Supplementary Information

Jasmonate Signaling Enhances Submergence Tolerance by Stabilizing ERF-VII Transcription Factor RAP2.12 in *Arabidopsis*

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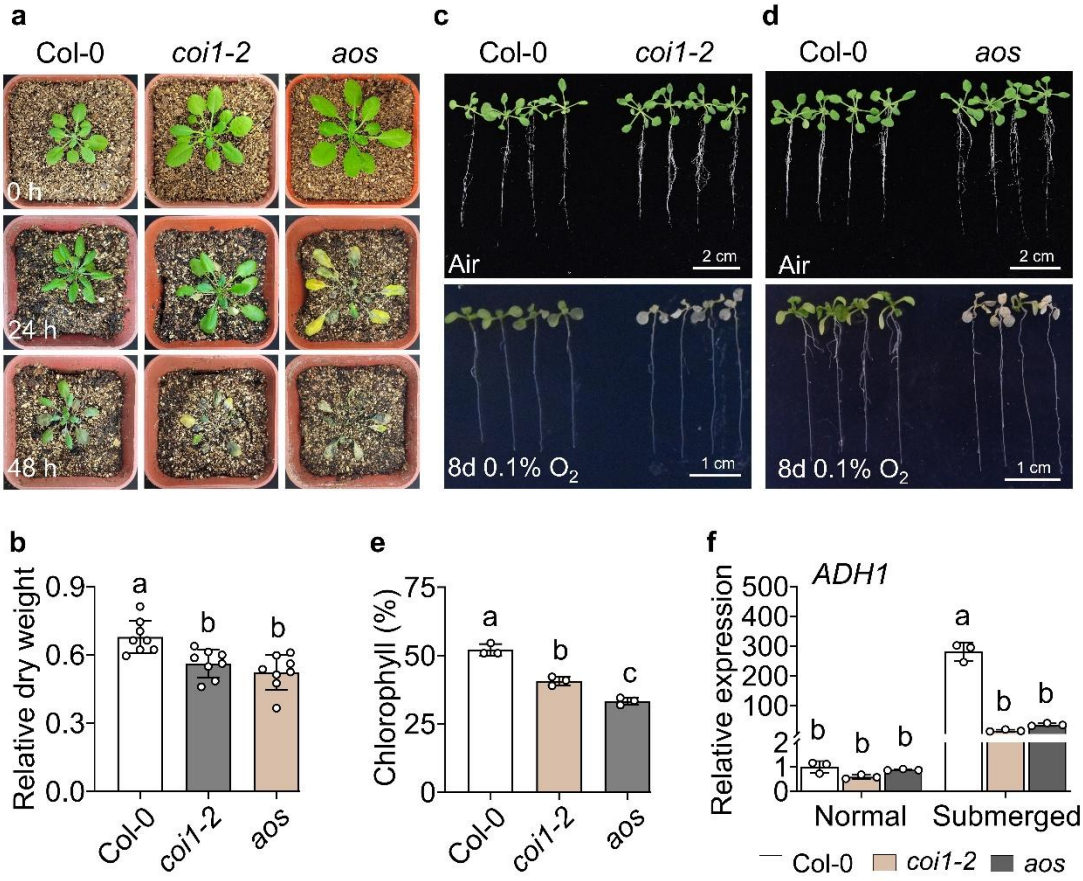
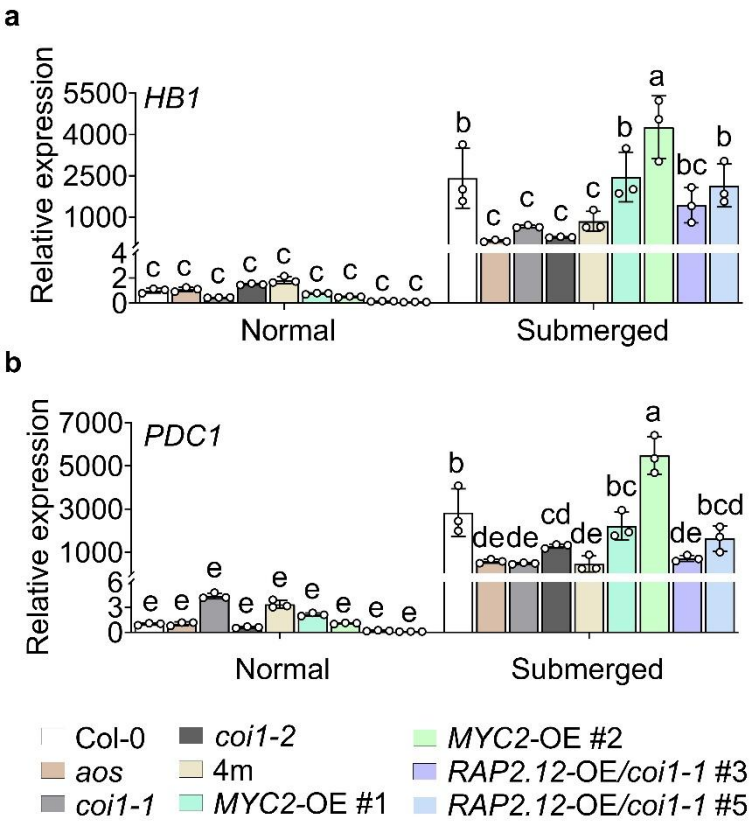


