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Supplementary information

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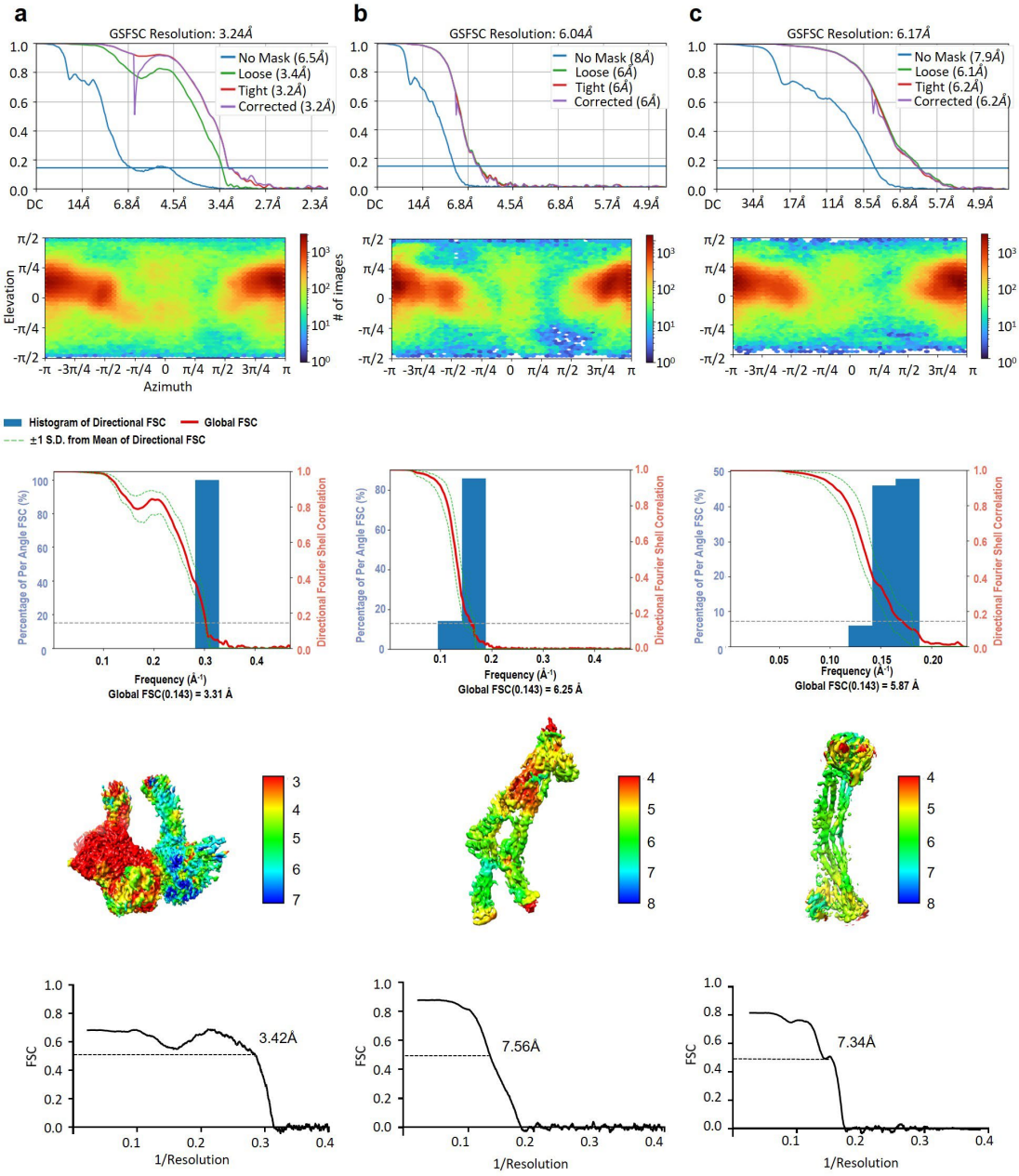
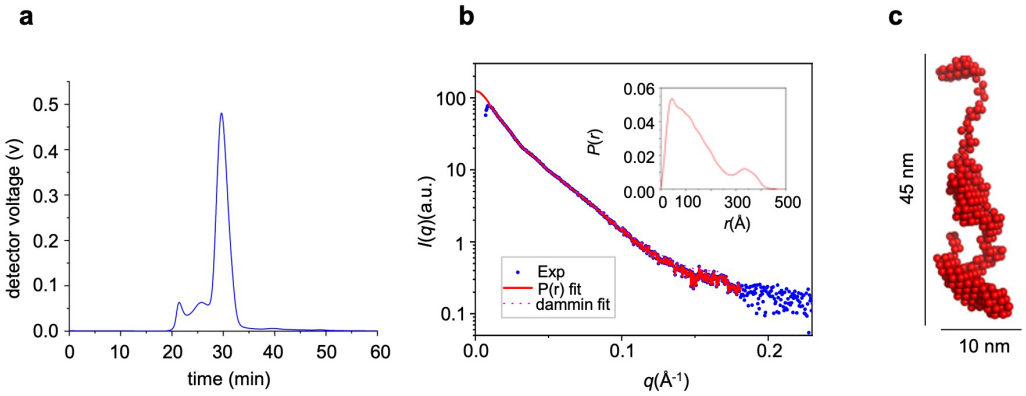
Cryo-EM structures of Smc5/6 in multiple states reveal its assembly and functional mechanisms

In the format provided by the authors and unedited



Supplementary Fig. 1 Resolution assessment of the cryo-EM maps of Smc5/6-8mer. (**a-c**) Global Fourier Shell Correlation (FSC) curve, angular distribution plot, 3D FSC curve, local resolution estimation, and model vs. map FSC of the focused refinement maps of the Head (**a**), Arm (**b**), and Hinge (**c**) regions of Smc5/6-8mer are displayed from top to bottom.

