

Supplemental Information

<i>S.cerevisiae</i> :	MSSQEYTFI	DIPVTRAQVE	HCSYSFWSSL	YPKYVPSIV	LKSLPKKFIQ	YLEQDGIKLP	QEENSRSVYT	70
<i>S.pombe</i> :	-----M	TLILTKNOVL	HCQFSSWYSL	FRKLTPKAKV	IKPIPATVLK	YLEDHSIYVE	QPMNTVEEV-	60
<i>D.rerio</i> :	-----	---MKKEQVV	NCQFSVWYPL	FKKHTIKSL-	ILPIEQNVID	YLLDDGTLVV	SGSENNNSQT	56
<i>H.sapiens</i> :	-----	---MKKEHVL	HCQFSAWYFP	FRGVTIKSV-	ILPLPQNVKD	YLLDDGTLVV	SGRDDPPTH	56
<i>A.thaliana</i> :	-----	---MKKEDEVN	RCQIQNWYPR	FKSLTIKTK-	FHKLEESFIN	YLLDDSGPFL	LPHSVTNEDA	56
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<i>S.cerevisiae</i> :	E--EIIRN-E	DNDYSDWEDD	EDTATEFVQE	VEPLIDFPPEL	HQKLDKDALNE	-LCAVAPKLN	WSAPRDATWI	136
<i>S.pombe</i> :	-----	-----DSEED	EESAPAYYPE	REA-----	IQLIEKAIKE	LGCYVVPKLN	WSTPKDALWI	108
<i>D.rerio</i> :	QANNSDSD--	EEDIQWT-DD	ETTTTIVT---	---APEFPEF	NVKVQEAINV	LGCYVVPKLN	WSAPRDANWI	117
<i>H.sapiens</i> :	QP-DSDDE--	AEEIQWS-DD	ENTATLT---	---APEFPEF	ATKVQEAINS	LGCYVVPKLN	WSAPRDAYWI	116
<i>A.thaliana</i> :	MPNRVHNFEE	EDDFQVPEEA	SDDEPLN---	---RPSFPPEL	EIEIRESIET	LGTIIPKLN	WSPKDAAWI	120
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<i>S.cerevisiae</i> :	LPNNTMKQNE	VNELYLLLNA	SNYIMHDLQR	AFKGCVDGDD	----IKGLK	FDLVLRQWCD	-MNPALVFRV	200
<i>S.pombe</i> :	TTTGS�KCTT	AEVLLLLKLS	SDFVAHDLNH	AFDDCKDFDN	ADGSVPKDFS	FELVLRKWF	-MHASTVFR	177
<i>D.rerio</i> :	ALNSSLQQS	LSEIFLLFKS	SDFITHTLTQ	PFLHCSDDSP	----DPTIN	YELVLRKWE	-LIPGGEFR	181
<i>H.sapiens</i> :	AMNSSLQCT	LSDIFLLFKS	SDFITRDFTQ	PFIHCDSDSP	----DPCIE	YELVLRKWE	-LIPGGEFR	180
<i>A.thaliana</i> :	SPSQNLSTC	FNEIALFRS	SDSLTHDLFN	AYDSCSKVKS	----SRPES	FYLALRKWYP	SLKPEMFR	185
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<i>S.cerevisiae</i> :	FVKNAHIVGA	TQRDL-NYVD	YLDELSDTFK	DLTDEIVHDV	VLPKFPDKSF	VLDVYIIPRF	NKIFVIDINP	269
<i>S.pombe</i> :	FVKSKRLIAF	QRDD-NYVE	FLKENIDCYE	KLISDLLKK-	-LDTEPDPDF	VFDVYIHK--	DRAWLIDINP	242
<i>D.rerio</i> :	FVKENKLI	CORDYTQHYQ	HIGKQEAIS	TSLQFFRDN	IQYQEPDEF	VLDVYRDS-	GRVWLDINP	250
<i>H.sapiens</i> :	FVKENKLI	SORDYTQYD	HISKQKEEIR	RCIQDFKHK	IQYKFLDEF	VFDIYRDS-	GKVWLDINP	249
<i>A.thaliana</i> :	FVKSNELVGI	QREVTTFP	VLLNEKDLLK	GLIEEFDDK	IRFEISENY	TFDVYVTK-	RRVWLDINP	254
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		<i>cdc123-1</i> E283K					Δ 327	
<i>S.cerevisiae</i> :	FARKTDSLLF	SWNEIAAIAP	PKNDVE----	----DYELRL	VTRHNTGRFA	SKEHSENV	QDLVEASLNP	331
<i>S.pombe</i> :	FYPRTDGLLF	SWSELESMS	ENM-----	----KPEIRL	IPKGSMPSTG	SAKYTYNRVP	EDMIAASE-G	300
<i>D.rerio</i> :	FGEVTDLLF	TWEELTSGKN	LITANQTQEE	ALPDGPAFRC	TNSEV--TVQ	PSPCLSYRIP	RDFLDLTT-G	317
<i>H.sapiens</i> :	FGEVTDLLF	TWEELISENN	LNGDFSEVDA	QEQDSPAFC	TNSEV--TVQ	PSPYLSYRIP	KDFVLDLST-G	316
<i>A.thaliana</i> :	WCGSTLPLMY	TWEELKIHG	EC---D----	----ELELRI	VESR---LS	VLPGLKTAVP	YDYLVDVSA-G	308
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<i>S.cerevisiae</i> :	EAIRELTKW	KELLSQAKE	ESSDSENET-	-				360
<i>S.pombe</i> :	ENLLEFAQKW	QDLTNSNE-	-----	-				319
<i>D.rerio</i> :	EDAYKLIDFL	KLKRGQEEE	EESNEEGEEP	Q				348
<i>H.sapiens</i> :	EDAHKLIDFL	KLKRNQEDD	-----	-				336
<i>A.thaliana</i> :	SGWAQVLKIV	EETF--QKDD	QSSDEVA---	-				333
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Figure S1. Alignment of Cdc123 orthologs.

