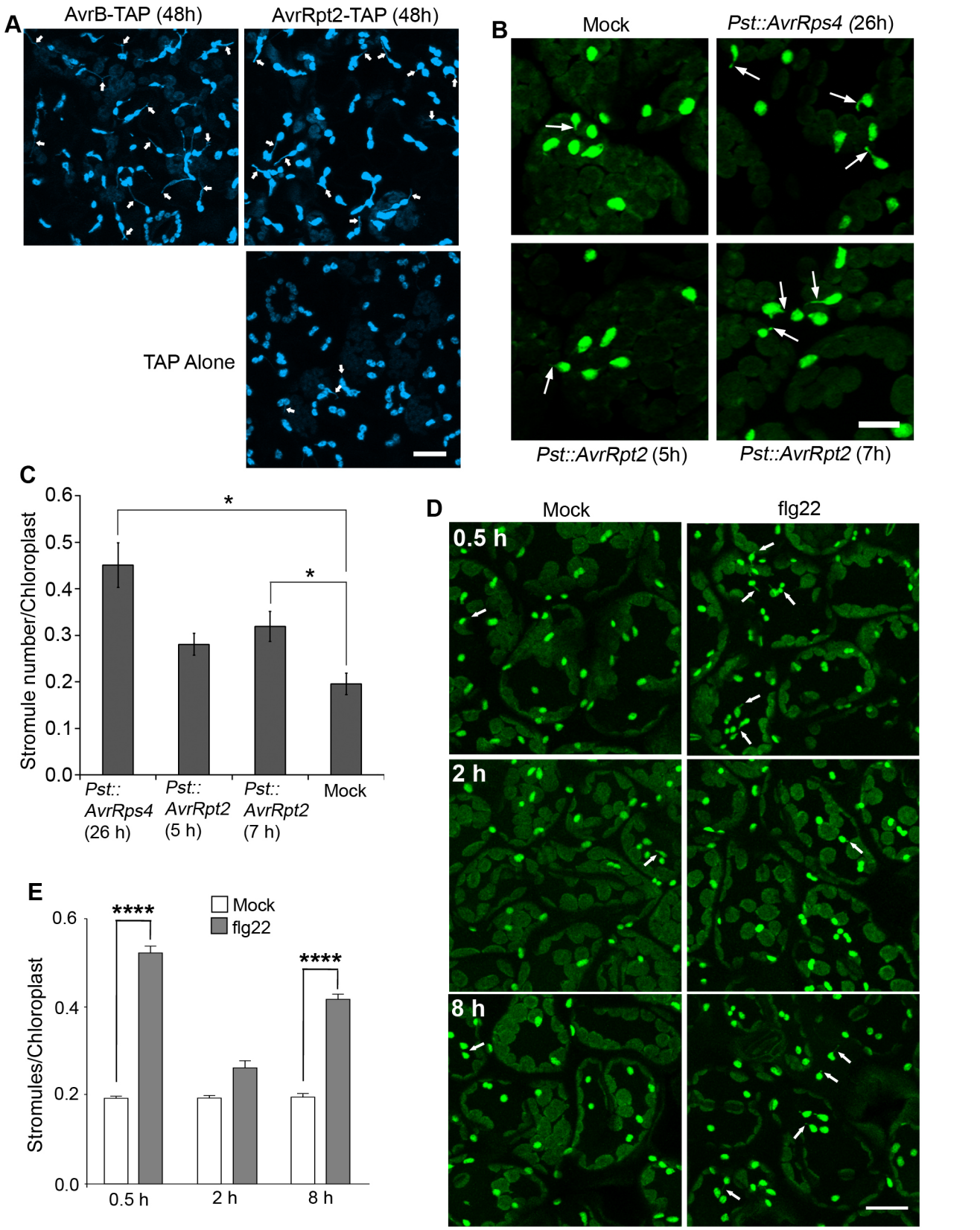

A
AvrB-TAP (48h)
AvrRpt2-TAP (48h)
TAP Alone
B
Mock
Pst::AvrRps4 (26h)
Pst::AvrRpt2 (5h)
Pst::AvrRpt2 (7h)
C
Stromule number/Chloroplast
0.0
0.1
0.2
0.3
0.4
0.5
0.6
*
*
Pst:: AvrRps4 (26 h)
Pst:: AvrRpt2 (5 h)
Pst:: AvrRpt2 (7 h)
Mock
D
Mock
flg22
0.5 h
2 h
8 h
E
Mock
flg22
Stromules/Chloroplast
0.0
0.2
0.4
0.6
****
****
0.5 h
2 h
8 h