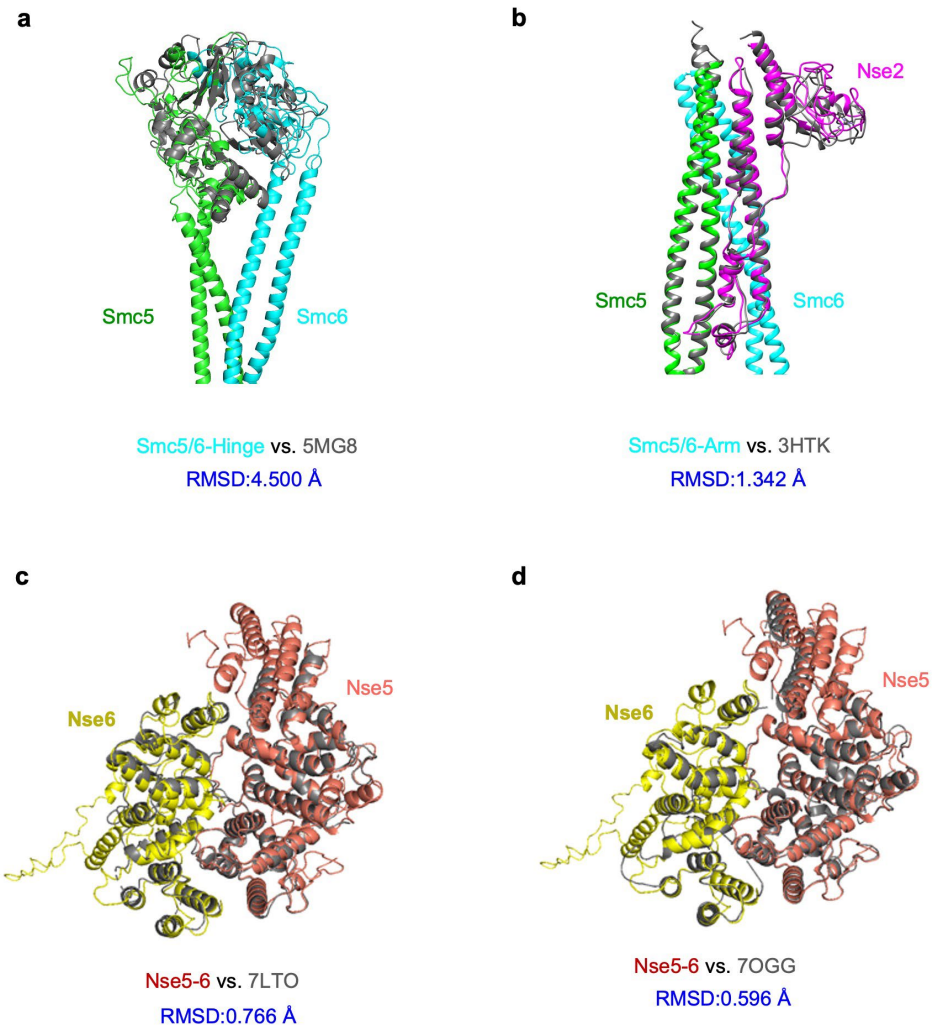


Supplementary Fig. 2 SEC-SAXS data of the Smc5/6-8mer complex.

(**a**) A SEC elution profile of the Smc5/6-8mer complex.

(**b**) A SAXS scattering profile of the Smc5/6-8mer complex. The back-calculated scattering profile of the *Ab initio* model (pink dash line) and atomic model (red line) were fitted to the experimental SAXS data (blue dot line). The inset shows the Pair distance distribution function *P*(r) curve.

(**c**) A low-resolution bead model calculated by DAMMIF based on the SAXS data.