Cdc123 orthologs from *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Danio rerio*, *Homo sapiens* and *Arabidopsis thaliana* were aligned with ClustalΩ (clustal consensus is shown below the aligned sequences). Residues that are conserved in 5 or 4 species are highlighted in black and grey, respectively. The amino acid substitution (E283K) resulting from the *cdc123-1* mutant allele (G847A) as well as the last residue of the truncated version encoded by the *cdc123Δ327* mutant allele are indicated.

Table S1. Yeast strains

name	relevant genotype	back-ground	used in Figure
BY4741	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0</i>	S288c	1A, 1B, 2D, 3A
W4050	<i>MATa cdc123-1-ha3-URA3</i>	S288c	1A
W3702	<i>MATa cdc123Δ327-ha3-natMX4</i>	S288c	1A, 2D
W3439	<i>MATa cdc123-1-ha3-URA3 can1Δ::MFA1pr-HIS3-MFAalpha1pr-LEU2</i>	S288c	1B
W3129	<i>MATa cdc123Δ327-ha3-natMX4 can1Δ::MFA1pr-HIS3-MFAalpha1pr-LEU2</i>	S288c	1B, 2B
YTH3 (1)	<i>MATa CDC33(-198,1)::kanMX4-tTA-tetO7</i>	S288c	1B
BY4742	<i>MATa his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 MET15</i>	S288c	1C
W13221	<i>MATa cdc123Δ327-ha3-natMX4 MET15</i>	S288c	1C
W13224	<i>MATa cdc123-1-ha3-URA3 MET15</i>	S288c	1C
W10897	<i>MATa p180</i>	S288c	1D, 1E
W10898	<i>MATa cdc123Δ327-ha3-natMX4 p180</i>	S288c	1D, 1E
W10899	<i>MATa gcn2Δ::kanMX6 p180</i>	S288c	1D, 1E
W10900	<i>MATa gcn2Δ::kanMX6 cdc123Δ327-ha3-natMX4 p180</i>	S288c	1D, 1E
W10901	<i>MATa gcn3Δ::kanMX4 p180</i>	S288c	1D, 1E
W10902	<i>MATa gcn3Δ::kanMX4 cdc123Δ327-ha3-natMX4 p180</i>	S288c	1D, 1E
W10903	<i>MATa pWS3396</i>	S288c	1D, 1E
W10904	<i>MATa cdc123-1-ha3-URA3 pWS3396</i>	S288c	1D, 1E
W10905	<i>MATa gcn2Δ::kanMX6 pWS3396</i>	S288c	1D, 1E
W10906	<i>MATa gcn2Δ::kanMX6 cdc123-1-ha3-URA3 pWS3396</i>	S288c	1D, 1E
BY4743	<i>MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 MET15/met15Δ0 LYS2/lys2Δ0</i>	S288c	2A
W4467	<i>MATa/α GCD11/GCD11-myc13-kanMX6</i>	S288c	2A
W5471	<i>MATa/α cdc123Δ327-ha3-natMX4/cdc123Δ327-ha3-natMX4 GCD11/GCD11-myc13-kanMX6</i>	S288c	2A
W5472	<i>MATa/α CDC123-ha3-HIS3MX6/CDC123-ha3-HIS3MX6 GCD11/GCD11-myc13-kanMX6</i>	S288c	2A
W4177	<i>MATa GCD11-myc13-kanMX6</i>	S288c	2B, 3A
K699	<i>MATa ade2-1 can1-100 his3-11 leu2-3,-112 trp1-1 ura3-2 ssd1</i>	W303	2C, 2E, 5C, 5D

