1   **IFN signaling and neutrophil degranulation transcriptional signatures are induced during**

2   **SARS-CoV-2 infection**

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25

26   **Abstract**

27   The novel virus SARS-CoV-2 has infected more than 14 million people worldwide resulting in the

28   Coronavirus disease 2019 (COVID-19). Limited information on the underlying immune

29   mechanisms that drive disease or protection during COVID-19 severely hamper development of

30   therapeutics and vaccines. Thus, the establishment of relevant animal models that mimic the

31   pathobiology of the disease is urgent. Rhesus macaques infected with SARS-CoV-2 exhibit

32   disease pathobiology similar to human COVID-19, thus serving as a relevant animal model. In

33   the current study, we have characterized the transcriptional signatures induced in the lungs of

34   juvenile and old rhesus macaques following SARS-CoV-2 infection. We show that genes

35   associated with Interferon (IFN) signaling, neutrophil degranulation and innate immune pathways

36   are significantly induced in macaque infected lungs, while pathways associated with collagen

37   formation are downregulated. In COVID-19, increasing age is a significant risk factor for poor

38   prognosis and increased mortality. We demonstrate that Type I IFN and Notch signaling pathways

39   are significantly upregulated in lungs of juvenile infected macaques when compared with old

40   infected macaques. These results are corroborated with increased peripheral neutrophil counts

41   and neutrophil lymphocyte ratio in older individuals with COVID-19 disease. In contrast, pathways

42   involving VEGF are downregulated in lungs of old infected macaques. Using samples from

43   humans with SARS-CoV-2 infection and COVID-19, we validate a subset of our findings. Finally,

44   neutrophil degranulation, innate immune system and IFN gamma signaling pathways are

45   upregulated in both tuberculosis and COVID-19, two pulmonary diseases where neutrophils are

46   associated with increased severity. Together, our transcriptomic studies have delineated disease

47   pathways to improve our understanding of the immunopathogenesis of COVID-19 to facilitate the

48   design of new therapeutics for COVID-19.

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50

INTRODUCTION

COVID-19, caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged as a pandemic disease during the end of 2019 and beginning of 2020. In the absence of a specific treatment or vaccine against SARS-CoV-2, infected individuals develop symptoms associated with a cytokine storm (*1*). This cytokine storm can initiate viral sepsis and inflammation-induced lung injury which lead to other complications including pneumonitis, acute respiratory distress syndrome (ARDS), respiratory failure, shock, organ failure and potentially death (*1, 2*).

By combining established principles of anti-viral immunity with analysis of immune responses in COVID-19 patients, a picture of the host defense response against SARS-CoV-2 is beginning to emerge (*3, 4*). Upon infection of the mucosal epithelium, SARS-CoV-2 is detected by intracellular pattern recognition receptors (PRRs) that bind viral RNA and DNA. PRR signaling triggers activation of transcription factors and induces Interferon (IFN) signaling, which in turn activates resident macrophages. Infected macrophages induce cytokine secretion that consequently triggers recruitment of myeloid cells, likely resulting in a feed-back loop that aggravates immunopathogenesis and promotes disease progression.

Analyses of transcriptomic response of host cells upon virus infection have potential to identify the host immune response dynamics and gene activated regulatory networks (*5, 6*). Recent studies have reported transcriptional changes in cells in the broncho-alveolar lavage (BAL) and peripheral blood mononuclear cells (PBMCs) of COVID-19 patients (*7*). Single cell RNA-seq has recently identified initial cellular targets of SARS-CoV-2 infection in model organisms (*8*) and patients (*9*) and characterized peripheral and local immune responses in severe COVID-19 (*10*), with severe disease being associated with a cytokine storm and increased neutrophil accumulation. However, most of these studies have mostly been performed in peripheral blood samples from a limited number of moderate or severe COVID-19 patients within limited age ranges (*10*). To overcome the limitations associated with obtaining samples from human subjects

77   and to get more in-depth understanding of the transcriptional changes during COVID-19, we have

78   developed a SARS-CoV-2 macaque model, where both juvenile and old macaques were infected

79   and exhibited clinical symptoms that reflect human COVID-19 disease that is self-limited. In the

80   current study, we have characterized the transcriptional signatures induced in the lungs of juvenile

81   and old rhesus macaques following SARS-CoV-2 infection. Our results show that genes

82   associated with Interferon (IFN) signaling, neutrophil degranulation and innate immune pathways

83   are significantly induced in the lungs in response to SARS-CoV-2 infection. Interestingly, this is

84   associated with a downregulation of genes associated with collagen formation and regulation of

85   collagen pathways. In COVID-19, increasing age is a significant risk factor for poor prognosis of

86   infection(*11*). We demonstrate that specific immune pathways, namely Type I IFN and Notch

87   signaling, are significantly upregulated in juvenile macaques when compared with old macaques

88   infected with SARS-CoV-2. These results are corroborated with increased peripheral neutrophil

89   counts and neutrophil lymphocyte ratio in older individuals with COVID-19 disease. In contrast,

90   the VEGF pathway is downregulated in old infected macaques. Incidently, levels of VEGF protein

91   are increased in plasma of older COVID-19 patients, emphasizing the importance of studying both

92   local and peripheral responses. Finally, we report that neutrophil degranulation, innate immune

93   system and IFN gamma (IFN-γ) signaling pathways are upregulated in both tuberculosis (TB) and

94   COVID-19, two pulmonary infectious diseases where neutrophils accumulation is associated with

95   increased severity. Together, our study has delineated disease pathways that can serve as a

96   valuable tool in understanding the immunopathogenesis of SARS-CoV-2 infection and

97   progressive COVID-19, and facilitate the design of therapeutics for COVID-19.

98

99   **MATERIALS AND METHODS**

100   **Macaques.** All of the infected animals were housed in Animal Biosafety Level 3 (ABSL3) at the

101   Southwest National Primate Research Center, Texas Biomedical Research Institute, where they

102   were treated per the standards recommended by AAALAC International and the NIH Guide for

103   the Care and Use of Laboratory Animals. Sham controls were housed in ABSL2. The animal

104   studies in each of the species were approved by the Animal Care and Use Committee of the

105   Texas Biomedical Research Institute and as an omnibus Biosafety Committee protocol.

106   **Animal studies, and tissue harvest for RNA sample preparation.** Rhesus macaques *(Macaca*

107   *mulatta)* animals enrolled in this study have been described in detail(*12*) (in review), and the

108   infection of these animals with 1.05×10^6 pfu SARS-CoV-2 isolate USA-WA1/2020 (BEI

109   Resources, NR-52281, Manassas, VA) has also been described earlier(*12*) (in review). Control

110   (SARS-CoV-2 uninfected) samples were obtained from opportunistic necropsies conducted on

111   rhesus macaques from the same colony in the past few months.   Infected animals were

112   euthanized for tissue collection at necropsy, including lung. specimens Lung tissue from three

113   juvenile (3 yrs old) and five old (average 17 yrs old) rhesus macaques (**Table S1**) were

114   homogenized, snap-frozen in RLT buffer, and DNAse-treated total RNA was extracted using the

115   Qiagen RNeasy Mini kit (Qiagen) for RNA-seq analysis as described earlier(*13*) .

116   **Viral RNA determination**. SARS-CoV-2 RNA isolation and measurement of viral RNA in lung

117   homogenates using RTqPCR has been described(*12*) (in review).

118   **RNA-sequencing and analysis.** cDNA libraries were prepared from RNA samples using the

119   Clontech SMARTer universal low input RNA kit to maximize yield, and samples were sequenced

120   on Illumina NovaSeq S4 XP (paired 150bp reads). After adapter trimming using Trimmomatic

121   v0.39(*14*), sequenced RNA-seq reads were aligned to the *Macaca mulatta* genome (version 10,

122   Ensembl release 100(*15*)) using the STAR aligner v2.7.3a(*16*) (2-pass mode, basic). All raw RNA-

123   Seq fastq files were uploaded to the NCBI Sequence Read Archive (SRA(*17*)), and complete

124   sample metadata and accession information are provided in **Table S1**. Read fragments (read

125   pairs or single reads) were quantified per gene per sample using featureCounts v1.5.1(*18*).

126   Significantly differentially expressed genes between naïve, controller and progressor sample sets

127   were identified using DESeq2 v1.4.5(*19*) with default settings, and a minimum P value

128   significance threshold of 0.01 (after False Discovery Rate [FDR(*20*)] correction for the number of

129   tests). Principal components analysis also was calculated using DESeq2 output (default settings,

130   using the top 500 most variable genes). FPKM (fragments per kilobase of gene length per million

131   reads mapped) normalization was performed using DESeq2-normalized read counts. Pathway

132   enrichment analysis among differentially expressed gene sets of interest was performed for (a)

133   Reactome(*21*) pathways, using the human orthologs as input into the WebGestalt(*22*) web server

134   (p ≤ 0.05 after FDR correction, minimum 3 genes per term) and (b) KEGG(*23*) pathways and

135   Gene Ontology(*24*) terms, using the g:profiler web server(*25*) which has a database of these

136   annotations matched to macaque ENSEMBL gene IDs (p ≤ 0.05 after FDR correction, minimum

137   3 genes per term). Mapped fragment counts, relative gene expression levels, gene annotations,

138   and differential expression data for every macaque gene are available in **Table S2**, along with

139   orthology matches to human genes retrieved from ENSEMBL(*15*) and identifications of

140   differentially expressed (DE) genes belonging to enriched pathways of interest, for genes of

141   interest in **Table S3**, and significant functional enrichment for Reactome, KEGG and Gene

142   Ontology pathways, among differentially gene sets of interest in **Table S4**. Additionally, genes

143   significantly differentially regulated during progression of tuberculosis (in both the macaque gene

144   and the corresponding mouse ortholog) were identified from a previous transcriptomic study of

145   tuberculosis-infected lung tissue(*13*), and the upregulated and downregulated gene sets were

146   intersected with the COVID-19 results from the current study.

