

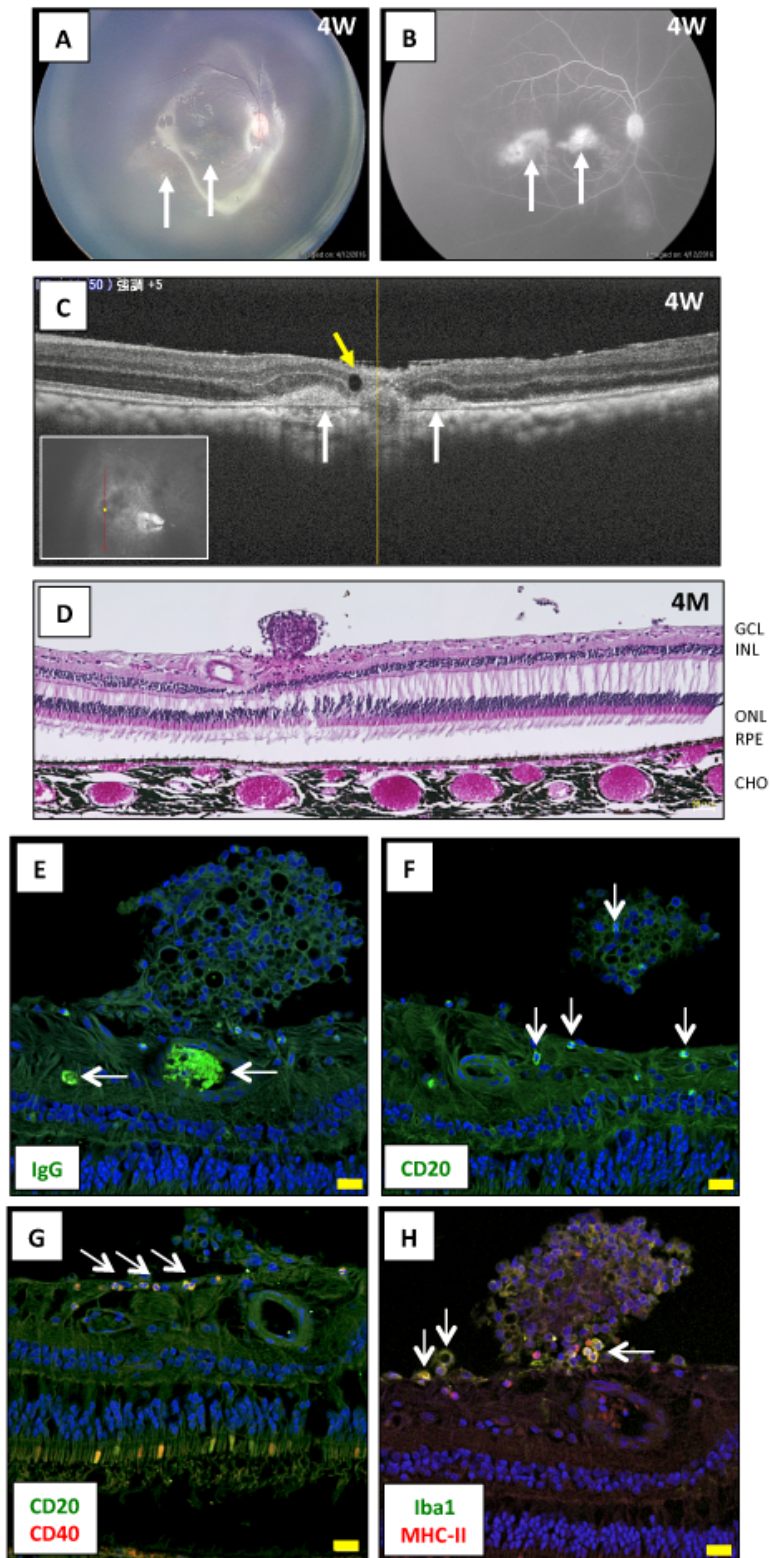
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Supplemental Information

