# Supplemental Materials Molecular Biology of the Cell

Eng et al.



### Supplemental Figure 1. Genetic linkage of mcd1-Q266 and smc3-K113R to suppression of temperature sensitivity of in mcd1-1, smc3-42, or smc3-AID<sup>608</sup> strains.

(A) Haploid yeast strain mcd1-1 (yVG3312-7A) was transformed with Stul digested plasmid pVG285/mcd1-Q266-URA3 (resulting strain yTE42) and resulting colonies were patched onto a ura- plate at 30°C. The outgrowth plate was replica plated to YPD plates, which were subsequently incubated at 23°C, 30°C, or 37°C. Growth of transformants was assessed three days after replica plating. mcd1-1 strains do not grow at 37°C, but mcd1-1 mcd1-Q266-URA3 strains show robust growth at 37°C. (B) Transformant genotyping. Independent transformants from (A) were genotyped using MCD1 primers flanking the 15 basepair insertion (and unique Pmel restriction endonuclease site) encoding the corresponding amino acids in the mcd1-Q266 allele. After colony PCR, ~1U of Pmel was added to the reaction and incubated for 1hr at 37°C in PCR buffer. The digested PCR reaction was run on a 1% TAE gel. The presence of the largest PCR product corresponds to the expected product size of a wild-type MCD1 amplicon. The two smaller bands are the expected digestion products resulting from a unique Pmel site inserted in the same MCD1 amplicon. Representative wild-type MCD1 and mcd1-Q266-URA3 mcd1-1 genotypes are shown. (C) Genetic linkage of temperature sensitivity to mcd1-Q266-URA3. Transformants from (A) were grown on FOA media to select for cells which had spontaneously lost mcd1-Q266-URA3. After a second round of selection on FOA, cells were patched onto YPD plates and incubated at the indicated temperature. Two parental clones of mcd1-1 mcd1-Q266-URA3 are shown as well as six independent popout isolates. (D) Genetic linkage of temperature sensitivity suppression to smc3-K113R-URA3. As processed in B, smc3-K113R-URA3 smc3-42 (yVG3473-1C) cells were grown on FOA to select for spontaneous urarevertants. Three independent "popout" clones are shown. Strains which have spontaneously lost smc3-K113R-URA3 fail to grow at 37°C. For comparison, smc3-K113R smc3-42 cells which still harbor smc3-K113R-URA3 still grow at 37°C (albeit poorly) when compared to SMC3 smc3-42 strains (yVG3377-IA). Parental smc3-42 (yVG3358-3B) grow well at 23°C but fail to grow at 37°C. (E) Linkage of auxin sensitivity in smc3-AID<sup>608</sup> strains to the presence of smc3-K113R-URA3. As described for (C), smc3-K113R-URA3 smc3-AID<sup>608</sup> cells were grown on FOA to select for spontaneous loss of smc3-K113R-URA3. These FOA resistant cells were then replica plated to YPD or YPD plates supplemented with auxin. Patches from nine independent transformants are shown. Loss of smc3-K113R-URA3 restores auxin sensitivity of smc3-AID<sup>608</sup> strains.



#### Supplemental Figure 2. Allele specificity of complementation pairs.

(A) Allelic specificity of complementation pairs in *MCD1*. Haploid yeast strains *mcd1-1* (yVG3312-7A), *MCD1 mcd1-1* (yTE396), *mcd1-V137K mcd1-1* (yTE388), or *mcd1-R135 mcd1-1* (yTE392) were grown and diluted as described in Figure 1A and plated onto YPD and incubated at either 23°C or 37°C for 3d. All dilutions shown are from the same YPD plate. All strain grew well at 23°C but *mcd1*, *mcd1-V137K mcd1-1* and *mcd1-R135 mcd1-1* failed to grow at 37°C strains.

(B) smc3-K113R does not support viability in the absence of wild-type *SMC3* at any temperature. All three haploid yeast strains contain *SMC3* shuffle plasmid pEU42 {*SMC3 URA3 CEN*} and are deleted for the genomic *SMC3*. They also contain a *CEN LEU2* plasmid bearing either *SMC3* (*SMC3*; yVG3486) or *smc3-K113R* (*smc3-K113R*; yVG3486-K113R), or no additional plasmid (yVG3464-16C).

