Supplementary Information

Supplementary Figures

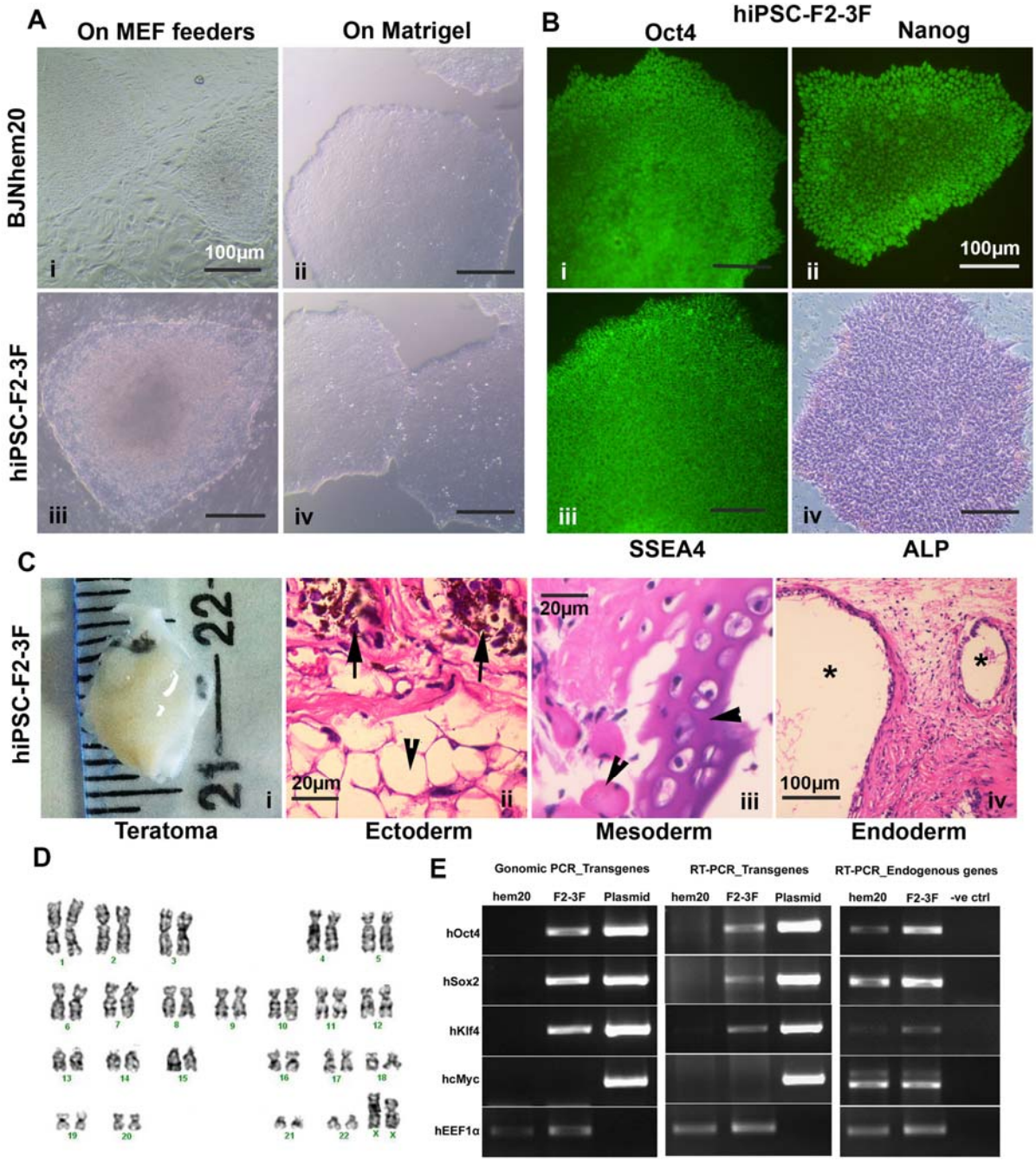


Figure S1. Derivation and characterization of a human induced pluripotent stem cell line. (A) Growing colony morphology of BJNhem20 cells (i-ii) and hiPSC-F2-3F1 cells at passage p0 on irradiated MEF feeders (iii) and at passage p20 on Matrigel coated plates (iv). (B) Immunocytochemistry of hiPSC-F2-3F1 cells at p20 on Matrigel coated chamber slides showing a homogenous expression of OCT4 (i), NANOG (ii), SSEA4 (iii) and alkaline phosphatase (iv). (C) A teratoma of about 8X8 mm, formed by hiPSC-F2-3F1 cells at p25, transplanted in the subcutaneous space of nude mice (i). H&E staining of tissue sections reveal the development of ectoderm derived RPE-like pigmented cell patches (arrow, ii), mesoderm derived adipose, cartilage and muscle tissues (arrow heads, iii) and endoderm derived gut epithelium like structures (asterisk, iv). (D) G-band karyotype of hiPSC-F2-3F1 cells at p20, confirmed a normal chromosomal pattern for this female line (n=2). (E) Genomic PCR profiles of transgene specific amplicons confirmed the genomic integration of human *OCT4, SOX2* and *KLF4* but not the *cMYC* transgenes (i). RT-PCR profiles of transgene-specific amplicons confirmed the expression of all transgenes except *cMYC,* at p20 (ii). RT-PCR profiles of endogenous gene specific amplicons confirmed the expression of all four transcripts, at levels comparable to that of BJNhem20, hESCs (iii). Plasmid DNA and no-RT samples were used as PCR controls. Genomic DNA and cDNA samples were normalized using eukaryotic elongation factor (*EEF1α*) as the loading control. Scale bars, 100 μm or as specified.

