SUPPLEMENTAL MATERIAL AND METHODS

Preparation of yeast strains

SPN1 was previously deleted in the parental BY4741 strain (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and the *dst1Δ*, and *rtf1Δ* deletion strains by gene replacement with the *LEU2* gene (1). The *nap1Δ, rtt106Δ, hir1Δ, hir2Δ, asf1Δ, cac1Δ, cac3Δ,* and *vps75Δ* deletion strains used in this study were purchased from Research Genetics and *SPN1* disrupted essentially as had been done with the other strains. Briefly, a *SPN1* covering plasmid (A *URA3* marked plasmid bearing the *SPN1* gene flanked by the TOA1 promoter and terminator) was introduced into the parental and deletion strains. A DNA fragment containing the *LEU2* gene flanked by *SPN1* promoter (468 bp) and terminator (485 bp) sequences was introduced into the strains. Following homologous recombination, the *LEU2* gene replaced genomic *SPN1*. Replacement of genomic *SPN1* in each of the strains was confirmed by PCR. The pCR311 or pAA344 plasmids were then introduced into the deletion strains by plasmid shuffling.

Yeast Media

YPD agar plates (1% Yeast extract, 2% Peptone, 2% Dextrose, 2% agar), YPD agar plates with 10 mM caffeine, 150 mM hydroxyurea, 2% formamide, 25 nM rapamycin and 3.5 mM H_2O_2, YPEG agar plates (1% Yeast extract, 2% Peptone, 3% Ethanol, 3% Glycerol, 2% agar), and synthetic complete plates without inostitol or with 3-aminotriazole were prepared as described in (3).

Spn1 expression and purification

DNA encoding wild type Spn1 and $Spn1^{141–305}$ was amplified from genomic DNA and inserted into pET15b expression vectors (Novagen). The expression plasmids were introduced into Rosetta 2 DE3 pLysS cells and wild type or $Spn1^{141–305}$ expressed and purified as follows. One liter of Luria Broth medium supplemented with ampicillin (100 µg/ml), chloramphenicol (34 µg/ml), and glucose (1%) was inoculated with cells from a 10ml overnight culture. For wild type Spn1, the cells were grown at 37 °C to an OD_{600} of ~0.4, transferred to 30 °C, and allowed to grow to an OD_{600} of ~0.6. Protein expression was

induced by adding IPTG to 1 mM and the cells incubated an additional 2 hours at 30°C. For Spn1$^{141–305}$ the cells were grown at 37 °C to an OD_{600} of ~0.5 and then transferred to 16 °C for 30 minutes before inducing protein expression with 1 mM IPTG. The cells were then incubated overnight at 16°C. After induction, the cells were put on ice for 30 minutes, collected by centrifugation at 4000 rpm for 15 minutes and the cell pellets stored at -80°C. For each protein a cell pellet from 1 L of medium was thawed and suspended in 40 ml of buffer (100 mM Tris (pH 7.5), 1 M NaCl, 10% glycerol, 50 mM imidazole, and 500 μM PMSF). The cells were lysed by sonication and the cellular debris pelleted by centrifugation at 15,000 rpm for 40 minutes. Recombinant wild type Spn1 was bound to a 5 mL Hi-Trap chelating column, the column extensively washed and Spn1 eluted using a linear gradient of imidazole from 50 mM to 500 mM in the same buffer as above. The appropriate fractions were pooled and concentrated to ~1 mL using an Amicon Ultra-15 centrifugal filter unit (Millipore). The concentrated pool was loaded onto a Superdex 200 (16/60) column (equilibrated and eluted with 25 mM MES pH 6.5, 200 mM NaCl, 10% glycerol) at 0.5 ml/minute. The appropriate fractions from the column were pooled and bound directly to a 5 mL Hi-Trap SP column. Spn1 was eluted from the column using a linear NaCl gradient from 50 mM to 600 mM in 25 mM MES pH6.5 and 10% glycerol. For Spn1$^{141-305}$, the same purification scheme was used except the Hi-Trap SP column was omitted. The histidine tag was left on both proteins for all of the experiments performed in this manuscript.

Co-immunoprecipitation Assay

The co-immunoprecipitation experiments were completed with the indicated strains as described previously (1) with one modification: 125 μg of soluble protein were used.

Chromatin Immunoprecipitation Assay

Triplicate samples (biological replicates) of BY4741, LZ0-1, and LZ0-3 cells were grown in 100 mL of YPD to an OD_{600} of 0.7 to 0.9. One half of each culture (50 mL) was immediately crosslinked (see below). Cells in the other half of each culture were collected by centrifugation, washed three times with 20 mL of YP, transferred to 50 mL of YP with 3% Ethanol and incubated at 30°C for an additional 4

hours. The cells were then crosslinked by adding Formaldehyde to a final concentration of 1% followed by incubation at room temperature for 15 minutes with swirling every 5 minutes. The crosslinking was quenched by the addition of 8 mL of 2.5 M Glycine followed by incubation at room temperature for 5 minutes. The cells were then collected by centrifugation, washed two times with 20 mL of ice-cold TBS (50 mM Tris-Cl pH7.5, 150 mM NaCl) and suspended in 2.5 mL of FA-lysis buffer (50 mM HEPES pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1 % Na-deoxycholate, and a 1X protease inhibitor cocktail of PMSF, benzamidine, pepstatin, leupeptin, and chymostatin). The cells were again collected by centrifugation, the supernatant removed and the cell pellets stored at -80°C. The next day the cells were briefly thawed on ice and suspended in 1 mL of FA-lysis buffer. All solutions, plastic tubes, and glassware used were ice-cold and the samples kept on ice between manipulations. An equal volume of acid washed glass beads (0.5 mm) was added to each sample and the cells broken by 15 cycles of vortexing for 1 minute followed by incubation on ice for 1 minute. The bottom of each tube was then punctured, the lysate collected by brief centrifugation and the glass beads washed 2 times with 2 mL of FA-lysis buffer. The lysates and corresponding washes for each sample were combined, transferred to a chilled 15 mL Corex tube and spun at 10,000 RPM for 20 minutes in a JA-20 rotor. The supernatants were discarded, the pellets washed 1 time with 2 mL FA-lysis buffer and the pellets suspended in 2 mL FA-lysis buffer. The suspended chromatin was sheared by sonication using a Branson W-350 model sonifier and a microtip (10 times for ten seconds each on continuous pulse at a power setting of 6 with a 90% output). The samples were chilled on ice for two minutes between each sonication cycle. Following sonication the samples were transferred to 2 mL microcentrifuge tubes and the samples spun at max for 20 minutes. The supernatants (chromatin material) were transferred to another tube, 50 μL samples were removed and then processed as the Input after reversal of the crosslinks and purification of the DNA. Chromatin material (500 μL) was combined with 10 μL of anti-RNA polymerase II (8W16G Covance MMs-126R) or anti-Myc (9E10, Millipore 05-419) antibodies and then incubated overnight at 4°C with rotation. Protein-A sepharose beads (Pharmacia) were prepared as a slurry according to the

manufacturer's directions, 50 μL of the beads were then added to each chromatin/antibody sample and the samples incubated for 3 hours at room temperature with rotation. The sepharose beads were collected by centrifugation and then washed four times with 1 mL each of: FA-lysis buffer, FA-lysis buffer with 500 mM NaCl, LiCl/detergent (50 mM Tris-Cl ph 8.0, 0.25 M LiCl, 1 mM EDTA, 0.5% NP-40, 0.5% sodium Deoxycholate) and TE buffer. Complexes were then eluted from the beads by suspending the washed beads in 100 μL of 50 mM Tris/HCl pH 8.0, 10 mM EDTA, 1%SDS followed by incubation at 65°C for 15 minutes. The beads were collected by brief centrifugation and the supernatant transferred to another tube. The beads were suspended in 150 μL of TE, 0.67% SDS, collected by brief centrifugation, the supernatant pulled off and combined with the supernatant from above. Proteinase K was then added to the samples (final concentration of 0.4 mg/mL) and the protein-DNA crosslinks reversed by incubating the samples overnight at 65°C. The samples were then cooled to room temperature, 5 μg of RNaseA added and the samples incubated at 37°C for 30 minutes. The samples were then extracted one time with an equal volume of Phenol/Chloroform and one time with Chloroform. DNA in the samples was ethanol precipitated, washed one time with 70% ethanol, air dried and suspended in 500 μL of sterile ddH2O.

