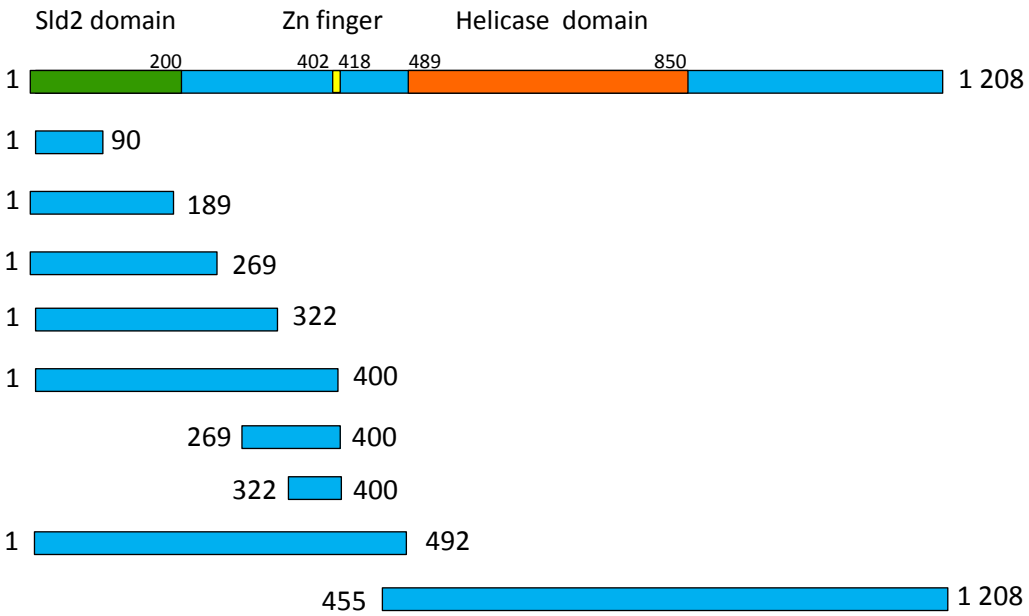
# Fig. 1

## A


Sld2 domain
Zn finger
Helicase domain
200
402
418
489
850
1
1 208
1
90
1
189
1
269
1
322
1
400
269
400
322
400
1
492
455
1 208

## B

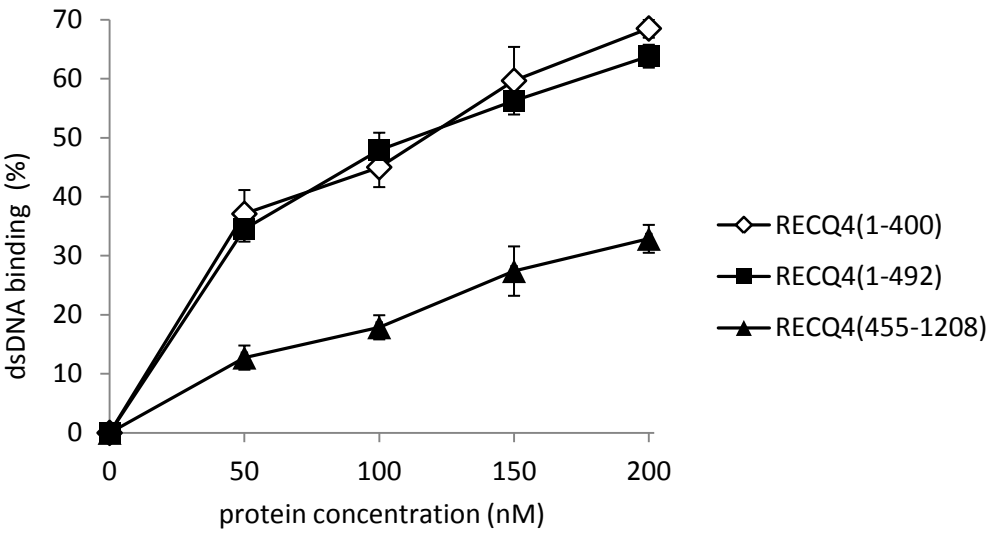
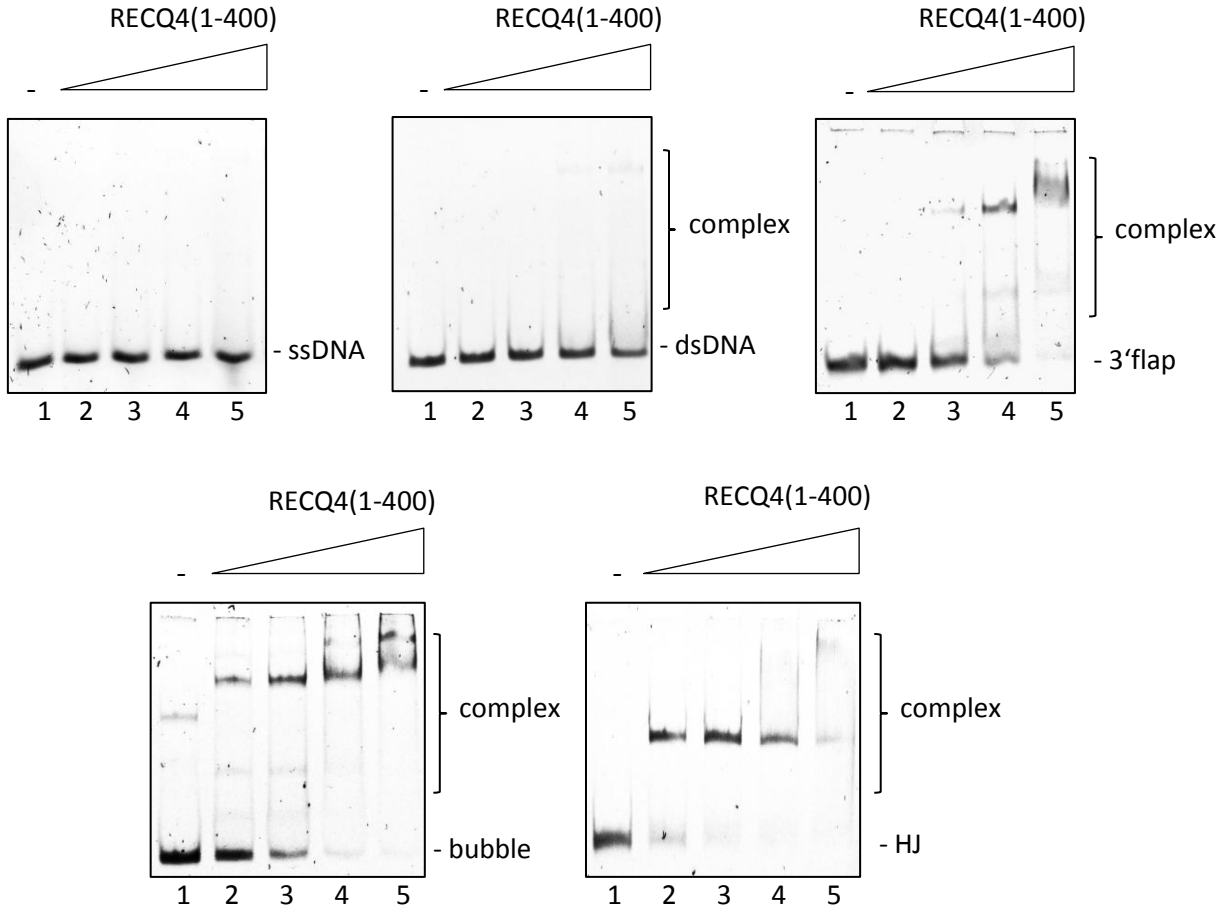

