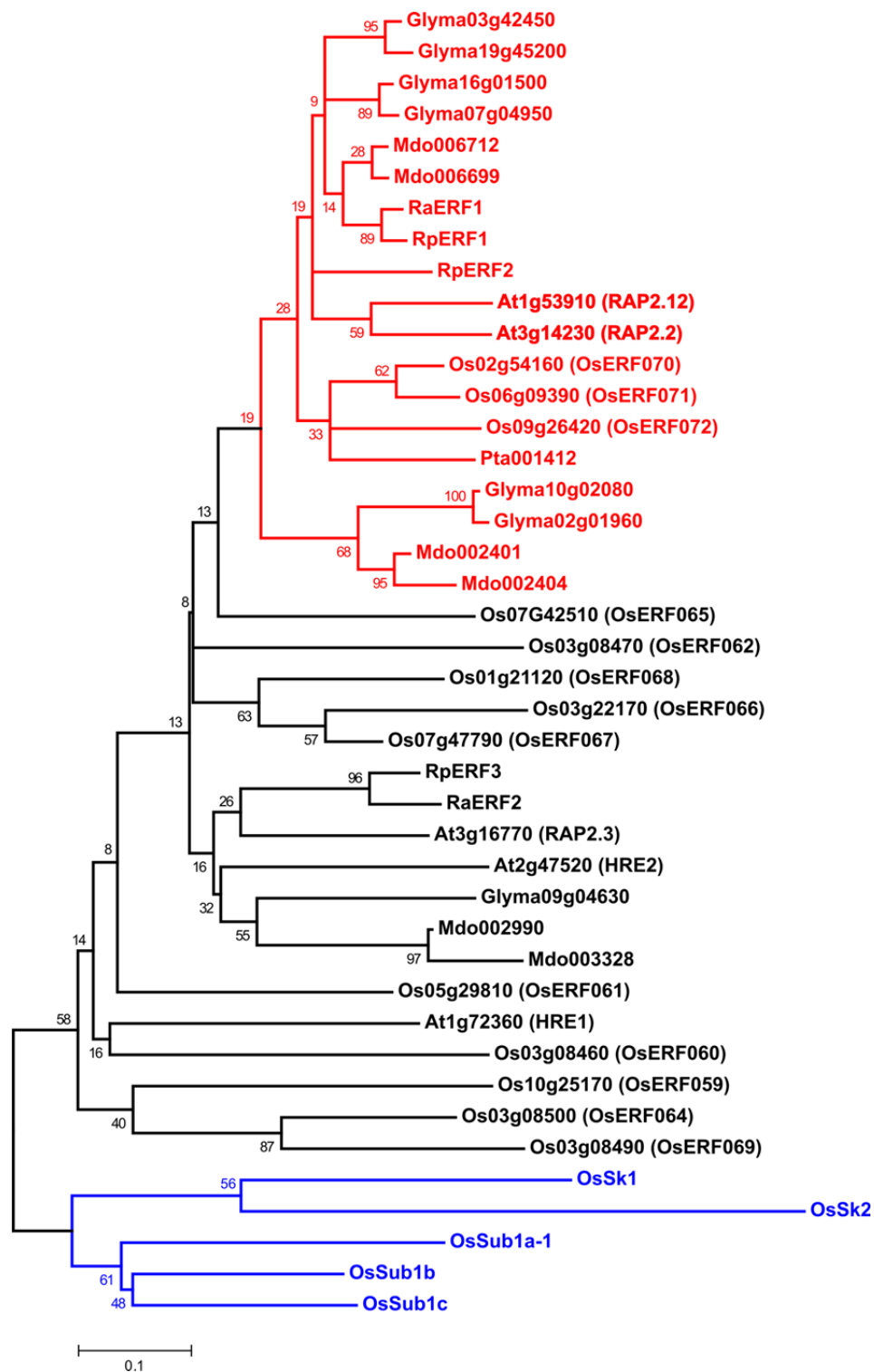


1. SUPPLEMENTARY FIGURES

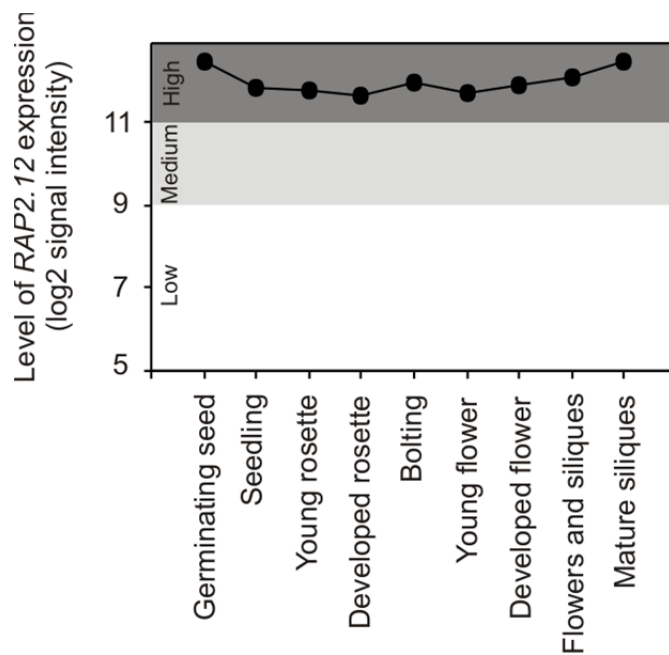


Supplementary Figure 1

Phylogenetic tree of sub-group VII of the ERF protein family among different plant species.

The phylogenetic tree was built using the Maximum Likelihood method, inferred from 100 bootstrap replicates using MEGA5³⁶. Branch lengths are on scale and represent the number of

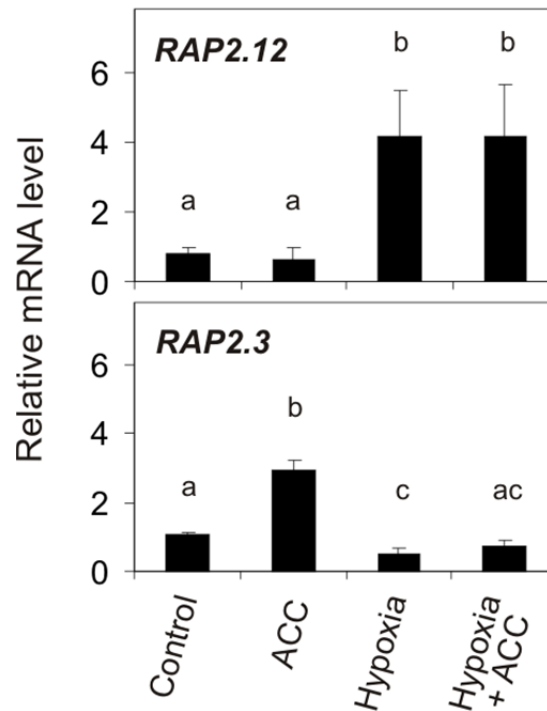
substitutions per site (distance is shown at the bottom of the tree). Analysis was performed using the Muscle algorithm³⁷. The level of position-specific residue conservation, the alignment quality and the consensus sequence were inferred using JalView³⁸. The following plant species were compared: *Arabidopsis thaliana* (At1g53910, At1g72360, At2g47520, At3g14230 and At3g16770), *Oryza sativa* (japonica subspecies: Os09g26420, Os02g54160, Os07g42510, Os03g08470, Os03g08460, Os03g08500, Os3g08490, Os10g25170, Os01g21120, Os03g22170 and Os07g47790; indica subspecies: OsSub1a, OsSub1b, OsSub1c, OsSK1 and OsSk2), *Rumex palustris* (RpERF1, RpERF2 and RpERF3), *Rumex acetosa* (RaERF1 and RaERF2), *Malus x domestica* (Mdo006712, Mdo006699, Mdo002401, Mdo002404, Mdo002990 and Mdo003328), *Glycine max* (Glyma16g01500, Glyma07g04950, Glyma03g42450, Glyma019g45200, Glyma10g02080, Glyma02g01960, Glyma05g29810), *Pinus taeda* (Pta001412). Two clearly separated clusters could be observed in the tree. A general one (highlighted in red) includes members from all the species considered. More than one member was present for each species, suggesting that evolution favored the duplication of RAP2.12-like genes. Interestingly, the flooding-related Snorkel and Sub1 proteins clustered together in a separate, rice-specific clade (highlighted in blue). Protein sequences from *Rumex* species are provided in Supplementary Table 8.



Supplementary Figure 2

***RAP2.12* mRNA is highly abundant in all tested *Arabidopsis* tissues and throughout the entire plant's lifecycle.**

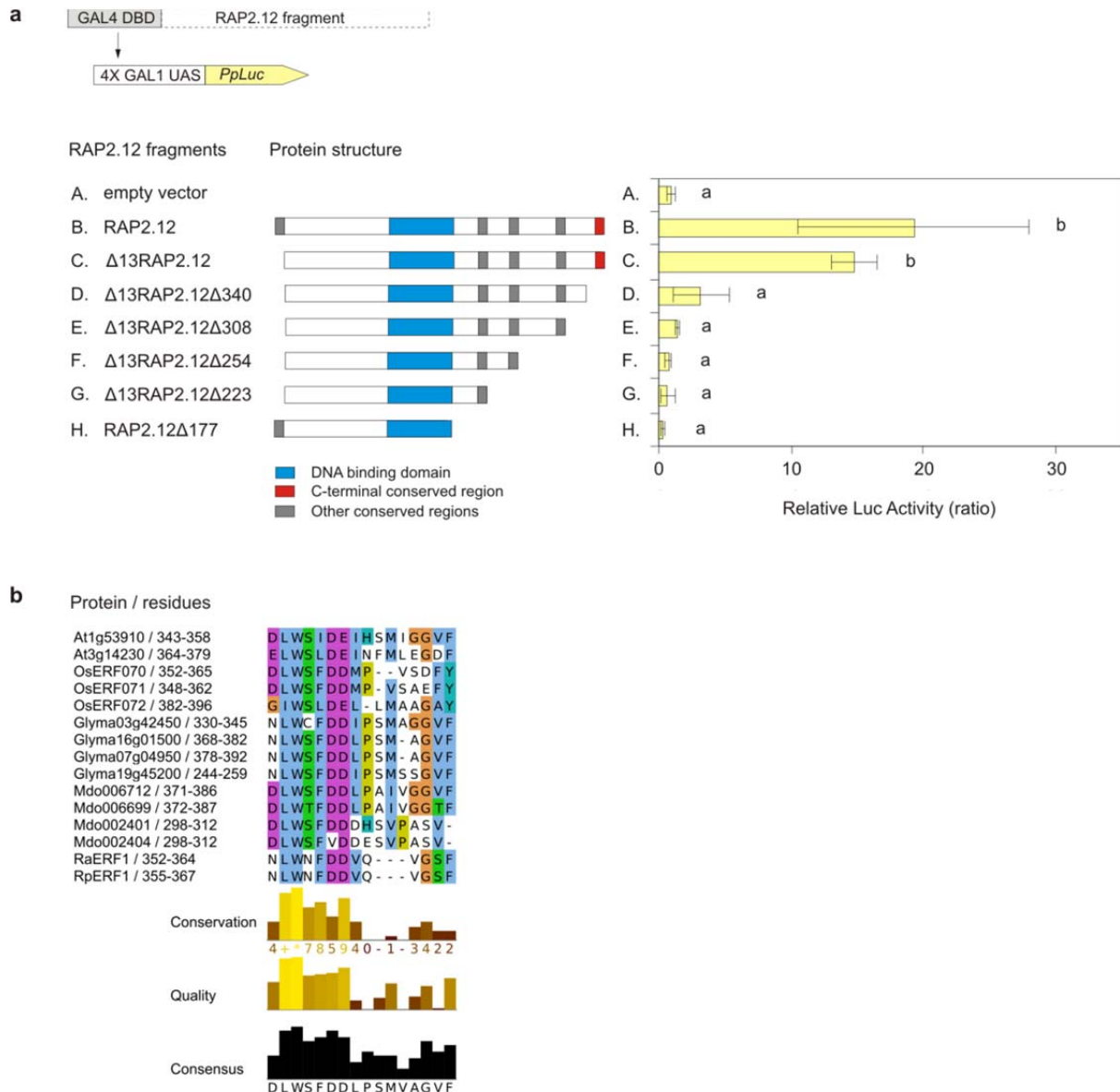
The average mRNA abundance of *RAP2.12* (*At1g53910*) was measured in various tissues and at different developmental stages using Affymetrix ATH1:22K microarrays. Data were retrieved from the Genevestigator webtool³⁹ on May 6th, 2011. The “medium” expression level corresponds to the interquartile region of the overall expression for all probesets on the array. “Low” and “High” expression levels correspond to the first and the fourth quartiles respectively. The data show that the gene *RAP2.12* is constitutively expressed and its mRNA is abundantly present in all tissues. Error bars are too small to be distinguished from the symbols.



