**SI Appendix**

## Materials and Methods:

### Devlopmental expression analysis of *GmbHLHm1*

Soybean (*Glycine max* cv. Djakal) seeds were planted in river sand and grown in growth chambers. Soybean nodules were collected every 5 days from 10 to 40 days after inoculation with *Bradyrhizobium japonicum* USDA110 at planting. Nodule tissue was frozen in liquid nitrogen and ground with a mortar and pestle. Total RNA was extracted from individual plant nodules with a Spectrum Plant Total RNA kit (Sigma-Aldrich) and treated with Turbo DNase (Ambion). 1 μg of total RNA was then used for cDNA synthesis with Superscript III (Invitrogen) and oligo-$dT_{20}$. qPCR was carried with IQ SYBR Green (Bio-Rad) in a Light Cycler (Bio-Rad) qPCR machine. After initial denaturation, the reaction was run for 36 cycles (96°C for 10sec, 55°C for 5 sec, 72°C for 20 sec). Either absolute or relative qPCR analysis was conducted for *GmbHLHm1* expression using primers GmbHLHm1-qPCR-F/R (Table S3). For absolute measurements, the molar concentrations of transcripts were calculated using DNA standards of known concentration generated from each respective gene. Ten-fold serial dilutions of the standards (from $10^{-3}$ to $10^{-8}$ fmol $\mu l^{-1}$) were amplified using qPCR, and the crossing points plotted against the log concentration to generate a standard curve. Reactions for the standard curve were performed under the same conditions as reactions containing cDNA, so that amplification efficiency was the same in all cases. For relative measurements, the mean $C_T$ value of three biological replicates was used to generate a $\Delta C_T$ value using *cons6* as an internal control

(1). The relative expression was then calculated by using the formula $2^{-\Delta CT}$ (2). The efficiency of each primer set was between 95 and 100%.

### *GmbHLHm1* expression in response to nitrogen treatment

RNA was isolated from soybean tissues grown in the absence of nitrogen fertilization for 14 days and thereafter under minus nitrogen conditions or supplied 5 mM $KNO_3^-$ daily in the irrigation supply. Amplified cDNA was then used in qPCR (Light Cycler) with primers specific to *GmbHLHm1* (bHLHm1LCF, bHLHm1LCR) and *GmRPS19* (GmRps19-fwd, GmRps19-rev) a ribosomal protein measured as a control (Table S3). In order to calculate the molar concentration of transcripts, DNA standards of known concentration were generated for each gene from the cloned cDNA segments. Ten-fold serial dilutions of the standards (from $10^{-3}$ to $10^{-8}$ fmol $\mu l^{-1}$) were amplified in Light Cycler reactions, and the crossing points plotted against the log concentration to generate a standard curve. Reactions for the standard curve were performed under the same conditions as reactions containing cDNA, so that amplification efficiency was the same in all cases.

### *GmbHLHm1* Promoter Analysis

The *GmbHLHm1* promoter (1926 bp upstream of start codon) was cloned with *GmbHLHm1*-Promoter-F/R primers using Platinum Taq High-Fidelity (Life Technologies). The fragment was ligated into pCR8-TOPO (Life Technologies) and recombined into the destination vector pKGWFS7 (3). The plasmids were then transformed into *Agrobacterium rhizogenes* K599. Hairy roots were generated according to Mohammadi-Dehcheshmeh *et al*. (4), except that transformed plants were incubated in glass jars to facilitate hairy root emergence. For GUS

analysis, hairy roots were fixed in 90% acetone on ice for 15 minutes, partially sectioned with a razor blade, then incubated in GUS staining buffer (0.1 M sodium phosphate buffer pH 7, 3% (w/v) sucrose, 0.5 mM EDTA, 0.5 mM potassium ferrocyanide, 0.5 mM potassium ferricyanide, 0.05% (w/v) X-Gluc). Nodules were then fixed in 5% (v/v) glutaraldehyde and embedded in Technovit 7100 (Heraeus Kulzer). 8 µm sections were cut on a Leica RM2265 microtome, stained with 0.05% (w/v) ruthenium red, and embedded in DPX (Sigma-Aldrich). Images were obtained with a Leica ASLMD laser-assisted micro-dissection microscope.

**GmbHLHm1 Immunogold Labeling**

Nodules were prepared for TEM analysis and immunogold labeling as described by Fedorova et al. (5). For immunogold labeling, nickel grids were placed specimen side down on drops of 50 mM glycine for 2 x 10 min followed by 1% (w/v) BSA in PBS for 15 min. Grids were incubated overnight at 4°C on 20 µl primary antibody, diluted 1:100 with 1% (w/v) BSA in PBS. Grids were rinsed 6 x 5 min on 1% (w/v) BSA in PBS, blotted on filter paper and incubated for 1 h at room temperature in a goat anti-rabbit 10 nm gold conjugated secondary antibody (Aurion), at 1:100 dilution in 1% (w/v) BSA in PBS. Grids were rinsed 6 x 5 min in PBS, washed briefly four times in dd$H_2O$ before being counter stained using 3% (w/v) uranyl acetate in 70% (v/v) ethanol for 3 min. Sections were observed using a Phillips CM100 transmission electron microscope.

**Localization of GmbHLHm1 in yeast**

mGFP5 was PCR amplified from the pCAMBIA 1302, minus the 6xHIS tag, with primers with ~ 30 bp 5' overhangs specific to pYES3 and *GmbHLHm1* sequences with primers N-term-GFP-F/R and C-term-GFP-F/R. pYES3-GmbHLHm1 vector was digested within the region to be recombined with suitable restriction endonucleases (*Sal*I for the N-terminal GFP-bHLHm1

construct, *Sph*I and *Not*I for the C-terminal bHLHm1-GFP construct). Both the PCR amplified GFP and digested pYES3-GmbHLHm1 were used to co-transform the yeast strain 26972c to allow for the recombination of the digested vector with the homologous regions of the GFP PCR product. Plasmid was extracted from putative positive yeast colonies and used to transform *E. coli* strain DH5α. Plasmid DNA was purified and its identity verified by restriction enzyme analysis and sequencing. GFP was visualized in mid-log phase yeast either directly using a Leica spectral confocal microscope or stained with the vital DNA stain Hoechst 33342 at a final concentration of 500 μM, and viewed using a BioRad MRC-1000UV microscope.

**Yeast Protein Expression and Western Blotting**

For anti-GmbHLHm1 western blots, yeast protein extracts were prepared from approximately 10 ml of exponentially growing cells. Cells were pelleted and washed once with 1 mM EDTA, pH 8.0. The pellet was then resuspended in 200 μl 2 M NaOH and transferred to a 1.5 ml microfuge tube and incubated for 10 min on ice. 200 μl 50% (w/v) trichloroacetic acid was added and the tube vortexed before being left on ice for 1 hr. Samples were spun at 14,000 rpm at 4°C for 20 min in a microcentrifuge. The pelleted protein was washed once with 100% acetone before being resuspended in 5% (w/v) SDS. An equal volume of SDS sample buffer (25 mM Tris-HCl (pH 6.8), 9 M urea, 1 mM EDTA, 1% (w/v) SDS, 0.7 M β-mercaptoethanol, 10% (v/v) glycerol) was then added to the resuspended protein. Proteins were transferred onto a nitrocellulose membrane, stained with Ponceau red to confirm protein transfer and then probed with an anti-GmbHLHm1 polyclonal antibody (1:5000 dilution) and detected with a BM Chemiluminescence Western Blotting Kit (Roche).

For anti-GFP western blots, 26972c containing GFP:GmbHLHm1-pYES3, GmbHLHm1-pYES3, GFP-pYES3 or empty pYES3 were grown in YNB with 2% (w/v) glucose. Cells were

then pelleted, washed in water and diluted to an $OD_{600}$ of 0.4 in YNB containing 2% (w/v) galactose. Cells were grown for 24 hours, pelleted and total protein was extracted according to (6). 20 µg of total protein was separated by 12% SDS-PAGE and transferred to nitrocellulose for blotting with a SNAPid system (Millipore). Rabbit anti-GFP antibody (Cell Signaling) was used at a dilution of 1:3000, followed by 1:6000 secondary anti-rabbit conjugated to peroxidase (Sigma). Signals were detected by chemiluminescence according to Haan and Behrmann (7).

**GFP-tagging of *ScAMF1***

The *GFP-ScAMF1* (ScAMF1 N-terminally tagged with GFP) fusion construct was synthesised by overlapping PCR. The *mgfp5* sequence without the stop codon was amplified from the pCAMBIA1302 vector (using the mGFP5-F and N-term-ScAMF1-GFP-R primers), while the complete *ScAMF1* sequence was amplified (using the N-term-ScAMF1GFP-F and ScAMF1-R primers) from the yeast expression vector YOR378W-BG1805 (Open Biosystems Yeast ORF Collection, Thermo Scientific) in separate PCR reactions with Phire Hot Start DNA polymerase (Finnzymes, Finland). The initial PCR products were combined in a second PCR to amplify the *GFP-ScAMF1* fusion using GFP-F and ScAMF1-R primers with HIFI 5 Prime PCR Extender DNA polymerase (5 Prime). The final product was cloned into pCR8-TOPO (Life Technologies) and recombined into pYES3-DEST. The pYES3- GFP-*ScAMF1* plasmid was transformed into 26972c yeast using a the LiAC/PEG method (8). To visualize GFP, pYES3-GFP-*ScAMF1* 26972c were grown in DIFCO yeast nitrogen base (YNB) with 2% glucose (w/v) for two days, then diluted to $OD_{600}$ 0.4 in YNB with 2% galactose (w/v) and grown for sixteen hours. GFP was monitored using a Leica SP5 confocal microscope (488 nm argon laser excitation, 505-520 BP filter detection). Cells were also counterstained using FM4-64 (Life

Technologies, USA). Cells were kept on ice, stain added and then observed by confocal microscopy within 10 min.

**Modified Split-Ubiquitin Experiments**

GmbHLHm1 was cloned into the *Pst* I / *Sac* I sites of pNCW, in frame with the N-terminal VP16/LexA-Cub construct using GmbHLHm1 specific primers with introduced Pst I / Sac I restriction sites (Table S3). GmbHLHm1 was also PCR amplified from pYES3-GmbHLHm1 with primers (pCMBV4-bHLHm1-FW/RV – see Table S3), which have 40 bp 5' overhangs homologous to upstream of the VP16/LexA-Cub regions of the pCMBV4 vector. The yeast reporter strain DSY-1 (MATa his3Δ200 trp1-901 leu2-3, 112 ade2 LYS2::(lexAop)4-HIS3 URA3::(lexAop)8-lacZ GAL4, Dualsystems TM) was co-transformed with *Sfi* I-digested pCMBV4 and the GmbHLHm1/pCMBV4 PCR product described above to allow recombination of the digested vector and homologous regions of the PCR product. Positive recombinants/transformants were initially selected by colony PCR and the construct isolated, used to transform DH5α *E. coli* and verified by restriction enzyme digests and sequencing. Beta-galactosidase activity was determined in the reporter yeast strain DSY-1 using an X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, Sigma) filter lift-off assay as per manufacturer's instructions (Dualsystems Biotech).

**Onion transformation**

GFP:GmbHLHm1 (primers GFP-pYES3-F and GmbHLHm1-CDS-R) and GFP:T1-GmbHLHm1 (primers T1-GmbHLHm1-F and GmbHLHm1-CDS-R) were amplified from a *GFP-GmbHLHm1*-pYES3 template. All fragments were inserted into pCR8-TOPO (Life Technologies). GFP:GmbHLHm1-pCR8 was recombined into the destination vector pK7WG2D

and T1-GmbHLHm1 was recombined into the destination vector pP7WGF2 (3). Both resulting plasmids were then digested with *Sac*I and *Xba*I and the resulting fragment (including a 35S promoter and terminator) was ligated into pBluescript. The plasma membrane marker pm-rk CD3-1007 (9) was digested with *Sac*I and *Eco*RI and inserted into pBluescript.

5 µg of purified plasmid DNA (in 10 µl) was combined with a 50 µl suspension (1.5 mg of 0.6 µm gold macrocarriers (Bio-Rad) in 50% (v/v) glycerol). Onion epidermal peels were bombarded with a PDS-1000/He particle delivery system (Bio-Rad) using a 1100 psi Rupture Disc. Epidermal peels were maintained on Murashige and Skoog basal medium (with vitamins, Austratec) supplemented with 9 g $L^{-1}$ tissue culture grade agar, 120 g $L^{-1}$ sucrose and 500 mg $L^{-1}$ tryptone. After bombardment, the peels were incubated in the dark for 24 h at room temperature before viewing. Images were obtained using a Zeiss LSM 5 Pascal confocal microscope. GFP fluorescence was monitored by excitation at 488 nm with an argon laser combined with a 505-520 nm bandpass filter. mCherry fluorescence was obtained by excitation at 543 nm with a helium/neon laser combined with 615 nm longpass filter.

***Nicotiana benthamiana* transformation**

*Agrobacterium tumefaciens* strain GV3101 harboring individual GFP fusion expression constructs were grown for 2 days at 28°C in liquid LB medium supplemented with rifampicin (50 mg $L^{-1}$), gentamycin (25 mg $L^{-1}$), tetracycline (5 mg $L^{-1}$), and kanamycin (50 mg $L^{-1}$). Cells were pelleted and resuspended at an $OD_{600}$ = 0.5 in infiltration medium (10 mM $MgCl_2$, 150 µM acetosyringone, 10 mM 2-(*N*-morpholino) ethanesulfonic acid (MES, pH 5.6) and left for 2 h in the dark, mixing occasionally. Approximately 100 µl of the *Agrobacterium* cell suspensions were infiltrated into the abaxial surface of 6-week-old *Nicotiana benthamiana* leaves using a 3 ml syringe. Leaves were viewed 2 – 3 days following infiltration using a confocal microscope

(LSM Pascal 410, Zeiss, Oberkochen, Germany) equipped with an argon laser with a 488 nm laser line. GFP and chlorophyll fluorescence were detected using 505-530 nm band-pass and 560 nm long-pass filters respectively. Images were captured and further analyzed using the LSM Pascal 5.0 software suite (Zeiss, Oberkochen, Germany).

