Supporting Information

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SI Materials and Methods

Suppression of Mechanical Injury–Induced Sporulation by 5-Fluorouracil. The assays of treatment with 5-fluorouracil (5-FU) was done as described previously by Galun and Gressel (1). Seven sheets of sterile Whatman no. 1 filter paper were soaked in 3 mL of potato dextrose broth (PDB) (Difco) in 8.5-cm Petri dishes, with a sheet of sterile hardened filter paper (Whatman; no. 50) placed at the bottom of the plate. Plugs of mycelium were placed in the center of the filter paper. Cultures were kept in the dark at 28 °C for 36 h, and then mycelium was injured and incubated for 4, 8, and 12 h. The filter paper with the mycelium adhered was immediately transferred 30 min to a plate containing 50 μg of 5-FU in PDB. The filter paper with the treated mycelium was washed with distilled water to remove excess 5-FU and transferred into a plate with fresh PDB. Cultures were incubated for an additional 48 h in the dark and photographed. For the 0-h treatment, the cultures were incubated for 10 min before and 20 min after injury. For the untreated control, the culture was transferred to a plate containing only PDB. Growth and treatments were carried out at 28 °C in the dark.

Effect of Antioxidants and DPI Inhibitor in Mechanical Injury–Induced Conidiation. For treatments with ascorbic acid, fungal colonies grown on filter paper with PDB (as described above for the effect of 5-FU) were transferred to plates containing a 30 mM ascorbic acid solution and incubated for 10 min before and 20 min after injury, and then the filter paper with the treated mycelium was washed with distilled water to remove excess AA and transferred to a plate with fresh PDB. Colonies were photographed after an additional 48 h of incubation. For this study, two controls were made, an untreated control and other treated with water. For assays with NAC, plugs of mycelium were inoculated in plates with MMV-CN supplemented with 60 mM NAC. Cultures were incubated for 40 h; mycelium was injured with a scalpel and photographed after an additional 48 h of incubation. Two controls were prepared: the first control consisted of only MMV-CN, and NAG (Sigma) was added to the second control.

To determine the effect of the NADPH oxidase inhibitor DPI in mechanical injury-induced conidiation, plugs of mycelium were microcultivated in MMV-CN on microscope slides. Microcultures were incubated in the dark at 28 °C for 24 h and then incubated in liquid MMV-CN with 50 mM DPI (DPI was dissolved in DMSO) for 10 min before and 20 min after injury. Microcultures were then transferred to a new plate and incubated for an additional 48 h. Two controls were used in the experiment, one untreated control and another control using MMV-CN with less than 1% of DMSO. Photographs in all assays were taken after an additional 48 h of incubation. All cultures and treatments were carried out in the dark and incubated at 28 °C.

Functional Annotation of Differentially Expressed Genes. For annotation of differential genes, a first analysis was performed using a BLASTP comparing against Fungal Functional Catalogue Database (FunCatDB) (2) with an E value of $\leq 1e^{-5}$. Additionally, a BLASTP was done against the proteins encode in the genomes of other fungi [*Trichoderma reesei* (*Tr*) (3) (http://genome.jgi-psf.org/Trire2/Trire2.home.html); *Neurospora crassa* (*Nc*) (http://www.broad.mit.edu/annotation/genome/neurospora/Home.html); *Fusarium graminearum* (*Fg*) (http://www.broad.mit.edu/annotation/genome/fusarium_graminearum/MultiHome.html); *Fussarium oxysporum* (*Fo*) (http://www.broadinstitute.org/annotation/genome/fusarium_group/MultiHome.html); and *Aspergillus nidulans* (*An*) (http://www.broadinstitute.org/annotation/genome/aspergillus_group/MultiHome.html)], using an E value of $\leq 1e^{-5}$. A second BLASTP was done against nonredundant database (nr) of the NCBI (http://www.ncbi.nih.gov) using an E value of $\leq 1e^{-10}$. Subsequently, all proteins deduced from genes regulated in early times by injury (933) and 592 genes regulated for late times were compared against the Pfam database using the Web Server Batch Sequence Search (http://pfam.sanger.ac.uk/search#tabview=tab1) with a cutoff of E value of $\leq 1e^{-2}$. Finally, all data were analyzed manually. Expression patterns identified by agglomerative hierarchical clustering, the clustering was performed using smooth correlation and average linkage clustering in GeneSpring GX 7.3.1 software (Agilent Technologies).

