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Supplementary Materials

Defining the domains of Cia2 required for its function in vivo and in vitro

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SUPPLEMENTAL FIGURES and TABLES



Figure S1. A) Sequence logo representing the alignment of 48 Cia2 homologs.¹ The N-terminal and C-terminal domains are indicated. The five conserved motifs are also indicated: two in the N-terminal domain (motifs 1 and 2 are red and orange, respectfully), two in the DUF59 domain (motifs 3 and 4 are green and blue respectfully with C161 yellow) and one following the DUF59 domain (motif 5 is colored purple with E208 red). B) Conserved regions mapped onto the two dimeric crystal forms of human Cia2a, the major dimer (Top, PDB ID 3UX2) and the major dimer (bottom).² Coloring as in part A and Figure 1.

Figure S2. Multiple sequence alignments and sequence logos of DUF59 proteins from bacteria and archaea (Panel A) Cia2a (Panel B), Cia2b (Panel C), Cia2 from organisms encoding one Cia2 ortholog (Panel D). In the alignments, regions predicted to be disordered by the MDFp2 server are red.³ In the sequence logos, conserved motifs are boxed as in Figure S2 as follows: Motif 1, red; Motif 2, orange; Motif 3, green; Motif 4, blue; Motif 5, purple. Although A. thaliana encodes three Cia2 homologs,⁴ their sequences cluster with the Cia2 and Cia2b sequences and were therefore all included in the Panel D alignments. The Uniprot numbers for the proteins used in the analysis are: A0CW13; A0DP48; A2DDP4; A7APH3; A7RPY9; A7SPP5; A8J757; A9SK08; A9UT22; B3RT11; B3RVK4; B8C104; C5L218; D2VP27; D3B7Q5; D3BHT1; D7FV08; E1ZKW8; E3KR97; F0Y5Q3; F0Z896; F0ZF45; I1J196; I7M608; M1VDD8; Q01GB5; Q4DHV8; Q6CEL6; R1EZP0; S9U937; V9VIF9; A0A067D6V7; B2WDH4; J3NI82; Q57YF8; Q6PBY9; Q6DHF2; B0EHV7; I1H8W2; A5KA37; G4YYC6; P38829; Q9V968; Q9Y3D0; Q9H5X1; O62252; Q9VTC4.

