Submission

Insights from biochemical reconstitution into the architecture and epigenetic specification of human kinetochores

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Chromosomes are carriers of the genetic material and their accurate transfer from a mother cell to its two daughters during cell division is of paramount importance for life. Kinetochores are crucial for this process, as they connect chromosomes with microtubules in the mitotic spindle¹. Kinetochores are multi-subunit complexes that assemble on specialized chromatin domains, the centromeres, whose unique feature is the enrichment of nucleosomes containing the histone H3 variant centromeric protein A (CENP-A)². A group of several additional CENPs, collectively known as constitutive centromere associated network (CCAN)³⁻⁶, establish the inner kinetochore, whereas a 10-subunit assembly known as the KMN network creates a microtubule-binding site in the outer kinetochore^{7,8}. Interactions between CENP-A and two CCAN subunits, CENP-C and CENP-N, have been previously described⁹⁻¹¹, but a comprehensive understanding of CCAN organization, of how it contributes to selective recognition of CENP-A, and of how it may contribute to propagate centromere identity through subsequent cell generations has been missing. Here, we use biochemical reconstitution to unveil fundamental principles of kinetochore organization and function. We show that cooperative interactions of a 7-subunit CCAN sub-complex, the CHIKLMN complex, determine binding selectivity for CENP-A over H3-nucleosomes. The CENP-A:CHIKLMN complex binds directly to the KMN network, resulting in a 21-subunit complex that forms a minimal high-affinity linkage between CENP-A nucleosomes and microtubules in vitro. This structural module is related to fungal point kinetochores, which bind a single microtubule. Its convolution with multiple CENP-A may give rise to the regional kinetochores of higher eukaryotes, which bind multiple microtubules. We also note that the specific arrangement of CCAN subunits around CENP-A suggests a model for the propagation of centromere identity through cell division.

The kinetochore is one of the largest and functionally intricate molecular machines of eukaryotic cells^{12,13}. It performs two related and essential functions. First, it binds to spindle microtubules to promote the bi-orientation of the sister chromatids in mitosis and meiosis II, and of the homologs in meiosis I. Second, it controls the spindle assembly checkpoint (SAC), a cell cycle checkpoint that prevents chromosome

segregation prior to completion of bi-orientation, thus ensuring genome stability during cell division^{1,14}.

The centromere is the genetic locus, unique to each chromosome, upon which the kinetochore is established^{2,15} (Figure 1a). In most eukaryotes, maintenance of centromere identity does not require specific DNA sequences, but rather relies on epigenetic mechanisms. Crucial for this process is the deposition of new CENP-A at mitotic exit, which repopulates the CENP-A pool after its equal partition to the sister chromatids during DNA replication. Elements of the machinery involved in this reaction have been identified, but a general molecular understanding of this process is missing^{2,15}.

Previous genetic and proteomic analyses of human mitotic kinetochores identified up to 100 or more proteins, only a fraction of which are core structural components, while others play accessory regulatory roles. Because CENP-A is considered crucial for the epigenetic specification of centromeres, understanding its recognition by other kinetochore proteins is important. Previous studies established that CENP-C and CENP-N recognise the divergent C-terminal tail and the CATD region of CENP-A, respectively^{10,11} (Extended data Figure 1). Whether CENP-C and CENP-N act in a complex on single or distinct CENP-A nucleosomes, and whether other CCAN subunits contribute to their interactions with CENP-A is not known. To shed light on this problem and on additional functional and structural aspects of kinetochore function, we embarked in a synthetic biology approach aiming to reconstitute human kinetochore function *in vitro* with purified components (Figure 1b, Extended data Figure 1). Here, we report the main conclusions emerging from this effort.

Similarly to their *S. cerevisiae* homologs¹⁶, human CENP-N and CENP-L formed a stoichiometric complex (Extended data Figure 2a). After immobilization on solid phase, CENP-LN interacted preferentially with octameric CENP-A mono-nucleosomes (CENP-A^{MN}) in comparison to H3 mono-nucleosomes (H3^{MN}) (Figure 1c). In size-exclusion chromatography (SEC) experiments, which separate macromolecules based on their size and shape, a recombinant construct encompassing residues 1-544 of CENP-C (CENP-C¹⁻⁵⁴⁴, which embeds the first nucleosome-binding motif of CENP-C, Extended data Figure 1), co-eluted with CENP-A^{MN} but not with H3^{MN} [both reconstituted on a 145-bp "601" DNA (ref. 17)] (Figure 1d and Extended data Figure 2e-f). SEC also demonstrated that CENP-LN binds directly and strongly to CENP-C¹⁻⁵⁴⁴ (Figure 1e, *light blue trace*), and directly but more weakly also to the 4-subunit CCAN sub-complex

CENP-HIKM (ref. 18) (Figure 1e, green trace). Together with our previous demonstration that CENP-C¹⁻⁵⁴⁴ binds CENP-HIKM (ref. 19), these interactions suggest that CENP-LN, CENP-HIKM, and CENP-C¹⁻⁵⁴⁴ bind in a single complex. SEC readily confirmed this hypothesis (Figure 1e, *orange trace*). We refer to this 7-subunit complex as the "CHIKLMN" complex.

Chemical crosslinking coupled with mass spectrometry (XL-MS)²⁰ identified an extensive network of interactions of the CHIKLMN subunits with themselves and with CENP-A^{MN}. CENP-C, which may be intrinsically disordered, emerged from this analysis as the backbone of CHIKLMN, as it contacts all other subunits, with the exclusion of histone H2A (Figure 2b, Extended Data Table I). Although the CENP-HIKM complex did not bind CENP-A^{MN} with affinity sufficient for co-elution in SEC experiments (not shown), we found several crosslinks between them, suggesting close proximity within the CENP-A^{MN}:CHIKLMN complex.

We asked if the network of interactions linking the CHIKLMN subunits and the CENP-A nucleosome correlated with localization co-dependencies in HeLa cells. Individual depletions of CENP-C, CENP-H, CENP-L, or CENP-M by RNA interference (RNAi) led to a near complete disappearance of the other CHIKLMN components from kinetochores during interphase. Significant levels of CENP-A were present on kinetochores at the time of fixation for indirect immunofluorescence, suggesting that the loss of CHIKLMN components is not caused by complete co-depletion of CENP-A, but rather by the co-dependency of CHIKLMN subunits for stable kinetochore recruitment (Figure 2c, Extended Data Figure 3a-b). Collectively, our observations indicate that the CHIKLMN complex forms a cooperative system whose stability depends on reciprocal interactions of its subunits.

The KMN network is made of three sub-complexes, the Knl1 complex (Knl1-C), the Mis12 complex (Mis12-C), and the Ndc80 complex (Ndc80-C). It forms the outer kinetochore and binds microtubules^{7,8} (Figure 1a). After characterizing CHIKLMN as the CENP-A^{MN} associated complex, we asked if it was also competent to recruit outer kinetochore components. The CENP-A^{MN}:CHIKLMN complex readily bound to a reconstituted 10-subunit KMN network complex and all components co-eluted from a SEC column, forming a 17-subunit kinetochore complex bound to an octameric CENP-A^{MN} (Figure 3a, *black trace*; Figure 3b). Probing the topology of this complex by XL-MS revealed an extensive network of interactions around a hub represented by the Mis12

complex (Extended Data Figure 4). The latter formed cross-links to several outer kinetochore subunits, as well as to inner kinetochore subunits, including CENP-C, CENP-K, and CENP-N.

We asked if reconstituted kinetochore particles could translocate centromeric chromatin onto microtubules. After immobilizing microtubules on a coverslip, we tested the ability of fluorescently labelled kinetochore components to interact with them (Figure 3c, Extended data Figure 5a). The KMN network bound microtubules in the absence of other components, as expected based on its well-established role as a microtubule receptor at the kinetochore^{7,8,21}. Neither CHIKLMN nor CENP-A^{MN}, together or in isolation, decorated microtubules in the absence of the KMN network (Figure 3c). Conversely, when KMN network was added together with the CHIKLMN complex and CENP-A^{MN}, the latter strongly decorated microtubules (Figure 3c), demonstrating that the KMN network and the CHIKLMN complex create a direct bridge between centromeres and microtubules. Only weak binding to microtubules was observed if CENP-A^{MN} were replaced with H3^{MN} (Extended data Figure 5b). Addition of CENP-A^{MN} strongly enhanced the intensity of the KMN on microtubules, suggesting either a stabilisation of binding, or more likely an effect of multi-valency arising from the 2-fold symmetry of the CENP-A^{MN} (Figure 3c). In this context, we note that the reconstitution of functional kinetochore particles on octameric CENP-A^{MN} may set a benchmark to resolve an on-going discussion on the actual structure of the CENP-A nucleosome²².

The point kinetochore of *Saccharomyces cerevisiae* consists of a single CENP-A (Cse4) nucleosome associated with proteins that are evolutionarily related to the CCAN subunits of humans¹³ (Figure 4a). Our finding that the human CCAN subunits and the KMN network form a single, apparently stoichiometric complex on a CENP-A^{MN} suggests that the human and *S. cerevisiae* kinetochores form a unit of similar architecture. At regional centromeres, which may extend over several megabases of genomic DNA, up to 200 CENP-A nucleosomes intersperse with conventional H3 nucleosomes at an approximate ratio of 1 CENP-A nucleosome over 25 H3 nucleosomes²³. We therefore propose that kinetochores built on regional centromeres represent the convolution with multiple CENP-A nucleosomes of the structural unit identified by our *in vitro* reconstitution (Figure 4a).

In metazoans, CENP-A deposition takes place early after cell division and before DNA replication²⁴. Doubling of CENP-A levels compensates for the subsequent halving of

CENP-A content on each sister chromatid during DNA replication, which proceeds without new CENP-A deposition. It is believed that the CENP-A vacancies created during DNA replication become occupied by "placeholder" H3.3 nucleosomes until new CENP-A deposition²⁵. The specific topology of the CENP-C protein and of its interactions suggests a molecular mechanism for this process. CENP-C⁵⁴⁵⁻⁹⁴³, like CENP-C¹⁻⁵⁴⁴, contains a nucleosome-binding element (Extended data Figure 1), reported to bind MNs with a moderate preference for CENP-A^{MN} over H3^{MN} [reference 11]. The Cterminal half of CENP-C also interacts directly with the Mis18 complex²⁶, which contributes to new CENP-A deposition by recruiting the CENP-A chaperone HJURP to kinetochores early after cell division²⁴. In vitro, we found that C-terminal segments of CENP-C interacts with CENP-A^{MN} but do not recapitulate any of the interactions with CHIKLMN subunits observed with the CENP-C N-terminal segment (Extended Data Figure 6a-b, summarized in Figure 4b). We speculate that the C-terminal region of CENP-C marks a second (H3.3) nucleosome, adjacent to the CENP-A^{MN}:CHIKLMN complex, for replacement with new CENP-A (Figure 4c). This di-nucleosome model, in which CENP-A^{MN}-CHIKLMN is viewed as a template for the transformation of a neighbouring H3 nucleosome, predicts that the number of newly deposited CENP-A molecules may match the number of CENP-A^{MN}:CHIKLMN complexes already existing within centromeric chromatin. Previous evidence, that CENP-HIKM recruits the FACT complex and the remodelling ATPase Chd1 to kinetochores for new CENP-A deposition, is consistent with this idea²⁷. In summary, the model identifies a CENP-A:H3 di-nucleosome structure bound to CHIKLMN as a minimal unit for the deposition of new CENP-A (Figure 4c).

