Extended Data figures



Supplementary Figure 1. Overexpression of Whi5 does not drastically alter doubling times, budding indices or cell cycle distributions. In all panels, haploid cells were induced with 30 nM β-estradiol, diploid cells with 50 nM. (a) Doubling times calculated from growth curves of exponentially growing cell populations for inducible and non-inducible (WT), untagged haploids (blue circles) and diploids (green circles). Black horizontal lines indicate the median of the distribution $(n_{haploid}^{WT} =$ 3, $n_{\text{haploid}}^{\text{not ind.}} = 4$, $n_{\text{haploid}}^{\text{ind.}} = 2$, $n_{\text{diploid}}^{\text{WT}} = 3$, $n_{\text{diploid.}}^{\text{not ind.}} = 3$, and $n_{\text{diploid.}}^{\text{ind.}} = 3$). Budding index (b) (percentage of budded cells), calculated by counting of budded and non-budded cells in exponentially growing cell populations using an optical microscope, for inducible and non-inducible (WT), untagged haploids (blue circles) and diploids (green circles). Black, horizontal lines indicate the median of the distribution $(n_{\text{haploid}}^{\text{WT}} = 3, n_{\text{haploid}}^{\text{not ind.}} = 5, n_{\text{haploid}}^{\text{ind.}} = 5, n_{\text{diploid}}^{\text{WT}} = 2, n_{\text{diploid.}}^{\text{not ind.}} = 1, n_{\text{diploid}}^{\text{ind.}} = 1, n_{\text{diploid}}^{\text{ind}} =$ 2, and $n_{\text{diploid.}}^{\text{ind}} = 2$). (c) Cell cycle distributions (percentage of cells in G1/S-phases (filled circles), or in G2/M-phases (open circles)) of inducible and non-inducible (WT), untagged haploid (blue) and diploid (green) cells, calculated from population distributions obtained through SYBR Green I staining of the DNA and flow cytometry. Black, horizontal lines indicate the median of the distribution $(n_{\text{haploid}}^{\text{WT}} = 6, n_{\text{haploid}}^{\text{not ind.}} = 6, n_{\text{haploid}}^{\text{ind.}} = 6, n_{\text{diploid}}^{\text{wT}} = 6, n_{\text{diploid.}}^{\text{not ind.}} = 6, \text{ and } n_{\text{diploid.}}^{\text{ind}} = 6).$ (d & e) Total first cell cycles of new-born cells (d) as well as individual G1- & G2/M-phases and histone production periods of new-born cells (e) for inducible and non-inducible (WT) *HTB2-mCitrine* haploids (blue), *HTB2-mCitrine* homozygous diploids (green) and *HTB2-mCitrine/htb2A* hemizygous diploids calculated from single cell fluorescent traces, measured with live cell fluorescence microscopy. G1 phases are defined as the time from birth to the first budding, histone production phases as the time between the first point of increase in fluorescent signal and the last point of increase, G2/M phases as the time from end of histone production phase to the separation of the cell from its bud. Black, horizontal lines indicate the median between single cells ($n_{haploid}^{WT} = 145$, $n_{haploid}^{not ind.} = 58$, $n_{homoz}^{ind.} = 71$, $n_{homoz.}^{not ind.} = 21$, $n_{homoz.}^{ind} = 85$, $n_{hemiz.}^{not ind.} = 48$ and $n_{hemiz.}^{ind.} = 43$), with notches indicating the 95% confidence interval. Colored boxes highlight the 25- and 75-percentiles, whiskers extend to $\pm 2.7\sigma$ of the distribution and magenta crosses highlight outliers. Note that during image acquisition all cells were grown in the absence of β -estradiol.



Supplementary Figure 2. Htb2-mCitrine amounts and concentrations increase with ploidy. (a) Htb2mCitrine amounts at birth for *HTB2-mCitrine* haploids and *HTB2-mCitrine* homozygous diploids estimated from fluorescence microscopy as a function of cell volume. Individual data points for the different conditions are shown in blue for haploids (triangles for 0 nM, circles for non-inducible, stars for 30 nM) and green for diploids (triangles for 0 nM, squares for non-inducible, stars for 50 nM). Lines connect binned means with error bars indicating standard errors. (b) Htb2-mCitrine concentrations at birth for *HTB2-mCitrine* homozygous diploids and *HTB2-mCitrine/htb2A* hemizygous diploids as a function of cell volume. Individual data points for the different conditions are shown in teal for hemizygotes (triangles for 0 nM, circles for 50 nM) and green for homozygotes (triangles for 0 nM, squares for non-inducible, stars for 50 nM). Lines connect binned means with error bars indicating standard errors.



Supplementary Figure 3. Decrease in histone transcript concentration with cell volume is specific to Whi5-dependent cell volume increase. (a) Mean cell volumes (grey circles) of exponentially growing cell populations measured with a Coulter counter are shown as a function of β -estradiol concentrations for non-inducible haploid cells, *whi5* Δ haploid cells with β -estradiol-dependent transcription factor (TF), and *whi5* Δ haploid cells with β -estradiol-dependent transcription factor (TF) and β -estradiol-inducible *WHI5*. Bars show the mean of the population with error bars indicating the standard deviation for $n_{non-ind.}^0 = 11$, $n_{non-ind.}^{30} = 4$, $n_{whi5\Delta+TF}^0 = 5$, $n_{whi5\Delta+TF}^{10} = 11$, $n_{ind.-WHI5}^{10} = 9$, $n_{ind.-WHI5}^{30} = 10$ biological replicates.

