Supplementary information

Supplementary Experimental Procedures

Measurement of stability of ternary complex – To estimate the off-rate of binding, a cold competitor was added to the preformed ternary complex. PriA protein, known to stably bind to fork-like DNA, was used as control. Binding reaction (with the labeled A-fork (3') as a substrate) was conducted as described (1), with the combination of Mrc1 (256 nM) and Swi1-Swi3 (16 nM) or PriA (4 nM) for 10 min at 30 ºC, followed by addition of 0.5 pmole (10-fold amounts) of cold A-fork (3') DNA and further incubation for 1, 5, and 10 min. Reactions were terminated and loaded onto 6% PAGE and the gel was dried and autoradiographed. Each band intensity was quantified and ratio of bound DNA to total DNA was calculated. The value at time zero was taken as 100 and the relative values at each timepoint were plotted.

Super-shift assays with antibodies – To confirm protein identity in ternary complex, 1 μg of antibody, as indicated, was added after standard binding reaction with the combination of Mrc1 (256 nM) and Swi1-Swi3 (16 nM), and incubation was continued for additional 10 min. Reactions were terminated and loaded onto 6% PAGE and the gel was dried and autoradiographed.

Competition assays for binding of Mrc1 and Swi1-Swi3 proteins – To examine binding specificity, various amounts of double-stranded DNA competitor, poly (dI-dC), were added to DNA binding reactions and binding reaction was conducted as described in "Experimental Procedures" in the main text. Substrates (double-stranded or Y-fork DNA) were present at 4 nM.

Native gel electrophoresis – Analysis of interaction of Mrc1 with the wild-type or mutant Swi1-Swi3 complex was conducted as described in "Experimental Procedures" except that the proteins were added at 32 nM.

Supplementary Reference

1. Tanaka, T., Mizukoshi, T., Sasaki, K., Kohda,D., and Masai, H. (2007) *J. Biol. Chem.* **282,** 19917-19927

Legends to Supplementary Figures

Supplementary Figure S1. **Purification of fission yeast Mrc1 and wild-type or mutant Swi1-Swi3 proteins.** (A) The purified fractions of Mrc1 and Swi1-Swi3 complex were analyzed on SDS-PAGE and the proteins were stained with silver. The final fractions from MonoQ column of Mrc1 (800 ng, lane 1), Swi1-Swi3 wild type (250 ng, lane 2), or Swi1 (E662K)-Swi3 (125 ng, lane 3) were loaded. (B) The Swi1-Swi3 complex is eluted in two peaks in MonoQ column fractionation. Purification procedures are described in "Experimental Procedures". In the final MonoQ fractionation, the Swi1-Swi3 complex was eluted with linear gradient of salt (0 – 1 M of KCl). Chromatogram shows two distinct peaks (upper left) and both peaks contain the Swi1-Swi3 complex (upper right, lanes 2 – 6). However, DNA binding activity could be detected only in the fraction number 13 (lower, lanes 13 – 16). The ternary complex was formed also only with fraction 13 (lane 17). The second peak (fractions 15 and 16) could not bind to DNA and did not support the ternary complex formation (lanes 18 – 27). Lanes 2 – 7 represent positive controls with a preparation containing the active Swi1-Swi3 protein. The second peak appears to contain a potent inhibitor for DNA binding, since addition of a small aliquot of this fraction inhibits DNA binding of an

active fraction.

Supplementary Figure S2. **Nuclease protection analyses of interaction of Swi1-Swi3 with arrested fork structures.** DNase I footprinting assays were conducted as described in "Experimental procedures". The substrates used, schematically drawn on top and at the sides of the panel, are A-fork (3') (lanes 1 – 7) and A-fork (3', 5') (lanes 8 – 14). Filled arrowheads indicate the positions of the branch point. The upper strand of the template was radioactively labeled with ^{32}P as indicated by asterisks.

Supplementary Figure S3. **Antibody supershift assays on the Mrc1-Swi complex.** Antibody supershift assays were conducted as described in the "Supplementary Experimental Procedures". Gel shift assays were conducted with Y-fork. Lane 1, no protein; lanes 2 – 4, Mrc1 (256 nM) + Swi1-Swi3 (16 nM). Lane 2, no antibody added; lane 3, anti-Mrc1 antibody; lane 4, anti-FLAG antibody. The positions of normal ternary complex, super-shifted complex, and Swi1-Swi3 complex are indicated by black, white, and gray arrows, respectively.

