Supplemental data

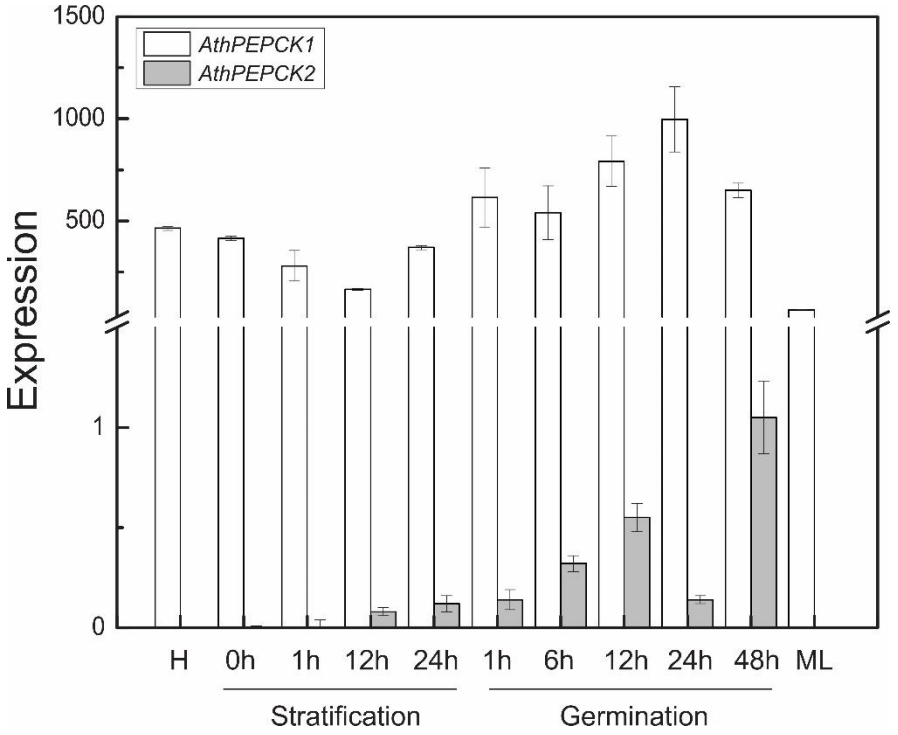

Fig. S1. Comparison of the *AthPEPCK1* and *AthPEPCK2* expression levels.

Transcriptomic data from germinating seeds were obtained from Narsai *et al.* (2011), while data from mature leaves (ML) were obtained from Klepikova *et al.* (2016), available at eFP Browser database (Winter *et al.*, 2007). The experiments were performed with Arabidopsis Col-0 seeds desiccated for 15 days (H) and then stratified at 4°C in the dark. Seeds were then plated onto Murashine and Skoog medium supplemented with 3% (w/v) sucrose and transferred to light for germination.

