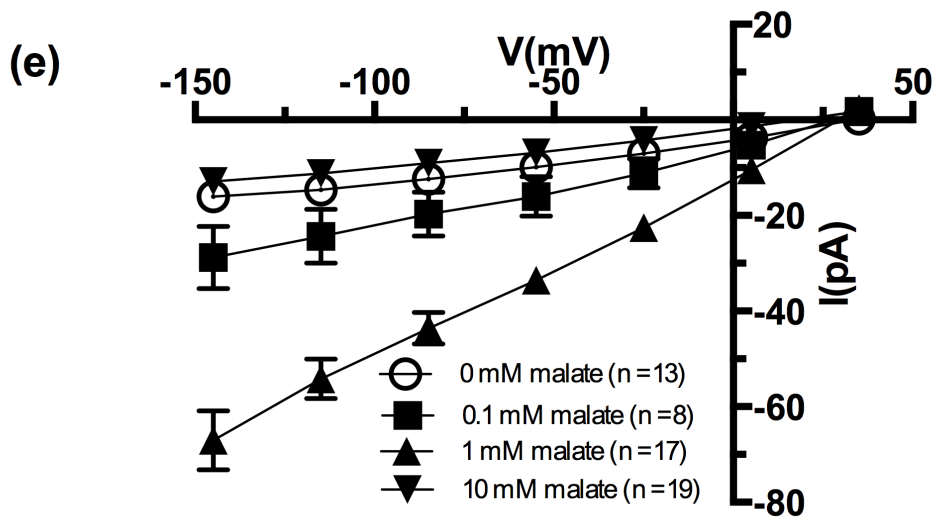
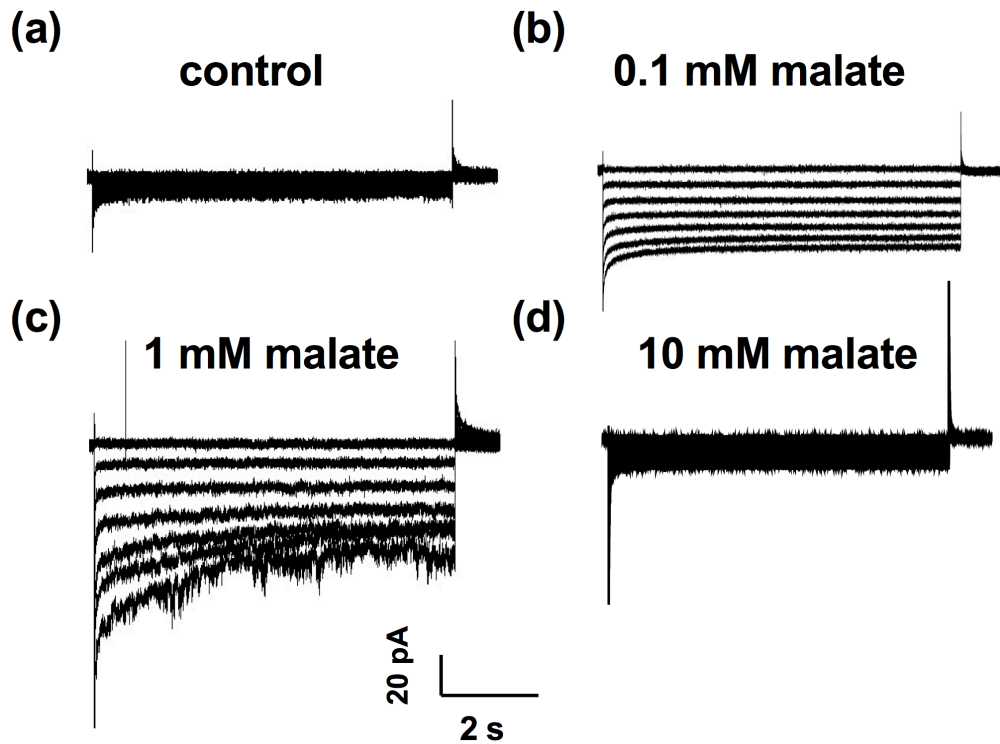


New Phytologist Supporting information Figs S1-S5.

