Supporting information for

**Non-canonical function of a small-molecular virulence factor coronatine against plant immunity: An In vivo Raman imaging approach**

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2 12 13 18 24 36
Figure S1. Venn diagram showing the overlap genes detected microarray analysis

(A) The numbers of upregulated gene expressions (more than twofold) by the treatment with 1, ent1 or 3-ent4, compared to mock treatment are presented (1, ent1 or 3-ent4 respectively). (B) The numbers of downregulated gene expressions (more than twofold) with 1, ent1 or 3-ent4, compared to mock treatment are presented (1, ent1 or 3-ent4 respectively).
Figure S2. Dicyne Raman signal of 5 and ent5.

Averaged Raman spectra of 5 and ent5 as crystal forms. The light intensity at the sample plane was calculated as 6.2 mW/µm² from the ratio of the measured laser power between the sample position and the area of the illumination line. The exposure time for each line was 10 s.
Figure S3. Diyne Raman signal of 10 mM of 5 in DMSO (a) and Standard curve for estimation of concentration (b).

The light intensity at the sample plane was calculated as 6.0 mW/µm² from the ratio of the measured laser power between the sample position and the area of the illumination line. The exposure time for each line was 120 s. (a) The averaged Raman spectrum obtained from 1×9 pixel region of the DMSO solution of diyne 5. Sample concentration was 10 mM. (b) Standard curve for estimation of concentration. The plotted intensity was based on the peak height calculated by subtracting the background at 2220 cm⁻¹ from the peak intensity at 2262 cm⁻¹ and averaged from a 9×1 pixel region of the DMSO solution of diyne 5. Bars represent mean and SE (n = 4).
Figure S4. Effects of 1, 5, and ent5 on stomatal reopening using closed stomata of coi1-1 (a) and anac (b).

IAA was used as a positive control. Dashed line indicates the mean stomatal aperture in the control experiment without test compound in which Arabidopsis leaf peels with closed stomata were incubated in MES buffer (pH 6.2) containing 2% EtOH. Bars represent the mean stomatal aperture with SE (n = 20 stomata). Different letters indicate significant differences between means (ANOVA: *P* < 0.05).
Figure S5. Low background autofluorescence from chloroplast was observed for *arc-6* guard cell.

DIC images (left), fluorescence images of chloroplast (>510 nm with 460-495 nm excitation light, center), and background Raman images (average of silent region: 1985-2315 cm\(^{-1}\), right) of *A. thaliana* wild-type (Col-0) and *arc6-1*. 
Figure S6. Effects of 1, 5, and ent5 on stomatal reopening using closed stomata of Ws (a) and arc6-1 (b) under dark.

IAA was used as a positive control. Dashed line indicates the mean stomatal aperture in the control experiment without test compound. Bars represent the mean stomatal aperture with SE (n = 20 stomata). Different letters indicate significant differences between means (ANOVA: P < 0.05).
Figure S7. Raman spectra in each area of arc6 guard cell indicating subcellular localization of 5.
Raman spectra in living guard cells of arc6-1 were obtained after 3-hour treatment by 100 µM 5 (left)/ent5 (right). Averaged Raman spectra were presented for each subcellular area (1.2 µm×1.1 µm: 3 × 3 = 9 pixels), nuclear region in red, perinuclear region in orange, vacuole in cyan or blue, plasma membrane (dorsal) in yellow or green and plasma membrane (ventral) in purple of the guard cell. The light intensity at the sample plane was calculated as 6.2 mW/µm² from the ratio of the measured laser power between the sample position and the area of the illumination line. The exposure time for each line was 120 s. Spectra were vertically offset for ease viewing.
Figure S8. Statistical analysis of five arc6-1 guard cells treated with 5

Average Raman spectra of each organelle region (1.2 µm × 1.1 µm: 3 × 3 = 9 pixels) of five arc6-1 guard cells treated with 100 µM of 5. The light intensity at the sample plane was calculated as 6.0-6.2 mW/µm² from the ratio of the measured laser power between the sample position and the area of the illumination line. The exposure time for each line was 120 or 150 s. Spectra were vertically offset for ease viewing.
Figure S9. Fluorescent images of nucleus and ER in living guard cells of Col-0 and arc6-1.
Fluorescence images and DIC images of arc6-1 (a) and Col-0 (b). ER was stained by ER tracker, and nucleus was stained by HoeFLAc2. Excitation wavelength was fixed at 488 nm, and images detected at 490-555 nm (left, green), at 610-735 nm (second left, red), DIC images (second right), merged images (right) were shown.
Figure S10. Competitive inhibition of 5 with 1 in Raman Imaging.

(a) Raman imaging in living guard cells of A. thaliana arc6-1 after three hours of co-treatment with 1 (100 µM) and 5 (100 µM). Diyne Raman signal of 5 was not detected. The light intensity at the sample plane was calculated as 6.0 mW/µm$^2$ from the ratio of the measured laser power between the sample position and the illumination line. Exposure time for each line was 120 s. Spectra were vertically offset for ease viewing. (b) Raman spectra of each organelle region (1.2 µm x 1.1 µm: 3 x 3 = 9 pixels) of (a).
**Scheme S1.** Synthesis of 5

L-Propargylglycine

1) Boc₂O, K₂CO₃, THF, H₂O
2) TMS-CH₂N₂, CHCl₃, MeOH
99% in 2 steps

1-iodo-1-butyne, Cul
piperidine, 70%

1) TFA, CH₂Cl₂
2) CFA (3), COMU, TEA, DMF
65% in 2 steps
3) HPLC purification
Table S1. Summary of the microarray analysis.

