Expanded View Figures

Figure EV1. CLRC interacts with shelterin without affecting ubiquitylation.

A Mass spectrometry analysis of proteins co-purified with Raf1 fused to the ubiquitin-associated (UBA) domain of the ubiquitin receptor Dsk2 from *S. cerevisiae* (UBA^{FLAG}Raf1). This method is based on the *Ubiquitin Ligase Trapping* method originally developed in *S. cerevisiae* [57], which stabilizes the binding of substrates to the ubiquitin ligase. The volcano plot depicts proteins enriched in UBA^{FLAG}Raf1 WT cells relative to UBA^{FLAG}Raf1 *rik1*Δ. Members of the CLRC and shelterin complexes are highlighted in red and blue, respectively. Bottom panel displays nuclear proteins enriched ($\log_2 \geq 5$ or $P \leq 0.01$).

B Silencing reporter assay at *imr1L::ura4*$^+$. Fivefold serial dilution on non-selective (EMM) and selective media (EMM + 5-FOA) of WT, *rik*Δ, and UBA^{FLAG}Raf1 cells. The N-terminal fusion does not interfere with the function of CLRC in heterochromatin formation. The *rik1*Δ strain was used as negative control.

C RT–qPCR analysis in strains expressing untagged Raf1, UBA^{FLAG}Raf1, and UBA^{FLAG}Raf1 *rik1*Δ (n = 3 independent experiments). Data are normalized to input and represented as mean ± SEM.

D Co-immunoprecipitation of Raf1 with Ccq1 (top and middle panel) and Tpz1 (bottom panel) in presence and absence of Rik1. Strains expressing endogenous levels of UBA^{FLAG}Raf1 and $Ccq1^{HA}$ or $Tpz1^{HA}$ were subjected to anti-HA immunoprecipitation in the presence of Benzonase (indicating that the interactions are independent of DNA or RNA; negative control: no antibody, noAb). Input and immunoprecipitated material were analyzed by anti-FLAG immunoblots. For $Ccq1^{HA}$, the reciprocal experiment is also shown.

E Ubiquitin pull-down experiments. Top panels shows *in vivo* ubiquitylation of $Ccq1^{HA}$ in WT and *rik1*Δ cells expressing 6His-ubiquitin. Left panels: precipitated 6His-ubiquitin conjugates (20%) analyzed by anti-His and anti-HA immunoblots. Right panel: input fraction (0.1%) analyzed by anti-HA immunoblot. The bottom panels show 6His-ubiquitin pull-down assays for $Rap1^{HA}$ in WT and *rik1*Δ cells (left) and $Epe1^{FLAG}$ in WT cells (right). Epe1 is shown as an example for poly-ubiquitylation. WT cells expressing untagged ubiquitin are used as negative control.

F Anti-HA immunoblot of epitope-tagged shelterin proteins expressed from their endogenous locus in WT and *rik1*Δ cells. The asterisks denote unspecific signals derived from cross-reactions with the anti-HA antibody. Note that Ccq1 is expressed at higher levels compared to other shelterin subunits; endogenous levels of Pot1 are low and not detected by the immunoblot. Estimated sizes (kDa) of the tagged proteins: Ccq1 (86 kDa), Tpz1 (61 kDa), Pot1 (67 kDa), Poz1 (34 kDa), and Rap1 (84 kDa).

