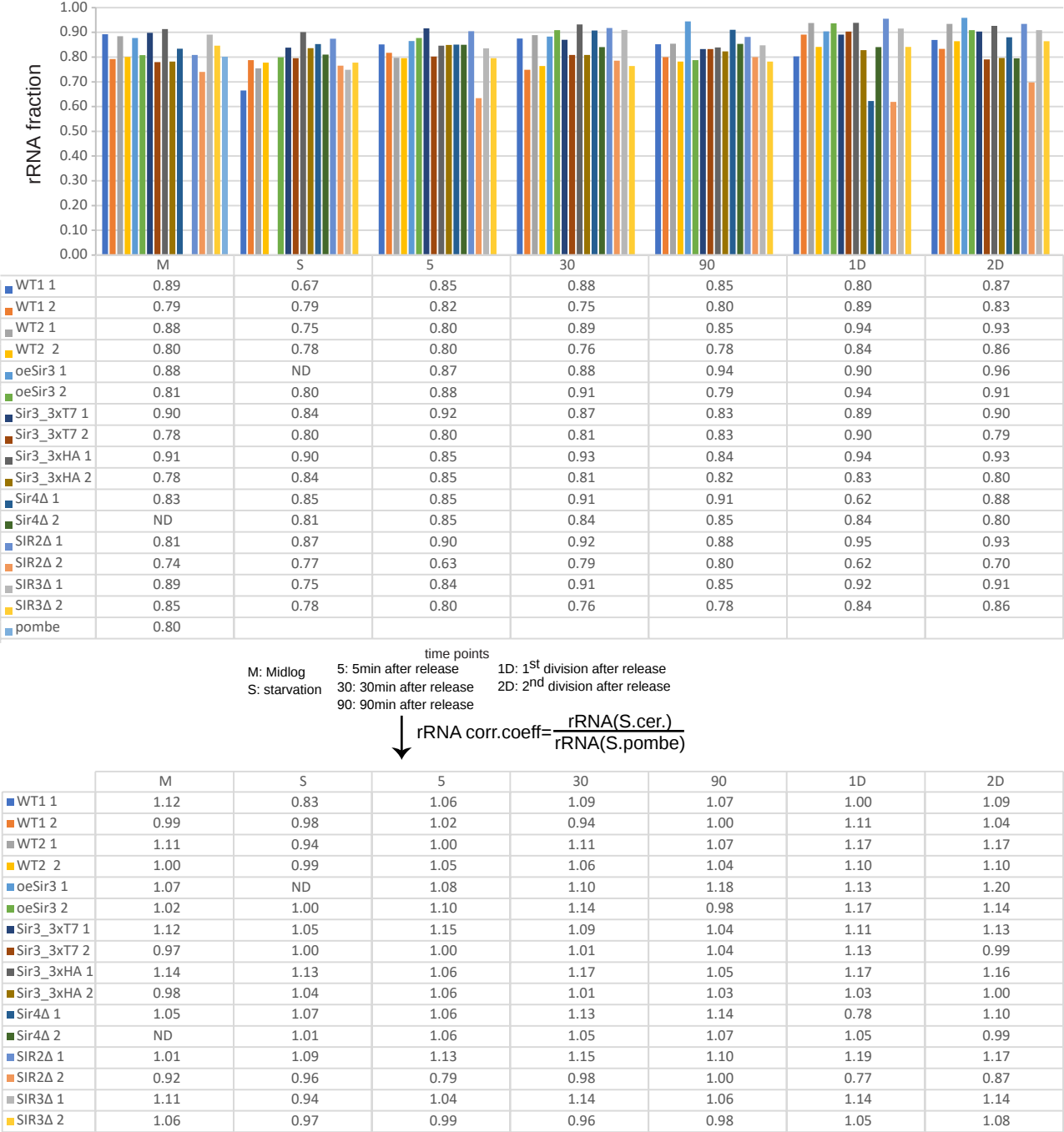


Figure S6: Calculation of the correction coefficient for variability in rRNA content between different strains and timepoints. A. We used Gel Analyzer (Istvan Lazar, www.gelanalyzer.com) to quantify the rRNA content from total RNA samples that were ran on the LabChip Nucleic Acid Analyzer. Total RNA from midlog WT1 cells is shown as an example. Background correction was performed with the rolling disk method (10% radius). The positions of 28S, 16S and 5.8S rRNA bands are indicated in the gel image below the pixel intensity profile. The rRNA bands are aligned with their corresponding pixel intensity peaks in the graph above the gel image. The rRNA fraction was determined with the formulas below the graph **B.** rRNA fraction for all RNAseq samples from Figure 7. The rRNA correction coefficient was obtained by dividing the rRNA fraction from all *S.cerevisiae* samples with the rRNA fraction from *S.pombe* midlog cells.

