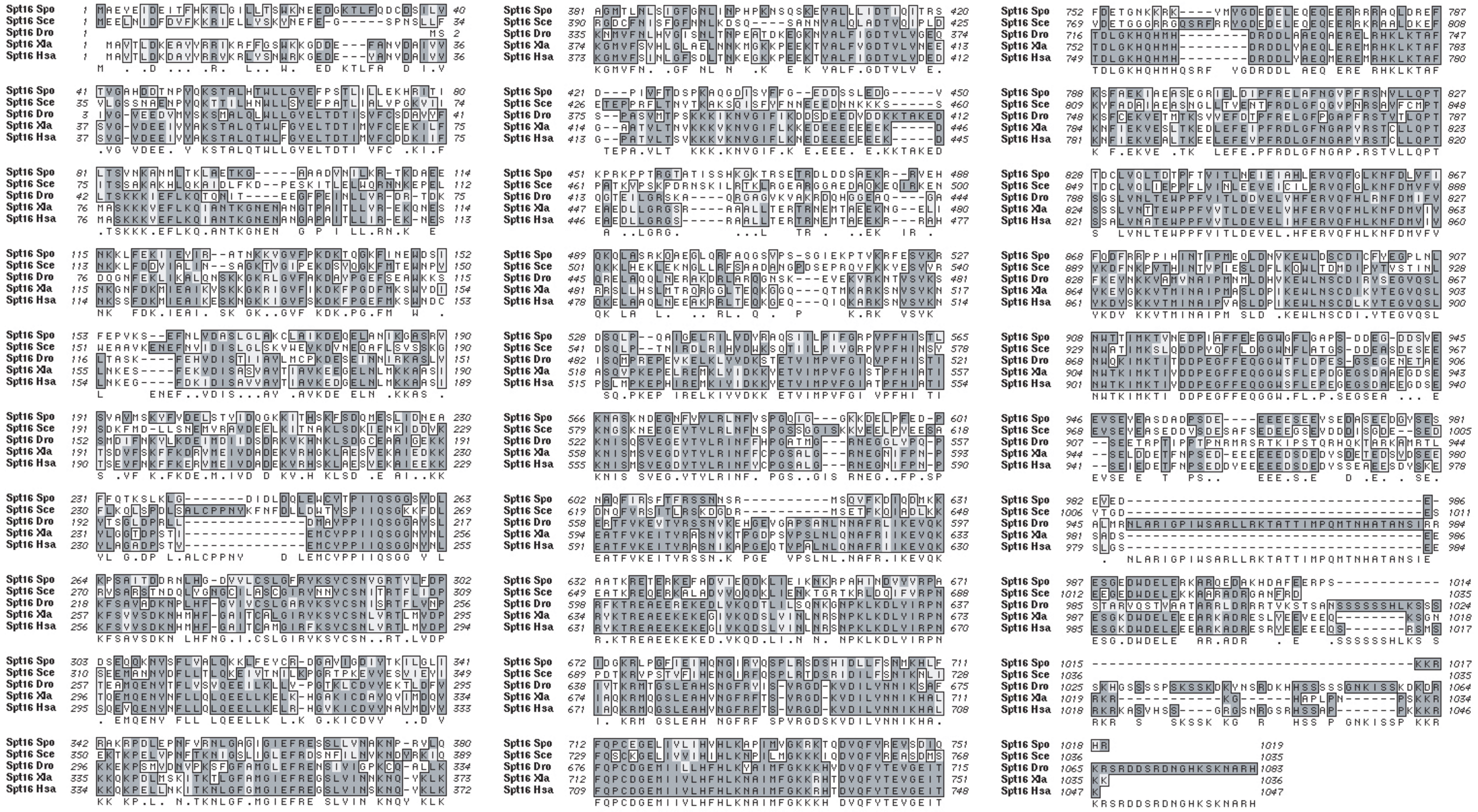
Figure S1. (A) Primary sequence alignments between *S. pombe* Spt16 and select eukaryotic orthologues



Lejeune *et al.* (2007)

Figure S1. (B) Primary sequence alignments between *S. pombe* Pob3 (SSRP1) and select eukaryotic orthologues