**Yeast Microarray Analysis**

Starter yeast cultures were initially prepared to ensure uniform cell growth. The 26972c yeast strain (*mep1-1, mep2*Δ*, Mep3, ura-*) (10) harboring *GmbHLHm1*-pYES3 or the pYES3 empty vector was inoculated in 20 ml of Grenson's minimal yeast media (11) supplemented with 2% w/v glucose and 0.1% (w/v) L-proline. The cultures were grown in 50 ml falcon tubes and incubated at 28°C and shaken at 200 rpm for 24 h. Ten replicate sterile 50 ml falcon tubes containing identical media were spiked with 1 ml of each yeast starter culture and incubated at 28°C at 200 rpm for 2 days. Log-phase cells were pelleted, washed in sterile Milli Q $H_2O$ twice and then resuspended in 20 ml of Grenson's minimal yeast media supplemented with 2% (w/v) galactose and 0.5 mM $(NH_4)_2SO_4$. The cells were incubated at 28°C and shaken at 200 rpm. The cell cultures were harvested after 12 h of incubation and washed once in 20 ml of ice-cold sterile Milli Q water. The cell pellets were transferred to 1.5 ml microcentrifuge tubes with 1 ml of sterile water, mixed and then centrifuged at 1400 x *g*. The supernatant was removed and the cell pellets snap frozen in liquid nitrogen for 30 sec and stored at -80°C. Total RNA was extracted using hot acidified phenol according to Collart et al (12). Frozen yeast cell pellets were resuspended in 400 µl of extraction buffer (10 mM Tris-Cl, 10 mM EDTA, 0.5 % w/v SDS, pH 7.5) solution. An equal volume of acidified phenol (pH 5) was added and vortexed for 10 sec and then incubated at 65° C for 60 min with intermitting light vortexing every 15 minutes. The cell solution was removed from the water bath and placed on ice for 5 min. The cooled cell solution

was centrifuged for 5 minutes at 14,000 x *g* at 4°C and the aqueous phase collected and re-extracted with 400 µl of acidified phenol. The solution was vortexed and then transferred to ice for 5 minutes and then centrifuged for 5 minutes at 16000 x *g* at 4°C. The aqueous phase was added to 400 µl of chloroform, mixed and the aqueous phase collected. RNA was ethanol precipitated and washed in 70% ethanol before resuspending in 50 µl of ice-cold DEPC-treated Milli Q water. RNA samples were diluted to a uniform concentration of 250 ng $\mu l^{-1}$ and analyzed on an Agilent 2100 Bioanalyser (Hanson Centre, Adelaide) and using a Nanodrop ND-1000 spectrometer. RNA labeling and hybridization to the Affymetrix Yeast Genome 2 Array and scanning was performed by the Ramaciotti Centre for Gene Function Analysis (University of New South Wales, Sydney, Australia) where all labeling kits and hybridization protocols followed those prescribed by Affymetrix. Raw CEL data files were imported into GeneSpring software and pre-processed using Robust Multichip Average (RMA) algorithm (13) where the variation between the chips was normalized at the probe set level to obtain the ratio of expression to the median expression for each sample. The mean value of the normalized gene absorbance measurements for each Affymetrix chip was calculated using a two-sample Bayesian *t*-test to provide statistical verification of changes in gene expression.

**Deletion of *ScAMF1* from 26972c Genome**

The *ScAMF1* (YOR378W) locus was replaced with the kanamycin resistance gene (*kanMX4*) through homologous recombination (26972c Δ*amf1*). The *kanMX4* gene was amplified from the pFA6a plasmid (14) using forward and reverse primers designed (YOR378wF-KanMx4 and YOR378wR-KanMx4) with overhangs specific to 15 nucleotides upstream and downstream of the *ScAMF1* locus, including the start and stop codons, respectively. High Fidelity Phusion DNA polymerase (Finnzymes, Finland) was used to amplify *kanMX4*. The PCR product was used in a

subsequent PCR reaction with primers (F-UP and R-DN) to extend the genomic flanking regions to 40 nucleotides. The 26972c yeast strain was transformed with the amplified *kanMX4* product using the LiAC/PEG/salmon sperm transformation protocol with the following modification: After the 42°C heat shock, cells were spun down, and resuspended in 40 ml YDP (10% (w/v) glucose, 10% (w/v) peptone and 5% (w/v) yeast extract) and incubated at 28°C with shaking at 200 rpm overnight before plating on 2xYPD supplemented with 200 μg $ml^{-1}$ geneticin (Sigma-Aldrich, USA). The deletion of *ScAMF1* was determined by PCR using primers internal to *kanMX4 (kanB and kanC)* and to the genomic sequence up and downstream of the *ScAMF1* locus (YOR378-KO-F and YOR378-KO-R) (Table S3).

**26972c plate assays and methylammonium uptake**

All yeast strains (26972c, 31019b (*MATa ura3 mep1*Δ *mep2*Δ*::LEU2 mep3*Δ*::KanMX2*), 26972c Δ*amf1*) were first transformed with pYES3, pYES3-*GmbHLHm1, pYES3-ScAMF1, pYES3-GmAMF3* and grown in 20 ml of liquid of a modified minimal yeast media (11) supplemented with 2%(w/v) glucose and 0.1% (w/v) L-proline in sterile 100 ml glass conical flasks. The cultures were incubated for approximately 2 days at 28°C and shaken at 200 rpm. The saturated yeast cultures were harvested by centrifugation at 4,000 rpm and washed twice in 50 ml of sterile Milli Q water. The yeast solutions were diluted to an $OD_{600}$ of 0.6. A 10-fold dilution series was carried out for each transformation combination. Dilution series were spotted out in 5μl aliquots onto solid Grenson's selective media. The yeast plates was incubated for 5 days at 28°C.

For MA uptake experiments, starter yeast cultures were grown overnight in 20 ml of liquid YNB media (0.67% (w/v) Yeast Nitrogen Base without amino acids (Difco) and 2% (w/v) glucose) in sterile 100 ml glass flasks. The cultures were incubated overnight at 28°C with shaking at 200 rpm, harvested at 4, 000 rpm for 2 min and washed twice in 50 ml sterile Milli Q water. The cells

were resuspended to an $OD_{600}$ of 0.2 in 20 ml of a modified minimal liquid media (11) at pH 6.5 supplemented with 1mM $NH_4Cl_2$ and 2% (w/v) D-galactose, and incubated overnight at 28°C with shaking at 200 rpm. The cells were harvested at an approximate $OD_{600nm}$ 1.0, and washed twice with 50 ml of sterile Milli Q water before being resuspended in room temperature $KPO_4$ buffer (20 mM $K_2HPO_4/KH_2PO_4$ buffer (pH 6.2) supplemented with 2% (w/v) D-galactose) to a uniform $OD_{600nm}$ 4.0.

The flux experiment consisted of 6 replicates for each transformed cell type staggered by 20 seconds: 100 µl of resuspended cells was added to 100 µl $KPO_4$ reaction buffer with $^{14}C$-methylammonium (Perkin-Elmer) in 1.5 ml microcentrifuge tubes. After 10 min, 100 µl of the cells was removed and collected by vacuum filtration on to a 0.45 µM nitrocellulose filter (Millipore, USA) and washed twice with 5ml of ice-cold $KPO_4$ buffer to prevent further $^{14}C$-methylammonium uptake. The cells collected on filters were washed twice with 5 ml of ice-cold $KPO_4$ buffer. The filters were placed into scintillation vials (Sarstedt) with 4 ml of scintillation fluid (StarScint-Perkin-Elmer). The radioactivity of the samples was determined with a liquid scintillation counter (Tri-Carb 2100TR, Packard). Counts were converted to equivalent amount of methylammonium and samples were normalized against total protein according to a modified Lowry method (15).

**GmbHLHm1 Mutagenesis**

GmbHLHm1 point mutations were generated by PCR of pYES3-*GmbHLHm1* using mutagenic primers (GmbHLHm1-R180K-F/R, GmbHLHm1-L191V-F/R, GmbHLHm1-L207V-F/R) (Table S3). The high fidelity polymerase *Pfu* (Promega) was used according to the manufacturer's instructions, using the following PCR conditions: 95°C 1 min; 30 cycles of 95°C 45 s, 50°C 45 s, 72°C 16 min. Reactions were spiked with 1 µl of *Dpn*I, a methylated DNA-specific restriction

endonuclease, and incubated at 37°C for 2 h to remove contaminating template plasmid DNA. *E. coli* strain DH5α was transformed with 5 µl of the PCR reaction and selected using restriction enzyme sites introduced or removed through the mutagenesis PCR.

**Electromobility Shift Assay**

A truncated version of GmbHLHm1 (amino acids 128-270) was amplified (GmbHLHm1-pMAL-F/R) with Phusion DNA polymerase (Finnzymes) and inserted into CloneJET (Fermentas). The fragment was then excised with *Nde* I and *Bam* HI and ligated into pMAL-c5X (New England Biolabs) to create a N-terminal fusion to the maltose-binding protein (MBP). MBP was expressed by using the empty pMAL-c5X plasmid. The plasmids were transformed into the *E. coli* strain NEB Express (New England Biolabs). MBP-GmbHLHm1$_{128\text{-}270}$ and MBP alone were expressed and purified according to the manufacturers instructions using an amylose resin (New England Biolabs).

A fragment of the YOR378W (-284 to ATG) promoter was amplified (YOR378W-Promoter-F/R primers) and ligated into pGEM-T Easy (Promega). The promoter fragment was then excised with *Not*I and end-filled with $^{32}$P dCTP (3000 ci/mmol, Easytide 5' Triphosphate γ-$^{32}$P, PerkinElmer) using the Klenow fragment (3'->5' exo-, New England Biolabs) in a 50 µl reaction (1 µl $^{32}$P-dCTP, 1 µl 1mM dGTP, 1 µl Klenow, 400 ng promoter fragment). Labeled probe was desalted with S-200 HR Microspin Columns (GE Healthcare). 1 µg of MBP-GmbHLHm1$_{128\text{-}270}$ or MBP alone was incubated with the labeled probe in a 20 µl reaction at room temperature for 25 min. The reaction contained 15 % (v/v) glycerol, 20 mM Tris pH 8.0, 175 mM NaCl, 5 mM EDTA, 50 mM KCl, 0.5 mM DTT, 0.5 µl Poly [d(I-C)] (1µg µl$^{-1}$) and 4 µl of probe. The reaction was then loaded on a 6% (w/v) native Tris-glycine polyacrylamide gel containing 10% (v/v) glycerol. After separation, the gel was vacuum dried and exposed to film for 24 h at -20°C.

**Yeast Growth Conditions for Quantitative PCR Analysis**

Three biological replicates of the experiment (pYES3-*GmbHLHm1* or pYES3-*GmbHLHm1*-mutation of interest) and the control (pYES3) were prepared for quantitative PCR (qPCR). The growth conditions were identical for all of the cell samples. The treatment was applied to all six samples and consisted of a 12 h 2% (w/v) D-galactose and 0.5 mM $(NH_4)_2SO_4$ induction.

Starter yeast cultures were initially prepared to ensure uniform cell growth. The 26972c yeast strain harbouring the pYES3-GmbHLHm1 (wild type or mutants) or the pYES3 empty vector was inoculated in 20 ml of Grenson's minimal yeast media supplemented with 2% (w/v) glucose and 0.1% (w/v) L-proline. The cultures were grown in 50 ml sterile falcon tubes and incubated at 28°C and shaken at 200 rpm for 24 h. Three replicate sterile 50 ml falcon tubes containing identical media were spiked with 1 ml of each yeast starter culture. The glucose proline yeast cultures were incubated at 28°C and shaken at 200 rpm for 2 days. The log-phase cells were transferred to 20 ml of Grenson's minimal yeast media supplemented with 2% (w/v) galactose and 0.5mM $(NH_4)_2SO_4$ after being washed twice with 20 ml of sterile Milli Q water. The cells were incubated at 28°C and shaken at 200 rpm. The cell cultures were harvested after 12 h of incubation and washed once in 20 ml of sterile Milli Q water. The cell pellets were transferred to 1.5 ml microcentrifuge tubes with 1 ml of sterile water, mixed and then centrifuged at 1,400 rpm. The supernatant was aspirated off and the cell pellets snap frozen in liquid nitrogen for 30 seconds. The frozen cell pellets were stored at -80°C until RNA extraction was required.

The frozen harvested yeast cell pellets were resuspended in 400µl of TES (10mM EDTA, 10mM Tris-Cl, pH 7.5 and 0.5% (w/v) SDS) solution before they began to thaw. To the TES cell solution, 400 µl of acidified phenol (pH 5.0) was added. The cell solution was vortexed for 10 s and then incubated at 65°C for 60 min with intermitting light vortexing every 15 min. The cell

solution was removed from the water bath and placed on ice for 5 minutes. The cooled cell solution was centrifuged for 5 minutes at 14,000 g at 4°C. The centrifugation resulted in the separation of organic and aqueous phases. The aqueous phase, containing the RNA, was collected and re-extracted with 400 µl of new acid phenol. The solution was vortexed for 10 seconds and then transferred to ice for another 5 minutes. After the incubation period the solution was centrifuged for 5 minutes at maximum speed at 4°C. The aqueous phase was once again collected into a new twice-autoclaved 1.5 mL microcentrifuge tube. 400 µl of chloroform was added to the twice-extracted aqueous phase, which was then vortexed and centrifuged for 5 min at maximum speed at 4°C.

The RNA was precipitated with 40 µl of 3M sodium acetate (pH 5.3) and 1 ml of ice-cold 100% ethanol was added to the collected aqueous phase of each sample. The samples were incubated at -80°C for 30 minute and then centrifuged for 5 minutes at maximum speed at 4°C. The supernatant was removed from the RNA pellet, which was then washed in 50 µl of 70% ice-cold ethanol. The samples were centrifuged for 5 minutes at maximum speed at 4°C. Most of the ethanol was carefully removed with a 2 µl pipette and the rest was evaporated, but the RNA pellet was allowed to remain wet. The resulting RNA pellet was resuspended in 50 µl of ice-cold DEPC-treated Milli Q water and heated at 65°C for approximately 10 min to dissolve the RNA entirely. The RNA was snap frozen in liquid nitrogen for 30 s and stored at -80°C.

**Yeast cDNA synthesis and qPCR**

The extracted total RNA (2µg) was treated with TURBO DNase (Ambion) to remove residual genomic DNA contamination prior to cDNA synthesis. 1 µg of the DNase-treated total RNA was converted to cDNA using the SuperScript III Reverse Transcriptase kit (Life Technologies). The cDNA was diluted 10-fold with sterile Milli Q water and stored at -20°C. qPCR with

performed with iQ SYBR Green Supermix reagent (Bio-Rad), 0.50 μM primers, 2 μL of cDNA in a 20 μl reaction volume. The cycling conditions were as follows: 2 min at 95°C, 40 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 1 min. Relative expression values were calculated by the $2^{-\Delta CT}$ method (16) using TUB1 and ALG9 as a reference (17).

**Cloning of GmAMF3**

Soybeans were planted in Waikerie sand (ten seeds per cm pot) and inoculated with *Bradyrhizobium japonicum* USDA110 on the day of planting and again the following day (100ml of a 1/10 dilution of a late log phase culture). Plants were grown in a glasshouse and watered daily, substituting nitrogen-free Herridge's nutrient solution (18) three times per week. Tissue was collected from nodules and frozen in liquid nitrogen. RNA was extracted using a RNeasy kit (Qiagen) and cDNA was synthesized from 1.25 μg of RNA using Superscript III (Invitrogen). GmAMF3 was amplified using primers GmMFS1.3 CDS F and R3 with 2 μl of a 1/10 dilution of nodule cDNA using Platinum Taq High Fidelity (Invitrogen). The full-length product was then inserted into pCR8 TOPO, but upon transformation into the *E. coli* strain TOP10 there were few colonies (grown at 37°C). Sequencing of the isolated plasmids found mutations in the *GmAMF3* coding sequence. Therefore, the pCR8 TOPO reaction was transformed into the strain XL1-Blue and grown at room temperature on LB media containing a reduced concentration of NaCl (5 g $L^{-1}$). After 4 days growth, numerous colonies appeared and sequencing found that these clones were mutation-free.

