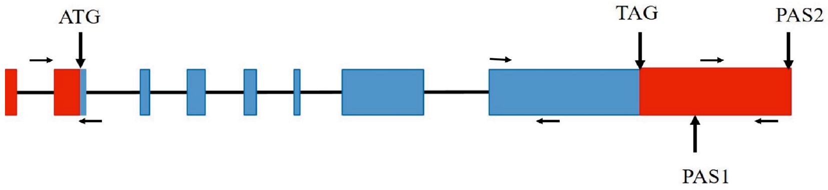

Figure S1. Gene structure of *Fm201*

Fm201 gene contains seven exons and seven introns. Blue boxes stand for exons, red boxes stand for the UTRs, lines between the boxes stand for introns; ATG and TAG indicate start codon and stop codon, respectively. PAS1 and PAS2 stand for the two polyadenylation signals, indicative of two distinct 3’ UTRs in *Fm201* gene, while the arrows indicate the specific primers for isolation of the sequence of *Fm201* UTRs.

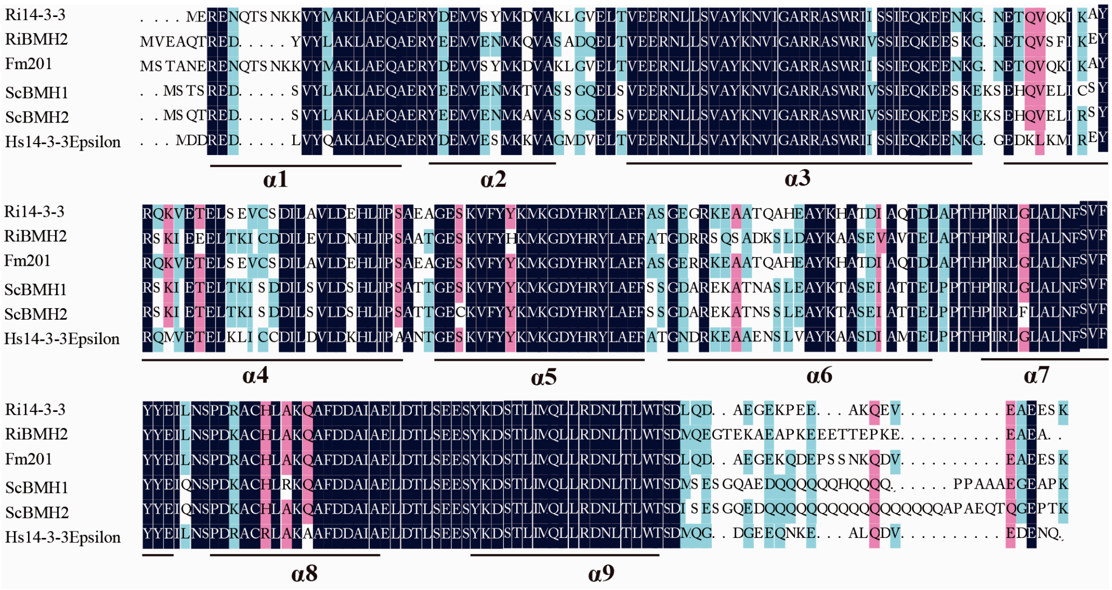

Figure S2. Multiple amino acid sequence alignment among Fm201, Ri14-3-3, RiBMH2, ScBMH1, ScBMH2 and Hs14-3-3ε.

Amino acid sequences with highlighted lines represent 9 predicted α-helixes.

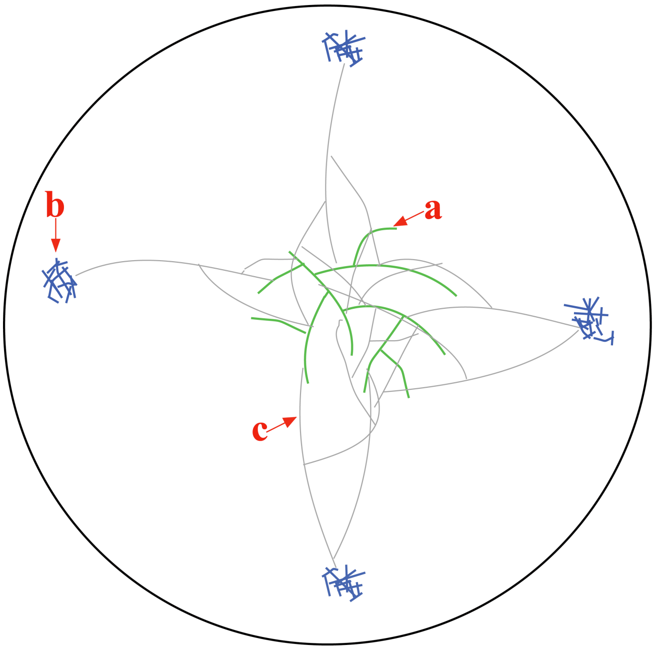

Figure S3. Schematic diagram of HIGS experiment design in petri dishes system.

Culture a represents hairy roots of *M. truncatula* harboring the RNAi vector grown on MS medium. Culture b indicates the infected hairy roots of *Petroselinum crispum* with *Rhizophagus irregularis* as the inocula. Culture c showes the growing extraradical hyphae from mycorrhizal roots of *Petroselinum crispum* toward the roots of *M. truncatula*. 30 days after inoculation, the mycorrhizal roots of *M. truncatula* with Ri14-3-3 RNAi vector were collected for subsequent colonization and gene expression analyses.