70
60
50
40
30
20
10
0
dsDNA binding (%)
0
50
100
150
200
protein concentration (nM)
RECQ4(1-400)
RECQ4(1-492)
RECQ4(455-1208)

**Fig. 2**

**A**


RECQ4(1-400)
-
- ssDNA
complex
- dsDNA
- 3'flap
- bubble
- HJ
1 2 3 4 5

**B**

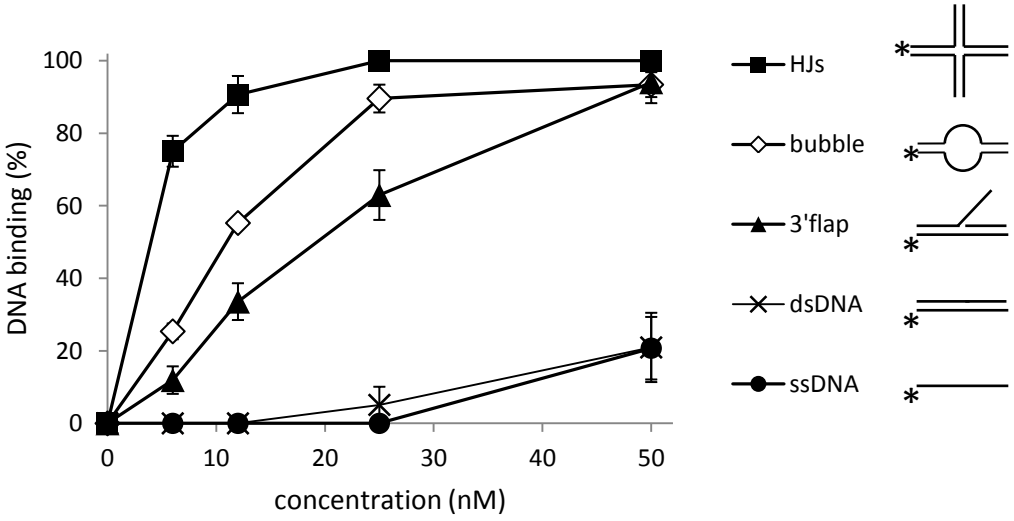

DNA binding (%)
concentration (nM)
0
20
40
60
80
100
0
10
20
30
40
50
HJs
bubble
3'flap
dsDNA
ssDNA
*

**Fig. 3**

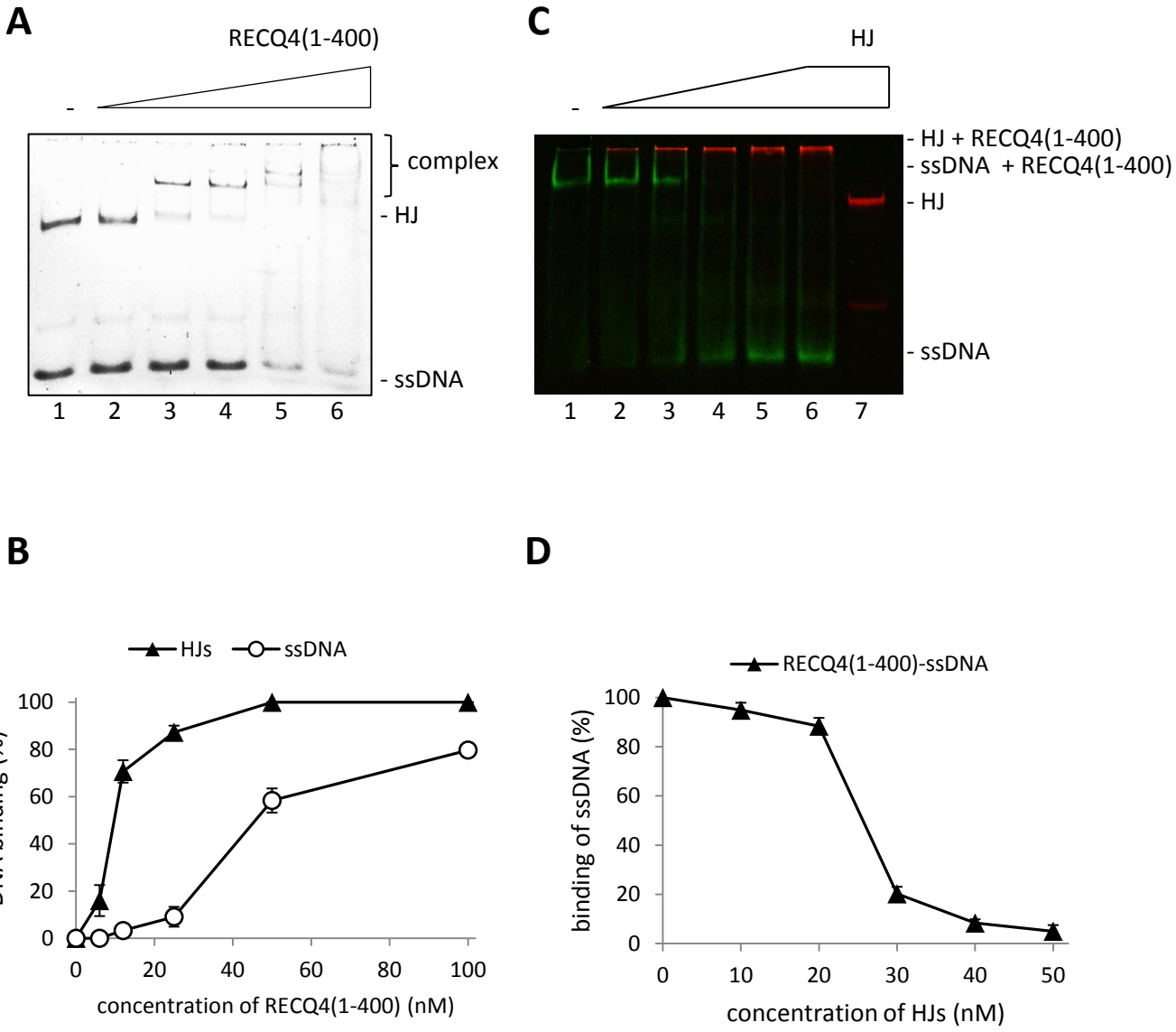
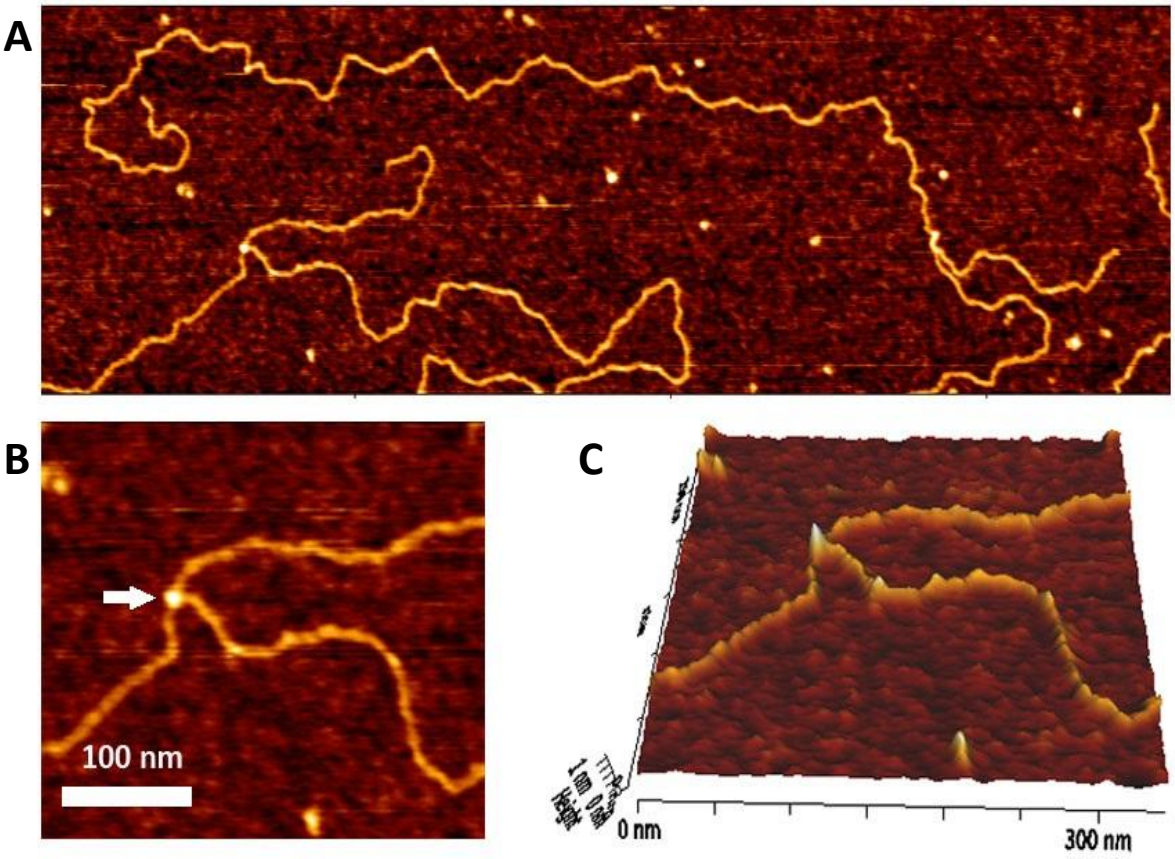
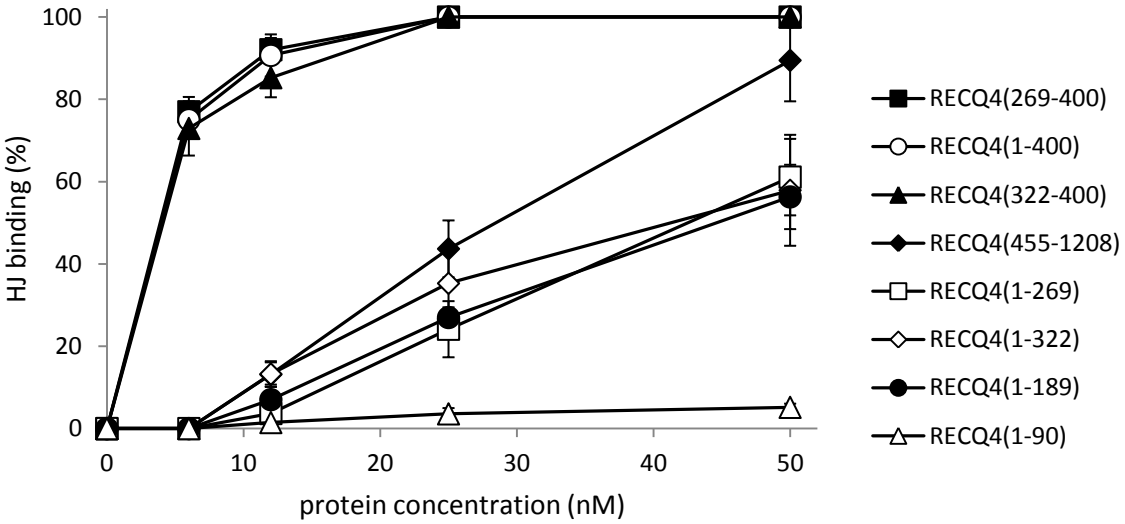

