



Introduction

The synergistic interaction of Maize chlorotic mottle virus (MCMV) and Sugarcane mosaic virus (SCMV) causes maize lethal necrosis disease. MCMV belongs to the genus Machlomovirus, while SCMV belongs to the genus Potyvirus. To condition susceptibility, viruses encode suppressors that counter gene silencing. Potyviral HC-Pro and VPg are silencing suppressors. Silencing suppressors in Machlomovirus have not been characterized. **We hypothesized that synergism between MCMV and SCMV is mediated by silencing suppressors with different mechanisms.** This hypothesis predicts that MCMV and SCMV have silencing suppressors that differentially affect gene silencing. To identify suppressors in MCMV and SCMV, cistrons coding for each one of the proteins were cloned. Silencing suppression activity was tested using a standard transient assay in *Nicotiana benthamiana*. Results showed that MCMV P50 and SCMV HC-Pro and VPg are silencing suppressors. To investigate the suppression mechanism, these proteins were tagged and co-expressed with the core components of the gene silencing pathway, with or without a GFP transgene sensor. P50 does not affect the accumulation of transgene-derived siRNAs nor the accumulation of argonaute (AGO) proteins or SGS3. In contrast, SCMV VPg induces degradation of AGO2, RDR6 and SGS3. These results show contrasting mechanisms by suppressors encoded by MCMV and SCMV.

Goal

- Characterize MCMV and SCMV:
 - Silencing suppressors proteins
 - Suppression mechanism.

Questions

- Which MCMV and SCMV proteins are the silencing suppressors?
- What are the mechanisms of suppression?

Model

- Synergism between MCMV and SCMV is mediated by silencing suppressors with different mechanisms.

