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capture antibodies according to the manufacturer's recommendations. The assays were read on a MAGPIX platform. The median fluorescence intensity of these beads was recorded for each bead and was used for analysis using a custom R script and a 5P regression algorithm.

Ferret infections

All procedures are described in our previous study (Liu et al., 2019). Ferrets were randomly assigned to the different treatment groups (naive, n = 2; SARS-CoV-2 infection, n = 6; influenza A virus (pH1N1) infection, n = 2; influenza A virus (H3N2) infection, n = 2). Both influenza A virus and SARS-CoV-2 infections of ferrets were performed simultaneously in the BSL-3 facility. For influenza A virus infections, all naive ferrets were infected intranasally with 10^5 PFU of influenza A/California/04/2009 (pH1N1) virus or 10^6 PFU of influenza A/Texas/71/2017 (H3N2) virus. Nasal washes were collected from anesthetized ferrets challenged with influenza A/California/04/2009 (pH1N1) virus on day 7 post infection and preserved at −80°C. Trachea were collected from euthanized ferrets challenged with influenza A/Texas/71/2017 (H3N2) virus on day 3 post infection and preserved at −80°C. For SARS-CoV-2 virus infections, all naive ferrets were infected with 5×10^4 PFU of SARS-CoV-2 isolate USA-WA1/2020. Nasal washes were collected from anesthetized ferrets on days 1, 3, 7 and 14 post-infection and trachea were collected from euthanized ferrets on day 3 post infection and preserved at −80°C. At the end of the study, anesthetized ferrets were euthanized by exsanguination followed by intracardiac injection of euthanasia solution (Sodium Pentobarbital). Total RNA from nasal washes and trachea was extracted using TRIzol (Invitrogen) and analyzed by RNA-Seq as described above.

QUANTIFICATION AND STATISTICAL ANALYSIS

Bioinformatic analyses

Raw reads were aligned to the human genome (hg19) using the RNA-Seq Aligment App on Basespace (Illumina, CA), following differential expression analysis using DESeq2 (Love et al., 2014). To diminish the noise introduced by variables inherent to the use of different cell types and systems, our differential expression analyses were always performed by matching each experimental condition with a corresponding mock treated sample that counted for the cell type, collection time, concurrent animal controls, etc. The raw sequencing data (fastq files) for the SARS-CoV-1 and MERS infections were downloaded from GEO (GSE56192, including their corresponding mock-treated controls) and processed in the same way as the rest of our experimental conditions. In order to capture the whole breadth of the response to IFNβ treatment we pooled samples from 4, 6 and 12 hr post treatment and compare them together to mock treated cells. Differentially expressed genes (DEGs) were characterized for each sample ($|L2FC| > 1$, p-adjusted-value < 0.05) and were used as query to search for enriched biological processes (Gene ontology BP) and network analysis of protein interactions using STRING (Szklarczyk et al., 2019). Heatmaps of gene expression levels were constructing using heatmap.2 from the gplot package in R (https://cran.r-project.org/web/packages/gplots/index.html). Sparse principal component analysis (sPCA) was performed on Log_2(Fold Change) values using SPC from the PMA package in R (Witten et al., 2009). Volcano plots, dot plots, scatterplots and linear regressions were constructed using ggplot2 (Wickham, 2016) and custom scripts in R. Heatmap of Type-I IFN responses was constructed on DEGs belonging to the following GO annotations: GO:0035457, GO:0035458, GO:0035455, GO:0035456, GO:0034340. Alignments to viral genomes was performed using bowtie2 (Langmead and Salzberg, 2012). Cell lineage profiling from SARS-CoV-2 unique gene signatures was generated using the Immunological Genome Project (https://www.immgen.org/; Yoshida et al., 2019). The genomes used for this study were: SARS-CoV-2 (GenBank: NC_045512.2), SARS-CoV-1 (GenBank: NC_004718.3), MERS-CoV (GenBank: NC_038294.1), HPIV3 (GenBank: Z11575.1), RSV (GenBank: NC_001803.1), IAV PR8 (GenBank: AF389115.1, AF389116.1, AF389117.1, AF389118.1, AF389119.1, AF389120.1, AF389121.1, AF389122.1) and IAV A/California/VRDL6/2010(H1N1) (GenBank: CY064994, CY064993, CY064992, CY064987, CY064990, CY064989, CY064988, CY064991). All RNA-Seq data performed in this paper can be found on the NCBI Gene Expression Omnibus (GEO) under accession number GSE147507. All non-RNA-seq statistical analyses were performed as indicted in figure legends using prism 8 (GraphPad Software, San Diego, California USA; https://www.graphpad.com/).

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