SUPPLEMENTAL INFORMATION

Supplemental information includes 3 tables and 6 figures and can be found with this article. Yeast strains used in this study are listed in Table S1. Details of plasmids and primers used in this study are specified in Table S2 and S3.

Table S1: Strains used is this study

| ***Strain*** | ***Genotype*** | ***Source*** |
|---|---|---|
| JC470 | *MAT***a** *ade2-1 trp1-1 his3-11 his3-15 ura3-1 leu2-3 leu2-112 Rad5+ (W303)* | R. Rothstein |
| JC471 | *MAT*$\alpha$ *ade2-1 trp1-1 his3-11 his3-15 ura3-1 leu2-3 leu2-112 Rad5+ (W303)* | R. Rothstein |
| JC1280 | *MAT*$\alpha$ *his3 trp1 ura3-52 leu2*::*proLEU2-lexAop6* | Golemis, et al, 1996 |
| JC1358 | JC471 with *smc6*-9::*KanMX6* | C. Boone Lab |
| JC1594 | JC470 with Smc6-6HIS-3FLAG::*KanMX4* | Bustard et al., 2012 |
| JC1595 | JC471 with Smc6-6HIS-3FLAG::*KanMX4* | this study |
| JC3032 | JC471 with *nse3*-1::*HYG* | Moradi-Fard et al., 2016 |
| JC3039 | JC470 with *smc6*-9::*KanMX6* | this study |
| JC3078 | JC1595 with *nse3*-1::*URA3* | this study |
| JC3084 | JC470 with *nse3*-1::*URA3* | this study |
| JC3467 | JC470 with Smc5-13MYC::*KanMX6* | this study |
| JC3483 | JC3467 with *nse3*-1::*URA3* | this study |
| JC 3728 | JC470 with Smc5-6HIS-3FLAG::*KanMX4* | this study |
| JC3787 | JC470 with *sir2*::*TRP1*, *nse3*-1::*HYG* | this study |
| JC3790 | JC471 with *lrs4*::*KanMX6* | this study |
| JC3791 | JC470 with *lrs4*::*KanMX6* | K. Mekhail lab |
| JC3796 | JC3791 with *nse3*-1::*HYG* | this study |
| JC4022 | JC470 with Heh1-13MYC::*KanMX6* | this study |
| JC4107 | JC470 with Heh1-TAP::*TRP1*, TELVR::*ADE2* | K. Mekhail lab |
| JC4205 | JC1595 with *heh1*::*HYG* | this study |
| JC4224 | JC471 with Heh1-13MYC::*KanMX6* | this study |
| JC4228 | JC4022 with *nse3*-1::*HYG* | this study |
| JC4233 | JC471 with Csm1-TAP::*TRP1* | K. Mekhail lab |
| JC4243 | JC1595 with *csm*1::*KanMX6* | this study |
| JC4251 | JC4233 with *nse3*-1::*HYG* | this study |
| JC4595 | JC3032 with *fob1::HIS3* | this study |
| JC4598 | JC1594 with Csm1-TAP::*TRP1* | this study |
| JC4648 | JC471 with *sir2*::*TRP1* | this study |
| JC4676 | JC471 with Nup49-*GFP*, Nop1-CFP::*URA3* | this study |
| JC4699 | JC1595 with *sir2*::*TRP1* | this study |
| JC4712 | JC4598 with *nse3*-1::*HYG* | this study |
| JC4729 | JC470 with Nup49-*GFP*, Nop1-CFP::*URA3*, *nse3*-1::*HYG* | this study |
| JC4731 | JC4676 with *lrs4*::*KanMX6* | this study |
| JC4733 | JC4676 with *sir2*::*TRP1* | this study |
| JC4735 | JC4676 with *heh1*::*HYG* | this study |
| JC4773 | JC4774 with *nse3*-1::*HYG* | this study |
| JC4774 | JC4224 with Csm1-TAP::*TRP1* | this study |
| JC4811 | JC1595 with Heh1-TAP::*TRP1* | this study |
| JC4813 | JC4811 with *nse3*-1::*URA3* | this study |
| JC4823 | JC3039 with *fob1::LEU2* | this study |
| JC4824 | JC1358 with *fob1::LEU2* | this study |
| JC4825 | JC471 with *fob1::LEU2* | this study |
| JC4929 | JC4824 *with* Csm1-TAP::*TRP1* | this study |
| JC4932 | JC4676 with *smc6*-9::*KanMX6* | this study |
| JC4937 | JC4233 with *fob1::LEU2* | this study |