The ratios of inducible or suppressed genes treated with 1, *ent1* or 3-*ent4*, compared to mock treatment, are presented (1/mock, *ent1/mock, 3-*ent4*/mock).

### Inducible genes both in 1/mock and 3-*ent4*/mock

| Accession No. | UniGeneID | GeneSymbol | 1/mock | *ent1/mock | 3-*ent4*/mock |
|---------------|-----------|------------|--------|------------|---------------|
| NM_114356     | At.36095  | AT3G44870  | 205.8  | 1.4        | 11.3          |
| NM_114355     | At.10101  | FAMT       | 135.2  | 1.4        | 8.9           |
| NM_102753     | At.40613  | JAZ8       | 100.3  | -1.2       | 11.6          |
| NM_001124015  | At.74131  | AT1G53903  | 81.9   | 1.5        | 14.2          |
| NM_121325     | At.26324  | JAZ10      | 81.5   | 1.2        | 6.4           |
| NM_203046     | At.26324  | JAZ10      | 72.8   | -1.1       | 6.1           |
| NM_106314     | At.51311  | AT1G76640  | 68.2   | 1.6        | 8.9           |
| NM_001085035  | At.28382  | MAPKK21    | 62.7   | -2.6       | 4.5           |
| NM_118304     | At.32596  | MSRB8      | 59.2   | -1.4       | 3.6           |
| NM_123658     | At.55327  | AT5G42930  | 35.2   | 1.6        | 3.4           |
| NM_106579     | At.34141  | MC7        | 33.5   | 1.9        | 2.6           |
| NM_125740     | At.28998  | CYP94B1    | 33.0   | 1.1        | 3.9           |
| NM_129014     | At.37783  | JAZ7       | 27.8   | 1.2        | 9.0           |
| NM_111830     | At.49602  | AT3G09950  | 24.8   | 1.1        | 2.1           |
| NM_124611     | At.29623  | CYP96A4    | 20.8   | 1.3        | 4.4           |
| NM_102616     | At.11829  | GRX480     | 20.4   | 1.0        | 9.7           |
| NM_119606     | At.48936  | RRTF1      | 20.3   | -1.6       | 4.0           |
| NM_101599     | At.27828  | JAZ5       | 20.2   | -1.2       | 3.3           |
| NM_128328     | At.13273  | CYP94C1    | 17.7   | 1.4        | 9.2           |
| NM_127138     | At.13860  | AT2G15760  | 15.4   | 1.7        | 2.6           |
| NM_118303     | At.25145  | MSRB7      | 15.2   | 1.2        | 2.5           |
| NM_001084589  | At.75339  | AT2G44578  | 15.2   | 1.4        | 3.1           |
| NM_128498     | At.12687  | GSTU6      | 15.0   | 1.5        | 2.3           |
| NM_124095     | At.29907  | NUDT8      | 14.7   | 1.2        | 8.1           |
| NM_101580     | At.10364  | GSTU26     | 14.6   | 1.3        | 2.0           |
| NM_121755     | At.31541  | RGL3       | 14.2   | 1.8        | 2.7           |
| NM_104167     | At.28621  | NAC019     | 14.1   | -2.0       | 4.9           |
| NM_126108     | At.49431  | MAPKKK19   | 14.1   | 1.2        | 5.9           |
| NM_001125571  | At.48901  | AT4G24350  | 14.0   | 1.4        | 2.5           |
| NM_119946     | At.31223  | AT4G37850  | 13.9   | 1.6        | 2.0           |
| Accession    | Gene ID  | Symbol | Log2 Fold Change | Old Log2 Fold Change | j enrichment (p<0.05) |
|--------------|----------|--------|------------------|----------------------|----------------------|
| BX813389     | At.28621 | NAC019 | 13.9             | -1.5                 | 5.3                  |
| NM_001036150 | At.71985 | AT1G64195 | 13.6             | 1.4                  | 2.3                  |
| NM_121915    | At.65529 | AT5G19100 | 13.1             | 1.2                  | 2.6                  |
| NM_129433    | At.11759 | ANNAT3 | 12.9             | 1.1                  | 2.7                  |
| NM_001085002 | At.44068 | AT4G29930 | 12.9             | 1.1                  | 2.9                  |
| NM_125071    | At.49801 | AT5G56880 | 12.7             | -1.2                 | 2.2                  |
| NM_121272    | At.65507 | AT5G12340 | 12.7             | 1.6                  | 5.5                  |
| NM_101823    | At.19779 | CLH1   | 12.3             | 1.2                  | 2.1                  |
| NM_114888    | At.53871 | AT3G50280 | 12.1             | 1.3                  | 2.5                  |
| NM_112418    | At.20460 | NAC3   | 11.2             | 1.3                  | 7.7                  |
| NM_115220    | At.49399 | AT3G53600 | 10.9             | 1.8                  | 5.1                  |
| NM_120770    | At.20009 | PGIP2  | 10.6             | 1.1                  | 2.7                  |
| NM_129837    | At.36972 | AT2G42760 | 10.0             | 1.6                  | 2.1                  |
| NM_129432    | At.20551 | ANNAT4 | 9.8              | -1.1                 | 2.5                  |
| NM_106153    | At.19896 | JAZ2   | 9.7              | 1.4                  | 3.5                  |
| NM_202133    | At.22658 | JAZ1   | 9.2              | 1.8                  | 5.0                  |
| NM_117855    | At.23185 | ERF-1  | 8.5              | 1.9                  | 4.4                  |
| AK221732     | At.73177 | AT2G43540 | 8.5              | 1.4                  | 3.7                  |
| NM_125136    | At.7483  | XTH25  | 8.5              | -1.7                 | 3.0                  |
| AI995133     | At.22648 | MYC2   | 8.3              | 1.4                  | 3.1                  |
| NM_125255    | At.29268 | AT5G58680 | 7.7              | -1.1                 | 2.9                  |
| NM_001035928 | At.700   | ESL1   | 7.6              | 1.1                  | 2.2                  |
| CD530941     | At.67560 | MSRB7  | 7.6              | 1.2                  | 2.7                  |
| NM_102998    | At.22648 | MYC2   | 7.3              | 1.5                  | 2.9                  |
| NM_120642    | At.8725  | AT5G05600 | 7.2              | 1.2                  | 2.8                  |
| NM_124093    | At.19731 | ERF2   | 7.1              | 1.7                  | 4.9                  |
| NM_115837    | At.54008 | AT3G59750 | 7.0              | 1.6                  | 6.4                  |
| NM_179700    | At.43434 | NAI1   | 6.7              | 1.2                  | 2.6                  |
