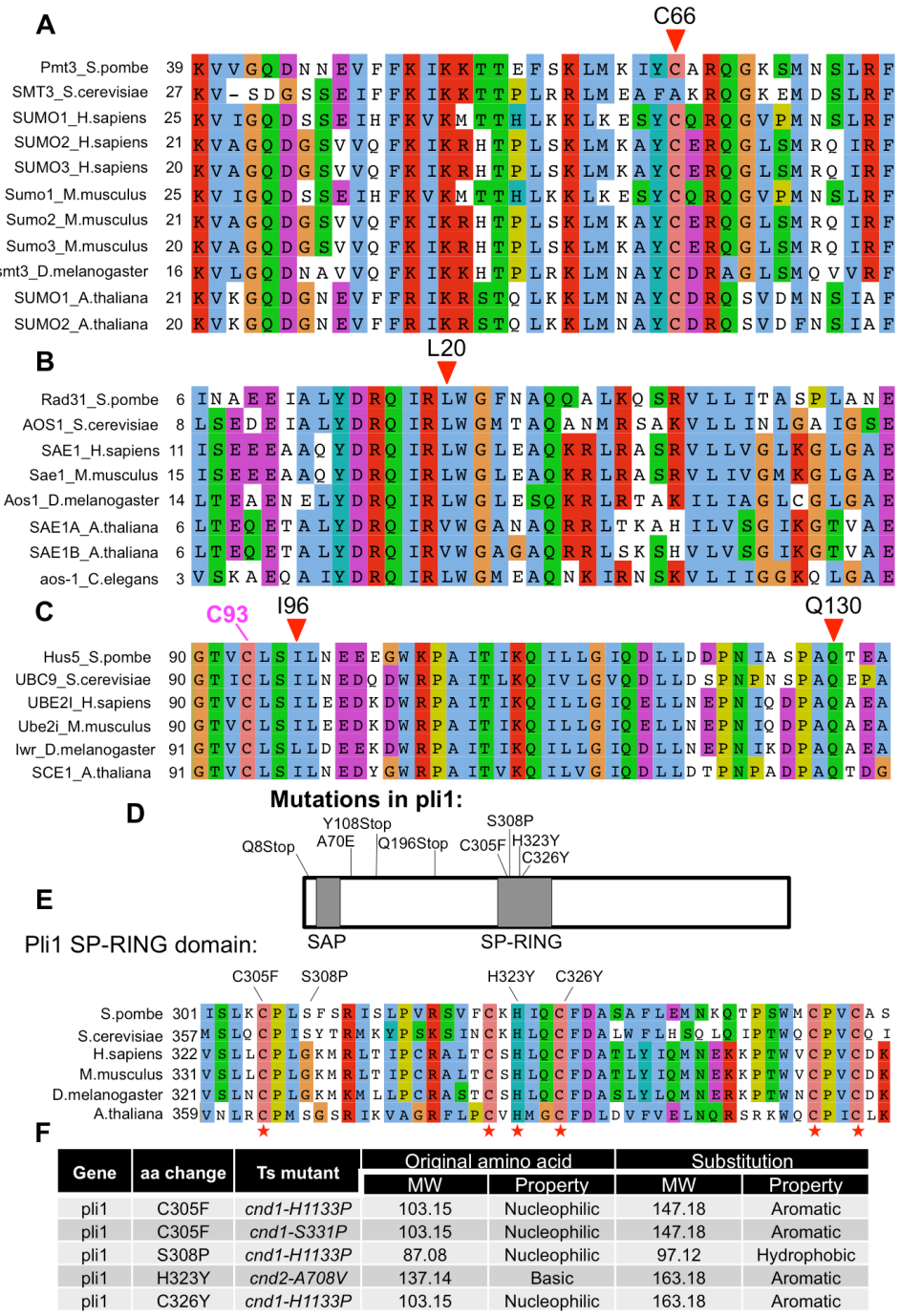


Gene	aa change	Ts mutant	Original amino acid		Substitution	
			MW	Property	MW	Property
pli1	C305F	*cnd1-H1133P*	103.15	Nucleophilic	147.18	Aromatic
pli1	C305F	*cnd1-S331P*	103.15	Nucleophilic	147.18	Aromatic
pli1	S308P	*cnd1-H1133P*	87.08	Nucleophilic	97.12	Hydrophobic
pli1	H323Y	*cnd2-A708V*	137.14	Basic	163.18	Aromatic
pli1	C326Y	*cnd1-H1133P*	103.15	Nucleophilic	163.18	Aromatic

Fig. S1. Condensin suppressors in SUMOylation pathway genes.

