Supplementary Figures

CNGC2 Negatively Regulates Stomatal Closure and Is not Required for flg22- and H_2O_2-Induced Guard Cell $[Ca^{2+}]_{cyt}$ Elevation in *Arabidopsis thaliana*

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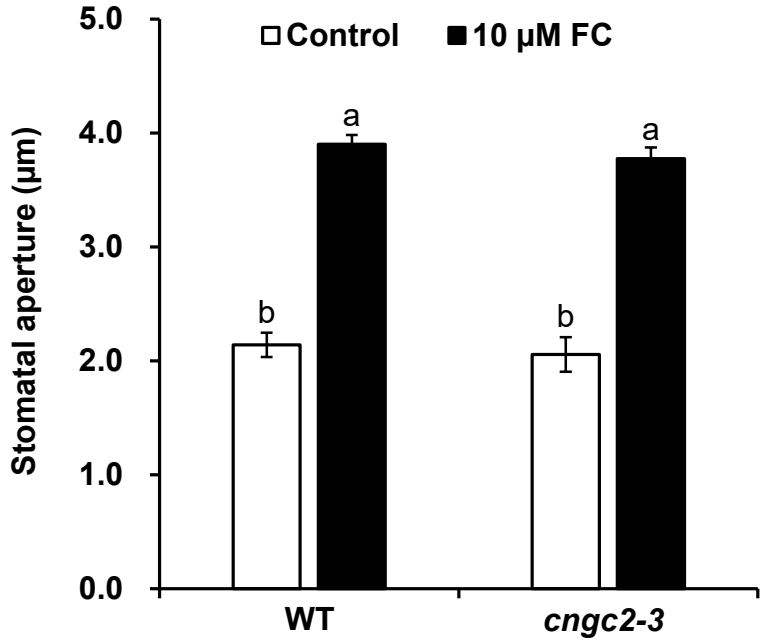
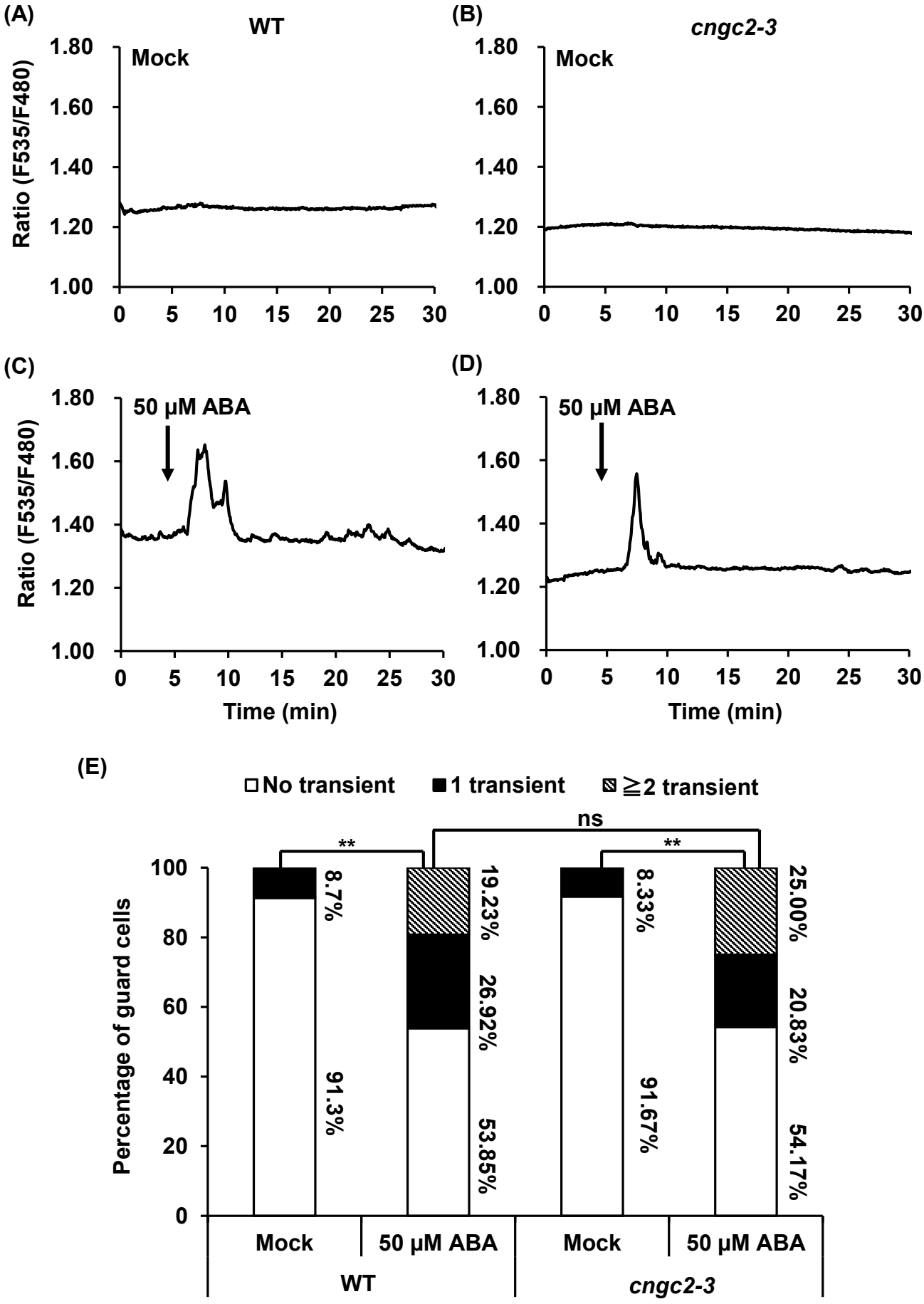
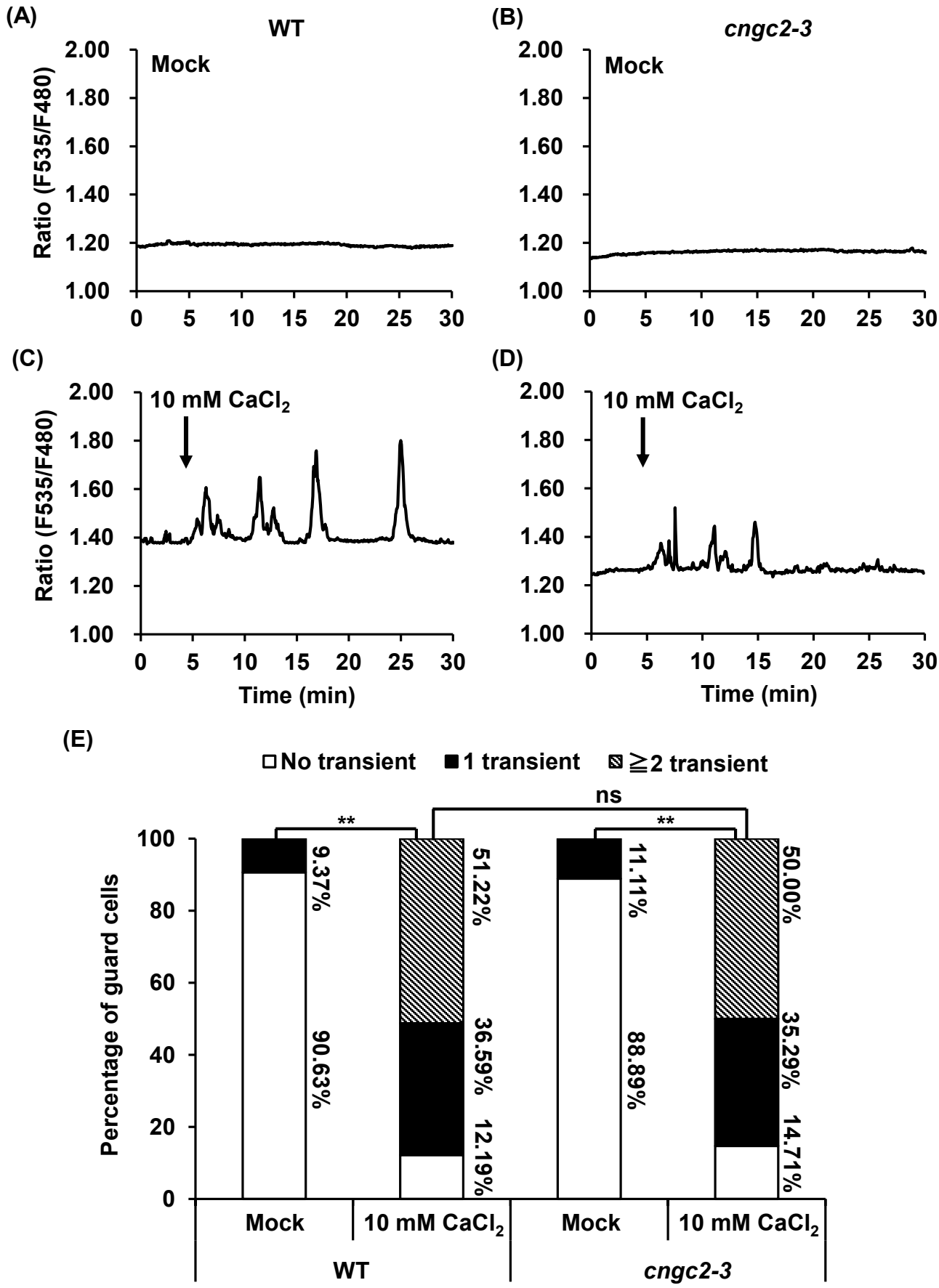


Supplementary Figure S1. Fusicoccin (FC)-mediated stomatal opening in WT and the *cngc2-3* mutant. Averages of stomatal apertures from three independent experiments (n=3, total 60 stomata) are shown. In this figure, Col-0 was used as wild-type (WT). Data are expressed as mean ± SE. Statistical differences were analyzed by one-way ANOVA with Tukey's test. Different letters represent significant differences ($P < 0.05$).


