Supplemental Materials

Molecular Biology of the Cell

Stieg et al.

Supplemental Table 1

Strain	Genotype*	Source
RSY10		(Strich *et al.*, 1989)
RSY1006	*slt2Δ::his5*$^{+}$	(Krasley *et al.*, 2006)
RSY1696	*cnc1Δ::KANMX6*	(Cooper *et al.*, 2000)
RSY1701	*med13Δ::HIS3*	(Khakhina *et al.*, 2014)
RSY1706	*med13Δ:: KANMX6*	(Khakhina *et al.*, 2014)
RSY1726	*cdk8Δ:: KANMX6*	(Khakhina *et al.*, 2014)
RSY1736	*kdx1Δ::KANMX6*	(Jin *et al.*, 2014)
RSY1737	*kdx1Δ::KANMX6 slt2Δ::his5*$^{+}$	(Jin *et al.*, 2014)
RSY1798	*Med13-myc::KANMX4*	(Khakhina *et al.*, 2014)
RSY1770	*grr1Δ::his5*$^{+}$	This study
RSY1771	*grr1Δ::his5+ MED13-13Myc:: KANMX6*	This study
RSY1787	*Med12-myc::KANMX4*	This study
RSY2066	*PTetO7-Ubi-Ile::3HA-Cdk8::NatMX4*	This study
PJ69-4a	*LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ gal4Δ gal80Δ*	(James *et al.*, 1996)

Yeast strains used in this study. Genotype of all strains is *MAT***a** *ade2 ade6 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 except* PJ69-4alpha which is *MAT***a** *trp1-901 leu2-3,112 ura3-52 his3-200 gal4*Δ *gal80*Δ

Supplemental Table 2

Plasmid Name	Gene	Epitope Tag	Marker	Promoter	2μ/ CEN/ int	Reference
pBK38	*CNC1*	GFP	*URA3*	*ADH1*	CEN	(Cooper *et al.*, 2012)
pKC337	*CNC1*	1myc	*TRP1*	*ADH1*	CEN	(Cooper *et al.*, 1997)
pKC800	*GAL4AD-MED13*	1HA	*LEU2*	*ADH1*	CEN	This study
pKC801	*MED13*	3HA	*URA3*	*ADH1*	CEN	This study
pKC802	*MED13*^{T210A}	3HA	*URA3*	*ADH1*	CEN	This study
pKC803	*MED13*	3HA	*LEU2*	*ADH1*	CEN	This study
pLR106	*BCK1-20*	None	*HIS3*	*ADH1*	CEN	(Krasley *et al.*, 2006)
pLR141	*CNC1*	1 myc	*URA*	*ADH1*	2μ	This study
pLR166	*CNC1*^{S266A}	1 myc	*TRP1*	*ADH1*	CEN	(Jin *et al.*, 2014)
pDS2	*GAL4AD -MED13*$^{1-305}$	1HA	*LEU2*	*ADH1*	CEN	This study
pDS4	*GAL4AD-MED13*$^{907-1421}$	1HA	*LEU2*	*ADH1*	CEN	This study
pDS5	*GAL4AD-MED13*$^{306-906}$	1HA	*LEU2*	*ADH1*	CEN	This study
pDS6	*GAL4AD-MED13*$^{1-906}$	1HA	*LEU2*	*ADH1*	CEN	This study