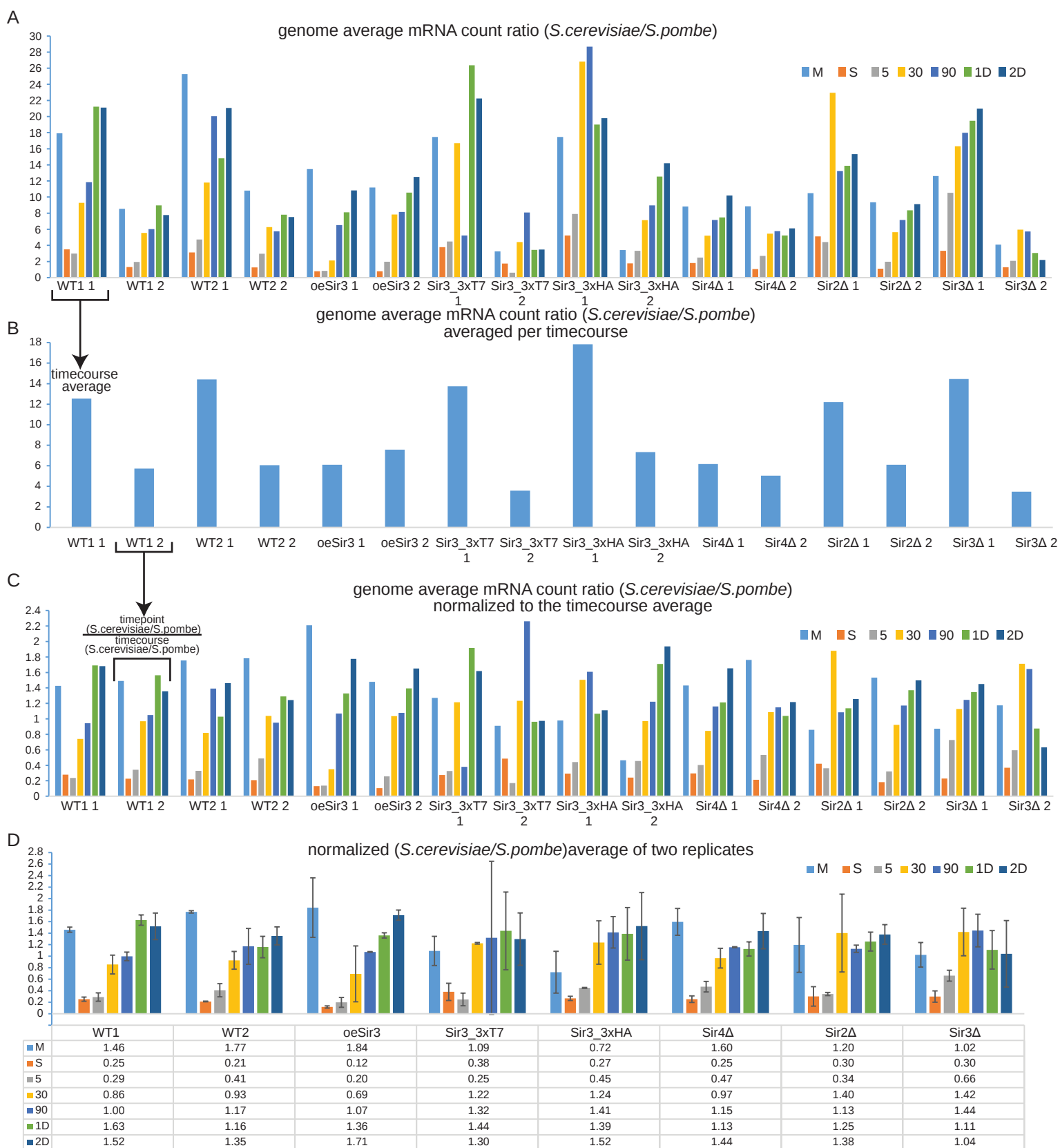


Figure S7: Faster restoration of global mRNA to pre-starvation midlog levels in Sir complex mutants and Sir3 hypomorphs A. ratios of *S.cerevisiae* and *S.pombe* genome-wide average mRNA read counts for each time point in each strain/replicate as indicated in the x-axis. **B.** average *S.cerevisiae*/*S.pombe* mRNA count ratios of all time points within each time course. These average ratios were used to normalize median mRNA densities of each gene for each time point within each timecourse from Fig. 7. **C.** The ratios of each time point from A were divided by the corresponding average ratio from the corresponding time course from C, to allow for direct comparisons between strains and replicates. **D.** The normalized time point ratios from C were averaged between biological replicates of the same strain. The error bars represent the standard error between two replicates. Pre-starvation midlog mRNA levels are restored 30 min after release from starvation in Sir mutants and Sir3 hypomorphs versus 90min for WT1, WT2 and oeSir3.