A
RECQ4(1-400)
-
complex
- HJ
- ssDNA
1 2 3 4 5 6
C
HJ
-
- HJ + RECQ4(1-400)
- ssDNA + RECQ4(1-400)
- HJ
- ssDNA
1 2 3 4 5 6 7
B
HJs
ssDNA
DNA binding (%)
0 20 40 60 80 100
0 20 40 60 80 100
concentration of RECQ4(1-400) (nM)
D
RECQ4(1-400)-ssDNA
binding of ssDNA (%)
0 20 40 60 80 100
0 10 20 30 40 50
concentration of HJs (nM)

**Fig. 4**



# Fig. 5

**A**


100
80
60
40
20
0
HJ binding (%)
0
10
20
30
40
50
protein concentration (nM)
RECQ4(269-400)
RECQ4(1-400)
RECQ4(322-400)
RECQ4(455-1208)
RECQ4(1-269)
RECQ4(1-322)
RECQ4(1-189)
RECQ4(1-90)

**B**

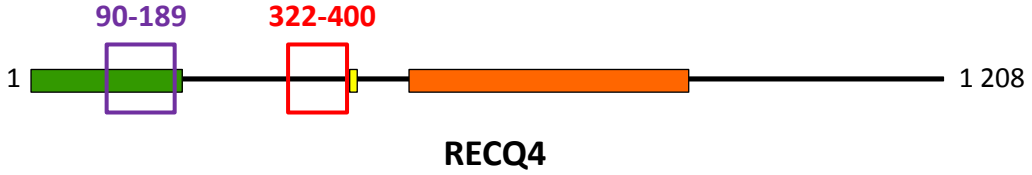
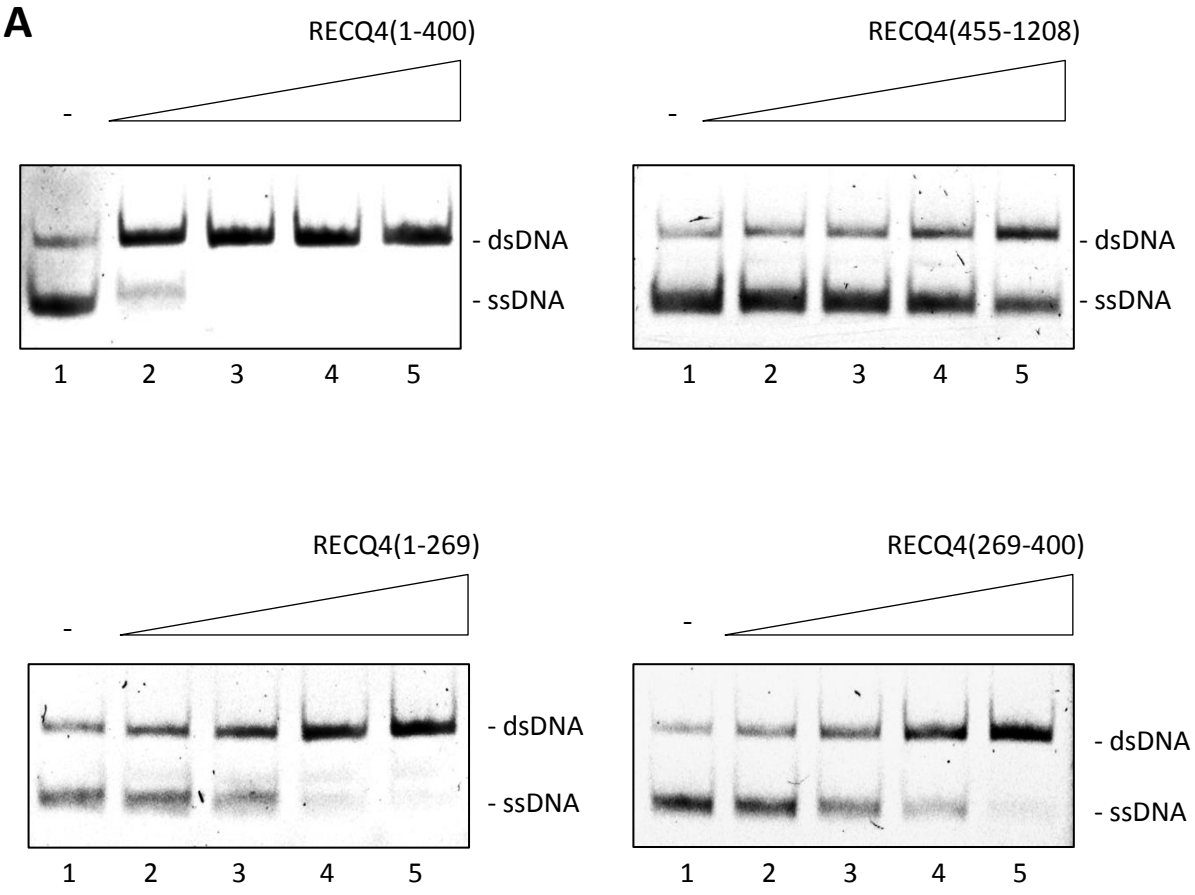