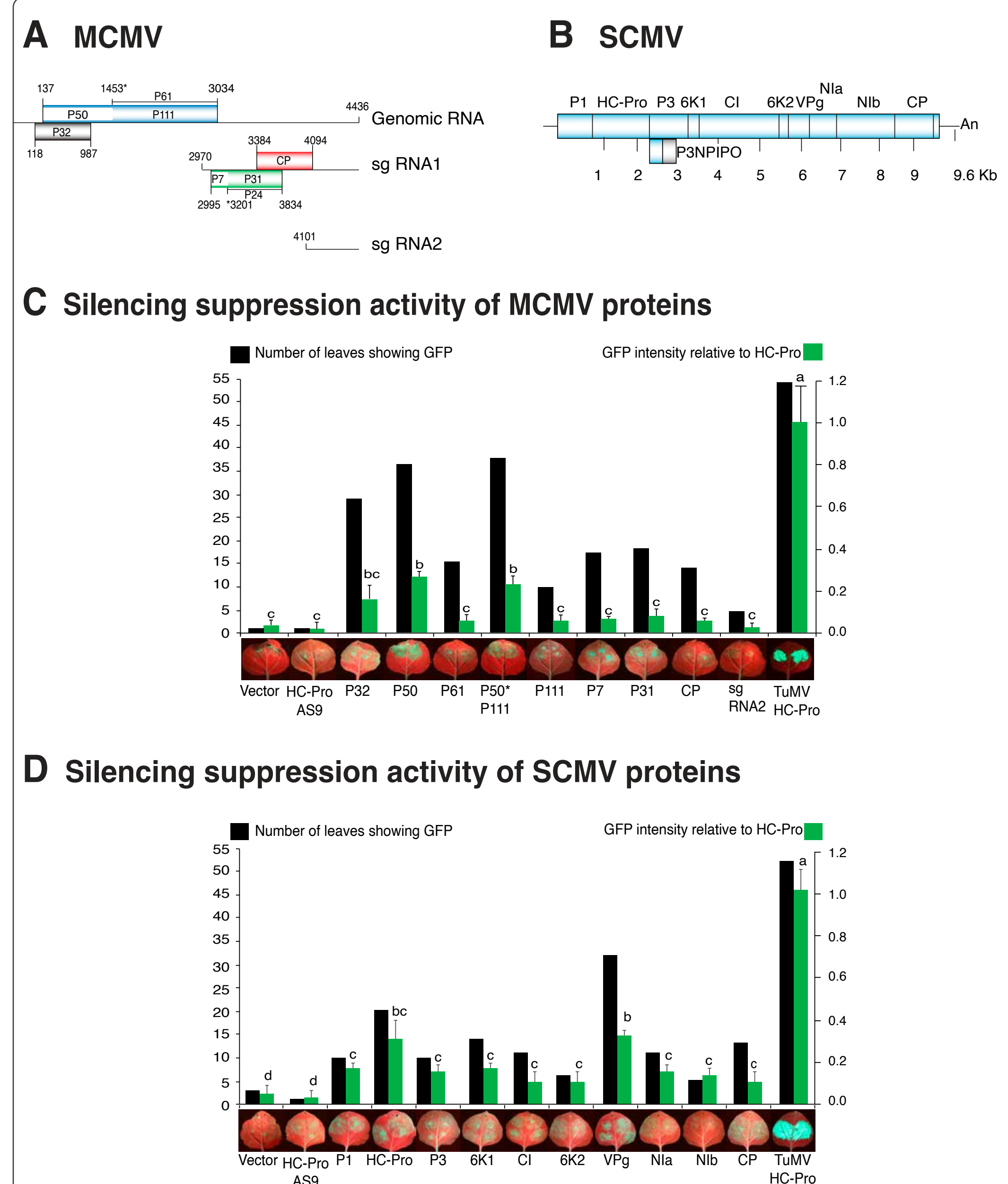
