

Supporting Information

Harries et al. 10.1073/pnas.0909239106

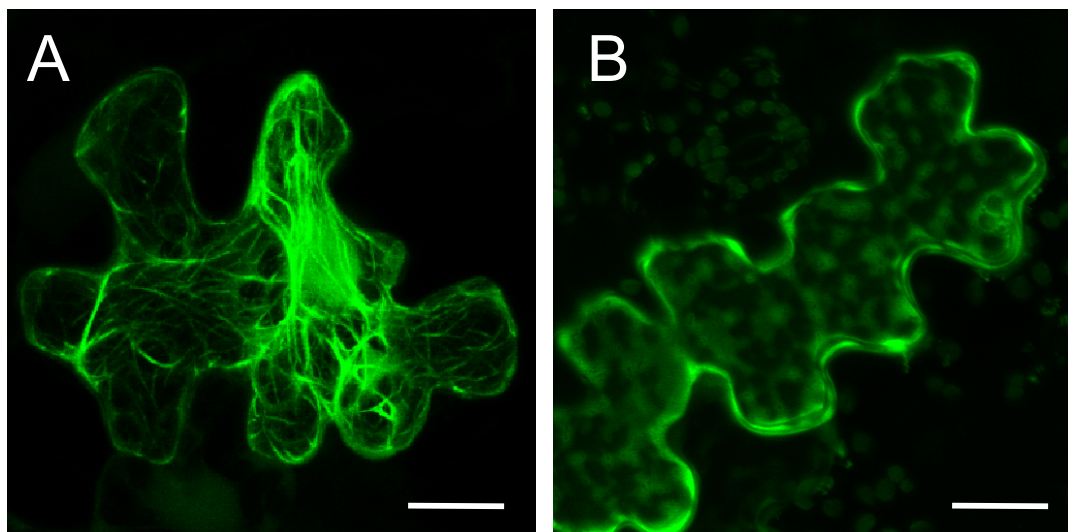


Fig. S1. Disruption of microfilaments within epidermal cells after treatment with 5 μM LatB. Images of *N. benthamiana* cells are from plants expressing the GFP-actin binding domain 2 of fimbrin-GFP fusion to label actin and were taken 3 h post-infiltration with DMSO (A) or DMSO with 5 μM LatB (B). Each image is a projection of approximately half of the cell combining section images separated by 0.5 μm . (Scale bar, 25 μm .)

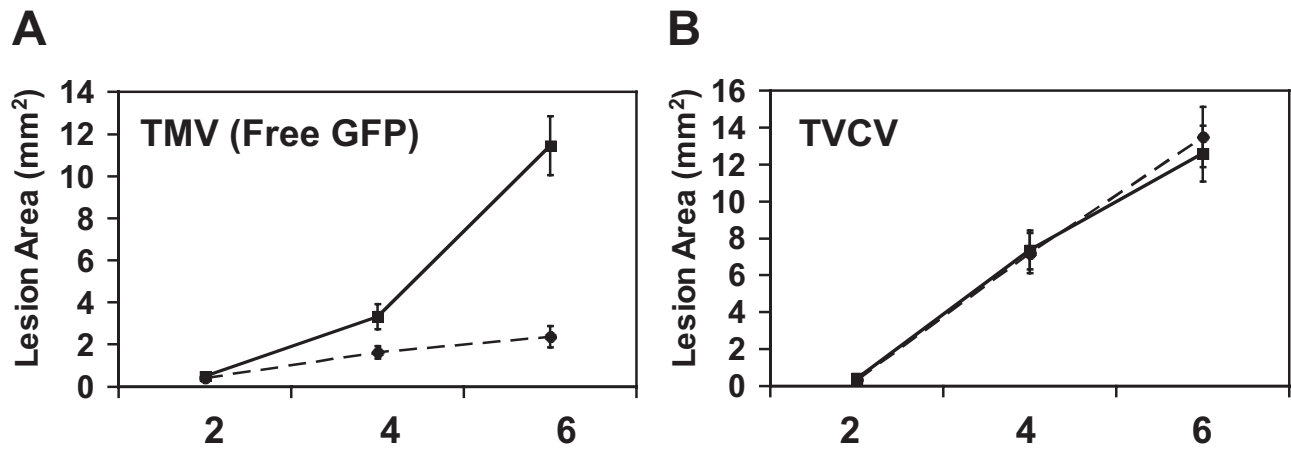


Fig. S2. Effect of LatB treatment on cell-to-cell spread of TMV expressing free GFP. Lesion areas were quantified to determine the effect of LatB on the cell-to-cell movement of (A) TMV expressing a free GFP and (B) TVCV at 2, 4, and 6 dpi. Lesion areas were determined in *N. benthamiana* leaf tissue infiltrated with either the actin inhibitor LatB (circles) or a DMSO buffer control (squares). Bars represent standard errors for 10 lesions per treatment.



