Genomic copy-number loss is rescued by self-limiting production of DNA circles

Andrés Mansisidor, Temistocles Molinar, Jr., Priyanka Srivastava, Demetri D. Dartis, Adriana Pino Delgado, Hannah G. Blitzblau, Hannah Klein, Andreas Hochwagen

Supplemental Material

Figure S1

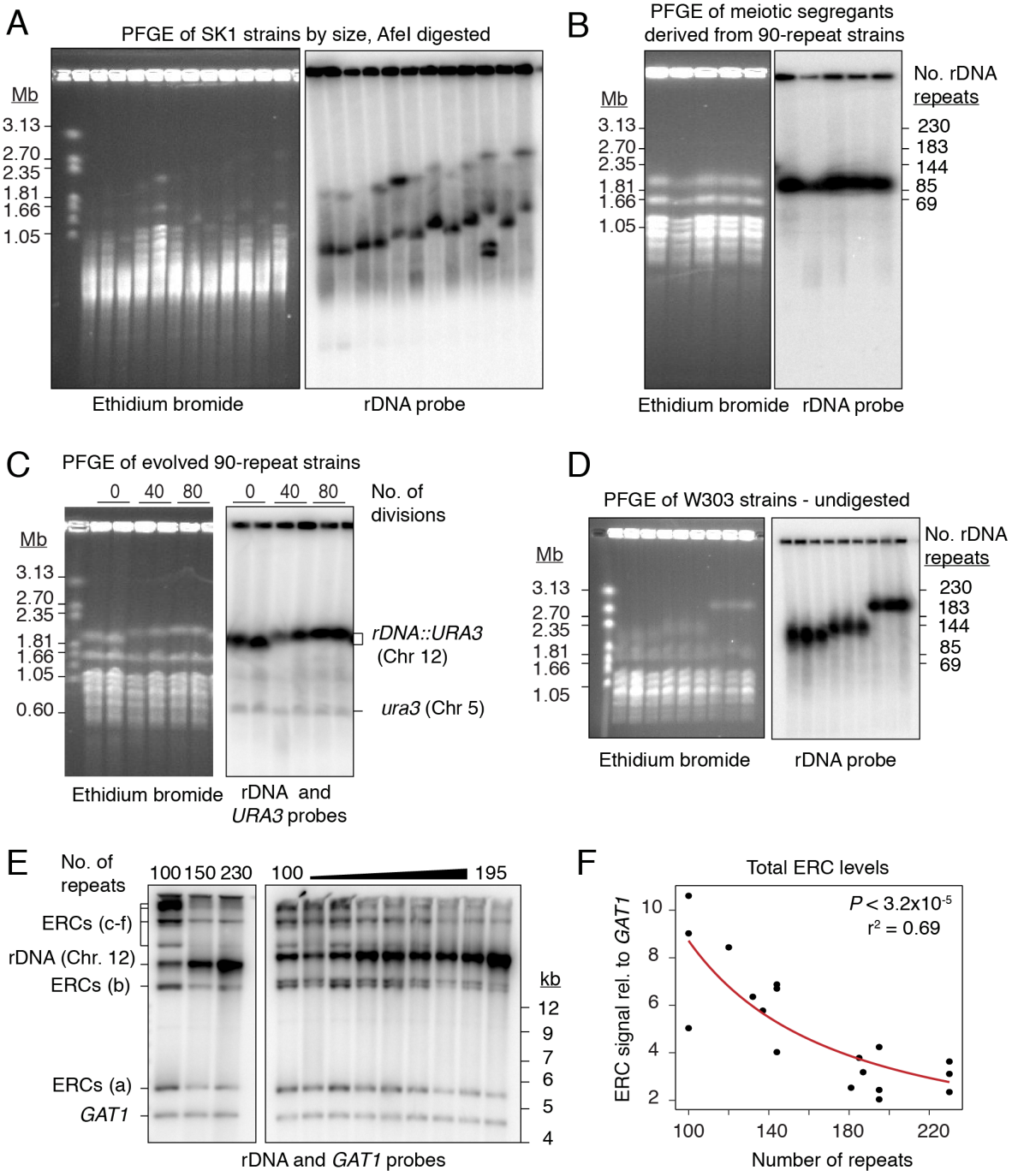
A
PFGE of SK1 strains by size, AfeI digested
Mb
3.13
2.70
2.35
1.81
1.66
1.05
Ethidium bromide
rDNA probe
B
PFGE of meiotic segregants
derived from 90-repeat strains
Mb
3.13
2.70
2.35
1.81
1.66
1.05
No. rDNA
repeats
230
183
144
85
69
Ethidium bromide
rDNA probe
C
PFGE of evolved 90-repeat strains
0 40 80
0 40 80
No. of
divisions
Mb
3.13
2.70
2.35
1.81
1.66
1.05
0.60
rDNA::URA3
(Chr 12)
ura3 (Chr 5)
Ethidium bromide
rDNA and
URA3 probes
D
PFGE of W303 strains - undigested
Mb
3.13
2.70
2.35
1.81
1.66
1.05
No. rDNA
repeats
230
183
144
85
69
Ethidium bromide
rDNA probe
E
No. of
repeats
100 150 230
100
195
ERCs (c-f)
rDNA (Chr. 12)
ERCs (b)
ERCs (a)
GAT1
kb
12
9
7
6
5
4
rDNA and GAT1 probes
F
Total ERC levels
P < 3.2x10-5
r2 = 0.69
ERC signal rel. to GAT1
10
8
6
4
2
100
140
180
220
Number of repeats