(*A*) Alignment of SUMO proteins from different species shows that Pmt3-C66 in *S. pombe* is conserved. (*B*) Alignment of SUMO-activating (E1) enzyme from different species shows that Rad31-L20 in *S. pombe* is conserved. (*C*) Alignment of SUMO E2 conjugating enzyme Hus5/Ubc9 from different species shows that Hus5-I96 and -Q130 in *S. pombe* are conserved. (*D*) Localization

of *cnd* suppressors in SUMO E3 ligase Pli1 protein sequence. Pli1 contains two domains: an amino-terminal SAP (SAF-A/B, Acinus and PIAS) domain and a central SP-RING (Siz/PIAS-RING) domain (1). (*E*) Alignment of SUMO E3 ligase Pli1 around its SP-RING domain. Asterisks indicate the positions of cysteiyl and histidyl residues forming the C2HC3 conserved SP-RING domain. The *cnd* suppressor mutations in Pli1's SP-RING domain are all single amino acid substitutions and three of the four (C305F, H323Y and C326Y) directly disrupt the C2HC3 motif. (*F*) Molecular weights (MW) and properties of the original amino acids and their substitutions. All the substitutions are bulkier than the original amino acids.

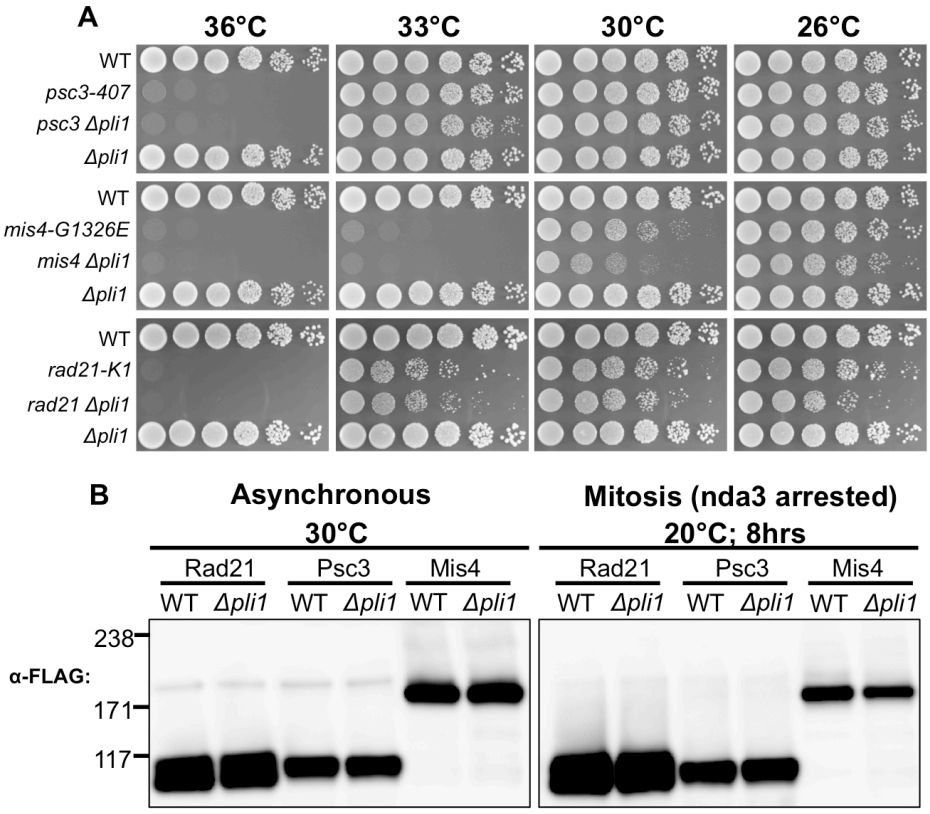
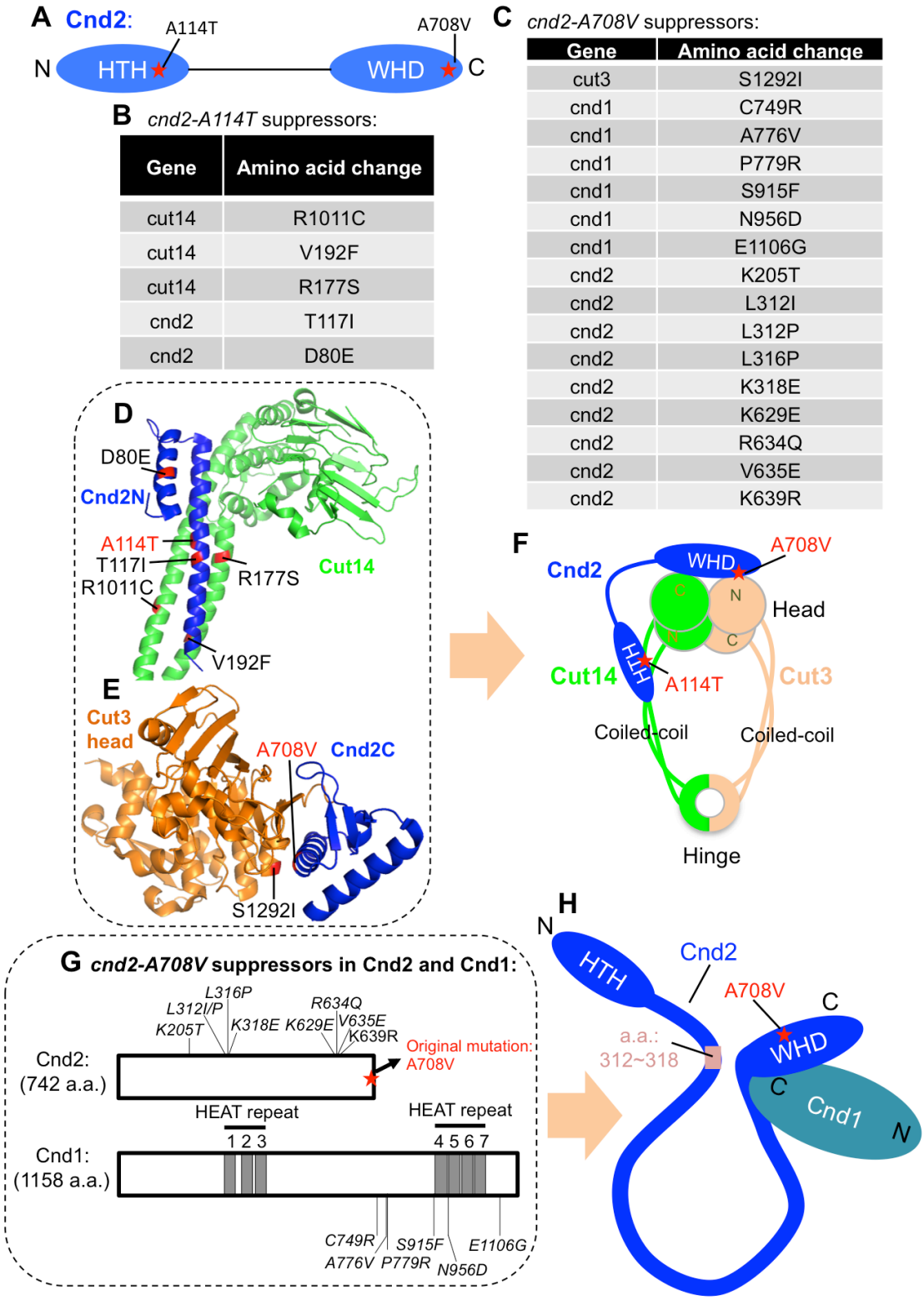