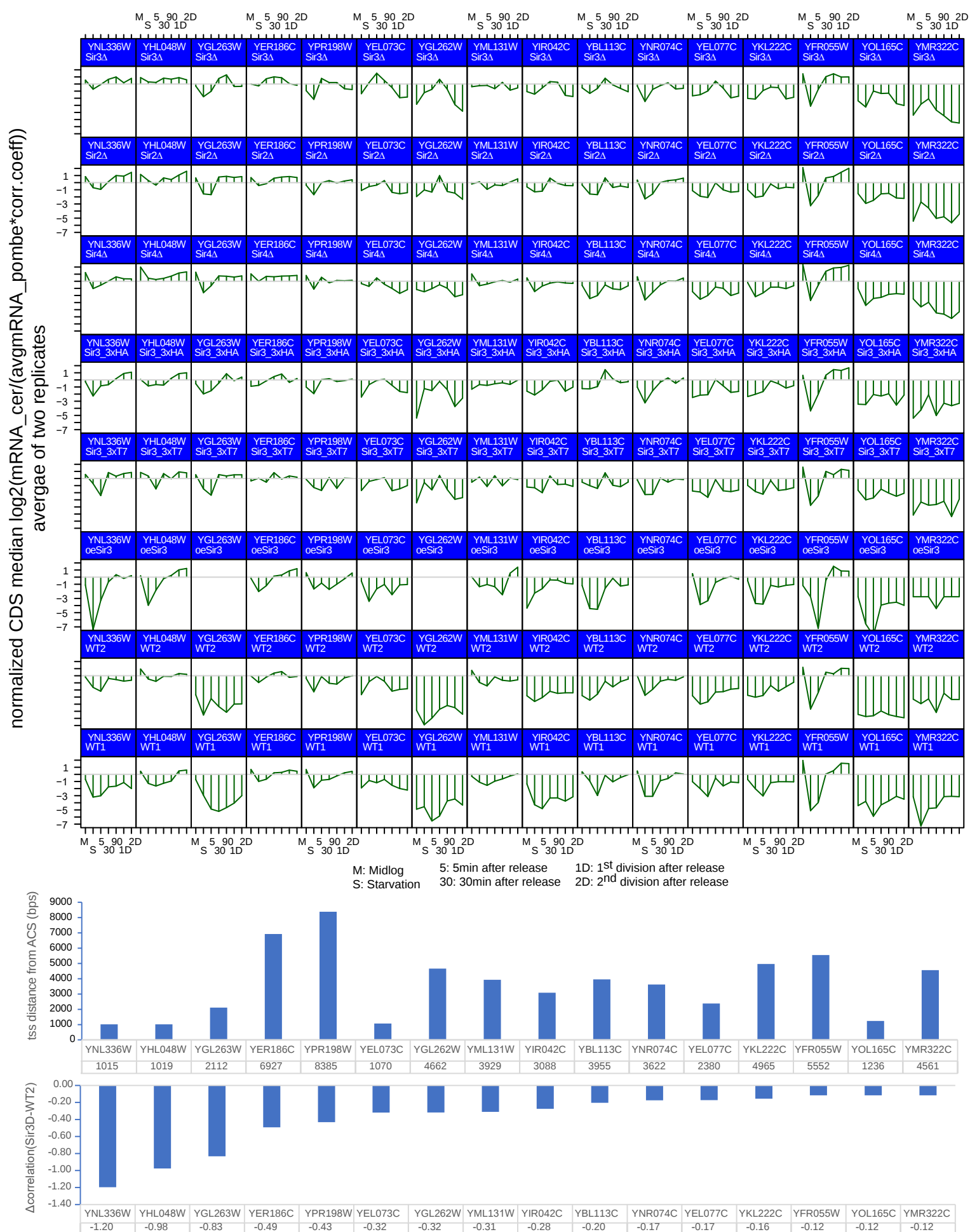
