Molecular analysis of importin α-mediated nucleocytoplasmic signaling in plant innate immunity

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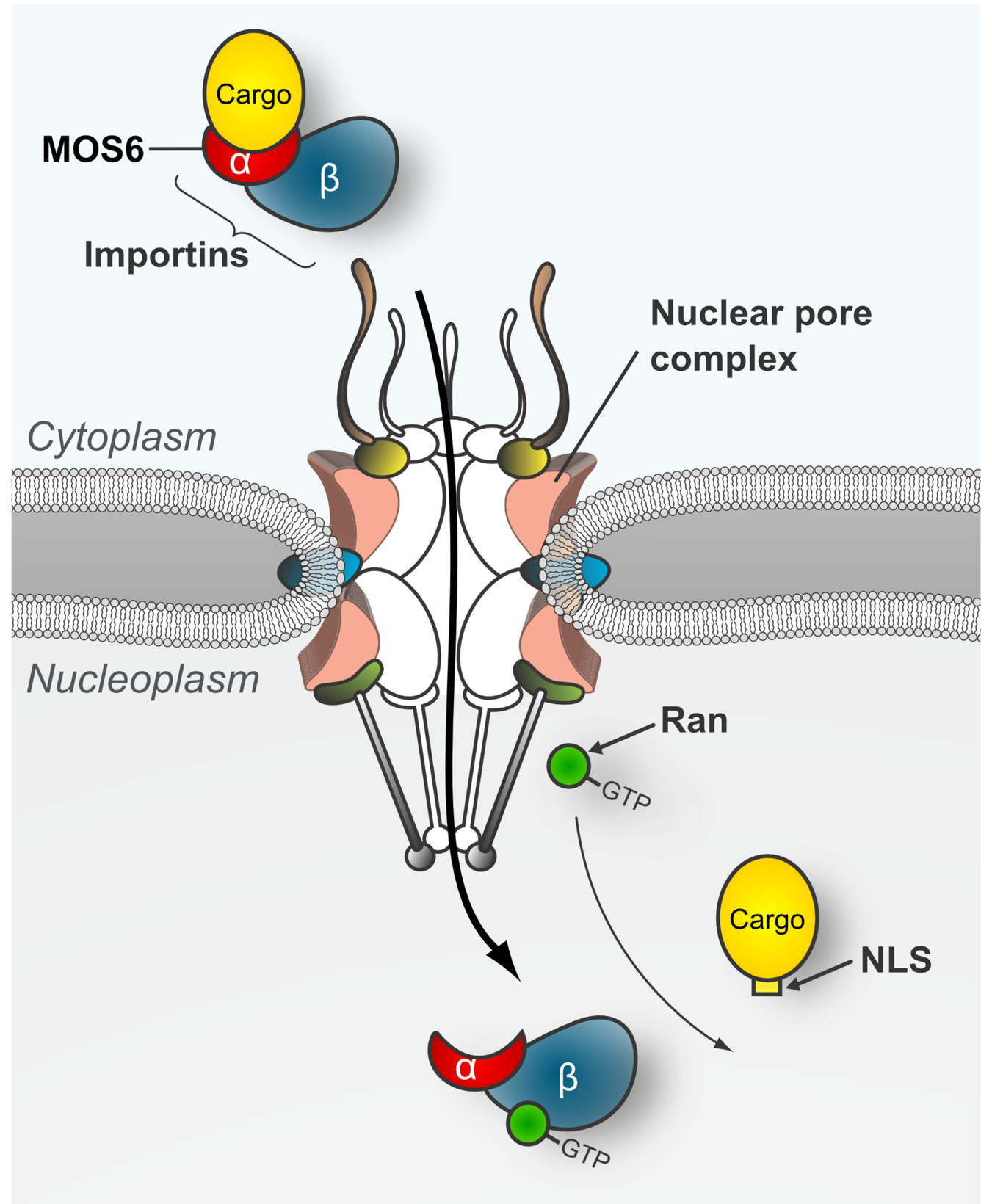
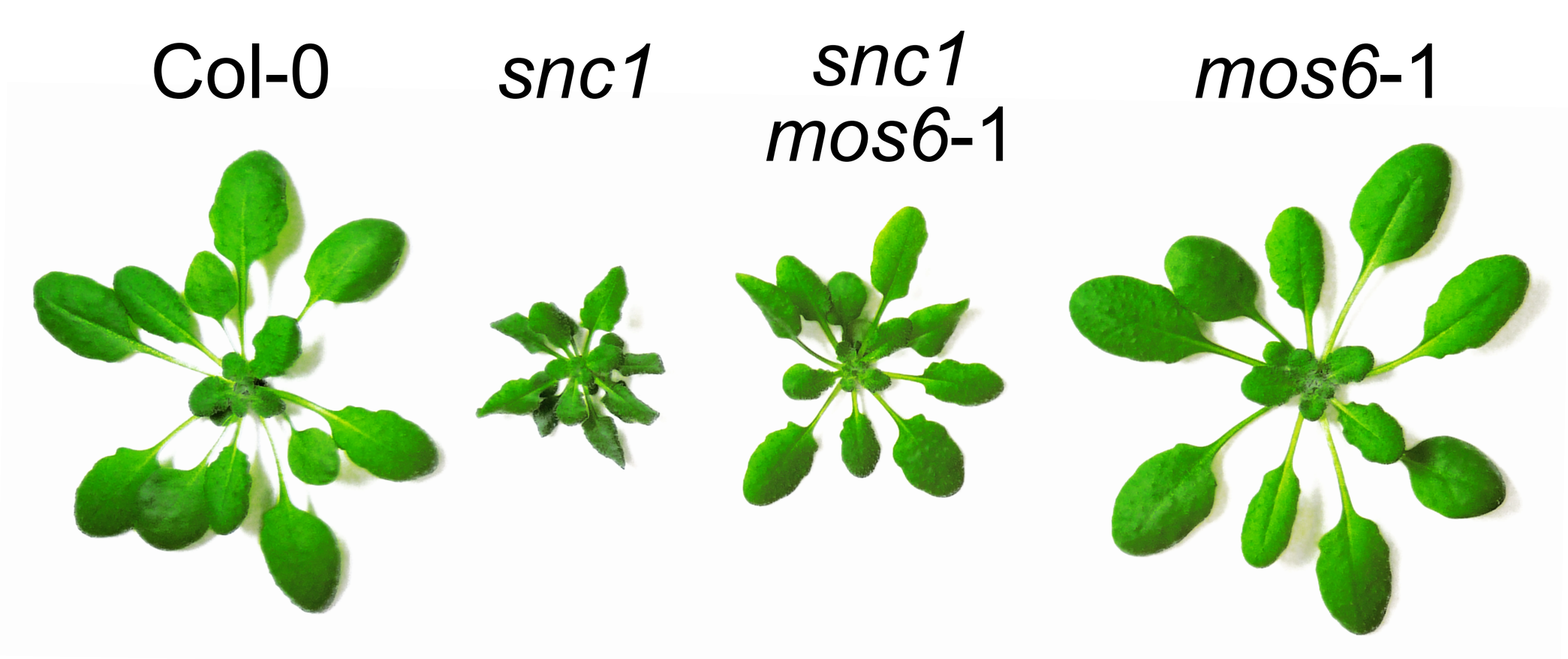
Introduction

