



Background

- Xylella fastidiosa* is a bacterial pathogen able to colonize more than 600 plant species and causing severe diseases such as Olive Quick Decline Syndrome (OQDS).
- The ecological niche of *X. fastidiosa* is defined by the host plant's xylem vessels and the foregut of xylem sap-feeding insect vectors.
- The absence of a Type III Secretion System (T3SS) raises the question of whether the bacterium releases effectors to enable colonization.

Aim

The objective of this research project is to predict effectors in the secretome of *Xylella fastidiosa*.

Methods

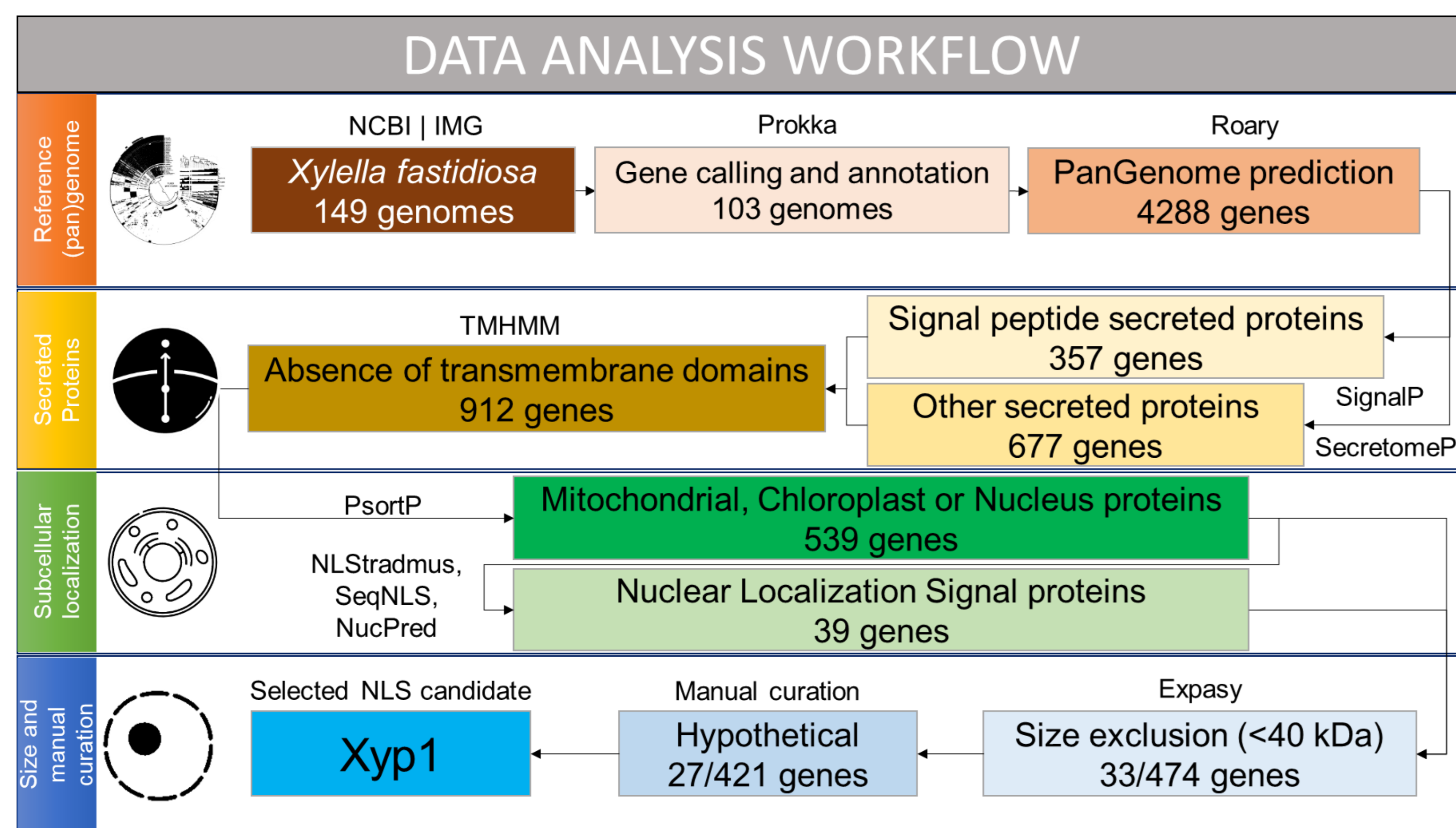


Figure 1. Pipeline that retrieved Xyp1 as one of the 27 NLS effector candidates in the *X. fastidiosa* pangenome.

