Supplemental Materials

Molecular Biology of the Cell

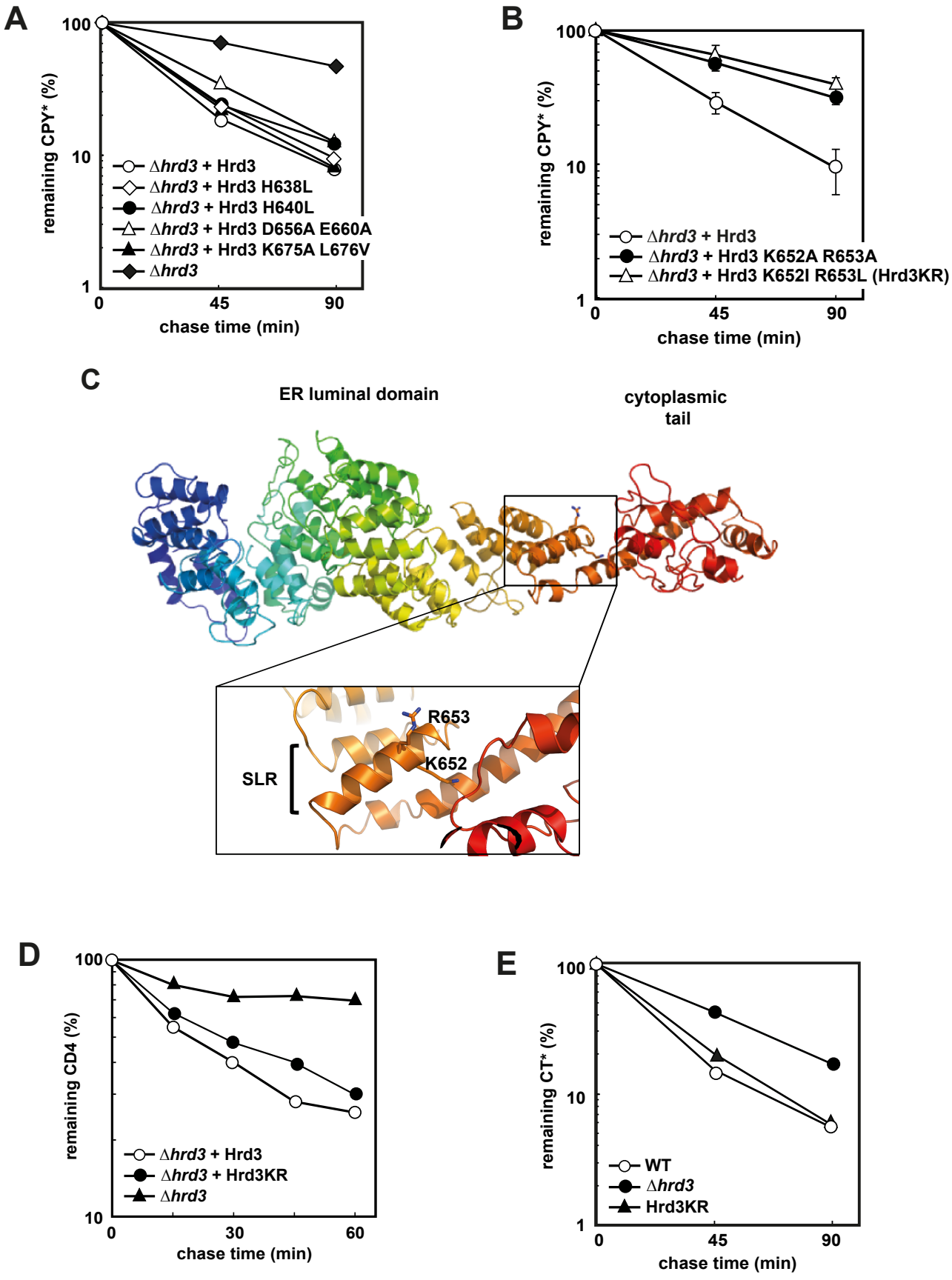
Mehnert et al.