**Synthesis of *ScAMF1 and GmAMF3* cRNA and injection into *X. laevis* oocytes**

*ScAMF1*-pCR8 *and GmAMF3-PCR8* was recombined individually into the expression vector pGEMHE-DEST (19). The cRNA was prepared by linearizing pGEMHE-*ScAMF1* and

pGEMHE-*GmAMF3* with *Nhe* I. Potential RNase contaminates were then eliminated by incubating the digestions with 1µl of proteinase K (20 mg $ml^{-1}$) (Life Technologies) in the presence of SDS to a final concentration of 0.5% (w/v) at 50°C for 1 h. The linearized plasmids were then extracted with 1 volume of phenol/chloroform/isoamyl-alcohol. The aqueous phase was then withdrawn and the DNA was precipitated with 1/10 volumes of 5 M ammonium acetate (pH 5.6) and 2 volumes of 100% (v/v) ethanol, incubated at -80°C for 15 min. The DNA was pelleted by centrifugation at maximum speed at room temperature for 15 min. The cRNA synthesis was carried out with a mMESSAGE mMACHINE T7 Kit (Ambion). The remaining plasmid template was digested with 1 µl of TURBO DNase (Ambion) at 37°C for 15 min. The RNA was precipitated by adding 30 µl of LiCl Precipitation solution (Ambion) and chilling at -80°C for 1 h. The cRNA was pelleted at 4°C for 15 min at maximum speed. The cRNA pellet was washed three times with 100 µl of 70% (v/v) ethanol. The quality of the RNA was observed by electrophoresis with a TBE denaturing agarose gel at 90V for 1 hour. The concentration of cRNA was calculated using a Quant-iT RiboGreen RNA assay kit (Life Technologies) with a VersaFluor Fluorometer (BioRad). The cRNA was diluted to a uniform concentration of 500 ng $\mu l^{-1}$ and stored at -80°C until required for injection into oocytes.

***Xenopus laevis* oocyte extraction and preincubation**

Oocytes were extracted and prepared from individual *Xenopus laevis* frogs (NASCO Biology) as described in Preuss et al. (20). Individual oocytes were injected with 23 ng (46 nl) of cRNA or nuclease-free water (control) and incubated in a modified ND10 buffer (200 mM mannitol, 10 mM $NaH_2PO_4$, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 10 mM HEPES, and pH 7.5 adjusted with Tris-base) supplemented with 8% (w/v) horse serum, 100 µg $ml^{-1}$ tetracycline, 1000 U $mL^{-1}$ penicillin

and 100 µg $mL^{-1}$ streptomycin at 18°C for 1-2 days. The oocytes were transferred to fresh solution on a daily basis.

### *Xenopus laevis* oocyte $^{14}$C-methylammonium uptake and electrophysiology

Prior to $^{14}$C-methylammonium uptake analysis, oocytes were incubated in MBS buffer (96 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM MES, pH adjusted to 6.5 with NaOH) for 20 minutes at room temperature. Single oocytes (10-12 replicates) were incubated at 18°C in 180 µl of MBS buffer supplemented with MA at 25 µM, 50 µM, 150 µM, 30 µM, 600 µM and 1000 µM supplemented with $^{14}$C-labeled MA (Perkin Elmer). After an incubation period of 16 hours, the oocytes were washed twice in 5ml of ice-cold MBS buffer with unlabelled MA. Individual oocytes were pipetted into scintillation vials with 50 µl of dilute $HNO_3$ and dissolved for 2 hours before adding 4 mL of IRGA-Safe Plus scintillation fluid (Perkin Elmer). The beta-decay was measured using a liquid scintillation counter (Tri-Carb 2100TR, Packard).

### *Xenopus laevis* oocyte electrophysiology

TEVC experiments were performed with a GeneClamp500 amplifier using Clampex8 (Axon instruments) as described by Preuss et al. (20). Standard recording solution (200 mM mannitol, 1 mM $MgCl_2$, 0.2 mM $CaCl_2$, 10 mM HEPES, and pH 7.5 adjusted with Tris-base) with or without $NH_4Cl$ (0 or 5 mM). The oocytes were clamped at stable resting potentials negative of -25 mV. Currents were measured at 20 mV increments from -140 mV to +60 mV for 3 s.

### RNAi silencing of *GmbHLHm1*

A 359 bp portion of the *GmbHLHm1* 3'UTR was amplified with the primers GmbHLHm1-RNAi-F/R, and inserted into pCR8-TOPO (Life Technologies). The fragment was then recombined into pK7GWIWG2D (II) (3) and transformed into *Agrobacterium rhizogenes* K599.

Soybean root transformation followed the method of Mohammadi-Dehcheshmeh *et al*. (4). Seeds were first germinated in petri dishes (15 cm dia). Seedlings with an emerged radicle were then inoculated and transferred individually to sterile Waikerie sand within 500 ml glass jars. The seedlings were provided with a modified Herridge nutrient solution (18) containing 1 mM $NH_4NO_3$ to facilitate hairy root emergence. After hairy roots emerged from the inoculation site, plants were transferred to germination trays (48-cell, cell size: 4.5 cm x 4 cm x 4.5 cm) filled with sand and covered with transparent lids. The plants were kept covered for the first 2 days and left to stabilize in the trays for 1 week. During this period, they were watered every 2 days with nutrient solution. After identifying positive hairy roots by GFP screening, the stem was cut just below the site of emergence and non-transgenic roots were removed. Plants were then transferred to individual pots containing river sand and watered with nitrogen-free Herridge's media. To generate nodules, plants were inoculated with 0.1 dilution of a saturated culture of *Bradyrhizobium japonicum* USDA110 grown in YEM media (21) at 28°C for 3 d. Non-inoculated hairy roots were supplied media containing 2.5 mM $NH_4NO_3$. For light microscopy, nodules were fixed in a combination of 3% glutaraldehyde and 4% paraformaldehyde, stained with 2% osmium tetroxide, dehydrated with ethanol and embedded in LR White (Sigma-Aldrich).

**Soybean Microarray Analysis**

Nodules from *GmbHLHm1* RNAi and empty vector control hairy roots were collected 24 days post inoculation with *Bradyrhizobium japonicum* USDA110. Total RNA was extracted from individual plant nodules with a Spectrum Plant Total RNA kit (Sigma-Aldrich). RNA was treated with Turbo DNase (Ambion), ethanol precipitated, and resuspended in water. 1 μg of total RNA was then used for cDNA synthesis with Superscript III (Life Technologies). Samples

showing similar *GmbHLHm1* RNA levels by qPCR (normalised to *cons6*) were then chosen for further analysis. RNA quality was then assessed by an Agilent 2100 Bioanalyzer. 250 ng of total RNA from four biological replicates was then converted to cDNA, biotin-labeled and fragmented with a WT Expression kit (Ambion) and a GeneChip terminal labeling kit (Affymetrix). Labeled cDNA was then hybridized to the Soybean 1.0 ST (22) whole transcript array (Affymetrix) according to the manufacturers protocol at the Ramiciotti Centre for Gene Function Analysis (The University of New South Wales, Sydney). Raw CEL files were then imported into the Partek Genomics Suite and preprocessed (pre-background adjustment for GC content, RMA background correction, quantile normalization, mean probeset summarization). FlexArray (genomequebec.mcgill.ca/FlexArray/, McGill University, Canada) was used for statistical analysis of microarray data where changes in expression between GmbHLHm1 RNAi (*bhlhm1*) and empty vector control tissues (*vector*) were calculated for significance using a two-sample Bayesian *t*-test (23). Subsequently, expression of a selection of genes from the array was analyzed by qRT-PCR (as described above) with primers from Table S3 to verify differential regulation (Fig S9). Fold changes were calculated by the $2^{-\Delta\Delta CT}$ method (16).

**Soybean Diurnal Expression**

Soybean (*Glycine max* cv. Djakal) plants were grown in river sand in a glass house (14/10 dark/light natural light cycle) and inoculated with *Bradyrhizobium japonicum* USDA110 at planting. Twenty-four days after sowing, nodules were collected at 6:00 am and every four hours until 6:00 am the following day. Total RNA was extracted from pooled plant nodules (from a single pot) with a Spectrum Plant Total RNA kit (Sigma-Aldrich). RNA was treated with Turbo DNase (Ambion) and reverse transcribed with Superscript III (Life Technologies) using and oligo-dt20 primer. qPCR was carried out with IQ SYBR green (BioRad) on a light

cycler (Bio-Rad). GmbHLHm1, GmGI1 [Glyma20g30980, (24)], GmGI2 (Glyma09g07240), GmPRR5 (Glyma04g40640), GmPRR7 (Glyma12g07860), GmLHY (Glyma16g01980), GmAMF3, (Glyma15g06660), GmAMF5 (Glyma09g33680), GS1γ2 (Glyma02g41120.1), GS1γ1 (Glyma14g39420.1), GS1β1 (Glyma11g33560.1), GmSS1 (Glyma20g31730.1), GmUO1 (Glyma10g23790.1), NifH (blr1769), FixU (bsr1757), GlnA 1 (blr4835). Expression was determined using primers (Table 3) relative to cons6 (1) or the average of SigA (bll7349) and GapA (bll1523), as calculated by the 2-ΔCT method (16). *cons6* expression was stable over the 24 h period.

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**Fig. S1.** ClustalW sequence alignment of bHLHm1-like proteins identified in plants. The bHLH (helix 1 and 2 and variable loop) and the C-terminal anchor domains are identified. GmbHLHm1 (Glyma15g06680), GmbHLHm2 (Glyma13g32650). Regions of interest include: 1) Alternate start codons (-21 and 1 aa); 2) Proposed cleaved N-terminal peptide required for nucleus entry; 3) The H-E-R motif that is the primary determinant for the binding of bHLH proteins to the palindromic CACGTG DNA sequence; 4) Residues substituted in this study (R180K, L191V and L207V) in the DNA binding bHLH motif; 5) Putative site 1 protease cleavage motif (RNVL) where substitutions L277A and L277I were introduced.

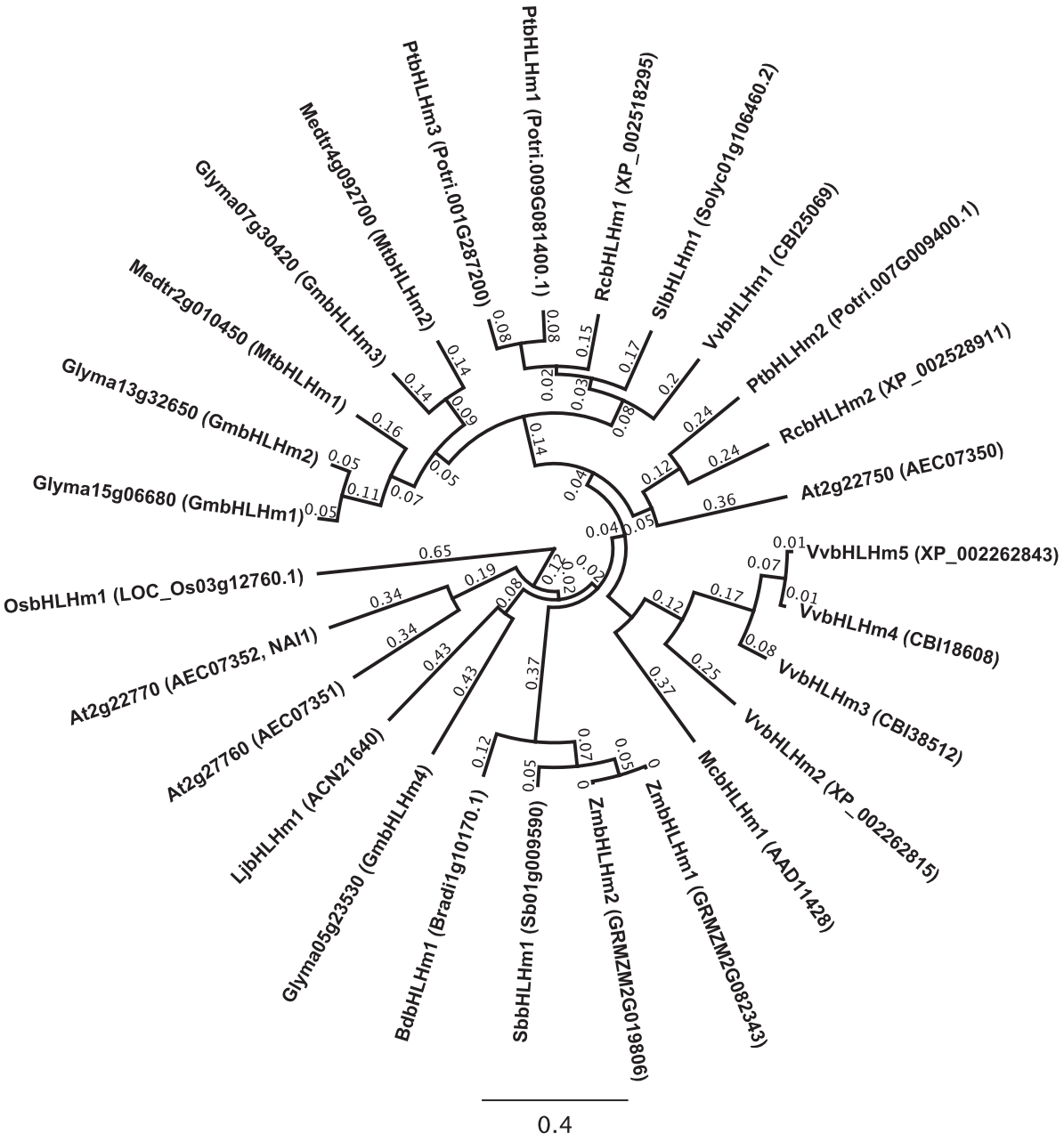



**Fig S2.** Phylogenetic tree of full-length bHLHm1-like bHLH containing proteins in plants including: *Glycine max* (Glyma), *Arabidopsis thaliana* (At), *Lotus japonicus* (Lj), *Medicago truncatula* (Mt), *Vitis vinifera* (Vv), *Zea mays* (Zm), *Oryza sativa* (Os), *Populas trichocarpa* (Pt), *Ricinius communis* (Rc), *Mesenbrantheum crystallanum* (Mc), *Brachypodium distachyon* (Bd), *Sorghum bicolor* (Sb), *Solanum lycopersicum* (Sl). The tree was developed using UPMGA best tree method with systemic tie breaking. Distances are absolute and gaps distributed proportionally.

