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Supplemental Information

Long-term survival in non-human primates of stem cell-derived, MHC-unmatched corneal epithelial cell sheets

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Supplemental Information

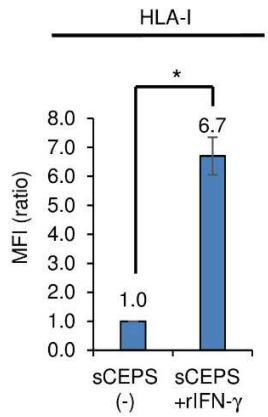

Figure S1

Figure S1. HLA-I expression intensity ratio of sCEPS. Related to Figure 1D. HLA-I expression intensity ratio from flow cytometric analyses of sCEPS with or without rIFN-γ (for 48 h). Means ± SEM of ten experiments are presented. *P < 0.05 compared to two groups using the Wilcoxon signed-rank test. MFI, Mean Fluorescence Intensity.

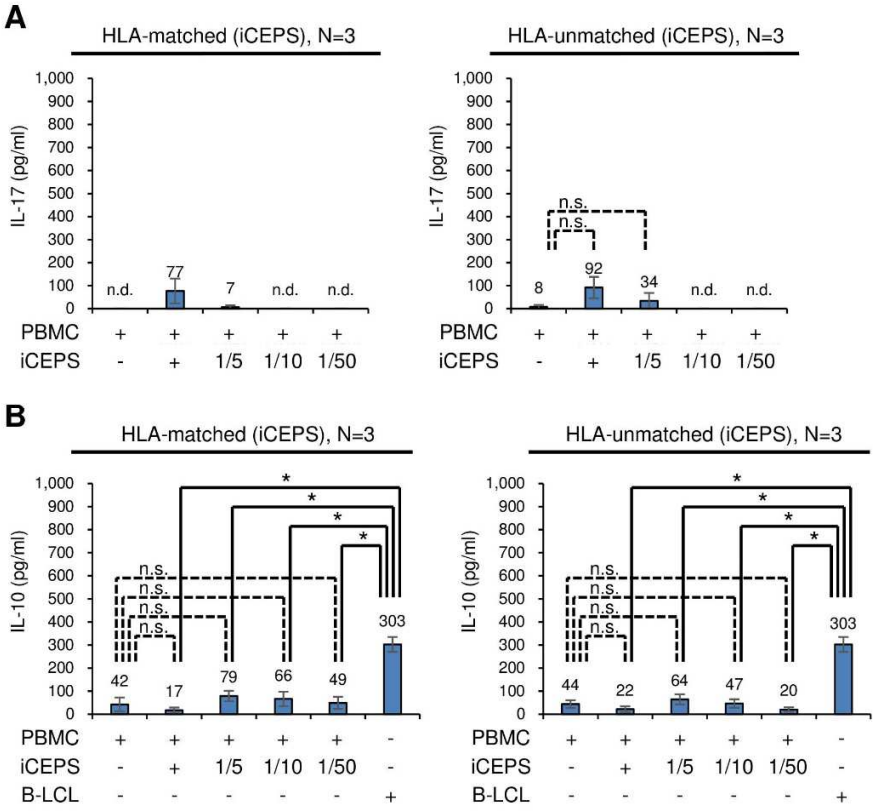

Figure S2

Figure S2. MLR of PBMCs and iCEPS. Related to Figure 1. (A and B) PBMCs were co-cultured with allogeneic iCEPS at a ratio of 1×10^5 (1:1) to 2×10^3 (1:1/50) cells/well. As a positive control, B-LCL were cultured at a concentration of 1×10^5 cells (B). After 7 days, supernatants were harvested and assessed for IL-17 (A) and IL-10 (B) secretion using ELISA. *P < 0.05 compared two groups using the Mann–Whitney U test. n.s., not significant. n.d., not detected.