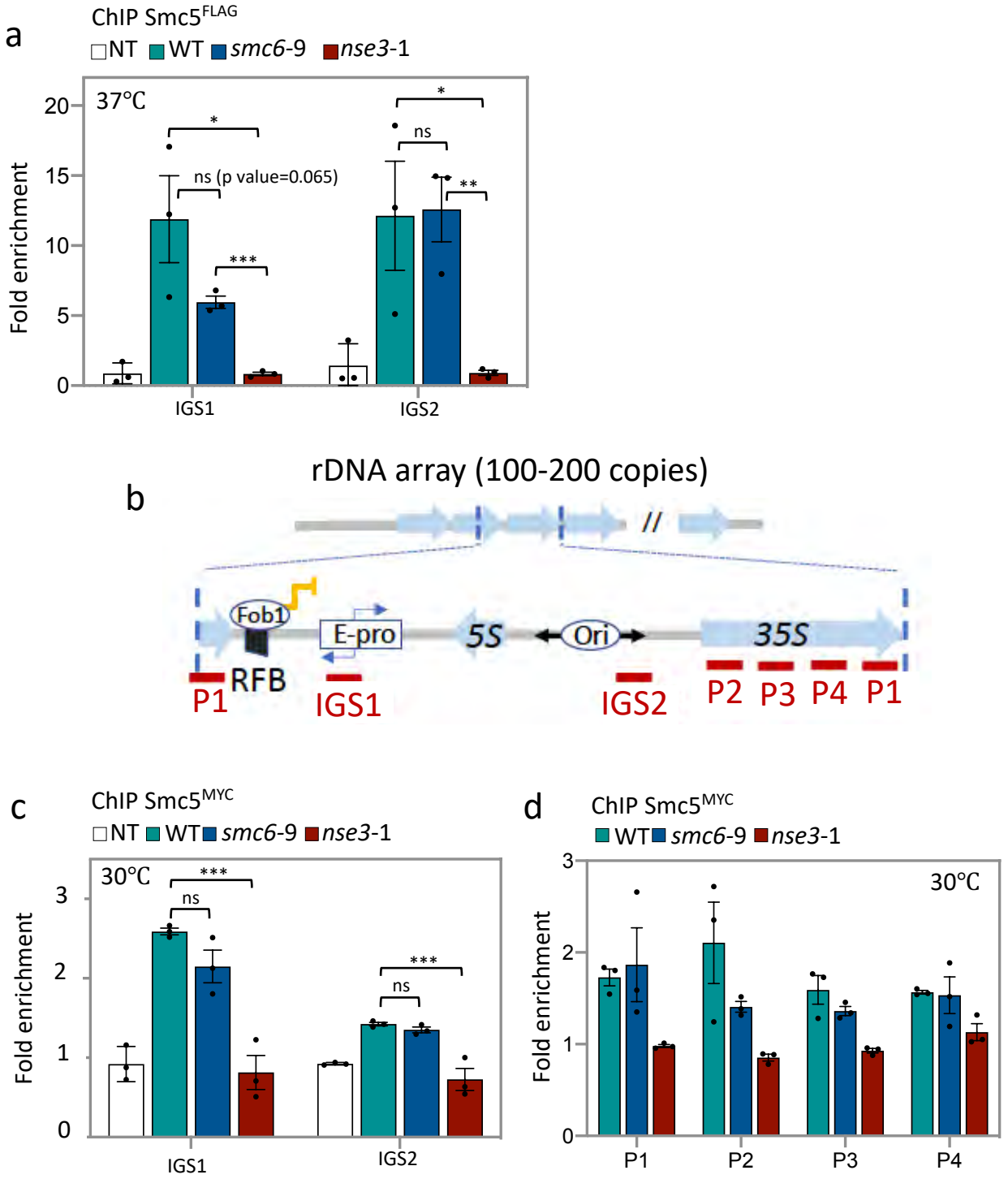
| | | |
|---|---|---|
| JC4938 | JC4233 with *smc6*-9::*KanMX6* | this study |
| JC4940 | JC4022 with *fob1::LEU2* | this study |
| JC4942 | JC4022 with *smc6*-9::*KanMX6* | this study |
| JC4943 | JC4022 with *smc6*-9::*KanMX6, fob1::LEU2* | this study |
| JC4973 | JC4233 with *fob1::LEU2*, *nse3*-1::*HYG* | this study |
| JC4975 | JC4022 with *fob1::LEU2*, *nse3*-1::*HYG* | this study |
| JC4976 | JC1358 with *lrs4*::*KanMX6* | this study |
| JC4978 | JC1358 with *sir2*::*HIS3* | this study |
| JC4979 | JC3790 with *sir2*::*HIS3* | this study |
| JC4980 | JC4979 with *nse3*-1::*HYG* | this study |
| JC4985 | JC5016 with *fob1::LEU2* | this study |
| JC5007 | JC471 with Fob1-3HA:: *HIS3* | this study |
| JC5008 | JC5007 with *nse3*-1::*HYG* | this study |
| JC5010 | JC5007 with *smc6*-9::*KanMX6* | this study |
| JC5014 | JC5016 with *smc6*-9::*KanMX6* | this study |
| JC5015 | JC5016 with *nse3*-1::*HYG* | this study |
| JC5016 | JC471 with Nop1-CFP::*URA3* | this study |
| JC5017 | JC5016 with *sir2*::*TRP1* | this study |
| JC5018 | JC5016 with *heh1*::*HYG* | this study |
| JC5019 | JC5016 with *lrs4*::*KanMX6* | this study |
| JC5039 | JC3467 with *smc6*-9::*KanMX6* | this study |
| JC5040 | JC5039 with *fob1::LEU2* | this study |
| JC5041 | JC3467 with *fob1::LEU2* | this study |
| JC5044 | JC3483 with *fob1::LEU2* | this study |
| JC5110 | JC5015 with *fob1::HIS3* | this study |
| JC5113 | JC5014 with *fob1::HIS3* | this study |
| JC5879 | JC3728 with *nse3*-1::*HYG* | this study |
| JC5882 | JC3032 with *fob1::HIS3* | this study |
| JC5883 | JC470 with *nse3*-1::*HYG, fob1::HIS3* | this study |
| JC5894 | JC3728 with *smc6*-9::*KanMX6* | this study |

