

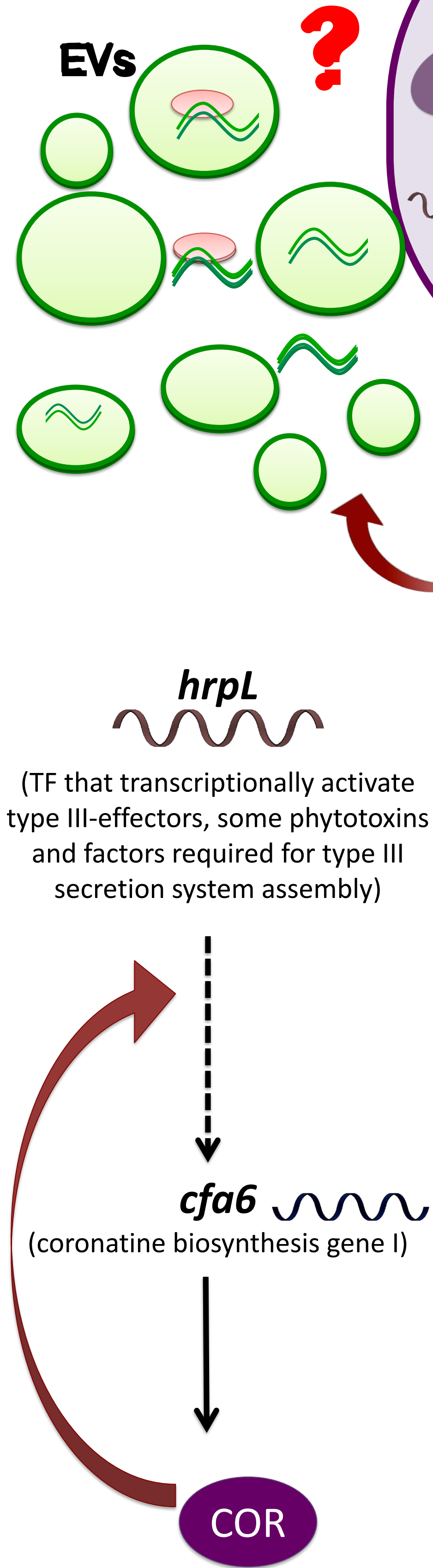
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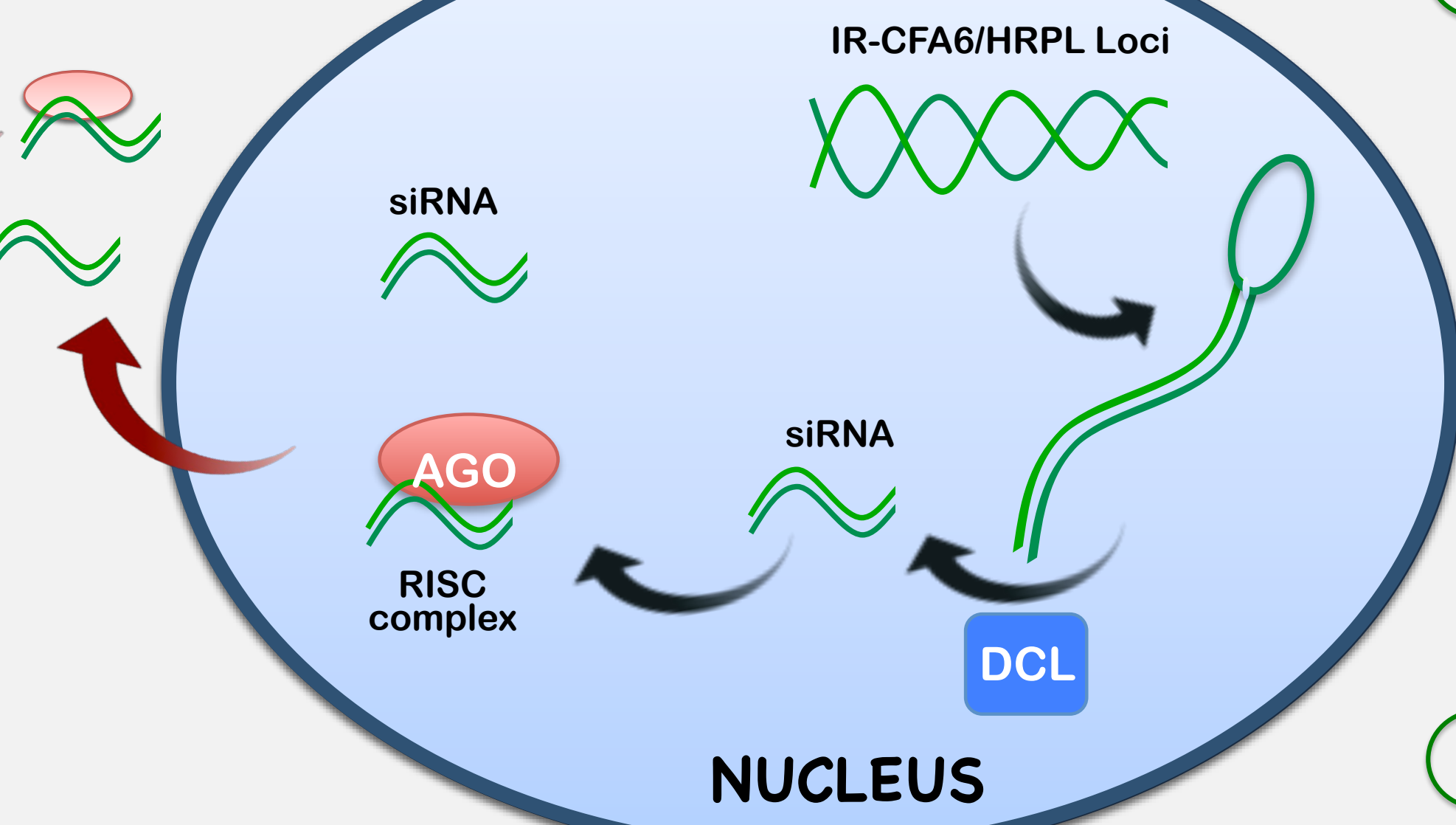


