Supplementary Information

Involvement of *RDR6* in short-range intercellular RNA silencing in *Nicotiana benthamiana*

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Supplementary Table S1 P values of Student's *t*-tests[1].

Comparisons	*RDR6*[2]	TCV
Mock		
Nb vs NbRDR6i	0.0001 (Nb > NbRDR6i)	-
GFP16c vs GFP16c/RDR6i	0.0003 (GFP16c > GFP16c/RDR6i)	-
TCV-GFPΔCP		
Nb vs NbRDR6i	0.0001 (Nb > NbRDR6i)	0.085[3]
GFP16c vs GFP16c/RDR6i	0.0001 (GFP16c > GFP16c/RDR6i)	0.31[3]
Nb vs GFP16c	-	0.0037 (Nb > GFP16c)[4]
NbRDR6i vs GFP16c/RDR6i	-	0.0001 NbRDR6i > GFP16c/RDR6i[4]

[1]Student's *t*-tests were carried out between the *RDR6* mRNA levels of wild-type (Nb) and *RDR6i* (NbRDR6i) *N. bentthamiana* plants, and that of GFP16c and GFP16c *RDR6i* (GFP16c/RDR6i) plants. For the TCV RNA, student's *t*-tests were performed between Nb and NbRDR6i, GFP16c and GFP16c/RDR6i, Nb and GFP16c, as well as NbRDR6i and GFP16c/RDR6i. Plants were mock-inoculated or inoculated with TCV-GFPΔCP.

[2]RNAi significantly down-regulated *RDR6* expression.

[3]Knock-down of *RDR6* gene expression by RNAi had no impact on accumulation of TCV-GFPΔCP RNA in plants.

[4]Virus-induced RNA silencing significantly reduced the TCV-GFPΔCP RNA levels. RNAi-mediated inhibition of *RDR6* did not affect intracellular RNA silencing.

Supplementary Table S2 Two types of short-range intercellular RNA silencing

	Transgene-induced RNA silencing[8-12]	Virus-induced RNA silencing (This study)
RNA silencing trigger	Transgene-originated Hairpin RNA	Viral RNA
Cell types	Companion to parenchyma	Epidermal to epidermal, mesophyll
Short-range spread	10 – 15 cells	6 – 10 cells
RDR6 gene	Independent	Dependent
Mobile signal	21-nt siRNA	unknown
Other factors	*RDR2*, *NRPD1a*	Virus movement proteins[14]
Plant species	*Arabidopsis thaliana*	*Nicotiana benthamiana*

Supplementary Table S3 Specific primers for qRT-PCR assays

Primers	Sequences (5’ – 3’)	RNA Targets
NbEF1α-qRT-F	TGCCTTGTGGAAGTTTGAGACC	*EF1α*
NbEF1α-qRT-R	GGTGGAGTCAATAATCAGGACAGC	*EF1α*
NbGAPDH-qRT-F	AGCTGGTGCTGATTTCGTTGTG	*GAPDH*
NbGAPDH-qRT-R	GAGCAGAGATCACAACCTTCTTGG	*GAPDH*
TCV-qRT-F	CGGAATTGAGTTCAGCGTCCTTCCG	TCV
TCV-qRT-R	GCAGGAGGGCCACCATTTAC	TCV
NbRDR6-qRT-F	CTTTGGATGAGAAGTGCCTA	*RDR6*
NbRDR6-qRT-R	TTTGGGACAAGCTCAAGTC	*RDR6*