

Supporting Information

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SI Materials and Methods

Yeast Strains. *Schizosaccharomyces pombe* cells were grown in the undefined medium yeast extract + supplements (YES) or in zinc-limited Edinburgh minimal medium (ZL-EMM) with or without the indicated zinc supplement (1). For all experiments with ZL-EMM, cells were pregrown to exponential phase in YES medium. Cells were then washed twice in ZL-EMM, diluted to a final OD₆₀₀ of 0.5 and grown for a further 14–16 h in ZL-EMM with or without zinc supplements. The generation of the *adh4*Δ, *adh1*Δ *adh4*Δ, *loz1*Δ, and *zrt1*Δ was performed by using standard gene replacement procedures (2) with the exception that additional homologous sequences were added by using two-step overlapping PCR to the *kanMX6* knockout cassette to generate *zrt1*Δ. Strains *loz1-1* and *adh1*Δ were derived from a cross of *adh1*Δ *loz1-1* to a wild-type strain of the opposite mating type.

Plasmid Generation. To generate a plasmid expressing a GFP-tagged Loz1 protein, eGFP was amplified from the vector JQW85 (3). The PCR product was digested with BamHI and SacI before cloning into similar sites in the vector JK148 (4) to create JK-GFP. To generate pLoz1-GFP, the *loz1* ORF was PCR amplified by using primers that contained EcoRI and BamHI, which facilitated cloning into EcoRI/BamHI-digested pBluescript SK (Stratagene/Agilent). The *loz1* ORF was then released as an EcoRI/BamHI fragment from pBluescript SK and was subcloned into similar sites in the vector JK-GFP. The *loz1* promoter was PCR amplified and introduced into the KpnI and EcoRI restriction sites of this plasmid to generate pLoz1-GFP. The construction of the *S. pombe adh4-lacZ* and *SPBC1348.06c-lacZ* reporters has been described (1). To generate the *loz1-lacZ* reporter, the *loz1* promoter was amplified by using primers that contained PstI/EagI restriction sites. The PCR product was cloned following a partial digest into the PstI/EagI sites of the vector *JK-lacZ* (1). The *adh4-nmt1-lacZ* reporter was generated by using two-step overlapping PCR. The overlap PCR product was cloned as a BamHI/EagI fragment into *JK-lacZ*. All of the truncated forms of *adh4-nmt1-lacZ* reporter were generated by using *adh4-nmt1-lacZ* as a PCR template. The indicated regions of the *adh4-nmt1* hybrid promoter were PCR amplified by using primers that contained EagI/BamHI site to facilitate cloning into EagI/BamHI-digested *JK-lacZ*. The *sod1-lacZ* reporter was generated by amplifying the *sod1* promoter using primers that contained EagI/BamHI site to facilitate cloning into EagI/BamHI-digested *JK-lacZ*. The Trx-His-Loz1 fusion construct was generated by amplifying the C-terminal of Loz1 with primers that contained EagI and BamHI sites. The resulting PCR product was digested with EagI/BamHI and was cloned into similar sites in the vector Pet32a (EMD Millipore). To generate *pgk1-adh4*, the *adh4* ORF was amplified by using primers that contained XhoI and SmaI sites. The PCR product was digested with XhoI/SmaI and was cloned into similar sites in the vector Rep3x (5) to generate pAdh4. To create *pgk1-adh4*, a KpnI/XhoI fragment containing the *nmt1* promoter was released from pAdh4 and was replaced with a KpnI/XhoI *pgk1* promoter fragment. The *loz1-1* and C490G mutations were introduced into respective plasmids by using QuikChange mutagenesis (Agilent Technologies). All plasmids containing a JK148 backbone were linearized with NruI, before integration into *leu1* in yeast. All plasmid constructs were confirmed by sequence analysis.

RNA Blot and Immunoblot Analysis. Total RNA was extracted by using hot acidic phenol. For RNA blot analysis, 7–15 μg of total RNA was denatured and separated on formaldehyde gels. Probes for RNA blots were generated from purified PCR products with the MAXIScript T7 kit (Ambion) according to manufacturer's instructions. PCR primers used for probe generation are found in Table S2, or have been described (1). Total protein extracts for immunoblot analysis were obtained by using a trichloroacetic acid protein precipitation (6). Proteins were separated by using 10% (wt/vol) SDS/PAGE gels, and immunoblot analysis was performed by using standard procedures. Immunoblots were incubated with the primary antibodies anti-myc (C3956 SIGMA) and Anti Act1 (ab3280-500; Abcam), washed, and then incubated with IR-Dye800CW conjugated anti-mouse IgG (LICOR) and IRDye680 conjugated anti-rabbit IgG (LICOR). Signal intensities were analyzed by using the Odyssey Infrared image system (LICOR).

