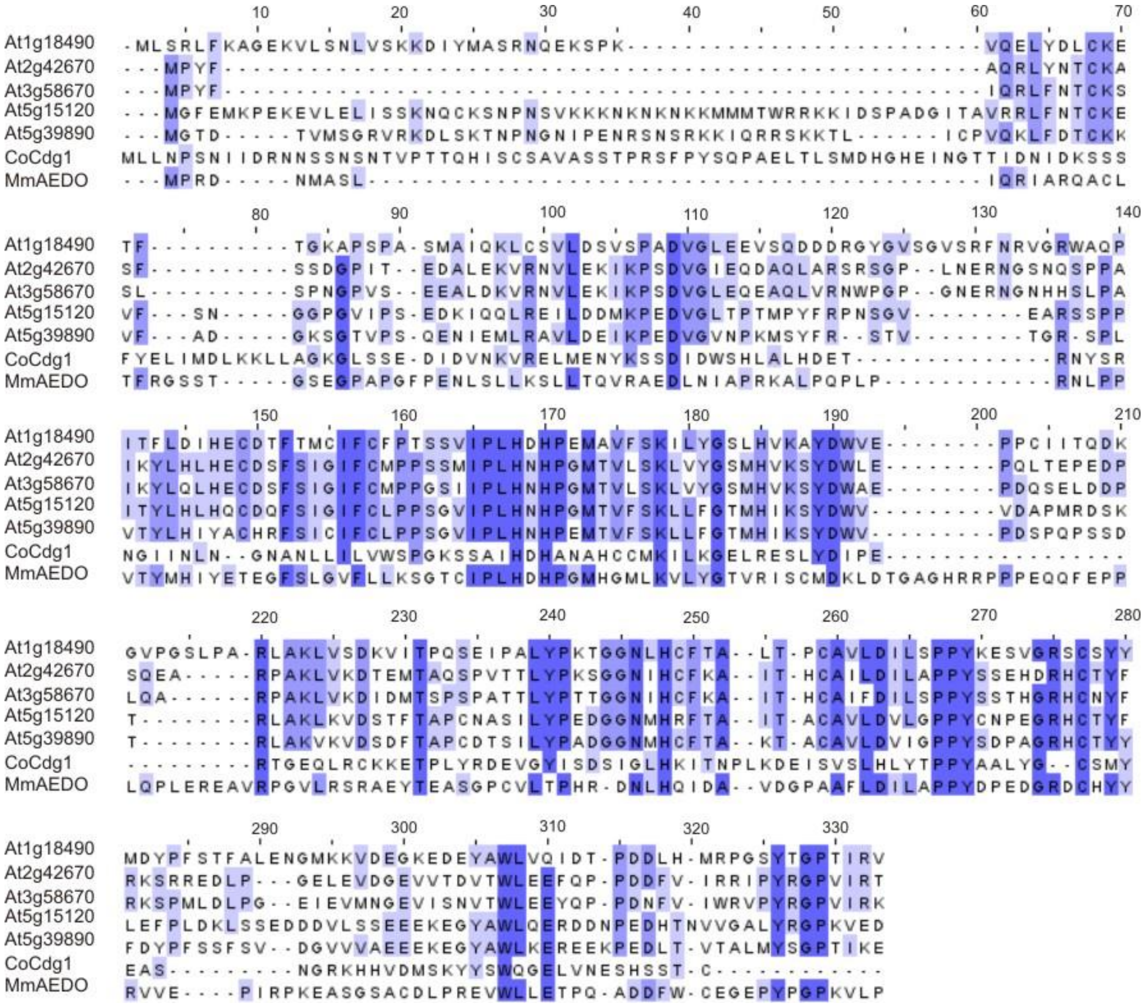
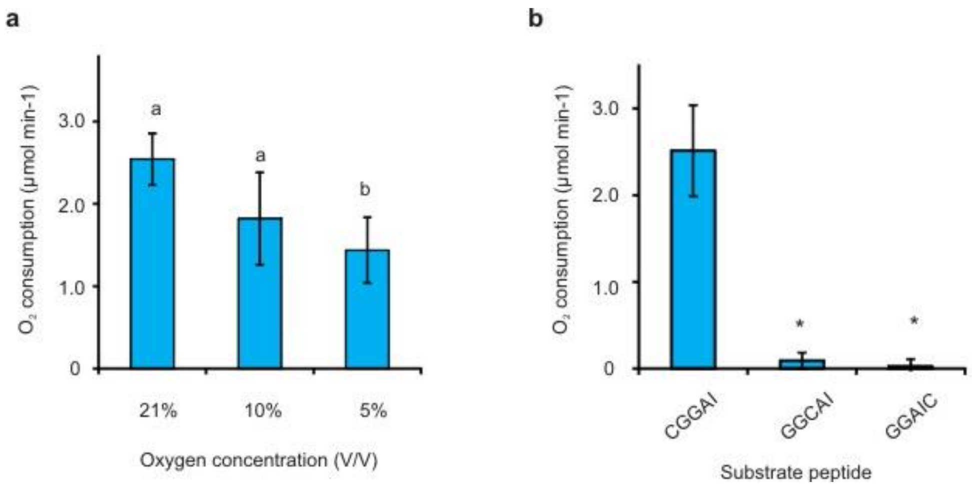
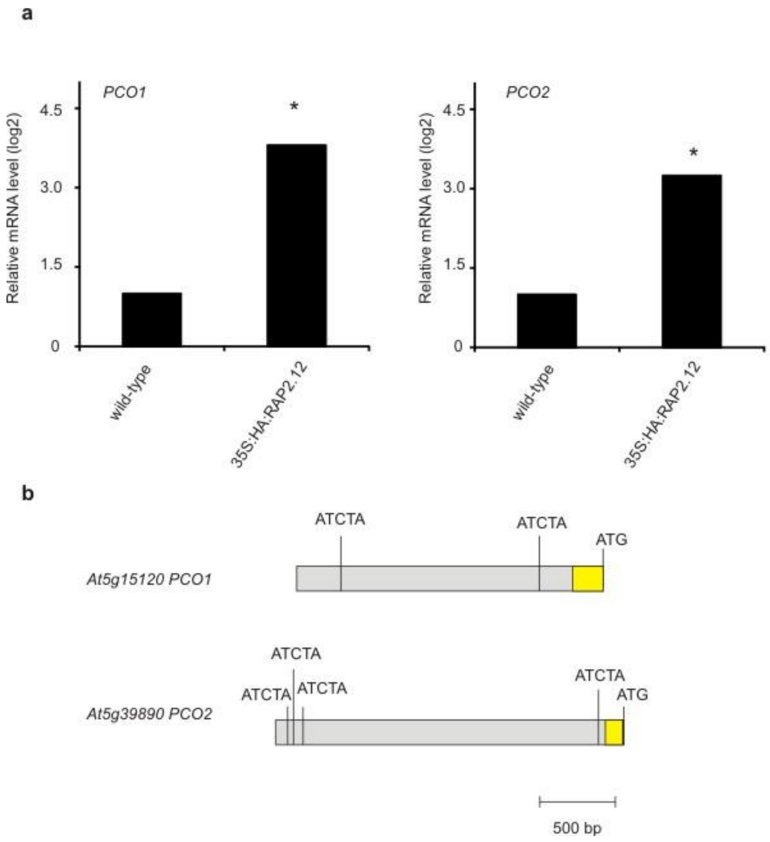


```
                   10        20        30        40        50        60        70
At1g18490  -MLSRLFKAGEKVLSNLVSKKDIYMASRNQEKSPK-------------------------VQELYDLCKE
At2g42670  ---MPYF-----------------------------------------------------AQRLYNTCKA
At3g58670  ---MPYF-----------------------------------------------------IQRLFNTCKS
At5g15120  ---MGFEMKPEKEVLELISSKNQCKSNPNSVKKKNKNKNKKMMMTWRRKKIDSPADGITAVRRLFNTCKE
At5g39890  ---MGTD-----TVMSGRVRKDLSKTNPNGNIPENRSNSRKKIQRRSKKTL------ICPVQKLFDTCKK
CoCdg1     MLLNPSNIIDRNNSSNSNTVPTTQHISCSAVASSTPRSFPYSQPAELTLSMDHGHEINGTTIDNIDKSSS
MmAEDO     ---MPRD-----NMASL-------------------------------------------IQRIARQACL

                   80        90       100       110       120       130       140
At1g18490  TF----------TGKAPSPA-SMAIQKLCSVLDSVSPADVGLEEVSQDDDRGYGVSGVSRFNRVGRWAQP
At2g42670  SF----------SSDGPIT--EDALEKVRNVLEKIKPSDVGIEQDAQLARSRSGP--LNERNGSNQSPPA
At3g58670  SL----------SPNGPVS--EEALDKVRNVLEKIKPSDVGLEQEAQLVRNWPGP--GNERNGNHHSLPA
At5g15120  VF---SN-----GGPGVIPS-EDKIQQLREILDDMKPEDVGLTPTMPYFRPNSGV--------EARSSPP
At5g39890  VF---AD-----GKSGTVPS-QENIEMLRAVLDEIKPEDVGVNPKMSYFR--STV--------TGR-SPL
CoCdg1     FYELIMDLKKLLAGKGLSSE-DIDVNKVRELMENYKSSDIDWSHLALHDET--------------RNYSR
MmAEDO     TFRGSST-----GSEGPAPGFPENLSLLKSLLTQVRAEDLNIAPRKALPQPLP------------RNLPP

                  150       160       170       180       190       200       210
At1g18490  ITFLDIHECDTFTMCIFCFPTSSVIPLHDHPEMAVFSKILYGSLHVKAYDWVE--------PPCIITQDK
At2g42670  IKYLHLHECDSFSIGIFCMPPSSMIPLHNHPGMTVLSKLVYGSMHVKSYDWLE--------PQLTEPEDP
At3g58670  IKYLQLHECDSFSIGIFCMPPGSIIPLHNHPGMTVLSKLVYGSMHVKSYDWAE--------PDQSELDDP
At5g15120  ITYLHLHQCDQFSIGIFCLPPSGVIPLHNHPGMTVFSKLLFGTMHIKSYDWV---------VDAPMRDSK
At5g39890  VTYLHIYACHRFSICIFCLPPSGVIPLHNHPEMTVFSKLLFGTMHIKSYDWV---------PDSPQPSSD
CoCdg1     NGIINLN--GNANLLILVWSPGKSSAIHDHANAHCCMKILKGELRESLYDIPE-----------------
MmAEDO     VTYMHIYETEGFSLGVFLLKSGTCIPLHDHPGMHGMLKVLYGTVRISCMDKLDTGAGHRRPPPEQQFEPP

                  220       230       240       250       260       270       280
At1g18490  GVPGSLPA-RLAKLVSDKVITPQSEIPALYPKTGGNLHCFTA--LT-PCAVLDILSPPYKESVGRSCSYY
At2g42670  SQEA-----RPAKLVKDTEMTAQSPVTTLYPKSGGNIHCFKA--IT-HCAILDILAPPYSSEHDRHCTYF
At3g58670  LQA------RPAKLVKDIDMTSPSPATTLYPTTGGNIHCFKA--IT-HCAIFDILSPPYSSTHGRHCNYF
At5g15120  T--------RLAKLKVDSTFTAPCNASILYPEDGGNMHRFTA--IT-ACAVLDVLGPPYCNPEGRHCTYF
At5g39890  T--------RLAKVKVDSDFTAPCDTSILYPADGGNMHCFTA--KT-ACAVLDVIGPPYSDPAGRHCTYY
CoCdg1     ---------RTGEQLRCKKETPLYRDEVGYISDSIGLHKITNPLKDEISVSLHLYTPPYAALYG--CSMY
MmAEDO     LQPLEREAVRPGVLRSRAEYTEASGPCVLTPHR-DNLHQIDA--VDGPAAFLDILAPPYDPEDGRDCHYY

                  290       300       310       320       330
At1g18490  MDYPFSTFALENGMKKVDEGKEDEYAWLVQIDT-PDDLH-MRPGSYTGPTIRV
At2g42670  RKSRREDLP---GELEVDGEVVTDVTWLEEFQP-PDDFV-IRRIPYRGPVIRT
At3g58670  RKSPMLDLPG--EIEVMNGEVISNVTWLEEYQP-PDNFV-IWRVPYRGPVIRK
At5g15120  LEFPLDKLSSEDDDVLSSEEEKEGYAWLQERDDNPEDHTNVVGALYRGPKVED
At5g39890  FDYPFSSFSV--DGVVVAEEEKEGYAWLKEREEKPEDLT-VTALMYSGPTIKE
CoCdg1     EAS---------NGRKHHVDMSKYYSWQGELVNESHSST-C------------
MmAEDO     RVVE----PIRPKEASGSACDLPREVWLLETPQ-ADDFW-CEGEPYPGPKVLP
```