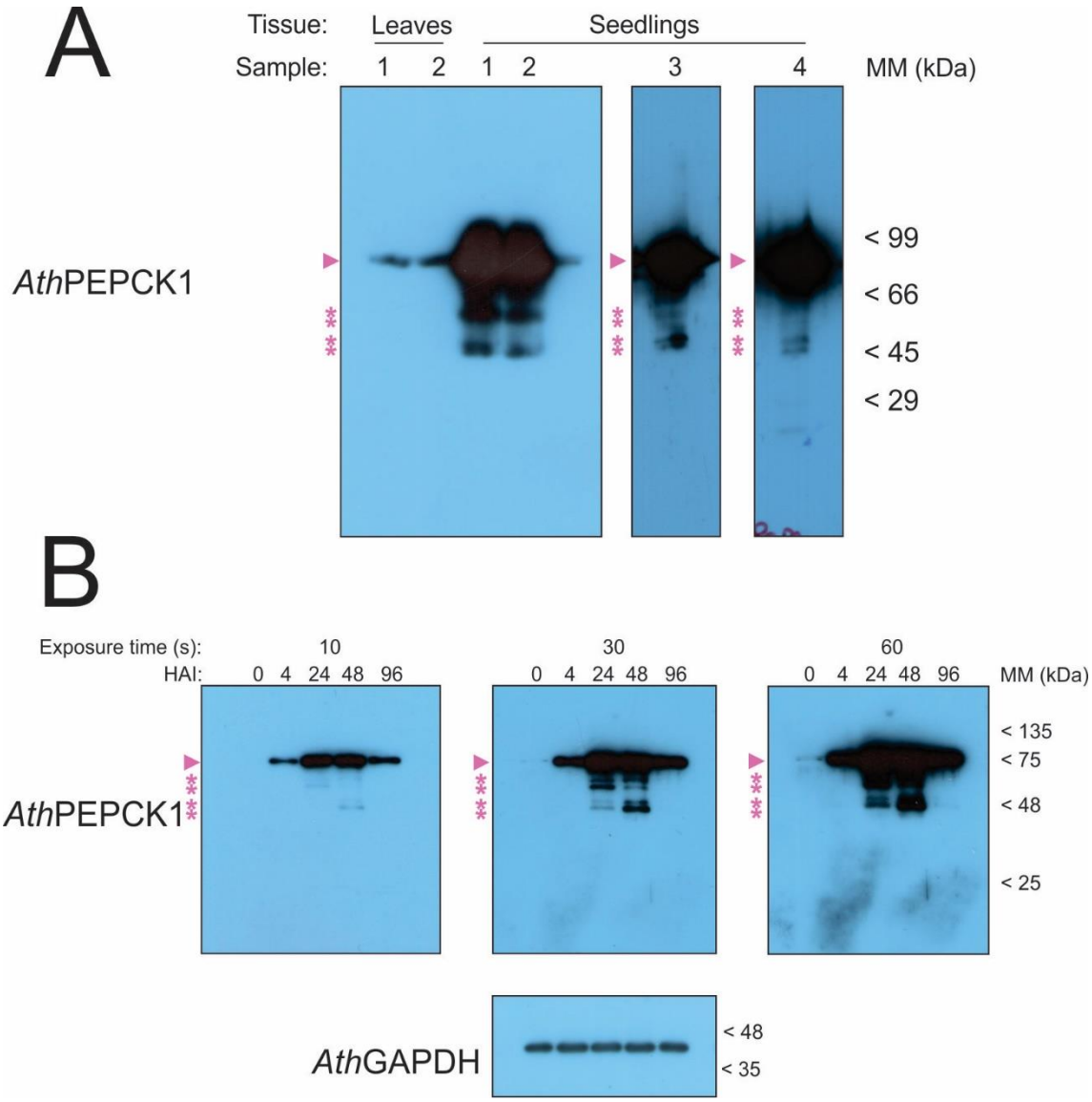

Fig. S2. Denaturing protein extraction from Arabidopsis leaves and seedlings.

A) Samples were extracted as indicated in the Materials and methods section, with denaturing sample buffer (1), denaturing buffer supplemented with 2 M urea (2), 10% (w/v) TCA (3), and 10% (w/v) TCA in acetone (4). In all cases, the gel was loaded with 0.8 mg FW tissue. Leaves, 32-days-old rosettes; Seedlings, 48 HAI germinating seedlings; MM, molecular mass marker. The arrow (►) indicates the full-length *Ath*PEPCK1 and the asterisk (*) its truncated forms.

B) Samples were extracted with denaturing sample buffer as indicated in the Materials and methods section. Then, the blot was developed under different exposures times. In all cases, the gel was loaded with 0.25 mg FW tissue.

