

Supplementary Information

Supplementary methods

Insects grazing videos

To analyze if collembolans were damaging *T. atroviride* mycelia, we recorded videos of the springtails on the mycelia. Briefly, we inoculated 1×10^3 conidia on Petri dishes (60mm x 15 mm) containing PDA. Plates were incubated for 30 h at 27° in darkness. Twenty 3rd Instar larvae and twenty adult collembolans were collected and cleaned as described above. We recorded ten 30 s videos of independent insects (larvae and collembolans) using a Leica EZ4 HD stereo microscope and edited them using the iMovie version 10.1.14 from the iOS Operative system.

T. atroviride consumption assays.

To determine if *O. folsomi* and *D. melanogaster* larvae were feeding on *T. atroviride* mycelia, we performed insect-fungus interactions, using 20 adult collembolans or 20 3rd instar larvae. After 30 min of feeding on mycelia, insects were collected and frozen at -20°C for two hours. The insect bodies were washed twice with ethanol (70%), once with sodium hypochlorite (20%), and three times with distilled sterile water to eliminate any living fungal structures from outside, and placed on *Trichoderma* selective culture medium (0.2 g/l MgSO₄·7H₂O, 0.9 g/l K₂ HPO₄, 0.15 g/l KCl, 1 g/l NH₄ NO₃, 3 g/l glucose, 0.075 g/l Rose Bengal, and 20 g/l agar) amended with 0.25 g/l chloramphenicol and 0.2 g/l quintozone. Plates were incubated in darkness for five days at 27°C. As a control, insects that never had contact with the fungus were used and treated similarly to the insect-fungus interaction. Five days later, we counted the larval bodies from which fungal colonies emerged. We used five replicates of 20 insects in three independent experiments. We compared the number of bodies from which *T. atroviride* emerged against the respective control for statistical analyses. We used a single-factor variance analysis (ANOVA), followed by Fisher's exact Test. Statistically significant differences in mean values at a $P > 0.05$.

Quantitative RT-qPCR

To confirm the RNA-seq results, specifically of genes related to the injury response, we performed RT-qPCR analyses of the expression of selected genes. To determine if there was any possible delay in the transcriptional response, we selected 15 min as an earlier time point and 30, and 90 min after mechanical injury and fungivory for the analyses. The primers used are enlisted in Supplementary Table 3. RT-qPCR was performed as previously described [25]. All experiments were repeated four times, comparing gene expression levels after the indicated treatment at a given time using a tail ANOVA and Tukey test with a $P < 0.05$.

Salivary gland treatments for regeneration assays

We dissected and homogenized 300 salivary glands (SG) as described in main text (aliquots of 10 dissected tissue). We incubated SGE for 15 min at 95°C or treated them with pronase (Boehringer Mannheim GmbH, Germany) at a final concentration of 200µg/ml. For pronase treatments, we added 2µl of the enzyme mix to 30 µl of PBS containing the SGE, incubated the sample for 40 min at 42°C, and inactivated the enzymes at 85°C for 10 min. For the protease inhibitor treatment, we used a combination of protease inhibitors cocktail (PI) (P9599 Sigma-Aldrich) and Phenylmethylsulfonyl fluoride (PMSF, P7626 Sigma-Aldrich) at 100mM. We added one microliter of each inhibitor to the SGE, incubated it for 30 min at room temperature, and inactivated the inhibitors by heating at 85°C for 10 min. As controls, we used 30 µl PBS aliquots treated as described for each treatment (heat, pronase, and protease inhibitors). We used one µl of treated SGE or PBS control per cut on the mycelia (four cuts per colony). We analyzed two hundred damaged hyphae in each experiment, and statistically significant differences in regeneration were determined using Two paired samples t-test and

