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Supplemental Figure S1. Relative metabolite contents of *pnp1-1* and WT grown under –P conditions for different time periods.

Supplemental Figure S2. Principal component analysis (PCA) of metabolite profiles.

Supplemental Table S1. Raw data for metabolite contents of *pnp1-1* and WT grown under +P or -P conditions, shown in Figures 7 and S1, respectively. See file Supplemental Table S1.xls.

Supplemental Table S2. Statistic analysis of metabolites data (PCA and 2 way ANOVA). See file Supplemental Table S2.xls.

Supplemental Figure S3. Pi uptake in *pnp1-1* and WT.

Supplemental Table S3. ATH1 microarray data for significantly regulated genes in the 3 hr P starvation experiment. See file Supplemental Table S3.xls.

Supplemental Table S4. ATH1 microarray data for significantly regulated genes in the one week P starvation experiment. See file Supplemental Table S4.xls.

Supplemental Table S5. Statistical significance of the functional categorization of regulated genes using the MapMan-defined BINs.

Supplemental Table S6. Genes encoding chloroplast-targeted proteins which are similarly regulated in *pnp1-1* +P vs. WT +P, and in WT -P vs. WT +P .

Supplemental Table S7. Normalized expression data for quantitative RT-PCR.

Supplemental Table S8. List of primers used for quantitative RT-PCR.