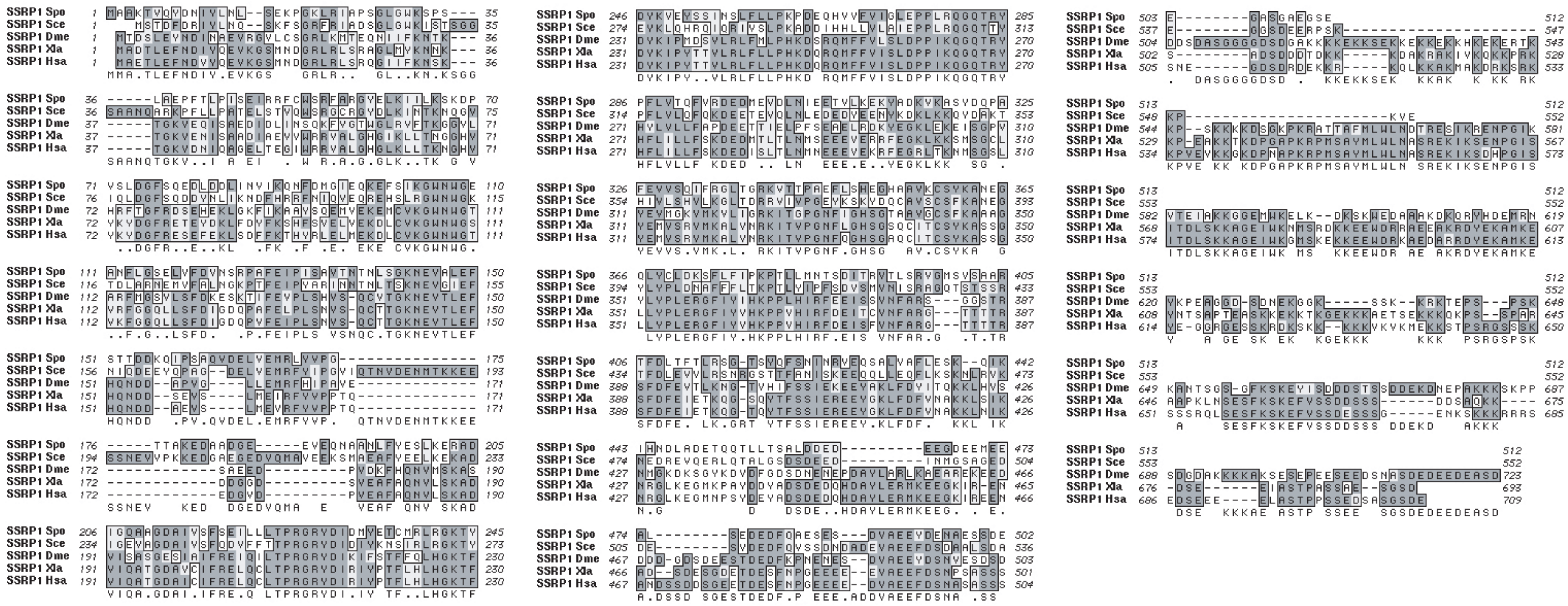


Figure S2

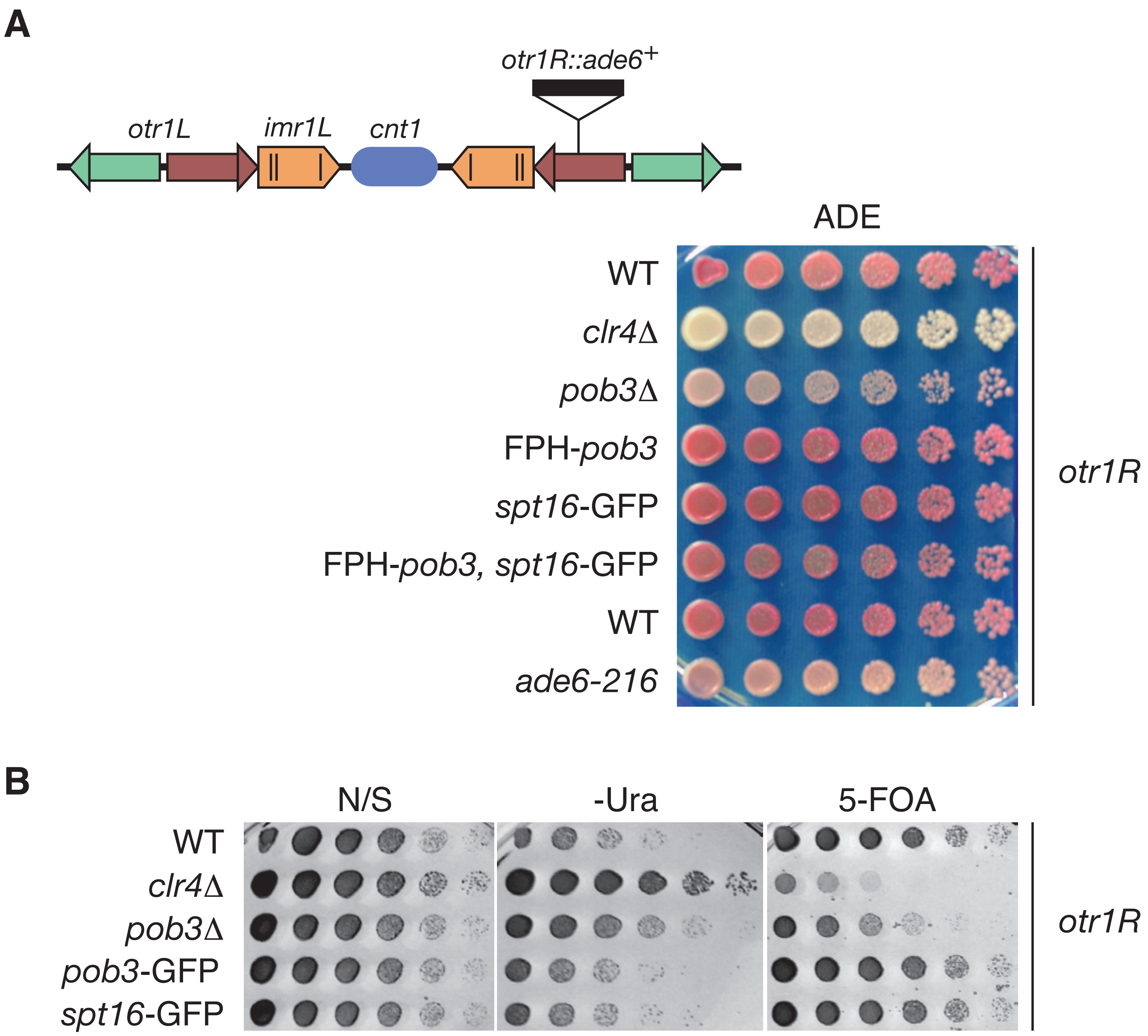


Figure S2. Tagged isoforms of the *S. pombe* Pob3 and Spt16 proteins are functional.

(A) Tagged FPH-Pob3, Spt16-GFP and double tagged strains are functional in the ADE-colorimetric centromere silencing assay.
(B) Pob3-GFP and Spt16-GFP fusion proteins do not affect centromeric silencing. Serial dilutions of