Fig. S2. Cohesin is not affected by loss of SUMO E3 ligase.

(*A*) cohesin ts mutants (*psc3-407*, *mis4-G1326E* and *rad21-K1*) cannot be rescued by *Δpli1*. No obvious genetic interaction can be observed. (*B*) Western blots cannot detect any band shift for Rad21-3FLAG, Psc3-3FLAG or Mis4-3FLAG in *Δpli1* compared to those in wild type (WT). Anti-FLAG antibody was used to detect protein bands. nda3-KM311 containing strains were used to arrest cells in mitosis by culturing them at 20°C for 8 hours. Cohesin (at least Rad21, Psc3 and Mis4) may not be SUMOylated.



Gene	Amino acid change
cut14	R1011C
cut14	V192F
cut14	R177S
cnd2	T117I
cnd2	D80E

Gene	Amino acid change
cut3	S1292I
cnd1	C749R
cnd1	A776V
cnd1	P779R
cnd1	S915F
cnd1	N956D
cnd1	E1106G
cnd2	K205T
cnd2	L312I
cnd2	L312P
cnd2	L316P
cnd2	K318E
cnd2	K629E
cnd2	R634Q
cnd2	V635E
cnd2	K639R

Fig. S3. Cut14 and Cut3 in condensin resemble Psm3 and Psm1 in cohesin respectively

(*A*) Two *cnd2* ts mutants, *cnd2-1* and *cnd2-A708V*, are available. Their responsible mutations are A114T and A708V respectively. A114 is located in