Significances were tested using a two-tailed, two-sample t-test at a confidence level $\alpha = 0.05$; ** $p_{whi5\Delta+TF}^{0nM vs 30nM} = 3.2 \cdot 10^{-3}$, *** $p_{Ind.-WHI5}^{0nM vs 10nM} = 8.3 \cdot 10^{-7}$, *** $p_{Ind.-WHI5}^{10nM vs 30nM} = 2.0 \cdot 10^{-5}$. (b – d) Relative mean mRNA concentration (grey circles) of exponentially growing haploid cell populations with and without β -estradiol addition. (b) Non-inducible cells, (c) whi5 Δ cells with β -estradioldependent transcription factor (TF), (d) whi5 Δ cells with β -estradiol-dependent transcription factor (TF) and β -estradiol inducible WHI5. For each gene, values are normalized on the mean mRNA concentration of the cell populations without β -estradiol addition (0 nM). Black horizontal lines indicate the median of the distribution (n = 4 except for $n_{30}^{HTB1} = 3$ (b), $n_0 = 5$, $n_{10} =$ 6, and $n_{30} = 5$, except for $n_0^{HTA1} = 4$, $n_{10}^{HTA1} = 5$, $n_{30}^{HTA1} = 4$, $n_0^{HTB1} = 3$, $n_{10}^{HTB1} = 3$, $n_{30}^{HTB1} =$ 3, $n_0^{HHT2} = 3$, $n_{10}^{HHT2} = 3$, and $n_{30}^{HHT2} = 3$ (c), $n_0 = 11$, $n_{10} = 9$, and $n_{30} = 10$, except for $n_0^{\text{ACT1}} = n_0^{\text{HTA1}} = n_0^{\text{HHT2}} = 10, n_0^{\text{HTA2}} = n_0^{\text{HHF1}} = 9, n_{10}^{\text{HTA2}} = n_{10}^{\text{HTB1}} = 8, n_{10}^{\text{HTA1}} = n_{10}^{\text{HHT2}} = 6,$ $n_{10}^{\text{ACT1}} = 7, n_{30}^{\text{HTA2}} = 9, n_{30}^{\text{HTA1}} = n_{30}^{\text{HHT2}} = 7 \text{ and } n_{30}^{\text{HHF1}} = 6 \text{ (d)}.$ Grey boxes highlight the 25- and 75-percentiles, whiskers extend to $\pm 2.7\sigma$ of the distribution and magenta crosses highlight outliers.



Supplementary Figure 4. Histone mRNA concentrations measured by RT-qPCR decrease with increasing cell volume. (a) Relative *RDN18* mRNA concentration for non-inducible and inducible haploid cells over mean cell volume, shown in a double logarithmic plot. mRNA concentrations are normalized on the mean mRNA concentration of non-inducible cells (green circles). (b – d) Raw data corresponding to Fig. 2d: Relative mRNA concentrations of *ACT1* and *ENO2* (b), *RPB1* and *RPB3* (b), as well as all core histone genes and *HHO1* (d) for non-inducible and inducible haploid cells over mean cell volume, shown in double logarithmic plots. mRNA concentrations are normalized on *RDN18* concentrations. For all panels, individual data points for the different conditions (down-

pointing triangles for 0 nM, circles for non-inducible, diamonds for 10 nM, left triangles for 30 nM) are shown in green (a), red (b), black (c) or blue (d). Lines show linear fits to the double logarithmic data, with volume-dependence parameters (VDPs) determined as the slope of the fit, with respective standard error.



Supplementary Figure 5. Direct feedback, Hir1-dependent feedback, as well as 3'- to 5'-end degradation by the nuclear exosome is not necessary for the decrease of histone mRNA concentrations with cell volume. (a) Summaries of the VDPs for an *HTB2* homozygous (green circles) and *HTB2/htb2* Δ hemizygous diploid (teal diamonds), determined by RT-qPCR for *HTB1*, *HTB2*, *HHF1* and *HHO1*. VDPs were determined as the slopes of the linear fits to the double logarithmic concentration over cell volume data (fit through $n_{\text{Homozygote}}^{\text{HTB1,HTB2}} = n_{\text{Hemizygote}}^{\text{HTB1,HTB2}} = 12$ biological replicates). Error bars indicate the standard error of the slope. (b) Relative *HTB1*, *HTA1* and *ACT1* mRNA concentrations (normalized on RDN18) for a wild-type haploid strain and an *htb2* Δ in the same background, measured by RT-qPCR. Concentrations are normalized on the respective median concentration in the wild-type. Biological replicates are represented as colored data points (circles), colored boxes highlight the 25- and 75-

percentiles, whiskers extend to $\pm 2.7\sigma$ of the distributions and magenta crosses highlight outliers. Black, horizontal lines indicate the median of the biological replicates (n = 9), notches indicate the 95% confidence interval. Significances were tested using a two-tailed, two-sample t-test at a confidence level $\alpha = 0.05$; *** $p_{HTB1} = 2.6 \cdot 10^{-4}$, *** $p_{HTA1} = 3.3 \cdot 10^{-5}$. (c – e) Raw data corresponding to Fig. 2f – h: Relative mRNA concentrations (normalized on *RDN18*) for inducible and non-inducible haploid cells over mean cell volume, shown in double logarithmic plots. Data corresponding to the *hir1* Δ (c), *rtt106* Δ (d) and *rrp6* Δ (e) cells are highlighted in blue for the different conditions (diamonds for non-inducible, left-pointing triangles for 0 nM, up-pointing triangles for 10 nM, and right-pointing triangles for 30 nM). Lines show the linear fits to the double logarithmic data. Grey dashed lines correspond to the linear fit for the wild-type cells, carrying no deletion, shown in Supplementary Fig. 4d.



Supplementary Figure 6. Concentration of mCitrine expressed from a single additional histone promoter in haploid and diploid cells measured with flow cytometry. mCitrine concentration, driven by an additional copy of the *HTB2* (a) or *HHF1* (b) promoter in haploid (blue filled circles) and diploid (blue open squares) cells, shown as a function of cell volume in a double logarithmic plot. Lines show linear fits to the double logarithmic data with volume-dependence parameters (VDPs) determined as the slope of the fit, with respective standard error (fit through $n_{\text{Haploid}} = 12$ and $n_{\text{Diploid}} = 8$ biological replicates).



Supplementary Figure 7. Cell-cycle-dependence does not explain cell-volume-dependence of expression from histone promoters. (a – c) Duration of mCitrine production period, determined by live-cell fluorescence microscopy, shown as a function of the cell volume at bud emergence for a diploid strain carrying an additional *HTB1* (a), *HTB2* (b) or *HHF1* (c) promoter driving *mCitrine* expression. Solid lines show linear fits to the data, dashed lines represent the 95% confidence intervals of the fit. Slopes of the fit are stated with respective standard error. (d – h) *mCitrine* mRNA concentration, estimated as the number of mRNA spots detected with smFISH in the whole cell including the bud and divided by the cell volume, shown as a function of the cell area ratio (cell area of the bud divided by the cell area of the mother cell) for diploid cells expressing *mCitrine* from an additional *HTB1* (d), *HTB2* (e), *HHF1* (f) or *ACT1* (g) promoter and a wild-type diploid strain, carrying no *mCitrine* allele (h). Individual data points correspond to cells in G1-phase (down-pointing triangles), S-phase (filled circles) or G2/M-phase (open circles).



