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Supplemental Information

SARS-CoV-2 infects human adult donor eyes and hESC-derived ocular epithelium

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Supplemental Material

Supplemental Figure 1

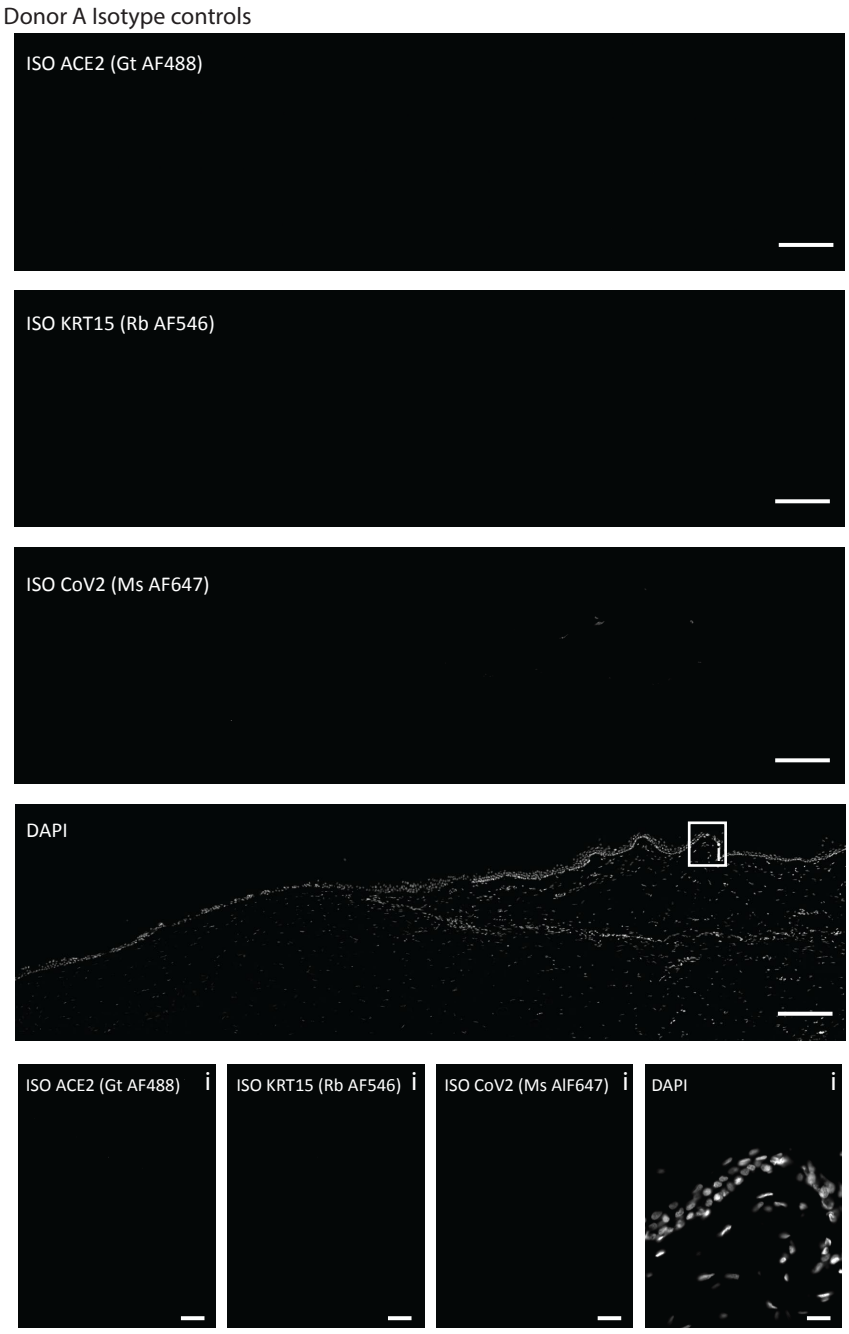


Figure S1. Isotype controls for images in Figure 1, corneal tissue from post-mortem donor who tested positive for SARA-CoV-2. Isotype controls for images of post-mortem human cornea/limbal/scleral tissue section found in Figure 1. Isotypes were obtained by staining with Alexa fluor (AF) conjugated IgG antibodies. Image 1-4 from the top represent stitched tile-scans, scale bar = 200µm. Bottom row of images show single tiles, scale bar = 20µm.