| NM_101603    | At.20467 | LOX3   | 6.7              | 1.1                  | 2.5                  |
| NM_202143    | At.15241 | OPCL1  | 6.5              | 1.1                  | 2.7                  |
| NM_106329    | At.28236 | AT1G76790 | 6.5              | -1.0                 | 2.3                  |
| NM_101507    | At.11316 | CYP79F1 | 6.3              | 1.3                  | 2.2                  |
| NM_202111    | At.11316 | CYP79F1 | 6.2              | 1.3                  | 2.0                  |
| NM_179009    | At.47204 | RAP2.9 | 6.2              | 1.2                  | 2.6                  |
| NM_001084415 | At.1135  | OPR3   | 6.1              | 1.0                  | 2.3                  |
| NM_128290    | At.13633 | AT2G27310 | 5.7              | 1.4                  | 2.7                  |
| Gene ID       | Accession | Symbol   | Log2 Fold Change | p-value | q-value |
|--------------|-----------|----------|------------------|---------|---------|
| NM_124454    | At.29715  | AT5G50760| 5.6              | 1.9     | 2.2     |
| NM_106732    | At.28188  | WRKY40   | 5.6              | -1.0    | 2.2     |
| NM_105738    | At.23705  | JAZ9     | 5.6              | 1.3     | 2.4     |
| NM_124172    | At.29871  | AT5G47980| 5.5              | 1.6     | 3.1     |
| NM_129313    | At.37407  | AT2G37580| 5.1              | 1.2     | 3.6     |
| NM_105604    | At.24346  | CM3      | 5.0              | 1.4     | 2.1     |
| NM_105657    | At.43696  | AT1G69890| 4.9              | -1.2    | 6.0     |
| NM_106569    | At.10915  | MYB63    | 4.7              | 1.5     | 3.5     |
| NM_119904    | At.22792  | CYP81F4  | 4.3              | 1.1     | 2.5     |
| DQ108691     | At.8519   | PGIP1    | 4.3              | 1.4     | 2.5     |
| NM_129917    | At.48587  | AT2G43550| 4.2              | -1.1    | 2.4     |
| DR381439     | At.68141  | ERF-1    | 4.2              | 1.3     | 2.6     |
| NM_117550    | At.23929  | APS3     | 4.2              | 1.3     | 3.1     |
| NM_128952    | At.53026  | AT2G34010| 4.2              | 1.7     | 2.4     |
| NM_202070    | At.42241  | RBOHB    | 3.9              | 1.2     | 2.5     |
| NM_201808    | At.64947  | AT2G26695| 3.9              | 1.7     | 3.0     |
| NM_113475    | At.26518  | AOC1     | 3.8              | 1.2     | 2.1     |
| NM_113476    | At.6411   | AOC2     | 3.7              | 1.4     | 2.8     |
| NM_121892    | At.54911  | AT5G18870| 3.6              | 1.6     | 2.0     |
| NM_101427    | At.28674  | IAA5     | 3.5              | 1.3     | 2.2     |
| BX814993     | At.15581  | AT1G03440| 3.4              | 1.2     | 2.3     |
| NM_001203302 | At.285    | ASA1     | 3.3              | 1.0     | 2.0     |
| NM_203005    | At.48988  | PAI2     | 3.3              | 1.6     | 2.2     |
| NM_148158    | At.45475  | FLS4     | 3.2              | 1.7     | 3.3     |
| NM_119813    | At.43740  | AT4G36500| 3.1              | 1.5     | 2.3     |
| NM_127881    | At.23258  | GH3.3    | 3.1              | 1.3     | 2.5     |
| BX820064     | At.67889  | AT1G20520| 3.0              | -1.1    | 2.7     |
| NM_102256    | At.10514  | UGT74B1  | 2.9              | 1.4     | 2.3     |
| NM_119047    | At.3405   | AT4G29030| 2.7              | 1.8     | 2.1     |
| NM_105366    | At.35712  | PDR11    | 2.6              | 1.1     | 2.4     |
| NM_106309    | At.19392  | AT1G76590| 2.6              | 1.2     | 2.8     |
| NM_119299    | At.24671  | CYP83B1  | 2.6              | 1.2     | 2.2     |
| NM_113478    | At.37325  | AT3G25790| 2.5              | 2.0     | 2.1     |
| NM_129921    | At.24529  | AT2G43590| 2.4              | 1.1     | 2.2     |
| NM_112507    | At.1206   | AHP4     | 2.3              | 1.3     | 2.2     |
| NM_103224    | At.39607  | MLP165   | 2.3              | 1.3     | 2.0     |
| Accession No. | UniGeneID | GeneSymbol | 1/mock | ent1/mock | 3-ent4/mock |
|--------------|-----------|------------|--------|-----------|-------------|
| NM_128001    | At.39164  | AT2G24400  | -5.4   | -1.2      | -2.6        |
| NM_105562    | At.26571  | bZIP       | -3.7   | -1.2      | -2.6        |
| NM_122241    | At.71067  | AT5G23350  | -3.7   | -1.2      | -2.2        |
| NM_101897    | At.69772  | AT1G20470  | -3.3   | -1.8      | -4.1        |
| NM_119296    | At.65443  | AT4G31470  | -3.2   | 1.3       | -2.1        |
| NM_123196    | At.30414  | AT5G38350  | -3.2   | -1.5      | -2.2        |
| NM_115373    | At.35048  | EXO70H1    | -3.1   | -1.1      | -2.1        |
| NM_105031    | At.50800  | AT1G63530  | -3.0   | -1.8      | -2.4        |
| NM_115310    | At.53931  | AT3G54530  | -2.9   | 1.4       | -2.0        |
| NM_120233    | At.49414  | LECRKA4.2  | -2.7   | 1.3       | -4.0        |
| NM_001123738 | At.49840  | MPK11      | -2.6   | 1.3       | -3.2        |
| NM_148384    | At.25207  | AT4G29905  | -2.6   | -1.4      | -2.1        |
| NM_125015    | At.29394  | EXP14      | -2.6   | -1.1      | -2.4        |
| NM_102442    | At.51777  | AT1G26790  | -2.5   | -1.3      | -14.1       |
| NM_113085    | At.37979  | AT3G21890  | -2.5   | -1.6      | -6.6        |
| NM_123329    | At.19755  | AT5G39670  | -2.5   | -1.7      | -2.8        |
| NM_113102    | At.6152   | AT3G22060  | -2.4   | 1.3       | -2.0        |
| NM_114694    | At.50254  | AT3G48240  | -2.4   | -1.4      | -2.6        |
| NM_120232    | At.28701  | LECRKA4.1  | -2.4   | -1.9      | -2.2        |
| NM_001085091 | At.72152  | IDL3       | -2.4   | -1.6      | -2.9        |
| NM_122316    | At.9177   | WRKY30     | -2.4   | 1.2       | -2.4        |
| NM_106211    | At.34755  | AT1G75590  | -2.4   | 1.2       | -2.2        |
| NM_101756    | At.41768  | AT1G18990  | -2.4   | -1.9      | -2.0        |
| Z35201       | At.71158  | AT1G80000  | -2.3   | -1.8      | -2.4        |
| NM_123433    | At.55282  | AT5G40680  | -2.3   | -1.5      | -2.1        |
| NM_001085043 | At.48962  | AT4G38560  | -2.3   | -1.3      | -2.1        |