Supplementary Figure 8. mCitrine expression driven by single additional histone promoter truncations and measured by flow cytometry decreases once part of the upstream activating sequences (UASs) are truncated. (a) Illustration of the full *HHF1* and *HTB1* promoter, as well as the 450 bp and 300 bp truncations. Green arrows show the location of the upstream activating sequences (UASs)³⁵, magenta boxes show the location of the NEG elements^{38,39}. (b - c) Normalized mCitrine concentration at 60 fL for different histone promoter truncations, integrated in haploid (blue filled circles) and diploid (green open squares) cells. (b) *mCitrine* driven by *HTB1* promoter truncations, (c) *mCitrine* driven by *HHF1* promoter truncations. Concentration at 60 fL were calculated by the linear fit to the double logarithmic dependence of concentration on cell volume (fit through $n_{\text{Haploid}}^{\text{Full}} = n_{\text{Haploid}}^{450\text{bp}} = 27$, $n_{\text{Diploid}}^{\text{Full}} = 18$, $n_{\text{Diploid}}^{50\text{cp}} = n_{\text{Diploid}}^{300\text{bp}} = 27$ and (b) $n_{\text{Haploid}}^{\text{Full}} = n_{\text{Haploid}}^{450\text{bp}} = 27$, $n_{\text{Diploid}}^{\text{Full}} = 18$, $n_{\text{Diploid}}^{300\text{bp}} = 17$ (c) biological replicates), and normalized to the maximum concentration calculated for haploid cells. Error bars are derived by error propagation of the 95% confidence interval of the linear fit at 60 fL.



Supplementary Figure 9. Change in behavior of truncated histone promoters is not due to a disruption of the cell-cycle-dependence. (a - d) Duration of mCitrine production period, determined by live-cell fluorescence microscopy, shown as a function of the cell volume at bud emergence for a diploid strain carrying an additional 450 bp *HHF1* (a), 300 bp *HHF1* (b), 450 bp *HTB1* (c) or 300 bp *HTB1* (d) promoter truncation driving *mCitrine* expression. Solid lines show linear fits to the data, dashed lines represent the 95% confidence intervals of the fit. Slopes of the fit are stated with respective standard error. (e & f) *mCitrine* mRNA concentration, estimated as the number of mRNA spots detected with smFISH in the whole cell including the bud and divided by the cell volume, shown as a function of the cell area ratio (cell area of the bud divided by the cell area of the mother cell) for diploid cells expressing *mCitrine* from an additional 450 bp *HTB1* (e) or an additional 300 bp *HTB1* (f) promoter. Individual data points correspond to cells in G1-phase (down-pointing triangles), S-phase (filled circles) or G2/M-phase (open circles).



Supplementary Figure 10. *HIR1*-dependent regulation on the transcript level might contribute to the cell-volume-dependence of histone expression. (a – d) Summary of volume-dependence parameters (VDPs) (a & c) and corresponding raw data (b & d) for a haploid strain carrying an additional *HTB1* promoter (a & b) or an additional *HTB2* promoter (c & d) driving *mCitrine* expression (grey circles), and a *hir1* Δ (blue triangles) in the same background, determined by RT-qPCR for *ACT1*, *HTA1/HTA2*, *HTB1*, *HTB2* and *mCitrine*. VDPs (a & c) were determined as the slope of the linear fit (b & d) to the double logarithmic dependence of concentration on cell volume. Error bars indicate the standard error of the slope. Deviation of the VDP from that of the 'wild-type' (carrying no deletion) was tested using linear regressions; ** $p_{\rm HTB1} = 7.4 \cdot 10^{-3}$, *** $p_{\rm mCitrine} = 7.3 \cdot 10^{-4}$ (a), *** $p_{\rm HTB1} = 1.0 \cdot 10^{-6}$, *** $p_{\rm HTB2} = 9.2 \cdot 10^{-4}$, $p_{\rm mCitrine} = 2.4 \cdot 10^{-4}$ (c).



Supplementary Figure 11. Illustration of the gating strategy used during all the flow cytometry experiments, shown for representative yeast populations of non-induced haploids (a), induced haploids (b), non-induced diploids (c), and induced diploids (d). Panels show screen shots of the data acquired with FACSDiva 8.0.1 (BD) software during experiments. To differentiate the yeast population from background noise, the first gate (Yeast) was manually traced by comparing the sidescatter (SSC) area signal with the forward scatter (FSC) area signal. Potential doublets were then eliminated by comparing the height of the FSC signal to the area of the signal (manually traced gate: singles-h), and then comparing the width of the FSC signal to the area of the signal (manually traced gate: singles-w). At least 10.000 cells were then measured in the final gate and the mCitrine (FITC)

area signal was analyzed (Citrine). For each experiments and the corresponding replicates, identical gates were used.

