Expanded View Figures

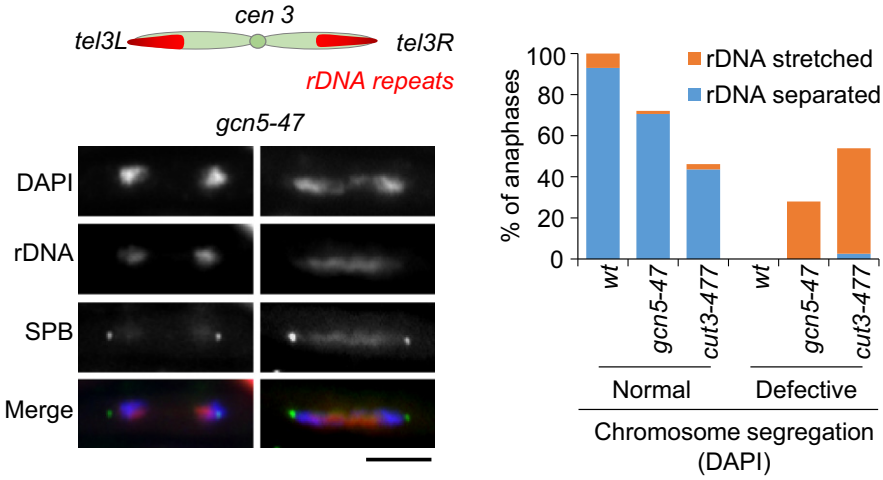

Figure EV1. Chromosomes III remain untangled during anaphase in *gcn5* mutant cells.
Scheme of chromosome III in which ~100 copies of 10 kb rDNA repeats, each consisting of 5.8S, 18S and 28S rDNA genes, are located adjacent to the telomeres. Fib1, which binds the rDNA repeats, was used to monitor the segregation of the arms of chromosome III in late anaphase cells. Fission yeast cells exponentially growing at 32°C and expressing Fib1-RFP (rDNA) and Cdc11-GFP (SPB) were fixed, stained with DAPI and examined for chromosome segregation (DAPI) and rDNA segregation ($n > 70$). Bar: 5 microns.