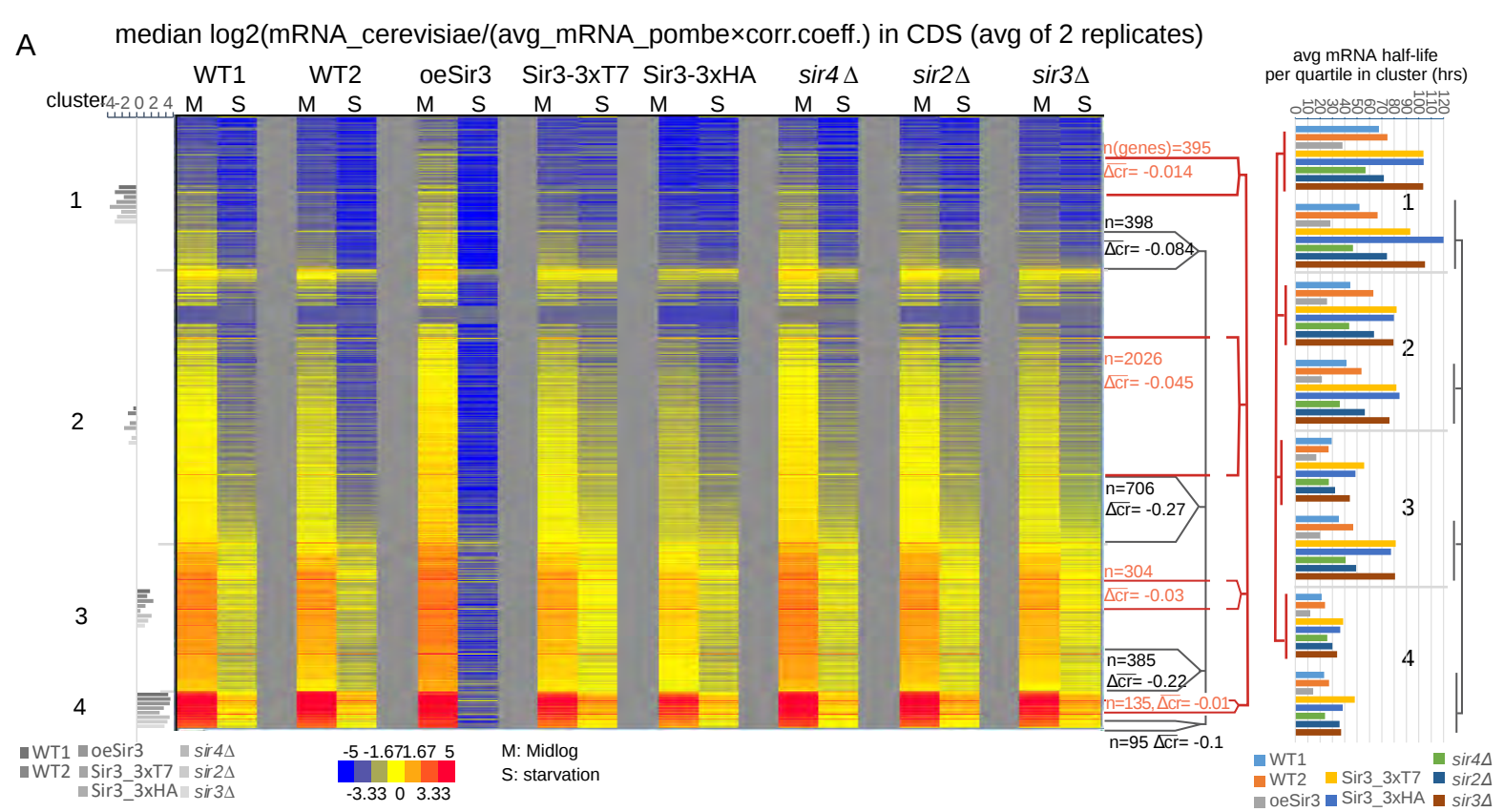


