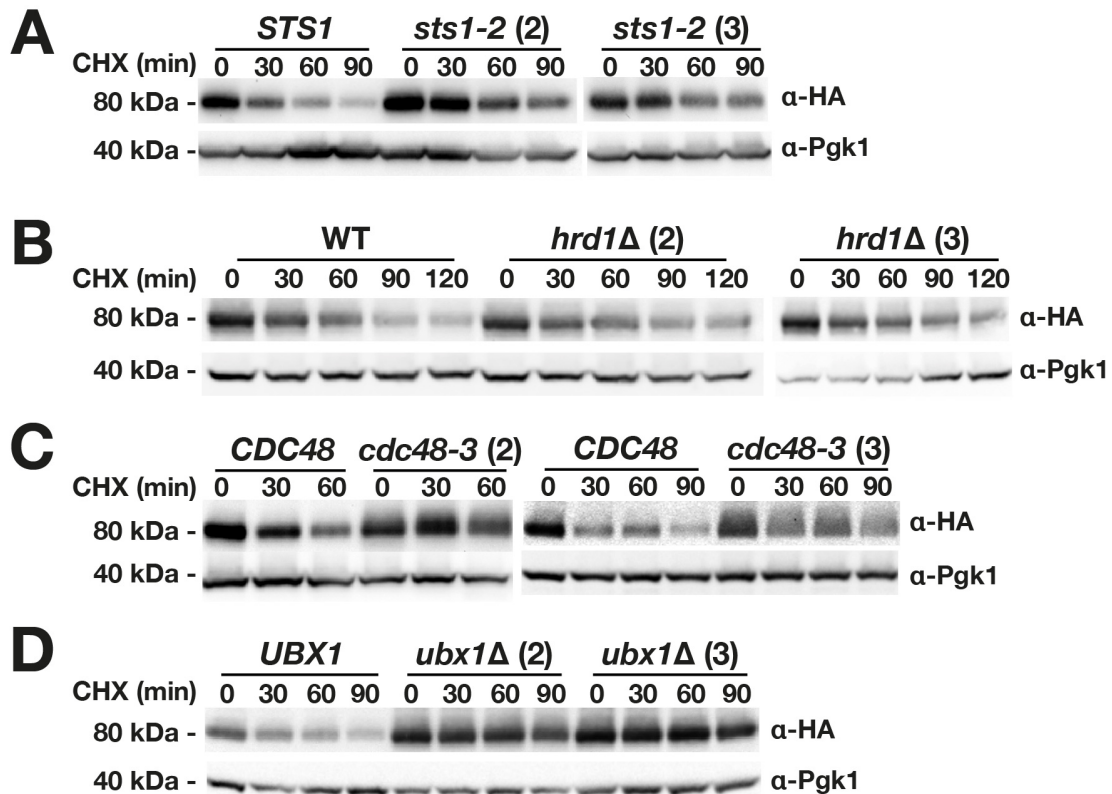


Supplemental Material



**Figure S1. Replicate immunoblots used to quantitate the dependence of *STS1*, *HRD1*, *CDC48* and *UBX1* on Asi1 stability.** (A) As in Fig. 2D (clone 1) - Asi1 is degraded in an *STS1*-dependent manner by proteasomes in the nucleus. The stability of Asi1 in *STS1* (NA10) and *sts1-2* (NA25) (independent clones 2 and 3) strains expressing Asi1-3HA (pPL1141). Cells were grown at 25 °C (permissive temperature) and shifted to 37 °C (non-permissive temperature) for 3 hours prior to CHX addition. (B) As in Fig. 4A (clone 1) - Asi1 turnover is independent of *HRD1*. CHX chase of Asi1-3HA (pPL1159) in WT (MBY154) and *hrd1Δ* (MBY155) (independent clones 2 and 3). (C) As in Fig. 5B (clone 1) - Asi1 turnover is impaired in *cdc48-3* temperature sensitive cells. CHX chase of Asi1-3HA (pPL1141) in *CDC48* (MHY1562) and *cdc48-3* (MHY3512) cells (independent clones 2 and 3). Cultures were grown at 25 °C (permissive temperature) and shifted to 37 °C (non-permissive temperature) 1 hour prior to CHX addition. (D) As in Fig. 5C (clone 1) - Asi1 turnover is impaired in *ubx1Δ* mutant cells. CHX chase of Asi1-3HA (pPL1141) in *UBX1* (BY4741) and *ubx1Δ* (BY4741<sub>ubx1Δ</sub>) cells (independent clones 2 and 3). Extracts from cells harvested at the indicated times were immunoblotted with anti-HA and anti-Pgk1.