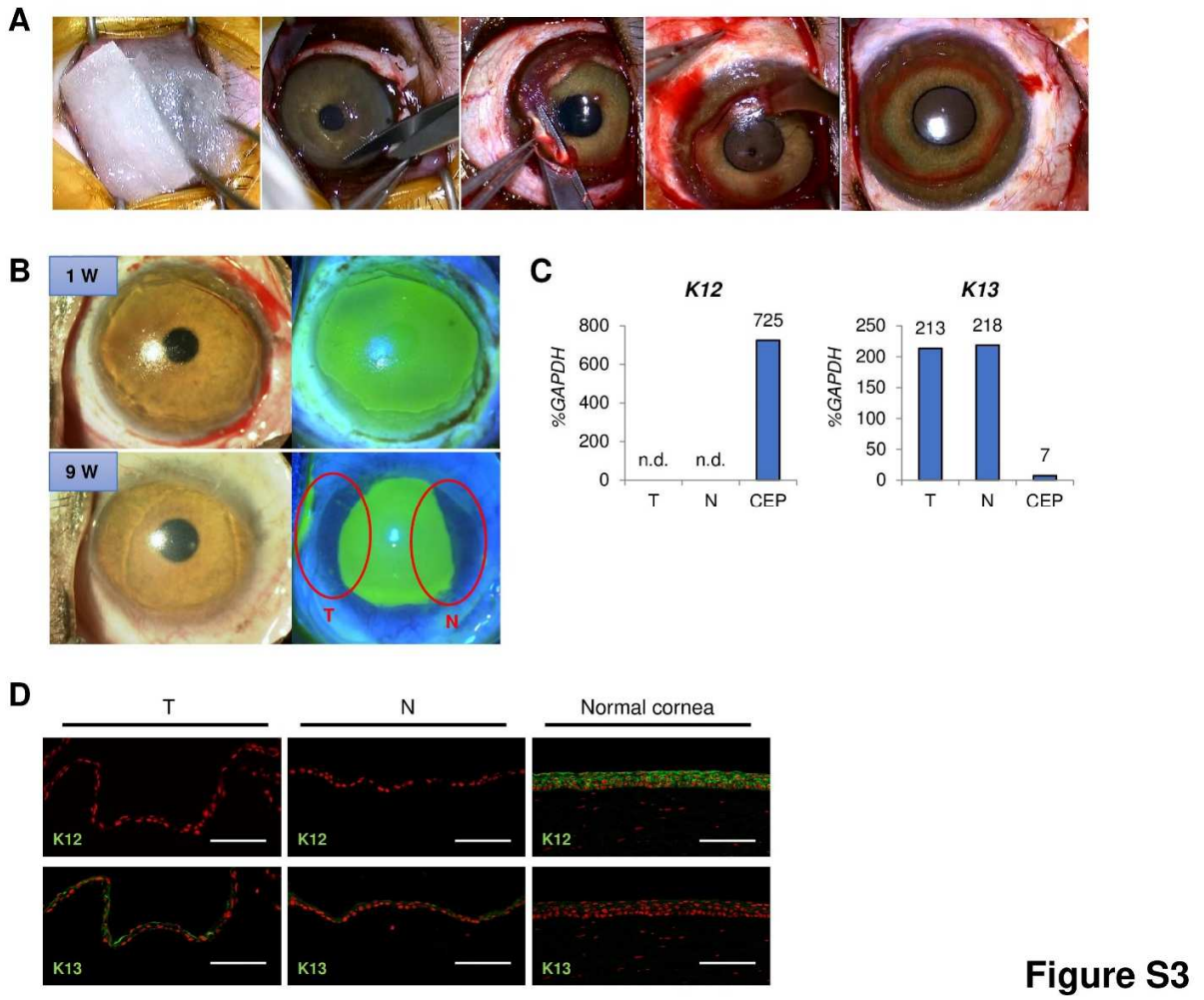

Figure S3. Cynomolgus monkey LSCD model. Related to Figure 2A. (A) Method of LSCD model preparation. (A) Soaked gauze with 90% EtOH was placed on the cornea for 30 s, then washed with 200 ml BSS PLUS® (Alcon Japan, ltd, Tokyo, Japan) (leftmost photograph). The conjunctiva was incised around the circumference (second photograph from the left). The limbus was resected (center photograph). The corneal epithelium was scraped using a scleral knife for 3 min, and a quick medical absorber was applied for 1 min (second photograph from the right). The rightmost photograph shows the anterior segment immediately after LSCD model preparation. (B) Photograph of the anterior segment 1 week (W, upper panels) or 9 W (lower panels) after LSCD model preparation using a slit lamp (left panels) and fluorescein staining (right panels). Areas of corneal surface without an epithelial barrier are stained by the fluorescein dye and appear green under blue light. At 9 W, epithelium invading cornea, which was not stained with fluorescein, was observed on the temporal (T) and nasal (N) sides (right lower panel). (C) *K12* or *K13* gene expression in temporal (T) or nasal (N) recovery epithelium. Normal corneal epithelium (CEP) of cynomolgus monkey was used as a positive control. n.d., not detected. (D) Immunostaining for K12 or K13 (green) in the temporal (T) or nasal (N) recovery epithelium. Normal cornea of cynomolgus monkey was used as a positive control. Nuclei, red. Scale bars, 100 μm.

