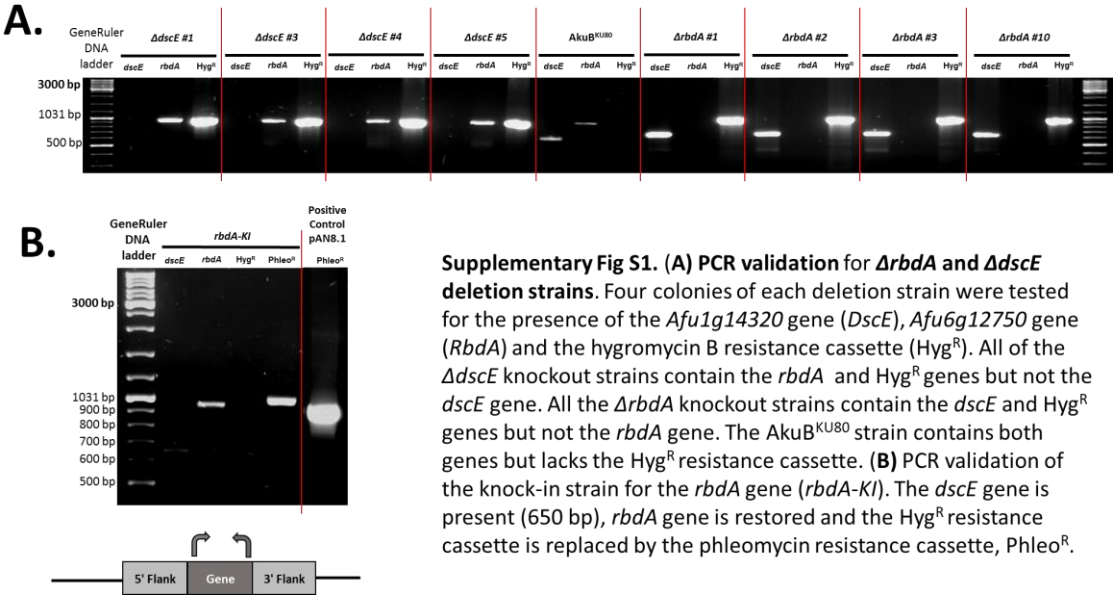
SUPPLEMENTARY METHODS

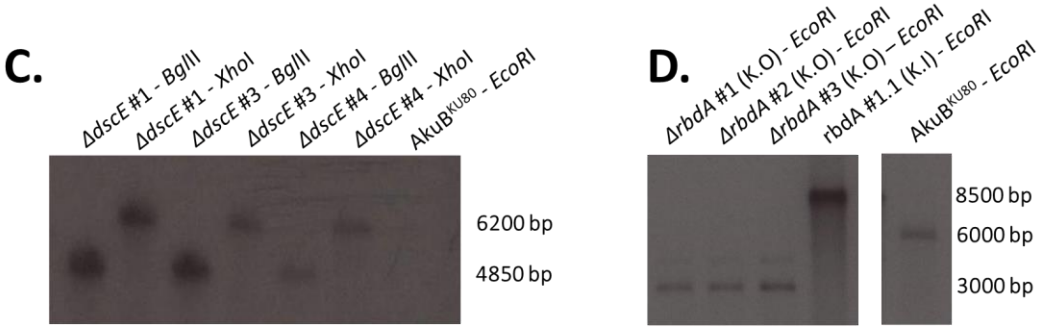
Generation and confirmation of *ΔrbdA*, *ΔdscE* and *ΔdigA* deletion mutants and *rbdA* reconstituted strain. *A. fumigatus* CEA17 *AkuB*^{KU80} (1) was used as a wild-type in all experiments. Deletion and reconstituted strains were created using the Yeast Gateway system. Briefly, to generate the knockout strains (*ΔrbdA*, *ΔdscE* and *ΔdigA*), a ~1 kb DNA fragment flanking the *A. fumigatus rbdA, dscE & digA* genes was generated by PCR, using the Expand high-fidelity PCR system (Roche Diagnostic, Penzberg, Germany) and primers designed to contain P1, P2 (for 5' flanks) and P3, P4 (for 3' flanks) sequences [Supplemental Table S2]. As a selection marker, we used the *hph* resistance cassette (~2300 bp), carrying a hygromycin B resistance gene with the *gpdA* promoter and terminator of *A. nidulans*. These fragments were transformed, together with a *Hind*III/*Xho*I linearized pYES-2 cloning vector, into BY7470 chemically competent *S. cerevisae* yeasts to yield the cloning vectors: pYES2-*ΔrbdA* (primers: P1-rbdA, P2-rbdA-KO, P3-rbdA-KO, P4-rbdA-KO)**,** pYES2-*ΔdscE* (primers: P1-dscE-KO, P2-dscE-KO, P3-dscE-KO, P4-dscE-KO) and pYES2-*ΔdigA* (primers: P1-digA-KO, P2-digA-KO, P3-digA-KO, P4-digA-KO) Supplemental Table S2 and Supplemental Fig. S2 A. Each of these plasmids was validated by PCR and sequencing. The validated plasmids were linearized, using the *Psi*I restriction enzyme, and transformed into the *AkuB*^{KU80} background strain protoplasts as previously described (2). PCR validation for the *ΔRbdA, ΔDscE* strains is shown in Supplemental Fig. S1 (A).