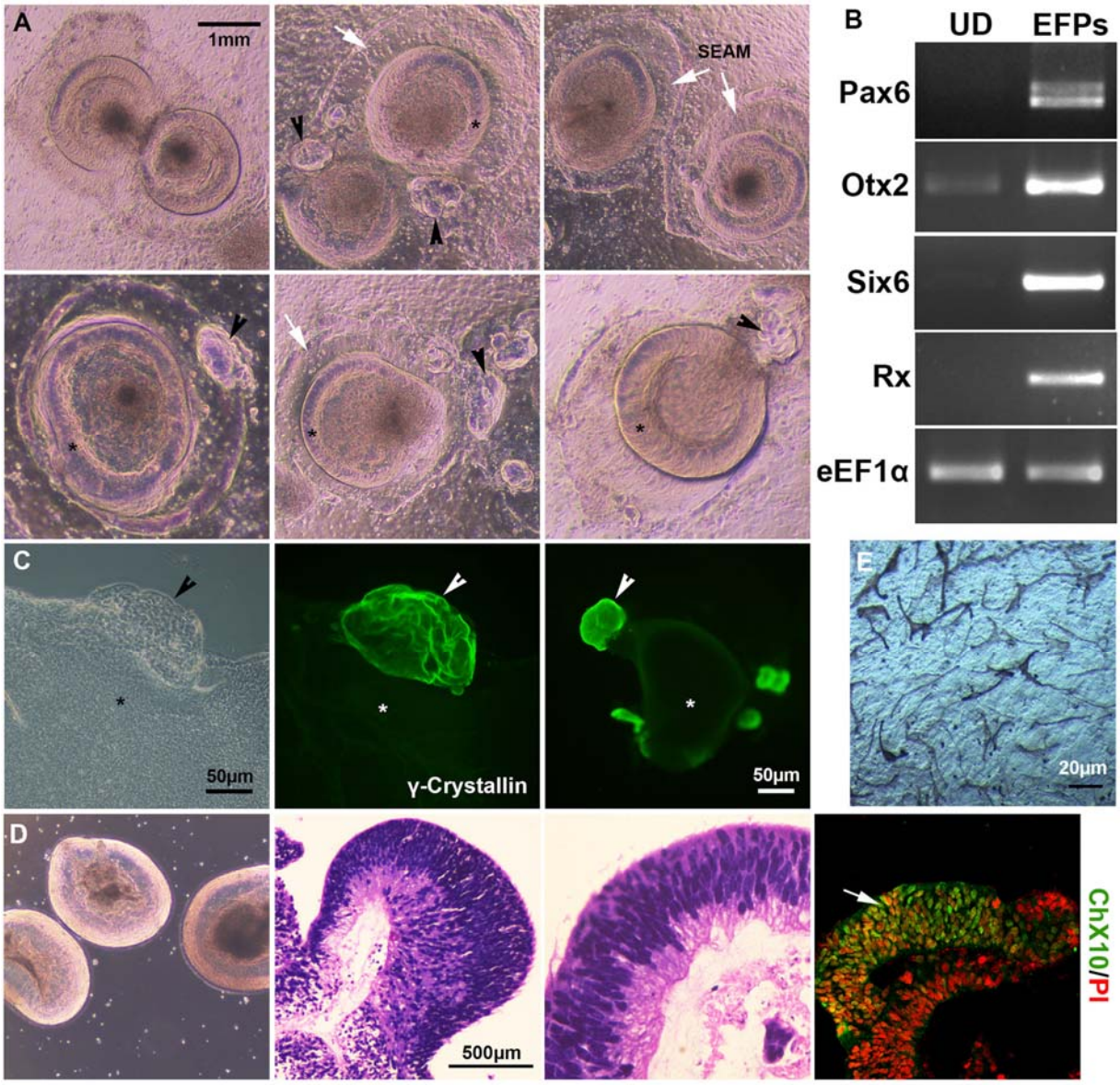


Figure S2. Morphology of adherent eye field clusters. (A) Distinct, oval to circular, three dimensional clusters of eye field primordial structures emerged at four weeks of differentiation. N=6. Upon continued adherent culture, the OSE cells migrate outwards to form a clear growth zone, as indicated by white arrows. Asterisks mark the central neuroretinal cups. OSE cells also gave rise to lens epithelium-like morphologically distinct clusters near the leading edge of the outgrowths as indicated by arrow heads. Scale bars, 1 mm. (B) RT-PCR profiles confirmed the

expression of early eye field commitment markers such as *PAX6, OTX2, SIX6* and *RX* in the cells that constitute EFP clusters. The cDNA samples were normalized using *EEF1α* as the loading control. (C) γ-Crystaline^{+} lentoid structures were observed adjacent to the neuroretinal cups. (D) NR clusters developed into optic vesicles, with layered arrangement of precursor cells in suspension cultures (H&E). They also formed double layered retinal cups, with Chx10^{+} neuroretinal layer (green) on the outer surface. (E) Spindle shaped, pigmented melanocytes were observed above the plane of epithelium in a bright field, confocal view.