## Major Highlights: Antibacterial Gene Silencing (AGS)

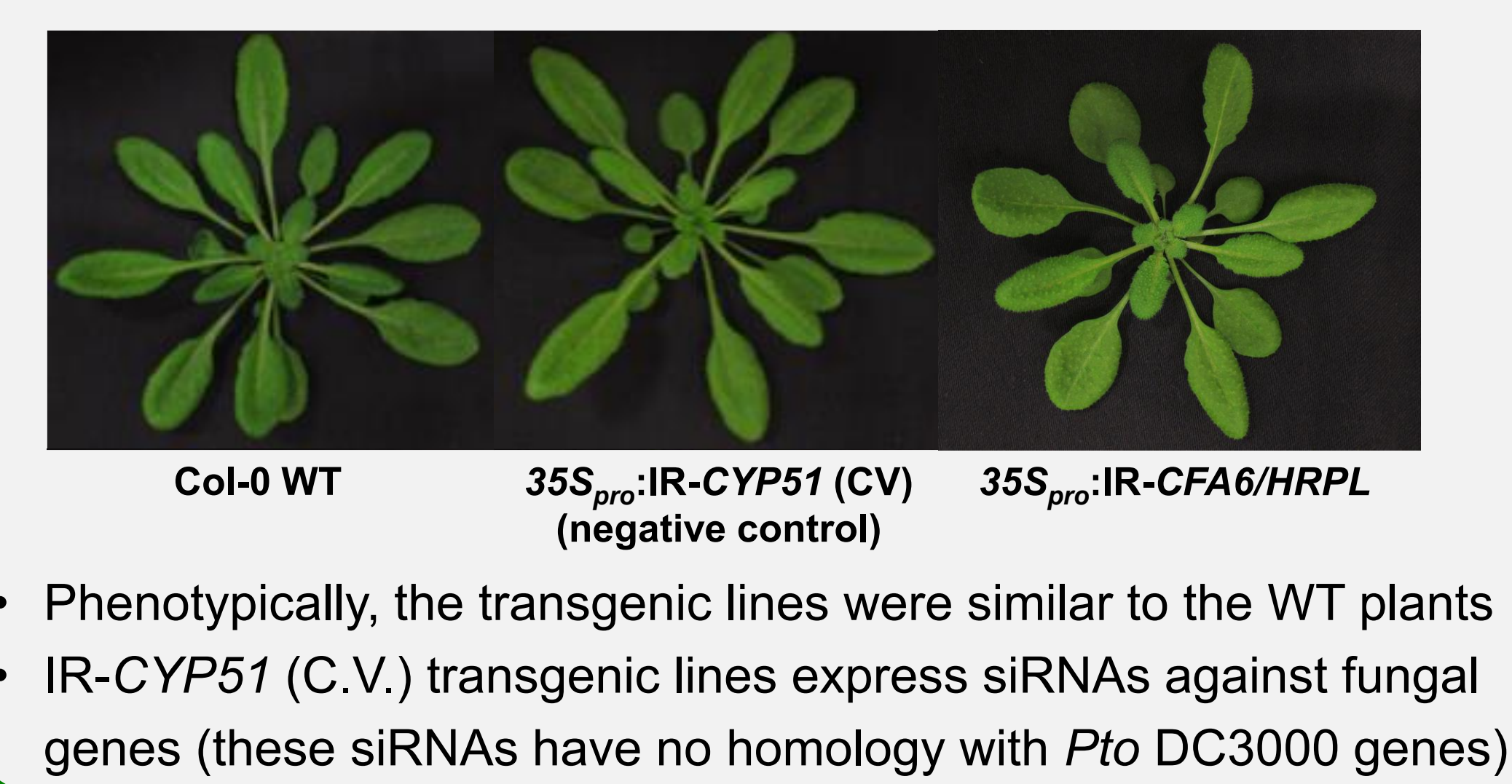
- In planta expression of small RNAs directed against pathogenicity factors of a virulent *Pseudomonas syringae* strain results in Antibacterial gene silencing (AGS) and disease protection (despite the absence of canonical eukaryotic-like RNAi factors expressed from this bacterium)
- This regulatory mechanism can be recapitulated upon exogenous application of total RNAs containing antibacterial small RNAs
- Extracellular vesicles (EVs) contribute to the trafficking of small RNAs from plants cells towards bacterial cells



