SI Appendix

Myristate as a carbon and energy source for the asymbiotic growth of the arbuscular mycorrhizal fungus *Rhizophagus irregularis*

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Extended Methods

Biological materials. Sterile spore suspension of the AM fungus *Rhizophagus irregularis* DAOM197198 (or DAOM181602, another voucher number for the same fungus) was purchased from Premier Tech. Contaminated hyphae in the spore suspension were removed by density-gradient centrifugation using gastrografin (972 mM amidotrizoate, 815 mM meglumine, and 157 mM sodium hydroxide). Five mL of 8%, 16%, 32%, and 48% gastrografin solution was carefully poured to form layers in a 50 mL centrifuge tube, and then 20 ml of spore suspension (approximately 4,000 spores ml^{-1}) was layered on top of the gastrografin solution. After centrifugation at 3,500 rpm for 15 min at room temperature using a swing rotor, middle layers containing spores were collected and transferred to a new tube. The collected layers were diluted with sterilized water and then centrifuged again. After the removal of the supernatant, spores were suspended in sterilized water, adjusted to approximately 10,000 spores ml^{-1}, and stored at 4 ºC for later use.

Asymbiotic culture. Approximately 300–400 spores of *R. irregularis* were inoculated on 0.3% Phytagel (Sigma-Aldrich) plates containing modified SC medium (1.7 g l^{-1} yeast nitrogen base [MP Biomedicals], 2.0 g l^{-1} complete SC mixture [Formedium], 5 mM ammonium sulfate, 1 mM glycerol, 5 mg l^{-1} thiamine hydrochloride, 5 mg l^{-1} nicotinic acid, 5 mg l^{-1} pyridoxal phosphate, and appropriate amounts of fatty acids) and then covered with 0.3% Phytagel dissolved in 3 mM magnesium sulfate in a 12-well culture plate. For the liquid culture, Phytagel was removed from the medium. All fatty acids and fatty acid salts were obtained from Sigma-Aldrich, Tokyo Chemical Industry, and Wako Pure Chemical Industries, and 2-myristoylglycerol and 2-palmitoylglycerol were purchased from Santa Cruz Biotechnology. Three types of fatty acids were added to the medium: fatty acid salts, fatty acids in an organic solvent, or fatty acids conjugated with BSA. Stock solutions of 100 mM free fatty acids and potassium salts of fatty acids were prepared by dissolving in ethanol and distilled water, respectively. For the preparation of fatty acid–BSA conjugates, an equal amount of 100 mM fatty acid was dissolved in ethanol, and 20% fatty acid-free BSA (Sigma-Aldrich) solution was mixed well and added with distilled water to generate 10 mM final concentration of fatty acid. More BSA solution was added to solubilize stearic acid. The plates were incubated at 28 ºC in the dark. Hyphal elongation was observed under the dissecting microscope Stemi 508 (Carl Zeiss) and light microscope Primovert (Carl Zeiss). Digital images were captured with a digital CCD camera AxioCam MRc5 (Carl Zeiss) operated with AxioVision (Carl Zeiss). Fully focused images were captured on the microscope with BZ-X800 (Keyence).

Immobilized cell culture. An overview of the immobilized cell culture system is represented

in the *SI Appendix* (Fig. S3*A*). Thirty-five mL of 0.75% Phytagel containing 3 mM magnesium sulfate was poured into a 90 mm Petri dish and solidified. A Phytagel tablet (6 mm high and 17.5 mm wide) with a circular incision (3 mm deep and 11.5 mm wide) was cut out using a sterile double cork borer (inner diameter: 11.5 and 17.5 mm, respectively). The gel within the circular incision on the top side of the gel tablet was removed to 3 mm deep using a spatula or disposable pipette tip connected to an aspirator to prepare a hole for spore inoculation. To flatten the bottom of the hole, a small amount of 0.75% Phytagel was added. Approximately 300–400 spores were placed in the hole and covered with 0.75% Phytagel containing 3 mM magnesium sulfate. The Phytagel tablets containing spores were transferred into a six-well culture plate. Each well was filled with 5 mL of full- or half-strength modified SC liquid medium with or without 0.5 mM potassium myristate and monosaccharides (glucose and xylose). AM fungi were grown at 28 ºC in the dark. During the culture, the medium was changed once a month.

Measurement of fungal biomass.
Gels containing fungal materials in wells of a six-well culture plate were cut using a knife. A 1.5× volume of citrate buffer (8.3 mM trisodium citrate, 1.7 mM citric acid, and 1% Triton X-100; pH 6.0) was added to the well and mixed using a spatula. The plate was incubated at 50 ºC for 20 min. The melted gel, including fungal materials, was transferred to a 5 mL tube and further incubated at 50 ºC for 20 min. The sample was centrifuged at 3,500 rpm for 10 min at room temperature using a swing rotor. After the removal of the supernatant, approximately 5 mL of the citrate buffer was added, incubated at 50 ºC for 10 min, and then centrifuged again. This step was repeated once. Fungal pellets were suspended in the remaining 1 mL of the citrate buffer and transferred to an antistatic 1.5 mL tube. After centrifugation at 3,500 rpm for 10 min, the supernatant was removed. The fungal pellet was washed with 1 mL of distilled water by centrifuging at 3,500 rpm for 10 min three times. After centrifugation at 12,000 rpm for 10 min using an angle rotor, the remaining water was completely removed. The pellet was dried at 70 ºC for 48 h. After cooling down in a desiccator, fungal materials were weighed using a micro analytical balance (BM-252, A&D). The number of inoculated spores in the well was counted in advance under a dissecting microscope. The standardized growth increment of *R. irregularis* was calculated by dividing the total fungal dry weight in a well by the number of inoculated spores and subtracting the mean dry weight of an inoculated spore.

Spore morphology and germination assay. Myristate-induced spores were produced by an immobilized cell culture system in the presence of 0.5 mM potassium myristate in a half-strength modified SC medium for approximately three months. Myristate-induced and symbiotically generated spores were mounted with polyvinyl alcohol–lactic acid–glycerol (PVLG) or Melzer's reagent for microscopic observation using a light microscope (Axio Imager

D1 microscope, Carl Zeiss). Spores were incubated with 10 μM SYTO 13 green fluorescent nucleic acid stain (Thermo Fisher) for 2 h and observed by epifluorescence microscopy (Axio Imager D1 microscope). The fluorescence was excited with the band-pass filter BP470/40, and emitted fluorescence was detected with BP525/50. For spore germination assay, myristate-induced and symbiotically generated spores were placed onto 0.3% Phytagel containing 3 mM magnesium sulfate and incubated at 28 ºC for one week. Spore germination was examined under a dissecting microscope.

Single spore inoculation. A single myristate-induced spore produced by an immobilized cell culture system in the presence of 0.5 mM potassium myristate and 5 mM xylose in a half-strength modified SC medium was placed onto carrot hairy root plates using a pipette according to Kameoka et al. (1). The production of daughter spores on extraradical hyphae emerging from hairy roots was observed under a dissecting microscope. AM fungal colonization was confirmed by trypan blue staining (2).