Figure S2
Caplan et al.,

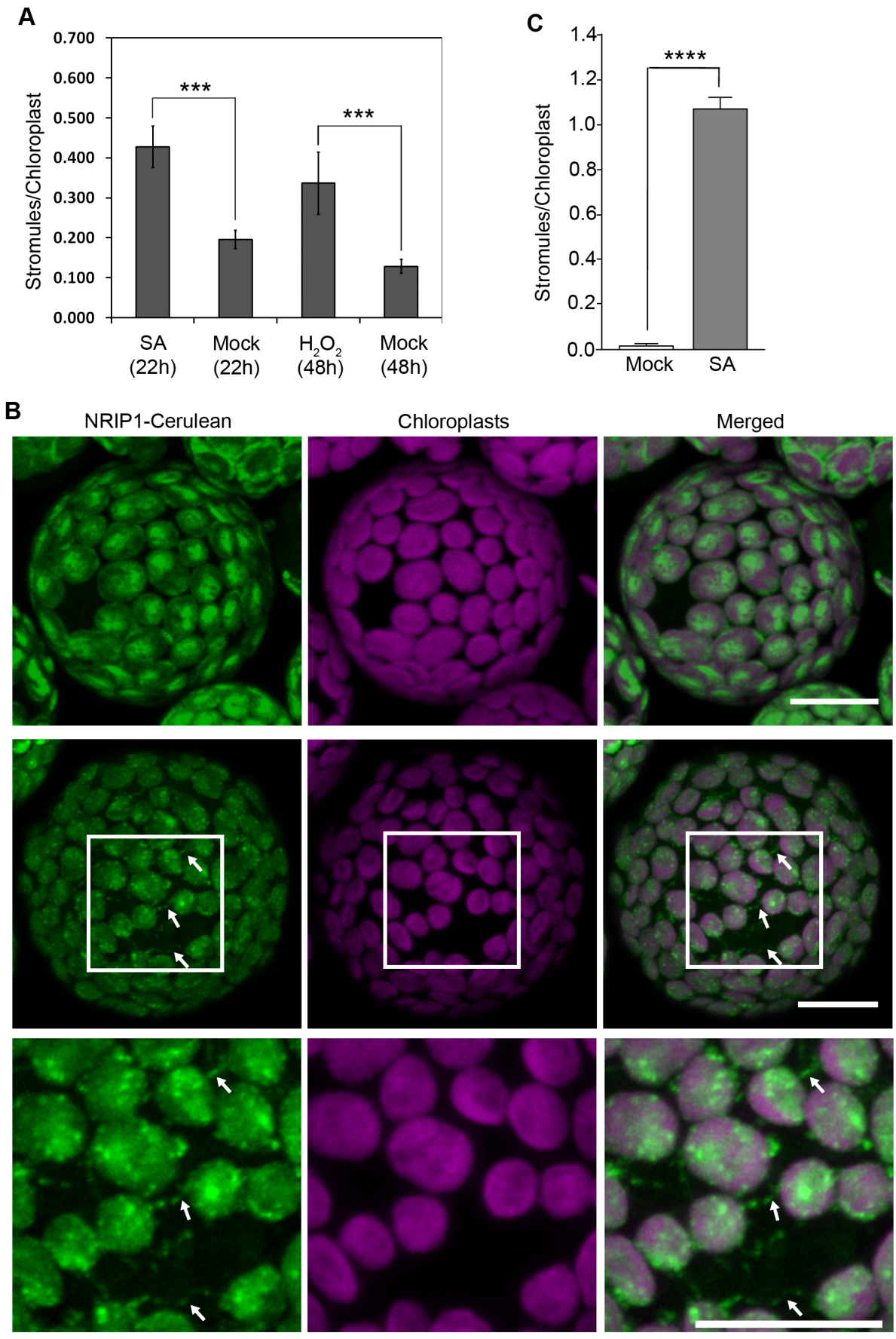


Figure S3
Caplan et al.,

