Science Advances
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Supplementary Materials for

ABA homeostasis and long-distance translocation are redundantly regulated by ABCG ABA importers

Yuqin Zhang, Himabindu Vasuki Kilambi, Jie Liu, Hamutal Bar, Shani Lazary, Aiman Egbaria, Dagmar Ripper, Laurence Charrier, Zeinu Mussa Belew, Nikolai Wulff, Suresh Damodaran, Hussam Hassan Nour-Eldin, Asaph Aharoni, Laura Ragni, Lucia Strader, Nir Sade, Roy Weinstain, Markus Geisler, Eilon Shani*

*Corresponding author. Email: eilonsh@post.tau.ac.il

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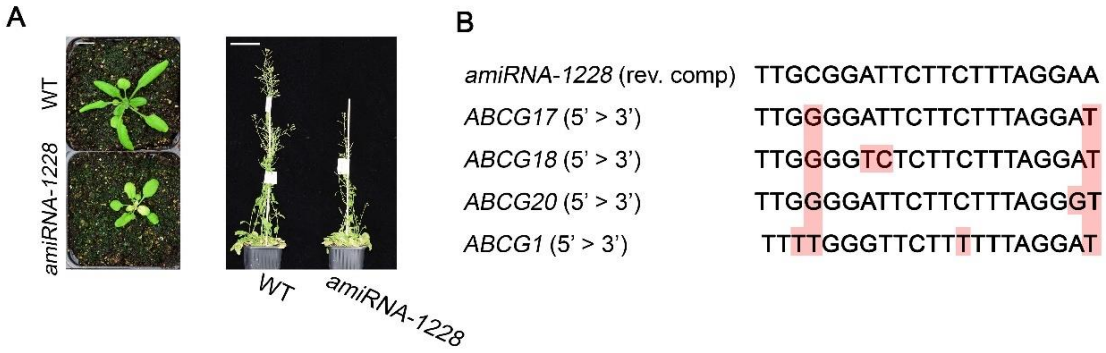


Fig. S1. Characterization of *amiRNA-1228* line. **A,** Shoot phenotypes of 20-day-old (left) and 50-day-old (right) WT and *amiRNA-1228* plants. Scale bar = 1 cm. **B,** *amiRNA-1228* sequence aligned to recognition sites of putative targets *ABCG17*, *ABCG18*, *ABCG20* and *ABCG1*. Red shading indicates mismatches with the *amiRNA-1228*. Photo Credit: Yuqin Zhang, Tel Aviv University.

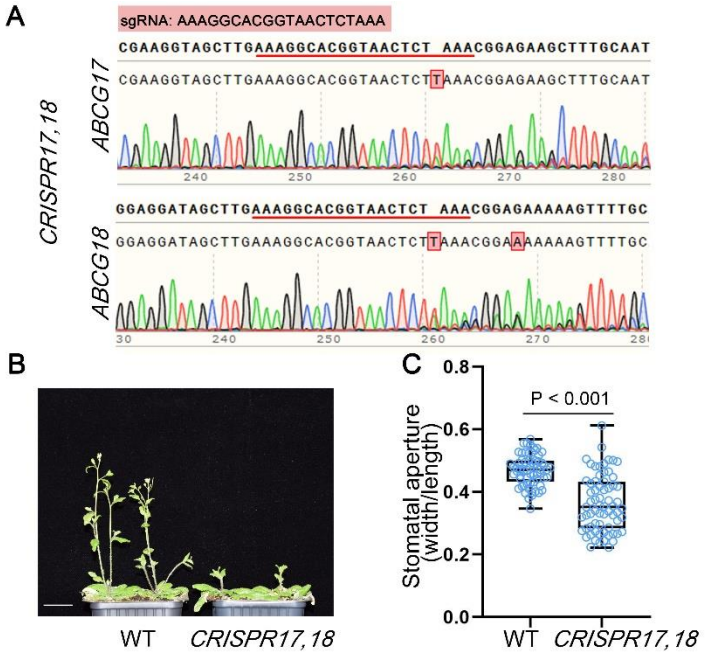


Fig. S2. *ABCG17* and *ABCG18* CRIPSR knockout plants show delayed growth and reduced stomatal aperture. A, Sequencing chromatograms of *CRISPR17,18*. CRISPR mutations are in position 291 bp for *ABCG17* CDS and position 412 bp for *ABCG18* CDS. The CRISPR mutation is a single-base insertion (AAAGGCACGGTAACTCTtAAA, lower case "t" and T in red box in the chromatogram indicates for the insertion) for both genes. Sequences marked with a red line indicate for sgRNA. Mutations were somatic and did not pass to the next generation. **B,** Shoot phenotypes of 35-day-old plants grown in soil under normal conditions. Scale bar = 1 cm. Photo Credit: Yuqin Zhang, Tel Aviv University. **C,** Stomatal aperture measurements of 35-day-old plants of the indicated genotypes. Shown are averages (±SD), n ≥ 64, P-value two-tailed Student's t-test is indicated.

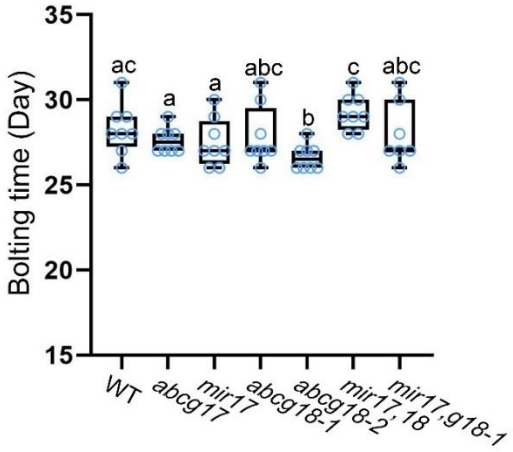


Fig. S3. *ABCG17,18* loss-of-function lines do not present strong bolting time phenotypes. Shown is the bolting time of the indicated *ABCG17,18* loss-of-function genotypes. Shown are averages (±SD), n ≥ 7, different letters represent significant differences, one-way ANOVA with student's t-test (P < 0.05).

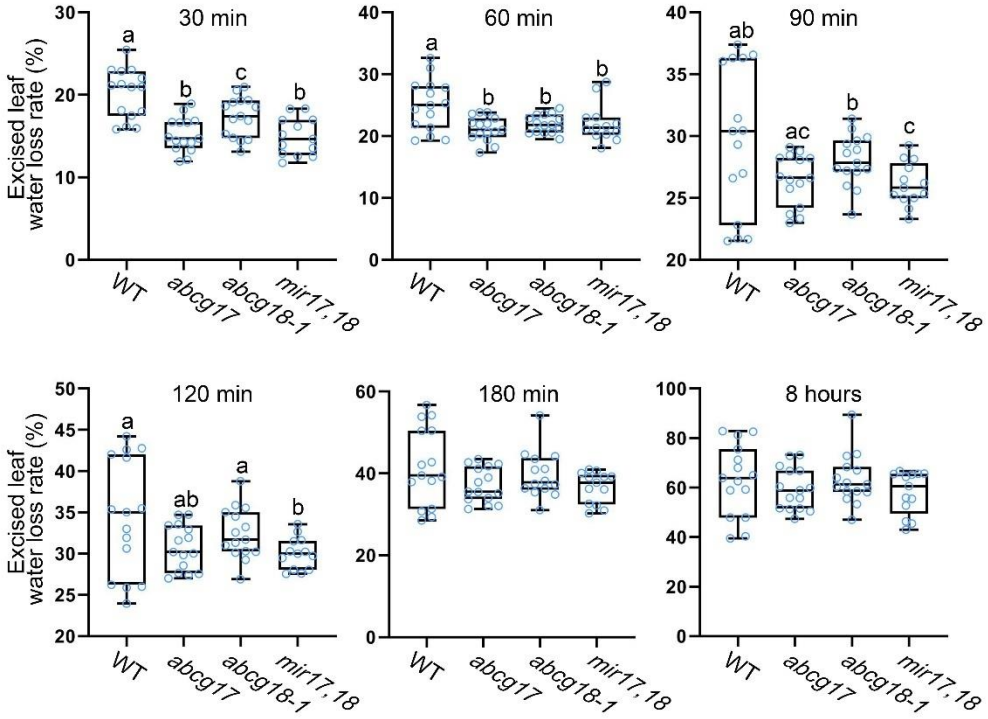


Fig. S4. *ABCG17* and *ABCG18* knockdowns result in a reduced water-loss rate. Percentage of water-loss rates of leaves excised from 30-day-old plants of the indicated genotypes at indicated time-points, exposed to room temperature air. Shown are averages (±SD), n ≥ 13, different letters represent significant differences, one-way ANOVA with student's t-test (P < 0.05). No significant differences were observed for 180 min and 8 hours time-points (P < 0.05).

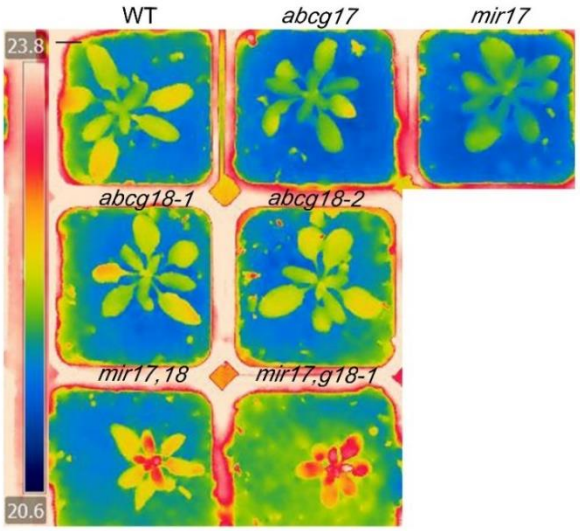


Fig. S5. *ABCG17* and *ABCG18* redundantly regulate leaf temperature. Thermal images of 25-day-old plants of the indicated genotypes grown on soil under normal conditions. The shown image was taken in a single take for all genotypes. *mir17,18* is *ABCG17, ABCG18* double-knockdown amiRNA line, *mir17,g18-1* is *mir17* (*amiRNA-ABCG17*) transformed into the background of *abcg18-1* T-DNA line. Color-coded scale bar indicates temperature. Black scale bar = 1 cm.

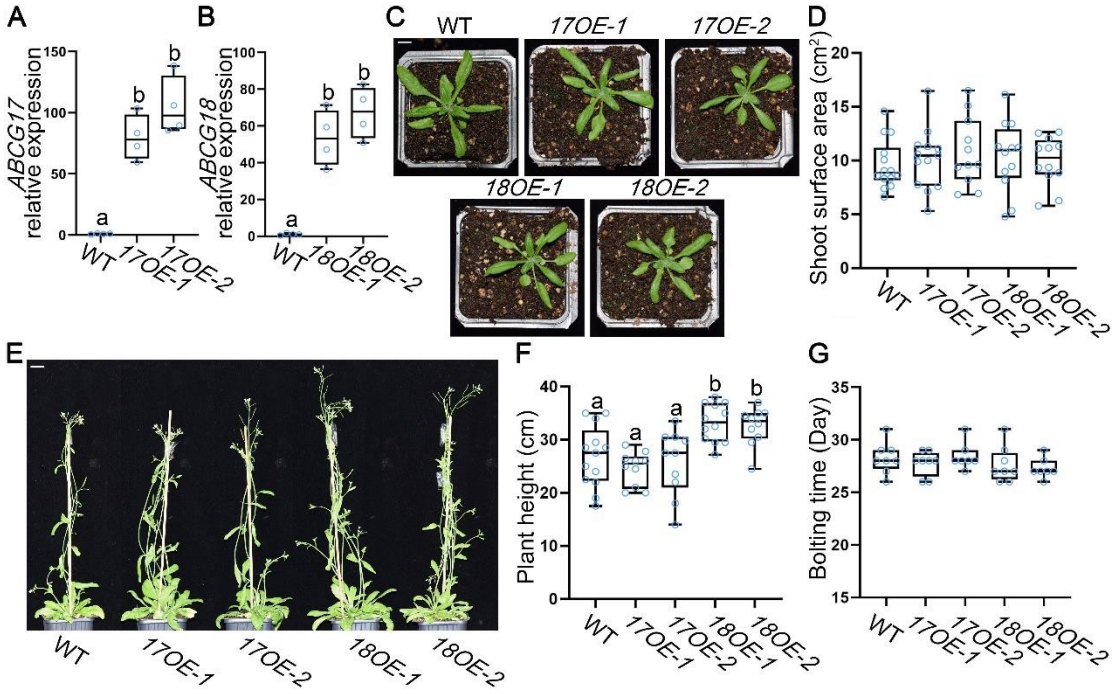


Fig. S6. Characterization of *ABCG17* and *ABCG18* overexpression lines. A, *ABCG17* mRNA relative expression in *ABCG17* overexpressing lines, quantified by qRT-PCR. Shown are averages (±SD), n = 4, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$). **B,** *ABCG18* mRNA relative expression in *ABCG18* overexpressing lines, quantified by qRT-PCR. Shown are averages (±SD), n = 4, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$). **C,** Shoot

phenotypes of 25-day-old plants of the indicated genotypes grown on soil under normal conditions. Scale bar = 1 cm. **D,** Quantification of shoot surface areas of the indicated lines. Shown are averages (±SD), n ≥ 11 plants. Results were not significant at P > 0.05 by one-way ANOVA with student's t-test (P < 0.05). **E,** Phenotypes of 45-day-old plants grown in soil under normal conditions. Scale bar = 2 cm. **F,** Heights of 45-day-old plants of the indicated genotypes. Shown are averages (±SD), n ≥ 11 plants, different letters represent significant differences, one-way ANOVA with student's t-test (P < 0.05). **G,** Bolting time of the indicated genotypes. Shown are averages (±SD), n ≥ 7. No significant differences were observed in one-way ANOVA with student's t-test (P < 0.05). Photo Credit: Yuqin Zhang, Tel Aviv University.

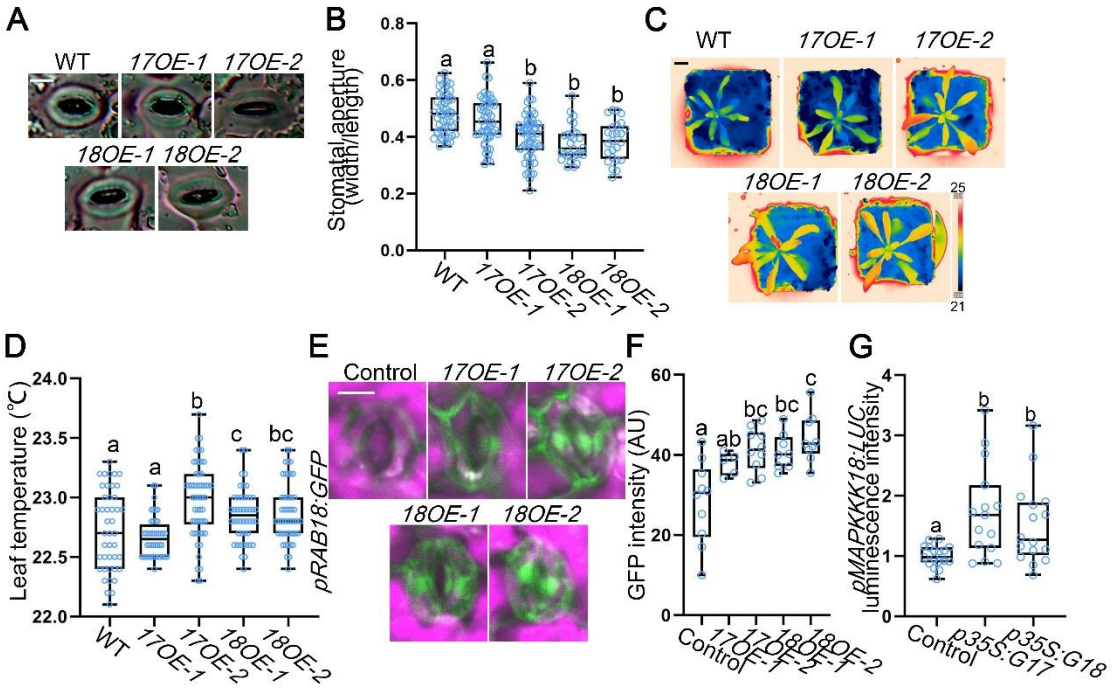


Fig. S7. *ABCG17* or *ABCG18* overexpression promotes ABA responses in shoots. A, Stomatal impressions of the indicated genotypes. Scale bar = 10 μm. **B,** Width/length ratio of stomatal apertures of 25-day-old plants of the indicated genotypes. Shown are averages (±SD), n ≥ 28, different letters represent significant differences, one-way ANOVA with student's t-test (P < 0.05). **C,** Thermal images of 25-day-old plants of the indicated genotypes grown on soil under normal conditions. Color-coded scale bar indicates temperature. Black Scale bar = 1 cm. **D,** Quantification of leaf temperature measurements of 25-day-old plants of the indicated genotypes. Shown are averages (±SD), n ≥ 43, different letters represent significant differences, one-way ANOVA with student's t-test (P < 0.05). **E,** *pRAB18:GFP* intensity in guard cells of 12-day-old lines expressing *p35S:ABCG17* (*17OE-1* and *17OE-2*) or *p35S:ABCG18* (*18OE-1* and *18OE-2*). Green stands for GFP, purple stands for chlorophyll. Scale bar = 10 μm. **F,** Quantification of respective GFP signal intensity in (E). Shown are averages (±SD), n ≥ 5, different letters represent significant differences, one-way ANOVA with student's t-test (P < 0.05). **G,** *pMAPKKK18:LUC* bioluminescence intensity in shoots of 12-day-old *ABCG17* (*p35S:G17*) and *ABCG18* (*p35S:G18*) overexpression lines. Shown are averages (±SD), n ≥ 15, different letters represent significant differences, one-way ANOVA with student's t-test (P < 0.05).

