

# **Cytoophidia Influence Cell Cycle and Size in *Schizosaccharomyces pombe***

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## **Supplementary information**

### **Supplementary Tables S1, S2**

### **Supplementary Figures S1-S5**

## Supplementary Tables S1, S2

Supplementary Table S1. *S. pombe* strains.

| Fission Yeast Strain | Genotype   |
|----------------------|--|
| JLL003S              | <i>h- his-D1 ura4-D18 leu1-32 ade6-M216</i>  |
| JLL005S              | <i>h- cts1-YFP (ura4+) ura4-D18 leu1-32 ade6-M216</i>                                      |
| JLL132R              | <i>h- cts1-H359A-YFP (ura4+) ura4-D18 leu1-32 ade6-M216</i>                                |
| JLL127R              | <i>h- cts1-YFP (leu2+) ura4-D18 leu1-32 ade6-M216</i>                                      |
| JLL128R              | <i>h- cts1-H359A-YFP (leu2+) ura4-D18 leu1-32 ade6-M216</i>                                |
| JLL122R              | <i>h- cts1-YFP (ura4+) ura4-D18 leu1-32 ade6-M216/ ade6<sup>+</sup>-htb1-mCherry</i>       |
| JLL123R              | <i>h- cts1-H359A-YFP (ura4+) ura4-D18 leu1-32 ade6-M216/ ade6<sup>+</sup>-htb1-mCherry</i> |
| JLL125R              | <i>h- cts1-YFP (ura4+) ura4-D18 leu1-32 ade6-M216/ leu<sup>+</sup>-cts1-mCherry</i>        |
| JLL126R              | <i>h- cts1-YFP (ura4+) ura4-D18 leu1-32 ade6-M216/ leu1<sup>+</sup>-cts1-H359A-mCherry</i> |
| JLL129R              | <i>h- cts1-YFP (ura4+) ura4-D18 leu1-32 ade6-M216/ leu<sup>+</sup>-mCherry</i>             |
| JLL134R              | <i>h- cts1-H359A-YFP (ura4+) ura4-D18 leu1-32 ade6-M216/ ade6<sup>+</sup>-slm9-mCherry</i> |
| JLL136R              | <i>h- cts1-H359A-YFP (ura4+) ura4-D18 leu1-32 ade6-M216/ ade6<sup>+</sup>-mCherry</i>      |
| JLL135R              | <i>h- cts1-YFP (ura4+) ura4-D18 leu1-32 ade6-M216/ ade6<sup>+</sup>-mCherry</i>            |

**Supplementary Table S2. List of primers.**

| Primer Name    | Sequence (5'-3')   | Use   |
|----------------|--|---|
| Ha-Cts1-59F    | CGGCTCTCGCGGTGTCGAAGGGATGATTGC<br>CGCAGCCAAGTGGGCTCGTGAATAATAAC<br>ACCCTACTTGGGCATTTGCCTTGGTATGC | Construction of H359A strain                  |
| Ha-ura4-55R    | CTATAGATAAAGAAAAACACACCAACAAAC<br>ACACATTATTTCTAATCCCGGAATCCCATA<br>TAATTAATGCTGAGAAAGTCTTTGCTG  | Construction of H359A strain                  |
| Cts1-check-58F | GTCACCATCGTCCTCGTAGG   | Verification of H359A strain                  |
| YFP-check-59R  | GACCATGTGATCGCGCTTCT   | Verification of H359A strain                  |
| KI864          | GCAGCCGACAAGAAGTACAGCATCGGCCTG<br>GCAATCGGCACCAACTCTGTG  | pdCas9 construction (D10A mutation)           |
| KI865          | GTCGTCTTCAGAAAGCTCTGAGGCACGAT<br>TGCCTCCACATCGTAGTCGGA   | pdCas9 construction (H840A mutation)          |
| KI866          | ATCGTGCCTCAGAGCTTTCTGAA  | pdCas9 construction (vector region)           |
| KI867          | CAGGCCGATGCTGTACTTCTTGT  | pdCas9 construction (vector region)           |
| cts1 sgRNA1-F  | cacc AATTTTTCTCTCCTCCACGT  | Targeting sequence for <i>cts1</i> Knock down |
| cts1 sgRNA1-R  | aaac ACGTGGAGGAGAGAAAAATT  | Targeting sequence for <i>cts1</i> Knock down |
| cts1 sgRNA2-F  | cacc CCTCCACGTACCACCAACTT  | Targeting sequence for <i>cts1</i> Knock down |
| cts1 sgRNA2-R  | aaac AAGTTGGTGGTACGTGGAGG  | Targeting sequence for <i>cts1</i> Knock down |
| cts1 sgRNA3-F  | cacc ATATGAAGTACGTTTTAGTA  | Targeting sequence for <i>cts1</i> Knock down |
| cts1 sgRNA3-R  | aaac TACTAAAACGTACTTCATAT  | Targeting sequence for <i>cts1</i> Knock down |
| cts1 sgRNA4-F  | cacc GTATCTGGAGGTGTTATTAG  | Targeting sequence for <i>cts1</i> Knock down |
| cts1 sgRNA4-R  | aaac CTAATAACACCTCCAGATAC  | Targeting sequence for <i>cts1</i> Knock down |
| cts1 sgRNA5-F  | cacc GGTGTAATTGCCTCTTCCAC  | Targeting sequence for <i>cts1</i> Knock down |
| cts1 sgRNA5-R  | aaac GTGGAAGAGGCAATTACACC  | Targeting sequence for <i>cts1</i> Knock down |
| cas9-r1-58R    | TCAGGGCAATCAGGTTTCCG   | Verification of pdcas9 expression             |
| nmt1-pro-57F   | TCTCACTTTCTGACTTATAGTCGCT  | Verification of pdcas9                        |

