

Appendix

A functional assay for SARS-CoV-2 N-antibodies

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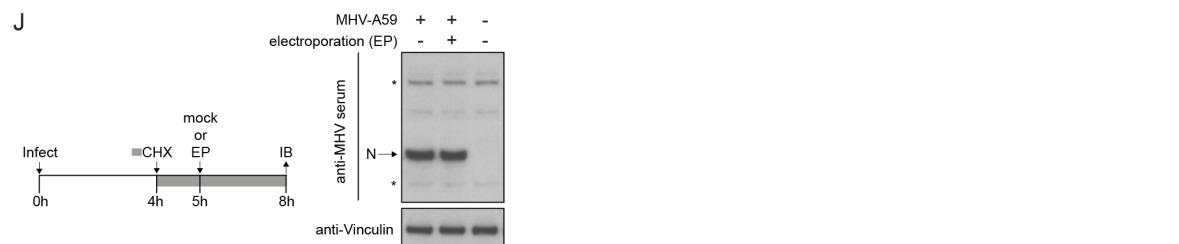
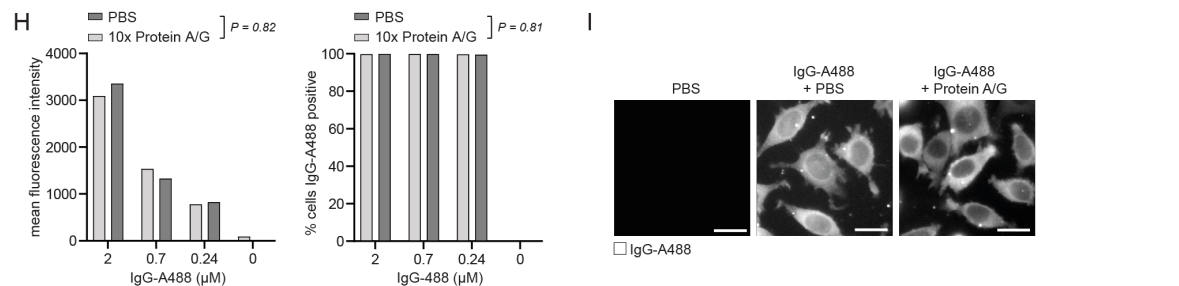
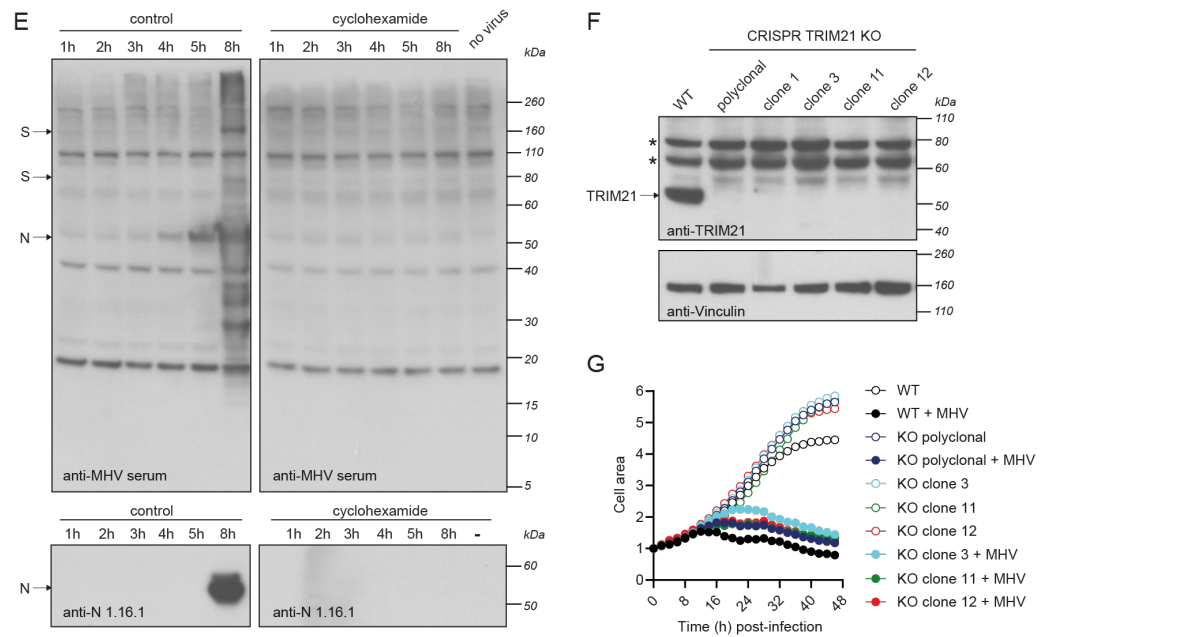
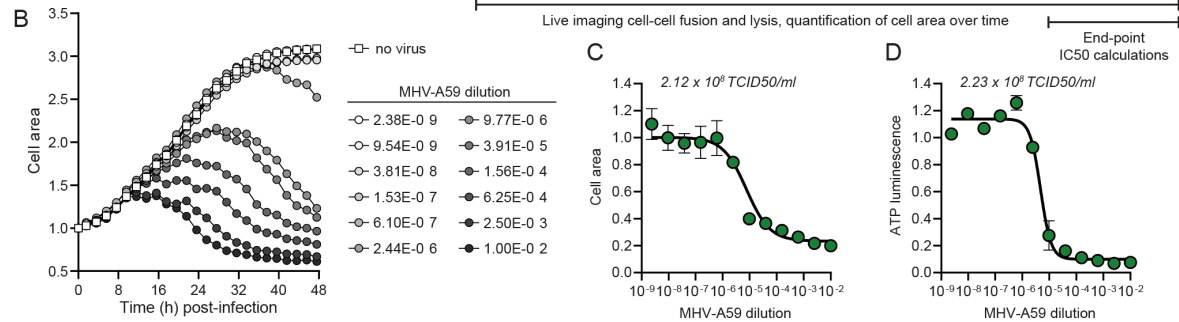