90-189
322-400
1
1 208
RECQ4

**Fig. 6**


A
RECQ4(1-400)
RECQ4(455-1208)
RECQ4(1-269)
RECQ4(269-400)
-
- dsDNA
- ssDNA
1 2 3 4 5
B
ssDNA annealing (%)
protein concentration (nM)
0 10 20 30 40
0 20 40 60 80 100
RECQ4(1-400)
RECQ4(269-400)
RECQ4(1-269)
RECQ4(455-1208)

**Supplementary Fig. 1** Purification of RECQ4 fragments. (A) Purification scheme for N-terminally MBP- and C-terminally 9-histidine-tagged RECQ4 fragments. (B) Purified RECQ4 fragments used in this study. Lane 1, RECQ4(1–90); Lane 2, RECQ4(1–189); Lane 3, RECQ4(1–269); Lane 4, RECQ4(269–400); Lane 5, RECQ4(1–322); Lane 6, RECQ4(1–400). Lane 7, RECQ4(1–492); Lane 8, RECQ4(455–1208); Lane 9, RECQ4(322-400); Lane 10, RECQ4(1–1208).

**Supplementary Fig. 2.** MBP has no effect on DNA binding of RECQ4 fragments. Increasing concentrations (3, 6, 12 and 25 nM, lanes 2–5) of MBP-RECQ4(1–400), RECQ4(1–400) and MBP were incubated with 3 nM FITC-labeled HJ at 37°C for 20 min.

**Supplementary Fig. 3.** DNA binding affinity of full-length RECQ4 and its various fragments for HJ. (A) Indicated amounts of RECQ4wt (6, 12, 25 and 50 nM, lanes 2–5) were incubated with 3 nM FITC-labeled HJ at 37°C for 20 min. (B) Quantification of DNA binding affinity of REC4wt and RECQ4(1–400) for HJ shown as mean ± SD based on three independent experiments. (C) Affinity of RECQ4 fragments for HJs in the EMSA. Fluorescently labeled HJ substrate (3 nM) was incubated with increasing amounts (6, 12, 25 and 50 nM, lanes 2–5) of RECQ4(1–90), RECQ4(1–189), RECQ4(1–269), RECQ4(1–322), RECQ4(1–400), RECQ4(269–400), RECQ4(322-400) and RECQ4(455–1208) for 20 min at 37°C.

**Supplementary Fig. 4.** DNA binding affinity of RECQ4(455–1208) for various DNA substrates. (A) 3 nM FITC-labeled ssDNA, dsDNA, 3′ flap and HJ were incubated with increasing concentrations of RECQ4(455–1208) (6, 12, 25 and 50 nM, lanes 2–5) at 37°C for 20 min. (B) Quantification of data in (A) shown as mean ± SD based on three independent experiments.

**Supplementary Fig. 5.** Control AFM experiment using the same amount of DNA isolated from replication extracts incubated without addition of RECQ4 (1-400). (A) Overview of a large area containing two DNA molecules. (B) Detail of a replication fork. (C) 3D profile demonstrating no significant enhancement of DNA height.

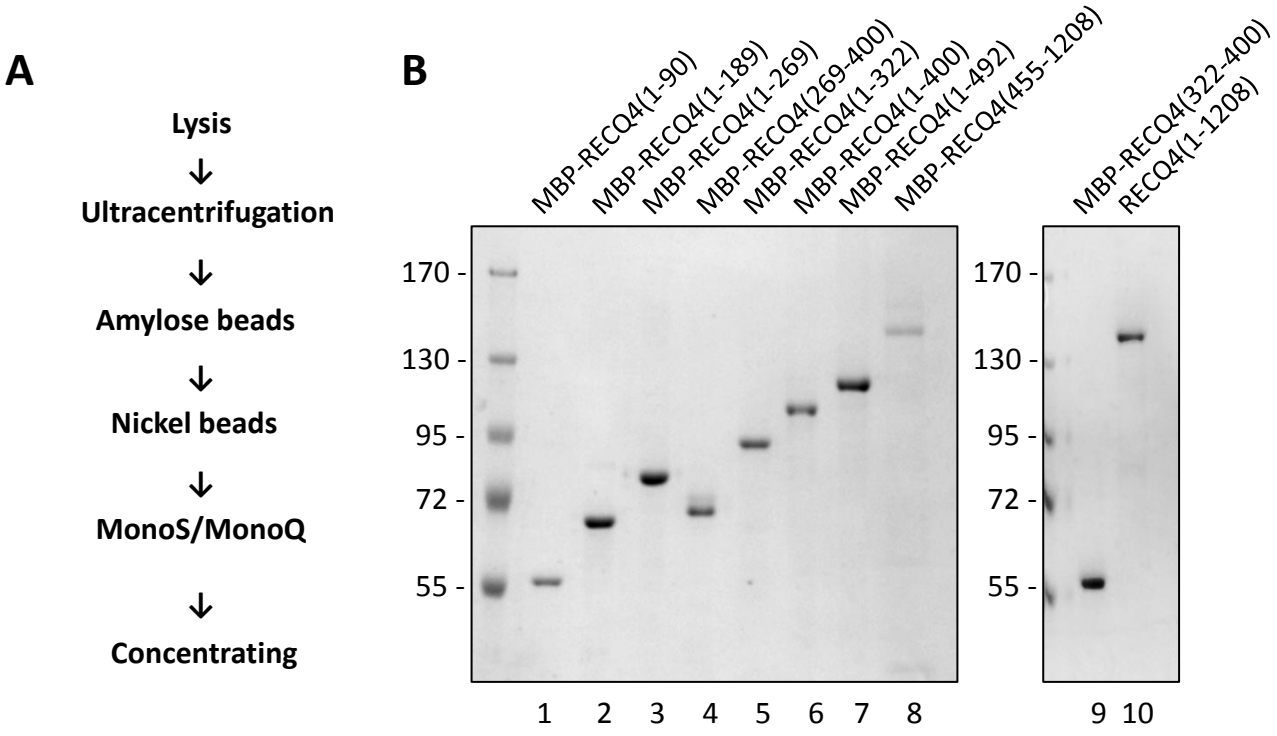
**Supplementary Fig. 6.** DNA binding affinity of RECQ4(1-189) for various DNA substrates. (A) 3 nM FITC-labeled ssDNA, 3' flap and HJ were incubated with increasing concentrations of RECQ4(1-189) (6, 12, 25 and 50 nM, lanes 2–5) at 37°C for 20 min. (B) Quantification of data in (A) shown as mean ± SD based on three independent experiments.

