**Supplemental Materials and Methods**

**Plasmid construction and fungal expression of tagged proteins**

Tagged GFP fusion proteins were expressed under the control of the clock-controlled gene, *ccg-1* promoter at the *his-3* locus in *N. crassa*. The ORFs of *ve-1, ve-2, vos-1* and *lae-1* were amplified by PCR as annotated by the *N. crassa* Ensemble Fungi genome database (https://fungi.ensembl.org/Neurospora\_crassa/Info/Index) using the primers listed in Table S2-S3 (AD oligos) and introduced into the vector pMF272 (Freitag *et al.* 2004). The final plasmids *Pccg-1::ve-1::sgfp, Pccg-1::ve-2::sgfp, Pccg-1::vos-1::sgfp, Pccg-1::lae-1::sgfp* were transformed into histidine auxotrophic strain and the transformants were selected on media lacking histidine. Modification of the endogenous *ve-2* and *lae-1* loci to allow expression of C-terminal GFP-tagged fusion proteins was achieved by PCR-amplification of the ORFs of both genes and 1 kbp fragments of their 3’UTRs using genomic DNA. The fragments were cloned into the vector pGFP::*hph::*loxP (Honda and Selker 2009; Dettmann *et al.* 2012) and transformed in ∆*mus-52::barR;his-3* to ensure homologous recombination with the endogenous *ve-2* and *lae-1* loci. Transformants were backcrossed with WTto remove the ∆*mus-52::barR* mutation. Right clones were selected by a diagnostic PCR reaction.

The generation of HA or FLAG tagged proteins in *N. crassa* was performed following the method described by (Honda and Selker 2009) with minor modifications. Generation of knock-in tagging cassettes involved the generation of four flanking regions of approximately 1.2 kb each, (I) upstream of the ATG codon (5’-UTR of *ve-1*), (II) the *veA* cDNA from *A. nidulans*, (III) 10xGly::3XFLAG::*hph* and (IV) (3’-UTR of *ve-1*). Each flank is obtained by PCR using primers with a tail complementary to the tagging fragment obtained by digestion of plasmids that contain a poly-glycine linker before the epitope that is followed by the selectable marker *hph* to confer hygromycin resistance. The four fragments (three PCR products and a linear digested fragment) and the linearized plasmid pRS426 were fused to make the knock-in cassettes by recombination in an auxotrophic strain of *S. cerevisiae* (Oldenburg *et al.* 1997). The *ve-1* gene from *N. crassa* was replaced by *veA* gene from *A. nidulans* under *ve-1* promoter. Strains expressing proteins VE-1, VE-2, LAE-1 and VeA tagged with the epitopes FLAG and HA were generated following the method described by (Honda and Selker 2009) with minor modifications. Double-tagged strains were obtained by sexual cross and descendent selected by Hygromycin resistance. In addition, all strains were verified by PCR and Western blot. Strains manipulation and growth media preparation followed standard procedures and protocols.

For heterologous expression of *ve-1*, *ve-2*, *vos-1*, *lae-1* in *A. nidulans*, corresponding 4.6 kbp *ve-1* locus (OSB285/286) and ORFs *ve-2* (OZG637/638), *vos-1* (OZG639/640), *lae-1* (OZG641/642) were amplified from genomic DNA (gDNA) of *N. crassa*. *ve-1* was cloned in *Swa*I site of pOSB113, leading to pOSB133 (*pve-1::ve-1*). *ve-2, vos-1* and *lae-1* were cloned under constitutively expressed *pgpdA* promoter in *Swa*I site of pOB257, resulting in pOB322 (*pgpdA::ve-1*), pOB323 (*pgpdA::ve-2*), pOB299 (*pgpdA::lae-1*). These plasmids were transformed into respective deletion strains of *veA*∆, *velB*∆, *vosA*∆, and *laeA*∆. In order to construct GFP fusions of the corresponding velvet complex genes, ORFs of *ve-1* (OZG1127/1128), *ve-2* (OZG891/1129), *vos-1* (OZG893/1130), *lae-1* (OSB297/OZG1131) were amplified from gDNA and fused to sGFP fragment (OZG908/750) and cloned under *pgpdA* promoter in *Swa*I site of pOB257, leading to pOB531 (*pgpdA::ve-1::sgfp*), pOB532 (*pgpdA::ve-2::sgfp*), pOB533 (*pgpdA::vos-1::sgfp*), pOB534 (*pgpdA::lae-1::sgfp*), which were transformed into respective deletion strains.

**Preparation of nuclear and cytoplasmic protein extracts**

For cellular fractionation, mycelia were pulverized to a fine powder in liquid nitrogen. Eight ml of buffer A [1 M sorbitol, 7% (w/v) Ficoll, 20% (v/v) glycerol, 5 mM magnesium acetate, 5 mM EGTA, 3 mM calcium chloride, 50 mM Tris-HCl pH 7.5] were added to the powder. Once homogenized, the mixture was filtered through gauze and two volumes of buffer B [10% (v/v) glycerol, 5 mM magnesium acetate, 5 mM EGTA, 25 mM Tris-HCl pH 7.5] were added to the filtrate. The mix of filtrate and buffer B was slowly added to 10.4 ml buffer C (1:1.7 Buffers A:B) as to prevent the liquid phases from mixing. This step was performed on 25 × 89 mm centrifuge tubes (Beckman). Centrifugation was performed at 3,000 × *g*, 4 °C. A 1 ml sample was collected from the supernatant and labelled total cell extract. The remainder was added to 5 ml of 1 M sucrose gradient [1 M sucrose, 10% (v/v) glycerol, 5 mM magnesium acetate, 1 mM DTT, 25 mM Tris-HCl, pH 7.5] slowly as to prevent mixing of liquid phases. Centrifugation was performed for 30 min at 9,400 × *g*, 4 ºC. The resulting supernatant was collected and labelled cytoplasmic fraction. The pellet was suspended in 500 µl buffer D [25% (v/v) glycerol, 5 mM magnesium acetate, 3 mM DTT, 0.1 mM EDTA, 25 mM Tris-HCl pH 7.5] and labelled as nuclear fraction. Samples for total cell extract, cytoplasmic fraction, and nuclear fraction were quantified