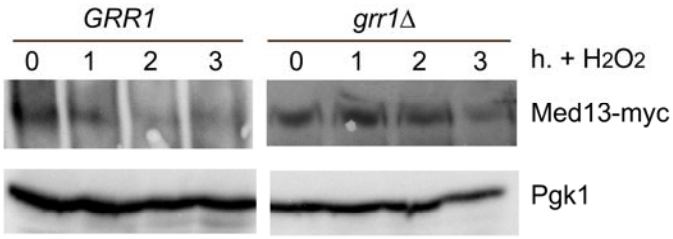
pDS7	*GAL4AD-MED13$^{306-570}$*	1HA	*LEU2*	*ADH1*	CEN	This study
pDS8	*GAL4AD-MED13$^{571-906}$*	1HA	*LEU2*	*ADH1*	CEN	This study
pDS10	*6HIS- MED13$^{571-906}$*	-	-	-		This study
pDS15	*GAL4AD-MED13$^{571-650}$*	1HA	*LEU2*	*ADH1*	CEN	This study
pDS16	*GAL4AD-MED13$^{651-906}$*	1HA	*LEU2*	*ADH1*	CEN	This study
pDS30	*GST-MED13$^{742-844}$*	GST	-	-	-	This study
pDS22	6His-*MED13$^{571-650}$*	6His	-	-	-	This study
pDS32	*GAL4AD-MED13$^{742-844}$*	1HA	*LEU2*	*ADH1*	2µ	This study
pDS33	*GAL4AD-MED13$^{742-844}$* *S748A*	1HA	*LEU2*	*ADH1*	2µ	This study
pDS34	*GAL4AD-MED13$^{742-844}$* *T781A*	1HA	*LEU2*	*ADH1*	2µ	This study
pDS36	*GAL4AD-MED13$^{742-844}$* *T835A, T837A*	1HA	*LEU2*	*ADH1*	2µ	This study
pDS40	*GAL4AD-MED13$^{742-844}$* *T801A*	1HA	*LEU2*	*ADH1*	2µ	This study
pDS41	*GAL4AD-MED13$^{742-844}$* *T781A, T801A*	1HA	*LEU2*	*ADH1*	2µ	This study
pDS42	*GAL4AD-MED13$^{742-844}$* *S748A, T781A*	1HA	*LEU2*	*ADH1*	2µ	This study
pSW108	*hCCNC1-GAL4^{BD}*	1HA	*TRP1*	*ADH1*	2µ	This study
GST-yCNC1	*GST-yCNC1*	GST	-	-	-	(Wang *et al.*, 2014)
6His-	6His-hCNC1	6His	-	-	-	This study

hCNC1						
6His-pACT2	6His-yCNC1 *GAL4AD*	6His 1HA	- *LEU2*	- *ADH1*	- CEN	This study (Van Criekinge and Beyaert, 1999)
pAS2	*GAL4BD*	1HA	*TRP*	*ADH1*	CEN	(Van Criekinge and Beyaert, 1999)
pAS2-Grr1	*GAL4BD-GRR1*	1HA	*TRP*	*ADH1*	CEN	(Wang and Solomon, 2012)
pAS2-Grr1ΔL	*GAL4BD-GRR1*$^{\Delta L}$-	1HA	*TRP*	*ADH1*	CEN	(Wang and Solomon, 2012)
pGrr1	*Grr1*	1HA	*TRP*	*ADH1*		(Hsiung *et al.*, 2001)
pGrr1ΔL	*Grr1ΔL*	1HA	*TRP*	*ADH1*		(Hsiung *et al.*, 2001)
Mt-dsRed	Mito-targeting	dsRed	*URA3*	*ADH1*	CEN	(Naylor *et al.*, 2006)
pHY1022	*MED13*	3HA	*URA3*	*ADH1*	2μ	(Chang *et*

						al., 2004)
pHY1089	*MED13*^{S608A,S1236A}	3HA	*URA3*	*ADH1*	2μ	(Chang *et al.*, 2004)
Slt2	*SLT2*	3HA	*LEU2*	*SLT2*	2μ	(Kim *et al.*, 2008)
Slt2^{K54R}	*SLT2*^{K54R}	3HA	*LEU2*	*SLT2*	2μ	(Kim *et al.*, 2008)
pCM188	*TET*	-	*URA3*	*ADH1-Tet02*	CEN	(Gari *et al.*, 1997)
pMK634	*Ubi-lLe-3HA*		*NAT-MX4*	*ADH1*	Int.	(Gnanasundram and Kos, 2015)

Plasmids used in this study.

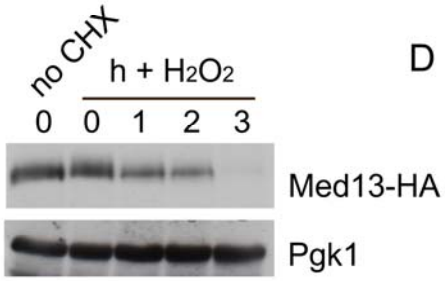
A



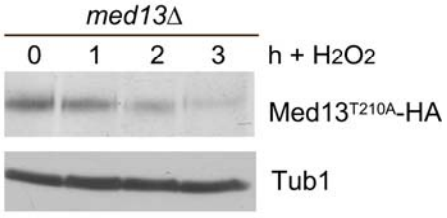
B

```
321 S S V T L T P P T S 330   human Med13
324 C G M P L T P P T S 330   human Med13L
205 Q G Y L I T P P K H 214   yeast Med13
```

