

Supplementary Table 1. *S. cerevisiae* strains used in this study

Strain name	Genotype
BY4742	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>
GSY188	BY4742 <i>ubc4::HIS3</i>
GSY90	BY4742 <i>ubc 13::kanMX</i>
GSY91	BY4742 <i>mms2::kanMX</i>
GSY92	BY4742 <i>ubc5::kanMX</i>
GSY93	BY4742 <i>ubc7::kanMX</i>
GSY94	BY4742 <i>ubc8::kanMX</i>
GSY95	BY4742 <i>ubc11::kanMX</i>
GSY96	BY4742 <i>pex4::kanMX</i>
GSY97	BY4742 <i>rad6::kanMX</i>
GSY98	BY4742 <i>chf1::kanMX</i>
GSY189	BY4742 <i>ubc1::natMX</i>
GSY190	BY4742 <i>ubc13::kanMX ubc4::HIS3</i>
GSY191	BY4742 <i>mms2::kanMX ubc4::HIS3</i>

Supplementary Table 2. Plasmids used in this study.

Name	Gene	Vector	Reference
pB299	<i>CHF1</i>	p425GAL1	(Bieganowski et al., 2004)
pC105	<i>CHF2</i>	p425GAL1	This study
pC110	<i>MMS2</i>	pSGAO4	This study
pC111	<i>UBC13</i>	pGEX4T-2	This study
pC114	<i>chf1-C345S,H350A</i>	pGEX4T-2	This study
pC115	<i>chf1-G192E</i>	pGEX4T-2	This study
pC116	<i>chf1-S220A,H223L</i>	pGEX4T-2	This study
pC117	<i>chf2-C451S,H456A</i>	pGEX4T-2	This study
pC118	<i>chf2-S326A,H329L</i>	pGEX4T-2	This study
pC119	<i>chf2-G298E</i>	pGEX4T-2	This study
pC145	<i>Z-CHF1</i>	p425GAL1	This study
pC146	<i>Z-CHF2</i>	p425GAL1	This study
pC153	<i>Z-chf1-K313R, K317R</i>	p425GAL1	This study
pC155	<i>Z-chf1-K260R</i>	p425GAL1	This study
pC156	<i>Z-chf1-K237R, K240R</i>	p425GAL1	This study
pC157	<i>Z-chf1-K204R</i>	p425GAL1	This study
pC158	<i>Z-chf1-K306R</i>	p425GAL1	This study
pC159	<i>Z-chf1-K306R,K313R,K317R</i>	p425GAL1	This study
pC160	<i>Z-chf1-K306R,K237R,K240R</i>	p425GAL1	This study

Bieganowski, P., Shilinski, K., Tsihchlis, P. N., and Brenner, C. (2004). Cdc123 and checkpoint forkhead associated with RING proteins control the cell cycle by controlling eIF2gamma abundance. *J Biol Chem* 279, 44656-44666.

Supplementary Table 3. Ubiquitinated peptides identified by LC-MS/MS

Chf1 + Ubc4				
Ub sites	Peptides	Charge (M+nH) ⁿ⁺	MMA (PPM)	XCorr
Chf1 K150	M*DK#HGLFSIR	2	0.91	2.48
Chf1 K204	EAISK#IPDQYHPVVK	2	5.42	3.83
Chf1 K217	THGCFK#VDDQGNWFLK	3	-2.73	2.99
Chf1 K237	VDDQGNWFLK#DVK	3	-3.95	5.19
Chf1 K240	DVK#SSSGTFLNHQR	2	3.78	3.91
Chf1 K260	LSSASTTSK#DYLLHDGDIIQLGM*DFR	3	3.07	5.86
Chf1 K300/K306	LK#ANAFNK#EALSR	3	-0.11	4.48
Chf1 K306	ANAFNK#EALSR	2	-4.72	3.83
Chf1 K313/K317	IK#NLQK#LTTGLEQEDCSICLNK	3	-0.07	6.83
Chf1 K317	NLQK#LTTGLEQEDCSICLNK	3	-1.82	6.72
Ub K48	LIFAGK#QLEDGR	2	0.73	3.68
Ub K63	TLSDYNIQK#ESTLHLVLR	3	-0.28	3.32
Chf1 + Ubc13/Mms2				
Chf1 K237	VDDQGNWFLK#DVK	3	9.13	3.95
Chf1 K306	ANAFNK#EALSR	2	11.73	2.73
Ub K63	TLSDYNIQK#ESTLHLVLR	3	3.8	2.88
Chf1 transubiquitination by Chf2 + Ubc4				
Chf1 K204	EAISK#IPDQYHPVVK	3	6.14	2.82
Chf1 K306	ANAFNK#EALSR	2	9.54	3.43
Ub K48	LIFAGK#QLEDGR	2	11.16	3.68
Chf2 + Ubc4				
Chf2 K211	NIVGGADGSTIVNNSQEMYK#NLR	3	-1.1	3.46
Chf2 K258	KDK#HGLFSIR	2	-3.71	3.15
Chf2 K288	K#AGPGSQLVIGR	2	-1.56	4.09
Chf2 K310	DAISK#IPEQYHPVVK	3	-3.01	3.43
Chf2 K310	VRDAISK#IPEQYHPVVK	3	-1.73	5.28
Chf2 K333	HGCFK#VDSQGNWYIK	3	-2.39	3.98
Chf2 K343	VDSQGNWYIK#DVK	2	-2.13	3.93
Chf2 K346	DVK#SSSGTFLNHQR	2	0.82	3.97
Chf2 K366	LSPASSLSK#DTPLR	3	-6.98	2.79
Chf2 K366	LSPASSLSK#DTPLRDGDILQLGM*DFR	3	-2.6	5.38
Chf2 K406,K412	LK#ANSFNK#EALQR	3	-6.59	3.20
Chf2 K412	ANSFNK#EALQR	2	1.28	3.28
Chf2 K423	LQNLQK#LTTGIEEEDCSICLCK	3	2.75	6.37

Ub K48	LIFAGK#QLEDGR	2	-0.62	3.97
Ub K63	TLSDYNIQK#ESTLHLVLR	3	-0.88	4.08
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Chf2 + Ubc13/Mms2				
Chf2 K258	KDK#HGLFSIR	3	10.88	3.34
Chf2 K310	DAISK#IPEQYHPVVK	3	10.66	3.00
Chf2 K346	DVK#SSSGTFLNHQR	3	9.39	2.80
Chf2 K366	LSPASSLSK#DTPLR	2	10.42	2.55
Ub K63	TLSDYNIQK#ESTLHLVLR	2	12.21	4.33
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Chf2 transubiquitination by Chf1 + Ubc4				
Chf2 K211	NIVGGADGSTIVNNSQEMYK#NLR	3	7.18	2.72
Chf2 K256	K#DKHGLFSIR	2	11.23	2.57
Chf2 K288	K#AGPGSQLVIGR	2	8.79	4.04
Chf2 K310	DAISK#IPEQYHPVVK	3	11.39	2.71
Chf2 K343	VDSQGNWYIK#DVK	3	9.2	3.52
Chf2 K366	LSPASSLSK#DTPLR	2	12.96	3.33
Chf2 K412	LKANSFNK#EALQR	3	8.98	2.81
Ub K48	LIFAGK#QLEDGR	2	10.41	3.86

* Oxidation (+/- 15.99644 Da)

GlyGly (114.05421 Da)