Complementation of *Δnox1* and *ΔnoxR* Mutants with the WT *nox1 and noxR* Genes. The entire ORFs of *Nox1* and *NoxR*, including 2.0 kb of native promoters and 1.5 kb terminator sequences, were amplified using Phusion High Fidelity Taq polymerase (New England Biolabs). The primer combination oAM-LU108/oAM-LU80 (Table S1) was used for Nox1 (PCR product, 5.3 kb) and oAM-LU92/oAM-LU11 for NoxR (PCR product 5.5-kb). The PCR products were cleaned up and dATP-tailed using Faststart polymerase (Roche) before subcloning in pCR2.1 vector. The resulting plasmids pCR2.1-NoxAc and pCR2.1-NoxRc were sequenced and cotransformed with plasmid pII99 (kindly provided by Barry Scott, Institute of Molecular BioSciences and National Bio-Protection Research Center, Massey University, New Zealand) in the *Δnox1-15* strain and *ΔnoxR-6*, respectively. pII99 contains a Geneticin-resistance cassette. Protoplasts were selected in 3 mg of Geneticin per milliliter of PDA. The resulting transformants were tested for their ability to conidiate after mechanical injury with a scalpel. The presence of the *nox1* and *noxR* genes was confirmed by PCR.

Confirmation of the Mutants *Δnox1*, *Δnox2*, and *ΔnoxR* by PCR. To screen for gene-replacement events, DNA of hygromycin-resistant colonies was subjected to a first PCR using primers ORFnox1-forward (F) and ORFnox1-reverse (R), ORFnox2-F and ORFnox2-R, ORFnoxR-F, and ORFnoxR-R to detect the ORFs of *nox1* (PCR product, 1.7 kb), *nox2* (PCR product, 1.8 kb), and *noxR* (PCR product, 1.8 kb), respectively. In a second PCR, gene-replacement events by double homologous recombination were confirmed using the following primers: for the nox1 mutant, the forward primer Pnox1-F and reverse primer hygR (PCR product 2.7 kb); for the nox2 mutant, the forward primer Pnox2-F and reverse primer hygR (PCR product 2.8 kb); and for the noxR mutant, the forward primer PnoxR-F and reverse primer hygR (PCR product, 2.6 kb). All colonies that showed no amplification of the ORF and produced the expected amplification product in the second PCR were considered confirmed mutants. For all PCR reactions, DNA of the WT strain was used as control. PCR was performed using Taq polymerase (Invitrogen) under the following thermal cycling conditions: 3 min at 95 °C, followed by a total of 35 cycles of 30 s at 95 °C, 30 s at 60 °C, 1 min per kilobase at 72 °C, and 5 min at 72 °C. The sequences of primers used are shown in the Table S1.

ROS Detection Assays. Plugs of mycelia of the WT strain were inoculated in plates of PDA or MMV-CN and incubated in the dark at 28 °C. After 36 h, the colony was cut and incubated under the same conditions for 0, 1, 3, and 5 min after injury. After each time, small mycelial samples were scraped, placed immediately on a slide with 0.3 mM nitroblue tetrazolium chloride (NBT) (Sigma) aqueous solution, and incubated for 30 min in the dark a 28 °C.

