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## Supplemental Information

# Live-Cell Imaging of Chromatin Condensation Dynamics by CRISPR

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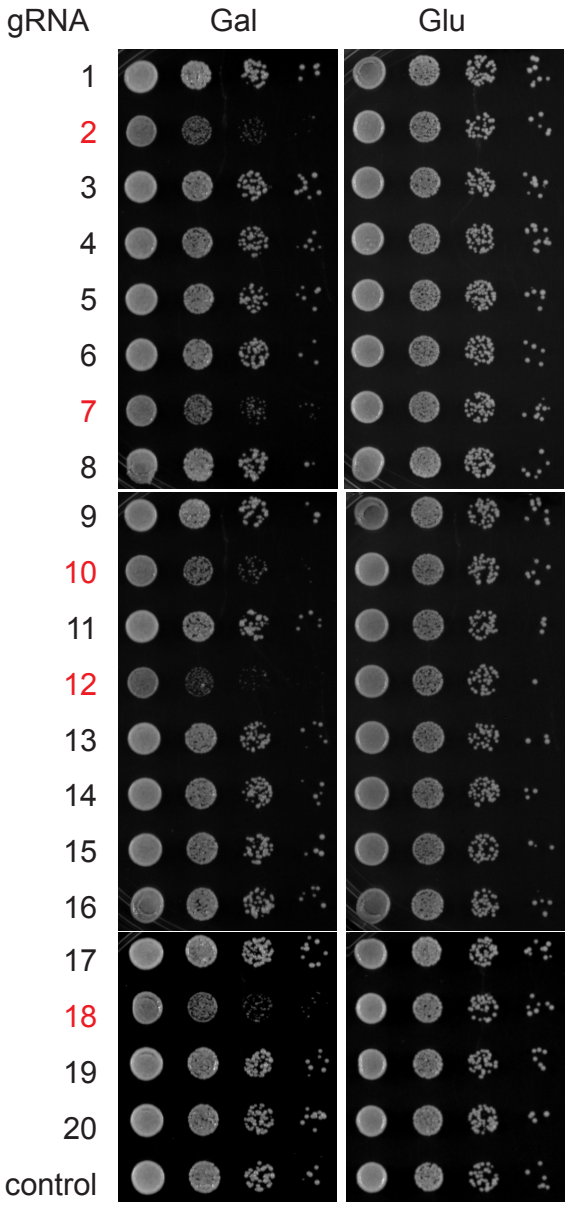


**Figure S1. Growth test of yeast cells overexpressing individual rDNA-targeting gRNAs. Related to Figure 1.**

Yeast cells were integrated with pGalL-dCas9-GFP and transformed with individual rDNA-targeting gRNAs on 2μ plasmids. A non-targeting gRNA plasmid was transformed as a control. Cells were serially diluted and spotted on plates supplemented with 2% glucose or galactose. Black: gRNAs binding to the template strand of the 18S gene. Red: gRNAs binding to the non-template strand.

