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Supplemental information

RIG-I-induced innate antiviral immunity protects mice from lethal SARS-CoV-2 infection

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Supplementary Methods

Viral gene expression analysis of oropharyngeal swab material

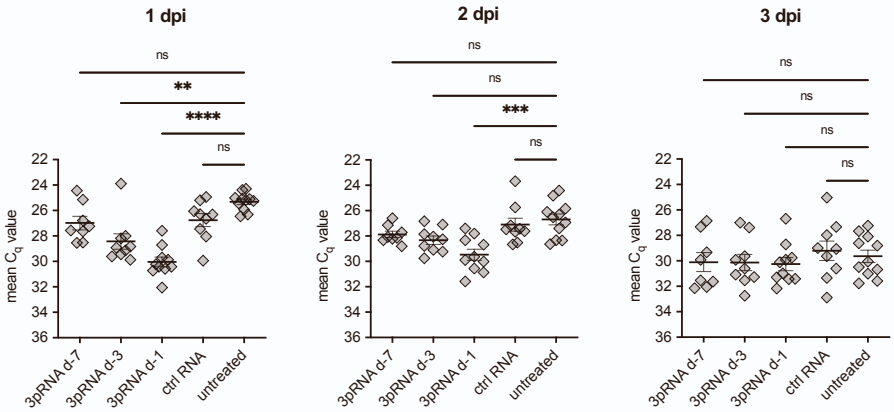
Viral RNA from oropharyngeal swab material was purified using the NucleoSpin RNA Virus kit (Macherey & Nagel) according to the manufacturer's instructions and used as a template for cDNA synthesis with RevertAid reverse transcriptase (ThermoFisherScientific, Waltham, MA). The resulting cDNA was used for amplification of selected genes by qPCR using EvaGreen QPCR-mix II (ROX) (Biobudget, Krefeld, Germany) on a Quantstudio 5 machine (ThermoFisherScientific, Waltham, MA). SARS-CoV-2 Spike RNA expression was determined using the commercial E.Sarbeco primer sets (IDT, Leuven, Belgium, 10006888 and 10006890). Viral expression from oropharyngeal swab material was shown as mean C_q values for each mouse.

Cytokine measurement

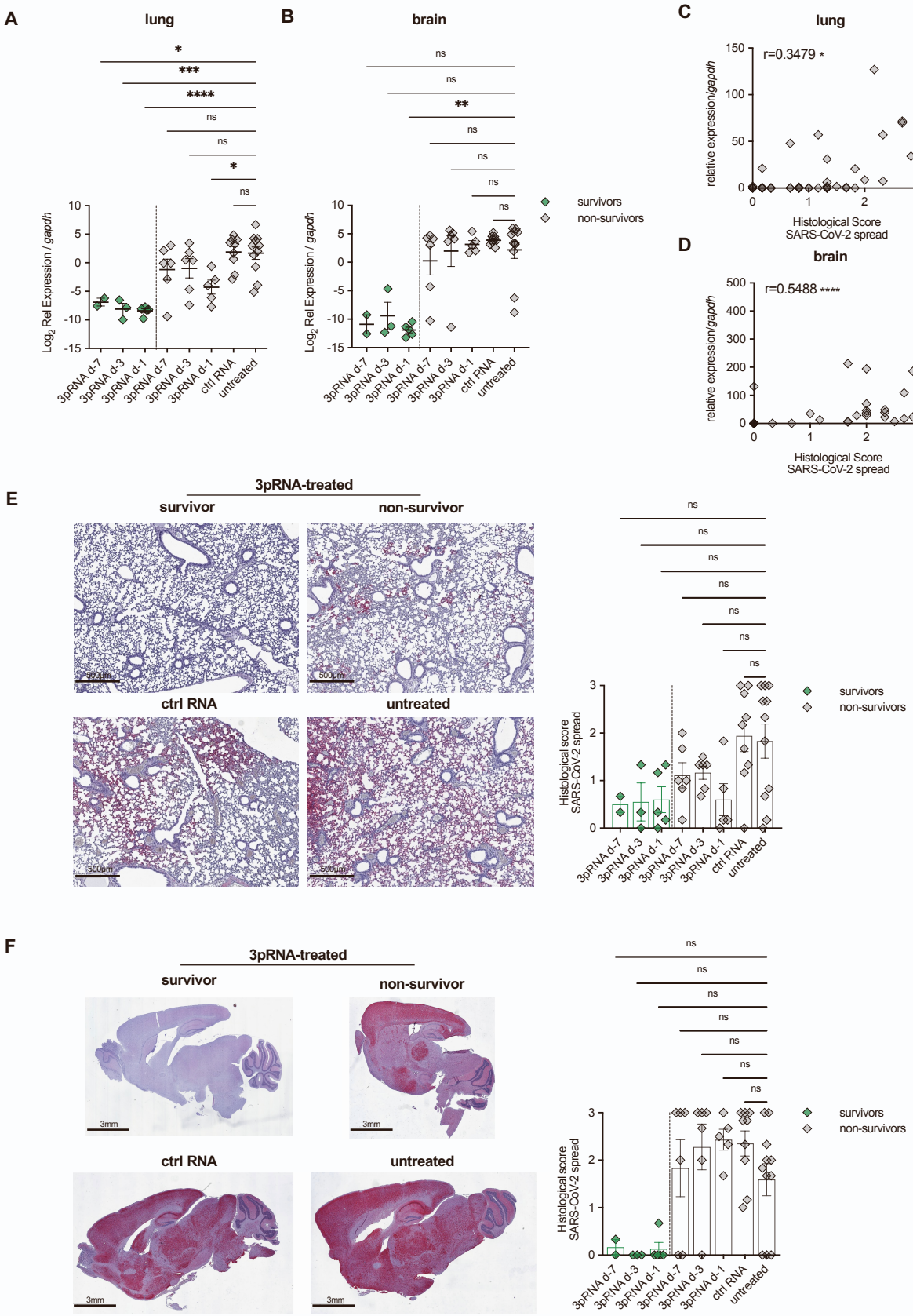
Serum cytokines were quantified with a mouse anti-virus response LEGENDplex bead assay (Biolegend) and measured on a Attune NXT flow cytometer (ThermoFisherScientific, Waltham, MA).