Supplementary Fig. 1 Mutants of JA signaling pathway displayed more sensitive phenotypes to submergence or hypoxia. (support Figure 1)

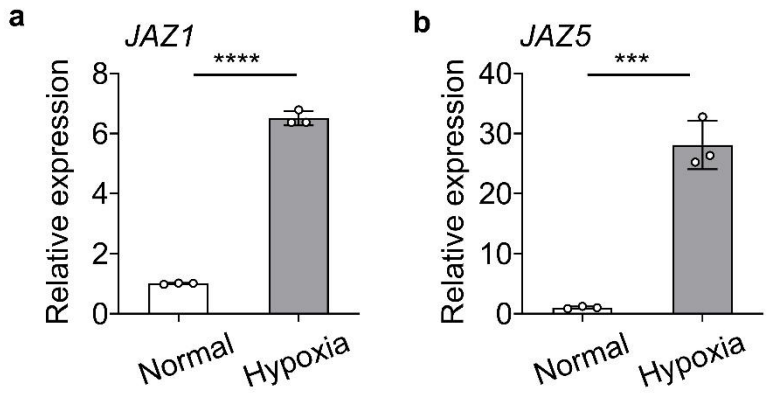
a) Phenotypes of 4-week-old Col-0, *coi1-2* and *aos* plants subjected to submergence for 0 h, 24 h or 48 h followed by a 7-d recovery under normal growth conditions. The phenotypes of Col-0, *coi1-2* and *aos* before submergence were shown as control. **b)** The relative dry weight for the genotypes shown in (a). 4-week-old Col-0, *coi1-2* and *aos* plants were subjected to 48-h submergence followed by a 7-d recovery under normal growth conditions comparing with plants in normal growth conditions for 9 days. Data are means ± SD (n = 8 independent plants). Different lowercase letters indicate significant differences at $P < 0.05$ (one-way ANOVA with Tukey's HSD test). **c)** Phenotypes of 1-week-old Col-0 and *coi1-2* seedlings subjected to hypoxia (0.1% [v/v] O_2) or normoxia conditions for 8 d followed by a 2-d recovery under normal conditions. **d)** Phenotypes of 1-week-old Col-0 and *aos* seedlings subjected to hypoxia