To generate the *rbdA* knockin strain (*rbdA*-KI), a ~2.5 kb DNA fragment flanking the *A. fumigatus RbdA* gene (1.1 kb upstream and 300 bp downstream to *rbdA*) was generated by PCR, using the Expand high-fidelity PCR system (Roche Diagnostic, Penzberg, Germany) and primers designed to contain P1, P5 (for the 5' flank + *rbdA*

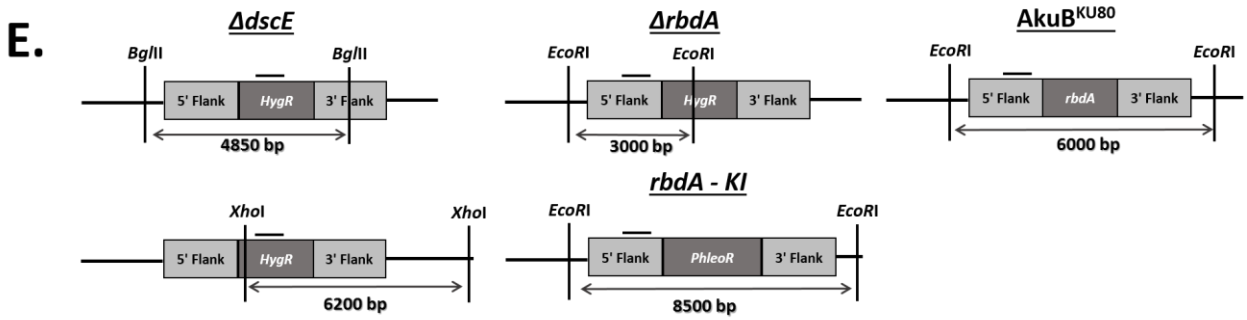
fragment) and P6, P7 (for 3' flanks) sequences (primers: P1-rbdA, P5-rbdA-KI, P6-rbdA-KI and P7-rbdA-KI) (Supplemental Table S2 and Supplemental Fig. S2B). As a selectable marker, we used the *phleo* resistance cassette (~2500 bp), carrying a phleomycin resistance gene with the *gpdA* promoter and terminator of *A.nidulans*. These fragments were transformed, together with *Hind*III /*Xho*I linearized pYES-2 cloning vector, into BY7470 chemically competent *S. cerevisae* yeasts to yield the cloning vector: pYES2-*rbdA*-KI. This plasmid was validated by PCR and sequencing. The validated plasmid was linearized, using *Psi*I restriction enzyme, and transformed into the *ΔrbdA* knockout strain protoplasts as mentioned. Three or more independent mutants were identified and verified for each strain by means of PCR, using the primers: rbdA-fwd-val and rbdA-rev-val, dscE-fwd-val and dscE-rev-val, Hyg-fwd and Hyg-rev, Phleo-fwd and Phleo-rev respectively (Supplemental Table S2). PCR validation for the *RbdA-KI* strain is shown if Supplementary Fig. 1 (B) and by Southern blot as described below.