Suppressed genes both in 1/mock and 3-ent4/mock

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| Gene ID         | Accession | Description          | Fold Change 1 | Fold Change 2 | Fold Change 3 |
|----------------|-----------|----------------------|---------------|---------------|---------------|
| NM_125360      | At.55626  | AT5G59680             | -2.3          | 1.5           | -2.6          |
| NM_001126029   | At.28918  | AT5G65140             | -2.3          | -2.0          | -2.2          |
| NM_104866      | At.66073  | AT1G61840             | -2.2          | -1.3          | -2.1          |
| NM_103127      | At.51926  | AT1G34050             | -2.2          | -1.4          | -2.7          |
| NM_124803      | At.9168   | MYB49                 | -2.2          | 1.0           | -2.4          |
| NM_001125961   | At.49174  | AT5G54130             | -2.2          | -1.9          | -3.8          |
| NM_001123865   | At.50783  | AT1G23110             | -2.2          | 1.1           | -2.0          |
| NM_179969      | At.50123  | AT2G38823             | -2.2          | 1.1           | -2.0          |
| NM_001125881   | At.69128  | AT5G41761             | -2.2          | -1.9          | -2.0          |
| NM_122435      | At.30922  | AT5G25260             | -2.2          | -1.8          | -2.2          |
| NM_116474      | At.34304  | AT4G02410             | -2.2          | -1.2          | -2.2          |
| NM_111779      | At.53235  | AT3G09450             | -2.1          | -1.4          | -9.4          |
| NM_124134      | At.29885  | AT5G47610             | -2.1          | -1.5          | -2.2          |
| NM_113088      | At.48689  | AT3G21920             | -2.1          | 1.2           | -2.2          |
| NM_111928      | At.27986  | AT3G10910             | -2.1          | 1.2           | -2.8          |
| NM_128058      | At.28320  | WRKY60                | -2.1          | -1.6          | -2.1          |
| NM_117436      | At.33355  | AT4G13620             | -2.0          | 1.1           | -2.1          |
| NM_113777      | At.53543  | AT3G28570             | -2.0          | 1.5           | -2.1          |
| NM_117199      | At.3654   | ACS6                  | -2.0          | -1.2          | -3.0          |
| NM_128762      | At.38127  | AT2G32020             | -2.0          | 1.3           | -2.3          |