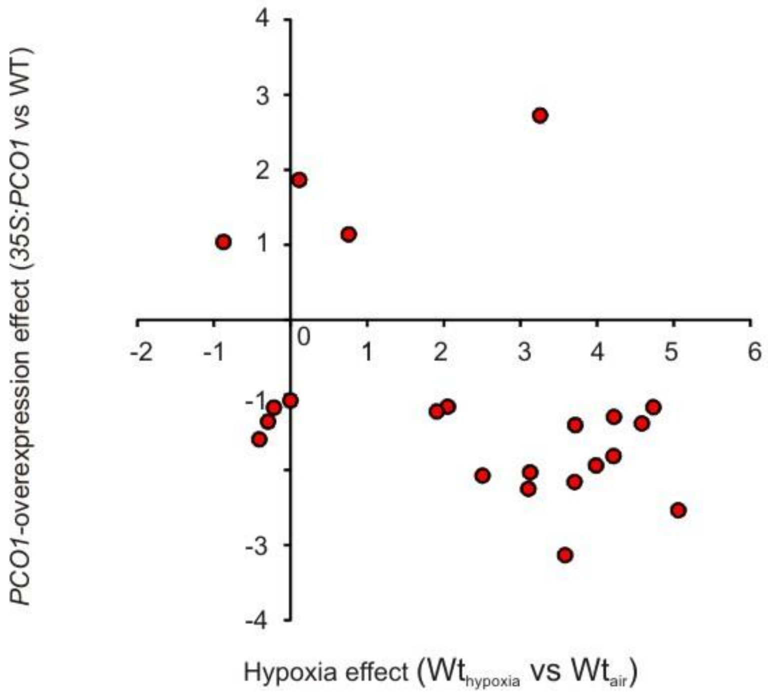
Supplementary Figure 1. Sequence comparison of thiol oxidase-like proteins. Multialignment of amino acidic sequences of *thiol*-oxidases from *Arabidopsis thaliana* (At1g18490, At2g42670, At3g58670, At5g15120 and At5g39890), *Mus musculus* (Cysteamine dioxygenase MmAEDO) and *Candida orthopsilosis* (Cysteine dioxygenase CoCDG). The alignment was performed using the Muscle algorithm[1]. The intensity of the blue-shadow is proportional to the conservation of residues in specific positions.