Supplementary Figure 3

RAP2.12 is induced under hypoxia but not by ethylene.

RAP2.12 mRNA level was measured by real time RT-qPCR in leaves of soil-grown 5-week old *Arabidopsis* plants treated for 1.5 h under hypoxic conditions [1% (v/v) oxygen in air] with or without spraying with 1mM of the ethylene precursor 1-aminocyclopropane-1-carboxylic-acid (ACC). *RAP2.12* was significantly up-regulated in rosette leaves during hypoxia, but ACC supplementation did not affect its expression either under aerobic or hypoxic conditions. The mRNA level of the ethylene responsive gene *RAP2.3* (*At3g16770*) was measured to prove the efficacy of the ACC treatment. Data are means relative to the reference (air, no ACC) \pm s.d. for n=3 [one-way ANOVA test; values that are significantly different ($p \leq 0.05$) from each other are marked with different lower-case letters in the graph].



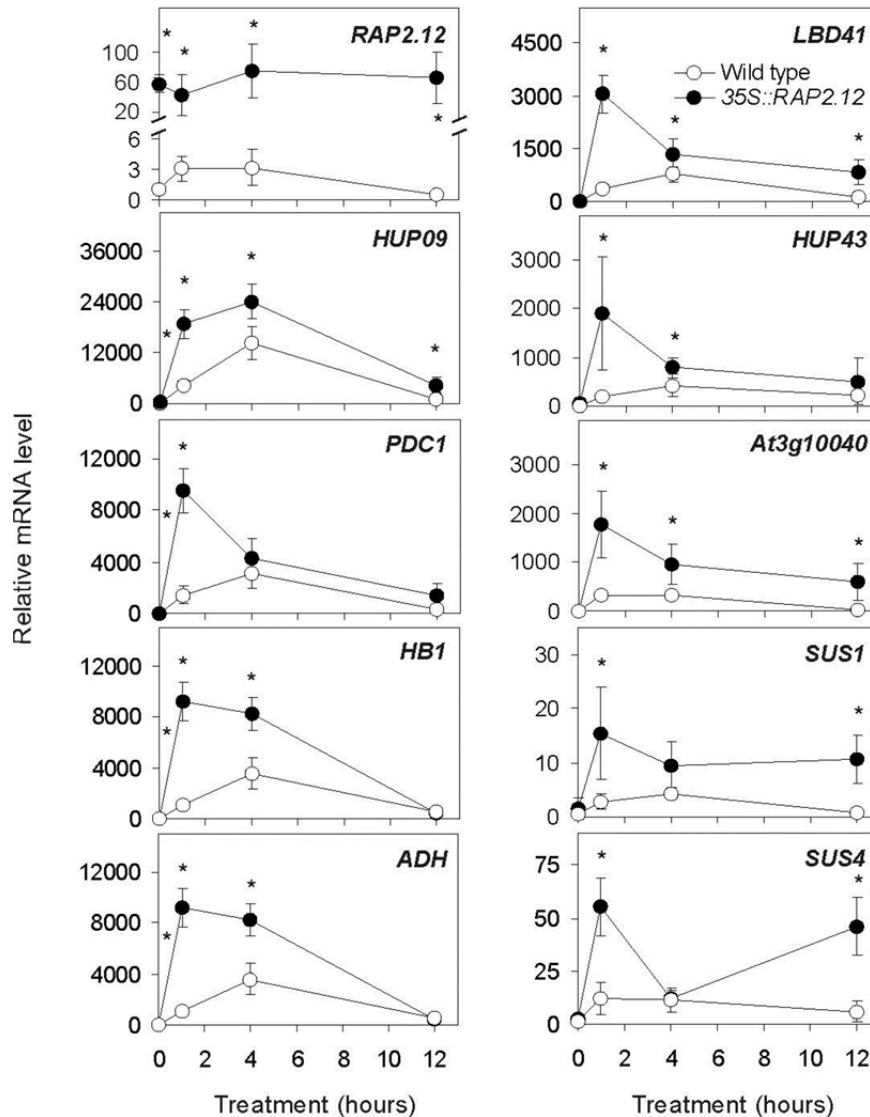
Supplementary Figure 4

RAP2.12 acts as a positive regulator of gene transcription *in planta*.

a Transactivation activity in *Arabidopsis* mesophyll protoplasts transfected with a *pGREEN::UAS::PpLuc* reporter and several different effector constructs expressing fragments of RAP2.12 fused with the GAL4 DNA binding domain (GAL4 DBD) from *Saccharomyces cerevisiae*. Data are mean of normalized (PpLuc/RrLuc) values, expressed relative to the reference (GAL4 DBD only) \pm s.d. for $n=5$ [one-way ANOVA test; values that are significantly different ($p \leq 0.05$) from each other are marked with different lower-case letters in the graph]. The results show that when RAP2.12 is fused to the GAL4 DBD, it activated transcription of the reporter *Luciferase* gene fused to a promoter that contains

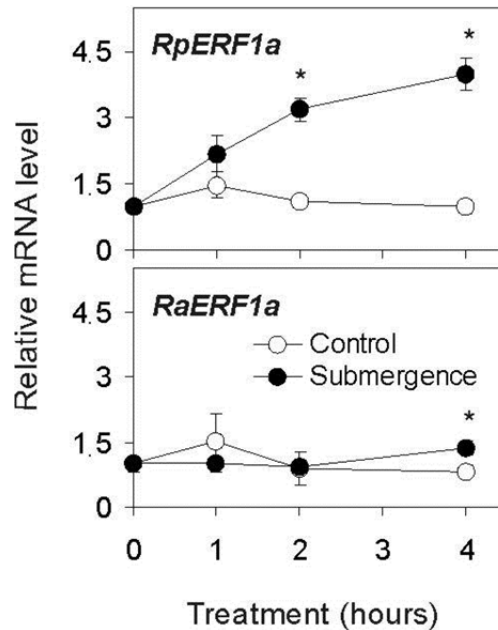
several GAL4 binding elements. Removal of the last 17 amino acid residues from RAP2.12, containing a conserved LWSFDD motif (see Supplementary Figure 3b) abolished the transactivation activity of RAP2.12.

b Multi-alignment showing a conserved LWSFDD motif present at the C-terminus of the RAP2.12 closest homologs in the same angiosperms considered in the phylogenetic analysis shown in Supplementary Figure 2. The analysis was performed using the Muscle algorithm³⁷. The level of position-specific residue conservation, the alignment quality and the consensus sequence were inferred using JalView³⁸. The highly conserved nature of this motif in the C-terminal part of RAP2.12, which was shown to be essential for transactivation (Supplementary Figure 3a), supports the hypothesis that this specific part of the protein acts as the activation domain of the transcription factor.



Supplementary Figure 5

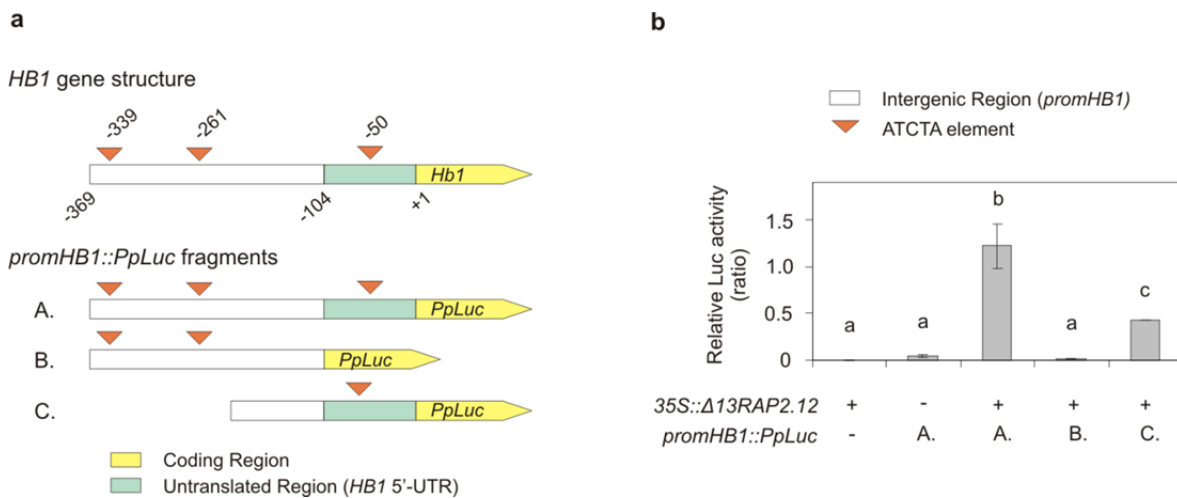
Time resolved expression pattern of hypoxia responsive genes during submergence of wild type and 35S::RAP2.12 plants. The expression of hypoxia-responsive genes and *RAP2.12* was analysed from the onset of flooding induced hypoxia (time point 0h) and in a time course of 12 hours of submergence. During the flooding treatment, plants were kept in darkness. Leaves from 4 week old, soil-grown plants were harvested and gene expression was analysed by realtime RT-qPCR. Data are means relative to the reference (Wt, 0h) ± s.d. for n=4 [Student t-test; values corresponding to 35S::RAP2.12 samples that differ significantly ($p \leq 0.05$) from the wt ones at different time points are marked with *].



Supplementary Figure 6

Differential expression of *ERF1* in *Rumex acetosa* and *R. palustris* during a 4 hour time course of complete flooding.

The *Rumex* species *R. palustris* and *R. acetosa* have two different strategies to tolerate flooding⁴. *R. palustris* fastly responds to flooding-induced hypoxia by rapid activation of fermentative metabolism⁴⁰ and shoot elongation (escape strategy), whereas *R. acetosa* follows a quiescence strategy that is characterised by late and low activation of fermentation and inhibition of shoot elongation. These different strategies have consequences for the distribution of the two species in flood-prone environments: *R. palustris* occurs at field sites characterised by long-lasting but undeeep floods, whereas *R. acetosa* completes its life cycle in rarely flooded sites⁴¹. Interestingly, the fast induction of the acclimation responses to flooding in *R. palustris* correlates with a rapid and strong induction of *ERF1*, which is the closest homologue of *RAP2.12* from *Arabidopsis* (see supplementary Fig. 2). In contrast, submergence-induced expression of *ERF1* in the slowly responding *R. acetosa* did not increase significantly until 4 hours after the onset of the flooding treatment. These data support the importance of *RAP2.12* orthologues in determining submergence-tolerance in wild plant species. Data are means relative to the reference (0h) \pm s.d. for n=6 [Student t-test; values corresponding to submerged samples that differ significantly ($p \leq 0.05$) from the corresponding aerobic ones at different time points are marked with *].

