Supporting information Methods S1

RNA sequencing analyses and PASs detection

De-multiplexed samples were trimmed to remove poly(A) tails and Illumina adapter sequences using the fastq-mcf utility from the ea-utils package (http://code.google.com/p/ea-utils/), keeping only reads with at least 17 nucleotides after trimming. Read-mapping was performed using STAR (Dobin *et al.*, 2013) with default parameters. The alignments obtained were later filtered removing low-quality mapping (MAPQ <30, see https://samtools.github.io/hts-specs/SAMv1.pdf), mappings with a high-level of A/Ts (>80%) and potential internal primings (more than seven As in the first 5' nucleotides or more than twelve As in the whole alignment (~2.5% of the alignments). The resulting alignments were assigned to the genomic features listed in the Ensembl Fungi gene annotation for *M. oryzae* version 29 (http://fungi.ensembl.org/), containing 13,218 annotated features. Each read was assigned to its overlapping feature (including protein-coding genes and the available ncRNAs), ambiguous cases were assigned to the closest 3' terminal end. To account for incomplete gene 3´UTR lengths, we extended all annotated genes by 400 bp. Reads that could not be assigned to any known features were treated separately.

One PAS was considered to be called with high-confidence if it was detected in at least two of the three replicates according to the following rule: its expression is considered distinct from basal noise if its standard score calculated against the whole gene expression (summing up all PASs expression from that gene) has a confidence level > 99% (z-score > 2.58 or z-score < 2.58). The standard score was calculated as: z-score = (value - mean) / std, where "value" is the number of supporting reads for the PAS, "mean" is the mean value of supporting reads for all the PASs in the gene, and "std" is the standard deviation of supporting reads for all the PASs in the gene. PASs with less than five supporting reads in every replicate were discarded. High-confidence PASs were subsequently merged if located less than 33bp from an adjacent one (roughly the span of the polyadenylation site regulatory region, including the A-rich and the U-rich regions upstream of the poly(A) cleavage), and only the most expressed PAS was retained. PASs that could not be assigned to any annotated genomic feature were classified as "orphan" PASs. The minimum folding energy (MFE) for every PAS region was predicted using the RNAfold tool from the Vienna package (Lorenz *et al.*, 2011).

Gene functional groups enrichment and sequence analysis

Roughly 60% of annotated genes in the *M. oryzae* genome have at least one Gene Ontology (GO) annotation. We used the FungiFun2 utility to perform gene group functional profiling and statistical

enrichment analysis of gene ontologies terms (Priebe *et al.*, 2015). FungiFun2 utilizes a variety of annotation/classification methods such FunCat (Functional Categories) (Ruepp *et al.*, 2004), GO (Gene Ontology) (Ashburner *et al.*, 2000) and KEGG (Kyoto Encyclopedia of Genes and Genomes) (Du *et al.*, 2014). FungiFun2 categorizes genes and proteins of fungal species on different levels and conducts an enrichment analysis. Enriched oligonucleotide motifs in the interesting regions were discovered using the DREME tool (Bailey, 2011).

Poly(A) tail length of mRNA transcripts

For size detection of poly(A) tails in total RNA we carried out a radioactive labelling with Klenow polymerase of 3' UTR for a total RNA samples (Lee *et al.*, 2014; Nilsen, 2015). The 3'-end of the poly(A) tail is radiolabelled followed by digestion of the body of the RNAs with both RNase A that cleaves single-stranded C and U residues and RNase T1 that cleaves G residues, leaving the poly(A) tail intact. Digested RNAs can therefore be separated on polyacrylamide gels, and the poly(A)-tail length can be visualized by autoradiography. 2 µg total RNA were denatured with 2 µl dT 14 GC template primer (100µM) at 80ºC for 5 minutes. Then 2 µl Klenow buffer (NEB 2), 1 µl α[^{32}P]-dCTP, and 1 µl Klenow polymerase (M0212S) were added. Incubate for 30 min at 37 °C. Heat inactivate at 80 °C for 5 min and spin. Then we add 2 µl Klenow buffer (NEB 2), 1 µl α[^{32}P]-dCTP, and 1 µl Klenow polymerase(M0212S). Incubate for 30 min at 37 °C. Heat inactivate at 80 °C for 5 minutes and spin. We removed unincorporated α-[^{32}P]-dCTP by G-25 handmade columns. The labelled RNAs were subjected to simultaneous degradation by RNase A (all C/U digestion) and RNase T1 (all G digestion) for 2h at 37°C, to cleave everything but the poly(A), in an 100 µl reaction mixture. After precipitation and phenolization samples were run in a UREA-PAGE (8M Urea, 16% polyacrylamide) dried and visualized by phosphor-image (Pharos). Images were digitalized and represented in a graph with ImageJ software (http://rsb.info. nih.gov/ij/).

