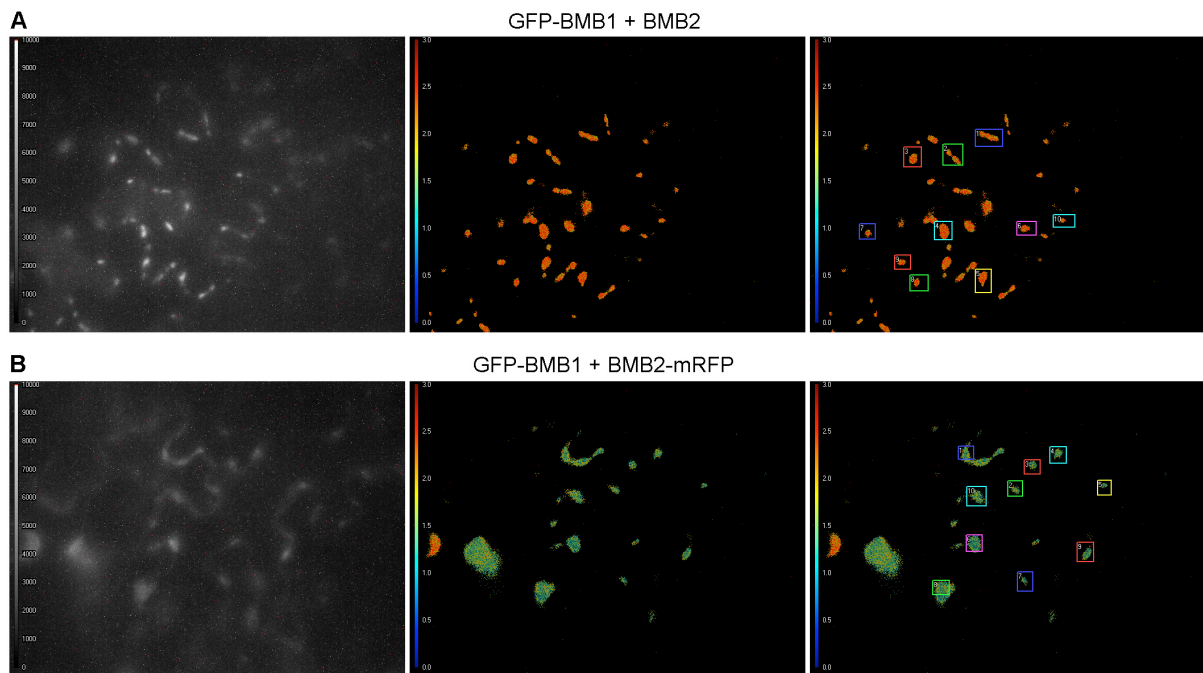
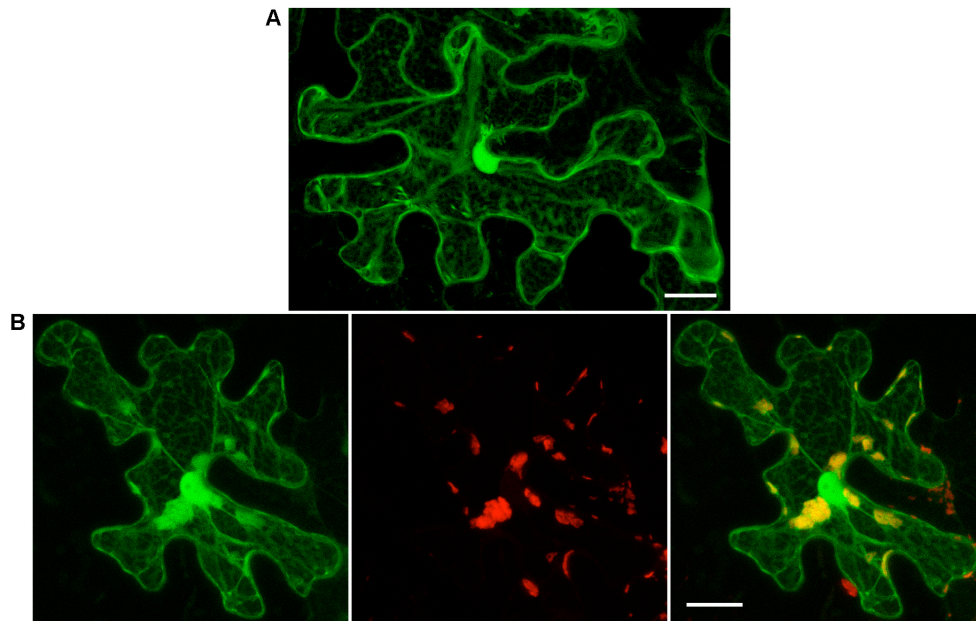