Table S2: Oligos used in this study

| ***Primer name*** | ***Primer number*** | ***Sequence 5'> 3'*** |
|---|---|---|
| NTS1 | C1577 | AGGGCTTTCACAAAGCTTCC |
| | C1578 | TCCCCACTGTTCACTGTTCA |
| NTS2 | C1795 | CCACCACACTCCTACCAATAAC |
| | C1796 | AGGTAGTCAGATGAAAGATGAATAGAC |
| P1 | C1791 | CACACTATCATCCTCATCGTATATT |
| | C1792 | AGAGAGAAGTAGACTGAACAAGT |
| P2 | C1799 | ACGATGAGAGACTGTTCAGGTTAAA |
| | C1800 | GGGTTGATGCGTATTGAGAGATA |
| P3 | C1801 | CCAATTGTTCCTCGTTAAGGTATTT |
| | C1802 | ATTCAGGGAGGTAGTGACAATAAA |
| P4 | C1807 | GTTTGAGAATAGGTCAAGGTCATTTC |
| | C1808 | GTTTCCCTCAGGATAGCAGAAG |
| ZN | C1275 | GCACTTAATTGGCGTAAGCTG |
| | C1276 | TCGCAGGAGCATATTTCGTA |
| Act1 | C1561 | TGTCCTTGTACTCTTCCGGT |
| | C1562 | CCGGCCAAATCGATTCTCAA |
| Smc5 | C1483 | GATCCCATGGATGACCAGTCTAATAGATTTGGGCAGATATG |
| | C1484 | GATCCTCGAGTTAATCGAATGAGTAGTTAGAAGTTTCACCG |
| Nse1 | C719 | CTAGGAATTCATGGAGGTACATGAAGAGC |
| | C720 | CTAGCTCGAGTTAAATAACGTATACGCCCTCTG |
| Nse3 | C723 | CTAGGAATTCATGAGTTCTATAGATAATGAC |
| | C724 | CTAGCTCGAGCTATATAGAATATGAATCGCC |
| Nse4 | C721 | CTAGGAATTCATGTCTAGTACAGTAATATC |
| | C722 | CTAGCTCGAGTAAGAATGGTGAAGTGATGTTG |
| Nse6 (pJ1493) | C609 | GATCGGATCCGTGTCACAAATGGGAAGCGTGAACTCATCACCG |
| | C610 | GATCCTCGAGCAGATCAATGTTCAGTCATCATGACTGTTACC TG |
| Nse6 (pJ965) | C892 | GATCGGATCCAAATGGGAAGCGTGAACTCATCACCG |
| | C610 | GATCCTCGAGCAGATCAATGTTCAGTCATCATGACTGTTACC TG |
| Csm1 | C1737 | GATC GAATTC ATGGATCCATTGACTGTATACAAAAACTCAGTGAAACA |
| | C1738 | GATC CTCGAG TTATGTAGCAGCTTACTCGGTTTCATCTTTTTTCTCTC |
| Lrs4 | C1735 | GATCGAATTCATGGAGCATGTAGATTCCGATTTTGCACCTATAAGGAG |
| | C1736 | GATC CTCGAG GATAGCTGTTACTCATACAAACTCGTCAACATTTAAAT |
| Heh1 | C1898 | GATCGCGGCCGCATGAATAGTGACTTGGAGTATTTAGAGGACGGTTTTGA |
| | C1915 | GATCCTCGAGTCATTTTGTGGGTTATATTTTGTTTTCAGCGGAATCCT |

