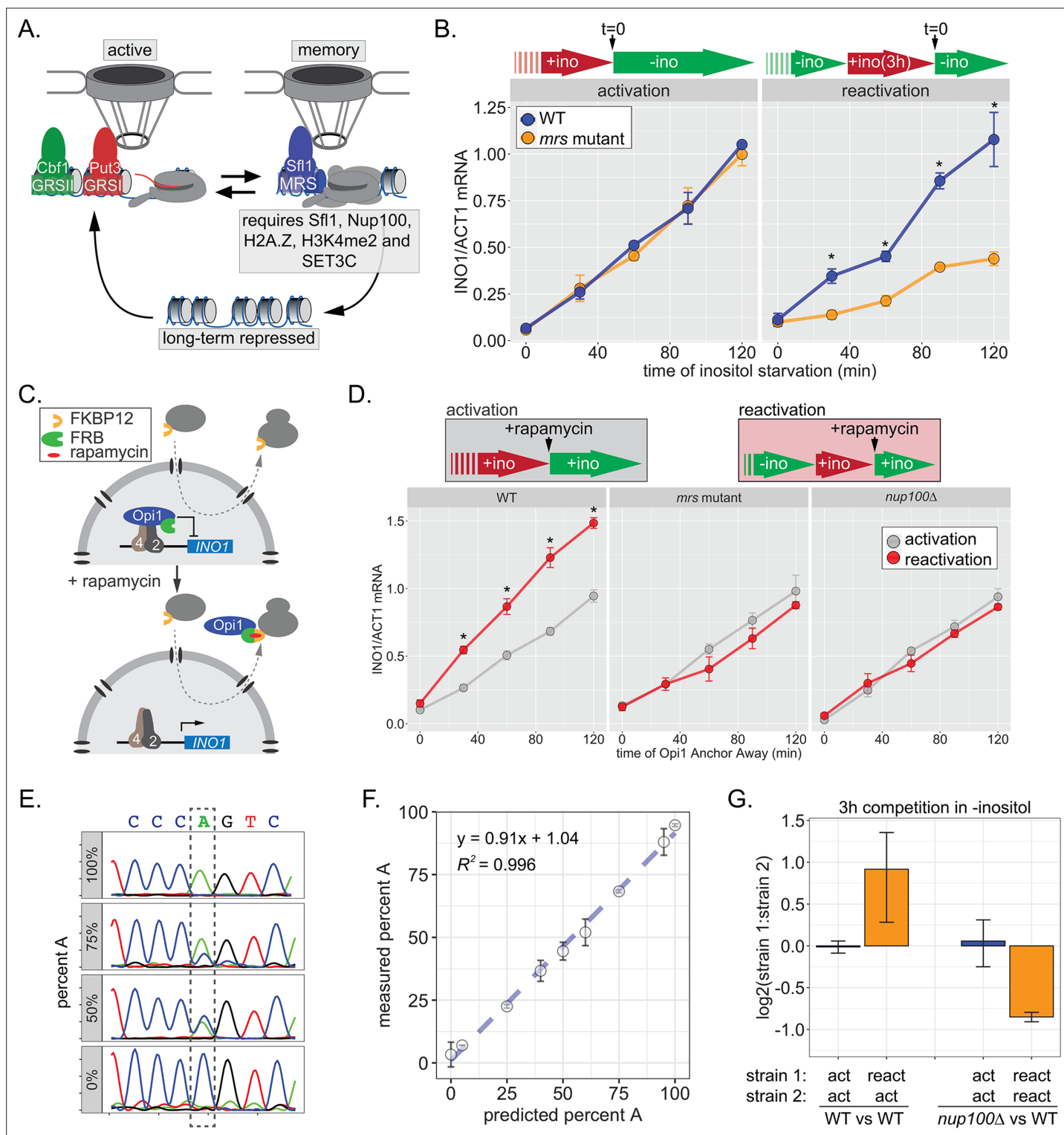


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## Figures and figure supplements

Mitotically heritable, RNA polymerase II-independent H3K4 dimethylation stimulates *INO1* transcriptional memory

**Bethany Sump et al**

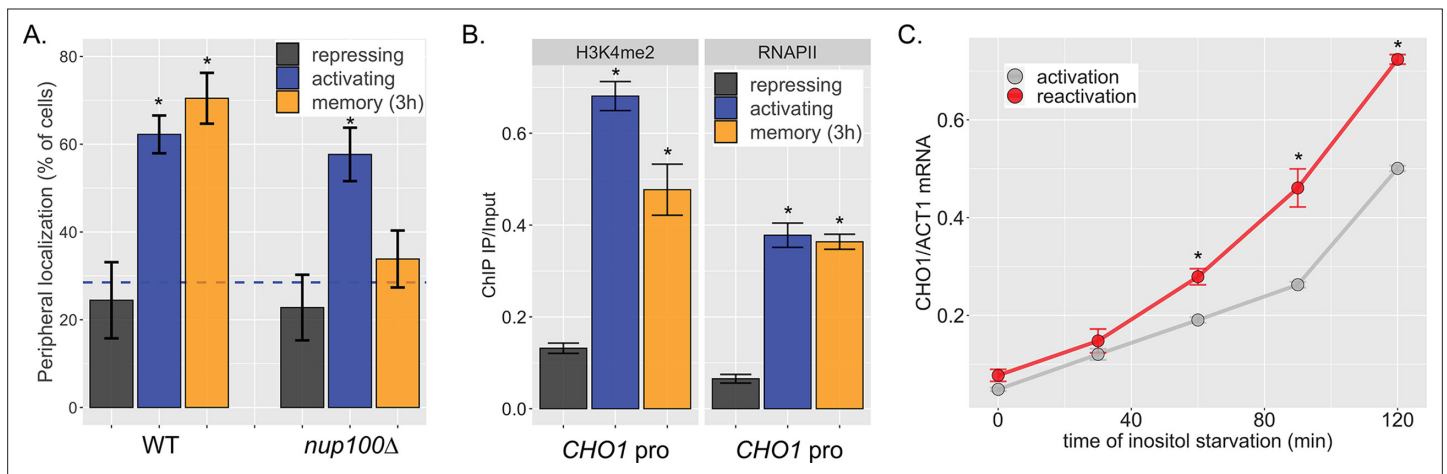


**Figure 1.** *INO1* transcriptional memory stimulates faster transcription and provides a competitive fitness advantage. (A) Model of *INO1* in the active, memory, and long-term repressed states, highlighting factors that are specifically required for memory. (B) Activation (left) and reactivation (right) of *INO1* in wild type (WT) and *mrs* mutant strains upon starvation of inositol. Cells were harvested at indicated time points and the *INO1* mRNA was quantified relative to *ACT1* mRNA by real time quantitative PCR (RT-qPCR) (\*p-value<0.05 from one-tailed t-test comparing WT and *mrs* mutant, alternative = greater). (C) Schematic of Anchor Away of Opi1 to induce *INO1*. (D) Top: experimental scheme for synthetic activation and reactivation of *INO1*. Activation and reactivation of *INO1* in WT (left), *mrs* mutant (middle), and *nup100Δ* (right) strains upon removal of Opi1 by Anchor Away in the presence of inositol. Bottom: Cells were harvested at indicated time points and *INO1* mRNA was quantified relative to *ACT1* mRNA by RT-qPCR (\*p-value<0.05 from one-tailed t-test comparing WT and *mrs* mutant, alternative = greater). (E) Percent A (0% to 100%) for the sequence C C C A G T C. The sequence is shown in the top panel, and the percent A is shown in the bottom panel. (F) Measured percent A (0 to 100) versus predicted percent A (0 to 100). The regression line is  $y = 0.91x + 1.04$  and  $R^2 = 0.996$ . (G) 3h competition in -inositol. The log2(strain 1:strain 2) is shown for WT vs WT and *nup100Δ* vs WT. The WT vs WT comparison shows a positive value, while the *nup100Δ* vs WT comparison shows a negative value.