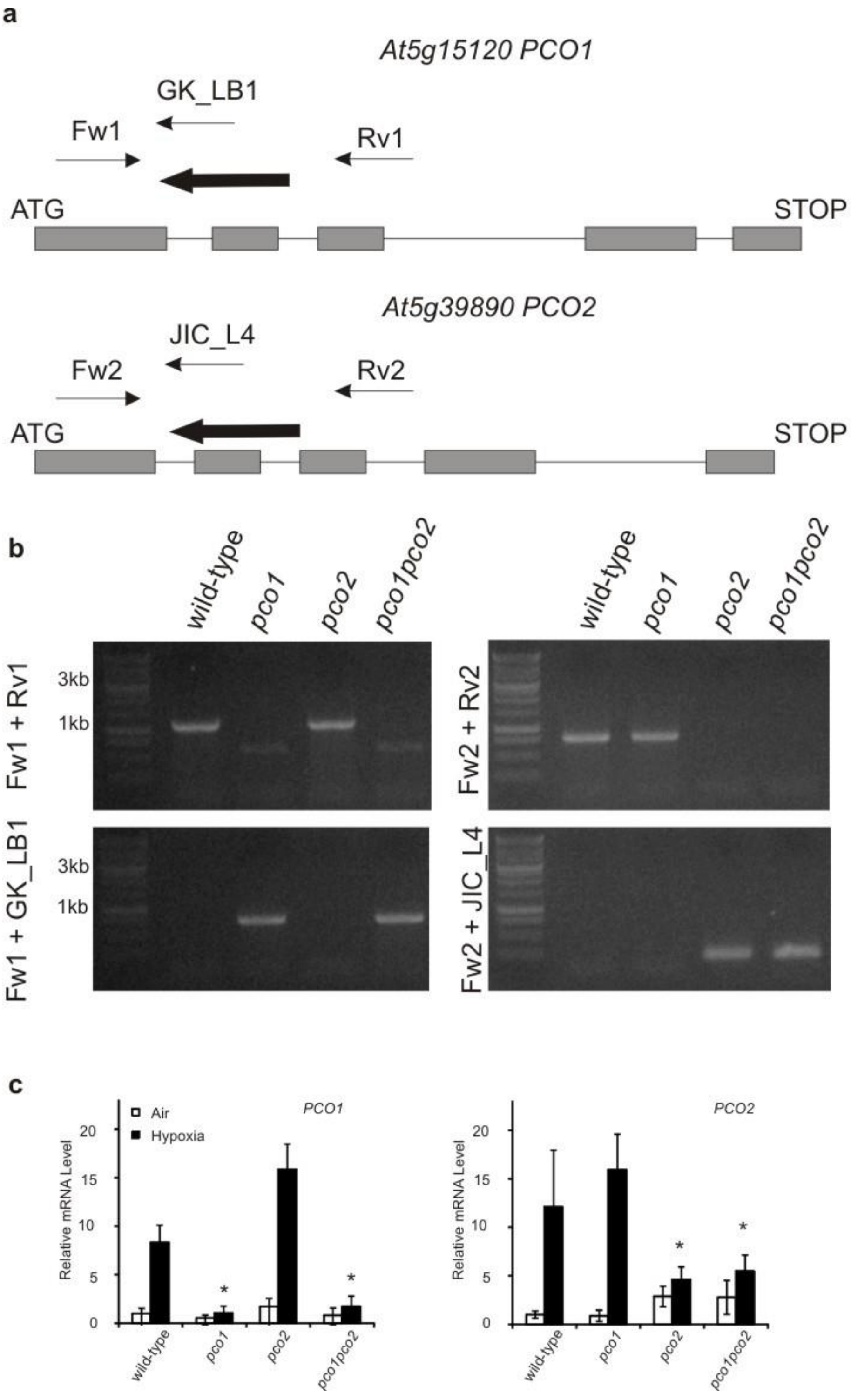
Supplementary Figure 2. Biochemical properties of the PCO1 enzyme. (a) Oxygen consumption rate and position specificity of PCO1 at different oxygen concentrations. A purified PCO1 protein was incubated with a CGGAI pentapeptide as the substrate and in presence of co-factors as described in the Materials and Methods section. The oxygen level in the solution was adjusted by flushing a gas mixture at the desired O_2 concentration. The oxygen tension was monitored by an optical phosphorescent sensor (Presens, Germany). Data are presented as mean ± s.d. (n = 4) Letters indicate statistically significant difference ($P<0.05$, one-way ANOVA). (b) Oxygen consumption by PCO1 incubated with three pentapetides differing for the position of a Cys residue: <u>C</u>GGAI, GG<u>C</u>AI or GGAI<u>C</u>. Data are presented as mean ± s.d. (n = 4). Asterisks indicate statistically significant difference ($P<0.05$, one-way ANOVA).



