Correspondence and materials. Supplementary data for web link.

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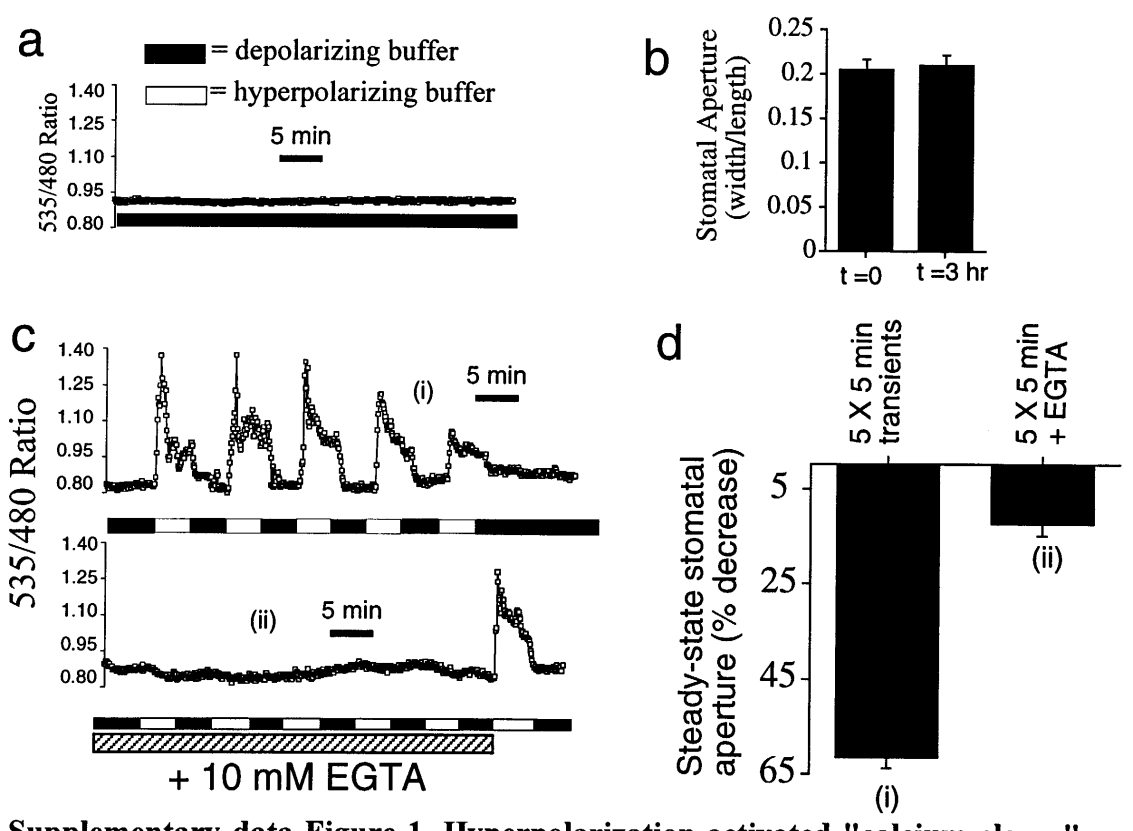
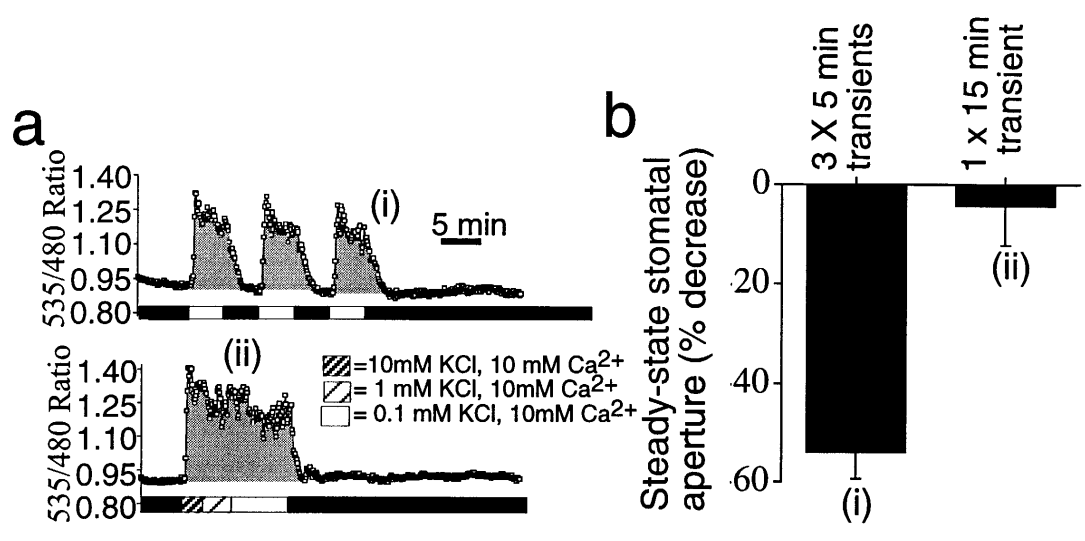


