**MATERIALS AND METHODS**

**Cohort**

Samples from hospitalized COVID-19 patients were obtained from an observational cohort study of hospitalized COVID-19 patients at UCLA. All participants signed informed consent to participate and the study was approved by the UCLA Institutional Review Board (#20-000473). Patients were recruited from two UCLA Health hospitals in Los Angeles, CA. Inclusion criteria included hospitalization for COVID-19, age greater than 18, and confirmed positive SARS-CoV-2 RT-PCR within 72 hours of admission. Exclusion criteria included pregnancy, hemoglobin less than 8 g/dL, inability to provide informed consent, or solid organ transplant. Upon enrollment blood samples, nasopharyngeal swab, and saliva were collected throughout hospitalization up to 6 weeks. Demographic and clinical data, including therapeutics, were collected from the electronic medical record. Clinical severity was scored using the NIAID 8-point ordinal scale[(21)](https://paperpile.com/c/6ZSIYW/YvSC8): 1, not hospitalized and no limitations; 2, not hospitalized but with limitations; 3, hospitalized no supplemental oxygen or ongoing medical care; 4, hospitalized no supplemental oxygen but with ongoing medical care; 5, hospitalized with supplemental oxygen; 6, hospitalized with non-invasive ventilation or high-flow oxygen; 7, hospitalized with invasive mechanical ventilation or ECMO; 8, death. For this study, mild COVID-19 included ordinal scale 3-4, moderate COVID-19 included ordinal scale 5, and severe COVID-19 included ordinal scale 6-7. Samples included in this study were collected from April 2020 through February 2021.

**Monocyte Isolation**

Cryopreserved PBMC from COVID-19-positive and healthy donors were thawed at 37 C and washed with RPMI supplemented with 10% FBS (RP10) to remove freezing medium. Cells from each donor were magnetically fractionated into CD14+ and CD14- populations using the Miltenyi MACS Human CD14+ MicroBead isolation kit (Miltenyi, cat. no. 130-050-201) according to the manufacturer’s instructions. CD14+ cells were then set aside in an incubator (37 C, 5% CO2) until the start of co-culture while CD14- cells were used for subsequent NK cell isolation.

**NK cell isolation and activation**

NK cells were isolated from CD14- cells from healthy donors and COVID-19 patients using the Miltenyi MACS Human NK Cell Isolation Kit (Miltenyi, cat. no. 130-092-657) according to the manufacturer’s instructions. 10% of NK cells from healthy and COVID-19-positive donors were set aside for phenotyping by flow cytometry. NK cells from two healthy donors (MJL01 and MJL03) were set aside for monocyte co-culture assays. The remaining NK cells were transferred to a round-bottom 96-well plate and resuspended in complete RPMI supplemented with 25 ng/mL (250 IU/mL) rhIL-2 (R&D Systems, cat. no. 202-IL-010), then placed in a 37C CO2 incubator for 12-16 hours. After incubation, the cells were washed twice to remove IL-2, counted, and resuspended in fresh RP10 before being transferred to BSL3 facilities for killing assays.

**NK cell phenotyping**

As described above, a sample of NK cells from healthy donors and COVID-19 patients were taken for phenotyping by flow cytometry. NK cells were washed in PBS and stained with eFluor 780 Fixable Viability Dye (eBioscience, cat. no. 65-0865-14) for 20 minutes. Cells were then washed in FACS buffer (PBS supplemented with 2% FBS) and stained for 30 minutes at room temperature with a panel of antibodies against surface antigens. Stained NK cells were washed, fixed for 15 minutes in 4% paraformaldehyde (PFA) (EIS, cat. no. 15710), and permeabilized (BD Biosciences, Cat. 340973). Permeabilized cells were stained with a panel of antibodies against intracellular proteins, then washed and analyzed on a Cytek Aurora spectral cytometer.

**Cell lines**

A549-ACE2 were a gift from Ralf Bartenschlager and were confirmed to be mycoplasma-free. A549-ACE2 cells were maintained in DMEM supplemented with 10% FBS and passaged every 2-3 days. Cell cultures were discarded and new cells were thawed after 25 passages.

**Infection of A549-ACE2 with SARS-CoV-2**

The day prior to infection, A549-ACE2 cells were seeded at a density of 100K cells/well in a 12-well plate. On the day of infection, cells were brought into the BSL3 and washed once with PBS to remove excess serum. PBS was then removed and mNeon Green SARS-CoV-2 was added in DMEM supplemented with 2% FBS (“D2”) at an MOI of 0.5 (final volume of 150 uL per well). The plate was rocked for 1 hour at 37C, after which time the virus was washed off with PBS and 0.5 mL D2 was added to each well. Plates were placed back in an incubator for 48 hours before being harvested for use in killing assays.