(A)
WT
Mock
(B)
cngc2-3
Mock
Ratio (F535/F480)
1.80
1.60
1.40
1.20
1.00
0
5
10
15
20
25
30
(C)
50 μM ABA
(D)
50 μM ABA
Time (min)
(E)
No transient
1 transient
≧2 transient
ns
**
**
Percentage of guard cells
100
80
60
40
20
0
8.7%
91.3%
19.23%
26.92%
53.85%
8.33%
91.67%
25.00%
20.83%
54.17%
Mock
50 μM ABA
Mock
50 μM ABA
WT
cngc2-3

Supplementary Figure S2. The *cngc2-3* mutant showed ABA-induced cytosolic calcium ($[Ca^{2+}]_{cyt}$) elevation in guard cells. The guard cell $[Ca^{2+}]_{cyt}$ elevation of wild-type (WT) and *cngc2-3* plants expressing Nuclear Export Signal (NES)-fused Yellow Cameleon3.6 (NES-YC3.6) was monitored. (A-D) Representative traces of fluorescence emission ratios (F535/F480) showing ABA-induced $[Ca^{2+}]_{cyt}$ transients in guard cells. In mock treatments, guard cells were incubated in stomatal assay buffer for 2 h in light, and no ABA applied during the measurement of fluorescence emission ratios (A and B). In ABA treatments, 50 μM ABA were applied to the guard cells in stomatal assay buffer 5 min after the measurement (C, D). (E) Percentage bar chart represents the percent (%) of guard cells showing transient $[Ca^{2+}]_{cyt}$ elevations. The number of transient $[Ca^{2+}]_{cyt}$ elevations under mock and 50 μM ABA treatment in WT and *cngc2-3* mutants were counted when the fluorescence ratio (F535/F480) increased ≥0.1 unit from the baseline. In this figure, Col-0 was used as wild-type (WT). The significance of differences between different treatments were determined by chi-squared ($\chi 2$) test, *$P < 0.05$, **$P < 0.01$. The “ns” indicates non-significant difference where $P > 0.05$.


(A)
WT
Mock
Ratio (F535/F480)
2.00
1.80
1.60
1.40
1.20
1.00
0
5
10
15
20
25
30
(B)
cngc2-3
Mock
(C)
10 mM CaCl2
Time (min)
(D)
10 mM CaCl2
Time (min)
(E)
No transient
1 transient
≧2 transient
ns
**
**
Percentage of guard cells
100
80
60
40
20
0
9.37%
90.63%
51.22%
36.59%
12.19%
11.11%
88.89%
50.00%
35.29%
14.71%
Mock
10 mM CaCl2
Mock
10 mM CaCl2
WT
cngc2-3

Supplementary Figure S3. The *cngc2-3* mutant showed high extracellular Ca^{2+}-induced cytosolic calcium ($[Ca^{2+}]_{cyt}$) elevation in guard cells. The guard cell $[Ca^{2+}]_{cyt}$ elevation of wild-type (WT) and *cngc2-3* plants expressing Nuclear Export Signal (NES)-fused Yellow Cameleon3.6 (NES-YC3.6) was monitored. (A-D) Representative traces of fluorescence emission ratios (F535/F480) showing extracellular Ca^{2+}-induced $[Ca^{2+}]_{cyt}$ transients in guard cells. In mock treatments, guard cells were incubated in stomatal assay buffer for 2 h in light, and no extracellular Ca^{2+} applied during the measurement of fluorescence emission ratios (A and B). In extracellular Ca^{2+} treatments, 10 mM $CaCl_2$ were applied to the guard cells in stomatal assay buffer 5 min after the measurement (C, D). (E) Percentage bar chart represents the percent (%) of guard cells showing transient $[Ca^{2+}]_{cyt}$ elevations. The number of transient $[Ca^{2+}]_{cyt}$ elevations under mock and 10 mM $CaCl_2$ treatment in WT and *cngc2-3* mutants were counted when the fluorescence ratio (F535/F480) increased ≥0.1 unit from the baseline. In this figure, Col-0 was used as wild-type (WT). The significance of differences between different treatments were determined by chi-squared ($\chi 2$) test, *$P < 0.05$, **$P < 0.01$. The “ns” indicates non-significant difference where $P > 0.05$.