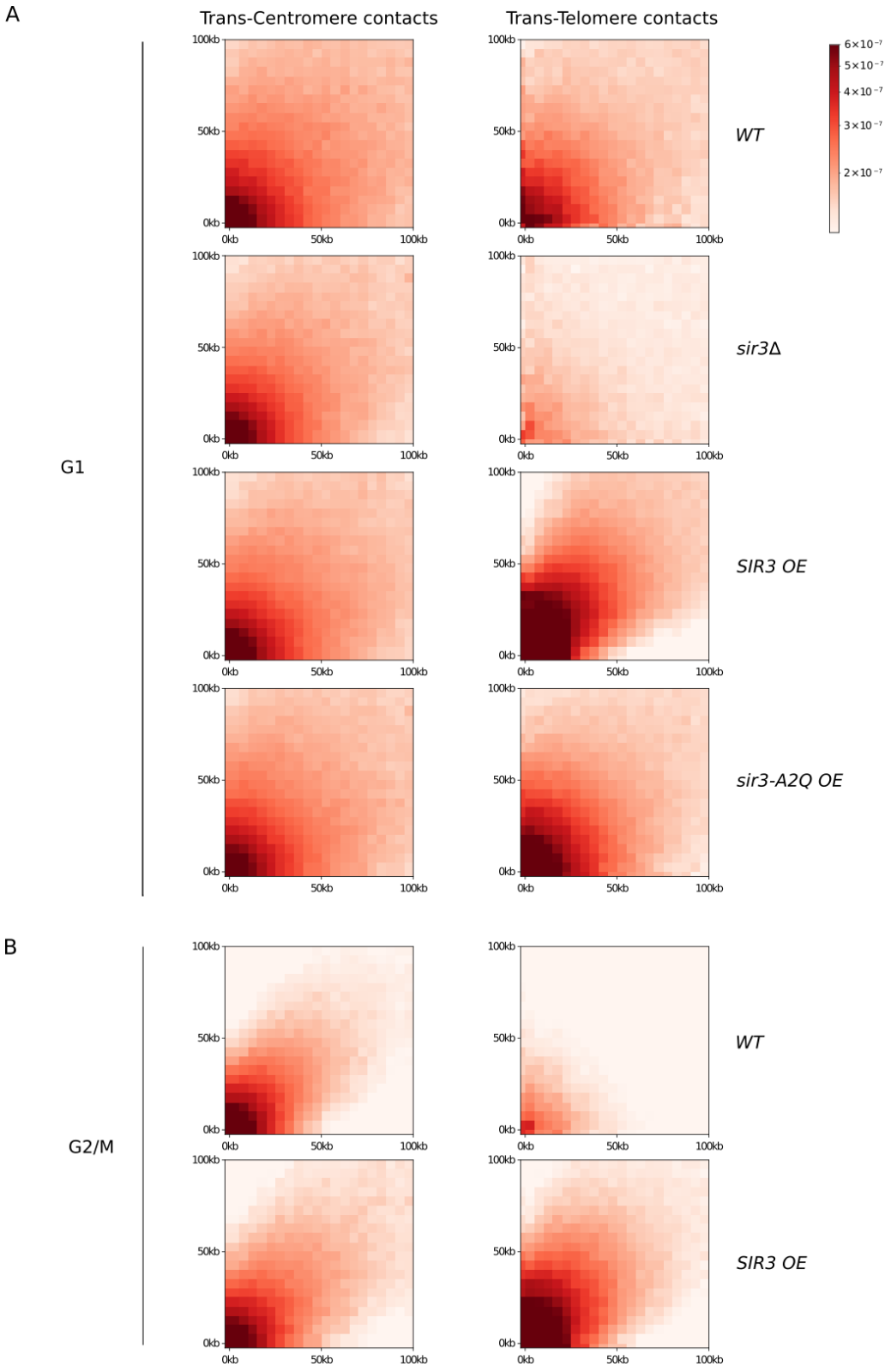
Supplemental information

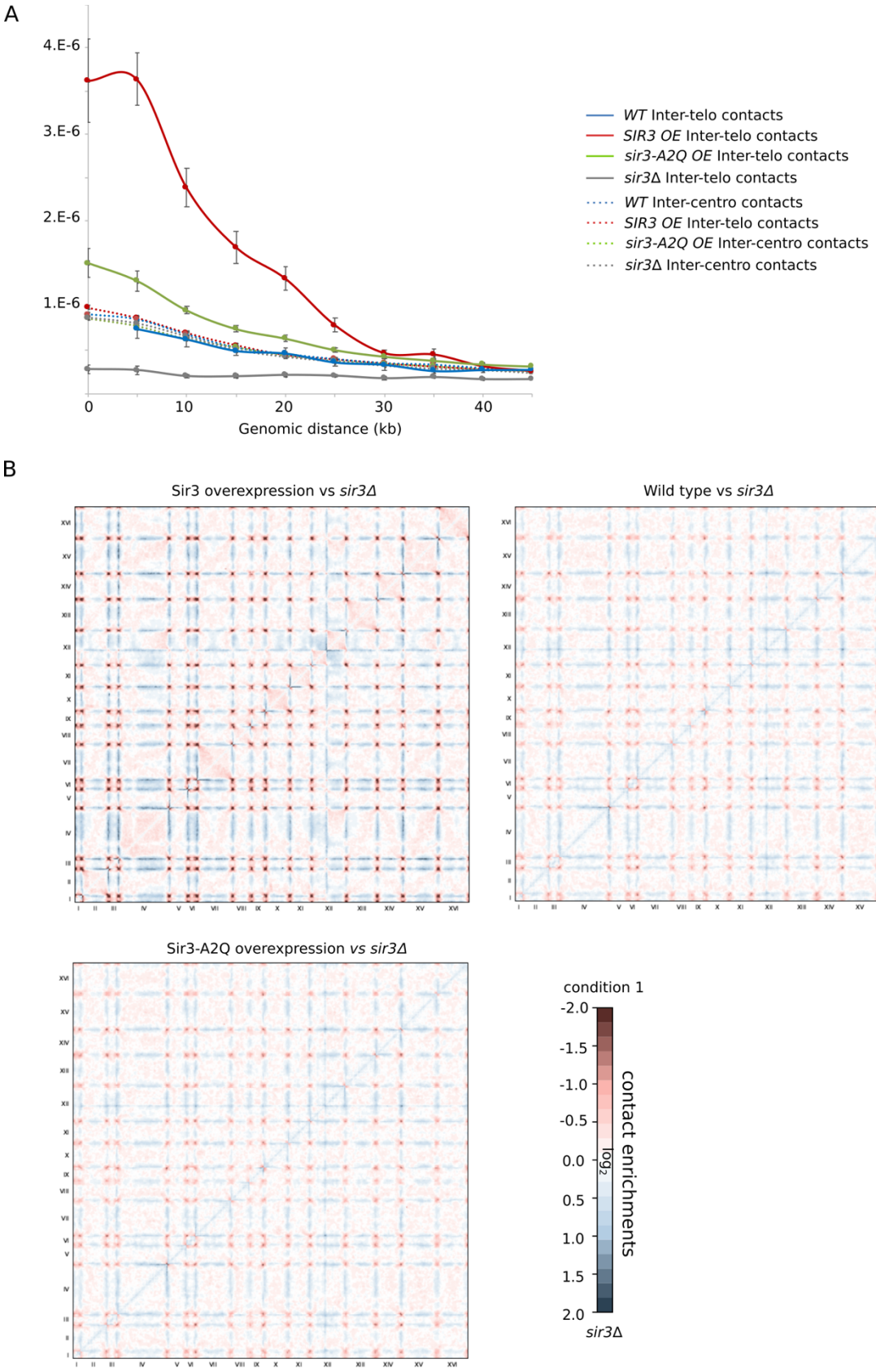
Sir3 mediates long-range chromosome interactions in budding yeast