Supplementary Fig S1. (**A) PCR validation** for ***ΔrbdA* and *ΔdscE* deletion strains**. Four colonies of each deletion strain were tested for the presence of the *Afu1g14320* gene (*DscE*), *Afu6g12750* gene (*RbdA*) and the hygromycin B resistance cassette (Hyg^R). All of the *ΔdscE* knockout strains contain the *rbdA* and Hyg^R genes but not the *dscE* gene. All the *ΔrbdA* knockout strains contain the *dscE* and Hyg^R genes but not the *rbdA* gene. The $AkuB^{KU80}$ strain contains both genes but lacks the Hyg^R resistance cassette. (**B)** PCR validation of the knock-in strain for the *rbdA* gene (*rbdA-KI*). The *dscE* gene is present (650 bp), *rbdA* gene is restored and the Hyg^R resistance cassette is replaced by the phleomycin resistance cassette, $Phleo^R$.



Supplementary Fig S1. (C) Southern Blot analysis for the *ΔdscE /Afu1g14320* null strains. Genomic DNA from each strain was digested with either *Bgl*II or *Xho*I restriction enzyme. The blot was hybridized with a radiolabeled Hyg^{R} probe. All colonies except for the AkuB^{KU80} were labeled as expected.
(D) Southern Blot analysis for the *ΔrbdA/Afu6g12750* null and *rbdA-KI* reconstituted strains. Genomic DNA from each strain was digested with *EcoR*I. The blot was hybridized with a radiolabeled probe matching a short sequence upstream to the *rbdA* gene. The original length of the digested fragment is 5968 bp as shown for the *AkuB^{KU80}* strain. Gene deletion yielded a shorter (2957 bp) fragment, while gene reconstitution in the *rbdA-KI* strain resulted in a larger (8487 bp) fragment.



Supplementary Fig 1. (**E)** Southern Blot restriction maps for the *ΔdscE /Afu1g14320* *ΔrbdA/Afu6g12750* null and *rbdA-KI* and *AkuB^{KU80}* strains. The radiolabled probe is designated by a line above the gene map.

Southern verification of *ΔRbdA, ΔDscE* and *RbdA-KI.* Southern analysis was performed as previously described (2). ^{32}P- *hph* -labeled DNA probe (generated by using Hyg-fwd and Hyg-rev primers- Supplemental Table S2). was used to verify correct integration of the deletion construct into the *dscE* gene locus of the *ΔdscE* strain, resulting in a single 4580 bp fragment (when the DNA was cut with *Bgl*II restriction enzyme) or a 6200 bp fragment (when the DNA was cut with *Xho*I restriction enzyme) (Supplemental Fig. S1C, E).

^{32}P- *rbdA* labeled DNA probe (generated by using P1-rbdA and P5-rbdA-KI primers Supplemental Table S2) was used to verify correct integration of the deletion construct and insertion construct into the *rbdA* gene locus of the *ΔrbdA* and *rbdA-KI* strains. The probe is complementary to the 5' flank locus of the constructs, resulting in a single 3000 bp band for the *ΔrbdA* strain, a single 8500 bp band for the *rbdA-KI* strain and a single 6000 bp band for the *AkuB*^{KU80} background strain when genomic DNA was cut with EcoRI restriction enzyme. (Supplemental Fig. 1D, E).

Generation and confirmation of c-myc tagged *srbA* gene expressing mutants.

Here we utilized the 'single cross-over' insertion method. We have prepared two myc-tagged constructs, one for a full-length *srbA* (termed srbA-FL) and one for a truncated and thus constitutively active *srbA* (termed N-srbA expressing the first 425 amino acids).