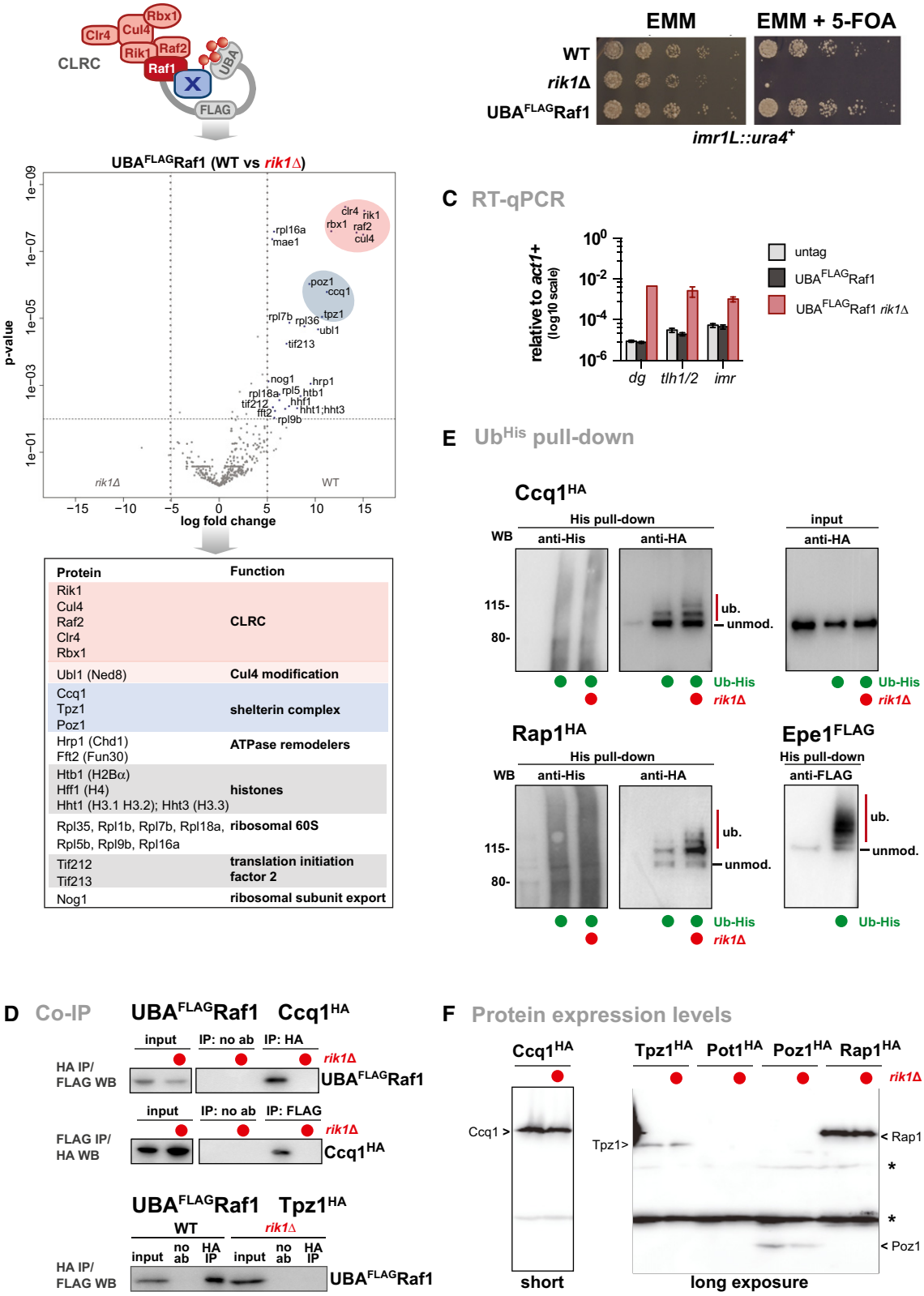
A LC-MS: UBA^FLAG Raf1
Rbx1
Cul4
Clr4
Rik1
Raf2
Raf1
CLRC
UBA
X
FLAG
UBA^FLAG Raf1 (WT vs rik1Δ)
p-value
1e−09
1e−07
1e−05
1e−03
1e−01
clr4
rik1
rbx1
raf2
cul4
rpl16a
mae1
poz1
ccq1
tpz1
rpl7b
rpl36
ubl1
tif213
nog1
hrp1
rpl18a
rpl5
htb1
tif212
hhf1
fft2
hht1;hht3
rpl9b
rik1Δ
WT
−15
−10
−5
0
5
10
15
log fold change
Protein
Function
Rik1
Cul4
Raf2
Clr4
Rbx1
CLRC
Ubl1 (Ned8)
Cul4 modification
Ccq1
Tpz1
Poz1
shelterin complex
Hrp1 (Chd1)
Fft2 (Fun30)
ATPase remodelers
Htb1 (H2Bα)
Hff1 (H4)
Hht1 (H3.1 H3.2); Hht3 (H3.3)
histones
Rpl35, Rpl1b, Rpl7b, Rpl18a,
Rpl5b, Rpl9b, Rpl16a
ribosomal 60S
Tif212
Tif213
translation initiation factor 2
Nog1
ribosomal subunit export
B Silencing assay
EMM
EMM + 5-FOA
WT