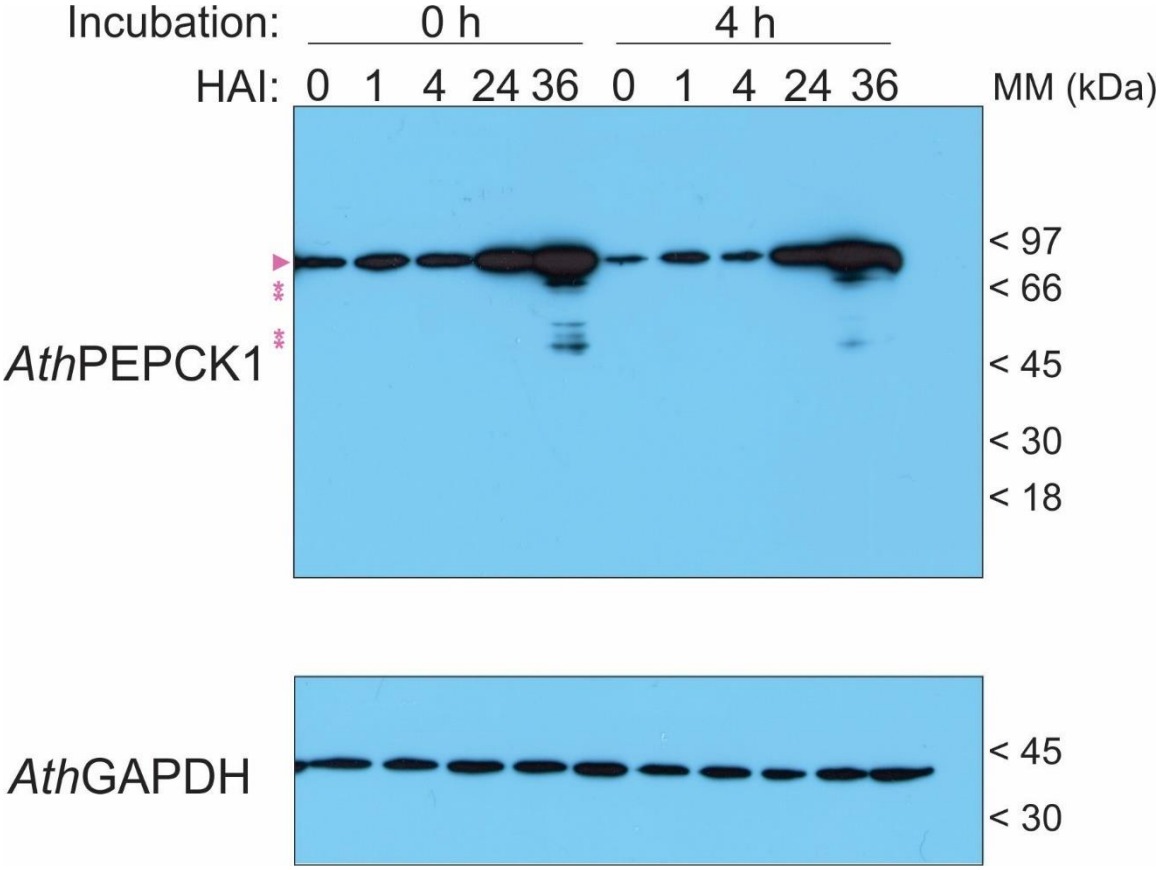

Fig. S3. Native extraction of *Ath*PEPCK1 from germinating seedlings.

To measure PEPCK activity, samples were extracted and used immediately or incubated on ice for 4 h (to check the stability of *Ath*PEPCK1). Afterwards, proteins were denatured by the addition of sample buffer, separated on a 12% SDS-PAGE, transferred to a nitrocellulose membrane and subjected to immunodetection with anti-*Ath*PEPCK1 or anti-*Tae*GAPDH antisera, as described in the Materials and methods section. HAI; hours after imbibition. MM; molecular mass marker. The arrow (►) indicates the full-length *Ath*PEPCK1 and the asterisk (*) its truncated forms. The gel was loaded with 3 µg of protein.