Samples were photographed under an inverted microscope. The same procedure was followed to detect hydrogen peroxide; samples of mycelium of 1 min after injury were incubated in the presence of 40 mM 5-(and 6)-carboxy-2′-7′-dichlorodihydrofluorescein diacetate (H2DCFDA) (Sigma) aqueous solution. H2DCFDA samples were observed using an Olympus 1× 71 inverted fluorescence microscope using 460- to 490-nm excitation, 500- to 550-nm emission, and a dichromatic mirror of 500-nm wavelengths. Nonspecific NBT and DCF staining was observed only after prolonged incubation with NBT or H2DCFDA.

1. Galun E, Gressel J (1966) Morphogenesis in *Trichoderma*: Suppression of photoinduction by 5-fluorouracil. *Science* 151:696–698.
2. Ruepp A, et al. (2004) The FunCat, a functional annotation scheme for systematic classification of proteins from whole genomes. *Nucleic Acids Res* 32:5539–5545.
3. Kubicek CP, et al. (2011) Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of *Trichoderma*. *Genome Biol* 12:R40.

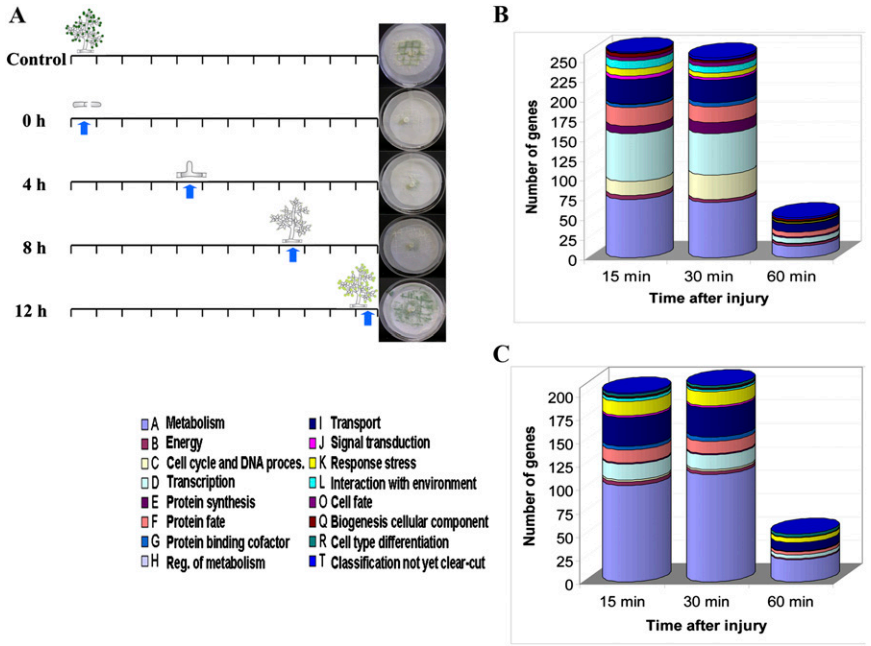

Fig. S1. Effect of 5-FU on injury induced conidiation and FunCat analysis of genes regulated in injury response. (*A*) Effect of 5-FU on injury induced conidiation. The inhibitor was applied at the indicated times (blue arrows) and photographed 48 h after injury. As control, the treatment was carried out without inhibitor. Drawings represent the stages at which the developmental program is interrupted. (*B*) Functional classification of injury induced genes. (*C*) Functional classification of injury repressed genes. (*B* and *C*) Graphs show the classification of genes differentially expressed at the indicated times after injury. Unclassified genes were not considered for the graph.