147

148   **Human sample collection.** Plasma samples were collected from COVID-19 patients that

149   attended the emergency room of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador

150   Zubirán (INCMNSZ), and the Instituto Nacional de Enfermedades Respiratorias Ismael Cosío

151   Villegas (INER) in Mexico City, from March to June of 2020. Detection of SARS-CoV-2 was

152   performed by real-time polymerase chain reaction (RT-PCR) in swab samples, bronchial

153   aspirates (BA), or bronchoalveolar lavage (BAL). For this purpose, viral RNA was extracted from

154   clinical samples with the MagNA Pure 96 system (Roche, Penzberg, Germany). The RT-PCR

155 reactions were performed in a total volume of 25 µL, containing 5µL of RNA, 12.5µL of 2 × reaction

156 buffer provided with the Superscript III one-step RT-PCR system with Platinum Taq Polymerase

157 (Invitrogen, Darmstadt, Germany; containing 0.4 mM of each deoxyribose triphosphates (dNTP)

158 and 3.2 mM magnesium sulfate), 1µL of reverse transcriptase/ Taq mixture from the kit, 0.4 µL of

159 a 50 mM magnesium sulfate solution (Invitrogen), and 1µg of nonacetylated bovine serum

160 albumin (Roche). All oligonucleotides were synthesized and provided by Tib-Molbiol (Berlin,

161 Germany). Thermal cycling was performed at 55 °C for 10 min for reverse transcription, followed

162 by 95 °C for 3 min and then 45 cycles of 95°C for 15 s, 58°C for 30s. Primer and probe sequences

163 are as follows: RdRP gene [RdRp-SARSr-F:GTGARATGGTCATGTGTGGCGG,RdRp-SARSr-

164 P2:

165 FAMCAGGTGGAACCTCATCAGGAGATGCBBQ,RdRP_SARSrP1:FAMCCAGGTGGWACRTC

166 ATCMGGTGATGCBBQ,RdRp_SARSrR:CARATGTTAAASACACTATTAGCATA], E gene

167 [E_Sarbeco_F:ACAGGTACGTTAATAGTTAATAGCGT,E_Sarbeco_P1:FAMACACTAGCCATC

168 CTTACTGCGCTTCGBBQ,E_Sarbeco_R:ATATTGCAGCAGTACGCACACA], N gene

169 [N_Sarbeco_F:CACATTGGCACCCGCAATC,N_Sarbeco_P1:FAMACTTCCTCAAGGAACAACA

170 TTGCCABBQ, N_Sarbeco_R:GAGGAACGAGAAGAGGCTTG]. Clinical and demographic data

171 were retrieved from the medical records of all participants. These data included age, gender,

172 anthropometrics, comorbidities, symptoms, triage vital signs, and initial laboratory test results.

173 Initial laboratory tests were defined as the first test results available (typically within 24 h of

174 admission) and included white blood cell counts (WBC), neutrophil and lymphocyte counts (**Table**

175 **S5**).

176

177 **Cytokine levels in human plasma samples**

178 Peripheral blood samples were obtained from all participants at hospital admission. Plasma levels

179 of interferon-gamma (IFN-γ) and vascular endothelial growth factor (VEGF), were determined by

180   Luminex assays using the Luminex platform Bio-Plex Multiplex 200 (Bio-Rad Laboratories, Inc.,

181   Hercules, CA, USA). Plasma samples from four healthy volunteer donors were used as controls.

182   **RESULTS**

183   **Genes up-regulated in COVID-19 infected macaques represent pathways characteristic of**

184   **neutrophil degranulation and IFN signaling**

185   We recently assessed the ability of SARS-CoV-2 to infect rhesus macaques during a longitudinal

186   two week infection study. This study included the effect of age on the progression of infection to

187   COVID-19. Indian-origin, SPF-rhesus macaques (*Macaca mulatta*) were infected by multiple

188   routes (ocular, intratracheal and intranasal) with sixth-passage virus at a target dose of 1.05×10^6

189   PFU/per animal and studied for two weeks. The macaques were grouped as naïve (uninfected),

190   and infected (juvenile or old) macaques. All infected animals developed clinical signs of viral

191   infection(*12*) (in review). Both juvenile and old macaques exhibited comparable clinical disease,

192   and equivalent longitudinal viral loads in the BAL, nasopharyngeal and buccopharyngeal swabs,

193   as well as lungs at endpoint. This was followed by comparable viral clearance. In order to fully

194   understand the immune pathways regulated upon SARS-CoV-2 infection, RNA was extracted and

195   RNA sequencing was carried out from a lung biopsy from juvenile macaques (n = 3, 1 male and

196   2 females) and old macaques infected with infected with SARS-CoV-2 (n = 5, 1 male and 4

197   females) and naive uninfected macaques (n = 4, 2 males and 2 females). An average of 68.6

198   million reads were generated, with an average of 20.3 million fragments (read pairs or orphaned

199   reads) mapping to macaque coding sequences, following analytical processing and mapping

200   (**Table S1**). Principal components analysis (PCA) based on whole-transcriptome gene expression

201   levels(*19*) showed that despite within-group variability for the COVID-19 infected samples, the

202   naïve samples grouped separately, suggesting substantial overall transcriptomic differences

203   resulting from the infection (**Figure 1A**). Differential gene expression analysis (DESeq2(*19*)) with

204   the juvenile and old COVID-19 samples grouped together identified 1,026 genes significantly (P

205   ≤ 0.01) up-regulated in response to infection, while 1,109 genes were significantly downregulated

206   (**Figure 1B**). Expression, annotation and differential expression data for all genes is available in

207   **Table S2**. Complete lists of differentially expressed genes for each comparison of interest

208   (described below) ranked by P value, with Z-scores for expression visualization are available in

209   **Table S3**, and significant pathway enrichment (Reactome(*21*), KEGG(*23*) and Gene

210   Ontology(*24*)) for all comparisons is shown in **Table S4**.

211   Evaluation of the top 30 most significantly up-regulated genes in the lungs of SARS-CoV-2-

212   infected macaques revealed significantly higher expression of CTSG (Cathepsin G),

213   ATP6AP2(ATPase H+ transporting accessory protein 2), IFNγR1 (Interferon Gamma Receptor),

214   CD36 and CD58, in comparison to expression in uninfected macaque lungs (**Figure 2A**).

215   Cathepsin G is a serine protease prominently found in neutrophilic granules. IFNγR1 associates

216   with IFNγR2 to form a receptor for the cytokine interferon gamma (IFNγ)(*26-29*), and required for

217   activation of antiviral responses, such as IRF3 (IFN regulatory factor-3), nuclear factor κB (NF-

218   κB) and JAK (Janus kinase)/STAT (signal transducer and activator of transcription) signaling

219   pathways (*30*). Reactome pathway analysis on up- and down-regulated genes in the lungs of

220   SARS-CoV-2 infected rhesus macaques showed that genes significantly up-regulated by

221   infection, included pathway enrichment for genes involved in "Neutrophil degranulation", "Innate

222   Immune system", "Immune system" and "IFN signaling" (**Table 1**; **Table S4A**). The up-regulation

223   of CD36 during COVID-19 in lungs is in conformity with these enriched pathways, since CD36, a

224   scavenger receptor expressed in multiple cell types, mediates lipid uptake, immunological

225   recognition, inflammation, molecular adhesion, and apoptosis (*31*), and is a Matrix

226   Metalloproteinase-9 substrate that induces neutrophil apoptosis. CD58 molecule (lymphocyte

227   function-associated antigen-3) is expressed on human hematopoietic and non-hematopoietic

228   cells, including dendritic cells, macrophages and endothelial cells (*32-35*), and interacts with its

229   receptor CD2 molecule (*36, 37*) on CD8^{+} cytotoxic T lymphocytes and NK cells to mediate

230　cytotoxic reactions (*38-40*). The complete ranked list of the 1,026 genes upregulated during

231　COVID-19 is shown in **Table S3A**.

232　ATP6AP2 was the most significantly up-regulated of the 65 genes upregulated within the enriched

233　"neutrophil degranulation" (R-HSA-6798695) pathway (**Table S3B**), and it interacts with renin or

234　prorenin to cause activation of intracellular signaling pathways, resulting in secretion of

235　inflammatory and fibrotic factors(*41*). CEACAM8 (Carcinoembryonic Antigen-Related Cell

236　Adhesion Molecule 8) is the gene that encodes for CD66b, a well characterized marker of

237　degranulation(*42*). Indeed, CD66b$^+$ neutrophils accumulate in the lungs of macaques infected

238　with SARS-CoV-2 (**Figure 2C**). We have also previously demonstrated that neutrophils are

239　heavily recruited early to the alveolar space following SARS-CoV-2 infection of macaques(*12*) (in

240　review). Additional genes strongly up-regulated during COVID-19 in the neutrophil degranulation

241　pathway are IDH-1(Isocitrate Dehydrogenase (NADP(+)) 1) which regulates neutrophil

242　chemotaxis, and FPR2 (Formyl Peptide Receptor 2), a G-coupled surface receptor which has a

243　deleterious role to play in viral infection including influenza (*43*). LTA4H (Leukotriene A4

244　hydrolase) is an enzyme that generates a neutrophil chemoattractant, leukotriene B4, a marker

245　for ARDS(*44*). Expression of 162 genes belonging to the "immune system" (R-HSA-168256)

246　pathway was upregulated in SARS-CoV-2 infected macaques (**Table S3C**). These included

247　LAMP-2(Lysosomal Associated Membrane Protein 2), and ATG7 (Autophagy Related 7), key

248　genes involved in autophagy. LAMP-2 is known to influence phagosomal maturation in neutrophil

249　(*45*). The IFN response constitutes the major first line of defense against viruses. Consistent with

250　this, we found up-regulation of genes associated with the IFN signaling pathways, specifically

251　Interferon Induced Protein with Tetratricopeptide Repeats 1 (IFIT3), IFN alpha receptor 1

252　(IFNAR1), IFN gamma receptor 1 (IFNGR1) and OAS 1 protein (2'-5'-

253　Oligoadenylate Synthetase 1). Together, these results suggest that upregulation of neutrophil

254　degranulation, Type I IFN signaling, and innate immune system is a characteristic feature of host

255　responses to SARS-CoV-2 infection.