β-Galactosidase Assays and Metal Analysis. For inductively coupled plasma mass spectrometry, 10 mL of cells with an OD₆₀₀ of 1.0 were spun down, washed with 0.5 M EDTA, and twice with ddH₂O. Cells were digested by boiling in 150 μL of metal-free nitric acid for 45 min, and metal content was measured by using a Thermo Finnigan Inductively coupled mass spectrometer. β-Galactosidase assays were performed as described, and activity units were calculated as follows: (ΔA₄₂₀ × 1000)/(min × ml of culture × culture absorbance at 600 nm). Errors bars represent SDs from three independent repeats (7).

Recombinant Protein Purification. Loz1 truncates and the empty Pet32a vector were expressed in BL21(DE3) pLysS cells grown at 31 °C to an A₆₀₀ of 0.5. Expression was induced with 1 mM isopropyl β-D-thiogalactopyranoside for 5 h at 31 °C in the presence of 10 mM MgCl₂, 50 μM ZnSO₄, and 1× of a yeast drop-out amino acid mix lacking histidine, leucine, methionine, and tryptophan (US Biological D9544-20). Cells were harvested and pellets resuspended in PBS supplemented with 10 mM Imidazole and 0.1% Tween-20. Cells were lysed by sonication, and the lysate was collected by centrifugation. The lysate was purified by using Ni-NTA Superflow (Qiagen) columns preequilibrated with lysis buffer. The column was washed with lysis buffer and protein eluted with 1× PBS supplemented with 0.05% Tween-20 and 250 mM Imidazole. Protein fractions were concentrated by using Vivaspin 5000 MCWO filters and were stored in 50% (vol/vol) glycerol at –20 °C. Following concentration proteins were >99% homogeneous as determined by SDS/PAGE.

EMSA. Pairs of oligonucleotides (Table S3) were end labeled with radioactive phosphate from [γ-³²P]dATP (PerkinElmer) by using T4 Polynucleotide kinase. The pairs of oligos were annealed to form double-stranded DNA. Typically, binding reactions were performed in buffer containing a final concentration of 10 mM Hepes at pH 7.4, 1 mg/mL BSA, 50 μM ZnSO₄ and the indicated amount of poly dIdC. Protein was incubated with radiolabeled oligonucleotide pairs for 20 min at room temperature, and protein–DNA complexes were resolved on 6% (wt/vol) acrylamide Tris-Borate-EDTA gels. Gels were dried and subjected to phosphorimager analysis.

1. Ehrensberger KM, et al. (2013) Zinc-dependent regulation of the Adh1 antisense transcript in fission yeast. *J Biol Chem* 288(2):759–769.

2. Bähler J, et al. (1998) Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. *Yeast* 14(10):943–951.

- Coffman VC, Wu P, Parthun MR, Wu JQ (2011) CENP-A exceeds microtubule attachment sites in centromere clusters of both budding and fission yeast. *J Cell Biol* 195(4):563–572.
- Keeney JB, Boeke JD (1994) Efficient targeted integration at *leu1-32* and *ura4-294* in *Schizosaccharomyces pombe*. *Genetics* 136(3):849–856.
- Forsburg SL (1993) Comparison of *Schizosaccharomyces pombe* expression systems. *Nucleic Acids Res* 21(12):2955–2956.

- Peter M, Gartner A, Horecka J, Ammerer G, Herskowitz I (1993) FAR1 links the signal transduction pathway to the cell cycle machinery in yeast. *Cell* 73(4):747–760.
- Guarente L (1983) Yeast promoters and *lacZ* fusions designed to study expression of cloned genes in yeast. *Methods Enzymol* 101:181–191.

