SUPPLEMENTARY INFORMATION

Structural Analysis of Fungi CENP-H/I/K Complex Reveals its Conserved Assembly Mechanism Underlying Faithful Chromosome Alignment

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SUPPLEMENTAL TABLE AND FIGURE LEGENDS

Table S1. Data collection, phasing and refinement statistics for crystal structure of *ct*CENP-I^{NT} alone and *ct*CENP-I^{NT} complex with *th*CENP-H^{CT}/K^{CT}

Figure S1. The phylogenetic tree of CENP-I (A), CENP-H (B) and CENP-K (C) from divergent species, and the reconstitution of thCENP-H/I^{NT}/K complex (D).

Figure S2. Sequence alignment of the CENP-I from multiple species
The alignment is generated using the online ESPript 3.0 server. Secondary structural elements predicted from PSI-PRED online server and found from crystal structure are indicated above the sequences, with the color blue and gray. The sequence of ctCENP-I shares high similarity (50%) with human CENP-I (E-value 7.65E-20) and high identity (62%) with thCENP-I.

Figure. S3 Sequence alignment of the CENP-K from multiple species
The sequence similarity between thCENP-K and hsCENP-K is 40%, based on the reciprocal Best BLAST result (E-value= 1.81E-06).

Figure. S4 Sequence alignment of the CENP-H from multiple species.
The thCENP-H exhibits a relative low sequence similarity (29%) with hsCENP-H, but the residues in involved the binding interfaces for the complex formation show higher conservation levels.

Figure S5. Density maps of *th*CENP-H^{CT} and *th*CENP-K^{CT}, and crystal structure of the ternary complex in one asymmetric unit
A. Cartoon view of fungi CENP-H/I/K complex structure. Even though two molecules of *ct*CENP-I^{NT} (cyan and green) were found in one asymmetric unit, only one binds to the heterodimer of *th*CENP-H^{CT} (magenta) and *th*CENP-K^{CT} (yellow).
B. SDS-PAGE electrophoresis analyses of the complex crystals, stained with Coomassie Blue.
C. Electron density for *th*CENP-H^{CT} in the complex structure, composite omit map calculated with Phenix, contoured at 1.0σ.

Figure S6. Analyses of the binding activities of *th*CENP-H WT or mutants bound to GST-*th*CENP-K$^{\Delta N75}$ and GST-*th*CENP-K^{FL}

A. *In vitro* binding assay were performed to examine the binding activity between thCENP-H^{N164} (ΔCT) and thCENP-K^{N143} (ΔCT) complex and ctCENP-I^{NT}.

B and **C.** Recombinant *th*CENP-H WT or mutant proteins were mixed with GST or GST-*th*CENP-K$^{\Delta N75}$ (**B**) and GST-*th*CENP-K^{FL} (**C**) proteins**.** GST pull-down assays were performed. The bead-bound proteins were resolved with SDS-PAGE and stained with Coomassie Blue. Quantification was summarized for both in **Figure 3B**.

Figure S7. Analyses of the binding activities of *hs*CENP-H WT or mutants bound to GST-*hs*CENP-K$^{\Delta N85}$ and GST-*hs*CENP-K^{FL}

A and **B.** Recombinant *hs*CENP-H WT or mutant proteins were mixed with GST or GST-*hs*CENP-K$^{\Delta N85}$ (**A**) and GST-*hs*CENP-K^{FL} (**B**) proteins**.** GST pull-down assays were performed. The bead-bound proteins were resolved with SDS-PAGE and stained with Coomassie Blue. Quantification was summarized for both (**A** and **B**) in **Figure 3D**.

Figure S8. Analyses of the binding activities of *th*CENP-H WT or mutants bound to *ct*CENP-I^{NT}, and Immuno-blotting analyses of gene knock-down and overexpression in cell experiment.

A. Recombinant *th*CENP-H WT or mutant proteins were mixed with GST or GST-*ct*CENP-I^{NT} proteins. GST pull-down assays were performed. The bead-bound proteins were resolved with SDS-PAGE and stained with Coomassie Blue. Quantification was available in **Figure 4B**.

B. Cell lysates of HeLa Tet-On cells treated with Luciferase, *hs*CENP-H or *hs*CENP-I siRNA were resolved with SDS-PAGE and blotted with the indicated antibodies.

C. Cell lysates of HeLa Tet-On cells in **Figure 5D** were resolved with SDS-PAGE and blotted with the indicated antibodies.

Data collection, phasing and refinement statistics

	*ct*CENP-I^{NT}(Se-Met)	*ct*CENP-I^{NT} complex with *th*CENP-H^{CT}/ K^{CT}
Wavelength	0.9778	0.986
Resolution range (Å)	46.197 - 2.298(2.38-2.30)*	46.46 - 2.199 (2.24-2.20)*
Space group	P 41212	P 41
Unit cell (*a*, *b*, *c* (Å))	59.671 59.671 145.971 90 90 90	67.847 67.847 186.269 90 90 90
Unique reflections	22388(2244)	42600 (4220)
Multiplicity	4.5 (4.5)	9.4 (7.6)
Completeness (%)	99.7 (99.9)	100 (99.5)
Mean I/sigma(I)	21.42(3.25)	29.64(1.44)
Wilson B-factor	47.39	46.37
R-meas†	0.070(0.588)	0.102 (1.562)
R-pim‡	0.033 (0.274)	0.033 (0.559)
$CC_{1/2}$§	0.866	0.694
Phase determination		
Anomalous scatterer	Selenium (1 of 1 possible sites)	
Figure of merit, 46.20–2.30 Å	0.246	
Refinement statistics		
Reflections used in refinement	12379(1202)	42479 (2611)
Reflections used for R-free	1238 (134)	2143 (228)
R-work	0.1739 (0.2132)	0.1877 (0.3134)
R-free	0.2277 (0.2867)	0.2298 (0.3549)
Number of non-hydrogen atoms	1790	4699
macromolecules	1700	4579
solvent	90	120
Protein residues	213	544
RMS(bonds)	0.007	0.008
RMS(angles)	0.961	0.901
Ramachandran favored (%)#	98.09	97.14
Ramachandran allowed (%)#	1.91	2.5
Ramachandran outliers (%)#	0	0.36
Rotamer outliers (%)	3.24	0.82
Clashscore	4.03	8.82
Average B-factor	54.73	69.44
macromolecules	54.44	69.79
solvent	60.28	56.11

*Data for the highest-resolution shell are shown in parentheses

†R-meas = $\Sigma_h(n/n\text{-}1)^{1/2}\,\Sigma_i\,|I_i(h) - \langle I(h)\rangle|/\Sigma_h\Sigma_i\,I_i(h)$, where $I_i(h)$ and $\langle I(h)\rangle$ are the ith and mean measurement of the intensity of reflection h.

‡R-pim = $100\,\Sigma_h\Sigma_i\,[1/(n_h\text{-}1)]^{1/2}|I_{h,i} - \langle I_h\rangle/\Sigma_h\Sigma_i\langle I_{h,i}\rangle$, where n_h is the number of observations of reflections h.