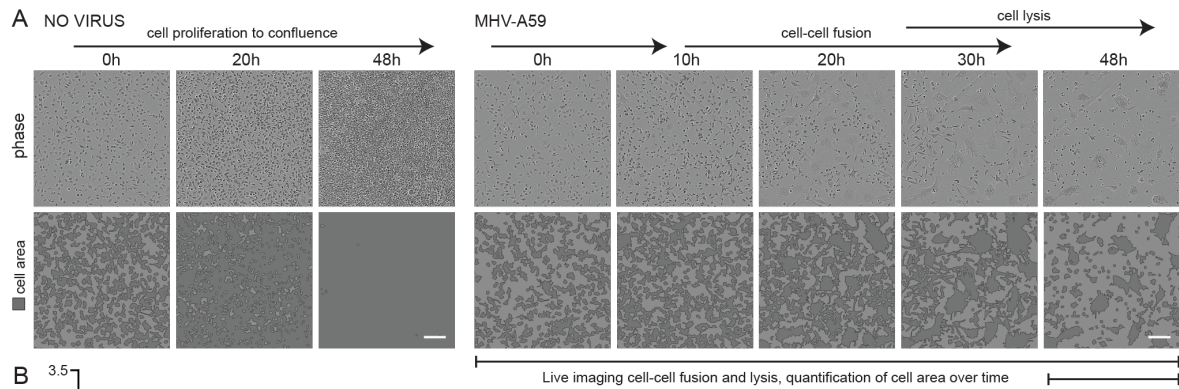
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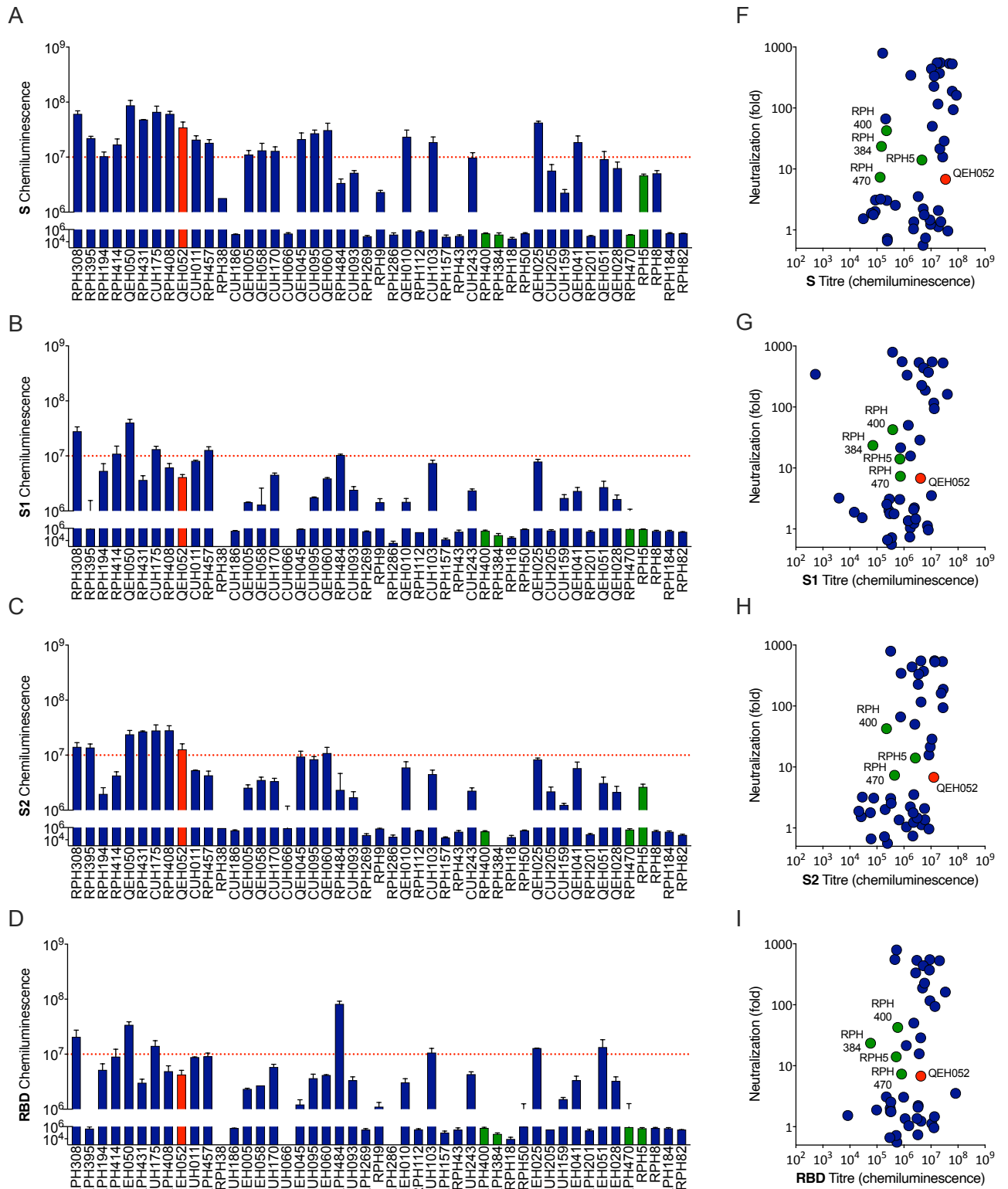
Appendix Figure S23