Figure 1 continued on next page

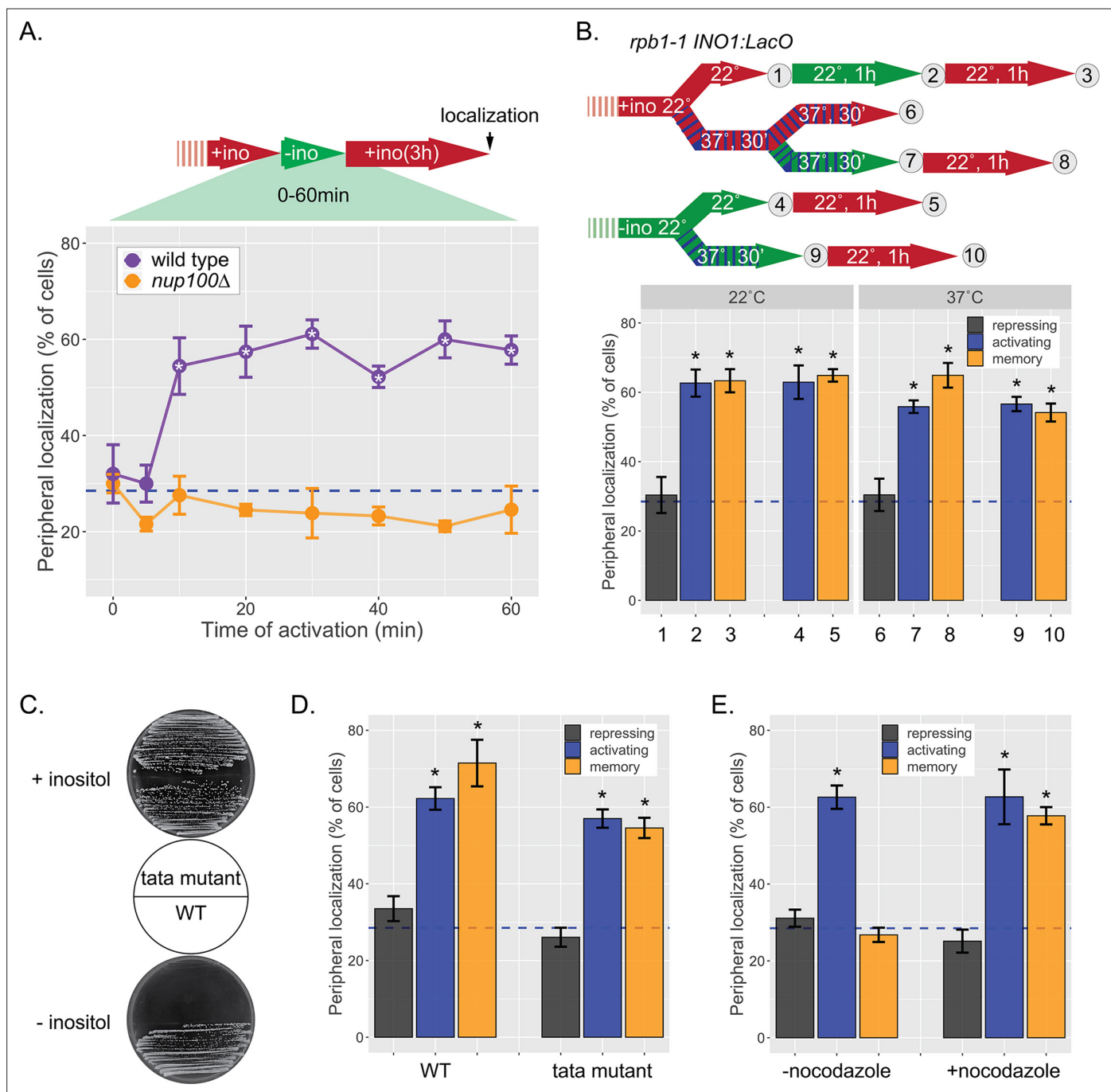
*Figure 1 continued*

value $<0.05$  from one-tailed t-test comparing reactivation and activation, alternative = greater). **(E)** Chromatograms resulting from sequencing mixtures of strains having either 'A' or 'C' SNP within an integrated plasmid, as indicated (dashed box). **(F)** Standard curve comparing the predicted percentage of strain A (as estimated by O.D.<sub>600</sub>) with the measured percentage of A (as quantified by the relative area under the peaks, as shown in E). **(G)** Relative abundance of competing strains, as indicated. The log<sub>2</sub> ratio of the abundance of the two strains after 3 hr of competition in media lacking inositol is shown. For panels B, D, F, and G, data are averages of three biological replicates  $\pm$  SEM.

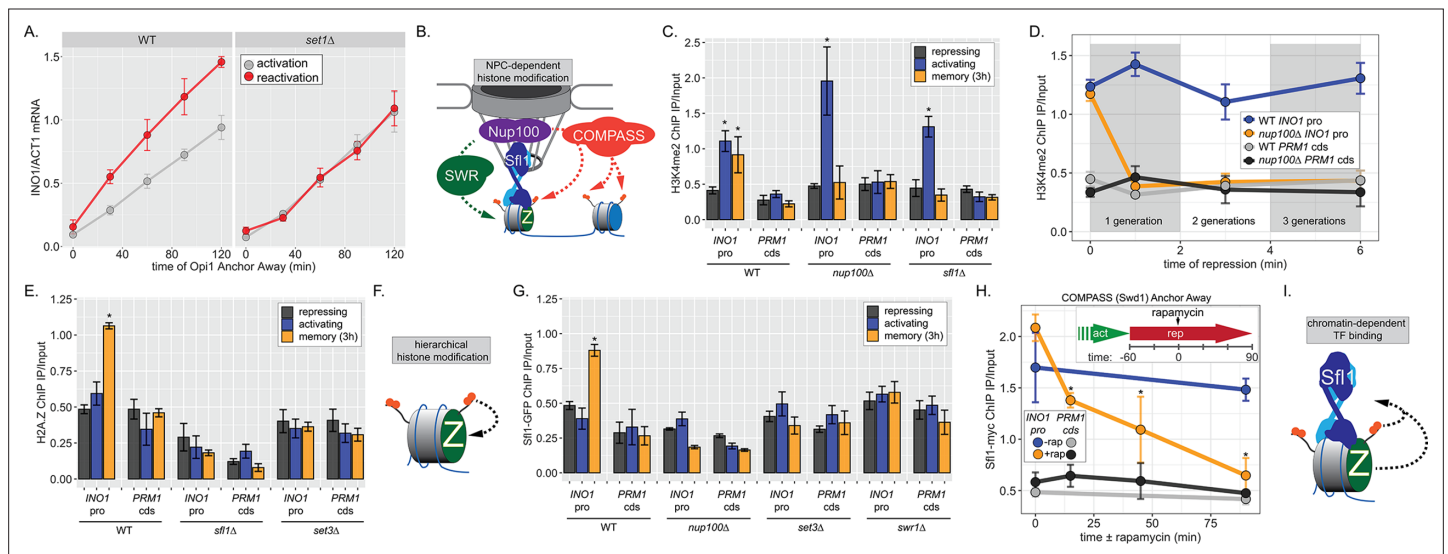


**Figure 1—figure supplement 1.** *CHO1* exhibits inositol transcriptional memory. **(A)** Peripheral localization of *CHO1* under repressing (+inositol), activating (−inositol), or memory (−inositol → +inositol, 3 hr) conditions in either a wild type (WT) or *nup100Δ* strain. The average of three biological replicates ± SEM; each biological replicate counted a population of ≥30 cells. **(B)** Chromatin immunoprecipitation (ChIP) against H3K4me2 (left) or RNA polymerase II (RNAPII) (right) of cells grown under repressing, activating, or memory conditions. **(C)** Cells were harvested at the indicated time points during inositol starvation (either initial starvation or second starvation after 3 hr of repression), and *CHO1* mRNA was quantified relative to *ACT1* mRNA by real time quantitative PCR. For all panels, \**p*<0.05 from one-tailed t-test comparing to repressed condition, alternative = greater.