Figure S1: ERC levels are dependent on chromosomal rDNA copy number, related to Figure 1.

(A) PFGE analysis of *URA3*-marked strains. Lanes are ordered roughly by rDNA array repeat no. from left to right (93, 89, 96, 100, 123, 118, 145, 133, 153, 179, 147, 183). **(B-C)** PFGE of strains with ~90 chromosomal rDNA copies. **(D)** PFGE analysis of W303 strains with different rDNA sizes. **(E)** Southern analysis of W303 strains probing against rDNA sequence and *GAT1* (loading control) after digestion by XhoI. ERC signals labeled "a-f" based on electrophoretic mobility. **(F)** Total ERC signal (bands "a-e") relative to *GAT1* as function of array size; $P < 3.2 \times 10^{-5}$ (Spearman's rank correlation) and r^2 fit to a power law function.

Figure S2

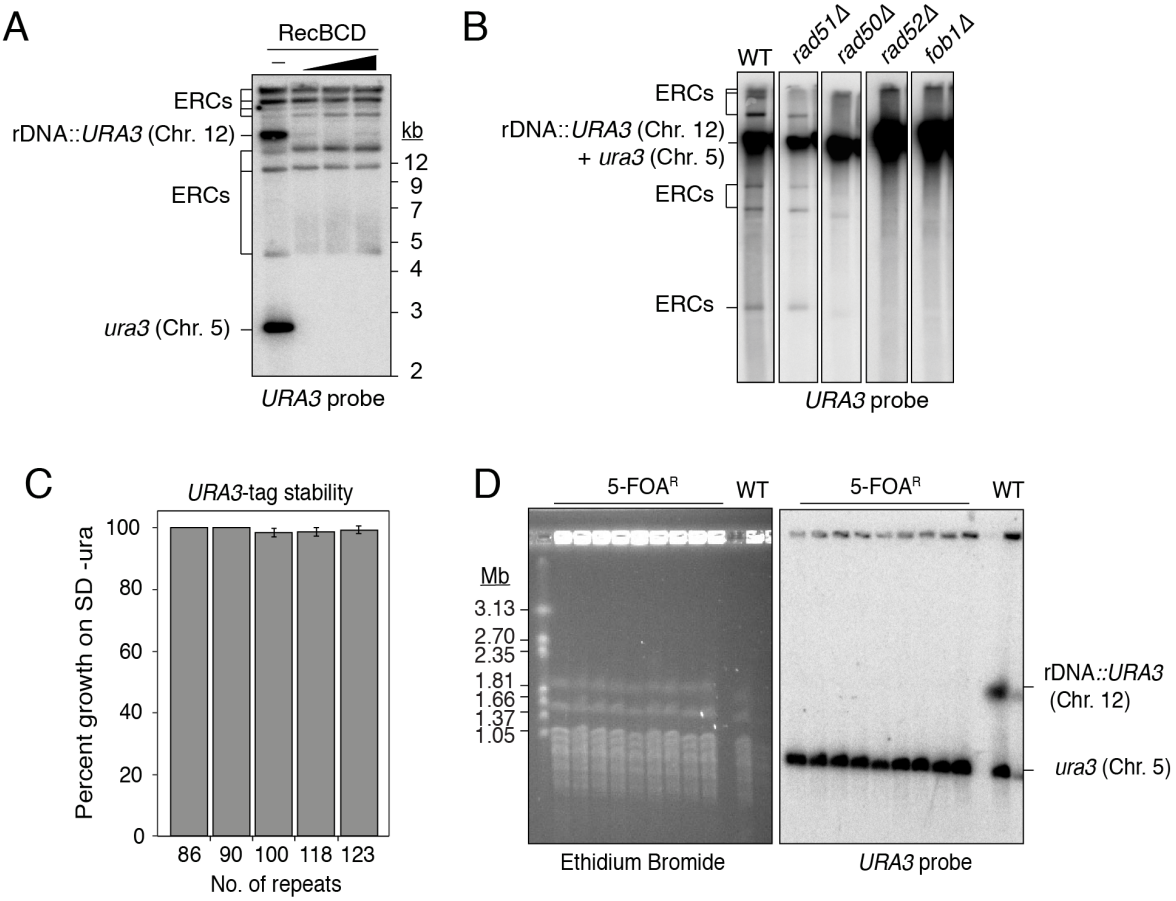
A
RecBCD
–
ERCs
rDNA::URA3 (Chr. 12)
ERCs
ura3 (Chr. 5)
kb
12
9
7
5
4
3
2
URA3 probe
B
WT
rad51Δ
rad50Δ
rad52Δ
fob1Δ
ERCs
rDNA::URA3 (Chr. 12)
+ ura3 (Chr. 5)
ERCs
ERCs
URA3 probe
C
URA3-tag stability
Percent growth on SD -ura
100
80
60
40
20
0
86
90
100
118
123
No. of repeats
D
5-FOA^R
WT
5-FOA^R
WT
Mb
3.13
2.70
2.35
1.81
1.66
1.37
1.05
rDNA::URA3
(Chr. 12)
ura3 (Chr. 5)
Ethidium Bromide
URA3 probe

