Fe-S protein assembly involves bipartite client binding and conformational flexibility in the CIA targeting complex

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Abstract

The cytosolic iron sulfur (Fe-S) assembly (CIA) pathway is required for the insertion of Fe-S clusters into cytosolic and nuclear client proteins, including many DNA metabolism proteins. The molecular mechanisms of client protein recognition and Fe-S cluster transfer remain unknown. Here we report crystal structures of the CIA targeting complex and cryo-EM reconstructions of the complex bound either to the DNA replication factor primase or the DNA helicase DNA2. The structures, combined with biochemical, biophysical and yeast complementation assays, reveal an evolutionarily conserved, bipartite client binding mode facilitated by the structural flexibility of the MMS19 subunit. The primase Fe-S cluster is located ~70 Å away from the catalytic cysteine in the CIA targeting complex, pointing to a conformationally dynamic mechanism of Fe-S cluster transfer. Altogether, our studies suggest a model for Fe-S cluster insertion and thus provide a mechanistic framework to understand the biogenesis of critical DNA replication and repair factors.

1 Main

2 Iron-sulfur (Fe-S) clusters, found in all three domains of life¹, stabilize protein folds,
3 facilitate electron transfer processes, and sense oxygen or iron levels in cells. Many
4 eukaryotic DNA replication and repair proteins, including B-family DNA polymerases
5 α , δ , ϵ and ζ , the DNA primase large subunit (PriL), and DNA helicases XPD, RTEL1,
6 FANCI, DNA2, and DDX11, contain Fe-S clusters²⁻⁸. Although the precise roles of
7 the Fe-S clusters in many of these proteins remain enigmatic, loss of the Fe-S cluster
8 often leads to reduced protein stability and functionality^{7,9,10}, and disease-causing
9 mutations have been mapped to the Fe-S domains of XPD, FANCI, and MUTYH¹¹.

10 The biogenesis of eukaryotic Fe-S proteins is a highly regulated, multi-step
11 process, which is conserved from yeast to human^{12,13}. The mitochondrial iron sulfur
12 cluster (ISC) machinery is required for the biogenesis of all mitochondrial Fe-S
13 proteins, while the cytosolic iron sulfur assembly (CIA) pathway is essential for the
14 maturation of cytosolic and nuclear Fe-S proteins. The CIA pathway depends on the
15 mitochondrial ISC machinery, which is thought to generate a sulfur-containing
16 precursor that is exported into the cytosol¹⁴. As a first step in the CIA pathway, the
17 [4Fe-4S] cluster is assembled on the NUBP1/NUBP2 scaffold complex (Nbp35-Cfd1
18 in yeast)¹⁵⁻¹⁸. The cluster is then thought to be transferred onto the intermediate
19 carrier protein CIAO3 (Nar1 in yeast)^{19,20}. In the next step, the CIA targeting complex
20 (CTC), consisting of MMS19, CIAO1, and CIAO2B (MET18, CIA1, and CIA2B in
21 yeast; Fig. 1a), recognizes client apo-proteins through direct physical interactions
22 and mediates the insertion of the Fe-S cluster. MMS19 is an adaptor subunit required
23 for the recognition of diverse client proteins, including essential DNA replication and
24 repair factors, by direct physical interactions²¹. Cells lacking MMS19 exhibit
25 pleiotropic phenotypes including genome instability due to reduced levels of Fe-S

1 cluster incorporation into DNA metabolism proteins and their consequent
2 destabilization^{22,23}.

3 CIAO2B, the smallest (17.6 kDa) CTC subunit, was previously shown to
4 contain a highly reactive cysteine residue (Cys93 in human and Cys161 in yeast),
5 and is therefore assumed to be the catalytically active component of the CTC²⁴.
6 Although structures of the CIAO2B-homolog FAM96A have been determined²⁵, it
7 remains unclear how exactly CIAO2B might facilitate the transfer of a preassembled
8 Fe-S cluster. CIAO1 contains a seven-bladed WD40-repeat β -propeller domain^{26,27},
9 while MMS19, for which there is no structural information currently available, is
10 predicted to be a large alpha-helical HEAT repeat protein^{10,28}.

11 Despite its essential cellular function, the overall architecture of the CTC, the
12 specific functions of its subunits, and the molecular mechanism of Fe-S cluster
13 transfer remain unclear. Given the structural and functional diversity of Fe-S proteins,
14 and the lack of common sequence motifs or folds for Fe-S cluster coordination, the
15 mechanisms by which the CTC specifically recognizes client proteins and assists in
16 their maturation remain enigmatic. Our integrative structural and biochemical study
17 provides detailed insights into the evolutionarily conserved mechanism of cytosolic
18 and nuclear Fe-S protein biogenesis, and suggests a model for the transfer of Fe-S
19 clusters from the intermediate carrier CIAO3 via the CTC and to diverse client
20 proteins.

21

22 **Results**

23 **Structure of CIAO1-CIAO2B catalytic core**

24 While most Fe-S proteins involved in DNA replication and repair require the
25 CTC adaptor protein MMS19 for their maturation, a subset of client proteins requires
26 only the minimal hetero-dimeric CIAO1-CIAO2B “core” complex^{10,28}. We determined

1 the structure of the *Drosophila melanogaster* minimal core complex by X-ray
 2 crystallography at a resolution of 2.0 Å (Fig. 1b, Extended Data Fig. 1 and Table 1).
 3 The structure reveals that CIAO2B interacts with the narrow-end face of the CIAO1 β-
 4 propeller, through contacts involving CIAO2B helices α3 and α4, with the N-terminal
 5 end of helix α4 positioned above the central cavity of the CIAO1 β-propeller. The
 6 CIAO2B interaction site, which is mostly formed by CIAO1 β-propeller blades 2, 3,
 7 and 4, is highly conserved from yeast to human (Extended Data Fig. 1b-e and Fig.
 8 1c). To verify the interaction, we systematically mutated residues on the narrow-end
 9 face of CIAO1 and performed pull-down assays using insect cells over-expressing
 10 Strep-tagged CIAO2B and His-tagged CIAO1 (Extended Data Fig. 1d). All residues
 11 involved in the interaction with CIAO2B are located in the evolutionarily conserved
 12 patch formed by blades 2, 3, and 4 of CIAO1 and interact with helix α4 of CIAO2B
 13 (Fig. 1c).

14 To assess the functional conservation of the CIAO1–CIAO2B interaction
 15 across species, we used a *Saccharomyces cerevisiae* strain in which expression of
 16 the endogenous *CIA1* gene is repressed in the presence of glucose. The resulting
 17 growth defect can be rescued by complementation with a plasmid containing a
 18 human CIAO1 transgene¹⁰. Complementation with the same set of mutants employed
 19 in our pull-down assay revealed that most residues lining the central cavity of the
 20 CIAO1 β-propeller narrow-end face are essential for yeast viability (Extended Data
 21 Fig. 1f). An additional conserved patch is found on the side of CIAO1 blade 3
 22 (Extended Data Fig. 1b), in close proximity to Cys86 of CIAO2B (human
 23 Cys93^{CIAO2B}). This residue was previously identified in a proteomic screen for highly
 24 reactive cysteines, is strictly required for yeast survival²⁴, and has been implicated in
 25 direct Fe-S cluster coordination and transfer²⁹. Together, these observations suggest

that the conserved patch on blade 3 of CIAO1 might mediate the recruitment of Fe-S client proteins and/or the Fe-S donor CIAO3^{19,30}.

MMS19 interaction with the core complex

Association of the CIAO1-CIAO2B core complex with the adaptor protein MMS19, which is required for the maturation of DNA replication and repair proteins, was shown to require the four C-terminal HEAT-like repeats in MMS19²¹. To visualize the interaction of MMS19 with the core complex, we crystallized a complex consisting of a C-terminal fragment of mouse MMS19 (residues 911-1031, hereafter referred to as MMS19^{CTD}) and *Drosophila* CIAO1-CIAO2B, and determined its structure at a resolution of 2.7 Å (Table 1). The interaction between the catalytic CIAO1-CIAO2B module and the MMS19 adaptor is mediated by the most C-terminal MMS19 HEAT-like repeat (helices $\alpha 45$ and $\alpha 46$), CIAO2B helices $\alpha 1$ and $\alpha 2$, and the loop connecting $\alpha 2$ and $\beta 3$ (Fig. 1d, e). There are no direct contacts between MMS19 and CIAO1, and CIAO2B acts as a bridge. A pull-down experiment using lysates from HEK293 cells expressing Flag-tagged wild-type and mutant human MMS19 and myc-tagged human CIAO1 and CIAO2B (Fig. 1f and Extended Data Fig. 2b) confirmed that the interaction requires helix $\alpha 46$ of MMS19. Two positively-charged patches in MMS19 were also critical for the interaction: K1007/K1008/K1009^{MMS19}, in the loop connecting helices $\alpha 45$ and $\alpha 46$, and R1012/K1013^{MMS19} within helix $\alpha 46$ (Fig. 1f and Extended Data Fig. 2b). These residues were tested in a yeast complementation assay. While yeast MMS19 knock-out strains are viable, they are sensitive to hydroxyurea (HU), a DNA-replication inhibitor¹⁰. Sensitivity was not restored by expression of MMS19 deletion constructs that lacked helix $\alpha 46$, both helices $\alpha 45$ and $\alpha 46$, or by the charge-reversal mutant R1013E/K1014E^{MMS19} (human R1012/K1013^{MMS19}). The effect was less pronounced for the mutant

1 K1008E/K1009E/R1010E^{MMS19} (human K1007/K1008/R1009^{MMS19}, Extended Data
2 Fig. 2c). Together, these results confirm that the molecular architecture of the CTC is
3 conserved across eukaryotes.