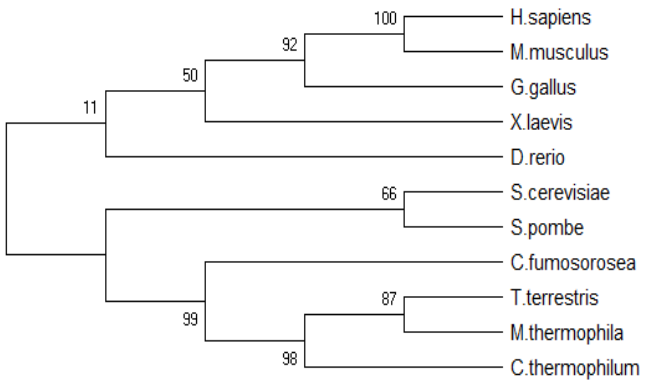
§$CC_{1/2}$ values shown are for the highest resolution shell.

#As defined by the validation suite MolProbity.

Table S1

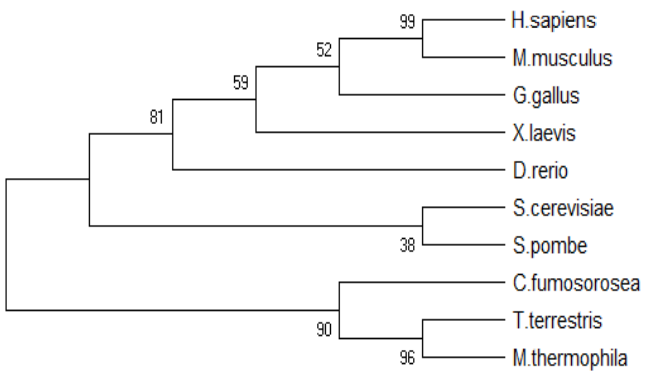
A

Maximum-Likelihood Phylogenetic Tree of CENP-I