Supplementary Figure S4. **Binding stability of Mrc1-Swi-DNA ternary complex.** Binding stability of Mrc1-Swi complex bound to a forked substrate was examined as described in the "Supplementary Experimental Procedures". Gel shift assays were conducted with A-fork (3'). Lane 1, no protein; lanes 2 – 5, Mrc1 (256 nM) + Swi1-Swi3 (16 nM); lanes 6 – 9, PriA (4 nM). After binding reaction, 10-fold excess of cold A-fork (3') was added and further incubation was continued as indicated. At each time point, reactions were applied onto 6% PAGE. Black and gray arrows indicate the positions of the complexes. Left graph shows the quantification of the data which was conducted as described in "Supplementary Experimental Procedures".

Supplementary Figure S5. **Effect of poly (dI-dC) on binding of Mrc1 and Swi1-Swi3 to substrate DNAs.** To examine binding specificity, double-stranded DNA competitor, poly (dI-dC), was added in each assay. (A) Substrates used were double-stranded 49 bp DNA (ds, lanes 1 – 14) or 49-mer Y-fork DNA (lanes 15 – 28). Swi1-Swi3 complex or Mrc1 was added at 16 nM or 512 nM, respectively, in the presence of increasing amounts of poly (dI-dC), as indicated in the figure. (B). Effects of poly (dI-dC) were examined in lower concentration ranges under the assay conditions identical to those in (A). The complexes generated were quantified and DNA binding level relative to that in the absence of competitor was plotted in the graph.

Supplementary Figure S6. **Amino acid substitutions in Swi1 or Swi3 mutant proteins generated.** A portion of the fission yeast Swi1 or Swi3 sequences are aligned with Tof1 (budding yeast) and Tim (human) or Csm3 (budding yeast) and Tipin (human), respectively. The conserved amino acids selected for substitution are indicated by bold letters.

Supplementary Figure S7. **Interaction of the Swi1-Swi3 complex containing a Swi3 mutant, W95A, with Mrc1.** Interaction of Swi1-Swi3 (W95A) mutant with Mrc1 was analyzed in native gel electrophoresis. Analysis was conducted as described in the legend to Fig. 7. Mrc1 (lane 1), Swi1-Swi3 wild type (lane 2), or Swi1-Swi3 W95A (lane 5), and combination of Mrc1 and Swi1-Swi3 (wild type in lane 3 and W95A in lane5) were examined in the presence of 4 nM of Y-fork substrate. Proteins were added at final concentration of 32 nM. The positions of molecular weight standards are indicated on the left of the gel. The Mrc1-Swi complex migrating above the Mrc1

band is not detected with the W95A mutant, suggesting that W95 of Swi3 plays an important role in interaction with Mrc1. Swi1-Swi3 (W95A) complex is only weakly visible (lane 4), presumably due to instability of this complex during the electrophoresis.