Here we have reported the production of entirely synthetic kinetochores that specifically bind centromeric chromatin while mediating a simultaneous connection to microtubules. Our efforts complement previous studies with kinetochore particles isolated from *S. cerevisiae*^{28,29} or reconstitutions on CENP-A arrays in extracts of *Xenopus laevis*³⁰. Synthetic kinetochores have the potential to drive new inroads into the structural characterization of kinetochore architecture, which remains largely unknown¹². The manipulation of recombinant kinetochores *in vitro* may allow gaining molecular insight into crucial kinetochore functions, including the regulation of microtubule binding, of the spindle assembly checkpoint, and of new CENP-A deposition.

Online content

Methods, along with additional Extended Data display items, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions

J.R.W., K.K., A.C.F. and A.M. designed the experiments. J.R.W, K.K., A.C.F., F.B., J.K., A.P., S.W., M.P. & S.P. purified proteins. D.V. and F.B. purified nucleosomes. J.R.W. carried out gel filtration experiments. K.K. carried out cell biology experiments. A.C.F. carried out microtubule binding experiments. J.F. and F.H. carried out cross-linking and mass-spec experiments. A.M. coordinated the working team. J.R.W. and A.M. wrote the paper.

Figure legends

Figure 1

Reconstitution of the CHICKLMN complex

a, Layered organisation of the kinetochore with schematic depiction of sub-complexes. Those in coloured boxes were included in the reconstitution. **b**, Coomassie-stained SDS-PAGE of recombinant proteins used in this study. **c**, CENP-NL binds preferentially to CENP-A over H3. GST-tagged CENP-L in complex with CENP-N on GSH-sepharose beads was combined with either CENP-A^{MN} or H3^{MN}. **d**, CENP-C¹⁻⁵⁴⁴ binds preferentially to CENP-A^{MN} over H3^{MN}. SEC elution profiles of CENP-C¹⁻⁵⁴⁴ (red trace), CENP-C¹⁻⁵⁴⁴ mixed to CENP-A^{MN} (purple), and CENP-C¹⁻⁵⁴⁴ mixed to H3^{MN} (grey). Shift in the elution profile indicate binding of CENP-C¹⁻⁵⁴⁴ to CENP-A^{MN}. **e**) CENP-NL (dark blue trace) binds CENP-C (light blue trace), and binds CENP-HIKM (light blue trace). These interactions are compatible and lead to formation of a 7-subunit 'CHIKLMN' complex (orange trace),

Figure 2

Selective cooperative binding of CHIKLMN to CENP-A mononucleosomes

a, CHIKLMN complex (red trace) binds to CENP-A (green trace) but not H3 (blue trace). The composition of the CENP-A^{MN}:CHIKLMN complex was confirmed by Western blotting of several subunits. **b**, topology of the CENP-A^{MN}:CHIKLMN complex. Chemical cross-linking with DSS, followed by mass spectrometry, revealed a network of cross-linked peptides. Intra protein cross-links are shown as red lines, intra-subcomplex cross-links are shown as orange lines, inter-subcomplex cross-links are shown as black lines. Proteins are coloured according to sub-complex (CENP-C, red; CENP-NL, blue; CENP-HIKM, green; CENP-A^{MN}, purple.) **c**, cooperative localization of CHIKLMN to kinetochores. Quantification of kinetochore levels of CENP-C (red bars), CENP-HK (green bars), and CENP-A (purple) measured by immunofluorescence (IF) in control HeLa cells (left) or after depletion of the indicated CCAN subunits by siRNAs. Localisations of CENP-C and CENP-HK are significantly perturbed, despite relatively high residual CENP-A levels. Representative IF images and number of cells used are shown in Extended Data Figure 3.

Figure 3

KMN and CHIKLMN connect CENP-A to microtubules

a, the CHIKLMN:CENP-A^{MN} complex (green trace) and the KMN (yellow trace) were mixed, run on a Superose 6 SEC column (black trace), and analysed by SDS-PAGE. All 17 components of the kinetochore and the 4 subunits of the CENP-A^{MN} shift together. **b**, analysis of peak fraction by Coomassie staining and WB demonstrates co-elution of all subunits. **c**, microtubule binding assay. Rhodamine-labelled microtubules (red channel) were tethered to glass coverslips and incubated in the presence of GFP-KMN (green), Alexa-488 labelled CHIKLMN (blue), or Alexa-647 labelled CENP-A^{MN} (purple), and combinations thereof.

Figure 4

Centromere and kinetochore assembly and propagation

a, similar subunit composition suggests conservation of a structural module that is used only once at "point" centromeres/kinetochores, which bind a single microtubule, and that is instead repeated several times at "regional" centromeres/kinetochores, which bind multiple microtubules¹³. As little as 1 nucleosome in 25 may contain CENP-A at centromeres²³. **b**, matrix of intra-kinetochore interactions. An observed physical interaction is highlighted in green, and lack of interaction is shown in red. c, a dinucleosome model of kinetochore assembly and new CENP-A deposition. The Nterminal region of CENP-C (exemplified by CENP-C¹⁻⁵⁴⁴) binds the KMN, the CHIKLMN, and CENP-A^{MN}. The C-terminal region of CENP-C (exemplified by CENP-C⁵⁴⁵⁻⁹⁴³) does not bind core kinetochore component (this study) but interacts with CENP-A loading machinery, including the Mis18 complex, which in turn recruits the CENP-A chaperone HJURP^{26,31}. Each half of CENP-C contains a nucleosome-binding motif, and has therefore potential to interact with two adjacent nucleosomes. After DNA replication, when CENP-A levels are halved, CENP-A is replaced with H3 (H3.3, references 24,25), and the C-terminal region of CENP-C binds to it, "marking" the nucleosome for future exchange with CENP-A. After mitosis, the C-terminal region of CENP-C contributes to recruit machinery that replaces H3 with CENP-A. Thus, the Cterminal region of CENP-C may bind to a nucleosome that "toggles" between H3 (in mitosis) and CENP-A (in interphase).



Weir et al. Figure 1



Weir et al. Figure 2



Weir et al. Figure 3



Weir et al. Figure 4

Methods Summary

Production of recombinant proteins CENP-NL was produced as a GST fusion construct from insect cells using a baculovirus expression system using the MultiBac system¹. Specifically, a coding sequence expressing 3C cleavable GST-tagged CENP-L was sub-cloned into MCS2, and the coding sequence of CENP-N was sub-cloned into MCS1 of pFL. Bacmid was then produced from EMBacY cells¹, and subsequently used to transfect Sf9 cells and produce baculovirus. Baculovirus was amplified through three rounds of amplification and used to infect Tnao38 cells². Cells infected with the GST-CENP-L/CENP-N virus were cultured for 72 hours before harvesting. Cells were washed and resuspended in lysis buffer (50 mM Na-HEPES, 300 mM NaCl, 10% glycerol, 4 mM 2-mercaptoethanol, 1 mM MgCl₂ pH 7.5). Resuspended cells were lysed by sonication in the presence of Benzonase before clearance at 100,000g at 4°C for 1 hour. Cleared lysate was passed over GSH-Sepharose, before extensive washing with lysis buffer. GST-CENP-L/CENP-N complex was then eluted in lysis buffer + 20 mM reduced glutathione. Eluted protein was concentrated in a 30 kDa Amicon-Ultra-15 Centrifugal Filter (Millipore) in the presence of GST-tagged 3C protease. Concentrated protein was then loaded onto a Superdex 200 16/600 column equilibrated in 20 mM Na-HEPES pH 7.5, 300 mM NaCl, 2.5% glycerol, pH 7.5. A 5 mL GSH-Sepharose FF column was connected in series after the Superdex 200 column to trap GST, un-cut GST-CENP-L/CENP-N and GST-tagged 3C protease. Peak fractions corresponding to CENP-L/CENP-N were collected and again concentrated in a 30 kDa MWCO concentrator to approximately 50-100 μM prior to being flash frozen in liquid N_2 and storage at -80°C. Other proteins were purified as described previously {Basilico:2014uc; Klare:2015co; Petrovic:2014cx; Tachiwana, 2015 #561}.

<u>H3 containing MNs</u> Plasmids for the production of *Xenopus laevis* H2A, H2B, H3 and H4 histones were a kind gift of Prof. Daniela Rhodes. *Xenopus laevis* histone expression and purification, refolding of histone octamers or H2A:H2B dimers, and reconstitution of H3 containing mononucleosomes were performed precisely as described³. Plasmids for the production of the "601" 145-bp DNA were a kind gift of Prof. Curt A. Davey at the Nanyang Technological University in Singapore. DNA production was performed as described³ with no modifications.

<u>CENP-A containing MNs</u> Plasmids for the production of human CENP-A:H4 histone tetramer were a kind gift of Prof. Aaron F. Straight's laboratory at Stanford University. Preparations of CENP-A-containing MNs were carried out precisely as described⁴.

<u>Analytical SEC analysis</u> Analytical size exclusion chromatography was carried out on a custom made Superose 6 5/200 in a buffer containing 20 mM HEPES, 300 mM NaCl, 2.5 % glycerol, 2 mM TCEP, pH 7.5 on an ÄKTAmicro system. As indicated, the following additional columns were used: Superdex 200 5/150 Increase and Superose 6 5/150. All samples were eluted under isocratic conditions at 4°C in SEC buffer (20 mM Hepes pH 7.5, 300 mM NaCl, 2.5 % Glycerol, 2 mM TCEP) at a flow rate of 0.2 ml/min. Elution of proteins was monitored at 280 nm. 100 µl fractions were collected and analysed by SDS-PAGE and Coomassie blue staining. To detect the formation of a complex, proteins were mixed at the indicated concentrations in 50 µl, incubated for at least 2 hr on ice and then subjected to SEC.

Kinetochore microtubule binding assay Cover slips and glass slides were cleaned by sonication in isopropanol and 1 M KOH or 1 % Hellmanex and 70 % ethanol, respectively. After functionalization of cover slips with 5 % biotinylated poly-L-lysine-PEG for 30 min, flow cells were created with a volume of 10-15 μ L. Flow cells were passivated with 1 % pluronic F-127 for 1 h and coated with avidin for 30-45 min. After incubation with 10 nM microtubules (10 % biotinylated, 10 % Rhodamine labeled, Cytoskeleton, Inc., polymerized according to manufacturer's instructions) for 10-20 min. Proteins (400 nM) were added in 80 mM Pipes (pH6.8), 125 mM KCl, 1 mM EGTA, 1 mM MgCl₂ and 20 µM Taxol). Flow cells were sealed with wax and imaged with spinning disk confocal microscopy on a 3i Marianas system (Intelligent Imaging Innovations, Göttingen, Germany) equipped with Axio Observer Z1 microscope (Zeiss, Oberkochen, Germany), a CSU-X1 confocal scanner unit (Yokogawa Electric Corporation, Tokyo, Japan), Plan-Apochromat 100x/1.4NA DIC oil objective (Zeiss), Orca Flash 4.0 sCMOS Camera (Hamamatsu, Hamamatsu City, Japan) and controlled by Slidebook Software 6.0 (Intelligent Imaging Innovations). Images were acquired as z- sections at 0.27 µm and maximal intensity projections were made with Slidebook Software 6.0 (Intelligent Imaging Innovations).

