

Figure S2. Enhanced accumulation of an RNAi-deficient Tobacco rattle virus (TRV) RNA1 replicon following co-expression with PPR3 in leaves of Nicotiana benthamiana. (A) Full-grown leaves of N. benthamiana were co-infiltrated with an Agrobacterium tumefaciens strain expressing PPR3 and a strain expressing the replicon TRV-M1 of Tobacco rattle virus (TRV) RNA1, which lacks the 16K gene coding for an RNAi suppressor [76], or were infiltrated only with the A. tumefaciens strain expressing TRV-M1 (control). (B) Leaves of N. benthamiana were co-infiltrated with three A. tumefaciens strains expressing PPR3, TRV-M1 and TRV-RNA2(GFP), respectively, and compared with controls, i.e., agroinfiltration for coexpression of TRV-M1 and TRV-RNA2(GFP). Control agroinfiltration was done to the opposite site of the mid-rib in the same leaf. Samples from infiltrated leaf tissues were collected for analysis 3 days post-infiltration (d.p.i.), before appearance of necrotic symptoms at 4 d.p.i. TRV RNA1 amounts were estimated using quantitative reverse transcription realtime PCR, as described [76]. RNA1 amounts (fold change) in (A) and (B) are shown for three independently tested leaves relative to the mean of the controls (no PPR3) of the three leaves (= 1.0). Blue bars indicate TRV RNA1 accumulation in the presence of PPR3, whereas red bars (-) show accumulation of RNA1 in controls lacking PPR3. TRV RNA2 influences accumulation of RNA1 by an unknown mechanism [76], which explains the less consistent results in (B). However, in all experiments, there was a consistent tendency for enhanced accumulation of TRV RNA1 in the presence of PPR3.