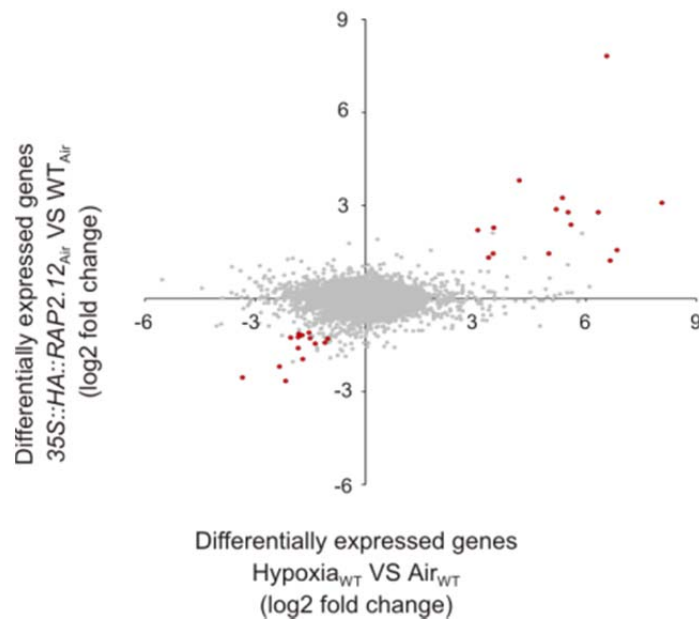


Supplementary Figure 7

RAP2.12 requires the presence of an ATCTA element in the *HB1* promoter to induce the expression of a *Luciferase* reporter gene.

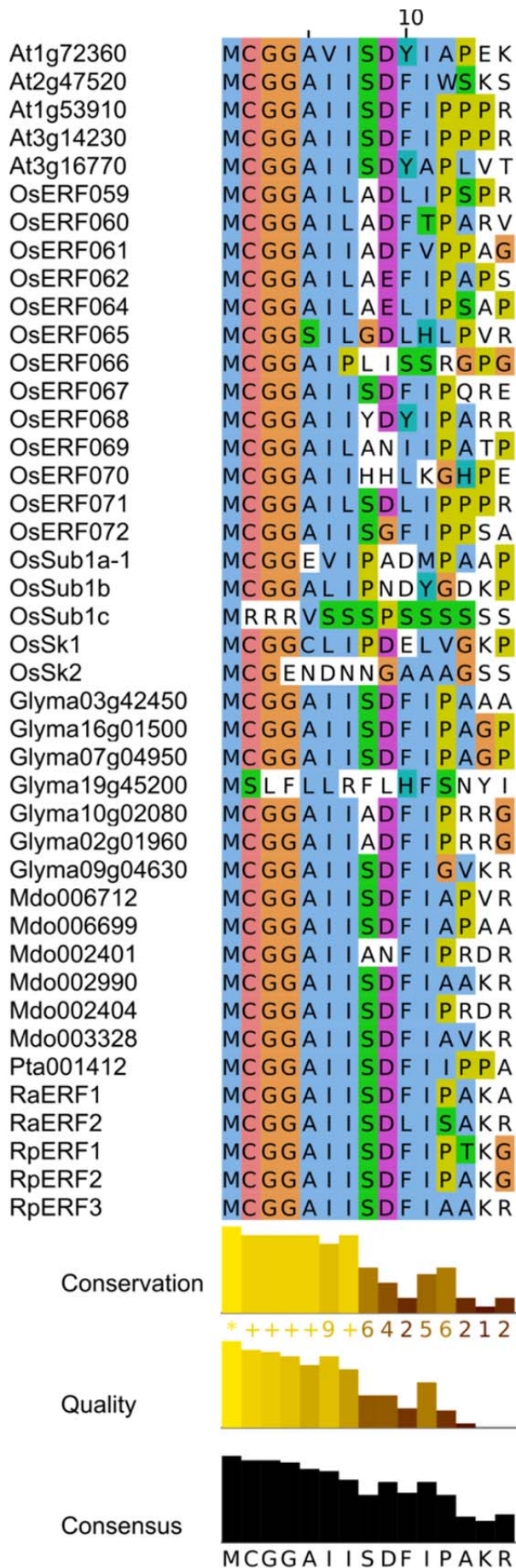
a A transactivation assay investigating the interaction between RAP2.12 and the promoter of the hypoxia inducible gene *HB1* was used to verify the function of the *cis*-element ATCTA in the promoter region. This element has previously been reported to be significantly overrepresented in the promoter of genes up-regulated by hypoxia¹⁷. RAP2.2 has also been shown to be able to bind the same element⁴². We used the genomic region upstream the Arabidopsis *HB1* gene that contains three copies of the ATCTA element to test the ability of RAP2.12 to bind this DNA motif. Fragments of the *HB1* promoter were generated to selectively exclude sequences containing the ATCTA element. The 5' intergenic region is colored in white, the UTR in green and the firefly *Luciferase* coding sequence in yellow. Red triangles mark the ATCTA elements.

b Regulation of a firefly *Luciferase* reporter gene under the control of different fragments of the genomic region upstream of the Arabidopsis *HB1* gene. Mesophyll protoplasts were transfected with a reporter construct *promHB1::PpLuc*, an effector vector *35S:: Δ 13RAP2.12* and a normalization vector *35S::RrLuc*. Removal of two ATCTA elements in the intergenic region significantly reduced RAP2.12-mediated induction of the reporter gene. Removal of a third ATCTA element located in the 5' untranslated region (UTR) completely abolished luciferase up-regulation by RAP2.12. Data are means relative to the reference (reporter construct only) after normalization \pm s.d. for n=5 [one-way ANOVA test; values that differ significantly ($p \leq 0.05$) from each other are marked with different lower-case letters].



Supplementary Figure 8

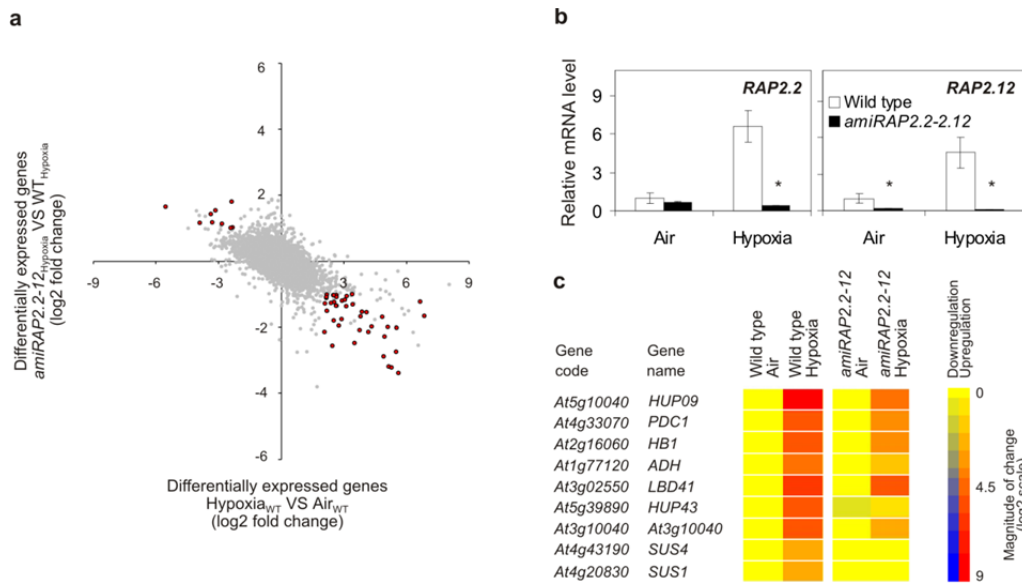
Correlation between differential gene expression as induced in wild type plants by hypoxia and overexpression of RAP2.12 in air. Microarray comparison of wild type plants treated with hypoxia (control treatment: wild type plants in air) and constitutive overexpression of HA::RAP2.12 in air (control: wild type plants in air). Red dots represent genes that are significantly (adj. p-value<0.05) up- or downregulated in both conditions. See also Supplementary Table 2 for a full list of the differentially expressed genes. The raw data of the microarrays can be downloaded from the Gene Expression Omnibus database (<http://www.ncbi.nlm.nih.gov/geo>; accession number: GSE29187).



Supplementary Figure 9

The N-terminal sequence of the ERF-VII family is highly conserved in plants

Multi-alignment showing a conserved MCGGAI I motif present at the N-terminus of most of the ERF protein sequences considered in the phylogenetic analysis shown in Supplementary Figure 2. Most of the flooding tolerance related ERF (Sub1A and OsSK2) proteins contain mutations in this region. OsSub1C completely lacks this motif. The analysis was performed using the Muscle algorithm³⁷. The level of position-specific residue conservation, the alignment quality and the consensus sequence were inferred using JalView³⁸.



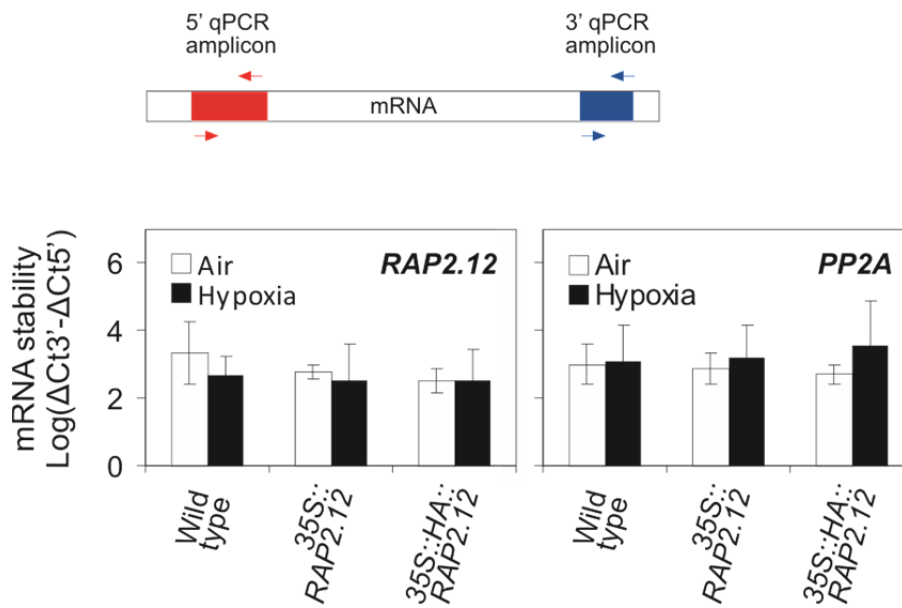
Supplementary Figure 10

Silencing *RAP2.2* and *RAP2.12* via an amiRNA approach reduces the molecular response to hypoxia

a The function of *RAP2.12* and its closest homolog *RAP2.2* was investigated by silencing their expression by overexpression of an artificial microRNA (see Supplementary Table 4). The graph shows a microarray comparison of the differential expression of genes in wild type plant caused by hypoxia (plotted on the horizontal axis) with the differential expression of genes resulting from silencing *RAP2.2* and *RAP2.12* under hypoxia (vertical axis). In red, the genes are marked that are significantly (adj. p-value <0.05) up- or down regulated by both hypoxia (log₂ fold change > +2 or <-2) and by silencing *RAP2.2* and *RAP2.12* (log₂ fold change > +2 or <-2). This analysis confirmed that these two transcription factors are required for the regulation of hypoxia responsive genes. A full list of the significant expression changes after *RAP2.2-12* silencing is provided in Supplementary Table 4

b Histogram to show the silencing of *RAP2.2* and *RAP2.12* via artificial miRNA in aerobic and hypoxic conditions. Data are mean values relative to the reference (wild-type, air) ± s.d. for n=6 [Student t-test; values corresponding to *amiRAP2.2-2.12* samples that differ significantly (p ≤ 0.05) from the corresponding wild-type are marked with *].