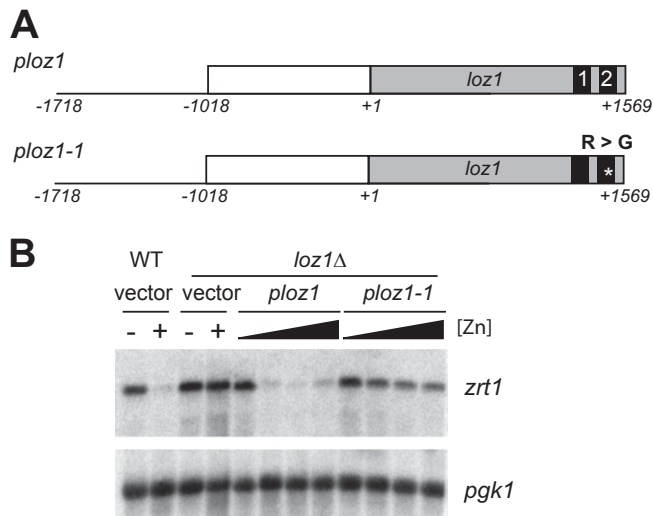


Fig. S1. The *loz1-1* mutation results in a partial loss of zinc-dependent gene expression. (A) Schematic representation of the plasmids *ploz1* and *ploz1-1*. The *loz1* ORF (gray box) and predicted 5' UTR (white box) are indicated. *ploz1-1* contains a single nucleotide substitution at position 1528 that leads to an arginine to glycine substitution in Loz1. (B) *loz1Δ* cells expressing *ploz1* or *ploz1-1* were grown were ZL-EMM supplemented with 0, 50, 200, or 500 μ M zinc before total RNA was isolated for RNA blot analysis. As controls, wild-type and *loz1Δ* cells containing the empty vector were grown in ZL EMM with no added zinc (-Zn) or with a 500 μ M zinc supplement (+Zn). RNA blots were probed for *zrt1* and *pgk1* transcripts.

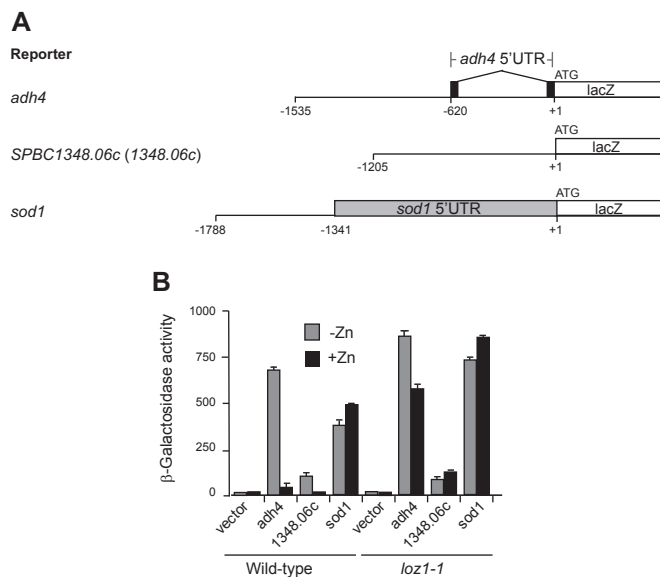


Fig. S2. *adh4-lacZ* and *SPBC1348.06c-lacZ* reporters are regulated by zinc in a manner that depends on Loz1. (A) Schematic representation of the reporter constructs. All reporters were generated by fusing the indicated promoter region, inclusive of the 5' UTR and translational ATG codon to the *lacZ* coding region. In each promoter fusion, the A from the translational ATG codon was designated +1. (B) Wild-type and *loz1-1* cells containing the above reporters genes were grown in ZL-EMM with or without a 100 μ M zinc supplement before cells were harvested, and β -galactosidase activity was measured by standard procedures.