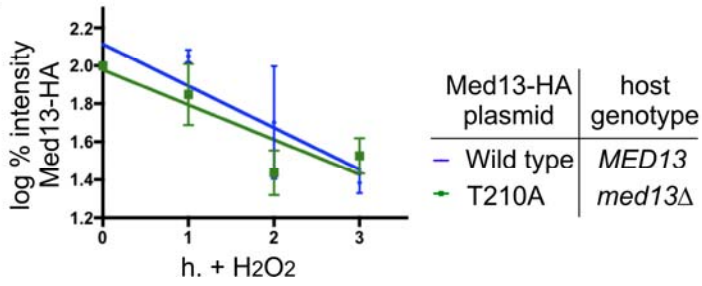
C

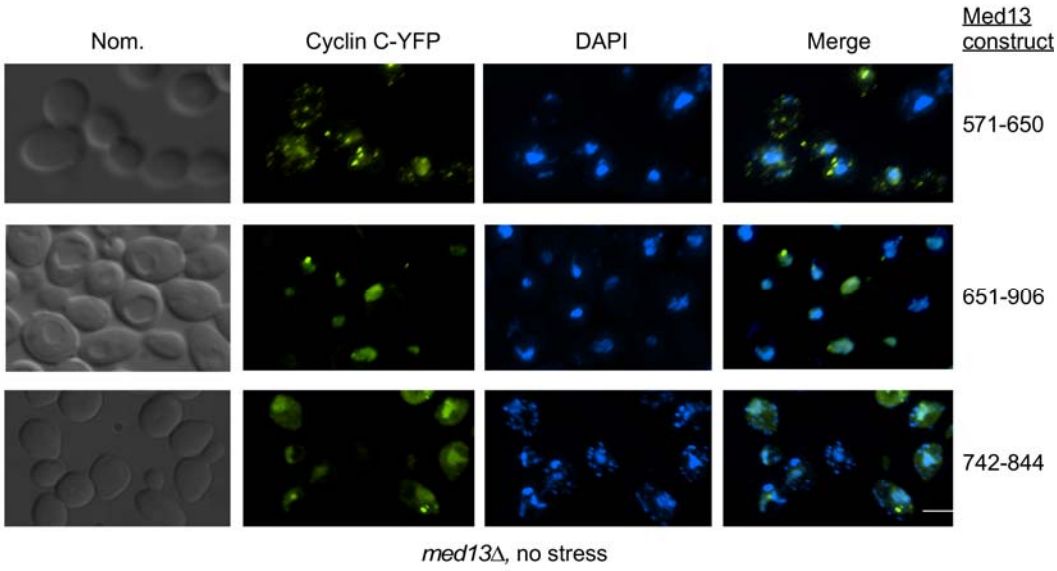


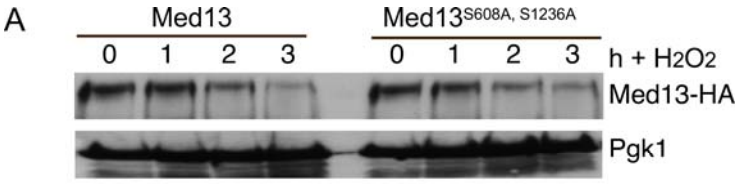
D

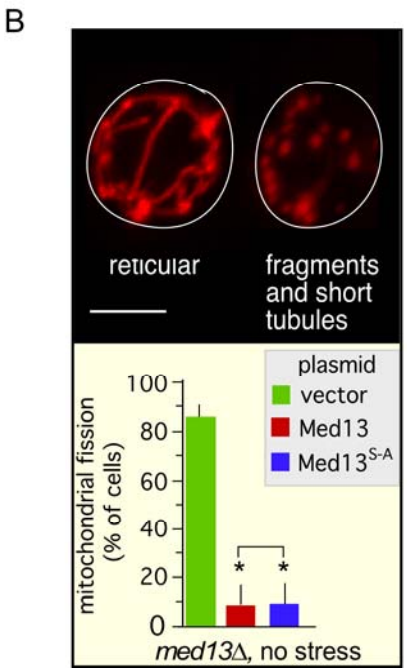


E

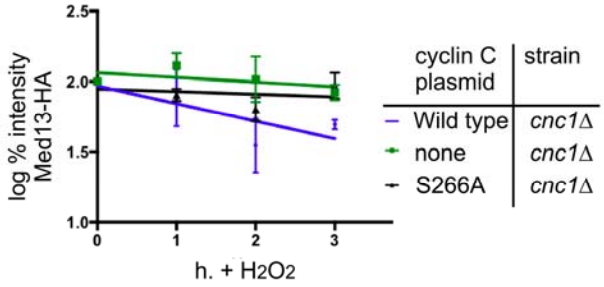



Nom.
Cyclin C-YFP
DAPI
Merge
Med13 construct
571-650
651-906
742-844
med13Δ, no stress