4 Helices α 45 and α 46 of MMS19 were previously shown to be a target for
5 ubiquitination by the MAGE-F1-NSE1 E3 ubiquitin ligase³¹, and ubiquitination was
6 blocked by mutating a cluster of five lysine residues (human K993, K1002, K1007,
7 K1008, K1013) to arginine. Based on our structure, ubiquitination of the MMS19^{CTD} at
8 any of these five positions is likely to preclude CIAO2B binding due to steric clashes
9 (Extended Data Fig. 2d). We therefore expect that ubiquitination of MMS19 is
10 incompatible with binding to CIAO1-CIAO2B, thus preventing formation of the CTC.
11 In addition to targeting MMS19 to the proteasome for degradation, leading to the
12 eventual shut-down of the CIA pathway, modification by ubiquitination might therefore
13 provide a regulatory mechanism that immediately inhibits Fe-S protein biogenesis
14 and prevents further transfer of Fe-S clusters into MMS19-dependent client proteins.

16 **Architecture of the CIA targeting complex**

17 To understand the architecture of the complete CTC, we determined the
18 crystal structure of a complex comprising full-length mouse MMS19 and *Drosophila*
19 CIAO1-CIAO2B at a resolution of 3.6 Å (Fig. 2). MMS19 consists of 46 α -helices
20 arranged in 23 tandem HEAT-like repeats, which form an overall left-handed super-
21 helical structure, positioning its N-terminus ~70 Å away from both its C-terminus and
22 the catalytic cysteine of CIAO2B. The structure confirms that CIAO2B interacts with
23 MMS19 solely via the last C-terminal HEAT-like repeat. The remainder of MMS19 is
24 thus accessible for interactions with client proteins or early-acting factors of the CIA
25 pathway.

1 The MMS19-CIAO1-CIAO2B crystal structure has a dimeric CTC
2 arrangement, mediated by the two MMS19 molecules wrapped around each other
3 with an interface area of 2,950 Å², and an extensive interface between the two
4 CIAO2B protomers (1,644 Å² buried surface area, Extended Data Fig. 2h).
5 Interestingly, CTC dimerization positions potential Fe-S cluster coordinating residues
6 C86, H85, and H119 in close proximity, hinting at how Fe-S cluster coordination
7 might be achieved by the CTC (Extended Data Fig. 2i). CTC dimerization is also
8 observed in solution, as indicated by the co-existence of monomeric and dimeric
9 species in an all-mouse CTC sample analyzed by negative-stain electron microscopy
10 (Extended Data Fig. 2j).

11 To gain initial insights into how client proteins might be recognized, we
12 mapped evolutionary conservation of amino acid residues on the surface of the CTC,
13 identifying two highly conserved surfaces patches: one in blade 3 of the CIAO1 β-
14 propeller, and the other within the N-terminal domain (NTD) of MMS19 (Extended
15 Data Fig. 2f). Notably, these two patches flank the presumed catalytic cysteine of
16 CIAO2B. These findings suggest that the CTC contains two binding sites for
17 recruitment of Fe-S client proteins: one located on the side of the β-propeller domain
18 of CIAO1, which might represent a general binding site for all Fe-S client proteins,
19 and a second site within the MMS19 NTD involved in recognizing Fe-S client proteins
20 whose biogenesis depends on the MMS19 adaptor (Extended Data Fig. 2g).
21 Alternatively, the CIAO1 site might be required for interactions with the Fe-S carrier
22 CIAO3, while the MMS19 site might be used solely for recruitment of client proteins.

23

24 **Cooperative binding of client proteins**

25 To assess the contributions of these conserved sites towards recruitment of client
26 proteins, we employed isothermal titration calorimetry (ITC) to analyze the interaction

1 between CTC and primase, a DNA-dependent RNA polymerase consisting of a
2 catalytic subunit (PriS) and a regulatory subunit (PriL) containing a Fe-S cluster. The
3 equilibrium dissociation constant between full-length CTC and primase was 35 nM
4 (Fig. 3a, Extended Data Table 1). In the absence of MMS19, primase binding affinity
5 for the CIAO1-CIAO2B core complex was reduced to 5.4 μ M (Fig. 3b). Without PriL,
6 the PriS subunit still bound to a CIAO1-CIAO2B core complex with an affinity of 3.1
7 μ M (Fig. 3c), indicating that CIAO1 does not directly recognize the primase Fe-S
8 domain. Primase affinity for the MMS19-CIAO2B sub-complex was below the level of
9 detection (Fig. 3d). CTC complexes containing N-terminally deleted MMS19
10 (constructs MMS19³⁸¹⁻¹⁰³⁰ or MMS19⁷¹⁰⁻¹⁰³⁰) displayed substantially reduced affinity
11 for primase (508 and 832 nM, respectively, Fig. 3e-f). Collectively, these
12 observations demonstrate that the interaction with the MMS19 NTD increases the
13 affinity of CTC for primase, and indicate that the bipartite binding mode results in a
14 high-affinity, cooperative interaction. This is consistent with previous studies showing
15 that the additional MMS19 binding surface is necessary for the maturation of DNA
16 replication and repair proteins, while the CIAO1 binding site is sufficient for certain
17 clients such as the metabolic enzyme GPAT^{10,28}.

18

19 **Cryo-EM reconstructions of CTC-client complexes**

20 To directly visualize CTC-client protein interactions, we used single-particle
21 cryo-EM to resolve the architecture of two client protein complexes in which human
22 CTC was bound to the primase PriL-PriS or the DNA helicase DNA2. 2D
23 classification revealed substantial conformational flexibility within MMS19 for both
24 complexes (Fig. 4a-c, Extended Data Fig. 4 and 5). Sorting of particles by 2D and 3D
25 classification followed by multi-body refinement resulted in reconstructions at
26 resolutions of 8.8 Å for CTC-primase, and 11.7 Å for CTC-DNA2 (Fig. 4d-e), which

1 enabled unambiguous docking of the three CTC subunits in both complexes. For the
2 CTC-primase reconstruction, the crystal structure of PriS (PDB 4RR2) was docked
3 into the density directly adjacent to CIAO1, while the N- and C-terminal domains of
4 PriL were docked into the remaining density. For the CTC-DNA2 reconstruction, the
5 precise orientation of DNA2 could not be resolved given its globular shape.
6 Nevertheless, both reconstructions confirm that the two CTC interaction sites in
7 CIAO1 blade 3 and the MMS19 NTD are engaged in client protein binding.

8 Analysis of the CTC-primase and CTC-DNA2 reconstructions reveals that
9 both primase and DNA2 interact with CIAO1 blade 3 (Fig. 4g-h). DNA2 contacts
10 CIAO1 within the conserved patch of blade 3 located towards the narrow-end face of
11 the β -propeller (Extended Data Fig. 1), whereas the charged patch located towards
12 the wide-end face of the β -propeller appears unoccupied (Extended Data Fig. 6b).
13 This is consistent with HEK293 cells pull-down assays, which indicate that mutations
14 in this part of CIAO1 do not abolish DNA2 binding (Extended Data Fig. 6a). In
15 contrast, charge reversal mutations in CIAO1 blade 3 prevent PriL-PriS binding to the
16 CTC (Extended Data Fig. 6). Mutation R125E^{CIAO1}, which reduces viability in yeast ²⁶,
17 abolished the interaction with both primase and DNA2.

18 Notably, comparisons of the CTC-primase and CTC-DNA2 reconstructions
19 reveal that while the conformation of MMS19 in the CTC-primase complex is similar
20 to its arrangement in the CTC crystal structure, MMS19 adopts a distinct
21 conformation in the CTC-DNA2 structure, with the NTD exhibiting a tighter curvature
22 within its helical repeats (Fig. 4f). This conformational change is necessary to
23 accommodate the more globular DNA2 protein, as compared to the elongated PriL-
24 PriS heterodimer. Our cryo-EM reconstructions therefore suggest that conformational
25 plasticity of MMS19 is important for client protein recognition, and likely responsible
26 for the ability of the CTC to recognize and stably interact with diverse client proteins.

1 In agreement with our ITC data, the CTC-primase reconstruction confirms that
2 the client protein binding site on CIAO1, which is directly adjacent to the proposed
3 catalytic cysteine of CIAO2B, is occupied solely by the PriS subunit and does not
4 directly recognize the client protein Fe-S domain. The Fe-S cluster-containing PriL
5 subunit, on the other hand, interacts with the MMS19 NTD. In this arrangement, the
6 Fe-S cluster is positioned ~ 70 Å away from Cys93^{CIAO2B}, an unexpected finding with
7 implications for the mechanism of Fe-S cluster transfer, as discussed below. Given
8 the observed conformational flexibility of MMS19, the CIA targeting complex might
9 undergo rearrangements to mediate Fe-S cluster transfer from CIAO2B into the client
10 protein, or alternatively accommodate the binding of a Fe-S carrier protein to act as
11 bridge between CIAO2B and the client Fe-S domain.

12 Altogether, the structures of CTC-client protein complexes demonstrate
13 considerable conformational plasticity of MMS19, which not only facilitates client
14 protein recognition but also likely contributes to the mechanism of Fe-S cluster
15 transfer from CIAO2B into the client.

16

17 Discussion

18 The targeted insertion of Fe-S clusters into cytosolic and nuclear proteins is a highly
19 regulated process that relies on the CIA pathway and constitutes an essential part of
20 their biogenesis. Despite its critical function in the biogenesis of key DNA replication
21 and DNA repair proteins, the molecular mechanism of the CTC has remained
22 unclear.