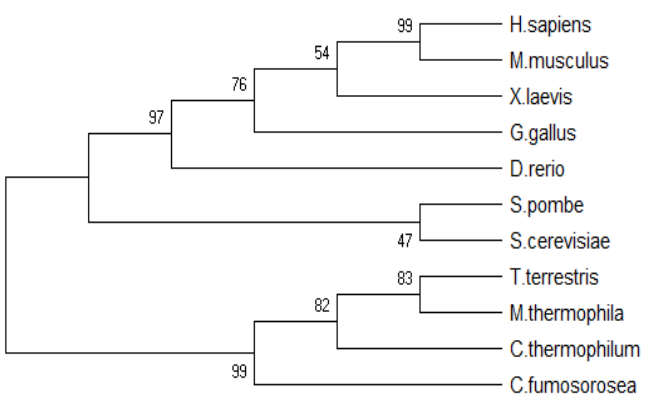
B

Maximum-Likelihood Phylogenetic Tree of CENP-H



C

Maximum-Likelihood Phylogenetic Tree of CENP-K



D

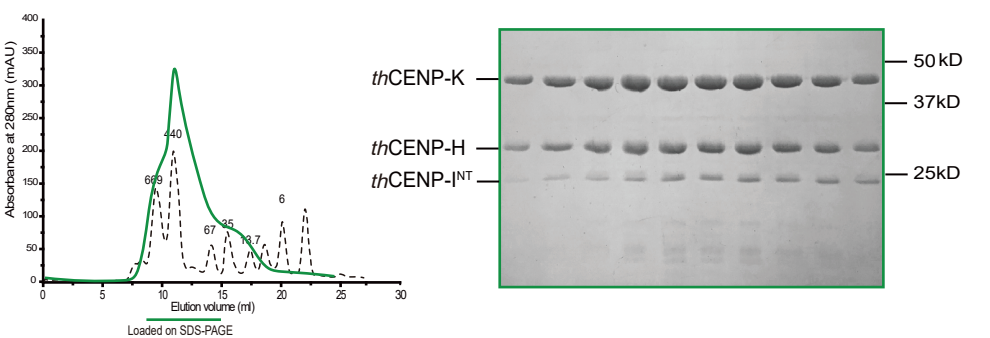
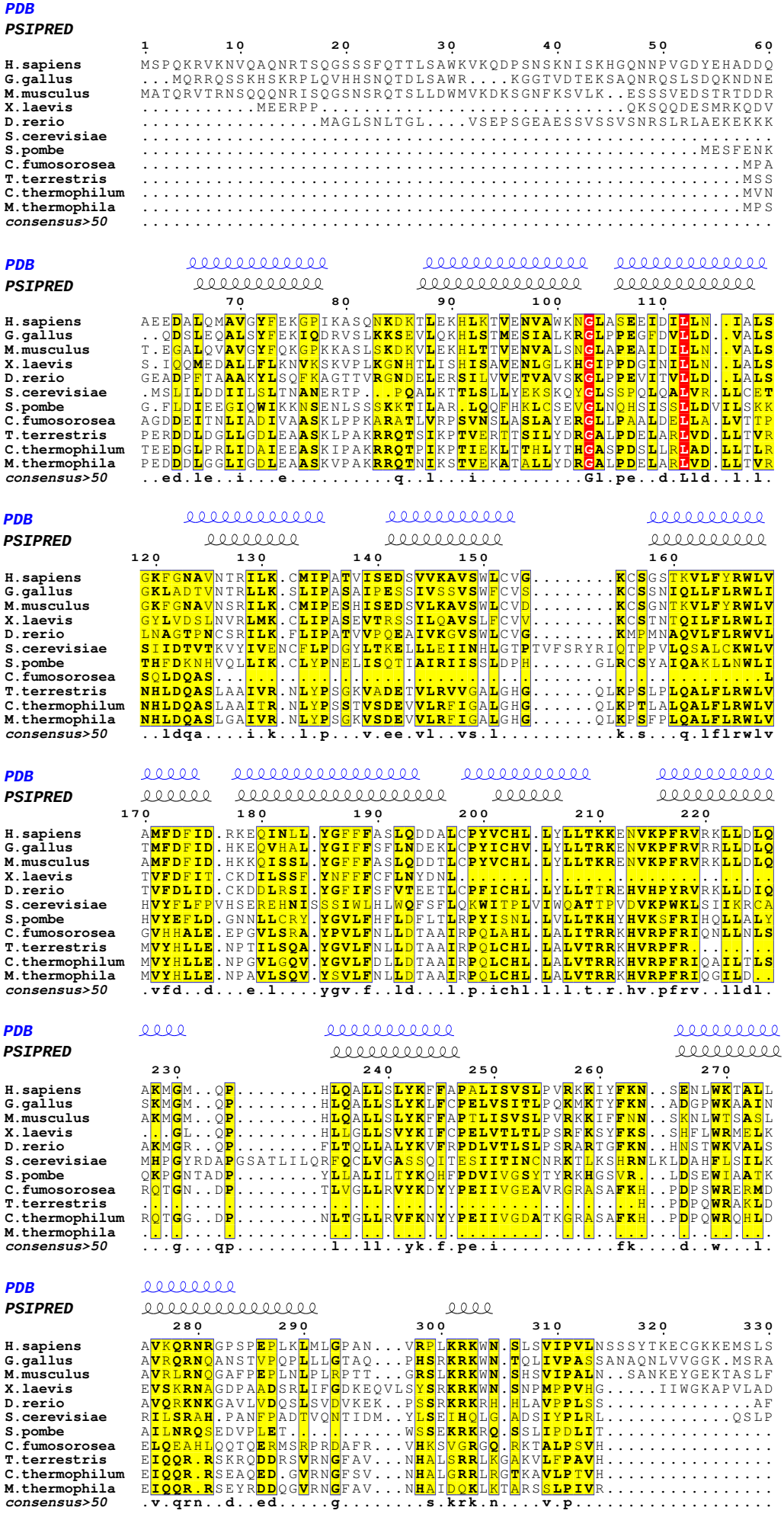
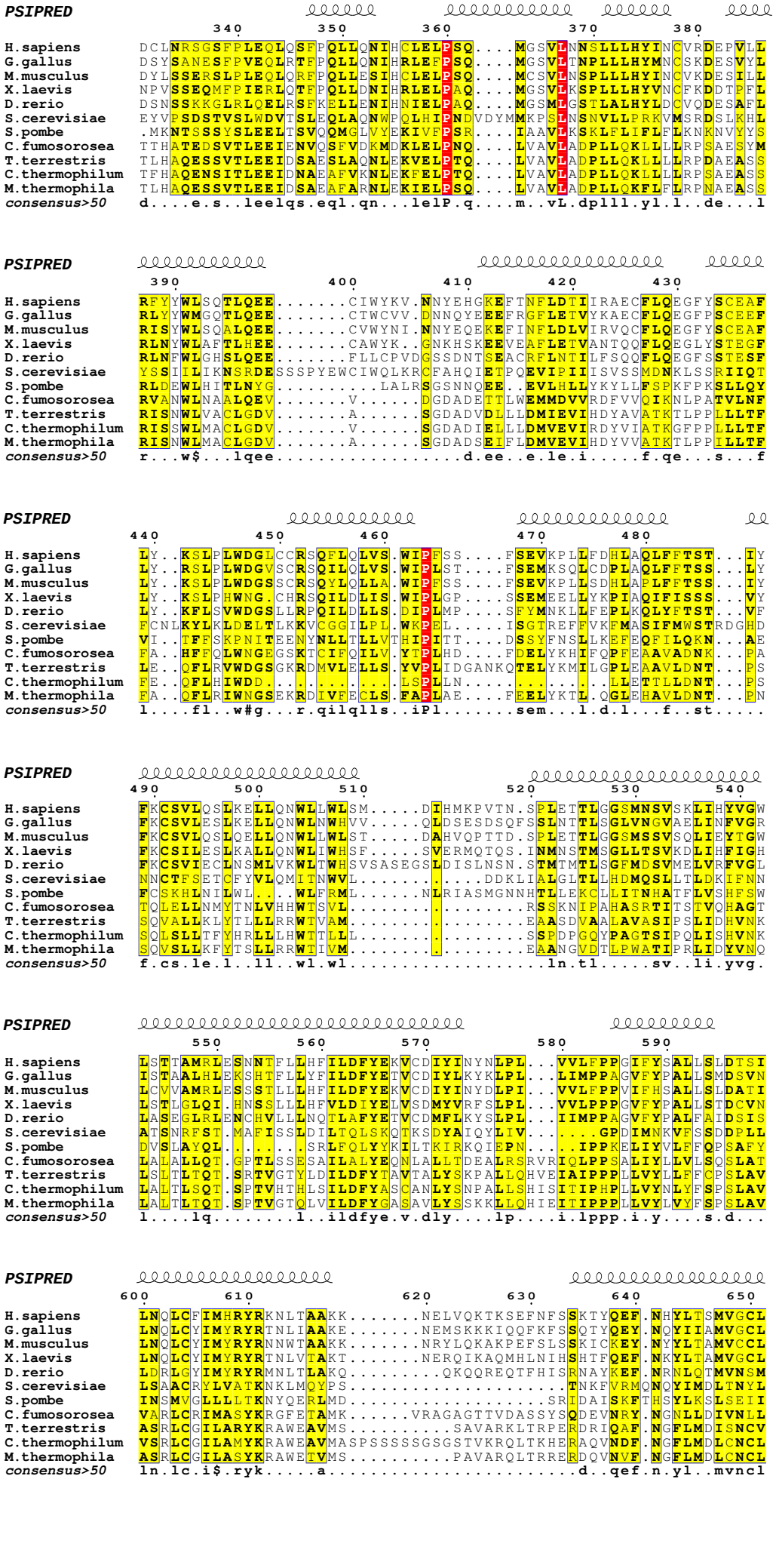