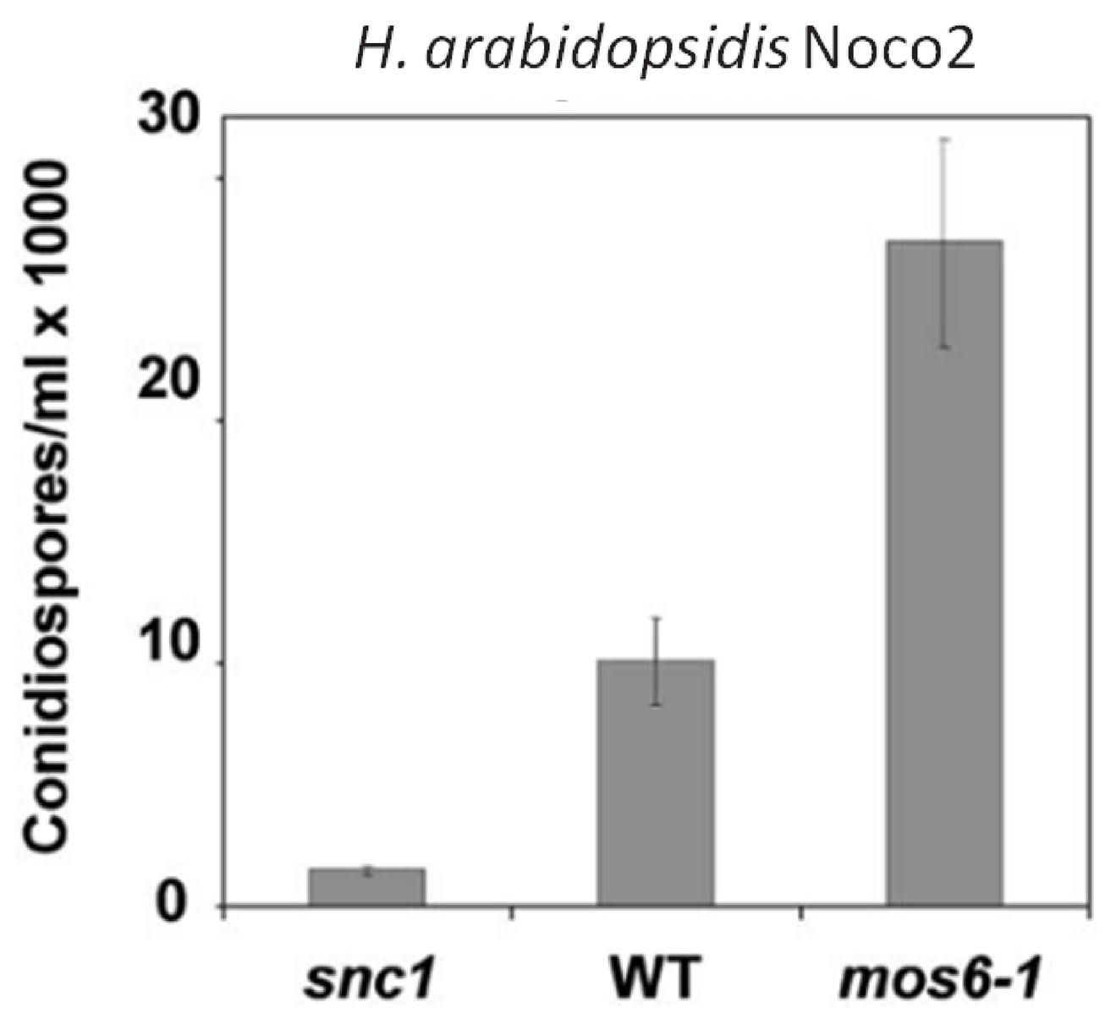
α-importins are a family of nuclear transport receptors. They mediate the translocation of nuclear localization signal (NLS)-containing cargo proteins from the cytoplasm into the nucleus through nuclear pores. The **importin-α3, MOS6 (for MODIFIER OF *snc1*, 6)**, is one of at least eight putative α-importins encoded by the *Arabidopsis* genome. MOS6 was identified as an **essential component of auto-immune responses caused by the constitutively active TIR-NB-LRR Resistance (R) protein variant, SNC1.** In addition, MOS6 is required for basal resistance. This suggests that MOS6 specifically or preferentially imports unknown cargo proteins involved in defense signaling into the nucleus. Here, we report our **approach to identify and characterize defense-related cargo substrates** and interaction partners of MOS6 *in planta*, using affinity purification of StrepII-tagged MOS6 coupled with mass spectrometry. In addition, our experiments address a possible functional redundancy of MOS6 with other α-importins.



