Supplementary information

Plasmid construction.

pESA1-KO for knocking out of *ESA1*, two PCR fragments, 0.53 kb containing the *ESA1* 5' region (primers F2 and R2) and 0.6 kb containing the *ESA1* 3' region (primers F3 and R3) were digested and inserted into pCUB6 at BglII and BamHI-SphI sites, respectively. pSAS2-KO for knocking out of *SAS2*, two PCR fragments, 0.55 kb containing the *SAS2* 5' region (primers F17 and R17) and 0.5 kb containing the *SAS2* 3' region (primers F18 and R18) were digested and inserted into pCUB6 at BglII and BamHI-SphI sites, respectively.

pCPC39, which contains a tet-on promoter and a *URA3* marker, *URA3* was constructed as follows: fragment A of *ADH1p-cartTA-GAL4AD* was amplified by PCR from pCaUME6-3 (3), which was originated from pNIM1 (2) with the primers TOF1 and TOR1. Fragment B of ACT1-terminator was amplified from genomic DNA of SC5314 with the primers TOF2 and TOR2. Fragment C1 of selective marker, *URA3*, was amplified by PCR from genomic DNA of SC5314 with the primers TOF3 and TOR3. Fragment D of tet-OP4 promoter was amplified from pCaUME6-3 (2) with the primers TOF4 and TOR4. The plasmid backbone E which contains the AmpR gene and pBR322 origin was amplified by PCR from pUC18 with the primers TOF5 and TOR5. Fragments A, B, D and E were assembled with C with the Cloneget Kit which applied in fusion strategy (4) to produce pCPC39 (Willget, Shanghai, China).

pCPC42, which contains a tet-off promoter and *URA3* marker, was constructed as follows: Fragment of a *C. albicans*-adapted catTA-Advance element was synthesized and amplified by the primers TOF6 and TOR6. The plasmid backbone was amplified from pCPC39 with the primers TOF7 and TOR7. The catTA-Advance fragment was assembled with backbones of pCPC39 with the Cloneget Kit (Willget, Shanghai, China) to produce pCPC42.

pCPC98 (pPtet-ESA1) for doxycycline controlled *C. albicans ESA1* expression was constructed as follows: The plasmid backbone fragment G which contains the AmpR gene and pBR322 origin was amplified by PCR from pUC18 with the primers TOF9 and TOR9. Fragment F containing -521 bp to +1979 bp of the *ESA1* was amplified from SC5314 with primers TOF8 and TOR8. The PCR products above were infused with the Cloneget Kit (Willget, Shanghai, China) to produce plasmid pCPC97. Primers TOF10 and TOR10 were used to amplify backbones fragment H from pCPC97, primers TOF11 and TOR11 to amplify fragment I (*URA3-Ptet*) from pCPC42, infusing these two fragments (H and I) to produce pCPC98 (pPtet-ESA1). A fragment containing *URA3-Ptet* flanked with *ESA1* 5' region and coding region was amplified from pCPC98 (pPtet-ESA1) using primers TOF12 and TOR12 integrated into *ESA1* locus to construct strains for doxycycline controlled *ESA1* gene expression (1, 4).

pBA1-WX1 (pBA1-ESA1) for the ectopic expression of *C. albicans ESA1* under *ADH1* promoter was constructed by inserting a 1.6 kb PCR fragment (primers F4 and R4) containing *CaESA1* coding sequence into the BglII-ClaI site of plasmid pBA1. The same strategy was used for construction of pBA1-WX2 to pBA1-WX10. A 1.2 kb PCR fragment (primers F5 and R4) of *CaESA1* deleting the chromo domain (CHD), 0.9 kb fragment (primers F4 and R6) of *CaESA1* deleting the MYST domain and 1.3 kb fragment (primers F7 and R7) of *CaESA1* deleting an insertion fragment were inserted into the BglII-ClaI site of pBA1. Primers F8 and R8 were used to amplify the full-length coding sequence of *S. cerevisiae ESA1* (*ScESA1*). Primers F9 and R9, F10 and R10 were used in construction of site-directed mutagenesis of ScEsa1 G315E and ScEsa1 L327S. Primers F11 and R11 were used to amplify the full-length human TIP60 isoform2 cDNA. Primers F12 and R12, F13 and R13 were used to construct hybrid fragments of TIP60 MYST+*Caesa1* CHD and TIP60 CHD+*Caesa1* MYST, respectively. All PCR fragments were inserted into the BglII-EcoRV site of pBA1 for the ectopic expression in *C. albicans* under control of *ADH1* promoter.