Extended Data tables

Nomo	Construng	Decomintion	Omicin	Ein
Iname	Genotype	Description	Origin	Fig.
ASY020-1	Mat a/a; ADE2/ADE2, URA3/ura3, leu2/LEU2	Non-inducible <i>WHI5</i> ,	Anika Seel,	1, 51,
		diploid strain.	Schmoller	S 2
		Also used as	lab	
		microscopy background	(unpublishe	
			d)	
ASY023-1	Mat α/a ; ADE2/ADE2, URA3/ura3,	Inducible <i>WHI5</i> , diploid	Anika Seel,	4, 7, S7,
	$WHI5/whi5\Delta::kanMX6-LexAprom-WHI5-$	strain.	Schmoller	S9
	ADH1term-LEU2, his3/his3::LexA-ER-AD-TF-	Used as microscopy	lab	
	HIS3	background	(unpublishe	
			d)	
DBY001-2	Mat a; ADE2, htb2∆::KlacURA3	Non-inducible WHI5,	This study	S5
		haploid $htb2\Delta$ strain		
DBY002-1	<i>Mat a; ADE2, whi5∆::kanMX6-LexAprom-</i>	Inducible WHI5,	This study	Table
	WHI5-ADH1term-LEU2, his3::LexA-ER-AD-	haploid <i>htb2</i> ∆ strain		S2
	<i>TF-HIS3, htb2∆::KlacURA3</i>			
DBY003-1	Mat a; ADE2, whi5∆∷kanMX6-LexAprom-	Inducible WHI5,	This study	Table
	WHI5-ADH1term-LEU2, his3::LexA-ER-AD-	haploid <i>hho1</i> ∆ strain		S2
	TF-HIS3, hho1∆::CglaTRP1	_		
DBY008-1	Mat a; ADE2, whi5 <i>A</i> ::kanMX6-LexAprom-	Inducible WHI5,	This study	Table
	WHI5-ADH1term-LEU2, his3::LexA-ER-AD-	haploid $hhfl \Delta$ strain		S2
	TF -HIS3, $hhfl \Delta$:: $CglaTRP1$	1 0		
DBY009-1	Mat α; ADE2, hhf2Δ::CglaTRP1	Non-inducible WHI5,	This study	Table
		haploid $hhf2\Delta$ strain	5	S2
DBY011-1	Mat α : ADE2, hht1 Δ ::CglaTRP1	Non-inducible <i>WHI5</i> .	This study	Table
-		haploid <i>hht1</i> /1 strain	j	S2
DBY013-1	Mat a: ADE2 hht2A::ColaTRP1	Non-inducible <i>WHI5</i>	This study	Table
2210101		haploid $hht_2/4$ strain	1 mb stady	S2
DBY020-2	Mat a: ADE2_ura3CelaTRP1-HTR1prom-	Non-inducible <i>WHI5</i>	This study	3 6 86
DD10202	mCitrine-ADH1term-URA3	haploid strain with	This study	S8
		additional copy of		50
		HTB1 promoter		
		expressing <i>mCitrine</i>		
DBY021-3	Mat a: ADE2_ura3CalaTRP1_HTB2prom_	Non-inducible WHI5	This study	3 86
DD1021-3	mai 0, MDE2, unusegiuriti 1-111152prom-	haploid strain with	This study	5,50
	metitine-ADIIIterm-OKA5	additional conv of		
		HTR2 promoter		
		expressing <i>mCitring</i>		
DPV022 1	Mat a: ADE2 una2uCalaTPD1 HHE1prom	Non inducible WHI5	This study	2686
DD 1022-1	Mai u, ADE2, uras. CgiarKF1-HHF1prom-	honloid strain with	This study	5, 0, 50,
	mCurine-ADIIIterm-OKA5	additional conv of		30
		HHE1 promotor		
		avprossing <i>mCitating</i>		
DRV027 11	Mata: ADE2 una2CalaTDD1 UTD1-na	Non inducible WIII5	This study	8
DD1027-11	main u, ADE2, uras: CguirKP1-HIB1prom-	honloid hth? 4 strain	This study	0
	mCulture-ADHIterm US2 14224. JEU2	uith additional conv of		
	$mKale2-ADIIIIeIm-miss, m02\Delta.:LEU2$	With authonal copy of		
		avprossing mCitring and		
		additional convert		
		ACT1 promotor		
		ACTT promoter		
DCV002 C		New index 111 WWV	This st 1	1
DCY003-6	Mat α; ADE2, htb1::HTB1-linker-mCitrine-	Non-inducible WHI5,	This study	1
	ADHIterm-UKA3	napioid strain with		
		HIBI tagged with		
DOMOGO		<i>mCitrine</i>		0
DCY008-8	Mat a; ADE2, ura3::CglaTRP1-HTB1prom-	Non-inducible <i>WHI5</i> ,	This study	8
1	mCitrine-ADH1term-URA3, his3::ACT1prom-	haploid strain with		1

Name	Genotype	Description	Origin	Fig.
	mKate2-ADH1term-HIS3	additional copy of		
		HTB1 promoter		
		additional copy of		
		ACT1 promoter		
		expressing <i>mKate2</i>		
KCY001-3	Mat a; ADE2, htb2::HTB2-linker-mCitrine-	Inducible WHI5,	This study	1, S1,
	$ADH1$ term-CglaTRP1, whi5 Δ ::kanMX6-	haploid strain with		S2
	LexAprom-WHI5-ADH1term-LEU2,	HTB2 tagged with		
	his3::LexA-ER-AD-TF-HIS3	mCitrine		1.01
KCY002-3	Mat α; ADE2, htb2::HTB2-linker-mCitrine-	Non-inducible <i>WHI5</i> ,	This study	1, S1,
	ADHIterm-CglaIKP1	HTR2 tagged with		52
		mCitrine		
KCY005-1	Mat α/a ; ADE2/ADE2,	Inducible <i>WHI5</i> , diploid	This study	1, 2, S1,
	$whi5\Delta::CglaTRP1/whi5\Delta::kanMX6-LexAprom-$	strain.		S2, S5,
	WHI5-ADH1term-LEU2, his3/his3::LexA-ER-	Also used as		S 7
	AD-TF-HIS3	microscopy background		
KCY006-1	Mat α/a ; ADE2/ADE2,	Inducible WHI5, diploid	This study	2, S5
	$htb2\Delta$::KlacURA3/HTB2,	strain with one HTB2		
	$WHI5_ADH1term_IEU2$ his3/his3··LexAprom-	allele deleted		
	AD-TF-HIS3			
KCY007-2	Mat α; ADE2, ura3::CglaTRP1-	Non-inducible WHI5,	This study	6
	150bpHHF1prom-mCitrine-ADH1term- URA3	haploid strain with	5	
		additional 150 bp of		
		HHF1 promoter		
		(truncated $5' - 3'$)		
KCV008 1	Mat a: ADE2 ura3::CalaTPD1	Non inducible WHI5	This study	6 88
KC 1000-1	300bpHHF1prom-mCitrine-ADH1term-URA3	haploid strain with	This study	0, 30
		additional 300 bp of		
		HHF1 promoter		
		(truncated $5' - 3'$)		
		expressing mCitrine		
KCY009-1	Mat α ; ADE2, ura3::CglaTRP1-	Non-inducible <i>WHI5</i> ,	This study	6, S8
	4506pHHF1prom-mCitrine-ADH1term-URA3	haploid strain with		
		HHF1 promoter		
		(truncated $5^{\circ} - 3^{\circ}$)		
		expressing mCitrine		
KCY010-2	Mat α; ADE2, ura3::CglaTRP1-	Non-inducible WHI5,	This study	6
	600bpHHF1prom-mCitrine-ADH1term-URA3	haploid strain with		
		additional 600 bp of		
		nnr 1 promoter (truncated 5' 2')		
		(u uncated 5 - 5) expressing <i>mCitrine</i>		
KCY011-1	Mat a; ADE2, ura3::CglaTRP1-	Inducible <i>WHI5</i> .	This study	6
	150bpHHF1prom-mCitrine-ADH1term-URA3,	haploid strain with		-
	whi5∆::kanMX6-LexAprom-WHI5-ADH1term-	additional 150 bp of		
	LEU2, his3::LexA-ER-AD-TF-HIS3	HHF1 promoter		
		(truncated $5' - 3'$)		
KCV012_1	Mat a ADE2 ura 2. CalaTDD1	expressing <i>mCitrine</i>	This start-	6 60
KC1012-1	Man a, ADE2, uras:: Cguirri- 300bnHHF1nrom-mCitrine_ADH1term_UPA3	hanloid strain with	This study	0, 30
	whi5 Δ ::kanMX6-LexAprom-WHI5-ADH1term-	additional 300 bp of		
	LEU2, his3::LexA-ER-AD-TF-HIS3	HHF1 promoter		
		(truncated $5' - 3'$)		

