A UNIVERSAL GENE EXPRESSION SYSTEM FOR FUNGI

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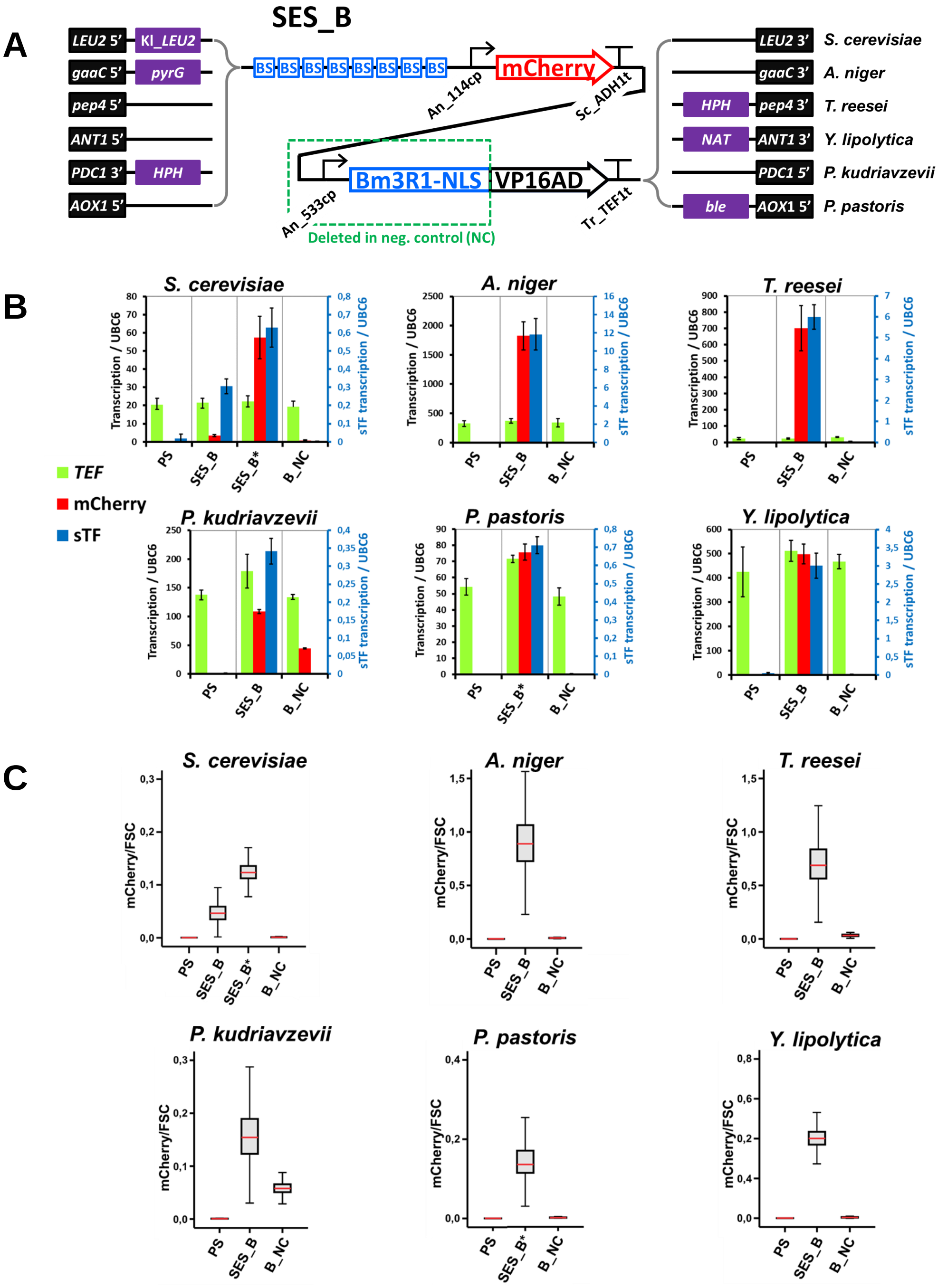


Supplementary Figure 1

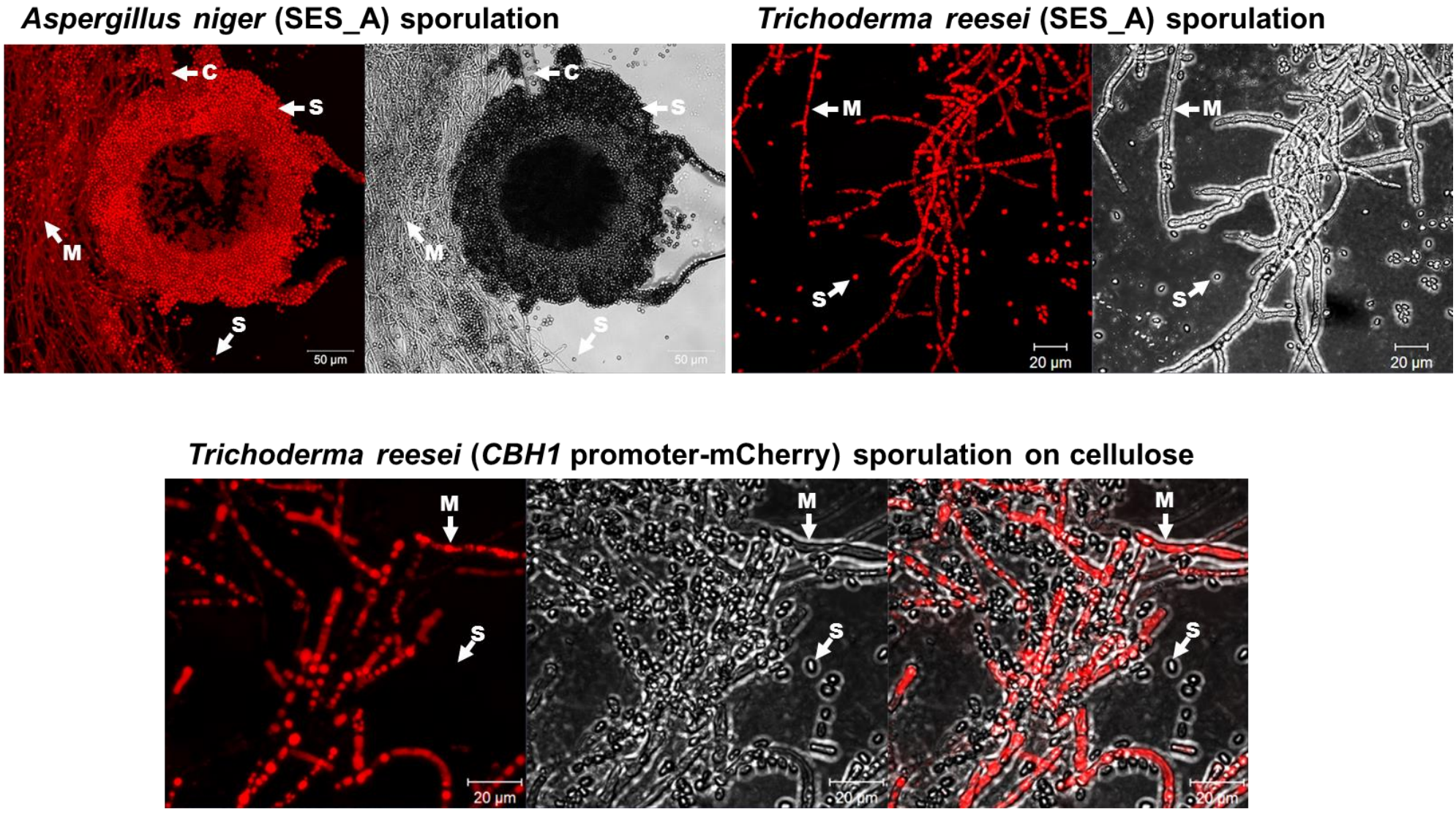
Alternative SES system (SES-B) and the quantification of its performance in fungal hosts.

Supplementary Figure 1. Alternative SES system (SES-B) and quantification of its performance in fungal hosts.

A) Schematic presentation of an alternative version of the SES system (SES-B), including positions of selection markers and genome-integration flanks for the listed species. The An_53301 core promoter (An_533cp) was used for the expression of the sTF (Bm3R1-NLS-VP16). The mCherry expression was controlled by the sTF via the sTF-specific binding sites (BS) positioned upstream of the An_1147651 core promoter (An_114cp). The negative control (NC) lacks the region spanning the core promoter (An_533cp) and a large portion of the sTF. Each cassette was integrated in the specified genomic locus in single copy.

B) Transcription analysis of the strains. The mCherry transcript levels were compared to the transcript levels of endogenous *TEF* genes in each host. Transcript levels of the sTF are shown on the secondary y-axis. PS denotes the parental strains (without SES). In *S. cerevisiae*, in addition to the SES-B, also a modified version was tested having the *S. cerevisiae*-codon-optimized sTF gene, which is indicated by an asterisk (SES-B*). In *P. pastoris*, only the SES-B* version was used. The transcription of the *UBC6* gene homologs (**Supplementary Table 9**) in each species were used for normalization. Values and error bars represent the mean and standard deviation from two biological (four technical) replicates.

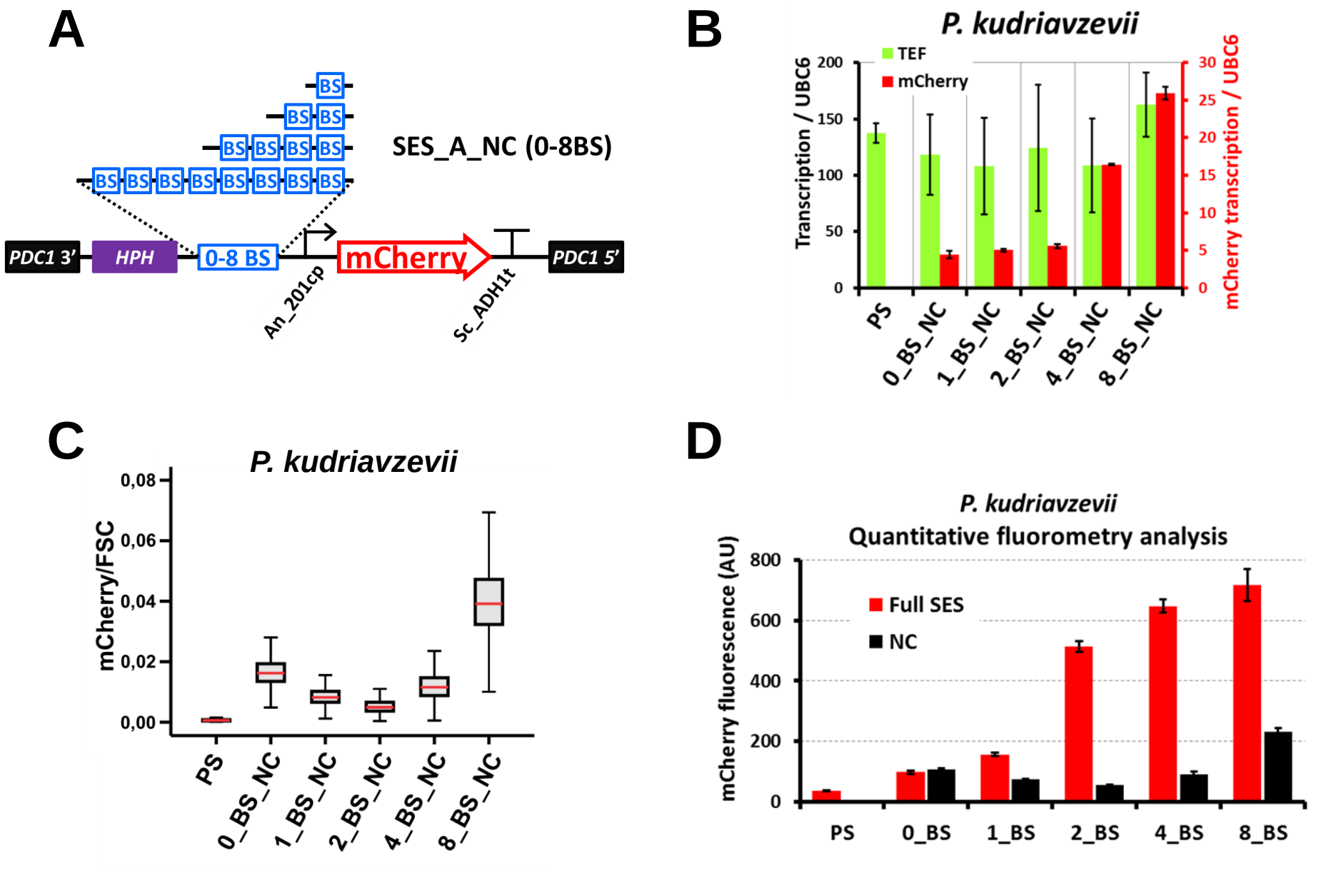
C) Analysis of mCherry expression by flow-cytometry. Flow-cytometry was performed on cells (yeast species) or conidia (filamentous fungi). The box plots show the fluorescence intensity (mCherry) normalized by the particle (cell/conidia) size (FSC – forward scatter) for ~10,000 cells/conidia from each strain. The horizontal red line (inside the grey box) represents the median value, the grey box represents the interquartile range (IQ range), the bottom line of grey box represents the 25% percentile value, the top line of grey box represents the 75% percentile value. The whiskers in box plot together with the IQ range, represent about 99% of all measured instances (cells/conidia) (for numerical values see **Supplementary Fig. 4A)**. Alternative fluorescence analysis of the strains was performed by quantitative fluorometry (**Supplementary Fig. 4B** and **4C**).



Supplementary Figure 2

Constitutive expression enabled by the SES in filamentous fungi; comparison with the widely used CBH1 promoter based expression system in *T. reesei.*

SES system confers highly constitutive expression as demonstrated by uniformly distributed mCherry fluorescence in different developmental stages in filamentous fungi: mycelia (arrow with M), conidiophore (arrow with C), and conidia/spores (arrow with S). In contrast, when the mCherry gene was placed under the control of a strong, inducible *cbh1* promoter in *T. reesei*, only mycelia were highly fluorescent and negligible expression was observed in conidia in the cellulase-inducing conditions in presence of cellulose (lower panel).



Supplementary Figure 3

Analysis of the intrinsic activation of SES-A-NC system in *P. kudriavzevii*.

