SUPPLEMENTAL DATA

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FIGURE LEGENDS

Figure S1: Effect of deletion of the F-box of Mdm30p expressed at endogenous levels on mitochondrial morphology, respiration and degradation of Fzo1p

(A) Mitochondrial morphology was assessed in wild type (vector) (W303 background), *mdm30Δ* (vector), or *mdm30Δ* cells expressing either Mdm30-HA or F-box deletion mutant of Mdm30-HA (Δ*fbox-HA*) from the *MDM30* promoter. (B) The same strains as described in panel A were grown at 37º C on selective media containing either dextrose or glycerol as the only carbon source. (C) Rate of Fzo1p degradation was analysed in the indicated strains (W303 background) after shift to 37°C and treatment with CHX. Yeast extracts were prepared at the indicated times and remaining Fzo1p or Mdm30-HA evaluated by immunoblotting. Levels of a stable protein, phosphoglucokinase (PGK) is shown as a loading control. (D) As Mdm30-HA is undetectable in whole cell lysates when expressed from the MDM30 promoter, immunoprecipitates with HA antibody (IP:HA) from strains used in C were immunoblotted with anti-HA antibody (IB:HA).

Figure S2: Ubiquitylated Fzo1p is localized to mitochondria.

(A) Localization of Fzo1p in sucrose gradient. A mitochondrial-enriched pellet was loaded onto a sucrose cushion (15%, 23%, 32% and 60% sucrose in MOPS buffer) as described (Meisinger *et al.*, 2000). After 1 h centrifugation at 134,000g, the majority of the mitochondria migrate at the 60/32% interface. Nine fractions surrounding this interface were collected and resolved by SDS-PAGE after TCA precipitation. Fzo1p, Tom20p (Mitochondrial marker) and Cue1p (ER marker) were analyzed in fractions 1 to 9, cytosolic fraction (S) and pellet fraction (P). Fzo1p co-localizes with Tom20p to fraction 7.

(B) Enlargement of Fzo1p immunoblot from fraction 7. Unmodified and modified forms of Fzo1p are indicated by thick and thin arrows respectively.

TABLES

Supplemental Data, Table 1. Strains and plasmids used in this study.