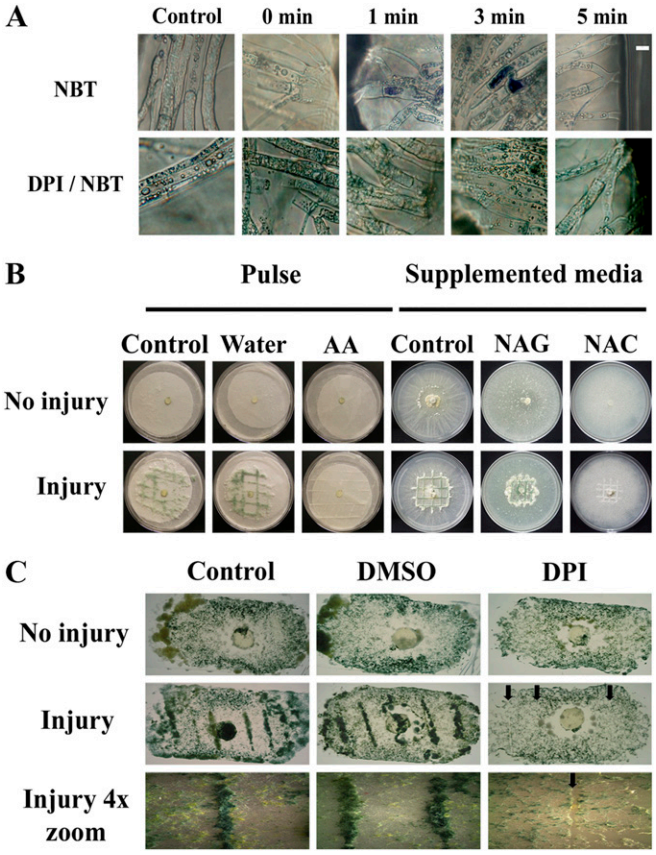

Fig. S2. ROS production and antioxidants affect injury-induced conidiation. (*A*) Production of superoxide in response to injury is blocked by DPI. Samples of mycelium were incubated in a 0.3 mM NBT solution; on the other hand, the samples were first incubated in 50 mM DPI for 10 min before and 20 min after injury and then were incubated in 0.3 mM NBT for 30 min. The samples were examined by bright-field microscopy (BF). A blue/purple coloration indicates the production of superoxide (Formazan generation). (*B*) Antioxidants repress injury-induced conidiation. (*Left*) A *T. atroviride* colony was injured after exposure to a pulse of 30 mM ascorbic acid (AA). (*Right*) Fungus grown in the presence of 60 mM NAC and then injured. A NAG-treated (60 mM), water-treated, and an untreated colony were used as controls. (*C*) The NADPH oxidase inhibitor DPI blocks injury-induced conidiation. A colony of *T. atroviride* was injured in the presence of DPI or DMSO as a negative control. Black arrows indicate the damaged area. Photographs of *T. atroviride* colonies were taken 48 h after injury. Photographs taken in a 4× stereo microscope are included in *B* for better appreciation.

