7000 (GE Healthcare). Membranes were stripped between hybridizations by washing with 1% SDS for 15 min at 80 °C and exposed for at least 24 h to verify complete removal of probe before re-hybridization. Blots in Figs 3b and 4b were performed similarly, except that 12 μg of total RNA was used. Probe sequences are listed in Supplementary Data 6.

5′-RNA ligase-mediated rapid amplification of cDNA ends. Five micrograms total RNA was ligated to 1 μg of a 44-nucleotide RNA adaptor (Supplementary Data 6) using a 20 μl T4 RNA ligase 1 reaction (NEB) per the manufacturer's instructions for a 1 h incubation at 37 °C. The reaction was then diluted with 68 μl water and 2 μl 0.5 M EDTA pH 8.0, and incubated at 65 °C for 15 min to inactivate the ligase. Sodium acetate pH 5.2 was added to a final concentration of 0.3 M, and the RNA was precipitated with ethanol. The precipitated and washed RNA was resuspended in 10 μl water; 3.33 μl of this sample was used as template in a reverse transcription reaction using random primers and Protoscript II reverse transcriptase (NEB) per the manufacturer's instructions. The resulting cDNA was used as template in first round PCR using a 5′ primer matching the RNA adaptor and a 3′ gene-specific primer (Supplementary Data 6); 1 μl of the product was used as template for nested PCR with nested primers (Supplementary Data 6). Gene-specific primers for *A. thaliana* cDNAs were based on the representative TAIR10 transcript models, while those for *N. benthamiana* cDNAs were based on the v0.4.4 transcripts (Sol Genomics Network[39]). In Fig. 4c, *N. benthamiana TIR1* is transcript ID *NbS00011315g0112.1*; *N. benthamiana ARF* is transcript ID *NbS00059497g0003.1*. Bands were purified from agarose gels and cloned into pCR4-TOPO (Life Tech). Inserts from individual clones were recovered by colony PCR and analysed by Sanger sequencing.

Quantitative reverse-transcription–PCR. Total RNA used for qRT–PCR was first treated with DNaseI (RNase-free; NEB) per the manufacturer's instructions, ethanol precipitated and resuspended. The treated total RNA (2 μg) was used for cDNA synthesis using the High Capacity cDNA Synthesis Kit (Applied Biosystems) per the manufacturer's instructions. PCR reactions used PerfeCTa SYBR Green FastMix (Quantabio) on a StepONE-Plus quantitative PCR system (Applied Biosystems) per the manufacturer's instructions. Primers (Supplementary Data 6) were designed to span the miRNA target sites to ensure that only uncleaved mRNAs were measured. Three reference mRNAs were used: *ACT2*, *AT1G13320* (which encodes PDF2, a subunit of PP2A), and *AT4G34270*[40]. Raw C_t values were used to calculate relative normalized expression values to each reference mRNA separately, and the final analysis used the median relative expression values between the *ACT12* and *AT4G34270*-normalized data.

***C. campestris* growth assays.** *C. campestris* seedlings were scarified, pre-germinated, and placed next to hosts in 0.125 ml water-filled tubes under cool-white fluorescent lighting supplemented with far-red-emitting LEDs (16-h day, 8-h night) at ~23 °C as described above. After a single attachment formed (four days), far-red light supplementation was removed to prevent secondary attachments. After 18 more days of growth, entire *C. campestris* vines were removed and weighed (Fig. 3c). Multiple additional growth trials were performed specifically on the *dcl4-2t* and *sgs2-1* mutant hosts under varying conditions (Extended Data Fig. 4).

miRNA target predictions. To find probable orthologues for *A. thaliana* genes of interest, the *A. thaliana* protein sequences were used as queries for a BLASTP analysis of the 31 eudicot proteomes available on Phytozome 11 (https://phytozome.jgi.doe.gov/pz/portal.html#). Transcript sequences for the top 100 hits were retrieved. In some cases no hits were found in a particular species; these are shown as 'NA' in Fig. 4a. The miRNA query set was all mature miRNA and miRNA* from the interface-induced, *C. campestris*-derived 21- or 22-nucleotide miRNAs (Supplementary Data 2). Probable targets from the 31 species were identified as those having a score of up to 4.5 using targetfinder.pl v0.1 (https://github.com/MikeAxtell/TargetFinder/).

N. benthamiana orthologues of *A. thaliana* proteins were found based on BLASTP searches against the v0.4.4 *N. benthamiana* protein models at Sol Genomics Network[39], and miRNA target sites predicted using targetfinder.pl as above.

Statistics and reproducibility. No statistical methods were used to predetermine sample size. The experiments were not randomized. 95% confidence intervals from Fig. 3a: 0.249 to 0.611 (*BIK1*), 0.267 to 0.781 (*SEOR1*), −0.122 to 0.649 (*HSFB4*), 0.385 to 0.894 (*TIR1*), 0.083 to 0.724 (*AFB2*), 0.071 to 0.678 (*AFB3*), −0.461 to −0.120 (*AT4G34270*). Note that these confidence intervals from unpaired Wilcoxon rank-sum tests are the estimators of the median of control stem minus interface for each gene. 95% confidence intervals from Fig. 3c: −0.580 to −0.200 (*seor1*), 0.220 to 0.400 (*bik1*), −0.070 to 0.150 (*scz2*), −0.170 to 0.060 (*tir1-1/afb2-3*), −0.440 to −0.180 (*afb3-4*). Note that these confidence intervals from unpaired Wilcoxon rank-sum tests are the estimators of the median of Col-0 minus the mutant for each comparison.

Code availability. ShortStack[35] (small-RNA-seq analysis), strucVis (visualization of predicted RNA secondary structures with overlaid small-RNA-seq depths), GSTAr.pl (prediction of miRNA targets) and Shuffler.pl/targetfinder.pl (prediction of miRNA targets controlling for false discovery rate) are all freely available at https://github.com/MikeAxtell. Cutadapt[33] is freely available at http://cutadapt.readthedocs.io/en/stable/index.html. The R package DESeq2[36] is freely available at http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html.

Data availability. Small-RNA-seq data from this work are available at NCBI GEO under accession GSE84955 and NCBI SRA under project PRJNA408115. The draft, preliminary *C. campestris* genome and transcriptome assemblies used in this study are available at the Parasitic Plant Genome Project website at http://ppgp.huck.psu.edu. *C. campestris* miRNA loci have been registered with miRBase. Source data availability: Fig. 1b, in Supplementary Data 2 and 3; Figs 1c, 2c, d, 3b, 4b and c, in Supplementary Fig. 1; Fig. 3a, c and Extended Data Fig. 4, included as Source Data; Fig. 4a, in Extended Data Table 1. There are no restrictions on data availability and the corresponding author will provide any data not already included as Supplementary Data or as Source Data upon request.

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