Appendix Table of Content

Appendix Figure S1. (related to Figure EV2E) Additional replicates of minigenome (MG) activity comparing the effects of expressing GFP versus GFP-RBBP6 peptide derived from human and bat RBBP6 proteins. (A, B) HEK293T cells were transfected with plasmids expressing components of the MG assay along with GFP-fused RBBP6 peptides or GFP alone. Reporter activity was read at 48h post-transfection and fold MG activity was calculated relative to a no VP30 control. # denotes statistical significance compared to RBBP6 peptide for each dose. The data represent the mean ± S.D. from one independent experiment in which each transfection condition was performed in triplicate (n=3). Statistical significance was calculated relative to the GFP control for each concentration tested. ****p<0.00005; ***p<0.0005, **p<0.005, *p<0.05

Appendix Figure S2. (related to Figure 5D) Additional replicates testing the effects on MG activity of expressing GFP-RBBP6 mutant peptides. (A, B) MG assays were performed in which plasmids encoding WT or mutant GFP-RBBP6 peptides were titrated. The data represent the mean ± S.D. from one independent experiment in which each transfection condition was performed in triplicate (n=3). Statistical significance was calculated relative to GFP control for each concentration tested. ****p<0.00005; ***p<0.0005, **p<0.005, *p<0.05

Appendix Figure S3. (related to Figure 6A) Representative immunoblots from the MG assay presented in Figure 6A and additional replicates of the same assay upon titration of the indicated host proteins.

(A) Immunoblots detecting the levels of over-expressed host proteins, VP30, VP35, NP and β-tubulin in representative cell lysates of from the MG assay shown in Fig 6A.

(B, C) HEK293T cells were transfected with plasmids encoding indicated host factors along with the plasmids of the MG assay. Empty expression plasmid (vector) was used as a control. Reporter activity was measured 48h post-transfection and fold MG activity was calculated relative to a no VP30 control. The data represent the mean ± S.D. from one independent experiment in which each transfection condition was performed in triplicate (n=3). ****p<0.00005; ***p<0.0005, **p<0.005, *p<0.05

Appendix Figure S4. (related to Figure 6B) Representative immunoblots from the MG assay presented in Figure 6B and additional replicates of the same assay upon expression of GFP-fused PPxPxY-containing peptides derived from the indicated host proteins.
(A) Representative immunoblots of cell lysates to detect expression of the GFP-peptide fusions, VP30 and β-tubulin. The peptides fused to GFP were derived from the indicated host proteins.
(B, C) MG activity was assessed upon over-expression of the indicated GFP-fused PPxPxY-containing peptides or GFP alone. The peptides fused to GFP were derived from the indicated host proteins. The data represent the mean ± S.D. from one independent experiment in which each transfection condition was performed in triplicate (n=3). Statistical significance was calculated relative to GFP control for each concentration tested. ****p<0.00005; ***p<0.0005, **p<0.005, *p<0.05

Appendix Figure S5. (related to Figure EV6A) Additional independent replicate of MG activity upon over-expression of each of the host proteins using WT or replication deficient MG systems.
A MG assay was performed with titration of the indicated host proteins. The data represent the mean ± S.D. from one independent experiment in which each transfection condition was performed in triplicate (n=3). Statistical significance was calculated relative to the vector control. ****p<0.00005; ***p<0.0005, **p<0.005, *p<0.05

Appendix Figure S6. (related to Figure EV6B) Additional independent replicate of a MG assay in which the effects of titrating the indicated host proteins was measured using a VP30-independent 5' UTR mutant minigenome.
MG activity was assessed upon expression of the host proteins. The data represent the mean ± S.D. from one independent experiment in which each transfection condition was performed in triplicate (n=3). ****p<0.00005; ***p<0.0005, **p<0.005, *p<0.05

Appendix Figure S7. (related to Figure EV6C) Additional replicate of a MG assay comparing the effects of over-expressing GFP, hnRNPUL1 peptide 3 fused to GFP and full-length hnRNPUL1.
A MG assay was performed with over-expression of GFP, or hnRNPUL1 peptide 3 fused to GFP of full-length (FL) hnRNPUL1. The data represent the mean ± S.D. from one independent

experiment in which each transfection condition was performed in triplicate (n=3). Statistical significance was calculated relative to the GFP control for each concentration tested. ****p<0.00005; ***p<0.0005, **p<0.005, *p<0.05

Appendix Figure S8. (related to Figure 6C) Representative immunoblots from the MG assay presented in Figure 6C and additional replicates of the same assay upon knockdown of the indicated host genes.