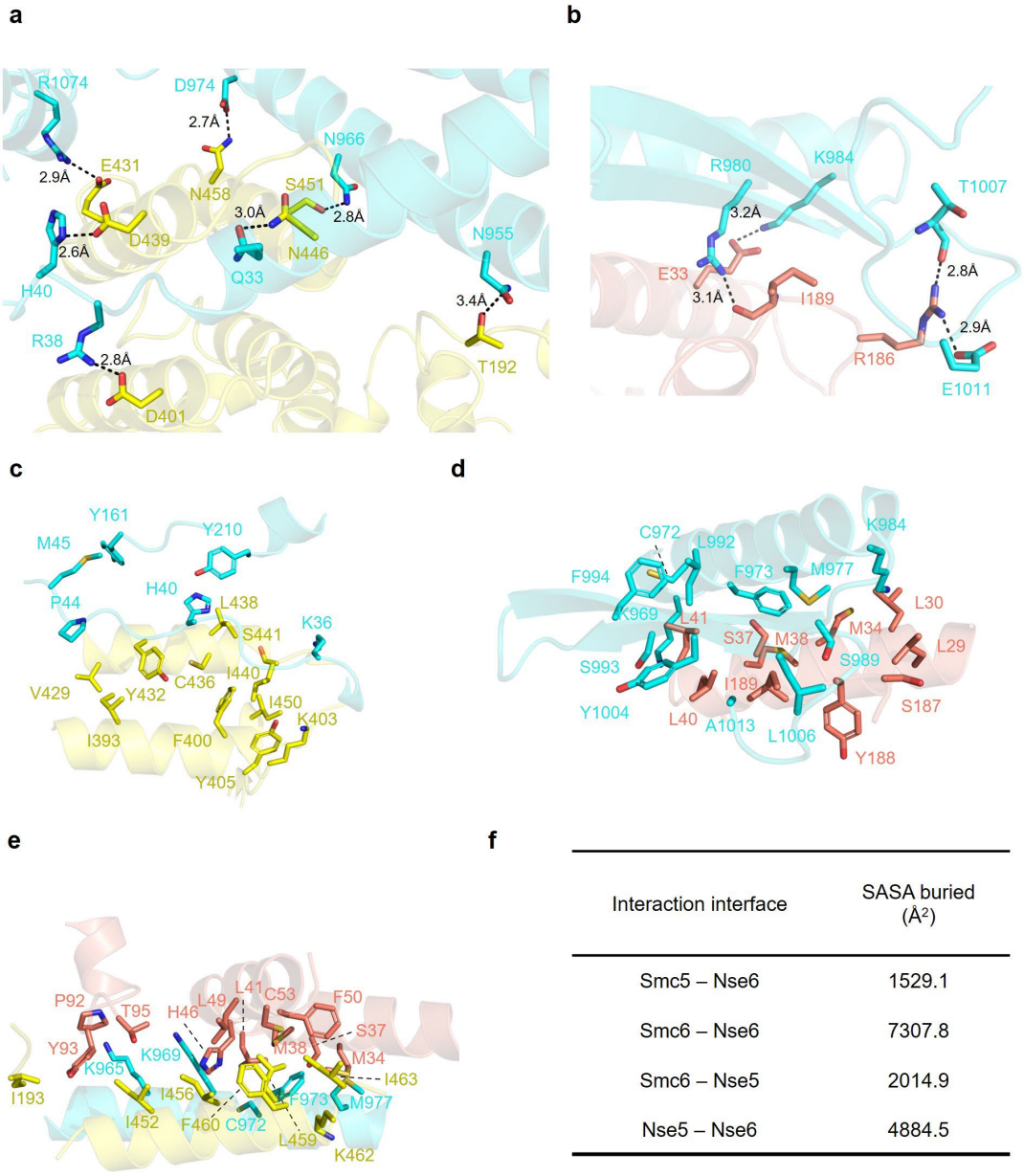
Supplementary Fig. 3 Comparison of the Smc5/6-8mer structure with published subcomplex structures.

(a) The published Smc5-6-Hinge structure (grey, PDB: 5MG8) from *Schizosaccharomyces pombe* was aligned to Smc5/6-8mer hinge structure.

(b) The published Nse2-Smc6 structure (grey, PDB: 3HTK) was aligned to the Smc5/6-8mer Arm structure (color).

(**c-d**) The compact regions of Nse5/6 (colored) from Smc5/6-8mer were aligned with the Nse5/6 subcomplex structures (grey, PDB: 7LTO (**c**); PDB: 7OGG(**d**)).

