A substrate of the ABC transporter PEN3 stimulates bacterial flagellin (flg22)-induced callose deposition in *Arabidopsis thaliana*

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**Supporting Information**

Supporting Figure S1: CID spectra of 4-methoxyindol-3-yl methanol and S-(4-methoxy-indol-3-ylmethyl) cysteine.

Supporting Figure S2: Scatter plots of the data shown in Fig. 3

Supporting Figure S3: Scatter plots of the data shown in Fig. 4

Table S1: Tentatively annotated features that were detected in the spore suspension without contact to leaves.

Table S2: Tentatively annotated features that accumulated to significantly higher levels in spore suspension droplets recollected from *pen3* mutants compared to WT.
Figure S1: Mass spectrum of 4-methoxy-indol-3-yl methanol.

A, MS adducts and fragments

B, MS2 of quantifier ion (MRM mode)

C, Fragmentation pattern (MRM mode) and possible fragments of S-(4-methoxy-indol-3-ylmethyl)cysteine

D, magnification of C
Supporting Figure S2: Scatter plots of the data shown in Fig. 3
Supporting Figure S3: Scatter plots of the data shown in Fig. 4
### Table S1: Tentatively annotated features that were detected in the spore suspension without contact to leaves. Annotation according to an in-house analyte list using m/z ratio and retention time. Allowed deviation: 15mDa, RT 0.2min. The feature table was acquired from the merged four repetitions containing measurements of 4 spore suspension samples. All features with a summed up intensity \(\geq 2000\) in the four spore suspension samples were taken into consideration.

