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

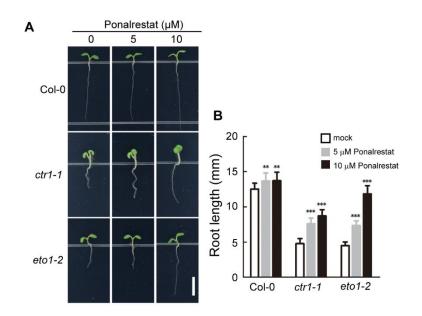


Figure S1. Ponalrestat promotes root elongation in de-etiolated seedlings. (A) Representative five-day-old Col-0, *ctr1-1*, and *eto1-2* seedlings in response to ponalrestat treatment. Scale bar = 5 mm. (B) Quantification of the primary root lengths of the five-day-old seedlings shown in (A). Bars represent means \pm 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.

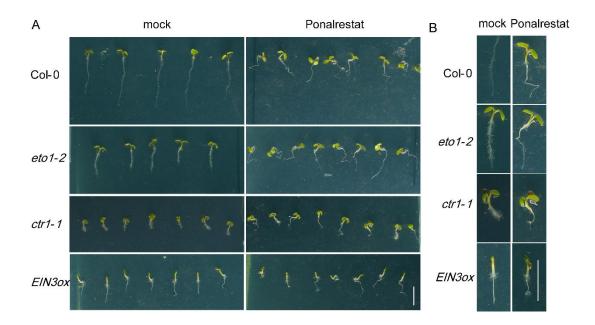


Figure S2. Ponalrestat attenuates the gravitropism of the primary root in ethylene-related mutants. After stratification for three days, the seeds were germinated and grown vertically for five days at 22 °C with light on 1/2 MS medium containing 10 μ M ponalrestat or DMSO as the mock control. (A) Col-0 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.

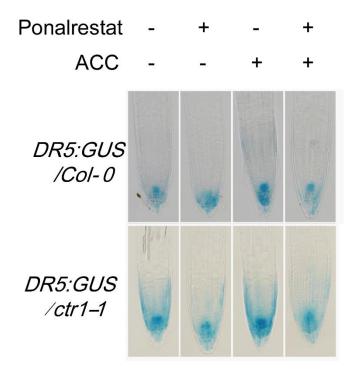


Figure S3. GUS staining of the *DR5:GUS* lines in the Col-0 or *ctr1-1* backgrounds. The seedlings were grown in the dark for three days on 1/2 MS medium containing ponalrestat (5 μ M) and/or ACC (10 μ M) or DMSO as the mock control followed by GUS staining. The experiment was repeated for at least three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

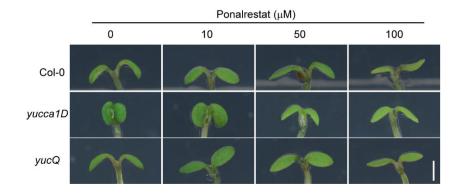


Figure S4. Observation of *YUC* mutants in response to ponalrestat.

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.

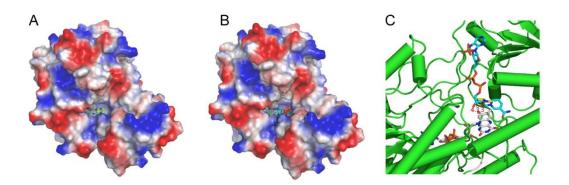


Figure S5. Structures of FMO and ligands. (A) Structure of FMO in complex with MET (in yellow) and FAD (in green) (PDB: 2GVC). (B) Structure of FMO in complex with NAD (in cyan) and FAD (in green) (PDB: 2GV8). (C) Stucture alignment of FMO (in green) in complex with MET (in yellow) and NAD (in cyan). 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.