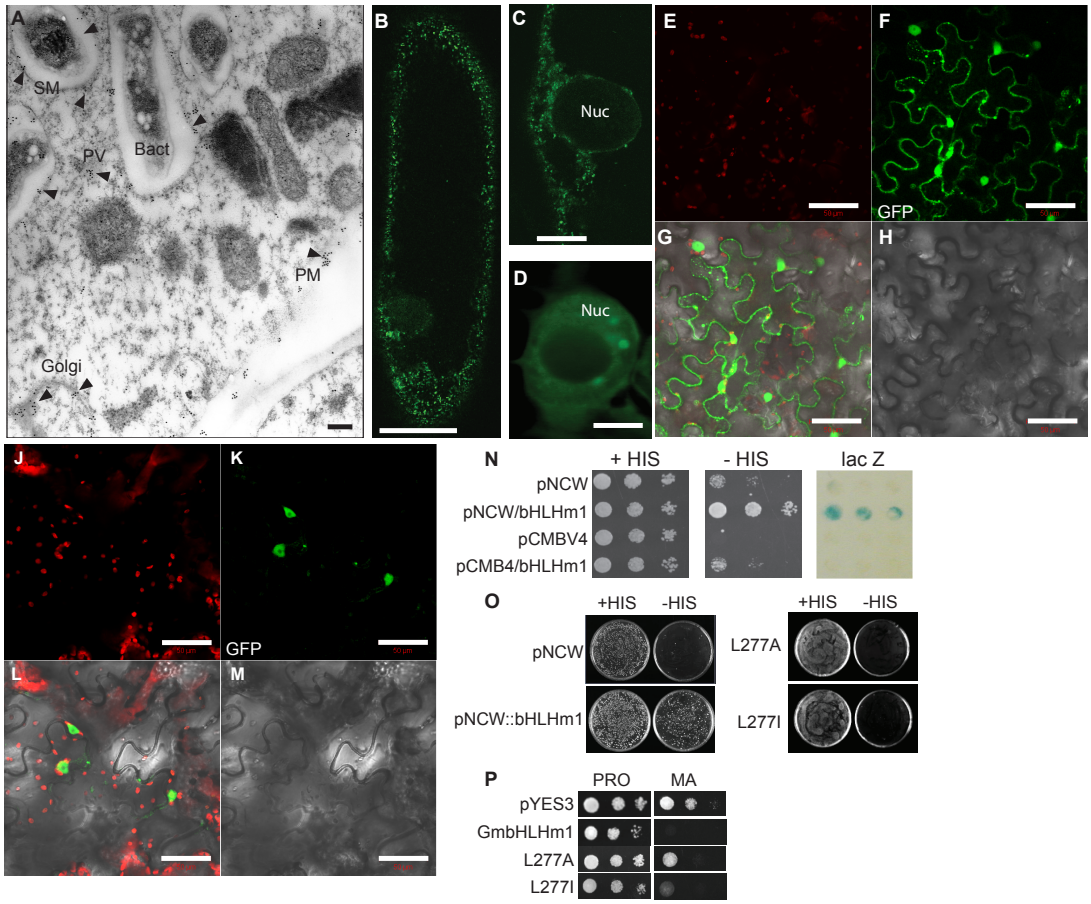
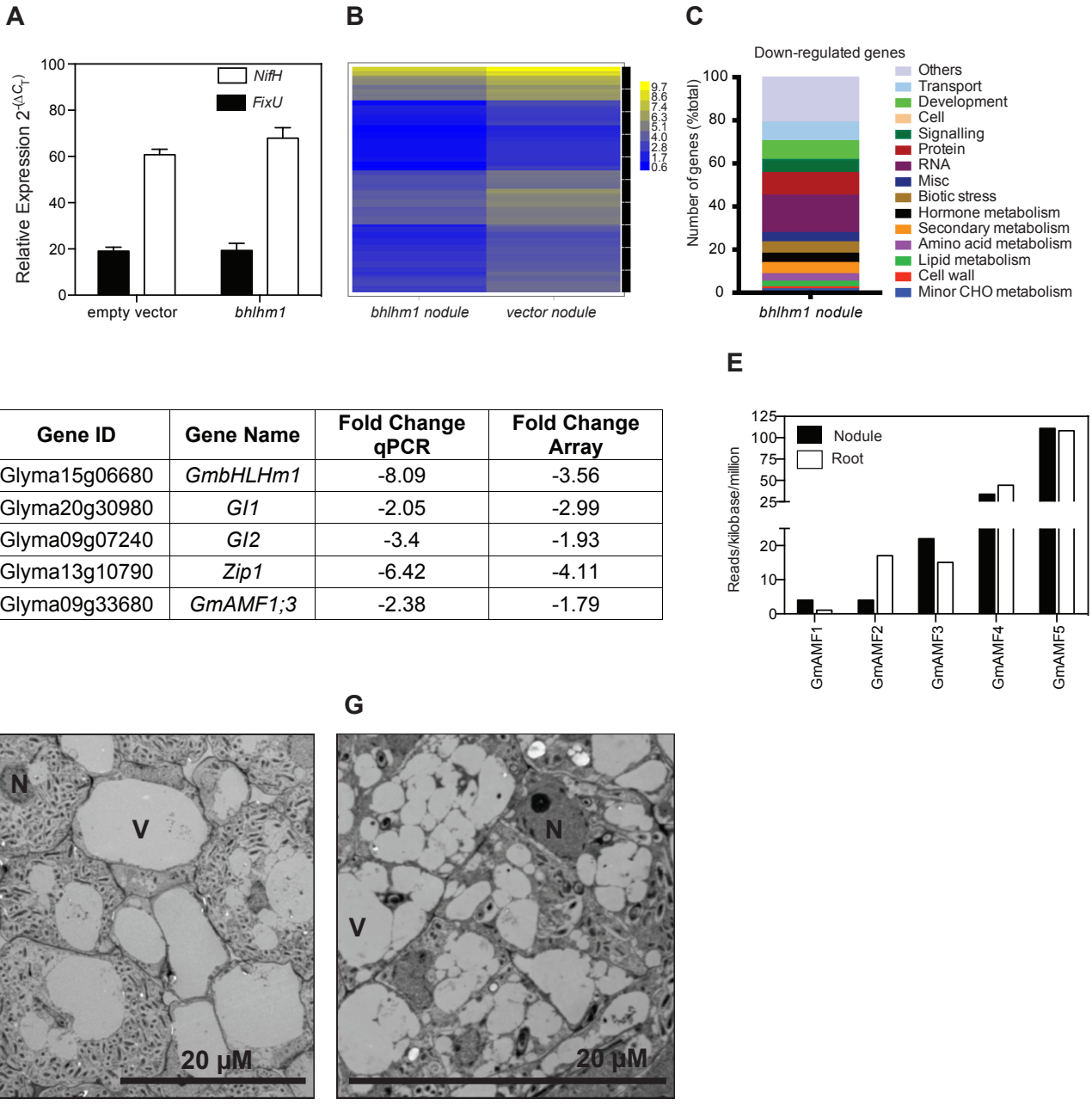



**Fig S3.** Release and delivery of GmbHLHm1 from its membrane location to the nucleus.
(A) Immunogold localisation of GmbHLHm1 (arrows) in infected soybean nodule cells incubated with anti-bHLHm1 antiserum (1:100) followed by 10 nm colloidal gold conjugated with anti-rabbit IgG (1:10). (B) Confocal image of N-terminal GFP GmbHLHm1 fusions in DNA bombarded onion epidermal cells. (C) Close-up of (B) showing GFP in the nucleus (Nuc) and peripheral punctate vesicles. (D) Close-up of a nucleus (Nuc) showing nuclear entry of GFP:GmbHLHm1 where 23 aa of the GmbHLHm1 N-terminus is deleted and fused with GFP. (E-H) Confocal images of a full-length N-terminal GmbHLHm1 GFP fusion cloned in pKGWFS7 that was transiently expressed in *Nicotiana benthamiana* leaves via Agrobacterium tumefaciens strain GV3101. (E) Chlorophyll autofluorescence identified with a 560 nm long-pass filter with 488 nm excitation. (F) GFP localisation at the plasma membrane and nucleus. (G) Overlay image of E, F and H. (H) DIC image of transfromed cells. (J-M) As described in (E-H) but with a N-terminal GmbHLHm1 GFP fusion which lacks the C-terminal transmembrane domain (S294-V347). (K, L) GFP is localised in the nucleus (Nuc). (N) LexA-VP16 fused to the N terminus (pNCW-bHLHm1) but not C terminus (pCMB-bHLHm1) of GmbHLHm1 complements the -HIS auxotrophy (i.e. growth on –HIS media) of the yeast strain DSY1 (Δhis3) and induces lac Z activity (blue colour). (O) Disruption in LexA-VP16::GmbHLHm1 delivery to the nucleus with amino acid substitutions at a predicted subtilisin site 1 proteolytic recognition site (RXXL - L277). (P) Reduction of MA sensitivity in 26972c yeast cells with GmbHLHm1 mutations, L277A and L277I. PRO refers to yeast minimal media containing 0.1% L-proline while MA refers to yeast minimal media containing 0.1% L-Proline and 0.1 M methylammonium. Bar = 200 µm (A), 50 µm (B, E-M), 30 µm (C, D).




| Gene ID | Gene Name | Fold Change qPCR | Fold Change Array |
|---|---|---|---|
| Glyma15g06680 | *GmbHLHm1* | -8.09 | -3.56 |
| Glyma20g30980 | *GI1* | -2.05 | -2.99 |
| Glyma09g07240 | *GI2* | -3.4 | -1.93 |
| Glyma13g10790 | *Zip1* | -6.42 | -4.11 |
| Glyma09g33680 | *GmAMF1;3* | -2.38 | -1.79 |

**Fig. S4.** (A) Expression analysis of *FixU* and *NifH* transcripts from bacteroid mRNA. Expression values were normalised to the average expression of *SigA* and *GapA*. Data represent the mean ± SE (n=4), as calculated by the $2^{-\Delta CT}$ method (48) with *SigA* and *GapA* as an endogenous control. (B) Comparison of the top 100 (nodule) down regulated genes (*bhlhm1*<*vector*, p<0.05). Differentially expressed genes from each array were normalised and then averaged (n=4 arrays). Data represents mean values. (C) Gene ontology (GO) terms of significantly down regulated genes in nodules and roots (P<0.05, fold change >2) (D) Expression analysis of selected transcripts from GmbHLHm1 nodule RNAi. qPCR was performed with primers shown in Table S4 on the four RNA replicates used for the microarray experiment. qPCR fold change values were generated using the calculation $2^{-\Delta\Delta CT}$ (Schmittgen and Livak, 2008) using cons6 (Libault et al., 2008) for normalization. Fold changes are expressed as a ratio of RNAi to empty vector control. All samples chosen showed at least a 2-fold downregulation by qPCR, in agreement with the microarray results. (E) Expression data was obtained from the soybean RNA-seq transcriptome database (http://www.soybase.org/soyseq/#). Data represents the reads per kilobase of exon model per million mapped reads (RPKM), as generated by Severin et al. (2010). (F, G) Transmission electron microscopy analysis of cross sections of infected cells from *bhlhm1* nodules. V: vacuole; Bact: bacteroid, N: nucleus.

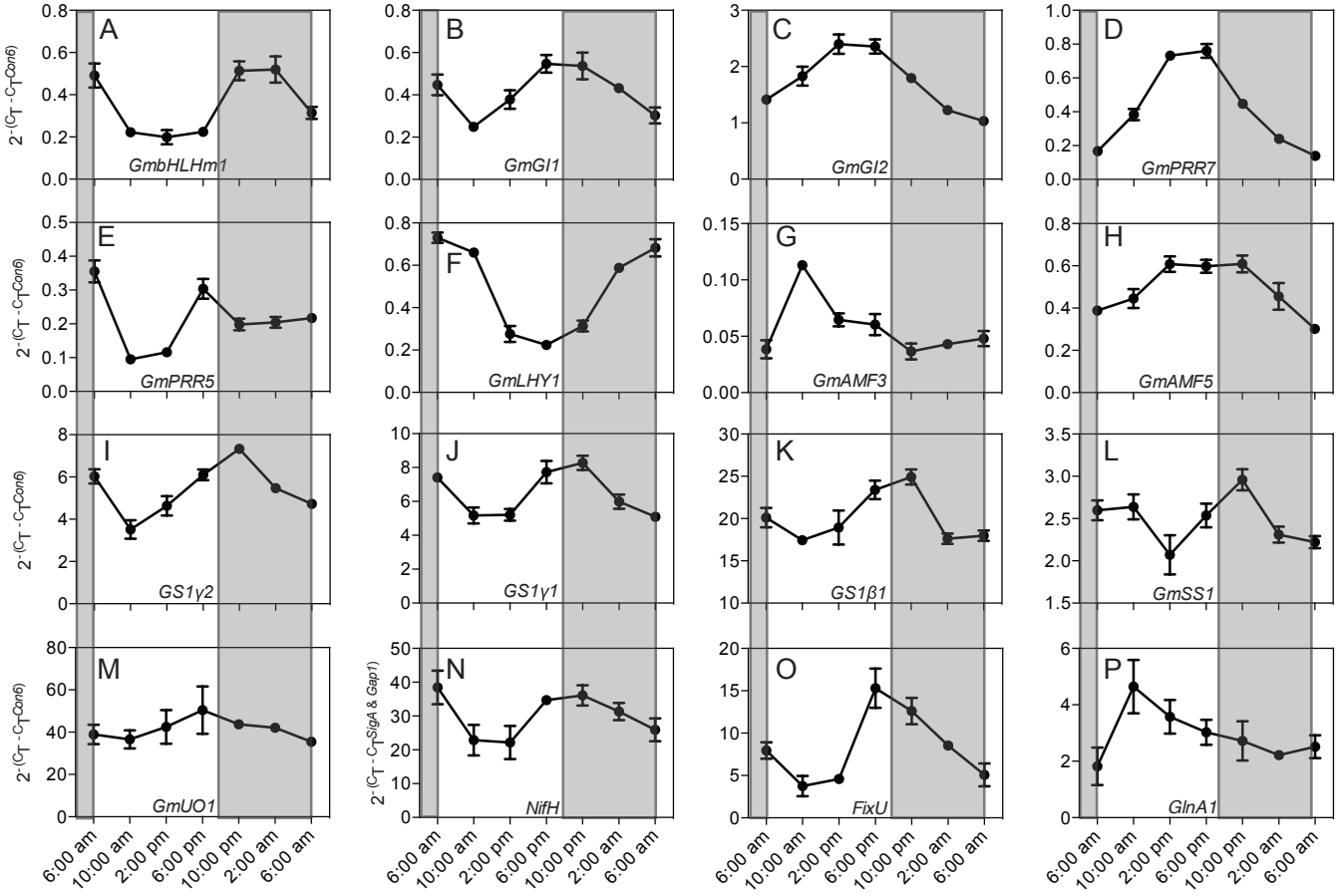



**Fig. S5.** Diel expression of selected soybean and rhizobium transcripts. Soybean plants were grown in the greenhouse under natural light (28/25°C day/night temperatures). At 24 days after planting, soybean nodules were harvested every 4 hours over a 24-hour period, starting at 6:00 am. Sunrise and sunset were at 5:57am and 8:07pm, respectively. Dark periods are highlighted as shaded columns. Tissue from six plants was pooled for RNA extraction and analyzed by qPCR. A, GmbHLHm1, B, GmGI1 (Glyma09g07240), C, GmGI2 (Glyma20g30980), D, GmPRR7 (Glyma12g07860), E, GmPRR5 (Glyma04g40640), F, GmLHY1 (Glyma16g01980), G, GmAMF3, (Glyma15g06660), H, GmAMF5 (Glyma09g33680), I, GS1γ2 (Glyma02g41120.1), J, GS1γ1 (Glyma14g39420.1), K, GS1β1 (Glyma11g33560.1), GmSS1 (Glyma20g31730.1), L, GmUO1 (Glyma10g23790.1), NifH (blr1769), FixU (bsr1757), GlnA 1 (blr4835). Data values represent the means of three independent biological replicates ± SE relative to cons6 (panels A-M) (Libault et al., 2008) or the average of SigA (bll7349) and GapA (bll1523) (panels N-P), as calculated by the 2-ΔCT method (Livak and Schmittgen, 2001).

