Supplementary Table I. Genotypes of strains used in this work.

Strain	Genotype	Reference
JKM139	*ho*Δ *MAT***a** *hml*Δ::*ADE1 hmr*Δ::*ADE1 ade1-100 leu2-3,112 trp1::hisG' lys5 ura3-52 ade3::GAL::HO*	(Lee et al. 1998)
JKM179	*ho*Δ *MAT*α *hml*Δ::*ADE1 hmr*Δ::*ADE1 ade1-100 leu2-3,112 trp1::hisG' lys5 ura3-52 ade3::GAL::HO*	(Lee et al. 1998)
SLY403	JKM179 *rsc2*Δ::*KAN*^{r}	(Shim et al. 2005)
SLY579	JKM179 *URA3*::*CMV-tTA KAN*^{r}*: TetO-TATA-STH1*	This work
SLY558	*ho*Δ *MAT*α *hml*Δ::*ADE1 hmr*Δ::*ADE1 ade1-100 leu2-3,112 trp1::hisG' lys5 ura3-52*	This work
SLY1103	*ho*Δ *MAT***a** *hml*Δ::*ADE1 hmr*Δ::*ADE1 ade1-100 leu2-3,112 trp1::hisG' lys5 ura3-52 ade3::GAL::HO RSC1-FLAG*	This work
SLY1101	*ho*Δ *MAT***a** *hml*Δ::*ADE1 hmr*Δ::*ADE1 ade1-100 leu2-3,112 trp1::hisG' lys5 ura3-52 ade3::GAL::HO RSC1-FLAG hta1-S129A hta2-S129A*	This work

Figure S1. Kinetics of DSB induction at the *MAT* locus.

HO expression was induced by the addition of 2% galactose for the indicated times in yeast grown in pre-induction medium (YEP-glycerol) with or without doxycycline, and DSB formation was monitored by qPCR using a set of primers targeting either side of the DSB. Percent HO cleavage was determined by the ratio of the uncut fragment signal relative to the control PCR with PRE1 primers normalized to the value obtained from the uninduced (0 hr) sample and is plotted as a function of duration of HO expression.

Figure S2. Mapping the nucleosome positions of chromatin remodeling intermediates.

Yeast cells grown and induced at 16ºC or 30ºC were harvested at various time intervals after HO expression for 1 hr. MNase-digested chromatin was then subjected to primer extension analysis using a primer corresponding to base pairs +120 to +144 with respect to the HO cut site (**A**), or that corresponding to base pairs +696 to +729 (**B**). Shown for reference are digestions of naked DNA (lanes 1 and 2), and no MNase digestion (lanes 11 and 12), as well as digestions of chromatin from cells grown and induced with HO for an hour at 30ºC (lane 10).

Figure S3. A single DSB does not induce global changes in chromatin structure. Nuclei were isolated from JKM179 cells before and after the induction of an HO break and incubated with increasing concentrations of MNase. The DNA was isolated, electrophoresed, and visualized by staining with ethidium bromide (A and E). To reveal local chromatin remodeling that only occurs at or near a DSB, the MNase digests were subjected to Southern blot hybridization using a radiolabeled probe that anneals 1 kb proximal (B and F) or 1 kb distal (C and G) to the DSB, or the *ACT1* gene (D and H) on another chromosome as a control.