Figure S2A – bacterial and archaeal DUF59 sequences

F8D2V7 HALXS		0	
M7AR11_9ACTN		0	
PAAD_ECOLI		0	
G1WCS9_9BACT	MF	2	
YITW_BACSU		0	
Q5GWP2_XANOR	MRLMECSAVVAWRDGRRRRRCGRLEWPTCARPARPHIQPMYSRSSEPVQFERDCDAVMVP	60	
F8D2V7 HALXS	MSETESDGRAGS	12	
M7AR11_9ACTN	HPSDT	23	
PAAD_ECOLI	MQRLATI	7	
G1WCS9_9BACT	RGKKERMPKTTRGSWQHGGRQKYGAWLLLFHIICYLCNDNDSKNMTQEEKT	53	
YITW_BACSU		1	
Q5GWP2_XANOR	QGDSVTLPAGSIGIITQALGGSTTVFVEGALFRIAGKDGDAIGREAPPGLELPANAS	117	
F8D2V7 HALXS	PAPTRAAVRDRLDRVTDPELDR-SIVELEYIDAIEIDGDRV-TVEFTLPTAWCSP	65	4-
M7AR11_9ACTN	AVALTDAVMAALDTVSDPELDQ-PITELRFVRSVLVDDEGV-TVHLRLPTSFCSP	76	
PAAD_ECOLI	APPQVHEIWALLSQIPDPEIPVLTITDLGMVRNVTQMGEGW-VIGFTPTYSGCP-	60	#2- I III III
G1WCS9_9BACT	KIEERIVDVLKTVYDPEIPV-NIWDLGMIYKIDVKDDGNVDLDMTFTAPNCP-	104	
YITW_BACSU	EEALKENIMGALEQVVDPELGV-DIVNLGLVYDVDMDEDGLTHITMTLTSMGCP-	54	
Q5GWP2_XANOR	DEEVEALVWQQLRTCFDPEIPF-NIVDLGLVYEAVVSHREEDNQRRVDVKMTLTAPGCG-	175	
F8D2V7 HALXS	AFAWMMAVDARDEVESLPAVEEARIVLDEHMHAEEINRGVNERRSFAESFPD-ADGDV	122	4-
M7AR11_9ACTN	${\tt NFAYLMCSDALDALEDIDGIGRV} QV{\tt MLDDHHDSDKINAGLAAHAGYKGTFTVEAEQDL}$	134	2
PAAD_ECOLI	- ATEHLIGAIREAM - TTNGFTPV QVVL QL DPAWTTDWMTPD ARERLREYGISPPAGHSCH	118	s.
G1WCS9_9BACT	-AADFILEDVRTKVDSVEGVAATNVNLVFEPAWDQSMMTEEARVELGFE	152	〒 2-
YITW_BACSU	-LAPIIVDEVKKALADLPEVKDTEVHIVWNPPWTRDKMSRYAKIALGIQ	102	1- V
25GWP2_XANOR	-MGEILVDDVRSKVEMIPTIAEADVELVFDPPWGRHMMSEAARLETGML	223	
F8D2V7 HALXS	EAVRADLDEKARVARQYDAVETLLEAGLEPETIVDLRPADLERYELEGADDGGAGAEAER	182	
M7AR11_9ACTN	DELRMTFLRKAHTAAMERAVSPMIAAGTTCVDDC	168	
PAAD_ECOLI	AHLPPEVRCPRCASVHTTLISEFGSTAC	146	
G1WCS9_9BACT		152	
YITW_BACSU		102	Motifs 3 and 4 are diverged in
Q5GWP2_XANOR		223	hastorial and grahagal DUFED
			Dacterial and archaeal DUF59
F8D2V7 HALXS	IAIYVQDRTVAVTVPAAPIDRYL-EKARETGALAAPTEPLFRTPDGEPIDLESFDLVHRR	241	proteins but these two motifs
M7AR11_9ACTN	AQLLLRDLPENPAKVALLRRRVQIGLGVCPNCRVVVDEDGSPLPAEAIPMRLRF	222	proteins but these two motils
PAAD_ECOLI	KALYRCDSCREPFDYFKCI	165	form the core of the DUF59
G1WCS9_9BACT		152	
YITW_BACSU		102	domain signature.
Q5GWP2_XANOR		223	
F8D2V7 HALXS	GRLAOVNMSSOGGICDGLREARERRFGGEGSATGDAETADD	282	
M7AR11 9ACTN	ARSVRISMEGNGHFCRGLLATRYADDTDCDGTSGPVVTDLRMLPMPTTRTAHTPEPATPG	282	
PAAD_ECOLI		165	
G1WCS9_9BACT		152	
YITW_BACSU		102	
Q5GWP2_XANOR		223	
F8D2V7 HALXS	282		
M7AR11 9ACTN	AHTGVPDPHTERSAS 297		
PAAD ECOLI	165		
G1WCS9_9BACT	152		
YITW_BACSU	102		
Q5GWP2_XANOR	223		



149

FA96A_HUMAN H2XPT5_CIOIN Q6PBY9_DANRE FA96A_DICDI V195B_DROME H3AYN3_LATCH F7F5F3_MONDO M3XNE9_MUSPF A7SPP5_NEMVE I7LXL0_TETTS K1QP22_CRAGI J3S4M7_CROAD F4X255_ACREC C3Y1R3_BRAFL A0CW13 PARTE -MORVSGLLSWTLSRVLWL 18 ___ ---MELVSGLLSKA----LFL----MLSYIKRKLSESDSGVSSVATVTSSCGGDSG _____ -MLSFFR ---MVDLASICRFSFNRSIFSTIT-A0CW13_PARTE Q38DX6_TRYB2

