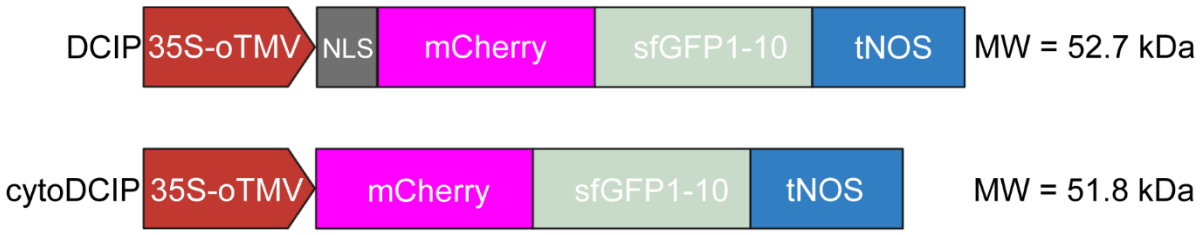
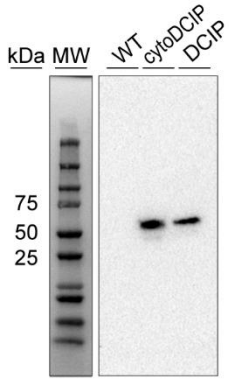
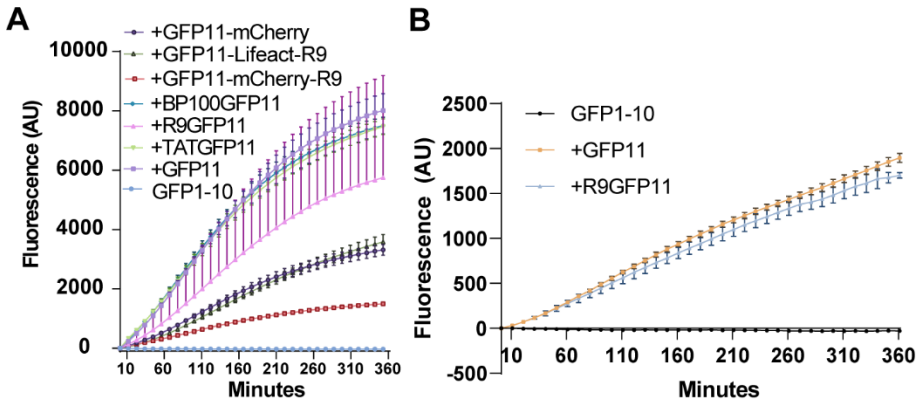