Fatty acid uptake. Fatty acid uptake was evaluated using a fluorescent fatty acid analogue, 4,4-difluoro-5-methyl-4-bora-3a,4a-diaza-*s*-indacene-3-dodecanoic acid (C_1-BODIPY 500/510 C_{12}, Thermo Fisher Scientific). *R. irregularis* was grown in an immobilized cell culture system with modified SC medium containing 0.5 mM potassium myristate without sugars for 4 or 6 weeks. Fungal hyphae were stained with 0.5 mM C_1-BODIPY 500/510 C_{12} in modified SC medium. After 10-minute incubation, fungal hyphae protruding outside a Phytagel tablet were observed using a laser scanning confocal microscope (Leica TCS SP2). For the samples incubated for 1 or 5 days in the medium containing the fluorescent probe, a Phytagel tablet containing fungal materials was melted by adding citrate buffer. Fluorescent signals were observed under an epifluorescence microscope (Axio Imager D1 microscope). For laser scanning confocal microscopy, the fluorescence of C_1-BODIPY 500/510 C_{12} was excited using a 488-nm wavelength argon laser, and fluorescence between 500 and 550 was detected. For epifluorescence microscopy, the fluorescence was excited with the band-pass filter BP470/40, and emitted fluorescence was detected with BP525/50. Digital images were captured with the digital CCD camera AxioCam MRm operated with AxioVision.

LC-MS analysis of glucosamine. [1-^{13}C]Myristic acid (Taiyo Nippon Sanso) and non-labeled myristic acid were dissolved in 200 mM potassium hydroxide to 100 mM final concentration of fatty acids. *R. irregularis* was cultured in a modified SC solid medium supplied with 1 mM neutralized myristate for two months. Approximately 1–2 mg dry fungal materials per sample were recovered from the solid medium as described above. Glucosamine derived from fungal biomass was extracted according to previous reports (3, 4) with some modifications. Briefly,

fungal materials were washed in cold 0.25 M hydrochloric acid solution twice for 5 and 35 min, respectively. After centrifugation at 12,000 rpm for 5 min, the fungal pellet was rinsed with Milli-Q water. The sample was then incubated in 1 mL of 0.2 M sodium hydroxide for 6 h at room temperature. After centrifugation, the pellet was further incubated in 1 mL of fresh 0.2 M sodium hydroxide at 70 ºC for 17 h. After cooling to room temperature, the sample was washed with Milli-Q water four times and dried at 70 ºC for several hours. Acid hydrolysis was carried out with 1 mL of 6 M hydrochloric acid solution at 70 ºC for 16 h. A total of 40 µL of each extract or glucosamine hydrochloride standard solution (10 µg ml^{-1}) was evaporated under reduced pressure, dissolved in 50% acetonitrile, and filtered through 0.45 µm membrane filters. The chromatographic separation was performed using the ACQUITY UPLC System (Waters), where 20 µL of sample was injected onto a COSMOSIL Sugar-D column (4.6 mm × 250 mm, Nacalai Tesque) at 30 ºC, and the solute was isocratically eluted using 75% acetonitrile containing 0.05% formic acid as the mobile phase at a flow rate of 0.75 mL min^{-1}. A glucosamine standard was detected with a retention time of 2.65 min. The mass spectrometer was operated in a positive ESI mode. The nebulizer and desolvation gas flows were 50 and 700 l h^{-1}, respectively. The capillary voltage was set at 2.8 kV, the cone voltage at 45 V, the source temperature at 120 ºC, and the desolvation gas temperature at 350 ºC. Data were collected using MassLynx version 4.1 (Waters). The relative intensities of the molecular ion peaks of glucosamine ($[M+H]^+$, *m/z* 180.19; $[M+1+H]^+$, *m/z* 181.19; and $[M+2+H]^+$, *m/z* 182.19) were monitored. The relative fractions of M+0, M+1, and M+2 in the glucosamine standard solution were 92.9%, 7.1%, and 0%, respectively.

^{13}C-NMR analysis of triacylglycerols. [1-^{13}C]Myristic acid (Cambridge Isotope Laboratories, Inc.) was dissolved in 200 mM potassium hydroxide to a 100 mM final concentration of fatty acids. *R. irregularis* was cultured in a modified SC solid medium supplied with 1 mM neutralized [1-^{13}C]myristate for 73 days. Approximately 43 mg of wet fungal materials were recovered from solid medium by gel solubilization as described above followed via vacuum filtration through filter paper. Lipids derived from fungal biomass were extracted with 3 mL of chloroform–methanol (2:1) via sonication for 30 min. After filtration, the lipid extract solution was concentrated by nitrogen gas. The concentrate was purified by preparative silica gel TLC (Kieselgel 60 F_{254}, Merck) using *n*-hexane–diethyl ether–acetic acid (80:30:1) as a developing solvent to yield 1.1 mg of triacylglycerol. For NMR analysis, the triacylglycerol was dissolved in 600 µL of $CDCl_3$. The ^{13}C-NMR spectra were recorded at 125 MHz on a JNM-ECZ500R spectrometer (JEOL) using the default parameter settings. The chemical shifts were referenced to the solvent peak ($CDCl_3$ δc 77.0) as an internal standard. 1.6 mg of triacylglycerol prepared from 27 mg of wet fungal materials of *R. irregularis* grown in a monoxenic root organ culture

(Premier Tech) was also subjected to ^{13}C-NMR analysis as above.

Determination of ATP content. Five hundred spores of *R. irregularis* were incubated in 100 µL of sterilized water at 28 ºC for 5 days. Potassium myristate was added to the germinating spores at a final concentration of 0.5 mM. For the control, the protonophore carbonyl cyanide m-chlorophenylhydrazone (CCCP) was simultaneously added at a final concentration of 50 µM. The germinating spores were incubated for 12 h. After centrifugation at 3,500 rpm for 10 min, 400 µL of phosphate buffered saline (PBS; pH 7.4) was added to the pellet. The spore suspension was transferred to a 2 mL tube containing a metal crusher (TAITEC) and placed on ice. The sample was crushed with a bead crusher (µT-12, TAITEC) at 2,200 rpm for 10 s three times. The crushed fungal materials were transferred to a 1.5 mL tube and centrifuged at 8,000×*g* for 2 min at 4 ºC. The supernatant was recovered and used for determination of ATP and protein concentration. ATP concentration was measured using the CellTiter-Glo Luminescent Cell Viability Assay Kit (Promega) following the manufacturer's instructions. Protein concentration was assayed using the Qubit Protein Assay Kits (Thermo Fisher Scientific). ATP content in the germinating spores was calculated as nmol per mg protein.

Quantitative RT-PCR. *R. irregularis* was grown in an immobilized cell culture system with modified SC medium containing 0.5 mM potassium myristate without sugars for 3 weeks. Subsequently, Phytagel tablets containing fungal materials were incubated in a modified SC medium without fatty acids for 11 days to induce fatty acid starvation. During the first three days of starvation, the culture medium was exchanged every day. After the starvation treatment, half of each sample was added to 100 mM potassium myristate to a final concentration of 0.5 mM, and the remaining half of each sample was added the same amount of sterilized water. After a 3 h incubation, fungal hyphae protruding outside a Phytagel tablet were recovered using forceps and immediately immersed in 500 µL of RNAiso Plus (Takara Bio). The fungal hyphae were crushed using the bead crusher µT-12 with a metal crusher at 2,200 rpm for 10 s three times with cooling on ice. RNA was extracted using RNAiso Plus following the manufacturer's instructions. Isolated RNA was treated with a TURBO DNA-free Kit (Thermo Fisher Scientific) to eliminate contaminating genomic DNA. cDNA was synthesized using a ReverTra Ace qPCR RT Kit (TOYOBO). Semiquantitative PCR was conduct using the StepOne Real-Time PCR System (Thermo Fisher Scientific) with a THUNDERBIRD SYBR qPCR Mix (TOYOBO). Primers used for the qRT-PCR are shown in *SI Appendix* (Table S4). Melting curve analysis confirmed the single peaks. The ΔΔCt method (5) was used to calculate the fold changes of gene expression levels between myristate- and water-treated groups with the *R. irregularis* elongation factor 1 beta (*EF-1β*) gene as an internal control using the R bioconductor package

pcr (6).

