

Electronic Supplementary Material

Cleavage of the Babuvirus Movement Protein B4 into Functional Peptides Capable of Host Factor Conjugation Is Required for Virulence

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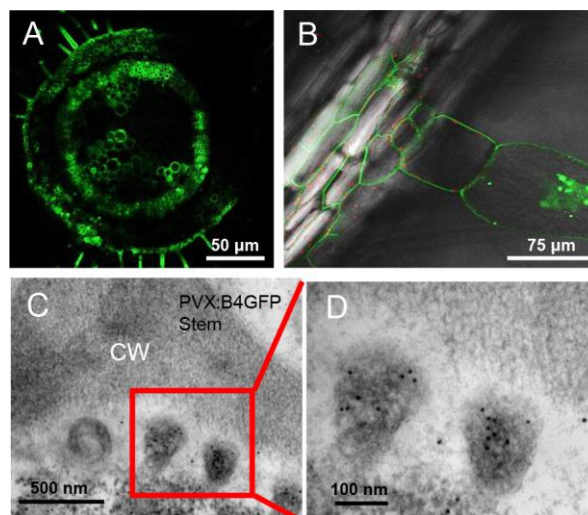


Figure S1. Cellular and subcellular localization of B4GFP in *Nicotiana benthamiana*. (A) The longitudinal section shows the aggregation of B4GFP in the peripheral membrane-associated region of stem cells, especially in the vasculature, in PVX: B4GFP-infected *N. benthamiana*. (B) B4GFP localized at the periphery of vascular bundle cells from the leaf mid-stem and entered vasculature-linking trichomes to form granules similar to those in mesophyll cells, indicating that mobile B4GFP is involved in symplastic transport. (C) Accumulation of B4GFP in vesicular tissue (stem) appeared in the apoplastic region; an enlarged image of immuno-labeled vesicles in (D) is shown. CM, cell wall.