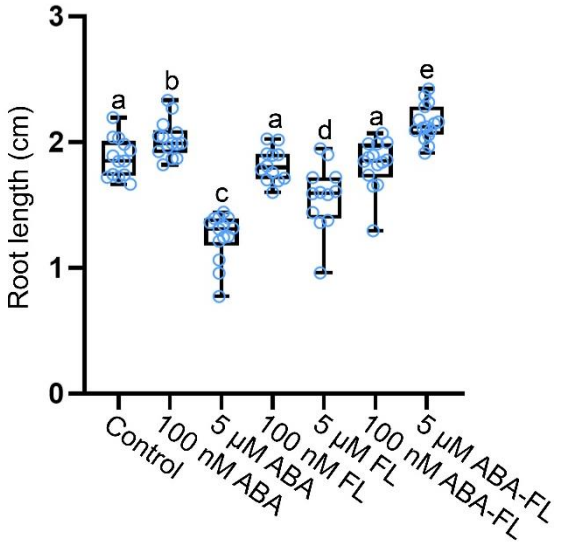


Fig. S8. ABA-FL has slight but significant activity in root growth assays. Characterization of synthetic ABA-FL activity in 7-day-old *Arabidopsis* root growth assays. Four-day-old seedlings were treated with the indicated concentration of ABA, FL, or ABA-FL for 3 days prior to measurements. Shown are averages (±SD), n ≥ 15, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$). ABA-FL: ABA-Fluorescein.

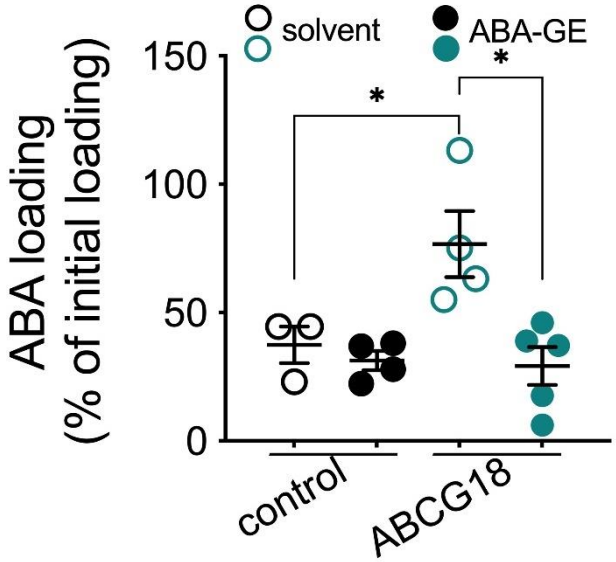


Fig. S9. ABA-GE competes with ABA in ABCG18 uptake assays. ABA-GE competition experiments with ABA uptake into protoplasts prepared from tobacco leaves transfected with *p35S:YFP-ABCG18* (*ABCG18*) or vector control. The solvent or ABA-GE in a 1000-fold access in comparison to [^{3}H]ABA (adjusted to 10 nM) was added; shown are mean ±SE (n ≥ 3), significant differences ($P < 0.05$) to solvent control were determined by a one-sample t and Wilcoxon test and are indicated by an asterisk.

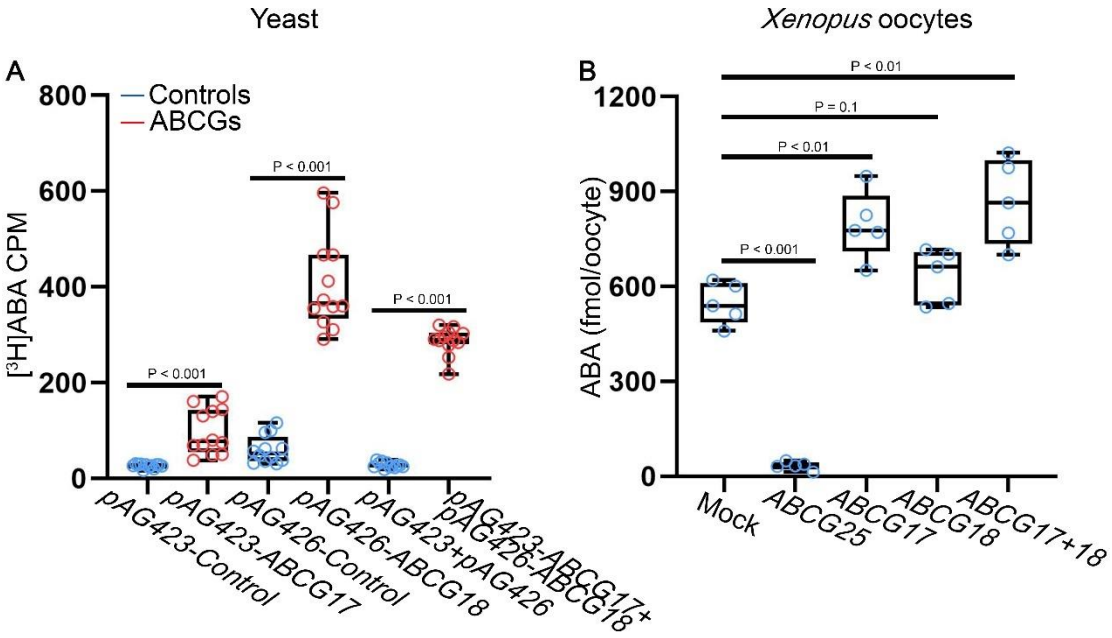


Fig. S10. ABCG17 and ABCG18 import ABA into yeast and *Xenopus* oocyte cells. A, ABA transport assay in yeast for the indicated ABCGs. Yeast strain YMM12 expressing *pAG423-ABCG17* (*ABCG17* in the pAG423 vector), *pAG426-ABCG18* (*ABCG18* in the pAG426 vector), and combination of both *ABCG17* and *ABCG18* with their corresponding controls were treated with 15 nM [^{3}H]ABA for 3 h. The cells were washed three times and resuspended in 0.1 M MES buffer, pH = 4.6. The radioactivity was analyzed by scintillation counting. Shown are boxplot data from n = 12. P-value two-tailed Student's t-test is indicated for each analysis. **B,** ABA transport assay in *Xenopus* oocytes for the indicated ABCGs. Shown are the average values of ABA content per oocyte (fmol/oocyte). Media ABA concentration is 10 µM (pH 5.8). Shown are averages (±SD), n = 5 X 4 oocytes. P-value two-tailed Student's t-test is indicated for each analysis.

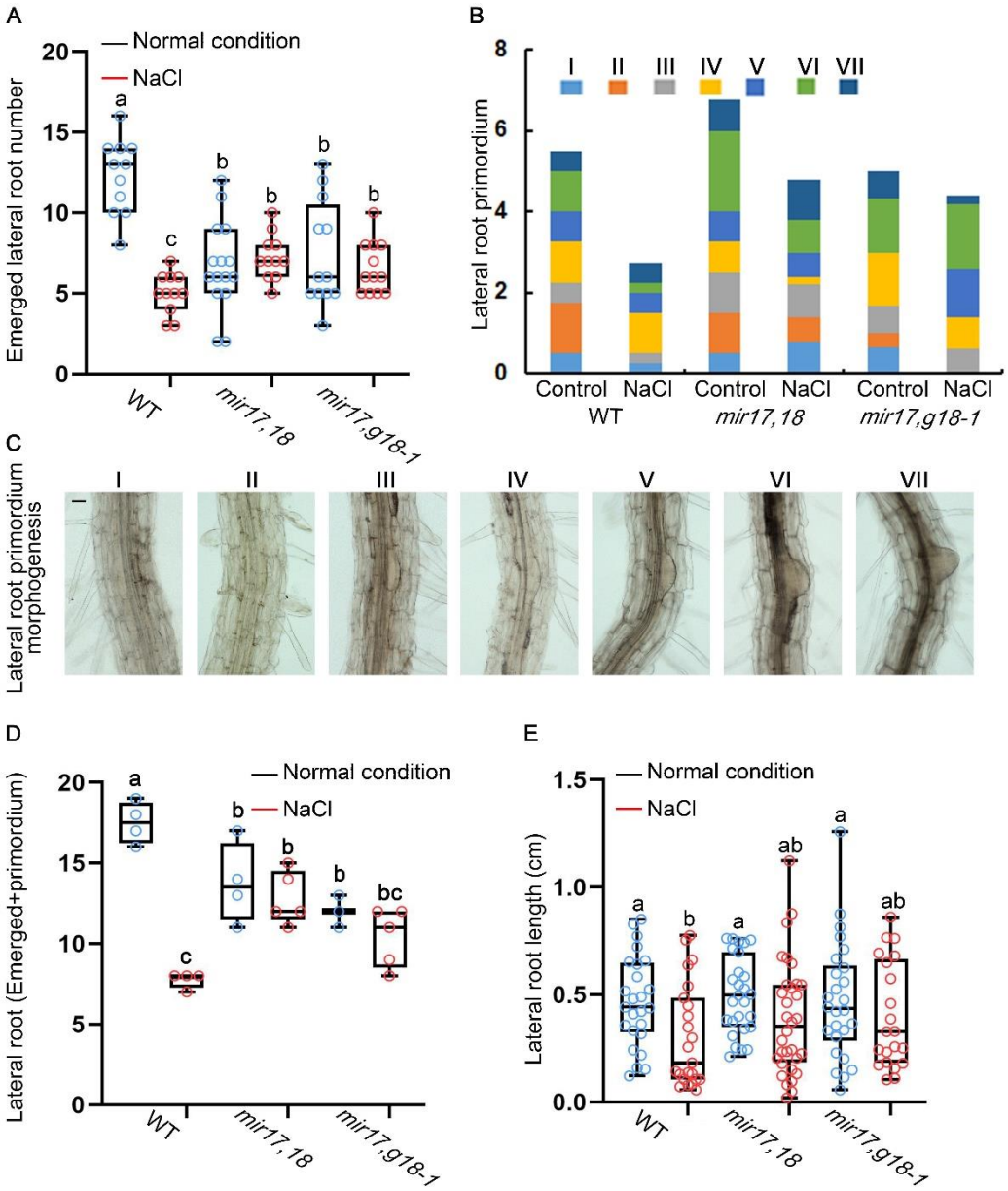


Fig. S11. Characterization of lateral root initiation, emergence and length of *ABCG17* and *ABCG18* double knockdown lines with and without NaCl treatment. A, Emerged lateral root number of 10-day-old seedlings of the indicated genotypes with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n ≥ 11, one-way ANOVA with student's t-test ($P < 0.05$). **B,** Lateral root primordium number of the indicated genotypes at different stages with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n ≥ 4. Values and statistics for each developmental stage are presented in **Supplementary Tables 2-3**. **C,** Developmental stages during *Arabidopsis* lateral root primordium morphogenesis. Scale bar = 25 µm. **D,** Lateral root initiation number including primordium and emerged lateral root of the indicated genotypes with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n = 4, one-way ANOVA with student's t-test ($P < 0.05$). **E,** Lateral root length of the first 3 roots (closest to the hypocotyl) of the indicated genotypes with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n ≥ 21, one-way ANOVA with student's t-test ($P < 0.05$). Different letters represent significant differences.

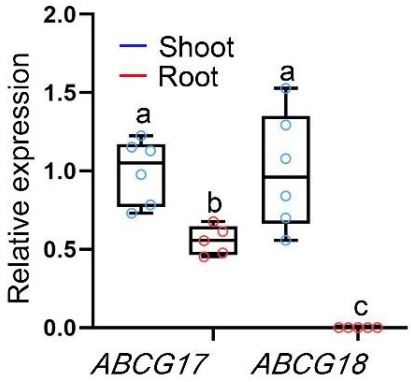


Fig. S12. Relative expression of *ABCG17* and *ABCG18* in *Arabidopsis* shoots and roots. Quantified by qRT-PCR. Shown are averages (±SD), n ≥ 5, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$).

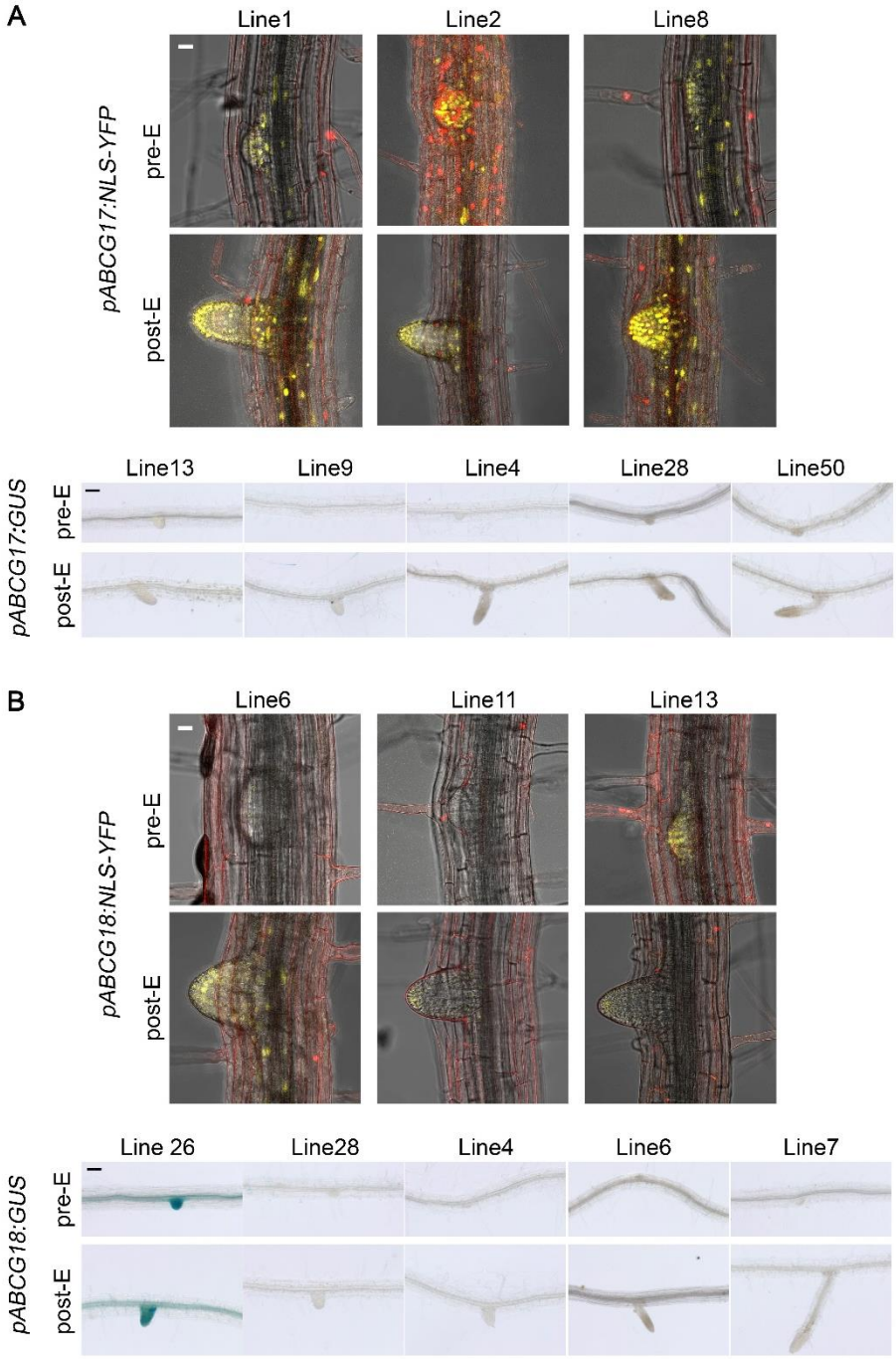


Fig. S13. *ABCG17* and *ABCG18* are weakly expressed during lateral root development. **A,** Images of lateral root pre-and post-emergence sites of *pABCG17:NLS-YFP* (YFP in yellow), and *pABCG17:GUS*. The presented images are additional independent lines to the ones presented in **Fig. 6D-E.** Scale bar for YFP images = 20 µm. Scale bar for GUS images = 100 µm. **B,** Images of lateral root pre-and post-emergence sites of *pABCG18:NLS-YFP* (YFP in yellow), and *pABCG18:GUS*. The presented images are additional independent lines to the ones presented in **Fig. 6D-E**. Scale bar for YFP images = 20 µm. Scale bar for GUS images = 100 µm. pre-E: pre-emergence, post-E: post-emergence.

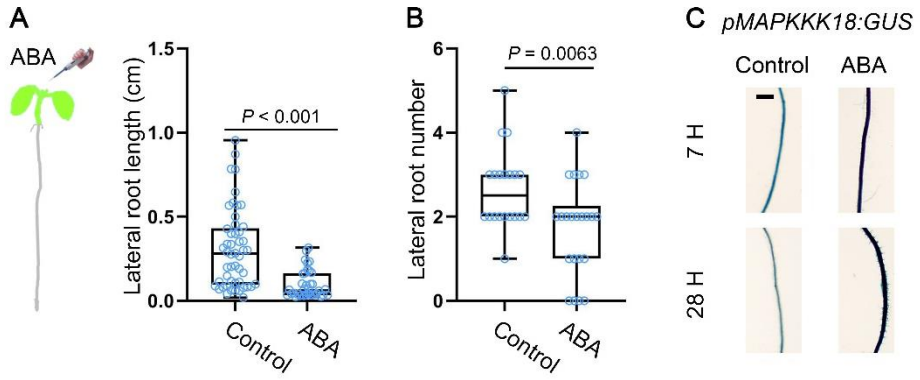


Fig. S14. Exogenous ABA application to WT shoot affects lateral root growth and induces ABA response in the root. A, Left, an illustration of shoot-specific ABA application. Right, lateral root length graph of 10-day-old WT seedlings without (Control) and with 5 µM ABA treatment of shoots of 5-day-old seedlings for 5 days. Shown are averages (±SD), n ≥ 20, P < 0.001 (P = 2.26685E-06) indicates significant differences, student's t-test. **B,** Lateral root number of 10-day-old WT seedlings without (Control) and with 5 µM ABA treatments to shoot of 5-day-old seedlings for 5 days. Shown are averages (±SD), n ≥ 20, P value indicates significant differences, student's t-test. **C,** *pMAPKKK18:GUS* reporter signal imaged in 5-day-old roots in response to 5 µM ABA shoot-specific application or no ABA treatment (Control) after 7 and 28 hours. Scale bar = 1 mm.

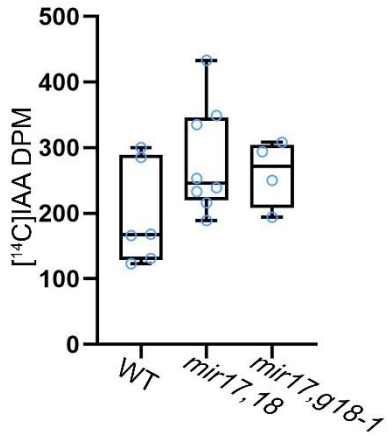


Fig. S15. Shoot-to-root IAA translocation is not affected in *ABCG17,18* loss-of-function lines. 0.1 µCi/ml [^{14}C]IAA was applied only to shoots. Shown are [^{14}C]IAA levels explicitly in roots, quantified as disintegration per minute (DPM) after background deduction. Shown are averages (±SD), n ≥ 6. No significant differences were observed in one-way ANOVA with student's t-test (P < 0.05).

