

1 **Histopathological assessments reveal retinal vascular changes, inflammation and gliosis in patients**  
2 **with lethal COVID-19**

3 Vijay K. Jidigam, PhD<sup>1</sup>, Rupesh Singh, PhD<sup>1</sup>, Julia C. Batoki<sup>1</sup>, Caroline Milliner<sup>1</sup>, Onkar B. Sawant,  
4 PhD<sup>2</sup>, Vera L. Bonilha, PhD<sup>1,3</sup>, Sujata Rao, PhD<sup>1,3</sup>.

5 <sup>1</sup> Department of Ophthalmic Research, Cole Eye Institute, Cleveland Clinic, 9500 Euclid Avenue,  
6 Cleveland, OH;

7 <sup>2</sup> Center for Vision and Eye Banking Research, Eversight, 6700 Euclid Ave, Suite 101, Cleveland, OH;

8 <sup>3</sup> Department of Ophthalmology, Cleveland Clinic Lerner College of Medicine of Case Western Reserve  
9 University, Cleveland, OH

10 **Corresponding Authors:** Sujata Rao, PhD and Vera L. Bonilha, PhD.

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19 **Running head:** Vascular and inflammatory changes in patients with lethal COVID-19.

20 **Address for reprints:** Department of Ophthalmic Research, Cole Eye Institute, Cleveland Clinic, 9500  
21 Euclid Avenue, Cleveland, OH, 44195 ([raos7@ccf.org](mailto:raos7@ccf.org) and [bonilhav@ccf.org](mailto:bonilhav@ccf.org))

22 **Key Words:** COVID-19, Retina, histopathology, vasculature, inflammation

23 **ABSTRACT**

24 **Purpose:** To assess for histopathological changes within the retina and the choroid and determine the  
25 long-term sequelae of the SARS-CoV-2 infection.

26 **Design:** Comparative analysis of human eyes.

27 **Subjects:** Eleven donor eyes from COVID-19 positive donors and similar age-matched donor eyes from  
28 patients with a negative test for SARS-CoV-2 were assessed.

29 **Methods:** Globes were evaluated ex-vivo with macroscopic, SLO and OCT imaging. Macula and  
30 peripheral regions were processed for epon-embedding and immunocytochemistry

31 **Main Outcome Measures:** Retinal thickness and histopathology, detection of SARS-CoV-2 Spike  
32 protein, changes in vascular density, gliosis, and degree of inflammation.

33 **Results:** Fundus analysis shows hemorrhagic spots and increased vitreous debris in several of the  
34 COVID-19 eyes compared to the control. OCT based measurements indicated an increased trend in  
35 retinal thickness in the COVID-19 eyes, however the difference was not statistically significant.  
36 Histology of the retina showed presence of hemorrhages and central cystoid degeneration in several of the  
37 donors. Whole mount analysis of the retina labeled with markers showed changes in retinal  
38 microvasculature, increased inflammation, and gliosis in the COVID-19 eyes compared to the controls.  
39 The choroidal vasculature displayed localized changes in density and signs of increased inflammation in  
40 the COVID-19 samples.

41 **Conclusions:** *In situ* analysis of the retinal tissue suggested that there are severe subclinical abnormalities  
42 that could be detected in the COVID-19 eyes. This study provides a rationale for evaluating the ocular  
43 physiology of patients that have recovered from COVID-19 infections to further understand the long-term  
44 effects caused by this virus.

45

## 46 INTRODUCTION

47 We are amid the human coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute  
48 respiratory syndrome coronavirus (SARS-CoV-2), which is of historic proportions, the likes of which we  
49 have not seen in 102 years. With >25 million cases confirmed, >490K deaths in the US (WHO COVID-  
50 19 Dashboard) it is one of the deadliest events in US history, and rates continuing to rise, the end is not in  
51 near sight. Despite being primarily a respiratory virus, COVID-19 can also present with non-respiratory  
52 signs, including ocular symptoms as conjunctival hyperemia, chemosis, epiphora, increased secretions,  
53 ocular pain, photophobia, and dry eye<sup>1-6</sup>. SARS-CoV-2 requires host cellular receptors (such as ACE2)  
54 for successful replication during infections. Immuno-histochemical studies and single-cell RNA-  
55 sequencing datasets have revealed both extra- and intra-ocular localization of SARS-CoV-2 receptors  
56 ACE2 receptor, and TMPRSS2 protease in human eyes<sup>1,7-9</sup>. The virus has also been detected within the  
57 anterior chamber and in the ocular fluids suggesting that ocular tissue may be directly affected due to  
58 Sars-CoV-2 infection<sup>1,10,11</sup>. Evidence of posterior eye involvement<sup>1</sup> in SARS-COV-2 infection is still  
59 scarce, though some recent optical coherence tomography angiography (OCTA) based findings show that  
60 retinal microvasculature is affected in patients that recovered from COVID-19 infection<sup>12,13</sup>. However, a  
61 detailed histopathological analysis of the retinal tissue and the impact of the SARS-CoV-2 infection on  
62 ocular health and function has not been examined. Here we report a comprehensive analysis of eyes from  
63 post-mortem patients infected with the SARS-CoV-2 virus. Fundus imaging provides evidence of  
64 increased hemorrhage in the eyes of COVID-19 patients. In some SARS-CoV-2 positive patients, there  
65 was evidence of vascular anomalies consistent with ocular vein occlusion and reduced capillary density.  
66 Additionally, there is an overall increase in the number of microglial cells within the retina of COVID-19  
67 patients. The microglial cells display abnormal morphological features indicative of microglial dystrophy.  
68 There is also increased gliosis in the COVID-19 eyes compared to the eyes from the age-matched control  
69 donors. To our knowledge, this is the first study that provides in vivo molecular information concerning  
70 the changes occurring within the retinal tissue of COVID-19 patients. We are still in the midst of the

71 coronavirus outbreak and there is constantly emerging information regarding its long-term sequelae on  
72 various systems in the body. The data reported here provide a rationale for longitudinal ocular  
73 assessments in recovered patients to truly gain insights into understanding the long-term effects caused by  
74 this virus.

75

## 76 **METHODS**

### 77 **Tissue acquisition and fixation**

78 Donor eyes were obtained through Eversight (Cleveland, OH). Eye bank records accompanying the donor  
79 eyes indicated whether the donor had COVID-19. Pathology analysis was performed with the approval of  
80 the Cleveland Clinic Institutional Review Board (IRB #20-755) and Institutional Biosafety Committee  
81 (IBC# 2018). The research adhered to the tenets of the Declaration of Helsinki. Tissue from eleven  
82 COVID-19 donor eyes from seven donors was analyzed. Additional information about the donors is  
83 provided in Table 1 (Fig 1). Cornea and anterior segment analysis for the COVID-19 donors were  
84 recently reported by Sawant et al.<sup>11</sup> For analysis, globes without the cornea were fixed and kept in 4%  
85 paraformaldehyde and 0.5% glutaraldehyde made in Dulbecco's Phosphate Buffered Saline (D-PBS)  
86 buffer for at least a month.

87

### 88 **Ex-vivo imaging of globes**

89 Globes were cut through the ora serrata, posterior poles were transferred to a chamber filled with D-PBS  
90 solution and imaged as previously described<sup>14</sup>. Briefly, fundus macrophotography (FM) images were  
91 collected using a Zeiss AxioCam MRC5 camera equipped with a macro zoom lens and AxioVision AC  
92 Software (Zeiss). Fundus autofluorescence was obtained using Blue Autofluorescence (BAF) mode of  
93 Heidelberg Spectralis confocal scanning laser ophthalmoscopy (SLO) (Heidelberg Engineering, Inc.).  
94 Optical Coherence Tomography images (OCT) were collected using spectral domain OCT system  
95 (Envisu R2210 UHR Leica Microsystems Inc.).

## 96 **Histopathology**

97 Fragments of retina-RPE-choroid were cut from the periphery to the optic nerve head and placed in 2.5%  
98 glutaraldehyde in 0.1M cacodylate buffer, sequentially dehydrated in ethanol and embedded in Epon as  
99 previously reported<sup>15</sup>. For light microscopy, semi-thin sections were cut with a diamond histotech knife,  
100 dried, and stained with toluidine blue. Slides were photographed with a Zeiss AxioImager.Z1 light  
101 microscope and the images were digitized using a Zeiss AxioCam MRc5 camera.

102

## 103 **Retinal flatmount and immunohistochemistry**

104 A piece of retina and choroid were cut from the posterior pole of each eyecup. The retina was dissected  
105 and washed overnight in TBS. The RPE/choroid was incubated in the disodium salt of  
106 ethylenediaminetetraacetic acid for 1.5 hours and RPE was removed using a pipette before washing with  
107 TBS overnight and wholemount staining was performed as described with the exception that the tissues  
108 were incubated with the antibodies and the UEA lectin for 4 days<sup>16</sup>. Retinas were stained with chicken  
109 anti-gial fibrillary acidic protein (GFAP; 1:500; Millipore, Burlington, MA, USA), mouse anti- SARS-  
110 CoV S Protein (1:100; NR-614, BEI Resources, NIAID, Manassas, VA, USA), rabbit anti-Iba-1 (1:250;  
111 Wako Chemicals USA, Inc., Richmond, VA) and Ulex europaeus agglutinin-FITC (UEA lectin1:100;  
112 Genetex, Irvine, CA, USA). Choroids were stained with UEA Lectin and Iba-1 antibody. The submacular  
113 regions of the retina were imaged using a Leica confocal microscope. Retinal images were acquired from  
114 three different zones, one near the ONH and Vein, one near the middle and one towards the periphery<sup>17,18</sup>.  
115 All care was taken to ensure that similar regions were represented in the images. The choroids were  
116 imaged with Bruch's membrane proximal to the objective.

117

## 118 **RESULTS**

### 119 **Imaging of posterior globes**

120 The combination of the fundus, confocal scanning laser ophthalmoscopy and optical coherence  
121 tomography imaging systems can provide a comprehensive characterization of retinal lesions before  
122 histopathology<sup>19</sup>. Therefore, these imaging techniques were performed on all COVID-19 and control eye  
123 donors; images obtained were qualitatively compared (Fig 1). Anatomical landmarks such as the optic  
124 nerve (ON) and fovea (Fig 1, black arrows) were identified in all donor's eyes using all three imaging  
125 modalities. Fundus images displayed differences between the eyes from both groups, which included  
126 several hemorrhage spots in the COVID-19 eyes (Fig 1A to 1F, white arrowheads). SLO BAF imaging  
127 revealed detached retina areas in the COVID-19 eyes (Fig 1H, 1J, and 1L, asterisks); further studies are  
128 needed to understand the cause of these retinal detachment areas. In addition, hemorrhage spots in the  
129 COVID-19 eyes were observed as hypofluorescent spots (Fig 1H, 1J, and 1L, white arrowheads).  
130 Quantification of SLO images showed decreased BAF signal in the COVID-19 eyes compared to  
131 controls, but the increase was not significant (Fig 1M to 1P). Finally, OCT based measures for retinal  
132 thickness and optic nerve head depth showed slight increase in the COVID-19 eyes compared to controls,  
133 but the increase was not significant (Supplemental Fig S1). In general, COVID-19 posterior poles had  
134 more vitreous debris, most likely due to detached epiretinal membranes or cellular floaters.