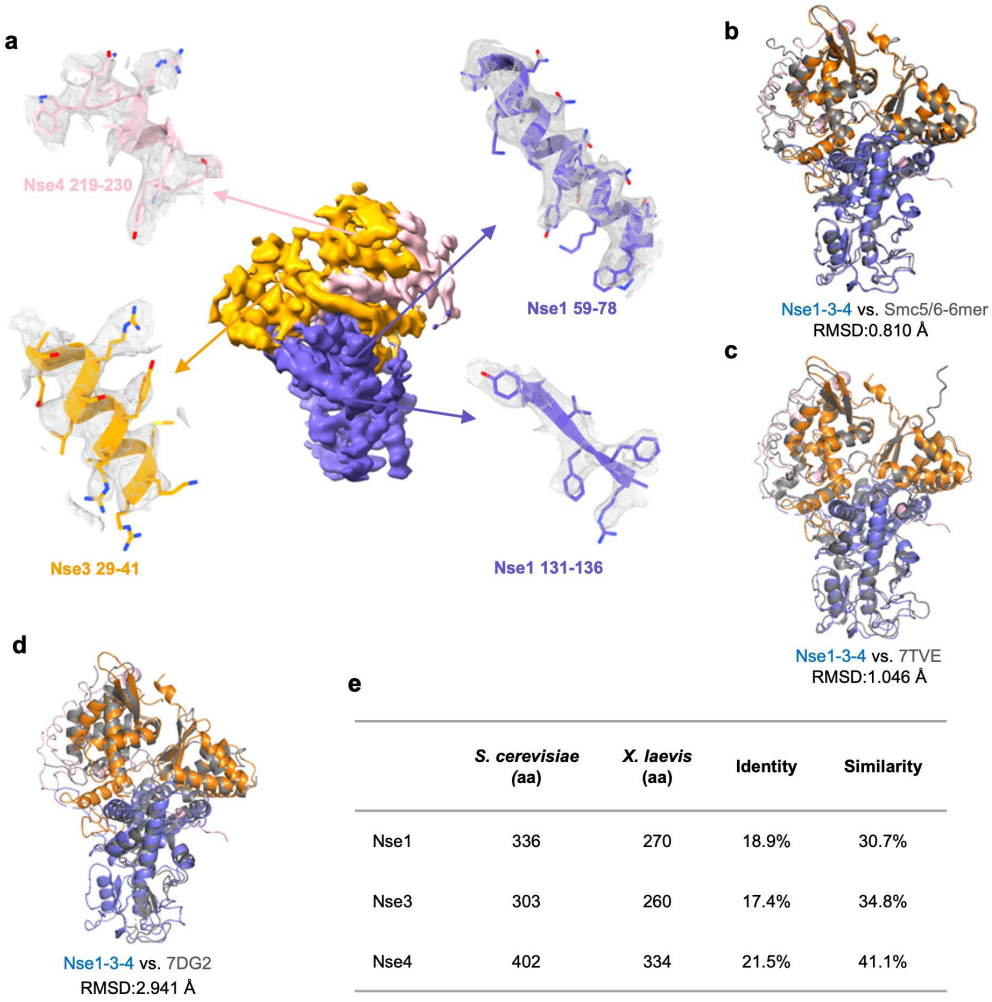
The RMSD value of each alignment is listed below each panel.



f

Interaction interface	SASA buried (Å²)
Smc5 – Nse6	1529.1
Smc6 – Nse6	7307.8
Smc6 – Nse5	2014.9
Nse5 – Nse6	4884.5

Supplementary Fig. 4 Interactions between Nse5-6 and the head regions of Smc5-6.
(**a-b**) Hydrogen bonds and salt bridges formed amongst the pairs of the subunits.
(**c-e**) Hydrophobic core interactions among the two pairs of subunits.
(**f**) Solvent accessible surface area (SASA) buried at the interface formed among indicated pairs of subunits. Sidechains of key residues are indicated as sticks.