23 Here we used an integrative structural and biochemical approach to uncover
24 the molecular architecture of the central component of the CIA pathway at atomic
25 resolution, and to visualize its interactions with client proteins. Our structural analysis
26 reveals that the CTC contains two interaction sites for recognition of client proteins, a

1 high-affinity binding site on CIAO1, which is likely shared among all client proteins,
2 and a second site within the MMS19^{NTD}, which facilitates the recruitment of DNA
3 repair and replication proteins by increasing their binding affinity due to cooperativity
4 between the two sites. Strikingly, the CTC-primase cryo-EM reconstruction reveals
5 that the high-affinity binding site on CIAO1 interacts with the catalytic PriS subunit,
6 which is located ~70 Å away from the Fe-S cluster in the PriL subunit. Thus, our
7 structural analysis reveals that at least for some client proteins, CIAO1 does not
8 directly recognize their Fe-S domains or any structural motifs proximal to the Fe-S
9 cluster. This binding mode is likely universal, given that prior interaction studies on
10 XPD showed that the CTC binding site resides within its arch domain and not within
11 the Fe-S domain³².

12 Comparisons of our cryo-EM reconstructions of CTC in complexes with DNA2
13 or PriL-PriS revealed that the conformational flexibility of MMS19 plays an important
14 role in accommodating diverse clients. While the CIAO1-CIAO2B catalytic core
15 represents a rigid unit, the plasticity of the alpha-helical subunit MMS19 allows the
16 CTC to adapt to diverse molecular shapes of client proteins, and potentially other co-
17 factors required for Fe-S cluster transfer. Thereby, MMS19 bears a striking similarity
18 to other α -helical repeat proteins that rely on structural flexibility to mediate protein-
19 protein interactions in cellular processes ranging from protein (de)phosphorylation to
20 ubiquitination and nuclear transport³³⁻³⁶. Karyopherins, HEAT-repeat proteins that
21 recognize diverse macromolecular cargoes and mediate their transport between the
22 nucleus and the cytoplasm, convert between two functionally and conformationally
23 distinct nuclear and cytoplasmic states, which facilitate cargo binding and
24 release^{34,37}. The HEAT-repeat comprising A subunit of protein phosphatase 2A, a
25 Ser/Thr phosphatase involved in cell cycle regulation, undergoes an extensive
26 conformational rearrangement that moves its NTD by ~60 Å upon interaction with the

1 regulatory B subunit³³. Similar parallels can be drawn between the CTC and other
2 molecular machineries involved in protein modification, such as cullin-like E3
3 ubiquitin ligases, which undergo conformational changes to enable recognition of
4 protein substrates and to facilitate ubiquitin transfer³⁵. In the context of the CIA
5 pathway, the structural plasticity of the CTC is likely not only critical for its ability to
6 recognize diverse client proteins, but may also facilitate large-scale (>50 Å)
7 molecular motions necessary for Fe-S cluster transfer and incorporation.

8 Our findings provide critical structural insights that have implications for the
9 Fe-S cluster transfer mechanisms of the CTC. The MMS19-CIAO1-CIAO2B crystal
10 structure has a dimeric CTC arrangement with an extensive interface between
11 CIAO2B protomers (Extended Data Fig. 2h), and analysis by negative stain EM
12 indicates that the CTC exists in a monomer-dimer equilibrium in solution (Extended
13 Data Fig. 2j). In the homologous CIAO1-CIAO2A system, which is specifically
14 required for the maturation of aconitase, a CIAO1-(CIAO2A)₂ heterotrimer was shown
15 to coordinate a Fe-S cluster under anaerobic conditions²⁹. A similar mode of cluster
16 coordination is likely to occur in the CIAO1-CIAO2B system, as supported by the
17 oligomerization states observed in our structural studies. We propose a mechanism
18 whereby the Fe-S cluster is transferred from an Fe-S carrier protein onto a MMS19-
19 CIAO1-(CIAO2B)₂ complex (Fig. 5). Although the dimeric holo-CTC is incompatible
20 with client protein recruitment due to steric clashes between the client and the
21 second MMS19 molecule, an additional CIAO2B molecule can be readily
22 accommodated by client-bound CTC (Extended Data Fig. 7). Given the distal
23 arrangement of the client Fe-S domain and the catalytic cysteine involved in cluster
24 transfer, CIAO2B dimerization would shield the Fe-S cluster until a client protein is
25 bound. Transfer can then occur either directly across the CTC, relying on MMS19
26 conformational flexibility, or possibly by involving a Fe-S carrier protein such as

1 CIAO3, which might additionally contribute to cluster stability. Upon Fe-S cluster
 2 transfer, CIAO2B would then be released, resulting in the MMS19-CIAO1-CIAO2B-
 3 client product complex observed in our cryo-EM reconstructions. Our model is in
 4 agreement with proteomic studies that showed an excess of CIAO2B over CIAO1
 5 and MMS19 in HeLa cells (176 nM MMS19, 455 nM CIAO1, 1253 nM CIAO2B)³⁸ and
 6 in mouse fibroblasts³⁹. The proposed transfer mechanism thus likely exploits the
 7 compositional and conformational dynamics of the CTC to facilitate client apo-protein
 8 recognition, Fe-S cluster loading, insertion, and finally Fe-S protein release.

9 In conclusion, our structural and functional studies of the CTC visualize the
 10 central component of the CIA machinery and reveal a critical role of conformational
 11 dynamics of its MMS19 subunit for client protein recognition and likely also Fe-S
 12 cluster transfer.

FIGURES AND FIGURE LEGENDS

Fig. 1. | Crystal structures of CIAO1-CIAO2B and MMS19^{CTD}-CIAO1-CIAO2B

complexes. **a**, Domain organization of MMS19, CIAO2B, and CIAO1. The MMS19 fragment contained in the crystal structure in **d** is indicated with a bar above the last three HEAT-like repeats. **b**, Crystal structure of the *Drosophila* CIAO1-CIAO2B complex; *left* side view *right* view from the bottom face of the CIAO1 β-propeller; sheets A through D are labeled in blade 1. **c**, Close-up view of the interface showing CIAO1 amino acid side chains required for interaction, and CIAO2B helix α4. **d**, Crystal structure of the MMS19^{CTD}-CIAO1-CIAO2B complex. **e**, Close-up view of the interface between MMS19^{CTD} and CIAO2B. **f**, Pull-down assay from HEK293 cells using Flag-tagged human MMS19 wt and mutants, and myc-tagged human CIAO2B/CIAO1.

Fig. 2. | Architecture of the full-length CIA targeting complex.

Crystal structure consisting of mouse MMS19 and Drosophila CIAO1-CIAO2B, shown in two orthogonal views.

Fig. 3. | The CIA targeting complex employs cooperative binding via two binding sites on CIAO1 and MMS19 for client protein recognition. ITC binding studies of full-length human CTC and its sub-complexes to full-length client protein primase, or its small subunit PriS. The upper part of each panel shows representative raw ITC data and the lower part shows integrated heat changes. K_d s estimated from the fitted curves are shown as an inset. Interaction between primase and full-length CIA targeting complex consisting of MMS19–CIAO1–CIAO2B (a), the catalytic core module consisting of CIAO1–CIAO2B (b), CIAO1–CIAO2B and PriS (c), MMS19–CIAO2B and primase (d), MMS19³⁸¹⁻¹⁰³⁰–CIAO1–CIAO2B (e), and MMS19⁷¹⁰⁻¹⁰³⁰–CIAO1–CIAO2B (f).

1

2

3 **Fig. 4. I Conformational plasticity of the CIA targeting complex enables**
4 **recognition of diverse client proteins.** **a**, Cryo-EM 2D class averages of CTC–
5 primase. **b**, For comparison, a model of CTC–primase with labelled subunits is
6 shown in cartoon representation. **c**, 2D class averages of CTC–DNA2. **d**,
7 Reconstruction of CTC–primase at 8.8 Å with docked crystal structures of MMS19,
8 CIAO2B, CIAO1, PriS and PriL. **e**, Reconstruction of CTC–DNA2 at 11.7 Å with
9 docked crystal structures of MMS19, CIAO2B, and CIAO1. **f**, View from the bottom of
10 the CTC–DNA2 reconstruction with rigid-body docked MMS19 illustrates the
11 structural rearrangement necessary to accommodate the more globular DNA2 client
12 protein. **g-h** View from the top of the CTC–primase (**g**) and CTC–DNA2 (**h**)
13 reconstruction; the location of blade 3 in CIAO1 is indicated.

14

15

Fig. 5 | Model of Fe-S cluster transfer and client protein binding by the CTC.

The Fe-S cluster is initially transferred from the Fe-S carrier to the CTC, likely involving a second molecule of CIAO2B for stable coordination of the Fe-S cluster (MMS19-CIAO1-(CIAO2B)₂-FeS). Upon dissociation of the Fe-S carrier, a client protein is recruited via the high-affinity binding site on CIAO1 and the second binding site on MMS19. The Fe-S cluster is then transferred from CIAO2B to the client, potentially aided by the highly reactive cysteine of CIAO2B. After transfer of the Fe-S cluster, the second CIAO2B molecule dissociates, resulting in the MMS19-CIAO1-CIAO2B-client complex observed in the cryo-EM reconstructions.