Supplemental Figure 2

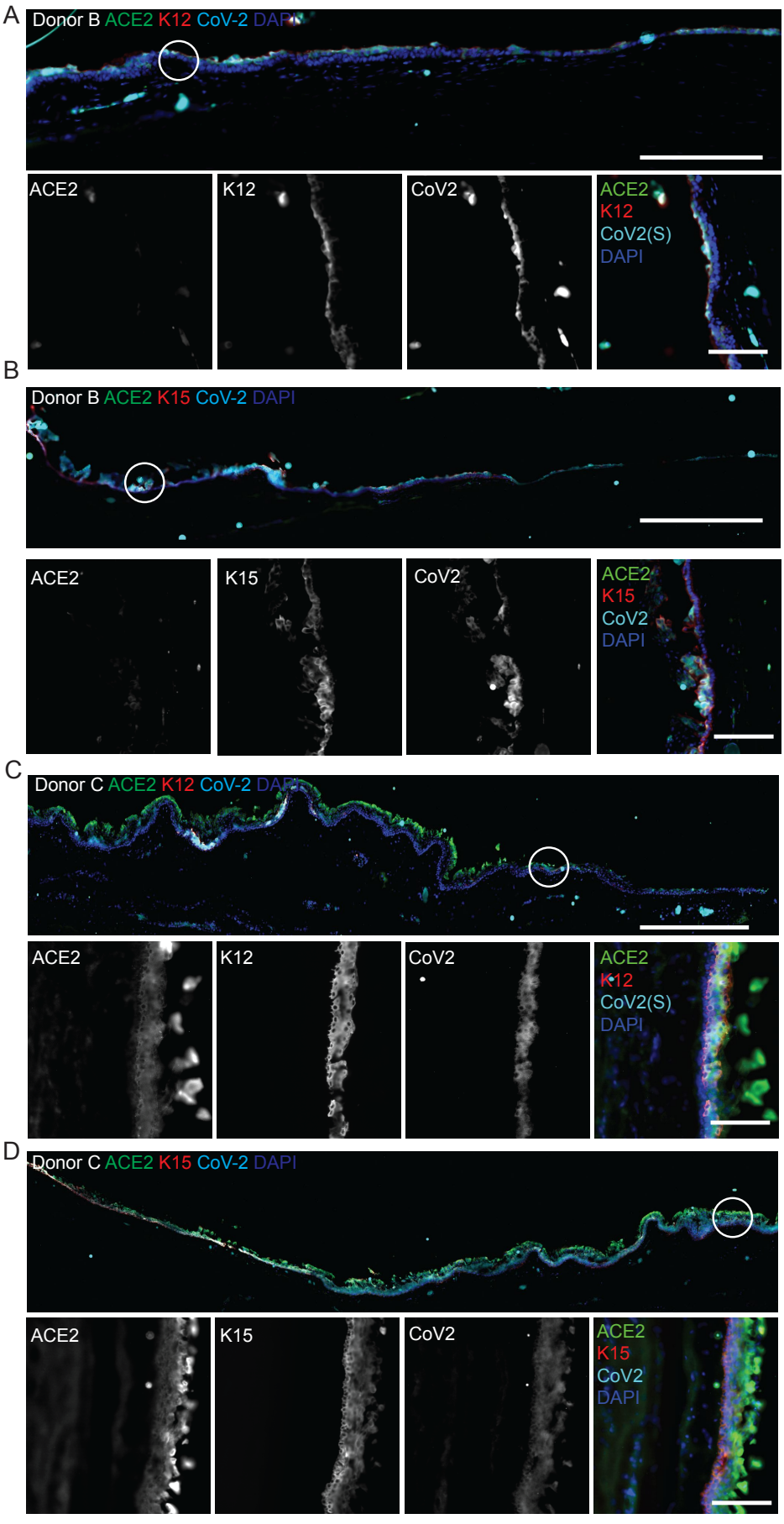


Figure S2. Immunofluorescence of post-mortem eye tissue from donors tested positive for SARS-CoV-2, related to Figure 1. A) Co-staining of SARS-CoV-2 (CoV-2) with cornea marker keratin 12, and ACE2, from donor one out of three, scale bar =200μm. White circle indicates region of zooms, scale bar in zooms = 50μm. Isotype controls in Figure S4A B) Co-staining of SARS-CoV-2 (CoV-2) with limbus marker keratin 15, and ACE2, from donor one out of three, scale bar =200μm. White circle indicates region of zooms, scale bar in zooms = 50μm. Isotype controls in Figure S4B C) Co-staining of SARS-CoV-2 (CoV-2) with cornea marker keratin 12, and ACE2, in two of the donors, scale bar =200μm. White circle indicates region of zooms, scale bar in zooms = 50μm. Isotype controls in Figure S4A. D) Co-staining of SARS-CoV-2 (CoV-2) with limbus marker keratin 15, and ACE2, from donor two out of three, scale bar =200μm. White circle indicates region of zooms, scale bar in zooms = 50μm. Isotype controls in Figure S4B.