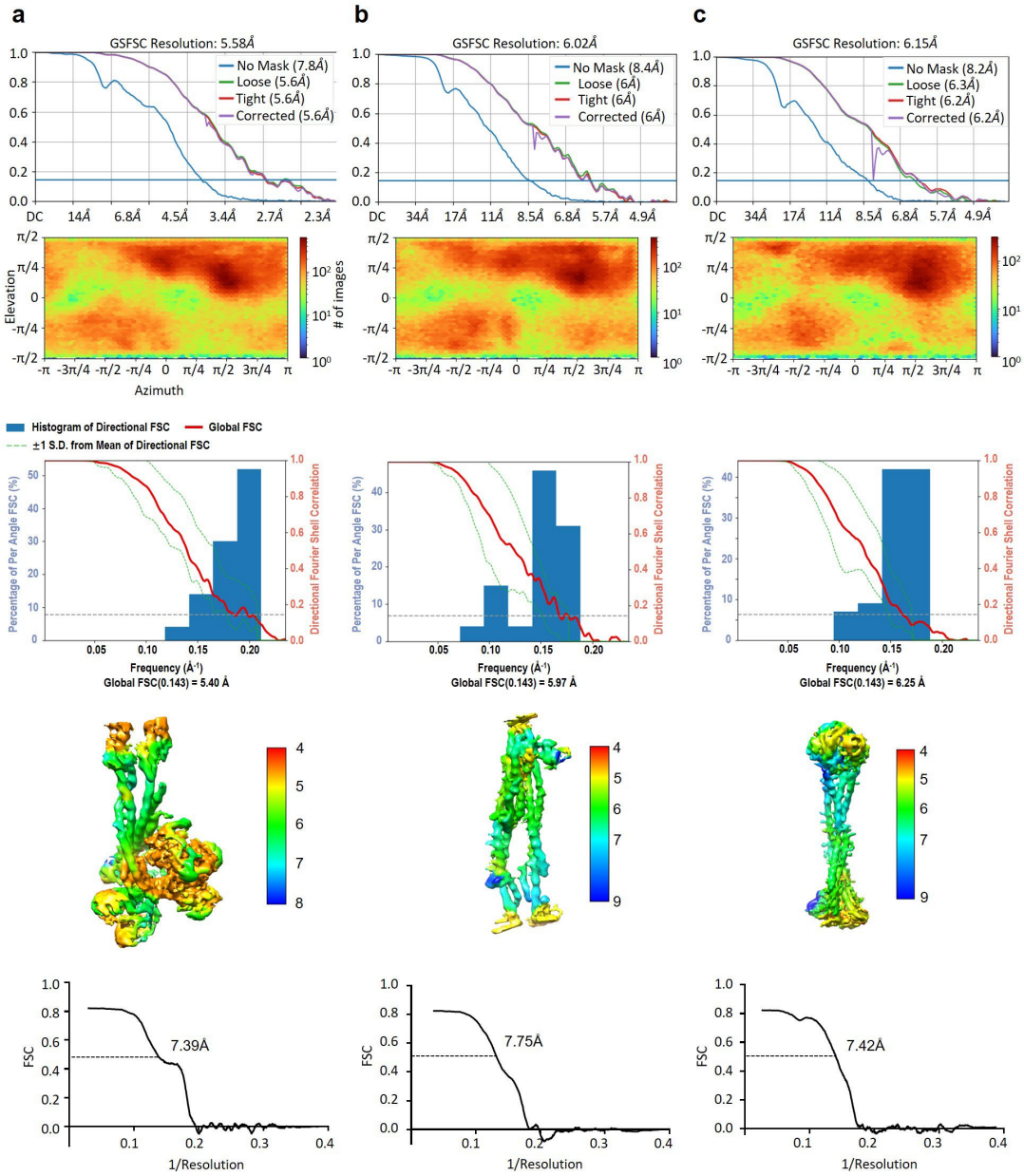
e

	S. cerevisiae (aa)	*X. laevis* (aa)	Identity	Similarity
Nse1	336	270	18.9%	30.7%
Nse3	303	260	17.4%	34.8%
Nse4	402	334	21.5%	41.1%

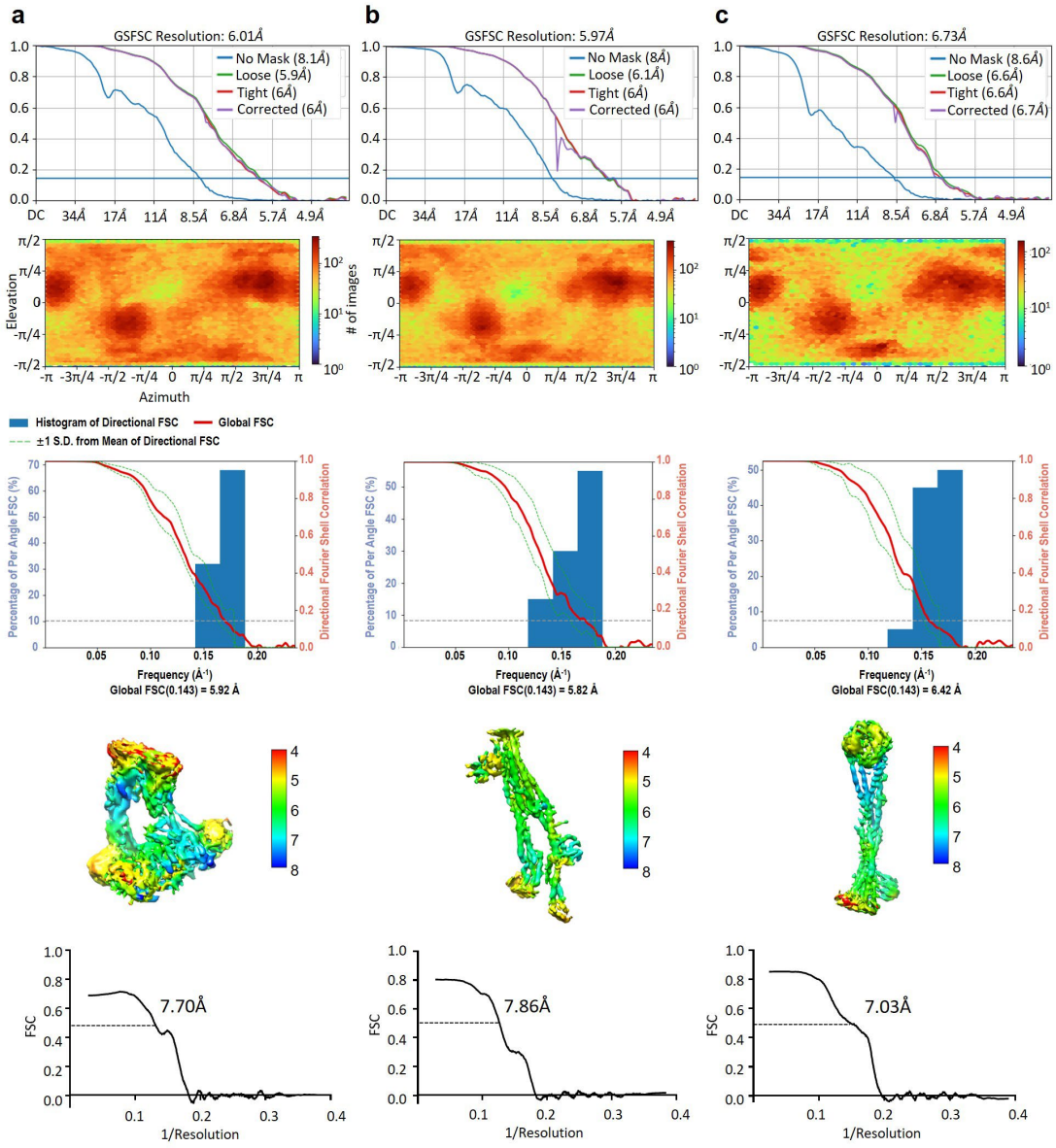
Supplementary Fig. 5 Local density maps and structural comparison of the Nse1-3-4 heterotrimer.

