Supplementary Information

Safety and efficacy of human ESC-derived corneal endothelial cells for corneal endothelial dysfunction

Juan Yu[1,3,5,6#] · Nianye Yu[1,3,5,6#] · Yao Tian[2,3,5,6#] · Yifan Fang[7#]· Bin An[2,5] · Guihai Feng[2,3,4,5] · Jun Wu[2,3,4,5] · Liu Wang[2,3,4,5] · Jie Hao[2,3,4,5] · Liqiang Wang[7] · Qi Zhou[2,3,4,5] · Wei Li[2,3,4,6*] · Yukai Wang[2,3,4,5*] · Baoyang Hu[1,2,3,4,6*]

[1] Savaid Medical School, University of Chinese Academy of Sciences, Beijing 100049, China.

[2] State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China.

[3] Institute of Stem Cell and Regeneration, Chinese Academy of Sciences, Beijing 100101, China.

[4] Beijing Institute for Stem Cell and Regenerative Medicine, Beijing, China.

[5] National Stem Cell Resource Center, Chinese Academy of Sciences, Beijing 100101, China.

[6] University of Chinese Academy of Sciences, Beijing 100864, China.

[7] Department of Ophthalmology, The First Center of the PLA General Hospital, Beijing, China.

Supplemental figures

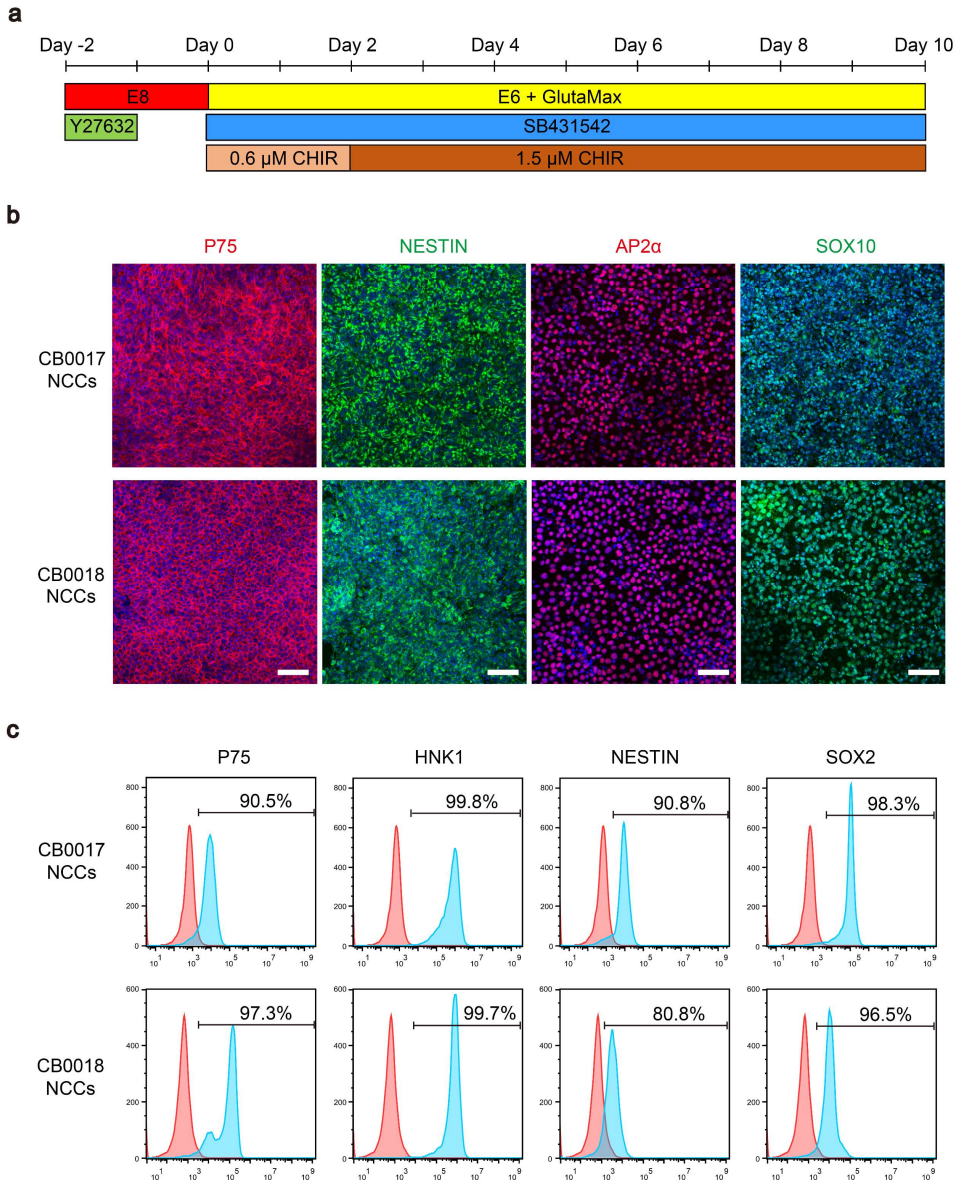

Fig. S1 Generation of NCCs from multiple hESC lines. **a** Schematic illustration of the differentiation conditions used to generate NCCs from multiple hESC lines. **b** Immunofluorescence staining showing that NCCs differentiated from hESC lines (CB0017 and CB0018) expressed P75, NESTIN, AP2α, and SOX10. Nuclei were stained with DAPI. Scale bars: 50 μm. **c** Flow cytometry analysis of P75, HNK1, Nestin and SOX2 expression in NCCs differentiated from hESC lines (CB0017 and CB0018). $n = 3$

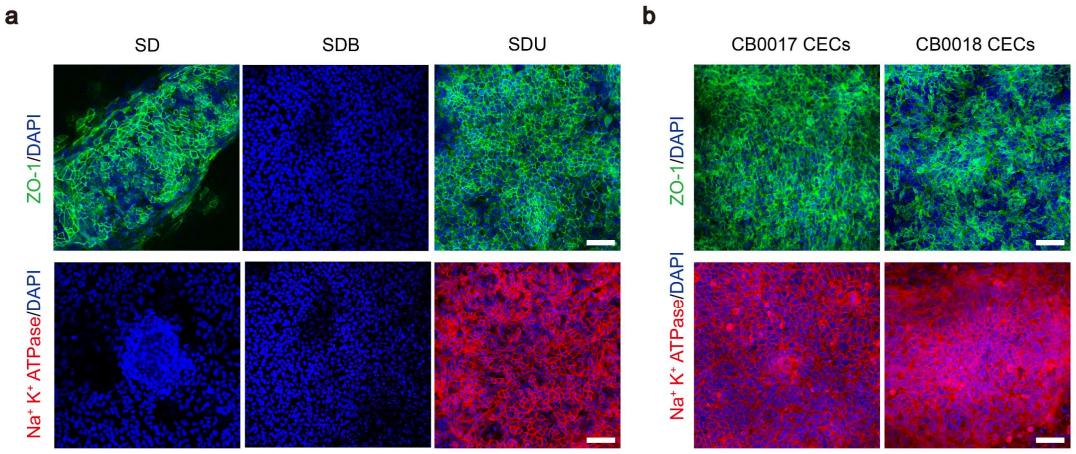

Fig. S2 Differentiation of multiple hESC-derived NCCs into multiple CECs. **a** Immunofluorescence staining images of small molecule screen of ZO-1 and Na^+ K^+ ATPase expression in CECs differentiated from CB0019-NCCs. Nuclei were stained with DAPI. Scale bars: 50 μm. **b** Immunofluorescence staining showing that CECs differentiated from hESC line (CB0017 and CB0018)-derived NCCs expressed ZO-1 and Na^+ K^+ ATPase. Nuclei were stained with DAPI. Scale bars: 50 μm

