

Supporting Information

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SI Results and Discussion

Microarray Analysis. To further analyze the global meaning of GcnE, the transcriptome present in the wild-type and the $\Delta gcnE$ mutant during standard cultivation and cocultivation was determined after 3 h by *A. nidulans* full-genome microarray analysis. Some of the data were verified by qRT-PCR analysis (Fig. S4 A–C). More than half of the differentially regulated genes ($P < 0.05$, $\text{absFC} < 2$) during cocultivation appear to be dependent on a functional Saga/Ada complex (Fig. S4D). The absolute fold change of differentially expressed fungal genes between wild type and $\Delta gcnE$ significantly increased upon contact with the bacterium when compared under noninducing conditions (Fig. S4E). A similar shift was observed for *S. cerevisiae* when the wild-type was compared with a *gcn5* mutant strain under osmotic stress (1). Our data suggest that GcnE is required for the fast fungal response to the bacteria that includes the activation of the *ors* cluster. We thus extend the importance of GcnE in response to abiotic alterations by a major function for biotic interactions.

SI Materials and Methods

Western Blot. For preparation of whole-cell extracts, mycelia was harvested and immediately frozen in liquid nitrogen. Mycelium was then ground under liquid nitrogen to a fine powder using mortar and pestle, resuspended in 1 mL phosphate buffer [50 mM KH_2PO_4 , 150 mM NaCl (pH 7), 1 mM PMSF, and protease inhibitor (Sigma Aldrich)], placed on ice for 10 min, and centrifuged at $10,000 \times g$ for 10 min. The supernatant was collected and the protein concentration determined using Coomassie Plus Protein Assay (Pierce). A total of 20 μg of protein was separated by SDS/PAGE and transferred to PVDF membrane. The membrane was incubated with the respective primary antibodies [goat polyclonal against S-tag (ab19321; Abcam), mouse monoclonal ANTI-FLAG M2 antibodies (Sigma; 1:2,000)] and detected with donkey anti-goat IgG-HRP (SC-2033) or goat anti-mouse IgG-HRP (sc-2005, 1:5,000; Santa Cruz Biotechnology) secondary antibodies and chemiluminescence detection kit for HRP (Applichem).

ChIP Coupled to qRT-PCR Analysis. ChIP was essentially performed as described previously (2). Antibodies used in this study are as follows: rabbit polyclonal against the C terminus of the human histone H3 (ab1791), rabbit polyclonal aniserum against histone H3 acetyl K9 (ab4441), rabbit polyclonal antiserum against histone H3 acetyl K14 (ab46984; Abcam), anti-acetyl K9-K14 histone H3 (06-599; Millipore), and mouse monoclonal ANTI-FLAG M2 antibodies (Sigma). A total of 2 μg of antibodies were used per reaction with 200 μg of total protein. The sonication buffer was prepared as described in Roze et al. (3), and for indirect sonication the Branson Sonifier 450 (G. Heinemann Ultraschall and Labortechnik) was used with the following settings: output control 10, duty cycle constant, 9×75 s on/30 s off. For amplification and quantification of precipitated DNA by qRT-PCR, the iQ SYBR Green Supermix (BioRad) or the MyTaq HS Mix 2 \times (Bioline) in combination Evagreen (Biotium) were used with the MyIQ Thermal Cycler (BioRad) or the StepOnePlus Real-Time PCR System (Applied Biosystems) according to the manufacturers' instructions. ChIP oligonucleotides amplified 150- to 200-bp-long DNA products within a region of maximal 350 bp upstream of the translational start of the gene of interest. Each PCR was carried out twice. The relative amount of DNA

was calculated by the ratio of precipitated DNA treated with antibodies targeted against acetylated H3 divided by the precipitated DNA treated with anti-C-terminus H3 antibody. For ChIP analysis with GcnE-3xFLAG, the relative amount of DNA was calculated by the ratio of immunoprecipitated DNA against input DNA. At least two biological replicates were analyzed, and SDs were calculated. Significance was determined using the Student *t* test.

Extraction, HPLC, and LC-MS Analysis of Secondary Metabolites.