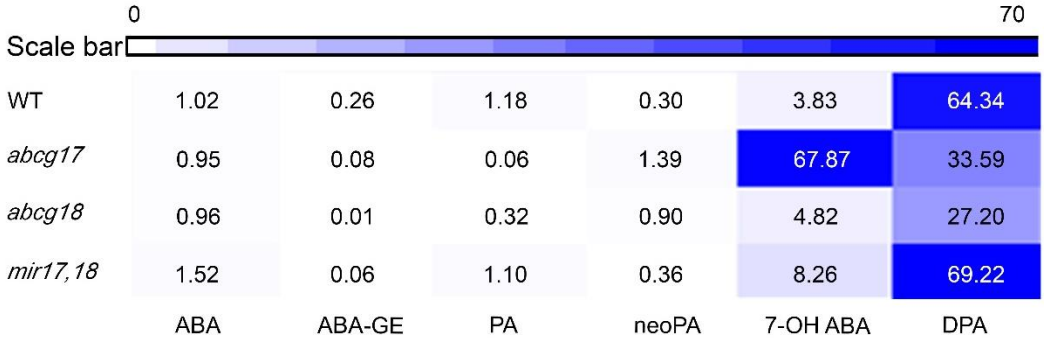


Fig. S16. *ABCG17* and *ABCG18* knockdown lines show alteration in shoot ABA homeostasis. Heat-map profile of absolute values (ng/g FW) for ABA and its metabolites in shoots of the indicated genotypes (12-day-old plants). Color-coded data is presented in absolute values and is similar to the normalized data shown in **Fig. 7C**. $n \geq 6$, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$). Abbreviations: ABA (Abscisic acid), ABA-GE (Abscisic acid-1-Beta-glucose ester), PA (Phaseic acid), neoPA (neo Phaseic acid), 7-OH ABA (7-hydroxy abscisic acid), DPA (Dihydrophaseic acid).

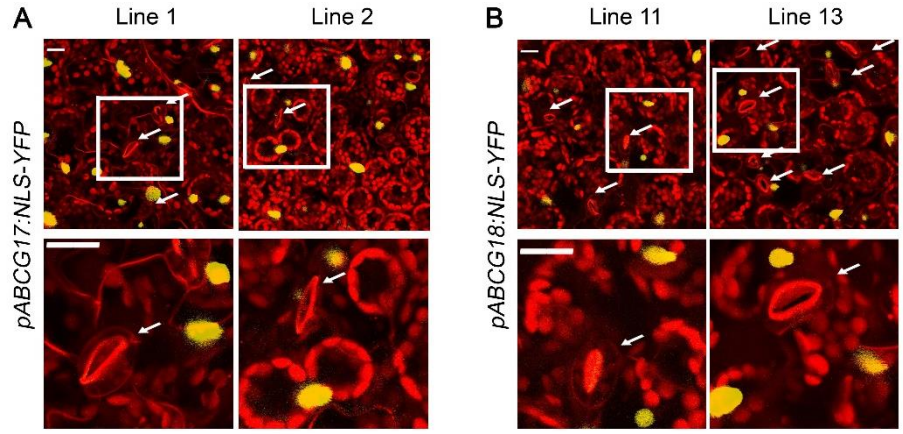


Fig. S17. *ABCG17* and *ABCG18* are primarily expressed in mesophyll cells. A-B, *pABCG17:NLS-YFP* (A) and *pABCG18:NLS-YFP* (B) signal (yellow) in leaves. The presented images are additional independent lines to the ones presented in **Fig. 7D**. The white arrows point to guard cells. Images at the bottom are magnifications of areas indicated by white boxes in the images above. Chlorophyll in red. All scale bars = 20 μm.

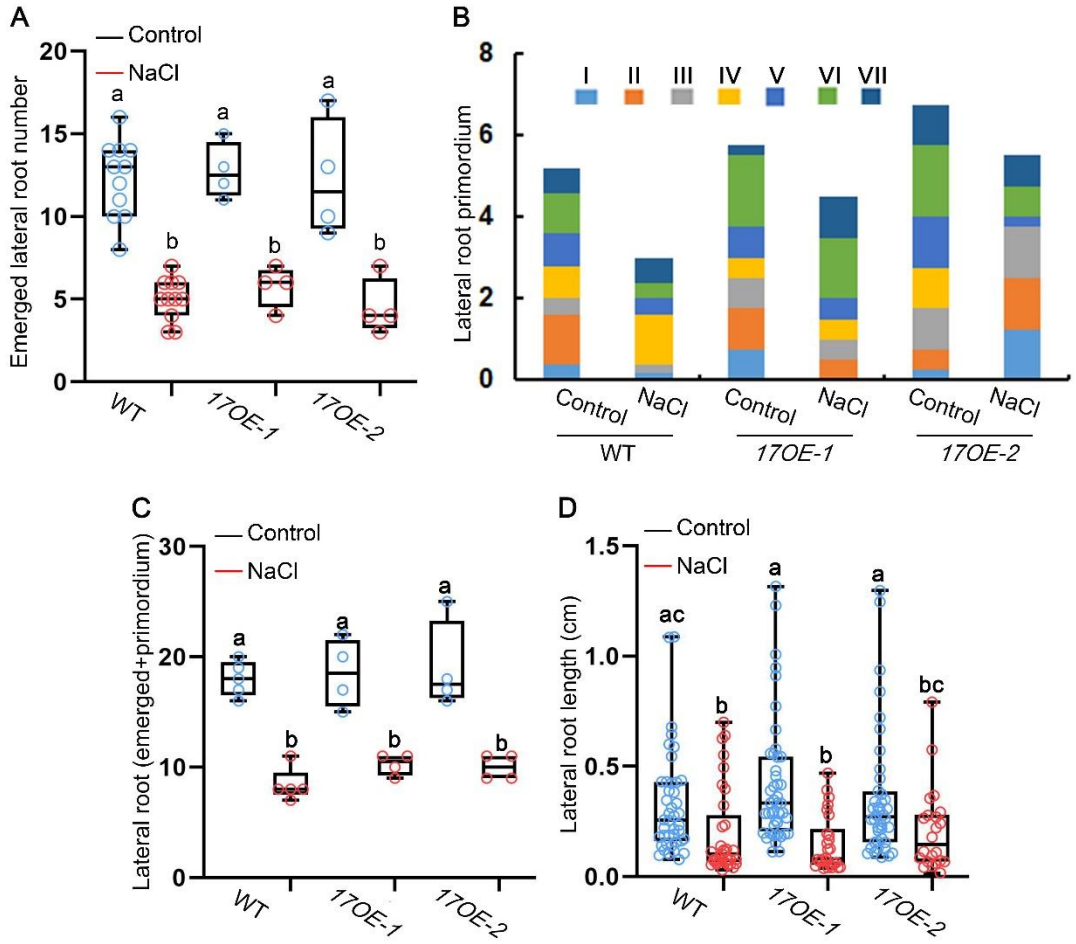


Fig. S18. Characterization of lateral root initiation, emergence and length of *ABCG17* overexpression lines with and without NaCl treatment. A, Emerged lateral root number of 10-day-old seedlings of the indicated genotypes with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n ≥ 4, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$). **B,** Lateral root primordium number of the indicated genotypes at different stages with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n ≥ 4. Values and statistics for each developmental stage are presented in **Supplementary Tables 5-6**. **C,** Lateral root initiation number including primordium and emerged lateral root of the indicated genotypes with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n = 4, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$). **D,** Lateral root length of the first 3 roots (closest to the hypocotyl) of the indicated genotypes with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n ≥ 22, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$).

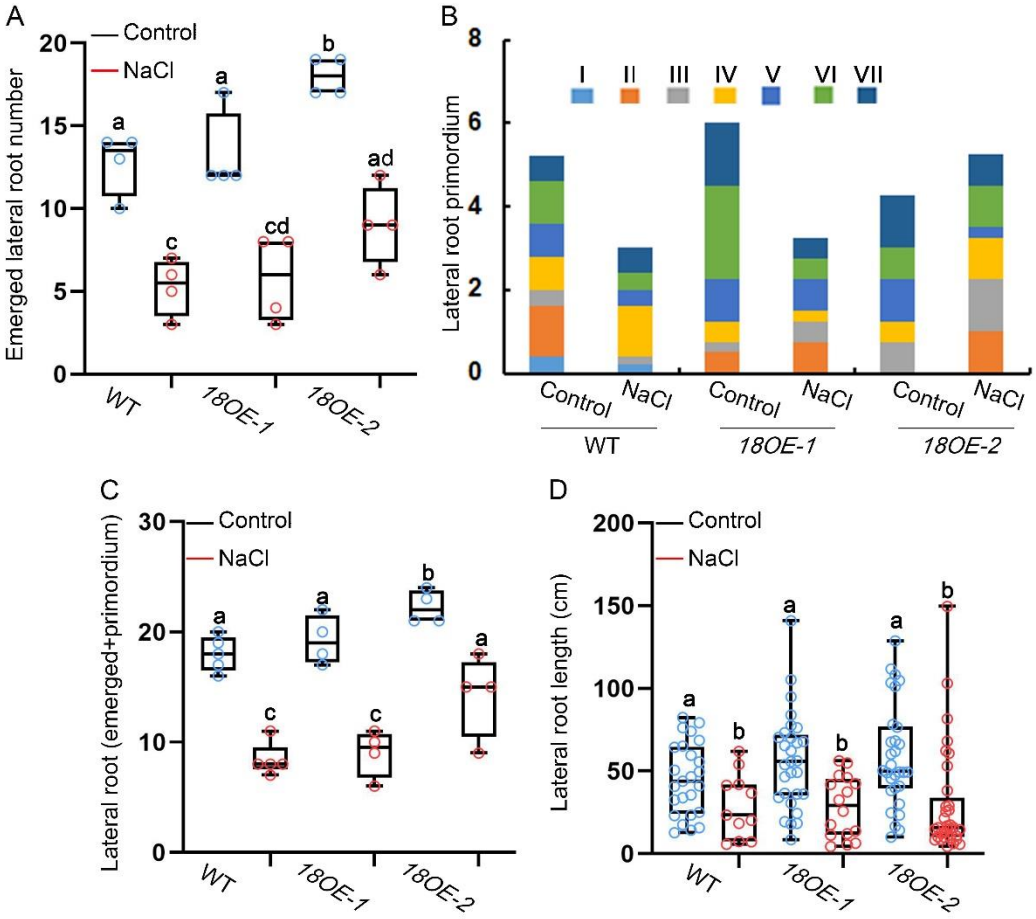


Fig. S19. Characterization of lateral root initiation, emergence and length of *ABCG18* overexpression lines with and without NaCl treatment. A, Emerged lateral root number of 10-day-old seedlings of the indicated genotypes with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n = 4, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$). **B,** Lateral root primordium number of the indicated genotypes at different stages with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n ≥ 4. Values and statistics for each developmental stage are presented in **Supplementary Tables 7-8**. **C,** Lateral root initiation number including primordium and emerged lateral root of the indicated genotypes with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n = 4, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$). **D,** Lateral root length of the first 3 roots (closest to the hypocotyl) of the indicated genotypes with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n ≥ 11, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$).

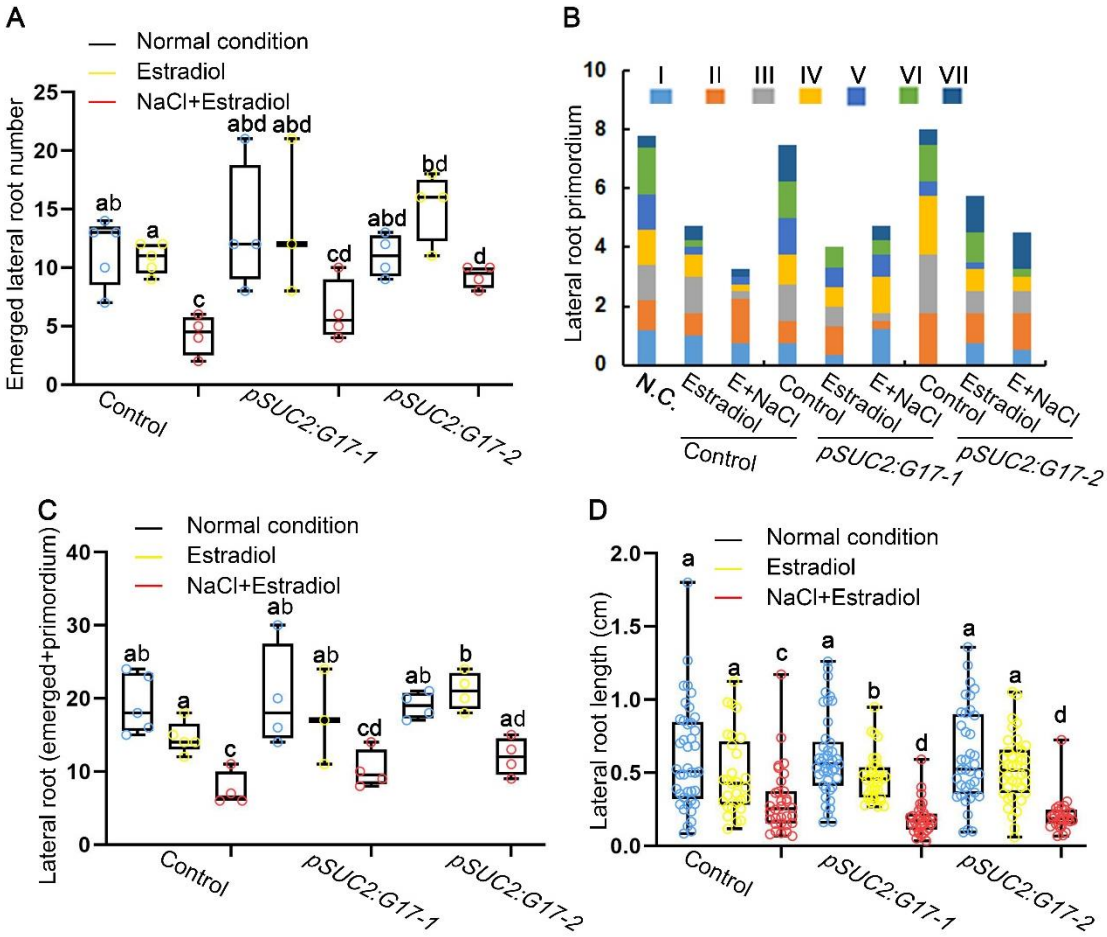


Fig. S20. Characterization of lateral root initiation, emergence and length of estradiol inducible *pSUC2: XVE:G17* lines with and without NaCl treatment. A, Emerged lateral root number of 10-day-old estradiol inducible seedlings of the indicated genotypes with and without 5-day 100 mM NaCl treatment. *pSUC2:XVE:ABCG17* induces *ABCG17* expression in an estradiol-dependent manner, specifically in the phloem companion cells. Shown are averages (±SD), n = 4, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$). **B,** Lateral root primordium number of the indicated estradiol inducible genotypes at different stages with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n ≥ 4. Values and statistics for each developmental stage are presented in **Supplementary Tables 9-11**. **C,** Lateral root initiation number including primordium and emerged lateral root of the indicated estradiol inducible genotypes with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n = 4, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$). **D,** Lateral root length of the first 3 roots (closest to the hypocotyl) of the indicated estradiol inducible genotypes with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n ≥ 26, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$). Estradiol concentration = 5 µM.

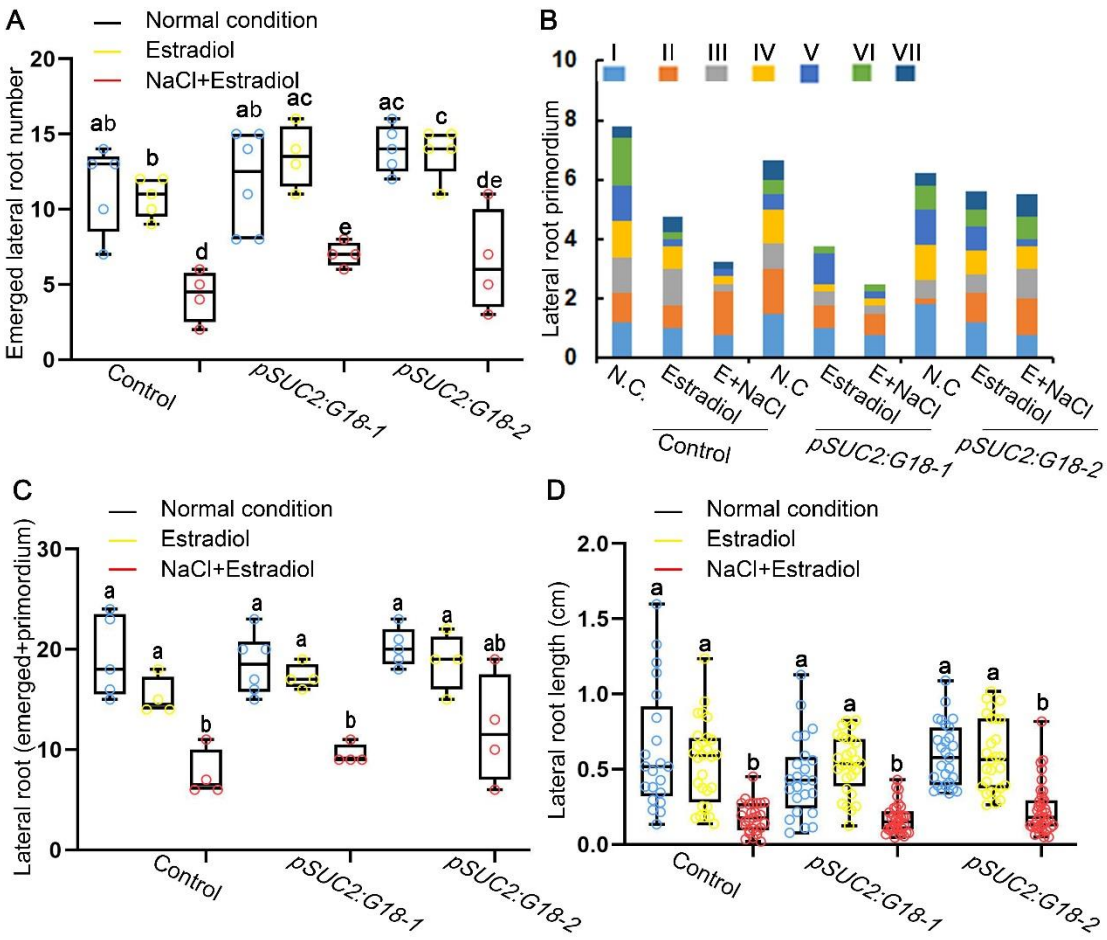


Fig. S21. Characterization of lateral root initiation, emergence and length of estradiol inducible *pSUC2: XVE:G18* lines with and without NaCl treatment. A, Emerged lateral root number of 10-day-old estradiol inducible seedlings of the indicated genotypes with and without 5-day 100 mM NaCl treatment. *pSUC2:XVE:ABCG18* induces *ABCG18* expression in an estradiol-dependent manner, specifically in the phloem companion cells. Shown are averages (±SD), n = 4, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$). **B,** Lateral root primordium number of the indicated estradiol inducible genotypes at different stages with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), $n \geq 4$. Values and statistics for each developmental stage are presented in **Supplementary Tables 12-14**. **C,** Lateral root initiation number including primordium and emerged lateral root of the indicated estradiol inducible genotypes with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), n = 4, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$). **D,** Lateral root length of the first 3 roots (closest to the hypocotyl) of the indicated estradiol inducible genotypes with and without 5-day 100 mM NaCl treatment. Shown are averages (±SD), $n \geq 21$, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$). Estradiol concentration = 5 µM.