wild-type (WT), *clr4*Δ, *pob3*Δ, pob3-GFP and spt16-GFP strains.

Figure S3

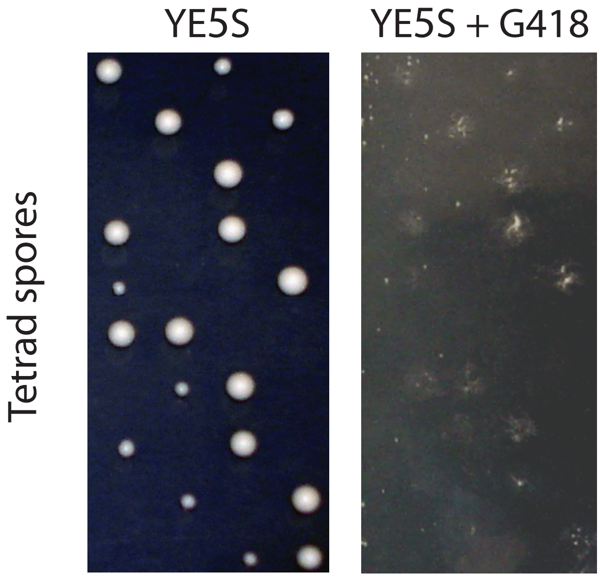
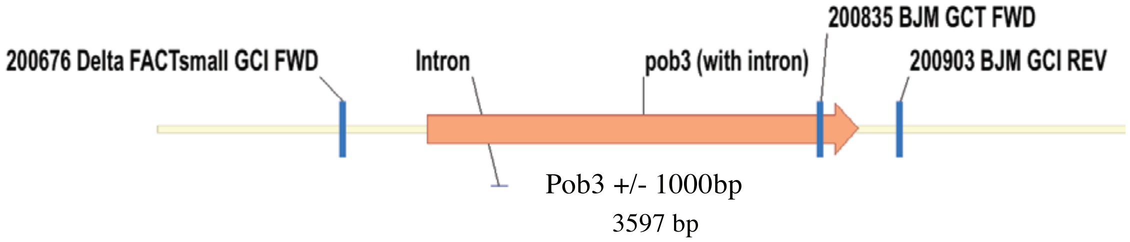


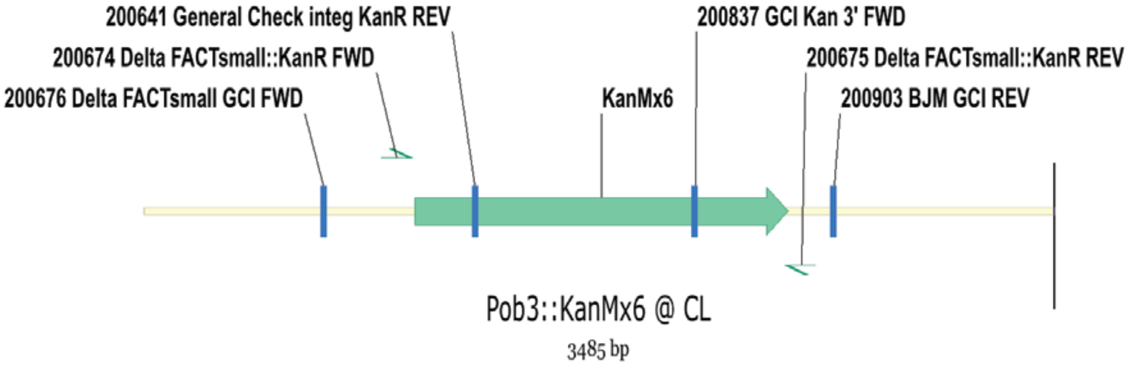
Figure S3. *spt16+* is an essential gene.

Tetrads dissected from the diploid *spt16::kanMX6/spt16+* were grown on YE5S media at 32 degrees Celsius for 2 days. All growing spore clones were G418 sensitive.