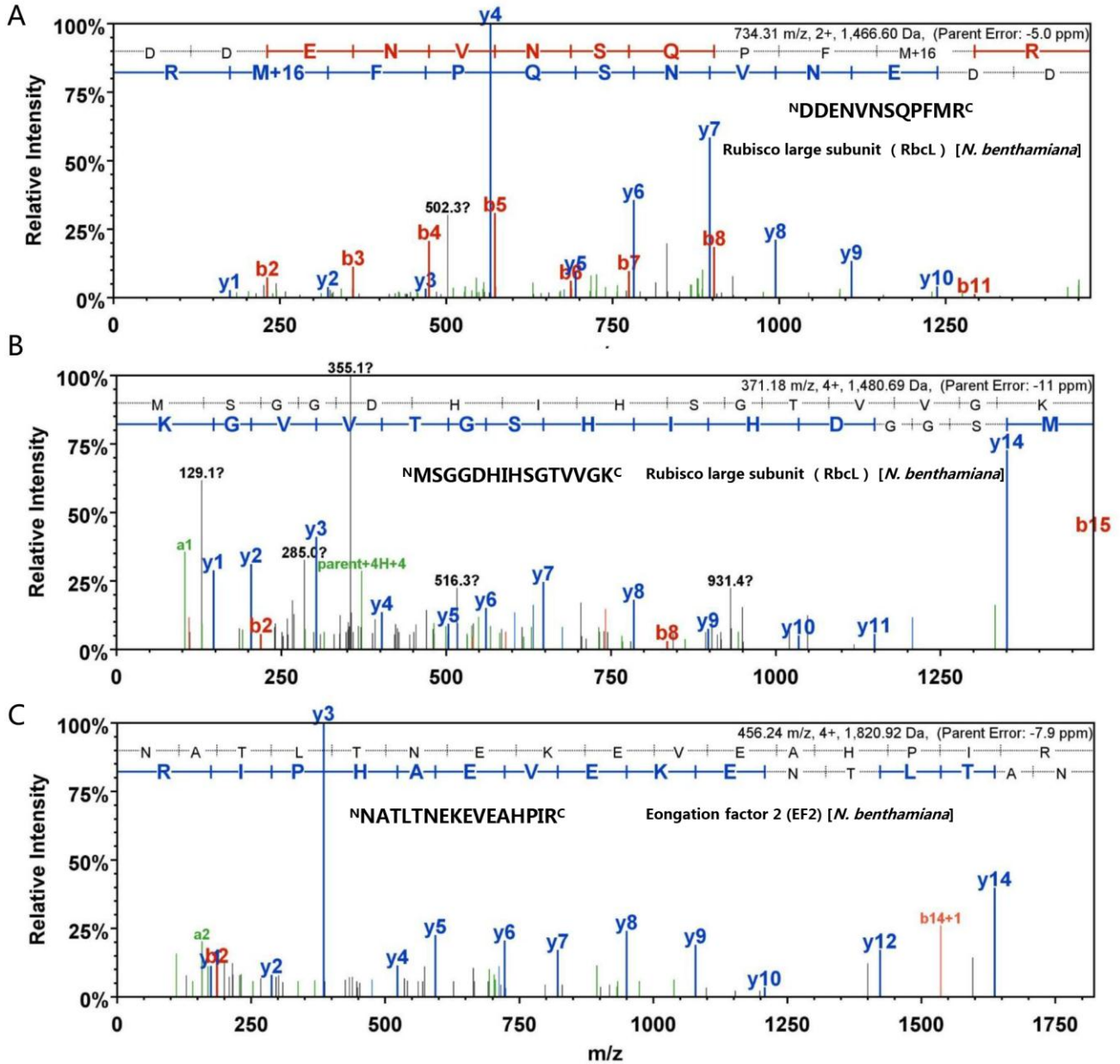


Figure S2. LC-MS spectra of peptides of RbcL and EF2 from HMW species. Three peptide sequences were determined in light of y and b ions by LC-MS. The “DDENVNSQPFMR” and “MSGGDHIHSGTVVGK” sequences were derived from RbcL (A and B). The “NATLTNEKEVEAHPIR” sequence corresponded to EF2-derived peptide (C). The individual y and b ions are specified in detail.

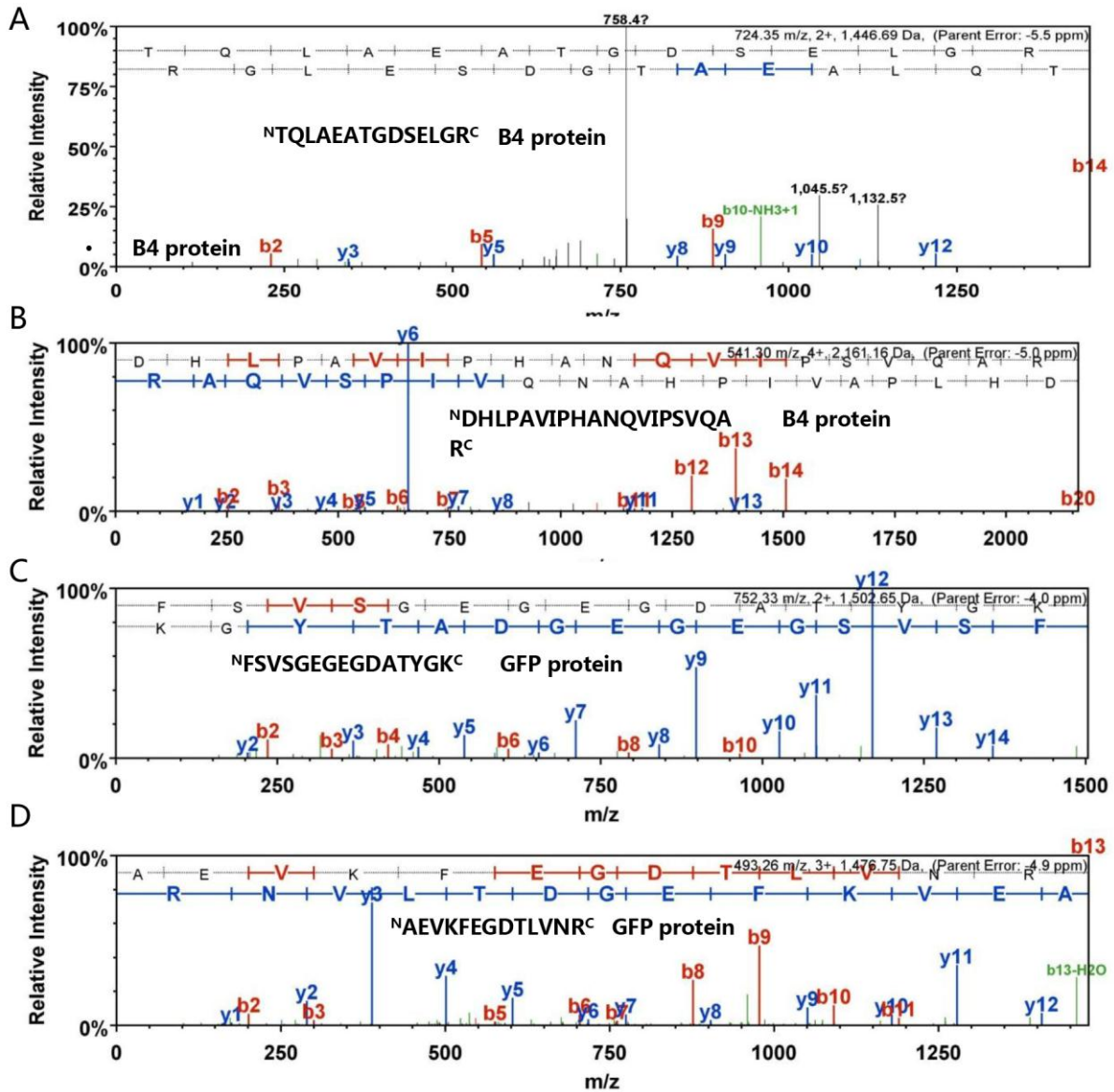


Figure S3. The LC-MS spectral data for peptides of B4 and GFP from HMW species. Three peptide sequences were determined in light of y and b ions by LC-MS. The “TQLAEATGDSELGR” and “DHLPAVIPHANQVIPSQAR” sequences were derived from Protein B4 (A and B). “FSVSGEGEGDATYGK” and “AEVKFEGDRTLVR” sequences belonged to GFP (C and D). The individual y and b ions are specified in detail.