Supplementary Figure legends

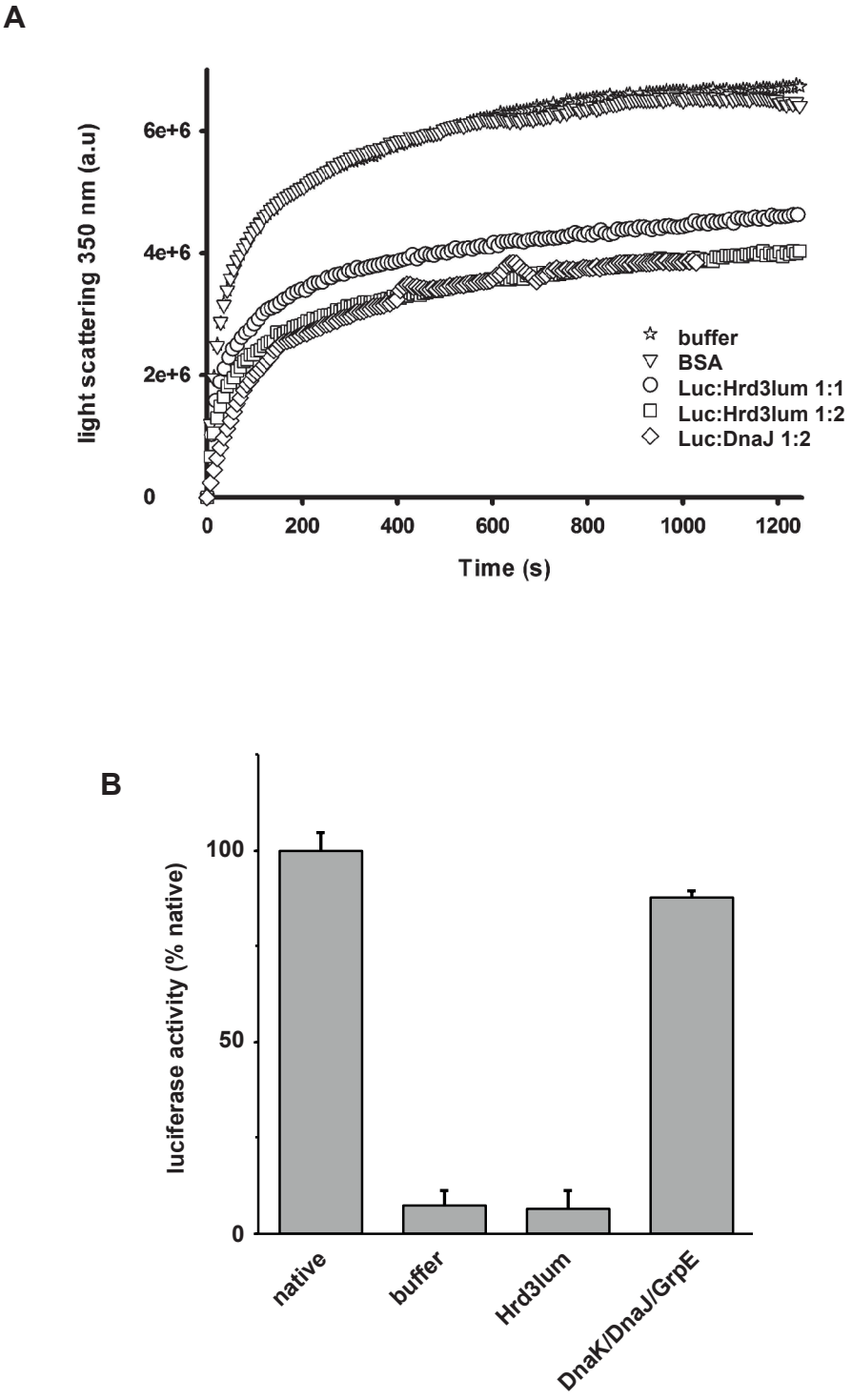
Supplementary Figure S1. (A) Yeast cells disrupted for *hrd3* and expressing the indicated plasmid-encoded Hrd3 variants were monitored for CPY* degradation by pulse chase analysis. **(**B) Turnover of CPY* was determined in Δ*hrd3* yeast strains containing empty vector or plasmids for the expression of Hrd3KR or Hrd3 K652A R653A by radioactive pulse chase analysis. Mean values of two independent experiments are shown. **(**C) Predicted structural model of Hrd3 as calculated by the Phyre2 program (Kelley and Sternberg, 2009). The insert depicts the relative position of residues K652 and R653 in the most carboxyterminal SLR. **(**D) Cycloheximide decay experiment to measure CD4 turnover in Δ*hrd3* cells containing plasmids for the expression of the indicated Hrd3 variants. Signals from the fluorescently-labeled secondary antibodies were quantified using a laser scanner. Mean values of two independent experiments are shown. **(**E) Radioactive pulse-chase analysis to investigate the degradation of plasmid-encoded CT* in yeast cells of the given genotype. (F) Pulse chase analysis of KWW-turnover in cells of the indicated genotype. Two independent experiments were quantified and mean values representing the percent of KWW left at the specified time points are given in the bottom line.