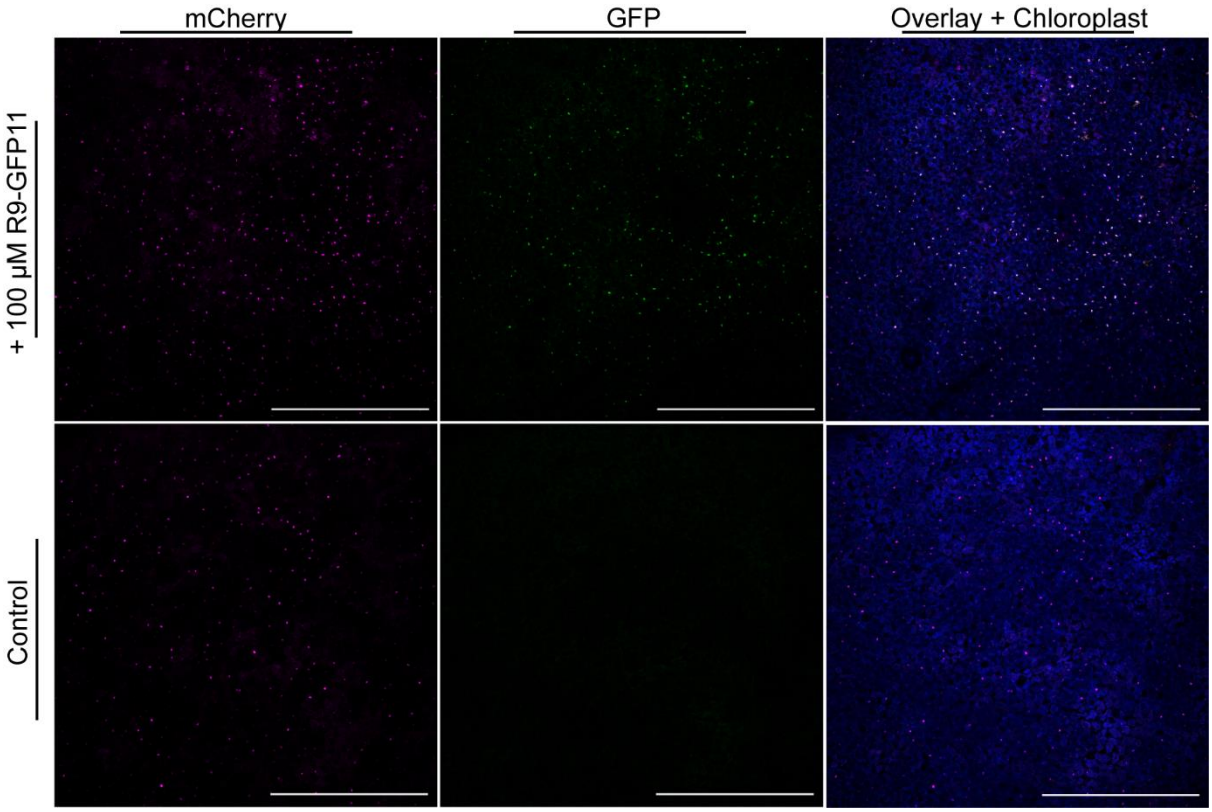
Supplemental Figure 1. Schematic of DCIP and cytoDCIP transcriptional unit. Expression is driven by a 35s promoter and terminated by tNOS. DCIP possess a SV40 NLS for nuclear localization whereas cytoDCIP does not and localizes to the cytosol. Both vectors were constructed as level-1 assemblies in Goldenbraid 2.0.



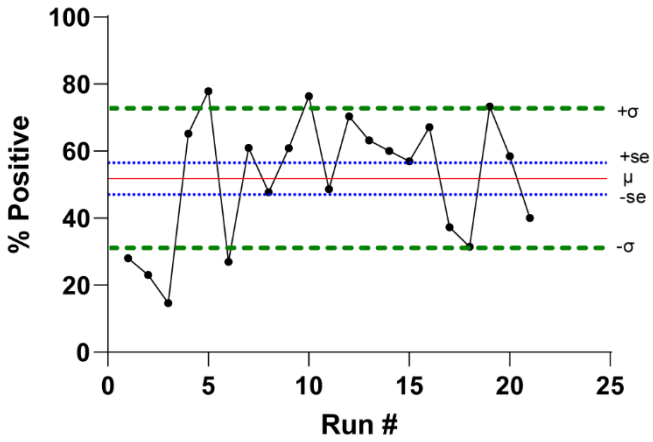
Supplemental Figure 2. Western Blot using anti-mCherry primary antibody and *N. benthamiana* leaf lysates 3 d.p.i. with either DCIP or cytoDCIP showing both protein fusions at the predicted molecular weight.