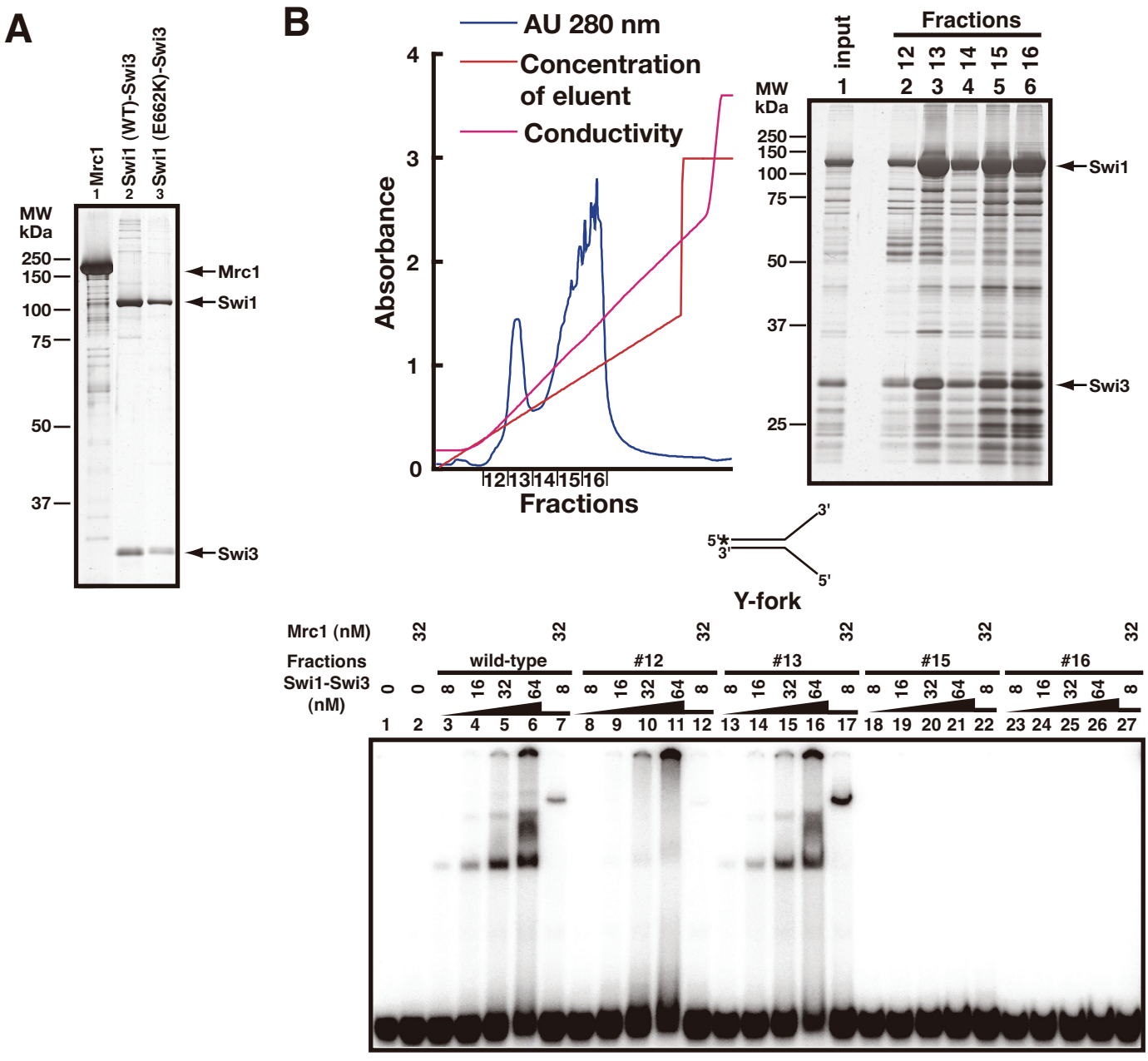
A
Mrc1
Swi1 (WT)-Swi3
Swi1 (E662K)-Swi3
MW kDa
250
150
100
75
50
37
Mrc1
Swi1
Swi3
B
AU 280 nm
Concentration of eluent
Conductivity
Absorbance
Fractions
12 13 14 15 16
input
Fractions
MW kDa
Swi1
Swi3
5'
3'
Y-fork
Mrc1 (nM)
Fractions
Swi1-Swi3 (nM)
wild-type
#12
#13
#15
#16