Quantitative PCR reactions were carried out in a 20 μL volume using iQ Supermix from BioRad (Catalog # 170-8882). The final concentration of each component in the reactions was: 0.1-0.2 μM primers, 20 mM Tris-Cl pH 8.4, 50 mM KCl, 0.2 mM each dNTP, 3 mM $MgCl_2$, 10 mM Fluoroscein, SYBT Green I dye and 50 U/mL iTaq DNA polymerase and contained 5 μL of the immunoprecipitated DNA. The reactions were completed using 96 well clear Multiplate PCR plates from BioRad (Catalog # MLL9601) and a CFX96 Real-Time System (BioRad). The annealing temperature for the primers (Table S1) was: 67°C for the *CYC1* promoter, 62°C for the *CYC1* ORF and 61°C for the Telomere proximal. The amplicon lengths are: 169 bp for the *CYC1* promoter, 148 bp for the *CYC1* ORF and 148 bp for the Telomere proximal. Primer specificity was monitored using melt curves and single peaks were detected with each primer set. Standard curves were generated using 10-fold serial dilutions of Input DNA and

were included on each plate. PCR efficiencies ranged from 90-110%, with a correlation coefficient greater than or equal to 0.98. CFX Manager Version 3.1, Microsoft Excel 2013 and GraphPad software was used to analyze the data.

Spheroplast Preparation

Cells were grown in 300 mL of YP +2% glucose or YP +3% ethanol until an OD_{600} of 0.7 to 0.9 was reached. The cells were collected by centrifugation in a JA-10 rotor at 5,000 RPM for 10 minutes at $4^{o}C$. The supernatant was decanted from above the cell pellets and the cells suspended in 20 mL of Sorbitol buffer (50 mM Tris-(3)7.5, 1.0 M Sorbitol, 10 mM $MgCl_2$, 10 mL 2-Mercaptoethanol and 1mM PMSF. The cell suspension was transferred to chilled 50 mL conical tubes and the cells collected by centrifugation in a GPKR centrifuge at 3,200 RPM for 5 minutes at $4^{o}C$. The supernatant was decanted from above the cell pellets and the cells washed 1 additional time with 20 mL of Sorbitol buffer. The mass of the cell pellets was determined and the cells suspended in 0.66 mL of Sorbitol buffer per gram of cells. Zymolase (10 $^{mg}/_{mL}$) was added as follows: 0.34 mL per gram of cells grown in glucose and 0.68 mL per gram of cells grown in ethanol. The cells plus Zymolase were incubated at $30^{o}C$ with gentle agitation until greater than 90% of the cells grown in glucose, or 60% of the cells grown in ethanol were converted to spheroplasts. The spheroplasts were collected by centrifugation in a GPKR centrifuge at 3,200 RPM for 8 minutes at $4^{o}C$ and then gently washed with 20 mL of Sorbitol buffer.

Micrococcal Nuclease Digestion

Spheroplasts were resuspended in 1 mL of MNase digestion buffer (10 mM Tris-Cl pH 7.5, 1 mM $CaCl_2$, 50 mM NaCl, 5mM $MgCl_2$ 0.5mM Spermidine, 0.75% NP-40, 1 mM DTT) per 0.175 gram of cells. The suspended spheroplasts were divided into aliquots and serial dilutions of MNase added to give a final MNase concentration range of 33-533 mU/µl for cells grown in glucose, and 2-33 mU/µl for cells grown in ethanol. The MNase reactions were incubated for 30 minutes at 37°C. Lower MNase concentrations were used for cells grown in ethanol due to increased sensitivity to MNase. Cleavage was quenched by the addition of stop solution (100 mM EDTA, 2.5% SDS, 0.3 mg/ml Proteinase K) to

yield a final concentration of 20 mM EDTA, 0.5% SDS, 0.6 mg/ml Proteinase K. The samples were incubated overnight at 37°C. The samples were extracted two times with Phenol:$CHCl_3$ and one time with $CHCl_3$. The DNA samples were ethanol precipitated, washed with 70% ethanol and dried at room temperature. The DNA was suspended in 100 μL of TE buffer (pH 7.5), 3 μL of RNaseA (10 $^{mg}/_{mL}$) added to each sample and the reactions incubated overnight at 37°C. The DNA samples were ethanol precipitated, washed with 70% ethanol and dried at room temperature. The DNA was suspended in 50 μL of 20 mM Tris-Cl (pH 7.5).

Indirect end labeling

A DNA fragment (366 bp) complimentary to the last 149 bp of the *CYC1* gene and 217 bp of the region 3' to the *CYC1* terminator codon was labeled with ^{32}P using a Random Primer DNA Labeling Kit (TaKaRa Bio Incorporated). The MNase cleaved DNA samples were digested with HindIII and then electrophoresed through a 1.5% agarose-TBE (0.045 M Tris-borate, 0.001 M EDTA) gel at 5.5 V/cm. The DNA was transferred to a Gene Screen membrane using capillary transfer according to the manufacturer's (PerkinElmer) protocol. The DNA was fixed to the membrane using ultra-violet light exposure for 5 minutes while the membrane was still wet. The membrane was incubated in prehybridization solution (6X SSC, 5X Denhardts reagent, 0.5% SDS, 100 ug/mL denatured Salmon Sperm DNA) at 65°C for at least 6 hours with constant rotation. The prehybridization solution was removed, hybridization solution (prehybridization solution plus ^{32}P labeled DNA) added and the membrane incubate at 65°C overnight with constant rotation. The membrane was washed as follows: one time quickly at room temperature with 2X SSC, two times for 15 minutes at 65°C with 2X SSC, 0.1% SDS, and two times for 15 minutes at room temperature with 0.1X SSC. The membrane was exposed a to phosphorimager screen overnight. Images were acquired using a Typhon FLA 9000 (GE Healthcare). The DNA markers were prepared by cleaving purified genomic DNA with EcoRI, NdeI, or EcoRV and HindIII.

SUPPLEMENTAL TABLES

Table S1: Plasmids, qPCR primer sequences and yeast strains used in this study.