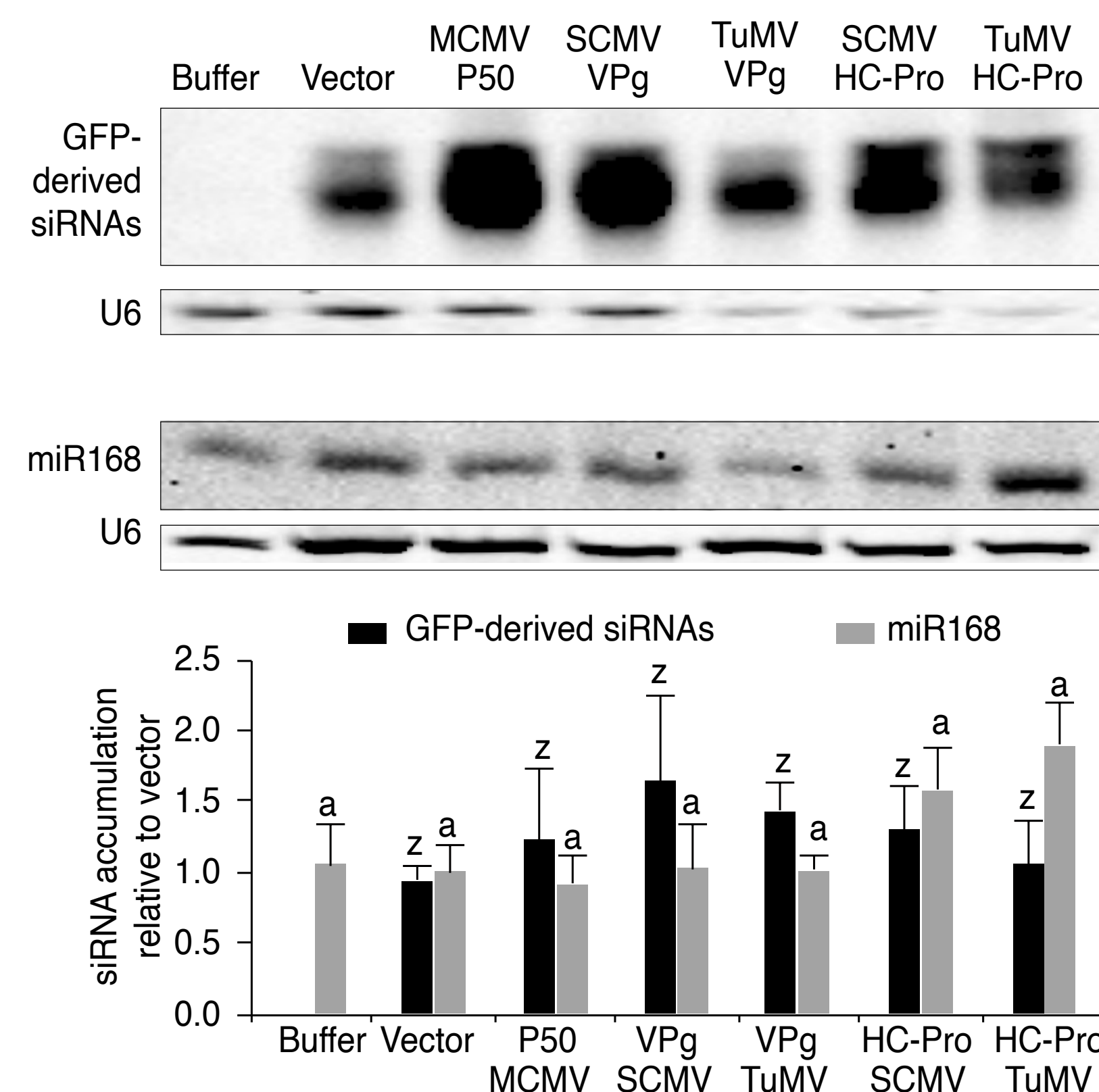


