Supplemental Data for:

A phenotype-directed chemical screen identifies ponalrestat as an inhibitor of the plant flavin monooxygenase YUCCA in auxin biosynthesis

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Running title: Ponalrestat is an inhibitor of YUCCA in *Arabidopsis*

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Supplemental Data included:

- **Supplemental figures (Figure S1-6)**
- **Supplemental text (File S1)**
- **Supplemental reference**

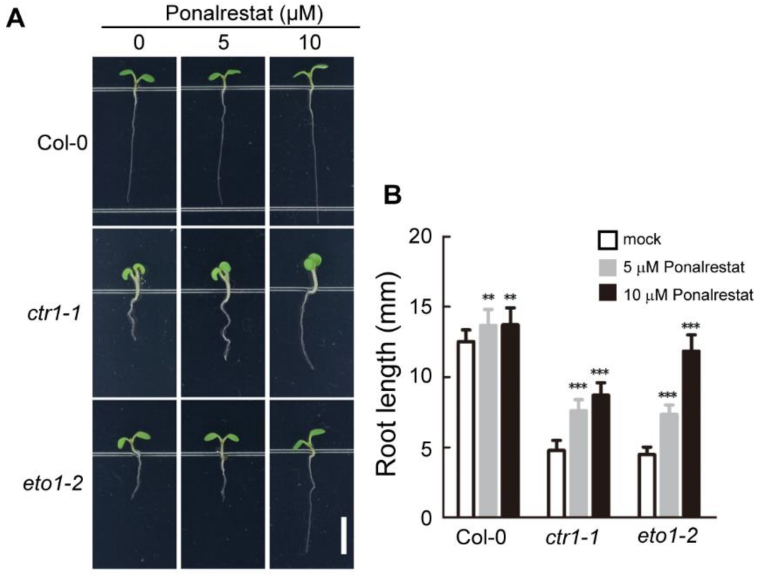

Figure S1. Ponalrestat promotes root elongation in de-etiolated seedlings. (A) Representative five-day-old Col-0, *ctr1-1*, and *eto1-2* seedlings in response to ponalrestat treatment. Scale bar = 5 mm. (B) Quantification of the primary root lengths of the five-day-old seedlings shown in (A). Bars represent means ± SD of at least 10 seedlings; a Student's *t*-test was used to compare ponalrestat-treated and mock-treated seedlings (***$P<0.001$). The experiment was repeated for at least three times with similar results.

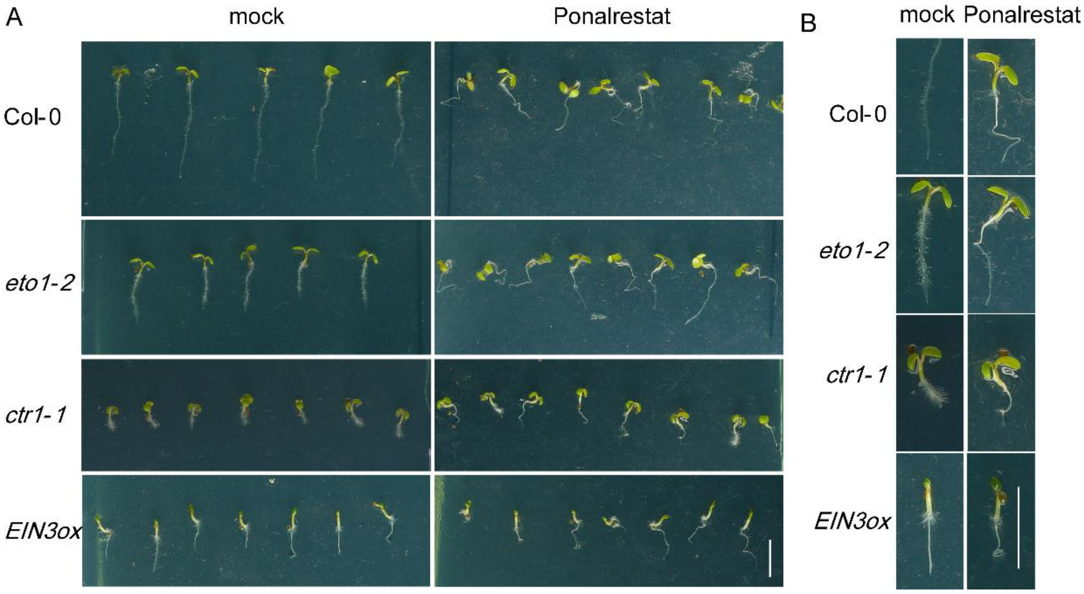

Figure S2. Ponalrestat attenuates the gravitropism of the primary root in ethylene-related mutants. After stratification for three days, the seeds were germinated and grown vertically for five days at 22 °C with light on 1/2 MS medium containing 10 μM ponalrestat or DMSO as the mock control. (A) Col-0 and mutant seedlings grown on vertical plates. (B) Representative seedlings from (A) showing the changes in root hair growth. Scale bars = 5 mm. The experiment was repeated for at least three times with similar results.

