

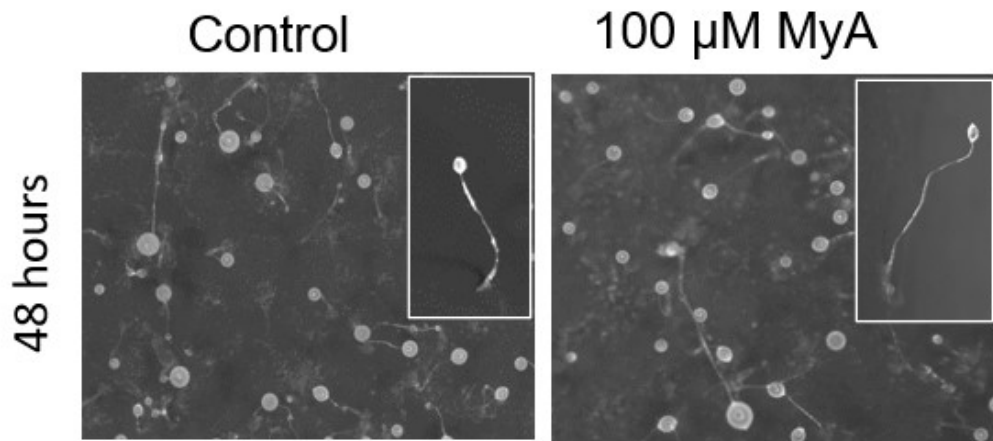
Plant Communications, Volume 5

Supplemental information

Functional mechanism study of the allelochemical myrigalone A identifies a group of ultrapotent inhibitors of ethylene biosynthesis in plants

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1 **Supplementary Information**



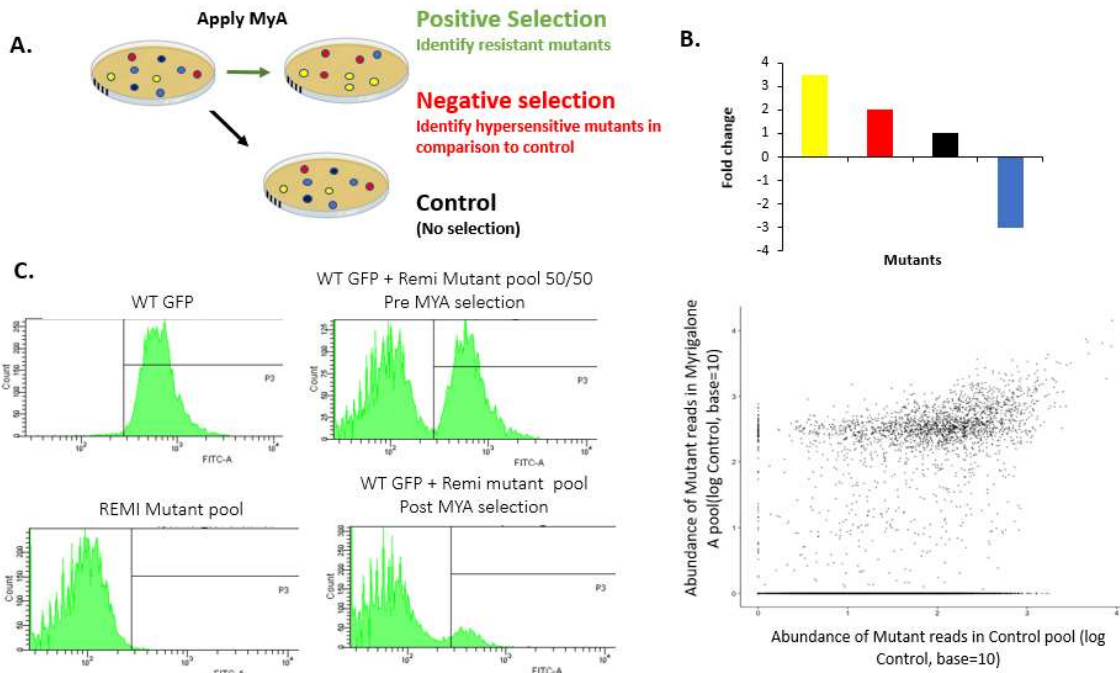
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3 ***Supplementary Figure S1: MyA-treatment delays Dictyostelium development.***

4 Developmental phenotypes, in the absence of MyA (control), showing fruiting body
5 morphology at 48 hours, from top-down view and individual fruiting bodies (side view
6 insert). Development in the presence of MyA (100 μ M) was restored at 48 hours.

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Supplementary Figure S2: MyA growth resistance screen. (A) *D. discoideum* growth resistance screens are commonly used to provide an unbiased search for genes controlling sensitivity to bioactive compounds. Here, different mutants are represented by different colours. Following exposure to a compound that reduces cell growth, two mutants (coloured red and yellow) continue growth, suggesting genes lost in these mutants regulate to sensitivity. (B) For each mutant, after compound exposure, fold change in levels of each mutant identified through next generation REMI-sequencing (REMI seq) (Gruenheit et al., 2019) provides a readout for resistance or sensitivity. (C) Following the third round of screening, a resistance check was performed using 5×10^5 cells per ml of WT GFP expressing cells grown together with 5×10^5 cells from the final round of screening for 48 hours in $100 \mu\text{M}$ of MyA. The ratio of WT GFP expressing cells to REMI-seq cells was calculated using a BD FACSCanto cell analyzer. A decrease in WT-GFP cells and an increase in REMI cells shows the presence of resistant mutants. (D) Mutant sequencing read counts which correlates towards mutant abundance was plotted for MyA treatment against control pools. Those mutants which showed a significant increase in growth against control pools were deemed to show selective resistance to MyA. This supplementary figure refers to main Figure 2.

| Resistant and hyper sensitive mutants identified in Myrtilone A growth screen | | | |
|---|---|---|-----------------------|
| D. discoideum Accession | D. discoideum Name | A. thaliana Homologue | A. thaliana Accession |
| Resistant Mutants | | | |
| DDB_G0272502 | DDB_G0272502 | N/A | N/A |
| DDB_G0267952 | DDB_G0267952 | protein disulfide-isomerase 5-1 | XP_020870842.1 |
| DDB_G0276245 | acyl-CoA oxidase | acyl-coenzyme A oxidase 2, peroxisomal | XP_00286650.1 |
| DDB_G0269448 | DDB_G0269448 | putative myotubularin | AAG51396.1 |
| DDB_G0284565 | ANK_REP_REGION domain-containing protein | Ankyrin repeat family protein | NP_178442.2 |
| DDB_G0271766 | pARTg | unnamed protein product | CA0275201.1 |
| DDB_G0270002 | DDB_G0270002 | kinesin-like protein KIN-4C | XP_002868397.1 |
| DDB_G0290635 | DDB_G0290635 | N/A | N/A |
| DDB_G0278769 | cat1 | Transducin/WD40 repeat-like superfamily protein | NP_974972.1 |
| DDB_G0283633 | Major facilitator superfamily domain-containing protein 5 (| molybdate-anion transporter | XP_020890378.1 |
| DDB_G0289183 | MTO1 homolog, mitochondrial | glucose-inhibited division family A protein | NP_178974.1 |
| DDB_G0287667 | DDB_G0287667 | receptor-like kinase 902 | NP_566589.1 |
| DDB_G0272298 | DDB_G0272298 | ervatamin-B | XP_002877667.1 |
| DDB_G0273593 | DDB_G0273593 | N/A | N/A |
| DDB_G0290743 | cyp51A41 | cytochrome P450 82G1 | XP_002883585.1 |
| DDB_G0287679 | TTRAY2 | Transducin/WD40 repeat-like superfamily protein | NP_197859.4 |
| DDB_G0275225 | FKBP12 | dihydroflavonol reductase | CAPO8819.1 |
| DDB_G0277497 | scd | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | NP_001030834.1 |
| DDB_G0268210 | BB_PF domain-containing protein | N/A | N/A |
| DDB_G0270848 | DDB_G0270848_ps | N/A | N/A |
| DDB_G0268986 | TRE3-B ORF2 | N/A | N/A |
| DDB_G0267848 | beta-lactamase-type transpeptidase fold containing protein | SRKp | AIPE1150.1 |
| DDB_G0288003 | EGF-like domain-containing protein | N/A | N/A |
| DDB_G0285385 | DDB_G0285385 | N/A | N/A |
| DDB_G0290079 | pgtB / putative glycosyltransferase | sulfoquinovosyl diacylglycerol 2 | NP_568085.2 |
| DDB_G0286871 | GCN5-related N-acetyltransferase | N/A | N/A |
| DDB_G0290627 | DDB_G0290627 | N/A | N/A |
| DDB_G0290833 | rabK1 / Rab GTPase | RAB GTPase homolog 7A | NP_565521.1 |
| DDB_G0280307 | beta-lactamase family protein | hypothetical protein AXX17_ATUG03410 | OAO89193.1 |
| DDB_G0286981 | DDB_G0286981 | N/A | N/A |
| DDB_G0272336 | DDB_G0272336_ps | unnamed protein product | CAA0370672.1 |
| DDB_G0269892 | DDB_G0269892 | N/A | N/A |
| DDB_G0276057 | UBiquitin regulatory X | Chain A, Thioredoxin h1 | 1XFL_A |
| DDB_G0275399 | DDB_G0275399 | hypothetical protein AXX17_AT3G49740 | OAP03484.1 |
| DDB_G0288061 | ATP-gated ion channel P2XE | N/A | N/A |
| DDB_G0277145 | pkd | serine/threonine-protein kinase AtPK2/AtPK19 | XP_020888566.1 |
| DDB_G0282133 | DDB_G0282133 | hypothetical protein AXX17_AT5G27870 | OAO90000.1 |
| DDB_G0281915 | transmembrane protein | N/A | N/A |
| DDB_G0274885 | Cell Division Cycle 73 | PHP, partial | AIU48804.1 |
| DDB_G0285599 | mcfB | adenine nucleotide transporter 1 | NP_192019.1 |
| DDB_G0269620 | DDB_G0269620 | N/A | N/A |
| DDB_G0277441 | rabj | Ras small GTP-binding family protein | NP_199326.1 |