Statistical analysis. All statistical analyses were performed using R version 3.5.2. To examine the differences among experimental groups, data were analyzed with Student's t-test, Tukey HSD test, and Fisher's exact test, as appropriate. Differences at $P < 0.05$ were considered to be significant.

References

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Movie S1. Translocation of the fluorescently labelled fatty acid derivative C_1-BODIPY 500/510 C_{12} taken up by *R. irregularis*. Fluorescent images of C_1-BODIPY 500/510 C_{12} and superimposed bright field images of *R. irregularis* cultivated using an immobilized cell culture system with the modified SC medium containing 0.5 mM of the fluorescent probe for 10 min.

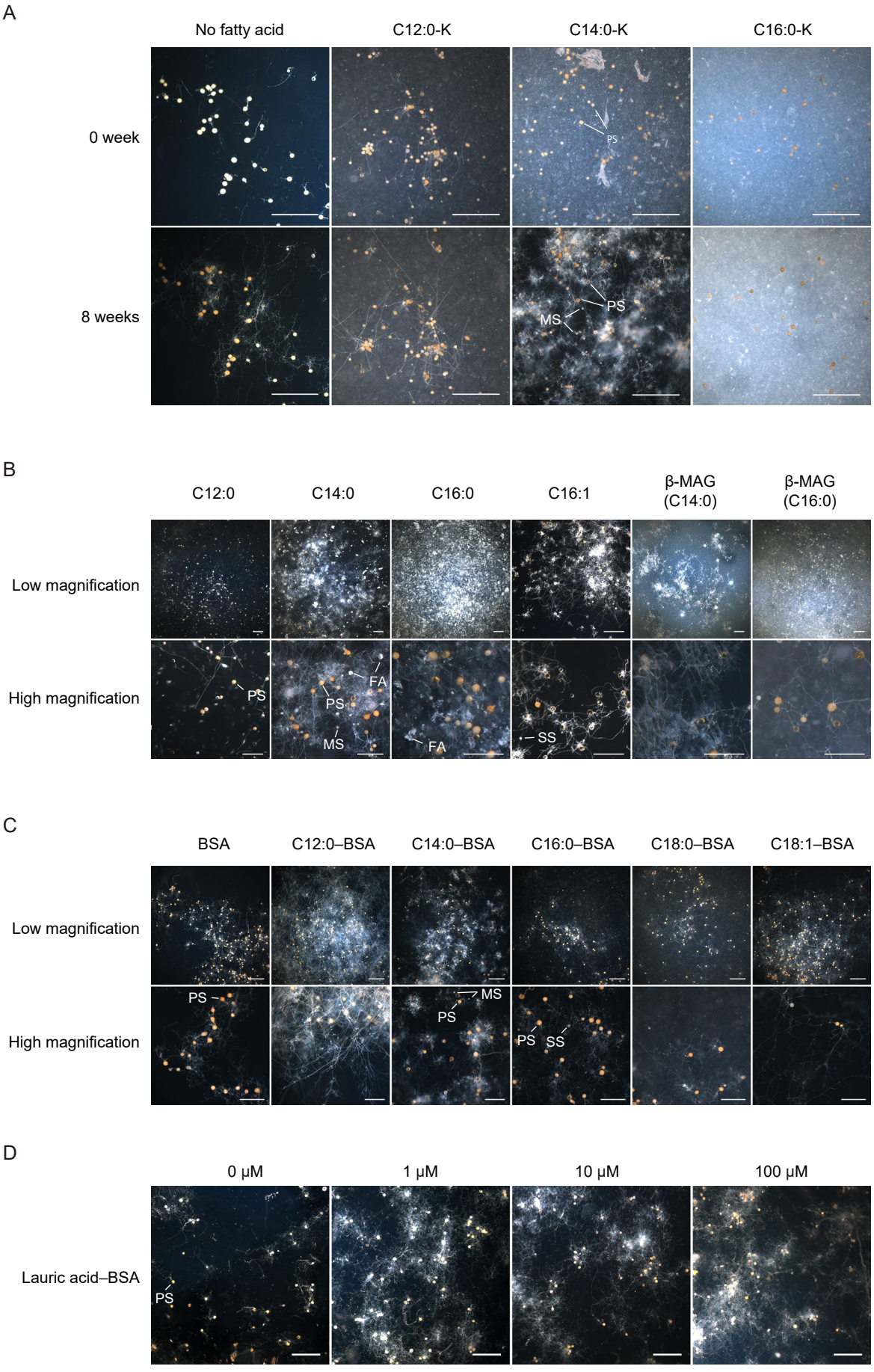

Fig. S1. *R. irregularis* cultured in the solid media supplemented with fatty acids. Fungal growth in modified SC medium with (*A*) 1 mM potassium salts of fatty acids, (*B*) 1 mM fatty acids or β-MAG, and (*C*) 0.5 mM fatty acid–BSA conjugates at 8 weeks after inoculation. (*D*) Effect of different concentrations of lauric acid–BSA conjugates on fungal growth at 8 weeks after inoculation. FA, fatty acid precipitates; MS, myristate-induced spore; PS, parent spore (inoculated) spore; and SS, secondary spore. Scale bars: 1 mm (*A*, upper panels in *B*, *C*, and *D*) and 0.5 mm (lower panels in *B* and *C*).