rik1Δ
UBA^FLAG Raf1
imr1L::ura4+
C RT-qPCR
relative to act1+ (log10 scale)
10^0
10^-2
10^-4
10^-6
dg
tlh1/2
imr
untag
UBA^FLAG Raf1
UBA^FLAG Raf1 rik1Δ
D Co-IP
UBA^FLAG Raf1 Ccq1^HA
input
IP: no ab
IP: HA
rik1Δ
HA IP/
FLAG WB
UBA^FLAG Raf1
input
IP: no ab
IP: FLAG
rik1Δ
FLAG IP/
HA WB
Ccq1^HA
UBA^FLAG Raf1 Tpz1^HA
WT
rik1Δ
input
no ab
HA IP
input
no ab
HA IP
HA IP/
FLAG WB
UBA^FLAG Raf1
E Ub^His pull-down
Ccq1^HA
His pull-down
WB
anti-His
anti-HA
input
anti-HA
115-
80-
ub.
unmod.
Ub-His
rik1Δ
Rap1^HA
His pull-down
WB
anti-His
anti-HA
115-
80-
ub.
unmod.
Ub-His
rik1Δ
Epe1^FLAG
His pull-down
anti-FLAG
ub.
unmod.
Ub-His
F Protein expression levels
Ccq1^HA
Tpz1^HA
Pot1^HA
Poz1^HA
Rap1^HA
rik1Δ
Ccq1 >
Tpz1>
< Rap1
*
*
< Poz1
short
long exposure