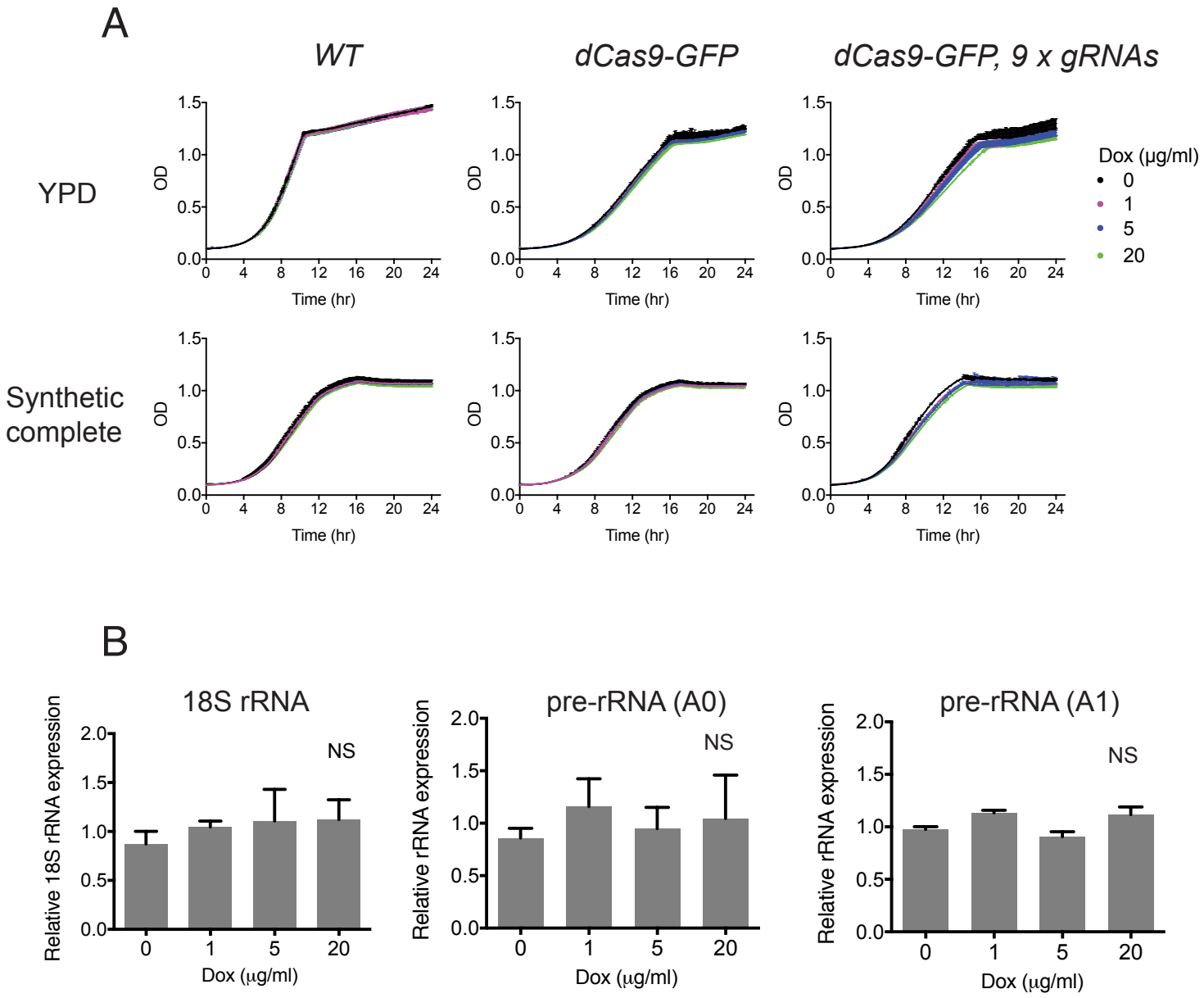


**Figure S2. Absence of major effects on cell growth and rRNA expression in cells co-expressing dCas9-GFP and 9x gRNAs. Related to Figure 1.**

(A) Growth curves of indicated strains. Cells were grown to log phase in YPD or synthetic complete media, diluted to OD=0.1, and incubated at 30°C with shaking in a BioTek microplate reader. OD600 values were measured every 3 min for 24 hrs. n = 2, error bars, mean ± s.e.m. (B) Quantitative RT-PCR analysis of 18S rRNA transcript levels and pre-rRNA levels. RNA was extracted from early-log phase cells grown in synthetic complete media with 2% glucose. For pre-rRNA analysis, qPCR primers were designed to span the A0 or A1 cleavage sites on the 5'ETS. Data was normalized to the expression level of the ALG9 control. n = 3, error bars, mean ± s.e.m. NS, not significant (one-way ANOVA, p>0.05).

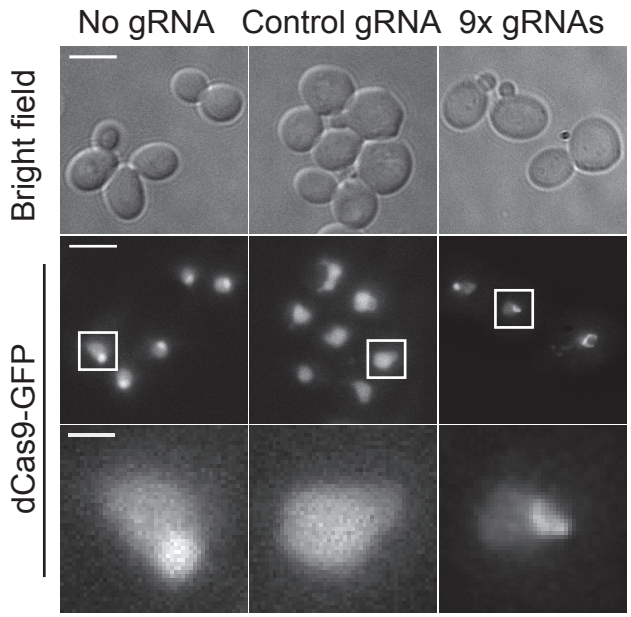


**Figure S3. CRISPR imaging of the rDNA chromatin using pGalL-dCas9-GFP. Related to Figure 1.** pGalL-dCas9-GFP and nine gRNAs that target the 18S rDNA locus were stably integrated into the genome. Cells were cultured in synthetic complete media supplemented with 2% galactose for 16hrs to induce dCas9-GFP expression. Deconvolved, maximum projected z series are shown. Scale bar, 5 μm (overview) or 1 μm (inset).

