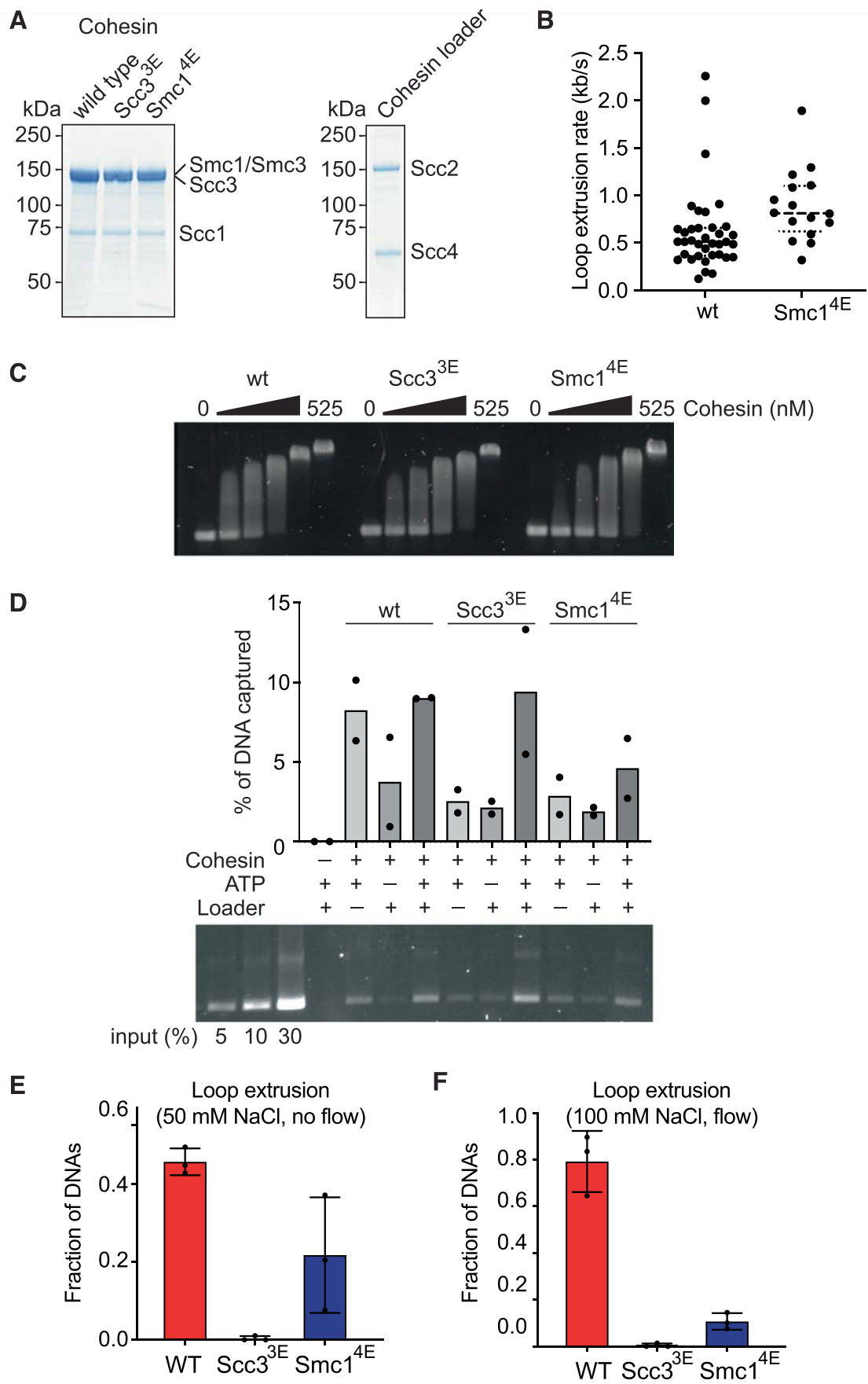
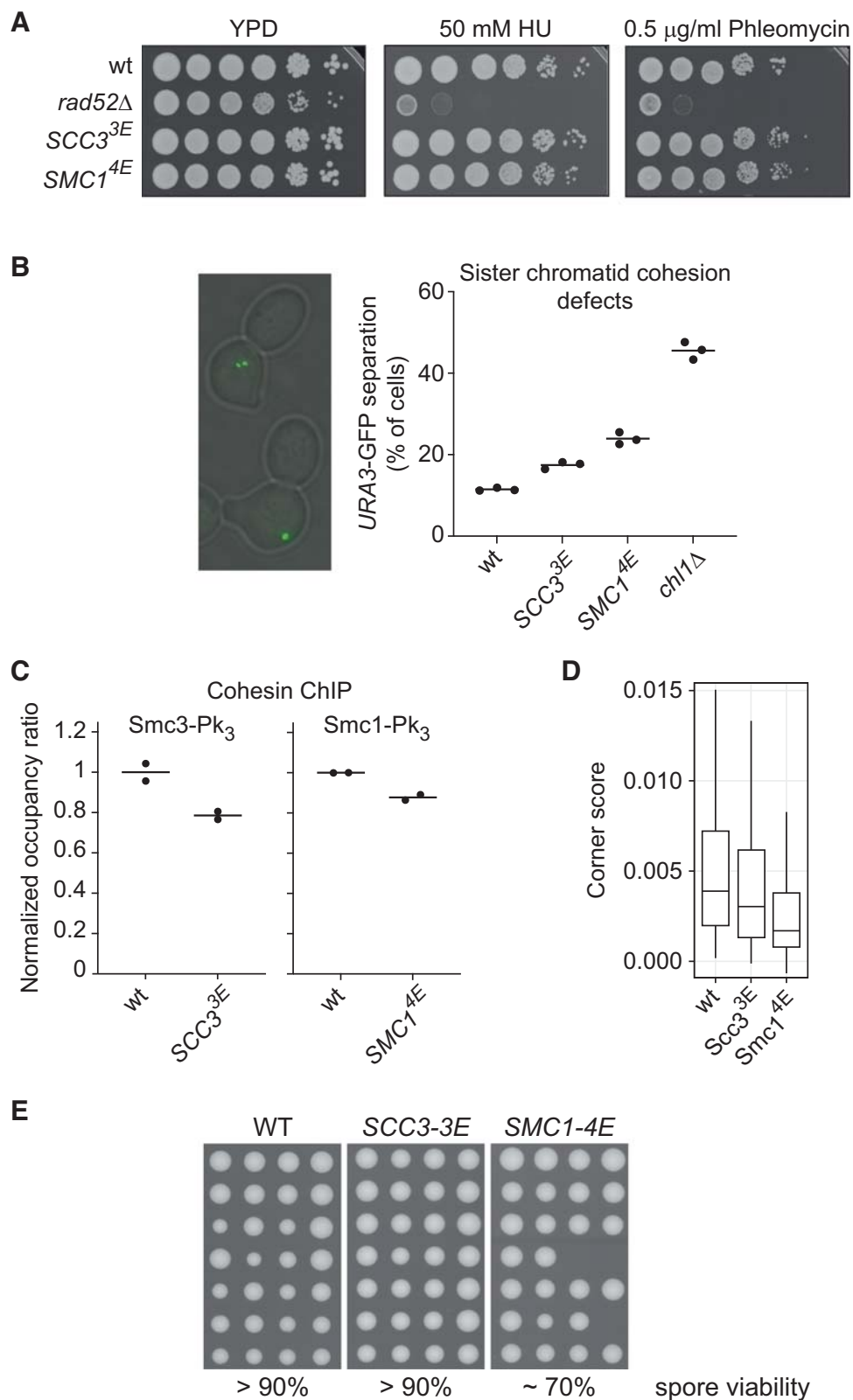


Expanded View Figures

Figure EV1. Characterisation of Scc3^{3E} and Smc1^{4E} loop extrusion defective cohesin complexes.

