Supplementary Figures Legends

Figure S1: Rap1 binding, as measured by ChIP, before (t = 0) or following HO cleavage adjacent to arrays of varying lengths of T_2AG_3 or $TG_{1\text{-}3}$ (TG) repeat tracts (top panel). Binding of tbf1-Δi protein, as measured by ChIP following HO induction of a DNA double-strand break adjacent to indicated T_2AG_3 arrays.

Figure S2: (A) Analysis by ChIP of the binding of Tbf1-myc in wild type and *vid22*-Δ *ygro071c*-Δ strains after galactose induction. **(B)** Southern blot monitoring behavior of 60 bp and 230 bp T_2AG_3 after cut on both sides of the break. **(C)** Percentage of large-budded cells (G2/M arrested) following HO cleavage in strains harbouring 230 bp T_2AG_3. Average restart time for each specific construct (indicated in parentheses) was estimated using a Kaplan-Meier survival analysis. Average restart time is not significantly different from wild type in *ygr071c*-Δ *vid22*-Δ, *tlc1*-Δ*48* and *cdc13-2* mutants but is significantly different between uncut *tbf1*-Δ*i* mutant and cut *tbf1*-Δ*i* mutant ($p=3.2x10^{-6}$). **(D)** Percentage of large-budded cells (G2/M arrested) following HO cleavage in strains harbouring 60 bp T_2AG_3. **(E)** Data for survival efficiency following HO induction is shown. Single cells forming colonies that have lost the *LYS2* marker are counted as viable. Cells that failed to form a colony are considered as inviable. Percent (%) viability in strains with 60 bp T_2AG_3 is significantly reduced (chi-square test) compared to wild type in *vid22*-Δ *ygr071*-Δ ($p<0.05$), *tbf1*-Δ*i* ($p<0.001$), *cdc13-2* ($p<0.001$), *tlc1*-Δ*48* ($p<0.001$) and *mre11*-Δ ($p<0.001$). No statistical difference (chi-square test) between wild type and mutants was found for strains with 230 bp T_2AG_3.

Figure S3: Arrays of Bas1, Abf1 or Reb1 repeats are not capped and are not elongated. **(A)** Southern blots monitoring cleavage at the HO site in a wild type strain containing arrays of Bas1, Abf1 or Reb1 binding sequences (See Materials and Methods for details). (INT) The internal loading control; (U) an uncut fragment; (C) the fragment resulting from "U" after induction of the HO cut. **(B)** Analysis by ChIP of the binding of Bas1-13myc in wild type strains after HO induction. **(C)** Analysis by ChIP of the binding of Est2-13myc at long or short Bas1 repeats in wild-type strains, both before and 2 hrs after galactose induction.

Figure S4: Cdc13 and Yku70 association, as measured by ChIP at the DNA ends indicated, before (t = 0) and 2 hrs following HO induction, in wild type strains.

Figure S5: Southern blot monitoring behavior of 60 bp and 230 bp T_2AG_3 tracts in *cdc13-2*, *tlc1*-Δ*48* and *yku70*-Δ mutants.

Figure S6: Southern blot monitoring behavior of 60 bp and 230 bp T_2AG_3 tracts in *sml1*Δ *mec1*Δ.

