Figure EV1. Alignment of human (h) and mouse (m) Cenp-F C-terminal domains.

Alignments between human and mouse Cenp-F (NCBI Reference Sequence: NP_057427.3 and NP_001074832.2, respectively) were performed based on EMBOSS Matcher. Arrows indicate the boundaries of hCenp-F-Ct, mCenp-F-Ct1, and mCenp-F-Ct2 fragments used in this study (bold font). Human and mouse Cenp-F-SID are in highlighted in yellow. Leucine residues involved in the leucine zippers [10] are highlighted in green; residues involved in the NLSs are in orange [10, 21], KEN7 protein degradation motif in dark blue [49], Rb-binding domain [36] in purple, and C-terminal CAAX farnesylation site [64] in cyan. The minimal kinetochore-binding domain of human Cenp-F is underlined in black [27]. Residues mutated in this study are in red, and the amino acid they are replaced by is indicated below (for mCenp-F) or above (for human Cenp-F) the sequence, along with their position within the sequence.
Figure EV2. Coiled-coil analysis and dimerization properties of mCenp-F C-terminal domain.

A COILS/PCOILS on Bioinformatics Toolkit [55] was launched for mCenp-F [AA 2,401–2,997(end)]. Probabilities of being coiled-coil region based on a window of 14, 21, and 28 residues are shown in green, blue, and red, respectively. The position of mCenp-F-Ct1, mCenp-F-Ct2, and mCenp-F-SID segments is represented. The gray box corresponds to the modeled peptide (mCenp-F-miniSID [aa 2,663–2,706]) presented in (B) and used in (D).

B Top: Heptad position information is shown above the amino acid sequence of mCenp-F-miniSID. Residues in buried positions α and δ of the coiled-coil heptad are highlighted in bold, and underlined when they correspond to leucine (L). Conserved residues (ConSurf server score greater than 6 [65]) are colored in gray. Arrowheads point to the residues mutated in this study using the same color code as in the model. Bottom: Model of the 3D-structure of mCenp-F [AA 2,663–2,706] coiled-coil domain, with the α- and δ-positions of the coiled-coil heptad highlighted as sticks. The side chains of all the residues mutated in the study are shown as spheres on the visible face of the coiled-coil: (i) buried residues L2668 and L2696 which contact themselves in the symmetric molecule of the parallel coiled-coil, (ii) residues L2681 and L2683 which are exposed and conserved hydrophobic residues, and (iii) residues K2678 and R2687 which form potentially stabilizing interactions in the interface of InterEvDock Model 3.

C In vivo crosslinking analyses. Whole-cell extracts from HeLa cells transiently expressing HA-tagged mCenp-F-Ct2-WT, mCenp-F-Ct2-LG/LG (L2668G/L2696G), or mCenp-F-Ct2-LE/LE (L2681E/L2683E) and treated or not with DSS were analyzed by Western blot using anti-HA antibodies. The Ponceau red staining is used as loading control. Molecular masses are indicated (kilodaltons). The arrowhead and arrow point to the position of the mCenp-F-Ct2 monomers and crosslinked dimers, respectively. Source data are available for this Western blot.

D (i) SEC-MALS analysis at 20°C of mCenp-F-miniSID-WT (in green), mCenp-F-miniSID-LE/LE (in blue), or mCenp-F-miniSID-LG/LG (in red) in 1 M NaCl-containing buffer. Differential refractive index curves normalized between 0 and 1 (right y-axis) are plotted for the three peptides as a function of the elution volume. The calculated molar masses (in kD, left y-axis) are plotted as bold lines. For each elution peak, the dashed horizontal line indicates the mass of the molecular species measured (indicated in the corresponding color on the left y-axis). Note that the calculated SEC-MALS molar mass of the WT and LE/LE peptides is intermediate between the theoretical molar mass expected for a monomer (5.1 kD) and a dimer (10.2 kD), most likely reflecting a fast exchange between monomeric and dimeric forms. The WT peptide also exhibits the presence of a minor (~5%) fraction of a tetrameric form. In contrast, the molar mass of the 2,668G/2,696G mutant is consistent with a monomeric form only. (ii) Calculated molar masses in kD as a function of NaCl concentration (0.15, 0.5, 1, and 2 M), reported as colored circles for the three peptides with the same color code as in panel (i). The box indicates the points that were extracted from the 1 M NaCl experiment shown in panel (i).

Source data are available online for this figure.
A The model was colored using Consurf server [65]. With default options, the server found 77 non-redundant proteins homologous to mNup133 with a sequence identity > 35%. Residue conservation is gradient-colored from white (variable) to red (conserved). The original alignment is provided as Appendix Fig S1.

B (i) Yeast co-expressing the indicated preys (pP6-GAL4-AD vectors; empty or containing Cenp-F [Ct1] or [Ct2]) and baits (pB27-LexA vectors, empty (-) or containing various mutant forms of Nup133 β-propeller [aa 67–513]) were grown on -LWH medium. Yeast growth on this medium indicates interaction. The following mutations were introduced within Nup133 [aa 67–513]: Δα1: aa [87–99] are replaced by GlyGlySerGly; ΔL2: aa [162–171] are replaced by GlyGly; Δα2/3: aa [313–335] are replaced by GlySerGlySerGly; ΔL4: aa [360–365] are replaced by GlySerGly. α1-mut: mNup133[aa 67–513]R92D/M96D/T96D; α2/3-mut: mNup133[aa 67–513]E317R/D321R/Y330A. (ii) The expression of the various LexA-Nup133 [aa 67–514] mutants was assessed by Western blot using anti-LexA antibody. Molecular masses are indicated (kilodaltons). Note that the replacement of helices α1 and α2/3 by short Gly-Ser linkers (GGSG and GSGSG, respectively, leading to Δα1 and Δα2/3) likely impaired the folding of the mutant proteins, ultimately resulting in their instability explaining their very low expression levels in yeast. In contrast, the mutation of these helices (α1-mut and Δα2/3-mut) does not alter the expression of the corresponding LexA-Nup133 [aa 67–514] fusions. Source data are available for this Western blot.