Strains	Genotypes	Reference
BY4742	MATα ; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0	Euroscarf
BY4742 *fzo1Δ*	MATα ; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0 YBR179C::kanMX4	Euroscarf
BY4742 *mdm30Δ*	MATα ; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0 YLR368w::kanMX4	Euroscarf
W303	MATa; ura3-52; trp1Δ2; leu2-3_112; his3–11; ade2-1; can1-100	
W303 FZO1-HA	MATa; ura3-52; trp1Δ2; leu2-3_112; his3–11; ade2-1; can1-100 FZO1-3HA-HIS3	This study
W303 *mdm30Δ*	MATa; ura3-52; trp1Δ2; leu2-3_112; his3–11; ade2-1; can1-100 YLR368w::kanMX6	This study
WT-CMY	MATa; his3-Δ200; ade2-101; lys2Δ1; ura3-52; lys2-801, trp1Δ62, bar1::HIS3	(Rinaldi *et al.*, 2004)
CMY-*mpr1-1*	MATa; his3-Δ200; ade2-101; lys2Δ1; ura3-52; lys2-801, trp1Δ62, bar1::HIS3, mpr1-1	(Rinaldi *et al.*, 2004)
WCG4	MATa ; ura3-52; leu2-3_112; his3-11	(Richter-Ruoff *et al.*, 1994)
WCG4 FZO1-HA	MATa ; ura3-52; leu2-3_112; his3-11 FZO1-3HA-KanMX6	This study
pre1-1 pre2-2	MATa ; ura3-52; leu2-3_112; his3-11; pre1-1 pre2-2	(Richter-Ruoff *et al.*, 1994)
pre1-1 pre2-2 FZO1-HA	MATa ; ura3-52; leu2-3_112; his3-11; pre1-1 pre2-2 FZO1-3HA-KanMX6	This study
Sub62	MATa; his3-Δ200; lys2-801; leu2-3_112; trp1-1; ura3-52	(Rubin *et al.*, 1998)
rpt2RF	MATa; his3-Δ200; lys2-801; leu2-3_112; trp1-1; ura3-52;rpt2RF	(Rubin *et al.*, 1998)
YPH499 (Cim WT)	MATa; ura3-52; lys2-801; ade2-101; trp1-Δ63; his3-Δ200; leu2-Δ1	(Ghislain *et al.*, 1993)
CMY763 *(Cim3-1)*	MATα ; ura3-52 ; leu2Δ1 his3-Δ200 ; cim3-1	(Ghislain *et al.*, 1993)
W303-1A	MATa; ura3-1; can1-100; GAL+; leu2-3_112; trp1-1; ade2-1; his3-11,15	
W303-1A FZO1-HA	MATa; ura3-1; can1-100; GAL+; leu2-3_112; trp1-1; ade2-1; his3-11,15 FZO1-3HA-KanMX6	This study
cdc53ts	MATa; ura3-1; can1-100; GAL+; leu2-3_112; trp1-1; ade2-1; his3-11,15; cdc53-1	(Willems *et al.*, 1996)
cdc53ts FZO1-HA	MATa; ura3-1; can1-100; GAL+; leu2-3_112; trp1-1; ade2-1; his3-11,15; cdc53-1 FZO1-3HA-KanMX6	This study
cdc34ts	MATa; ura3-1; can1-100; GAL+; leu2-3_112; trp1-1; ade2-1; his3-11,15; cdc34-2	(Willems *et al.*, 1996)
cdc34ts FZO1-HA	MATa; ura3-1; can1-100; GAL+; leu2-3_112; trp1-1; ade2-1; his3-11,15; cdc34-2 FZO1-3HA-KanMX6	This study
SUB280	MATa; lys2-801; leu2-3, 2-112; ura3-52; his3-D200; trp1-1; ubi1HTRP1; ubi2-D2Hura3; ubi3-Dub2; ubi4-D2HLEU2; [pUB39 wild-type ubiquitin] [pUB100]	D. Finley
SUB592	MATa; lys2-801; leu2-3, 2-112; ura3-52; his3-D200; trp1-1; ubi1HTRP1; ubi2-D2Hura3; ubi3-Dub2; ubi4-D2HLEU2; [pUB221: His6-cMyc-Ubiquitin] [pUB100]	D. Finley
SUB592 FZO1-HA	MATa; lys2-801; leu2-3, 2-112; ura3-52; his3-D200; trp1-1; FZO1-3HA-KanMX6; ubi1HTRP1; ubi2-D2Hura3; ubi3-Dub2; ubi4-D2HLEU2; [pUB221: His6-cMyc-Ubiquitin] [pUB100]	This study
Plasmids		**Reference**
pUb70(empty vector)	2 micron, CUP1 promoter, LYS2, Amp	D. Finley
pUb39 (Ub WT)	2 micron, CUP1 promoter, Ub , LYS2, Amp	D. Finley
pUb115 (Ub K48R)	2 micron, CUP1 promoter, UbK48R , LYS2, Amp	D. Finley
pUb197 (Ub K63R)	2 micron, CUP1 promoter, UbK63R , LYS2, Amp	D. Finley
pRS316	CEN, URA3, Amp	(Sikorski and Hieter, 1989)
pMDM30-HA	CEN, MDM30 promoter-MDM30-HA, URA3, Amp	This study
pΔfbox-HA	CEN, MDM30 promoter- *mdm30-HA fboxΔ*, URA3, Amp	This study
p426-TEF	2 micron, TEF promoter, URA3, Amp	(Mumberg *et al.*, 1995)
pTEF-MDM30-HA	2 micron, TEF promoter-MDM30-HA, URA3, Amp	This study
pTEF-fbox-HA	2 micron, TEF promoter-*mdm30-HA fbox*, URA3, Amp	This study
pYX232-mtGFP	2 micron, TPI promoter-mtGFP, TRP1, Amp	B.Westermann

Supplemental Data, Table 2. Numerical data from Figure 1B.

