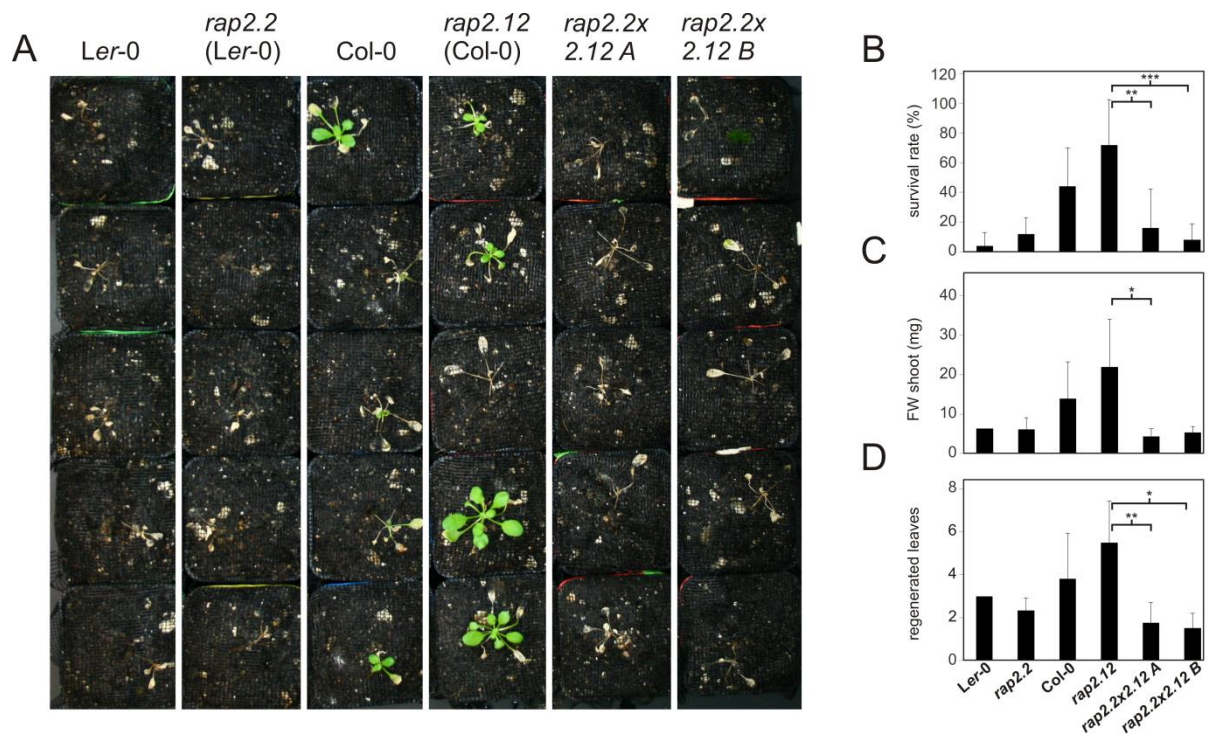


Supplemental Figure 1: T-DNA insertion mutations studied were null alleles (transcript undetectable).

Representative result of standard RT-PCR with *RAP2.2*- and *RAP2.12*-specific primers in Ler-0 and Col-0 ecotypes and respective T-DNA insertion lines. Quality and relative quantity of cDNA was verified by reference gene *TUBULIN* (*TUB*).



Supplemental Figure 2: Submergence phenotypes of *rap2.2* and *rap2.12* single and double mutants.

(A) Representative phenotype after 3-week-old plants were submerged for 3 days in darkness and recovered for two weeks under SD conditions.