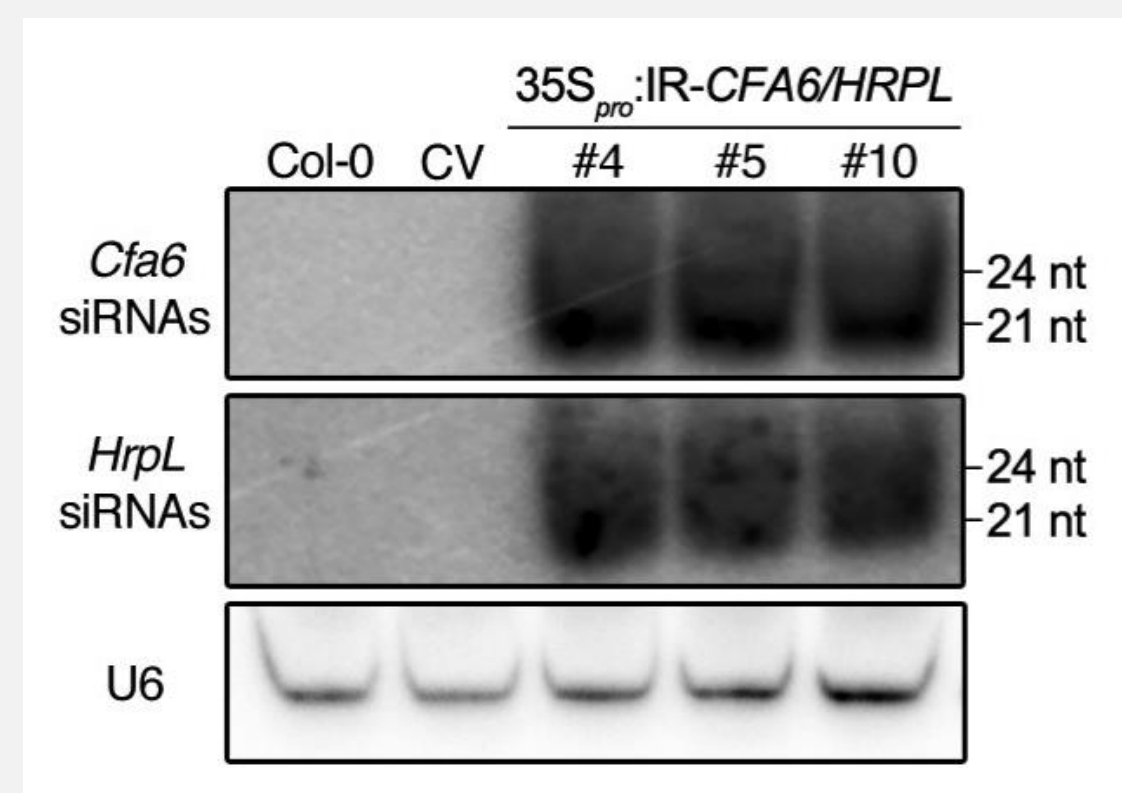
### 1 Generation of Arabidopsis stable transgenic lines expressing the chimeric hairpin IR-CFA6/HRPL



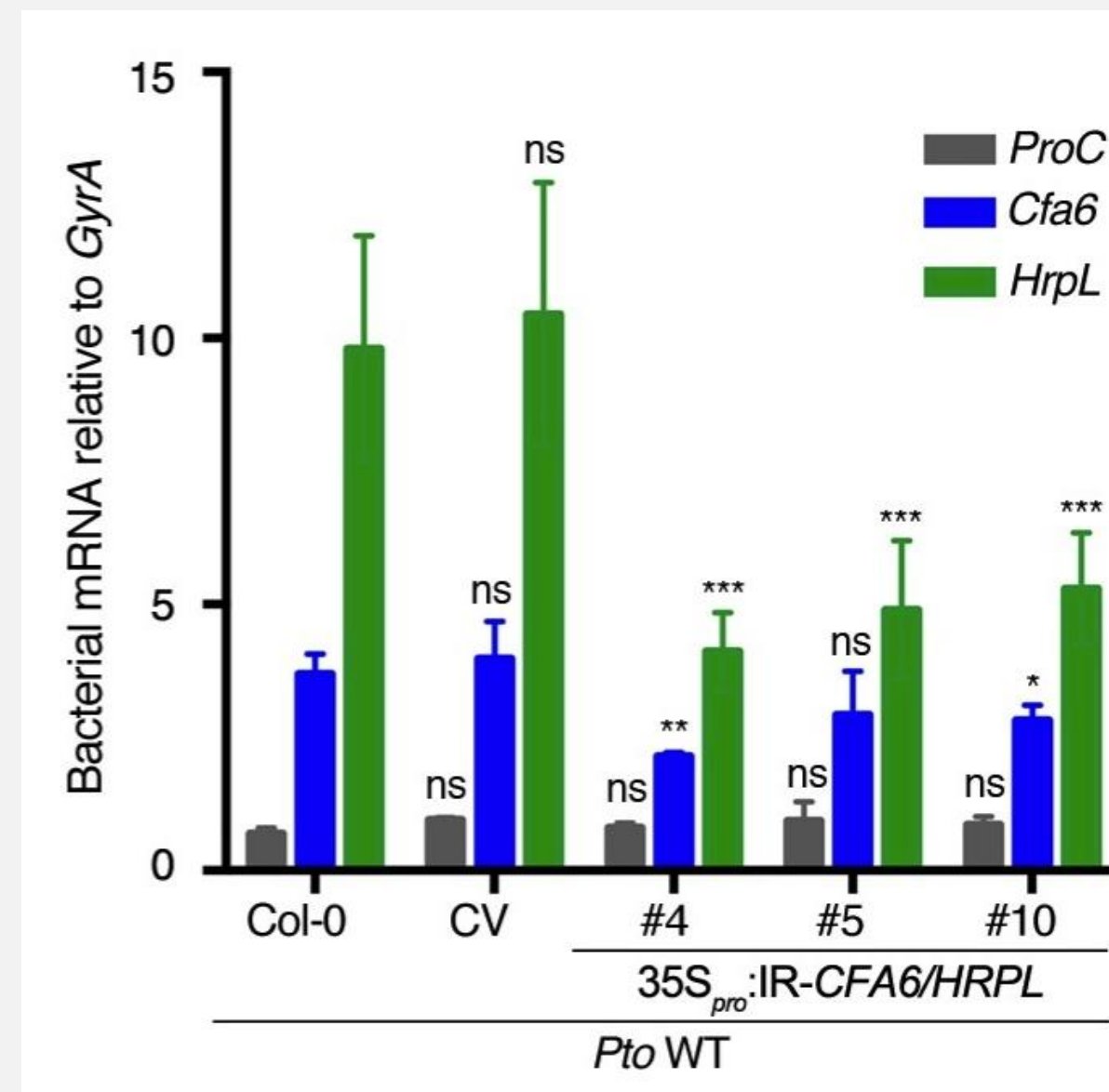
Phenotypically, the transgenic lines were similar to the WT plants