(A) Purified wild type (wt), Scc3^{3E}-, and Smc1^{4E}-cohesin and cohesin loader were analysed by SDS-PAGE followed by Coomassie Blue staining. (B) Loop extrusion rates, measured as described (Higashi et al, 2021), of wt and Smc1^{4E}-cohesin, in the presence of loader and ATP ($n_{wt} = 37$, $n_{Smc1^{4E}} = 16$). Dashed and dotted lines represent the median and quartile ranges, respectively. Processive extrusion by Smc1^{4E}-cohesin suggests that this variant is defective in loop initiation but less so loop extension. Indeed, Smc1^{4E}-cohesin shows a greater median extrusion rate, which might arise if the small number of loop extrusion events by this variant are biased towards DNAs under low tension on which extrusion proceeds relatively faster. (C) DNA affinity of wt, Scc3^{3E}- and Smc1^{4E}-cohesin as measured by an electrophoretic mobility shift assay. Increasing cohesin concentrations were between 32 and 525 nM in 2-fold steps. (D) Assay to measure topological (high-salt-resistant) loading of wt, Scc3^{3E}- and Smc1^{4E}-cohesin onto DNA (Minamino et al, 2018), in the presence of the indicated components. An example agarose gel of the recovered DNA is shown, as well as quantification of the individual results from two independent repeat experiments. Bars show the means. (E) Loop extrusion assay as in Fig. 1C, but the flow cell was incubated with wt, Scc3^{3E}- or Smc1^{4E}-cohesin, loader and ATP in the absence of flow, before flow was applied to visualise loops. The fractions of DNA with loops were counted in three independent repeat experiments. Individual data points are shown, bars represent the mean and error bars the standard deviation ($n_{wt} = 224$, $n_{Scc3^{3E}} = 269$, $n_{Smc1^{4E}} = 633$). (F) As Fig. 1C in the presence of flow, but a buffer containing 100 mM NaCl was used. See the Methods for complete buffer descriptions. Bars represent the mean and error bars the standard deviation ($n_{wt} = 452$, $n_{Scc3^{3E}} = 295$, $n_{Smc1^{4E}} = 242$).





**Figure EV2. Life without loop extrusion.**

(A) 10-fold serial dilutions of cultures of the indicated genotypes were plated onto YPD agar plates containing the indicated compounds and grown at 30 °C for 2 days. A wt and a DNA repair deficient (*rad52Δ*) strain were included as controls. (B) Sister chromatid cohesion in G2/M arrested cells was monitored at the GFP-marked *URA3* locus (Michaelis et al, 1997). A representative image of two G2/M arrested cells with intact (left) or defective (right) sister chromatid cohesion is shown. The percentage of cells ($n = 100$) with two separated GFP dots were recorded in three independent repeat experiments. The means are represented by horizontal bars. A wild type (wt) and a cohesion establishment defective (*chl1Δ*; Samora et al, 2016) strain served as controls. (C) Overall cohesin ChIP enrichment ratios of wt, compared to *Scc3^{3E}*- and *Smc1^{4E}*-cohesin, relative to a *C. glabrata* spike-in. Cohesin ChIP used *Smc3-Pk₃* in the *Scc3^{3E}* strain, or *Smc1^{4E}-Pk₃*, normalised against *Smc3-Pk₃* and *Smc1-Pk₃* wt control strains. (D) Corner score distributions of loops identified in the wild-type micro-C contact map and linked to cohesin anchors, sampled in the *Scc3^{3E}*- and *Smc1^{4E}*-maps ($n = 1060$). Box plots represent the median (centre), quartiles (box) and range (whiskers). (E) Tetrad dissection following sporulation of homozygous diploid wild type, *SCC3^{3E}* and *SMC1^{4E}* strains. Spore viability was calculated based on $n = 118/128$, $104/100$ and $82/120$ germinating and colony forming spores, respectively.