|            |                       |   |
|------------|-----------------------|---|
|            |                       | expression                                      |
| sp-cst1-F  | ATGATGGCGGTGAAGTCGAT  | qPCR for <i>cts1</i><br>transcription analysis  |
| sp-cst1-R  | ATAATCACCGCGACGTTCT   | qPCR for <i>cts1</i><br>transcription analysis  |
| sp-slm9-F  | TCACCTACACAAGGGCAACC  | qPCR for <i>slm9</i><br>transcription analysis  |
| sp-slm9-R  | GTTGGGTGAGGAACAGGAGG  | qPCR for <i>slm9</i><br>transcription analysis  |
| sp-cdc25-F | ACAAGCTACCCGCCATTTGA  | qPCR for <i>cdc25</i><br>transcription analysis |
| sp-cdc25-R | AGCAACCTTGCCCATCGTAA  | qPCR for <i>cdc25</i><br>transcription analysis |
| sp-cdr1-F  | TCGAGTACGTCCCTGATGGT  | qPCR for <i>cdr1</i><br>transcription analysis  |
| sp-cdr1-R  | GCGATGACAATGCGCTACAG  | qPCR for <i>cdr1</i><br>transcription analysis  |
| sp-cdr2-F  | AGCTTTCCGGCTCACCTTTT  | qPCR for <i>cdr2</i><br>transcription analysis  |
| sp-cdr2-R  | CCGATACCAAGAAGCTGCCA  | qPCR for <i>cdr2</i><br>transcription analysis  |
| sp-wee1-F  | TTATCGACTTCCACACCCGC  | qPCR for <i>wee1</i><br>transcription analysis  |
| sp-wee1-R  | GTAAGTGGACGAGCGGTAGG  | qPCR for <i>wee1</i><br>transcription analysis  |
| sp-sty1-F  | TCTTGCGAGGGCTCAAGTTT  | qPCR for <i>sty1</i><br>transcription analysis  |
| sp-sty1-R  | GAGCCAAACCGAAATCGCAG  | qPCR for <i>sty1</i><br>transcription analysis  |
| sp-pom1-F  | AGCCGTCGTCCTTTTAGCAA  | qPCR for <i>pom1</i><br>transcription analysis  |
| sp-pom1-R  | GGCGCAGTGTTAGGTCTTCT  | qPCR for <i>pom1</i><br>transcription analysis  |
| sp-tea1-F  | GTCACGCAGCTTCTGTTGTG  | qPCR for <i>tea1</i><br>transcription analysis  |
| sp-tea1-R  | GTGAGGGAGTGAACGGAAGG  | qPCR for <i>tea1</i><br>transcription analysis  |
| sp-bl1-F   | ACGGTTCATCCATCGTTTTGT | qPCR for <i>bl1</i><br>transcription analysis   |
| sp-bl1-R   | GAGTAGACTGGGCGTTTTCTG | qPCR for <i>bl1</i><br>transcription analysis   |
| sp-fin1-F  | GAAAATGCGCCTGCTTGTGA  | qPCR for <i>fin1</i><br>transcription analysis  |