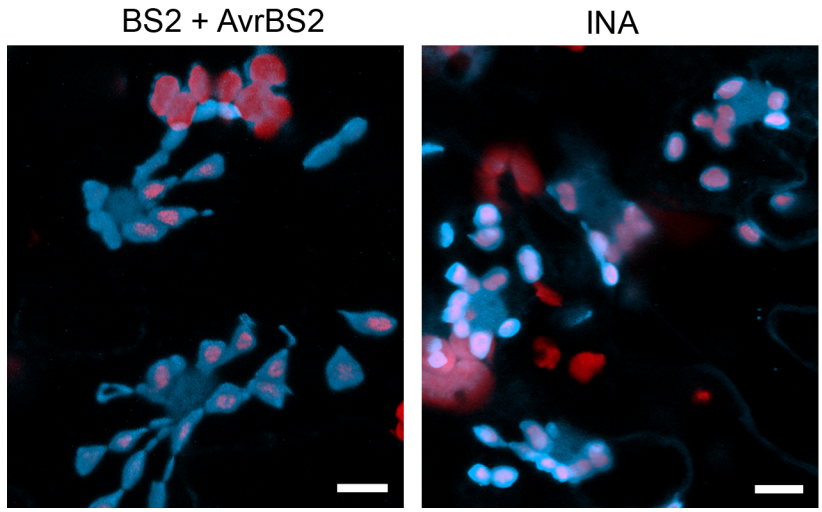
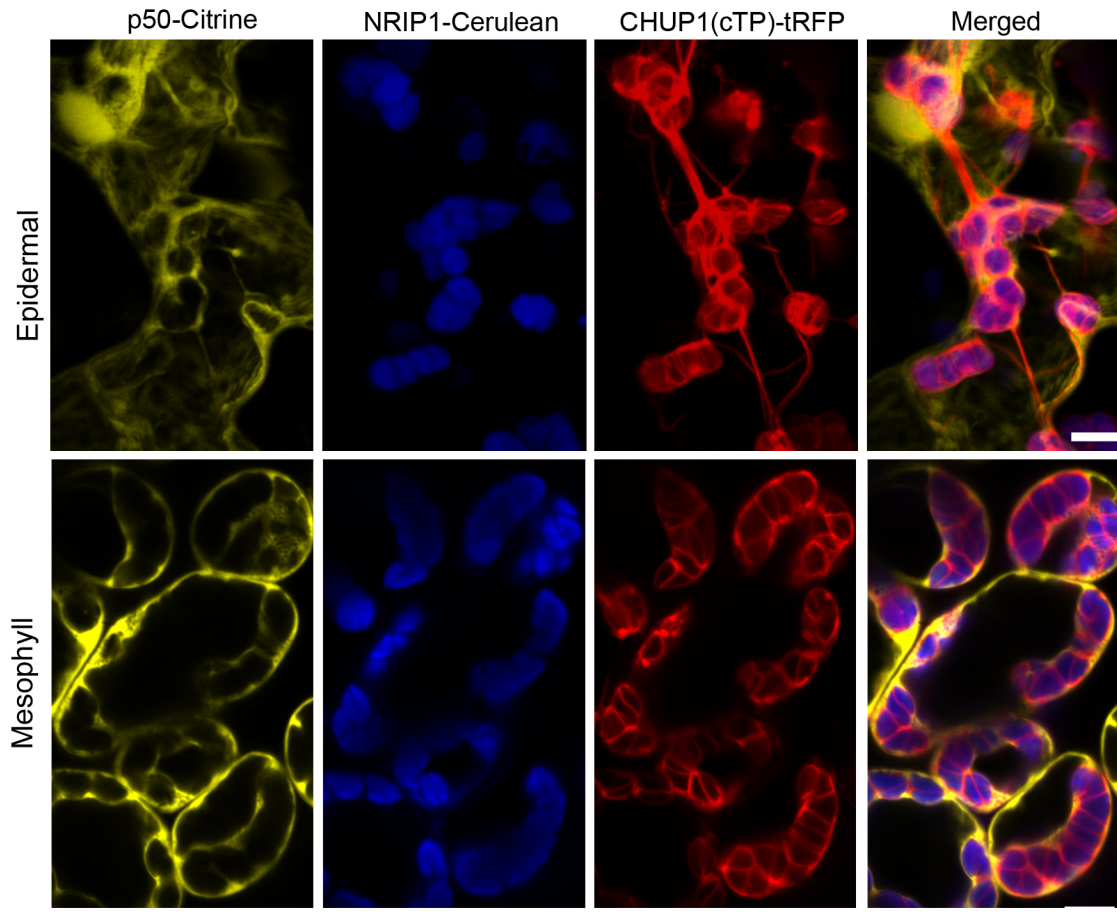