Supplemental Figure S1



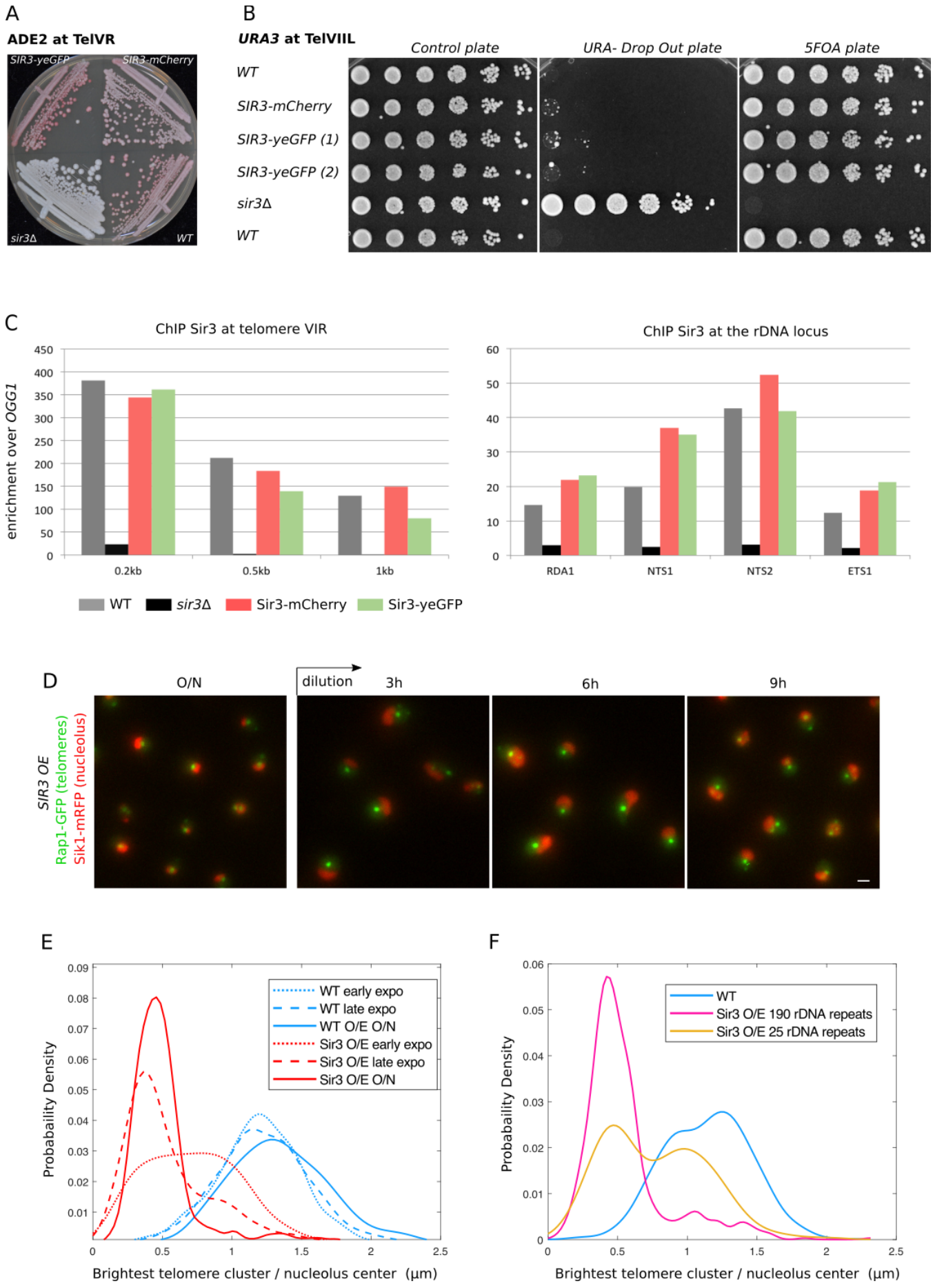
Supplemental Figure S1: Cumulated inter-chromosome contacts maps for the 100kb peri-centromeric and subtelomeric regions, for the strains shown in Figure 1 in G1 (A) or G2/M (B). Chromosomal arms are oriented to have centromere-centromere/telomere-telomere contacts in the lower-left corner of the maps.

Supplemental Figure S2



Supplemental Figure S2: (A) Comparison of Inter-telomere and Inter-centromere contacts as a function of the distance from the telomeres or centromeres respectively. The frequency is evaluated as the mean over all 480 couples of chromosomal arms, the error bar is the standard error. **(B)** Genome-wide ratio maps processed by Serpentine. Corresponding to the magnification displayed in Figure 2D.