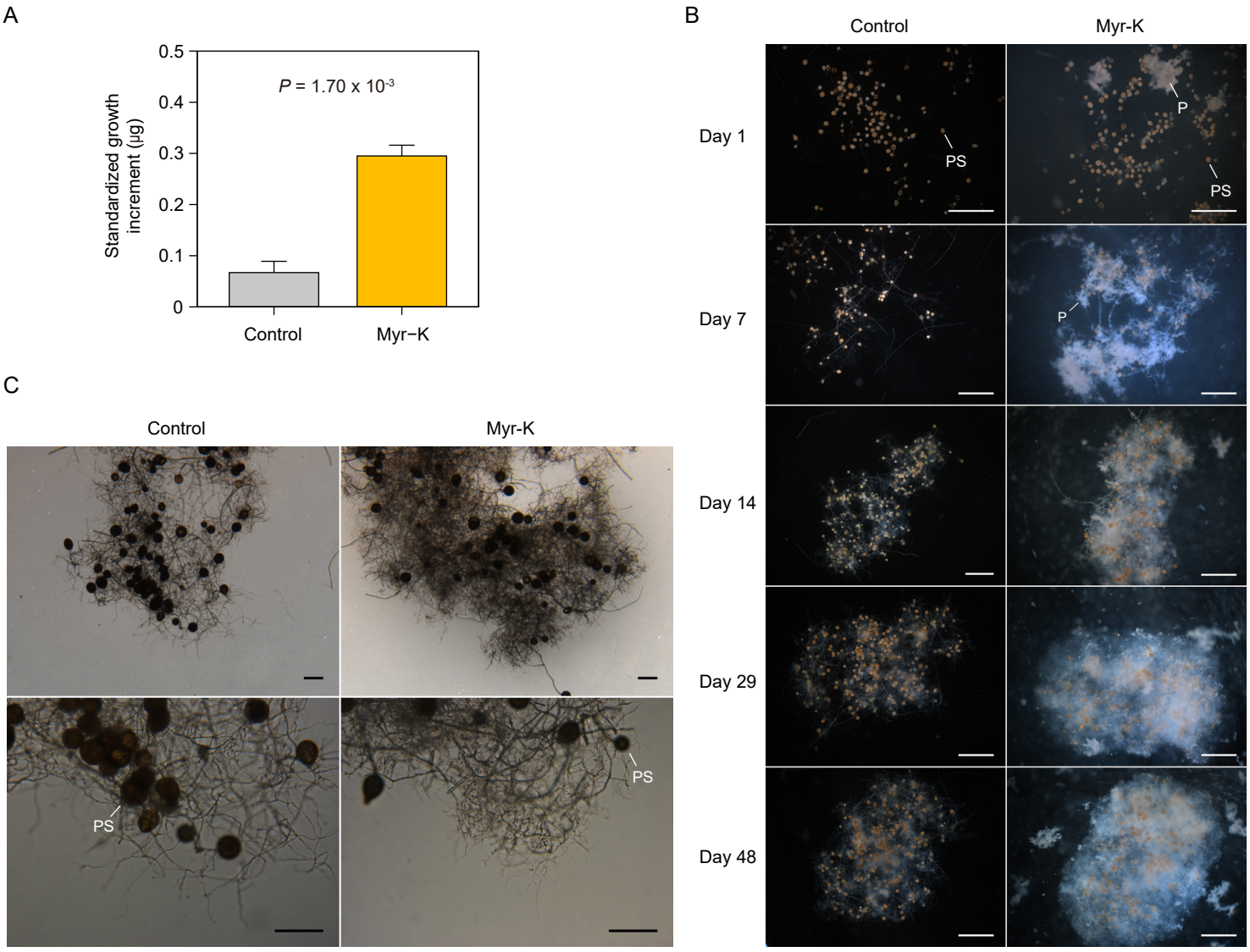

Fig. S2. Liquid culture of *R. irregularis*. (*A*) Biomass production in the modified SC liquid medium supplemented with or without 0.5 mM potassium myristate at 8 weeks after inoculation. Mean + s.e. (n = 3). The P value is based on a t-test. (*B*) Time course of fungal growth in the liquid medium without fatty acids or with potassium myristate. When potassium myristate was added to the medium, precipitates of the metal soaps were apparent, which attached to the hyphal surface. (*C*) Fungal growth in the liquid medium at 8 weeks after inoculation. To remove the metal soaps attached to the hyphal surface, fungal hyphae were washed with citrate buffer including 1% Triton X-100. Images were captured using an inverted microscope. Lower panels are magnified images of fungal hyphae. See *SI Appendix* (Table S2) for sample details. P, precipitate of metal soaps; and PS, parent spore (inoculated spore). Scale bars: 1,000 μm (*B*) and 200 μm (*C*).

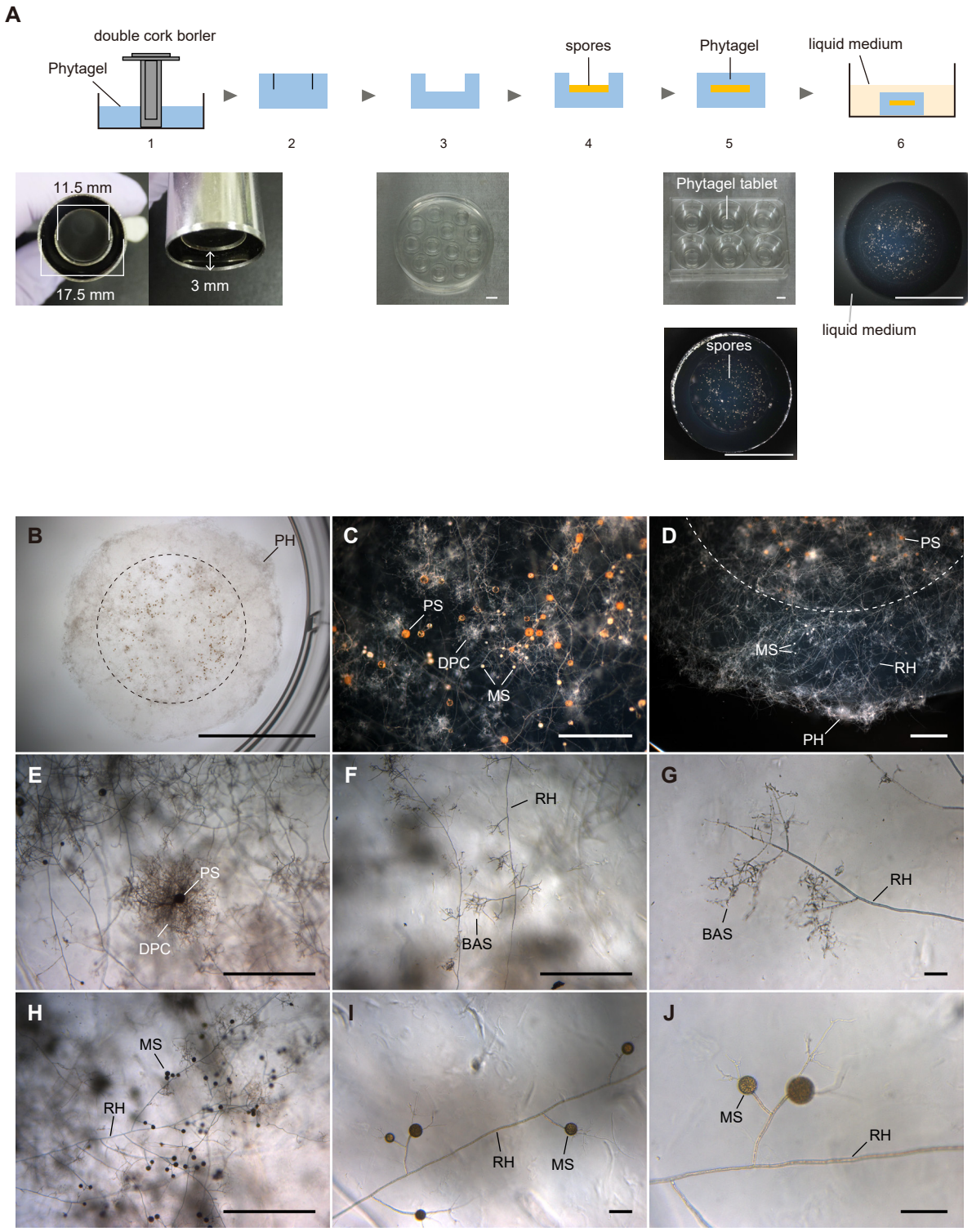

Fig. S3. Immobilized cell culture of *R. irregularis*. (*A*) Schematic presentation of the immobilized cell culture system. Step 1 and 2: Phytagel tablets with a circular incision were cut out using a sterile double cork borer. Step 3: The gel within the circular incision on the top side of the gel tablet was removed for spore inoculation. Step 4 and 5: Spores were placed in the hole and covered with Phytagel. Step 6: The phytagel tablets containing spores were transferred into a six-well culture plate. Each well was filled with the modified SC liquid medium with fatty acids. (*B*) Fungal growth by immobilized cell culture containing half-strength modified SC medium supplemented with 0.5 mM potassium myristate at 8 weeks after inoculation. A dotted circle shows the area in which fungal spores are inoculated. Magnified images of (*C*) the inoculated region and (*D*) peripheral area of a Phytagel tablet. (*E*) DPC-like structures formed around the inoculated spore. (*F*) BAS formed along the runner hyphae. (*G*) Magnified image of BAS. (*H–J*) Myristate-induced secondary spores formed along the runner hyphae. (*I* and *J*) Magnified images of myristate-induced spores. See *SI Appendix* (Table S2) for sample details. BAS, branched absorbing structure; DPC, densely packed coil; MS, myristate-induced spore; PH, hypha protruding outside a phytagel tablet; PS, parent spore (inoculated spore); and RH, runner hypha. Scale bars: 10 mm (*A* and *B*), 1 mm (*C–F* and *H*), and 0.1 mm (*G*, *I*, and *J*).