**Table S2** Sequences of all primers used for quantitative PCR

- **Allene oxide synthase** *(AOS: AT5G42650)*
  5’ CTCCGTTAATTTCTCGTC 3’
  3’ GCAGCAACAGATTATAAAC 5’

- **Vegetative Storage Protein 2** *(VSP2: AT5G24770)*
  5’ AGATCAATGGGCTGATTTGG 3’
  3’ GTGTATACAAGGGGACAATGC 5’

- **Tubulin-alpha 5** *(TUA: AT5G19780)*
  5’ GGTGAGTATGATGTTGAAGA 3’
  3’ AGAGATTTCAGAGTCG 5’

**Table S3** Sequences of all primers used for Y2H

- **Jasmonate ZIM domain protein 9** *(JAZ9: AT1G70700)*
  5’ CACCATGGAAAGAGATTTCTGG 3’
  3’ TGAGAAGATGAAGATGTATT 5’

- **CoronatineInsensitive 1** *(COI1: AT2G39940)*
  5’ CACCATGGAGATCCTGATTTC 3’
  3’ TCAGGACTTTCCTCGGTTAT 5’
**SI Materials & Methods**

**Microarray analysis**

Total RNA was extracted from roots from 7-day-old seedlings using RNeasy Plant Mini Kit (QIAGEN, Germany). cDNAs were synthesized using 1.0 µg of total RNA and labeled with one color (Cy3) using a Quick Amp labeling kit (Agilent Technologies, USA), followed by fragmentation and hybridization to the *Arabidopsis* Oligo 44K DNA microarray (Ver. 4.0, Agilent Technologies, USA). Following fragmentation, 1.65 µg of cRNA were hybridized to the Agilent expression microarray according to the protocols provided by the manufacturer. All arrays were scanned with a microarray scanner (G2505B, Agilent Technologies, USA) and analyzed using Agilent Feature Extraction v11 (Agilent Technologies, USA). For microarray analysis, raw data were first filtered by a flag signal detected in all samples. Filtered raw data were processed using the Limma Bioconductor package ([http://www.bioconductor.org/](http://www.bioconductor.org/)) in the R statistical environment ([http://www.r-project.org/](http://www.r-project.org/)). After quantile normalization of data, miRNAs with twofold or greater differential expression were identified, with *P*-values of <0.05 being considered statistically significant.

**Experimental Procedures for Raman Imaging and Spectroscopy**

Experimental procedures used for **Figure S3**: Raman spectra obtained with a RAMAN-11 slit-scanning Raman microscope (Nanophoton, Japan) at 532 nm excitation. Samples were placed on a quartz substrate during the measurements. The laser output was focused into the sample by a 60X/1.2 NA UPLSAPO 60XW water immersion objective lens (Olympus Corp., Japan). The slit width of the spectrograph was 70 µm. The light intensity at the sample plane was calculated as 6.0 mW/µm² from the ratio of the measured laser power between the sample position and the area of the illumination line. The exposure time for each line was 120 s/line. Each sample solution was measured at 4 times.

Experimental procedures used for **Figure 4**: The abaxial leaf epidermis of 6- to 8-week-old Col-0 or *coi1-16s* was peeled and cut to about 2 mm². The peels were submerged in buffer (10 mM MES-KOH, pH 6.2, 50 mM KCl) at 22 °C for 3 h in the dark to close the stomata. After incubation for 3 h with 100 µM 5/ent5 at 22 °C in the dark, peels were washed and then used for observation of bright-field images and Raman spectra obtained with a RAMAN-11 slit-scanning Raman microscope at 532 nm excitation. Samples were placed on a quartz substrate during the measurements. The laser output was focused into the sample by a 60X/1.2 NA UPLSAPO 60XW water immersion objective lens. The slit width of the spectrograph was 50 µm. The exposure time for each line was 120s/line. The laser intensity was calculated from the ratio of the measured laser power at the sample position and the illumination line. The light intensity at the sample plane was calculated as 6.2 mW/µm² (Col-0 with 5), 6.1
mW/µm² (Col-0 with ent5), 5.9 mW/µm² (coil-16s with 5), and 5.8 mW/µm² (coil-16s with ent5). Each Raman Spectra was Smoothed using a moving average method.