<u>GST-pulldown</u> assays GST pulldown experiments were performed using preblocked GSH Sepharose beads in pulldown buffer (10 mM Hepes pH 7.5, 200 mM NaCl, 0.05 % Triton, 2.5 % Glycerol, 1 mM TCEP). GST-CENP-NL as bait at a 1 µM concentration

was incubated with MNs as prey at a 3 μ M concentration. The bait was loaded to 12 μ l preblocked beads, before the prey was added. At the same time, 1 μ g of each protein was added into Laemmli sample loading buffer for the input gel. The reaction volume was topped up to 40 μ l with buffer and incubated at 4°C for 1 h under gentle rotation. Beads were spun down at 500 g for 3 minutes. The supernatant was removed and beads washed twice with 250 μ l buffer. Supernatant was removed completely, samples boiled in 15 μ l Laemmli sample loading buffer and run on a 14 % SDS-PAGE gel. Bands were visualized with Commassie brilliant blue staining. Preblocking of GSH sepharose beads 750 μ l of GSH Sepharose beads were washed twice with 1 ml washing buffer (20 mM Hepes pH 7.5, 200 mM NaCl) and incubated in 1 ml blocking buffer (20 mM Hepes pH 7.5, 500 mM NaCl, 500 μ g/ μ l BSA) over night at 4°C rotating. Beads were washed 5 times with 1 ml washing buffer and resuspended in 500 μ l washing buffer to have a 50/50 slurry of beads and buffer.

RNAi interference For CENP-C silencing, we used a single siRNA (target sequence: 5'-GGAUCAUCUCA- GAAUAGAA-3' obtained from Sigma-Aldrich), targeting the coding region of endogenous CENP-C mRNA. For an efficient depletion, siRNA for CENP-C was transfected at a concentration of 60 nM for 72 h. For CENP-A silencing a single siRNA (target sequence: 5'-CACAGUCGGCGGAGACAAGTT-3' obtained from Dharmacon) was used, as previously published⁵. To obtain an efficient depletion of CENP-A, 100 nM siRNA was transfected for 120 h. For CENP-M silencing, we used a combination of three siRNA duplexes 5'sequences: (target 5'-UUAAGCAGCUGGCGUGUUA-3'; ACAAAAGGUCUGUGGCUAA-3'; 5'-GUGCUGACUCCAUAAACAU-3'; purchased from Thermo Scientific, Carlsbad, CA) targeting the 3'-UTR of endogenous CENP-M. CENP-M siRNA duplexes were used at 20 nM each for 72 h as published⁶. For CENP-H a single siRNA (target sequence: 5'-CUAGUGUCUCAUGGAUAA-3' obtained from Dharmacon) targeting the coding region of endogenous CENP-H mRNA was used at 100 nM for 72 h. For CENP-L a single siRNA (target sequence: 5'- UUUAUCAGCCACAAGAUUA-3' obtained from Dharmacon) targeting the coding region of endogenous CENP-L was used at 100 nM for 72 h. Transfections of RNAi were performed with HyPerFect (QIAGEN) according to the manufacturer's instructions.

<u>Mammalian plasmids</u> Constructs were created by cDNA subcloning in pcDNA5/ FRT/TO-mCherry-IRES vector, a modified version of pcDNA5/FRT/TO vector (Invitrogen). pcDNA5/FRT/TO vector (Invitrogen) is a tetracycline-inducible expression vector designed for use with the Flp-In T-REx system. It carries a hybrid human cytomegalovirus/TetO2 promoter for high-level, tetracycline-regulated expression of the target gene. The control plasmid for mCherry expression was created by PCR amplifying the mCherry sequence and cloning it into the pcDNA5/FRT/TO vector previously modified to carry an internal ribosomal entry site (IRES) sequence to obtain the pcDNA5/FRT/TO mCherry-IRES vector. All plasmids used in the study for mammalian expression were derived from the pCDNA5/FRT/TO-mCherry-IRES and used for genomic integration and expression of human CENP-N o -L proteins. To create all N-terminally tagged mCherry fusions, we amplified CENP-N or CENP-L fragments by PCR from human cDNA and subcloned them in frame with the mCherry tag into the pcDNA5/FRT/TO mCherry-IRES vector using the restriction sites BamHI and XhoI.

Cell culture and transfection Parental Flp-In T-REx HeLa cells used to generate stable doxycycline-inducible cell lines were a gift from Stephen Taylor (University of Manchester, Manchester, England, UK). They were grown at 37°C in the presence of 5% CO2 in Dulbecco's Modified Eagle's Medium (DMEM; PAN Biotech) supplemented with 10 % TET-free Fetal Bovine Serum (Invitrogen) and 2 mM L-glutamine (PAN-Biotech). Flp-In T-REx HeLa cells⁷ and maintained in DMEM supplemented with 10% tetracycline-free FBS, 2 mM L-glutamine, 250 µg/ml hygromycin (Invitrogen, Carlsbad, CA) and 4 µg/ml blastidicin (Invitrogen, Carlsbad, CA). GFP-CENP-C fusions were expressed by addition of 25 ng/ml doxycycline (Sigma, St. Louis, MO) for 24 or 48 hr. For CENP-C silencing, we used a single siRNA (target sequence: 5'-GGAUCAUCUCAGAAUAGAA-3' from Sigma, St. Louis, MO), targeting the coding region of endogenous CENP-C mRNA. Transfections were performed with HyPerFect (Qiagen) according to the manufacturer's instructions. To obtain an efficient depletion of CENP-C, 60 nM siRNA was transfected 3 times within 72 hr. In the last 24 hr 25 ng/ml doxycycline was added to induce GFP-CENP-C construct expression. The siRNA did not effect expression of GFP-CENP. Phenotypes were analysed 96 hr after first siRNA addition and protein depletion was monitored by Western blotting or immunofluorescence.

Immunoblotting RNAi depleted cells for various CCAN components were harvested by trypsinisation and lysed by incubation in lysis buffer (75 mM HEPES pH 7.5, 150 mM KCl, 1.5 mM EGTA, 1.5 mM MgCl₂, 10% glycerol, 0.075% NP-40, 90 U/ml benzonase (Sigma), protease inhibitor cocktail (Serva) at 4°C for 15 min followed by sonication and

centrifugation. Cleared lysate was washed with lysis buffer, resuspended in Laemmli sample buffer, boiled and analyzed by western blotting using 12% NuPAGE gels (Life technologies). The following antibodies were used: anti-Vinculin (mouse monoclonal, clone hVIN-1; 1:15000; Sigma-Aldrich, V9131), anti α-tubulin (mouse monoclonal, Sigma-Aldrich T9026), anti-CENP-C [rabbit polyclonal antibody SI410 raised against residues 23-410 of human CENP-C; 1:1200; reference ⁸], anti-CENP-HK (rabbit polyclonal antibody SI0930 raised against the full length human CENP-HK complex; 1:1000), anti-CENP-M (rabbit polyclonal antibody raised against the full length human CENP-M), anti-CENP-L (rabbit polyclonal, Acries antibodies 17007-1-AP). Secondary antibodies were affinity purified anti-mouse (Amersham, part of GE Healthcare), anti-rabbit or anti-mouse (Amersham) conjugated to horseradish peroxidase (1:10000). After incubation with ECL western blotting system (GE Healthcare), images were acquired with ChemiDocTM MP System (BioRad). Levels were adjusted with ImageJ and Photoshop and images were cropped accordingly

Immunofluorescence and quantification Flp-In T-REx HeLa cells (parental or mCherry-CENP-L inducible) were grown on coverslips pre-coated with 0.01% poly-L-Lysine (Sigma). Cells were fixed with PBS/PHEM-paraformaldehyde 4 % followed by permeabilisation with PBS/PHEM-Triton 0.5%. The following antibodies were used for immunostaining: CREST/anti-centromere antibodies (human auto-immune serum, 1:100; Antibodies Inc., Davis, CA), .), anti-CENP-C (SI410; 1:1,000, or the directly Alexa488 conjugated form of this antibody 1:400), anti-CENP-A mouse monoclonal (Gene Tex GTX13939, 1:500) anti-CENP-HK (SI0930; 1:800 or the Alexa488 directly conjugated form of this antibody 1:800). Rodamine Red-conjugated, DyLight405-conjugated secondary antibodies were purchased from Jackson ImmunoResearch Laboratories, West Grove, PA. Alexa Fluor 647-labeled secondary antibodies were from Invitrogen. Coverslips were mounted with Mowiol mounting media (Calbiochem). All experiments were imaged at room temperature using the spinning disk confocal microscopy of a 3i Marianas system (Intelligent Imaging Innovations, Denver, CO) equipped with an Axio Observer Z1 microscope (Zeiss, Oberkochen, Germany), a CSU-X1 confocal scanner unit (Yokogawa Electric Corporation, Tokyo, Japan), Plan-Apochromat 63x or 100x/1.4NA objectives (Zeiss) and Orca Flash 4.0 sCMOS Camera (Hamamatsu, Hamamatsu City, Japan) and converted into maximal intensity projections TIFF files for illustrative purposes. Quantification of kinetochore signals was performed on unmodified Z-series images using Imaris 7.3.4 software (Bitplane, Zurich, Switzerland). After background

subtraction, all signals were normalized to CREST where indicated and values obtained for control cells were set to 1. In some cases (e.g. CENP-L RNAi treatment, the levels of CREST were decreased below detection limit, probably to loss of proteins CREST antibody recognizes). In these cases protein levels were only substracted by the background and total proteins levels were set in relation to the untreated control cells. Quantifications are based on three independent experiments where 5 to 10 cells were analysed.

Chemical cross-linking and mass spectrometry Cross-linking analysis of CENP-A^{MN}:CHIKLMN:KMN complex was performed with an equimolar mixture of light and heavy-labeled (deuterated) bis[sulfosuccinimidyl] suberate (BS3- d_0/d_{12} , Creative Molecules). The complex was incubated with 0.8 mM BS3 for 30 min at 30 °C and the crosslinking reaction was quenched by adding ammonium bicarbonate to a final concentration of 100 mM. Digestion with lysyl enodpeptidase (Wako) was performed at 35 °C, 6M Urea for 2 h (at enzyme-substrate ratio of 1:50 w/w) and was followed by a second digestion with trypsin (Promega) at 35°C overnight (also at 1:50 ratio w/w). Digestion was stopped by the addition of 1% (v/v) trifluoroacetic acid (TFA). Crosslinked peptides were enriched on a Superdex Peptide PC 3.2/30 column (300×3.2 mm) at a flow rate of 25 µl min⁻¹ and water/acetonitrile/TFA, 75:25:0.1 as a mobile phase. Fractions were analysed by liquid chromatography coupled to tandem mass spectrometry using a hybrid LTQ Orbitrap Elite (Thermo Scientific) instrument. Cross-linked peptides were identified using xQuest⁹. False discovery rates (FDRs) were estimated by using xProphet¹⁰ and results were filtered according to the following parameters: FDR =0.05, min delta score = 0.85, MS1 tolerance window of -4 to 4 ppm, ld-score > 22. The crosslinks were visualised using the webserver xVis (http://xvis.genzentrum.lmu.de/) [reference¹¹].