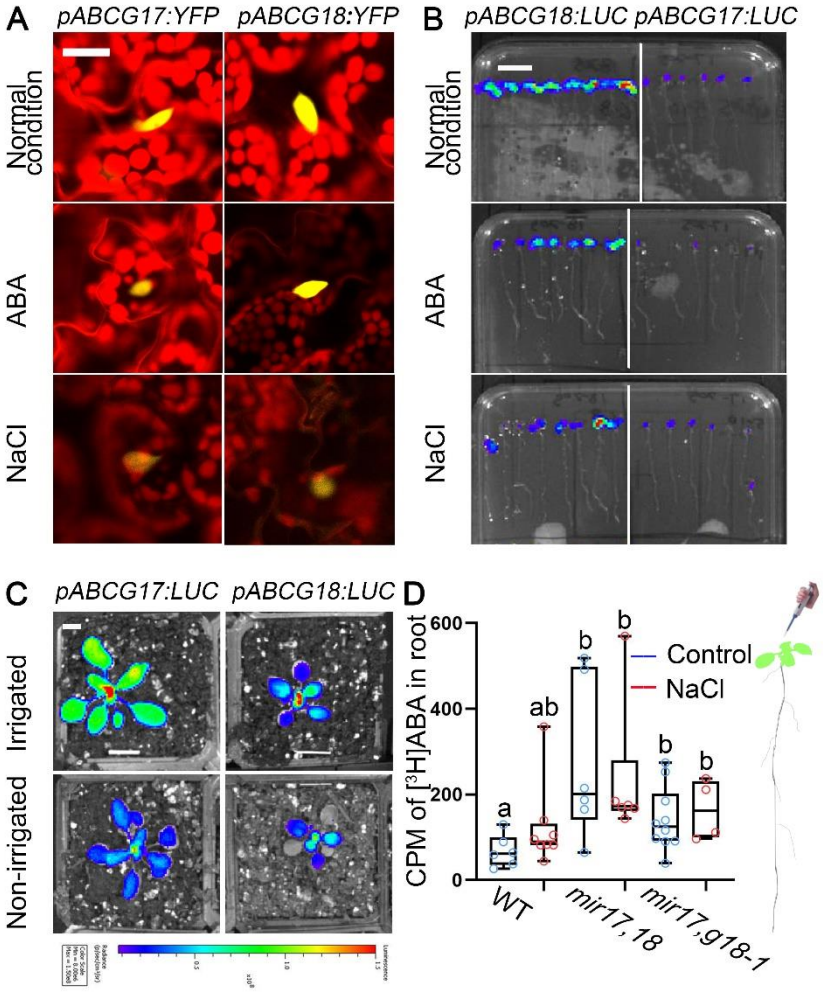
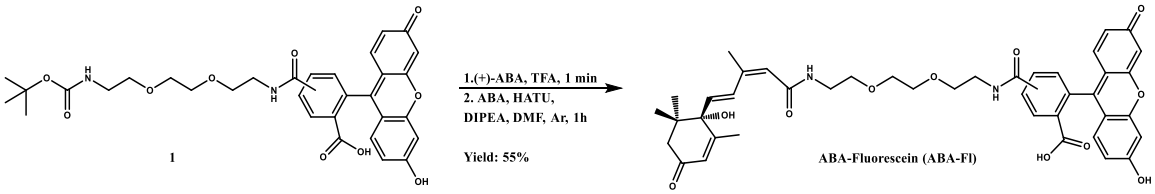


Fig. S22. Abiotic stresses repress *ABCG17* and *ABCG18* expression in the shoot. A, *pABCG17:YFP* (*pABCG17:NLS-YFP*) and *pABCG18:YFP* (*pABCG18:NLS-YFP*) signal (yellow) in mesophyll cells with 5 µM ABA or 100 mM NaCl treatments for 3 days. Chlorophyll is in red. Scale bar = 20 µm. **B,** Luciferase bioluminescence signal of *pABCG17:LUC* and *pABCG18:LUC* transgenic in 5-day-old plants treated with 5 µM ABA or 100 mM NaCl for 3 days. Scale bar = 1 cm. **C,** Luciferase bioluminescence signal of *pABCG17:LUC* or *pABCG18:LUC* transgenic plants with and without irrigation. Plants were irrigated for 15 days following by water withhold for 1 week. Control plants (irrigated) were watered throughout the experiment. Color-coded scale bar indicates luciferase bioluminescence intensity. White scale bar = 1 cm. **D,** [^{3}H]ABA counts per minute (CPM) in roots, after background deduction, following 24-hour [^{3}H]ABA application to shoot with and without 100 mM NaCl treatment in the media (roots were isolated as indicated by a black line in the illustration on the right). Control samples were not treated with NaCl. Shown are averages (±SD), n ≥ 4, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$).

General synthetic procedures

(+)-ABA was purchased from Duchefa Biochemie. All other chemicals were purchased from Merck or Combi-Blocks and were used as received unless otherwise stated. Anhydrous solvents and reagents (DMF) were obtained as SureSeal bottles from Merck. Thin-layer chromatography and flash chromatography were performed using Merck KGaA pre-coated silica gel 60 F-254 plates and Silicycle silica gel 40-63 (230-400 mesh), respectively. UV absorbance spectra were recorded on Agilent Cary 60 UV-Vis Spectrophotometer. Fluorescence spectra were recorded on a Fluorolog 2 (Spex) fluorimeter. Low-resolution ESI mass spectrometry was performed on LC/MS Acquity QDa detector coupled with a Waters HPLC. High-resolution ESI mass spectrometry was performed on a Waters SYNAPT system. ^{1}H and ^{13}C NMR spectra were collected in DMSO-d6 (Cambridge Isotope Laboratories) at 25 ^{o}C using a Bruker Advance III spectrometer at 400 MHz and 100 MHz, respectively, at the Department of Chemistry NMR Facility at Tel-Aviv University. All chemical shifts are reported in the standard δ notation of parts per million using either TMS or residual solvent peak as an internal reference. Abbreviations: DMF, dimethylformamide; HATU, hexafluorophosphate azabenzotriazole tetramethyl uronium; TFA, trifluoroacetic acid; DIPEA, *N*,*N*-diisopropylethylamine.

Synthesis of compound 2 (ABA-FL)



ABA conjugated to fluorescein was synthesized using a previously described protocol (*57*). Briefly, compound **1** (10.5 mg, 1 eq) was dissolved in 0.5 mL TFA and stirred for 1 minute. TFA was immediately removed under reduced pressure. The residue was dissolved in dry DMF under argon atmosphere, and DIPEA (3.3 µL, 1.1 eq) was added. In a separate 1.5-mL Eppendorf tube, (+)-ABA (5 mg, 1.1 eq) was dissolved in dry DMF, then DIPEA (7.5 µL, 1.1 eq) and HATU (7.2 mg, 1.1 eq) were added. The mixture was vortexed for 1 minute and then was added to the TFA-treated compound **1**. The reaction was stirred at room temperature for 1 hour, and the solvent was removed under reduced pressure. The residue was dissolved in 2 mL acetonitrile:water (2:1, v/v) and the desired product was purified using preparative HPLC (see "Preparative HPLC purification conditions" section). Preparative HPLC retention time: 13.52 minutes. The desired ABA-FL was obtained as a yellow solid (7.0 mg, yield 55%). ^{1}H NMR (400 MHz, DMSO-d6) δ = 10.20 (s, 1H), 8.91 (t, J = 5.5 Hz, 1H), 8.77 (t, J = 5.6 Hz, 1H), 8.46 (d, J = 1.5 Hz, 1H), 8.25 (dd, J_1 = 8.1, J_2 =1.6 Hz, 1H), 8.17 (dd, J_1 = 8.0, J_2 = 1.4 Hz, 1H), 8.06 (d, J = 8.0 Hz, 1H), 7.95 (dt, J_1 = 14.2, J_2 = 5.6 Hz, 2H), 7.88 (d, J = 4.6 Hz, 1H), 7.84 (d, J = 4.6 Hz, 1H), 7.69 (s, 1H), 7.37 (d, J = 8.1 Hz, 1H), 6.70 (t, J = 2.6 Hz, 3H), 6.56 (dt, J_1 = 8.4, J_2 = 3.0 Hz, 7H), 6.08 (s, 1H), 6.03 (s, 1H), 5.80 (d, J = 5.1 Hz, 2H), 5.72 (d, J = 7.9 Hz, 2H), 5.13 (d, J = 2.2 Hz, 2H), 3.55 (dd, J_1 = 7.1, J_2 = 4.9 Hz, 6H), 3.48 – 3.40 (m, 10H), 3.20 (dd, J_1 = 19.0, J_2 = 5.8 Hz, 6H), 2.09 (s, 1H), 2.04 (s, 1H), 1.97 (d, J = 1.3 Hz, 1H), 1.89 (t, J = 1.5 Hz, 5H), 1.82 (d, J = 1.4 Hz, 6H), 1.24 (d, J = 4.3 Hz, 7H), 0.93 (dd, J_1 = 13.5, J_2 = 3.1 Hz, 13H).; ^{13}C NMR (101 MHz, DMSO-d6) δ = 197.34, 168.12, 165.56, 165.55, 164.82,

164.67, 163.61, 159.72, 151.90, 143.91, 140.63, 136.25, 135.37, 129.27, 129.19, 128.04, 125.99, 125.88, 122.37, 121.41, 112.83, 112.77, 109.20, 109.14, 102.35, 78.45, 69.64, 69.55, 69.18, 68.86, 68.71, 49.45, 41.38, 24.21, 23.24, 20.86, 19.06.; LC/MS: Retention time 9.77 min, 753.47 $[M+H]^+$, HR-MS(ESI) calcd. for formula $C_{42}H_{44}N_2O_{11}Na$ $[M+Na]^+$: 775.2843; Found: 775.2841.

HPLC-MS analysis conditions

HPLC-MS analysis was performed on a Waters HPLC equipped with an XBridge C18 column (100 X 3 mm, 5 µm) starting with 2 min of solvent A (0.1% TFA in water), followed by a gradient from 0% to 100% solvent B (0.1% TFA in acetonitrile) over 15 min, then 1 minute at 100% solvent B, and ending with 2 min 100% solvent A at flow rate of 1 mL/min. Mass spectrometry was performed on LC/MS Acquity QDa detector coupled with Waters HPLC.

Preparative HPLC purification conditions

Preparative HPLC was performed on a Waters 2545 HPLC equipped with an XBridge C18 column (100 X 19 mm, 5 µm) starting with 2 min of solvent A (0.1% TFA in water), followed by a gradient from 0% to 80% solvent B (0.1% TFA in acetonitrile) in 20 min, continuing with 3 min gradient of 80% to 100% solvent B, then 3 min at 100% solvent B, and ending with 2 min 100% solvent A at a flow rate of 15 mL/min.

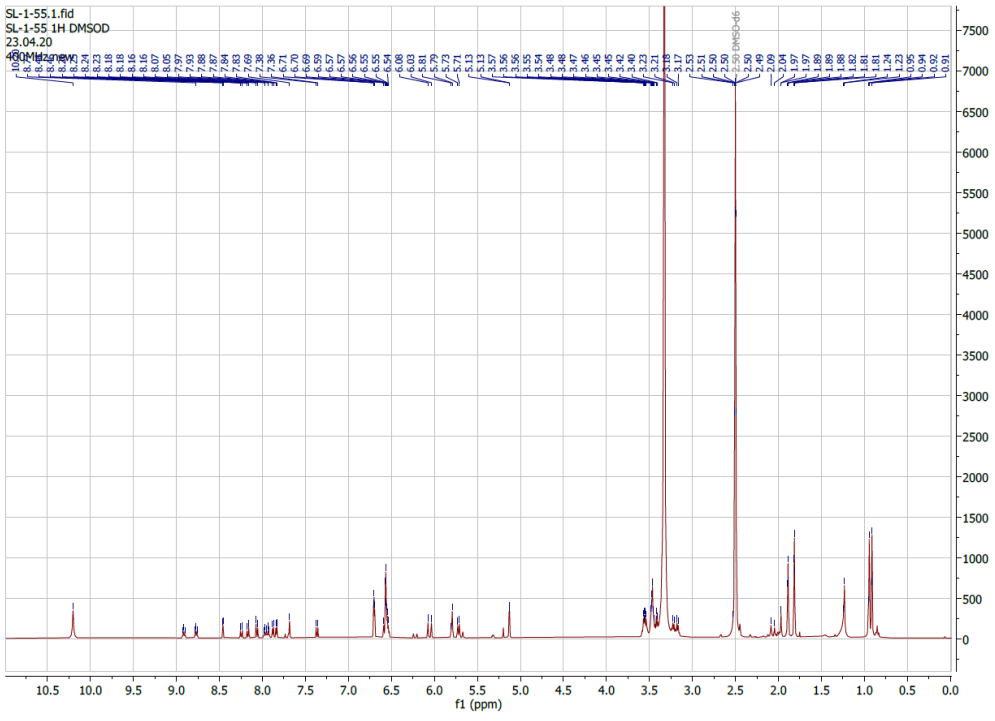


Fig. S23. ^{1}H-NMR spectrum of ABA-FL.

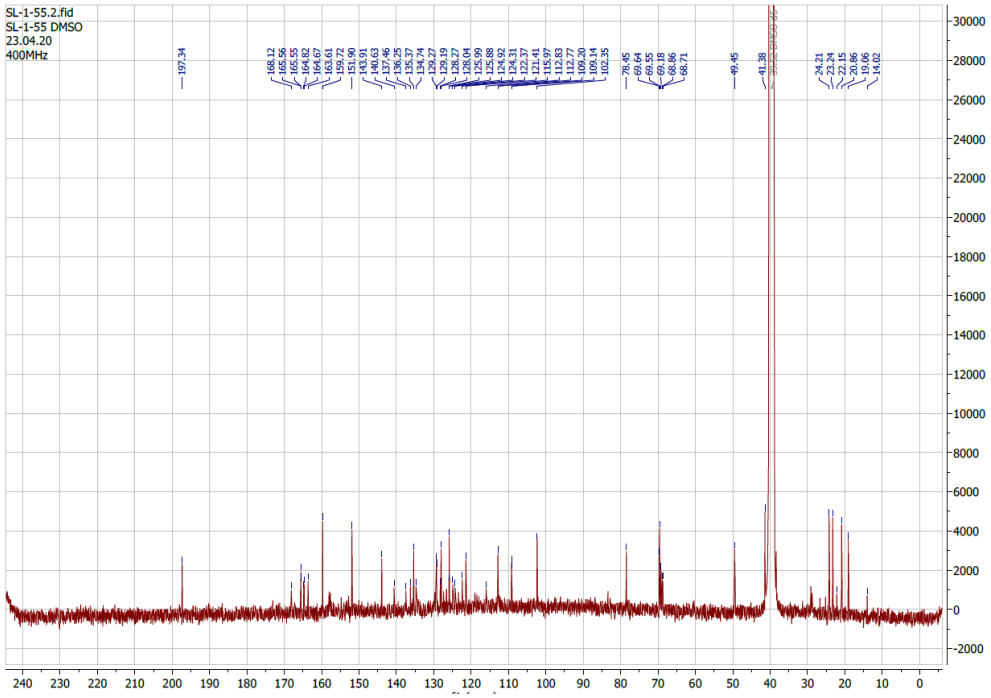


Fig. S24. ^{13}C-NMR spectrum of ABA-FL.

Quantitifying ABA by LC-MS/MS (oocytes transport assays).

Oocyte samples were subjected to analysis by LC-MS/MS. Chromatography was performed on an Advance UHPLC system (Bruker, Bremen, Germany). Separation was achieved on a Kinetex 1.7 u XB-C18 column (100 x 2.1 mm, 1.7 µm, 100 Å, Phenomenex, Torrance, CA, USA). Formic acid (0.05 %) in water and acetonitrile (supplied with 0.05 % formic acid) were employed as mobile phases A and B respectively. The elution profile was: 0-0.1 min, 5% B; 0.1-1.0 min, 5-45% B; 1.0-3.0 min 45-100% B, 3.0-3.5 min 100% B, 3.5-3.55 min, 100-5% B and 3.55-4.7 min 5% B. The mobile phase flow rate was 400 µl min-1. The column temperature was maintained at 40 °C. The liquid chromatography was coupled to an EVOQ Elite TripleQuad mass spectrometer (Bruker, Bremen, Germany) equipped with an electrospray ion source (ESI). The ion spray voltage was maintained at +5000 V and -3000 V, in positive and negative ion mode, respectively. MRM was used to monitor analyte molecular ion → fragment ion transitions. MRM transitions were optimized by direct infusion experiments into the MS source or taken from literature. Detailed values for mass transitions and references are listed in **Supplementary Table 1**.

Table 1. MRM transitions for LC-MS/MS analysis of ABA oocyte samples.