**Table S1. Yeast strains and plasmids used in this study**

Strain	Genotype	Reference
CAY220	<i>MATa ura3-52 leu2Δ1 ssd1 cim3-1::RPT6</i>	(Pfirrmann et al., 2010)
MBY150	<i>MATa ura3-52 leu2-3,112 lys2Δ201 asi1Δ::kanMX</i>	This study
MBY151	<i>MATa ura3-52 leu2-3,112 lys2Δ201 asi1Δ::kanMX ubc6Δ::LEU2</i>	This study
MBY152	<i>MATa ura3-52 leu2-3,112 lys2Δ201 asi1Δ::kanMX ubc7Δ::LEU2</i>	This study
MBY153	<i>MATa ura3-52 leu2-3,112 lys2Δ201 asi1Δ::kanMX ubc6Δ::LEU2 ubc7Δ::natMX</i>	This study
MBY154	<i>MATa ura3-52 lys2Δ201 asi1Δ::kanMX</i>	This study
MBY155	<i>MATa ura3-52 lys2Δ201 asi1Δ::kanMX hrd1Δ::URA3</i>	This study
MBY156	<i>MATa ura3-52 lys2Δ201 asi1Δ::kanMX doa10Δ::natMX</i>	This study
MBY157	<i>MATa ura3-52 lys2Δ201 asi1Δ::kanMX doa10Δ::natMX hrd1Δ::URA3</i>	This study
PLY123	<i>MATa ura3-52 leu2-3,112 lys2Δ201</i>	Ljungdahl lab
PLY127	<i>MATa ura3-52 lys2Δ201</i>	Ljungdahl lab
PLY1314	<i>MATa ura3-52 asi1Δ::hphMX</i>	(Boban et al., 2006)
PLY1321	<i>MATa ura3-52 asi1Δ::hphMX asi3Δ::kanMX</i>	(Zargari et al., 2007)
PLY1327	<i>MATa ura3-52 lys2Δ201 asi1Δ::hphMX</i>	Ljungdahl lab
PLY1343	<i>MATa ura3-52 asi1Δ::hphMX asi2Δ::hisG</i>	(Zargari et al., 2007)
PLY1346	<i>MATa ura3-52 asi1Δ::hphMX asi2Δ::hisG asi3Δ::kanMX</i>	(Zargari et al., 2007)
PLY1348	<i>MATa ura3-52 leu2Δ1 ssd1 cim3-1</i>	(Pfirrmann et al., 2010)
PLY1581	<i>MATa ura3-52 trp1Δ101::loxP ASI3-6HA::Kl-TRP1</i>	Zargari et al., 2007)
PLY1630	<i>MATa ura3-52 asi1Δ::hphMX ssy5Δ::natMX,</i>	(Zargari et al., 2007)
PLY1811	<i>MATa ura3-52 lys2Δ201 ASI1-6HA::hphNT1</i>	This study
PLY1947	<i>MATa ura3-52 asi1Δ::hphMX asi2Δ::hisG asi3Δ::kanMX doa10Δ::natMX</i>	This study
YAZ101	<i>MATa ura3-52 lys2Δ201 asi1Δ::hphMX pep4Δ</i>	Ljungdahl lab
BY4741	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	M. Hochstrasser
BY4741 <sub>ubc7Δ</sub>	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ubc7Δ</i>	M. Hochstrasser
BY4741 <sub>cue1Δ</sub>	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 cue1Δ</i>	M. Hochstrasser
BY4741 <sub>ubx1Δ</sub>	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ubx1Δ</i>	M. Hochstrasser
MHY1562	<i>MATa his3Δ200 leu2-3,112 ura3-52 trp1 ade2-1</i>	(Ravid and Hochstrasser, 2007)
MHY3512	<i>MATa his3Δ200 leu2-3,112 ura3-52 trp1 ade2-1 cdc48-3</i>	(Ravid and Hochstrasser,

Strain	Genotype	Reference
NA10	<i>MATa ura3-1 trp1-1 ade2-1 leu2-3, 112 his3-11 (STS1)</i>	2007) (Chen et al., 2011)
NA25	<i>MATa ura3-1 trp1-1 ade2-1 leu2-3, 112 his3-11 sts1-2</i>	(Chen et al., 2011)
Plasmid	Description	References
pAZ013	pRS202 (2 $\mu$ <i>URA3</i> ) containing <i>AS11</i>	Ljungdahl lab
pPL1136	pRS202 (2 $\mu$ <i>URA3</i> ) containing <i>AS11-3HA</i>	(Zargari et al., 2007)
pPL1141	pRS316 (CEN/ARS <i>URA3</i> ) containing <i>AS11-3HA</i>	(Zargari et al., 2007)
pPL1142	pRS316 (CEN/ARS <i>URA3</i> ) containing <i>3HA-AS12</i>	(Zargari et al., 2007)
pPL1159	pRS317 (CEN/ARS <i>LYS2</i> ) containing <i>AS11-3HA</i>	Ljungdahl lab
pPL1307	YEpl95-based (2 $\mu$ <i>URA3</i> replaced with <i>LYS2</i> ) containing <i>P<sub>CUP1</sub>-6HIS-UBI</i>	(Omnus et al., 2011)