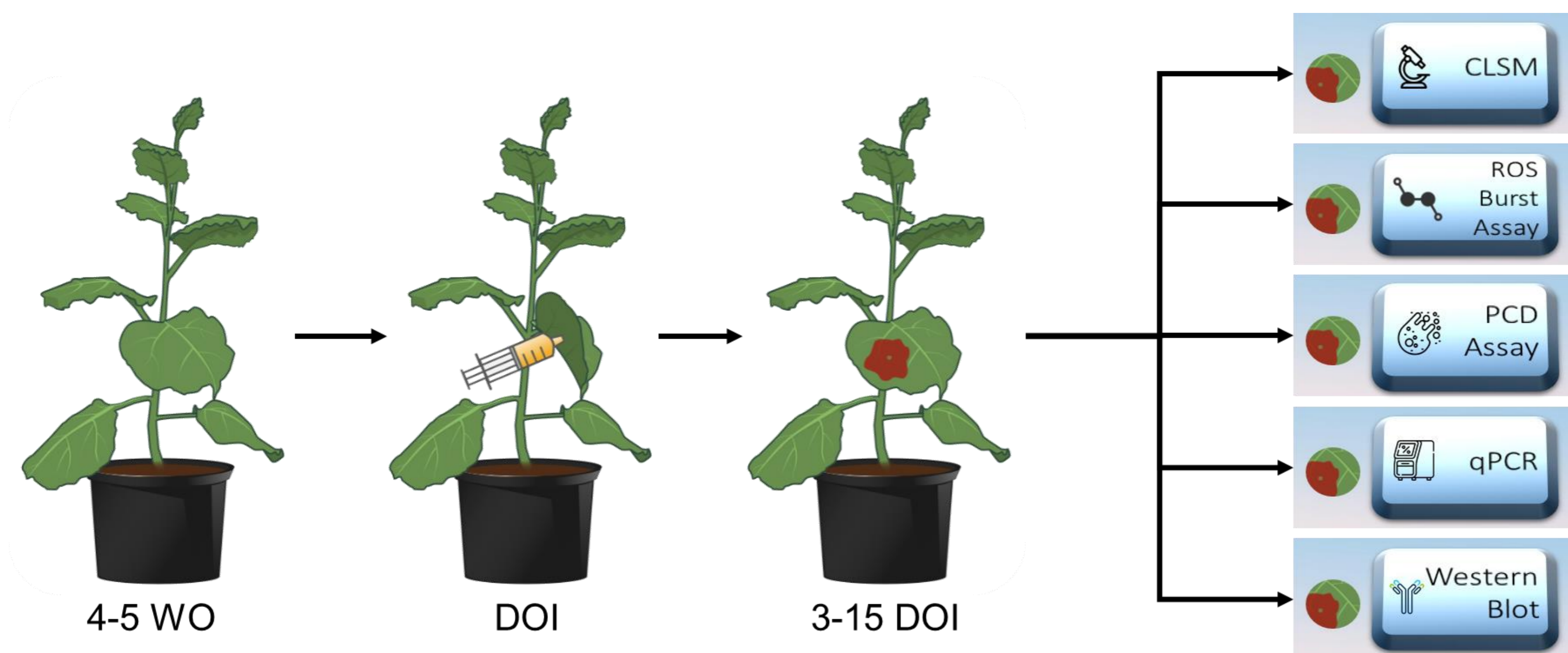


Figure 2. Experiment design to challenge Xyp1 as a putative effector in Tobacco plants.

Highlights

- Pipeline used for screening revealed 421 effector candidates, from which 27 have predicted NLS motifs
- N-NLS motif seems to be responsible for Xyp1 colocalization with SV40 large T antigen in *N. benthamiana* cell nucleus
- Xyp1 induces mild PCD in different *Nicotiana* species up to 15 DPI
- ROS burst in response to flg22 is attenuated by Xyp1 (C1) in *Nicotiana* species.

References

Bai, X. D. et al. AY-WB *Phytoplasma* Secretes a Protein That Targets Plant Cell Nuclei. *Mol Plant Microbe In* 22, 18-30, doi:10.1094/Mpmi-22-1-0018 (2009).