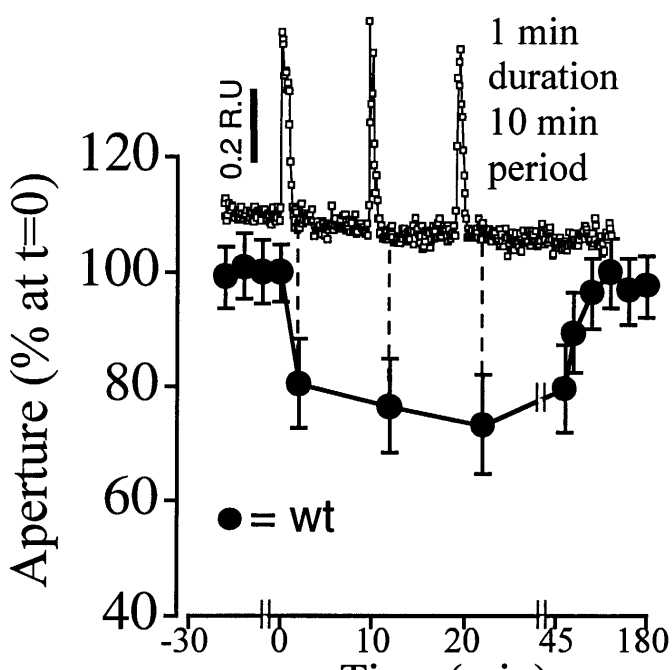
Supplementary data Figure 1. Hyperpolarization-activated "calcium-clamp" and $[Ca^{2+}]_{cyt}$ oscillations in *Arabidopsis* guard cells expressing yellow cameleon 2.1.

(a) Stomata were opened in high KCl (100 mM) (depolarizing) buffer for 2.5h in the light. Guard cells subsequently exhibited a stable resting $[Ca^{2+}]_{cyt}$ level in the 100 mM KCl buffer. **(b)** Stomatal aperture was stable after the 2.5 h opening period (t=0) and for a subsequent 3 h period (t=3) when cells were maintained in the 100 mM KCl buffer. **(c)** Repetitive exchanges to low KCl (0.1 mM) hyperpolarizing buffer induced a series of $[Ca^{2+}]_{cyt}$ transients composing a $[Ca^{2+}]_{cyt}$ oscillation (upper panel). Oscillations were abolished if extracellular calcium was removed with 10 mM EGTA (lower panel). **(d)** Steady-state stomatal closure was induced when five hyperpolarizations of 5 min duration and a 10 min period (5 x 5) were applied in the presence of extracellular calcium, but was reduced in the absence of extracellular calcium (5 x 5 + EGTA). Apertures were measured 3 h following the onset of oscillations imposed as (i) and (ii) in (c). Apertures are mean ±SEM of 160 stomates from n=4 replicates.