Detection of Retinal Pigment Epithelium-Specific Antibody in iPSC-Derived Retinal Pigment Epithelium Transplantation Models

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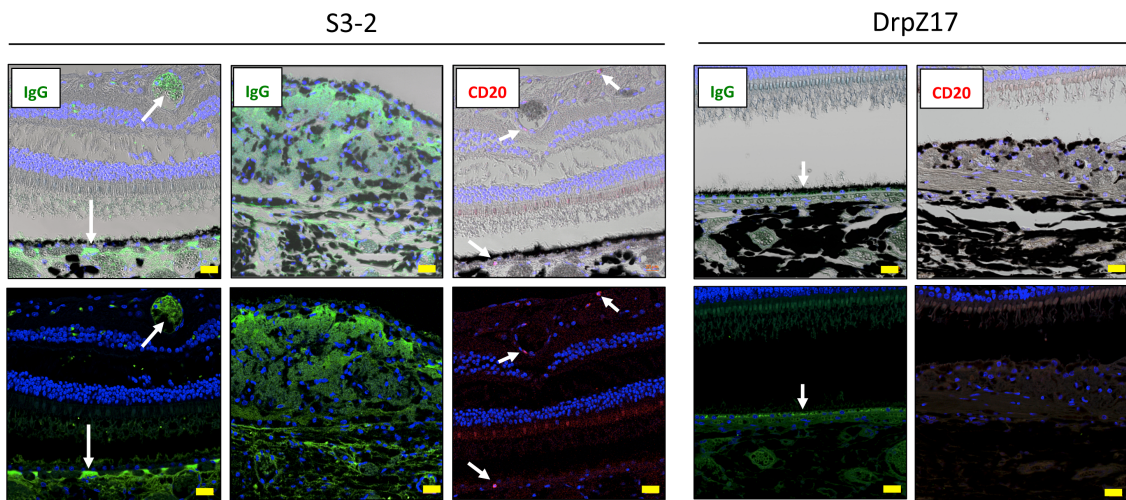
Supplementary Figure 1.



Allogeneic transplantation of iPSC-derived RPE cells into the subretinal space of a monkey and B cell related immunostaining. Transplantation of monkey 46a iPS-RPE sheets (two sheets into the right

