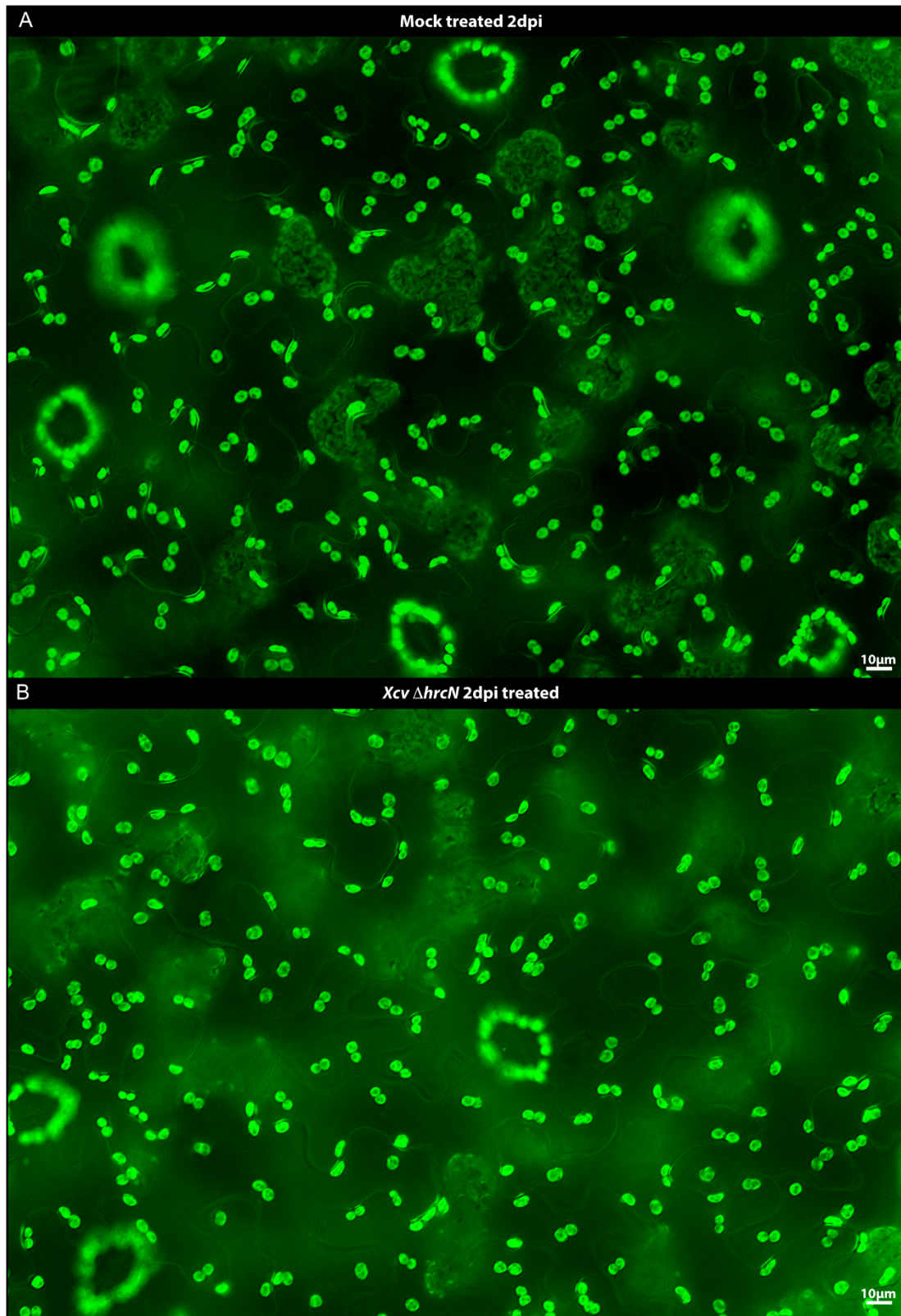
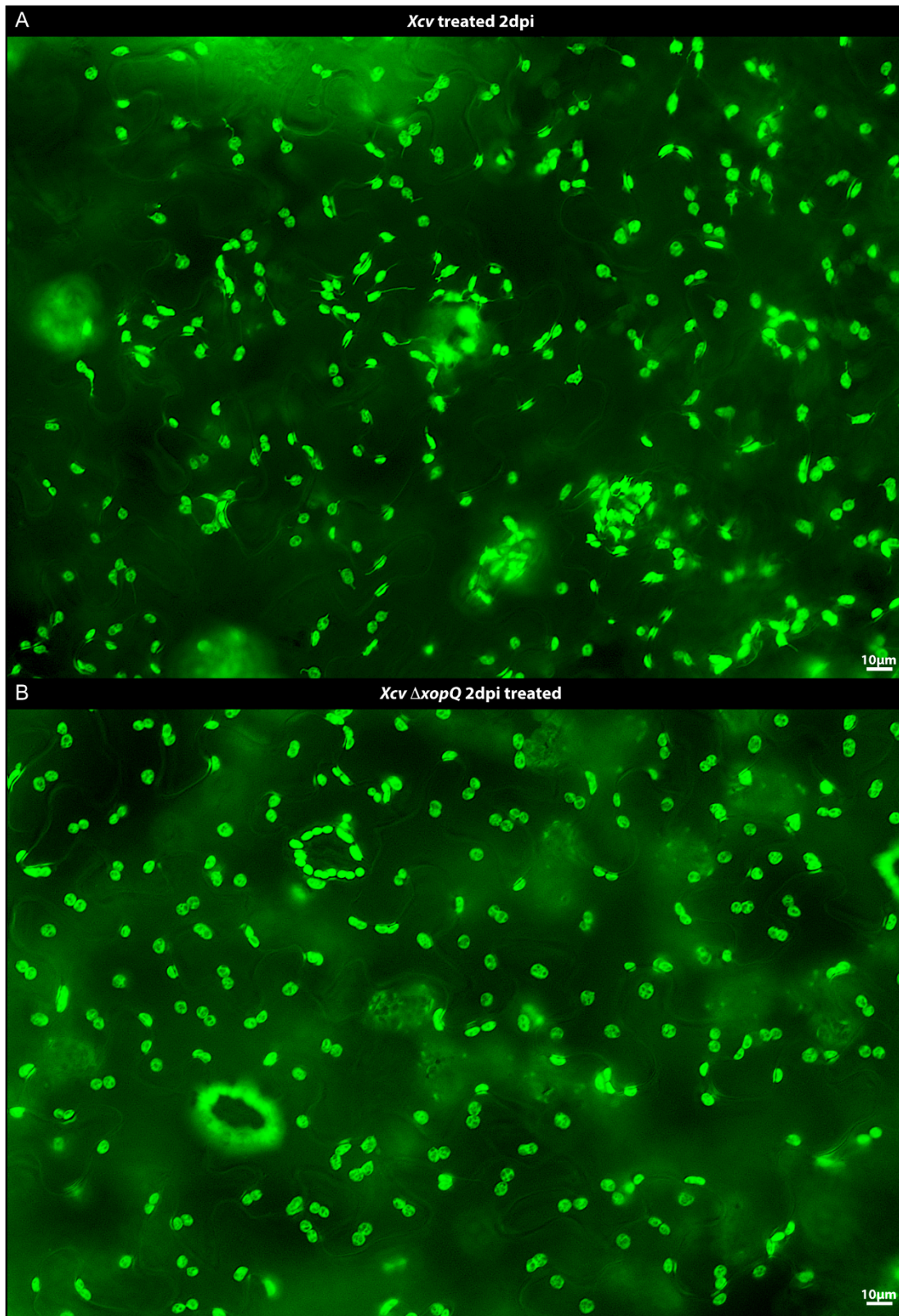


## Supplemental – Figures

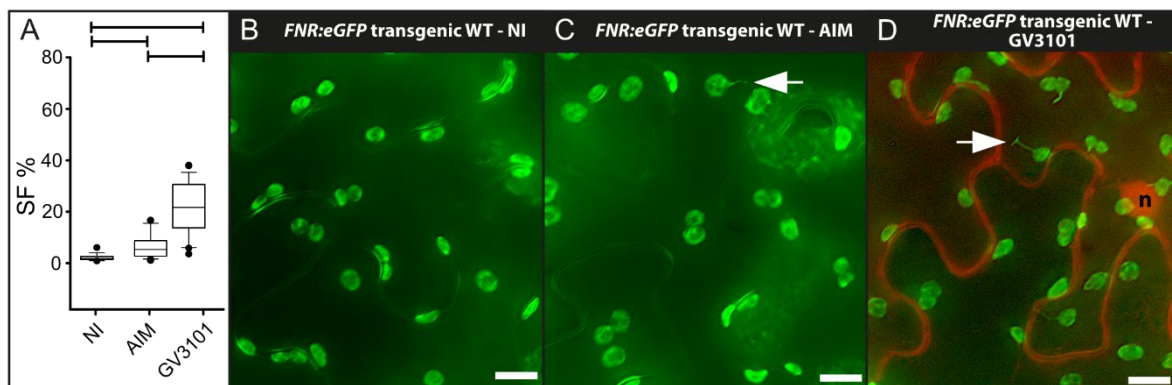
**Supplemental Figure S1 Full frames of stacked fluorescence images of a mock and *Xanthomonas campestris* pv. *vesicatoria*  $\Delta$ *hrcN* infiltrated *Nicotiana benthamiana* plant. Representative full-frame images used for stromule counts in *FNR:eGFP* transgenic plants in response to the mock treatment (A) and treatment with *Xcv hrcN* (B). Scale bar is 10  $\mu$ m.**



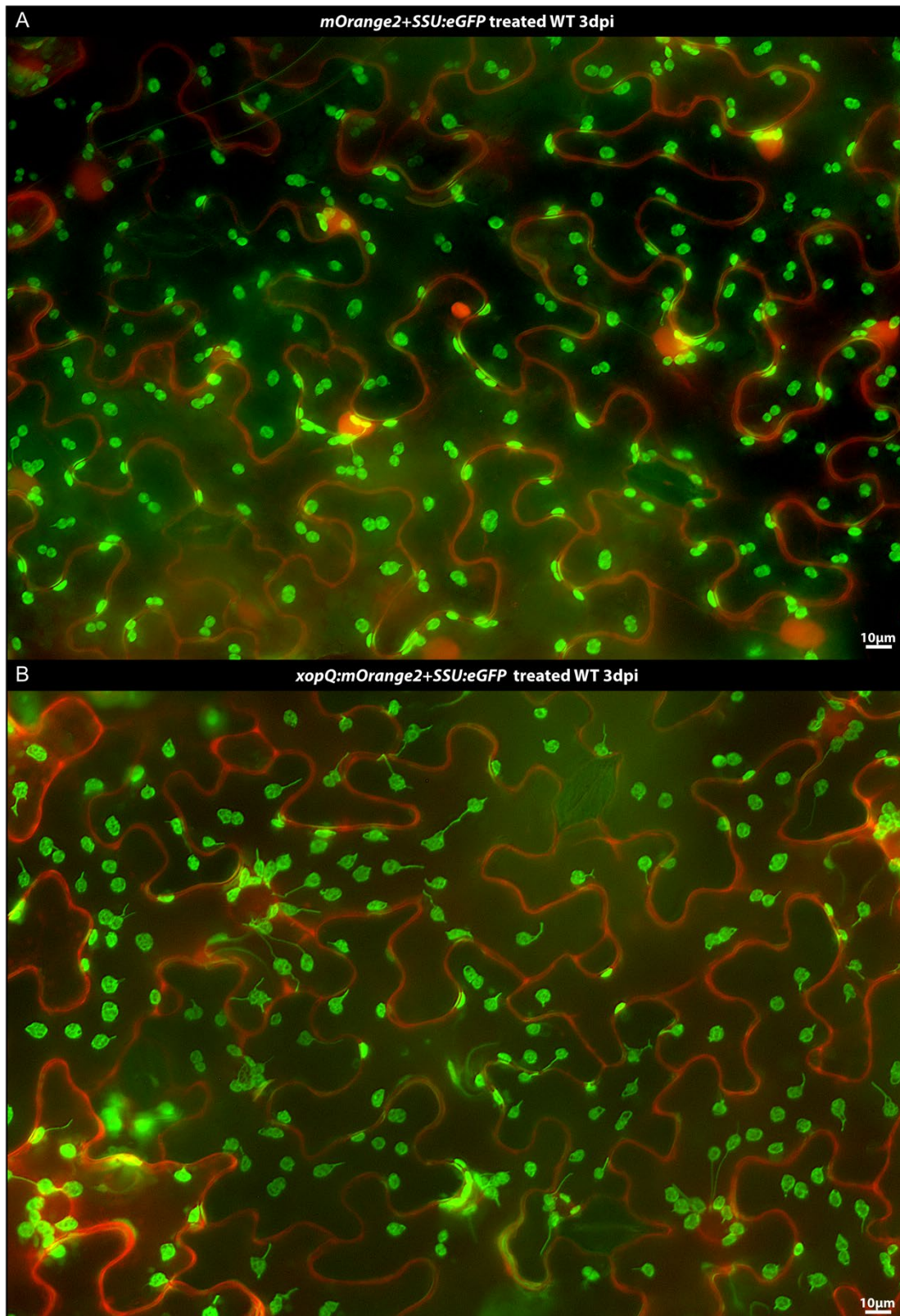
**Supplemental Figure S2 Full-frame of stacked fluorescence images of a *Xanthomonas campestris* pv. *vesicatoria* 85-10 and *Xcv*  $\Delta xopQ$  inoculated *Nicotiana benthamiana* plants. Representative full frame images used for stromule counts in *FNR:eGFP* transgenic plants in response to treatment with *Xcv* 85-10 (A) and treatment with *Xcv*  $\Delta xopQ$  (B).**