Landa, B. B. et al. *Xylella fastidiosa*'s relationships: the bacterium, the host plants, and the plant microbiome. *New Phytol* 234, 1598-1605, doi:10.1111/nph.18089 (2022).

Results and Discussion

Xyp1 constructs

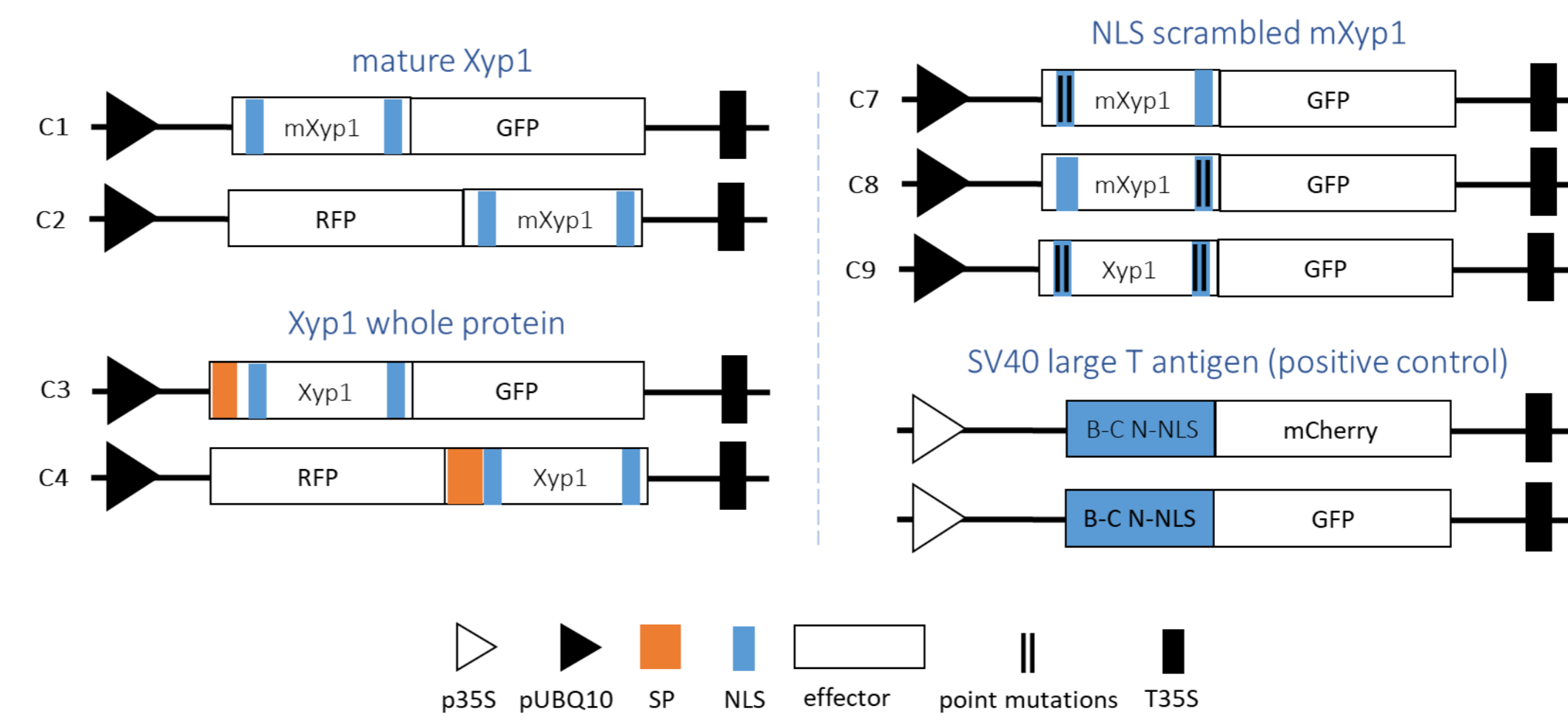


Figure 3. Constructs 1 to 4 (C1-4) show the strategy to challenge whole or mature protein sequences and attaching fluorescent protein in N- or C-terminal. We scrambled N-, C-terminal NLS motifs or both (C7-9). We used the NLS of SV40 large T antigen (B-C N-NLS) sequence as a positive NLS.