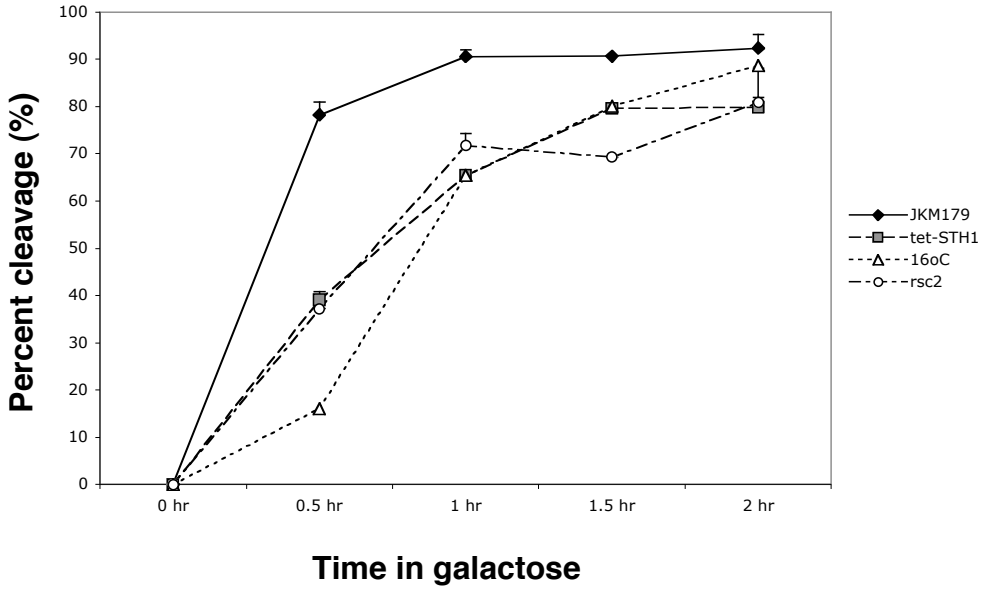
Figure S4. Accessibility of DNA surrounding the DSB to restriction enzymes.

Permeabilized spheroplasts were digested with the enzymes listed in Figure 3a and subjected to Southern blot hybridization using a probe specific for the *MAT* locus as described in the Materials and Methods. Percent cleavage was determined as described in Figure 3. U, uncleaved; C, cleaved; *, no HO cleavage.

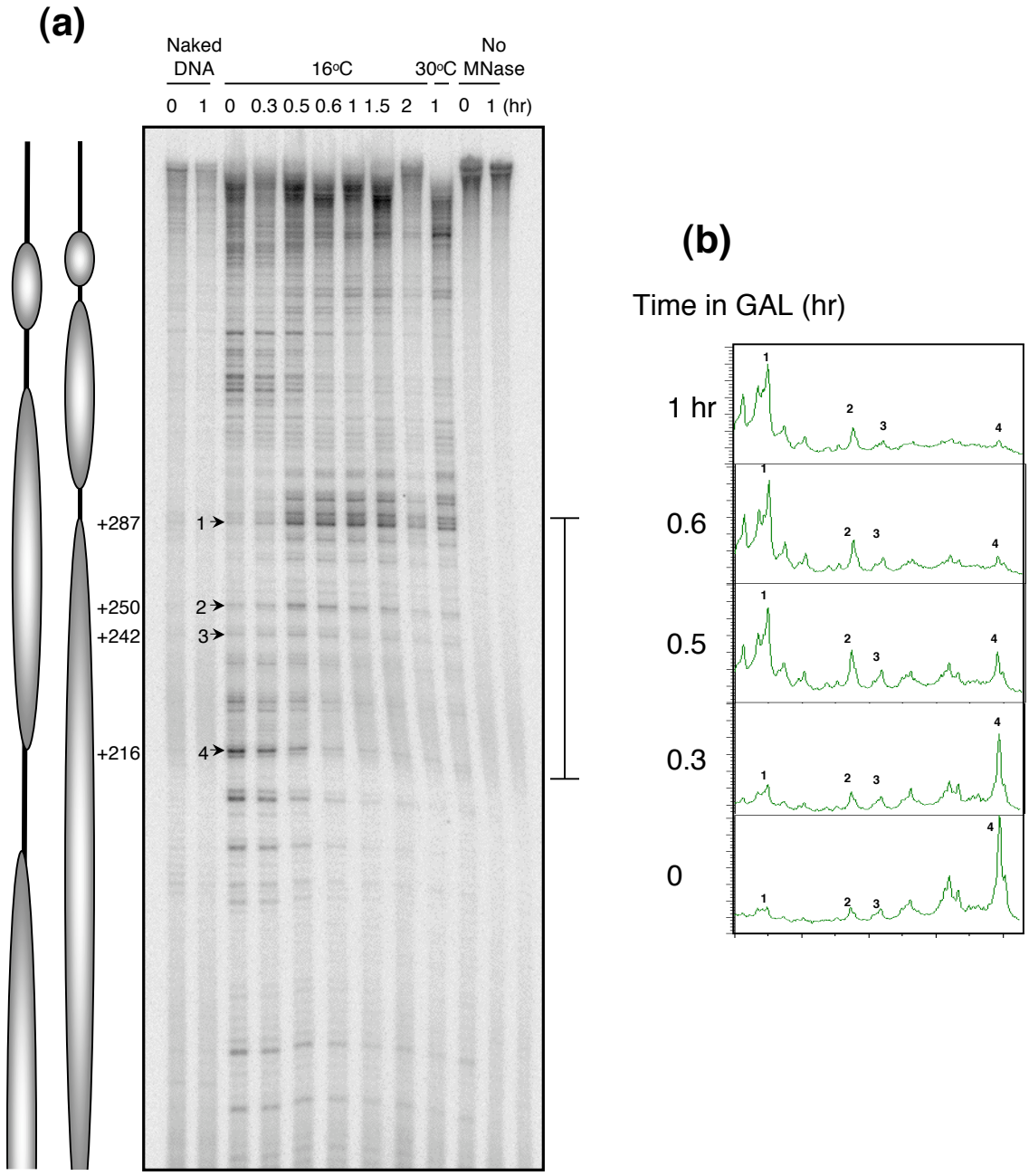
Figure S5. Repression of Sth1 expression does not alter the level of Yku or Mre11 proteins