**Cytosolic Malate and Oxaloacetate Activate S-type Anion Channels in *Arabidopsis*
Guard Cells**

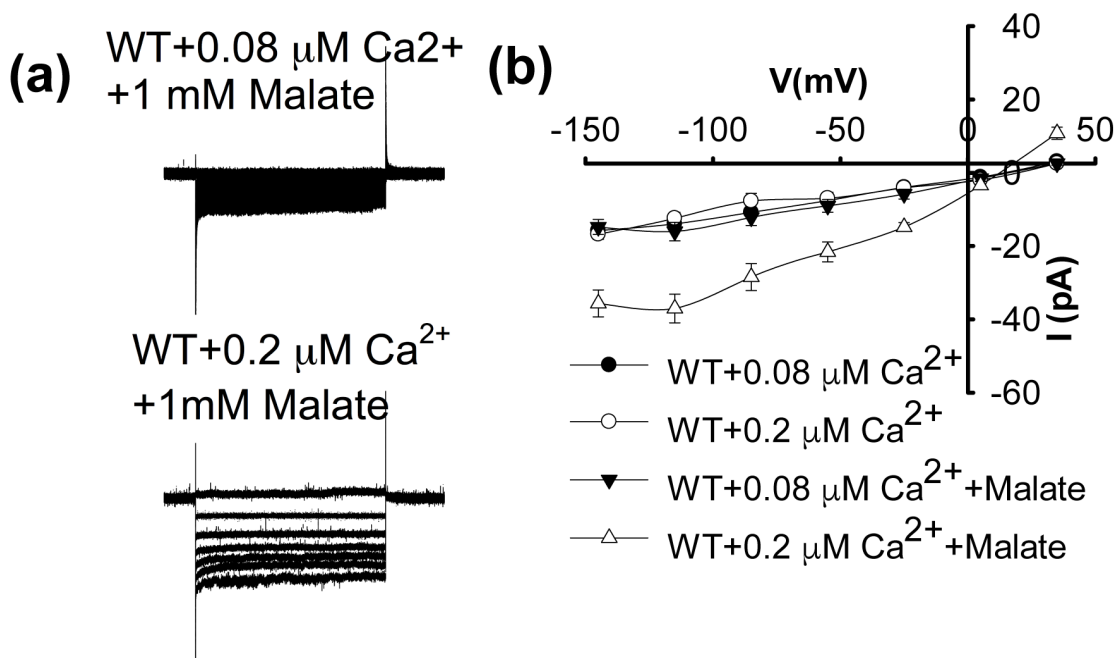
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Supplemental Fig. 1. Cytosolic malate at 1 mM activates ionic currents in *Arabidopsis thaliana* wild-type (WT) guard cells with 0.1 mM malate showing partial activation in the depicted experimental set, whereas 10 mM malate showed no activation of currents.