(A) Western blots to assess the protein levels of host proteins, VP30, VP35, NP and β-tubulin upon transfection of siRNAs to the indicated proteins.

(B, C) HEK293T cells were transfected with siRNA targeting RBBP6, hnRNP L, hnRNPUL1 or PEG10 along with scrambled siRNA. Twenty-four hours post-transfection, cells were transfected with plasmids for the MG assay, including VP30 at 12.5 and 25ng doses. The data represent the mean ± S.D. from one independent experiment in which each transfection condition was performed in triplicate (n=3). ****p<0.00005; ***p<0.0005, **p<0.005, *p<0.05

Appendix Figure S9. (related to Figure 6D) Representative immunoblots from the MG assay presented in Figure 6D and additional replicates of the same assay upon single or double knockdown of RBBP6 and hnRNP L.

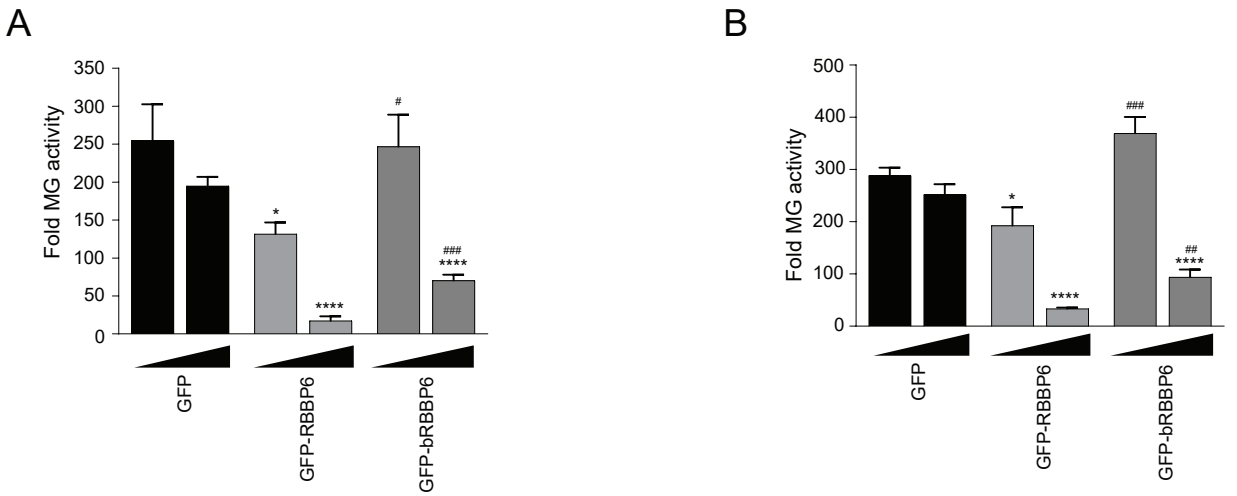
(A) Immunoblots to detect levels of host proteins and VP30 upon knockdown of the host genes.

(B, C) MG assays was performed upon single or simultaneous double knockdown of RBBP6 and hnRNP L in HEK293T cells. Twenty-four hours post siRNA-transfection, cells were transfected with plasmids for the MG assay, including VP30 at 12.5 and 25ng doses. The data represent the mean ± S.D. from one independent experiment in which each transfection condition was performed in triplicate (n=3). ****p<0.00005; ***p<0.0005, **p<0.005, *p<0.05