the amino-terminal HTH motif (Helix-Turn-Helix motif) and A708V is located in the carboxyl-terminal WHD domain (Winged Helix Domain). (*B*) List of *cnd2-A114T* suppressors that were mapped in *cut14* and *cnd2* itself. (*C*) List of *cnd2-A708V* suppressors that were mapped in *cut3*, *cnd1* and *cnd2* itself. (*D*) Localization of *cnd2-A114T* suppressors in a structure of the kleisin-N SMC interface in prokaryotic condensin (PDB ID: 3ZGX) based on homolog alignment. (*E*) Localization of *cnd2-A708V* suppressors in a structure of Kleisin-C SMC interface (PDB ID: 4I99) based on homolog alignment. (*F*) Predicted relationships among Cnd2/Kleisin, Cut14 and Cut3 based on (*D*) and (*E*). Condensin structure may resemble cohesin structure in that the N-terminus of Cnd2/Kleisin may bind the Cut14 head-coiled coil junction and the C-terminus of Cnd2/Kleisin may bind the Cut3 head domain. Judging from kleisin's binding pattern with SMC heads, Cut14 and Cut3 in condensin may resemble Psm3 and Psm1 in cohesin respectively**.** (*G*) *cnd2-A708V* suppressors in Cnd1 and Cnd2 protein sequences. *cnd2-A708V* intragenic suppressors were enriched in two narrow regions (a.a.: 312~318 and a.a.: 629~639). a.a.: 312~318 is far from the original ts mutation (A708V), while a.a.: 629~639 is close to it. All *cnd2-A708V* suppressors in Cnd1 are located in the Cnd1 C-terminus. (*H*) Based on (*G*), Cnd2 may form a hooked structure and the neck of it may interacts with the Cnd1 C-terminus.

A ***cnd3-L269P*** **suppressors:**

Gene	Amino acid change	Gene product
mts4	P852S	19S proteasome regulatory subunit Mts4
mts4	-	19S proteasome regulatory subunit Mts4
rpt1	E375G	19S proteasome regulatory subunit Rpt1
pre9	L147Q	20S proteasome component alpha 3
pre9	R143P	20S proteasome component alpha 3
rpn11	W120C	19S proteasome regulatory subunit Rpn11
pam1	A28T	20S proteasome component beta 6
scl1	G18R	20S proteasome component alpha 1
rpn3	H268P	19S proteasome regulatory subunit Rpn3
rpt1	E193K	19S proteasome regulatory subunit Rpt1
pup1	G9V	20S proteasome component beta 2
nas2	Q6Stop	26S proteasome regulatory particle assembly protein

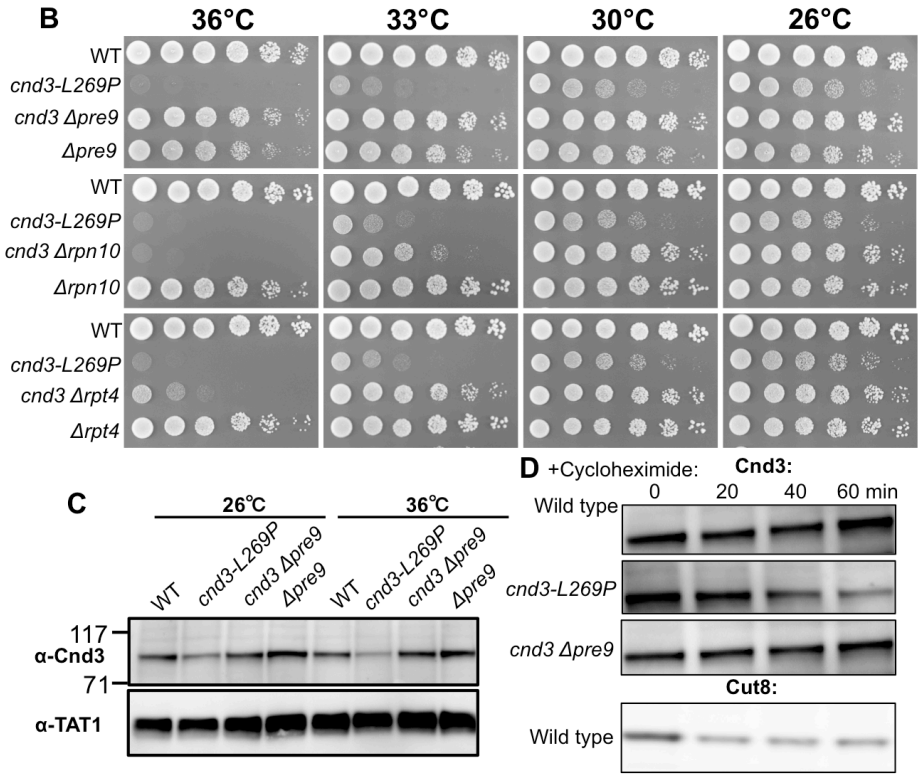


Fig. S4. Cnd3 may be targeted for degradation by 26S proteasome

(*A*). Multiple *cnd3-L269P* suppressors were identified in 26S proteasome complex genes. The 26S proteasome is a multiprotein complex consisting of two distinct sub-complexes: the 20S catalytic core and the 19S regulatory complex. (*B*) Deletion mutants of 26S proteasome complex genes (*Δpre9*, *Δrpn10* and *Δrpt4*) rescued *cnd3-L269P* too. Rpn10 and Rpn13 have redundant roles in recognition of ubiquitinated proteins (2), therefore, suppression of *cnd3-L269P* by *Δrpn10* is weaker than that by *Δpre9* or *Δrpt4*.