FA96A_HUMAN	SGLSEPGAAROPRIMEEKALEVIDLIRTIRDPERPN-TLEELEVV
H2XPT5_CIOIN	MEDYEGTIYDIIRTIKDPEKPG-SLEDLDVV
Q6PBY9_DANRE	TGL8NETNDKRRKKMEEKALEVYDVIRTIRDPEKPN-TLEELDVV
FA96A_DICDI	MIMNYNVIDKIDVFDIIRHIKDPEFPK-TLEELKVV
F0Z896_DICPU	MINYNNNIIDSIDVFDIIRHIKDPEYPN-TLEQLKVV
U195B DROME	VRKTSQMSMDDEAIAFGEDALLHELGYKNQTELQETIYDLLRGIRDPEKPC-TLEDLNVV
H3AYN3_LATCH	SGLSNRNNARKSKKMEEKALEVYDIIRTVRDPEKPN-TLEELDVV
F7F5F3_MONDO	SGRSERGAARQPRIMEEKALEVYDIIRTIRDPEKPN-TLEELEVV
M3XNE9_MUSPF	TGLFERGAARQPRIMEEKALEVYDLIRTIRDPEKPN-TLEELEVV
A7SPP5_NEMVE	DPSFGASRIDNDVNSQSNRNLALDVYDLIKDIKDPEKPQ-TLEDLKVV
I7LXL0_TETTS	MERLKTQEEIESILDDTFYIISTIRDPEFPQ-TLGDLNVI
KlQP22_CRAGI	MSEAFENDDNGDLKOMRELVYDLIRGIIDPEKPE-TLEELNVV
J3S4N7_CROAD	AAPRRAGESGRAAAMAQAQERALEVYDLIRTIRDPEKPN-TLEELEVV
F4X255_ACREC	MAKNQTLKTDIELKESVYGKFCALFVSYLLRTIKDPEKPQ-TLEQLDVV
C3Y1R3_BRAFL	KVNLCVLSMSKDAVLRQELDDLSDIVYDLIRDIRDPEKDN-TLEELDVV
A0CW13_PARTE	MQNIIDDIYYIIYNIRDPEIPQ-TLGQLEVI
Q38DX6_TRYB2	MTDRLTAEDVFYELSTIRDPERPDCTLADLDVV
	: : *** :* :*.*:
FA96A HUMAN	SESCVEVQEINE-EEYLVIIRFTPTVPHCSLATLIGLCLRVKLQ
UDYDE CTOTH	