Figure S1

CENP-I Alignment




PSIPRED
340 350 360 370 380
H.sapiens DCLNRSGSFPLEQLQSFPQLLQNIHCLELPSQ....MGSVHNNSLLLHYINCVRDEPVLL
G.gallus DSYSANESFPVEQLRTFPQLLQNIHRLEFPSQ....MGSVLTNPLLLHYMNCSKDESVYL
M.musculus DYLSSERSLPLEQLQLQRFPQLLESIHCLELPSQ....MCSVLNSPLLLHYINCVKDESILL
X.laevis NPVSSEQMFPIERLQTFPQLLDNIHRLELPAQ....MGSVLKSPLLLHYVNCFKDDTPFL
D.rerio DSNSSKKGLRLQELRSFKELLENIHNIELPAQ....MGSMLGSTLALHYLDCVQDESAFL
S.cerevisiae EYVPSDSTVSLWDVTSLEQLAQNWPQLHIPNDVDYMMKPSLNSNVLLPRKVMSRDSLKHL
S.pombe .MKNTSSSYSLEELTSVQQMGLVYEKIVFPSR....IAAVLKSKLFLIFLFLKNKNVYYS
C.fumosorosea TTHATEDSVTLEEIENVQSFVDKMDKLELPNQ....LVAVLADPLLQKLLLLRPSAESYM
T.terrestris TLHAQESSVTLEEIDSAESLAQNLEKVELPTQ....LVAVLADPLLQKLLLLRPDAEASS
C.thermophilum TFHAQENSITLEEIDNAEAFVKNLEKFELPTQ....LVAVLADPLLQKLLLLRPSAEASS
M.thermophila TLHAQESSVTLEEIDSAEAFARNLEKIELPSQ....LVAVLADPLLQKFLFLRPNAEASS
consensus>50 d....e.s..leelqs.eql.qn...lelP.q....m..vL.dplll.yl.l..de...l
PSIPRED
390 400 410 420 430
H.sapiens RFYYWLSQTLQEE.......CIWYKV.NNYEHGKEFTNFLDTIIRAECFLQEGFYSCEAF
G.gallus RLYYWMGQTLQEE.......CTWCVV.DNNQYEEEFRGFLETVYKAECFLQEGFPSCEEF
M.musculus RISYWLSQALQEE.......CVWYNI.NNYEQEKEFINFLDLVIRVQCFLQEGFYSCEAF
X.laevis RLNYWLAFTLHEE.......CAWYK..GNKHSKEEVEAFLETVANTQQFLQEGLYSTEGF
D.rerio RLNFWLGHSLQEE.......FLLCPVDGSSDNTSEACRFLNTILFSQQFLQEGFSSTESF
S.cerevisiae YSSIILIKNSRDESSSPYEWCIWQLKRCFAHQIETPQEVIPIISVSSMDNKLSSRIIQT
S.pombe RLDEWLHITLNYG..........LALRSGSNNQEE..EVLHLLYKYLLFSPKFPKSLLQY
C.fumosorosea RVANWLNAALQEV.......V......DGDADETTLWEMMDVVRDFVVQIKNLPATVLNF
T.terrestris RISNWLVACLGDV.......A......SGDADVDLLLLDMIEVIHDYAVATKTLPPLLLTF
C.thermophilum RISSWLMACLGDV.......V......SGDADIELLLLDMVEVIRDYVIATKGFPPLLLTF
M.thermophila RISNWLMACLGDV.......A......SGDADSEIFLDMVEVIHDYVVATKTLPPILLTF
consensus>50 r...w$...lqee..................d.ee..e.le.i.....f.qe...s...f
PSIPRED
440 450 460 470 480
H.sapiens LY..KSLPLWDGLCCRSQFLQLVS.WIPFSS....FSEVKPLLFDHLAQLFFTST...IY
G.gallus LY..RSLPLWDGVSCRSQILQLVS.WIPLST....FSEMKSQLCDPLAQLFFTSS...LY
M.musculus LY..KSLPLWDGSSCRSQYLQLLA.WIPFSS....FSEVKPLLSDHLAPLFFTSS...IY
X.laevis LY..KSLPHWNG.CHRSQILDLIS.WIPLGP....SSEMEELLYKPIAQIFISSS...VY
D.rerio LY..KFLSVWDGSLLRPQILDLLS.DIPLMP....SFYMNKLLFEPLKQLYFTST...VF
S.cerevisiae FCNLKYLKLDELTLKKVCGGILPL.WKPEL.....ISGTREFFVKFMASIFMWSTRDGHD
S.pombe VI..TFFSKPNITEENYNLLTLLVTHIPITT....DSSYFNSLLKEFEQFILQKN...AE
C.fumosorosea FA..HFFQLWNGEGSKTCIFQILV.YTPLHD....FDELYKHIFQPFEAAVADNK...PA
T.terrestris LE..QFLRVWDGSGKRDMVLELLS.YVPLIDGANKQTELYKMILGPLEAAVLDNT...PS
C.thermophilum FE..QFLHIWDD.....................LSPLLN.....LLETTLLDNT...PS
M.thermophila FA..QFLRIWNGSEKRDIVFECLS.FAPLAE....FEELYKTL.QGLEHAVLDNT...PN
consensus>50 l....fl..w#g...r.qilqlls..iPl.......sem...l.d.l...f..st.....
PSIPRED
490 500 510 520 530 540
H.sapiens FKCSVLQSLKELLQNWLLWLSM.....DIHMKPVTN.SPLETTLGGSMNSVSKLIHYVGW
G.gallus FKCSVLESLKELLQNWLNWHVV.....QLDSESDSQFSSLNTTLSGLVNGVAELINFVGR
M.musculus FKCSVLQSLQELLQNWLLWLST.....DAHVQPTTD.SPLETTLGGSMSSVSQLIEYTGW
X.laevis FKCSILESLKALLQNWLIWHSF.....SVERMQTQS.INMNSTMSGLLTSVKDLIHFIGH
D.rerio FKCSVIECLNSMLVKWLTWHSVSASEGSLDISLNSN.STMTMTLSGFMDSVMELVRFVGL
S.cerevisiae NNCTFSETCFYVLQMITNWVL.....DDKLIALGLTLLHDMQSLLTLDKIFNN
S.pombe FCSKHLNILWL....WLFRML.....NLRIASMGNNHTLLEKCLLITNHATFLVSHFSW
C.fumosorosea TQLELLNMYTNLVHHWTSVL.....RSSKNIPAHASRTITSTVQHAGT
T.terrestris SQVALLKLYTLLLRRWTVAM.....EAASDVAALAVASIPSLIDHVNK
C.thermophilum SQLSLLTFYHRLLLHWTTLL.....SSPDPGQYPAGTSIPQLISHVNK
M.thermophila SQVSLLKFYTSLLRRWTIVM.....EAANGVDTLPWATIPRLIDYVNQ
consensus>50 f.cs.le.l..ll..wl.wl.....ln.tl.....sv..li.yvg.
PSIPRED
550 560 570 580 590
H.sapiens LSTTAMRLESNNTFLLHFILDFYEKVCDIYINYNLPL...VVLFPPGIFYSALLSLDTSI
G.gallus ISTAALHLEKSHTFLLYFILDFYETVCDIYLKYKLPL...LIMPPAGVFYPALLSMDSVN
M.musculus LCVVAMRLESSSTLLLHFILDFYEKVCDIYINYDLPI...VVLFPPVIFHSALLSLDATI
X.laevis LSTLGLQI.HNSSLLLHFVLDIYELVSDMYVRFSLPL...VVLPPPGVFYPALLSTDCVN
D.rerio LASEGLRLENCHVLLLNQTLAFYETVCDMFLKYSLPL...IIMPPAGVFYPALFAIDSIS
S.cerevisiae ATSNRFST.MAFISSLDILTQLSKQTKSDYAIQYLIV.....GPDIMNKVFSSDDPLL
S.pombe DVSLAYQL.........SRLFQLYYKILTKIRKQIEPN.....IPPKELIYVLFFQPSAFY
C.fumosorosea LALALLQT.GPTLSSESAILALYEQNLALLTDEALRSRVRIQLPPSALIYLLVLSQSLAT
T.terrestris LSLTLTQT.SRTVGTYLDILDFYTAVTALYSKPALLQHVEIAIPPPLLVYLLFFCPSLAV
C.thermophilum LALTLSQT.SPTVHTHLSILDFYASCANLYSNPALLSHISITIPHPLLVYNLYFSPSLAV
M.thermophila LALTLTQT.SPTVGTQLVILDFYGASAVLYSSKKLLQHIEITIPPPLLVYLVYFSPSLAV
consensus>50 l....lq........l..ildfye.v.dly....lp....i.lppp.i.y....s.d...
PSIPRED
600 610 620 630 640 650
H.sapiens LNQLCFIMHRYRKNLTAAKK.....NELVQKTKSEFNFSSKTYQEF.NHYLTSMVGCL
G.gallus LNQLCYIMYRYRTNLIAAKE.....NEMSKKKIQQFKFSSQTYQEY.NQYIIAMVGCL
M.musculus LNQLCYIMYRYRNNWTAAKK.....NRYLQKAKPEFSLSSKICKEY.NYYLTAMVCCL
X.laevis LNQLCYIMYRYRTNLVTAKT.....NERQIKAQMHLNIHSHTFQEF.NKYLTAMVGCL
D.rerio LDRLGYIMYRYRMNLTLAKQ.....QKQQREQTFHISRNAYKEF.NRNLQTMVNSM
S.cerevisiae LSAACRYLVATKNKLMQYPS.....TNKFVRMQNQYIMDLTNYL
S.pombe INSMVGLLLLTKNYQERLMD.....SRIDAISKFTHSYLKSLSEII
C.fumosorosea VARLCRIMASYKRGFETAMK.....VRAGAGTTVDASSYSQDEVNRY.NGNLLDIVNLL
T.terrestris ASRLCGILARYKRAWEAVMS.....SAVARKLTRPERDRIQAF.NGFLMDISNCV
C.thermophilum VSRLCGILAMYKRAWEAVMASPSSSSSGSGSTVKRQLTKHERAQVNDF.NGFLMDLCNCL
M.thermophila ASRLCGILASYKRAWETVMS.....PAVARQLTRRERDQVNVF.NGFLMDLCNCL
consensus>50 ln.lc.i$.ryk.....a.....d..qef.n.yl..mvncl