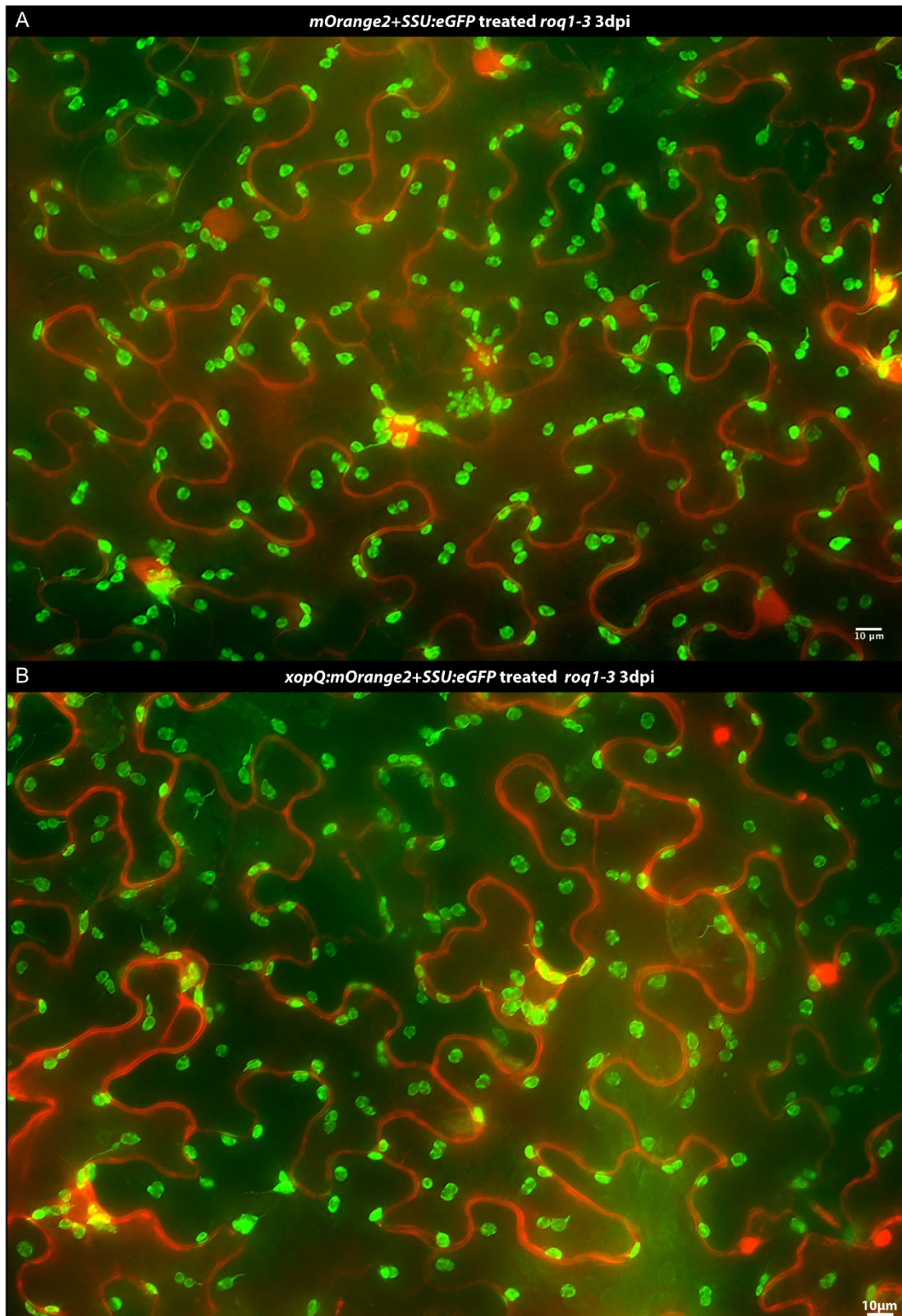
**Supplemental Figure S3 Moderate optical densities of GV3101 (pMP90) induce moderate stromule frequencies at 3 dpi.** (A) Box plot of stromule frequency in % (SF%), bars indicate significant differences based on a One-Way ANOVA analysis on transformed data. (SF%) in lower leaf epidermis cells of non-infiltrated (NI), agrobacterium infiltration medium (AIM)-infiltrated and GV3101 (pMP90)-infiltrated *N. benthamiana* *FNR:eGFP* plants. GV3101 (pMP90) mediates the expression of *mOrange2*. (B), (C) and (D) are representative sectors of images taken for stromule quantification. “n” = nucleus, arrow = stromule; scale bar corresponds to 10 $\mu$ m; green fluorescence originates from the *SSU:eGFP* plastid stroma marker (b, c and d) and the red fluorescence originates from the mORANGE2 fluorescence protein (d).



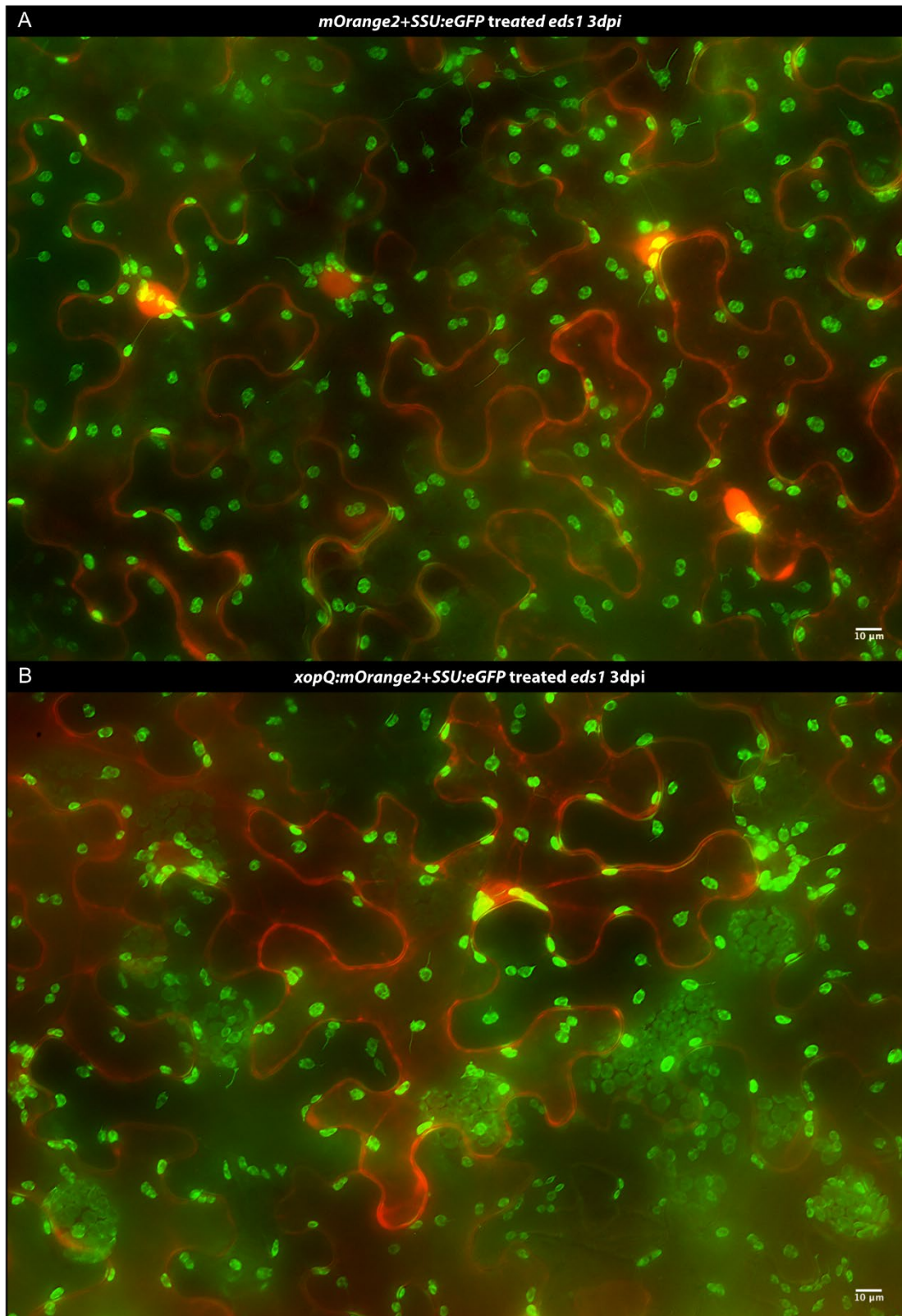
**Supplemental Figure S4 Full-frame stacked fluorescence images of an inoculated *Nicotiana benthamiana* wild-type plant.** Representative full frame images from the dataset used for stromule counts in WT in response to (A) *mOrange2+SSU:eGFP* and (B) *xopQ:mOrange2+SSU:eGFP*. Scale bars correspond to 10  $\mu$ m.