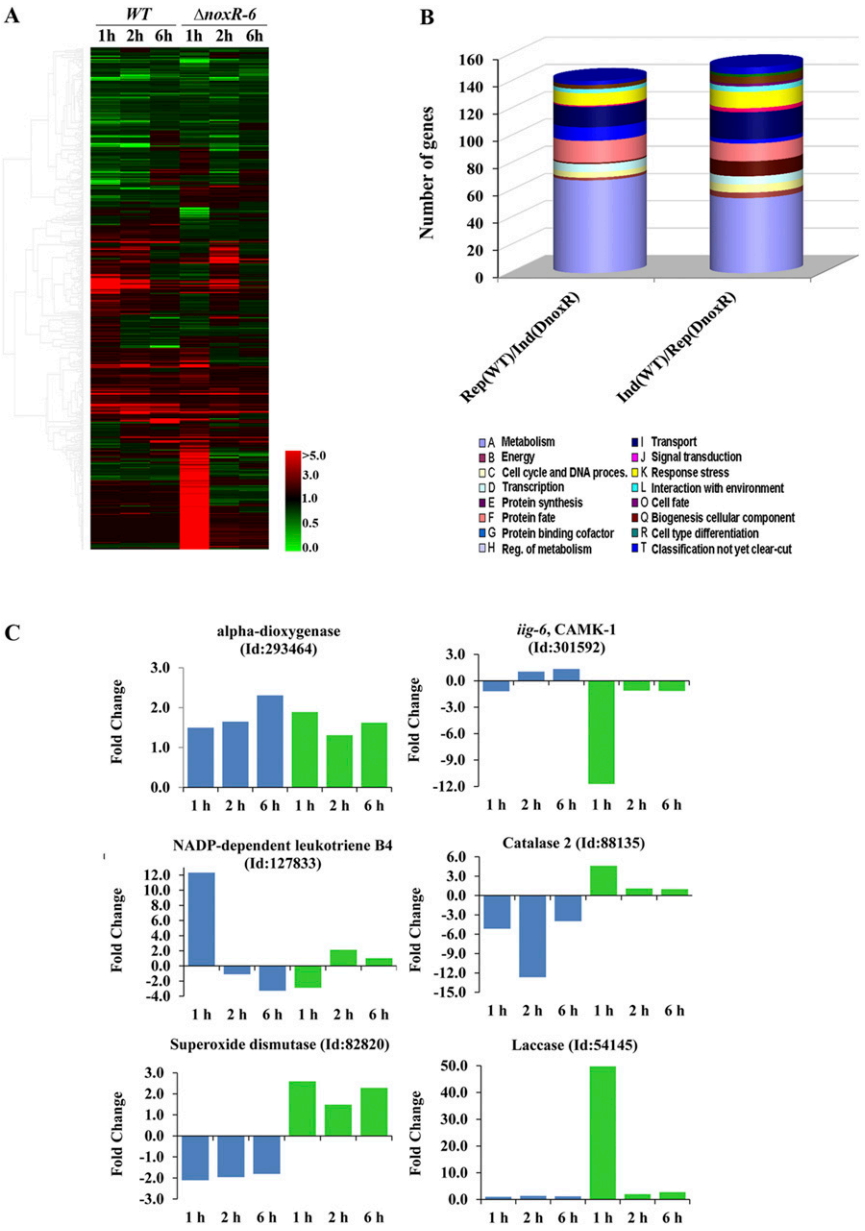

Fig. S3. Transcriptome analysis of WT and *ΔnoxR* strains in response to mechanical injury in late times. (*A*) Transcriptome comparison of WT and *ΔnoxR* strains. Hierarchical clustering of temporal expression of 592 injury-responsive genes was performed using smooth correlation and average linkage clustering in GeneSpring GX 7.3.1 software (Agilent Technologies). A total of 189 genes are induced in response to injury only in the WT strain and are repressed or not responsive in the Δ*noxR* mutant; in contrast, 300 genes are repressed or not responsive in the WT strain and induced in the *ΔnoxR* mutant. (*B*) Functional classification of injury regulated genes. Graphs show the classification of total repressed genes or not responsive in WT strain and induced in the *ΔnoxR* mutant [Rep(WT)/Ind(*ΔnoxR*)] and total induced genes in WT strain and repressed or not responsive in the Δ*noxR* mutant [Ind(WT)/Rep(*ΔnoxR*)]. Unclassified genes were not considered for the graph. (*C*) Comparison of level expression of some genes regulated in WT and *ΔnoxR* in injury response. The graphs represent the fold change that have genes at the times indicated in WT strain (blue bars) and *ΔnoxR* mutant (green bars) in response to mechanical injury.