Plasmid	Description	
pCR311	Full length wild type *SPN1* with 403 bp of upstream sequence and 116bp of downstream sequence, myc2 tagged at the amino terminus, pRS313 (*CEN*, *HIS3*)	
pCR312	$Spn1^{K192N}$ with with 403 bp of upstream sequence and 116bp of downstream sequence, myc2 tagged at the amino terminus, pRS313 (*CEN*, *HIS3*)	
pAA344	$spn1^{141\text{-}305}$ with 403 bp of upstream sequence and 116bp of downstream sequence, myc2 tagged at the amino terminus, pRS313 (*CEN*, *HIS3*)	
qPCR Primers	**Sequence**	**Chromosome: coordinates**
STA 633 (*CYC1* Promoter)	TCATATGGCATGCATGTGCT	X[1]: 526185-526204
STA 634 (*CYC1* Promoter)	CGGCCTTGAATTCAGTCATT	X: 526334-526353
STA 635 (*CYC1* ORF)	AAGGCCGGTTCTGCTAAGAA	X: 526347-526366
STA 636 (*CYC1* ORF)	ACGAATACCCTTCAGCTTGAC	X: 526474-526494
STA 555 (Telomere Proximal)	CGTAACAAAGCCATAATGCC	VI[2]: 269501-269520
STA 556 (Telomere Proximal)	CAGAAAGTAGTCCAGCCG	VI: 269567-269584
Strain	**Genotype**	**Source**
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Research Genetics
LZ0	BY4741 *spn1*::LEU2 +YCp50-*SPN1* (*URA3*)	Fishbeck *et al*. (2002)
LZ0-1	LZ0 + pCR311 lacking YCp50-*SPN1* (*URA3*)	This Study
LZ0-2	LZ0 + pCR312 YCp50-*SPN1* (*URA3*)	This Study
LZ0-3	LZ0 + pAA344 lacking YCp50-*SPN1* (*URA3*)	This Study
dst1Δ	BY4741 *dst1::kanMX4*	Research Genetics
LZ1	BY4741 *dst1::kanMX4 spn1*::LEU2 +YCp50-*SPN1* (*URA3*)	Zhang *et al*. (2008)
LZ1-1	LZ1 + pCR311 lacking YCp50-*SPN1* (*URA3*)	This Study
LZ1-2	LZ1 + pCR312 YCp50-*SPN1* (*URA3*)	This Study
LZ1-3	LZ1 + pAA344 lacking YCp50-*SPN1* (*URA3*)	This Study
rtf1Δ	BY4741 *rtf1::kanMX4*	Research Genetics
LZ2	BY4741 *rtf1::kanMX4 spn1*::LEU2 +YCp50-*SPN1* (*URA3*)	Zhang *et al*. (2008)

LZ2-1	LZ2 + pCR311 lacking YCp50-*SPN1* (*URA3*)	This Study
LZ2-2	LZ2 + pCR312 YCp50-*SPN1* (*URA3*)	This Study
LZ2-3	LZ2 + pAA344 lacking YCp50-*SPN1* (*URA3*)	This Study
nap1Δ	BY4741 *nap1::kanMX4*	Research Genetics
CR26	BY4741 *nap1::kanMX4 spn1*::*LEU2* +YCp50-*SPN1* (*URA3*)	This study
CR26-1	CR26 + pCR311 lacking YCp50-*SPN1* (*URA3*)	This study
CR26-2	CR26 + pCR312 lacking YCp50-*SPN1* (*URA3*)	This study
CR26-3	CR26 + pAA344 lacking YCp50-*SPN1* (*URA3*)	This study
rtt106Δ	BY4741 *rtt106::kanMX4*	Research Genetics
CR30	BY4741 *rtt106::kanMX4 spn1*::*LEU2* +YCp50-*SPN1* (*URA3*)	This study
CR30-1	CR30 + pCR311 lacking YCp50-*SPN1* (*URA3*)	This study
CR30-2	CR30 + pCR312 lacking YCp50-*SPN1* (*URA3*)	This study
CR30-3	CR30 + pAA344 lacking YCp50-*SPN1* (*URA3*)	This study
hir1Δ	BY4741 *hir1::kanMX4*	Research Genetics
CR10	BY4741 *hir1::kanMX4 spn1*::*LEU2* +YCp50-*SPN1* (*URA3*)	This study
CR10-1	CR10 + pCR311 lacking YCp50-*SPN1* (*URA3*)	This study
CR10-2	CR10 + pCR312 lacking YCp50-*SPN1* (*URA3*)	This study
CR10-3	CR10 + pAA344 lacking YCp50-*SPN1* (*URA3*)	This study
hir2Δ	BY4741 *hir2::kanMX4*	Research Genetics
CR11	BY4741 *hir2::kanMX4 spn1*::*LEU2* +YCp50-*SPN1* (*URA3*)	This study
CR11-1	CR11 + pCR311 lacking YCp50-*SPN1* (*URA3*)	This study
CR11-2	CR11 + pCR312 lacking YCp50-*SPN1* (*URA3*)	This study
CR11-3	CR11 + pAA344 lacking YCp50-*SPN1* (*URA3*)	This study
asf1Δ	BY4741 *asf1::kanMX4*	Research Genetics
CR28	BY4741 *asf1::kanMX4 spn1*::*LEU2* +YCp50-*SPN1* (*URA3*)	This study
CR28-1	CR28 + pCR311 lacking YCp50-*SPN1* (*URA3*)	This study
CR28-2	CR28 + pCR312 lacking YCp50-*SPN1* (*URA3*)	This study
CR28-3	CR28 pAA344 lacking YCp50-*SPN1* (*URA3*)	This study
Vps75Δ	BY4741 *vps75::kanMX4*	Research Genetics
CR50	BY4741 *vps75::kanMX4 spn1*::*LEU2* +YCp50-*SPN1* (*URA3*)	This study
CR50-1	CR50 + pCR311 lacking YCp50-*SPN1* (*URA3*)	This study
CR50-2	CR50 + pCR312 lacking YCp50-*SPN1* (*URA3*)	This study
CR50-3	CR50 + pAA344 lacking YCp50-*SPN1* (*URA3*)	This study
cac1Δ	BY4741 *cac1::kanMX4*	Research Genetics
CR51	BY4741 *cac1::kanMX4 spn1*::*LEU2* +YCp50-*SPN1* (*URA3*)	This study
CR51-1	CR51 + pCR311 lacking YCp50-*SPN1* (*URA3*)	This study
CR51-2	CR51 + pCR312 lacking YCp50-*SPN1* (*URA3*)	This study
CR51-3	CR51 + pAA344 lacking YCp50-*SPN1* (*URA3*)	This study
cac3Δ	BY4741 *cac3::kanMX4*	Research Genetics
CR52	BY4741 *cac3::kanMX4 spn1*::*LEU2* +YCp50-*SPN1* (*URA3*)	This study
CR52-1	CR52 + pCR311 lacking YCp50-*SPN1* (*URA3*)	This study
CR52-2	CR52 + pCR312 lacking YCp50-*SPN1* (*URA3*)	This study
CR52-3	CR52 + pAA344 lacking YCp50-*SPN1* (*URA3*)	This study

[1]Accession number NC-001142; [2]Accession number NC-001138

Table S2: Predicted secondary structure for Spn1 and Spn1$^{141-305}$ from the CD analysis.

Protein	α helix	β sheet	β turn	Unstructured
Spn1	135 to 157	47 to 51	88 to 92	117 to 137
Spn1$^{141-305}$	100 to 120	8 to 16	28 to 34	12 to 16

Table S3: Hydrodynamic properties of wild type Spn1 and Spn1$^{141-305}$.

WT Spn1				
NaCl (mM)	**S_{20W}[1]**	**M.W.**	**f/f_0**	**RMSD**
150	2.60	54.7	2.05	.005045
300	2.54	50.2	1.98	.005062
500	2.45	50.1	2.05	.004986
Spn1$^{141-305}$				
NaCl (mM)	**$S_{20,W}$**	**M.W.**	**f/f_0**	**RMSD**
150	1.84	21.2	1.43	.006612
300	1.82	21.1	1.44	.005762
500	1.81	21.6	1.47	.006671

[1]Abreviations used in the table: $S_{20,W}$ is the sedimentation coefficient corrected to 20°C and the density of water, M.W. is predicted molecular weight, f/f_0 is the frictional ratio and RMSD is the root-mean-square deviation.