Fig. S3. LatB inhibits the formation of necrotic lesions in *Nicotiana tabacum* cv. Xanthi-NN. Representative image comparing TMV local necrotic lesion formation in half leaves infiltrated with 5 μ M LatB (bottom half) compared with half leaves infiltrated with a DMSO control (top half). Infiltration with LatB or DMSO was carried out 3 h before inoculation of the entire leaf surface with TMV U1 virions. The image was taken at 3 dpi with TMV. Arrows indicate necrotic lesions. In some areas of the leaf, the lesions induced confluent collapse of plant tissue. Asterisks indicate infiltration point damage.

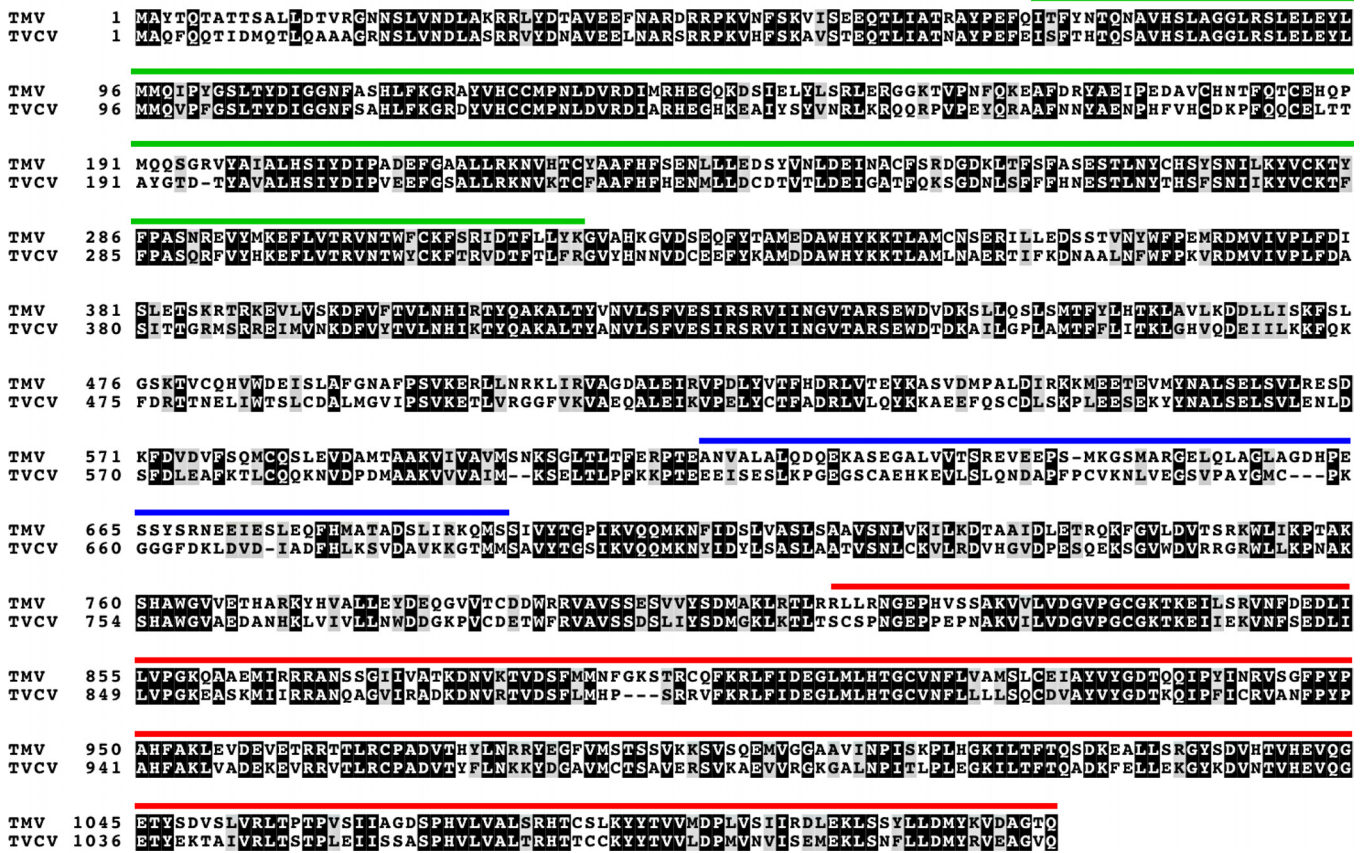


Fig. S4. Comparison of amino acid sequences between the TMV 126-kDa (TMV) and TVCV 125-kDa (TVCV) proteins. Identical amino