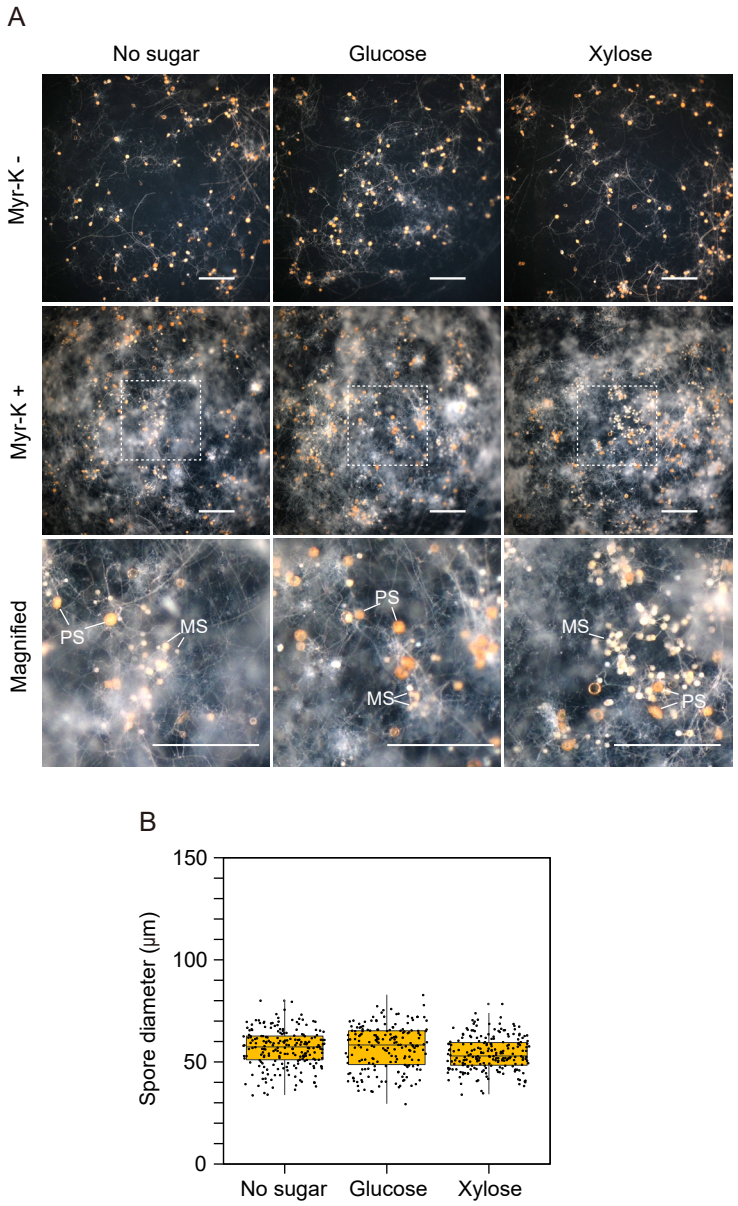

Fig. S4. Effects of sugars in combination with myristate on the growth and sporulation of *R. irregularis*. (*A*) Fungal growth in the immobilized cell culture containing half-strength modified SC medium supplemented with combinations of 0.5 mM potassium myristate and 5 mM sugars at two months after inoculation. Images were taken by a dissecting microscope. Lower panels show the magnified images of dotted areas in the middle panels. Yellow-orange spores are parent spores (PS) and small spores in white to light-yellow are myristate-induced spores (MS). Scale bars: 1 mm. (*B*) Size of the myristate-induced spores in immobilized cell culture system. Boxes show the first, median, and third quartiles; the whiskers extend to 1.5× interquartile range, and data points for each treatment are displayed.