A) Schematic presentation of the DNA cassette, containing the SES-A-NC versions with zero to eight sTF-binding sites, used for genome integration in *P. kudriavzevii*. The positions of selection marker (HPH) as well as the integration DNA-flanks (*PDC1* locus) are shown.

B) Transcription analysis of the strains. The mCherry transcript levels were compared to transcript levels of the endogenous *TEF1* gene. PS denotes parental strain (without SES). The values and error bars represent the mean and standard deviation from two biological (four technical) replicates.

C) Analysis of the mCherry fluorescence by flow-cytometry measurements of *P. kudriavzevii* strains. The box plots show the same features as in **Fig. 3B**.

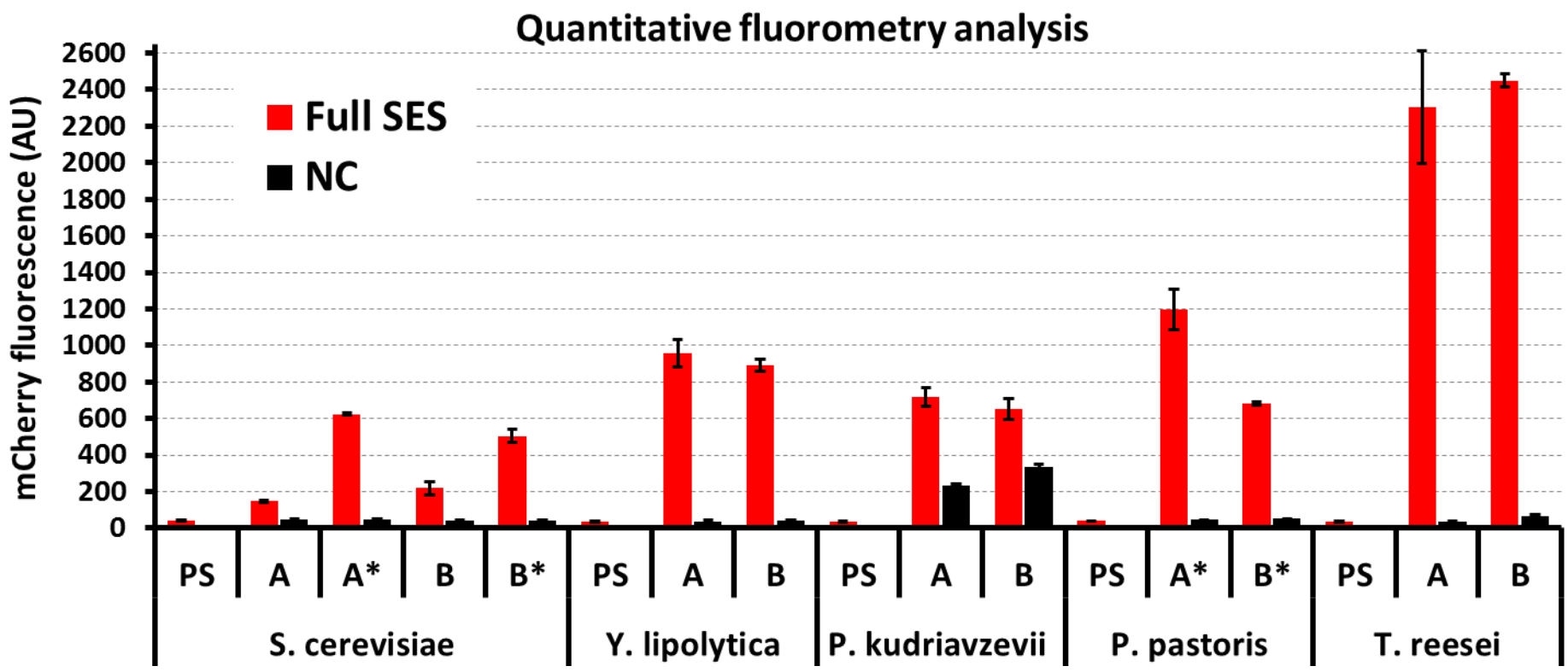
D) Quantitative fluorometry analysis of the strains. The graphs show the fluorescence intensity (mCherry) normalized by the optical density of the cell suspensions used for the fluorometry analysis. The strains of *P. kudriavzevii* harboring the SES-A_0-8BS versions are also included and these strains correspond to those shown in **Fig. 4B** and **4C.** The values and error bars represent the mean and standard deviation from three biological replicates. The cultivation conditions in (B-D) were identical to those used in the transcription analysis of *P. kudriavzevii* (**Fig. 3A**). The impact of the diverse number of the sTF binding sites on the mCherry production (in absence of the sTF) indicates an intrinsic activation of the expression by an unidentified native TF in *P. kudriavzevii*.

A

Numerical values (×10^{-3}) for the flow cytometry analysis (Fig. 3b)

	PS	SES-A	SES-A-NC	SES-B	SES-B-NC
S. cerevisiae	0.3 ± 0.3 (n=9998)	28.8 ± 16.7 (n=9995)	2.7 ± 1.3 (n=9992)	48.2 ± 20.5 (n=9992)	1.1 ± 1.1 (n=9993)
		166.9 ± 35.6* (n=9997)		126.8 ± 32.8* (n=9991)	
Y. lipolytica	0.4 ± 0.4 (n=9987)	476.9 ± 91.8 (n=9995)	4.1 ± 5.6 (n=9990)	407.3 ± 74.4 (n=9994)	5.3 ± 4.5 (n=9996)
P. kudriavzevii	0.7 ± 0.6 (n=9962)	201.5 ± 47.0 (n=9982)	16.9 ± 5.4 (n=9970)	159.0 ± 57.0 (n=9979)	59.2 ± 15.3 (n=9979)
P. pastoris	0.3 ± 0.2 (n=9832)	235.7 ± 97.5* (n=9836)	0.6 ± 1.0 (n=9834)	155.3 ± 89.9* (n=9837)	1.7 ± 1.8 (n=9863)
T. reesei	0.3 ± 2.5 (n=9716)	750.7 ± 372.3 (n=9820)	141.0 ± 145.4 (n=9779)	761.5 ± 444.0 (n=9745)	34.3 ± 14.3 (n=9787)
A. niger	0.6 ± 0.1 (n=9485)	884.3 ± 219.4 (n=9812)	4.2 ± 13.5 (n=9397)	893.1 ± 252.9 (n=9822)	9.5 ± 3.8 (n=9697)

B



C

Numerical values of the fluorometry analysis

	PS	SES-A	SES-A-NC	SES-B	SES-B-NC
S. cerevisiae	41,2 ± 2,3	146,8 ± 5,3 624,6 ± 8,3*	49,3 ± 0,8	218,6 ± 33,6 504,8 ± 36,4*	42 ± 0,8
Y. lipolytica	34,6 ± 1,5	959,2 ± 75,5	35,5 ± 9,8	890,8 ± 32,5	40,6 ± 5,1
P. kudriavzevii	35,5 ± 0,9	716,8 ± 52	230,1 ± 14,2	650,8 ± 57,8	336,0 ± 15,5
P. pastoris	39,5 ± 2,0	1198,0 ± 110,8*	45,4 ± 1,3	683,0 ± 10,5*	50,5 ± 1,4
T. reesei	34,4 ± 3,6	2303 ± 308,6	34,2 ± 5,8	2448,6 ± 35,7	67,3 ± 6,0

Supplementary Figure 4

Numerical values for the flow cytometry analysis and fluorometry analysis of the strains harboring the SES.

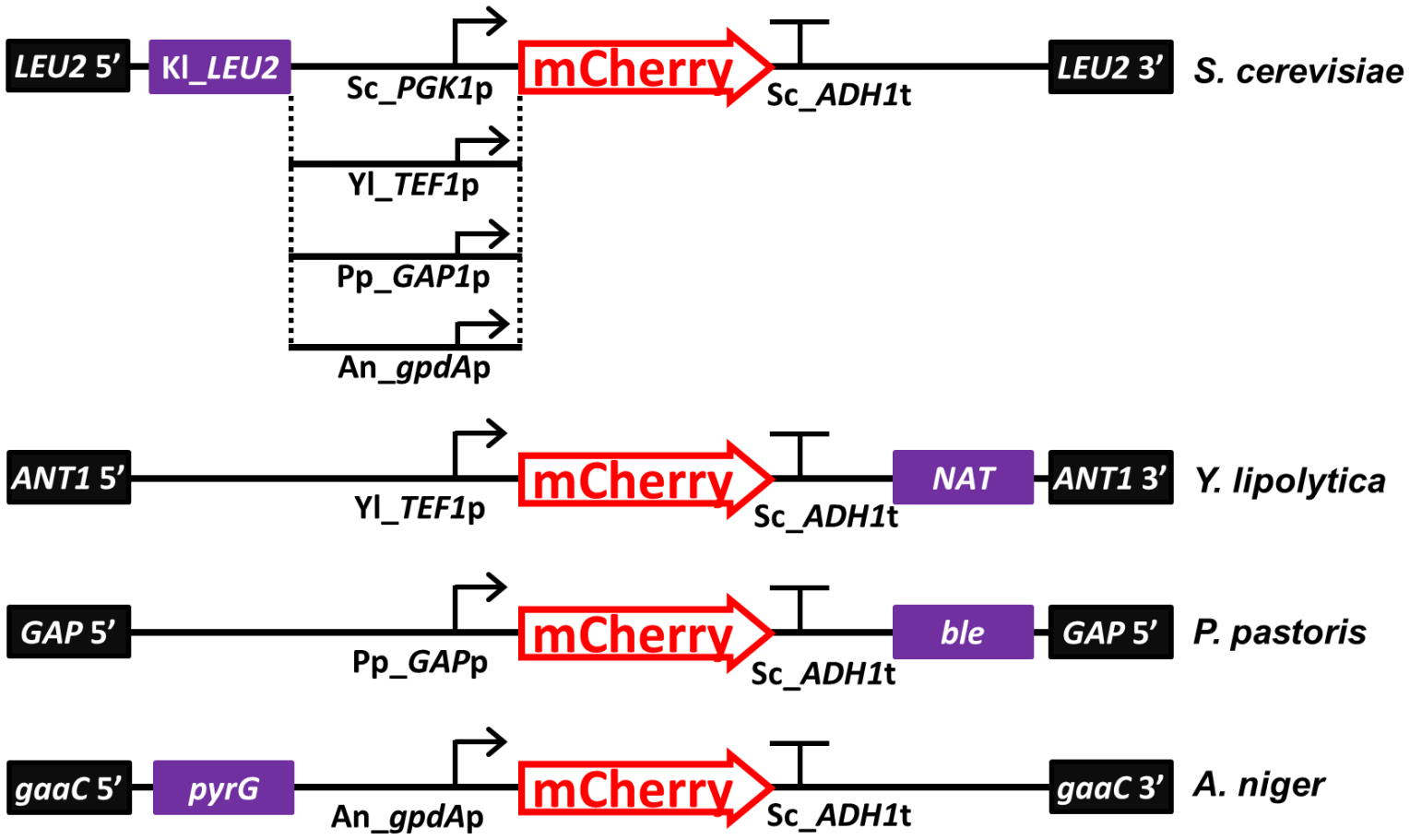
Supplementary Figure 4. Numerical values for the flow cytometry analysis and the fluorometry analysis of the strains harboring the SES.

A) The table shows the mean values with the standard deviations (and number of analysed cells/conidia) of the flow cytometry analysis (shown in **Fig. 3B**).

B) Quantitative fluorometry analysis of mCherry expression in the SES-containing yeasts and *T. reesei* strains. This analysis was not conducted for *A. niger*, because the morphology and other specific properties of its mycelia prohibit reproducible sample handling in the fluorometry analysis setup. The graphs show fluorescence intensity (mCherry) normalized by the optical density of the cell/mycelia suspensions. Values and error bars represent the mean and standard deviation from at least three independent cultivations.

C) The table showing the mean values with the standard deviations of the fluorometry measurements shown in (B). The values with asterisk represent use of the SES systems (SES-A* and SES-B*) in *S. cerevisiae* and *P. pastoris* with *S. cerevisiae*-codon-optimized version of the sTF.

A



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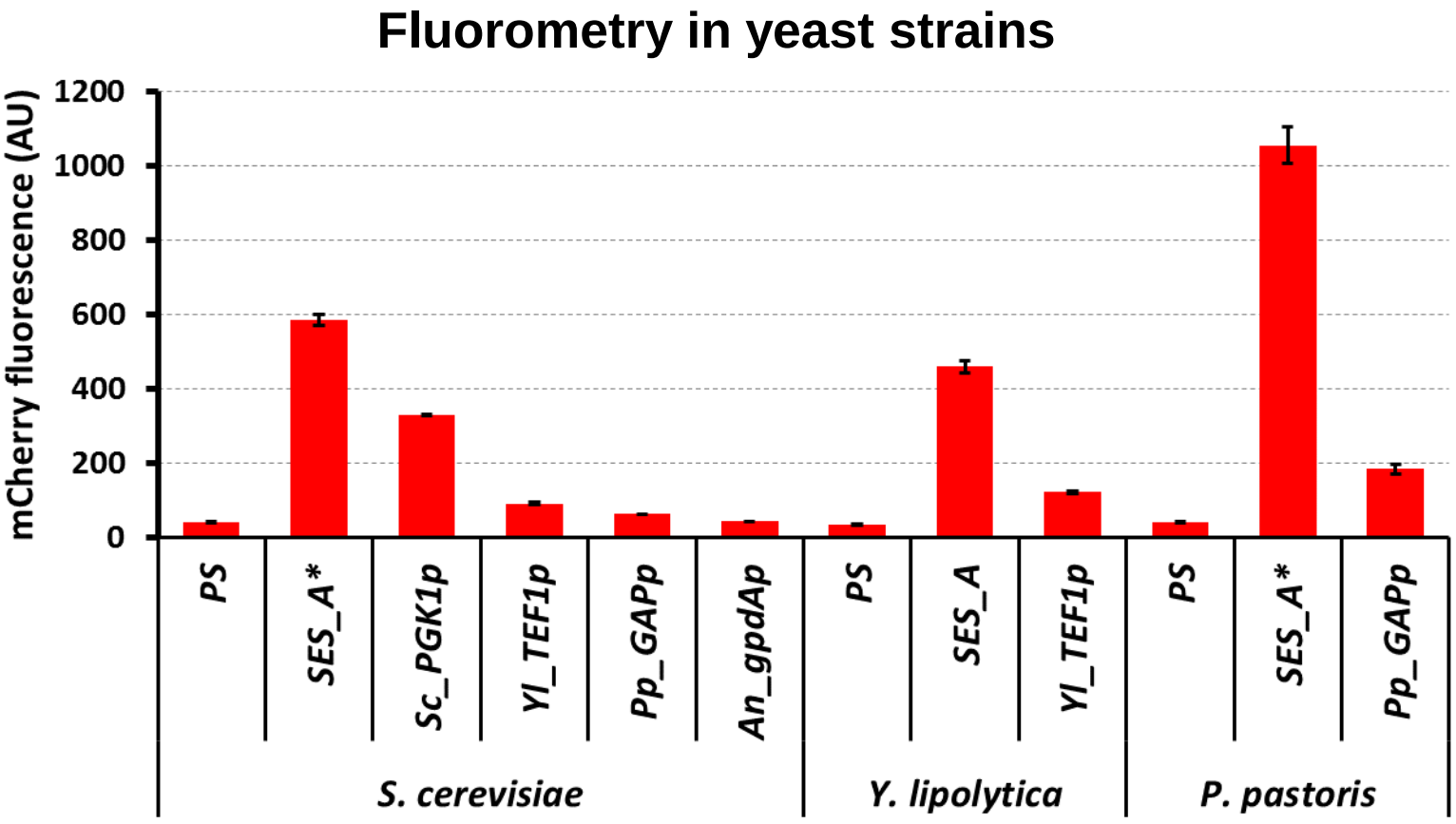