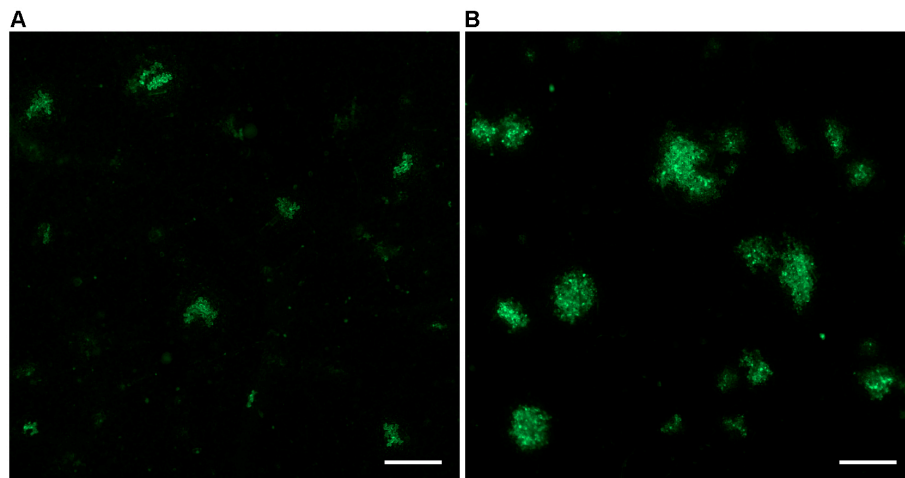
**Figure S1.** Schematic representation of HGSV-specific constructs used in this study. Expression cassettes of binary vectors are presented. Genes are shown as boxes. 35S, the *Cauliflower mosaic virus* 35S promoter; ter, *Cauliflower mosaic virus* 35S terminator.



**Figure S2.** Analysis of interaction between BMB1 and BMB2 by FRET-FLIM. Representative FRET-FLIM images of cells coexpressing either the GFP-BMB1 donor with BMB2 (a no-acceptor control) (A), or the GFP-BMB1 donor with the BMB2-mRFP acceptor (B) are shown. Left, donor fluorescence intensity. Center, donor excited-state lifetime map; the color bar indicates lifetime in ns. Right, PAMB-containing areas of measurement shown on the lifetime map.



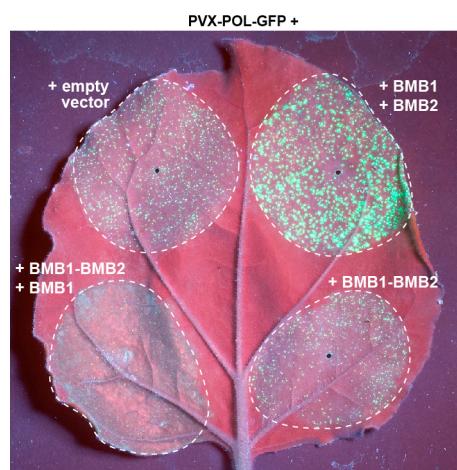
**Figure S3.** Subcellular localization of GFP+22. Confocal microscopy of cells either expressing GFP+22 (A), or coexpressing GFP+22 and BMB2-mRFP (B). Scale bar, 20  $\mu\text{m}$ .