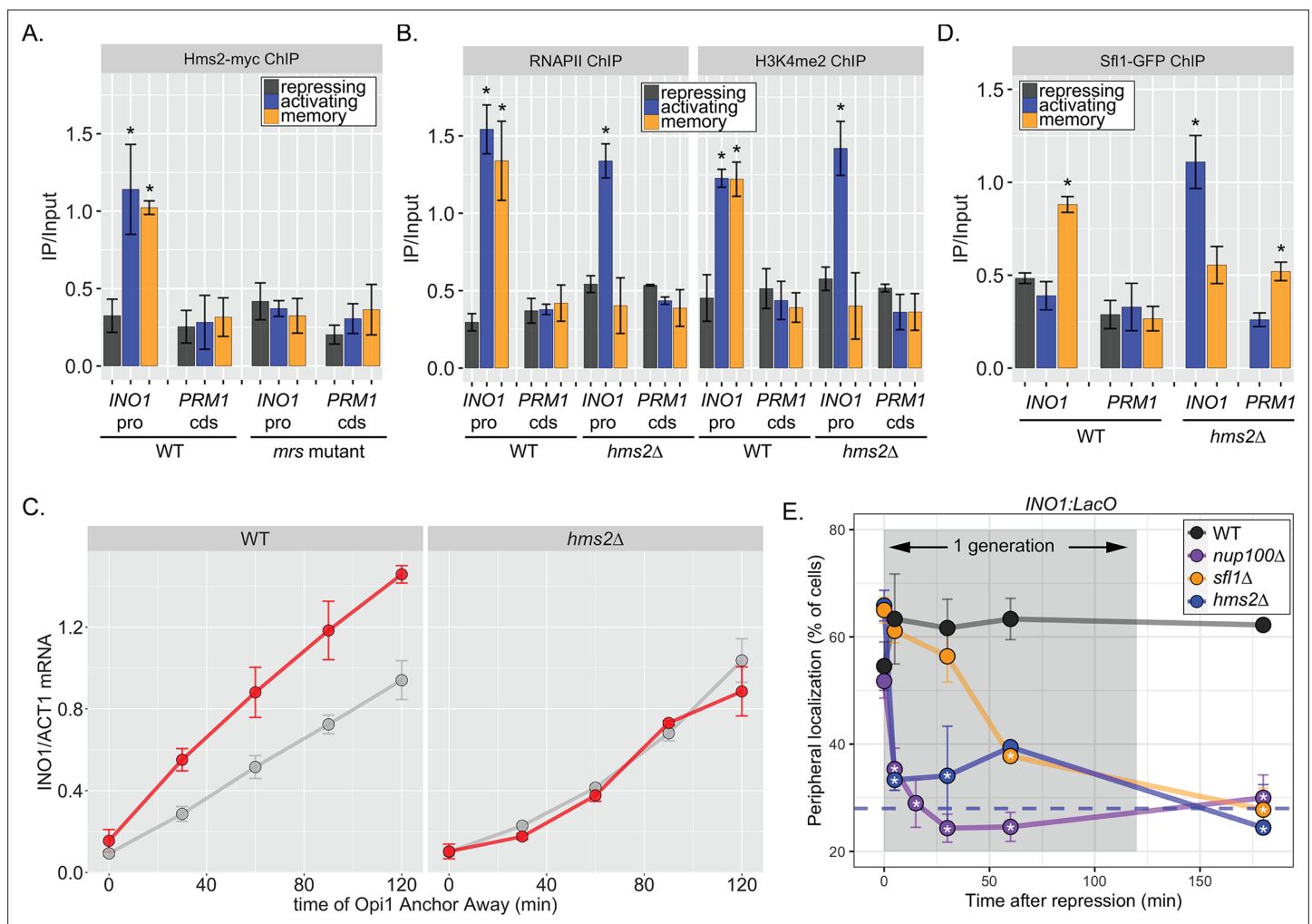




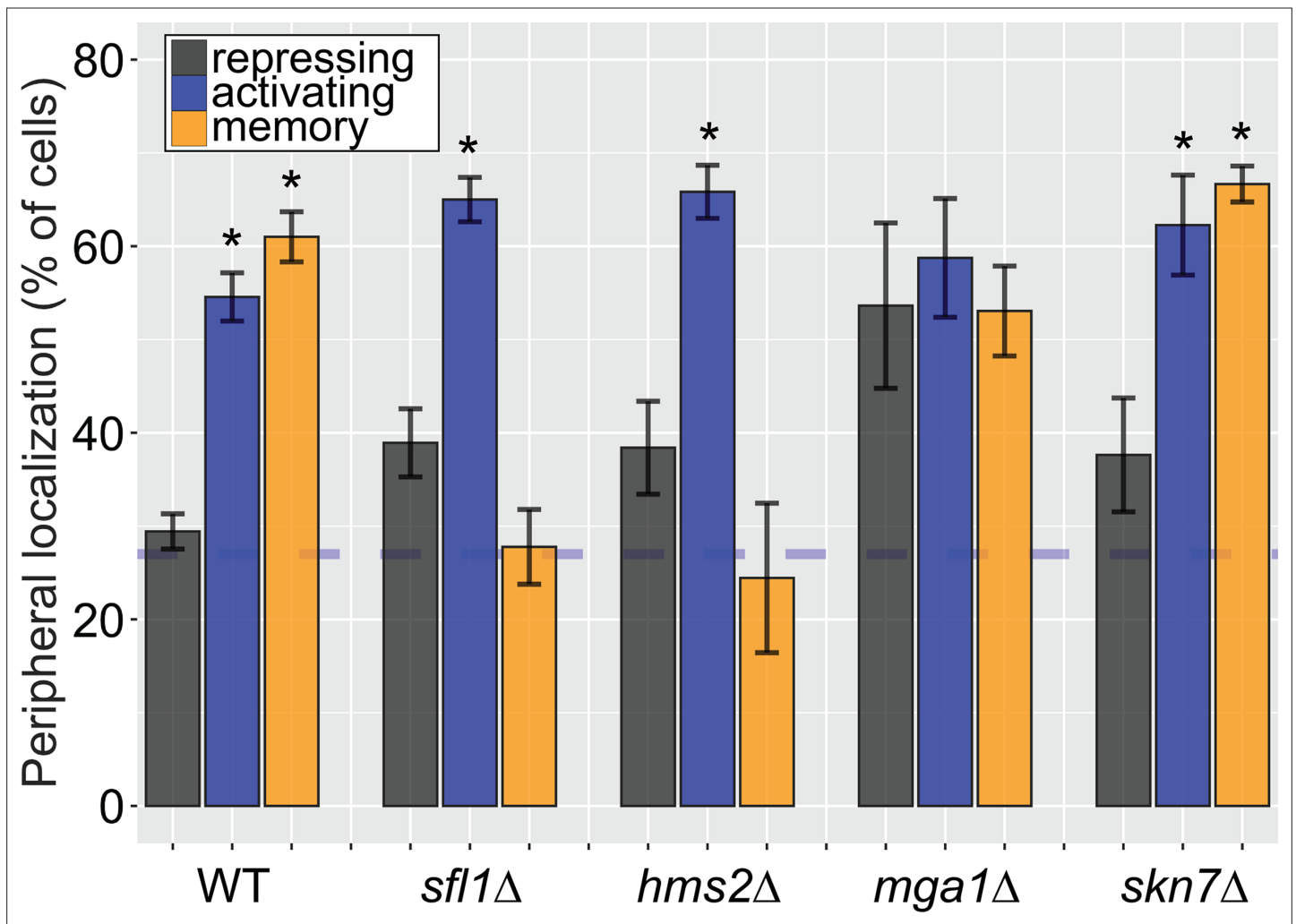
**Figure 1—figure supplement 2.** *INO1* memory does not require transcription. **(A)** Cells grown in the repressing (+inositol) condition, switched to the activating conditions (−inositol) for the indicated amount of time before being returned to the repressing condition for 3 hr. After 3 hr of repression, peripheral localization was scored. \* $p < 0.05$  from one-tailed t-test comparing wild type (WT) to *nup100*, alternative = greater. **(B)** Peripheral localization of *INO1* in a *rpb1-1* temperature sensitive mutant strain. The permissive temperature is 22°C and the restrictive temperature is 37°C. Top: schematic of experimental design; cells were grown under either repressing (red) or activating (green) at either 22°C (solid) or 37°C (hatched) before scoring for peripheral localization. **(C)** Growth of WT and *tata* mutant cells on SDC (synthetic dextrose complete) +inositol (top) and SDC −inositol (bottom). The TATA box (5′ – TATATAAATT – 3′) at the endogenous *INO1* gene was replaced with 5′ – GCGCGCCCGG – 3′ using CRISPR Cas9. **(D)** Peripheral localization of WT and *tata* mutant *INO1* was scored following 1 hr of inositol starvation and 3 hr of growth in +inositol. **(E)** Peripheral localization of *INO1* under repressing (+inositol), activating (−inositol), or memory (−inositol → +inositol, 18 hr) conditions, with or without addition of nocodazole for 18 hr. Note: nocodazole-treated cells divided  $\leq 4$  times, while the untreated cells divided  $\sim 9$  times. For all experiments, average of three biological replicates  $\pm$  SEM; each biological replicate counted a population of  $\geq 30$  cells. For panels B, D, and E, \* $p < 0.05$  from one-tailed t-test comparing to repressed condition, alternative = greater. Blue hatched line: expected peripheral localization for a randomly localized gene.



