

## SUPPLEMENTARY INFORMATION

### Outcome of SARS-CoV-2 reinfection depends on genetic background in female mice

Gagandeep Singh<sup>1,2,#</sup>, Juan García-Bernalt Diego<sup>1,2,#</sup>, Prajakta Warang<sup>1,2</sup>, Seokchan Park<sup>1,2</sup>, Lauren A. Chang<sup>1,2,3</sup>, Moataz Nouredine<sup>1,2,3</sup>, Gabriel Laghlali<sup>1,2,4</sup>, Yonina Bykov<sup>1,2,3</sup>, Matthew Prellberg<sup>1,2,3</sup>, Vivian Yan<sup>1,2,3</sup>, Sarabjot Singh<sup>5</sup>, Lars Pache<sup>6</sup>, Sara Cuadrado-Castano<sup>1,2,7,8</sup>, Brett Webb<sup>9</sup>, Adolfo García-Sastre<sup>1,2,10,11</sup>, Michael Schotsaert<sup>1,2,7,8,\*</sup>

# These two authors contributed equally.

\* Corresponding author: Michael Schotsaert (michael.schotsaert@mssm.edu)

### AFFILIATIONS

<sup>1</sup>Department of Microbiology, Icahn School of Medicine at Mount Sinai New York, NY, USA

<sup>2</sup>Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai New York, NY, USA

<sup>3</sup>Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

<sup>4</sup>Department of Pharmaceutics, Ghent University, Ghent, Belgium

<sup>5</sup>RT-PCR COVID-19 Laboratory, Civil Hospital, Moga, Punjab, India

<sup>6</sup>NCI Designated Cancer Center, Sanford-Burnham Prebys Medical Discovery Institute, 10901 N Torrey Pines Rd, La Jolla, CA 92037, USA

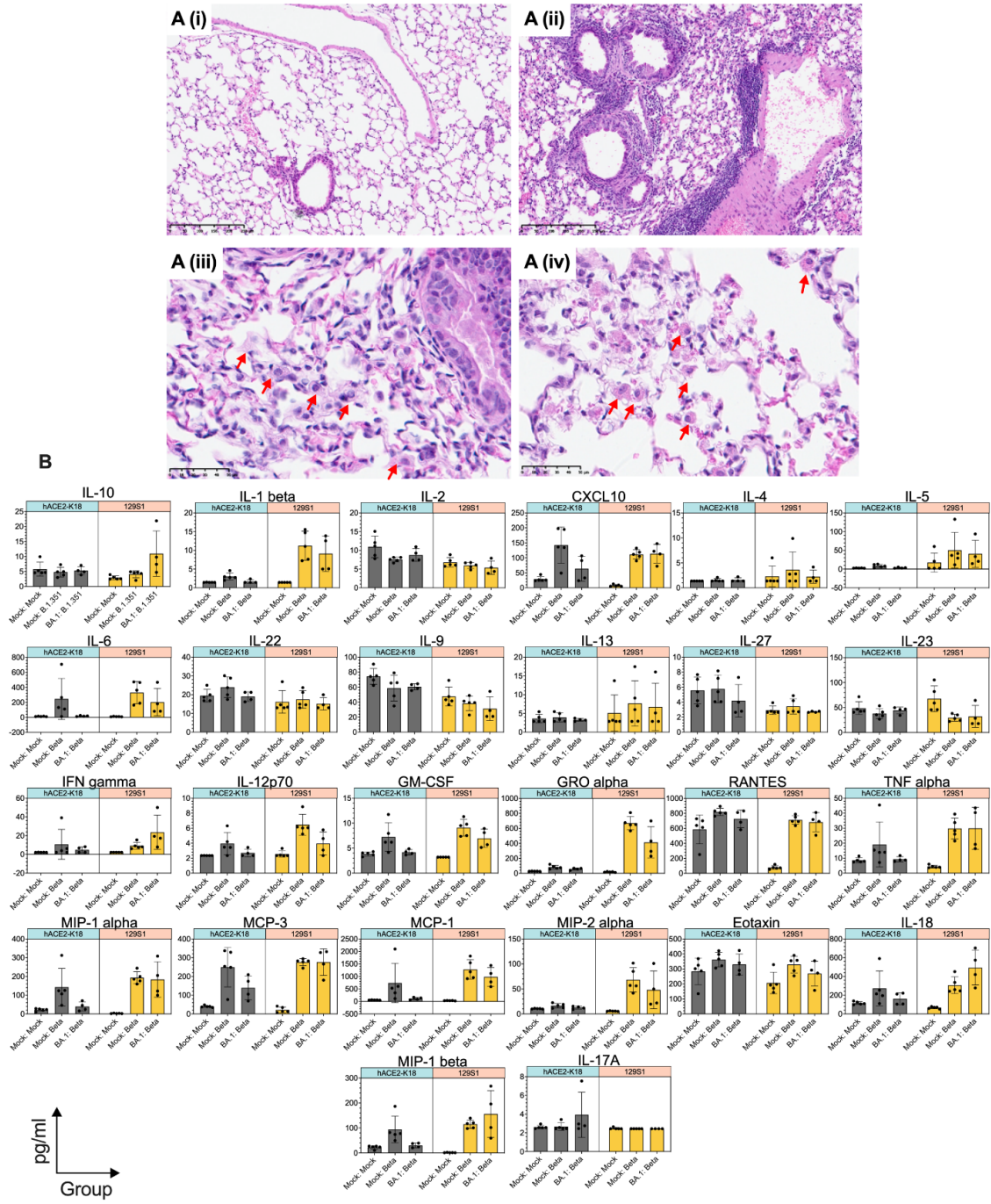
<sup>7</sup>Lipschultz Precision Immunology Institute (PrIISM), Icahn School of Medicine at Mount Sinai, New York, NY, USA