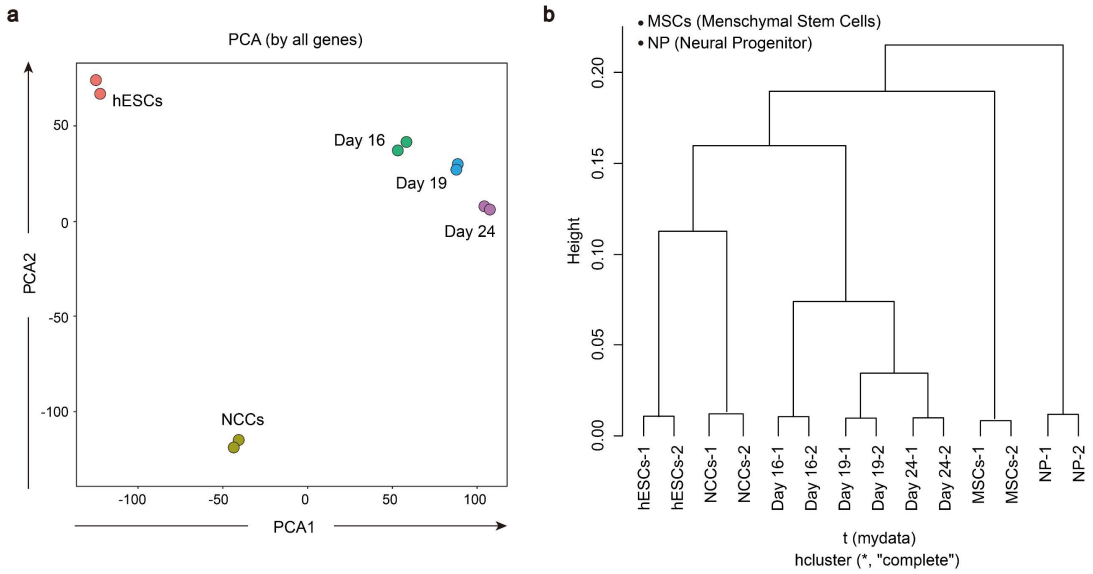

Fig. S3 Global expression profiles of the cells during CEC differentiation of hESCs. **a** Principal component analysis (PCA) was carried out to evaluate the similarities of the gene expression profiles between undifferentiated hESCs, hESC-NCCs and differentiated CECs at different time points. **b** Cluster dendrogram analysis for RNA expression among undifferentiated hESCs, hESC-NCCs, different time points of CEC differentiated cells, mesenchymal stem cells (MSCs) and neural progenitor (NP). $n = 2$