yuc3 1 yuc4 1 yuc5 1 yuc6 1 yuc6 1	LMEFVTETLGKRIHDPYVETRCLMIPGPIIVGSGPSGLATAACIKSRDIPSLILERSTCIASLWQHKTYDRLRLHUPKDPC MYGNNNKKSINITSMPQNLIPEGSDIFSRCIWNGPVIVGAGPSGLAVAACISRGVPFIILERANCIASLWQHKTYDRLKLHUPKOPC MGTCRESEPTQIFVPGPIIVGAGPSGLAVAACISNRGVPSVILERTDCIASLWQKKTYDRLKLHUPKOPC MEMFRLMGSEDSSDRRCIWNGPVIVGAGPSGLATAACIREGVPFVULERADCIASLWQKKTYDRLKLHUPKOPC MDFCWKREMEGKLAHDHRGMTSPRRICVVTGPVIVGAGPSGLAVAACISNRGVPSVILERADCIASLWQKKTYDRLKLHUPKOPC MCNNNNTSCVNISSMLQPEDIFSRCIWNGPVIVGAGPSGLAVAACISKRGYDFVULERANCIASLWQKKTYDRLKLHUPKOPC MEMFRLMGQDQLTNNRCIWNGPVIVGAGPSGLAVAACIKERGITSVLLERANCIASLWQKKTYDRLKLHUPKOPC MENMFRLMDQDQLTNNRCIWNGPVIVGAGPSGLAVAACIHEONVPFVVLERADCIASLWQKKTYDRLKLHUPKOPC MENMFRLMDQDQLTNNRCIWNGPVIVGAGPSGLATAACIHEORVPFVVLERADCIASLWQKKTYDRLKLHUPKOPC MENMFRLMASEEYFSERRCVWNGPVIVGAGPSGLATAACIHEORVPFVVLERADCIASLWQKKTYDRLKLHUPKOPC	74 81 90 70 78 85 85 78 78 78 58 62
yuc4 71 yuc5 79 yuc6 86 yuc7 86 yuc8 79 yuc8 79 yuc9 79	5 ELPIIPFPGDPTYPTROOFIEYLEDYARRDIKEEFNOTVESAAFDENLGMWRVTSVGEEGTTEYVCRWLVAATGENAEPVVER 5 OLENLPFPEDIEYFTKYOFIEYLESYATHDDLEVENETVOSAKYDKRFGUWRVOTVLKSELGYCFFYICRWLVVATGENAEKVVE 9 OLEKMPFPEDPEYFTKROFIDYLESYATRBEINEKFNEGVOTARFDETSGUWRVTVSKSESTOT-EVEYICRWLVVATGENAERVME 9 OLEKMPFPEDPEYFTKROFIDYLESYATRBIKEFNKSVESARFDETSGUWRVT-TSOE-EMEYICRWLVVATGENAERVME 9 OLEFMPFPENVEFTFMSKELEVNYLDAYVARBDIKEFNKSVESARFDETSGUWRVTATSOE-EMEYICRWLVVATGENAERVME 9 OLEFMPFPENVEFTFMSKELEVNYLDAYVARBDIKERNKTVKSSTFDESNKWRVVAENTVTGETEVYWSEFLVVATGENAERVME	151
yuc2 169 yuc3 181 yuc4 152 yuc5 167 yuc6 171 yuc7 176 yuc8 168 yuc9 164 yuc10 146	 DET-PDFCGPTLHTSSYKSCFIFSEKKILVVCCGNSGMEVCLDICNFNALPSLVVRDSVHVLPQEMLGISTFGISTSLLKWPPVHVV FEGL-EDDCGDVLHAGDVKSCGRYQCKKVLVVCGCNSGMEVSIDIYHCGNPSMVVRSVHVLPREIFGKSTFELGVTMMKYMPVLA PPGL-KKB-T-C9VVHTSAYKSCSAFANRKVLVVCGCNSGMEVSIDICRYNALPHMVVRSVHVLPREIFGKSTFELGVTMMKYMPVLA PPGL-KKB-T-GPVVHTSAYKSCSAFANRKVLVVCGCNSGMEVSIDICRYNALPHMVVRSVHVLPREIFGKSTFELGVTMMKYMPVLA PPGL-KKB-T-GPVVHTSAYKSCSAFANRKVLVVCGCNSGMEVSIDICRYNALPHMVVRSSVHVLPREIFGKSTFELSVTMMLMWPELMLV FEGL-EDDCGDVLEACDVKSCERYRGKRVLVVCGCNSGMEVSIDICNFGAOPSLVVRDAVHVLPREITGKSTFELSVTMMLMWPVLV FEGL-EDDCGDVLEACDVKSCERYRGKRVLVVCGCNSGMEVSIDICNHDASPSMVVRSSVHVLPREVLGKSTFELSVTMMKWPVLV TOGL-SEDSGEVIHACDVKSCERYRGKRVLVVCGCNSGMEVSIDICNHDASPSMVVRSSLHWMPREVMGKSTFELAMKNLRWPELMLV INGL-SEDSGEVIHACDVKSCERFRGKRVLVVCGCNSGMEVSIDIANHRAFSMVVRSSLHWMPREVMGKSTFELAMKNLRWPELMLV VCG-IDTCGEVIHACDVKSCERFRGKRVLVVCGCNSGMEVSIDIANHRAFSMVVRSSLHWMPREVMGKSTFGISVMMKWLELWLV VCG-IDTCGEVIHACDVKSCERFRGRVLVVCGCNSCMEVSIDIANHRAFSMVVRSSVHVLPREIMGKSTFGISVMMKWLEWLV VCG-IDTCGEVIHACDVKSCERFRGRVLVVCGCNSCMEVSIDIANHRAFSMVVRSSVHVLPREIMGKSTFGISVMMKWLEWLV VCG-IDTCGEVIHACDVKSCERFRGRVLVVCGCNSCMEVSIDIANHRAFSMVVRSSVHVLPREIMGKSTFGISVMMKWLEWLV VCG-IDTCGEVIHACDVKSCERFRGRVLVVCGCNSCMEVSIDIANHRAFSMVVRSSVHVLPREIMGKSTFGISVMMKWLEWLV VCG-IDTCGEVIHACDVKSCERFRGRVLVVCGCNSCMEVSIDIANHRAFTSVVRSSVHVLPREIMGKSTFGISVMMKWLEWLV VCG-IDTCGEVIHACDVKSCERFFGISVMKVLVVCGCNSCMEVSIDIANHRAFTSVVRSSVHVLPREIMGKSTFGISVMMKVLPREIMLKV 	244 255 267 238 254 259 262 254 251 227 230