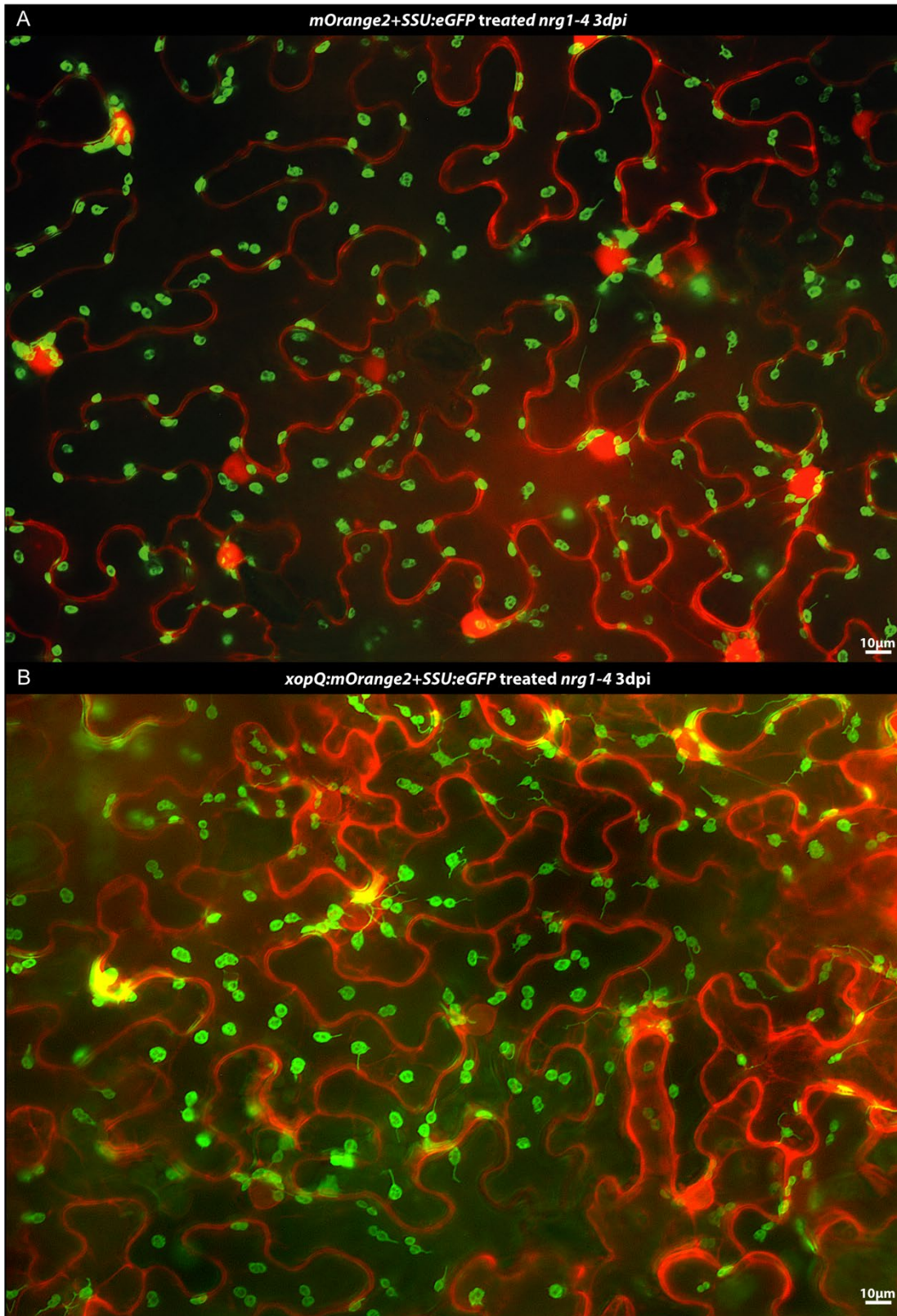
**Supplemental Figure S5 Full frame stacked fluorescence images of an inoculated *Nicotiana benthamiana roq1* plant.** Representative full-frame images used for stromule counts in *roq1-3* mutant in response to (A) *mOrange2+SSU:eGFP* and (B) *xopQ:mOrange2+SSU:eGFP*. Scale bars correspond to 10  $\mu$ m.



**Supplemental Figure S6 Full-frame stacked fluorescence images of an inoculated *Nicotiana benthamiana eds1* plant.** Representative full frame images from the dataset used for stromule counts in the *eds1a* mutant in response to (A) *mOrange2+SSU:eGFP* and (B) *xopQ:mOrange2+SSU:eGFP*. Scale bar corresponds to 10  $\mu\text{m}$ .

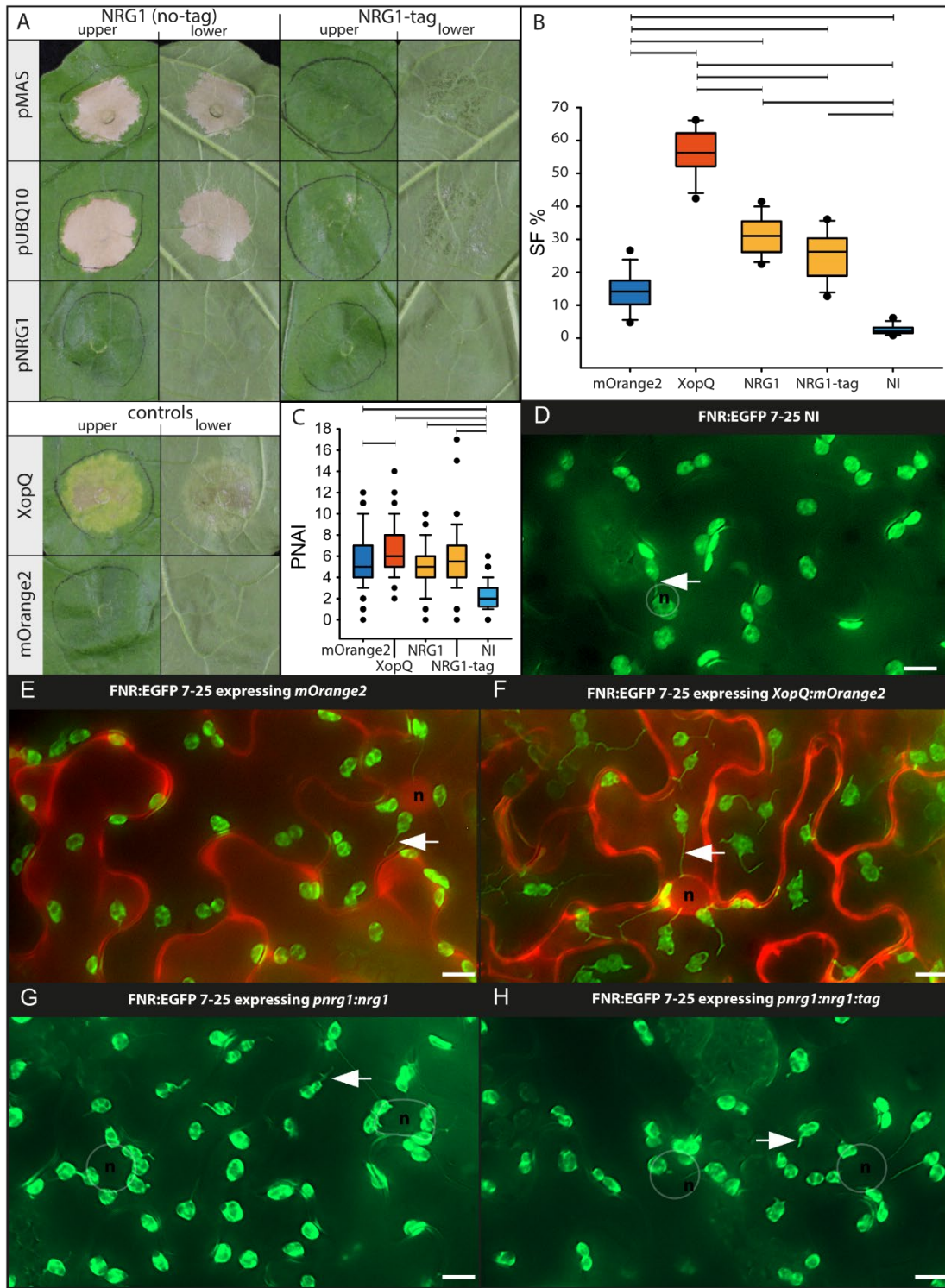


**Supplemental Figure S7 Full-frame stacked fluorescence images of an inoculated *Nicotiana benthamiana nrg1* plant.** Representative full-frame images used for stromule counts in the *nrg1-4* mutant in response to (A) *mOrange2+SSU:eGFP* and (B) *xopQ:mOrange2+SSU:eGFP*. Scale bar corresponds to 10  $\mu$ m.

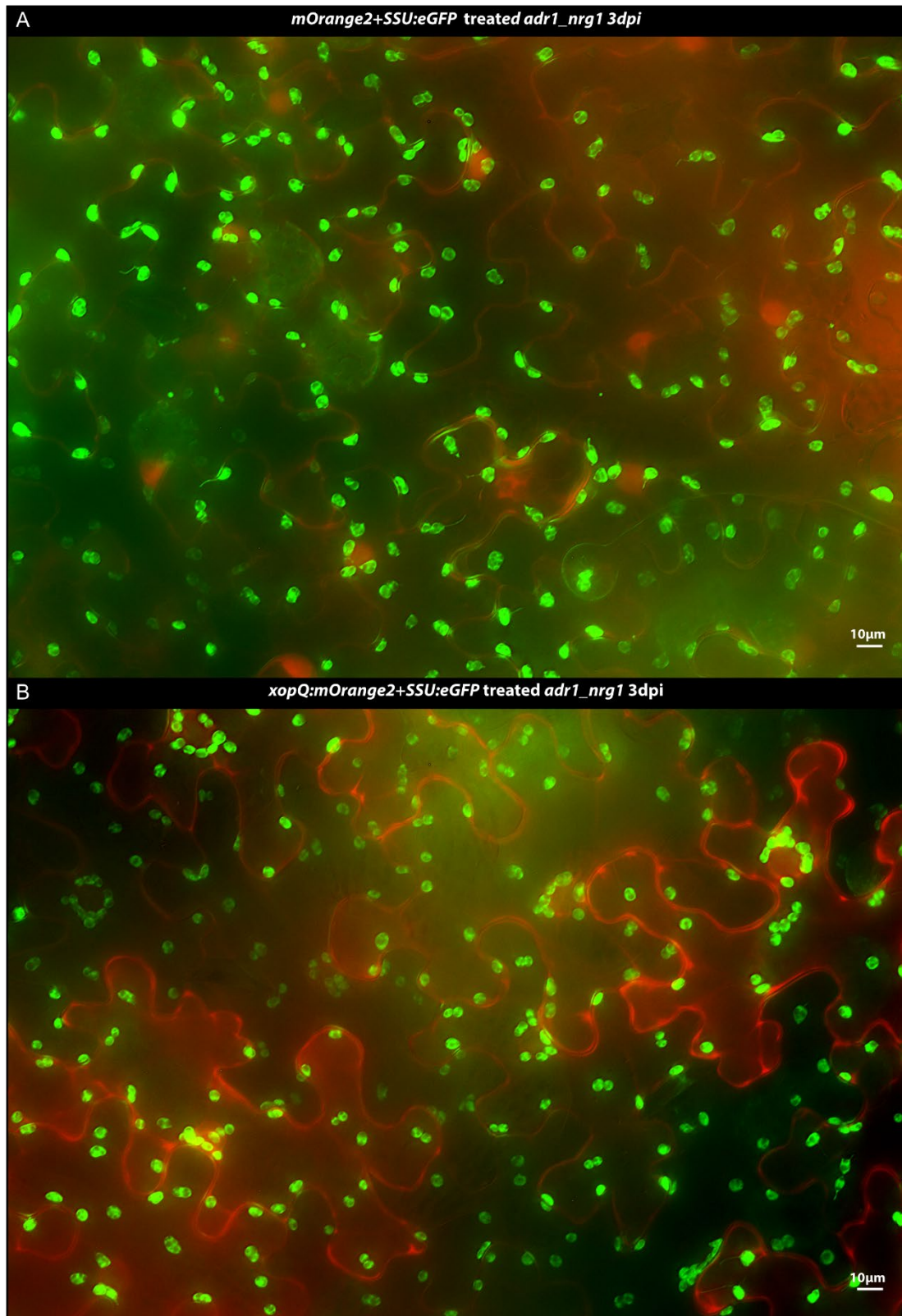




**Supplemental Figure S8 Macroscopic phenotype, SF% and PNAI in response to NRG1 over-expression in FNR:eGFP transgenic WT plants.** NI = not infiltrated, mOrange2 = *p35S::mOrange2*, XopQ = *p35S::XopQ:mOrange2*, NRG1 = *pNRG1::NRG1*, NRG1-tag = *pNRG1::NRG1:tag*; **(A)** macroscopic phenotype of infiltrated leaf tissue 10 dpi, lower = abaxial view of infiltrated leaf, upper = adaxial view of infiltrated leaf; strong cell death is visible by grey areas, mild cell death symptoms are visible by darker areas and are only visible from the abaxial side; **(B)** Box plot of stromule frequency in % (SF%), bars indicate significant differences based on a One-Way ANOVA analysis on transformed data. **(C)** plastid nucleus association index (PNAI), bars indicate significant differences based on a Kruskal-Wallis One Way analysis on ranks; **(D-H)** representative sections of stacked fluorescence images of infiltrated (**E-H**) and not infiltrated (**D**) tissue, GFP fluorescence of FNR:eGFP depicted in green (**D-H**) mOrange2 fluorescence depicted in red (**E-F**), arrow heads = plastids with stromules, white circles = position of nuclei, located by transmitted light images, n = nuclei, scale bar corresponds to 10  $\mu\text{m}$ .