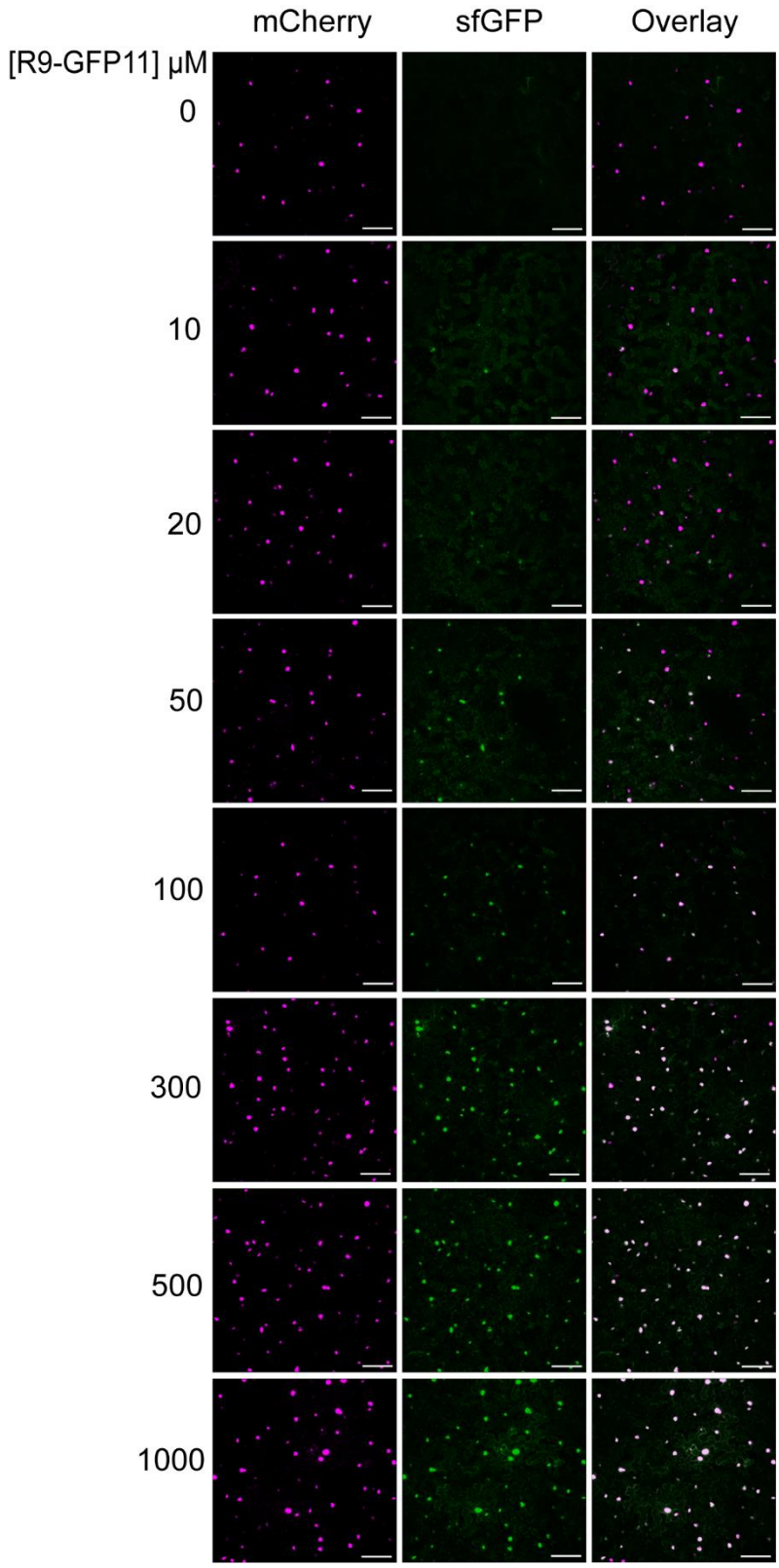
Supplemental Figure 3. *In vitro* GFP complementation assay using recombinantly expressed and purified sfGFP1-10 and all GFP11 containing constructs used in this study. A final concentration of 5μM GFP1-10 his-tag eluate and 10μM of GFP11 containing protein or peptide were incubated for up to 6 hours. Fluorescence was measured every minute on a Biorad CFX96 qPCR machine set at (A) 21°C or (B) 4°C.



