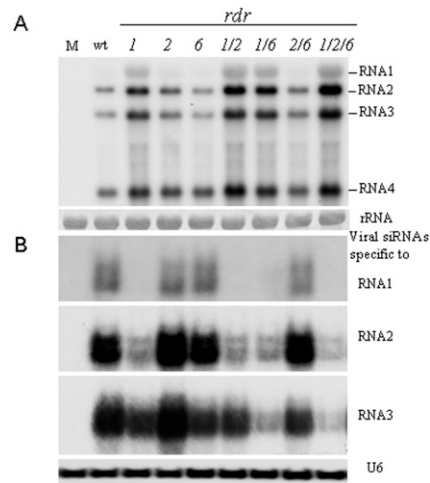
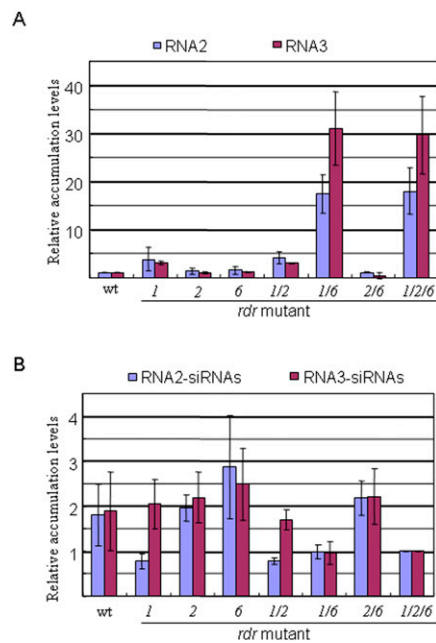