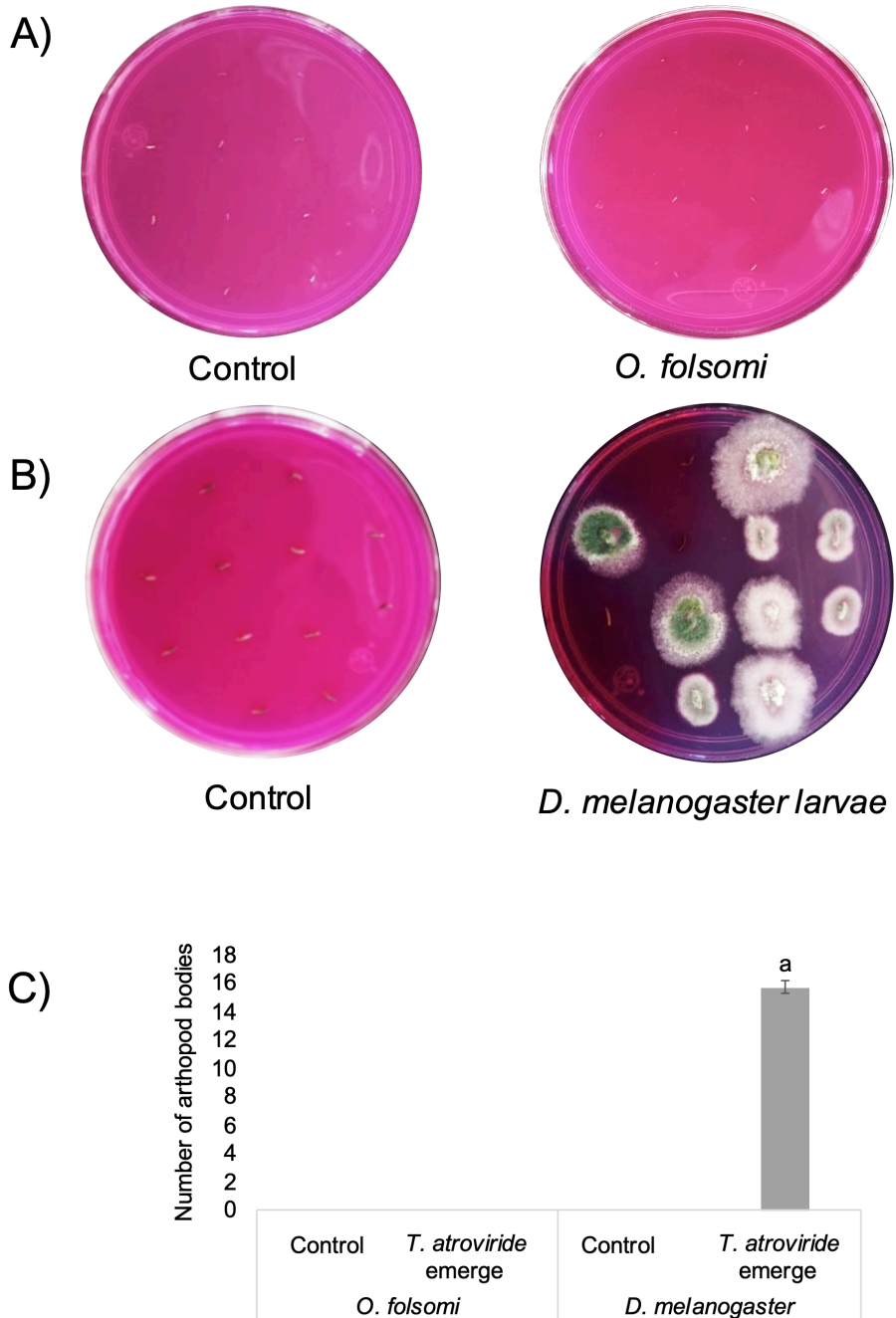
a Fisher Test $P > 0.05$. For injury and SGE control, we cut hyphae as described in the previous section. We performed each experiment using four independent biological and three technical replicates. We counted regenerated hyphae under a Leica DM6000-B microscope fitted with a 100x objective HCX PL Fluotar (0.75 N. A).

Supplementary videos description

Video S1. *D. melanogaster* larvae grazing on *T. atroviride* mycelia. This video shows how larvae pull the *T. atroviride* mycelia to eat of it.

