

Supplementary data

Interaction of Bunyamwera Virus Non-Structural Protein NSm with Cellular BNIP1 is Required for Efficient Viral Gene Expression and Replication

Rosalind F. Wartnaby,^a Juan Fontana,^b John N. Barr^{a*}

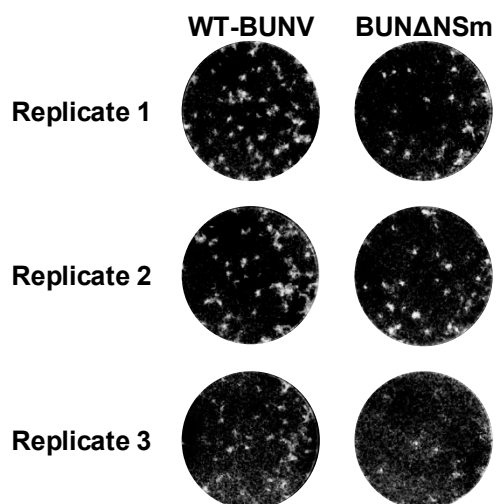
^aSchool of Molecular and Cellular Biology, Faculty of Biological Sciences and Astbury Centre for Structural and Molecular Biology, University of Leeds, UK

^bInstituto Biofisika, CSIC-UPV/EHU, Leioa, Spain

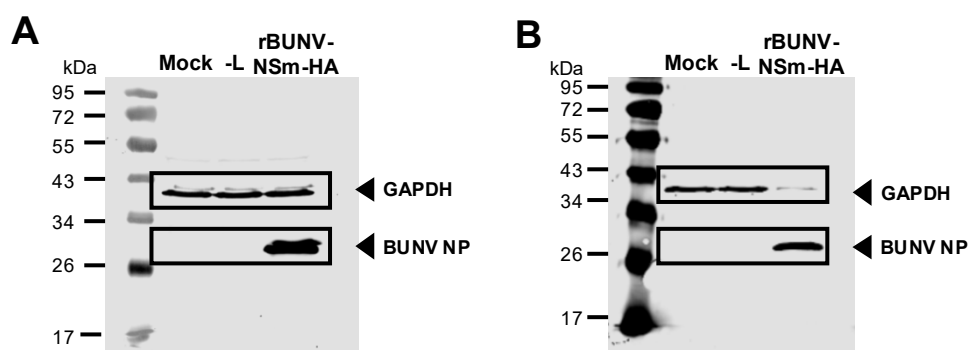
Running Title: NSm-BNIP1 interaction in Bunyamwera virus replication

*Corresponding author

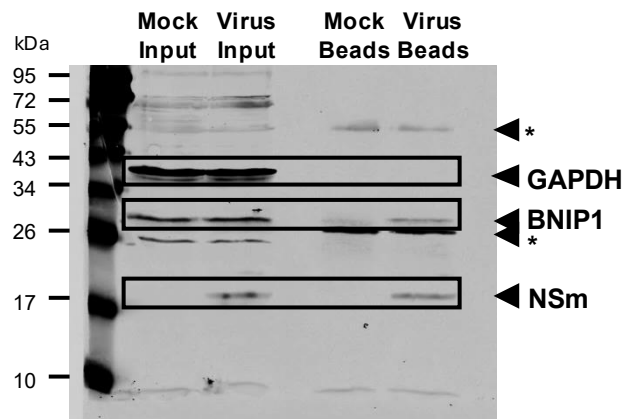
Email address for correspondence: J.N.Barr@leeds.ac.uk



