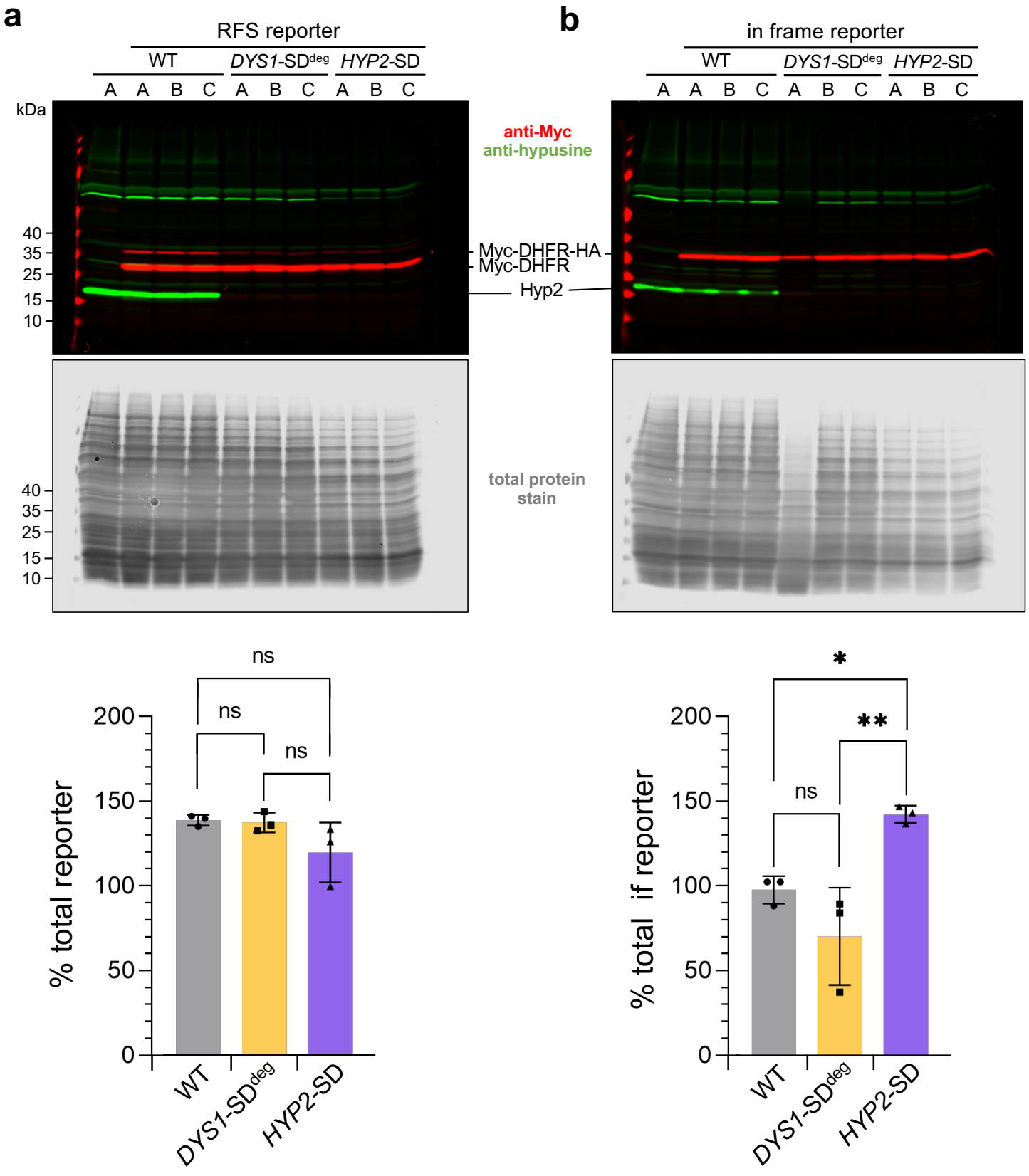
Halwas, K., Döring, L.-D., Oehlert, F.V. and Dohmen, R.J. Supplementary Material