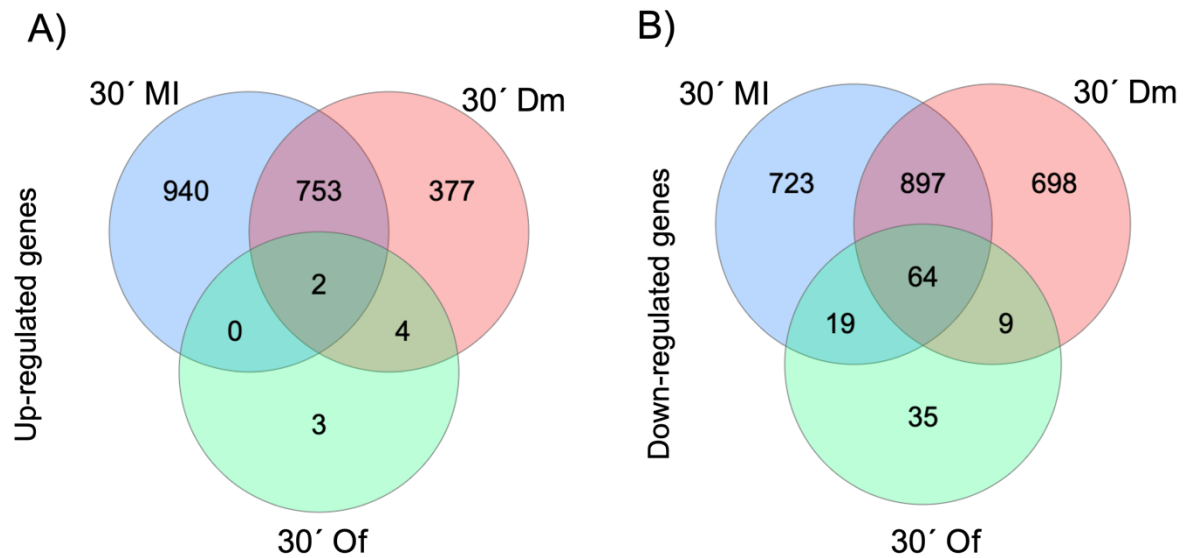
Video S2. *O. folsomi* walking on *T. atroviride* mycelia. This video shows how the Collembola walk on the *T. atroviride* mycelial mat but, no obvious consumption is observed.

Supplementary Figures



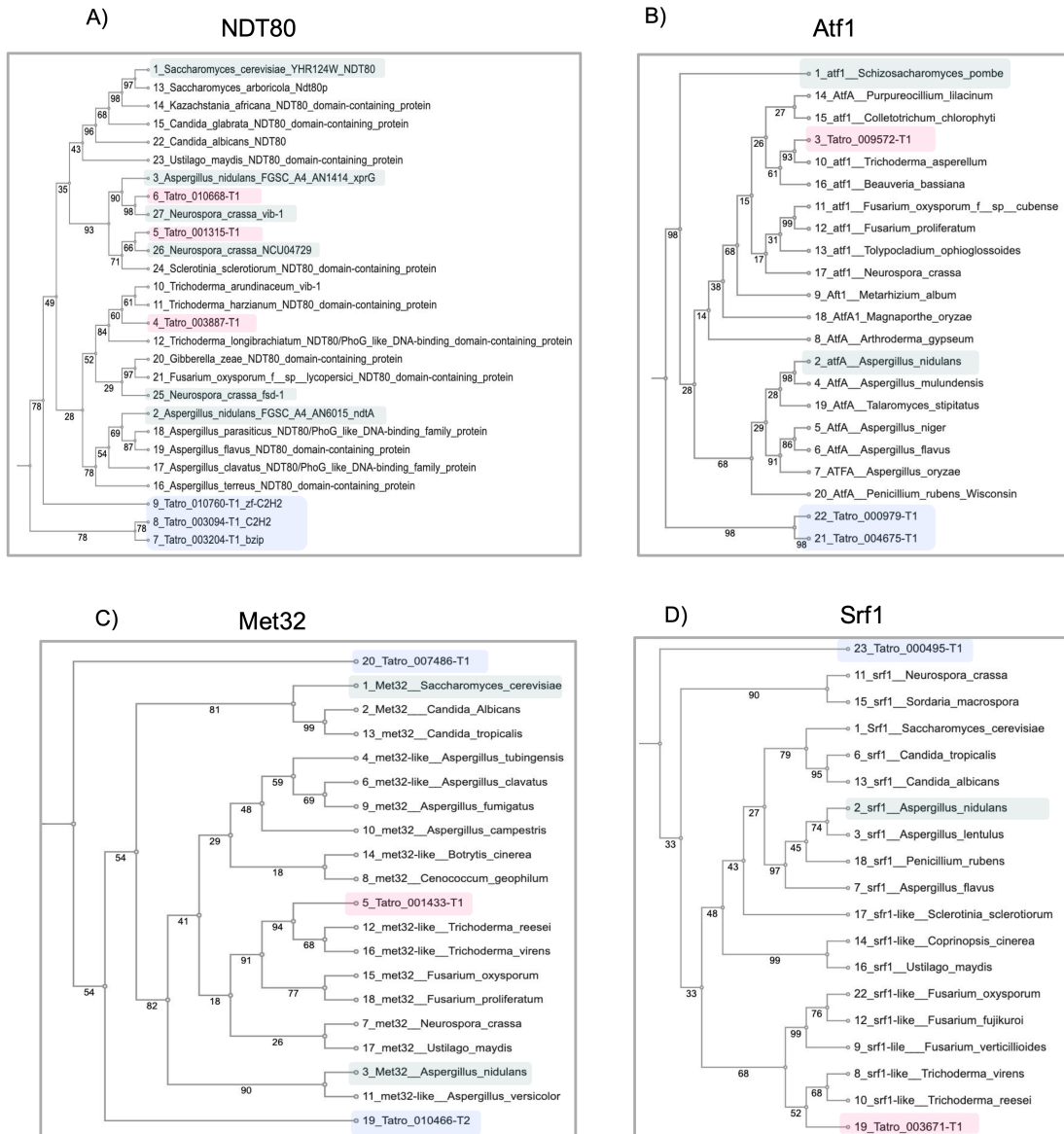
Supplementary Figure 1. Analysis of *T. atroviride* consumption by the collembola *O. folsomi*, compared to *D. melanogaster* larvae.