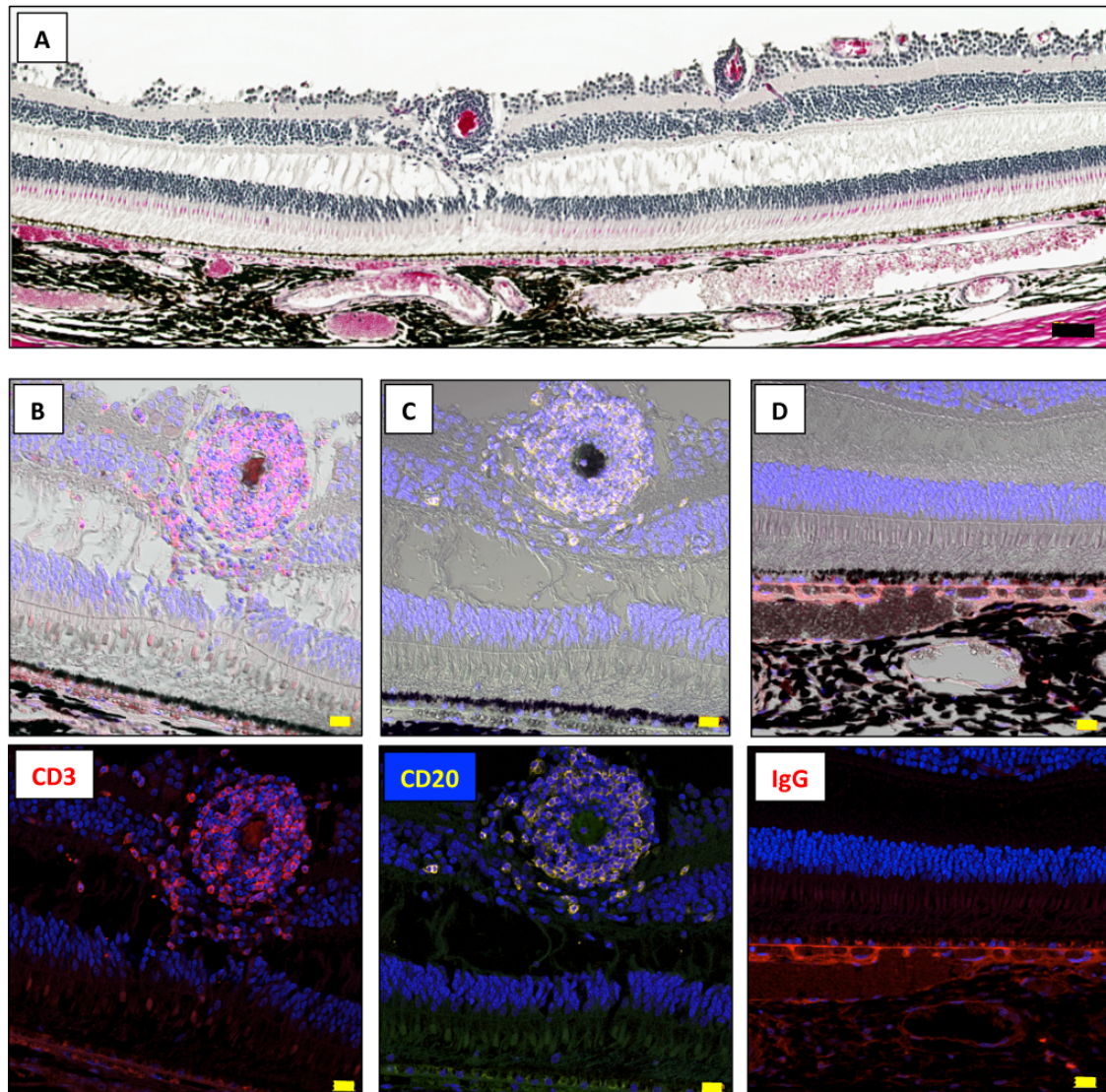
eye) into the subretinal space in a TLHM6 normal monkey was performed without immunosuppression. Fibroblastic scar-like sheets in a fundus photograph (**A**, arrows) and leakage from the graft sheets in fluorescein angiography (FA; **B**, arrows) were observed at the 4-week (4W) evaluation. Optical coherence tomography (OCT; **C**) revealed the presence of retinal cystic edema (yellow arrow) and retinal infiltration (white arrows) in the subretinal space. (**D**) Hematoxylin-eosin (HE)-stained section of the TLHM6 monkey eye [section at 4 months (4M) after transplantation] for histological interpretation. There was an inflammatory nodule on the retina. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; RPE, retinal pigment epithelium; CHO, choroid. Scale bar, 50 μm . (**E-H**) Photomicrographs showing labeling of the retina in the right eye of a TLHM6 monkey with anti-IgG, CD20/CD40, and MHC class II (MHC-II) antibodies using retinal sections. In the eye, there was enhanced IgG staining in the retina (**E**, white arrows) under the mass of the retinal nodules. Numerous CD20⁺ cells (**F**, white arrows) and CD20⁺/CD40⁺ double-positive cells (**G**, white arrows) were observed in the retina. In addition, we found Iba1⁺/MHC-II⁺ double-positive cells (antigen-presenting cells) around the retinal nodules (**H**, white arrows). Scale bars, 20 μm .

Supplementary Figure 2.