(C) smc3-K112/3-RR Fails to Complement smc3-42 at all temperatures. Strains from Figure 1B and two additional strains similarly generated with smc3-K112/3-RR integrated at *LEU2* (*smc3-42 smc3-K112/3-RR*: yTE580) and wild-type *SMC3* (*smc3-42 SMC3*: yTE581) were prepared essentially as described in Figure 1B. Note that *smc3-K113R smc3-42* strains show very weak growth at 37°C, and *smc3-K112/3-RR smc3-42* show a subtle growth defect at 23°C.

Supplemental Figure 3.





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Cohesin Interallelic Complementation

### Supplemental Figure 3. The double mutant *smc3-K113R smc3-AID*<sup>608</sup> strain Shows Temperature Dependence For Auxin Sensitivity.

(A)Haploid *SMC3-AID*<sup>608</sup> strain alone *SMC3-AID*<sup>608</sup> (yVG3651-3D) or bearing a second integrated *SMC3* allele, either *SMC3* (*SMC3 SMC3-AID*<sup>608</sup>; yMB81-1A), or *smc3-K113R* (*smc3-K113R SMC3-AID*<sup>608</sup>; yTE440) were grown at 23°C and diluted as described in Figure 1A then plated on YPD or YPD containing auxin (YPD AUX) at 23°C and 30°C for 2d.

(B) Auxin resistance of *SMC3-AID*<sup>608</sup> is not observed in *smc3-K112/3-RR smc3-AID*<sup>608</sup> (yMB79-1A) strains. Haploid *SMC3-AID*<sup>608</sup> strains bearing an second integrated *SMC3* allele, either SMC3 (*SMC3 SMC3-AID*<sup>608</sup>; yMB84-1A) or *smc3-K112/3-RR* (*smc3-K112/3-RR SMC3-AID*<sup>608</sup>; yMB79-1A) were grown at 23°C and diluted as described in Figure 1A then plated onto YPD or YPD supplemented with auxin (YPD AUX). Plates were incubated at 23°C for 2 days. Compare growth to *smc3-K113R smc3-AID*<sup>608</sup> serial dilutions in Figure 1C.



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### Supplemental Figure 4. Analysis of GAL expression in *MCD1* or *SMC3* over-expression strains on viability and cohesion.

(A) Analysis of Mcd1-1 protein levels in galactose inducible *mcd1-1* strains. Haploid *mcd1-1* (yVG3312-7A) and *pGAL-mcd1-1 mcd1-1* cells (yTE95) were grown in lactic-acid-glycerol rich media to early-log-phase at 30°C. The culture was split into three aliquots. One aliquot was incubated for an additional 1h with no additional changes. Galactose was added to the other two aliquots (2% final) then one aliquot incubated at 30°C and the other at 37°C and both incubated an additional 1h. Five ODs of each aliquot was harvested, and total protein extracts subjected to SDS-PAGE and Western blot analysis. Top Panel shows Mcd1-1p levels as detected using Rb anti-Mcd1p antibodies (anti-Mcd1p) and Bottom panel shows Tubulin used as a loading control and detected via Rb anti-Tubulin antibodies (anti-Tubulin).

(B) RNA expression analysis in galactose inducible *smc3* strains. Strains from Figure 2B were grown in lactic acid glycerol-containing media to early log phase at 30°C. Twenty-five ODs of cells were collected. Galactose was added (2% final) to the remaining cells and incubated 1h to allow pGAL induction. Twenty-five ODs of cells were collected. The pre and post galactose treated cells were processed for RNA expression analysis following nucleic acid extraction with Trizol and lithium acetate precipitation. *SMC3* mRNA levels were analyzed by RT-qPCR using primers at the 5' end of *SMC3* in triplicate comparing fold expression over each pre-induction sample and normalized against tubulin.

(C) Cohesion analysis in inducible *smc3* and *mcd1* strains. Strains listed in Figure 2A and 2B were grown on 5-FOA media at 23°C to counterselect against the CEN URA plasmid and then grown to mid-log in lactic-acid-glycerol media. Cells were then allowed to arrest in mid-M in the presence of nocodazole and galactose for 3.5 hours. Afterwards, cells were upshifted to 34°C for one additional hour. Data is assembled from two biological replicates and at least 200 cells were counted from each replicate. No cohesion defect was observed in any of the strains indicated at 23°C.