(**a**) Representative local cryo-EM density maps of the Nse1-3-4 structure described in this work. (**b-d**) Structural comparison of the Nse1-3-4 heterotrimer. The budding yeast Nse1-3-4 trimer structure obtained in this work (color) was aligned with the structure of the same subcomplex extracted from Smc5/6-6mer structure described in this work (grey) (**b**), with another budding yeast Nse1-3-4 structure (PDB: 7TVE, grey) (**c**), and with the *X. laevis* Nse1-3-4 structure (PDB: 7DG2, grey) (**d**).

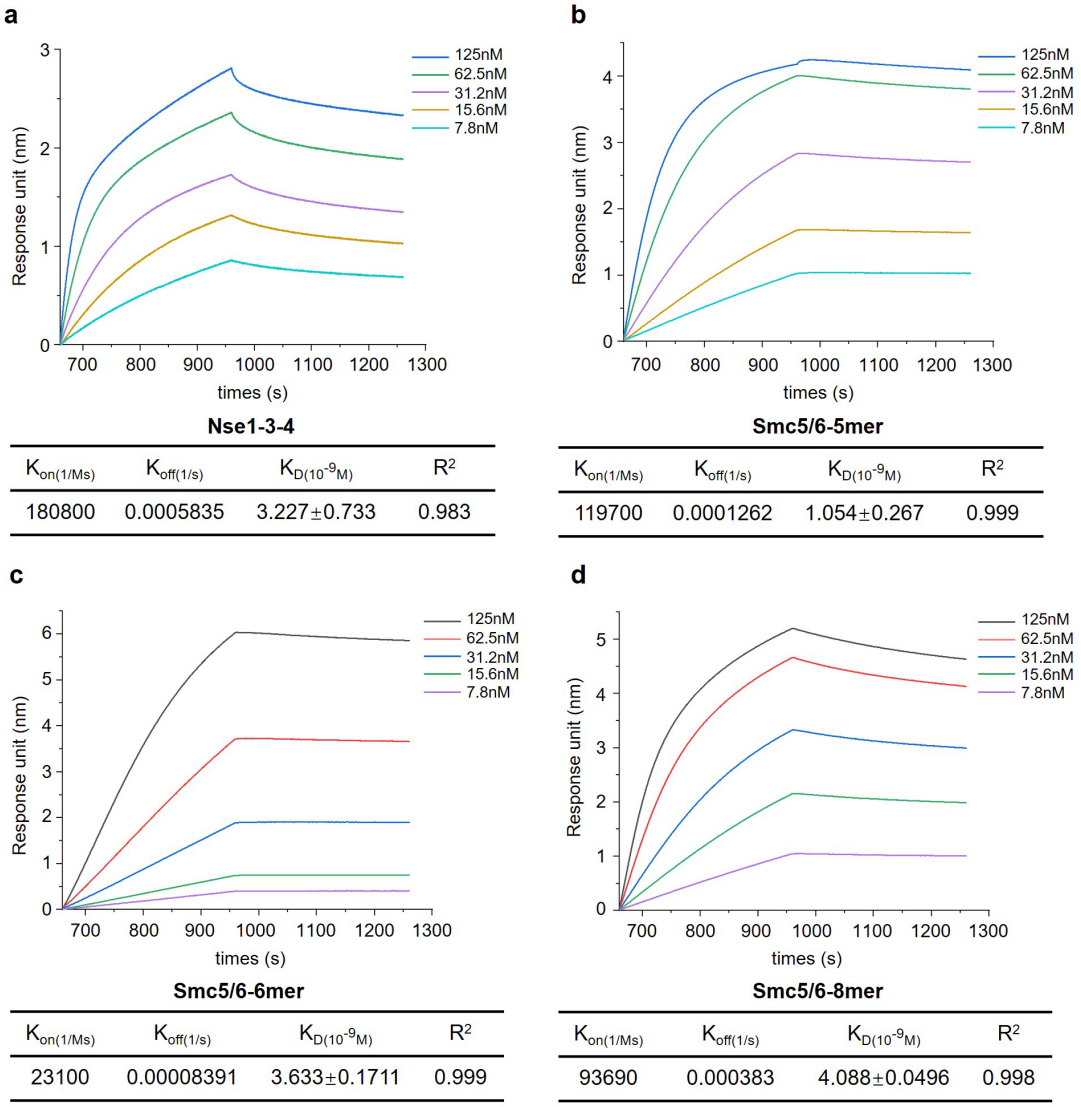
(**e**) Indicated protein properties of the Nse1, Nse3, and Nse4 proteins from *S. cerevisiae* and *X. laevis* are compared.



Supplementary Fig. 6 Resolution assessment of the cryo-EM maps of Smc5/6-6mer. (**a-c**) Global Fourier Shell Correlation (FSC) curve, angular distribution plot, 3D FSC curve, local resolution estimation, and model vs. map FSC of the focused refinement maps of the Head (**a**), Arm (**b**), and Hinge (**c**) regions of Smc5/6-6mer are displayed from top to bottom.