| feature                          | m/z meas. | M meas. | RT [min] |
|----------------------------------|-----------|---------|----------|
| L-arginine                       | 175.11808 | 174.1108| 0.4      |
| D-glucose-6-phosphate RT1        | 283.03293 | 282.02565| 0.41     |
| D-glucurono-3,6-lactone, adduct  | 184.04966 | 366.08476| 0.43     |
| shikimic acid (dimer)            | 175.05054 | 348.08653| 0.43     |
| D-galacturonic acid, dimer       | 411.06729 | 410.06002| 0.43     |
| D-xylitol, xylite                | 175.05609 | 174.04882| 0.43     |
| L-aspartic acid, dimer           | 156.04064 | 310.06672| 0.44     |
| fructose, dimer                  | 383.11141 | 382.10413| 0.44     |
| ureidosuccinic acid, dimer RT1   | 375.08929 | 374.08201| 0.44     |
| myo-inositol                     | 219.02651 | 180.06327| 0.44     |
| betaine, trimethylglycine; dimer | 235.16428 | 234.157  | 0.46     |
| D(-)-raffinose                   | 543.13193 | 504.16826| 0.46     |
| D-sucrose                        | 365.10357 | 364.09629| 0.46     |
| L-valine                         | 118.08387 | 117.07659| 0.46     |
| L-proline                        | 138.05346 | 115.06265| 0.47     |
| L-cysteine, dimer                | 263.02117 | 262.01389| 0.48     |
| pshikimic acid, dimer            | 156.04064 | 310.06672| 0.44     |
| adduct of ascorbic acid, charge 2+| 230.99011 | 192.02729| 0.49     |
| L-proline + H2O                  | 130.08402 | 129.07674| 0.48     |
| cytidine RT1                     | 244.09146 | 243.08418| 0.48     |
| L-methionine, dimer              | 321.09147 | 320.08419| 0.48     |
| D-galacturonic acid, dimer       | 206.02689 | 410.03975| 0.49     |
| nicotinic acid - dimer           | 247.07374 | 246.06646| 0.49     |
| adduct of ascorbic acid, charge 2+| 230.99011 | 192.02729| 0.49     |
| L-proline + H2O                  | 130.04975 | 129.04247| 0.49     |
| uridine RT1                      | 245.07036 | 244.06308| 0.49     |
| L(-)-glutamic acid               | 148.05935 | 147.05207| 0.49     |
| D(-)-ribose, adduct              | 265.08538 | 264.0781 | 0.49     |
| 3-hydroxy-3-methylglutaric acid  | 163.05979 | 162.05251| 0.5      |
| L-methionine, dimer              | 321.09121 | 320.08393| 0.52     |
| D-sucrose                        | 343.1219  | 342.11462| 0.56     |
| D-glucronic acid, dimer          | 208.04233 | 414.07011| 0.57     |
| L-aspartic acid, dimer           | 267.07034 | 266.06306| 0.58     |
| L-glutamic acid, dimer           | 170.02877 | 338.04299| 0.59     |
| ureidosuccinic acid; dimer RT2   | 353.09132 | 352.08404| 0.6      |
| alpha-ketoglutaric acid          | 147.03136 | 146.02408| 0.62     |
| D-sucrose RT2                    | 325.11249 | 342.11607| 0.62     |
| uridine RT2                      | 245.07147 | 244.06419| 0.62     |
| D-sucrose RT2                    | 343.12374 | 342.11646| 0.62     |
| ureidosuccinic acid              | 199.03734 | 198.03007| 0.63     |
| cytidine 5'-monophosphate        | 324.06385 | 323.05658| 0.63     |
| Compound                                                                 | Mass 1 | Mass 2  | Error  |
|-------------------------------------------------------------------------|--------|---------|--------|
| D-galacturonic acid, dimer                                               | 206.02738 | 410.0402 | 0.63   |
| cytosine                                                                | 112.04865 | 111.04137 | 0.63   |
| D-glucose 6-phosphate RT2                                               | 261.03338 | 260.0261 | 0.63   |
| uric acid                                                               | 169.03559 | 168.02831 | 0.64   |
| citric acid                                                             | 230.99024 | 192.02691 | 0.64   |
| nicotinic acid, niacin                                                  | 124.03673 | 123.02946 | 0.64   |
| cytidine RT2                                                            | 244.0919 | 243.08462 | 0.64   |
| adenosine 5'-monophosphate (AMP), RT2                                   | 348.0686 | 347.06133 | 0.64   |
| xanthine, 2,6-dihydropurine                                             | 153.03921 | 152.03194 | 0.65   |
| glutathione                                                             | 308.09017 | 307.08289 | 0.65   |
| uracil                                                                  | 113.03346 | 112.02618 | 0.66   |
| L-proline + H2O                                                         | 130.04903 | 129.04175 | 0.66   |
| nicotinic acid - dimer                                                  | 247.07443 | 246.06715 | 0.66   |
| uridine RT2                                                             | 245.07771 | 244.07043 | 0.66   |
| L-tyrosine, peak 2, -NH2                                                | 165.05419 | 164.04691 | 0.68   |
| L-tyrosine                                                              | 182.0808 | 181.07353 | 0.68   |
| adenosine, peak 2                                                       | 268.10326 | 267.09598 | 0.69   |
| 3,4-dihydroxyphenylalanine, L-DOPA, RT2                                 | 198.07287 | 197.0656  | 0.7    |
| hypoxanthine                                                            | 137.04486 | 136.03759 | 0.76   |
| guanosine                                                               | 284.0993 | 283.09203 | 0.76   |
| inosine                                                                 | 269.09146 | 268.08419 | 0.78   |
| L-leucine                                                               | 132.10024 | 131.09297 | 0.78   |
| cis-aconitic acid                                                       | 175.02604 | 174.01877 | 0.81   |
| hypoxanthine; 6-hydroxypurine, dimer                                     | 295.07341 | 294.06614 | 0.84   |
| 2-O-methyladenosine                                                     | 282.11743 | 281.11015 | 1.01   |
| phenylalanine, in-source fragment                                        | 120.07876 | 119.07149 | 1.23   |
| L-phenylalanine                                                         | 166.08518 | 165.07791 | 1.24   |
| D-pantothenic acid                                                      | 220.11746 | 219.11019 | 1.57   |
| D-panthenol                                                             | 206.13806 | 205.13078 | 1.59   |
| H-Leu-Pro-OH, leucyl-proline                                            | 229.15413 | 228.14685 | 2.51   |
| H-Phe-Val-OH, phenylalanyl-valine                                        | 265.14299 | 264.13571 | 2.97   |
| kinetin (internal standard)                                             | 216.08761 | 215.08034 | 3      |
| beta-carbocine-3-carboxylic acid                                        | 217.09601 | 216.08873 | 3.1    |
| Ile-Phe, isoleucyl-phenylalanine, in-source fragment phenylalanine      | 166.0853 | 165.07803 | 3.81   |
| N-phenylacetyl-aspartic acid                                            | 252.08647 | 251.0792 | 4.1    |
| trans-sinapic acid                                                      | 207.06756 | 224.06954 | 4.52   |
| 3,4-dimethoxyhydrocinnamic acid                                        | 211.09379 | 210.08651 | 5.15   |
| herniarin, 7-methoxycoumarin                                             | 177.05472 | 176.04744 | 6.01   |
| IAA-valine; IAA-Val; Indole-3-acetyl-valine (internal standard)         | 297.12012 | 274.13108 | 6.22   |
| apigenin                                                                | 271.05889 | 270.05161 | 6.79   |
| corchorifatty acid F                                                    | 351.21336 | 328.22396 | 7.11   |
| 9,12,13-trihydroxyoctadec-10-enoic acid                                | 353.22862 | 330.23908 | 7.38   |
| biochanin (Na-Addukt) (internal standard)                               | 307.05667 | 284.06706 | 8.82   |
| biochanin A, 5,7-dihydroxy-4'-methoxyisoflavone (internal standard)     | 285.07504 | 284.06777 | 8.82   |
| Compound                                      | M/Z 1   | M/Z 2   | Retention Time |
|-----------------------------------------------|---------|---------|----------------|
| oxo-phytodienoic acid | 12-OPDA  | 293.21004 | 292.20271 | 10.26 |
| L-alpha-lecithin, in-source fragment      | 454.29081 | 453.28354 | 11.2          |
| lauric acid, n-dodecanoic acid             | 201.18387 | 200.1766 | 12.11         |
| eicosapentaenoic acid                      | 303.23051 | 302.22068 | 13.69         |
| myristic acid, tetradecanoic acid          | 229.21506 | 228.20735 | 13.78         |
| linoleic acid                               | 281.24635 | 280.23882 | 14.64         |
Table S2: Tentatively annotated features that accumulated to significantly higher levels in spore suspension droplets recollected from pen3 mutants compared to WT. Annotation according to an in-house analyte list using m/z ratio and retention time. Allowed deviation: 15mDa, RT 0.2min. The feature table was acquired from four repetitions containing measurements of 4 spore suspension samples each.