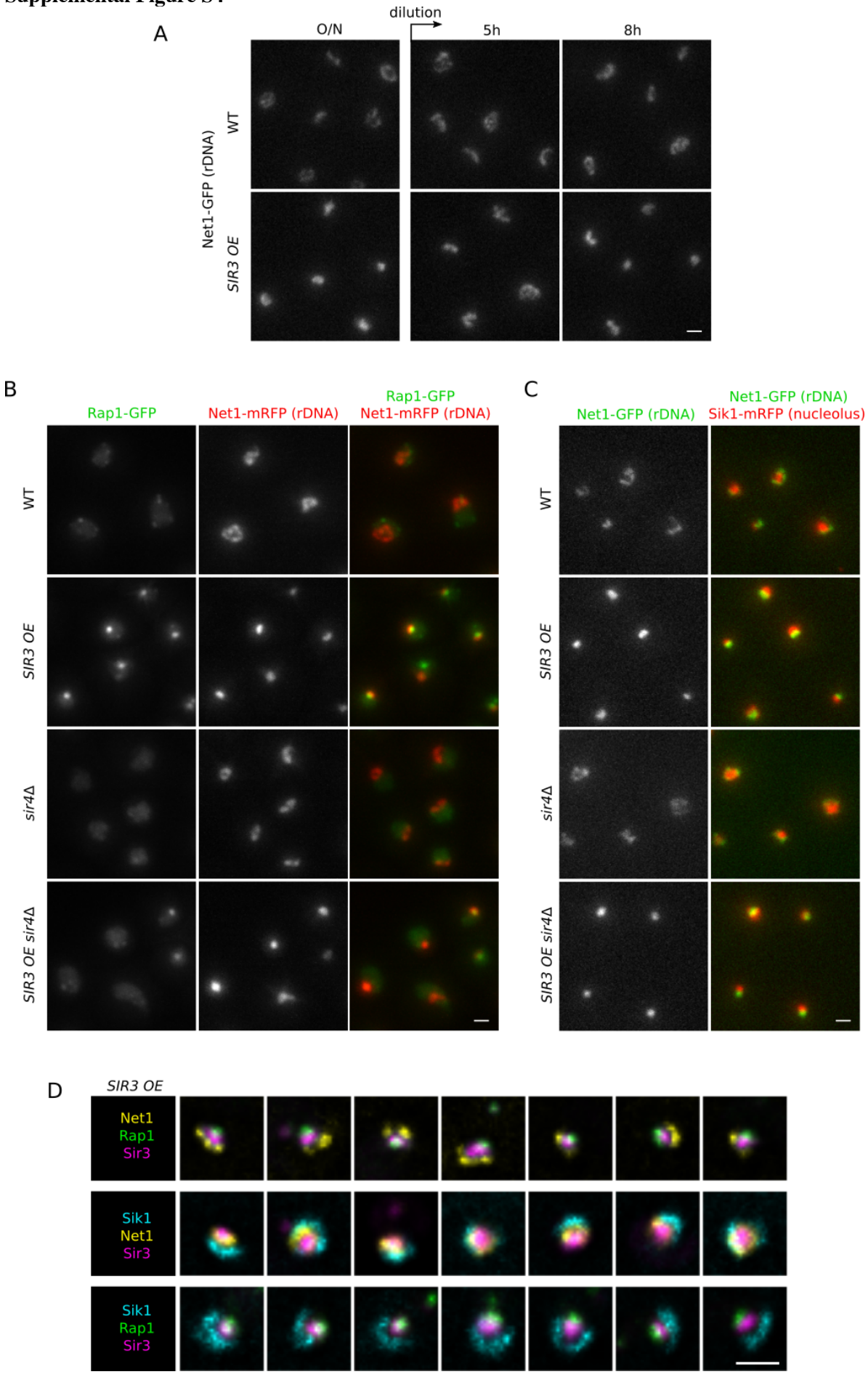
Supplemental Figure S3



Supplemental Figure S3. (A-C) Functional assessment of Sir3-Cterminal tagged proteins. (A) Telomeric silencing