YPGAL 37°C

YPGAL 23°C

YPD 23°C

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### Supplemental Figure 5. *mcd1-Q266* and *mcd1-1* Alleles in the *cis* Configuration Fail to Restore Viability.

(A) Schematic showing position of mutants in Mcd1p. Wild-type Mcd1p is shown on the left. Mcd1-Q266p has an insertion mutation in the linker domain of Mcd1. The mcd1-1p has a mutation in the Mcd1 C terminus. The "chimera" mcd1-1,Q266 contains both mutations. The location of each mutation is marked with (\*) in red.

(B) Haploid *MCD1-AID* strains carrying an additional 6MYC tagged copy of *MCD1* (yTE428), *pGAL-MCD1* (yTE75), *pGAL-mcd1-1*,Q266 (yTE484), or *mcd1-1*,Q266 (yTE575) and a haploid yeast strain carrying two different *MCD1* alleles, *mcd1-1* and *mcd1-Q266-6MYC* (yTE42) were grown and diluted as described in Figure 1A. Cells were plated onto YPD, and YPD containing auxin (YPD AUX), or YP galactose containing auxin (YPGAL AUX) then incubated at 23°C for 2d.

(C) Haploid *mcd1-1* (*mcd1-1*; yVG3312-7A) strain alone or carrying an additional *MCD1* allele, either *MCD1* (*MCD1 mcd1-1*; yTE396), *pGAL-mcd1-1-6MYC* (*pGAL-mcd1-1 mcd1-1*; yTE523), *mcd1-1*, Q266-6MYC (*mcd1-1*, Q266 *mcd1-1*; yTE521), or pGAL-mcd1-1, Q266 (*pGAL-mcd1-1*, Q266 *mcd1-1*; yTE519) were and diluted as described in Figure 1A. Cells were plated onto YPD and incubated at 23°C 2d or onto YP galactose (YPGAL) and incubated at 23°C or 37°C for 2d. Note that like *mcd1-Q266*, the chimeric allele *mcd1-1*, Q266 is dominant negative over *mcd1-1* when overexpressed.



## Supplemental Figure 6. Analysis of Cohesion Establishment Kinetics of *smc3-K113R* in *smc3-42* or *smc3-AID*<sup>608</sup> strain backgrounds.

(A) Kinetic analysis of sister chromatid cohesion. Haploids *smc3-42* alone (*smc3-42*; yTE494) or carrying either *SMC3* (*SMC3*; yTE496) or *smc3-K113R* (*smc3-K113R smc3-42*; yTE500) integrated at URA3 were released from G1 into mid-M phase arrest as describe in Figure 3B except the temperature was shifted to 30°C and no auxin was added. Cell aliquots were fixed and processed for cohesion analysis and for FACS at various time points (Material and methods). Top panel is cohesion analysis at the *CEN* proximal *TRP1* locus (Material and methods). The percentage of cells where sisters had separated (2 GFP spots) is plotted. Bottom panel, flow cytometry analysis. All cells entered S phase by 30 minutes and no further replication was observed around 60 minutes. The shape of the peaks are different because cells were prepared by 4% PFA fixative instead of 70% Ethanol.

(B) Endpoint analysis of sister chromatid cohesion. Strains in (A) were examined for sister chromatid cohesion at the *CEN* proximal *TRP1* locus using the G1 arrest and released into mid-M phase arrest regimen as in (A), except cells were split into three aliquots at the G1 arrest stage. One aliquot was kept at 23°C, one shifted to 30°C and the third to 34°C then cells released into mid-M phase arrest at the same temperature they were upshifted to in G1, 23°C, 30°C or 34°C. The data from 180 minute time point from the graph above in (A) is replotted for comparison.