Figure S2: ERC formation is not linked to the chromosomal loss of rDNA repeats, related to Figure 2.

(A-B) Southern analysis of *URA3*-marked ERCs in ~90-repeat strains after **(A)** digestion with RecBCD, AfeI, and AvrII. AfeI and AvrII do not cut within the rDNA sequence or the *URA3*-marked repeat. **(B)** Analysis of non-digested DNA. Panels are from the same blot but are cropped to reduce redundancy and are shown at different signal levels to clearly demonstrate that there are no ERCs present in *rad52*Δ and *fob1*Δ mutants. **(C)** Proportion of *URA3*-marked cells that grow on uracil-depleted media. Data represents mean $\pm$ standard deviation of three clonal replicates (SD -ura; n=250 for each strain). **(D)** PFGE of 5-FOA^R strains derived from ~90-repeat *URA3*-marked strain; n=9.

Figure S3

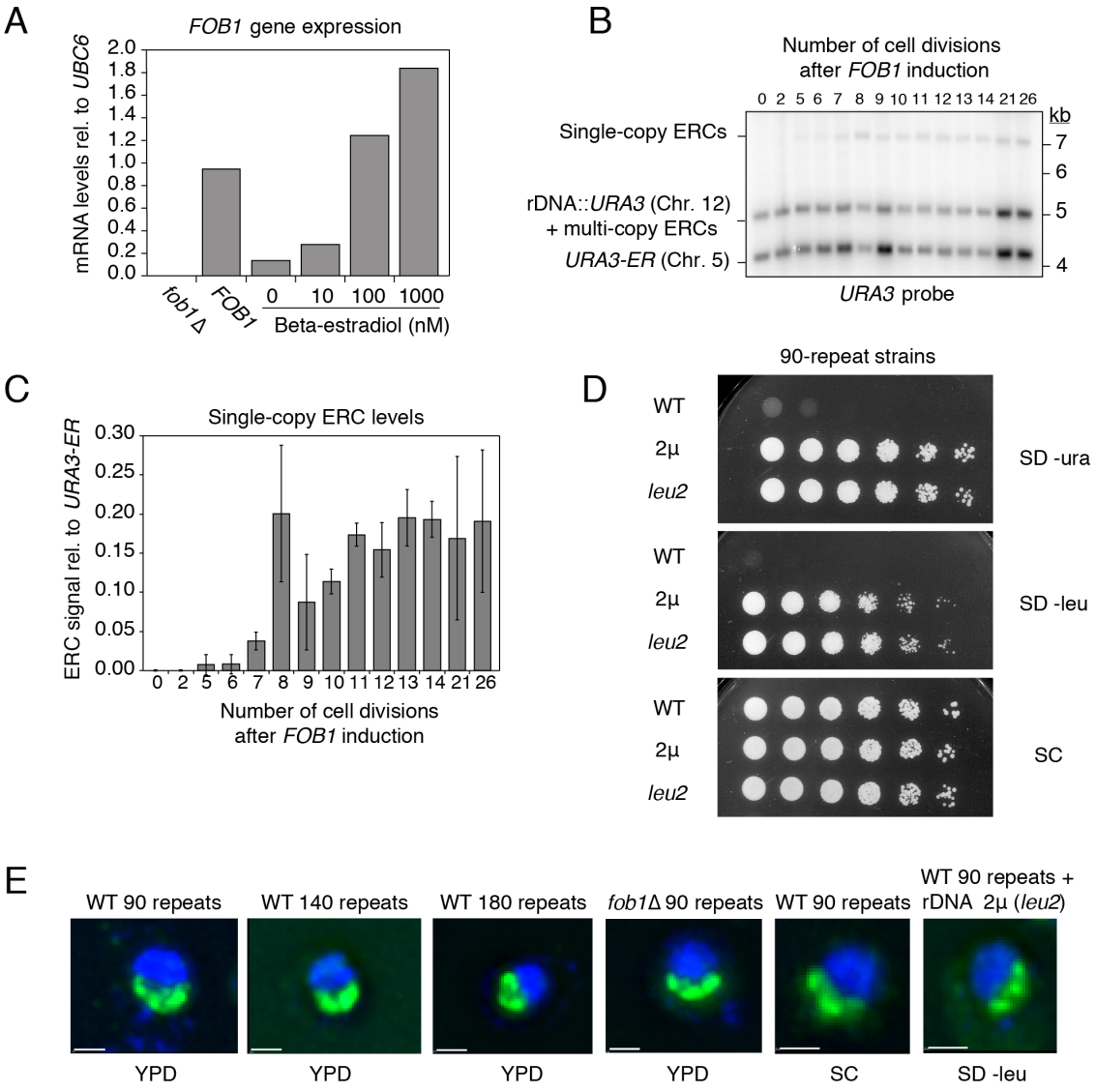
A
FOB1 gene expression
mRNA levels rel. to UBC6
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
fob1Δ
FOB1
0
10
100
1000
Beta-estradiol (nM)
B
Number of cell divisions
after FOB1 induction
0 2 5 6 7 8 9 10 11 12 13 14 21 26
kb
Single-copy ERCs
rDNA::URA3 (Chr. 12)
+ multi-copy ERCs
URA3-ER (Chr. 5)
7
6
5
4
URA3 probe
C
Single-copy ERC levels
ERC signal rel. to URA3-ER
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0 2 5 6 7 8 9 10 11 12 13 14 21 26
Number of cell divisions
after FOB1 induction
D
90-repeat strains
WT
2µ
leu2
SD -ura
WT
2µ
leu2
SD -leu
WT
2µ
leu2
SC
E
WT 90 repeats
WT 140 repeats