Fungal cultures were homogenized and exhaustively extracted with ethyl acetate. The resulting extracts were dried over sodium sulfate and concentrated under reduced pressure. For HPLC analysis, the extracts were dissolved in 2 mL of methanol. Analytical HPLC was performed on a Shimadzu LC-10Avp series HPLC system consisting of an autosampler, high-pressure pumps, column oven, and PDA. HPLC conditions: C18 column (Eurospher 100-5, 250×4.6 mm) and gradient elution [MeCN/0.1% (vol/vol) TFA (H_2O) 0.5/99.5 in 30 min to MeCN/0.1% (vol/vol) TFA 100/0, MeCN 100% for 10 min], flow rate, $1 \text{ mL} \cdot \text{min}^{-1}$; injection volume, 20 μL . LC-MS measurements were performed using an Exactive Orbitrap High Performance Benchtop LC-MS Mass Spectrometer with an electrospray ion source (Thermo Fisher Scientific). HPLC conditions: C18 column (Betasil C18 3 μm , 150×2.1 mm) and gradient elution [MeCN/0.1% (vol/vol) HCOOH (H_2O) 5/95 for 1 min, going up to 98/2 in 15 min, then 98/2 for another 3 min], flow rate, $0.2 \text{ mL} \cdot \text{min}^{-1}$; injection volume, 3 μL . Compounds were identified by UV and high-resolution electrospray ionization mass spectrometry (HRESI-MS) or by comparison with an authentic standard.

Generation of Transformation Cassettes. Transformation cassettes for gene deletion and gene replacement were constructed according to Szewczyk et al. (4). For gene deletion, $\sim 1,500$ -bp-long DNA fragments flanking the ORF of interest were amplified using genomic DNA of *A. nidulans* as a template and fused to the *argB* deletion cassette (5). The deletion primers marked with a Q (Table S3) were used for fusion PCR to assemble flanking regions and *argB* cassette. For *gcnE* replacement, transformation cassettes consisted of the *gcnE* coding sequence fused at the 3' end with the codon-optimized 3xFLAG-tag or S-tag, the *pabaA1* gene, and $\sim 1,500$ -bp-long DNA regions flanking *gcnE*. Southern blot analysis and DNA sequencing proved the correct fusion of GcnE with the tag and the integration of the encoding cassette into the *gcnE* locus.

Microarrays. Custom-made *A. nidulans* full-genome microarrays were applied [platform data available at the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GPL11193)]. Cultivation of *A. nidulans*, RNA preparation, cDNA synthesis and labeling, hybridization, and microarray scanning was performed as described in Schroeckh et al. (5). Data were processed using robust spline and quantile normalization provided by the Linear Models for Microarray Data (LIMMA) packages (6) from Bioconductor (<http://www.bioconductor.org/>). *P* values were calculated with the empirical Bayes method and subsequently adjusted for multiple testing using the false discovery rate.

