

***New Phytologist* Supporting Information**

Article title: **A Role for Ethylene Signaling and Biosynthesis in Regulating and Accelerating CO₂- and ABA-mediated Stomatal Movements in Arabidopsis.**

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Article acceptance date: 05 March 2023

The following Supporting Information is available for this article:

Dataset S1: Gas exchange raw data. (See separate file.)

Fig. S1 Ethylene production is lower in the high-order *acs* mutants in response to elevated and low [CO₂].

Fig. S2 CO₂-induced stomatal movements are severely affected in *acs* octuple mutant plant leaves but not in the *acs* sextuple and *acs* septuple mutants.

Fig. S3 Leaves of the ethylene overproducer, *eto1-1*, show intact CO₂-induced stomatal conductance responses.

Fig. S4 Leaves of the ethylene insensitive signaling mutant, *ein2-1*, show intact CO₂-induced stomatal conductance responses.

Fig. S5 Leaves of the dominant ethylene insensitive receptor mutants, *etr1-1* and *etr2-1*, show intact CO₂-induced stomatal conductance responses.

Fig. S6 Leaves of intact *etr1-6;etr2-3* double mutant and *etr1-6* single mutant plants show accelerated stomatal opening and closing and an enhanced magnitude of stomatal conductance responses to [CO₂] shifts.

Fig. S1

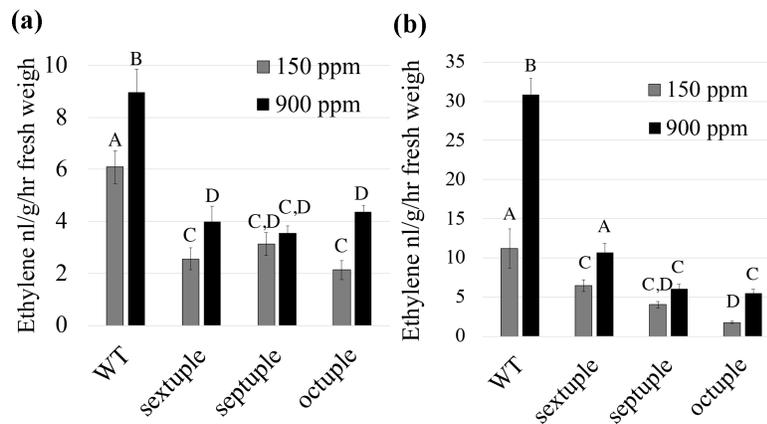


Fig. S1 Ethylene production is lower in the high-order *acs* mutants in response to elevated and low [CO₂]. Five-week-old *A. thaliana* wild-type (WT), ACC synthase (*acs*) sextuple, septuple and octuple mutant plants were incubated for 90 min under low (150 ppm) or high (900 ppm) [CO₂]. Two independent experimental data sets, different from that shown in Figure 2, are shown here. Ethylene production in *A. thaliana* rosettes was quantified using gas chromatography (n=6 replicates per each line and each treatment, where 2 whole *A. thaliana* rosettes were measured in each replicate). Different letters above bars mean significant statistical differences between lines and treatment (Two-way ANOVA, $P < 0.05$). Similar results were found in 3 independent experimental sets. See Fig. 2 for an additional independent experimental set.