1 **Table 1. Summary of data collection and refinement statistics of the CIAO1-**
2 **CIAO2B, MMS19^{CTD}-CIAO1-CIAO2B, and MMS19-CIAO1-CIAO2B crystal**
3 **structures.**

	CIAO1-CIAO2B	MMS19 ^{CTD} -CIAO1-CIAO2B	MMS19-CIAO1-CIAO2B
Data collection			
Space group	<i>I</i> 4	<i>P</i> 4 ₂ 2	<i>P</i> 2 ₁ 2 ₁ 2 ₁
Cell dimensions			
<i>a</i> , <i>b</i> , <i>c</i> (Å)	125.58, 125.58, 85.49	144.07, 144.07, 166.43	107.40, 140.70, 327.33
α , β , γ (°)	90, 90, 90	90, 90, 90	90, 90, 90
Resolution range (Å)	19.86-2.00 (2.07-2.00) *	19.99-2.65 (2.74-2.65) *	20-3.6 (3.73-3.60) *
<i>R</i> _{sym} or <i>R</i> _{merge}	0.52 (7.74)	0.21 (6.22)	0.28 (10.79)
<i>I</i> / σ <i>I</i>	14.80 (0.72)	23.53 (0.83)	12.09 (0.43)
Completeness (%)	99.38 (95.11)	99.64 (99.98)	99.9 (99.7)
Redundancy	53.9 (47.5)	53.1 (50.4)	41.7 (40.7)
Refinement			
No. reflections			
Total	2,406,129 (202,774)	2,726,174 (252,812)	4,622,817 (454,946)
Unique	44,669 (4,240)	51,309 (5,018)	110,930 (11,172)
<i>R</i> _{work} / <i>R</i> _{free}	18.05 / 20.84	19.77 / 24.22	25.30 / 27.87
No. atoms			
Protein	3,774	9,452	22,972
Water	149	10	
Ligand	1	14	
<i>B</i> -factors			
Protein	53.52	102.99	205.28
Water	45.63	78.18	
Ligand	40.65	108.53	
R.m.s. deviations			
Bond lengths (Å)	0.006	0.002	0.017
Bond angles (°)	0.71	0.51	1.79

*Values in parentheses are for highest-resolution shell.

1 **METHODS**

2 No statistical methods were used to predetermine sample size. The experiments
3 were not randomized. The investigators were not blinded to allocation during
4 experiments and outcome assessment.

5

6 **Protein expression and purification**

7 Full-length human, mouse and *Drosophila* MMS19, CIAO2B and CIAO1 were sub-
8 cloned into pAC-derived vectors⁴⁰, adding either a Strep II or a hexa-histidine tag to
9 their N-terminus, followed by a tobacco etch virus (TEV) protease cleavage site.
10 Point mutants were generated using the Quikchange Site-directed Mutagenesis Kit
11 (Stratagene) according to the manufacturer's protocol. Baculoviruses were produced
12 through recombination in *Spodoptera frugiperda* (Sf9) insect cells (unauthenticated
13 and untested for mycoplasma contamination, Thermo Fisher Scientific B82501)
14 grown in EX-CELL 420 medium (Sigma) by co-transfection of the pAC-derived vector
15 containing the gene of interest and viral DNA⁴¹ using Cellfectin II Reagent (Thermo
16 Fisher Scientific). Following virus amplification in Sf9 insect cells, recombinant CIA
17 complexes and subunits were expressed in *Trichoplusia ni* High-Five insect cells
18 (unauthenticated and untested for mycoplasma contamination, Expression Systems
19 94-002F) infected at a density of 2.0×10^6 ml⁻¹ with the appropriate virus or
20 combination of viruses, and grown in Sf-900 II medium (Thermo Fisher Scientific) at
21 27 °C and 120 rpm. Cells were harvested after 40-48 hours, resuspended in buffer
22 containing 50 mM Tris pH 8.0, 200 mM NaCl, 1 mM TCEP, 2 mM PMSF, and
23 Complete EDTA-free protease inhibitor tablets (Roche), and lysed by sonication. The
24 lysate was clarified by ultracentrifugation at 40,000 g for 40 min. The supernatant
25 was then filtered through Miracloth (Merck) and passed once through an empty

1 Econo-Pac chromatography column (Bio-Rad) before being loaded onto a column
2 packed with the appropriate affinity chromatography matrix.

3 ***MmMMS19-DmCIAO1-CIAO2B***. Full-length mouse MMS19, and *Drosophila*
4 *melanogaster* CIAO2B and CIAO1 were sub-cloned into pAC-derived vectors⁴⁰,
5 adding a Strep II-tag to the N-terminus of MMS19 and a hexa-histidine tag to the N-
6 termini of CIAO2B and CIAO1. The protein complexes were purified using Ni-NTA
7 agarose resin (Qiagen), resulting in a mixture of MMS19-CIAO2B-CIAO1 and
8 CIAO2B-CIAO1 complexes. His- and Strep-tags were removed by cleavage with TEV
9 protease during overnight dialysis against buffer containing 50 mM Tris pH 8.0, 100
10 mM NaCl, 1 mM TCEP. The complex was further purified by Poros HQ ion exchange
11 chromatography (Thermo Fisher Scientific) and a subtractive Ni-NTA affinity step.
12 Trimeric MMS19-CIAO2B-CIAO1 was separated from dimeric CIAO1-CIAO2B by
13 size exclusion chromatography on a Superdex 200 column (GE Life Sciences) in 20
14 mM HEPES pH 7.4, 200 mM NaCl, 1 mM TCEP. The two peaks were pooled
15 separately and concentrated to 27 mg/ml (MMS19-CIAO2B-CIAO1 complex) and 35
16 mg/ml (CIAO1-CIAO2B complex).

17 ***HsMMS19-CIAO2B-CIAO1 and its subcomplexes and truncations***. Human
18 MMS19-CIAO2B-CIAO1, its subcomplexes, truncations, and individual subunits for
19 ITC measurements and electron microscopy analysis, were expressed recombinantly
20 in High-Five insect cells using the baculovirus expression system, and purified as
21 described above for the *MmMMS19-DmCIAO2B-CIAO1* complex by Ni-NTA affinity,
22 Poros HQ ion exchange chromatography, and size exclusion chromatography. Prior
23 to ITC measurements, samples were purified on a Superdex 200 column (GE Life
24 Sciences) in assay buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 3% glycerol, 0.5
25 mM TCEP).

1 ***MmDNA2***. Full-length mouse DNA2 was sub-cloned into a pAC-derived vector,
2 adding a hexa-histidine tag followed by a TEV cleavage site to its N-terminus. The
3 protein was recombinantly expressed in High-Five insect cells using the baculovirus
4 expression system and purified by Ni-NTA affinity chromatography. DNA2-containing
5 fractions were pooled and further purified on a heparin column (HiTrap, GE Life
6 Sciences), followed by size exclusion chromatography on a Superdex 200 column
7 (GE Life Sciences) in buffer containing 20 mM HEPES pH 7.4, 100 mM NaCl, and 1
8 mM TCEP.

9 ***HsPriL-PriS and HsPriS***. Human primase was expressed and purified as
10 described^{42,43}, with minor modifications. For expression of PriL-PriS, plasmids
11 pBG100-p48 and pETDuet-p58 (a kind gift from W. Chazin) were transformed into
12 BL21 Star (DE3) cells (Thermo Fisher). The culture was grown in TB medium at 37
13 °C and the temperature was lowered to 18 °C when the cell density approached
14 OD₆₀₀ of 0.6. Expression was induced with 1 mM IPTG and continued for 20 hours at
15 18 °C. Upon induction, the culture medium was supplemented with 0.1 mg/ml ferric
16 citrate. Cells were harvested, resuspended in buffer containing 50 mM Tris pH 8.0,
17 150 mM NaCl, 3% glycerol, 1 mM TCEP, 2 mM PMSF, Complete EDTA-free
18 protease inhibitor tablets (Roche) and snap frozen in liquid nitrogen. Cells were lysed
19 using an Emulsiflex C3 homogenizer (Avestin). The lysate was cleared by
20 ultracentrifugation and by passing it through a 5 µm filter before being loaded onto a
21 Ni-NTA affinity column (Qiagen). The eluate was further purified on a heparin column
22 and a Superdex 200 column (GE Life Sciences), eluting in buffer containing 20 mM
23 HEPES pH 7.4, 150 mM NaCl, 3% glycerol, and 1 mM TCEP. For expression of
24 human PriS, plasmid pBG100-p48 was transformed into BL21 Star (DE3) cells
25 (Thermo Fisher). The culture was grown in TB medium as described above for the
26 PriL-PriS complex. Expression was induced with 1 mM IPTG and continued for 20

1 hours at 18 °C. Cells were harvested, resuspended and lysed, and PriS was purified
2 as described above for the PriL-PriS complex.

3

4 **Crystallization and data collection**

5 Crystallization experiments were performed in MRC 2-well crystallizations plates
6 (Swissci) with a Phoenix dispensing robot (Art Robbins) using the sitting-drop vapor
7 diffusion method at 20 °C.

8 X-ray diffraction data were collected at beam lines X06DA (PXIII) and X10SA (PXII)
9 of the Swiss Light Source (Paul Scherrer Institute, Villigen, Switzerland) at a
10 wavelength of 1 Å (unless noted otherwise below) from crystals cooled to 100 K.
11 Diffraction data were processed using XDS or autoproc^{44,45}. Data collection and
12 refinement statistics are summarized in Extended Data Table 1.