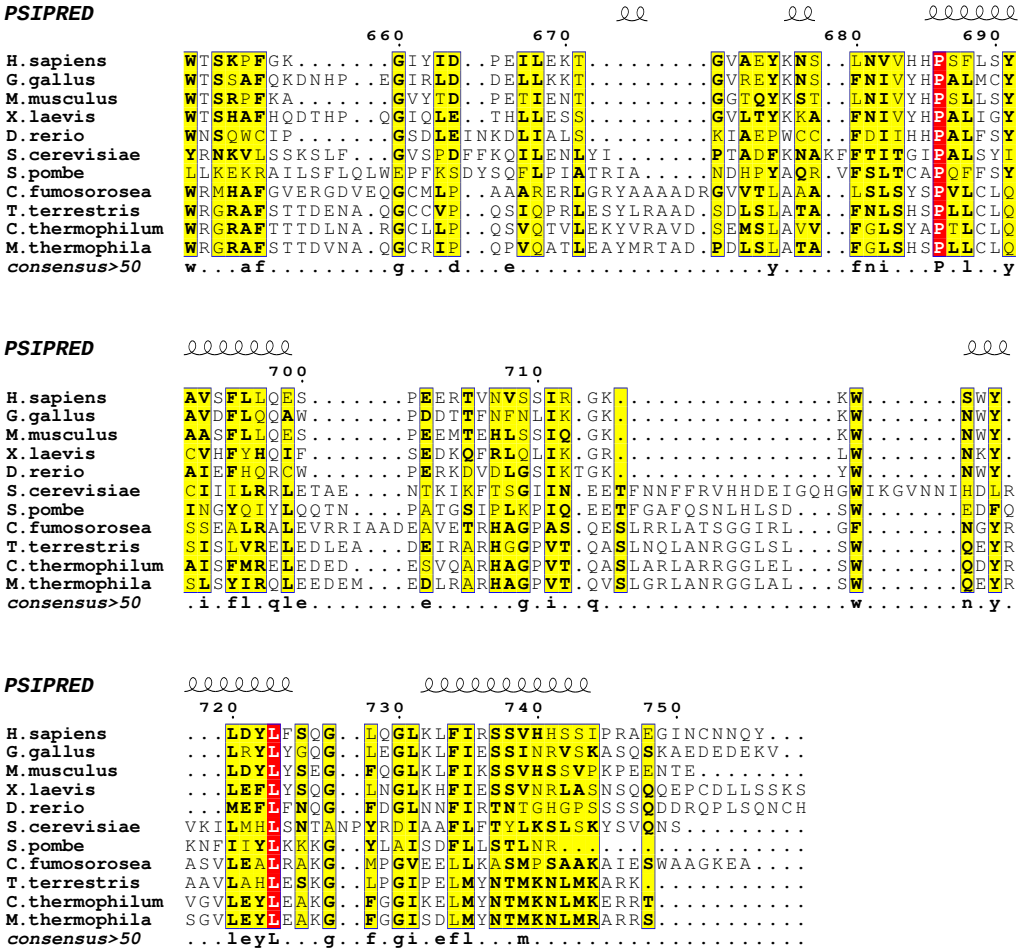

PSIPRED
660
670
680
690
H.sapiens
G.gallus
M.musculus
X.laevis
D.rerio
S.cerevisiae
S.pombe
C.fumosorosea
T.terrestris
C.thermophilum
M.thermophila
consensus>50
PSIPRED
700
710
H.sapiens
G.gallus
M.musculus
X.laevis
D.rerio
S.cerevisiae
S.pombe
C.fumosorosea
T.terrestris
C.thermophilum
M.thermophila
consensus>50
PSIPRED
720
730
740
750
H.sapiens
G.gallus
M.musculus
X.laevis
D.rerio
S.cerevisiae
S.pombe
C.fumosorosea
T.terrestris
C.thermophilum
M.thermophila
consensus>50