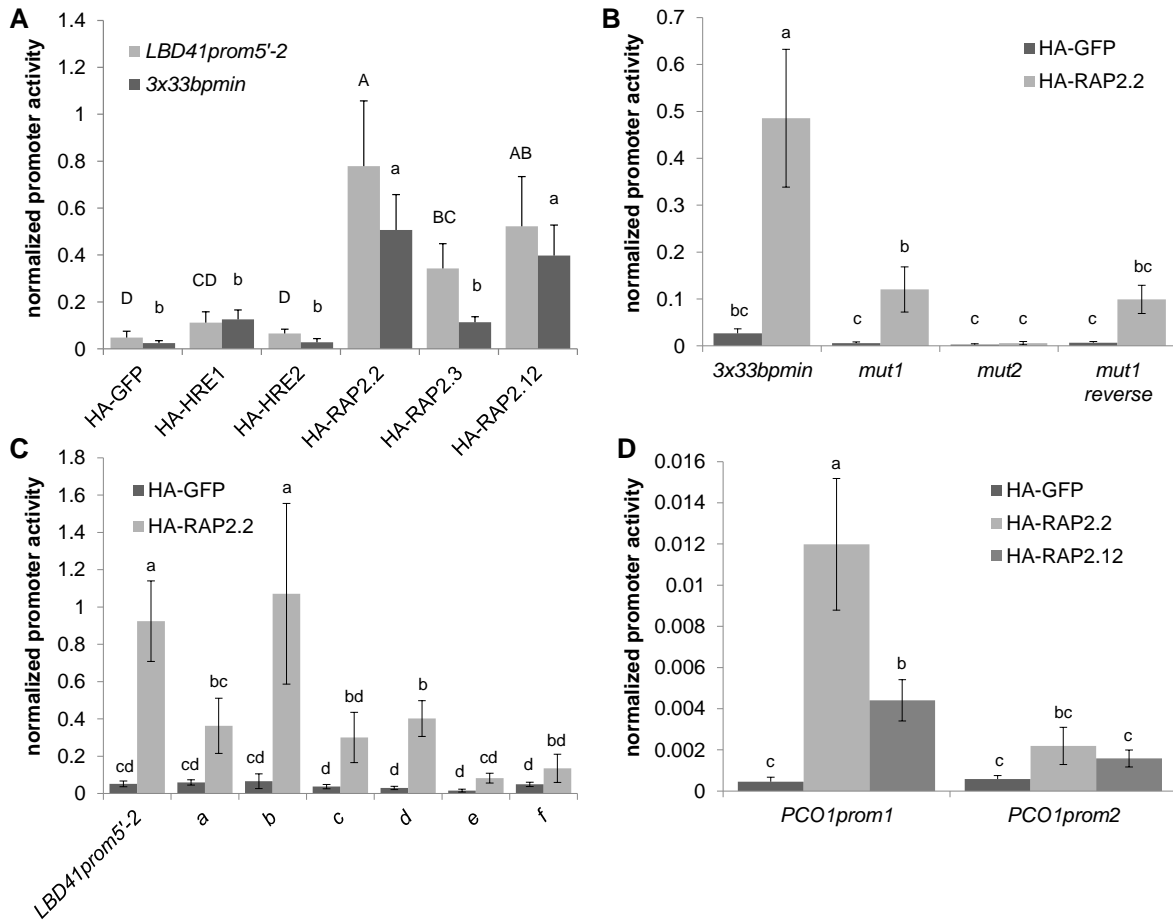
(B) Mean survival rate +/- SD [%] of 25 individuals out of 5 replicates. ** indicates significant differences at $p < 0.01$ and *** at $p < 0.001$ (Tukey HSD test).

(C) Mean fresh weight from regenerated shoots \pm SD [mg] of 1 to 18 surviving individuals per genotype out of 5 replicates. * indicates significant difference at $p < 0.05$ (Tukey HSD test).

(D) Mean leaf number (leaf size $> 1 \text{ mm}^2$) from regenerated shoots \pm SD of 1 to 18 individuals per genotype out of 5 replicates. * indicates significant difference at $p < 0.05$ and ** at $p < 0.01$ (Tukey HSD test).

Supplemental Figure 3 (next page): Comparative phylogenetic footprinting can detect expected *cis*-elements.

Tree shows phylogenetic footprints for each of 49 highly low phosphate-responsive genes from *A. thaliana* shoots (Bustos et al., 2010), grouped by similarity to seven distinct clusters (Clusters1-7). Clusters were defined as groups of at least five motifs with a branch length of less than 0.01 between two nodes, representing one consensus sequence. Sequence logos display consensus sequences and names of known matching *cis*-elements are indicated. Cluster 7 represents the well-characterized *PHR1* promoter-binding site (P1BS).



Supplemental Figure 4: Normalized promoter activities for Figures 5 and 6.

