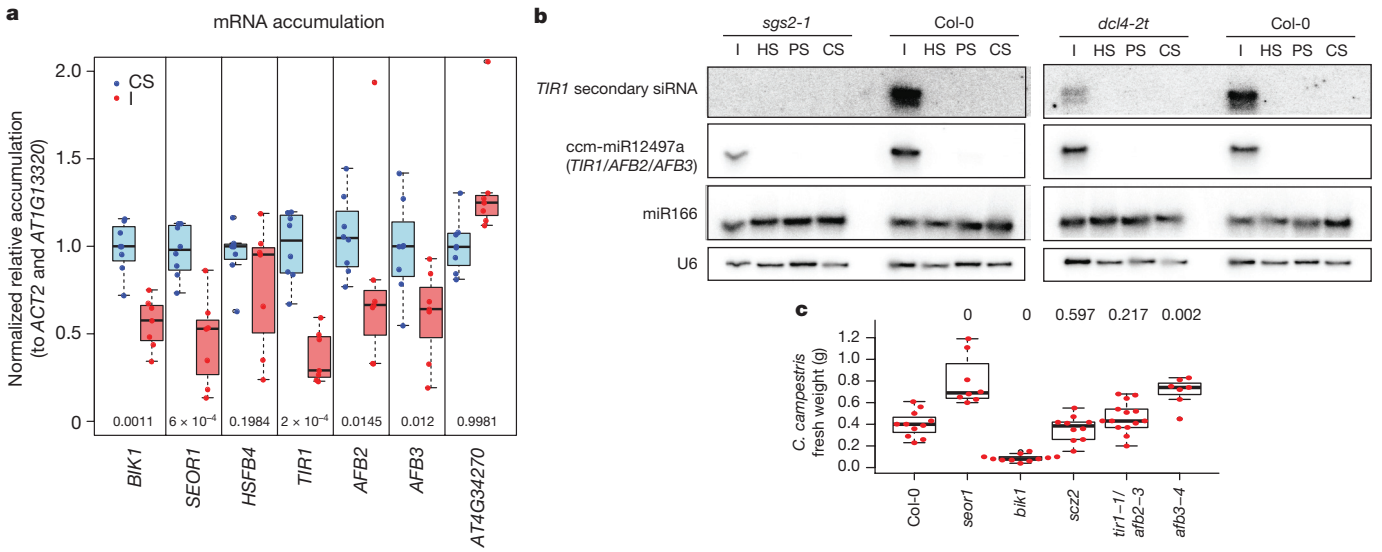

Figure 3 | Effects of *C. campestris* miRNAs and their targets. **a**, Accumulation of *A. thaliana* mRNA in interface versus control stems, shown by quantitative reverse-transcriptase polymerase chain reaction (qRT–PCR). Control stems, $n = 8$; interface, $n = 7$ biologically independent samples. Box plots show the median, box edges represent the first and third quartiles, and the whiskers extend to 1.5× interquartile range. *P* values are displayed above the *x* axis; Wilcoxon rank-sum tests, unpaired, one-tailed. *AT4G34270* (also known as TIP41-like protein), control. **b**, RNA blots from *C. campestris* infestations of the indicated *A. thaliana* genotypes. Full gels are shown in Supplementary Fig. 1. The experiment was performed twice with similar results. **c**, *C. campestris* biomass on *A. thaliana* hosts of the indicated genotypes. *P* values and plotting conventions as in **a**, except two-tailed tests were used; $n = 11$, 8, 11, 10, 14, and 7 biologically independent samples (left to right).