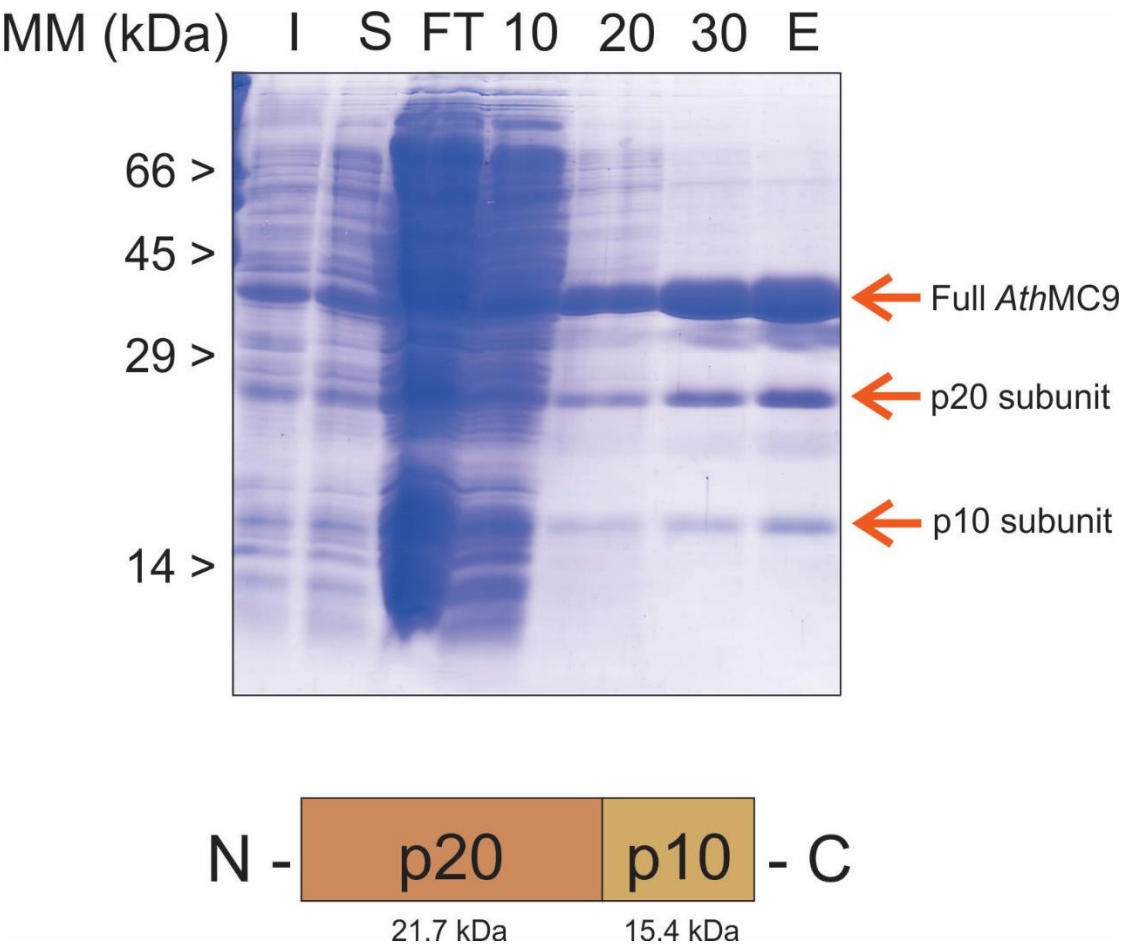

Fig. S4. Purification of *Ath*MC9 by IDA-Ni^{2+}.

Fractions obtained during *Ath*MC9 purification were analyzed in a 15% SDS-PAGE. I, insoluble fraction; S, soluble fraction; FT, flow-through; 10-30, imidazole concentration (in mM) used to wash the column; E, elution with 300 mM imidazole; MM, molecular mass marker. At the bottom of the gel a scheme of *Ath*MC9 subunits is shown.