WT 180 repeats
fob1Δ 90 repeats
WT 90 repeats
WT 90 repeats +
rDNA 2µ (leu2)
YPD
YPD
YPD
YPD
SC
SD -leu

Figure S3: Rapid accumulation of *URA3*-marked ERCs, related to Figure 3.

(A) Quantification of *FOB1* mRNA levels by qPCR of strains with *fob1*Δ mutation, WT *FOB1* gene, and the Gal-inducible *FOB1* strain; biological replicates, n=2. The latter strain was treated with varying concentrations of Beta-estradiol, whereas the former two were subject to mock treatment with ethanol. Each strain tested contains ~90 chromosomal rDNA repeats. **(B)** Southern analysis of single-copy ERC kinetics in a ~90-repeat strain over the course of 26 cell divisions before (t=0) and after treatment with 100nM Beta-estradiol; biological replicates, n=3. **(C)** Quantification of single copy ERCs relative to the single-copy Estradiol Receptor::*GAL4* gene that was inserted at the *URA3* locus on Chr. 5 (*URA3-ER*). Data represents mean $\pm$ standard deviation of three clonal replicates. **(D)** Spot assay of 90-repeat strains with or without the rDNA-containing 2μ plasmid, onto uracil dropout media, leucine dropout media, and synthetic complete (SC) media (3.5-fold dilutions). **(E)** Immunofluorescence of Nop1 (FITC, green) and DAPI (blue) for strains with different chromosomal rDNA copy numbers, as well as with and without the rDNA-containing 2μ plasmid grown on synthetic complete (SC) and leucine dropout media (SD -leu), respectively. Scale bars equal 1μm in length.

