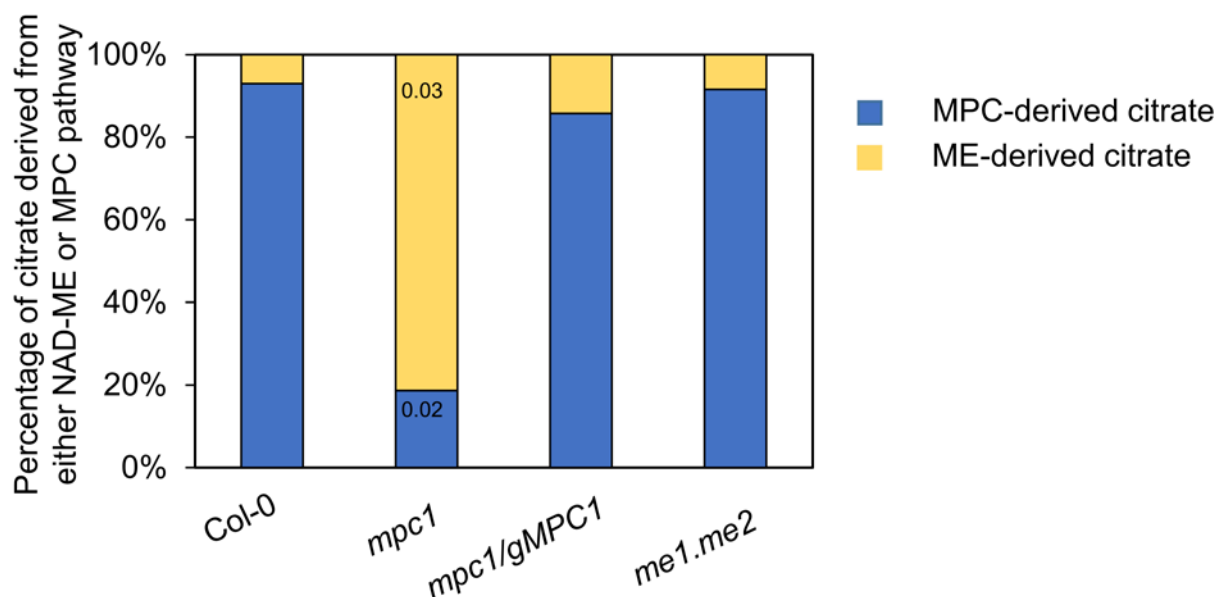

Supplementary information

Metabolic evidence for distinct pyruvate pools inside plant mitochondria

In the format provided by the
authors and unedited



Supplemental Figure S1. The proportion of citrate derived from difference sources of pyruvate. Isolated mitochondria incubated with 500 μM $^{13}\text{C}_3$ -pyruvate and 500 μM malate with or without the addition of glutamate in the presence of ADP at pH 6.4 to initiate substrate uptake and consumption to initiate substrate uptake and consumption via MPC and NAD-ME pathways. Metabolic reaction was stopped by centrifugation through a single silicon oil layer by which the mitochondrial pellet was separated from the extra-mitochondrial medium. The amount of citrate (labelled and unlabelled) in the extra-mitochondrial medium were quantified using LC-SRM-MS. Bar graphs show the percentage of $^{13}\text{C}_2$ -citrate (blue) and citrate (yellow) derived from the MPC and NAD-ME pathways, respectively. Each bar is the averaged value from three biological replicates ($n=3$). Significant differences between wildtype and mutants are denoted by asterisks based on two-sided Student's t-tests (P-values are shown as discrete numbers)

Supplemental Table 5. Mitochondrial integrity assessed as percentage of cytochrome c oxidase (Complex IV) activity before and after detergent. 100 µg isolated mitochondria were mixed with 10 mM ascorbate and 25µM cytochrome c in a final volume of 1 ml respiration buffer. 0.05% (v/v) Triton X-100 was added after 5 minutes. Oxygen consumption rates before ascorbate, before and after Triton were recorded. % integrity = $100 - 100 \times (\text{Before Triton} - \text{Before Ascorbate}) / (\text{After Triton} - \text{Before Ascorbate})$

		Before Ascorbate (nmol/min/mg protein)	Before triton (nmol/min/mg protein)	After triton (nmol/min/mg protein)	% integrity
WT	Rep 1	12.00	45.00	390.00	91.27
	Rep 2	11.00	28.00	240.00	92.58
	Rep 3	12.00	23.00	193.00	93.92
	Average				92.59 ± 1.33
<i>mpc1</i>	Rep 1	13.00	44.00	370.00	91.32
	Rep 2	11.00	32.00	270.00	91.89
	Rep 3	8.00	22.50	175.00	91.32
	Average				91.51 ± 0.33

Supplemental Table 6. Mitochondrial quality assessed as respiratory control ratio (State 3(+ADP) / State 4(-ADP) oxygen consumption rate). 100 µg isolated mitochondria were mixed with 10 mM pyruvate and 10 mM malate as substrates with cofactors (2 mM NAD⁺, 0.2 mM TPP and 0.012 mM CoA) in a final volume of 1 ml respiration buffer. State 3 was recorded after 1 mM ADP was added. The mitochondria entered state 4 when ADP was fully converted into ATP. Respiratory control ratio (RCR) was calculated as the ratio of State 3 rate to State 4 rate.

		State 3 (nmol/min/mg protein)	State 4 (nmol/min/mg protein)	After KCN (nmol/min/mg protein)	RCR
WT	Rep1	174.20	73.80	14.00	2.36
	Rep2	171.40	70.00	26.00	2.45
	Rep3	133.40	51.70	17.00	2.58
	Average				2.46 ± 0.11
<i>mpc1</i>	Rep1	178.95	74.80	25.00	2.39
	Rep2	188.20	79.00	28.00	2.38
	Rep3	157.00	65.00	17.00	2.42
	Average				2.40 ± 0.017