**Supplementary Fig. 7.** Single-strand annealing activity of RECQ4. (A) SSA reactions containing increasing amounts of RECQ4(1–492) (5, 10, 20 and 40 nM, lanes 2–5) were incubated with a mixture of 3 nM FITC-labeled ssDNA and non-labeled complementary ssDNA at 37°C for 20 min. (B) Time-course of the single-strand annealing experiment in which 7.5 nM RECQ4(1–400) or RAD52 was incubated with 3 nM FITC-labeled ssDNA and non-labeled complementary ssDNA at 37°C for indicated time (0, 2, 4, 6 and 8 min; lanes 1–5 and 6-10). (C) Quantification of data in (B) shown as mean ± SD based on three independent experiments.

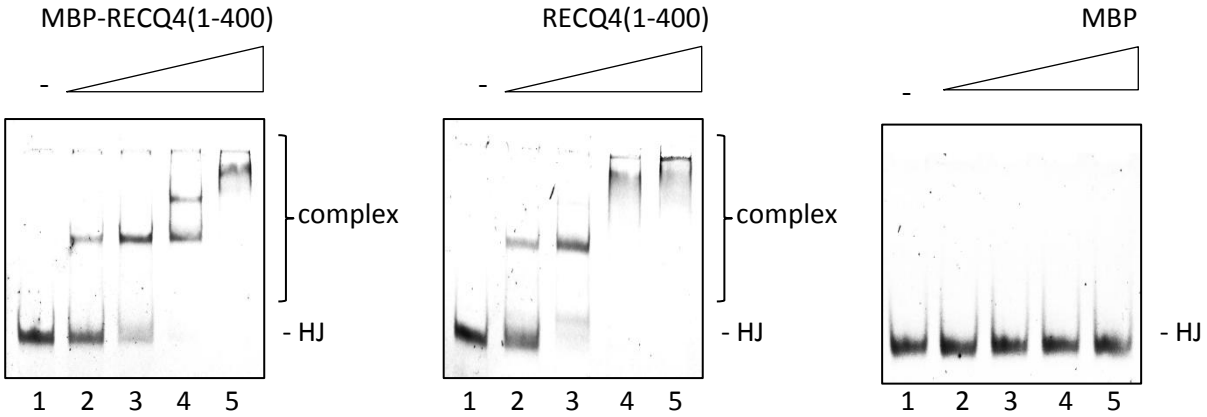
**Supplementary Fig. 8.** ATPase activity of RECQ4. (A) RECQ4wt (150 nM) or Srs2 (75 nM) was incubated with 83-nt ssDNA (75 µM nucleotides), mixture of unlabeled 10 mM ATP and 148 Bq/µl $\gamma$-$^{32}$P-ATP for indicated times at 37°C. (B) Quantification of ATP hydrolysis of RECQ4wt and RECQ4(455–1208) shown as mean ± SD based on three independent experiments. The kcat of ATP hydrolysis for RECQ4 was calculated to be ~7 $min^{-1}$.

**Supplementary Fig. 9.** Helicase activity of RECQ4 on 3'-overhang substrate. (A) Fluorescently labeled 3'-overhang (6 nM) was mixed with indicated amounts of RECQ4(455–1208) (12.5, 25, 50 and 100 nM, lanes 2–5) or BLM (12.5 nM, lane 7) and incubated at 37°C for 20 min. Lane 6, control reaction with the highest concentration of RECQ4 (100 nM) in the absence of ATP. (B) RECQ4wt (lanes 2-5), RECQ4(455-1208) (lanes 6-9) (12.5, 25, 50 and 100 nM) or 12.5 nM BLM (lane 10) were incubated with FITC-3'-overhang (6 nM) at 37°C for 20 min.