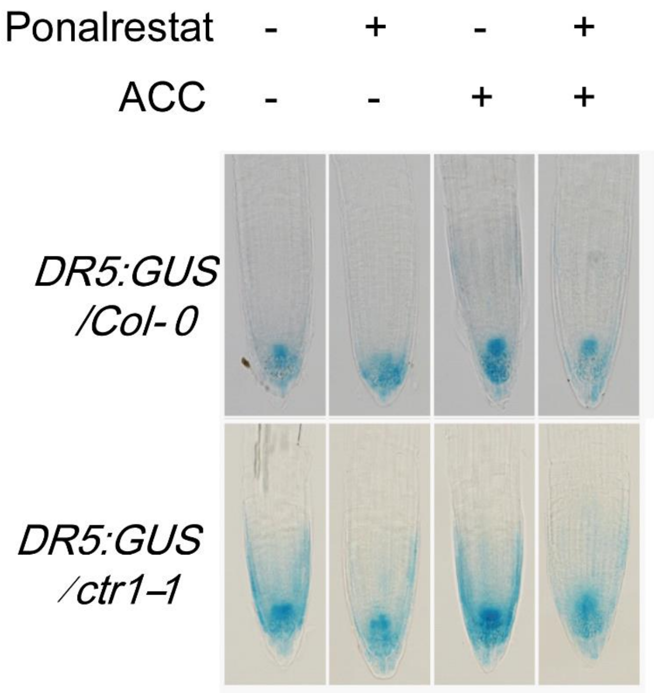

Figure S3. GUS staining of the *DR5:GUS* lines in the Col-0 or *ctr1-1* backgrounds. The seedlings were grown in the dark for three days on 1/2 MS medium containing ponalrestat (5 μM) and/or ACC (10 μM) or DMSO as the mock control followed by GUS staining. The experiment was repeated for at least three times with similar results.

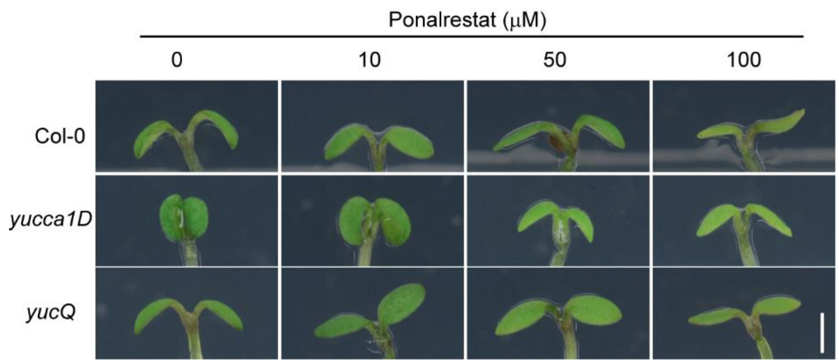

Figure S4. Observation of *YUC* mutants in response to ponalrestat.
Col-0, *yucca1D*, and *yucQ* seeds were germinated and grown for five days on 1/2 MS medium supplemented with ponalrestat. Scale bar = 2 mm. The experiment was repeated for at least three times with similar results.