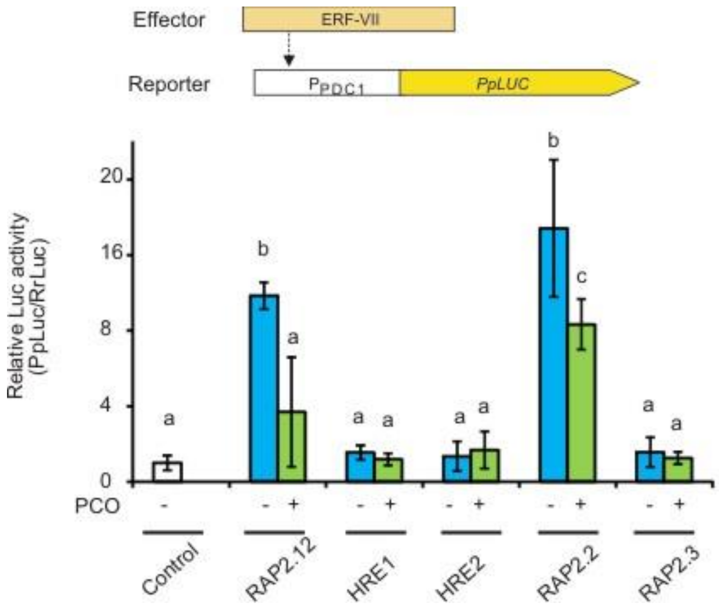
Supplementary Figure 3. *PCO1* and *PCO2* are targets of RAP2.12. (a) Relative mRNA level of *PCO1* (*At5g15120*) and *PCO2* (*At5g39890*) in leaves of wild-type and *35S:HA:RAP2.12* plants. These transgenic plants carry a chimeric version of the anaerobic activator RAP2.12 that is stabilized independently of the oxygen concentration, as the Cys in penultimate position is shifted by 18 positions due to the insertion of an N-terminal HA-epitope followed by a gateway linker. Both *PCO1* and *PCO2* are up-regulated as consequence of RAP2.12 stabilization. Data are obtained from the microarray dataset deposited at the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo; accession number: GSE29187). *adj. P<0.05 (with a Benjamini–Hochberg P-value correction). **(b)** Relative position of ATCTA elements, a DNA element putatively recognized by RAP2.12[2], is shown in the upstream sequence of *PCO1* and *PCO2* (gray: intergenic region, yellow: untranslated region)



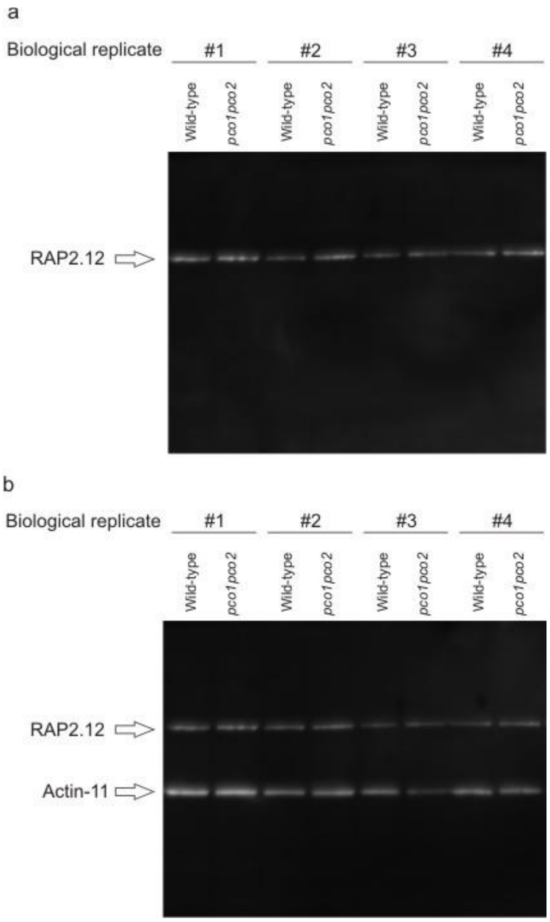
Supplementary Figure 4. Correlation between differential gene expression as induced in wild-type plants by hypoxia and overexpression of *PCO1* in hypoxia. Microarray comparison of wild type plants treated with hypoxia (control treatment: wild-type plants in air, average of all the experiments carried out with hypoxic conditions present in the Genvestigator[3] database) and constitutive overexpression of *PCO1* in hypoxia (PCO1 hypoxia: wild-type hypoxia). Red dots represent genes that are significantly (adj. P-value<0.05) up- or downregulated by *PCO1* overexpression. A full list of the differentially expressed genes is provided in Supplementary Table 4. The raw data of the microarrays can be downloaded from the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo; accession number: GSE44343).