(*C*). Mutant Cnd3-L269P protein level in *cnd3-L269P* was reduced, especially at the restrictive temperature. The Mutant Cnd3-L269P protein level was rescued by *Δpre9*. (*D*). Mutant Cnd3-L269P protein level in *cnd3-L269P* decreased gradually in the presence of cycloheximide (a protein synthesis inhibitor; 300 mg/mL), but the reduction did not occur when *Δpre9* was introduced. The nuclear proteasome tether protein Cut8 (3), which was decayed rapidly, was loaded as a control.

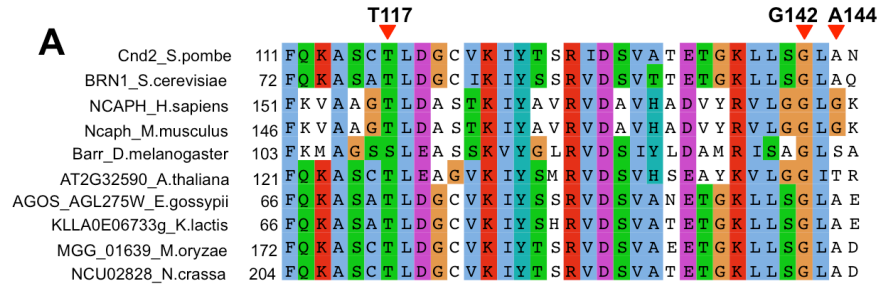


Fig. S5. Suppression of condensin and cohesin hinge ts mutants by mutations of head-associated factors

(*A*). Conservation of condensin hinge suppressors in Cnd2/kleisin among various species. (*B*) Molecular weights (MW) and properties of the original amino acids and their substitutions. All substitutions are bulkier than the original amino acids. Original condensin hinge ts mutants used for screening

are described too. (*C*). Conservation of cohesin hinge suppressors in Rad21/kleisin among various species. (*D*). The substitution of asparagine for aspartate (Psc3-D388N) may affect its interaction with double-stranded DNA (dsDNA). *psc3-D388N* was identified as a suppressor of a cold sensitive mutant, *psm3-G653E*, in which DNA binding was impaired. Localization of Psc3-D388N, into an atomic model of Scc3/Psc3 bound to a fragment of the Scc1/Rad21 kleisin subunit and DNA (PDB ID: 6H8Q), was indicated. The mutation faces the bound dsDNA, therefore it may affect its interaction with dsDNA. (*E*). Molecular weights (MW) and properties of the original amino acids and their substitutions.