F

HEAFID_CIOIN	IBBG454KI5B====================================
Q6PBY9_DANRE	TEKCVEVQELGD-DEYLIVIKFSPTVPHCSLATLIGLCLQVKLQ
FA96A_DICDI	NEDWITVIDNNDINDSDDINN-NNNENYKGYCFIKILFQPTVPHCHLAPTIALCIREKIK
F0Z896_DICPU	NEDWITVEDNINDKKDCCYIKIYFTPTVPHCHLAPTIALCIREKIN
U195B_DROME	YEDGIFVMPPTRSNVSVVRIEFNPTVPHCSLATLIGLCIRVKVE
H3AYN3_LATCH	SEDSVEVQEIDE-DEYLIIIRFTPTVPHCSLATLIGLCLRVKLQ
F7F5F3_MONDO	TESCVEVKEIHE-EDYLVIIRFTPTVPHCSLATLIGLCLRVKLQ
M3XNE9_MUSPF	TESSVEVQERNE-DDYLVIIRFTPTVPHCSLATLIGLCLRVKLQ
A7SPP5_NEMVE	YESCVEVQKVAGQDHITITFTPTVPHCSLATLIGLCIRVKLE
I7LXL0_TETTS	QRENLRFQQVQISS-GQKRNEIYLGIIQIIWVPTVPHCHLASQIGLSIITKLQ
K10P22 CRAGI	SEEDVSVSRLNKDYLIKVVFVPTVPHCSLASLIGLSIRTKLE
J3S4N7_CROAD	SESCVEVVEIGP-GESLITIRFTPTVPHCSLATLIGLCLRIKLQ
F4X255_ACREC	YEDCIKVCHSTPGGVSVIRVEFNPTVPHCSLATLIGLCIRVKLE
C3Y1R3 BRAFL	YESGVHVEPWGE-DKFHISIEFTPTVPHCSLATLIGLCLRVKLE
A0CW13_PARTE	QKEFINVEGPRITIYWKPTVKHCSFALQIALSIRVKLS
Q38DX6_TRYB2	AMNRCRVEYIESSADFQSLRGQGCNDSGKPSVVVKVILQPTVPHCSLMEFICLCVYVRLR
	: **: ** : * *.: ::
FA96A_HUMAN	RCLPFKHKLEIYISEGTHSTEEDINKQINDKERVAAAMENPNLREIVEQCVLEPD
H2XPT5_CIOIN	RTLPTTHKIRVFVKEG8HNTEDEVNKQINDKERIAAAMENPNIRKMVENCIKEPD
Q6PBY9_DANRE	RCLPFKHKLEIYITEGTHSIEEDINKQINDKERVAAAMENPNLREIVEQCVTEPDD
FA96A_DICDI	EYLPKRSKIEIYIKKGTHQTEDEINKQINDKERIIAALENPEIFQLVKKCIKEDDY
F0Z896_DICPU	QYLPKRSKIEIYITPGSHQTEEEINKQINDKERIIAALENPEIYDLVQKCIKEDEY
U195B_DROME	RGLPHNIKLDIYIKKGAHQTEEEINKQINDKERIAAAMENPNLRDLVENCIKDEE
H3AYN3_LATCH	RCLPFKHKVEIYISEGTHSTEEDINKQINDKERVAAAMENPNLREIVEQCVTEPDD
F7F5F3_MONDO	RCLPFKHKLEIYISEGTHSTEEDINKQINDKERVAAAMENPNLREIVEQCVIEPD
M3XNE9_MUSPF	RCLPFKHKLEIYISEGTHSTEEDINKQINDKERVAAAMENPNLREIVEQCVLEPD
A7SPP5_NEMVE	KSLPEKFKLDIYLKKGTHSTENEINKQINDKERIAAAMENPNLRKIVENCIDEDNGYD
I7LXL0_TETTS	QELPNYDEYKIEILVKEGTHQDKHQLDKQINDKERVCAAQENEHLMQFIQNLISYE
K1QP22_CRAGI	TSIPDKFKLDIFIKEGTHETADDINKQINDKERIAAAMENPNLQRIVNQCLENS
J3S4N7_CROAD	RCLPFKHKLEIYISEGAHSIEEDINKQINDKERVAAAMENPSLREIVDQCVLDPD
F4X255_ACREC	RQLSASFKLDIYIKKGAHSTEQEINKQINDKERIAAAMENPNLRELVEKCILEEDY
C3Y1R3_BRAFL	NNLPQHYKLDITVKEGTHSTGPEINKQINDKERIAAAMENPDLRAVVNKCVQDPE
A0CW13_PARTE	QELLNYKSYKIHIIVKDNLHNQKSQIDKQVNDKERYLAAMENEYLMNFINQLIY
O38DX6 TRYB2	EVFSLSNNAKFDITLVDGSHVRQRELEKQVADKERLAAAMEDKALLQEVERHINCE
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EVFSLSNNAKFDITLVDGSHVRQRELEKQVADKERLAAAMEDKALLQEVERHINCE-: *. :: * :::**: **** ** *: : :.. :

Figure S2B – Cia2a sequences



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MIP18_HUMAN	M
F6T485_CIOIN	
Q6DHF2_DANRE	
FA96B DICDI	
F0ZF45_DICPU	
U195A_DROME	
H3AYU6_LATCH	
F7CX16_MONDO	MVG
M3Y1R4_MUSPF	М
A7RPY9_NEMVE	
I7M608_TETTS	MLYMYVFLQNKGLKFKRLDQIGVCIYHLFYNFYAFFNSIFLIERIFIIYLKSLLIKIARK
klp752_CRAGI	
J3S061_CROAD	
F4W6S7_ACREC	
C3XU95 BRAFL	
A0DP48 PARTE	
Q4DHV8 TRYCC	