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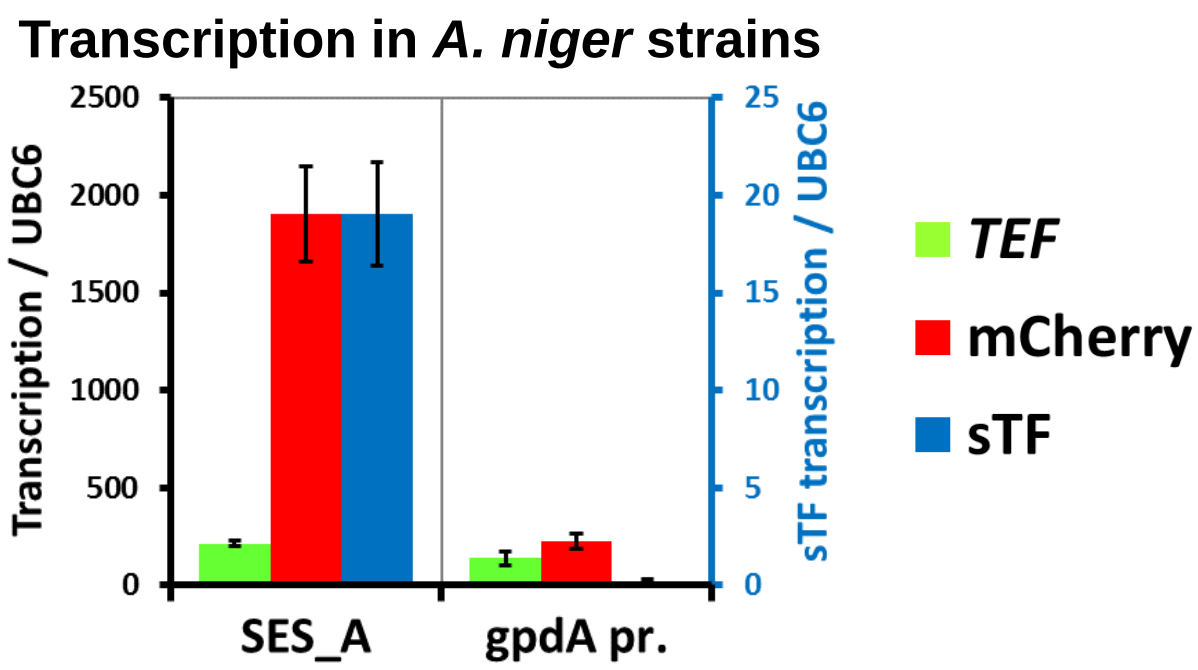


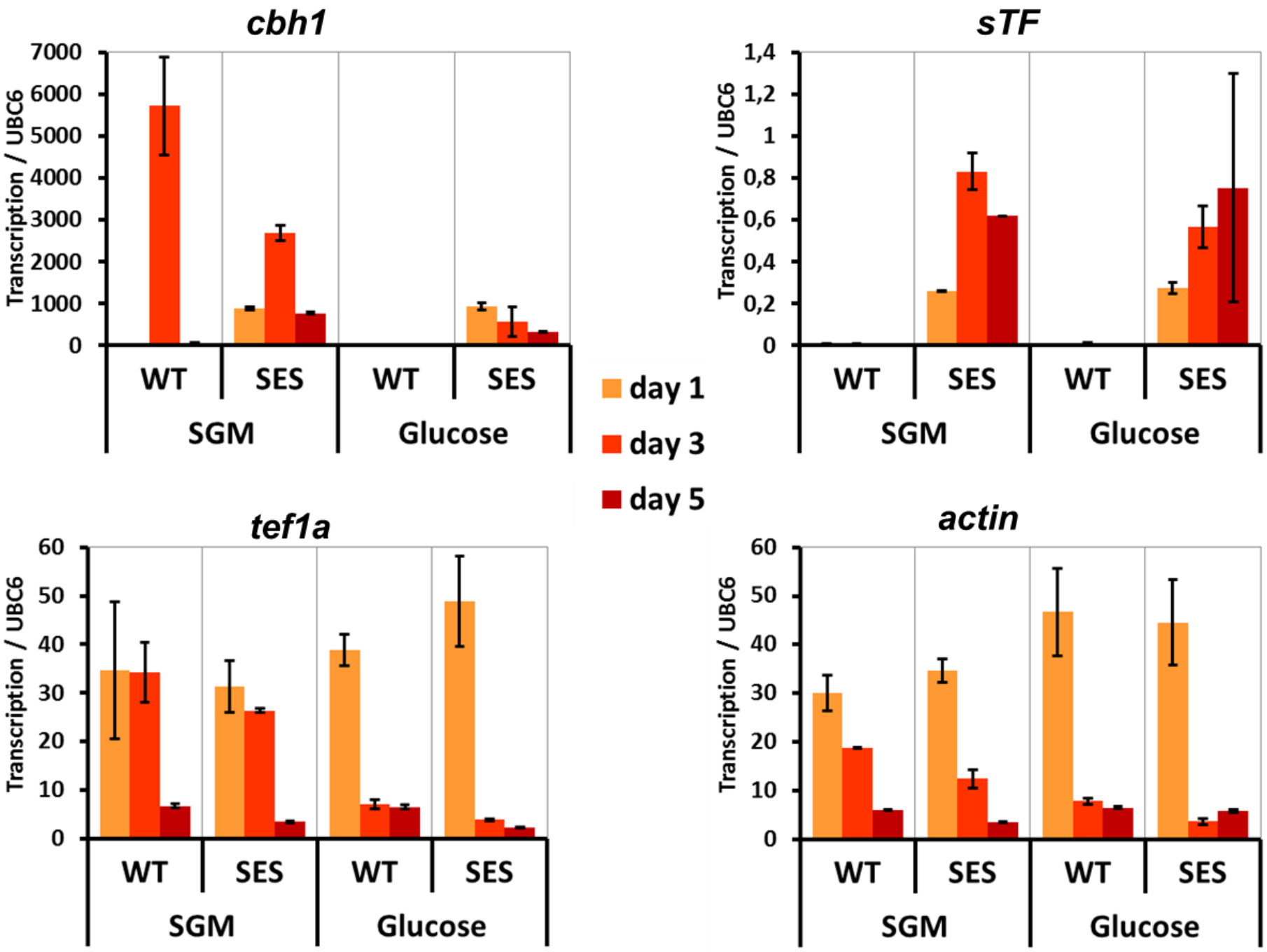
Supplementary Figure 5
Comparison of the SES and established expression tools (native promoters) in selected fungi

Supplementary Figure 5. Comparison of the SES-A system with commonly used gene expression tools

A) Schematic presentation of the DNA-cassettes, including positions of selection markers and genome-integration flanks, used for testing the established fungal promoters in the listed species. The cassettes tested in *S. cerevisiae* contained: 1) *S. cerevisiae PGK1* promoter; 2) *Y. lipolytica TEF1* promoter; 3) *P. pastoris GAP1* promoter; and 4) *A. nidulans gpdA* promoter. The cassette for *Y. lipolytica* contained its native *TEF1* promoter, the cassette for *P. pastoris* (mCherry gene cloned into the pGAPZ_A vector) contained its native *GAP1* promoter, and the *A. niger* cassette contained the *A. nidulans gpdA* promoter which is frequently used in this organism for heterologous gene expression.

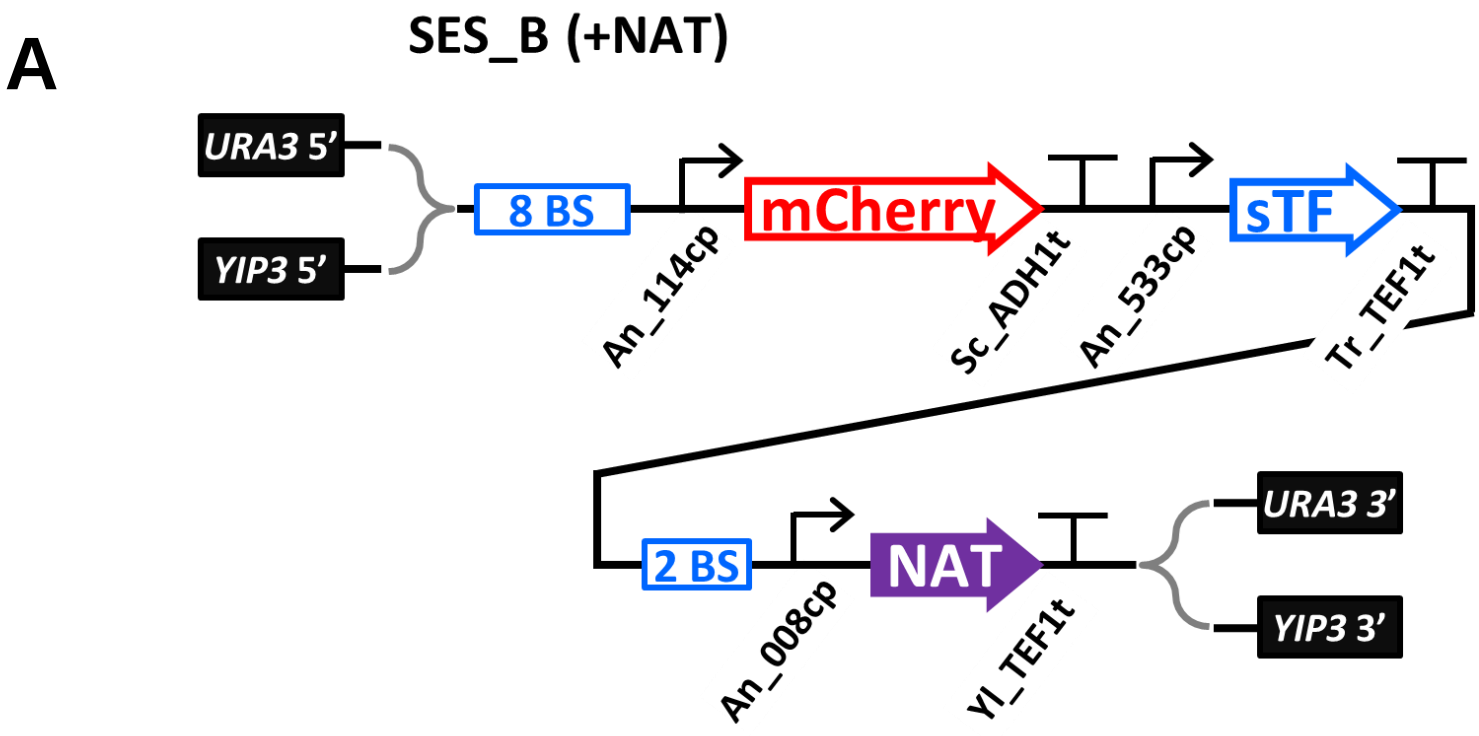
B) Fluorometry analysis of the yeast strains carrying single-copy genome integrated SES-A/SES-A* cassettes, or the cassettes with the established fungal promoters. PS denotes the parental strains. Values and error bars represent the mean and standard deviation from three independent cultivations.

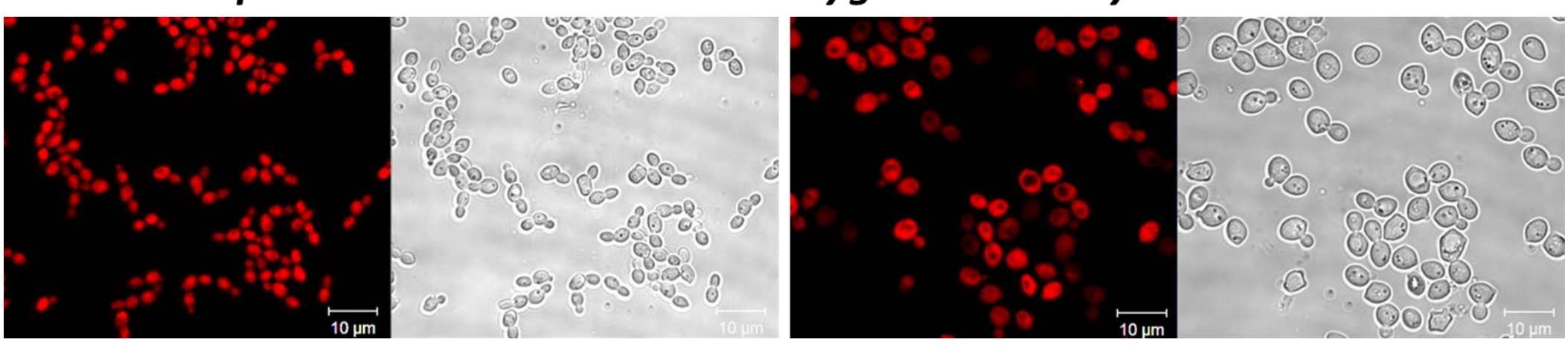
C) Transcription analysis of the *A. niger* strains carrying single-copy genome integrated SES-A cassette, or the cassette with the *A. nidulans gpdA* promoter. The mCherry, the *tefA* gene, and the sTF (on the secondary y-axis) transcript levels were analyzed and normalized to signal of the *UBC6* homolog gene transcription. Values and error bars represent the mean and standard deviation from two biological (four technical) replicates.