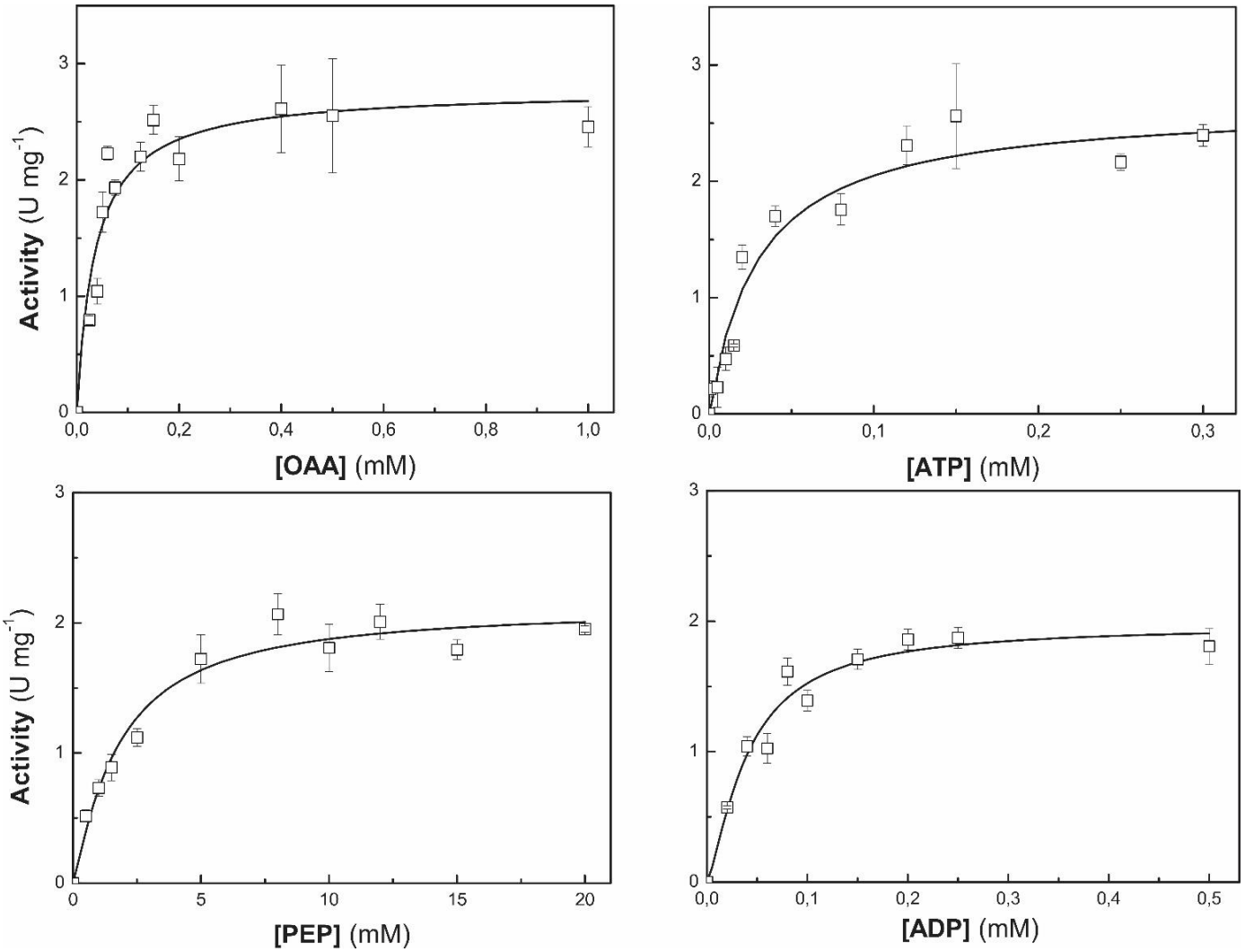

Fig. S5. Substrate saturation curves for the *Ath*PEPCK1 Δ19 mutant.

Each curve was done using the variable substrate concentration described in the figure and a fixed substrate concentration of: A, 1.0 mM ATP; B, 0.5 mM OAA; C, 0.5 mM ADP; D, 5.0 mM PEP. Each point represents the mean ± standard error of four independent datasets. Values were adjusted to the Michaelis-Menten equation, and calculated parameters are shown in Table 1.