Generation of the N-srbA construct. To generate the N-srbA construct, two fragments were cloned. The first includes a ~1.35 kb DNA fragment (the first 1275 bases of the *srbA* gene that encode the first 425 amino acids, 2 *myc*-tag sequences, the stop codon sequence TAG and a *BamH*I restriction site), generated by Phusion high-fidelity PCR. The reverse primer was designed to contain 2 *myc*-tag sequences, the stop codon TAG and a *BamH*I restriction site. (primers: N-SrbA-Fwd, N-SrbA-myc-Rev) (Supplemental Table S2). The second fragment includes ~0.5kb DNA fragment (downstream to the *A. fumigatus srbA* gene, flanked by *BamH*I and *Pst*I restriction sites respectively), generated by Phusion high-fidelity PCR. The forward primer was designed to contain a *BamH*I restriction site and the reverse primer was designed to contain a *Pst*I restriction site. (primers: N-SrbA-peptide-Fwd, N-SrbA-term-Rev) (Supplemental Table S2). These fragments was cloned into the pGEM-T easy vector separately, cut using their designated restriction enzymes and cloned into pAN8.1

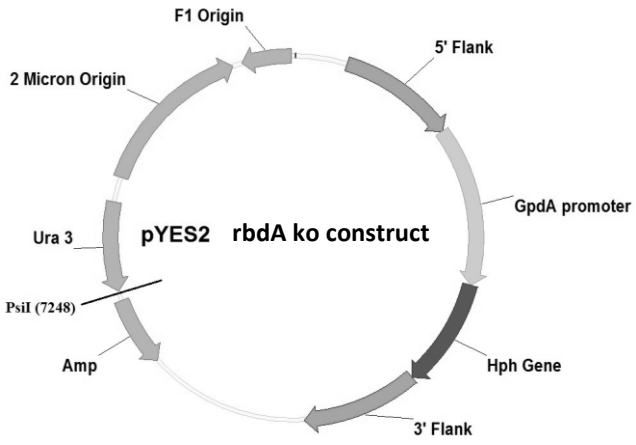
vector linearized with *Eco*RI and *Pst*I restriction enzymes. This vector contains the *phleo* resistance cassette (~2500 bp), as a selectable marker (Supplemental Fig. 2C).

Generation of the srbA-FL-myc construct: To generate the srbA-FL-myc construct, two fragments were cloned. The first, a ~2.6 kb DNA fragment (2.5 kb upstream to the *A. fumigatus srbA* gene, the first 12 bases of the gene, 2 myc tags sequences and a BamHI restriction site) generated by Phusion high-fidelity PCR with SrbA-promoter-Fwd, SrbA-myc-Rev primers (Supplemental Table S2). The second fragment includes ~3.2 kb DNA fragment (3 kb of the rest of the *A. fumigatus srbA* gene, 207 bases downstream to the gene and a BstBI restriction site), generated by Phusion high-fidelity PCR system with primers SrbA-peptide-Fwd, SrbA-term-Rev) (Supplemental Table S2). The two fragments were first subcloned into the pGEM-T easy vector (Promega, USA) and then cut with their designated restriction enzymes and cloned into the pAN8.1 vector that was linearized using EcoRI and BstBI restriction enzymes. This vector contains the *phleo* resistance cassette (~2500 bp), as a selectable marker (Supplemental Fig. 2D).

Generation of the *AkuB^{KU80}GFP-srbA N-term*, *AkuB^{KU80}GFP-srbA* and *ΔrbdA GFP- srbA N-term* strains. *A. fumigatus* CEA17 *AkuB^{KU80}* (1) was used as a wild-type in all experiments. To generate fungal strains expressing a GFP-fused N-terminal domain of SrbA (GFP-SrbA$_{Nterm}$) the xylose promoter sequence and the *gfp* gene were first amplified from the plasmid pXylpsGFP_GEM–hph (a kind gift from Hubertus Haas)(3) using the primers P5-gfp-srbA and P6-gfp-srbA (Supplemental Table S2). Primers P3-gfp-srbA and P4-gfp-were used to amplify the pyrithiamin resistance cassette from pSK275 (4). Primer pairs P1/P2 and P7/8 amplified an upstream fragment and the first 813 bp of the *srbA* gene, respectively. The latter encoded the N-terminal 271 amino acids of SrbA. An overlap extension PCR (oePCR) was used to