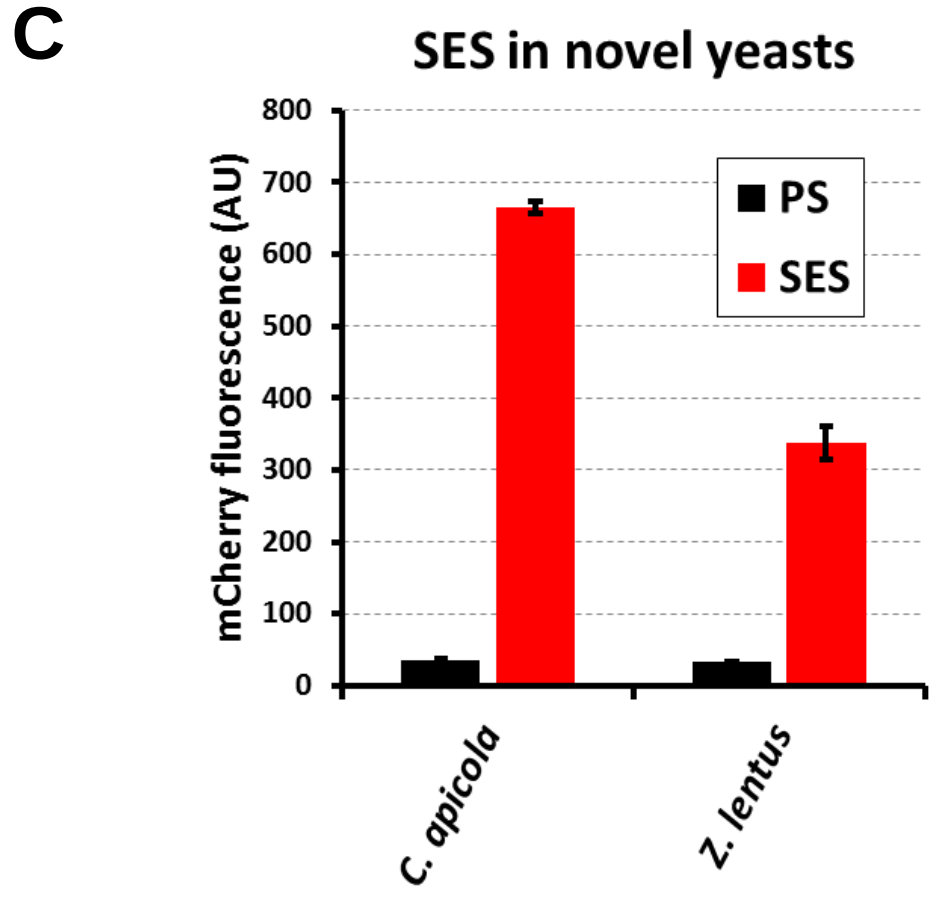


Supplementary Figure 6
Transcription of *cbh1*, sTF, *tef1a* and actin-encoding genes in strains cultivated in bioreactors.

Time course transcription profiles are shown for parental (PS) and the SES-C-containing strains. Values and error bars represent the mean and standard deviation from two biological (four technical) replicates.







Supplementary Figure 7
Universal SES system for novel hosts.

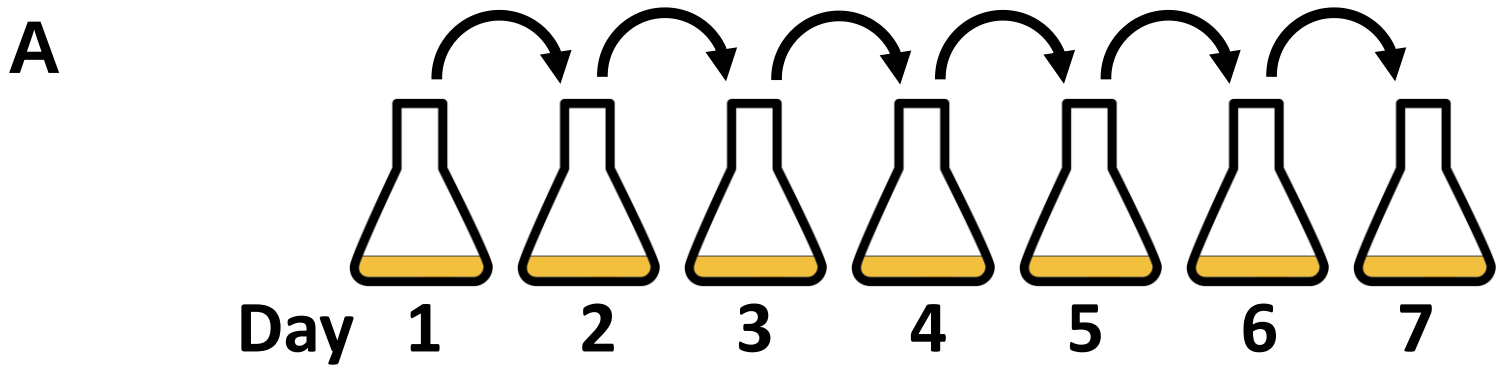
Supplementary Figure 7. Universal SES system for novel hosts.

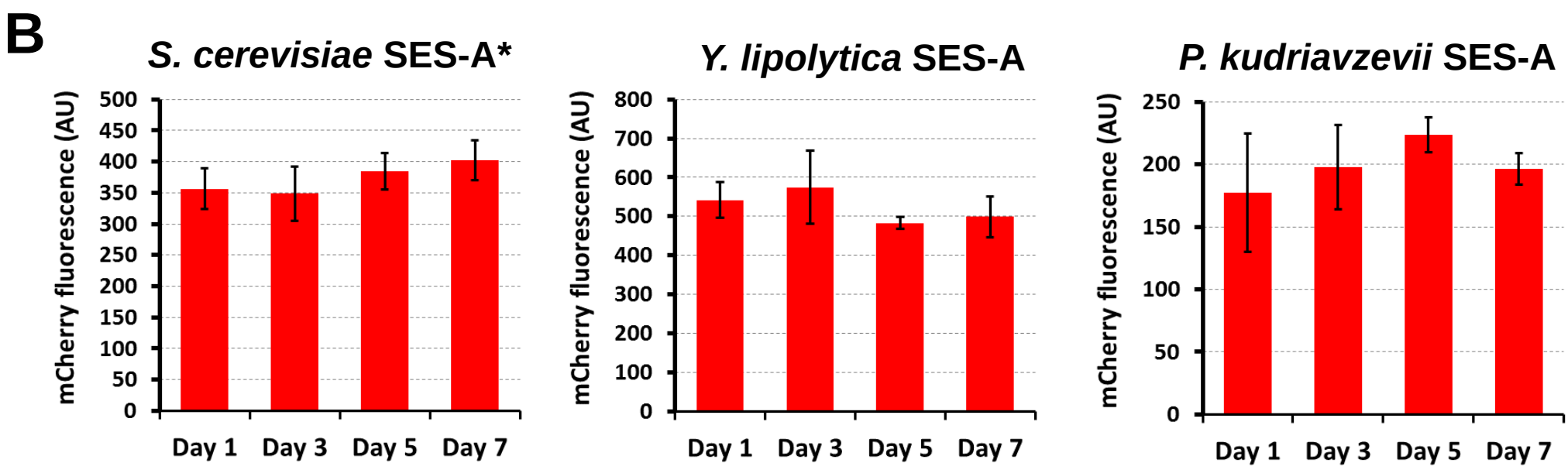
A) Schematic presentation of the SES cassettes (SES-B+NAT) used for *C. apicola* and *Z. lentus*. The selection marker and mCherry expression was controlled by the sTF (Bm3R1-NLS-VP16). The genome integration flanks are also indicated.

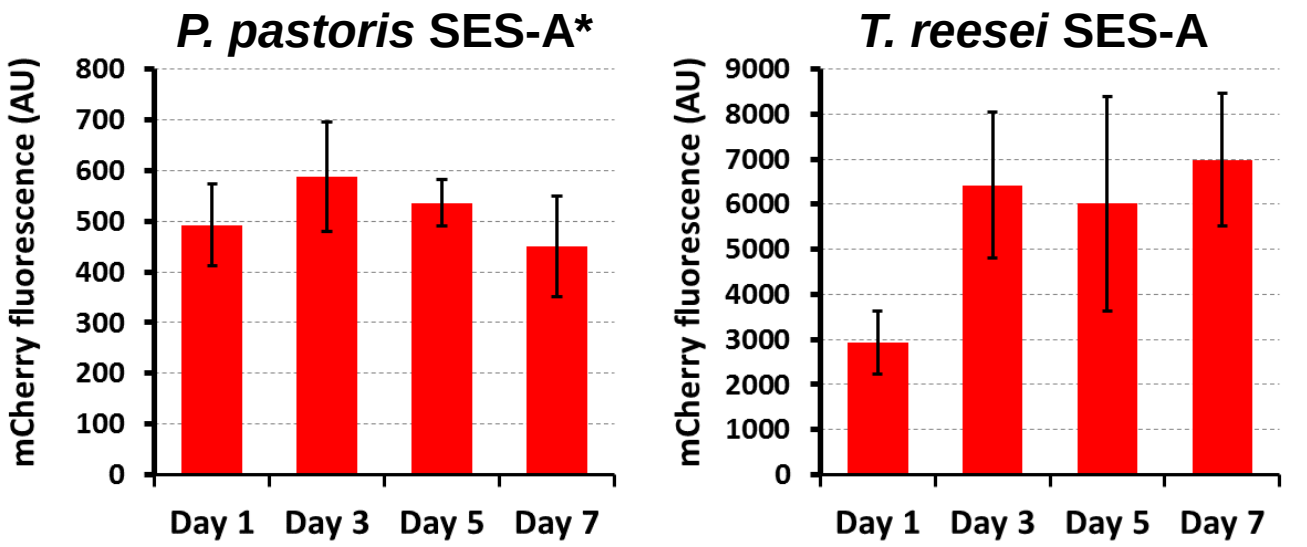
B) Fluorescent microscopy of the two species harboring the SES-B (+NAT). Uniform mCherry fluorescence signal was observed and the corresponding bright field image is shown for both species. The micrograph acquisition settings and the contrast were adjusted to maximize the visualization of the red fluorescence.

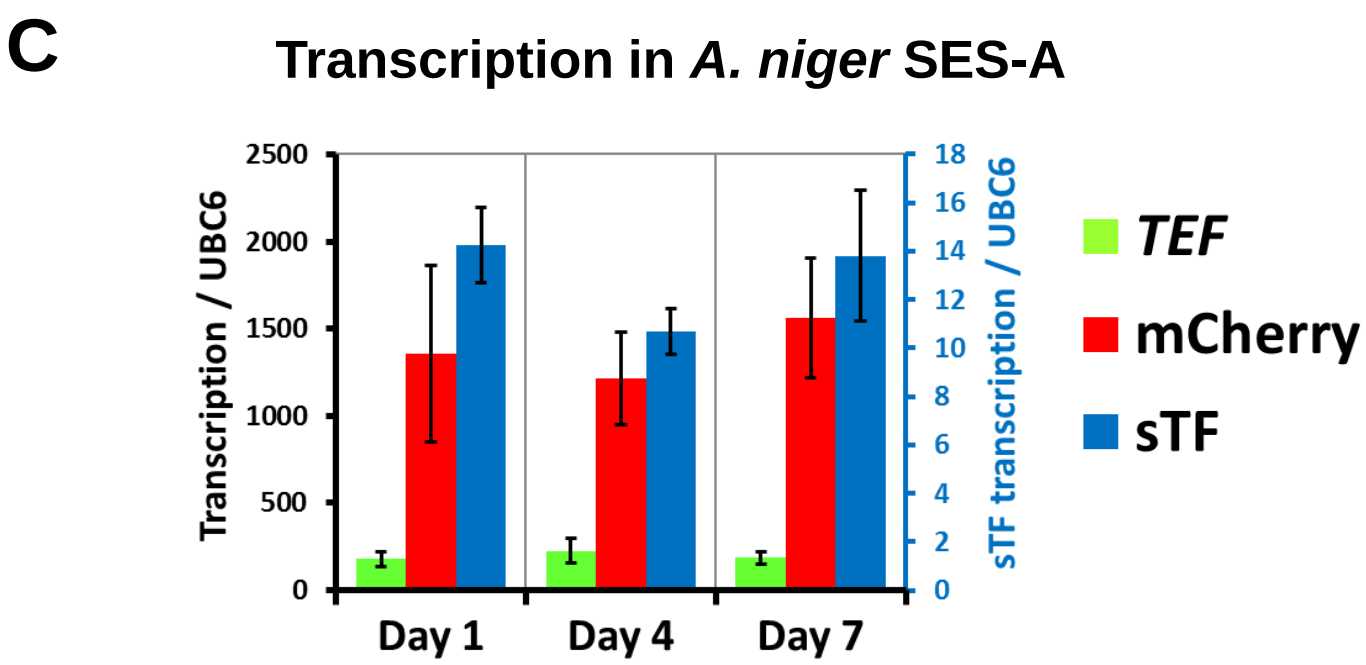
C) Quantitative fluorometry analysis of the mCherry expression in the yeast strains. The graphs show fluorescence intensity (mCherry) normalized by the number of cells. Values and error bars represent the mean and standard deviation from three independent cultivations. PS denotes parental strain (without SES).

Supplementary Figure 8









Supplementary Figure 8
Test of genetic stability of the SES system

Supplementary Figure 8. Genetic stability test of the SES-A system in the fungal strains.

A) Schematic presentation of the cultivations. The 24-hour cultures were diluted each day to a fresh medium (~1:160 for the yeast strains; ~1:20 for the filamentous fungi).