Fig. S2

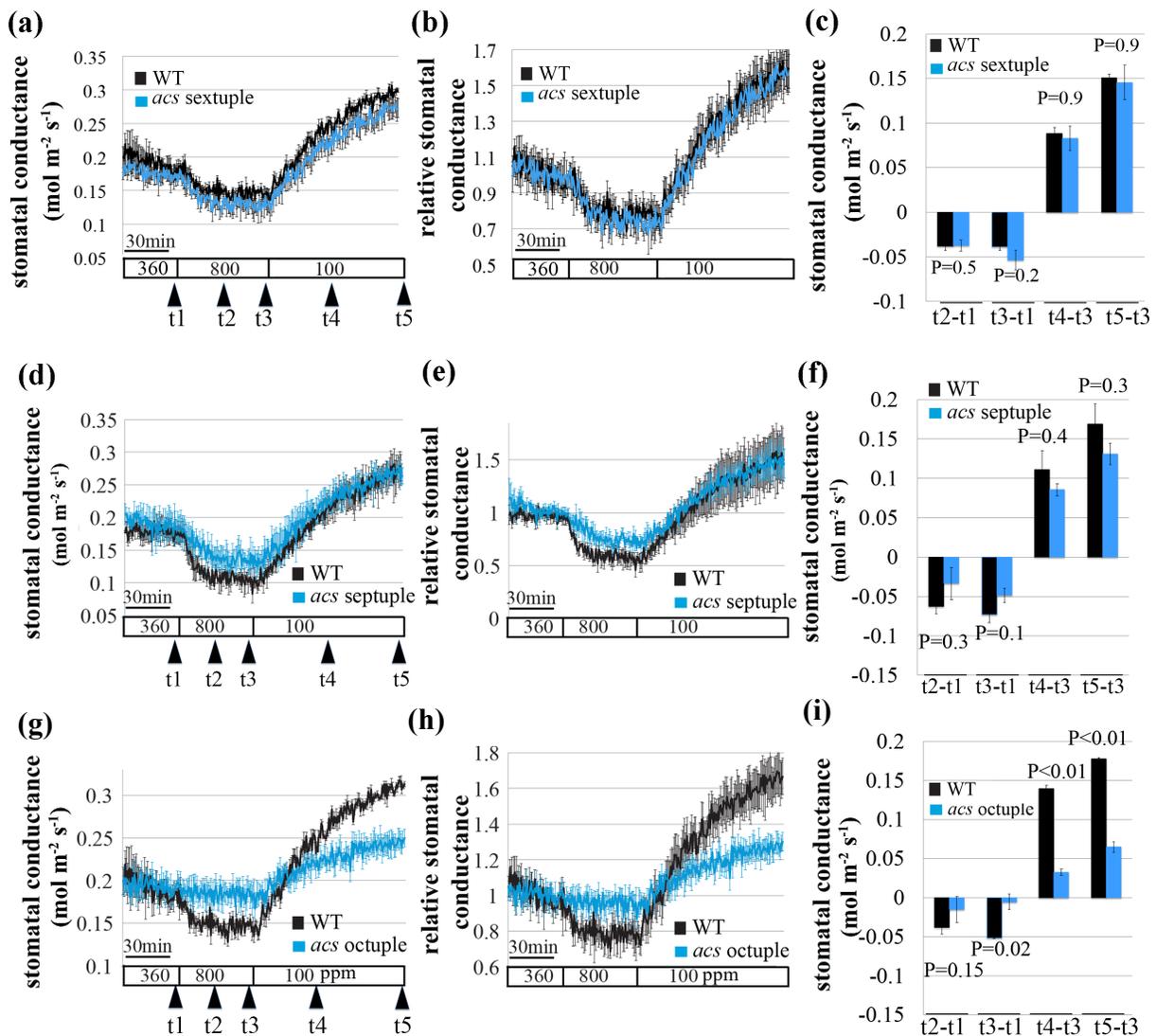


Fig. S2 CO₂-induced stomatal movements are severely affected in *acs* octuple mutant plant leaves but not in the *acs* sextuple and *acs* septuple mutants. The graphs show average stomatal conductance of (a-c) wild-type (WT, Col-0) (n=4) and *acs* sextuple (n=3), (d-f) WT (n=3) and *acs* septuple (n=4), and (g-i) WT (n=4) and *acs* octuple (n=4) *A. thaliana* mutant leaves from intact plants in response to shifts in imposed [CO₂] as indicated at the bottom (ppm). (a, d and g) show stomatal conductance means (±SEM) of intact leaves from individual plants per genotype. (b, e, and h) Stomatal conductance (in panels a, d and g) were normalized to the

stomatal conductance at 360 ppm [CO₂] before shifting to 800 ppm [CO₂]). (**c, f and i**) Changes in absolute stomatal conductance (mean ± SEM) were calculated at the indicated time points based on the data presented on panels **a, d** and **g** (t₁=stomatal conductance at 360 ppm [CO₂], t₂=15 min after shifting to 800 ppm [CO₂], t₃=30 min after shifting to 800 ppm [CO₂], t₄=40 min after shifting to 100 ppm [CO₂], t₅=80 min after shifting to 100 ppm [CO₂]). Statistical analyses were done using unpaired Student's *t* tests between the wild-type and the mutant line, P-value is presented above/under columns. Note, wild-type control gas exchange data presented in panels **a-c** and **d-f** are the same as shown in Fig. **3g-I**, as these mutants were investigated within the same experimental set. Comparable results are shown in Fig. **3**.