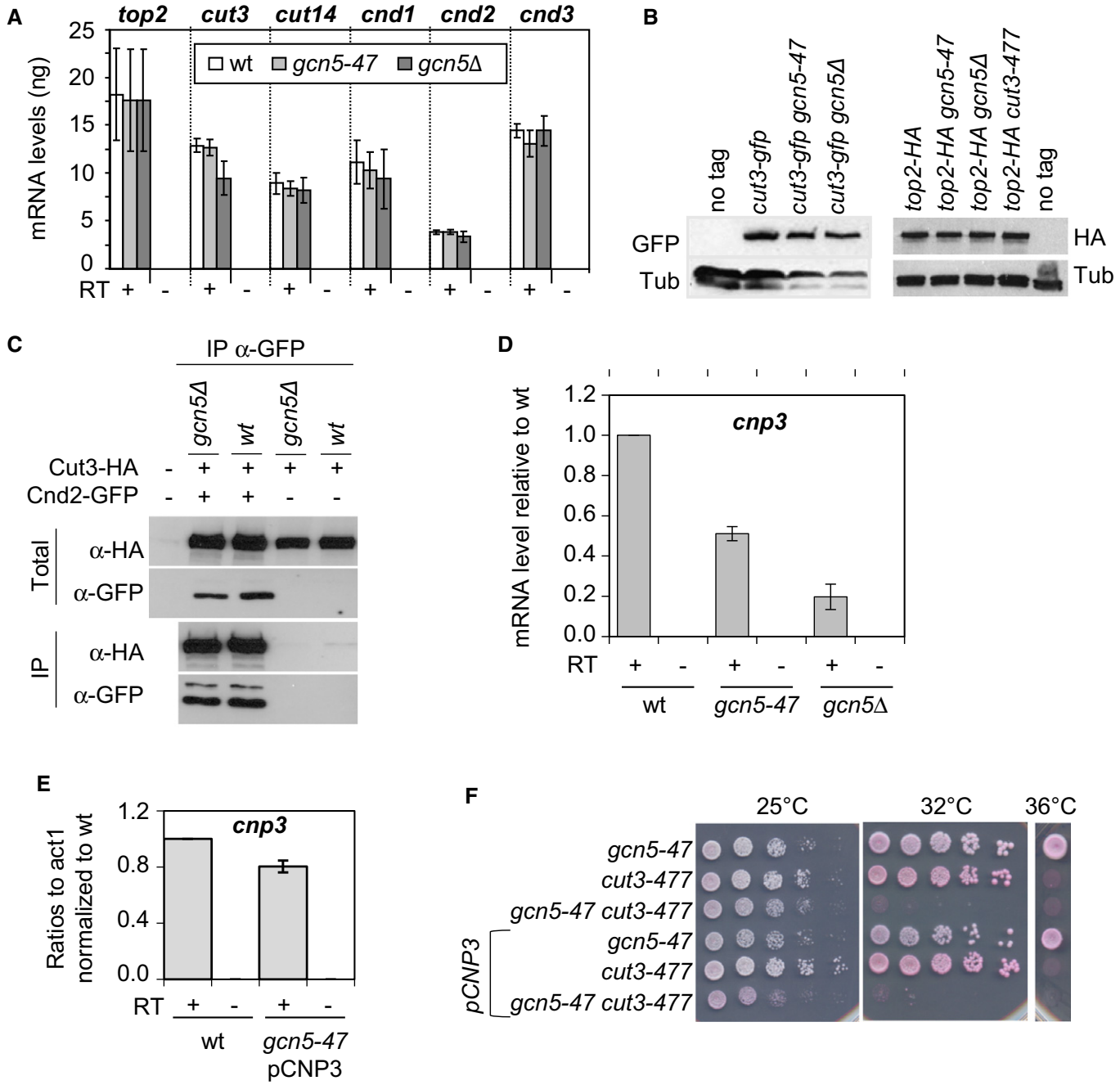

Figure EV2. The link between Gcn5 and Cut3 is direct.

A Steady-state level of condensin and Topo II mRNA in cells lacking functional Gcn5. About 500 ng of total RNA extracted from fission yeast cells exponentially growing at 32°C was reverse-transcribed in the presence (+) or absence (–) of reverse transcriptase (RT) and cDNAs were quantified by real-time qPCR. Indicated values correspond to the average and mean deviation from two independent experiments.

B Steady-state levels of Cut3-GFP and Top2-HA detected by Western blotting. α-Tubulin (Tub) was used a loading control.

C Integrity of the condensin complex as judged by co-immunoprecipitation. Cnd2-GFP was immunoprecipitated from indicated strains arrested in mitosis (septation indexes <4%). Levels of Cnd2-GFP and Cut3-HA in total and immunoprecipitated fractions were assessed by Western blotting.

D, E Steady-state level of *cnp3* mRNA measured by RT-qPCR. About 500 ng of total RNA was reverse-transcribed in the presence (+) or absence (–) of RT. pCNP3 indicates that the eponym plasmid bearing the *cnd3* gene was inserted into the genome. Averages and s.d. calculated from 2 biological replicates are shown.

F Restoring *cnp3* mRNA level does not suppress the negative genetic interaction between *gcn5-47* and *cut3-477*. Cells of indicated genotype were serially diluted fivefold and spotted onto complete media supplemented with phloxin B.