O. folsomi bodies growing on Trichoderma selective media (Rose Bengal). Left plate, insects' control, without any contact with Trichoderma mycelia. Right plate, insects after interaction with Trichoderma mycelia. B) *D. melanogaster* larvae bodies growing on Trichoderma selective media (Rose Bengal). Left plate, control of larvae without any contact with Trichoderma mycelia. Right plate, larvae after interaction with Trichoderma mycelia. C) Graph showing the number of bodies of collembola and larvae where *T. atroviride* emerged. One tail ANOVA and Fisher Test $P > 0.05$. (N=3).

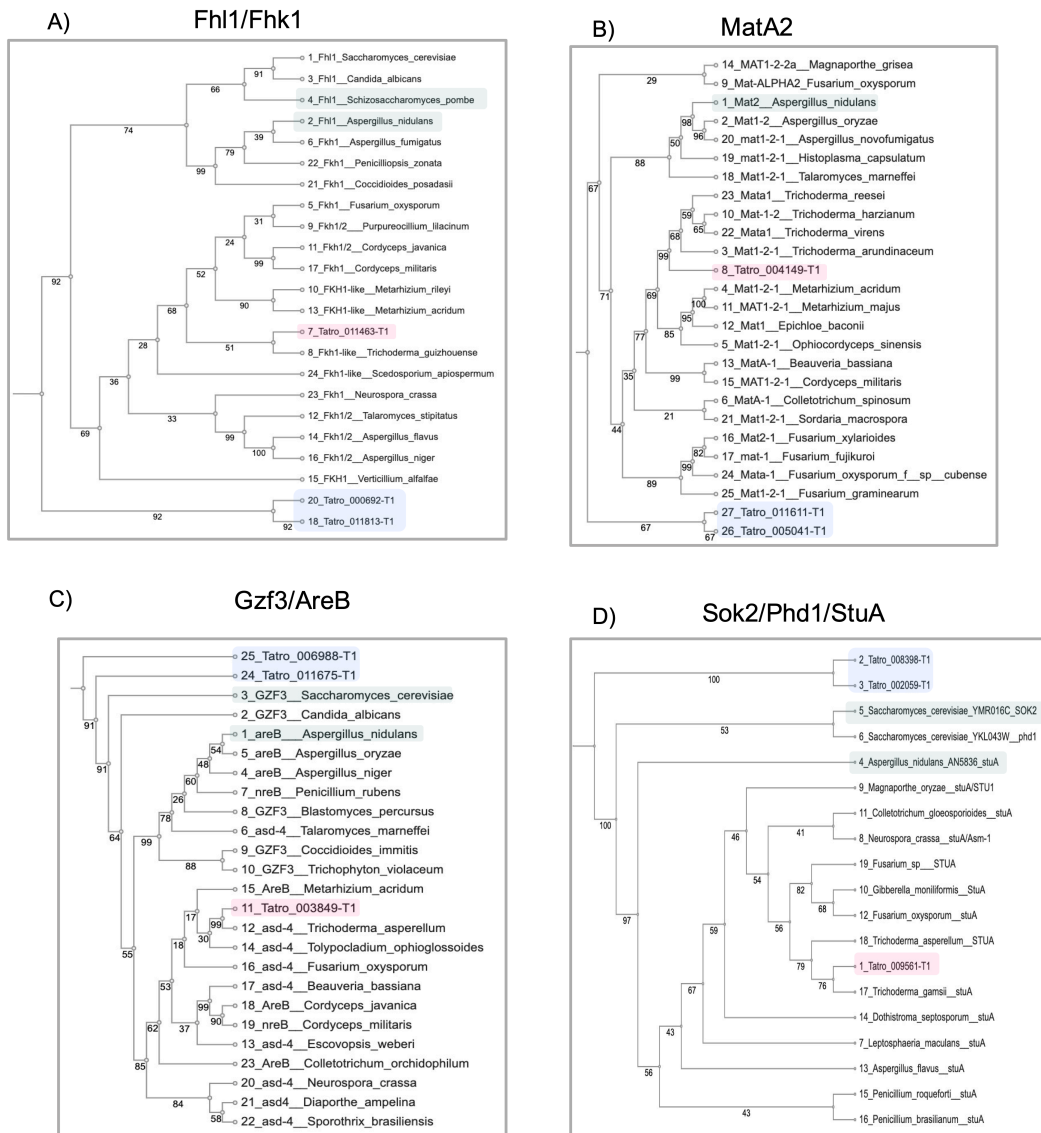