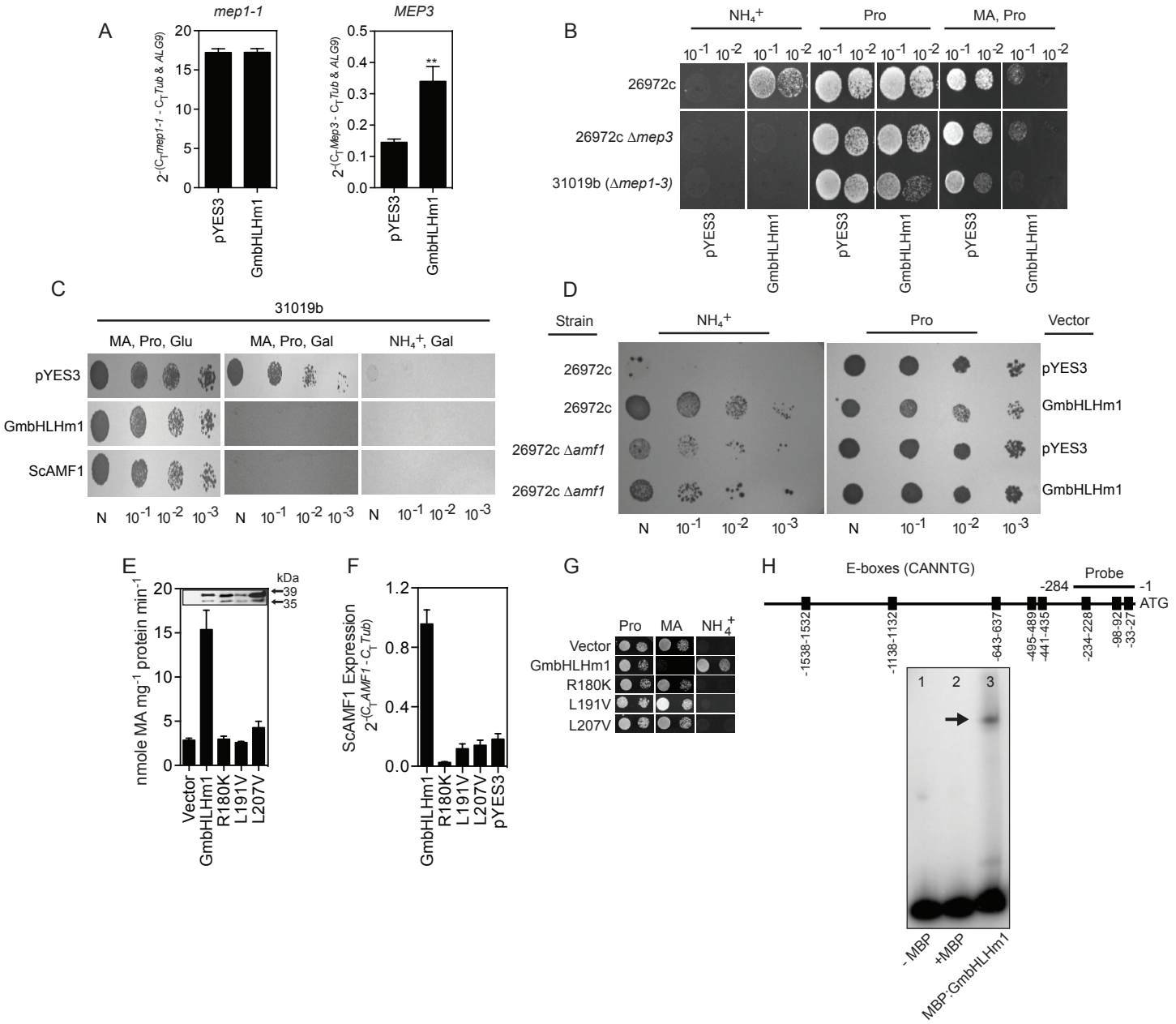



**Fig. S6.** Influence of GmbHLHm1 on *Mep* expression and associated growth responses on galactose media containing $NH_4^+$ or MA. In all yeast experiments, heterologous gene expression is through the galactose inducible expression vector, pYES3. (A) QPCR analysis of *mep1-1* and *MEP3* expression in 26972c cells with or without GmbHLHm1. Data presented is $2^{-\Delta C_T}$ with *Tubilin* and *ALG9* as endogenous controls for both pYES3 and GmbHLHm1-transformed cells. Data represents the mean ± SE (n=10 biological replicates). Data marked as ** are significantly different (one-way anova, Tukey's multiple comparison, p=0.0002). (B) Spot plates of 26972c (*mep1-1*, *mep2*Δ, MEP3), *amf1*Δ), 26972c Δ*mep3* and 31019b (Δ*mep1-3*) cells expressing *GmbHLHm1* on media containing either 1 mM $NH_4^+$ ($NH_4^+$), 0.1% L-proline (PRO), 0.1% L-proline + 0.1 M MA (MA, PRO). (C) Spot plates of 31019b cells containing pYES3, GmbHLHm1 or ScAMF1 on media containing 0.1% L-proline + 0.1 M MA with either 2% Glucose (MA, Pro, Glu) or 2% Galactose (MA, Pro, Gal). (D) Spot plates of 26972c and 26972cΔ*amf1* (*mep1-1*, *mep2* Δ, MEP3, Δ*amf1*) cells expressing *GmbHLHm1* or *pYES3* (control) on media containing 1 mM $NH_4^+$ ($NH_4^+$) or 0.1% L-proline (PRO). All spot plates were grown for 6 days at 28°C. (E-G) Disruption of amino acids (R180K, L191V, and L207V) in the bHLH region of GmbHLHm1 influences ScAMF1 activity and expression in 26972c cells. (E) Uptake of 1 mM $^{14}$C-MA by 26972c cells containing *GmbHLHm1* or disrupted *GmbHLHm1* mutations (R180K, L191V, and L207V) (n=6). Inset figure (E), western blot analysis of whole cell protein fractions of 26972c cells (GmbHLHm1, pYES3 or R180K, L191V, and L207V mutants) with anti-GmbHLHm1 antibodies (H) Electromobility shift analysis of $^{32}$P-labelled *ScAMF1* promoter. Cartoon represents a predcited full length *ScAMF1* promoter (-1707 bp) with identified E-BOX domains identified (black boxes) and the truncated Y3 (-284 to -1 bp) probe used in the binding experiment. $^{32}$P-labelled Y3 probe was mixed without MPB (-MPB, lane 1), with MPB (+MPB, lane 2) or with MPB:GmbHLHm1 (lane 3). MBP: maltose binding protein.

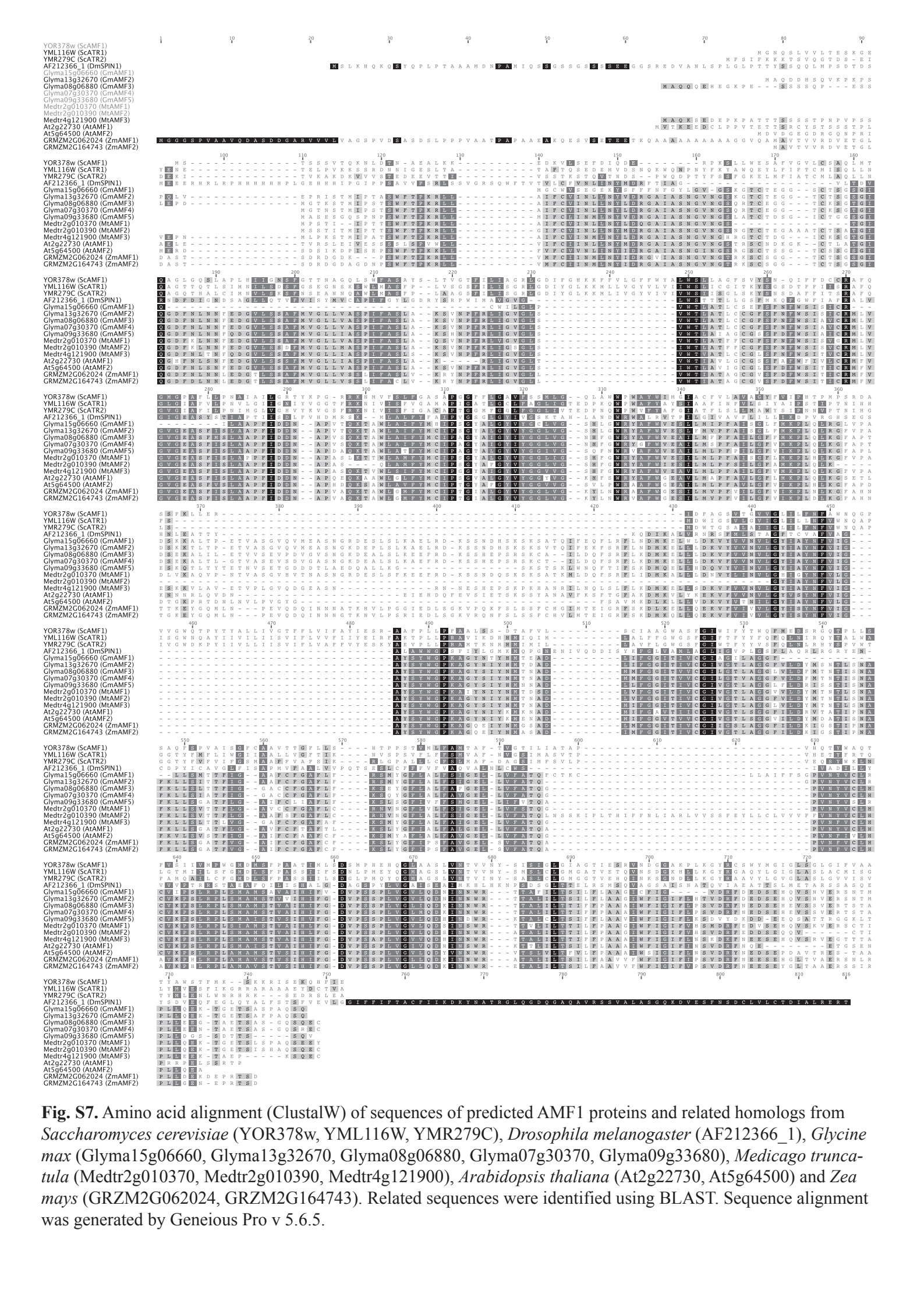


**Fig. S7.** Amino acid alignment (ClustalW) of sequences of predicted AMF1 proteins and related homologs from *Saccharomyces cerevisiae* (YOR378w, YML116W, YMR279C), *Drosophila melanogaster* (AF212366_1), *Glycine max* (Glyma15g06660, Glyma13g32670, Glyma08g06880, Glyma07g30370, Glyma09g33680), *Medicago truncatula* (Medtr2g010370, Medtr2g010390, Medtr4g121900), *Arabidopsis thaliana* (At2g22730, At5g64500) and *Zea mays* (GRZM2G062024, GRZM2G164743). Related sequences were identified using BLAST. Sequence alignment was generated by Geneious Pro v 5.6.5.

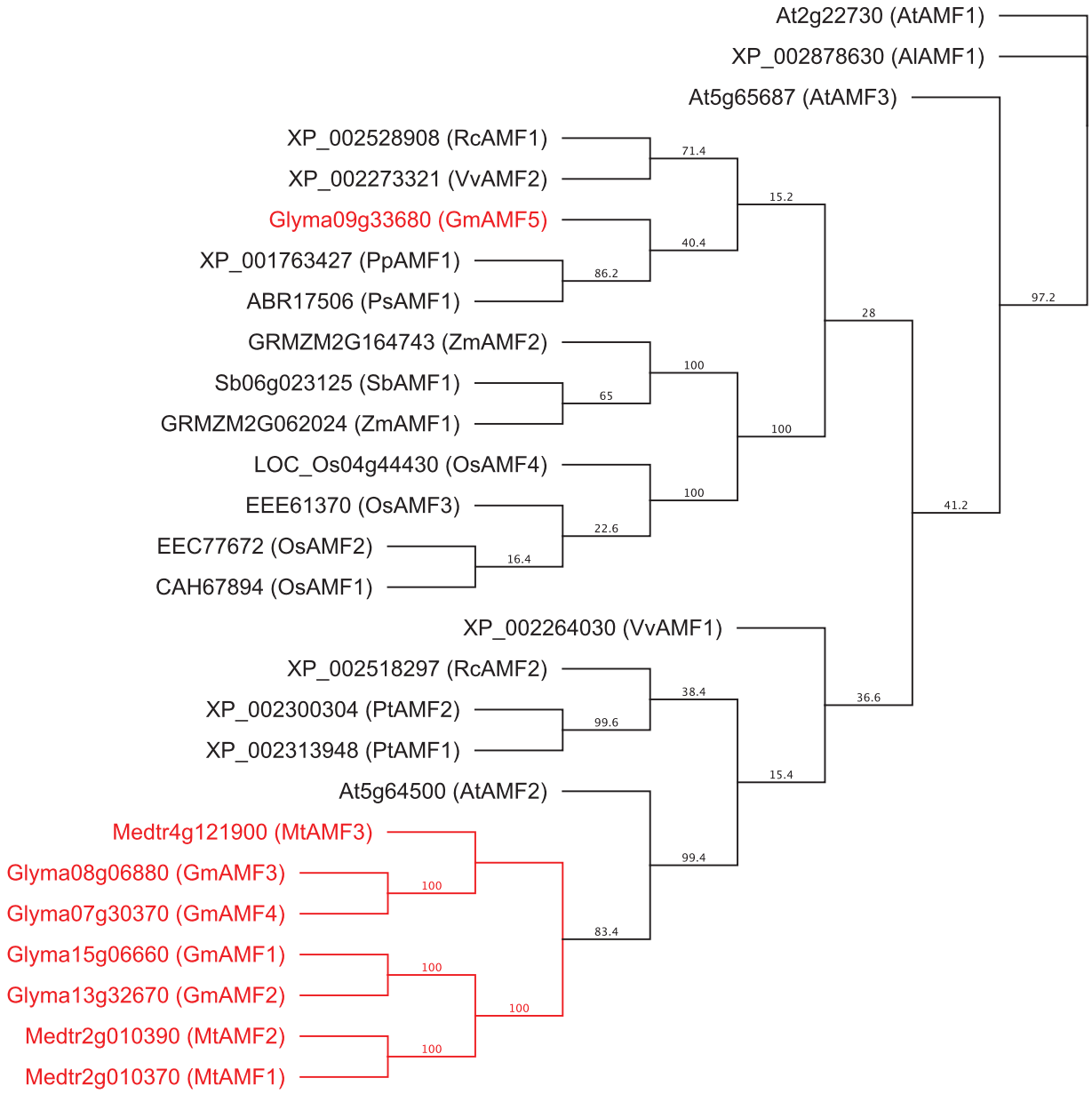



**Fig S8.** Phylogenetic tree of AMF1 proteins in plants including: *Glycine max* (Glyma), *Arabidopsis thaliana* (At), *Arabidopsis lyrata*, *Medicago truncatula* (Mt), *Vitis vinifera* (Vv), *Zea mays* (GRMZM), *Oryza sativa* (Os), *Populus trichocarpa* (Pt), *Ricinius communis* (Rc), *Sorghum bicolor* (Sb), *Picea sitchensis* (Ps), *Physcomitrella patens* (Pp). The tree was developed using PHYML with MTREV substitution model with Bootstrap (500) branch support. The tree was drawn with equal branch lengths with bootstrap proportions indicated.