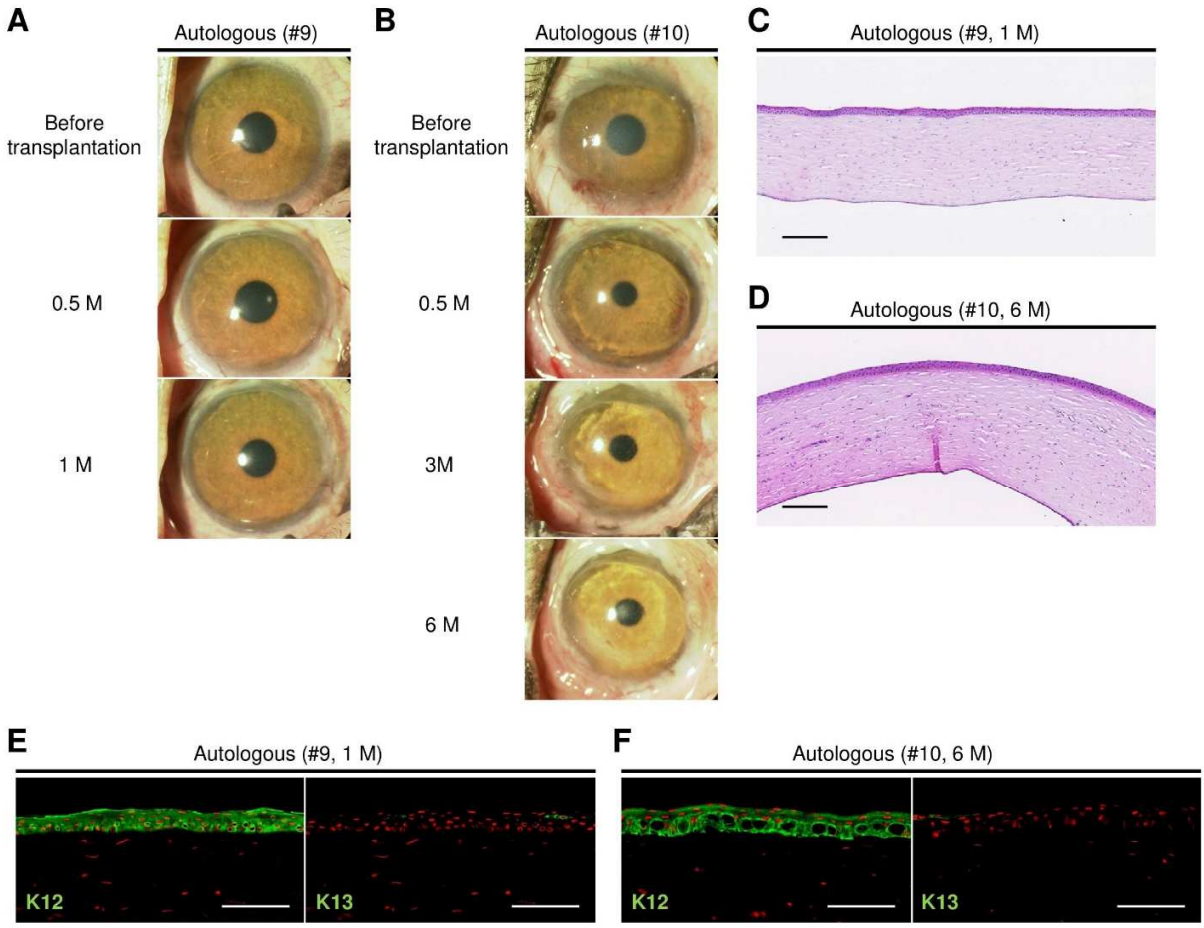

Figure S4. Autologous sCEPS transplantation in cynomolgus monkeys. Related to Figures 2A and 4F. (A and B) Anterior segment photograph of #9 (A) and #10 (B) after autologous sCEPS transplantation. The transplanted sCEPS remained stable with slight ocular surface inflammation in both monkeys examined by ophthalmoscopy during the observation period. M, months. (C and D) H&E staining of the central cornea of #9 (C) and #10 (D) after autologous sCEPS transplantation. A uniformly stratified epithelium on the central corneal surface and infiltrated inflammatory cells in the corneal stroma were observed in both monkeys, indicating that a certain degree of immune response occurred. Scale bars, 200 μm. (E and F) Immunostaining of the central cornea of #9 (E) and #10 (F) for K12 and K13 (green) after autologous sCEPS transplantation. The stratified epithelium in the central cornea was positive for K12 and locally positive for K13 in the outermost layer, similar to sCEPS in both #9 and #10, indicating that the donor remained. Nuclei, red. Scale bars, 100 μm. See also Figure S6.