(C) Kinetic analysis of sister chromatid cohesion. Haploid *SMC3-AID*<sup>608</sup> strain alone (*SMC3-AID*<sup>608</sup>; yVG3460-2A) or bearing a second *SMC3* allele integrated at *URA3*, either *SMC3* (*SMC3*; yTE466) or *smc3-K113R* (*smc3-K113R SMC3-AID*<sup>608</sup>; yTE471) were released from G1 into mid-M phase arrest as described in (A) except auxin was added in G1 arrested cells and kept in media through mid-M phase arrest. Cohesion loss was monitored and DNA content measured as described in (A). Top panel is cohesion analysis at the *CEN* proximal *TRP1* locus. Bottom panel, flow cytometry analysis. All cells entered S phase by 30 minutes and no further replication was observed around 60 minutes.

(D) Endpoint analysis of sister chromatid cohesion. Strains in (C) were examined for sister chromatid cohesion in mid-M pase arrest at 23°C, 30°C, and 34°C as described in (B). An independent biological replicate for the 30°C endpoint assay is shown in D.



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### Supplemental Figure 7. Global chromosomal binding of Mcd1p or Smc3p in Interallelic Complementation Pairs.

(A) Haploid *MCD1-3FLAG MCD1-AID* (yTE171), *mcd1-1-3FLAG* (yTE103), and double mutant *mcd1-1-3FLAG mcd1-Q266-6MYC* (yTE181) strains were grown to early-log-phase then arrested in mid-M phase at 37°C as described in Figure 3B, except auxin was not added to yTE103 or yTE181. Cells were prepared for chromosome spreads (Materials and methods). FLAG tagged Mcd1 protein was detected using M $\alpha$ FLAG antibodies (anti-FLAG) via indirect immunofluorescence and DNA stained with DAPI to detect chromosomes.

(B) Haploid wild-type *SMC3-6HA* (yGC1-8A), *smc3-42-6HA* (yVG3523-1A) and double mutant *smc3-42-6HA smc3-K113R* (yVG3527-1A) strains were grown to early-log-phase then arrested in mid-M phase at 34°C as described in Figure 3C, except without auxin addition. Cells were processed for spreads (Materials and methods). HA tagged Smc3 protein was detected using a M $\alpha$ HA antibodies (anti-HA) via indirect immunofluorescence and DNA stained with DAPI to detect chromosomes. Note that smc3-42-6HA forms a diffuse "halo" pattern by chromosome spreads in a region larger than that detected by DAPI staining.



## Supplemental Figure 8. Analysis of Mcd1-Q266-6MYCp Binding by ChIP in *mcd1-1-3FLAG mcd1-Q266-6MYC* strains.

Haploid mcd1-1-3FLAG mcd1-Q266-6MYC (yTE181) strain was arrested in mid-M phase at 37°C as described in Figure 6A. Cells were processed for ChIP using M $\alpha$ MYC antibodies to detect binding of mcd1-Q266-6MYC protein. Chromosome binding at cohesin-associated regions are described in Figure 6A. The percentage of mcd1-Q266-6MYC MYC immunoprecipitated is plotted as compared to total input (% MYC IP). A representative experiment is shown.



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### Supplemental Figure 9. Deletion of *WPL1* Does Not Promote Viability in *smc3-42* nor *mcd1-1* cells.

(A) Haploid yeast cells (WT: yVG3202-2B; *wpl1* $\Delta$ : yVG3297-7C *smc3-42*: yVG3300-3D; *wpl1* $\Delta$  *smc3-42*: yVG3295-11B) were grown and diluted as described in Figure 1A. Cells were plated onto YPD, and then incubated at the indicated temperature for 2d. All cells are from the same plate as indicated below each photomicrograph. A dashed line indicates where the photomicrograph was spliced. (B) As in A, haploid yeast cells (WT: yVG327-1C; *wpl1* $\Delta$ : yVG3297-2D; *mcd1-1*: yVG3312-7A; *wpl1* $\Delta$  *mcd1-1*: yVG3324-2C) were grown and diluted as described in Figure 1A. Cells were plated onto YPD, and then incubated the indicated temperature for 2d. Note that *wpl1* $\Delta$  *mcd1-1* cells are are very sick even at 23°C, when *mcd1-1* cells show no growth defects.

