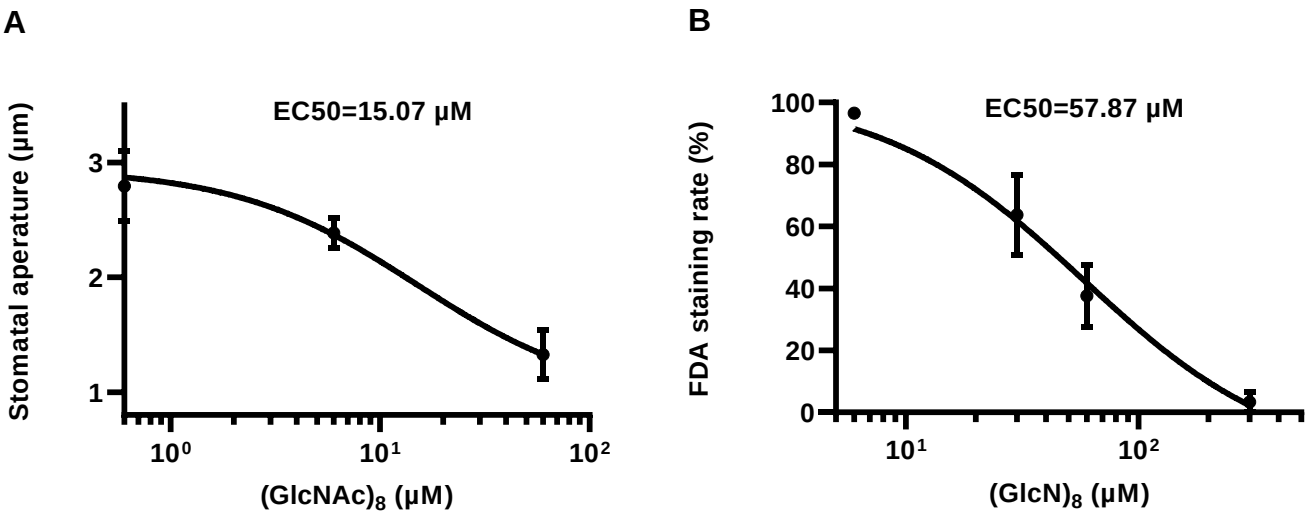

Figure S1. EC50 values for $(GlcNAc)_8$-induced stomatal closure and $(GlcN)_8$-induced reduction of FDA staining rate

The data of stomatal closure and reduction of FDA staining rate are from Figure 1A and Figure 7A, respectively. The dose response curves and EC50 values were calculated via non-linear regression using Prism 8.2.1 software (GraphPad software).

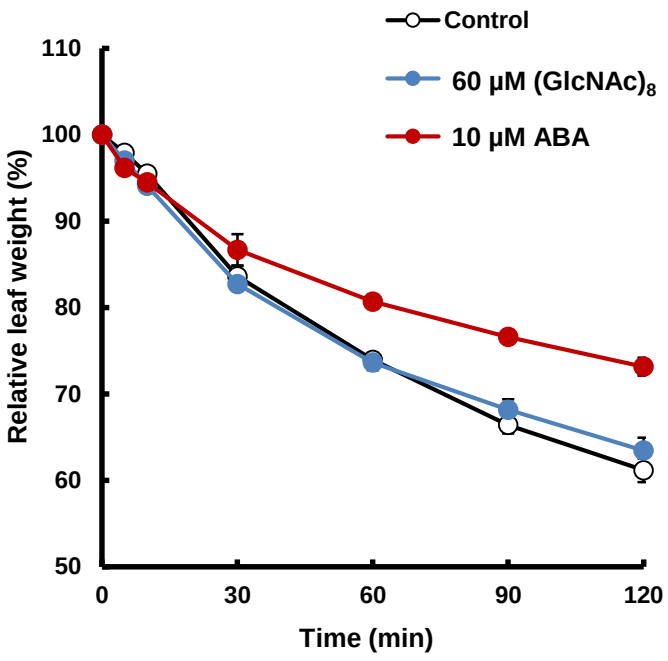

Figure S2. Water loss from detached leaves treated with ABA and $(GlcNAc)_8$

Relative water loss was represented by the weight of the leaves at various time points divided by the original weight. Data are mean ± SEM (*n*=3).