13 ***DmCIAO1-CIAO2B***. Crystals of *Drosophila* CIAO1-CIAO2B were grown in 400 nl
14 hanging drops containing equal volumes protein solution at 35 mg/ml and a reservoir
15 solution consisting of 0.1 M Tris pH 8.5 and 25% PEG MME 2k. Crystals appeared
16 within hours, grew to their final size within 2 days, and were harvested after 7 days
17 and flash cooled in liquid nitrogen.

18 ***MmMMS19^{CTD}-DmCIAO1-CIAO2B***. Crystals of a chimeric complex composed of
19 mouse MMS19^{CTD} (residues 911-1031) and the *Drosophila* CIAO1-CIAO2B complex
20 were grown in 600 nl hanging drops containing 400 nl protein solution at 7.8 mg/ml
21 and 200 nl reservoir solution consisting of 0.2 M KSCN and 20% PEG 3350. Crystals
22 appeared after 4 days and were harvested after 6 days using 20% ethylene glycol as
23 a cryo-protectant.

24 ***MmMMS19-DmCIAO1-CIAO2B***. Crystals of a chimeric complex composed of mouse
25 MMS19 and the *Drosophila* CIAO1-CIAO2B complex were grown in 600 nl hanging
26 drops containing 400 nl protein solution at 27 mg/ml and 200 nl reservoir solution

1 consisting of 100 mM HEPES pH 7.5 and 25% PEG 3350. Crystals appeared after 2
2 days, grew to their final size within 4 days, and were harvested after 6 days. Crystals
3 were cryo-protected in a buffer containing 100 mM HEPES pH 7.5, 30% PEG 3350,
4 and 10% ethylene glycol, and flash cooled in liquid nitrogen.

5 ***MmMMS19-MmCIAO1-CIAO2B***. For experimental phasing, crystals of the all-mouse
6 *MmMMS19-MmCIAO1-CIAO2B* complex were grown in 600 nl hanging drops
7 containing 400 nl protein solution at 12 mg/ml and 200 nl reservoir solution consisting
8 of 100 mM HEPES pH 7.0, 207 mM ammonium citrate, 21% PEG 3350, and 0.5%
9 jeffamine ED-2001. For a native sulfur single wavelength anomalous dispersion (S-
10 SAD) experiment, data were collected at 5975 eV (2.0751 Å). For phasing, crystals
11 were derivatized by soaking in mother liquor supplemented with hexatantalum
12 tetradecabromide (Jena Bioscience) prior to harvesting. Diffraction data were
13 collected at the Ta L-III peak absorption edge (9880.5 eV, 1.255 Å, $f' = -17.27$, $f'' =$
14 23.25).

16 **Crystal structure determination and refinement**

17 Crystals of the *DmCIAO1-CIAO2B* complex diffracted to 2.0 Å and belonged to the
18 tetragonal space group I4. The structure was solved by molecular replacement in
19 Phaser, as implemented in Phenix^{46,47}, using the structure of human CIAO1 as a
20 search model (PDB 3FM0,²⁷). Model building was performed in Coot⁴⁸. The final
21 model was refined in Phenix to an R_{work} factor of 18.05% and an R_{free} factor of
22 20.84%, and contains residues 2-17 and 32-155 of CIAO2B, and 2-335 of CIAO1
23 (Table 1). The asymmetric unit contained one copy of the *DmCIAO1-CIAO2B*
24 heterodimer, with residues 2-17 of CIAO2B packing against a crystallographic
25 symmetry-related copy of CIAO2B. Analysis with Molprobity^{49,50} shows 98.5% of all
26 residues within favoured regions of the Ramachandran plot with one outlier.

1 Crystals of the *MmMMS19*^{CTD}-*DmCIAO1*-CIAO2B complex diffracted to 2.7 Å and
 2 belonged to the tetragonal space group P4₂2₁. The structure was determined by
 3 molecular replacement in Phaser as implemented in Phenix^{46,47}, using the structure
 4 of the *Drosophila* CIAO1-CIAO2B complex as a search model. The asymmetric unit
 5 contains two copies of the *MmMMS19*^{CTD}-*DmCIAO1*-CIAO2B complex. Iterative
 6 model building and refinement were carried out with the programs Coot and Phenix.
 7 The model was refined to an R_{work} factor of 19.77% and an R_{free} factor of 24.22%
 8 (Table 1), and contains residues 2-21 and 28-155 of CIAO2B, residues 2-335 of
 9 CIAO1, and residues 913-1031 of MMS19. Analysis with Molprobity shows 97.9% of
 10 all residues within favoured regions of the Ramachandran plot with no outliers.

11 Crystals of the *MmMMS19*-*DmCIAO1*-CIAO2B and the *MmMMS19*-*MmCIAO1*-
 12 CIAO2B complex belonged to the orthorhombic space group P2₁2₁2₁ and contained
 13 two copies of the MMS19-CIAO2B-CIAO1 complex in the asymmetric unit. Best
 14 diffraction was obtained from a crystal of *MmMMS19*-*DmCIAO1*-CIAO2B complex to
 15 3.6 Å.

16 The structure was solved by single-wavelength anomalous dispersion (SAD) phasing
 17 using a dataset obtained from Ta₆Br₁₂-derivatized crystals of the *MmMMS19*-
 18 *MmCIAO1*-CIAO2B complex. Heavy atom search was performed in SHELXD and
 19 phasing was carried out in SHARP^{51,52}. The resulting phases were improved by
 20 density modification with Parrot as implemented in ccp4⁵³. The resulting map
 21 revealed clear density corresponding to MMS19, into which ideal poly-serine α-
 22 helices were placed. The model was then refined against using deformable elastic
 23 network (DEN) restraints in CNS solve^{54,55}, which led to major improvement of the
 24 maps, enabling placement of CIAO2B, CIAO1 and the *MmMMS19*^{CTD} fragment using
 25 the higher-resolution *DmCIAO1*-CIAO2B and *MmMMS19*^{CTD}-*DmCIAO1*-CIAO2B
 26 structures.

1 The resulting model was completed by iterative building and refinement against the
 2 native *MmMMS19-DmCIAO1-CIAO2B* crystal data. The amino acid sequence of
 3 MMS19 was assigned based on bulky density visible for large hydrophobic side
 4 chains, as well as information on the location of cysteine and methionine residues
 5 determined using native sulfur SAD phasing data obtained from a crystal of the
 6 *MmMMS19-MmCIAO1-CIAO2B* complex. Further iterative cycles of refinement and
 7 model building were carried out initially with autoBUSTER⁵⁶, applying NCS restraints,
 8 target restraints to the MMS19^{CTD}-CIAO1-CIAO2B structure applied as LSSR, and
 9 TLS refinement. Subsequent refinement in Phenix, using NCS and target restraints
 10 as well as TLS refinement, produced a final model with an R_{work} factor of 25.3% and
 11 an R_{free} factor of 27.9% (Extended Data Table 1). The final model contains residues
 12 2-155 of CIAO2B, 2-335 of CIAO1, and 15-43, 46-539, 548-638, 649-699 (chain C),
 13 649-696 (chain F), and 702-1028 of MMS19. Analysis with Molprobity shows 95.3%
 14 of all residues within favoured regions of the Ramachandran plot with 0.07% outliers.

16 **Electron microscopy sample preparation and data collection**

17 For negative stain analysis, human MMS19-CIAO2B-CIAO1 complex was mixed with
 18 a 1.4-molar excess of mouse DNA2 or human PriL-PriS, incubated on ice for 30 min,
 19 and purified on a Superose 6 10/300 column (GE Healthcare) in 20 mM HEPES, 75
 20 mM NaCl, and 1 mM TCEP. The chromatogram and analysis by SDS-PAGE
 21 indicated that the complexes were stoichiometric and homogeneous (Extended Data
 22 Fig. 4a-b and 5a-b). However, initial characterization of a uranyl-formate stained
 23 sample from the peak fraction revealed that the complex was extremely fragile and
 24 prone to disintegration on the EM grid. The complex was stabilized by cross-linking
 25 on ice for 10 min with 0.5% (final) glutaraldehyde (Ted Pella). Continuous carbon
 26 grids (Ted Pella, 01840-F) were glow-discharged for 90 sec at 15 mA using an

1 easiGlow glow discharge cleaning system (PELCO). The sample was diluted to ~70
2 nM and 4 μ l were incubated on an EM grid for 1 min. After blotting with filter paper,
3 the grids were subsequently laid onto 5 drops of 1% (w/v) uranyl formate solution.
4 Data were collected on a Tecnai Spirit transmission electron microscope (Thermo
5 Fisher) operating at 120 kV with a nominal magnification of 49,000x using EPU
6 (Thermo Fisher), and recorded on a FEI Eagle 4k CCD camera, with a final pixel size
7 of 1.52 Å.

8 For cryo-EM analysis, the sample was prepared and cross-linked as above, and
9 diluted to 0.3 mg/ml (CTC-primase) or 0.4 mg/ml (CTC-DNA2). 4 μ l sample were
10 applied onto an UltraAuFoil gold grid (R 1.2/1.3, Quantifoil) prepared with a thin
11 home-made carbon film floated on top, and glow-discharged using the easiGlow glow
12 discharge cleaning system for 90 s at 15 mA. The sample was incubated on the grid
13 for 30 s, blotted for 3 s at force setting -1, and plunge frozen in liquid ethane using a
14 Vitrobot Mark IV (Thermo Fisher).

15 Data were collected on a Cs-corrected (CEOS GmbH) Titan Krios transmission
16 electron microscope (Thermo Fisher) operating at 300 kV using EPU (Thermo
17 Fisher). The CTC-primase dataset was recorded at a nominal magnification of 130k
18 on a Falcon II direct electron detector, resulting in a final pixel size of 0.87 Å.