(0.1% [v/v] O_2) or normoxia conditions for 8 d followed by a 2-d recovery under normal conditions. **e)** Relative chlorophyll contents for the genotypes shown in (c) and (d). Data are means ± SD (n = 3 biological replicates with each replicate of 4 independent seedlings). Different lowercase letters indicate significant differences at $P < 0.05$ (one-way ANOVA with Tukey's HSD test). **f)** RT-qPCR analysis of hypoxia response gene *ADH1* in 4-week-old Col-0, *coi1-2* and *aos* plants subjected to submergence or normal conditions for 4 h. Transcripts were normalized to the expression levels of *ACTIN8*. The Col-0 data was from Figure. 1G. Error bars represent means ± SD (n = 3 biological replicates with each replicate of 8 independent plants). For each biological replicate, three technical repeats were used for analyses. Different lowercase letters indicate significant differences at $P < 0.05$ (one-way ANOVA with Tukey's HSD test).



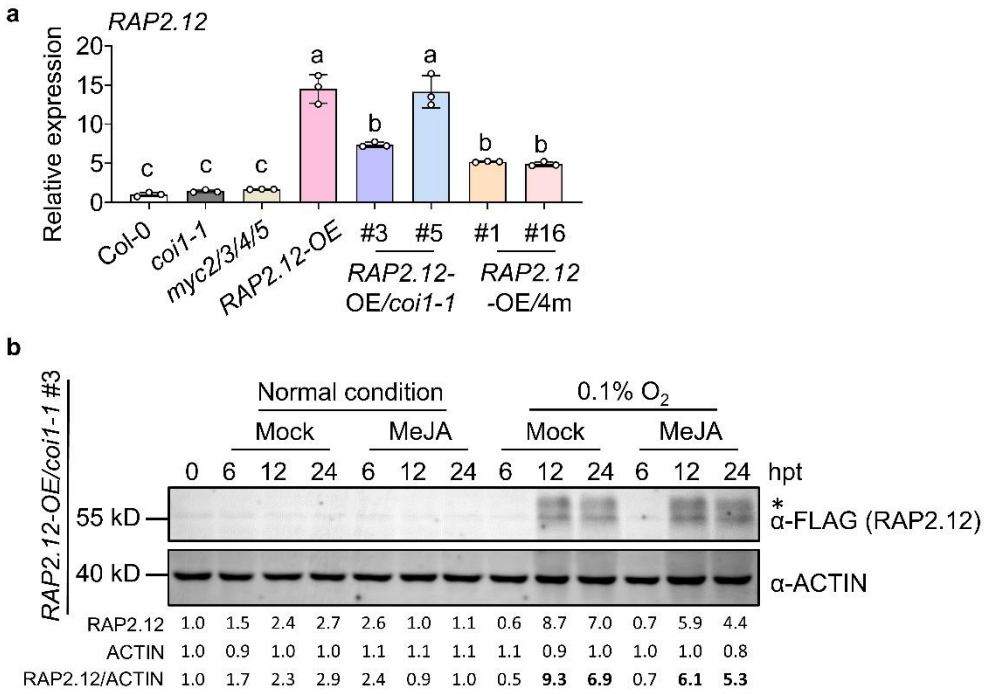
Supplementary Fig. 2 JA signaling mutants displayed lower expression level of *HRG*s under submergence treatment. (support Figure 1, 3)

a) and **b)** RT-qPCR analysis of hypoxia response gene *HB1* (a) and *PDC1* (b) in 4-week-old Col-0, *aos, coi1-1, coi1-2, myc2/3/4/5* (4m), *MYC2*-OE (#1 and #2) and *RAP2.12*-OE/*coi1-1* (#3 and #5) plants subjected to submergence or normal conditions for 4 h. Transcripts were normalized to the expression levels of *ACTIN8*. Error bars represent means ± SD (n = 3 biological replicates with each replicate of 8 independent plants). For each biological replicate, three technical repeats were used for analyses. Different lowercase letters indicate significant differences at $P < 0.05$ (one-way ANOVA with Tukey's HSD test).