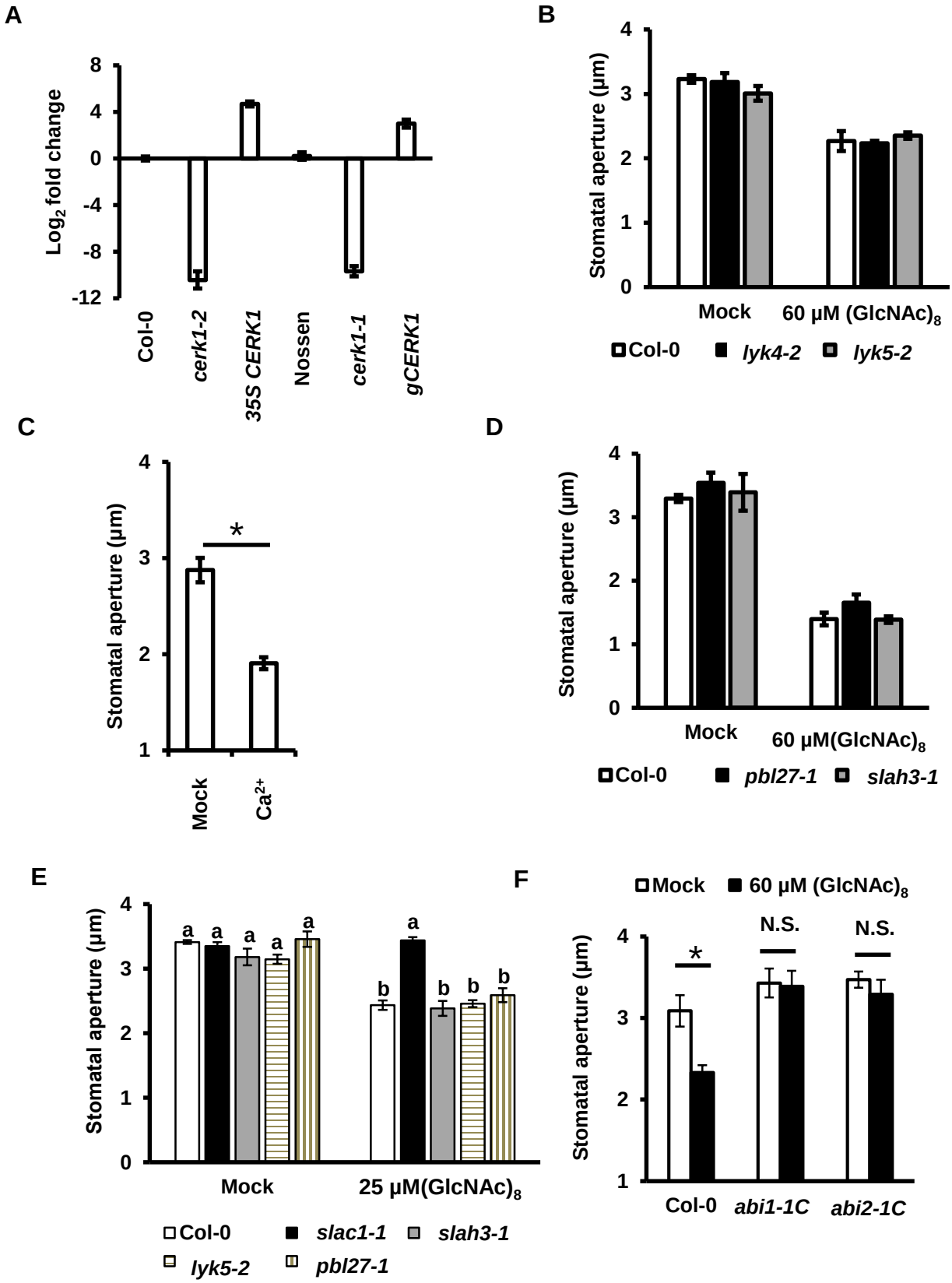
A
Log2 fold change
8
4
0
-4
-8
-12
Col-0
cerk1-2
35S CERK1
Nossen
cerk1-1
gCERK1
B
Stomatal aperture (µm)
4
3
2
1
0
Mock
60 µM (GlcNAc)8
Col-0
lyk4-2
lyk5-2
C
Stomatal aperture (µm)
4
3
2
1
*
Mock
Ca2+
D
Stomatal aperture (µm)
4
3
2
1
0
Mock
60 µM(GlcNAc)8
Col-0
pbl27-1
slah3-1
E
Stomatal aperture (µm)
4
3
2
1
0
a
a
a
a
a
b
a
b
b
b
Mock
25 µM(GlcNAc)8
Col-0
slac1-1
slah3-1
lyk5-2
pbl27-1
F
Mock
60 µM (GlcNAc)8
Stomatal aperture (µm)
4
3
2
1
*
N.S.
N.S.
Col-0
abi1-1C
abi2-1C

Figure S3. $(GlcNAc)_8$-induced stomatal closure in *lyk4*, *lyk5*, *pbl27*, *slah3, abi1-1C* and *abi2-1C*

(A) Transcript levels of *CERK1.* The levels of *CERK1* transcript with *UBQ10* as a reference in leaves of different genotypes were normalized to that of Col-0. Data are mean ± SEM (*n*=3).

