SUPPLEMENTARY INFORMATION

Fission yeast Cactin restricts telomere transcription and elongation by promoting Rap1 pre-mRNA splicing and protein stabilization.

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FIGURE LEGENDS

Figure S1. Screening of a complete *S. pombe* deletion library for telomeric RNA regulators. (A) Representative image of a dot-blot hybridization experiment performed in 96-well format. Membranes were first hybridized to a double stranded telomeric probe, stripped and re-hybridized to a 18S rRNA probe for normalization. Each 96-well plate included two wt strains (wt^A in red and wt^B in orange), the *rap1*Δ strain (yellow; as a control for hybridization) and an empty well (blue; for background correction). The bar graph shows the quantification of the dot blot shown above. **(B)** Dot-blot experiment using total RNA from eight selected UP-TERRA mutants. The membrane was first hybridized to a double stranded telomeric probe, stripped and re-hybridized to a U6 snRNA probe for normalization. Quantifications of this and similar experiments are shown in **Figure 1A**.

Figure S2. Transcriptome analysis in *cay1*Δ cells. (A) Enrichment of transcripts in *cay1*Δ relative to wt at subtelomeric sequences (pNSU71), *Tf2-9* LTR retrotransposons, *tlh1+* gene and cen1, cen2 and cen3 centromeres. Probe positions on chromosomes and on telomeric contig-containing pNSU71 plasmid (starting at the telomere boundary) are indicated. T1, T2 and T3: subtelomeric TAS1, TAS2 and TAS3 sequences. **(B)** Scatterplot showing normalized expression profiles of *cay1*Δ (y-axis) versus wt (x-axis) on a natural log scale. Genes are represented by red dots, LTR sequences by blue dots. **(C)** Normalized expression of all exons and introns in wt and *cay1*Δ cells.

Figure S3. *cay1*Δ cells fail to silence subtelomeric reporter genes. *cay1+*, *taz1+* and *rap1+* genes were deleted in a previously established reporter strain carrying a *his3+* gene integrated approximately 300 bp away from the telomere of chromosome I and an *ade6+* gene integrated within the centromere of chromosome I. Serial dilutions of the different strains were spotted on non-selective minimal medium, medium lacking histidine or medium with 10 mg/l adenine (low adenine) and grown at 30°C. Strains with silenced centromeric *ade6+* grow in red colonies on low adenine medium. *clr4-s5*: Clr4 histone H3K9 methyltransferase mutant.

Figure S4. Effects of histone H3 levels on telomeric silencing, telomere length and cold sensitivity. (A) Western blot analysis using total protein extracts from the indicated strains and antibodies against total histone H3, H3K9ac and Act1 (loading control). Numbers at the bottom are ratios between H3K9ac and Act1 (H3K9ac/Act1), total H3 and Act1 (H3/Act1) and H3K9ac and total H3 (H3K9ac/H3). Values are expressed as fold increase over wt. **(B)** Western blot analysis using antibodies against total histone H3, Rap1 and Act1 (loading control). Numbers at the bottom are ratios between total H3 and Act1 (H3/Act1) and Rap1 and Act1 (Rap1/Act1). Values are expressed as fold

increase over wt. **(C)** Northern blot analysis of ARIA, detected with a double stranded telomeric probe, ARRET, *Tf2* retrotransposons and 18S rRNA (loading control) in the indicated strains. **(D)** Telomere length analysis of *Apa*I digested DNA from the indicated strains. **(E)** Serial dilutions of the indicated strains were spotted on complete medium and grown at 30°C and 20°C.

Figure S5. Telomeric Rap1 levels are reduced in *cay1*Δ cells. (A) Western blot analysis of strains carrying the indicated Myc-tagged proteins. Act1 was used as loading control. **(B)** Quantification of telomeric DNA isolated in ChIP experiments using strains carrying the indicated Myc-tagged proteins. Immunoprecipitated DNA is normalized to input DNA and expressed as fold increase over the tagged wt. Bars and error bars are averages and s.d. from at least 7 independent experiments. Statistical significance was assayed using the unpaired, two-tailed Student's t-test. *P < 0.05, **P < 0.01, ***P < 0.001 relative to tagged wt strain.

Figure S6. Analysis of telomeric G-overhangs, length and transcriptome in shelterin protein mutants. (A) Telomere overhang analysis using *Hind*III digested DNA hybridized under native and denaturing conditions with a telomeric C-rich oligonucleotide. Numbers are quantifications of native signals normalized to corresponding denaturing signals and expressed as fold increase over wt. *Exo*I treatment was included to assure that native signals mostly derived from G-overhangs. **(B)** Telomere length analysis using *Apa*I digested DNA from the indicated strains. **(C)** Northern blot analysis of telomeric transcriptome species in the indicated strains using strand-specific probes for TERRA, ARIA, ARRET, αARRET and 18S rRNA (loading control). TERRA is essentially undetectable in all strains. **(D)** Northern blot analysis of ARIA, detected with a double stranded telomeric probe, ARRET, *Tf2* retrotransposons and 18S rRNA (loading control) in the indicated strains.

Figure S7. Characterization of the O*trt1*Δ*cay1*Δ strain. (A) Sketch of *S. pombe* chromosomes. After *trt1+* deletion, telomeres progressively shorten leading to chromosome circularization in O*trt1*Δ. To generate the O*trt1*Δ*cay1*Δ strain, *cay1+* was deleted in O*trt1*Δ cells. C, I, L and M represent the terminal *Not*I chromosome fragments and L+I and M+C correspond to their fusion products. **(B)** PFGE analysis of chromosome circularization in the indicated strains. Genomic DNA was digested with *Not*I and hybridized to a telomeric probe and to C,I,L and M probes. Electrophoresed DNA was visualized by GelRed staining.

TABLES

Table S1. Strains identified in the screening for telomeric regulators.
List and GO term enrichment analysis of all gene deletion strains showing at least a two-fold increase or three-fold decrease in TERRA/ARIA signal over wt after normalization through 18S rRNA signal.

Table S2. Differentially expressed genes in *cay1*Δ cells.
List of all genes differentially expressed in *cay1*Δ as compared to wt (P<0.05) and GO term enrichment analysis of genes expressed > 2-fold or < 0.7-fold in *cay1*Δ as compared to wt.

Table S3. Fission yeast strains used in this study.

Table S4. Probes and oligonucleotides used in this study.