Name	Genotype	Description	Origin	Fig.
		expressing <i>mCitrine</i>		
KCY013-1	Mat a; ADE2, ura3::CglaTRP1-	Inducible <i>WHI5</i> ,	This study	6, S8
	450bpHHF1prom-mCitrine-ADH1term-URA3,	haploid strain with		
	whi5A::kanMX6-LexAprom-WHI5-ADH1term-	additional 450 bp of		
	LEU2, his3::LexA-ER-AD-TF-HIS3	HHF1 promoter		
		(truncated $5' - 3'$)		
TOTAL 1		expressing <i>mCitrine</i>		-
KCY014-1	Mat a; ADE2, ura3::CglaTRP1-	Inducible WHI5,	This study	6
	6000bpHHF1prom-mCitrine-ADH1term-URA3,	haploid strain with		
	$wnis\Delta::kanMXO-LexAprom-wHIS-ADHIterm-$	additional 600 bp of		
	LEU2, MISS::LeXA-EK-AD-IF-HISS	<i>HHF1</i> promoter		
		(truncated 5 - 3)		
KOV015_1		expressing <i>mCurrine</i>	This is a l	6
KC Y015-1	Mat a; ADE2, ura5::Cgla1KP1-	Inducible <i>WHIS</i> ,	This study	0
	1500pH1B1prom-mCltrine-ADH1term-UKAS,	napioid strain with		
	$wnis\Delta::kanMXO-LexAprom-wHIS-ADHIterm-$	additional 150 bp of		
	LEU2, hiss::LexA-ER-AD-IF-Hiss	HIBI promoter		
		(truncated 5 - 5)		
KOV016 1		expressing <i>mCurrine</i>	This is a l	6 69
KC Y016-1	Mat a; ADE2, ura5::Cgla1KP1-	Inducible <i>WHIS</i> ,	This study	0, 58
	SUDDPHIBIProm-mCurine-ADHIlerm-UKAS,	additional 200 km of		
	$Wnis\Delta$: :kanimizo-Lexaprom- $Wnis$ -ADHIIerm-	<i>LITP</i> 1 momentar		
	LEU2, MISS::LeXA-EK-AD-IF-HISS	HIBI promoter		
		(truncated 5 - 3)		
KOV017 1		expressing <i>mCurrine</i>	This is a	6 69
KCY017-1	Mat a; ADE2, ura5::Cgla1KP1-	Inducible WHIS,	This study	0, 58
	4500pH1B1prom-mCitrine-ADH1term-URA3,	haploid strain with		
	$whis\Delta::kanMXO-LexAprom-WHIS-ADHIterm-LEU2 1: 2 L A ED AD TE US2$	additional 450 bp of		
	LEU2, MISS::LeXA-EK-AD-IF-HISS	(true pasted 5', 2')		
		(truncated 5 - 5)		
VCV019_1	Mat a. ADE2 uma ² CalaTDD1	Inducible WIII5	This study	6
KC 1018-1	Mai a; ADE2, uras: CgiarKP1-	hanloid strain with	This study	0
	whi5AkanMY6 LaxAprom WHI5 ADH1tarm	additional 600 hp of		
	I EU2 his2 Lard EP AD TE HIS2	HTR1 promotor		
	LEU2, MISSLEXA-EK-AD-IF-HISS	(truncated 5' 2')		
		(Indicated 5 - 5)		
KCV010_1	Mat a. ADE2 una2CalaTPD1	Inducible WHI5	This study	6
KC 1019-1	Mai a, ADE2, aras. CgiarKF1- 750hpHTP1ppopp mCitring ADH1torm UPA2	hanloid strain with	This study	0
	vision which when MY6 Lord prom WHIS ADHItem	additional 750 hp of		
	IEU2 his2 Lard EP AD TE HIS2	HTR1 promotor		
	LE02, <i>msjLexa-EK-AD-11-11155</i>	(truncated 5' 3')		
		$\left(\frac{1}{1} \frac$		
KCV020-1	Mat a: ADE2 ura3CalaTPP1	Non inducible WHI5	This study	6
KC 1020-1	150hpHTR1prom-mCitring-ADH1term-URA3	haploid strain with	This study	0
		additional 150 bp of		
		HTR1 promoter		
		(truncated 5' - 3')		
		expressing <i>mCitrine</i>		
KCY021-1	Mat a: ADE2 ura3::CalaTRP1.	Non-inducible <i>WHI</i> 5	This study	6.58
Re10211	300bnHTB1nrom-mCitrine-ADH1term-URA3	haploid strain with	This study	0, 50
		additional 300 bp of		
		HTR1 promoter		
		(truncated 5' - 3')		
		expressing $mCitrino$		
KCY022-1	Mata: ADF2 ura3···ColaTRP1_	Non-inducible WHI5	This study	6.58
1101022-1	450bnHTR1nrom-mCitring_ADH1torm_URA3	hanloid strain with	i mo study	0,00
		additional 450 bp of		