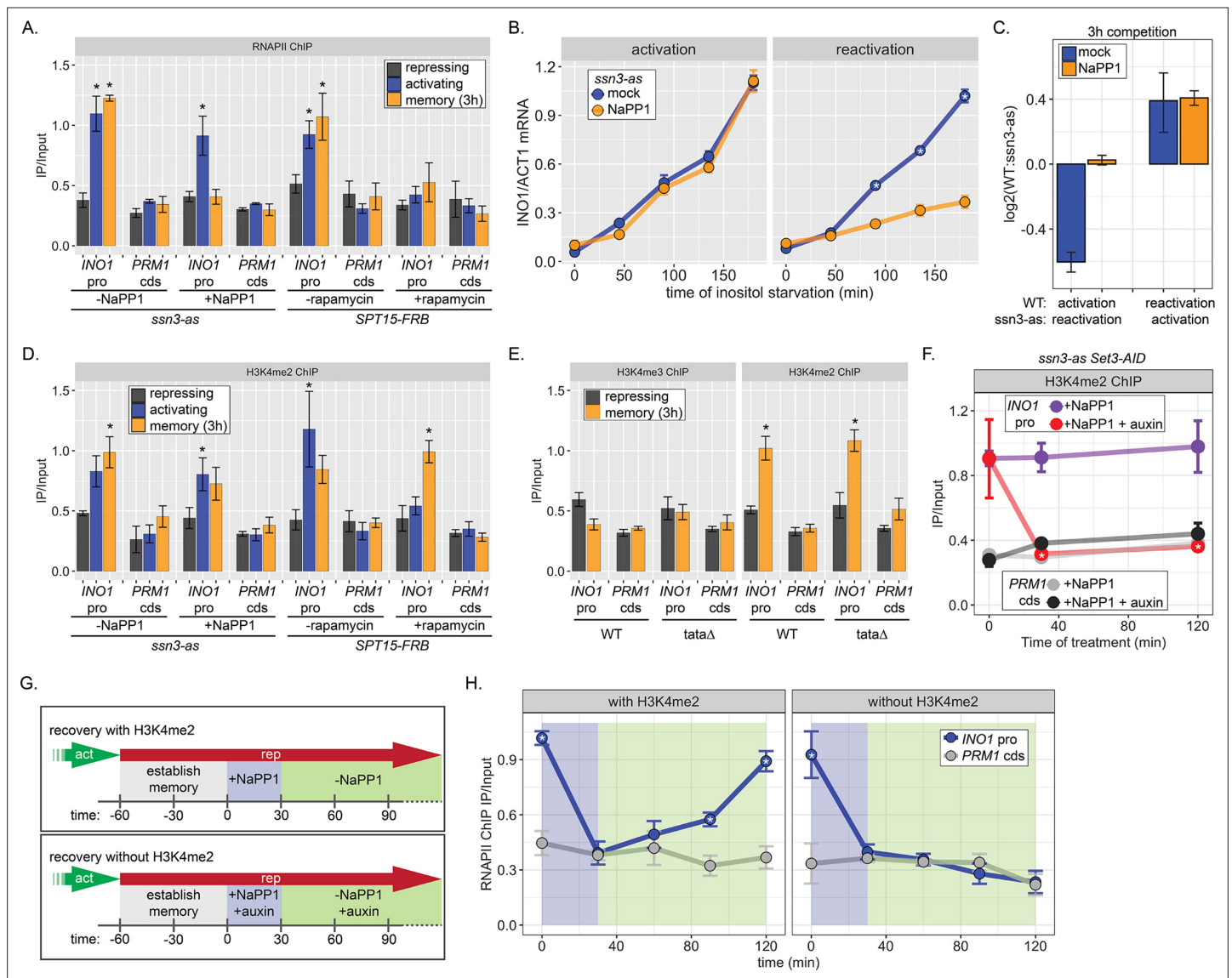
**Figure 2.** *INO1* transcriptional memory requires a positive feedback loop. **(A)** Activation and reactivation of *INO1* in wild type (WT) (left) and *set1Δ* (right) strains upon removal of Opi1 by Anchor Away. Cells were harvested at indicated time points and *INO1* mRNA was quantified relative to *ACT1* mRNA by real time quantitative PCR (RT-qPCR) (\*p-value<0.05 from one-tailed t-test comparing reactivation and activation, alternative = greater). Data for the WT strain is the same as **Figure 1D** and is shown for comparison. **(B)** Model for transcription factor (TF)- and NPC-dependent H3K4 dimethylation by COMPASS (orange circles) and SWR (Sui/Snf Related)-dependent H2A.Z incorporation (green Z). For the chromatin immunoprecipitation (ChIP) experiments in panels C, D, E, G, and H: recovery of either the *INO1* promoter or the repressed *PRM1* coding sequence was quantified by RT-qPCR. The average of ≥3 replicates ± SEM is plotted (\*p-value<0.05 from one-tailed t-test compared with the repressing condition, alternative = greater). **(C)** ChIP against H3K4me2 in WT, *nup100Δ*, and *sfi1Δ* strains grown under repressing, activating, and memory (3 hr) conditions. **(D)** ChIP against H3K4me2 at the indicated times after switching to repressing conditions in WT and *nup100Δ* strains. The gray and white bars indicate doubling times. **(E)** ChIP against H2A.Z from WT, *sfi1Δ*, or *set3Δ* strains under repressing, activating, or memory (3 hr) conditions. **(F)** Schematic for hierarchical relationship between H2A.Z incorporation and H3K4me2. **(G)** ChIP against Sfi1-GFP in WT, *nup100Δ*, *set3Δ*, and *swr1Δ* strains grown under repressing, activating, or memory (3 hr) conditions. **(H)** ChIP against Sfi1-myc at the indicated times ± 1 μg/ml rapamycin in a Swd1 (COMPASS) Anchor Away strain, 1 hr after shifting from activating to repressing conditions. \*p-value<0.05 from one-sided t-test compared with the time = 0 min time point, alternative = less. **(I)** Schematic of the requirement for H2A.Z incorporation and H3K4me2 for Sfi1 binding to the *INO1* promoter during memory.