**Figure S4.** Complementation of PVX-POL-GFP cell-to-cell transport. Representative images showing individual fluorescent cells (A) observed in the absence of cell-to-cell transport in a leaf agroinfiltrated for expression of PVX-POL-GFP and multicellular fluorescent loci (B) observed upon complementation of PVX-POL-GFP cell-to-cell transport in the presence of HGSV MPs. Scale bar, 500  $\mu\text{m}$ .

	mN	mHydr	mMid
	** * *	* *	*** * * *
HGSV	---MPIFQQAASNA	VLFV <b>CASIC</b> VIAYFFF	----NPGNPYNVPLSPGHVFPFGGEYA
PisVX	---MPRFVDNSASSK	LILVL <b>CF</b> CMLVSAVYIMY	---HPGNPYSVPMAPGHVFPFGGRYA
<i>L. sativus</i>	--MVLVRTPDKKDDTK	VIIVYGFMVLIG <b>CCFFI</b> YNSVNP	PGNPYNVGLAPGHVFPFHGGRLI
<i>L. chinensis</i>	MAILNAPGNNVSLNLN	IV <b>CF</b> CCV <b>CL</b> CFICWCM	---NNPGNPYDVGFANGFVFPNGGRYM
<i>Q. castanea</i>	---MPIISNTQGKGS	LGLIV <b>CV</b> LVVGF <b>CFWCVC</b>	---HPGNPFNVGISPGHVFPFHGGSYE
HGSV	VHARFNGPSFPLS--MGGSS	VGLLM <b>LCVILLVVV</b> THM	-----
PisVX	ITAEFYPPRGTVW--RDSSN	LMVLFICLTICCYLRFR	-----
<i>L. sativus</i>	VTAEYNSPPGWFQ-FKECPS	LNFLLMFVMALITV <b>IFF</b> SSPRLENNR	
<i>L. chinensis</i>	VQAEFLPPKFSLFNFRGNGS	FSLVLICV <b>GILVYL</b> RF	-----
<i>Q. castanea</i>	IKATFLPQQRSFY-FRESPG	LFL <b>LL</b> FLCVFLAFVVSKLAI	-----

**Figure S5.** Amino acid sequence alignment of BMB2 proteins. The alignment includes proteins encoded by HGSV, *Pistachio virus X* (PisVX), and virus-like RNAs identified in transcriptomes of *Lathyrus sativus*, *Litchi chinensis* and *Quercus castanea*. Membrane-associated sequence segments predicted by the TMHMM algorithm (<https://services.healthtech.dtu.dk/service.php?TMHMM-2.0>) are shown in yellow color. Residues conserved in the central hydrophilic region are indicated by gray shading. Cysteine residues in the first membrane-binding hydrophobic region are shown in blue color. Residues affected by mutations in mutants mN, mHydr and mMid are shown by asterisks.



**Figure S6.** Complementation of cell-to-cell movement of PVX-POL-GFP by BMB1-BMB2 and BMB1-BMB2 combination with BMB1. The leaf was coinfiltrated for expression of PVX-POL-GFP and the following constructs as indicated, empty vector (negative control), BMB1 + BMB2 (positive control), BMB1-BMB2, and BMB1-BMB2 + BMB1. The leaf was imaged under UV light at 5 d.p.a. Dashed lines encircle infiltrated areas.