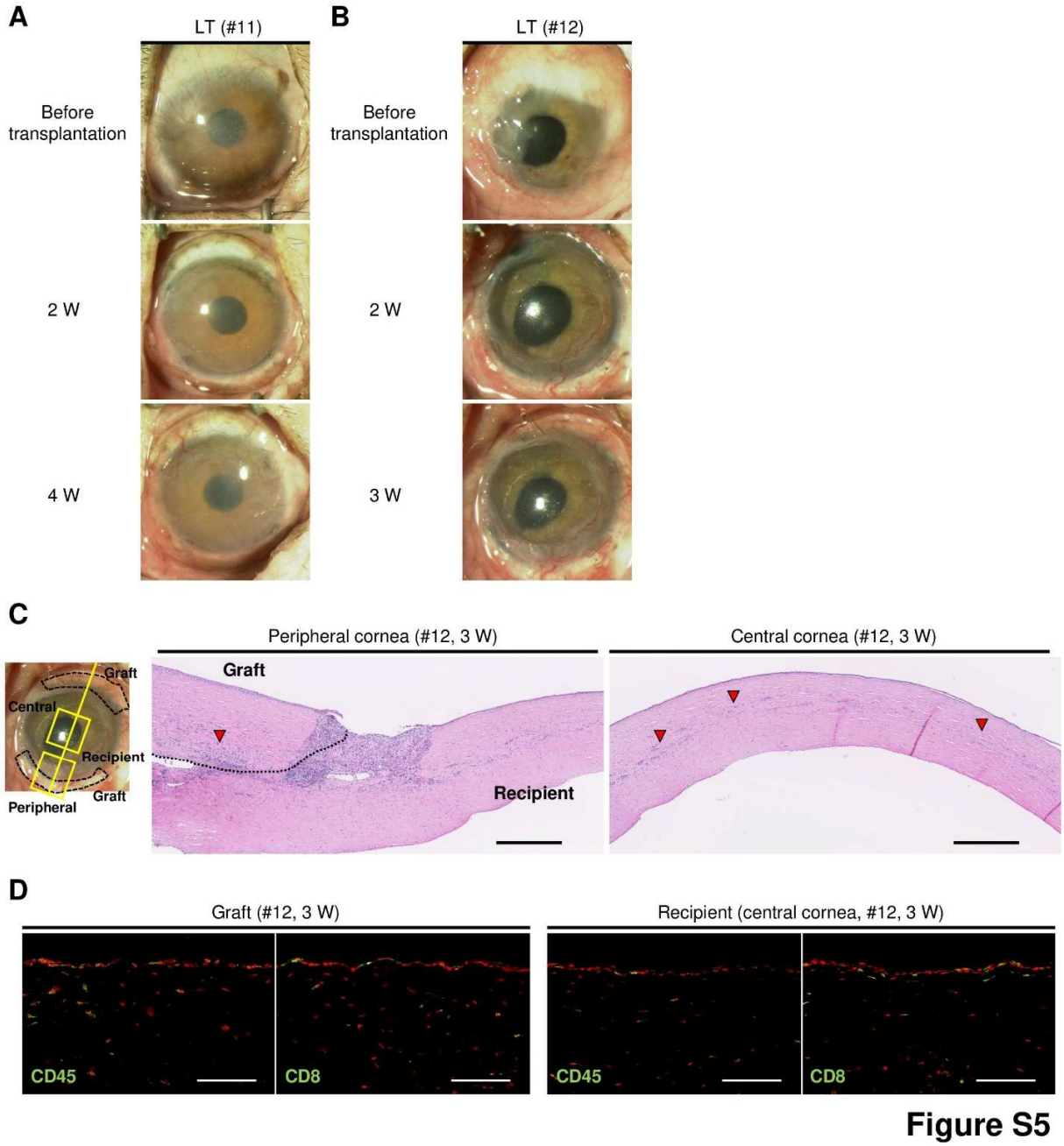

Figure S5

Figure S5. Allogeneic LT in cynomolgus monkeys. Related to Figure 2A. (A and B) Anterior segment photograph after LT. Corneal neovascularization (CNV) was observed preoperatively in both #11 (A) and #12 (B) after LSCD model preparation. The entire cornea was epithelialized, and the ocular surface inflammation was reduced 14 D after transplantation in both monkeys. Sharply increased central corneal thickness (CCT), risen conjunctival injection (CI) grade, and expanded CNV area were observed simultaneously at 28 D in #11 or at 20 D in #12. In #12, the eyeball was removed to perform histological analyses at 20 D. W, weeks. (C) H&E staining of the central cornea of #12 after LT. More infiltrated inflammatory cells in the recipient cornea and much more in the donor limbal tissue (graft) were observed (arrowheads), compared to those in the corneal stroma in #9 (autologous sCEPS transplantation, see Figure S4C). Scale bars, 500 μm. W, weeks. (D) Immunostaining of the central cornea of #12 for CD45 and CD8 (green) after LT. Many infiltrated CD8 positive cells were observed in both graft and recipient epithelium and the underlying stroma, indicating that rejection had occurred. Nuclei, red. Scale bars, 100 μm. See also Figure S6 and Table S3.