Xyp1 is colocalized with SV40 large T antigen in *N. benthamiana* cell nucleus

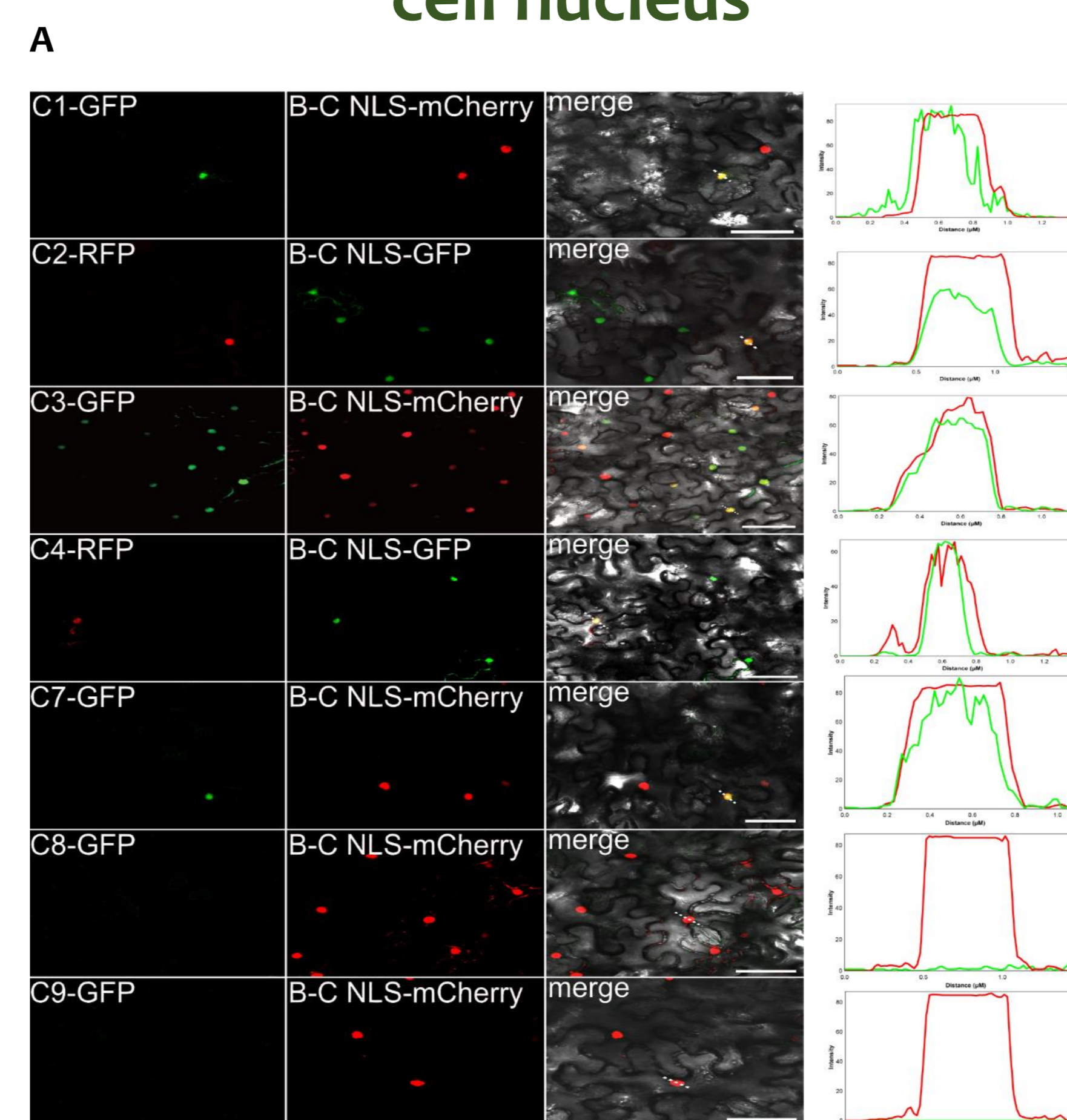
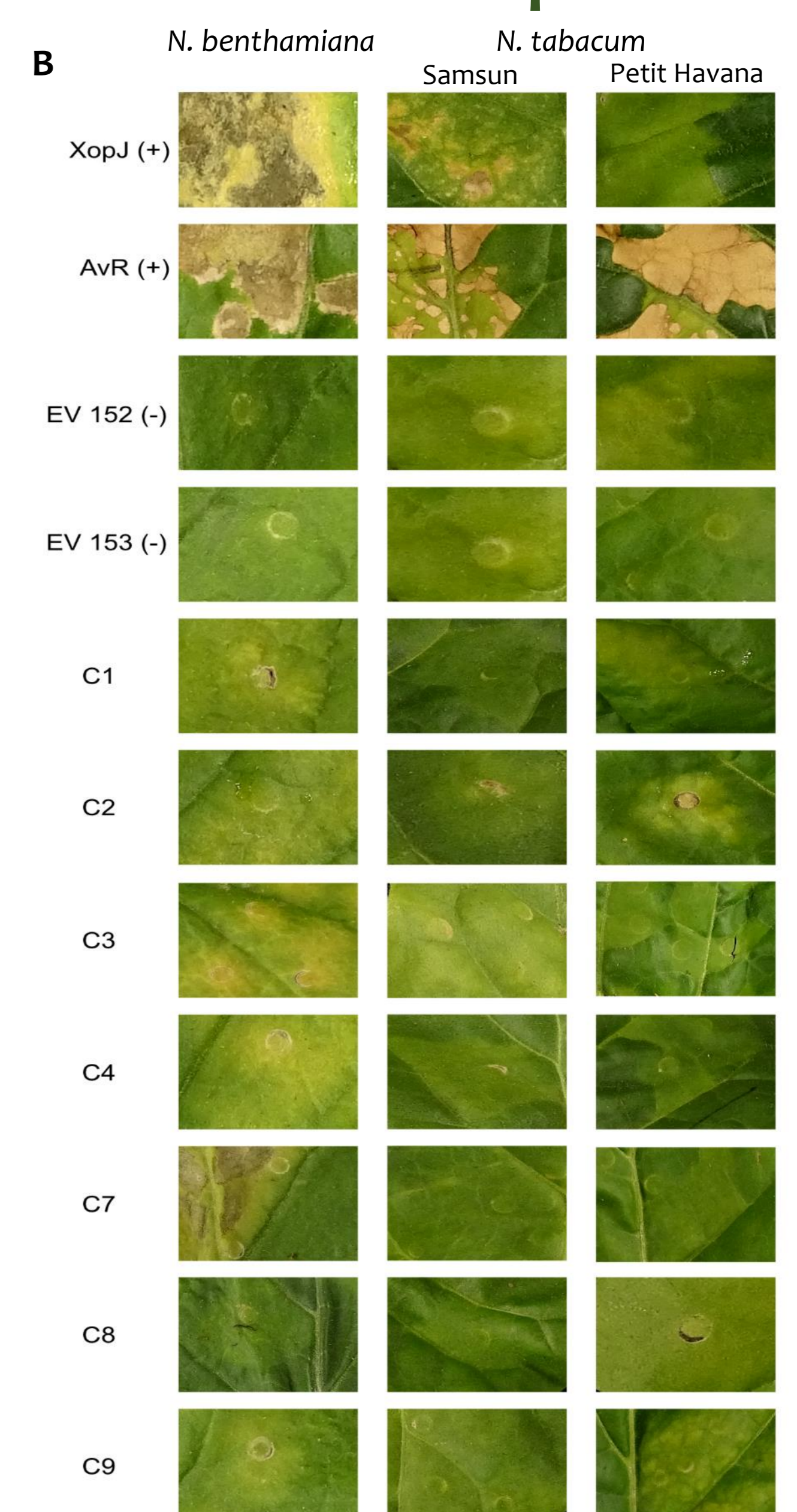


