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Supplemental Information

Antagonism of the Interferon-Induced OAS-RNase L Pathway by Murine Coronavirus ns2 Protein Is Required for Virus Replication and Liver Pathology

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Virus; For Figure S1
Recombinant murine coronavirus ns2-H46A (previously called inf-ns2-H46A) was obtained from Dr. Stuart Siddell (University of Bristol) and has been described previously (Roth-Cross et al., 2009; Schwarz et al., 1990). Recombinant ns2-L94P (previously called VUSS1) was obtained from Dr. Mark Denison (Vanderbilt University) and has been described previously (Sperry et al., 2005).

Transfection of HEK 293T cells; to supplement Experimental Procedures section RNase L-mediated ribosomal RNA (rRNA) cleavage in intact cells
HEK 293T monolayer cells at about 80 % confluence were transfected with 0.8 μg of pCAGGS (pC), pC-ns2 or pC-ns2-H126R using Lipofectamine 2000 (Invitrogen). At 24h post transfection, cells were fixed in 4% paraformaldehyde for immunofluorescent staining, described below. For rRNA cleavage or 2-5A quantification, cells were treated with 1000 U/ml universal type I IFN (PBL) for another 24 h and then transfected with 10 μg/ml poly (I:C) (EMD Biosciences). Four hours later, cells were harvested and used either for RNA isolation or quantification of 2-5A, as described in the Experimental Procedures of the main part of the manuscript.

Immunofluorescent staining; Figure S2
BMM or 293T cells were fixed in 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. Cells were stained with monoclonal antibodies directed against the MHV N protein [MAb 1.16.1; kindly provided by Dr. Julian Leibowitz] or the MHV ns2 protein (obtained from Dr. Stuart Siddell, University of Bristol, UK) at a dilution of 1:200, followed by a AlexaFluor 488 goat anti-mouse antibody (dilution 1:400) combined with Hoechst or DAPI (dilution 1:100) (Molecular Probes, Eugene, OR) as indicated in the figure legends. Samples were examined under a Nikon Eclipse fluorescence microscope (Nikon Inc., Melville, NY).

Expression and Purification of ns2, ns2-H126R and mu-2'PDE Recombinant Proteins; to supplement Experimental Procedures section 2'-PDE activity assay
pMal-ns2 and pMal-ns2-H126R were amplified in E. coli strain TB1 and the recombinant proteins with maltose binding protein (MBP) tags were purified by binding to an amylose resin (NEB, Inc.). Following cleavage of the MBP tag with thrombin, ns2 protein was subjected to another round of amylose resin purification followed by ion exchange chromatography using monoQ GL100 column (GE LifeSciences, Inc.) to remove the MBP tag and the proteases. Peak fractions were pooled and concentrated using centiprep MWCO 15 kDa centrifugal filter units. The pET-SUMO clone containing the full-length His_6-SUMO-tagged mu-2'PDE ORF was amplified in E. coli BL21DE3pLysS and purified on a HisPrep FF16/60 column (GE Life Sciences). The eluted fractions were further purified on a Sephacryl S 200 HR 16x100 size

exclusion chromatography column and concentrated using centiprep MWCO 10 kDa centrifugal filter units (Millipore Inc., Billerica, MA). The SUMO tag was removed using SUMO protease (Invitrogen Inc) according to manufacturer's protocol.

SUPPLEMENTAL REFERENCES

Wang, W.B., Levy, D.E., and Lee, C.K. (2011). STAT3 negatively regulates type I IFN-mediated antiviral response. J Immunol *187*, 2578-2585.