MIP18_HUMAN	VGGGGVGGGLLENANPLIYQRSGERPVTAGEEDEQVPDSIDAREIFDL	49
F6T485_CIOIN	MNETQKDNLNPLVHQRITPRTRIKQPAELDNDVRDPFDRREIFD	44
Q6DHF2_DANRE	MSGGTRLENANPLIFQRSGERLLTSTDEDEDVADPIDVREIFD	43
FA96B DICDI	MSDNPNPVIYVDNENCKSFEDNENSFNSSRYSIEEDQIDEFDEQEIFD	48
F0ZF45_DICPU	MSDNPNPVIYVQENEIKDEEDVLMSSRYSLGEDELDPFDEEEIFD	45
U195A_DROME	MPTEIENINPNVYDRIKERVLTANEEDENVPDPFDKREIFD	41
H3AYU6_LATCH	MVGGARLENANPLIYHRSGERQVTDEEVDEEIPDRIDDREIFD	43
F7CX16 MONDO	AG8GAGLGGLLENANPLIYQR8GERPVTAGEEDDQVPDSIDDREIFDIHC	53
M3Y1R4 MUSPF	VGGGGGMGGGLLENANPLIYERSGERPVTAGEEDEQVPDSIDAREIFDILP	51
A7RPY9_NEMVE	MAAVTDRLENVNPTVFQRLKERVVLAEEEDDNIVDKIDDREIFD	44
17M608 TETTS	ILRQQAIMSKVDNPNPQIHEIKQTISEAQRKKRDLLEQNEEIEDEIDQLEIFD	113
K1P752 CRAGI	BAGKLDNANPVIFEQSKERQVLPEEEDDDVTDKIDDREVFD	41
J3S061_CROAD	MVGP8GGGLENANPLVYRRQGERPTTAREQDEGLPDAIDDREIFD	45
F4W6S7_ACREC	MSENLENINPKLYKKLDDREITVEEQDEDVADEFDAREIFD	41
C3XU95 BRAFL	MSSPDRLQNANPQLYGRTSEREVTPEELNEDVEDAIDAREIFDILS	46
A0DP48 PARTE	MQLENPKPQVFQTGNFEFTQQYIKRKQQEYDLDVEDPIDEYEIFD	45
Q4DHV8 TRYCC	MTELVNPNPTVFRDALQHQPQRSAEELLQEQDESFRDPIDSLEVFH	46
17 (T)	* ;* ; * ;* *;*.	

MIP18 HUMAN	IRSINDPEHPLTLEELNVVEQVRVQVSDPESTVAVAFTPTIPH	92
F6T485 CIOIN	LIRDINDPEHPLTLEDLRVVSENDIEVDDEKSFIKVSFTPTIPH	88
Q6DHF2 DANRE	LIRSINDPEHPLSLEELNVVEQVRVNVNDEESTVSVEFTPTIPH	87
FA96B DICDI	LVRSITDPEHPLTLEQLNVVRIENVNINLENSYILLYFTPTVPH	92
F0ZF45 DICPU	LVRNITDPEHPLTLEQLNVVRVENINIDIKKSYIRLYFTPTVPH	89
U195A DROME	LIRNINDPEHPLTLEELHVVQEDLIRINDSQNSVHISFTPTIPH	85
H3AYU6 LATCH	LIRSINDPEHPLTLEELNVVEQVRVEVSDEESAVSVEFTPTIPH	87
F7CX16 MONDO	GRRRLEIKFSFTSHSHLIRSINDPEHPLTLEELNVVEQVRVKVNDRESTVAVEFTPTIPH	113
M3Y1R4 MUSPF	HLIRSINDPEHPLTLEELNVVEQVRVQVSDPESTVAVAFTPTIPH	96
A7RPY9 NEMVE	MIRSINDPEHPLTLEELNVVEQALIDVSDDESYVKVQFTPTIPH	88
I7M608_TETTS	LIRHIDDPEHPLTLEQLNVLQPENIKVNIDHKLVTVLFTPTIPH	157
K1P752 CRAGI	MIRNINDPEHPLTLEELNVVENARVKVDDENNYVGIEFTPTIPH	85
J3S061 CROAD	LIRGIHDPEHPLTLEELNVVEQLRVQVSDAQSAVSVEFTPTIPH	89
F4W6S7 ACREC	IIRNINDPEHPLTLEELNVVEQNLIEVDDKRNRVDVKFTPTIPH	85
C3XU95_BRAFL	WHREPKPDNDSAWSINDPEHPLTLEELNVIEQSRITVDEDNNHVSVEFTPTIPH	100
A0DP48 PARTE	GNRVMVYFTPTIPH	87
Q4DHV8 TRYCC	HIRSIRDPEHPNTLEELKVVEPELIRVDEVKQTVRVQFTPTVPH	90
	* **** :**:*.:: : : : ****:**	