256

Genes down-regulated following SARS-CoV-2 infection in macaques represent pathways characteristic of collagen degradation and TFG-β signaling

It is thought that up to 40% of patients with COVID-19 develop ARDS, and 20% of ARDS cases are severe (*46*). A well-documented sequela of ARDS is the development of fibrotic disease (*47, 48*). We found that the 1,109 genes downnegulated in SARS-CoV-2-infected macaques were significantly enriched for collagen degradation, regulation and formation (**Figure 2B**; **Table 2**; **Table S3D**; **Table S4B**). For example, among the "collagen degradation" (R-HSA-1442490) enriched pathway (**Table S3E**), COLA1 (collagen type I chain), other members of the collagen gene family (COL4A2 COL16A1 COL4A4 COL6A2 COL6A1 COL5A1 COL9A1 COL13A1 COL12A1 COL1A2) and Matrix metalloproteases such as MMP23B (Matrix Metallopeptidase 23B), MMP15 and MMP14 were all significantly dowregulated in COVID-19 diseased lungs when compared with expression in lungs of uninfected controls. Additionally, Reactome pathway enrichment prominently featured pathways down-regulated in COVID-19 disease in macaques comprised of "collagen degradation", "collagen chain trimerization", "degradation of extracellular matrix" and "collagen formation" (**Table 2**). Increased collagen degradation is essential for the prevention of fibrosis, a sequelae of COVID-19 and ARDS. Therefore, regulation of collagen degradation and extracellular matrix modeling suggest that this may be a feature of SARS-CoV-2 infection of rhesus macaques being a self-limiting model with early and robust anamnestic responses. TGFβ (Transforming Growth Factor Beta 1) is involved in normal tissue repair following lung injury, and in mediating fibrotic tissue remodeling by increasing the production and decreasing the degradation of connective tissue (*49*). Our results indicate a downregulation of genes associated with TGFβ signaling (**Table 2**), including the genes PARD3 (par-3 family cell polarity regulator) and PARD6A (par-6 family cell polarity regulator alpha), which are involved in regulating epithelial cell apico-basolateral polarization, SMURF (SMAD specific E3 ubiquitin protein ligase 1), a negative regulator of TGFβ pathway, and FURIN, which is a TGFβ converting

282 enzyme (**Table S3F**). While the interaction of the genes within these pathways is complex, our

283 results project a broad downregulation of mechanisms that contribute to lung repair and

284 remodeling in animals with anamnestic control of SARS-CoV-2 infection.

285

286 **Type I interferon signaling and Notch signaling pathways are upregulated in young**

287 **macaques but not old macaques with COVID-19 disease**

288 Age is a significant risk factor for increased morbidity and mortality in COVID-19 disease (*11*). In

289 order to identify the differential immune responses associated with SARS-CoV-2 infection in old

290 macaques, we carried out differential expression analysis between the groups; namely between

291 juvenile (n=3) vs naive (n=4), and old (n=5) vs naive (n=4). In order for a gene to be considered

292 to be differentially expressed only in the juvenile macaques, we required a stringent P value for

293 significance ≤ 0.01 in the juvenile COVID-19 vs naive, and a P value for significance ≥ 0.1 in the

294 old COVID-19 vs naive comparison. This approach identified 86 genes significantly up-regulated

295 (**Figure 3A**; **Table S3G**) and 96 genes significantly down-regulated (**Figure 3B**; **Table S3H**) with

296 COVID-19 disease only in juveniles. Note that no genes were significantly upregulated in juveniles

297 and significantly downregulated in old, and vice-versa. Of these genes, the top 30 most

298 significantly differential between juvenile and old are shown for up-regulated genes in **Figure 4A**

299 and for down-regulated genes in **Figure 4B**. No pathways were found to be significantly enriched

300 among the 96 genes significantly downregulated only in juveniles, but the Reactome and KEGG

301 pathways significantly enriched among the 86 genes upregulated only in juveniles are shown in

302 **Table 3**. Complete gene lists per pathway, and all significant pathways enrichment results

303 including for Gene Ontology (GO) are available in **Table S4C**.

304 The genes with significantly upregulated expression in SARS-CoV-2 infected juvenile but not old

305 macaques included MX1 (MX Dynamin Like GTPase 1), MX2 (MX Dynamin Like GTPase 2) and

306 USP18 (Ubiquitin Specific Peptidase 18) (**Figure 5**). This is consistent with and highlights the role

307 of the Reactome pathway "interferon alpha/beta signaling" being enriched in juvenile macaques

308   during SARS-CoV-2 infection (**Table 3, Table S4C**). Other genes in this pathway which exhibited

309   increased expression included IFIT1 and IFIT2. Additionally, by KEGG analysis, the Notch

310   signaling pathway was observed to be significantly upregulated in juvenile infected macaques

311   when compared with old infected macaques. ADAM17 (ADAM Metallopeptidase Domain 17), a

312   key component of the Notch signaling pathways is known to be involved in shedding of the surface

313   protein ACE2 (Angiotensin converting enzyme 2) (*50*). Therefore, it is interesting that a linear

314   correlation in the expression of ACE2 and ADAM17 exists in infected macaques (**Figure 4C**).

315   Note that we also see a significant upregulation of ACE2 across all samples (4.2-fold, $P = 4.9 \times 10^{-3}$),

316   and a substantially larger upregulation among the juvenile samples (7.1-fold, $P = 3.4 \times 10^{-4}$).

317   Additionally, the induction of DLL4, a Notch ligand, was increased in the infected juvenile

318   macaques. Finally, the differential induction of DTX3L (Deltex E3 Ubiquitin Ligase 3L) in juvenile

319   infected macaques compared to old infected macaques is important because Deltex stabilizes

320   the receptor in the endocytic compartment allowing signal transduction to proceed in Notch

321   signaling(*52*). Of the Hepatitis-induced pathway genes that are upregulated in juvenile COVID-19

322   diseased lungs, CXCL-10 (C-X-C Motif Chemokine Ligand 10) is a chemokine associated with

323   severe disease in COVID-19 in humans (*53*), but can also be involved in recruitment of CXCR3

324   (C-X-C Motif Chemokine Receptor 3) expressing immune cells. 14-3-3 (otherwise called YWHAG)

325   interacts with MDA5 (melanoma differentiation-associated protein 5), which belongs to the RIG-I-

326   like receptor family and drive anti-viral immunity. Together, these results suggest that specific

327   pathways including Type I IFN and Notch signaling are highly induced in juvenile macaques

328   during SARS-CoV-2 infection, when compared to similarly infected old macaques.

329

330   **Genes related to VEGF signaling are downregulated in old macaques but not juvenile**

331   **macaques during COVID-19-disease**

332   Using the same approach as for the juvenile macaque-specific differentially regulated genes, we

333   identified 97 genes significantly up-regulated (**Figure 3A**; **Table S3I**) and 160 genes significantly

334   down-regulated (**Figure 3B**; **Table S3J**) with COVID-19 disease only in infected old macaques,

335   and not infected juveniles. Pathway enrichment analysis only identified significant functional

336   enrichment among the down-regulated gene set (**Table 4**; **Table S4D**). Our results show that in

337   the lungs of old macaques, the only Reactome pathways enriched among genes downregulated

338   during COVID-19 included genes involved in the "VEGF-VEGFR2 Pathway" and "Signaling by

339   VEGF" (**Figure 6, 7**). Vascular endothelial growth factor (VEGF) is a signaling protein that

340   promotes angiogenesis, and is a key factor that promotes ARDS. Previous research showed that

341   ACE2 antagonizes and down-regulates VEGFA(*54*), improving lung function following acute lung

342   injury (*55*). Here, we observe both a significant increase in ACE2 in response to COVID-19 and

343   a significant decrease in VEGF pathways in old macaques, which may be due to this antagonistic

344   relationship. VEGFA, p21-activated kinase (PAK2), cytoplasmic tyrosine kinase (SRC),

345   RhoA/ROCK signaling [ROCK1(Rho Associated Coiled-Coil Containing Protein Kinase 1) and

346   WASF2(WASP Family Member 2) are all essential for multiple aspects of VEGF-mediated

347   angiogenesis and are all significantly downregulated in old macaques with COVID-19 (**Figure 7**).

348   Overall, despite juvenile and old macaques having a comparable clinical course with resolution,

349   our data suggest that there are significant differenes in signaling pathways, especially those

350   related to VEGF signaling that may ultimately result in differences is long term outcomes. Thus,

351   our results suggest that down-regulation of VEGF pathways is associated with increasing age, in

352   a macaque cohort of self-limiting disease model, and protect from serious lung injury during

353   COVID-19 disease.

354   **Aged COVID-19 patients exhibit increased plasma VEGF protein levels and high peripheral**

355   **neutrophil to lymphocyte ratio**

356   To further address if our findings were relevant in the human setting of SARS-CoV-2 infection, we

357   stratified COVID-19 patients into aged group (>60 years) and a group of COVID-19 patients <60

358   years (**Table S5**). We found that with increasing age, there were increased association of disease

359   parameters and comorbidities (**Table S5**). We measured the levels of human plasma proteins

360   levels for IFN-α, IFN-β and IFN-γ. While levels of plasma IFN-α, and IFN-β were below the levels

361   of reliable detection, we found that the COVID-19 patients who were <60 years expressed

362   significantly higher plasma IFN-γ levels when compared to levels in plasma of healthy controls

363   (**Fig. 8A**). Although plasma levels of IFN-γ protein was also increased in aged

364   COVID-19 patient group, levels were not significantly different from healthy controls (**Fig. 8A**).

365   This was in contrast to plasma protein levels of VEGF, which was significantly higher in aged

366   individuals with COVID-19 disease when compared with levels in individuals with COVID-19

367   disease who were <60 years old (**Fig. 8B**). The increased levels of VEGF in aged COVID-19

368   patients coincided with significantly increased peripheral neutrophil counts as well as increased

369   peripheral neutrophil to lymphocyte ratios, when compared with both healthy controls and COVID-

370   19 group <60 years old (**Fig. 8C,D**). These results show that plasma protein levels of VEGF and

371   accumulation of peripheral neutrophils is increased in aged individuals with COVID-19 disease,

372   when compared to younger individuals with COVID-19 disease.

373   **Neutrophil degranulation and IFN pathways overlap between COVID-19 and TB disease**.

374   Tuberculosis (TB) is a pulmonary granulomatous disease caused by infection with *Mycobacterium*

375   *tuberculosis.* TB disease in humans and macaques is associated with a neutrophil and IFN

376   signature(*13*). Thus, we next compared and contrasted the transcriptional profile of genes and

377   pathways that are shared by the two diseases, and those that are unique to COVID-19.There was

378   not a substantial overlap between differentially expressed genes in response to COVID-19 and

379   TB. However, of the 97 genes that were commonly upregulated in TB and COVID-19 (**Figure**

380   **9A**, **Table S3K**), the Reactome pathway enrichment was well featured in "Neutrophil

381 degranulation", "Innate immune response", and "Interferon gamma signaling" (**Figure 9B, Table**

382 **S4E**). Nearly as many genes (76) had opposite differential expression patterns (upregulated in

383 COVID-19, downregulated in TB), as genes upregulated in both (**Figure 10A**, **Table S3L**). These

384 genes were associated with blood vessel morphogenesis and angiogenesis including leptin

385 receptor (LEPR), TGFβ2 (**Figure 10B, Table S4F**). These results suggest that both TB and

386 COVID-19 share features of neutrophil accumulation of IFN signaling, but that COVID-19 disease

387 immunopathogenesis uniquely features vascularization of the lung.