Table S3: Plasmids Used in this study

| ***Plasmid number*** | ***Plasmid description*** | ***Source*** |
|---|---|---|
| J 965 | pGAL-lexA | S. Gasser lab |
| J 1493 | pJG4-6 | S. Gasser lab |
| J 359 | pSH18-34 lexAGal1-lacZ | |
| J 1805 | J 965 with Nse1 | |
| J 102 | J 965 with Mms21 | |
| J 138 | J 965 with Nse3 | |
| J 1804 | J 965 with Nse4 | |
| J 038 | J 965 with Nse5 | |
| J 141 | J 965 with Nse6 | |
| J1902 | J 965 with Smc5 | |
| J 1874 | J 965 with Csm1 | |
| J 1875 | J 965 with Lrs4 | |
| J 048 | J 1493 with Smc5 | |
| J 063 | J 1493 with Nse6 | |
| J 050 | J 1493 with Mms21 | |
| J 1881 | J 1493 with Heh1 | |
| J 187 | pFN4 pNOP1-CFP | 1742 S. Gasser lab |
| J 1830 | pNOY373, containing 9.1 Kb rDNA repeats | (Unal et al., 2011) |

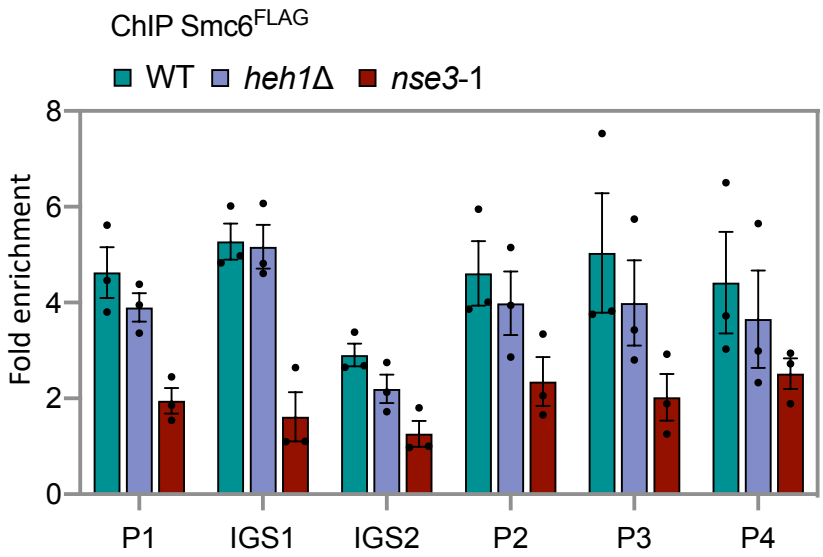



**Supplementary Figure 1, Related to Figure 1.**

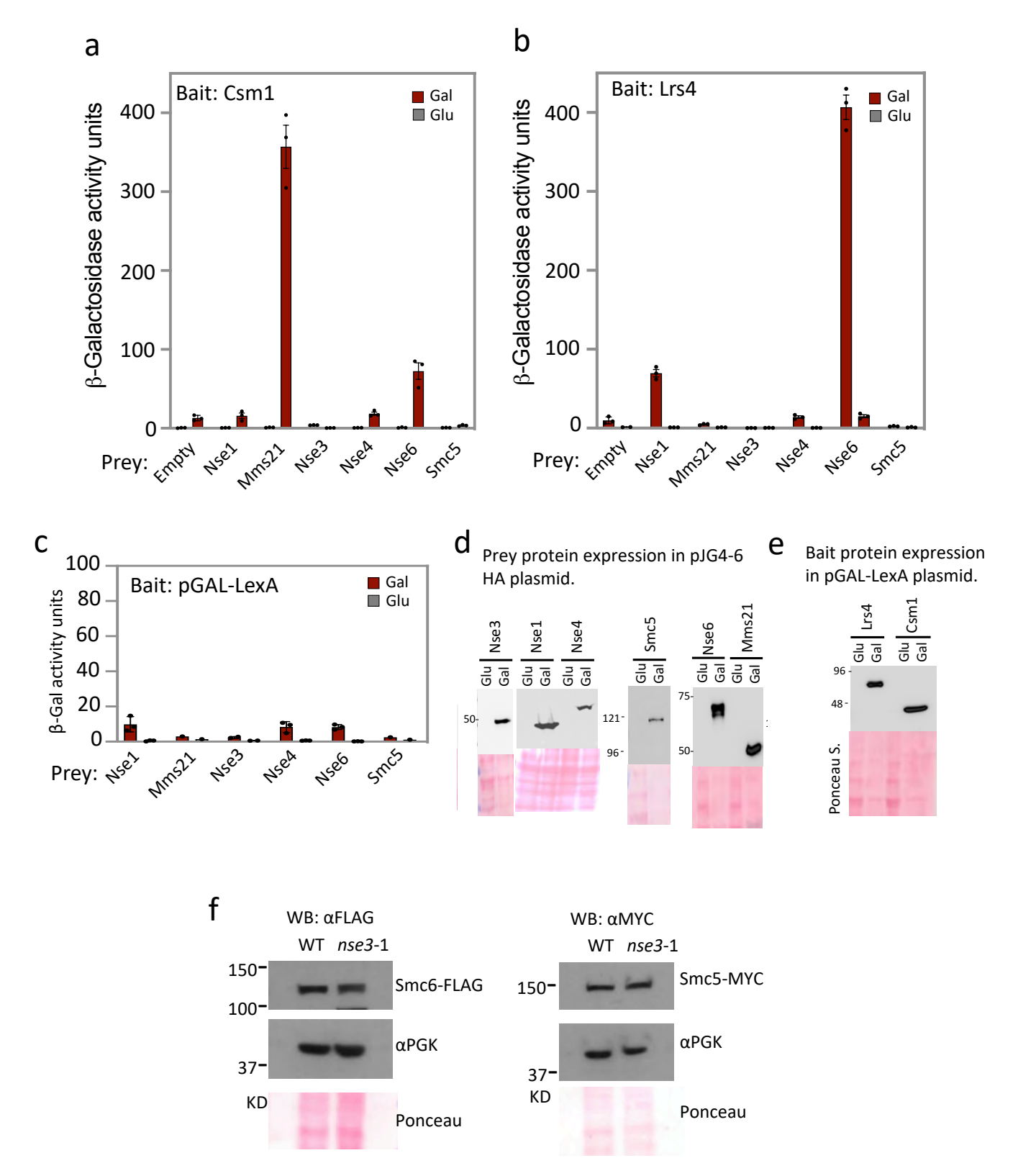
**a.** Enrichment of Smc5$^{MYC}$ at IGS1 and IGS2 with α-FLAG at 37℃ in non-tagged control (JC 470), WT (JC 3728), *nse3*-1 (JC 5879) and *smc6*-9 (JC 5894). Fold enrichment is based on normalization to negative control region as described in Fig.1. Analysis was performed using at least three biological replicates. Statistical analysis is described in methods.