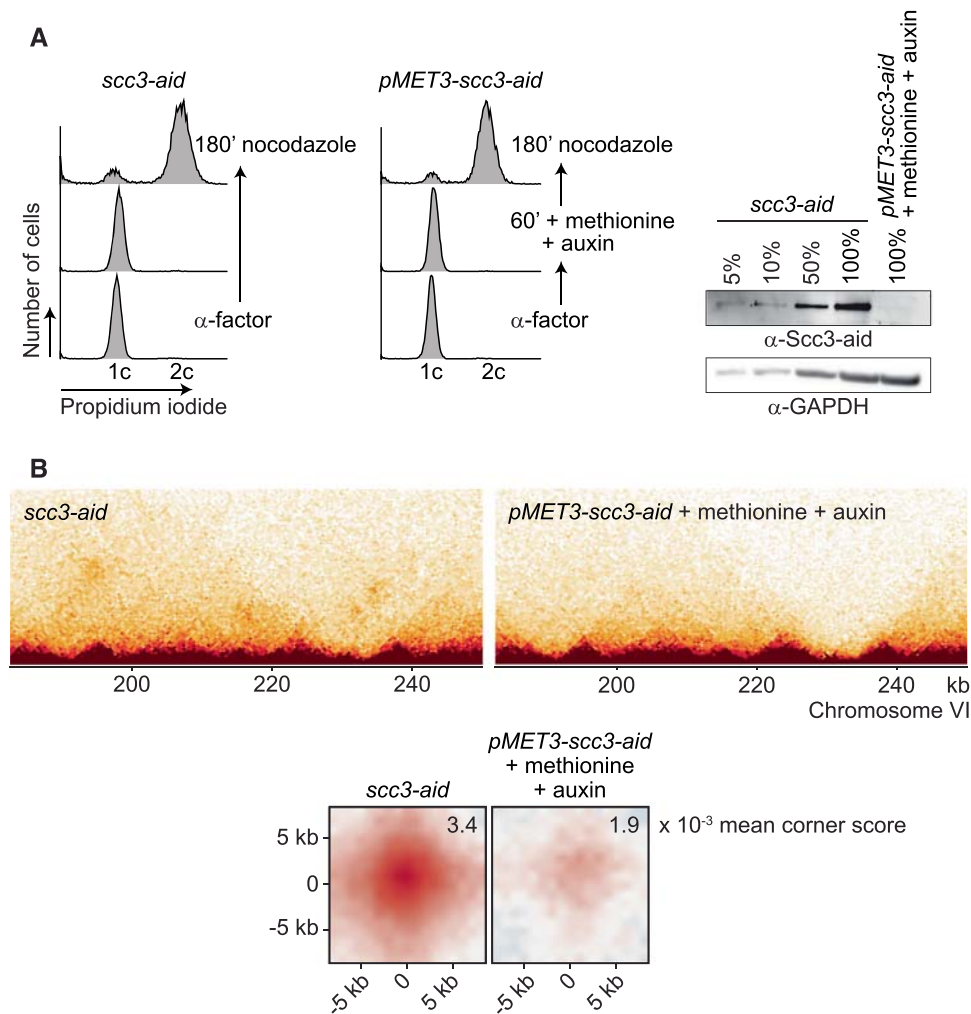


Figure EV3. Scc3 is required for chromatin loop formation.