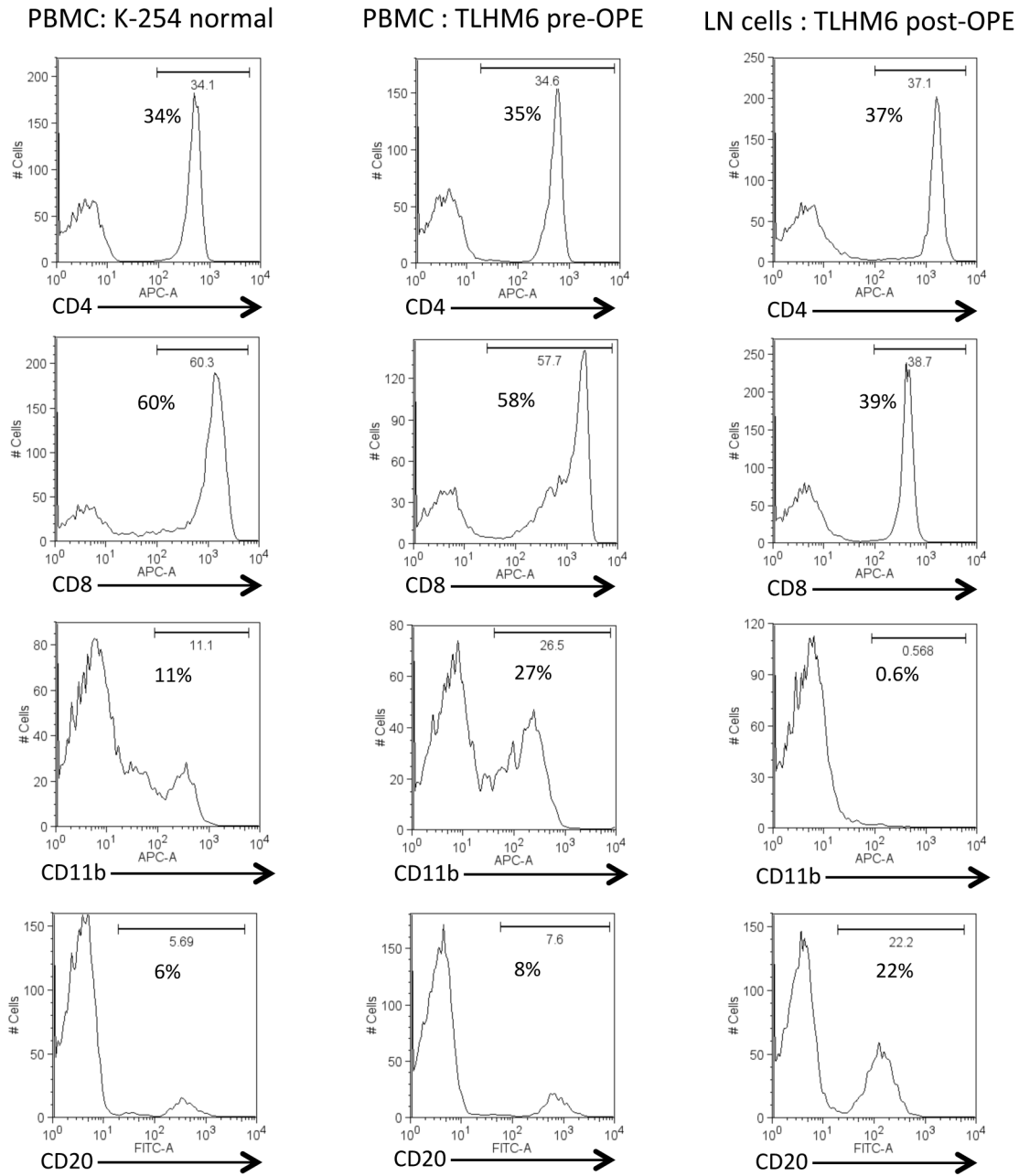
B cell related immunohistochemistry in monkeys that received allogeneic transplantation of iPSC-derived RPE cells: an MHC haplotype-mismatched monkey (S3-2) and an MHC haplotype-matched monkey (DrpZ17). Photomicrographs showing labeling of the retina in the left eye of an S3-2 or DrpZ17 monkey with anti-IgG or CD20 antibodies using paraffin-embedded sections. In the S3-2 eye, there was enhanced IgG staining in the retina and under the host RPE layer (left images, white arrows) as well as in the mass of the retinal nodules (middle images). In addition, numerous CD20⁺ cells in the retina and RPE layer (right images, white arrows) were observed. Conversely, in the DrpZ17 monkey (MHC haplotype-matched allograft; 1121A1 MHC homozygote iPS-RPE sheets → DrpZ17 MHC control monkey), weak IgG staining (left images, white arrow) was seen in the RPE layer. The iPS-RPE cell sheet seemed to be intact with no infiltration of CD20⁺ B cells into the DrpZ17 monkey eye (right images). Scale bars, 20 μ m.