Supplementary Figure 2. Comparison between the differential expressed genes (DEG) at 30min after mechanical injury, grazing by *D. melanogaster* larvae and exposure to the springtail *O. folsomi*.

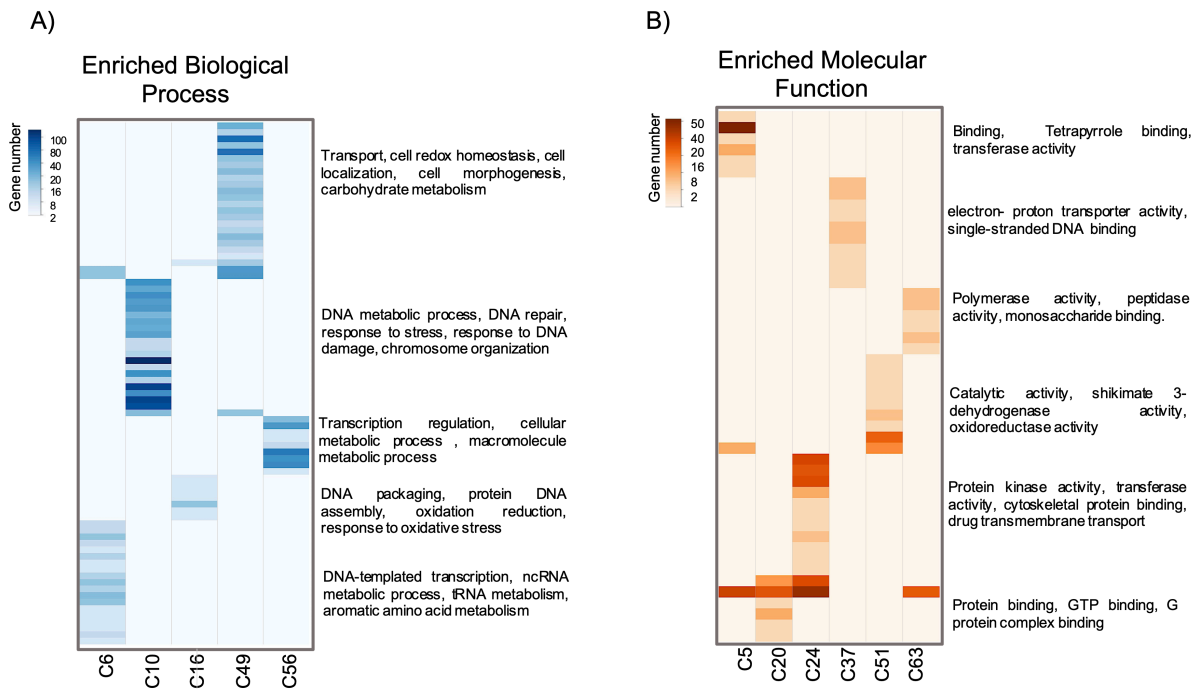
A) Venn diagram showing the number of up regulated genes at 30 min after the indicated stimulus. B) Venn diagram showing the number of down regulated genes at 30 min after the indicated stimulus. MI: after mechanical injury. Dm: after attack by *D. melanogaster* larvae. Of: after exposure to *O. folsomi*. DEG were obtained using an FDR >0.005.