Extended Data Figure 1

Building blocks of the kinetochore

Schematic organization of protein and sub-complexes used in this study, with essential structural features. CENP-A is a histone H3 variant. Crucial to its function in kinetochore assembly are the so-called CATD box and the C-terminal region, which are believed to interact with CENP-N and CENP-C, respectively¹²⁻¹⁴. CENP-C is a blueprint for kinetochore assembly, with binding motifs for outer and inner kinetochore subunits ordered from the N- to the C-terminus. The N-terminal region starts with a binding site for the Mis12 complex^{15,16}, followed by a binding site for the CENP-HIKM complex⁶. Two related nucleosome-binding motif have been identified, in the so-called "central region" and "CENP-C motif" [reference ¹³]. The nucleosome-binding motifs interact with the H2A:H2B dimer and with the C-terminal region of CENP-A13. Finally, the dimerization motif has a cupin-like fold¹⁷. The C-terminal region also binds to M18BP1^{18,19}, which is involved in CENP-A deposition. The two subunits of the CENP-LN complex have similar size and are structurally related, as revealed by the crystal structure of their S. cerevisiae's homologs²⁰. The 4-subunit CENP-HIKM complex contains a tight sub-complex of the CENP-H and CENP-K subunits⁶. CENP-M is a pseudo Ras-like small GTPase that has lost the ability to bind GTP⁶. It interact with CENP-I and is required for its stability⁶, but no CENP-M ortholog has been identified in S. cerevisiae, whereas Ctf3 is the CENP-I ortholog in this organism (see Figure 4a). Structurally, CENP-I may resemble the HEAT-repeat α -solenoid structure of Importin- β [reference ⁶]. The 4-subunit Ndc80 complex is crucial for microtubule binding by kinetochores ^{21,22}. It is a dumbbell-shaped, elongated protein with large coiled-coil domains^{23,24}. Calponin-homology (CH) domains near the N-terminus of the Ndc80 and Nuf2 subunits have been implicated in microtubule binding^{23,24}. The Spc24 and Spc25 subunits target the Ndc80 complex to the kinetochore^{25,26} through interactions with the Mis12 complex. The 4-subunit Mis12 complex remains structural uncharacterized, except for low resolution negative-stain electron microscopy analyses²⁶⁻²⁹. It is a hub of interactions, including interactions with the CENP-C complex (discussed above), the Ndc80 complex (also discussed above), and the Knl1 subunit of the Knl1 complex²⁸. The 2-subunit Knl1 complex plays a crucial role in spindle assembly checkpoint signalling³⁰. The C-terminal region of Knl1, the largest known core kinetochore subunits, consists of tandem RWD domains and is sufficient with an interaction with the Mis12 complex^{26,28}.

A longer region, approximately comprising the last 300 residues, is also sufficient for tight binding to Zwint. For our studies, we used a construct encompassing residues Knl1²⁰⁰⁰⁻²³¹¹, and endowed with the ability to bind the Mis12 complex and Zwint.

Extended Data Figure 2

Size-exclusion chromatography analyses

The indicated samples (at a concentration of 10 μ M, 5 μ M for mononucleosomes) were loaded on the indicated size-exclusion chromatography column and the resulting elution fractions were analysed by SDS-PAGE. **a**, CENP-NL complex. **b**, CENP-C¹⁻⁵⁴⁴ complex. **c**, CENP-HIKM. **d**, KMN network. **e**, CENP-A^{MN}; the lower panel is a MidoriGreen stained agarose gel of the same fractions analysed by SDS-PAGE in the upper panel. **f**, H3^{MN}; lower panel as in **e**.

Extended Data Figure 3

Kinetochore localization studies

Representative images showing kinetochore levels in interphase cells of CENP-A, CENP-C, and CENP-HK (with an antibody raised against the CENP-HK complex) in Flp-In T-REx HeLa cells upon treatment with the indicated siRNA. Kinetochores were visualized with anti-CENP-A sera. Scale bar = 10 μ m. Magnification = 630x.

Extended Data Figure 4

Controls for fluorescent MT binding experiment

a, Additional controls for the MT binding assays shown in Figure 3c. Rhodaminelabelled microtubules (red channel) were tethered to glass coverslips and incubated in the presence of GFP-KMN (green), Alexa-488 labelled CHIKLMN (blue), or Alexa-647 labelled CENP-A MN (purple), and combinations thereof. **b**, only CENP-A^{MN} were translocated to microtubules, whereas H3^{MN} were not. The upper panel was already shown in Figure 3**c**.

Extended Data Figure 5

Topology of the kinetochore

Kinetochore topology. Using chemical cross-linking and mass spec the inter-peptide interactions within the kinetochore sample were analysed. Intra protein cross-links are shown in red, intra-subcomplex cross-links are shown in green, inter-subcomplex cross-links in blue. Proteins are coloured according to sub-complex Proteins are coloured according to their sub-complex. CENP-A^{MN}, purple; CENP-C, red; CENP-HIKM, green; CENP-NL, blue; Mis12, peach; Knl1, orange; Ndc80, yellow.

Extended Data Figure 6

CENP-C⁵⁴⁵⁻⁹⁴³ does not interact with CCAN subunits

a, SEC analysis of CENP-A^{MN} (purple), CENP-C⁶³¹⁻⁹⁴³ (red trace), and their combination (green trace) shows a stoichiometric interaction. **b,** SEC analysis of CENP-NL (blue trace), CENP-HIKM (green trace), CENP-C⁵⁴⁵⁻⁹⁴³ (red trace), and their combination (orange trace). No apparent shift of CENP-C was observed.

Extended Data Table I

Table of cross-links identified by mass spectrometry on the CENP-A^{MN}:CHIKLMN complex

Extended Data Table II

Table of cross-links identified by mass spectrometry on the CENP-A^{MN}:CHIKLMN:KMN complex

Extended data References

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Western blot depletion control goes here