**Supplemental Figure S9 Full-frame stacked fluorescence images of an inoculated *Nicotiana benthamiana* *adr1\_nrg1* plant.** Representative full-frame images used for stromule counts in the *adr1\_nrg1* mutant in response to (A) *mOrange2+SSU:eGFP* and (B) *xopQ:mOrange2+SSU:eGFP*. Scale bar corresponds to 10  $\mu$ m.



## **Supplemental – tables**

**Supplemental Table S1 Summary of SF% values used for stromule frequency bar blots in the main manuscript.** Values used for drawing box plots in Figure 1B, 2B, 3B, 4B and 5B; n = total number of plants used for respective treatment and genotype; SF% Mean = arithmetic average of stromule frequency values expressed in %; 25% = value for the 25 percentile; 75% = value of the 75 percentile; Median in %.

	n	SF% Mean	Median	25%	75%
<b>Figure 1B</b>					
Mock	4	3.00	2.99	2.30	5.24
<i>Xcv 85-10ΔhrcN</i>	4	3.80	2.82	1.24	8.81
<i>Xcv 85-10ΔxopQ</i>	4	3.90	4.10	2.30	5.41
<i>Xcv 85-10</i>	4	32.3	32.40	23.80	39.70
<b>Figure 2B</b>					
<i>roq1-3</i> / mORANGE2	13	30.0	32.10	23.65	35.05
<i>roq1-3</i> / XopQ	13	27.1	29.80	23.25	36.40
<i>roq1-4</i> / mORANGE2	13	23.4	26.60	17.05	31.50
<i>roq1-4</i> / XopQ: mORANGE2	13	22.8	24.30	18.60	25.95
WT / mORANGE2	13	25.0	22.40	18.00	32.40
WT / XopQ: mORANGE2	13	55.1	62.90	37.10	69.20
<b>Figure 3B</b>					
<i>eds1a-1</i> / mORANGE2	11	12.5	13.90	7.16	17.80
<i>eds1a-1</i> / XopQ	11	8.8	7.97	5.96	15.10
WT / mORANGE2	11	11.4	12.30	7.18	19.50
WT / XopQ: mORANGE2	11	47.9	48.60	42.20	54.10
<b>Figure 4B</b>					
<i>nrg1-4</i> / mORANGE2	15	22.8	24.00	19.40	26.50
<i>nrg1-4</i> / XopQ: mORANGE2	15	43.7	43.30	41.10	46.90
<i>nrg1-5</i> / mORANGE2	15	24.6	23.60	20.70	31.00
<i>nrg1-5</i> / XopQ: mORANGE2	15	47.1	43.40	40.30	50.20
WT / mORANGE2	15	28.1	25.50	21.60	35.10
WT / XopQ: mORANGE2	15	63.7	64.70	55.30	72.10
<b>Figure 5B</b>					
<i>adr1_nrg1</i> / mORANGE2	15	27.8	26.90	22.16	30.14
<i>adr1_nrg1</i> / XopQ: mORANGE2	15	28.8	27.58	23.50	36.30
WT / mORANGE2	9	32.5	28.53	27.78	38.72
WT / XopQ: mORANGE2	9	54.9	56.57	46.83	63.15
<b>Figure S3A</b>					
NI	16	2.1	1.7	1.3	2.6
AIM	22	6.3	5.3	2.7	8.7
GV3101	23	20.7	21.7	13.7	30.6

**Supplemental Table S2 summary of PNAI values used for box blots in the main**

**manuscript.** Values displayed in the PNAI box blots of Figure 6B. Infiltration = constructs co-infiltrated; plant line = genetic background used for experiment; n = number of nuclei included in the analysis. Lower numbers for *XopQ:mOrange2*-related experiments result from lower expression levels and therefore lower numbers of clearly defined nuclei. Median PNAI = median of the plastid nucleus association values; 25% = value for the 25 percentile; 75% = value of the 75 percentile.

Expressed constructs	plant line	n	median PNAI	25%	75%
<i>mOrange2 + SSU:eGFP</i>	wild-type	464	4	4	6
<i>XopQ:mOrange2 + SSU:eGFP</i>	wild-type	297	6	5.75	7
<i>mOrange2 + SSU:eGFP</i>	roq1-3	95	5	4	6
<i>XopQ:mOrange2 + SSU:eGFP</i>	roq1-3	61	7	6	9
<i>mOrange2 + SSU:eGFP</i>	eds1a-1	90	5	3	7
<i>XopQ:mOrange2 + SSU:eGFP</i>	eds1a-1	66	8	5	10
<i>mOrange2 + SSU:eGFP</i>	nrg1-4	126	4	3	5
<i>XopQ:mOrange2 + SSU:eGFP</i>	nrg1-4	113	6	5	7
<i>mOrange2 + SSU:eGFP</i>	nrg1_adr1	249	5	3	6
<i>XopQ:mOrange2 + SSU:eGFP</i>	nrg1_adr1	102	8	6	10.25

**Supplemental Table S3 Values used for SF% bar plots in supplemental figure Figure S3A.** n = total number of plants used for respective treatment and genotype; SF% Mean = arithmetic average of stromule frequency values expressed in %; C.I. of Mean 95% lower = absolute value of the lower 95% confidence interval of the presented mean; C.I. of Mean 95% upper = absolute value of the upper 95% confidence interval of the presented mean; difference C.I. Mean 95% lower = difference of mean and the absolute value of the lower 95% confidence interval; difference C.I. Mean 95% upper = difference of the absolute value of the 95% upper confidence interval and the mean; the difference values have been used to blot the whiskers in the bar blots.

	n	SF% Mean	C.I. of Mean 95% lower	C.I. of Mean 95% upper	difference C.I. Mean 95% lower	difference C.I. Mean 95% upper
None infiltrated	16	2	1.4	2.6	0.5	0.6
AIM – buffer control	22	5.7	3.9	7.7	1.7	2
mORANGE2	23	19.7	15.4	24.4	4.3	4.7

**Supplemental Table S4 Values used for SF% box plots in supplemental figure Figure S9B.** Expressed constructs = constructs infiltrated; plant line = genetic background used for experiment; n = number of plants included in the analysis. Median SF% = median of stromule frequency values expressed in %; 25% = value for the 25 percentile; 75% = value of the 75 percentile.

Expressed constructs	plant line	n	median SF%	25%	75%
<i>p35S:mOrange2</i>	FNR EGFP 7-25	13	14.2	10.2	17.5
<i>p35S:XopQ:mOrange2</i>	FNR EGFP 7-25	13	56.3	52.2	62.3
<i>pNRG1:NRG1</i>	FNR EGFP 7-25	13	31.0	26.1	35.5
<i>pNRG1:NRG1:tag</i>	FNR EGFP 7-25	13	26.2	18.9	30.3
<i>NI</i>	FNR EGFP 7-25	13	2.1	1.5	3.2



**Supplemental Table S5 Values used for PNAI box plots in supplemental figure Figure S9D.** Expressed constructs = constructs infiltrated; plant line = genetic background used for experiment; n = number of nuclei included in the analysis. Median PNAI = median of stromule frequency values expressed in %; 25% = value for the 25 percentile; 75% = value of the 75 percentile.

Expressed constructs	plant line	N	median PNAI	25%	75%
<i>p35S:mOrange2</i>	FNR EGFP 7-25	85	5	4	7
<i>p35S:XopQ:mOrange2</i>	FNR EGFP 7-25	83	6	5	8
<i>pNRG1:NRG1</i>	FNR EGFP 7-25	60	5	4	6
<i>pNRG1:NRG1:tag</i>	FNR EGFP 7-25	64	5.5	4	7
<i>NI</i>	FNR EGFP 7-25	67	2	1.25	3

**Supplemental material – statistics**

**Supplemental Statistics S1** - SF was treated as a measurement variable. The different treatments were treated as nominal variable. Because SF is a proportion the values were arcsine transformed before further testing.

**Descriptive Statistics:**

Column	Size	Missing	Mean	Std Dev	Std. Error	C.I. of Mean
xcv trans	12	0	0.894	0.139	0.0400	0.0880
harcN trans	12	0	0.448	0.126	0.0364	0.0802
XopQ trans	12	0	0.460	0.0691	0.0199	0.0439
moc trans	12	0	0.411	0.141	0.0406	0.0893

Column	Range	Max	Min	Median	25%	75%
xcv trans	0.493	1.136	0.644	0.891	0.795	0.972
harcN trans	0.357	0.662	0.305	0.422	0.340	0.579
XopQ trans	0.233	0.576	0.343	0.468	0.400	0.506
moc trans	0.562	0.562	0.000	0.424	0.391	0.497

Column	Skewness	Kurtosis	K-S Dist.	K-S Prob.	SWilk	WSWilk	Prob
xcv trans	0.116	-0.000154	0.116	0.795	0.981	0.988	
harcN trans	0.592	-1.077	0.179	0.340	0.903	0.173	
XopQ trans	-0.171	-0.559	0.124	0.761	0.980	0.984	
moc trans	-2.505	7.736	0.347	<0.001	0.706	<0.001	

Column	Sum	Sum of Squares
xcv trans	10.733	9.810
harcN trans	5.378	2.585
XopQ trans	5.522	2.593
moc trans	4.927	2.241

**One Way Analysis of Variance**

Normality Test (Shapiro-Wilk) Passed (P = 0.080)  
 Equal Variance Test: Passed (P = 0.399)

Group Name	N	Missing	Mean	Std Dev	SEM
xcv trans	12	0	0.894	0.139	0.0400
harcN trans	12	0	0.448	0.126	0.0364
XopQ trans	12	0	0.460	0.0691	0.0199
moc trans	12	0	0.411	0.141	0.0406

Source of Variation	DF	SS	MS	F	P
Between Groups	3	1.877	0.626	41.972	<0.001
Residual	44	0.656	0.0149		
Total	47	2.533			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

**All Pairwise Multiple Comparison Procedures (Tukey Test):**

Comparison	Diff of Means	p	q	P	P<0.050
xcv trans vs. moc trans	0.484	4	13.725	<0.001	Yes
xcv trans vs. harcN trans	0.446	4	12.660	<0.001	Yes
xcv trans vs. XopQ trans	0.434	4	12.320	<0.001	Yes
XopQ trans vs. moc trans	0.0495	4	1.405	0.754	No
XopQ trans vs. harcN trans	0.0120	4	0.340	0.995	Do Not Test
harcN trans vs. moc trans	0.0375	4	1.065	0.875	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

**Supplemental Statistics S2 - SF** was treated as a measurement variable. The different treatments were treated as nominal variable. Because SF is a proportion the values were arcsine transformed before further testing.