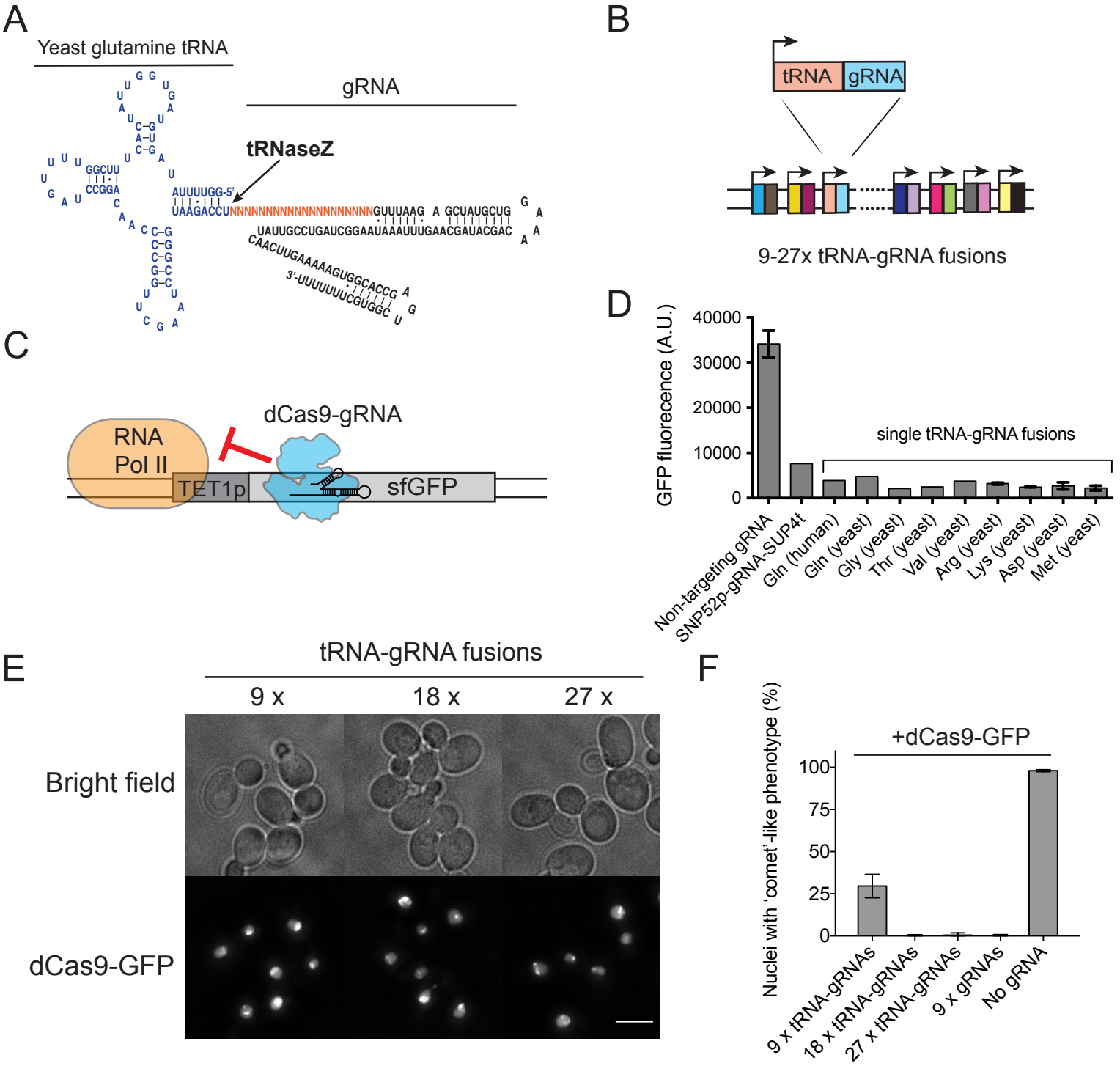


**Figure S4. Alternative tRNA-gRNA fusion system for CRISPR imaging. Related to Figure 1.**
(A) Schematic of a yeast glutamine tRNA-gRNA fusion. tRNA (blue), target base pairing region (orange) and structural gRNA sequence (black) are shown. The predicted tRNase Z cleavage site is indicated. (B) The tRNA-gRNA expression system. 9x tRNAs of the yeast and human origin were fused with individual gRNAs. The resulting tRNA-gRNA fusions were assembled by two-rounds of goldengate assembly to obtain the 9x, 18x, and 27x tRNA-gRNA plasmids. The resulting plasmids were then integrated into the genome. (C) Design of a CRISPR-silencing reporter assay for testing the functionality of tRNA-gRNA fusions. A super-folder GFP (sfGFP) reporter was placed under a TEF1 promoter and integrated into the genome. A single gRNA was designed to target the non-template strand of the sfGFP open reading frame at position 87-110nt to block RNA pol II activity. sfGFP expression should be silenced in the presence of dCas9 and functional gRNAs. (D) Functional test of individual tRNA-gRNA fusions by the assay described in (C). All strains were integrated with GalLp-dCas9 and transformed with 2μ plasmids expressing either control gRNAs or tRNA-gRNA fusions. Cells were cultured in 2% galactose media overnight to induce dCas9 expression, and analyzed for sfGFP fluorescence by FACS. n = 3, error bars, mean ± SD. (E) CRISPR imaging of the rDNA gene with increasing number of tRNA-gRNA fusions. gRNAs were designed to target the 18S and 25S rDNA on the template strand. (F) Fraction of cells showing 'comet'-like dCas9 binding pattern in indicated strains. n = 3, error bars, mean ± SD.