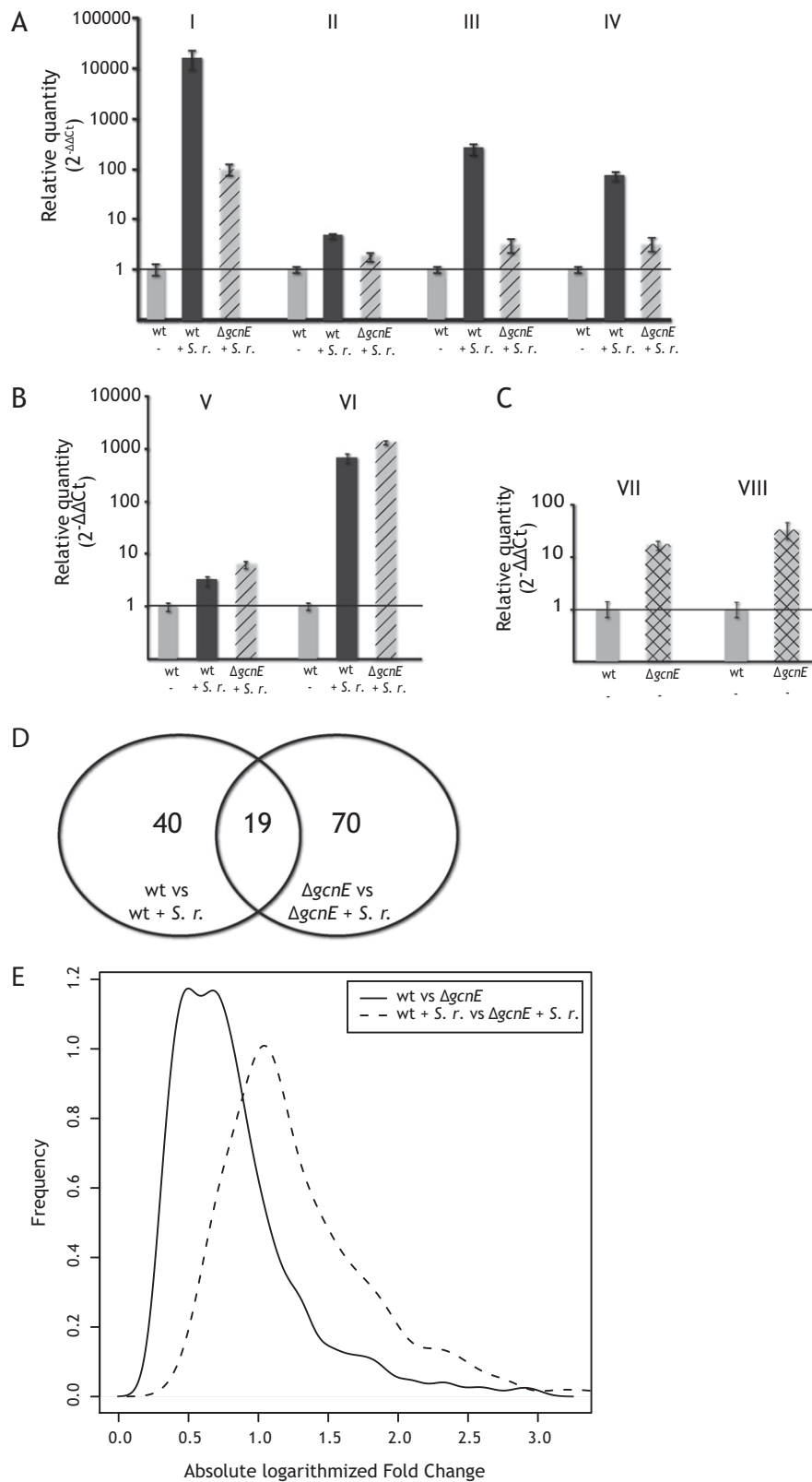


Fig. S4. Full-genome microarray analysis of wild type and $\Delta gcnE$ during standard cultivation and cocultivation with *S. rapamycinicus*. (A) Quantification of GcnE-dependent gene expression in *A. nidulans*. Relative transcript levels of AN9315 (I), AN2846 (II), AN5831 (III), and AN4905 (IV) increased in a GcnE-dependent manner when *A. nidulans* was cocultivated with *S. rapamycinicus*. The investigated genes were found to be differentially regulated in full genome microarray. Relative quantities of mRNA steady-state levels were determined by qRT-PCR. Relative quantities are given as \log_2 of $-\Delta\Delta Ct$. (B) Quantification of GcnE-independent gene expression in *A. nidulans*. The enhanced expression of genes AN3974 (V) and AN9397 (VI) in cocultivation is independent of GcnE. The investigated genes were found to be differentially regulated in full genome microarray. Relative quantities of mRNA steady-state levels were determined by