Table S4: Growth of the *dst1Δ* and *rtf1Δ* strains with *SPN1* and *spn1*$^{141-305}$ under various conditions as described in the materials and methods section.

Parental Strain	Spn1 Allele	30°C	38°C	Caff.	HU	Form.	YPEG	Rap.	H_2O_2	-Ino	3AT
BY4741	*SPN1*	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	spn1$^{141-305}$	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
dst1Δ	*SPN1*	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	spn1$^{141-305}$	++++	½+	½+	½+	++	++++	++++	++++	++++	++++
rtf1Δ	*SPN1*	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	spn1$^{141-305}$	++	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	++

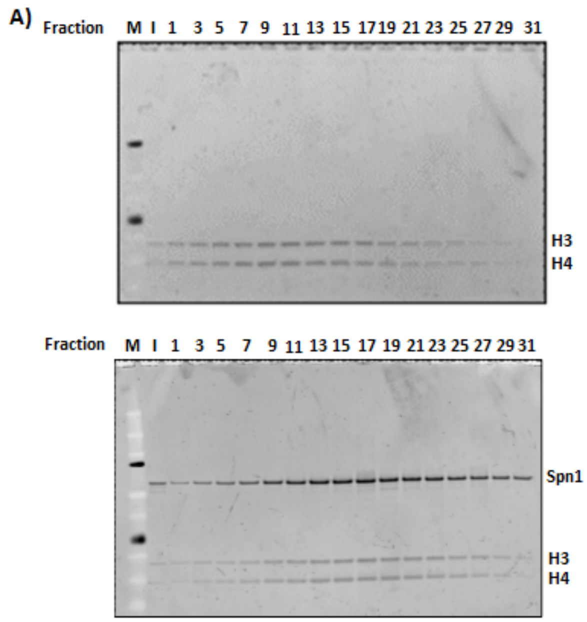
N.G. = No Growth; Red = Differential phenotype from parental strain

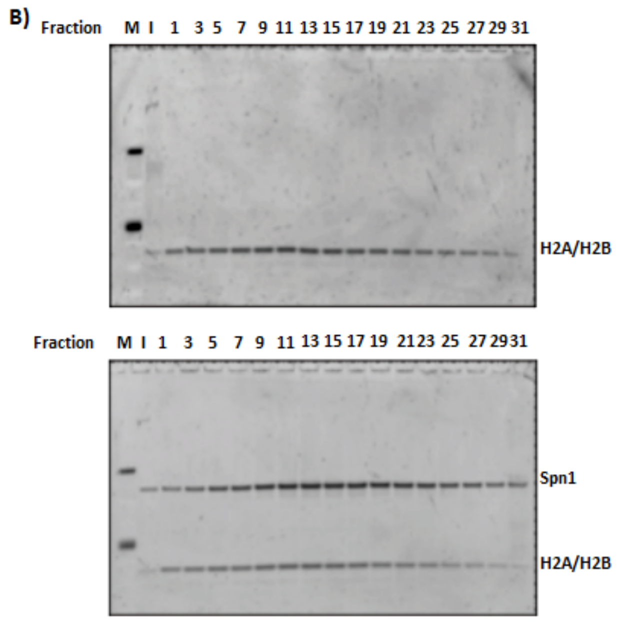
Table S5: Growth of the histone chaperone gene deletion strains with *SPN1*, *spn1*^{K192N}, and *spn1*$^{141-305}$ under various conditions as described in the materials and methods section.

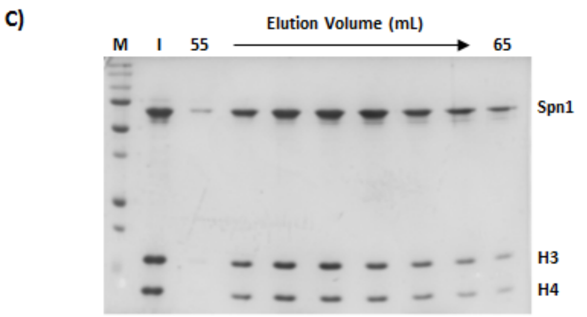
Parental Strain	Spn1 Allele	30°C	38°C	Caff.	HU	Form.	YPEG	Rap.	H_2O_2	-Ino	3AT
BY4741	*SPN1*	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	spn1^{K192N}	++++	N.G.	++++	++++	++++	++++	++++	++++	++++	++++
	spn1$^{141-305}$	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
nap1Δ	*SPN1*	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	spn1^{K192N}	++++	N.G.	++++	++++	++++	++++	++++	++++	++++	++++
	spn1$^{141-305}$	++++	++++	+++[2]	++++	++++	++++	++++	++++	++++	++++
rtt106Δ	*SPN1*	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	spn1^{K192N}	++++	N.G.	++++	++++	++++	++++	++++	++++	++++	++++
	spn1$^{141-305}$	++++	N.G.	+½	++	++	++++	++++	++++	++++	++++
hir1Δ	*SPN1*	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	spn1^{K192N}	++++	N.G.	++++	+++	+++	++++	+++	++++	++++	++++
	spn1$^{141-305}$	½+	N.G.	N.G.	N.G.	N.G	N.G.	N.G.	N.G.	N.G.	N.G.
hir2Δ	*SPN1*	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	spn1^{K192N}	++++	N.G.	++++	+++	+++	++++	+++	++++	++++	++++
	spn1$^{141-305}$	N.G.	N.G.	N.G.	N.G.	N.G	N.G.	N.G.	N.G.	N.G.	N.G.
asf1Δ	*SPN1*	++++	+++	++++	N.G.	++++	++++	++++	++++	N.G.	++++
	spn1^{K192N}	++++	N.G.	++++	N.G.	++++	++++	++++	++++	N.G.	++++
	spn1$^{141-305}$	++++	N.G.	++	N.G.	++++	++++	++++	++++	N.G.	++++
cac1Δ	*SPN1*	++++	+++	++++	++++	++++	++++	++++	++++	+++	++++
	spn1^{K192N}	++++	N.G.	++++	++++	++++	++++	++++	++++	+++	++++
	spn1$^{141-305}$	++++	N.G.	+++	++	++++	++++	++++	++++	+++	++++
cac3Δ	*SPN1*	++++	+++	++++	++++	++++	++++	++++	++++	++++	++++
	spn1^{K192N}	++++	N.G.	++++	++++	++++	++++	++++	++++	++++	++++
	spn1$^{141-305}$	++++	+++	++++	++++	++++	++++	++++	++++	++++	++++
vps75Δ	*SPN1*	++++	+++	++++	++++	++++	++++	++++	++++	++++	++++
	spn1^{K192N}	++++	N.G.	++++	++++	++++	++++	++++	++++	++++	++++
	spn1$^{141-305}$	++++	+++	++++	++++	++++	++++	++++	++++	++++	++++

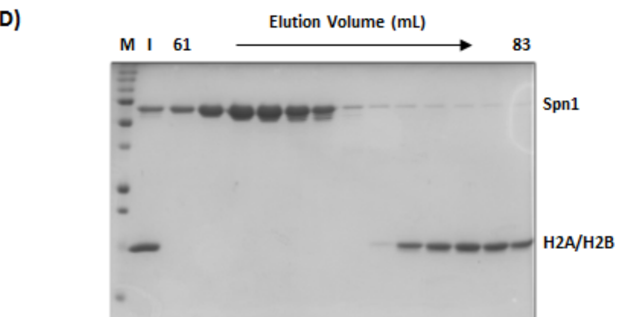
N.G. = No Growth; Red = Differential phenotype from parental strain