388

389 **DISCUSSION**

390 Lack of understanding of the complexity of COVID-19 immunopathogenesis hampers

391 identification of therapeutic strategies for COVID-19. While studies using immune profiling in

392 COVID-19 patients have shed light on related immune mechanisms of this disease, these have

393 primarily involved peripheral samples obtained from moderate to severe COVID-19 patients, who

394 are generally also older. To overcome these limitations, we have generated a nonhuman primate

395 model (rhesus macaques) of SARS-CoV-2 infection that reflects several features of the

396 immunopathogenesis of human COVID-19, and provides a platform to interrogate the immune

397 pathways that mediate disease versus protection, especially in the context of young versus older

398 hosts. In this study, we show that upregulation of pathways characteristic of neutrophil

399 degranulation and IFN signaling are characteristic of COVID-19 disease in infected hosts.

400 Importantly, the significantly higher induction of genes associated with Type I IFN signaling

401 pathway and Notch signaling in young macaques infected with SARS-CoV-2 is a key determinant

402 that distinguishes them from infected old macaques. Lungs of old macaques infected with COVID-

403 19 however, uniquely feature downregulation of VEGF signaling pathways. Importantly, in PBMCs

404 of humans infected with SARS-CoV-2 we found increased levels of VEGF and peripheral

405 neutrophil counts in individuals >60 years when compared to younger individuals. These results

406   together provide novel insights into the immunopathogenesis of COVID-19 disease, especially

407   from the unique perceptive of age as a contributing factor.

408   As we learn more about the pathophysiology of COVID-19, it is becoming clear that disease

409   severity is associated with hyperinflammation which in turn induces lung and multiorgan injury

410   and mortality via a cytokine storm (*1, 2, 56*). While therapeutic options that focus on

411   immunomodulatory agents such as corticosteroids are being considered and used, a risk exits

412   that immunomodulators may also inhibit protective pathways. Therefore, a thorough

413   understanding of the host inflammatory responses during SARS-CoV-2 infection is needed before

414   precise immunomodulators can be specifically designed to limit inflammation without regulating

415   protective mechanisms of action. The distinct role of myeloid cells in COVID-19 lung injury and

416   immunopathogenesis is just beginning to be described, and we have clearly shown that

417   neutrophils are intensely recruited to the lung compartment in macaques after SARS-CoV-2

418   infection (12) (in review). Neutrophils can play a protective role contributing to early antiviral

419   defense (*57*), but also can be pathological due to processes associated with degranulation and

420   lysis, thereby promoting lung inflammation. Consistent with this notion, in current COVID-19

421   literature, an increased peripheral neutrophil-to-lymphocyte ratio is observed in severe COVID-

422   19 cases, and in some studies is also associated with unfavorable prognosis (*58*). These results

423   in human studies are consistent with our macaque studies that describe neutrophil degranulation

424   as one of the top transcriptional pathways up-regulated in the lungs of COVID-19 macaques when

425   compared to uninfected controls. In this regard, expression of Cathepsin G is northworthy since

426   it is prominent serine protease that amplifies inflammation by stimulating the production of

427   cytokines and chemokines that drive immune cell recruitment to the lung (*59*), and activates

428   metalloproteases to cleave extracellular matrix proteins, thereby promoting neutrophil migration

429   (*60*). Cathepsin G also induces potent chemotactic recruitment of monocytes, neutrophils and

430   antigen presenting cells in addition to promoting endothelial and epithelial permeability (*61*). The

431   latter function of Cathepsin G could be important in enhancing viral invasion to extra-alveolar sites

432   while increased epithelial permeability might also explain the gastrointestinal route of transmission

433   (12) (in review). Additionally, ATP6AP2, causes secretion of inflammatory and fibrotic factors

434   (*41*), CD36, that induces neutrophil apoptosis, and CECAM8 whose cross-linking induces IL-8

435   production , all of which are highly expressed in COVID-19 diseased lungs. In patients with severe

436   COVID-19, neutrophils express higher frequency of CD66b$^+$ neutrophils(*62*). These different

437   genes that are up-regulated as part of the neutrophil degranulation/innate immune response

438   pathways suggest a prominent role for neutrophils that can promote inflammation and a cytokine

439   storm leading to COVID-19 disease pathogenesis. Furthermore, our studies shed light on the

440   importance of the membrane glycoprotein, CD36 in the response to SARS-CoV-2 infection. CD36

441   is expressed on platelets, macrophages and even epithelial cells. In addition to its well

442   characterized apoptotic function, CD36 is also a receptor for thrombospondin-1 and related

443   proteins and can function as a negative regulator of angiogenesis(*78*). This is particularly

444   important given that angiogenesis is an important feature in patients with COVID-19 and

445   associated ARDS (*79*). CD36 also binds long-chain fatty acids and facilitates their transport into

446   cells, leading to muscle utilization, coupled with fat storage. This contributes to the pathogenesis

447   of metabolic disorders, such as diabetes and obesity and atherothrombotic disease (*79*). A recent

448   single-cell analysis revealed significantly higher CD36 expression in association with ACE2-

449   expressing human lung epithelia cells (*80*). Increased CD36 expression may therefore provide a

450   protective role from extreme lung injury during COVID-19, which is observed in the macaques.

451   Our novel findings that CD36 (as well as other prominent signaling pathways) may be involved in

452   the pathogenesis of COVID-19 has implicaitons for host-direc ted therapy for SARS-CoV-2

453   infection. In contrast, neutrophils are recruited into the lung very early following macaque infection

454   with SARS-CoV-2(*12*) (in review). Additionally, in lungs of deceased individuals with severe

455   COVID-19 disease neutrophil infiltration occurred in pulmonary capillaries and was accompanied

456   with extravasation of neutrophils into the alveolar space, and neutrophilic mucositis(*63*). In the

457   case of COVID-19, neutrophils could also be a source of excess neutrophil extracellular traps

458   (*64*). Cytokine storm characterized by increased plasma concentrations of IL1β, IL2, IL6, IL7, IL8,

459   IL10, IL17, IFNγ, IFNγ-inducible protein 10, monocyte chemoattractant protein 1 (MCP1), G-CSF,

460   macrophage inflammatory protein 1α, and TNFα seen in severe COVID-19 patients can regulate

461   neutrophil activity by upregulating the expression of chemoattractants that recruit myeloid cells to

462   the lung. These results are also consistent with upregulation of pathways associated with immune

463   and innate signaling, especially IFN signaling. These results together suggest a scenario in the

464   lung where induction of the cytokine storm drives the recruitment of neutrophils, thereby

465   contributing to inflammation. Thus, degranulation of neutrophils and formation of NETs may

466   further promote cytokine responses and inflammation and disease immunopathogenesis.

467   The IFN response constitutes the major first line of defense against viruses. Recognition of viral

468   infections by innate immune sensors activates both the type I and type III IFN response. While

469   some studies have shown that serum of COVID-19 patients contains increased expression of pro-

470   inflammatory cytokines and chemokines, without detectable levels of type I and III IFNs(*65*), other

471   studies suggest that the IFN response may be delayed. Importantly, elevated IFNs correlate with

472   more severe disease(*66, 67*). However, it is not fully clear if type I IFNs are protective or

473   pathological in COVID-19(*68*). Thus, it is possible that severe infection drives the higher

474   expression of genes in the IFN pathways, but may not lead to viral containment, but instead drives

475   pathological damage. On the other hand, increased induction of type I IFN signaling pathways in

476   SARS-CoV-2 infected macaques, as well as increased induction in juvenile macaques, could

477   support a role for IFN signaling in protection rather than disease progression. Our studies provide

478   data to support the recently proposed hypothesis that that IFN induction may be compromised in

479   older hosts(*68*). When the early IFN response is not optimal to control viral infection, it is possible

480   that delayed or inadequate IFN responses may lead to inflammation mediated damage. Not all

481   animal models, especially mice fully mimic the spectrum of human disease caused by SARS-

482   CoV-2, likely due to the regulatory responses of IFNs on viral entry receptors such as ACE2 which

483   are differentially regulated in humans compared to mice. Further testing the protective versus

484   pathological roles of IFNs in the macaque model with the availability of IFNAR blocking reagents

485   should further clarify the specific role of IFN pathways in COVID-19.

486   ARDS in influenza, MERS and SARS have been associated with fibrotic irreversible interstitial

487   lung disease(*69, 70*). Pulmonary fibrosis is a recognized sequelae of ARDS(*47*). Pulmonary

488   fibrosis can develop either following chronic inflammation or as a consequence of genetically

489   associated and age-related fibroproliferative process, as in idiopathic pulmonary fibrosis

490   (IPF)(*71*). Fibrosis is the hardening, and/or scarring of tissues due to excess deposition of

491   extracellular matrix components including collagen. Fibrosis is often the terminal result of

492   inflammatory insults induced by infections, autoimmune or allergic responses and others. It is

493   thought that the mechanisms driving fibrogenesis are divergent from those modulating

494   inflammation. The key cellular mediator of fibrosis is the excessive accumulation of fibrous

495   connective tissue (components of the ECM such as collagen and fibronectin) in and around

496   inflamed or damaged tissue. Since a significant proportion of COVID-19 patients develop severe

497   ARDS, it is predicted that a similar outcome of fibrosis will be associated with COVID-19. Also,

498   since the risk factors associated with COVID-19 including increasing age, male and associated

499   co-morbidities coincide with IPF risk factors, it is expected that COVID-19 patients will experience

500   fibrotic lung disease. Despite these associations, there is no evidence currently that "scarring of

501   the lung" experienced by COVID-19 patients is fibrotic or progressive and an outcome of COVID-

502   19 disease post recovery. Therefore, our results provide unique insights into the role of fibrosis

503   during SARS-CoV-2 infection. Most notably, we find significant downregulation of collagen

504   degradation pathways, as well as pathways associated with collagen formation, collagen

505   trimerization and assembly. Furthermore, the role for TGF-β and ECM degradation is well

506   documented in fibrosis. Indeed, the genes associated with these pathways are also significantly

507   down-regulated. These results for the first time provide novel insights into the early pathological

508   events occurring during COVID-19 in the lungs with relevance to underlying immune mechanisms

509   associated with canonical fibrosis pathways. While long term consequences of the pulmonary

510   COVID-19 such as fibrosis remain to be determined, our results on down-regulation of collagen

511   degradation and TGF-β pathways may represent important early events on the lungs of SARS-

512   CoV-2 infected individuals.   We speculate that such events may protect individuals from

513   progression to ARDS and fibrosis, while it is possible that in individuals with early activation of

514   collagen degradation progress more severe outcomes may ensue.