135

### 136 **Histopathological and immunohistological findings in the retina of COVID-19 patients**

137 Little is known about the ocular implications of COVID-19 disease. To gain insight into the disease's  
138 retinal pathology, semi-thin sections of epon-embedded tissue were analyzed and compared to matched  
139 controls. The control donors' retinas displayed each of the usual retinal lamina and the RPE (Fig 2A). The  
140 COVID-19 donors' retina displayed retinal edema compared to the control retinas (Fig 2B). Interestingly,  
141 a few control retinas displayed variable areas of cystoid change and atrophy in the retina's far periphery  
142 (Fig 2C). However, cystoid changes and atrophies were observed in the central area of the COVID-19  
143 donors (Fig 2D). In our cohort of COVID-19 samples, we also observed hemorrhages of various sizes in  
144 the outer plexiform layer around the optic nerve head in a few COVID-19 donors (Fig 2E and F,  
145 asterisks); these were also autofluorescent in cryosections. The frequency of observed morphological

146 findings detected in our cohort is provided in Table 2. Immunostaining of retinas with SARS-CoV-2 S  
147 protein antibody reacted with round cells within the retina of all the COVID-19 eyes close to the optic  
148 nerve head but not in the control retinas. However, in the retinas of two of the COVID-19 positive cases  
149 several cells were identified in the retina, choroid and optic nerve head (Fig 2H, and 2I, arrows) but not in  
150 the controls.

151

### 152 **Retinal microvasculature anomalies in COVID-19 patients:**

153 To gain insight into the disease's retinal pathology, flatmounts of both retina and choroid were labeled  
154 with a lectin that labels the endothelium of vessels. In 4 out of 7 COVID-19 positive donor eyes, there  
155 were signs of major vein occlusion, indicated by constriction of the vein and increased signs of  
156 hemorrhages upstream of the constrictions (Supplemental Fig S2). Additionally, in the COVID-19  
157 positive eyes, microvasculature density was severely reduced closer to the optic disc and around the veins  
158 (Fig 3A to 3F) compared to the age-matched control donor eyes. In several of the COVID-19 eyes, there  
159 was an observable loss of microvasculature and distinct thinning of the microcapillaries compared to the  
160 control eyes. In the choroidal plexus, vasculature did not appear to be different between the COVID-19  
161 eyes and the age matched control cohorts. Though in some areas there was reduced focal lectin staining  
162 indicative of capillary dropouts unlike in the retinal vasculature, it was difficult to determine whether  
163 there is an overall reduction in the choroidal vasculature density in the COVID-19 patients (Supplemental  
164 Fig S3). Nevertheless, our analysis suggests that there are distinct histological changes in the retinal  
165 microvasculature. Though it is not possible to conclusively determine whether all reported vascular  
166 features can be completely attributed to the virus, based on the comparative analysis of the age-matched  
167 control donor eyes, it is important to note that other comorbidities cannot solely explain the observed  
168 differences.

### 169 **Gliosis and increased infiltration of microglial cells in the retina of COVID-19 patients.**

170 SARS-CoV-2 infection can lead to multisystem inflammatory syndrome. Ocular tissue, like many other  
171 tissues can be affected by this inflammatory process. To determine whether signs of increased  
172 inflammation can be detected, we assessed for gliosis and inflammation in the retina of COVID-19  
173 patients. Retinal and choroidal preparations were examined using GFAP, a marker for astrocytes (Fig 4A  
174 to 4L) and Iba-1, to label the microglial cells (Fig 4A' to 4L'). Irrespective of whether the eyes were from  
175 COVID-19 positive or negative individuals, Iba-1 positive microglial cells could be detected in all the  
176 eyes. As a result of aging, an increasing proportion of microglial cells display abnormal morphological  
177 features such as shortened, gnarled, beaded, or fragmented cytoplasmic processes, and loss of fine  
178 ramifications and formation of spheroidal swellings; these changes are designated collectively as  
179 microglial dystrophy<sup>20</sup>. These changes are determined to be different than what occurs during microglial  
180 activation which is defined as hypertrophic microglia<sup>20-23</sup>. In all the eyes examined, there was evidence  
181 for both hypertrophic and dystrophic microglia, though there was evidence of microglial dystrophy in  
182 more of the COVID-19 eyes compared to the controls. (Fig 4E'-4L', hypertrophic are indicated with red  
183 arrow heads, while dystrophic features are indicated with blue and yellow arrowheads; magenta is normal  
184 morphology) Additionally, in all the COVID-19 eyes examined, there was also an overall increase in the  
185 number of microglial cells (Fig 4C', 4D', 4G', 4H', 4K', 4L'). This increase was observed closer to the  
186 optic nerve head and towards the middle and the peripheral retina. An overall assessment of the increased  
187 microglial cells is also indicated in Table 2. Besides the differences in the numbers, microglial cells also  
188 displayed activated microglia characteristics with enlarged spheroidal morphology and retracted  
189 processes. Similar to our retinal observations, there was evidence of increased microglial cells in the  
190 choroid (Supplemental Fig S2). A direct or systemic inflammation can result in the activation of the  
191 astrocytes along with the microglial cells. Astrocyte network were visualized with GFAP. GFAP  
192 immunoreactivity was highly variable with temporal differences within macular and perimacular regions  
193 that were examined. In general, closer to the optic nerve, the GFAP immunoreactivity was increased in  
194 the COVID-19 patients compared to their age matched controls. The astrocyte networks also appear to be  
195 different, with more elongated cell processes closer to the ONH (Fig 4C, 4G,4K) but towards the mid and



196 peripheral regions, these elongated bundles appear to overlap and form dense mesh like structures (Fig  
197 4D,4H, 4L). The increased GFAP immunoreactivity suggests signs of increased gliosis within the retina  
198 of patients infected with SARS-CoV-2 virus. However, due to the limited number of eyes examined, it is  
199 difficult to conclusively assess the influences of other comorbidities such as age and sex for the analysis.

200

## 201 **DISCUSSION**

202 Though the SARS-CoV-2 is primarily a respiratory tract virus, there is sufficient evidence that the virus  
203 can be detected in several other tissues like the peripheral and central nervous systems. The presence of  
204 the ACE2 receptor and the cofactor TMPRSS2 in several anatomical parts of the anterior surface suggest  
205 that the SAR-CoV-2 infection of the eye tissues, especially at the limbus and the cornea is possible. In  
206 agreement with this observation, a recent report estimated the prevalence of ocular manifestations in  
207 COVID-19 patients to range between 2 to 32%<sup>24</sup>. The ophthalmic manifestations appear to be associated  
208 with the disease severity of COVID-19<sup>1,25</sup>. Several groups have reported varying viral RNA and protein  
209 levels within the tears, conjunctiva, cornea, and vitreous<sup>11,26</sup>. The presence of viral ribonucleic acid  
210 (RNA) of SARS-CoV-2 was reported in three of fourteen human retinas of deceased patients with  
211 confirmed COVID-19 by real-time reverse transcriptase-polymerase chain reaction (RT-PCR)<sup>27</sup>. In this  
212 study, SARS-CoV-2 S protein immunoreactivity showed different degrees of distinct and specific  
213 localization in round cells within the retina of all the COVID-19 eyes close to the optic nerve head. The  
214 anterior segments of our COVID-19 cohort's eyes were previously analyzed<sup>11</sup>. Among eleven eyes  
215 recovered from seven COVID-19 donors: three conjunctival, one anterior corneal, five posterior corneal,  
216 and three vitreous swabs tested positive for SARS-CoV-2 RNA. Cases of SARS-CoV-2 have been known  
217 to induce anterior segment pathologies such as conjunctivitis and anterior uveitis and posterior  
218 pathologies, including retinitis, optic neuritis, choroiditis with retinal detachment and retinal

219 vasculitis<sup>25,28</sup>. These studies further illustrate the importance of long-term assessments of ocular  
220 physiology of individuals that have recovered from COVID-19.

221 To date, there is no detailed cellular and molecular characterization of the retina in SARS-CoV-2 infected  
222 patients. Previous studies reported retinal lesions in outpatients after confirmed SARS-CoV-2 infection  
223 with mild to moderate symptoms. Findings included non-specific and controversial hyper-reflective OCT  
224 lesions in the ganglion cell and inner plexiform layers, microhemorrhages, and nerve fiber infarcts<sup>29</sup>.  
225 Other studies identified hemorrhages, cotton wool spots, dilated veins and tortuous vessels in fundus  
226 photographs<sup>13,30</sup>. These are commonly seen as manifestations of diabetes mellitus and systemic  
227 hypertension but are also associated with several other etiologies, including ischemic, embolic,  
228 connective tissue, neoplastic, and infectious<sup>31-33</sup>. CWSs compromise localized accumulations of  
229 axoplasmic debris at the level of retinal ganglion cell axons resulting from axoplasmic flow interruption  
230 due to vascular or mechanical causes<sup>34</sup>. In our histological study, we detected amorphous debris in the  
231 outer plexiform layer corresponding to hemorrhages. Another exciting finding observed in COVID-19  
232 eyes was the presence of a large number of cystoid lesions spread across the entire retina that closely  
233 resemble the retinal alterations of patients suffering from cystoid macular edema (CME)<sup>35,36</sup> is observed  
234 in human retinal diseases like AMD, diabetic retinopathy, retinal vein occlusion, retinitis pigmentosa<sup>35,37</sup>,  
235 optic atrophy<sup>38</sup>, among others<sup>36,39</sup>. The presence of cysts causes a thickening of the affected retina and  
236 decreases visual acuity. Moreover, the neuroretina's compression, the nerve fibers and capillaries by the  
237 cystic alterations further contribute to retinal degeneration and aggravation of hypoxic conditions.