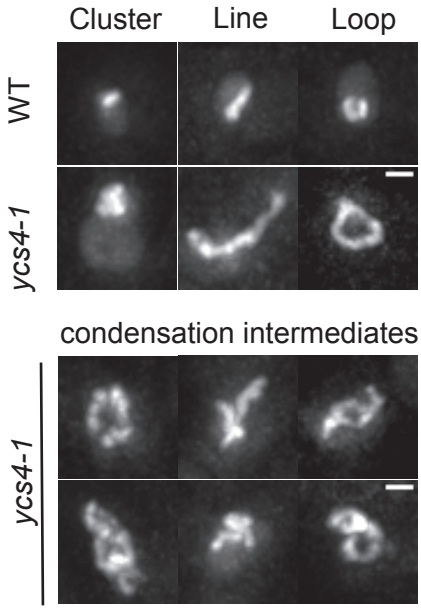


**Figure S5. rDNA morphologies during condensation in WT versus ycs4-1 cells grown in YPD media. Related to Figure 4.**

Cells were grown overnight in YPD media at 25°C until early-log phase, then were shifted to 37°C for 3hrs. Glucose was removed at time 0 and samples were collected after 30min incubation at 37°C. Prior to live-cell imaging, YPD media was replaced with synthetic complete media (glucose-free) to reduce background for better imaging. Scale bar, 1 μm.

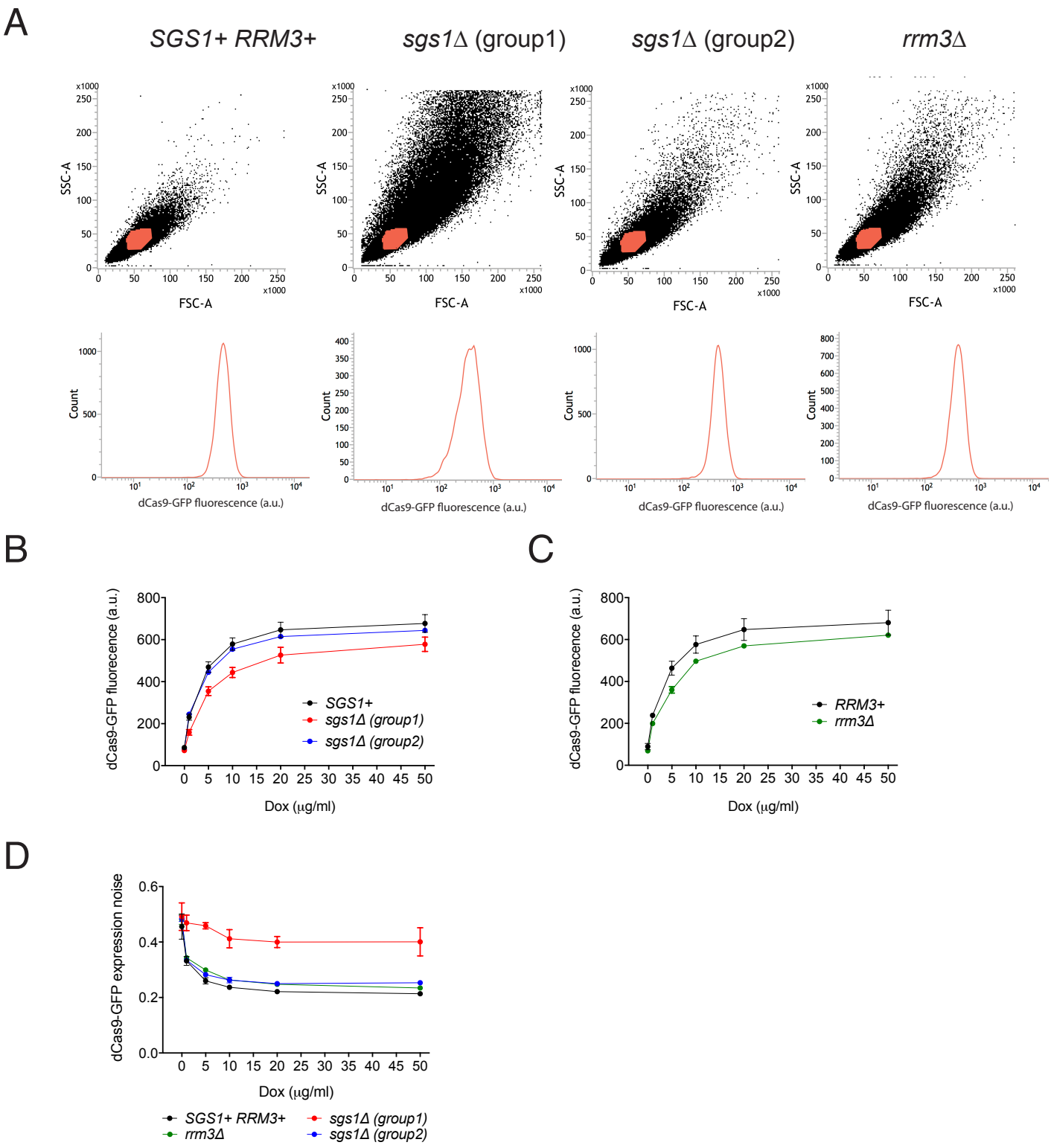


**Figure S6. FACS analysis of the *sgs1Δ* and *rrm3Δ* mutants. Related to Figure 7.**
(A) FACS analysis of the size, granularity, and dCas9-GFP fluorescence levels in indicated strains. All strains were stably integrated with pTet-dCas9-GFP and 9x gRNAs. Cells were grown in synthetic complete media supplemented with 5 μg/ml doxycycline for 16hrs until OD reached 0.1-0.2. 10,000 cells were collected from the red polygon gate area and their fluorescence was plotted as histograms (BD FACSDiva software) and displayed below the corresponding scatter plot. FSC: Forward SCatter, SSC: Side SCatter. (B-C) Mean dCas9-GFP fluorescence levels in *sgs1Δ* (B) and *rrm3Δ* (C) mutants. Cells were induced with 0-50 μg/ml doxycycline in synthetic complete media for 16hrs. FACS experiments were performed as in (A). Data are presented as mean ± SD (n = 3). (D) Noise in dCas9-GFP expression in *sgs1Δ* and *rrm3Δ* mutants. Noise was calculated as standard deviation divided by the mean (n = 3, error bars, mean ± SD).