Name	Genotype	Description	Origin	Fig.
		HTB1 promoter		
		(truncated $5^{2} - 3^{2}$)		
KCV023 /	Mata: ADE2 ura3: CalaTPD1	Non inducible WHI5	This study	6
KC1023-4	600hpHTR1prom-mCitrine-ADHterm-URA3	hanloid strain with	This study	0
		additional 600 bp of		
		HTB1 promoter		
		(truncated $5' - 3'$)		
		expressing mCitrine		
KCY024-1	Mat α; ADE2, ura3::CglaTRP1-	Non-inducible WHI5,	This study	6
	750bpHTB1prom-mCitrine-ADH1term-URA3	haploid strain with		
		additional 750 bp of		
		(trupposted 5', 2')		
		(function 5 - 5)		
KCY027-4	Mat a/a: ADE2/ADE2_hth2:·HTB2-linker-	Non-inducible <i>WHI5</i>	This study	1 S1
1101027	mCitrine-ADH1term-CglaTRP1 /http2::HTB2-	diploid strain with both	This study	S2
	linker-mCitrine-ADH1term-KlacURA3	HTB2 alleles tagged		
		with <i>mCitrine</i>		
KCY028-1	<i>Mat α/a; ADE2/ADE2, htb2::HTB2-linker-</i>	Inducible WHI5, diploid	This study	1, S1,
	mCitrine-ADH1term-KlacURA3/htb2::HTB2-	strain with both <i>HTB2</i>		S2
	linker-mCitrine-ADHIterm-CglaTRPI,	alleles tagged with		
	$whi5\Delta::Cgla1KP1/whi5\Delta::kanMXO-LexAprom-$	mCitrine		
	WHIS-ADHITERM-LEU2, MISS/MISS::LEXA-EK-			
KCY029-1	Mat a/a: ADE2/ADE2.	Inducible WHI5, diploid	This study	1. S1.
110102/1	$htb2\Delta::KlacURA3/htb2::HTB2-linker-$	strain with one <i>HTB2</i>	This study	S2
	mCitrine-ADH1term-CglaTRP1,	allele deleted and the		
	$whi5\Delta::CglaTRP1/whi5\Delta::kanMX6-LexAprom-$	other HTB2 allele		
	WHI5-ADH1term-LEU2, his3/his3::LexA-ER-	tagged with mCitrine		
WOLLOOL 1	AD-TF-HIS3	x 1 11 xxxxxx 11 1 1 1		
KCY031-1	Mat α/a; ADE2/ADE2, ura3::CglaTRP1-	Inducible <i>WHI5</i> , diploid	This study	3, 4, 6, 7, 86
	HIBIPIOM-mCurine-ADHIlerm-OKAS/urus, WHI5/whi5/ $\Delta \cdots$ kanMY6-Ler/arom-WHI5-	conv of HTR1 promoter		7, 50,
	ADH1term-LEU2, his3/his3::LexA-ER-AD-TF-	expressing <i>mCitrine</i>		57,50
	HIS3	enpressing memorie		
KCY032-2	Mat α/a; ADE2/ADE2, ura3::CglaTRP1-	Inducible WHI5, diploid	This study	3, 4, 6,
	HHF1prom-mCitrine-ADH1term-URA3/ura3,	strain with additional	-	7, S6,
	WHI5/whi5∆::kanMX6-LexAprom-WHI5-	copy of HHF1 promoter		S7, S8
	ADH1term-LEU2, his3/his3::LexA-ER-AD-TF-	expressing mCitrine		
VCV022.2	HISS Mata/a: ADE2/ADE2	Inducible WILLS distants	This start-	2 4 67
KC1033-2	Mai a/a; ADE2/ADE2, ura5::Cgla1KP1- HTP2prom mCitying ADH1tama UDA2/	strain with additional	i nis study	3, 4, 87, 88
	WHI5/whi5/kanMY6 LarAprom WHI5	copy of HTB2 promoter		20
	ADH1term-LEU2, his3/his3::LexA-ER-AD-TF-	expressing <i>mCitrine</i>		
	HIS3	enpressing memorie		
KCY035-3	Mat α/a; ADE2/ADE2, ura3::CglaTRP1-	Inducible WHI5, diploid	This study	3, 4, S7,
	ACT1prom-mCitrine-ADH1term-URA3/ura3,	strain with additional		S8
	WHI5/whi5∆::kanMX6-LexAprom-WHI5-	copy of ACT1 promoter		
	ADH1term-LEU2, his3/his3::LexA-ER-AD-TF-	expressing <i>mCitrine</i>		
VCV029 1	HISS Mata/a: ADE2/ADE2	Non indusible WIII5	This start-	6 00
KU I USÖ-1	Mai u/a; ADE2/ADE2, Ura5:: Ugla1KP1- 300bpHTR1prom_mCitring ADH1term	diploid strain with	i nis study	0, 38
	IIRA 3/ura 3 leu 2/LEU 2	additional 300 hp of		
		HTB1 promoter		
		(truncated $5' - 3'$)		
		expressing mCitrine		
KCY039-1	Mat α/a; ADE2/ADE2, ura3::CglaTRP1-	Inducible WHI5, diploid	This study	6, 7, S8,