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Appendix Figure S47

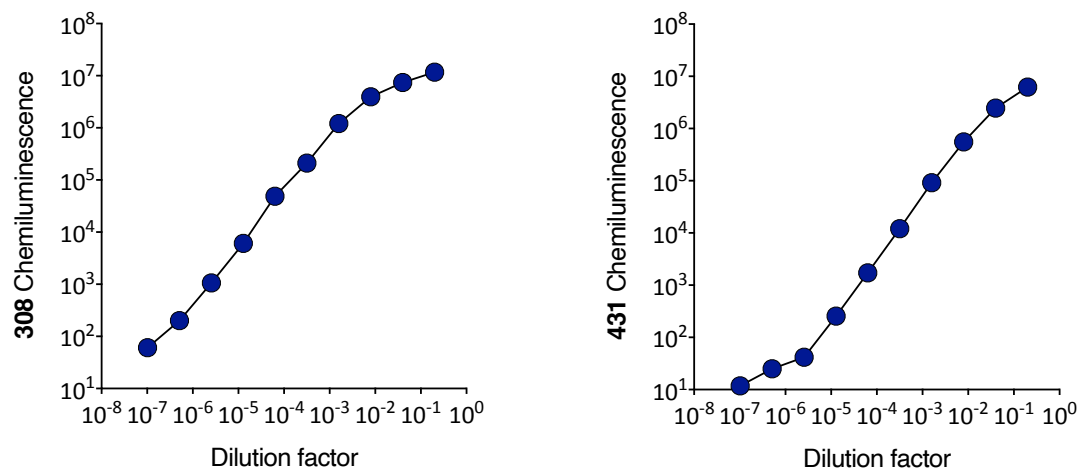


Appendix Figure S1: Kinetics of MHV-A59 infection can be determined using live cell microscopy. (A) Time-course of L929 cells either mock infected or infected with MHV-A59, showing either phase contrast (upper row) and fitted cell area (lower row, darker grey). Scale bar = 200µm. (B) Quantification of cell area at different timepoints post-infection with a titration of viral doses. (C) Cell area at 48h post-infection at a range of MHV-A59 doses, fitted with a non-linear regression curve to calculate a TCID50 value. (D) Same as (C), except using ATP luminescence instead of cell area. (E) Western blot at different timepoints post-infection with MHV-A59 using anti-MHV polyclonal antisera or anti-N monoclonal antibody 1.16.1. Note the increase in viral proteins, particularly S and N, by 8h post-infection. Treatment with cyclohexamide to prevent protein expression demonstrates that this increase is the result of de novo protein synthesis. (F) Western-blot of L929 cells that have undergone CRISPR-Cas9 for TRIM21 knockout. Both polyclonal cells and clones show loss of TRIM21 protein. The presence of non-specific bands detected by the anti-TRIM21 antibody is indicated by an asterisk. (G) Growth of WT and TRIM21 KO cells in the presence (filled circles) or absence (empty circles) of MHV-A59. (H) L929 cells were electroporated with different concentrations of fluorescent antibody (IgG-A488) in the absence (PBS) or presence of 10x antibody concentration of Protein A/G and analysed 1h later by flow cytometry. (I) Microscopy images of cells from (H) at 6h post-electroporation. Scale bar = 20µm. Protein A/G does not interfere with cytosolic delivery of antibody. Data was analysed using a variable slope nonlinear fit (C&D) or a Students *t*-test (H). Error bars depict the mean +/- SEM. All data represents at least two independent replicates. (J) L929 Cells were infected with MHV-A59 for 4h, then cyclohexamide (CHX) was added to block further viral protein synthesis. After 5h, cells were electroporated with buffer and left for a further 3h before being western blotted for cellular N protein levels (*denotes a non-specific band).