B) Quantitative fluorometry analysis of mCherry expression in the yeast and *T. reesei* strains. The *S. cerevisiae* and *P. pastoris* strains contained the SES-A* version with the *S. cerevisiae*-codon-optimized sTF. The *Y. lipolytica*, *P. kudriavzevii*, and *T. reesei* strains contained the basic SES-A system (with *A. niger* codon optimized sTF). The analysis was performed in days 1, 3, 5, and 7. The graphs show the fluorescence intensity (mCherry) normalized by the number of cells/mycelia in the suspension. Values and error bars represent the mean and standard deviation from at least three independent cultivations.

C) Transcription analysis of the *A. niger* strain with the SES-A system. The analysis was performed in days 1, 4, and 7. The mCherry, the *tefA* gene, and the sTF (on the secondary y-axis) transcript levels were analyzed and normalized to signal of the *UBC6* homolog gene transcription. Values and error bars represent the mean and standard deviation from two biological (four technical) replicates.

Supplementary tables

Supplementary Table 1

Parental strains used in this study.

Organism	Strain	VTT code	Source
Saccharomyces cerevisiae	CEN.PK111-32D	H3891	*
Saccharomyces cerevisiae	CEN.PK102-5B	H3900	*
Saccharomyces cerevisiae	CEN.PK102-3A	H3899	*
Pichia kudriavzevii	ATCC 32196	VTT C-79090T	VTT culture collection
Yarrowia lipolytica		VTT C-00365	VTT culture collection
Pichia pastoris	X-33		Thermo Fisher Scientific
Aspergillus niger	ATCC1015		ATCC
Trichoderma reesei	VTT strain based on M124	M2068	VTT culture collection
Trichoderma reesei	*cbh1*::mCherry (native CDS of the *CBH1* replaced by the mCherry CDS)	M717	VTT culture collection
Candida apicola		VTT-C-87174	VTT culture collection
Zygosaccharomyces lentus		VTT-C-09840	VTT culture collection

*Strains kindly provided by Dr. P. Kötter (Institute of Microbiology, J.W. Goethe University, Germany)

Supplementary Table 2

Candidate core promoters tested in *S. cerevisiae* CP-screen (**Fig. 1C**). The shaded sequences (9bp) are regions added to the native core promoter sequences (replacing its original 9bp 3'-end sequence) for screening and cloning purposes. The ATG, start codon of the mCherry coding sequence, is underlined. Sc - *Saccharomyces cerevisiae* origin; An – *Aspergillus niger* origin; Tr – *Trichoderma reesei* origin.

DNA sequences of the selected UCPs and other CPs used for constructing the expression systems	
Sc-THI4cp	ATCATGAAATTGATTTTTTGATTTTCAATTTATGAACTACCCAGATATATAAATATTGGAATAAATTGTGTATTAAGTAGTCGGGAAATATCT TTTATGTTCTCTTTCTTATCATCTAGAAATAATAAATCACAACCAAAAAAATCAACTAACTTAATTAAAATG
Sc-TEF1cp	CTCTTTCGATGACCTCCCATTGATATTTAAGTTAATAAACGGTCTTCAATTTCTCAAGTTTCAGTTTCATTTTTCTTGTTCTATTACAACTTTTT TTACTTCTTGCTCATTAGAAAGAAAGCATAGCAATCTAATCTAAGTTTTTAATTAAAATG
Sc-PGK1cp	AAGGGGGTGGTTTAGTTTAGTAGAACCTCGTGAAACTTACATTTACATATATATAAACTTGCATAAATTGGTCAATGCAAGAAATACATATT TGGTCTTTTCTAATTCGTAGTTTTTCAAGTTCTTAGATGCTTTCTTTTTCTCTTTTTTACAGATCATCAAGGAAGTAATTATCTACTTTTTACAA CAAATTAATTAAAATG
An-201205cp (An_201cp)	TTCTCTTTTCTTAAGAATATGTTCAAAGACTAGGATGGATAAATGGGGTATATAAAGCACCCTGACTCCCTTCCTCCAAGTTCTATCTAACCA GCCATCCTACACTCTACATATCCACACCAATCTACTACAATTATTAATTAAAATG
An-53301cp (An_533cp)	CGCCCCAAGAGAGCTGAAGATGCTGAGTAGGGTTGTCCAGGCAGCACATATATAAGATGCTTCGTCCCCTCCCATCGAGTCCTTCTTTTCTC TCTCTCATCAATCACTCTACTTCCTACTCTACCTTAAACTCTTCACTACTTCATACATTAATTAAAATG
An-205017cp (An_205cp)	TATAGTACTATTGATTTAGTATTGTTGTTGGATGTGCTGGTAGGTGTGTAGTATATATAGGAGATAGTAGAGGCAGATGATGATGATGGT ACTATTTTGAATCACCTCAAACGATACTATTCGCATCTTTGATAAAGATATCAAGAAACCAGAACAATCATTACTACTCTCCATAAGGATATA TATATACTTTACATCTTAATTAAAATG
An-00850cp (An_008cp)	AACCCAAAGTAATAAGTCTGTAGTAATTGGTCTCGCCCTGAATTCCAAACTATAAATCAACCACTTTCCCTCCTCCCCCCCGCCCCCACTTGG TCGATTCTTCGTTTTCTCTCTACCTTCTTTCTATTCGGTTTTCTTCTTCTTTTATTTTCCCTCTCCCATCAATCAAATTCATATTTGAAAAAAATTA ACATTAATTAAAATG
An-1114556cp (An_111cp)	GGGGCGGAAACTTGAAACTGGACGCCTTGTGAACGGCGTATGTGGTATATAAGGAACCAAGTCCCGCTGTAGTCTTCGGTTCATCAGACC CAGCACAGCACAGCAACACAACATTACAGCATAGCAAGCACTTCTCTATATTTCTACACATCACAGCACATTTCTATACAGTTTACGTCTAAT TATCTCCTGTTAATTAAAATG
An-1147651cp (An_114cp)	GCCCTGCAGTGCCTGATCACCTTATCAAGTGGCCAAATATCCCACTATAAAAGGCTTGGGAACCCCTCGTTCTGTCTTACCTTCTATCATCTT ACCAAATCCACTCCTCTTCCTTCATACATCAATCTTACCAATCAACTACCTCTACAACTCCAATACACTTAATTAAAATG
An-1178623cp	GGCTACTCGGGTTTTAAGCCGTCTTAAAAGCCGACACGAATTAGTTATAAAAGACTCTGTACTTGAGCAGGATATTCCTTCATTCTTTTCATT TAGATTGATATCGAATTCATTCTACAAGGATCGGATACTCTTCCATCCTTTATTTTGTCTCTGTGAATCAAACTTAATTAAAATG
An-11310cp	AATTCTGCTTCTTTTGCGGACTCAGGATCAACTGAGTATTTGCGAGCTAGTATAAGTAGCGCGCCTCCCTCGTCACCGCGTCTCCCTCATTCT TATCCTCATCTTCACTCTATCCTCTGTCGACGCCAACGCAACCACCGTATCCTATACCCTTATCACAACTTTCTTCACCTTTCACACCTAATCTC AAAATTAATTAAAATG

An-57241cp	AGGTAATGAATATTGGTTGCTGGCGGGCTGATCTTCTCCCGACACGTCTATATAAACTGGTCACCTTCTGGCCCTTCCTTTCTATCTCTTCCT TCTCATCATCAGTCTCAAACAAGCCTCTTTCTCTCCTACCTTCACTCTCCACTTTCTCCTTTCGAAAGGGATAAAACTCTCCTCCTCATTCTCAC CTATATATACCTTGTGCTTTAATTAAAATG
An-06590cp	GATTTCTAGAAATTTCTGCCCTTTACTTGCCTTCCCTCTTTGTCAACAAATATAAAGAGACTCCAATTCCCCTTCTCTGATTTCCAACATTTTTC ATTCTCCACTTCAGAACCATCTGAAGGAGCTTGGCTGTCTCGCTTCTTCTTCTTTCCTTCTTTACTAACATCCCTACCCCTCCTTAGAAAACCA AGTCTCTCCTCCTTTAATTAAAATG
An-53540cp	GCGCTGGTCAGCTGGGTCTTCAGCTTACTTTTTCTTCTGATCACGTAGGGTATATCGGTGGAATCTGGGTGGCGAGGAAGCCTGAATCCTC CAACATAAATAGGGCCACTCCCTCTCCCTCTTTTTCTGTAAACAAACACACACTAGACACCGACGACACTTTTCTCTCCAAGTCAAATCCTCT GACAGATCTTTCTCGCTAAAGAGGCAATAAAAGAAGAACTCATCGCTTTAATTAAAATG
An-1141688cp	ACTTGGATGATGGAGGAGTTGATCGAGGTCAATGAGGAGAGGCTTGCAAGTATAAGAAGAGACTGCTCGACCAGCAGAATGGATCTTCT TGTTCATCAACCAAGAGTCCAAGGCTTCTTTGTCTGGTTCTATCTCTTCTCCGAACTCTCTTGCTTGACATTCTCTTAATTAAAATG
An-07850cp	CCGTCCCCCAGGTTTTGGGTAGAATTGGAATATCATTAGATCTGTCGTCTTATATTGTTTATTTTAGAGAGATAAAGTACGACCAATCCCTT GCACACATCCTCGACAGGATGTGAATTGTTTCCCAAAAAATGGCACCATTGGTTAGCCAGCCACACACAGTGACTAACGCGCGTGATGAT GAATAGATCCAGAAGTTAATTAAAATG
An-11300cp	GGTGATCTGAAAGGCTCCCGGGTAGTCCAACGTCACTCTGATCGCGTTGTTATATATGTCCTCCGCTCCCTCGCGTTGGCCGTCAACCCTTT AGCACCATATCACGCTTATCGCCATCGTCACTTGTTAACTACAACTCCCTAATAAATTAAACACCTTCAAAAGGAAAAAAAAAAAAACCTCAC ATACCATTAATTAAAATG
An-138407cp	GGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAAC CTTGGGTCTGGCTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGTTAATTAAAATG
An-14130cp	GTGGCTCCGGTGTCGGCGCGGGGAGGCTCGCCAACTGCCCCGCCAGTCCGTATAAGTTGTAGTTTATACCTCGCTCTCCTCCGCTCAATCG TCCACCTTCTCCGTGCCAGACTATCTTCCCTGCTTTTAATTAAAATG
An-1158310cp	CGGTACGGCAGGGCACGCGGGGTAAACATGAGGGGTGGTCTACTATATAAGTCCATCGAGTGATGCACAACGCCAACTACAGCTTCGCA ACACACATATCCCATTAATTGAAAGTTATAGACACATATACTCTACAGATCAGTCAAGTACATCTACCACACCCCCTGAACCCAACCCCAAA ACTAACAGCATTAATTAAAATG
Tr-112258cp (Tr_112cp)	CACATCCTTGGAGATCAGTTGCAGTCTATTCATTCAGGCTCAACATATAAAGATGGGATACTTCCAACAGATGATAGTTGTCAAACAACCTC TTTGATCCTACACAATTTGGCCCAAGACACACAAGACGCTCACATCTCCTACCTAACCAAACAAAGAAAAAAACATCCACCAACTTAATTAA AATG
Tr-123236cp (Tr_123cp)	ATCTTACAAAGTTGCTTGGCAGTAAACCGTGCAATGGACACCAGGTATAAAGTCAGTGATATCCTCCCCGAATTCAAAGTTTCATCACCAA GCTCCTCAATCAACTCTACTTGAACAATACTACAAACAACCAAACCTCATTCAACAACTTAATTAAAATG
Tr-123989cp	TAAACGGAATGAGCTAGTAGGCAAAGTCAGCGAATGTGTATATATAAAGGTTCGAGGTCCGTGCCTCCCTCATGCTCTCCCCATCTACTCA TCAACTCAGATCCTCCAGGAGACTTGTACACCATCTTTTGAGGCACAGAAACCCAATAGTCAACCGCGGACTTAATTAAAATG
Tr-119989cp	AACAGCCTGCGAGAGCTGGAAGATGAAGAGGGCCAGAAAAAAAAAGTATAAAGAAGACCTCGATTCCCGCCATCCAACAATCTTTTCCATC CTCATCAGCACACTCATCTACAACCATCACCACATTCACTCAACTCCTCTTTCTCAACTCTCCAAACACAAACATTCTTTGTTGAATACCAACC ATCACCACTTAATTAAAATG
Tr-123232cp	GGTCTGGATGAAACGTCTTGGCCAAATCGTGATCGATTGATACTCGCATCTATAAGATGGCACAGATCGACTCTTGATTCACAGACATCCG TCAGCCCTCAAGCCGTTTGCAAGTCCACAAACACAAGCACAAGCATATTAATTAAAATG
Tr-73638cp	CCGGCACAAATCAGGAGCAACAGGCACTGCAAAATGACCTGGCAGTATATATAGACCTGACCGTATGAGTCTATTGTAGACATTCTAGCTA AGAGATCCGAGCCTAGTTCATAATACAGTAGTTGAGTTCATAGCAACTTCACTCTCTAGCTGAACAAATTATCTTTAATTAAAATG