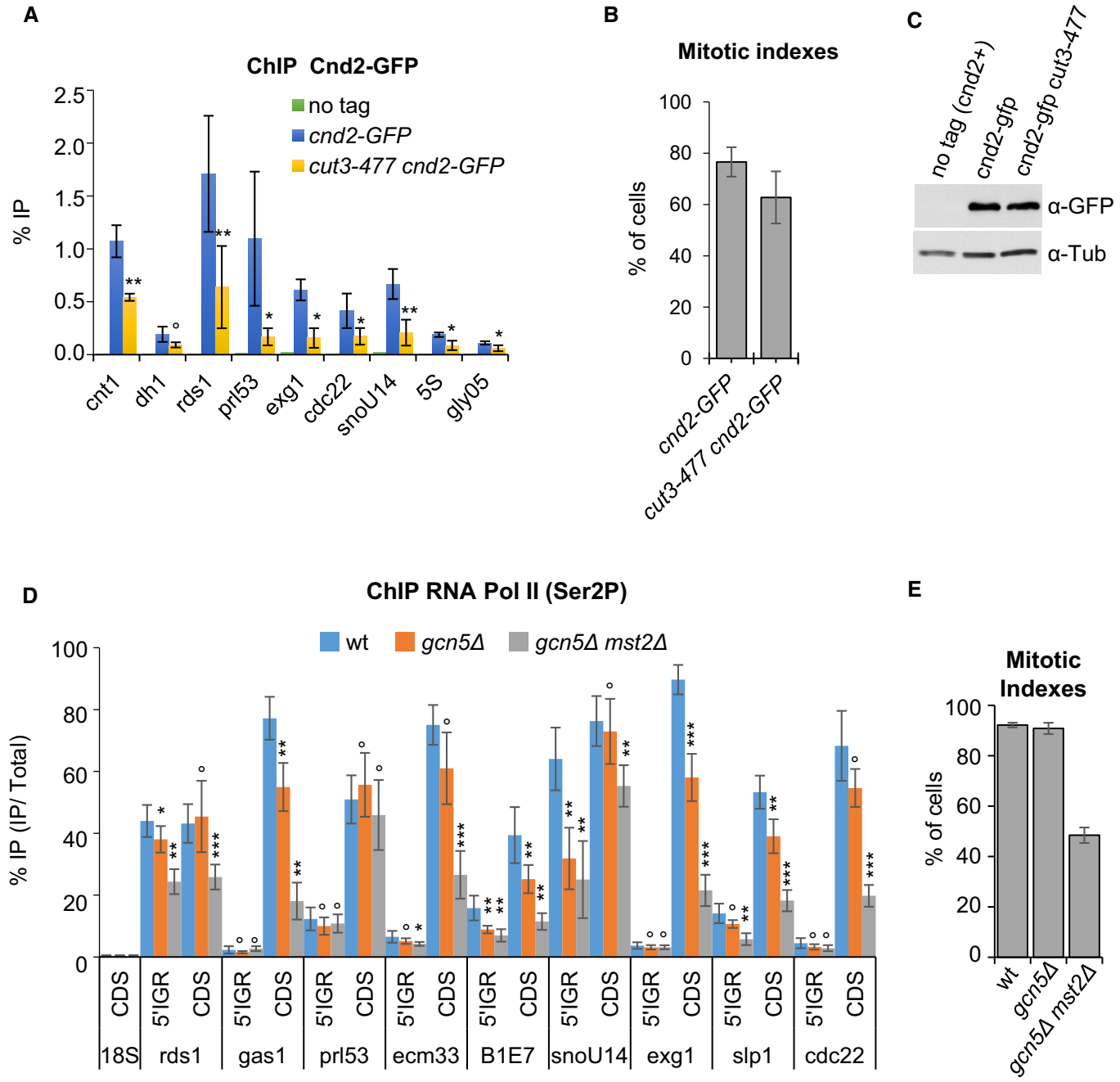

Figure EV3. Condensin association sites in fission yeast and their transcription.

A Condensin binding assessed by ChIP. Fission yeast cells were arrested in pro/metaphase at 19°C by the *nda3-KM311* mutation and processed for ChIP against Cnd2-GFP. % IP correspond to the averages, and s.d. calculated from 6 ChIPs performed on 3 biological replicates.

B Mitotic indexes. Averages and s.d. calculated from the 3 biological replicates used in (A) are shown.