We performed additional small-RNA-seq from *C. campestris* on *A. thaliana* hosts, and from *C. campestris* on *N. benthamiana* hosts. Both sets of experiments were designed identically to the original small-RNA-seq study (two biological replicates each of host stem, interface and parasite stem samples). The interface-induced set of *C. campestris* miRNA loci was highly reproducible across both of the *A. thaliana* experiments as well as the *N. benthamiana* experiment (Extended Data Fig. 5). Induction of several *C. campestris* miRNAs during *N. benthamiana* parasitism was confirmed by RNA blots (Fig. 4b). Several *N. benthamiana* mRNAs both contained plausible target sites for *C. campestris* miRNAs and showed accumulation of phased, secondary siRNAs in the interface samples, including orthologues of *TIR1* and *BIK1* (Extended Data Fig. 6). Analysis of uncapped RNA ends provided strong evidence for miRNA-directed cleavage of one of the *N. benthamiana TIR1* orthologues (Fig. 4c, d). This is direct evidence that the same *C. campestris* miRNAs target orthologous host mRNAs in multiple species. None of the interface-induced miRNAs we tested were detectable in *C. campestris* pre-haustoria from seedling tips that had coiled around dead bamboo stakes instead of a live host (Fig. 4b, Extended Data Fig. 7). This suggests that contact with a living host is a requirement for expression of these miRNAs.

These data demonstrate that *C. campestris* induces a large number of miRNAs at the haustorium, and that some of these miRNAs target and reduce accumulation of host mRNAs. Many of the induced miRNAs are 22 nucleotides in length, and are associated with secondary siRNA production from their host targets using the host's secondary siRNA machinery. Several of the targets are linked to plant pathogenesis: manipulation of levels of *TIR1, AFB2,* and *AFB3* mRNA affects bacterial pathogenesis and defence signalling[27], and BIK1 is a central regulator of pathogen-induced signalling[28]. Perhaps the most intriguing target is *SEOR1*, which encodes a very abundant protein that is present in large agglomerations in phloem sieve-tube elements[18]. *seor1* mutants show an increased loss of sugars from detached leaves[19], and our data show that *seor1* mutants also support increased *C. campestris* growth. A key function of the haustorium is to capture nutrients from the host phloem; targeting *SEOR1* could be a strategy to increase sugar uptake from host phloem. Overall, these data suggest that *C. campestris* trans-species miRNAs might function as virulence factors to remodel host gene expression to the parasite's advantage. Further experiments that directly disrupt the delivery or function of these miRNAs will be needed to test this hypothesis directly.

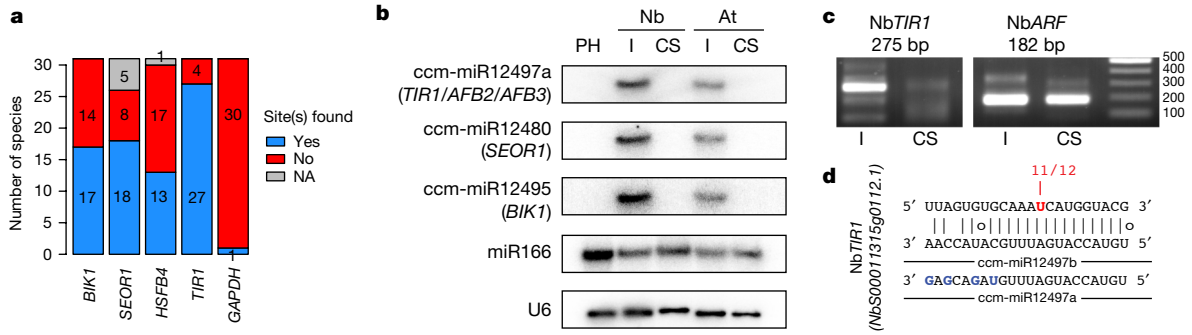

Figure 4 | Conservation of host mRNA targeting by *C. campestris*. **a**, Predicted targets of interface-induced *C. campestris* miRNA–miRNA*. NA, no orthologous genes found. **b**, RNA blots from interface and control stem of *C. campestris*-infested *N. benthamiana* (*Nb*), *A. thaliana* (*At*), and *C. campestris* pre-haustoria (PH). The experiment was performed once. **c**, 5′-RLM-RACE products for the indicated *N. benthamiana* cDNAs. *ARF*, positive control. The image was cropped to remove irrelevant lanes. Full gels are shown in Supplementary Fig. 1. The experiment was performed once. **d**, Complementary site and 5′-RLM-RACE results from a *N. benthamiana TIR1* orthologue.