Figure S4
Caplan et al.,



Supplemental Figure Legends

Figure S1. Stromule induction during bacterial immunity in *Nicotiana*, Related to Figure 2

A. Transient expression of *Pseudomonas syringae* bacterial effector AvrB (left panel) and AvrRpt2 (right top panel) fused to a TAP tag and TAP alone (right bottom panel) in transgenic *N. benthamiana* plants expressing NRIP1-Cerulean (blue). Stromules were visualized by creating maximum intensity projections through Z-stacks acquired by confocal microscopy. Increase in stromules (arrows) after 48 h of expression of AvrB (left panel) and AvrRpt2 (right top panel) were observed compared to TAP alone control (right bottom panel). The larger chloroplasts with lower fluorescence intensity are in the mesophyll. Scale bar equals 10 μm.

B. *P. syringae pv tomato* (*Pst*) DC3000 bacteria expressing *AvrRps4* and *AvrRpt2* effectors at $1x10^6$ cfu/ml were infiltrated onto cTP-GFP *Arabidopsis* plants (green). Stromules were visualized by creating maximum intensity projections of confocal microscopy Z-stacks. Stromules were visible in plants expressing *Pst::AvrRps4* for 26 h (top right) or *Pst::AvrRpt2* for 7 h (bottom right) compared to *Pst::AvrRpt2* for 5h (bottom left) or the mock infiltration control (top left). Scale bar equals 10 μm.

C. Stromules per chloroplasts induced in experiments described in B were counted by confocal microscopy. Infection with *Pst::AvrRps4* for 26 h or *Pst::AvrRpt2* for 7 h resulted in significant stromule induction compared to the mock infiltration control. Data represented as the mean ± SEM, *P<0.05 (Student's *t*-test).

D. NRIP1-Cerulean Col-0 transgenic Arabidopsis plant leaves were mock treated (left panels) or treated with 1 μM flg22 peptide (right panels) and stromules were visualized at indicated time point. Increased number of stromules (arrows) was observed at 0.5 h (top panels) and 8 h

(bottom panels) flg22-treated samples compared to 2 h treatment (middle panels). Sacle bar equals 20 μm.

E. Stromules induced in experiments described in D were quatnitified and data represented as the mean ± SEM, ****P<0.001 (Student's *t*-test).

Figure S2. Stromules are induced in Arabidopsis by exogenous application of pro-defense molecules H_2O_2 and INA, Related to Figure 3

A. Infiltration of the SA analog, INA, or H_2O_2 in Col-0 *Arabidopsis* plants expressing chloroplast targeted GFP (cpGFP) resulted in the induction of stromules compared to buffer mock control. Data represented as the mean ± SEM, ***P<0.005 (Student's *t*-test).