Supplementary Figure 3.



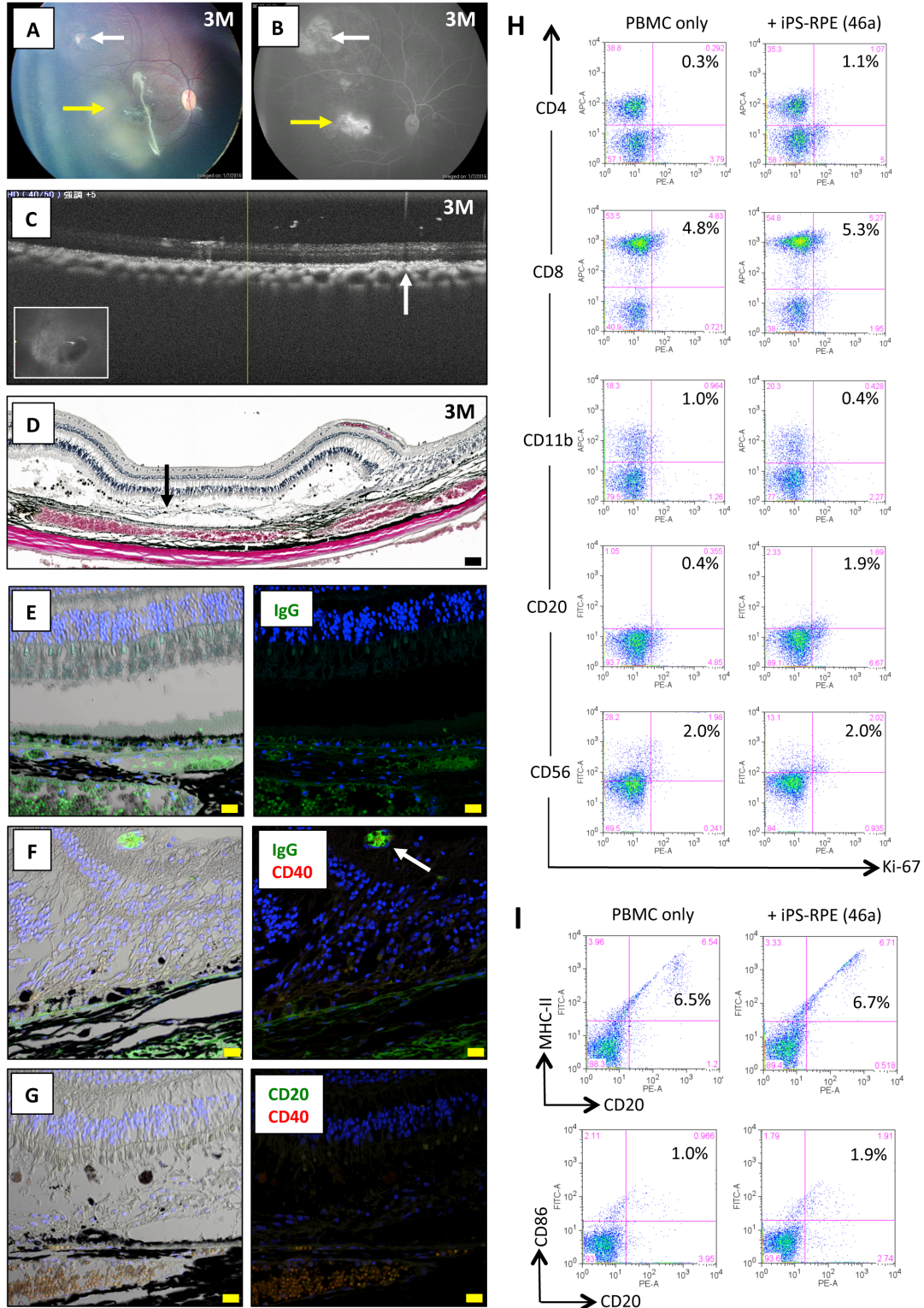
Transplantation of human iPSC-derived RPE cells (xenografts) into the subretinal space of a monkey and B/T cell related immunostaining. We prepared a human iPSC-RPE cell suspension (MTEV19 cells: 5×10^4 cells) for the transplantation. (A) Hematoxylin-eosin-stained section of the K-189 monkey right eye (section at 6 months after transplantation) for histological interpretation. There was an inflammatory nodule on the retina. Photomicrographs showing labeling of the retina in the right eye of the monkey with anti-CD3 (B), anti-CD20 (C), or anti-IgG antibody (D) using paraffin-embedded sections. In the eye, numerous CD3⁺ T cells (left image) and CD20⁺ B cells (middle image) were observed in the mass of the retinal nodules. In addition, there was enhanced IgG staining under the host RPE layer (left image). Scale bars, 20 μm .