515   Finally, we provide novel insights into the transcriptional regulation of immune pathways that are

516   induced and regulated by age, an important risk factor for COVID-19 disease and outcome. This

517   is a significant component of risk for disease and prognosis of COVID-19. We find higher induction

518   of genes associated with Type I IFN signaling and Notch signaling in the old mecaque. Up-

519   regulation of these significant Type I IFN signaling genes suggest that in a model of self-limited

520   clinical disease in macaques, Type I IFN induction may be differentially regulated by age-

521   associated factors. Age-specific regulation of this pathway has been demonstrated in the murine

522   model of TB(*72*). There is also a well-documented relationship between Notch signaling and viral

523   infections. For example, Human Papilloma Virus and Simian Virus 40 can highjack the cellular

524   machinery, including components of Notch signaling, and these events re associated with cancer

525   progression(*73*). Most studies thus far have only followed SARS-CoV-2 infected macaque for up

526   to two weeks, and it was initially thought that this virus causes acute infection. However, details

527   are now emerging from both animal models(*12*) (in review) and patients, that the virus can persist

528   for longer periods, leading to persistent shedding from tissues, and exhaustion of adaptive

529   responses. While innate and T cell responses are comparable between juvenile and old

530   macaques following infection, SARS-CoV-2 specific antibody is generated at significantly higher

531   levels in the plasma of juveniles, relative to old macaques(*12*) (in review). Since Notch signaling

532   regulates multiple stages of B-cell differentiation and shapes the antibody repertoire(*74*), higher

533   expression of many of the Notch pathway member genes in juvenile macaques may be

534   responsible for the development of stronger antibody responses in these animals, impacting

535   disease progression. Alternatively, it is possible that the differences in Notch signaling and

536   production of virus-specific antibody between jouvenile and old macaques may impact disease

537   progression over a longer period of time, or be particularly relevant in models of co-morbidity,

538   such as diabetes. Similarly, Type I IFN responses are critical for the downstream breadth of

539   antibody production and recognition (*75-77*). Thus, while T cell responses are comparable in

540   juvenile and old macaques, differences in critical signaling pathways uncovered by our RNA-seq

541   analysis potentially explain why juvenile macaques mount significantly stronger antibody

542   responses, and consequently why younger subjects have reduced susceptibility to COVID-19.

543   While this has not been recapitulated in the macaque model, older patients of COVID-19 are more

544   susceptible to progression. This is consistent with increased disease progression when COVID-

545   19 patients were stratified based on age. A previous study found that peripheral VEGF

546   concentrations were significantly higher in COVID-19 patients than in healthy controls(*81*). We

547   also find this effect in our human samples (**Figure 8B**) where people with COVID-19 that are older

548   than 60 years of age have more VEGF protein in their peripheral blood. However, we also find

549   significantly lower levels of VEGF pathway gene transcripts in the lungs of macaques with SARS-

550   CoV-2 infection, especially older macaques (**Figure 6, 7**). Our study further demonstrates that the

551   changes in VEGF signaling may be associated with increasing age rather than just with disease

552   severity. VEGF pathways promote angiogenesis and induce vascular leakiness and permeability.

553   Our results therefore suggest that higher levels of VEGF in the periphery, while a biomarker for

554   COVID-19, may be driven as a compensatory mechanism due to lower levels of VEGF signaling

555   at the site of infection, i.e, the lung. These results further underscore the value of studying

556   responses to SARS-CoV-2 infection in the lung compartment. By uncovering new aspects of the

557   role of these signaling pathway in SARS-CoV-2 infection in the lung compared to the periphery

558   using animal models and human samples, will shed further light on pathways that can be

559   harnessed for therapeutics for COVID-19 disease.

560 TB and COVID-19 both primarily affect lung function. TB was already one of the leading causes

561 of death due to an infectious disease prior to emergence of COVID-19. In the current scenario

562 the clinical management of both TB and COVID together, particularly in the endemic regions is

563 another rapidly emerging healthcare challenge needing immediate attention. In order to properly

564 address the solution for this emerging crisis a better understanding of the comparative

565 immunological manifestations of both the diseases must be understood. Our results are the first

566 to clearly demarcate the main differences in the manifestation of both the diseases in the alveolar

567 niche. Neutrophil degranulation was one of the most significantly enriched pathways in both the

568 disease conditions and therefore appears as a promising druggable target for efficient

569 management of severe co-morbid TB COVID-19 condition. However, the selective enrichment of

570 angiogenesis and vascular permeability in observed in the lungs of SARS-CoV-2 infected

571 macaques is not seen in models, or patients of TB. These results have the potential to generate

572 additional, specific druggable targets for COVID-19.

573 Overall, we interrogated transcriptional profiles of lungs from juvenile and old macaques infected

574 with SARS-CoV-2. This study has provided fundamentally new information on the host response

575 in young and old macaques infected with SARS-CoV-2, a model that provides relevant insights

576 necessary for further vaccine and therapeutic development for COVID-19 and a subset of these

577 observations confirmed in human samples with control of SARS-CoV-2 infection as well as

578 COVID-19 disease, and as a function of age.

579

600

601   **Author Contributions**. B.A.R., M.A., D.S., J.C., B.S., J.M., O. G, J.A.C-P., L.A.J-A., T.S.R-R.,

602   J.Z. carried out experiments, analysed data; J.Z., L.S.S., J.T., R.C., M.M., D.K., and S.A.K

603   designed the study, provided funding or reagents; M.A., B.A.R., D.K., and S.A.K wrote the paper;

604   all authors read, edited and approved the manuscript.

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799   **Figure Legends**

800   **Figure 1**: **Genes upregulated in COVID-19-infected macaques represent pathways**

801   **characteristic of neutrophil degranulation and IFN signaling.** Differential gene expression

802   between naive and COVID-19 samples. (**A**) PCA plot showing the clustering of samples based

803   on overall transcriptomic profiles. (**B**) Gene expression plot showing the relative normalized gene

804   expression levels (FPKM) for each gene, with genes significantly differentially regulated by

805   COVID-19 indicated.

806   **Figure 2: Genes downregulated in COVID-19-infected macaques represent pathways**

807   **characteristic of collagen degradation and TFG-β signaling.** The top 30 most significantly (**A**)

808   upregulated genes and (**B**) downregulated genes in COVID-19 infected macaque lungs.

809   Expression values are visualized by Z scores of normalized expression data (FPKM) per sample,

810   and Log_2 Fold Change and -Log P values are from the DESeq2 output. Genes are sorted by P

811   value. (**C**) Multilabel confocal immunofluorescence microscopy of FFPE lung sections from SARS

812   CoV-2 infected rhesus macaques with SARS CoV-2 Spike specific antibody (green), neutrophil

813   marker CD66abce (red) and DAPI (blue) at 10X magnification.

814   **Figure 3: 86 genes significantly upregulated and 96 genes significantly downregulated with**

815   **COVID-19 only in juvenile macaques.** Scatterplots visualizing the significance values of COVID-

816   19 upregulated (**A**) and downregulated (**B**) genes, in juvenile and old macaques. Green shaded

817   areas contain genes significant only in juveniles, and red shaded areas contain genes significant

818   only in old macaques.

819   **Figure 4: Genes related to Type I interferon signaling are upregulated in juvenile macaques**

820   **compared to old macaques during COVID-19-infection.** The top 30 most significantly (**A**)

821   upregulated genes and (**B**) downregulated genes in COVID-19 infected juvenile macaque lungs

822   but not in old macaques. Expression values are visualized by Z scores of normalized expression

823   data (FPKM) per sample, and Log_2 Fold Change and -Log P values are from the DESeq2 output.

824   Genes are sorted by P value. (**C**) The relative gene expression of ACE2 and ADAM17 among

825   naive, juvenile and old COVID-19 infected macaques.

826   **Figure 5**: **Interferon alpha signaling genes are significantly upregulated in juvenile COVID-**

827   **19-infected macaques but not old COVID-19-infected macaques.** The relative expression

828   levels (FPKM) for the five "interferon alpha signaling" (HSA-909733) genes belonging to this gene

829   set are shown. P values represent FDR-corrected significance values from DESeq2.

830 **Figure 6: Genes related to VEGF signaling are downregulated in old macaques compared**

831 **to juvenile macaques during COVID-19.** The top 30 most significantly (**A**) upregulated genes

832 and (**B**) downregulated genes in infected old macaque lungs but not in juvenile macaques.

833 Expression values are visualized by Z scores of normalized expression data (FPKM) per sample,

834 and Log_2 Fold Change and -Log P values are from the DESeq2 output. Genes are sorted by P

835 value.

836 **Figure 7: VEGF pathway genes are significantly downregulated in old COVID-19-infected**

837 **macaques but not juvenile COVID-19-infected macaques.** The relative expression levels

838 (FPKM) for the seven "Signaling by VEGF" (R-HSA-194138) genes belonging to this gene set are

839 shown. P values represent FDR-corrected significance values from DESeq2.

840 **Figure 8. VEGF and peripheral neutrophil counts are higher in old COVID-19 patients.**

841 Peripheral blood samples were obtained from a cohort of patients with laboratory-confirmed

842 SARS-CoV-2 infection at hospital admission. Levels of different immune markers were

843 determined by Luminex assay in plasma samples from COVID-19 and healthy volunteer controls.

844 COVID-19 patients were stratified by age as younger than or older than 60 years. (A) Levels of

845 IFN-γ and (B) levels of VEGF proteins were measured in plasma of COVID-19 and healthy

846 controls. Peripheral neutrophil counts (C) and neutrophil to lymphocyte ratio (NLR) values (D)

847 were retrieved from the medical records of COVID-19 patients and compared between age

848 groups.