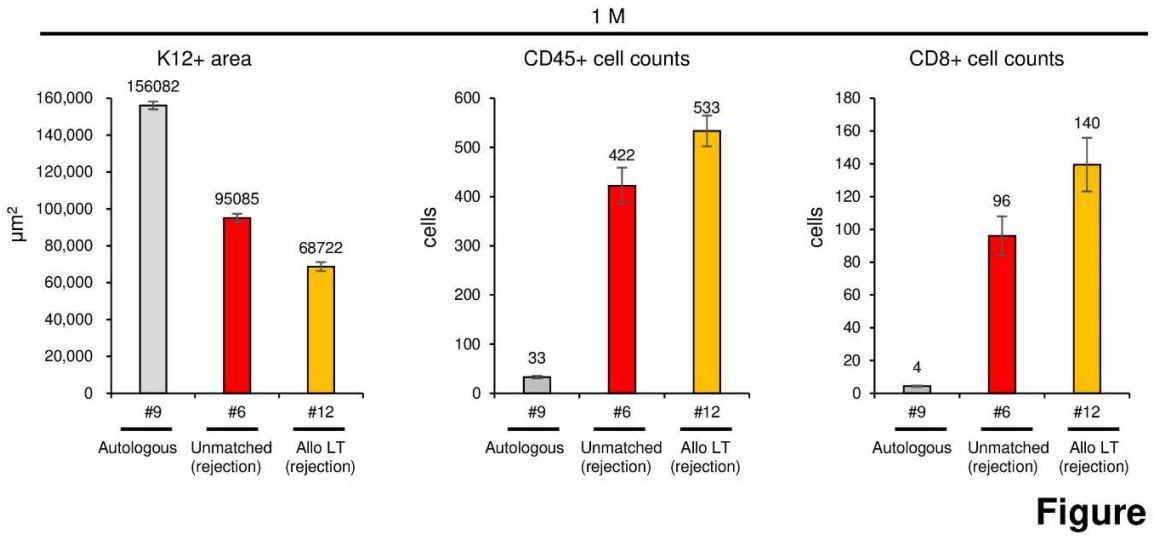

Figure S6

Figure S6. K12 positive area and CD45 and CD8 positive cell count in the central cornea 1 month (M) after transplantation in cynomolgus monkeys. Related to Figures 4, S4, and S5. K12 positive area and CD45 and CD8 positive cell counts in the central cornea (4 mm diameter central zone) at 1 M after transplantation. Means ± SEM of the data from ten slides are presented. The intervals between each sample were more than 70 μm. #6 (MHC-unmatched monkey with rejection) and #12 (LT with rejection) had less extensive K12 positive areas and more CD45 and CD8 positive cells than #9 (autologous sCEPS transplantation).

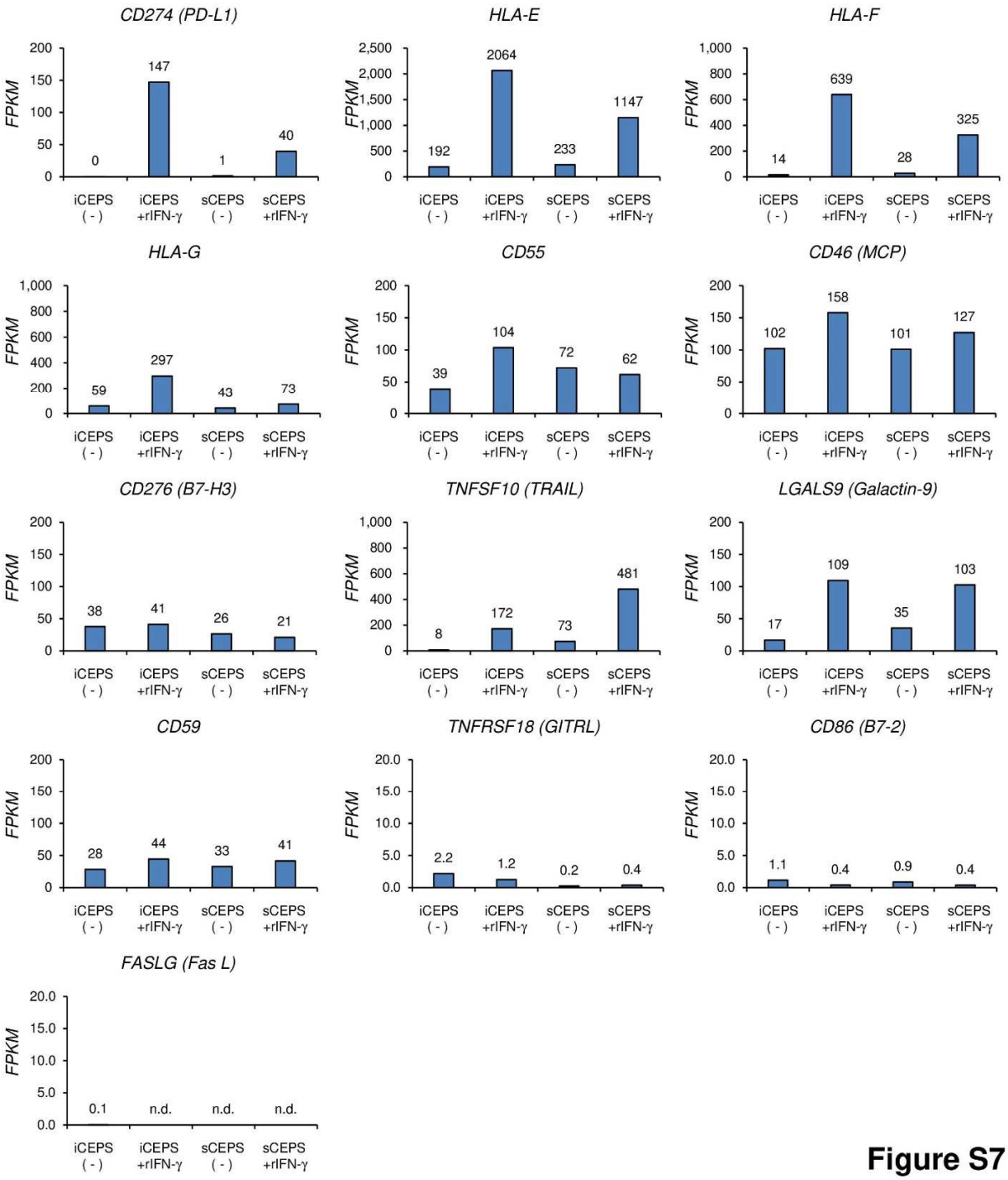

Figure S7

Figure S7. RNA-sequencing of iCEPS and sCEPS with or without rIFN-γ treatment (48 h), focusing on immunomodulatory molecules expressed on the cell surface of the anterior segment of the eye. Related to Figure 5. FPKM, fragments per kilobase of exon per million mapped fragments. n.d., not detected.