C Steady-state level of Cnd2-GFP. Exponentially growing cells were shifted at 36°C for 2.5 h to inactivate *cut3-477*, and whole cell extracts assessed for Cnd2-GFP levels by Western blotting using anti-GFP (A11122) antibody. Tubulin (Tub) served as loading control.

D, E RNA Pol II occupancy assessed by ChIP in mitotically arrested cells. Cells expressing Cnd2-GFP were arrested in mitosis and processed for ChIP against RNA Pol II phosphorylated on serine 2 (Ser2P). % IP correspond to averages, and s.d. calculated from 8 ChIPs performed on 4 biological replicates. (E) Mitotic indexes. Averages and s.d. calculated from the 4 biological replicates used in (D) are shown.

Data information: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ and °$P > 0.05$.

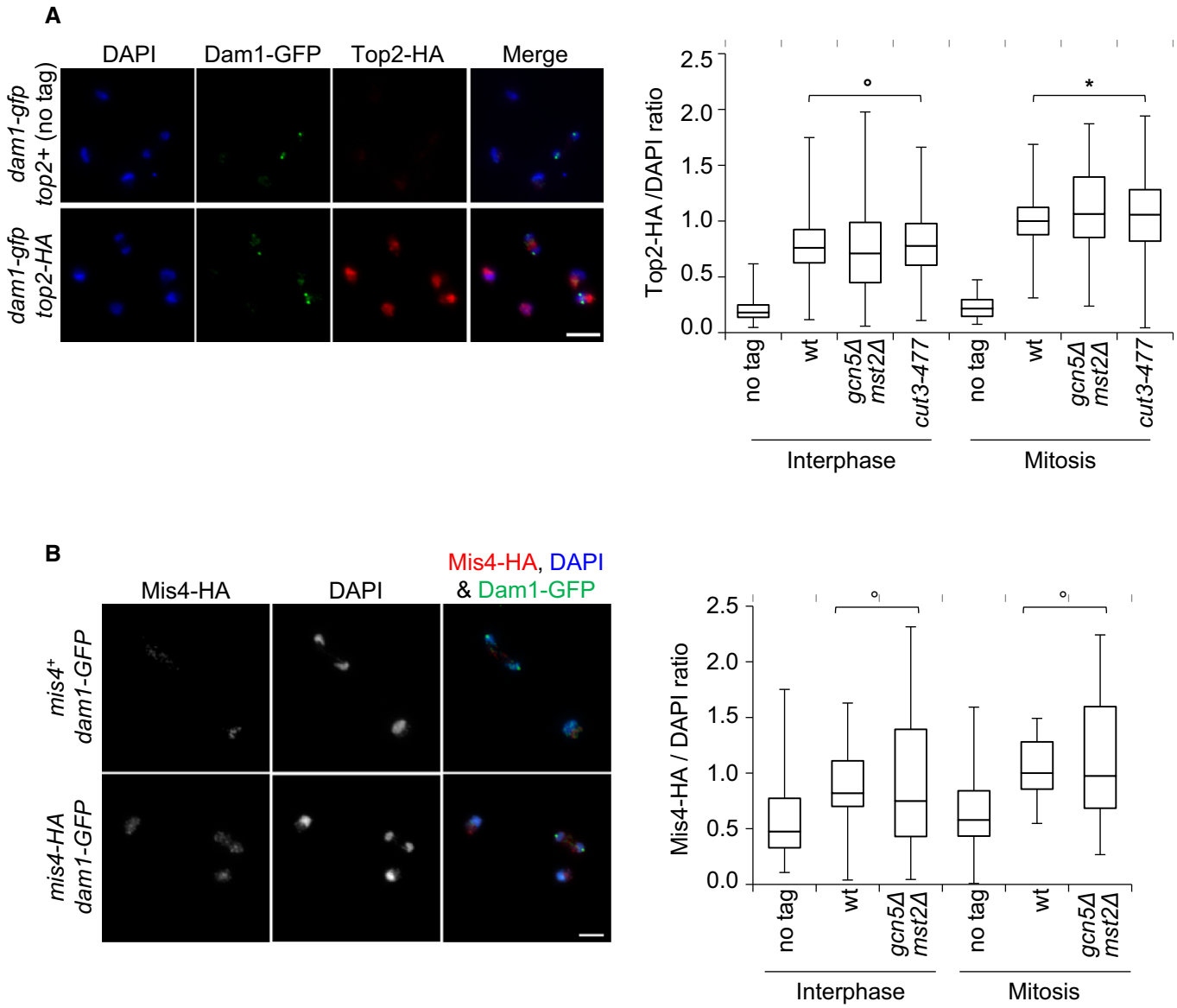

Figure EV4. Gcn5 and Mst2 are not required for the chromosomal association of Top2 and Mis4.