Table S1 (continued). Yeast strains

name	relevant genotype	back-ground	used in Figure
W12768	<i>MATa kanMX4-pGCD11-flag3-GCD11</i>	W303	2C
W12770	<i>MATa kanMX4-pGCD11-flag3-GCD11 cdc123Δ327-ha3-HIS3MX6</i>	W303	2C
W4444	<i>MATα cdc123Δ::kanMX4 ura3::pGALL-cdc123-1-ha3-URA3</i>	W303	2E
W5294	<i>MATa CDC123-myc13-kanMX6</i>	S288c	3A
W4887	<i>MATa GCD1-myc13-HIS3MX6</i>	S288c	3A
W4884	<i>MATα GCD11-ha3-HIS3MX6</i>	S288c	3B
W5288	<i>MATα CDC123-ha3-HIS3MX6</i>	S288c	3B
W5290	<i>MATα SUI2-ha3-HIS3MX6</i>	S288c	3B
W276	<i>MATα his3 trp1 ura3::lexAop-lacZ leu2::lexAop-LEU2</i>	W303	4A
W12626	<i>MATa/α GCD11/gcd11Δ::kanMX6</i>	W303	4C, 5A
W12784	<i>MATa/α GCD11/gcd11Δ::kanMX6 his3/his3::pGCD11-flag3-GCD11-tCYC1-HIS3</i>	W303	4C, 4D, 5A
W12786	<i>MATa/α GCD11/gcd11Δ::kanMX6 his3/his3::pGCD11-flag3-GCD11(aa1-523)-tCYC1-HIS3</i>	W303	4C, 4D
W12788	<i>MATa/α GCD11/gcd11Δ::kanMX6 his3/his3::pGCD11-flag3-GCD11(aa1-519)-tCYC1-HIS3</i>	W303	4C, 4D, 5A
W12790	<i>MATa/α GCD11/gcd11Δ::kanMX6 his3/his3::pGCD11-flag3-GCD11(aa1-514)-tCYC1-HIS3</i>	W303	4C, 4D
W12833	<i>MATa/α GCD11/gcd11Δ::kanMX6 his3/his3::pGCD11-flag3-GCD11(aa1-519)-tCYC1-HIS3 leu2/leu2::pTEF2-CDC123-tCYC1-LEU2</i>	W303	5A, 5B
W12949	<i>MATa/α GCD11/gcd11Δ::kanMX6 leu2/leu2::pTEF2-CDC123-tCYC1-LEU2</i>	W303	5A
W12950	<i>MATa/α GCD11/gcd11Δ::kanMX6 his3/his3::pGCD11-flag3-GCD11-tCYC1-HIS3 leu2/leu2::pTEF2-CDC123-tCYC1-LEU2</i>	W303	5A
W5539	<i>MATa cdc123Δ::kanMX4 ura3::pTEF2-SUI2-tCYC1-URA3</i>	W303	5C, 5D
W5495	<i>MATa cdc123Δ::kanMX4 leu2::pTEF2-GCD11-tCYC1-LEU2</i>	W303	5C, 5D
W5540	<i>MATα cdc123Δ::kanMX4 ura3::pTEF2-SUI2-tCYC1-URA3 leu2::pTEF2-GCD11-tCYC1-LEU2</i>	W303	5C, 5D
W5544	<i>MATa cdc123Δ::kanMX4 trp1::pTEF2-SUI3-tCYC1-TRP1 leu2::pTEF2-GCD11-tCYC1-LEU2</i>	W303	5C, 5D
W5592	<i>MATα cdc123Δ::kanMX4 ura3::pTEF2-SUI2-tCYC1-URA3 trp1::pTEF2-SUI3-tCYC1-TRP1 leu2::pTEF2-GCD11-tCYC1-LEU2</i>	W303	5C, 5D

