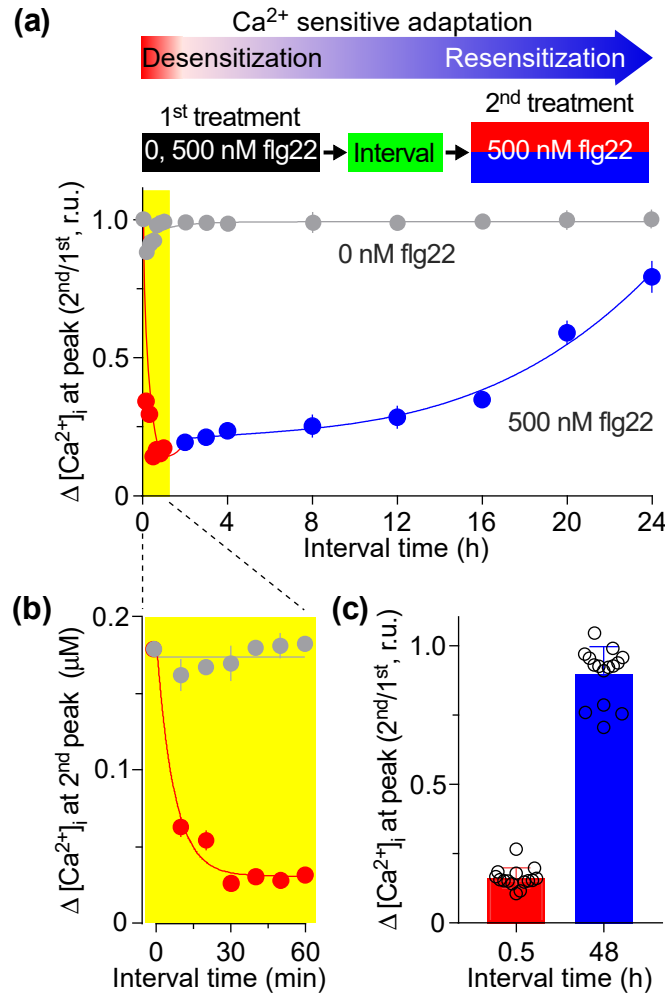


**FIGURE 1. Desensitization and resensitization of flg22-induced  $[Ca^{2+}]_i$  increases (FICA).**

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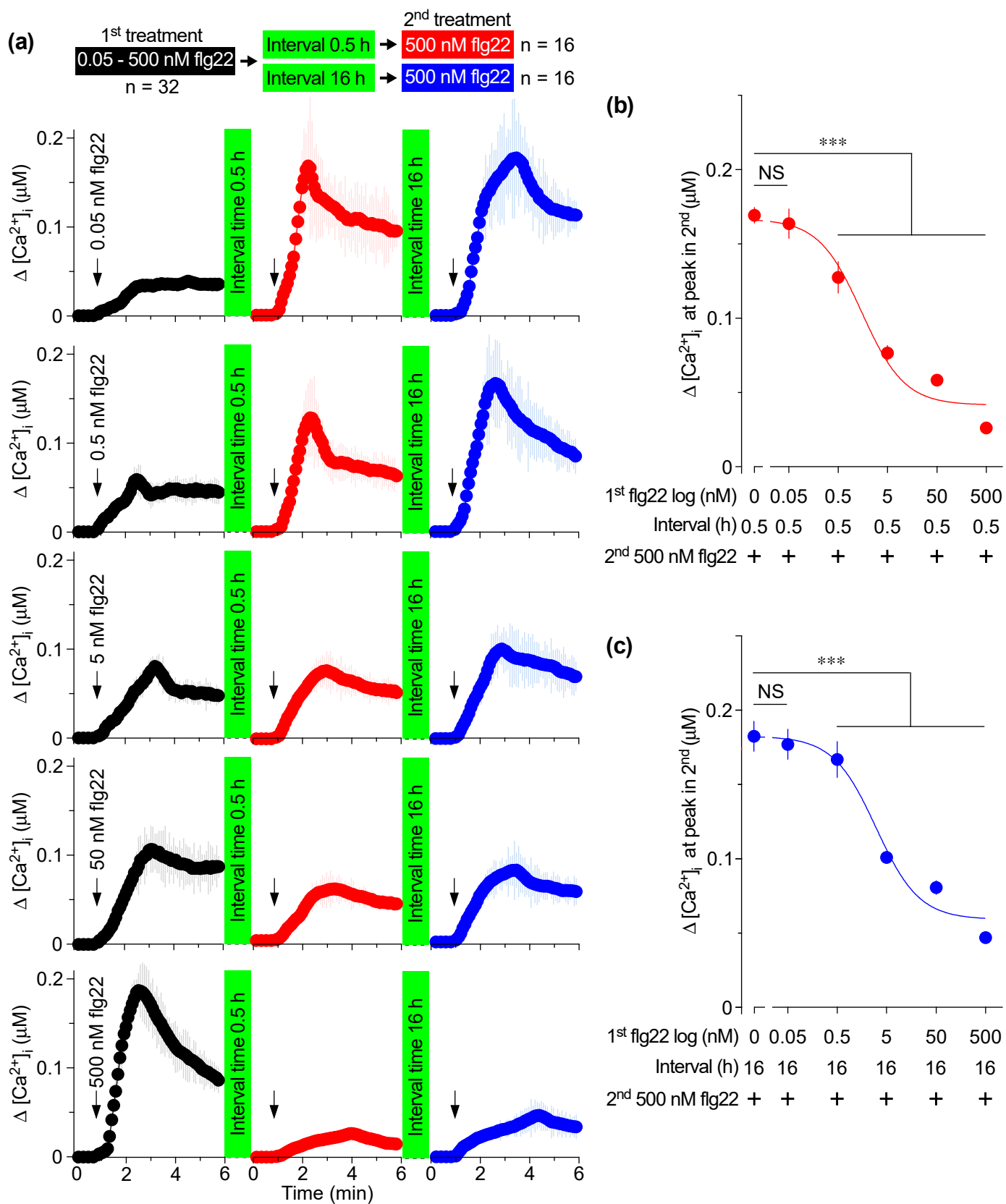
- (a)** Peak values of flg22-induced  $[Ca^{2+}]_i$  increases (FICA) ( $\Delta [Ca^{2+}]_i$ ) in *Arabidopsis* seedlings plotted against the applied concentration of flg22. Five-days-old seedlings expressing the  $Ca^{2+}$ -indicator protein aequorin were transplanted to 96-well plate and treated with a solution containing flg22, and  $[Ca^{2+}]_i$  was analyzed using aequorin luminometry spectroscopy. Values are mean  $\pm$  s.e.m.,  $n = 16$ .
- (b)** Schematic of experimental designs for analyzing desensitization and resensitization of FICA. The seedlings were treated by flg22 (1<sup>st</sup> treatment), and flg22 washed out for a period of time (Interval); subsequently seedlings were treated with flg22 again (2<sup>nd</sup> treatment) to analyze desensitization and resensitization of FICA.
- (c)** Increase of  $\Delta [Ca^{2+}]_i$  in the 1<sup>st</sup> round of 0 nM flg22 elicitation (gray) and the 2<sup>nd</sup> round of 500 nM flg22 elicitation with the interval time of 0.5 h as a control for desensitization analyses (black). Values are mean  $\pm$  s.d.,  $n = 16$ .
- (d)** Increase of  $\Delta [Ca^{2+}]_i$  in the 1<sup>st</sup> round of 500 nM flg22 elicitation (black) and the 2<sup>nd</sup> round of 500 nM flg22 elicitation with the interval time of 0.5 h for desensitization analyses (red). Values are mean  $\pm$  s.d.,  $n = 16$ .
- (e)** Increase of  $\Delta [Ca^{2+}]_i$  in the 1<sup>st</sup> round of 500 nM flg22 elicitation (black) and 2<sup>nd</sup> round of 500 nM flg22 elicitation with the interval time of 24 h for resensitization analyses (blue). Values are mean  $\pm$  s.d.,  $n = 16$ .