Tr-123818cp	GAGACGAGGCAAGCTTGATGAGGCCAAATTATCCGTCAACTGTCTTATAAAGGAGCCCATGCCAAACCCCCCCTAAAGACTCAAGAAGCCAAACCTGAACAACCCCAGCACCTGAACAGTCATACAACCCCTCCAAGCCCAAAAGACACAACAACTCCTACTAGCTGAAGCAAGAAGTTAATTAAATG
Tr-123979cp	CAGCAGTGAAGAAGAGGGGAAGAAGATAAACCTGTAGGTTGGACAGAGTGTATAAAAGGGAGGGCTGTGCCCAACGAGGAGCGAGATTAACTTTGGATTTGGAGCAGAACAATATTGGAATCACAAGAAGAAGGATCTCTGTCTTTAATTAAAATG
Tr-69465cp	GAAAAATGGTGAGGAGATCTGCCTTCGAGTGCGTGTAGAAAAATGTATATAAGGATGTGTTTCACTCAACTTGTCTTAAGAATCGGTTCTCTAGCCGCGCTTTCAATTACTTCGAGACTTTCGCTTAAAATCGCCCTGCCATTTAATTAAAATG
Tr-49976cp	TGCCCCTGGCGTTGCAAGCCGCGTACAACTGCCCTTTTACCTAGGTATAAAAGACCTGTAGTAACCAACTACTATTGCAATTCTTCTTCACGTGGGCATCTATTCGTATCTTACACAAGGGCGCTGCAACTAATTGACTTGATCTTCCATCTCGTGTCTTGCTTGTAACCATTAATTAAAATG
Tr-123946cp	CTGTTAGGCTGTGAGTTATAAAGGTTGATGGATTGGGTCGAGGTTGTCAATGTCAGAGCATCTTACCTCTCACGCTTCAATCTTACCTACACGCTTCCTCTCAATCCTTGAACACCAATTGTTGCTCTAGCGCCTATCCTTCACTCATCACTCGCCTCGTACACTAAACTCTTCATCCCGAACAGACACGGCTTAATTAAAATG
Tr-79202cp	GGACGGCGGTCGTCCTCATCACAGCGACGTGAGCACACCTGCAACTATACTATGGCAGTCACCGAGACTCAGACAACGGTCGAAGACGCAATCGAGATTGCAGATCTGACGCAAACTGGCCGTCAAGATGCTGCTGGTCCAGAGCCAGAAGCCGAGAGCCGTAGCCTCGTTAATTAAAATG
Tr-121350cp	GGTGAGGCCTCTAACCCCTCTTGAGGAGCGTGGCAGCTTGAAGCTTATAAATAGCCCTTTGTTCTCCCTCAGAAACCTTCCTCTTCTTCTCCCCTTCAAGCAAACACTCCTCACACAAACCACACACAACACTCAACTTCTCTTCATTAATTAAAATG
Tr-123009cp	AGTTGTGGTTTTGGTCTCGATTTGGGGGTATATAAGGCGTGAGGATTCCCGGTTGATGGAATTTGGATTTTTCTGTCTTCTCTTCAGCGAGAAAAATCGAGGGTTGCTGAGATACTGTTTCCCGCTTGCTCTATAACTTCTTCTTTTTTTTTTGCTCTTTTGGCCTTTAACGTTCTTGAAGGCGTTGGTTTTAATTAAAATG
Tr-120311cp	ATTCCAGTTTGCGGATAGCGTGGCTCAGGAGAGCGAACACGAAATTATAAAAGAGGCCATGGCGAGCTCCCTGGGGAGATTCTGCTCTGTATCACAACCCACCAACATTTCCAAAGTTTACAACCTCCTTGAACACCCTTTCCCTTGTCAATCGACTTAATTAAAATG
Tr-107784cp	AGCTGGGCTTGAAATCGATCATACTGCAGCCATAAGAGCAAGGGATATAACTAGAAGATGTCTCGAACTCGATAGAGAGAGGAAAACTTTTCGACGACAAACAACAACAATCTGAGCACGAGACTACAGACAACGCGCATTCGACGGCCTCCATACATCAGAAACTACTTTCCGCCTTGGATTTAATTAAAATG
Tr-80980cp	GTAATACCTGAAAGCAAGGAAAAGAGAATTCGCACCGGAGATGGATATAAAAAGCTTGCACTGGTCGCGACAATGAAGGGTCAATCTCAGAGCTCATCAGATCACCACCTCGACAACCCTCTCAACATTTTGCCTAGTTGCTTATTCCTAGGCGTACCTTCTCCAATCAATATCAACATCATTAATTAAAATG

Supplementary Table 3

DNA sequences of mCherry, *CBH1* and sTFs. The part of the sTF encoding the DNA-binding protein (Bm3R1) is codon-optimized for *A. niger* (A.n.) or *S. cerevisiae* (S.c.). The *cbh1* sequence is identical to the *T. reesei* genome sequence of the gene (contains introns).

Gene	Sequence
mCherry	ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAAC GGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGG TGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCC CGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACC CAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCA GAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGC TGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCT ACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCA CTCCACCGGCGGCATGGACGAGTTATACAAGTAA
Bm3R1 (A.n.)-NLS- VP16	ATGGAGTCCACACCCACGAAACAAAAAGCTATTTTTTCTGCCTCGCTCCTTCTGTTCGCCGAACGCGGGTTTGACGCCACTACGATG CCGATGATCGCTGAAAATGCTAAGGTCGGCGCAGGAACGATTTACCGATACTTTAAGAATAAGGAGAGTCTGGTCAACGAGCTGT TCCAGCAGCACGTTAATGAATTTTTGCAATGTATCGAGAGTGGCTTGGCGAACGAAAGGGACGGTTATCGCGATGGGTTCCATCAT ATCTTCGAGGGAATGGTCACATTCACAAAGAACCATCCGCGCGCCTTGGGATTTATCAAGACACATTCCCAAGGTACATTCCTAACC GAAGAGTCACGCCTTGCATACCAAAAACTTGTTGAGTTCGTCTGCACCTTCTTTCGAGAGGGACAGAAACAGGGCGTAATTCGAAA CTTGCCCGAGAATGCCCTGATCGCCATCCTATTCGGATCGTTTATGGAGGTCTATGAGATGATCGAAAACGATTATCTCTCTCTAAC GGATGAGTTGCTTACGGGGGTAGAGGAATCGCTCTGGGCTGCTCTCTCCCGACAATCGGCTAGCCCTCCCAAGAAGAAGCGCAAG GTCAGCACGGCCCCCCCCACGGACGTCTCCCTCGGCGACGAGCTCCACCTGGACGGCGAGGACGTCGCCATGGCCCACGCCGACG CCCTCGACGACTTCGACCTCGACATGCTGGGCGACGGCGACAGCCCCGGCCCCGGCTTTACCCCCCACGACTCCGCCCCCTACGGC GCCCTGGACATGGCCGACTTCGAGTTTGAGCAGATGTTCACCGACGCCCTGGGCATTGACGAGTACGGCGGCTGA
Bm3R1 (S.c.)-NLS- VP16	ATGGAATCTACTCCTACTAAGCAAAAAGCCATCTTCTCTGCCTCCTTGTTGTTGTTCGCTGAGAGAGGTTTCGACGCTACCACTATGC CAATGATTGCCGAAAACGCTAAGGTTGGTGCTGGTACTATCTACAGATACTTCAAGAACAAAGAATCCTTAGTTAATGAGTTGTTTC AACAACACGTCAATGAATTTTTGCAATGTATCGAATCTGGTTTGGCTAACGAAAGAGATGGTTACAGAGACGGTTTCCACCACATC TTCGAAGGTATGGTCACCTTCACCAAGAACCATCCTAGAGCCTTAGGTTTCATCAAGACCCACTCTCAAGGTACTTTTTTGACCGAA GAATCCAGATTGGCTTATCAAAAGTTGGTCGAATTTGTCTGTACTTTCTTCAGAGAAGGTCAAAAGCAAGGTGTCATCAGAAATTT GCCAGAAAACGCTTTGATTGCTATCTTGTTCGGTTCTTTCATGGAAGTCTACGAAATGATTGAAAACGATTATTTGTCTTTGACTGAT GAATTATTAACCGGTGTTGAGGAATCCTTGTGGGCTGCCTTGTCTCGTCAATCTGAATTCCCTCCCAAGAAGAAGCGCAAGGTCAG CACGGCCCCCCCCACGGACGTCTCCCTCGGCGACGAGCTCCACCTGGACGGCGAGGACGTCGCCATGGCCCACGCCGACGCCCTC GACGACTTCGACCTCGACATGCTGGGCGACGGCGACAGCCCCGGCCCCGGCTTTACCCCCCACGACTCCGCCCCCTACGGCGCCCT GGACATGGCCGACTTCGAGTTTGAGCAGATGTTCACCGACGCCCTGGGCATTGACGAGTACGGCGGCTGA
LexA-NLS- VP16	ATGAAAGCGTTAACGGCCAGGCAACAAGAGGTGTTTGATCTCATCCGTGATCACATCAGCCAGACAGGTATGCCGCCGACGCGTG CGGAAATCGCGCAGCGTTTGGGGTTCCGTTCCCCAAACGCGGCTGAAGAACATCTGAAGGCGCTGGCACGCAAAGGCGTTATTGA AATTGTTTCCGGCGCATCACGCGGGATTCGTCTGTTGCAGGAAGAGGAAGAAGGGTTGCCGCTGGTAGGTCGTGTGGCTGCCGGT GAACCACTTCTGGCGCAACAGCATATTGAAGGTCATTATCAGGTCGATCCTTCCTTATTCAAGCCGAATGCTGATTTCCTGCTGCGC GTCAGCGGGATGTCGATGAAAGATATCGGCATTATGGATGGTGACTTGCTGGCAGTGCATAAAACTCAGGATGTACGTAACGGTC AGGTCGTTGTCGCACGTATTGATGACGAGGTTACCGTTAAGCGCCTGAAAAAACAGGGCAATAAAGTCGAACTGTTGCCAGAAAA TAGCGAGTTTAAACCAATTGTCGTAGATCTTCGTCAGCAGAGCTTCACCATTGAAGGGCTGGCGGTTGGGGTTATTCGCAACGGCG ACTGGCTGGAATTCCCTCCCAAGAAGAAGCGCAAGGTCAGCACGGCCCCCCCCACGGACGTCTCCCTCGGCGACGAGCTCCACCT GGACGGCGAGGACGTCGCCATGGCCCACGCCGACGCCCTCGACGACTTCGACCTCGACATGCTGGGCGACGGCGACAGCCCCGG CCCCGGCTTTACCCCCCACGACTCCGCCCCCTACGGCGCCCTGGACATGGCCGACTTCGAGTTTGAGCAGATGTTCACCGACGCCCT GGGCATTGACGAGTACGGCGGCTGA
cbh1	ATGTATCGGAAGTTGGCCGTCATCTCGGCCTTCTTGGCCACAGCTCGTGCTCAGTCGGCCTGCACTCTCCAATCGGAGACTCACCCG CCTCTGACATGGCAGAAATGCTCGTCTGGTGGCACGTGCACTCAACAGACAGGCTCCGTGGTCATCGACGCCAACTGGCGCTGGA CTCACGCTACGAACAGCAGCACGAACTGCTACGATGGCAACACTTGGAGCTCGACCCTATGTCCTGACAACGAGACCTGCGCGAA GAACTGCTGTCTGGACGGTGCCGCCTACGCGTCCACGTACGGAGTTACCACGAGCGGTAACAGCCTCTCCATTGGCTTTGTCACCC AGTCTGCGCAGAAGAACGTTGGCGCTCGCCTTTACCTTATGGCGAGCGACACGACCTACCAGGAATTCACCCTGCTTGGCAACGAG TTCTCTTTCGATGTTGATGTTTCGCAGCTGCCGTAAGTGACTTACCATGAACCCCTGACGCTATCTTCTTGTTGGCTCCCAGCTGACT GGCCAATTCAAGGTGCGGCTTGAACGGAGCTCTCTACTTCGTGTCCATGGACGCGGATGGTGGCGTGAGCAAGTATCCCACCAAC ACCGCTGGCGCCAAGTACGGCACGGGGTACTGTGACAGCCAGTGTCCCCGCGATCTGAAGTTCATCAATGGCCAGGCCAACGTTG AGGGCTGGGAGCCGTCATCCAACAACGCGAACACGGGCATTGGAGGACACGGAAGCTGCTGCTCTGAGATGGATATCTGGGAGG CCAACTCCATCTCCGAGGCTCTTACCCCCCACCCTTGCACGACTGTCGGCCAGGAGATCTGCGAGGGTGATGGGTGCGGCGGAACT TACTCCGATAACAGATATGGCGGCACTTGCGATCCCGATGGCTGCGACTGGAACCCATACCGCCTGGGCAACACCAGCTTCTACGG CCCTGGCTCAAGCTTTACCCTCGATACCACCAAGAAATTGACCGTTGTCACCCAGTTCGAGACGTCGGGTGCCATCAACCGATACTA TGTCCAGAATGGCGTCACTTTCCAGCAGCCCAACGCCGAGCTTGGTAGTTACTCTGGCAACGAGCTCAACGATGATTACTGCACAG CTGAGGAGGCAGAATTCGGCGGATCCTCTTTCTCAGACAAGGGCGGCCTGACTCAGTTCAAGAAGGCTACCTCTGGCGGCATGGT TCTGGTCATGAGTCTGTGGGATGATGTGAGTTTGATGGACAAACATGCGCGTTGACAAAGAGTCAAGCAGCTGACTGAGATGTTA CAGTACTACGCCAACATGCTGTGGCTGGACTCCACCTACCCGACAAACGAGACCTCCTCCACACCCGGTGCCGTGCGCGGAAGCTG CTCCACCAGCTCCGGTGTCCCTGCTCAGGTCGAATCTCAGTCTCCCAACGCCAAGGTCACCTTCTCCAACATCAAGTTCGGACCCAT TGGCAGCACCGGCAACCCTAGCGGCGGCAACCCTCCCGGCGGAAACCCGCCTGGCACCACCACCACCCGCCGCCCAGCCACTACC ACTGGAAGCTCTCCCGGACCTACCCAGTCTCACTACGGCCAGTGCGGCGGTATTGGCTACAGCGGCCCCACGGTCTGCGCCAGCG GCACAACTTGCCAGGTCCTGAACCCTTACTACTCTCAGTGCCTGTAA

Supplementary Table 4

Sequences of sTF binding sites. The Bm3R1 binding sites are used in all SES constructs, the LexA binding sites are used in the centromeric plasmid in the *S. cerevisiae* CP screen.

Binding site	**Sequence**
Bm3R1 ver 1	CGGAATGAACATTCATTCCG
Bm3R1 ver 2	CGGAATGAAGGTTCATTCCG
Bm3R1 ver 3	CGGAATGAACTTTCATTCCG
LexA ver 1	CTGTATGTACATACAG
LexA ver 2	CTGTATATATATACAG
LexA ver 3	CTGTATGCGCATACAG
LexA ver 4	CTGTATATATATACAG

Supplementary Table 5

Selection markers, integration loci, and length of the integration flanks which were used in transformations of different host organisms.