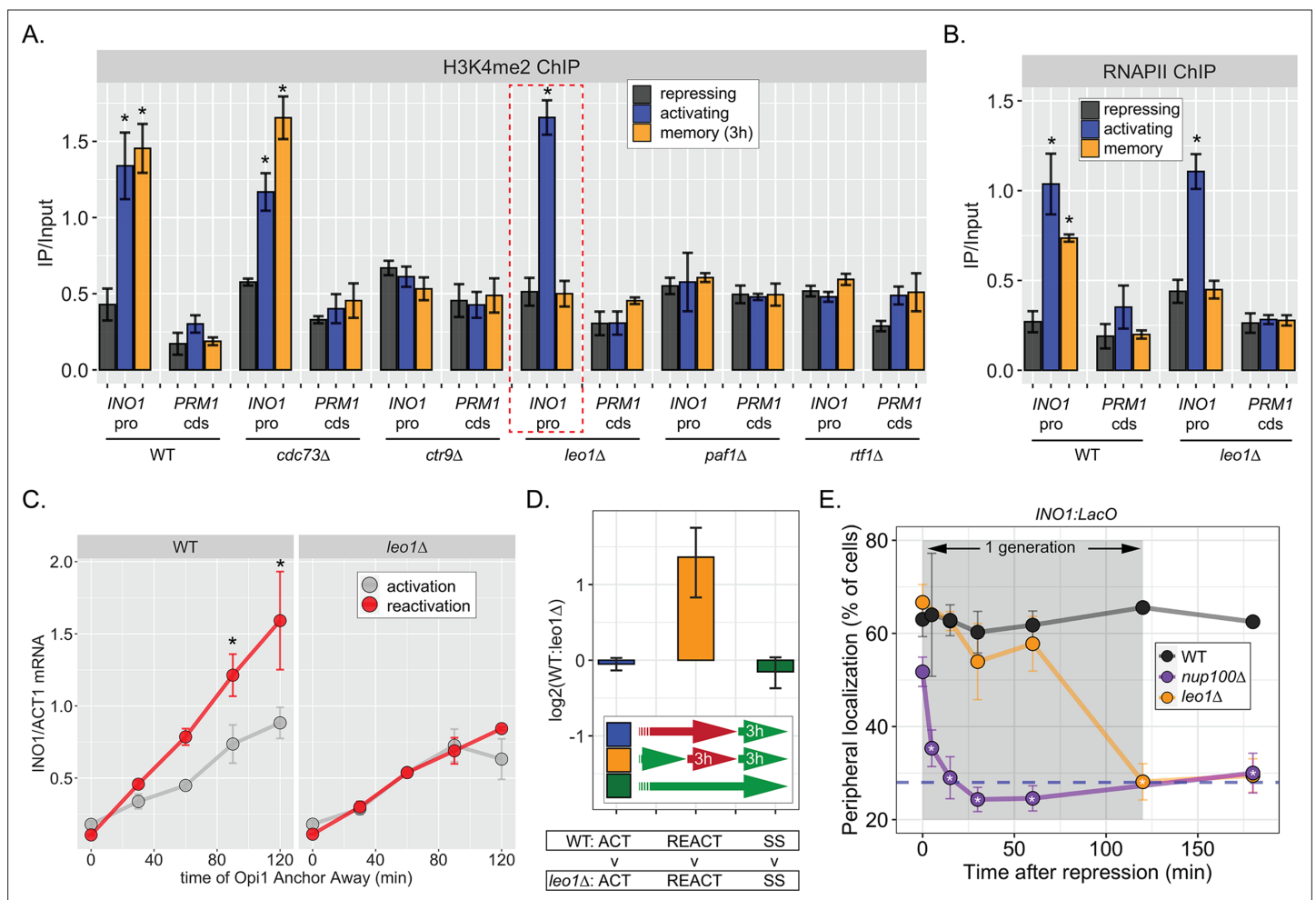
**Figure 3.** Two different Hsf1-like TFs are required for inositol memory. (A–C) Chromatin immunoprecipitation (ChIP) against Hms2-myc (A), RNAPII (B, left), H3K4me2 (B, right), or Sfl1-GFP (D) in the indicated strains grown under activating, repressing, or memory (3 hr) conditions. Recovery of either the *INO1* promoter or the repressed *PRM1* coding sequence was quantified by real time quantitative PCR (RT-qPCR) and the average of  $\geq 3$  replicates  $\pm$  SEM is plotted (\*p-value < 0.05 from one-tailed t-test compared with the repressing condition, alternative = greater). (C) Activation and reactivation of *INO1* in wild type (WT) (left) and *hms2Δ* (right) strains upon removal of Opi1 by Anchor Away. Cells were harvested at indicated time points and *INO1* mRNA was quantified relative to *ACT1* mRNA by RT-qPCR (\*p-value < 0.05 from one-tailed t-test comparing reactivation and activation, alternative = greater). Data from the WT strain is the same as Figure 1D and is shown for comparison. (E) Peripheral localization of *INO1* in either WT, *sfl1Δ*, *hms2Δ*, or *nup100Δ* strains. At t=0, inositol was added to cells growing without inositol and peripheral localization was scored at the indicated times. The doubling time of this strain (~120 min) is indicated. The average of three biological replicates  $\pm$  SEM is plotted and each biological replicate  $\geq 30$  cells. Blue hatched line: expected peripheral localization for a randomly localized gene. \*p-value < 0.05 from one-tailed t-test compared with time = 0, alternative = less.