|            |                        |  |
|------------|------------------------|--|
| sp-fin1-R  | CTGTAAGGGCTGCTCCGAAT   | qPCR for <i>fin1</i><br>transcription analysis     |
| sp-pop3-F  | CAACATGCGGACTCTCAGGT   | qPCR for <i>pop3</i><br>transcription analysis     |
| sp-pop3-R  | GTTCCATCCTCGCTCGATGT   | qPCR for <i>pop3</i><br>transcription analysis     |
| sp-cdc13-F | TTCGTTTCCGACTGCTTCC    | qPCR for <i>cdc13</i><br>transcription analysis    |
| sp-cdc13-R | GAAGGGCACATCACCTCCTC   | qPCR for <i>cdc13</i><br>transcription analysis    |
| sp-tor1-F  | GATGCCGCTGTAGGTATGCT   | qPCR for <i>tor1</i><br>transcription analysis     |
| sp-tor1-R  | TGATCCCAGTCACCCAAAGC   | qPCR for <i>tor1</i><br>transcription analysis     |
| sp-tor2-F  | AACTCCAACAACCAGACGCA   | qPCR for <i>tor2</i><br>transcription analysis     |
| sp-tor2-R  | ATCCCATTACCCAAAGCGT    | qPCR for <i>tor2</i><br>transcription analysis     |
| taf10-F    | GGAAGAAGAGGTGACCGTGG   | qPCR for <i>taf10</i><br>transcription analysis    |
| taf10-R    | AACGGAAGAAATCGGGCCTT   | qPCR for <i>taf10</i><br>transcription analysis    |
| 18s rRNA-F | TGCCCCGAGCTTCTAAGTGTG  | qPCR for <i>18s rRNA</i><br>transcription analysis |
| 18s rRNA-R | ACCCCATCCTGACCCCATTA   | qPCR for <i>18s rRNA</i><br>transcription analysis |
| gpd3-F     | TGCCTACACTGCTTCCAAGG   | qPCR for <i>gpd3</i><br>transcription analysis     |
| gpd3-R     | CCCAAAACCGAACGCATGTT   | qPCR for <i>gpd3</i><br>transcription analysis     |
| nda2-F     | ACAATTACGCCCGTGGACAT   | qPCR for <i>nda2</i><br>transcription analysis     |
| nda2-R     | TGTTCAAGCGCTCCAGAAGT   | qPCR for <i>nda2</i><br>transcription analysis     |
| pda1-F     | CCCACCATAACGGTGCTACA   | qPCR for <i>pda1</i><br>transcription analysis     |
| pda2-R     | AGCAAACAAGTCAATCAAGCGT | qPCR for <i>pda1</i><br>transcription analysis     |
| tdh1-F     | GTGTCGTTGACTTGTTGCC    | qPCR for <i>tdh1</i><br>transcription analysis     |
| tdh1-R     | CACCCACCCGACGTTTCTTA   | qPCR for <i>tdh1</i><br>transcription analysis     |
| actin-F    | GGTCAAGATTGTTGCTCCTC   | qPCR for <i>actin</i>                              |

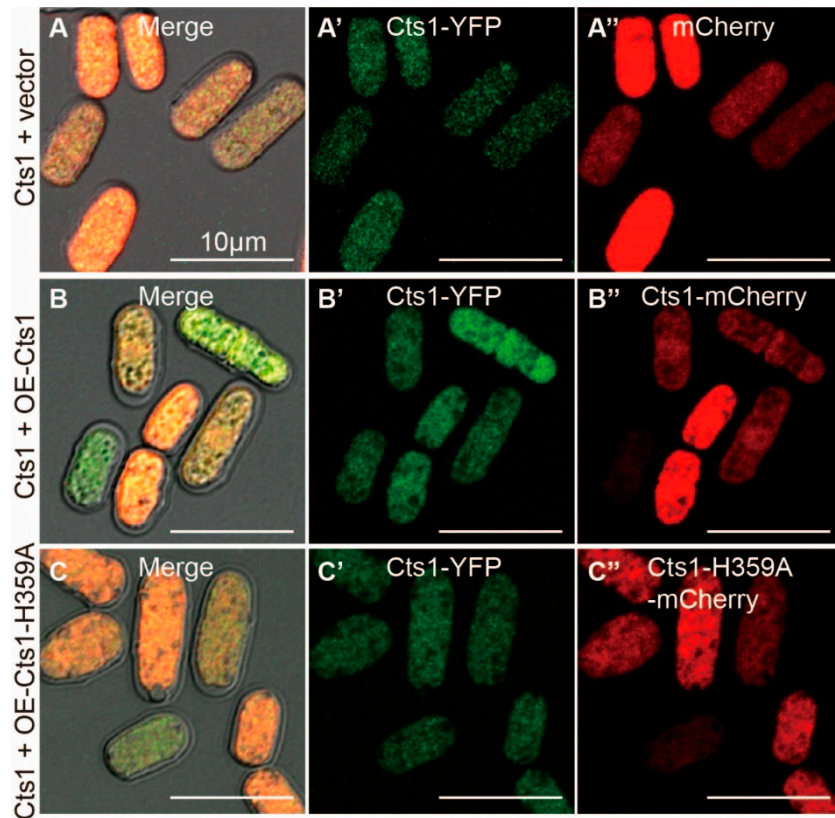
|         |                      |   |
|---------|----------------------|---|
|         |                      | transcription analysis                          |
| actin-R | CGCTCTCATCATACTCTTGC | qPCR for <i>actin</i><br>transcription analysis |