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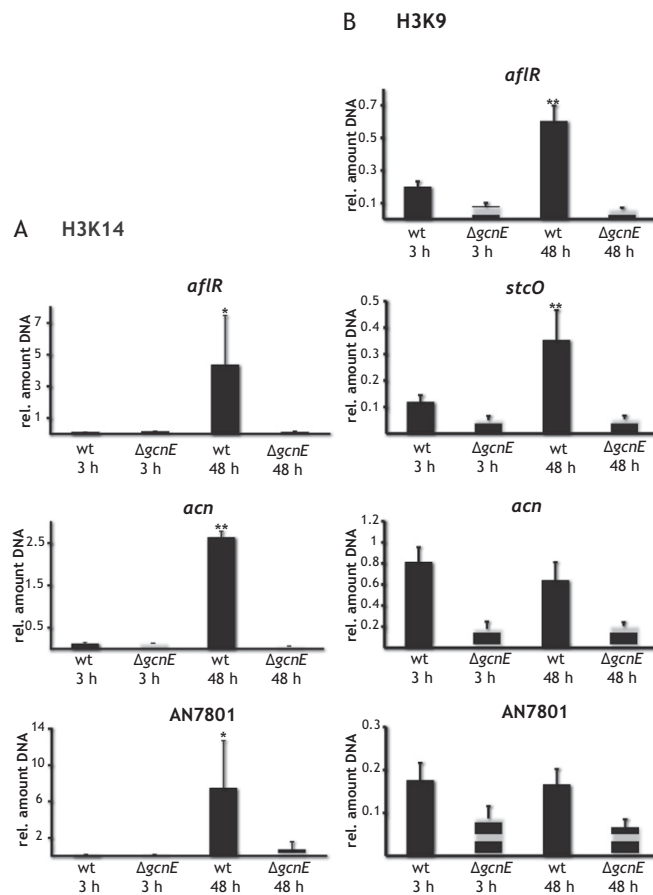


Fig. S6. H3K9 and H3K14 acetylation of sterigmatocystin cluster genes. ChIP experiment of wild-type and $\Delta gcnE$ deletion strains incubated under sterigmatocystin nonproducing (3-h incubation) and producing (48-h incubation) conditions. Promoter regions of *aflR*, *stcO*, AN7801, and *acn* were analyzed by qRT-PCR. SDs of the mean of two (K14) and three (K9) biological replicates are shown. Data are given as the relative amount of DNA obtained from acetylated H3 protein relative to the total amount of H3 protein. The low DNA amounts in the $\Delta gcnE$ samples under producing conditions represent the involvement of Saga/Ada in H3K9 and H3K14 acetylation of these secondary metabolite cluster genes. Statistical significance of data obtained for *aflR*, *stcO*, AN7801, and *acn* promoters of wild type incubated under nonproducing or producing conditions is given by the P value (* $P < 0.05$; ** $P < 0.01$). (A) Acetylation of H3K14. (B) Acetylation of H3K9.

Table S1. Deletion of genes encoding putative acetyltransferases

Gene number	Name	Homologous proteins human*	Homologous proteins yeast*	Deletion	Phenotype under conditions tested
Putative GNAT-type acetyltransferases					
AN2161.4	<i>ngn1</i>			Yes	—
AN2102.4	<i>ngn2</i>			Yes	—
AN2294.4	<i>elp3</i>	Elp3 (hKat9)	Elp3 (yKat9)	Yes	Slight differences [†]
AN3621.4	<i>gcnE</i>		Gcn5 (yKat2)	Yes	Major differences [‡]
AN3879.4	<i>spt10</i>		Spt10	No	
AN1328.4	<i>nnaA</i>	Naa10	Ard1	No	
AN4993.4	<i>nnaC</i>	Naa30	Mak3	Yes	Slight differences [†]
AN7944.4	<i>ngn3</i>			Yes	—
AN3162.4	<i>ngn4</i>			Yes	—
AN7374.4	<i>ngn5</i>			Yes	—
AN9204.4	<i>ngn6</i>			Yes	—
AN0743.4	<i>ngn7</i>			Yes	—
AN2745.4	<i>nnaB</i>	Naa20	Nat3	Yes	Major differences [‡]
AN1621.4	<i>ngn8</i>			Yes	—
AN8005.4	<i>ngn9</i>			Yes	—
AN10234.4	<i>ngn10</i>			Yes	—
AN8373.4	<i>ngn11</i>			Yes	—
AN9493.3	<i>ngn12</i>			Yes	—
AN8706.4	<i>gna1</i>			Yes	Major differences [‡]
AN7171.4	<i>ngn13</i>			Yes	—
AN11810.4	<i>ngn14</i>			Yes	—
AN2870.4	<i>ngn15</i>			Yes	—
AN2479.4	<i>ngn16</i>			Yes	—
AN0969.4	<i>ngn17</i>			Yes	—
AN11841.4	<i>ngn18</i>			Yes	Slight differences [†]
AN1360.4	<i>ngn19</i>			Yes	—
AN5866.4	<i>ngn20</i>			Yes	—
AN10238.4	<i>ngn21</i>			Yes	—
AN0626.4	<i>ngn22</i>			Yes	—
AN8604.4	<i>ngn23</i>			Yes	—
AN8684.4	<i>ngn24</i>			Yes	Major differences [‡]
AN3825.4	<i>ngn25</i>			Yes	—
AN8539.4	<i>ngn26</i>			Yes	—
AN3344.4	<i>ngn27</i>			Yes	—
AN6411.4	<i>ngn28</i>			Yes	—
Putative MYST-type acetyltransferases					
AN10956.4	<i>esa1</i>	Tip60/PLIP (hKat5)	Esa1 (yKat5)	No	
AN5640.4	<i>nmy1</i>	HBO1/MYST2 (hKat7)	Sas3 (yKat6)	No	
AN3071.4	<i>nmy2</i>	HMOF/MYST1 (hKat7)	Sas3 (yKat6)	Yes	Slight differences [†]
Putative Rtt109-type acetyltransferase					
AN8807.4	<i>rtt109</i>			Yes	Major differences [‡]
Putative Hat1 acetyltransferase					
AN6214.4	<i>nha1</i>	HAT1 (hKat1)	Hat1	Yes	Slight differences [†]
No putative p300/CBP-type acetyltransferase					