The level of Sth1 protein expression was analyzed by Western blot analysis of whole-cell extracts using anti-Sth1 antiserum (a gift from B. Laurent) (**A**). To detect the level of Yku (**B**) or Mre11 (**C**) protein, yeast extracts (200 μg) were immunoprecipitated with an anti-Yku or anti-Mre11 antibody, separated with SDS-PAGE, transferred to a polyvinylidene difluoride membrane, and then probed with anti-Yku or anti-Mre11 antiserum. Immunoblotting with an anti-CpY antibody is shown as an input control.

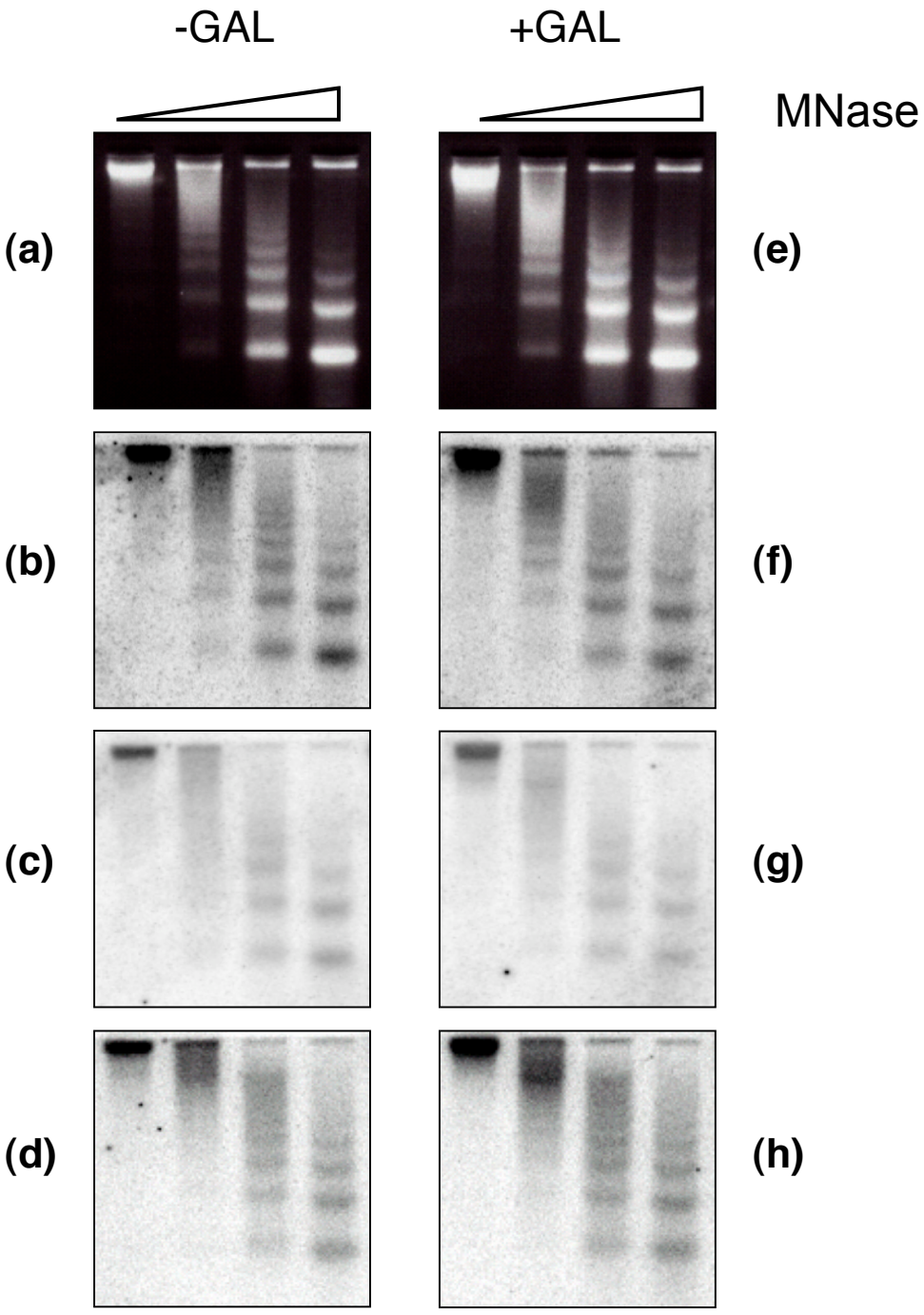
Supp Fig. 1


Percent cleavage (%)
100
90
80
70
60
50
40
30
20
10
0
0 hr
0.5 hr
1 hr
1.5 hr
2 hr
Time in galactose
JKM179
tet-STH1
16oC
rsc2