Table S1. Primers used in this study

Primers	Sequence	Purpose and features
F1	5'-ACTGTCCATCAATCAATCAAAAACGAAAAAGACAATAACACCGAC AACAAGTAATACATC Gttttcccagtcacgacgtt	KO *ESA1* by PCR method
R1	5'-TCTGGTTTAAGATTATTGACATGCCATGGCGATAAAAGTATTAGAAT ATGGGGAACATCtgtggaattgtgagcggata	
F2	5'-tgca agatct ctgcag GAAATGCTTTTGTGCAGTGAAC	pESA1-KO-5' region
R2	5'-tgca agatct GATGTATTACTTGTTGTCGGTG	
F3	5'-cgat ggatcc GATGTTCCCCATATTCTAATAC	pESA1-KO-3' region
R3	5'-cgat gcatgc ctgcag CTGATTATAACGATCCACAGGT	
F4	5'-gatc agatct ATGGCGGTAGCAGAAATCAAA	pBA1-WX1
R4	5'-gatc atcgat TTACCACCCAAACCGTAATTG	(pBA1-ESA1)
F5	5'-gatc agatct ATGAAACTGAAAAAGAAGAGCAAATC	pBA1-WX2
R4	5'-gatc atcgat TTACCACCCAAACCGTAATTG	(pBA1-*caesa1* d CHD)
F4	5'-gatc agatct ATGGCGGTAGCAGAAATCAAA	pBA1-WX3
R6	5'-gatc atcgat CTAATTTTCCTTTGTGTCTGCTT	(pBA1-*caesa1* d MYST)
F7	5'-AATGGTAATTTCTCTTTCTACCAATAGCTTTA	pBA1-WX4
R7	5'-TTGGTAGAAAGAGAAATTACCATTGAGGACATT	(pBA1-*caesa1* d Ins)
F8	5'-gatc agatct ATGTCCCATGACGGAAAAGAAG	pBA1-WX5
R8	5'-gatc gatatc TTACCAGGCAAAGCGTAACTGA	(pBA1-ScESA1)
F9	5'-CAATACCAAAGGATGGaATATGGTAAGTTATTGA	pBA1-WX6
R9	5'-TCAATAACTTACCATATtCCATCCTTTGGTATTG	(pBA1-scesa1 G315E)
F10	5'-GAATTTTCGTATGAATcGTCGAAAAAGGAAAACA	pBA1-WX7
R10	5'-TGTTTTCCTTTTTCGACgATTCATACGAAAATTC	(pBA1-scesa1 L327S)
F11	5'-gatc agatct ATGGCGGGAGGTGGGGAGATA	pBA1-WX8
R11	5'-gatc gatatc TCACCACTTTCCTCTCTTGCTCCA	(pBA1-hTIP60)
F12	5'-gtgcccagcgagacagccccaAAACTGAAAAAGAAGAGCAAA	pBA1-WX9
R12	5'-TTTGCTCTTCTTTTTCAGTTTtggggctgtctcgctgggcac	(pBA1-TIP60MYST +caesa1 CHD)
F13	5'-AAAGCAGACACAAAGGAAAATgcctcggttttccctcagaat	pBA1-WX10
R13	5'-attctgagggaaaaccgaggcATTTTCCTTTGTGTCTGCTTT	(pBA1-TIP60CHD +caesa1 MYST)
F14	5'-GCCAACATATCCATAGTTAAcGCTTTTTCCATATTTTGTTT	HindIII mutation in *AGR4*
R14	5'-AAACAAAATAGGAAAAAGCgTTAACTATGGATATGTTGGC	in p671
F15	5'-gac aagctt GTTTTCCCAGTCACGACGTT	*HIS1* to *AGR4*
R15	5'-gac aagctt TGTGGAATTGTGAGCGGATA	substitution in p671
F16	5'-ACATTAGCTTCGAAAACACTAAGTTTTTGTTTTACTTCGGGTATAAA GCCACTAAATTCTgttttcccagtcacgacgtt	KO *SAS2* by PCR method
R16	5'-TCAAGTGGAATGTGGAAGAAAACAAACTAGCTAGTGGTGGCAACG ATAATAATTTGTTTGtgtggaattgtgagcggata	
F17	5'-tgca agatct ctgcag CTCTTTCTAATGCTGCCAACAT	pSAS2-KO-5' region
R17	5'-tgca agatct GATATTAGATGTAGAAATTAAC	
F18	5'-cgat ggatcc TGCTCCTAACAAAACCGAAGCA	pSAS2- KO-3' region
R18	5'-cgat gcatgc ctgcag CAAACTAGCTAGTGGTGGCAAC	
TOF1	5'- ACAGTCTGCCAGACTGCCAGCGAGAAAGAGGAGTAT	
TOR1	5'- GTAGCGATGCACGGTTTACTCTTTTTTTGGGTTTGGTGG	
TOF2	5'- ACCGTGCATCGCTACCATTGTTCACCACAAATGTTTCT	