238         Though, data on histopathological analysis of retinal vasculature and choriocapillaris is scarce,  
239 there have been a few studies on OCTA based findings in patients with SARS-Cov-2. In a recent study by  
240 Abrishami et al,<sup>12</sup> OCTA was performed 2 weeks after recovery from systemic COVID-19 and mean  
241 vessel density in the superficial and deep plexus were significantly reduced in the COVID-19 cohort  
242 versus the age matched controls. In another study by Turker et al<sup>40</sup>, a reduction in retinal vessel density of  
243 the superficial and deep capillary plexus was reported. In their study, the measurements were done within

244 one week of discharge after complete recovery. Another OCTA based study reported several retinal  
245 findings including hemorrhages, cotton wool spots, dilated veins, tortuous vessel and changes in mean  
246 arterial and vein diameter in patients with COVID-19<sup>13</sup>. Although the authors stated that such findings  
247 indicating microangiopathy might be secondary to COVID-19 or incidental, they also speculated that the  
248 virus itself or the systemic treatments used might have triggered microangiopathy in patients with  
249 systemic vascular disease. In the present study, all retinal and choroicapillaris changes were examined in  
250 post-mortem tissue. We detect similar reduction in retinal vascular density in COVID-19 patients along  
251 with increased vessel tortuosity, vein occlusions and hemorrhages. However, unlike the retinal  
252 vasculature, the choroidal vasculature did not appear to be severely affected as a result of the COVID-19  
253 infection. It is important to note that there are studies that have found little or no change in retinal  
254 vascular density<sup>41</sup>. These apparent differences in reported observations, could be due to the disease  
255 severity, study populations, diagnostic criteria and methodologies used in the different studies. It still  
256 remains to be determined whether the changes in the retinal vasculature is due to a direct infection of the  
257 retina or whether these are part of a common systemic vascular diseases such as diabetes mellitus, chronic  
258 kidney disease and hypertension.

259 We also find evidence of increased microglial cells in the retina of the COVID-19 eyes. There is  
260 no reported evidence yet for increased infiltration of inflammatory cells in the retina, though a recent case  
261 study reported a possible association between COVID-19 and Papillophlebitis, a rare condition that  
262 occurs due to a consequence of inflammation of the retinal vein<sup>42</sup>. Chronological aging is associated with  
263 a significant increase in the total numbers of both hypertrophic as well as dystrophic microglia. A recent  
264 study showed that dystrophy are the disease associated microglia morphology<sup>21</sup>. In the present study we  
265 see evidence for increased microglial cells in the COVID-19 eyes and several of these appear to show the  
266 characteristic of microglial dystrophy and hypertrophy. Hypertrophic microglia are associated with  
267 increased microglial activation. However, due to the very low numbers of eyes examined and the  
268 subjectivity associated with classifying a hypertrophic, dystrophic and normal microglia purely based on  
269 morphological attributes, it is difficult to infer whether these dystrophic/hypertrophic morphological

270 features is associated with changes in the microglia. While there is likely more than one explanation for  
271 the differences detected in the microglial morphology, whether this is a direct consequence of the SARS-  
272 CoV-2 infection remains to be determined. However, these preliminary findings are interesting and  
273 suggest that there could be an increase in the secretion of the pro-inflammatory molecules as a result of  
274 the microglial dystrophy and warrant further investigation. Though, there is no report on activation of  
275 microglia in the retina, there is some evidence from neuropathological findings in patients who have died  
276 from COVID-19 that in a significant number of these patients, various gliosis stages with diffuse  
277 activation of microglia and astrocytes could be detected. In our study we also find evidence of increased  
278 astrocyte activation as indicated by the increase in GFAP immunoreactivity. The astrocyte morphology  
279 also appears to be different with more elongated morphology closer to the ONH and dense mesh like  
280 networks toward the middle and the periphery. Such phenotypic heterogeneity has been associated with  
281 different responses of the astrocytes to an injury and their adaptive functions. Importantly, the dense mesh  
282 like network is indicative of scar formation after an injury<sup>43,44</sup>. GFAP has been also detected in the plasma  
283 of patients with COVID-19 and the amount is directly correlated with the severity of the disease<sup>45,46</sup>.  
284 Whether the observed activation is temporary and is resolved after the infection is gone, remains to be  
285 seen. However, even short-term increase in GFAP can be detrimental to the underlying neuronal cells and  
286 can result in focal damage. Despite the small sample size, the work presented here raises the possibility  
287 of subclinical vascular deficits combined with increased inflammation in patients with severe disease who  
288 have recovered from COVID-19 infection.

289         This study has some limitations, including the small sample size and the broad inclusion criteria.  
290 The severity of the viral infection is unknown, and the duration of hospitalization could severely impact  
291 the histopathological findings, thus limiting the generalization of the findings. Owing to the small  
292 numbers of available control eyes available, the cause of death for these control cohorts will notably have  
293 a huge impact on all the assessments reported. All care was taken to ensure that the investigated cohorts  
294 were closely matched in terms of age as well as the duration that these patients were maintained on  
295 ventilators. These limitations do not appear to change the results as many of the reported findings could

296 only be observed in the deceased patients with COVID-19. Further evaluation with a much larger sample  
297 size is needed to determine the effects of SAR-CoV-2 infection on retinal morphology, vasculature,  
298 inflammation and gliosis.

299 In conclusion, we observed several ocular anomalies the COVID-19 cohorts compared to the  
300 control cohorts. Surprisingly, despite the small sample size, there were some consistent differences  
301 detected between the patient cohorts and the COVID-19 negative patients. Of note are the subclinical  
302 microvasculature features that we observed. As some of these observations have not been noted  
303 previously these histopathological analyses of the post-mortem eyes from the COVID-19 patients suggest  
304 that as more individuals recover from the COVID-19 infections, depending on the severity of their illness  
305 these individuals may present with ocular clinical symptoms that have not been examined previously.  
306 Therefore, a heightened vigilance for the long -term disease sequelae in other tissues like the eye is  
307 warranted.

308

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316

## 317 **AUTHORS CONTRIBUTIONS**

318 VLB, and SR contributed to study conception and design, and accessed and verified the data. VKJ drafted  
319 the manuscript. All authors contributed to data acquisition, interpreted the data, critically revised the  
320 manuscript, and provided approval for the final version of the manuscript to be published.

321

## 322 FINANCIAL DISCLOSURE(S)

323 None.

324

325

326

## 327 FIGURE LEGENDS

328

329 **Figure 1.** Ex-vivo imaging of COVID-19 donor eyes. Representative fundus (**A-F**) and SLO images (**G-**

330 **L**) collected from COVID-19 and an age-similar controls. Fovea (black arrow) and optic nerve head (ON)

331 were visible in all eyes. Hemorrhage spots (white arrowheads) were visible in most of the COVID-19

332 eyes. BAF images of COVID-19 (**H, J, L**) and control (**G, I, K**) eyes revealed a pattern that matched the

333 fundus images. Also, in the COVID-19 eyes, the detached retinas were apparent with SLO (**H, J, L, \***).

334 Mean intensity calculation using BAF SLO images with all age groups (**M**), age group 80-90 (**N**), age

335 group 70-75 (**O**), and age group < 45 (**P**). Scale bar A-F= 0.3cm, G-L= 200 $\mu$ m.

336

337 **Figure 2.** Histology and immunohistology of COVID-19 donor eyes. Representative toluidine blue-

338 stained plastic 1  $\mu$ m sections of retinas from COVID-19 donors (**B, D, E, F**) and an age-similar controls

339 (**A, C**). Morphology of the control retina displayed typical retinal lamina. A few control retinas displayed

340 cystoid degeneration (**C**) in the far periphery, while these were observed in the central retina of several

341 COVID-19 eyes (**D**). Two of the COVID-19 retinas showed a cotton-wool exudate (**E, F, asterisk**) in the

342 outer plexiform layer close to the optic nerve head. Immunofluorescence of two COVID-19 retinas

343 labeled with antibodies to SARS-CoV S protein (**H-I**, Alexa488, green) showed the presence of several

344 positive cells (arrows) when compared to control (**G**). GCL ganglion cell layer, INL inner nuclear layer,

345 ONL outer nuclear layer, POS photoreceptor outer segments, RPE retinal pigment epithelium, Ch

346 Choroid. Scale bar A-D, F= 50 $\mu$ m, E= 100 $\mu$ m, G-I= 40 $\mu$ m.

347 **Figure 3.** Retinal vascular abnormalities in COVID-19 patients. Representative images of retinal  
348 vasculature visualized using rhodamine-conjugated UEA lectin to label the blood vessels. (**A-R**).  
349 Representative images are from three different age groups. Age group 80-90 (**A-F**), Age group 70-75 (**G-**  
350 **L**) and age group <45 (**M-R**). To illustrate the spatial differences in vascular density of the retinal  
351 microvasculature, the retinal preparations were subdivided into three different zones, one near the optic  
352 nerve head closer to the vein (**A,D,G,J,M,P**) one near the middle closer to the vein (**B,E,H,K,N,Q**) and one  
353 between the middle and the periphery (**C,F,I,L,O,R**). Vessel density was severely reduced together with  
354 several capillaries showing signs of atrophy(white arrowheads) in the eyes from the COVID 19 patients (**E,**  
355 **F, L, R, Q**) compared to age matched controls (**B, C, I, O, N**). Most noticeable difference were observed  
356 in regions distal to the optic nerve head however in one of the COVID positive sample (**P-R**), regressing  
357 vessels could be detected in the entire retina. White arrowheads indicate the severe capillary dropout. ONH,  
358 Optic nerve head, V, Vein, Mid, Middle region, Scale bar: 100um.

359  
360 **Figure 4.** Retinal vascular anomalies are accompanied by gliosis and inflammation. Retinal whole mounts  
361 stained with an antibody for GFAP for glial cells (**A- L**; green) and Iba1 for microglia (**A'-L'**, grey). There  
362 is an overall increase in GFAP immunoreactivity near the ONH regions (**G,K**) in the COVID-19 patients  
363 when compared to age matched controls (**E, I**). However, in one COVID negative case (**A, B**) there is a  
364 similar increase in GFAP immunoreactivity near the ONH area (**A**), but not near the middle regions (**B**). In  
365 general the GFAP positive cells appear to form dense networks and overlapping cable like structures  
366 indicative of increased gliosis (**A'-L'**) Representative images of retinal tissues stained with Iba1 to visualize  
367 the microglial cells. In all the COVID positive eyes, in the regions distal to the optic nerve and right next  
368 to the vein. (**C', D' G' H'K' L'**) there is an increase in the total number of microglial cells irrespective of  
369 the age of the deceased patient compared to the control eyes (**B',F',J'**). In general, in all of the eyes  
370 examined, several of the microglial cells displayed hypertrophic morphology (red arrowhead, **D'-L'**) with  
371 some showing fragmented processes (black arrowhead **A', B', G', H', L'**), beady, processes (blue  
372 arrowheads, **K', L'**) indicative of dystrophic microglia with very few normal looking microglia only seen

373 in one eye examined (Magenta arrowheads, C',D'). ONH, Optic nerve head, V, Vein, Mid, Middle region,