## Supplementary Figures S1-S4



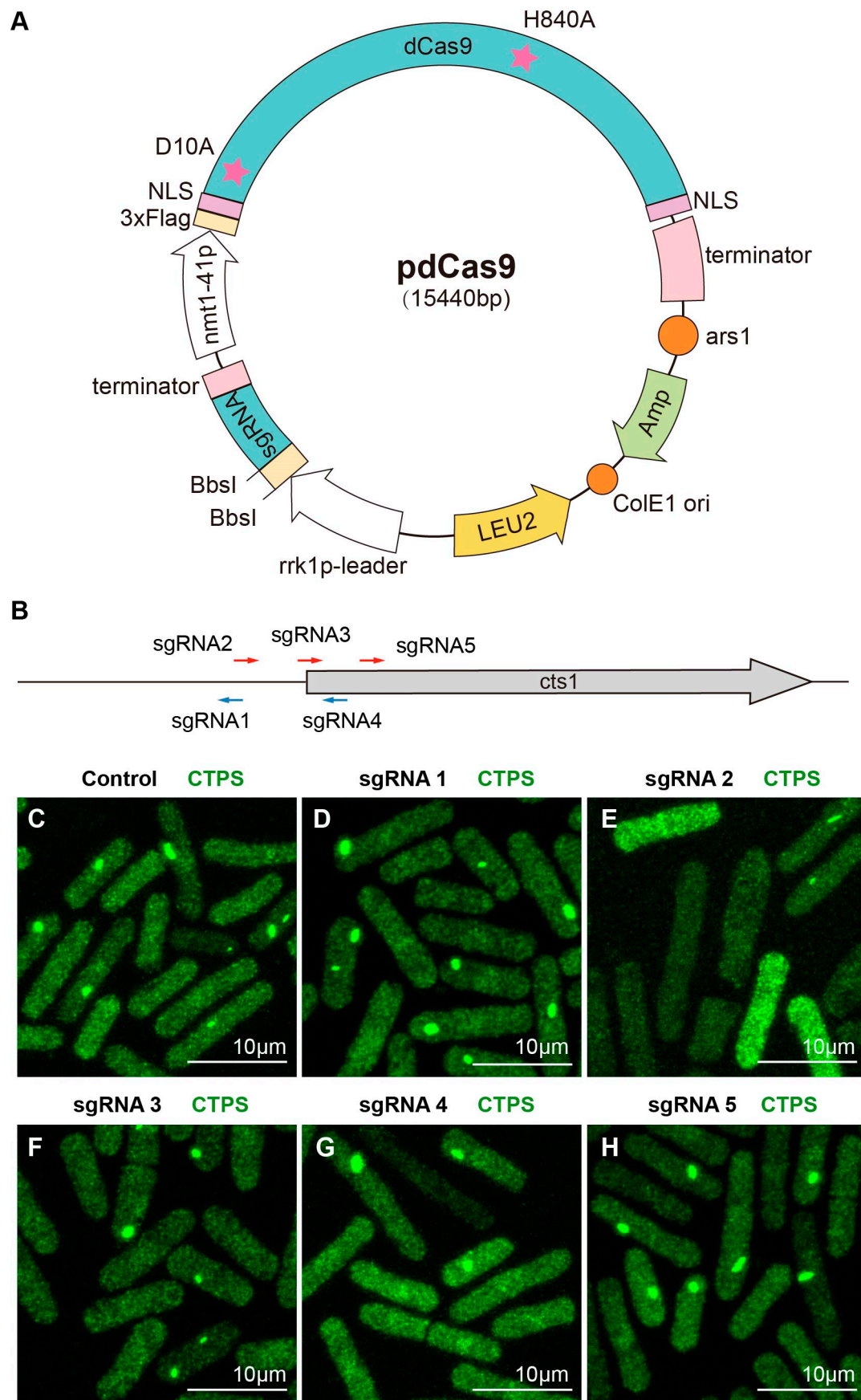
**Supplemental Figure S1. Sequencing analysis.**

Sequencing analysis of genomic DNA to confirm CTPS H359A mutation in Cts1-H359A strain.