MIP18_HUMAN	CSMATLIGLSIKVKLLRSLPQRFKMDVHITPGTHASEHAVNKQLADKERVAAALENT-HL	151
F6T485_CIOIN	CSMATLIGLAIRVRLLRSLPPRFKVEVEISPGSHQSEKAVNKQLGDKERVAAALENN-HL	147
Q6DHF2 DANRE	CSMATLIGLSIKVKLLRSLPDRFKIDVHITPGTHASEDAVNKQLADKERVAAALENS-QL	146
FA96B_DICDI	CSMANLIGLSIKEKLARSLPKRFKVDVIVTPGSHSSESSVNKQLNDKERVSAALDTSSSI	152
F0ZF45 DICPU	CSMANLIGLSIKEKLARSLPKRFKVDVIVTPGSHSSESSGKYIYTIVNYY	139
U195A DROME	CSMATLIGLSIRVKLLRSLPPRFKVTVEITPGTHASELAVNKQLADKERVAAALENN-HL	144
H3AYU6 LATCH	CSMATLIGLSIKVKLLRSLPDRFKVDVHITPGTHVSELAVNKQLSDKERVAAALENS-HL	146
F7CX16 MONDO	CSMATLIGLSIKVKLIRSLPERFKMDVHITPGTHASEHAVNKQLADKERVAAALENS-HL	172
M3Y1R4 MUSPF	CSMATLIGLSIKVKLLRSLPORFKMDVHITPGTHASEHAVNKQLADKERVAAALENT-HL	155
A7RPY9 NEMVE	CSMATLIGLAIRVRLLRSLPDRFKVDVKITPGTHQSEIAVNKQLADKERVAAALENN-HL	147
17M608 TETTS	CSLAQIIGLMIKVKLIRSLPRDYKVDVYITPGTHVQELSVNKQINDKERVMAAIENP-SI	216
K1P752 CRAGI	CSMATLIGLSIRVKLLRSLPPRFKVDVSITPGTHASEVAVNKQLADKERVAAALENS-HL	144
J3S061 CROAD	CSMATLIGLSIKVKLIRSLPERFKVDVHITPGTHASEHAVNKQLADKERVAAALENM-HL	148
F4W6S7 ACREC	CSMATLIGLSIRVOLLRALPSRFKVSVEISPGTHVSEAAVNKOLADKERVAAALENS-ML	144
C3XU95 BRAFL	CSMATLIGLSIRVKLLRALPTRFKVDVHITPGTHQSEHAVNKQLADKERVAAALENQ-HL	159
A0DP48 PARTE	CSMAQTIGLTLKIKLMRSLPKNYKVYVEIKQNMHIKEVELNKLFQDKERVLAAIENQ-QL	146
04DHV8 TRYCC	CSMTTLIGLCISLKLORSLPRGTKVDVYVTPGSHEQEEQVNKQLNDKERVAAALENK-NL	149
	**** * ** **** *** *** *** *** ****	

