

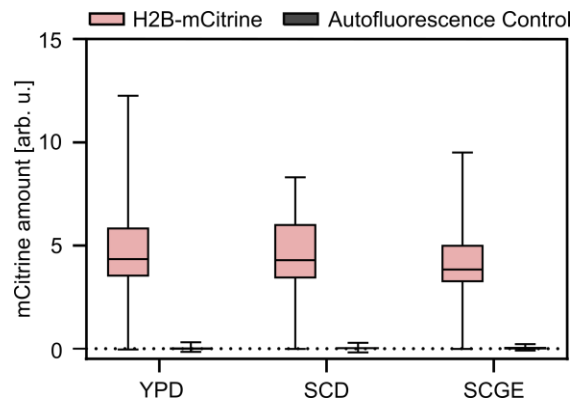
Appendix for:

Decoupled transcript and protein concentrations ensure histone homeostasis in different nutrients

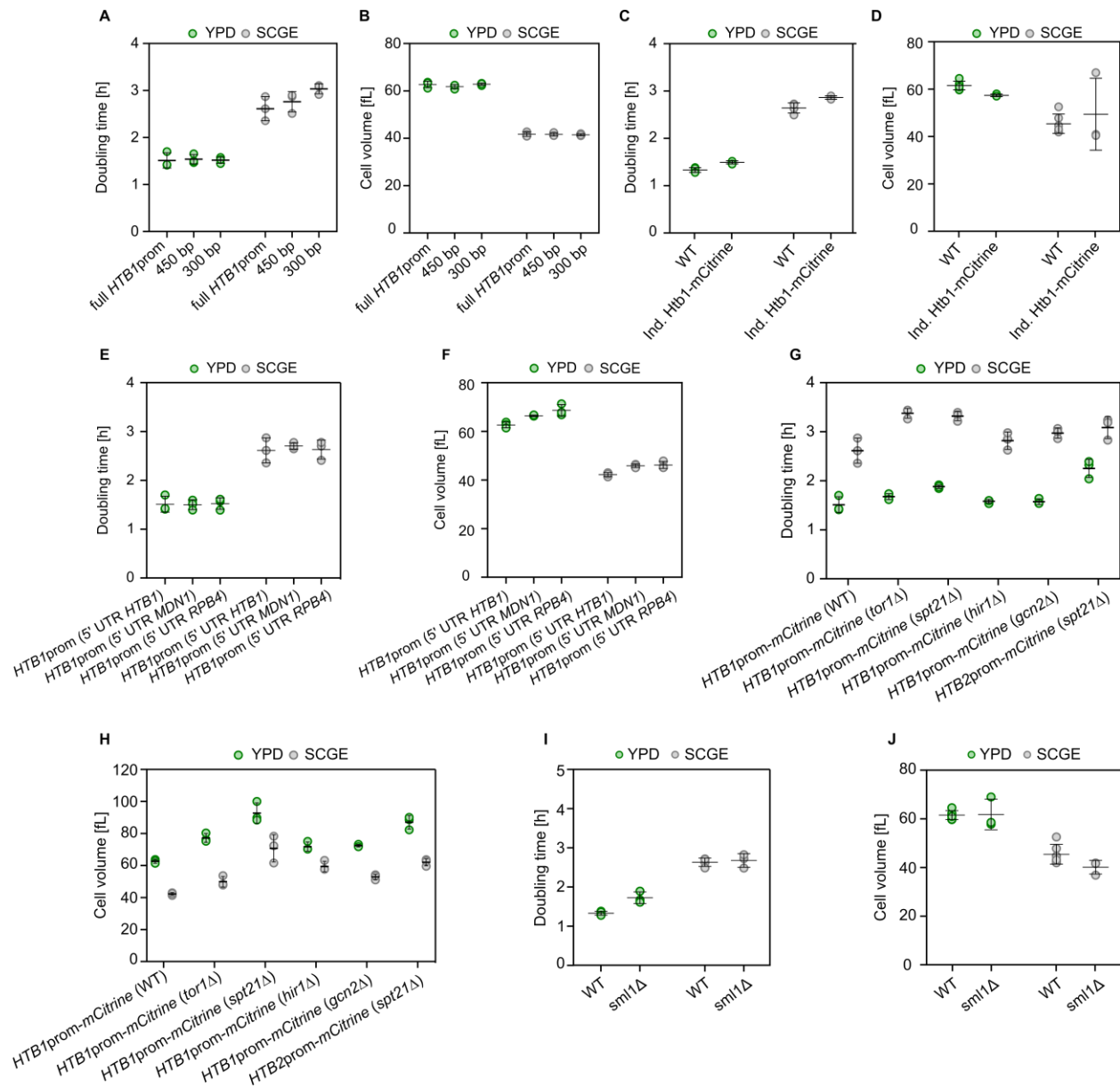
Dimitra Chatzitheodoridou¹, Daniela Bureik¹, Francesco Padovani¹, Kalyan Varma Nadimpalli¹, Kurt M. Schmoller¹,