**FIGURE 2. Time course of desensitization and resensitization of flg22-induced  $[Ca^{2+}]_i$  increases.**

- (a) Sensitive adaptation of FICA undergoes rapid desensitization (red) and **slow** resensitization (blue), and is independent of the control of  $[Ca^{2+}]_i$  increases induced by mechanical stimuli (1<sup>st</sup> round of 0 nM flg22 elicitation, gray);  $[Ca^{2+}]_i$  increases were calculated by the second peak value ratio to the control value, and normalized to  $[Ca^{2+}]_i$  increases induced by water at time 0 as 1 (r.u. relative unit). Values are mean  $\pm$  s.d.,  $n = 16$ . The experiments were repeated 3 times with similar results.
- (b)  $\Delta[Ca^{2+}]_i$  peak values in the 2<sup>nd</sup> round of flg22 elicitation from the data as in **a** were plotted as a function against the intervals, which covered the whole desensitization adaptation (red). Values are mean  $\pm$  s.d.,  $n = 16$ .

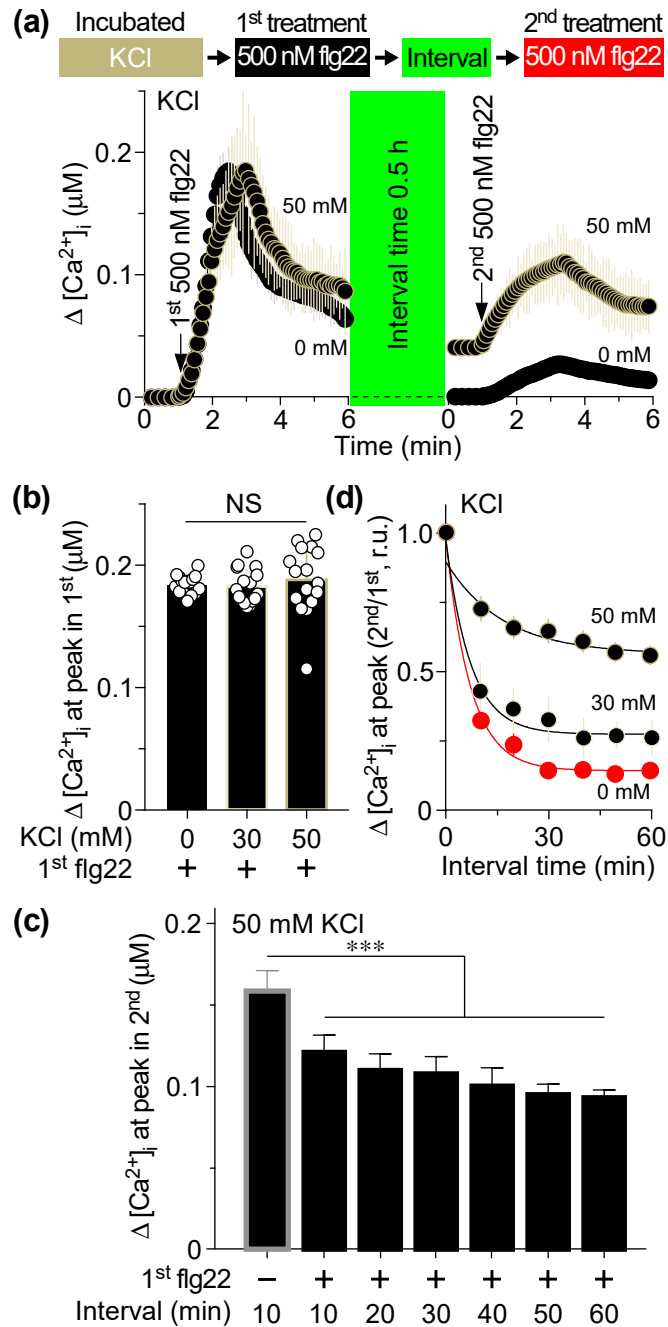
- (c) The competency of desensitization (0.5 h, red) and resensitization (48 h, blue) of FICA from the data as in **a**. Values are mean  $\pm$  s.d.,  $n = 16$ .