Supplementary Figure 4.



Detection of B cells in PBMCs or lymph nodes from a monkey with immune rejection. PBMCs were collected from a normal control monkey (K-254) and a TLHM6 monkey (pre-operation, pre-OPE) in order to evaluate the percentage of B cells in the periphery. Lymph node (LN) cells were also collected from the TLHM6 monkey after transplantation (4 months post-OPE). Briefly, PBMCs or LN cells were stained with anti-CD4, anti-CD8, anti-CD11b, anti-CD20, and each isotype control antibody at 4°C for 30 min and these cells were then analyzed on a FACS flow cytometer. Numbers (%) in the histograms indicate the percentage of cells positive for the indicated antigen.

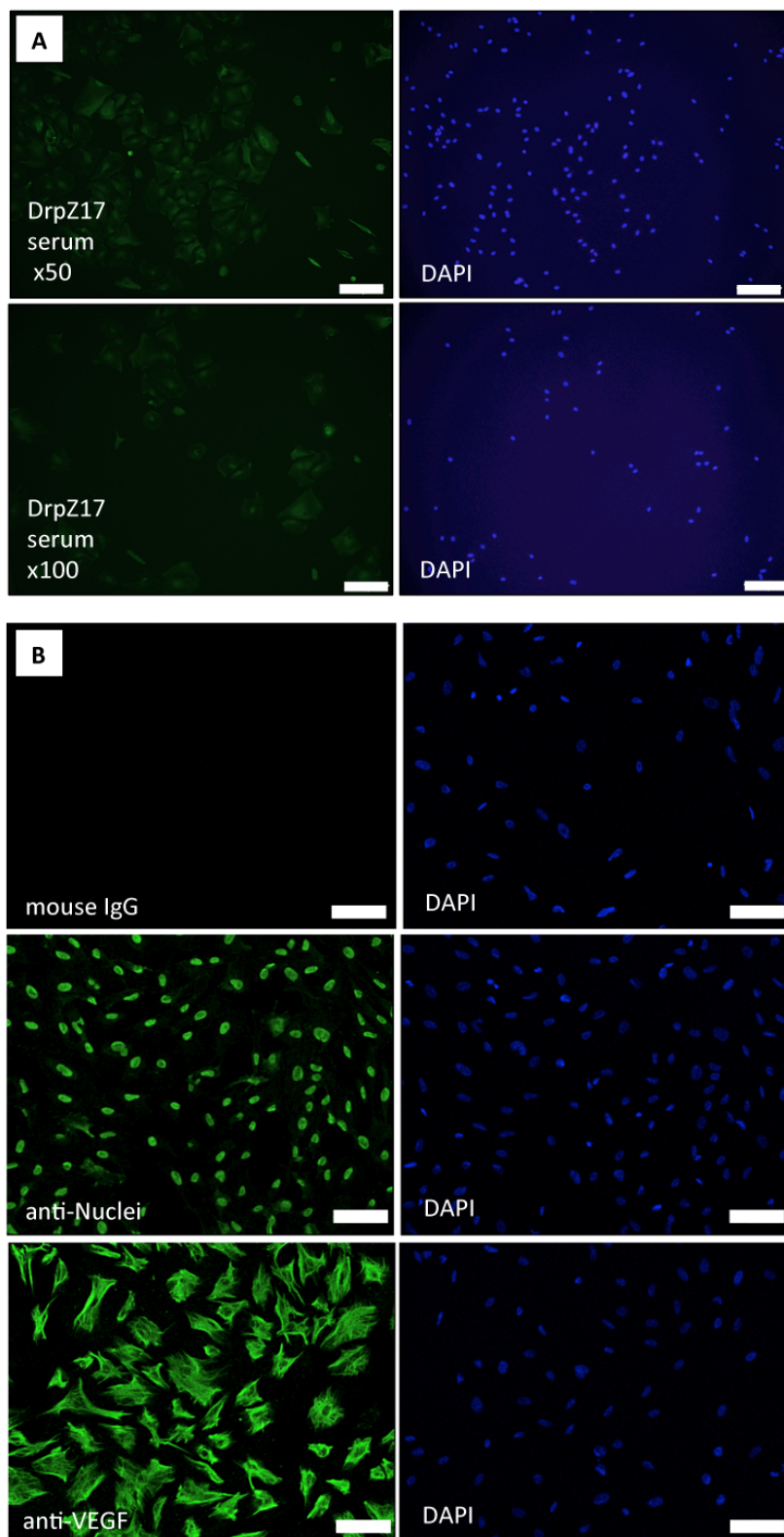
Supplementary Figure 5.