**b.** Location of P1-P4 probes in the rDNA.

**c., d.** Enrichment of Smc5$^{Myc}$ at IGS1 and IGS2 (b) or P1-P4 (c) by ChIP with α-MYC at 30℃ in non-tagged control (JC 470), WT (JC 3467), *nse3*-1 (JC 3483) and *smc6*-9 (JC 5039).




**Supplementary Figure 2, Related to Figure 2.**
Enrichment of Smc6$^{FLAG}$ at IGS1 and IGS2 by ChIP with α-FLAG in WT (JC 1595), *heh1*Δ (JC 4205) and *nse3*-1 (JC 3078). Fold enrichment is based on normalization to negative control region as described in Fig.1. Analysis was performed using at least three biological replicates. Statistical analysis is described in methods.


a
Bait: Csm1
Gal
Glu
β-Galactosidase activity units
400
300
200
100
0
Prey:
Empty
Nse1
Mms21
Nse3
Nse4
Nse6
Smc5
b
Bait: Lrs4
Gal
Glu
β-Galactosidase activity units
400
300
200
100
0
Prey:
Empty
Nse1
Mms21
Nse3
Nse4
Nse6
Smc5
c
Bait: pGAL-LexA
Gal
Glu
β-Gal activity units
100
80
60
40
20
0
Prey:
Nse1
Mms21
Nse3
Nse4
Nse6
Smc5
d
Prey protein expression in pJG4-6 HA plasmid.
Nse3
Nse1
Nse4
Smc5
Nse6
Mms21
Glu
Gal
50
121
96
75
50
e
Bait protein expression in pGAL-LexA plasmid.
Lrs4
Csm1
Glu
Gal
96
48
Ponceau S.
f
WB: αFLAG
WT
nse3-1
150
100
37
Smc6-FLAG
αPGK
KD
Ponceau
WB: αMYC
WT
nse3-1
150
37
Smc5-MYC
αPGK
KD
Ponceau