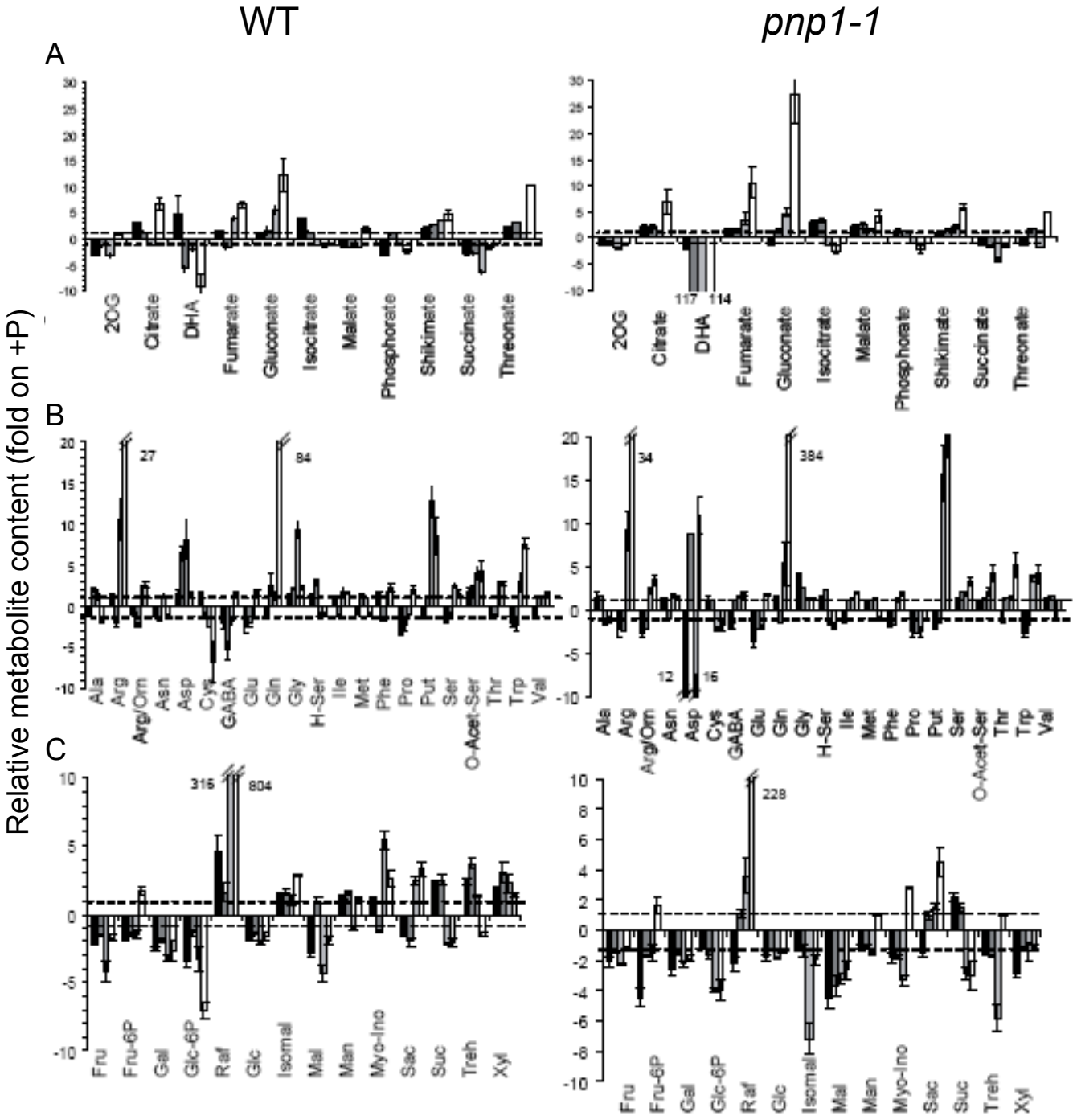

Figure S1. Relative content of *pnp1-1* and WT grown in the absence of phosphate for different time periods compared to +P conditions.
Metabolites were determined as described in Materials and Methods. Black and dark grey bars represent 3 and 6 hours of phosphate starvation; light grey and white bars represent 1 and 3 weeks of starvation. Data of amino acids (A), sugars (B) and organic acids (C) measurements are presented as average fold change ± SE normalized on +P values of each genotype, namely WT or *pnp1-1* seedlings grown in the presence of phosphate. Values are the mean of six independently sampled bulked seedlings. The data set and statistical significance are presented in Table S1.

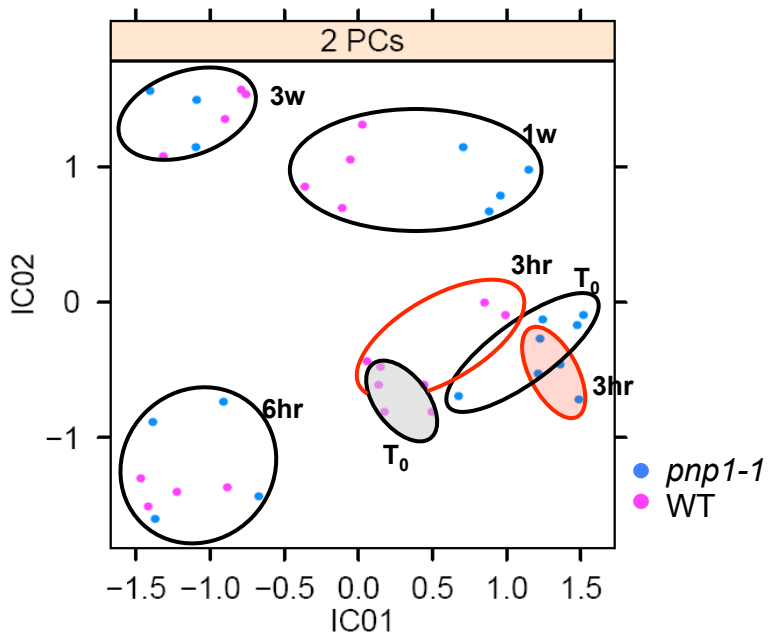

Figure S2. Principal component analysis of metabolite profiles.
WT (pink dots) and *pnp1-1* (blue dots) when grown with phosphate (T_0) and without phosphate for 3 hr, 6 hr, 1 week and 3 weeks. PCA is presented as the combinations of the first two dimensions which together comprise 65% of the metabolite variance. Each data point represents an independent sample. The analysis of the data was performed using MeV software (Saeed AI et al., 2003 or http://www.tm4.org/mev.html). Further analysis was performed using MetaGeneAlyse (Scholz, 2004) available at http://metagenealyse.mpimp-golm.mpg.de. Details of this analysis are presented in Table S2.