Figure S1

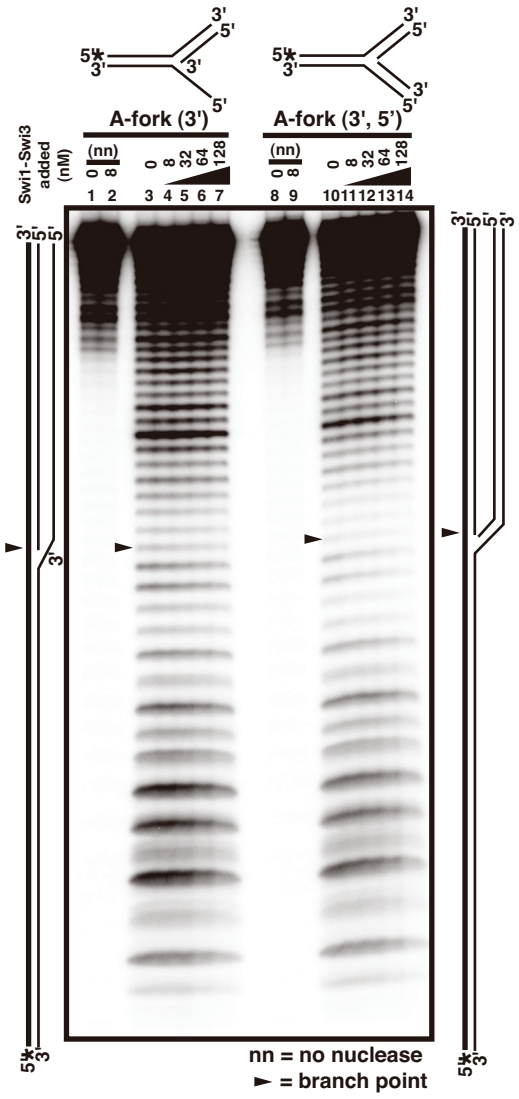
A-fork (3')
A-fork (3', 5')
Swi1-Swi3 added (nM)
(nn)
0
8
0
8
32
64
128
1 2 3 4 5 6 7 8 9 10 11 12 13 14
nn = no nuclease
► = branch point

Figure S2

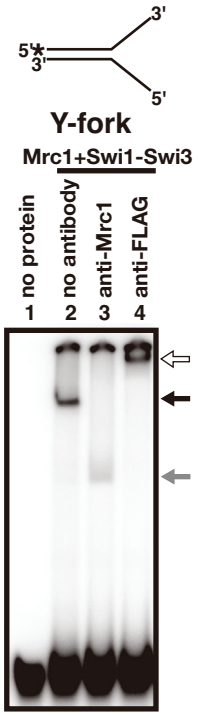
3'
5'
*
3'
5'
Y-fork
Mrc1+Swi1-Swi3
no protein
no antibody
anti-Mrc1
anti-FLAG
1
2
3
4

Figure S3

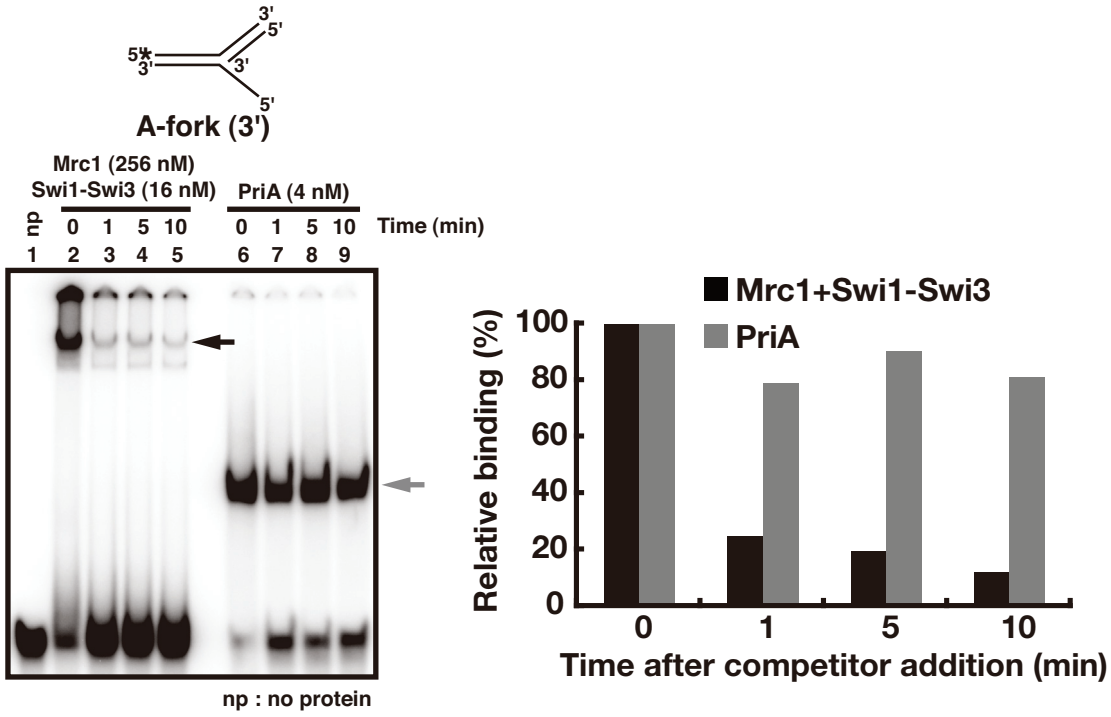
3'
5'
5'*
3'
3'
5'
A-fork (3')
Mrc1 (256 nM)
Swi1-Swi3 (16 nM)
PriA (4 nM)
np
0 1 5 10
0 1 5 10
Time (min)
1 2 3 4 5 6 7 8 9
np : no protein
Mrc1+Swi1-Swi3
PriA
Relative binding (%)
100
80
60
40
20
0
0 1 5 10
Time after competitor addition (min)