849 **Figure 9: Genes higher in expression during both COVID-19 and TB share common**

850 **pathways.** (**A**) The top 50 (of 97) most significantly upregulated genes in COVID-19 infected and

851 TB infected macaques. Expression values are visualized by Z scores of normalized expression

852 data (FPKM) per sample, and Log_2 Fold Change and -Log P values are from the DESeq2 output.

853 Genes are sorted by P value. (**B**) Significant Reactome pathway enrichment among the 97 genes.

854 **Figure 10: Genes higher in expression during COVID-19 than TB are related to blood**

855 **morphogenesis pathways.** (**A**) The top 50 (of 76) most significantly upregulated genes in

856 COVID-19 infected compared to TB-infected macaques. Expression values are visualized by Z

857 scores of normalized expression data (FPKM) per sample, and Log_2 Fold Change and -Log P

858 values are from the DESeq2 output. Genes are sorted by P value. (**B**) Significant Gene Ontology

859 pathway enrichment among the 76 genes.

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Symbol	Gene name	Log₂ FC	-Log P
SH3BGRL2	SH3 domain binding glutamate rich protein like 2	2.23	13.0
ATP6AP2	ATPase H+ transporting accessory protein 2	1.67	13.0
PPT1	palmitoyl-protein thioesterase 1	1.92	12.4
IFNGR1	interferon gamma receptor 1	1.96	12.2
SPCS2	signal peptidase complex subunit 2	1.52	11.6
C1GALT1C1	C1GALT1 specific chaperone 1	1.91	11.2
LMBRD1	LMBR1 domain containing 1	1.73	11.2
CLDND1	claudin domain containing 1	1.63	11.0
GFM1	G elongation factor mitochondrial 1	1.54	10.9
TTC5	tetratricopeptide repeat domain 5	1.87	10.8
JKAMP	JNK1/MAPK8 associated membrane protein	2.18	10.8
CCDC126	coiled-coil domain containing 126	2.07	10.8
ARL6IP5	ADP ribosylation factor like GTPase 6 interacting 5	1.67	10.8
CD46	CD46 molecule	1.63	10.7
TSC22D1	TSC22 domain family member 1	1.89	10.8
SPCS2	signal peptidase complex subunit 2	1.44	10.2
CTSG	cathepsin G	4.89	9.7
CD36	CD36 molecule	2.29	9.5
IDH1	isocitrate dehydrogenase (NADP(+)) 1	1.70	9.5
OSER1	oxidative stress responsive serine rich 1	1.48	9.4
ZDHHC6	zinc finger DHHC-type palmitoyltransferase 6	1.69	9.4
CD58	CD58 molecule	1.45	8.8
SELENOT	selenoprotein T	2.06	8.8
IGFBP3	insulin like growth factor binding protein 3	1.25	8.7
TMEM267	transmembrane protein 267	2.62	8.7
PIGK	phosphatidylinositol glycan anchor biosynthesis K	1.75	8.7
TMEM128	transmembrane protein 128	1.60	8.7
PLSCR1	phospholipid scramblase 2	1.75	8.7
SPTLC1	serine palmitoyltransferase long chain base subunit 1	1.54	8.5
COPB1	COPI coat complex subunit beta 1	1.23	8.4

Naive uninfected — COVID-19 (Juvenile, Old)

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Symbol	Gene name	Log₂ FC	-Log P
PALM	paralemmin	-3.53	12.8
COL1A1	collagen type I alpha 1 chain	-1.92	11.2
PURA	purine rich element binding protein A	-2.15	11.0
RRBP1	ribosome binding protein 1	-2.30	10.8
ZFP36	ZFP36 ring finger protein	-1.70	10.6
LENG8	leukocyte receptor cluster member 8	-1.89	10.3
DBN1	drebrin 1	-1.66	10.2
MYH14	myosin heavy chain 14	-2.95	10.1
INTS1	integrator complex subunit 1	-2.64	9.9
TCIRG1	T cell immune regulator 1, ATPase H+ transporting V0	-1.69	9.7
RABL6	RAB, member RAS oncogene family like 6	-4.00	9.7
CHD3	chromodomain helicase DNA binding protein 3	-2.22	9.7
MFSD12	major facilitator superfamily domain containing 12	-2.41	9.6
STK11	serine/threonine kinase 11	-2.36	9.6
SH3PXD2A	SH3 and PX domains 2A	-3.64	9.4
NCLN	nicalin	-1.89	9.4
SNX29	sorting nexin 29	-1.88	9.3
NIBAN2	niban apoptosis regulator 2	-2.36	9.1
AURKAIP1	aurora kinase A interacting protein 1	-2.25	9.0
FBXW5	F-box and WD repeat domain containing 5	-2.00	8.8
CEP104	centrosomal protein 104	-1.87	8.8
HSPG2	heparan sulfate proteoglycan 2	-2.28	8.7
PTH1R	parathyroid hormone 1 receptor	-3.70	8.7
ARHGAP45	Rho GTPase activating protein 45	-2.82	8.6
CUEDC1	CUE domain containing 1	-3.08	8.6
INF2	inverted formin, FH2 and WH2 domain containing	-2.13	8.5
MMP23B	matrix metallopeptidase 23B	-2.47	8.5
TNK2	tyrosine kinase non receptor 2	-2.42	8.4
CDC42BPG	CDC42 binding protein kinase gamma	-2.54	8.3
VPS9D1	VPS9 domain containing 1	-2.68	8.2

Naive uninfected — COVID-19 (Juvenile, Old)

Z score of expression: <-1 -0.5 0 0.5 >1 >2

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Symbol	Gene name	Log$_2$ FC (Juv/Old)	-Log P Juv	-Log P Old
RIN2	Ras and Rab interactor 2	1.23	4.5	0.2
-	ENSMMUG00000016082: zinc finger protein 252	1.44	3.5	0.0
AXL	AXL receptor tyrosine kinase	1.21	3.9	0.2
MX1	MX dynamin like GTPase 1	1.54	2.4	0.1
ST6GAL1	ST6 beta-galactoside alpha-2,6-sialyltransferase 1	1.74	4.4	0.5
MX2	MX dynamin like GTPase 2	1.53	2.2	0.1
NRIP1	nuclear receptor interacting protein 1	0.87	2.1	0.0
ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase	0.96	2.1	0.0
MS4A4E	membrane-spanning 4-domains subfamily A member 4A-like	2.84	2.7	0.1
BMP3	bone morphogenetic protein 3	2.02	3.4	0.4
LILRA2	leukocyte immunoglobulin-like receptor, subfamily A 2	2.66	2.2	0.0
GRAMD2B	GRAM domain containing 2B	0.90	2.2	0.1
WBP1L	sideroflexin 2	0.85	2.8	0.1
-	ENSMMUG00000050862	1.47	4.2	0.9
-	ENSMMUG00000059006	1.76	2.0	0.1
KAT1	kynurenine aminotransferase 1	1.43	2.2	0.1
L3MBTL3	L3MBTL histone methyl-lysine binding protein 3	1.39	2.3	0.1
CD74	CD74 molecule	0.74	3.5	0.6
FOXJ3	forkhead box J3	0.94	2.8	0.3
GNAQ	G protein subunit alpha q	0.80	2.6	0.2
FGD2	FYVE, RhoGEF and PH domain containing 2	1.65	2.8	0.3
KIR3DH	Macaca mulatta killer-cell Ig-like receptor KIR3DL17 (KIR3DL17)	1.79	3.8	0.8
RELL1	RELL like 1	1.17	3.8	0.8
ABHD17B	abhydrolase domain containing 17B	1.23	2.3	0.2
-	ENSMMUG00000049530: zinc finger protein 626-like	1.48	2.3	0.2
C20H16orf54	chromosome 20 C16orf54 homolog	1.31	2.0	0.1
USP18	ubiquitin specific peptidase 18	1.27	2.5	0.3
GGA2	golgi associated, gamma adaptin ear containing, ARF binding 2	1.47	2.0	0.1
-	ENSMMUG00000057791	1.47	2.8	0.5
CD47	CD47 molecule	1.08	2.4	0.3

Heatmap columns: Naive uninfected | COVID-19 Juvenile | COVID-19 Old

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Symbol	Gene name	Log$_2$ FC (Juv/Old)	-Log P Juv	-Log P Old
SLC27A6	solute carrier family 27 member 6	-4.40	2.2	0.5
UCHL1	ubiquitin C-terminal hydrolase L1	-1.30	3.4	0.1
-	ENSMMUG00000028699	-1.86	5.1	0.9
PPP1R14A	protein phosphatase 1 regulatory inhibitor subunit 14A	-1.68	2.7	0.1
CBR1	carbonyl reductase 1	-1.41	3.0	0.2
CXCL14	C-X-C motif chemokine ligand 14	-2.64	2.8	0.1
SFI1	SFI1 centrin binding protein	-1.55	3.3	0.3
ATAD3A	ATPase family AAA domain-containing protein 3A-like	-1.54	2.7	0.1
NT5C3B	5'-nucleotidase, cytosolic IIIB	-1.23	2.4	0.1
NEFL	neurofilament light	-4.06	3.5	0.4
ZFHX4	zinc finger homeobox 4	-4.20	2.8	0.2
FRMD3	FERM domain containing 3	-2.51	4.1	0.8
ALOX15	arachidonate 15-lipoxygenase	-3.03	2.1	0.0
GADD45G	growth arrest and DNA damage inducible gamma	-1.74	2.4	0.1
EFNB1	ephrin B1	-1.28	2.9	0.3
SPAG7	sperm associated antigen 7	-1.46	2.7	0.2
SIVA1	SIVA1 apoptosis inducing factor	-1.07	2.8	0.3
PRSS16	serine protease 16	-2.21	2.4	0.2
ZNF511	zinc finger protein 511	-1.92	2.0	0.1
SERPINF1	serpin family F member 1	-1.10	2.6	0.2
TMED1	transmembrane p24 trafficking protein 1	-1.01	3.1	0.5
ND1	NADH dehydrogenase subunit 1	-1.53	3.2	0.5
FUOM	fucose mutarotase	-1.10	2.5	0.3
TRIP10	thyroid hormone receptor interactor 10	-0.76	2.8	0.4
NUDT16	U8 snoRNA-decapping enzyme-like	-0.99	3.8	0.9
FAM167B	family with sequence similarity 167 member B	-1.80	2.2	0.2
RANBP1	RAN binding protein 1	-0.91	2.8	0.5
MSLN	mesothelin	-3.40	2.8	0.5
THAP7	THAP domain containing 7	-1.16	2.8	0.5
-	ENSMMUG00000006283: 60S ribosomal protein L18a-like	-2.04	2.8	0.5

Heatmap columns: Naive uninfected | COVID-19 Juvenile | COVID-19 Old

Z score of expression: <-1 -0.5 0 0.5 >1 >2

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Legend: ○ Naive · ○ COVID-19, Juvenile · ○ COVID-19, Old

Scatter plot — y-axis: ADAM17 expression level (FPKM), 0–35; x-axis: ACE2 expression level (FPKM), 0–3.