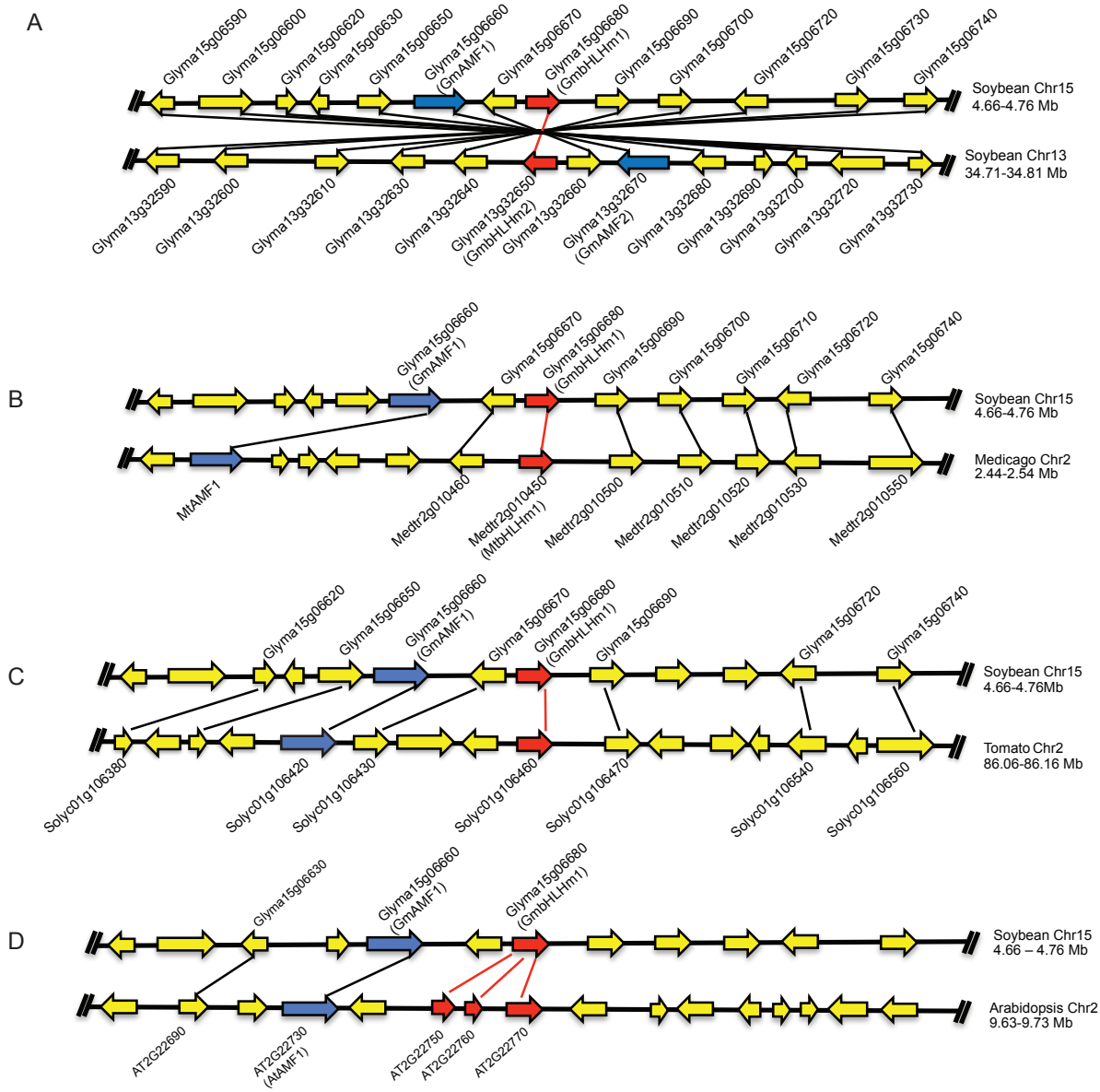



**Fig S9.** Conserved alignment of soybean Chr15, 100 Kb either side of GmbHLHm1 and (A) soybean Chr 13, (B) Medicago Chr2, (C) Tomato Chr2 and (D) Arabidopsis Chr2. Alignments were obtained from the Plant Genome Duplication Database (http://chibba.agtec.uga.edu/duplication). bHLHm1 homologs shown in red and AMF1 homologs shown in blue.

**Table S1.** Affymetrix microarray analysis of soybean gene expression in *bhlhm1* silenced nodules. Ratio is defined as the fold-change difference (up to -1.75) in gene expression between *bhlhm1* and empty vector controls (n=4 arrays in both cases). Significance between samples was calculated using the Bayesian two-sample *t*-test.

| Probe ID | Gene ID | Gene Name | Gene Description | Fold Change | P-value |
|---|---|---|---|---|---|
| 11764628 | Glyma01g00980.1 | NRPC2 | Nuclear RNA polymerase C2 | -7.03 | 1.86E-05 |
| 12161099 | Glyma18g38670.1 | ELI3-2 | Elicitor-activated gene 3-2 | -5.34 | 1.62E-12 |
| 11857831 | Glyma05g21680.1 | BRU6 | Auxin-responsive GH3 family protein | -4.75 | 2.17E-04 |
| 12067348 | Glyma13g31690.1 | Scpl31 | Serine carboxypeptidase-like 31 | -4.75 | 7.20E-08 |
| 12193838 | Glyma19g32860.1 | | Unknown | -4.66 | 1.64E-04 |
| 12043203 | Glyma13g01140.1 | TCH4 | Xyloglucan endotransglucosylase | -4.62 | 3.37E-06 |
| 12061345 | Glyma13g10790.1 | ZIP1 | Zinc transporter 1 precursor | -4.11 | 6.88E-04 |
| 11826846 | Glyma03g29440.1 | SIP2 | Seed imbibition 2 | -3.66 | 1.79E-04 |
| 12093726 | Glyma15g06680.3 | GmbHLHm1 | Basic helix-loop-helix (bHLH) DNA-binding protein | -3.56 | 5.82E-07 |
| 12103513 | Glyma15g02110.1 | | Unknown | -3.47 | 2.88E-04 |
| 12014411 | Glyma11g05510.1 | GH3.1 | Auxin-responsive GH3 family protein | -3.46 | 1.30E-06 |
| 11988514 | Glyma10g44170.1 | HPT1 | homogentisate phytyltransferase 1 | -3.43 | 2.39E-05 |
| 11825214 | Glyma03g24490.1 | | Unknown | -3.35 | 8.42E-06 |
| 12145011 | Glyma17g06560.1 | | Unknown | -3.25 | 1.87E-06 |
| 12146522 | Glyma17g10050.1 | | Gibberellin-regulated family protein | -3.21 | 2.27E-06 |
| 12149276 | Glyma17g18040.1 | BRU6 | Auxin-responsive GH3 family protein | -3.20 | 1.31E-03 |
| 12063707 | Glyma13g21410.1 | | Unknown | -3.09 | 1.04E-03 |
| 12188292 | Glyma19g01120.1 | | Oxidoreductase, zinc-binding dehydrogenase | -3.04 | 2.27E-05 |
| 11989313 | Glyma10g02210.1 | SAG21 | Senescence-associated gene 21 | -3.04 | 5.77E-03 |
| 12177419 | Glyma19g01150.1 | | Oxidoreductase, zinc-binding dehydrogenase | -3.00 | 1.20E-03 |
| 12215520 | Glyma20g30980.1 | GI | Gigantea protein (GI) | -2.99 | 3.29E-04 |
| 11823277 | Glyma03g08020.1 | | Unknown | -2.98 | 7.18E-03 |
| 12020757 | Glyma11g31530.1 | | RNA-binding KH domain-containing protein | -2.95 | 1.84E-08 |
| 11898990 | Glyma06g44120.1 | | Unknown | -2.88 | 2.04E-04 |
| 12117935 | Glyma16g16800.1 | | Unknown | -2.88 | 3.34E-05 |
| 11852130 | Glyma04g40640.1 | PRR5 | Pseudo-response regulator 5 | -2.86 | 1.36E-07 |
| 12129338 | Glyma16g28570.1 | | Disease resistance family protein | -2.82 | 2.27E-04 |
| 11783910 | Glyma01g42640.1 | | Winged-helix DNA-binding transcription factor | -2.80 | 5.71E-04 |
| 12177426 | Glyma19g01200.1 | FDH | Formate dehydrogenase | -2.73 | 7.02E-04 |
| 11993298 | Glyma10g20390.1 | SEC14 | SEC14 cytosolic factor family protein | -2.73 | 3.81E-03 |
| 12136833 | Glyma17g14680.1 | Beta-VPE | Beta vacuolar processing enzyme | -2.72 | 7.95E-05 |
| 11767597 | Glyma01g14740.1 | | Translation elongation factor EFG | -2.65 | 1.62E-04 |
| 11844686 | Glyma04g04270.1 | | HXXXD-type acyl-transferase family protein | -2.62 | 4.23E-03 |
| 12132673 | Glyma17g03860.1 | | ABCG37 ABC transporter G family member | -2.62 | 1.08E-02 |
| 11857509 | Glyma05g18360.1 | | Unknown | -2.61 | 8.92E-03 |
| 12220878 | g91214122-72 | ndhE | NADH dehydrogenase subunit 4L | -2.60 | 7.23E-04 |
| 11943863 | Glyma08g14070.1 | | Beta-1,4-N-acetylglucosaminyltransferase | -2.58 | 3.69E-05 |
| 12128130 | Glyma16g25080.1 | | Disease resistance protein (TIR-NBS-LRR class) | -2.56 | 7.69E-03 |
| 12133610 | Glyma17g05920.1 | | NF-YA8 // nuclear factor Y, subunit A8 | -2.53 | 4.00E-03 |
| 12088298 | Glyma14g27020.1 | | Unknown | -2.50 | 1.91E-07 |
| 11933429 | Glyma08g26570.1 | | Unknown | -2.50 | 3.66E-04 |
| 11823685 | Glyma03g11610.1 | | NAD(P)-linked oxidoreductase | -2.47 | 5.95E-05 |
| 11856612 | Glyma05g09130.1 | GLCAK | Glucuronokinase G | -2.46 | 2.89E-03 |
| 12188569 | Glyma19g01980.1 | | ABC transporter family protein | -2.45 | 4.47E-04 |
| 12111422 | Glyma15g33040.1 | | Unknown | -2.44 | 4.92E-04 |
| 11959985 | Glyma09g29240.1 | | Unknown | -2.44 | 4.20E-04 |
| 11769271 | Glyma01g28790.1 | | Unknown | -2.44 | 3.20E-04 |
| 12121849 | Glyma16g32100.1 | | Unknown | -2.44 | 4.24E-04 |
| 11980213 | Glyma10g14830.1 | | Unknown | -2.44 | 1.49E-04 |
| 12089440 | Glyma14g35810.1 | | Sequence-specific DNA binding transcription factors | -2.44 | 2.45E-03 |
| 12090661 | Glyma14g39560.1 | | HSP20-like chaperones superfamily protein | -2.43 | 1.79E-03 |
| 11896919 | Glyma06g35580.1 | | Tyrosine transaminase family protein | -2.42 | 4.23E-03 |
| 11857317 | Glyma05g15860.1 | | Unknown | -2.40 | 1.72E-05 |
| 11889065 | Glyma06g05280.1 | BCAT-2 | Branched-chain amino acid transaminase 2 | -2.40 | 6.24E-03 |
| 12034768 | Glyma12g05080.1 | | Unknown | -2.39 | 1.54E-06 |

| | | | | | |
|---|---|---|---|---|---|
| 11832737 | Glyma04g02220.1 | | ACT-like protein tyrosine kinase family protein | -2.39 | 4.51E-03 |
| 11898057 | Glyma06g40740.1 | | Disease resistance protein (TIR-NBS-LRR class) | -2.38 | 8.55E-04 |
| 11889851 | Glyma06g07160.1 | | Unknown | -2.37 | 2.62E-05 |
| 11783686 | Glyma01g42030.1 | PME1 | Pectin methylesterase inhibitor 1 | -2.34 | 1.34E-02 |
| 12150573 | Glyma17g26660.1 | | Unknown | -2.33 | 9.03E-06 |
| 12167721 | Glyma18g05710.1 | | Leucine-rich repeat protein kinase family protein | -2.31 | 5.41E-05 |
| 12173883 | Glyma18g44390.1 | | Unknown | -2.30 | 2.13E-03 |
| 12014253 | Glyma11g05030.1 | HHP4 | Heptahelical protein 4 | -2.29 | 2.15E-04 |
| 12050976 | Glyma13g25270.1 | TPS04 | Terpene synthase 04 | -2.28 | 3.38E-02 |
| 11924761 | Glyma08g04150.1 | | Unknown | -2.28 | 1.79E-03 |
| 12200874 | Glyma20g11250.1 | | Unknown | -2.25 | 1.87E-04 |
| 12090313 | Glyma14g38800.1 | ATM3 | ABC transporter of the mitochondrion 3 | -2.24 | 3.40E-03 |
| 11836423 | Glyma04g11230.1 | | Tetratricopeptide repeat (TPR)-containing protein | -2.24 | 1.45E-06 |
| 12174598 | Glyma18g46610.1 | | Acyl-CoA thioesterase family protein | -2.23 | 1.01E-02 |
| 11891260 | Glyma06g10700.1 | EXO | Phosphate-responsive 1 family protein | -2.23 | 2.09E-02 |
| 11892151 | Glyma06g13280.1 | GLT1 | NADH-dependent glutamate synthase 1 | -2.22 | 7.19E-03 |
| 12026233 | Glyma12g07860.1 | PRR7 | Pseudo-response regulator 7 | -2.22 | 1.69E-03 |
| 11948749 | Glyma08g27810.1 | | Unknown | -2.21 | 3.68E-04 |
| 12053597 | Glyma13g32240.1 | | Unknown | -2.21 | 1.10E-05 |
| 11798864 | Glyma02g04770.1 | SERAT3;2 | Serine acetyltransferase 3;2 | -2.20 | 4.15E-02 |
| 11966192 | Glyma09g04750.1 | ATL2 | TOXICOS EN LEVADURA 2 | -2.20 | 3.06E-02 |
| 12144042 | Glyma17g04360.1 | PDR9 | Pleiotropic drug resistance 9 | -2.20 | 6.20E-04 |
| 12002281 | Glyma11g04220.1 | | Unknown | -2.19 | 1.04E-02 |
| 12149223 | Glyma17g17840.1 | | Unknown | -2.19 | 2.64E-03 |
| 11899262 | Glyma06g45180.1 | ULT1 | Developmental regulator, ULTRAPETALA | -2.17 | 3.96E-03 |
| 12147171 | Glyma17g11590.1 | | Leucine-rich repeat family protein | -2.17 | 5.26E-03 |
| 12128071 | Glyma16g24940.1 | | Disease resistance protein (TIR-NBS-LRR class) | -2.15 | 7.94E-04 |
| 12096460 | Glyma15g14000.2 | FAH1 | Fatty acid hydroxylase 1 | -2.15 | 6.89E-04 |
| 11830446 | Glyma03g39100.1 | | Adenine nucleotide alpha hydrolases-like protein | -2.13 | 2.93E-04 |
| 11903624 | Glyma07g08500.1 | RPS2 | NB-ARC domain-containing disease resistance protein | -2.12 | 1.93E-02 |
| 11778825 | Glyma01g23460.1 | | Unknown | -2.11 | 6.88E-05 |
| 12028725 | Glyma12g17020.1 | | Unknown | -2.11 | 2.62E-05 |
| 11766227 | Glyma01g05550.1 | SBH2 | Sphingoid base hydroxylase 2 | -2.11 | 6.75E-03 |
| 12043198 | Glyma13g01130.1 | XTR6 | Xyloglucan endotransglycosylase 6 | -2.11 | 3.55E-02 |
| 12135561 | Glyma17g11370.1 | | RING | -2.11 | 5.68E-03 |
| 11941496 | Glyma08g07580.1 | WBC11 | White-brown complex homolog protein 11 | -2.11 | 1.51E-02 |
| 11844827 | Glyma04g04750.1 | LBD39 | LOB domain-containing protein 39 | -2.11 | 1.00E-02 |
| 12030466 | Glyma12g30240.1 | FRU | FER-like regulator of iron uptake | -2.10 | 2.38E-02 |
| 11908549 | Glyma07g31200.1 | scpl31 | Serine carboxypeptidase-like 31 | -2.10 | 7.97E-03 |
| 11812567 | Glyma03g11580.1 | | NAD(P)-linked oxidoreductase superfamily protein | -2.09 | 8.67E-04 |
| 11899234 | Glyma06g45020.1 | | Unknown | -2.09 | 1.73E-02 |
| 12213252 | Glyma20g24510.1 | | F1F0-ATPase inhibitor protein, putative | -2.08 | 1.95E-02 |
| 12194695 | Glyma19g35270.1 | PDR12 | Pleiotropic drug resistance 12 | -2.08 | 2.67E-07 |
| 11896573 | Glyma06g30920.1 | | Unknown | -2.08 | 4.11E-03 |
| 12109393 | Glyma15g19380.1 | | Unknown | -2.07 | 5.19E-03 |
| 12173852 | Glyma18g44250.1 | PYD4 | PYRIMIDINE 4 | -2.06 | 4.54E-02 |
| 12003488 | Glyma11g07270.1 | | Unknown | -2.06 | 3.20E-04 |
| 12201584 | Glyma20g17440.1 | | Uricase / urate oxidase / nodulin 35, putative | -2.04 | 1.28E-05 |
| 12162104 | Glyma18g42430.1 | | Unknown | -2.03 | 1.50E-02 |
| 12162019 | Glyma18g42290.1 | | RING | -2.03 | 1.86E-03 |
| 12094050 | Glyma15g07600.1 | scpl31 | Serine carboxypeptidase-like 31 | -2.01 | 1.56E-03 |
| 12157936 | Glyma18g14100.1 | | Unknown | -2.00 | 3.21E-03 |
| 11960302 | Glyma09g30390.1 | NUDT4 | Nudix hydrolase homolog 4 | -2.00 | 3.00E-02 |
| 11875838 | Glyma06g05140.1 | | Unknown | -1.99 | 2.89E-02 |
| 12027636 | Glyma12g12260.1 | | Unknown | -1.98 | 2.99E-02 |
| 12195756 | Glyma19g38490.1 | | Alpha | -1.98 | 1.00E-04 |
| 11886154 | Glyma06g45030.1 | | Unknown | -1.98 | 2.13E-02 |
| 11801772 | Glyma02g12710.1 | | Unknown | -1.98 | 5.80E-03 |
| 12158617 | Glyma18g17150.1 | | Unknown | -1.98 | 1.65E-02 |
| 11988345 | Glyma10g43850.1 | TT5 | Chalcone-flavanone isomerase family protein | -1.97 | 9.14E-03 |
| 12008867 | Glyma11g22950.1 | | Unknown | -1.97 | 7.73E-03 |
| 12017024 | Glyma11g12510.2 | | Unknown | -1.97 | 9.81E-05 |
| 12080899 | Glyma14g38130.1 | | Unknown | -1.96 | 8.52E-04 |
| 11811601 | Glyma03g05270.1 | | Unknown | -1.96 | 1.65E-02 |
| 11915648 | Glyma07g10140.1 | | DNAse I-like superfamily protein | -1.96 | 3.66E-02 |