**FIGURE 3. The adaptation of FICA is dependent on the concentrations of flg22 applied.**

**FIGURE 3. The adaptation of FICA is dependent on the concentrations of flg22 applied.**

- (a) Dose effects of flg22 on the sensitive adaptation of FICA. To allow direct comparisons, all seedlings were analyzed using the same 96-well plate and elicited with flg22 concentrations ranging from 0.05 nM to 500 nM for the 1<sup>st</sup> round of elicitation ( $n = 32$ ). These seedlings were washed, and then treated with 500 nM flg22 at 0.5 h interval for desensitization analyses (red,  $n = 16$ ) and at 16 h interval for resensitization analyses (blue,  $n = 16$ ). The intervals were calculated according to the time when the seedlings were washed with H<sub>2</sub>O after the 1<sup>st</sup> round of flg22 elicitation. Values are mean  $\pm$  s.d..
- (b) Peak values of  $\Delta [\text{Ca}^{2+}]_i$  in the 2<sup>nd</sup> round of flg22 elicitation at intervals of 0.5 h plotted as a function against the concentrations of applied flg22 in the 1<sup>st</sup> round. Values are mean  $\pm$  s.d.,  $n = 16$ . NS,  $P = 0.0792$ ; \*\*\*,  $P < 0.001$ ; one-way ANOVA.
- (c) Peak values of  $\Delta [\text{Ca}^{2+}]_i$  in the 2<sup>nd</sup> round of flg22 elicitation at intervals of 16 h plotted as a function against the concentrations of applied flg22 in the 1<sup>st</sup> round. Values are mean  $\pm$  s.d.,  $n = 16$ . NS,  $P = 0.2104$ ; \*\*\*,  $P < 0.001$ ; one-way ANOVA.



**FIGURE 4. The desensitization of FICA partly depends on the depolarization of the plasma membrane.**

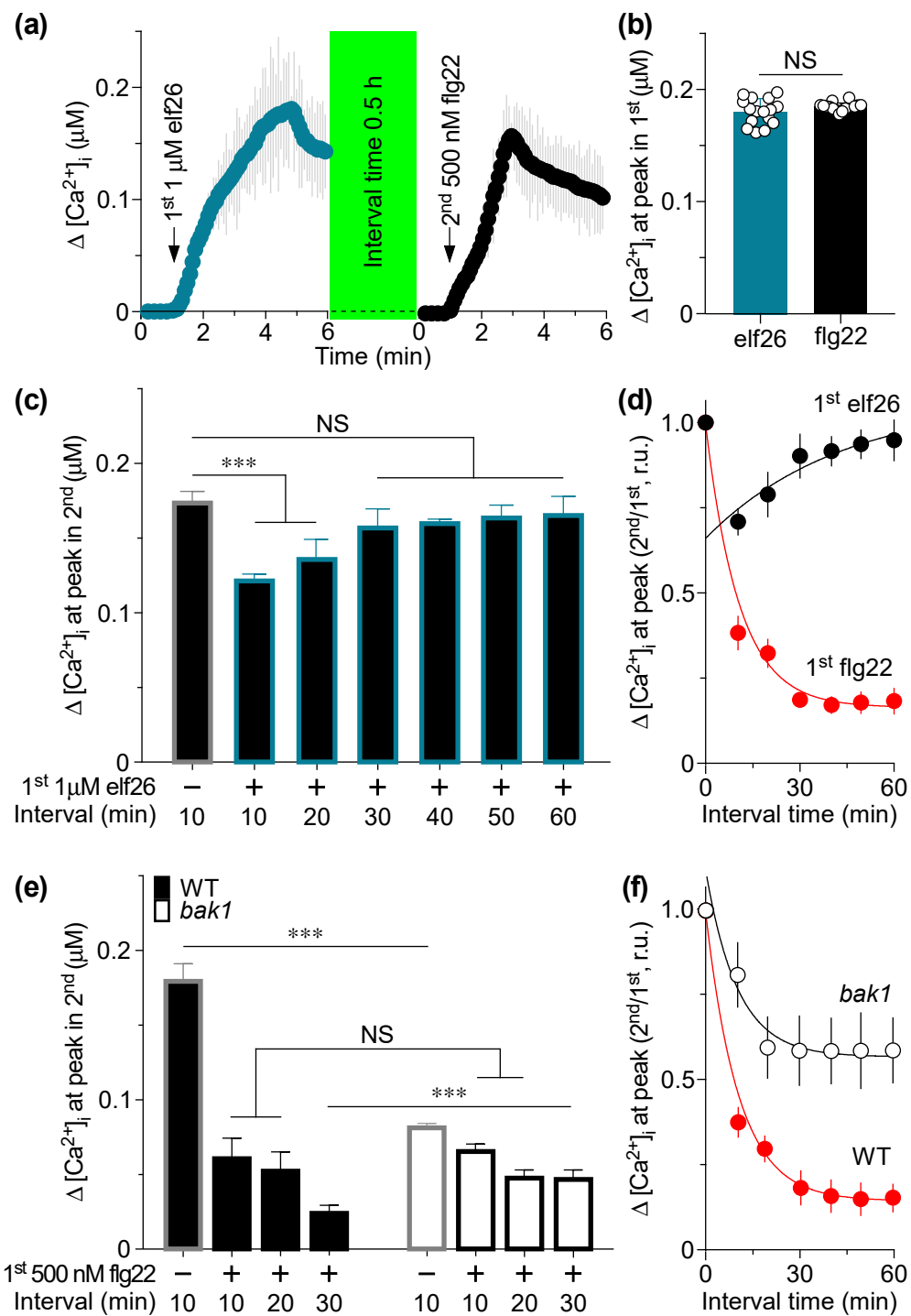
(a) Kinetics of FICA in seedlings preincubated with 50 mM KCl (50 mM) or with H<sub>2</sub>O (0 mM KCl); to allow direct comparisons, all seedlings were performed in the same 96-well plate