Analyte	Retention Time [min]	Q1 [m/z]	Q3 [m/z]	CE [eV]
ABA [M-H]	2.00	263.0	153.1^{Qt}	7
		263.0	151.0	7

Qt = quantifier ion, additional transitions were used for identification only. CE = collision energy; Q = quadrupole.

Table 2. Lateral root primordia number at different developmental stages of the double *ABCG17* and *ABCG18* knockdown lines under normal conditions. n ≥ 4 plants.

MS	I	II	III	IV	V	VI	VII
WT	0.5±0.58	1.25±1.26	0.5±0.57	1±0.82	1.75±0.5	1±0.82	0.5±0.58
mir17,18	0.5±0.58	0.82±0.75	1±0.82	0.75±0.96	0.75±0.5	2±0.82	0.75±0.96
mir17,g18-1	0.67±0.58	0.33±0.58	0.67±0.58	1.33±1.53	0±0	1.33±0.58	0.67±0.58

Table 3. Lateral root primordia number at different developmental stages of the double *ABCG17* and *ABCG18* knockdown lines with 100 mM NaCl treatment. n ≥ 4 plants.

NaCl	I	II	III	IV	V	VI	VII
WT	0.25±0.5	0±0	0.25±0.5	1±0.82	0.5±0.58	0.25±0.5	0.5±0.58
mir17,18	0±0	0±0	0±0	0.5±0.58	0.5±0.58	0.25±0.5	0±0
mir17,g18-1	0±0	0±0	0.6±0.55	0.8±1.3	1.2±0.84	1.6±1.52	0.2±0.45

Table 4. Accuracy and precision parameters for ABA and ABA metabolites. Determined by UPLC-ESI -MS method. S-Shoot, R-Root.

S. No.	Compound	LOD (µg/g)	LOQ (µg/g)	Spiked standard (ng/ml; low/ medium/high)	Internal standard (ng/ml)	Method precision (RSD%, n = 5)			Method accuracy (%, n = 5)		
						Low	Medium	High	Low	Medium	High
1	ABA	0.002	0.01	0.5 /10/ 900	1250	0.05(S) 0.8 (R)	1.2 (S) 1.7 (R)	1.4 (S) 1.3 (R)	90.2 (S) 88.2 (R)	91.8 (S) 94.6 (R)	97.7 (S) 97.8 (R)
2	Neo PA	0.08	0.4	0.5 /10/ 900	150	3.0 (S) 5.5 (R)	4.1 (S) 7.4 (R)	2.7 (S) 4.0 (R)	92.6 (S) 94.0 (R)	88.9 (S) 92.0 (R)	96.0 (S) 96.8 (R)
3	PA	0.6	2.5	0.5 /10/ 900	60	1.5 (S) 2.6 (R)	0.8 (S) 5.4 (R)	2.3 (S) 2.7 (R)	88.8 (S) 85.7 (R)	88.3 (S) 91.0 (R)	91.2 (S) 98.2 (R)
4	7-hydroxy ABA	0.2	1.0	0.5 /10/ 900	150	6.1 (S) 7.7 (R)	4.1 (S) 7.1 (R)	3.3 (S) 4.2 (R)	96.4 (S) 89.0 (R)	98.8 (S) 97.7 (R)	97.3 (S) 95.3 (R)
5	DPA	0.24	1.2	0.5 /10/ 900	150	3.3 (S) 4.9 (R)	5.9 (S) 4.7 (R)	2.2 (S) 1.7 (R)	97.1 (S) 95.1 (R)	90.1 (S) 99.1 (R)	92.1 (S) 99.4 (R)
6	ABA GE	0.02	0.1	0.5 /10/ 900	52.5	0.7 (S) 1.5 (R)	0.5 (S) 0.4 (R)	0.7 (S) 2.4 (R)	95.0 (S) 99.3 (R)	89.3 (S) 92.3 (R)	93.3 (S) 97.0 (R)

Abbreviations: ABA (Abscisic acid), ABA-GE (Abscisic acid-1-Beta-glucose ester), PA (Phaseic acid), neoPA (neo Phaseic acid), 7-OH ABA (7-hydroxy abscisic acid), DPA (Dihydrophaseic acid). LOD is limit of detection, LOQ is limit of quantification, RSD is relative standard deviation.

Table 5. Lateral root primordia number at different developmental stages of *ABCG17* overexpression lines under normal conditions. n ≥ 4 plants.

MS	I	II	III	IV	V	VI	VII
WT	0.4±0.55	1.2±1.1	0.4±0.54	0.8±0.84	0.8±0.45	1±0.71	0.6±0.55
17OE -1	0.75±0.5	1±0.82	0.75±0.96	0.5±0.58	0.75±0.96	1.75±0.96	0.25±0.5
17OE -2	0.25±0.5	0.5±0.58	1±0.82	1±0.82	1.25±0.5	1.75±0.5	1±1.15

Table 6. Lateral root primordia number at different developmental stages of *ABCG17* overexpression lines with 100 mM NaCl treatment. n ≥ 4 plants, different letters represent significant differences, one-way ANOVA with student's t-test ($P < 0.05$)

NaCl	I	II	III	IV	V	VI	VII
WT	0.2±0.45 a	0±0 a	0.2±0.45	1.2±0.84 a	0.4±0.55	0.4±0.55	0.6±0.55
17OE-1	0±0 a	0.5±1 ab	0.5±1	0.5±0.58 ab	0.5±0.58	1.5±1	1±0.82
17OE-2	1.25±0.5 b	1.25±0.96 b	1.25±0.96	0±0 b	0.25±0.5	0.75±0.96	0.75±0.5

Table 7. Lateral root primordia number at different developmental stages of *ABCG18* overexpression lines under normal conditions. n ≥ 4 plants, different letters represent significant differences, one-way ANOVA with student's t-test (P < 0.05)

MS	I	II	III	IV	V	VI	VII
WT	0.4±0.55	1.2±1.1	0.4±0.55	0.8±0.84	0.8±0.45	1±0.71 a	0.6±0.55 a
18OE-1	0±0	0.5±1	0.25±0.5	0.5±0.58	1±1.4	2.25±0.5 b	1.5±0.78 b
18OE-2	0±0	0±0	0.75±0.5	0.5±0.58	1±0.82	0.75±0.96 a	1.25±0.5 ab

Table 8. Lateral root primordia number at different developmental stages of *ABCG18* overexpression lines with 100 mM NaCl treatment. n ≥ 4 plants, different letters represent significant differences, one-way ANOVA with student's t-test (P < 0.05)

NaCl	I	II	III	IV	V	VI	VII
WT	0.2±0.45	0±0	0.2±0.45 a	1.2±0.84	0.4±0.55	0.4±0.55	0.6±0.55
18OE-1	0±0	0.75±1.5	0.5±0.58 ab	0.25±0.5	0.75±0.5	0.5±1	0.5±0.58
18OE-2	0±0	1±1.15	1.25±0.5 b	1±0.82	0.25±0.5	1±0.82	0.75±0.96

Table 9. Lateral root primordia number at different developmental stages of *pSUC2: XVE:ABCG17* overexpression lines under normal condition. n ≥ 4 plants, different letters represent significant differences, one-way ANOVA with student's t-test (P < 0.05)

MS	I	II	III	IV	V	VI	VII
Control	1.2±1.64 ab	1±1	1.2±1.1	1.2±1.1	1.2±1.1	1.6±0.89	0.4±0.55
pSUC2: G17-1	0.75±0.5 a	1.75±0.5	1.25±1.26	1±1.2	1.25±0.5	1.25±1.26	1.25±1.26
pSUC2: G17-2	0±0 b	1.75±0.96	2±1.4	2±0.82	0.5±1	1.25±0.96	0.5±0.58

Table 10. Lateral root primordia number at different developmental stages of *pSUC2: XVE: ABCG17* overexpression lines with 5 µM estradiol treatment. n ≥ 4 plants.

Estradiol	I	II	III	IV	V	VI	VII
Control	1±0.82	0.75±0.5	1.25±0.5	1.75±0.5	0.25±0.5	0.25±0.5	0.5±0.58
pSUC2: G17-1	0.33±0.58	1±1	0.67±0.58	0.67±0.58	0.67±1.2	0.67±0.58	0±0
pSUC2 :G17-2	0.75±0.5	1±1.4	0.75±0.96	0.75±0.96	0.25±0.5	1±0.82	1.25±1.5

Table 11. Lateral root primordia number at different developmental stages of *pSUC2: XVE: ABCG17* overexpression lines with 5 µM estradiol and 100 mM NaCl treatment. n ≥ 4 plants.

Estradiol +NaCl	I	II	III	IV	V	VI	VII
Control	0.75±0.5	1.5±0.58	0.25±0.5	0.25±0.5	0.25±0.5	0±0	0.25±0.5
pSUC2: G17-1	1.25±0.5	0.25±0.5	0.25±0.5	1.25±1.5	0.75±0.96	0.5±0.58	0.5±0.58
pSUC2: G17-2	0.5±0.58	1.25±1.3	0.75±0.96	0.5±1	0±0	0.25±0.5	1.25±1.5

Table 12. Lateral root primordia number at different developmental stages of *pSUC2: XVE: ABCG18* overexpression lines under normal condition. n ≥ 4 plants, different letters represent significant differences, one-way ANOVA with student's t-test (P < 0.05)

MS	I	II	III	IV	V	VI	VII
Control	1.2±1.64	1±1	1.2±1.1	1.2±1.1	1.2±1.1	1.6±0.9 a	0.4±0.55
pSUC2: G18-1	1.5±1.1	1.5±1.4	0.83±0.75	1.2±1.2	0.5±0.84	0.5±0.55 b	0.67±0.52
pSUC2: G18-2	1.8±1.1	0.2±0.45	0.6±0.55	1.2±0.84	1.2±0.84	0.8±0.45 ab	0.4±0.55

Table 13. Lateral root primordia number at different developmental stages of *pSUC2: XVE:ABCG18* overexpression lines with 5 µM estradiol treatment. n ≥ 4 plants, different letters represent significant differences, one-way ANOVA with student's t-test (P < 0.05)

Estradiol	I	II	III	IV	V	VI	VII
Control	1±0.82	0.75±0.5	1.25±0.5	0.75±0.5	0.25±0.5 a	0.25±0.5	0.5±0.58
pSUC2: G18-1	1±0.82	0.75±0.5	0.5±.58	0.25±0.5	1±0 b	0.25±0.5	0±0
pSUC2: G18-2	1.2±1.1	1±0.71	0.6±0.55	0.8±0.84	0.8±0.45 ab	0.6±0.55	0.6±0.55

Table 14. Lateral root primordia number at different developmental stages of *pSUC2: XVE:ABCG18* overexpression lines with 5 µM estradiol and 100 mM NaCl treatment. n ≥ 4 plants, different letters represent significant differences, one-way ANOVA with student's t-test (P < 0.05)

Estradiol +NaCl	I	II	III	IV	V	VI	VII
Control	0.75±0.5	1.5±0.58	0.25±0.5 a	0.25±0.5	0.25±0.5	0±0	0.25±0.5
pSUC2: G18-1	0.75±0.96	0.75±0.5	0.25±0.5 a	0.25±0.5	0.25±0.5	0.25±0.5	0±0
pSUC2: G18-2	0.75±0.96	1.25±0.96	1±0 b	0.75±0.96	0.25±0.5	0.75±0.96	0.75±0.96

Table 15. T-DNA insertions.

Gene	Gene accession	T-DNA line	Insertion
ABCG17	*AT3G55100*	CS332619	Chr3 20421138
ABCG18	*AT3G55110*	SALK_100187	Chr3 20425486
ABCG18	*AT3G55110*	GK-544E01	Chr3 20424746

Table 16. Primers used for genotyping T-DNA lines.

Gene	Primer name	Primer sequence (5'-3')
abcg17	*abcg17*-LP	GCAGAACAGCTTCGTAGGGATACT
	abcg17-RP	TGATGCATTAGCAGGACA
	BP	ATTTTGCCGATTTCGGAAC
abcg18-1 SALK_100187	*abcg18-1*-LP	AGAAGAGACCCCAAGCTAACG
	abcg18-1-RP	TCACAGAGTTCGCACTTGATG
	BP	ATTTTGCCGATTTCGGAAC
abcg18-2 GK-544E01	*abcg18-2*-LP	CAGCTGATTCATGGCTCCTAG
	abcg18-2-RP	CAACACACTTGCATGGTTACG
	BP	ATAATAACGCTGCGGACATCTACATTTT

Table 17. Cloning primers.

Promoter/gene	Forward primer (5'-3')	Reverse primer (5'-3')
pABCG17	CACCTCACGCCCTCTTATTCTT GCTTCC	TCACGCCCTCTTATTCTTGC TTCC
pABCG18	CACCTCACGCCCTCTTATTCTT GCTTCC	TCACGCCCTCTTATTCTTGC TTCC
ABCG17 CDS	CACCATGCTGCAAAGAGACGC CGT GATC	TCACGCCCTCTTATTCTTGC TTCC
ABCG18 CDS	CACCATGCCACGTGTTTCGGC GGAAATT	TCACGTCCTCTTATTCTTAC TCCC
ABCG19 CDS	CACCATGAATCTATCACTCAGC GGTAGA	TCACGTCCTCTTATTCCTGC TCCC
pABCG17: ABCG17	AGGCGCGCCATGCTGCAAAGA GACGCCGTG	TCACGCCCTCTTATTCTTGC TTCCAAGC
pABCG18: ABCG18	AGGCGCGCCATGCCACGTGTT TCGGCGG	CGGCGCGCCCACCCTTTCA

Table 18. *mir* sequences.

Targeted gene	mir sequences
ABCG17	TTATTTGTCCTGCTAACGCAT
ABCG18	TAAGATAAACGTTTCCGGCAA
ABCG17,18	TGTTTAGAGTTACCGTGGCTT

Table 19. sgRNA for *ABCG17* and *ABCG18*.

CRISPR17,18	Primer sequences
Forward	ATTGAAAGGCACGGTAACTCTAAA
Reverse	AAACTTTAGAGTTACCGTGCCTTT

Table 20. Sequencing primers for *CRISPR17,18*.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
ABCG17	ATGCTGCAAAGAGACGCCGTGATC	GCCTTCTTTCTCCCCCGGAGACAC
ABCG18	GCTCCGACTCAACACATATTGGAT	GTCGCTCTCCGCCAGATACTCCAC

Table 21. qRT-PCR primers.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
ABCG1	GTGAAGTACCCGTATGAAGCG	GTAGTTCCCCTAATGGCGTG
ABCG17	CCCGAAGGAACAAGAGGTTT	GTGAGGTTCTTGACTAACTCTTA GCT
ABCG18	GTATCCGATCCCGGTTGAT	AATTCCGAATGTCATGATGAGTTA
ABCG20	AAGACAACCAGAGCTATTCGG	AGCGTTCTTGTATACCTCTTGG

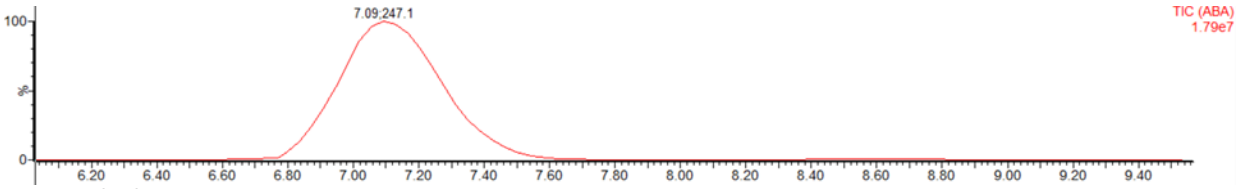
Supplementary Dataset 1

ABA homeostasis and long-distance translocation are regulated by redundant ABCG ABA importers