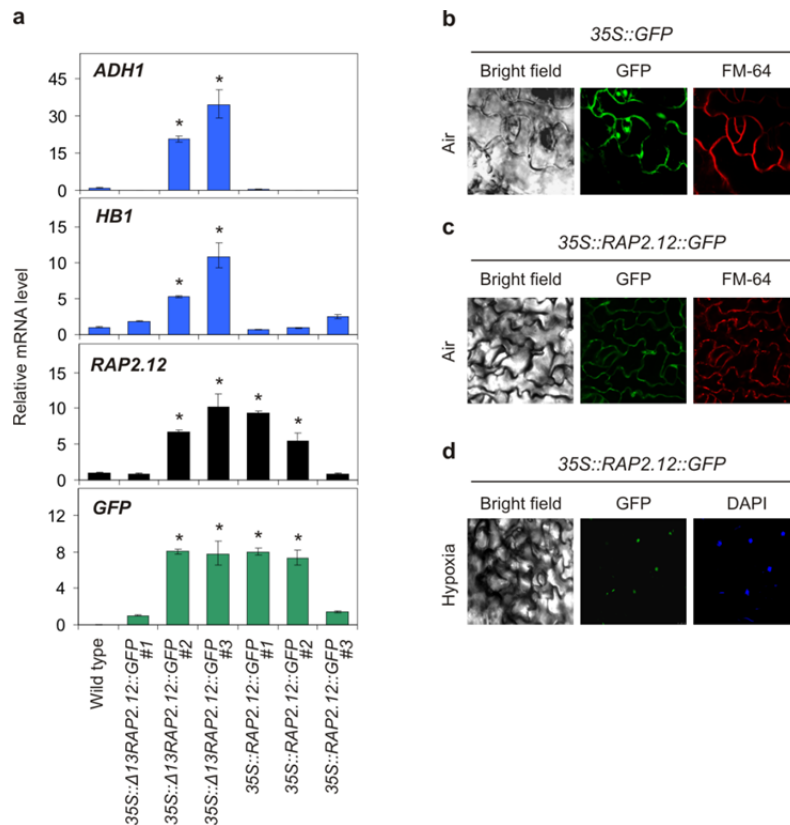
c Heat map indicating differential expression in air and hypoxia of a selection of genes involved in the hypoxic acclimation response in plants. Real time RT-qPCR was used to measure mRNA abundance in plants in which the expression of *RAP2.2* and *RAP2.12* was reduced by overexpressing an artificial microRNA. The data show a reduced induction of anaerobic genes in the amiRNA lines. Numerical values are provided in Supplementary Table 3.



Supplementary Figure 11

Stability of *RAP2.12* mRNA is not affected by fusion of an HA peptide-coding sequence.

The mRNA stability of *RAP2.12* and the control housekeeping gene *PP2A* (*At1g13320*) was assessed by real time RT-qPCR as the ratio between the amplification of a 3' and 5' region of the transcripts. The stable values for the calculated ratios show that *RAP2.12* mRNA integrity was not affected by hypoxia nor by the addition of the HA tag sequence at the 5' end.



Supplementary Figure 12

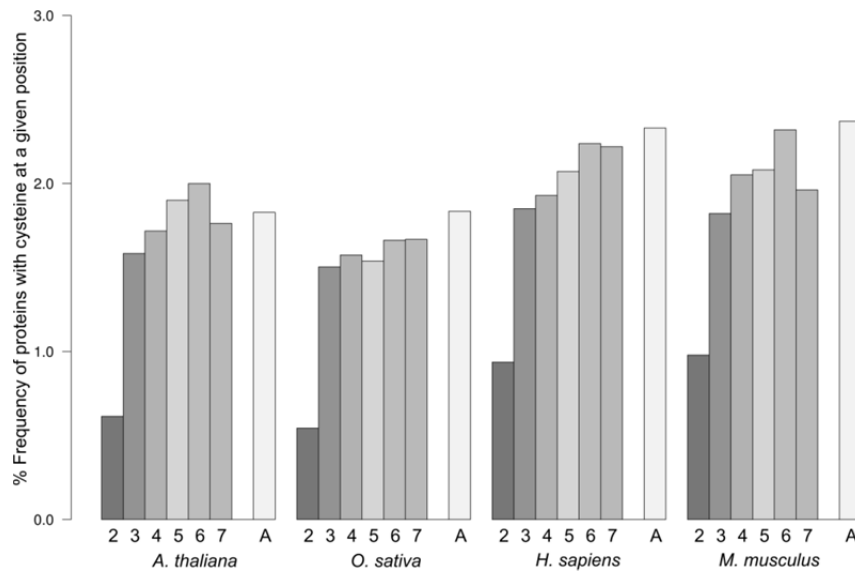
RAP2.12 is localized at the plasma membrane under aerobic conditions and in the nucleus under hypoxia.

a Real time RT-qPCR was used to measure the mRNA level of two hypoxia marker genes (*ADH* and *HBI*), *GFP* and *RAP2.12* in leaves of plants overexpressing C-terminal tagged versions of RAP2.12. *RAP2.12::GFP* indicates the full version of RAP2.12 fused to GFP; *Δ 13RAP2.12::GFP* indicates a deleted version of RAP2.12 in which the first 13 amino acids at the N-terminus were removed. The results show that fusing GFP to the C-terminus of full-length RAP2.12 did not affect the activity of RAP2.12 and could therefore be used to analyse the cellular localisation of RAP2.12 under aerobic and hypoxic conditions. Data are means \pm s.d. for n=3 (two-way ANOVA test). Three independent T0 plants of each construct (marked with #1, #2, and #3) are shown. Asterisks indicate significant differences after one-way ANOVA test (p<0.05).

b Non-overlapping localization of GFP and FM-64 fluorescence in *35S::GFP* plants under aerobic conditions.

c Co-localization of GFP and FM-64 fluorescence in *35S::RAP2.12::GFP* leaves under aerobic conditions.

d Co-localization of GFP and DAPI fluorescence in *35S::RAP2.12::GFP* leaves under hypoxic conditions.

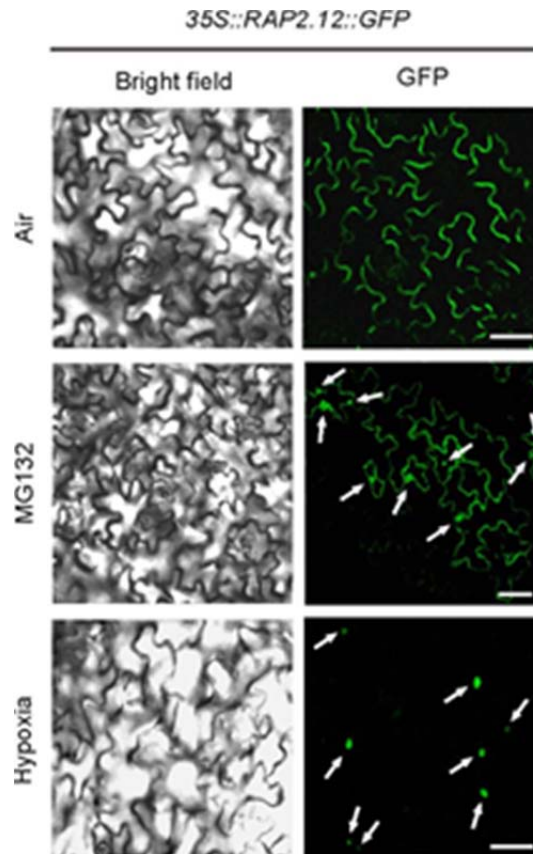


Supplementary Figure 13

Frequency of proteins possessing a cysteine in a specific protein sequence position.

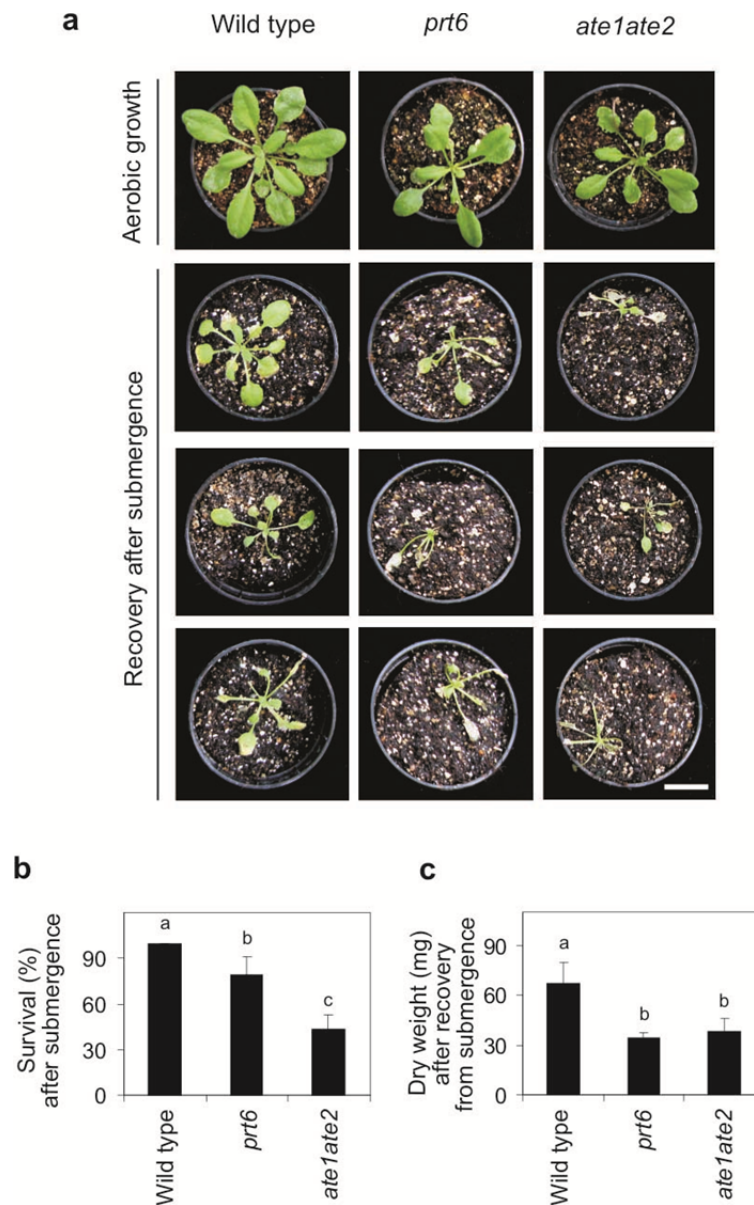
Methionine is always assumed to be the first amino acid. In the bars named "A" the average proteomic cysteine abundance is indicated. Although Cys-dependent protein destabilization by the N-end rule pathway occurs generally in eukaryotes, only in plants and animals Cys oxidation is required for subsequent protein degradation. In fact, the number of proteins with Cys as penultimate N-terminal residue occur significantly less frequently than expected by chance.

Sequences were obtained from the inParanoid database⁴³ and were originally provided by TAIR for *Arabidopsis thaliana* (<http://arabidopsis.org/>), the Rice Genome Annotation Project for *Oryza sativa* (<http://rice.plantbiology.msu.edu/>), Ensembl for *Homo sapiens* and *Mus musculus* (<http://dec2008.archive.ensembl.org/>). Numerical values and statistical assessment of the occurrence significance are provided in Supplementary Table 5.



Supplementary Figure 14

Effect of the proteasome inhibitor MG132 on the stability and localisation of RAP2.12::GFP in air. For comparison, also RAP2.12::GFP localisation under hypoxic conditions without MG132 is shown. Arrows indicate nuclei.