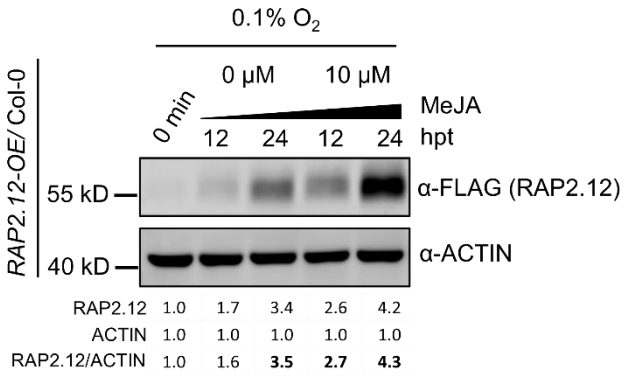
Supplementary Fig. 3 Hypoxic treatment induces the activation of JA signaling. (support Figure 1)

a) and **b)** RT-qPCR analysis of JA responsive gene, *JAZ1* (a) and *JAZ5* (b) in 1-week-old Col-0 seedlings under hypoxia treatment for 15 min. Transcripts were normalized to the expression levels of *ACTIN8*. Error bars represent means ± SD (n = 3 biological replicates with each replicate of 8 independent seedlings). For each biological replicate, three technical repeats were used for analyses. Asterisks indicate significant differences between the samples (****$P < 0.0001$ and ***$P < 0.001$ by Student's t-test).



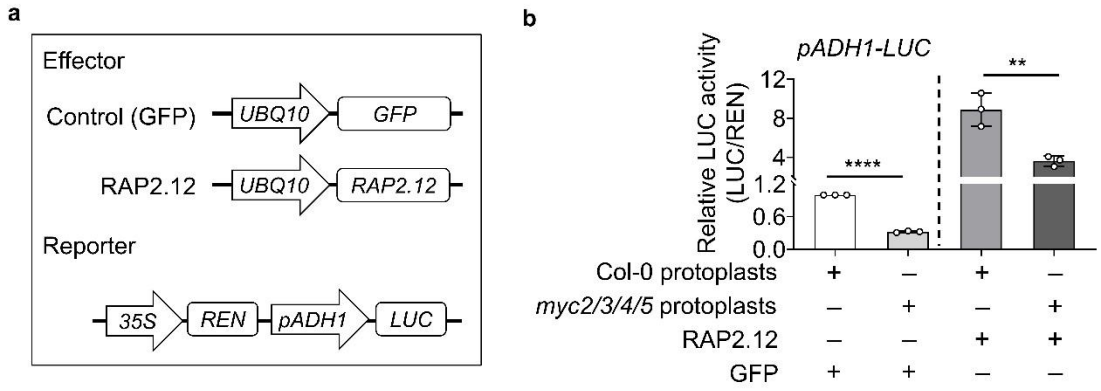
Supplementary Fig. 4 JA signaling play critical roles in RAP2.12 stabilization. (support Figure 1, 2, 5)

a) RT-qPCR analysis of the expression of *RAP2.12* in 1-week-old Col-0, *coi1-1*, *myc2/3/4/5, RAP2.12*-OE, *RAP2.12*-OE/*coi1-1* #3, #5 and *RAP2.12*-OE/4m #1, #16 seedlings. Transcripts were normalized to levels of *ACTIN8*. Error bars represent means ± SD (n = 3 biological replicates with each replicate of 8 independent seedlings). For each biological replicate, three technical repeats were used for analyses. Different lowercase letters indicate significant differences at $P < 0.05$ (one-way ANOVA with Tukey's HSD test). **b)** RAP2.12 protein abundance in *RAP2.12*-OE/*coi1-1* #3 seedlings treated with 100 μM MeJA or DMSO (Mock) under normal condition or hypoxia (0.1% [v/v] O_2) for 0 h, 6 h, 12 h, and 24 h. ACTIN was used as a loading control. The relative quantification of RAP2.12 intensity was shown below. hpt, hours post treatment.