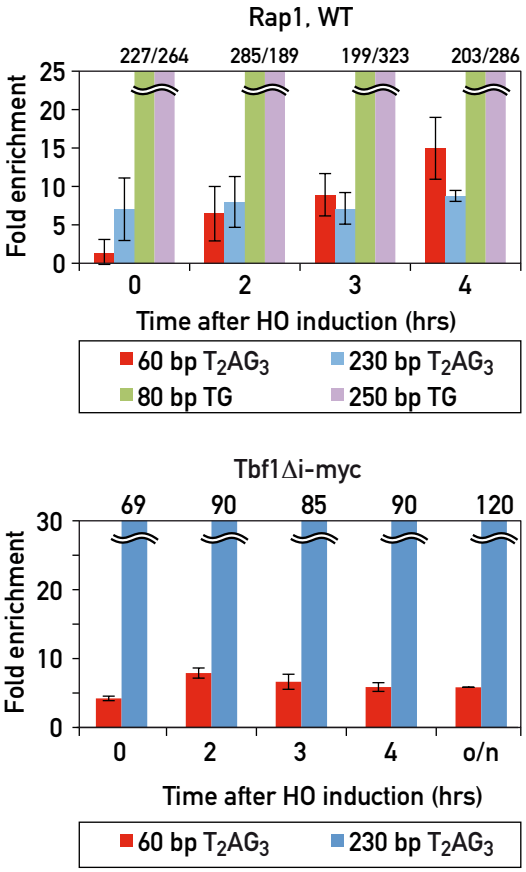
Figure S7: Survival frequency after HO cut in a strain without T_2AG_3 tracts (DSB). Cells were plated on rich medium after 6 hrs or overnight cutting in galactose-containing medium. About 1% of the cells formed a

colony. In order to distinguish between uncut cells and survivors due to telomere formation, colonies were replica plated on plates without lysine. Survival frequency is statistically different ($p<0.001$, chi-square test) in *mec1*-Δ compared to the *MEC1* control strain.

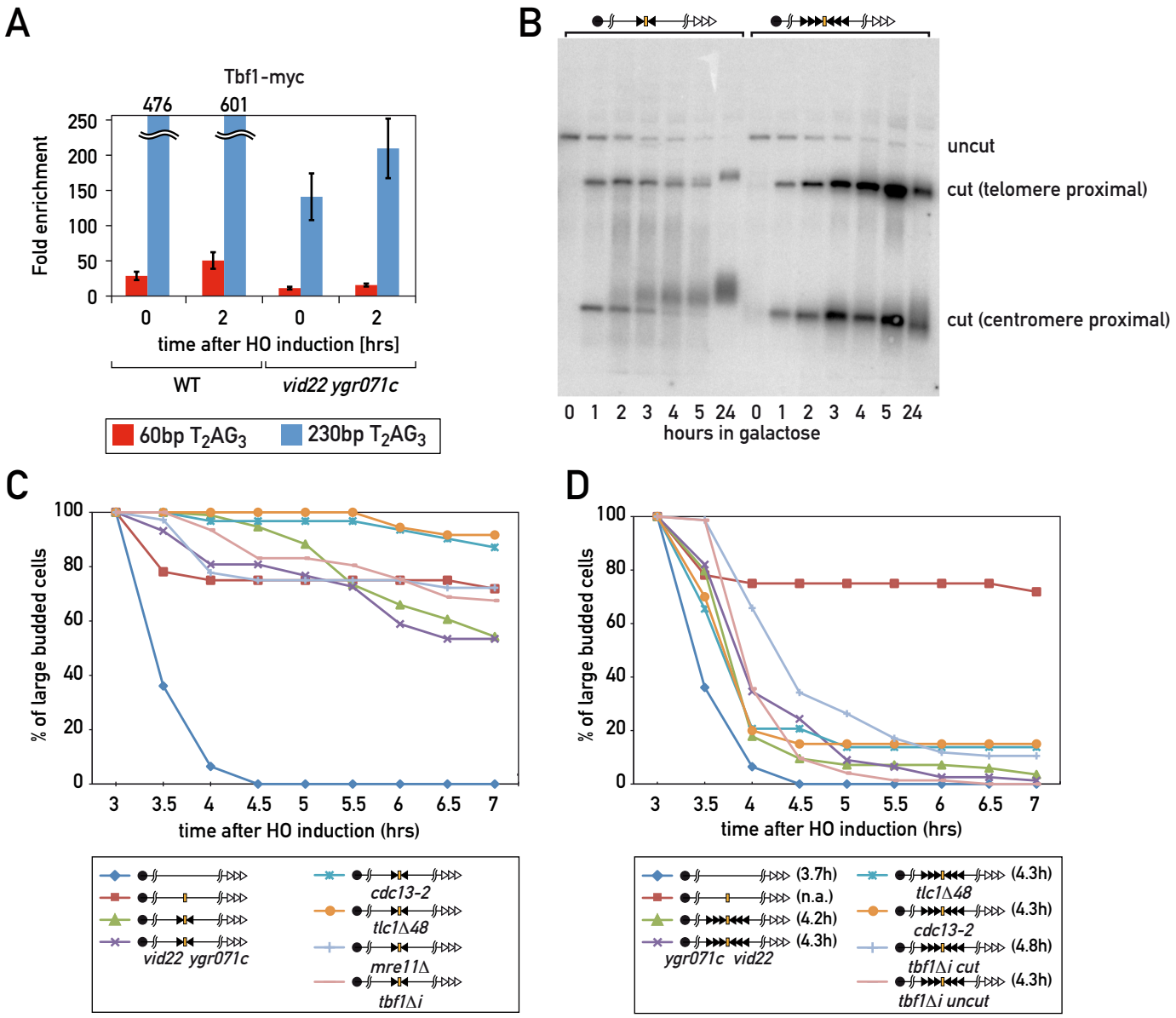
Figure S8: Southern blot monitoring behavior of endogenous telomeres in WT and *tbf1*-Δ*i* mutants. The number of successive re-streaks in indicated. Single colonies from YPD plates were picked and grown overnight in YPD liquid medium prior to preparation of genomic DNA. DNA was digested by *Xho*I and analyzed by Southern-blot using a TG probe.