Figure S8: Gene expression dynamics after exit from starvation of subtelomeric genes responsive to Sir3 levels (top). We selected genes within 15kbp of subtelomeric Sir3p nucleation sites from the RNA-seq data-sets in Figure 7 (middle panel) and kept the genes whose expression profiles were the most different between wt and Sir3 Δ ($\Delta\text{correlation}(\text{Sir3}\Delta\text{-WT2})$ between -0.12 and -1.2) as shown in the bar graph on the bottom.



$$2^{\text{mRNA}_S} = (2^{\text{mRNA}_M}) * e^{(k_S - k_D) * t}$$

$\text{mRNA}_S = \log_2(\text{mRNA}_{\text{cerevisiae}}/(\text{avg_mRNA}_{\text{pombe}} * \text{corr.coeff.}))$ in starvation
 $\text{mRNA}_M = \log_2(\text{mRNA}_{\text{cerevisiae}}/(\text{avg_mRNA}_{\text{pombe}} * \text{corr.coeff.}))$ in midlog

for synthesis rate $k_S(\text{starvation}) \sim 0$ and $t=72\text{hrs} \Rightarrow$

$$\left[\begin{array}{l} \text{apparent decay rate (hrs}^{-1}\text{)} = k_{da} = \frac{\ln(2^{\text{mRNA}_S - \text{mRNA}_M})}{-72\text{hrs}} \\ \text{half-life (hrs)} = \frac{\ln(2)}{k_{da}} \end{array} \right.$$