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| D. discoideum Accession | D. discoideum Name | A. thaliana Homologue | A. thaliana Accession |
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| DDB_G0285031 | DDB_G0285031 | N/A | N/A |
| DDB_G0281349 | PH domain-containing protein | N/A | N/A |
| DDB_G0283373 | Sulfhydryl oxidase | N/A | N/A |
| DDB_G0275155 | DDB_G0275155 | N/A | N/A |
| DDB_G0271700 | thioredoxin-like protein | protein disulfide-isomerase 5-1 | XP_020870842.1 |
| DDB_G0288573 | DDB_G0288573 | N/A | N/A |
| DDB_G0285201 | Late secretory pathway protein AVL9 homolog | N/A | N/A |
| DDB_G0285271 | DDB_G0285271 | metalloendopeptidase / zinc ion binding protein | NP_001190451.1 |
| DDB_G0284853 | DDB_G0284853 | Aminotransferase-like, plant mobile domain family protein | NP_193340.2 |
| DDB_G0284279 | DDB_G0284279_ps | N/A | N/A |
| DDB_G0281087 | gtaV | DNA-binding protein with MIZ/SP-RING zinc finger, PHD-finger and SAP domain-containing protein | NP_001032108.1 |
| DDB_G0267492 | DDB_G0267492 | ATP-dependent zinc metalloprotease FTSH 4 | XP_002880747.1 |
| DDB_G0274045 | 3B-2 | N/A | N/A |
| DDB_G0273543 | DDB_G0273543 | N/A | N/A |
| DDB_G0268138 | putative glutathione S-transferase alpha-1 | GSTF11 | OAP02577.1 |
| DDB_G0286295 | DDB_G0286295 | N/A | N/A |
| DDB_G0275615 | DDB_G0275615_ps | N/A | N/A |
| DDB_G0293854 | DDB_G0293854 | N/A | N/A |
| DDB_G0289617 | DDB_G0289617 | N/A | N/A |
| DDB_G0291664 | putative protein kinase | Mitogen activated protein kinase kinase kinase-like protein [Arabidopsis thaliana] | NP_567072.1 |
| DDB_G0287565 | DDB_G0287565_ps | N/A | N/A |
| DDB_G0290273 | DDB_G0290273 | N/A | N/A |
| DDB_G0290709 | pk32 | Chain A, 3-OXOACYL CARRIER PROTEIN SYNTHASE | 1W0L_A |
| DDB_G0291758 | DDB_G0291758 | N/A | N/A |
| DDB_G0270730 | fsiB | N/A | N/A |
| DDB_G0273951 | DDB_G0273951 | expressed protein | EFH43206.1 |
| DDB_G0277843 | DG1105 | N/A | N/A |
| DDB_G0268290 | DDB_G0268290 | uncharacterized protein LOC110224440 | XP_020866161.1 |
| DDB_G0291123 | Glycogen Phosphorylase | alpha-glucan phosphorylase 2 | NP_190281.1 |
| DDB_G0295773 | DDB_G0295773 | DUF1077 family protein | EU04121.1 |
| DDB_G0278677 | DDB_G0278677 | N/A | N/A |
| DDB_G0273005 | DDB_G0273005 | N/A | N/A |
| DDB_G0269132 | ecmB | N/A | N/A |
| DDB_G0276919 | PhosphoLipase D, GPI-specific | N/A | N/A |
| DDB_G0285347 | DDB_G0285347 | N/A | N/A |
| DDB_G0272304 | aspS2 | N/A | N/A |
| DDB_G0279431 | DDB_G0279431 | N/A | N/A |
| DDB_G0281767 | DDB_G0281767 | Regulator of chromosome condensation (RCC1) family protein [Arabidopsis thaliana] | NP_186900.3 |
| DDB_G0277577 | DDB_G0277577 | E3 ubiquitin-protein ligase dbi4 | XP_002880863.1 |
| DDB_G0292560 | racJ | ROP9 [Arabidopsis thaliana] | OAO98596.1 |
| DDB_G0284683 | DDB_G0284683 | N/A | N/A |

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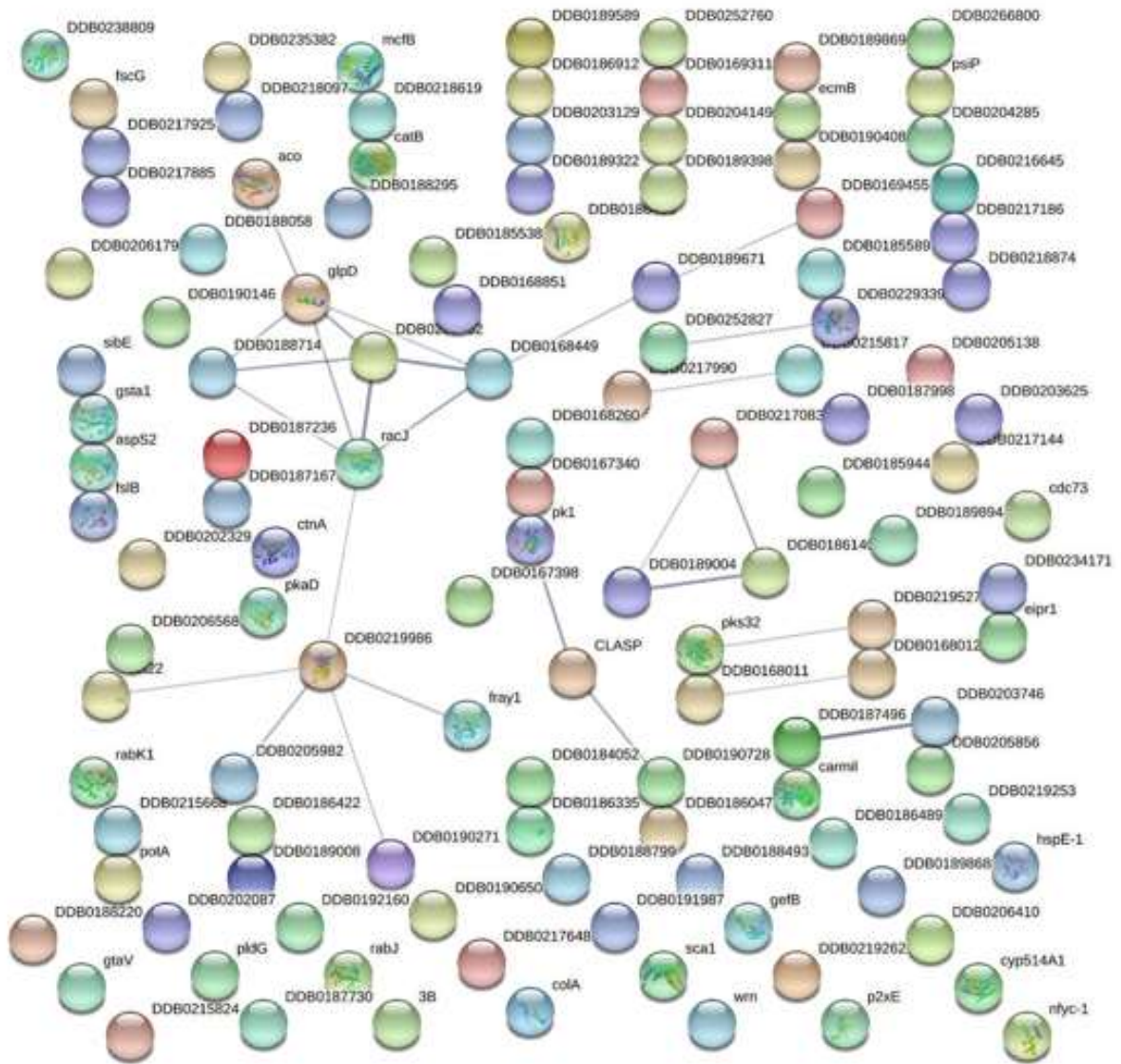
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| D. discoideum Accession | D. discoideum Name | A. thaliana Homologue | A. thaliana Accession |
|-------------------------|--------------------|---|--------------------------------|
| DDB_G0292156 | fscG | N/A | N/A |
| DDB_G0276527 | DDB_G0276527 | N/A | N/A |
| DDB_G0284511 | DDB_G0284511 | N/A | N/A |
| DDB_G0279679 | DDB_G0279679 | N/A | N/A |
| DDB_G0292696 | colA | N/A | N/A |
| DDB_G0267602 | DDB_G0267602 | Regulator of chromosome condensation (RCC1) family protein | NP_201191.1 |
| DDB_G0283467 | DDB_G0283467 | N/A | N/A |
| DDB_G0285299 | DDB_G0285299 | Protein kinase superfamily protein [Arabidopsis thaliana] | NP_188511.1 |
| DDB_G0290689 | DDB_G0290689 | diadenosine 5'-5''-P1,P4-tetraphosphate hydrolase, putative | AAF76368.1 |
| DDB_G0269222 | gefB | N/A | N/A |
| DDB_G0287013 | DDB_G0287013 | phytochrome and flowering time regulatory protein (PFT1) | NP_001077596.1 |
| DDB_G0282291 | potA | N/A | N/A |
| DDB_G0288239 | sibE | N/A | N/A |
| DDB_G0268512 | wrn | DNA helicase (Recq14A) | NP_172562.2 |
| DDB_G0280171 | psiP | N/A | N/A |
| DDB_G0275023 | act22 | actin-11 | NP_187818.1 |
| DDB_G0273545 | nfyC-2 | nuclear factor Y, subunit C1 | NP_190428.1 |
| DDB_G0287501 | DDB_G0287501 | Jojoba acyl CoA reductase-related male sterility protein | NP_187805.1 |
| DDB_G0276823 | DDB_G0276823 | N/A | N/A |
| DDB_G0269278 | DDB_G0269278 | DECAPPING NUCLEASE DXO1 | SDKN_A |
| DDB_G0279573 | DDB_G0279573 | TOM3 | QAP08400.1 |
| DDB_G0271286 | clasp | CUP-associated protein | NP_849997.2 |
| DDB_G0293542 | DDB_G0293542 | phytanoyl-CoA dioxygenase (PhyH) family protein | NP_001325094.1 |
| DDB_G0281567 | fhkC | SNF1 family protein kinase, partia | AAD00542.1 |
| DDB_G0280155 | DDB_G0280155_ps | N/A | N/A |
| DDB_G0278863 | fray1 | protein kinase-like protein | CAC01871.1 |
| DDB_G0277055 | DDB_G0277055 | N/A | N/A |
| DDB_G0273623 | hspE-2 | heat shock protein 70B | NP_173055.1 |
| DDB_G0277301 | DDB_G0277301 | N/A | N/A |
| DDB_G0277597 | gpt3 | N/A | N/A |
| DDB_G0290125 | DDB_G0290125 | N/A | N/A |
| DDB_G0268212 | DDB_G0290125 | RNA helicase - like protein | CAA16726.1 |