Supplemental Figure 3

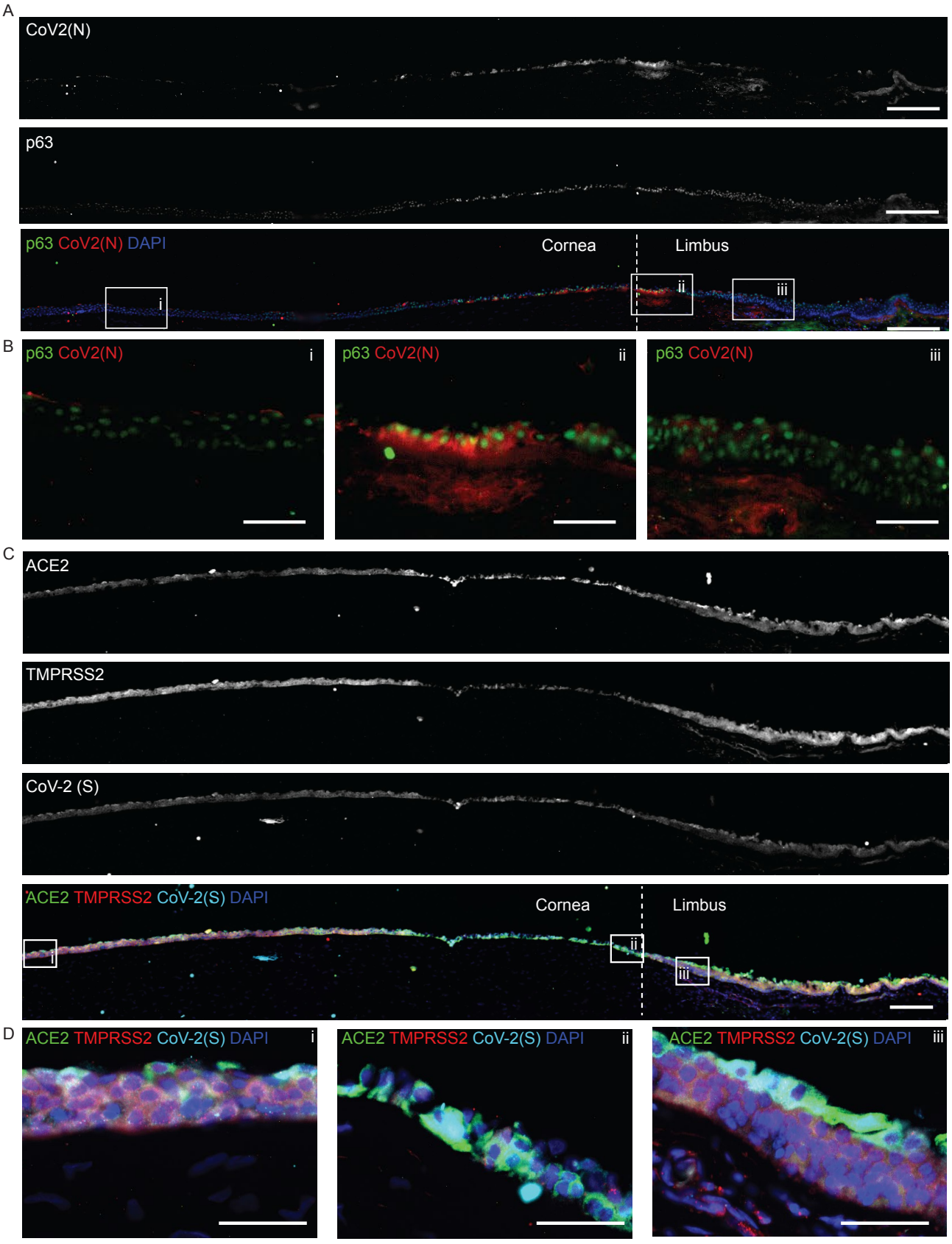


Figure S3. Immunofluorescence of post-mortem eye tissue from donors who tested positive for SARS-CoV-2, stained