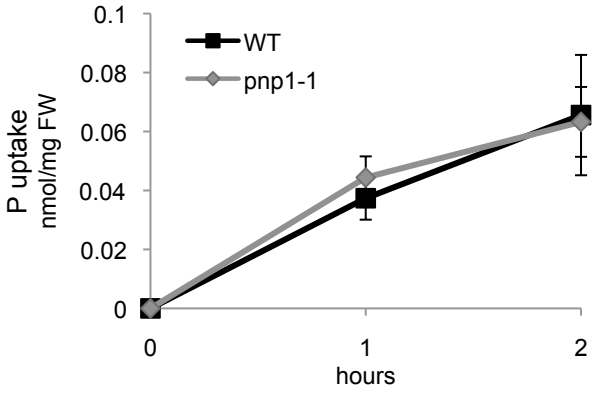

Figure S3. Pi uptake in *pnp1-1* and WT plants.
Seedlings were germinated and grown for 2 weeks on nylon meshes on the surface of semisolid MS medium containing 500 µM of Pi, 0.5% sucrose and 0.4% agar. For the experiment, meshes were rinsed once in the same liquid medium and then transferred onto fresh liquid medium containing 1 µCi/ml of ^{33}P as radiotracer. After one or two hours, plants were briefly rinsed twice in the same non-radioactive media and then blot-dried onto Whatman paper. Approximately ten plants were pooled, weighed and dried overnight at 65˚C in scintillation vials. The Pi uptake was calculated from scintillation counting. At least three replicates in two independent assays were averaged; error bars represent standard deviations. The method is based on the following publication:

Shin H, Shin HS, Dewbre GR, Harrison MJ (2004) Phosphate transport in *Arabidopsis*: Pht1;1 and Pht1;4 play a major role in phosphate acquisition from both low- and high-phosphate environments. Plant J. **39**: 629-642

pnp1-1 +P *vs* WT +P

bin[a]	name	elements	p-value
26	misc	1242	0
1	PS	162	3.48E-19
27	RNA	2318	7.53E-18
31	cell	637	1.95E-14
20	stress	756	1.18E-10
9	mitochondrial electron transport / ATP synthesis	116	1.48E-08
28	DNA	917	1.70E-08
16	secondary metabolism	345	6.07E-06
5	fermentation	13	6.79E-06
19	tetrapyrrole synthesis	43	7.22E-05
4	glycolysis	64	2.73E-04
8	TCA / org. transformation	72	0.007
10	cell wall	439	0.011
35	not assigned	7981	0.015
6	gluconeogenese/ glyoxylate cycle	10	0.021
2	major CHO metabolism	89	0.021
23	nucleotide metabolism	128	0.025
17	hormone metabolism	456	0.050
14	S-assimilation	13	0.093
29	protein	2997	0.104
3	minor CHO metabolism	122	0.121
11	lipid metabolism	339	0.164
7	OPP	31	0.166
24	Biodegradation of Xenobiotics	23	0.233
30	signalling	1095	0.310
12	N-metabolism	24	0.368
22	polyamine metabolism	14	0.557
34	transport	880	0.584
18	Co-factor and vitamine metabolism	42	0.600
32	micro RNA, natural antisense etc	44	0.606
15	metal handling	58	0.689
13	amino acid metabolism	212	0.770
33	development	556	0.789
21	redox.regulation	183	0.829
25	C1-metabolism	30	0.968

WT -P *vs* WT +P

bin[a]	name	elements	p-value
20	stress	756	0
26	misc	1242	0
1	PS	162	6.97E-24
29	protein	2997	1.23E-22
27	RNA	2318	1.12E-18
31	cell	637	1.20E-15
10	cell wall	439	9.82E-13
34	transport	880	1.16E-10
16	secondary metabolism	345	2.32E-10
35	not assigned	7981	3.05E-08
17	hormone metabolism	456	7.99E-08
4	glycolysis	64	8.43E-08
19	tetrapyrrole synthesis	43	1.81E-07
5	fermentation	13	2.69E-04
30	signalling	1095	5.20E-04
14	S-assimilation	13	6.82E-04
15	metal handling	58	0.001
28	DNA	917	0.001
25	C1-metabolism	30	0.014
9	mitochondrial electron transport / ATP synthesis	116	0.026
18	Co-factor and vitamine metabolism	42	0.043
6	gluconeogenese/ glyoxylate cycle	10	0.048
8	TCA / org. transformation	72	0.087
2	major CHO metabolism	89	0.132
7	OPP	31	0.184
24	Biodegradation of Xenobiotics	23	0.190
12	N-metabolism	24	0.196
32	micro RNA, natural antisense etc	44	0.284
11	lipid metabolism	339	0.286
3	minor CHO metabolism	122	0.293
21	redox.regulation	183	0.375
22	polyamine metabolism	14	0.462
33	development	556	0.546
23	nucleotide metabolism	128	0.620
13	amino acid metabolism	212	0.939