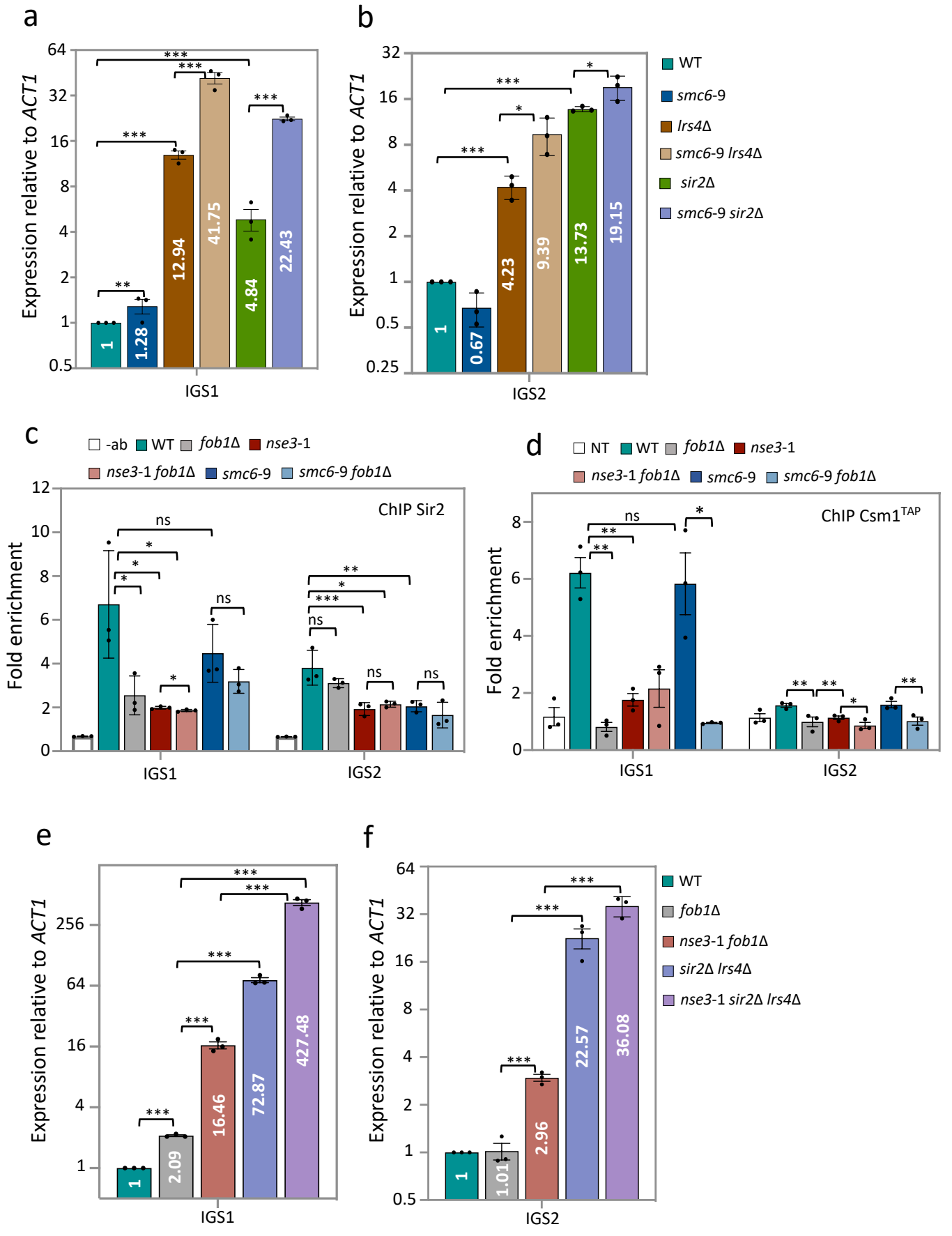
**Supplemantry Figure 3, Related to Figure 3.**

**a., b.** Yeast two-Hybrid analysis between Smc5/6 (Nse1, Mms21, Nse3 Nse4, Nse6 and Smc5) as prey vectors and Cohibin components (a) Csm1 or (b) Lrs4 as bait vectors.

**c.** Smc5/6 components as preys (Nse1, Mms21, Nse3 Nse4, Nse6 and Smc5) with empty vector (pGAL-LexA) control represent background β-galactosidase activity units.

**d., e.** Western blots with (d) α-HA and (d) α-LexA shows the expression levels of proteins with their corresponding epitope tags from Yeast-two hybrid vectors after induction in Galactose-containing media in comparison with Glucose-containing media as control. Glu: Glucose; Gal: Galactose. The expression vectors are listed in Table S3.

**f.** Western blots with α-FLAG for $Smc6^{FLAG}$ in WT (JC 1595) and *nse3*-1 (3078) used for ChIP in Fig. 3f, 3g and α-MYC for $Smc5^{MYC}$ in WT (JC 3467) and *nse3*-1 (3483) used for ChIP in Supplementary Fig. 1a,b.