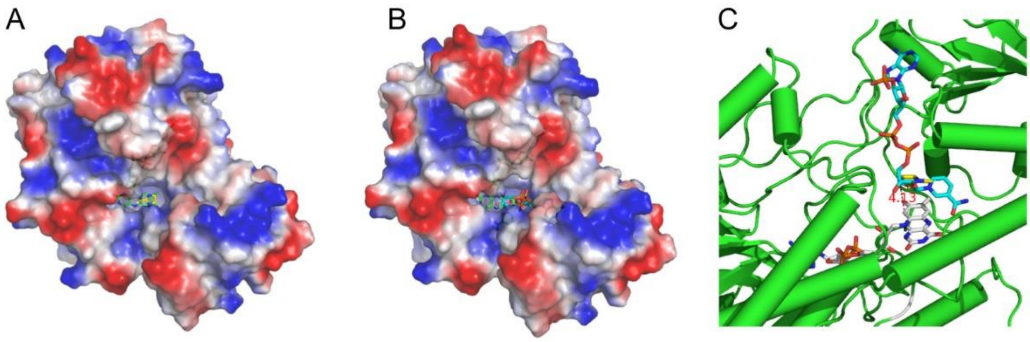

Figure S5. Structures of FMO and ligands. (A) Structure of FMO in complex with MET (in yellow) and FAD (in green) (PDB: 2GVC). (B) Structure of FMO in complex with NAD (in cyan) and FAD (in green) (PDB: 2GV8). (C) Stucture alignment of FMO (in green) in complex with MET (in yellow) and NAD (in cyan). The the distance from overlap site of FMO and MET to flavin group of FAD (in gray) is 4.13 Å indicated with red dash line.

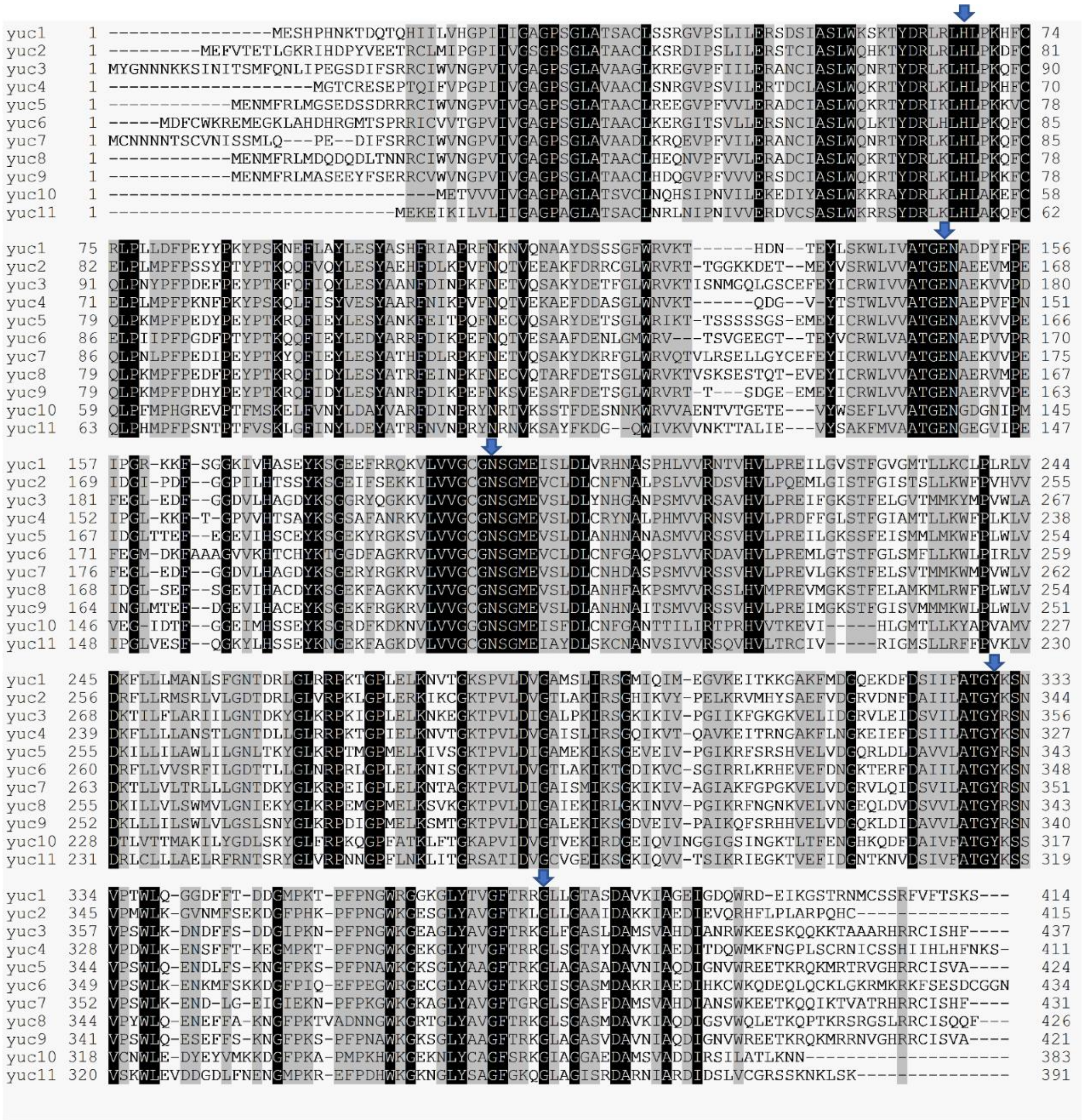

Figure S6. Sequence alignment of YUCCA family members. Blue arrowheads indicate conserved amino acids in the YUCCA family and that correspond to the key amino acids in the active enzymatic pocket.