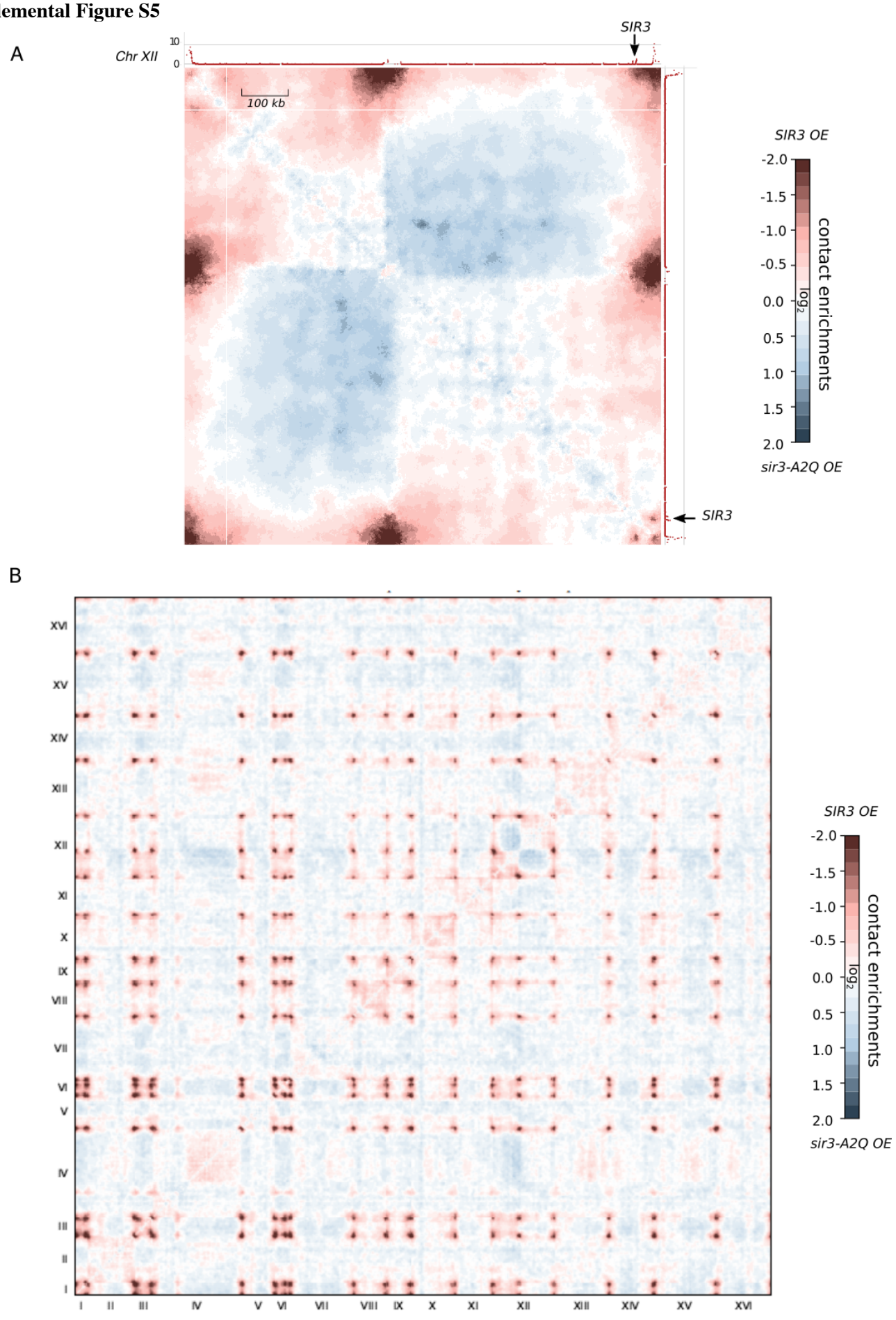
assay at *telVR::ADE2*. To assess the functionality of Sir3-Cterminal tagged proteins, wild-type (WT; yAT71), *sir3Δ* (yAT1196), *SIR3-mCherry* (yAT52), and *sir3-yeGFP* (yAT4064) cells were streaked on a YPD plate. After 4 days, the plates were stored for 4 days at 4°C before taking the pictures of the plates. The color of the colonies is indicative of the state of silencing of the *ADE2* reporter gene at *telVR*: the *ADE2* gene is expressed in white colonies and repressed in pink colonies. **(B)** Telomeric silencing assay at *telVIIL::URA3*. The same strains than in (A) were grown in YPD, cultures were normalized to 1.2×10^7 cells / ml and fivefold serial dilutions were plated either onto CSM, URA- drop out or 5-FOA plates. Growth on URA- plates and decreased growth on 5-FOA plates reflect a disruption of telomeric silencing. **(C)** The graphs represent Sir3 occupancy along subtelomere VIR and the rDNA locus probed by ChIP-qPCR using an anti-Sir3 antibody (Ruault *et al*., 2011) in WT (yAT1684), *sir3Δ* (yAT1710), *SIR3-mCherry* (yAT3653), and *SIR3-yeGFP* (yAT2803) strains. The bar graph represents the Sir3 enrichment over *OGG1*. **(D-F) Association of the telomere hypercluster and the rDNA in strains overexpressing Sir3 is regulated by the physiology of the cell and the size of the rDNA array. (D)** Representative fluorescent images of a double tagged strain Rap1-GFP / Sik1-mRFP overexpressing Sir3 under the GPD promoter (yAT1046) in different physiological conditions: after an overnight culture and 3h, 6h and 9h after dilution in fresh medium. Cells were grown in CSM 2% glucose. Scale bar is 1 μm. **(E)** Distance between the brightest Rap1-GFP cluster and the nucleolus center is plotted for a wild-type (yAT1782) and a strain overexpressing Sir3 (yAT1827) measured in different conditions: after an overnight culture (n=757 for yAT1782, n=596 for yAT1827), in early (n=652 for yAT1782, n=546 for yAT1827) and late exponential phase (n=739 for yAT1782, n=513 for yAT1827). Cells were grown in CSM 2% galactose. Imaged were analyzed using the Nucloc software (Berger et al., 2008). **(F)** Distance between the brightest Rap1-GFP cluster and the nucleolus center is plotted for a wild-type (yAT340, n=581, same data than in Figure 3E), for a long rDNA strain overexpressing Sir3 (190 repeats, yAT1778, n= 588) and for a short rDNA strain overexpressing Sir3 (25 repeats, yAT1780, n= 367). Cells were grown in CSM 2% galactose. Data were acquired after an overnight culture and analyzed using the Nucloc software (Berger et al., 2008).

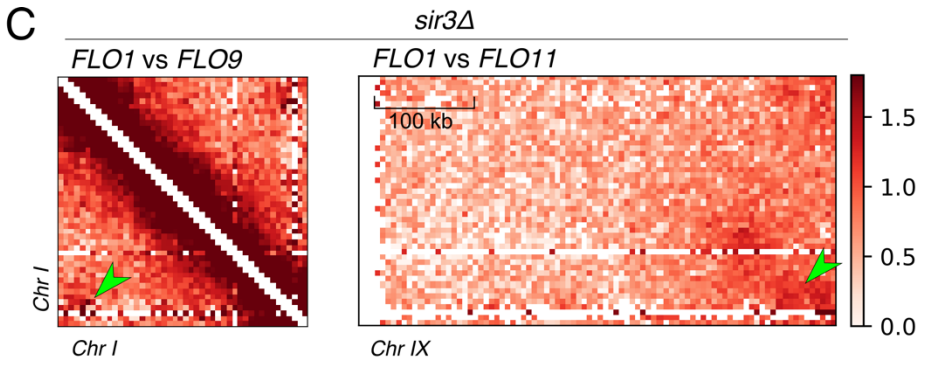
Supplemental Figure S4



Supplemental Figure S4: rDNA spatial reorganization upon Sir3 overexpression is independent of the formation of the telomere hypercluster and is regulated by the physiology of the cell. (A) Representative fluorescent images of Net1-GFP in wild-type strain (yAT1004) and in a strain expressing high Sir3 levels (yAT1724). Images were taken in different physiological conditions: after an overnight culture and 5h and 8h after dilution in fresh medium. **(B)** Representative fluorescent images of a double tagged strain Rap1-GFP / Sik1-mRFP in a wild-type strain (yAT3729), in a strain expressing high Sir3 levels (yAT3730), in a *sir4Δ* (yAT3765) and in a *sir4Δ* expressing high Sir3 levels (yAT3743). Cells were grown in synthetic CSM 2% galactose and imaged after an overnight culture. **(C)** Representative fluorescent images of a double tagged strain Net1-GFP / Sik1-mRFP in wild-type strain (yAT1004), in a strain expressing high Sir3 levels (yAT1724), in a *sir4Δ* (yAT1723) or in a *sir4Δ* strain expressing high Sir3 levels (yAT2124). Cells were grown in CSM 2% glucose and imaged after an overnight culture. **(D)** Additional representative cells related to Figure 4C. scale bar is 1 μm for all the panels.