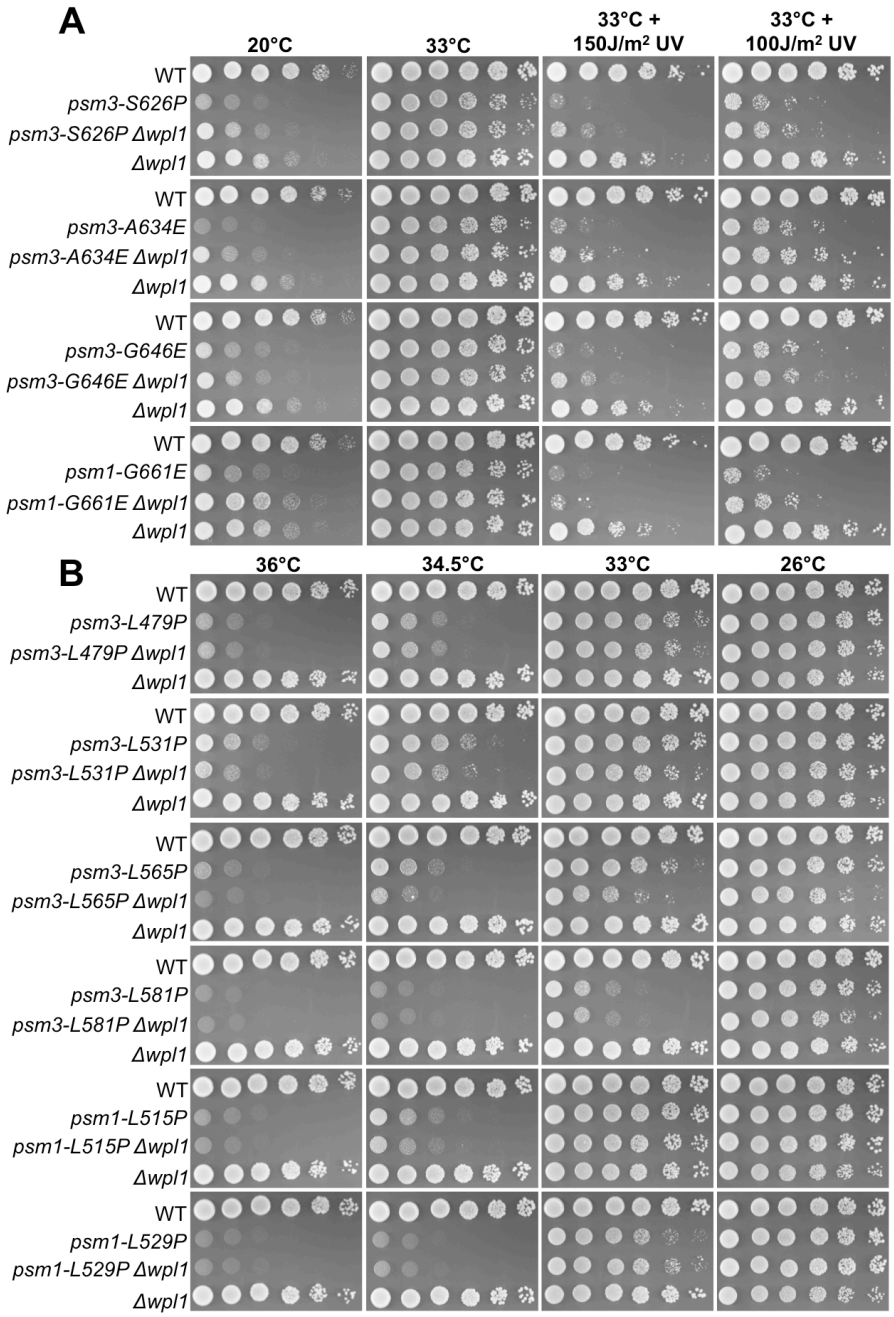


Fig. S6. Cohesin cs mutants, but not ts mutants, were rescued by loss of Wpl1

(*A*). Suppression of cohesin cs mutants by *Δwpl1*. Multiple mutations in *wpl1* were identified as suppressors of cohesin cs mutants, double mutants (cohesin cs mutants in combination with *Δwpl1*) confirmed the suppression. Cohesin cs mutants were sensitive to ultraviolet (UV), and the UV sensitivity

was rescued by *Δwpl1* too. See **Fig. 5** also. (*B*). Cohesin ts mutants cannot be rescued by *Δwpl1*.

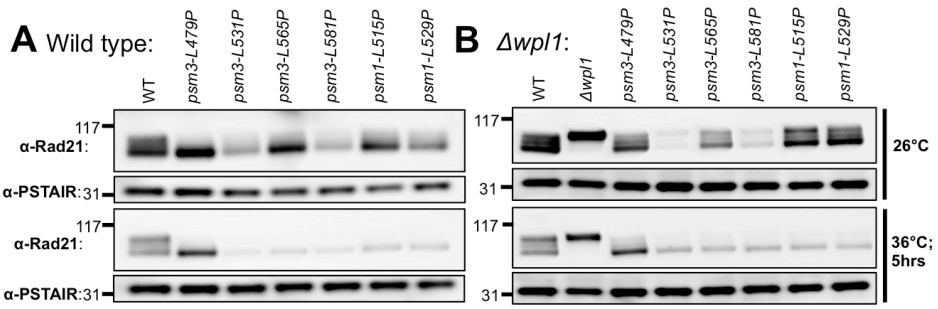
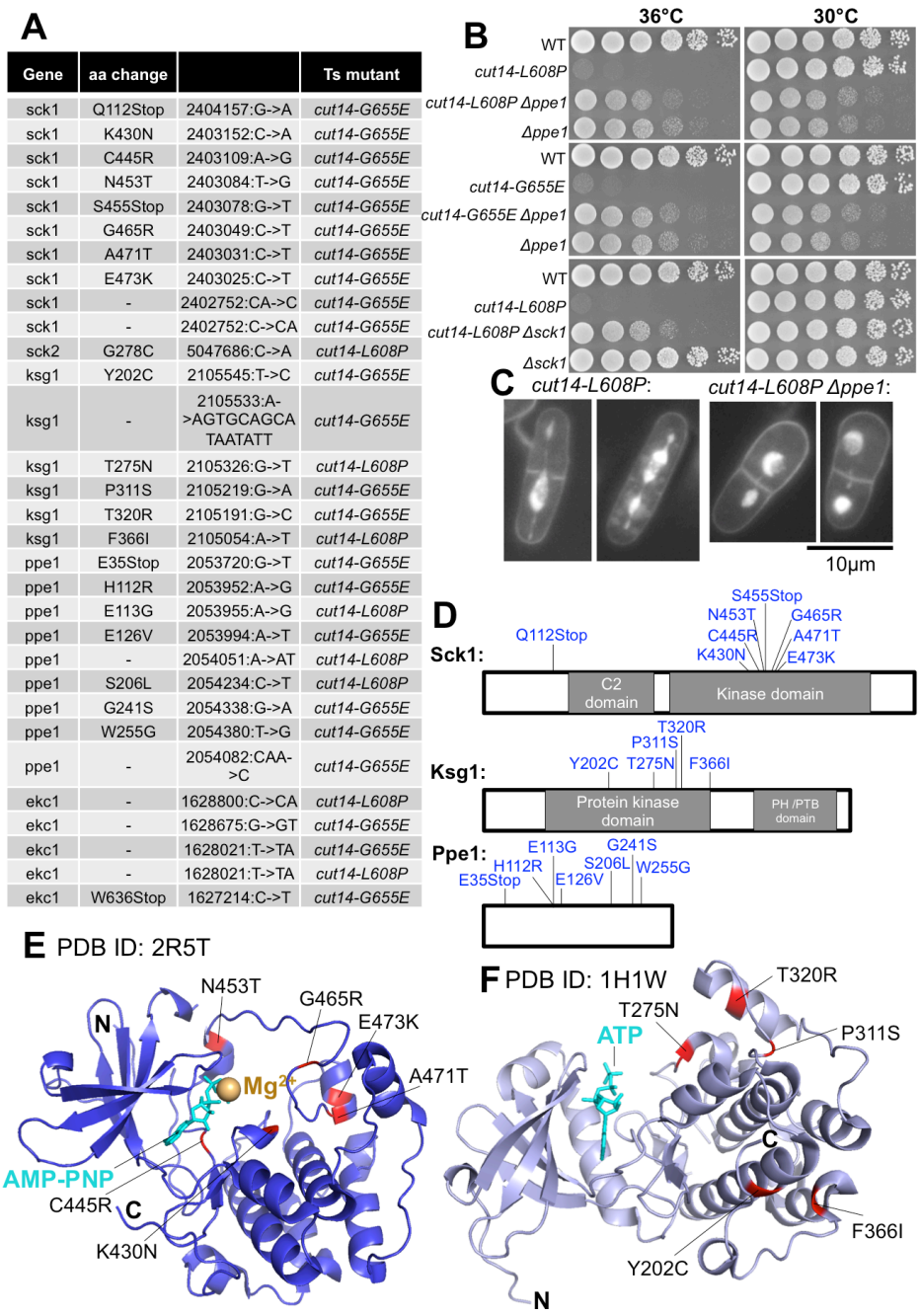


