

Figure 6–Figure supplement 1

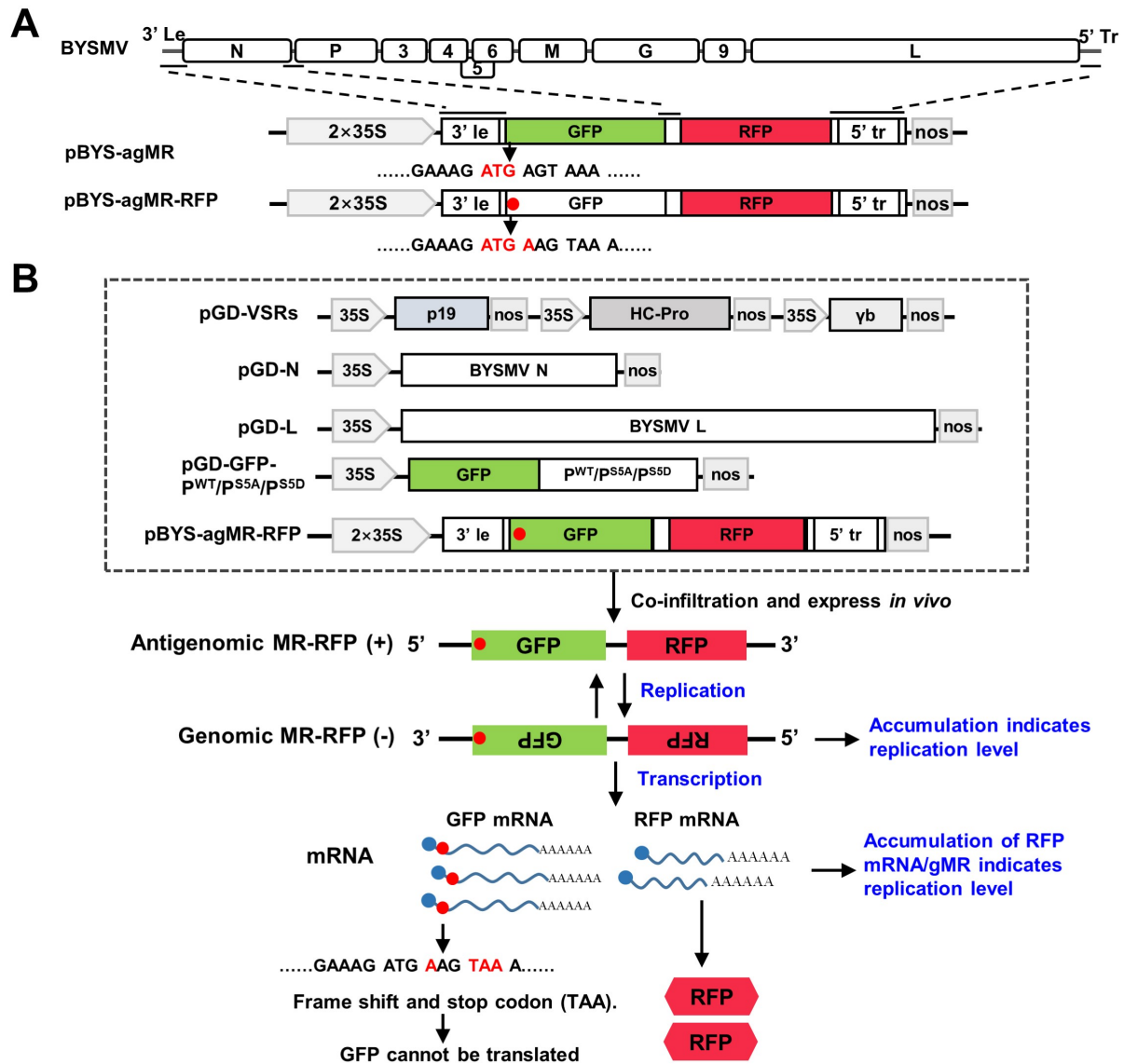


Figure 6–Figure supplement 1. Illustration of BYS-agMR-RFP infection in epidermal cells of *N. benthamiana* leaves. (A) The binary vectors of the pBYS-agMR and pBYS-agMR-RFP plasmids. In the pBYS-agMR plasmid, the ORFs of BYSMV N and P were replaced with GFP and RFP, respectively, and were flanked by 3' leader (le) and 5' trailer (tr) sequences outside the N gene and the L gene. Based on the pBYS-agMR plasmid, the pBYS-agMR-RFP plasmid was obtained by introducing a A after the start codon of the GFP ORF. (B) Illustration of BYS-agMR-RFP replication and transcription. pGD-VSRs contains three expression cassettes to express tomato bushy stunt virus p19, the tobacco etch virus HC-Pro, and the barley stripe mosaic virus γ b simultaneously. The ORFs of N, L, and GFP-P^{WT}/GFP-P^{SSA}/GFP-P^{SSD} were inserted into the pGD vector for expression of N, L, and GFP-P^{WT}/GFP-P^{SSA}/GFP-P^{SSD}. BYS-agMR-RFP was rescued by co-expression of pBYS-agMR-RFP, N, L, GFP-P^{WT}/GFP-P^{SSA}/GFP-P^{SSD}, and VSRs in *N. benthamiana* leaves. After co-infiltration, the agMR-RFP was first transcribed and formed replication complexes with co-expressed N, L, and GFP-P^{WT}/GFP-P^{SSA}/GFP-P^{SSD}. Then, the full-length gMR-RFP was produced through replication. Subsequently, the full-length gMR-RFP serves as a template to transcribe into mRNA of GFP and RFP. Accumulation of the gMR-RFP represents replication level. The normalization of RFP mRNA levels relative to the gMR template indicates transcription level. Note that GFP mRNA cannot be translated because A-insertion-induced frame shift.