Supplemental Figure S5

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FLO1 vs FLO11
100 kb
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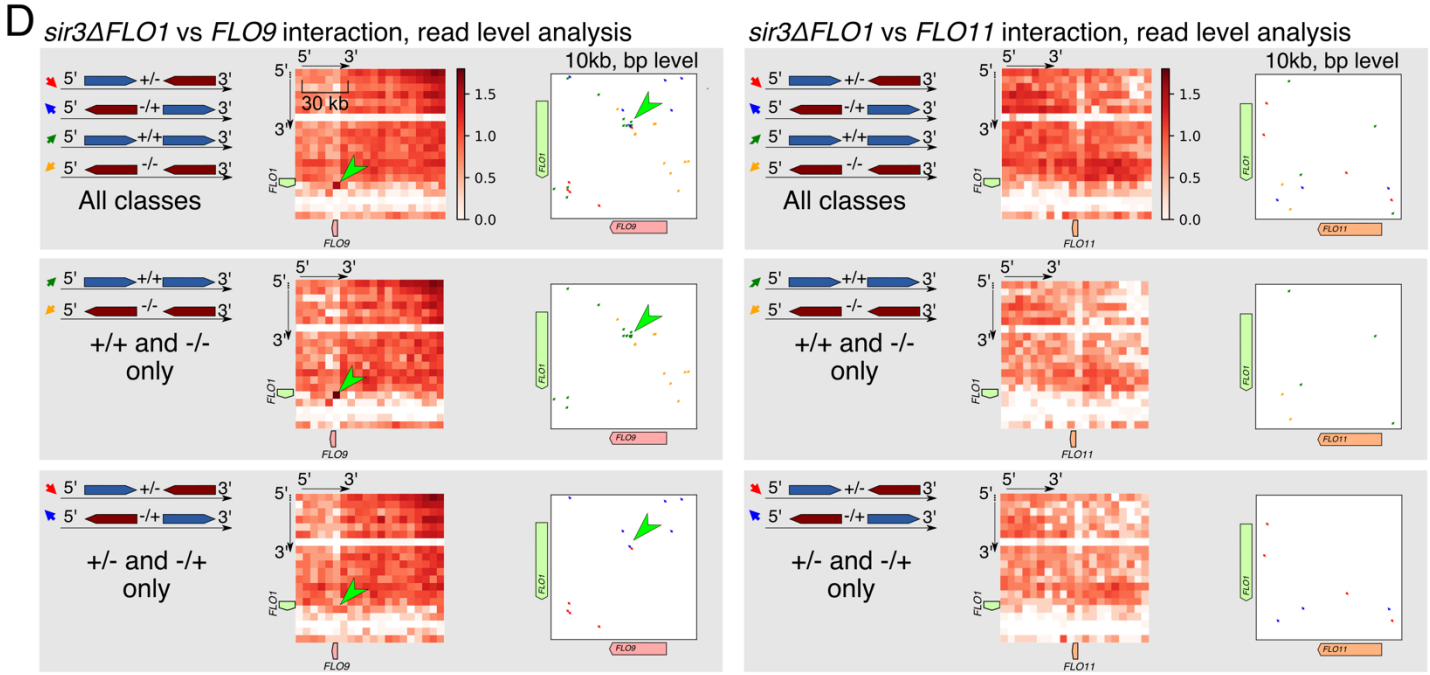
1.0
0.5
0.0
Chr I
Chr I
Chr IX