374 Scale bar: 100um.

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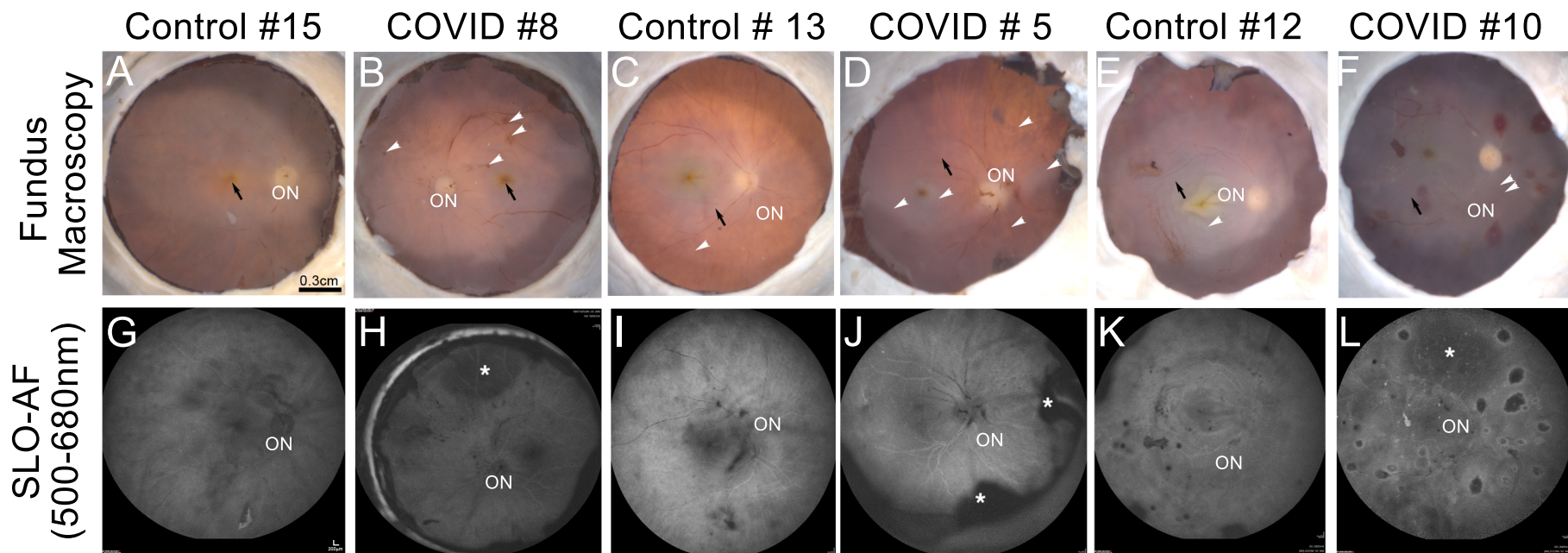
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**Table 1. Human Donor Information.**

CASE	Age Range <sup>b</sup>	Gender <sup>c</sup>	Race <sup>d</sup>	PMI <sup>e</sup>	SARS-CoV-2 Testing
N <sup>a</sup>					
2	80-90	F	C	12	Positive <sup>f</sup>
4	60-65	M	SA	7	Positive <sup>f</sup>
5	70-75	F	C	27	Positive <sup>f</sup>
6	65-70	M	H	20	Positive <sup>f</sup>
7	55-60	F	C	11	Positive <sup>f</sup>
8	80-90	F	H	16	Positive <sup>f</sup>
10	45-50	M	H	13	Positive <sup>f</sup>
11	75-80	M	C	5	Negative
12	35-40	F	C	17	Negative
13	70-75	M	C	7	Negative
15	80-90	M	C	24	Negative
16	60-65	F	C	13	Negative
17	60-65	M	C	7	Negative

<sup>a</sup> Cornea and anterior segments analysis were reported in Sawant et al., *Ocul Surf.* 2020 Nov 8;S1542-0124(20)30168-3. doi: 10.1016/j.jtos.2020.11.002.

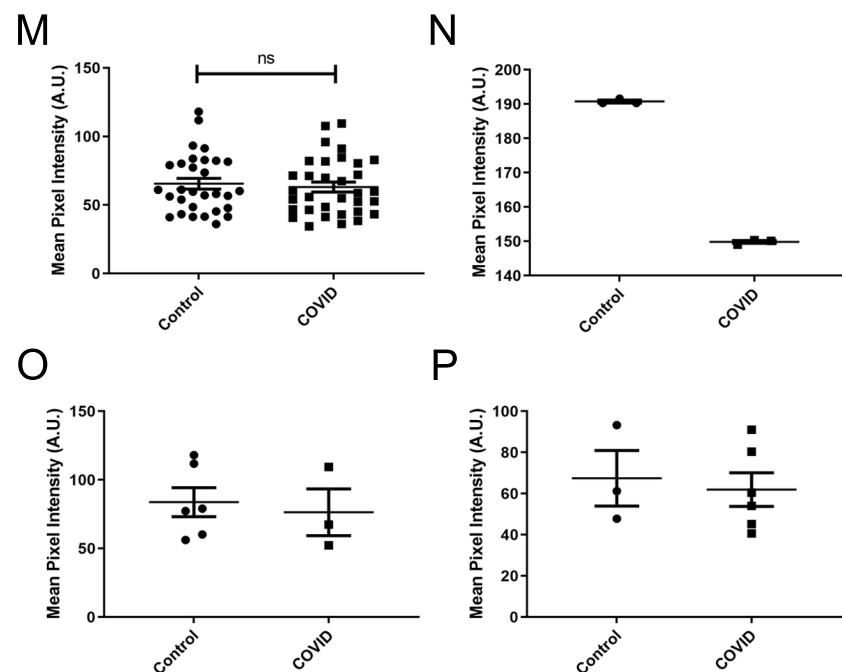
<sup>b</sup> Age: age at death (years);

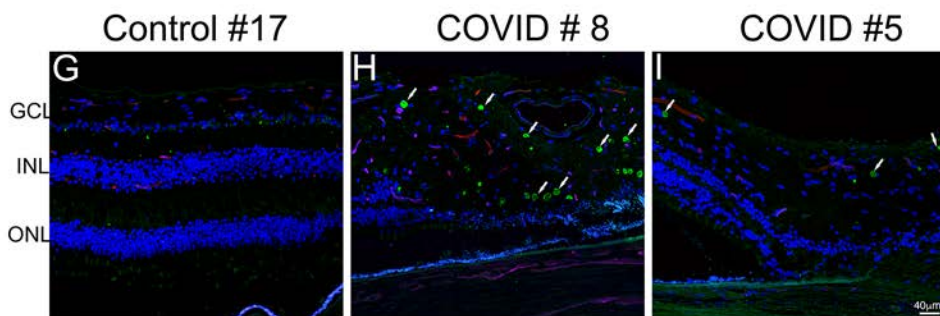
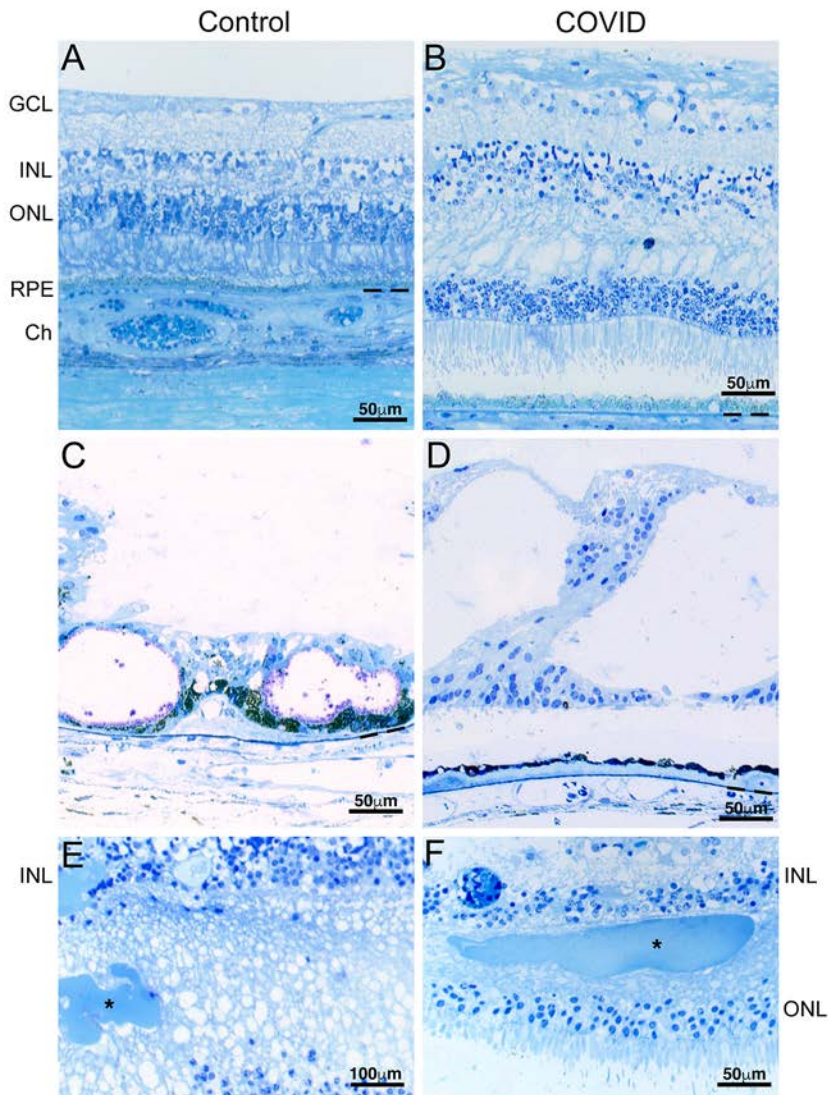
<sup>c</sup> Gender: M = male, F = female;

<sup>d</sup> Race: AA = African American, C = caucasian, H = hispanic, SA = south asian

<sup>e</sup> Interval from death to preservation (hrs).

<sup>f</sup> Results reported in Sawant et al., *Ocul Surf.* 2020 Nov 8;S1542-0124(20)30168-3. doi: 10.1016/j.jtos.2020.11.002.