Table S1. MHC typing of cynomolgus monkeys of MHC-matched sCEPS transplantation. Related to Figures 2B, 3, and 4.

Group	MHC almost homozygous donor		MHC-matched sCEPS transplantation					
No.			#1		#2		#3	
Mafa-F	F-like4	F-like1	F-like4	F-like1	F-like4	F-like1	F-like4	F-like1
Mafa-A1	A1*052:02	A1*089:03	A1*052:02	A1*089:03	A1*052:02	A1*089:03	A1*052:02	A1*089:03
Mafa-A2~A5	A4*01:04	A2*05:50 A3*13:03:01	A4*01:04	A2*05:50 A3*13:03:01	A4*01:04	A2*05:50 A3*13:03:01	A4*01:04	A2*05:50 A3*13:03:01
Mafa-E	E-like5 E-like11	E-like3 E-like10	E-like5 E-like11	E-like3 E-like10	E-like5 E-like11	E-like3 E-like10	E-like8 E-like9	E-like3 E-like10
Mafa-B	B*033:02 B*095:01 B*098:10	B*033:02 B*095:01 B*098:10	B*033:02 B*095:01 B*098:10	B*041:01 B*050:08 B*072:01 B*098:08 B*101:01:02	B*033:02 B*095:01 B*098:10	B*050:08 B*056:02:01 B*060:03:01 B*072:01 B*089:01:02 B*157:01	B*033:02 B*095:01 B*098:10	B*007:01:01 B*085:01 B*098:08 B*158:01 B*159:01
Mafa-I	I*01:11	I*01:11	I*01:11	I*01:12:01	I*01:11	I*01:12:01	I*01:11	I*01:13:01
Mafa-DRB	DRB1*03:21 DRB1*10:07	DRB1*03:21 DRB1*10:07	DRB1*03:21 DRB1*10:07	DRB1*03:19 DRB*W33:04	DRB1*03:21 DRB1*10:07	DRB1*03:21 DRB1*10:07	DRB1*03:21 DRB1*10:07	DRB*W1:07 DRB*W6:02 DRB*W6:03:01 DRB3*04:02:01
Mafa-DQA1	DQA1*01:07:01	DQA1*01:07:01	DQA1*01:07:01	DQA1*24:05	DQA1*01:07:01	DQA1*01:07:01	DQA1*01:07:01	DQA1*24:10
Mafa-DQB1	DQB1*06:08	DQB1*06:08	DQB1*06:08	DQB1*18:05	DQB1*06:08	DQB1*06:08	DQB1*06:08	DQB1*18:26
Mafa-DPA1	DPA1*02:05	DPA1*02:05	DPA1*02:05	DPA1*07:04	DPA1*02:05	DPA1*02:05	DPA1*02:05	DPA1*04:02
Mafa-DPB1	DPB1*15:04	DPB1*15:04	DPB1*15:04	DPB1*21:01	DPB1*15:04	DPB1*15:04	DPB1*15:04	DPB1*03:04

sCEPS, somatic stem cell-derived corneal epithelial cell sheets.

Table S2. MHC typing of cynomolgus monkeys of MHC-unmatched sCEPS transplantation. Related to Figures 2B, 3, and 4.