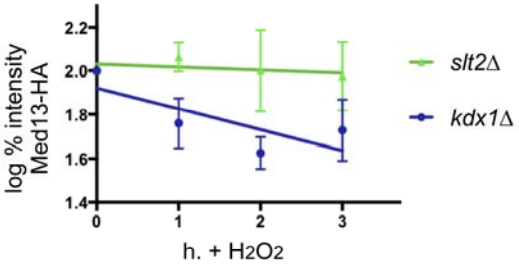

A
Med13
Med13S608A, S1236A
0 1 2 3
0 1 2 3
h + H2O2
Med13-HA
Pgk1


B
reticular
fragments and short tubules
plasmid
vector
Med13
Med13S-A
mitochondrial fission (% of cells)
100
80
60
40
20
0
*
*
med13Δ, no stress

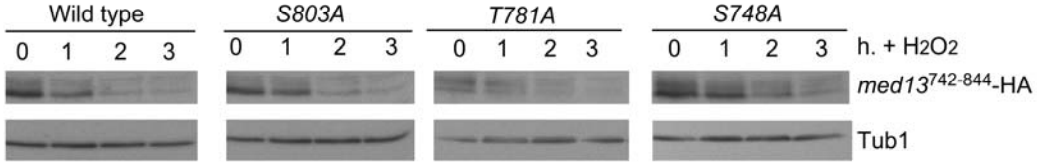
A


log % intensity Med13-HA
2.5
2.0
1.5
1.0
0
1
2
3
h. + H2O2
cyclin C plasmid
strain
Wild type
cnc1Δ
none
cnc1Δ
S266A
cnc1Δ