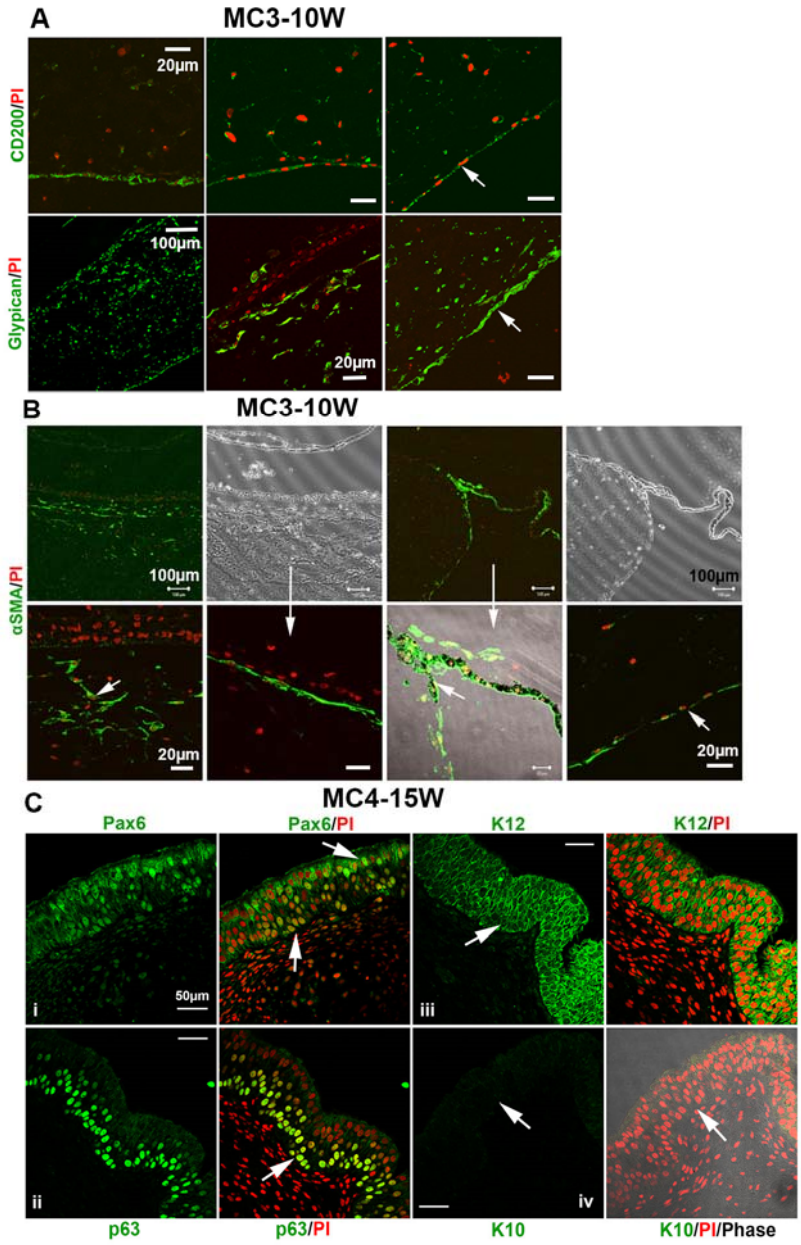


Figure S3. Expression of corneal endothelium and smooth muscle markers in minicorneas. (A) The endothelium like single cell lining on the basal side for the stroma expressed CD200 (i) and Glypican 4 (ii). (B) The peripheral and anterior stromal cell infiltrates were αSMA^{+} (i-ii). Peripheral endothelium and the surrounding adnexal tissues also expressed αSMA (arrows). PI counterstain marked the nuclei in red. (C) The surface epithelium of 15W old corneal organoids were Pax6^{+}, p63^{+}, K12^{+}, K10^{-}. Scale bars, 20 μm or as specified.