B

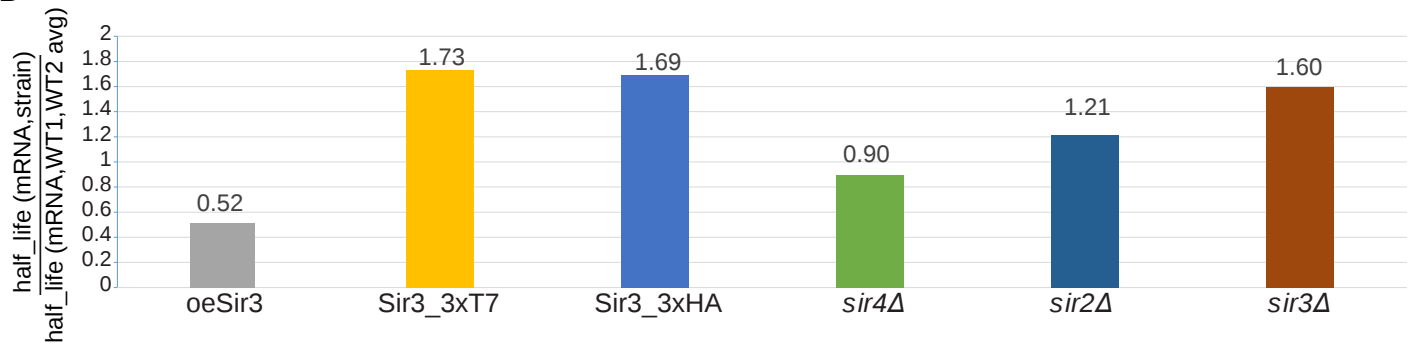
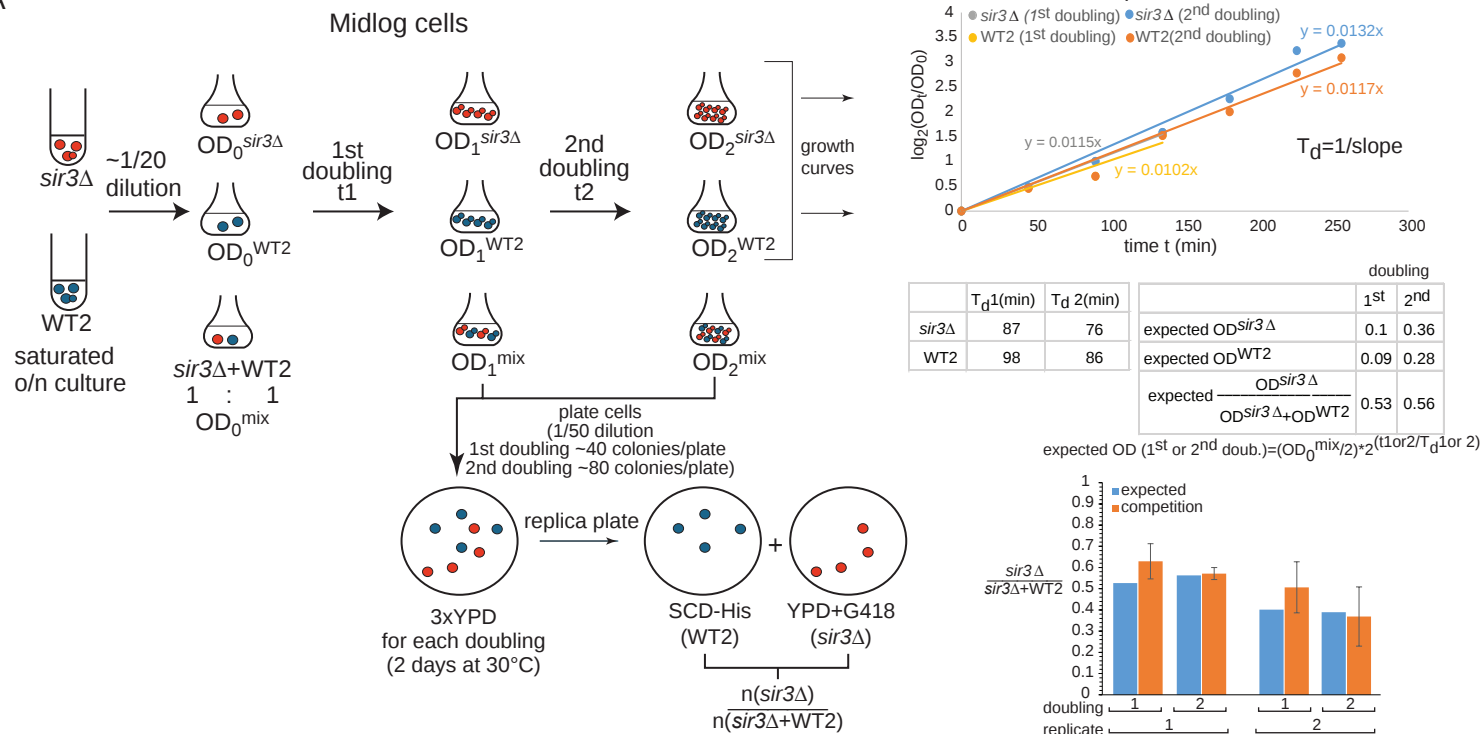