yuc4 239 yuc5 255 yuc6 260 yuc7 263 yuc8 255 yuc9 252 yuc9 252 yuc10 228 yuc11 233	5 DRFLLRMSRLVLGDTDRLGIVREKLGELERKIKCEKTPVLDVETLAKTRSCHIKVY-PELKRVMHYSAEFVDCRVDNFDAIILATGYKS 3 DRFLLLANSTLGNTDKYGLKRKIGELELNNECKTPVLDVATLSKIRSCHIKVY-PELKRVMHYSAEFVDCRVDNFDAIILATGYRSM 5 DRFLLLIANSTLGNTDLIGTRRHGTGTELKNISCKTPVLDVATSIRSCOIKVT-OAVKEITNGARFLNEKEKTGFTELIDSVILATGYRSM 5 DRFLLVVSRFILGDTTLLGTNRRIGTELELNISCKTPVLDVATISK SKGVEIV-PGIKRFSRSHVELVDCORLDLOAVVLATGYRSM 6 DRFLLVVSRFILGDTTLLGTNRRIGTELELNISCKTPVLDVATISK SKGVEIV-PGIKRFSRSHVELVDCORLDLOAVVLATGYRSM 7 DRFLLVVSRFILGDTTLLGTNRRIGTELELNISCKTPVLDVATISK SKGVEIV-PGIKRFSRSHVELVDCORLDLOAVVLATGYRSM 8 DRTLVVSRFILGDTTLLGTNRRIGTELELNISCKTPVLDIGAISK SKGVEIV-PGIKRFSRSHVELVDCORLDLOAVVLATGYRSM 9 DRTLLVSRFILGDTTLLGTNRRIGTELELNISCKTPVLDIGAISK SKGVEVPV-PGIKRFSRSHVELVDCORVLDISVILATGYRSM 9 DRTLLVSRFILGDTSKYGKREIGSELEKNTSKKTPVLDIGAISK SKGVEVPV-PGIKRFNGNKVELVNCEQLDVDSVVLATGYRSM 9 DKILLVLSWMVLGSLSNYGKREDIGBMELKSVKKKTPVLDIGAIEKTRLCKINVV-PGIKRFNGNKVELVNCEQLDVDSVVLATGYRSM 9 TLVTHAKLIYGDLSKYGFRRAGGFARKFFKRFTFRUFTGRAPUDVTOVERVER RDCFQVUSGGGSINCKTTFFENGHKQOFDAIVPATGYRSM 9 TLVTHAKLIYGGLSKTGFRRAGGFARKFFTGRAPUDVTOVERVER RDCFQVUSGGGSINCKTTFFENGHKOPDISVPATYFXS 9 DRLCLLLAELRFRNTSRYGIVRENNGFFINKLITSRSATIDVCCVGEVSKSKIQVV-TSIKRIEGKTVEFIDENTKNVDSIVFATGYRS	333 344 356 327 343 348 351 343 340 317 319
yuc1 334 yuc2 345 yuc3 357 yuc4 328 yuc5 344 yuc6 349 yuc7 352 yuc8 344 yuc9 341 yuc10 318 yuc11 320	5 VPMULA-GVNMFSEKDGFPHK-PFPNGMKGESGLYAVGFTKLCLLGAAIDAKKIAEDIEVQRHFLPLARPQHC	414 415 437 411 424 434 431 426 421 383 391

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 reference¹.

PRT:¹H-NMR (400 MHz, CDCl₃): $\delta = 12.70$ (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 C₁₇H₁₃BrFN₂O₃ [M+H]⁺: 391.00881, found 391.00888.

Compound 1: ¹H-NMR (400 MHz, CDCl₃): $\delta = 12.92$ (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 C₂₄H₁₇Br₂F₂N₂O₃ [M+H]⁺: 576.95685, found 576.95715.

Compound **2**: ¹H-NMR (400 MHz, CDCl₃): $\delta = 8.46$ (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): C₁₉H₁₇BrFN₂O₃ [M+H]⁺: 419.1.

Compound **3**: ¹H-NMR (400 MHz, CDCl₃): 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: ¹H-NMR (400 MHz, CDCl₃): 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: ¹H-NMR (400 MHz, CDCl₃): 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**: ¹H-NMR (400 MHz, CDCl₃): $\delta = 12.79$ (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 C₁₇H₁₄BrN₂O₃ [M+H]⁺: 373.01823, found 373.01848.

Compound 7: ¹H-NMR (400 MHz, CDCl₃): 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