A, B The chromosomal association of Topo II (A) or of the cohesin loader Mis4 (B) assessed by chromosome spreading. Fission yeast cells exponentially growing at 32°C were processed for chromosome spreading and immunofluorescence against Dam1-GFP and Top2-HA or Mis4-HA. Chromatin was stained with DAPI. Dam1-GFP, recruited at kinetochores during mitosis, was used as a mitotic marker of isolated nuclei. Top2-HA or Mis4-HA signals associated with interphase (Dam1-GFP negative) or mitotic nuclei (Dam1-GFP positive) were quantified and divided by the intensity of the DAPI signal. Ratios were normalized to the median value given by the wt control in mitosis. Boxes indicate the 25th percentile, median and 75th percentile and whiskers the min and max values calculated from $n = 3$ experiments. °$P > 0.05$ and *$P < 0.05$. Bars: 5 microns.

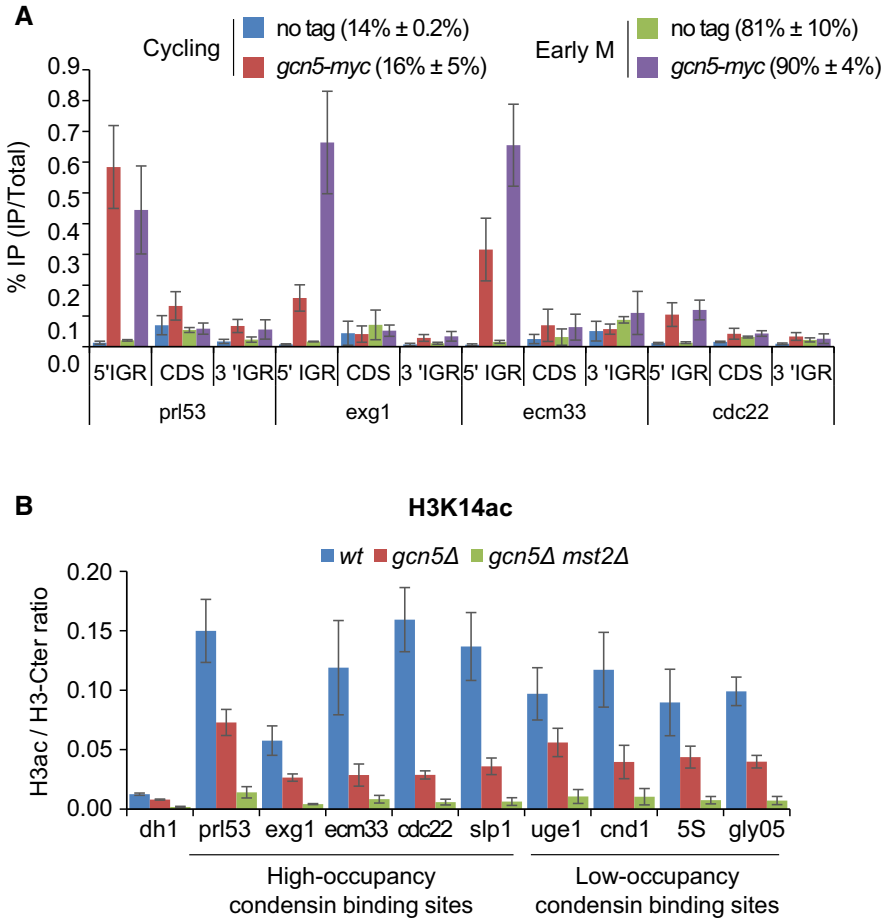

Figure EV5. Gcn5 localization across transcribed genes during mitosis.

