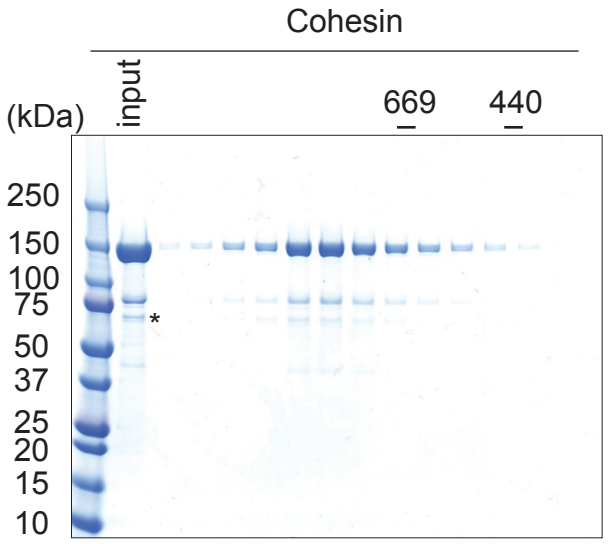
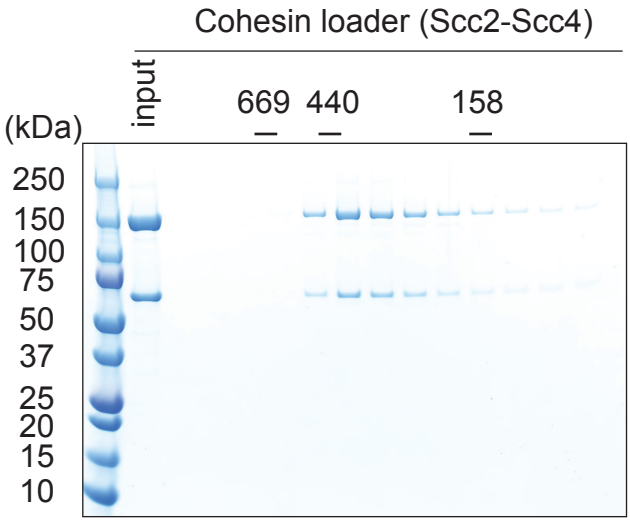
A

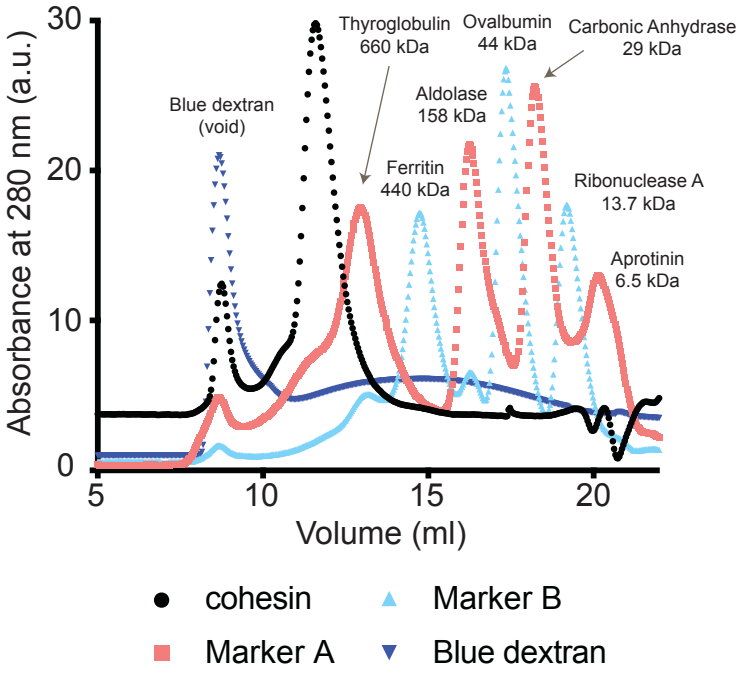
Cohesin
(Smc1, Smc3, Scc1 and Scc3)
IgG agarose
↓
Heparin HP
↓
Superose 6