Name	Genotype	Description	Origin	Fig.
	300bpHTB1prom-mCitrine-ADH1term-	strain with additional		S 9
	URA3/ura3, WHI5/whI5\Delta::kanMX6-LexAprom- WHI5 ADH1tarm LEU2 his3/his3::LaxA ER	300 bp of <i>HTB1</i>		
	AD-TF-HIS3	-3') expressing		
		mCitrine		
KCY040-1	Mat α/a; ADE2/ADE2, ura3::CglaTRP1-	Non-inducible WHI5,	This study	6, S8
	450bpHTB1prom-mCitrine-ADH1term-	diploid strain with		
	URA3/ura3, leu2/LEU2	additional 450 bp of		
		(truncated $5' - 3'$)		
		(u uncated 5' = 5') expressing <i>mCitrine</i>		
KCY041-1	Mat α/a; ADE2/ADE2, ura3::CglaTRP1-	Inducible <i>WHI5</i> , diploid	This study	6, 7, S8,
	450bpHTB1prom-mCitrine-ADH1term-	strain with additional		S9
	URA3/ura3, WHI5/whi5∆::kanMX6-LexAprom-	450 bp of <i>HTB1</i>		
	WHI5-ADH1term-LEU2, his3/his3::LexA-ER-	promoter (truncated 5'		
	AD-IF-HIS5	-3°) expressing		
KCY043-1	Mat α/a: ADE2/ADE2. ura3::CglaTRP1-	Inducible <i>WHI5</i> , diploid	This study	6, 7, S8,
	300bpHHF1prom-mCitrine-ADH1term-	strain with additional		S9
	URA3ura3, WHI5/whi5∆::kanMX6-LexAprom-	300 bp of <i>HHF1</i>		
	WHI5-ADH1term-LEU2, his3/his3::LexA-ER-	promoter (truncated 5'		
	AD-TF-HIS3	(-3^{\prime}) expressing		
KCY045-1	Mat a/a: ADE2/ADE2 ura3CalaTRP1-	Inducible WHI5 diploid	This study	678
KC 1045 1	450bpHHF1prom-mCitrine-ADH1term-	strain with additional	This study	S9
	$URA3/ura3$, $WHI5/whi5\Delta$::kanMX6-LexAprom-	450 bp of <i>HHF1</i>		
	WHI5-ADH1term-LEU2, his3/his3::LexA-ER-	promoter (truncated 5'		
	AD-TF-HIS3	-3') expressing		
KCV040-1	Mat a: ADE2 ura2CalaTPD1 HTP1prom	mCitrine Inducible WHI5	This study	\$10
KC 1049-1	mai a, $ADE2$, $urascgarKi 1-IIIB1prom-mCitrine-ADH1term-URA3 hir1\Lambda··natMX6$	hanloid <i>hir</i> $I\Lambda$ strain	This study	510
	whi5 Δ ::kanMX6-LexAprom-WHI5-ADH1term-	with additional copy of		
	LEU2, his3::LexA-ER-AD-TF-HIS3	HTB1 promoter		
		expressing <i>mCitrine</i>		
КСҮ050-2	Mat α/a ; ADE2/ADE2, htb1::HTB1-linker-	Non-inducible WHI5,	This study	1
	mCurine-ADH11erm-KlacUKA5/nlb1::H1B1- linker_mCitrine-ADH1term_ColaTRP1	HTR1 alleles tagged		
		with <i>mCitrine</i>		
KCY051-1	Mat a; ADE2, ura3::CglaTRP1-HTB2prom-	Inducible WHI5,	This study	S10
	$mCitrine-ADH1 term-URA3$, $hir1\Delta$:: $natMX6$,	haploid $hirl\Delta$ strain		
	$whi5\Delta$:: $kanMX6$ -LexAprom-WHI5-ADH1term-	with additional copy of		
	LEU2, his3::LexA-ER-AD-1F-HIS3	HIB2 promoter		
KSY212-2	Mat a: ADE2 rrn6A…CølaTRP1	Non-inducible <i>WHI5</i>	This study	2.85
1012122		haploid <i>rrp6</i> ⊿ strain	This study	2, 55
KSY213-6	Mat a; ADE2, rrp6Д::CglaTRP1,	Inducible WHI5,	This study	2, S5
	$whi5\Delta$:: $kanMX6$ -LexAprom-WHI5-ADH1term-	haploid <i>rrp6</i> ⊿ strain		
1/03/01/4 1	LEU2, his3::LexA-ER-AD-TF-HIS3	NY ' 1 '11 W/////	TD1 • 1	2.05
KSY214-1	Mat α ; ADE2, htr1 Δ ::Cgla1KP1	Non-inducible WHIS, haploid <i>hirl A</i> strain	This study	2, 85
KSY215-2	Mat a: ADE2. hir1A::CelaTRP1	Inducible <i>WHI5</i>	This study	2, 85
	whi5 Δ ::kanMX6-LexAprom-WHI5-ADH1term-	haploid <i>hirl</i> Δ strain	<i>Soud j</i>	_, _,
	LEU2, his3::LexA-ER-AD-TF-HIS3	-		
KSY219-3	Mat a; ADE2, rtt106∆::CglaTRP1,	Inducible <i>WHI5</i> ,	This study	2, S5
	$whib\Delta::kanMXb-LexAprom-WHI5-ADH1term-$	haploid $rtt106\Delta$ strain		
KSY208-3	LEU2, MISS::LexA-EK-AD-IF-HISS Mat a: ADF2 ura3mCitrine_ADH1term_	Non-inducible WHI5	This study	
10012003	URA3	haploid strain with	1 mo study	