Supplementary Figure 15

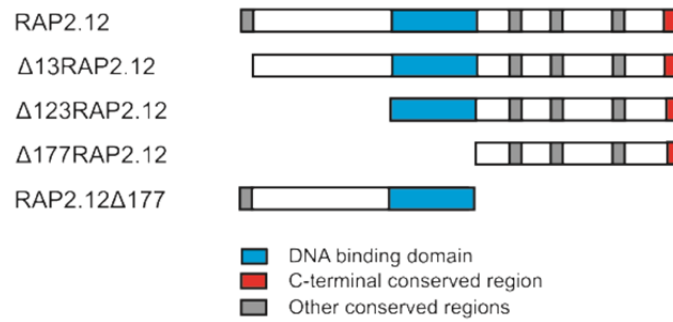
Plant tolerance to flooding decreases when the N-end rule pathway is impaired in both *prt6* and *ate1ate2* knockout lines.

a Phenotype of plants (wild-type, *prt6* and *ate1ate2* mutants) grown in air, or after a 84h submergence-induced hypoxic treatment. Scale bar = 2 cm. The photographs of the plants were taken after one week of aerobic recovery after the treatment.

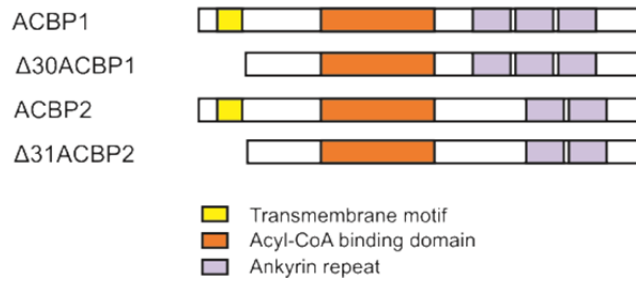
b Percentage of plants that survived 84 h of flooding-induced hypoxia (mean values \pm s.d. obtained from three biological replicate experiments with 7 plants each).

c Dry-weight of leaves harvested after two weeks of recovery from the submergence treatment (mean values \pm s.d., n=4).

GAL4 AD-RAP constructs



GAL4 DBD-ACBP constructs

**Supplementary Figure 16**

Schematic overview of the various constructs that were used in the Yeast-two-Hybrid analysis shown in Fig. 2d.

Supplementary Table 1. Comparison of the mRNA levels of hypoxia responsive genes in wild-type, 35S::RAP2.12, 35S::HA::RAP2.12 and 35S:: Δ 13::RAP2.12 under aerobic conditions; after 4h submergence in the dark and 12h after reoxygenation. Data are means relative to the reference (WT, air) \pm s.d. for n=4 (Student's T test, asterisks indicate $P \leq 0.05$). Two independent transgenic lines for each construct were used.

Gene name	Aerobic conditions										
	Wt	St. Dev.	35S::RAP2.12	St. Dev.	p-value \leq 0.05	HA::RAP2.12	St. Dev.	p-value \leq 0.05	Δ 13RAP2.12	St. Dev.	p-value \leq 0.05
<i>HUP09</i>	1	0.999	1.351	0.355	no	8.346	2.437	yes	55706.425	31863.313	yes
<i>PDC</i>	1	0.851	1.053	0.435	no	6.247	0.934	yes	23236.266	13046.033	yes
<i>HB1</i>	1	0.702	1.482	0.774	no	12.171	4.068	yes	637.384	311.346	yes
<i>ADH</i>	1	0.369	1.306	0.960	no	3.767	0.336	yes	319.712	173.379	yes
<i>LBD41</i>	1	0.356	2.839	0.970	yes	6.147	1.239	yes	2784.292	1580.313	yes
<i>HUP43</i>	1	0.170	2.021	0.765	yes	24.688	6.865	yes	5657.972	3123.867	yes
<i>At3g10040</i>	1	0.144	1.098	0.407	no	2.821	0.712	yes	2858.914	1627.423	yes
<i>SUS4</i>	1	0.364	0.985	0.698	no	1.634	0.476	yes	67.638	7.076	yes
<i>SUS1</i>	1	0.192	0.915	0.421	no	1.269	0.183	yes	19.655	3.305	yes
Gene name	Submergence										
	Wt	St. Dev.	35S::RAP2.12	St. Dev.	p-value \leq 0.05	HA::RAP2.12	St. Dev.	p-value \leq 0.05	Δ 13RAP2.12	St. Dev.	p-value \leq 0.05
<i>HUP09</i>	4338.350	1278.159	8757.547	3130.088	yes	5427.439	1810.178	no	5698.389	1573.690	no
<i>PDC</i>	2336.303	414.865	3739.303	781.257	yes	3626.421	47.480	yes	3206.081	1668.352	no
<i>HB1</i>	1573.346	123.184	1892.992	169.557	yes	1813.761	213.814	no	1385.214	402.157	no
<i>ADH</i>	578.685	101.584	722.441	168.781	yes	744.435	93.199	no	100.065	12.105	yes
<i>LBD41</i>	400.622	38.025	554.644	110.786	yes	484.726	128.284	no	393.841	163.173	no
<i>HUP43</i>	175.449	87.251	341.099	164.976	no	372.347	54.525	yes	431.636	97.027	yes
<i>At3g10040</i>	159.487	19.724	167.866	39.406	yes	158.596	64.270	no	271.305	49.498	yes
<i>SUS4</i>	28.935	9.391	37.642	12.465	yes	73.353	48.001	yes	9.503	2.332	yes
<i>SUS1</i>	10.449	3.505	19.682	5.702	yes	15.113	1.883	no	6.028	0.469	yes
Gene name	Reoxygenation										
	Wt	St. Dev.	35S::RAP2.12	St. Dev.	p-value \leq 0.05	HA::RAP2.12	St. Dev.	p-value \leq 0.05	Δ 13RAP2.12	St. Dev.	p-value \leq 0.05
<i>HUP09</i>	1.142	0.594	2.669825216	0.9610168	yes	51.044	10.701	yes	10661.668	2989.246	yes
<i>PDC</i>	1.767	0.809	3.515	2.415	no	32.130	13.751	yes	10402.025	4269.986	yes
<i>HB1</i>	1.404	0.360	1.326	0.804	no	21.456	2.359	yes	215.098	68.848	yes
<i>ADH</i>	3.520	0.962	4.582	2.504	no	35.358	21.441	yes	528.129	194.543	yes
<i>LBD41</i>	0.944	0.634	2.746	1.474	no	18.128	4.607	yes	2834.914	1257.576	yes
<i>HUP43</i>	0.486	0.193	1.910	0.738	yes	37.616	7.992	yes	7433.902	3327.095	yes
<i>At3g10040</i>	0.690	0.263	0.695	0.282	no	5.071	1.641	yes	2791.611	1280.793	yes
<i>SUS4</i>	2.570	2.391	6.038	6.836	no	14.601	6.004	yes	37.172	10.361	yes
<i>SUS1</i>	1.548	0.556	1.540	0.472	no	4.219	2.088	yes	20.188	14.089	yes

Supplementary Table 2. List of genes that are differentially regulated by overexpressing *HA::RAP2.12* in air and the effect of a hypoxic treatment on their expression.

		<i>35S::HA::RAP2.12</i> vs WT (air)		Hypoxia vs Air (WT)		
Affy ID	AGI ID	log ₂ FC	adj.P.Val	log ₂ FC	adj.P.Val	Annotation
260869_at	At1g43800	7.8197	8.8813E-05	6.5673	8.0375E-06	Plant stearyl-acyl-carrier-protein desaturase family protein
249384_at	At5g39890	3.8034	3.9695E-04	4.1772	1.8841E-06	Protein of unknown function (DUF1637)
250152_at	At5g15120	3.2446	1.2737E-04	5.3641	3.2375E-09	Protein of unknown function (DUF1637)
255807_at	At4g10270	3.0794	1.8269E-06	8.0614	4.4036E-13	Wound-responsive family protein
263096_at	At2g16060	2.8727	1.8269E-06	5.1882	1.1250E-11	Non symbiotic hemoglobin 1
247024_at	At5g66985	2.7874	7.3108E-03	5.5201	5.3236E-08	Unknown protein
264846_at	At2g17850	2.7829	2.8950E-02	6.3275	1.3562E-07	Cell cycle control phosphatase superfamily protein
261567_at	At1g33055	2.3732	1.8269E-06	5.5915	8.0903E-13	Unknown protein
252882_at	At4g39675	2.2770	9.8405E-03	3.4906	1.9319E-06	Unknown protein
258879_at	At3g03270	2.2069	3.2678E-04	3.0593	8.5771E-08	Adenine nucleotide alpha hydrolases superfamily protein
245317_at	At4g15610	1.8938	3.2678E-04	0.3386	3.2075E-01	Uncharacterised protein family (UPF0497)
253298_at	At4g33560	1.5629	4.6451E-02	6.8471	1.1245E-10	Wound-responsive family protein
245951_at	At5g19550	1.4492	4.3822E-03	3.4795	1.7091E-09	Aspartate aminotransferase 2
258487_at	At3g02550	1.4466	1.3260E-03	4.9920	4.0046E-12	LOB domain-containing protein 41
245998_at	At5g20830	1.3244	4.6451E-02	3.3424	1.1074E-07	Sucrose synthase 1
258930_at	At3g10040	1.2288	1.1282E-02	6.6548	4.4036E-13	sequence-specific DNA binding transcription factors
254667_at	At4g18280	1.0501	2.0431E-02	1.4467	2.5028E-05	glycine-rich cell wall protein-related
253050_at	At4g37450	1.0279	7.0086E-03	-0.9081	3.5454E-04	Arabinogalactan protein 18
254592_at	At4g18880	-1.0094	3.2161E-02	0.1688	6.4263E-01	Heat shock transcription factor A4A
260592_at	At1g55850	-1.0341	2.2922E-02	1.5614	1.1977E-05	Cellulose synthase like E1
245193_at	At1g67810	-1.0591	3.9100E-02	1.3074	2.3520E-04	SUFE2__sulfur E2
264314_at	At1g70420	-1.0889	1.1541E-02	0.5251	5.2996E-02	Protein of unknown function (DUF1645)
256185_at	At1g51700	-1.0926	7.0086E-03	-0.5303	3.2496E-02	DOF zinc finger protein 1
267036_at	At2g38465	-1.1101	3.2843E-02	-1.5369	5.3966E-05	Unknown protein
249850_at	At5g23240	-1.1360	1.3927E-02	-1.8083	2.4733E-06	DNAJ heat shock N-terminal domain-containing protein