D
sir3ΔFLO1 vs FLO9 interaction, read level analysis
sir3ΔFLO1 vs FLO11 interaction, read level analysis
All classes
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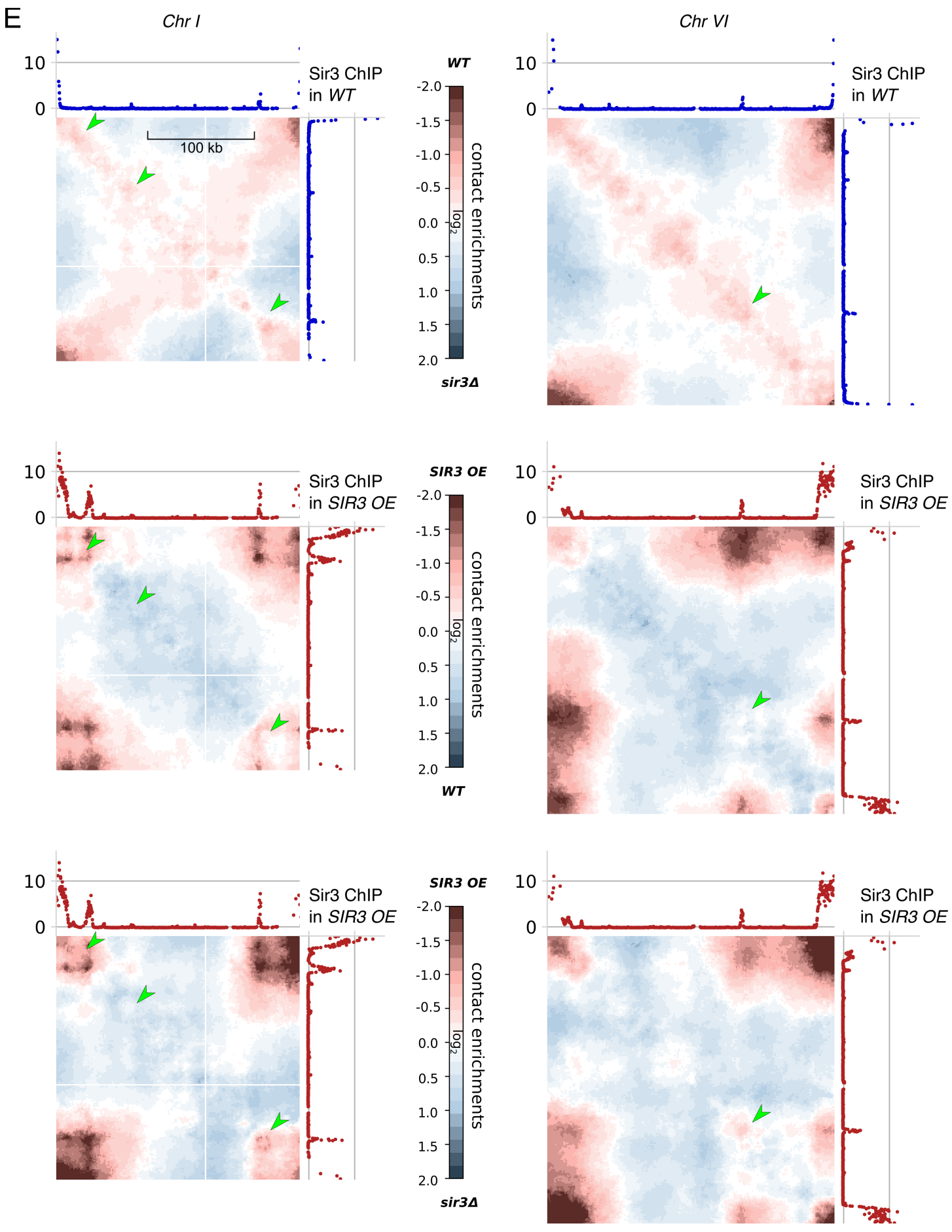
+/- and -/+ only
30 kb
10kb, bp level
FLO1
FLO9
FLO11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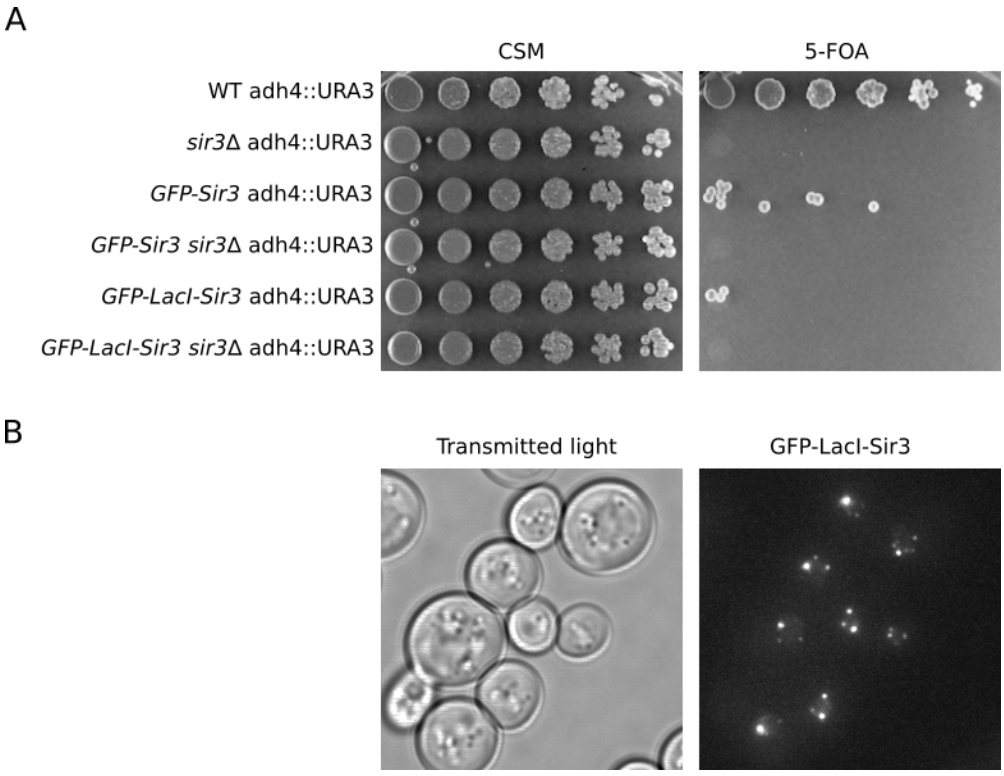
Chr I
Chr VI
10
0
Sir3 ChIP
in WT
100 kb
WT
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
log2
contact enrichments
sir3Δ
Sir3 ChIP
in SIR3 OE
SIR3 OE
WT
Sir3 ChIP
in SIR3 OE
SIR3 OE
sir3Δ
Sir3 ChIP
in SIR3 OE

Supplemental Figure S5: (A). Effects of Sir3 binding on *SIR3*. Intrachromosomal ratio plot of Chromosome XII contact maps generated in *sir3-A2Q OE* and *SIR3 OE* conditions, and processed by Serpentine binning. The ChIP deposition profile of Sir3 in overexpressing conditions are plotted along the top and right axis. Intrachromosomal loci enriched in Sir3 are indicated by the closest gene name. **(B) Genome-wide ratio maps processed by Serpentine.** Corresponding to the magnification displayed in Figure 5 and S5A **(C-D) Contacts between *FLO1, FLO9* and *FLO11* genes**. **(C)** Normalized contact maps binned at 5kb, showing the interactions between Chromosome I with itself and Chromosome I with IX, in *sir3Δ* strains grown in exponential conditions. Green arrows point to the bins connecting *FLO1, FLO9* and *FLO11* genes. **(D)** Zoom of the maps shown in panel A, for two different classes of aligned reads (same direction vs convergent + divergent reads). Contacts that result from alignment artefacts, often display a bias toward one of the two class of reads with respect to the other. This can be seen in the *FLO1 vs FLO9* interaction (green arrow left panel), and not in the *FLO1 vs FLO11* interactions (green arrow right panel). Finally, arrow plots show contact maps at read level. The green arrow on the arrow plots of the left panel points to the couple of sites that account for the alignment bias between different read classes. This is localized between a couple of regions that present high degree of homology between the *FLO1* and *FLO9* genes. **(E) Contacts between internal binding sites and subtelomeres in wild type conditions.** Serpentine maps of Chromosome I and VI, comparing Hi-C maps obtained in overnight culture, showing contact enrichments between wild type and *sir3Δ* mutant; cells overexpressing Sir3 to wild type, and cells overexpressing Sir3 to *sir3Δ* mutant. Green arrows represent the position along the diagonal of internal Sir3 binding sites.