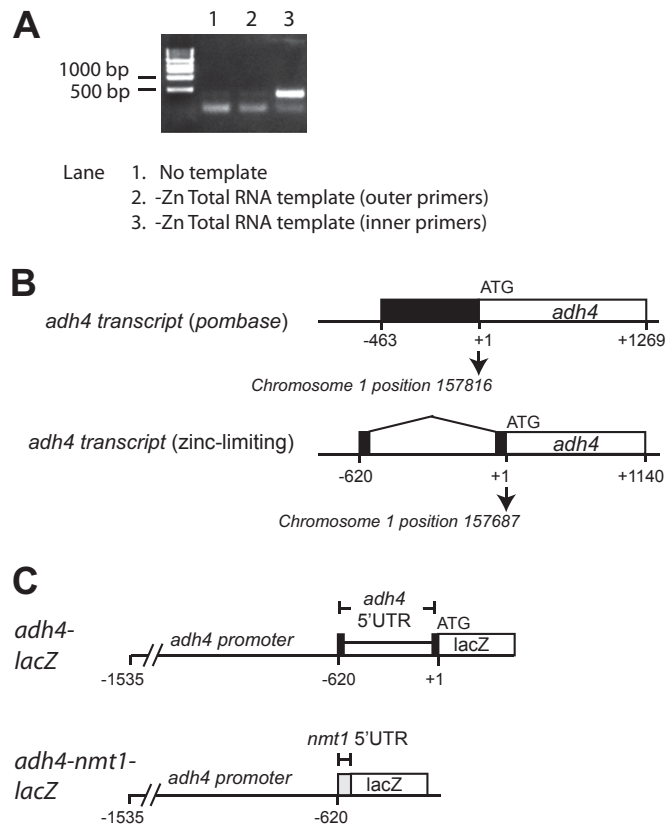


Fig. S3. The *adh4* 5' UTR is not necessary for zinc-dependent regulation of *adh4-lacZ* reporter activity. (A) To map the transcriptional start of the *adh4* transcript, an RNA ligase-mediated 5' rapid amplification of cDNA ends (RLM-RACE) was performed. The 5' RLM-RACE used total RNA purified from wild-type cells grown in ZL-EMM and a reverse primer specific to the *adh4* coding region. A nested PCR with outer primers and inner primers was performed according to manufacturer's instructions (Ambion). A PCR without template was performed as a control. Sequencing of the 5' RACE inner product revealed the presence of an unannotated exon and intron that alters the position of the transcriptional and translational start sites. (B) A schematic diagram of the *adh4* 5' UTR (black rectangles) and coding regions (white rectangles) in zinc-limited cells and the annotated *adh4* transcript described in pombase (www.pombase.org). (C) Schematic representations of *adh4-lacZ* and *adh4-nmt1-lacZ* reporter constructs. *adh4-nmt1-lacZ* contains the *adh4* promoter and *nmt1* 5' UTR.

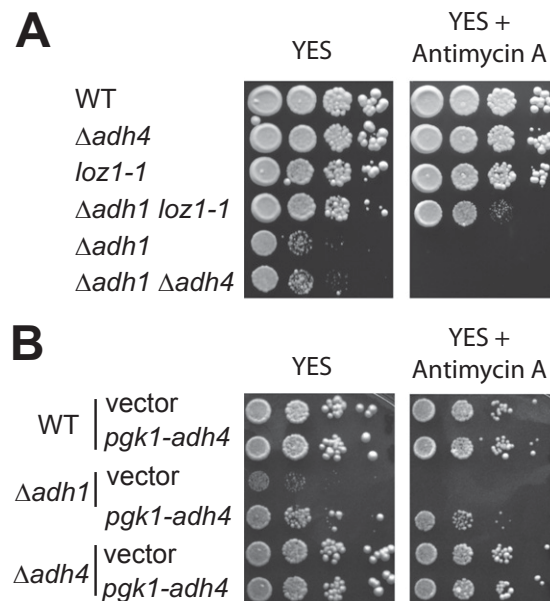


Fig. S4. Increased *adh4* expression confers a growth advantage to *adh1* Δ cells. (A) Wild-type, *adh4* Δ , *loz1-1*, *adh1 loz1-1*, *adh1* Δ , and *adh1* Δ *adh4* Δ cells were grown overnight in YES medium before cells were spotted in 10-fold serial dilutions onto YES medium with or without a 10 μ M antimycin A supplement. Plates were incubated for 3 d at 31 $^{\circ}$ C before photography. (B) Wild-type, *adh1* Δ , and *adh4* Δ cells containing the vector Rep3x or the plasmid *pgk1-adh4* were grown overnight in YES medium and were plated as described in A.

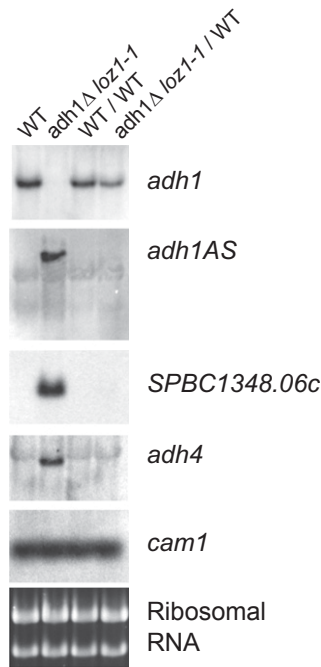


Fig. S5. *loz1-1* is a recessive mutation. Haploid wild-type and *adh1Δ loz1-1* cells, and the corresponding diploids strains obtained by mating to a wild-type strain were grown overnight in YES medium. Cells were harvested and total RNA purified for RNA blot analysis. RNA blots were probed for *adh1*, *adh1AS*, *SPBC1348.06c*, and *adh4* transcripts as described in Fig. 1. Blots were also probed for Calmodulin 1 (*cam1*) as a loading control.

