Isw2p and Itc1p (ISW2 complex) are required for complete derepression of *INO1-lacZ*. ISWI proteins have been reported to be recruited to promoters by specific transcription factors such as Cbf1p (1). Because we found that Cbf1p regulated *INO1-lacZ* (Fig. 2) and *INO1* (Fig. 3A) we tested mutants in other proteins reported to be involved in ISWI complexes (ISW1a, ISW1b, and ISW2 complexes). We transformed *isw1Δ*, *ioc2Δ*, *ioc3Δ*, *ioc4Δ*, *isw2Δ*, and *itc1Δ* strains with pJH330 and assayed the transformants for β-galactosidase activity as described above. The data show that members of both ISW1 (ISW1a: Ioc3p and Isw1p; ISW1b: Ioc2p, Ioc4p, and Isw1p) and ISW2 (Isw2p and Itc1p) complexes affected transcription from the *INO1* promoter (Fig. S1). However, while ISW1 components affected repression, ISW2 complex proteins (Isw2p and Itc1p) were required for complete derepression of transcription from the *INO1* promoter as was the case for the *cbf1Δ* strain (Fig. 2).

Isw2p and Cbf1p work through the same pathway to aid *INO1* transcription. We reasoned that if Cbf1p and Isw2p act in the same pathway to transcribe *INO1* then a *cbf1Δ isw2Δ* double mutant and an *isw2Δ* mutant should have the same effect *INO1* expression. To test this possibility we created isogenic strains, containing *isw2ΔURA3* and *isw2ΔURA3 cbf1Δ* mutants, by replacing the *ISW2* ORF with *URA3* gene in wild type (BY4742) and *cbf1Δ* strains. This was accomplished by amplifying the *URA3* gene flanked by 200 bp of promoter sequences from the YEp357R plasmid (2) using the ISW2 URA3 primer pair (Table 1). The amplified fragment containing the *URA3* gene flanked by 45 bp of sequence homologous to the *ISW2* ORF was cloned into pGEM-T (Promega, Madison, WI) to create pGEM-*ISW2* and sequenced. This fragment was re-amplified from pGEM-*ISW2* using the same primers, gel purified and used to

transform the BY4742 and *cbf1Δ* strains. The *INO1* transcript levels were quantified by QRT-PCR as described above. The data show that *INO1* expression is nearly identical in both strains indicating that Cbf1p and Isw2p likely act in the same pathway to regulate *INO1* transcription.