# Supporting Information

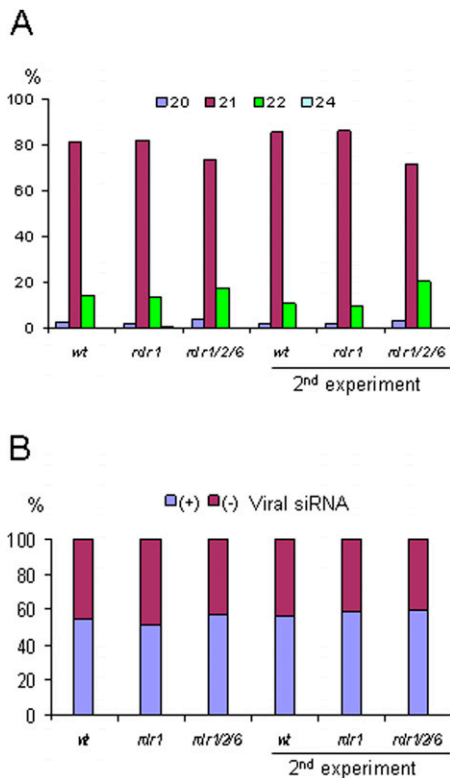
Wang et al. 10.1073/pnas.0904086107



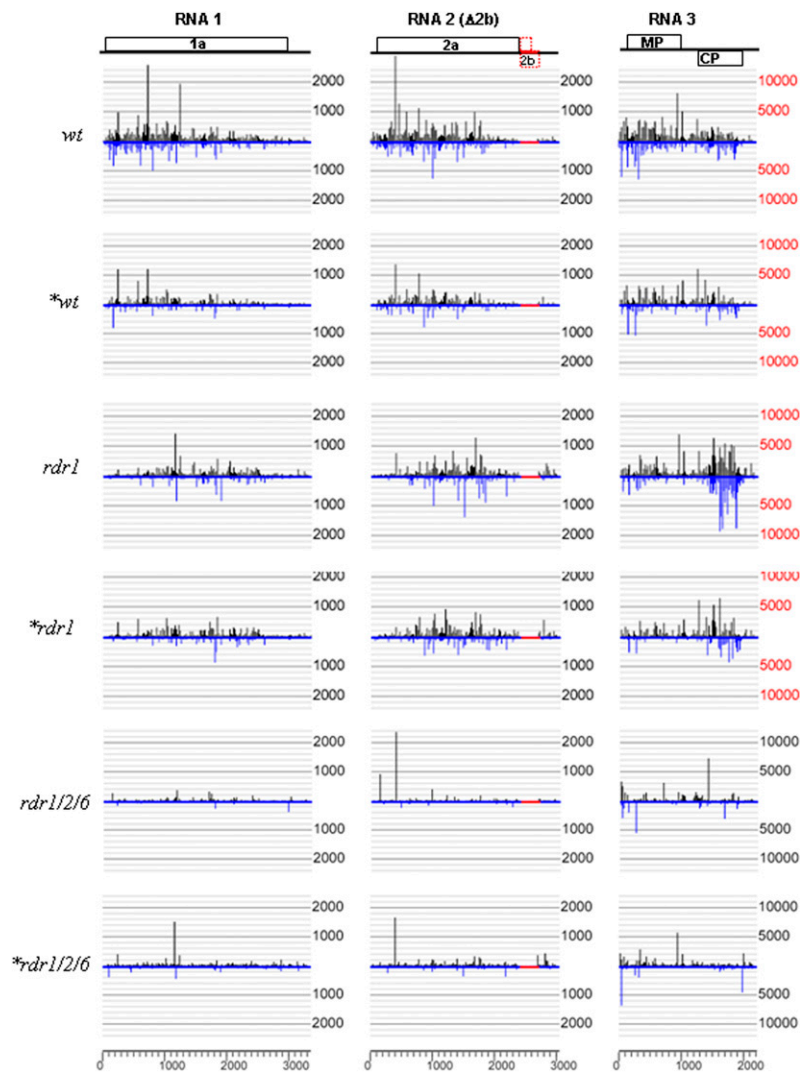
**Fig. S1.** Accumulation of (A) viral genomic/subgenomic RNAs and (B) viral siRNAs in the inoculated leaves of WT and *rdr* mutant plants 5 days after inoculation; 25S rRNA and U6 RNA served as loading controls. M, mock.



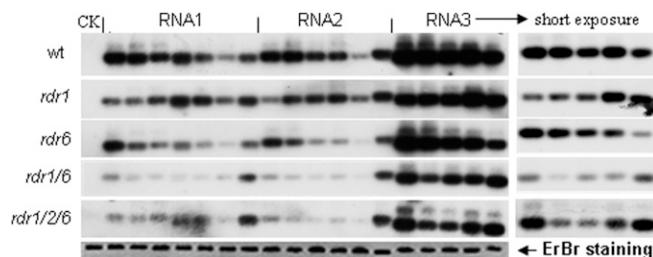
**Fig. S2.** The relative accumulation levels for (A) viral genomic RNAs 2 and 3, and (B) viral siRNAs specific to RNAs 2 and 3 measured for three independent experiments. The accumulation levels of RNAs 2 and 3 in WT plants and of RNA2-siRNAs and RNA3-siRNAs in *rdr1 rdr2 rdr6* plants were all set as 1.