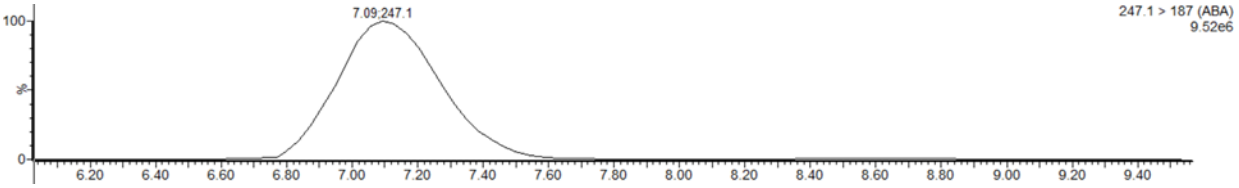
Zhang et al., 2021

Peak chromatograms for ABA and ABA related metabolites

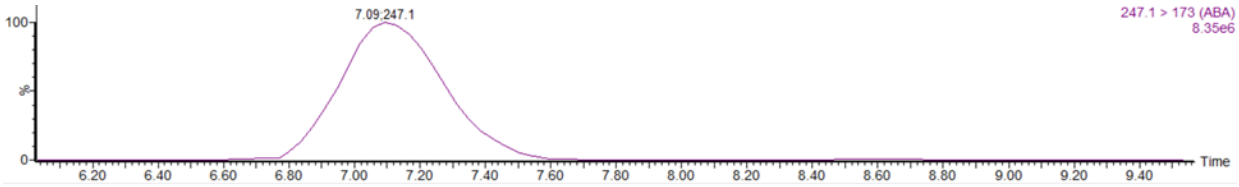
ABA standard 2.5 μg/ml



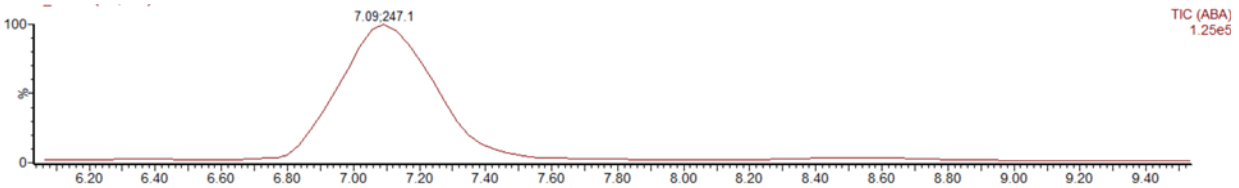
ABA standard MRM transition 1



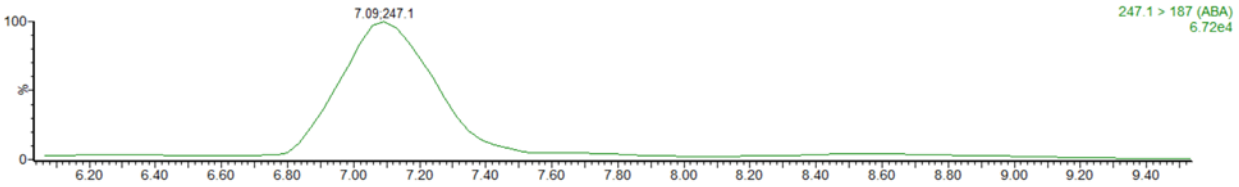
ABA standard MRM transition 2



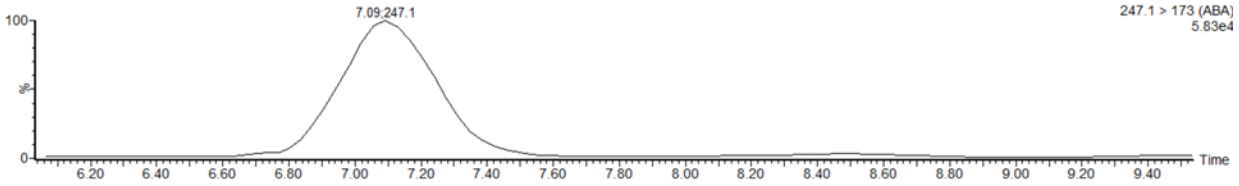
Sample-ABA



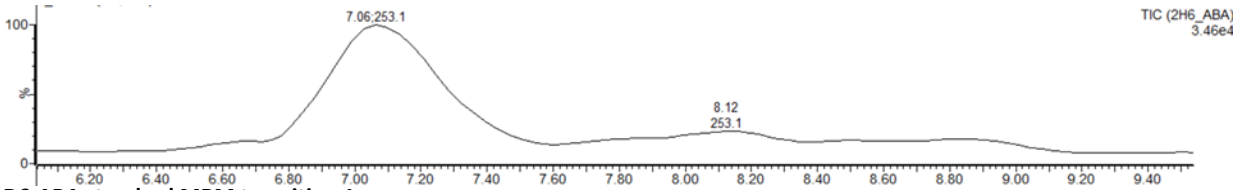
Sample-ABA MRM transition 1



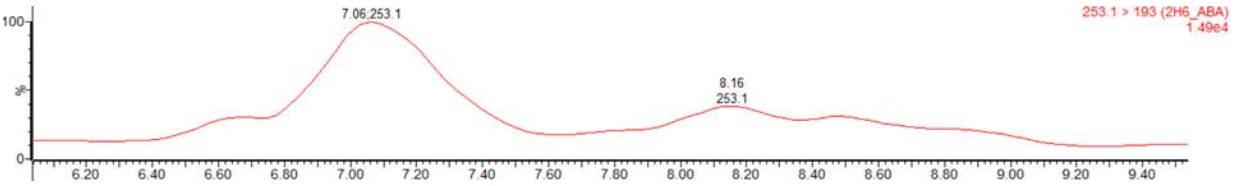
Sample-ABA MRM transition 2



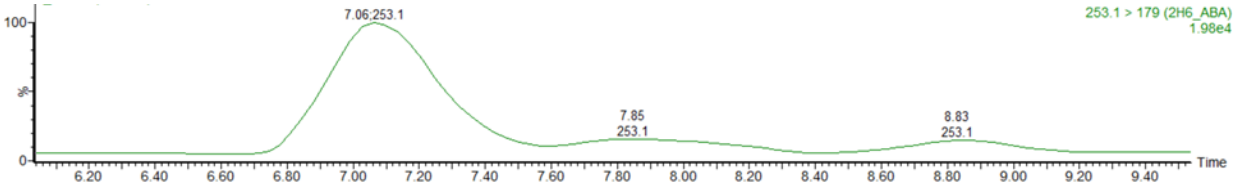
D6-ABA standard



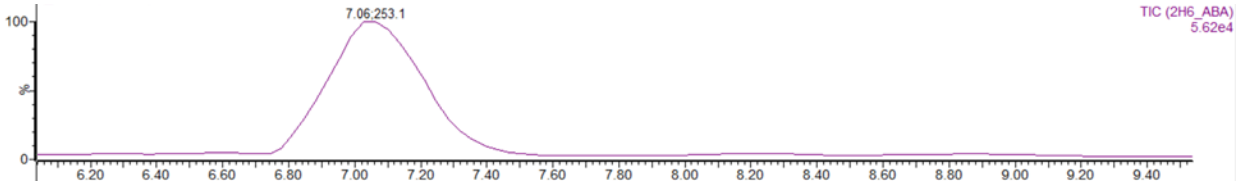
D6-ABA standard MRM transition 1



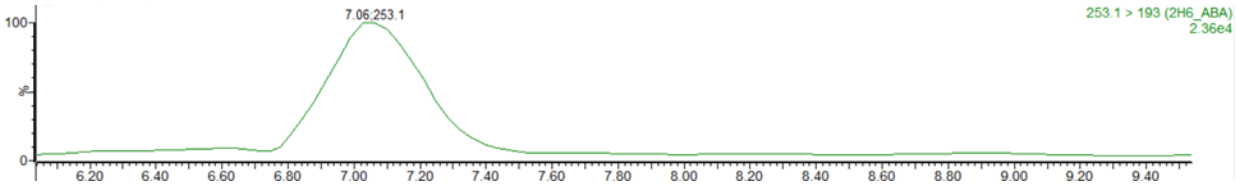
D6-ABA standard MRM transition 2



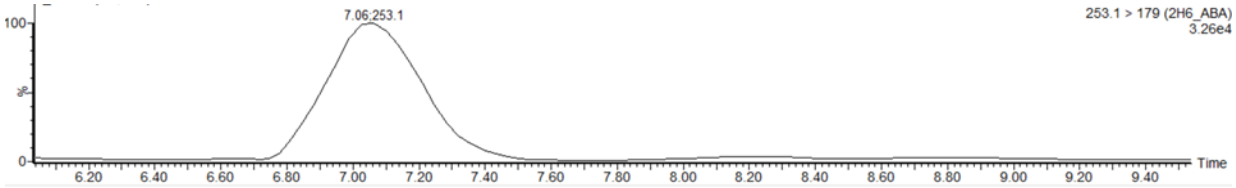
D6-ABA-sample



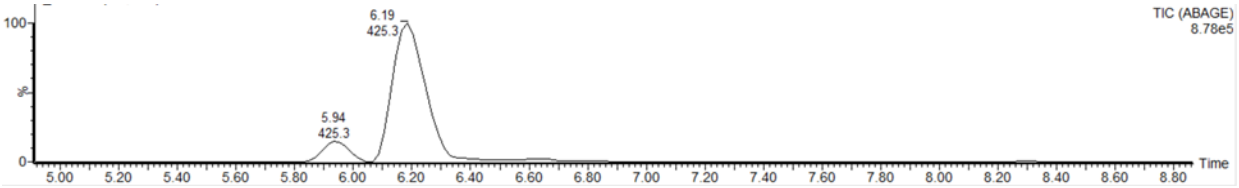
D6-ABA-sample-MRM transition1



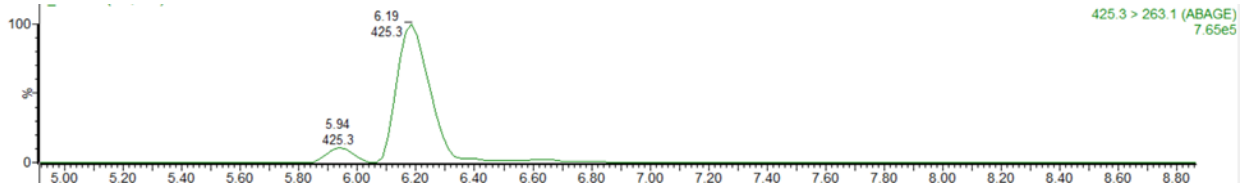
D6-ABA-sample-MRM transition2



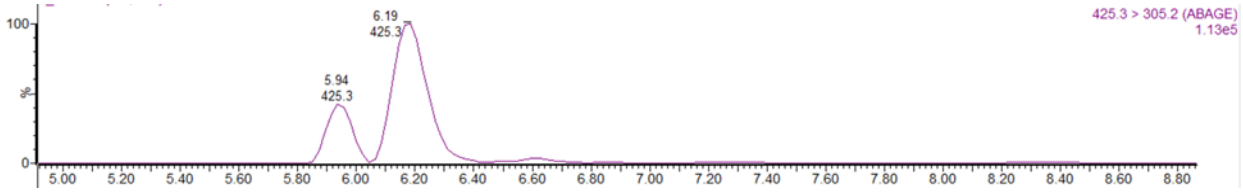
ABA-GE standard 2μg/ml



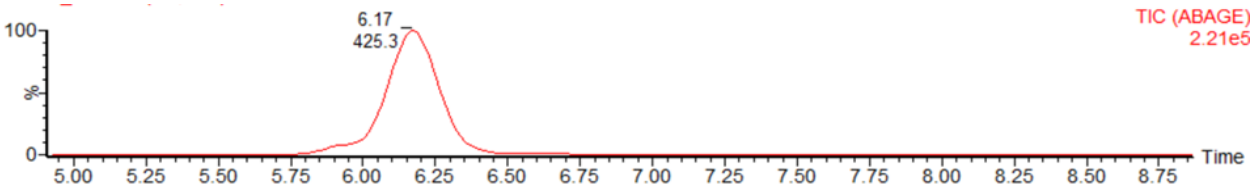
ABA-GE standard MRM transition1



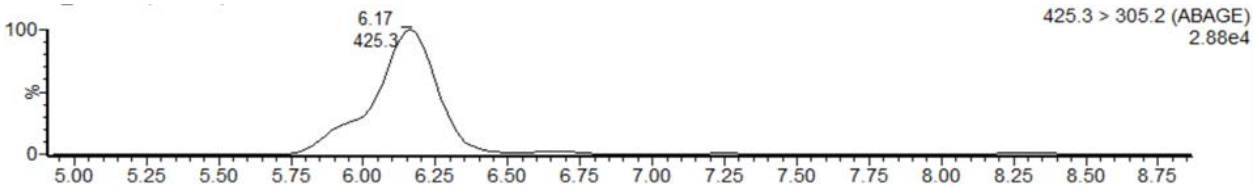
ABA-GE standard MRM transition2



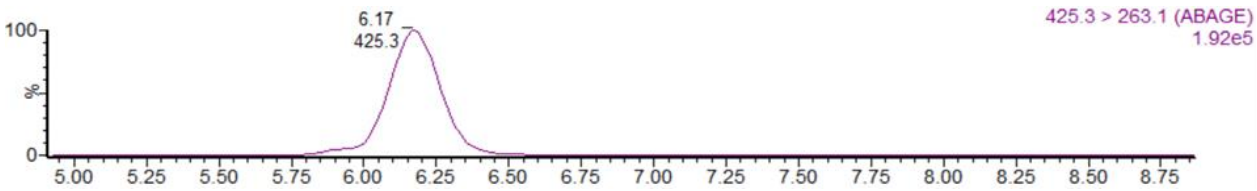
ABA-GE sample



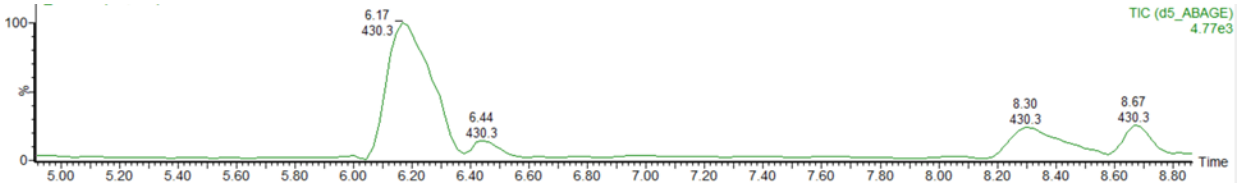
ABA-GE sample MRM transition1



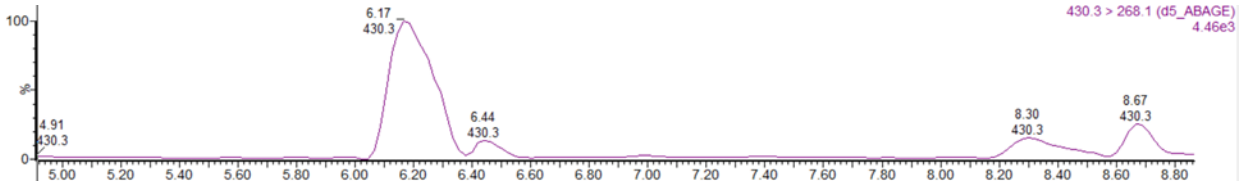
ABA-GE sample MRM transition2



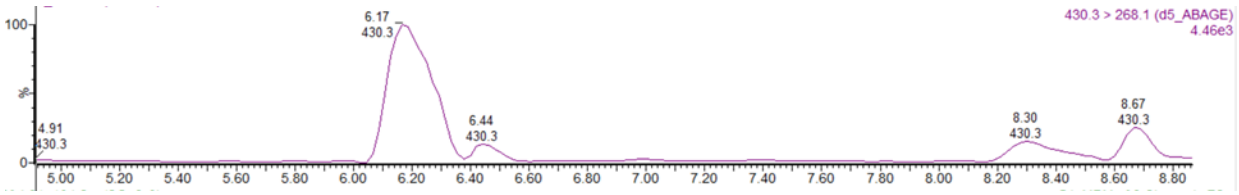
D5-ABA-GE Standard 52.5 ng/ml



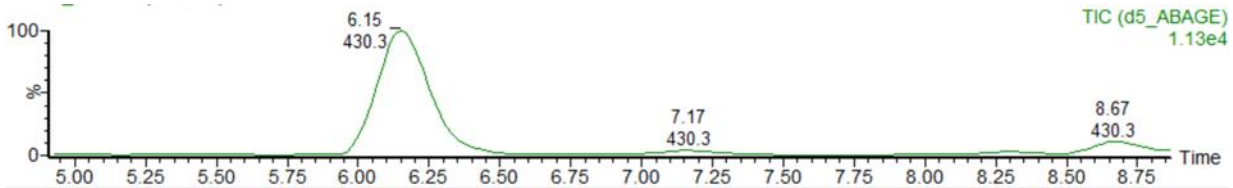
D5-ABA-GE MRM transition1



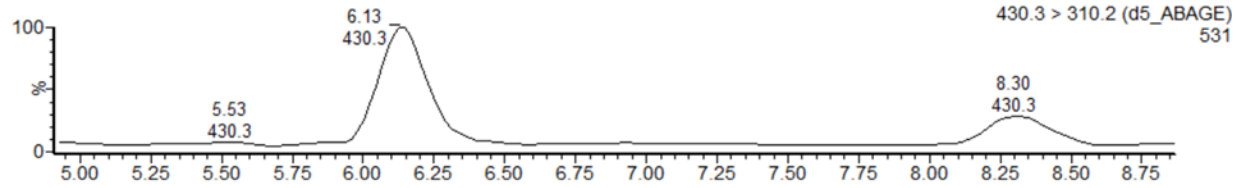
D5-ABA-GE MRM transition2



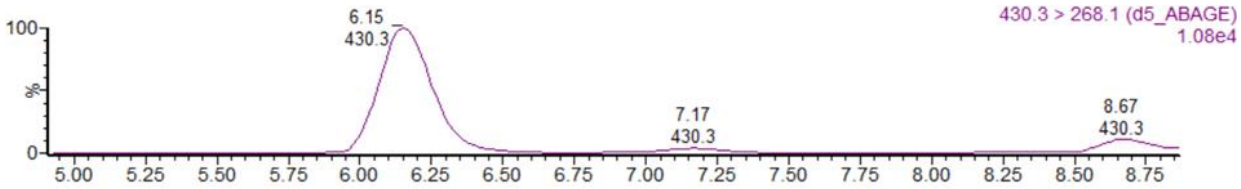
D5-ABA-GE Sample



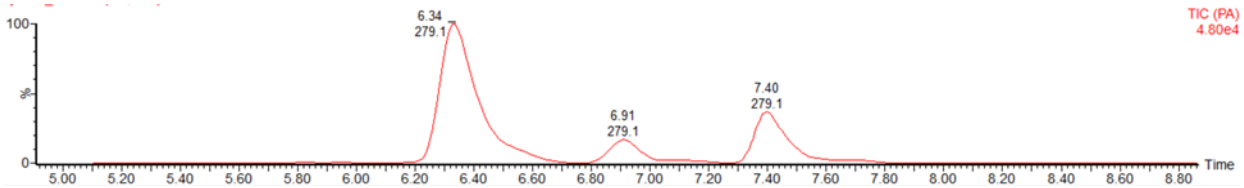
D5-ABA-GE Sample MRM transition1



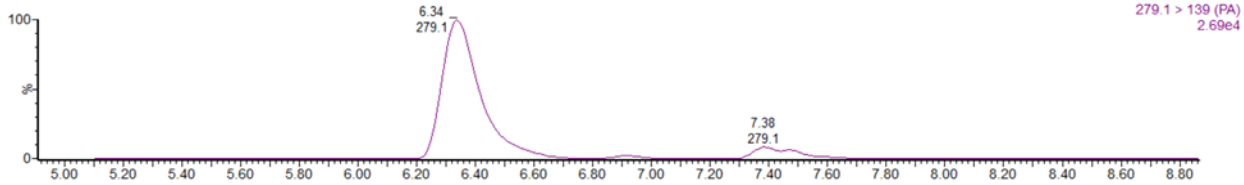
D5-ABA-GE Sample MRM transition2