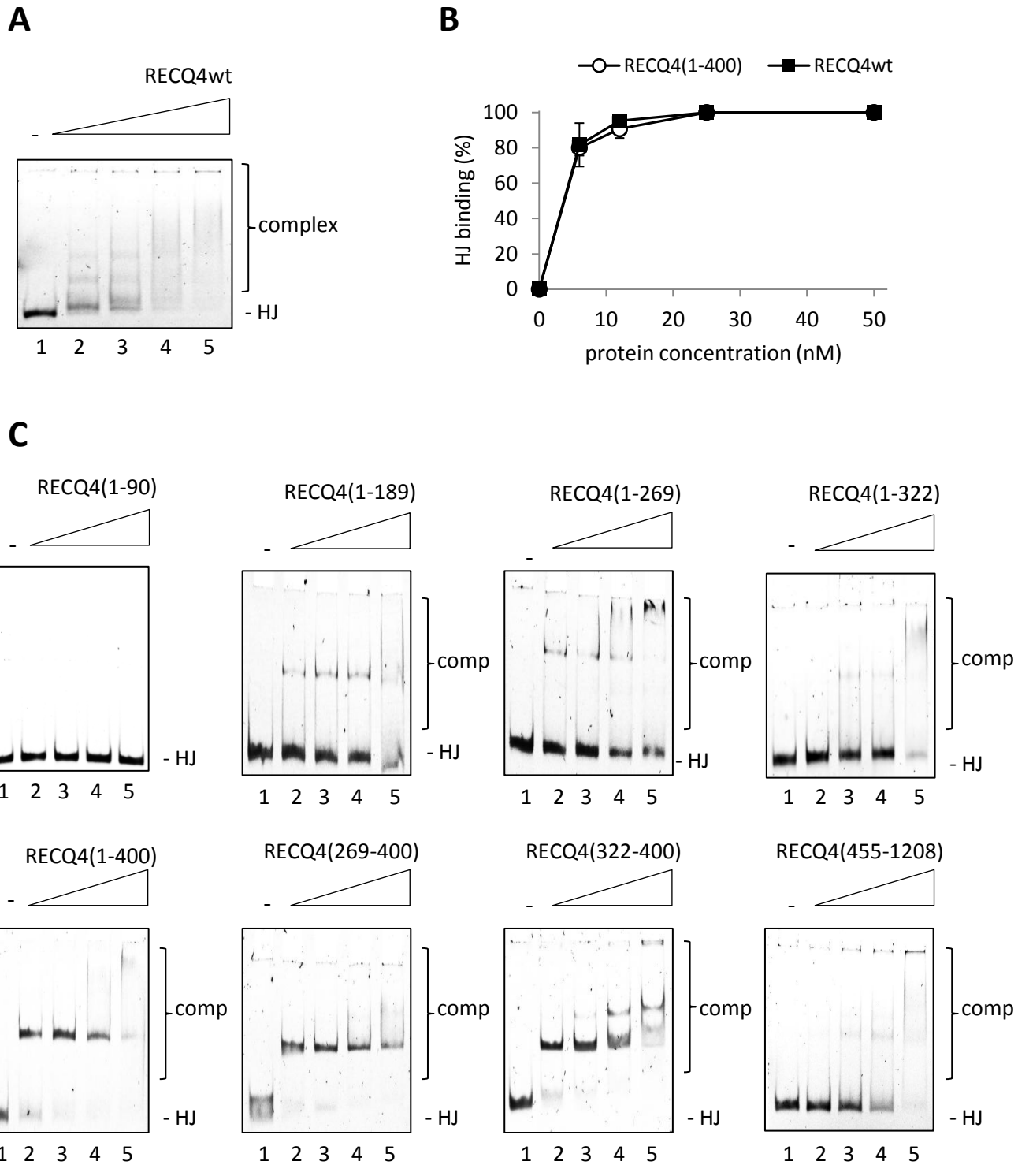
# Supplementary Fig. 1



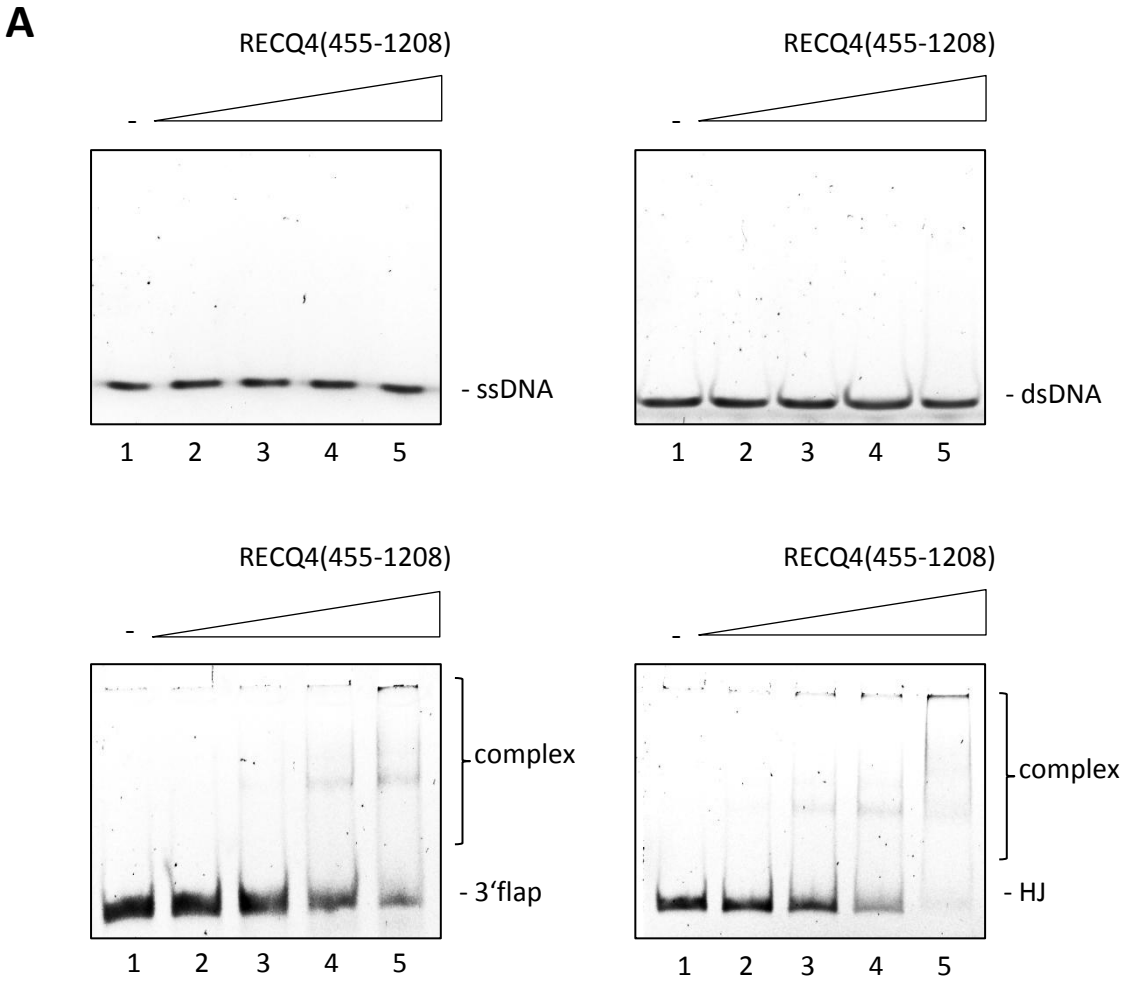

# Supplementary Fig. 2

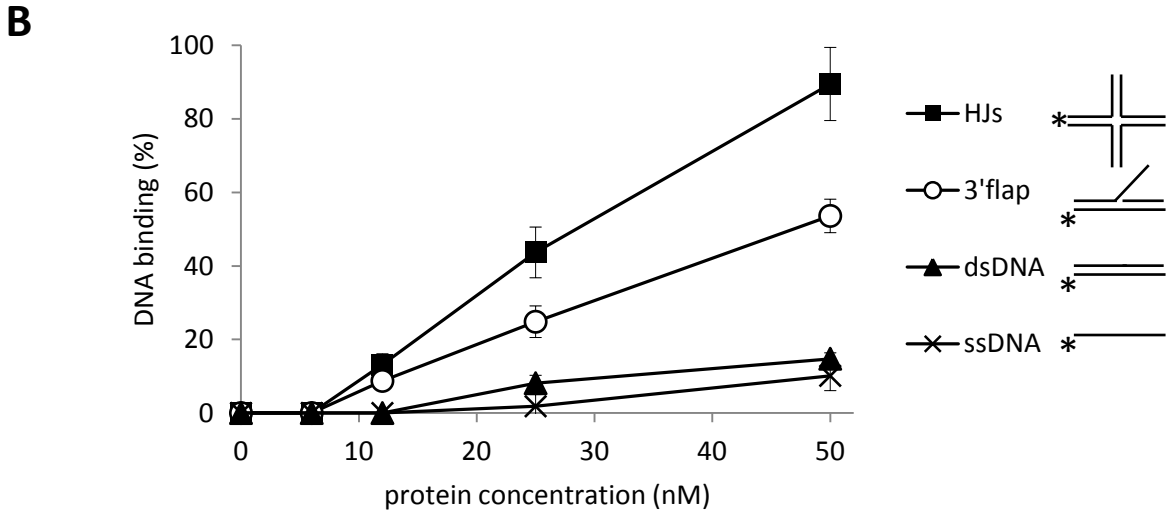


# Supplementary Fig. 3

