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online data

SUPPLEMENTARY ONLINE DATA

In vivo regulatory phosphorylation of the phosphoenolpyruvate carboxylase AtPPC1 in phosphate-starved *Arabidopsis thaliana*

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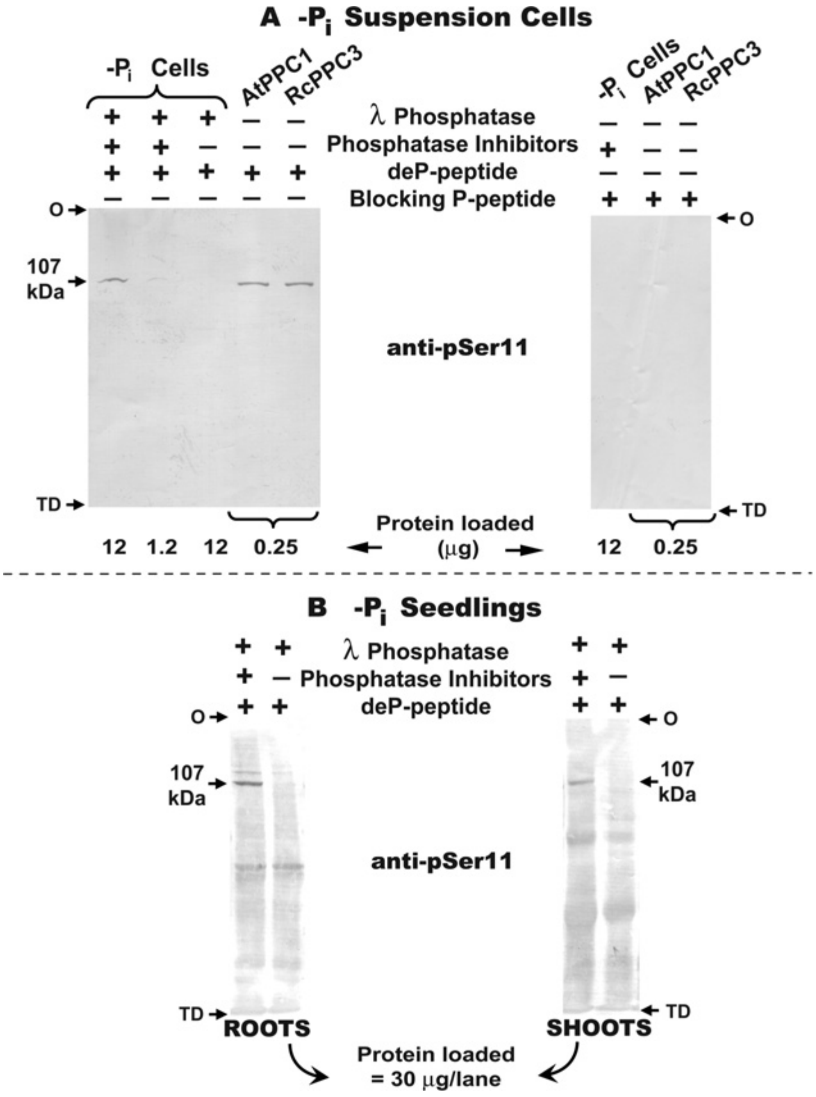

Figure S1 Anti-pSer-11 antibody specificity

Clarified extracts from 7-day-old $-\mathrm{P_i}$ suspension cells (**A**) or roots and shoots of 21-day-old $-\mathrm{P_i}$ seedlings (**B**) were incubated for 1 h with λ-phosphatase with or without protein phosphatase inhibitors as described in the Experimental section in the main article, subjected to SDS/PAGE and electroblotted on to a PVDF membrane. Immunoblots were probed with anti-pSer-11 IgG in the presence of 10 $\mu\mathrm{g} \cdot \mathrm{ml}^{-1}$ of the corresponding dephosphopeptide or blocking phosphopeptide (P-peptide) [28] as indicated. AtPPC1 and RcPPC3 denote homogeneous native Class 1 PEPC preparations isolated from the $-\mathrm{P_i}$ *Arabidopsis* suspension cells (see Table 1 and Figure 2 in the main article) or the endosperm of developing castor-oil seeds respectively [26]. deP-peptide, dephosphopeptide; O, origin; P-peptide, phosphopeptide; TD, tracking-dye front.