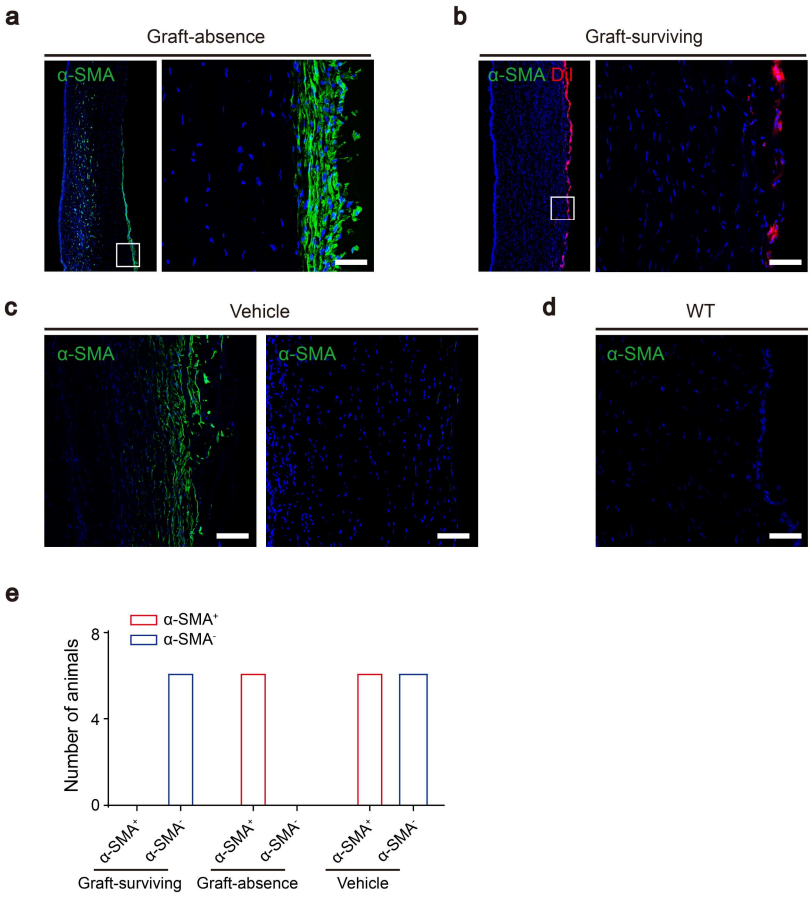

Fig. S4 Analysis of CEC product survival in the graft group 28 days after surgery. **a** – **d** The expression of α-SMA in graft, vehicle and WT corneas. **a** Immunofluorescence staining showing that the edge of the corneal stroma in the graft-absence group expressed α-SMA, which indicated no surviving cells, while **b** red DiI was observed in the graft-surviving group, in which CECs survived. **c** Some corneas expressed α-SMA, while others did not in the vehicle group. **d** No expression of α-SMA in WT corneas. Nuclei were stained with DAPI. Rectangles indicate areas of magnification shown in the right panels. Scale bars: 50 μm. **e** Quantification of α-SMA expression in graft and vehicle groups

Supplemental Tables

Table. S1 Quality control and in-process tests for the manufacturing of hESC-derived CECs

Target	Test	Method	Release Criteria	Test Result	Pass or Fail?
hESCs	Pluripotent markers	FACS	SSEA-4 ≥ 90%	SSEA-4: 95.9%	PASS
	Sterility test	Rapid sterility testing method	No bacterial or fungal growth	No bacterial or fungal growth	PASS
	Mycoplasma	ELISA method	Negative	Negative	PASS
NCCs	NCCs markers	IF	AP2α ≥ 80%	AP2α: 85.30%	PASS
			SOX10 ≥ 80%	SOX10: 87.25%	
		FACS	P75 ≥ 90%	P75: 95.2%	
			HNK1 ≥ 90%	HNK1: 99.7%	
			NESTIN ≥ 80%	NESTIN: 98.6%	
			SOX2 ≥ 90%	SOX2: 98.9%	
	Viability	AO/PI staining	Cell count ≥ 80%	Cell count ≥ 90%	PASS
	Sterility	Rapid sterility testing method	No bacterial or fungal growth	No bacterial or fungal growth	PASS
	Mycoplasma	ELISA method	Negative	Negative	PASS
CECs	CEC markers	IF	AQP1 ≥ 60%	AQP1: 68.7%	PASS
		FACS	ZO-1/ Na^+ K^+ ATPase ≥ 90%	ZO-1/ Na^+ K^+ ATPase: 96.4%	
			N-Cadherin ≥ 90%	N-Cadherin: 97.5%	
			CD166 ≥ 90%	CD166: 96.6%	
	Impurities makers	IF	KI67≤ 1%	KI67≤ 0.1%	PASS
	Undifferentiation markers (*OCT4*)	qPCR method	Cq > 30	Cq: 33.86	PASS
	Morphology	Microscopic observation	Cobblestone or hexagonal morphology can be observed	Cobblestone or hexagonal morphology can be observed	PASS
	Viability	AO/PI staining	Cell count ≥ 80%	Cell count ≥ 80%	PASS
	Sterility	Rapid sterility testing method	No bacterial or fungal growth	No bacterial or fungal growth	PASS
	Mycoplasma	ELISA method	Negative	Negative	PASS
	Endotoxin	LAL[b] turbidimetry test	≤ 10 EU/ml	< 1 EU/ml	PASS