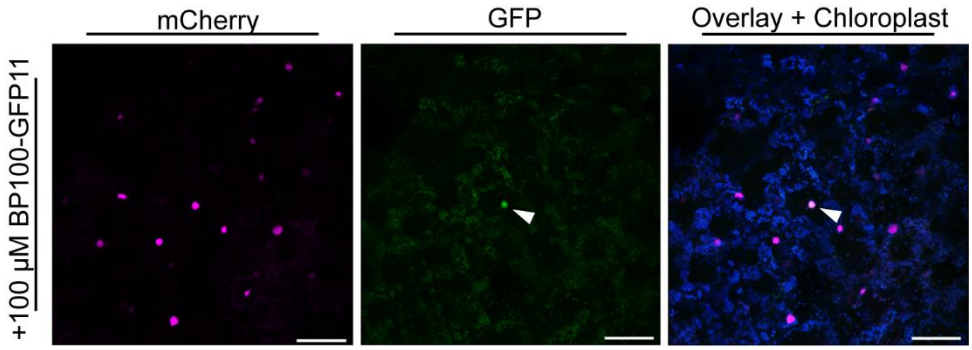
Supplemental Figure 4. DCIP expressing plant infiltrated with either 100µM R9-GFP11 (above) or water control (below) and imaged using confocal microscopy at 4-5H using a 5x objective. mCherry fluorescence is pseudocolored magenta and sfGFP fluorescence is colored green. Chloroplast autofluorescence is colored blue. Scale bar is 1mm.



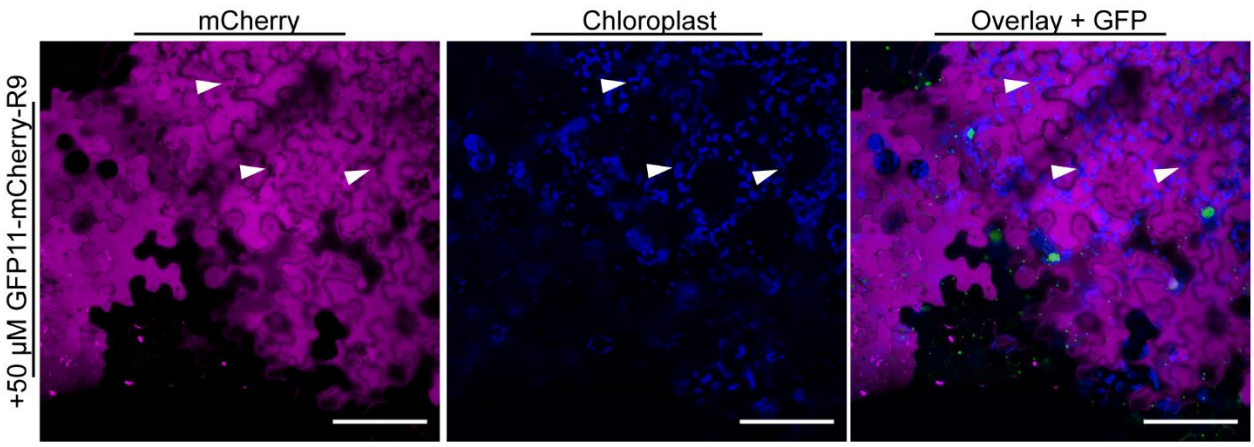
Supplemental Figure 5. Percentage of GFP positive nuclei in DCIP expressing *N. benthamiana* treated with 100μM R9-GFP11 for 4-5H in the first 21 DCIP experiments performed. Data were pooled across several experiments to probe the innate variability in R9 delivery across multiple plants. Standard error and standard deviation are marked with a dotted and dashed line respectively. The mean, 52%, is marked with a solid line.