**Descriptive Statistics:**

Column	Size	Missing	Mean	Std Dev	Std. Error	C.I. of Mean
tr Roq1/3 mOrange	13	0	0.580	0.0700	0.0194	0.0423
tr Roq1/3 XopQ	13	0	0.547	0.132	0.0366	0.0797
tr Roq1/4 mOrange	13	0	0.505	0.115	0.0319	0.0696
tr Roq1/4 XopQ	13	0	0.498	0.0665	0.0184	0.0402
tr WT mOrange	13	0	0.524	0.127	0.0353	0.0769
tr WT XopQ	13	0	0.836	0.183	0.0509	0.111

Column	Range	Max	Min	Median	25%	75%
tr Roq1/3 mOrange	0.222	0.709	0.487	0.602	0.508	0.633
tr Roq1/3 XopQ	0.444	0.699	0.255	0.577	0.502	0.647
tr Roq1/4 mOrange	0.373	0.657	0.284	0.542	0.425	0.595
tr Roq1/4 XopQ	0.228	0.603	0.375	0.515	0.446	0.534
tr WT mOrange	0.466	0.787	0.321	0.492	0.437	0.605
tr WT XopQ	0.540	1.131	0.591	0.915	0.655	0.982

Column	Skewness	Kurtosis	K-S Dist.	K-S Prob.	SWilk	WSilk
tr Roq1/3 mOrange	0.235	-0.908	0.164	0.414	0.941	0.469
tr Roq1/3 XopQ	-1.087	0.902	0.177	0.309	0.897	0.121
tr Roq1/4 mOrange	-0.695	-0.406	0.165	0.404	0.941	0.470
tr Roq1/4 XopQ	-0.426	-0.252	0.159	0.456	0.958	0.716
tr WT mOrange	0.461	0.271	0.138	0.633	0.969	0.883
tr WT XopQ	-0.0613	-1.607	0.213	0.108	0.882	0.076

Column	Sum	Sum of Squares
tr Roq1/3 mOrange	7.536	4.427
tr Roq1/3 XopQ	7.109	4.096
tr Roq1/4 mOrange	6.564	3.473
tr Roq1/4 XopQ	6.469	3.272
tr WT mOrange	6.813	3.765
tr WT XopQ	10.874	9.500

**One Way Analysis of Variance**

Normality Test (Shapiro-Wilk) Passed (P = 0.395)  
 Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
tr Roq1/3 mOrange	13	0	0.580	0.0700	0.0194
tr Roq1/3 XopQ	13	0	0.547	0.132	0.0366
tr Roq1/4 mOrange	13	0	0.505	0.115	0.0319
tr Roq1/4 XopQ	13	0	0.498	0.0665	0.0184
tr WT mOrange	13	0	0.524	0.127	0.0353
tr WT XopQ	13	0	0.836	0.183	0.0509

Source of Variation	DF	SS	MS	F	P
Between Groups	5	1.071	0.214	14.310	<0.001
Residual	72	1.078	0.0150		
Total	77	2.149			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).  
 Power of performed test with alpha = 0.050: 1.000

**All Pairwise Multiple Comparison Procedures (Tukey Test):**

Comparison	Diff of Means	p	q	P	P<0.050
tr WT XopQ vs. tr Roq1/4 XopQ	0.339	6	9.984	<0.001	Yes
tr WT XopQ vs. tr Roq1/4 mOrange	0.332	6	9.769	<0.001	Yes
tr WT XopQ vs. tr WT mOrange	0.312	6	9.205	<0.001	Yes
tr WT XopQ vs. tr Roq1/3 XopQ	0.290	6	8.534	<0.001	Yes
tr WT XopQ vs. tr Roq1/3 mOrange	0.257	6	7.566	<0.001	Yes
tr Roq1/3 mOrange vs. tr Roq1/4 XopQ	0.0821	6	2.418	0.530	No
tr Roq1/3 mOran vs. tr Roq1/4 mOran	0.0748	6	2.203	0.629	Do Not Test
tr Roq1/3 mOrange vs. tr WT mOrange	0.0556	6	1.639	0.855	Do Not Test
tr Roq1/3 mOrange vs. tr Roq1/3 XopQ	0.0328	6	0.968	0.983	Do Not Test
tr Roq1/3 XopQ vs. tr Roq1/4 XopQ	0.0492	6	1.451	0.908	Do Not Test
tr Roq1/3 XopQ vs. tr Roq1/4 mOrange	0.0419	6	1.235	0.952	Do Not Test
tr Roq1/3 XopQ vs. tr WT mOrange	0.0228	6	0.671	0.997	Do Not Test
tr WT mOrange vs. tr Roq1/4 XopQ	0.0265	6	0.780	0.994	Do Not Test
tr WT mOrange vs. tr Roq1/4 mOrange	0.0192	6	0.564	0.999	Do Not Test
tr Roq1/4 mOrange vs. tr Roq1/4 XopQ	0.00731	6	0.215	1.000	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

**Supplemental Statistics S3 - SF** was treated as a measurement variable. The different treatments were treated as nominal variable. Because SF is a proportion the values were arcsine transformed before further testing.

**Descriptive Statistics:**

Column Size	Missing	Mean	Std Dev	Std. Error	C.I. of Mean		
tr eds1 mO	11	0	0.368	0.100	0.0302	0.0673	
tr eds1 XQ	11	0	0.308	0.0846	0.0255	0.0568	
tr WT mO	11	0	0.359	0.124	0.0372	0.0830	
tr WT XQ	11	0	0.765	0.0727	0.0219	0.0488	

Column Range	Max	Min	Median	25%	75%	
tr eds1 mO	0.341	0.546	0.205	0.381	0.271	0.435
tr eds1 XQ	0.246	0.457	0.210	0.286	0.247	0.399
tr WT mO	0.361	0.546	0.185	0.359	0.271	0.457
tr WT XQ	0.241	0.877	0.635	0.771	0.707	0.826

Column Skewness	Kurtosis	K-S Dist.	K-S Prob.	SWilk	WSWilk	Prob
tr eds1 mO	-0.00880	-0.219	0.132	0.737	0.975	0.934
tr eds1 XQ	0.913	-0.575	0.282	0.014	0.860	0.057
tr WT mO	0.261	-0.940	0.129	0.750	0.947	0.601
tr WT XQ	-0.0396	-0.323	0.158	0.551	0.972	0.908

Column Sum	Sum of Squares	
eds1 mOrange SF	1.500	0.249
eds1 XopQ SF	1.068	0.132
WT mOrange SF	1.471	0.265
WT XopQ SF	5.276	2.583
tr eds1 mO	4.052	1.593
tr eds1 XQ	3.389	1.116
tr WT mO	3.951	1.571
tr WT XQ	8.413	6.488

**One Way Analysis of Variance**

Normality Test (Shapiro-Wilk) Passed (P = 0.432)  
 Equal Variance Test: Passed (P = 0.279)

Group Name	N	Missing	Mean	Std Dev	SEM
tr eds1 mO	11	0	0.368	0.100	0.0302
tr eds1 XQ	11	0	0.308	0.0846	0.0255
tr WT mO	11	0	0.359	0.124	0.0372
tr WT XQ	11	0	0.765	0.0727	0.0219

Source of Variation	DF	SS	MS	F	P
Between Groups	3	1.476	0.492	52.148	<0.001
Residual	40	0.377	0.00944		
Total	43	1.853			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).  
 Power of performed test with alpha = 0.050: 1.000

**All Pairwise Multiple Comparison Procedures (Tukey Test):**

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
tr WT XQ vs. tr eds1 XQ	0.457	4	15.595	<0.001	Yes
tr WT XQ vs. tr WT mO	0.406	4	13.852	<0.001	Yes
tr WT XQ vs. tr eds1 mO	0.397	4	13.539	<0.001	Yes
tr eds1 mO vs. tr eds1 XQ	0.0602	4	2.056	0.474	No
tr eds1 mO vs. tr WT mO	0.00918	4	0.313	0.996	Do Not Test
tr WT mO vs. tr eds1 XQ	0.0510	4	1.743	0.610	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

**Supplemental Statistics S4 - SF** was treated as a measurement variable. The different treatments were treated as nominal variable. Because SF is a proportion the values were arcsine transformed before further testing.

Descriptive Statistics:

Column	Size	Missing	Mean	Std Dev	Std. Error	C.I. of Mean
tr nrg1-4 mOrange2	15	0	0.498	0.0672	0.0174	0.0372
tr nrg1-4 XopQ	15	0	0.722	0.0420	0.0108	0.0232
tr nrg1-5 mOrange2	15	0	0.519	0.0825	0.0213	0.0457
tr nrg1-5 XopQ	15	0	0.756	0.0841	0.0217	0.0466
tr WT mOrange2	15	0	0.559	0.105	0.0270	0.0579
tr WT XopQ	15	0	0.924	0.117	0.0302	0.0648

Column	Range	Max	Min	Median	25%	75%
tr nrg1-4 mOrange2	0.227	0.595	0.367	0.512	0.456	0.541
tr nrg1-4 XopQ	0.138	0.791	0.652	0.718	0.696	0.754
tr nrg1-5 mOrange2	0.252	0.638	0.386	0.507	0.472	0.591
tr nrg1-5 XopQ	0.258	0.918	0.660	0.720	0.688	0.787
tr WT mOrange2	0.350	0.752	0.402	0.529	0.484	0.634
tr WT XopQ	0.402	1.118	0.715	0.934	0.838	1.014

Column	Skewness	Kurtos.	K-S Dist.	K-S Prob.	SWilk	WSWilk
tr nrg1-4 mOrange2	-0.766	0.187	0.178	0.220	0.920	0.194
tr nrg1-4 XopQ	-0.145	-0.889	0.144	0.504	0.959	0.683
tr nrg1-5 mOrange2	-0.220	-1.132	0.153	0.421	0.942	0.403
tr nrg1-5 XopQ	0.947	-0.227	0.199	0.108	0.875	0.040
tr WT mOrange2	0.408	-0.733	0.145	0.495	0.963	0.738
tr WT XopQ	-0.381	-0.649	0.192	0.141	0.955	0.602

Column	Sum	Sum of Squares
tr nrg1-4 mOrange2	7.473	3.787
tr nrg1-4 XopQ	10.834	7.850
tr nrg1-5 mOrange2	7.792	4.142
tr nrg1-5 XopQ	11.336	8.666
tr WT mOrange2	8.384	4.839
tr WT XopQ	13.866	13.008

One Way Analysis of Variance

Normality Test (Shapiro-Wilk) Passed (P = 0.977)  
 Equal Variance Test: Passed (P = 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
tr nrg1-4 mOrange2	15	0	0.498	0.0672	0.0174
tr nrg1-4 XopQ	15	0	0.722	0.0420	0.0108
tr nrg1-5 mOrange2	15	0	0.519	0.0825	0.0213
tr nrg1-5 XopQ	15	0	0.756	0.0841	0.0217
tr WT mOrange2	15	0	0.559	0.105	0.0270
tr WT XopQ	15	0	0.924	0.117	0.0302

Source of Variation	DF	SS	MS	F	P
Between Groups	5	2.085	0.417	55.863	<0.001
Residual	84	0.627	0.00747		
Total	89	2.712			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001). Power of performed test with alpha = 0.050: 1.000