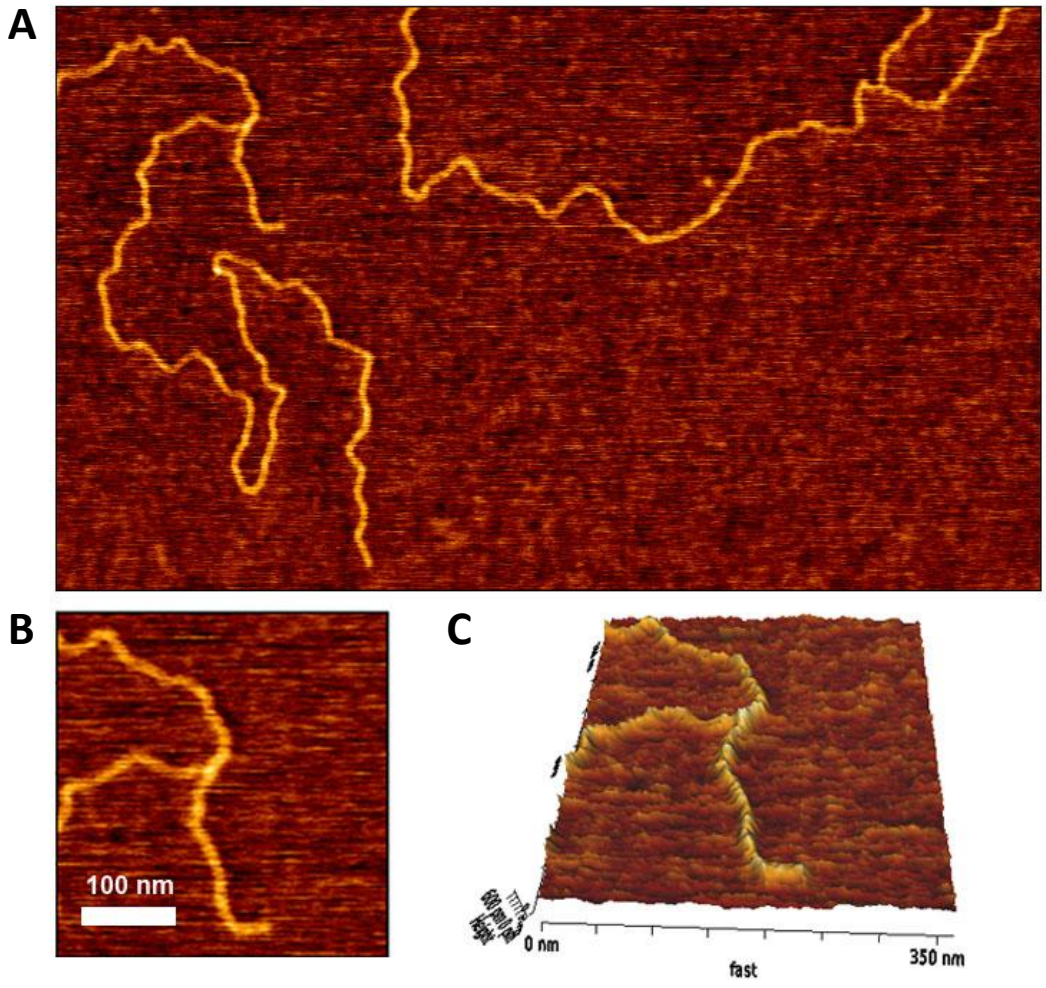


# Supplementary Fig. 4

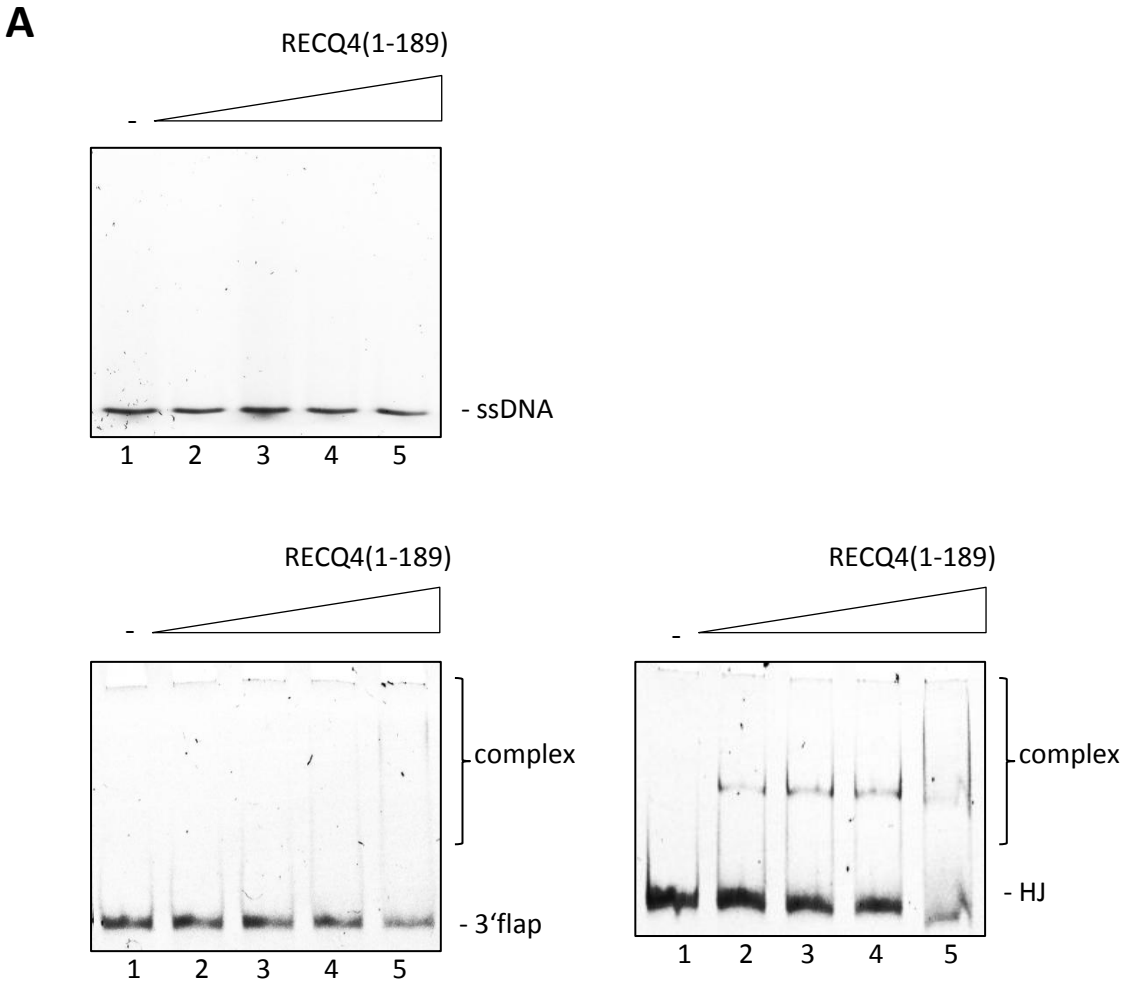


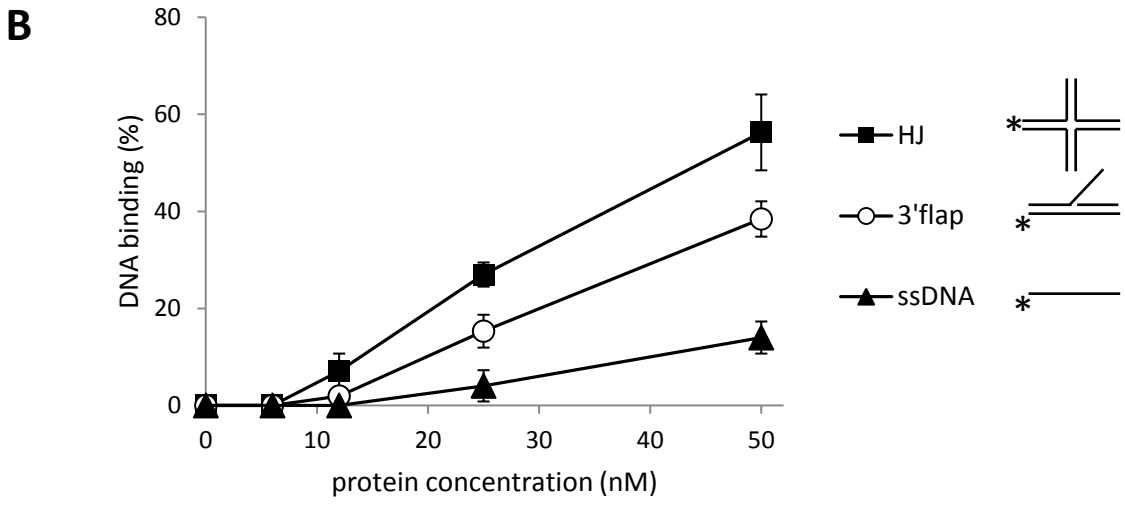



## Supplementary Fig. 5

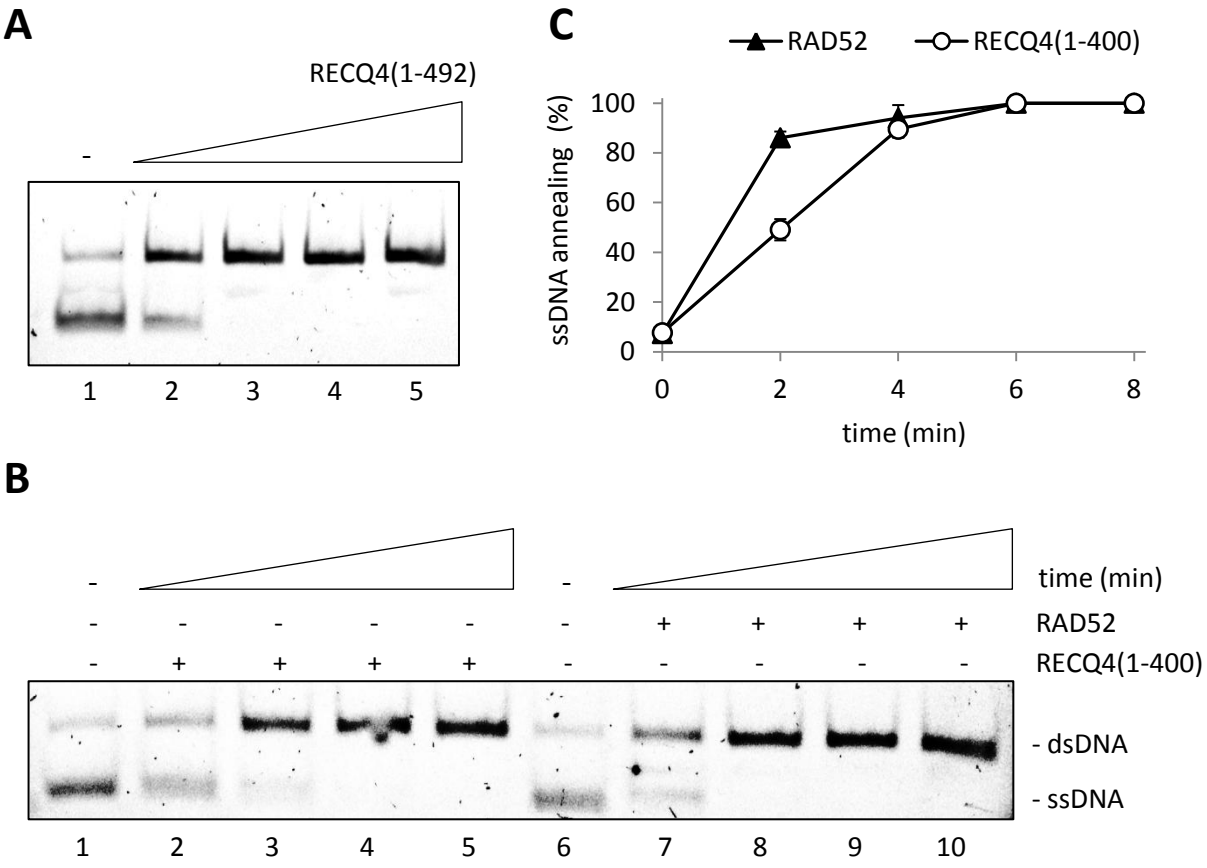