(B) Stomatal closure induced by 60 µM $(GlcNAc)_8$ in Col-0, *lyk4-2* and *lyk5-2*. Averages from three independent experiments (90 total stomata per bar) are shown. Data are mean ± SEM (*n*=3).

(C) Effect of 1mM Ca^{2+} on cerk1-2 stomatal aperture.

(D) Stomatal closure induced by 60 µM $(GlcNAc)_8$ in Col-0, *pbl27-1* and *slah3-1*. Averages from three independent experiments (90 total stomata per bar) are shown. Data are mean ± SEM (*n*=3).

(E) Stomatal closure induced by 25 µM $(GlcNAc)_8$ in Col-0, *slac1-1*, *slah3-1*, *lyk5-2* and *pbl27-1*. Averages from four independent experiments (120 total stomata per bar) are shown. Data are mean ± SEM (*n*=4). Different letters indicate statistical significance (*P*<0.05, ANOVA with Tukey's test).

(F) Stomatal closure induced by 60 µM $(GlcNAc)_8$ in Col-0, *abi1-1C* and *abi2-1C*. Averages from three independent experiments (90 total stomata per bar) are shown. Data are mean ± SEM (*n*=3). Student's t test: *, *P*<0.05. N.S., No significant difference.

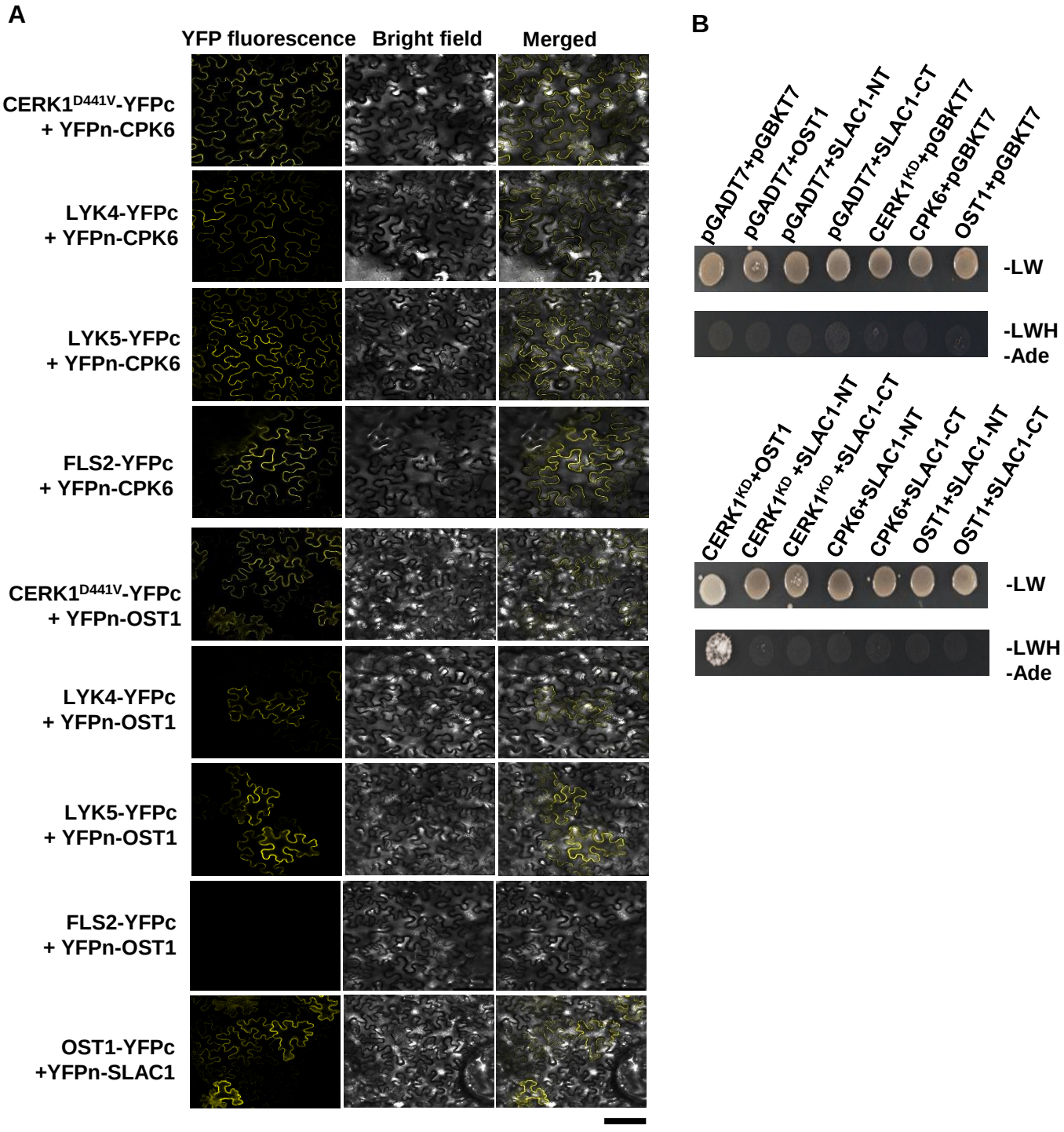

Figure S4. Additional BiFC and yeast-two hybrid experiments

(A) Confocal microscopy of *N. benthamiana leaves* transiently expressing the indicated split-YFP constructs. Representative images are shown. Scale bar, 100 µm.

(B) Yeast two-hybrid assays with the indicated expressing combinations.

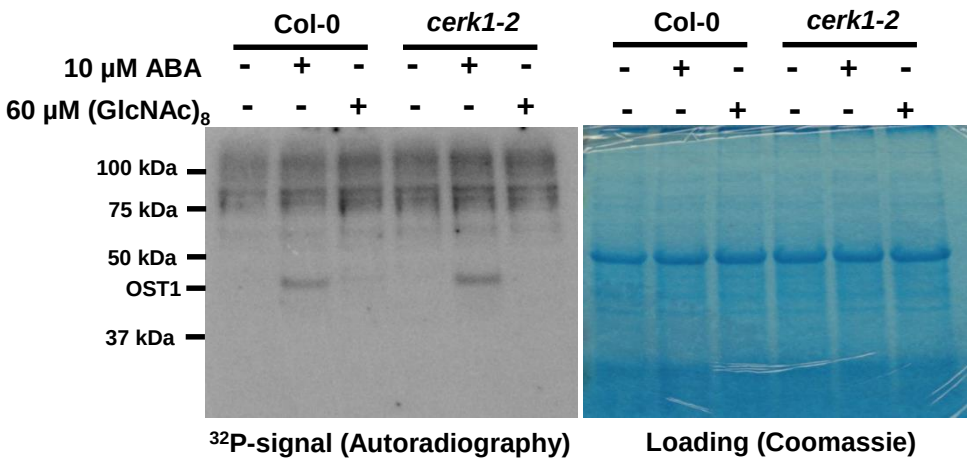

Figure S5. Kinase activity of OST1 in response to ABA and $(GlcNAc)_8$

In-gel kinase assays with Histone-III as substrate for whole seedling protein. The experiment has been repeated three times with similar results.