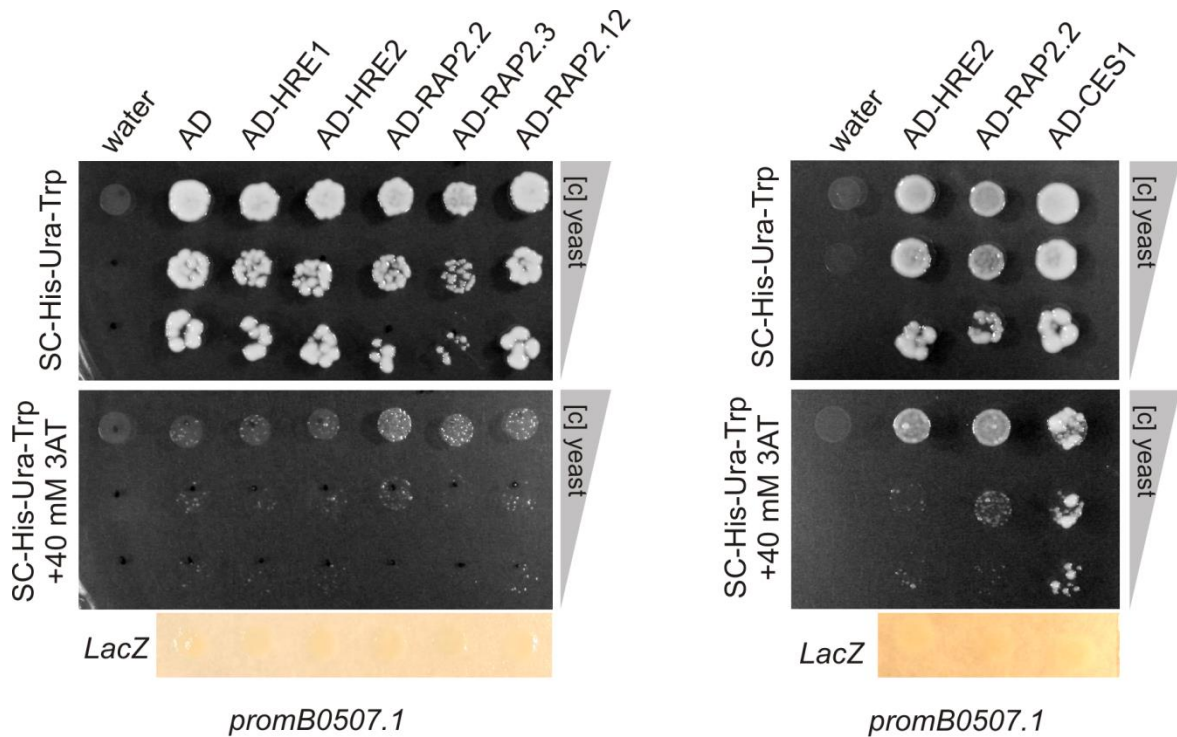
(A) Comparison of LUC activity from the 589 bp long *LBD41prom5'-2:LUC* with the *3x33bpmin:LUC* reporter construct in the presence of transiently expressed HA-ERF-VIIs.

(B) Comparison of HA-RAP2.2-induced LUC activity of wild type and mutated versions of *3x33bpmin:LUC*.

(C) Comparison of HA-RAP2.2-induced LUC activity of the 589 bp *LBD41prom5'-2:LUC* reporter and point mutations in two C9motifs.

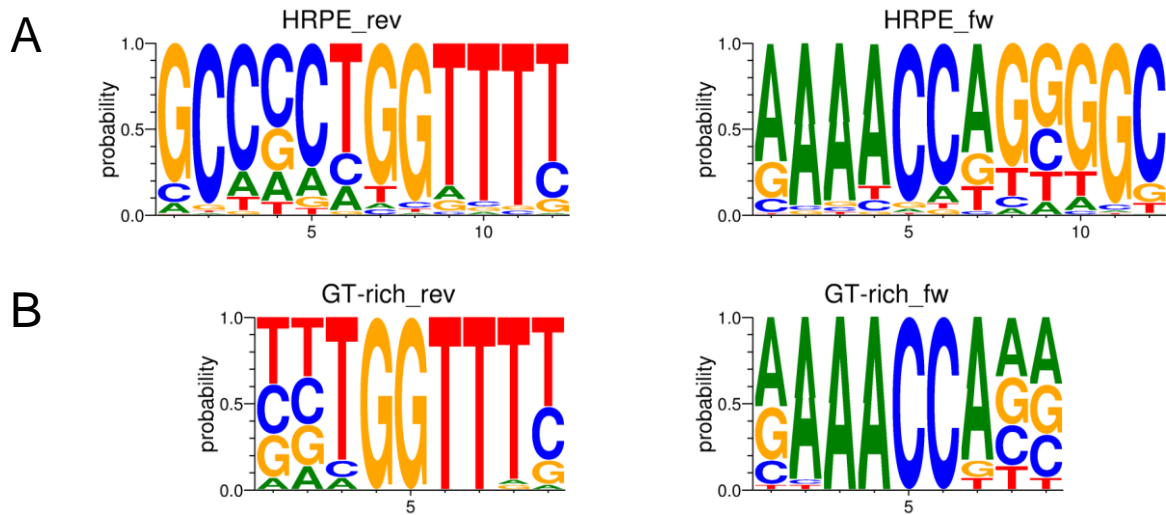
(D) Effect of HA-RAP2.2 and HA-RAP2.12 on C9motif-containing and C9motif-less *PCO1* promoter versions.