Figure S4

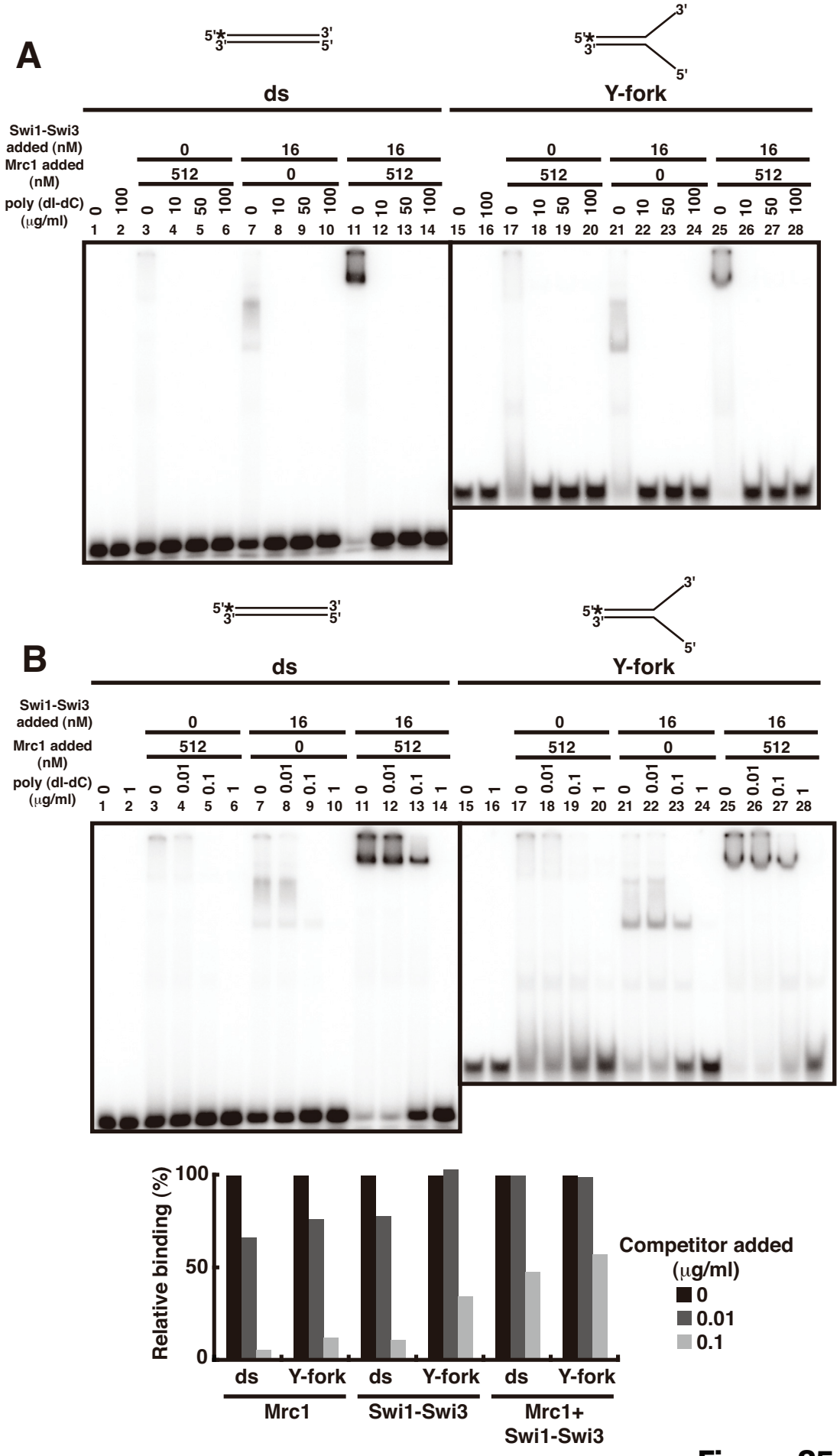
A
ds
Y-fork
Swi1-Swi3 added (nM)
Mrc1 added (nM)
poly (dI-dC) (μg/ml)
B
Relative binding (%)
100
50
0
Competitor added (μg/ml)
0
0.01
0.1
ds
Y-fork
Mrc1
Swi1-Swi3
Mrc1+ Swi1-Swi3