Fig. S3

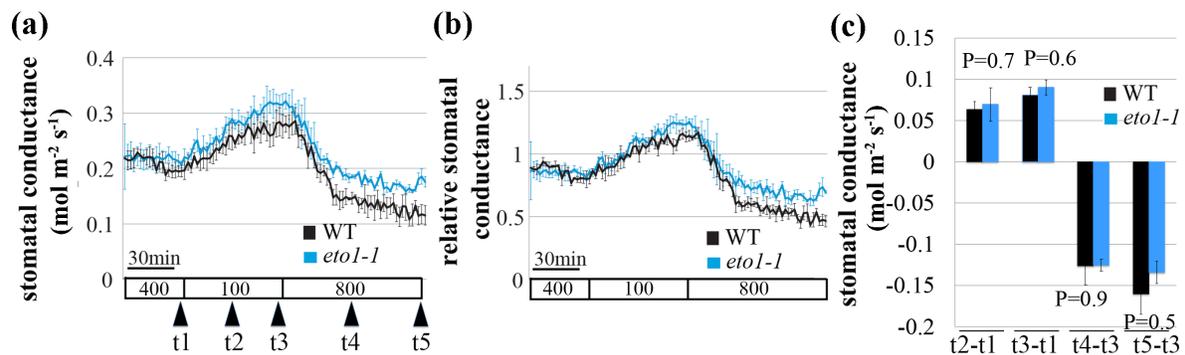


Fig. S3 Leaves of the ethylene overproducer, *eto1-1*, show intact CO₂-induced stomatal conductance responses. Stomatal conductance of wild-type (WT, Col-0) and *eto1-1 A. thaliana* mutant leaves from intact plants in response to shifts in imposed [CO₂] as indicated at the bottom (ppm). **(a)** Shown are mean (\pm SEM) of n=3 leaves from individual plants per genotype. **(b)** Stomatal conductance (in panel a) was normalized to the stomatal conductance at 400 ppm [CO₂]. **(c)** Changes in absolute stomatal conductance (mean \pm SEM) were calculated at the indicated time points based on the data presented on panel a (t1 = stomatal conductance under 400 ppm [CO₂], t2=20 min following exposure to 100 ppm [CO₂], t3=40 min following exposure to 100 ppm [CO₂], t4=22 min following exposure to 800 ppm [CO₂], t5=44 min following exposure to 800 ppm [CO₂]). Statistical analyses were done using unpaired Student's *t* tests between the wild-type and the mutant line, P-value is presented above/under columns. Note, wild-type control gas exchange data presented are the same as shown in Fig. S5, as these mutants were investigated within the same experimental set. Experimental sets were repeated 3 times leading to similar findings. See Fig. 4 for an additional independent experimental set.