positive for p63 and SARS-CoV2 and Co-expression of ACE2 and TMPRSS2, related to Figure 1 . A) Tile scan of cornea-limbal region of eye from donor A, From top: Rb-anti-SARS-CoV2 N-capsid (CoV2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

Supplemental Figure 4

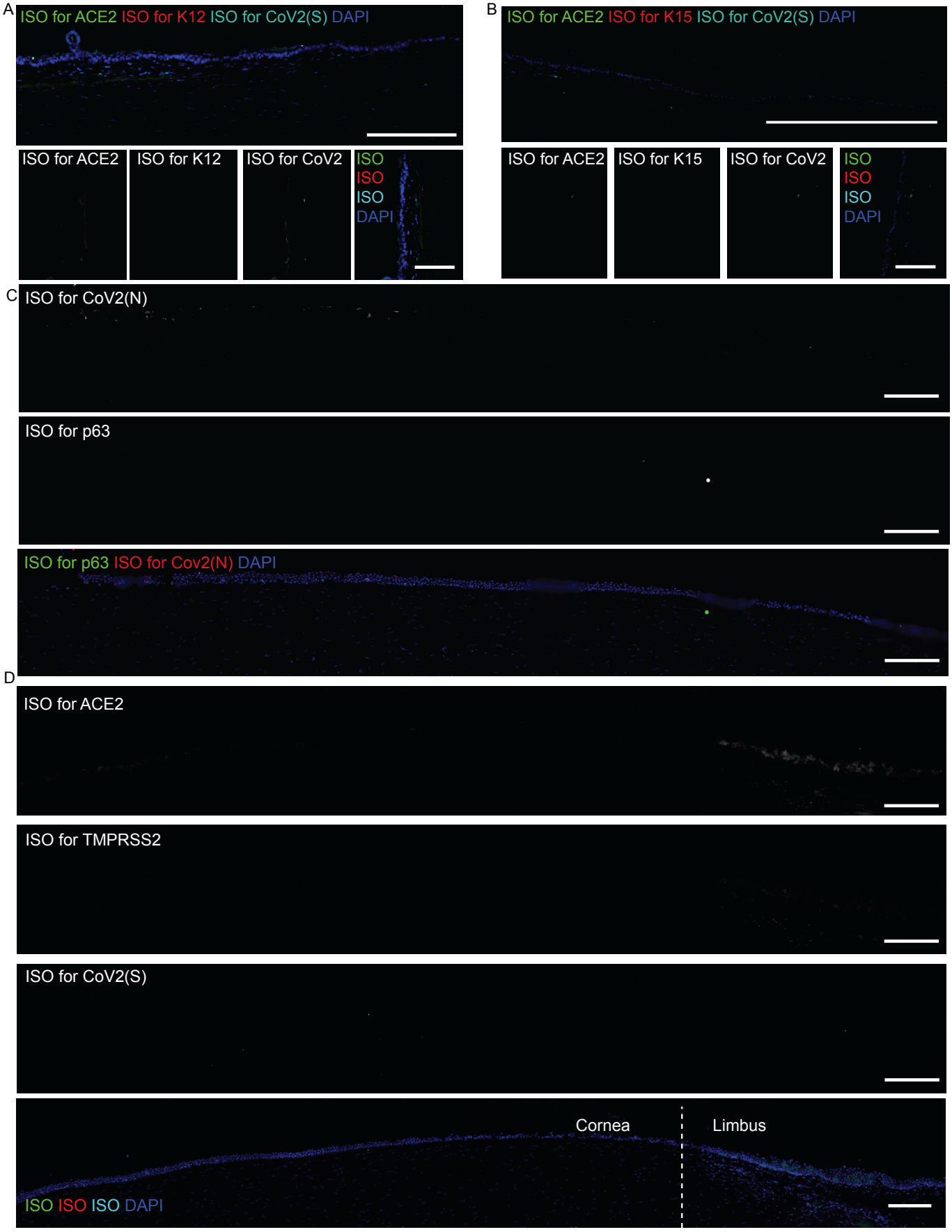
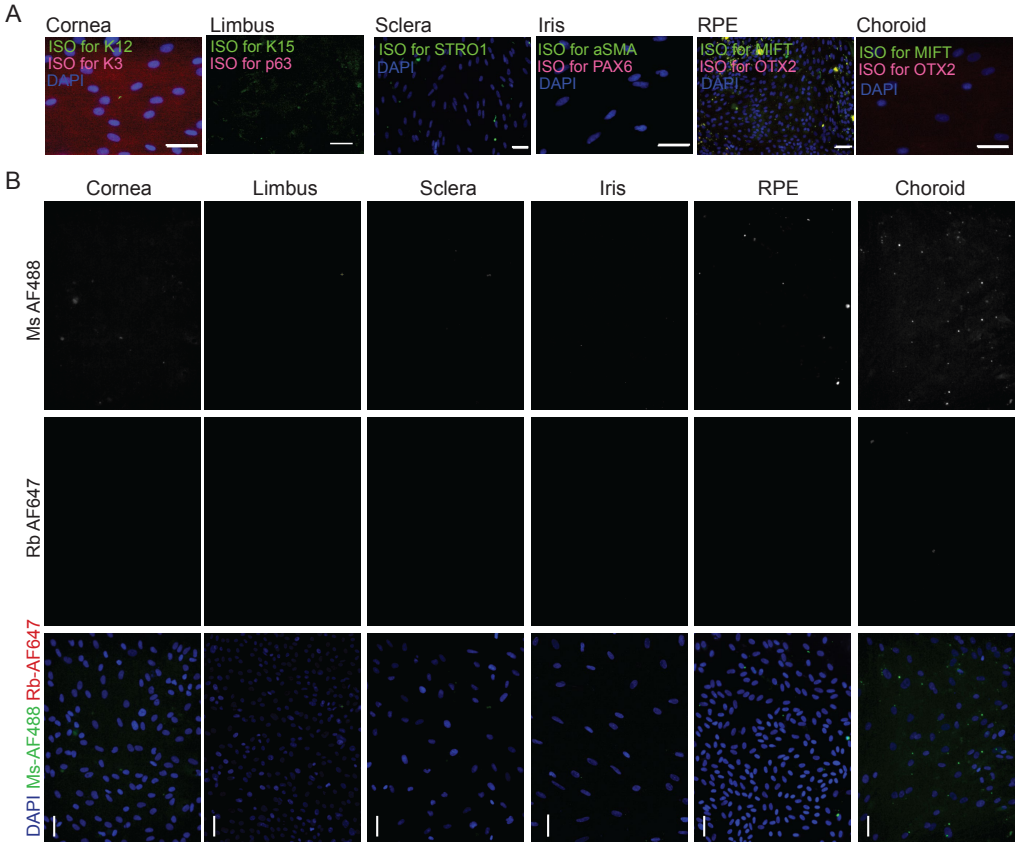