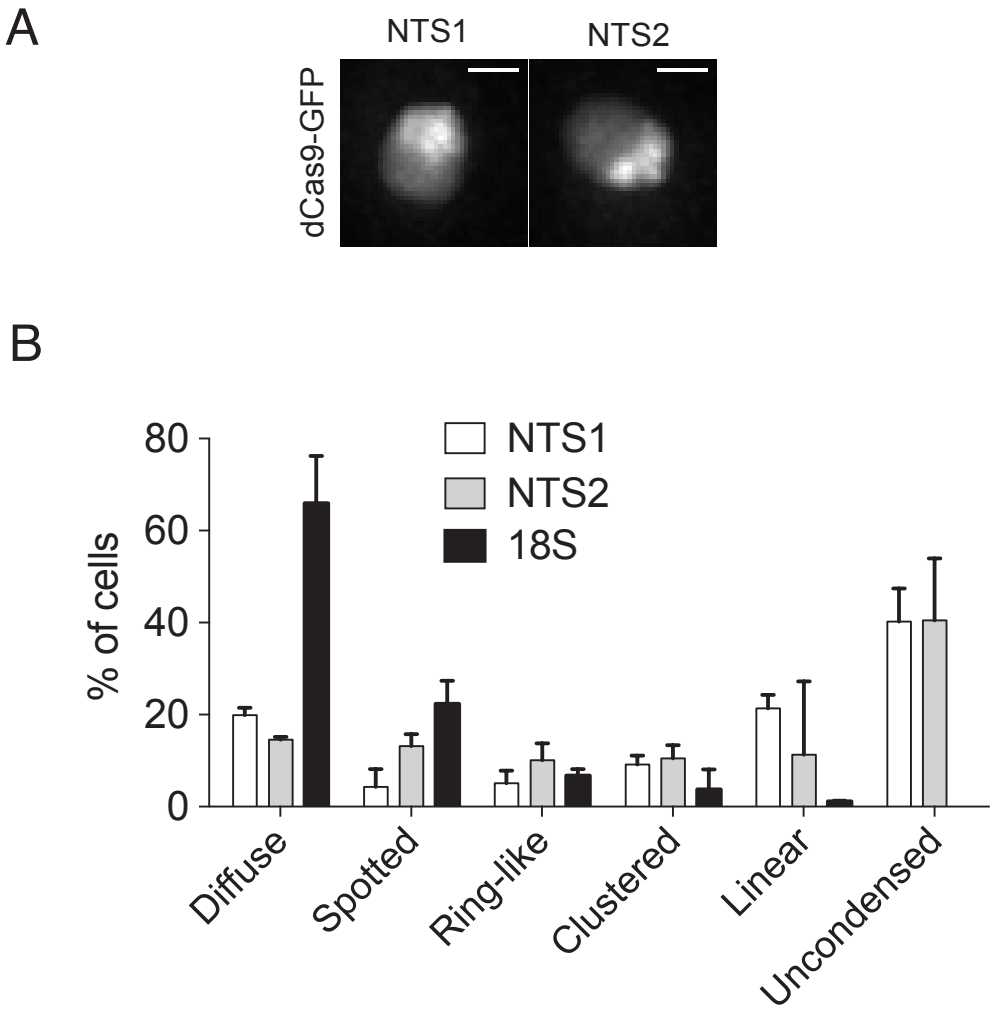


**Figure S7. rDNA phenotypic classes in cells expressing dCas9-GFP and gRNAs targeting non-transcribed spacer regions. Related to Figure 1.**
(A) Uncondensed rDNA in dCas9-GFP cells expressing NTS1- or NTS2- targeting gRNAs. Cells were grown to $OD_{600}$=~0.2. Scale bar, 1 μm. (B) Distribution of rDNA phenotypic classes in dCas9-GFP cells expressing gRNAs targeting the non-transcribed spacers NTS1 or NTS2 versus the 18S rDNA. n = 3, error bars, mean ± s.e.m. All strains were grown to similar densities ($OD_{600}$=~0.2).

| Plasmid | Source |
|---|---|
| pRS415-pTet-dCas9-EGFP-CENΔARSΔ | this study |
| pRS415-pGalL-dCas9-EGFP-CENΔARSΔ | this study |
| pRS423-SNP52-SUP4t gRNA recipient plasmid | this study |
| pRS423-control gRNA-HIS3 | this study |
| YIp9 x gRNA-HIS3 | this study |
| YIp9 x tRNA-gRNA fusion-HIS3 | this study |
| YIp18 x tRNA-gRNA fusion-HIS3 | this study |
| YIp27x tRNA-gRNA fusion-HIS3 | this study |
| ptetO2-YFP-CYCt-spHIS5 | Murat Acar (Alias Y23) |
| pRS306-pMyo2-rtTA | Thomas Young (Alias pTY18a) |