Source data are available online for this figure.
Figure EV4. The mCenp-FR2687E mutant interacts, likely in a non-specific manner, with full-length Nup133 but not with its C-terminal domain and localizes in GLFG bodies.

A, B Y2H interactions between LexA alone (−) or fused to mNup133 full-length (FL, [aa 1–1155]) or C-terminal domain (CTD, [aa 501–1155]) and GAL4-AD either alone (−) or fused to mCenp-F-SID (WT or bearing the indicated mutations) or Nup107 [aa 784–925] were assayed based on growth on -LWH medium supplemented, when indicated, by 1 mM 3-Aminotriazole (3AT). Note in (A) that the mCenp-FR2687E mutant, but none of the other mCenp-F mutants assayed in Fig 2B, interacts with full-length mNup133. However, unlike WT mCenp-F-SID, the interaction of the R2687E mutant with full-length mNup133 is abrogated by the addition of 1 mM 3-Aminotriazole, indicating that the mCenp-FR2687E mutant may interact with the full-length mNup133 in a rather weak manner. In (B), note that the lexA-mNup133-CTD construct is transactivating when used in -LWH medium. Under conditions required to prevent this transactivation (i.e., addition of 1 mM 3-Aminotriazole), the interaction of Nup133-Cterm with its established C-terminal partner, Nup107, is preserved, while no interaction was detected between Nup133-Cterm and mCenp-F-SID, either WT or R2687E. This observation is consistent with our previous studies using human Nup133 constructs that indicated that the C-terminal domain of Nup133 does not interact with Cenp-F [13].

C HeLa-E cells transiently transfected with GFP-mCenp-F-C12 R2687E were fixed 2 days after transfection and stained with an antibody directed against Elys that labels the NPCs and the GLFG bodies in interphase cells. The corresponding Western blot is presented in Appendix Fig S3. Scale bar, 10 μm. Note that consistent with the Y2H interaction observed with full-length Nup133 [aa 1–1155], GFP-mCenp-F-C12 R2687E localizes in the GLFG bodies.

Figure EV5. Unlike GFP-mCenp-F-C12 and GFP-mCenp-F-C12, full-length Cenp-F is not targeted to Nup133-labeled intranuclear GLFG bodies present in HeLa-E cells.

A HeLa-E or HEp-2 cells transiently transfected with GFP-mCenp-F-C12 or GFP-mCenp-F-C12 were fixed 2 days after transfection and immunolabeled with rabbit anti-Nup133 and anti-Nup98 antibodies. DNA was stained with DAPI. Projections of three consecutive z-sections covering 0.6 μm are shown. The star indicates that exposure time was increased in the GFP channel for cells transfected with GFP-mCenp-F-C12. Note that GFP-mCenp-F-C12 may reach the nucleus by passive diffusion through the NPC due to its rather small size (47 kDa), whereas the GFP-mCenp-F-C12 fusion that features a bipartite NLS [10,23] (Fig 1A) further accumulates in the nucleolus. Black and white arrowheads indicate GLFG bodies containing or not Nup133. Scale bar, 10 μm.

B, C HeLa-E cells transfected with GFP-mCenp-F-C12 (B) or GFP-hCenp-F (C) were fixed 2 and 1 day after transfection, respectively, and immunolabeled with the indicated antibodies. A single plane is shown except for panels (C, ii) where a projection of 11 z-sections is presented to better visualize the kinetochores (marked by the CREST serum). In (B), threefold magnifications of the marked areas are also presented. Arrows in the C2 cells shown in (B, ii) and (C, ii) (identified based on their strong Cenp-F levels) point to GLFG bodies stained by anti-Nup133 that contain GFP-mCenp-F-C12 but neither endogenous Cenp-F (the anti-Cenp-F antibody used here does not recognize the transfected GFP-mCenp-F-C12 construct) nor GFP-hCenp-F. Arrowheads in the metaphase cell (B, ii) highlight co-localization of GFP-mCenp-F-C12, Nup133, and endogenous Cenp-F at kinetochores. Note that a clear localization of the KT-core domain of hCenp-F was previously reported to require either the internal repeats or a longer C-terminal extension including the C-terminal CAAX farnesylation site that are absent from our construct [27,28]. This minor discrepancy likely reflects the improved detection due to our pre-extraction conditions and the use of a GFP tag that does not rely on antibody detection. The star indicates that the gamma was altered on that image to improve the visualization of GFP-mCenp-F-C12 kinetochore localization. Scale bars, 10 μm.
A

HeLa-E + GFP-mCep-F-Ct1

HeLa-E + GFP-mCep-F-Ct2

Hep2 + GFP-mCep-F-Ct2

B

HeLa-E + GFP-mCep-F-Ct2

GFP/Nup133/Cep-F

GFP

Nup133

Cep-F

DAPI

(i)

(ii)

C

HeLa-E + GFP-hCep-F

(i)

GFP

Nup133

Cep-F

DAPI

(ii)

GFP

CREST

Phospho-H3

DAPI

GFP/CREST

Figure EV5.