join fragments P1-P2 with P3-P4 and P5-P6 with P7-P8. The resulting fragments P1-P4 were cloned into pJET1.2 (Thermo Scientific, Schwerte Germany) according to the manufacturer`s instructions and fragment P5-P8 was introduced in the resulting plasmid using *Avr*II and *Asc*I restriction sites (Supplemental Fig. 2E). To generate the N-terminal *gfp*-fusion with full length *srbA* the same primers were used, except that P10-srbA-fl was used to amplify full length *srbA*. The entire fusion construct of Pxyl-gfp-srbA-fl was amplified using P9 and P10 as primers and cloned into pSK275 using *Not*I restriction sites (Supplemental Fig. 2F). The resulting plasmids pJET-GFP-NsrbA and pSK-GFP-srbA were purified from *E. coli* and used for the transformation of protoplasts from *A. fumigatus* CEA17 *AkuB*^{KU80} and Δ*rbdA*. Transformants were restreaked twice on MM agar selecting for pyrithiamine resistance and genomic integration of the cassettes was verified by PCR.

A



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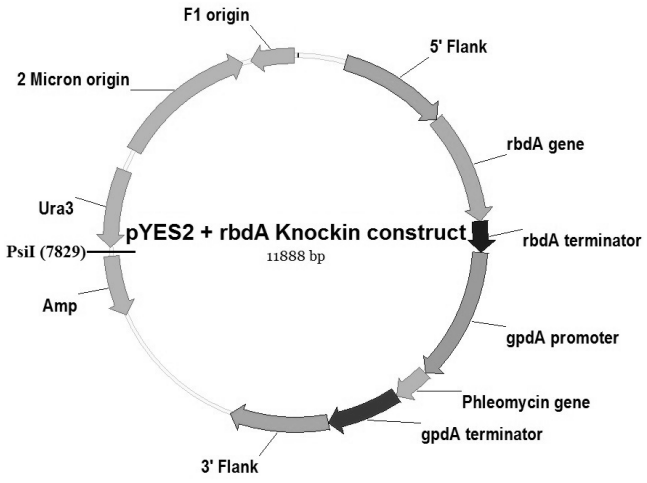

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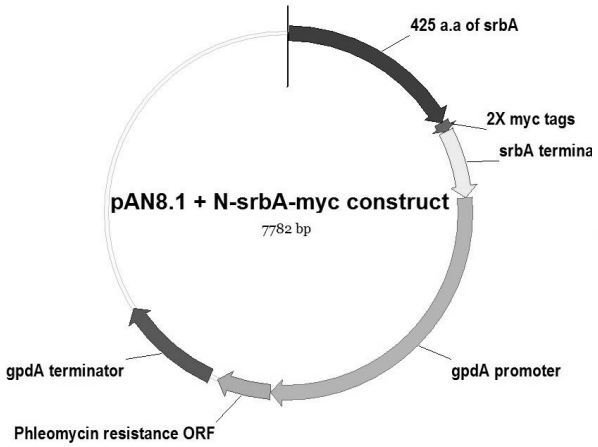

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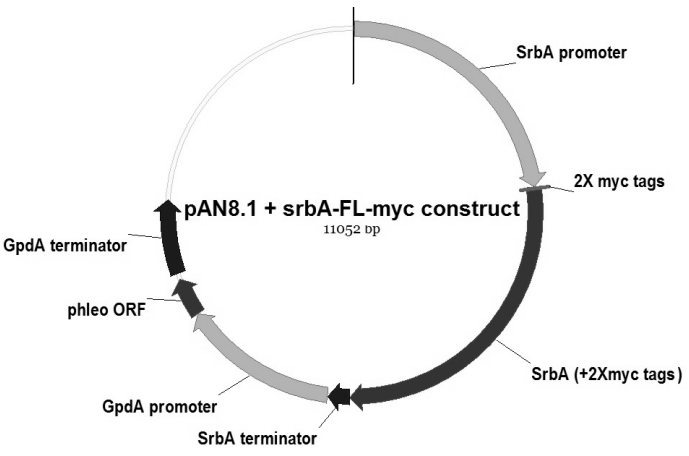