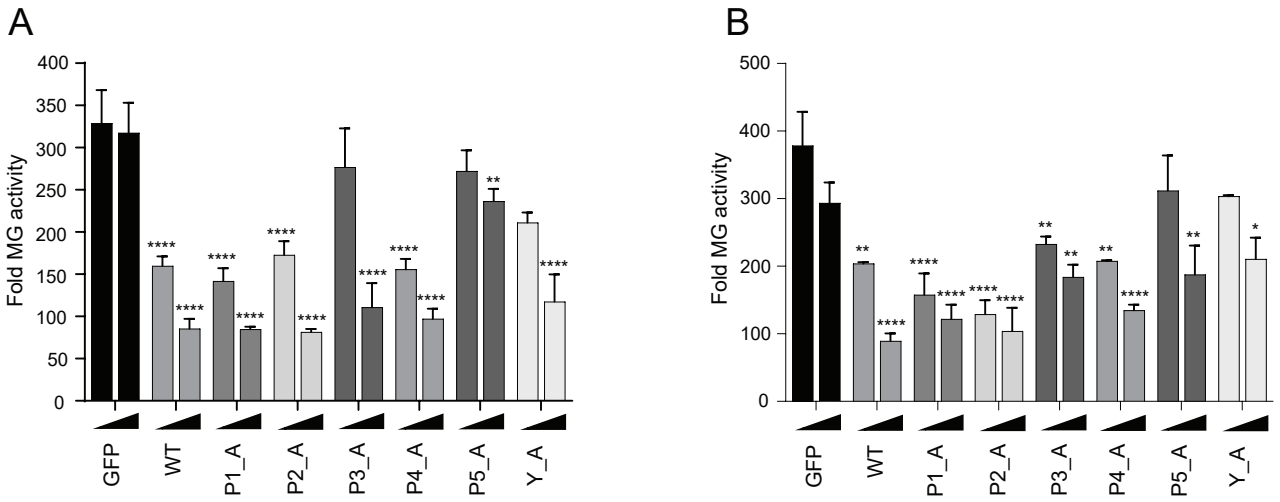
Appendix Figure S10. (related to Figure 7A) Western blots to assess knockdown efficiency in lysates from EBOV infected cells.

Western blots assessing levels of (A) hnRNP L, PEG10 and (B) hnRNPUL1 in siRNA knockdown, EBOV infection experiments. HeLa cells were transfected with scrambled siRNA (SCR si) or siRNAs targeting hnRNP L, hnRNPUL1 or PEG10. Seventy-two hours post-transfection, western blots were performed on whole cell lysates using antibodies to the indicated proteins.

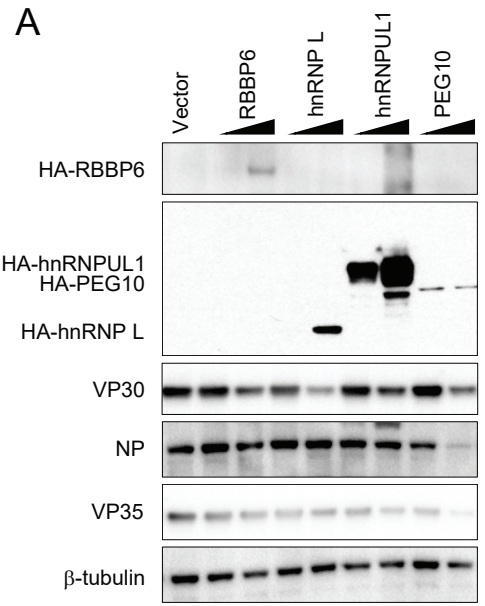
Appendix Figure S1. Additional replicates of minigenome (MG) activity comparing the effects of expressing GFP versus GFP-RBBP6 peptide derived from human and bat RBBP6 proteins.