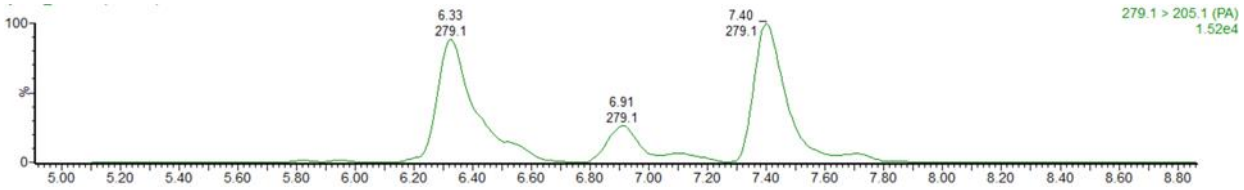
PA standard 15ng/ml



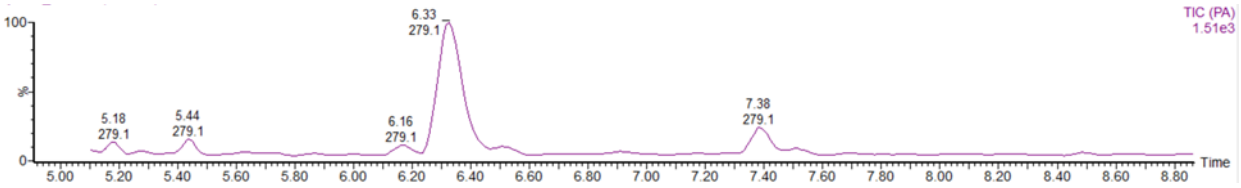
PA standard MRM transition1



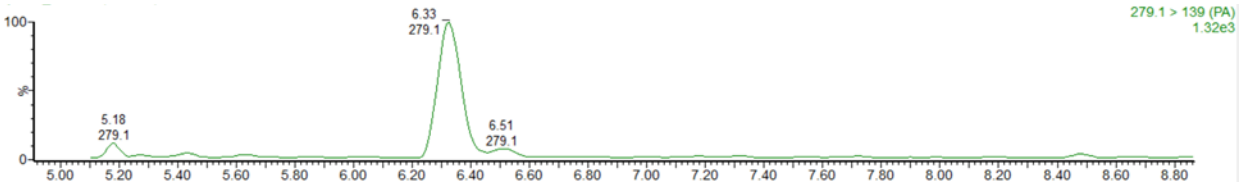
PA standard MRM transition2



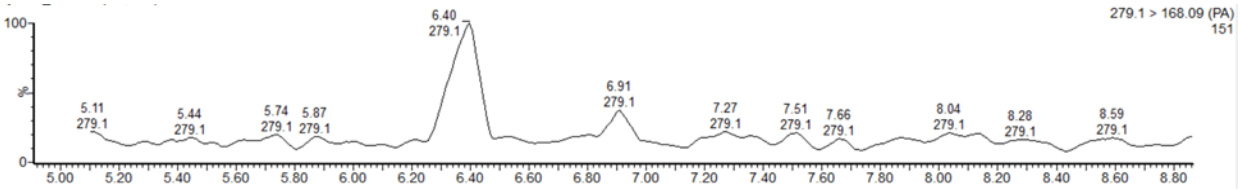
PA sample



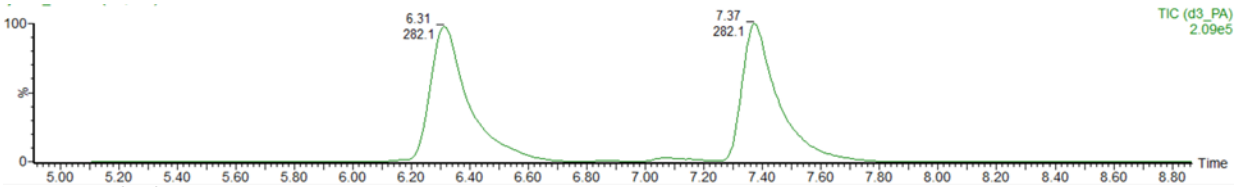
PA sample MRM transition1



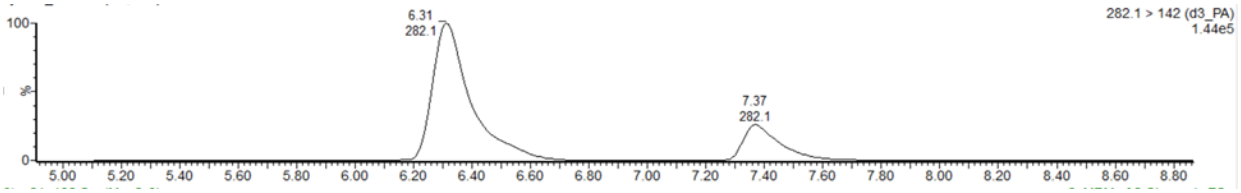
PA sample MRM transition2



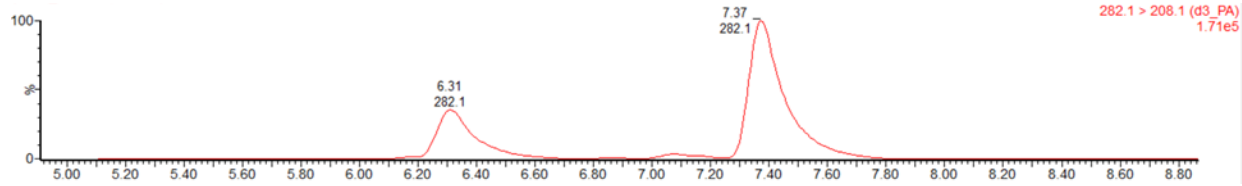
D3-PA standard 150 ng/ml



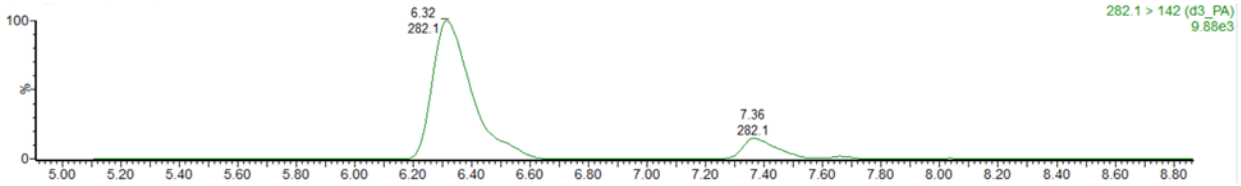
D3-PA standard MRM transition1



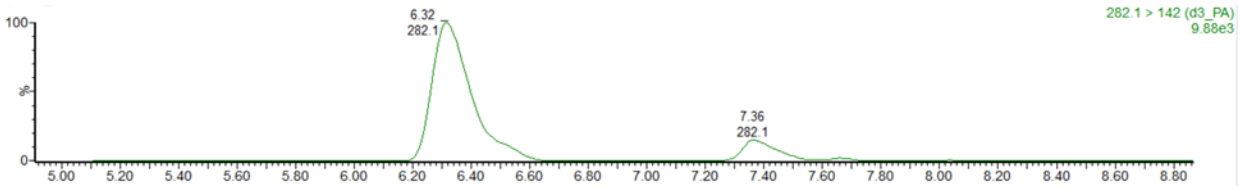
D3-PA standard MRM transition2



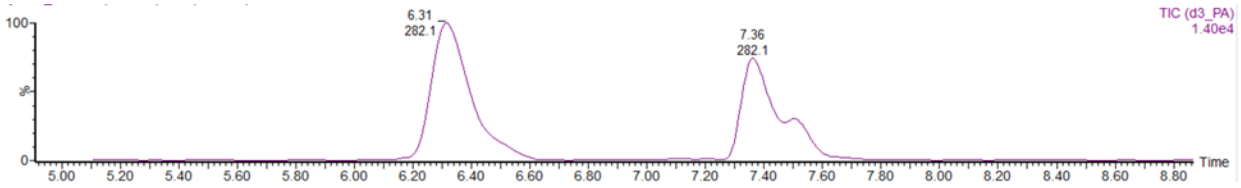
D3-PA sample



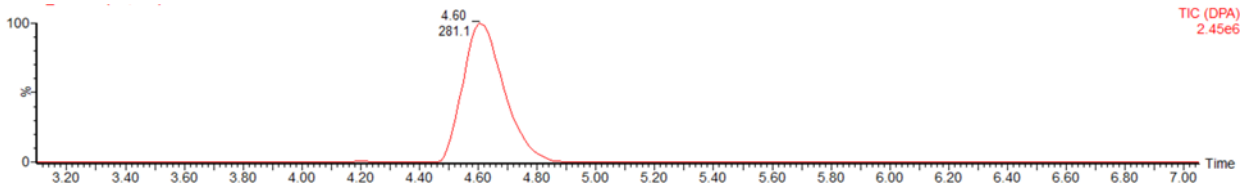
D3-PA sample MRM transition1



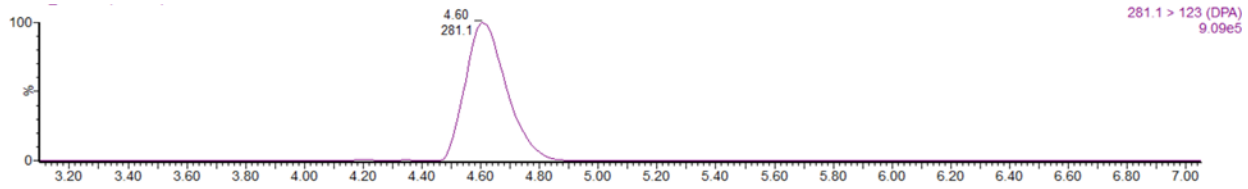
D3-PA sample MRM transition2



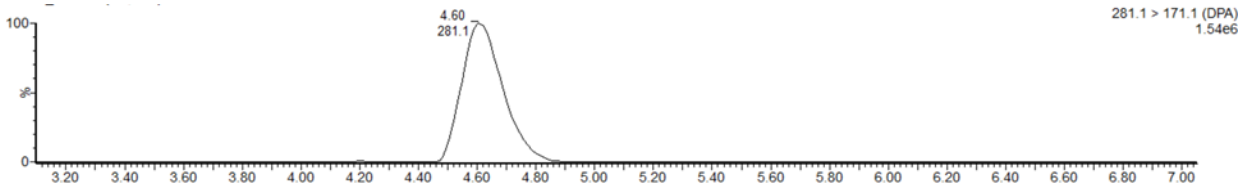
DPA standard 300 ng/ml



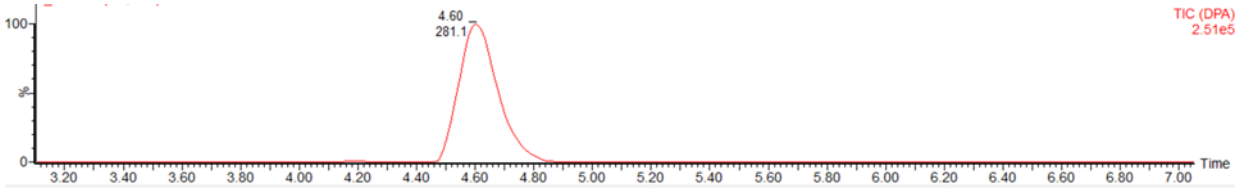
DPA standard MRM transition1



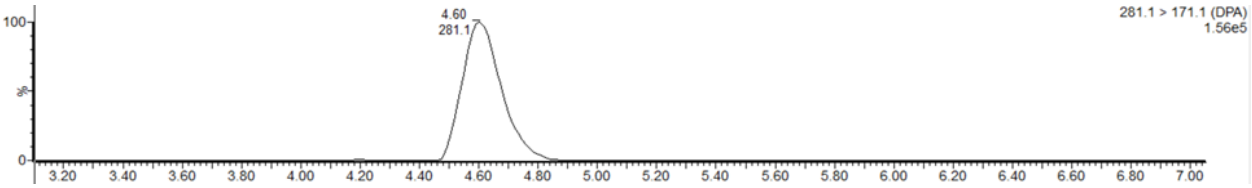
DPA standard MRM transition2



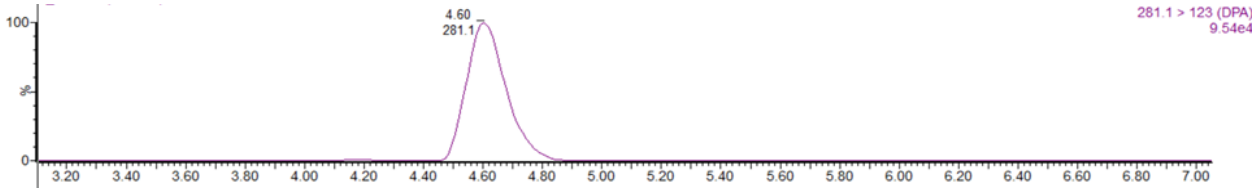
DPA sample



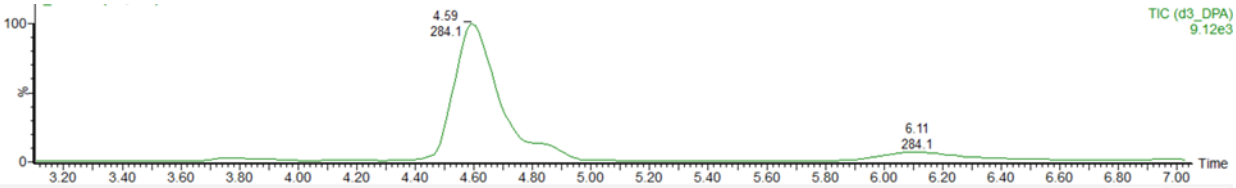
DPA sample MRM transition1



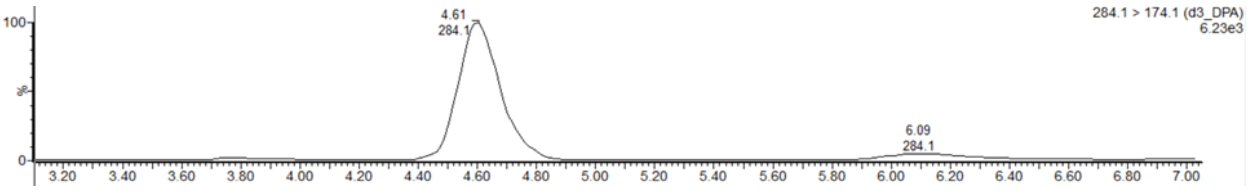
DPA sample MRM transition2



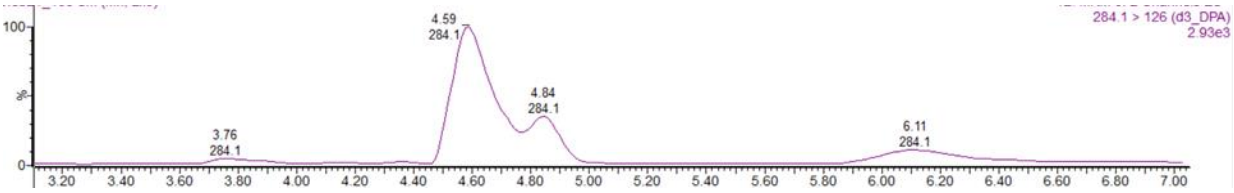
D3-DPA Standard 150 ng/ml



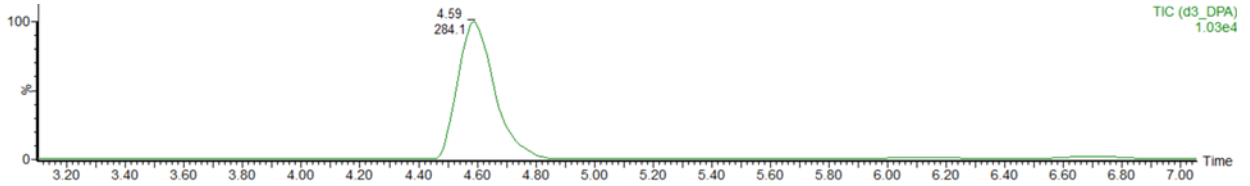
D3-DPA Standard MRM transition1



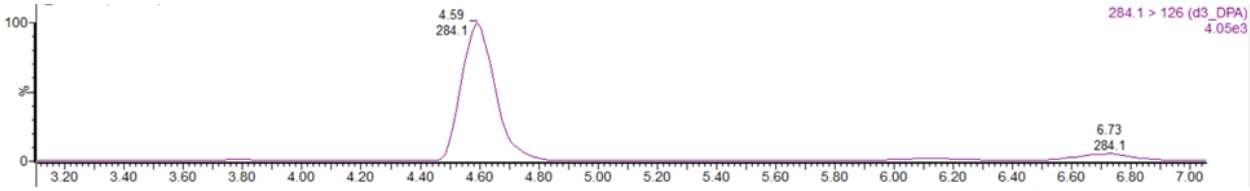
D3-DPA Standard MRM transition2



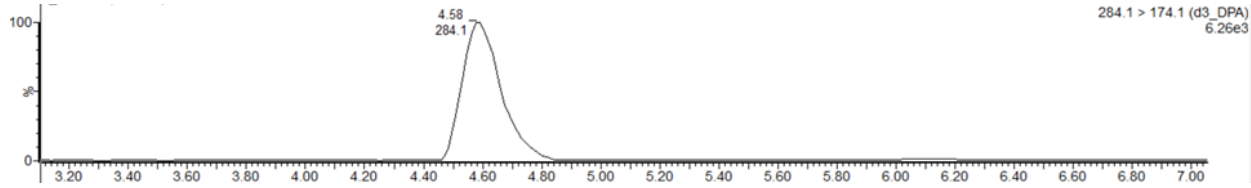
D3-DPA Sample



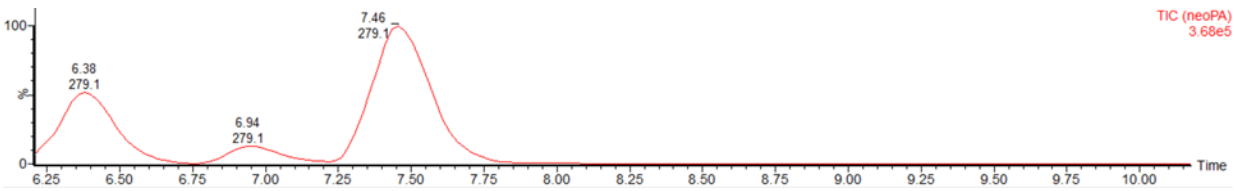
D3-DPA Sample MRM transition1



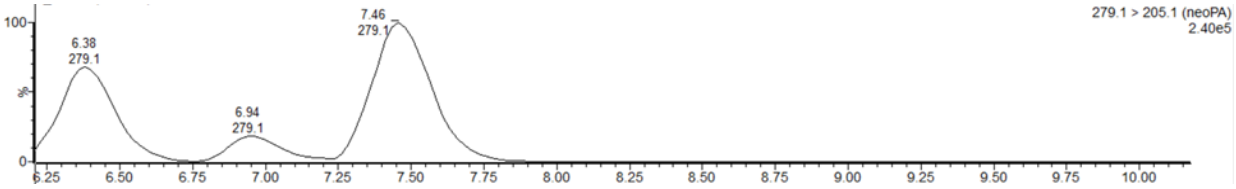
D3-DPA Sample MRM transition2



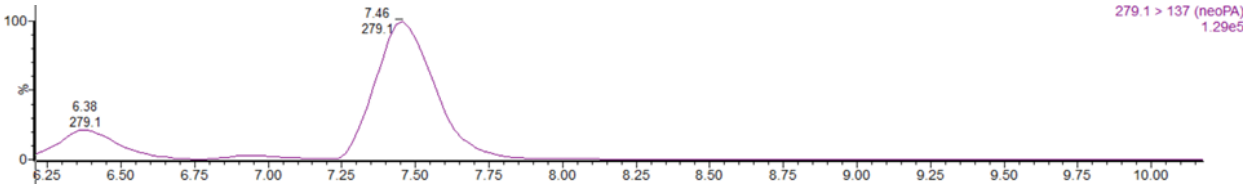
Neo PA standard 2.5 µg/ml



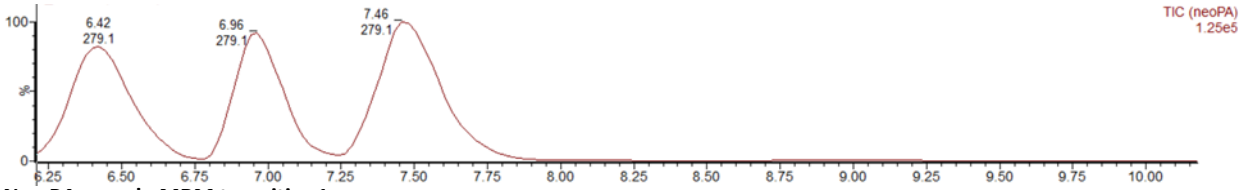