Supplementary Figure S2. (A) Hrd3 prevents aggregation of a malfolded protein *in vitro*. The ER-luminal part of Hrd3 (Hrd3lum) expressed in insect cells was purified and added in the given molar ratio to chemically denatured firefly luciferase (Luc) that was diluted in buffer. Aggregation of luciferase was measured in a time course by light scattering at 350 nm. Units on the x-axis refer to light absorption of the sample. Buffer (●), BSA (▽) and DnaJ purified from *E.coli* (◇; kindly provided by J. Brodsky) served as controls. (B) Chemically denatured firefly luciferase was incubated in refolding buffer (Promega) containing the indicated proteins. The enzymatic activity was determined in a luminometer according to the manufacturer's protocol. Mean values from three independent experiments in percent activity of the non-denatured protein (native) and standard deviation of mean are given.

Supplementary Figure S1; Mehnert *et al.*



Supplementary Figure S2; Mehnert *et al.*



Supplementary Table 1

Name	Genotype	Resource
YBM70	∆hrd3::LEU2, prc1-1, trp1-1(am), his3- 200, ura3-52, lys2-801, leu2-3,-112, MATa	Meusser *et al.*, 2004
YCH232	6xHA-HRD3, YOS9-myc7xHIS(kanMX6), prc1-1, trp1-1 (am), his3-200, leu2-3,-112, lys2-801, ura3-52, MATa	Gauss *et al.*, 2006b
YFZ004	6xHA-hrd3(M637V), prc1-1, trp1-1 (am), his3- 200, leu2-3,-112, lys2-801, ura3-52, MATa	this work
YFZ005	6xHA-hrd3(H638L), YOS9-myc7xHIS(kanMX6), prc1-1, trp1-1 (am), his3- 200, leu2-3,-112, lys2-801, ura3-52, MATa	this work
YFZ006	6xHA-hrd3(M637V), YOS9-myc7xHIS(kanMX6), prc1-1, trp1-1 (am), his3- 200, leu2-3,-112, lys2-801, ura3-52, MATa	this work
YFZ007	6xHA-hrd3(KL(675/676)IV), YOS9-myc7xHIS(kanMX6), prc1-1, trp1-1 (am), his3- 200, leu2-3,-112, lys2-801, ura3-52, MATa	this work
YFZ008	6xHA-hrd3(E639A), YOS9-myc7xHIS(kanMX6), prc1-1, trp1-1 (am), his3- 200, leu2-3,-112, lys2-801, ura3-52, MATa	this work
YFZ015	URA3::6xmyc-Hmg2, ∆hrd3::LEU2, ∆prc1::LEU2, prc1-1, trp1-1(am), his3- 200, ura3-52, lys2-801, leu2-3,-112, MATa	this work
YFZ022	∆pra1::TRP1, prc1-1, trp1-1(am), his3- 200, ura3-52, lys2-801, leu2-3,-112, MATa	this work
YFZ023	∆pra1::TRP1, ∆hrd3::LEU2, prc1-1, trp1-1(am), his3- 200, ura3-52, lys2-801, leu2-3,-112, MATa	this work
YFZ024	∆pra1::TRP1, 6xHA-Hrd3, YOS9-myc7xHIS(kanMX6), prc1-1, trp1-1(am), his3- 200, ura3-52, lys2-801, leu2-3,-112, MATa	this work
YFZ025	∆pra1::TRP1, 6xHA-Hrd3 KR652/653IL, YOS9-myc7xHIS(kanMX6), prc1-1, trp1-1(am), his3- 200, ura3-52, lys2-801, leu2-3,-112, MATa	this work
YFZ028	∆pra1::TRP1, ∆scj1::HIS3, prc1-1, trp1-1(am), his3- 200, ura3-52, lys2-801, leu2-3,-112, MATa	this work
YFZ044	∆hrd3::LEU2, ∆scj1::HIS3, prc1-1, trp1-1(am), his3- 200, ura3-52, lys2-801, leu2-3,-112, MATa	this work
YRG020	6xHA-HRD3, prc1-1, trp1-1(am), his3- 200, leu2-3,-112, lys2-801, ura3-52, MATa	Gauss *et al.*, 2006b
YRG146	SCJ1-9xMyc (TRP1), prc1-1,_trp1-1(am), his3-∆200, ura3-52, lys2-801, leu2-3,-112, MATalpha	this work
YRG175	6xHA-HRD3, SCJ1-9xMyc(TRP1), prc1-1, trp1-1(am), his3-∆200, ura3-52, lys2-801, leu2-3,-112, MAT not determined	this work
YRG182	∆jem1::kanMX6, prc1-1, trp1-1(am), his3- 200, ura3-52, lys2-801, leu2-3,-112, MATa	this work
YRG183	∆scj1::HIS3, prc1-1, trp1-1(am), his3- 200, ura3-52, lys2-801, leu2-3,-112, MATa	this work
YRG190	∆jem1::kanMX6, scj1::HIS3, prc1-1, trp1-1(am), his3- 200, ura3-52, lys2-801, leu2-3,-112, MATa	this work
YRG277	∆erj5::HIS3, prc1-1, trp1-1(am), his3- 200, ura3-52, lys2-801, leu2-3,-112, MATa	this work
YRG284	6xHA-HRD3, ∆scj1::HIS5, prc1-1, trp1-1 (am), his3- 200, leu2-3,-112, lys2-801, ura3-52, MATa	this work
YRG280	6xHA-HRD3, ∆jem1::kanMX6, prc1-1, trp1-1 (am), his3- 200, leu2-3,-112, lys2-801, ura3-52, , MATa	this work
YRG362	6xHA-hrd3(KR652/653IL), YOS9-myc7xHIS(kanMX6), prc1-1, trp1-1 (am), his3- 200, leu2-3,-112, lys2-801, ura3-52, MATa	this work
YRG365	6xHA-hrd3(NR645/646II), YOS9-myc7xHIS(kanMX6), prc1-1, trp1-1 (am), his3- 200, leu2-3,-112, lys2-801, ura3-52, MATa	this work
YRG386	SCJ1-9xMyc (TRP1), ∆hrd3::LEU2, prc1-1, trp1-1(am), his3-∆200, ura3-52, lys2-801, leu2-3,-112, MATalpha	this work
YTX557	∆hrd3::LEU2, Der1-13xmyc (TRP), prc1-1, trp1-1(am), his3-∆200, ura3-52, lys2-801, leu2-3,-112, MATa	this work
YTX485	HRD1-3xHA (TRP1), ∆hrd3::LEU2, prc1-1, trp1-1(am), his3-∆200, ura3-52, lys2-801, leu2-3,-112, MATalpha	this work