Group	MHC-unmatched sCEPS transplantation				sCEPS donor for #8	MHC-unmatched sCEPS transplantation with local steroids
No.	#4	#5	#6	#7		#8
Mafa-F	F-like1_like	F-like1_like	F-like1_like	F-like1_like	F-like1 F-like4	F*01:06:01
Mafa-A1	A1*010:02:01/02 A1*102:01_like	A1*077:01_like	A1*001:01:02 A1*032:03_like	A1*058:02 A1*090:01	A1*052:02 A1*089:03	A1*015:01/03
Mafa-A2~A5	A2*05:06:01 A3*13:14	A4*14:03/04	A4*14:03/04	A2*05:31	A2*05:50 A3*13:03:01 A4*01:04	A3*13:16
Mafa-E	E*02:01/02_like E*02:04 E-like5	E*02:03	E*02:03_like E*02:04	ND	E-like3 E-like5 E-like10 E-like11	E*02:03 E*02:04
Mafa-B	B*030:02 B*043:01 B*045:03_like B*051:05 B*057:03 B*073:01 B*079:02 B*092:01 B*136:02/04	B*001:01 B*030:01/03 B*030:08N B*044:0101 B*051:06 B*079:02 B*085:01 B*184:01_like	B*007:01_like B*007:05 B*030:02 B*043:01 B*046:03/11 B*056:01 B*057:03 B*073:01 B*082:01 B*092:01	B*004:03_like B*007:01 B*007:05 B*039:01 B*060:03_like B*060:04 B*082:01 B*098:03	B*033:02 B*045:05 B*050:08 B*072:01 B*095:01 B*098:10 B*099:01 B*108:01 B*114:02	B*007:01:01/04 B*030:01/03/07 B*039:01 B*060:01 B*060:03 B*060:04
Mafa-I	I*01:06 I*01:08/09	I*01_like I*01:08	I*01:06 I01_like	I*01_like I*01:18	I*01:11 I*01:12:01	I*01
Mafa-DRB	DRB*W25:03 DRB*W4:03 DRB*W6:01/06/07_like DRB1*03:12	DRB1*04:05 DRB4*01:06	DRB*W6:01/06/07_like DRB*W7:02/07 DRB1*04:14_like DRB5*03:04	DRB*W1:01 DRB*W3:02 DRB1*03:03/30 DRB1*03:06 DRB5*03:09	DRB1*03:21 DRB1*10:07 DRB*W1:08 DRB*W3:01 DRB*W36:01	DRB1*03:03/30 DRB1*04:12 DRB*W3:02 DRB*W4:01
Mafa-DQA1	DQA1*01:12 DQA1*05:04	DQA1*26:04 DQA1*05:06	DQA1*24:02 DQA1*01:07	DQA1*01:03 DQA1*26:04_like	DQA1*01:07:01 DQA1*26:03	
Mafa-DQB1	DQB1*06:30 DQB1*17:05	DQB1*18:13_like DQB1*15:04	DQB1*17:08 DQB1*18:08	DQB1*06:16 DQB1*18:02	DQB1*06:08 DQB1*18:07:02	DQB1*06:16 DQB1*18:01:01
Mafa-DPA1	DPA1*02:09_like DPA1*07:03_like	DPA1*04:02 DPA1*02:01	DPA1*02:20_like DPA1*07:04_like	DPA1*02:12 DPA1*06:01	DPA1*02:05 DPA1*07:04	
Mafa-DPB1	DPB1*19:06 DPB1*01:02	DPB1*01:01/07 DPB1*02:04	DPB1*15:05 DPB1*15:04	DPB1*05:02 DPB1*01:02	DPB1*15:04 DPB1*21:01	DPB1*01:01/07 DPB1*18:01

sCEPS, somatic stem cell-derived corneal epithelial cell sheets.

Table S3. MHC typing of cynomolgus monkeys of allogeneic LT. Related to Figures 2B and S5.

Group	LT donor 1	LT recipient 1	LT donor 2	LT recipient 2
No.		#11		#12
Mafa-F	F-like1	F-like1	F-like1	F-like1
Mafa-A1	A1*028:01 A1*065:03	A1*015:01/02/03	A1*007:03 A1*053:01	A1*007:03 A1*066:03
Mafa-A2~A5	A3*13:07	A2*24:04 A4*14:03/04	A2*05:21 A4*01:07 A6*01:02	A2*05:22 A2*05:12/26 A6*01:01 A6*01:02
Mafa-E	E*02:04 E*02:01/02 E*02:03 E-like5	E*02:03	E-like5 E-like10 E*02:04	E-like1 E-like5 E-like9 E-like4
Mafa-B	B*013:03 B*056:02/03 B*056:02 B*104:03 B*109:02 B*137:03 B*162:01 B11L*01:05	B*001:01 B*015:03 B*044:01 B*051:04 B*062:01N B*068:03 B*079:02 B*085:01 B*098:04 B*104:03 B*109:03 B*115:02	B*017:02 B*018:01 B*048:05 B*051:01 B*056:02/03 B*060:03 B*068:08 B*072:03 B*104:03 B*108:01 B*002:04/B*136:02 B*136:03 B*159:01	B*030:11 B*033:03 B*048:05 B*051:03 B*060:03 B*068:04 B*068:08 B*072:03 B*088:01 B*104:03 B*108:01 B*109:03 B*161:04 B11L*01:03
Mafa-I	I*01 I*01:18	I*01 I*01:18	I*01:06 I*01	I*01
Mafa-DRB	DRB*W25:03 DRB*W27:04 DRB*W4:03 DRB1*07:02	DRB*W6:01/06/07 DRB5*03:11 DRB5*03:12	DRB*W49:01 DRB*W7:01 DRB*W7:02/07 DRB1*04:12 DRB1*04:14 DRB1*10:02	DRB*W1:04 DRB*W2:02 DRB1*03:03/30 DRB1*10:02
Mafa-DQA1	DQA1*01:12 DQA1*01:12	DQA1*01:03 DQA1*26:04/05	DQA1*26:01:01	DQA1*01:13
Mafa-DQB1	DQB1*06:30 DQB1*18:04	DQB1*18:08	DQB1*15:01 DQB1*16:01	DQB1*06:17 DQB1*16:01
Mafa-DPA1	DPA1*02:09 DPA1*10:01	DPA1*08:01	DPA1*02:01	DPA1*02:10/14
Mafa-DPB1	DPB1*15:01 DPB1*18:01	DPB1*01:04 DPB1*06:01	DPB1*01:01/07	DPB1*01:01/07 DPB1*01:06

LT, limbal transplantation.

Supplemental Experimental Procedures

MLR with human iCEPS

MLR was performed as described in the Experimental Procedures. As a positive control of IL-10 secretion, B-LCL were incubated with 50 μg/ml mitomycin C for 45 min at 37 °C to inactivate their proliferative activity before MLR, and were further cultured in 1×10^5 cells. Supernatants were harvested after 7 days and assessed for IL-17 or IL-10 secretion using ELISA (Bio-Techne).