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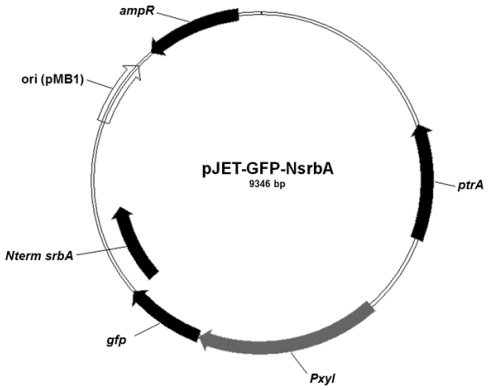
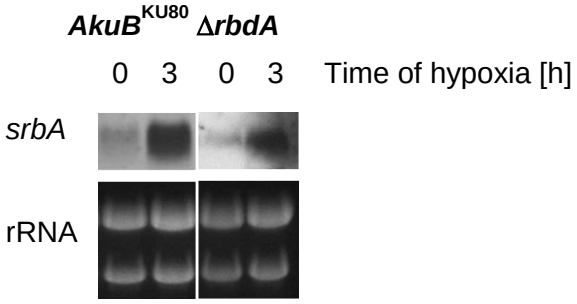


F.

Supplemental Figure S2. Schematic drawing of plasmids (A-F) used in this work



Supplemental Figure S3. Northern blot analysis of the regulation of *srbA* in the wildytpe strain (*KU80*) and Δ*rbdA* deletion strain. The strains were cultivated for 16 h in MM ($1 \cdot 10^6$spore/ml inoculum) under normoxic coniditions and were then shifted for 3 h to hypoxic conditions (0.2% O_2). 10 µg RNA was loaded per lane. rRNA was used as loading control. The blot was hybridized with an srbA-specific probe prepared by PCR amplification with primers:
Forward 5‘ CGAGATTCGTAACAAGAGATTGG
Reverse 3’ CCTCGAAATACCTGAGAAGAGC

Supplemental Table S1. Strains used in this study

Strain	Organism	Genotype	Source
AkuB^{KU80}	*A. fumigatus*	*CEA17 Afu2g02620::pyrG*	da Silva Ferreira *et al.*, 2006
Δ*rbdA*	*A. fumigatus*	*Afu6g12750::hph*	This work
Δ*dscE*	*A. fumigatus*	*Afu1g14320::hph*	This work
Δ*srbA*	*A. fumigatus*	*Afu2g01260::hph*	This work
Δ*rbdA-KI*	*A. fumigatus*	*Afu6g12750::hph Afu6g12750-phl*	This work
Δ*stuA*	*A. fumigatus*	*Afu6g14090::hph*	Sheppard *et al., 2005*
*Δ*rbdA-NsrbA-myc*	*A. fumigatus*	*Afu6g12750::hph NsrbA-myc-phl*	This work
*Δ*rbdA-srbA-myc*	*A. fumigatus*	*Afu6g12750::hph srbA-myc-phl*	This work
*Δ*dscE-NsrbA-myc*	*A. fumigatus*	*Afu1g14320::hph NsrbA-myc-phl*	This work
*Δ*dscE-srbA-myc*	*A. fumigatus*	*Afu1g14320::hph srbA-myc-phl*	This work
AkuB^{KU80}GFP-srbA N-term	*A. fumigatus*	*Pxyl-gfp-NsrbA-ptrA*	This work
*Δ*rbdA GFP- srbA N-term*	*A. fumigatus*	*Afu6g12750::hph Pxyl-gfp-NsrbA-ptrA*	This work
AkuB^{KU80}GFP-srbA-fl	*A. fumigatus*	*Pxyl-gfp-srbA-ptrA*	This work
*Δ*rbdA GFP-srbA-fl*	*A. fumigatus*	*Afu6g12750::hph Pxyl-gfp-srbA-ptrA*	This work