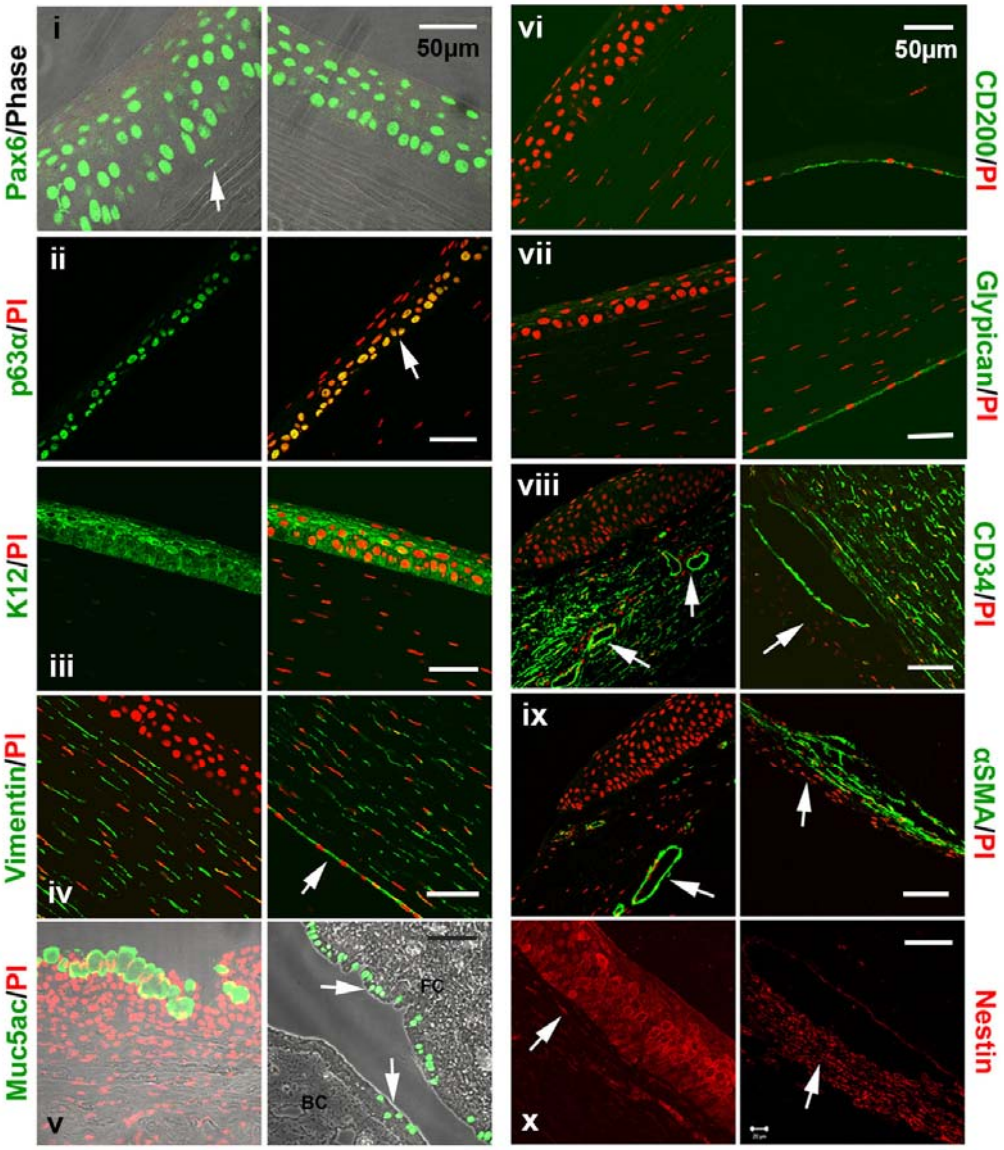


Figure S4. Tissue-specific marker expression profiles of adult corneas. Confocal images of adult donor corneal tissues sections showing the expression of various markers such as PAX6 (i), P63α (ii), K12 (ii), Vimentin (iv), MUC5AC (v), CD200 (vi), GPC4 (vii), CD34 (viii), αSMA (ix) and Nestin (x). The sections were counter stained with PI (red). Corneal epithelial cells were PAX6^{+} and K12^{+}. The basal epithelial cells were P63α^{+}. The VIM^{+} NCCs and CD34^{+} MSCs contribute equally to the corneal stroma. αSMA^{+} cells lines the lumen of Schlemm's canal. Trabecular meshwork cells were NES^{+}. Endothelial cells were CD200^{+}, GPC4^{+} and VIM^{+}. Scale bars are as indicated.