**All Pairwise Multiple Comparison Procedures (Tukey Test):**  
 Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
tr WT XopQ vs. tr nrg1-4 mO	0.426	6	6	19.101	<0.001 Yes
tr WT XopQ vs. tr nrg1-5 mO	0.405	6	6	18.151	<0.001 Yes
tr WT XopQ vs. tr WT mOrange2	0.365	6	6	16.381	<0.001 Yes
tr WT XopQ vs. tr nrg1-4 XopQ	0.202	6	6	9.058	<0.001 Yes
tr WT XopQ vs. tr nrg1-5 XopQ	0.169	6	6	7.560	<0.001 Yes
tr nrg1-5 Xo vs. tr nrg1-4 mO	0.257	6	6	11.542	<0.001 Yes
tr nrg1-5 Xo vs. tr nrg1-5 mO	0.236	6	6	10.591	<0.001 Yes
tr nrg1-5 Xo vs. tr WT mOrang	0.197	6	6	8.821	<0.001 Yes
tr nrg1-5 Xo vs. tr nrg1-4 Xo	0.0334	6	6	1.498	0.896 No
tr nrg1-4 Xo vs. tr nrg1-4 mO	0.224	6	6	10.043	<0.001 Yes
tr nrg1-4 Xo vs. tr nrg1-5 mO	0.203	6	6	9.093	<0.001 Yes
tr nrg1-4 Xo vs. tr WT mOrang	0.163	6	6	7.323	<0.001 Yes
tr WT mOrang vs. tr nrg1-4 mO	0.0607	6	6	2.721	0.395 No
tr WT mOrang vs. tr nrg1-5 mO	0.0395	6	6	1.770	0.810 Do Not Test
tr nrg1-5 mO vs. tr nrg1-4 mO	0.0212	6	6	0.950	0.985 Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

**Supplemental Statistics S5 - SF** was treated as a measurement variable. The different treatments were treated as nominal variable. Because SF is a proportion the values were arcsine transformed before further testing.

#### Descriptive Statistics

Column	Size	Missing	Mean	Std Dev	Std. Error	C.I. of Mean
tr WT_mOrange	9	0	0.607	0.607	0.0807	0.0269 0.0620
tr Adrl/Nrg1_mOrange	15	0	0.556	0.556	0.0885	0.0228 0.0490
tr WT_XopQ	9	0	0.835	0.835	0.116	0.0388 0.0894
tr Adrl/Nrg1_XopQ	15	0	0.567	0.567	0.0798	0.0206 0.0442

Column	Range	Max	Min	Median	25%	75%
tr WT_mOrange	0.240	0.757	0.517	0.560	0.548	0.670
tr Adrl/Nrg1_mOrange	0.289	0.747	0.458	0.539	0.489	0.580
tr WT_XopQ	0.365	0.989	0.624	0.854	0.752	0.920
tr Adrl/Nrg1_XopQ	0.245	0.703	0.458	0.549	0.513	0.632

Column	Skewness	Kurtosis	K-S Dist.	K-S Prob.	Swilk	WSwilk	Prob
tr WT_mOrange	0.823	-0.394	0.275	0.048	0.893	0.212	
tr Adrl/Nrg1_mOrange	1.127	0.432	0.193	0.138	0.872	0.036	
tr WT_XopQ	-0.773	0.105	0.225	0.207	0.935	0.527	
tr Adrl/Nrg1_XopQ	0.360	-0.908	0.119	0.723	0.944	0.436	

Column	Sum	Sum of Squares
tr WT_mOrange	5.465	3.370
tr Adrl/Nrg1_mOrange	8.346	4.753
tr WT_XopQ	7.513	6.380
tr Adrl/Nrg1_XopQ	8.500	4.906

#### One Way Analysis of Variance

Normality Test (Shapiro-Wilk) Passed (P = 0.485)  
 Equal Variance Test: Passed (P = 0.795)

Group Name	N	Missing	Mean	Std Dev	SEM
tr WT_mOrange	9	0	0.607	0.607	0.0269
tr Adrl/Nrg1_mOrange	15	0	0.556	0.556	0.0228
tr WT_XopQ	9	0	0.835	0.835	0.0388
tr Adrl/Nrg1_XopQ	15	0	0.567	0.567	0.0206

Source of Variation	DF	SS	MS	F	P
Between Groups	3	0.520	0.173	21.242	<0.001
Residual	44	0.359	0.00816		
Total	47	0.879			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).  
 Power of performed test with alpha = 0.050: 1.000

#### All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
tr WT_XopQ vs. tr Adr1/Nrg1	0.278	4	10.338	<0.001	Yes
tr WT_XopQ vs. tr Adr1/Nrg1	0.268	4	9.955	<0.001	Yes
tr WT_XopQ vs. tr WT_mOrange	0.228	4	7.559	<0.001	Yes
tr WT_mOrange vs. tr Adr1/Nrg1	0.0508	4	1.887	0.547	No
tr WT_mOrange vs. tr Adr1/Nrg1	0.0405	4	1.503	0.714	Do Not Test
tr Adr1/Nrg1 vs. tr Adr1/Nrg1	0.0103	4	0.443	0.989	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

## Supplemental Statistics S6 - PNAI was treated as a measurement variable. The different treatments were treated as nominal variable.

### Descriptive Statistics:

Column	Size	Missing	Mean	Std Dev	Std. Error	C.I. of Mean
WT XopQ all	297	0	6.084	2.318	0.135	0.265
WT mOrange all	464	0	4.360	2.239	0.104	0.204
adr1nrg1 XopQ	102	0	8.696	3.598	0.356	0.707
adr1 nrg1 mOrange2	249	0	4.723	2.804	0.178	0.350
nrg1-4 XopQ	113	0	5.867	1.868	0.176	0.348
nrg1-4 mOrange2	126	0	3.897	1.710	0.152	0.302
eds1 XopQ	66	0	8.076	2.276	0.280	0.559
eds1 mOrange	90	0	5.633	1.917	0.202	0.401
Roq-1-3 XopQ	61	0	7.246	2.357	0.302	0.604
Roq1-3 mOrange	95	0	5.063	2.004	0.206	0.408

Column	Range	Max	Min	Median	25%	75%
WT XopQ all	13.000	14.000	1.000	6.000	4.000	7.000
WT mOrange all	13.000	13.000	0.000	4.000	3.000	6.000
adr1nrg1 XopQ	27.000	27.000	0.000	8.000	6.000	10.250
adr1 nrg1 mOrange2	15.000	15.000	0.000	5.000	3.000	6.000
nrg1-4 XopQ	8.000	10.000	2.000	6.000	5.000	7.000
nrg1-4 mOrange2	8.000	9.000	1.000	4.000	3.000	5.000
eds1 XopQ	10.000	14.000	4.000	8.000	6.000	10.000
eds1 mOrange	9.000	10.000	1.000	5.000	4.000	7.000
Roq-1-3 XopQ	10.000	13.000	3.000	7.000	5.500	9.000
Roq1-3 mOrange	10.000	10.000	0.000	5.000	4.000	6.000

Column	Skewness	Kurtosis	K-S Dist.	K-S Prob.	Swilk	WSWilP
WT XopQ all	0.734	0.860	0.141	<0.001	0.950	<0.001
WT mOrange all	0.503	0.335	0.135	<0.001	0.967	<0.001
adr1nrg1 XopQ	1.473	5.671	0.136	<0.001	0.904	<0.001
adr1 nrg1 mOrange2	0.440	0.602	0.0992	<0.001	0.961	<0.001
nrg1-4 XopQ	-0.105	-0.504	0.117	<0.001	0.967	0.007
nrg1-4 mOrange2	0.251	-0.214	0.143	<0.001	0.956	<0.001
eds1 XopQ	0.243	-0.527	0.121	0.017	0.967	0.075
eds1 mOrange	0.0213	-0.402	0.141	<0.001	0.970	0.034
Roq-1-3 XopQ	0.382	-0.268	0.132	0.010	0.966	0.088
Roq1-3 mOrange	0.0249	0.00775	0.123	0.001	0.973	0.050

Column	Sum	Sum of Squares
WT XopQ all	1807.000	12585.000
WT mOrange all	2023.000	11141.000
adr1nrg1 XopQ	887.000	9021.000
adr1 nrg1 mOrange2	1176.000	7504.000
nrg1-4 XopQ	663.000	4281.000
nrg1-4 mOrange2	491.000	2279.000
eds1 XopQ	533.000	4641.000
eds1 mOrange	507.000	3183.000
Roq-1-3 XopQ	442.000	3536.000
Roq1-3 mOrange	481.000	2813.000

### One Way Analysis of Variance

Normality Test (Shapiro-Wilk) Failed (P < 0.050)  
 Test execution ended by user request, ANOVA on Ranks begun



Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Missing	Median	25%	75%
Roq1-3 mOrange	95	0	5.000	4.000	6.000
Roq1-3 XopQ	61	0	7.000	5.500	9.000
eds1 mOrange	90	0	5.000	4.000	7.000
eds1 XopQ	66	0	8.000	6.000	10.000
nrg1-4 mOrange2	126	0	4.000	3.000	5.000
nrg1-4 XopQ	113	0	6.000	5.000	7.000
adr1 nrg1 mOrange2	249	0	5.000	3.000	6.000
adr1nrg1 XopQ	102	0	8.000	6.000	10.250
WT mOrange all	464	0	4.000	3.000	6.000
WT XopQ all	297	0	6.000	4.000	7.000

H = 369.128 with 9 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :

Comparison	Diff of Ranks	Q	P<0.05
adr1nrg1 XopQ vs nrg1-4 mOrang	765.133	11.963	Yes
adr1nrg1 XopQ vs WT mOrange al	668.019	12.721	Yes
adr1nrg1 XopQ vs adr1 nrg1 mOr	587.146	10.401	Yes
adr1nrg1 XopQ vs Roq1-3 mOrang	515.110	7.523	Yes
adr1nrg1 XopQ vs eds1 mOrange	399.535	5.753	Yes
adr1nrg1 XopQ vs nrg1-4 XopQ	347.932	5.305	Yes
adr1nrg1 XopQ vs WT XopQ all	339.626	6.163	Yes
adr1nrg1 XopQ vs Roq1-3 XopQ	132.459	1.704	No
adr1nrg1 XopQ vs eds1 XopQ	2.490	0.0328	Do Not Test
eds1 XopQ vs nrg1-4 mOrange2	762.643	10.452	Yes
eds1 XopQ vs WT mOrange all	665.528	10.535	Yes
eds1 XopQ vs adr1 nrg1 mOr	584.656	8.794	Yes
eds1 XopQ vs Roq1-3 mOrange	512.620	6.662	Yes
eds1 XopQ vs eds1 mOrange	397.044	5.102	Yes
eds1 XopQ vs nrg1-4 XopQ	345.442	4.643	Yes
eds1 XopQ vs WT XopQ all	337.136	5.159	Yes
eds1 XopQ vs Roq1-3 XopQ	129.969	1.524	Do Not Test
Roq1-3 XopQ vs nrg1-4 mOrang	632.674	8.447	Yes
Roq1-3 XopQ vs WT mOrange all	535.560	8.189	Yes
Roq1-3 XopQ vs adr1 nrg1 mOr	454.687	6.628	Yes
Roq1-3 XopQ vs Roq1-3 mOrange	382.652	4.857	Yes
Roq1-3 XopQ vs eds1 mOrange	267.076	3.354	Yes
Roq1-3 XopQ vs nrg1-4 XopQ	215.473	2.824	No
Roq1-3 XopQ vs WT XopQ all	207.167	3.069	Do Not Test
WT XopQ all vs nrg1-4 mOrange2	425.507	8.334	Yes
WT XopQ all vs WT mOrange all	328.393	9.203	Yes
WT XopQ all vs adr1 nrg1 mOr	247.520	5.999	Yes
WT XopQ all vs Roq1-3 mOrange	175.485	3.100	No
WT XopQ all vs eds1 mOrange	59.909	1.037	Do Not Test
WT XopQ all vs nrg1-4 XopQ	8.306	0.156	Do Not Test
nrg1-4 XopQ vs nrg1-4 mOrange2	417.201	6.706	Yes
nrg1-4 XopQ vs WT mOrange all	320.087	6.354	Yes
nrg1-4 XopQ vs adr1 nrg1 mOr	239.214	4.392	Yes
nrg1-4 XopQ vs Roq1-3 mOrange	167.178	2.501	Do Not Test
nrg1-4 XopQ vs eds1 mOrange	51.603	0.761	Do Not Test
eds1 mOrange vs nrg1-4 mOrang	365.598	5.516	Yes
eds1 mOrange vs WT mOrange all	268.484	4.854	Yes
eds1 mOrange vs adr1 nrg1 mOr	187.611	3.176	No
eds1 mOrange vs Roq1-3 mOrange	115.576	1.636	Do Not Test
Roq1-3 mOrang vs nrg1-4 mOrang	250.023	3.832	Yes
Roq1-3 mOrang vs WT mOrange al	152.908	2.828	No
Roq1-3 mOrang vs adr1 nrg1 mOr	72.035	1.244	Do Not Test
adr1 nrg1 mOr vs nrg1-4 mOrang	177.987	3.390	Yes
adr1 nrg1 mOr vs WT mOrange al	80.873	2.144	Do Not Test
WT mOrange al vs nrg1-4 mOrang	97.114	2.013	No

Note: The multiple comparisons on ranks do not include an adjustment for ties.