¹Institute of Functional Epigenetics, Molecular Targets and Therapeutics Center, Helmholtz Zentrum München, 85764 Neuherberg, Germany *correspondence: kurt.schmoller@helmholtz-munich.de

Table of contents	Page
Appendix Figure S1	2
Appendix Figure S2	3
Appendix Table S1	5
Appendix Table S2	6
Appendix Table S3	9
Appendix Table S4	10



Appendix Figure S1. Autofluorescence control for the H2B-mCitrine strain in different growth media. Compared to H2B-mCitrine intensity, autofluorescence is negligible. Total mCitrine fluorescence intensity after background correction was measured in cells with mCitrine-tagged H2B ($n_{\text{YPD}} = 492$, $n_{\text{SCD}} = 392$, $n_{\text{SCGE}} = 275$) and untagged H2B ($n_{\text{YPD}} = 227$, $n_{\text{SCD}} = 285$, $n_{\text{SCGE}} = 215$). Box plots represent median and 25th and 75th percentiles, whiskers are extending to the minimum and maximum values.



Appendix Figure S2. Growth phenotypes of strains used in figures 4, 5 and 6. (A) Nutrient-specific population doubling times and (B) mean volumes of cells carrying an additional copy of the full-length or truncated *HTB1* promoter driving the expression of mCitrine. Lines and error bars represent the means and standard deviations of $n = 3$ independent measurements shown as individual dots. (C) Nutrient-specific population doubling times and (D) mean cell volumes of wildtype cells and cells with β -estradiol inducible *HTB1-mCitrine*. Lines and error bars represent the means and standard deviations of $n = 3-4$ independent measurements shown as individual dots. (E) Nutrient-specific population doubling times and (F) mean volumes of cells expressing mCitrine with the *HTB1*, *MDN1* or *RPB4* 5' UTR. Lines and error bars represent the mean and standard deviation of $n = 3$ independent measurements shown as individual dots. (G) Population doubling times and (H) mean cell volumes of wildtype, *tor1Δ*, *spt21Δ*, *hir1Δ*, and *gcn2Δ* cells

expressing *HTB1*prom-mCitrine or *HTB2*prom-mCitrine in YPD and SCGE. Lines and error bars represent the means and standard deviations of n = 3 independent measurements shown as individual dots. **(I)** Population doubling times and **(J)** mean cell volumes of wildtype and *sm11Δ* cells growing in YPD and SCGE. Lines and error bars represent the means and standard deviations of n = 3-6 independent measurements shown as individual dots.

Appendix Table S1. Promoter sequences used for the experiments shown in Fig. 5, in which *mCitrine* mRNA is expressed from the *HTB1* promoter with *HTB1*, *MDN1* or *RPB4* 5' UTR.

Gene feature	Position relative to start codon
<i>HTB1</i> promoter	- 817 bp to -127 bp relative to <i>HTB1</i> ORF
<i>HTB1</i> 5' UTR	127 bp upstream of <i>HTB1</i> ORF
<i>MDN1</i> 5' UTR	150 bp upstream of <i>MDN1</i> ORF
<i>RPB4</i> 5' UTR	125 bp upstream of <i>RPB4</i> ORF

Appendix Table S2. Strains used in this study.