Host organism (SES versions and/or control-promoter system versions)	**Used selection marker (selection condition)**	**Integration locus (ID/database)**	**Length and target location of 5' integration flank**	**Length and target location of 3' integration flank**
S. cerevisiae (SES-A, -A*, -A-NC, -B, -B*, -B-NC, Sc_PGK1p, Yl_TEF1p, Pp_GAP1p, An_gpdAp)	LEU2 (absence of Leucine)	LEU2 (protein ID in JGI*: 626)	52 bp (-82 to -30 bp upstream of start codon)	50 bp (+1 to +50 bp downstream of stop codon)
P. pastoris (SES-A*, -A-NC, -B*, -B-NC)	*ble* (100 µg/ml Zeocin)	AOX promoter (protein ID in JGI*: 40209)	463 bp (-527 to -64 bp upstream of start codon)	413 bp (-940 to -528 bp upstream of start codon)
P. pastoris (GAP1p)	*ble* (100 µg/ml Zeocin)	GAP promoter (protein ID in JGI*: 37283)	293 bp (-302 to -10 bp upstream of start codon)	186 bp (-488 to -303 bp upstream of start codon)
P. kudriavzevii (SES-A, -A-NC, -A(0-8BS), -A-NC(0-8BS), -B, -B-NC)	HPH (500 µg/ml Hygromycin B)	PDC1 (gene ID in EFD**: JL09_g917)	624 bp (-998 to -374 bp upstream of start codon)	720 bp (-528 to +192 bp downstream of stop codon)
Y. lipolytica (SES-A, -A-NC, -B, -B-NC; Yl_TEF1p)	NAT (400 µg/ml Nourseothricin)	ANT1 (protein ID in JGI*: 68263)	755 bp (-1000 to -246 bp upstream of start codon)	294 bp (+7 to +300 downstream of stop codon)
A. niger (SES-A, -A-NC, -A(0-8BS), -B, -B-NC, An_gpdAp)	pyrG (absence of uracil)	gaaC (protein ID in JGI*: 1158310)	1955 bp (-1974 to -20 upstream of start codon)	1692 bp (+165 to 1846 downstream of stop codon)
T. reesei (SES-A, -A-NC, -B, -B-NC)	HPH (150 µg/ml Hygromycin B)	PEP4 (protein ID in JGI*: 77579)	958 bp (-1029 to -69 bp upstream of start codon)	992 bp (+3 to 995 bp downstream of stop codon)
T. reesei (SES_C)	HPH (150 µg/ml Hygromycin B)	CBHI (protein ID in JGI*: 123989)	2186 bp (-2184 to +2 bp upstream of start codon)	1746 bp (+692 to 2438 bp downstream of stop codon)

C. apicola (SES-B+NAT)	NAT (200 µg/ml Nourseothricin)	*S. cerevisiae* URA3 homolog ***	799 bp (-872 to -73 bp upstream of start codon)	807bp (+4 to 811 bp downstream of stop codon)
Z. lentus (SES-B+NAT)	NAT (200 µg/ml Nourseothricin)	*S. cerevisiae* YIP3 homolog ***	549 bp (-642 to -93 bp upstream of start codon)	499 bp (+370 bp of ORF to +291 downstream of stop codon)

*JGI = Joint Genome Institute's database (http://genome.jgi.doe.gov/programs/fungi/index.jsf)
** EFD = Ensembl Fungi database (http://fungi.ensembl.org/index.html)
*** - no public genome database available

Supplementary Table 6

GaaC targeting sequences used for crRNA design for *A. niger* transformation, and URA3 targeting sequences used for crRNA design for *C. apicola* transformation.

Target	**Sequence**
gaaC_1	TGAAGGGTCGAAGAAAGTCA
gaaC_2	GCCAGCGAACAATCCATTCG
gaaC_3	GAATTGCACTCTTCGTACCG
Ca_URA3	GGATATCGGCAGTACTGTGA

Supplementary Table 7

Primers used for RT-PCR-based analysis of single copy SES system (or promoter-control system) genome-integration. The RT-PCR signals of mCherry and /or the sTF coding sequences were compared to the signals of reference genes in each transformed SES-strain. In cases of the control promoter strains, only the mCherry coding sequence was used.

Primer number	Target	ID in database	Primer name	Sequence
890	mCherry		mCherry_qPCR_F	GTGATGAACTTCGAGGACGG
891			mCherry_qPCR_R	TTCAGCCTCTGCTTGATCTC
1366	Bm3R1 (A.n.)		An_Bm3R1_qPRC_F	GTCACATTCACAAAGAACCATCC
1367			An_Bm3R1_qPRC_R	GACCTCCATAAACGATCCGA
1308	Bm3R1 (S.c.)		Bm3R1_qPCR_F	TCACCAAGAACCATCCTAGAG
1309			Bm3R1_qPCR_R	GAAAGAACCGAACAAGATAGCA
1439	*Y. lipolytica* actin (reference gene)	protein ID in JGI*: 70741	Yl_ACT_qPCR_F	ACTCAATGCCGACACAAAGAC
1440			Yl_ACT_qPCR_R	TCAGATCCTACATCCAGTACCAG
484	*S. cerevisiae IPP1* (reference gene)	protein ID in JGI*: 254	Sc_IPP1_qPCR_F	ACTTTGAACCCAATCATCCA
485			Sc_IPP1_qPCR_R	CACCAACTGCCTTAGTTTCTG
1441	*P. kudriavzevii* actin (reference gene)	gene ID in EFD**: JL09_g438	Pk_ACT_qPCR_F	TAACGAAAGATTCAGAGCACCAG
1442			Pk_ACT_qPCR_R	AGCCAATGCAGTGATTTCCT
1732	*P.pastoris IPP1* (reference gene)	protein ID in JGI*: 35302	Pp_IPP_qPCR_F	GTAACTGTTTCCCTCACCAC
1733			Pp_IPP_qPCR_R	CAACAAAGCCATAACACCGA
1784	*T.reesei* actin (reference gene)	protein ID in JGI*: 44504	Tr_act_qPCR_gDNA_F	GCCTTCTATGTCTCCATCCAG
1785			Tr_act_qPCR_gDNA_R	CTCAGCCAGGATCTTCATCAG
480	*A. niger sdhA* (reference gene)	protein ID in JGI*: 53356	An_sdhA_qPCR_gDNA_F	GGTTGTTGACATTAACTCCGA
481			An_sdhA_qPCR_gDNA_R	CCACAGTTCATACAAGGCTC
X-67	*C. apicola ubc7* homolog (ref. gene)	***	Ca_UBC7_qPCR_F	GGTGATGACCCAAACATGTATGAG
X-68			Ca_UBC7_qPCR_R	AGTGCCCATAATATCTGAGCGA
X-90	*Z. lentus act1* homolog (ref. gene)	***	Zl_ACT1_qPCR_F	GTTACTCGTTCTCGACCACC
X-91			Zl_ACT1_qPCR_R	GCTCTGAACCTCTCGTTACC

*JGI = Joint Genome Institute's database (http://genome.jgi.doe.gov/programs/fungi/index.jsf)

** EFD = Gene ID in Ensembl Fungi database (http://fungi.ensembl.org/index.html)

*** - no public genome database available

Supplementary Table 8

The detector's voltages which were used for quantification of forward scatter (FSC) and mCherry in flow cytometry analysis.

Organism	**FSC voltage (V)**	**mCherry voltage (V)**
S. cerevisiae/ Y. lipolytica/ P. kudriavzevii	43	527
P. pastoris	5	450
T. reesei	110	429
A. niger	43	429

Supplementary Table 9

Primers and gene targets used in transcription analysis.

Universal primers				
Primer number	Target (amplicon length)	ID in database	Primer name	Sequence
890	mCherry (211 bp)		mCherry_qPCR_F	GTGATGAACTTCGAGGACGG
891			mCherry_qPCR_R	TTCAGCCTCTGCTTGATCTC
1366	Bm3R1 (A.n.) (209 bp)		An_Bm3R1_qPRC_F	GTCACATTCACAAAGAACCATCC
1367			An_Bm3R1_qPRC_R	GACCTCCATAAACGATCCGA
1308	Bm3R1 (S.c.) (193 bp)		Bm3R1_qPCR_F	TCACCAAGAACCATCCTAGAG
1309			Bm3R1_qPCR_R	GAAAGAACCGAACAAGATAGCA
***Y. lipolytica* primers**				
Primer number	Target	ID in database	Primer name	Sequence
1483	*UBC6* (204 bp)	Protein ID in JGI*: 69371	Yl_UBC_qPCR_F	GCAGGAAGTATGACATCCAC
1484			Yl_UBC_qPCR_R	TGATGACTGTGCTCTTAGCC
1445	*TEF1* (204 bp)	Protein ID in JGI*: 66455	Yl_TEF_qPCR_F	CGACTCTTTCAACGCTCAGG
1446			Yl_TEF_qPCR_R	GACCATCTTGACAATGGCGG
***S. cerevisiae* primers**				
Primer number	Target	ID in database	Primer name	Sequence
1189	*UBC6* (201 bp)	Protein ID in JGI*: 2053	Sc_UBC6_qPCR_F	ACTTTCCCGTCTGATTATCCA
1190			Sc_UBC6_qPCR_R	TAATTGATCCTGTCGTGGCT
1419	*TEF1* (189 bp)	Protein ID in JGI*: 6442	Sc_TEF1_qPCR_F	AACATGATTGAAGCTACCACC
1420			Sc_TEF1_qPCR_R	GCACAGTACCAATACCACCA
***P. kudriavzevii* primers**				
Primer number	Target	ID in database	Primer name	Sequence
1489	*UBC6* (207 bp)	Transcript ID in EFD**: KGK40836	Pk_UBC_qPCR_F	CCTTCATGACTGGAGATGAGAG
1490			Pk_UBC_qPCR_R	GTGGATTCGGATTGCTTCTG
1443	*TEF1* (214 bp)	Transcript ID in EFD**: KGK37491	Pk_TEF_qPCR_F	CTTGGATTGTCACACTGCCC
1444			Pk_TEF_qPCR_R	TTTGTCTCATATCTCTGACTGCGA
***P. pastoris* primers**				
Primer number	Target	ID in database	Primer name	Sequence
1730	*UBC6* (208 bp)	Protein ID in JGI*: 36033	Pp_UBC_qPCR_F	ACCACCGGATCTATAAGCAC
1731			Pp_UBC_qPCR_R	ATAGCACCTTCAGCAGTATCAG
1728	*TEF1* (201 bp)	Protein ID in JGI*: 36802	Pp_TEF_qPCR_F	CGAGTTGATTGAGAAGATTGACAG
1729			Pp_TEF_qPCR_R	GGACTTGATAACACCGACAG

***T. reesei* primers**				
Primer number	Target	ID in database	Primer name	Sequence
1487	*UBC6* (207 bp)	Protein ID in JGI*: 77732	Tr_UBC_qPCR_F	CCAACATCCTCGAATGGCAC
1488			Tr_UBC_qPCR_R	GCTGGGTTGAAGGATTTCGG
1417	*tef1a* (193 bp)	Protein ID in JGI*: 46958	Tr_TEF_qPCR_F	AAGTCTACCACCACTGGTCAC
1418			Tr_TEF_qPCR_R	TTGGGAGTCTCGAACTTCCAG
1481	Actin (195 bp)	Protein ID in JGI*: 44504	Tr_act_qPCR_F	CAACATTGTCATGTCTGGTGG
1482			Tr_act_qPCR_R	CTGCTTGGAGATCCACATCTG
1479	*cbh1* (201 bp)	Protein ID in JGI*: 123989	Tr_CBH1_qPCR_F	AGAATGGCGTCACTTTCCAG
1480			Tr_CBH1_qPCR_R	TTGGCGTAGTAATCATCCCAC
***A. niger* primers**				
Primer number	Target	ID in database	Primer name	Sequence
1485	*UBC6* (204 bp)	Protein ID in JGI*: 1180989	An_UBC_qPCR_F	ATACTCGAATGGCACTACATCCT
1486			An_UBC_qPCR_R	ATGCGGGATTAAATGACTTCGG
1415	*tefA* (181 bp)	Protein ID in JGI*: 1147607	An_TEF_qPCR_F	CGTCATCATGGGTAAGGAGG
1416			An_TEF_qPCR_R	AAGCGTACTTGAAGGAACCC

*JGI = Joint Genome Institute's database (http://genome.jgi.doe.gov/programs/fungi/index.jsf)
** EFD = Gene ID in Ensembl Fungi database (http://fungi.ensembl.org/index.html)

Supplementary Table 10

Z. lentus ACT1 – partial CDS

CCCGAGGAGCACCCAGTTCTGTTGACCGAGGCGCCCATGAACCCCAAGTCGAACAGAGAGAAGATGACGCAGATCATGTTCGAGACTTTCAA
CGTGCCTGCGTTCTACGTCTCGATCCAGGCCGTCTTGTCGCTATACTCGTCCGGTAGAACGACCGGTATCGTGTTGGACTCCGGTGACGGTGT
GACCCACGTCGTGCCCATCTACGCCGGTTTCTCGATGCCTCACGCCATTTTGAGAATCGACTTGGCCGGTAGAGACTTGACCGACTACTTGATG
AAGATTTTGAGCGAAAGGGGTTACTCGTTCTCGACCACCGCCGAGAGGGAAATCGTGCGTGACATCAAGGAGAAGCTGTGCTACGTCGCGCT
CGACTTCGAGCAGGAGATGCAGACCGCGGCCCAGTCCTCGTCCGTGGAGAAGTCGTACGAGTTGCCCGACGGTCAGGTGATCACCATCGGTA
ACGAGAGGTTCAGAGCCCCTGAGGCGCTGTTCCACCCATCCGTTTTGTCGTTGGAGTCCTCCGGTGTTGACCAGACCACTTACAACTCCATCAT
GAAGTGCGACGTCGACGTCCGTAAGGACTTGTACGGTAACATCGTCATGTCCGGTGGTACCACGATGTTCCCCGGTATCGCCGAGAGAATGC
AGAAGGAGATCACCGCGTTGGCTCCATCCTCCATGAAGGTCAAAATCATCGCTCCACCAGAGAGAAAATACTCCGTCTGGATCGGTGGCTCCA
TCTTGGCCTCTTTGACCACTTTCCAACAGATGTGGATCTCGAAACAAGAGTACGA