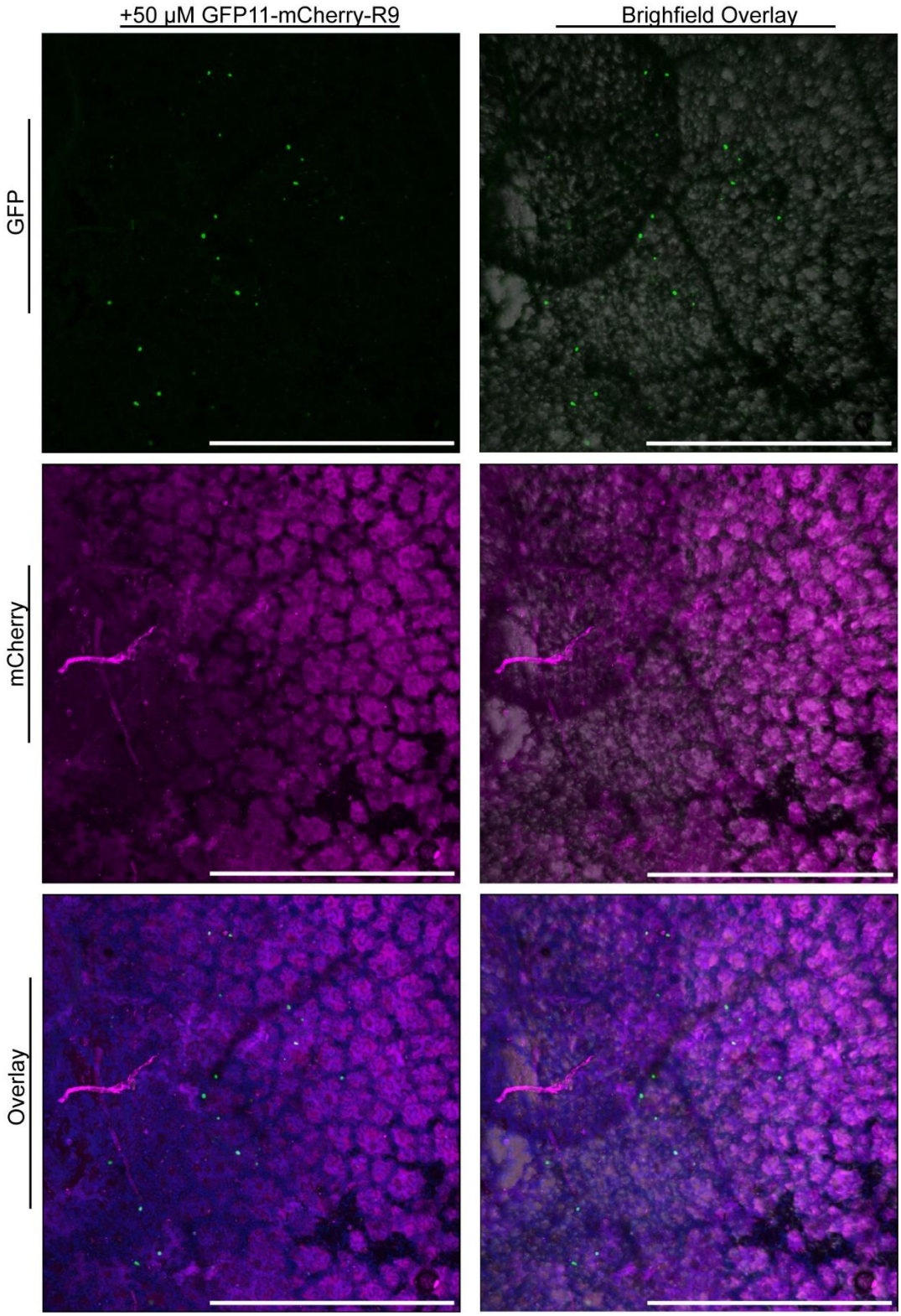
Supplemental Figure 6. Representative two-color maximum intensity projections of DCIP expressing leaves infiltrated with 0-1000 µM R9-GFP11 and incubated for 4-5 hours. Scale bar is 100µm. mCherry is pseudocolored magenta and sfGFP is pseudocolored green. Overlay results in white coloration



Supplemental Figure 7. Example maximum intensity projection of sfGFP complementation as resulting from 100µM BP100-GFP11 incubation in a DCIP expressing leaf disc for 4-5H. Scale bar is 100µm. sfGFP fluorescent nucleus is marked by a white triangle. mCherry is pseudocolored magenta, sfGFP is pseudocolored green, and chloroplast autofluorescence is pseudocolored blue. Overlay results in white coloration.