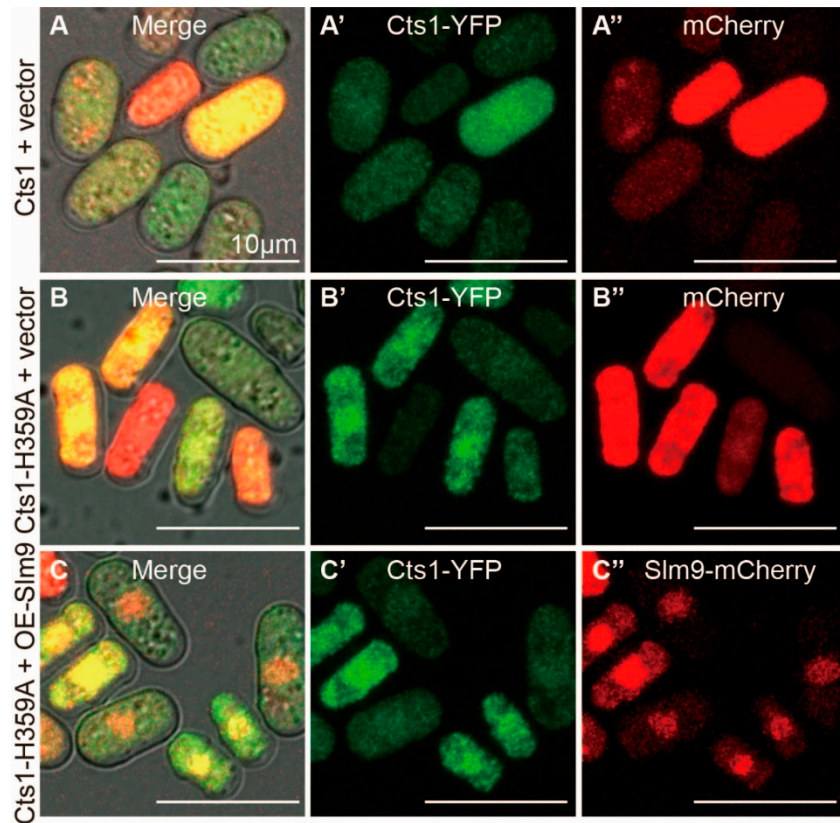


**Supplemental Figure S2. Cytophidia do not form at stationary phase in various fission yeast strains. (A-C'')** Photos of Cts1 strains that overexpress mCherry (A-A''), CTPS-mCherry (OE-Cts1, B-B''), and Cts1-H359A-mCherry (OE-Cts1-H359A, C-C'') at the stationary phase. Channels: green (CTPS), red (mCherry) and bright field. Scale bars, 10  $\mu$ m.