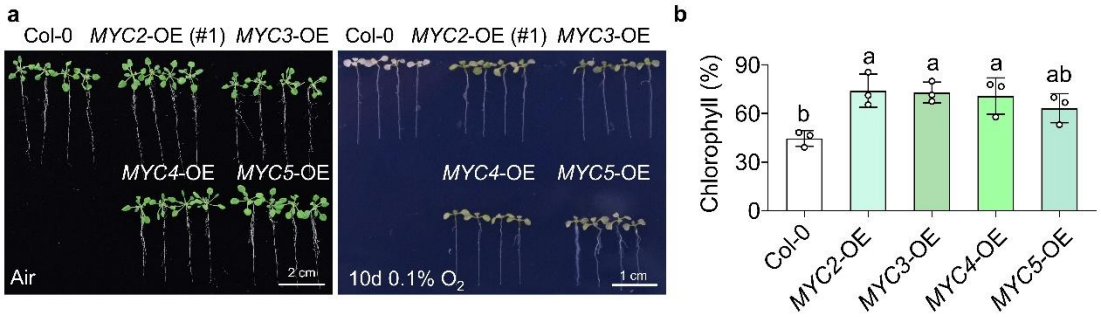
Supplementary Fig. 5 MeJA treatment stabilize RAP2.12. (support Figure 2)

RAP2.12 protein abundance in 1-week-old *RAP2.12-OE* seedlings in the background of Col-0 treated with different concentration of MeJA under hypoxia (0.1% [v/v] O_2) for 0 h, 12 h, and 24 h. ACTIN was used as a loading control. The relative quantification of RAP2.12 intensity was shown below. hpt, hours post treatment.



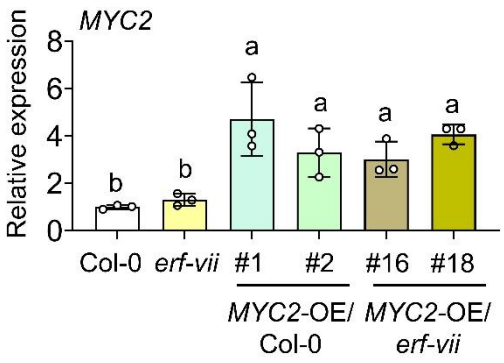
Supplementary Fig. 6 The activation of *ADH1* transcription is significantly reduced in *myc2/3/4/5* protoplasts. (support Figure 3)

a) Schematic diagrams showing the effector and reporter constructs used in (a). *RAP2.12* driven by *UBQ10* promoter was used as the effector, and *GFP* driven by *UBQ10* promoter was used as a control. The dual-luciferase reporter constructs consist of the *Renilla luciferase* (*REN*) gene driven by *35S* promoter for internal normalization and *firefly LUC* gene driven by the promoter of *ADH1* as reporter. **b)** Dual-luciferase (LUC) reporter assays showing the activation of *ADH1* transcription by RAP2.12. Effector and reporter constructs used in the transient LUC assays are displayed in schematic diagrams (b). The effector and the reporter were co-expressed in the Col-0 or *myc2/3/4/5* protoplasts. Error bars represent SD (n = 3 biological replicates). For each biological replicate, three technical repeats were used for analyses. Asterisks indicate significant differences between the samples (****$P < 0.0001$ and **$P < 0.01$ by Student's t-test).