SUPPLEMENTAL FIGURES

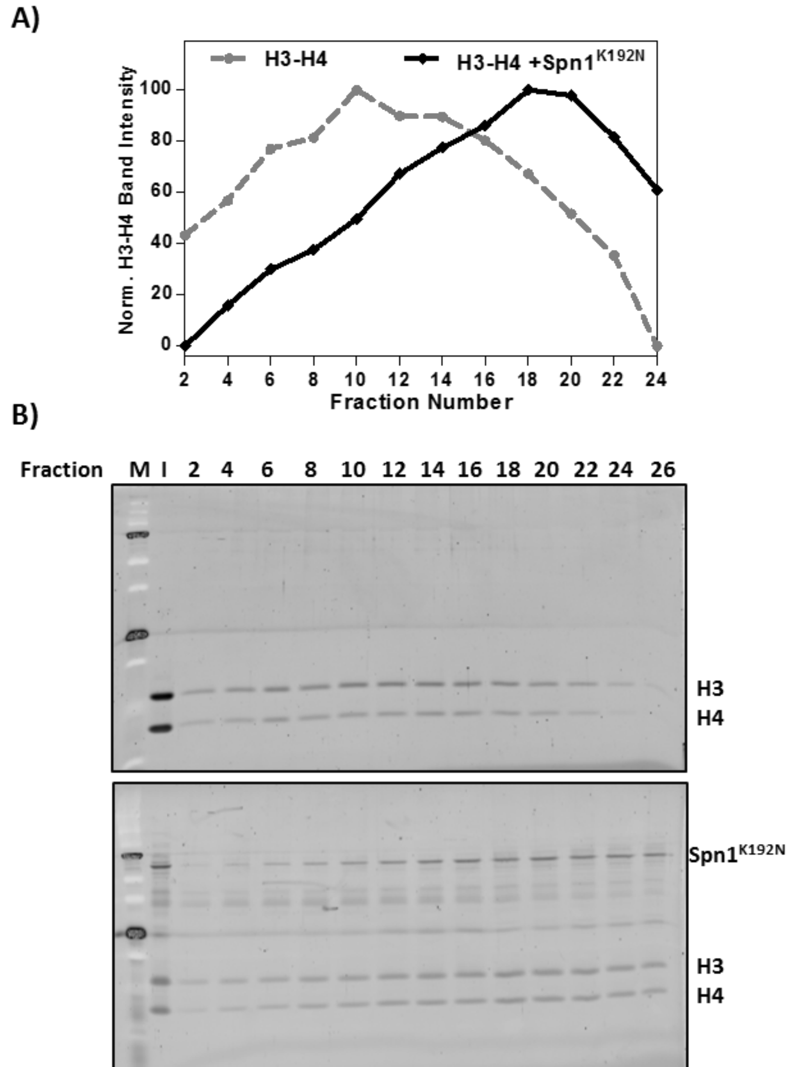




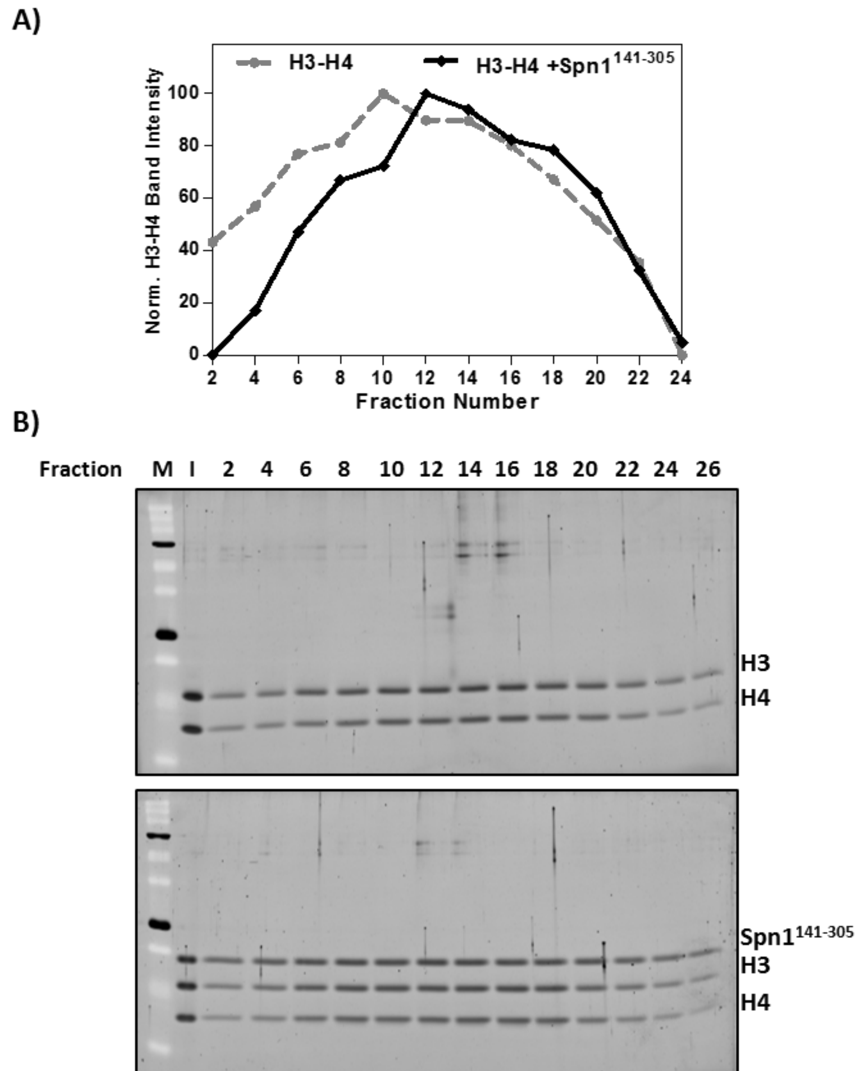




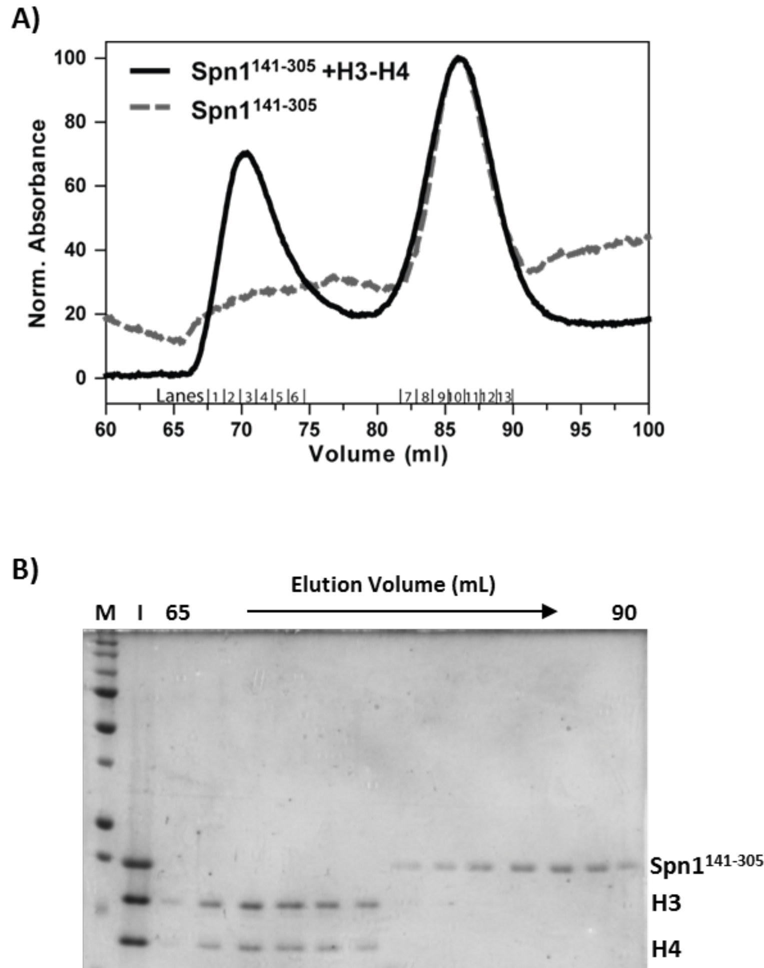
Supplemental Figure S1: Aliquots of the fractions collected following sucrose gradient sedimentation (A and B) or size exclusion chromatography (C and D) of Spn1 and histones H3-H4 or Spn1 and histones H2A-H2B were analyzed by SDS-PAGE. Following electrophoresis, proteins in the gels were stained with Sypro Ruby. Images of the gels with Spn1 and H3-H4 (left panel) or Spn1 and H2A-H2B (right panel) are shown above.



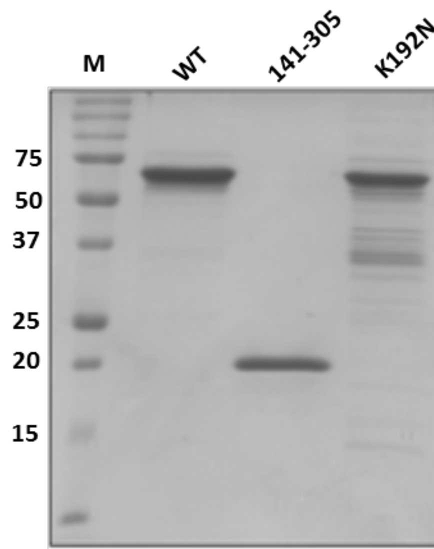
Supplemental Figure S2: Spn1^{K192N} has retained the ability to bind histones H3-H4. The binding of Spn1^{K192N} to histones H3-H4 was assessed by sucrose gradient sedimentation. Histones H3-H4 alone, or H3-H4 with Spn1^{K192N} were sedimented through 5-25% sucrose gradients and proteins in each fraction from the gradient were then separated by SDS-PAGE and visualized in the gels by Sypro Ruby staining. (A) The sedimentation profiles for H3-H4 alone or H3-H4 with Spn1^{K192N}. (B) Images of the stained gels for H3-H4 alone or H3-H4 with Spn1^{K192N}.



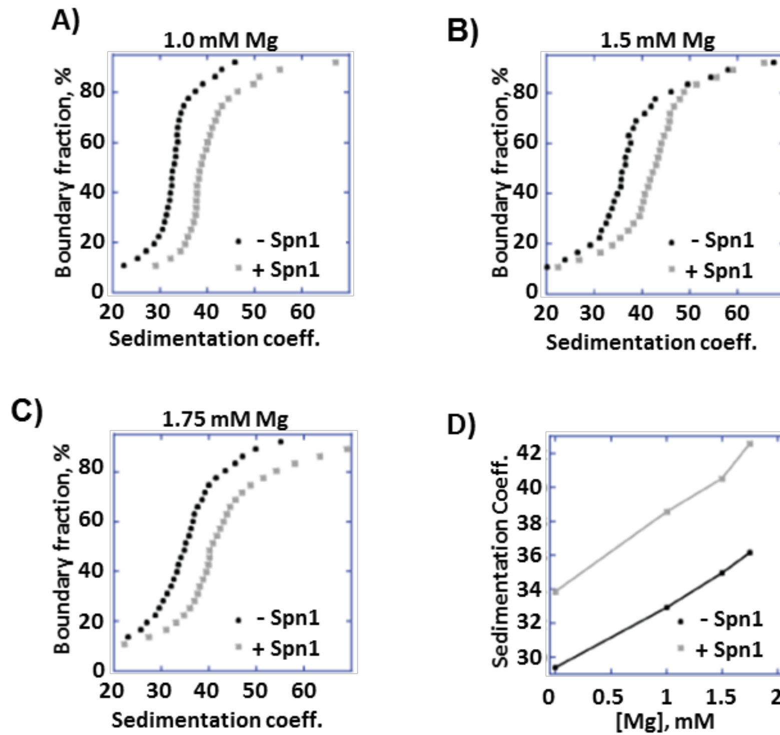