All data are means \pm SD of 6 replicates, different letters indicate significant differences at $p < 0.05$ (Tukey HSD test). Normalized promoter activity is LUC activity normalized to *p35S:RUC* activity. Data are the basis for calculation of fold-change in Figure 5B and Figure 6C-E.



Supplemental Figure 5: ERF-VIIs do not activate unrelated Y1H reporters.

Yeast-one-hybrid (Y1H) assays with stable *promB0507:HIS promB0507:LacZ* yeast strain transiently expressing Activation Domain (AD)-ERF-VII fusions (baits). Water and an AD-only expressing vector were used as negative controls. *AD-CES1* was used as positive control. Panels show equally concentrated yeast transformants and 1:10 and 1:100-fold dilutions after 5 d of growth on selective medium in the absence and presence of 40 mM of the *HIS* gene inhibitor 3-aminotriazole (3AT).



C

AtLBD41_C9_A	1	-----AAATCCAGGGGC-----	12
AtLBD41_C9_B	1	-----AAAACCAGGAGC-----	12
AtLBD41_C9_C	1	-----AAAAACAGCGGC-----	12
AtADH_C9_RSAT_rev	1	-----AATACTAGGGGC-----	12
ZmADH_C9_RSAT	1	-----AAAACCACGGTC-----	12
ZmLBD41_C9_RSAT	1	-----GAAACCACAGGC-----	12
ARE_MAIZE_sub1_rev	1	-----GCGAAACCGGGGCTGCAGCC--	20
ARE_MAIZE_sub2_rev	1	-----GGGCAAGCAAACCACGGCGCGGC----	23
ARE_ATH	1	AGAGAACAAGGCCAAAACCAAATACGCCCTAG	32

Supplemental Figure 6: Comparison of the HRPE and AREs.

(A) Graphical display of the HRPE in both orientations.

(B) Graphical display of the GT-rich region from the At ARE (Hoeren et al., 1998).

(C) Alignment of predicted HRPEs from At *LBD41*, At *ADH1*, Zm *LBD41* and Zm *ADH1*, and previously identified Zm *Adh1* ARE subregions. The GC pair that is important for TF binding (Figure 6D) is highlighted in red.

Yellow: HRPE motifs predicted by RSAT

Italic: 5'UTR

Magenta: ATCTA element

Underlined: ARE region

Green: ARE GC-rich subregion (Walker et al., 1987; Dolferus et al., 1994)

Blue: ARE GT-rich subregion (Walker et al., 1987; Dolferus et al., 1994)

ATG: start codon

>AtADH1

ATAATACTATTAAAGAGCTATTTAAGATGAAACCGCCCG~~AAACCAA~~AAGCATTTCGATGGGTA
CACCGATTACTGCTTTTAGCAACACCACGGCGTGACCATCAAGACTAATTAAGTAAAGACCAC
ATTTTAAAAAACTATTAATAATTACTACAATTTGTAATTAAAAAGATCAACGAGAAATGCC
ACGTGGACGAATACTAGCAACGCCAAGTGGAAAGAGCGTTTCGAGAGAACAAGGCA~~AAACCAA~~
ATAC~~GCCCC~~~~TAGTATT~~CTACAGATGTGCGACTGGATAATTACAAAAGATTTCAATAAACAGTA
CTAATTAATTTCTAGTGGTGAGTTTTTGTAAAT~~ATCTA~~CTTCTTCCAATTACCAGCTGCTAT
ATAAATCCCCTTCTCTGTTTCTCTTTTCTTACATCACAATCACACAAAACCTAACAAAAGATC
AAAAGCAAGTTCTTCACTGTTGATA~~ATG~~