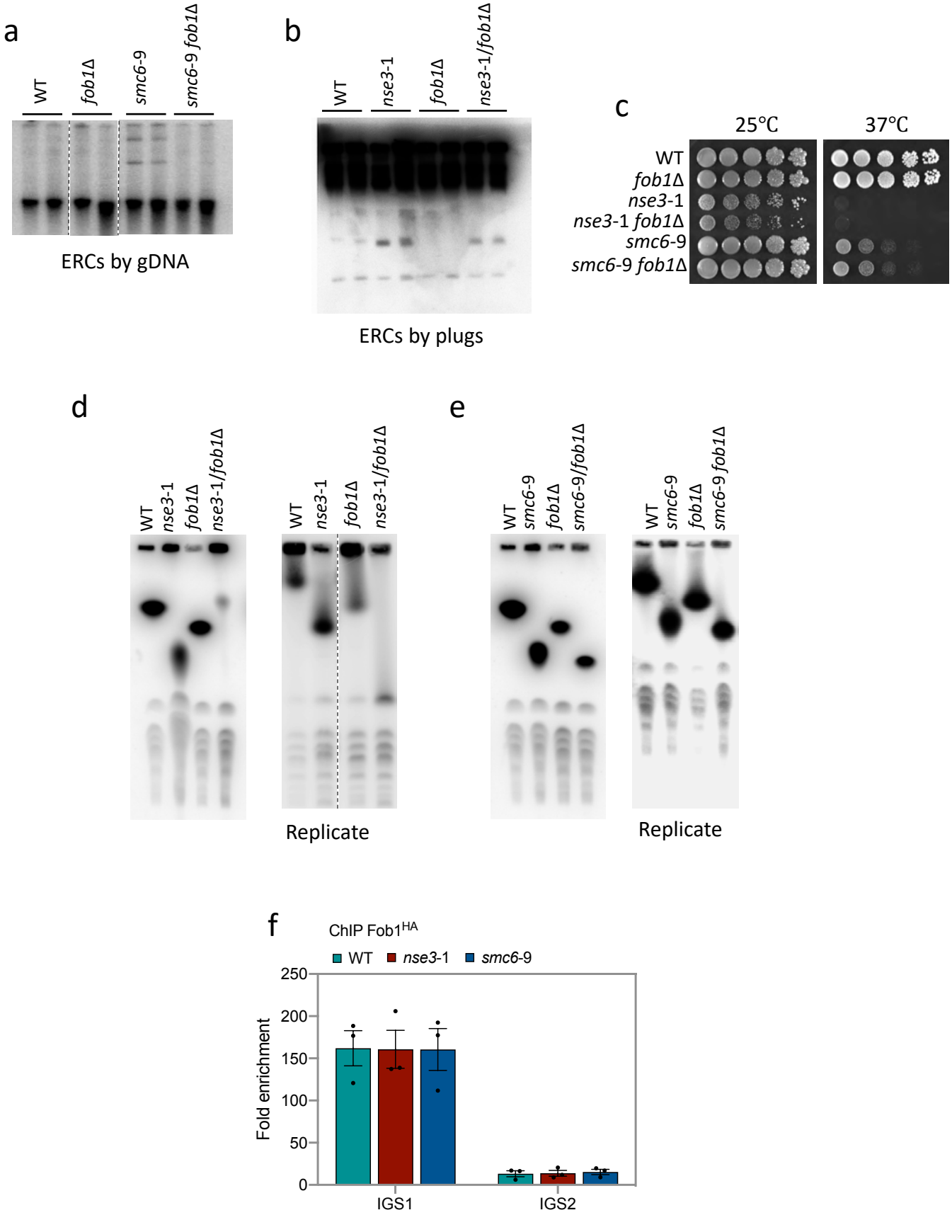

a
Expression relative to ACT1
IGS1
1
1.28
12.94
41.75
4.84
22.43
b
IGS2
0.67
4.23
9.39
13.73
19.15
WT
smc6-9
lrs4Δ
smc6-9 lrs4Δ
sir2Δ
smc6-9 sir2Δ
c
-ab
fob1Δ
nse3-1
nse3-1 fob1Δ
smc6-9 fob1Δ
ChIP Sir2
Fold enrichment
d
NT
ChIP Csm1TAP
e
427.48
72.87
16.46
2.09
f
2.96
22.57
36.08
1.01

**Supplemantry Figure 4, Related to Figures 3 and 4.**
**a., b.** Transcription of (a) IGS1 and (b) IGS2 measured and represented as relative to WT cells after normalization to *ACT1* expression for WT (JC 471), *smc6*-9 (JC 1358), *lrs4*Δ (JC 3791), *smc6*-9 *lrs4*Δ (JC 4976), *sir2*Δ (JC 4648) and *smc6*-9 *sir2*Δ (JC 4978) strains**.**

**c.** Enrichment of Sir2 at IGS1 and IGS2 by ChIP with α-Sir2 in WT (JC 471), *fob1*Δ (4825), *nse3*-1(JC 3032), *nse3*-1 *fob1*Δ (JC 4595), *smc6*-9 (JC 1358) and *smc6*-9 *fob1*Δ (JC 4824). Fold enrichment is based on normalization to negative control region relative to no antibody control.

**d.** Enrichment of $Csm1^{TAP}$ at IGS1 and IGS2 by ChIP with α-TAP in Non-tagged control (JC 470), WT (JC 4233), *fob1*Δ (JC 4937), *nse3*-1 (JC 4251), *nse3*-1 *fob1*Δ (JC 4973), *smc6*-9 (JC 4938) and *smc6*-9 *fob1*Δ (JC 4929) at IGS1 and IGS2. Fold enrichment is based on normalization to negative control region as described in Fig.1. Analysis was performed using at least three biological replicates. Statistical analysis is described in methods.