**Table S1. Plasmids used in this study. Related to Figure 1.**

| gRNA | Target | gRNA spacer sequence | PAM |
|---|---|---|---|
| gRNA1 | 18S | GCTTAAAATCTCGACCCTT | TGG |
| gRNA2 | 18S | GAATAAGGGTTCGATTCCGGAGA | GGG |
| gRNA3 | 18S | GCAAATTACCCAATCCTAATTCA | GGG |
| gRNA4 | 18S | GCAAGTCTGGTGCCAGCAGCCG | CGG |
| gRNA5 | 18S | GTAGTTGAACTTTGGGCCCGGT | TGG |
| gRNA6 | 18S | GAATAATAGAATAGGACGTT | TGG |
| gRNA7 | 18S | GGGCATCAGTATTCAATTGTCAG | AGG |
| gRNA8 | 18S | GTCTTAACCATAAACTATGCCGACTA | GGG |
| gRNA9 | 18S | GATTTTGTGGGTGGTGGTGCA | TGG |
| gRNA10 | 18S | GCTGGTTATCCACTTCTTAGA | GGG |
| gRNA11 | 18S | GCCGCACGCGCGCTACACTGA | CGG |
| gRNA12 | 18S | GTGAAACTCCGTCGTGCTG | GGG |
| gRNA13 | 18S | GCCCGTCGCTAGTACCGATTGAA | TGG |
| gRNA14 | 18S | GTTTCCGTAGGTGAACCTGCGGA | AGG |
| gRNA15 | 18S | GCATGGCCTTGTGCTGGCGA | TGG |
| gRNA16 | 18S | GTCCTTGTGGCTCTTGGCGAACC | AGG |
| gRNA17 | 18S | **G**CTACTGCGAAAGCATTTGCCA | AGG |
| gRNA18 | 18S | GACGGAAGGGCACCACCAGGAG | TGG |
| gRNA19 | 25S | GTTCCTGCCGAAGTTTCCCTC | AGG |
| gRNA20 | 25S | GCGAATGATTAGAGGTTCCG | GGG |
| gRNA21 | 25S | GGTAAGCAGAACTGGCGATG | CGG |
| gRNA22 | 25S | **G**AGACAGCCGGACGGTGGCCA | TGG |
| gRNA23 | 25S | GCCCTGACGAGTAGGCAGGCG | TGG |
| gRNA24 | 25S | GGCCTCTAGTGCAGATCTTGG | TGG |
| gRNA25 | 25S | **G**CGTCAACAGCAGTTGGACGT | GGG |
| gRNA26 | 25S | GGCCACCATCGAAAGGGAATC | CGG |
| gRNA27 | 25S | GGAGACGTCGGCGCGAGCCCT | GGG |
| Control gRNA | None-targeting | GATCTCGAGCTCGATATCGGATCCATT | N/A |
| sfGFP silencing gRNA | sfGFP | GCATCACCTTCACCCTCTCCA | CGG |

**Table S2. gRNAs designed for this study. Related to Figure 1.**

Only gRNA spacer sequences are shown; the structural gRNA component is described in the Result section. G in bold at the beginning of some gRNA sequences were additionally added to facilitate gRNA transcription from the SNR52 promoters.

| tRNA Promoter | Sequence |
|---|---|
| Human Gln | GGTTCCATGGTGTAATGGTTAGCACTCTGGACTCTGAATCCA GCGATCCGAGTTCAAATCTCGGTGGAACC |
| Yeast Gln | GGTTTTATAGTGTAGTGGTTATCACTTTCGGTTTTGATCCGGACAACCCCGGTTCGAATCCGGGTAAGACCT |
| Yeast Gly | GCGCAAGTGGTTTAGTGGTAAAATCCAACGTTGCCATCGTTGGGCCCCCGGTTCGATTCCGGGCTTGCGCA |
| Yeast Thr | GCTTCTATGGCCAAGTTGGTAAGGCGCCACACTAGTAATGTGGAGATCATCGGTTCAAATCCGATTGGAAGCA |
| Yeast Val | GGTTTCGTGGTCTAGTCGGTTATGGCATCTGCTTAACACGCAGAACGTCCCCAGTTCGATCCTGGGCGAAATCA |
| Yeast Arg | GCTCGCGTGGCGTAATGGCAACGCGTCTGACTTCTAATCAGAAGATTATGGGTTCGACCCCCATCGTGAGTG |
| Yeast Lys | GCCTTGTTGGCGCAATCGGTAGCGCGTATGACTCTTAATCATAAGGTTAGGGGTTCGAGCCCCCTACAGGGCT |
| Yeast Asp | TCCGTGATAGTTTAATGGTCAGAATGGGCGCTTGTCGCGTGCCAGATCGGGGTTCAATTCCCCGTCGCGGAG |
| Yeast Met | GCTTCAGTAGCTCAGTAGGAAGAGCGTCAGTCTCATAATCTGAAGGTCGAGAGTTCGAACCTCCCCTGGAGCA |