>ZmADH1

GCGCGGCGCATCCGACGGCCACGACAGCGCAGTGCCGTCCTCCGCCGCCACCGCTTGGCGAT
TGTCCGCACCCCACCAGTCCACCACCTCCCCACGAGCG~~AAAACCACGGTC~~CACGGACCACG
GCTATGTTCCACTCCAGGTGGAGGCTGCA~~GCCCCGGTTT~~CGCAAG~~GCCGCG~~CCGT~~GGTTT~~GCT
TGCCCACAGGCGGCCAAACCGCACCCCTCCTTCCCGTCGTTTCCCATCTCTTCCTCCTTTAGA
GCTACCACTATATAAATCAGGGCTCATTTTCTCGCTCCTCACAGGCTCATCTCGCTTTGGAT
CGATTGGTTTCGTAACCTGGTGGAGGACTGAGGGTCTCGGAGTGGATTGATTTGGGATTCTGT
TCGAAGATTTGCGGAGGGGGGCA~~ATG~~

Supplemental Figure 7: Promoter of *ADH1* from *Arabidopsis* and maize with marked motifs, as described in the legend.

Yellow: HRPE motifs predicted by RSAT

Italic: 5'UTR

Magenta: ATCTA element

Underlined: primers used

ATG: start codon

>At3g02550 (LBD41)

TTCTCAAGTCTTTGTA AATTGTAATCTTGATAAATTCAAAATGCTTTGTATTATTCGAACAA
GCTTCCGTCTTTAGAGATTCTAATTGTCTTGTCTCTCTGGAATGGACAAGAGAAACGAAACG
GAAAATAATGCGAATGAAACGGAGCCATCAGCGTTGACTACGTAACCTTGACACTTACGAATT
ACGTACGGTCTATTCAACGAACGAAGATTCCCTTCAATATTGTCGTGAACAAGAACAATAATC
TCATGGACGCATTAATGAATGGAAATAGTATTTGACTAGTTAATGTCGTTTAGAACTTAGT
AAATTTTGAACCAAACCCATGGATATGAATTGCAGTAGAATCATAATACCAATCGGAAATAC
TAGTTAATTATTTTTTACAACCTATTAAATTTTGAACCAAAGACATGAATATGAATTGATGT
ATGAAACGCTACATTTTTGTCTTTTTGCTACGTGGTTTGTGTTAAATGTACATGTTTTTTT
AATAGAAAACAAGGATTTAACTCACGTTTTACCGCGGGCAATCGGTTTTATTTGGTTTTTTATA
CTAAATATTTGAGTAAATTTGATATCCTAACTTATAAATATAAATTTTTTAAATCAAACATA
ACTAAAAATAAATTTGGATTTTTTTTTGTTGTTATTTAGTGATAAGAAGAATGTGTTAAAAATG
TGATTAATTTTTTTTTGCTTGTATTTATTTATTTTTGTTGAACGGTTTAGATTGAAGGGAA
TTAATTTTATTGAACGGTTTAGATTTAATCGAATCATATAAAGTTTAGAAGATTGTTAAGAC
CAAATTACCCTTAATGATTTTTGAATAACAAAGAACAAAATCCAAATTTGAAATAAAAATTA
GATCAAATTAGACAAAATTC AATTTGTACTTCCCTTATAATAATAAGAGGATACATAACAGA
ATCCACTAACTCAAATCAAATGTGCTACAAAATGGGTCTCACAATATTGTAATGTAATCTG
GTACAAAGTTAAATTTAAGTGGATTACGTAAGAAAGAATATCAAAGCTGAGTTTTGTTGTC
TGGATTAACTATAAGGTGGGAAAATAAGAATTAATCTAAGGTGATTAAAGATAAAAATTT
ATTAAGTAAAAGATGATTTAAGATTACGTTTTTTACGAAAGCGTCCGCTAACAGAGAGAGTC
ACAAAGATCCGCCACAGGGAGAGAAGAAGAAAAGATCAAAAACCGGAAACCATAAAACCAG
AAACCAGTCAATAGTTTTGGACATAGTTGAAAGCAGTATTGTTAAAACAGATTCTTTTCCAG
AAAACCAGGAGCTAAGTGTGGGCCCCAGTTCTTCCAAATGCTCCTCAAATGACTCAAATCCC
ATCTGGATGACTTACGTGGCGATTTCGACCAAATTTCTTACTCGCGAGCGTGGAGGCCACGTG
TCTTATCACAGTCTCTTCAAACACATTTAAGACAATTTAACCAAATCCTACTCCAATAGATA
CTCTGAATTGGTCAGATACTTTAATCAACAACCATATTAATCGTTTTAGAACGAATTAGATT
TGTATAATCCAGAATTTATAGACTATTCACTAGTAATTA AAAAATGGTAACTTGATATGTCA
CGTGTATGACACGCGCATTTGGATAGAGTGGGGACAAAACATAACTTGGGGAAGAAACACAA
GAAGCAAACCTTGAATCAGGGTGAAGAAAAACAGCGGC GGTTCGTTGGGAAAATAAAGGAAGG
ATTTTGT AATTAAGACAAAACAACCCTTGTCTTTAGAGAAAACAGCTCTTCTTATCTTG
TTTTCTTTGTCTTCTGCCTTCTTCCCTTGGTTTTATAATCTCTCTCCGCTTCCCCCAA
CCAGGGGCTTCTTACACGCACACAATCCTTTAAAGCTATCGTCTTTCTTATATAATCAGTGA
ATCTTCATTAACACCTCCACAAAATCTCAGAAATAACTTTCACAACAGAGTCAAAGAGTTC
CAAATCGTTTTGTTTGTATTTGTACGATCAAAGTTGTTGGTACTTGTAAGATAATCGAAACC
AAAGATG