Figure EV1.

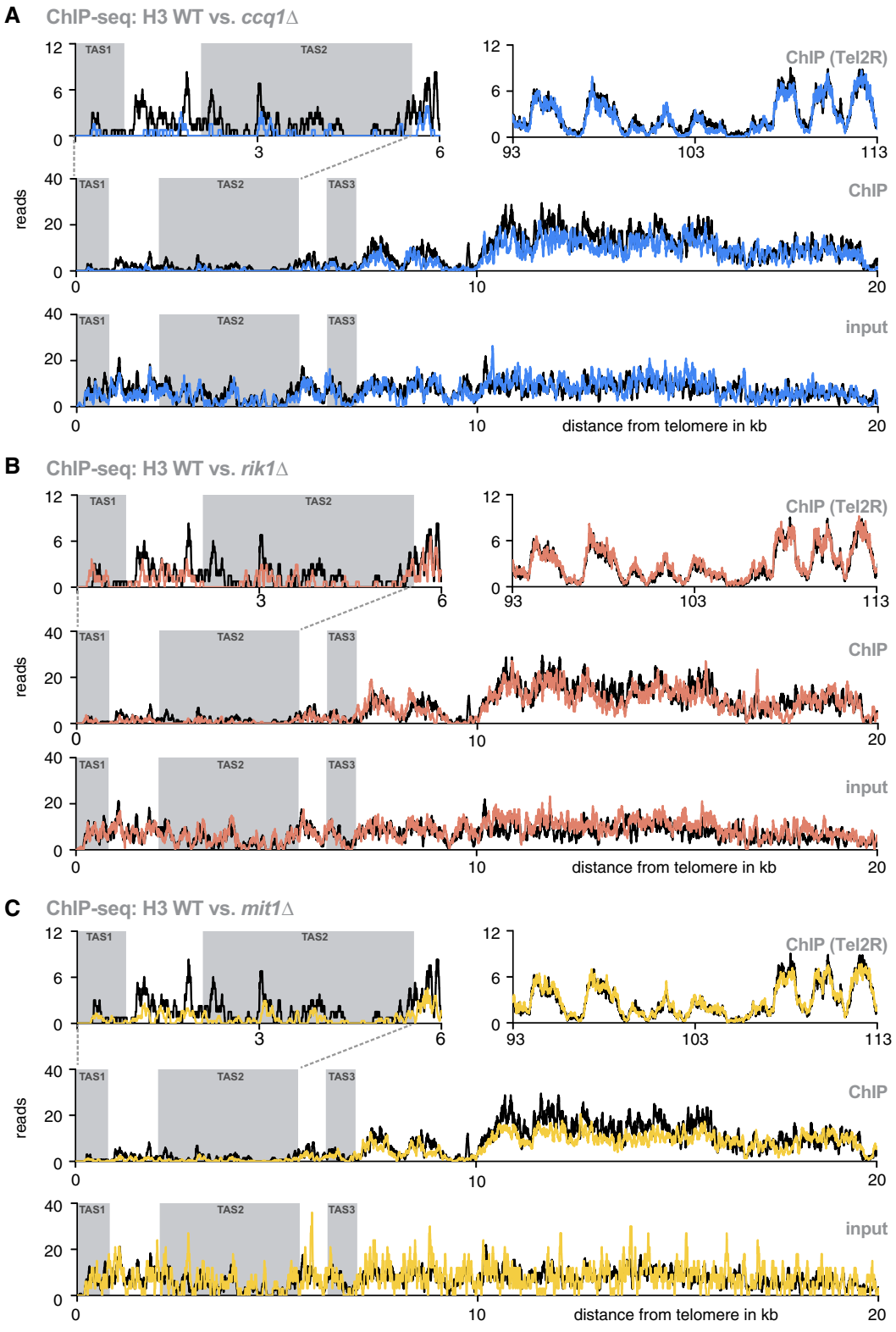

Figure EV2. Subtelomeric H3 profiles in WT cells and mutants deficient in Ccq1, CLRC, and SHREC.

A–C ChIP-seq reads mapped to TEL2R (shown in reverse orientation for consistency) for WT (black) vs. *ccq1*Δ (blue) (A), *rik1*Δ (red) (B), and *mit1*Δ (yellow) cells (C). Shown are reads of ChIP data for TAS1 and TAS2 (top left panel), a nucleosome-free region reported by Tashiro *et al* [12] (top right panel), and the subtelomeres including the TAS1-3 and *tlh1*$^{+}$ (middle panel). The bottom panel shows input samples for the same region. Gray shaded areas are TAS regions.