B. Protoplasts isolated from NRIP1-Cerulean *Arabidopsis* leaves were mock treated (top panels) or treated with 100 μM SA for 1 hour (middle and bottom panels). Stromules (arrows) were induced in SA-treated protoplasts compared to the mock control. Bottom panels are enlarged images of the rectangle region of middle panel images. All images are 3D projections of the protoplast. Sacle bar equals 15 μm.

C. Stromules induced in B were quatnitified and data represented as the mean ± SEM, ****P<0.001 (Student's *t*-test).

Figure S3. Accumulation of chloroplast-localized NRIP1 protein in the nucleus during BS2-AvrBS2 induced response and application of INA, Related to Figure 4

Transient co-expression of BS2 immune receptor and the AvrBs2 bacterial effector (left panel) or infiltration of the SA analog INA (right panel) resulted in increased stromules and chloroplasts

around nuclei and an increase in NRIP1-Cerulean in nuclei. Images are transparent projections of confocal microscopy z-stacks with a 0.45 gamma correction. Scale bar equals, 10 μm.

Figure S4. Disruption of chloroplast outer envelope protein abolishes stromule induction, Related to Figure 7

p50-Citrine (yellow; column 1) and CHUP1(cTP)-tRFP (red; column 3) were transiently coexpressed in N-containing *N. benthamiana* leaves expressing NRIP1-Cerulean (blue, column 2) for 42 h. CHUP1(cTP)-tRFP signal surrounded chloroplast autofluorescence, suggesting it localizes to the chloroplast envelope (red; column 3). 2D maximum intensity projections of confocal microscopy z-stacks show long stromules-like connections between chloroplasts (top row). Chloroplasts also appeared to cluster and either fuse or tightly associate to exclude cytosol marked by p50-Citrine (bottom row). Scale bars equal 10 μm.

Supplemental Movie Legends

Movie S1, Related to Figure 4

Time lapsed movie of stromules-to-nuclear connections. Tight connections can be seen between stromules (blue) and the nucleus (yellow). An increased concentration of NRIP1-Cerulean was observed at the points of stromules-to-to nuclear connections, and as the nucleus moves right the tight connections are maintained.

Movie S2, Related to Figure 4

Stromules-to-nuclear connections can form complex networks. 3D rotation of a transparent projection of stromules (blue) surrounding a nucleus (yellow). The nucleus is 9.5 μm in diameter.

Movie S3, Related to Figure 6

Time lapsed movie of HyPer sensor of H_2O_2. Ratiometric relative measurement of H_2O_2 via the HyPer sensor shows higher concentrations of H_2O_2 in the nucleus at the sites of stromules-to-nuclear and chloroplast-to-nuclear connections.

Supplemental Experimental Procedures

Plasmid constructs

Plasmids, p50-TAP, p50-Citrine and Citrine alone are described in (Burch-Smith et al., 2007; Caplan et al., 2008). To create the VIGS vector TRV2-*CHUP1*, 500 bp of the *NbCHUP1* was PCR amplified from *NbCHUP1* cDNA and cloned into pYL156 (Liu et al., 2002). The primer pair 5'-tctagaggttgatgaacgagctgtcctcaag-3' and 5'-gagctctgacacgactccttaattcttcaaaag-3' were used to amplify *NbCHUP1* from *N. benthamiana cDNA* and cloned into TRV2 cut with XbaI-SacI to generate TRV2-*CHUP1*. The HIV-REV NES sequence (5-CTT CAG CTA CCA CCG CTT GAG AGA CTT ACT CTT GAT-3') was added to the C-terminus of Cerulean to generate Cerulean-NES. The NES sequence was added to the N-terminus of NRIP1-Cerulean to generate NES-NRIP1-Cerulean. NES was added to the C-terminus of Cerulean-cTP to generate Cerulean-cTP-NES. Details of all the plasmids used in this study are available upon request.