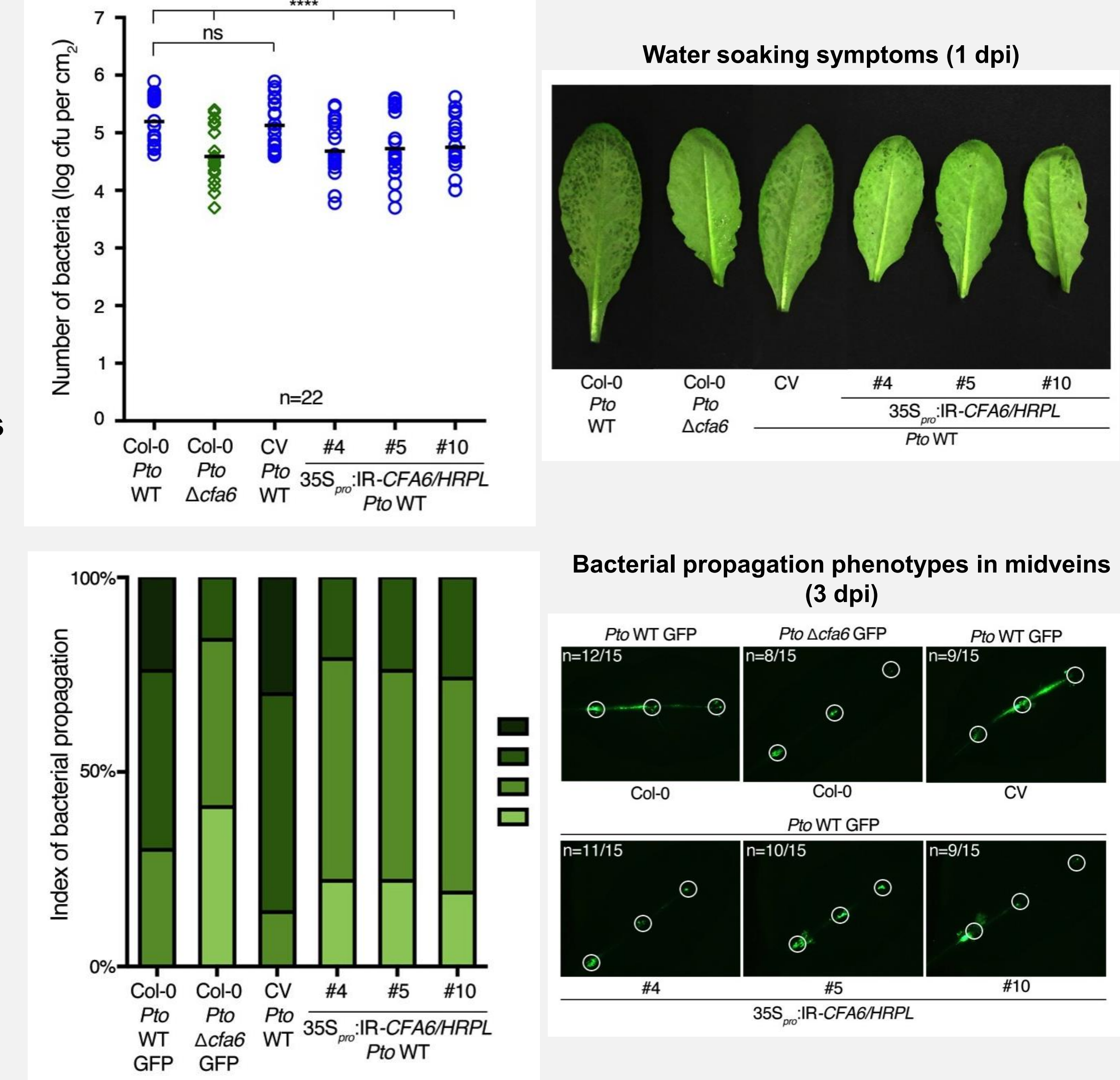
### 2 LMW Northern blot analysis of siRNAs produced from IR-CFA6/HRPL plants