(A) FACS analysis of DNA content, as well as experimental outline, of the experiment to deplete Scc3 by promoter shut-off and an auxin-inducible degron (*pMET-scc3-aid* cells). As a control, we used cells in which *scc3-aid* is expressed under control of its endogenous, methionine-insensitive promoter and to which we added methionine but not auxin during G1 arrest, before release into nocodazole-containing medium for arrest in G2/M. Scc3 depletion was confirmed by Western blotting. Serial dilutions of the control sample without auxin addition were loaded, as well as the depleted sample. Scc3 was detected using an α -aid-tag antibody (Cosmo Bio, CAC-APC004AM). GAPDH, detected by an α -GAPDH antibody (abcam, clone GA1R, ab125247) served as a loading control. (B) 500 bp-resolution merged micro-C contact maps from two independent experiments with Scc3-depleted *pMET-scc3-aid* and control *scc3-aid* cells. Aggregate chromatin loop profiles, detected by chromosight and linked to cohesin anchors in a wild-type strain without any cohesin alteration (Fig. 1C), were recorded in both present maps.

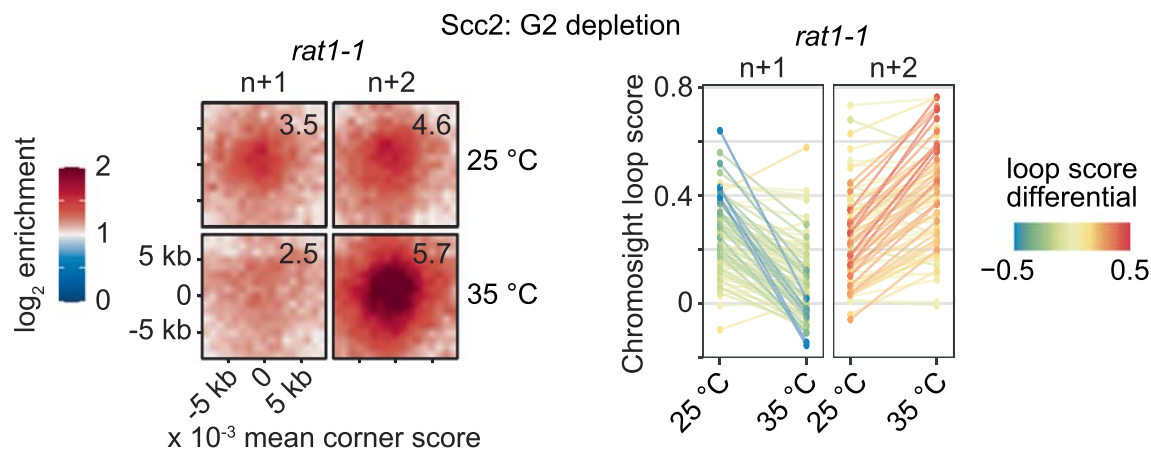
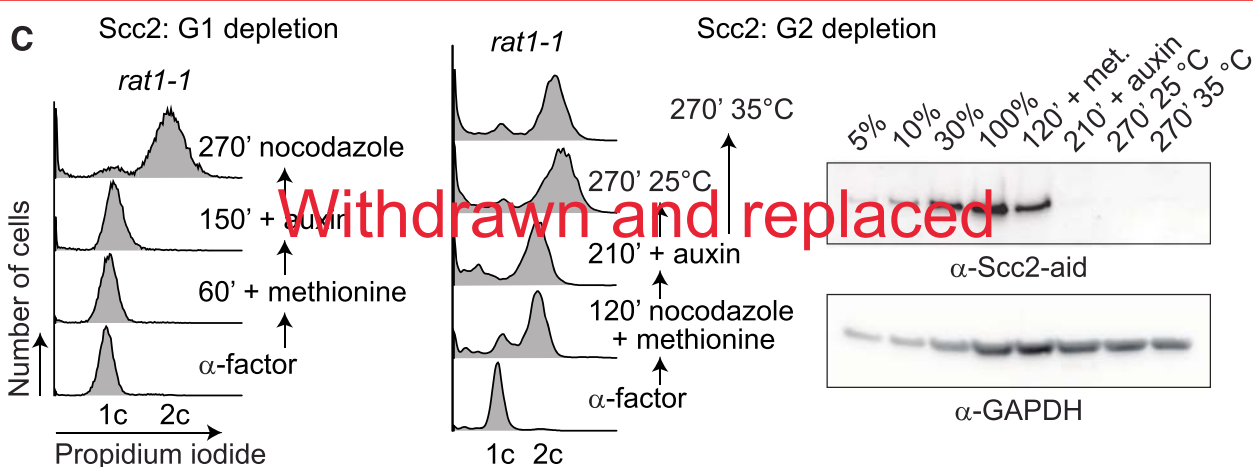
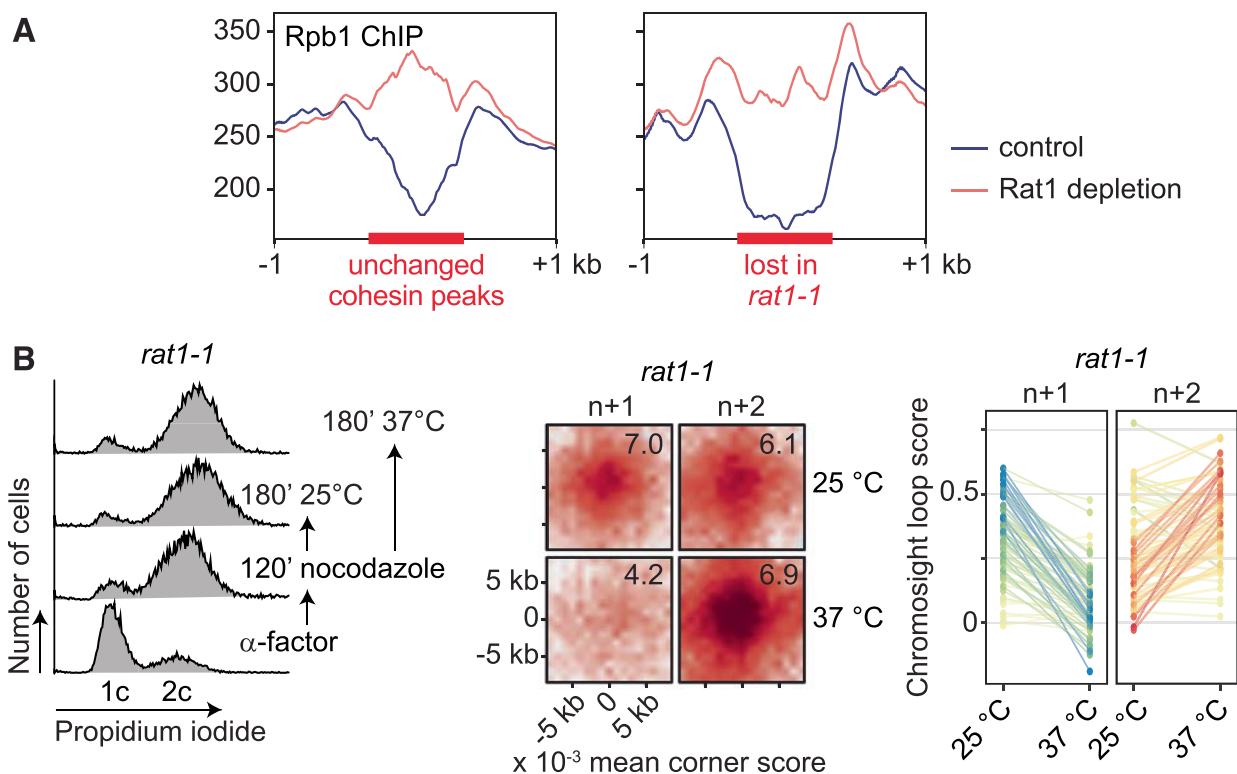
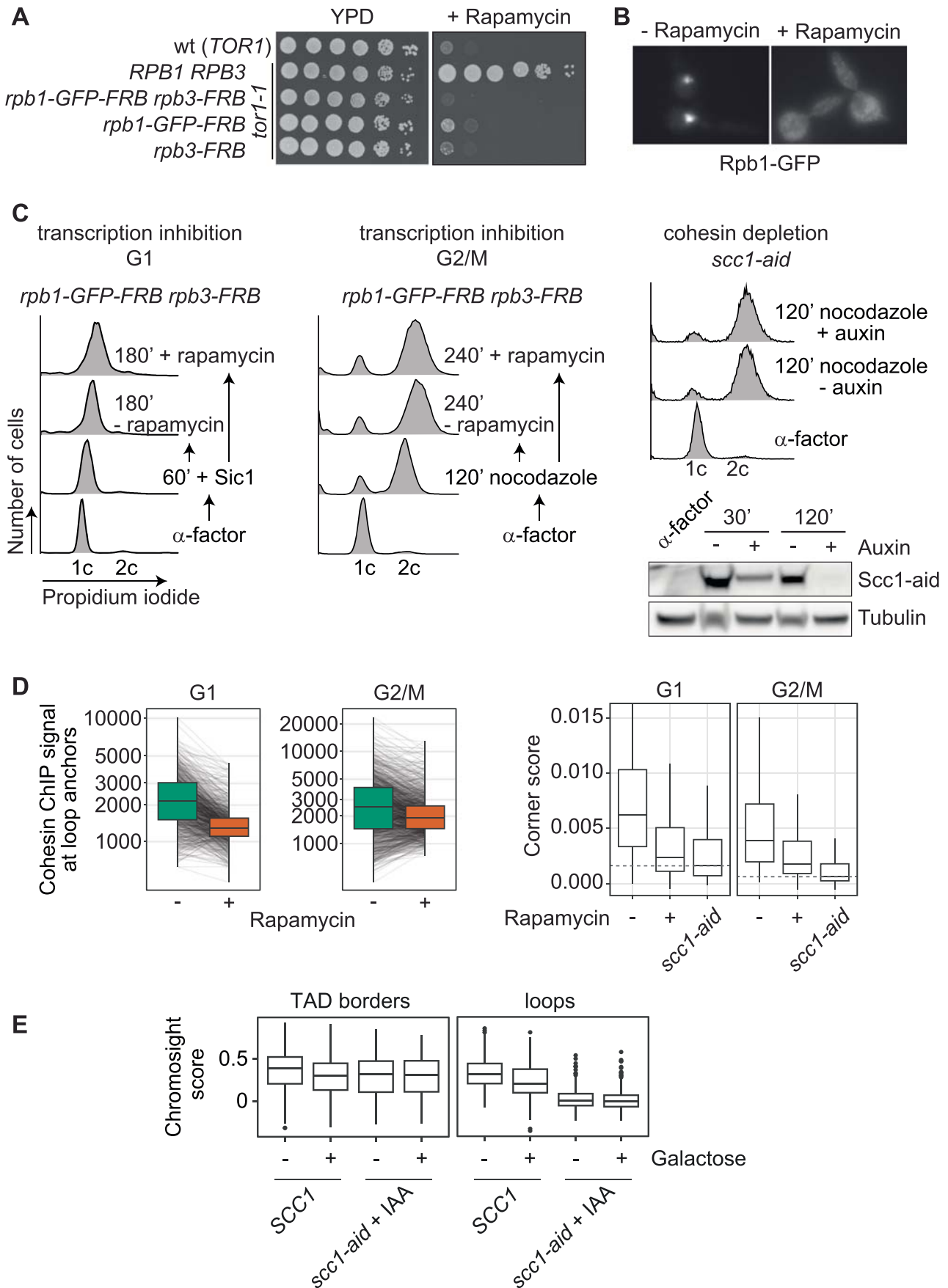



Figure EV4. Transcription expands cohesin-mediated chromatin loops.