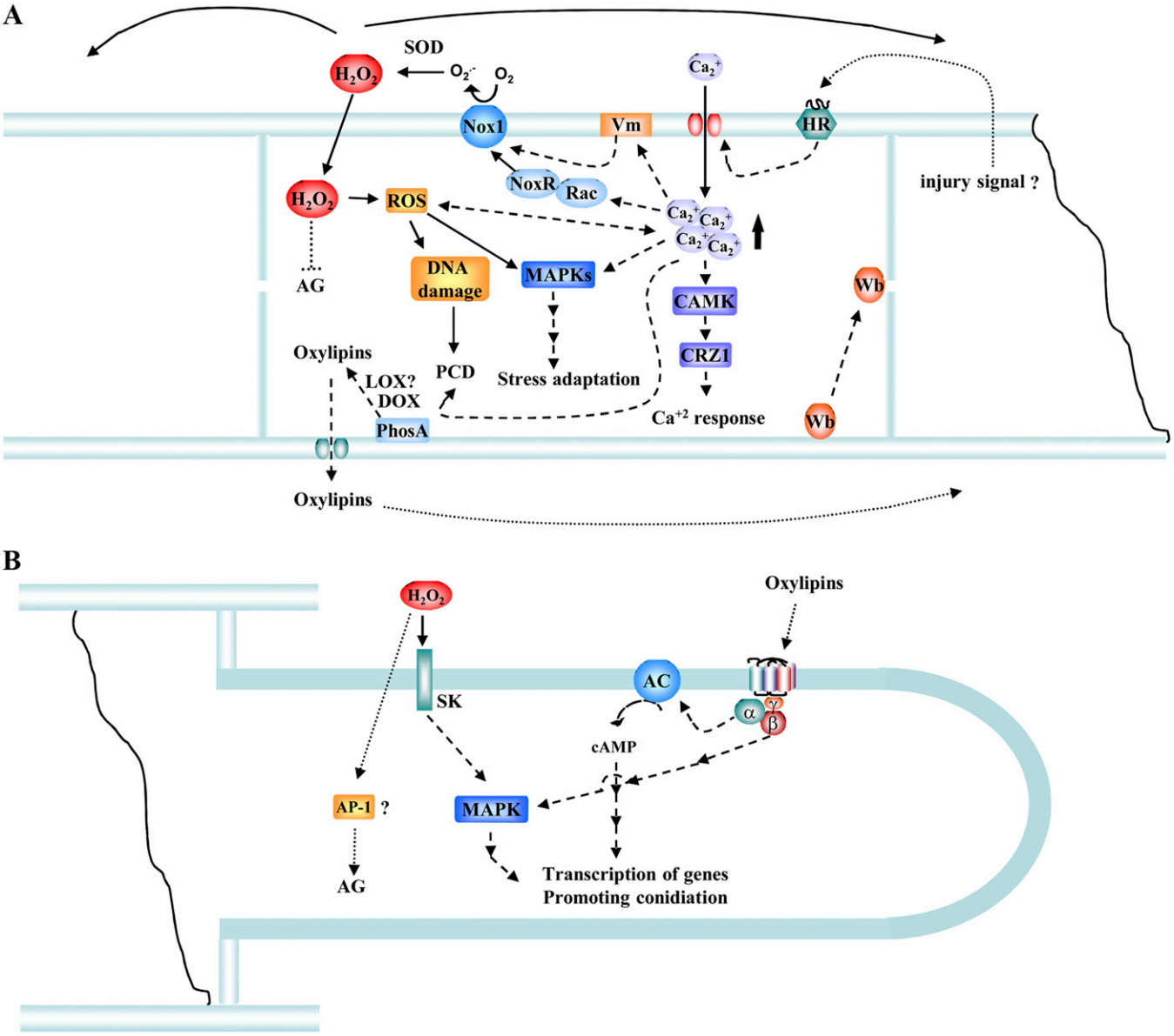

Fig. S4. Schematic representation of the events occurring in response to injury in *T. atroviride*. (*A*) Model for the events taking place in the cell adjacent to the injured cell. Injury provokes the production of ROS and increases in intracellular calcium and oxylipin synthesis. α, β, and γ, subunits of a heterotrimeric G protein; AG, antioxidant genes; DOX, dioxygenases; HR, hypothetical receptor; phosA, phospholipase A-like; SOD, Superoxide dismutase; Vm, membrane potential; Wb, Woronin body. (*B*) Hypothetical model of the response of the newly formed cell. H_2O_2 and oxylipins serve as signal for entry into conidiation through MAPK and cAMP pathways, respectively. In addition, H_2O_2 could activate the transcription factor activating protein (AP)-1 to induce the antioxidant response. AC, adenyl cyclase; SK, sensor kinase. Solid arrows indicate data supported by our own experiments, discontinuous arrows indicate data experimentally supported in other fungal systems, and dotted arrows indicate completely hypothetical steps.