Figure S4

Strain EL44 = *pob3+::KanMX6* construction & verification





(a) Primers to amplify the KanR deletion cassette for *pob3*+::KanMX6 strain

200674 Delta FACTsmall::KanR FWD

AATGTGTTAATGTGTGAATTTGAGAATAACCTGGTATAGCTGTTTTCTGACCA
AATACTTAATACTTCACCAGTTATAAAAAATGCGGATCCCCGGGTTAATTAA

(includes the pob3's ATG) anneals on the Kanamycin-containing plasmid

200675 Delta FACTsmall::KanR REV

AATTTGTAATAGCAAAAGACAAGGCTGAAAAGTTGATCCAAATAGACTTCCA
ATTTAACATTGCTCAATTTTATAGATCATTAGAATTCGAGCTCGTTTAAAC

(includes the pob3's TAA) anneals on the Kanamycin-containing plasmid

(b) Primers to check *pob3*+::KanMX6 deletion by PCR on the **5' end** and **3' end**

200676 Delta FACTsmall GCI FWD

GCT GAA CCT CGT TCC TCA G

200641 General Check integ KanR REV

CAA TTC AAC GCG TCT GTG AGG

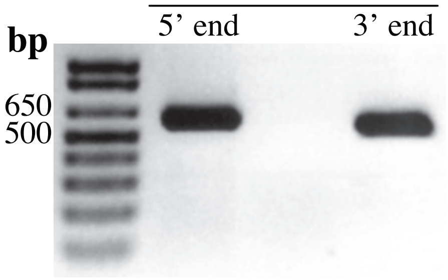
200837 GCI KAN 3' FWD

TAT GGA ACT GCC TCG GTG AG

200903 BJM GCI REV

TTT ACC ACT CGG ATT CAT CG

PCRs on strainEL44



5' end (200676/200641) expected amplicon: 604bp
3' end (200903/200837) expected amplicon: 552bp