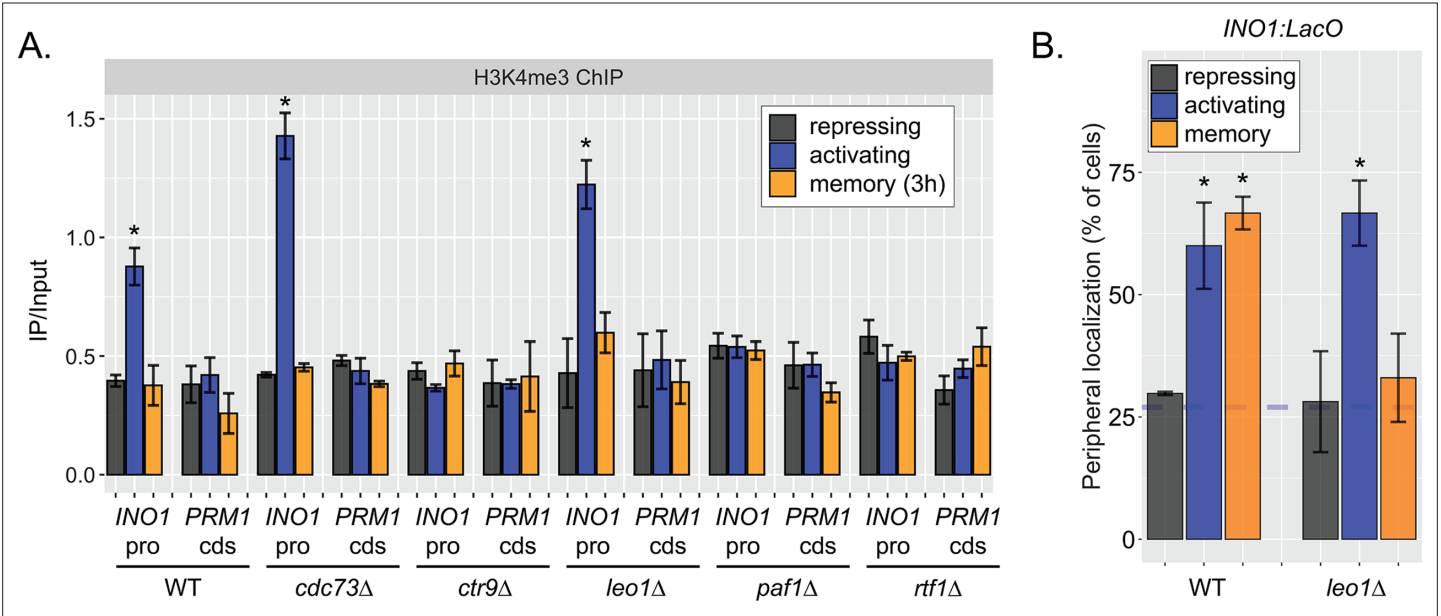
**Figure 3—figure supplement 1.** Some, but not all, Hsf1-like TFs are required for *INO1* transcriptional memory. Peripheral localization of *INO1* in either wildtype (WT), *sfl1Δ*, *hms2Δ*, *mga1Δ*, and *skn7Δ* strains. Cells grown without inositol were switched to complete media and then imaged after 3 hr. The average of three biological replicates  $\pm$  SEM is plotted and each biological replicate  $\geq 30$  cells. Blue hatched line: expected peripheral localization for a randomly localized gene. \* $p < 0.05$  from one-tailed t-test comparing to the repressing condition, alternative = greater.



**Figure 4.** RNA polymerase II (RNAPII) binding during *INO1* memory requires H3K4me2, but H3K4 dimethylation does not depend on RNAPII. Chromatin immunoprecipitation (ChIP) against RNAPII (A) or H3K4me2 (D) in *ssn3-as* and *SPT15-FRB* Anchor Away strains upon addition of either 1-Naphthyl-PP1 (NaPP1) or rapamycin for 1 hr as indicated. Cells were grown in repressing, activating, or memory (3 hr) conditions. \* $p < 0.05$  from one-tailed t-test compared with repressing condition, alternative = greater. (B) Activation (left) and reactivation (right) of *INO1* in the *ssn3-as* strain treated either with DMSO (dimethyl sulfoxide; mock) or NaPP1 for upon inositol starvation (1 hr pretreatment or treatment). Cells were harvested at indicated time points and the *INO1* mRNA was quantified relative to *ACT1* mRNA by real time quantitative PCR. \* $p < 0.05$  from one-tailed t-test comparing between mock and NaPP1, alternative = greater. (C) The  $\log_2$  ratio of the indicated strains to each other after competition for 3 hr in the absence of inositol. (E) ChIP against H3K4me3 (left) or H3K4me2 (right) in wild type (WT) and *ino1-tata* strains. Cells were grown in repressing (+inositol) or memory conditions (+inositol → -inositol, 1 hr → +inositol, 3 hr). \* $p < 0.05$  from one-tailed t-test compared with repressing condition, alternative = greater. (F) ChIP against H3K4me2 after establishing memory for 1 hr, followed by addition of either NaPP1 or NaPP1 and auxin at  $t = 0$ . \* $p$ -value  $< 0.05$  from one-tailed t-test comparing NaPP1 treated and NaPP1+auxin treated samples at each time, alternative = less. (G) Schematic for experiment in (H) to monitor RNAPII recruitment with (top) or without (bottom) H3K4me2. NaPP1 was added with or without 0.5 mM auxin for 30 min before removing NaPP1. (H) ChIP against RNAPII, following the experimental set up in (G) with cells crosslinked at the indicated times. For panels A, D, E, F, and H, recovery of the *INO1* promoter or the *PRM1* coding sequence (negative control locus) was quantified relative to input by qPCR the averages of three biological replicates  $\pm$  SEM were plotted; \* $p$ -value  $< 0.05$  from one-tailed t-test comparing ChIP of *INO1* promoter to *PRM1* cds at each time, alternative = greater.

