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Supplemental Information

SARS-CoV-2 infects human adult donor eyes

and hESC-derived ocular epithelium

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1 Supplemental Material

2 Supplemental Figure 1

Donor A Isotype controls



3

4 Figure S1. Isotype controls for images in Figure 1, corneal tissue from post-mortem donor who

- 5 **tested positive for SARA-CoV-2.** Isotype controls for images of post-mortem human
- 6 cornea/limbal/scleral tissue section found in Figure 1. Isotypes were obtained by staining with Alexa
- 7 fluor (AF) conjugated IgG antibodies. Image 1-4 from the top represent stitched tile-scans, scale bar =
- 8 200 μ m. Bottom row of images show single tiles, scale bar = 20 μ m.
- 9



2 Figure S2. Immunofluorescence of post-mortem eye tissue from donors tested positive for SARS-

CoV-2, related to Figure 1. A) Co-staining of SARS-CoV-2 (CoV-2) with cornea marker keratin 12, and 3 ACE2, from donor one out of three, scale bar =200µm. White circle indicates region of zooms, scale bar in 4 zooms = 50µm. Isotype controls in Figure S4A B) Co-staining of SARS-CoV-2 (CoV-2) with limbus marker 5 keratin 15, and ACE2, from donor one out of three, scale bar =200µm. White circle indicates region of 6 zooms, scale bar in zooms = 50µm. Isotype controls in Figure S4B C) Co-staining of SARS-CoV-2 (CoV-2) 7 with cornea marker keratin 12, and ACE2, in two of the donors, scale bar =200µm. White circle indicates 8 region of zooms, scale bar in zooms = 50µm. Isotype controls in Figure S4A. D) Co-staining of SARS-CoV-2 9 (CoV-2) with limbus marker keratin 15, and ACE2, from donor two out of three, scale bar =200µm. White 0 circle indicates region of zooms, scale bar in zooms = 50μ m. Isotype controls in Figure S4B. 1

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4 Supplemental Figure 3

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6	Figure S3. Immunofluorescence of post-mortem eye tissue from donors who tested positive for
7	SARS-CoV-2, stained positive for p63 and SARS-CoV2 and Co-expression of ACE2 and TMPRSS2,
8	related to Figure 1 . A) Tile scan of cornea-limbal region of eye from donor A, From top: Rb-anti-SARS-
9	CoV2 N-capsid (CoV2 (N)), Ms-anti-p63, overlay showing CoV2(N) in red, p63 in green and DAPI in blue.
0	Scale bare = $200\mu m$. ISO-type controls in Figure S4B B) Magnified images from interest regions of the
1	cornea stained for CoV2 (N) and p36, i = central cornea, ii and iii = limbus region. Scale bar = 50μ m. C)
2	Tile scan of cornea-limbal region of eye from donor A, From top: Gt-anti-ACE2, Rb-anti-TMPRSS2, Ms-
3	anti-SARS-CoV2-spike (CoV2(S)), overlay showing ACE2 in green, TMPRSS2 in red, CoV2(S) in cyan, DAPI
4	in blue. Scale bare = $200\mu m$. ISO-type controls in Figure S4C D) Magnified images from interest regions of
5	the cornea stained with ACE2, TMPRSS2 and CoV2(S), i and ii = central cornea, iii = limbus region. Scale
6	bar = 50μm.