and preincubated with KCl or H<sub>2</sub>O for 0.5 h, and the  $\Delta [Ca^{2+}]_i$  increases were analyzed.

Values are mean  $\pm$  s.d.,  $n = 16$ .

- (b) Quantification of peak values of  $\Delta [Ca^{2+}]_i$  in the 1<sup>st</sup> round of flg22 elicitation in seedlings preincubated with 0 mM, 30 mM or 50 mM KCl. Values are mean  $\pm$  s.d.,  $n = 16$ . NS,  $P = 0.6043$ ;  $t$ -test.
- (c) Quantification of peak values of  $\Delta [Ca^{2+}]_i$  in the 2<sup>nd</sup> round of flg22 elicitation at indicated intervals in seedlings preincubated with 50 mM KCl before the 1<sup>st</sup> round of with (+) or without 500 nM flg22 (–) elicitation. Values are mean  $\pm$  s.d.,  $n = 16$ . \*\*\*,  $P < 0.001$ ;  $t$ -test.
- (d) The desensitization competency of  $[Ca^{2+}]_i$  plotted as a function against the increased intervals in seedlings preincubated with 0 mM, 30 mM or 50 mM KCl. Values are mean  $\pm$  s.d.,  $n = 16$ .

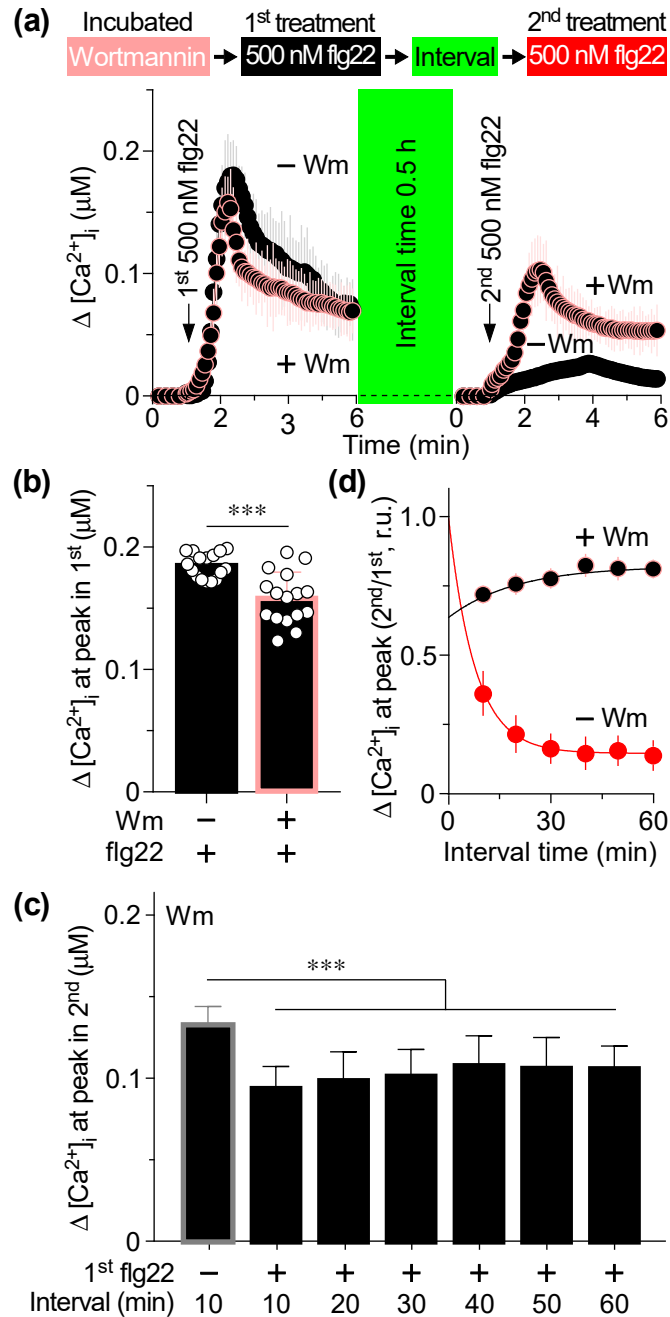




**FIGURE 5. The desensitization of FICA is flg22 ligand-specific and depends on the activity of flg22 binding to receptors.**

**FIGURE 5. The desensitization of FICA is flg22 ligand-specific and depends on the activity of flg22 binding to receptors.**

- (a)**  $\Delta [\text{Ca}^{2+}]_i$  evoked by 1<sup>st</sup> round of 1  $\mu\text{M}$  elf26 elicitation, and the 2<sup>nd</sup> round of 500 nM flg22 elicitation at 0.5 h interval for desensitization analyses. Values are mean  $\pm$  s.d.,  $n = 16$ .