Figure S4

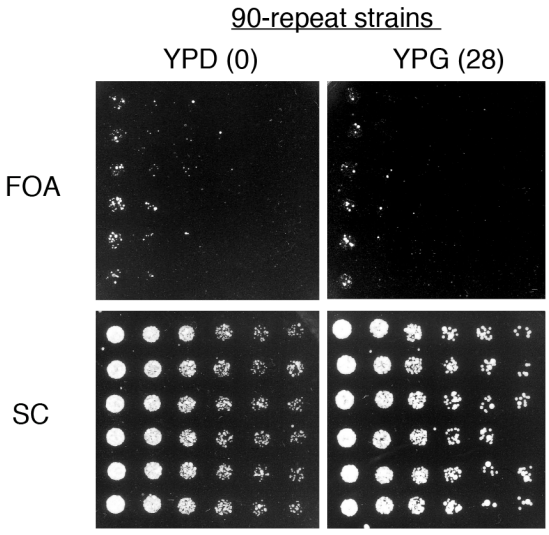

Figure S4: rDNA::*URA3* insertion tags are stable upon growth in glycerol, related to Figure 4. (A) Spot assay of ~90-repeat, *URA3*-marked strains on synthetic complete (SC) media with and without 5-FOA (3.5-fold dilutions) before (YPD) and after 28 cell divisions on glycerol (YPG) (biological replicates, n=6).

Figure S5

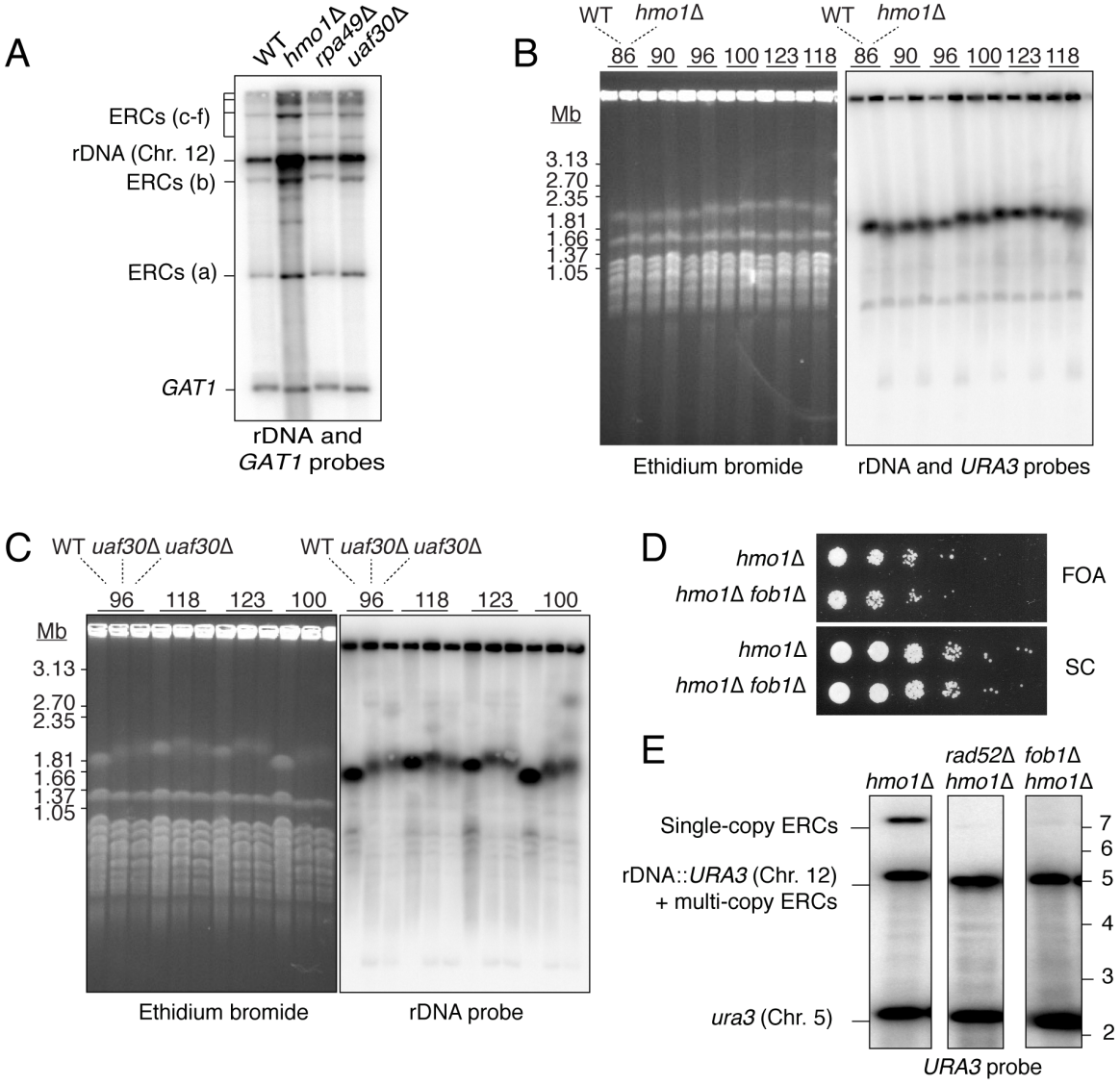
A
WT
hmo1Δ
rpa49Δ
uaf30Δ
ERCs (c-f)
rDNA (Chr. 12)
ERCs (b)
ERCs (a)
GAT1
rDNA and
GAT1 probes
B
WT
hmo1Δ
WT
hmo1Δ
86 90 96 100 123 118 86 90 96 100 123 118
Mb
3.13
2.70
2.35
1.81
1.66
1.37
1.05
Ethidium bromide
rDNA and URA3 probes
C
WT uaf30Δ uaf30Δ
WT uaf30Δ uaf30Δ
96 118 123 100 96 118 123 100
Mb
3.13
2.70
2.35
1.81
1.66
1.37
1.05
Ethidium bromide
rDNA probe
D
hmo1Δ
hmo1Δ fob1Δ
FOA
hmo1Δ
hmo1Δ fob1Δ
SC
E
hmo1Δ
rad52Δ hmo1Δ
fob1Δ hmo1Δ
Single-copy ERCs
rDNA::URA3 (Chr. 12)
+ multi-copy ERCs
ura3 (Chr. 5)
7
6
5
4
3
2
URA3 probe