Fig. S4

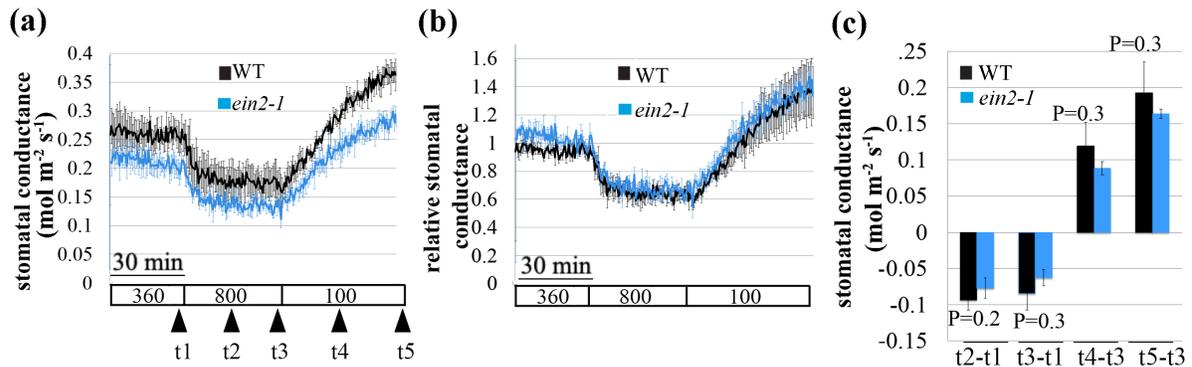


Fig. S4 Leaves of the ethylene insensitive signaling mutant, *ein2-1*, show intact CO₂-induced stomatal conductance responses. The graphs show average leaf stomatal conductance of wild-type (WT, Col-0) and *ein2-1* *A. thaliana* mutant from intact plants in response to shifts in response to [CO₂] shifts, as indicated at the bottom (ppm). **(a)** Shown are stomatal conductance means (\pm SEM) of n=3 leaves from different plants for each genotype. **(b)** Stomatal conductance (in panel a) was normalized to the stomatal conductance at 360 ppm [CO₂] before shifting to 800 ppm [CO₂]. **(c)** Changes in absolute stomatal conductance (mean \pm SEM) were calculated at the indicated time points based on the data presented on panel a (t1= stomatal conductance at 360 ppm [CO₂], t2=20 min following exposure to 800 ppm [CO₂], t3=40 min following exposure to 800 ppm [CO₂], t4=25 min following exposure to 100 ppm [CO₂], t5=50 min following exposure to [CO₂]). Statistical analyses were done using unpaired Student's *t* tests between the wild-type and the mutant line, P-value is presented above/under columns. Independent experimental sets were carried out twice for each genotype with comparable results. See Fig. 5 for additional independent experiments.