**Flow cytometry-based killing assay**

The morning of the killing assay, IL-2-activated NK cells were counted and brought into the BSL3. SARS-CoV-2-infected A549-ACE2s were washed with PBS, harvested using TrypLE, and counted before being resuspended in fresh RP10. Target cells and NK cells were plated in V-bottom 96-well plates at an effector:target (E:T) ratio of 10:1. The plate containing target cells and NK cells was spun down for 1 minute at 1000 RPM to bring cells together, then placed in the 37 C incubator for 3 hours. After 3 hours, cells were washed with PBS and stained with eFluor 780 Fixable Viability Dye for 25 minutes. Cells were then washed in PBS and fixed for 30 minutes in 4% PFA before being transferred to fresh tubes, decontaminated, and removed from the BSL3 and analyzed on a Cytek Aurora spectral cytometer.

**Allogeneic NK cell/monocyte co-culture**

Purified CD14+ cells in RP10 were added to a round-bottom 96 well plate or the bottom of a 24-well transwell plate for a final concentration of 1.5x106 CD14+ cells/mL. Two replicates were plated for each monocyte donor; each of these replicates then received NK cells from one of two healthy donors (MJL01 or MJL03) at a final concentration of 0.75x106 NK cells/mL. These NK cells were not preactivated with IL-2 or otherwise treated prior to co-culture. NK cells were added directly to monocytes in round-bottom 96 well plates or to the top of a 0.4 um transwell insert in transwell plates. Both culture systems had a monocyte:NK ratio of 2:1 and the final concentration of cells in media was kept consistent between the two culture systems. Once the cells had been added, 96 well culture plates were spun down for 1 minute at 1000 RPM to bring the cells together. Spun-down 96 well plates and transwell culture plates were then placed in a 37 C incubator for 2 hours. After 2 hours, NK cells from the transwell inserts were collected and transferred to a fresh 96-well plate. NK cells from all cultures were then stained for flow cytometry and analyzed in the manner described above (under “NK cell phenotyping”). The transwell and direct cultures for each donor were performed simultaneously to minimize batch effects.

**O-link**

After the NK cells were harvested from the transwell cultures, the remaining transwell culture supernatants were saved for O-link analysis. The supernatants were collected in microcentrifuge tubes and centrifuged to remove any cells and cell debris in the sample. Once clarified, the supernatants were transferred to fresh tubes and frozen at -80C until analyzed. Samples did not undergo any freeze-thaw cycles other than when they were thawed for final analysis. O-link was performed in technical duplicate according to the manufacturer’s instructions using the 92-analyte Inflammation Panel from O-link[(22)](https://paperpile.com/c/6ZSIYW/8USCT).

***Scriabin* analysis**

scRNA-seq data from Wilk et al. 2021[(3)](https://paperpile.com/c/6ZSIYW/3P8a) was first passed through a denoising algorithm (“ALRA”), which uses low-rank matrix approximation to impute expression levels of lowly-expressed genes, thereby partially alleviating the sparsity of the gene expression matrix[(23)](https://paperpile.com/c/6ZSIYW/DI2ju). Scriabin was then used to identify sets of highly co-expressed ligand-receptor pairs and group them into Interaction Programs (“IPs”) whose expression could be compared between sample conditions and cell types. More detail regarding the detailed methodology underpinning *Scriabin* can be found in the manuscript describing the method (https://doi.org/10.1038/s41587-023-01782-z).

***MultiNicheNet* analysis**

*MultiNicheNet* was applied to the scRNA-seq dataset from Wilk et al. 2021[(3)](https://paperpile.com/c/6ZSIYW/3P8a). *MultiNicheNet* identifies active ligand-receptor interactions between cell types of interest by taking into account not only expression of ligands and receptors themselves but also the expression of gene targets known to be regulated by those ligand-receptor pairs[(24)](https://paperpile.com/c/6ZSIYW/r8va). *MultiNicheNet* was used to identify the top fifty most active ligand-receptor interactions in the dataset in which monocytes were the sender cell and NK cells were the receiver cell (using the default *MultiNicheNet* parameters). We then manually pruned the list of the top fifty interactions to remove any interactions that were erroneously included in the *NicheNet* interaction database or those that would be highly unlikely to occur between NK cells and monocytes (eg: proteins that only interact with each other within the same cell). We manually removed twenty-five interactions, leaving us with twenty-five remaining ligand-receptor pairs. We then used *MultiNicheNet* to visualize expression of the target genes downstream of these interactions that were differentially expressed in severe COVID-19 and healthy donors. More detail regarding the detailed methodology underpinning *MultiNicheNet* can be found in the manuscript describing the method (https://doi.org/10.1101/2023.06.13.544751).

**Quantification and statistical analysis**

Flow cytometry data visualization was performed using FlowJo v10.7.1. Figures were generated in R using the *ggplot2*, *Seurat*, and *Scriabin* packages. Colors for figures were generated using the *NatParksPalettes* package. Statistical analyses were performed as described in figure legends and plotted using the R *ggpubr* package.