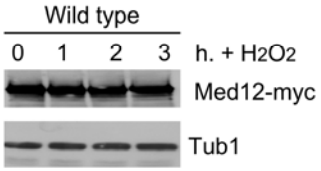
B


log % intensity Med13-HA
2.2
2.0
1.8
1.6
1.4
0
1
2
3
h. + H2O2
slt2Δ
kdx1Δ

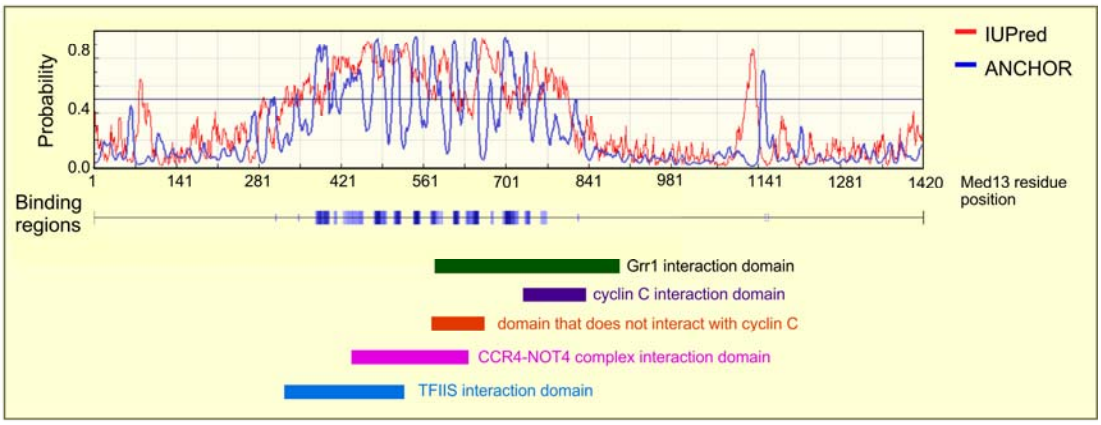
C


Wild type
S803A
T781A
S748A
0 1 2 3
h. + H2O2
med13742-844-HA
Tub1

D


Wild type
0 1 2 3
h. + H2O2
Med12-myc
Tub1

A



B

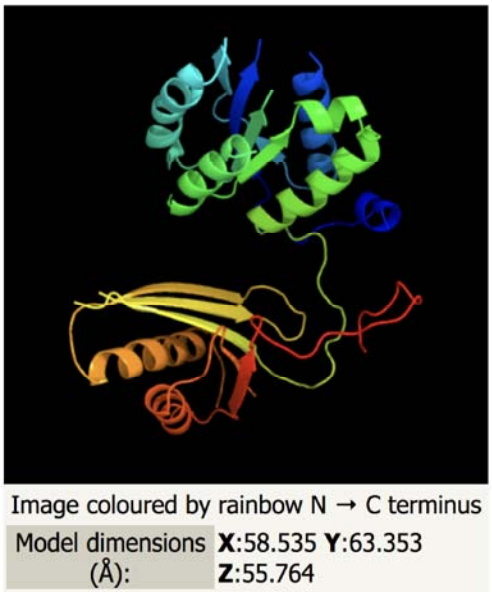


FIGURE S1. The human Fbw7 degron on Med13 is not required for Med13 degradation. (A) RSY1771 (*grr1Δ::HIS3 MED13-myc::KAN*) harboring either *ADH1$_{PRO}$-GRR1* or a vector control were treated with 0.4 mM H_2O_2 for the timepoints indicated and Med13 levels analyzed by Western blot. Tub1 levels were used as loading controls. (B) Conservation of the human Med13 SCF^{Fbw7} degron in yeast. (C) Degradation of Med13-HA after treatment of the cells with cycloheximide. Wild type cells (RSY10) harboring Med13-HA (pKC801) were treated with cycloheximide as previously described (Jin *et al.*, 2014) and Med13-HA degradation analyzed as described. Pgk1 was used as a loading

control. (D) *med13*Δ cells (RSY1701) harboring Med13^{T210A} as the only copy of Med13 were treated with 0.4 mM H_2O_2 for the timepoints indicated and Med13 levels analyzed by Western blot. Tub1 levels were used as a loading control. (E) Quantification of Med13-HA degradation from C and D.

FIGURE S2. Fluorescence microscopy of mid-log phase *med13*Δ cells (Y1701) harboring the Gal^{AD}-Med13 fusion constructs shown and cyclin C-YFP (pBK38). Cells were stained with Dapi to visualize the nucleus. Bar = 10μM

FIGURE S3. PKA mediated phosphorylation of Med13 is not required for its degradation following H_2O_2 stress. (A) Wild type (RSY10) cultures expressing either PHY1066 (2μ Med13-HA) or PHY2081 (2μ Med13-HA plasmid with PKA sites, S608 and S1236 mutated to alanine) were grown to mid-log phase (0 hr) then treated with 0.4 mM for the indicated times. Med13-HA levels were determined by Western blot analysis. Tub1 levels were used as a loading control. (B) The Med13^{S808A,S1236A} mutant complements *med13*Δ mitochondrial morphology. *med13*Δ cells (RSY1701) expressing either PHY1066 ,PHY2081 or a vector and the DsRed mitochondrial targeting plasmid (mt-DsRed) were grown to mid-log. Mitochondrial morphology was examined using fluorescence microscopy of living cells. The left hand panel shows representative images of reticular or fragmented mitochondria are shown. Bar = 10μM. The percent of cells (mean ± s.e.m.) within the population displaying mitochondrial fission is given * $p<0.05$ difference from wild type.

FIGURE S4. (A) Quantification of Med13-HA degradation following 0.4mM H_2O_2 stress for the experiments shown in Fig. 6 (A) and Fig. 7 (B). (C) Wild type (RSY10) cultures harboring either wild type Gal4AD-Med13$^{742-844}$ or the various point mutations indicated

were grown to mid-log phase (0 hr) then treated with 0.4 mM H_2O_2 for the indicated times. Med13-HA levels were determined by Western blot analysis. Tub1 levels were used as a loading control. (D) Med12 is not destroyed in H_2O_2 stress. A strain harboring endogenous Med12-myc (RSY1787) was treated with 0.4 mM H_2O_2 for the timepoints indicated and Med12 levels analyzed by Western blot. Tub1 levels were used as loading controls.

FIGURE S5. (A). IUPred and ANCHOR plot analysis of Med13. These programs generates a plot with the profiles calculated by IUPred (Dosztanyi *et al.*, 2005), a general disorder prediction method (in red), and ANCHOR (Dosztanyi *et al.*, 2009), a prediction of disordered binding regions (in blue). Underneath the profile, predicted binding regions are indicated by the horizontal bars. The bar is shaded according to the prediction score. Regions that are filtered out are marked by empty bars. Grr1 and cyclin C binding domains identified in this work are also shown. In addition, the domain of Med13 that does not interact with cyclin C is marked as well as the CCR4-NOT4 complex and TFIIS interaction domains (Liu *et al.*, 2001; Wery *et al.*, 2004). (B) Phyre2 (Kelley *et al.*, 2015) plot analysis of yeast Med13.