Figure S5

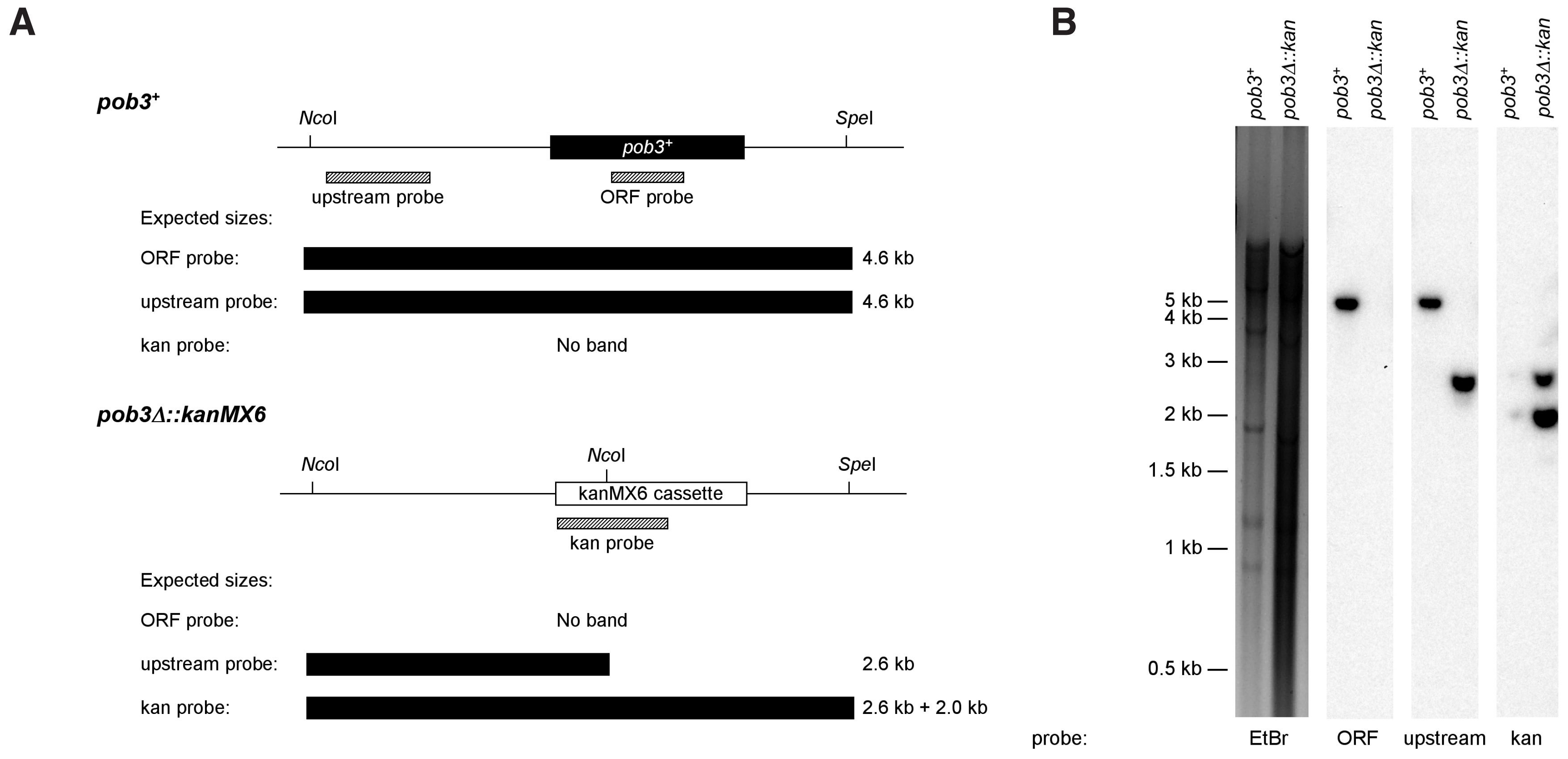


Figure S5. Soutern blot verification of *pob3*+ gene deletion in *S. pombe.*

(A) Diagram showing the wild-type *pob3*+ strain and *pob3Δ::kanMX6* knockout strain, including the probes and expected sizes of DNA fragments obtained by restriction enzymes NcoI and SpeI.
(B) Southern blot for *pob3*+ and *pob3Δ::kanMX6* strains using the indicated radiolabelled ORF, upstream and kan probes.

Figure S6

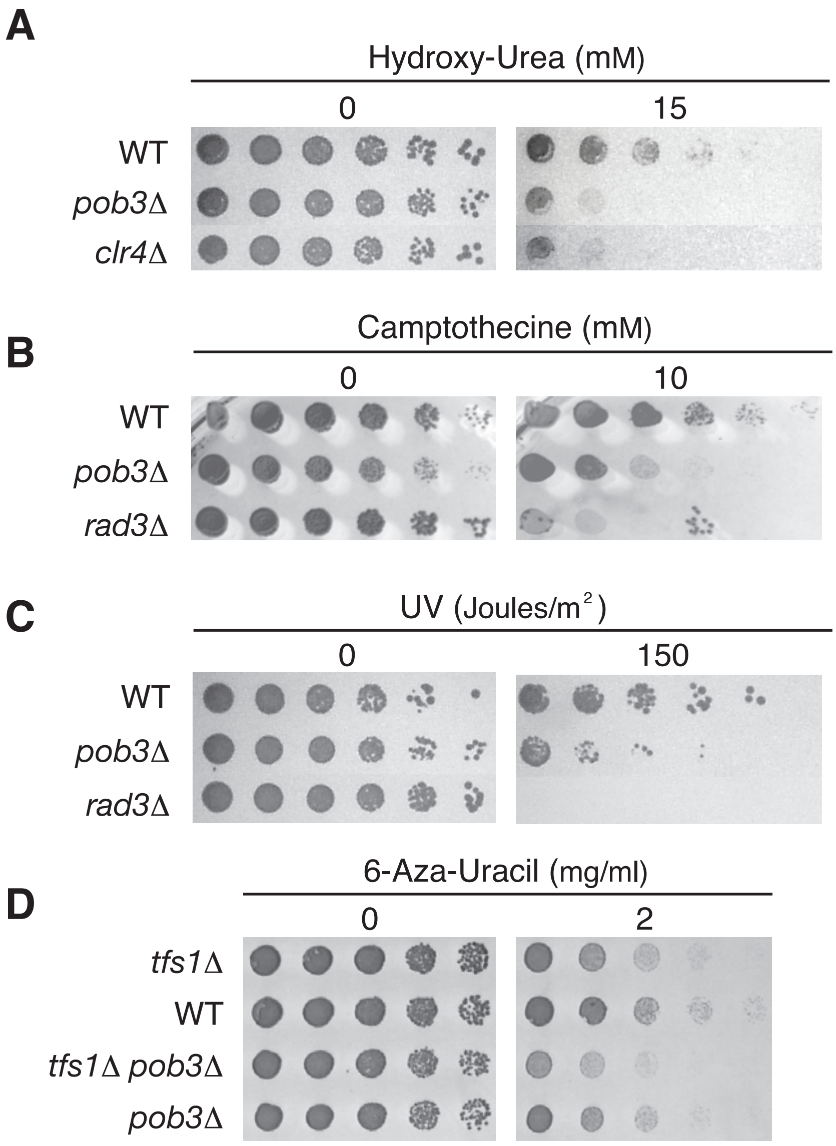


Figure S6. The small subunit of the *S. pombe* chromatin remodeling complex FACT (Pob3) has a role in genome stability.

Sensitivity of wild-type, *pob3*Δ deletion and reference yeast strains to:
(A) Hydroxyurea (HU), which reveals defects in DNA replication.
(B) camptothecine (CPT) and (C) ultra-violet (UV) radiation, both of which reveal defects in controlling induced DNA damage.
(D) 6-aza-uracil (6AU), a small molecule that affects intracellular GTP levels and is commonly used to screen for mutants affecting transcript elongation.