Targets for gene deletion were chosen according to the Broad Institute *Aspergillus* Comparative Database feature search for the following gene families: GNAT, MYST, p300/CBP, and Rtt109. Targets represent the major families of acetyltransferases (1). The deletion strains were obtained by homologous recombination using *argB* as a selectable marker. Correct integration of the deletion cassette was verified by Southern blot as shown for $\Delta gcnE$ and $\Delta adaB$ in Fig. S1. All deletion strains were phenotypically tested on different media and exposed to stress conditions. Strains showing a phenotype different from the wild type are indicated.

*NCBI blast score >130.

[†]In at least two conditions, minor changes in color or growth compared with the wild type.

[‡]Deletion strain phenotype strongly differs from wild-type strain.

1. Sterner DE, Berger SL (2000) Acetylation of histones and transcription-related factors. *Microbiol Mol Biol Rev* 64:435–459.

Table S2. Bacterial and fungal strains

Strain	Genotype	Ref.
<i>Streptomyces rapamycinicus</i>		(1)
<i>Aspergillus nidulans</i>		(2)
A1153	<i>yA1, pabaA1; argB2; pyroA4, nkuA::bar</i>	This study
A1153Δ <i>gcnE</i>	<i>yA1, pabaA1; gcnE::argB2; pyroA4, nkuA::bar</i>	This study
A1153Δ <i>adaB</i>	<i>adaB::argB2, yA1, pabaA1; pyroA4, nkuA::bar</i>	This study
A1153Δ <i>laeA</i>	<i>yA1, pabaA1; pyroA4, nkuA::bar; laeA::argB2</i>	This study
A1153 <i>gcnE</i> ^C	<i>yA1, pabaA1; (gcnE::argB2)::gcnEp-gcnE-pabaA1; pyroA4, nkuA::bar</i>	This study
A1153 <i>gcnE</i> -3xflag	<i>yA1, pabaA1; gcnE::gcnEp-gcnE-3x-flag-pabaA1; pyroA4, nkuA::bar</i>	This study
A1153 <i>gcnE</i> -S-tag	<i>yA1, pabaA1; gcnE::gcnEp-gcnE-S-tag-pabaA1; pyroA4, nkuA::bar</i>	This study

1. Kumar Y, Goodfellow M (2008) Five new members of the *Streptomyces violaceusniger* 16S rRNA gene clade: *Streptomyces castelarensis* sp. nov., comb. nov., *Streptomyces himastatinicus* sp. nov., *Streptomyces mordarskii* sp. nov., *Streptomyces rapamycinicus* sp. nov. and *Streptomyces ruanii* sp. nov. *Int J Syst Evol Microbiol* 58:1369–1378.
2. Nayak T, et al. (2006) A versatile and efficient gene-targeting system for *Aspergillus nidulans*. *Genetics* 172:1557–1566.