| D. discoideum Accession | D. discoideum Name | A. thaliana Homologue | A. thaliana Accession |
|-------------------------|--------------------|---|-----------------------------|
| Hypersensitive mutants | | | |
| DDB_G0292386 | carmil | N/A | N/A |
| DDB_G0268248 | DDB_G0268248 | N/A | N/A |
| DDB_G0288695 | DDB_G0288695 | N/A | N/A |
| DDB_G0274597 | ctnA | N/A | N/A |
| DDB_G0269108 | catB | CAT2 | OAO97606.1 |
| DDB_G0289031 | DDB_G0289031 | alpha/beta-Hydrolases superfamily protein | NP_192960.1 |
| DDB_G0273949 | DDB_G0273949 | AT5G41080 | BAH57127.1 |
| DDB_G0274279 | tssc1 | Transducin/WD40 repeat-like superfamily protein | NP_173478.2 |

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Supplementary Figure S3: MyA resistance mutants identified in growth resistance screen. Following MyA growth resistance screen, 125 mutants were identified showing enhanced growth (green, resistant) or reduced growth (red, sensitive), shown here with Dictybase gene ID, gene name (if known), GO terminology, and potential *A. thaliana* homologue and accession number. This supplementary figure refers to main Figure 2.



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53 **Supplementary Figure S4: String analysis of all MyA resistant mutants.** String Analysis for all
 54 proteins ablated in 112 MyA resistant mutants identified during mutant library screening. Lines
 55 connecting individual mutants indicate connectivity. This supplementary figure refers to main Figure
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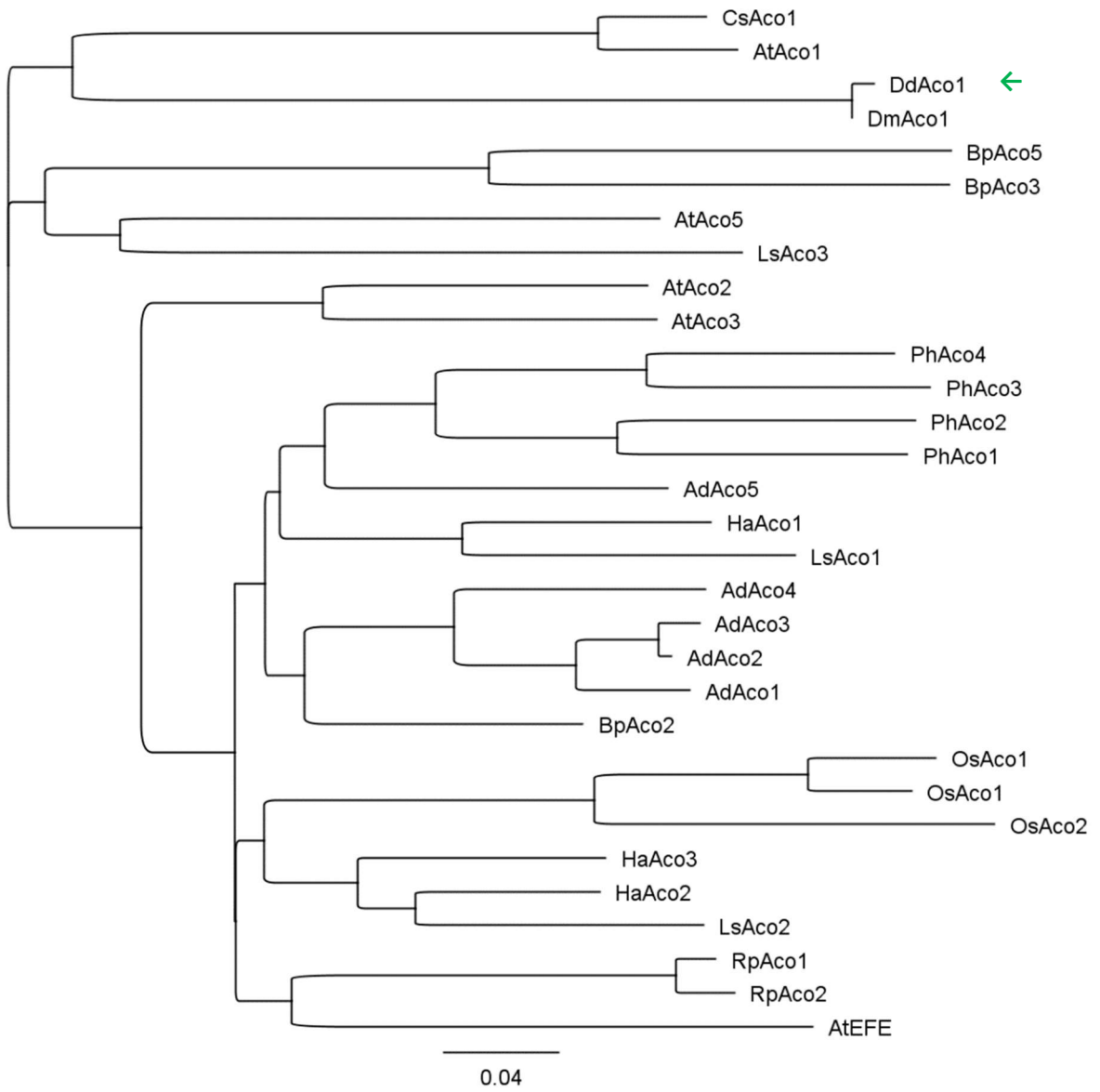
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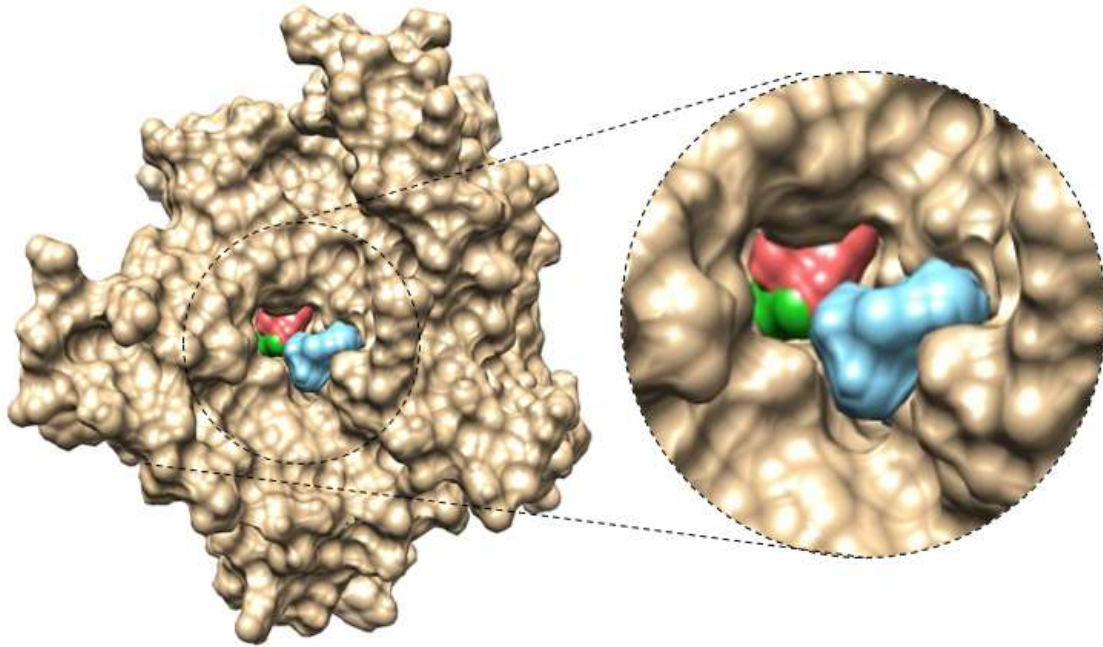
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| Name | Accession | Description | Organism |
|--------------|----------------|-------------|------------------------------|
| ACTACCOXI | M97961.1 | AdAco1 | Actinidia deliciosa |
| HQ293205 | HQ293205.1 | AdAco2 | Actinidia deliciosa |
| HQ293207 | HQ293207.1 | AdAco3 | Actinidia deliciosa |
| HQ293209 | HQ293209.1 | AdAco4 | Actinidia deliciosa |
| HQ293211 | HQ293211.1 | AdAco5 | Actinidia deliciosa |
| NM_127517 | NM_127517.5 | AtAco1 | Arabidopsis thaliana |
| NM_104918 | NM_104918.5 | AtAco2 | Arabidopsis thaliana |
| NM_101073 | NM_101073.3 | AtAco3 | Arabidopsis thaliana |
| NM_106382 | NM_106382.3 | AtAco5 | Arabidopsis thaliana |
| NM_100380 | NM_100380.4 | AtEFE | Arabidopsis thaliana |
| AY154649 | AY154649.1 | BpAco2 | Betula pendula |
| X97992 | X97992.1 | BpAco3 | Betula pendula |
| X97994 | X97994.1 | BpAco5 | Betula pendula |
| XM_010490852 | XM_010490852.2 | CsAco1 | Camelina sativa |
| XM_637519 | XM_637519.1 | DdAco1 | Dictyostelium discoideum AX4 |
| AB291210 | AB291210.1 | DmAco1 | Dictyostelium mucoroides |
| HNNACC | L29405.1 | HaAco1 | Helianthus annuus |
| HAU62554 | U62554.1 | HaAco2 | Helianthus annuus |
| HAU62555 | U62555.1 | HaAco3 | Helianthus annuus |
| AB158345 | AB158345.1 | LsAco1 | Lactuca sativa |
| AB158346 | AB158346.1 | LsAco2 | Lactuca sativa |
| AB158347 | AB158347.1 | LsAco3 | Lactuca sativa |
| AF049888 | AF049888.1 | OsAco1 | Oryza sativa |
| X85747 | X85747.1 | OsAco1 | Oryza sativa Indica Group |
| AF049889 | AF049889.1 | OsAco2 | Oryza sativa |
| PETACO1A | L21976.2 | PhAco1 | Petunia x hybrida |
| PETACO2A | L21977.1 | PhAco2 | Petunia x hybrida |
| PETACO3A | L21978.1 | PhAco3 | Petunia x hybrida |
| PETACO4A | L21979.1 | PhAco4 | Petunia x hybrida |
| RpACO1 | Y10034.1 | RpAco1 | Rumex palustris |
| AF041479 | AF041479.1 | RpAco2 | Rumex palustris |