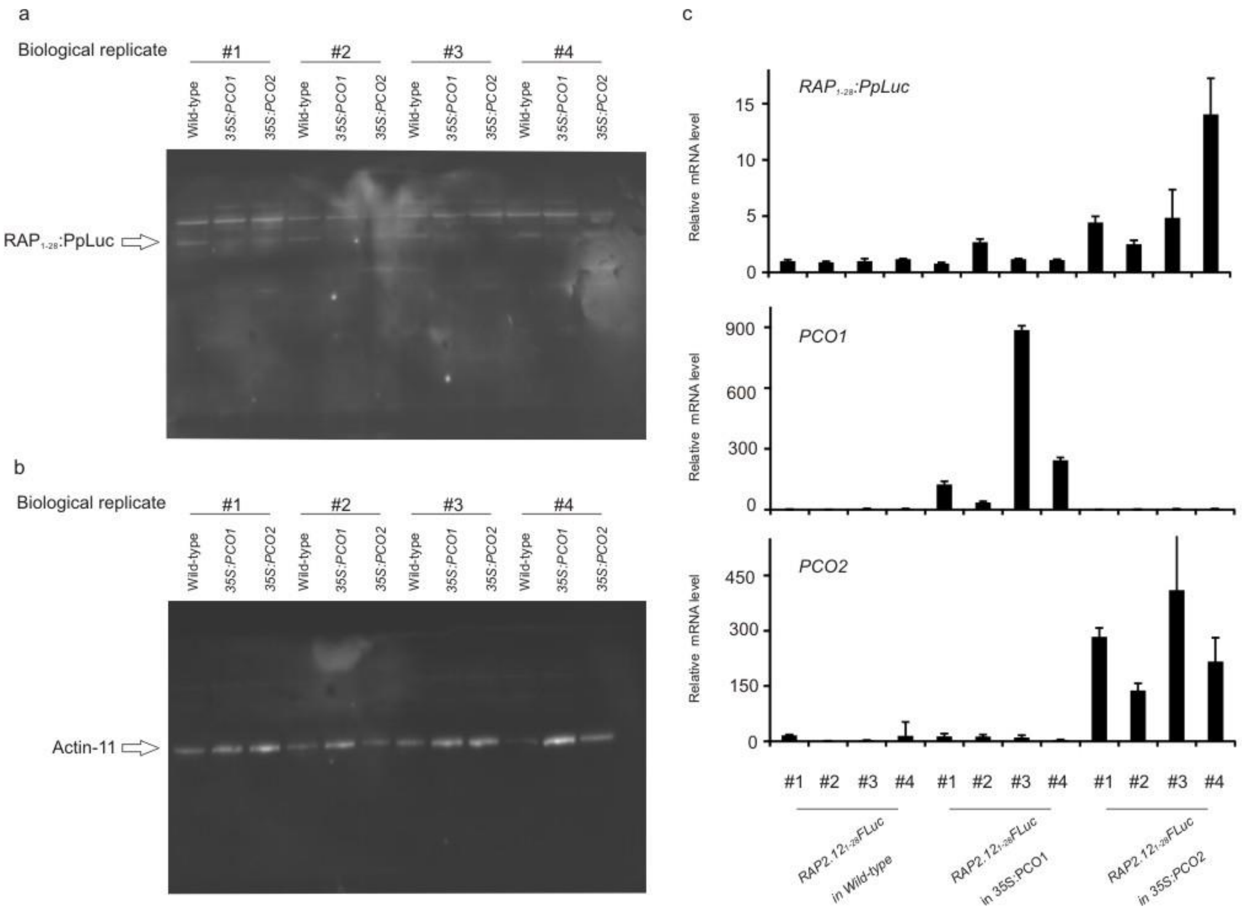
Supplementary Figure 5. Genotypization of *pco1, pco2* and *pco1pco2* double mutants. (a) Position of the insert of the *pco1* and *pco2* T-DNA insertion lines and the location of the annealing sequence of the primers used in PCR. **(b)** The result of a PCR screening is displayed, as analyzed using gel electrophoresis. **(c)** mRNA levels of *PCO1* (left) and *PCO2* (right) in wild-type, *pco1* (N451210), *pco2* (N116554) and *pco1pco2* double knock-out mutants under aerobic and hypoxic (5% O_2, 3h) is shown. Data are presented as mean ± s.d. (n=4) and referred to the aerobic wild-type samples (wt, air = 1), $^*P<0.05$, two-way ANOVA.



Supplementary Figure 6. Transactivation assay investigating the effect of PCO on ERF-VII mediated activation of the *PDC1* promoter driving expression of *Photinus pyralis Luciferase* (*PpLuc*). PCO reduced the transactivation activity of RAP2.12 and RAP2.2 on the *PDC1* promoter, but had no effect on HRE1, HRE2 and RAP2.3. Differently from RAP2.2 and RAP2.12, the hypoxia responsive ERFs (HRE1 and HRE2[4]) and RAP2.3 could not alter significantly the expression of the reporter gene, suggesting that either the HREs and RAP2.3 are more sensitive to the N-end rule mediated degradation or their activity is lower than the one of RAP2.2 and RAP2.12. Values are expressed relatively to the Control, set as 1. Data are presented as mean ± s.d. (n=4), letters indicate statistically significant significance P<0.05, one-way ANOVA.



Supplementary Figure 7. Original blots from which portions are shown in figure 5d. (a) Immunodetection of the RAP2.12 protein in wild-type and *pco1pco2* plants using a polyclonal anti-RAP2.12 antibody, as indicated by the white arrow. The band corresponding to RAP2.12 has an apparent molecular mass of about 65 kD, whereas the calculated molecular mass of AtRAP2.12 is only 39.8 kD. This discrepancy is in agreement with what observed for RAP2.2 by Welsh et al. [5]**(b)** Immunodetection of Actin-11 using a monoclonal antibody. Four biological independent replicates for each genotype were tested. The second biological replicate was used as representative examples in Figure 5d.



Supplementary Figure 8. Original blots from which portions are shown in figure 5f. (a) Immunodetection of the RAP$_{1\text{-}28}$:PpLuc protein stably expressed in wild-type, *35S:PCO1* and *35S:PCO2* plants using a polyclonal anti-PpLuc antibody, as indicated by the white arrow. **(b)** Immunodetection of Actin-11 using a monoclonal antibody. Four biological independent replicates (#1-#4) were tested. The first biological replicate was used as representative examples in Figure 5f. **(c)** mRNA levels of *RAP2.12* $_{1\text{-}28}$ *FLuc*, *PCO1* and *PCO2* in wild-type and *35S:PCO* plants transformed with *35S:RAP2.12* $_{1\text{-}28}$ *FLuc*. Data are presented as mean ± s.d. (n=3) and referred to the first *RAP2.12* $_{1\text{-}28}$ *FLuc* in wild-type sample.

Supplementary Figure 9. Phenotypic alteration caused by *RAP2.12* overexpression in a *pco1pco2* mutant. Phenotypes caused by the ectopic expression of a wild-type version of RAP2.12 in the *pco1pco*2 double mutant and its comparison with those due to ectopic expression of a stabilized version of RAP2.12[2] (obtained by deletion of the first 13 amino acid) and *pco1pco2* double mutants. In *pco1pco2* plants supertransformed with *35S:RAP2.12* growth is delayed, leaf size and number is decreased, and leaves tend to bend laterally, similar to what was observed in *35S:Δ13RAP2.12* plants.