A ChIP against Gcn5-myc on fission yeast cells cycling or arrested in prometaphase. Mitotic indexes indicated in parentheses were determined by scoring binucleated cells and hypercondensed nuclei. % IP are averages, and s.d. calculated from 6 ChIPs performed on 3 biological replicates.

B The chromosomal occupancy of H3K14ac during mitosis. Cells expressing Cnd2-GFP were blocked in early mitosis, mitotic indexes measured (see Fig 4D) and cells processed for ChIP against total H3 (H3-Cter) and H3K14ac. Transcriptionally silent pericentric heterochromatin (dh1) served as negative control. H3ac/H3-Cter average ratios and standard deviations calculated from 6 ChIPs performed on 3 biological replicates are shown.

Figure EV6. Condensin accumulates at nucleosome-depleted regions dependent upon Gcn5.

A Fission yeast cells expressing Cnd2-GFP were arrested in early mitosis, mitotic indexes determined (see Fig 5A) and processed for MNase digestion. Mitotic chromatin was digested with increasing amounts of MNase to obtain mononucleosomes to dinucleosomes ratios of ~80:20. Mononucleosomal DNA excised from the gel was subjected to massive parallel sequencing (MNase-seq).

B Overlap between nucleosome-depleted regions (NDR) or non-NDRs identified during mitosis by MNase-seq and condensin peaks identified by ChIP-seq against Cut14-pk9 (Sutani *et al*, 2015). NDRs (red) are enriched in the population of DNA fragments co-immunoprecipitated with Cut14-pk9 (IP/Input > 1) and depleted from the flow through (IP/Input < 1).

C Nucleosome scanning (MNase-qPCR) assay for nucleosome occupancy at the *prl53* promoter. Mitotic chromatin was digested by MNase as described in (A). Input (undigested) and mononucleosomal DNA was purified, and the % of input DNA, which was protected from MNase digestion, was assessed by qPCR. Averages and s.d. calculated from 3 experiments on 3 biological replicates are shown. Nucleosome patterns obtained by MNase-seq analysis (related to Fig 5B) are indicated for comparison.

D Representative MNase-seq nucleosome patterns at the 5S rRNA, *uge1* and *cnd1* genes (see Fig 5B). Lack of Gcn5 or both Gcn5 and Mst2 does not significantly modifies nucleosome occupancy at these three low-occupancy condensin-binding sites.