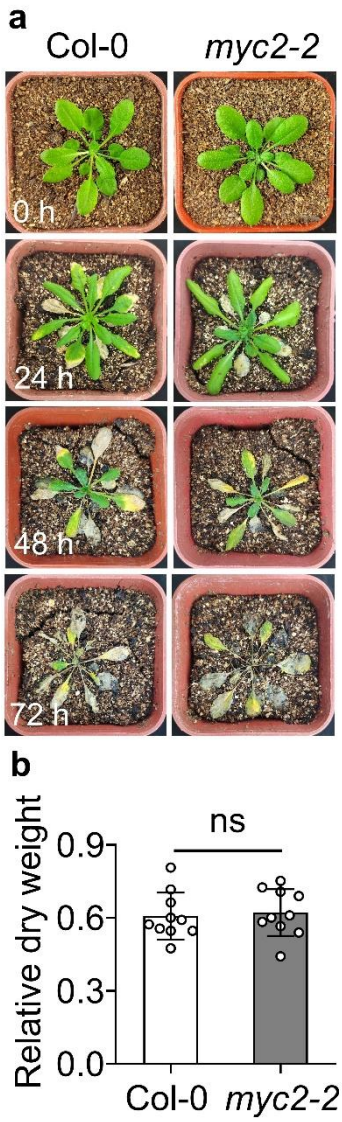
Supplementary Fig. 7 MYC proteins positively regulate plant resistance to hypoxia. (support Figure 3)

a) Phenotypes of 1-week-old Col-0 and *MYC2, MYC3, MYC4* or *MYC5* overexpression seedings (*MYC2*-OE *#1*, *MYC3*-OE, *MYC4*-OE, *MYC5*-OE) subjected to hypoxia (0.1% [v/v] O_2) or normoxia conditions for 10 d followed by a 2-d recovery under normal conditions **b)** Relative chlorophyll contents for the genotypes shown in (a) under 8 d hypoxia and 2 d recovery. Data are means ± SD (n = 3 biological replicates with each replicate of 4 independent seedlings). Different lowercase letters indicate significant differences at $P < 0.05$ (one-way ANOVA with Tukey's HSD test).



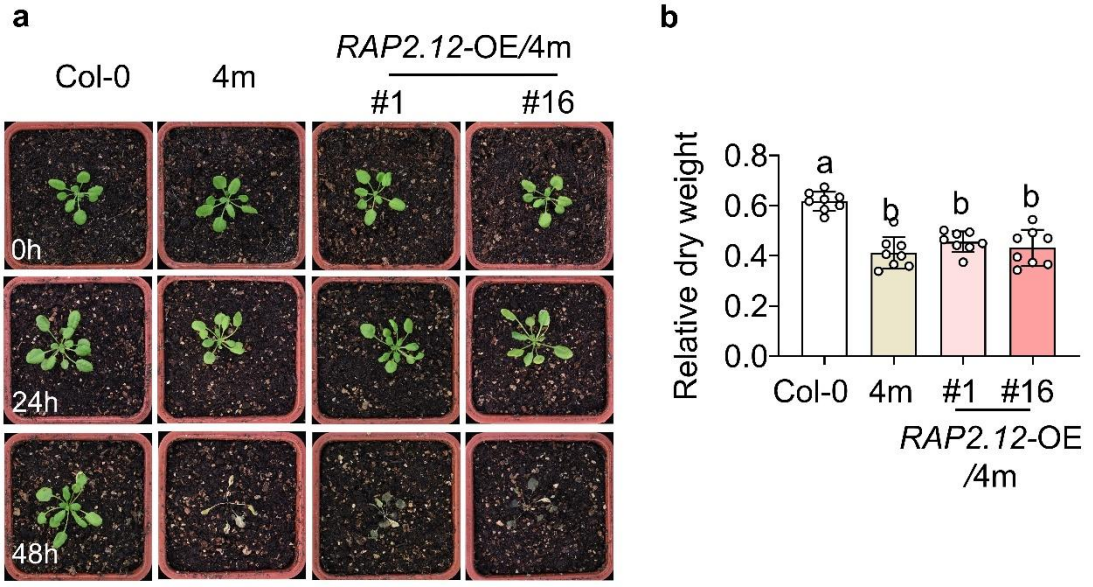
Supplementary Fig. 8 *MYC2* expression in transgenic lines. (support Figure 3, 6)