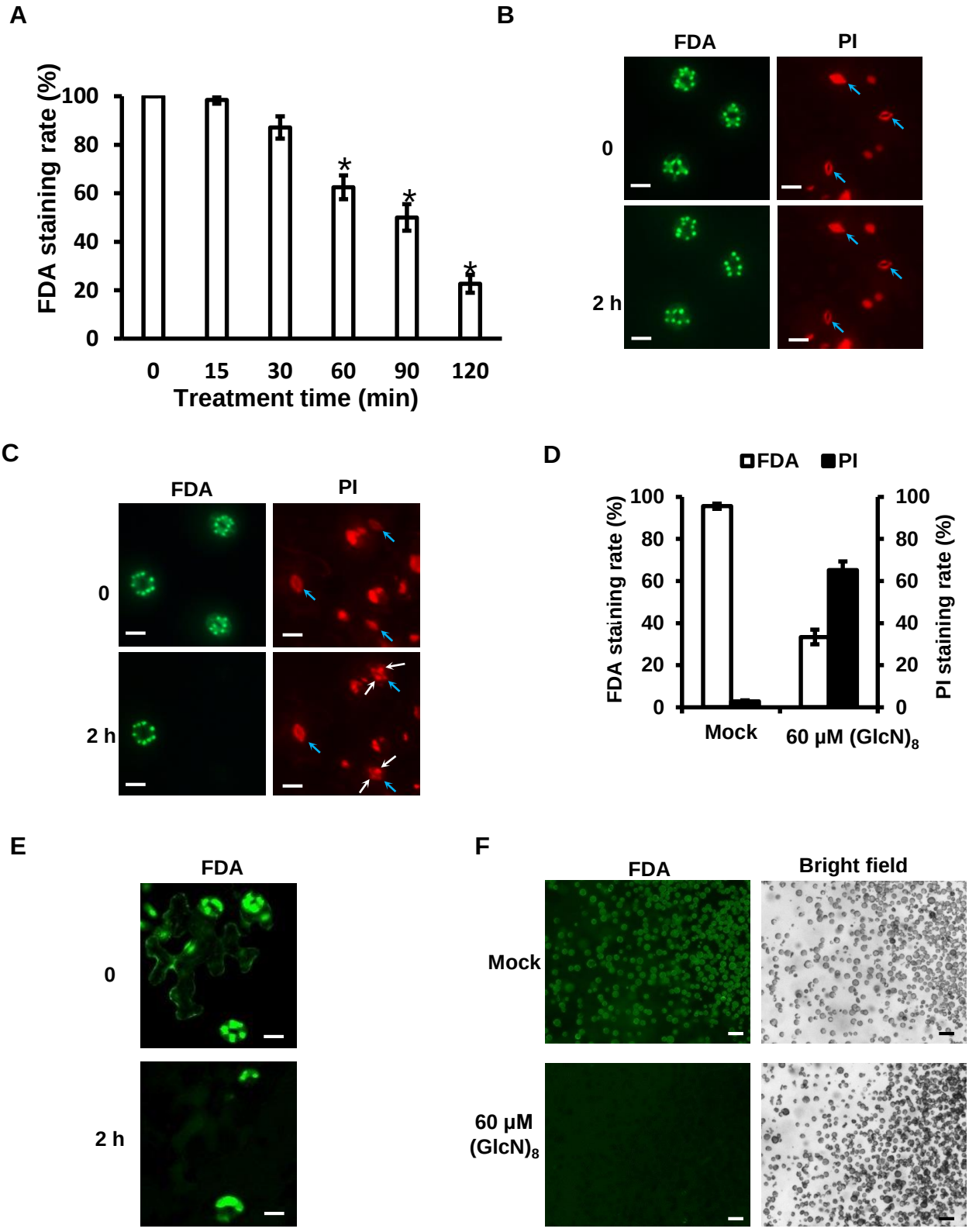
A
FDA staining rate (%)
100
80
60
40
20
0
0
15
30
60
90
120
Treatment time (min)
B
FDA
PI
0
2 h
C
FDA
PI
0
2 h
D
FDA
PI
FDA staining rate (%)
PI staining rate (%)
Mock
60 μM (GlcN)8
E
FDA
0
2 h
F
FDA
Bright field
Mock
60 μM
(GlcN)8

Figure S6. $(GlcN)_8$-induced cell death in Col-0

(A) Time course of $(GlcN)_8$-induced guard cell death. Guard cell death was evaluated by FDA staining. The time starting $(GlcN)_8$ treatment was considered as 0 time point. Averages from three independent experiments (360 guard cells in total) are shown. Data are mean ± SEM (*n*=3). Asterisks indicate statistical significance compared to that of 0 time point (*P*<0.05, Student's t test).

(B-D) FDA and PI double staining of guard cell treated with 60 µM $(GlcN)_8$. Epidermal tissues were double stained with FDA and PI to find live guard cells. After 2 h $(GlcN)_8$ treatment, the same epidermal tissues were stained with FDA and PI again to distinguish live and dead guard cells. The same guard cells before and after $(GlcN)_8$ treatment were used for quantification. Representative staining images were shown in (B) for Mock treatment and (C) for $(GlcN)_8$ treatment. Blue arrowheads, stomatal pores stained by PI; white arrowheads, nuclei of dead guard cells stained by PI; scale bar, 20 µm. Quantification of staining rates was shown in (D). Averages from three independent experiments (200 to 250 guard cells per bar) are shown. Data are mean ± SEM (*n*=3).