**e., f.** Transcription of (e) IGS1 and (f) IGS2 measured and represented as relative to WT cells after normalization to *ACT1* expression for WT (JC 471), *fob1*Δ (JC 4825), *nse3*-1 *fob1*Δ (JC 4595), *sir2*Δ *lrs4*Δ (JC 4979), *nse3*-1 *sir2*Δ *lrs4*Δ (JC 4980) strains**.**


a
WT
fob1Δ
smc6-9
smc6-9 fob1Δ
ERCs by gDNA
b
WT
nse3-1
fob1Δ
nse3-1/fob1Δ
ERCs by plugs
c
25°C
37°C
WT
fob1Δ
nse3-1
nse3-1 fob1Δ
smc6-9
smc6-9 fob1Δ
d
WT
nse3-1
fob1Δ
nse3-1/fob1Δ
Replicate
e
WT
smc6-9
fob1Δ
smc6-9/fob1Δ
Replicate
f
ChIP Fob1HA
WT
nse3-1
smc6-9
Fold enrichment
250
200
150
100
50
0
IGS1
IGS2

**Supplementary Fig. 5, Related to Figure 4.**

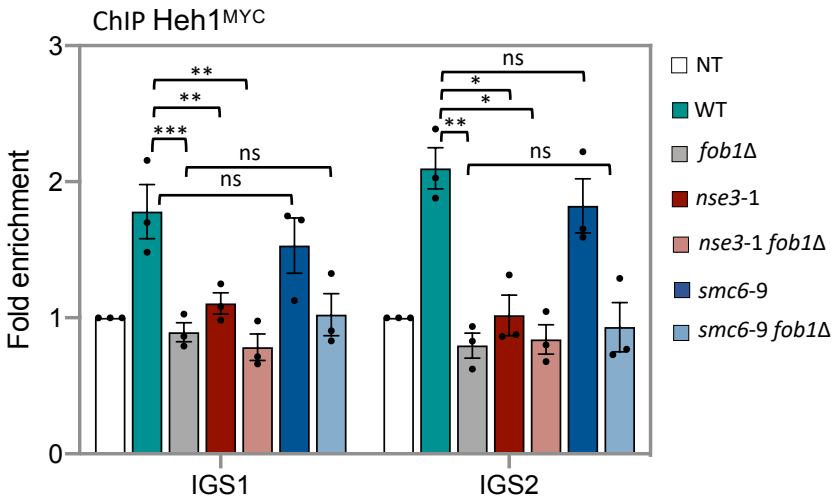
**a.** ERCs for WT (JC 470, JC 471), *fob1*Δ (JC 4825, JC 4825), *smc6*-9 (JC 1358, JC 3039) and *smc6*-9 *fob1*Δ (JC 4824, JC 4823) strains were visualized by running ≅ 10ug gDNA at low voltage for ≅ 24hours followed by southern blotting and probing for rDNA repeats.

**b.** ERCs for WT (JC 470, JC 471), *nse3*-1(JC 3032, JC 3084), *fob1*Δ (JC 4825, JC 4825) and *nse3*-1 *fob1*Δ (JC 5882, JC 5883) strains were visualized by running plugs containing 5x $10^7$ cells/plug at low voltage for ≅ 24hours followed by southern blotting and probing for rDNA repeats.

**c.** Drop assay to check the temperature sensitivity of the strains - WT (JC 471), *fob1*Δ (JC 4825), *nse3*-1(JC 3032), *nse3*-1 *fob1*Δ (JC 4595), *smc6*-9 (JC 1358) and *smc6*-9 *fob1*Δ (JC 4824) on YPAD at 37°C.

**d., e.** rDNA repeats for WT (JC 471), *fob1*Δ (JC 4825), *nse3*-1(JC 3032), *nse3*-1 *fob1*Δ (JC 4595), *smc6*-9 (JC 1358) and *smc6*-9 *fob1*Δ (JC 4824) strains were visualized by PFGE, followed with southern blotting and probing for rDNA repeats. Independent replicate experiments were performed.

**f.** Enrichment of $Fob1^{HA}$ at IGS1 and IGS2 by ChIP with α-HA in WT (JC 5007), *nse3*-1 (JC 5008) and *smc6*-9 (JC 5010). Fold enrichment is based on normalization to negative control region (ZN). Analysis was performed using at least three biological replicates. Statistical analysis is described in methods.




**Supplementary Fig. 6.**
Enrichment of Heh1$^{MYC}$ at IGS1 and IGS2 by ChIP with α-MYC in non-tagged control (JC 470), WT (JC 4022), *fob1*Δ (JC 4940), *nse3*-1 (JC 4228), *nse3*-1 *fob1*Δ (JC 4975), *smc6*-9 (JC 4942) and *smc6*-9 *fob1*Δ (JC 4943) at IGS1 and IGS2. Fold enrichment is based on normalization to negative control region relative to non-tagged control. Analysis was performed using at least three biological replicates. Statistical analysis is described in methods.