>At5g15120 (PCO1)

CTATTGATGTTTTAAGGGAAAGACAATTTTTTCAACATTTTGTATTTTTGCTTCTAATCTAG
TTCAGGAAATAAATAATTT CAGGGGTGAAAATGATTA AAAAAAAAAATCTAATTTGTAAAGTT
ATTTTTTGTGTTATGAAAACAAGTTATATAAAGAGTAAGCCATAACAGCTGTTTCTTGTAAT
ACATAATCACATTTTGTGTTAACAACCAAACTAAATCATCAAATACTTTTTTTTGGTA
TTCAAATCTGCATAAAACATGCTGGTAAACAAAATATAATGATAATGATATTAGAGGTTTT
TAGACATTATGATATGTTTTCTTAATATCATATCATTAGAATCATATAATCAACATCTTCT
GATTTGTTATGTGCTTAATTGCTTTATTTGTC AAGATCTCAACTATAAATGACAATTATACT
GCATGACAAAATAAGATATACACAAAATAATTTGCCAAAGATAACAAGTGATACCAAAACA
AATATTTATCAATGAAAAGAGAAAAGAAATAACAAAACAAAGATTCCTAGTACTCATTCC

CATATAATCCGGTGAAGAAAAAAGATTTTCAGGGTCAAAGACGACATTTTTAAATTATGTAT
ATCAAAGTAAACAACACACAAAACAATCCTATGATTTTAACATATTTCAAATTAAATACATG
TATTTTTATTTGTCAAATTTATATAAATGAATCTATGAAATTGACAAATTATGAGTGAAATA
GTAATTCAGCCAGTGTAAGTAAATAAAACAAATCTAATAAAACCGGCTATTTATAACCAACCG
GGTTAGAGTAAACCGATAAAACATATGATAAATATTTTAATAAAGTGTATGTGTTTCCAAATT
TGCCCCTTTGCGAAGACCGAAACGGATAAAAAAGAATCCGCTGTTTGAAAAAACACAGCC
CATAGGAAACAAAAGAATGGCCCCTGGTTTTGTAATCGATAATGGTATAATCCTCTTTTCTT
ATTTTTCCCTGTTTTTATATCTCATTCTTATTCTTCTTAAATCCATTCCCAGATTCACACTT
TTGATTTCGTGATACACTGTTACATTTTATTTTTTTTTGTTGGGTTGGTGAGACTTTTATG

Supplemental Figure 8: Promoters of *LBD41* and *PCO1* from *Arabidopsis* with marked motifs and underlined primers, as described in the legend.