Table S1. Primers used in this work

Id Prot. Tav2*	Gene name	Primer name	Sequence 5′ to 3′	Analysis
127833	*iig-1*	Ficont02053	TGGTCTCGCCGATCTGGACGGG	Northern blot
		Ricont02053	GGATGGAGAGATTCTGGTTGAGG	Northern blot
297699	*iig-2*	Ficont20013	ATTCTGCTTGCAGCCCAGC	Northern blot
		Ricont20013	CAACACCAAGATGAAGTCCGTCG	Northern blot
297381	*iig-3*	Ficont16107	CCGTTGCAAGAGAACTTGACGCC	Northern blot
		Ricont16107	ACGATGCCTGCGGCTGAG	Northern blot
33350	*iig-4*	Ficont01196	TGGAGCAGATCCTAGCTCTTGTG	Northern blot
		Ricont01196	CATTTCCGAGGTTTGGTCGAG	Northern blot
36070	*iig-5*	Ficont04483	AGCTAGCGAAGGATTGCGCATC	Northern blot
		Ricont04483	ATCGCGCCAACCGGAAGC	Northern blot
301592	*iig-6*	camk301592F	TTCGCCAACATGCTCAATC	Northern blot
		camk301592R	GAGGCGTCGCTAAAGCTAC	Northern blot
126859	*iig-7*	cytb126859F	GCTTCATAACCATGCCAGAG	Northern blot
		cytb126859R	TGTAATCTTTGCAACAGCGCCTG	Northern blot
314604	*iig-8*	bzip314604F	GGTTGTGGCAGACCGTAGTC	Northern blot
		bzip314604R	CTCATCCATACCAACAAGCGTCTC	Northern blot
219770	*iig-9*	metA219770F	TGGTGGTTACGGATACGACAAC	Northern blot
		metA219770R	AGGTCGCGTCACAGTTCAAG	Northern blot
297389	*irg-1*	Frcont03522	TCTGGCGCCGAGCGCTGTTG	Northern blot
		Rrcont03522	ATAGCCACTGGCGCCCACAC	Northern blot
297668	*irg-3*	Frcont17073	CTTGGCATGGACGACACGC	Northern blot
		Rrcont17073	CCAGGCACCATTGGACCTCTGC	Northern blot
300386	*irg-4*	Frcont15790	AAATTCCCGCATGGCAAGG	Northern blot
		Rrcont15790	GAGGTATGGGTTATCCAGCTC	Northern blot
297186	*irg-5*	Frcont18209	GGTTAACCCCCCTGCCTGATGTG	Northern blot
		Rrcont18209	TGACAAGCCCTGGATCGGC	Northern blot
300960	*irg-6*	Frcont20402	TCTCTGCAAGACGAGAGTTGC	Northern blot
		Rrcont20402	TTCGTTGGGGATGCTCGTAAGC	Northern blot
253020	*irg-7*	Ficont01499	CTCAAAGCTGCAAAGCAACTCG	Northern blot
		Ricont01499	GCGCCCACATGGTGGTAG	Northern blot
155960	*irg-8*	cat2155960F	ACTCGAAATAAGGATTGGTGG	Northern blot
		cat2155960R	CCGACATCATATTTACAAGC	Northern blot
Hygromycin	*hph*	hygF	GATCGACGTTAACTGATATTGAAGGAG	Gene replacement
	hph	hygR	CTATTCCTTTGCCCTCGGACGAGTGCTG	Gene replacement
315943	Promoter	PnoxR-F	GAC TCG TCT CTC ATA CGG AGA G	Gene replacement