R² = 0.60
P = 0.003

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Figure 6

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Symbol	Gene name	Naive uninfected / COVID-19 (Juvenile / Old)	Log₂ FC	-Log P	Tuberculosis (Naive / Controller / Progressor)	Log₂ FC	-Log P
FCGR1A	Fc fragment of IgG receptor Ia		1.08	3.0		2.42	23.5
GBP7	guanylate-binding protein 6-like		1.84	2.6		3.20	6.2
PPT1	palmitoyl-protein thioesterase 1		1.92	12.4		0.90	5.3
STT3A	STT3 oligosaccharyltransferase complex catalytic A		1.32	7.9		0.68	3.3
CTBS	chitobiase		1.59	7.7		0.72	2.2
FPR2	formyl peptide receptor 2		5.28	6.7		3.54	8.9
GCNT1	glucosaminyl (N-acetyl) transferase 1		2.54	6.7		1.37	4.0
NECAP1	NECAP endocytosis associated 1		1.44	6.6		0.64	2.7
TEFM	transcription elongation factor, mitochondrial		1.72	5.8		1.06	4.1
B2M	beta-2-microglobulin		1.62	5.8		1.39	5.5
HEXA	hexosaminidase subunit alpha		0.87	5.5		1.03	7.3
KIT	KIT proto-oncogene, receptor tyrosine kinase		2.11	5.4		2.53	9.6
GLA	galactosidase alpha		1.16	5.2		1.27	8.9
COX15	cytochrome c oxidase assembly homolog COX15		1.15	5.1		0.39	1.4
SLAMF6	SLAM family member 6		1.66	5.1		2.49	12.5
KCNJ2	potassium inwardly rectifying channel subfamily J2		2.30	5.1		1.35	10.0
EDEM2	ER degradation enhancing alpha-mannosidase like 2		1.17	4.9		0.91	4.4
ADGRE1	adhesion G protein-coupled receptor E1		2.04	4.7		0.94	1.9
M6PR	mannose-6-phosphate receptor, cation dependent		1.19	4.7		0.50	1.7
DERL2	derlin 2		1.19	4.6		0.46	2.0
SCPEP1	serine carboxypeptidase 1		1.31	4.4		1.06	4.7
SLC38A6	solute carrier family 38 member 6		1.90	4.3		1.16	3.0
CPQ	carboxypeptidase Q		1.13	4.3		0.71	1.6
LRR1	leucine rich repeat protein 1		1.15	4.2		1.01	2.9
ARHGAP25	Rho GTPase activating protein 25		0.96	4.2		0.77	3.7
SH2D1A	SH2 domain containing 1A		1.62	4.1		2.01	1.9
SRGN	serglycin		1.23	4.0		1.51	6.1
CTSS	cathepsin S		1.43	4.0		1.91	10.3
RNF34	ring finger protein 34		0.91	4.0		0.42	2.2
WARS1	tryptophanyl-tRNA synthetase 1		1.01	3.9		3.34	25.8
CD40	CD40 molecule		0.98	3.8		1.90	12.0
PRPF4	pre-mRNA processing factor 4		0.59	3.8		0.44	1.6
CCRL2	C-C motif chemokine receptor like 2		1.42	3.7		0.79	1.6
VDAC2	voltage dependent anion channel 2		0.71	3.5		0.43	2.3
DHRS9	dehydrogenase/reductase 9		2.68	3.5		2.65	9.3
CDCA7	cell division cycle associated 7		3.68	3.4		2.51	7.4
PDIA6	protein disulfide isomerase family A member 6		0.88	3.4		1.16	5.5
RNFT1	ring finger protein, transmembrane 1		1.45	3.4		0.51	1.7
SLC31A2	solute carrier family 31 member 2		1.43	3.3		1.97	13.2
CD44	CD44 molecule (Indian blood group)		0.88	3.3		1.17	9.4
EED	embryonic ectoderm development		1.39	3.2		1.07	2.3
TM6SF1	transmembrane 6 superfamily member 1		1.07	3.2		0.48	1.4
RTCB	RNA 2',3'-cyclic phosphate and 5'-OH ligase		0.66	3.2		0.63	2.3
PRCP	prolylcarboxypeptidase		1.32	3.2		1.06	3.3
NARS1	asparaginyl-tRNA synthetase 1		0.99	3.2		0.38	2.1
AGTRAP	angiotensin II receptor associated protein		0.83	3.1		1.29	5.9
AGA	aspartylglucosaminidase		1.33	3.1		0.76	3.9
PSAP	prosaposin		1.06	3.0		1.19	6.1
RNF114	ring finger protein 114		0.73	3.0		1.05	8.0
CLEC12A	C-type lectin domain family 12 member A		1.42	2.9		1.69	5.6

Z score of expression: <-1 -0.5 0 0.5 >1 >2

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Pathway ID	Pathway description	Total pathway size	# Significant genes	FDR-corrected P value
R-HSA-168256	Immune System	1997	35	2.0E-05
R-HSA-6798695	Neutrophil degranulation	479	15	5.7E-04
R-HSA-168249	Innate Immune System	1053	21	2.6E-03
R-HSA-877300	Interferon gamma signaling	92	6	0.017
R-HSA-1236975	Antigen processing-Cross presentation	99	6	0.021
R-HSA-1280218	Adaptive Immune System	756	15	0.045

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951   Figure 9

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Symbol	Gene name	COVID-19 Log₂ FC	COVID-19 -Log P	Tuberculosis Log₂ FC	Tuberculosis -Log P
CD46	CD46 molecule	1.63	10.7	-0.63	2.5
TSC22D1	TSC22 domain family member 1	1.89	10.6	-0.55	1.9
CD36	CD36 molecule	2.29	9.5	-0.93	1.4
SPTLC1	serine palmitoyltransferase long chain base subunit 1	1.54	8.5	-0.42	2.7
EIF4A2	eukaryotic translation initiation factor 4A2	1.17	7.9	-0.46	1.8
MYCT1	MYC target 1	1.55	7.5	-0.96	2.7
BCHE	butyrylcholinesterase	1.39	7.3	-1.39	4.8
RNASE4	ribonuclease, RNase A family, 4	1.26	7.0	-1.18	4.7
MEIS1	Meis homeobox 1	1.52	6.4	-1.74	7.8
ADGRF5	adhesion G protein-coupled receptor F5	1.36	5.8	-1.47	6.4
CLK1	CDC like kinase 1	1.59	5.6	-1.29	4.5
BBS9	Bardet-Biedl syndrome 9	1.62	5.5	-1.03	3.0
PIGG	phosphatidylinositol glycan anchor biosynthesis G	1.25	5.5	-0.36	2.3
S1PR1	sphingosine-1-phosphate receptor 1	1.83	5.5	-1.33	6.4
SPARCL1	SPARC like 1	1.35	5.1	-1.89	7.1
-	ENSMMUG00000012978	1.35	5.1	-0.94	3.9
OMD	osteomodulin	1.86	5.1	-1.28	7.5
TCP11L2	t-complex 11 like 2	1.65	5.0	-1.03	7.1
LEPR	leptin receptor	2.47	4.8	-3.59	10.8
GJA5	gap junction protein alpha 5	1.48	4.7	-1.72	5.3
TMEM100	transmembrane protein 100	1.70	4.2	-2.49	10.0
SGCE	sarcoglycan epsilon	1.45	4.2	-2.02	19.0
ARMCX1	armadillo repeat containing X-linked 1	1.21	4.2	-1.06	8.8
EDNRB	endothelin receptor type B	2.18	4.2	-1.16	3.3
ILK	integrin linked kinase	0.63	4.1	-0.55	2.3
TMEM236	transmembrane protein 236	4.40	4.0	-1.07	7.9
BMX	BMX non-receptor tyrosine kinase	1.38	3.9	-2.72	8.7
PLPP1	phospholipid phosphatase 1	0.92	3.7	-0.65	2.0
RAB33B	RAB33B, member RAS oncogene family	1.78	3.7	-0.54	2.2
KLHDC1	kelch domain containing 1	2.15	3.7	-1.89	5.6
PRPF18	pre-mRNA processing factor 18	1.21	3.7	-0.75	4.5
NQO1	NAD(P)H quinone dehydrogenase 1	1.63	3.6	-0.67	1.5
ADGRL4	adhesion G protein-coupled receptor L4	1.23	3.6	-0.91	1.8
TEK	TEK receptor tyrosine kinase	1.56	3.5	-1.65	6.8
CLIC4	chloride intracellular channel 4	1.49	3.5	-0.57	2.0
MMUT	methylmalonyl-CoA mutase	1.52	3.4	-0.59	2.1
LRRC4C	leucine rich repeat containing 4C	2.93	3.4	-1.81	2.2
MAGEH1	MAGE family member H1	1.10	3.4	-0.64	1.5
CNOT8	CCR4-NOT transcription complex subunit 8	0.93	3.4	-0.61	2.4
DDX47	DEAD-box helicase 47	0.93	3.3	-0.42	1.7
FAM120B	family with sequence similarity 120B	0.71	3.2	-0.33	2.6
NDUFA9	NADH:ubiquinone oxidoreductase subunit A9	0.71	3.2	-1.74	11.5
EMCN	endomucin	1.99	3.1	-1.18	2.0
MAMDC2	MAM domain containing 2	1.07	3.1	-1.70	5.4
FAM221A	family with sequence similarity 221 member A	1.26	3.0	-1.36	4.2
PLS3	plastin 3	0.75	2.9	-1.01	3.7
GABARAPL2	GABA type A receptor associated protein like 2	0.80	2.9	-1.07	10.6
GULP1	GULP PTB domain containing engulfment adaptor 1	1.00	2.9	-2.69	9.8
SLCO2A1	solute carrier organic anion transporter family 2A1	1.61	2.9	-1.47	5.5
KDR	kinase insert domain receptor	1.06	2.8	-1.58	5.3