acids are highlighted in black, and similar residues are highlighted in gray. The methyltransferase and helicase domains are overscored with green and red lines, respectively. The blue line, within the intervening region, denotes a stretch of 80 aa where the two proteins share only 15% identity.

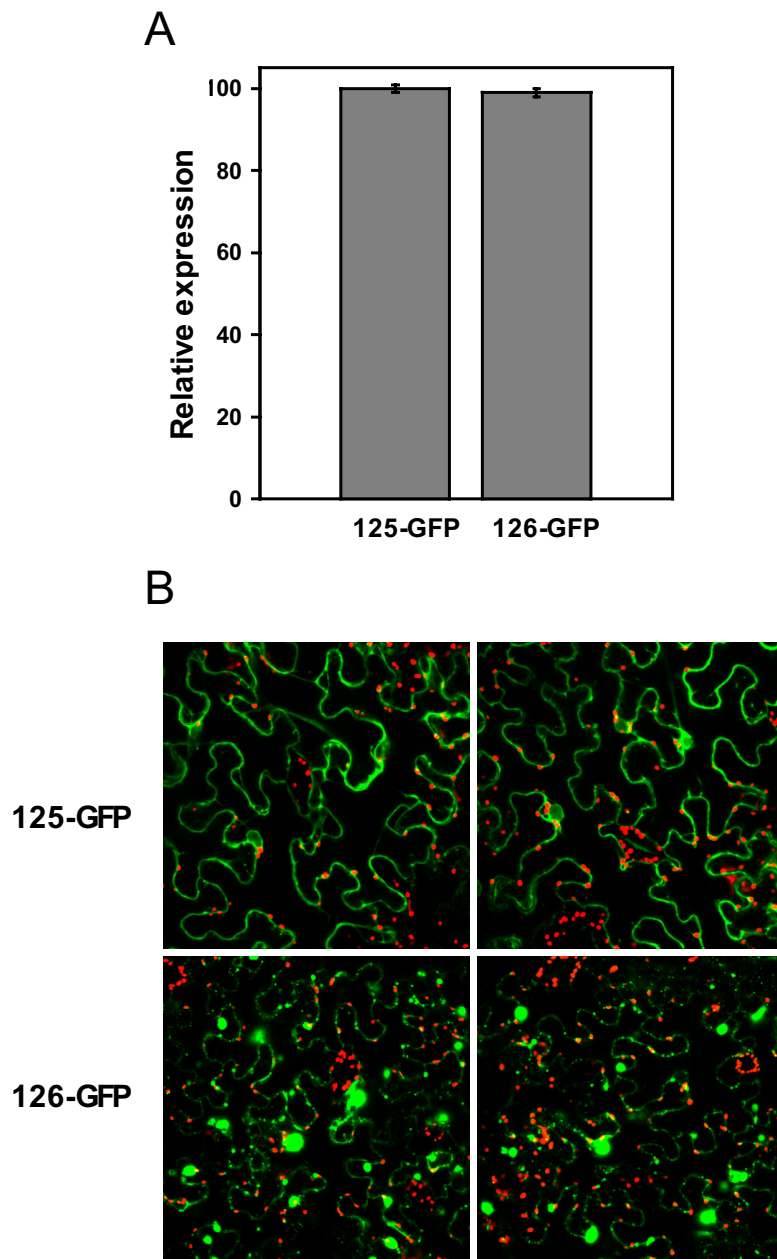
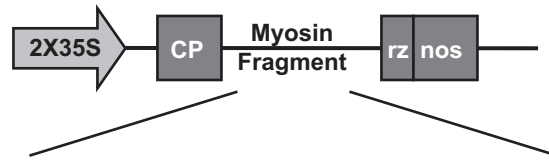


Fig. S5. Transcript and protein levels for TVCV 125-kDa protein-GFP and TMV 126-kDa protein-GFP fusions were similar after agroinfiltration. Transcript (A) and protein (B) levels for the 125/126-kDa proteins were either measured from extracts (by quantitative RT-PCR for transcripts) or visualized in leaf epidermal cell sections (by fluorescence for protein) at 3 days post-agroinfiltration.