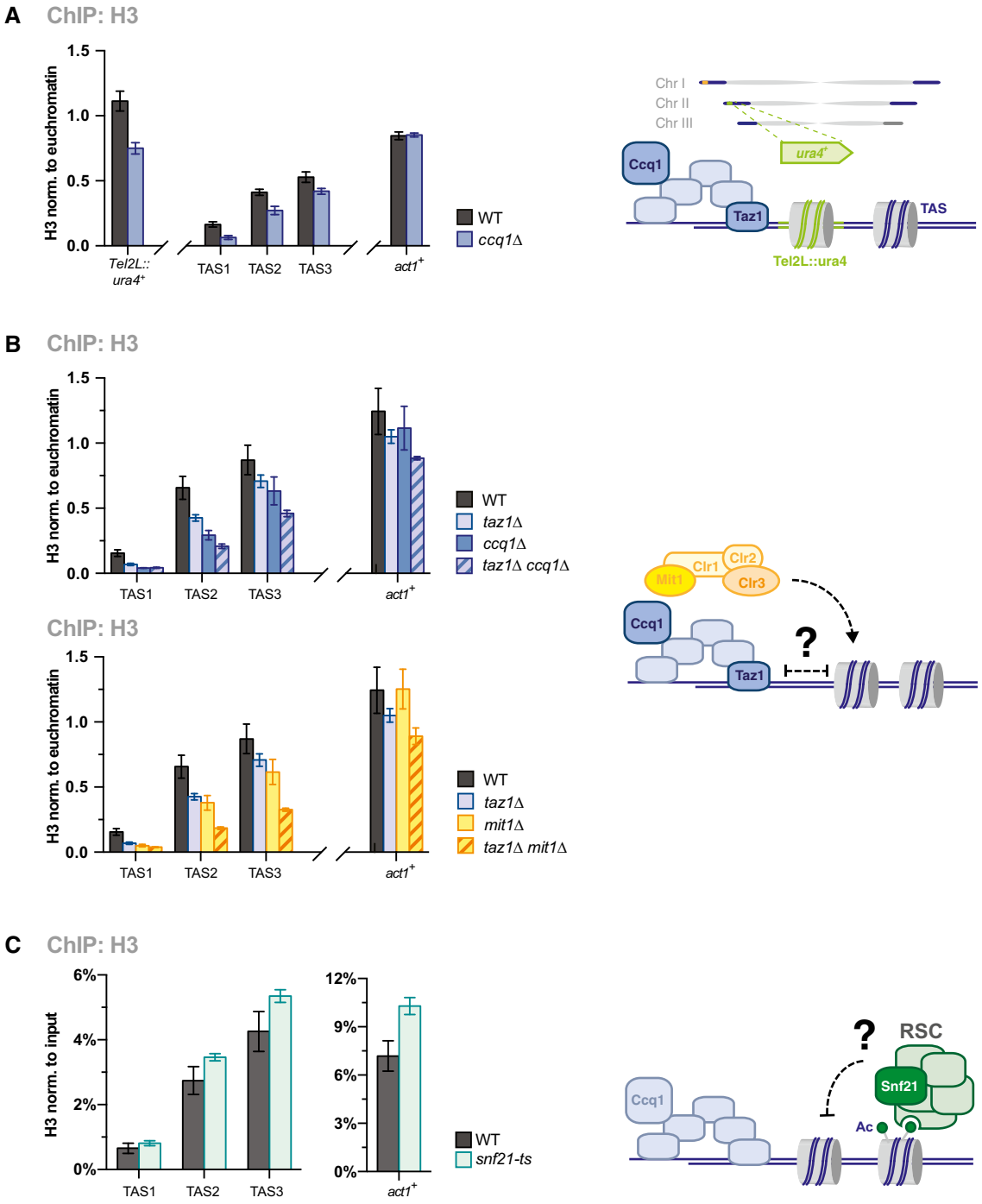

Figure EV3. Low nucleosome occupancy at TAS is not caused by proximity of telomeric repeats or shelterin binding, RSC.

A ChIP-qPCR analysis of H3 at reporter gene (*ura4*$^+$) and TAS regions WT and *ccq1*Δ strains (see scheme) (n = 3 independent experiments).

B ChIP-qPCR analysis of H3 in WT, *ccq1*Δ, and *taz1*Δ (top) or *mit1*Δ (bottom) and corresponding double mutants (see scheme) (n = 3 independent experiments).

C ChIP-qPCR analysis of H3 in WT and *snf21-ts* cells (see scheme) (n = 3 independent experiments each derived from 2 to 3 parallel ChIP samples).

Data information: In (A, B), data are represented as mean ± SEM (normalization as in Fig 2B and C). In (C), data are normalized to input and represented as mean ± SEM, note that the global role of RSC precludes using internal controls (i.e., EC) for normalization.