# Supplementary Fig. 6



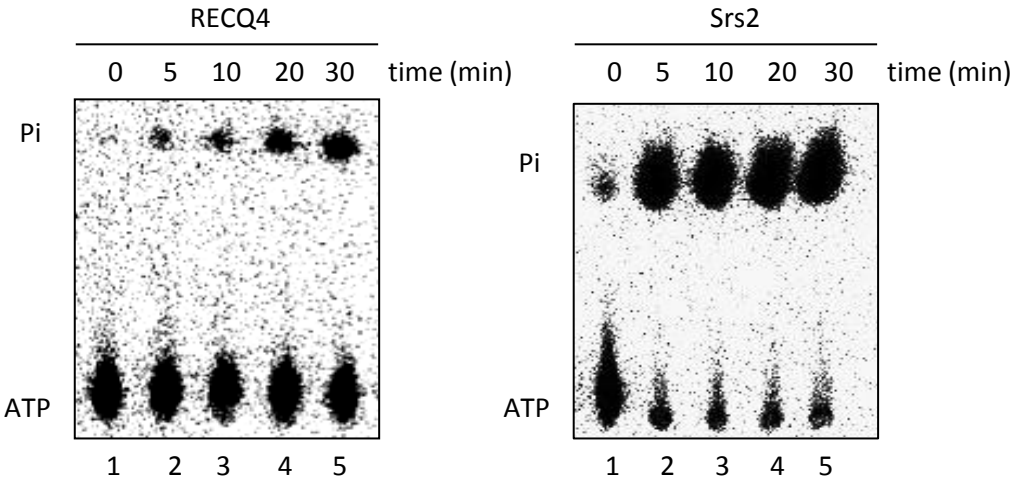



# Supplementary Fig. 7

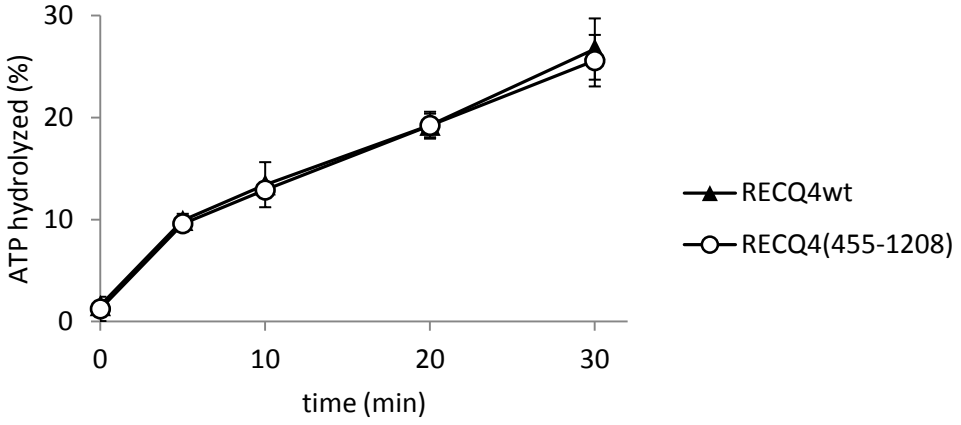


# Supplementary Fig. 8

**A**




**B**



# Supplementary Fig. 9