### 3 RT-qPCR analysis on bacterial mRNAs from infected plants (3dpi)

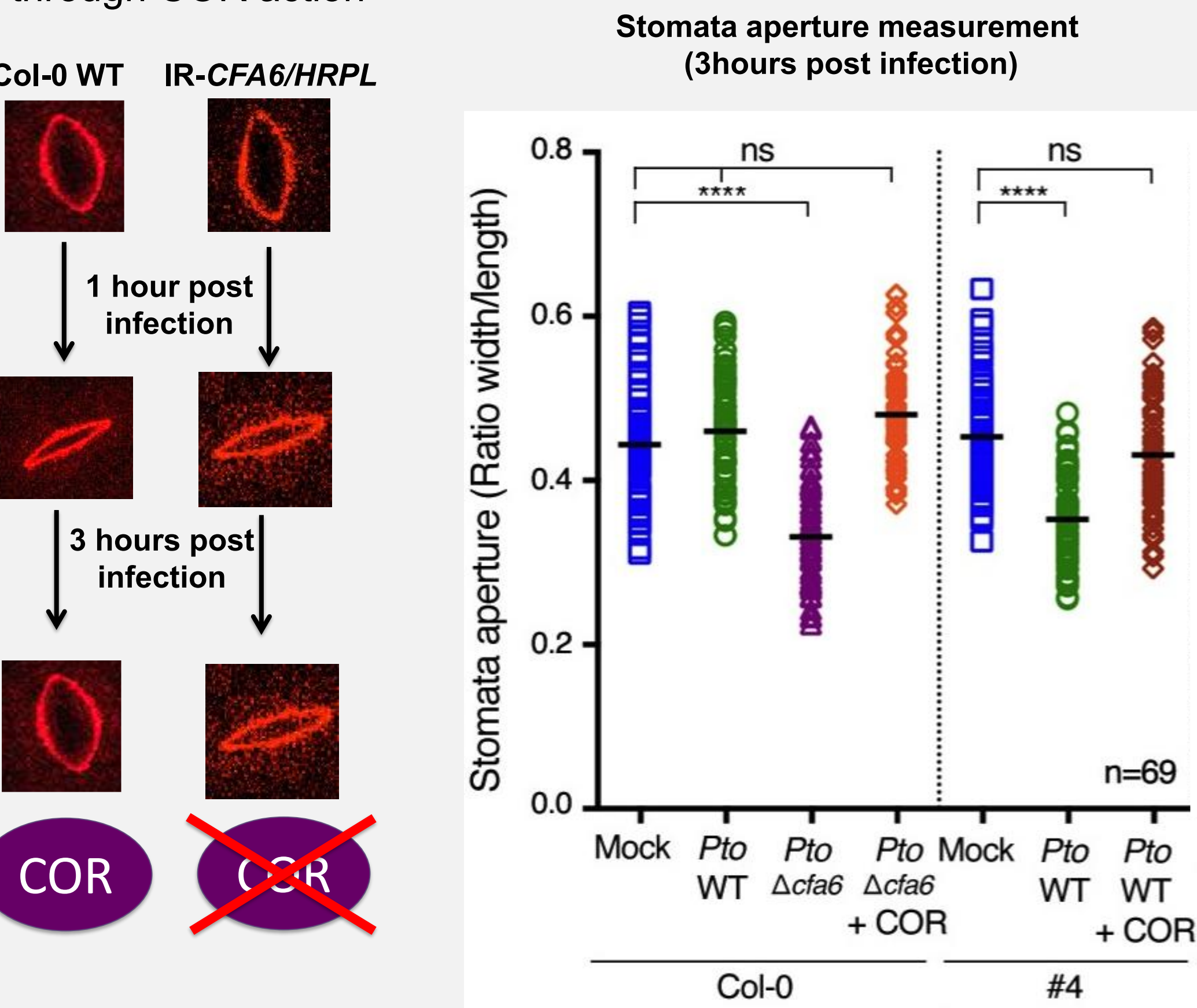


### 4 Effect of siRNAs on bacterial virulence & disease symptoms



### 5 Coronatine (COR)-dependent stomata reopening is impaired in IR-CFA6/HRPL transgenic plants

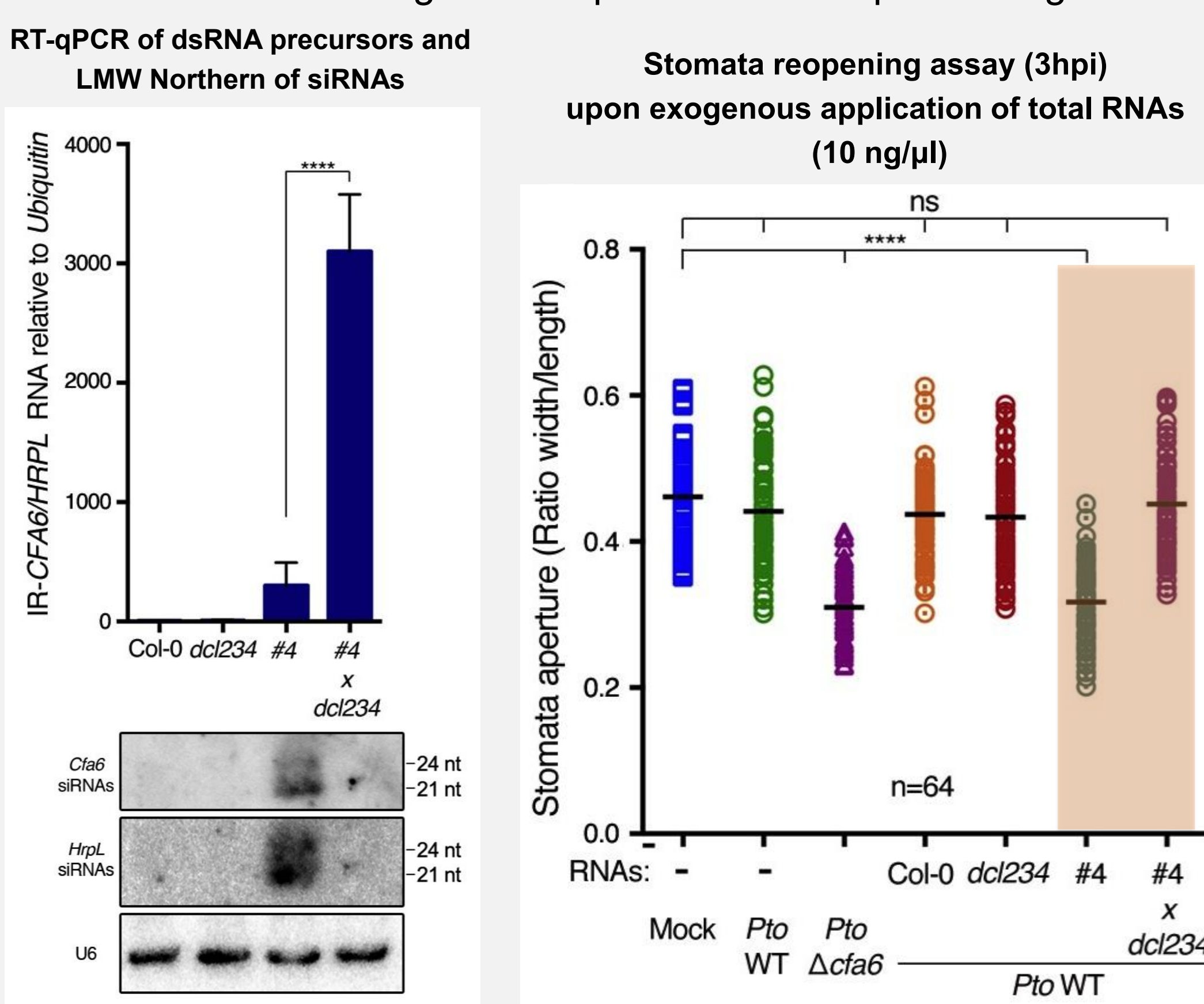
- Stomata are the major entry sites for *Pto* DC3000