Figure S4. Isotype controls from images of postmortem corneas, related to Figure 1. A) Isotype controls for tile scans and magnified images in Figure S2 A and C, Isotype obtained by staining directly with secondary antibodies conjugated to Alexa fluor. Gt-Alexa fluor 488 (ISO ACE2(green)), Rb-Alexa fluor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

Supplemental Figure 5



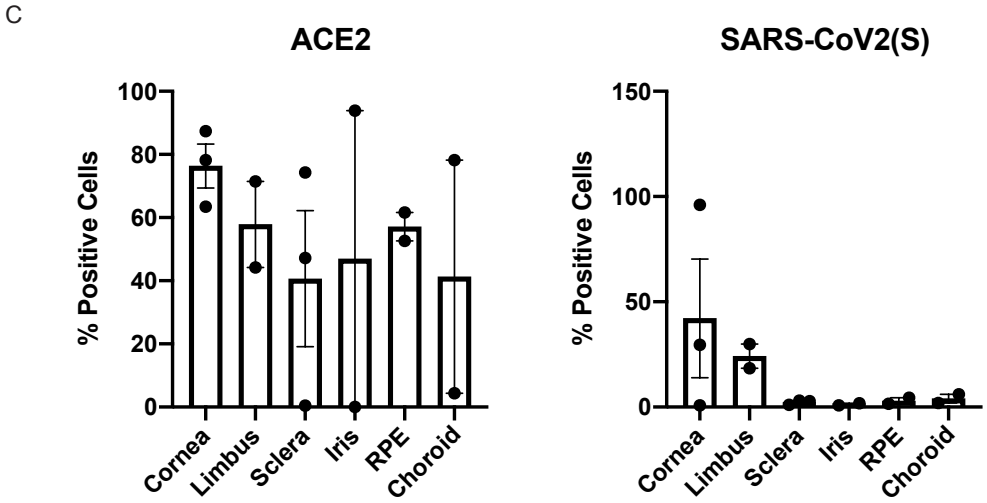
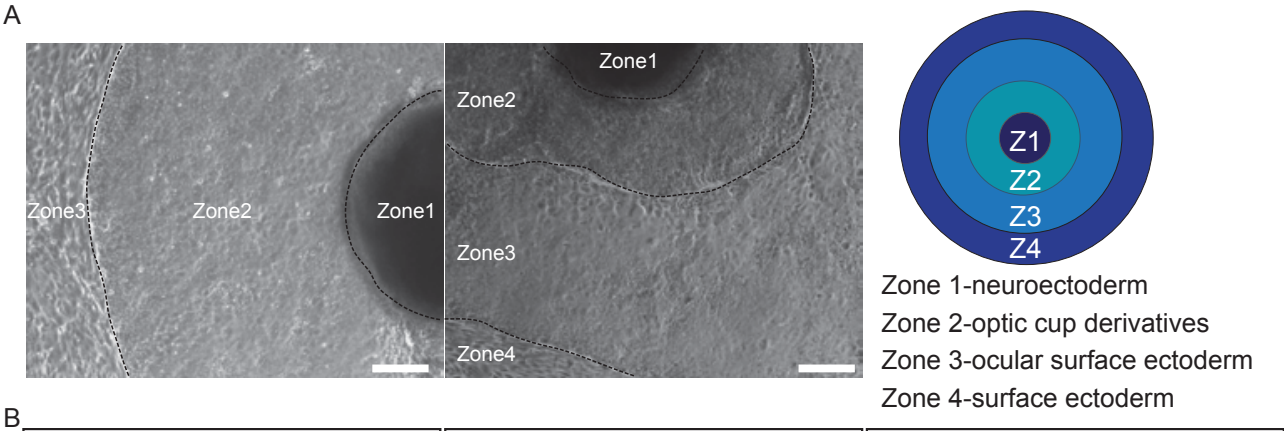


Figure S5. Isotype controls from imunoflourencense of cell-type markers for human donor cells, SARS-CoV-2 infected cells and quantification of IF for ACE2 and CoV2(s) in donor ocular cells, related to Figure 2. A) Isotype control for cell type markers in primary cell cultures from human cadaver donor cells. Green =Ms-Alexa fluor (AF) 488 (ISO), red = Rb- AF647 (ISO), blue = DAPI, scale bar = 50μm. B) Iso-type controls for images of SARS-CoV2 and ACE-2 in primary cells cultures from human cadaver donors, scale bar=50μm. C) Quantification of ACE2 positive cells (left) and SARS-CoV2(S) positive cells (right) from IF images of postmortem donor cells infected with SARS-CoV2 at an M.O.I.=1. Images of cells were taken with fixed settings and brightness thresholds for ACE2 (red fluorescents) and SARS-CoV2(S) (green fluorescents) were set to fixed values in all images prior to counting, using FiJi software. Dots represent biological replicates and between 4 and 7 randomly picked images were counted per replica, counting 776±687 cells per sample (mean ± SD), columns and error-bars show mean ± SEM.