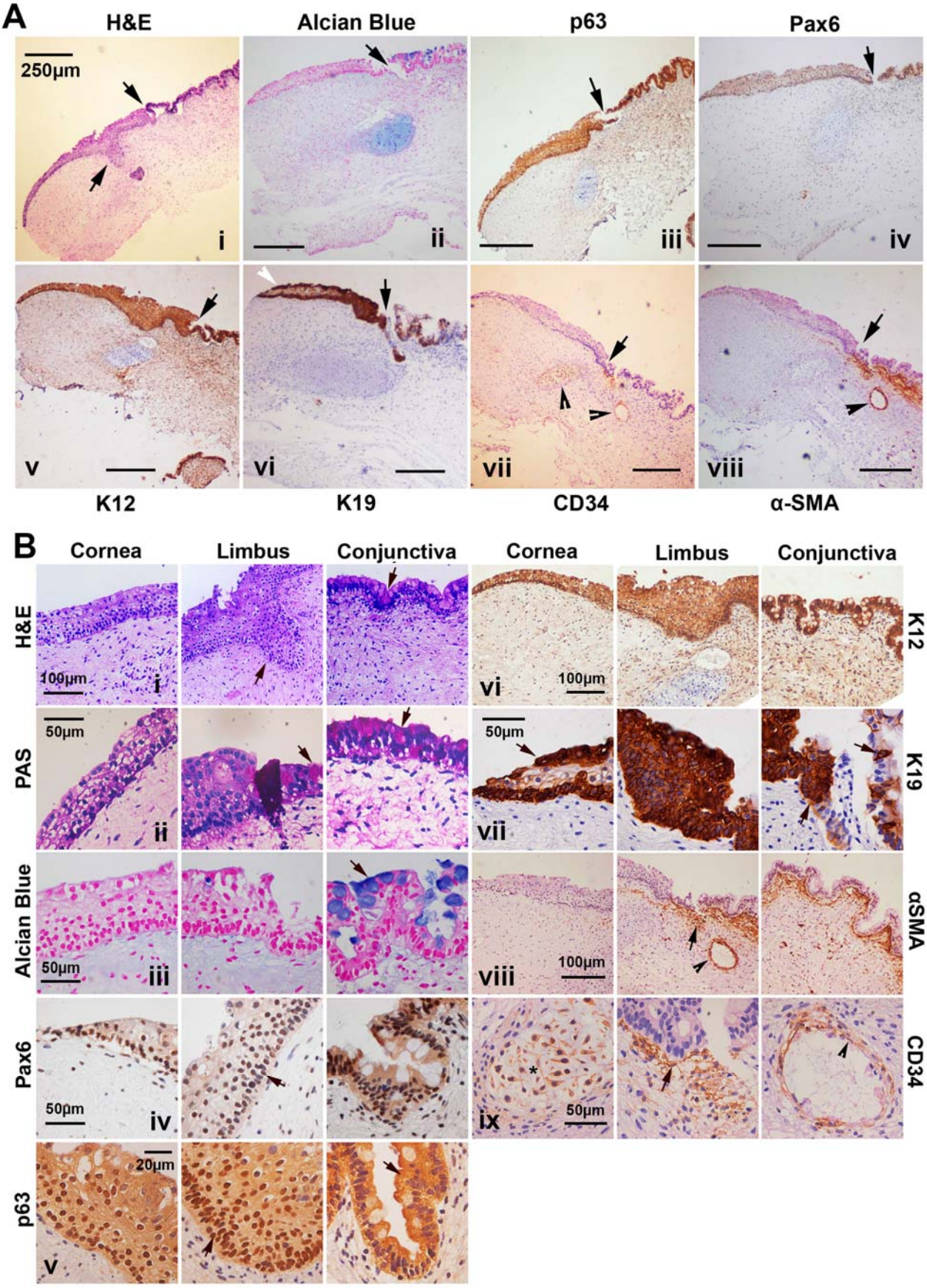


Figure S5. Gross morphology and IHC characterization of the minicornea, MC4. (A) Low magnification bright field IHC images of 15 weeks old MC4. Scale bars, 250 μm. (B) Higher magnification view of the sections shown above. H&E stained images displayed the gross tissue morphology (i) PAS and Alcian blue staining indicate the presence of goblet cells (ii-iii). Immunostained sections were labeled with DAB to mark the expression of different corneal markers such as the PAX6 (iv), P63 (v), K12 (vi), K19 (vii), αSMA (viii) and CD34 (ix). Limbal margins are marked by arrows. αSMA^{+}, $CD34^{+}$ vasculature like structures are marked by arrowheads. Another $CD34^{+}$ mesenchymal stromal cell patch is marked by asterisk. Note the intense expression of K19 in the basal and surface epithelium, separated by a layer of no expression. Scattered $K19^{+}$ cells were also found interspersed within the developing conjunctival epithelium (arrows). Hematoxylin and nuclear fast red were used as blue and pink counter stains respectively. Scale bars, 50 μm or as specified.