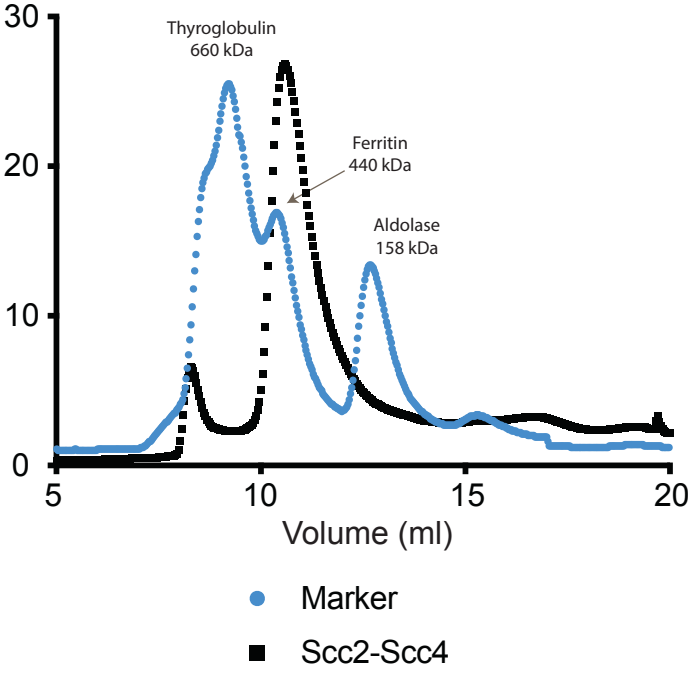
B

Cohesin loader (Scc2 and Scc4)
IgG agarose
↓
Heparin HP
↓
Superdex 200