<sup>8</sup>Icahn Genomics Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

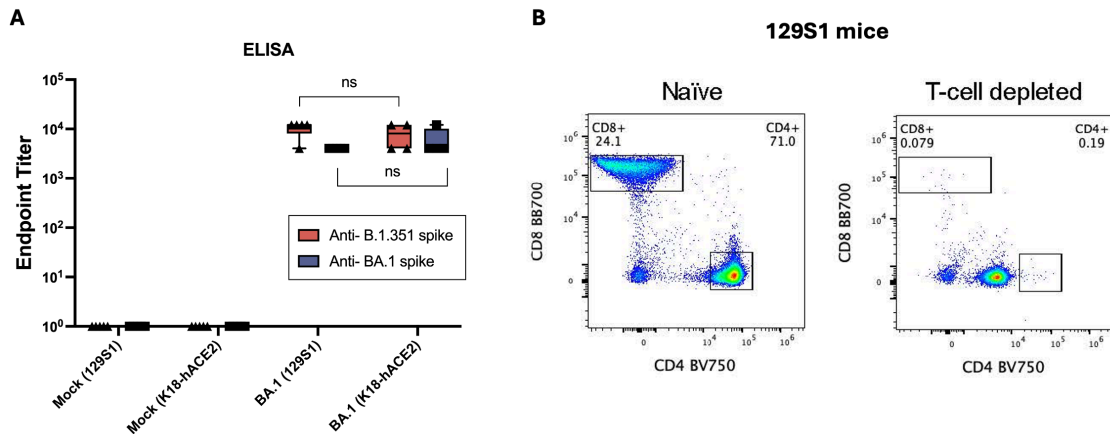
<sup>9</sup>Department of Veterinary Sciences, University of Wyoming, Laramie, WY, USA

<sup>10</sup>Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai New York, NY, USA

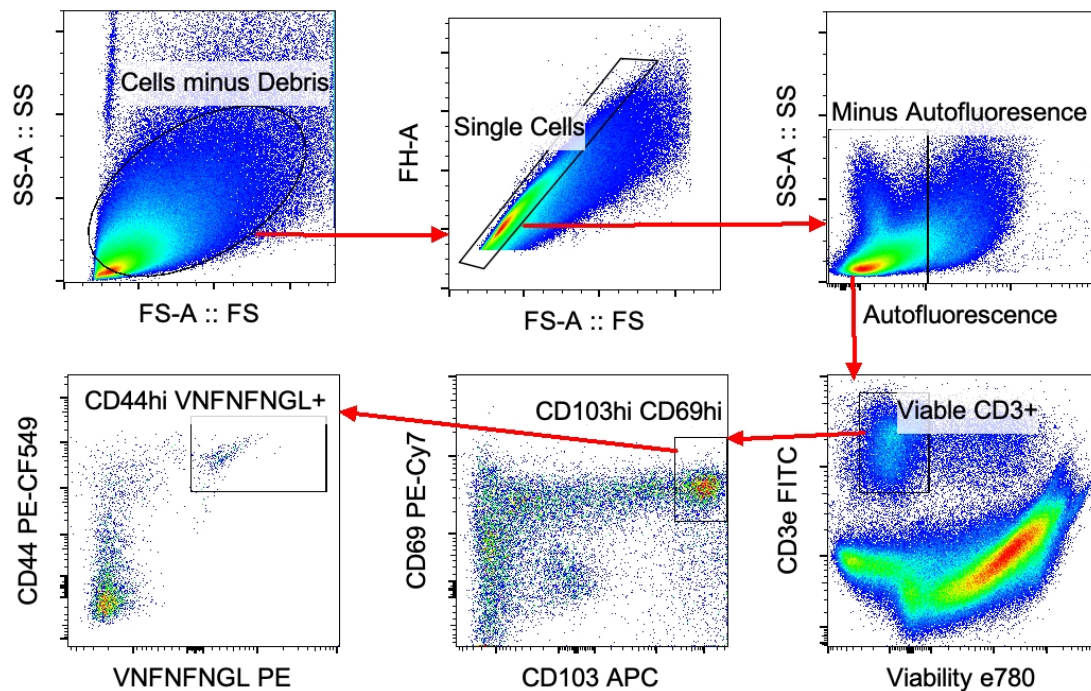
<sup>11</sup>The Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai New York, NY, USA