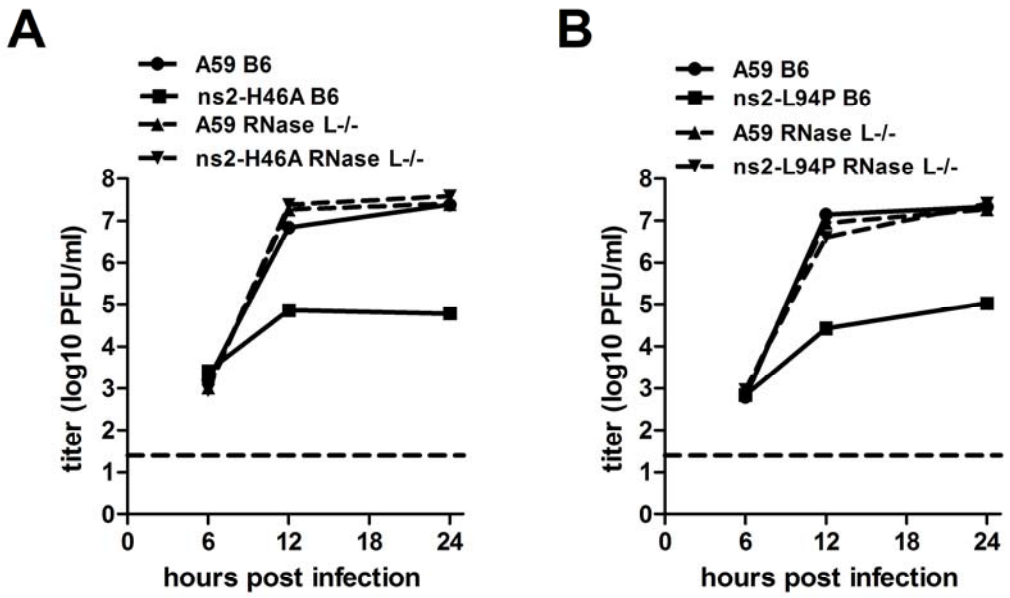

Figure S1. The MHV ns2 Mutants, ns2-H46A and ns2-L94P, Replicate to a Minimal Extent in BMM from B6 mice, but Recover wt Replication in BMM Derived from RNase $L^{-/-}$ Mice, Related to Figure 3

BMM, derived from B6 or RNase $L^{-/-}$ mice were infected at an MOI of 0.01 PFU/cell. At the indicated times post infection, titers of viruses in the cell lysates combined with supernatants were determined by plaque assay on L2 cells (n=3). Error bars represent s.e. of means.

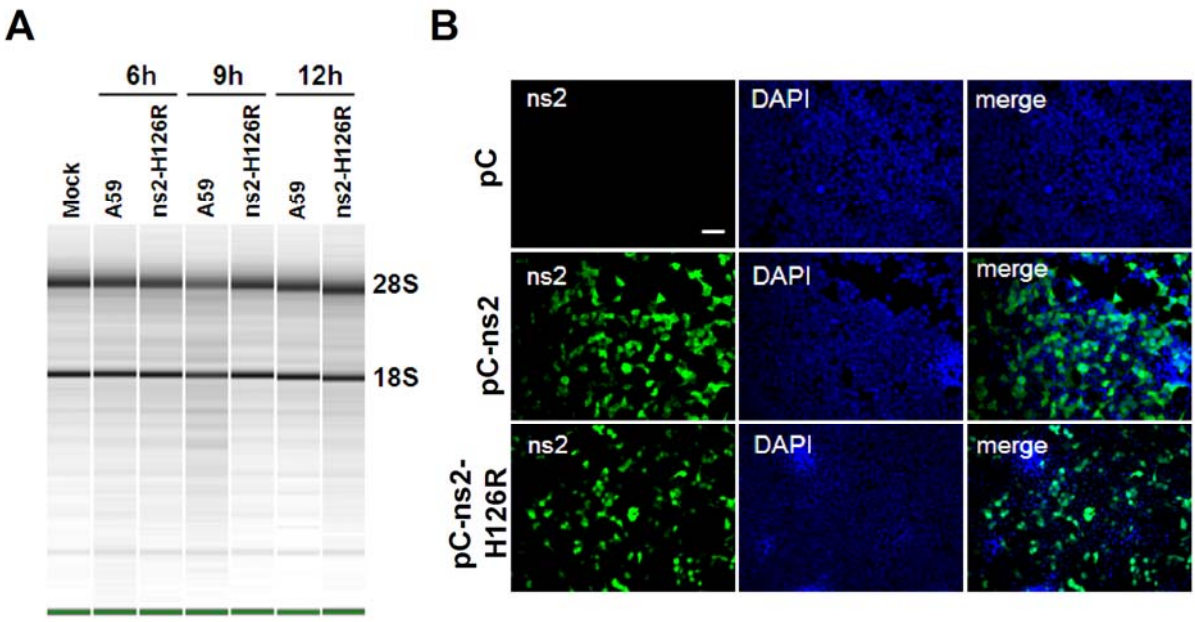

Figure S2. Analysis of rRNA in infected BMM derived from RNase L-/- mice; Expression of wt ns2 and Mutant ns2-H126R Proteins in HEK 293T Cells, Related to Figure 4