71 **Supplementary Figure S5. Phylogenetic analysis of Dictyostelium and plant ACO proteins.**
72 **(A)** Neighbor-joining phylogenetic tree is based on similarity of proteins. Alignment of sequences
73 and neighbour joining tree was constructed in Geneious R.9 using Jukes-Cantor as the genetic
74 distance model. Scale bar indicates bootstrap values (100 replications). **(B)** Protein sequences
75 used for phylogenetic analysis.



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77 **Supplementary Figure S6. Tertiary structure analysis of *D. discoideum* ACO enzymes and**
78 **MyA binding.** Space-filling model of *D. discoideum* ACO enzyme, shown with binding by existing
79 ACO inhibitors AIB (pink), POA (green) and MyA (light blue) within the catalytic pocket.

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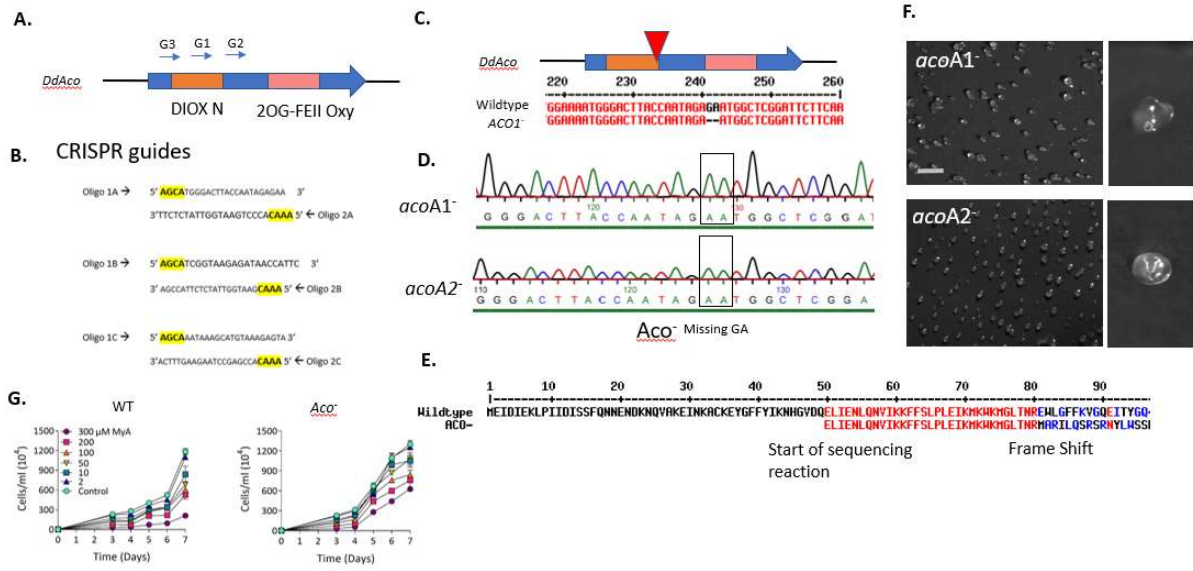
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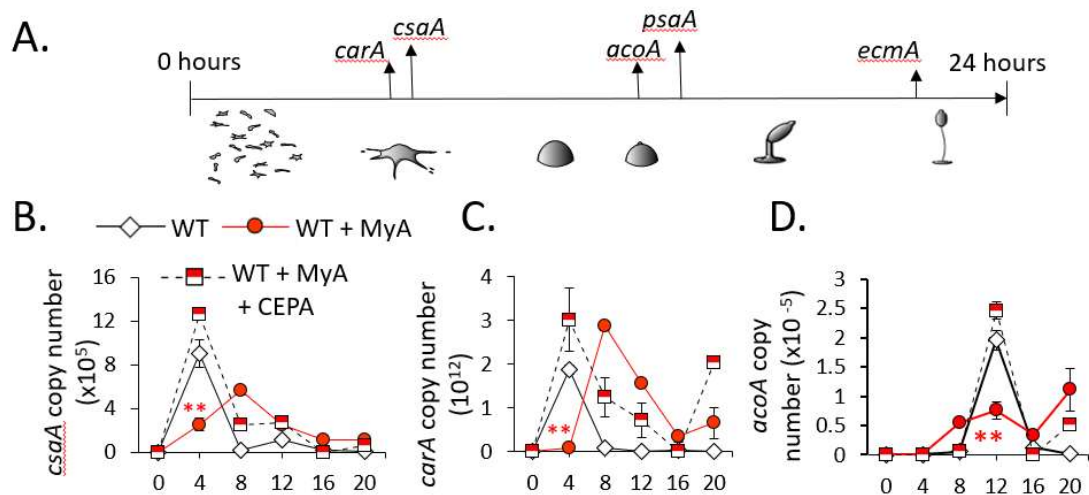
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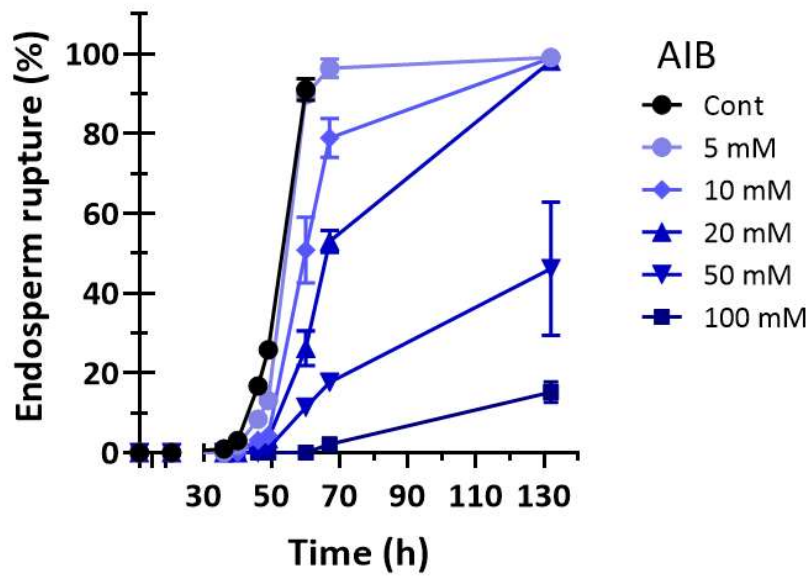
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Supplementary Figure S7: Genetic ablation of *D. discoideum acoA* gene. (A) Schematic of *Dictyostelium ACO* gene showing location of Crispr guides and (B) Crispr guide sequences. (C, D) Schematic showing mutated site (red triangle) and sequence analysis for two independent mutations, leading to (E) a frame shift from AA80. (F) Both independent mutants show common block in development at the mound stage (size bar = 1mm). (G) Growth sensitivity of Wild type (Ax3) and *Aco*⁻ cells were analysed as described in the methods. Growth rates were calculated, for each MyA concentration in cell type, and used to provide the secondary plot shown in Figure 3. Data represents 3 biological repeats analysed with 3 technical repeats. This supplementary figure refers to main Figure 2.