(E) Effect of $(GlcN)_8$ on leaf epidermal cell viability. The same cells were imaged before and after treatment of 60 µM $(GlcN)_8$. Scale bar, 20 µm.

(F) Effect of $(GlcN)_8$ on mesophyll protoplast viability. Mesophyll protoplasts were stained with FDA after 2 h $(GlcN)_8$ treatment. Scale bar, 100 µm.

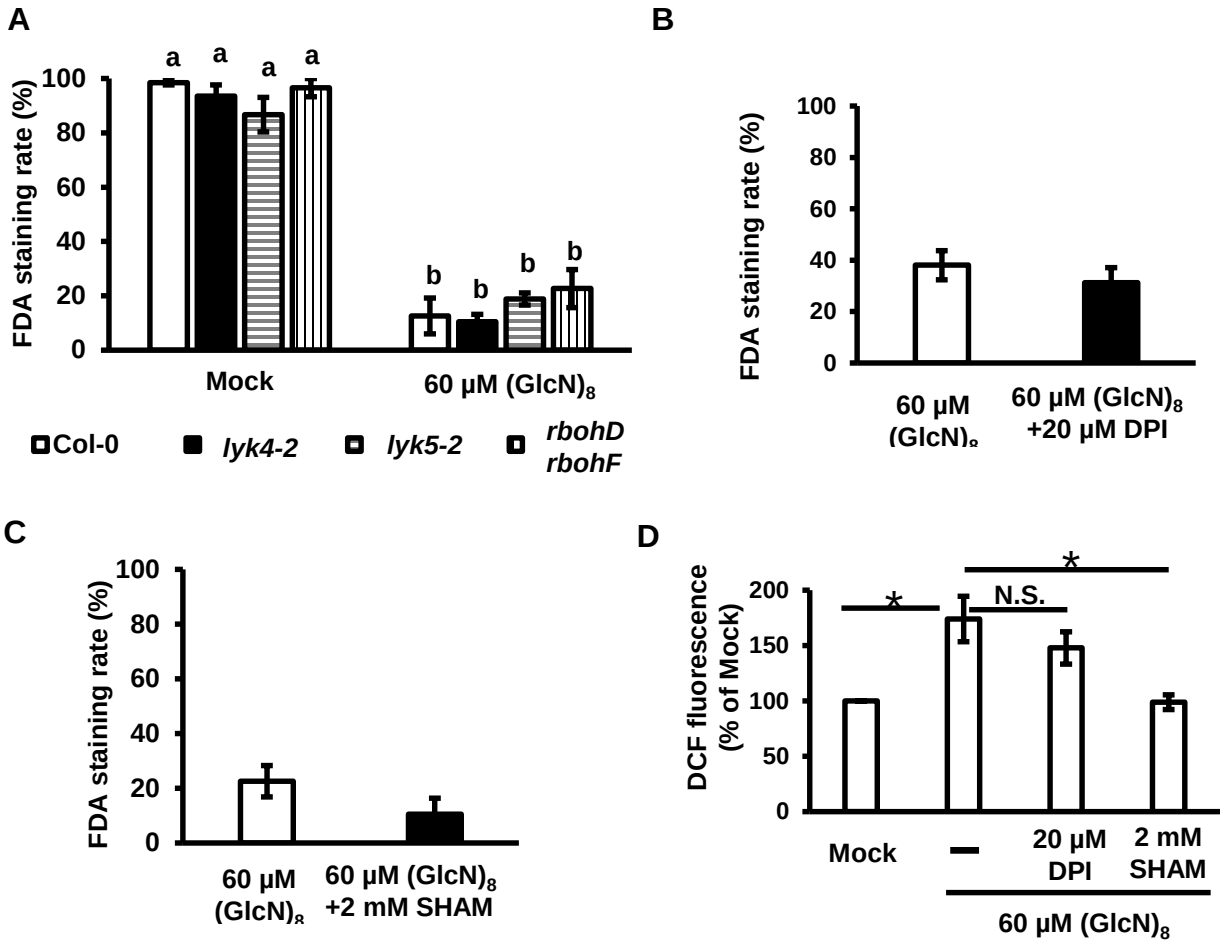

Figure S7. Effect of DPI, SHAM, *lyk4*, *lyk5* and *rbohD rbohF* mutations on $(GlcN)_8$-induced guard cell death

(A) Effect of *lyk4*, *lyk5* and *rbohD rbohF* mutations on $(GlcN)_8$-induced guard cell death. Averages from three independent experiments (200 to 250 guard cells per bar) are shown. Data are mean ± SEM (*n*=3). Different letters indicate statistical significance (*P*<0.05, ANOVA with Tukey's test).