pnp1-1 -P *vs pnp1-1* +P

bin[a]	name	elements	p-value
20	stress	756	1.54E-05
4	glycolysis	64	6.71E-05
35	not assigned	7981	1.82E-04
26	misc	1242	2.81E-04
29	protein	2997	3.80E-04
8	TCA / org. transformation	72	3.12E-03
9	mitochondrial electron transport / ATP synthesis	116	0.009
30	signalling	1095	0.014
12	N-metabolism	24	0.046
23	nucleotide metabolism	128	0.046
19	tetrapyrrole synthesis	43	0.052
14	S-assimilation	13	0.058
34	transport	880	0.078
21	redox.regulation	183	0.099
25	C1-metabolism	30	0.107
28	DNA	917	0.140
1	PS	162	0.150
11	lipid metabolism	339	0.158
6	gluconeogenese/ glyoxylate cycle	10	0.201
15	metal handling	58	0.214
32	micro RNA, natural antisense etc	44	0.232
18	Co-factor and vitamine metabolism	42	0.258
16	secondary metabolism	345	0.442
10	cell wall	439	0.445
3	minor CHO metabolism	122	0.450
22	polyamine metabolism	14	0.597
27	RNA	2318	0.603
31	cell	637	0.634
5	fermentation	13	0.786
7	OPP	31	0.813
17	hormone metabolism	456	0.920
2	major CHO metabolism	89	0.930
33	development	556	0.939
13	amino acid metabolism	212	0.955
24	Biodegradation of Xenobiotics	23	0.987

pnp1-1 -P *vs* WT -P

bin[a]	name	elements	p-value
9	mitochondrial electron transport / ATP synthesis	116	6.50E-10
34	transport	880	1.80E-09
1	PS	162	6.92E-08
29	protein	2997	2.75E-07
10	cell wall	439	1.24E-05
2	major CHO metabolism	89	6.15E-05
7	OPP	31	6.17E-04
8	TCA / org. transformation	72	9.87E-04
28	DNA	917	0.004
17	hormone metabolism	456	0.011
20	stress	756	0.015
11	lipid metabolism	339	0.020
23	nucleotide metabolism	128	0.028
4	glycolysis	64	0.036
33	development	556	0.047
26	misc	1242	0.055
15	metal handling	58	0.073
14	S-assimilation	13	0.078
13	amino acid metabolism	212	0.092
24	Biodegradation of Xenobiotics	23	0.109
35	not assigned	7981	0.140
5	fermentation	13	0.147
6	gluconeogenese/ glyoxylate cycle	10	0.147
27	RNA	2318	0.226
30	signalling	1095	0.251
3	minor CHO metabolism	122	0.261
18	Co-factor and vitamine metabolism	42	0.394
22	polyamine metabolism	14	0.504
25	C1-metabolism	30	0.559
32	micro RNA, natural antisense etc	44	0.597
12	N-metabolism	24	0.683
31	cell	637	0.776
16	secondary metabolism	345	0.807
21	redox.regulation	183	0.817
19	tetrapyrrole synthesis	43	0.863

Table S5. Statistical significance of the functional categorization of the microarray data using MapMan-defined bins.

The full dataset of each comparison (*pnp1-1* +P relative to WT on +P, etc.) was loaded to perform this analysis. As described in the MapMan manual, results are based on the Wilcoxon Rank Sum Test, with the goal of predicting BINs that exhibit a different behavior in terms of expression profile compared to all the other remaining BINs. The lists here represent only the major bins. [a] Indicates the number associated with each bin (or functional category) in the MapMan program. The number of elements of each category and the p-values are also indicated.

Table S6. Genes encoding chloroplast-targeted proteins which are similarly regulated in *pnp1-1* +P vs. WT +P, and in WT -P vs. WT +P.