Supplemental Figure S3: The core domain of Spn1 does not appear to bind histones H3-H4. The binding of $Spn1^{141-305}$ to histones H3-H4 was assessed by sucrose gradient sedimentation. Histones H3-H4 alone, or H3-H4 with $Spn1^{141-305}$ were sedimented through 5-25% sucrose gradients and proteins in each fraction from the gradient were then separated by SDS-PAGE and visualized in the gels by Sypro Ruby staining. (A) The sedimentation profiles for H3-H4 alone or H3-H4 with $Spn1^{141-305}$ and (B) images of the stained gels for H3-H4 alone or H3-H4 with $Spn1^{141-305}$.



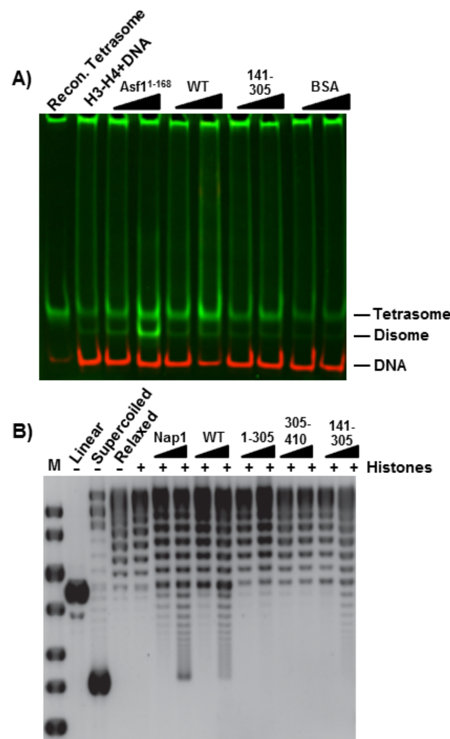
Supplemental Figure S4: The core domain of Spn1 does not bind histones H3-H4. The binding of Spn1$^{141\text{-}305}$ to histones H3-H4 was assessed by size exclusion chromatography. Spn1$^{141\text{-}305}$, and Spn1$^{141\text{-}305}$ with H3-H4 were passed through a Superdex 200 column. Proteins in each fraction from the column were separated by SDS-PAGE and visualized in the gels by Sypro Ruby staining. (A) Overlays of the size exclusion chromatograms for Spn1$^{141\text{-}305}$ alone and Spn1$^{141\text{-}305}$ with H3-H4. (B) An image of the gel from the SDS-PAGE analysis of the fractions.