**Table S1. Primers used in this study.**

BMB2-mN-P	GCCTCGAGACCATGCCAATTGCTGGAGGTTTCAGCGGGGGGTAACGCGCCCGTGT TGTTT
BMB2-mHydr-P	CGCTCGAGACCATGCCGATATTTCAAGGTCAAGCGAAGAGTAACGCGCCCGTGT GTTTGTGGTTGCTAGCATAGCTGTTATAGCG
BMB2-mMid-ovl-P	TATAATGTACCTTTGAGTCCTGGTACCGTGTTTCGGTTTCAGCGCAGAGTACGC
BMB2-mMid-ovl-M	ACCAGGACTCAAAGGTACATTATAAGAACCTGCAGGATTAAAGAAG
BMB1C-BglII-P	GTCCTGATGTTGAGTTTGTGCG
BMB1C-d22-M	CGCTCTAGATTACTGGAAATTATCATGGTCTACCAC
BMB2-C22-P	GCGGATCCCCGGCATATGAAGATTCTCTT
Right	CCTTATCTGGGAACTACTCACACATT
Left	TATCCTTCGCAAGACCCTTCCTCT

**Table S2. Synthetic genes used in this study.**

Gene	Sequence
BMB1	GGATCCGATGGAAAGCTTCAACTACGTTACCTCTGCGGATCTGGTTTGCTCCCTGTCTCGTTTTCG TGACTTCTACAAGGAACGTAGCGCGTCTCTGTCTGACGACGACGTTTACGCGGACACCACCCGTGA ATACTCTGCTGCCCTGGACAGCACCGAATTCGTAGATTACAACCTCCAGATCTTCTGCTGAACGG TGTGCCAGGTGGCGGTAAAACCAAATTCGTCTACGAAAACGTTGAAGTGCGTAACTCTTGCGTTGT TGTTCCGTTTAAAGCTCTGAAAGACGAGTACCGCAAACGTGGTTACCGTTCCTTCACCCAGTTCCG TGCACTGCCGTCCGTTTCGTTCCGCAGACATCCTGGTAATCGATGAATACACCTGCGTGTGCTACTC TGTTCTGGTGAGCCTGGTTTACAAACTGCGTCCGGCAACCGTTGCCCTGATCGGTGACTTTAATCA GTGCTGGATTTCGTGACGGCGAGGGTTTCTCTATGGAACCGTTTGTAAGCACCCCTGGTGGTGAACAA GTACCTGTCCGTTTGCTACCGCTGTCCGTGTCCGGACGTAGAGTTTGTTTCGTAACCTACCTGGACCT GGACATTACCCCGGGCAAATGCGGCTCCACCTGTAGCTGCACCGGCTTCAACGTAGAAACCCCTGGT TGAAGGTATGACCTTCGATTACCGCGGTGAAGTTGCGCTGGTGTTCAGCGGCGCGAGCAAATCCTA CCTGGAGTCTATCGGCGTTTCCGCTGTAACCGTCCGTAGCTTCATGGGTAAGCAGGCAGATACCGT TGCGCTGTTTCATCCTGAAAGATCACGACATCCGTCTGCTGGGCGTTTCTTCTCTGAAGCTGGTAGC ATTTACCCGTCACGTTAGCAAACCTGGTTGTTTACAGCGATATGTCCACGATGATGTGGTTCGCTCT GATCGAAGGTGCGACTGAGTCTGGCGAAGTGGTTGACCACGATAAATTCCAGCCGGCGTACGAGGA CAGCCTGGAACAGTACTTCGACCGCCTGCTGCGTGCGGACTTCTACGTTCTGTAACCTCGAG
BMB2-QTY	CTCGAGACCATGCCGATATTTCAAGGTCAAGCGAAGAGTAACGCGCCCACTCAATATACATGTGCT AGTACATGTACTACAGCGTATTACTACTATAATCCTGGTAATCCTTATAATGTACCTTTGAGTCCT GGTCACGTGTTCCCTTTTCGGGGGAGAGTACGCGGTTTCATGCTAGGTTCAATGGACCGAGTTTCCCT CTTAGTATGGGTGGTTCTAGTACAGGACAGCAAATGCAGTGTACTACACAACAACTACTACTACG CATATGTAATCTAGA