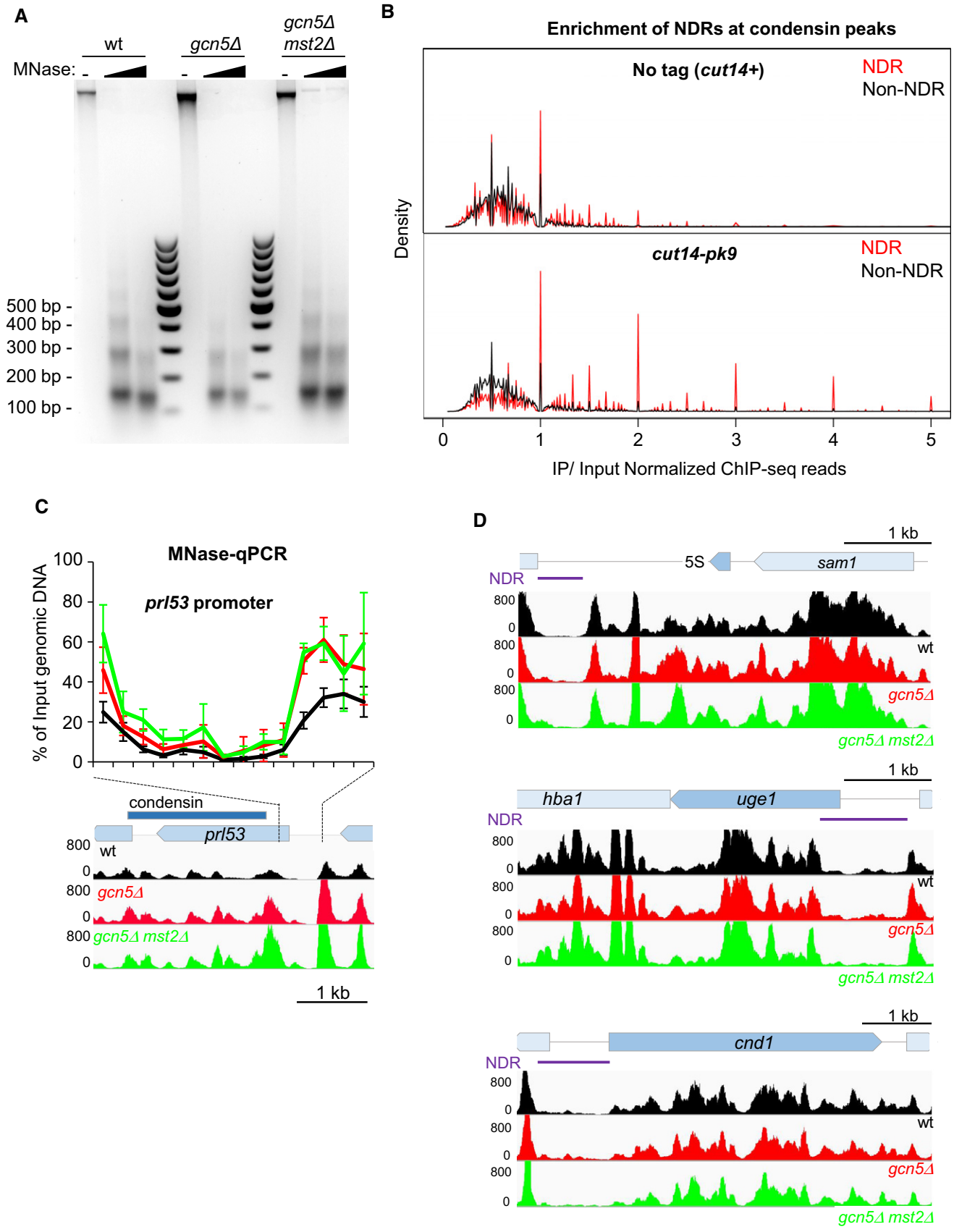

Figure EV6.