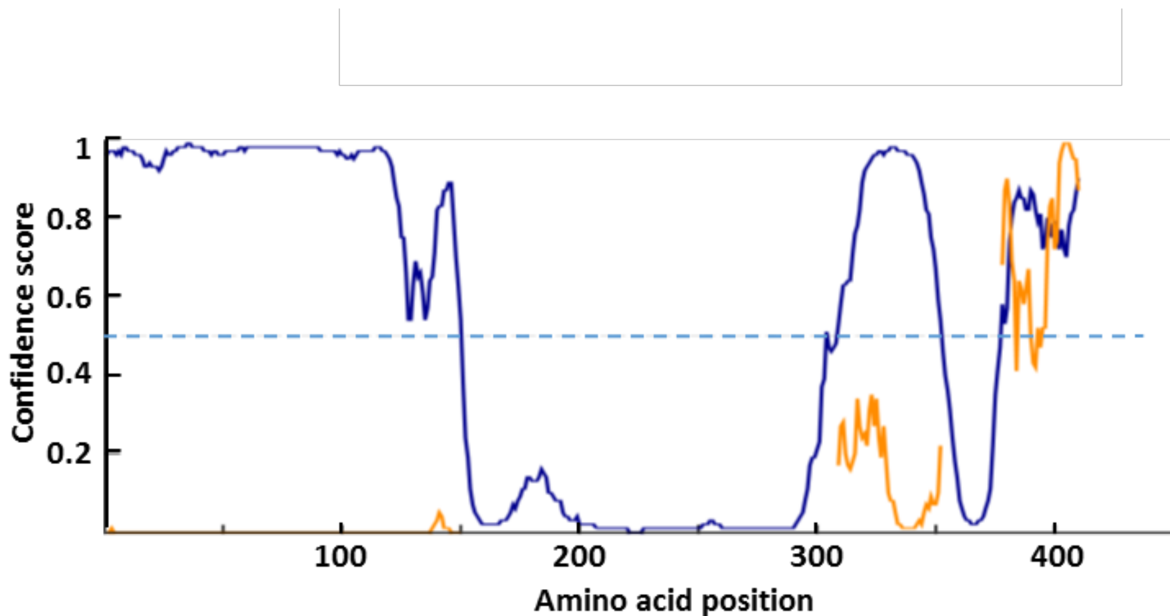
Supplemental Figure S5: Spn1^{K192N} degrades during purification. Equal amounts (0.5 μg) of wild type Spn1 (WT), Spn1$^{141-305}$ (141-305) and Spn1^{K192N} (K192N) were electrophoresed through a 15% SDS-polyacrylamide gel and the proteins stained with Coomassie Brilliant Blue R250.



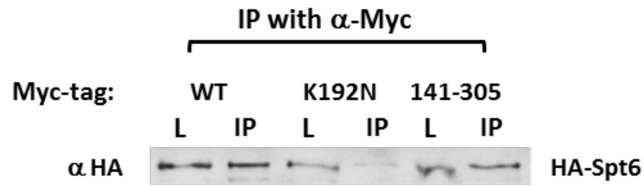
Supplemental Figure S6: The binding of Spn1 to nucleosomal arrays in the presence of magnesium does not affect intrinsic chromatin folding. The binding of wild type Spn1 to 207-12 nucleosomal arrays in the presence of increasing Mg concentrations was assessed by AUC. The Spn1 to nucleosome ratio was 1.0. (A) The sedimentation profile at 1 mM Mg, (B) The sedimentation profile at 1.5 mM Mg, (C) The sedimentation profile at 1.75 mM Mg, (D) A plot of the sedimentation coefficients for the nucleosomal arrays with and without wild type Spn1 versus Mg concentration.



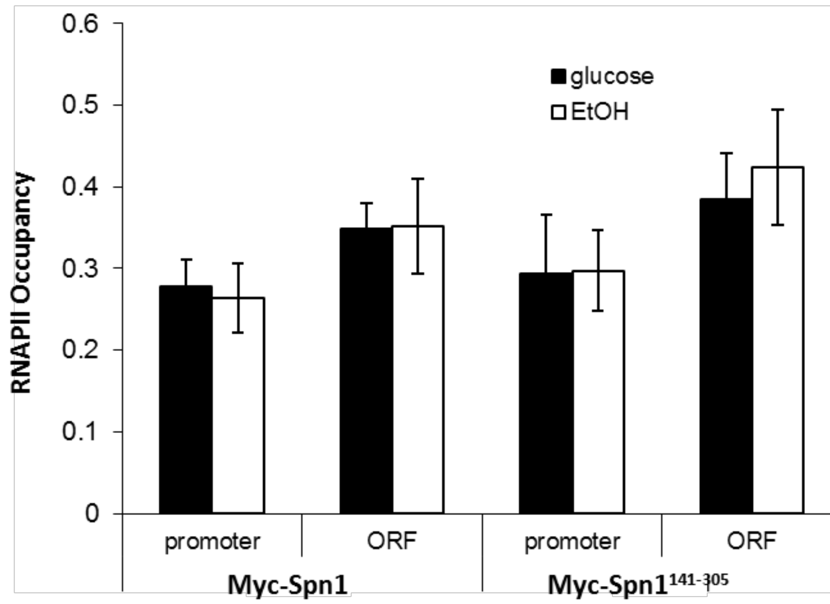
Supplemental Figure S7: Full length Spn1 facilitates H3-H4 deposition onto DNA and has weak nucleosome assembly activity that is compromised in Spn1$^{1\text{-}305}$, Spn141$^{141\text{-}410}$, and Spn1$^{141\text{-}305}$. (A) Histones H3-H4 labeled with Alex488 and a 79 bp DNA containing the 601 nucleosome positioning sequence (5) were incubated alone or with Asf1$^{1\text{-}168}$, Spn1 (WT) or Spn1$^{141\text{-}305}$ (141-305). DNA and DNA-protein complexes were resolved by electrophoresis through native 5% polyacrylamide gels, the gels stained with Ethidium Bromide and bands visualized using a Typhoon FLA 9500. A representative gel is shown. The position of the tetrasome, disome and free DNA are indicated at the right of the gel image. (B) Nucleosome assembly activity of Nap1, full length Spn1 (WT), Spn1$^{1\text{-}305}$ (1-305), Spn141$^{141\text{-}410}$ (141-410), or Spn1$^{141\text{-}305}$ (141-305) as indicated, was analyzed using a plasmid supercoiling assay as described in the methods.