Material includes:

Figure S1


a
RFS reporter
WT
DYS1-SDdeg
HYP2-SD
A A B C A B C A B C
kDa
40
35
25
15
10
anti-Myc
anti-hypusine
Myc-DHFR-HA
Myc-DHFR
Hyp2
total protein stain
b
in frame reporter
% total reporter
% total if reporter
ns
*
**
WT
DYS1-SDdeg
HYP2-SD

Legend to Figure S1:

Western blot analyses for RFS as well as in-frame reporters, and total protein staining relating to Figure 4. (**a**) Shown are the additional data for the same blots shown in figure 4a, in which wild type (wt), *HYP2*-SD, and *DYS1*-SD^{deg} expressing the RFS reporter were analyzed. The top panel shows the full scan of the blot after anti-MYC and anti-hypusine detection. The lower panel shows the total protein stain of the same blot. Below the quantification of the total reporter signal (signals for both bands added together) after normalization to total protein signals is shown. Means, standard deviations and significance were calculated as in figure 4. (b) same as in (a) but for cells expressing the in frame (if) reporter. For the quantification, the signals of one apparently underloaded lane with one of the *DYS1*-SD^{deg} spore clones was not considered.

Figure S2: Plasmid sequences

pFO1/2 pUC21–NotI–P_{URA3}–***URA3***–P_{ADH1}–2xMyc–DHFR–RFS_{OAZ1}(t)/if–**2xHA**–T_{CYC1}–T_{URA3}–NotI

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pKH15 [pUC19–P_{HYP2}–P_{TEF}–*his5*–T_{TEF}–P_{GAL1}–tc3–Ub–R–HA–ORF_{HYP2}–T_{HYP2}]

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pKH16 [pUC19–P_{HYP2}–P_{TEF}–*his5*–T_{TEF}–P_{GAL1}–tc3–HA–ORF_{HYP2}–T_{HYP2}]

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Table S1: Quantification of anti-Myc western blot signals of blot shown in Fig. 4

Strain	Band(s)	Signal	%	% RFS	Mean	SD	T-test vs. WT (p)
Wild type 1	Full-length	1391147	14,47	14,47			
	truncated	8222782	85,53				
	Total	9613929	100,00				
Wild type 2	truncated	1395516	14,01	14,01	14.48	0,47	
	Full-length	8565407	85,99				
	Total	9960923	100,00				
Wild type 3	truncated	1518780	14,95	14,95			
	Full-length	8642364	85.05				
	Total	10161144	100,00				
***DYS1*-SD 1**	Full-length	434763	4,91	4,91			
	truncated	8416616	95,09				
	Total	8851379	100,00				
***DYS1*-SD 2**	truncated	508256	5,42	5,42	5,27	0,32	0,0000296
	Full-length	8860794	96,58				
	Total	9369050	100,00				
***DYS1*-SD 3**	truncated	478043	5,49	5,49			
	Full-length	8222782	94,51				
	Total	8700825	100,00				
***HYP2*-SD 1**	Full-length	256957	4,42	4,42			
	truncated	5551927	95,58				
	Total	5808884	100,00				
***HYP2*-SD 2**	truncated	433732	5,46	5,46	5,08	0,57	0,0000337
	Full-length	7504955	94,54				
	Total	7938687	100,00				
***HYP2*-SD 3**	truncated	430305	5,35	5,35			
	Full-length	7611366	94.65				
	Total	8041671	100,00				