Figure S5

Mutants of SpSwi1 generated

```
                        Y26A
                                          Y42A
Tof1          41   dpppYKLGDD CLACLKDLKR WFKLVDDQQK RWDVAMAVAE YRILTDDLLP
SpSwi1        24   kv--YVLGDE ALACLKDLKR YLQVVDEKYK VWQIRSLLSS LQLVTNDICP
huTimeless    31   ---------- ---CLESVKD LIRYLRHEDE TRDVRQQLGA AQILQSDLLP

                                    Y127A,K128A
Tof1         151   TLYGELKKHQ LV---YKKTI LSMESGKVLR AAIRLALDVI KIDRLSRTPR
SpSwi1       115   DVLYNLRQAQ SN---YKNSI LSYKKGSVLS AILAVLLKPL stpaESRTLR
huTimeless   105   sfrhhflqvl tylqaYKEAF ASEKAFGVLS ETLy---ELL QLGWEERQEE

                             R268A,H269A
                                 R271A,F272A,G273A
Tof1         348   NVIKHTSARH SRFGGLLSIQ TPDKtrltvs gsqalvdeki aLQKLDDSKK
SpSwi1       260   YLKRNAHTRH NRFGTMLSVQ TEDRrftias qniktdg--- -LDELDSHKR
huTimeless   266   TRALQRGNRH SRFGGSYIVQ glksigerdl ifhkglhnlr nyssdlgkqp

                             F660A,I661A
                                E662K
Tof1         752   RLKKSPAWFV GLLFPPLhnS EVGFYQRYGE YNVLNNESMY AAPASQFKPi
SpSwi1       652   ALKDVPALFI ELPFPKL--T DTFYYLEYGK SPLFSIHGSR KGPLYETVPg
huTimeless   780   LAAVNQKAFV ELLFWKN--T AVVREMTEGY GSLDDRSSSR RAPTWSpeee
```

Mutants of SpSwi3 generated

```
                             W95A
                               H97A
                                        F105A
ScCsm3        88   LSNIIQFYQL WAHELFPKAK FKDFMKICQT VGKtd----- ----------
SpSwi3        85   LKQLLGMYHI WTHELYPRAT FDDSISYLKT LGKHRSVKvr rrgwineiav
huCsm3       100   LKMLIRHMEH WAHRLFPKLQ FEDFIDRVEY LGSKKEVQtc lkrirl----
```

Figure S6

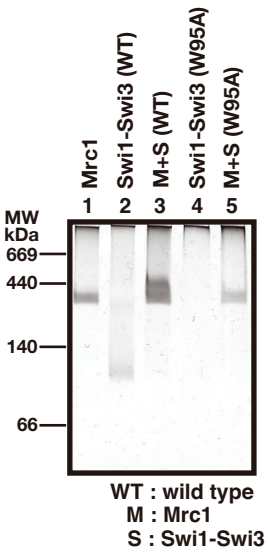
Mrc1
Swi1-Swi3 (WT)
M+S (WT)
Swi1-Swi3 (W95A)
M+S (W95A)
1 2 3 4 5
MW
kDa
669
440
140
66
WT : wild type
M : Mrc1
S : Swi1-Swi3

Figure S7