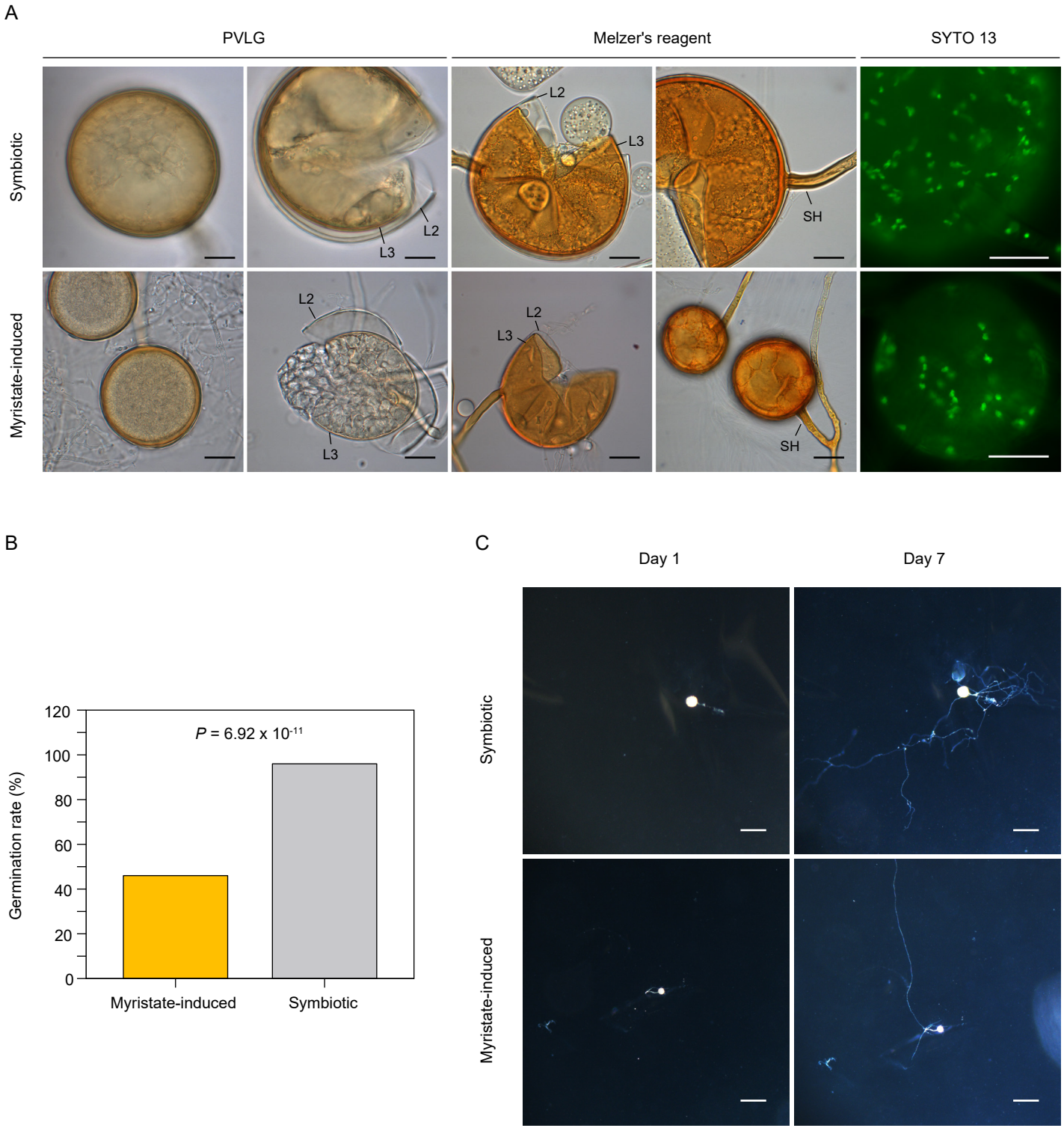

Fig. S5. Spore morphology and germination. (*A*) Myristate-induced and symbiotically generated spores of *R. irregularis*. Intact or crushed spores were mounted in PVLG or Melzer's reagent. Both spores appear yellow-brown in color and have apparent L2 and L3 spore walls. Only L3 was stained with Melzer's reagent. Spore size and thickness of L2 and L3 in the myristate-induced spores are less than those in the symbiotically generated spores. Subtending hyphae of both spores exhibit a cylindrical shape. Nuclei in the spores were stained with SYTO 13 green fluorescent nucleic acid stain and observed by epifluorescence microscopy. Many nuclei were observed in both spores. SH, subtending hypha. (*B*) Germination rate of the myristate-induced and symbiotically generated spores. In total, 50 myristate-induced spores and 77 symbiotic spores were tested on 0.3% Phytagel for 7 days. Myristate-induced spores were generated using an immobilized cell culture with a half-strength SC medium and 0.5 mM potassium myristate at 3 months after inoculation. The *P* value is based on Fisher's exact test. (*C*) Images of the germinating spores. Scale bars: 20 μm (*A*) and 200 μm (*C*).

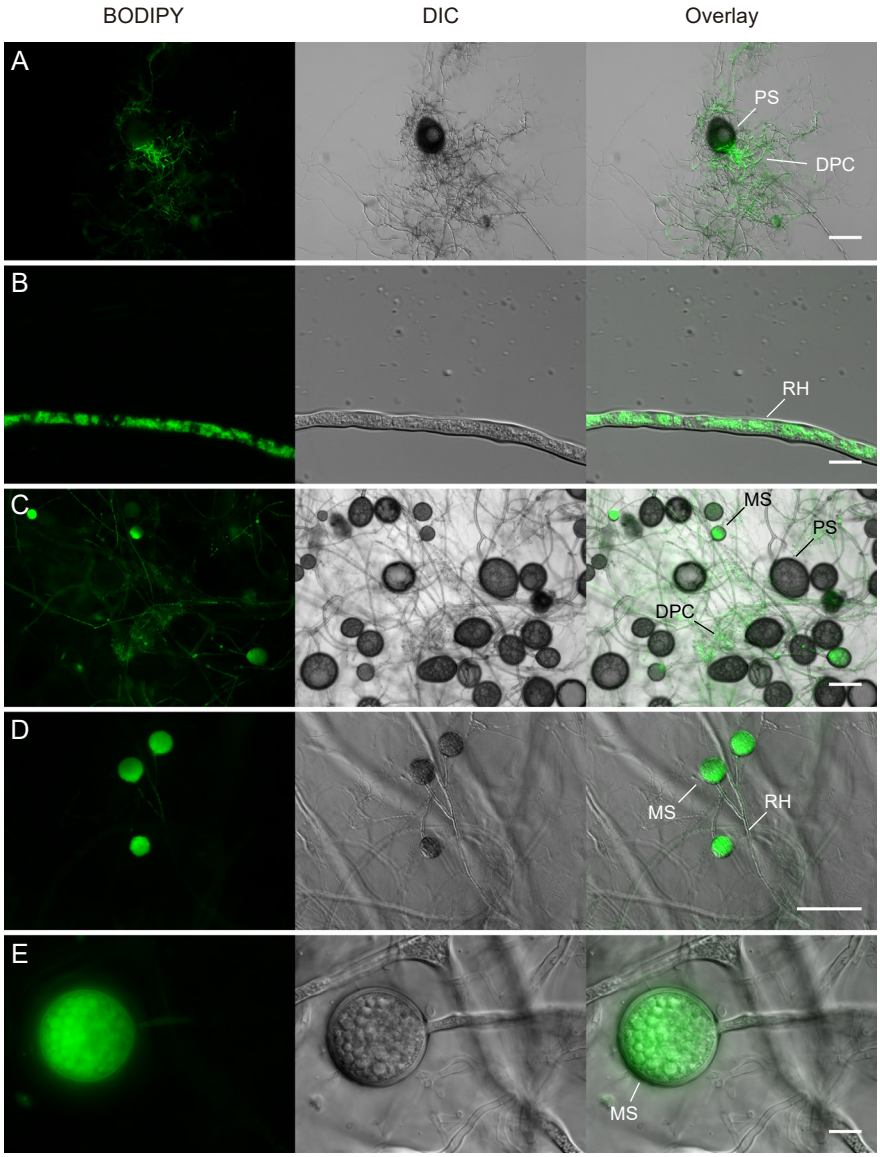

Fig. S6. Uptake of the fluorescently labelled fatty acid derivative C_1-BODIPY 500/510 C_{12} by *R. irregularis*. Fluorescent images of C_1-BODIPY 500/510 C_{12} and superimposed bright field images of *R. irregularis* cultivated using an immobilized cell culture system with the modified SC medium containing 0.5 mM of the fluorescent probe for 1 day (*A*, *C*, and *D*) or 5 days (*B* and *E*). (*A*) Parent spore and DPCs. (*B*) Runner hypha. (*C*) Parent spores and myristate-induced secondary spores. (*D*) Secondary spores formed along runner hypha. (*E*) Secondary spore. DPC, densely packed coil; MS, myristate-induced spore; PS: parent spore (inoculated spore); and RH, runner hypha. Scale bars: 100 μm (*A*, *C*, and *D*) and 10 μm (*B* and *E*).

Table S1. Composition of the modified SC medium without fatty acids.

Chemicals	mg L^{-1}	Chemicals	mg L^{-1}
$(NH_4)_2SO_4$	660	Alanine	38
KH_2PO_4	1000	Arginine, HCl	38
$MgSO_4$	500	Asparagine	38
NaCl	100	Aspartic acid	38
$CaCl_2$	100	Cysteine	38
Boric acid	0.5	Glutamine	38
$CuSO_4$	0.04	Glutamic acid	38
KI	0.1	Glycine	38
$FeCl_3$	0.2	Histidine	38
$MnSO_4$	0.4	Isoleucine	38
Na_2MoO_5	0.2	Leucine	190
$ZnSO_4$	0.4	Lysine	38
Glycerol	92	Methionine	38
Biotin	0.02	Phenylalanine	38
Ca pantothenate	0.4	Proline	38
Folic acid	0.002	Serine	38
Inositol	40	Threonine	38
Niacin	5.4	Tryptophan	38
Para-aminobenzoic acid	4.2	Tyrosine	38
Pyridoxine, HCl	0.4	Valine	38
Pyridoxial phosphate	5	Phytagel	3000
Riboflavin	0.2		
Thiamine, HCl	5.4		
Adenine sulfate	9		
Uracil	38		

Table S2. Samples used in the present study.

[a]Immobilized cell culture, [b]*in vitro* culture using carrot hairy roots, [c]weeks post-inoculation, [d]C, confocal laser scanning microscope; D, dissecting microscope; E, epifluorescence microscope; F, fully focused composite image; I, inverted microscope; and L, light microscope.