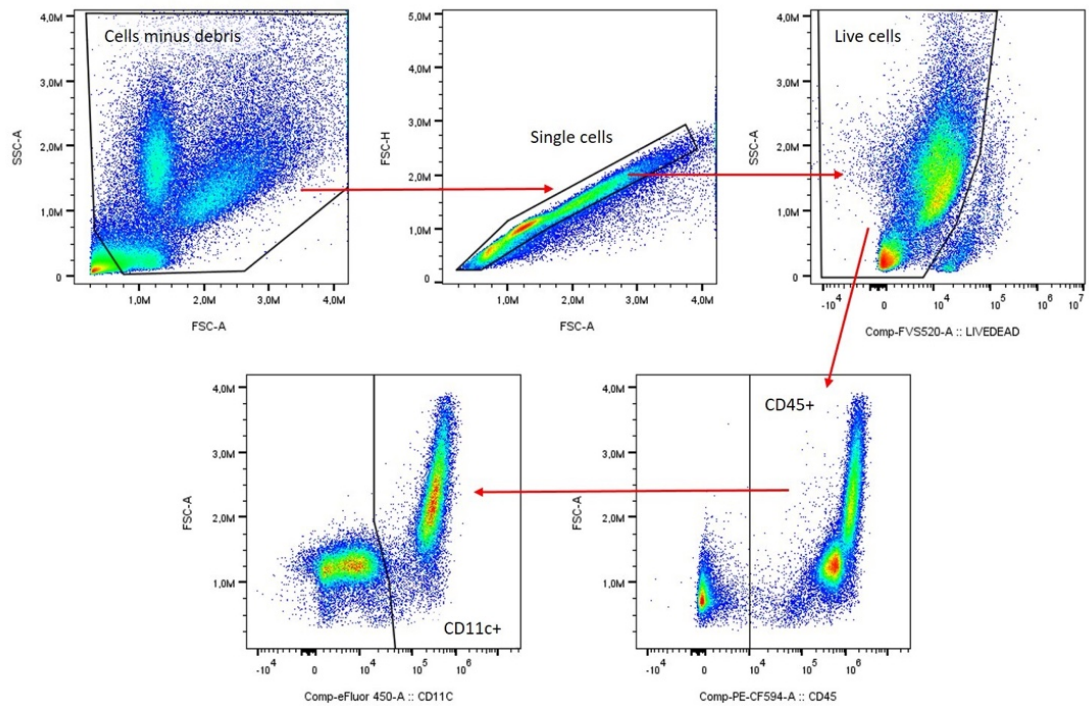
**Supplementary figure 1: Histopathology and lung homogenate supernatant cytokine/chemokine data after B.1.351 re-infection.** Mock inoculated K18-hACE2 (n=10) or 129S1 (n=10) mice were either inoculated with either mock (n=5) or B.1.351 (n=5) after 30 days. BA.1 inoculated K18-hACE2 (n=5) or 129S1 (n=5) mice were either inoculated with B.1.351 after 30 days. A(i) Representing histology images from mock infected K18-hACE2 inoculated with mock with no histology lesions, A(ii) BA.1 infected 129S1 inoculated with B.1.351 with perivascular and peribronchiolar lymphoid hyperplasia, A(iii) B.1.351 infected K18-hACE2 exhibiting alveolar macrophages with foamy or light eosinophilic cytoplasm. A(iv) BA.1 infected 129S1 inoculated with B.1.351 exhibiting alveolar macrophages with abundant homogeneous eosinophilic cytoplasm. B) Bar graphs (Mean±SD) of absolute concentration (pg/ml) of cytokines and chemokines in lungs of infected K18-hACE2 (dark grey) or 129S1 (yellow) mice. Source data are provided as a Source Data file.