Table S3. Oligonucleotides used to create probes for Northern blots, gene knockout cassettes, CHIP experiments, and qRT-PCR

Oligonucleotide	Sequence
Oligonucleotides used to generate transformation cassettes	
<i>gcnE</i>	
3621lbfor	GAGAGGGTAAAGTATGGTTCC
3621lbforQ	GATTGCATTGTGCAGCCAGG
3621lbrev	GCAAACAGAACTTTGACTCCC ATTGTAAGCTTGTGCTATGCGAG
3621rbfor	ATTGACATGTTCTCTCGTCCCTTGCATCGCGTGGC
3621rbrev	CTGCTATTGACATTGTCCG
3621rbrevQ	GAGGAAAGCTGACAGTCTCTG
<i>AdaB</i>	
10763lbfor	CGAGACACATCAAACCTGAATGC
10763lbforQ	CGTACGGAAGGTACTTGGTTG
10763lbrev	GCAAACAGAACTTTGACTCCCAT TGCTTGATGTCTCCGAGC
10763rbfor	ATTGACATGTTCTCTCGCTTCC TTACAAGTTTTATCAAATATCATTTCAAGT
10763rbrev	GAACCTGCTAAGATTCTCAAGG
10763rbrevQ	GAGAACTGTTCAACAAGAGTCC
argBcassfor	ATGGGAGTCAAAGTCTGTTTGCC
argBcassrev	GGAAGCGAGAGAACATGTCAATTAG
<i>gcnE^C</i>	
3621komplbrev	GACCTTTCTACAGATCTGGCAGTAGTAGACTGGATC CAAAGG
3621komprbfor	CTCCGTCGCATGTCCAGATTATAGTTGGCTGGTGGT GGAG
3621komprbrevQ	GACGTGCCATTATCGATAAGG
3621komprbrev	CCAGCAACGAATGTTTCTTGG
<i>gcnE-3xflag, gcnE-S-tag</i>	
3621stagntermlbrev	GCGGCGGCGGTCTCCTTCATTGTAAG CTTGTTGCTATGCGAG
3621stagntermorffor	ATGAAGGAGACCGCCGCCCAAGTTCCG AGCGCCAGCACATGGACTCCACCGGAACCACCGGAAC CACTGAAAGTGGGTTGCAGGC
3621stagctermlbrev	GGAGTCCATGTGCTGGCGCTCGA ACTTGCGGCGGCGGTCTCCTTGTTCC GGTGGTCCGGTAATTGACTTACCGACCACTCG
3621stagctermdsfor	AGCGCCAGCACATGGACTCCTGACCTTGC ATCGCGTGGC
36213flagtagntermlbrev	CCGTCGTGGTCTTGTAGTCCATTGT AAGCTTGTGCTATGC
36213flagtagntermorffor	ATGGACTACAAGGACCACGACGGTGACTACAAG GACCACGACATCGACTACAAGGACGACGACGACAAGA CCGGAACCACCGGAACCACTGAAAGTGGGTTGCAGGC
36213flagtagctermlbrev	GGTTCGGTGGTCCGGTCTTGTGCTGCTGCTGCTCCTTGT AGTCGATGTCGTGGTCTTGTAGTACCGTCTGTTGGTCC TTGTAGTCGGTCCGGTGGTCCGGTAATTGTACTTAC CGACCA
36213flagtagctermdsfor	AGACCGGAACCACCGGAACCTGACCCTTGCATCG CGTGGC
Oligonucleotides for probes used in Northern blots	
aatbfor	GTAAACCTAATCCAGCTCACC
aatbrev	CAATGTCGCTAGATCGCTTCC
aflRfor	GAGAGTTGCATCTCGTGCTC
aflRrev	GAGAGTGACCACGTAATCATC
stcOfor	CCAGTGACCTCACAGTGAATG
stcOrev	CCTTGCCATGCAGTCTATC
7801for	CATCGACCAAGTGAGACAGG
7801rev	CACTCCATGATCTTCAATCAAG
7826for	CTGATATGCGTGACAAGCAGG
7826rev	GAAGTCAGTGTGCGAACCTG
tdiAfor	GTATACCACCAACAGCATC
tdiArev	GGTGTCTGCTTATCAGGTTAAG

Table S3. Cont.