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Supplemental Figure 4

A ISO for ACE2 ISO for K12 ISO for CoV2(S) DAPI	B ISO for ACE2 ISO for K15 ISO for CoV2(S) DAPI
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2	Figure S4. Isotype controls from images of postmortem corneas, related to Figure 1. A) Isotype
3	controls for tile scans and magnified images in Figure S2 A and C, Isotype obtained by staining directly
4	with secondary antibodies conjugated to Alexa fluor. Gt-Alexa fluor 488 (ISO ACE2(green)), Rb-Alexa
5	fluor 546 (ISO for K12 (red)) and Ms-Alexa fluor 647(ISO for CoV2 (Cyan)), DAPI is shown in blue. Scale
6	bar in tile scan (top) = $200\mu m$, scale bar in magnified images (bottom) = $50\mu m$. B) Isotype controls for tile
7	scans and magnified images in Figure S2 B and D Isotype obtained by staining directly with secondary
8	antibodies conjugated to Alexa fluor. Gt-Alexa Fluor 488 (ISO ACE2(green)), Rb-Alexa fluor 546 (ISO for
9	K15 (red)) and Ms-Alexa fluor 647(ISO for CoV2 (Cyan)), DAPI is shown in blue. Scale bar in tile scan
0	(top) = $200\mu m$, scale bar in magnified images (bottom) = $50\mu m$. C) Iso-type controls for the images in
1	Figure S3C obtained be staining the tissue directly with Alexa fluor (AF) conjugated anti-Rabbit IgG (Rb-
2	647) as ISO for CoV2(N) and anti-Mouse IgG (Ms-488) ISO for p63. Scale bare = $200\mu m$. D) Iso-type
3	controls for the images in Figure S3D. obtained be staining the tissue directly with AF conjugated
4	antibodies; AF-anti-Goat IgG (Gt-488) ISO for ACE2, AF-anti-Rabbit IgG (Rb-546) as ISO for TMPRSS2, and
5	AF-anti-Mouse (Ms-647) as ISO for CoV2(s). Scale bar = $200\mu m$.



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2 Figure S5. Isotype controls from imunoflourencense of cell-type markers for human donor cells,

3 SARS-CoV-2 infected cells and quantification of IF for ACE2 and CoV2(s) in donor ocular cells,

4 related to Figure 2. A) Isotype control for cell type markers in primary cell cultures from human cadaver

5 donor cells. Green =Ms-Alexa fluor (AF) 488 (ISO), red = Rb- AF647 (ISO), blue = DAPI, scale bar = 50μm.

6 B) Iso-type controls for images of SARS-CoV2 and ACE-2 in primary cells cultures from human cadaver

7 donors, scale bar=50μm. C) Quantification of ACE2 positive cells (left) and SARS-CoV2(S) positive cells

8 (right) from IF images of postmortem donor cells infected with SARS-CoV2 at an M.O.I.=1. Images of cells

9 were taken with fixed settings and brightness thresholds for ACE2 (red fluorescents) and SARS-CoV2(S)

0 (green fluorescents) were set to fixed values in all images prior to counting, using FiJi software. Dots

1 represent biological replicates and between 4 and 7 randomly picked images were counted per replica,

2 counting 776±687 cells per sample (mean ± SD), columns and error-bars show mean ± SEM.

3



7 **Figure S6. Pluripotent stem cell derived SEAM, related to Figure 4**. A) Phase images of SEAM

8 differentiation. B) Expression pattern of proteins marking the differentiation of the following lineage

9 progenitors in specific SEAM zones; TUBB3 marks the neuroectoderm lineage in Zone1, CHX10 marks the

0 neural retina progenitors in Zone 2, PAX6 marks the optic cup in Zone 2, OTX2 marks retinal pigment

- 1 epithelium. E-Cad marks ocular and surface ectoderm in Zone 3 and 4, respectively. Scale bar = 100μm.
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- 3



- 7 Figure S7. Annotation of SEAM differentiation, related to Figure 4. Distal optic cup (DOC), Non-
- 8 melanocytic periocular mesenchyme (NM-POM), retinal pigment epithelium (RPE), Intestine (Ints),
- 9 Ocular Surface Ectoderm (OCE), Macrophages (Macro), Lymphocyte (Lymph), Surface ectoderm (SrfEct),
- 0 Bone Marrow (BM), Melanocytic Periocular Mesenchyme (M-POM), Neural (Neur).
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