Fig. S5

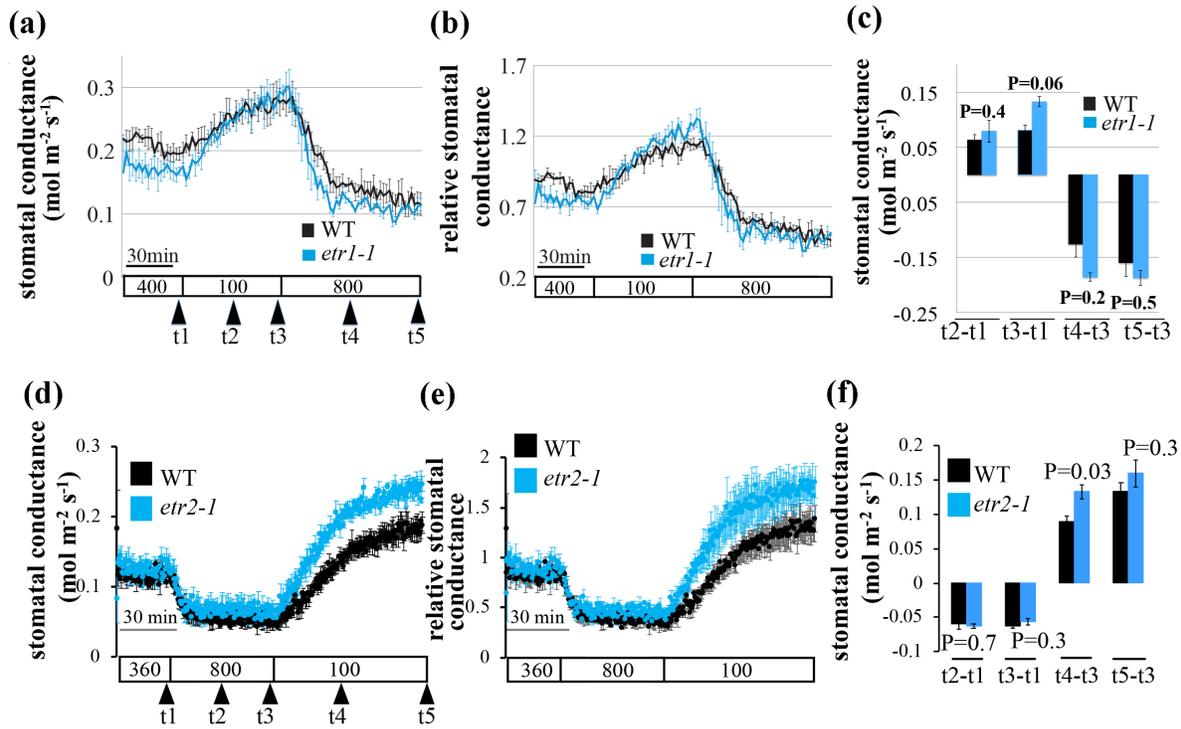


Fig. S5 Leaves of the ethylene insensitive receptor mutants, *etr1-1* and *etr2-1*, show intact CO₂-induced stomatal conductance responses. Stomatal conductance of wild-type (WT, Col-0), (a-c) *etr1-1* or (d-f) *etr2-1* *A. thaliana* mutant leaves from intact plants in response to shifts in imposed [CO₂] as indicated at the bottom (ppm). (a, d) Shown are mean (±SEM) of n=3 leaves from individual plants per genotype. (b, e) Stomatal conductances (in panels a and d) were normalized to the stomatal conductances at 400/360 ppm [CO₂] before shifting to 800/100 ppm [CO₂]. (c, f) Changes in absolute stomatal conductance (mean ± SEM) were calculated at the indicated time points based on the data presented on panel a (t1= stomatal conductance at 400 ppm [CO₂], t2=20 min following exposure to 100 ppm [CO₂], t3=40 min following exposure to 100 ppm [CO₂], t4=22 min following exposure to 800 ppm [CO₂], t5=44 min following exposure to 800 ppm [CO₂]) or panel d (t1 = stomatal conductance at 360 ppm [CO₂], t2=15 min following exposure to 800 ppm [CO₂], t3=30 min following exposure to 800 ppm [CO₂], t4=40

min following exposure to 100 ppm [CO₂], t5=80 min following exposure to 100 ppm [CO₂]). Statistical analyses were done using unpaired Student's *t tests* between the wild-type and the mutant line, P-value is presented above/under columns. Note, wild-type control gas exchange data presented in panels **a-c** are the same as shown in Fig. **S3**, as these mutants were investigated within the same experimental set. Independent experimental sets were carried out twice for each genotype with similar findings. See Fig. **6** for an additional independent study.

Fig. S6

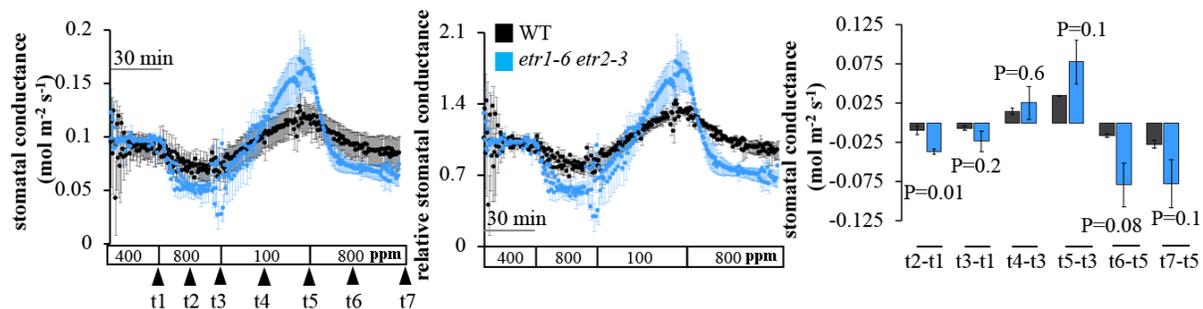


Fig. S6 Leaves of intact *etr1-6;etr2-3* double mutant plants show accelerated stomatal opening and closing and an enhanced magnitude of stomatal conductance responses to [CO₂] shifts. **(a)** The graph shows average stomatal conductance (n=4 independent leaves per genotype) ± SEM of wild-type (WT, Col-0) and *etr1-6;etr2-3* *A. thaliana* mutant leaves from intact plants in response to shifts in imposed [CO₂] as indicated at the bottom (ppm). **(b)** Stomatal conductance (in panels **a**) was normalized to the average of the first 30 minutes of stomatal conductance values at 400 ppm [CO₂]. **(c)** Changes in absolute stomatal conductance (mean ± SEM) are shown (t1= stomatal conductance at 400 ppm [CO₂], t2=15 min following exposure to 800 ppm [CO₂], t3=30 min following exposure to 800 ppm [CO₂], t4=20 min following exposure to 100 ppm [CO₂], t5=45 min following exposure to 100 ppm [CO₂], t6=30 min after a second exposure to 800 ppm [CO₂], t7=60 min after a second exposure to 800 ppm [CO₂]). Statistical analyses were done using unpaired Student's *t* tests between the wild-type and the mutant line, P-value is presented above/under columns. See Fig. 7 for additional independent study.