Suppl Fig.2

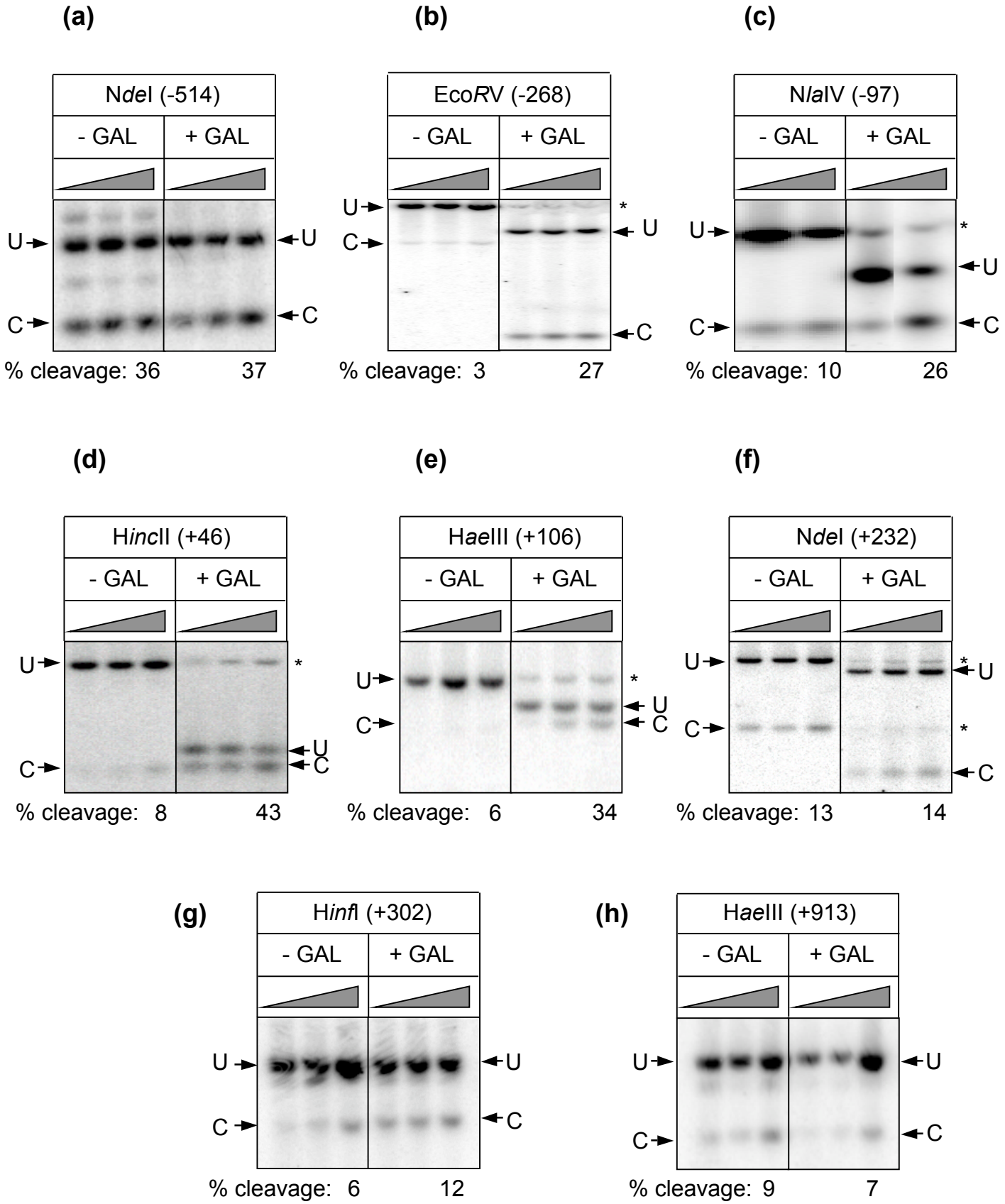

(a)
Naked DNA
16°C
30°C
No MNase
0 1 0 0.3 0.5 0.6 1 1.5 2 1 0 1 (hr)
+287
+250
+242
+216
1
2
3
4
(b)
Time in GAL (hr)
1 hr
0.6
0.5
0.3
0