Strain Name	Genotype
yGC01-8A	MATa SMC3-F864-6HA-HIS3:his3-11,15 smc3∆::HPH
	leu2-3,112 ura3-52 bar1 GAL+
yMB079-1A	MATa smc3-K112/3-RR-LEU2:leu2-3,112 SMC3-3V5-AID <sup>608</sup>
	TIR1-CaTRP1 LacO-NAT::lys4 pHIS3-GFP-LacI-HIS3:his3-
	11,15 ura3-52 bar1 GAL+
yMB081-1A	MATa SMC3-LEU2:leu2-3,112 SMC3-3V5-AID <sup>608</sup> TIR1-
	CaTRP1 LacO-NAT::lys4 pHIS3-GFP-LacI-HIS3:his3-11,15
	ura3-52 bar1 GAL+
yMB084-1A	MATa SMC3-LEU2:leu2-3,112 SMC3-3V5-AID <sup>608</sup> TIR1-
	CaTRP1 LacO-NAT::trp1-1 pHIS3-GFP-LacI-HIS3:his3-11,15
	ura3-52 bar1
vTF042	<b>MATa</b> mcd1-Q266-6MYC-URA3::ura3-52 mcd1-1 in yVG3312-
J	7A
yTE043	MATa mcd1-1 {pVG201 CEN/ARS URA3 MCD1}
yTE045	MATa TIR1-URA3::ura3-52 in yVG3349-1B
yTE048	MATa TIR1-CaLEU2 in yVG3349-1B
	MATa pGAL-MCD1-6MYC-URA3::ura3-52 MCD1-
yTE075	AID::KanMx6 pGPD1-OsTIR1-CaLEU2:leu2-3,112 in
	yVG3349-1B
yTE095	MATa mcd1-1-6MYC-URA3::ura3-52 in yVG3312-7A
	MATa
vTE103	mcd1-1-[3FLAG-352]::HpHmx6
,	LacO(DK)-NAT::lys4 pHIS3-GFP-LacI-HIS3::his3-11,15 trp1-1
	ura3-52 leu2-3,112 bar1 GAL+
yTE149	
	MC01-Q266-6MYC-URA3:Ura3-52 MCD1-AID1::KanMX6 LacO-
	NAT::Iys4 OSTIR1-CaLEU2:ieu2-3,112 in yVG3349-1B
	MATa MCD1-3ELAG-URA3ura3-52 MCD1-AID1KapMy6
yTE171	OsTIR1-Cal FII2 leu2-3 112 in v//G3349-1B
yTE181	МАТа
	mcd1-Q266-6MYC-URA3:ura3,52 mcd1-1-[3FLAG-
	352]::HpHMx6 LacO(DK)-NAT::lys4 pHIS3-GFP-Lacl-
	HIS3::his3-11,15 trp1-1 leu2-3,112 bar1 GAL+
yTE285	MATa
	mcd1-Q266-6MYC-URA3:ura3,52 MCD1-AID::KanMx6 LacO-
	NAT::trp1 OsTIR1-CaLEU2:leu2-3.112 in vVG3349-1B