(B and C) Effect of DPI and SHAM on $(GlcN)_8$-induced guard cell death. Averages from three independent experiments (200 to 250 guard cells per bar) are shown. Data are mean ± SEM (*n*=3).

(D) ROS accumulation induced by $(GlcN)_8$ in guard cells. ROS accumulation was expressed as the percentage of DCF fluorescence levels to that of Mock treatment. Averages from three independent experiments (more than 120 total guard cells per bar) are shown. Data are mean ± SEM (*n*=3). Student's t test: *, *P*<0.05. N.S., No significant difference.

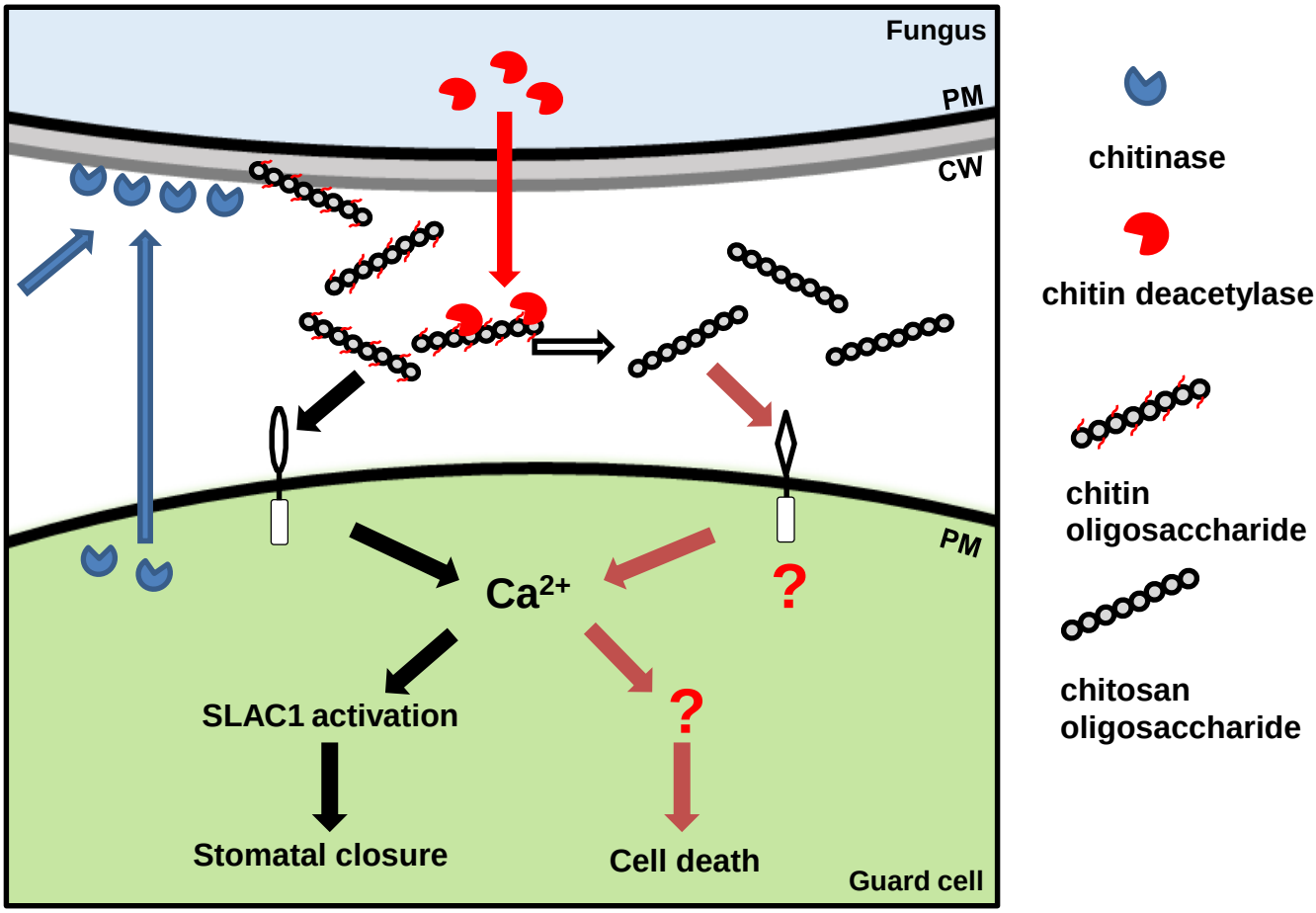

Figure S8. Proposed model of guard cell-fungus interaction

During fungal penetration through stomata, chitin in the cell wall of fungi is digested by chitinases preexisted in the apoplast or secreted by plant cells, leading to the release of CTOS. Guard cells then perceive the CTOS through its receptor, CERK1, which induces SLAC1 activation through a Ca^{2+}-dependent pathway and consequently stomatal closure to prevent fungal invasion. On the other hand, fungi secrete chitin deacetylases, converting CTOS to CSOS, to evade CTOS-triggered immune response. When CSOS accumulates over a threshold, it is sensed by yet-unknown receptors in the plasma membrane, which triggers cell death also in a Ca^{2+}-dependent manner to inhibit fungal infection. PM, plasma membrane; CW, cell wall. For simplicity, plant cell wall is not illustrated.

Table S1. Primers for vector construction

Assay	Constructs	Primer name	Primer sequence (5'→3')