Figure 1. Silencing suppression activity of MCMV and SCMV. A and B) Each cistron of MCMV and SCMV were 6HIS3FLAG tagged and cloned. C and D) MCMV and SCMV each protein were transiently expressed in *N. benthamiana* WT plants in the presence of the transgene GFP. Pictures were taken at 4 dpi under UV light. GFP fluorescence accumulation was evaluated.

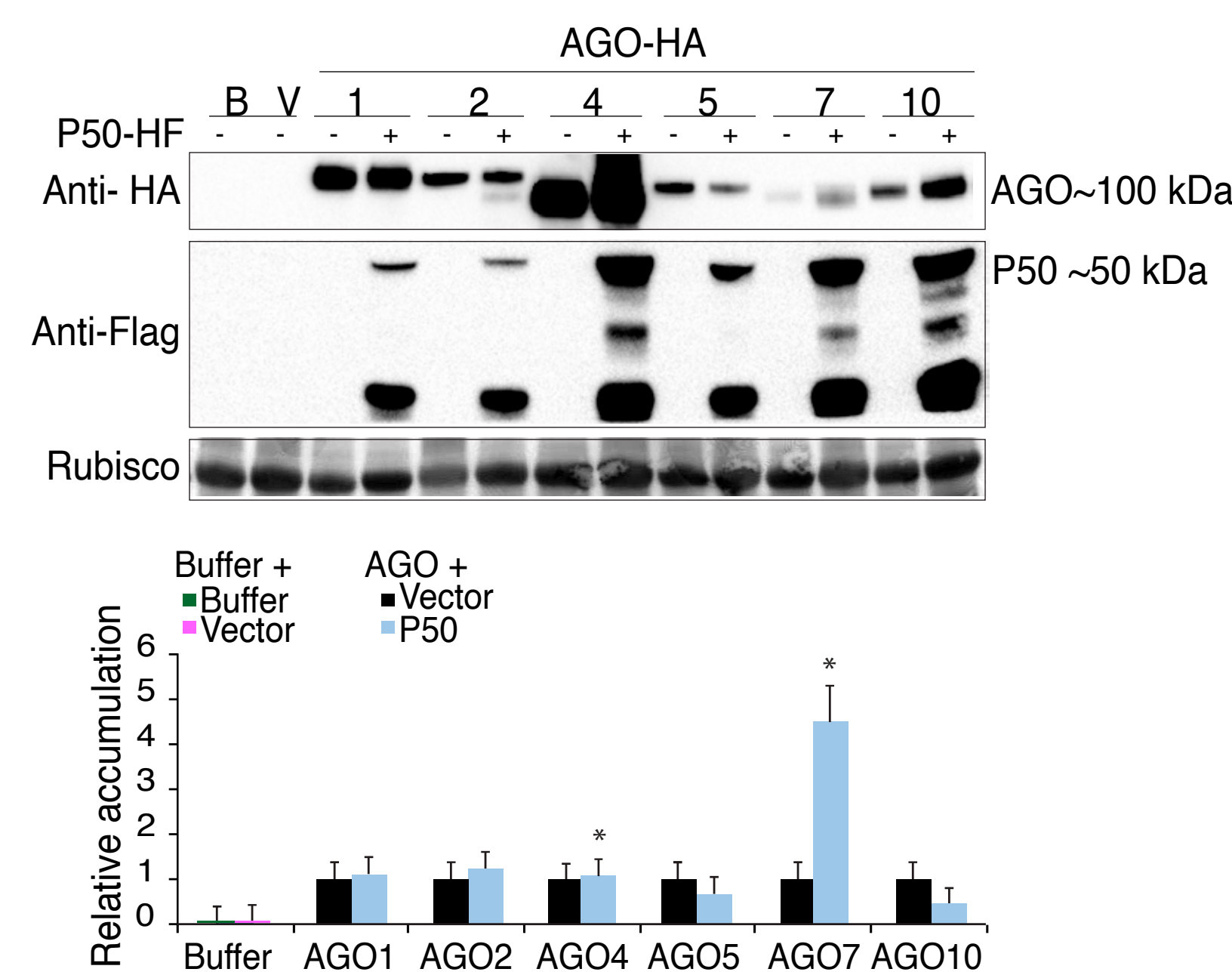
Potential Impacts

Discovery of the unknown silencing suppressors involved in the MLND. Contributing to the understanding of the molecular mechanisms behind the synergistic interaction of MCMV and SCMV.