249928_at	At5g22250	-1.1410	4.6267E-02	0.2537	5.4701E-01	Polynucleotidyl transferase superfamily protein
265184_at	At1g23710	-1.1469	8.7855E-03	-0.9165	1.2892E-03	Protein of unknown function (DUF1645)
248964_at	At5g45340	-1.1800	4.0863E-02	-1.7307	4.3485E-05	Cytochrome P450, family 707, subfamily A, polypeptide 3
258787_at	At3g11840	-1.1954	2.2922E-02	-0.2579	5.0507E-01	Plant U-box 24
247252_at	At5g64770	-1.2010	4.3822E-03	-0.4145	1.1075E-01	Root meristem growth factor (RGF)
249719_at	At5g35735	-1.2085	2.2922E-02	-0.9524	5.4719E-03	Auxin-responsive family protein
259479_at	At1g19020	-1.2173	2.0269E-02	0.3191	3.7529E-01	Unknown protein
249918_at	At5g19240	-1.2200	4.6040E-02	0.0106	9.8577E-01	Glycoprotein membrane precursor GPI-anchored
259211_at	At3g09020	-1.2356	2.8950E-02	-0.9174	1.0238E-02	alpha 1,4-glycosyltransferase family protein
252474_at	At3g46620	-1.2460	3.5620E-02	-0.1085	8.3461E-01	zinc finger (C3HC4-type RING finger) family protein
249383_at	At5g39860	-1.2492	2.2603E-02	-0.8143	1.8243E-02	basic helix-loop-helix (bHLH) DNA-binding family protein
253322_at	At4g33980	-1.2547	9.6506E-03	-1.8263	3.3745E-06	Unknown protein
267623_at	At2g39650	-1.2605	4.8506E-02	-2.0342	2.3215E-05	Protein of unknown function (DUF506)
251745_at	At3g55980	-1.2753	2.2514E-02	-1.5062	1.4949E-04	Salt-inducible zinc finger 1
263379_at	At2g40140	-1.2939	2.1334E-02	-1.0198	4.7307E-03	Zinc finger (CCCH-type) family protein
248164_at	At5g54490	-1.4077	4.5548E-02	-0.6818	1.2433E-01	Pinoid-binding protein 1
260804_at	At1g78410	-1.4186	3.4870E-02	-1.1078	8.7367E-03	VQ motif-containing protein
266800_at	At2g22880	-1.4239	3.1605E-02	0.5899	1.6455E-01	VQ motif-containing protein
263182_at	At1g05575	-1.4336	1.1282E-02	-0.9918	5.7567E-03	Unknown protein
261459_at	At1g21100	-1.4394	3.8612E-02	0.0823	8.9933E-01	O-methyltransferase family protein
245711_at	At5g04340	-1.4413	1.1282E-02	-0.0329	9.5392E-01	Zinc finger of Arabidopsis thaliana 6
264000_at	At2g22500	-1.4571	3.7629E-02	-1.3601	2.5436E-03	Uncoupling protein 5
261892_at	At1g80840	-1.5074	2.1147E-03	-0.5424	6.9820E-02	WRKY DNA-binding protein 40
246777_at	At5g27420	-1.5606	7.1182E-03	0.5174	1.5693E-01	Carbon/nitrogen insensitive 1
247573_at	At5g61160	-1.5880	1.2648E-02	0.7909	4.8302E-02	Anthocyanin 5-aromatic acyltransferase 1
262382_at	At1g72920	-1.6023	2.1746E-02	-1.8309	1.9754E-04	Toll-Interleukin-Resistance (TIR) domain family protein
262381_at	At1g72900	-1.6275	1.9202E-02	-0.3510	4.7917E-01	Toll-Interleukin-Resistance (TIR) domain-containing protein
248799_at	At5g47230	-1.6552	3.6809E-02	-0.6713	1.8626E-01	Ethylene responsive element binding factor 5
258792_at	At3g04640	-1.6958	9.3780E-03	-0.6328	1.2278E-01	glycine-rich protein

261470_at	At1g28370	-1.8496	1.1313E-02	-0.7428	1.0838E-01	ERF domain protein 11
246923_at	At5g25100	-1.8737	1.8207E-08	-0.5279	8.9590E-04	Endomembrane protein 70 protein family
256442_at	At3g10930	-1.9273	4.7973E-02	-0.9506	1.2176E-01	Unknown protein
265725_at	At2g32030	-1.9492	1.1313E-02	-1.7053	9.2210E-04	Acyl-CoA N-acyltransferases (NAT) superfamily protein
266010_at	At2g37430	-2.1939	2.2622E-03	-2.3485	1.4661E-05	C2H2 and C2HC zinc fingers superfamily protein
253643_at	At4g29780	-2.5432	1.0631E-02	-3.3453	1.2482E-05	Unknown protein
254120_at	At4g24570	-2.6662	7.1182E-03	-2.1670	9.1271E-04	Dicarboxylate carrier 2

Supplementary Table 3. Comparison of the mRNA levels of hypoxia responsive genes in wild-type and 35S::amiRAP2.2-12 under aerobic conditions and after 1.5 h hypoxic treatment. Data are means relative to the reference (WT, air) \pm s.d. for n=3 (Student's T test, asterisks indicate $P \leq 0.05$). Three independent transgenic lines for each construct were used.

	Wt		amiRAP2.2-12		p-value ≤ 0.05	Wt		amiRAP2.2-12		p-value ≤ 0.05
	Air	St. Dev.	Air	St. Dev.		Hypoxia	St. Dev.	Hypoxia	St. Dev.	
<i>RAP2.12</i>	1	0.390	0.155	0.045	yes	4.778	1.251	0.069	0.004	yes
<i>RAP2.2</i>	1	0.262	0.681	0.145	no	6.589	0.324	0.374	0.233	yes
<i>PDC1</i>	1	0.906	1.087	0.482	no	440.853	708.348	95.569	38.232	no
<i>ADH</i>	1	0.476	1.630	0.471	no	203.211	57.990	8.796	3.418	yes
<i>Sus1</i>	1	0.823	1.381	0.951	no	38.263	12.633	2.241	3.085	yes
<i>Sus4</i>	1	0.684	1.477	0.104	no	21.503	4.023	0.769	0.656	yes
<i>Hb1</i>	1	0.354	0.550	0.055	no	637.309	206.613	133.813	17.674	yes
<i>LBD41</i>	1	0.380	0.456	0.027	no	1540.563	173.576	516.456	72.907	yes
<i>At5g39890</i>	1	0.739	0.206	0.040	no	768.471	7.633	6.167	7.132	yes
<i>At5g10040</i>	1	0.043	0.432	0.184	yes	17341.686	8778.689	303.850	180.270	yes
<i>At3g10040</i>	1	0.416	0.494	0.139	no	519.005	110.176	33.905	10.846	yes

Supplementary Table 4. List of genes that are differentially regulated by simultaneous silencing of *RAP2.2* and *RAP2.12* via an amiRNA approach and the effect of a hypoxic treatment on their expression. In bold, genes whose expression is up- or down-regulated by both hypoxia (log₂ fold change > +2 or <-2) and by silencing *RAP2.2* and *RAP2.12* (log₂ fold change > +2 or <-2)

		35S::amiRAP2.2-12 vs WT (hypoxia)		Hypoxia vs Air (WT)		
Affy ID	AGI ID	log₂FC	adj.P.Val	log₂FC	adj.P.Val	Annotation
258537_at	At3g04210	1.8021	5.0671E-04	-2.3635	4.8978E-07	Disease resistance protein (TIR-NBS class)
266693_at	At2g19800	1.6546	4.3840E-03	-5.5316	8.9351E-11	Myo-inositol oxygenase 2
265722_at	At2g40100	1.5614	1.5491E-04	-1.4400	6.0809E-06	Light harvesting complex photosystem II
244973_at	AtCg00690	1.5584	4.9118E-03	-1.0871	4.4260E-03	Photosystem II reaction center protein T
255064_at	At4g08950	1.5409	1.4068E-02	-3.1401	5.0652E-07	Phosphate-responsive 1 family protein
264261_at	At1g09240	1.4366	5.3338E-03	-0.3823	3.1056E-01	Nicotianamine synthase 3
247925_at	At5g57560	1.4183	8.1933E-03	-3.3636	2.8609E-08	Xyloglucan endotransglucosylase/hydrolase family protein
254416_at	At4g21380	1.3881	4.9477E-03	0.4201	2.2863E-01	Receptor kinase 3
244965_at	AtCg00590	1.3688	4.2514E-02	0.0156	9.8412E-01	ORF31__electron carriers
245052_at	At2g26440	1.3564	3.1294E-03	-1.7501	5.9532E-06	Plant invertase/pectin methylesterase inhibitor superfamily
252076_at	At3g51660	1.3299	2.3944E-02	-0.9443	2.4083E-02	Tautomerase/MIF superfamily protein
245265_at	At4g14400	1.3153	1.5653E-02	-0.5885	1.2999E-01	ACD6__ankyrin repeat family protein
244933_at	AtCg01070	1.3051	1.1757E-02	-0.2885	4.9042E-01	NADH-ubiquinone/plastoquinone oxidoreductase chain 4L
245346_at	At4g17090	1.2906	2.0492E-03	-1.9290	5.3649E-07	Chloroplast beta-amylase 3
260804_at	At1g78410	1.1891	4.2753E-02	-1.1078	8.7367E-03	VQ motif-containing protein
247866_at	At5g57550	1.1777	2.4259E-02	-3.2935	3.5343E-08	Xyloglucan endotransglucosylase/hydrolase 25
251642_at	At3g57520	1.1624	2.3954E-03	-3.8856	2.6792E-11	AtSIP2_SIP2__seed imbibition 2
265400_at	At2g10940	1.1259	2.0588E-03	-2.8291	8.6847E-10	Lipid-transfer protein/seed storage 2S albumin superfamily protein
267076_at	At2g41090	1.1223	1.7599E-04	-0.7828	1.7252E-04	Calcium-binding EF-hand family protein
247814_at	At5g58310	1.1194	1.1276E-02	-1.0575	1.2335E-03	Methyl esterase 18
248888_at	At5g46240	1.1154	3.9571E-02	-1.3424	1.0797E-03	Potassium channel in Arabidopsis thaliana 1
249777_at	At5g24210	1.1025	1.6055E-02	-0.0462	9.2725E-01	Alpha/beta-Hydrolases superfamily protein
252373_at	At3g48090	1.0386	1.1276E-02	-0.4061	1.6866E-01	Alpha/beta-Hydrolases superfamily protein
262374_s_a	At1g72910	1.0313	2.0842E-02	-0.5053	1.1624E-01	Toll-Interleukin-Resistance (TIR) domain-containing protein
261032_at	At1g17430	1.0271	1.6493E-02	-1.3834	7.7243E-05	Alpha/beta-Hydrolases superfamily protein

255080_at	At4g09030	1.0137	1.3699E-03	-2.3195	1.4511E-09	Arabinogalactan protein 10
258920_at	At3g10520	1.0046	4.4023E-03	-2.4221	5.5371E-09	Non symbiotic hemoglobin 2
248037_at	At5g55930	1.0025	1.0546E-02	-1.5045	1.0358E-05	Oligopeptide transporter 1
247488_at	At5g61820	-1.0061	8.1933E-03	3.3975	3.5805E-10	Unknown protein
253987_at	At4g26270	-1.0173	8.1933E-03	2.1883	9.1837E-08	Phosphofructokinase 3
258203_at	At3g13950	-1.0220	2.4259E-02	0.7539	2.0001E-02	Unknown protein
252168_at	At3g50440	-1.0264	1.6655E-02	2.6464	4.2291E-08	Methyl esterase 10
249015_at	At5g44730	-1.0281	3.3492E-02	1.6731	4.2570E-05	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
257844_at	At3g28480	-1.0294	5.3338E-03	2.5163	7.4352E-09	Oxoglutarate/iron-dependent oxygenase
259040_at	At3g09270	-1.0322	1.1083E-02	1.0015	9.5024E-04	Glutathione S-transferase TAU 8
253264_at	At4g33950	-1.0342	2.3954E-03	0.7469	1.5297E-03	Sucrose non fermenting receptor kines 2.6
254300_at	At4g22780	-1.0347	8.1933E-03	2.5099	2.0941E-08	ACT domain repeat 7
257516_at	At1g69040	-1.0450	3.5468E-02	0.6012	9.1996E-02	ACT domain repeat 4
262805_at	At1g20900	-1.0476	4.3381E-03	0.8921	7.2502E-04	Predicted AT-hook DNA-binding family protein
248719_at	At5g47910	-1.0526	3.6367E-03	1.9537	9.6687E-08	Respiratory burst oxidase homologue D
247760_at	At5g59130	-1.0538	3.1294E-03	1.2804	1.2215E-05	Subtilase family protein
263374_at	At2g20560	-1.0559	2.6333E-02	3.1420	2.0329E-08	DNAJ heat shock family protein
249234_at	At5g42200	-1.0594	4.0945E-02	1.8738	2.5779E-05	RING/U-box superfamily protein
249645_at	At5g36910	-1.0643	4.3840E-03	0.7106	5.3256E-03	THI2.2__thionin 2.2
261570_at	At1g01120	-1.0688	1.9544E-02	1.1239	1.1338E-03	KCS1__3-ketoacyl-CoA synthase 1
253513_at	At4g31760	-1.0723	7.0567E-03	1.3857	2.8306E-05	Peroxidase superfamily protein
245724_at	At1g73390	-1.0733	1.7599E-04	1.3583	1.8882E-07	Endosomal targeting BRO1-like domain-containing protein
266502_at	At2g47720	-1.0804	1.7859E-02	2.1644	1.0911E-06	Unknown protein
253404_at	At4g32840	-1.0888	6.1467E-03	2.6458	1.1616E-08	Phosphofructokinase 6
248218_at	At5g53710	-1.0964	9.7062E-03	1.5140	2.3283E-05	Unknown protein
262518_at	At1g17170	-1.1072	1.3705E-03	1.2357	1.0177E-05	ATGSTU24_GST_GSTU24__glutathione S-transferase TAU 24
259879_at	At1g76650	-1.1203	3.7642E-02	2.1675	8.1528E-06	CML38__calmodulin-like 38
247716_at	At5g59350	-1.1302	5.3338E-03	1.1722	1.6320E-04	Unknown protein
265920_s_a	At2g15120	-1.1440	6.1467E-03	0.7950	6.4867E-03	Unknown protein
258879_at	At3g03270	-1.1698	2.4259E-02	3.0593	8.5771E-08	Adenine nucleotide alpha hydrolases-like superfamily protein
247151_at	At5g65640	-1.1958	2.4259E-02	2.9261	1.8882E-07	beta HLH protein 93
253638_at	At4g30470	-1.2081	7.9471E-04	1.7305	2.7610E-07	NAD(P)-binding Rossmann-fold superfamily protein
245317_at	At4g15610	-1.2211	7.3756E-03	0.3386	3.2075E-01	Uncharacterised protein family (UPF0497)