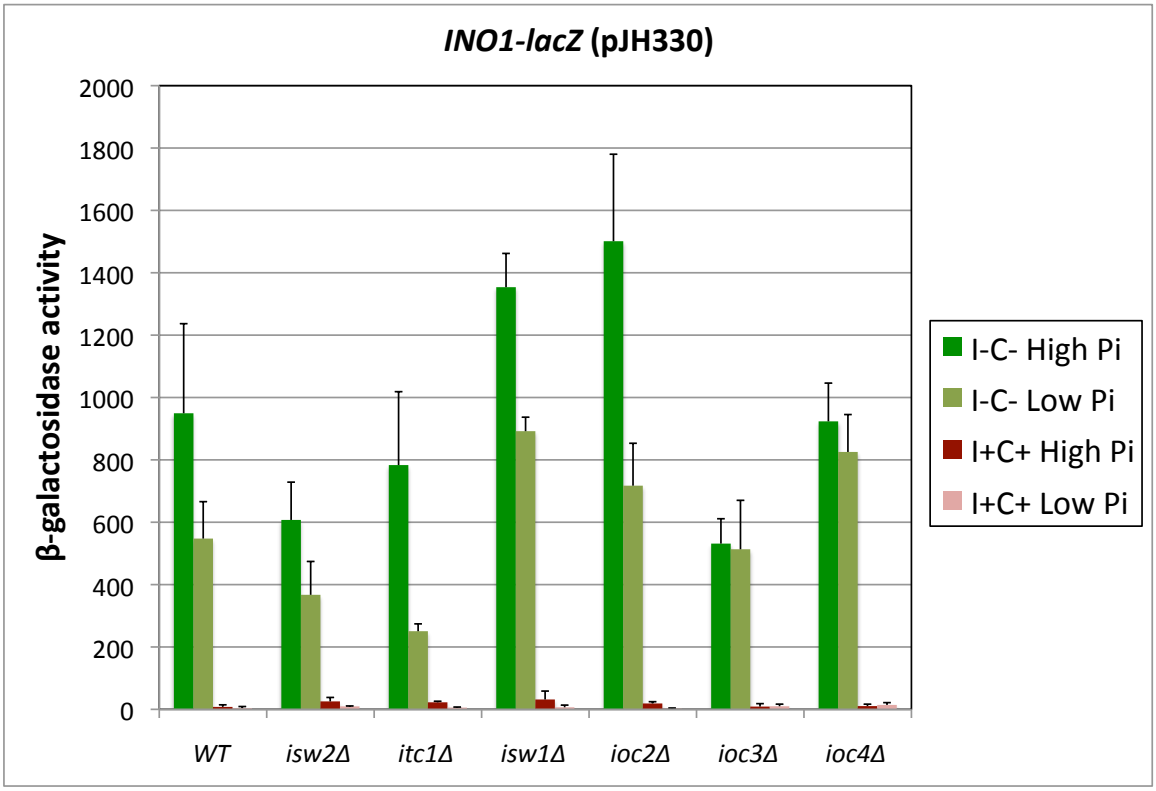

Fig. S1: WT and isogenic ISWI complex knockout strains in yeast were transformed with the pJH330 plasmid. Transformants were grown in four media as described above. Green and red bars indicate derepressing and repressing conditions whereas solid and faded bars indicate High and Low P_i respectively. Cells were harvested in mid-log phase and assayed for β-galactosidase. The data represents means and standard errors of means of at least three different experiments.

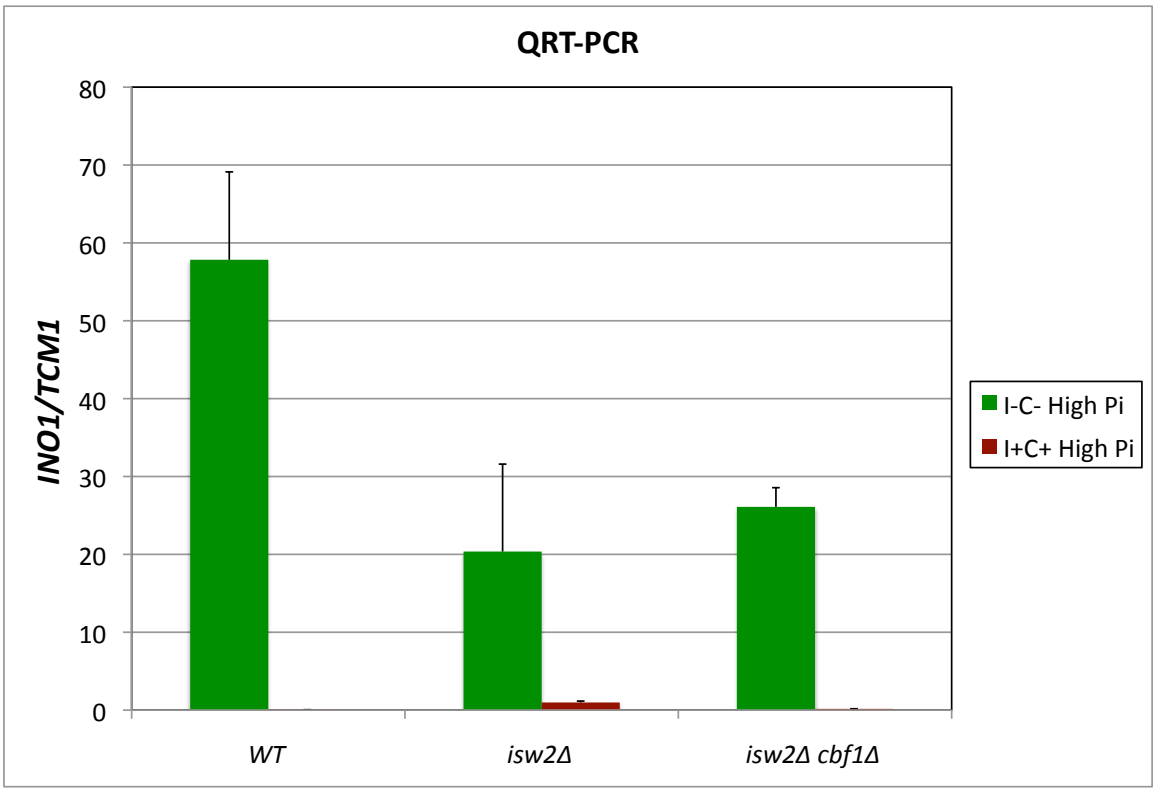

Fig. S2. WT, *isw2Δ* and *isw2Δ cbf1Δ* strains were grown to mid log phase in I+C+ High Pi (solid red bars) and I-C-High Pi (solid green bars) conditions and *INO1* transcript levels were quantified using real time PCR. *INO1* transcript levels were normalized to *TCM1* transcript levels. The data represents means and standard errors of means of at least three different experiments.

References

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