5 Supplemental Figure 6



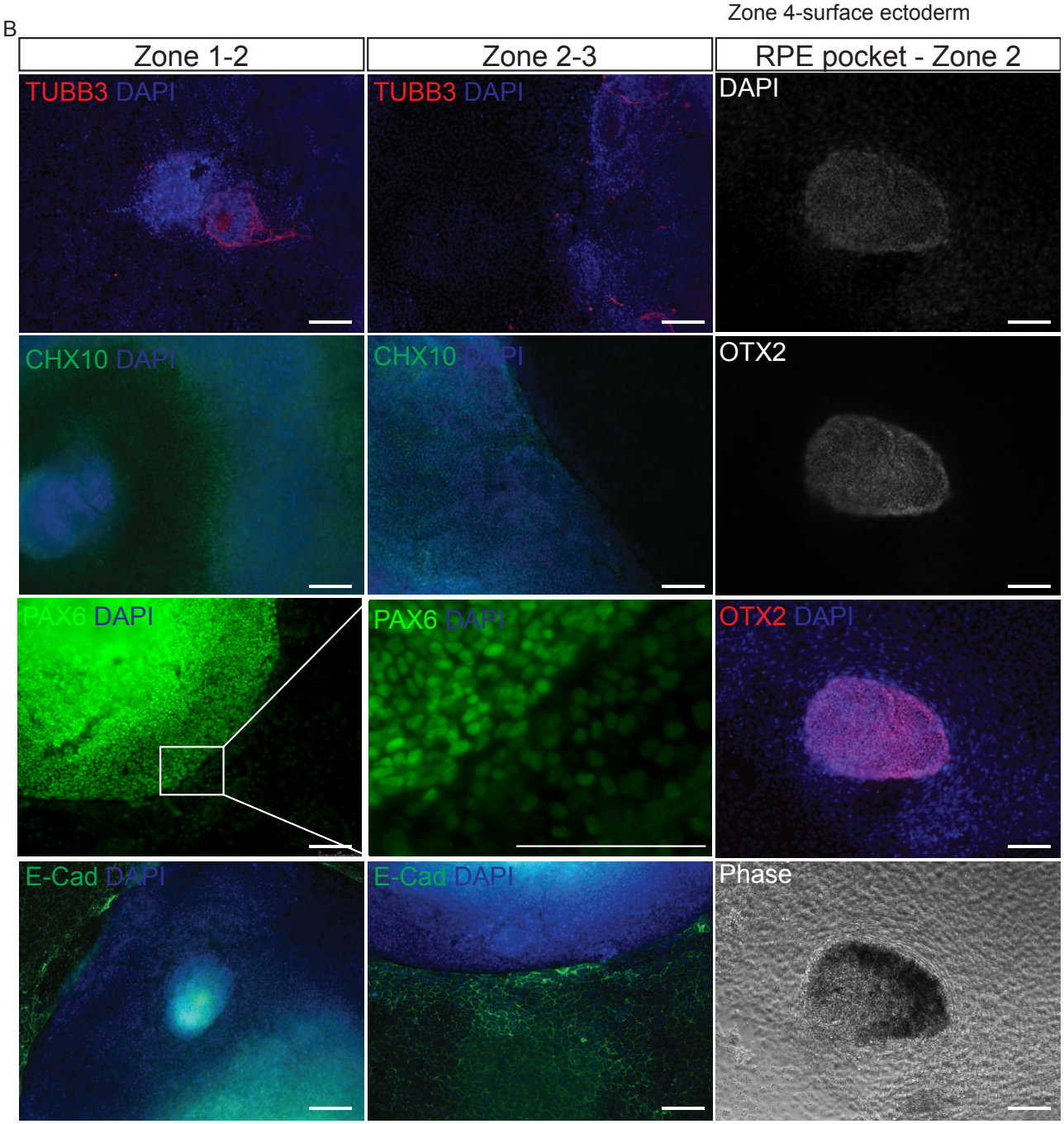


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Figure S6. Pluripotent stem cell derived SEAM, related to Figure 4. A) Phase images of SEAM differentiation. B) Expression pattern of proteins marking the differentiation of the following lineage progenitors in specific SEAM zones; TUBB3 marks the neuroectoderm lineage in Zone1, CHX10 marks the neural retina progenitors in Zone 2, PAX6 marks the optic cup in Zone 2, OTX2 marks retinal pigment epithelium. E-Cad marks ocular and surface ectoderm in Zone 3 and 4, respectively. Scale bar = 100μm.