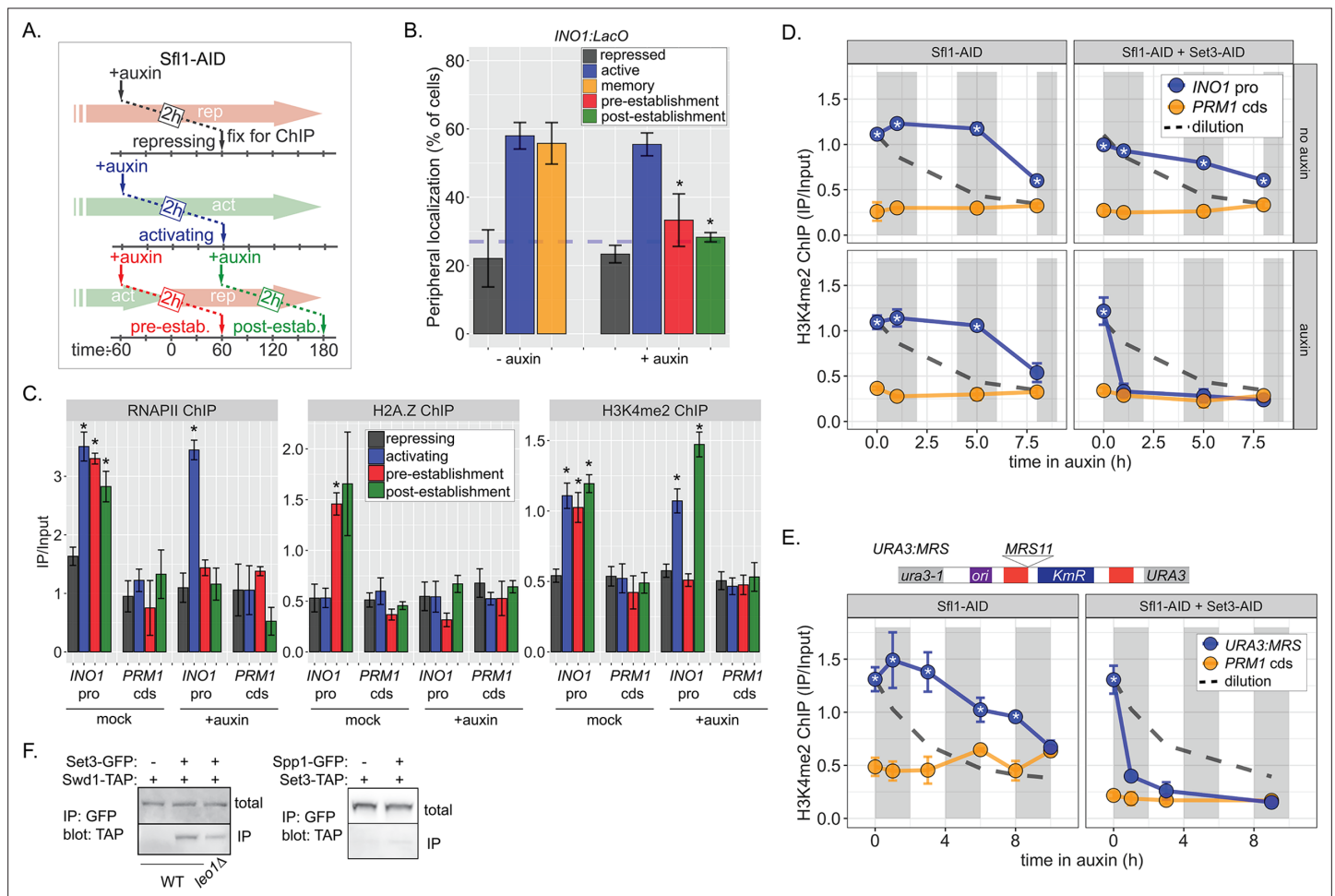


**Figure 5.** The Leo1 protein of the Paf1 complex is specifically required for *INO1* memory. **(A)** Chromatin immunoprecipitation (ChIP) against H3K4me2 in the indicated strains under repressing (+inositol), activating (−inositol), or memory (−inositol → +inositol, 3 hr) conditions, highlighting the effects of the *leo1Δ* mutation (dashed red box). **(B)** ChIP against RNA polymerase II in wildtype (WT) and *leo1Δ* strains repressing, activating (−inositol), or memory (−inositol → +inositol, 3 hr) conditions. For A & B: recovery of *INO1* promoter or *PRM1* coding sequence (negative control) were quantified by quantitative PCR and the averages of three biological replicates ± SEM are plotted. For A and B, \**p* < 0.05 from one-tailed t-test comparing against repressed condition, alternative = greater. **(C)** Activation and reactivation of *INO1* in WT (left) or *leo1Δ* (right) strains upon Anchor Away of Opi1. Cells were harvested at indicated times and *INO1* mRNA was quantified relative to *ACT1* mRNA by real time quantitative PCR. \**p* < 0.05 from one-tailed t-test comparing activation to reactivation, alternative = greater. **(D)** Competitive fitness of WT vs *leo1Δ* strains competed for 3 hr in the absence of inositol during activation (ACT, +inositol → −inositol, blue), reactivation (REACT, −inositol → +inositol (3 hr) → −inositol, orange), or steady state (SS, −inositol → −inositol, green). The ratio of the relative abundance of the WT:*leo1Δ* strains was quantified and expressed as a log<sub>2</sub> ratio ± SEM. **(E)** Peripheral localization of *INO1* in WT, *nup100Δ* (replotted from **Figure 3**), and *leo1Δ* strains shifted from activating (−inositol) to repressing (memory) conditions for the indicated times. The gray box indicates the approximate doubling time of this strain. The average of ≥3 biological replicates ± SEM is plotted and each biological replicate ≥30 cells.



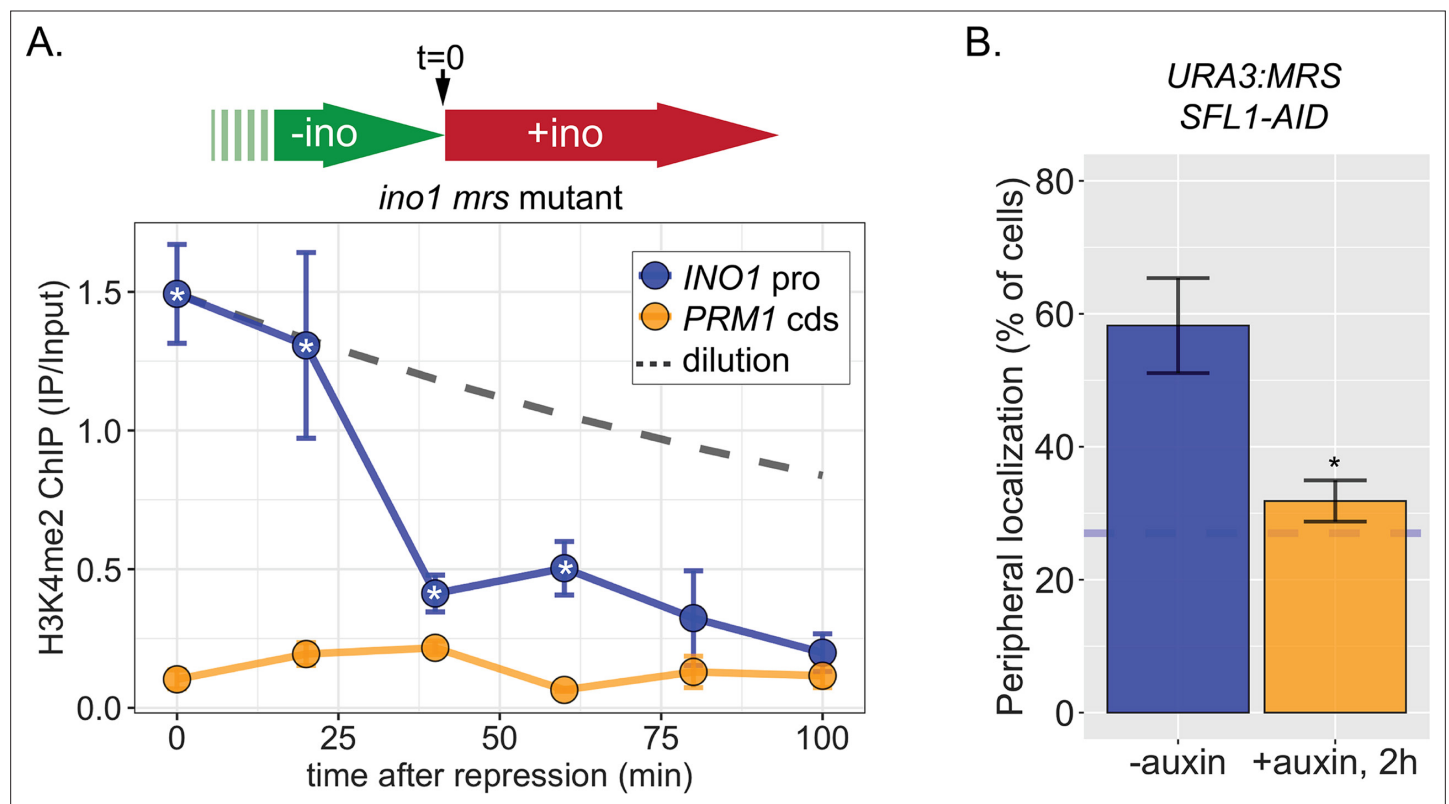
**Figure 5—figure supplement 1.** Effects of loss of Leo1 on H3K4me3 and *INO1* localization. **(A)** Chromatin immunoprecipitation (ChIP) against H3K4me3 in wild type (WT), *cdc73Δ*, *ctr9Δ*, *leo1Δ*, *paf1Δ*, and *rtf1Δ* strains in repressing (+inositol), activating (–inositol), memory (–inositol → +inositol [3 hr]) conditions. Recovery of the *INO1* promoter or the *PRM1* coding sequence was quantified by quantitative PCR relative to input and are the averages of three biological replicates ± SEM. **(B)** Peripheral localization of WT and *leo1Δ* strains under repressing (+inositol), activating (–inositol), or memory (–inositol → +inositol, 3 hr) conditions. The average of ≥3 biological replicates ± SEM; each biological replicate ≥30 cells is plotted. \*p<0.05 from one-tailed t-test against repressing condition, alternative = greater.