Supplementary Table 11

Z. lentus YIP3 – genomic fragment (CDS highlighted)

TCTGGAGCGGCGCATCACGGGTGCGGTGCATGGGGAACCAGCACGTCACCTGAGCGCCCTGATCGGGTGGAACGATCTCTCGGAATCGATTG
CCGCCATGACCGACCCGGACAGGTTTTCGAAGCTGCTGCAGGACTTGGGTCAAGCCGGTGATCCAGATGAGGCGTTCTCGTCCGTGCCATAC
GAGAAGGGATTCAACCTTTTGTTCCACCTGGAACAGGTGGTAGGCGGCAAGCAGCAGTTCGACCCGTTTGTGAGACACTATTTTACCAAGTTT
GCCAGGCAGTCGCTGGACACGTTCCAGTTCCTGGACACTTTGTTCGAGTTTTACCGGGACAAGAGAGGGGCACTCGATGGCGTGGACTGGGA
GACGTGGCTGTACAGGCCGGGATTGCCTCCAAAGCCGCAGTTCGACACTTCGTTGGCAGACGACGTGTACGCGGTGGCAGAGCGGTGGTTCG
ATGCGGCGCACGAGGGGCAGTACGATGGGTTCTCATCCGAGGATCTTGCAAACGTGAGCACTACGCAACTGGTGTTGTTCTTGGACACTTTG
GTGCAGGCCAAGCGGCTGGACTGGACCAAGCATACGGATGCCGCTAGACGGCTGCTGGATGTCTATCACGAAAGGGTAGTCGCTCCGCAGA
ATGCAGAAATTGTGTTTAGGAAATTTAGGCTACAGGTTGAGGCAAGAATGCAGGAGGCTTACGGGCGTCTGGCACAGTGGTTGGGCACTGTG
GGGCGCATGAAGTTCGTGAGGCCAGGTTACCGGCTCCTAAACAGAGTGGACAGACCGCTGGCGTTGGAAACGTTTGCCAAATTCAAAGACAC
CTATCACCCCATCTGCAAGGCACTCGTGAAGCAGGATTTGAAATTGTAGCGGTCTTTGTGACCATGTGTACGTACAAAATGCGCTGCTCCGCCT
ATTTCGCGTTAGCCTTGCGCGCTGTTGACAACAAACACTTCCTCATCACACAGTTGAATCAGAAGACCCAACCAGCAAAACCAACCATGAACCA
GATAGGCGCTCTAAGTGTATGTGCAGTGACGGATGCCCCACACCCTACCGCCCTCCTTTGATCCACCGTACTAACTTTTCCTCCCAGCAATTCTC
CAATCTCACCGAGAACCTCTCTGTGGAACGCCTCAAGAGCGAGATCCAAAACGTGCAGAGCAAACTCTCCTCCGTGCGCCCACCGCAGGAGTT
CTTCAACGTCAAGAACTTTTCCAAACCGCAAAATTTCGCAGAGTTGCAATCTAGGGTCTCCTACAACATCAAGTACTACCAGTCCAACTATGCCC
TCATCGTCGGAGCCCTCAGCGTGTACAGCCTCTTGACCAACCTCCTGCTCCTGTTCGTGATAGCACTCGTCGTCGCTGGTGTCGCAGGCATCAG
CAGGCTTAGGGGCGAAGACTGGGTGACACCTTTCGGCACGCTGAAGGCCACACAGCTGTACACGGTGCTGCTTTGTGTTACGCTGCCCCTTGG
CTTTTTGGCATCTCCCTTCACCACCATCCTGTGGCTCGTGGGCGCCTCCTCCGTCACCGTCATGGGTCACGCCTCCTTCATGGAGAAACCCATCC
AGGTCGTCTTCGAGGAGGAAACTGTGTAGTTCAGCATATATATCAGCGTATGGACCTTTTAATAGATTCTCCAACAGGGTTTCCGTACCGGCCG
CCGCCCTGAGTTGGATATTCAAGTGTTCCGAAGCAAATACCGGGGACTCCACCGCTCTCCATCACAGCTATTCAATAGAATAGAAATGTGTTTC
TTTTGGTATAGCTGTCTAGGATCTTCGCACGTGACTTATTTTGTCGAAGTACATACCATCGTTGAACCGGACGTGGTACACTACTGCGTCGGCC
GGCTCTCTCCGATGGAGAAAGAGCTTTAACAAGCCGGATGTCCCTTTCAGTATAGATATAGTACCCCACCCCGATCCCCCCCCCCCACAATGAC
TATCTATCCCAATGGAGCTAATCGCTTCATACGTCATTCTTCCGATCTGCGGG

Supplementary Table 12

DNA sequences of the established fugal promoters commonly used for gene expression control. These sequences were used for comparisons with the SES-A system in selected yeast and filamentous fungi species.

Promoter	Sequence
S. cerevisiae PGK1 promoter	GACTTCAACTCAAGACGCACAGATATTATAACATCTGCATAATAGGCATTTGCAAGAATTACTCGTGAGTAAGGAA AGAGTGAGGAACTATCGCATACCTGCATTTAAAGATGCCGATTTGGGCGCGAATCCTTTATTTTGGCTTCACCCTCA TACTATTATCAGGGCCAGAAAAAGGAAGTGTTTCCCTCCTTCTTGAATTGATGTTACCCTCATAAAGCACGTGGCCT CTTATCGAGAAAGAAATTACCGTCGCTCGTGATTTGTTTGCAAAAAGAACAAAACTGAAAAAACCCAGACACGCTC GACTTCCTGTCTTCCTATTGATTGCAGCTTCCAATTTCGTCACACAACAAGGTCCTAGCGACGGCTCACAGGTTTTGT AACAAGCAATCGAAGGTTCTGGAATGGCGGGAAAGGGTTTAGTACCACATGCTATGATGCCCACTGTGATCTCCAG AGCAAAGTTCGTTCGATCGTACTGTTACTCTCTCTCTTTCAAACAGAATTGTCCGAATCGTGTGACAACAACAGCCTG TTCTCACACACTCTTTTCTTCTAACCAAGGGGGTGGTTTAGTTTAGTAGAACCTCGTGAAACTTACATTTACATATAT

	ATAAACTTGCATAAATTGGTCAATGCAAGAAATACATATTTGGTCTTTTCTAATTCGTAGTTTTTCAAGTTCTTAGAT GCTTTCTTTTTCTCTTTTTTACAGATCATCAAGGAAGTAATTATCTACTTTTTACAACAAATTA
Y. lipolytica *TEF1* promoter	GAAAAGTCTCTACAAGTAGTCTTGTAAAAACATTCCCCACAGTAACCATCTATATTGCAAAACAATACGACTGCAAT TGGGGATTGTTGCTCGTATCGCACCCGTACCGTGCCACCGTAACACCCCCCAGACACCAATCACTACGACCGATCCA ACTCCACGTTTCTGCTACCCAGTCTGCTGGCCGGCTTGGCTTTGCGTTTCGCTCCCACACTACACCGTAATACCACGT CACTCTCATTGCAGGTTACCCTGCCCGTAGTCGCTCGATCCACCTCCTCCTTCTCTCGTGTGTGCAGCAAAGAGGCA GAGATGGAGCCCGTATGGTGAGCCGTAGAGTGGAGCGAGGGGCGATCTTACAACGGCTGTCGGCGGATATAAAC GGTATTTTCACAATTGCACCCCAGCCAGACCGATAGCCGGTCGCAATCCGCCACCCACAACCGTCTACCTCCCACAG AACCCCGTCACTTCCACCCTTTTCCACCAGATCATATGTCCCAACTTGCCAAATTAAAACCGTGCGAATTTTCAAAAT AAACTTTGGCAAAGAGGCTGCAAAGGAGGGGCTGGTGAGGGCGTCTGGAAGTCGACCAGAGACCGGGTTGGCGG CGCATTTGTGTCCCAAAAAACAGCCCCAATTGCCCCAATTGACCCCAAATTGACCCAGTAGCGGGCCCAACCCCGGC GAGAGCCCCCTTCTCCCCACATATCAAACCTCCCCCGGTTCCCACACTTGCCGTTAAGGGCGTAGGGTACTGCAGTC TGGAATCTACGCTTGTTCAGACTTTGTACTAGTTTCTTTGTCTGGCCATCCGGGTAACCCATGCCGGACGCAAAATA GACTACTGAAAATTTTTTTGCTTTGTGGTTGGGACTTTAGCCAAGGGTATAAAAGACCACCGTCCCCGAATTACCTTT CCTCTTCTTTTCTCTCTCTCCTTGTCAACTCACACCCGAAATCTTAAGCATTTCCTTCTGAGTATAAGAATCATTCAAA
P. pastoris *GAP1* promoter	TTTTTGTAGAAATGTCTTGGTGTCCTCGTCCAATCAGGTAGCCATCTCTGAAATATCTGGCTCCGTTGCAACTCCGAA CGACCTGCTGGCAACGTAAAATTCTCCGGGGTAAAACTTAAATGTGGAGTAATGGAACCAGAAACGTCTCTTCCCTT CTCTCTCCTTCCACCGCCCGTTACCGTCCCTAGGAAATTTTACTCTGCTGGAGAGCTTCTTCTACGGCCCCCTTGCAG CAATGCTCTTCCCAGCATTACGTTGCGGGTAAAACGGAGGTCGTGTACCCGACCTAGCAGCCCAGGGATGGAAAA GTCCCGGCCGTCGCTGGCAATAATAGCGGGCGGACGCATGTCATGAGATTATTGGAAACCACCAGAATCGAATATA AAAGGCGAACACCTTTCCCAATTTTGGTTTCTCCTGACCCAAAGACTTTAAATTTAATTTATTTGTCCCTATTTCAATC AATTGAACAACTAT
A. nidulans *gpdA* promoter	CCTTGTATCTCTACACACAGGCTCAAATCAATAAGAAGAACGGTTCGTCTTTTTCGTTTATATCTTGCATCGTCCCAA AGCTATTGGCGGGATATTCTGTTTGCAGTTGGCTGACTTGAAGTAATCTCTGCAGATCTTTCGACACTGAAATACGT CGAGCCTGCTCCGCTTGGAAGCGGCGAGGAGCCTCGTCCTGTCACAACTACCAACATGGAGTACGATAAGGGCCA GTTCCGCCAGCTCATTAAGAGCCAGTTCATGGGCGTTGGCATGATGGCCGTCATGCATCTGTACTTCAAGTACACCA ACGCTCTTCTGATCCAGTCGATCATCCGCTGAAGGCGCTTTCGAATCTGGTTAAGATCCACGTCTTCGGGAAGCCAG CGACTGGTGACCTCCAGCGTCCCTTTAAGGCTGCCAACAGCTTTCTCAGCCAGGGCCAGCCCAAGACCGACAAGGC CTCCCTCCAGAACGCCGAGAAGAACTGGAGGGGTGGTGTCAAGGAGGAGTAAGCTCCTTATTGAAGTCGGAGGAC GGAGCGGTGTCAAGAGGATATTCTTCGACTCTGTATTATAGATAAGATGATGAGGAATTGGAGGTAGCATAGCTTC ATTTGGATTTGCTTTCCAGGCTGAGACTCTAGCTTGGAGCATAGAGGGTCCTTTGGCTTTCAATATTCTCAAGTATCT CGAGTTTGAACTTATTCCCTGTGAACCTTTTATTCACCAATGAGCATTGGAATGAACATGAATCTGAGGACTGCAAT CGCCATGAGGTTTTCGAAATACATCCGGATGTCGAAGGCTTGGGGCACCTGCGTTGGTTGAATTTAGAACGTGGCA CTATTGATCATCCGATAGCTCTGCAAAGGGCGTTGCACAATGCAAGTCAAACGTTGCTAGCAGTTCCAGGTGGAAT GTTATGATGAGCATTGTATTAAATCAGGAGATATAGCATGATCTCTAGTTAGCTCACCACAAAAGTCAGACGGCGT AACCAAAAGTCACACAACACAAGCTGTAAGGATTTCGGCACGGCTACGGAAGACGGAGAAGCCACCTTCAGTGGA CTCGAGTACCATTTAATTCTATTTGTGTTTGATCGAGACCTAATACAGCCCCTACAACGACCATCAAAGTCGTATAGC TACCAGTGAGGAAGTGGACTCAAATCGACTTCAGCAACATCTCCTGGATAAACTTTAAGCCTAAACTATACAGAATA AGATAGGTGGAGAGCTTATACCGAGCTCCCAAATCTGTCCAGATCATGGTTGACCGGTGCCTGGATCTTCCTATAG AATCATCCTTATTCGTTGACCTAGCTGATTCTGGAGTGACCCAGAGGGTCATGACTTGAGCCTAAAATCCGCCGCCT CCACCATTTGTAGAAAAATGTGACGAACTCGTGAGCTCTGTACAGTGACCGGTGACTCTTTCTGGCATGCGGAGAG ACGGACGGACGCAGAGAGAAGGGCTGAGTAATAAGCCACTGGCCAGACAGCTCTGGCGGCTCTGAGGTGCAGTG GATGATTATTAATCCGGGACCGGCCGCCCCTCCGCCCCGAAGTGGAAAGGCTGGTGTGCCCCTCGTTGACCAAGAA TCTATTGCATCATCGGAGAATATGGAGCTTCATCGAATCACCGGCAGTAAGCGAAGGAGAATGTGAAGCCAGGGG TGTATAGCCGTCGGCGAAATAGCATGCCATTAACCTAGGTACAGAAGTCCAATTGCTTCCGATCTGGTAAAAGATTC ACGAGATAGTACCTTCTCCGAAGTAGGTAGAGCGAGTACCCGGCGCGTAAGCTCCCTAATTGGCCCATCCGGCATC TGTAGGGCGTCCAAATATCGTGCCTCTCCTGCTTTGCCCGGTGTATGAAACCGGAAAGGCCGCTCAGGAGCTGGCC AGCGGCGCAGACCGGGAACACAAGCTGGCAGTCGACCCATCCGGTGCTCTGCACTCGACCTGCTGAGGTCCCTCA GTCCCTGGTAGGCAGCTTTGCCCCGTCTGTCCGCCCGGTGTGTCGGCGGGGTTGACAAGGTCGTTGCGTCAGTCCA ACATTTGTTGCCATATTTTCCTGCTCTCCCCACCAGCTGCTCTTTTCTTTTCTCTTTCTTTTCCCATCTTCAGTATATTCA TCTTCCCATCCAAGAACCTTTATTTCCCCTAAGTAAGTACTTTGCTACATCCATACTCCATCCTTCCCATCCCTTATTCC TTTGAACCTTTCAGTTCGAGCTTTCCCACTTCATCGCAGCTTGACTAACAGCTACCCCGCTTGAGCAGACATCA