Heatmap column groups: Naive uninfected | COVID-19 (Juvenile, Old) | Tuberculosis (Naive, Con-troller, Progressor)

Z score of expression: <-1 -0.5 0 0.5 >1 >2

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Gene Ontology ID	GO description	Total pathway size	# Significant genes	FDR-corrected P value
blood vessel morphogenesis	GO:0048514 (BP)	435	14	2.0E-06
tube development	GO:0035295 (BP)	790	17	1.1E-05
blood vessel development	GO:0001568 (BP)	507	14	1.4E-05
angiogenesis	GO:0001525 (BP)	353	12	2.0E-05
vasculature development	GO:0001944 (BP)	530	14	2.5E-05
cardiovascular system development	GO:0072358 (BP)	538	14	3.0E-05
tube morphogenesis	GO:0035239 (BP)	641	15	3.5E-05
circulatory system development	GO:0072359 (BP)	791	15	5.5E-04
peptidyl-tyrosine phosphorylation	GO:0018108 (BP)	285	8	0.029
peptidyl-tyrosine modification	GO:0018212 (BP)	287	8	0.031
endothelial cell differentiation	GO:0045446 (BP)	82	5	0.034
cell junction organization	GO:0034330 (BP)	211	7	0.036
anatomical structure formation involved in morphogenesis	GO:0048646 (BP)	846	13	0.039
protein tyrosine kinase activity	GO:0004713 (MF)	107	5	0.040

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956   Figure 10

957 **Table 1**: Significant Reactome and KEGG pathway enrichment among the 1,026 genes

958 significantly upregulated by COVID-19.

Pathway ID	Pathway description	Total pathway size	# Significant genes	FDR-corrected P value
Reactome				
R-HSA-6798695	Neutrophil degranulation	479	65	1.1E-07
R-HSA-168256	Immune System	1997	162	1.0E-03
R-HSA-168249	Innate Immune System	1053	95	3.1E-03
R-HSA-162906	HIV Infection	232	31	4.3E-03
R-HSA-72766	Translation	291	36	4.3E-03
R-HSA-162587	HIV Life Cycle	151	22	0.016
R-HSA-5368287	Mitochondrial translation	93	16	0.019
R-HSA-8953854	Metabolism of RNA	673	63	0.019
R-HSA-72306	tRNA processing	106	17	0.021
R-HSA-5389840	Mitochondrial translation elongation	87	15	0.021
R-HSA-162599	Late Phase of HIV Life Cycle	138	20	0.021
R-HSA-9018679	Biosynthesis of EPA-derived SPMs	6	4	0.024
R-HSA-913531	Interferon Signaling	197	25	0.024
R-HSA-8978868	Fatty acid metabolism	177	23	0.027
R-HSA-6781823	Formation of TC-NER Pre-Incision Complex	54	11	0.027
R-HSA-5696399	Global Genome Nucleotide Excision Repair (GG-NER)	84	14	0.034
R-HSA-159231	Transport of Mature mRNA Derived from an Intron less Transcript	40	9	0.040
R-HSA-5419276	Mitochondrial translation termination	87	14	0.044
R-HSA-159234	Transport of Mature mRNAs Derived from Intron less Transcripts	41	9	0.044
R-HSA-1236975	Antigen processing-Cross presentation	99	15	0.048
R-HSA-6781827	Transcription-Coupled Nucleotide Excision Repair (TC-NER)	79	13	0.048
KEGG				
KEGG:01100	Metabolic pathways	1391	131	1.9E-04
KEGG:00020	Citrate cycle (TCA cycle)	32	10	2.4E-03

KEGG:03060	Protein export	24	8	0.010
KEGG:01212	Fatty acid metabolism	55	12	0.018
KEGG:00280	Valine, leucine and isoleucine degradation	48	11	0.021

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961 **Table 2**: Significant Reactome and KEGG pathway enrichment among the 1,109 genes

962 significantly downregulated by COVID-19.

Pathway ID	Pathway description	Total pathway size	# Significant genes	FDR-corrected P value
Reactome				
R-HSA-5653656	Vesicle-mediated transport	667	74	2.5E-05
R-HSA-199991	Membrane Trafficking	628	66	6.1E-04
R-HSA-1442490	Collagen degradation	64	14	6.4E-03
R-HSA-73887	Death Receptor Signaling	141	22	6.4E-03
R-HSA-194315	Signaling by Rho GTPases	444	47	7.8E-03
R-HSA-8948216	Collagen chain trimerization	44	11	7.8E-03
R-HSA-2022090	Assembly of collagen fibrils and other multimeric structures	61	13	7.9E-03
R-HSA-1474290	Collagen formation	90	16	9.5E-03
R-HSA-170834	Signaling by TGF-beta Receptor Complex	73	14	0.011
R-HSA-1650814	Collagen biosynthesis and modifying enzymes	67	13	0.013
R-HSA-3247509	Chromatin modifying enzymes	275	32	0.013
R-HSA-4839726	Chromatin organization	275	32	0.013
R-HSA-194840	Rho GTPase cycle	138	20	0.014
R-HSA-2214320	Anchoring fibril formation	15	6	0.014
R-HSA-9006934	Signaling by Receptor Tyrosine Kinases	455	45	0.022
R-HSA-9006936	Signaling by TGF-beta family members	102	16	0.022
R-HSA-446353	Cell-extracellular matrix interactions	18	6	0.033
R-HSA-2243919	Crosslinking of collagen fibrils	18	6	0.033
R-HSA-1474228	Degradation of the extracellular matrix	140	19	0.033
R-HSA-193704	p75 NTR receptor-mediated signaling	97	15	0.033
R-HSA-193648	NRAGE signals death through JNK	59	11	0.037
R-HSA-416482	G alpha (12/13) signaling events	79	13	0.038

R-HSA-3000480	Scavenging by Class A Receptors	19	6	0.038
R-HSA-5140745	WNT5A-dependent internalization of FZD2, FZD5 and ROR2	13	5	0.040
R-HSA-9007101	Rab regulation of trafficking	124	17	0.047
KEGG				
KEGG:04144	Endocytosis	231	37	3.0E-06
KEGG:05165	Human papillomavirus infection	314	42	6.3E-05
KEGG:04510	Focal adhesion	194	29	4.3E-04
KEGG:04530	Tight junction	150	24	9.1E-04
KEGG:05135	Yersinia infection	116	20	1.7E-03
KEGG:05132	Salmonella infection	205	26	0.023
KEGG:04390	Hippo signaling pathway	146	20	0.045

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965   **Table 3:** Significant Reactome and KEGG pathway enrichment among the 86 genes significantly

966   upregulated by COVID-19 only in Juvenile macaques.

Pathway ID	Pathway description	Total pathway size	# Significant genes	FDR-corrected P value
Reactome				
R-HSA-909733	Interferon alpha/beta signaling	69	5	0.033
KEGG				
KEGG:04330	Notch signaling pathway	53	4	9.9E-03
KEGG:05160	Hepatitis C	140	5	0.047

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969 **Table 4:** Significant Reactome and KEGG pathway enrichment among the 160 genes significantly

970 downregulated by COVID-19 only in Old macaques.

Pathway ID	Pathway description	Total pathway size	# Significant genes	FDR-corrected P value
Reactome				
R-HSA-4420097	VEGFA-VEGFR2 Pathway	99	7	0.037
R-HSA-194138	Signaling by VEGF	107	7	0.037
KEGG				
KEGG:04611	Platelet activation	122	8	2.5E-03
KEGG:05206	MicroRNAs in cancer	158	8	0.016

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988 **Supplementary Tables**

989 **Table S1:** Read processing and mapping statistics, and download accessions for all RNA-seq

990 samples.

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992 **Table S2**: Fragment counts, relative gene expression levels, gene annotations, and differential

993 expression data for every macaque gene.

994

995 **Table S3:** Complete lists of significantly differentially expressed gene sets of interest (including

996 gene names, relative expression data, fold change and P values). Gene sets include: (**A**) 1,026

997 genes significantly up-regulated with COVID-19 vs Naive, (**B**) 65 "neutrophil degranulation" (R-

998 HSA-6798695) genes significantly up-regulated during COVID-19, (**C**) 162 "neutrophil

999 degranulation" (R-HSA-6798695) genes significantly up-regulated during COVID-19, (**D**) 1,109

1000 genes significantly down-regulated with COVID-19 vs Naive, (**E**) 14 "collagen degradation" (R-

1001 HSA-1442490) genes significantly up-regulated during COVID-19, (**F**) 14 "Signaling by TGF-beta

1002 Receptor Complex" (R-HSA-170834) genes significantly up-regulated during COVID-19, (**G**) 86

1003 genes significantly up-regulated with COVID-19 vs Naive only in Juvenile macaques, (**H**) 96

1004 genes significantly down-regulated with COVID-19 vs Naive only in Juvenile macaques, (**I**) 97

1005 genes significantly up-regulated with COVID-19 vs Naive only in Old macaques, (**J**) 160 genes

1006 significantly down-regulated with COVID-19 vs Naive only in Old macaques, (**K**) 97 genes

1007 significantly up-regulated by both COVID-19 and TB and (**L**) 76 genes significantly up-regulated

1008 by COVID-19 but down-regulated by TB.

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1010 **Table S4**: Significant functional enrichment for Reactome, KEGG and Gene Ontology pathways,

1011 among differentially gene sets of interest. Gene sets include: (**A**) 1,026 genes up-regulated in

1012 COVID-19 vs Naive, (**B**) 1,109 genes down-regulated in COVID-19 vs Naive, (**C**) 86 genes

1013 signficantly up-regulated by COVID-19 only in Juvenile macaques, (**D**) 160 genes signficantly

1014   down-regulated by COVID-19 only in Old macaques, (**E**) 97 genes significantly up-regulated by

1015   both COVID-19 and TB, and (**F**) 76 genes significantly up-regulated by COVID-19 but down-

1016   regulated by TB.

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1018   **Table S5**. Clinical characteristics and lab parameters of COVID-19 patients. Clinical and

1019   demographic data were retrieved from the medical records of all participants. These data included

1020   age, gender, anthropometrics, comorbidities, symptoms, triage vital signs, and initial laboratory

1021   test results.

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