Histology

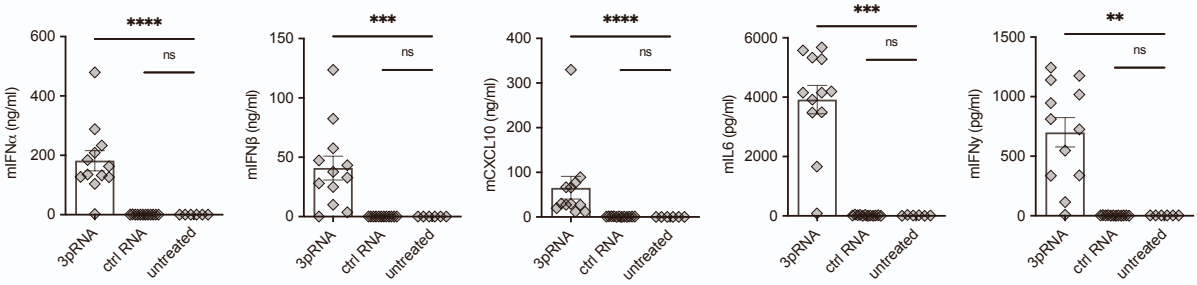
Half of the lungs and one mid-sagitally-cut half of the brain were fixed in 6% neutral buffered formalin (Carl Roth, Karlsruhe, Germany) for at least 48 hours. Tissues were embedded in paraffin. For SARS-CoV-2 antigen immunohistochemistry, slides were incubated with blocking reagent (10% normal goat serum) followed by rabbit monoclonal antibody against SARS-CoV-2 nucleocapsid protein (Biozol, Eching, Germany, SIN-40143-R019, 1:20000). The secondary antibody and the chromogen from the Dako REAL detection system (Agilent Technologies, Santa Clara, California) was used for the staining according to the manufacturer's protocol. Tissue sections were visualized using an Aperio SlideScanner CS2 and the Aperio Imagescope 12.4 software (Leica, Mannheim, Germany). Three scientists scored the sections in a blinded fashion as follows: 0 no staining; 1 weak staining, <5% brain area or <10% of lung area; 2 strong staining, 5-33% brain area or 10-50% of lung area; 3 strong staining >33% of brain area or >50% lung area.