The importin-α3, MOS6, plays a role in *snc1* auto-immunity and plant defense



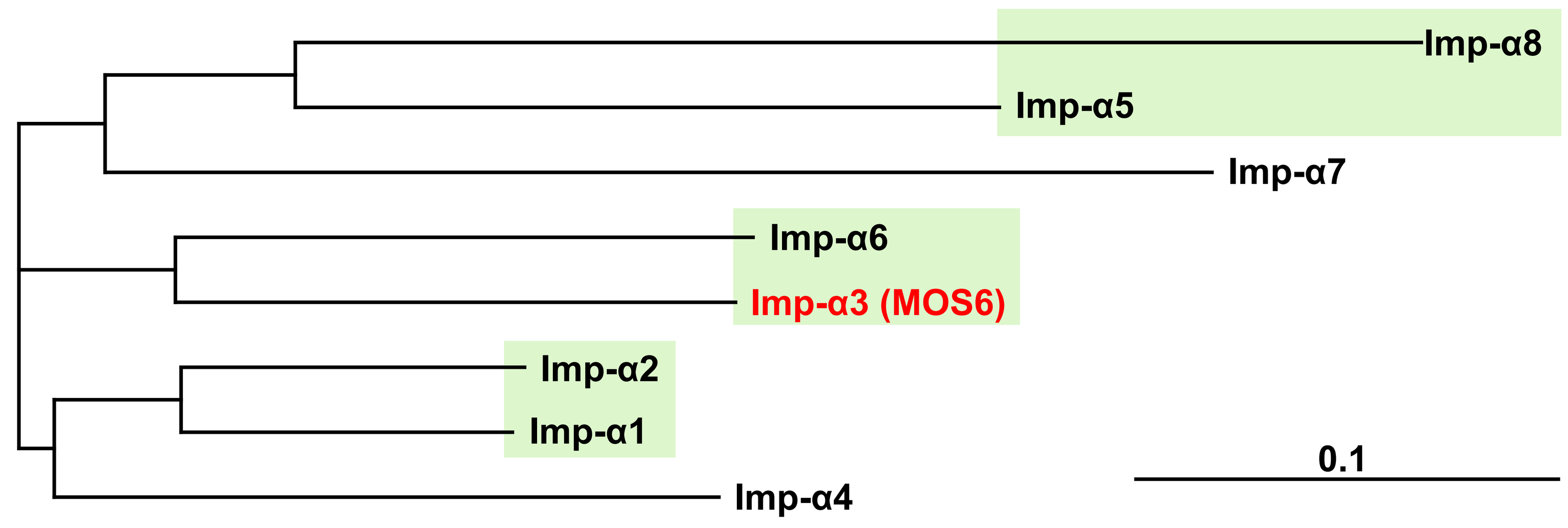
***mos6*-1 partially suppresses the auto-immunity and related growth inhibition in *snc1*.**



***mos6*-1 single mutants show impaired resistance against virulent *H. arabidopsidis* Noco2 (Palma *et al.*, 2005)**

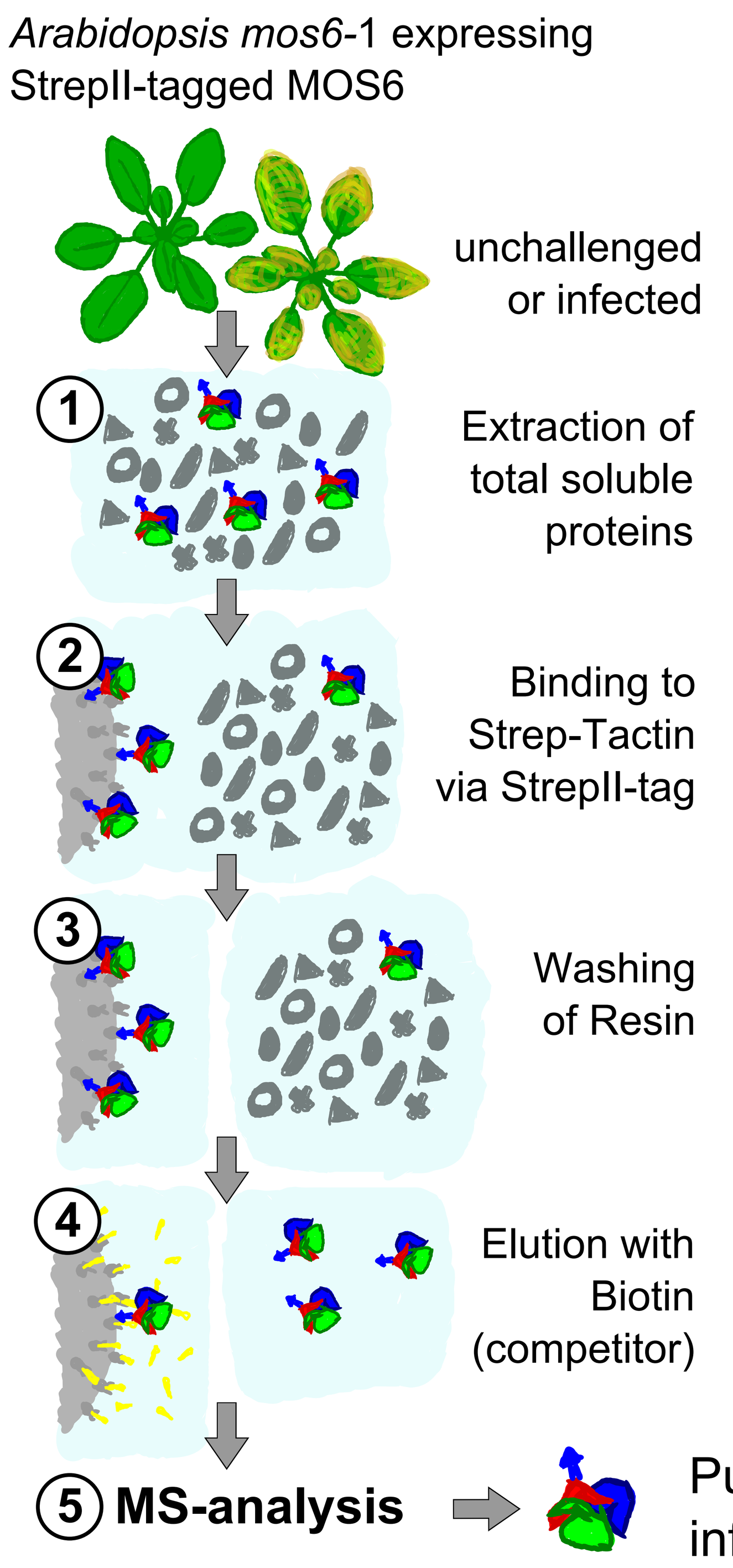
MOS6 may function redundantly with other α-importins