A Myosin TRV2 Constructs



Myosin	Start (bp)	End (bp)	Length	% Identity
VIII-1	2012	2363	351	39
VIII-2	2165	2474	309	53
XI-2	2315	2625	310	57
XI-F	2309	2620	311	56

B Primers used for myosin detection

Myosin	Sequences
VIII-1	5'-GCCCGAGAGAGCAATGGA-3' 5'-CCTCAGCTAATCGGCTTATAACACT-3'
VIII-2	5'-ACTCCTATTGAATTTGCCAGCAA-3' 5'-CTGCACATAAACTGCCATTATTCC-3'
XI-2	5'-CAACTCCTACCCGCAAACCA-3' 5'-TCCCATTGTCATTCTCCCAA-3'
XI-F	5'-GCACAGGGTTTTCGCTCAA-3' 5'-CCCTCAATTCGCTGTATCC-3'

Fig. S6. TRV-myosin VIGS constructs used in this study. Schematic showing (A) TRV2 VIGS constructs used to silence the individual myosin genes in *N. benthamiana* and (B) the primers used to amplify fragments from individual myosin genes. The highest percent nucleotide identity found between regions and other known *N. benthamiana* myosin sequences are noted. 2 × 35S, a double CaMV 35S promoter; CP, TRV2 coat protein; rz, ribozyme; nos, nos terminator sequence.