- (b)** Peak values of  $\Delta [\text{Ca}^{2+}]_i$  evoked by 1  $\mu\text{M}$  elf26 (cyan) or 500 nM flg22 (black) in the 1<sup>st</sup> round of elicitation from data as in **a**. Values are mean  $\pm$  s.d.,  $n = 16$ . NS,  $P = 0.149$ ;  $t$ -test.
- (c)** Peak values of  $\Delta [\text{Ca}^{2+}]_i$  in the 2<sup>nd</sup> round of 500 nM flg22 elicitation at indicated intervals after 1<sup>st</sup> round of 0  $\mu\text{M}$  elf26 (–) or 1  $\mu\text{M}$  elf26 (+) elicitation from experiments similar to these in **a**. Values are mean  $\pm$  s.d.,  $n = 16$ . NS,  $P > 0.05$ ; \*\*\*,  $P < 0.001$ ;  $t$ -test.
- (d)** The desensitization of  $[\text{Ca}^{2+}]_i$  plotted as a function against the increased intervals from data as in **c**, which showed the competency for nonspecific ligands elf26 (black), and the specific ligands flg22 (red, as in Figure 2b). Values are mean  $\pm$  s.d.,  $n = 16$ .
- (e)** Peak values of  $\Delta [\text{Ca}^{2+}]_i$  in the 2<sup>nd</sup> round of 500 nM flg22 elicitation at indicated intervals in wild type (WT, black) and *bak1* mutant (white) seedlings. Values are mean  $\pm$  s.d.,  $n = 16$ . NS,  $P > 0.05$ ; \*\*\*,  $P < 0.001$ ; one-way ANOVA, multiple comparisons.
- (f)** The desensitization competency of  $[\text{Ca}^{2+}]_i$  plotted as a function against the increased intervals in *bak1* (black) and WT (red, as in Figure 2b as a control) seedlings. Values are mean  $\pm$  s.d.,  $n = 16$ .