Supplementary Fig. 7 Resolution assessment of the cryo-EM maps of Smc5/6-5mer. (**a-c**) Global Fourier Shell Correlation (FSC) curve, angular distribution plot, 3D FSC curve, local resolution estimation, and model vs. map FSC of the focused refinement maps of the Head (**a**), Arm (**b**), and Hinge (**c**) regions of Smc5/6-5mer are displayed from top to bottom.



$K_{on(1/Ms)}$	$K_{off(1/s)}$	$K_{D(10^{-9}M)}$	R^2
180800	0.0005835	3.227±0.733	0.983

$K_{on(1/Ms)}$	$K_{off(1/s)}$	$K_{D(10^{-9}M)}$	R^2
119700	0.0001262	1.054±0.267	0.999

$K_{on(1/Ms)}$	$K_{off(1/s)}$	$K_{D(10^{-9}M)}$	R^2
23100	0.00008391	3.633±0.1711	0.999

$K_{on(1/Ms)}$	$K_{off(1/s)}$	$K_{D(10^{-9}M)}$	R^2
93690	0.000383	4.088±0.0496	0.998

Supplementary Fig. 8 DNA binding activity results.

DNA binding data for purified Nse1-3-4 (**a**), Smc5/6-5mer (**b**), -6mer (**c**), and -8mer (**d**) are presented for the association with 30 bp dsDNA using Octet RED 96.

Supplementary Table 1. SAXS data collection parameters for Smc5/6-8mer.

(a) Sample details		
	Smc5/6-8mer	
Organism	*Saccharomyces cerevisiae* (strain ATCC 204508 / S288c) (Baker's yeast)	
Source (Catalogue No. or reference)	Spodoptera frugiperda Sf9 cells expressed	
Description: sequence (including Uniprot ID + uncleaved tags), bound ligands/modifications, etc.	Q08204, Q12749, P38632, P40026, Q03718, Q07913, Q05541, P43124	
Extinction coefficient ε (wavelength and units)	0.610	
Molecular mass M from chemical composition (Da)	520478.92	
loading volume/concentration, (mg ml^{-1})	10	
injection volume (µl)	100	
flow rate (ml min^{-1})	0.4	
Concentration measure method	UV	
Solvent composition and source	20 mM HEPES-NaOH, pH 8.0, 300 mM NaCl	
(b) SAXS data collection parameters		
Source, instrument and description or reference	BL19U2 beamline at SSRF	
Wavelength (Å)	1.033	
Sample-to-detector distance	2.2 m	
q-measurement range ($Å^{-1}$)	0.007-0.45	
Exposure time	1.5 s	
number of exposures	2000	
Sample configuration including path length	1.5 mm	
Sample temperature (ºC)	23	
(c) Software employed for SAXS data reduction, analysis and interpretation		
SAXS data reduction to sample–solvent scattering	RAW	
Guinier	AutoRG (ATSAS)	
P(r)	GNOM	
Scattering particle volume	RAW	
Shape/bead modelling	DAMMIF	
Molecular graphics	PyMOL	
(d) Structural Parameters		
From Guinier fit	I(0) (cm^{-1})	120.03±2.71
	Rg (Å)	122.92±2.46
From *P*(r)	I(0) (cm^{-1})	121.4±4.5
	Rg (Å)	122.1±4.4
	Dmax (Å)	480.0
MW (kDa) from sequence	520.5	
From volumn of correlation (Vc)	444.9	
Using the porod volumn (Vp)	403.8	
Modeling		
	χ^2	1.023
DAMMIN	NSD	0.910±0.029
	Ensemble Resolution	66±5

Supplementary Table 2. Primers used in this study.