TOR2	5'- AGTCTGGCAGACTGTAGATATAGAAATGCCTTGGG	
TOF3	5'- ACAGTCTGCCAGACTCAAAACCGGATCTTCAGAAC	Fragment A to E for pCPC39 construction
TOR3	5'- CTGACTGACTGACTGCTACTATCCCAGCTACTTCG	
TOF4	5'- CAGTCAGTCAGTCAGGCTTGGACAGCATCAGCAGTAG	
TOR4	5'- TATGACCATGATTACCTATTTATATTTGTATGTGTGTAGGAGT	
TOF5	5'- gtaatcatggtcata CCGCGGAGGCCTGGGCCCGATAACGCAGGAAAGAACAT	
TOR5	5'-AGTCTGGCAGACTGTCTTAAGGGATCCAAGCTTCCCGGGTTCTTAG ACGTCAGGTGGCA	
TOF6	5'- AATACAAAAACAATTATGTCAAGATTAGATAAATCAAAAGTTATTAAT	cartTA-advance fragment for pCPC42 construction
TOR6	5'- ACCAAACAAAGTCGAACCACCTGATTCACATTTTAATTGT	
TOF7	5'- TCGACTTTGTTTGGTGGCGCCAATTTTAATCAAAGT	pCPC39 fragment for pCPC42 construction
TOR7	5'- AATTGTTTTTGTATTTGTTGTTGTTGTTGTATGACAAT	
TOF8	5'- ACAGTCTGCCAGACTGAACGTTTGCGGTATGACCT	
TOR8	5'- CTGACTGACTGACTGAACGGCGAAATAAAGCAATG	
TOF9	5'-CAGTCAGTCAGTCAGGATAACGCAGGAAAGAACAT	
TOR9	5'-TCTAGAGTCGACCTGGGGCCCAGGCCTCCGCGGCTCGAGTTCTTAG ACGTCAGGTGGCA	Fragment F to I for pCPC98 construction
TOF10	5'- AACGTTTCGTGGACGTTGTCTCAACTGGCGAAATG	
TOR10	5'- AGGTAATCATTGTCAATGGCGGTAGCAGAAATCAA	
TOF11	5'- CGTCCACGAAACGTTGAGAAAGAGGAGTATTGGCATTG	
TOR11	5'- TGACAATGATTACCTATTTATATTTGTATGTGTGTAGGAGT	
TOF12	5'-GGGAATCACAAAACTCCTGAA	*Ptet-ESA1* insertion
TOR12	5'-TGTGGGGTACCGGTTGTACT	
CF1	5'-GACAGTTGCTAGTTGAAAAGA	verify deletion of first copy *ESA1* at 5' region
CR1	5'-ttcttcaattcgtccattaca	
CF2	5'-gatctacttcaatttgactca	verify deletion of first copy *ESA1* at 3' region
CR2	5'-GATGCTGGGATTTGGAAAGAA	
CF3	5'-GATCTCCGTTTTATAAGGGAA	verify deletion of second copy *ESA1* at 5' region
CR3	5'- gacttcgacagaaccatttaa	
CF4	5'- gtttaagcgatgattcacgag	verify deletion of second copy *ESA1* at 3' region
CR4	5'- GGTGGGAAAGACTGTGTCGTA	
CF5	5'-ATGGCGGTAGCAGAAATCAA	amplify *ESA1* ORF
CR5	5'-TTACCACCCAAACCGTAATT	
CF6	5'-GTACTATTTTCTTCATCGTCT	verify deletion of first copy *SAS2* at 5' region
CR5	5'-gatatcagctgcaggcaagaa	
CF7	5'-gtacattacttttgctataca	verify deletion of first copy *SAS2* at 3' region
CR7	5'-GGTACCCATTTGGCAATAGGA	
CF8	5'-GCAACATTAGCTTCGAAAACA	verify deletion of second copy *SAS2* at 5' region
CR8	5'- gacttcgacagaaccatttaa	
CF9	5'- gtttaagcgatgattcacgag	verify deletion of second copy *SAS2* at 3' region
CR9	5'-GAATTGAAGATCACTTTGTTA	
CF10	5'-GAAATATCAATAAAGTCACA	amplify *SAS2* ORF
CR10	5'-TCATAGTATTAAACATTCTG	