Table S2. Plasmids

name	description	vector	insert	used in Figure
p180 (2)	<i>pGCN4-lacZ, URA3, ARS/CEN</i>			1D, 1E
pWS3396	<i>pGCN4-lacZ, LEU2, ARS/CEN</i>	pRS315 (SalI/PstI)	SalI/PstI fragment of p180	1D, 1E
pWS1389	<i>prha-his6-CDC123</i>	pJOE4056.2 (BamHI/HindIII)	<i>CDC123</i> (BamHI/HindIII)	3C
pWS1483	<i>prha-mbp-GCD11</i>	pJOE2955 (BamHI/HindIII)	<i>GCD11</i> (BamHI/HindIII)	3C
pWS1957	<i>prha-mbp-GCD11-RBS-his6-CDC123</i>	pWS1483 (HindIII)	<i>RBS-his6- CDC123</i> (HindIII)	3C
pEG202 (3)	<i>pADH-lexA-MCS, HIS3, 2μ</i>			4A
pJG4-5 (3)	<i>pGAL-NLS-AD-HA-MCS, TRP1, 2μ</i>			4A
pWS1463	<i>pADH-lexA-CDC123</i>	pEG202 (EcoRI/XhoI)	<i>CDC123</i> (EcoRI/XhoI)	4A
pWS1513	<i>pGAL-NLS-AD-HA-GCD11</i>	pJG4-5 (EcoRI/XhoI)	<i>GCD11</i> (<i>bp1-1584</i>) (EcoRI/SalI)	4A
pWS1535	<i>pADH-lexA-SUI3</i>	pEG202 (EcoRI/XhoI)	<i>SUI3</i> (MunI/XhoI)	4A
pWS1537	<i>pADH-lexA-SUI2</i>	pEG202 (EcoRI/XhoI)	<i>SUI2</i> (EcoRI/XhoI)	4A
pWS3413	<i>pGAL-NLS-AD-HA-GCD11(aa410-527)</i>	pJG4-5 (EcoRI/XhoI)	<i>GCD11</i> (<i>bp1228-1584</i>) (EcoRI/SalI)	4A
pWS3473	<i>pGAL-NLS-AD-HA-GCD11(aa1-514)</i>	pJG4-5 (EcoRI/XhoI)	<i>GCD11(bp1-1542+Stop)</i> (EcoRI/SalI)	4A
pWS3917	<i>pADH-lexA-hD123</i>	pEG202 (EcoRI/XhoI)	<i>hD123</i> (EcoRI/XhoI)	4A

Table S3. Antibodies

antibody	description	used for immunoprecipitation in Figure	used for Western blot in Figure
anti-Cdc123	affinity-purified polyclonal rabbit antiserum (this study)	-	3A, 4C, 5A
anti-flag	M2, mouse monoclonal (Sigma-Aldrich)	2C, 4C, 5A (agarose conjugate)	2C, 4C, 5A
anti-Gcd11	polyclonal rabbit antiserum (4)	-	2D, 2E, 5D
anti-Gcd11	affinity-purified polyclonal rabbit antiserum (this study)	-	3A, 3C
anti-ha	12CA5, mouse monoclonal	-	3B, 4A
anti-his	Tetra-His antibody, mouse monoclonal (Qiagen)	-	3C
anti-myc	9E10, mouse monoclonal	2A, 3A	2A, 3A
anti-Sui2	polyclonal rabbit antiserum (5)	2D, 2E	1E, 2A, 2D, 2E, 3A, 5D
anti-Sui2	polyclonal rabbit antiserum (this study)	-	2C, 4C, 5A
anti-Sui2- P	anti-eIF2 α [phosphoS52] polyclonal rabbit antibody (Invitrogen, 44728G)	-	1E
anti-Sui3	polyclonal rabbit antiserum (6)	2D, 2E	2A, 2D, 2E, 3A, 5D
anti-Sui3	polyclonal rabbit antiserum (this study)	-	2C, 4C, 5A

Supplemental References

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