Figure S7

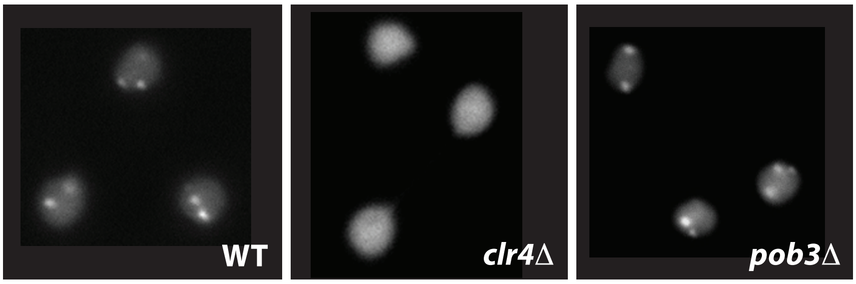


Figure S7. GFP-Swi6 delocalization assay.

GFP-Swi6 is functional and a good marker for centromeres and telomeres (Pidoux *et al*., 2000).

GFP-Swi6 fluorescence images of wild-type (WT), *clr4*Δ and *pob3*Δ cells. GFP-Swi6 is delocalized from centromeres and telomeres in *clr4*Δ mutants, as opposed to WT and *pob3*Δ cells.

Table S1. Gene expression profiling of a *pob3*Δ strain relative to wild-type *S. pombe*.

LOWER expression in *pob3*Δ *versus* WT. 2-fold cut-off (3 of 4 data points): 15 genes (total)	
Gene Name	**Gene dB Product description**
SPCC965.14c	putative cytidine and deoxycytidylate deaminase zinc-binding protein
SPAC644.05c	putative deoxyuridine 5'-triphosphate nucleotidohydrolase (EC 3.6.1.23); dUTP pyrophosphatase
rps10-2	40S ribosomal protein S10
sou1	sorbitol utilization protein sou1; short chain dehydrogenase
SPAC926.05c	dnaj protein
SPBC609.05 = *pob3*	similar to *S. cerevisiae* POB3 protein that binds to DNA polymerase I; putative structure specific recognition protein
SPAC1B3.16c	MFS transporter of unknown specificity
SPAC5D6.05	Protein that plays a role in cell separation, mating and differentiation
SPAC694.03	hypothetical protein; similar to *S. cerevisiae* YCL047C
SPAC29B12.04	putative pyridoxine (vitamin B6) biosynthetic enzyme; by similarity to yeast snz1
SPCC622.19	hypothetical protein; (conserved in fungi- Gaillardin et al)
SPBC146.08c	putative elongation initation factor eIF1a
SPBP23A10.03c	hypothetical protein; similar to *S. cerevisiae* YDR511W
vti1	putative vesicle transport V-snare protein
SPBC1709.06	conserved hypothetical protein; UPF0034 family
HIGHER expression in *pob3*Δ *versus* WT. 2-fold cut-off (3 of 4 data points): 44 genes (total)	
Gene Name	**Gene dB Product description**
fio1	iron transport multi-copper oxidase precursor
hsp9	heat shock protein hsp9
hsp16	heat shock protein hsp16
SPAC29A4.06c	hypothetical protein; contains Pfam-B_33047; shares conserved region with *Drosophila* CG15747; *Arabidopsis* F12K2.14; and C. elegans C16C10.6; predicted coiled-coil
cox1	
lys7	putative alpha-aminoadipate reductase small chain, sixth step in lysine biosynthesis pathway- from ACPS domain
fip1	iron permease
frp1	ferric reductase transmembrane componen
SPAC212.06c	pseudogene; truncated C-terminal of DNA helicase in rearranged telomeric region
SPAC212.08c	hypothetical protein; possibly *S. pombe* specific; duplicated in telomeric region; contains N-term predicted signal sequence
SPAC212.11	DNA helicase possibly pseudo
SPAC1F8.03c	MFS efflux transporter of unknown specificity
SPAC750.07c	very hypothetical protein
SPAC1834.10c	hypothetical protein; sequence orphan

SPAC1782.01	similar to *S. cerevisiae* Ecm29 cell wall stucture/biosynthesis protein
SPAC22F8.12c	hypothetical protein; serine-rich protein; sequence orphan; predicted N-terminal signal sequence
pim1	putative peptide transporter
SPAC1002.19	putative GTP cyclohydrolase; riboflavin biosynthesis
SPCC338.12	putative proteinase precursor
SPAC9E9.04	conserved hypothetical protein; putative endoplasmic reticulum transmembrane protein; similar to *S. cerevisiae* YET1; contains predicted N-term signal sequence; contains 2 predicted transmembrane helices; contains predicted C-term coiled-coil
SPCC965.07c	Glutathione S-transferase
srp14	signal recognition particle 14 kda protein
SPAC11H11.05c	very hypothetical protein
SPAC1002.17c	probable uracil phosphoribosyltransferase
SPBPJ4664.02	hypothetical protein; sequence orphan; low similarity to glycoamylases and other cell surface proteins; contains ~250-270 copies of a 13 AA repeat, NSSTPITSSSIL; contains predicted N-terminal signal sequence
SPAC22F8.05	putative alpha,alpha-trehalose-phosphate synthase
SPBC1921.06c	hypothetical protein; sequence orphan; contains predictd N-term signal sequence
SPBC27B12.03c	putative c-5 sterol desaturase
SPCC1739.08c	short chain dehydrogenase; putative sorbitol utilization
SPAPB1A11.03	putative FMN dependent dehydrogenase; similar to lactate dehydrogenase
SPAC19G12.09	putative aldose reductase
SPBC557.06c	
leu2	3-isopropylmalate dehydratase
rpn10	19s proteasome regulatory subunit
klp8	kinesin-like protein
SPAC12B10.10	hypothetical protein; sequence orphan; predicted C-term coiled-coil region
sec24	putative component of the COPII coat; involved in endoplasmic reticulum to Golgi transport
cdc16	cell division control protein 16; spindle assembly checkpoint component; GTPase-activating protein (GAP) for Spg1p GTPase; essential;two-component GEF for the GTPase spg1 (with byr4)
SPBPB2B2.01	putative amino-acid permease
SPAC637.03	hypothetical protein; sequence orphan; contains 8 predicted transmembrane segments contains predicted N-term signal sequence
SPBC21D10.10	bromodomain protein
SPBC887.16	very hypothetical protein
SPAC27D7.05c	hypothetical protein; sequence orphan; has transcript from microarray
SPAC27D7.06	putative electron transfer flavoprotein alpha-subunit precursor