Name	Genotype	Description	Origin	Figure
ASY020-1	<i>Mat α/a;</i> <i>ADE2/ADE2,</i> <i>URA3/ura3,</i> <i>leu2/LEU2</i>	Diploid wildtype strain	Anika Seel, Schmoller Lab	6
CY14093	<i>Mat α;</i> <i>sml1Δ::hphMX3</i>	Haploid <i>sml1Δ</i> strain	Christopher Bruhn	6, S2
CY14098	<i>Mat α;</i> <i>sml1Δ::hphMX3,</i> <i>rad53Δ::natMX6</i>	Haploid <i>sml1Δrad53Δ</i> strain	Christopher Bruhn	6
CY15164	<i>Mat α;</i> <i>sml1Δ::hphMX3,</i> <i>rad53Δ::natMX6,</i> <i>spt21Δ::kanMX6</i>	Haploid <i>sml1Δrad53Δ spt21Δ</i> strain	Christopher Bruhn	6
DBY020-2	<i>Mat α; ADE2,</i> <i>ura3::CglaTRP1-</i> <i>HTB1prom-mCitrine-</i> <i>ADH1term-URA3</i>	Haploid strain with additional copy of <i>HTB1</i> promoter driving mCitrine	This study	5
DBY021-3	<i>Mat α; ADE2,</i> <i>ura3::CglaTRP1-</i> <i>HTB2prom-mCitrine-</i> <i>ADH1term-URA3</i>	Haploid strain with additional copy of <i>HTB2</i> promoter driving mCitrine	This study	5
DBY054-8	<i>Mat a/α ;</i> <i>ADE2/ADE2, leu2-</i> <i>3/LEU2, URA3/ura3-</i> <i>1, htb1Δ::CglaTRP1/</i> <i>/HTB1, htb2Δ::HIS3</i> <i>/htb2Δ::NatMX6</i>	Diploid <i>HTB1/htb1Δ,</i> <i>htb2Δ/htb2Δ</i> strain	This study	6
DBY064-1	<i>Mat α; ADE2,</i> <i>ura3::CglaLEU2-</i> <i>HTB1prom-mCitrine-</i> <i>ADH1term-URA3</i>	Haploid strain with additional copy of <i>HTB1</i> promoter driving mCitrine	This study	4
DBY065-2	<i>Mat α ; ADE2,</i> <i>ura3::CglaLEU2-</i> <i>HTB1prom (mutated</i> <i>UAS3/UAS4)-</i> <i>mCitrine-ADH1term-</i> <i>URA3</i>	Haploid strain with additional copy of <i>HTB1</i> promoter, with mutated Spt10 binding sites in UAS3 and UAS4, driving mCitrine	This study	4
DCY001-1	<i>Mat α; ADE2,</i> <i>htb2::Htb2-linker-</i> <i>mCitrine-ADH1term-</i> <i>CglaTRP1,</i> <i>htb1::Htb1-linker-</i> <i>mCitrine-ADH1term-</i> <i>KlacURA3</i>	Haploid strain with <i>HTB1</i> and <i>HTB2</i> tagged with mCitrine	This study	1, EV1

DCY002-2	<i>Mat α; ADE2, ura3::CglaTRP1-HTB2prom-mCitrine-ADH11term-URA3,his3::ACT1prom-mKate2-ADH1term-HIS3</i>	Haploid strain with additional copy of <i>HTB2</i> promoter driving mCitrine and <i>ACT1</i> promoter driving mKate2	This study	3, EV3, EV4
DCY008-8	<i>Mat α; ADE2, ura3::CglaTRP1-HTB1prom-mCitrine-ADH1term-URA3,his3::ACT1prom-mKate2-ADH1term-HIS3</i>	Haploid strain with additional copy of <i>HTB1</i> promoter driving mCitrine and <i>ACT1</i> promoter driving mKate2	This study	3, 4, 5, EV3, EV4, EV5, S2
DCY011-1	<i>Mat α; ADE2, ura3::CglaTRP1-HTB1prom- MDN1 5'UTR-mCitrine-ADH1term-URA3</i>	Haploid strain with additional copy of <i>HTB1</i> promoter expressing mCitrine with <i>MDN1</i> 5' UTR	This study	5,S2
DCY012-1	<i>Mat α; ADE2, ura3::CglaTRP1-HTB1prom- RPB4 5'UTR-mCitrine-ADH1term-URA3</i>	Haploid strain with additional copy of <i>HTB1</i> promoter expressing mCitrine with <i>RPB4</i> 5' UTR	This study	5,S2
DCY013-4	<i>Mat α; ADE2, ura3::CglaTRP1-HTB1prom-mCitrine-ADH1term-URA3, his3::ACT1prom-mKate2-ADH1term-HIS3, tor1Δ::hphMX6</i>	Haploid <i>tor1Δ</i> strain with additional copy of <i>HTB1</i> promoter driving mCitrine and <i>ACT1</i> promoter driving mKate2	This study	5, S2
DCY14-3	<i>Mat α; ADE2, LexApr-HTB1-mCitrine-ADH1term-URA3, his3::LexA-ER-AD-TF-HIS3</i>	Haploid strain expressing β-estradiol inducible Htb1-mCitrine	This study	EV5
DCY15-1	<i>Mat α; ADE2, ura3::CglaTRP1-HTB1prom-mCitrine-ADH1term-URA3, his3::ACT1prom-mKate2-ADH1term-HIS3, spt21Δ:: LEU2</i>	Haploid <i>spt21Δ</i> strain with additional copy of <i>HTB1</i> promoter driving mCitrine and <i>ACT1</i> promoter driving mKate2	This study	5, S2
DCY16-1	<i>Mat α; ADE2, ura3::CglaTRP1-HTB1prom-mCitrine-</i>	Haploid <i>hir1Δ</i> strain with additional copy of <i>HTB1</i> promoter	This study	5, S2