Experimental procedures used for Figure S5: The abaxial leaf epidermis of 6- to 8-week-old Col-0 or arc6-1 was peeled and cut to about 2 mm². The peels were submerged in buffer (10 mM MES-KOH, pH 6.2, 50 mM KCl) at 22 °C for 3 h in the dark to close the stomata. After incubation for 3 h with 100 µM 5/ent5 at 22 °C in the dark, peels were washed and then used for observation of bright-field images and Raman spectra obtained with a RAMAN-11 slit-scanning Raman microscope at 532 nm excitation. Samples were placed on a quartz substrate during the measurements. The laser output was focused into the sample by a 60X/1.2 NA UPLSAPO 60XW water immersion objective lens. The slit width of the spectrograph was 50 µm. The exposure time for each line was 120 s/line. The laser intensity was calculated from the ratio of the measured laser power at the sample position and the illumination line. The laser intensity of Col-0 was 6.2 mW/µm², arc6-1 was 5.8 mW/µm². For Raman images in Fig. S3, the Raman spectral data set was un-processed Raman images were reconstructed using the peak intensity of average intensity of silent region (1985-2315 cm⁻¹). The final Col-0 images consist of 79 × 37 pixels and the final arc6-1 images consists of 69 × 31 pixels.

Experimental procedures used for Figure 5 and Figures S7 and S8: The abaxial leaf epidermis of 6- to 8-week-old arc6-1 was peeled and cut to about 2 mm². The peels were submerged in buffer (10 mM MES-KOH, pH 6.2, 50 mM KCl) at 22 °C for 3 h in the dark to close the stomata. After incubation for 3 h with 100 µM 1 and 100 µM 5 at 22 °C in the dark, peels were washed and then used for observation of bright-field images and Raman spectra obtained with a RAMAN-11 slit-scanning Raman microscope at 532 nm excitation. Samples were placed on a quartz substrate during the measurements. The laser output was focused into the sample by a 60X/1.2 NA UPLSAPO 60XW water immersion objective lens. The slit width of the spectrograph was 50 µm. The exposure time for each line was 120-150 s/line. The laser intensity was calculated from the ratio of the measured laser power at the sample position and the illumination line. The light intensity at the sample plane was calculated as 6.0-6.2 mW/µm² (arc6-1 with 5), 6.2 mW/µm² (arc6-1 with ent5).

For Raman Spectra in Figures S7 and S8, each Raman Spectra was smoothed using a moving average method.

Experimental procedures used for Figure S10: The abaxial leaf epidermis of 6- to 8-week-old arc6-1 was peeled and cut to about 2 mm². The peels were submerged in buffer (10 mM MES-KOH, pH 6.2, 50 mM KCl) at 22 °C for 3 h in the dark to close the stomata. After co-incubation for 3 h with 100 µM 1 and 100 µM 5 at 22 °C in the dark, peels were washed and then used for observation of bright-field images and Raman spectra obtained with a RAMAN-11 slit-scanning Raman microscope at 532 nm excitation. Samples were placed on a quartz
substrate during the measurements. The laser output was focused into the sample by a 60X/1.2 NA UPLSAPO 60XW water immersion objective lens. The slit width of the spectrograph was 70 µm. The exposure time for each line was 120 s/line. The laser intensity was calculated from the ratio of the measured laser power at the sample position and the illumination line. The light intensity at the sample plane was 6.0 mW/µm². Each Raman Spectra was smoothed using a moving average method.

For Raman images in Figures 5 and S10, the Raman spectral data set was further processed using the singular value decomposition (SVD) technique for noise reduction. Then we used a narrow spectral region (1985-2315 cm⁻¹) in the calculation procedure for SVD to avoid artifacts in constructed images. A modified polyfit technique was then used at each pixel to determine the autofluorescence baseline signal, which was subtracted from the original Raman spectrum. After SVD processing, Raman images were reconstructed using the peak intensity of diyne at 2,258 cm⁻¹. The final 5 images consist of 58 × 28 pixels and the final 5 images consists of 78 × 34 pixels (Figure 5). The final 1 and 5 co-incubated images consist of 57 × 27 pixels (Figure S10).

Experimental Procedures for Fluorescence Imaging

Experimental procedures used for Figure S5: To examine nuclear and ER localization, the abaxial leaf epidermis of 6- to 8-week-old Col-0 or arc6-1 was peeled and cut to about 2 mm². Light micrographs and fluorescent images were taken using an IX71 microscope (Olympus Corp., Japan) equipped with DP72 CCD camera (Olympus Corp., Japan) and WIB filter (Olympus Corp., Japan).

Experimental procedures used for Figures 5 and S9: To examine nuclear and ER localization, the abaxial leaf epidermis of 6- to 8-week-old Col-0 or arc6-1 was peeled and cut to about 2 mm². The peels were incubated with test compounds in buffer (10 mM MES-KOH, pH 6.2, 50 mM KCl) containing 0.5% DMSO at 22 °C in the dark a) for 90 min without compounds b) for 90 min with 5 µM of HoeFLAc² and c) for 90 min with 5 µM ER-Tracker Green (Thermo Fisher Scientific, Inc., USA). Light micrographs and fluorescent images were taken using or an LSM-710 confocal microscope system (Carl Zeiss, Germany).