Allogeneic transplantation of an iPS-RPE cell sheet into the subretinal space of a monkey with local steroid injection and B cell related immunohistochemistry. Transplantation of a 46a iPS-RPE sheet (right eye) into the subretinal space in a TLHM15 monkey was performed with local immunosuppression

by IVTA/STTA injection. **(A)** In the operated right eye [3-month (3M) evaluation], the transplanted RPE sheet (white arrow) is shown in a fundus photograph. The yellow arrow indicates macular damage due to operation error. **(B)** No leakage from the graft sheet was observed in fluorescein angiography (**A**, arrows), and **(C)** OCT evaluation showed a graft sheet without inflammatory signs. **(D)** Hematoxylin-eosin analysis at 3M showed the RPE sheet with few inflammatory cells in the subretinal space. Scale bar, 50 μm . **(E-G)** Photomicrographs showing labeling of the TLHM15 monkey retina with anti-IgG, anti-CD20, or anti-CD40 antibody. Little IgG staining (**E**, **F**), and no CD20⁺ or CD40⁺ cells (**F**, **G**) were seen in paraffin-embedded sections of the retina. Scale bars, 20 μm . **(H)** MLR assay using PBMCs from the TLHM15 monkey and transplanted iPS-RPE cells. In the PBMC-RPE MLR assay *in vitro*, CD4⁺/Ki-67⁺ (proliferated helper T cells), CD8⁺/Ki-67⁺ (proliferated cytotoxic T cells), CD11b⁺/Ki-67⁺ (proliferated monocytes), CD20⁺/Ki-67⁺ (proliferated B cells), and CD56⁺/Ki-67⁺ (proliferated NK/NKT cells) cells were evaluated using flow cytometry. Compared with PBMCs only, all types of PBMCs that were exposed to transplanted 46a iPS-RPE cells were poorly proliferated. Numbers (%) in the histogram indicate double-positive cells (e.g., CD4⁺/Ki-67⁺). **(I)** Expression of MHC-II and CD86 molecules on B cells exposed to iPS-RPE cells. PBMCs in the presence of iPS-RPE cells were stained with anti-MHC-II, anti-CD86, and anti-CD20 antibodies. Compared with PBMCs only, expression of these molecules on CD20⁺ B cells in PBMCs exposed to 46a iPS-RPE cells was almost the same, suggesting that PBMCs from the TLHM15 monkey do not include activated memory B cells. Numbers (%) in the histogram indicate double-positive cells (e.g., CD20⁺/MHC-II⁺).