- Stomata closes in response to bacterial PAMPs and are reopened through COR action



The impaired ability of *Pto* D3000 to reopen stomata on IR-CFA6/HRPL plants is likely caused by a compromised COR production by *Pto* DC3000 cells

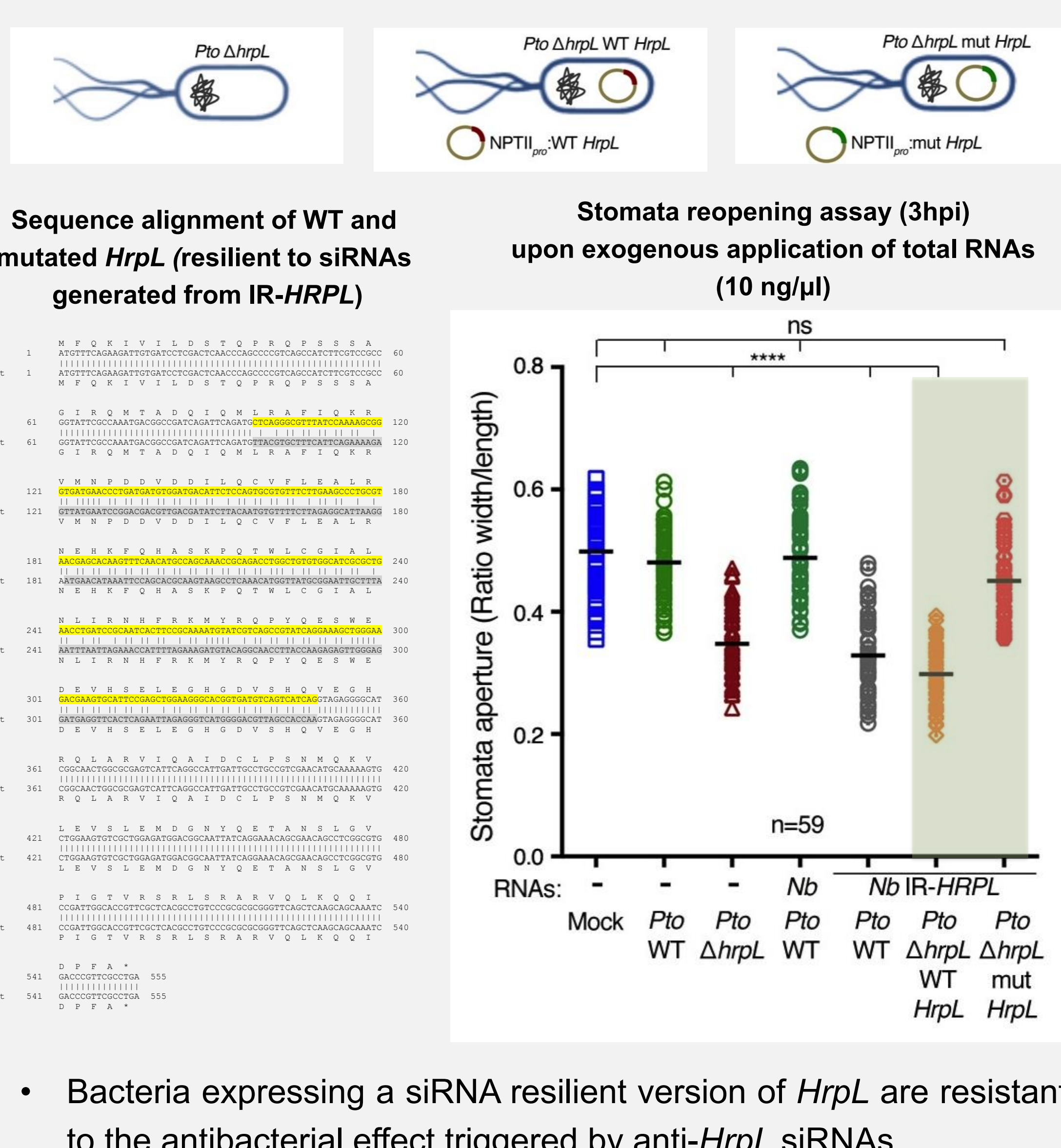
### 6 siRNAs, but not dsRNA precursors, trigger AGS and the dampening of pathogenesis