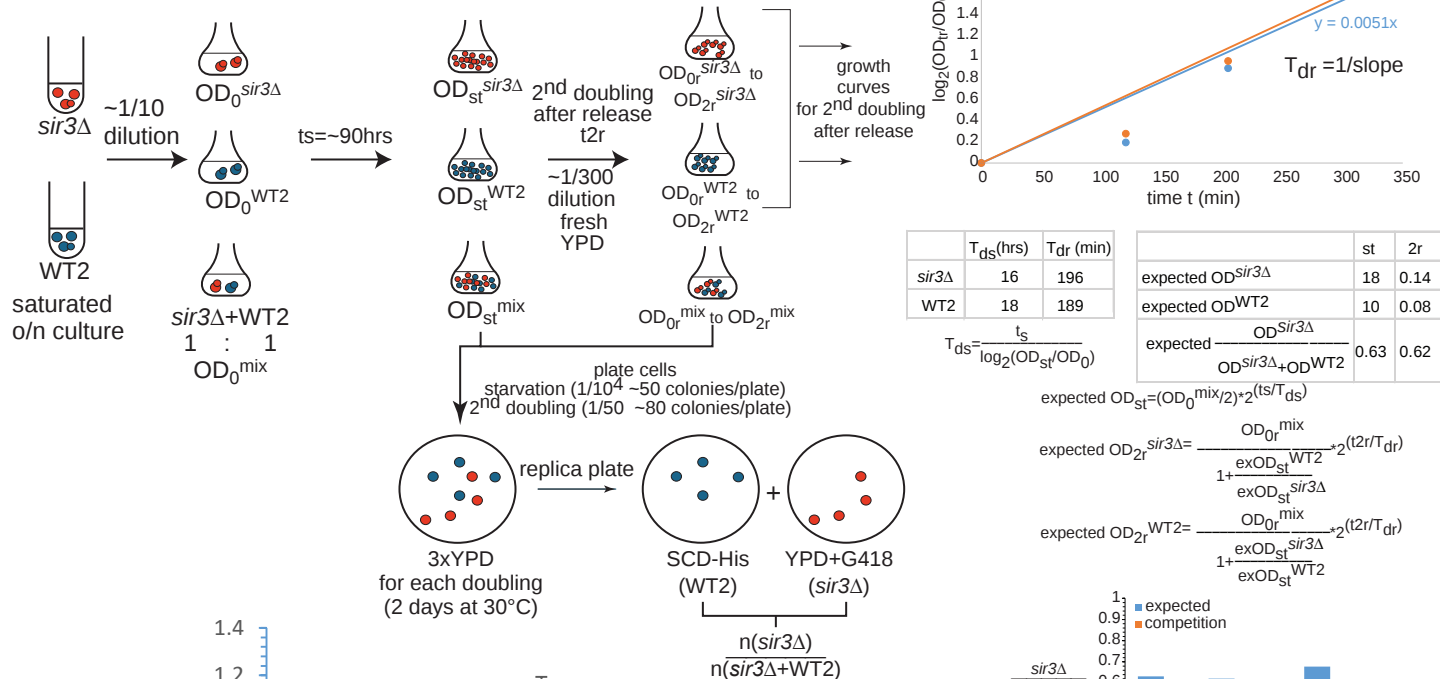
Figure S9: Apparent mRNA half-lives during growth arrest caused by starvation. A. The heat map shows mRNA levels in midlog (M) and starvation (S) from the RNA-seq experiment from Figure 7. Genes are organized as in Figure 7A. mRNA decay rates (k_d) and half lives for each gene were determined from the decrease in mRNA levels between midlog (mRNA_M) and starvation (mRNA_S) according to the equation shown in red below the heat map. We assumed that mRNA synthesis and decay are exponential and that the mRNA synthesis rate (k_s) is close to 0 during growth arrest (starvation) for the majority of yeast genes. The average mRNA half lives in gene quartiles with *sir3*Δ most similar to WT1/2 (framed in red) or least similar to WT1/2 (framed in grey) in each cluster are shown in the bar graph on the right. The apparent mRNA half lives in *sir3*Δ and *Sir3* hypomorphs (3xT7 and 3xHA) are longer than in WT1 and 2 in all clusters and gene quartiles. **B.** genome average apparent mRNA half life ratios between indicated strains and the average of WT1 and WT2 strains. If we assume that the true genome average mRNA decay rate (k_d) is the same in all strains, the differences in the measured genome average apparent decay rate (k_{da}) between different strains will be determined by $k_s > 0$ in each strain except oeSir where $k_s \geq 0$. It consequently follows from $k_{da}(\text{oeSir3}) > k_{da}(\text{sir4}\Delta) > k_{da}(\text{wt}) > k_{da}(\text{sir2}\Delta) > k_{da}(\text{sir3}\Delta) > k_{da}(\text{sir3_3xHA}) > k_{da}(\text{Sir3_3xT7})$ that the genome average mRNA synthesis rates during starvation are $k_s(\text{oeSir3}) < k_s(\text{sir4}\Delta) < k_s(\text{wt}) < k_s(\text{sir2}\Delta) < k_s(\text{sir3}\Delta) < k_s(\text{Sir3_3xHA}) < k_s(\text{Sir3_3xT7})$.strain