Supplemental Table 1: Overview of group VII ERF T-DNA insertion lines used in the literature

Name of line	ID of line	Ecotype	References
hre1-1	SALK_039484	Col-0	Hess et al., 2011; Gibbs et al., 2014
hre1-2	SALK_018181	Col-0	Hess et al., 2011
hre1-3	SALK_023445	Col-0	Licausi et al., 2010
hre2-1	SALK_052858	Col-0	Licausi et al., 2010; Hess et al., 2011; Park et al., 2011, Gibbs et al., 2014
rap2.12-1	SALK_019873	Col-0	Zhao et al., 2012
rap2.12-2	SAIL_1215_H10	Col-0	Papdi et al., 2015; this paper
rap2.12-3	FLAG_525G09	WS	Bui et al., 2015
rap2.12-4	GK_503A1_11	Col-0	Gibbs et al., 2014 (named "rap2.12-1" in that publication)
rap2.2-1	SAIL_184_G12	Col-0	Hinz et al., 2010; Gibbs et al., 2014
rap2.2-2	SAIL_799_D10	Col-0	Hinz et al., 2010
rap2.2-3	SALK_010265	Col-0	Zhao et al., 2012
rap2.2-4	SAIL_18_G09	Col-0	Bui et al., 2015; Papdi et al., 2015
rap2.2-5	AY201781/ GT5337	Ler-0	this paper
rap2.3-1			Ogawa et al., 2007; Gibbs et al., 2014; Papdi et al., 2015

Supplemental Table 2: Majority of C9motif containing core genes are early N-end rule targets.

P-values indicate hypergeometric distribution probability as a measure for overrepresentation of Cluster motif-containing core genes among indicated datasets in the promoter regions in comparison to the promoters of all *Arabidopsis* genes. The 3 kb upstream of the ATG start codon for the gene groups were screened for the 9 cluster motifs by use of RSAT, with a significance cutoff of > 4.5 . Low P-values indicate enrichment of cluster motifs among the groups of genes.

Cluster motif	Sample size	1	2 (lbox)	3	4 (GCC-box)	5	6	7 (ABRE)	8	9 (HRPE)
49-core genes (Mustroph et al., 2009)	49	0.91	0.10	0.0184	0.32	0.62	0.0042	0.20	0.02	7.65E-06
30 min, 1% vs. 21% O ₂ (van Dongen et al., 2009) ¹	66	0.92	0.08	0.0027	0.63	0.76	0.0689	0.11	0.23	6.56E-05
<i>ate1/2</i> and <i>prt6</i> vs. Col-0 (Gibbs et al., 2011) ²	79	0.89	0.57	0.0006	0.79	0.59	0.0001	0.25	0.78	6.42E-04

¹ 10-day-old roots, 12h light cycle, grown on 0.5xMS, 1% Suc, SLR > 1

² 7-day-old whole seedlings, 16h light cycle, grown on 0.5xMS, 1% Suc, SLR > 1 , Padj < 0.05

SUPPLEMENTAL DATASETS

Supplemental Data Set 1: Overview over homologous genes of the 49 core hypoxia-response genes in 25 species from the PLAZA 2.5 database

Supplemental Data Set 2: Overview over Position-specific scoring matrices (PSSMs) identified in this paper. **(A)** PSSMs for all cluster motifs. **(B)** List of all sequence motif pattern matches.

Supplemental Data Set 3: Occurrence of the nine motifs in the 49 core hypoxia-response genes, together with published microarray data.

Supplemental Data Set 4: Occurrence and overlap of the HRPE and the GT-rich region of At ARE (Hoeren et al. 1998) in the 49 core hypoxia-response genes, and in Zm *LBD41* and Zm *Adh1*.

Supplemental Data Set 5: **(A)** List of constructs used in this paper. **(B)** List of primers used in this paper. **(C)** List of AGI codes used in the paper.