- Introduction of *dcl2 dcl3 dcl4* triple mutations in a IR-HRPL/CFA6 line #4 background to prevent dsRNA processing



Small RNA entities trigger suppression of *Pto* DC3000-induced stomatal reopening while dsRNA precursors do not interfere with this process

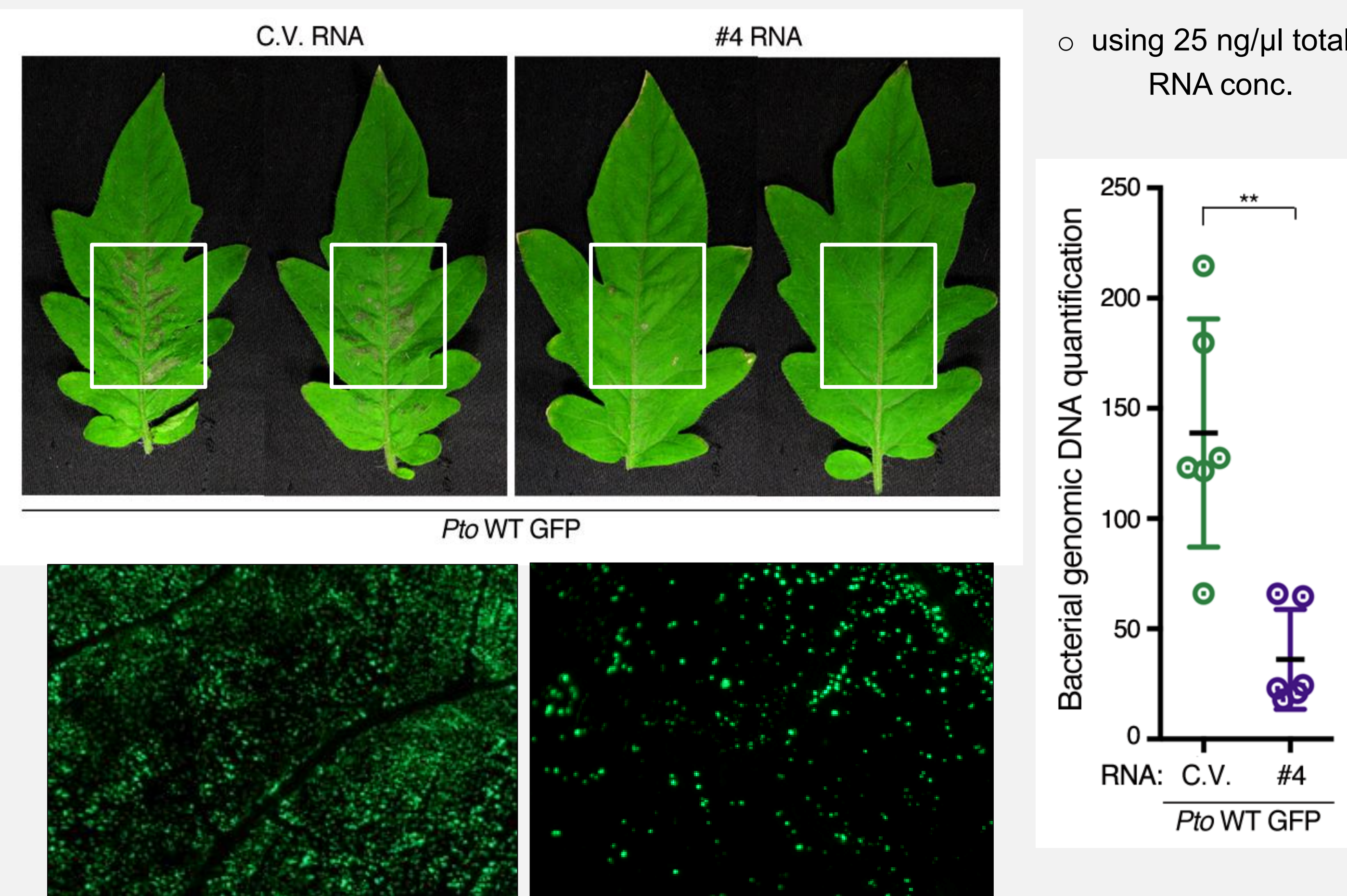
### 7 Small RNAs are likely uptaken and causal for the dampening of *Pto* DC3000 pathogenicity