MIP18_HUMAN	LEVVNQCLSARS	163
F6T485_CIOIN	LNVVNQCLTIKGNSYETTV	166
Q6DHF2 DANRE	LEVVNQCLSSRGV	159
FA96B DICDI	LTIVNECIKQN	163
F0ZF45_DICPU		139
U195A_DROME	AEVINQCIAAKG	156
H3AYU6 LATCH	LEVVNQCLTARS	158
F7CX16 MONDO	LEVVNQCLSARS	184
M3Y1R4_MUSPF	LEVVNQCLSARS	167
A7RPY9_NEMVE	LDVIDQCLVSKK	159
I7M608_TETTS	LRVVNKGVSNSDRLDNCC-	234
K1P752 CRAGI	IEVVNQCLATRK	156
J3S061_CROAD	LQVVNQCLSGRS	160
F4W6S7_ACREC	LGVINQCLAPKD	156
C3XU95_BRAFL	LEVVNQCLSTRN	171
A0DP48_PARTE	LKIINFGIQGLR	158
Q4DHV8_TRYCC	LNVVESCLNEFE	161

Figure S2C - Cia2b proteins



Figure S2D – Cia2 sequences that are not part of a Cia2a/Cia2b paralogous pair.



Figure S3. MALDI-TOF MS spectrum of refolded Cia2. The experimentally determined mass is 25,630.3 Da, which is in close agreement with the expected mass of 25,660.23 Da for the [M+H]+ ion.



Figure S4. Anti-MYC Western blot of empty vector (EV), wild-type (WT), E208A-Cia2, and Δ 102-Cia2. WT and E208A-Cia2 have bands at around 37kDa, consistent with the SDS-PAGE migration for Cia2. Δ 102-Cia2 contains a 15kDa band, consistent with its predicted molecular weight. The relative migration of molecular weight markers in kDa are indicated to the left. Both WT and E208-Cia2 have additional lower molecular weight bands that are likely due to proteolysis of Cia2.



Figure S5. The structural similarity of NFU-domain proteins and DUF59 proteins. A) The structure of arabidopsis CnfU iron sulfur cluster biosynthesis protein⁵ (PDB 2Z51). The FeS-binding cysteines, two from each polypeptide, are yellow spheres. The two polypeptide chains are colored blue and green. Each CnfU polypeptide has two NifU domains, colored light and dark green in the green colored polypeptide, where the N-terminal NifU domain has the CxxC motif. B) The FeS binding domain of CnfU (light green in panel A) overlaid with human Cia2a⁶ (2M5H, orange). C) The FeS binding domain of CnfU colored by secondary structure and secondary structure map. The location of the CxxC motif is indicated and cysteine sulfurs are yellow spheres. D) Cia2a DUF59 domain (residues 27-119) colored by secondary structure map. The absolutely conserved cysteine of the DUF59 domain is shown as a yellow sphere. E) All of the known Nfu structures in the protein data bank including the mouse (1VEH), rice (2JNV), and human (5M5O) Nfu proteins. The cysteines of the CxxC motif is shown as spheres. F) All of the known Duf59 structures in the protein data bank including *T. maritima* (1UWD), *T. thermophiles* (2CU6), *B. anthracis* (3LNO), and *M. tuberculosis* (5IRD). The cysteine corresponding to the absolutely conserved cysteine of the DUF59 domain to the absolutely conserved cysteine corresponding to the absolutely conserved cysteine of the DUF59 domain to the absolutely conserved cysteine corresponding to the absolutely conserved cysteine of the DUF59 domain to the absolutely conserved cysteine of the DUF59 domain to the absolutely conserved cysteine corresponding to the absolutely conserved cysteine of the DUF59 domain is shown as spheres.



Figure S6. UV-Vis spectra of as isolated SUMO-Cia2 (A), as isolated dtCia2 (B), and chemically reconstituted dtCia2 (C). Features in the low 400 nm region are consistent with $[Fe_2S_2]$ or $[Fe_4S_4]$ binding.

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