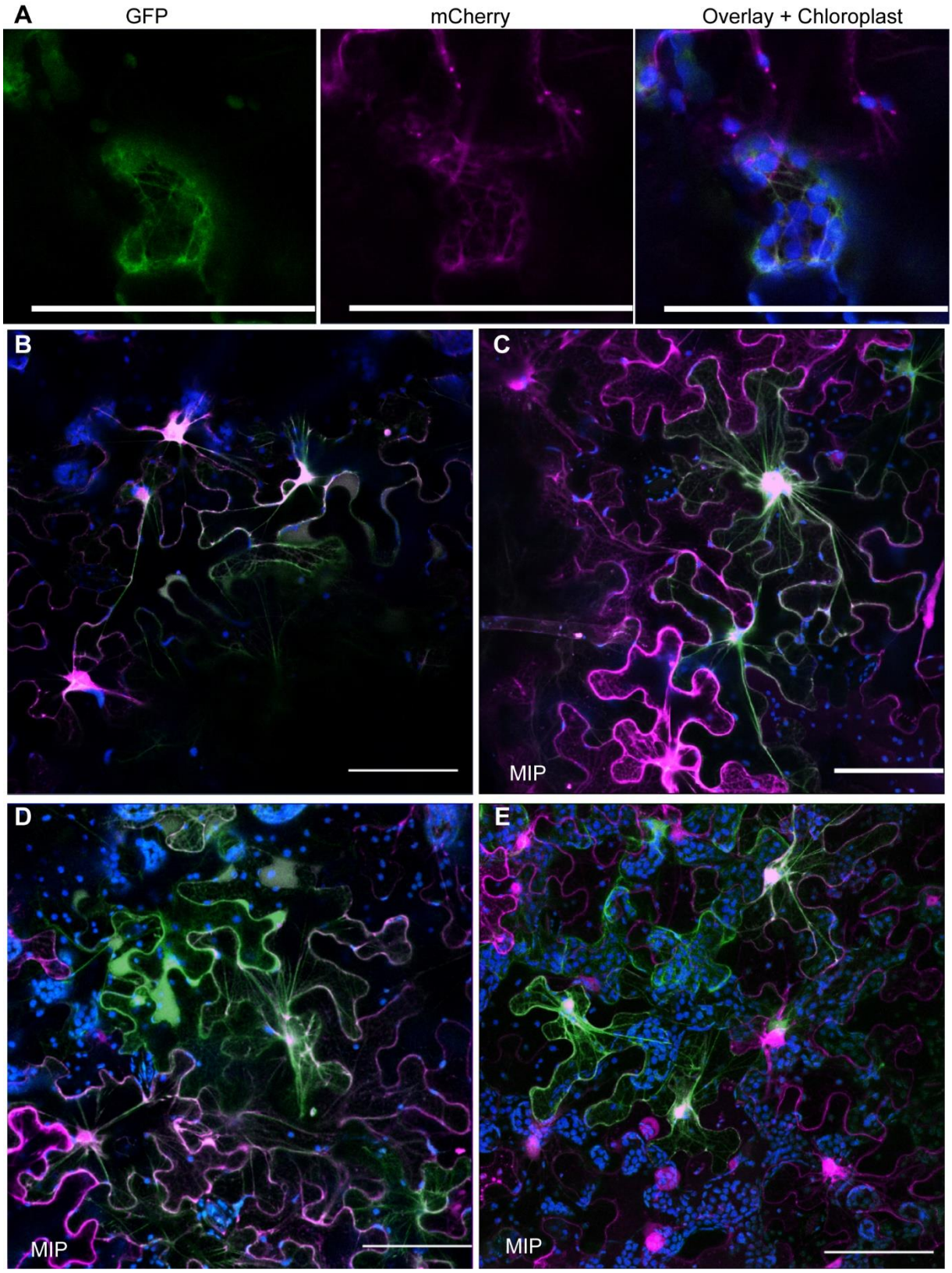


Supplemental Figure 8. Single confocal imaging slice at 5H after infiltration with 50 µM GFP11-mCherry-R9 into a DCIP expressing *N. benthamiana* leaf. White triangles mark excluded regions in the mCherry fluorescence channel (magenta) and the chloroplast autofluorescence (blue) channels. Both channels overlaid with sfGFP fluorescence (green, right image). Scale bar is 100 µM.