Bacteria expressing a siRNA resilient version of *HrpL* are resistant to the antibacterial effect triggered by anti-*HrpL* siRNAs

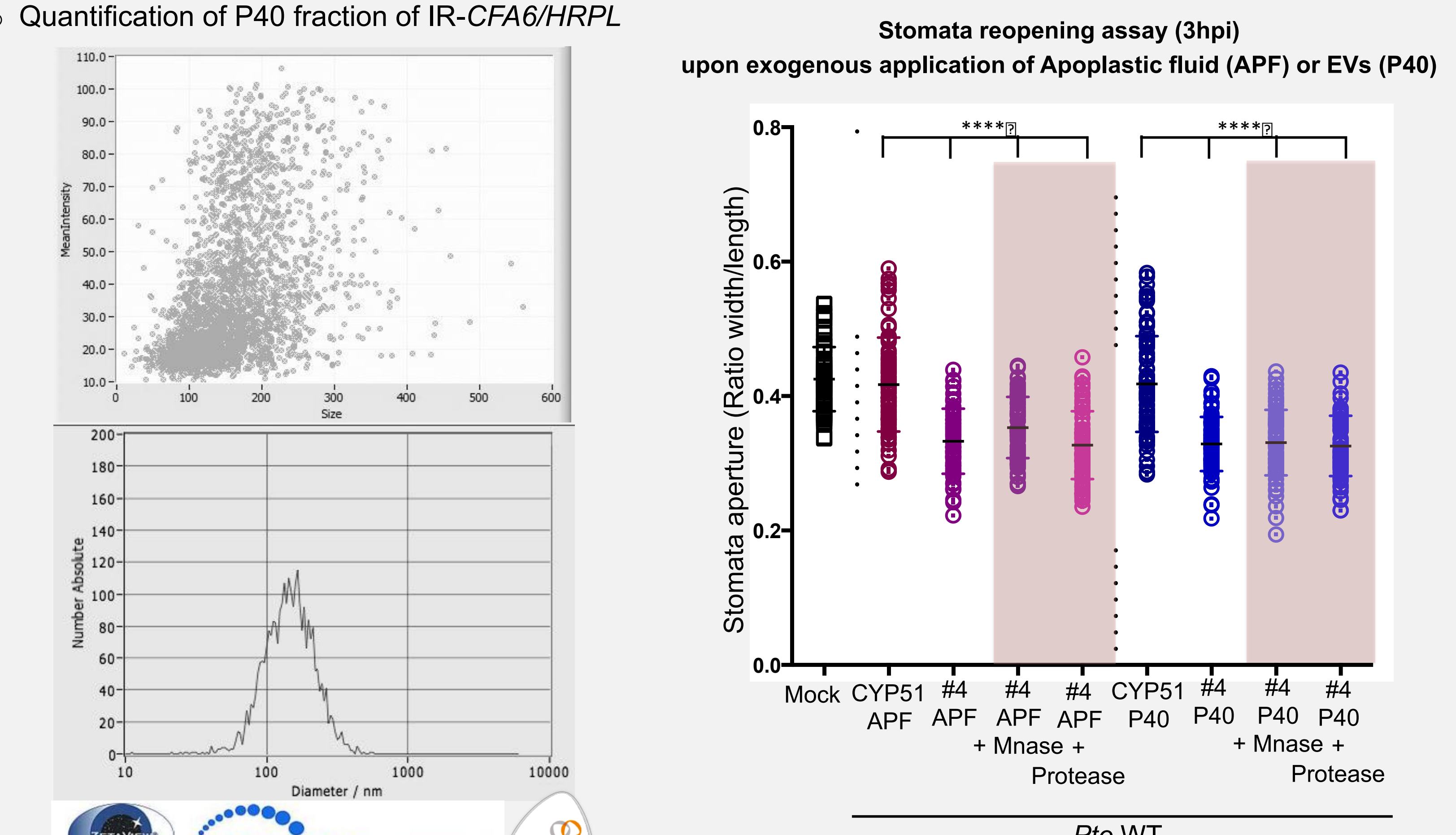
### 8 Exogenous application of antibacterial RNAs protect WT tomato plants against Bacterial Speck

- Exogenous application of total RNAs extracted from Arabidopsis transgenic plants expressing antibacterial siRNAs on four-week-old WT tomato plants leads to disease protection



### 9 Apoplastic fluids as well as apoplastic Extracellular vesicles (EVs) possess AGS activity

- Extraction of Apoplastic fluid (APF) and extracellular vesicles (P40) using differential ultracentrifugation and testing the efficiency of EVs for AGS



## Acknowledgements

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