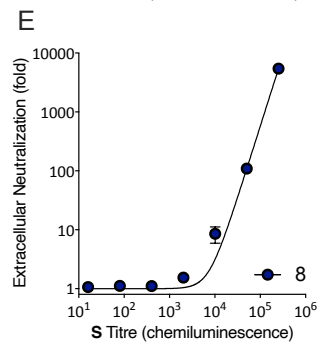
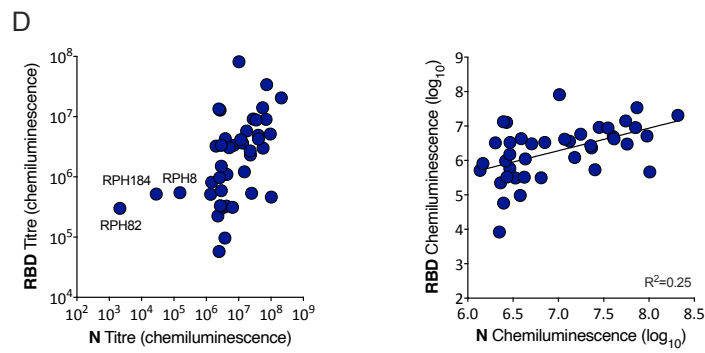
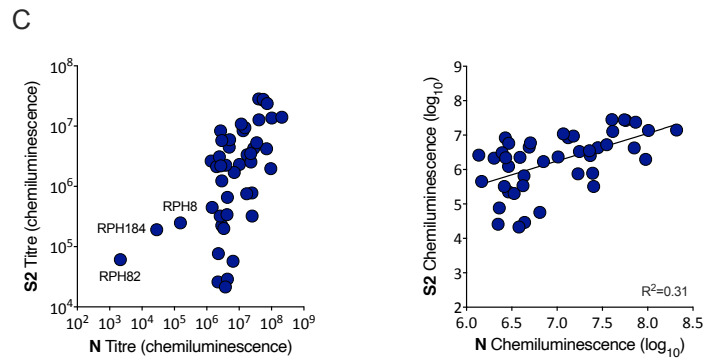
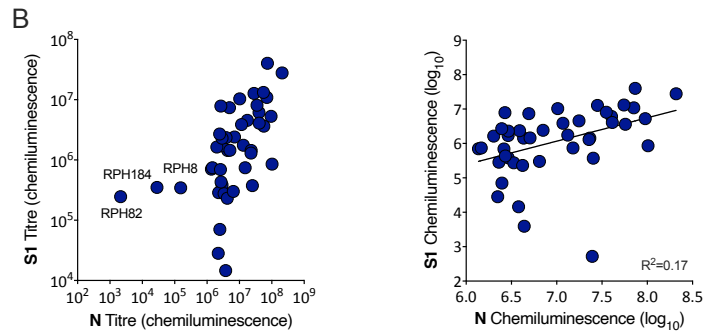
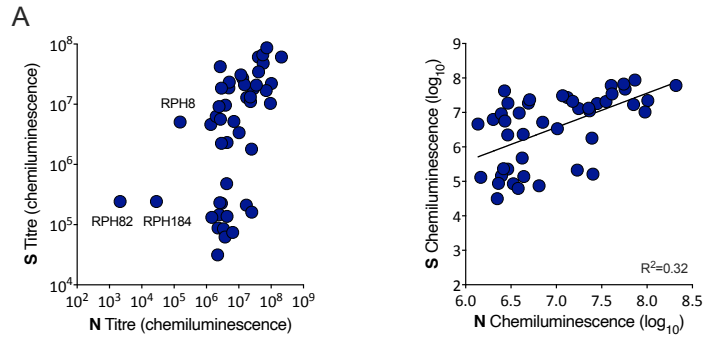


Appendix Figure S2: Specific antibody levels in SARS-CoV-2 convalescent sera and correlation with intracellular neutralization activity. (A-D) Capillary-based protein

detection (Jess) of antibodies against SARS-CoV-2 antigens in convalescent sera, quantified by chemiluminescence. **(F-I)** Correlation between intracellular neutralization (fold decrease in SARS-CoV-2 replication), as quantified by EDNA, and antibody titre, as quantified by chemiluminescence by Jess. Error bars depict the mean $\pm$ SEM. All data represents at least three independent replicates.



Appendix Figure S3: Quantification of anti-N chemiluminescent signal in a titration of serum from two SARS-CoV-2 convalescents. Jess is able to detect the presence of N-antibodies in serum that is diluted > million-fold.



Appendix Figure S4: Correlation between N-antibody levels and antibodies against other SARS-CoV-2 antigens in convalescent sera. (A-D) Levels of antibodies against different antigens in individual sera, as quantified by chemiluminescence. In each case, levels are plotted against the N-antibody titre. Both raw and log10-transformed data are shown, with the R^2 correlation coefficient calculated in each case in the absence of outliers RPH8, RPH82 and RPH184. **(E)** Extracellular neutralization (ie no electroporation) of a titration of strongly S positive but weakly N positive serum. Data was analysed using a linear regression.