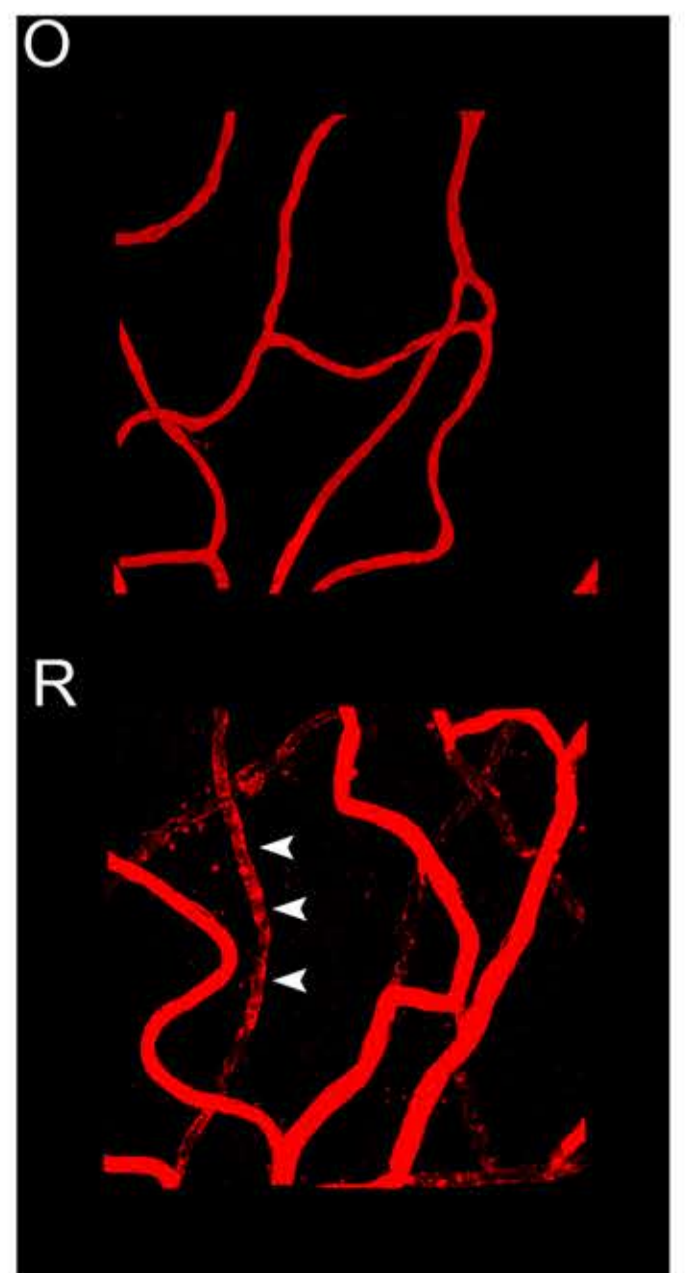
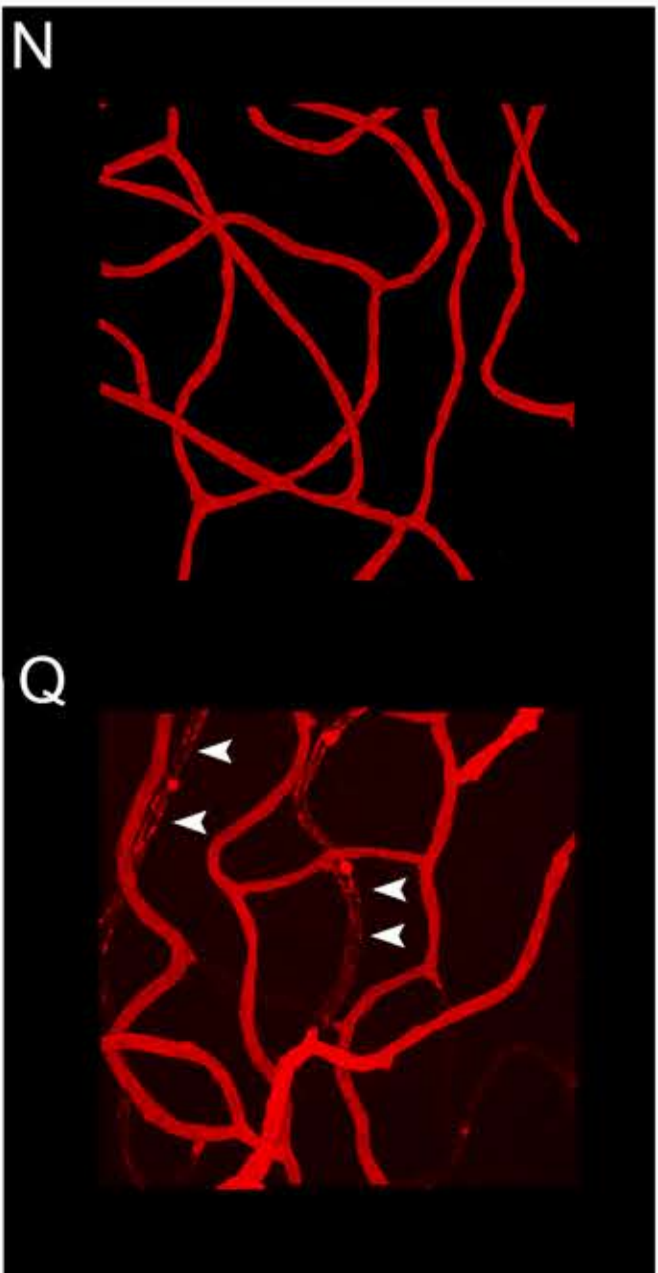
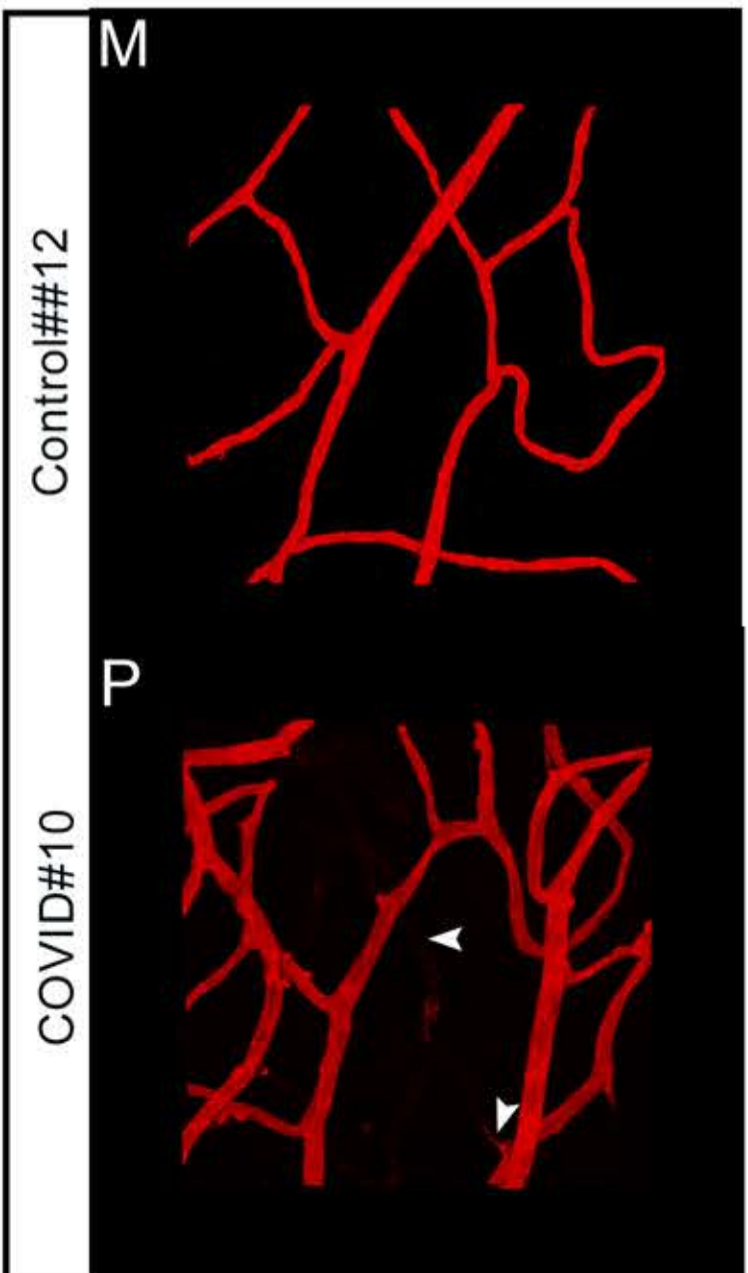
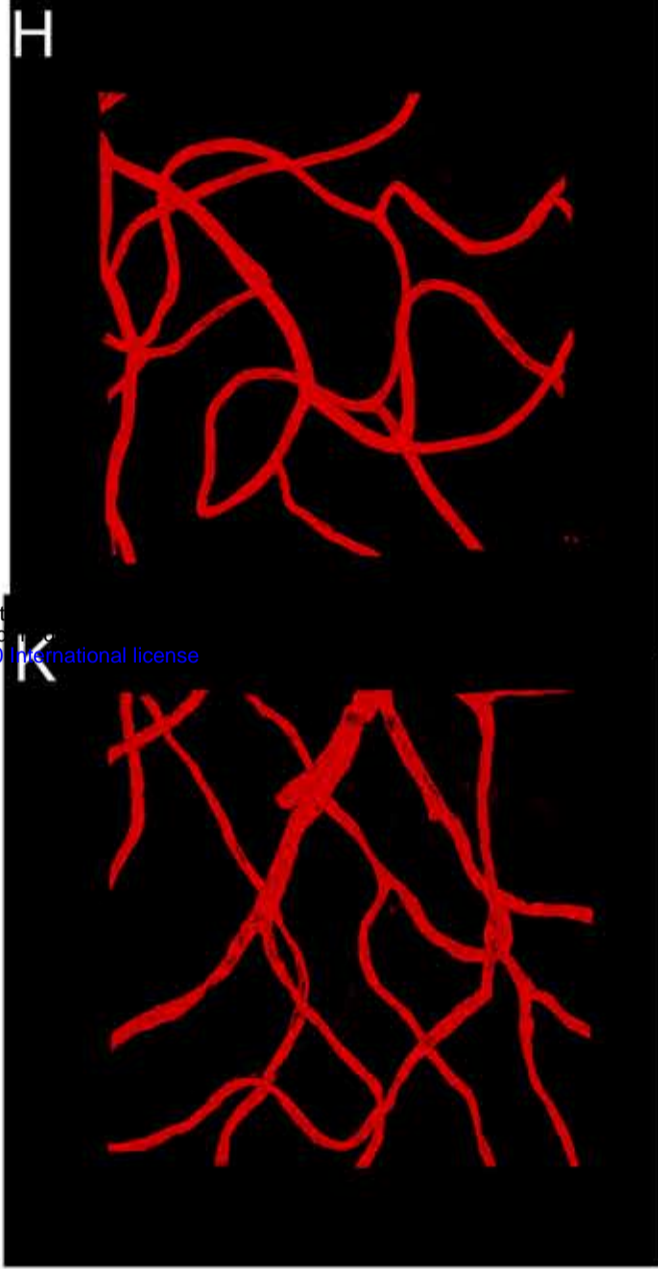