SUPPLEMENTAL REFERENCES

1. Bui, L.T., Giuntoli, B., Kosmacz, M., Parlanti, S., and Licausi, F. (2015) Constitutively expressed ERF-VII transcription factors redundantly activate the core anaerobic response in *Arabidopsis thaliana*. *Plant Science* 236: 37–43.
2. Bustos, R., Castrillo, G., Linhares, F., Puga, M.I., Rubio, V., Pérez-Pérez, J., Solano, R., Leyva, A., and Paz-Ares, J. (2010). A central regulatory system largely controls transcriptional activation and repression responses to phosphate starvation in *Arabidopsis*. *PLoS Genet.* 6: e1001102.
3. Dolferus, R., Jacobs, M., Peacock, W.J., and Dennis, E.S. (1994). Differential interactions of promoter elements in stress responses of the *Arabidopsis* Adh gene. *Plant Physiol.* 105: 1075–1087.
4. Gibbs, D.J., Isa, N.M., Movahedi, M., Lozano-Juste, J., Mendiondo, G.M., Berckhan, S., Marín-de la Rosa, N., Vicente Conde, J., Sousa Correia, C., Pearce, S.P., et al. (2014). Nitric oxide sensing in plants is mediated by proteolytic control of group VII ERF transcription factors. *Mol. Cell* 53: 369–379.
5. Gibbs, D.J., Lee, S.C., Isa, N.M., Gramuglia, S., Fukao, T., Bassel, G.W., Correia, C.S., Corbineau, F., Theodoulou, F.L., Bailey-Serres, J., and Holdsworth, M.J. (2011). Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. *Nature* 479: 415–418.
6. Hess, N., Klode, M., Anders, M., and Sauter, M. (2011). The hypoxia responsive transcription factor genes ERF71/HRE2 and ERF73/HRE1 of *Arabidopsis* are differentially regulated by ethylene. *Physiol Plant* 143: 41–49.
7. Hinz, M., Wilson, I.W., Yang, J., Buerstenbinder, K., Llewellyn, D., Dennis, E.S., Sauter, M., and Dolferus, R. (2010). *Arabidopsis* RAP2.2: an ethylene response transcription factor that is important for hypoxia survival. *Plant Physiol.* 153: 757–772.
8. Hoeren, F.U., Dolferus, R., Wu, Y., Peacock, W.J., and Dennis, E.S. (1998). Evidence for a role for AtMYB2 in the induction of the *Arabidopsis* alcohol dehydrogenase gene (ADH1) by low oxygen. *Genetics* 149: 479–490.
9. Licausi, F., van Dongen, J.T., Giuntoli, B., Novi, G., Santaniello, A., Geigenberger, P., and Perata, P. (2010). HRE1 and HRE2, two hypoxia-inducible ethylene response factors, affect anaerobic responses in *Arabidopsis thaliana*. *Plant J.* 62: 302–315.
10. Mustroph, A., Zanetti, M.E., Jang, C.J.H., Holtan, H.E., Repetti, P.P., Galbraith, D.W., Girke, T., and Bailey-Serres, J. (2009). Profiling translatoemes of discrete cell populations resolves altered cellular priorities during hypoxia in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 106: 18843–18848.
11. Ogawa, T., Uchimiya, H., Kawai-Yamada, M. (2007) Mutual regulation of *Arabidopsis thaliana* ethylene-responsive element binding protein and a plant floral homeotic gene, APETALA2. *Ann Bot.* 99: 239–244.
12. Papdi, C., Pérez-Salamó, I., Joseph, M.P., Giuntoli, B., Bögre, L., Koncz, C., and Szabados, L. (2015). The low oxygen, oxidative and osmotic stress responses synergistically act through the Ethylene Response Factor-VII genes RAP2.12, RAP2.2 and RAP2.3. *Plant J.* 82: 772–784.
13. Park, H.Y., Seok, H.Y., Woo, D.H., Lee, S.Y., Tarte, V.N., Lee, E.H., Lee, C.H., Moon, Y.H. (2011) AtERF71/HRE2 transcription factor mediates osmotic stress response as well as hypoxia response in *Arabidopsis*. *Biochem Biophys Res Commun.* 414: 135–141.
14. van Dongen, J.T., Fröhlich, A., Ramírez-Aguilar, S.J., Schauer, N., Fernie, A.R., Erban, A., Kopka, J., Clark, J., Langer, A., Geigenberger, P. (2009) Transcript

- and metabolite profiling of the adaptive response to mild decreases in oxygen concentration in the roots of Arabidopsis plants. *Ann Bot.* 103: 269–280.
15. Walker, J.C., Howard, E.A., Dennis, E.S., and Peacock, W.J. (1987). DNA sequences required for anaerobic expression of the maize alcohol dehydrogenase 1 gene. *Proc. Natl. Acad. Sci. U.S.A.* 84: 6624–6628.
16. Zhao, Y., Wei, T., Yin, K.Q., Chen, Z., Gu, H., Qu, L.J., Qin, G. (2012) Arabidopsis RAP2.2 plays an important role in plant resistance to *Botrytis cinerea* and ethylene responses. *New Phytol.* 195:450–460.