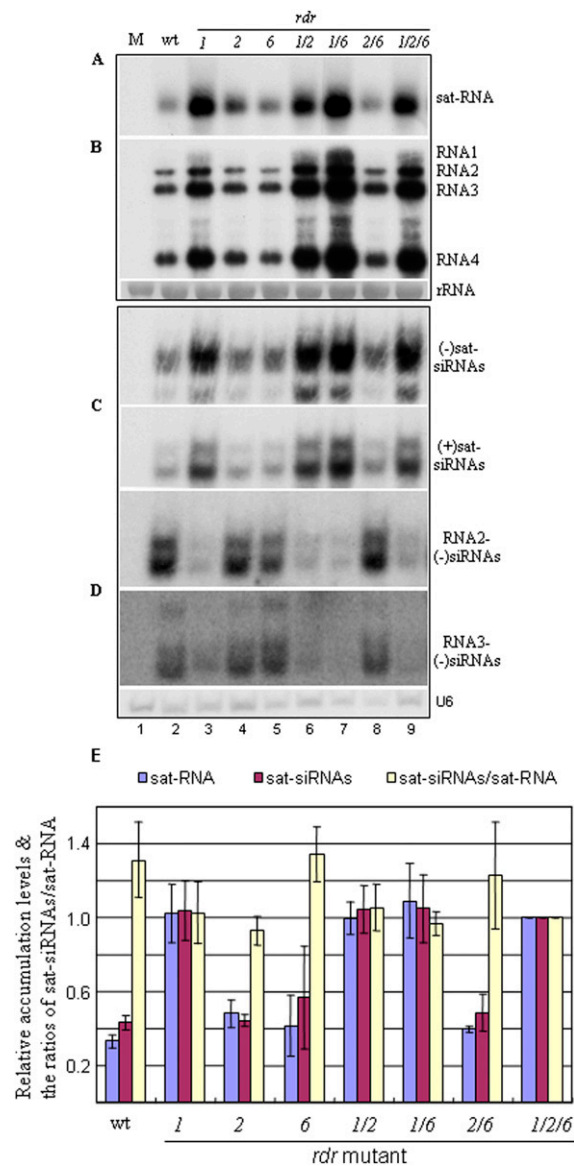
**Fig. S3.** The profiles of virus-derived siRNAs in WT, *rdr1*, *rdr6*, and *rdr1/2/6* plants infected with CMVf- $\Delta$ 2b. (A) The size distribution of cloned viral siRNAs from infected plants. (B) The percentages of positive- and negative-strand viral siRNAs completely identical (+) or complementary (-) to the genomic RNAs. The results of independently constructed and sequenced small RNA libraries made from CMVf- $\Delta$ 2b-infected wt, *rdr1*, and *rdr1 rdr2 rdr6* plants are presented on the right.



**Fig. S4.** Three small RNA libraries (marked by \*) were independently constructed from systemically infected leaves of WT and *rdr1* plants 2 weeks after inoculation with CMVf- $\Delta$ 2b, sequenced by Illumina platform, and aligned for comparison with the previously sequenced WT and *rdr1* libraries, as described in Fig. 3. Part of the analyses of the three libraries were presented in Fig. S3.



**Fig. S5.** Relative abundance of viral siRNAs targeting the evenly divided regions of the individual genomic RNAs of CMVf- $\Delta$ 2b. Equal amount of DNA fragments specific to RNA1 (divided into seven fragments of 480 bp each), RNA2 (six fragments, 500 bp each) and RNA3 (five fragments, 450 bp each), respectively, was fractionated and hybridized to the labeled total small RNAs harvested and gel-purified from WT, *rdr1*, *rdr6*, *rdr1 rdr6*, or *rdr1 rdr2 rdr6* plants infected with CMVf- $\Delta$ 2b.



**Fig. S6.** The accumulation levels for sat-RNA (A), the helper virus CMV (B) sat-siRNAs (C), and CMV RNA2/RNA3-derived siRNA (D), as well as the ratios of sat-siRNAs versus sat-RNA measured for three independent experiments (E). The accumulation levels of sat-RNA and sat-RNA-derived siRNAs (sat-siRNAs), as well as the ratio of sat-siRNAs/sat-RNA in *rdr1 rdr2 rdr6* plants, were all set as 1. The differences of the ratios of sat-siRNAs/sat-RNA between *rdr1 rdr2 rdr6* plants and the remaining six genotypes of plants were not statistically significant with  $P$ -values  $>0.05$ .

**Table S1. Relative abundance of siRNAs (19- to 25-nt) derived from the RNA1, RNA2, and RNA3 of CMVf- $\Delta$ 2b in WT and mutant plants**

		Normalized reads per million (relative to that in WT plants)			
		Total viRNAs	RNA1	RNA2	RNA3
Repeat 1	wt	373,235 (1.00)	58,641 (1.00)	53,173 (1.00)	261,421 (1.00)
	<i>rdr1</i>	348,039 (0.93)	31,660 (0.54)	37,657 (0.71)	278,722 (1.07)
	<i>rdr1/2/6</i>	160,233 (0.43)	23,235 (0.40)	22,388 (0.42)	114,610 (0.44)
Repeat 2	wt	221,328 (1.00)	27,616 (1.00)	29,503 (1.00)	164,209 (1.00)
	<i>rdr1</i>	216,645 (0.98)	29,484 (1.07)	34,375 (1.17)	152,786 (0.93)
	<i>rdr1/2/6</i>	81,786 (0.37)	6,719 (0.24)	7,933 (0.27)	67,134 (0.41)