Figure S9: Southern blot monitoring behavior of 11 bp TG with or without 60 bp T_2AG_3 upstream. Strains were transformed with pTG11 plasmid (Zhang et al. 2010) or pCRS100 (derived from pTG11 with 60 bp T_2AG_3 added). DNA was digested by *Eco*RV and analyzed by Southern-blot using a *URA3* probe.

FigureS1


Rap1, WT
227/264
285/189
199/323
203/286
Fold enrichment
25
20
15
10
5
0
0
2
3
4
Time after HO induction (hrs)
60 bp T2AG3
230 bp T2AG3
80 bp TG
250 bp TG
Tbf1Δi-myc
69
90
85
90
120
Fold enrichment
30
20
10
0
0
2
3
4
o/n
Time after HO induction (hrs)
60 bp T2AG3
230 bp T2AG3

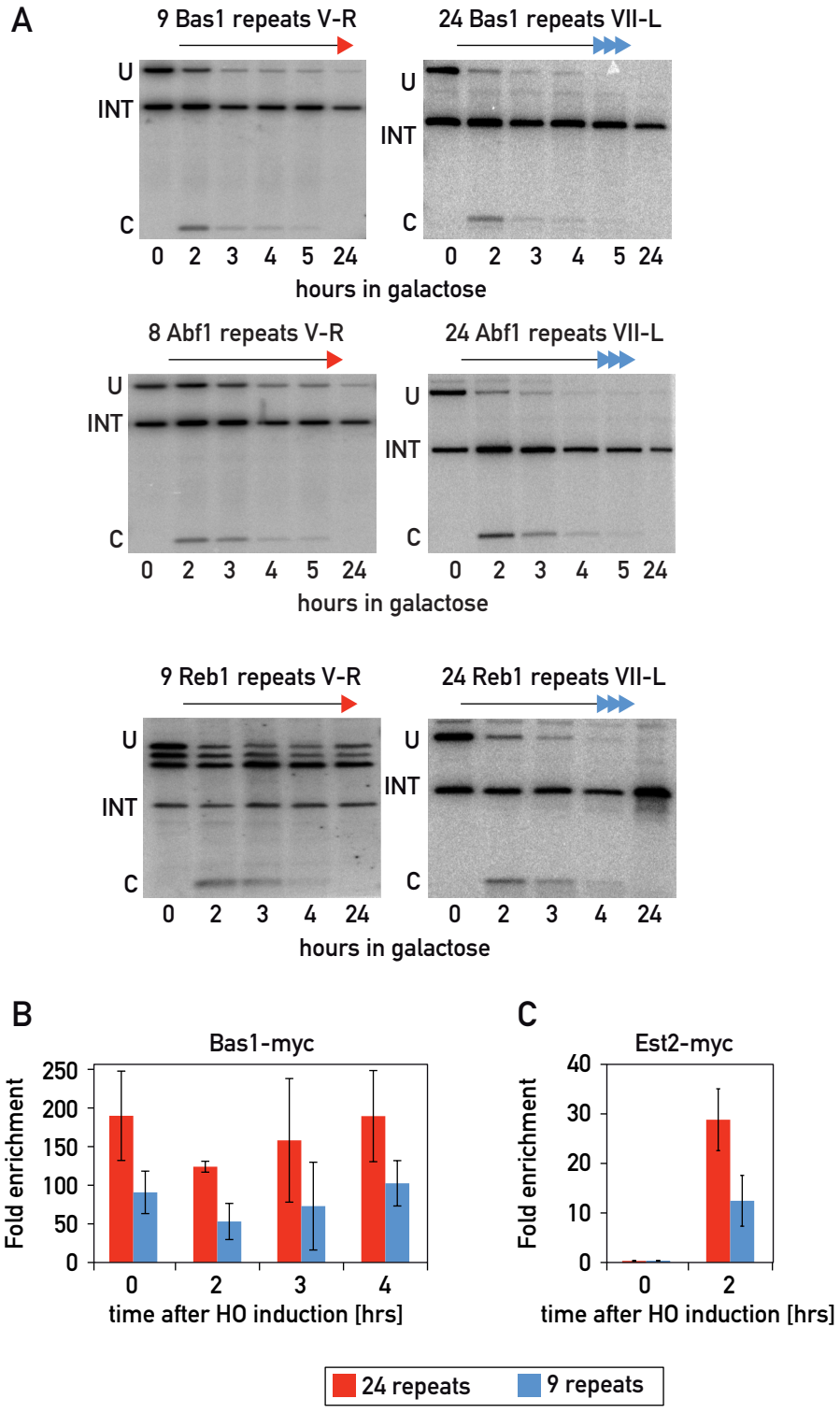
FigureS2



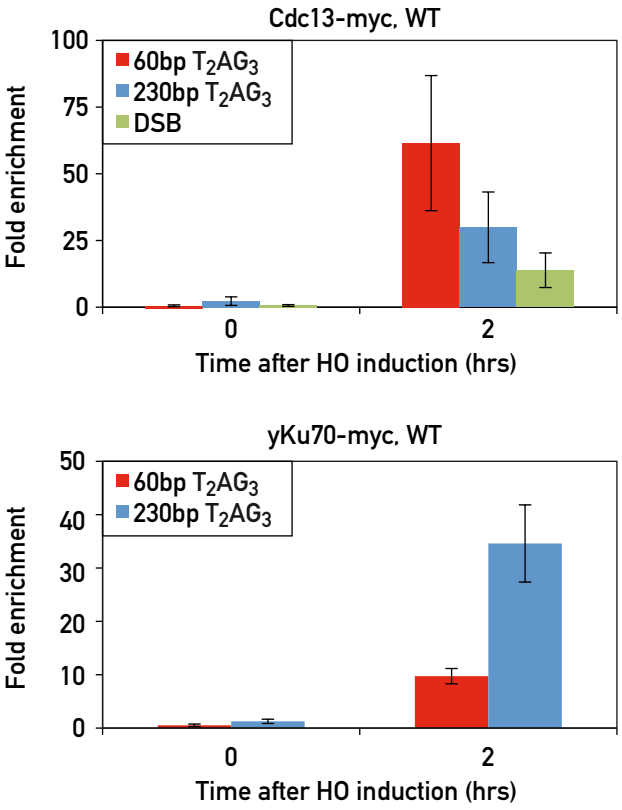
E

	Genotype	Total	Not Viable	% Viability
60bpT2AG3-HO-60bpT2AG3	WT	94	16	83.0%
	vid22Δ ygr071cΔ	73	23	68.5%
	tbf1-Δi	77	38	50.6%
	cdc13-2	31	21	32.3%
	tlc1-Δ48	36	29	19.4%
	mre11Δ	36	34	5.6%
230bpT2AG3-HO-230bpT2AG3	WT	84	2	97.6%