File S1

The synthetic procedure was followed the published reference[1].
PRT:^{1}H-NMR (400 MHz, $CDCl_3$): δ = 12.70 (s br, 1H), 8.15 (d, *J* = 7.6 Hz, 1H), 7.99-7.87 (m, 3H), 7.56 (dd, *J* = 9.6, 1.6 Hz, 1H), 7.36 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.21 (t, *J* = 8.4 Hz, 1H), 5.34 (s, 2H), 3.99 (s, 2H); HRMS (ESI): m/z calcd for $C_{17}H_{13}BrFN_2O_3$ $[M+H]^+$: 391.00881, found 391.00888.

Compound **1**: ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 12.92 (s br, 1H), 8.28 (d, *J* = 7.6 Hz, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.96 (t, *J* = 7.6 Hz, 1H), 7.88 (t, *J* = 7.6 Hz, 1H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.34-7.31 (m, 2H), 7.15 (d, *J* = 8.0 Hz, 1H), 7.08 (t, *J* = 8.0 Hz, 1H), 6.98 (t, *J* = 8.0 Hz, 1H), 5.34 (d, *J* = 14.8 Hz, 1H), 5.25 (d, *J* = 14.8 Hz, 1H), 4.65 (t, *J* = 7.6 Hz, 1H), 3.31 (s peak is overlapped by water peak, 2H,); HRMS (ESI): m/z calcd for $C_{24}H_{17}Br_2F_2N_2O_3$ $[M+H]^+$: 576.95685, found 576.95715.

Compound **2**: ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.46 (d, *J* = 1.2 Hz, 1H), 7.84-7.69 (m ,3H), 7.26-7.21 (m, 3H), 5.41 (s, 2H), 4.18 (q, *J* = 7.2 Hz, 2H), 3.96 (s, 2H), 1.23 (t, *J* = 7.2 Hz, 3H); GC-MS (ESI): $C_{19}H_{17}BrFN_2O_3$ $[M+H]^+$: 419.1.

Compound **3**: ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 12.61 (s, 1H), 8.27 (d, *J* = 8.0 Hz, 1H), 7.95-7.84 (m, 3H), 3.95 (s, 2H).

Compound **4**: ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 12.69 (s br, 1H), 8.31 (d, *J* = 7.6 Hz, 1H), 7.98-7.87 (m, 3H), 7.32-7.26 (m, 5H), 5.32 (s, 2H), 4.00 (s, 2H).

Compound **5**: ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 12.72 (s br, 1H), 8.31 (d, *J* = 7.6 Hz, 1H), 7.99-7.87 (m, 3H), 7.36-7.24 (m, 2H), 7.04 (t, *J* = 8.4 Hz, 1H), 5.35 (s, 2H), 3.99 (s, 2H).

Compound **6**: ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 12.79 (s br, 1H), 8.31 (d, *J* = 8.0 Hz, 1H), 7.97-7.87 (m, 3H), 7.52 (d, *J* = 7.2 Hz, 2H), 7.28 (t, *J* = 7.6 Hz, 2H), 5.29 (s, 2H), 3.99 (s, 2H); HRMS (ESI): m/z calcd for $C_{17}H_{14}BrN_2O_3$ $[M+H]^+$: 373.01823, found 373.01848.

Compound **7**: ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 12.79 (s br, 1H), 8.31 (d, *J* = 7.6 Hz, 1H), 8.00-7.88 (m, 3H), 7.36-7.31 (m, 1H), 7.24-7.11 (m, 3H), 5.38 (s, 2H), 3.99 (s, 2H)

Supplemental reference

1. Sriam, D., Yogeeswari, P., Senthilkumar, P., Sangaraju, D., Nelli, R., Banerjee, D., Bhat, P., Manjashetty, H. (2010) Synthesis and antimycobacterial evaluation of novel Phthalazin-4-ylacetamides against log- and starved phase cultures. *Chem Biol Drug Des*. **75**, 381–391