19 Exposures were fractionated into 50 frames, with a total dose of 50 $e^- \text{Å}^{-2}$. A total of
20 3,128 movies were acquired at a defocus range from -0.6 to -3 μ m. The CTC-DNA2
21 dataset was recorded using a Volta phase plate on a Gatan K2 summit direct
22 electron detector with a Quantum LS energy filter (slit width 20 eV), at a nominal
23 magnification of 105k, resulting in a final pixel size of 1.1 Å. Exposures were
24 fractionated into 40 frames, with a total dose of 39.9 $e^- \text{Å}^{-2}$. A total of 1,948 movies
25 were acquired at a defocus range from -0.4 to -0.8 μ m.

26

1 **Electron microscopy data processing**

2 For negative stain data, particles were picked automatically using DoG picker⁵⁷.
 3 Particles were extracted, and 2D classification, initial model generation and 3D
 4 refinement performed in SPARX and Relion⁵⁸⁻⁶⁰. For cryo-EM data, particles were
 5 picked with Gautomatch (<http://www.mrc-lmb.cam.ac.uk/kzhang/>), using templates
 6 based on low-pass filtered 2D class averages from a previous data set collected
 7 using a Volta phase plate. Concomitantly with data collection, movies were motion
 8 corrected using MotionCor2⁶¹, and CTF parameters were estimated using Gctf⁶².
 9 Using cryoflare, an in-house software wrapper for on-the-fly processing, data quality
 10 was assessed and suitable micrographs were selected for further processing
 11 (www.cryoflare.org). 2D and 3D classification, as well as 3D refinement and multi-
 12 body refinement were performed in Relion. For multi-body refinement, partially
 13 overlapping masks were employed that contained the CIAO1-CIAO2B-DNA2
 14 subcomplex and MMS19-CIAO2B, or the CIAO1-CIAO2B-PriS-PriL and MMS19-
 15 CIAO2B subcomplexes, respectively. For details of the data processing scheme, see
 16 Extended Data Figure 4 (CTC-primase) and Extended Data Figure 5 (CTC-DNA2).
 17 Both CTC-primase and CTC-DNA2 samples showed preferred orientation (Extended
 18 Data Fig. 4g and 5g), which could not be overcome in the presence of a range of
 19 different detergents, or by glow-discharging in the presence of amylamine.

20

21 **ITC measurements**

22 Protein samples were purified on a Superdex 200 column (GE Life Sciences) in
 23 assay buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 3% glycerol, 0.5 mM TCEP)
 24 prior to ITC measurements. ITC measurements were performed at 25 °C using a
 25 Microcal VP-ITC Calorimeter (Malvern) with a stirring speed of 307 rpm and a
 26 reference power of 10 $\mu\text{Cal s}^{-1}$. Experimental parameters for calorimetric titrations of

the CIA complex and its subcomplexes (in the syringe) and primase (in the cell) were optimized for each sample. Data were analyzed with MicroCal ITC Origin 7 analysis software (Origin-based software provided by Malvern).

Pull-down assays in HEK293 cells

HEK Expi 293 cells (unauthenticated and untested for mycoplasma contamination, Thermo Fisher Scientific 14527) were grown in FreeStyle 293 expression medium (Thermo Fisher Scientific) at 37 °C, 85% humidity, and 135 rpm. All plasmids used for transfection were based on the GATEWAY system (Invitrogen), contained a cytomegalovirus (CMV) promoter, and expressed either Flag- or myc-tagged protein constructs (see Extended Data Table 3 for details). Cells were seeded at 0.6×10^6 ml⁻¹ 24 h prior to transfection. For transfection of 10 ml cells in suspension, 10 µg of DNA were diluted into 400 µl of Opti-MEM reduced serum media (Thermo Fisher Scientific). In a separate tube, 25 µl of polyethylenimine (Polysciences) at 1 mg/ml were diluted into 400 µl of Opti-MEM reduced serum media and then added to the DNA solution. The transfection mix was added drop-wise to the cells. Cells were harvested after 42 hours, resuspended in a buffer containing 50 mM Tris pH 8.0, 200 mM NaCl, 5% glycerol, 1 mM TCEP, 0.1% Triton-X-100, 2 mM PMSF, Complete EDTA-free protease inhibitor tablets (Roche), and lysed by sonication. Clarified lysates were incubated with Flag-M2 beads (Sigma-Aldrich) for 2 hours at 4 °C. After extensive washing, bound proteins were eluted in buffer containing 0.5 mg/ml 1x Flag peptide (Sigma-Aldrich) for 2 hours at 4 °C. Samples were analyzed by SDS-PAGE and Western blotting using Anti-Flag M2-peroxidase (HRP) antibody (Sigma-Aldrich), or c-Myc tag antibody (Genescript) and Anti-mouse IgG-peroxidase antibody (Sigma-Aldrich), using Clarity ECL Western Blotting Substrate (Bio-Rad).

1 **Yeast drop assays**

2 Yeast were grown in YPAD or minimal medium with appropriate selection markers at
3 30 °C. For details of haploid *S. cerevisiae* strains see Extended Data Table 4.
4 Plasmids were transformed into yeast using a standard protocol using lithium
5 acetate, single-stranded carrier DNA and PEG 4000. For drop assays, overnight
6 cultures of yeast cells were diluted to an OD₆₀₀ of 0.7. Five 10-fold serial dilutions
7 were prepared, and 2 µl each were dropped onto selective plates. Plates were
8 incubated at 30 °C and imaged after 2-3 days.

9

10 **Illustrations and figures**

11 Sequence alignments were generated using Clustal Omega⁶³ and colored with
12 ESPript⁶⁴. Structure figures were generated with PyMOL (www.pymol.org) and
13 Chimera⁶⁵.

14

15 **Data availability**

16 The atomic coordinates and structure factors reported in this study have been
17 deposited in the Protein Data Bank (PDB) under accession numbers 6TBN (CIAO1-
18 CIAO2B complex), 6TBL (MMS19^{CTD}-CIAO1-CIAO2B complex), and 6TC0 (MMS19-
19 CIAO1-CIAO2B complex). The cryo-EM density maps have been deposited in the
20 Electron Microscopy Data Bank (EMDB) under accession codes EMD-XXX (CTC-
21 primase) and EMD-XXX (CTC-DNA2).

22

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24 acquisition, S.K. and N.T; Investigation, S.K.; Methodology, S.K.; Project
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1 and N.T; Visualization, S.K.; Writing - original draft, S.K.; Writing - review and editing,
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6

7 **Competing interests**

8 The authors declare no competing interests.

9

10 **Supplementary Information** is available for this paper.

SUPPLEMENTARY INFORMATION

Extended Data Fig. 1 | Crystal structure of the CIAO1-CIAO2B core complex. a, Coomassie-stained SDS-PAGE of purified *Drosophila melanogaster* CIAO1-CIAO2B complex. **b,** Surface representation of CIAO1 colored according to amino acid conservation across species based on the alignment in **d**. **c,** Surface representation of CIAO1 colored according to electrostatic potential from -3 to +3 k_BT/e. **d,** CIAO1 sequence alignment used to map evolutionary conservation in **a**. Secondary structure elements of human CIAO1 are indicated above the alignment. Numbering is relative to the human sequence. **e,** Strep pull-down assay from Hi5 insect cells expressing

1 Strep-CIAO2B and His-CIAO1 wt or indicated mutants. **f**, Yeast drop assay of CIAO1
2 point mutations located at the top face of the beta-propeller. A Gal-CIA1 strain was
3 transformed with either empty vector, wt human CIAO1, or a CIAO1 point mutant; a
4 dilution series of each culture was spotted on agar plates in the presence of glucose
5 or galactose.

Extended Data Fig. 2 | Crystal structures of the MMS19-CIAO2B-CIAO1 CIA targeting complex. **a**, Coomassie-stained SDS-PAGE gels of purified MMS19^{CTD}-CIAO2B-CIAO1 and MMS19-CIAO2B-CIAO1 complexes. **b**, Pull-down assay from HEK293 cells using Flag-tagged human MMS19 wt and mutants, and myc-tagged human CIAO2B/CIAO1. WCE (right) and eluted proteins (IP:Flag, left) were analyzed by Western blot. Corresponding to Fig. 1f. **c**, Yeast drop assay of MMS19 mutations. A MMS19 knockout strain was transformed with either empty vector, wt MMS19, or a MMS19 mutant; a dilution series of each culture was spotted on agar plates in the presence or absence of galactose and 20 mM HU. **d**, Lysine residues implicated as targets for ubiquitination by MAGE-F1-NSE1 are shown in green. **e**, Crystal structure

1 of the CIA targeting complex shown in the same orientation as in **f**. **f**, Conservation of
2 amino acid residues across species mapped onto the surface of the CTC structure.
3 **g**, Schematic indicating the location of the two client-protein binding sites as deduced
4 from analysis of the crystal structure of the CIA targeting complex. **h**, Cartoon
5 representation of the dimeric crystal structure of *Mm*MMS19-*Dm*CIAO2B-CIAO1
6 complex as observed in the crystal. **i**, Close-up view of the CIAO2B-CIAO2B dimer
7 interface. Potential Fe-S cluster-coordinating residues H85, C86, and H119 are
8 shown as sticks. **j**, Reference-free 2D class averages of a negatively stained mouse
9 MMS19-CIAO2B-CIAO1 sample showing monomeric (top) and dimeric (bottom) CIA
10 targeting complexes. *Scale bar represents 100 Å*.