258930_at	At3g10040	-1.2297	5.6669E-03	6.6548	4.4036E-13	sequence-specific DNA binding transcription factors
248138_at	At5g54960	-1.2299	9.3168E-03	1.8579	8.0187E-06	Pyruvate decarboxylase-2
262164_at	At1g78070	-1.2347	5.0671E-04	2.5417	1.7186E-09	Transducin/WD40 repeat-like superfamily protein
247224_at	At5g65080	-1.2698	9.3168E-03	0.3015	4.3205E-01	K-box region and MADS-box transcription factor family protein
267024_s_a	At2g29870	-1.2739	2.4259E-02	2.3818	4.7958E-06	Aquaporin-like superfamily protein
256376_s_a	At1g66690	-1.2746	5.3338E-03	0.7783	1.2941E-02	S-adenosyl-L-methionine-dependent methyltransferases
251072_at	At5g01740	-1.2765	1.0771E-02	2.0949	3.9809E-06	Nuclear transport factor 2 (NTF2) family protein
255110_at	At4g08770	-1.2850	1.3069E-02	1.0697	3.9852E-03	Peroxidase superfamily protein
264217_at	At1g60190	-1.3044	7.5352E-03	3.4255	6.3339E-09	ARM repeat superfamily protein
245035_at	At2g26400	-1.3466	2.5713E-02	2.6599	2.9011E-06	Acireductone dioxygenase 3
265675_at	At2g32120	-1.3495	3.0132E-02	2.6371	4.4200E-06	Heat-shock protein 70T-2
258366_at	At3g14230	-1.3537	1.6455E-02	0.0225	9.7174E-01	Related to AP2 2
266290_at	At2g29490	-1.3597	4.6678E-05	3.1142	1.1250E-11	Glutathione S-transferase TAU 1
259384_at	At3g16450	-1.3881	3.6380E-02	0.9950	3.5100E-02	Mannose-binding lectin superfamily protein
261420_at	At1g07720	-1.4178	2.0492E-03	0.8441	5.5489E-03	3-ketoacyl-CoA synthase 3
266988_at	At2g39310	-1.4675	5.1140E-03	1.0871	2.9999E-03	Jacalin-related lectin 22
254819_at	At4g12500	-1.4684	3.1027E-02	0.7892	1.0743E-01	Seed storage 2S albumin superfamily protein
245393_at	At4g16260	-1.5052	6.3989E-03	2.1982	6.1767E-06	Glycosyl hydrolase superfamily protein
254200_at	At4g24110	-1.5259	1.7637E-02	3.8646	6.2424E-08	Unknown protein
261020_at	At1g26390	-1.5444	1.3699E-03	1.1073	8.4142E-04	FAD-binding Berberine family protein
253799_at	At4g28140	-1.5456	1.3705E-03	4.0974	2.7321E-10	Integrase-type DNA-binding superfamily protein
260568_at	At2g43570	-1.5499	4.2762E-02	1.6034	4.1315E-03	Chitinase, putative
267567_at	At2g30770	-1.5962	1.9605E-02	1.4531	3.6876E-03	Cytochrome P450, family 71, subfamily A, polypeptide 13
260015_at	At1g67980	-1.6001	5.0195E-03	0.7167	6.2503E-02	Caffeoyl-CoA 3-O-methyltransferase
253298_at	At4g33560	-1.6602	1.6544E-02	6.8471	1.1245E-10	Wound-responsive family protein
265200_s_a	At2g36790	-1.6720	1.7948E-04	3.8066	1.4405E-10	UDP-glucosyl transferase 73C6
265418_at	At2g20880	-1.6896	2.1486E-04	4.8408	8.9683E-12	Integrase-type DNA-binding superfamily protein
262307_at	At1g71000	-1.7602	1.3510E-02	2.8966	5.8646E-06	Chaperone DnaJ-domain superfamily protein
254059_at	At4g25200	-1.7935	3.9298E-03	2.5315	3.1459E-06	Mitochondrion-localized small heat shock protein 23.6
267178_at	At2g37750	-1.7965	3.5796E-03	1.6407	2.8713E-04	Unknown protein
244940_at	AtCg00905	-1.9040	1.1083E-02	-0.4641	4.3177E-01	Ribosomal protein S12C
253505_at	At4g31970	-1.9230	6.3253E-05	1.5611	7.7254E-06	Cytochrome P450, family 82, subfamily C, polypeptide 2
250296_at	At5g12020	-1.9508	1.4704E-02	2.7575	3.6681E-05	17.6 kDa class II heat shock protein

245173_at	At2g47520	-1.9731	3.1451E-02	4.3355	1.1959E-06	Hypoxia Responsive Factor 2
257925_at	At3g23170	-1.9951	1.5464E-02	5.1154	3.6143E-08	Unknown protein
247024_at	At5g66985	-2.0280	2.4259E-02	5.5201	5.3236E-08	Unknown protein
260101_at	At1g73260	-2.0424	3.7081E-03	0.9223	4.8671E-02	Kunitz trypsin inhibitor 1
250476_at	At5g10140	-2.0743	6.2623E-03	1.4698	5.8236E-03	Flowering locus C
246854_at	At5g26200	-2.0929	7.0567E-03	3.7614	6.2443E-07	Mitochondrial substrate carrier family protein
249384_at	At5g39890	-2.1434	1.9605E-02	4.1772	1.8841E-06	Protein of unknown function (DUF1637)
249780_at	At5g24240	-2.1435	7.5858E-06	2.0771	6.3825E-08	Phosphatidylinositol 3- and 4-kinase
245148_at	At2g45220	-2.2227	2.0492E-03	1.6839	7.6938E-04	Plant invertase/pectin methylesterase inhibitor superfamily
261266_at	At1g26770	-2.2837	4.9046E-04	4.9571	8.4195E-10	Expansin A10
262197_at	At1g53910	-2.3836	3.1027E-02	-0.0633	9.5905E-01	Related to AP2 12
260978_at	At1g53540	-2.4803	4.1120E-03	3.5250	3.1029E-06	HSP20-like chaperones superfamily protein
256243_at	At3g12500	-2.5698	6.2442E-03	2.4545	5.0484E-04	Basic chitinase PR3
250464_at	At5g10040	-2.7448	2.4259E-02	5.5000	2.1369E-06	Unknown protein
266385_at	At2g14610	-2.7506	2.1279E-03	1.2313	3.4697E-02	Pathogenesis-related gene 1
254832_at	At4g12490	-2.7642	2.5713E-02	0.7798	4.3553E-01	Seed storage 2S albumin superfamily protein
256589_at	At3g28740	-2.8912	8.1807E-03	4.9127	1.5962E-06	Cytochrome P450 superfamily protein
252515_at	At3g46230	-3.1982	5.3338E-03	5.1524	1.2463E-06	AHeat shock protein 17.4
266294_at	At2g29500	-3.2150	1.0771E-02	5.2954	3.7957E-06	HSP20-like chaperones superfamily protein
250351_at	At5g12030	-3.3929	3.5170E-02	5.6240	3.8078E-05	Heat shock protein 17.6A
254889_at	At4g11650	-3.8074	3.7571E-05	1.7209	1.2694E-03	Osmotin 34

Supplementary Table 5. Proteomic analysis of sequences possessing a cysteine in different N-terminal positions in four different organisms, assuming methionine as the first amino acid. For all organisms, there is a significantly lower frequency of Cys at position 2 than expected by chance.

Organism	Total proteins	Number of proteins with cysteine in position <i>n</i> (p-value)						Expected	Number of cysteines in the proteome (relative abundance)	Number of aminoacids in the proteome
		<i>n</i> =2	<i>n</i> =3	<i>n</i> =4	<i>n</i> =5	<i>n</i> =6	<i>n</i> =7			
<i>Arabidopsis thaliana</i>	26,207	161 (8.6x10 ⁻⁶⁵)	415 (0.003)	450 (0.174)	498 (0.407)	524 (0.045)	462 (0.420)	479	201,375 (1.831%)	10,996,310
<i>Oryza sativa</i>	56,797	309 (2.4x10 ⁻¹⁵⁹)	854 (1.4x10 ⁻⁹)	894 (2.2x10 ⁻⁶)	874 (6.9x10 ⁻⁸)	944 (0.002)	947 (0.003)	1042	476,079 (1.835%)	25,939,952
<i>Homo sapiens</i>	21,673	203 (1.6x10 ⁻⁵³)	401 (1.4x10 ⁻⁶)	418 (6.7x10 ⁻⁵)	449 (0.012)	485 (0.380)	481 (0.290)	505	266,049 (2.330%)	11,420,212
<i>Mus musculus</i>	23,497	230 (1.5x10 ⁻⁵⁶)	428 (8.9x10 ⁻⁹)	482 (0.001)	489 (0.003)	545 (0.622)	461 (2.4x10 ⁻⁵)	557	272,629 (2.372%)	11,494,162

Sequences were obtained from the inParanoid database⁴³ and were originally provided by TAIR for *Arabidopsis thaliana* (<http://arabidopsis.org/>), the Rice Genome Annotation Project for *Oryza sativa* (<http://rice.plantbiology.msu.edu/>), Ensembl for *Homo sapiens* and *Mus musculus* (<http://dec2008.archive.ensembl.org/>), SGD for *Saccharomyces cerevisiae* (<http://www.yeastgenome.org/>), and the Sanger Institute for *Schizosaccharomyces pombe* (http://www.sanger.ac.uk/Projects/S_pombe/). Statistical significance for divergence between expected and observed frequency of Cys was calculated using the binomial exact test⁴⁴, with a two-sided alternative hypothesis.

Supplemental Table 6. Full list of the vectors used with references.