A



B

Starvation



C

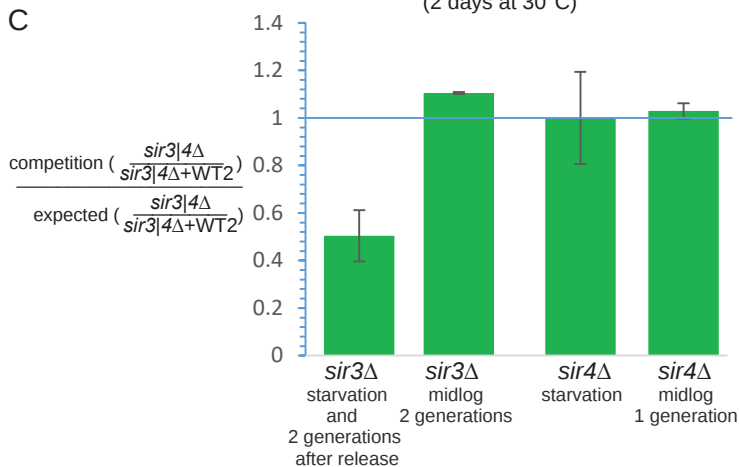


Figure S10: Competitive fitness test between *sir3Δ* or *sir4Δ* and WT2. A. Competition test during exponential growth. Left: outline of the experiment. Saturated *sir3Δ* and WT2 cultures were diluted 20fold in fresh YPD separately or mixed at a 1:1 OD ratio and grown for 2 generations. Individual cultures are used to calculate individual growth rates for the 1st and 2nd doubling (scatter plot on the right), used to determine the expected *sir3Δ* fraction in the mix (expected $(OD_{sir3Δ}/(OD_{sir3Δ}+OD_{WT2}))$ if neither strain is influenced by the presence of the other strain in the mix (the tables on the right below the scatter plot shows values obtained from the first biological replicate). To determine the *sir3Δ* fraction when the two strains are competing with each other, the mixed culture was grown in parallel with the individual cultures and cells from the mix were plated on 3 YPD plates after the 1st and the 2nd doubling. Each YPD plate was then replica plated on a SCD-His (selects for WT2) and a YPD+G418 plate (selects for *sir3Δ*) and individual colonies were counted on each selection plate to get the experimental *sir3Δ* fraction ($n(sir3Δ)/n(sir3Δ+WT2)$). The bar graph on the bottom right shows the expected (blue) and the experimental (competition, orange) *sir3Δ* fractions in the mixed culture for two biological replicates and two generations. The error bars represent the standard error from the average of 3 plates. **B.** As in A but for starvation conditions. Saturated o/n *sir3Δ* and WT2 cultures were diluted (OD_0) and mixed before starvation (cells were left at 30°C for $ts \sim 90$ hrs). An aliquot was plated on 3xYPD plates after 90 hrs of starvation (the st time point) and another aliquot from the starved culture was diluted (OD_{0r}) into fresh YPD. After two doublings the mixed culture was plated on 3xYPD plates. Individual *sir3Δ* and WT2 cultures were grown in parallel in the same conditions as the mix to determine expected *sir3Δ* fractions as in A. The bar graph on the bottom left shows expected and experimental *sir3Δ* fraction for two biological replicates at indicated time points (the time point after release from starvation was only taken in the first replicate). The error bars represent the standard error from the average of 3 plates as in A. **C.** Ratio between experimental (competition) and expected *sir3Δ* or *sir4Δ* fraction in the mixed population during starvation and exponential growth. The *sir3Δ* ratios are averaged over two biological replicates and time points for each replicate from B and A respectively (the starvation condition is averaged from two starvation time points from two replicates and 1 release time point from 1 replicate). The *sir4Δ* ratios are averaged over two biological replicates. The starvation condition is averaged from two starvation time points: 70.25 hrs and 90 hrs for replicates 1 and 2, respectively. *sir4Δ* colonies are also selected on YPD+G418 plates. The error bars represent the standard error from the average of replicates and time points.