Table S2. **Hyper-geometric probability comparisons of genes either up- or down-regulated in *pob3* deletion cells.**

HIGH expression in *pob3Δ* cut 2.0 (3 of 4)		**44 genes in list**		
P-value	**Similar List Name**	**Genes in list**	**Genes in overlap**	**Reference for gene list**
1,07e-7	HIGH expression in *rpb7-G150D* cut 1.5 (6 of 8)	62	10	Djupedal *et al* (2005), *Genes Dev*, 19, 2301
2,56e-6	HIGH expression in *clr3D* cut 1.5	61	9	Hansen *et al* (2005), *Mol Cell Biol*, 25, 590
0,000136	HIGH expression in pSwi6 cut 1.5 (3 of 4)	254	13	Wiren *et al* (2005), *EMBO J*, 17, 2906
0,00162	HIGH expression in *clr6-1* cut 2.0 (6 of 10)	63	7	Wiren *et al* (2005), *EMBO J*, 17, 2906
0,00318	HIGH expression in *clr6-1 clr3D* cut 1.5	570	17	Hansen *et al* (2005), *Mol Cell Biol*, 25, 590
0,00472	HIGH expression in *clr1D* cut 1.5	47	8	Hansen *et al* (2005), *Mol Cell Biol*, 25, 590
0,00518	HIGH expression in *clr4-681* cut1.5	107	8	Hansen *et al* (2005), *Mol Cell Biol*, 25, 590
0,0109	LOW IGR WT H4K16Ac H3 Cter corr cut 1.5	1267	24	Wiren *et al* (2005), *EMBO J*, 17, 2906
0,0115	LOW IGR WT H4K5Ac H3 Cter corr cut 1.5	1110	22	Wiren *et al* (2005), *EMBO J*, 17, 2906
0,0115	HIGH expression in *clr6-1* (6 of 10) cut 1.5	253	11	Wiren *et al* (2005), *EMBO J*, 17, 2906
0,0309	LOW IGR WT H4K12Ac H3 Cter corr cut 1.5	1083	21	Wiren *et al* (2005), *EMBO J*, 17, 2906
0,0356	HIGH expression in *clr3-735* cut1.5	183	9	Hansen *et al* (2005), *Mol Cell Biol*, 25, 590

LOW expression in *pob3Δ* cut 2.0 (3 of 4)		**15 genes in list**		
P-value	**Similar List Name**	**Genes in list**	**Genes in overlap**	**Ref. for gene list**
0,0229	LOW expression in *rpb7-G150D* cut 1.5 (6 of 8)	47	4	Djupedal *et al* (2005), *Genes Dev*, 19, 2301

For methods section: Similar gene lists were identified using the automatic hyper-geometric distribution tests in the gene list inspector function of Gene Spring v7.2 software (Silicon Genetics). The hyper-geometric distribution test calculates the probability of overlap corresponding to k or more genes between a list of n genes compared against another gene list of m fragments when randomly sampled from a universe of u genes:

$$\frac{1}{\binom{u}{m}} \sum_{i=k}^{n} \binom{m}{i}\binom{u-m}{n-i}$$

Table S3

Strain list (main figures)