Neo PA standard MRM transition1



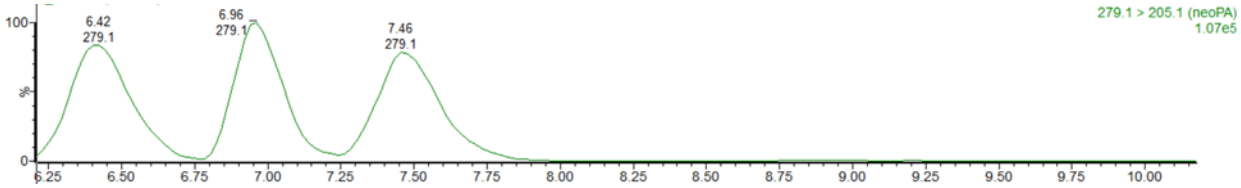
Neo PA standard MRM transition2



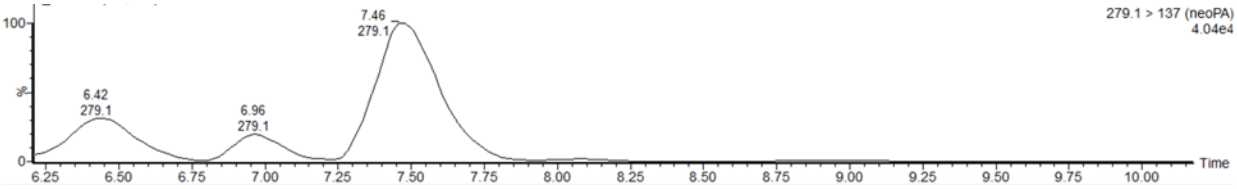
Neo PA sample



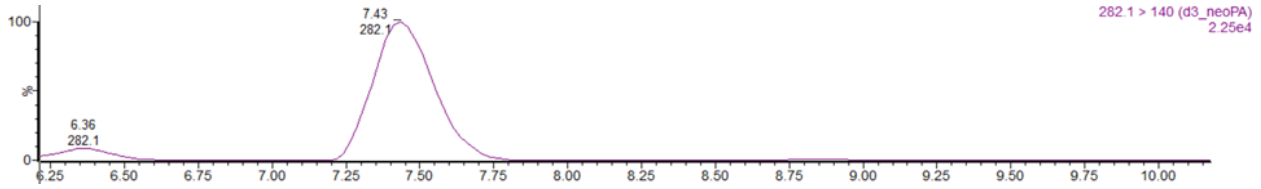
Neo PA sample MRM transition1



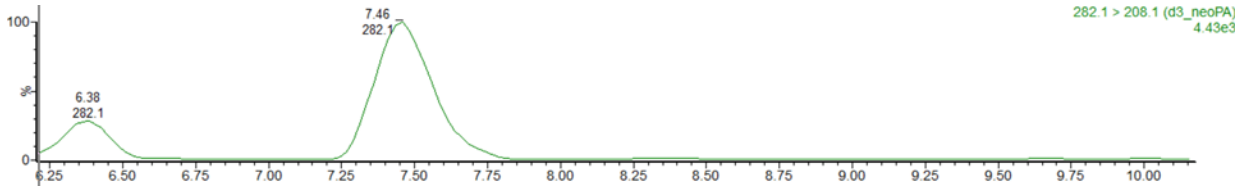
Neo PA sample MRM transition2



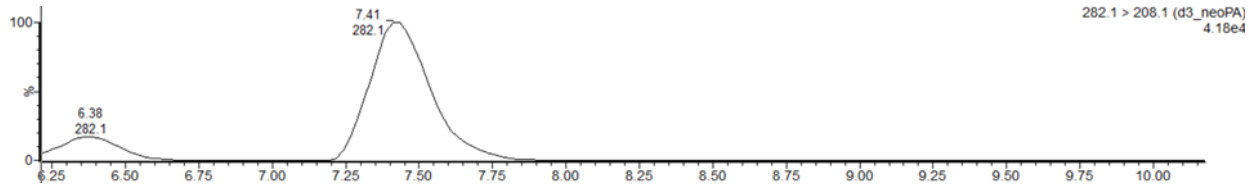
D3-neo PA Standard 150 ng/ml



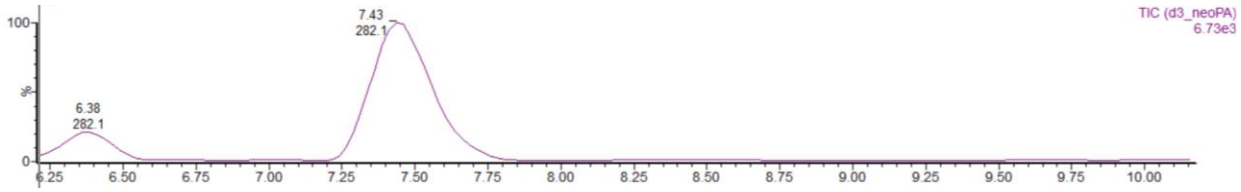
D3-neo PA Standard MRM transition1



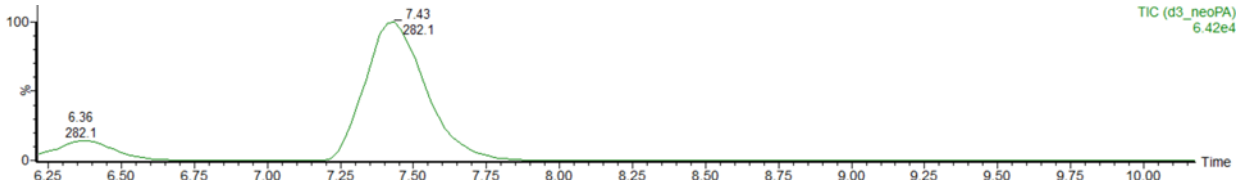
D3-neo PA Standard MRM transition2



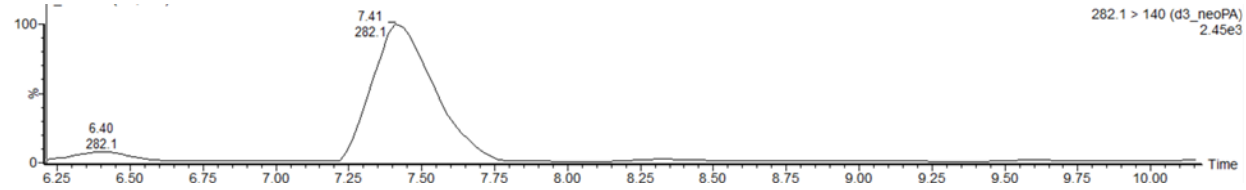
D3-neo PA Sample



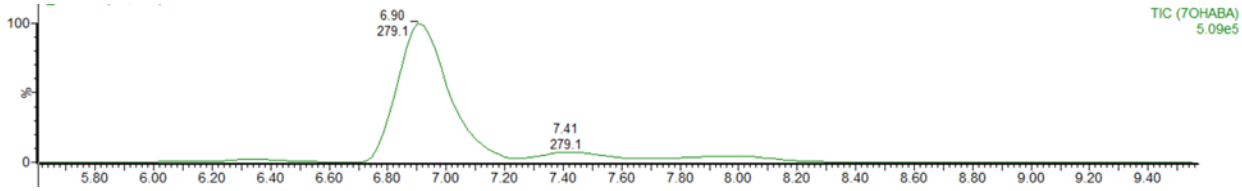
D3-neo PA Sample MRM transition1



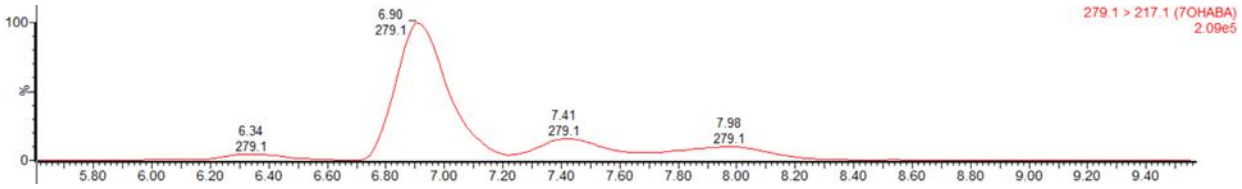
D3-neo PA Sample MRM transition2



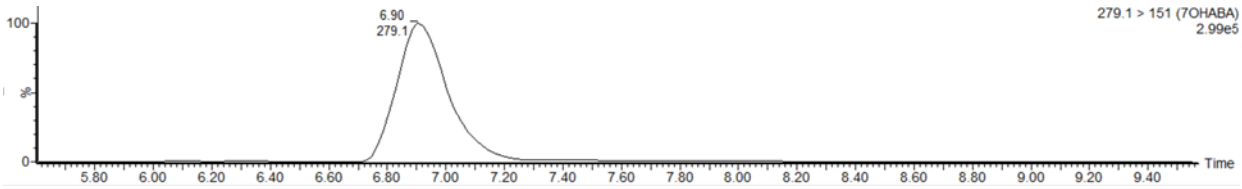
7-hydroxy ABA standard 600 ng/ml



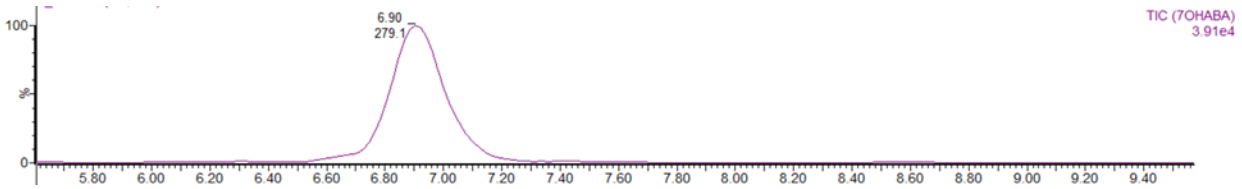
7-hydroxy ABA standard MRM transition1



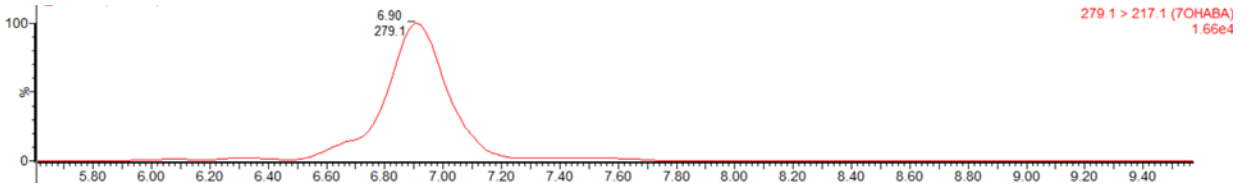
7-hydroxy ABA standard MRM transition2



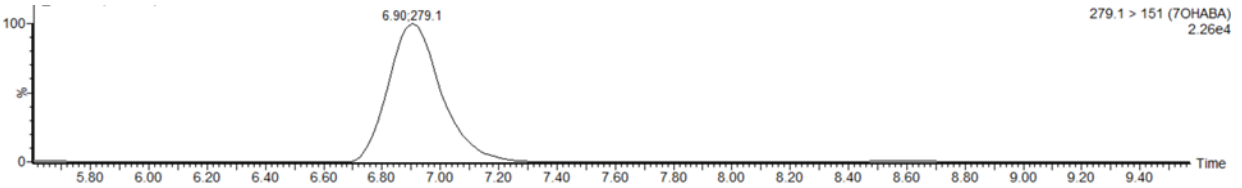
7-hydroxy ABA-sample



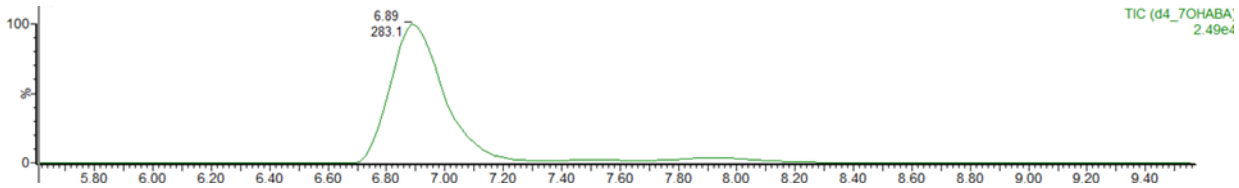
7-hydroxy ABA sample MRM transition1



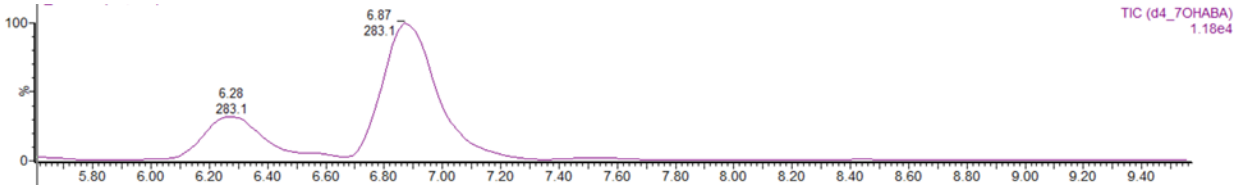
7-hydroxy ABA sample MRM transition2



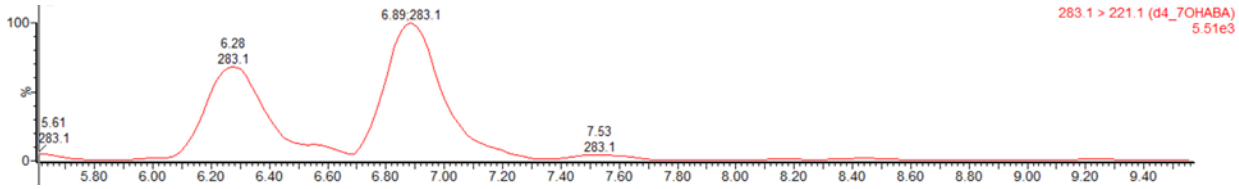
D4-7-hydroxy ABA standard 150 ng/ml



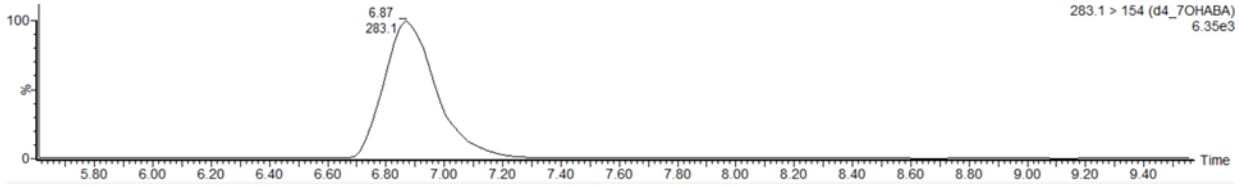
D4-7-hydroxy ABA standard MRM transition1



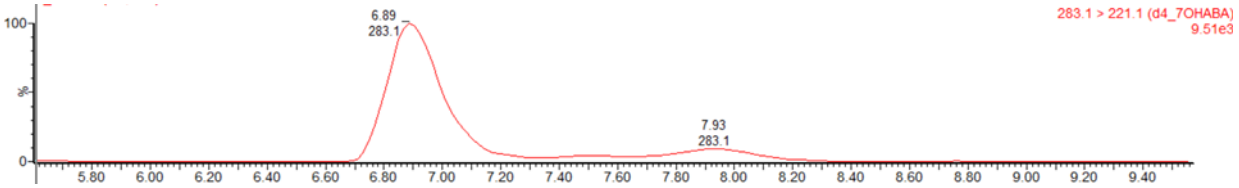
D4-7-hydroxy ABA standard MRM transition2



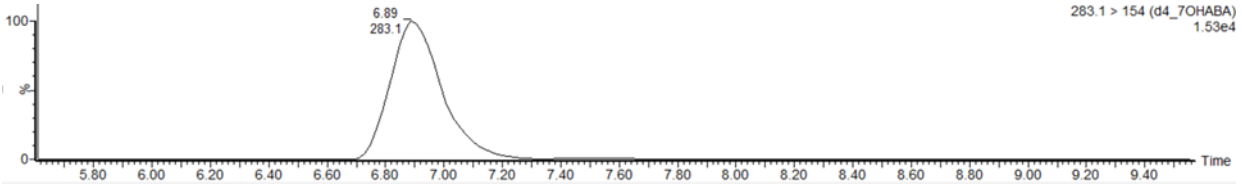
D4-7-hydroxy ABA sample



D4-7-hydroxy ABA sample MRM transition1



D4-7-hydroxy ABA sample MRM transition2



D- refers to the deuterated standard, i.e. deuterium labelled internal standard, D3- means three hydrogens are replaced by three deuterium atoms, similarly for 4, 5 and 6.