Fig. S7. Both Rad21 phosphorylation and Rad21 protein level were diminished in cohesin hinge ts mutants

(*A*). Western blot of Rad21 using an anti-Rad21 polyclonal antibody. Rad21 phosphorylation (smeary bands above the major non-phosphorylated Rad21 band) (4, 5) detected in wild type strain was diminished in cohesin hinge ts mutants at permissive temperature (26°C). Rad21 protein levels were decreased in *psm3-L531P* and *psm3-L581P* even at permissive temperature (26°C). Rad21 phosphorylation and Rad21 protein level were diminished (or greatly decreased) in cohesin ts mutants at restrictive temperature (36°C, 5hrs). (*B*). Rad21 phosphorylation and Rad21 protein level in double (*Δwpl1* containing) mutants. Rad21 was completely phosphorylated in the *Δwpl1* single mutant. Recovery of Rad21 phosphorylation was observed in *hinge Δwpl1* double mutants at the permissive temperature (26°C). However, diminished Rad21 phosphorylation and Rad21 protein level observed in cohesin hinge ts mutants at restrictive temperature (36°C, 5hrs) could NOT be rescued by *Δwpl1*. Both Rad21 phosphorylation and the Rad21 protein level were diminished (or greatly decreased) in double (*Δwpl1* containing) mutants.



Gene	aa change		Ts mutant
sck1	Q112Stop	2404157:G->A	*cut14-G655E*
sck1	K430N	2403152:C->A	*cut14-G655E*
sck1	C445R	2403109:A->G	*cut14-G655E*
sck1	N453T	2403084:T->G	*cut14-G655E*
sck1	S455Stop	2403078:G->T	*cut14-G655E*
sck1	G465R	2403049:C->T	*cut14-G655E*
sck1	A471T	2403031:C->T	*cut14-G655E*
sck1	E473K	2403025:C->T	*cut14-G655E*
sck1	-	2402752:CA->C	*cut14-G655E*
sck1	-	2402752:C->CA	*cut14-G655E*
sck2	G278C	5047686:C->A	*cut14-L608P*
ksg1	Y202C	2105545:T->C	*cut14-G655E*
ksg1	-	2105533:A->AGTGCAGCATAATATT	*cut14-G655E*
ksg1	T275N	2105326:G->T	*cut14-L608P*
ksg1	P311S	2105219:G->A	*cut14-G655E*
ksg1	T320R	2105191:G->C	*cut14-G655E*
ksg1	F366I	2105054:A->T	*cut14-L608P*
ppe1	E35Stop	2053720:G->T	*cut14-G655E*
ppe1	H112R	2053952:A->G	*cut14-G655E*
ppe1	E113G	2053955:A->G	*cut14-L608P*
ppe1	E126V	2053994:A->T	*cut14-G655E*
ppe1	-	2054051:A->AT	*cut14-L608P*
ppe1	S206L	2054234:C->T	*cut14-L608P*
ppe1	G241S	2054338:G->A	*cut14-G655E*
ppe1	W255G	2054380:T->G	*cut14-G655E*
ppe1	-	2054082:CAA->C	*cut14-G655E*
ekc1	-	1628800:C->CA	*cut14-L608P*
ekc1	-	1628675:G->GT	*cut14-G655E*
ekc1	-	1628021:T->TA	*cut14-G655E*
ekc1	-	1628021:T->TA	*cut14-L608P*
ekc1	W636Stop	1627214:C->T	*cut14-G655E*