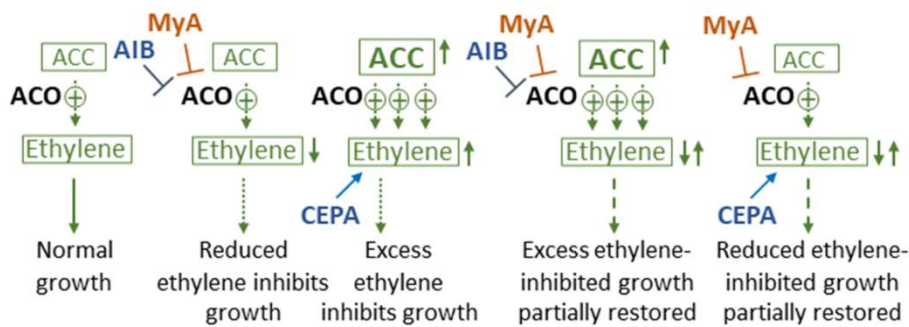
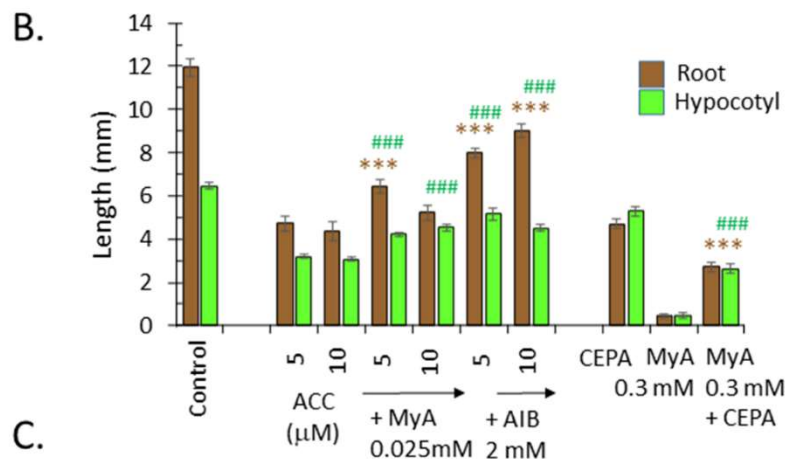
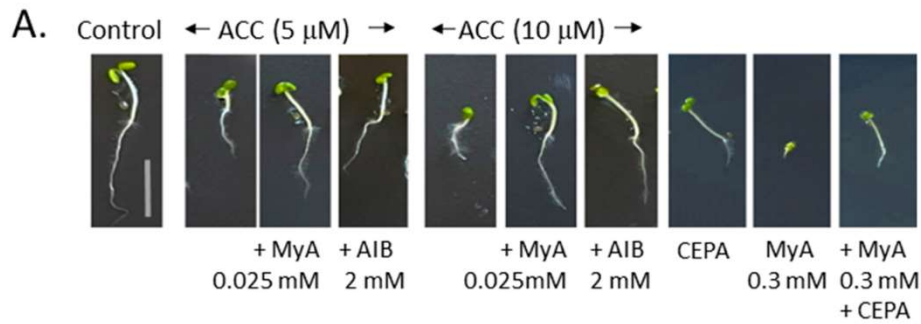
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 102 **Supplementary Figure S8: MyA regulates *D. discoideum* development through loss**
 103 **of ethylene.** (A) To quantify developmental effects of MyA, expression of specific
 104 developmental genes can be monitored, including (B) *csA* (Contact site A), (C) *cAR1*
 105 (*cAMP* receptor 1) and (D) *AcoA*. Here, wild type cells, in solvent only conditions, or with
 106 MyA (100 μ M) alone or in the presence of CEPA, were allowed to develop for the
 107 indicated time periods, RNA was extracted, and gene expression determined using
 108 quantitative PCR, expressed as absolute copy number. Data is derived from 3
 109 independent experiments analysed in triplicate, and shown as mean \pm SEM, with
 110 statistical analysis performed using a Student's *t*-test comparing WT with WT + MyA with
 111 ** (red) $P < 0.01$, and WT + MyA with WT + MyA + CEPA ** (black) $P < 0.01$ and
 112 *** $P < 0.001$ at the point of greatest difference.

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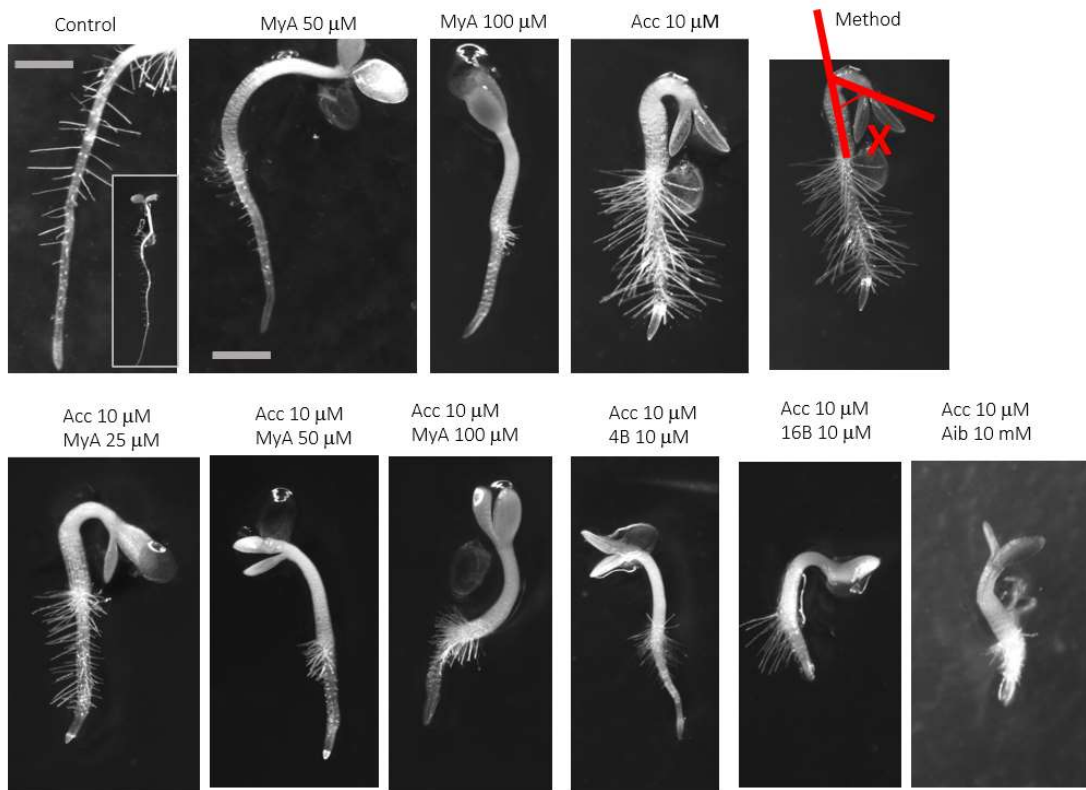
Supplementary Figure S9: Dose dependent reduction in *A. thaliana* endosperm rupture following ethylene inhibition by AIB. This supplementary figure refers to main Figure 5.



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Supplementary Figure S10: MyA inhibits ethylene response in *A. thaliana* seedlings. (A, B)

To provide further evidence for the effect of MyA on inhibition of ethylene production in *A. thaliana* Col-0 seedlings, root and hypocotyl growth was assessed in the presence of increased ethylene by addition of ACC (5 and 10 μ M) and with the addition of MyA (25 μ M) or AIB (2mM), or through CEPA treatment \pm MyA (300 μ M)(n=3). Statistical analysis is shown for root (brown asterisk) and hypocotyl (green hash) comparing ACC with corresponding MyA or AIB treatments or comparing MyA with MyA and CEPA. (C) Schematic showing the effects of MyA on *A. thaliana* seedling growth, reducing effects of ACC-dependent ethylene production by ACO, and reducing seedling growth with effects partially rescued by exogenous ethylene provided by CEPA. Statistical analysis performed using a Student's *t*-test for control and the lowest concentration of compounds showing an effect (brown asterisk for root, green hatch for hypocotyl) *** or ###P<0.001. Data derived from three independent experiments, with at least 5 seedlings per experiments shown as mean \pm SEM.



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Supplementary Figure S11: MyA blocks ethylene promoted Apical Hook curvature. MyA, 4B and 16B treatment of *A. thaliana* seedlings was assessed for the inhibition of apical hook curvature as part of the ethylene triple response after 4 days of growth. MyA caused a concentration dependant decrease in the both ACC promoted ACC induced apical hook curvature. Images shows representative seedling from 3 independent experiments, each with 5 seedlings. Ethylene response can be seen for ACC treatment, with increased root hair density and length together with an exaggerated apical hook. Scale bar = 0.3 mm. Data represents average and SEM (error bars) shown with statistical analysis performed using a student t test. *** Indicate $P < 0.005$). Statistical analysis is performed for Acc treatment against Acc plus MyA, 4B and 16B treatment. Apical hook angle was measured as Angle X shown in (A).