Final construct	Original destination vector	Reference
35S::HA::RAP2.12	pE-35S-HA-GW	Weiste et al. 2007 ⁴⁵
35S::RAP2.12	pK7WG2	Karimi et al. 2005 ⁴⁶
35S::Δ13RAP2.12	pK7WG2	Karimi et al. 2005 ⁴⁶
35S::amiRAP2.2-12	pB7WG2	Karimi et al. 2005 ⁴⁶
RAP2.12::GFP	pK7FWG2	Karimi et al. 2005 ⁴⁶
Δ13RAP2.12::GFP	pK7FWG3	Karimi et al. 2005 ⁴⁶
GAL4 DBD::ACBP1	pDEST TM 32	ProQuest TM Two-Hybrid System Instruction Manual
GAL4 DBD::ACBP2	pDEST TM 32	ProQuest TM Two-Hybrid System Instruction Manual
GAL4 DBD::Δ30ACBP1	pDEST TM 32	ProQuest TM Two-Hybrid System Instruction Manual
GAL4 DBD::Δ31ACBP2	pDEST TM 32	ProQuest TM Two-Hybrid System Instruction Manual
GAL4 AD::Δ13RAP2.12	pDEST TM 22	ProQuest TM Two-Hybrid System Instruction Manual
GAL4AD::Δ123RAP2.12	pDEST TM 22	ProQuest TM Two-Hybrid System Instruction Manual
GAL4AD::Δ184RAP2.12	pDEST TM 22	ProQuest TM Two-Hybrid System Instruction Manual
GAL4AD::RAP2.12Δ177	pDEST TM 22	ProQuest TM Two-Hybrid System Instruction Manual
ACBP1::nYFP	pDEST ^{GW} VYNE	Gehl et al. 2009 ³⁵
ACBP2::nYFP	pDEST ^{GW} VYNE	Gehl et al. 2009 ³⁵
RAP2.12::cYFP	pDEST ^{GW} VYCE	Gehl et al. 2009 ³⁵
35S::PpLuc	pGreenII-800Luc	Hellens et al. 2005 ⁴⁷
35S::MCGGAI::PpLuc	pGreenII-800Luc	Hellens et al. 2005 ⁴⁷
GBD::RAP2.12	p35S-GBD-GW	Weiste et al. 2007 ⁴⁵
GBD::Δ13RAP2.12	p35S-GBD-GW	Weiste et al. 2007 ⁴⁵
GBD::RAP2.12Δ177	p35S-GBD-GW	Weiste et al. 2007 ⁴⁵
GBD::RAP2.12Δ223	p35S-GBD-GW	Weiste et al. 2007 ⁴⁵
GBD::RAP2.12Δ254	p35S-GBD-GW	Weiste et al. 2007 ⁴⁵
GBD::RAP2.12Δ308	p35S-GBD-GW	Weiste et al. 2007 ⁴⁵
GBD::RAP2.12Δ340	p35S-GBD-GW	Weiste et al. 2007 ⁴⁵
35S::RrLuc-AUS::PpLuc	pGreenII-800Luc	Hellens et al. 2005 ⁴⁷
Prom _{HB1} ::PpLuc	pGreenII-800Luc	Hellens et al. 2005 ⁴⁷
Prom _{HB1A} ::PpLuc	pGreenII-800Luc	Hellens et al. 2005 ⁴⁷
Prom _{HB1B} ::PpLuc	pGreenII-800Luc	Hellens et al. 2005 ⁴⁷
Prom _{HB1C} ::PpLuc	pGreenII-800Luc	Hellens et al. 2005 ⁴⁷
35S::Δ13RAP2.12small	p2GW7	Karimi et al. 2005 ⁴⁵

Supplementary Table S7. Full list of the primers used in this study. (Fw = forward primer; Rv = reverse primer)

Primer name	Sequence 5'→3'
gwRAP2.12Fw	caccatgtgtggaggagctataat
gwMAGRAP2.12Fw	caccatggctggaggagctataat
gwRAP2.12Rv	tcagaagactcctccaatcatg
gwRAP2.12ΔstopRv	gaagactcctccaatcatg
gwΔ13RAP2.12	caccatgcgaggtctcgccgtgt
amiRAP2.2-12I	gatgttgccctaatccctcggctcctctctctcttttgtattcc
amiRAP2.2-12II	gaggaccgagggattaggcaacatcaaagagaatcaatga
amiRAP2.2-12III	gaggcccgagggatttggcaacttcacaggtcgtgatatg
amiRAP2.2-12IV	gaagttgccaaatccctcgggcctctacatatatattcct
gwmiRNAFw	cacccaaacacacgctcggacgca
gwmiRNARv	catggcgatgccttaataaaga
gwACBP1Fw	caccatggctgattggtatcagct
gwΔ30ACBP1Fw	caccatgtttaagacgagaat
gwACBP1Rv	ctaattggaatccttcttctctcgcgat
gwACBP1ΔstopRv	attggaatccttcttctctcgcgat
gwACBP2Fw	caccatgggtgattgggctcaact
gwΔ31ACBP2Fw	caccatgaaagaagacaatctctctctt
gwACBP2Rv	ttagtctgcctgctttgcagaatct
gwACBP2ΔstopRv	gtctgcctgctttgcagaatct
Δ123RAP2.12Fw	caccatgaatcagtaccgagggatt
Δ184RAP2.12Fw	atgtccgatttcattccaccgcc
RAP2.12Δ177Rv	cttagcttttagatccacggattctc
RAP2.12Δ223Rv	tcaattttcaaaggagatggt
RAP2.12Δ254Rv	tcaataccattacatccagc
RAP2.12Δ308Rv	tcaggcttctcctcaaagaacag
RAP2.12Δ340Rv	tcagtttgcaccattgtcctg
gw35SFw	caccactagagccaagctgatctcc
gw35SRv	tcgactagaatagtaaattgtaatgt
gw35SMCGAIRv	tacaatcttactattcttagtcgaatgtgtggaggagctataata
gwUASFw	caccagcttggatccatcgatg
gwUASRv	tggcactcgagcgtgtcctc
gwPHB1Fw	caccaatctctgttctgccaat
gwPHB1Rv	aatatctcacaacctctaatgat
gwPHB1BRv	cttttgtgttaccgaagagacagttc
gwPHB1CFw	caccgttttagactaataattggtc
sgAt1g77120Fw	tattcgatgcaaagctgctgtg
sgAt1g77120Rv	cgaacttcgtgttctcgcggt
sgAt2g16060Fw	tttgaggtggccaagtatgca
sgAt2g16060Rv	tgatcataagcctgaccccaa
sgAt3g43910Fw	cgcagaacgtgtaataacgcg
sgAt3g43910Rv	caacccttgagagcaaagcaaa
sgAt4g33070Fw	cgattatggcactaacggatt
sgAt4g33070Rv	tgttcaccaccgctgataac
sgAt5g10040Fw	tcatcggcggacatagcaa
sgAt5g10040Rv	atcatcaaccaccaacctcc
sgAt3g10040Fw	acaaccaccgcaacagaatcc
sgAt3g10040Rv	tctccgcaattctcgccat

sgAt5g39890Fw	cttcgagccgttttggatga
sgAt5g39890Rv	acgtcactaacggagatcgtcc
sgAt3g02550Fw	tgaagcgcaagctaacgca
sgAt3g02550Rv	atcccaggacgaaggtgattg
sgAt4g20830Fw	acgctgaacgtatgataacgcg
sgAt4g20830Rv	aaccctggaaagcaaggcaag
sgAt1g53910Fw	actgaatgggacgcttcactgg
sgAt1g53910Rv	agggtttgcaccattgtcctgag
sgAt3g14230Fw	ttcgtcttcaccgcaactactaag
sgAt3g14230Rv	ttcttggcatatgctgaacctacc
sgAt3g16770Fw	aactcacggctgaggaactctg
sgAt3g16770Rv	acttggttggtgggatggagtttg
sgRAP2.125'Fw	aaagagataacaggaacggaaacatagt
sgRAP2.125'Rv	cgtcgaaatcatcatcgcaa
sgRAP2.123'Fw	gctcaagtctatggatctcgaga
sgRAP2.123'Rv	gcaccattgtcctgagtcgtta
sgPP2A5'Fw	tagatcgctcggaaacttggaaa
sgPP2A5'Rv	cctcaccaaaaactcaaactactcc
sgPP2A3'Fw	taacgtggccaaaatgatgc
sgPP2A3'Rv	gttctccacaaccgcttgggt
sgUBQ10Fw	ggccttgtataatccctgatgaataag
sgUBQ10Rv	aaagagataacaggaacggaaacatagt
sgRpERF1sFw	aggggttctctaagcctgcg
sgRpERF1aRv	tcaccatcggactcgtcctt
sgRaERF1sFw	aggggttctctaagcctgcg
sgRaERF1aRv	tcaccatcggactcgtcctt
sgRspTUB1Fw	ccatactccaccagtgttgc
sgRspTUB1Rv	catcatcatcaccctcaacg

Supplementary Table 8

The ERF sequences from *Rumex palustris* and *Rumex acetosa* in this table are deposited at the NCBI database (RaERF1, JF968115; RaERF2, JF968116; RpERF1, JF968117; RpERF2, JF968118; RpERF3, JF968119) and will become available soon.

>RaERF1

MCGGAIISDFIPAKASGRLTATELWSGISGEVTKKKGLSKPARSHVSDVFNDYVNFADFLFKDES DGEEMED
 VKPFAAKGFNPISFNSSQGSKATEFNSSQAESAKRKRKNQYRGIRQRPWGKWA AEIRDPRKGV RVWLGT FNTAE
 EAARAYDAEARRIRGNKAKVNF PENFPVSNRRTVKVNHQKSSSNCKSDSNPAPQNW NQSYNNNVEFVAEEALTY
 TNSLADVEKVEPINF TSPNPCNASPLYPSSGQSSNSFDYADFLV LGANGPQTPEISSYFSAILEGEDEQCNAKKM
 KVSTSSSEEDNGKISRSDLSDFEAEMQLVQTPFLDENWAVDAFLGGNGTQDDL SMMNLWNFDDVQVGSF

>RaERF2

MCGGAIISDLISAKRCRQVSTQDLWSELDASDFFSFSGVGDEKTAGTGDEKKNKKQRSGGGDGDGKQTAP EPAK
 PARKNVYRGIRRRPWGKWA AEIRDPKKGVRVWLGT FSTAE EAARAYDEAARKIRGDKAKLNF PDSAPAKNIAAS
 ADKESRPSFADVGLEYGYGLGETGRMDPIWNLERWLGLEEEVGPSESKGSSTEFDGSDSVDLWCLDQFQFSSM
 IGGGGGGSGSGGGFM

>RpERF1

MCGGAIISDFIPTKGSRLTAGELWSGIPSEVTRQKGF SKPARSHMSDVFNDYVNFADFLFKDES DGEEL ED
 VKPFAVKGLNPNFNFSSQGLKSAEFTSQAESAKRKRKNQYRGIRQRPWGKWA AEIRDPRKGV RVWLGT FNTAE
 EAARAYDAEARRIRGNKAKVNF PVDIPASNRTIKVNHQKSNANAKTNQKPAPQNW NQSYNNIGFVAEEPLNYT
 NSLTDVGVKVEPILFTAPNPCNASPIYLSGQSSNSFDYADLGFGENGPQTPEISSYFSAILEGEEAQITEQSNA
 KKMKMSTSSSEEDNGKISCSDLSDFEAEMLSIETPFLDENWAVDAFLGGNGTQDCISP MNLWNFDDVQVGSF

>RpERF2

MCGGAIISDFIPAKGSRRLVASDLWSEGLAGEGKKKKRSSGGYSKPARSEVIDVVKDEYDFEADFVDFVDECDDDE
VFGDVKPFSEFRPVNTFNSSSTQGPKPVEFTGQTEKSAKRKRKNQYRGIRQRPWGKWAAEIRDPKKGVRVWLGTF
DTAEAAARAYDSEARRIRGKKAKVNFPEDESAPVTSKRRTTIKVSQ

>RpERF3

MCGGAIISDFIAAKRSRQLTTQDLWSELDASDFFGFSGVDENITAGAEKKNKKQRGGGGDGGSGKQMASDPA
KPQRKNVYRGIRKRPWGKWAAEIRDPKKGVRVWLGTFNTAEAAARAYDQAARKIRGDKAKLNFPDTPAPKRRL
GVAEDNNTHSVAVNELTRTSHPPSLPNVDAGLGYGHGHGETGRVDPIWNLESWLGLDEEVGTSESKGSSTEFDE
SDSVELWGLDQFQFSSMMVGGGGDCSGGGPGGGGGYGF

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