Fig. S8. Suppression of condensin hinge mutants by loss of kinases or a phosphatase complex

(*A*). Two condensin hinge ts mutants (*cut14-L608P* and *cut14-G655E*) were rescued by multiple mutations in kinase genes (*sck1*, *sck2* and *ksg1*) and Ppe1/PP6 phosphatase complex genes (*ppe1* and *ekc1*). (*B*). Spot test results showing *cut14-L608P* and *cut14-G655E*'s suppression by *Δppe1* or *Δsck1*. (*C*). Chromosome segregation defects of *cut14-L608P* were partially

rescued by *Δppe1*: sister chromatids were segregated, but unequal, as large and small daughter nuclei were observed frequently. (*D*). Mutation localization mapping in protein sequence indicated that mutations were enriched in the kinase domains of Sck1 and Ksg1, therefore loss of their kinase activities rescued condensin hinge ts mutants. (*E* and *F*). Sck1 mutations were located in the cleft that binds AMP-PNP directly, as illustrated by structural analysis (6), while Ksg1 mutations may not affect ATP binding directly (7). Whether these kinases and phosphatase directly affect condensin hinge phosphorylation remains to be clarified.

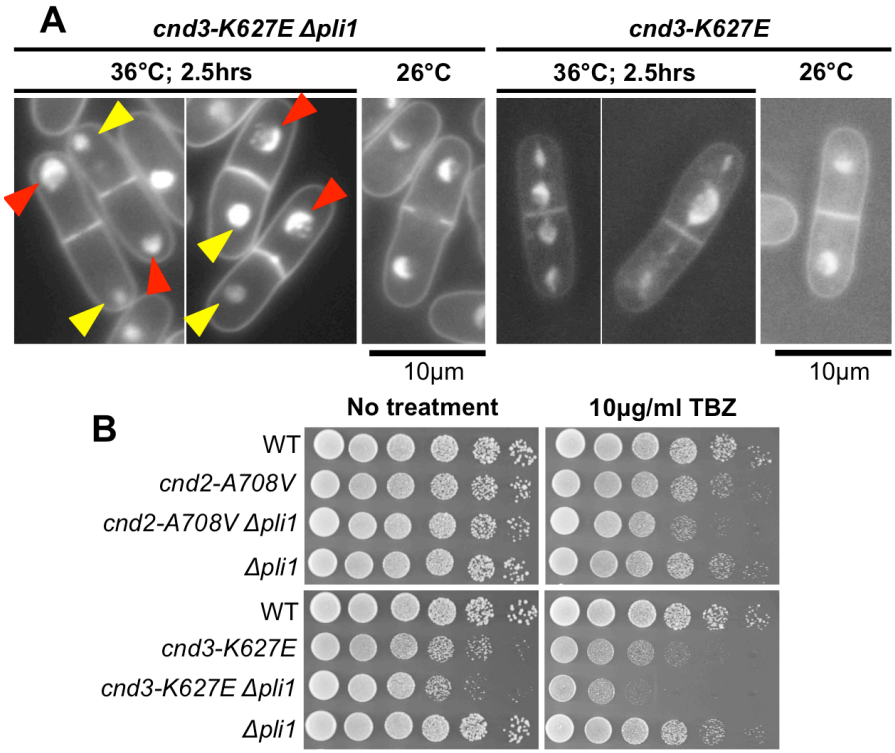


Fig. S9. SUMOylation may act distinctively at chromosome arms and centromeres

(*A*). DAPI-staining of the *cnd3-K627E* single mutant and the *cnd3-K627E Δpli1* double mutant at permissive and restrictive temperatures. Although the chromosome segregation defect in *cnd3-K627E* was rescued by loss of SUMO E3 ligase mutant *Δpli1*, the large and small nuclei phenotype, which was commonly observed in centromeric mutants, appeared. (*B*). Although *Δpli1* rescued the temperature sensitivity of condensin non-SMC ts mutants, sensitivity of *cnd3-K627E Δpli1* to a microtubule destabilizing drug, TBZ, was even more severe than that of the *cnd3-K627E* single mutant.

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