Table S2 – *S. cerevisiae* strains used in this study

Strain*	Relevant Genotype	Source / Reference
WCG4a or -α	*his3-11,15, leu2-3,112, ura3-52*	[63]
KH27-1B,-5C,-9C**	*hyp1Δ::KanMX6, pKH16::His3MX6 (in HYP2), pFO1::URA3, LEU2, his3-11,15*	This work
KH29-3A	*hyp1Δ::KanMX6, pKH16::His3MX6 (in HYP2), pFO2::URA3, LEU2, his3-11,15*	This work
KH29-2B	*hyp1Δ::KanMX6, pKH16::His3MX6 (in HYP2), LEU2, his3-11, ura3-52*	This work
KH32-10D,-11D,-11B**	*hyp1Δ::KanMX6, pKH13::His3MX6 (in DYS1), pFO2::URA3, LEU2, his3-11,15*	This work
KH33-4C,-5D,-9B**	*hyp1Δ::KanMX6, pKH13::His3MX6 (in DYS1), pFO1::URA3, LEU2, his3-11,15*	This work
KH30	*hyp1Δ::KanMX6, pFO2::URA3, LEU2, HIS3*	This work
KH31	*hyp1Δ::KanMX6, pFO1::URA3, LEU2, HIS3*	This work
JD411-1A,4B**	*pFO1::URA3, LEU2, HIS3*	This work
JD403-12C	*pFO2::URA3, LEU2, HIS3*	This work

**All strains are congenic derivatives of WCG4a or WCG4-α*
***Congenic spore clones obtained by tetrad dissection*

Table S3 –Plasmids used in this study

Name	Relevant characteristics	Derivative of / reference
pKH11	P_{HYP2}-*HYP2*, *CEN/LEU2*	pRS315
pKH12	P_{HYP2}-*HA-HYP2*, *CEN/LEU2*	pRS315
pKH18	P_{HYP2}-$hyp2^{K51R}$, *CEN/LEU2*	pRS315
pKH13	5´Δ-P_{DYS1}-*His3MX6*-$P_{GAL1\text{-}tc3}$-Ub-R-HA-*DYS1*-3´Δ	pJET1.2
pKH14	5´Δ-P_{DYS1}- *His3MX6*-$P_{GAL1\text{-}tc3}$-HA-*DYS1*-3´Δ	pJET1.2
pKH15	5´Δ-P_{HYP2}- *His3MX6*-$P_{GAL1\text{-}tc3}$-Ub-R-HA-*HYP2*-T_{HYP2}	pUC19
pKH16	5´Δ-P_{HYP2}- *His3MX6*-$P_{GAL1\text{-}tc3}$-HA-*HYP2*-T_{HYP2}	pUC21
pFO1	P_{ADH1}-*2xMYC-DHFR-RFS-2xHA*-T_{CYC1}::*URA3*	pLD57
pFO2	P_{ADH1}-*2xMYC-DHFR-IF-2xHA*::T_{CYC1}::*URA3*	pLD57
pLD57	P_{URA3}-*URA3*-$T_{URA3\ (with\ polylinker)}$	pUC21
pRS315	*CEN/LEU2*	[64]
pUC19	*E. coli* cloning vector with Amp^R	[66]
pUC21	*E. coli* cloning vector with Amp^R	[65]
pJET1.2	*E. coli* cloning vector with Amp^R	Thermofisher
pFA6-His3MX6	*His3MX6*	[56] Longtine 1998
pFA6-KanMX6	*KanMX6*	[56] Longtine 1998
pPW66R	*Ub-R-*$DHFR^{td}$*-CDC28*	[51] Dohmen 1994

Table S4 – Antibodies used in this study

Antibody	Host	Dilution	Source
Anti-HA (3F10)	rat	1:1000	Merck
Anti-Myc (9B11)	mouse	1:1000	Cell Signaling Technology
Anti-Hypusine	rabbit	1:2000	Merck
Anti-Rabbit, Alexa Fluor Plus 800 (A32735)	goat	1:5000	Thermo Fisher
Anti-Mouse, Alexa Fluor Plus 680 (A32729)	goat	1:20000	Thermo Fisher
Anti-Mouse, Alexa Fluor 680 (A21096)	goat	1:5000	Thermo Fisher