1
2 **Extended Data Fig. 4 | Cryo-EM reconstruction of a CTC-primase complex.** **a**,
3 Size exclusion chromatography profile of assembled CTC-primase complex. **b**,
4 Coomassie-stained SDS-PAGE of CTC-primase complex. **c**, Negative stain 2D class
5 averages of the CTC-primase complex. **d**, Cryo-EM micrograph of CTC-primase
6 sample. **e**, Representative 2D class averages of the CTC-primase data set. **f**,
7 Overview of data processing and classification scheme. **g**, Angular distribution of
8 particle orientations in the reconstruction. **h**, FSC plot for half-maps of the
9 reconstruction, 0.143 FSC criterion is indicated.

Extended Data Fig. 5 | Cryo-EM reconstruction of a CTC-DNA2 complex. **a**, Size exclusion chromatography profile of assembled CTC-DNA2 complex. **b**, Coomassie-stained SDS-PAGE of CTC-DNA2 complex. **c**, Negative stain 2D class averages of the CTC-DNA2 complex. **d**, Cryo-EM micrograph of CTC-DNA2 sample. **e**, Representative 2D class averages of the CTC-DNA2 data set. **f**, Overview of data processing and classification scheme. **g**, Angular distribution of particle orientations in the reconstruction. **h**, FSC plot for half-maps of the reconstruction, 0.143 FSC criterion is indicated.

1

2 **Extended Data Fig. 6 | Pull-down assays in mammalian cells pinpoint blade 3 in**
3 **CIAO1 as the main interaction site for recruitment of client proteins. a**, Co-
4 expression and co-IP of Flag-MMS19 with myc-CIAO2B, wild-type or mutant myc-
5 CIAO1, and either myc-PriL/S or myc-DNA2 in HEK293 cells. **b**, Location of mutated
6 amino acid residues around blade 3 of CIAO1. The inset shows the location of the
7 conserved and charged patches in blade 3 of CIAO1.
8

Extended Data Fig. 7 | Superposition of the CTC dimer found in the crystal structure and the CTC-primase complex observed by cryo-EM. a, Superposition of the CTC dimer with the CTC-primase model results in significant steric clashes between the second MMS19 molecule (grey) and the large subunit of primase. **b**, Superposition of the CIAO1-CIAO2B complex shows that the catalytic core can be readily accommodated in between the CTC and primase without any steric clashes.

1 **Extended Data Table 1 | Summary of isothermal titration calorimetry**
2 **experiments.**

3

Syringe	Cell	K _d [μM]	N	Δ H [kcal/mol]	Δ S [cal/mol/deg]
MMS19-CIAO1-CIAO2B	PriS-PriL	0.04	1.02	- 46.5	- 122
CIAO1-CIAO2B	PriS-PriL	4.3	1.08	- 25.4	- 60.9
CIAO1-CIAO2B	PriS	3	0.95	- 33.23	- 86.2
MMS19-CIAO2B	PriS-PriL	n.d.	n.d.	n.d.	n.d.
MMS19 ³⁸¹⁻¹⁰³⁰ -CIAO1-CIAO2B	PriS-PriL	0.56	0.97	- 32.16	- 79.3
MMS19 ⁷¹⁰⁻¹⁰³⁰ -CIAO1-CIAO2B	PriS-PriL	1.08	1.11	- 32.25	- 80.9

4

1 **Extended Data Table 2 | Plasmids used in this study.**
2

Plasmid	Description	Source
pSK23	Human MMS19 with N-terminal Strep-tag and TEV site in pAC8Red vector. Expression vector for full-length human MMS19.	This study.
pSK26	Human CIAO2B with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length human CIAO2B.	This study.
pSK28	Human CIAO1 with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length human CIAO1.	This study.
pSK69	Mouse MMS19 with N-terminal Strep-tag and TEV site in pAC8Red vector. Expression vector for full-length mouse MMS19.	This study.
pSK72	Mouse CIAO2B with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length mouse CIAO2B.	This study.
pSK76	Mouse CIAO1 with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length mouse CIAO1.	This study.
pSK129	Human MMS19 ^{381-end} with N-terminal Strep-tag and TEV site in pAC8Red vector. Expression vector for human MMS19 residues 381-end.	This study.
pSK131	Human MMS19 ^{710-end} with N-terminal Strep-tag and TEV site in pAC8Red vector. Expression vector for human MMS19 residues 710-end.	This study.
pSK204	Drosophila melanogaster CIAO2B with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length Drosophila CIAO2B.	This study.
pSK206	Drosophila melanogaster CIAO1 with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length Drosophila CIAO1.	This study.
pSK259	Drosophila melanogaster CIAO1 F79A with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length Drosophila CIAO1 with F79A mutation.	This study.
pSK260	Drosophila melanogaster CIAO1 W18A with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length Drosophila CIAO1 with W18A mutation.	This study.
pSK261	Drosophila melanogaster CIAO1 Y256A with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length Drosophila CIAO1 with Y256A mutation.	This study.
pSK262	Drosophila melanogaster CIAO1 W198A with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length Drosophila CIAO1 with W198A mutation.	This study.
pSK263	Drosophila melanogaster CIAO1 Y168A with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length Drosophila CIAO1 with Y168A mutation.	This study.
pSK264	Drosophila melanogaster CIAO1 R123E with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length Drosophila CIAO1 with R123E mutation.	This study.
pSK265	Drosophila melanogaster CIAO1 R63E with N-terminal His-tag and TEV site in pAC8Red vector. Expression	This study.

	vector for full-length <i>Drosophila</i> CIAO1 with R63E mutation.	
pSK266	<i>Drosophila melanogaster</i> CIAO1 D324K with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length <i>Drosophila</i> CIAO1 with D324K mutation.	This study.
pSK267	<i>Drosophila melanogaster</i> CIAO1 D305K with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length <i>Drosophila</i> CIAO1 with D305K mutation.	This study.
pSK268	<i>Drosophila melanogaster</i> CIAO1 N307A with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length <i>Drosophila</i> CIAO1 with N307A mutation.	This study.
pSK269	<i>Drosophila melanogaster</i> CIAO1 K152E with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length <i>Drosophila</i> CIAO1 with K152E mutation.	This study.
pSK270	<i>Drosophila melanogaster</i> CIAO1 K107E with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length <i>Drosophila</i> CIAO1 with K107E mutation.	This study.
pSK328	Mouse DNA2 with N-terminal His-tag and TEV site in pAC8Red vector. Expression vector for full-length mouse DNA2.	This study.
pSK358	Yeast MMS19 (MET18) in p415GAL1 vector. Expression of full-length yeast MMS19 in yeast.	This study.
pSK434	Human CIAO1 in p416-CYC1 vector. Expression of full-length human CIAO1 in yeast.	This study.
pSK448	Human CIAO1 E137A, D138A, E139A, E141A in p416-CYC1 vector. Expression of full-length human CIAO1 with mutations E137A, D138A, E139A, E141A in yeast.	This study.
pSK450	Human CIAO1 E137K, D138K, E139K, E141K in p416-CYC1 vector. Expression of full-length human CIAO1 with mutations E137K, D138K, E139K, E141K in yeast.	This study.
pSK453	Human CIAO1 W20A in p416-CYC1 vector. Expression of full-length human CIAO1 with mutation W20A in yeast.	This study.
pSK454	Human CIAO1 R65E in p416-CYC1 vector. Expression of full-length human CIAO1 with mutation R65E in yeast.	This study.
pSK455	Human CIAO1 F81A in p416-CYC1 vector. Expression of full-length human CIAO1 with mutation F81A in yeast.	This study.
pSK456	Human CIAO1 K109E in p416-CYC1 vector. Expression of full-length human CIAO1 with mutation K109E in yeast.	This study.
pSK457	Human CIAO1 R125E in p416-CYC1 vector. Expression of full-length human CIAO1 with mutation R125E in yeast.	This study.
pSK458	Human CIAO1 K154E in p416-CYC1 vector. Expression of full-length human CIAO1 with mutation K154E in yeast.	This study.
pSK459	Human CIAO1 Y170A in p416-CYC1 vector. Expression of full-length human CIAO1 with mutation Y170A in yeast.	This study.
pSK460	Human CIAO1 W198A in p416-CYC1 vector. Expression of full-length human CIAO1 with mutation W198A in yeast.	This study.