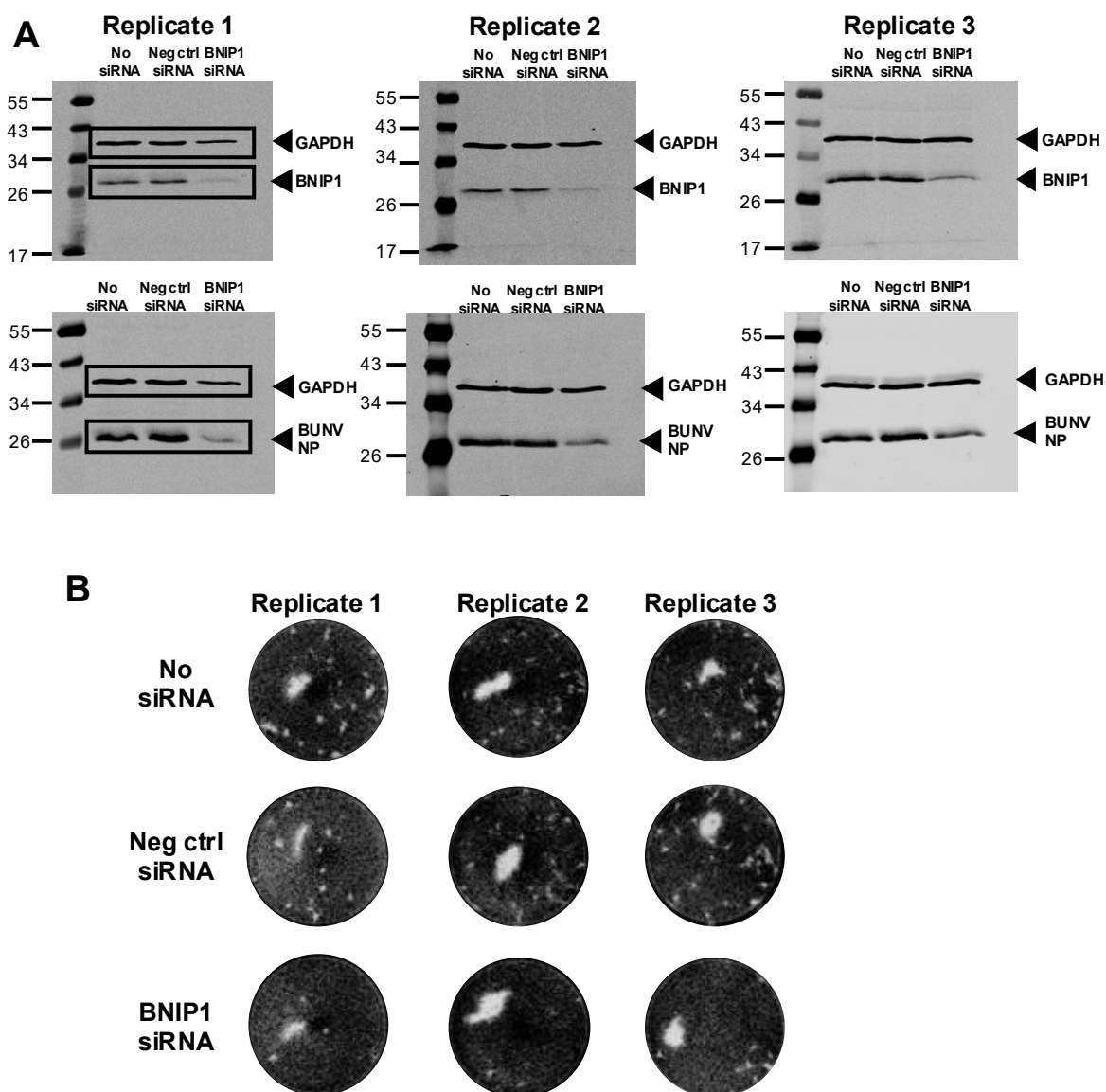
Supplementary Fig S1. Representative images of plaque assay wells infected with virus dilutions of 10^{-4} , corresponding to the graph comparing peak virus yields of rWT-BUNV and rBUNV Δ NSm in Fig. 1B.