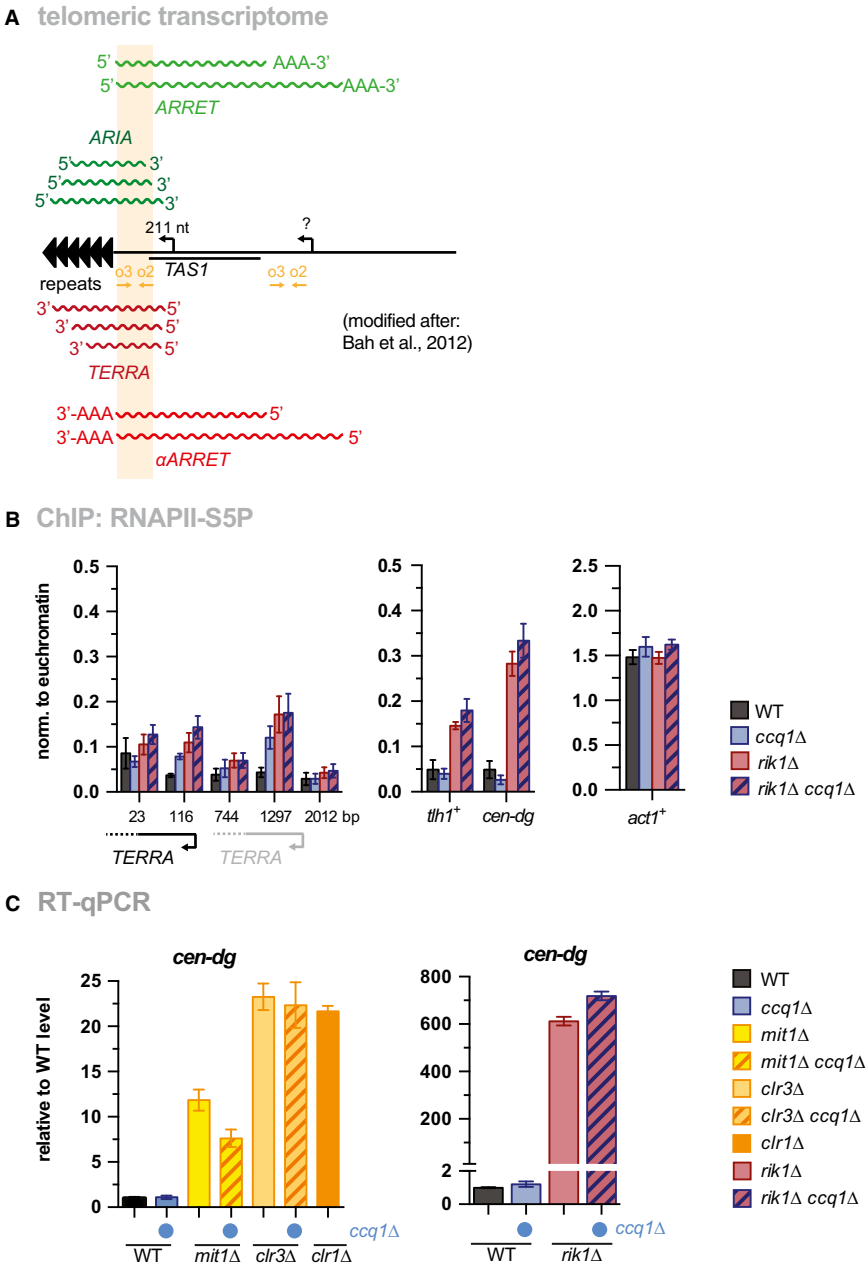

Figure EV4. Expression of telomeric transcripts in the absence of CLRC and SHREC.

A Scheme of telomeric transcripts (modified after [36]). Whereas *ARRET* and *αARRET* RNAs have a poly-A tail, only a small percentage of *TERRA* and *ARIA* transcripts are poly-adenylated. The amplicon (primers o2 + o3) used for RT–qPCR and ChIP-qPCR analysis anneals to all telomeric transcripts without discriminating strand specificity or shorter species that lack transcribed parts from the telomeric repeats. For simplicity, we refer to these transcripts as "*TERRA*". An identical sequence of the o2/o3 amplicon is present in a telomere-distal region, but it is unknown whether this region also contains transcription start sites.

B ChIP-qPCR analysis of RNAPII-S5P in WT, *ccq1Δ*, *rik1Δ*, and the corresponding double mutant (n = 3 independent experiments).

C Ccq1 is not involved in silencing of pericentromeric heterochromatin. RT–qPCR analysis of transcript levels of *cen-dg* in WT, *ccq1Δ*, *mit1Δ*, *clr3Δ*, *rik1Δ*, double mutants with *ccq1Δ* (indicated by blue dot), and *clr1Δ* cells (n = 3 independent experiments)

Data information: In (B), data are shown relative to the average of three euchromatic genes (EC) and represented as mean ± SEM. In (C), data are represented as mean ± SEM and shown relative to WT level.