pSK461	Human CIAO1 Y256A in p416-CYC1 vector. Expression of full-length human CIAO1 with mutation Y256A in yeast.	This study.
pSK462	Human CIAO1 D305K in p416-CYC1 vector. Expression of full-length human CIAO1 with mutation D305K in yeast.	This study.
pSK463	Human CIAO1 N307A in p416-CYC1 vector. Expression of full-length human CIAO1 with mutation N307A in yeast.	This study.
pSK464	Human CIAO1 D324K in p416-CYC1 vector. Expression of full-length human CIAO1 with mutation D324K in yeast.	This study.
pSK465	Human CIAO1 E133A, E137A, D138A, E139A, E141A in p416-CYC1 vector. Expression of full-length human CIAO1 with mutations E133A, E137A, D138A, E139A, E141A in yeast.	This study.
pSK466	Human CIAO1 E133K, E137K, D138K, E139K, E141K in p416-CYC1 vector. Expression of full-length human CIAO1 with mutations E133K, E137K, D138K, E139K, E141K in yeast.	This study.
pSK468	Human DNA2 in pDEST-myc vector, for expression of N-terminally myc-tagged human DNA2 in human cells.	This study.
pSK472	Human PriS in pDEST-myc vector, for expression of N-terminally myc-tagged human PriS in human cells.	This study.
pSK474	Human PriL in pDEST-myc vector, for expression of N-terminally myc-tagged human PriL in human cells.	This study.
pSK475	Human MMS19 K1007A, K1008A, R1009A in pDEST-Flag-pCR3 vector, for expression of N-terminally Flag-tagged MMS19 with mutations K1007A, K1008A, R1009A in human cells. Based on pKG101.	This study.
pSK476	Human MMS19 K1007E, K1008E, R1009E in pDEST-Flag-pCR3 vector, for expression of N-terminally Flag-tagged MMS19 with mutations K1007E, K1008E, R1009E in human cells. Based on pKG101.	This study.
pSK477	Human MMS19 R1012A, K1013A in pDEST-Flag-pCR3 vector, for expression of N-terminally Flag-tagged MMS19 with mutations R1012A, K1013A in human cells. Based on pKG101.	This study.
pSK478	Human MMS19 R1012E, K1013E in pDEST-Flag-pCR3 vector, for expression of N-terminally Flag-tagged MMS19 with mutations R1012E, K1013E in human cells. Based on pKG101.	This study.
pSK495	Yeast MMS19 (MET18) K1008A, K1009A, R1010A in p415GAL1 vector. Expression of full-length yeast MMS19 with mutations K1008A, K1009A, R1010A in yeast.	This study.
pSK496	Yeast MMS19 (MET18) K1008E, K1009E, R1010E in p415GAL1 vector. Expression of full-length yeast MMS19 with mutations K1008E, K1009E, R1010E in yeast.	This study.
pSK497	Yeast MMS19 (MET18) R1013A, K1014A in p415GAL1 vector. Expression of full-length yeast MMS19 with mutations R1013A, K1014A in yeast.	This study.
pSK498	Yeast MMS19 (MET18) R1013E, K1014E in p415GAL1 vector. Expression of full-length yeast MMS19 with mutations R1013E, K1014E in yeast.	This study.
pSK511	Human CIAO1 E133A, E137A, D138A, E139A, E141A in pDEST-myc vector, for expression of N-terminally myc-tagged human CIAO1 with mutations E133A,	This study.

	E137A, D138A, E139A, E141A in human cells. Based on pKG203.	
pSK512	Human CIAO1 E133K, E137K, D138K, E139K, E141K in pDEST-myc vector, for expression of N-terminally myc-tagged human CIAO1 with mutations E133K, E137K, D138K, E139K, E141K in human cells. Based on pKG203.	This study.
pSK513	Yeast MMS19 (MET18) ¹⁻¹⁰¹⁰ in p415GAL1 vector. Expression of yeast MMS19 residues 1-1010 in yeast.	This study.
pSK514	Yeast MMS19 (MET18) ¹⁻⁹⁸⁸ in p415GAL1 vector. Expression of yeast MMS19 residues 1-988 in yeast.	This study.
pSK515	Human MMS19 ¹⁻¹⁰¹⁰ in pDEST-Flag-pCR3 vector, for expression of N-terminally Flag-tagged MMS19 residues 1-1010 in human cells. Based on pKG101.	This study.
pSK516	Human MMS19 ¹⁻⁹⁸⁵ in pDEST-Flag-pCR3 vector, for expression of N-terminally Flag-tagged MMS19 residues 1-985 in human cells. Based on pKG101.	This study.
pSK527	Human CIAO1 R62E in pDEST-myc vector, for expression of N-terminally myc-tagged human CIAO1 with mutation R62E in human cells. Based on pKG203.	This study.
pSK528	Human CIAO1 D82K in pDEST-myc vector, for expression of N-terminally myc-tagged human CIAO1 with mutation D82K in human cells. Based on pKG203.	This study.
pSK529	Human CIAO1 E102K in pDEST-myc vector, for expression of N-terminally myc-tagged human CIAO1 with mutation E102K in human cells. Based on pKG203.	This study.
pSK530	Human CIAO1 E105K, N106A, E107K in pDEST-myc vector, for expression of N-terminally myc-tagged human CIAO1 with mutations E105K, N106A, E107K in human cells. Based on pKG203.	This study.
pSK531	Human CIAO1 H104A in pDEST-myc vector, for expression of N-terminally myc-tagged human CIAO1 with mutation H104A in human cells. Based on pKG203.	This study.
pSK532	Human CIAO1 W130A in pDEST-myc vector, for expression of N-terminally myc-tagged human CIAO1 with mutation W130A in human cells. Based on pKG203.	This study.
pSK533	Human CIAO1 Q151A in pDEST-myc vector, for expression of N-terminally myc-tagged human CIAO1 with mutation Q151A in human cells. Based on pKG203.	This study.
pSK534	Human CIAO1 D171K in pDEST-myc vector, for expression of N-terminally myc-tagged human CIAO1 with mutation D171K in human cells. Based on pKG203.	This study.
pSK535	Human CIAO1 R125E, K127E in pDEST-myc vector, for expression of N-terminally myc-tagged human CIAO1 with mutations R125E, K127E in human cells. Based on pKG203.	This study.
pSK536	Human CIAO1 R125E in pDEST-myc vector, for expression of N-terminally myc-tagged human CIAO1 with mutation R125E in human cells. Based on pKG203.	This study.
pSK537	Human CIAO1 E102K, E105K, N106A, E107K in pDEST-myc vector, for expression of N-terminally myc-tagged human CIAO1 with mutations E102K, E105K, N106A, E107K in human cells. Based on pKG203.	This study.

pSK538	Human CIAO1 E102K, E105K, N106A, E107K, R125E, K127E in pDEST-myc vector, for expression of N-terminally myc-tagged human CIAO1 with mutations E102K, E105K, N106A, E107K, R125E, K127E in human cells. Based on pKG203.	This study.
pSK539	Human CIAO1 R62E in p416-CYC1 vector, for expression of human CIAO1 with mutation R62E in yeast.	This study.
pSK540	Human CIAO1 D82K in p416-CYC1 vector, for expression of human CIAO1 with mutation D82K in yeast.	This study.
pSK541	Human CIAO1 E102K in p416-CYC1 vector, for expression of human CIAO1 with mutation E102K in yeast.	This study.
pSK542	Human CIAO1 E105K, N106A, E107K in p416-CYC1 vector, for expression of human CIAO1 with mutations E105K, N106A, E107K in yeast.	This study.
pSK543	Human CIAO1 H104A in p416-CYC1 vector, for expression of human CIAO1 with mutation H104A in yeast.	This study.
pSK544	Human CIAO1 W130A in p416-CYC1 vector, for expression of human CIAO1 with mutation W130A in yeast.	This study.
pSK545	Human CIAO1 Q151A in p416-CYC1 vector, for expression of human CIAO1 with mutation Q151A in yeast.	This study.
pSK546	Human CIAO1 D171K in p416-CYC1 vector, for expression of human CIAO1 with mutation D171K in yeast.	This study.
pSK547	Human CIAO1 R125E, K127E in p416-CYC1 vector, for expression of human CIAO1 with mutation R125E, K127E in yeast.	This study.
pSK548	Human CIAO1 E102K, E105K, N106A, E107K in p416-CYC1 vector, for expression of human CIAO1 with mutations E102K, E105K, N106A, E107K in yeast.	This study.
pSK549	Human CIAO1 E102K, E105K, N106A, E107K, R125E, K127E in p416-CYC1 vector, for expression of human CIAO1 with mutations E102K, E105K, N106A, E107K, R125E, K127E in yeast.	This study.
pSK550	Human MMS19 in pDEST-myc vector, for expression of N-terminally myc-tagged human MMS19 in human cells.	This study.
pSK551	Human DNA2 in pDEST-Flag-pCR3 vector, for expression of N-terminally Flag-tagged DNA2 in human cells.	This study.
pSK552	Human PriS in pDEST-Flag-pCR3 vector, for expression of N-terminally Flag-tagged PriS in human cells.	This study.
pSK553	Human PriL in pDEST-Flag-pCR3 vector, for expression of N-terminally Flag-tagged PriL in human cells.	This study.
pBG100-p48	Human PriS in pBG100 vector, for recombinant expression of human PriS in bacteria. <i>A gift from W. Chazin.</i>	⁴³
pETDuet-p58	Human PriL in pETDuet vector, for recombinant expression of human PriL in bacteria. <i>A gift from W. Chazin.</i>	⁴³

pKG101	Human MMS19 in pDEST-Flag-pCR3 vector, for expression of N-terminally Flag-tagged MMS19 in human cells. <i>A gift from K. Gari.</i>	¹⁰
pKG203	Human CIAO1 in pDEST-myc vector, for expression of N-terminally myc-tagged human CIAO1 in human cells. <i>A gift from K. Gari.</i>	¹⁰
pKG241	CIAO2B-pDEST-myc, expression of N-terminally myc-tagged human CIAO2B in human cells. <i>A gift from K. Gari.</i>	¹⁰
pKG274	YIplac128-pGAL-Flag5'CIA1, Yeast integrative plasmid for replacement of CIA1 endogenous promoter by pGAL. Marked with LEU2. Used for generation of Gal-CIAO1 yeast strain. <i>A gift from K. Gari.</i>	¹⁰

1

1 **Extended Data Table 3 | Yeast strains.**

2

Strain	Parental strain	Genotype	Source
<i>Saccharomyces cerevisiae</i> MET18 knockout	BY4741	MATa, his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	Dharmacon
<i>Saccharomyces cerevisiae</i> P _{GAL} FlagCIA1-LEU2	W303	MATa, RAD5 leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15	this study, based on ¹⁰ , using plasmid pKG274

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