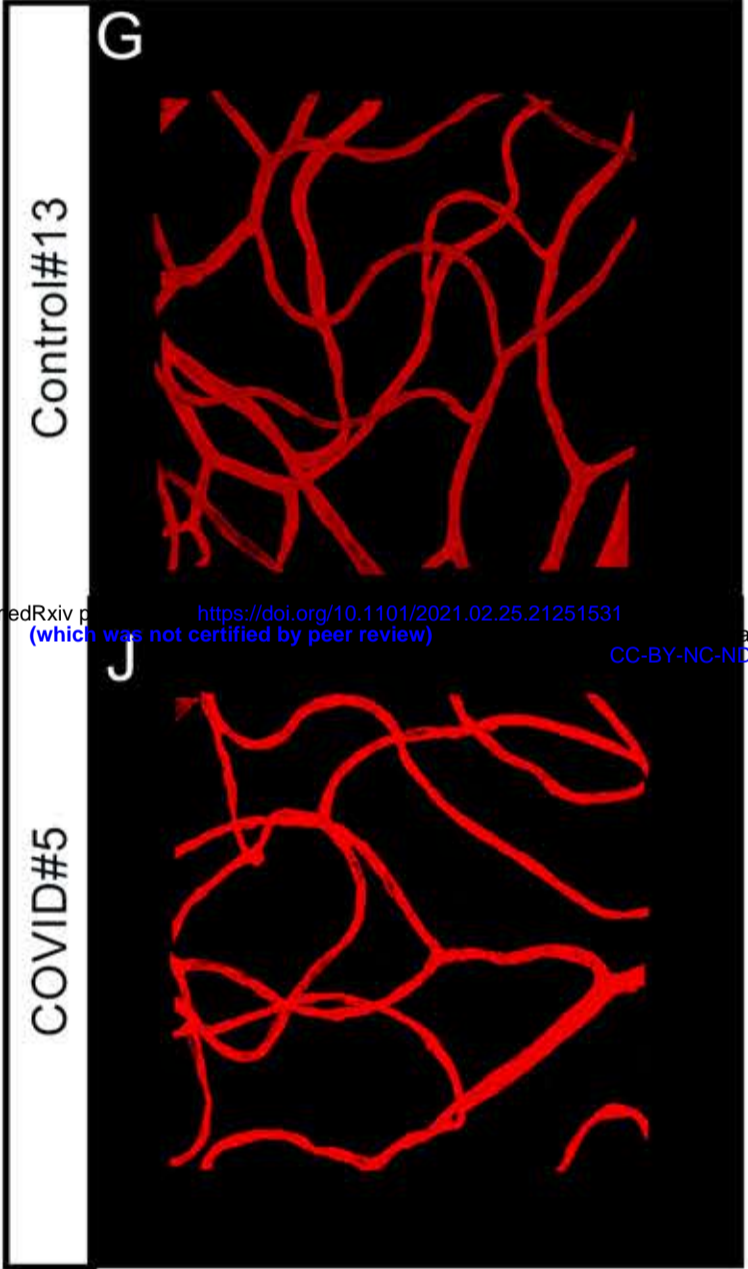
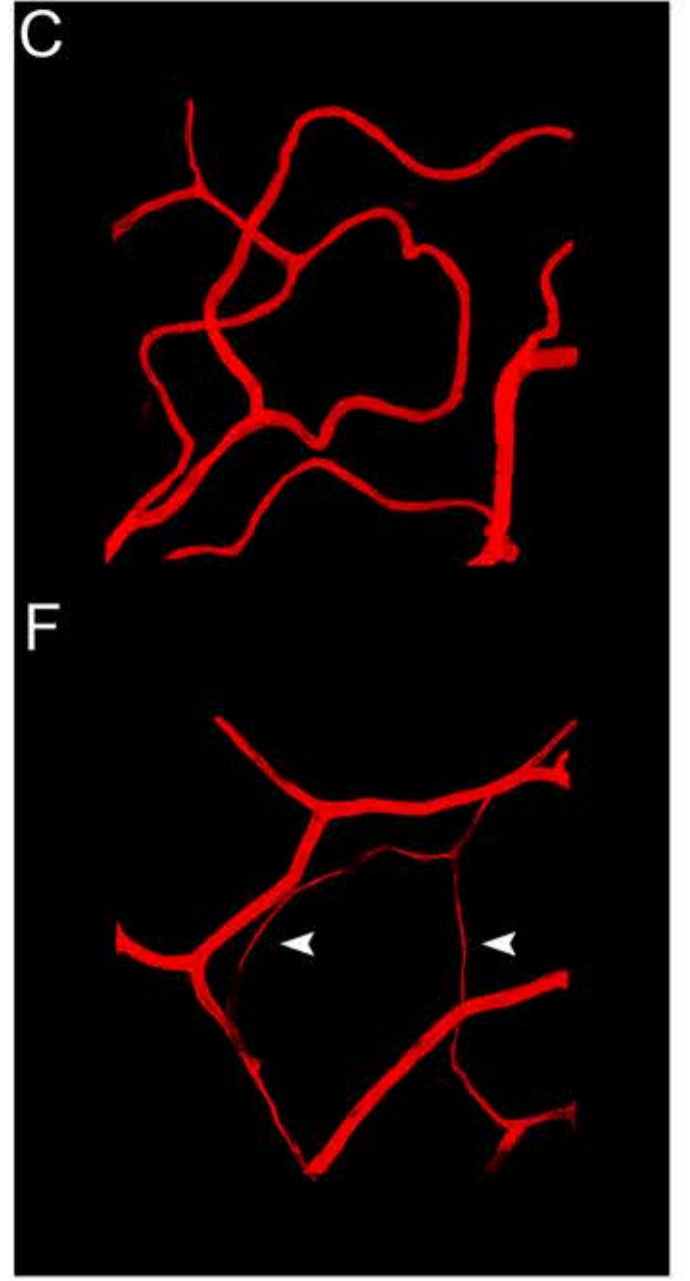
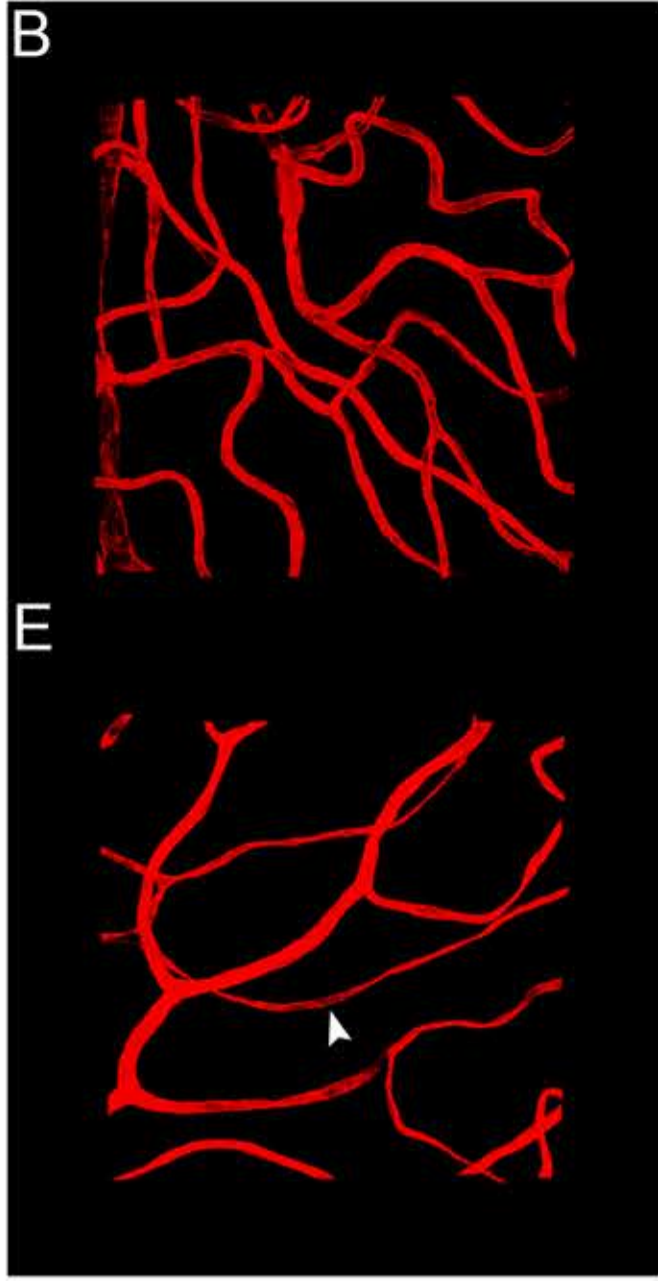
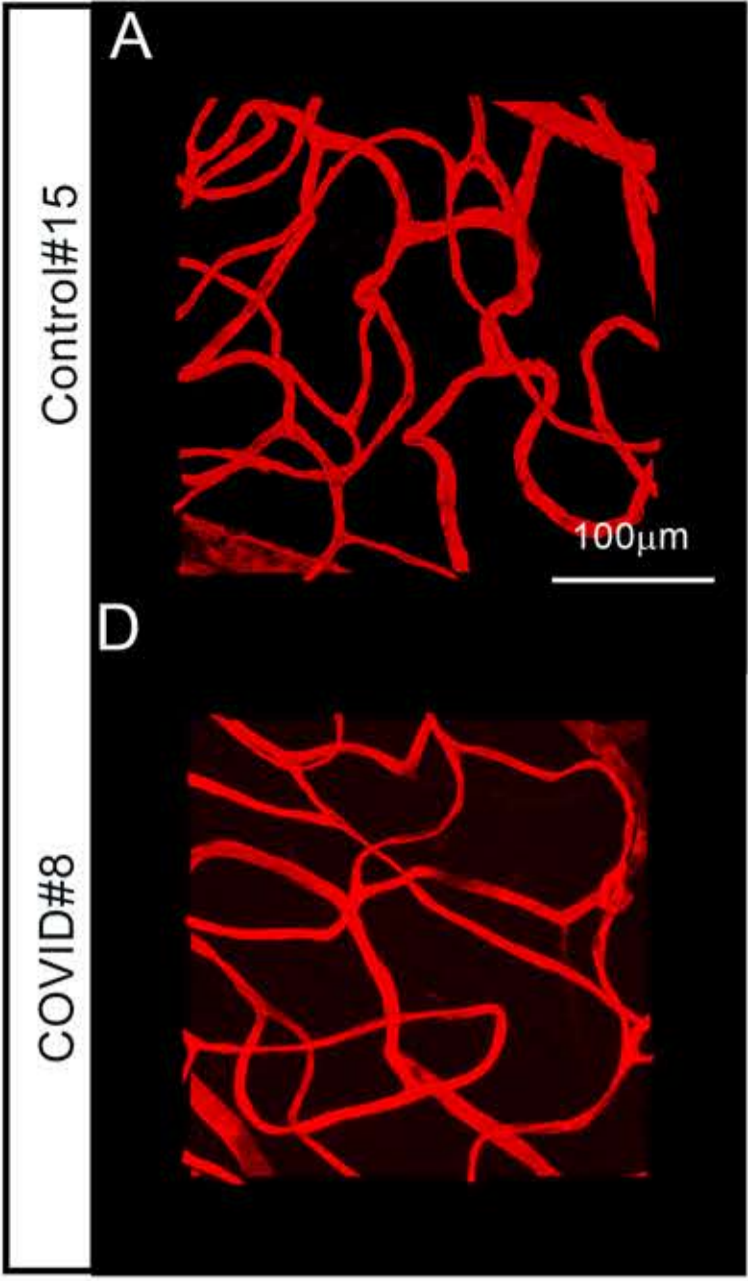