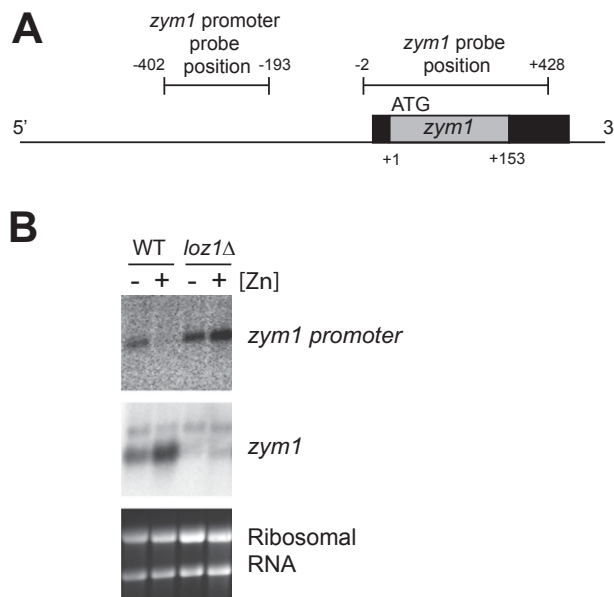


Fig. S6. A zinc-regulated intergenic transcript is generated at the *zym1* locus. (A) A diagrammatic representation of the *zym1* locus. The *zym1* ORF (gray box), UTRs (black boxes), and regions that are complementary to the *zym1* and *zym1* promoter probes have been highlighted. Numbers represent nucleotide position based on the A of the translational ATG start codon being designated +1. (B) Wild-type and *loz1Δ* cells were grown in ZL-EMM supplemented with 0 (-Zn) or 500 (+Zn) μ M zinc and total RNA purified for RNA blot analysis. RNA blots were probed with *zym1* and *zym1* promoter probes. Ribosomal RNAs are shown as a loading control.

Table S1. Linkage analysis to map the position of the *loz1-1* mutation

Chromosome no.	Gene name of mutant	Common name	Start	PDT	T	NPD	No. of tetrads
1	<i>SPAC25B8.01</i>	<i>dap1</i>	4155473	6	0	0	6
	<i>SPAC25B8.19c</i>	<i>loz1</i>	4192642	N/A	N/A	N/A	N/A
	<i>SPAC694.02</i>	—	4200185	6	0	0	6
	<i>SPAC694.04c</i>	—	4206795	8	3	0	11
	<i>SPAC1F7.11c</i>	—	4245302	11	1	0	12
	<i>SPAC2C4.09</i>	—	4276024	5	1	1	7
	<i>SPAC25G10.02</i>	<i>cce1</i>	4297129	4	3	0	7
	<i>SPAC25G10.05c</i>	<i>his1</i>	4304689	4	2	0	6
	<i>SPAC27F1.08</i>	<i>pdt1</i>	4331339	4	7	0	11
	<i>SPAC23D3.04c</i>	<i>gpd2</i>	4342487	8	8	0	16
	<i>SPAC1527.03</i>	—	4384659	5	7	1	13
2	<i>SPBC577.02</i>	<i>rpl3801</i>	754652	0	2	5	7
	<i>SPBC18E5.11c</i>	<i>edc3</i>	2094393	0	2	6	8
3	<i>SPCC162.01c</i>	—	1587869	1	7	3	11
	<i>SPCC1442.16c</i>	<i>zta1</i>	1794979	1	3	0	4
	<i>SPCC126.09</i>	—	2131761	1	7	1	9
	<i>SPAC22E12.11c</i>	<i>set3</i>	5039902	1	4	0	5

In the mapping analysis, *loz1-1* cells (strain ABY84) were crossed to the above mutant strains from the *S. pombe* deletion collection. In the deletion collection, the respective gene is replaced with the *KanMX4* cassette that confers resistance to the antibiotic G418. Following mating, diploids were plated onto malt extract. After 2 d, tetrads were dissected by using standard microdissection and individual spores were grown on YES medium at 31 °C. Mutant segregants containing the *KanMX4* cassette were identified by growth on YES medium supplemented with G418. Segregants containing the *loz1-1* allele were distinguished by the expression of *adh4* in zinc-replete medium. To measure *adh4* expression, individual spores were grown overnight in YES medium and total RNA purified for Northern blot analysis. Northern blots were hybridized with a probe specific to the *adh4* mRNA. Based on the *adh4* expression and G418 phenotypes, the numbers of tetrads that had (i) no recombination events (parental ditype or PDT); (ii) one recombination event (tetratype or T) or (iii) had a double crossover event (nonparental ditype or NPD) was determined. Because *S. pombe* has three chromosomes, mutant markers were initially chosen at different points along the length of each of the chromosomes. When data were obtained suggesting that *loz1-1* was linked to genes located on the right arm of chromosome 1, new markers were chosen in this region to confirm and refine mapping of the *loz1-1* mutation. The predominance of PDTs when ABY84 was crossed to *SPAC25B8.01* and *SPAC694.02* suggested linkage of *loz1-1* to these genes. Sequencing of all of the genes located within between these loci revealed a single nucleotide substitution in *loz1*. All mutant strains were obtained from Bioneer.