Figure S2

CENP-K Alignment

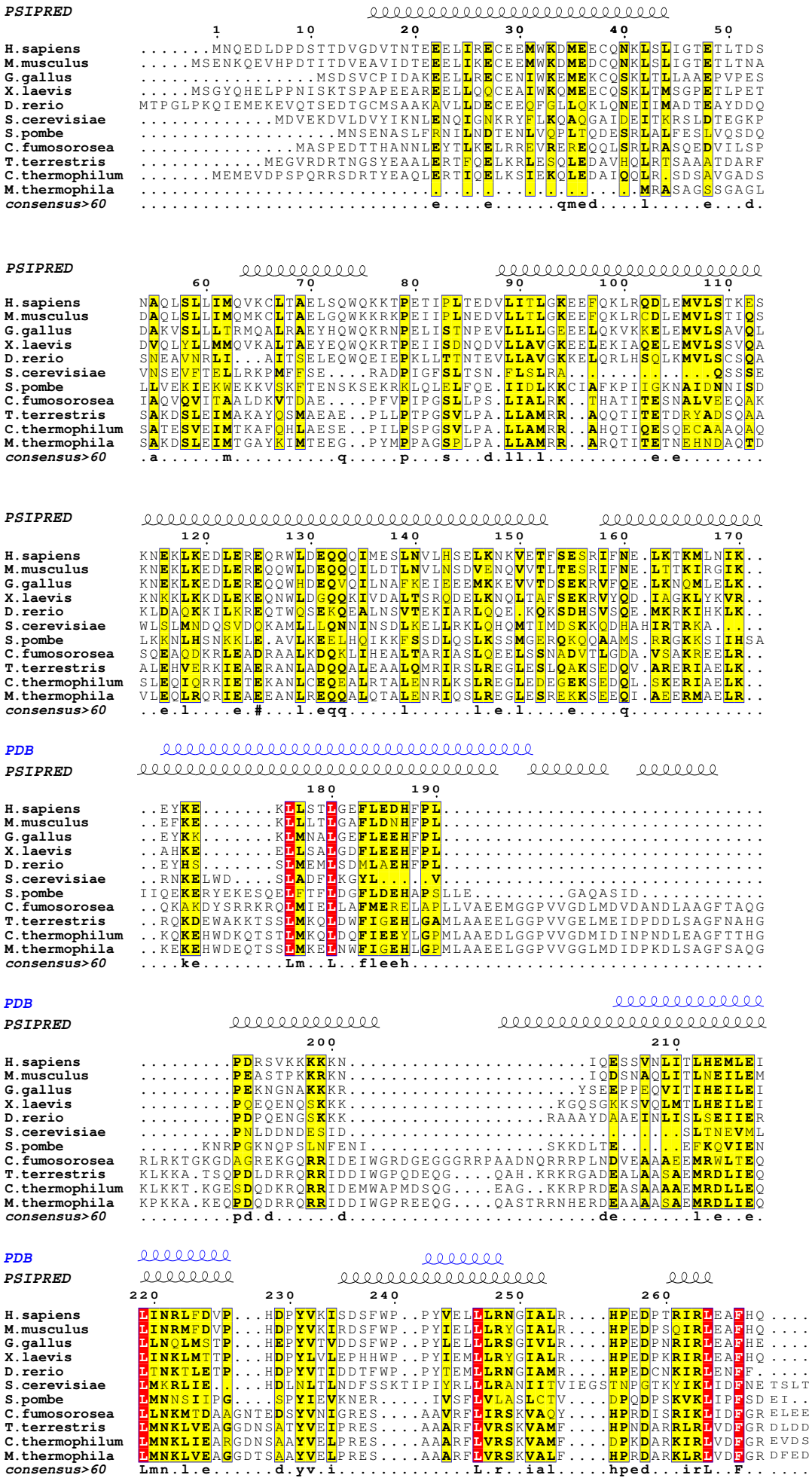


Figure S3

CENP-H Alignment

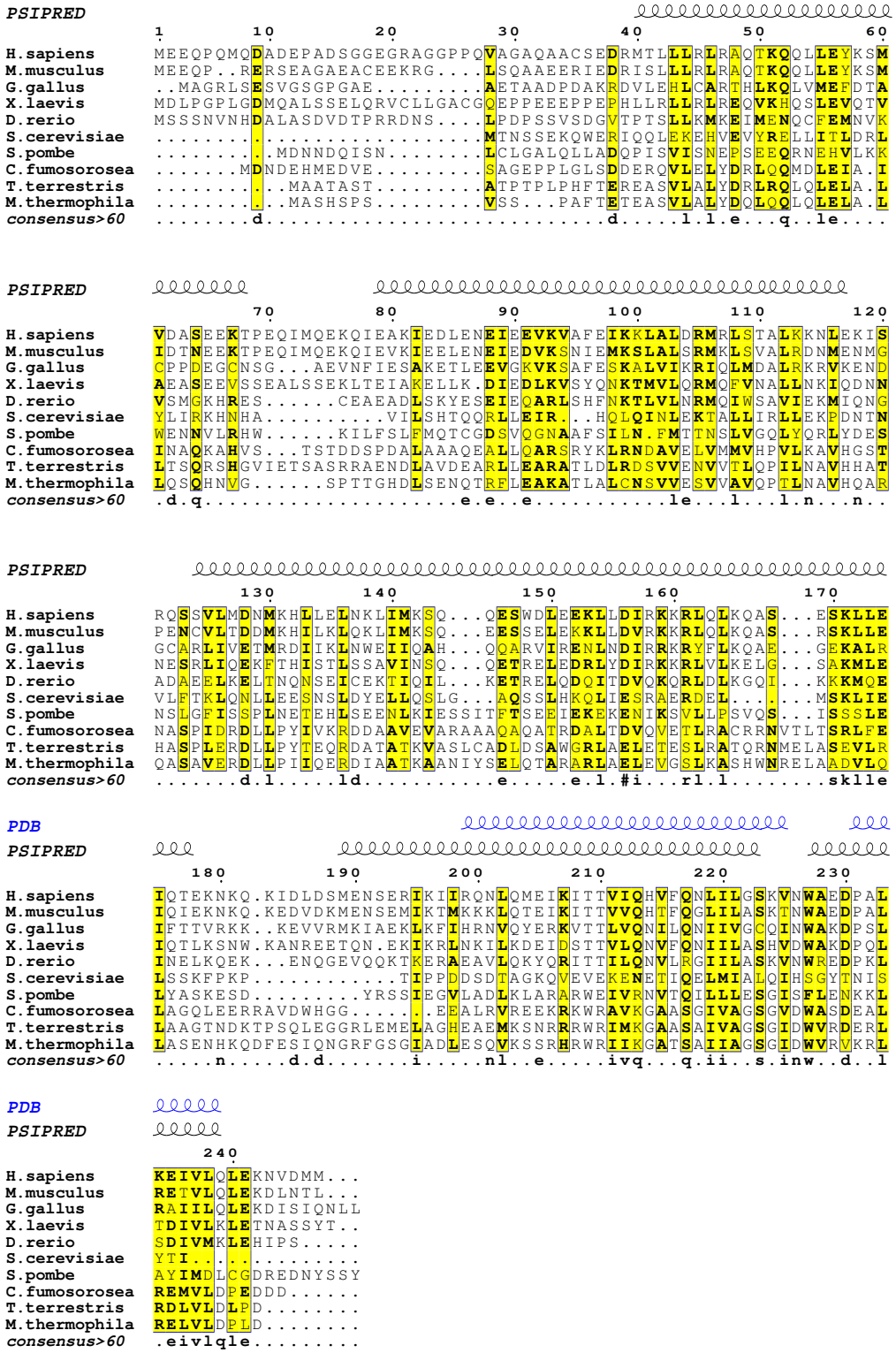


Figure S4

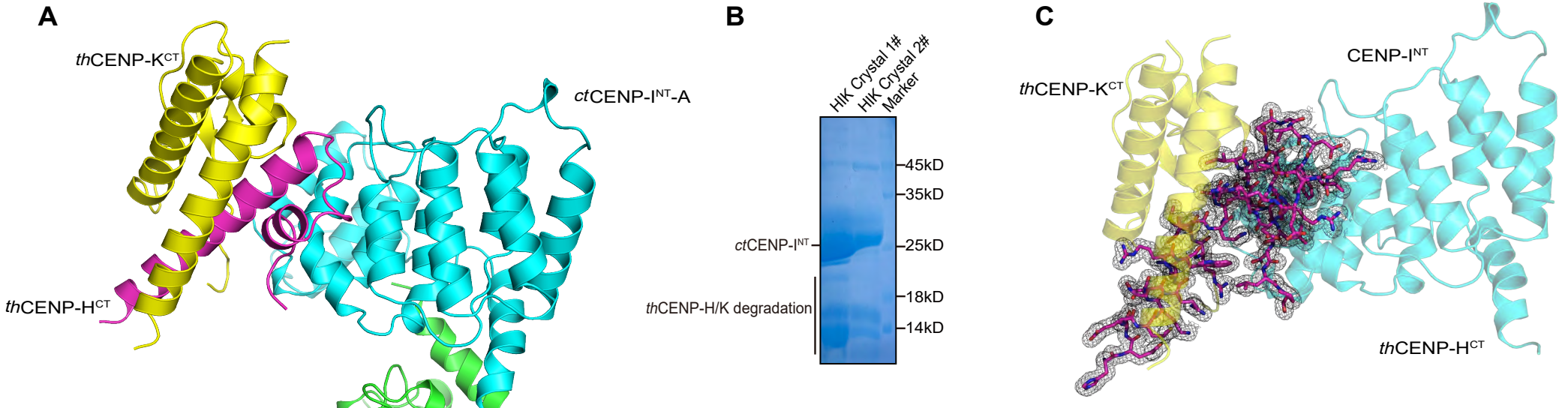

A
thCENP-KCT
ctCENP-INT-A
thCENP-HCT
ctCENP-INT-B
B
HIK Crystal 1#
HIK Crystal 2#
Marker
45kD
35kD
25kD
18kD
14kD
ctCENP-INT
thCENP-H/K degradation
C
thCENP-KCT
CENP-INT
thCENP-HCT