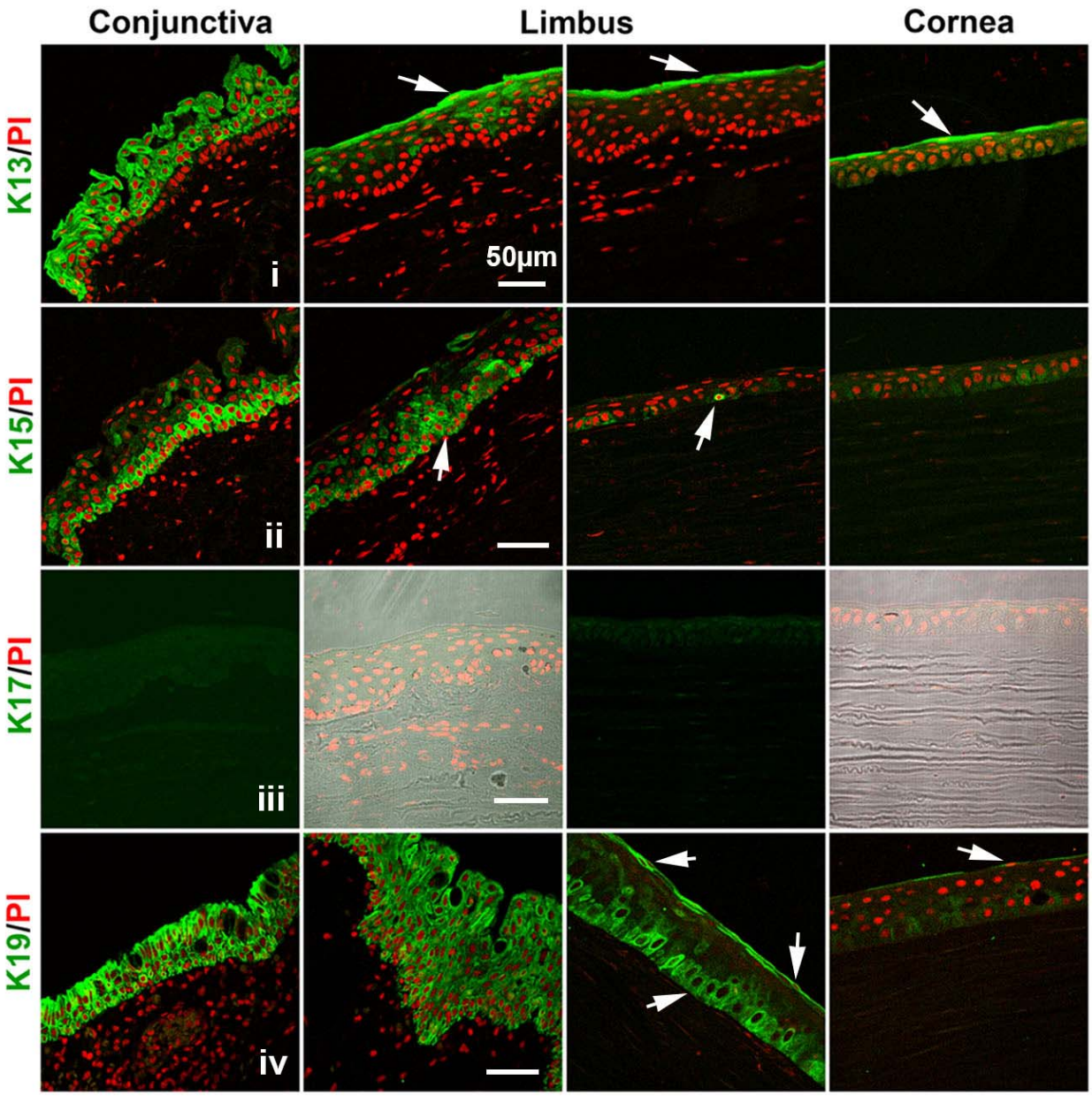


Figure S6.

Confocal images of tissues sections showing the expression of keratins such as K13 (i), K15 (ii), K17 (iii) and K19 (iv). Note the surface and suprabasal epithelial expression of K13, basal epithelial expression of K15, conjunctival, limbal basal and corneal surface epithelial staining of K19. K17 expression was found to be absent in adult corneal tissues. The sections were counterstained with PI (red). All scale bars, 50 μm.

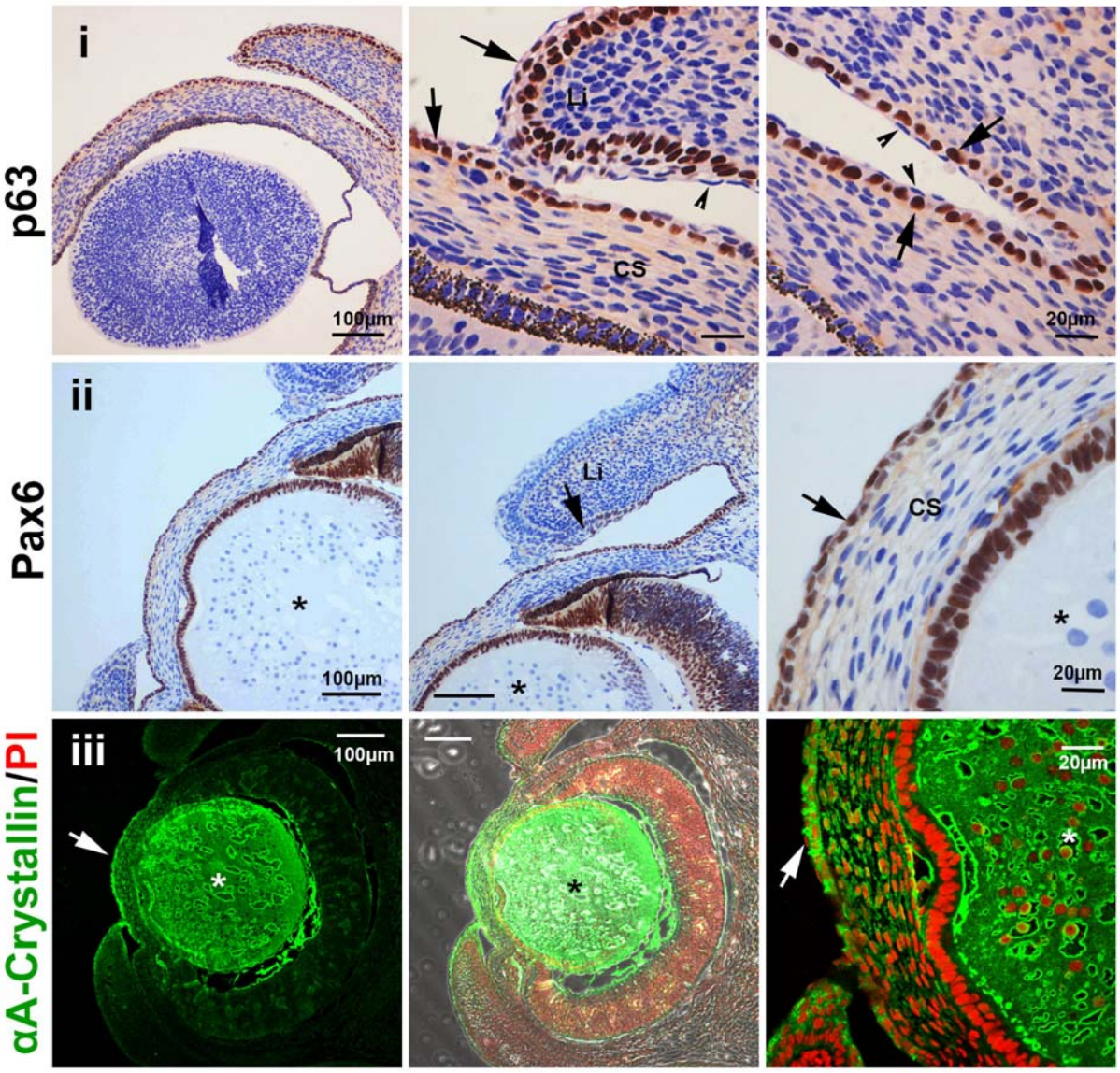


Figure S7. Marker expression patterns in developing eyes of mouse embryos.