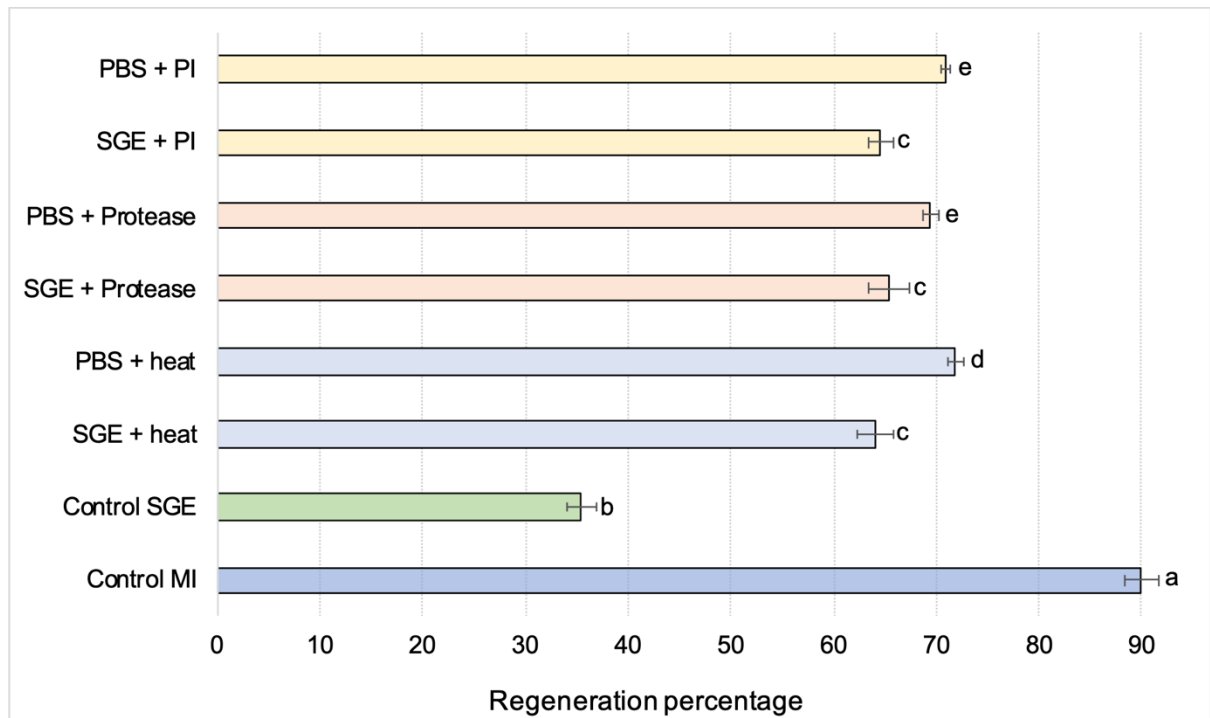
Supplementary Figure 3. Neighbor-Joining trees for the NDT80, Atf1, Met32 and srf1 transcription Factors. A) Neighbor-joining tree showing the phylogenetic relationship between *T. atroviride* NDT80 candidates (pink boxes) and those reported in other fungi. As outgroup we selected three different transcription factors in *T. atroviride*, a zf-C2H2, C2H2 and a bZIP (Blue box). B) Neighbor-joining tree showing the phylogenetic relationship between *T. atroviride* Atf1 candidate (pink box) and those reported in other fungi. As outgroup we selected two transcription factors of the same bZIP family in *T. atroviride* (Blue box). C) Neighbor-joining tree showing the phylogenetic relationship between *T. atroviride* Met32 candidate (pink box) and those reported in other fungi. As outgroup we selected two transcription factors of the same ZnF-C2H2-type family in *T. atroviride* (Blue boxes). D) Neighbor-joining tree showing the phylogenetic relationship between *T. atroviride* Srf1 candidate (pink box) and those reported in other fungi. As outgroup we selected one transcription factors of the same MADS family in *T. atroviride* (Blue box). Numbers in the nodes indicate Bootstrap values. Numbers at the left side of the gene names mean the order in the Fasta file submitted to MAFFT. Sequences for *A. nidulans*, *S. pombe* and *S. cerevisiae* were obtained from AspGD (AspGD.org) and Yeasttract Data base (Yeasttract.org), respectively. Protein sequences from other fungi were obtained by performing BLAST on UNIPROT (uniprot.org) and Fungi DB (FungiDB.org). A-D) *S. cerevisiae*, *A. nidulans* and/or *N. crassa* (green boxes).