Importin-α **double mutants** have been generated to address potential functional redundancies in plant immunity.

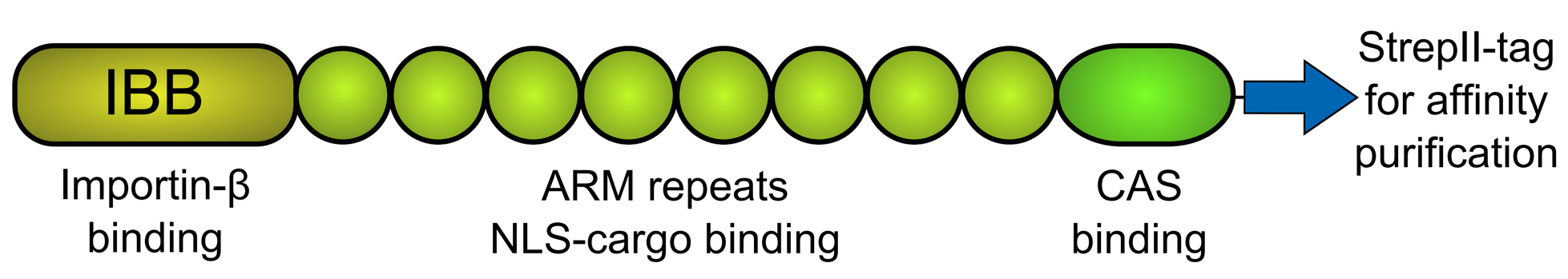


Dendogram based on full length amino acid sequences, ClustalW

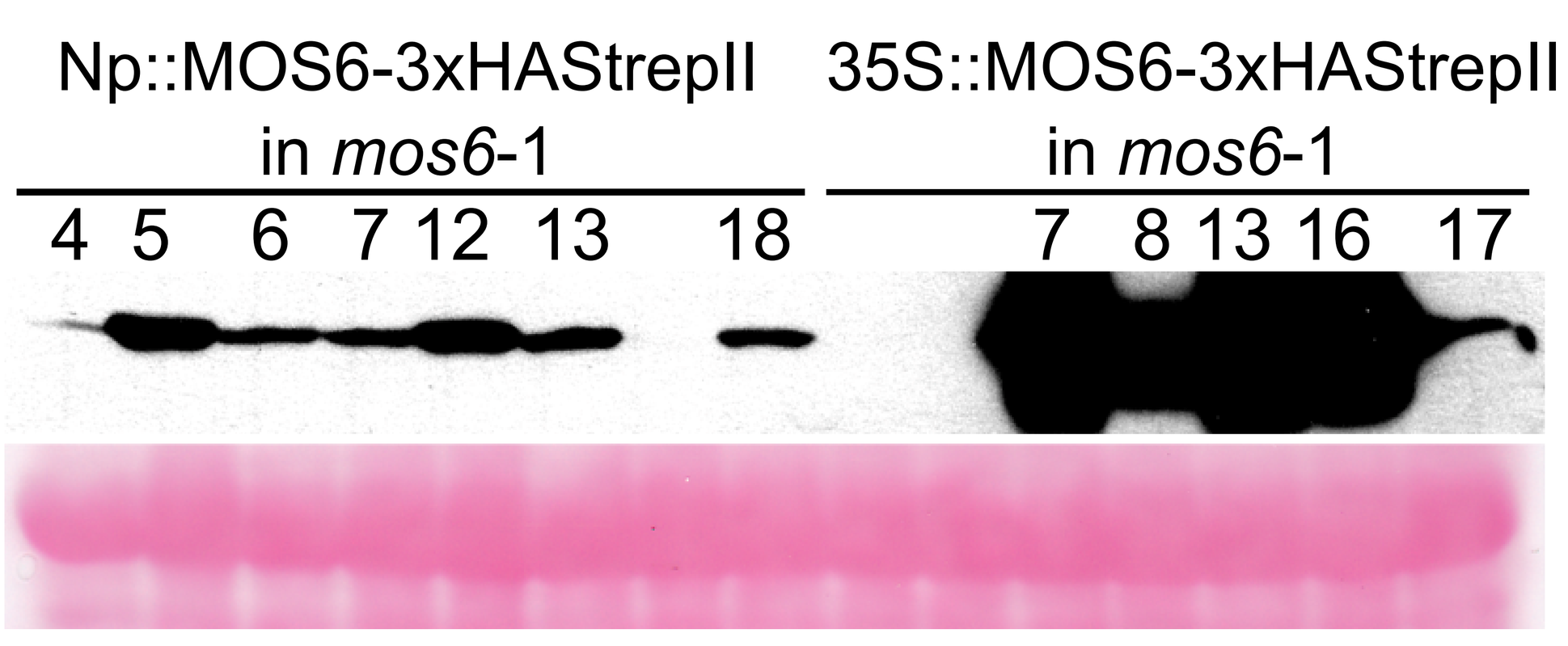