Table. S2 Primers used for qRT-PCR

Gene	Direction	Sequences
POU5F1	Forward	5’ AAA CGA CCA TCT GCC GCT TTG A 3’
	Reverse	5’ GGT TGC CTC TCA CTC GGT TCT C 3’
NANOG	Forward	5’ CAG CCC AGA TTC TTC CAC CAG TCC C 3’
	Reverse	5’ CGG AAG CTT CCC AGT CGG GTT CAC C 3’
NGFR	Forward	5’ CCT CAT CCC TGT CTA TTG CTC C 3’
	Reverse	5’ GTT GGC TCC TTG CTT GTT CTG C 3’
B3GAT1	Forward	5’ GAA AGC AGC CTC CTT CGA GAA C 3’
	Reverse	5’ CCT CAT TCA CCA GCA CTG GCT T 3’
NESTIN	Forward	5’ TCA AGA TGT CCC TCA GCC TGG A 3’
	Reverse	5’ AAG CTG AGG GAA GTC TTG GAG C 3’
TFAP2A	Forward	5’ GAC CTC TCG ATC CAC TCC TTA C 3’
	Reverse	5’ GAG ACG GCA TTG CTG TTG GAC T 3’
SOX10	Forward	5’ ATG AAC GCC TTC ATG GTG TGG G 3’
	Reverse	5’ CGC TTG TCA CTT TCG TTC AGC AG 3’
TJP1	Forward	5’ AGT AAG AGC ACA GCA ATG GAG 3’
	Reverse	5’ TCA CTA TTG ACG TTT CCC CAC 3’
CDH2	Forward	5’ CCC AAG ACA AAG AGA CCC AG 3’
	Reverse	5’ GCC ACT GTG CTT ACT GAA TTG 3’
AQP1	Forward	5’ TGG CTG TGG GAT TAA CCC TG 3’
	Reverse	5’ GGT TGC TGA AGT TGT GTG TGA TC 3’
GAPDH	Forward	5’ TTG AGG TCA ATG AAG GGG TC 3’
	Reverse	5’ GAA GGT GAA GGT CGG AGT CA 3’

Table. S3 List of antibodies used in FACS.

Antigen	Label	Dilution	Company	Cat. No.
Anti-human CD271	PE	20 µl / test	BD Pharmingen	560927
Anti-human HNK1	PE	5 µl / test	BD Pharmingen	560844
Anti-human NESTIN	PE	5 µl / test	BD Pharmingen	561230
Anti-human SOX2	PE	5 µl / test	BD Pharmingen	562195
Anti-human CD90	PE	5 µl / test	Invitrogen	12-0909-42
Anti-human CD105	PE	5 µl / test	Biolegend	800504
Anti-human CD73	PE	5 µl / test	Invitrogen	12-0739-42
Anti-human CD29	PE	5 µl / test	Biolegend	303004
Anti-human CD19	PE	20 µl / test	BD Pharmingen	555413
Anti-human CD34	PE	20 µl / test	BD Pharmingen	555822
Mouse IgG1 Isotype Control	PE	20 µl / test	BD Pharmingen	555749
Anti-human ZO-1	/	1:50	Thermo Fisher Scientific	61-7300
Anti-Human Na^+ K^+ ATPase	/	1:50	EMD Millipore	05-369
Anti-Human N-cadherin	/	1:50	Santa Cruz	Sc-59987
Anti- Human CD166	PE	20 µl / test	BD Pharmingen	559263

Table. S4 Antibodies used in Immunofluorescence Staining

Antigen	Host	Dilution	Company	Cat. No.
P75	mouse	1:100	ATS	AB-N07
NESTIN	rabbit	1:1000	EMD Millipore	ABD69
SOX10	rabbit	1:500	abcam	ab108408
AP2α	mouse	1:200	DSHB	3B5
TUJ1	rabbit	1:1000	Biolegend	802001
Peripherin	mouse	1:100	Santa Cruz	sc-377093
S100β	rabbit	1:100	abcam	ab52642
ZO-1	rabbit	1:250	Thermo Fisher Scientific	61-7300
Na^+ K^+ ATPase	rabbit	1:500	abcam	ab76020
AQP1	rabbit	1:200	abcam	ab168387
N-cadherin	mouse	1:100	Santa Cruz	sc-59987
OCT4	rabbit	1:200	Santa Cruz	sc-9081
KI67	rabbit	1:200	Thermo Fisher Scientific	710229
STEM121	mouse	1:200	Clontech	Y40410
α-SMA	mouse	1:500	Sigma	F3777
Donkey anti-mouse IgG Alexa 488	Donkey	1:200	Jackson ImmunoResearch	715-545-151
Donkey anti-rabbit IgG Cy™ 3	Donkey	1:200	Jackson ImmunoResearch	711-165-152
Donkey anti-rabbit IgG Alexa 488	Donkey	1:200	Jackson ImmunoResearch	711-545-152
Donkey anti-mouse IgG Cy™ 3	Donkey	1:200	Jackson ImmunoResearch	715-165-151