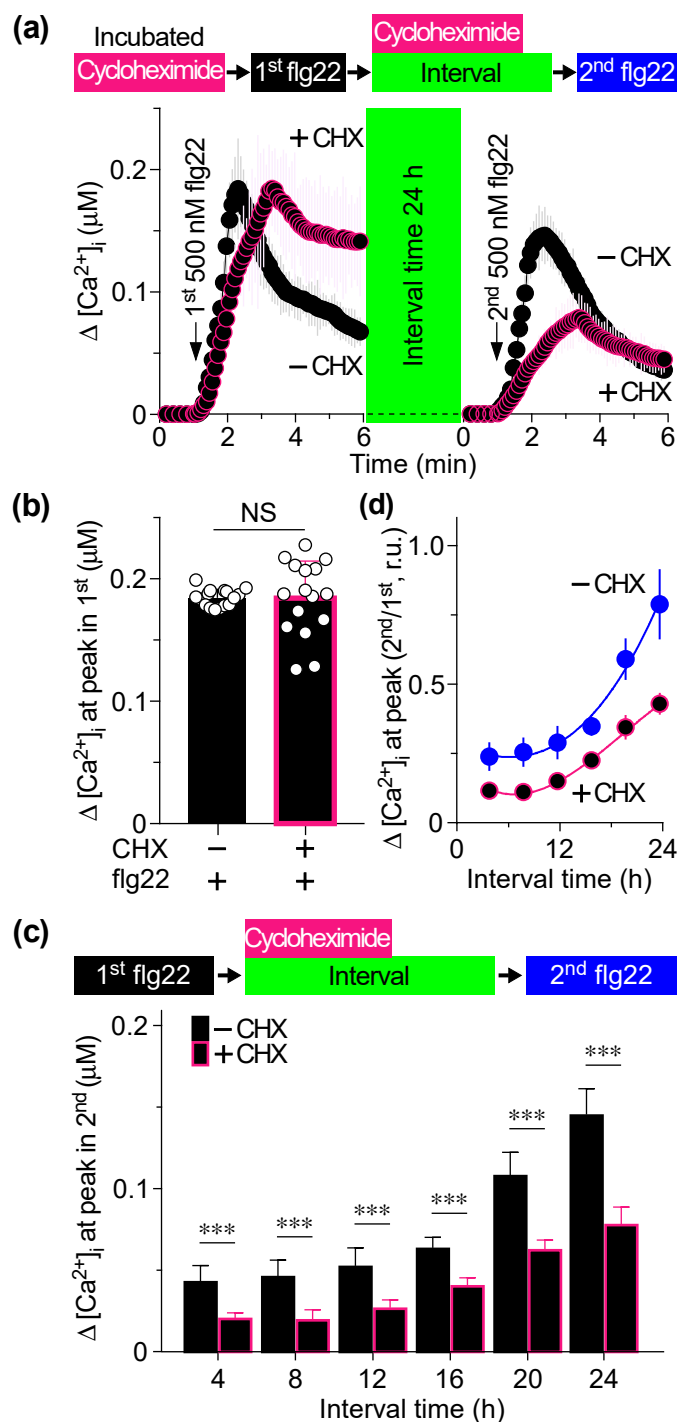


**FIGURE 6. The desensitization of FICA depends on the endocytosis of FLS2.**

(a) Kinetics of FICA in seedlings preincubated with DMSO (– Wm) or with wortmannin (+ Wm). To allow direct comparisons, all seedlings were performed in the same 96-well plate and preincubated with 30  $\mu\text{M}$  Wm or DMSO for 1 h, washed, and elicited with the 1<sup>st</sup> round of 500 nM flg22. These same seedlings were rewashed with water, and then treated with the

2<sup>nd</sup> round of 500 nM flg22 at interval of 0.5 h for desensitization analyses. Values are mean  $\pm$  s.d.,  $n = 16$ .

- (b) Quantification of peak values of  $\Delta [\text{Ca}^{2+}]_i$  in the 1<sup>st</sup> round of flg22 elicitation with DMSO or 30  $\mu\text{M}$  Wm preincubated from data as in **a**. Values are mean  $\pm$  s.d.,  $n = 16$ . \*\*\*,  $P < 0.001$ ;  $t$ -test.
- (c) Peak values of  $\Delta [\text{Ca}^{2+}]_i$  in the 2<sup>nd</sup> round of flg22 elicitation at indicated intervals with 30  $\mu\text{M}$  Wm preincubated before the 1<sup>st</sup> round of 0 nM flg22 (–) or 500 nM flg22 (+) elicitation. Values are mean  $\pm$  s.d.,  $n = 16$ . \*\*\*,  $P < 0.001$ ;  $t$ -test.
- (d) The desensitization competency of  $[\text{Ca}^{2+}]_i$  plotted as a function against the increased intervals with (+) or without (–) Wm preincubated from data similar to these in **c** and Figure 2b. Values are mean  $\pm$  s.d.,  $n = 16$ .