Supp Fig.3

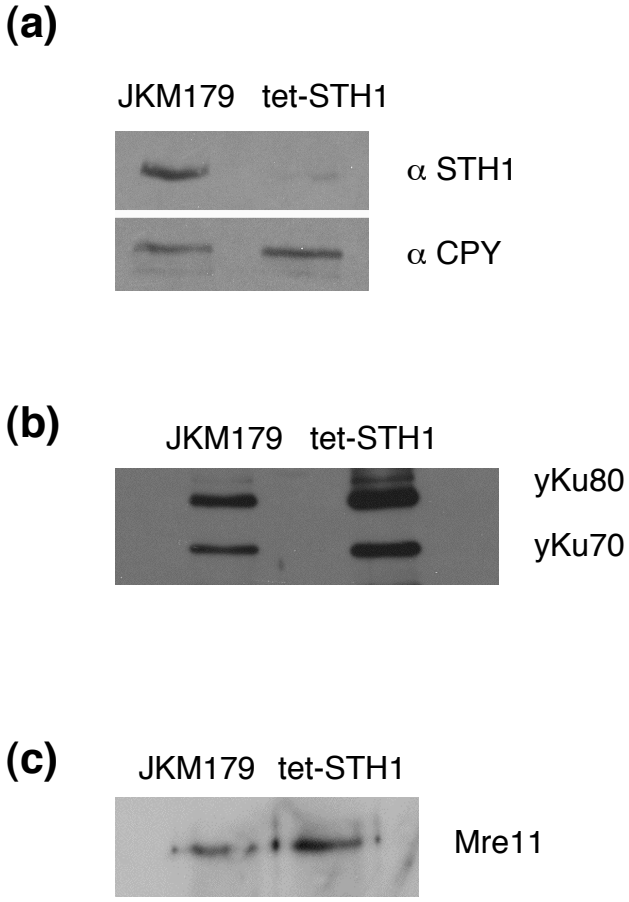


Suppl Fig.4

Shim *et al.*, 2006



Supp Fig. 5


(a)
JKM179
tet-STH1
α STH1
α CPY
(b)
JKM179
tet-STH1
yKu80
yKu70
(c)
JKM179
tet-STH1
Mre11