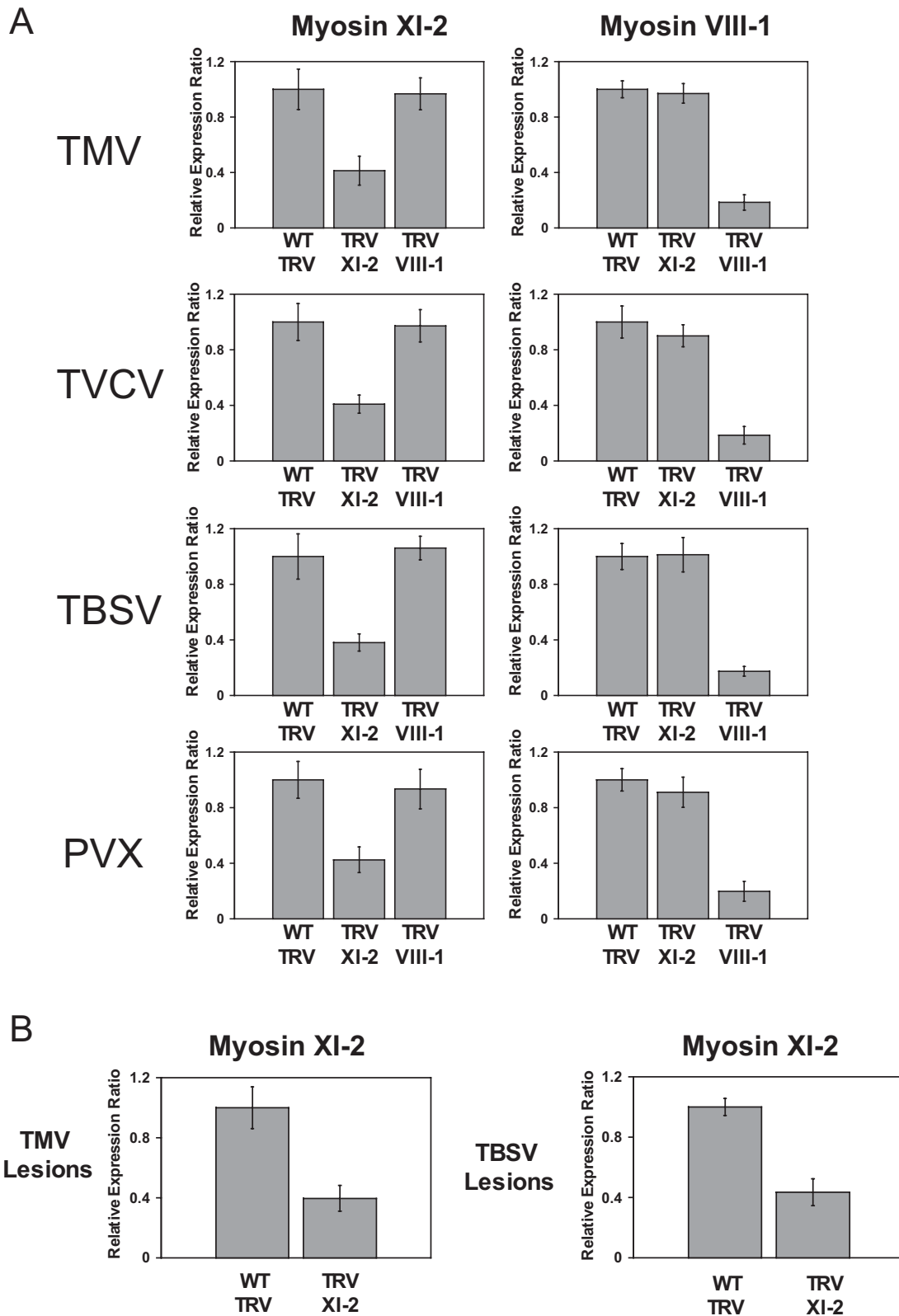


Fig. S7. Secondary virus infection does not significantly influence TRV-induced silencing of myosin transcripts. Myosin VIII-1 (A) and XI-2 (A and B) transcript levels were determined from whole leaf extracts (A) and virus lesions (B) by quantitative RT-PCR at 3 dpi with the indicated viruses for tissues carrying a systemic infection by the TRV VIGS vector (approximately 18 dpi). Bars represent means \pm standard errors for three replicates per treatment. Note that for results shown in (A), lesions covered \approx 60% of the leaf surface for tissue challenged with TBSV, 40% for TMV and TVCV, and 20% for PVX.

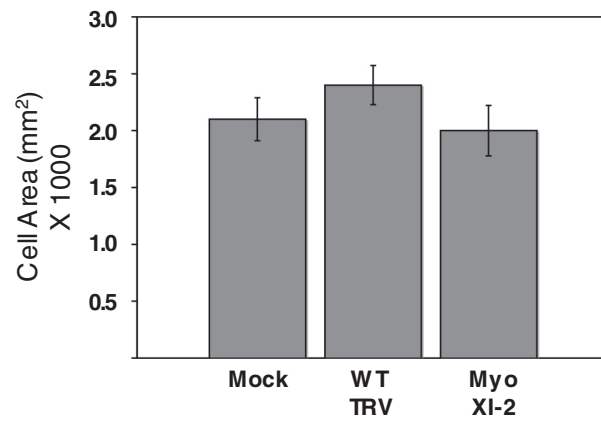


Fig. S8. Epidermal cell size in myosin XI-2 silenced *N. benthamiana* plants was unaltered compared with controls. Epidermal cell areas were measured in plants inoculated (through agroinfiltration) with buffer (Mock), TRV without insert (WT TRV), and TRV expressing a fragment of myosin XI-2 (Myo XI-2). Measurements were taken at 18 days post-infiltration in systemic (upper non-infiltrated) tissue equivalent to those observed for TMV spread in other experiments. Bars represent means \pm standard errors for 10 replicates per treatment. Analysis of variance followed by an lsd calculation indicated no difference between treatment means ($P = 0.05$).

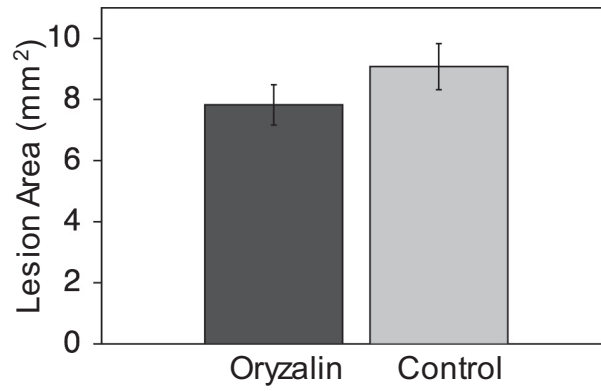


Fig. S9. Oryzalin treatment does not inhibit TVCV intercellular spread. Half leaves of *N. benthamiana* were infiltrated with 20 μ M oryzalin in DMSO (Oryzalin) or DMSO buffer alone (Control). At 3 h post-infiltration, the entire leaf surface was inoculated with TVCV-GFP, and lesion areas were determined at 2 dpi. Bars represent standard errors for 12 lesions from each treatment. A 2 mM oryzalin stock was prepared in DMSO and diluted to 20 μ M in water. An equivalent dilution of DMSO (1:100) was used as a control. Analysis of variance followed by an lsd calculation indicated no difference between treatment means ($P = 0.05$).