A MCMV-P50 and SCMV-VPg effect on siRNA



B MCMV-P50 effect on Argonautes



C SCMV-VPg effect on Argonautes

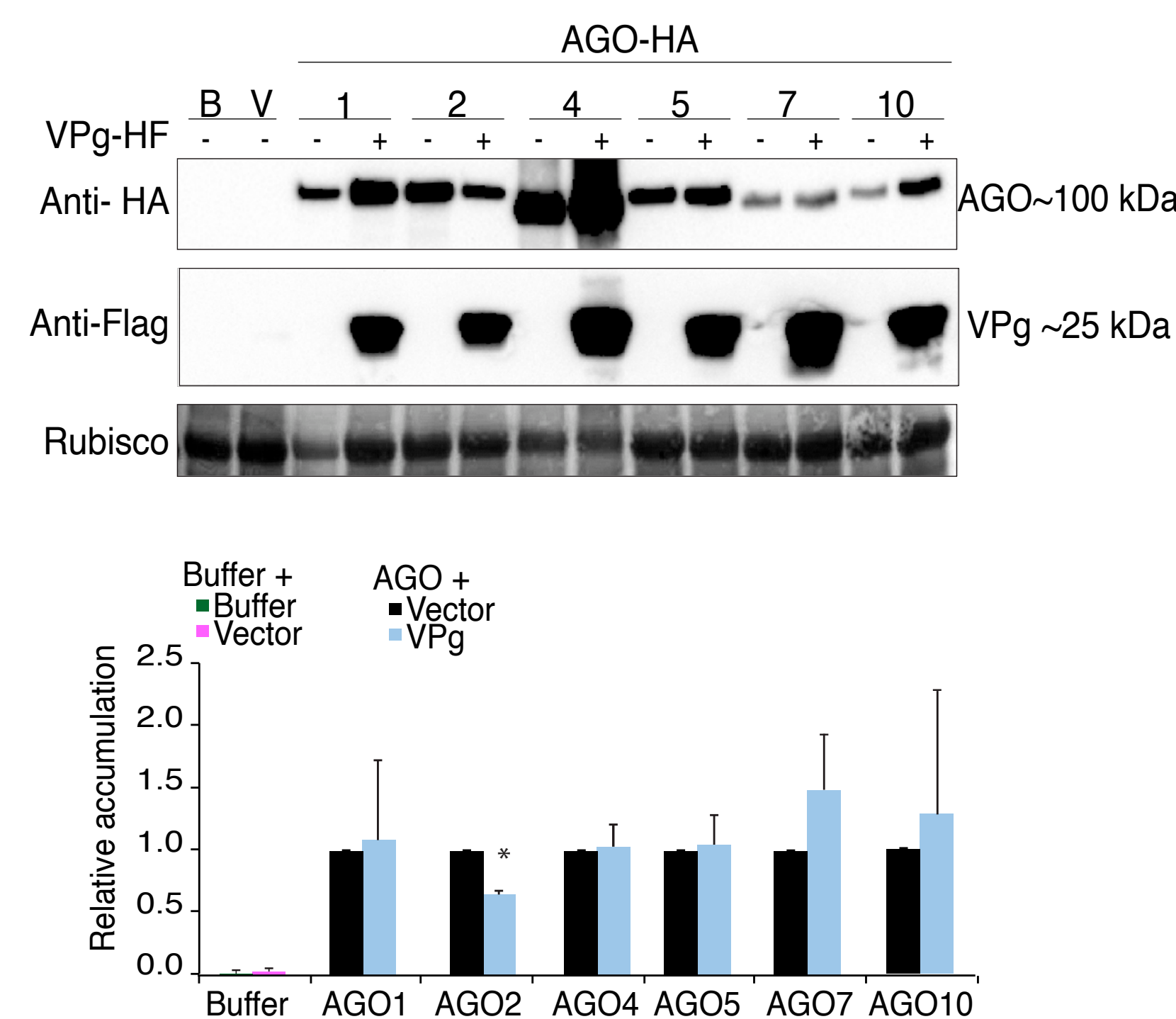
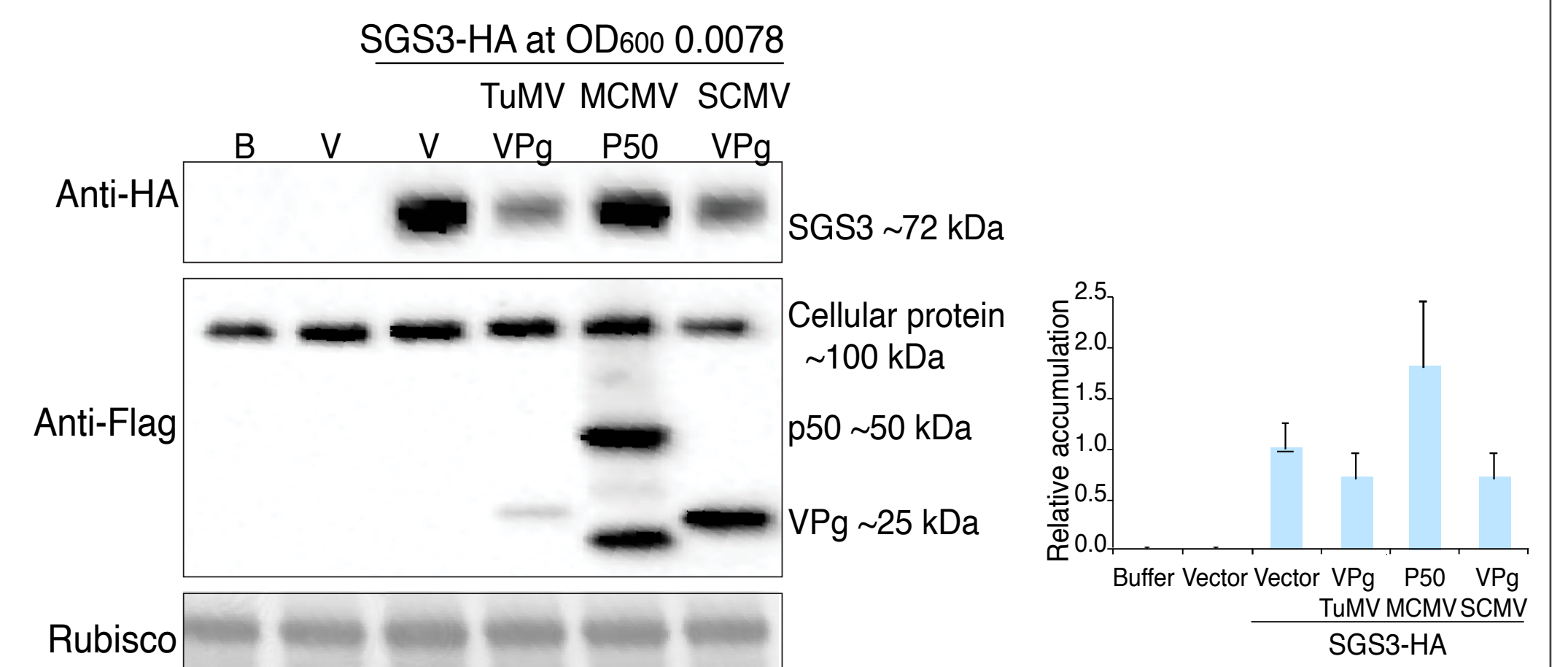
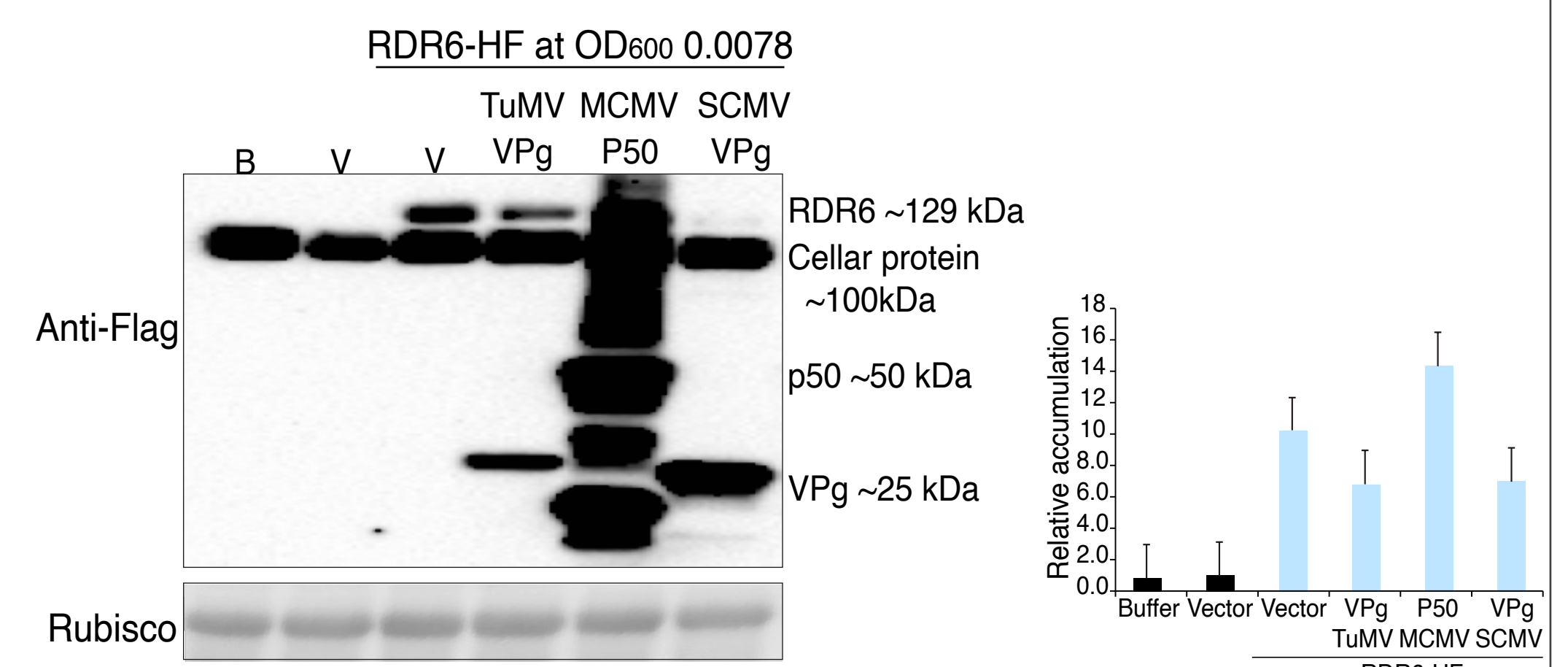