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b





Rank	Id Protein1	Protein2	Туре	XLType Spectrum	AbsPos1	AbsPos2	deltaAA	Mr I	Mz z	:	Error_rel[pinseen	mions	тіс	WT	C ints	um del	taS lo	d-Score MS2	%Δ	comment
	1 KGNYAER-LH2A1_XENLA 1 LLEIQTEKNI CENPH_HUMAN	H2B11_XENLA CENPK_HUMAN	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	37 179	86 149	49 30	1874,97 2548,339	625,998 638,092	3	-2,6	14 6	10 14	0,74 0,63	0,12 0,1	175 297	0,79 0,3	41,78 40,02	6	
	1 LQNIIDNQK Ndc80 1 KATLIK-LKEI Ndc80	Nuf2 Spc25 His	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	360 633	243 52	117 581	3171,666 1612.94	1058,23 404,243	3	-0,2	6 11	12 11	0,51 0.37	0,08	421 354	0,3 0,7	39,28 39,12	5	
	1 TLKEEVQK-INdc80	Nuf2	xlink	inter-protei AG_Herzog	504	348	156	1842,092	461,531	4	1,3	2	8	0,63	0,11	234	0	39,01	5	candidate
	1 HAVSEGTK/H2B11_XENLA	H2A1_XENLA	xlink	inter-protei AG_Herzog	117	200	110	1938,053	485,521	4	-1,1 -0,1	2	9	0,38	0,05	469	0,8	38,49	3	candidate
	1 AFAEKLSVK Spc25_His 1 VELECETIKC Ndc80	Nuf2 Nuf2	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	46 346	461 243	415 103	1872,974 2901,459	625,332 726,373	3	0,3	2	9 11	0,43 0,54	0,07 0,08	280 685	0,56 0,7	38,35 37,71	4 3	
	1 LQKSTHLLIFCENPA_HUMAN 1 SRKPFADR-JCENPA_HUMAN	H2A1_XENLA	xlink	inter-protei AG_Herzog	56	128	72	1865,113	467,286	4	1,6	1	7	0,4	0,07	271	0,46	37,47	4	candidate
	1 AFAEKLSVK Spc25_His	Nuf2	xlink	inter-protei AG_Herzog	46	456	410	1846,05	616,358	3	-3,5	4	7	0,40	0,03	281	0,30	37,43	4	candidate
	1 LKEEER-DK\Spc25_His 1 GSTEAKITE\Dsn1_His	Ndc80 Mis12	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	52 248	629 129	577	1621,816 2341,209	406,462 781,411	4	-2,5	7	7	0,4 0,46	0,05 0,07	455 679	0 0,44	36,68 36,6	4	candidate
	1 TPETIPLTED CENPK_HUMAN 1 KGNYAER-KH2A1 XENLA	CENPH_HUMAN H2B11 XENLA	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	94 37	118 28	24 9	3496,906 1548,823	875,234 388,214	4	-1.9	8 1	8 5	0,46 0.46	0,06	388 434	0,39 0.54	36,54 36.4	5 2	candidate
	1 KESYAIYVYI H2B11_XENLA	H2A1_XENLA	xlink	inter-protei AG_Herzog	35	37	2	2237,131	560,291	4	-2,4	8	8	0,43	0,06	358	0,42	36,33	3	
	1 AVTKYTSAK H2B11_XENLA	H2A1_XENLA H2A1_XENLA	xlink	inter-protei AG_Herzog	121	6	115	1678,924	447,873 560,649	3	-0,9	4	7	0,43	0,08	251	0,66	36,04	4	
	1 IIHENIVEKE CENPN_HUMAN 1 DAAEREKLK Nuf2	CENPL_HUMAN Ndc80	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	245 416	321 577	76 161	2427,365 2280,264	486,481 571,074	5	-1 -0,9	6 1	7 11	0,6 0,55	0,09 0,09	309 323	0,41 0,79	35,99 35,91	3 6	
	1 DSIKAFAEK-Spc25_His 1 OKIDI DSMI CENPH HUMAN	Ndc80 CENPK HUMAN	xlink xlink	inter-protei AG_Herzog	41 183	629 149	588 34	1826,932 2897 365	609,985 725 349	3	0,8	4	6 15	0,5	0,07	328 612	0,66	35,87	4	candidate
	1 DKYEKK-LKENdc80	Spc25_His	xlink	inter-protei AG_Herzog	629	52	577	1749,91	438,485	4	-2,9	3	8	0,39	0,06	262	0	35,49	3	
	1 KPEAPR-SALCENPA_HUMAN 1 AVTKYTSAKH2B11_XENLA	CENPA_HUMAN	xlink	inter-protei AG_Herzog inter-protei AG_Herzog	121	128	119	1353,76	452,261 410,034	3	-0,9	2	9	0,37 0,38	0,06	342 198	0,68	35,44 35,33	4	candidate
	1 EKHHNILPK CENP-C_1-544_His 1 EIRDKYEK-L Ndc80	CENPK_HUMAN Spc25 His	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	349 629	163 52	186 577	2244,267 2020,047	449,861 674,357	5	1,7	1 14	9 8	0,42 0,38	0,07 0,06	416 459	0,61 0,75	35,27 35,1	3 4	
	1 YKTELCTK-/ Mis12 1 LONIIDNOK Ndc80	Nsl1 Nuf2	xlink	inter-protei AG_Herzog	129	144	15	2008,096	503,032	4	2,1	5	8	0,36	0,06	237	0,32	34,99	4	candidate
	1 DSIKAFAEK-Spc25_His	Ndc80	xlink	inter-protei AG_Herzog	41	629	588	2225,156	742,727	3	-0,8	3	10	0,34	0,06	341	0,73	34,56	4	
	1 FVQKLGDAINSI1 1 QTKDCPQR Nsl1	Dsn1_His Dsn1_His	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	262	322	108	3479,881 2055,084	696,984 514,779	4	-1,7	2	13 9	0,55	0,09	308	0,81	34,13 33,98	5	
	1 DTYKDSIK-TSpc25_His 1 SQEIFLNLK1Nuf2	Nuf2 Ndc80	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	37 427	456 577	419 150	1822,967 2698,504	608,664 675,634	3	-0,3	5 4	8 6	0,46 0.59	0,08	258 279	0	33,97 33.81	4	
	1 VELECETIKC Ndc80	Nuf2	xlink	inter-protei AG_Herzog	346	266	80	2486,286	622,579	4	2,3	3	5	0,48	0,07	378	0,74	33,78	3	
	1 HAVSEGTK/H2B11_XENLA	CENPA_HUMAN	xlink	inter-protei AG_Herzog	117	200	108	2061,116	516,287	4	-2,8	2	8	0,38	0,07	311	0,89	33,64	3	
	1 VLKQVHPD H2B11_XENLA 1 LLLPGELAKI H2B11_XENLA	H2A1_XENLA CENPA_HUMAN	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	47 109	6	41	2219,229 2596,454	444,854 650,121	5	-1,8	1	6 7	0,5 0,63	0,09 0,08	349 208	0,45 0,81	33,5 33,19	2	
	1 TSDVVLKR-INSI1 1 KLOKSTHLL CENPA HUMAN	Mis12 H2A1 XENLA	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	257 56	187 128	70 72	1825,076 1993,207	457,277 499.31	4	-1,6	2 1	9 4	0,52 0.25	0,08	199 414	0,68 0.83	33,07 33.07	2	candidate
	1 LLNAFIVAEI CENPL_HUMAN	CENPN_HUMAN	xlink	inter-protei AG_Herzog	103	245	142	2889,637	723,417	4	1,3	2	8	0,6	0,1	246	0,34	33,03	3	candidate
	1 IPSNILLPED Mis12	Nsl1	xlink	inter-protei AG_Herzog	108	43	24	2354,265	589,574	4	-0,6	2	5	0,56	0,08	266	0,85	33,02	2	candidate
	1 TVTAMDVVH4_HUMAN 1 IPSNILLPED Mis12	H2A1_XENLA Nnf1	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	92 103	37 116	55 13	2440,276 2592,423	611,077 649,114	4	-1	1	6 9	0,65 0,66	0,08 0,13	183 152	0,52 0,37	32,94 32,77	1 3	
	1 DAAEREKLK Nuf2	Ndc80	xlink	inter-protei AG_Herzog	416	577	161	2124,166	709,063	3	0,4	4	10	0,44	0,07	269	0,57	32,73	4	
	1 HAVSEGTK/ H2B11_XENLA	CENPA_HUMAN	xlink	inter-protei AG_Herzog	117	9	108	2304,26	577,073	4	2,2	4	7	0,35	0,05	381	0,78	32,67	3	
	1 USINKPISEHNOCSU 1 VTLFPKDVCCENPA_HUMAN	CENP-C_1-544_His	xlink	inter-protei AG_Herzog inter-protei AG_Herzog	124	505	381	2297,258 2449,321	613,338	4	-0,3	3	6	0,34 0,55	0,05	189	0,47	32,62 32,6	4	
	1 KAGSLKQR-CENP-C_1-544_His 1 AGSLKQR-S CENP-C 1-544 His	CENPK_HUMAN CENPK HUMAN	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	328 328	196 197	132 131	1612,999 1484,903	404,258 495,976	4	-0,1	1	4 7	0,51 0,41	0,08 0,07	184 173	0 0,83	32,49 32,34	3 5	candidate candidate
	1 KTNNFSAFL Spc25_His	Nsl1 Nuf2	xlink	inter-protei AG_Herzog	203	257	54	2535,387	634,855	4	2,5	2	7	0,45	0,06	896 636	0,29	32,27	3	
	1 TSDVVLKR-INsl1	Zwint	xlink	inter-protei AG_Herzog	257	210	47	1712,976	429,252	4	-1,5	3	6	0,38	0,06	259	0,78	31,87	2	candidate
	1 LUNIIDNUK NOCSU 1 LMLGPANV CENPI_HUMAN_57_C	CENPK_HUMAN	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	360 250	252 149	108	2820,502 2797,53	706,133 700,39	4	-0,1	1	4 10	0,35 0,48	0,04	365	0,48	31,79 31,51	5	
	1 KGNYAER-KH2A1_XENLA 1 EDCASELKV CENPM HUMAN	H2B11_XENLA CENP-C 1-544 His	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	37 45	17+117 273	20 228	1519,831 2638,363	380,966 528,68	4	-3,1 -0,1	1	3 12	0,43 0,25	0,06 0,05	457 172	0,54 0,7	31,44 31,23	1	candidate
	1 KLQKSTHLL CENPA_HUMAN	H2A1_XENLA	xlink	inter-protei AG_Herzog	56 179	75 164	19	2234,325	447,873	5	1,1	1	6	0,3	0,05	327	0,81	31,21	3	
	1 KTSDVVLK-INsl1	Mis12	xlink	inter-protei AG_Herzog	250	187	63	1797,07	450,275	4	-1,2	3	10	0,24	0,04	137	0,74	31,09	3	
	1 QTKDCPQR Nsl1	Dsn1_His	xlink	inter-protei AG_Herzog inter-protei AG_Herzog	262	322	19	2369,333 2183,177	593,341 546,802	4	-3,3	6	8	0,65	0,1	403	0,81	30,95 30,83	4	
	1 IKEAEEEDEI Ndc80 1 SQQESWDL CENPH_HUMAN	Nuf2 CENPK_HUMAN	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	519 153	362 119	157 34	2111,027 3027,543	528,765 1010,189	4	0,1	2	5 6	0,46 0,46	0,08 0,06	300 666	0 0,29	30,71 30,65	4 3	