Figure S5: RNAPI mutants increase ERC levels while maintaining array size, related to Figure 5.

(A) Detection of total ERCs in WT and mutants affecting RNAPI-dependent transcription (*hmo1*Δ, *rpa49*Δ, and *uaf30*Δ) by Southern blotting and probing with rDNA sequence. **(B-C)** PFGE of *hmo1*Δ and *uaf30*Δ strains used in Fig. 5A and Fig. S5A; probed against rDNA and *URA3* sequences. Samples were grouped by repeat number with WT followed by *hmo1*Δ samples or WT followed by two *uaf30*Δ clones. **(D)** Southern analysis of *URA3*-marked ERCs in *hmo1*Δ, *hmo1*Δ *fob1*Δ, and *hmo1*Δ *rad52*Δ, after digestion with NdeI. **(E)** Spot assay of *URA3*-marked *hmo1*Δ and *hmo1*Δ *fob1*Δ strains on synthetic complete (SC) media with and without 5-FOA (3.5-fold dilutions).

Figure S6

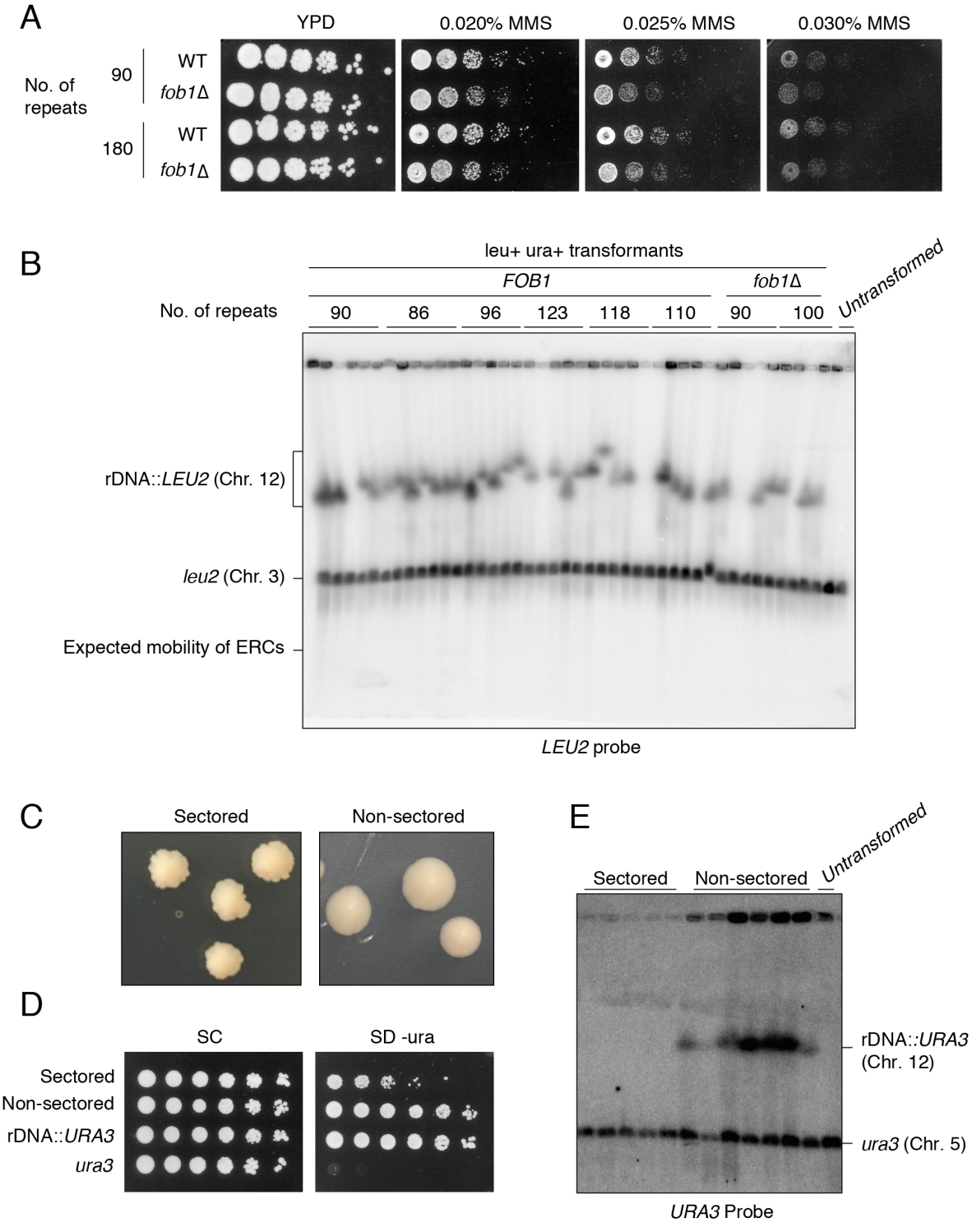
A
YPD
0.020% MMS
0.025% MMS
0.030% MMS
No. of repeats
90
180
WT
fob1Δ
WT
fob1Δ
B
leu+ ura+ transformants
FOB1
fob1Δ
Untransformed
No. of repeats
90
86
96
123
118
110
90
100
rDNA::LEU2 (Chr. 12)
leu2 (Chr. 3)
Expected mobility of ERCs
LEU2 probe
C
Sectored
Non-sectored
D
SC
SD -ura
Sectored
Non-sectored
rDNA::URA3
ura3
E
Sectored
Non-sectored
Untransformed
rDNA::URA3 (Chr. 12)
ura3 (Chr. 5)
URA3 Probe

Figure S6: ERC levels are linked to ERC re-insertion frequency, related to Figure 6.

(A) Spot assay of 90- and 180-repeat *URA3*-marked strains on rich media containing varying amounts of methyl methanesulfonate (MMS); 5-fold dilutions. **(B)** Confirmation of integration of *LEU2* construct at the rDNA (Chr. 12) by PFGE of undigested plugs and probing for *LEU2* sequence. **(C)** Colony morphology of cells after transforming cells with a *URA3*-marked ERC. **(D)** Spot assay of sectored and non-sectored strains onto synthetic complete (SC) and synthetic dextrose (SD) -ura media with 5-fold dilutions. Micromanipulation of single colonies were performed in parallel to determine ~ 2% frequency of sectored colony growth on SD -ura media. **(E)** PFGE analysis of whole undigested chromosomes probed against *URA3* sequence, n=5 sectored and n=7 non-sectored.