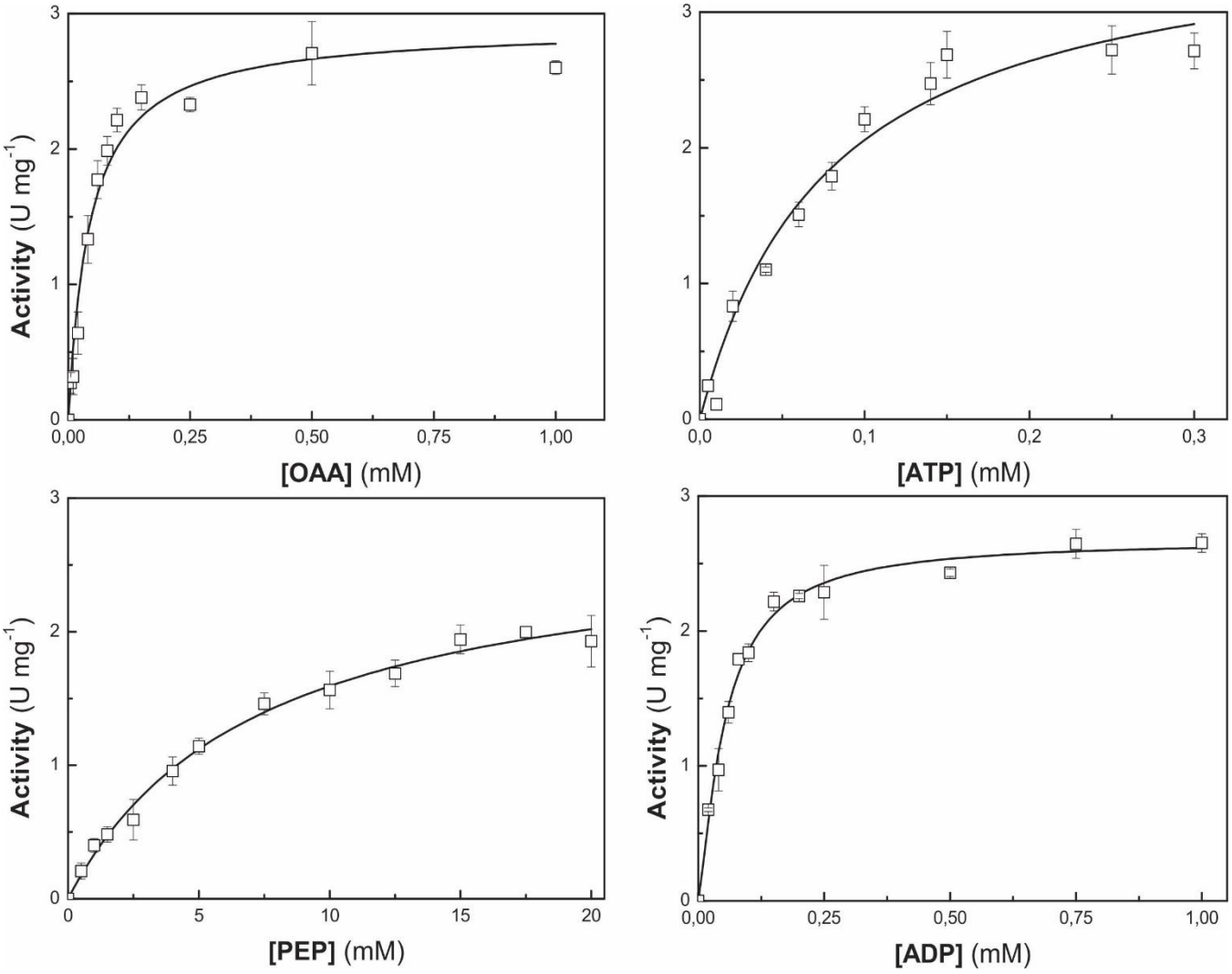

Fig. S6. Substrate saturation curves for the *Ath*PEPCK1 Δ101 mutant.

Each curve was done using the variable substrate concentration described in the figure and a fixed substrate concentration of: A, 0.5 mM ATP; B, 0.5 mM OAA; C,0.5 mM ADP; D, 15.0 mM PEP. Each point represents the mean ± standard error of four independent datasets. Values were adjusted to the Michaelis-Menten equation, and calculated parameters are shown in Table 1.