Supplemental Figure 7

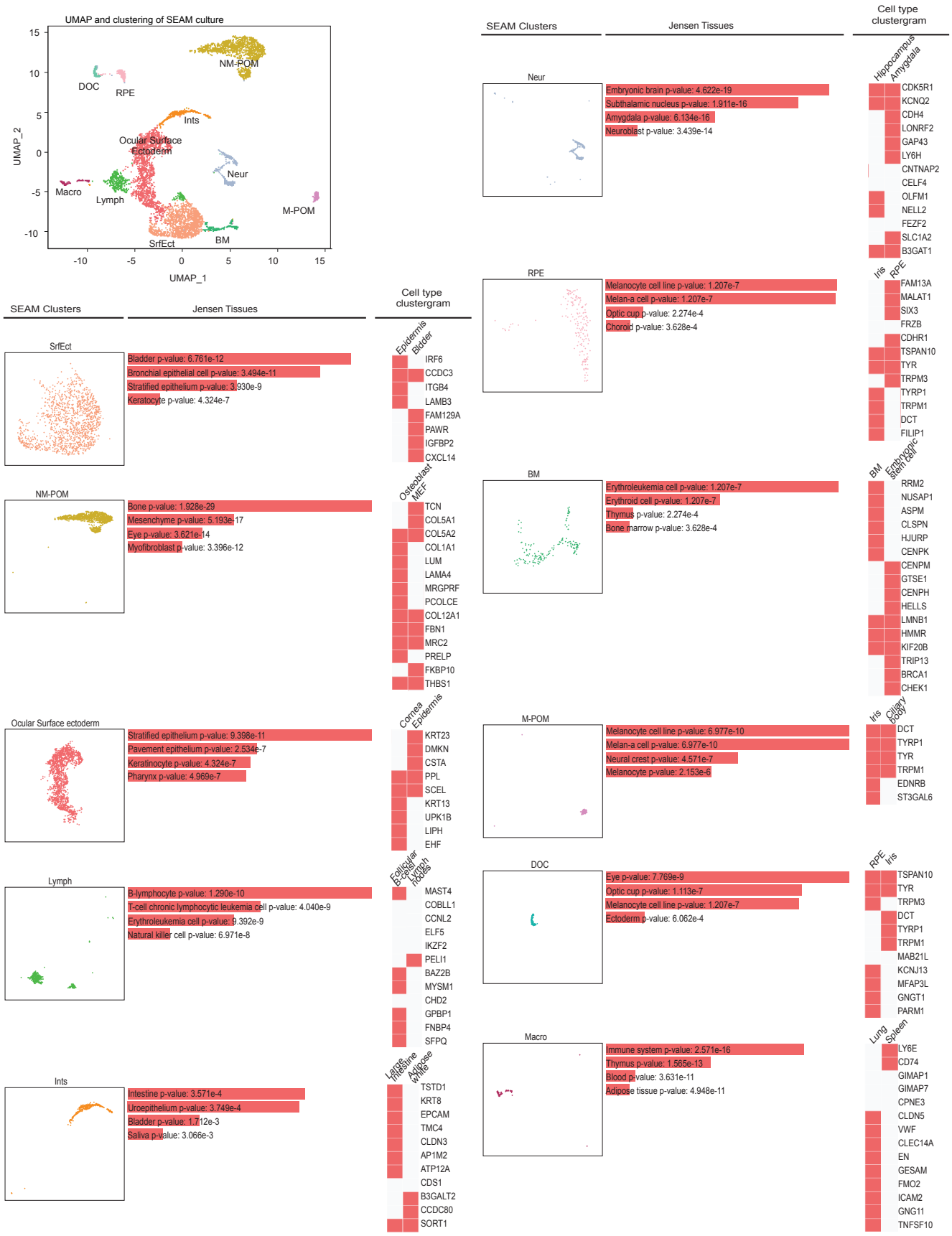

Figure S7. Annotation of SEAM differentiation, related to Figure 4. Distal optic cup (DOC), Non-melanocytic periocular mesenchyme (NM-POM), retinal pigment epithelium (RPE), Intestine (Ints), Ocular Surface Ectoderm (OCE), Macrophages (Macro), Lymphocyte (Lymph), Surface ectoderm (SrfEct), Bone Marrow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