Figure S5

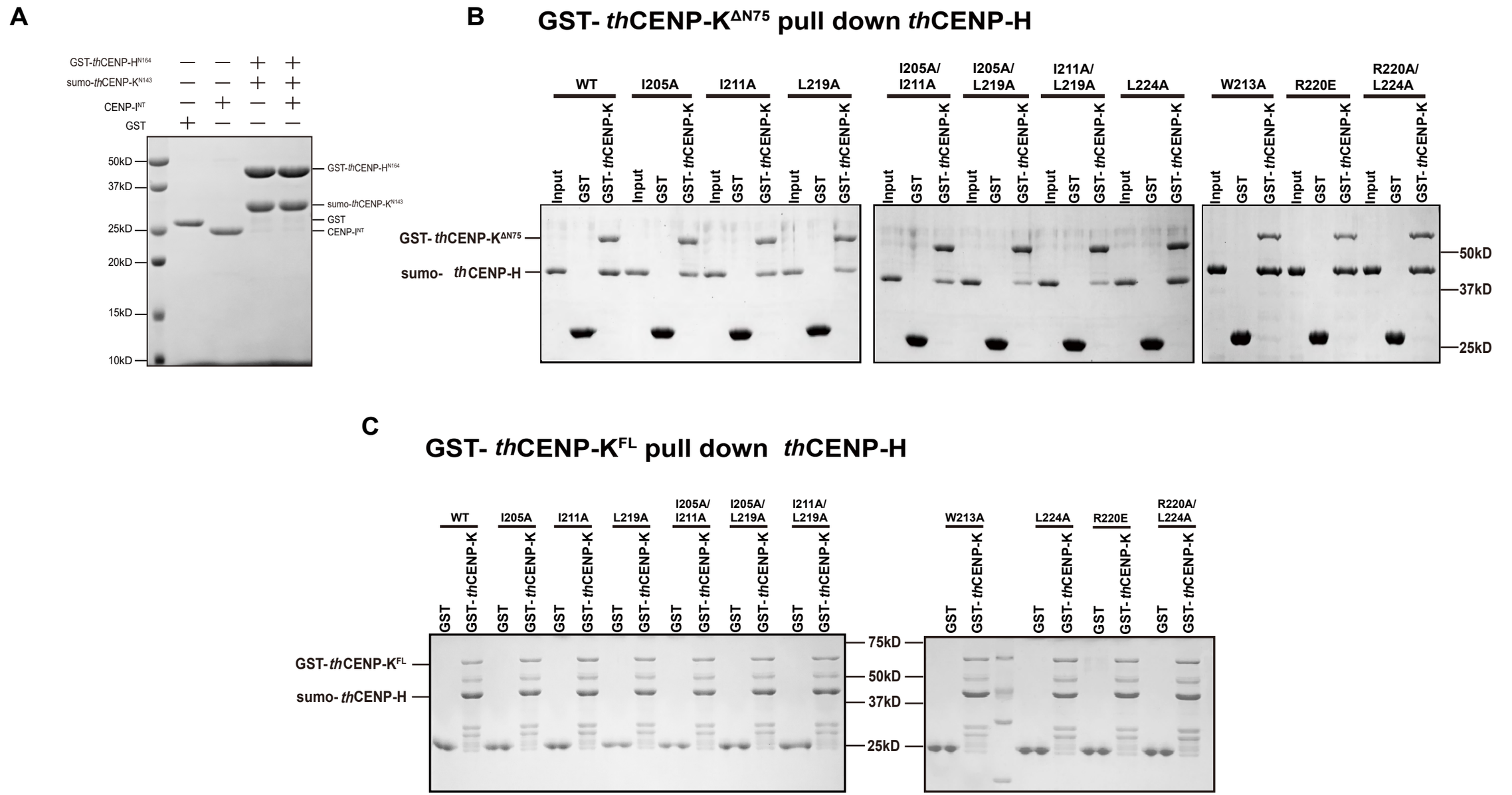

A
GST-thCENP-H^N164
sumo-thCENP-K^N143
CENP-I^NT
GST
50kD
37kD
25kD
20kD
15kD
10kD
GST-thCENP-H^N164
sumo-thCENP-K^N143
GST
CENP-I^NT
B
GST- thCENP-K^ΔN75 pull down thCENP-H
WT
I205A
I211A
L219A
I205A/ I211A
I205A/ L219A
I211A/ L219A
L224A
W213A
R220E
R220A/ L224A
Input
GST
GST- thCENP-K
GST- thCENP-K^ΔN75
sumo- thCENP-H
50kD
37kD
25kD
C
GST- thCENP-K^FL pull down thCENP-H
GST- thCENP-K^FL
sumo- thCENP-H
75kD
50kD
37kD
25kD