| | | Smiles | Pubchem ID | Cluster in binding pocket | Full fitness (kcal/mol) | Estimated ΔG (kcal/mol) | | Smiles | Pubchem ID | Cluster in binding pocket | Full fitness (kcal/mol) | Estimated ΔG (kcal/mol) | |
|---------|----|---|------------|---------------------------|-------------------------|-------------------------|---------|--------|--------------------------------------|---------------------------|-------------------------|-------------------------|-------|
| MYA | | CC1=C(O)C(C)=O[C@H](C1=O)C(C)=O[C@@H](C)C=CC=C | 11722329 | 0 | -2016 | -7.21 | | | | | | | |
| POA | | C1=CC=C(C=C1)C=C1C=O | 1047 | 0 | -1993 | -6.89 | | MYA | 11722329 | 0 | -2016 | -7.21 | |
| Z-AMB | | CC(C)C=C(O)N | 6119 | 0 | -1946 | -5.98 | | | | | | | |
| ETHi-1 | 1 | [NH+]C(=O)O[C@H](C)C(=O)C=C1O=C1O=C1C2=CC=CC=C2 | 70181725 | 0 | -1973 | -6.23 | ETHi-42 | 42 | CN(C)C1=CC=C(C=C1)C(C)=O[C@H](C)C1=O | 4804033 | 0 | -1996 | -7.16 |
| ETHi-2 | 2 | CN(C)C(=O)C1=CC=C(C=C1)C=C1C=O | 179008 | 0 | -2101 | -7.54 | ETHi-43 | 43 | CC1=CC=C(C=C1)C(C)=O | 641785 | 0 | -1949 | -6.85 |
| ETHi-3 | 3 | O=C1C=CC(=C1)C=C1C=O | 7180 | 0 | -1952 | -7.58 | ETHi-44 | 44 | CC1=CC=C(C=C1)C(C)=O | 39898991 | 0 | -1996 | -6.89 |
| ETHi-4 | 4 | O=C1C=CC(=C1)C=C1C=O | 85172 | 0 | -1972 | -6.90 | ETHi-45 | 45 | CC1=CC=C(C=C1)C(C)=O | 487535 | 0 | -1967 | -6.69 |
| ETHi-5 | 5 | CC(=O)C(O)C1=CC=C(C=C1)C=O | 12617805 | 0 | -2003 | -6.57 | ETHi-46 | 46 | CC1=CC=C(C=C1)C(C)=O | 242389 | 0 | -1940 | -6.16 |
| ETHi-6 | 6 | O=C1C=CC(=C1)C=C1C=O | 10776860 | 2 | -2112 | -6.78 | ETHi-47 | 47 | CC(=O)C1=CC=C(C=C1)C=O | 3624793 | 0 | -2000 | -6.22 |
| ETHi-7 | 7 | CN1C(O)C(C)=O[C@H](C1=O)C=C1C=O | 71685841 | 0 | -2100 | -6.87 | ETHi-48 | 48 | CC(=O)C1=CC=C(C=C1)C=O | 2834748 | 0 | -2028 | -6.67 |
| ETHi-8 | 8 | CC(=O)C(O)C1=CC=C(C=C1)C=O | 78914998 | 0 | -2123 | -7.08 | ETHi-49 | 49 | CC1=CC=C(C=C1)C(C)=O | 5133076 | 0 | -1999 | -7.56 |
| ETHi-9 | 9 | CN1C(O)C(C)=O[C@H](C1=O)C=C1C=O | 4121330 | 0 | -2050 | -6.89 | ETHi-50 | 50 | CC1=CC=C(C=C1)C(C)=O | 73245192 | 0 | -1994 | -7.06 |
| ETHi-10 | 10 | CC(=O)C(O)C1=CC=C(C=C1)C=O | 4661376 | 2 | -2004 | -6.26 | ETHi-51 | 51 | CC1=CC=C(C=C1)C(C)=O | 4829690 | 1 | -1958 | -7.34 |
| ETHi-11 | 11 | [H]C(=O)C(O)C1=CC=C(C=C1)C=O | 459585 | 1 | -1954 | -6.02 | ETHi-52 | 52 | CC1=CC=C(C=C1)C(C)=O | 4827180 | 0 | -1988 | -7.16 |
| ETHi-12 | 12 | OC1=CC=C(C=C1)C=C1C=O | 138005 | 0 | -1955 | -7.21 | ETHi-53 | 53 | CC1=CC=C(C=C1)C(C)=O | 3886373 | 0 | -1995 | -7.41 |
| ETHi-13 | 13 | CC1=CC=C(C=C1)C=C1C=O | 74882511 | 0 | -1996 | -7.39 | ETHi-54 | 54 | CC1=CC=C(C=C1)C(C)=O | 5179811 | 0 | -1954 | -7.73 |
| ETHi-14 | 14 | CC1=CC=C(C=C1)C=C1C=O | 4366209 | 0 | -1940 | -7.11 | ETHi-55 | 55 | CN1C(O)C(C)=O | 7187007 | 1 | -2048 | -7.55 |
| ETHi-15 | 15 | C1=CC=C(C=C1)C=C1C=O | 154739961 | 0 | -1972 | -7.17 | ETHi-56 | 56 | CC1=CC=C(C=C1)C(C)=O | 520301 | 0 | -1995 | -7.24 |
| ETHi-16 | 16 | O=C1C=CC(=C1)C=C1C=O | 518917 | 0 | -1963 | -7.26 | ETHi-57 | 57 | CN1C(O)C(C)=O | 671273 | 0 | -2117 | -7.33 |
| ETHi-17 | 17 | CN1C(O)C(C)=O | 2783882 | 0 | -2005 | -6.96 | ETHi-58 | 58 | CN1C(O)C(C)=O | 7424300 | 0 | -2028 | -7.34 |
| ETHi-18 | 18 | CC(=O)C(O)C1=CC=C(C=C1)C=O | 240733 | 0 | -2000 | -6.26 | ETHi-59 | 59 | CN1C(O)C(C)=O | 56817789 | 0 | -1943 | -7.03 |
| ETHi-19 | 19 | CC(=O)C(O)C1=CC=C(C=C1)C=O | 270038 | 0 | -1947 | -6.80 | ETHi-60 | 60 | CC1=CC=C(C=C1)C(C)=O | 4842748 | 0 | -1988 | -6.90 |
| ETHi-20 | 20 | O=C1C=CC(=C1)C=C1C=O | 948375 | 0 | -1965 | -7.56 | ETHi-61 | 61 | CC1=CC=C(C=C1)C(C)=O | 4034774 | 0 | -1985 | -7.50 |
| ETHi-21 | 21 | CN1C(O)C(C)=O | 1487848 | 0 | -2108 | -7.21 | ETHi-62 | 62 | CN1C(O)C(C)=O | 2109821 | 0 | -2129 | -7.59 |
| ETHi-22 | 22 | CC(=O)C(O)C1=CC=C(C=C1)C=O | 9932496 | 0 | -1937 | -6.88 | ETHi-63 | 63 | CC1=CC=C(C=C1)C(C)=O | 4850886 | 0 | -1967 | -7.54 |
| ETHi-23 | 23 | CC(=O)C(O)C1=CC=C(C=C1)C=O | 12414561 | 0 | -2000 | -6.56 | ETHi-64 | 64 | CC1=CC=C(C=C1)C(C)=O | 4038300 | 0 | -1941 | -6.13 |
| ETHi-24 | 24 | CC1=CC=C(C=C1)C=C1C=O | 788371 | 0 | -1991 | -7.12 | ETHi-65 | 65 | CC(=O)C(O)C1=CC=C(C=C1)C=O | 72914501 | 0 | -2009 | -7.28 |
| ETHi-25 | 25 | CC1=CC=C(C=C1)C=C1C=O | 5961012 | 0 | -1928 | -8.13 | ETHi-66 | 66 | CC1=CC=C(C=C1)C(C)=O | 2109710 | 0 | -1928 | -7.08 |
| ETHi-26 | 26 | O=C1C=CC(=C1)C=C1C=O | 13359793 | 0 | -1957 | -6.78 | ETHi-67 | 67 | CC(=O)C(O)C1=CC=C(C=C1)C=O | 2834747 | 0 | -2022 | -6.83 |
| ETHi-27 | 27 | O=C1C=CC(=C1)C=C1C=O | 13989020 | 0 | -1570 | -6.86 | ETHi-68 | 68 | CC1=CC=C(C=C1)C(C)=O | 1116074 | 0 | -1969 | -7.82 |
| ETHi-28 | 28 | CC1=CC=C(C=C1)C=C1C=O | 1123127 | 0 | -1952 | -7.87 | ETHi-69 | 69 | CN1C(O)C(C)=O | 2437215 | 0 | -2035 | -8.12 |
| ETHi-29 | 29 | CC1=CC=C(C=C1)C=C1C=O | 45835562 | 0 | -1995 | -7.31 | ETHi-70 | 70 | CC1=CC=C(C=C1)C(C)=O | 72839790 | 0 | -1999 | -7.80 |
| ETHi-30 | 30 | CN1C(O)C(C)=O | 179008 | 0 | -2101 | -7.54 | ETHi-71 | 71 | CC1=CC=C(C=C1)C(C)=O | 5023048 | 0 | -1995 | -7.52 |
| ETHi-31 | 31 | CC1=CC=C(C=C1)C=C1C=O | 6082788 | 0 | -1927 | -7.18 | ETHi-72 | 72 | CC1=CC=C(C=C1)C(C)=O | 72079420 | 0 | -2002 | -7.60 |
| ETHi-32 | 32 | CC1=CC=C(C=C1)C=C1C=O | 18278259 | 0 | -1995 | -7.31 | ETHi-73 | 73 | CC1=CC=C(C=C1)C(C)=O | 72079415 | 0 | -1995 | -7.48 |
| ETHi-33 | 33 | CC1=CC=C(C=C1)C=C1C=O | 1787203 | 0 | -1966 | -6.67 | ETHi-74 | 74 | CC1=CC=C(C=C1)C(C)=O | 72079384 | 0 | -1996 | -7.78 |
| ETHi-34 | 34 | CC1=CC=C(C=C1)C=C1C=O | 1478699 | 0 | -1969 | -7.04 | ETHi-75 | 75 | CC1=CC=C(C=C1)C(C)=O | 4867013 | 0 | -1990 | -7.91 |
| ETHi-35 | 35 | CC1=CC=C(C=C1)C=C1C=O | 3611702 | 0 | -1920 | -7.61 | ETHi-76 | 76 | CC1=CC=C(C=C1)C(C)=O | 3932217 | 0 | -1988 | -7.76 |
| ETHi-36 | 36 | CC(=O)C(O)C1=CC=C(C=C1)C=O | 2786945 | 0 | -2002 | -6.47 | ETHi-77 | 77 | O=C1C=CC(=C1)C=C1C=O | 2990624 | 0 | -1959 | -6.55 |
| ETHi-37 | 37 | CC1=CC=C(C=C1)C=C1C=O | 1226045 | 0 | -1974 | -6.98 | ETHi-78 | 78 | O=C1C=CC(=C1)C=C1C=O | 4853743 | 0 | -1977 | -7.56 |
| ETHi-38 | 38 | O=C1C=CC(=C1)C=C1C=O | 307506 | 1 | -1996 | -7.03 | ETHi-79 | 79 | CC1=CC=C(C=C1)C(C)=O | 3544001 | 0 | -2001 | -6.70 |
| ETHi-39 | 39 | CC1=CC=C(C=C1)C=C1C=O | 5957910 | 0 | -1917 | -7.74 | ETHi-80 | 80 | CC1=CC=C(C=C1)C(C)=O | 5953811 | 0 | -1991 | -7.69 |
| ETHi-40 | 40 | CC(=O)C(O)C1=CC=C(C=C1)C=O | 2834748 | 0 | -2021 | -7.08 | ETHi-81 | 81 | CC1=CC=C(C=C1)C(C)=O | 750021 | 0 | -2025 | -7.32 |
| ETHi-41 | 41 | CN1C(O)C(C)=O | 3800750 | 0 | -2049 | -6.91 | ETHi-82 | 82 | CC1=CC=C(C=C1)C(C)=O | 743463 | 0 | -1948 | -7.38 |
| | | | | | | | ETHi-83 | 83 | CC1=CC=C(C=C1)C(C)=O | 4585549 | 0 | -2009 | -7.22 |
| | | | | | | | ETHi-84 | 84 | CC1=CC=C(C=C1)C(C)=O | 788371 | 0 | -1989 | -7.25 |

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Supplementary Figure S12: *In silico* analysis of novel compounds (ETHi) binding to *Petunia Aco*, showing similes, Pubchem ID, binding pocket, full fitness, delta G for 83 compounds related to MyA. This supplementary figure refers to main Figure 7.