**Table S3. tRNA promoters used in this study. Related to Figure 1.**

| Strains | Genotype |
|---|---|
| YYX 103 | *Matα, pGalL-dCas9-EGFP::LEU2* |
| YYX 252 | *Matα, pTet-dCas9-EGFP::LEU2, rTTA::URA3* |
| YYX 281 | *Matα, pTet-dCas9-EGFP::LEU2, rTTA::URA3, 9 x gRNA::HIS3* |
| YYX 257 | *Matα, pTet-dCas9-EGFP::LEU2, rTTA::URA3, 9 x gRNA::HIS3, Net1-mRFPmars::natNT2* |
| YYX 404 | *Matα, pTet-dCas9-EGFP::LEU2, rTTA::URA3, control gRNA-2μ::HIS3* |
| YYX 334 | *Matα, pTet-dCas9-EGFP::LEU2, rTTA::URA3, 9 x tRNA-gRNA::HIS3* |
| YYX 382 | *Matα, pTet-dCas9-EGFP::LEU2, rTTA::URA3, 18 x tRNA-gRNA::HIS3* |
| YYX 383 | *Matα, pTet-dCas9-EGFP::LEU2, rTTA::URA3, 27 x tRNA-gRNA::HIS3* |
| YYX 390 | *Matα, pTet-dCas9-EGFP::LEU2, rTTA::URA3, sgs1::hphNT1* |
| YYX 387 | *Matα, pTet-dCas9-EGFP::LEU2, rTTA::URA3, 9 x gRNA::HIS3, rrm3::hphNT1* |
| YYX 393, YYX 394, YYX 396 | *Matα, pTet-dCas9-EGFP::LEU2, rTTA::URA3, 9 x gRNA::HIS3, sgs1::hphNT1* (group1) |
| YYX 399, YYX 400, YYX 403 | *Matα, pTet-dCas9-EGFP::LEU2, rTTA::URA3, 9 x gRNA::HIS3, sgs1::hphNT1* (group2) |
| YYX 458 | *Mata, pTet-dCas9-EGFP::LEU2, rTTA::URA3, 9 x gRNA::HIS3, ycg1-2::kanMX* |
| YYX 459 | *Mata, pTet-dCas9-EGFP::LEU2, rTTA::URA3, 9 x gRNA::HIS3, ycs4-1::kanMX* |
| YYX 460 | *Matα, pTet-dCas9-EGFP::LEU2, rTTA::URA3, 9 x gRNA::HIS3, mcd1::kanMX, mcd1-1::TRP1* |
| YYX 461 | *Matα, pTet-dCas9-EGFP::LEU2, rTTA::URA3, 9 x gRNA::HIS3, top2::kanMX, top2-4::TRP1* |
| YYX 462 | *Matα, pTet-dCas9-EGFP::LEU2, rTTA::URA3, 9 x gRNA::HIS3, top1::hphNT1* |

**Table S4. Yeast strains constructed for this study. Related to Figures 1-7.**

# Transparent Methods

## EXPERIMENTAL PROCEDURES

### Yeast strains and plasmids

All yeast strains are in S288C background (Supplementary Table 4). Plasmids, guide RNAs, tRNA-gRNA fusions generated for this study are listed in Supplementary Table 1-3. pRS415-pGalL-dCas9-EGFP-CENΔARSΔ plasmid was constructed using Gibson assembly (NEB) based on plasmids pSLQ1658-dCas9-EGFP (a gift from Bo Huang and Stanley Qi, Addgene plasmid #51023 (Chen *et al.*, 2013)) and p415-GalL-Cas9-CYC1t (a gift from George Church, Addgene plasmid #43804 (Dicarlo *et al.*, 2013)). The yeast centromere (CEN) and autonomously replicating sequence (ARS) were removed from this plasmid to convert it into a yeast integrating plasmid. pRS415-pTet-dCas9-EGFP-CENΔARSΔ plasmid was constructed by swapping the GalL promoter with a Tet promoter obtained from plasmid ptetO2-YFP-CYCt-spHIS5. The resulting plasmid was then linearized at the HpaI site, and integrated as a single copy in yeast cells at the *LEU2* locus using lithium acetate (LiAc) transformation. rtTA was amplified by PCR with a *URA3* marker from the plasmid pRS306-pMyo2-rtTA, and integrated at the *TRP1* locus by homologous recombination. The gRNA donor plasmid was constructed based on pRS423. Individual gRNAs were cloned between the SNR52 promoter and the SUP4 terminator in the donor plasmid at the BamH1 restriction site using Gibson assembly. Nine gRNAs were amplified by PCR from each donor plasmid using primers carrying 4bp complimentary overhangs, purified, and assembled into a receptor plasmid by goldengate assembly (Engler *et al.*, 2009). ~500ng of the assembled 9x gRNAs plasmid was linearized at the NdeI site and integrated into yeast cells at the *HIS3* locus. qPCR was performed to ensure only one copy of the plasmid was integrated. To generate tRNA-gRNA fusions, nine different tRNA promoters along with the structural gRNA components were synthesized as gBlocks (IDT) and cloned into pRS423 plasmids. These plasmids were cut at the internal BpiI sites where individual gRNAs were inserted. The resulting 9 donor plasmids (Level 0 plasmids) were assembled into a Level 1 recipient plasmid by goldengate method using BsaI digestion. Three groups of 9x tRNA-gRNA assemblies were generated this way, and further assembled into Level 2 recipient plasmids to obtain 9x, 18x, and 27x tRNA-gRNA fusion plasmids. The obtained plasmids were linearized with NdeI and integrated at the *HIS3* locus by LiAc transformation. To construct the temperature sensitive *mcd1-1 and top2-4* mutants, we introduced these mutations on genes on plasmids and integrated the plasmids as a single copy at the *TRP1* locus in the strain carrying pTet1-dCas9-GFP and 9x gRNAs, then deleted the endogenous genes using kanMX4 marker. *ycs4-1* and *ycg1-2* mutants were obtained from Dr. Charlie Boone's lab (University of Toronto) and pTet-dCas9-GFP, pMyo2-rtTA and 9x gRNAs were integrated into these strains as described above.