(A) Characterisation of cohesin peaks that are displaced following *rat1-1* inactivation. Aggregated Rpb1 ChIP profiles (Baejen et al, 2017) are shown over scaled cohesin peak regions, and their surroundings, that remained either unchanged or that were lost following *rat1-1* inactivation. Before Rat1 depletion (anchor away was used by Baejen et al, 2017), cohesin peaks that will be displaced show strict Rpb1 avoidance. In contrast, cohesin peaks that will remain unchanged were already partly Rpb1 occupied. Following Rat1 depletion, Rpb1 broadly overlapped with both type of regions. We confirmed that differing cohesin peak widths did not cause these differences. To conduct these analyses, raw sequences from (Baejen et al, 2017) were aligned to the S288C genome for analysis using the standard nf core chipseq procedure. Bam files were then converted to BigWigs using bamCoverage with normalizeUsing RPKM and ignoreDuplicates parameters. Binsize was selected at 20 bp and data were smoothed over 3 bins. For comparison we overlaid our previous cohesin (Scc1) ChIP microarray analysis (Ocampo-Hafalla et al, 2016) and selected peaks exclusive to control cells. Peaks longer than 4000 bp or shorter than 500 bp were excluded from the analysis. (B) FACS analysis of DNA content of the cells in the experiment shown in Fig. 2C, together with an experimental outline. Aggregate chromatin profiles of loops ($n = 91$), identified as in Fig. 2B, and a graph depicting the *rat1-1* dependent loop score changes. (C) FACS analyses of DNA content of the cells in the experiment shown in Fig. 2D, together with experimental outlines. Western blot analysis confirmed Scc2 depletion by an auxin-inducible degron. Samples at the indicated times in the experiment are shown. Scc2 was detected using the aid-tag antibody. Tubulin served as a loading control. Aggregate loop profiles ($n = 52$) and a graph depicting the *rat1-1* dependent loop score changes are shown.



◀ **Figure EV5. Transcription inhibition and its effect on cohesin-mediated chromatin loops.**

(A) 10-fold serial dilutions of cultures of the indicated genotypes were plated onto YPD agar plates, with or without 2 µg/ml added rapamycin, and grown at 30 °C for 2 days. A strain in which both Rpb1 and Rpb3 subunits of RNA polymerase II were fused to FRB showed a tighter response to rapamycin, as compared to strains with either one of the fusions. (B) An example of Rpb1-GFP-FRB relocation from the nucleus to the cytoplasm after one hour 2 µg/ml rapamycin treatment. Cells show the typical elongated bud shape of Sic1-induced late G1 arrest (Lopez-Serra et al, 2013). (C) FACS analysis of DNA content of the cells in the experiment shown in Fig. 3, as well as an experimental outline. Western blot analysis confirmed Scc1-aid depletion by its auxin-inducible degron, at 30 min and 120 min (the time of cell harvest) after release from α -factor synchronisation. Scc1 was detected with the α -aid antibody, tubulin served as the loading control and was detected with a mouse monoclonal α -Tub1 antibody (clone TAT-1). (D) Cohesin ChIP signal intensity distributions at loop anchors (normalised mean reads), in the absence or presence of rapamycin, in both the G1 and G2/M synchronised cultures. Grey lines connect individual ChIP signal intensities under the two conditions (G1: $n = 1059$, G2: $n = 1447$). Corner score distributions of the corresponding loops (G1: $n = 788$, G2: $n = 1060$), before and after transcription inhibition, as well as of the same loop positions sampled following Scc1 depletion, are shown alongside. Box plots represent the median (centre), quartiles (box) and range (whiskers). Baseline corner scores in the absence of cohesin are indicated by dashed lines. (E) Chromosight TAD boundary ($n = 822$) and Chromosight loop score ($n = 1300$) distributions, detected in SCC1 cells grown in glucose (Fig. 5) and recorded from the maps under the indicated experimental conditions. Box plots represent the median (centre), quartiles (box) and range (whiskers).