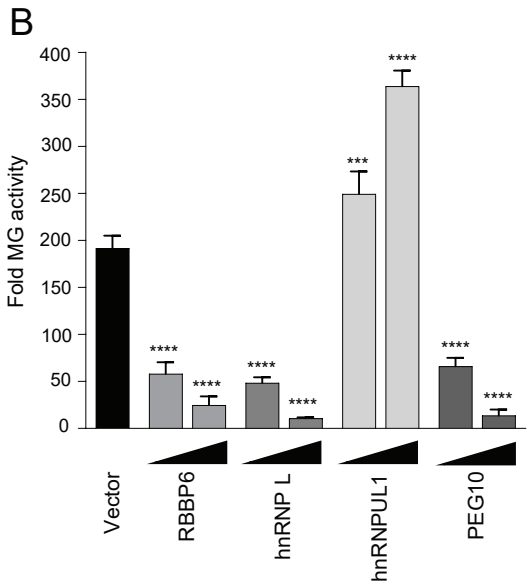


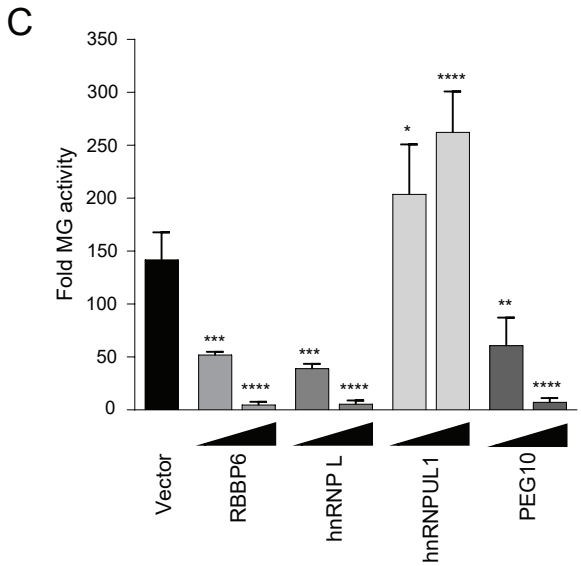
Appendix Figure S2. Additional replicates testing the effects on MG activity of expressing GFP-RBBP6 mutant peptides.



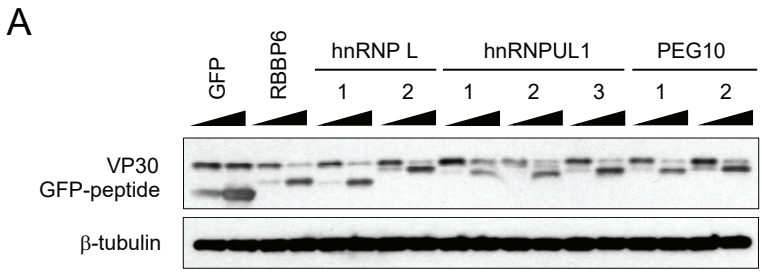
Appendix Figure S3. Representative immunoblots from the MG assay presented in Figure 6A and additional replicates of the same assay upon titration of the indicated host proteins.