	vid22Δ ygr071cΔ	78	0	100.0%
	tbf1-Δi	76	5	93.4%
	cdc13-2	29	1	96.6%
	tlc1-Δ48	20	1	95.0%

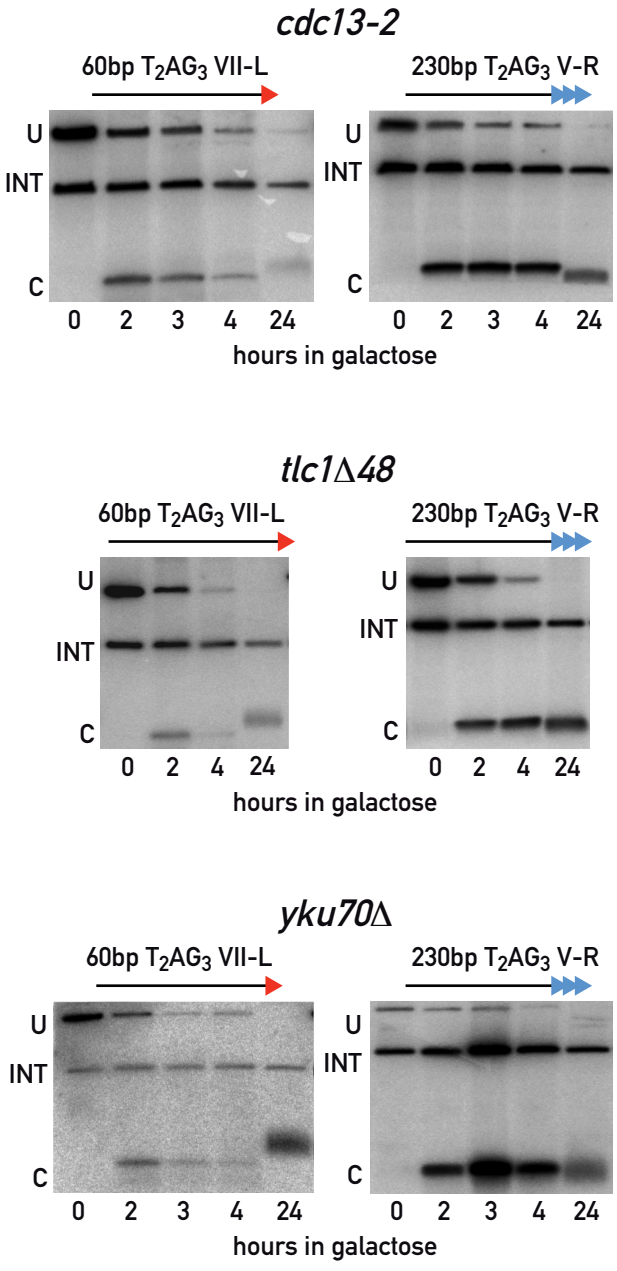
FigureS3


A
9 Bas1 repeats V-R
24 Bas1 repeats VII-L
U
INT
C
0 2 3 4 5 24
hours in galactose
8 Abf1 repeats V-R
24 Abf1 repeats VII-L
9 Reb1 repeats V-R
24 Reb1 repeats VII-L
B
Bas1-myc
Fold enrichment
250
200
150
100
50
0
0 2 3 4
time after HO induction [hrs]
C
Est2-myc
40
30
20
10
0
0 2
24 repeats
9 repeats