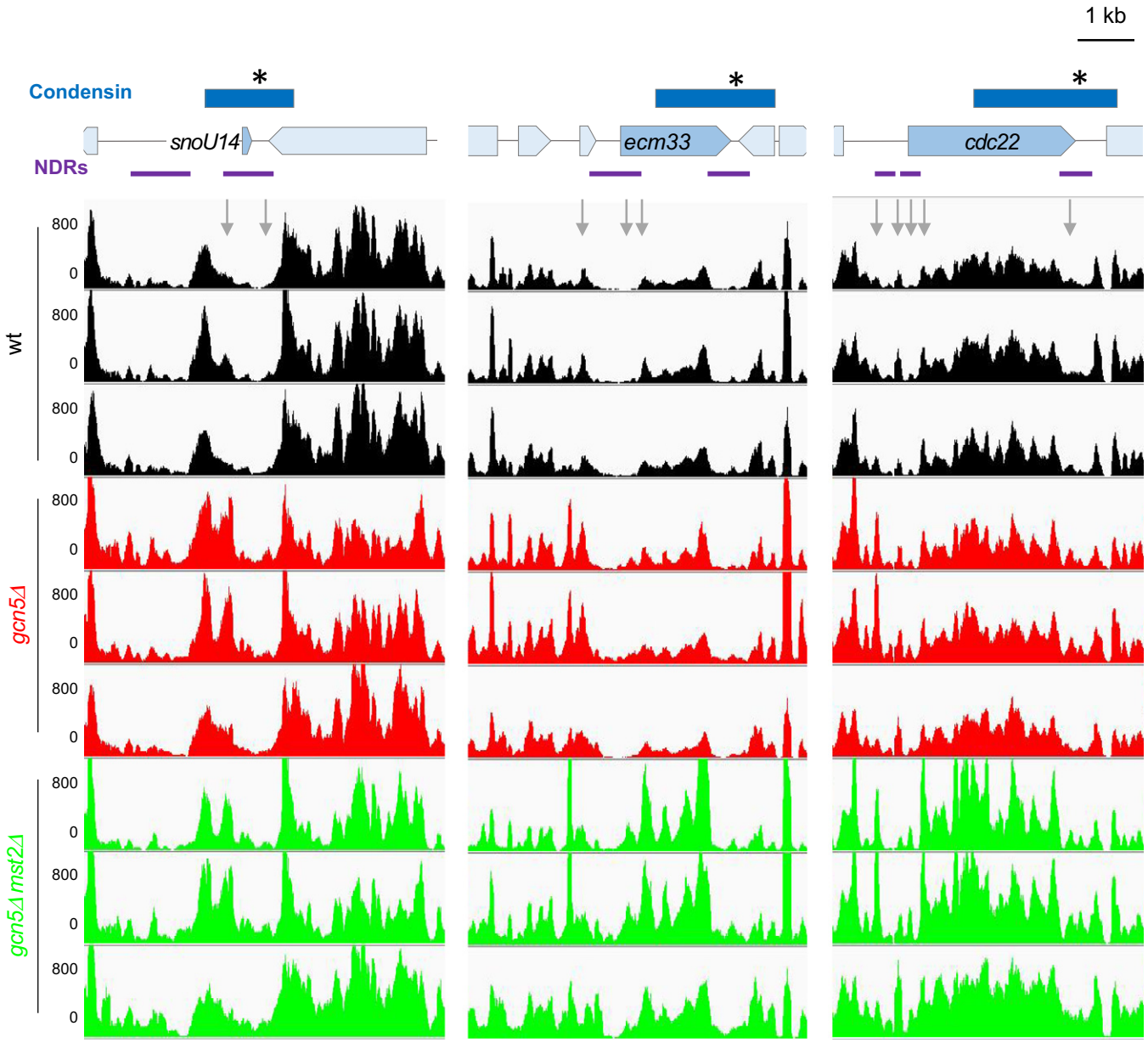

Figure EV7. MNase-seq patterns at three high-occupancy condensin-binding sites in replicates.
Related to Figs 5A and EV6. MNase-seq nucleosome patterns at the *snoU14*, *ecm33* and *cdc22* genes in the three wt, *gcn5Δ* or *gcn5Δ mst2Δ* biological and technical replicates. For each gene and each replicate, results shown in Figs 5A and EV6 correspond to the first line.

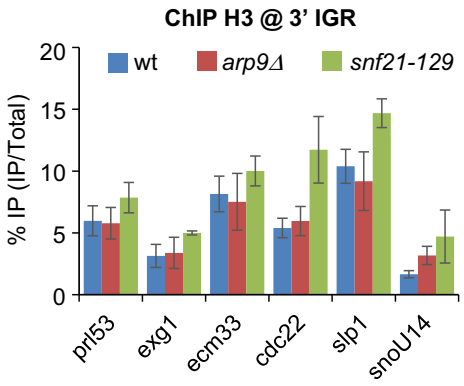

Figure EV8. The impact of RSC loss of function on histone H3 occupancy at the 3′ end of genes bound by condensin.
Fission yeast cells expressing Cnd2-GFP were arrested in early mitosis, mitotic indexes determined (see Fig 6A) and cells processed for ChIP against H3. % IP correspond to averages, and s.d. calculated from 3 ChIPs performed on 3 biological replicates (see Fig 6 for additional information).