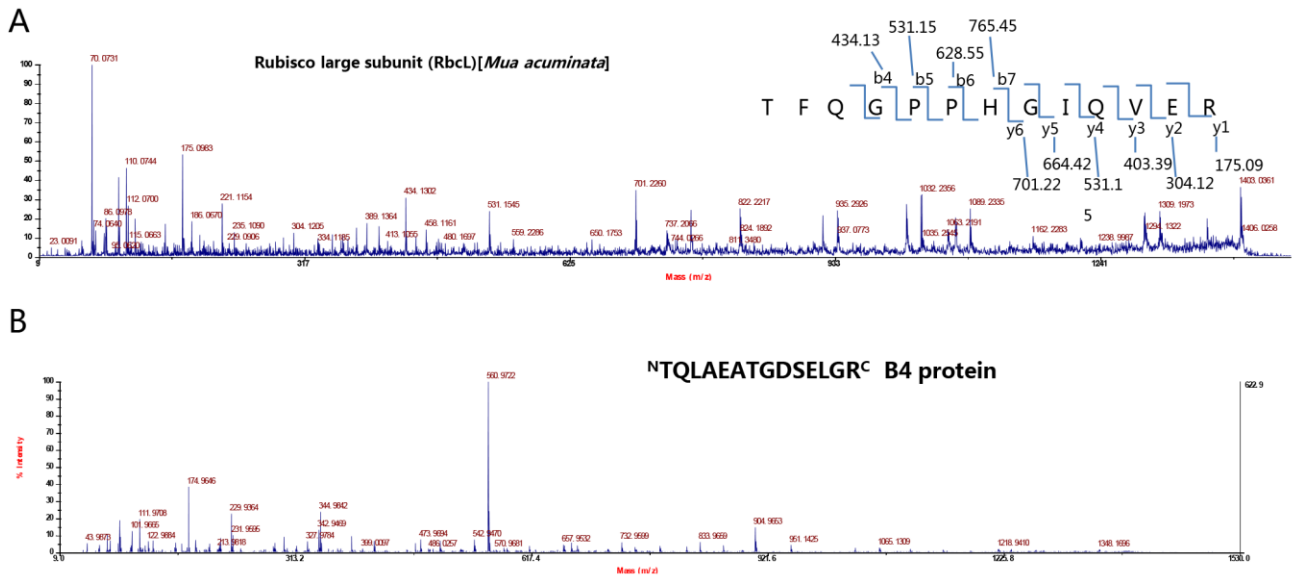


Figure S4. MS data for RbcL and B4 from HMW species in BBTV-infected immunoprecipitate. The two peptide sequences were determined according to y and b ions by MALDI-TOF/TOF MS. “TFQGGPHGIQVER” belonged to *Musa* RbcL (A) and “TQLAEATGDSELGR” was derived from Protein B4 (B).

Table S1. The primers for B4-derived mutants and related constructs

Primers' names	Sequences(5'-3')	Usage
Mut1 F	GCGGAGATTGTGGCGTATCTC GTAGAATACCTGACC	For Mut 1 position
Mut1 R	GAGATACGCCACAATCTCCGCAATATATGCGGGAAC CTC	
Mut2 F	ACC GCA GCA GCT GTA TGG ATG CAG AAA ACG	For Mut 2 position
Mut2 R	CAG TACAGCTGCTGCGGTCAGGTATTCTACGAGATA	
Mut5 F	GGAGATTCAGAGCTCGGCGCAGGTAGTGTGGATGAC	For Mut 5 position
Mut5 R	GCCGAGCTCTGAATCTCCAGTTGCCTCCGCAACTG	
Mut6 F	GCGGATCATCTACCGGCTGTTATACCACATGCAAATC	For Mut 6 position
Mut6 R	AGCCGGTAGATGATCCGCTGCGTCATCCACACTACC	
Mut7 F	ATCCCTTCAGTTCAAGCTGCAGCGGATGAACAAGGA	For Mut 7 position
Mut7 R	AGCTTGAAGTGAAGGGATAACCTGATTTGCATGTGG	
Mut8 F	GATGAACAAGGAGCAGCAGGAAACGCAGGACCTAT G	For Mut 8 position
Mut8 R	TCCTGCTGCTCCTTGTTTCATCCCTTCTAGCTTGAAC	
deltaTMB4F	CCATCGATATGGCATTGACAACAGAGCGGGTGAAC TATTC	ΔTMB4GFP
deltaTMB4R	TTCGAATTGCTCTTTGAGGTTCCCAAG	