	1 TLKEEVQKL Ndc80 1 LNEDDKVM CENR.C 1.544 His	Nuf2 CENPK HUMAN	xlink	inter-protei AG_Herzog	504	348	156	2845,594	712,406	4	2,2	3	6	0,5	0,08	357	0 75	30,64	3	
	1 EKLQIK-KLC Knl1_2000_2311	Mis12	xlink	inter-protei AG_Herzog	13	187	174	1666,009	417,51	4	-3,2	1	8	0,25	0,04	330	0,86	30,47	3	
	1 RKSLHPIHQ USN1_HIS 1 IIHENIVEKE CENPN_HUMAN	CENPL_HUMAN	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	108 245	43 296	51	2659,431 2725,507	444,246 546,109	5	-1,2	3	4 9	0,39 0,48	0,05	495 350	0,73	30,47 30,25	6	candidate
	1 SQQESWDLCENPH_HUMAN 1 KKEASLQFVCENP-C 1-544 His	CENPK_HUMAN Nsl1	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	153 93	119 276	34 183	3155,633 2872,508	632,134 719.135	5	-0,6	3 4	5 5	0,35 0.47	0,05	137 709	0,55 0.83	30,24 30,22	1	
	1 TLKEEVQKL Ndc80	Nuf2	xlink	inter-protei AG_Herzog	504	348	156	3001,698	751,432	4	3	1	6	0,34	0,05	807	0,45	29,57	3	
	1 EDCASELKV CENPM_HUMAN	CENP-C_1-544_His	xlink	inter-protei AG_Herzog	402	273	228	2794,471	699,626	4	2,2	1	8	0,28	0,04	533	0,52	29,42	4	
	1 CSGSTKVLF CENPI_HUMAN_57_C	CENPA_HUMAN CENP-C_1-544_His	xlink	inter-protei AG_Herzog inter-protei AG_Herzog	109	212	32 102	2951,619 2804,463	738,913 468,418	4	1,2	3	8 9	0,44 0,35	0,09	771	0,88	29,37 29,29	4	
	1 KSLHPIHQG Dsn1_His 1 KVGNNLQR Ndc80	Mis12 Nuf2	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	108 577	62 456	46 121	2517,339 1782,006	504,476 446,509	5	0,5 -3,2	4 1	4 5	0,37 0,23	0,05 0,04	560 266	0,55 0,52	28,82 28,82	3 4	candidate
	1 IIHENIVEKE CENPN_HUMAN 1 KTSDVVI KR Nsl1	CENP-C_1-544_His Mis12	xlink xlink	inter-protei AG_Herzog	245 257	349 187	104	2631,452	527,298 489 302	5	1	2	7	0,38	0,06	351 184	0,39	28,76	4	
	1 LASILKESLN Nuf2	Ndc80	xlink	inter-protei AG_Herzog	315	423	108	3578,999	716,808	5	2,2	4	4	0,28	0,04	362	0,58	28,39	2	
	1 TPETIPLTED CENPK_HUMAN	CENPH_HUMAN	xlink	inter-protei AG_Herzog	41 94	118	24	3624,998	726,007	5	3,2	9	6	0,34	0,05	215	0,51	27,96	4	
	1 NKVETFSES CENPK_HUMAN 1 KTNNFSAFL Spc25_His	CENPI_HUMAN_57_C Nsl1	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	149 203	160 257	11 54	2350,226 2663,481	588,564 666,878	4	-0,8	1	4 5	0,37 0,38	0,05 0,05	367 747	0,26 0,9	27,87 27,79	0	candidate candidate
	1 KSLHPIHQGDsn1_His 1 KNI FKISR-F CENPH HUMAN	CENP-C_1-544_His CENP-C_1-544_His	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	108 118	212	104 379	3202,744 1872 067	534,799 469.025	6	-3	1	7	0,25	0,04	550 135	0,55	27,64	2	candidate
	1 DKYEKK-LKENdc80	Spc25_His	xlink	inter-protei AG_Herzog	632	52	580	1749,908	438,485	4	-4	3	4	0,3	0,05	229	0,43	27,44	3	
	1 LLLPGELAKIH2B11_XENLA	CENPA_HUMAN	xlink	inter-protei AG_Herzog	109	203	100	2839,593	568,926	5	0,4	3	4	0,81	0,00	192	0,33	27,36	2	
	1 YQAYMSNL Ndc80 1 KTNNFSAFL Spc25_His	Nut2 Nsl1	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	327 203	213 275	114	4149,073 2705,476	830,823 677,377	5	-1,1	3	4 5	0,38 0,41	0,05 0,06	389 422	0,35 0,84	27,23 27,17	1 3	
	1 RFSSESKNK CENP-C_1-544_His 1 DAVTYTEH/H4 HUMAN	H4_HUMAN H2B11 XENLA	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	505 78	45 86	460 8	1891,039 2328.199	473,768 583.058	4	0,3	1	4 6	0,35 0.47	0,05	335 281	0,65 0.85	27,16 26.89	3 4	
	1 DNLLKLIAE\Spc25_His	CENP-C_1-544_His	xlink	inter-protei AG_Herzog	84	134	50	2366,338	395,397	e	-1,8	1	7	0,23	0,04	261	0,88	26,75	4	
	1 KTPETIPLTE CENPK_HUMAN	CENPH_HUMAN	xlink	inter-protei AG_Herzog	94	118	24	3624,993	726,006	5	1,7	3	4	0,33	0,04	307	0,53	26,5	2	
	1 EESKKK-DGI CENP-C_1-544_His 1 AGSLKQR-S CENP-C_1-544_His	H2B11_XENLA CENPK_HUMAN	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	496 328	28 196	468 132	1487,834 1484,903	496,952 495,975	3	-0,8	1	5 5	0,12 0,35	0,02 0,05	247 150	0,48 0,89	26,49 26,3	4	candidate candidate
	1 KTSDVVLK-INsl1 1 KTSDV// KR Nsl1	Zwint	xlink	inter-protei AG_Herzog	250	210	40	1684,967 1841 073	422,25	4	-3,1	2	4	0,13	0,02	50 317	0,65	26,24	1	candidate
	1 QKIDLDSMICENPH_HUMAN	CENPI_HUMAN_57_C	xlink	inter-protei AG_Herzog	183	160	23	2718,363	680,599	4	-0,7	1	5	0,2	0,03	364	0,48	25,79	1	
	1 KSLHPIHQGDsn1_His	CENPI_HUMAN_57_C	xlink	inter-protei AG_Herzog	154	33	75	2642,408	529,489	5	-3,2 -2,2	2	4	0,28	0,04	289	0,29	25,43	1	candidate
	1 FKSGLNGSIICENPN_HUMAN 1 NSFKKILQR-CENPN HUMAN	CENPL_HUMAN Nsl1	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	276 110	296 142	20 32	3361,826 1830,113	561,312 458,536	6	0 2,1	4 1	5 4	0,28 0,46	0,04 0,1	442 132	0,4 0	25,17 25	2	candidate
	1 KSESSPIVR- CENP-C_1-544_His 1 KSESSPIVR- CENP-C_1-544_His	CENPK_HUMAN	xlink	inter-protei AG_Herzog	273	163	110	2131,182	533,803	4	-3,2	1	6	0,22	0,04	269	0,51	24,96	3	
	1 ESVVQHLIH CENPN_HUMAN	H2B11_XENLA	xlink	inter-protei AG_Herzog	59	117	58	3240,692	811,181	4	0	2	10	0,37	0,06	353	0,57	24,88	6	
	1 ELEELKEIEA Spc24	Spc25_His	xlink	inter-protei AG_Herzog	112	79	33	4281,044 2582,373	861,799	3	1,3	1	7	0,35	0,07	395	0,47	24,83	2	
	1 NKVETFSES CENPK_HUMAN 1 ASMKETNR Dsn1_His	CENPH_HUMAN Mis12	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	149 102	196 62	47 40	1975,107 1893,939	659,377 474,492	3	-1,8	2	4 6	0,3 0,16	0,04 0,03	215 196	0,9 0,83	24,54 24,38	2	candidate candidate
	1 QQAEQERDZwint 1 HVOKOFAE Nnf1	Nsl1 Nsl1	xlink xlink	inter-protei AG_Herzog	210 154	250 149	40	2554,367 4597 484	639,6 920 505	4	-1,7	2	8	0,14	0,02	147 741	0,66	24,2 24.08	3	
	1 KKIYFK-QG(CENPI_HUMAN_57_C	H2A1_XENLA	xlink	inter-protei AG_Herzog	208	10	198	1608,931	403,24	4	-2,8	1	4	0,23	0,04	296	0,76	24,04	2	candidate
	1 IVEEGKVRK Nnf1	SPC25_HIS CENP-C_1-544_His	xiink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	606 116	21 409	585 293	4932,254 1995,118	987,459 666,047	5	-u,5 3,1	1	6	0,25	0,05	749 172	0 0,64	24,01 23,99	3	
	1 KETISTANK/Spc25_His 1 QQAEQERDZwint	Ndc80 Nsl1	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	122 210	638 257	516 47	2646,371 2582,371	530,282 517,482	5	0 -2,4	7 3	8 7	0,23 0,16	0,04 0,03	371 133	0,78 0,86	23,51 23,34	4 5	
	1 LLLPGELAKI H2B11_XENLA 1 DNVEKESKFMIs12	CENP-C_1-544_His Spc25_His	xlink xlin⊭	inter-protei AG_Herzog	109	519 52	410	3444,921	689,992 512 018	5	3,7	2	10 5	0,48	0,08	247 50	0,4	23,32 22.96	4	
	1 LLETQDGAESpc24	Spc25_His	xlink	inter-protei AG_Herzog	50	73	23	2637,432	660,366	4	-0,7	1	4	0,22	0,03	200	0,68	22,37	2	
	1 LQIKIDEMD Knl1_2000_2311	CENPK_HUMAN	xlink xlink	inter-protei AG_Herzog	154 17	144 119	10 102	4597,465 2642,379	441,404	6	-2,6	2	6	0,41	0,07	046 72	0,88 0,9	22,17 21,95	3	candidate
	1 LLLPGELAKIH2B11_XENLA 1 AQVYVPLKENdc80	CENPA_HUMAN Nuf2	xlink xlink	inter-protei AG_Herzog inter-protei AG_Herzog	109 462	9 299	100 163	2995,702 3840,999	600,148 961,258	5	3,1 -0,2	1	5 5	0,53 0,46	0,07 0,06	204 163	0,64 0,48	21,54 20,91	1 2	
	1 NKLVPEEVT CENP-C_1-544_His 1 QQAEQERD Zwint	CENPA_HUMAN Nsl1	xlink xlink	inter-protei AG_Herzog inter-protei AG Herzog	507 210	124 250	383 40	3067,718 2710,476	767,937 543,103	4	0,4	2 2	8 7	0,34 0,23	0,05 0,04	447 371	0,34 0,71	20,53 20,38	3 1	
	1 NSFKKILQR-CENPN_HUMAN 1 YKTELCTKO MIs12	H4_HUMAN Dsn1 His	xlink xlin⊭	inter-protei AG_Herzog	110	21	89 112	2078,267	416,661	5	3,8	2	4 7	0,17	0,03	160 584	0,85	19,6 18 99	2	candidate
	1 FVQKLGDAINsl1	CENP-C_1-544_His	xlink	inter-protei AG_Herzog	59	57	2	4343,249	869,658	5	2,2	1	7	0,32	0,05	358	0,43	18,91	1	