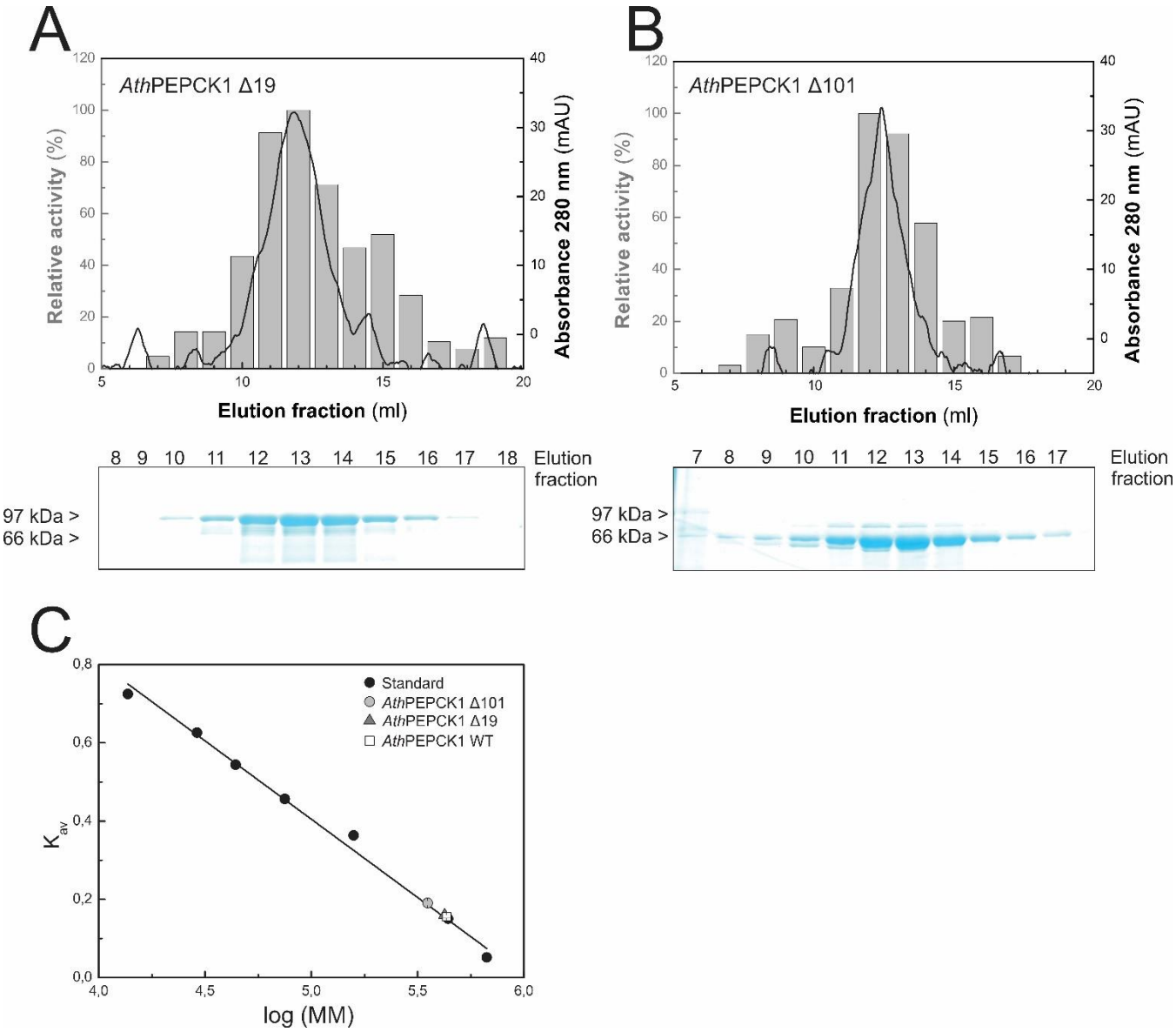

Fig. S7. Size exclusion chromatography for the *Ath*PEPCK1 truncated mutants.