Supplementary Tables

		AGI code				
		AT5G15120	*AT5G39890*	*AT1G18490*	*AT2G42670*	*AT3G58670*
		PCO1	*PCO2*	*PCO3*	*PCO4*	*PCO5*
Developmental stage	germinated seed	12.60	12.02	11.10	10.50	11.40
	seedling	10.94	10.54	10.97	10.40	11.02
	young rosette	10.37	9.96	10.95	10.41	10.64
	developed rosette	9.48	9.34	11.25	10.62	11.00
	bolting	10.77	10.27	11.24	10.61	10.75
	young flower	9.80	9.45	11.08	10.60	10.75
	developed flower	10.16	9.71	11.18	10.48	10.91
	flowers and siliques	10.02	9.58	11.16	10.77	10.72
	mature siliques	12.02	12.36	11.47	10.31	10.68
	senescence	10.92	11.32	12.20	10.76	12.33

Supplementary Table 1. *PCO* mRNA is high throughout the entire plant's lifecycle. The average mRNA abundance of *PCO1* (*At5g15120*), *PCO2* (*At5g39890*), *PCO3* (*At1g18490*), *PCO4* (*At2g42670*) and *PCO5* (*At3g58670*), expressed in log2 scale, was measured in various tissues and at different developmental stages using Affymetrix ATH1:22K microarrays. Data were retrieved from the Genevestigator webtool [3] on March 11th, 2013.

Affy ID	**AGI ID**	**Annotation**	**FC in air (*35S:PCO1* VS WT)**	**adj.P Val**	**Hypoxia effect**	**Germination**
250152_at	*At5g15120*	PCO1	22.69	0.00	**9.06**	0.17
262719_at	*At1g43590*	Transposable element gene	3.51	0.00	1.05	0.61
260181_at	*At1g70710*	Glycosyl hydrolase 9B1	0.49	0.01	0.86	**5.75**
247151_at	*At5g65640*	beta HLH protein 93	0.49	0.01	**2.08**	1.91
255149_at	*At4g08150*	KNOTTED-like 1	0.48	0.05	0.89	**3.73**
264953_at	*At1g77120*	Alcohol dehydrogenase 1	0.48	0.00	**15.55**	0.11
263096_at	*At2g16060*	Hemoglobin 1	0.48	0.03	**8.09**	0.21
251886_at	*At3g54260*	TRICHOME BIREFRINGENCE-LIKE 36	0.46	0.02	1.04	1.69
266223_at	*At2g28790*	Pathogenesis-related thaumatin	0.45	0.02	0.85	0.61
260869_at	*At1g43800*	Plant stearoyl-acyl-carrier-protein	0.44	0.04	**11.97**	**20.13**
252612_at	*At3g45160*	Putative membrane lipoprotein	0.44	0.03	1.20	1.45
257066_at	*At3g18280*	Seed storage 2S albumin	0.44	0.02	1.02	**10.75**
261826_at	*At1g11580*	Methylesterase PCR A	0.40	0.00	**2.25**	**2.26**
258487_at	*At3g02550*	LOB domain-containing protein 41	0.39	0.01	**12.61**	1.01
245688_at	*At1g28290*	Arabinogalactan protein 31	0.39	0.01	0.94	**48.48**

261335_at	*At1g44800*	Nodulin MtN21	0.39	0.03	0.69	**17.04**
265588_at	*At2g19970*	Cysteine-rich secretory protein	0.37	0.02	0.91	1.12
265948_at	*At2g19590*	ACC oxidase 1	0.35	0.04	**5.87**	**12.81**
255517_at	*At4g02290*	Glycosyl hydrolase 9B13	0.32	0.01	0.87	**2.29**
257952_at	*At3g21770*	Peroxidase superfamily protein	0.29	0.02	0.86	**18.82**
265117_at	*At1g62500*	Seed storage 2S albumin	0.16	0.01	0.89	**2.17**

Supplementary Table 2. List of genes that are differentially regulated by PCO1 overexpression in air and the effect of hypoxia on their expression in wild-type plants. The “Hypoxia-effect” value is calculated as the average of the effect caused by the treatment defined as “hypoxia” in the Genevestigator database[3] on March 11th 2013. The “Germination effect”value is the difference in expression of displayed genes in the comparison 24h germination against desiccation downloaded from the Genevestigator database[3] on March 11th 2013.

	Number of differentially expressed genes (35S:PCO1 vsWildtype)	% differentially expressed genes (35S:PCO1 vsWildtype)
Total	21	100%
Up-regulated	2	10%
Down-regulated	19	90%
Hypoxia induced	8	38%
Germination induced	11	52%
Hypoxia & Germination	3	14%

Supplementary Table 3. Absolute and relative number of genes that are differentially regulated by overexpressing PCO1 in air and the effect of hypoxia and germination on their expression. The hypoxia effect is calculated as average of the effect caused by treatment defined as “hypoxia” in the Genevestigator database[3] on March 11th 2013. The “Germination effect”value is the difference in expression of displayed genes in the comparison 24h germination against desiccation downloaded from the Genevestigator database[3] on March 11th 2013.

ID	**AGI ID**	**Annotation**	**FC in hypoxia (35S:PCO1 VS WT)**	**adj.P Val**	**Hypoxia effect**
250152_at	At5g15120	Protein of unknown function (DUF1637)	6.60	0.00	**9.57**
262719_at	At1g43590	Transposable element gene	3.63	0.04	1.08
259037_at	At3g09350	Fes1A	2.20	0.01	1.70
245325_at	At4g14130	Xyloglucanendotransglucosylase/hydrolase 15	2.05	0.02	0.55
247224_at	At5g65080	AGL68 MAF5 MADS-box transcription factor	0.47	0.01	1.00
253404_at	At4g32840	Phosphofructokinase 6	0.45	0.00	**4.16**
253416_at	At4g33070	Pyruvate decarboxylase 1	0.45	0.00	**26.69**
251065_at	At5g01870	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin	0.44	0.01	0.86
245951_at	At5g19550	Aspartate aminotransferase 2	0.43	0.00	**3.76**
259879_at	At1g76650	Calmodulin-like 38	0.41	0.00	**18.66**
265117_at	At1g62500	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin	0.39	0.00	0.82
258930_at	At3g10040	sequence-specific DNA binding transcription factors	0.38	0.00	**24.00**
257153_at	At3g27220	Galactose oxidase	0.38	0.00	**13.16**
254327_at	At4g22490	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin	0.33	0.01	0.75
264846_at	At2g17850	Rhodanese/Cell cycle control phosphatase	0.28	0.01	**18.59**
264953_at	At1g77120	Alcohol dehydrogenase 1	0.26	0.00	**15.89**

261567_at	At1g33055	unknown protein	0.24	0.00	**8.76**
260668_at	At1g19530	unknown protein	0.24	0.02	**5.68**
258487_at	At3g02550	LOB domain-containing protein 41	0.22	0.00	**13.06**
263096_at	At2g16060	Non symbiotic hemoglobin 1	0.21	0.00	**8.60**
255807_at	At4g10270	Wound-responsive family protein	0.17	0.00	**33.48**
260869_at	At1g43800	Plant stearoyl-acyl-carrier-protein desaturase	0.11	0.00	**11.99**

Supplementary Table 4. List of genes that are differentially regulated by PCO1 overexpression under hypoxia (4h, 5% O_2 V/V in air) as compared to wild-type plants and the effect of hypoxia on their expression in wild-type plants. Hypoxia-effect is calculated as average of the effect caused by treatment defined as “hypoxia” in the Genevestigator database[3] on March 11th 2013.