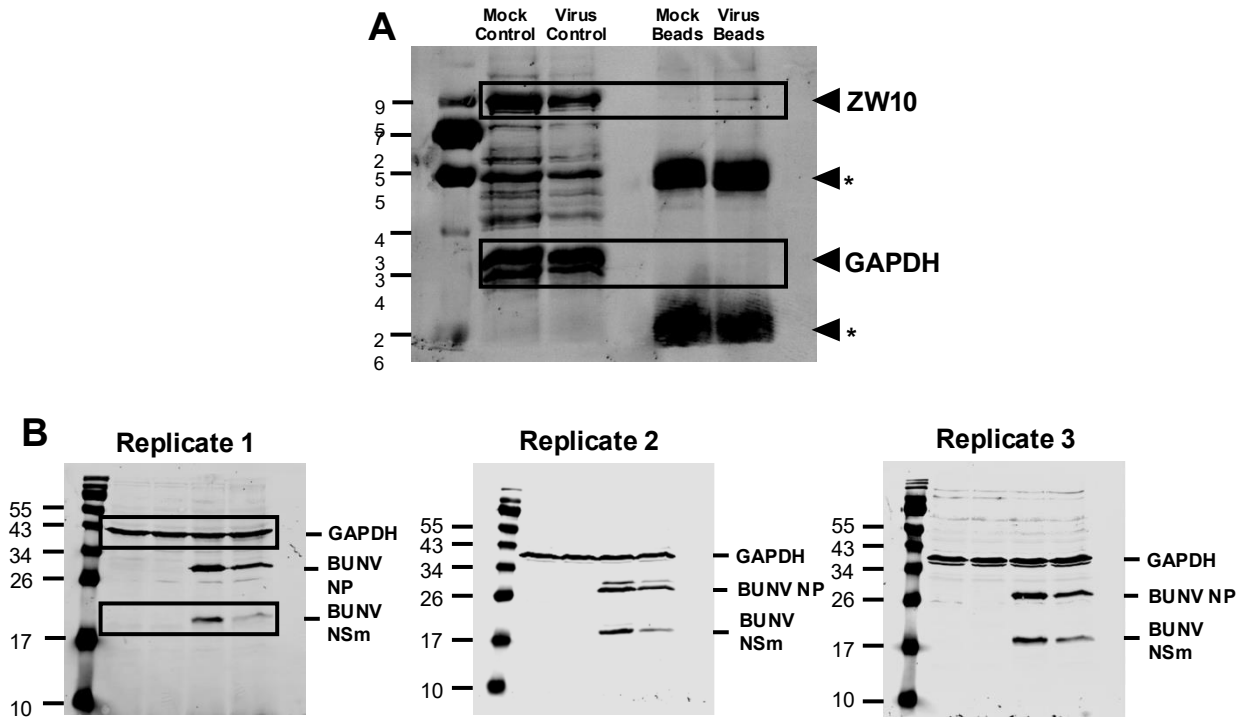
Supplementary Fig S2. Uncropped western blots of those displayed in Fig. 2C, with regions shown in the main figure indicated by boxed areas.



Supplementary Fig S4. Uncropped western blot of that displayed in Fig. 5A, with regions shown in the main figure indicated by boxed areas. Asterisks indicate non-specific bands corresponding to antibody heavy and light chains (~50 kDa) and (~25 kDa) from the antibody used for immunoprecipitation.



Supplementary Fig S5. Effects of siRNA-mediated knockdown of host cell BNIP1 on BUNV gene expression and replication. **(A)** Uncropped western blots of those displayed in Fig. 6A and B, with regions shown in the main figure indicated by boxed areas, alongside uncropped western blots corresponding to the other two biological replicates. **(B)** Representative images of plaque assay wells infected with virus dilutions of 10^{-5} for replicate 1, and 10^{-4} for replicates 2 and 3, corresponding to the graph comparing viral titers in Fig. 6E.



Supplementary Fig S6. Analysis of the NSm-ZW10 interaction and the effect of BFA treatment on NSm protein expression during BUNV infection in mammalian cells. **(A)** Uncropped western blot of that displayed in Fig. 7A, with regions shown in the main figure indicated by boxed areas. Asterisks indicate non-specific bands corresponding to antibody heavy and light chains (~50 kDa) and (~25 kDa) from the antibody used for immunoprecipitation. **(B)** Uncropped western blot of that displayed in Fig. 7C, with regions shown in the main figure indicated by boxed areas, alongside uncropped western blots corresponding to the other two biological replicates.

Gene	Peak Area Rank	Peak Area	# PSMs	# Unique Peptides
ALG1	1	830014060.875	21	11
BNIP1	2	815843529.864583	28	14
CANX	3	802892685.208333	22	15
SRSF1	4	688937643	2	1
TMEM109	5	684565750	1	1
NPC1	6	325738097.1875	1	1
SCAMP2	7	309151510.25	3	2
POMK	8	251739886.6875	8	8
TM9SF1	9	232744882.458333	5	4
TM9SF2	10	227749698.875	17	7
ENSG00000286268	11	205004681.5	2	2
SSR3	12	187973128.5	3	1
SETDB1	13	173429791	1	1
PHB1	14	170238238.125	7	6
RNF121	15	145350561.3125	3	2
TUBB2B	16	130429300	183	1
SLC35B2	17	117355633	1	1
STT3A	18	113528322.625	5	3
CLCA2	19	110619594	1	1
TNPO1	20	102767660.791667	5	5

Supplementary table S1. Details of the top 20 gene hits by peak area alongside their peptide-spectrum match (PSM) and unique peptide counts from LC-MS/MS analysis of a co-immunoprecipitation, identifying host cell proteins that may interact with NSm in rBUNV-NSm-HA infected A549 cells at 24 hpi at an MOI of 0.5. Before ranking, hits were first filtered by the removal of genes with a peak area of above zero in the non-infected mock samples, as well as those identified as likely laboratory contaminants such as human keratins.