Figure 4 A. Confocal microscopy (CLSM) data suggests that Xyp1 can colocalize to plant nuclei with NLS of SV40 large T antigen (B-C NLS); scale bars indicate 50 μm. Intensity of merged protein spots were compared and plotted in Fiji software. B. PCD Assay in different Tobacco plants shows mild induction of HR by Xyp1. Plants were observed up to 15 days post infiltration (15 DPI). EV 152 and EV 153 correspond to vectors pUBC-GFP-Dest and pUBN-RFP-Dest, respectively.

Xyp1 elicits PCD in *Nicotiana* species



Xyp1 attenuates ROS burst in *Nicotiana* species

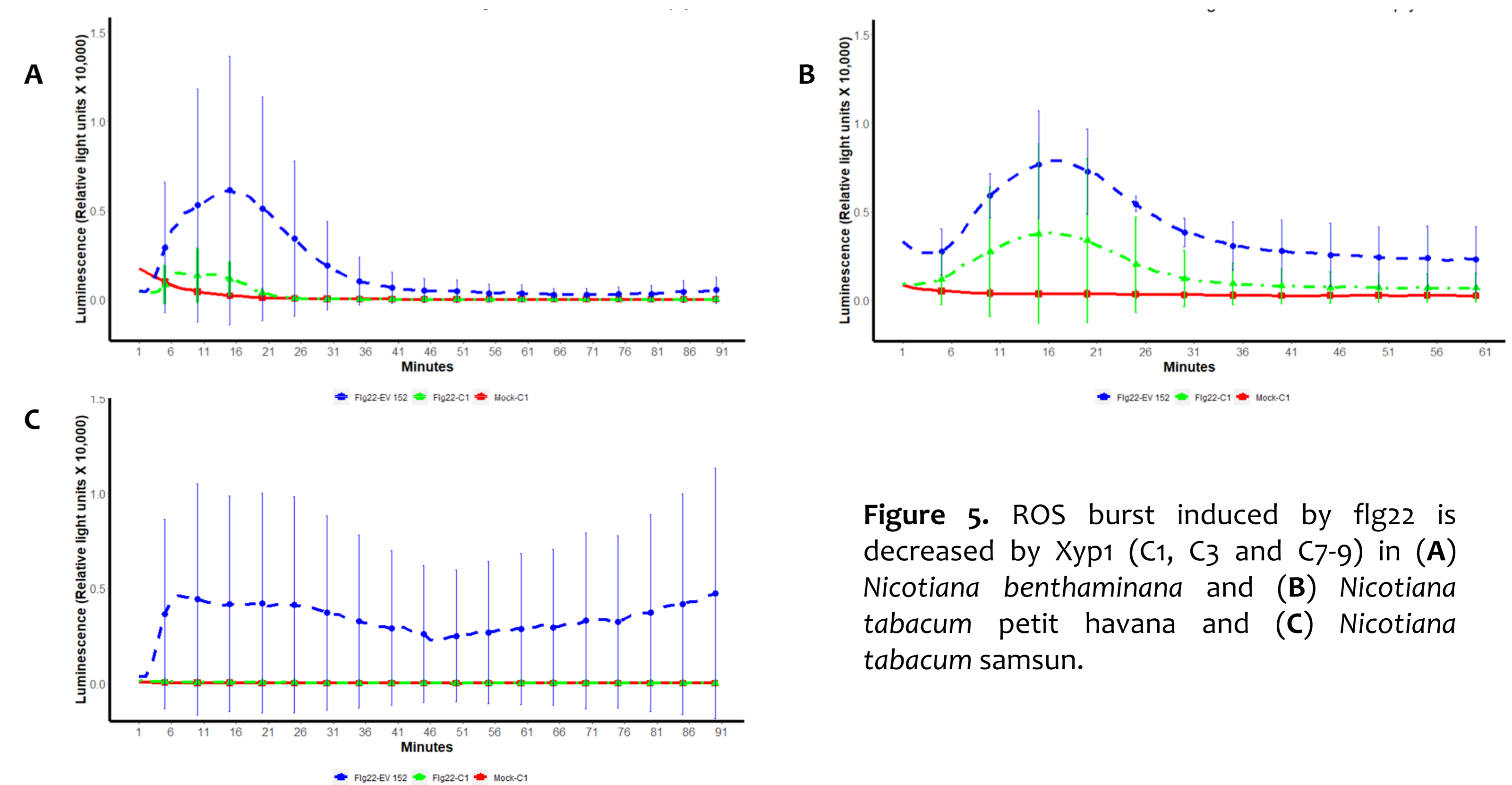


Figure 5. ROS burst induced by flg22 is decreased by Xyp1 (C1, C3 and C7-9) in (A) *Nicotiana benthamiana* and (B) *Nicotiana tabacum* petit havana and (C) *Nicotiana tabacum* samsun.

Outlook

- Assess immunomodulation of Xyp1 and its correlation with nuclear localization
- Generate and analyze stable Xyp1 expressing *At* lines

References

Bai, X. D. et al. AY-WB *Phytoplasma* Secretes a Protein That Targets Plant Cell Nuclei. *Mol Plant Microbe In* 22, 18-30, doi:10.1094/Mpmi-22-1-0018 (2009).

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