BiFC	pXY106-CPK6	CPK6-BclI-F	CGTGATCAATGGGCAATTCATGTCGTGG
		CPK6-SalI-R	CGCGTCGACCTACACATCTCTCATGCTGAT
	pXY106-OST1	OST1-BamHI-F	CGGGATCCATGGATCGACCAGCAGTGA
		OST1-SalI-R	CGCGTCGACTCACATTGCGTACACAATCTC
	pXY106-SLAC1	SLAC1-BamHI-F	CGGGATCCATGGAGAGGAAACAGTCAAAT
		SLAC1-SalI-R	CGCGTCGACTCAGTGATGCGACTCTTCCTC
	pXY104-LYK4	LYK4-Sal1-F	GGGATCCTCTAGAGTCGACATGATCTCGTTTTCATTTC
		LYK4-Sal1-R	CGCTGCCACCGCCGTCGACGTACGACGATTCTTCCCAG
	pXY104-LYK5	LYK5-Sal1-F	GGGATCCTCTAGAGTCGACATGGCTGCGTGTACACTC
		LYK5-Sal1-R	CGCTGCCACCGCCGTCGACGTTGCCAAGAGAGCCGGAAC
	pXY104-FLS2	FLS2-Sal1-F	GGGATCCTCTAGAGTCGACATGAAGTTACTCTCAAAG
		FLS2-Sal1-R	CGCTGCCACCGCCGTCGACAACTTCTCGATCCTCGTTAC
	pXY104-CERK1^{D441V}	CERK1-BamHI-F1	CGGGATCCATGAAGCTAAAGATTTCTCTAAT
		CRRK1-SalI-R1	CGCGTCGACCCGGCCGGACATAAGACTGAC
		CERK1-F2	GTTTATGTCCATAGGGtCATTAAATCTGCCAAT
		CERK1-R2	ATTGGCAGATTTAATGaCCCTATGGACATAAAC
Yeast two-hybrid	pGADT7-CREK1^{KD}	CREK1^{KD}-EcoRI-F	CATGGAGGCCAGTGAATTCAATTTGTCTTTTAAGATTG
		CREK1^{KD}-EcoRI-R	GCCCACCCGGGTGGAATTCCCGGCCGGACATAAGACTG
	pGBKT7-CPK6	CPK6-EcoRI-F	GCCATGGAGGCCGAATTCATGGGCAATTCATGTCGTG
		CPK6-SalI-R	TGCGGCCGCTGCAGGTCGACGCACATCTCTCATGCTGATG
	pGBKT7-OST1	OST1-EcoRI-F	GCCATGGAGGCCGAATTCATGGATCGACCAGCAGTGA
		OST1-SalI-R	TGCGGCCGCTGCAGGTCGACGCATTGCGTACACAATCTC
	pGBKT7-SLAC1 NT	SLAC1 NT-EcoRI-F	GCCATGGAGGCCGAATTCATGGAGAGGAAACAGTCAAATG
		SLAC1 NT-SalI-R	TGCGGCCGCTGCAGGTCGACGTAGGAGAAACGGCCATTG
	pGBKT7-SLAC1 CT	SLAC1 CT-EcoRI-F	GCCATGGAGGCCGAATTCTTTGTCTGGCAAACGTTG
		SLAC1 CT-SalI-R	TGCGGCCGCTGCAGGTCGACGGTGATGCGACTCTTCCTC