| | | | | | |
|---|---|---|---|---|---|
| 12078505 | Glyma14g24140.1 | ALDH3H1 | Aldehyde dehydrogenase 3H1 | -1.95 | 2.64E-02 |
| 12214668 | Glyma20g28230.1 | MSS1 | Major facilitator superfamily protein | -1.95 | 6.60E-04 |
| 12176448 | Glyma18g52250.1 | | NAD(P)-linked oxidoreductase superfamily protein | -1.95 | 2.63E-02 |
| 11826491 | Glyma03g28650.1 | | Calcium-binding EF-hand family protein | -1.95 | 3.78E-03 |
| 12042575 | Glyma12g36510.1 | | NB-ARC domain-containing disease resistance protein | -1.95 | 3.11E-02 |
| 12022261 | Glyma11g36240.1 | | Unknown | -1.94 | 3.93E-04 |
| 12130482 | Glyma16g32540.1 | AGL6 | AGAMOUS-like 6 | -1.94 | 8.84E-03 |
| 12105961 | Glyma15g08940.1 | | Splicing factor, putative | -1.93 | 1.32E-02 |
| 11967103 | Glyma09g07240.1 | GI | Gigantea protein (GI) | -1.93 | 6.67E-04 |
| 12061775 | Glyma13g16070.1 | | Unknown | -1.93 | 1.38E-03 |
| 11961400 | Glyma09g33530.1 | | Unknown | -1.93 | 1.42E-03 |
| 12141219 | Glyma17g34880.1 | | Protein phosphatase 2C family protein | -1.93 | 5.47E-03 |
| 11782657 | Glyma01g39150.1 | | Unknown | -1.93 | 9.48E-08 |
| 12112117 | Glyma15g37600.1 | LRP1 | Lateral root primordium (LRP) protein-related | -1.93 | 1.46E-02 |
| 12132685 | Glyma17g03880.1 | | Unknown | -1.93 | 7.59E-03 |
| 12041825 | Glyma12g34340.1 | | HSP20-like chaperones superfamily protein | -1.93 | 1.63E-02 |
| 12193827 | Glyma19g32850.1 | | Myb-like HTH transcriptional regulator family protein | -1.92 | 1.94E-02 |
| 11861537 | Glyma05g32850.1 | ATAF1 | NAC domain transcriptional regulator | -1.92 | 3.75E-02 |
| 12052090 | Glyma13g28090.1 | | Alpha | -1.91 | 4.47E-03 |
| 11962409 | Glyma09g36210.1 | RTFL12 | ROTUNDIFOLIA like 12 | -1.91 | 7.63E-04 |
| 11926179 | Glyma08g07900.1 | J20 | DNAJ-like 20 | -1.91 | 6.85E-03 |
| 11927117 | Glyma08g10330.1 | | RNA-binding KH domain-containing protein | -1.90 | 2.59E-04 |
| 11987928 | Glyma10g42960.1 | TRN1 | Transportin 1 | -1.90 | 4.83E-03 |
| 11978781 | Glyma10g07860.1 | | IQ calmodulin-binding motif family protein | -1.89 | 3.26E-02 |
| 11844949 | Glyma04g05330.1 | | KH domain-containing protein | -1.89 | 4.19E-03 |
| 11922792 | Glyma07g39650.1 | SCL13 | SCARECROW-like 13 | -1.88 | 3.45E-02 |
| 12041894 | Glyma12g34580.1 | | Calcium-binding EF-hand family protein | -1.88 | 4.08E-03 |
| 12186745 | Glyma19g42040.1 | SWEET17 | Nodulin MtN3 family protein | -1.87 | 4.67E-02 |
| 12210083 | Glyma20g04840.1 | | Transmembrane amino acid transporter | -1.87 | 4.48E-04 |
| 12204473 | Glyma20g28500.2 | | Protein of unknown function (DUF581) | -1.87 | 1.12E-03 |
| 11792350 | Glyma02g31370.1 | | MATE efflux family protein | -1.86 | 8.68E-05 |
| 12156608 | Glyma18g09000.1 | MRP3 | Multidrug resistance-associated protein 3 | -1.86 | 3.35E-02 |
| 12089442 | Glyma14g35820.1 | CPUORF37 | Conserved peptide upstream open reading frame 37 | -1.86 | 3.21E-03 |
| 11993838 | Glyma10g24130.1 | | Unknown | -1.85 | 1.70E-02 |
| 11886701 | Glyma06g46760.1 | | Cytochrome P450, family 709, subfamily B | -1.84 | 1.22E-06 |
| 12080015 | Glyma14g35490.1 | | Unknown | -1.84 | 1.84E-03 |
| 12177373 | Glyma19g01040.1 | | Unknown | -1.84 | 3.18E-02 |
| 11893961 | Glyma06g17810.1 | | Pyridoxal phosphate (PLP)-dependent transferase | -1.84 | 7.92E-04 |
| 12213448 | Glyma20g24980.1 | | Unknown | -1.84 | 3.11E-03 |
| 11837496 | Glyma04g15810.1 | | Unknown | -1.84 | 1.28E-02 |
| 12113627 | Glyma15g42740.1 | | A20 | -1.83 | 4.45E-03 |
| 11784171 | Glyma01g43420.1 | WRKY41 | WRKY family transcription factor | -1.83 | 3.96E-02 |
| 12137322 | Glyma17g16130.1 | | Protein of unknown function (DUF581) | -1.83 | 7.56E-04 |
| 12179482 | Glyma19g08980.1 | | Unknown | -1.83 | 2.08E-03 |
| 12154108 | Glyma18g01720.1 | | RING | -1.83 | 5.76E-03 |
| 12003703 | Glyma11g07750.1 | TRX1 | Thioredoxin H-type 1 | -1.83 | 2.49E-02 |
| 11981862 | Glyma10g27430.1 | | Unknown | -1.82 | 5.35E-03 |
| 12103991 | Glyma15g03590.1 | ARI7 | IBR domain-containing protein | -1.82 | 2.27E-04 |
| 12007764 | Glyma11g19020.1 | TGA9 | bZIP transcription factor family protein | -1.82 | 8.53E-03 |
| 11885071 | Glyma06g41220.1 | | Unknown | -1.81 | 3.10E-02 |
| 12136645 | Glyma17g14190.1 | AGL14 | AGAMOUS-like 14 | -1.81 | 4.03E-02 |
| 12155162 | Glyma18g04530.1 | ALY4 | ALWAYS EARLY 4 | -1.81 | 2.56E-02 |
| 12067192 | Glyma13g31410.1 | | Aluminium induced protein, YGL and LRDR motifs | -1.80 | 3.94E-03 |
| 12181644 | Glyma19g28520.1 | | Arabidopsis NAC domain containing protein 87 | -1.80 | 2.66E-02 |
| 12176044 | Glyma18g50820.1 | ANX2 | Malectin | -1.80 | 1.17E-02 |
| 12136042 | Glyma17g12540.1 | | Aspartate-glutamate racemase family | -1.80 | 9.15E-04 |
| 11927490 | Glyma08g11260.1 | | Unknown | -1.80 | 1.68E-02 |
| 12012458 | Glyma11g00260.1 | | Cyclase family protein | -1.80 | 3.69E-03 |
| 12113035 | Glyma15g40690.1 | | Unknown | -1.79 | 4.69E-02 |
| 12054497 | Glyma13g35060.1 | ALF5 | MATE efflux family protein | -1.79 | 1.08E-02 |
| 12106817 | Glyma15g11410.1 | | MATE efflux family protein | -1.79 | 8.63E-04 |
| 12150891 | Glyma17g29800.1 | | CHY-type | -1.79 | 4.46E-03 |
| 11975881 | Glyma09g42120.1 | | TCP family transcription factor | -1.79 | 1.66E-03 |
| 11961474 | Glyma09g33680.1 | GmAMF5 | Major facilitator superfamily protein | -1.79 | 1.36E-03 |

| | | | | | |
|---|---|---|---|---|---|
| 12208693 | Glyma20g38930.1 | HPT1 | Homogentisate phytyltransferase 1 | -1.79 | 6.47E-03 |
| 11816534 | Glyma03g31410.1 | | Unknown | -1.79 | 4.88E-04 |
| 11930715 | Glyma08g19850.1 | | Unknown | -1.79 | 1.12E-03 |
| 11822940 | Glyma03g06950.1 | RPP5 | Disease resistance protein (TIR-NBS-LRR class) | -1.79 | 2.55E-03 |
| 11886159 | Glyma06g45040.1 | | Unknown | -1.79 | 2.42E-02 |
| 11913530 | Glyma07g04050.1 | NF-YA3 | Nuclear factor Y, subunit A3 | -1.79 | 2.52E-03 |
| 12162747 | Glyma18g44600.1 | | Leucine-rich receptor-like protein kinase | -1.78 | 8.01E-03 |
| 12133547 | Glyma17g05790.1 | | DENN (AEX-3) domain-containing protein | -1.78 | 3.40E-02 |
| 11949143 | Glyma08g29470.1 | | Regulator of Vps4 activity in the MVB pathway | -1.78 | 7.97E-03 |
| 11879111 | Glyma06g12640.1 | SKP2A | F-box | -1.78 | 3.13E-03 |
| 12077591 | Glyma14g16700.1 | | CHY-type | -1.78 | 5.47E-03 |
| 11866676 | Glyma05g12850.1 | | Unknown | -1.77 | 2.12E-02 |
| 12112796 | Glyma15g39970.1 | | Unknown | -1.77 | 3.00E-03 |
| 11895236 | Glyma06g21210.1 | IBS1 | Protein kinase superfamily protein | -1.77 | 3.63E-02 |
| 11885509 | Glyma06g42910.1 | | Unknown | -1.77 | 2.16E-03 |
| 12015405 | Glyma11g08730.1 | SEN1 | Rhodanese | -1.77 | 2.44E-04 |
| 11842308 | Glyma04g41540.1 | GLT1 | NADH-dependent glutamate synthase 1 | -1.77 | 3.58E-03 |
| 11798007 | Glyma02g02070.1 | SAG21 | Senescence-associated gene 21 | -1.76 | 1.59E-02 |
| 11798767 | Glyma02g04500.1 | | MATE efflux family protein | -1.76 | 1.90E-04 |
| 12121514 | Glyma16g31050.1 | | Unknown | -1.76 | 4.90E-02 |
| 11957462 | Glyma09g15820.1 | SCL30A | SC35-like splicing factor 30A | -1.76 | 3.30E-03 |
| 11882117 | Glyma06g20730.1 | GSTU9 | Glutathione S-transferase tau 9 | -1.76 | 3.03E-03 |
| 12068174 | Glyma13g33970.1 | TPS1 | Trehalose-6-phosphate synthase | -1.76 | 1.48E-02 |
| 11788291 | Glyma02g09390.1 | MAP70-2 | Microtubule-associated proteins 70-2 | -1.76 | 3.42E-04 |
| 11941146 | Glyma08g06760.1 | ZTP29 | ZIP metal ion transporter family | -1.76 | 3.73E-02 |
| 11975371 | Glyma09g40880.1 | | Leucine-rich repeat protein kinase family protein | -1.76 | 1.58E-03 |
| 12176943 | Glyma18g53760.1 | | Phosphoenolpyruvate carboxylase family protein | -1.76 | 1.60E-02 |
| 11961410 | Glyma09g33540.1 | | Protein of unknown function (duplicated DUF1399) | -1.75 | 1.12E-02 |
| 11769067 | Glyma01g27440.1 | | Disease resistance protein (TIR-NBS-LRR class) | -1.75 | 1.06E-02 |
| 12032785 | Glyma12g36570.1 | CEV1 | Cellulose synthase family protein | -1.75 | 1.41E-03 |

**Table S2.** Microarray analysis of yeast gene expression in 26972c cells containing GmbHLHm1 or an empty vector control (pYES3). Cells were initially grown on minimal media containing 0.1% L-proline and 2% glucose. At mid-log phase, cells were transferred to new minimal media containing 1 mM ammonium and 2% galactose and grown for a further 12 h. Fold change (> 2-fold) is defined as the increase in yeast gene expression with GmbHLHm1 over pYES3 containing cells (n=3 biological replicates in both cases).