mRNA half-life experiments using thiolutin and RNA Dot Blotting

For mRNA half-life analysis, mycelial samples were taken at different times after thiolutin (6 mg/ml) or DMSO addition and were frozen in liquid nitrogen. Total RNA was extracted from these samples by LiCl standard procedure and used for either dot–blot experiments to estimate the proportion of poly(A) mRNA in the total RNA or qPCRs. Total RNA was applied to a positively charged nylon membrane using a dot-blotting tool. 1 µg of RNA was blotted into separate wells in 200 µl-dots. The membrane was exposed to UV (1 min) for RNA crosslinking, then incubated in a prehybridization buffer (6x saline-sodium citrate, 5x Denhardt's solution, 0.05% sodium pyrophosphate, 0.5% SDS, 100 µg/ml sheared salmon sperm) for 1 h at 37ºC and probed overnight

at room temperature (RT) using hybridization buffer (6x saline-sodium citrate, 1x Denhardt's solution, 0.05% sodium pyrophosphate) with 50 ng/ml of the biotinylated oligo(dT) probe (5'-biotin- TTTTTTTTTTTTTTTTTTTTTV-3') synthesized by Sigma–Aldrich. After hybridization, the membrane was washed four times (5 min/wash) with washing buffer (6x saline-sodium citrate and 0.05% sodium pyrophosphate). For biotin detection, the membrane was blocked with 25 mL of blocking buffer and incubated for 15 min shaking gently. To detect biotinylated oligo(dT)s, the membrane was incubated in the conjugate/blocking buffer (1:400 dilution of streptavidin antiboby) and washed four times (5 min/wash) in 20mL of 1x washing solution shaking gently. The membrane was transferred to a new container with substrate equilibration buffer (1xPBS), and incubated for 5 min. ECL substrate (Pierce) was used for HRP/Luminol chemiluminescence assays.

Polysomes fractionation and analysis

To isolate polysomes *M.oryzae* ground mycelia were cultured for 48h in liquid CM, then washed with distilled water and transferred into fresh liquid CM or MM-C for 16h. To block ribosomal complexes cycloheximide was added to 0.1 mg/ml and incubated in ice for 15 min. Using 2 ml tubes and some crystal washed beads, mycelia were lysed in 1000 µL of ice-cold polysomal lysis buffer (10 mM NaCl, 2 mM Tris-HCl pH 7.5, 0.5 mM MgCl2 and 1% Triton X-100) supplemented with protease inhibitors and cycloheximide 0.1 mg/ml. Cell extracts were vortexed for 5 min. The lysate was separated by centrifugation at 13000 rpm for 20 min at 4°C in a 2 ml tube. The supernatant was transferred to a clean 1.5 mL tube, and was cleared by an additional centrifugation step at 14,000 rpm for 15 minutes at 4°C. Aliquots corresponding to 800 µg of total RNA (measured by Qubit fluorometric quantitation) were loaded onto an 11 mL linear sucrose gradient (15 to 50% w/v) prepared using polysomal lysis buffer without Triton X-100 and supplemented with cycloheximide 0.1 mg/ml. The sucrose gradients were loaded in a Beckman SW 41Ti rotor and centrifuged at 40,000 rpm for 160 min at 4°C. The gradients were fractionated using a Bio-rad BioLogic LP System with a long needle at the bottom of the gradient at 0.5 ml/min and fractionated in 900 µl into fraction collectors. BioRad Econo UV monitor was used to measure UV of each fraction. Then, RNA was isolated from each tube using a LiCl method.

Protein purification and RNA-binding assays

Purification Rbp35 was carried out as previously described (Franceschetti *et al.*, 2011). For purification of CFI25, its cDNA (798bp) was amplified by PCR using mycelial RNA, a high fidelity Q5 DNA polymerase (NEB) and primers MD17/MD18 (Supplementary Table S8). The cDNA was cloned *Eco*RI- *Xho*I into a modified pET28 vector (5,667bp; Novagen), which contains a

thioredoxin A N-terminal tag to enhance protein solubility and a 6xHis tag for purification, and transformed in *E. coli* Rosetta DE3 (Novagen). Protein expression was induced 4 h at 28°C with 1 mM IPTG (Sigma-Aldrich). Centrifuged cell pellets (30 min at 7000g) were resuspended in lysis buffer (20 mM sodium phosphate pH 8, 300 mM NaCl and one tablet of PIC/50 ml, 1 mM PMSF and 50 µg/ml Dnase I), lysed by sonication and pelleted at 4°C for 20 min at 20,000g. Recombinant proteins were purified from clear lysate by metal affinity chromatography (HisTrap HP 1 ml, # 17-5247-01 GE Healthcare) in buffer 20 mM sodium phosphate pH 8, 500 mM NaCl and 5% glycerol, and eluted with imidazole containing buffer at 250 mM. Both Rbp35 and CFI25 purified protein samples were desalted on PD10 columns (#17085101 GE Healthcare) using buffer (20 mM sodium phosphate pH 7.5, 50mM NaCl, 10% glycerol, 1mM DTT and PIC) and concentrated on Amicon® Ultra 4 mL Centrifugal Filters (Merck Millipore #UFC801008). HPLC-purified 5'end-biotynilated RNA probes of 42nt (Sigma-Aldrich) were used in EMSA ssays (Supplementary Table S8). Binding reactions (20 µl) included 10 mM Tris HCl pH 7.5, 50 mM KCl, 10 mM DTT, 1 mM $MgCl_2$, 1% Glycerol, 2 µg Yeast tRNA (#AM7119 Ambion), 20U RNase inhibitor (#100021540 AB), 10 µg BSA, and 2 pmol of biotinylated probe. Proteins were added at 0-20 µg according to each binding assay. RNA probes were boiled 2 min at 95ºC. After their immersion in ice, binding buffer and proteins were added and the mix was incubated 30 min before loading in 4% native polyacrylamide gels prepared with TBE 0.5x in DEPC-treated water. Gels were run 40 min at 100V in TBE 0.5x, then transfrered on Hybond-XL nylon membrane (#RPN203S GE Healthcare) at 400 mA for 1 hour. The membrane was UV crosslinked at 120mJ/cm^2. Detection was performed with stabilized Streptavidin-Horseradish peroxidase conjugate (#21134 Thermo-Scientific) and Enhanced Chemiluminescent subtrates (#32106 Thermo-Scientific) following LightShift Chemiluminescent EMSA procedure (#20148 Thermo-Scientific).