Rank	Id	Protein1	Protein2	XLType Spectrum	AbsPos1	AbsPos2	deltaAA	Mr	Mz :	z	Error_rel[p;nseen	mions	TIC	intsu	m delt	aS Id-	Score MS2	%Δ	comment	FDR (estimate)
	1 AGSLKQR-MNYFKIR-a5-b5	CENP-C_1-544	sp Q96H22 CENPN_HUMAN	inter-protei AG_Herzog	328	335	2	1867,01	467,76	4	-1,8	3 :	.0 0	,43	387	0,62	36,72	6		0,0540541
	1 KPSGSSQNR-SRKPEAPR-a1-b3	CENP-C_1-544	sp P49450 CENPA_HUMAN	inter-protei AG_Herzog	238	9	229	2037,067	510,275	4	-2,2	2	9 0	,35	293	0	35,74	5		
	1 KPSGSSQNR-NLEKISR-a1-b4	CENP-C_1-544	sp[Q9H3R5]CENPH_HUMAN	inter-protei AG_Herzog	238	118	120	1956,036	653,02	3	-1,6	5 3	.0 0	,39	521	0.21	34,12	5		
	1 KSESSPIVR-IENELKTK-a1-b6	CENP-C_1-544	spl09BS16ICENPK_HUMAN	inter-protei AG_Herzog	273	163	110	2131 183	533,804	4	-2.7	2	4 U 8 O	36	324	0,21	32,81	4		
	1 KSESSPIVR-IKUR-a1-b2	CENP-C 1-544	spl09H3R5ICENPH HUMAN	inter-protei AG_Herzog	273	196	7	7 1781 075	594.7	3	-13	4	8 0	28	274	0.88	30.88	4		
	1 KAGSLKOR-MNYFKIR-a6-b5	CENP-C 1-544	sp1Q96H221CENPN_HUMAN	inter-protei AG Herzog	328	335		1995.108	499.785	4	-0.5	1	5 0	.58	256	0.65	29.47	2	candidate	
	1 RFSSESKNK-RGGVKR-a7-b5	CENP-C_1-544	sp P62805 H4_HUMAN	inter-protei AG_Herzog	505	45	460	1891,043	473,769	4	2,5	3	4 C	,37	308	0,61	27,83	2		
	1 KAGSLKQR-MLNIKEYK-a6-b5	CENP-C_1-544	sp Q9BS16 CENPK_HUMAN	inter-protei AG_Herzog	328	170	158	8 2062,157	516,547	4	-1,7	2	4 0	,52	217	0,59	25,64	2		
	1 LNFDDKVMLKK-LLDIRKK-a6-b6	CENP-C_1-544	sp Q9H3R5 CENPH_HUMAN	inter-protei AG_Herzog	212	159	53	3 2372,386	475,485	5	-0,4	2	7 0	,18	203	0,78	23,03	3		
	1 NKLVPEEVISIVIK-VILFPKDVQLAR-a2-bb	CENP-C_1-544	sp P49450 CENPA_HUMAN	inter-protei AG_Herzog	507	124	38:	3067,719	767,938	4	0,7	1	7 0	,39	268	0,63	22,88	2		
	1 AUTKYTSAK-GKOGGK-s4.b2	sp P02281 H2B11_XENLA	sp[P49450]CENPA_HUMAN	inter-protei AG_Herzog	109	9	100	1679.972	4/4,2/3	2	-0,4 1	5	9 0	,83	257	0,53	42,54	5		
	1 AVTKYTSAK-SRKPFAPR-ad-b3	sp1P022811H2B11_XENLA	sniP494501CENPA_HUMAN	inter-protei AG_Herzog	121	9	112	2045 122	512 288	4	-2.3	8 1	0 0	57	261	0.37	38.7	4		
	1 VLKOVHPDTGISSK-GKOGGK-a3-b2	sp1P022811H2B11_XENLA	sp1P068971H2A1_XENLA	inter-protei AG Herzog	47	6	4	2219.226	444.853	5	-0.5	4	7 0	.52	381	0.46	37.86	3		
	1 AVTKYTSAK-QGGKTR-a4-b4	sp P02281 H2B11_XENLA	sp P06897 H2A1_XENLA	inter-protei AG_Herzog	121	10	111	1750,952	438,746	4	-3,2	8	8	0,3	196	0,35	37,53	4		
	1 AVTKTQK-KGNYAER-a4-b1	sp P02281 H2B11_XENLA	sp P06897 H2A1_XENLA	inter-protei AG_Herzog	21	37	16	5 1748,939	438,243	4	-1,8	2	9 0	,46	181	0	36,15	3		
	1 KESYAIYVYK-KGNYAER-a1-b1	sp P02281 H2B11_XENLA	sp P06897 H2A1_XENLA	inter-protei AG_Herzog	35	37		2237,134	560,291	4	-1,2	8	9 0	,45	335	0,24	34,9	3		
	1 VLKQVHPDTGISSK-SRKPEAPR-a3-b3	sp/P02281/H2B11_XENLA	sp[P49450]CENPA_HUMAN	inter-protei AG_Herzog	47	9	38	3 2585,425	431,912	6	-1,5	5	7 0	,51	213	0,45	34,61	4		
	1 HAVECCTVAUTV VDCADD +2 h1	sp P02281 H2B11_XENLA	sp[P49450]CENPA_HUMAN	inter-protei AG_Herzog	121	9	104	2 1801,991	451,500	4	-1,/	3	8 U 7	,34	238	0.00	34,31	2		
	1 HAVSEGTKAVTK-KPEAPK-30-01 1 HAVSEGTKAVTK-SPKDEADR-98-62	sp[P02281]H2B11_XENLA	CENERA HUMAN	inter-protei AG_Herzog	117	9	100	2001,117	577 071	-	-2,2	6	5	0,4	244	0,00	22.8	1		
	1 LLLPGELAKHAVSEGTK-EICVKFTR-a9-b5	sp P02281 H2B11_XENLA	sp1P494501CENPA_HUMAN	inter-protei AG Herzog	109	77	32	2951.615	738,912	4	-0.2 1	ő	6 0	.39	181	0.66	31.59	3		
	1 AVTKYTSAK-TALLAVKQR-a4-b7	sp P02281 H2B11_XENLA	sp Q92674 CENPI_HUMAN	inter-protei AG_Herzog	121	277	156	5 2104,224	527,064	4	-0,6	2	8 0	,25	250	0,51	30,82	4		
	1 LLLPGELAKHAVSEGTK-LVPEEVTSTVTKSR-a9-b12	sp P02281 H2B11_XENLA	CENP-C_1-544	inter-protei AG_Herzog	109	519	410	3444,902	689,988	5	-1,6	3	9 0	,62	205	0,79	28,46	4		
	1 LLLPGELAKHAVSEGTK-FSSESKNK-a9-b6	sp P02281 H2B11_XENLA	CENP-C_1-544	inter-protei AG_Herzog	109	505	396	2825,517	707,387	4	-0,2	2	6 0	,58	201	0,82	27,8	4		
	1 ESYAIYVYKVLK-ECQKSHPK-a9-b4	sp P02281 H2B11_XENLA	CENP-C_1-544	inter-protei AG_Herzog	44	80	30	2625,353	526,078	5	0,7 1	1	8 0	,27	238	0,48	26	4		
	1 HAVSEGTKAVTK-KRLNFDDK-88-51	sp P02281 H2B11_XENLA	CENP-C_1-544	inter-protei AG_Herzog	117	205	100	3 2399,272	800,765	3	-3,6	2	5 0	,34	280	0.04	25,17	2	condidate	
	1 VI KOVHPDTGISSK-KPFAPR-a3-b1	sp[P02281]H2B11_XENLA	sni P494501 CENPA_HUMAN	inter-protei AG_Herzog	47	9	33	2333,034	586 583	4	1.4	2	8	0,0	348	0,54	23,13	3	candidate	
	1 KGNYAER-LAHYNKR-a1-b6	spi P06897 i H2A1 XENLA	sp1P022811H2B11_XENLA	inter-protei AG Herzog	37	86	49	1874,973	625,999	3	-1.4 2	9	- 8 0	.76	199	0.58	40.53	4	candidate	
	1 KGNYAER-KGSKK-a1-b4	sp P06897 H2A1_XENLA	sp P02281 H2B11_XENLA	inter-protei AG_Herzog	37	16	2:	1520,829	507,951	3	-1,2	1	8 0	,44	359	0,75	35,5	5		
	1 KGNYAER-KDGKK-a1-b4	sp P06897 H2A1_XENLA	sp P02281 H2B11_XENLA	inter-protei AG_Herzog	37	28	9	1548,824	517,282	3	-1,3	3	6 0	,34	295	0,9	32,29	3	candidate	
	1 KGNYAER-DGKKR-a1-b3	sp P06897 H2A1_XENLA	sp P02281 H2B11_XENLA	inter-protei AG_Herzog	37	28	9	1576,827	526,617	3	-3,5	2	5 0	,48	185	0,66	31,45	4		
	1 SRKPEAPR-SAKSK-a3-b3	sp P49450 CENPA_HUMAN	sp P06897 H2A1_XENLA	inter-protei AG_Herzog	9	128	119	1596,891	400,231	4	-2,4	2	7 0	,48	173	0	40,12	5		
	1 SRKPEAPR-RGGVKR-a3-b5	sp[P49450]CENPA_HUMAN	sp P62805 H4_HUMAN	inter-protei AG_Herzog	9	45	30	5 1749,008	438,26	4	-2,6	8	9 0	,31	540	0,88	37,41	5		
	1 QGWLKEIK-SAKSK-85-03 1 SPKPEADD-GKOGGK-92-b2	spip49450 CENPA_HUMAN	sp P06897 H2A1_XENLA	inter-protei AG_Herzog	49	128	/5	1650 015	422,495	4	2,1	4 2	6 L 9	,26	140	0,82	30,53	2		
	1 VTLEPKDVOLAR-ESSESKNK-a6-b6	spir49450 CENPA HUMAN	CENP-C 1-544	inter-protei AG_Herzog	124	505	381	2449 316	613 337	4	-2.3	3	7 0	51	250	0,45	33.57	ŝ		
	1 EICVKFTR-GKGGK-a5-b2	sp P49450 CENPA HUMAN	sp P62805 H4 HUMAN	inter-protei AG Herzog	77	6	7:	1634,879	545,967	3	-1,5	4	4 0	,38	285	0,46	32,52	2		
	1 SRKPEAPR-KDKEESK-a3-b3	sp P49450 CENPA_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	9	492	483	1940,031	486,016	4	-1	1	8 0	,19	265	0,84	31,63	3		
	1 RSRKPEAPR-GGVKR-a4-b4	sp P49450 CENPA_HUMAN	sp P62805 H4_HUMAN	inter-protei AG_Herzog	9	45	36	5 1749,009	438,26	4	-1,8	2	4 C	,21	568	0,95	31,33	3	candidate	
	1 LAREICVKFTR-LLDIRKK-a8-b6	sp P49450 CENPA_HUMAN	sp Q9H3R5 CENPH_HUMAN	inter-protei AG_Herzog	77	159	82	2 2414,424	483,893	5	1,7	2	5 0	,87	221	0	30,61	3		
	1 SRKPEAPR-KGNYAER-a3-b1	sp P49450 CENPA_HUMAN	sp P06897 H2A1_XENLA	inter-protei AG_Herzog	9	37	28	3 1914,002	479,508	4	-2,5	1	7 0	,32	387	0,61	30,14	1		
	1 KPEAPR-RGGVKR-a1-b5	sp P49450 CENPA_HUMAN	sp P62805 H4_HUMAN	inter-protei AG_Herzog	9	45	30	5 1505,876	502,966	3	-2,1	2	4 0	,34	434	0,77	25,74	1	candidate	
	1 SKKPEAPK-ESKNEK-83-03 1 TV/TAMDV/VALKD.KGNVAED.a12.b1	sp[P49450]CENPA_HUMAN	spiQ98516 CENPK_HUMAN	inter-protei AG_Herzog	9	114	103	5 1810,949 5 2440 274	453,745	4	-2,4	5	4 U 6 0	,18	143	0.69	23	1		
	1 DNIOGITKPAIR-KPFAPR-a8-b1	spiP628051H4_HUMAN	sn1P494501CENPA_HUMAN	inter-protei AG_Herzog	32	9	2:	2159 205	540,809	4	-0.7	1	6 0	51	525	0.36	34.04	3		
	1 TVTAMDVVYALKR-KESYAIYVYK-a12-b1	sp1P628051H4_HUMAN	sp P02281 H2B11_XENLA	inter-protei AG Herzog	92	35	57	2866.511	717.636	4	-2.9	4	9 0	.74	178	0.77	31.33	3		
	1 DNIQGITKPAIR-SRKPEAPR-a8-b3	sp P62805 H4_HUMAN	sp P49450 CENPA_HUMAN	inter-protei AG_Herzog	32	9	23	3 2402,341	601,593	4	0,9	3	6 0	,37	462	0,59	30,7	3		
	1 DAVTYTEHAKRK-LAHYNKR-a10-b6	sp P62805 H4_HUMAN	sp P02281 H2B11_XENLA	inter-protei AG_Herzog	78	86	8	2456,292	615,081	4	-0,2	2	6 0	,39	404	0,8	28,71	4		
	1 DAVTYTEHAKR-LAHYNKR-a10-b6	sp P62805 H4_HUMAN	sp P02281 H2B11_XENLA	inter-protei AG_Herzog	78	86	8	3 2328,196	583,057	4	-0,8	4	6 0	,44	289	0	25,03	4		
	1 KVLRDNIQGITKPAIR-ALKNVTVSFR-a12-b3	sp P62805 H4_HUMAN	sp Q96H22 CENPN_HUMAN	inter-protei AG_Herzog	32	117	85	5 3092,823	619,572	5	1,5	1	7 0	,36	319	0,8	23,9	3		
	1 LINAEN/AEKOK-IIHENIVEKER-910-60	CORNOSCICENRI HUMAN	sp[P45450]CENPA_HUMAN	inter-protei AG_Herzog	102	245	143	2999.622	578 924	5	01 1		5 0	,55	441	0,85	22,87	4		
	1 LLNAFIVAEKOK-TALLAVKOR-a10-b7	splQ8N056ICENPL HUMAN	sp10926741CENPI HUMAN	inter-protei AG Herzog	103	277	174	2509,496	628.382	4	-1.4	2 2	4 0	.46	546	0,30	37.88	7		
	1 LMLGPANVRPLKR-NKVETFSESR-a12-b2	spl Q92674 ICENPI HUMAN	splQ9BS16ICENPK HUMAN	inter-protei AG Herzog	301	149	152	2797.519	560,512	5	-2.9	7 :	.0 0	.54	350	0.46	34.38	5		