**Supplemental Statistics S7** - SF was treated as a measurement variable. The different treatments were treated as nominal variable. Because SF is a proportion the values were arcsine transformed before further testing.

**Descriptive Statistics:**

Column	Size	Missing	Mean	Std Dev	Std. Error	C.I. of Mean
ni	16	0	0.141	0.0381	0.00953	0.0203
AIM	22	0	0.240	0.0920	0.0196	0.0408
mOrange	23	0	0.460	0.131	0.0272	0.0565

Column	Range	Max	Min	Median	25%	75%
ni	0.157	0.248	0.0907	0.132	0.119	0.156
AIM	0.321	0.421	0.0997	0.233	0.170	0.298
mOrange	0.476	0.665	0.188	0.484	0.379	0.579

Column	Skewness	Kurtosis	K-S Dist.	K-S Prob.	SWilk	WSWilk	Prob
ni	1.500	3.256	0.197	0.098	0.878	0.036	
AIM	0.537	-0.392	0.137	0.328	0.948	0.290	
mOrange	-0.358	-0.475	0.0940	0.769	0.967	0.625	

**One Way Analysis of Variance**

Normality Test (Shapiro-Wilk) Passed (P = 0.271)  
 Equal Variance Test: Failed (P < 0.050)

Group Name	N	Missing	Mean	Std Dev	SEM
ni	16	0	0.141	0.0381	0.00953
AIM	22	0	0.240	0.0920	0.0196
mOrange	23	0	0.460	0.131	0.0272

Source of Variation	DF	SS	MS	F	P
Between Groups	2	1.077	0.538	54.298	<0.001
Residual	58	0.575	0.00992		
Total	60	1.652			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).  
 Power of performed test with alpha = 0.050: 1.000  
 All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
mOrange vs. ni	0.320	3	13.947	<0.001	Yes
mOrange vs. AIM	0.221	3	10.504	<0.001	Yes
AIM vs. ni	0.0991	3	4.285	0.010	Yes

**Supplemental Statistics S8** - SF was treated as a measurement variable. The different treatments were treated as nominal variable. Because SF is a proportion the values were arcsine transformed before further testing.

**Descriptive Statistics:**

Column	Size	Missing	Mean	Std Dev	Std. Error	C.I. of Mean
mO/tr	13	0	0.379	0.0854	0.0237	0.0516
Xq/tr	13	0	0.849	0.0721	0.0200	0.0436
NRG1/tr	13	0	0.590	0.0602	0.0167	0.0364
NRG1/tr	13	0	0.521	0.0856	0.0237	0.0517
NI/tr	13	0	0.152	0.0435	0.0121	0.0263

Column	Range	Max	Min	Median	25%	75%
mO/tr	0.323	0.542	0.219	0.386	0.325	0.432
Xq/tr	0.241	0.950	0.709	0.849	0.807	0.909
NRG1/tr	0.205	0.699	0.494	0.591	0.536	0.638
NRG1 tag tr	0.280	0.644	0.364	0.537	0.449	0.583
NI/tr	0.160	0.250	0.0894	0.145	0.123	0.179

Column	Skewness	Kurtosis	K-S Dist.	K-S Prob.	SWilk	WSWilk	Prob
mO/tr	-0.0950	0.313	0.114	0.792	0.983	0.991	
Xq/tr	-0.388	-0.210	0.161	0.439	0.957	0.709	
NRG1/tr	0.103	-0.538	0.102	0.833	0.977	0.960	
NRG1 tag/tr	-0.399	-0.603	0.134	0.662	0.965	0.828	
NI/tr	0.772	0.948	0.143	0.595	0.950	0.595	

Column	Sum	Sum of Squares
mO/tr	4.922	1.951
Xq/tr	11.039	9.436
NRG1/tr	7.665	4.563
NRG1 tag/tr	6.774	3.618
NI/tr	1.970	0.321

### One Way Analysis of Variance

Normality Test (Shapiro-Wilk) Passed (P = 0.803)

Equal Variance Test: Passed (P = 0.194)

Group Name	N	Missing	Mean	Std Dev	SEM
mO/tr	13	0	0.379	0.0854	0.0237
Xq/tr	13	0	0.849	0.0721	0.0200
NRG1/tr	13	0	0.590	0.0602	0.0167
/tr	13	0	0.521	0.0856	0.0237
NI/tr	13	0	0.152	0.0435	0.0121

Source of Variation	DF	SS	MS	F	P
Between Groups	4	3.465	0.866	170.929	<0.001
Residual	60	0.304	0.00507		
Total	64	3.769			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

### All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Means	p	q	P	P<0.050
Xq/tr vs. NI/tr	0.698	5	35.333	<0.001	Yes
Xq/tr vs. mO/tr	0.471	5	23.833	<0.001	Yes
Xq/tr vs. /tr	0.328	5	16.616	<0.001	Yes
Xq/tr vs. NRG1/tr	0.260	5	13.144	<0.001	Yes
NRG1/tr vs. NI/tr	0.438	5	22.189	<0.001	Yes
NRG1/tr vs. mO/tr	0.211	5	10.689	<0.001	Yes
NRG1/tr vs. /tr	0.0685	5	3.472	0.115	No
NRG1 tag/tr vs. NI/tr	0.370	5	18.717	<0.001	Yes
NRG1 tag/tr vs. mO/tr	0.142	5	7.217	<0.001	Yes
mO/tr vs. NI/tr	0.227	5	11.500	<0.001	Yes

**Supplemental Statistics S9 - PNAI was treated as a measurement variable. The different treatments were treated as nominal variable.**

### Descriptive Statistics:

Column	Size	Missing	Mean	Std Dev	Std. Error	C.I. of Mean
mOrange2	85	0	5.624	2.521	0.273	0.544
XopQmOrange2	83	0	6.614	2.241	0.246	0.489
NRG1	60	0	5.183	2.095	0.270	0.541
NRG1/tag	64	0	5.656	2.885	0.361	0.721
NI	76	0	2.408	1.338	0.154	0.306

Column	Range	Max	Min	Median	25%	75%
mOrange2	12.000	12.000	0.000	5.000	4.000	7.000
XopQmOrange2	12.000	14.000	2.000	6.000	5.000	8.000
NRG1	10.000	10.000	0.000	5.000	4.000	6.000
NRG1/tag	17.000	17.000	0.000	5.500	4.000	7.000
NI	6.000	6.000	0.000	2.000	1.250	3.000

Column	Skewness	Kurtosis	K-S Dist.	K-S Prob.	SWilk	WSWilk	Prob
mOrange2	0.529	0.310	0.158	<0.001	0.957	0.006	
XopQmOrange2	0.725	0.676	0.130	0.001	0.949	0.002	
NRG1	0.000361	0.246	0.165	<0.001	0.964	0.075	
NRG1/tag	1.395	3.932	0.155	<0.001	0.891	<0.001	
NI	0.545	-0.265	0.251	<0.001	0.914	<0.001	

Column	Sum	Sum of Squares
mOrange2	478.000	3222.000
XopQmOrange2	549.000	4043.000
NRG1	311.000	1871.000
NRG1/tag	362.000	2572.000

NI 183.000 575.000

**One Way Analysis of Variance**

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Missing	Median	25%	75%
mOrange2	85	0	5.000	4.000	7.000
XopQmOrange2	83	0	6.000	5.000	8.000
NRG1	60	0	5.000	4.000	6.000
NRG1/tag	64	0	5.500	4.000	7.000
NI	76	0	2.000	1.250	3.000

H = 127.244 with 4 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

**All Pairwise Multiple Comparison Procedures (Dunn's Method):**

Comparison	Diff of Ranks	Q	P<0.05
XopQmOrange2 vs NI	179.492	10.628	Yes
XopQmOrange2 vs NRG1	54.476	3.022	Yes
XopQmOrange2 vs NRG1/tag	46.145	2.608	No
XopQmOrange2 vs mOrange2	42.536	2.591	Do Not Test
mOrange2 vs NI	136.955	8.155	Yes
mOrange2 vs NRG1	11.940	0.666	No
mOrange2 vs NRG1/tag	3.608	0.205	Do Not Test
NRG1/tag vs NI	133.347	7.389	Yes
NRG1/tag vs NRG1	8.331	0.436	Do Not Test
NRG1 vs NI	125.016	6.805	Yes

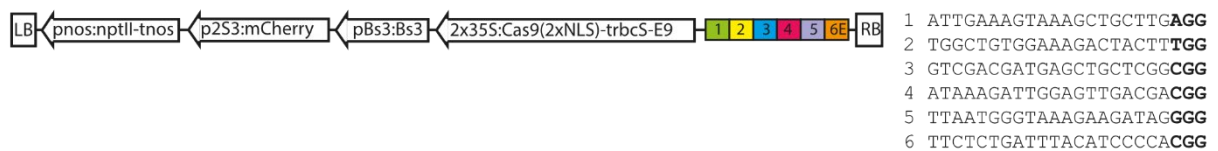
Note: The multiple comparisons on ranks do not include an adjustment for ties.

## **Supplemental – Materials and Methods**

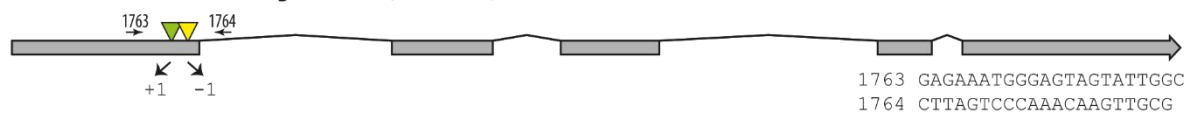
## Supplemental Materials and Methods S1 Generation of *Nb nrg1 adr1* double mutant line.

(A) Scheme of pDGE365 used for generation of *Nb nrg1 adr1* double mutant plants; based on pDGE311 (Stuttman *et al.*, 2021). Guide RNAs were expressed under control of an *Arabidopsis* U6 promotor fragment. Target sites are depicted, color code corresponds to panel b. (B) Gene models of *nrg1* and *adr1* genes targeted for editing. *NRG1* contains +1 and -1 nt mutations in the line used in this study; the +1 mutation at target site 1 induces a STOP codon directly downstream. The +1 insertion at target site 4 in *adr1* (magenta) induces an early STOP 20 codons downstream, before target site 5 (grey).