Table S1. Yeast Strains used in this study

Strain	Genotype
BY4741	MATa.his3Δ1.leu2Δ0.met15Δ0.ura3Δ0
BY4741-pMRI-12	MATa.his3Δ1.leu2Δ0.met15Δ0.ura3Δ0.KanMX
Bmh1-Fm201	MATa.his3Δ1.leu2Δ0.met15Δ0.ura3Δ0.BMH1::KanMX(Gal7[*Fm201*])
bmh1-Fm201-pSH47	MATa.his3Δ1.leu2Δ0.met15Δ0.ura3Δ0.BMH1::KanMX(Gal7[*Fm201*]).ura3
bmhs-Fm201	MATa.his3Δ1.leu2Δ0.met15Δ0.ura3Δ0.BMH1::KanMX(Gal7[*Fm201*]).BMH2::ura3
bmh1-Ri14-3-3	MATa.his3Δ1.leu2Δ0.met15Δ0.ura3Δ0.BMH1::KanMX(Gal7[*Ri14-3-3*])
bmh1-Ri14-3-3-pSH47	MATa.his3Δ1.leu2Δ0.met15Δ0.ura3Δ0.BMH1::KanMX(Gal7[*Ri14-3-3*]).ura3
bmhs-Ri14-3-3	MATa.his3Δ1.leu2Δ0.met15Δ0.ura3Δ0.BMH1::KanMX(Gal7[*Ri14-3-3*]).BMH2::ura3
Bmh1-RiBMH2	MATa.his3Δ1.leu2Δ0.met15Δ0.ura3Δ0.BMH1::KanMX(Gal7[*RiBMH2*])
bmh1-RiBMH2-pSH47	MATa.his3Δ1.leu2Δ0.met15Δ0.ura3Δ0.BMH1::KanMX(Gal7[*RiBMH2*]).ura3
bmhs-RiBMH2	MATa.his3Δ1.leu2Δ0.met15Δ0.ura3Δ0.BMH1::KanMX(Gal7[*RiBMH2*]).BMH2::ura3

Table S2. Primers used in this study

Primers	Sequences（5’-3’）	Primers	Sequences（5’-3’）
1433F	CGCTGAGCAAGCCGAACG	201ATGF3	GATTCCCCATGGTGACCTCTAATAAAAAGGT
1433R	AGTCCAAAGGGTCAAGTTATC T	201TAGR2	CTAATTATGGATCCTTTGATTCTTCAGCTTCG
201spF1	TGGGGAGGAGAAAGGAATGTTTGTTG	201F	TCACTGTGGAGGAACGTAATCT
P_{Gal7}F	CCCGAGCTCTTTGCCAGCTTACTATCCTTC	201spR2	AACAAACATTCCTTTCTCCTCCCCAT
P_{Gal7}R	CTCACTAGTTTTTGAGGGAATATTCAACTGTT	pMRI-12F1	CTTTATTCCGGCCTGGAGTCAAAAGAGGAAGCTCGGTGGCAAAT TTTGCCAGCTTACTATCCTTCTT
FmAct	CCTCACGCCATTCTTCGAC	pMRI-12R1	ACATATATAGATATAGATATATAATTGCAATAATGAACTACAAATTATTACATGTTCTTTCCTGCGTTATCCCCTG
FmAct	CAGCGGTTTGCATTTCTTGT	pSH47F	GGGGGAAAAGCAAAGAACGAGAAAACTTGGACAGAAGGTTAATACTCTAGATTGTACTGAGAGTGCAC
201F	TCACTGTGGAGGAACGTAATCT	pSH47R	CTGGAGTGGTAAATCTTCATTTCCCCTTGTATTTCTCAGCGCTCTTATTTGGTTGTGTGCGGTATTTCACACCGC
201R	TCAGCAATGGCATCATCAAA	BMH1upF	GCTTACTAGCCGGCTAGG
QT	CCAGTGAGCAGAGTGACGAGGACTCGAGCTCAAGCTTTTTTTTTTTTTTTTT	BMH1downR	GTGAAGATACAGGTGCGACAAAC
QC	CCAGTGAGCAGAGTGACGAGGACTCGAGCTCAAGCCCCCCCCCCCCCCCCC	BMH2upF	GAGATAATAATACAGCG
QO	CCAGTGAGCAGAGTGACG	BMH2downR	GAGGTGCGTATACAATGG
QI	GAGGACTCGAGCTCAAGC	201PR2	GGTCACCATGGTGTGAAAAAAAACGGAATAATTT
Ri14-3-3F	GCAAGCCGAACGTTATGATG	Fm201RTF	CGGGTGAAAGACGAAAAGAAGC
Ri14-3-3R	GGCAAGGATATCCGAGCATAC	Fm201RTR	CAAGTCCGAGACGAATAGGATGAGT
RiBMH2ATG	ATGGTCGAAGCTCAAACAC	UraF	AGGCGGCAGAAGAAGTAACA
RiBMH2TAA	TTAAGCTTCTGCTTCTTCCTTTG	UraR	AATGCGTCTCCCTTGTCATC