Figure 2. Mechanisms of MCMV-P50 and SCMV-VPg silencing suppression. A) MCMV-P50 and SCMV-VPg were agroinfiltrated with and without GFP. *N. benthamiana* WT plants were used. At 2 dpi RNA extraction was conducted. GFP-derived siRNA was evaluated by the northern blot assay. U6 was used as a loading control. B and C) MCMV-P50 and SCMV-VPg were expressed with and without Argonautes proteins. Protein samples were harvested at 2 dpi. Western blot analysis was conducted to detect changes in Argonautes accumulation in the presence of P50 or VPg.

D MCMV-P50 and SCMV-VPg effect on SGS3



E MCMV-P50 and SCMV-VPg effect on RDR6



F MCMV-P50 and SCMV-VPg effect on RDR1

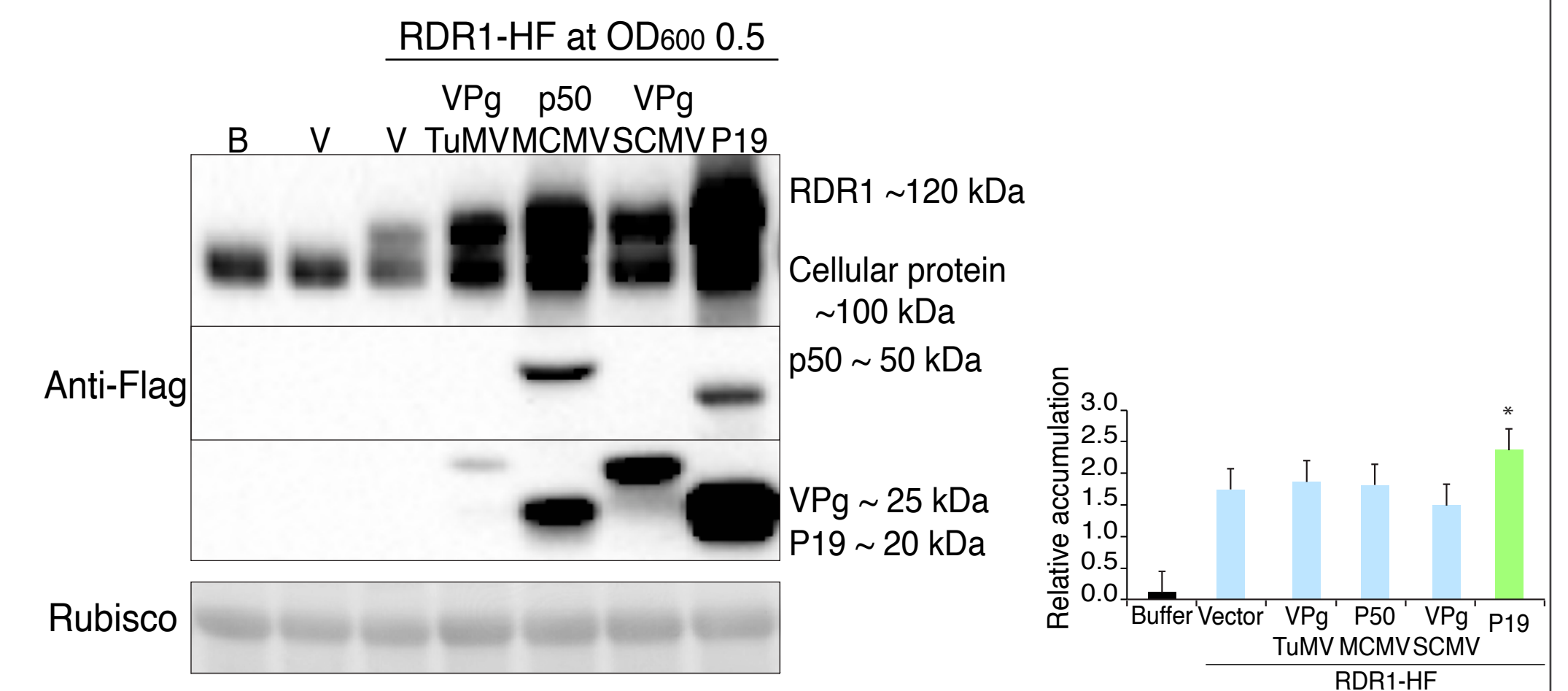


Figure 2. Mechanisms of MCMV-P50 and SCMV-VPg silencing suppression. D) MCMV-P50 and SCMV-VPg effect on SGS3, E) on RDR6 and F) on RDR1. Proteins were extracted at 2 dpi. Standard Western blot protocol was developed.

Preliminary conclusion

Silencing Suppressors	Mechanisms			
	siRNA	AGO2	SGS3	RDR6
MCMV P50	Needs further study	No degradation	No degradation	No degradation
SCMV VPg	No effect	Degradation	Degradation	Degradation

Acknowledgment

I would like to acknowledge the funding agency National Institute of Health, and I would like to thank Dr. Garcia-Ruiz for his guidance and support.

References

- Elmayan, T., et al., A neomorphic sgs3 allele stabilizing miRNA cleavage products reveals that SGS3 acts as a homodimer. *Febs J.* 2009. 276(3): p. 835-44.
- Garcia-Ruiz, H., et al., Roles and Programming of Arabidopsis ARGONAUTE Proteins during Turnip Mosaic Virus Infection. *PLoS Pathog.* 2015. 11(3): p. e1004755
- Wamaitha, M.J., et al., Metagenomic analysis of viruses associated with maize lethal necrosis in Kenya. *Viro J.* 2018. 15(1): p. 90.
- Hafren, A., et al., Turnip Mosaic Virus Counteracts Selective Autophagy of the Viral Silencing Suppressor HC-Pro. *Plant Physiol.* 2018. 176(1): p. 649-662.