Supplementary Figure 6.



Detection of RPE-specific antibody (RSA) in the iPSC-derived RPE transplanted animal model with MHC haplotype-matched identity. Serum was collected from the operated MHC

haplotype-matched monkey, DrpZ17, at 12 weeks after surgery, and transplanted iPS-RPE cells (1121A1 MHC homozygote lines) were prepared. After RPE cell fixation, immunohistochemistry was performed using the diluted serum for RSA detection. **(A)** iPS-RPE cells + serum from the DrpZ17 monkey, **(B)** negative control staining (mouse IgG: upper image); anti-nuclei, positive control staining (middle image); anti-VEGF, RPE control staining (lower image). DAPI, nuclear staining (blue). Scale bars, 200 μm .

Supplementary Table 1.

Supplementary Table 1. Results of MHC allele typing in transplanted monkeys

MHC antigens	Name	TLHM1 monkey	TLHM6 monkey	TLHM15 monkey
	DOB	4/3/2010	5/19/2009	2/26/2008
	Age	6 Y	7 Y	8 Y
	Country	Japan	China	China
	Sex	Male	Male	Male
Mafa class I (MHC class I)	Mafa-A1	A1*015:01/02/03	A1*032:04 A1*065:04:02	A1*022:01
	Mafa-A2-A5	A4*14:01/08/10_new	A3*13:07 A4*14:03/04	A4*14:03/04
	Mafa-B sub-region	B*007:01	B*041:01	B*013:04_new
		B*039:01	B*060:02	B*013:06_new
		B*050:02	B*065:02	B*013:10
		B*060:04	B*069:03	B*014:01
		B*064:03_new	B*072:03_new	B*036:01
		B*065:02	B*095:01	B*037:01_new
B*069:03_new		B*101:01	B*041:01_new	
B*117:01/02_new	B*104:03	B*045:03_new		
B*148:01_new	B*154:01	B*050:02		
	B*155:01	B*072:03_new		
		B*115:04:02_new		
Mafa class II (MHC class II)	Mafa-DRB sub-region	DRB3*04:03	DRB*W37:01	DRB*W1:01
		DRB*W4:04	DRB*W49:01	DRB*W3:02
		DRB*W20:03	DRB1*04:13	DRB1*03:03/30
		DRB*W25:01	DRB1*10:02	DRB1*04:02
		DRB*W27:04		DRB1*W4:01
Mafa-DQA1	DQA1*23:01_new	DQA1*05:03_new	DQA1*01:03	
	DQA1*24:04	DQA1*05:04	DQA1*01:04	
Mafa-DQB1	DQB1*18:04	DQB1*16:01	DQB1*06:01	
	DQB1*18:16/21	DQB1*17:05	DQB1*06:16	
Mafa-DPA1	DPA1*10:01	DPA1*02:06/08_new DPA1*10:01	DPA1*02:20_new DPA1*10:01_new	
Mafa-DPB1	DPB1*18:01	DPB1*07:01	DPB1*15:05	
	DPB1*18:02	DPB1*18:02	DPB1*18:01	
Grafts (right eye / left eye)		46a iPS-RPE sheets/ sheets	46a iPS-RPE sheets/-	46a iPS-RPE sheets/-
MHC matched to 46a iPS-RPE cells		No	No	No
Immune rejection (right eye / left eye)		Yes / Yes	Yes /-	No /-
Medication (right eye / left eye)		None	None	IVTA/STTA injection /-
Obsevation period (right eye / left eye)		6M / 4W	4M /-	3M /-

New in MHC results indicates new allele gene. IVTA - intravitreal triamcinolone acetamide. STTA - sub-Tenon triamcinolone acetamide.