Table S2. Yeast strains used in this study

Yeast	Strain name	Genotype	Source
<i>S. pombe</i>	JW81	<i>h- ade6-M210 leu1-32 ura4-D18</i>	1
	ABY11	<i>h- ade6-M210 leu1-32 ura4-D18 psod1-lacZ::leu1+</i>	This study
	ABY46	<i>h- ade6-M210 leu1-32 ura4-D18 pJK148::leu1+</i>	2
	ABY54	<i>h- ade6-M210 leu1-32 ura4-D18 adh4Δ::ura4+</i>	This study
	ABY65	<i>h- ade6-M210 leu1-32 ura4-D18 adh4Δ::ura4+ adh1Δ::kanMX6</i>	This study
	ABY66	<i>h- ade6-M210 leu1-32 ura4-D18 adh1Δ::kanMX6, loz1-1</i>	This study
	ABY80	<i>h- ade6-M210 leu1-32 ura4-D18 padh4-lacZ::leu1+</i>	2
	ABY81	<i>h- ade6-M210 leu1-32 ura4-D18 padh4-nmt1-lacZ::leu1+</i>	This study
	ABY83	<i>h+ ade6-M210 leu1-32 ura4-D18 adh1Δ::kanMX6</i>	This study
	ABY84	<i>h- ade6-M210 leu1-32 ura4-D18 loz1-1</i>	This study
	ABY87	<i>h- ade6-M210 leu1-32 ura4-D18 zrt1Δ::kanMX6</i>	This study
	ABY98	<i>h- ade6-M210 leu1-32 ura4-D18 pSPBC1348.06c-lacZ::leu1+</i>	2
	ABY134	<i>h- ade6-M210 leu1-32 ura4-D18 loz1-1 pSPBC1348.06c-lacZ::leu1+</i>	This study
	ABY140	<i>h- ade6-M210 leu1-32 ura4-D18 loz1-1 padh4-lacZ::leu1+</i>	This study
	ABY141	<i>h- ade6-M210 leu1-32 ura4-D18 loz1-1 pJK148::leu1+</i>	This study
	ABY285	<i>h- ade6-M210 leu1-32 ura4-D18 loz1-1 psod1-lacZ::leu1+</i>	This study
	<i>loz1Δ</i>	<i>h+ ade6-M210 leu1-32 ura4-D18 loz1Δ::kanMX4</i>	Bioneer
	ABY385	<i>h+ ade6-M210 leu1-32 ura4-D18 loz1Δ::kanMX4 pJK148::leu1+</i>	This study
	ABY387	<i>h+ ade6-M210 leu1-32 ura4-D18 loz1Δ::kanMX4 psod1-lacZ::leu1+</i>	This study
	ABY393	<i>h+ ade6-M210 leu1-32 ura4-D18 loz1Δ::kanMX4 padh4::leu1+</i>	This study
	ABY395	<i>h+ ade6-M210 leu1-32 ura4-D18 loz1Δ::kanMX4 pSPBC1348.06c-lacZ::leu1+</i>	This study
	ABY425	<i>h- ade6-M210 leu1-32 ura4-D18 loz1-13xmyc::KanMX6</i>	This study
	ABY450	<i>h- ade6-M210 leu1-32 ura4-D18 loz1Δ::kanMX6 ploz1-GFP::leu1+</i>	This study
	ABY451	<i>h- ade6-M210 leu1-32 ura4-D18 loz1-1 ploz1-GFP::leu1+</i>	This study
	ABY463	<i>h- ade6-M210 leu1-32 ura4-D18 ploz1-lacZ::leu1+</i>	This study
	ABY540	<i>h- ade6-M210 leu1-32 ura4-D18 loz1Δ::kanMX6</i>	This study
	ABY583	<i>h- ade6-M210 leu1-32 ura4-D18 loz1-1 ploz1-lacZ::leu1+</i>	This study
	ABY600	<i>h- ade6-M210 leu1-32 ura4-D18 loz1Δ::kanMX6 ploz1-lacZ::leu1+</i>	This study
	ABY653	<i>h- ade6-M210 leu1-32 ura4-D18 loz1Δ::kanMX6 ploz1::leu1+</i>	This study
	ABY655	<i>h- ade6-M210 leu1-32 ura4-D18 loz1Δ::kanMX6 ploz1-1::leu1+</i>	This study
	ABY658	<i>h- ade6-M210 leu1-32 ura4-D18 padh4-1050-nmt1-lacZ::leu1+</i>	This study
	ABY659	<i>h- ade6-M210 leu1-32 ura4-D18 padh4-713-nmt1-lacZ::leu1+</i>	This study
	ABY660	<i>h- ade6-M210 leu1-32 ura4-D18 loz1Δ::kanMX6 padh4-1050-nmt1-lacZ::leu1+</i>	This study
	ABY666	<i>h- ade6-M210 leu1-32 ura4-D18 loz1Δ::kanMX6 padh4-nmt1-lacZ::leu1+</i>	This study
	ABY674	<i>h- ade6-M210 leu1-32 ura4-D18 loz1Δ::kanMX6 padh4-713-nmt1-lacZ::leu1+</i>	This study
	ABY737	<i>h- ade6-M210 leu1-32 ura4-D18 padh4-693-nmt1-lacZ::leu1+</i>	This study
	ABY739	<i>h- ade6-M210 leu1-32 ura4-D18 loz1Δ::kanMX6 padh4-693-nmt1-lacZ::leu1+</i>	This study