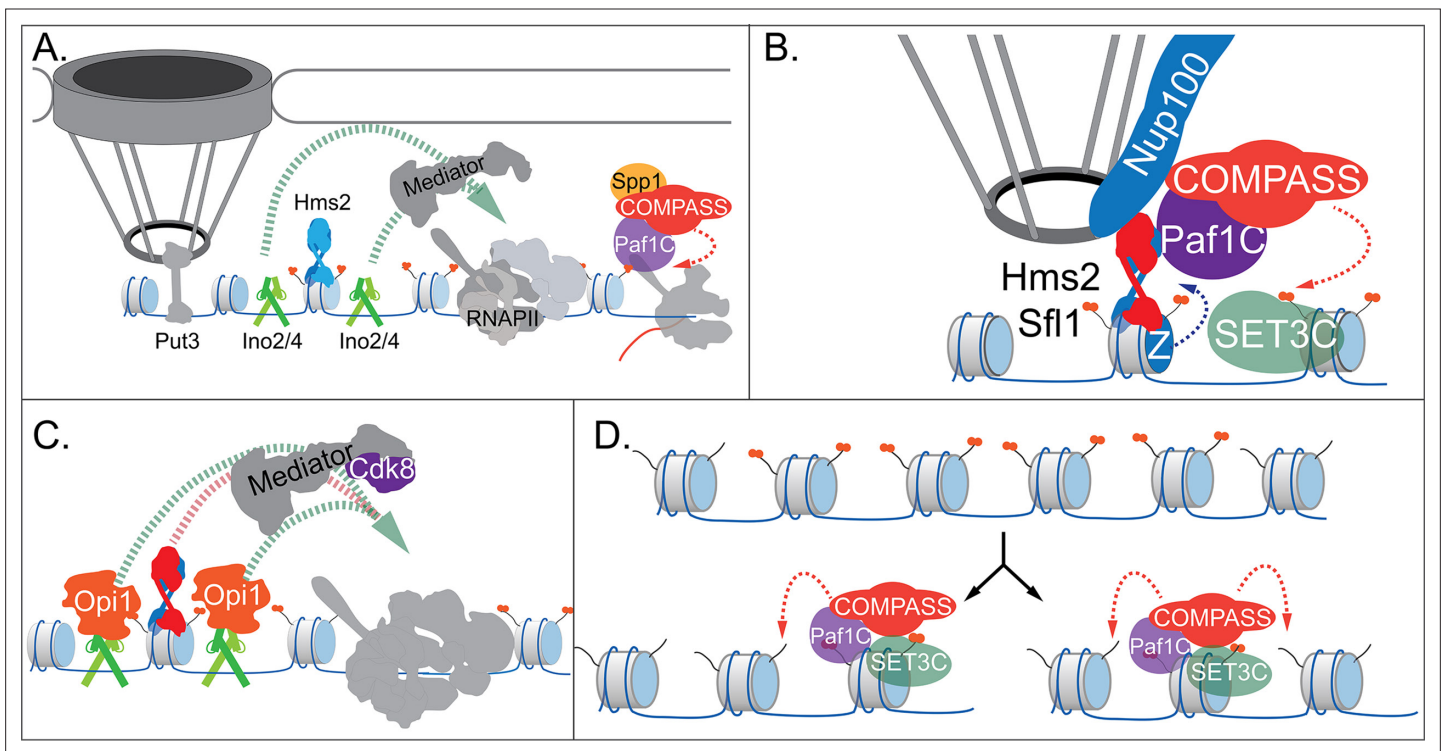


**Figure 6.** Distinct molecular requirements for establishment and inheritance of H3K4me2 during *INO1* memory. (**A**) Experimental set-up to test the role of Sfl1 in establishment and inheritance of *INO1* memory, using auxin-inducible degradation of Sfl1 before or after establishing memory. The top arrow indicates when auxin was added; the bottom arrow indicates when cells were fixed for chromatin immunoprecipitation (ChIP). Using this approach, peripheral localization of *INO1* (**B**) or ChIP against RNAPII (**C**, left), H2A.Z (**C**, middle), or H3K4me2 (**C**, right) was measured under the indicated conditions  $\pm 0.5$  mM auxin. Peripheral localization is the average of three biological replicates  $\pm$  SEM; each biological replicate  $\geq 30$  cells. Blue hatched line: expected peripheral localization for a randomly localized gene. \* $p < 0.05$  from one-tailed t-test comparing auxin treated to untreated, alternative = less. (**D**) ChIP against H3K4me2 in *Sfl1-AID* (left) and *Sfl1-AID+Set3 AID* (right) strains either without auxin (top) or after addition of auxin (lower). Auxin was added after 1 hr of repression. For panels D and E, vertical gray and white bars represent estimated generation times and dashed line represents the expectation from perfect retention of H3K4me2, followed by dilution through DNA replication (i.e.  $t_{1/2}$  = the doubling time). (**E**) Schematic of insertion of 11 bp MRS at the *URA3* locus in the *URA3:MRS* strain (top) and ChIP against H3K4me2 in *URA3:MRS Sfl1-AID* (left) and *URA3:MRS Sfl1-AID+Set3 AID* (right) strains after addition of auxin (lower). Panels C, D, and E: recovery of *INO1* promoter or *PRM1* coding sequence or *URA3:MRS* region was quantified by quantitative PCR relative to input and are the averages of three biological replicates  $\pm$  SEM. \* $p < 0.05$  from one-tailed t-test comparing to recovery of each DNA in the repressed condition (C) or to *PRM1* cds (D & E), alternative = greater. (**F**) Co-immunoprecipitation of Set3-GFP and Swd1-TAP (left), Spp1-GFP and Set3-TAP (right) from the indicated strains. The Green fluorescent protein (GFP)-tagged proteins were immunoprecipitated with anti-GFP nanobodies; recovery of Swd1-TAP and Set3-TAP were monitored by immunoblotting with anti-TAP antibody.





**Figure 6—figure supplement 1.** Rapid loss of H3K4me2 from the *INO1* promoter in the absence of transcriptional memory. Time course chromatin immunoprecipitation (ChIP) against H3K4me2 in the *mrs* mutant strain. Cells were switched from activating (–inositol) to repressing (+inositol) medium at  $t=0$  and fixed for ChIP at the indicated times. The dashed line represents the expectation from perfect retention of H3K4me2, followed by dilution through DNA replication (i.e.  $t_{1/2}$  = the doubling time). Recovery of *INO1* promoter and *PRM1* coding sequence was quantified by quantitative PCR relative to input and are the averages of three biological replicates  $\pm$  SEM. \* $p < 0.05$  from one-tailed t-test comparing to recovery of *INO1* promoter to *PRM1* cds, alternative = greater.



**Figure 7.** Models for *INO1* transcriptional memory. **(A).** *INO1* under activating conditions. Interaction with the nuclear pore complex (NPC) is mediated by Put3 (and, potentially, Cbf1; Ahmed et al., 2010; Randise-Hinchliff et al., 2016). Ino2/Ino4 heterodimers bind UAS<sub>INO</sub> elements flanking the memory recruitment sequence (MRS) and recruit coactivators such as Mediator to promote RNA polymerase II (RNAPII) recruitment and transcription. Hms2 binds to the MRS element but does not contribute to *INO1* transcription or localization. **(B)** Upon repression, *INO1* transcriptional memory is established by Hms2-dependent recruitment of Sfl1, leading to Nup100-dependent interaction with the NPC. Nup100, Sfl1, and Hms2 are required for both Spp1<sup>+</sup> COMPASS-dependent H3K4 dimethylation and H2A.Z incorporation near the MRS. SET3C associates with H3K4me2 and is required for its persistence. **(C)** During memory, Cdk8<sup>+</sup> Mediator is recruited to the *INO1* promoter. This requires Sfl1/Hms2 but may also require other TFs such as Ino2 and Ino4, despite their repression by Opi1. **(D)** H3K4me2 inheritance after DNA replication. Following DNA replication, only half of the nucleosomes bear H3K4me2. SET3C recognizes the H3K4me2-marked nucleosomes and recruits Spp1<sup>+</sup> COMPASS, which methylates adjacent nucleosomes. In the presence of Sfl1/Hms2, this re-establishment will likely emanate from the MRS outwards.