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| Paper name | No. | Activity* | MolPort ID | IUPAC Name |
|------------|-----|-----------|-------------|---|
| ETHi-57-1 | 1 | - | 002-088-741 | 1,3-dimethyl-5-[2-oxo-2-(piperidin-1-yl)ethyl]-1,3,5-triazinane-2,4,6-trione |
| ETHi-28-2 | 2 | - | 001-545-115 | N-[(4-chlorophenyl)methyl]-2,6-dimethoxybenzamide |
| ETHi-46-3 | 3 | - | 000-165-364 | (2E)-1-(2-hydroxy-4,6-dimethoxyphenyl)-3-phenylprop-2-en-1-one |
| ETHi-21-4 | 4 | - | 002-852-891 | 1,3-dimethyl-5-(3-oxobutanoyl)-1,3-diazinane-2,4,6-trione |
| ETHi-70-5 | 5 | - | 028-724-729 | 2-(5,5-dimethyl-2,4-dioxo-1,3-oxazolidin-3-yl)-N-[(2-methoxyphenyl)methyl]acetamide |
| ETHi-68-6 | 6 | - | 001-893-172 | 2-[(2E)-3-(4-iodophenyl)prop-2-enoyl]-2,3-dihydro-1H-indene-1,3-dione |
| ETHi-77-7 | 7 | * | 000-263-283 | 5-benzoyl-4,5,6,7-tetrahydro-1-benzofuran-4-one |
| ETHi-67-8 | 8 | * | 002-137-080 | methyl 5-acetyl-2,2-dimethyl-4,6-dioxocyclohexane-1-carboxylate |
| ETHi-62-9 | 9 | - | 004-271-067 | 6-amino-1,3-dimethyl-5-[2-[(1-methyl-1H-1,2,3,4-tetrazol-5-yl)sulfanyl]acetyl]-1,2,3,4-tetrahydropyrimidine-2,4-dione |
| ETHi-69-10 | 10 | * | 028-826-119 | 6-amino-5-[2-[(3,4-dichlorophenyl)amino]acetyl]-1,3-dimethyl-1,2,3,4-tetrahydropyrimidine-2,4-dione |
| ETHi-54-11 | 11 | ** | 044-278-648 | 2,6-dimethoxyphenyl 3-(5-methylfuran-2-yl)prop-2-enoate |
| ETHi-74-12 | 12 | *** | 044-251-366 | 3-[3-(3-bromo-4-fluorophenyl)prop-2-enoyl]-6-methyl-3,4-dihydro-2H-pyran-2,4-dione |
| ETHi-72-13 | 13 | ND | 044-250-800 | 3-[3-(3-chlorophenyl)prop-2-enoyl]-6-methyl-3,4-dihydro-2H-pyran-2,4-dione |
| ETHi-25-14 | 14 | - | 000-658-456 | (2E)-3-(4-chlorophenyl)-1-(2,4,6-trimethoxyphenyl)prop-2-en-1-one |
| ETHi-39-15 | 15 | ND | 003-803-656 | (2E)-3-(2-chlorophenyl)-1-(2,4,6-trimethoxyphenyl)prop-2-en-1-one |
| ETHi-84-16 | 16 | *** | 002-137-086 | 5,5-dimethyl-2-(2-phenylacetyl)cyclohexane-1,3-dione |

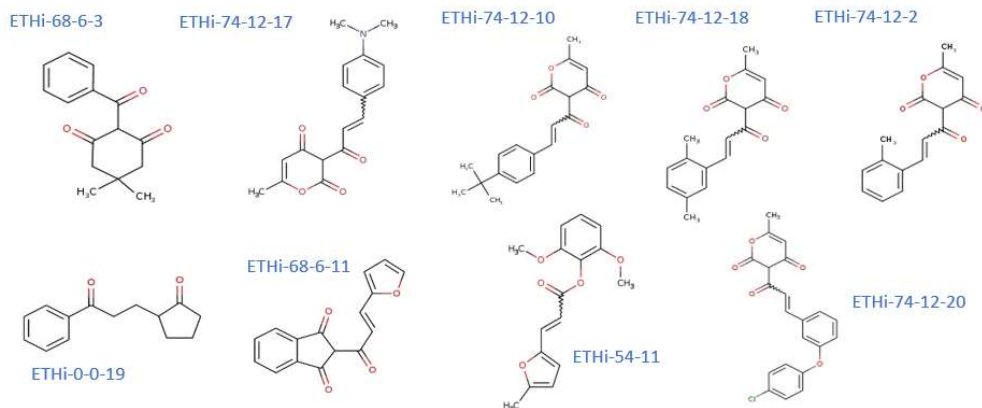
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Supplementary Figure S13: Bioactivity analysis of novel ethylene inhibitors (ETHi) for efficacy in reducing the phenotype identified in blocking ethylene production in *D. discoideum*. Compounds predicted by molecular modelling to bind to the plant ACO enzyme (Supplementary Fig. 7) were tested for developmental delay effects in *D. discoideum*, to mimic that shown for established ethylene inhibitors and MyA, blocking development at the mound stage, where “**” = delayed to mounds (MyA-like), “***” = mostly mounds several breakthrough, “**” = less delayed development and “-” = no effect on development. Compounds are listed as preliminary names, activity related to inhibition of development, MolPort ID, and IUPAC name. This supplementary figure refers to main Figure 7.**

178A.