1. Wu JQ, Kuhn JR, Kovar DR, Pollard TD (2003) Spatial and temporal pathway for assembly and constriction of the contractile ring in fission yeast cytokinesis. *Dev Cell* 5(5):723–734.
2. Ehrensberger KM, et al. (2013) Zinc-dependent regulation of the *Adh1* antisense transcript in fission yeast. *J Biol Chem* 288(2):759–769.

Table S3. Primers used in this study

Function	Name	Sequence	
Cloning <i>S. pombe</i>	GFPF	5'-GCTTCGTACGCTGCAGGTCGAC-3'	
	GFP-R	5'-GATATCGAGCTCTGCCGGTAGAGGTGTGGTCAATAAG-3'	
	Loz1EcoRI	5'-GTCGTGAGAATTCATGAGCTCAGATAACACTCCTTC-3'	
	Loz1BamHI	5'-CCCGGGGATCCGCAAACCATGAATGCCTTGATGTC-3'	
	Loz1promEcoRI	5'-TACAATGAATTCACCTTTTAAAAACAGCAAAAGCC-3'	
	Loz1promKpnI	5'-GTGACTGGTACCACTGAACGGGAAGATGTCTCTTG-3'	
	Nmt1adh4-lacZ1	5'-CTGGGATCCATGATTAAACAAAGCGACTATAAG-3'	
	Nmt1adh4-lacZ2	5'-CAAGACGCTTCATTGAATAAGTTGAATTAATTATTTTC-3'	
	Nmt1adh4-lacZ3	5'-cttattcaaTGAGCGTCTTGAGTATGAAGAGC-3'	
	Nmt1adh4-lacZ4	5'-GATCTGCGCCGGTATGAAGTTAAGACTGTCTATGC-3'	
	Adh4-1050F	5'-GATCTGCGCCGATGAACAGCGATAATGCGAT-3'	
	Adh4-713F	5'-GATCTGCGCCGGTAAAAGAAACGGAATGTGACC-3'	
	Adh4-693F	5'-GATCTGCGCCGACCATTCATATGCTTGGAA-3'	
	Sod1-lacZF	5'-CGAACCCGGCCGTGAAGCTACGCAGTTTGG-3'	
	Sod1-lacZR	5'-CTGGATCCATTTTGTATGACGATAATTTACTTTA-3'	
	Loz1-lacZF	5'-CGGTGGCGCCGAATTCAGCATTGCCACTACCGTCG-3'	
	Loz1-lacZR	5'-CTTGGCTGCAGCTCATCACTTTTAAAAACAGCAAAAGCC-3'	
	Adh4Xhol	5'-GATCTCGAGATGTCCATTCTCGTTCTCCATTTTC-3'	
	Adh4Smal	5'-GATCTACCCGGTTACATAGCCGATTTGAAAATAG-3'	
	Protein purification and EMSAs	Loz1-T3-BamHI	5'-GGATCCGAATTCATCGGATCCGAATTCATTTCTGTATCATCACAA-3'
		Loz1-T3-EagI	5'-GGTGCTCGAGTGCAGCCGCAACCATGAATGCCTTGATG-3'
		Probe 5F	5'-TCAGCATCGACGACGATCAGCTCAAATAACGAAAAAACCTTTTGGAGA-3'
		Probe 5R	5'-TCTCAAAAAGGGTTTTTTTCGTTATTTGACGTGATCGTCGATGCTGA-3'
		Probe 5mutF	5'-TCAGCATCGACGACAATTACGTCAAATAACGAAAAAACCTTTTGGAGA-3'
Probe 5mutR		5'-TCTCAAAAAGGGTTTTTTTCGTTATTTGACGTAATTTGTCGTCGATGCTGA-3'	
Probe 8F		5'-CCGGAAGATGAACAGCGATAATGCGATTGCTTTGACCGTATATCCCT-3'	