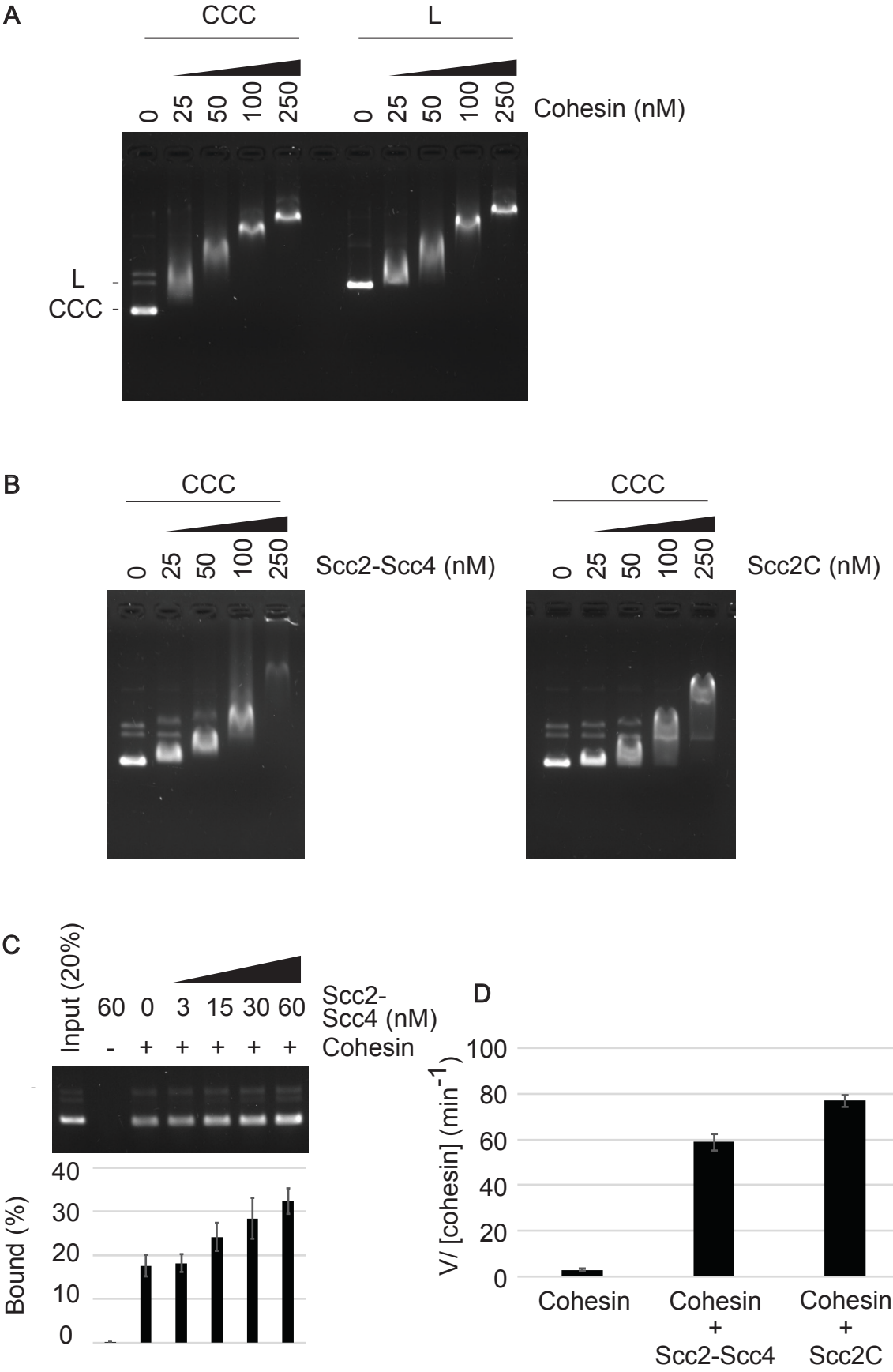
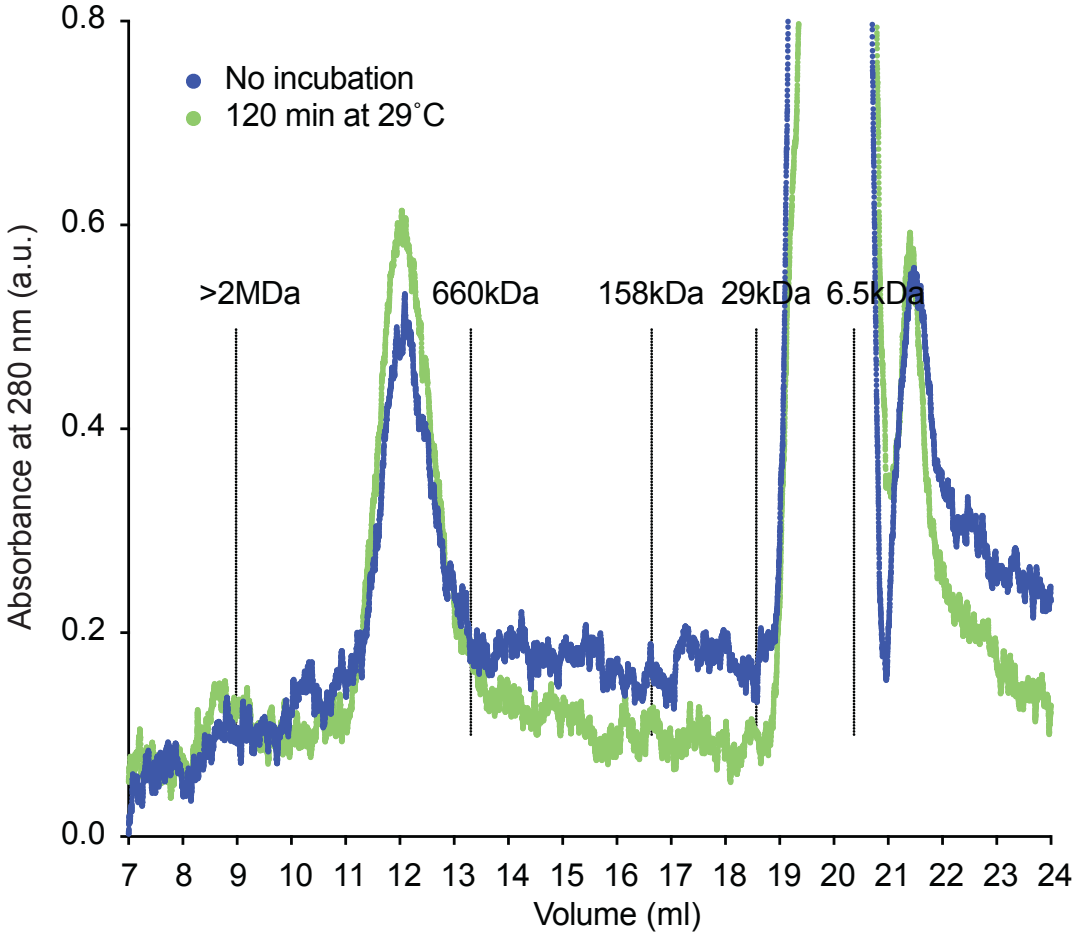
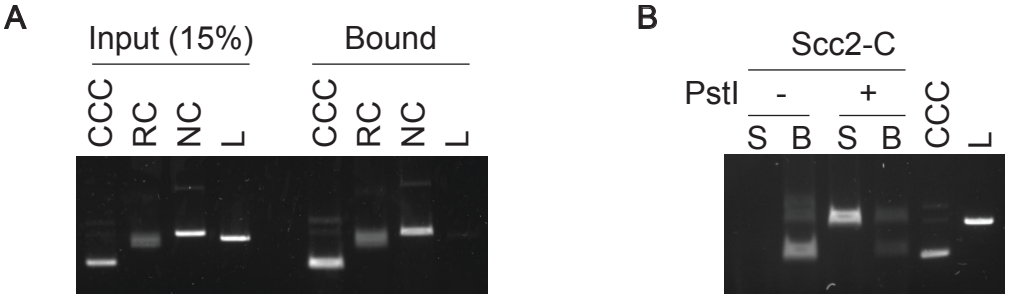
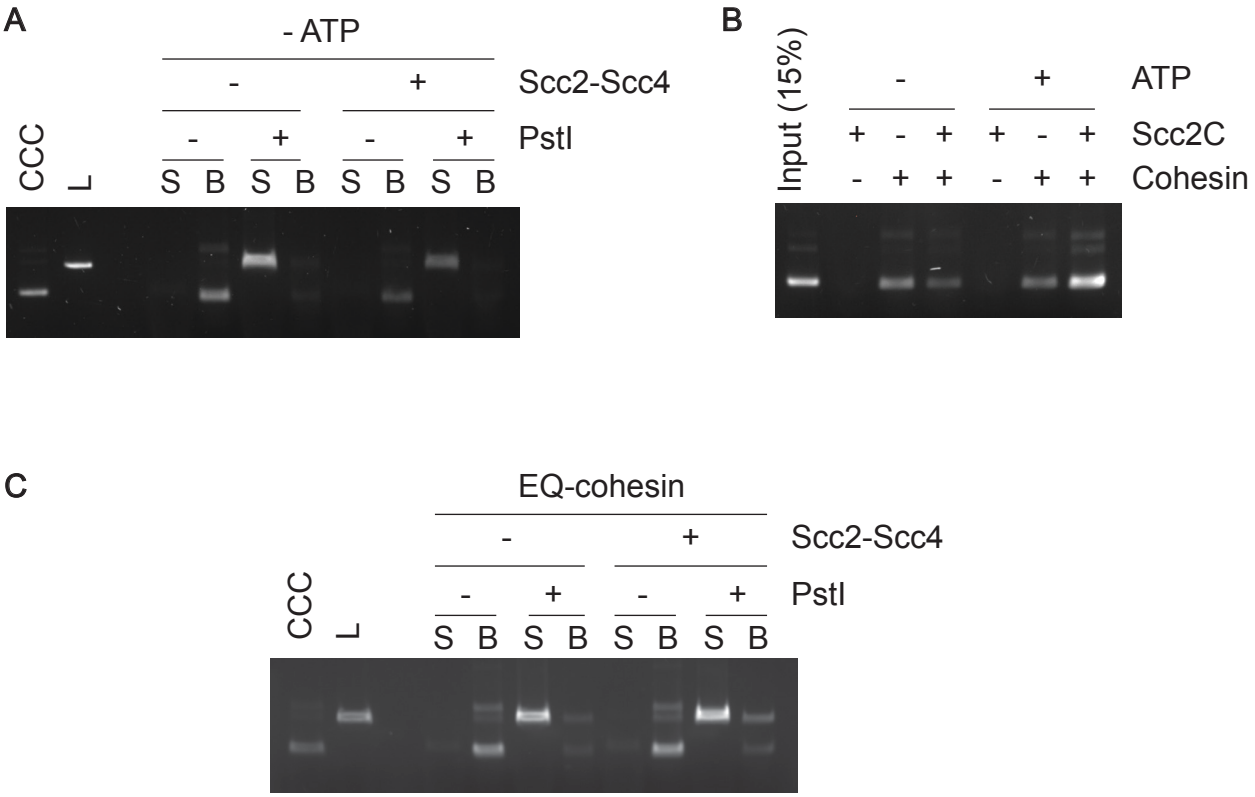