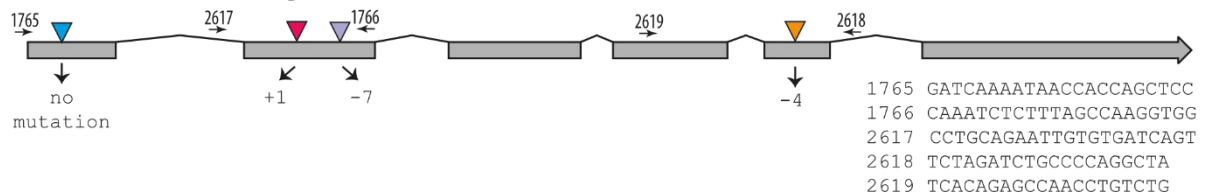
**A**



**B** Niben101Scf02118g00018 (*NbNRG1*)



Niben101Scf02422g02015 (*NbADR1*)



<b>Primer</b>	<b>Sequence, 5' -&gt;3'</b>	<b>Use</b>
<b>AC509</b>	GGTTTCCGCAATGATCCCTC	see AC509
<b>AC510</b>	AAGCTCCTTGATGCCTTCCT	ZmCas9 intron; check for the transgene; with AC510; WT=523bp
<b>JS1763_NRG1screen-F</b>	GAGAAATGGGAGTAGTATTGGC	JS1764
<b>JS1764_NRG1screen-R</b>	CTTAGTCCCAAACAAGTTGCG	genotyping of nrg1 mutation; with JS1763; WT=360bp
<b>JS1765_ADR1screen1-F</b>	GATCAAAATAACCACCAGCTCC	see JS1766
<b>JS1766_ADR1screen1-R</b>	CAAATCTCTTTAGCCAAGGTGG	genotyping of adr1 mutation over sgRNA1, 2 and 3; with JS1765; WT=1089 bp
<b>AC455</b>	CCTGCAGAATTGTGTGATCAGT	genotyping of adr1 mutation over sgRNA1; with JS1766; WT=481bp
<b>AC456</b>	TCTAGATCTGCCCCAGGCTA	see AC457
<b>AC457</b>	TCACAGAGCCAACCTGTCTG	genotyping of adr1 mutation over sgRNA2; with AC456; WT=644bp
<b>AC511</b>	TCCACTCTGTTTAAGCAGCA	additional sequencing of the ADR1 locus
<b>AC512</b>	CACCCAAGTCGAGGAAACAC	additional sequencing of the ADR1 locus

## Supplemental Materials and Methods S2 Cloning of plasmids

The promotor and terminator module were available in the plasmid collection of Engler et al. (2014). The transit peptide of RUBISCO from *Nicotiana benthamiana* was created as a SP module and eGFP as a CDS2\* module (Engler *et al.*, 2014; Marillonnet & Werner, 2015). The following primers were used to clone the two modules: L0-SP-ssu-for = ttga aga caa aatg gct tcc tca gtt ctt tc; L0-SP-ssu-rev = ttg aag aca aac ctg agc tca aat cag gaa ggt atg; L0-CDS2\*-eGFP-for = ttg aag aca aag gtg tga gca agg gcg agg; L0-CDS2\*-eGFP-rev = ttg aag aca aaa gct tac ttg tac agc tcg tcc atg c. For the amplification of the transit peptide a plastid marker construct described in (Nelson *et al.*, 2007) was used as a template. eGFP was amplified from pICSL30006 (Engler *et al.*, 2014).

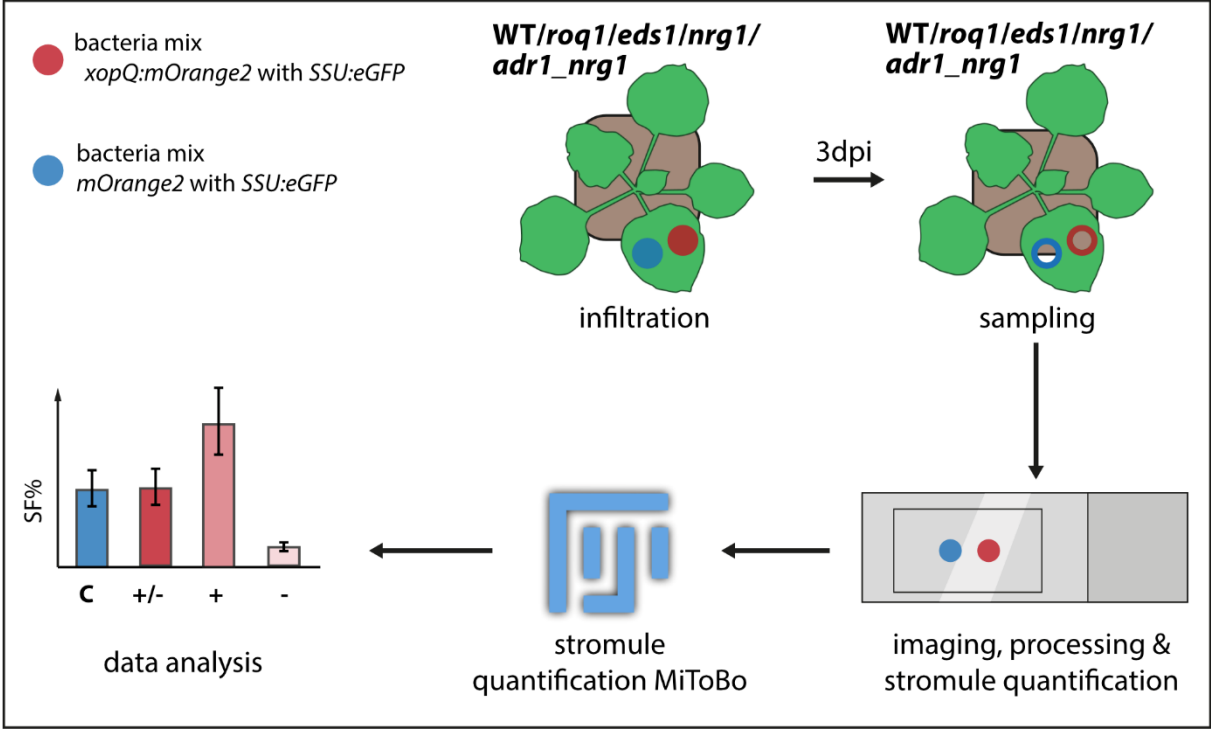
NRG1 overexpression constructs were assembled using the Modular Cloning (MoClo) system and modules from the Plant Parts I and II collections (Engler et al., 2014; Gantner et al., 2018). The *NbNRG1* coding sequence without STOP codon was cloned into pAGM1287; internal *BsaI* and/or *BpiI* restriction sites were eliminated (pJOG1210). The coding sequence with STOP codon was amplified from this construct and cloned into pICH41308 (pJOG1373). A 606 bp fragment upstream of *NbNRG1* was cloned as promoter fragment by PCR amplification and ligation into pICH41295 (pJOG1369). Constructs for expression NRG1 with or without a 6xHA-2xStrep tag (HS) were assembled as following:

Expression cassette	Acceptor	Promotor	CDS	tag	terminator
<i>pMAS:NRG1:tOCS</i>	pICH47732 L1-P1f	pICH85281	pJOG1373	None	pICH41432
<i>pMAS:NRG1:HS:tOCS</i>	pICH47732 L1-P1f	pICH85281	pJOG1210	pJOG331	pICH41432
<i>pUBQ10:NRG1:tOCS</i>	pICH47732 L1-P1f	pJOG684	pJOG1373	None	pICH41432
<i>pUBQ10:NRG1:HS:tOCS</i>	pICH47732 L1-P1f	pJOG684	pJOG1210	pJOG331	pICH41432
<i>pNRG1:NRG1:tOCS</i>	pICH47732 L1-P1f	pJOG1369	pJOG1373	None	pICH41432
<i>pNRG1:NRG1:HS:tOCS</i>	pICH47732 L1-P1f	pJOG1369	pJOG1210	pJOG331	pICH41432



**Supplemental Materials and Methods S3 Experimental procedure utilized for *A. tumefaciens* infiltration experiments.**

Depiction of the experimental procedure for assessing stromule frequency in different mutant backgrounds. Bacteria mix = 1:1 mixes of *A. tumefaciens* of an  $OD_{600nm} = 0.2$ .



**Supplemental Materials and Methods S4 Information on sample sizes and data analysis for stromule frequencies and PNAI values.** Stromule frequency measurements in WT, *roq1*, *eds1*, *nrg1* and *adr1\_nrg1* mutant plant lines in response to *xopQ-mOrange2* and *mOrange2* expression were performed in 3 independently grown batches of 3-5 plants each. Stromule frequencies presented in Figure 2, 3, 4, 5 and Fig S9 represent the average values of all experiments. SF% in Figure 1 represents the average of 5 plants. For calculation of stromule frequencies in % (SF%) the number of plastids with one or more stromules was counted and divided by the total number of plastids. The resulting data was arcsine transformed, and statistical analysis was performed on the transformed data using SigmaPlot 12 (Systat Software GmbH, Erkrath, Germany). 95% confidence intervals and arithmetic averages were calculated and back-transformed data was represented in bar graphs (transformations completed using Microsoft Excel). For evaluating statistical significance between SF% values One-Way ANOVA and a subsequent Post Hoc Test (Tukey Test) were performed using SigmaPlot 12 (Systat Software GmbH, Erkrath, Germany). Test results can be found in supplemental materials “notes on stats”.

PNAI was measured with the help of the Fiji/ImageJ MTBCellcounterPlugIn (Franke *et al.*, 2015). For evaluating statistical significance between the PNAI of the various treatments a Kruskal Wallis One-Way ANOVA analysis on ranks and a Post Hoc test (Dunn’s Method) was performed using SigmaPlot 12 (Systat Software GmbH, Erkrath, Germany).

## Supplemental Materials and Methods S5 Naming conventions.

### Genes

Names for plant genes are given in italic capital letters (e.g. *ROQ1*). Names of mutant alleles are printed in small italic letters (e.g. *roq1-3*). Bacterial genes are printed in small italic letters, with the exception of the letter designation which is capitalized (e.g. *xopQ* = the gene *Xanthomonas outer protein Q*). Genes which are missing in a given bacterial strain are indicated by the Greek letter delta (e.g.  $\Delta xopQ$  = strain missing the *xopQ* gene). Cloned DNA sequences are handled like the respective genes. Fusion sites of DNA sequences are indicated by “:” (e.g. *FNR:eGFP; xopQ:mOrange2*).

### Proteins

Plant proteins are not italicized and in all capital letters (e.g. ROQ1). Bacterial proteins are not italicized and start with a capital letter (e.g. XopQ = the protein Xanthomonas outer protein Q). Fluorescence proteins and genes are treated as plant genes and proteins when the name is abbreviated, such as *eGFP* (gene) or eGFP (Protein). Such protein names often consist of information about the species, the name of a color, descriptive words or oligomerization or other properties (this is indicated in small letters). In cases where the full name of the fluorescence protein is used, rather than the abbreviation, the first letter is capitalized (e.g. *mOrange2* = monomeric (property of protein=small) Orange fluorescence protein (full fluorescence protein name) 2 (numerical designation)). The protein is addressed in all capital not italicized letters except for the oligomerization descriptor (e.g. mORANGE2). Fusion sites of protein sequences are indicated by “-” (e.g. XopQ-mORANGE2).

## Supplemental References

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