Supplementary Figure 4. Neighbor-Joining trees for the Fhl1/Fkh1, MatA2, Gzf3/areB and Sok2/Phd1/StuA transcription Factors. A) Neighbor-joining tree showing the phylogenetic relationship between *T. atroviride* Fhl1/Fkh1 candidate (pink boxes) and those reported in other fungi such. As outgroup we selected two transcription factors of the same Fork-Head family in *T. atroviride* (Blue box). B) Neighbor-joining tree showing the phylogenetic relationship between *T. atroviride* MatA2 candidate (pink box) and those reports in other fungi such. As outgroup we selected two transcription factors of the same HTH family in *T. atroviride* (Blue box). C) Neighbor-joining tree showing the phylogenetic relationship between *T. atroviride* Gzf3/areB candidate (pink box) and those reported in other fungi. As outgroup we selected two transcription factors of the same GATA family in *T. atroviride* (Blue boxes). D) Neighbor-joining tree showing the phylogenetic relationship between *T. atroviride* Sok2/Phd1/StuA candidate (pink box) and those reported in other fungi. As outgroup we selected one transcription factors of the same HTH family in *T. atroviride* (Blue box). Numbers in the nodes indicate Bootstrap values. Numbers to the left side of the gene names correspond to the order in the Fasta submitted to MAFFT. Trees were constructed in MAFT V.7 supported by 1000 Bootstrap resampling. A-D) *S. cerevisiae*, *A. nidulans* and/or *N. crassa* (green boxes).



Supplementary Figure 5. Enriched GO terms for the eleven selected cores after K-means and Hierarchical clustering analysis. A) Heatmap showing the Enriched Biological Process (BP) in the selected clusters (6, 10, 16, 49 and 56). Blue scale colors indicate the log₂ of the number of genes belonging to the mentioned process. Interesting BPs are highlighted at the right side of the heatmap. B) Heatmap showing the Molecular Functions (MF) enriched in the selected clusters (5, 20, 24, 37, 51, 63). Orange scales indicate the log₂ of the number of genes belonging to the mentioned process. Row names correspond to the enriched MF. Relevant MF are highlighted at right side of the heatmap.



Supplementary Figure 6. Effect of enzymatic treatments on the *T. atroviride* hyphal regeneration inhibitory activity of the SGE extract content. Salivary glands of *D. melanogaster* larvae were treated with heat, proteases and Protease inhibitors. As control 1X PBS was subjected to the indicated treatment. As regeneration control, mechanical injury and injury + SGE were used. Error bars represent the standard error value. Different letters above the error bars indicate the statistically significant differences. For statistical analysis a two paired samples t-test and Fisher test $P > 0.05$ was performed. MI: Mechanical injury. SGE: salivary gland extract. PI: protease inhibitors. PBS: Phosphate Buffer Solution. (N=4).

Supplementary Table 1. List of conditions and RNA-seq libraries used in this work. Libraries are deposited at NCBI GEO under the accession number GEO: GSE152652

Library name	Condition	Total of reads
CT0-1	Control Time 0	11 886 832
CT0-2	Control Time 0	6 160 389
CT0-3	Control Time 0	11 322 756
CT8-1	Control 8h	13 061 548
CT8-2	Control 8h	17 739 132
CT8-3	Control 8h	11 128 133
30MI-1	30 min after mechanical injury	14 890 821
30MI-2	30 min after mechanical injury	15 029 776
30MI-3	30 min after mechanical injury	12 779 955
90MI-1	90 min after mechanical injury	13 603 699
90MI-2	90 min after mechanical injury	12 588 206
90MI-3	90 min after mechanical injury	14 019 333
4hMI-1	4h after mechanical injury	12 562 991
4hMI-2	4h after mechanical injury	14 662 169
4hMI-3	4h after mechanical injury	13 152 496
8hMI-1	8h after mechanical injury	12 340 894
8hMI-2	8h after mechanical injury	11 387 514
8hMI-3	8h after mechanical injury	11 248 740
30Dm1-1	30 min after fungivory by <i>D. melanogaster</i> larvae	12 619 491
30Dm1-2	30 min after fungivory by <i>D. melanogaster</i> larvae	14 173 398
30 Dm1-3	30 min after fungivory by <i>D. melanogaster</i> larvae	11 128 259
90 Dm1-1	90 min after fungivory by <i>D. melanogaster</i> larvae	15 158 853
90 Dm1-2	90 min after fungivory by <i>D. melanogaster</i> larvae	13 389 660
90 Dm1-3	90 min after fungivory by <i>D. melanogaster</i> larvae	12 790 200
4h Dm1-1	4h after fungivory by <i>D. melanogaster</i> larvae	14 638 995
4h Dm1-2	4h after fungivory by <i>D. melanogaster</i> larvae	16 743 244
4h Dm1-3	4h after fungivory by <i>D. melanogaster</i> larvae	13 386 733
8h Dm1-1	8h after fungivory by <i>D. melanogaster</i> larvae	13 444 906
8h Dm1-2	8h after fungivory by <i>D. melanogaster</i> larvae	10 203 896
8h Dm1-3	8h after fungivory by <i>D. melanogaster</i> larvae	11 323 663
30 Of2-1	30 min after fungivory by <i>O. folsomi</i>	12 163 991
30 Of2-2	30 min after fungivory by <i>O. folsomi</i>	10 831 625
30 Of2-3	30 min after fungivory by <i>O. folsomi</i>	12 420 561
90 Of2-1	90 min after fungivory by <i>O. folsomi</i>	13 997 315
90 Of2-2	90 min after fungivory by <i>O. folsomi</i>	14 690 665
90 Of2-3	90 min after fungivory by <i>O. folsomi</i>	10 448 459
4h Of2-1	4h after fungivory by <i>O. folsomi</i>	12 429 452
4h Of2-2	4h after fungivory by <i>O. folsomi</i>	11 468 811
4h Of2-3	4h after fungivory by <i>O. folsomi</i>	14 934 409
8h Of2-1	8h after fungivory by <i>O. folsomi</i>	13 744 234
8h Of2-2	8h after fungivory by <i>O. folsomi</i>	13 133 967
8h Of2-3	8h after fungivory by <i>O. folsomi</i>	13 330 159