Transient expression by Agroinfiltration

4-5 week-old *N. benthamiana* plants grown on light carts or in environmentally controlled growth chambers were infiltrated with GV2260 *Agrobacterium* cultures containing expression vectors as described in (Caplan et al., 2008). *Agrobacterium* cultures containing expression vectors for p50-TAP, TAP alone, p50-Citrine, Citrine, BS2, AvrBS2, AvrB, AvrRpt2, TRV1 and TRV2 were adjusted to an OD_{600} = 1.0. For co-infiltration, *Agrobacterium* cultures were mixed 1:1.

HR-PCD assay in silenced plants

TRV-based silencing of *CHUP1* was performed using *N. benthamiana* and N-containing transgenic *N. benthamiana* plants as described in (Liu et al., 2002). Approximately 10 days post silencing, plants were infiltrated with *Agrobacterium* expressing p50-TAP or TAP alone. All pictures were taken using a Canon 40D camera and a 100 mm macro lens.

Small signaling molecule stromule induction

INA at 1mM, H_2O_2 at 5mM, SNAP at 1mM, and xanthine at 200μM with xanthin oxidase at 0.2U/ml was syringe infiltrated into *N. benthamiana* and Arabidopsis leaves. Stromules were quantified as described above 26 to 72 h post infiltration.

Generation of Arabidopsis Col-0 and *chup1* plants expressing NRIP1-Cerulean

N. benthamiana NRIP1 fused to Cerulean fluorescent protein described in (Caplan et al., 2008) was transformed into Arabidopsis Col-0 using the floral dip method (Zhang et al., 2006). Homozygous NRIP1-Cerulean plants were identified for further analyses.

Homozygous *chup1* mutant (SALK_129128C; (Schmidt von Braun and Schleiff, 2008) were obtained from the Arabidopsis Biological Resource Center (ABRC). Homozygous plants were confirmed by PCR. NRIP1-Cerulean was transformed into *chup1* mutants and homozygous lines were generated.

Arabidopsis protoplast isolation and SA treatment

Protoplasts were isolated from Arabidopsis mesophyll cells as described in (Yoo et al., 2007). After incubation on ice for 2 hours, approximately 1 X 10^3 protoplasts in MMG buffer

were treated either with 0.01% DMSO (mock) or 100 μM salicylic acid (Simga). Images were captured one-hour post-treatment using a Zeiss LSM710 confocal microscope.

Flg22 treatment

Synthetic flg22 peptide (GeneScript, Scotch Plains, NJ) was dissolved in sterile H_2O at a final concentration of 1μM. Leaves from 4 week old Col-0::NRIP1-Cerulean plants were infiltrated with 1μM flg22 or H_2O. Stromule induction was observed using a Zeiss 710 confocal microscope at 30 min, 2 hours, and 8 hours after infiltration. Total 18-24 images were analyzed from 5-6 plants. Statistical analysis was performed and graphs were generated by PRISM 6 (graphPad).

Constitutive stromule induction visualization in *chup1* mutant

Col-0::NRIP1-Cerulean and *chup1*::NRIP1-Cerulean plants were grown in short-day condition for 4 weeks and leaves were imaged using a Zeiss LSM710 confocal microscope.

Cell death assays in *chup1* mutant

Col-0::NRIP1-Cerulean and *chup1*::NRIP1-Cerulean plants were grown in short-day condition for 4 weeks and leaves were infiltrated with $1X10^6$ CFU/mL Pst DC3000 expressing AvrRpt2 using a needless syringe. At 6 hours post-infiltration, leaves were stained by heating for 10 minute in alcoholic lactophenol trypan blue solution followed by boiling for 1 minute (Koch and Slusarenko, 1990). Leves were destained in chloral hydrate solution and processed for imaging. One 10 mm^2 area close to the infiltration site and one 10 mm^2 area distal to the infiltration site were photographed using stereomicroscope (3.4X digital zoom). Trypan blue

stained dead cells were counted. At least 5-7 plants and 2-3 leaves per plant were included in this analysis.

Supplemental References

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