Probe 8R		5'-AGGGGAATATACGGTCAAAGCAATCGCATTATCGTGGTTCATCTCCGG-3'	
Site-directed mutagenesis	loz1-1F	5'-CGGTTCATGTAGGCAGTAATATG-3'	
	loz1-1R	5'-CATATTACTGCCTACATTGAACGC-3'	
	loz1C470GF	5'-GTTTCGATATAGAGGTACGGAATGTTTAC-3'	
Northern probes	loz1C470GR	5'-GTAACATTCGGTACCTCTATATCGAAC-3'	
	Zrt1F	5'-GGCAGCTGGTTTAGGTGTTTCGTG-3'	
	Zrt1RT7	5'-TAATACGACTCACTATAGGGAGCACCAAAATCAAGCCATTTACC-3'	
	Vel1F	5'-GTCCGCATTGTGGTACGTATC-3'	
	Vel1RT7	5'-TAATACGACTCACTATAGGGAGCGTTGTAAATGTCAAGGGAG-3'	
	Zym1F	5'-CGATGGAACACACTACCCAATGTAAG-3'	
	Zym1RT7	5'-TAATACGACTCACTATAGGGAGcGATGATGCCCTTACTCTACGC-3'	
	Zym1promF	5'-CCTTAACAGGACCCAGAATC-3'	
	Zym1promT7	5'-TAATACGACTCACTATAGGGAGcGTTGTACACAAATCATGCTGTTG-3'	
	Adh1ASF	5'-GACTACCTCGAGATGAAGATGTGTATGAACTGAATATC-3'	
	Adh1ASRT7	5'-TAATACGACTCACTATAGGGAGGTCATTATACTACTAATGCAATGC-3'	
	Loz1F	5'-CTTATCCAGTCTCGGTTCCGCTT-3'	
	Loz1RT7	5'-TAATACGACTCACTATAGGGAGATGGCTACAAACCATGAATGC-3'	
	Strain generation	Adh4uraF	5'-ATGTCATTTCTTCGTTCTCCATTTTCGTTTAAATTCGATCGCCTGCTCGATTCTTTCCGTCACTCTTTTCATTTCCCGCCAGGGTTTTCCAGTCAACGAC-3'
		Adh4uraR	5'-CGTTCATTTACATAGCCGATTTGAAAATAGCCTTTACCTCGTCATGTGTAGGCTGAATAGGGTTAGTAGCACCACAGCGGATAACAATTTACACAGGA-3'
		Adh1F	5'-GAGAATTCATTTGTCTTGACTATCACAACTTTTAAAGTCTTTTCTTTTCTAACCACATAATGACTATTCCGGATCCCCGGGTTAATTA-3'
		Adh1R	5'-CTATAGGCATGTATTCAATAGGAATTAAGTGGATCACATTCTCATTCCCTTACTTGGAAAGGTCCAAGGAATTCGAGCTCGTTTAAAC-3'
Zrt1-1		5'-CTTTAACGTTTTTTCGTTGAAAACAACCTTGGCCCTGTTGAGTTATATCTTGTATCTTCTCGTACATTAACCGGATCCCCGGGTTAATTA-3'	
Zrt1-2		5'-CATCGAAAAAGCCACCAAAATCAAGCGAATTCGAGCTCGTTTAAAC-3'	
Zrt1-3		5'-GCTTGATTTGGTGGCTTTTTCGATG-3'	
Zrt1-4		5'-GAGACTTCTTAGTTTTGTAAGTGC-3'	
Loz1-KO-F		5'-ACTCCGAATAGAACGATCTGACTTACTTTGGTTCAGTTCAATTTTTTCATTTCTTTAGGCTTTTGCTGTTTTTAAAGTGCAGATCCCCGGGTTAATTA-3'	
Loz1-myc F		5'-TTGTCTGCGATTACGCTGGATGTGGAAGGCGTTCAATGTA CGCAGTAATATGCGCGGACATCAACGCATTATGTTTTCGCGATCCCCGGGTTAATTA-3'	
Loz1R		5'-TATCGTCAGAAGTTGATGATGCGCAATAAATGGCTTTCAAGTAACGGCTAATAATTGCTATCAGACAGGTTAAATTTGGGAATTCGAGCTCGTTTAAAC-3'	