Supplemental Figure S6



Supplemental Figure S6: Trans-interactions are independent of Sir3 silencing function.
(A) Growth assay, telomeric silencing assay at *telVIIL::URA3* were carried out with the following strains: wild-type (yAT69), *sir3Δ* (yAT1010), *pHIS3-GFP-Sir3* (yAT1011), *pHIS3-GFP-SIR3 sir3Δ* (yAT1676), *pHIS3-GFP-LacI-Sir3* (yAT821) and *pHIS3-GFP-LacI-Sir3 sir3Δ* (yAT921). To monitor telomeric silencing at *telVIIL::URA3*, strains were grown in YPD overnight and plated in 5-fold serial dilutions starting at OD_{600nm}= 1 (corresponding to 1×10^7 cells/ml) onto appropriate plates: complete CSM without uracil as a growth control plate and onto a 5-FOA plate to assess telomeric silencing. Decreased growth on the 5-FOA plate reflects the disruption of telomeric silencing (expression of the *URA3* gene at telo *VIIL*). **(B)** Representative images (Transmitted-light image and the GFP channel fluorescent image) of a strain expressing the *pHIS3-GFP-LacI-Sir3* construct (yAT788) grown in complete synthetic medium (exponential phase).

Materials and Methods for Supplemental Figure 3 and 6

Silencing assays

For telomeric silencing assays, cultures were grown in liquid YPD and plated in five-fold serial dilutions starting at OD_{600nm} =1 (1.2×10^7 cells /ml) onto appropriate plates. 5-fluoroorotic acid (5-FOA; Zymo Research) plates were prepared by adding 5-FOA to a final concentration of 0.1% to supplemented synthetic medium.

Supplemental Table S1. Yeast strains used in this study.

W303-1 derived strains : MATa ade2-1 can1-100 leu2-3,112 his3-11,15 trp1-1 ura3-1 (W303-1)

yAT208 MATα ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)$_n$ ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) sir3::pGAL1-SIR3(KanMX)
yAT232 MATa ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)$_n$ ppr1Δ::HIS3 rap1::RAP1-GFP(LEU2)
yAT340 MATα ade2-1::ADE2 rap1::GFP-RAP1(LEU2) sik1::SIK1-mRFP(KanMX)
yAT341 MATa ade2-1::ADE2 rap1::GFP-RAP1(LEU2) sik1::SIK1-mRFP(KanMX) sir3::pGAL1-SIR3(KanMX)
yAT772 MATα ade2-1::ADE2 rap1::RAP1-GFP(LEU2) sir2Δ::KanMX sir3::pGAL1-SIR3(NAT)
yAT1004 MATα ade2-1::ADE2 net1::NET1-GFP(HIS3MX) sik1::SIK1-mRFP(KanMX)
yAT1008 MATα ade2-1::ADE2 net1::NET1-GFP(HIS3MX) sik1::SIK1-mRFP(KanMX) sir3::pGAL1-SIR3(NAT)
yAT1198 MATα ade2-1::ADE2 rap1::GFP-RAP1(LEU2) sik1::SIK1-mRFP(KanMX) sir3::pGal1-sir3-A2Q(NAT)
yAT1205 MATa ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)$_n$ ppr1Δ::HIS3 rap1::RAP1-GFP(LEU2) sir3::pGal1-sir3-A2Q(KanMX)
yAT1470 MATa ade2-1::ADE2 adh4::URA3 his3::GFP-LacI-SIR3(HIS3) leu2::LacOp(LEU2) lys2::LacOp(TRP1) rap1-17 sir3Δ::kanMX
yAT1476 MATα ade2-1::ADE2 his3::GFP-LacI(HIS3) leu2::LacOp(LEU2) lys2::LacOp(TRP1) rap1-17 sir3Δ::kanMX
yAT1541 MATα ade2-1::ADE2 net1::NET1-GFP(HIS3MX) sik1::SIK1-mRFP(KanMX) sir3::pGAL1-sir3-A2Q(NAT)
yAT1864 MATα ade2-1::ADE2 his3::GFP-LacI-SIR3(HIS3) leu2::LacOp(LEU2) lys2::LacOp(TRP1) rap1-17 sir3Δ::kanMX sir4Δ::NAT
yAT2213 MATa Net1::NET1-GFP(HIS3MX) sik1::SIK1-BFP2(HPH) sir3::pGPD-SIR3(KanMX) sir3::SIR3-mcherry(kan::ADE2)
yAT2803 MATa ade2-1::ADE2 hmlΔ::HPH net1::NET1-TagRFP-T(HIS5sp) sir3::SIR3-yEGFP(TRP1)
yAT3113 MATa ade2-1::ADE2 hmlΔ::HPH net1::NET1-TagRFP-T(HIS5sp) sir3::pGPD-SIR3(NAT) sir3::SIR3-yeGFP(TRP1)
yAT3666 MATa hmlΔ::NAT rap1::GFP-RAP1(LEU2) sir3::pGPD-SIR3(KanMX) sir3::SIR3-mCherry(kan::ADE2) sik1::SIK1-BFP2(HPH) LacO at RPL9A ::TRP1
yAT3729 MATa ade2-1::ADE2 hmlΔ::HPH rap1::RAP1-GFP(LEU2) net1::NET1-mCherry(KanMX)
yAT3730 MATa ade2-1::ADE2 hmlΔ::HPH rap1::RAP1-GFP(LEU2) net1::NET1-mCherry(KanMX) sir3::pGPD-Sir3(NAT)
yAT3733 MATa ade2-1::ADE2 hmlΔ::HPH rap1::RAP1-GFP(LEU2) net1::NET1-mCherry(KanMX) sir3::pGPD-sir3-A2Q(NAT)
yAT3901 MATa net1::Net1-BFP2(HPH) RAD5 rap1::GFP-RAP1(LEU2) sir3::pGPD-SIR3(KanMX) sir3::SIR3-mcherry(kan::ADE2)

BY4741 derived strains: MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0

yAT2476 MATa rap1::GFP-RAP1(LEU2) sir3::pGPD-SIR3(NAT)
yAT2583 MATa hmlΔ::HPH rap1::RAP1-GFP(LEU2)
yAT2584 MATa hmlΔ::HPH rap1::RAP1-GFP(LEU2) sir3Δ::KanMX
yAT2822 MATa hmlΔ::HPH rap1::RAP1-GFP(LEU2) sir3::pGPD-SIR3-A2Q(NAT)
RSGY584 MATα hmlΔ::HPH; sir3Δ::KanMX; IV(715448-845757)::synIV(715448-845757 LEU2)

Table S2. Primers used in this study.