YTX1145	∆scj1::HISMX6, CPY*-3xHA (kanMX6), trp1-1(am), his3-∆200, ura3-52, lys2-801, leu2-3,-112, MAT not determined	this work
YTX140	prc1-1, trp1-1(am), his3- 200, ura3-52, lys2-801, leu2-3,-112, MATa	Biederer *et al.*, 1997
YTX365	CPY*-3xHA (kanMX6), trp1-1(am), his3-∆200, ura3-52, lys2-801, leu2-3,-112, MAT not determined	this work
YTX1213	URA3:6xMyc-Hmg2, ∆jem1::kanMX6, prc1-1, trp1-1(am), his3-∆200, ura3-52, lys2-801, leu2-3,-112, MATa	this work
YTX1214	URA3:6xMyc-Hmg2, ∆scj1::HIS3, prc1-1, trp1-1(am), his3-∆200, ura3-52, lys2-801, leu2-3,-112, MATalpha	this work
YMM072	∆hrd3::LEU2, ∆der1::HIS3, prc1-1, trp1-1(am), his3-∆200, ura3-52, lys2-801, leu2-3,-112, MAT not determined	this work
YMM224	Hrd3 K652I/R653L, prc1-1, trp1-1(am), his3-∆200, ura3-52, lys2-801, leu2-3,-112, MATa	this work
YMM225	Hrd3 K652I/R653L, CPY*-3xHA (KanMX), trp1-1(am), his3-∆200, ura3-52, lys2-801, leu2-3,-112, MATa	this work

Supplementary Table 2

Name	Insert and/or purpose	Backbone	Resource
pBM108	CD4	pRS414	Meusser *et al*., 2004
pGK1-pBpa	3SUP4-tRNA CUA for pBpa incorporation		Chen, *et al*., 2007
pFastBac1™	Baculovirus cloning		Invitrogen
pRH244	6xMyc-Hmg2 for genomic integration		Hampton *et al*., 1996
pFZ029	Hrd3 KR652/653IL	pJU301	this work
pFZ060	Hrd3 KR652/653AA	pRS317	this work
pFZ063	6xHA-Hrd3 (–278 … 2690)	pRS316	this work
pFZ064	6xHA-Hrd3 KR652/653IL (–278 … 2690)	pRS316	this work
pTX191	CPY*-CUP	pRS413	this work
pTX339	PrA*	pRS415	this work
pWO804	CT*	pRS316	Taxis *et al*., 2003
pSM101	KWW	pRS316	Vashist and Ng, 2004
pJU301	Hrd3 (-386...2706)	pRS317	this work
pMM075	Der1 (1...706)-13xMyc	pRS425-CUP	Mehnert *et al*., 2014
pMM083	1xHis-Scj1	pRS426-CUP	this work
pMM084	Hrd3 KR652/653IL	pRS406	this work
pMM100	1xHis-Scj1	pRS414-CUP	this work
pMM101	1xHis-Scj1	pRS424-CUP	this work