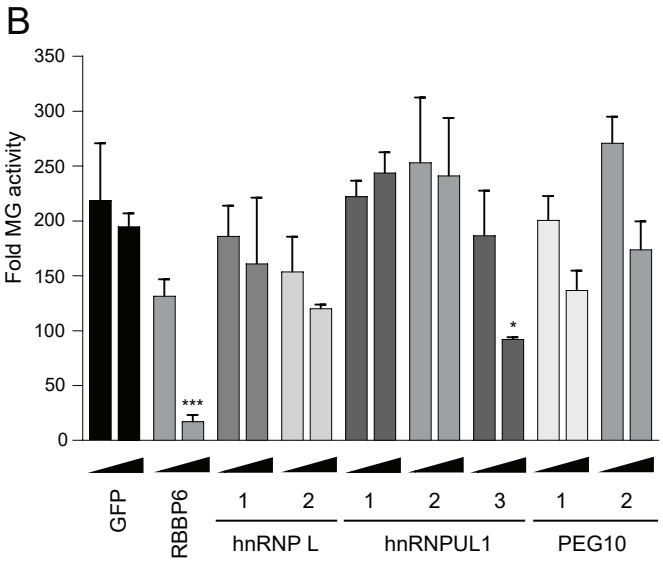


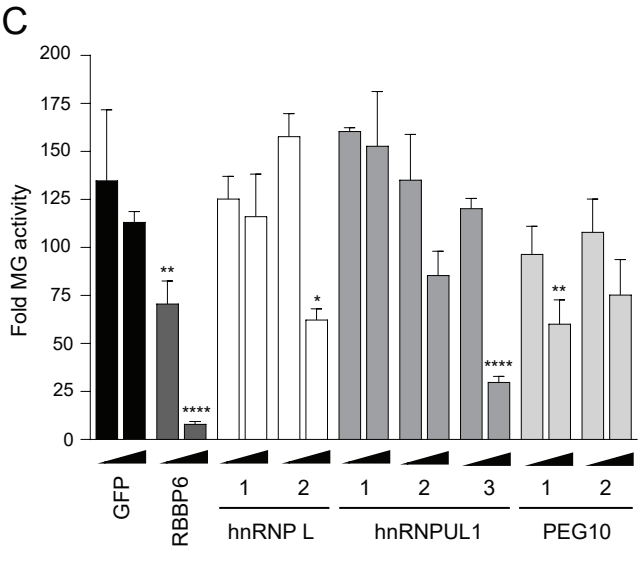




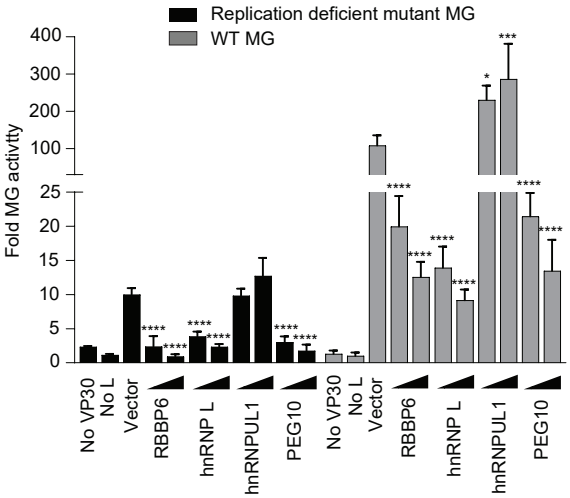
Appendix Figure S4. Representative immunoblots from the MG assay presented in Figure 6B and additional replicates of the same assay upon expression of GFP-fused PPxPxY-containing peptides derived from the indicated host proteins.



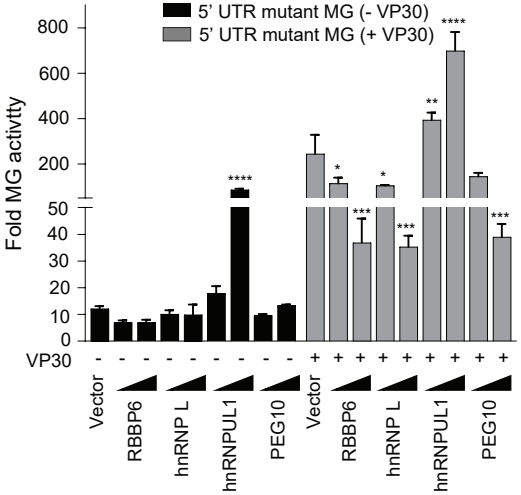




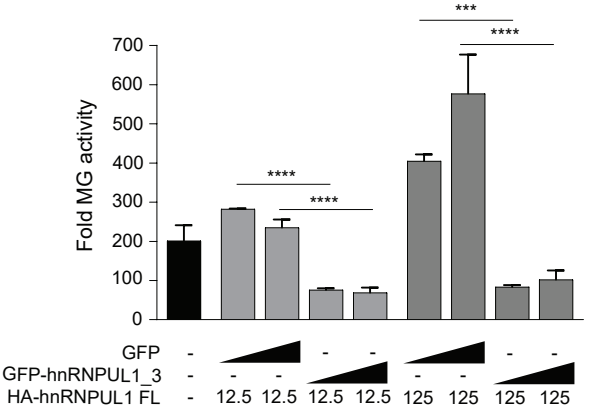
Appendix Figure S5. Additional independent replicate of MG activity upon over-expression of each of the host proteins using WT or replication deficient MG systems.