RT-qPCR analysis of the *MYC2* expression in 1-week-old Col-0 or *erf-vii* transgene seedlings over-expressing *MYC2* (*MYC2*-OE #1, #2; *MYC2*-OE/*erf-vii* #16, #18). Transcripts were normalized to levels of *ACTIN8*. Error bars represent SD (n = 3 biological replicates with each replicate of 8 independent seedlings). For each biological replicate, three technical repeats were used for analyses. Different lowercase letters indicate significant differences at $P < 0.05$ (one-way ANOVA with Tukey's HSD test).



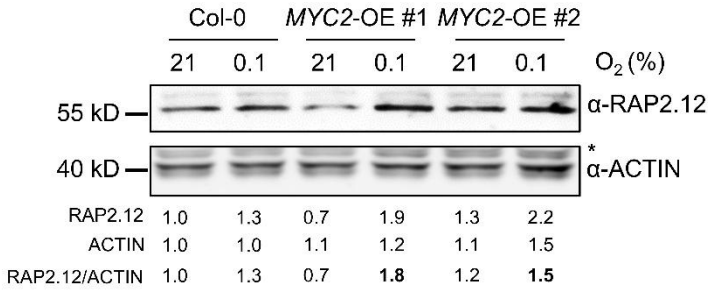
Supplementary Fig. 9 There is no significant difference in submergence resistance between *myc2-2* mutant and Col-0. (support Figure 3)

a) Phenotypes of 4-week-old Col-0 and *myc2-2* mutant plants subjected to submergence for 48 h, 72 h or 96 h followed by a 7-d recovery under normal growth conditions. The phenotypes of Col-0 and *myc2-2* before submergence were shown as control. **b)** The relative dry weight for the genotypes shown in (a). Col-0 and *myc2-2* plants were subjected to 48-h submergence and 7-d recovery under normal growth conditions comparing with plants in normal growth conditions for 9 d. Data are means ± SD (n = 10 independent plants).



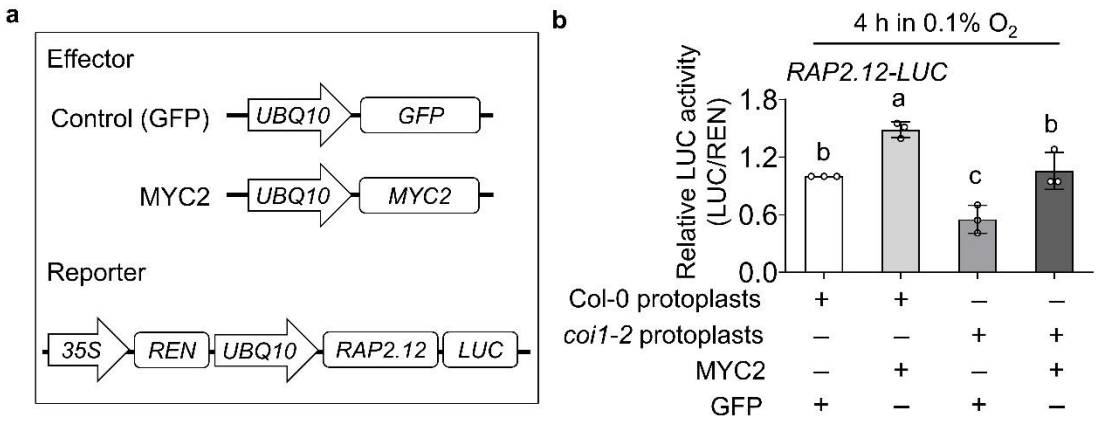
Supplementary Fig. 10 Phenotypes of RAP2.12 overexpression liens in *myc2/3/4/5* backgrounds under submergence. (support Figure 3)