| feature                                      | m/z meas. | M meas. | RT [min] | fold change pen3/Col-0 |
|----------------------------------------------|-----------|---------|----------|------------------------|
| acetylagmatine                               | 173.13945 | 172.13217 | 0.48     | 5.0                    |
| 3,3 dimethylglutaric acid                    | 161.07041 | 160.06314 | 3.48     | 2.3                    |
| 1-O-sinapoyl-beta-glucose                    | 189.04766 | 206.05231 | 3.52     | 10.3                   |
| kaempferol 3-O-Rha(1-2)Glc 7-O-Rha           | 741.21924 | 740.21197 | 3.79     | 2.6                    |
| 5-hydroxycamalexin                           | 217.0428  | 216.03544 | 3.85     | 4.8                    |
| beta-d-glucosyl indole-3-carboxylate         | 306.09651 | 323.0984  | 4        | 2.8                    |
| scopoletin                                   | 193.04282 | 192.03555 | 4.29     | 5.2                    |
| kaempferol deoxyhex Hex                      | 595.16563 | 594.15836 | 4.33     | 2.0                    |
| unknown camalexin metabolite #1             | 217.04267 | 234.04616 | 4.43     | 14.7                   |
| kaempferitrin, kaempferol 3,7-di-O-alpha-Rha | 579.16939 | 578.16211 | 4.7      | 3.2                    |
| 1-methylindole-3-carboxylic acid (1Me-I3COOH) | 176.07081 | 175.06353 | 5.91     | 2.4                    |
| 8-methylsulphinoaryl                         | 266.12397 | 265.11667 | 5.95     | 10.6                   |
| 2-formamidophenyl-2'-thiazolylketone         | 233.03819 | 232.03055 | 6.00     | 3.2                    |
| (-)-jasmonic acid                            | 211.13333 | 210.12587 | 6.74     | 7.5                    |