yTE388	MATa mcd1-V137K-TRP1::trp1-1 mcd1-1 in yVG3312-7A
yTE392	MATa mcd1-RID-R135-TRP1::trp1-1 mcd1-1 in yVG3312-7A
yTE396	MATa MCD1-TRP1::trp1 mcd1-1 in yVG3312-7A
yTE420	MATa smc3A {pEU42 CEN/ARS URA3 SMC3} pGAL-smc3-
	42-[S182L]-LEU2::leu2-3,112
yTE424	MATa smc3∆ {pEU42 CEN/ARS URA3 SMC3} pGAL-SMC3-
	LEU2::leu2-3,112
yTE428	MATa MCD1-6MYC-URA3::ura3-52 MCD1-3V5-AID2::KanMx6
	TIR1-CaLEU2::leu2-3,112 pHIS3-LacI-GFP LacO-(DK)-
	NAT:lys4
yTE440	MATa smc3-K113R-URA3::ura3-52 SMC3-3V5-AID <sup>608</sup> TIR1-
	CaTRP1::trp1-1 LacO-NAT::lys4 pHIS3-GFP-LacI-HIS3:his3-
	11,15 ura3-52 bar1
yTE449	MATa smc3∆ {pEU42 CEN/ARS URA3 SMC3} pGAL-smc3-
	K113R-LEU2::leu2-3,112
VTE452	MATa mcd1-1-3V5-307:HpHmx6 LacO-NAT::trp1 pHIS3-GFP-
y12400	Lacl-HIS3:his3-11,15 ura3-52 bar1
	MATa mcd1-1-3V5-307:HpHmx6 mcd1-Q266-6MYC-
yTE456	URA3::ura3-52 LacO-NAT::trp1 pHIS3-GFP-LacI-HIS3:his3-
	11,15 bar1
	MATa SMC3-URA3::ura3-52 SMC3-3V5-AID <sup>608</sup> TIR1-
yTE466	CaTRP1::trp1-1 LacO-NAT::trp1 pHIS3-GFP-LacI-HIS3:his3-
	11,15
	MATa smc3-K113R-URA3::ura3-52 SMC3-3V5-AID <sup>608</sup> TIR1-
yTE471	CaTRP1::trp1-1 LacO-NAT::trp1 pHIS3-GFP-LacI-HIS3:his3-
	11,15
	MATa {CEN TRP1 MCD1} {pVG201 CEN/ARS URA3 MCD1}
yTE474	mcd1∆::KanMx6 in yVG3349-1B
yTE478	MATa {CEN IRP1 mcd1-Q266} {pVG201 CEN/ARS URA3
	MCD1} mcd1\Delta::KanMx6 in yVG3349-1B
yTE480	MATA (CENTRP1 pGAL1-MCD1) {pVG201 CEN/ARS URA3
,	
yTE482	MATA (CEN TRP1 pGAL1-mcd1-Q266) {pVG201 CEN/ARS
уТЕ484 уТЕ491	<b>WATA</b> $p_{GAL}$ -mc01-1,Q266-6WYC-URA3::Ura3,52 MCD1-3V5-
	MCD1 mod1 1 in vVC2212 74
yTE494	<b>WATA</b> SMC3-42 LacO-NAT::TP1 PHIS3-GFP-LacI-HIS3:his3-
	11,15 leu2-3,112 Dar1 trp1-1 GAL+

yTE496	MATa SMC3-URA3 smc3-42 LacO-NAT::trp1 pHIS3-GFP-
	Lacl-HIS3:his3-11,15 leu2-3,112 bar1 trp1-1 GAL+
yTE500	MATa smc3-K113R-URA3 smc3-42 LacO-NAT::trp1 pHIS3-
	GFP-Lacl-HIS3:his3-11,15 leu2-3,112 bar1 trp1-1 GAL+
yTE502	MATa mcd1∆::KanMx6 {CEN/ARS URA3 MCD1} {CEN/ARS
	TRP1 pGAL-mcd1-1}
yTE505	MATa smc3-K113R-LEU2::leu2-3,112 smc3-42 LacO-
	NAT::lys4 pHIS3-GFP-Lacl-HIS3:his3-11,15 leu2-3,112 bar1
	trp1-1 GAL+
yTE519	MATa pGAL-mcd1-1,Q266-6MYC-URA3::ura3-52 in yVG3312-
	7A
yTE521	MATa mcd1-1,Q266-6MYC-URA3::ura3-52 in yVG3312-7A
yTE523	MATa mcd1-1-6MYC-URA3::ura3-52 in yVG3312-7A
yTE575	MATa mcd1-1,Q266-6MYC-URA3::ura3-52 MCD1-
	AID::KanMx6 pGPD1-OsTIR1-CaLEU2:leu2-3,112 in
	yVG3349-1B
	MATa smc3-42 {pEU42 CEN/ARS URA3 SMC3} LacO-
yTE576	NAT::lys4 pHIS3-GFP-Lacl-HIS3:his3-11,15 leu2-3,112 bar1
-	trp1-1 GAL+
	MATa smc3-42 smc3-K113R-LEU2::leu2-3,112 {pEU42
yTE578	CEN/ARS URA3 SMC3} LacO-NAT::lys4 pHIS3-GFP-Lacl-
	HIS3:his3-11,15 leu2-3,112 bar1 trp1-1 GAL+
	MATa smc3-42 smc3-K112/3-RR-LEU2::leu2-3,112 {pEU42
yTE580	CEN/ARS URA3 SMC3} LacO-NAT::lys4 pHIS3-GFP-Lacl-
	HIS3:his3-11,15 leu2-3,112 bar1 trp1-1 GAL+
	MATa smc3-42 SMC3-LEU2::leu2-3,112 {pEU42 CEN/ARS
yTE581	URA3 SMC3} LacO-NAT::lys4 pHIS3-GFP-LacI-HIS3:his3-
	11,15 leu2-3,112 bar1 trp1-1 GAL+
yVG3202-3B	Matx LacO-NAT::Lys4 GFPLacI-HIS3:his3-11,15 trp1-1
	leu2-3,112 ura3-52 bar1 GAL+
	Matx smc3-42 wpl1A::HPH trp1-1 leu2-3.112 ura3-52
yVG3295-11B	······································
yVG3297-2D	Mata wpl1/\::HPH LacO-NAT::lvs4 GEPLacI-HIS3:his3-
	11 15 $tro 1-1 leu 2-3 112 ura 3-52 bar1 GAI +$
yVG3297-7C	Maty woll A: HPH LacO-NAT: lyc4 GEPL acl-HIS3: bic3-
	14 15 tro1 1
	11,15 up 1-1
	1euz-3,112 uta3-52 bart GAL+
yvG3300-3D	
yVG3312-7A	MAIA mcd1-1 LacO-NAI: Iys4 trp1-1 bar1 pHIS3-GFP-
	Lacihis3:nis3-11,15 ura3-52 GAL+
yVG3324-2C	Matx mcd1-1 wpl1A::HPH LacO-NAT::lys4 trp1-1 ura3-52
	bar1