Identification and molecular characterization of MOS6 defence-related cargo proteins and interaction partners



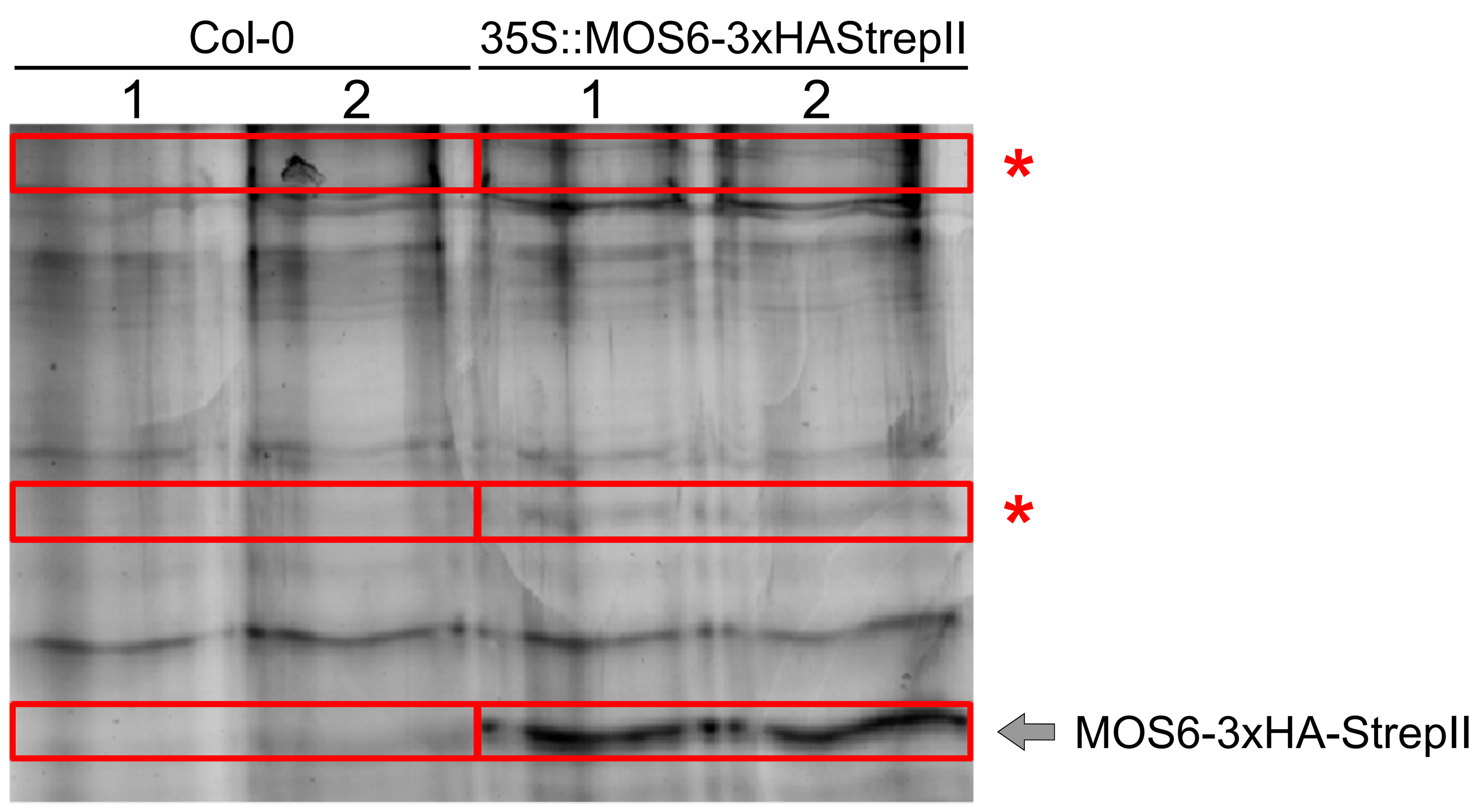
Purified native protein complexes from unchallenged and infected tissues are compared for protein composition.