Probeset ID	AGI code	*pnp1-1*+P vs. WT+P		WT-P vs. WT+P		Description
		Fold Change	FDR	Fold Change	FDR	
250515_at	At5g09570	39.15	0.000	78.71	0.000	Expressed protein
265674_at	At2g32190	9.20	0.003	8.18	0.006	Expressed protein
262930_at	At1g65690	7.06	0.000	7.55	0.000	Harpin-induced protein-related
260399_at	At1g72520	6.98	0.002	3.03	0.035	Lipoxygenase
258182_at	At3g21500	5.82	0.000	8.75	0.000	1-deoxy-d-xylulose 5-phosphate synthase
250090_at	At5g17330	4.98	0.018	4.34	0.045	Glutamate decarboxylase 1 (GAD 1)
265670_s_at	At2g32210	4.81	0.005	3.27	0.029	Expressed protein
257174_at	At3g27190	3.72	0.015	6.69	0.004	Uracil phosphoribosyltransferase
264400_at	At1g61800	3.49	0.016	10.56	0.002	Glucose-6-phosphate/phosphate translocator
267592_at	At2g39710	3.12	0.005	2.80	0.014	Aspartyl protease family protein
252652_at	At3g44720	2.90	0.005	2.30	0.024	Prephenate dehydratase family protein
256793_at	At3g22160	2.71	0.005	2.36	0.013	VQ motif-containing protein
267496_at	At2g30550	2.68	0.009	2.12	0.044	Lipase class 3 family protein
260602_at	At1g55920	2.49	0.009	3.23	0.003	Serine O-acetyltransferase
254331_s_at	At4g22710	2.27	0.010	2.12	0.021	Cytochrome P450 family protein
246870_at	At5g26030	2.24	0.003	2.22	0.004	Ferrochelatase I
253830_at	At4g27652	2.10	0.006	2.05	0.008	Expressed protein
246517_at	At5g15760	0.46	0.006	0.47	0.009	Plastid-specific 30S ribosomal protein 3
248402_at	At5g52100	0.45	0.014	0.44	0.023	Dihydrodipicolinate reductase family protein
263136_at	At1g78580	0.45	0.027	0.43	0.035	Trehalose-6-phosphate synthase
246346_at	At3g56810	0.43	0.009	0.49	0.030	Expressed protein
259707_at	At1g77490	0.43	0.004	0.42	0.004	L-ascorbate peroxidase, thylakoid-bound (tapx)
259996_at	At1g67910	0.41	0.002	0.28	0.000	Expressed protein
261196_at	At1g12860	0.40	0.023	0.43	0.048	Basic helix-loop-helix (bHLH) family protein
249472_at	At5g39210	0.39	0.006	0.35	0.004	Expressed protein
266616_at	At2g29680	0.39	0.028	0.18	0.004	Cell division control protein CDC6
254954_at	At4g10910	0.32	0.020	0.25	0.011	Expressed protein
265312_at	At2g20240	0.29	0.001	0.29	0.001	Expressed protein
260770_at	At1g49200	0.21	0.003	0.15	0.001	Zinc finger (C3HC4-type RING finger) family protein
263981_at	At2g42870	0.10	0.003	0.17	0.010	Expressed protein

Table S7. Normalized expression data of the quantitative RT-PCR experiment.

Annotation	*PHR1*			*RNS1*			*PHT1;1*			*PHT1;4*		
AGI	At4g28610			At2g02990			At5g43350			At2g38940		
	qPCR fold	SEM	array fold	qPCR fold	SEM	array fold	qPCR fold	SEM	array fold	qPCR fold	SEM	array fold
WT +P	0.88	0.07	1.17	0.11	0.02	0.10	0.04	0.01	0.02	0.02	0.00	0.002
WT -P	0.95	0.07	1.00	0.84	0.08	0.63	0.64	0.06	0.68	0.79	0.09	0.76
pnp1-1 +P	0.84	0.06	1.10	0.33	0.08	0.38	0.13	0.02	0.13	0.04	0.00	0.02
pnp1-1 -P	1.00	0.06	1.00	1.00	0.06	1.00	1.00	0.11	1.00	1.00	0.07	1.00