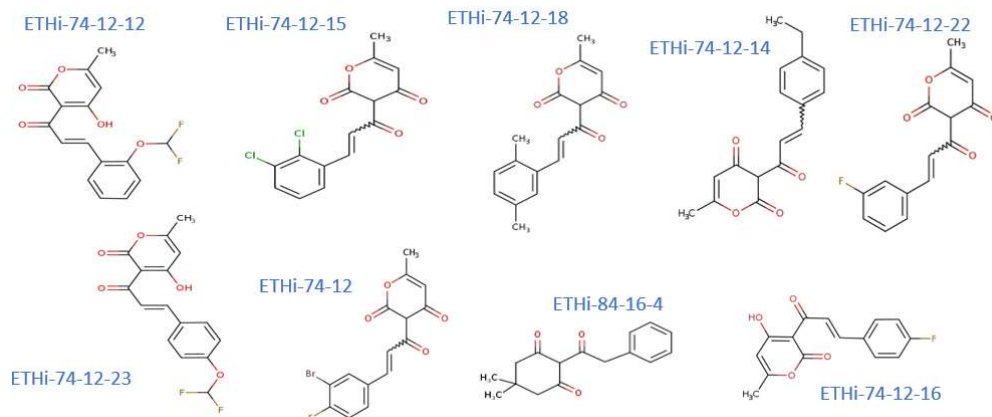
| Paper name | No. | Activity* | SMILES | MolPort id | IUPAC Name |
|---------------|-----|-----------|---|-------------|--|
| MyA | | *** | CC1C(=O)C(C(=O)C(C1=O)C(C)C(=O)CCC=C=CC=C2 | | 2,2,4-trimethyl-6-[3-phenylpropoxy]cyclohexane-1,3,5-trione |
| ETHi-74-12-1 | 1 | - | COc1ccc(C=C(C(=O)C2C(=O)OC(C)=CC2=O)cc1OC | 044-402-240 | 3-[3-(3,4-dimethoxyphenyl)prop-2-enoyl]-6-methyl-3,4-dihydro-2H-pyran-2,4-dione |
| ETHi-74-12-2 | 2 | ** | CC1=C(C(=O)C(C(=O)C=C2c1ccc2)C(=O)O1 | 044-250-811 | 6-methyl-3-[3-(2-methylphenyl)prop-2-enoyl]-3,4-dihydro-2H-pyran-2,4-dione |
| ETHi-68-6-3 | 3 | - | O=C(C(=C1c1ccccc1)C1C(=O)c2ccccc2)C1=O | 002-577-535 | 2-[(2E)-3-phenylprop-2-enoyl]-2,3-dihydro-1H-indene-1,3-dione |
| ETHi-84-16-4 | 4 | *** | CC1(C)CC(=O)C(C(=O)C2c1ccc2)C(=O)C1 | 002-137-086 | 5,5-dimethyl-2-(2-phenylacetyl)cyclohexane-1,3-dione |
| ETHi-68-6-5 | 5 | - | Cc1ccc(C(=C(C(=O)C2C(=O)c3ccccc3C2=O)cc1 | 002-161-837 | 2-[(2E)-3-(4-methylphenyl)prop-2-enoyl]-2,3-dihydro-1H-indene-1,3-dione |
| ETHi-68-6-6 | 6 | - | O=C(C(=C2c1ccc2)C(=O)c2ccccc12 | 000-489-111 | 2-(phenylmethylidene)-2,3-dihydro-1H-indene-1,3-dione |
| ETHi-68-6-3 | 7 | ** | CC1(C)C(=O)C(C(=O)c2ccccc2)C(=O)C1 | 001-957-279 | 2-benzoyl-5,5-dimethylcyclohexane-1,3-dione |
| ETHi-0-0-28 | 28 | - | CC1(CCCC1)C(=O)CCc1ccccc1 | 016-631-668 | 1-(1-methylcyclohexyl)-3-phenylpropan-1-one |
| ETHi-74-12-9 | 9 | - | CCOC1cc(C=C(C(=O)C2C(=O)OC(C)=CC2=O)ccc1OC | 044-251-050 | 3-[3-(3-ethoxy-4-methoxyphenyl)prop-2-enoyl]-6-methyl-3,4-dihydro-2H-pyran-2,4-dione |
| ETHi-74-12-10 | 10 | ** | CC1=C(C(=O)C(C(=O)C=C2cc1ccc2)C(C)C(C(=O)O)1 | 044-250-863 | 3-[3-(4-tert-butylphenyl)prop-2-enoyl]-6-methyl-3,4-dihydro-2H-pyran-2,4-dione |
| ETHi-68-6-11 | 11 | ** | O=C(C(=C1c1ccc1)C1C(=O)c2ccccc2)C1=O | 001-917-147 | 2-[(2E)-3-(furan-2-yl)prop-2-enoyl]-2,3-dihydro-1H-indene-1,3-dione |
| ETHi-74-12-12 | 12 | *** | Cc1ccc(O)c(C(=O)C=C2c1ccc2O(C(F)F)C(=O)O)1 | 002-759-778 | 3-[(2E)-3-[2-(difluoromethoxy)phenyl]prop-2-enoyl]-4-hydroxy-6-methyl-2H-pyran-2-one |
| ETHi-0-0-13 | 13 | - | Cc1ccc(cc1)C(=O)CCCCC1=O | 000-805-492 | 2-[3-(4-methylphenyl)-3-oxopropyl]cyclohexan-1-one |
| ETHi-74-12-14 | 14 | *** | CCc1ccc(C=C(C(=O)C2C(=O)OC(C)=CC2=O)cc1 | 044-250-851 | 3-[3-(4-ethylphenyl)prop-2-enoyl]-6-methyl-3,4-dihydro-2H-pyran-2,4-dione |
| ETHi-74-12-15 | 15 | *** | CC1=C(C(=O)C(C(=O)C=C2cc1ccc2)C2C(C)C(=O)O1 | 044-250-754 | 3-[3-(2,3-dichlorophenyl)prop-2-enoyl]-6-methyl-3,4-dihydro-2H-pyran-2,4-dione |
| ETHi-74-12-16 | 16 | ** | CC1=C(C(=O)C(C(=O)C=C2cc(F)cc2)C(=O)O)1 | 005-287-602 | 3-[(2E)-3-(4-fluorophenyl)prop-2-enoyl]-4-hydroxy-6-methyl-2H-pyran-2-one |
| ETHi-74-12-17 | 17 | ** | CN(C)c1ccc(C=C(C(=O)C2C(=O)OC(C)=CC2=O)cc1 | 044-250-746 | 3-[3-[4-(dimethylamino)phenyl]prop-2-enoyl]-6-methyl-3,4-dihydro-2H-pyran-2,4-dione |
| ETHi-74-12-18 | 18 | ** | CC1=C(C(=O)C(C(=O)C=C2cc(F)cc2)C(=O)O)1 | 044-207-442 | 3-[3-(2,5-dimethylphenyl)prop-2-enoyl]-6-methyl-3,4-dihydro-2H-pyran-2,4-dione |
| ETHi-0-0-19 | 19 | - | O=C(C(C1CCC1=O)C)C1=O | 001-782-962 | 2-(3-oxo-3-phenylpropyl)cyclopentan-1-one |
| ETHi-74-12-20 | 20 | ** | CC1=C(C(=O)C(C(=O)C=C2c1ccc(O)c3cc(O)cc3)C2(=O)O1 | 044-250-673 | 3-[3-(3-(4-chlorophenoxyl)phenyl)prop-2-enoyl]-6-methyl-3,4-dihydro-2H-pyran-2,4-dione |
| ETHi-74-12-21 | 21 | - | CC1=C(C(=O)C(C(=O)C=C2c1ccc(C)cc2)C(=O)O1 | 044-250-849 | 6-methyl-3-[3-(3-methylphenyl)prop-2-enoyl]-3,4-dihydro-2H-pyran-2,4-dione |
| ETHi-74-12-22 | 22 | *** | CC1=C(C(=O)C(C(=O)C=C2c1ccc(F)cc2)C(=O)O1 | 044-250-804 | 3-[3-(3-fluorophenyl)prop-2-enoyl]-6-methyl-3,4-dihydro-2H-pyran-2,4-dione |
| ETHi-74-12-23 | 23 | *** | Cc1ccc(O)c(C(=O)C=C2c1ccc(O)C(F)cc2)C(=O)O)1 | 017-264-417 | 3-[(2E)-3-[4-(difluoromethoxy)phenyl]prop-2-enoyl]-4-hydroxy-6-methyl-2H-pyran-2-one |
| ETHi-74-12-24 | 24 | - | CC1(C)C(=O)C(C(=O)C2c1ccc2)C(=O)C1 | 004-947-021 | 2-benzyl-5,5-dimethylcyclohexane-1,3-dione |
| ETHi-74-12-25 | 25 | - | CC1=C(C(=O)C(C(=O)C=C2c1ccc(cc2)[N+](=O)C(=O)O)1 | 046-695-572 | 6-methyl-3-[(2E)-3-(4-nitrophenyl)prop-2-enoyl]-3,4-dihydro-2H-pyran-2,4-dione |
| ETHi-74-12-26 | 26 | - | CC1=C(C(=O)C(C(=O)C=C2c1ccc2)C(=O)O)1 | 003-285-478 | 6-methyl-3-[(2E)-3-phenylprop-2-enoyl]-3,4-dihydro-2H-pyran-2,4-dione |
| ETHi-68-6-27 | 27 | - | O=C1(C)C(C2c1ccc2)C(=O)c1ccccc1 | 001-835-266 | (2E)-2-(phenylmethylidene)-2,3-dihydro-1H-inden-1-one |

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181C.



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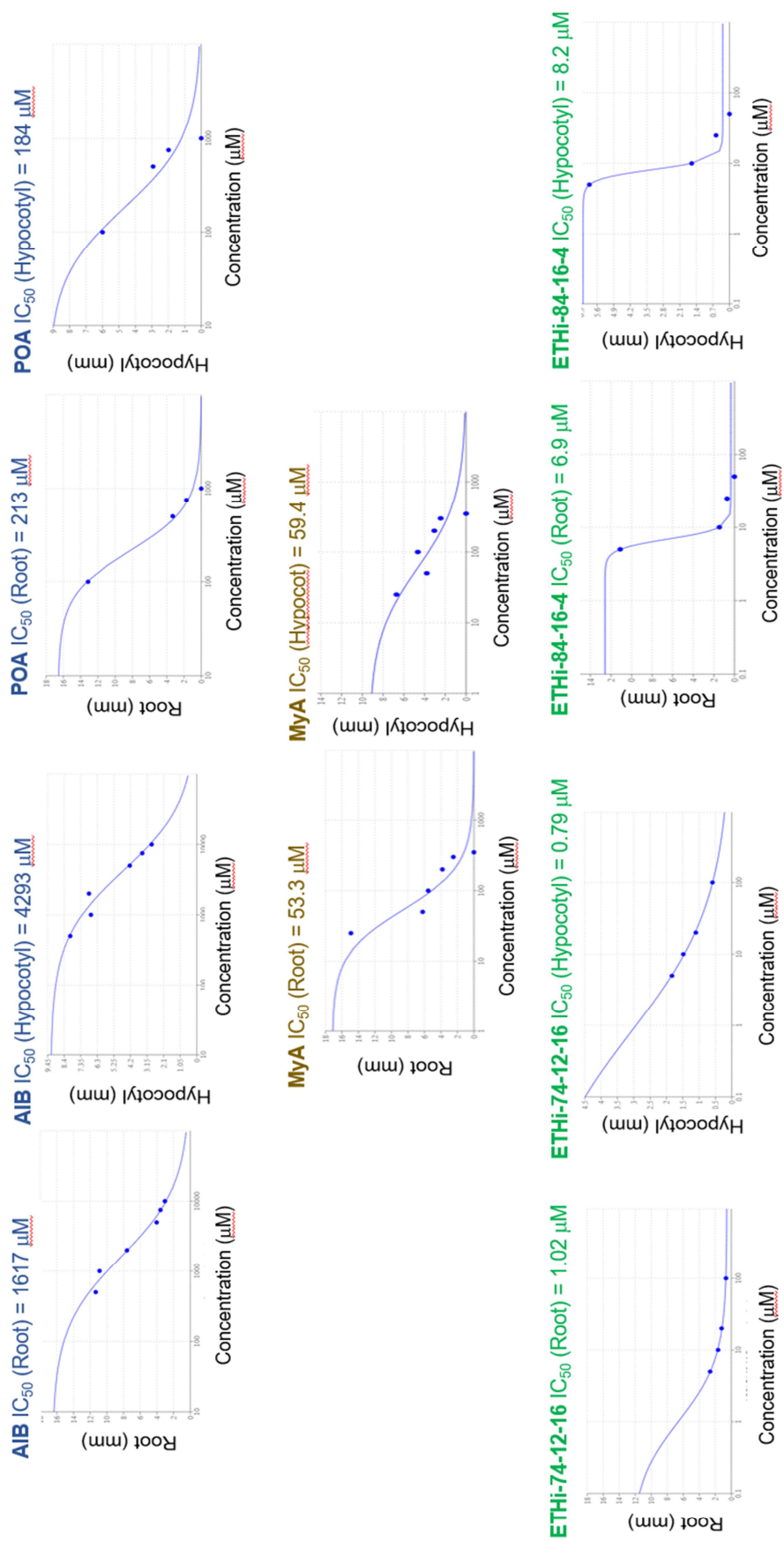
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Supplementary Figure S14: Second round of bioactivity analysis of novel ethylene inhibitors (ETHi) for efficacy in reducing the phenotype identified in blocking ethylene production in *D. discoideum*. A. Compounds identified in the first round of testing (Sup. Fig. 13) were used to identify related compounds, that were tested for developmental delay effects in *D. discoideum*, to

187 *mimic that shown for established ethylene inhibitors and MyA, blocking development at the*
188 *mound stage, where '****' = delayed to mounds (MyA-like) , '***' = mostly mounds several*
189 *breakthrough, '**' = less delayed development and '-' = no effect on development. Compounds are*
190 *listed as preliminary names, activity related to inhibition of development, MolPort ID, and IUPAC*
191 *name. B. and C. Compound structures are indicated for highly potent and intermediary potency*
192 *respectively. This supplementary figure refers to main Figure 7.*



Supplementary Figure S15: Comparison root and hypocotyl extension inhibition of established ACO inhibitors, MyA and novel compounds in *A. thaliana* seedlings. This supplementary figure refers to main Figure 8.