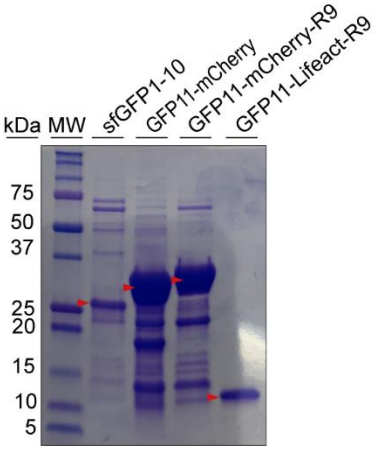


Supplemental Figure 9. DCIP expressing plant infiltrated with 50µM GFP11-mCherry-R9 and imaged using confocal microscopy at 5H using a 5x objective. mCherry fluorescence is pseudocolored magenta and sfGFP fluorescence is colored green. Green

nuclear fluorescence resulting from successful delivery appear as small circular objects. Chloroplast autofluorescence is colored blue. Transmitted light is overlayed in the right column to show anatomy. Scale bar is 1mm.


A
GFP
mCherry
Overlay + Chloroplast
B
C
MIP
D
MIP
E
MIP

Supplemental Figure 10. Image gallery of representative cytoDCIP expressing plants infiltrated with 60μM GFP11-Lifeact-R9 and imaged using confocal microscopy at 6H. (A) Example of fine filamentous delivery-mediated actin labeling and scaffolding of cytoDCIP. (B-E) Representative images of delivery-mediated cytoDCIP-actin binding and labeling. mCherry fluorescence is pseudocolored magenta, chloroplast autofluorescence is pseudocolored blue, and sfGFP fluorescence is colored green. Scale bars are 100μM. Maximum intensity projections were gamma adjusted and are marked MIP.



Supplemental Figure 11. SDS-PAGE of recombinant proteins used in this study. Red triangles indicate the protein of interest. Proteins were stained with Coomassie R-250.

Supplemental Table 1. Parent plasmids and newly constructed plasmids from this study. The predicted protein sequences are provided in expressed plasmids.

Plasmid Name	Notes	Source	Protein Product Sequence	Length (AA)
DCIP	35s::NLS:mCherry:sfGFP1-10::tNOS fusion for nuclear localized expression using agrobacterium in pDGB1-a1R vector backbone.	Generated in this work	MPKKKRKVGVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKGGGGSGGGSLQMIDSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQTVLSKDPNEK*	471
cytoCDCIP	35s::mCherry:sfGFP1-10::tNOS fusion for cytosolic expression using agrobacterium in pDGB1-a1R vector backbone.	Generated in this work	MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKGGGGSGGGSLQMIDSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQTVLSKDPNEK*	463
1B-GFP1-10	sfGFP1-10 inserted into 1B for E. coli expression and Hisx6 tag purification.	Generated in this work	MGSSHHHHHHENLYFQSNAMIDSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQTVLSKDPNEKGIGSG*	240
1BR9-mCherry	mCherry insertion of 1BR9 for E. coli expression and Hisx6 tag purification.	Generated in this work	MGSSHHHHHHENLYFQSNARDHMVLHEYVNAAGITGGGGSGGGGSYFQSNAVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKGIGSGSNGSSGSVSRRRRRRRRR*	309

1BR9-mCherrySTOP	mCherry insertion of 1BR9 for E. coli expression and Hisx6 tag purification with stop codon to prevent R9 tagging.	Generated in this work	MGSSHHHHHHENLYFQSNARDHMVLHEYVNAAGITGGGGSGGGGSYFQSNAVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK*	286
1BR9-Lifeact	Lifeact peptide insertion of 1BR9 for E. coli expression and Hisx6 tag purification.	Generated in this work	MGSSHHHHHHENLYFQSNARDHMVLHEYVNAAGITGGGGSGGGGSYFQSNAMGVADLIKKFESISKEEGIGSGSNGSSGSVSRRRRRRRRRR*	91
PEP101E-NLS-mCherry	UBQ10 driven expression of NLS-mCherry-sfGFP1-10 and Hisx6 tag purification.	Generated in this work	MPKKKRKVGVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKGGGGSGGGSLQMIDSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQTVLSKDPNEK*	471
1BR9	LIC backbone derivative from 1B for expression of N-terminal GFP11 and C-terminal R9 tagged proteins in E. coli	Generated in this work	MGSSHHHHHHENLYFQSNARDHMVLHEYVNAAGITGGGGSGGGGSYFQSN(A(yORF)G)IGSGSNGSSGSVSRRRRRRRRR*	72
PEP101E	Cytosolic UBQ10 driven expression of sfGFP1-10 Plasmid from Prof. Dinesh Kumar (Park et al Plant Cell. 2017 Jul;29 (7):1571-1584.)	Addgene #97387	N/A	N/A
1B	LIC backbone for Hisx6 tagged expression of proteins in E. coli from Scott Gradia at UC Berkeley MacroLab	Addgene #29653	N/A	N/A
H6-mCherry	mCherry coding sequence from Scott Gradia at UC Berkeley MacroLab	Addgene #29722	N/A	N/A