(A) BMM derived from RNase L-/- mice were infected (MOI=1 PFU/cell). At the indicated times, RNA was purified and analyzed on a Bioanalyzer. The positions of 18S and 28S rRNA are indicated. **(B)** HEK 293T cells were transfected with 0.8 µg of pCAGGS, pC-ns2 or pC-ns2-H126R and after 24 hours stained with anti-ns2 monoclonal antibody followed by a secondary antibody conjugated with AlexaFluor-488. The scale bar represents 100 µm.

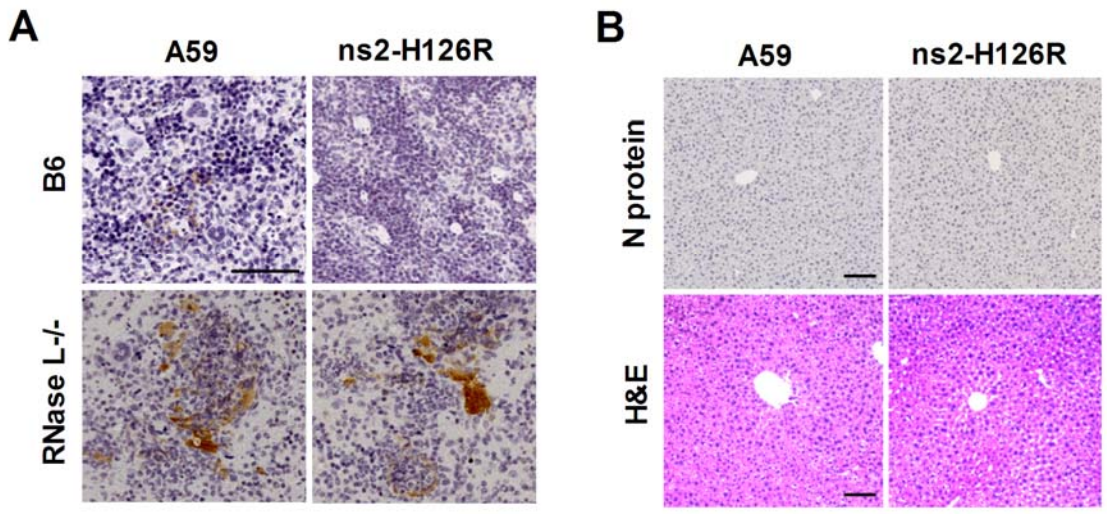

Figure S3. Virus Replication and pathology in Spleens and Livers of B6 and RNase L-/- Mice, Related to Figure 6

(A) Spleen sections from mice sacrificed at 5 d.p.i. were stained with anti-MHV N monoclonal antibody to detect viral antigen expression. Sections are representative of 2 sections from each of 3 animals from each group. The scale bar represents 100 μm. **(B)** Liver sections from RNase L -/- mice sacrificed at 30 d.p.i. were stained with anti-MHV N monoclonal antibody to detect viral antigen expression or H&E to evaluate the pathology. Sections are representative of 2 sections from each of 3 animals from each group. The scale bars represent 100 μm.

Table S1. Primers used in qRT-PCR, Related to Figures 2 and 7

Genes	Sequences
MHV nucleocapsid	F 5'-GGCGTCCGTACGTACCC-3' R 5'- GGTCAGCCCAAGTGGTC-3'
EMCV 2A-2B region	F 5'-AATGCCCACTACGCTGGT-3' R 5'-GTCGTTCGGCAGTAGGGT-3'
SeV nucleocapsid	F 5'-TGCCCTGGAAGATGAGTTAG-3' R 5'-GCCTGTTGGTTTGTGGTAAG-3'
TNF-α	F 5'-TCACTGGAGCCTCGAATGTC-3' R 5'-GTGAGGAAGGCTGTGCATTG-3'
IFN-γ	F 5'-GACTGTGATTGCGGGGTTGT-3' R 5'-GGCCCGGAGTGTAGACATCT-3'

EMCV primers are taken from (Wang et al., 2011). SeV primers were obtained from Dr. Carolina Lopez.