IHC images of eye balls of E13.5 mouse embryos showing p63 expression in the basal epithelial cells of corneal, conjunctival and lid surface epithelium (i). PAX6 expression is limited to the corneal and conjunctival epithelium. Arrows points to the lip region of the lid epithelium (Li) showing diminished PAX6 expression (ii). αA-crystallin is expressed in the lens (asterisk) and the opposing surface ectoderm derived corneal epithelium (iii). Arrow heads point to PAX6$^-$ apical wing cells. CS indicates the corneal stroma. All scale bars, 20 μm or as specified.

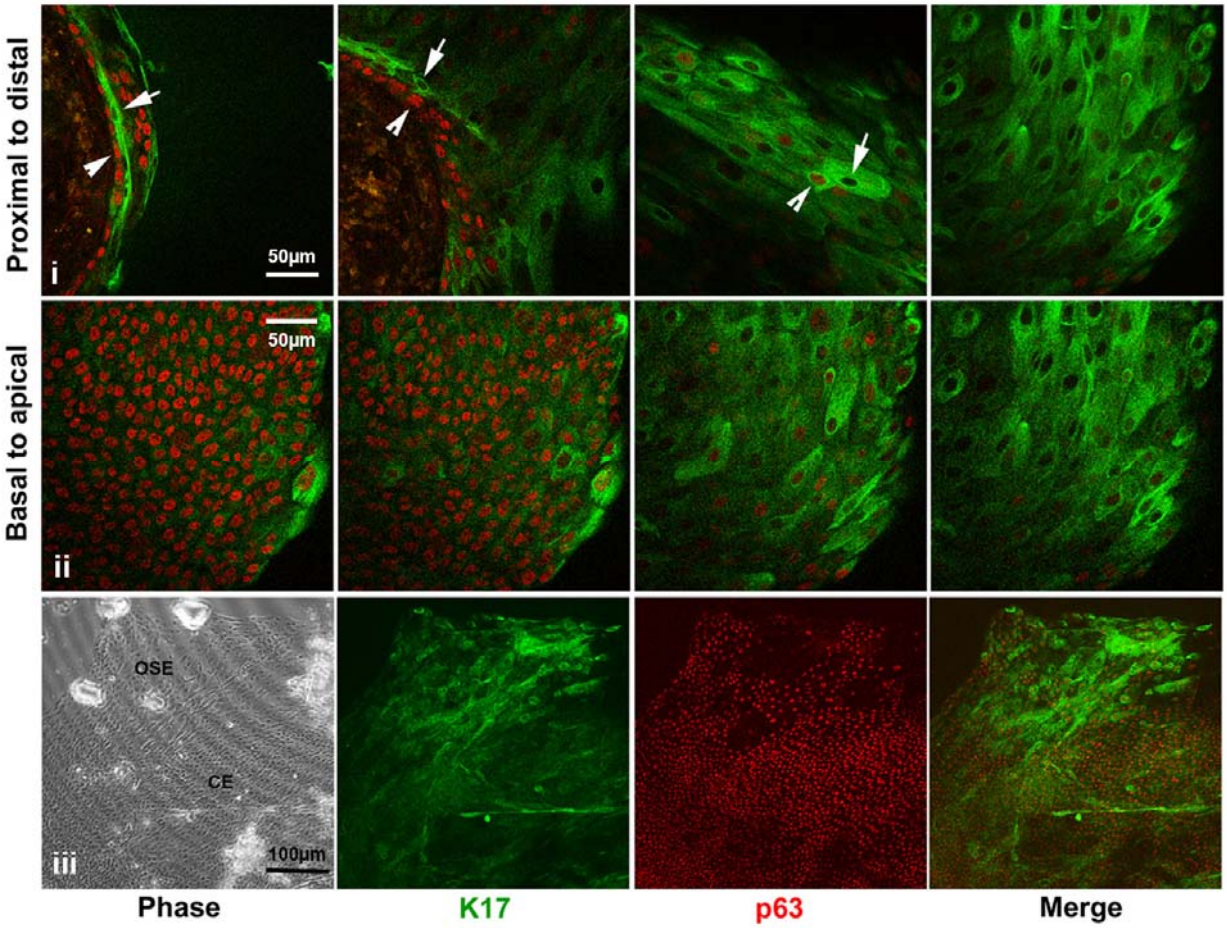
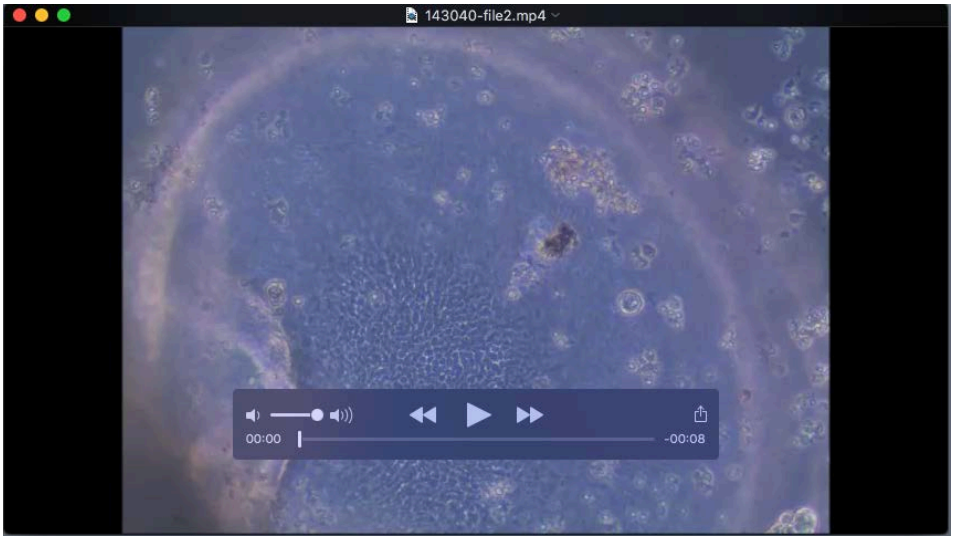