Supplemental Table S2. Oligonucleotides used in the study

name	Sequence 5‘-3‘
FHp_fw	GCTCACTGCCCATCCCG
FHp_rev	CCTTGTCCTCCTCCGTGG
5G06240_fw	GCACCGAGTTTCTCGCACAT
5G06240_rev	GACAATCCCAGACAAGCAATG
erg25A_fw	CATCCCAGTGCCTAAACGAAC
erg25A_rev	CACGAAATCATTACTTCAGCC
erg11A_fw	CACACCAAGGTTGACATAAGC
erg11A_rev	GGACTATCTGCGCGATTCAC
P1-gfp-srbA	GGTGATTGATGAAGAAGTGGC
P2-gfp-srbA	CAAGAGGCCATCTAGGCCATCTTTCACTGCTGGTCTTGGACC
P3-gfp-srbA	GATGGCCTAGATGGCCTCTTG
P4-gfp-srbA	AAACCTAGGAAATTTGGCGCGCCAATTGATTACGGGATCC
P5-gfp-srbA	AAAGGCGCGCCACTGATGCGAGCAACAGTATG
P6-gfp-srbA	CTTGTACAGCTCGTCCATGC
P7-gfp-srbA	GCATGGACGAGCTGTACAAGTCCACCCCTGGCATTGGTGG
P8-gfp-srbA	AAAACCTAGGTAACAATCTGATCAGCGG
P9-gfp-srbA-fl	AAAGCGGCCGCCACTGATGCGAGCAACAG
P10-srbA-fl	GCGGCCGCTTACTACAGATCCTCCTCGGAAATCAGCTTCTGCTCATCGGAGAGCAGTAG
P1-dscE-KO	GCTGTAATACGACTCACTATAGGGAATATTGCAAGCGTAGCACCTTTAT
P2-dscE-KO	CAATAGTGCCACGTTCTAAATTCAACCAAGGTGATCCATACAACGACAGTAATA
P3-dscE-KO	CCCAGCACTCGTCCGAGGGCAAAGGAATAGGGGCTCCTGTTGATGTATATG
P4-dscE-KO	CATAACTAATTACATGATGCGGCCCTCTAGCGTCGTTACAGTTCGGTTT
dscE-fwd-val	CTCTACTACGCCGATCACAATG
dscE-rev-val	ACCCAGGCGTTTGTATCATC
P1-rbdA-KO	GCTGTAATACGACTCACTATAGGGAATATTGCTTCGGTGGAAAGGTAAG
P2-rbdA-KO	CAATAGTGCCACGTTCTAAATTCAACCAAGTGGCCGATGGAGGAATAA
P3-rbdA-KO	CCCAGCACTCGTCCGAGGGCAAAGGAATAGACTTGTCAACAGGTCCTTATTT
P4-rbdA-KO	CATAACTAATTACATGATGCGGCCCTCTAGTATCGACCCACGTTTAAAGTATC
rbdA-fwd-val	CGTATATGCTCCGATTACCACTT
rbdA-rev-val	TCAGCTTTCCTTCAATCCATCT
P1-digA-KO	GCTGTAATACGACTCACTATAGGGAATATTCTCCAGACATTGACGACAGATAA
P2-digA-KO	CAATAGTGCCACGTTCTAAATTCAACCAAGACGAGCCTAGCAGGTAAGA
P3-digA-KO	CCCAGCACTCGTCCGAGGGCAAAGGAATAGGACAAGCTCCGCAAGAAATTG
P4-digA-KO	CATAACTAATTACATGATGCGGCCCTCTAGTCCTAACGCTTGCATGATAGTT
digA-fwd-val	ACGAATCGCATCCTTCGTATAG
digA-rev-val	CGAACTCTTTCTCAGCGTAACT
P5-rbdA-KI	ATACAATGCTCATCATAACATTCCACCTGGCTGCGCGTGTTTCTGATTT
P6-rbdA-KI	CGTTTACCCAGAATGCACAGGTACACTTGTATTCTTGGTTTGTCCGCGACAT