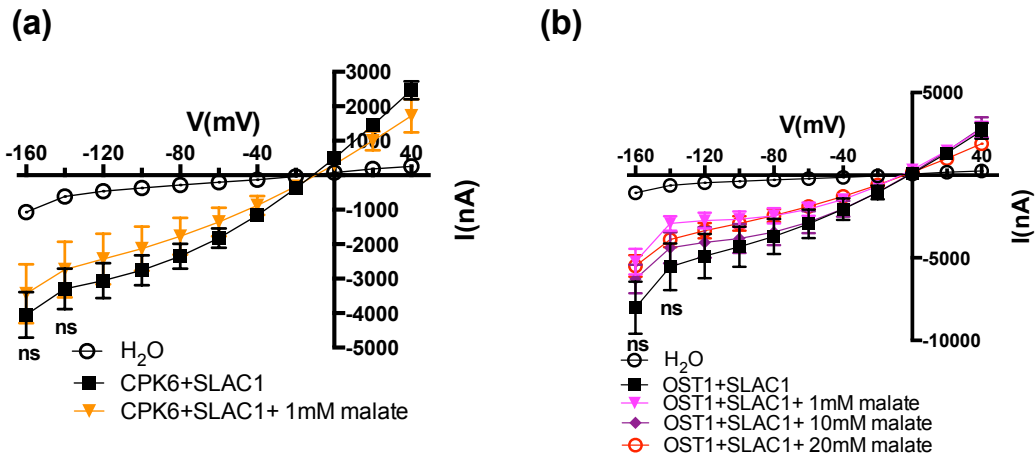
(a, b, c and d) Typical whole-cell recordings of ionic currents in guard cell protoplasts of wild type plants without malate or with 0.1 mM, 1 mM and 10 mM malate as indicated added to the pipette solution that dialyzes the cytosol of guard cells. (e) Steady-state current-voltage relationships. The number of guard cells for each condition (n= 8 to 19) is indicated in the figure. Data are mean \pm s.e.m Two-way ANOVA and Tukey's test give P values at -145 mV (P = 0.02 for 0 mM malate vs 0.1 mM malate; P < 0.001 for 0 mM malate vs 1 mM malate; P = 0.8 for 0 mM malate vs 10 mM malate).



Supplemental Fig. 2 Cytosolic malate (1 mM) did not activate S-type anion channel currents in wild-type (WT) *Arabidopsis thaliana* guard cells at 0.08 μM free cytosolic Ca^{2+} .

(a) Typical whole-cell recordings of S-type anion channel currents in guard cell protoplasts of wild type plants with 0.08 μM free Ca^{2+} , 0.2 μM free Ca^{2+} , 0.08 μM free Ca^{2+} + 1 mM malate and 0.2 μM free Ca^{2+} +1 mM malate in the pipette solution. (b) Steady state current-voltage relationships recorded as in (a). The number of guard cells

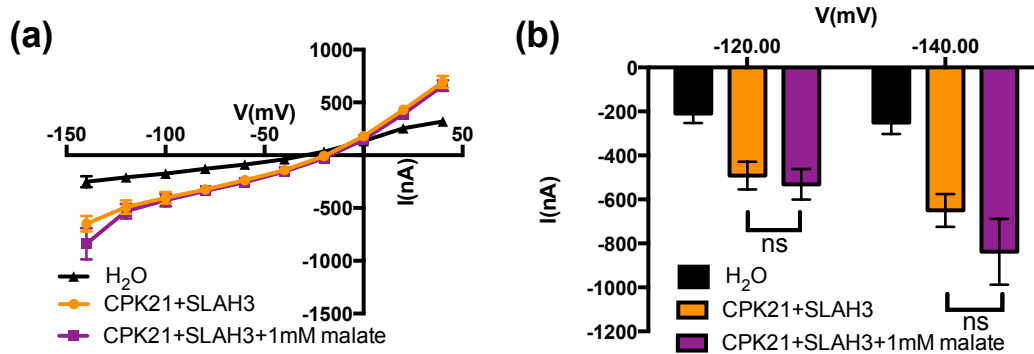
was from 6 to 8 for each condition. Data are mean \pm s.e.m ($P < 0.01$: WT + 0.08 $\mu\text{M Ca}^{2+}$ +1 mM malate versus WT+ 0.2 $\mu\text{M Ca}^{2+}$ + 1 mM malate at -145 mV. One-way ANOVA and Tukey's test).