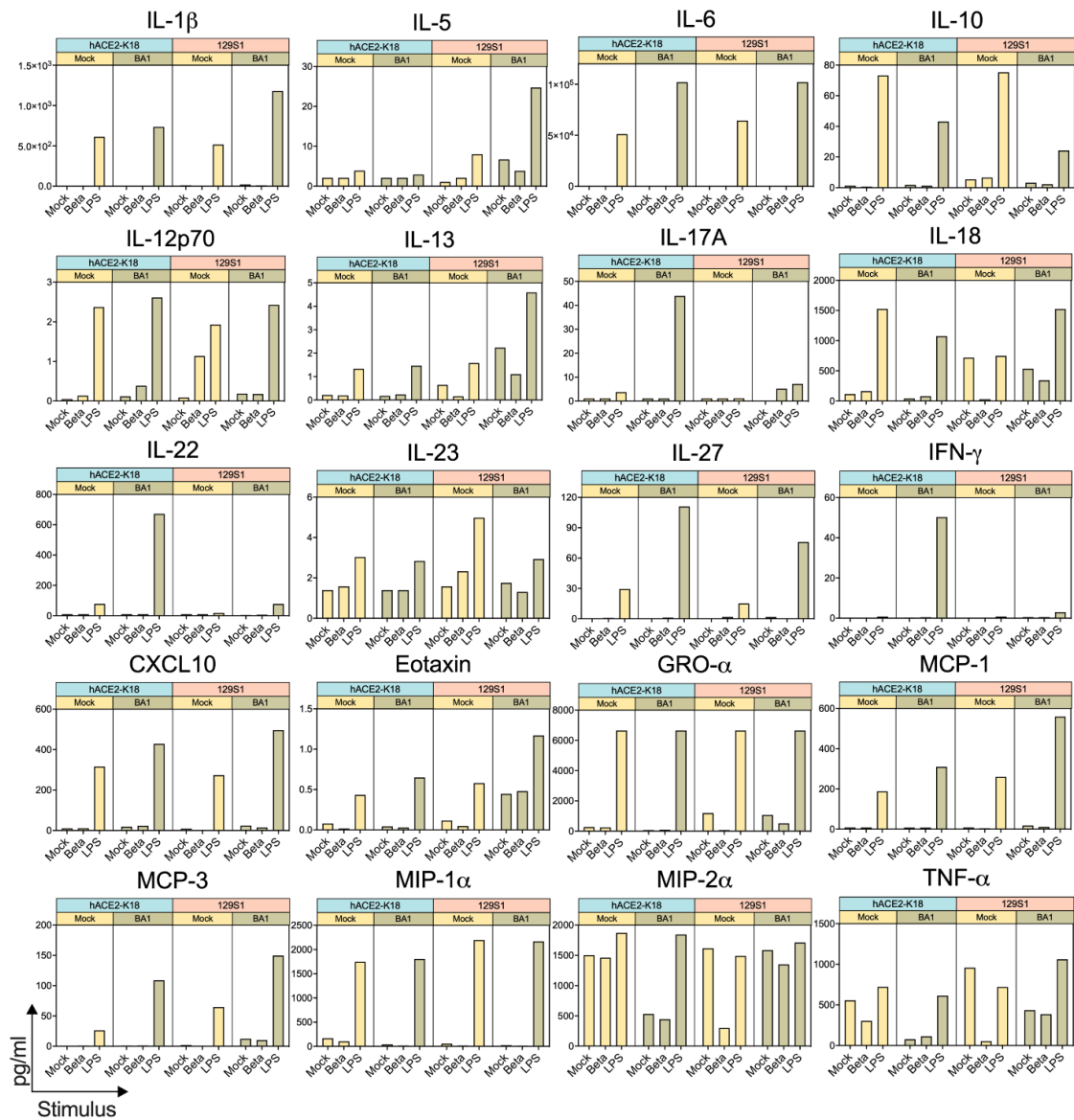
**Supplementary figure 2: ELISA titers after BA.1 infection and T-cell depletion.** A: ELISA end point titers calculated from Figure 3 A. Representation: Box-plot with median as centre, 25th to 75th percentile-bound box and whiskers representing maximum and minimum values. Statistical analysis: Two tailed t-test was used to determine statistical significance of the results, where p-values > 0.05 are denoted as 'ns'. B: Flow cytometry analysis from blood obtained from 129S1 mice of the T-cell depletion experiment, four days after challenge with B.1.351. Cell populations presented are pre-gated as: Live cells>CD3+ cells. CD8+ T cell depletion was complete, however, by this time point, a CD4 dim population was observed with a lower mean fluorescence intensity when compared to the original CD4+ population. Source data are provided as a Source Data file.