Strain	Genotype
EL18	h+ leu1-32, ade6-216, ura4D18, imr1R(NcoI)::ura4+,
EL32	h+ clr4::kanMX6+, imr1R(NcoI)::ura4+, leu1-32, ade6-216, ura4D18
EL44	h+ pob3::kanMX6+, imr1R(NcoI)::ura4+, ade6-216, leu1-32, ura4D18
EL87	h- pob3::kanMX6, imr1R(NcoI):ura4+, leu1-32, ade6-210, ura4D18
EL93	h+ pob3::kanMX6, otrIR(SphI):ura4, ade6-210, his3D1?, leu1-32, ura4DS/E
EL102	h? pob3::kanMX6, mat3M::ura4, ade6-210, arg3D4, leu1-32, ura4D18
EL105	h? pob3::kanMX6, (rDNA:ura4) Ylp2.4pUCura4.7, ade6-210, leu1-32, his3D1, ura4DS/E,
EL107	h? pob3::kanMX6, otr2:ura4+ ?, TM1:arg3+, TM3:ade6+ ?, tel:his3+, ade6-210, arg3D4, leu1-32, his3D1, ura4D18/DS/E ?
EL127	h- spt16-GFP-kanMX6, otr1R (SphI)::ura4, arg3D4, his3D1, leu1-32, ura4DS/E
EL130	h- pob3-GFP-kanMX6, otr1R (SphI):ura4, arg3D4, his3D1, leu1-32, ura4DS/E
EL238	h- pob3::natMX6, ade6-210, arg3D4, his3D1, leu1-32, ura4DS/E
EL243	h? pob3::kanMX6, otrIR(SphI):ade6, ade6-210, leu1-32, ura4D18/DS-E?
EL306	h+ FPH-pob3, otrIR(SphI):ade6, ade6-210, leu1-32, ura4-D18
EL319	h? pob3::kanMX6, dcr1::natMX6, otrIR:ura4, ade6-210, his?, leu?, ura4D18/DS-E?
EL332	h? pob3::kanMX6, [CM3112 sup3e], ade6-704, arg3D4?, leu1-32, his3D1, ura4-D18
EL344	h? FPH-pob3, spt16-GFP-kanMX6, otrIR(SphI):ade6, ade6-210, arg3D4?, his3D1?, leu1-32, ura4D18
EL348	h? pob3::natMX6, [Ch16 ade6-216 m23::ura4], ade6-210, arg3D4?, his3D1?, leu1-32, ura4DS/E
FY511	h90 mat3-M::ura4, ade6-216, leu1-32, ura4-D18
FY521	h- [Ch16 ade6-216 m23::ura4], ade6-210, leu1-32, ura4DS/E
FY1034	h? swi6::his1, otr1R (dg-glu BamHI-Spe1 fragment) SphI::ura4, ade6-210, his1-102, leu1-32, ura4-DS/E
FY1180	h+ otr1R(dg-glu)SphI:ade6, ade6-210, leu1-32, ura4-D18
FY2116	h- [CM3112 sup3e], ade6-704, his3D1, leu1-32, ura4-D18
FY3027	h+ TM1::arg3, TM3::ade6, tel::his3, otr2::ura4, ade6-210, arg3D4, his3D1, leu1-32, ura4-D18/DS-E?
FY3606	h? rik1::leu2, TM1::arg3, TM3::ade6, otr2::ura4, his3::tel, ade6-210, arg3D4, his3D1, leu1-32, ura4D18
FY4134	h- ade6-210, arg3D4, his3D1, leu1-32, ura4DS/E
FY4841	h- otr1R(SphI)::ura4, arg3D4, his3D1, leu1-32, ura4DS/E
FY6084	h- clr4::LEU2+, otr1R(SphI):ura4 ura4D/SE, leu1-32, ade6-210/216?
FY6603	h+ Ylp2.4pUCura4.7, ura4-DS/E, leu1-32, ade6-216
FY6802	h+ tas3::kanMX6, imr1R(Nco1)::ura4+, ade6-216, leu1-32, ura4D18/DS-E?
FY7036	h- dcr1::natMX6, otr1R(dg-glu)SphI:ade6, ade6-210, his3D1?, leu1-32?, ura4D18/DS-E?

Strain list (Supplementary figures)

Strain	Genotype
EL63	h+ ade6-210 leu1-32
EL90	h+ pob3::kanMX6, ade6-210, arg3D4, his3D1, leu1-32, ura4D18
EL98	h? pob3::kanMX6, ars1(MluI)::pREP81X-gfp-swi6-LEU2+, ade6-210/216?, leu1-32, ura4-D18
EL111	h+ pob3::kanMX6, ade6-210, leu1-32
EL115	h? pob3::kanMX6, tfs1::LEU2+, ade6-210, leu1-32, can1-1?
EL173	h+ tfs1::natMX6, ade6-210, leu1-32
EL229	h? clr4::LEU2+ otr1R(Sph1):ade6+, ade6-210/216?, leu1-32, ura4D18/DS-E?
EL345	h? FPH-pob3, spt16-GFP-kanMX6, otrIR(dg-glu)SphI:ade6, ade6- 210, arg3D4?, his3D1?, leu1-32, ura4D18
EL351	h? spt16-GFP-KanMX6, otr1R(dg-glu)Sph1:ade6, ade6-210, arg3D4?, his3D1?, leu1-32, ura4D18/DS-E?
EL355	h+/h+ spt16::KanMX6/ORF, ade6-M210/ade6-M216, ura4D18/ura4D18, leu1-32/leu1-32
FY1665	h- rad3::ura4+, ade6-704, leu1-32, ura4-D18
FY2214	h- ars1(MluI)::pREP81Xgfpswi6 LEU2+, ade6-210, leu1-32, ura4-D18
FY3300	h? clr4 :: ura4 ars1(MluI)::pREP81Xgfpswi6-LEU2+, ade6-210/216?, leu1-32, ura4D18/DS-E?