Normoxia	*Wt*		*35S:PCO1*			*35S:PCO2*		
	Value	**st.Dev.**	**Value**	**st.Dev.**	**P-value<0.05**	**Value**	**st.Dev.**	**P-value<0.05**
PCO1	1.00	0.33	105.89	68.01	yes	0.06	0.02	no
PCO2	1.00	0.93	0.28	0.29	no	511.81	193.38	yes
ADH	1.00	0.31	0.44	0.15	no	0.47	0.54	no
HB1	1.00	0.00	0.59	0.19	yes	0.49	0.36	yes
PDC1	1.00	0.43	1.22	1.08	no	1.64	0.98	no
At3g10040	1.00	0.26	1.46	0.48	no	0.64	0.21	no
HUP9	1.00	0.27	3.41	4.23	no	0.87	0.34	no
LBD41	1.00	0.25	0.48	0.45	no	0.76	0.22	no
HUP7	1.00	0.76	0.34	0.35	no	0.27	0.12	no
SUS1	1.00	0.45	4.65	2.02	yes	0.64	0.44	no
SUS4	1.00	0.55	3.35	2.51	no	0.73	0.37	no

Hypoxia	*Wt*		*35S:PCO1*			*35S:PCO2*		
	Value	**st.Dev.**	**Value**	**st.Dev.**	**P-value<0.05**	**Value**	**st.Dev.**	**P-value<0.05**
PCO1	17.27	2.65	134.48	96.42	yes	1.25	0.62	no
PCO2	6.52	1.51	0.31	0.02	no	1052.21	285.23	yes
ADH	22.73	5.22	1.75	1.46	yes	1.79	0.58	yes
HB1	15.91	4.95	1.40	0.97	yes	0.17	0.07	yes
PDC1	27.74	7.75	4.16	2.88	yes	1.93	1.05	yes
At3g10040	18.38	6.20	3.75	2.63	yes	4.02	5.35	yes
HUP9	297.37	177.13	34.13	26.83	yes	0.55	0.37	yes
LBD41	31.34	4.62	1.09	0.41	yes	0.38	0.49	yes
HUP7	14.39	8.44	1.19	0.90	yes	0.32	0.26	yes
SUS1	4.06	2.20	1.96	1.72	no	3.05	3.04	no
SUS4	5.25	4.03	1.00	0.77	yes	0.69	0.20	yes

Anoxia	*Wt*		*35S:PCO1*			*35S:PCO2*		
	Value	**st.Dev.**	**Value**	**st.Dev.**	**P-value<0.05**	**Value**	**st.Dev.**	**P-value<0.05**
PCO1	41.77	2.76	24.20	15.07	no	44.98	9.33	no
PCO2	61.25	15.76	33.31	10.45	no	58.62	13.51	yes
ADH	106.76	32.80	89.96	12.08	no	121.51	8.33	no
HB1	207.23	31.16	203.87	39.25	no	179.13	25.98	no
PDC1	167.13	15.96	121.09	9.82	no	221.75	22.20	no
At3g10040	28.62	3.40	34.69	7.71	no	32.87	7.08	no
HUP9	2951.33	534.81	2071.19	820.78	no	3128.07	365.31	no
LBD41	100.63	4.94	149.09	65.65	no	111.66	36.72	no
HUP7	10.17	2.42	16.96	5.01	no	17.82	5.59	no
SUS1	40.80	23.78	27.74	22.24	no	29.21	18.36	no
SUS4	643.72	250.13	382.29	257.03	no	578.46	262.76	no

Supplementary Table 5. Comparison of the mRNA levels of hypoxia responsive genes in wild-type, *35S:PCO1*, *35S:PCO2* under aerobic conditions and after 4h hypoxia (5% O_2 V/V in air) in the dark. Data are means relative to the reference (WT, air) ± s.d. for n=5 (one-way ANOVA test). Two independent transgenic lines for each construct were used.

	wt			***ate1ate2***			***prt6***		
	Value	**St.Dev.**		**Value**	**St.Dev.**	**P-value<0.05**	**Value**	**St.Dev.**	**P-value<0.05**
PCO1	1.00	0.57		106.27	61.66	no	190.38	88.41	yes
ADH	1.00	0.35		2.08	1.11	no	2.65	0.70	yes
HB1	1.00	0.54		5.32	2.20	yes	7.21	2.90	yes
PDC1	1.00	0.53		2.72	1.40	no	2.34	1.01	no
At3g10040	1.00	0.24		3.55	1.41	yes	4.66	1.47	yes
LBD41	1.00	0.17		55.33	30.52	yes	76.52	45.63	yes
HUP7	1.00	0.40		139.55	62.10	no	557.26	217.12	yes

	35S:PCO1			***35S:PCO1 ate1ate2***			***35S:PCO1 prt6***		
	Value	**St.Dev.**		**Value**	**St.Dev.**	**P-value<0.05**	**Value**	**St.Dev.**	**P-value<0.05**
PCO1	537.33	176.46	yes	199.97	40.18	yes	251.61	56.82	yes
ADH	1.25	0.62	no	2.70	0.78	yes	2.03	0.82	no
HB1	2.12	1.51	no	6.54	2.14	yes	3.37	0.53	no
PDC1	0.94	0.50	no	4.45	1.91	yes	2.98	2.11	no
At3g10040	1.20	0.36	no	4.01	1.42	yes	3.31	0.31	yes
LBD41	1.15	0.68	no	64.12	21.41	yes	78.02	35.47	yes
HUP7	2.49	1.95	no	553.45	196.87	yes	296.85	122.14	yes

Supplementary Table 6 Comparison of the mRNA levels of hypoxia responsive genes in wild-type, *prt6, ate1ate2, 35S:PCO1, 35S:PCO1* in the *ate1ate2* background and *35S:PCO1* in the *prt6* background. Four-week old plants grown in soil were used. Data are means relative to the reference (WT) ± s.d. for n=4 (one-way ANOVA test).