Supplemental Fig. 3 cytosolic malate at 1, 10 and 20 mM does not enhance or inhibit SLAC1-mediated ion currents in *Xenopus* oocytes co-expressing OST1 or CPK6

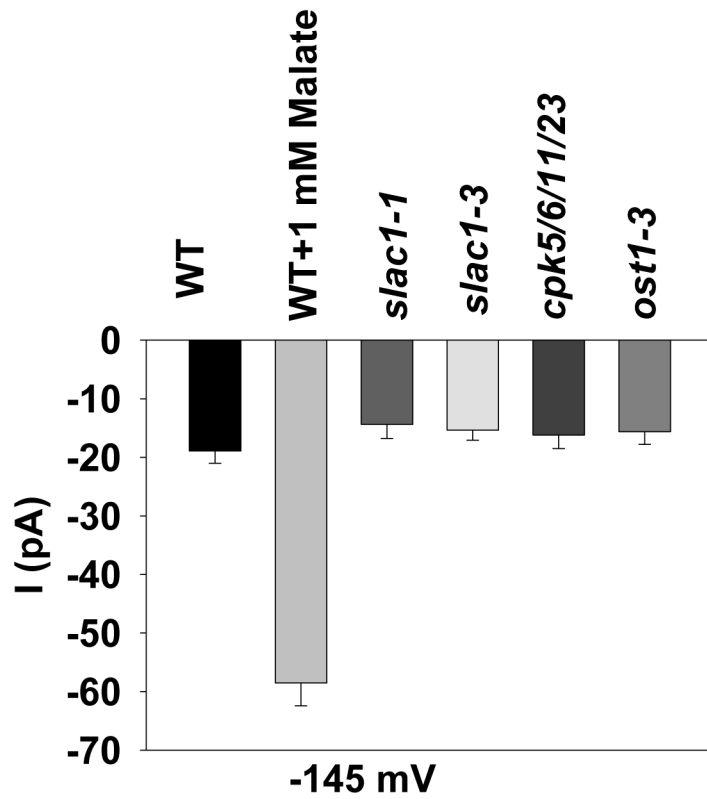
(a) Mean current-voltage curves of oocytes co-expressing CPK6 and SLAC1, in the presence or absence of 1 mM injected malate as final concentration. “ns” indicates that there is no significant difference between the presence and absence of 1 mM malate based on two-way ANOVA and Tukey’s test. $P = 0.98$ at -160 mV and $P = 0.99$ at -140 mV. (b) Mean current-voltage curves of oocytes co-expressing OST1 and SLAC1, in the absence or presence of 1 mM, 10 mM and 20 mM injected malate as final concentrations. “ns” indicates that there is no significant difference between the presence and absence of 1, 10 and 20 mM malate based on two-way ANOVA and Tukey’s test. ($P = 0.15$ for 0 mM malate vs 1 mM malate at -160 mV and $P = 0.12$ at -140 mV; $P = 0.14$ for 0 mM

malate vs 10 mM malate at -160 mV and $P = 0.44$ at -140 mV; $P = 0.1$ for 0 mM malate vs 20 mM malate at -160 mV and $P = 0.13$ at -140 mV). The number of oocytes was $n = 8-12$. Error bars denote mean \pm s.e.m.



Supplemental Fig. 4 Cytosolic malate at 1 mM does not enhance SLAH3-mediated ion currents in *Xenopus* oocytes.

(a) Mean current-voltage curves of oocytes co-expressing CPK21 and AtSLAH3, in the presence or absence of 1 mM cytosolic malate. (b) Average SLAH3-mediated currents at -120 and -140 mV, co-expressing CPK21, in the presence or absence of 1 mM cytosolic malate. “ns” indicates that there is no significant difference between the presence and absence of 1 mM malate based on two-way ANOVA and Tukey’s test ($P = 0.94$ at -120 mV and $P = 0.31$ at -140 mV). The number of oocytes was $n = 8-14$. Error bars denote mean \pm s.e.m.



Supplemental Fig. 5 Average S-type anion channel currents recorded at -145 mV from different genotypes as shown in Figure 4 and Figure 6 under control conditions (0 mM malate). Note the wild-type (WT) and WT+1 mM malate are the same data as shown in Figure 4. The number of guard cell was from 5 to 6 for each condition. Data are presented as means \pm SE.