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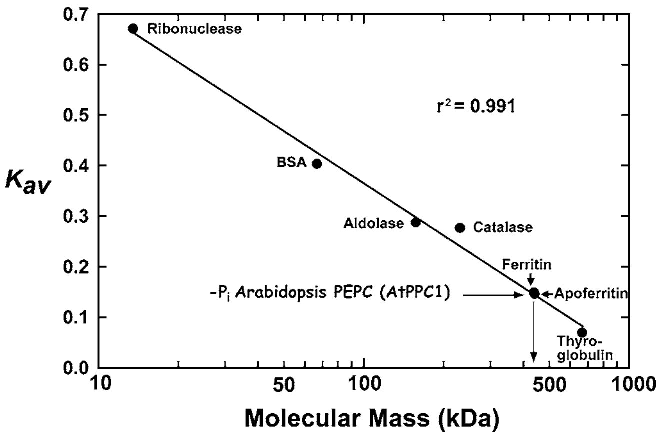

Figure S2 Native molecular-mass estimation for PEPC from $-P_i$ *Arabidopsis* suspension cells

Native molecular-mass estimation was performed during FPLC on a calibrated Superdex-200 HR 16/60 gel-filtration column as described in the Experimental section in the main article. r^2, correlation coefficient.

Table S1 MALDI–QqTOF-MS identified tryptic peptides (derived from purified native PEPC from $-P_i$ *Arabidopsis* suspension cells) matching the deduced amino-acid sequence of AtPPC1

MS/MS-sequenced peptides are in bold font, with those unique to AtPPC1 italicized. Calc., calculated; meas., measured.

Position	*m/z* (Meas.)	[MH+] (Calc.)	Delta	Peptide sequence
734–739	**860.431**	**860.430**	**+0.001**	**FVEYFR**
726–733	961.506	961.510	−0.004	SVVFQEPR
494–503	***1129.628***	***1129.636***	***−0.008***	***RPLFGSDLPK***
153–162	1148.587	1148.583	+0.004	LATPELEYGR
564–576	1267.686	1267.700	−0.014	LADLEAAPAAVAR
553–563	***1325.785***	***1325.794***	***−0.009***	***QPLRVVPLFEK***
189–199	1350.698	1350.719	−0.021	IRDCLAQLYAK
482–493	**1445.761**	**1445.774**	**−0.013**	**RQEWLLSELSGK**
603–615	**1478.766**	**1478.775**	**−0.009**	**DAGRLSAAWQLYK**
770–781	**1489.783**	**1489.795**	**−0.012**	**AIPWIFAWTQTR**
782–795	***1599.872***	***1599.879***	***−0.007***	***FHLPVWLGFGSAIR***
711–725	**1709.835**	**1709.841**	**−0.006**	**ALLDEMAVVATEEYR**
726–739	**1802.916**	**1802.922**	**−0.006**	**SVVFQEPRFVEYFR**
360–375	***1815.970***	***1815.996***	***−0.026***	***SIPTTEPYRVILGDVR***
690–706	***1852.956***	***1852.960***	***−0.004***	***FTAATLEHGMRPPISPK***
163–179	1878.976	1878.976	0.000	NQTVDLVLTAHPTQSVR
908–923	***1919.028***	***1919.061***	***−0.033***	***IRDPSYHVTLRPHISK***
734–749	**1989.989**	**1990.006**	**−0.017**	**FVEYFRLATPELEYGR**
163–180	**2035.091**	**2035.104**	**−0.013**	**NQTVDLVLTAHPTQSVRR**
360–377	***2059.102***	***2059.118***	***−0.016***	***SIPTTEPYRVILGDVRDK***
889–906	**2159.085**	**2159.116**	**−0.031**	**LRDSYITTLNVCQAYTLK**
644–665	***2201.132***	***2201.167***	***−0.035***	***GGGPTHLAILSQPPDTINGSLR***
476–493	***2262.100***	***2262.114***	***−0.014***	***EWSEERRQEWLLSELSGK***
97–116	2290.134	2290.164	−0.030	AFSHMLNLANLAEEVQIAYR
97–116	2306.159	2306.159	0.000	AFSHMLNLANLAEEVQIAYR
666–685	**2364.100**	**2364.128**	**−0.028**	**VTVQGEVIEQSFGEEHLCFR**
690–710	2421.215	2421.249	−0.034	FTAATLEHGMRPPISPKPEWR
97–117	2446.239	2446.266	−0.027	AFSHMLNLANLAEEVQIAYRR
97–117	2462.225	2462.260	−0.035	AFSHMLNLANLAEEVQIAYRR
207–227	***2503.256***	***2503.278***	***−0.022***	***QELDEALQREIQAAFRTDEIK***
272–294	2577.202	2577.230	−0.028	VPYNAPLIQFSSWMGGDRDGNPR
229–250	2622.243	2622.211	+0.032	TPPTPQDEMRAGMSYFHETIWK
711–733	2652.307	2652.333	−0.026	ALLDEMAVVATEEYRSVVFQEPR
934–957	2690.323	2690.384	−0.061	ELIELNPTSEYAPGLEDTLILTMK
228–250	**2778.299**	**2778.312**	**−0.013**	**RTPPTPQDEMRAGMSYFHETIWK**
30–54	2984.489	2984.572	−0.083	LVEYDALLLDRFLDILQDLHGEDLR
302–330	3626.669	3626.562	+0.107	DVCLLARMMAATMYFNQIEDLMFEMSMWR