Gene Name	Sequence
OLI1	F: GAGCAGGTATTGGTATTGCTATCG
	R: TTGATGGGTTTCTTGATACACCAT
OGG1	F: CAATGGTGTAGGCCCCAAAG
	R: ACGATGCCATCCATGTGAAGT
RDN25	F: CGCCGACGTCTCCACATT
	R: GATTCCGGAACCTGGATATGG
NTS1	F: GGCTTCCTATGCTAAATCCCATAAC
	R: GCAGCTGGATAGTGCGAATTTT
NTS2	F: TGCCGCCGACATTCTGT
	R: GGATGCGGGCGATAATGAC
ETS1	F: GCGACTCTCTCCACCGTTTG
	R: CGAGTAGGCTTGTCGTTCGTT

Table S3. Yeast strains used in Supplemental material.

W303-1 derived strains : MATa ade2-1 can1-100 leu2-3,112 his3-11,15 trp1-1 ura3-1 (W303-1)

yAT69 MATα adh4::URA3-4xUASG-$(C1\text{-}3A)_n$
yAT340 MATα ade2-1::ADE2 rap1::GFP-RAP1(LEU2) sik1::SIK1-mRFP(KanMX)
yAT788 MATa ade2-1::ADE2 his3::GFP-LacI-SIR3(HIS3) sir3Δ::KanMX
yAT821 MATα adh4::URA3-4xUASG-$(C1\text{-}3A)_n$ pHIS3-GFP-LacI-SIR3(HIS3)
yAT921 MATα adh4::URA3-4xUASG-$(C1\text{-}3A)_n$ pHIS3-GFP-LacI-SIR3(HIS3) sir3Δ::KanMX
yAT1004 MATα ade2-1::ADE2 net1::NET1-GFP(HIS3MX) sik1::SIK1-mRFP(KanMX)
yAT1010 MATα adh4::URA3-4xUASG-$(C1\text{-}3A)_n$ sir3Δ::kanMX
yAT1011 MATα adh4::URA3-4xUASG-$(C1\text{-}3A)_n$ pHIS3-GFP-SIR3(HIS3)
yAT1046 MATα ade2-1::ADE2 rap1::GFP-RAP1(LEU2) sik1::SIK1-mRFP(KanMX) sir3::pGPD-SIR3(NAT)
yAT1676 MATα adh4::URA3-4xUASG-$(C1\text{-}3A)_n$ pHIS3-GFP-SIR3(HIS3) sir3Δ::KanMX
yAT1684 MATa hmlΔ::HPH RAD5+ rap1::RAP1-GFP(LEU2) RDN1::ADE2
yAT1710 MATa hmlΔ::HPH RAD5+ rap1::RAP1-GFP(LEU2) RDN1::ADE2 sir3Δ::KanMX
yAT1723 MATα ade2-1::ADE2 net1::NET1-GFP(HIS3MX) sik1::SIK1-mRFP(KanMX) sir4Δ::HPH
yAT1724 MATα ade2-1::ADE2 net1::NET1-GFP(HIS3MX) sik1::SIK1-mRFP(KanMX) sir3::pGPD-SIR3(NAT)
yAT1778 MATa fob1Δ::HIS3 rDNA copy number 190 rap1::GFP-RAP1(ADE2) sik1::SIK1-mRFP(KanMX) sir3::pGAL1-SIR3(NAT)
yAT1780 MATa fob1Δ::HIS3 rDNA copy number 25 rap1::GFP-RAP1(ADE2) sik1::SIK1-mRFP(KanMX) sir3::pGAL1-SIR3(NAT)
yAT1782 MATa fob1Δ::HIS3 rDNA copy number 190 rap1::GFP-RAP1(ADE2) sik1::SIK1-mRFP(KanMX)
yAT1827 MATa fob1Δ::HIS3 rDNA copy number 190 rap1::GFP-RAP1(ADE2) sik1::SIK1-mRFP(KanMX) sir3::pGAL1-SIR3(NAT)
yAT2124 MATα ade2-1::ADE2 net1::NET1-GFP(HIS3MX6) sik1::SIK1-mRFP(KanMX) sir3::pGPD-SIR3(NAT) sir4Δ::HPH
yAT2803 MATa ade2-1::ADE2 hmlΔ::HPH net1::NET1-TagRFP-T(HIS5sp) sir3::SIR3-yEGFP(TRP1)
yAT3653 MATa hmlΔ::NAT ppr1Δ::HIS3 sir3::SIR3-mCherry(kan::ADE2)
yAT3729 MATa ade2-1::ADE2 hmlΔ::HPH rap1::RAP1-GFP(LEU2) net1::NET1-mCherry(KanMX)
yAT3730 MATa ade2-1::ADE2 hmlΔ::HPH rap1::RAP1-GFP(LEU2) net1::NET1-mCherry(KanMX) sir3::pGPD-Sir3(NAT)
yAT3743 MATa ade2-1::ADE2 hmlΔ::HPH rap1::RAP1-GFP(LEU2) net1::NET1-mCherry(KanMX) sir3::pGPD-SIR3(NAT) sir4Δ::HIS3MX6
yAT3765 MATa ade2-1::ADE2 hmlΔ::HPH net1::NET1-mCherry(KanMX) rap1::RAP1-GFP(LEU2) sir4Δ::HIS3MX6

YPH499 derived strains: MATa ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1

yAT52 MATa adh4::URA3 ppr1Δ::HIS3 rap1::RAP1-GFP(LEU2) sir3::SIR3-mCherry(KanMX) TelVR::ADE2
yAT71 MATa adh4::URA3 ppr1Δ::HIS3 TelVR::ADE2
yAT1196 MATa adh4::URA3 ppr1Δ::HIS3 rap1::RAP1-GFP(LEU2) sir3Δ::KanMX TelVR::ADE2
yAT4064 MATa adh4::URA3 ppr1Δ::HIS3 rap1::RAP1-GFP(LEU2) sir3::SIR3-yEGFP(TRP1) TelVR::ADE2