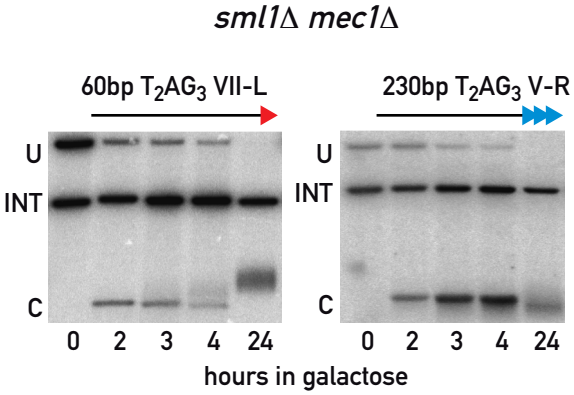
FigureS4


Cdc13-myc, WT
60bp T2AG3
230bp T2AG3
DSB
Fold enrichment
100
75
50
25
0
0
2
Time after HO induction (hrs)
yKu70-myc, WT
60bp T2AG3
230bp T2AG3
Fold enrichment
50
40
30
20
10
0
0
2
Time after HO induction (hrs)

FigureS5


cdc13-2
60bp T2AG3 VII-L
230bp T2AG3 V-R
U
INT
C
0 2 3 4 24
hours in galactose
tlc1Δ48
60bp T2AG3 VII-L
230bp T2AG3 V-R
U
INT
C
0 2 4 24
hours in galactose
yku70Δ
60bp T2AG3 VII-L
230bp T2AG3 V-R
U
INT
C
0 2 3 4 24
hours in galactose