	1 ECGKK-IKIIR-a4-b2	sp Q92674 CENPI_HUMAN	sp Q9H3R5 CENPH_HUMAN	inter-protei AG_Herzog	324	196	128	1399,82	467,614	3	-1,6	1	6 0	,54	177	0,87	31,26	2		
	1 TALLAVKQR-NLEKISR-a7-b4	sp Q92674 CENPI_HUMAN	sp Q9H3R5 CENPH_HUMAN	inter-protei AG_Herzog	277	118	159	9 1995,184	499,804	4	0,2	1	5 0	,24	257	0,43	27,95	2		
	1 LMLGPANVRPLKR-LLEIQTEKNK-a12-b8	sp Q92674 CENPI_HUMAN	sp Q9H3R5 CENPH_HUMAN	inter-protei AG_Herzog	301	179	122	2816,622	564,332	5	-3,2	3	9 0	,32	176	0,4	26,25	5		
	1 LMLGPANVRPLKR-ESKNEK-a12-b3	sp Q92674 CENPI_HUMAN	sp Q9BS16 CENPK_HUMAN	inter-protei AG_Herzog	301	114	187	2335,302	584,833	4	-0,9	2	5	0,5	157	0,87	25,66	3		
	1 MNYFKIR-IKIIR-a5-b2	sp Q96H22 CENPN_HUMAN	sp Q9H3R5 CENPH_HUMAN	inter-protei AG_Herzog	335	196	139	9 1750,035	438,516	4	1,1	2	8 0	,69	252	0,85	42,23	3		
	1 IIHENIVEKER-NAEKPSR-89-04	splQ96H22 [CENPN_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	245	409	104	2317,238	1/3,42	3	-0,5	4 c	9 0	,45	195	0,26	36,64	2		
	1 IIHENIVEKER-KSESSPIVR-a9-b1	spl Q96H22 CENPN_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	243	273	25	3 2518 366	504 681	5	-2,8	1 1	2 0	35	700	0,04	35.6	5		
	1 FKSGLNGSILAEREEPLR-HFKIHLSATR-a2-b3	spl Q96H22 ICENPN HUMAN	sp108N0561CENPL HUMAN	inter-protei AG Herzog	276	296	20	3361.832	841,466	4	1.9 1	3 3	4 0	.44	484	0.53	33.86	7		
	1 IIHENIVEKER-STKYEMYSK-a9-b3	sp Q96H22 CENPN_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	245	399	154	2652,345	664,094	4	-0,6	2 :	.0 0	,36	512	0,13	33,5	4		
	1 IIHENIVEKER-IKILCHK-a9-b2	sp Q96H22 CENPN_HUMAN	sp Q8N0S6 CENPL_HUMAN	inter-protei AG_Herzog	245	321	76	5 2427,361	486,48	5	-2,8	5	6 0	,59	323	0,48	33,5	3		
	1 IIHENIVEKER-AGSLKQR-a9-b5	sp Q96H22 CENPN_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	245	328	83	3 2275,261	569,823	4	-1,5	2 :	.1 0	,33	476	0,4	33,33	5		
	1 ALKNVTVSFR-NAEKPSR-a3-b4	sp Q96H22 CENPN_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	117	409	292	2072,139	519,043	4	0,7	3	9 0	,36	470	0,74	32,58	4		
	1 IIHENIVEKER-HFKIHLSATR-a9-b3	splQ96H22 CENPN_HUMAN	sp Q8N056 CENPL_HUMAN	inter-protei AG_Herzog	245	296	51	2725,501	455,258	6	-0,7	3	7 0	,56	308	0,76	32,46	4		
	1 IIHENIVEKER-FKHHNII PK-a9-b2	spl Q96H22 CENPN_HUMAN	CENP-C 1-544	inter-protei AG_Herzog	243	349	104	2508,551	439 583		-1,0	6	8 U	,32	276	0,03	31.55	3		
	1 HHOIVKMDLR-KSESSPIVR-a6-b1	spl Q96H22 ICENPN HUMAN	CENP-C 1-544	inter-protei AG Herzog	190	273	83	2415.307	484.069	5	0.6	2	8 0	.26	313	0.63	30.86	3		
	1 IIHENIVEKER-KPSGSSQNR-a9-b1	sp Q96H22 CENPN_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	245	238	1	2476,301	496,268	5	-1	4	9 0	,37	396	0,24	30,71	4		
	1 FKSGLNGSILAER-HFKIHLSATR-a2-b3	sp Q96H22 CENPN_HUMAN	sp Q8N0S6 CENPL_HUMAN	inter-protei AG_Herzog	276	296	20	2737,501	685,383	4	-0,6	5	7 0	,39	472	0,3	30,5	2		
	1 HHQIVKMDLR-ESKNEK-a6-b3	sp1Q96H22 CENPN_HUMAN	sp[Q9BS16]CENPK_HUMAN	inter-protei AG_Herzog	190	114	76	2147,114	537,786	4	-0,7	3	8	0,2	151	0,44	30,37	4		
	1 IIMENIVEKER-TALLAVKQR-a9-b7 1 IIMENIVEKER-KAGSLKOP 40-b6	spiue6H22[CENPN_HUMAN	SPIUS2674 CENPL HUMAN	inter-protei AG_Herzog	245	277	33	2515,453	601 847	4	1,7	1	5 0	,54 41	321 22F	0,85	29,21	1		
	1 ESVVOHLIHLCEEKR-HAVSFGTKAVTK-a1d-h9	spl Q96H22 CENPN HIIMAN	sp P02281 H2R11_XFNIA	inter-protei AG Herror	245 50	528	8:	32403,357	649 146	4	-1,1	4	5 L 8	0.3	510	0,34	28.63	3		
	1 IIHENIVEKER-SRKPEAPR-a9-b3	sp1Q96H221CENPN_HUMAN	sp1P494501CENPA HUMAN	inter-protei AG Herzog	245	9	236	2456.351	615.096	4	0.5	3	7 C	.44	257	0.41	27.95	4		
	1 MNYFKIR-KAGSLK-a5-b1	sp Q96H22 CENPN_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	335	323	12	1710,943	571,322	3	-3,8	2	з с	,41	197	0	27,93	0		
	1 QFKNSFKK-TIKQK-a3-b3	sp Q96H22 CENPN_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	105	418	313	1780,028	446,015	4	2,1	1	6 0	,23	243	0,85	27,68	4	candidate	
	1 IFNELKTK-MNYFKIR-a6-b5	sp Q9BS16 CENPK_HUMAN	sp Q96H22 CENPN_HUMAN	inter-protei AG_Herzog	163	335	172	2100,145	526,044	4	0,4	4 :	.0 0	,54	341	0,54	37,69	4		
	1 NKVETFSESR-IKIIR-a2-b2	sp Q9BS16 CENPK_HUMAN	sp Q9H3R5 CENPH_HUMAN	inter-protei AG_Herzog	149	196	41	1975,108	494,785	4	-0,9	4	4 0	,54	310	0,85	34,51	1	candidate	
	1 TRETIRI TEDVI ITI GREEGOK-NI EKISR-a17-b4	spi Q98516 CENPK_HUMAN	splugghzzicenpn_HUMAN	inter-protei AG_Herzog	1/0	335	100	2146,127	537,539 975 222	4	-2,4	1	9 U 6 O	39	238	0,71	34,2	4		
	1 NKVETFSESR-KENVKPFR-a2-b1	spl Q9BS16 CENPK_HUMAN	sp10926741CENPI HUMAN	inter-protei AG Herzog	149	211	62	2 2350.231	784.418	3	1.3	6 :	.0 0	.34	466	0,35	31.62	5		
	1 KTPETIPLTEDVLITLGKEEFQK-KNLEKISR-a18-b5	sp Q9BS16 CENPK_HUMAN	sp Q9H3R5 CENPH_HUMAN	inter-protei AG_Herzog	94	118	24	3753,067	751,621	5	-3,8	2	4 0	,46	667	0,47	29,55	2		
	1 CLTAELSQWQKK-AQTKQQLLEYK-a11-b4	sp Q9BS16 CENPK_HUMAN	sp Q9H3R5 CENPH_HUMAN	inter-protei AG_Herzog	76	51	25	5 2977,553	745,396	4	-1,8	1	8 C	,35	246	0,42	27,03	6		
	1 IFNELKTKMLNIK-FSSESKNK-a8-b6	sp Q9BS16 CENPK_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	165	505	340	2654,444	885,822	3	3,2	1	7 0	,13	147	0,8	23,29	3	candidate	
	1 LLEIQTEKNK-NKVETFSESR-a8-b2	sp Q9H3R5 CENPH_HUMAN	sp Q9BS16 CENPK_HUMAN	inter-protei AG_Herzog	179	149	30	2548,334	638,091	4	-1,8 2	1 :	.3 0	,54	356	0,38	37,58	5		
	1 LLEIQTEKNK-KENVKPFR-a8-b5	SPLU9H3R5 [CENPH_HUMAN	SPI U92674 [CENPI_HUMAN	inter-protei AG_Herzog	179	215	30	2369,326	593,339	4	-2,3	1 2 ·	/ C	,/1	198	0,53	36,81	3		
	1 SOOFSWDI FEKI I DIRK-LKEDI FR-a11-52	spicerisks [CENPH_HUMAN spicerisks]CENPH_HUMAN	spi/OBBS16/CENPK_HUMAN	inter-protei AG_Herzog	152	238	1/1	1 3155 621	795,13	4	-2,2	∠ : 8	.u () 8	,++2 0.4	44b 269	0,52	33,63	4		
	1 LLEIOTEKNK-KENVKPFR-a8-b1	spl Q9H3R51CENPH_HUMAN	spl092674 CENPI HUMAN	inter-protei AG Herzog	179	211	34	2369.379	790,784	3	-1.2	2	- 9 n	.49	651	0.73	33.27	3		
	1 SQQESWDLEEKLLDIR-LKEDLER-a11-b2	sp Q9H3R5 CENPH_HUMAN	sp Q9BS16 CENPK_HUMAN	inter-protei AG_Herzog	153	119	34	3027,536	1010,187	3	-1,3	2	6	0,5	867	0,32	32,85	2		
	1 QKIDLDSMENSER-NKVETFSESR-a2-b2	sp Q9H3R5 CENPH_HUMAN	sp Q9BS16 CENPK_HUMAN	inter-protei AG_Herzog	183	149	34	2897,371	725,351	4	0,1	5 :	2 0	,45	463	0,29	31,96	4		
	1 AQTKQQLLEYK-KSESSPIVR-a4-b1	sp Q9H3R5 CENPH_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	51	273	222	2488,356	623,097	4	1,1	2	8 0	,25	487	0,32	27,56	2		
	1 LLEIQTEKNK-KSESSPIVR-a8-b1	sp Q9H3R5 CENPH_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	179	273	94	2354,304	589,584	4	-0,9	1	8 0	,39	216	0,64	26,93	1		
	1 QKIDLDSMENSER-KENVKPFR-a2-b5	sp Q9H3R5 CENPH_HUMAN	sp Q92674 CENPI_HUMAN	inter-protei AG_Herzog	183	215	. 33	2718,356	680,597	4	-3,2	1	4 0	,25	201	0,3	26,28	2		
	1 AUTRUULLEYK-KPSGSSQNR-84-b1 1 EIVLOLEKN/DMM-LMLCOANU/001K0 -9 512	spiceH3R5[CENPH_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	51	238	187	2446,277	612,577	4	-1,8	1	5 0	27	373	0,38	23,8	4		
	1 SOOESWDLEEKLLDIRK-I KEDI FREOR-a11-b?	spl Q9H3R5 [CENPH_HUMAN	spl Q9BS16 CENPL_HUMAN	inter-protei AG_Herzog	242	301	55	3568.82	714 774	4	-2	3	4 0	.42	456	0,43	23.08	2	candidate	
	1 EDCASELKVHLAK-KSESSPIVR-a8-b1	sp Q9NSP4 CENPM HUMAN	CENP-C 1-544	inter-protei AG Herzog	45	273	225	2638.359	660.597	4	-1,9	8 1	.5 0	,31	443	0,65	36,66	5	Lannandate	
	1 VHLAKSLPLPSSVNRPR-IIHENIVEKER-a5-b9	sp Q9NSP4 CENPM_HUMAN	sp Q96H22 CENPN_HUMAN	inter-protei AG_Herzog	50	245	195	3386,915	565,494	6	0 1	0	7 0	,49	310	0,87	31,18	2		
	1 EDCASELKVHLAK-RKSESSPIVR-a8-b2	sp Q9NSP4 CENPM_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	45	273	228	2794,462	699,623	4	-1,1	7	7 C	,39	502	0,57	30,66	2		
	1 EDCASELKVHLAK-KPSGSSQNR-a8-b1	sp Q9NSP4 CENPM_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	45	238	193	2596,29	650,08	4	-0,7	3	7 0	,27	395	0,52	28,25	2		
	1 VHLAKSLPLPSSVNRPR-KPSGSSQNR-a5-b1	sp[Q9NSP4]CENPM_HUMAN	CENP-C_1-544	inter-protei AG_Herzog	50	238	188	2967,631	594,534	5	-1,7	1	7 0	,37	334	0,38	25,08	4	1000 T	
	1 EDCASELKVHLAK-SESTLELETVKR-88-B11 1 EDCASELKVHLAK-KSESTLELETVKR-88-B1	SPICENEM_HUMAN	CENP-C_1-544 CENP.C_1.544	inter-protei AG_Herzog	45	2/1	226	3003,591	700,906 620.24F	4	-1,2	-	3 C	,48 26	345	0,55	24,98	1	candidate	
	JERNYTHERN NUM STEPLETYNN 80°UT	-p. across + comm_noMAN			40	200	213		003,345	5	- 4,4	-		,	JA 3	0,05	,	-		