	<i>ADH1term-URA3, his3::ACT1prom-mKate2-ADH1term-HIS3, hir1Δ::LEU2</i>	driving mCitrine and <i>ACT1</i> promoter driving mKate2		
DCY17-1	<i>Mat α; ADE2, ura3::CglaTRP1-HTB1prom-mCitrine-ADH1term-URA3, his3::ACT1prom-mKate2-ADH1term-HIS3, gcn2Δ::LEU2</i>	Haploid <i>gcn2Δ</i> strain with additional copy of <i>HTB1</i> promoter driving mCitrine and <i>ACT1</i> promoter driving mKate2	This study	5, S2
JE621	<i>Mat α, ADE2, TRP1, LEU2, his3::LexO transcription factor-HIS3, cdc20::natMX-lexOPr-Cdc20</i>	Haploid strain expressing β-estradiol inducible Cdc20	Jennifer Ewald Lab	2, EV2
KCY021-1	<i>Mat α; ADE2, ura3::CglaTRP1-300bpHTB1prom-mCitrine-ADH1term-URA3</i>	Haploid strain with additional 300 bp truncation of <i>HTB1</i> promoter driving mCitrine	Kora-Lee Claude, Schmoller Lab	4, S2
KCY022-1	<i>Mat α; ADE2, ura3::CglaTRP1-450bpHTB1prom-mCitrine-ADH1term-URA3</i>	Haploid strain with additional 450 bp truncation of <i>HTB1</i> promoter driving mCitrine	Kora-Lee Claude, Schmoller Lab	4, S2
KSY229-1	<i>Mat α; ADE2, ura3::CglaTRP1-ACT1prom-mCitrine-ADH1term-URA3</i>	Haploid strain with additional copy of <i>ACT1</i> promoter driving mCitrine	This study	3, EV3, EV4
MMY116-2C	<i>Mat α; ADE2</i>	Haploid wildtype strain	Skotheim lab stock	1, 2, 3, EV1, EV2, EV3, EV4, S1, S2

Appendix Table S3. Sequences of qPCR primers used in this study.

Gene	qPCR primer direction	qPCR primer sequence (5'-3')
HTB1	forward	TACACACATACAATGTCTGCTAAAG
	reverse	AGTGTCAGGGTGAGTTTGCTT
HTB2	forward	CCTCTGCCGCCGAAAAGAAA
	reverse	TCTTACCATCGACGGAGGTTG
HTA1	forward	GTTGCCAAAGAAGTCTGCCA
	reverse	CAGTTTAGTTCCCTCCGCCTT
HTA2	forward	TCGCCAAGGTGGTGT TTT
	reverse	TGATTTGCTTTGTTTCTTTTCAACT
HHF1	forward	TACACCGAACACGCCAAGAG
	reverse	TTGCTTGTTGTTACCGTTTTCTT
HHF2	forward	ACGAAGAAGTCAGAGCCGTC
	reverse	ACCGATTGTTTAACCAACCGATTG
HHT1	forward	CAATCTTCTGCCATCGGTGC
	reverse	ACTGATGACAATCAACAACTATGA
HHT2	forward	AGCAAACACTCCACAATGGC
	reverse	CAAGGCAACAGTACCTGGCT
ACT1	forward	AGTTGCCCCAGAAGAACACC
	reverse	GGACAAAACGGCTTGATGG
MDN1	forward	CATCAACAAACCTGACCAACTAATCC
	reverse	CATCAAGGTTTTCCAAAGTGGGC
mCitrine	forward	GAGCTGAAGGGCATCGACTT
	reverse	TTCTGCTTGTCGGCCATGAT
RDN18	forward	AACTCACCAGGTCCAGACACAATAAGG
	reverse	AAGGTCTCGTTCGTTATCGCAATTAAGC

Appendix Table S4. Sequences of qPCR primers used for mRNA stability measurements shown in figure 2B.

Gene	qPCR primer direction	qPCR primer sequence (5'-3')
HTB1	forward	TGGCTGCGTATAACAAGAAGTCT
	reverse	CCAAAGGAAGTGATTTTCATTATGC
HTB2	forward	TGCTCTATACTCAAACCAACAACA
	reverse	ATCTCTTCTTACCATCGACGGA
ACT1	forward	TATGTGTAAAGCCGGTTTTGC
	reverse	GACAATAC CGTGTTCAATTGGG