Strain	Number of cells (%)				TOTAL
	Aggregated	Fizzed	Tubular	Fused	
WT vector	25 (7.6)	25 (7.6)	237 (71.8)	43 (13)	330
mdm30Δ vector	203 (67)	15 (5)	67 (22.1)	18 (5.9)	303
mdm30Δ MDM30-HA	36 (10.1)	77 (21.5)	193 (53.9)	52 (14.5)	358
mdm30Δ fbox-HA	189 (66.1)	27 (9.4)	60 (21)	10 (3.5)	286

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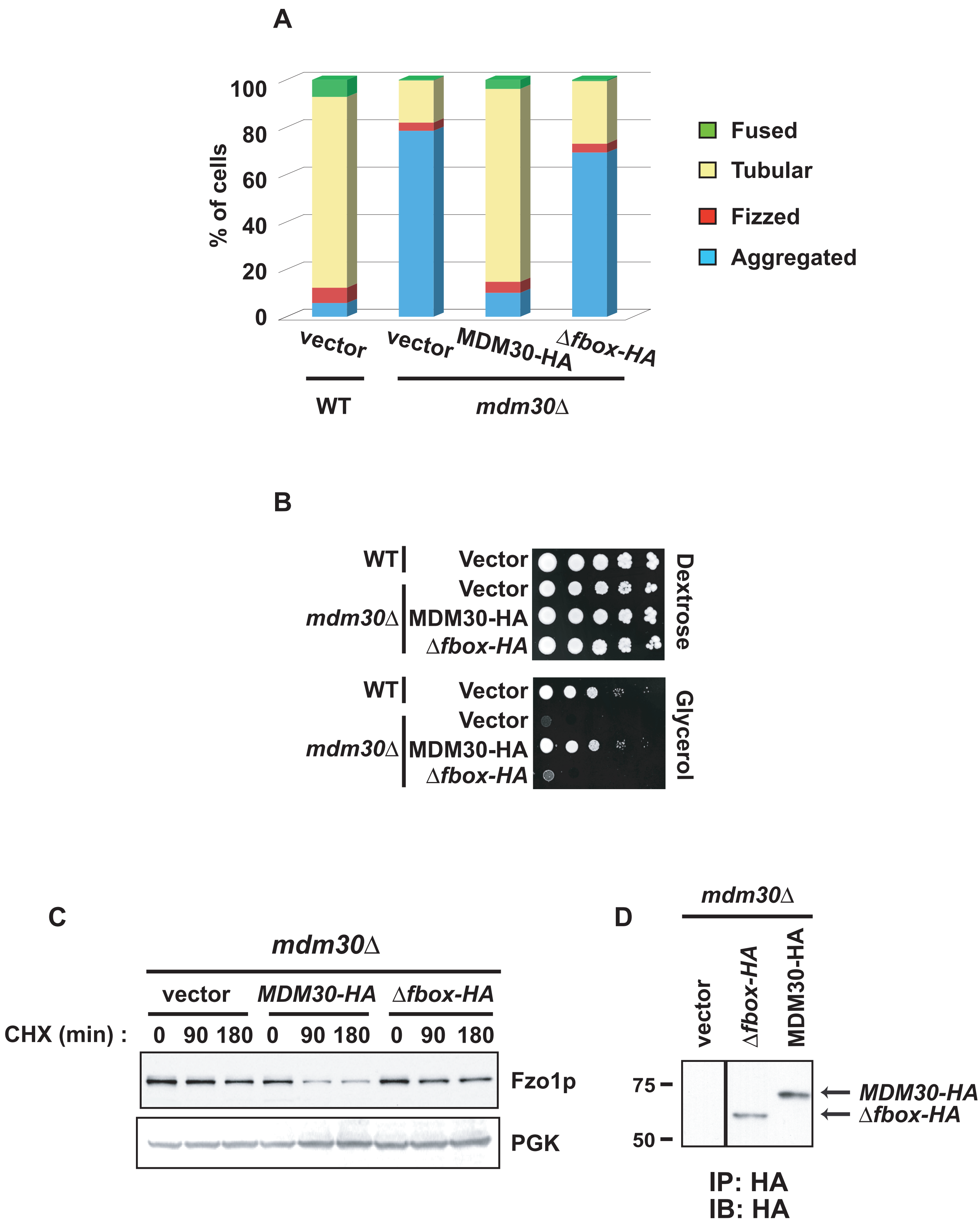

Cohen et al. Figure S2