Figure S6

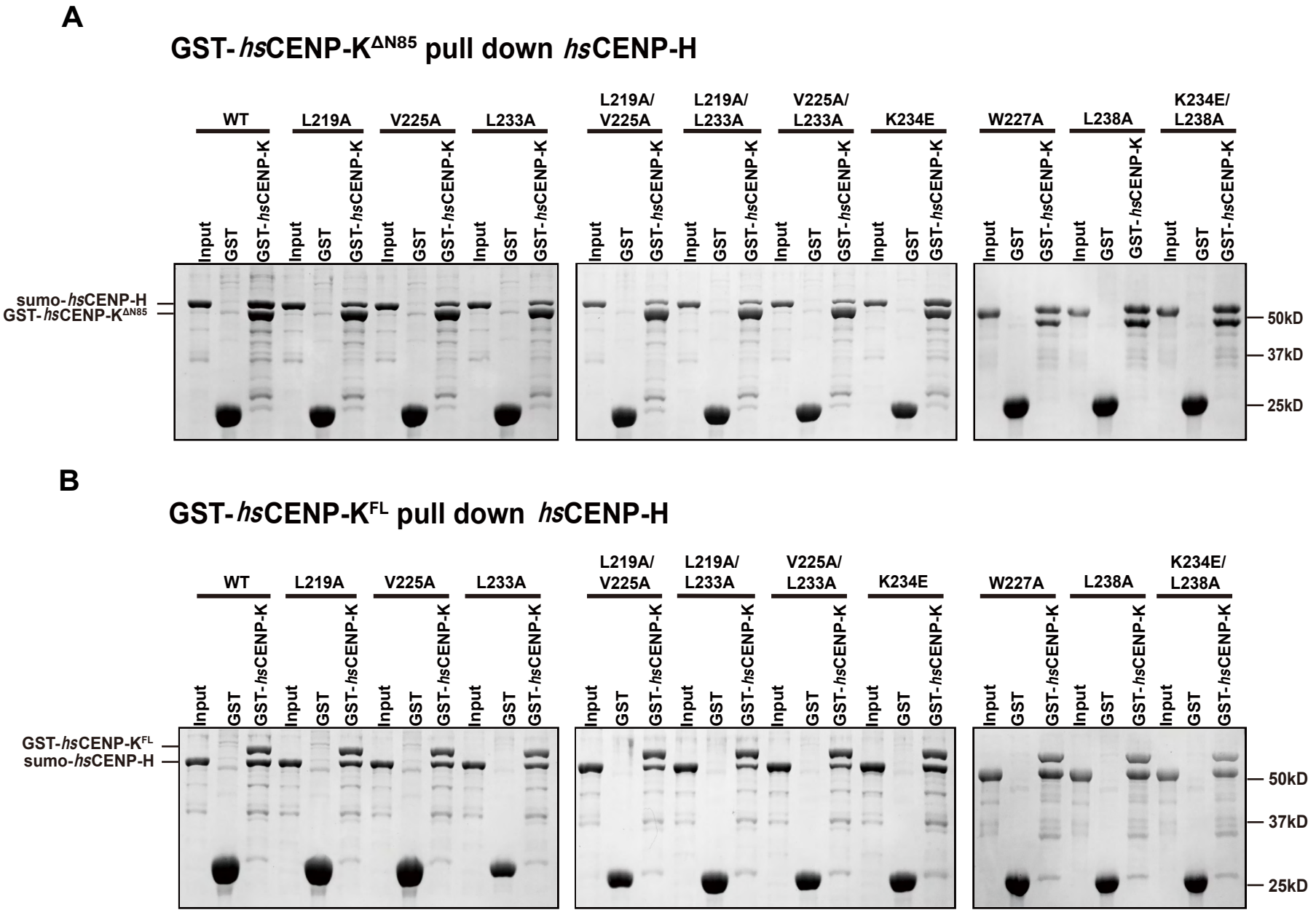

A
GST-hsCENP-K^ΔN85 pull down hsCENP-H
WT
L219A
V225A
L233A
L219A/ V225A
L219A/ L233A
V225A/ L233A
K234E
W227A
L238A
K234E/ L238A
Input
GST
GST-hsCENP-K
sumo-hsCENP-H
GST-hsCENP-K^ΔN85
50kD
37kD
25kD
B
GST-hsCENP-K^FL pull down hsCENP-H
GST-hsCENP-K^FL
sumo-hsCENP-H
50kD
37kD
25kD

Figure S7

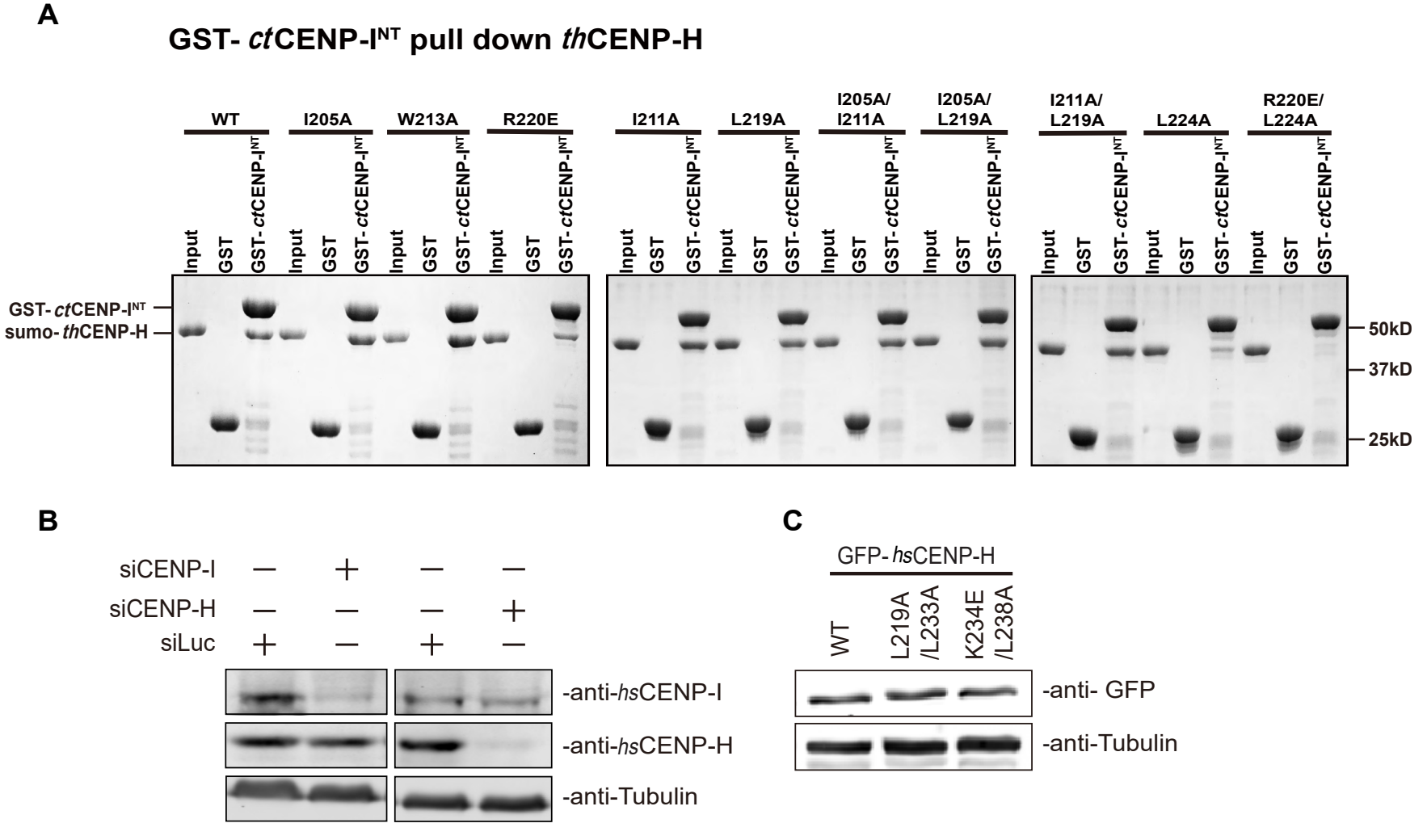

A
GST- ctCENP-I^NT pull down thCENP-H
WT
I205A
W213A
R220E
I211A
L219A
I205A/ I211A
I205A/ L219A
I211A/ L219A
L224A
R220E/ L224A
Input
GST
GST- ctCENP-I^NT
GST- ctCENP-I^NT
sumo- thCENP-H
50kD
37kD
25kD
B
siCENP-I
siCENP-H
siLuc
-anti-hsCENP-I
-anti-hsCENP-H
-anti-Tubulin
C
GFP- hsCENP-H
WT
L219A /L233A
K234E /L238A
-anti- GFP
-anti-Tubulin

Figure S8