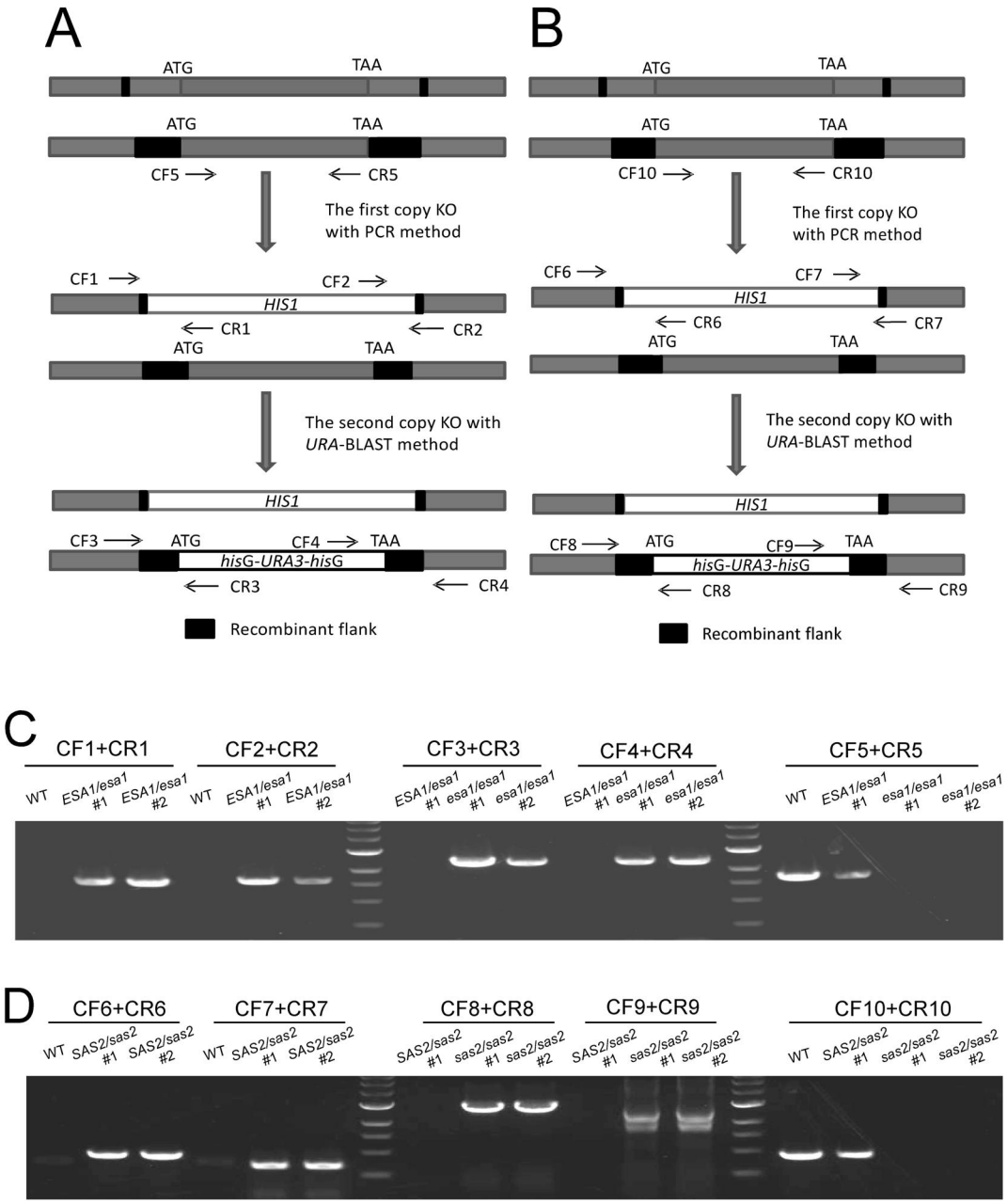

Figure S1. Disruption of the *C. albicans ESA1* and *SAS2* genes. (A) Strategy for disruption of *ESA1* and verification of *esa1* mutants. (B) Strategy for disruption of *SAS2* and verification of *sas2* mutants. PCR analysis of *ESA1* deletion (C) and *SAS2* deletion (D) constructs. Genomic DNA from the recipient strain WT (BWP17), *ESA1* deletion or *SAS2* deletion mutants was amplified with primers indicated in Supplementary Table S1 for verification.