Supplemental Table 2. DNA primers and synthetic sequences used for molecular cloning.

DNA Set	FW 5->3' Sequence	RV 5->3' Sequence	Notes
1	AAACTGCAGATGCCAAAGAAAAAGCGGAAAGTCGGAGTGAGCAAGGGCGAGGAG	AAACTGCAGGCTGCCACCGCCGCTACCGCCACCGCCCTTGTACAGCTCGTCCATGC	Primer set for amplification of mCherry from h6 mCherry Vector with PSTI overhangs for 5' insertion into Dinesh-Kumar sfGFP1-10 vector with an NLS sequence and disordered linker.
2	TACTTCCAATCCAATGCAGTGAGCAAGGGCGAGGAG	CTCCCACTACCAATGCCTTACTTGTACAGCTCGTCCATG	Primer set for amplification of mCherry from h6 mCherry Vector for 1BR9 cloning (with stop codon).
3	TACTTCCAATCCAATGCAGTGAGCAAGGGCGAGGAG	CTCCCACTACCAATGCCCTTGTACAGCTCGTCCATG	Primer set for amplification of mCherry from h6 mCherry Vector for 1BR9 cloning (no stop codon).
4	TACTTCCAATCCAATGCCACCATGATCGATAGCAAAGGAGAAG	TTATCCACTTCCAATGTTATTATCACTTTTCGTTGGGATCTTTC	Primer set for amplification of sfGFP1-10 from plasmid from Dinesh-Kumar for cloning into 1B
5	GCGCCGTCTCGCTCAAAGCTCACTTTT	GCGCCGTCTCGCTCGAATGCCAAAGA	Primer set for amplification of NLS:mCherry:GFP1-10 for Goldenbraid cloning into pUPD2.
6	GCGCCGTCTCGCTCGAATGGTGAGCA	GCGCCGTCTCGCTCGAATGCCAAAGA	Primer set for amplification of mCherry:GFP1-10 for Goldenbraid cloning into pUPD2.
7	TACTTCCAATCCAATGCACGCGATCACATGGTCCTGCACGAGTACGTGAACGCCGCCGGGATCACTGGTGGCGGAGGTTCTGGAGGCGGTGGATCGTACTTCCAATCCAATATTGGTAGTGGGAGCAACGGCAGCAGCGGATCCGTGAGCCGCCGTCGCCGTCGCCGTCGCTAATAACATTGGAAGTGGATAA	TTATCCACTTCCAATGTTATTAGCGACGGCGACGGCGACGGCGACGGCGGCTCACGGATCCGCTGCTGCCGTTGCTCCCACTACCAATATTGGATTGGAAGTACGATCCACCGCCTCCAGAACCTCCGCCACCAGTGATCCCGGCGGCGTTCACGTACTCGTGCAGGACCATGTGATCGCGTGCATTGGATTGGAAGTA	Double stranded LIC insert for conversion of 1B to 1BR9.
8	TACTTCCAATCCAATGCAATGGGCGTGGCCGACCTGATCAAGAAGTTCGAGAGCATCAGCAAGGAAGAGGGCATTGGTAGTGGGAG	CTCCCACTACCAATGCCCTCTTCCTTGCTGATGCTCTCGAACTTCTTGATCAGGTCGGCCACGCCCATTGCATTGGATTGGAAGTA	Double stranded Lifeact insert for LIC into 1BR9.