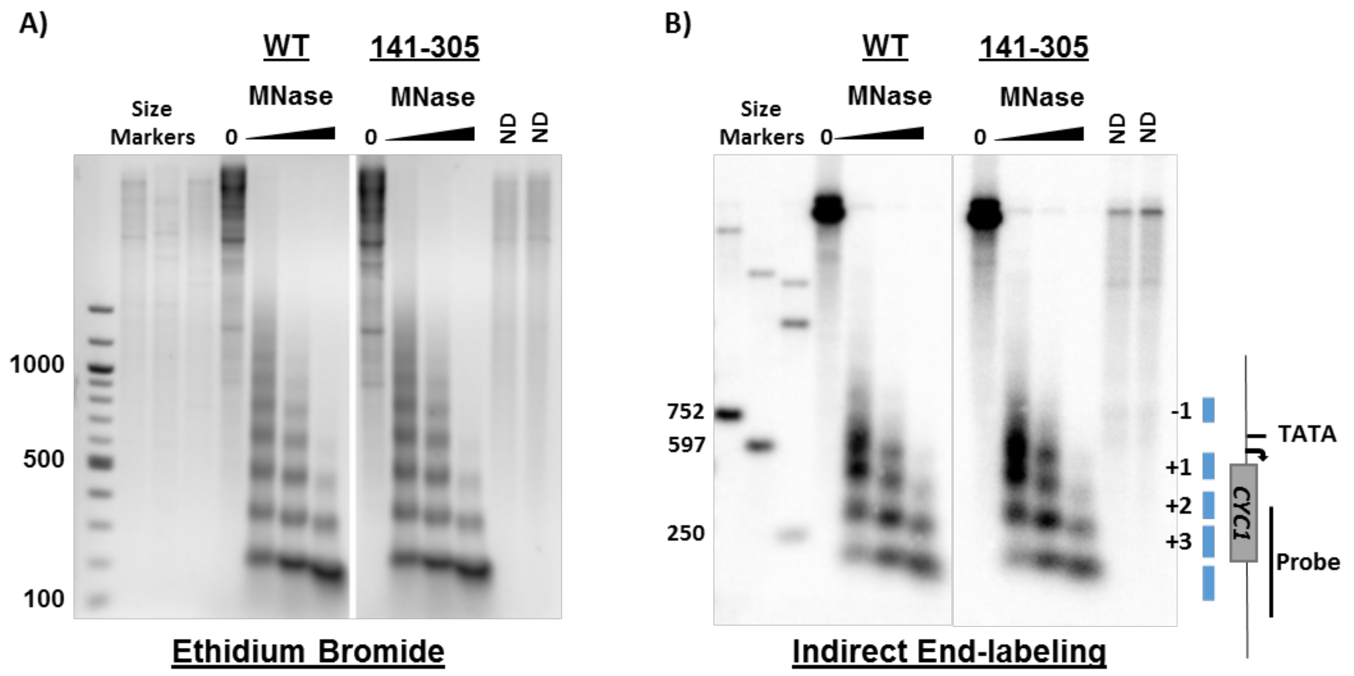
Supplemental Figure S8: The N- and C-termini of Spn1 are predicted to be predominantly Structurally disordered. The primary sequence of the Spn1 protein was analyzed using the DISOPRED3 software (2) on the PSIPRED server (4). The disorder profile plot of the Spn1 sequence from the analysis is shown above. The solid blue line traces the disorder confidence versus the sequence position. The blue dashed line indicates the threshold above which amino acids are predicted to be disordered. The solid orange line traces the confidence of disordered residues being involved in protein:protein interactions.



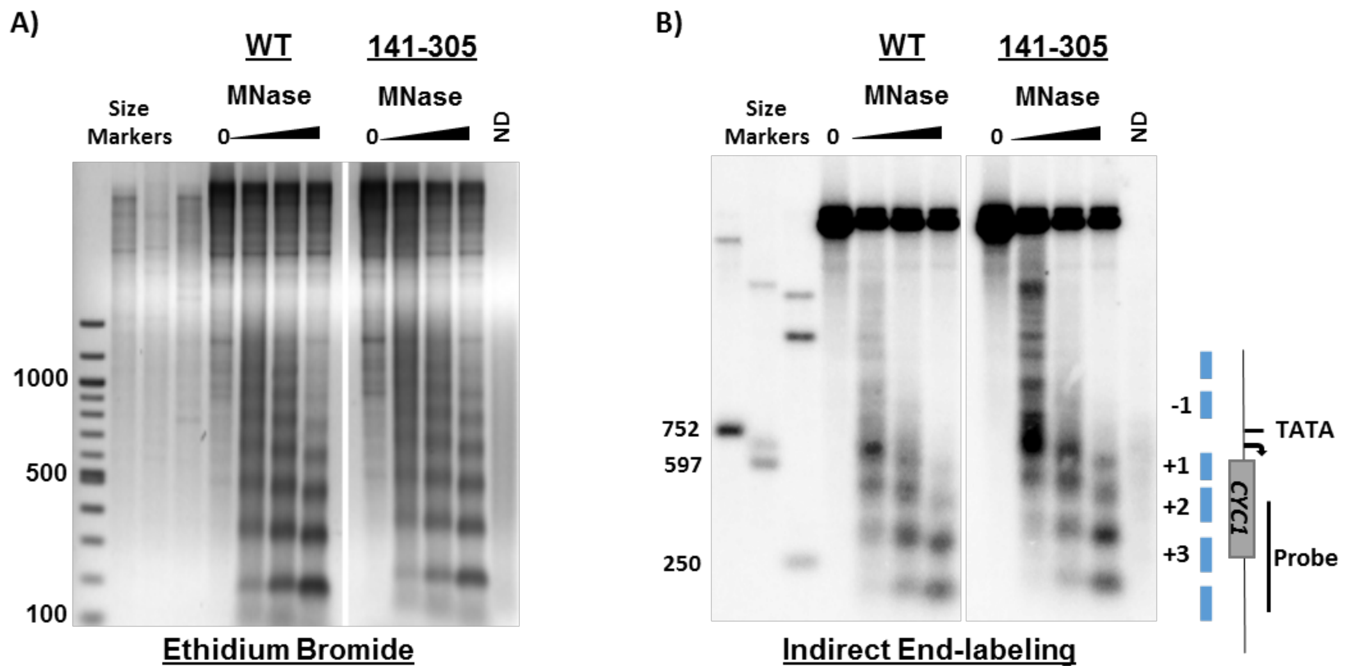
Supplemental Figure S9: The core domain of Spn1 is sufficient for interaction with Spt6 *in vivo*. Cell lysates prepared from strains expressing HA-Spt6, and Myc-Spn1, Myc-Spn1^{K192N}, or Myc-Spn1$^{141\text{-}305}$ (L) were immunoprecipitated (IP) with anti-Myc antibodies. HA-Spt6 was detected in the immunoprecipitates by immunoblot analysis with monoclonal anti-HA antibodies.



Supplemental Figure S10: RNAPII occupancy at the *CYC1* gene promoter and open reading frame is the same in the *spn1*$^{141\text{-}305}$ strain as it is in the *SPN1* strain. ChIP analysis of RNAPII occupancy at the *CYC1* promoter under repressive (glucose) and activating (ethanol) growth conditions. Occupancies were normalized to an un-tagged stain and then to the telomere.



Supplemental Figure S11: When transcription is repressed, Spn1 occupancy at the *CYC1* locus does not affect nucleosome positioning or relative occupancy. MNase digested chromatin DNA from *SPN1* and *spn*$^{141\text{-}305}$ cells grown in glucose was digested with HinDIII and analyzed by indirect end labeling as described in the methods section. (A) Image of the MNase digested DNA samples following electrophoresis and staining with ethidium bromide. (B) Image of the MNase digested *CYC1* locus. The lengths of select DNA fragments in the markers are shown at the left of each panel. MNase digested naked DNA was electrophoresed in the lanes labeled ND. A schematic of the *CYC1* locus with the positions of the nucleosomes (numbered blue boxes), TATA sequence, *CYC1* ORF and the ^{32}P-probe is shown at the right of panel B.



Supplemental Figure S12: Spn1's chromatin functions are required to maintain proper nucleosome occupancy at the *CYC1* locus when transcription is occurring. MNase digested chromatin DNA from *SPN1* and $spn^{141\text{-}305}$ cells grown in ethanol was digested with HinDIII and analyzed by indirect end labeling as described in the methods section. (A) Image of the MNase digested DNA samples following electrophoresis and staining with ethidium bromide. (B) Image of the MNase digested *CYC1* locus. The lengths of select DNA fragments in the markers are shown at the left of each panel. MNase digested naked DNA was electrophoresed in the lanes labeled ND. A schematic of the *CYC1* locus with the positions of the nucleosomes (numbered blue boxes), TATA sequence, *CYC1* ORF and the ^{32}P-probe is shown at the right of panel B.

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