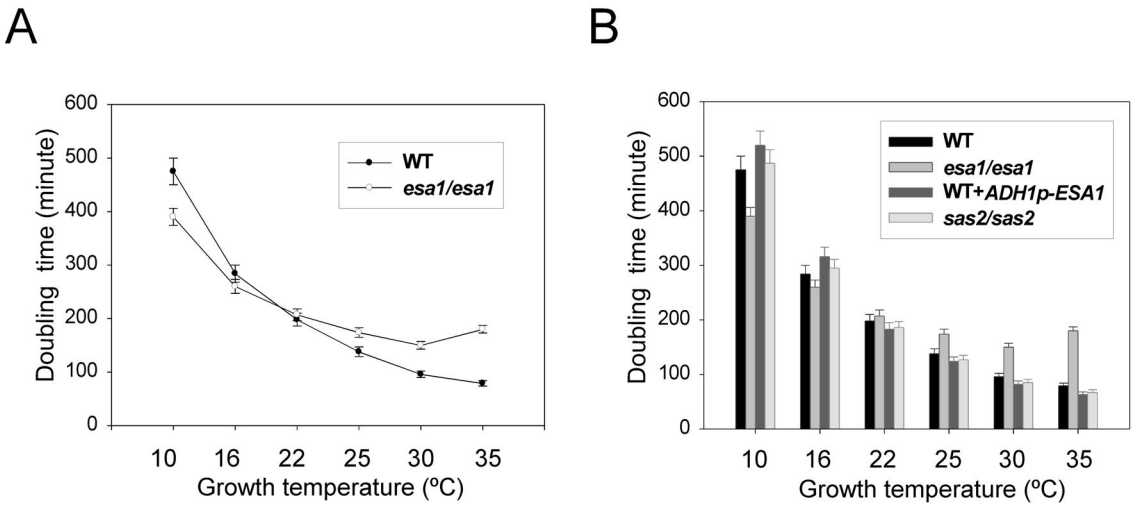

Figure S2. Sas2 and Esa1 have opposite effects on cell growth. (A) Doubling time of wild type and *esa1/esa1* mutant at different temperatures. The doubling time in minutes were drawn by sigmaplot10 data statistical analysis. (B) Doubling time of wild type (BWP17+ pBA1), wild type+ *ADH1*p-*ESA1* (BWP17+ pBA1-ESA1), *esa1/esa1* (CWX3+ pBA1) and *sas2/sas2* (CWX6+ pBA1). Overnight cultures were re-inoculated into fresh liquid YPD and grown at different temperatures, OD_{600} values were measured during log phase at various time points (every 0.5 h at 35°C and 30°C, 1 h at 25°C and 22°C, 2.5 h at 16°C and 4 h at 10°C) and used for calculation of generation time.