Figure S8. IHC characterization of cell outgrowth from corneal organoids on glass coverslips. Confocal images of 10 weeks old MC explants grown as confluent cultures on glass coverslips, showing the expression of K17 and P63. Serial images taken at proximal and distal positions from the explants show that $K17^{+}$, $P63^{-}$ cells (arrows) emerge first and forms the leading edge of the wave front, while $K17^{+}$, $P63^{+}$ cells (arrow heads) emerge later (i). Serial Z-sections from basal to apical surface of the outgrowths show $K17^{+}$, $P63^{+}$ cells on the basal side and $K17^{+}$, $P63^{-}$ cells (arrows) on the apical surface (ii). Low magnification images of epithelial outgrowth showing K17 and P63 expression, as described above (iii). Scale bars, 50 μm or as specified.

Supplementary Movies



Movie 1

Phase-contrast images of a minicornea at 8 weeks of development. A basal to apical view series.

Movie 2

Phase-contrast images of cell outgrowths from EFPs. A proximal to distal view series.

Movie 3

Fluorescence images of P63+ cell outgrowths from EFPs. A proximal to distal view series.

Supplementary Tables

Table S1. Details of primers used in the study

Human gene name	Primer Sequence 5'→ 3'	Amplicon size (bp)	NCBI Accession number
	RT-PCR primer sets		
P63	F: GCTGGAGACATGAATGGACT R: GGTGAATCGCACAGCATCAA	399 (α) 305 (β)	NM_003722 NM_001114978
K12	F- ATTGGAAATGCCCAGCTCCT R-TCTGCTCAGCGATGGTTTCA	352	NM_000223
PAX6	F1- ATAACCTGCCTATGCAACCC R1- GGAACTTGAACTGGAACTGAC	208	NM_000280
PAX6	F2: GAAGATTGTAGAGCTAGCTCACAGCG R2: TGTTGCTTTTCGCTAGCCAGGTTG	369 (5a) 327 (wt)	NM_001604 NM_000280
OTX2	F- ACTTCGGGTATGGACTTGCT R- GTTCCACTCTCTGAACTCAC	350 (a) 326 (b)	NM_021728 NM_172337
SIX6	F- ATTTGGGACGGCGAACAGAA R- TGGATGGGCAACTCAGATGT	381	NM_007374
RX	F- GCAAGGTCAACCTACCAGA R- TCGTCCAGCGGGAACTTGT	439	NM_013435
EEF1A	F- GAAGTCTGGTGATGCTGCCATTGT R- TTCTGAGCTTTCTGGGCAGACTTG	198	NM_001402
OCT3/4-endo	F3: TCCCTTCGCAAGCCCTCATTT R2- TCTGCAGAGCTTTGATGTCC	486	NM_002701
OCT3/4-transgene	F- CCTCACTTCACTGCACTGTACTC L3205- CCCTTTTTCTGGAGACTAAATAAA	335	
SOX2-endo	F- CCCAGCAGACTTCACATGTCC R- GCGTGAGTGTGGATGGGATTG	287	NM_003106
SOX2-transgene	F- CCCAGCAGACTTCACATGTCC L3205- CCCTTTTTCTGGAGACTAAATAAA	348	
KLF4-endo	F- GATCGTGGCCCCGGAAAAGGAC R- GATTGTAGTGCTTTCTGGCTGG	394	NM_004235
KLF4-transgene	F- GATCGTGGCCCCGGAAAAGGAC L3205- CCCTTTTTCTGGAGACTAAATAAA	455	
MYC- endo	F2- AGCTTGTACCTGCAGGATCT R2- CTGCGTAGTTGTGCTGATGT	409	NM_002467
MYC-transgene	F- GAACAGCTACGGAACTCTTGTGC L3205- CCCTTTTTCTGGAGACTAAATAAA	419	

Table S2. Details of antibodies used in the study

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Table S3. Dimensions of the minicorneas at different stages of *in vitro* development

Age of MCs	**Culture method**	**Tissue ID**	**Length/ Diameter**	**Total width/ thickness**	**Epithelial thickness**
6 weeks	Suspension	MC1-1	1.1 mm		15-25 µm
8 weeks	Suspension	MC1-2	2.7 mm		15-42 µm
10 weeks	Suspension	MC2	1.15 mm		70-80 µm
10 weeks	Adherent	MC3	7.2 mm	1.3 mm	46-51 µm
		MC3 (lid)	1.8-2.0 mm	150-220 µm	
15 weeks	Adherent	MC4	5.6 mm	1 mm	85-90 µm
		MC4 (Limbus to limbus)	**1.92 mm**	1 mm	
15 weeks	Suspension	MC5	1.5 mm		15-25 µm
Adult cornea		Limbus to limbus	**11-11.75 mm**	600 µm (central)	53 µm (central) 67 µm (limbal)