Air

	wt		***pco1***			***pco2***			***pco1pco2***		
	Value	**st.Dev.**	**Value**	**st.Dev.**	**P-value<0.05**	**Value**	**st.Dev.**	**P-value<0.05**	**Value**	**st.Dev.**	
PCO1	1.00	0.53	0.54	0.28	no	1.72	0.82	no	0.81	0.75	no
PCO2	1.00	0.38	0.87	0.58	no	2.87	1.06	yes	2.77	0.87	yes
ADH	1.00	0.48	1.60	1.46	no	2.02	1.03	no	5.32	1.52	yes
HB1	1.00	0.33	1.46	2.12	no	3.71	2.30	no	8.26	0.40	yes
PDC1	1.00	0.56	1.51	1.25	no	3.25	2.15	no	3.85	1.50	no
At3g10040	1.00	0.20	1.71	1.28	no	2.78	1.10	no	9.82	3.85	yes
HUP9	1.00	0.30	1.82	0.45	no	3.00	1.38	no	57.81	36.55	yes
LBD41	1.00	0.15	1.17	0.86	no	1.17	0.40	no	3.75	1.51	yes
HUP7	1.00	0.81	0.87	1.48	no	7.42	6.44	no	16.52	9.20	yes
SUS1	1.00	0.40	0.69	0.39	no	1.18	0.66	no	3.07	0.20	yes
SUS4	1.00	0.69	0.97	0.94	no	1.11	0.62	no	3.53	0.27	yes

Hypoxia

	wt		***pco1***			***pco2***			***pco1pco2***		
	Value	**st.Dev.**	**Value**	**st.Dev.**	**P-value<0.05**	**Value**	**st.Dev.**	**P-value<0.05**	**Value**	**st.Dev.**	
PCO1	8.33	0.21	1.05	0.71	yes	15.86	2.61	yes	1.74	1.04	yes
PCO2	12.10	0.48	15.94	3.65	no	4.60	1.30	yes	5.49	1.65	yes

ADH	16.71	0.39	23.47	3.47	no	29.34	11.02	no	57.27	20.71	yes
HB1	6.56	0.16	6.70	0.89	no	6.52	2.48	no	8.99	3.47	no
PDC1	15.48	0.33	20.56	4.56	no	39.53	9.86	yes	20.71	4.47	no
At3g10040	11.07	0.17	20.88	3.53	no	38.63	8.06	yes	71.76	12.10	yes
HUP9	111.85	0.35	141.27	29.08	no	216.38	69.37	no	258.91	103.44	yes
LBD41	11.74	0.17	18.60	1.77	no	27.30	5.43	yes	44.59	15.09	yes
HUP7	22.28	0.69	24.94	7.35	no	27.78	21.49	no	68.60	25.74	yes
SUS1	4.27	0.36	5.01	1.10	no	5.31	2.05	no	7.40	2.52	no
SUS4	6.74	0.62	11.87	6.63	no	9.49	4.30	no	8.90	2.96	no

Supplementary Table 7. Comparison of the mRNA levels of hypoxia responsive genes in wild-type, *pco1*, *pco2* and double *pco1pco2* ko plants under aerobic conditions and after 4h hypoxia (5% O_2 V/V in air) in the dark. Data are means relative to the reference (WT, air) ± s.d. for n=4 (one-way ANOVA test).

Final Construct	Original Destination Vector	Reference
DEST-PCO1	pDEST17	Life technologies manual
DEST-PCO2	pDEST17	Life technologies manual
35S:GFP:PCO1	pK7WGF2	6
35S:GFP:PCO2	pK7WGF2	6
P_{PCO1}:GUSGFP	pKGWFS7	6
P_{PCO2}:GUSGFP	pKGWFS7	6
35S:PCO1	pEARLEYGATE202	7
35S:PCO2	pEARLEYGATE202	7
35S:RAP2.12	p2GW7	6
P_{PDC1}:PpLuc	pGWL7	8
small35S:PCO1	p2GW7	6
small35S:PCO2	p2GW7	6
RAP:G4D	p2GW7	6
(C2A)RAP:G4D	p2GW7	6
UAS:PpLuc	pGreenII-800Luc	9
RAPΔ33:RLuc	p2GW7	6
G4D:PCO1	p35S-GBD-GW	10
G4D:PCO2	p35S-GBD-GW	10
small35S:HRE1	p2GW7	8
small35S:HRE2	p2GW7	8
small35S:RAP2.2	p2GW7	6
small35S:RAP2.3	p2GW7	6
small35S:RAP2.12	p2GW7	6
small35S:PpLuc	p2GW7	6
small35S:RrLuc	p2GW7	6

Supplementary Table 8. Full list of the vectors used with references

Primer name	**Sequence 5'-->3'**
ins_PCO1fw	AATGGTGGTCCTGGTGTTATTC
ins_PCO1rv	GCAAGGTAACAACGACAAACAA
LB1_GK	ATATTGACCATCATACTCATTGC
ins_PCO2fw	TGTTCTTTTGCCCTCTTCTCTC
ins_PCO2rv	TCCGGGTGATGTACAAATACAA
dspm5	CGGGATCCGACACTCTTTAATTAACTGACACTC
gwPCO1fw	CACCATGGGGTTTGAGATGAAACC
gwPCO1rv	TCAATCTTCAACCTTTGGGCCT
gwPCO2fw	CACCATGGGAACTGATACAGTTAT
gwPCO2rv	TCATTCTTTGATGGTTGGTCC
gwPPDC1fw	CACCCTTGAATTACACTA
gwPPDC1rv	GGAAATGGGAAGGTTTGAGTT
gwPPCO1fw	CACCAAAATTTCTTTTAATTTACTATTGATG
gwPPCO1rv	TCTCTGGTTTCATCTCAAACCCCAT
gwPPCO2fw	CACCTTTTATCCATTTTTTCACAAC
gwPPCO2rv	CAGACATAACTGTATCAGTTCCCAT
gwRAP2.12fw	CACCATGTGTGGAGGAGCTATAAT
gwRAP2.12rv	TCAGAAGACTCCTCCAATCATGGAATG
gwC2ARAP2.12fw	CACCATGGCTGGAGGAGCTATAAT
RAPD33RLUCfw	AAGAAGAATTTGAAATGGCTTCGAAAGTTTAT
RAPD33RLUCrv	ATAAACTTTCGAAGCCATTTCAAATTCTTCTT
gwRAP2.3fw	CACCATGTGTGGCGGTGCTATTAT
gwRAP2.3rv	TTACTCATACGACGCAATGACATCA

Supplementary Table 9. List of primers used in this work (fw = forward primer, rv = reverse primer)

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