a) Phenotypes of 4-week-old Col-0, *myc2/3/4/5* (4m) and *RAP2.12*-overexpression in 4m background plants (*RAP2.12*-OE/4m#1 and #16) subjected to submergence for 24 h or 48 h followed by a 7-d recovery under normal growth conditions. The phenotypes of Col-0, 4m and *RAP2.12*-OE/4m (#1 and #16) plants before submergence were shown as control. **b)** The relative dry weight for the genotypes shown in (a). 4-week-old Col-0, 4m and *RAP2.12*-OE/4m (#1 and #16) plants were subjected to 48-h submergence followed by a 7-d recovery under normal growth conditions comparing with plants in normal growth conditions for 9 days. Data are means ± SD (n = 8 independent plants). Different lowercase letters indicate significant differences at $P < 0.05$ (one-way ANOVA with Tukey's HSD test).



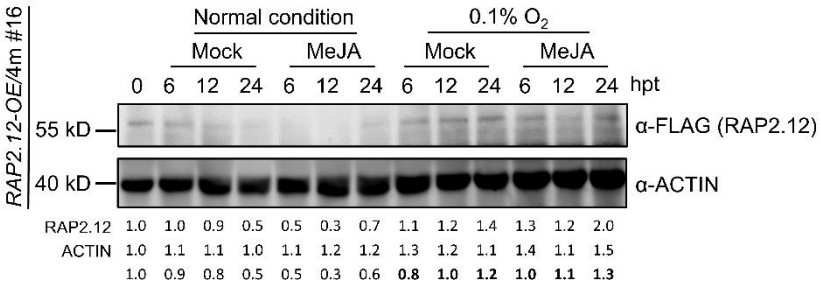
Supplementary Figure 11. MYC proteins stabilize native RAP2.12 in transgenic plant.

Native RAP2.12 protein abundance in Col-0 and *MYC2*-OE (#1, #2) seedings under 21% or 0.1% O_2 (v/v) treatment for 8h. ACTIN was used as a loading control. The relative quantification of RAP2.12 intensity was shown below.



Supplementary Fig. 12 MYC2 co-expression induces RAP2.12 accumulation in JA mutant cell. (support Figure 5)

a) Schematic diagrams show the effector and reporter constructs used in (b). *MYC2* driven by *UBQ10* promoter was used as the effector, and *GFP* driven by *UBQ10* promoter was used as a control. The dual-luciferase reporter constructs consist of the *Renilla luciferase* (*REN*) gene driven by *35S* promoter for internal normalization and and *RAP2.12* gene with a *firefly LUC* reporter sequence fused to the C terminal (*RAP2.12-LUC*) driven by the *UBQ10* promoter as reporter **b)** Dual-luciferase (LUC) activity assays showing MYC2 stabilized RAP2.12 protein. Effector and reporter constructs used in the transient LUC assays are displayed in schematic diagrams (a). The effector and the reporter were co-expressed in the protoplasts of Col-0 or *coi1-2* plants and then subjected to hypoxia (0.1% [v/v] O_2) for 4 h after cultured for 16 h. The LUC activity in Col-0 protoplasts co-expressing *GFP-FLAG* and *RAP2.12-LUC* was used to standardize the data in different groups. Error bars represent SD (n = 3 biological replicates). For each biological replicate, three technical repeats were used for analyses. Different lowercase letters indicate significant differences at $P < 0.05$ (one-way ANOVA with Tukey's HSD test).



Supplementary Fig. 13 RAP2.12 stabilization in another *RAP2.12*-overexpression line in 4m background. (support Figure. 6)

RAP2.12 protein abundance in *RAP2.12*-OE/*4m* #16 seedlings treated with 100 μM MeJA or DMSO (Mock) under normal condition or hypoxia (0.1% [v/v] O_2) for 0 h, 6 h, 12 h, and 24 h. ACTIN was used as a loading control. The relative quantification of RAP2.12 intensity was shown below. hpt, hours post treatment.