Annotation	*JACALIN*			*RNaseH*			*PORA*			*PSBP2*		
AGI	At1g52100			At1g24090			At5g54190			At2g30790		
	qPCR fold	SEM	array fold	qPCR fold	SEM	array fold	qPCR fold	SEM	array fold	qPCR fold	SEM	array fold
WT +P	0.03	0.01	0.04	0.24	0.02	0.16	0.62	0.08	1.40	0.57	0.06	0.97
WT -P	0.04	0.01	0.05	0.40	0.05	0.18	1.00	0.11	1.00	1.00	0.09	1.00
pnp1-1 +P	1.00	0.09	1.00	0.88	0.07	1.12	0.08	0.01	0.13	0.15	0.01	0.13
pnp1-1 -P	0.64	0.08	0.86	1.00	0.06	1.00	0.09	0.01	0.13	0.18	0.02	0.21

Annotation	*At4*		*IPS1*	
AGI	At5g03545		At3g0922	
	qPCR fold	SEM	qPCR fold	SEM
WT +P	0.010	0.001	0.004	0.00
WT -P	1.00	0.06	1.00	0.12
pnp1-1 +P	0.009	0.001	0.007	0.00
pnp1-1 -P	0.45	0.03	0.74	0.14

Annotation	*PNP*			*IPS1*	
AGI	At3g03710			At3g0922	
	qPCR fold	SEM	array fold	qPCR fold	SEM
WT +P	1.00	0.15	1	0.000	0.000
WT -P	0.48	0.11	0.50	1.00	0.28
phr1-1 +P	0.99	0.16		0.000	0.000
phr1-1 -P	0.48	0.06		0.000	0.000

The fold change expression of at least 3 biological replicates (tested by at least 2 technical replicates for each) was normalized to *ACT2*. The data were adjusted to the sample with the highest expression level of each gene. SEM stands for standard error of the mean. The relative expression from the microarray experiment was calculated relative to the sample with the highest expression in the qRT-PCR assay and indicated for the relevant genes.

Table S8. Primers used for quantitative RT-PCR

AGI	Annotation	Forward primer (5'-3')	Reverse primer (5'-3')
At3g18780	*ACT2*	ATTCTTGCTTCCCTCAGCAC	CCCCAGCTTTTTAAGCCTTT
At4g28610	*PHR1*	AGTCTTGGCGGTAGTGAAAGAG	AGCGGTGTCAACTTCCTTTCTGG
At2g02990	*RNS1*	TGATGCCTCTAAACCATTCGAT	TACCATGCTTCTCCCATTCG
At5g43350	*PHT1;1*	AATTTCTCCTGCCAAGCTGA	AGGCATCGGTAAAGAAACCC
At2g38940	*PHT1;4*	TCAATGGCGTTGCCTTCTGT	ATCACCAAGCCACCCGAAA
At1g52100	Jacalin	TATATGTAGGACAAGGCGATGTTGG	AGCAATGTTTCCTTTCCATGTCTTT
At1g24090	RNase H	CGCCACATAGTCATACACCG	GGTCTCGGCTTTTACCATCA
At5g54190	*PORA*	AAGATTGTTGATTGATGACTTGAAGAA	AATGTATTAGTGTTTCCGGTTATGGAT
At2g30790	*PSBP2*	ATCGTCGTTTCGTCATGTGTCC	CAGCACCAACGAGTAAGGTAAGG
At5g03545	*At4*	CTGAAGCTCAAGAACCCTCTGAA	CCTCTCAAAACCCTTTATTGGTGA
At3g09922	*IPS1*	AGACTGCAGAAGGCTGATTCAGA	TTGCCCAATTTCTAGAGGGAGA
At3g03710	*PNP*	GGAGCGATTCTCAGCTGTAGGTC	GGCGTAGAGGTCTATCGATCAACC

Legends to Supplemental Data presented as separate files

Supplemental Table S1. Dataset of relative changes in metabolite levels of *pnp1-1* and WT under +P conditions and during P starvation (after three hours, six hours, one week and three weeks). Data are normalized with respect to the internal standard and the FW.

Supplemental Table S2. Metabolite two way ANOVA and PCA statistical details.

Supplemental Table S3. ATH1 microarray data for three hour P starvation experiment. The significantly regulated genes in the different comparisons are listed. The fold change was calculated as the ratio of mean of the signal intensities (≥ 2-fold change, FDR < 0.05).

Supplemental Table S4. ATH1 microarray data for the one week P starvation experiment. The significantly regulated genes in the different comparisons are listed. The fold change was calculated as the ratio of mean of the signal intensities (≥ 2-fold change, FDR < 0.05).