Oligonucleotide	Sequence
tdiBfor	CTACAGAATACTGGTCCCGTC
tdiBrev	CATGAGTTCGGACACATCCTG
laeAfor	GTATCGGAGAGTCTGATCTAC
laeArev	CCTAGCCTGGTATATGTGCAG
aatAfor	GATTACAGAAGCTGGTATCATTGG
aatArev	GCTAAAGGTCATGACAAAGGTC
acvAfor	ACATTCCCGAGAGCCAATTTGCG
acvArev	GTCGTGCGGAATCATGTATCGG
orsAfor	CAGCGATCTTATCCGGACATGCAGG
orsArev	CATCGACATTGAACCGGCTCTCGG
orsBfor	TGGACCCAGTGCCGAAGCCTGC
orsBrev	CGTTGATTATGTCCCATTCGACAGTGC
orsDfor	TTGCACGACAAGATGCCCCAGTGAG
orsDrev	TCGCAGACCCTCACCAGATGTGTGGC
AN7915for	TTAACGAGCAGGAGACCAACCGGACCG
AN7915rev	AACCCTCGACTGAAGTGCATGTTAG G
AN7918for	GACCACAGCGCGGAAGCACGTC
AN7918rev	CTTCCGCCATATCCACCAGACTATCTGC
actfor	ATCGGTATGGGTGAGAAGGACTC
actrev	AGTGGTGACGTGGATACCACCGC
Oligonucleotides used for ChIP experiments	
7909pchipfor	CAGCTTCCAGCCATGATTAAG
7909pchiprev	GCACTGCTGTTCTATTGCC
7913pchipfor	GTGAGTCCAGAGTATCCCAG
7913pchiprev	AATGAGAAGAATGCCAAGCC
7915pchipfor	CGGTAGACCCTAAACTAAATGG
7915pchiprev	GCGGTAAAAGGAACGGAAG
7918pchipfor	AGAATGAAGACGAGACGGAC
7918pchiprev	ATTTGGCTGTGGAAGGGTG
stcOpFchip	CCTGATGTAGGATTAGGATAGAATG
stcOpRchip	AAGGCTGGGCTGAAGATTATCGAG
AN7801pFchip	TGTACCCAATAGCGTCCCATCATAAG
AN7801pRchip	CACACTTCAACTCTGTAATCCAAGG
aflRpromfwdchip	GTACAGCAAAGACAAAGATATGCG
aflRpromrevchip	GTAAGTGTAAAGCTGTACTTGGG
acnchipfor	CTGGGCTGAACCATCATTAC
acnchiprev	ACTACACAAAGACTGCTACG
tdiAchipfor	CGTCAGCGTGTAATATGAAC
tdiAchiprev	CAGAAGGGTCTCAATGAGTG
tdiBchipfor	GTAAGTCCAATCAGGCCCG
tdiBchiprev	ACTGTCCATCATATACGCCCC
Oligonucleotides used for qRT-PCR	
9315qrtfor3	GGAGCAAGTTACGGCAAAG
9315qrtrev3	TAAGGAACCGTCGAGGAAG
5831qrtfor	TTGGGGAGCATATCACGGAG
5831qrtrev	GGTAGTCGTGTCGGATCATC
4905qrtfor	ATGTGCCTAGTCCGCATAC
4905qrtrev	GCATCATTCTTCATCCCCTC
2846qrtfor	TCAACGGAGATAATGCGAAC
2846qrtrev	CTTTATCCTCTTCAACCCCAAC
9397qrtfor	GCGACGAAGAAGAACCTTG
9397qrtrev	GTTGAACTCCGTGAACTCC
3974qrtfor	GACGAAGAACATCTGAATCCC
3974qrtrev	TCAGCCTCTACAGCAATCC
6669qrtfor	GCATCATTGCTGCATCACC
6669qrtrev	AGCCCCAGATGAAGAACACC
2839qrtfor	TCCTCAATGCCGCAGAAAC
2839qrtrev	ACCAGAAAGAACCCATCTCC