**FIGURE 7. The resensitization of FICA depends on the *de novo* synthesis of FLS2.**

(a) The effect of CHX on FICA. As illustrated, seedlings were preincubated with 50  $\mu$ M CHX (+) or DMSO (–) for 1 h, and analyzed for the  $\Delta$   $[Ca^{2+}]_i$  increases in the 1<sup>st</sup> round of 500 nM flg22 elicitation. These seedlings were washed and incubated with 50  $\mu$ M CHX or DMSO

for 2 h. After re-washed with water, these same seedlings were analyzed for the  $\Delta [\text{Ca}^{2+}]_i$  increases with the 2<sup>nd</sup> round of 500 nM flg22 elicitation at interval of 24 h. Values are mean  $\pm$  s.d.,  $n = 16$ .

- (b) Quantification of peak values of  $\Delta [\text{Ca}^{2+}]_i$  in the 1<sup>st</sup> round of 500 nM flg22 elicitation in seedlings pretreated with DMSO (–) or 50  $\mu\text{M}$  CHX (+) from experiments as in **a**. Values are mean  $\pm$  s.d.,  $n = 16$ . NS,  $P = 0.9833$ ;  $t$ -test.
- (c) Analyses of peak values of  $\Delta [\text{Ca}^{2+}]_i$  in the 2<sup>nd</sup> round of 500 nM flg22 elicitation at indicated intervals from experiments similar to these in **a**. Values are mean  $\pm$  s.d.,  $n = 16$ . \*\*\*,  $P < 0.001$ ,  $t$ -test.
- (d) The resensitizing competency of  $[\text{Ca}^{2+}]_i$  plotted as a function against the increased intervals with (+) or without (–) CHX incubated. Values are mean  $\pm$  s.d.,  $n = 16$ .

Table S1. Time course of early signaling events evoked by flg22 and the main components of resensitization of FICA.

Early signaling event	Start time	Peak time	Decay time	Resensitization time	Resensitization components	Reference
Ca <sup>2+</sup> channels/Ca <sup>2+</sup> increases	0.67-1 min	2-6 min	30 min	<b>16-48 h*</b>	1, 2, 3, 4, 5, 6, 7, 8, 9	(Jeworutzki et al., 2010; Ma et al., 2020; Ranf et al., 2011)
PM depolarization/ Ca <sup>2+</sup> , H <sup>+</sup> , Cl <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , K <sup>+</sup>	1 min	1-3 min	60 min	1-1.5 h	1, 2, 7, 9	(Jeworutzki et al., 2010; Yu et al., 2017)
MAPK cascade	5 min	15 min	40-60 min	2 h	1, 9	(Ranf et al., 2011; Robatzek et al., 2006; Smith et al., 2014)
Phosphorylation RbohD/ROS burst	4-6 min	10-15 min	30 min	3-16 h	1, 2, 3, 4, 6, 8, 9	(Ben Khaled et al., 2015; Boller & Felix, 2009; Ma et al., 2020; Smith et al., 2014)
FLS2 endocytosis	20-30 min	45-60 min	60-75 min	2-3 h	1, 9	(Beck et al., 2012; Jeworutzki et al., 2010; Mbengue et al., 2016)

\* Our current study.

Components in the flg22 signal transduction pathway:

1. Ligand-dependent activation of FLS2.
2. Activation of the co-receptor BAK1.
3. Ca<sup>2+</sup> influx and cytosolic Ca<sup>2+</sup> increases via the opening of Ca<sup>2+</sup> channels.
4. ROS burst resulted from the activation of Ca<sup>2+</sup>-dependent NADPH oxidases.
5. Activation of MAPK cascade.
6. BIK1 phosphorylated and dissociated from the FLS2/BAK1 complex.
7. The PM depolarization caused by the Ca<sup>2+</sup>-dependent activation of anion channels.
8. Endocytosis of ligand-activated FLS2.
9. The local accumulation of FLS2 at the PM mediated by the secretory trafficking of the SYP121.

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