MOS6 has the typical architecture of all importin-α proteins.



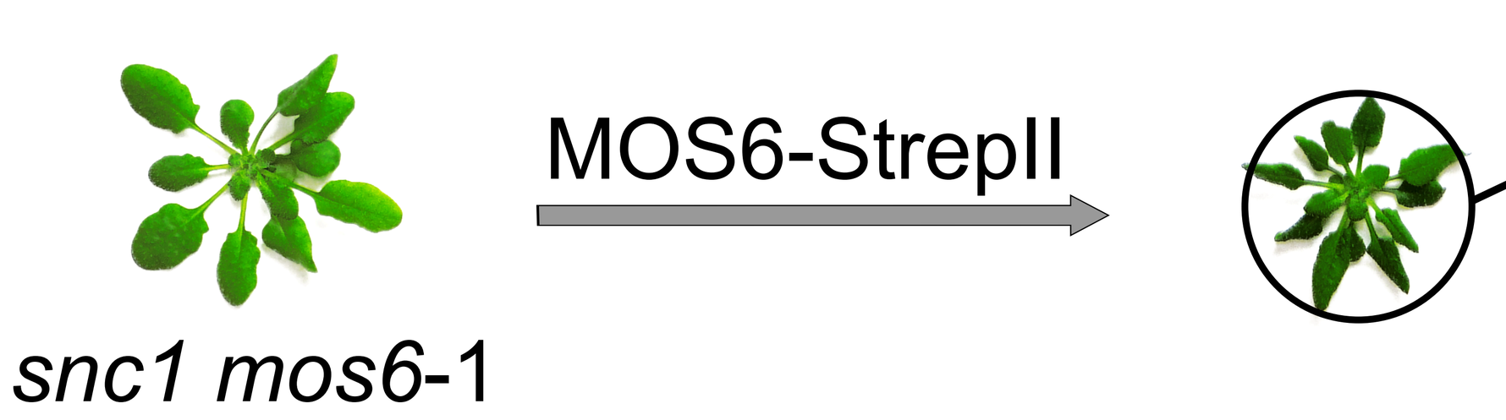
Western blot of transgenic lines expressing 3xHA-StrepII tagged MOS6 under control of the native promoter (Np) or 35S promoter.



Silver stained SDS-Gel of StrepII affinity-purified protein complexes from a MOS6-3xHA-StrepII overexpressing line in comparison to Col-0. Asterisks indicate differential bands in addition to MOS6-3xHA-StrepII.

Interactions will be validated and identified host components will be analyzed by reverse genetics (i.e. knockout and overexpression analyses) to test their requirement for innate immunity.

Complementation of *snc1 mos6*-1 with StrepII-tagged MOS6



snc1-like stunted plant, expressing MOS6-StrepII

identification of MOS6 cargo substrates in an auto-immune mutant background

Literature

Palma *et al.* (2005), Current Biology 15: 1129-35.
Wiermer *et al.* (2007), Cellular Microbiology 7: 1880-90.

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