yVG3327-1C	Mata GFPLacl-HIS3:his3-11,15 LacO-NAT::lys4 trp1-1
	leu2-3,112 ura3-52 bar1 GAL+
yVG3349-1B	MATa LacO-NAT:lys4 trp1-1 bar1 pHIS3-GFP-LacIHIS3:his3-
	11,15 leu2-3,112 ura3-52 GAL+
yVG3358-3B	MATa smc3-42 LacO-NAT::lys4 pHIS3-GFP-LacI-HIS3:his3-
	11,15 leu2-3,112 bar1 trp1-1 GAL+
yVG3460-2A	MATa LacO-NAT:trp1 trp1-1 bar1 pHIS3-GFP-LacIHIS3:his3-
	11,15 leu2-3,112 ura3-52 GAL+
yVG3464-1C	MATa {pEU42 CEN/ARS URA3 SMC3} smc3∆::HPH leu2-
	3,112 ura3-52 bar1 GAL+
yVG3473-1C	MATa smc3-K113R-URA3 smc3-42 LacO-NAT::lys4 pHIS3-
	GFP-Lacl-HIS3:his3-11,15 leu2-3,112 bar1 trp1-1 GAL+
yVG3486-00	MATa smc3∆ {pEU42 CEN/ARS URA3 SMC3} {CEN/ARS
	LEU2 SMC3}
yVG3486-	MATa smc3∆ {pEU42 CEN/ARS URA3 SMC3} {CEN/ARS
K113R	LEU2 smc3-K113R}
yVG3523-1A	<b>MATa</b> smc3-42-6HA-HIS3::his3-11,15 smc3∆::HpHMx6
	LacO(DK)-NAT::lys4 pHIS3-LacI-GFP-TRP-HIS trp1-1 leu2-
	3,112 ura3-52 GAL+ bar1
yVG3527-1A	MATa smc3-K113R-LEU2::leu2-3,112 smc3-42-6HA-
	HIS3::his3-11,15 smc3∆::HpHMx6 LacO(DK)-NAT::lys4 pHIS3-
	Lacl-GFP-TRP-HIS trp1-1 leu2-3,112 ura3-52 GAL+ bar1
yVG3651-3D	MATa SMC3-3V5-AID <sup>608</sup> TIR1-TRP1 LacO-NAT::lys4 pHIS3-
	GFP-Lacl-HIS3:his3-11,15 ura3-52 bar1 GAL+