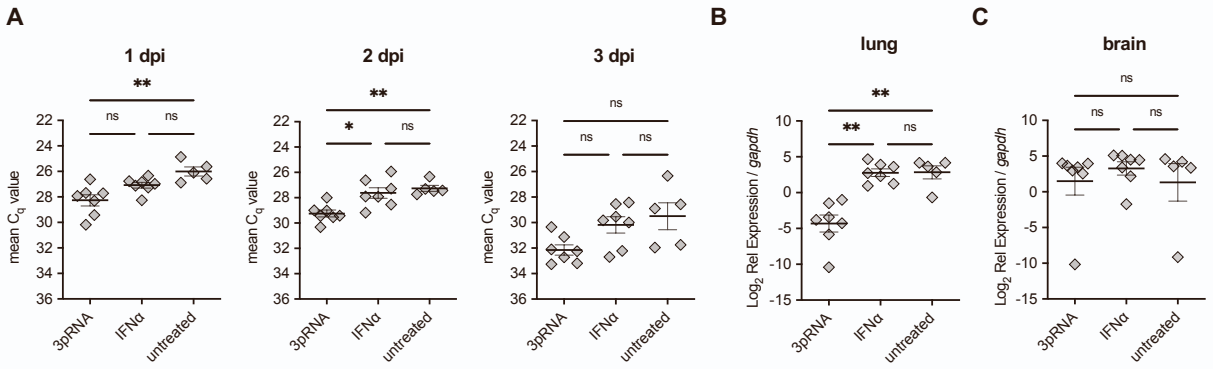
Supplementary Figure 1: **Gene expression analysis of oropharyngeal swab material upon prophylactic 3pRNA treatment.** Expression of SARS-CoV-2 viral RNA in oropharyngeal swab material on 1-3 dpi. Plotted are the mean + SEM (3pRNA d-7 n=8, 3pRNA d-3 & ctrl RNA n=9, 3pRNA d-1 n=10, untreated n=11). Data are pooled from two independent experiments. Statistical significance was calculated by non-parametric Kruskal−Wallis test with Dunn's multiple testing. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.



Supplementary Figure 2: RIG-I prophylaxis reduces viral manifestation into the lungs and brains. (A,B) SARS-CoV-2 viral burden in the lungs (B) and brains (B) quantified by qPCR relative to murine *gapdh* expression. (C,D) Pearson correlation (n=47) of histological scores of SARS-CoV-2 spread (x-axis) and relative expression qPCR values of SARS-CoV-2 spike expression in lungs (C) and brains (D). (E,F) Representative pictures and scoring of immunohistochemical staining of the SARS-CoV-2 nucleocapsid protein in lung (E) and brain (F) sections. Plotted are the mean + SEM (in A,B,D,E 3pRNA d-7 n=8, 3pRNA d-3 & ctrl RNA n=9, 3pRNA d-1 n=10, untreated n=11). Data are pooled from two independent experiments. Statistical significance was calculated by one-way ANOVA with Dunnett's multiple testing for lognormally-distributed data (A) or non-parametric Kruskal-Wallis test and Dunn's multiple testing (B,E,F). * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.



Supplementary Figure 3: Induction of cytokines in the sera of RIG-I ligand-treated mice. C57BL/6J mice were i.v. injected with 20 µg 3pRNA or control RNA complexed to *in vivo j*etPEI. After 4h, serum was collected, and cytokines were measured using a mouse anti-viral LegendPLEX panel. Plotted are the mean + SEM (3pRNA and ctrl RNA n=12, untreated n=6). Statistical significance was calculated by non-parametric Kruskal−Wallis test with Dunn's multiple testing. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.



Supplementary Figure 4: SARS-CoV-2 viral burden in oropharyngeal swab material and in organs from mice upon 3pRNA or IFNα treatment. (A) Expression of SARS-CoV-2 viral RNA in oropharyngeal swab material of 3pRNA or IFNα-treated animals 1-3 dpi. (B+C) SARS-CoV-2 viral burden in the lungs (B) and in the brain (C) quantified by qPCR relative to murine *gapdh* expression. Plotted are the mean + SEM (3pRNA and IFNα n=7, untreated n=5). Statistical significance was calculated by one-way ANOVA (Welch) with Dunnett's T3 multiple testing for lognormally distributed data and otherwise with a non-parametric Kruskal-Wallis test with Dunn's multiple testing. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.