| Yeast ID | Gene Symbol | Fold Change | P value | Gene Description |
|---|---|---|---|---|
| YOR378W | | 56.5 | 8.6E-14 | ScAMF1 |
| YML123C | PHO84 | 38.7 | 0.0E+00 | High-affinity inorganic phosphate (Pi) transporter |
| YDR046C | BAP3 | 17.6 | 3.9E-10 | Amino acid permease |
| YHL024W | RIM4 | 14.8 | 7.3E-14 | Putative RNA-binding protein |
| YDR281C | PHM6 | 13.4 | 1.2E-11 | Protein of unknown function |
| YMR122W-A | | 10.3 | 0.0E+00 | |
| YKR013W | PRY2 | 5.6 | 3.6E-15 | Protein of unknown function |
| YLR414C | | 5.3 | 8.7E-11 | |
| YCR098C | GIT1 | 5.2 | 2.1E-06 | Glycerophosphoinositol and glycerophosphocholine permease |
| YJR150C | DAN1 | 5.1 | 2.1E-07 | Cell wall mannoprotein |
| YDL055C | PSA1 | 5.0 | 0.0E+00 | GDP-mannose pyrophosphorylase |
| YGL041C-B | | 4.8 | 2.3E-10 | |
| YER001W | MNN1 | 4.6 | 1.1E-13 | Alpha-1,3-mannosyltransferase |
| YLR300W | EXG1 | 4.3 | 0.0E+00 | Major exo-1,3-beta-glucanase |
| YNL217W | | 4.3 | 6.2E-13 | |
| YCL069W | VBA3, VBA5 | 4.2 | 6.5E-09 | Permease of basic amino acids in the vacuolar membrane |
| YJR153W | PGU1 | 4.1 | 3.0E-09 | Endo-polygalacturonase |
| YEL021W | | 4.1 | 6.3E-08 | |
| YPL061W | ALD6 | 4.0 | 5.6E-15 | Cytosolic aldehyde dehydrogenase |
| YOL155C | HPF1 | 4.0 | 1.0E-08 | Haze-protective mannoprotein |
| YGL253W | HXK2 | 3.9 | 0.0E+00 | Hexokinase isoenzyme 2 |
| YJR114W | | 3.7 | 5.6E-07 | |
| YMR305C | SCW10 | 3.7 | 2.3E-10 | Cell wall protein with similarity to glucanases |
| YMR069W | NAT4 | 3.7 | 6.8E-06 | N alpha-acetyl-transferase |
| YPL019C | VTC3 | 3.5 | 1.5E-13 | Vacuolar polyphosphate permease |
| YER064C | | 3.4 | 1.0E-11 | |
| YIL169C | HPF1 | 3.3 | 2.3E-08 | Haze-protective mannoprotein |
| YHR183W | GND1 | 3.2 | 5.4E-11 | 6-phosphogluconate dehydrogenase |
| YBR093C | PHO5 | 3.0 | 2.0E-12 | Repressible acid phosphatase |
| YLR267W | BOP2 | 2.8 | 2.7E-08 | Protein of unknown function |
| YLR332W | MID2 | 2.7 | 6.6E-12 | O-glycosylated plasma membrane sensor |
| YDL039C | PRM7 | 2.7 | 6.4E-09 | Pheromone-regulated protein |
| YEL021W | URA3 | 2.5 | 3.2E-07 | Orotidine-5'-phosphate (OMP) decarboxylase |
| YNL145W | MFA2 | 2.5 | 8.8E-09 | Mating pheromone a-factor |
| YDL038C | | 2.5 | 4.0E-09 | |
| YER145C | FTR1 | 2.5 | 1.1E-12 | High affinity iron permease |
| YBR092C | PHO3 | 2.5 | 5.4E-10 | Constitutively expressed acid phosphatase similar to Pho5p |
| YIL169C | | 2.4 | 2.2E-05 | |
| YHR179W | OYE2 | 2.4 | 2.0E-10 | NADPH oxidoreductase containing flavin mononucleotide (FMN) |
| YKL109W | HAP4 | 2.4 | 2.4E-12 | Subunit of the Hap2p/3p/4p/5p CCAAT-binding complex |
| YKL198C | PTK1 | 2.4 | 2.4E-13 | Putative serine/threonine protein kinase |
| YDL245C | HXT15 HXT16 | 2.4 | 4.2E-04 | Similar to hexose transporter family members |
| YJL159W | HSP150 | 2.4 | 7.8E-16 | O-mannosylated heat shock protein |
| YGR286C | BIO2 | 2.3 | 9.4E-10 | Biotin synthase |
| YJL158C | CIS3 | 2.3 | 8.0E-11 | Mannose-containing glycoprotein |
| YGL116W | CDC20 | 2.2 | 4.1E-10 | Cell-cycle regulated activator of anaphase-promoting complex/cyclosome (APC/C) |
| YAR071W | PHO11, PHO12 | 2.2 | 6.0E-15 | Repressible acid phosphatases |

| | | | | |
|---|---|---|---|---|
| YER045C | ACA1 | 2.2 | 4.9E-09 | bZIP transcription factor of the ATF/CREB family |
| YER037W | PHM8 | 2.2 | 8.6E-03 | Protein of unknown function, expression is induced by low phosphate |
| YBR187W | GDT1 | 2.2 | 3.1E-14 | Putative protein of unknown function |
| YBR240C | THI2 | 2.2 | 1.9E-07 | Zinc finger protein of the Zn(II)2Cys6 type |
| YOR032C | HMS1 | 2.1 | 8.9E-08 | bHLH protein with similarity to myc-family transcription factors |
| YHR137W | ARO9 | 2.1 | 5.4E-10 | Aromatic aminotransferase II |
| YNR075W | COS10 | 2.1 | 5.4E-03 | Protein of unknown function |
| YEL021W | | 2.1 | 1.6E-07 | |
| YDR481C | PHO8 | 2.0 | 9.6E-12 | Repressible alkaline phosphatase |
| YHR079C-A | SAE3 | 2.0 | 1.7E-03 | Meiosis specific protein involved in DMC1-dependent meiotic recombination |
| YCR021C | HSP30 | 2.0 | 9.8E-13 | Hydrophobic plasma membrane localized, stress-responsive protein |
| YJL068C | | 2.0 | 0.0E+00 | |
| YIL011W | TIR3 | 2.0 | 1.2E-08 | Cell wall mannoprotein of the Srp1p/Tip1p family |
| YDR275W | BSC2 | 2.0 | 3.3E-11 | Protein of unknown function |
| YPL018W | CTF19 | 2.0 | 1.2E-06 | Outer kinetochore protein |
| YEL035C | UTR5 | 2.0 | 6.9E-04 | Protein of unknown function |

**Table S3. PCR Primers used in the study**

| Primer Name | Sequence |
|---|---|
| *GmbHLHm1-qPCR-F* | TTATTGCTCAGATGGATATGGAA |
| *GmbHLHm1-qPCR-R* | GACGACCGAAAAATGCATAAC |
| *cons6-qPCR-F* | AGATAGGGAAATGGTGCAGGT |
| *cons6-qPCR-R* | CTAATGGCAATTGCAGCTCTC |
| *GmbHLHm1-Promoter-F* | GATTTAACCTAAGAAAACCAATTCC |
| *GmbHLHm1-Promoter-R* | ATACTCAAACTACAACATCCCATG |
| *N-term-GFP-FW* | ATCGGACTACTAGCAGCTGTAATACGACTCACTATAGGGAATA TTATGGTAGATCTGACTAGTAAAGG |
| *N-term-GFP-RV* | AGGTAGCCCTCTGATTGATGAAATCTCCATATGAGAACTCCTCA TAGCTTTGTATAGTTCATCCATGCC |
| *C-term-GFP-FW* | AAGGATCTTGTGAGAAGTTTACGCTCAGCTTTTTCATATTTCGT GATGGTAGATCTGACTAGTAAAGG |
| *C-term-GFP-RV* | AGGGCGTGAATGTAAGCGTGACATAACTAATTACATGATGCGG CCTCAGCTAGCTTTGTATAGTTCATC |
| *bHLHm1-RNAi-FW* | TGCTTGTAAAAGTTTGGTTGAAA |
| *bHLHm1-RNAi-RV* | GCAACATGTCGAGCATATACACAAG |
| *SNRK2.4-qPCR-F* | TGAGAAGTGAGTCAAGGAAGCA |
| *SNRK2.4-qPCR-R* | CAGGCAAAACATTCGCCTTA |
| *GmLHY-qPCR-F* | AAAGGCAACCTTTGGTTTTTG |
| *GmLHY-qPCR-R* | ACGACAAGTTTCGTGAGCTG |
| *GmGIb-qPCR-F* | CCCACAACCCCTCTTATCAATAC |
| *GmGIb-qPCR-R* | GGCAGCGGTATCAAGAAAGT |
| *GmGIc-qPCR-F* | GAGTCCTAAGCCACTGCAAAAG |
| *GmGIc-qPCR-R* | GCTTCCCATGTCAAGCAGTT |
| *GmZIP1 qPCR F* | TCTATTACAATAATGGGATTGTTCTTC |
| *GmZIP1 qPCR R* | AGGGCAGTTGGACTGTTTTC |
| *GmbHLHm1-R180K-F* | GAAAGGAAGAAAAGAGAGAAGC |
| *GmbHLHm1-R180K-R* | GTTCTCTCTTTTCTTCCTTTC |
| *GmbHLHm1-L191V-F* | AACGGTTCATAGCTGTATCTGCTCTTGTTC |
| *GmbHLHm1-L191V-R* | GAACAAGAGCAGATACAGCTATGAACCGTT |
| *GmbHLHm1-L207V-F* | GATGGACAAAGCGTCGGTTGTTGGAGAAGC |
| *GmbHLHm1-L207V-R* | GCTTCTCCAACAACCGACGCTTTGTCCATC |
| *GmbHLHm1 pMAL-F* | CATATGCCTAAAATTGACAACAATGCTCTTG |
| *GmbHLHm1 pMAL-R* | GATCCTTATCTTGCTTCAATTTCAGGTAGTG |
| *YOR378W-Promoter-F* | GGGTTGTCGCTACGAATGTT |
| *YOR378W-Promoter-R* | ATTGATTTTATGTTCTTTATGTTCCAG |
| *GFP-F* | ATGGTAGATCTGACTAGTAAAG |
| *N-term-ScAMF1-GFP-R* | GGAGCTAGTTGACATAGCTTTGTATAGTTC |
| *N-termScAMF1-GFPF* | GAACTATACAAAGCTATGTCAACTAGCTCC |
| *ScAMF1-R* | TTCTATAAAATGCTGCTTTTCG |
| *YOR378wF-KanMx4* | GAACATAAAATCAATATGCGTACGCTGCAGGTCGAC |
| *YOR378wR-KanMx4* | CTCTATATATGAACTTAATCGATGAATTCGAGCTCG |
| *F-UP* | TACATTCATCAATTGCTGGAACATAAAGAACATAAAATCAATA TG |
| *R-DN* | TTGGGTTATATACCACGATGAAGTATTTCTCTATATATGAACTT A |
| *YOR378-KO-F* | GGGTTGTCGCTACGAATGTT |
| *YOR378-KO-R* | TTTCGGACTCTTTGTCGCTT |
| *kanB* | CTGCAGCGAGGAGCCGTAAT |
| *kanC* | TGATTTTGATGACGAGCGTAAT |

| | |
|---|---|
| *ScAMF1-qPCR-F* | TGT GCT CTG CCC AAC TAA TG |
| *ScAMF1-qPCR-R* | GCG GAA GCA AAC CAA CTA AG |
| *ScTUB1-qPCR-F* | AGG AGG ACG CGG CTA ATA AT |
| *ScTUB1-qPCR-R* | AAC CCA TCA CAT TGG TCT GC |
| *ScALG9F* | CACGGATAGTGGCTTTGGTGAACAATTAC |
| *ScALG9R* | TATGATTATCTGGCAGCAGGAAAGAACTTGGG |
| *Mep1-qPCR-F* | GCGATGCTCTTCTACGGTTGTC |
| *Mep1-qPCR-R* | CGTGTCCGCAGAGATAAGCAAC |
| *Mep3-qPCR-F* | GGTGGTTGGTTGACGCATAAC |
| *Mep3-qPCR-R* | CGGCTTCCTCAGTGACTCTTAG |
| *pNCW-bHLHm1-PST1-FW* | GGCCCTGCAGCATGAGGAGTTCTCATATGGAGAT |
| *pNCW-bHLHm1-SAC1-RV* | GGCCCCGCGGTCACACGAAATATGAAAAAGCTGA |
| *pCMBV4-bHLHm1-FW* | TACTTCTATAGACACGCAAACACAAATACACACACTAATCTAG ACATGAGGAGTTCTCATATGGAG |
| *pCMBV4-bHLHm1-RV* | CATGGTCGACGGTATCGATAAGCTTGATATCGAATTCCTGCAG ATCACGAAATATGAAAAAGCTGA |
| *GmbHLHm1 T1 F* | ATGGCTAACTTCCTCCATCAGTGG |
| *GFP pYES3 F* | ATGGTAGATCTGACTAGTAAAGGAG |
| *GmbHLHm1 CDS R* | TCACACGAAATATGAAAAAGC |
| *GmAMF1;3qPCRF* | ATGTGCCTTCCTCACCTCTC |
| *GmAMF1;3qPCRR* | GACTTACCTATAAACCATATTGCAG |
| *GmAMF1;3 qPCR2 F* | GCTAAAATTTGTTTCACACAATGATG |
| *GmAMF1;3 qPCR2 R* | CAGAGTAGAGGGCACGTACA |
| *GmAMF1;5 qPCR F* | ATGTGGGGCTGATCACAAGT |
| *GmAMF1;5 qPCR R* | AGCGGTGGAAGAATCAGCAG |
| *GmAMF1;3 cds.F* | ATGGCACAACAACAAGAACATGA |
| *GmAMF1;3 cds.R* | TCATAATGACTTACCTATAAACCAT |
| *GmAMF1;3 cds.R2* | TAGCAAATCAATAGGAAGTTGTCA |
| *GmAMF1;3 cds.R3* | TCAGCATTCTTGGGATTGACC |
| *GmAMF1;3 Promoter F* | ACTCTTTATATTACTTGATTTCTCTCAA |
| *GmAMF1;3 Promoter R* | TTGTTGTTGTGCCATACCAATATAAT |
| *bHLHm1 LCF* | CTAAGGTGGAGATGGCGTGT |
| *bHLHm1 LCR* | CGTTGGCTGAGCTTCTCTCT |
| *GmRps19-fwd* | GCTTAGGGCTCTAACCAAGC |
| *GmRps19-rev* | CGATCTCCGTGACCAAATGG |
| *GmUO1 F* | AGTTCGAGCAGAGGCACGGGA |
| *GmUO1 R* | CCCACGCGCCACTCCACAAT |
| *NifH F* | TGCGTCGCATGACGGTGCTT |
| *NifH R* | GGATGATGCCCTTGCCGCCA |
| *GS1y2F* | TGTTGCTGCTGAAGAACCCT |
| *GS1y2R* | CCAAGAGGCCATCCAAGAGG |
| *GS1y1F* | GCCTCTAGGATGGCCTCTTG |
| *GS1y1R* | ATATCACGCCCGAAAGCCTT |
| *GS1B1F* | TGACCTGTGCTGGAACCATC |
| *GS1B1R* | CCTGAGGAGCAAAGCAAGGA |
| *SS1F* | TTGGTCTTGCGAGAGCGGGC |
| *SS1R* | CGGCAACCATGGGGTGAGGC |
| *FixU F* | ATGATCCGCCGCTCTCCGGA |
| *FixU R* | CCAGCCGCCCCATAGGGTCT |
| *GlnA 1 F* | TGAAGAACACCGCGCATCAG |
| *GlnA 1 R* | GGCGTAGTCACCTTTGTCGT |
| *SigA F* | ACACCGGCTCGGAGCTCGAT |
| *SigA R* | CGCAGCGAGCTGATGCACCT |
| *GapA F* | GCGCCACCGACGTGAAGGAA |
| *GapA R* | CGATCGAGACGTTCGGCGCA |

**Table S4. Nutrient solution for hairy root transformation. pH was adjusted to 6.2-6.4.**

| Chemical | Concentration |
|---|---|
| **Macro Elements** | |
| $MgSO_4.7H_2O$ | 500 µM |
| $KH_2PO_4$ | 200 µM |
| $K_2HPO_4$ | 50 µM |
| KCl | 250 µM |
| Fe-Na-EDTA | 100 µM |
| $CaCl_2$ | 250 µM |
| **Trace Elements** | |
| $H_3BO_3$ | 46 µM |
| $MnSO_4.H_2O$ | 8 µM |
| $ZnSO_4.7H_2O$ | 8 µM |
| $CuSO_4.5H_2O$ | 2 µM |
| $Na2MoO_4.2H_2O$ | 2 µM |
| **Nitrogen source** | |
| $NH_4NO_3$ | 1 mM |