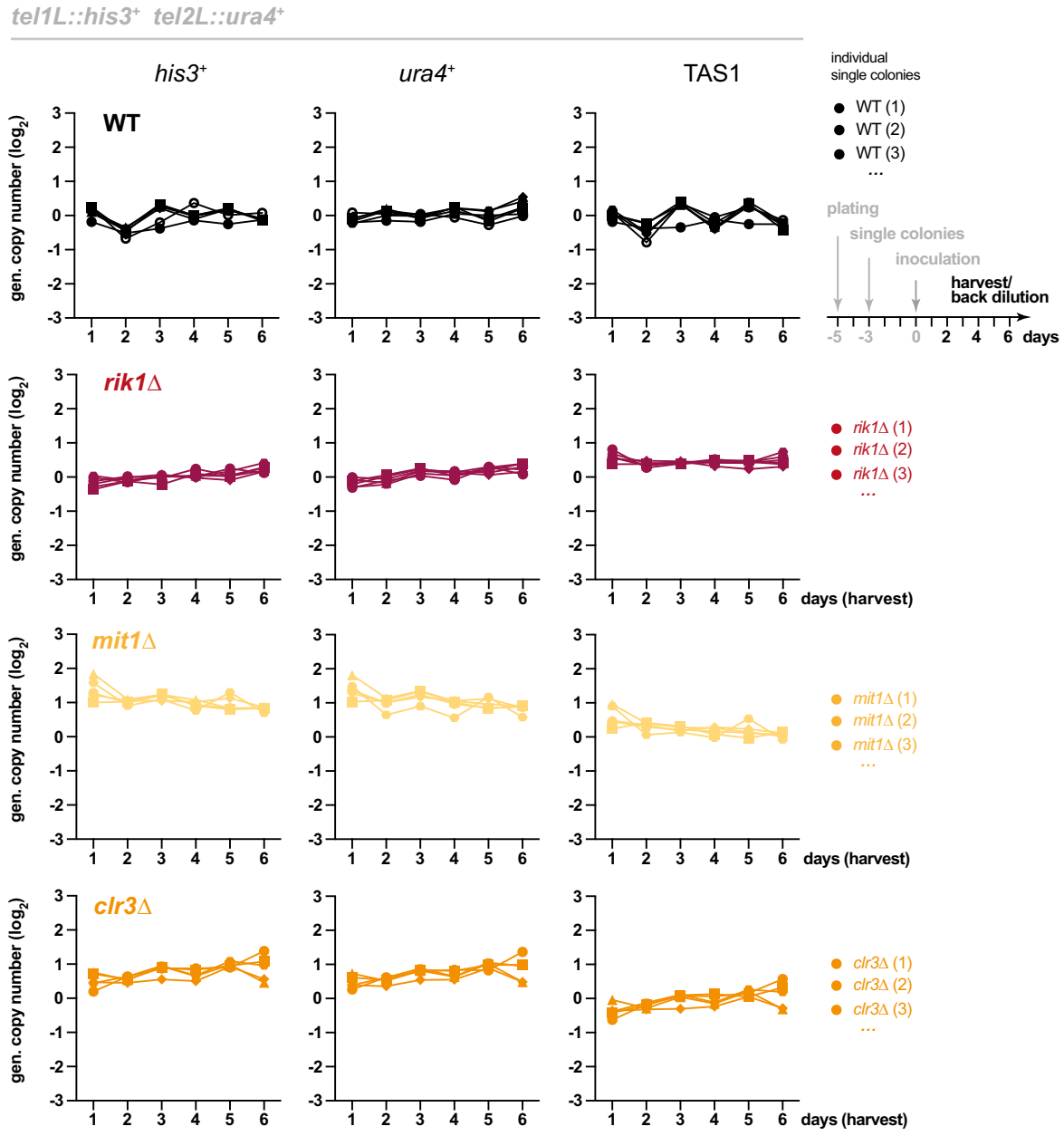

Figure EV5. Subtelomeres do not undergo recombination in the absence of CLRC and SHREC.

Genomic copy number of *his3*$^+$, *ura4*$^+$, and TAS1 in indicated strains harboring the reporter genes *tel1L::his3*$^+$ and *tel2L::ura4*$^+$ cells. Cultures from individual WT and knockout clones (WT, $n = 6$; *rik1Δ*, $n = 6$; *mit1Δ*, $n = 5$; *clr3Δ*, $n = 5$) were inoculated at day 0 to grow in liquid media with regular back-dilution (every 24 h, approximately 7 generations). Samples were taken at indicated harvest times, and relative copy numbers of genomic regions were assessed by qPCR (normalization against intrachromosomal loci).