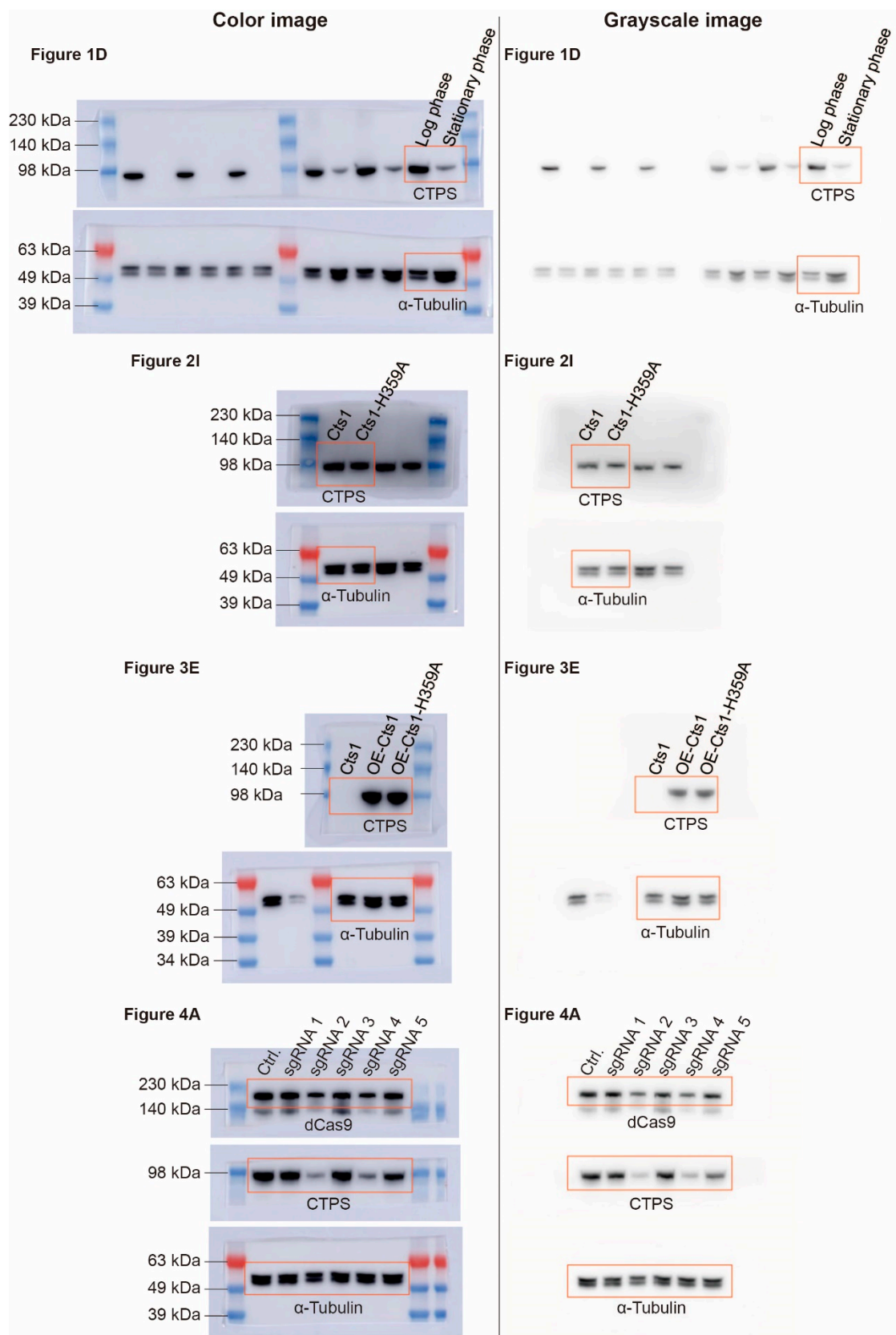




**Supplemental Figure S3. knockdown the CTPS by using the CRISPR-dCas9 system.** (A) Plasmid map of pdCas9. dCas9, humanized dCas9, originally from *Streptococcus pyogenes*. The pentagram indicated mutations introduced in the dCas9 gene to cause the indicated amino acid substitutions. nmt1-41p, a moderate-strength variant of the nmt1 promoter, the transcription of which is inducible. When the targeting sequence is not replaced, pdCas9 expresses a sgRNA with a nonsense targeting sequence in fission yeast. (B) Diagram of the designed targeting sequences with the *cts1* gene. Red and blue arrows indicate targeting sequences of sgRNAs that base-pair with the template and non-template strand of the targeted DNA, respectively. (C) Photographs of the CTPS-YFP strains which expressed the pdCas9 plasmid carried nonsense sgRNA (as a control group), sgRNA1, sgRNAs2, sgRAN3, sgRAN4, sgRAN5, respectively, at log phase. Scale bars, 10  $\mu$ m.



**Supplemental Figure S4. Overexpression of *slm9* in Cts1-H359A strain.** (A-A'') Photographs of Cts1 strain that overexpressed only mCherry at the stationary phase. Scale bars, 10  $\mu$ m. (B-B'') Photographs of Cts1-H359A strain that overexpressed only mCherry at the stationary phase. Scale bars, 10  $\mu$ m. (C-C'') Photographs of Cts1-H359A strain that overexpressed Slm9-mCherry at the stationary phase. Scale bars, 10  $\mu$ m. Channels: green (CTPS), red (mCherry) and bright field.



**Supplemental Figure S5. Uncropped original Western Blotting images in figure 1D, 2I, 3E, 4A.**