Primer #	Purpose	Direction	Primer sequence
1	To PCR *Nse1* and assemble into 9A vector	forward	TTTAAGAAGGAGATATAGATCATGGAGGTACATGAAGAGCA
2		reverse	TTATGGAGTTGGGATCTTATTATTAAATAACGTATACGCCCTCT
3	To PCR *Nse3* and assemble into 9C vector	forward	TACTTCCAATCCAATGCAATGAGTTCTATAGATAATGACAGC
4		reverse	TTATCCACTTCCAATGTTATTATATAGAATATGAATCGCCAATGCT
5	To PCR *Nse4* and assemble into 9C vector	forward	TACTTCCAATCCAATGCAATGTCTAGTACAGTAATATCTAGAA
6		reverse	TTATCCACTTCCAATGTTATTAGTCTAAGAATGGTGAAGTGAT
7	To PCR *Nse1* and assemble into 438-A vector	forward	TACTTCCAATCCAATCGATGGAGGTACATGAAGAGCA
8		reverse	TTATCCACTTCCAATGTTATTAAATAACGTATACGCCCTCTG
9	To PCR *Nse2* and assemble into 438-A vector	forward	TACTTCCAATCCAATCGATGGCCTTGAACGATAATCC
10		reverse	TTATCCACTTCCAATGTTATTATAAAACATCGATGGCTTGACTA
11	To PCR *Nse3* and assemble into 438-A vector	forward	TACTTCCAATCCAATCGATGAGTTCTATAGATAATGACAGC
12		reverse	TTATCCACTTCCAATGTTATTATATAGAATATGAATCGCCAATGCT
13	To PCR *Nse4* and assemble into 438-A vector	forward	TACTTCCAATCCAATCGATGTCTAGTACAGTAATATCTAGAA
14		reverse	TTATCCACTTCCAATGTTATTAGTCTAAGAATGGTGAAGTGAT
15	To PCR *Nse5* and assemble into 438-A vector	forward	TACTTCCAATCCAATCGATGGATGGTGCGTTGATAAATT
16		reverse	TTATCCACTTCCAATGTTATTATTCTATAATTACACTTTTATCATGAACG
17	To PCR *Nse6* and assemble into 438-A vector	forward	TACTTCCAATCCAATCGATGGGAAGCGTGAACTCAT
18		reverse	TTATCCACTTCCAATGTTATTACCTGATTTTAGAAAACAAATTTAATATTCC
19	To PCR *Smc5* and assemble into 438-A vector	forward	TACTTCCAATCCAATCGATGACCAGTCTAATAGATTTGG
20		reverse	TTATCCACTTCCAATGTTATTAATCGAATGAGTAGTTAGAAGTTTC
21	To PCR *Smc6* and assemble into 438-C vector	forward	TACTTCCAATCCAATGCAATGATTTCGACTACGATTAGTG
22		reverse	TTATCCACTTCCAATGTTATTAATTATAAAAATTGGAATTATTCTGTCTCTC
23	To PCR *nse2-3A* fragment	forward	GTAATACCGGATCTGCAAAACCCCGCAGACG
24		forward	ATGGCCTTGAACGATAATCC
25		reverse	GCAGATCCGGTATTACACAA
26	To confirm *nse2-3A*	forward	CCTTTGATTCTGGTTACTCC
27		reverse	GACGGTTTGAATGTAGAGAG
28	To PCR *nse6-RAAE* and *nse6-9A* fragments	forward	GTAGCCAAAAGTGAGTTACAAAAAAGCATAAAG
29		forward	ATGGGAAGCGTGAACTCATC
30		reverse	CTCACTTTTGGCTACAGTGT
31	To confirm *nse6-RAAE* and *nse6-9A*	forward	GTCTTCTTTGTATTTGTTTTAG
32		reverse	GAAAATATTGTTACTAAGGACC

Supplementary Table 3. Yeast strains used in this work.

Name	Genotypes
T2271-2-7C	*nse6-D86R,I88A,L89A,R91E-AID-3FLAG::KAN*
T2272-1-4C	*nse6-D86A,P87A,I88A,L89A,K90A,R91A,T92A,I93A,I94A-AID-3FLAG::KAN*
T2273-14-6C	*nse2-K20A,H23A,H26A-3HA::KAN*
X8974	*NSE6-AID-3FLAG::KAN SMC5-13MYC::HIS3*
X8975	*nse6-D86R, I88A, L89A, R91E-AID-3FLAG::KAN RAD5 SMC5-13myc::HIS3*
X8978	*NSE2-3HA::KAN SMC6-13myc::HIS*
X8979	*nse2-K20A,H23A,H26A-3HA::KAN SMC6-13MYC::HIS3*
T294	*SMC5-13MYC::HIS3*
X3254	*SMC6-13 MYC:HIS3*
X8981-1A	*nse2-K20A,H23A,H26A-3HA::KAN nse6-D86R,I88A,L89A,R91E-AID-3FLAG::KAN*
X8984-5B	*NSE2-3HA::KAN NSE6-AID-3FLAG::KAN*
T1596-2-9A	*mms4Δ::KAN*
X8990-10A	*NSE2-3HA::KAN mms4Δ::KAN*
X8982-1B	*nse2-K20A,H23A,H26A-3HA::KAN mms4Δ::KAN*
X9037-7D	*NSE6-AID-3XFLAG::KAN mms4Δ::KAN*
X8991-2A	*nse6-D86R,I88A,L89A,R91E-AID-3FLAG::KAN mms4Δ::KAN*
T766-1	*esc2Δ::KAN*
X8987-6A	*NSE2-3HA::KAN esc2Δ::KAN*
X8983-2B	*nse2-K20A,H23A,H26A-3HA::KAN esc2Δ::KAN*
X9038-7B	*NSE6-AID-3FLAG::KAN esc2Δ::KAN*
X8992-6B	*nse6-D86R,I88A,L89A,R91E-AID-3FLAG::KAN esc2Δ::KAN*
G172	*sgs1Δ::HIS3*
X8989-7A	*NSE2-3HA:KAN sgs1Δ::HIS3*
X8985-9A	*nse2-K20A,H23A,H26A-3HA::KAN sgs1Δ::HIS3*
X8997-1A	*NSE6-AID-3FLAG::KAN sgs1Δ::HIS3*
X8980-1C	*nse6-D86R,I88A,L89A,R91E-AID-3FLAG::KAN sgs1Δ::HIS3*
X3952-1B	*rrm3Δ::KAN*
X8988-2B	*NSE2-3HA::KAN rrm3Δ::KAN*
X8986-2B	*nse2-K20A,H23A,H26A-3HA::KAN rrm3Δ::KAN*
X8998-3B	*NSE6-AID-3FLAG::KAN rrm3Δ::KAN*
X8993-17C	*nse6-D86R,I88A,L89A,R91E-AID-3FLAG::KAN rrm3Δ::KAN*