	Promoter	PQnoxR-R	TGC TCC TTC AAT ATC AGT TAA CGT CGA TCA GGG GGG TGA GAG GCA AAG AC	Gene replacement
302802	Terminator	TQnoxR-F	CCC AGC ACT CGT CCG AGG GCA AAG GAA TAG TGA CAT CTT GGC GAC ATG GC	Gene replacement
	Terminator	TnoxR-R	CAG ATC GCA GAC GCA GAG G	Gene replacement
	Next 5′	N5′-noxR-F	GTT AGC AGC AGC ATC TGA GC	Gene replacement
	Next 3′	N3′-noxR-R	GGA TCG CTG TGA GTG TGT GC	Gene replacement
	Promoter	Pnox1-F	CCG TTA CAA GTG CAC CTC TGT C	Gene replacement
	Promoter	PQnox1-R	TGC TCC TTC AAT ATC AGT TAA CGT CGA TCA GGA CGC CTC GGG ATT TGA CC	Gene replacement
300495	Terminator	TQnox1-F	CCC AGC ACT CGT CCG AGG GCA AAG GAA TAG TCT GGA AGG AAG CAT GAA GAC G	Gene replacement
	Terminator	Tnox1-R	GCA CCA TCG AGC ACT CAT ACC	Gene replacement
	Next 5′	N5′-nox1-F	CAC CGC AGC GAG CAA ATC C	Gene replacement
	Next 3′	N3′-nox1-R	TCA AGC ACC TTC ATC ACC ACG	Gene replacement
	Promoter	Pnox2-F	GTA TAA GCC ACG GAA TGC ACG	Gene replacement
	Promoter	PQnox2-R	TGC TCC TTC AAT ATC AGT TAA CGT CGA TCA GTC TCC TTC TGT AGT ATC CCA G	Gene replacement
	Terminator	TQnox2-F	CCC AGC ACT CGT CCG AGG GCA AAG GAA TAG TCT GTG GAC CCA AGG GAT TGG	Gene replacement
	Terminator	Tnox2-R	CCT CGC TGG AGT TGT GCT G	Gene replacement
	Next 5′	N5′-nox2-F	GTC ACA GCT CTT GTC GCA TCG	Gene replacement
	Next 3′	N3′-nox2-R	CAA TGC CTC CGC CAC AGC C	Gene replacement
302802	*Δnox1*	ORFnox1-F	TCTAGATGGATGGTAAGCCCTTCCTC	Confirmation
		ORFnox1-R	TTAGAAATGCTCTTTCCAGAAGCG	Confirmation

Table S1. Cont.

Id Prot. Tav2*	Gene name	Primer name	Sequence 5′ to 3′	Analysis
300495	*Δnox2*	ORFnox2-F	TCTAGACAATGGGATACGAAGAGTATGAG	Confirmation
		ORFnox2-R	TTAGAAGTTTTCCTTGCCCCAGAC	Confirmation
315943	*ΔnoxR*	ORFnoxR-F	CCGCGGCACAATGTCGCTGAAACAGG	Confirmation
		ORFnoxR-R	AAGCTTGCGATCTAGACTTCAATAACCC	Confirmation
302802	*nox1+*	oAM-LU108	GGCACCATTGGCGAGGAATC	Complementation
		oAM-LU80	GGCACCATTGGCGAGGAATC	Complementation
315943	*noxR+*	oAM-LU92	CAGAATCTCGCAGATCGCAGAC	Complementation
		oAM-LU111	GTGTGCTTGTCCACGTCTATGC	Complementation

**T. atroviride* Genome Version 2 Database Protein Indentifier.

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)
Dataset S3 (XLSX)