P7-rbdA-KI	CATAACTAATTACATGATGCGGCCCTCTAGAGTTGCCCGTTGACACCATC
Hyg-fwd	GTTTCTGATCGAAAAGTTCGACAG
Hyg-rev	CGTCGGTTTCCACTATCGGCG
Phleo-fwd	TAATTGGCCCATCCGGCATCTGTA
Phleo-rev	AGCTGACATCGACACCAACGATCT
srbAFL-prm-fwd	ATCAATTGCTCCAGGGACCTTC
srbAFL-myc-rev	ATGGATCCCAAGTCCTCTTCAGAAATGAGCTTTTGGCCGCCCAAGTCCTCTTCAG
	AAATGAGCTTTTGGCCGCCGGGGGTGGACATCGTGTTTC
srbAFL-pep-fwd	ATGGATCCGGCATTGGTGGGGATTTCCA
srbAFL-term-rev	ATTTCGAACCGAGAGAGAAACGCCTTGTA
N-srbA-fwd	ATGTCCACCCCCGGCATTGG
N-srbA-myc-rev	ATGGATCCCTACAAGTCCTCTTCAGAAATGAGCTTTTGGCCGCCCAAGTCCTCTT
	CAGAAATGAGCTTTTGGCCGCCAATTGCGCGCGCGTGCCAA
N-srbA-term-fwd	ATGGATCCTGATGATGACGCTACTGCCAG
N-srbA-term-rev	ATCTGCAGGCGTAAGTCTAGTCCCGTGAAA
srbA-myc-val-fw	TGAACTTAAACCCGCAGTTAGCC
srbA-myc-val-re	TTTTGGCCGCCCAAGTCCTC
SrbA-prom-fwd	ATCAATTGCTCCAGGGACCTTC
SrbA-myc-Rev	ATGGATCCCAAGTCCTCTTCAGAAATGAGCTTTTGGCCGCCCAAGTCCTCTTCAGAAATGAGCTTTTGGCCG CCGGGGGTGGACATCGTGTTTC
SrbA-pept-fwd	ATGGATCCGGCATTGGTGGGGATTTCCA
SrbA-term-Rev	ATTTCGAACCGAGAGAGAAACGCCTTGTA
N-SrbA-fwd	ATGTCCACCCCCGGCATTGG
SrbA-myc-Rev	ATGGATCCCTACAAGTCCTCTTCAGAAATGAGCTTTTGGCCGCCCAAGTCCTCTTCAGAAATGAGCTTTTGG CCGCCAATTGCGCGCGCGTGCCAA
N-SrbA-term-fwd	ATGGATCCTGATGATGACGCTACTGCCAG
N-SrbA-term-rev	ATCTGCAGGCGTAAGTCTAGTCCCGTGAAA

Gene name	**Primer sequence**
Mpo	Forward, 5′-TTACACCCCAGGCATAAAAA-3′
	Reverse, 5′-TTCCATACAGCTCAGCACAA-3′
Cxcl1	Forward, 5′-CCGCTCGCTTCTCTGTGC-3′
	Reverse, 5′-CTCTGGATGTTCTTGAGGTGAATC-3′
Tnfa	Forward, 5′-CGAGTGACAAGCCTGTAGCC-3′
	Reverse, 5′-AAGAGAACCTGGGAGTAGACAAG-3′
Ifng	Forward, 5′-ACTGGCAAAAGGATGGTGAC-3′
	Reverse, 5′-TGAGCTCATTGAATGCTTGG-3′
Il17a	Forward, 5′-GACTACCTCAACCGTTCCAC-3′
	Reverse, 5′-CCTCCGCATTGACACAGC-3′
Il4	Forward, 5′-CGGCATTTTGAACGAGGTCACAGG-3′
	Reverse, 5′-AGCACCTTGGAAGCCCTACAGACG-3′
Il10	Forward, 5′-CCCTTTGCTATGGTGTCCTT-3′
	Reverse, 5′-TGGTTTCTCTTCCCAAGACC-3′
Il1b	Forward, 5′-TGACGGACCCCAAAAGATGAAGG-3′
	Reverse, 5′-CCACGGGAAAGACACAGGTAGC-3′
Rage	Forward, 5′-GGAATTGTCGATGAGGGGAC-3′

Gene name	Primer sequence
	Reverse, 5′-CAACAGCTGATTGCCCTCTG -3′
S100	Forward, 5′-GCCCTCATTGATGTCTTCCACC -3′

Supplemental References

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