Relative PEPCK activity obtained for the fractions from the gel filtration column for *Ath*PEPCK1 Δ19 (A) and *Ath*PEPCK1 Δ101 (B). No activity was detected in the fractions without bars. The fractions obtained from the gel filtration column were analyzed by 12% SDS-PAGE (results are shown at the bottom of each panel). (C) Superdex 200 gel filtration calibration curve constructed using the protein standards described in the Materials and methods section. Data presented for the truncated mutants are the mean of two independent experiments.

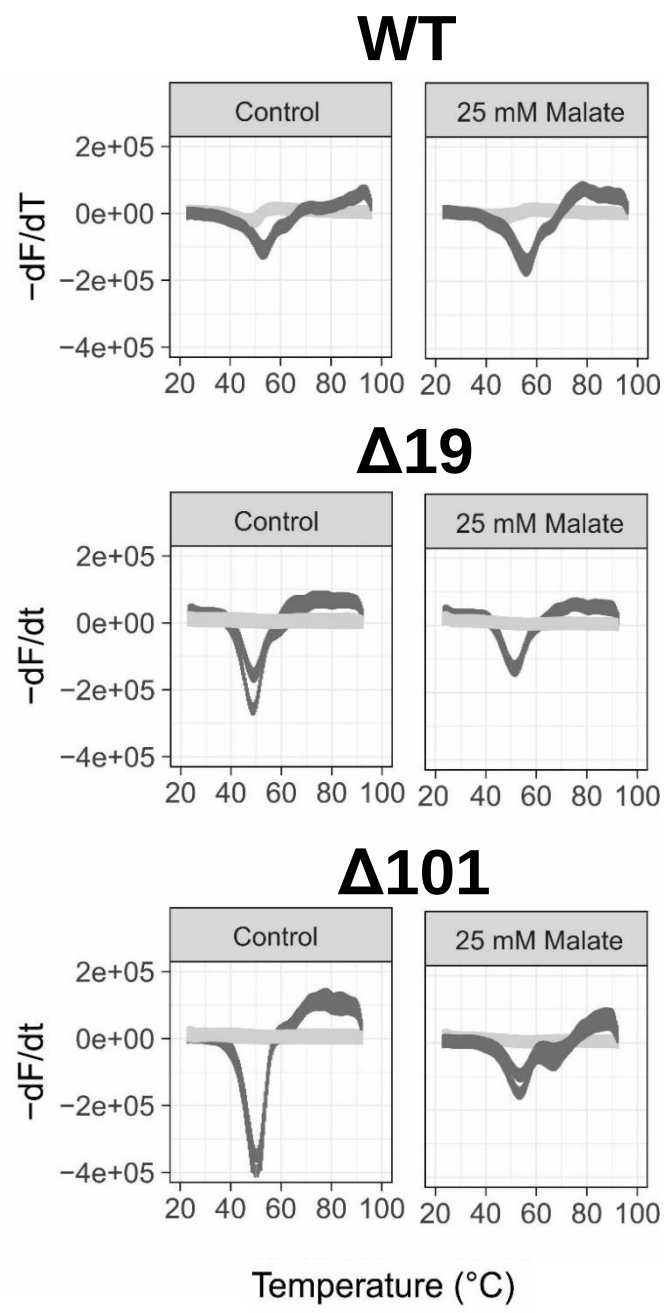

Fig. S8. Thermal shift assay coupled to differential scanning fluorometry for the *Ath*PEPCK1 truncated mutants.

The first derivative of the fluorescence emission (−dF/dT) was plotted as a function of the temperature for the reactions without protein (light grey) or with the corresponding enzyme (dark grey). The T_m is calculated from the minimum of the derivative curve.

Supplemental references

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Winter D, Vinegar B, Nahal H, Ammar R, Wilson G V, Provart NJ. 2007. An 'Electronic Fluorescent Pictograph' browser for exploring and analyzing large-scale biological data sets. PloS one **2**, e718.