General Experimental Procedures for Chemical Synthesis

¹H NMR and ¹³C NMR spectra in CDCl₃ were recorded on a JNM-ECS-400 NMR spectrometer (JEOL Inc., Japan). High-resolution electrospray ionization mass spectrometry was carried out on a micrOTOF II mass spectrometer (Bruker Daltonics Inc., Germany). Chemical reagents and solvents were purchased from Kanto Chemical Co. Ltd. (Japan), Wako
Pure Chemical Industries Co. Ltd. (Japan), and Nacalai Tesque, Inc. (Japan). All anhydrous solvents were dried by standard techniques and freshly distilled before use or purchased in anhydrous form. Flash chromatography was carried out using dry-packed Chromatorex PSQ 100B silica gel (Fuji Silysia Chemical Ltd., Japan). All reactions were carried out under air unless stated otherwise. FT/IR spectra were recorded on a JASCO FT/IR-4100 spectrometer (JASCO Inc., Japan). Optical rotation was measured by a JASCO DIP-1000 polarimeter. High performance liquid chromatography was carried out with a combination of a JASCO PU-2086 Plus pump and JASCO UV-2075 detector equipped with a Develosil RP-AQUEOUS φ20 × 250 mm column (Nomura Chemical, Co., Ltd., Japan). Freeze-drying was performed using a EYELA FDU-830 freeze dryer system (Tokyo Rikakikai Co., Ltd., Japan).

**Experimental Procedures for Synthesis and compound data**

Methyl (S)-2-((tert-butoxycarbonyl)amino)pent-4-ynoate (6)

To a solution of L-propargylglycine [CAS No.23235-01-0] (113.1 mg, 1.00 mmol) in THF/DMF/H$_2$O (1/1/1, 12.0 mL) was added di-tert-butyl dicarbonate (250 µL, 1.09 mmol) and K$_2$CO$_3$ (139 mg, 1.00 mmol) at room temperature under argon atmosphere. After the reaction mixture was stirred for 2 h, the mixture was extracted by saturated aqueous NaHCO$_3$ (3 × 30 mL). The aqueous layer was mixed with 5% aqueous KH$_2$SO$_4$ (150 mL) and extracted with EtOAc (3 × 50 mL). The organic layer was dried over $\text{Ns}_2\text{SO}_4$, and filtered. After evaporation, the residue was dissolved in CHCl$_3$/MeOH (3/1, 8.0 mL) and mixed with 1.6 M trimethylsilyldiazomethane in $n$-hexane (2.0 mL) at room temperature under argon atmosphere. After the reaction was stirred for 10 min, the reaction was quenched by acetic acid and then the mixture was evaporated. The residue was purified by silica gel column chromatography ($n$-hexane/EtOAc = 15/1) to give 6 (225.3 mg, 0.991 mmol, 99%) as a colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ$_H$: 5.35 (d, $J$ = 8.4, 1H), 4.48 (dt, $J$ = 8.4, 4.8, 1H), 3.78 (s, 3H), 2.78–2.68 (m, 2H), 2.04 (t, $J$ = 2.8, 1H), 1.46 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ$_C$: 171.1, 155.1, 80.2, 78.5, 71.6, 52.6, 51.9, 28.2(3C), 22.8; IR (film) cm$^{-1}$: 3294, 2978, 2958, 2123, 1750, 1713, 1506, 1439, 1358, 1064, 1025, 994; HRMS (ESI, positive) m/z [M+Na]$^+$ calcd. for C$_{11}$H$_{17}$NO$_4$Na : 250.1050, Found : 2501059; [α]$_D$$^{21}$ +57.8° ( c = 0.89, CHCl$_3$)

Methyl (R)-2-((tert-butoxycarbonyl)amino)pent-4-ynoate (ent6)

Ent6 was prepared from D-propargylglycine [CAS No. 23235-03-2] according to the same
method as 6. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H: 5.35 (d, $J = 8.4$, 1H), 4.48 (dt, $J = 8.4$, 4.8, 1H), 3.78 (s, 3H), 2.78–2.68 (m, 2H), 2.04 (t, $J = 2.8$, 1H), 1.46 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C: 171.1, 155.1, 80.2, 78.5, 71.6, 52.6, 51.9, 28.3(3C), 22.8; IR (film) cm$^{-1}$: 3294, 2978, 2958, 2123, 1750, 1713, 1506, 1439, 1358, 1064, 1025, 994; HRMS (ESI, positive) m/z [M+Na]$^+$ calcd. for C$_{11}$H$_{17}$NO$_4$Na : 250.1050, Found : 250.1042; $\left[\alpha\right]_{D}^{21}$–58.9° (c = 0.77, CHCl$_3$)

Methyl (S)-2-((tert-butoxycarbonyl)amino)nona-4,6-diynoate (7)