Supplementary Table 2. Shared and specific Up and Down regulated genes for MI and Dm time points.

	Up-regulated genes			Down-regulated genes		
	MI		Dm	MI		Dm
Time	Unique genes	Shared genes	Unique genes	Unique genes	Shared genes	Unique genes
30 min	940	755	381	742	961	707
90 min	737	485	165	577	899	422
4 h	307	157	124	221	113	148
8 h	203	450	200	66	139	322

Supplementary Table 3. List of primers used in this work. Table shows name, gene ID and sequence for primers used in this study.

Primer name	gene ID in <i>T. atroviride</i> genome	Forward sequence	Reverse Sequence
Het 1 (Medina-Castellanos et al., 2018)	Tatro_003823-T1	CGTCTATGGCTTGGATGTTGCC	GTCACAGGTGCGCGAAGCC
Het 2	Tatro_007840-T1	TGGCATCGGCCTGATTGTGG	TGCCTCTCAACGTCCTCCCA
Calmodulin (Medina-Castellanos et al., 2018)	Tatro_010495-T1	GCACGAAAAATGAAGGACAC	CGCGAATCATCTCATCGAC
Calmodulin Dependent Protein Kinase (CAMK1)	Tatro_005111-T1	CAGAGAACACGAAGAAGACC	CCTCCTTTCCCATCTCTTCC
Cyclin	Tatro_011781-T1	TTCCCGCCATCACCATCTTG	TCGTCGTCACCTTCGTCGTC
Cyclin Dependent Kinase (CDK)	Tatro_011459-T1	ACCTCAAATACTTCATCCCCTC	TGCCAAATGCTACGTTTCC
Nacht 1	Tatro_002543-T1	ATGGCGCCAGTATTCGCCTC	AAGGCCGCAACCTCGTCAAA
Nacht 2	Tatro_005315-T1	ATGGCGCCAGTATTCGCCTC	AAGGCCGCAACCTCGTCAAA
DNA pol B	Tatro_010695-T1	GAGTGGCGTATGTCATGGTG	GCGTCTTCTTGGCAAACCTC

Supplementary Data sets

Supplementary data sets are available as Excel books at: <https://drive.google.com/drive/folders/171CfEo2fkspiDXv05yfv0rawivb9uIgd?usp=sharing>

Supplementary Data includes the following:

Supplementary Data 1. List of DEG at 30' after grazing by collembolan *O. folsomi*, showing their logFC, count per million (CMP) value and gene annotations. DEG were obtained using contrasts and filtered with an FDR > 0.005 against control condition.

Supplementary Data 2. List of GO terms enriched on the eleven selected cores after K-means and hierarchical clustering analysis

Supplementary Data 3. List of the enriched motifs on the eleven selected cores.

Supplementary Data 4. List of enriched GO terms for Figure 3. Lists are organized in twelve groups, six for the Figure 3D and six for the Figure 3E. Each list contains GO Id, Go name, Number of genes per GO term and P-value.

Supplementary Data 5. List of genes extracted from Groups 5 (Fig. 3D) and Group 2 (Fig. 3E). Genes were divided in seven groups: 1. Signaling (Ca²⁺, MAPKs, ATP and Lectins), 2. Cell cycle and Cell death, 3. Stress response, 4. Gene expression regulation, 5. Secondary metabolism, 6. DNA metabolism and DNA repair, and 7. Putative innate immune system. Each list includes the expression value in logFC units, at 30 min, 90 min. In addition, expression values for late times 4 and 8h after each stimulus, were added to list. Blue-Red scale color indicated the up and down regulation values for each gene.