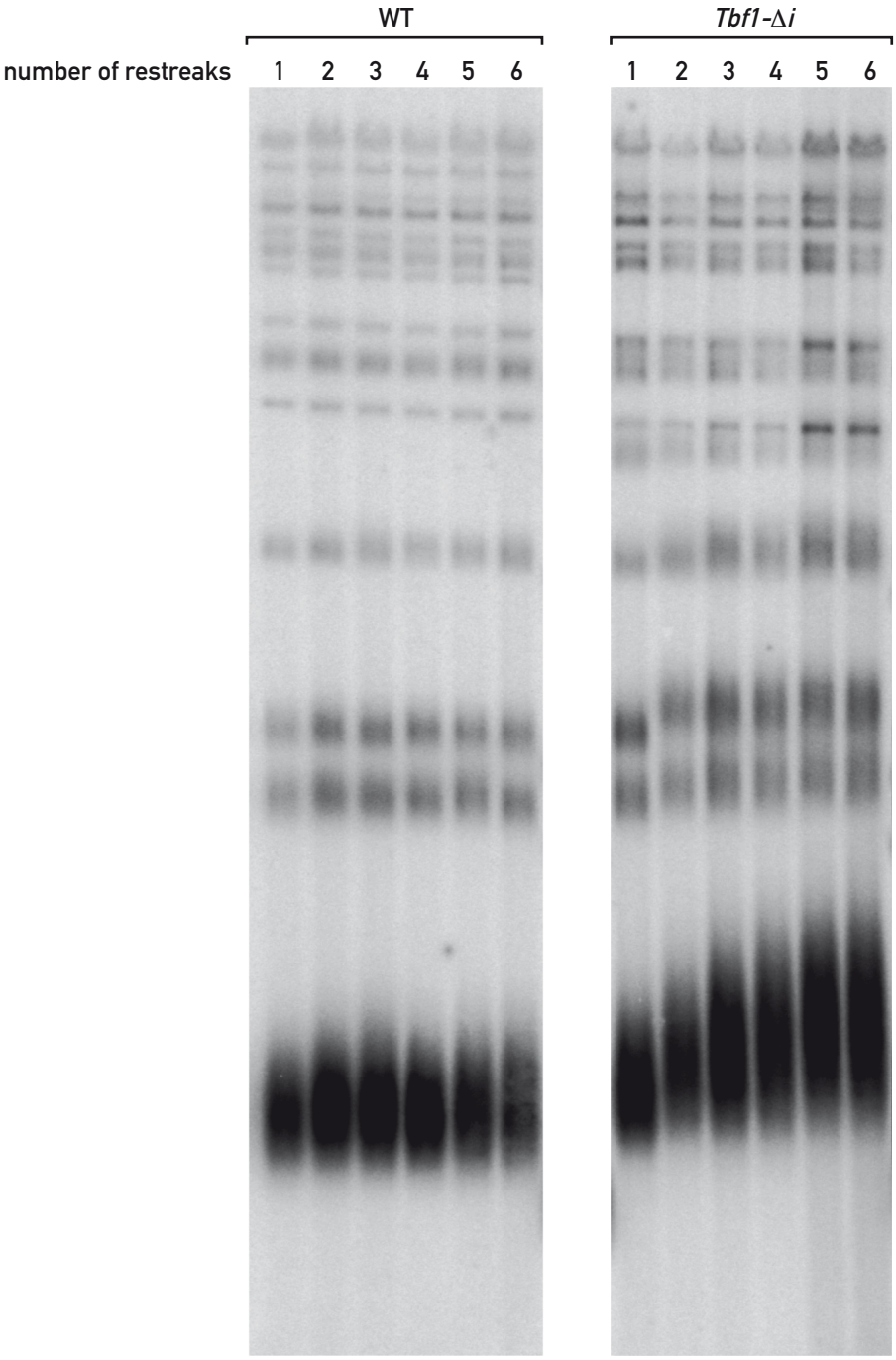
FigureS6


sml1Δ mec1Δ
60bp T2AG3 VII-L
230bp T2AG3 V-R
U
INT
C
U
INT
C
0 2 3 4 24
0 2 3 4 24
hours in galactose

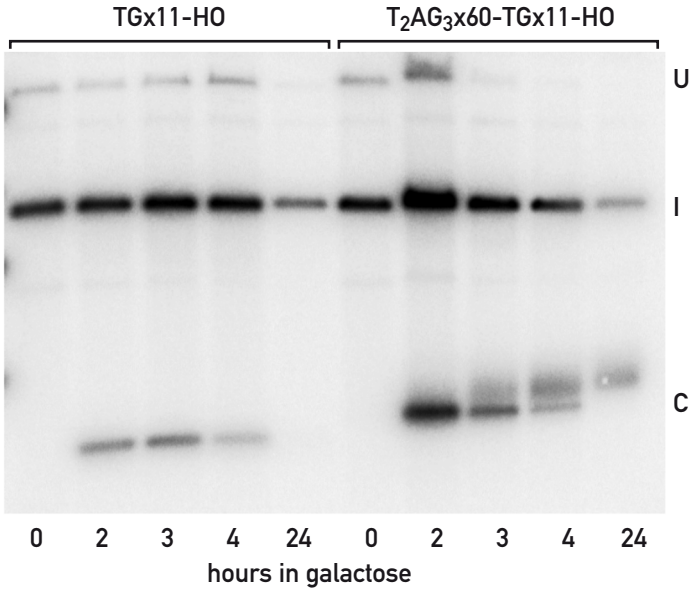
FigureS7

	lys-/total (%)	
	6 hrs cut	o/n cut
sml1Δ *rad52*Δ	322/1666 (19)	366/2152 (17)
mec1Δ *sml1*Δ *rad52*Δ	464/1782 (26)	490/1584 (31)

FigureS8



FigureS9


TGx11-HO
T2AG3x60-TGx11-HO
U
I
C
0 2 3 4 24 0 2 3 4 24
hours in galactose