Quantification and statistical analyses

Differential gene expression analysis was performed using the R/Bioconductor package DESeq2 (Love *et al.*, 2014), using the whole reads count for each feature (the number of reads that have been assigned to that feature) by triplicate. A gene was considered differentially expressed if the p-*value* adjusted for false discovery rate control (the padj column in DESeq2) is less than 0.05. Differential PAS expression analysis was performed using the R/Bioconductor package DEXSeq (Anders *et al.*, 2012), using the reads count for high-confidence PASs for each feature by triplicate. A PAS was considered differentially expressed if the p-*value* adjusted for false discovery rate control (the padj column in DEXSeq) is less than 0.05. To estimate the differential usage of 3'UTRs during plant

infection we fitted a statistical model to the observed data using Stan (Carpenter *et al.*, 2017). 3'UTR expression is assumed to derive from a binomial distribution: 3'UTRexpr ~ Binomial (TotalGeneExpr, *p*), where *p* is the parameter to estimate. Partial pooling was also implemented in order to account for the two replicates present in the experiment. The difference between the posterior distributions of the stages to be contrasted was used to assess significant changes, posterior distributions whose 95% quantiles did not overlap with zero were considered to represent significant changes. Gene set enrichments were calculated using the hyper geometric/Fisher's exact test, using a *p*-value of 0.05 as threshold. For multiple comparisons in polysomal fractions, a Fisher's Least Significant Difference (LSD) test was used to compare every possible pair of samples, and significance levels were set at a p-value < 0.05.

Genomic data of the selected organisms in Figure S3G were retrieved from the NCBI using the genome assemblies indicated in the following table:

Species	**Strain**	**Genome assembly**
Arabidopsis thaliana	TAIR10	GCA_000001735.1
Blumeria graminis	DH14	GCA_000151065.1
Candida albicans	WO-1	GCA_000149445.2
Coprinopsis cinerea	okayama7#130	GCA_000182895.1
Homo sapiens	-	GCA_000001405.22
Laccaria bicolor	S238N-H82	GCA_000143565.1
Magnaporthe oryzae	70-15	GCA_000002495.2
Neurospora crassa	ATCC 24698	GCA_000182925.2
Pleurotus ostreatus	PC15	GCA_000697685.1
Podospora anserina	S mat+	GCA_000226545.1
Rhizophagus irregularis	DAOM 181602	GCA_000439145.2
Saccharomyces cerevisiae	R64-1-1	GCA_000146045.2
Schizosaccharomyces pombe	972h-	GCA_000002945.2
Tuber melanosporum	Mel28	GCA_000151645.1
Ustilago maydis	521	GCA_000328475.2

Polyadenylation sites were predicted from the EST datasets downloaded from the Genbank website using the PASA pipeline (Haas *et al.*, 2003).

Construction of plasmids and generation of *M. oryzae* Δ*14-3-3b* mutant

Gene deletion constructs were generated using multisite gateway technology (Invitrogen) as previously described (Tucker *et al.*, 2010). The 5' and 3' gene regions of *14-3-3B* (MGG_13806) were amplified by PCR using the primers described in Table S8. Selection of deletion mutants was carried out using simultaneously 200 mg/ml hygromycin and 5-fluoro- 2'-deoxyuridine (50 µM). For the construction of 14-3-3B:mCherry variants, primers were designed to amplify the ORF including 1.5 kb upstream of the start codon, and a full 3'UTR or a short 3'UTR including just the first PAS. Visualization of fungal cells containing mCherry constructs was performed with a Leica TCS SP8 confocal microscope. mCherry was excited using the 561-nm laser line, and the emission was captured using a 575–615 nm band-pass filter. Sequence data and gene numbers used in this study were taken from EnsemblFungi (Magnaporthe oryzae MG8; http://fungi.ensembl.org/index.html).

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