Name	Genotype	Description	Origin	Fig.
		additional <i>mCitrine</i>		
		copy (not expressed)	T1 1	2 4 56
KSY222-1	Mat a; ADE2, ura3::Cgla1RP1-H1B1prom-	Inducible WHIS,	This study	3, 4, 86, \$10
	$m_{\text{Clirine-ADH1}term-UKAS}, whis \Delta$::kan $m_{\text{XO-}}$	napioid strain with		510
	LexAprom-whis-Admiterm-LEU2, $his^{2}\cdots LexA=EPAD=TEHIS^{2}$	HTR1 promotor		
	nissLexA-EK-AD-IF-HISS	avprossing <i>mCitring</i>		
KSV222 2	Mat a: ADE2 ura3: CalaTPD1 HHE1prom	Inducible WHI5	This study	3 1 86
KS 1 223-3	mai a, ADE2, arasCguarki 1-inin 1prom-	haploid strain with	This study	5, 4, 50
	LarAnrow WHIS ADHItarm LEU?	additional conv of		
	his ³ ··· LarA FR AD TE HIS ³	HHE1 promoter		
	nissLexA-ER-AD-II-IIIss	avprossing <i>mCitring</i>		
KSV225 2	Mat a: ADE2 ura3::CalaTRP1 HTR2prom	Inducible WHI5	This study	3 1 86
KS1225-2	mai a, ADE2, arasCgarKi 1-111D2prom- mCitring_ADH1term_URA3_whi5AkanMX6_	hanloid strain with	This study	5, 4, 50, \$10
	I erAprom-WHI5-ADH1term-I FII?	additional copy of		510
	his3LexA_FR_AD_TF_HIS3	HTB2 promoter		
		expressing <i>mCitrine</i>		
KSY226-3	Mat a: ADE? ura3::mCitrine_ADH1term_	Inducible <i>WHI</i> 5	This study	
KB1220 5	I/RA3 whi5A··kanMX6-LexAprom-WHI5-	haploid strain with	This study	
	ADH1term-LEU2 his3LexA-ER-AD-TE-HIS3	additional <i>mCitrine</i>		
		copy (not expressed)		
KSY229-1	Mat a: ADE2. ura3::CglaTRP1-ACT1prom-	Non-inducible <i>WHI5</i> .	This study	3. S6
	mCitrine-ADH1term-URA3	haploid strain with	j	- ,
		additional copy of		
		ACT1 promoter		
		expressing <i>mCitrine</i>		
KSY230-1	Mat a; ADE2, ura3::CglaTRP1-ACT1prom-	Inducible WHI5,	This study	3, S6
	$mCitrine-ADH1term-URA3$, whi5 Δ ::kanMX6-	haploid strain with		
	LexAprom-WHI5-ADH1term-LEU2,	additional copy of		
	his3::LexA-ER-AD-TF-HIS3	ACT1 promoter		
		expressing <i>mCitrine</i>		
MMY116-2C	Mat a; ADE2	Non-inducible WHI5,	Skotheim	1, 2, 3,
		haploid strain.	lab stock	S1, S2,
		Also used as		S3, S4,
		microscopy background		S5
MS62-1	Mat a; ADE2, whi5 Δ ::kanMX6, his3::LexA-	β-estradiol dependent	Matthew	S3
	ER-AD-TF-HIS3	transcription factor,	Swaffer,	
		haploid <i>whi5</i> ⊿ strain	Skotheim	
			lab	
MS63-1	<i>Mat a; ADE2, whi5</i> Δ <i>::kanMX6-LexAprom-</i>	Inducible WHI5,	Matthew	1, 2, 3,
	WHI5-ADH1term-LEU2, his3::LexA-ER-AD-	haploid strain.	Swaffer,	S1, S2,
	TF-HIS3	Also used as	Skotheim	S3, S4,
		microscopy background	lab	S5
				1

Supplementary Table 1. Yeast strains used in this work. All strains are based on W303. *CglaTRP1* denotes the *TRP1* gene of the organism *C. glabrata*, *KlacURA3* denotes the *URA3* gene of the organism *K. lactis*.

	MS63-1	Δ strain
qPCR primer	[mean C_p^{Gene}]	[<i>Cp</i> range]
HHO1	20.3 <u>+</u> 0.1	по атр
HTB2	16.8 <u>+</u> 0.1	40.9 – <i>no amp</i>
HHF1	17.7 <u>+</u> 0.3	34.6 – no amp
HHF2	19.5 <u>+</u> 0.1	33.6 - 35.9
HHT1	18.0 ± 0.1	32.9 – no amp
HHT2	19.0 <u>+</u> 1.4	33.7 – no amp

Supplementary Table 2. Results of qPCR measurements on deletion strains to test for primer specificity. C_p^{Gene} for the MS63-1 strain are the mean of n = 3 technical replicates, with standard deviation of the mean. For the deletion strains, C_p -ranges reach from the minimum C_p^{min} to maximum C_p^{max} values of n = 3 technical replicates for *HHO1*, *HTB2*, *HHT2* and n = 6 technical replicates for *HHF1*, *HHF2*, *HHT1*. No detectable amplification curve over threshold is denoted as "no amp".

Gene	qPCR primer direction	qPCR primer sequence (5' - 3')
ACT1	forward	AGTTGCCCCAGAAGAACACC
ACTI	reverse	GGACAAAACGGCTTGGATGG
ENO2	forward	TTGTTCCATCTGGTGCCTCC
ENO2	reverse	ACGAAAGCAGCAGCAATGAC
	forward	TACACCGAACACGCCAAGAG
	reverse	TTGCTTGTTGTTACCGTTTTCTT
инер	forward	ACGAAGAAGTCAGAGCCGTC
ΠΠΓΖ	reverse	ACCGATTGTTTAACCACCGATTG
	forward	ACCAGCAAAGGCAAGGAGAA
ппот	reverse	AAAGCCGTGAGCCCTTCAAT
	forward	CAATCTTCTGCCATCGGTGC
	reverse	ACTGATGACAATCAACAAACTATGA
	forward	AGCAAACACTCCACAATGGC
	reverse	CAAGGCAACAGTACCTGGCT
	forward	GTTGCCAAAGAAGTCTGCCA
ΠΙΑΙ	reverse	CAGTTTAGTTCCTTCCGCCTT
UTAD	forward	TCGCCCAAGGTGGTGTTTT
IIIA2	reverse	TGATTTGCTTTGTTTCTTTTCAACT
	forward	TACACACATACAATGTCTGCTAAAG
	reverse	AGTGTCAGGGTGAGTTTGCTT
	forward	CCTCTGCCGCCGAAAAGAAA
ΠΙΔΖ	reverse	TCTTACCATCGACGGAGGTTG
m Citaria a	forward	GAGCTGAAGGGCATCGACTT
mCurine	reverse	TTCTGCTTGTCGGCCATGAT
	forward	AACTCACCAGGTCCAGACACAATAAGG
KDN10	reverse	AAGGTCTCGTTCGTTATCGCAATTAAGC
	forward	CCAGAAGTGGTCACACCATATAA
KPD1	reverse	GGTCTCCGCTATCACGAATG
RPB3 —	forward	TGTGGGGTCTATTCCCGTTG
	reverse	CGCCCGTCATCATTACGTCT

Supplementary Table 3. Sequences of qPCR primers used in this work.