Table S2 MALDI QqTOF MS identified Asp-N peptides (derived from purified native PEPC from $-\mathrm{P_i}$ *Arabidopsis* suspension cells) matching AtPPC1

MS/MS-sequenced peptides are in bold font, with those unique to AtPPC1 italicized. Calc., calculated; meas., measured.

Position	*m/z* (Meas.)	[MH+] (Calc.)	Delta	Peptide sequence
333–339	830.466	830.447	−0.019	DELRARA
500–508	1015.546	1015.530	+0.016	DLPKTEEIA
340–348	1055.581	1055.522	+0.059	DEVHANSRK
470–478	***1112.521***	***1112.500***	***+0.021***	***DIGSYREWS***
801–809	**1125.601**	**1125.583**	**+0.018**	**DVRNLHMLQ**
149–158	1191.621	1191.589	+0.032	DLNKSPEEIF
512–522	1212.642	1212.625	+0.017	DTFHVIAELPA
52–61	**1265.660**	**1265.637**	**+0.023**	**DIRETVQELY**
138–148	1278.706	1278.694	+0.012	DLEETFKKLVG
224–234	***1281.690***	***1281.679***	***+0.011***	***DEIKRTPPTPQ***
2–12	1302.738	1302.719	+0.019	ANRKLEKMASI
290–301	1340.709	1340.691	+0.018	DGNPRVTPEVTR
880–890	1457.884	1457.869	+0.015	DPYLKQRLRLR
566–580	***1529.851***	***1529.832***	***+0.019***	***DLEAAPAAVARLFSV***
13–26	***1576.893***	***1576.880***	***+0.013***	***DVHLRQLVPGKVSE***
210–223	**1647.857**	**1647.844**	**+0.013**	**DEALKREIQAAFRT**
13–27	1691.888	1691.907	−0.019	DVHLRQLVPGKVSED
810–822	1739.861	1739.836	+0.025	DMYQHWPFFRVTI
434–449	**1851.064**	**1851.048**	**+0.016**	**DFLRQVSTFGLSLVRL**
696–713	***2114.132***	***2114.132***	***0.000***	***EHGMRPPISPKPEWRAIL***
581–598	**2201.089**	**2201.080**	**+0.009**	**DWYKNRINGKQEVMIGYS**
376–394	2262.162	2262.148	+0.014	DKLYHTRERAHQLLSNGHS
891–909	**2315.232**	**2315.217**	**+0.015**	**DSYITTLNVCQAYTLKRIR**
138–158	2451.258	2451.265	−0.007	DLEETFKKLVGDLNKSPEEIF
479–499	***2517.331***	***2517.320***	***+0.011***	***EERRQEWLLSELSGKRPLFGS***
167–190	2781.612	2781.607	+0.005	DLVLTAHPTQSVRRSLLQKHGRIR
349–372	2836.469	2836.283	+0.186	DAAKHYIEFWKSIPTTEPYRVILG
235–259	3054.611	3054.581	+0.030	DEMRAGMSYFHETIWKGVPKFLRRV
260–287	3106.553	3106.566	−0.013	DTALKNIGIEERVPYNAPLIQFSSWMGG
470–499	3610.807	3610.803	+0.004	DIGSYREWSEERRQEWLLSELSGKRPLFGS

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