Figure	Culture	Medium	Fatty acid	Sugar	Wpi[c]	Replication	Microscopy[d]	Scale (μm)
Fig. 1A	Solid	SC	None	None	8	4	-	-
	Solid	SC	(0.05% BSA)	None	8	3	-	-
	Solid	SC	1 mM pottasium laurate	None	8	4	-	-
	Solid	SC	1 mM lauric acid	None	8	4	-	-
	Solid	SC	0.5 mM lauric acid-BSA	None	8	4	-	-
	Solid	SC	1 mM pottasium myristate (Myr-K)	None	8	4	-	-
	Solid	SC	1 mM myristic acid	None	8	4	-	-
	Solid	SC	0.5 mM myristic acid-BSA	None	8	4	-	-
	Solid	SC	1 mM pottasium palmitate	None	8	4	-	-
	Solid	SC	1 mM palmitic acid	None	8	3	-	-
	Solid	SC	0.5 mM palmitic acid-BSA	None	8	4	-	-
	Solid	SC	1 mM palmitoleic acid	None	8	3	-	-
	Solid	SC	0.5 mM stearic acid-BSA	None	8	4	-	-
	Solid	SC	0.5 mM oleic acid-BSA	None	8	4	-	-
	Solid	SC	1 mM C14:0 *sn* -2 monoacylglycerol	None	8	4	-	-
	Solid	SC	1 mM C16:0 *sn* -2 monoacylglycerol	None	8	4	-	-
Fig. 1B	Solid	SC	None	None	2, 4, 6, 8	6	-	-
	Solid	SC	0.2 mM Myr-K	None	2, 4, 6, 8	5-6	-	-
	Solid	SC	0.5 mM Myr-K	None	2, 4, 6, 8	5-6	-	-
	Solid	SC	1.0 mM Myr-K	None	2, 4, 6, 8	5-6	-	-
Fig. 1C	ICC[a]	0.5× SC	None	None	8	6	-	-
	ICC	0.5× SC	0.5 mM Myr-K	None	8	6	-	-
	ICC	0.5× SC	None	5 mM glucose	8	6	-	-
	ICC	0.5× SC	0.5 mM Myr-K	5 mM glucose	8	6	-	-
	ICC	0.5× SC	None	5 mM xylose	8	6	-	-
	ICC	0.5× SC	0.5 mM Myr-K	5 mM xylose	8	6	-	-
Fig. 1D	Solid	SC	None	None	2	-	I	200
Fig. 1E	Solid	SC	None	None	8	-	D	200
Fig. 1F	Solid	SC	1 mM Myr-K	None	8	-	D	200
Fig. 1G	Solid	SC	1 mM Myr-K	None	2	-	I	200
Fig. 1H	Solid	SC	1 mM Myr-K	None	8	-	D	1,000
Fig. 1I	Solid	SC	1 mM Myr-K	None	5	-	I, F	200
Fig. 1J	Solid	SC	1 mM Myr-K	None	5	-	I, F	200
Fig. 1K	Solid	SC	1 mM Myr-K	None	5	-	I, F	200
Fig. 1L	Solid	SC	1 mM Myr-K	None	5	-	I, F	200
Fig. 1M	Solid	SC	1 mM Myr-K	None	5	-	I, F	200
Fig. 1N	Solid	SC	1 mM Myr-K	None	8	-	D	200
Fig. 1O	Solid	SC	1 mM Myr-K	None	5	-	I	200
Fig. 1P	Solid	SC	1 mM Myr-K	None	5	-	I	200
Fig. 1Q	ICC	0.5× SC	None	None	8	-	D	1,000
Fig. 1R	ICC	0.5× SC	0.5 mM Myr-K	None	8	-	D	1,000
Fig. 2A	Solid	SC	None	None	2, 4, 6, 8	3	-	-
	Solid	SC	0.2 mM Myr-K	None	2, 4, 6, 8	5-6	-	-
	Solid	SC	0.5 mM Myr-K	None	2, 4, 6, 8	5-6	-	-
Fig. 2B	Solid	SC	None	None	8	4	-	-
	Solid	SC	0.2 mM Myr-K	None	8	205	-	-
	Solid	SC	0.5 mM Myr-K	None	8	268	-	-
	Liquid	SC	None	None	0	206	-	-
Fig. 2C	ICC	0.5× SC	0.5 mM Myr-K	None	8	6	-	-
	ICC	0.5× SC	0.5 mM Myr-K	5 mM glucose	8	6	-	-
	ICC	0.5× SC	0.5 mM Myr-K	5 mM xylose	8	6	-	-
Fig. 2D	Monogenic	M	None	1% sucrose	1	-	D	500
Fig. 2E	Monogenic	M	None	1% sucrose	5	-	D	500
Fig. 2F	Monogenic	M	None	1% sucrose	14	-	D	500
Fig. 2G	Monogenic	M	None	1% sucrose	11	-	L	500
Fig. 3A	ICC	SC	0.5 mM Myr-K (6 weeks) -> 0.5 mM C_1-BODIPY 500/510 C_{12} (10 min)	None	3	-	C	200
Fig. 3B	Solid	SC	1 mM $^{13}C_1$-myristic acid	None	10	1	-	-
Fig. 3C	Solid	SC	1 mM myristic acid	None	8	5	-	-
	Solid	SC	1 mM $^{13}C_1$-myristic acid	None	8	5	-	-
Fig. 3D	Liquid	Water	None (5 days) -> none (12 h)	None	5 days	4	-	-
	Liquid	Water	None (5 days) -> 0.5 mMMyr-K (12 h)	None	5 days	4	-	-
	Liquid	Water	None (5 days) -> 50 μM CCCP (12 h)	None	5 days	4	-	-
	Liquid	Water	None (5 days) -> 50 μM CCCP + 0.5 mM Myr-K (12 h)	None	5 days	4	-	-
Fig. 3E	ICC	SC	0.5 mM Myr-K (3 weeks) -> none (11 days) -> none (3 h)	None	4	5-6	-	-
	ICC	SC	0.5 mM Myr-K (3 weeks) -> none (11 days) -> 0.5 mM Myr-K (3 h)	None	4	5-6	-	-

Table S2. Continued.