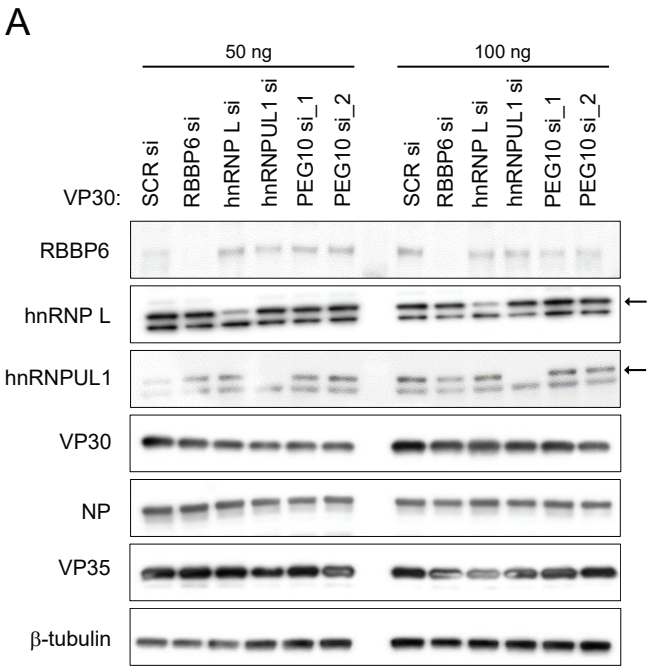
Appendix Figure S6. Additional independent replicate of a MG assay in which the effects of titrating the indicated host proteins was measured using a VP30-independent 5' UTR mutant minigenome.

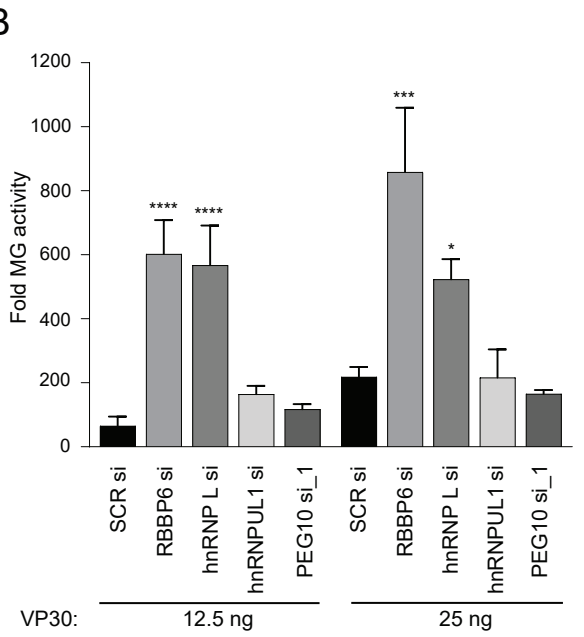


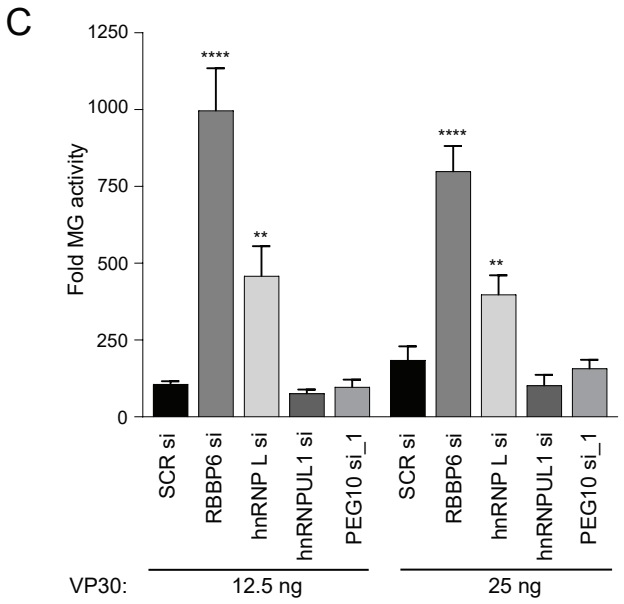
Appendix Figure S7. Additional replicate of a MG assay comparing the effects of over-expressing GFP, hnRNPUL1 peptide 3 fused to GFP and full-length hnRNPUL1.