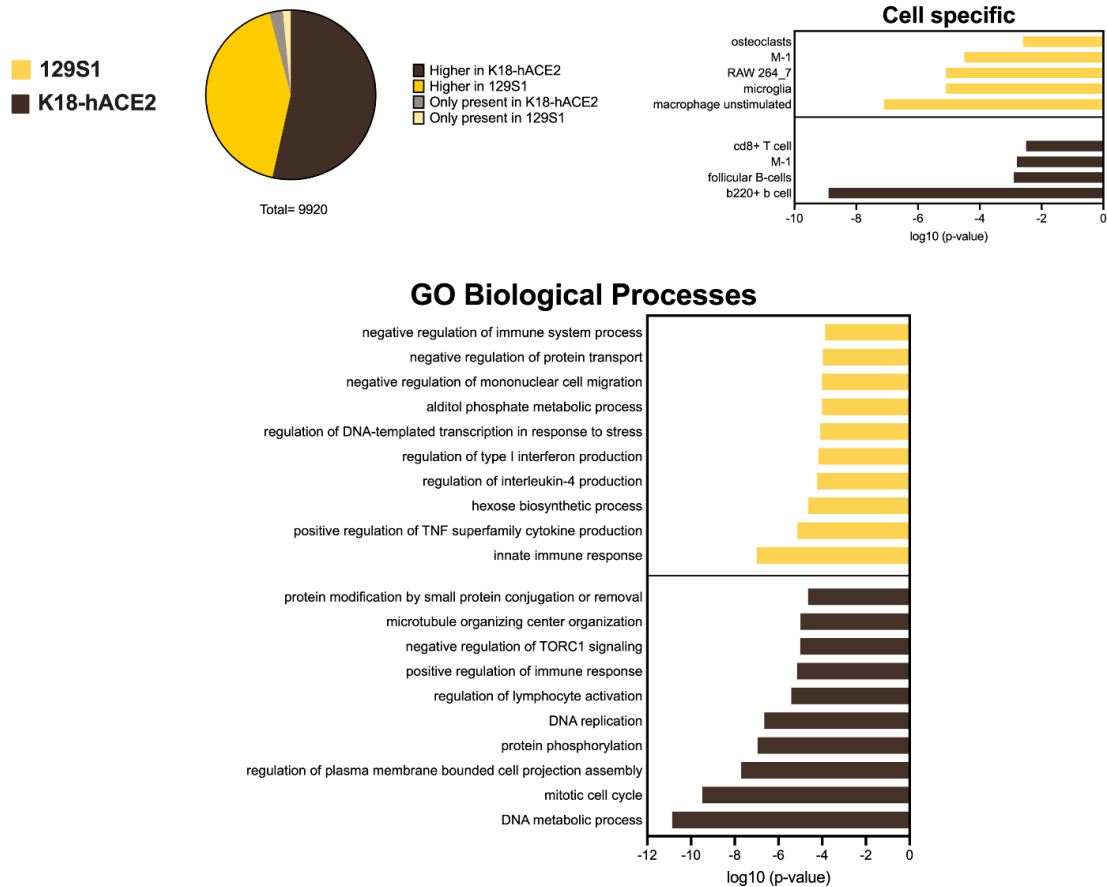
**Supplementary figure 3: Gating scheme for T-cell analysis.**



**Supplementary figure 4: Gating scheme for CD11c+ cell analysis in BALF**



**Supplementary figure 5: CD11c+ cells Luminex multiplex ELISA results.** Mock or BA.1 inoculated K18-hACE2 (n=8) or 129S1 (n=9) mice were euthanized after 30 days post-infection, Lungs (n=4 or 3) or BALF (n=5) were collected. CD11c cells were enriched from the BALF and used for trained immunity experiment. Source data are provided as a Source Data file.



**Supplementary figure 6: Long-read RNAseq results.** Mock or BA.1 inoculated K18-hACE2 (n=8) or 129S1 (n=9) mice were euthanized after 30 days post-infection, Lungs (n=4 or 3) or BALF (n=5) were collected. CD11c cells were enriched from the BALF and used for trained immunity experiment. Results from the long-read-RNAseq analysis from CD11c BALF cells. Pie-chart of total gene analyzed, and their distribution based on relative expression (upper left), bar graph showing cell specific hits in each BA.1 infected strain after gene enrichment analysis (upper right), bar graph showing GO biological pathways in each BA.1 infected strain after gene enrichment analysis (lower left). Source data are provided as a Source Data file.