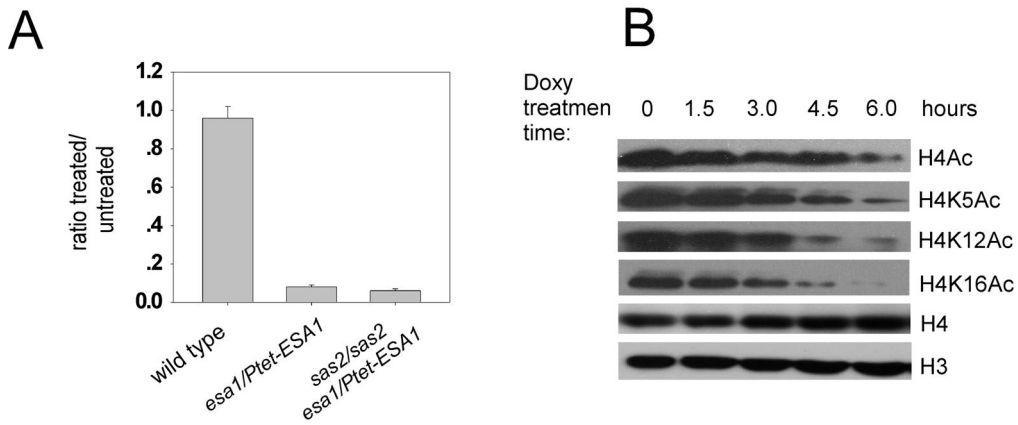

Figure S3. Down-regulating the expression of *ESA1* in a tet-off system. (A) Quantitative RT-PCR analyses showed that the transcriptions of *ESA1* in *esa1/Ptet-ESA1* (CWX7) and *sas2/sas2 esa1/Ptet-ESA1* (CWX8) mutants were efficiently repressed by doxycycline treatment. The cells were pretreated with 50 μg/ml doxycycline for 24 h, and re-inoculated into fresh YPD media containing 50 μg/ml doxycycline and grown for 6 h. (B) Western analyses showed that the histone H4 acetylation levels in CWX8 cells were greatly reduced by doxycycline treatment. Antibodies that specifically recognize acetylated H4 (H4Ac), H4 acetylated at lysine residues 5 (H4K5Ac), 12 (H4K12Ac), 16 (H4K16Ac), and histone H4 (H4) (Abcam).

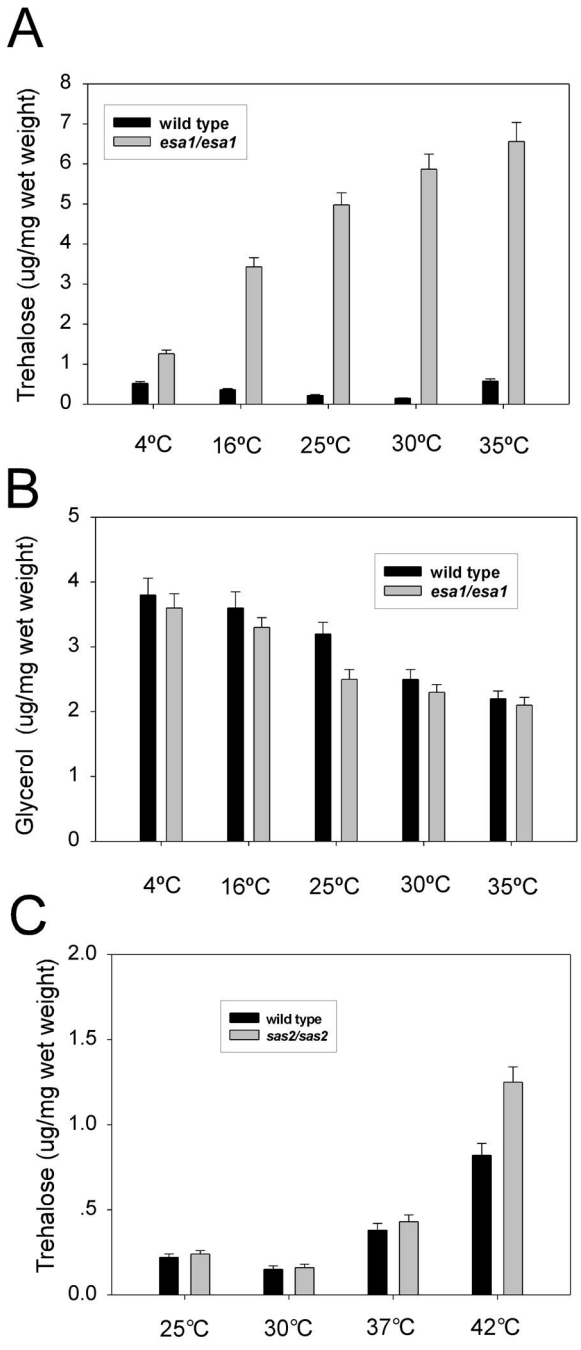

Figure S4. Determination of trehalose and glycerol levels. Trehalose (A) but not glycerol (B) was highly accumulated in *esa1/esa1* mutants. The growing cells were spread onto YPD plates and incubated at 4°C for 2 weeks, 16°C for 5 days, 25°C for 3 days, 30°C and 35°C for 2 days respectively. Cells were harvested and washed three times with ice-cold water and frozen in liquid nitrogen for assay. Trehalose level was measured by a trehalose assay kit (Sigma). Glycerol level was determined by glycerol assay kit (Abcam). About 20 mg wet weight of cells were used for each assay. Data represent means of three independent experiments. Strains are wild type (BWP17+pBES116) and *esa1/esa1* mutant (CWX3+ pBES116). (C) Trehalose concentration in *sas2/sas2* mutant (CWX6+ pBES116) cells. The growing cells were spread onto YPD plates and incubated at 25°C for 3 days, 30°C, 37°C and 42°C for 2 days respectively, and harvested for determination of trehalose levels.

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