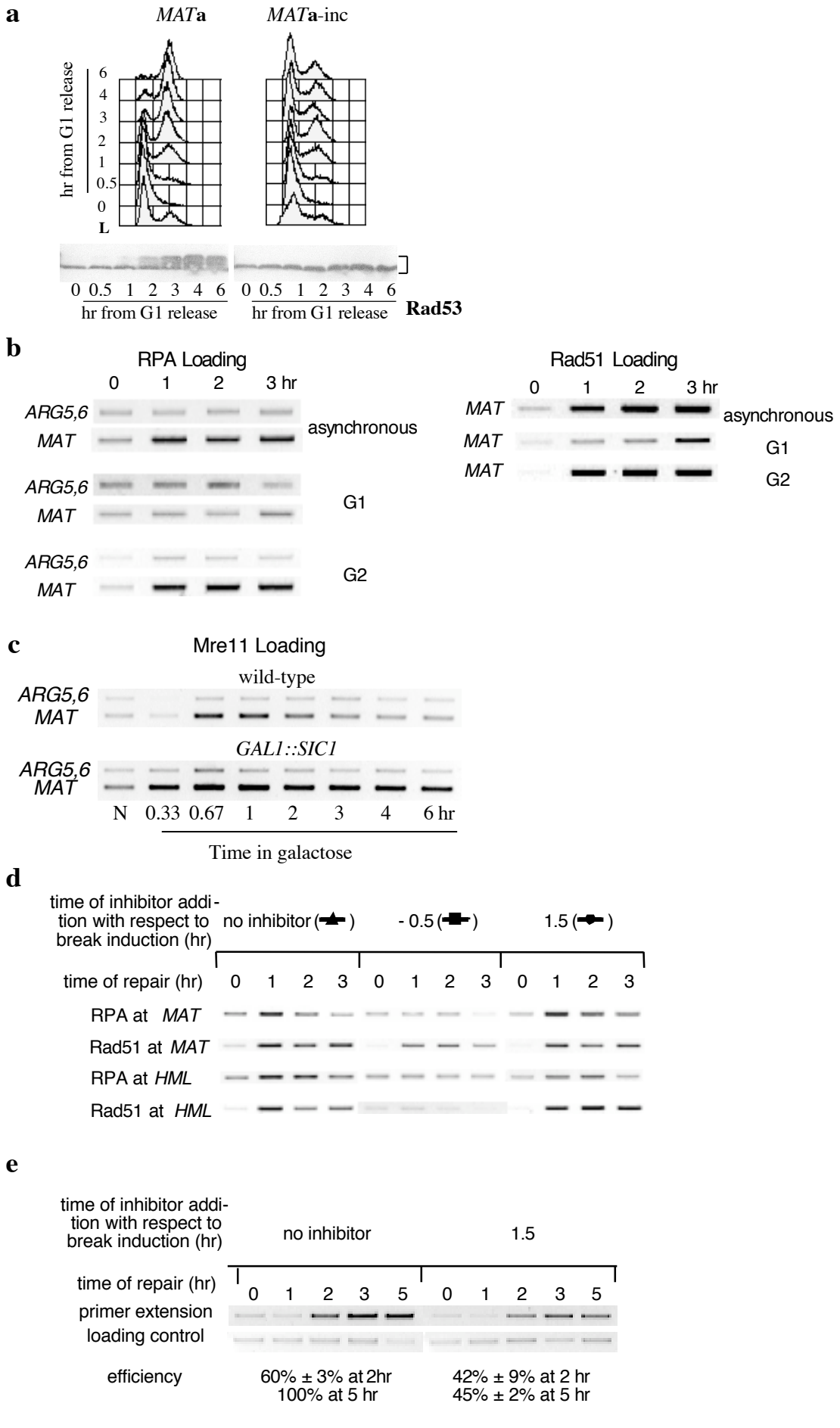
Supplemental Fig. S1

**a**, Rad53 phosphorylation in response to a unrepaired DSB break . G1 cells of strain JKM179 were arrested by α-factor were released into galactose-containing medium to induce expression of HO endonuclease. The DSB at *MAT* cannot be repaired by homologous recombination. Checkpoint activation was monitored by Rad53 hyperphosphorylation on a Western blot. A control strain, treated the same way, carries a *MAT***a**-inc mutation that cannot be cleaved. FACS analysis is shown. **b**, Chromatin immunoprecipitation (ChIP) of RPA and Rad51 at DSB ends in cells at different stages of the cell cycle. Experimental details are described in Fig 3b. **c**, ChIP of Mre11 bound to DSB ends in nocodazole-arrested G2 cells, after induction of a DSB. **d,** ChIP of RPA and Rad51 at both *MAT* and the donor *HML* during induction of *MAT* switching in cells in which CDK1 was inhibited at the times indicated. These data are shown graphically in Fig. 5b. **e**, New DNA synthesis primed by the 3' end of *MAT* after strand invasion into the donor *HMLα* locus was measured by PCR using the primers indicated in Fig. 5d.


a
MATa
MATa-inc
hr from G1 release
6
4
3
2
1
0.5
0
L
0 0.5 1 2 3 4 6
hr from G1 release
0 0.5 1 2 3 4 6
hr from G1 release
Rad53
b
RPA Loading
0 1 2 3 hr
ARG5,6
MAT
asynchronous
G1
G2
Rad51 Loading
0 1 2 3 hr
MAT
asynchronous
G1
G2
c
Mre11 Loading
wild-type
GAL1::SIC1
ARG5,6
MAT
N 0.33 0.67 1 2 3 4 6 hr
Time in galactose
d
time of inhibitor addition with respect to break induction (hr)
no inhibitor
- 0.5
1.5
time of repair (hr)
0 1 2 3 0 1 2 3 0 1 2 3
RPA at MAT
Rad51 at MAT
RPA at HML
Rad51 at HML
e
time of inhibitor addition with respect to break induction (hr)
no inhibitor
1.5
time of repair (hr)
0 1 2 3 5 0 1 2 3 5
primer extension
loading control
efficiency
60% ± 3% at 2hr
100% at 5 hr
42% ± 9% at 2 hr
45% ± 2% at 5 hr