MHC genotyping of cynomolgus monkey MHC genes

DNA typing of the cynomolgus monkey MHC class I genes (Mafa-A, Mafa-B, Mafa-E, Mafa-F, and Mafa-I) and Mafa class II genes (Mafa-DRB, Mafa-DQA1, Mafa-DQB1, Mada-DPA1, and Mafa-DPB1) were performed with MHC locus-specific primer sets and PCR conditions(Shiba et al., 2016; Shiina et al., 2015). MHC typing results of the 13 recipients or donors are shown in Tables S1-S3.

Preparation of non-human primate LSCD models

All cynomolgus monkey LSCD models were prepared with the right eye. To make the LSCD model, gauze soaked with 90% EtOH was placed on the cornea for 30 s. The cornea was then washed with BSS PLUS® (Alcon Japan, ltd, Tokyo, Japan), incised all around the conjunctiva, and the limbus was resected. After the LSCD model was prepared, the ocular surface condition was observed weekly using a slit lamp and fluorescein staining; areas of corneal surface without an epithelial barrier are stained by the dye and appear green under blue light. Epithelium invading the cornea, which was not stained with fluorescein, was collected 2-4 W after model preparation, and the absence of corneal epithelium regeneration and conjunctival invasion into the cornea were confirmed by assessing K12 and K13 expression using PCR and immunostaining.

Gene expression analyses

Total RNA was obtained from the abraded corneal epithelium of cynomolgus monkeys (#1-12) using the RNeasy total RNA kit and the QIAzol reagent (Qiagen, California, USA). Reverse transcription was performed using the SuperScript III First-Strand Synthesis System for qRT-PCR (Thermo Fisher Scientific) according to the manufacturer's protocol, and cDNA was used as a template for PCR. qRT-PCR was performed using the ABI Prism 7500 Fast Sequence Detection System (Thermo Fisher Scientific) per the manufacturer's instructions. Quantitative PCR was performed with Taq-man Universal PCR Mastermix and preformulated primers for K12 (assay ID Mf02827482_g1), K13 (assay ID Mf02841662_m1), Tp63 (assay ID Hs00978339_m1), and GAPDH (assay ID Mf04392546_g1) (Thermo Fisher Scientific), according to the manufacturer's instructions. Thermocycling was performed with an initial cycle at 95 °C for 20 s, followed by 45 cycles at 95 °C for 3 s and 60 °C for 30 s.

Transplantation of autologous sCEPS or allogeneic limbus

Autologous sCEPS transplantation was performed as negative controls of rejection (#9 and #10), and allogeneic LT was performed as positive controls of rejection (#11 and #12). LSCD model preparation was performed as described in Experimental Procedures. For autologous sCEPS transplantation, sCEPS was prepared from the fellow eye (left eye) and transplantation was performed as described in Experimental Procedures. For LT, the cornea containing euthanized cynomolgus monkey limbus was collected, and the central part of the cornea was removed with a trephine blade (Inami, Tokyo, Japan). The graft was prepared, divided into two, and transplanted to the recipient's upper and lower corneal border regions.

Systemic steroid was administered for 4 (#9, #10, and #11) or 5 days (#12) from the preoperative day to the 2nd or 3rd post-operative day. After transplantation, ocular surface observation, CI grade assessment, and CNV area measurement were performed as described in Experimental Procedures. Histological analyses were performed as described in Experimental Procedures.

RNA-sequencing

Total RNA from iCEPS (2×10^5 cells) or sCEPS (1×10^5 cells) was provided to the NGS core facility of the Genome Information Research Center at the Research Institute for Microbial Diseases, Osaka University. Library preparation was performed using a TruSeq stranded mRNA sample prep kit (Illumina, California, USA) according to the manufacturer's instructions. Sequencing was performed on an Illumina HiSeq 2500 platform in a 75-base single-end mode. Illumina Casava1.8.2 software used for base-calling. Sequenced reads were mapped to the human reference genome sequences (hg19) using TopHat v2.1.1 in combination with Bowtie2 ver. 2.2.3 and SAMtools ver. 0.1.19. The fragments per kilobase of exon per million mapped fragments (FPKMs) were calculated using Cuffdiff v2.2.1. RNA-sequencing data of this study have been deposited in the NCBI/GEO database (https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE159831.

Supplemental References

Shiba, Y., Gomibuchi, T., Seto, T., Wada, Y., Ichimura, H., Tanaka, Y., Ogasawara, T., Okada, K., Shiba, N., Sakamoto, K., et al. (2016). Allogeneic transplantation of iPS cell-derived cardiomyocytes regenerates primate hearts. Nature *538*, 388–391.

Shiina, T., Yamada, Y., Aarnink, A., Suzuki, S., Masuya, A., Ito, S., Ido, D., Yamanaka, H., Iwatani, C., Tsuchiya, H., et al. (2015). Discovery of novel MHC-class I alleles and haplotypes in Filipino cynomolgus macaques (Macaca fascicularis) by pyrosequencing and Sanger sequencing: Mafa-class I polymorphism. Immunogenetics *67*, 563–578.