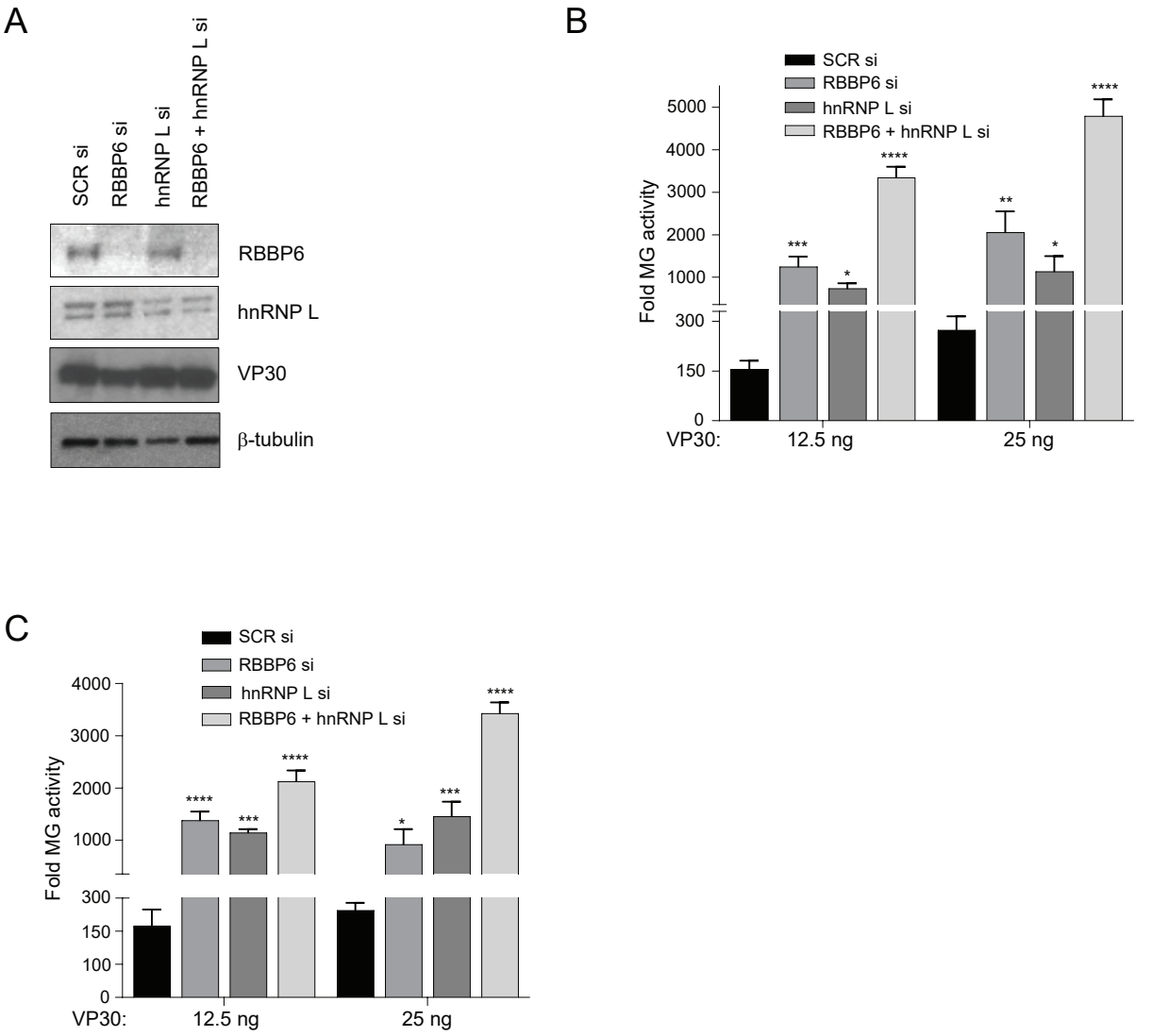
Appendix Figure S8. Representative immunoblots from the MG assay presented in Figure 6C and additional replicates of the same assay upon knockdown of the indicated host genes.







Appendix Figure S9. Representative immunoblots from the MG assay presented in Figure 6D and additional replicates of the same assay upon single or double knockdown of RBBP6 and hnRNP L.



Appendix Figure S10: Western blots to assess knockdown efficiency in lysates from EBOV infected cells.