**GFP-TRAP and LC-MS/MS protein identifications**

2 ml pulverized frozen fungal mycelia grown in liquid GMM media at 37 °C for 24h was mixed with 1 ml B buffer [200 mM NaCl, 50 mM Tris pH 7.5, 1 mM EDTA, 0.1% (v/v) NP-40, 1.5 mM DTT, 10% Glycerol, 1.25 mM Benzamidine, 1 mM PMSF, 1x Phosphatase inhibitor cocktail pill (Roche) and 2x complete EDTA free protease inhibitor pills (Roche) (for 100 ml B buffer)] and vortexed. Mixture was centrifuged at 13,000 rpm at 4 °C for 10-15 min. Supernatant was poured into a new 1.5 ml eppendorf tube. 20 μl GFP-TRAP magnetic beads were first washed with in 200 μl B buffer and mixed with protein extracts from GFP fusion expressing strains (*N. crassa* and *A. nidulans*). The protein extracts and beads were incubated on the rotator for 2h at 4 °C. The beads were separated from protein extract by using DynaMag-2 rack (ThermoFisher) and washed three times with 1 ml B buffer. For final wash step, no protease inhibitor was used in B buffer to facilitate trypsin digestion on beads. Half of the beads were used to run on 10% SDS polyacrylamide and stained with silver to visualize the GFP pulldowns. The rest was used for trypsin digestion. Proteins attached to magnetic beads were digested directly on the beads by addition of protease max and trypsin and followed by C-18 zip tip purification before running in LC-MS as described (O'Keeffe *et al.* 2014). LC-MS identifications of GFP fusions and their interaction partners were carried out as described in detail in by using Proteome Discoverer Version 1.4 (Thermo Fisher). Specific interaction partners of the VE-1GFP, VE-2GFP, VOS-1GFP, and LAE-1GFP were determined by filtering out the proteins found in only GFP control samples. Each experiment was performed at least as two biological replicates.

**Microscopy**

Spinning disc confocal microscopy of *N. crassa* was performed as described in (Dettmann *et al.* 2012) using an inverted Axio Observer Z1 microscope (Zeiss) equipped with a CSU-22 confocal scanner unit and a CCD camera (Axiocam MRm Rev.3). ZEN Blue 2012 software (Zeiss) was used for image/video acquisition and image analysis. Plasma membrane was stained with FM4-64 (1 mg/ml). Microscopy of *A. nidulans* was performed as described in (Bayram *et al.* 2012). *A. nidulans* strains expressing VEL1, VEL2, VOS1, LAE1-GFP fusion proteins were grown in liquid GMM in 8-well borosilicate cover glass chambers at 30 °C for 14-18h (Nunc). Confocal images of GFP fused proteins were recorded with an Axiovert Observer Z1 (Zeiss) microscope in combination with CSU-X1 A1 confocal scanner unit (Yokogawa) and QuantEM:512SC (Photometrics) camera. Confocal images were processed by using the SlideBook 5.0 software package (3i). Nuclei were stained by using 5 μM DRAQ5 dye (Abcam) for 10-15 minutes.

**Supernatant preparation and organic extraction for *N. crassa***

Supernatants were stored at -20 °C until direct analysis or organic extraction. For siderophore analysis, supernatants were brought to 1.5 mM FeSO4 using 50 mM FeSO4 and left to chelate iron at RT for 20 min. Supernatants were centrifuged at 13,400 rpm 10 min prior to HPLC injection (100 μl). For organic extraction, 20 ml supernatant was mixed with 20 ml ethyl acetate with agitation for 3 min. Samples were centrifuged at 1,000 or 4,500 rpm for 10 min. Aqueous and lipid layers were removed with a Pasteur pipette and organic layer evaporated under a vacuum at 60 °C until dryness. Dried extracts were washed with 1-2 ml methanol. Samples were stored at -20 °C overnight then centrifuged at 13,400 rpm 10 min and supernatant moved to a fresh eppendorf. Methanol volume was reduced by evaporation in a Speedivac and final extracts were brought to a 200 μl volume in methanol. Samples were stored at -20 °C until analysis.

**Biomass determination**

Harvested mycelia from *N. crassa* in 15 ml falcons were moved from -80 °C into liquid nitrogen and then immediately lyophilised for 2 overnights. Dry biomass was determined by weighing in original 15 ml falcons.

**Carotenoid analysis**

Mycelia were collected from the liquid medium by filtration and were immediately frozen in liquid nitrogen and freeze-dried for 24h. Carotenoids were extracted with acetone in a FastPrep-24 device (MP Biomedicals), dried in a Concentrator Plus equipment (Eppendorf) and dissolved in 0.1-1 ml of n-hexane. Spectrophotometrical measurements were recorded from 350-650 nm in a Shimadzu UV spectrophotometer 1800. Total amounts of carotenoids were estimated according to the maximal absorbance at 480 nm ca. of the sample and an average maximal E of (1 mg l-1, 1 cm) of 200. Final values were corrected to the dry weight and dilution of the sample.