**Live-cell imaging**

Yeast cells were grown in synthetic complete media supplemented with 2% glucose. Doxycycline was added to the culture at 0.5 µg/ml for ~16hr to induce dCas9 expression. Cells were grown to early-log phase (OD=0.2, unless stated otherwise) before imaging. For short-term live imaging, cells were collected by gentle centrifugation (2000 rpm, 2min), and quickly mounted on a coverslip and imaged immediately. For the rDNA condensation assay, the strain integrated with the dCas9-GFP and gRNAs was grown overnight to early-log phase in synthetic complete media supplemented with 2% glucose and 0.5 µg/ml doxycycline. Cells were collected by gentle centrifugation and concentrated to ~5 x $10^8$ cells/ml. 7 µl of the concentrated culture was pipetted on a 18mm x 18mm coverslip and mounted on a microscope slide. The edges of the coverslip were sealed by nail polish to prevent evaporation. For stress-induction experiments, early-log phase cells were collected by centrifugation, washed twice in different nutrient depleted media and grown for an extra hour at 30°C with shaking. UV treatment was done with a Stratalinker UV Crosslinker using 254nm UV light on yeast culture in synthetic complete media, and cells were grown for an extra hour at 30°C before imaging.

Live-cell imaging was performed on a Leica DMi8 widefield microscope equipped with 100x NA 1.44 HCX PL APO CS Oil objective lens (with 1.6x zoom) and Andor EMCCD iXon Ultra 888 camera. dCas9-GFP fluorescence was excited with 479nm LED light from Spectrax 6 light source (Lumencor) and collected with a 495nm dichroic mirror and a 500-550nm emission filter (Chroma). Net1-mRFPmars was excited with 575nm light and collected with a triple band pass dichroic mirror and 600-700nm emission filter. For time-lapse microscopy experiments, reduced LED light power was applied to minimize photobleaching, and Leica's adaptive focus system was applied for maintenance of focus over time. Z stacks were collected with a step-size of 180 nm over 5.11 µm. 16 bit images were collected and deconvolved using the Leica LAS X software (blind optimization algorithm, 10 iterations). Z-series were displayed as maximum projections, and brightness and contrast were adjusted using the FIJI software.

**Structured illumination microscopy**

Super-resolution 3D-SIM images were acquired on a DeltaVision OMX SR (GE Healthcare) equipped with a 60x/1.42 NA PlanApo oil immersion lens (Olympus), and sCMOS cameras (pco.edge). Samples were prepared as described in the 'rDNA condensation assay' and mounted on a coverslip in synthetic complete media. dCas9-GFP fluorescence was excited with 488 nm solid state laser with 5% power. Image stacks of 4 µm with 125 nm thick z-sections and 15 images per optical slice (3 angles and 5 phases) were acquired using immersion oil with a refractive index 1.518. Images were reconstructed using Wiener filter settings of 0.005 and optical transfer functions (OTFs) measured specifically for each channel with SoftWoRx 6.5.2 (GE Healthcare). The width of the dCas9-bound chromatin fiber profile was estimated using the 'full width at half-max intensity' method (Murray, 2011) using FIJI software.

**CHIP-qPCR**

CHIP experiments were performed as previously described (Xue, Rushton and Maringele, 2011) with some modifications. Briefly, ~4 x $10^8$ cells were collected for each CHIP reaction, and cells were pre-adjusted to a density of ~1x $10^7$ cells/ml in 40 ml culture. Formaldehyde was added directly to the culture at a final concentration of 1%. To stop crosslinking, 6 ml of 2.5 M glycine was added, and samples were incubated at room temperature for 5 min before harvesting. CHIP enrichment values was calculated as (IP-secondary antibody)/Input * 100%. Anti-GFP (Roche 11814460001) and anti-mouse (ab6728, Abcam) antibodies were used. Primers used for amplification of the rDNA genes were AAACGGCTACCACATCCAAG (18S, forward) and GGCCCAAAGTTCAACTACGA (18S, reverse); CCGAATGAACTAGCCCTGAA (25S, forward) and CGACTAACCCACGTCCAACT (25S, reverse); GGTTGCGGCCATATCTACC (5S, forward) and ATTGCAGCACCTGAGTTTCG (5S, reverse).

**Western blot**

Whole cell extracts were prepared using the trichloroacetic acid (TCA) method. Protein lysate were separated on a 7.5% Tris-Tricine gel and blotted with a Rad53 antibody (Abcam ab104232). Membrane was imaged using a Fujifilm LAS-4000 system.

## Supplemental References


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