ONH/V

Mid/V

Mid



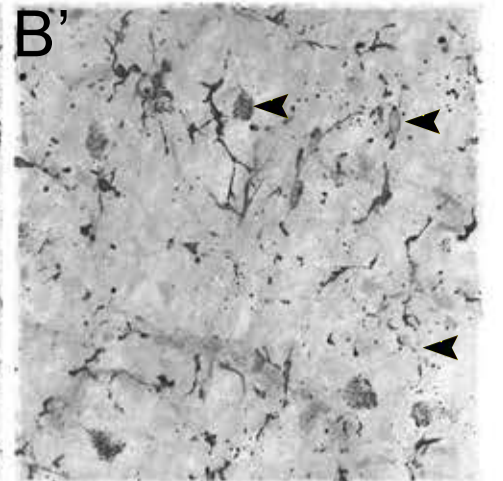
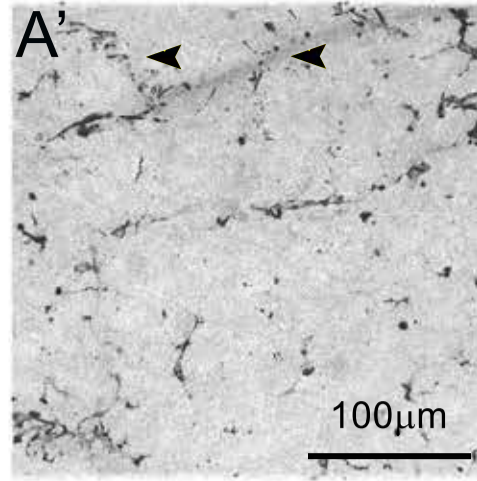
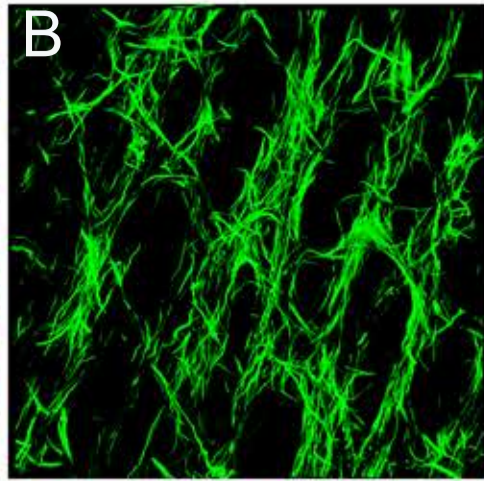
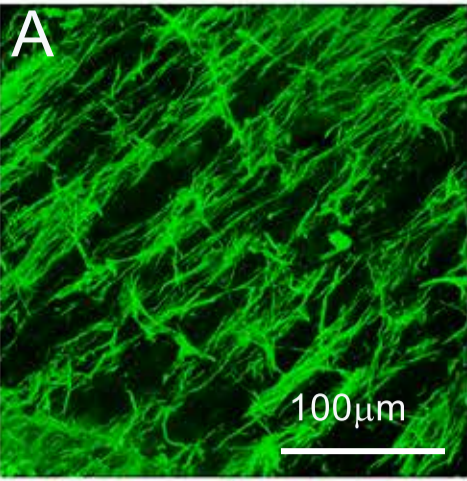
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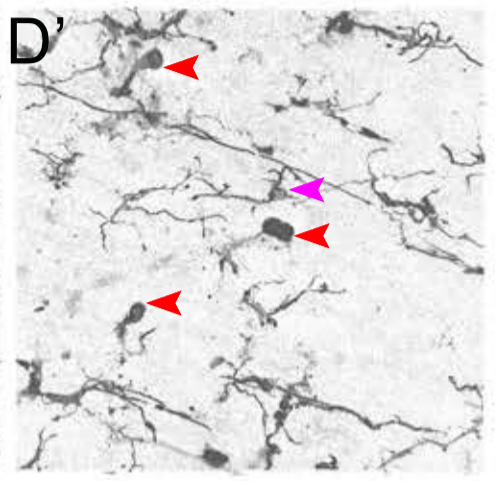
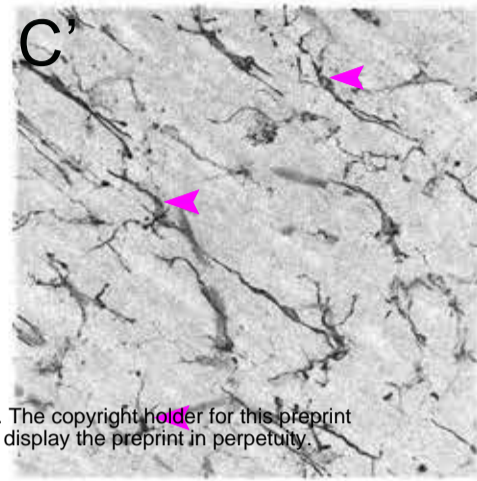
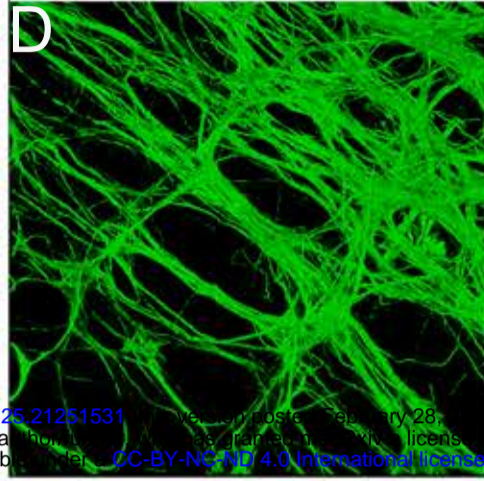
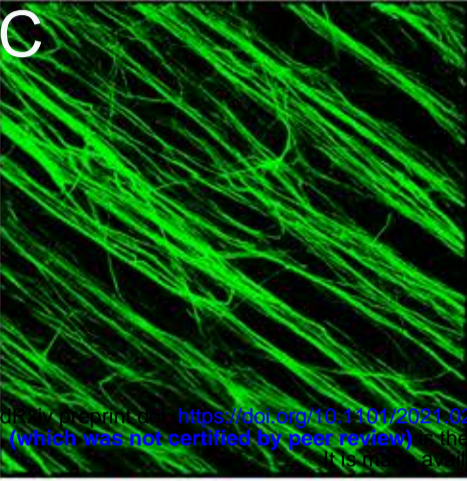
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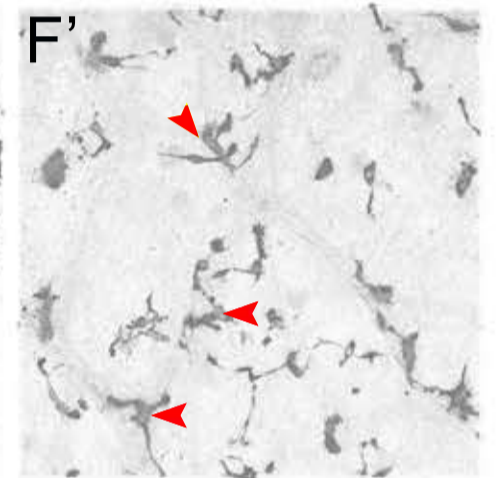
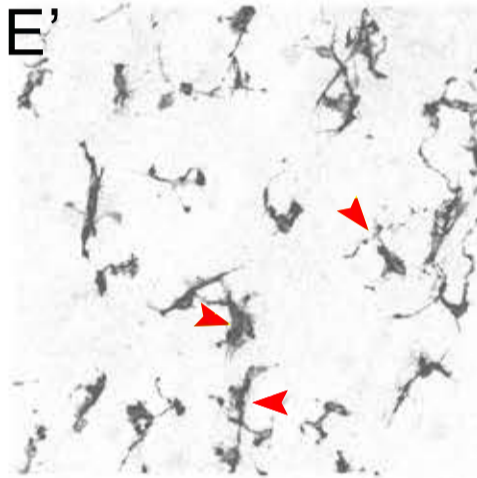
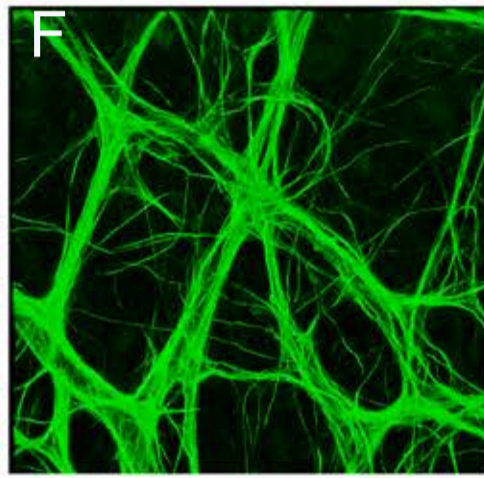
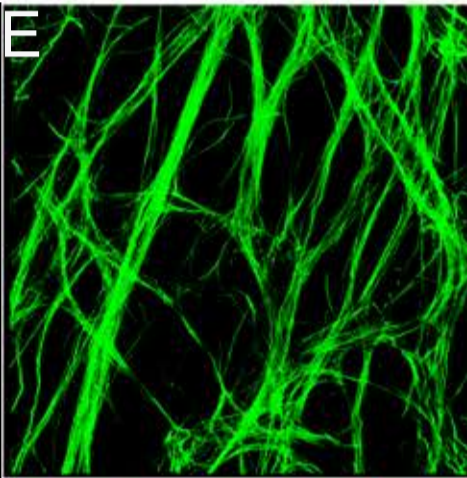
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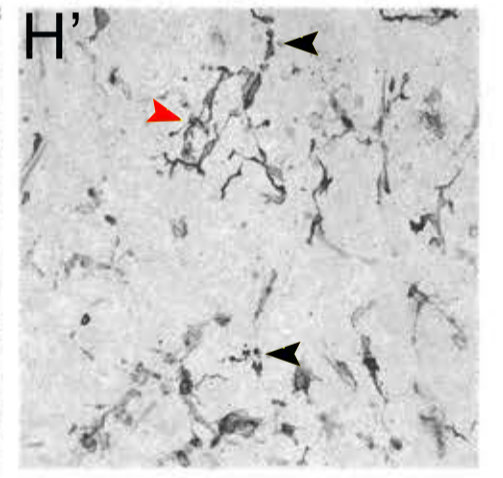
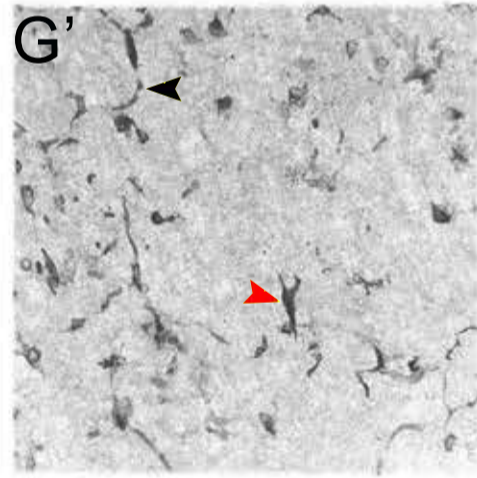
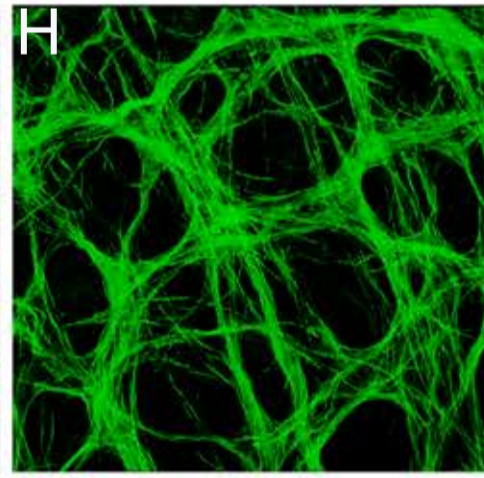
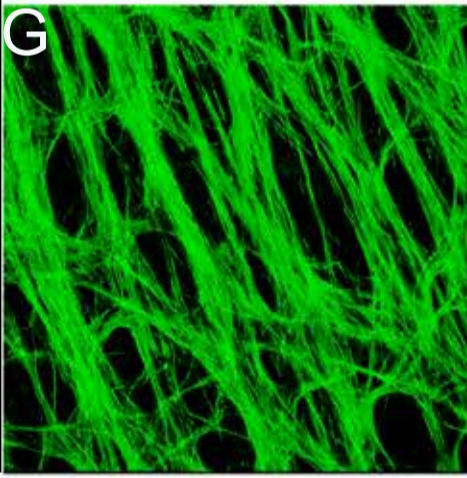
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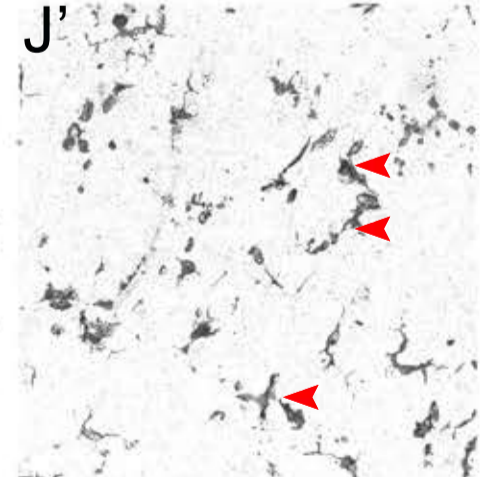
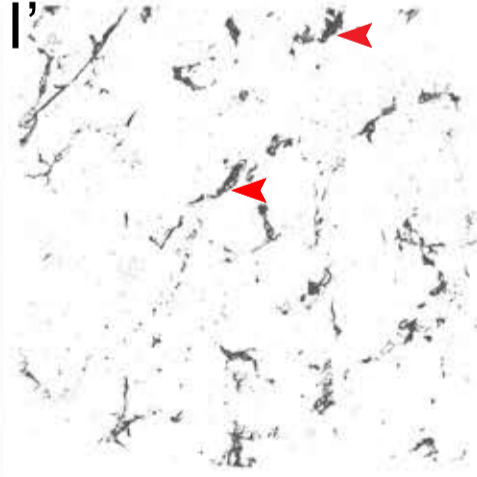
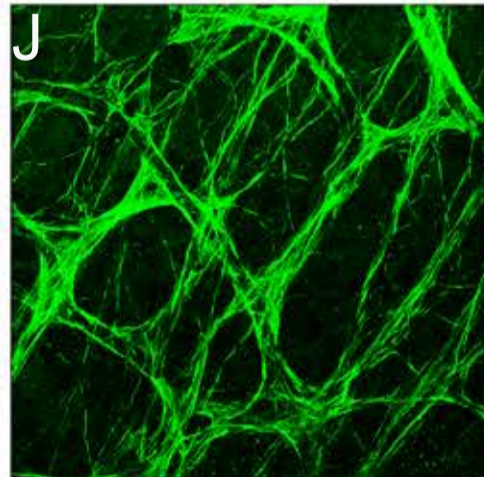
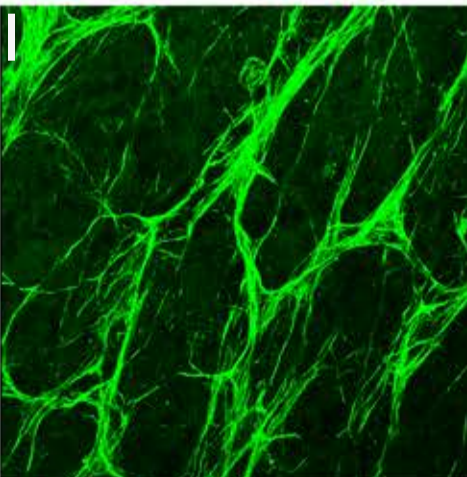
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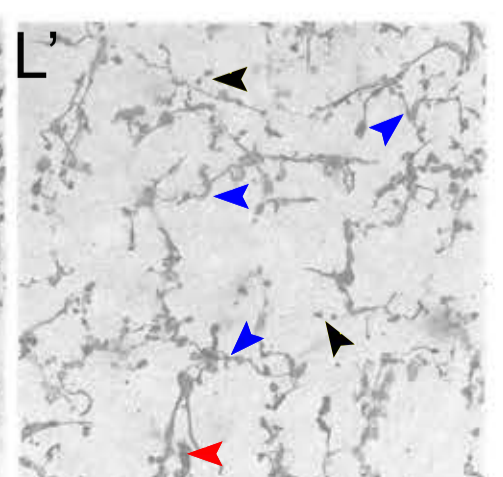
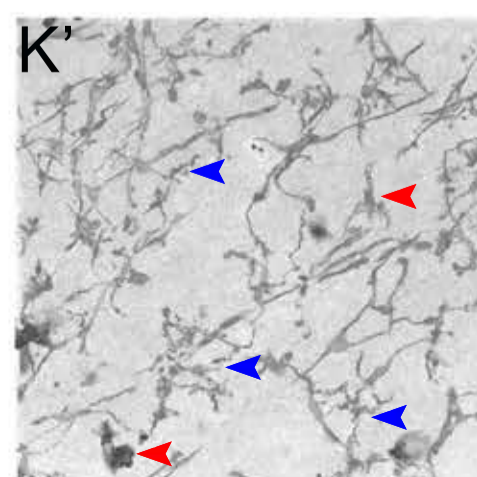
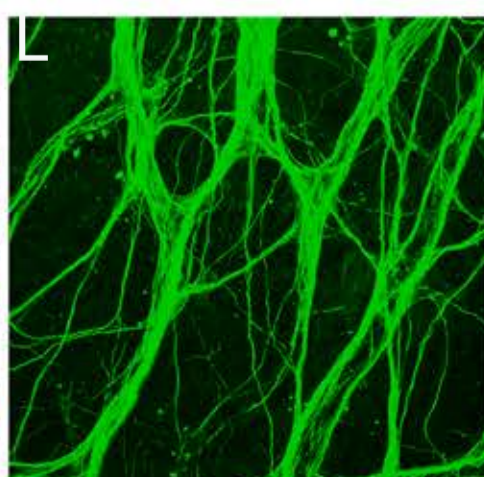
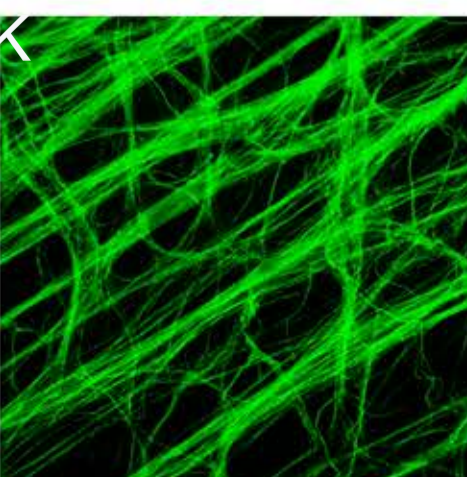
COVID#5



