A
B


| Thiamine ( $\mu \mathrm{M}$ ) | 20 | 0 | 20 | 0 | 20 | 0 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Targeting seq. | - | nonsense | $a 4$ |  |  |  |
| pSPdCas9 | - | + | + |  |  |  |



| Thiamine ( $\mu \mathrm{M}$ ) | 20 | 0 | 20 | 0 | 20 | 0 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Targeting seq. | - | nonsense | $a 4$ |  |  |  |
| pSPdCas9 | - | + | + |  |  |  |

Figure S1. mRNA levels of the $d$ Cas 9 gene in the presence or absence of thiamine with parallel assessment of adeb $^{+}$mRNA levels.
Fission yeast strains carrying indicated plasmids were grown with or without $20 \mu \mathrm{M}$ thiamine. Results of parallel assessments of ade $6^{+}$and dCas 9 mRNA levels by RT-qPCR are shown. (A) mRNA levels of the ade6 ${ }^{+}$gene. (B) mRNA levels of the $d$ Cas 9 gene. Values relative to the mean of the $d \operatorname{Cas} 9 \mathrm{mRNA}$ level measured on the nonsense control without thiamine are shown as percentages. The chart is in logarithm scale. (A, B) circles indicate measured values from two biological replicates. Black lines indicate means of the two biological replicates. A wild-type fission yeast strain $972\left(h^{-}\right)$was used for the no-plasmid control. These data are independent of those shown in the other figures.


Figure S2. Phenotype tests of fission yeast cells subjected to ura4 $^{+}$gene repression by dCas9-mediated CRISPRi.
Serial dilution ( 10 -fold) spot test growth assays of fission yeast cells bearing pSPdCas9 plasmids with the indicated targeting sequences on differentially supplemented media as shown. The ura4 mutant (strain sp168, $h^{-}$ura4) is defective in uracil biogenesis. Cells were spotted after induction of dCas9-mediated CRISPRi (see the Materials and Methods section for details).


Figure S3. Transcriptional repression of the his2 ${ }^{+}$or $\boldsymbol{\text { his }} 7^{+}$gene by dCas9-mediated CRISPRi.
Quantification of his $2^{+}(\mathrm{A})$ or $h i s 7^{+}(\mathrm{B})$ mRNA by RT-qPCR with sgRNAs that have the indicated targeting sequence. (A, B) Results of two biological replicates are shown. Diagrams are labeled as Figure 2A. A white triangle indicates 100 bp from the TSS. (C, D) Serial dilution (10-fold) spot test growth assays of fission yeast cells bearing pSPdCas9 plasmids with the indicated targeting sequences on differentially supplemented media as shown. The his5-303 mutant (strain sp152, $h^{+}$his5-303) is defective in histidine biogenesis. Cells were spotted after induction of CRISPRi (see the Materials and Methods section for details).