To a solution of 6 (132.1 mg, 0.581 mmol) in piperidine (2.5 mL) was added 1-butynyl iodide [CAS No.66794-29-4] (100 µL, 0.966 mmol) and copper (I) iodide (57 mg, 29.9 µmol) at 0 °C under argon atmosphere. After 4 h stirring, the reaction was quenched with 5% aqueous KHSO$_4$ (30 mL). The reaction mixture was extracted with EtOAc (3 × 20 mL), and then the organic layer was dried over Na$_2$SO$_4$ and filtered. After evaporation, the residue was purified by silica gel column chromatography ($n$-hexane/EtOAc = 15/1) to give 7 (111.4 mg, 0.408 mmol, 70%) as a white solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H: 5.33 (d, $J = 8.4$, 1H), 4.46 (dt, $J = 8.4$, 4.8, 1H), 3.78 (s, 3H), 2.86–2.75 (m, 2H), 2.26 (q, $J = 7.2$, 2H), 1.46 (s, 9H), 1.15 (t, $J = 7.2$, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C: 170.9, 155.0, 80.2, 79.9, 71.0, 68.3, 64.2, 52.7, 52.0, 28.2(3C), 23.7, 13.2, 12.8; IR (film) cm$^{-1}$: 3366, 2980, 2939, 2260, 1749, 1715, 1508, 1436, 1267, 1251, 1218, 1168, 1062, 778; HRMS (ESI, positive) m/z [M+Na]$^+$ calcd. for C$_{15}$H$_{21}$NO$_4$Na : 302.1363, Found : 302.1360; $\left[\alpha\right]_{D}^{21}$+94.5° (c = 0.78, CHCl$_3$).

Methyl (R)-2-((tert-butoxycarbonyl)amino)nona-4,6-diynoate (ent7)

Ent7 was prepared from ent6 according to the same method as 7. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H: 5.33 (d, $J = 8.4$, 1H), 4.46 (dt, $J = 8.4$, 4.8, 1H), 3.78 (s, 3H), 2.86–2.75 (m, 2H), 2.26 (q, $J = 7.2$, 2H), 1.46 (s, 9H), 1.15 (t, $J = 7.2$, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C: 170.9, 155.0, 80.2, 79.9, 71.0, 68.3, 64.3, 52.7, 52.0, 28.3(3C), 23.7, 13.2, 12.8; IR (film) cm$^{-1}$: 3366, 2980, 2939, 2260, 1749, 1715, 1508, 1436, 1267, 1251, 1218, 1168, 1062, 778; HRMS (ESI, positive) m/z [M+Na]$^+$ calcd. for C$_{15}$H$_{21}$NO$_4$Na : 302.1363, Found : 302.1351; $\left[\alpha\right]_{D}^{21}$–94.5° (c = 0.90 in CHCl$_3$).

Methyl (S)-2-(((3aS,6R,7aS)-6-ethyl-1-oxo-2,3,3a,6,7,7a-hexahydro-1H-indene-4-carboxamido)nona-4,6-diynoate (5)
To a solution of 7 (6.6 mg, 23.6 µmol) in CH₂Cl₂ (0.8 mL) was added TFA (0.2 mL) at room temperature under argon atmosphere. After 30 min stirring, the reaction mixture was evaporated and the residue was dissolved in DMF (1.0 mL). To this solution, CFA (3) 22 (5.2 mg, 25.0 µmol), COMU (12.9 mg, 30.1 µmol) and TEA (7.26 mg, 71.7 µmol) were added at room temperature under argon atmosphere. After overnight stirring, the reaction was quenched by 5% aqueous KHSO₄ (5 mL), and then the mixture was extracted with EtOAc (10 mL). The organic layer was washed with 5% aqueous KHSO₄ (2 × 3 mL), saturated aqueous NaHCO₃ (2 × 3 mL), and brine (3 mL), and then dried over Na₂SO₄ and filtered. After evaporation, the residue was purified by silica gel column chromatography (n-hexane/EtOAc = 4/1) to give 5 (6.0 mg, 15.3 µmol, 65%). Moreover, the 5 (3.9 mg) was purified by HPLC (mobile phase: CH₃OH / H₂O = 60 / 40, flow rate: 8.0 mL/min) on Develosil RPAQUEOUS (φ20 × 250 mm, Nomura Chemicals Co. Ltd., Japan) to give 5 (3.6 mg, Rₜ = 71–75 min) as a colorless crystal. 

\[ \text{1H NMR (400 MHz, CDCl}_3\] \δ_H: 6.59 (d, J = 7.6 Hz, 1H), 6.49 (s, 1H), 4.80 (dt, J = 7.6, 4.4 Hz, 1H), 3.93 (s, 3H), 3.17 (dt, J = 11.6, 6.8 Hz, 1H), 2.90 (dd, J = 17.2, 4.4 Hz, 2H), 2.52–2.12 (m, 7H), 1.90 (dt, J = 13.2, 4.8 Hz, 1H), 1.69–1.36 (m, 3H), 1.15 (t, J = 7.6 Hz, 3H), 1.08 (td, J = 13.2, 10.8 Hz, 1H), 1.01 (t, J = 7.6 Hz, 3H); \text{13C NMR (100 MHz, CDCl}_3\] \δ_C: 220.2, 170.9, 167.5, 139.1, 135.0, 80.1, 70.9, 68.6, 64.1, 53.0, 50.7, 46.5, 39.2, 37.4, 36.1, 28.0, 27.8, 26.0, 23.3, 13.2, 12.9, 11.3.; IR (film) cm⁻¹: 3335, 2961, 2939, 2879, 2858, 2260, 1741, 1654, 1624, 1521, 1457, 1437, 1350, 1317, 1262, 1219, 1148, 1069; HRMS (ESI, positive) m/z [M+Na]^+ calcd. for C₂₂H₂₇NO₄Na: 392.1832, Found: 392.1825; [α]D²¹ +115° ( c = 0.18 in CHCl₃).
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