A
CCC
L
0 25 50 100 250
0 25 50 100 250
Cohesin (nM)
L
CCC
B
CCC
CCC
0 25 50 100 250
Scc2-Scc4 (nM)
0 25 50 100 250
Scc2C (nM)
C
Input (20%)
60 0 3 15 30 60
Scc2-Scc4 (nM)
- + + + + +
Cohesin
Bound (%)
40
30
20
10
0
D
V/ [cohesin] (min-1)
100
80
60
40
20
0
Cohesin
Cohesin + Scc2-Scc4
Cohesin + Scc2C

Figure S3


No incubation
120 min at 29˚C
>2MDa
660kDa
158kDa
29kDa
6.5kDa
Absorbance at 280 nm (a.u.)
0.0
0.2
0.4
0.6
0.8
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
Volume (ml)


A
Input (15%)
Bound
CCC
RC
NC
L
CCC
RC
NC
L
B
Scc2-C
PstI
-
+
S
B
S
B
CCC
L


A
- ATP
-
+
Scc2-Scc4
-
+
-
+
PstI
CCC
L
S B S B S B S B
B
Input (15%)
-
+
ATP
+ - + + - +
Scc2C
- + + - + +
Cohesin
C
EQ-cohesin
-
+
Scc2-Scc4
-
+
-
+
PstI
CCC
L
S B S B S B S B