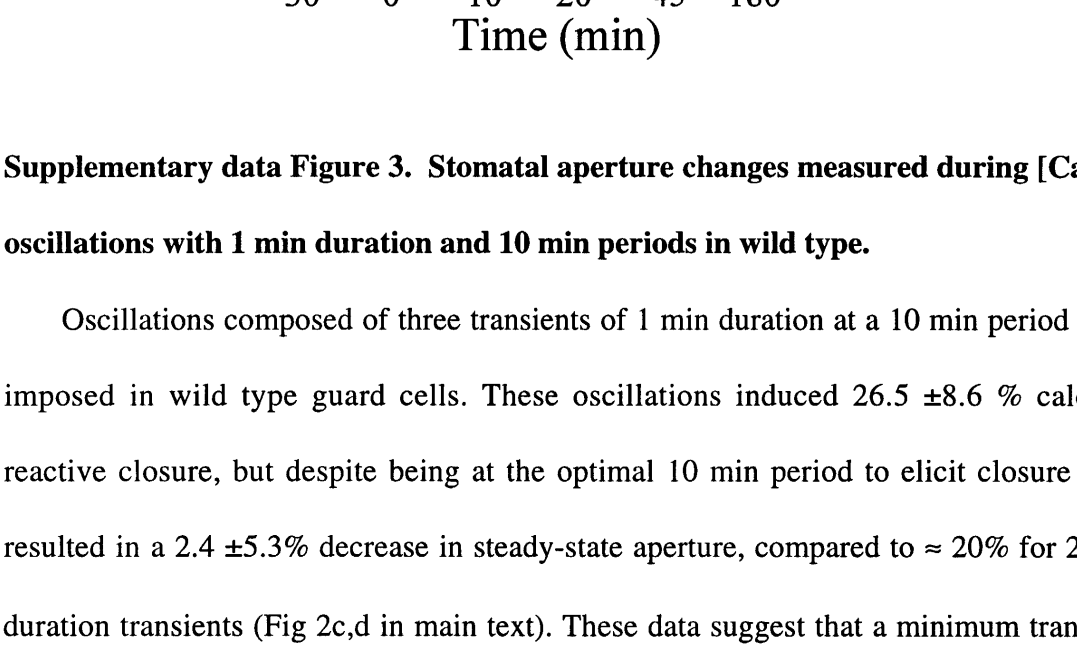
Supplementary data Figure 2. Steady-state stomatal closing is enhanced when $[Ca^{2+}]_{cyt}$ elevations oscillate.

(a) Three 5 min $[Ca^{2+}]_{cyt}$ transients imposed in guard cells (upper panel) induced a total integrated $[Ca^{2+}]_{cyt}$ increase of 3332 ±422 nM•min (see shaded area, n=22 guard cells). A 15 min $[Ca^{2+}]_{cyt}$ increase induced by stepwise decreases from 100 to 10, 1 and 0.1 mM KCl at the indicated points in the presence of 10 mM Ca^{2+} (lower panel) induced a total integrated $[Ca^{2+}]_{cyt}$ increase of 3678 ±97 nM•min (see shaded area, n=30 guard cells). **(d)** Steady-state stomatal closure was greater ($P< 0.001$) following three 5 min $[Ca^{2+}]_{cyt}$ transients (left bar, i) than one 15 min $[Ca^{2+}]_{cyt}$ elevation (right bar, ii) even though the total $[Ca^{2+}]_{cyt}$ increases were similar ($P> 0.56$).



Supplementary data Figure 3. Stomatal aperture changes measured during $[Ca^{2+}]_{cyt}$ oscillations with 1 min duration and 10 min periods in wild type.

Oscillations composed of three transients of 1 min duration at a 10 min period were imposed in wild type guard cells. These oscillations induced 26.5 ±8.6 % calcium reactive closure, but despite being at the optimal 10 min period to elicit closure only resulted in a 2.4 ±5.3% decrease in steady-state aperture, compared to ≈ 20% for 2 min duration transients (Fig 2c,d in main text). These data suggest that a minimum transient duration greater than 1 min is required to elicit significant calcium programmed aperture changes.



Supplementary Data Figure 4. Reducing the amplitude of $[Ca^{2+}]_{cyt}$ oscillations in guard cells reduces stomatal aperture.

(a) Three 5 min $[Ca^{2+}]_{cyt}$ transients imposed in guard cells in the absence (upper panel) or presence (middle panel) of 50 µM of the Ca^{2+} channel blocker La^{3+} (n=16 cells). Transients were also imposed by 5 min exchanges between the high (100 mM) KCl buffer and a buffer containing 5 mM KCl, 50 µM Ca^{2+} (n=16 cells) (lower panel). Limiting calcium influx with 50 µM La^{3+} (a, ii) or by reducing the hyperpolarization step and extracellular calcium (a, iii) reduced the amplitude of $[Ca^{2+}]_{cyt}$ transients by ≈ 70 %. **(b)** Steady state stomatal closure was only 2 ±7 % when the amplitude of $[Ca^{2+}]_{cyt}$ transients was reduced with La^{3+} and 6 ±6 % when the amplitude was reduced by 5 mM KCl and 50 µM Ca^{2+} buffers. These data together suggest that the amplitude of $[Ca^{2+}]_{cyt}$ transients composing oscillations needs to reach a critical level before closure is elicited.