Figure S7

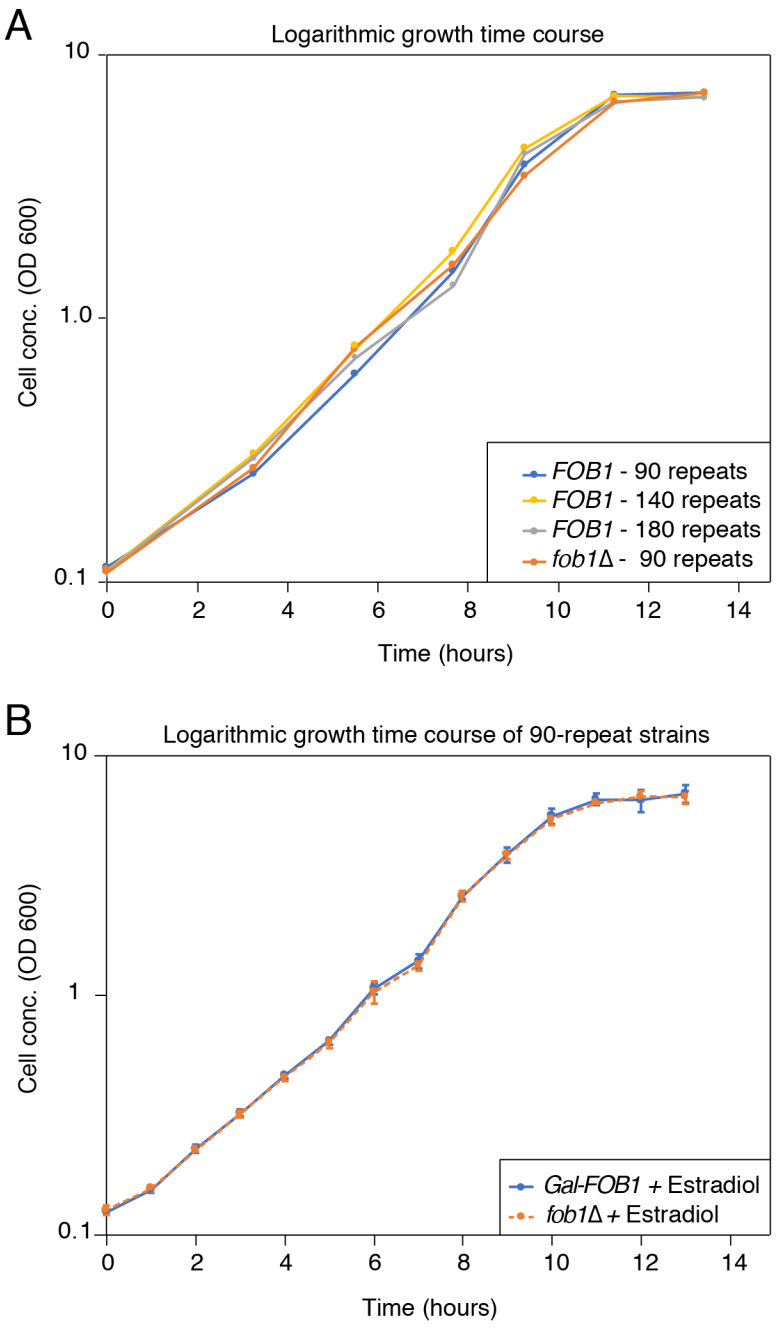
A
Logarithmic growth time course
Cell conc. (OD 600)
10
1.0
0.1
0
2
4
6
8
10
12
14
Time (hours)
FOB1 - 90 repeats
FOB1 - 140 repeats
FOB1 - 180 repeats
fob1Δ - 90 repeats
B
Logarithmic growth time course of 90-repeat strains
Cell conc. (OD 600)
10
1
0.1
0
2
4
6
8
10
12
14
Time (hours)
Gal-FOB1 + Estradiol
fob1Δ + Estradiol

Figure S7: Growth rates are independent of chromosomal rDNA copy number or ERC production, related to Figure 7.

(A-B) Growth curves for strains grown in rich media. **(A)** WT *FOB1* and *fob1*Δ strains with varying number of chromosomal rDNA copy number. **(B)** *URA3-ER* Gal-inducible *FOB1* strain (blue line; biological replicates, n=2) and *URA3-ER fob1*Δ (orange dotted line; biological replicates, n=3) strain were grown in the presence of 100nM Beta-estradiol. Both strains have ~90 chromosomal rDNA repeats.