Control#12



COVID#10



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**Table 2. Assessment of retinal fundus and histological findings in donor eyes.**

CASE N°	Hemorrhages <sup>a</sup>	Cystoid Degeneration <sup>b</sup>	Increased GFAP Staining	Increased infiltration of Iba-1+ cells	Central vein occlusion
2	Not Observed	X	X	X	Not observed
4	X	X	X	X	X
5	X		X	X	X <sup>e</sup>
6	Not Observed	X	X	X <sup>d</sup>	X <sup>f</sup>
7	X		X	X	X <sup>f</sup>
8	X		X	X	X
10	X	X	X	X	X
11	X	X	Not observed	Not observed	Not observed
12	X	X	Not observed	Not observed	Not observed
13	Not Observed	X	Not observed	X <sup>d</sup>	Not observed
15	X	X	X <sup>c</sup>	X <sup>d</sup>	X
16	Not Observed	X	Not observed	Not observed	X <sup>f</sup>
17	X	X	X	X <sup>d</sup>	X <sup>f</sup>

<sup>a</sup> In at least one of the eyes;

<sup>b</sup> Observed in both epon-embedded sections and cryosections;

<sup>c</sup> Slightly elevated GFAP staining observed near the optic nerve head but not towards the middle and the peripheral retina.

<sup>d</sup> Higher numbers of Iba-1 positive cells detected in the middle and peripheral retina.

<sup>e</sup> Vascular Tortuosity in arteries and veins and aneurysms.

<sup>f</sup> Though vein occlusion was not evident, the vascular staining was patchy in the veins and venules.