Figure	Culture	Medium	Fatty acid	Sugar	Wpi[c]	Replication	Microscopy[d]	Scale (μm)
Fig. S1A	Solid	SC	None	None	0, 8	-	D	1,000
	Solid	SC	1 mM pottasium laurate	None	0, 8	-	D	1,000
	Solid	SC	1 mM Myr-K	None	0, 8	-	D	1,000
	Solid	SC	1 mM pottasium palmitate	None	0, 8	-	D	1,000
Fig. S1B	Solid	SC	1 mM lauric acid	None	8	-	D	500, 1,000
	Solid	SC	1 mM myristic acid	None	8	-	D	500, 1,000
	Solid	SC	1 mM palmitic acid	None	8	-	D	500, 1,000
	Solid	SC	1 mM palmitoleic acid	None	8	-	D	500, 1,000
	Solid	SC	1 mM C14:0 *sn* -2 monoacylglycerol	None	8	-	D	500, 1,000
	Solid	SC	1 mM C16:0 *sn* -2 monoacylglycerol	None	8	-	D	500, 1,000
Fig. S1C	Solid	SC	(0.05% BSA)	None	8	-	D	500, 1,000
	Solid	SC	0.5 mM lauric acid-BSA	None	8	-	D	500, 1,000
	Solid	SC	0.5 mM myristic acid-BSA	None	8	-	D	500, 1,000
	Solid	SC	0.5 mM palmitic acid-BSA	None	8	-	D	500, 1,000
	Solid	SC	0.5 mM stearic acid-BSA	None	8	-	D	500, 1,000
	Solid	SC	0.5 mM oleic acid-BSA	None	8	-	D	500, 1,000
Fig. S1D	Solid	SC	(0.05% BSA)	None	8	-	D	1,000
	Solid	SC	0.001 mM lauric acid-BSA	None	8	-	D	1,000
	Solid	SC	0.01 mM lauric acid-BSA	None	8	-	D	1,000
	Solid	SC	0.1 mM lauric acid-BSA	None	8	-	D	1,000
Fig. S2A	Liquid	SC	None	None	8	3	-	-
	Liquid	SC	0.5 mM Myr-K	None	8	3	-	-
Fig. S2B	Liquid	SC	None	None	0, 1, 2, 4, 8	-	D	1,000
	Liquid	SC	0.5 mM Myr-K	None	0, 1, 2, 4, 8	-	D	1,000
Fig. S2C	Liquid	SC	None	None	8	-	I	200
	Liquid	SC	0.5 mM Myr-K	None	8	-	I	200
Fig. S3A	ICC	0.5× SC	0.5 mM Myr-K	None	0	-	D	10,000
Fig. S3B	ICC	0.5× SC	0.5 mM Myr-K	None	8	-	D	10,000
Fig. S3C	ICC	0.5× SC	0.5 mM Myr-K	None	8	-	D	1,000
Fig. S3D	ICC	0.5× SC	0.5 mM Myr-K	None	8	-	D	1,000
Fig. S3E	ICC	0.5× SC	0.5 mM Myr-K	None	8	-	I	1,000
Fig. S3F	ICC	0.5× SC	0.5 mM Myr-K	None	8	-	I	1,000
Fig. S3G	ICC	0.5× SC	0.5 mM Myr-K	None	8	-	I	100
Fig. S3H	ICC	0.5× SC	0.5 mM Myr-K	None	8	-	I	1,000
Fig. S3I	ICC	0.5× SC	0.5 mM Myr-K	None	8	-	I	100
Fig. S3J	ICC	0.5× SC	0.5 mM Myr-K	None	8	-	I	100
Fig. S4A	ICC	0.5× SC	None	None	8	-	D	1,000
	ICC	0.5× SC	0.5 mM Myr-K	None	8	-	D	1,000
	ICC	0.5× SC	None	5 mM glucose	8	-	D	1,000
	ICC	0.5× SC	0.5 mM Myr-K	5 mM glucose	8	-	D	1,000
	ICC	0.5× SC	None	5 mM xylose	8	-	D	1,000
	ICC	0.5× SC	0.5 mM Myr-K	5 mM xylose	8	-	D	1,000
Fig. S4B	ICC	0.5× SC	0.5 mM Myr-K	None	8	211	-	-
	ICC	0.5× SC	0.5 mM Myr-K	5 mM glucose	8	218	-	-
	ICC	0.5× SC	0.5 mM Myr-K	5 mM xylose	8	188	-	-
Fig. S5A	ICC	0.5x SC	0.5 mM Myr-K	None	12	-	L, E	20
Fig. S5B	ICC	0.5x SC	0.5 mM Myr-K (12 weeks) -> Phytagel plate (1 week)	None	12	50, 77	-	-
Fig. S5C	ICC	0.5x SC	0.5 mM Myr-K (12 weeks) -> Phytagel plate (0 or 1 week)	None	12	-	D	200
Fig. S6A	ICC	SC	0.5 mM Myr-K (6 weeks) -> 0.5 mM C_1-BODIPY 500/510 C_{12} (1 days)	None	6	-	E	100
Fig. S6B	ICC	SC	0.5 mM Myr-K (6 weeks) -> 0.5 mM C_1-BODIPY 500/510 C_{12} (5 days)	None	6	-	E	10
Fig. S6C	ICC	SC	0.5 mM Myr-K (6 weeks) -> 0.5 mM C_1-BODIPY 500/510 C_{12} (1 days)	None	6	-	E	100
Fig. S6D	ICC	SC	0.5 mM Myr-K (6 weeks) -> 0.5 mM C_1-BODIPY 500/510 C_{12} (1 days)	None	6	-	E	100
Fig. S6E	ICC	SC	0.5 mM Myr-K (6 weeks) -> 0.5 mM C_1-BODIPY 500/510 C_{12} (5 days)	None	6	-	E	10
Movie S1	ICC	SC	0.5 mM Myr-K (6 weeks) -> 0.5 mM C_1-BODIPY 500/510 C_{12} (10 min)	None	3	-	C	25

Table S3. Infection capability of myristate-induced spores. Carrot hairy roots were inoculated with a single myristate-induced spore and grown for two months. Six independent experiments were performed.

Trial	Number of inoculated spores	Germination rate (%)	Rate of spores which colonize to roots and produce next generation spores (%)	
			per inoculated spore	per germinated spore
Trial 1	20	30	15	50
Trial 2	40	33	18	54
Trial 3	29	7	3	50
Trial 4	40	20	13	63
Trial 5	60	12	2	14
Trial 6	56	5	4	67

Table S4. Primers used in this study.

Gene	RIR ID[a]	Annotation	Sequence	Reference
FAD1	RIR_0930900	Acyl-CoA dehydrogenase	TGGTATTCATTCGTGCCATGA GCAGCGCGTGCAAGTG	This study
FAL3	RIR_0764900	Acyl-CoA ligase	TCAAAAAGATGCCAAAGTGACAAT AACCTTGTGTTACGAGCATCCA	This study
FAL4	RIR_2539500	Acyl-CoA ligase	ATTGTAGAGGGTTATGGGCAGACT TTTCGCCTCGAAGACCAACT	This study
ICL1	RIR_1924000	Isocitrate lyase	CCGCGCCATAGCGTATG TAGGTTTTGCAGTCTCCATCCA	This study
PCK2	RIR_2338300	Phosphoenolpyruvate carboxykinase	AAAAACTTGGAAAGGTCCTCAAGA AGAAATCTCGCAGCTAACGAATCT	This study
FBP1	RIR_2783400	Fructose-1,6-bisphosphate phosphatase	CGCGTATCCCGCTGATAAAA GGAAAGCCATAGGAAAACATTCA	This study
CIT1	RIR_0822400	Citrate synthase	CTACATGGCCTTGCTAATCAAGAA CACCAATCGCGTCTCTCATCT	This study
ACH1	RIR_2973500	Aconitate hydratase	CTTCCCGGGTGGTCTTATGA ACCCAAACCACCAGCGTTAG	This study
ICD1	RIR_1281200	Isocitrate dehydrogenase	AGCGGATCGGCCAGAAC CAAGGGTAAAATCGGTTGTTGTAGA	This study
KGD2	RIR_2870300	2-Ketoglutarate dehydrogenase	TTGTACGGTGATCGCGAAGA GGCAATACGTAGACGCATCCTAT	This study
MDH1	RIR_0715300	Malate dehydrogenese	GAAGTTGTTAAAGCCAAGGATGGT ACCAGCCTGTGCCATTGAA	This study
NMT1	RIR_3261900	*N* -myristoyltransferase	CGATGCGATGTTTCGCTTT GGTGGTTGAAGAGCCCATTTT	This study
EF-1β	RIR_3067400	Translation elongation factor 1 subunit beta	CCCATGCAGCTCGATGGTA TGCCAGGAAGTGAAGAAAATGA	Kobae et al., 2015[b]

[a]Maeda et al., Evidence of non-tandemly repeated rDNAs and their intragenomic heterogeneity in *Rhizophagus irregularis. Commun. Biol.* **1**, 87 (2018).

[b]Kobae et al., Up-regulation of genes involved in *N* -acetylglucosamine uptake and metabolism suggests a recycling mode of chitin in intraradical mycelium of arbuscular mycorrhizal fungi. *Mycorrhiza* **25**, 411-417 (2015).