

Supplementary Data

Title: Cornea organoids from human induced pluripotent stem cells

James W. Foster¹, Karl Wahlin⁵, Sheila M. Adams⁴, David E. Birk⁴, Donald J. Zack³, *Shukti

Chakravarti^{1,2,3}

Supplementary table 1.

<i>Gene</i>	<i>Accession number</i>	<i>Forward Primer</i>	<i>Reverse Primer</i>	<i>Product size</i>
<i>CDH2</i>	NM_001792.3	AAATTGAGCCTGAAGCCAAC	GTGGCCACTGTGCTTACTGA	102
<i>CD34</i>	NM_001025109	CTTGGGCATCACTGGCTATT	TCCACCGTTTTCCGTGTAAT	97
<i>PAX6</i>	NM_001310161	AAAGCAACAGATGGGCGCAGAC	TCTTTGCAGCTTCCGCTTCAGC	238
<i>ABCG2</i>	NM_001257386	TCCACTGCTGTGGCATTAAA	CCTGCTTGGAAGGCTCTATG	70
<i>NP63</i>	NM_003722.4	TGAGCCACAGTACACGAACC	TGCGCGTGGTCTGTGTTATA	99
<i>KERA</i>	NM_007035	TATTCCTGGAAGGCAAGGTG	ACCTGCCTCACACTTCTAGACC	102
<i>KRT14</i>	NM_000526.4	CGGCCTGCTGAGATCAAAGA	TCTGCAGAAGGACATTGGCA	106
<i>COL8A1</i>	NM_001850.4	AGATGCTGCTCTGCCTTCAC	AAGGGAGCTCACACGTTTCAC	93
<i>KRT12</i>	NM_000223.3	CCATTGATGTCTGGCCTCTAC	TTTCAGCCAGCATTGGAAA	130
<i>F11R</i>	NM_144502	GAAGAAAAGCCCGAGTAGGC	TCGAGAGGAAACTGTTGTGC	91
<i>S100A4</i>	NM_032034.3	ATGGAGGAGTTGGCAGTGTC	CTGCTTCCCTTGCAGAAAAC	148
<i>POLR2A</i>	NM_000937.4	CATCATCCGAGACAATGGTG	AACAATGTCCCCATCACACA	115
<i>ΔNp63</i>		GAA AAC AAT GCC CAG ACT CAA TTT	TCT GCG CGT GGT CTG TGT TAT	127
<i>P63α</i>		ATG TCG AAA TTG CTC AGG GAT TTT CAG A	TGA CCA CCA TCT ATC AGA TTG AGC ATT ACT	77

All quantitative PCR reactions were carried out under standard cycling conditions (40 cycles) with a 60°C annealing temperature.

P63 Primer sequences were obtained from Cavanagh et. al Experimental eye research, 2008, <http://dx.doi.org/10.1016/j.exer.2007.12.007>

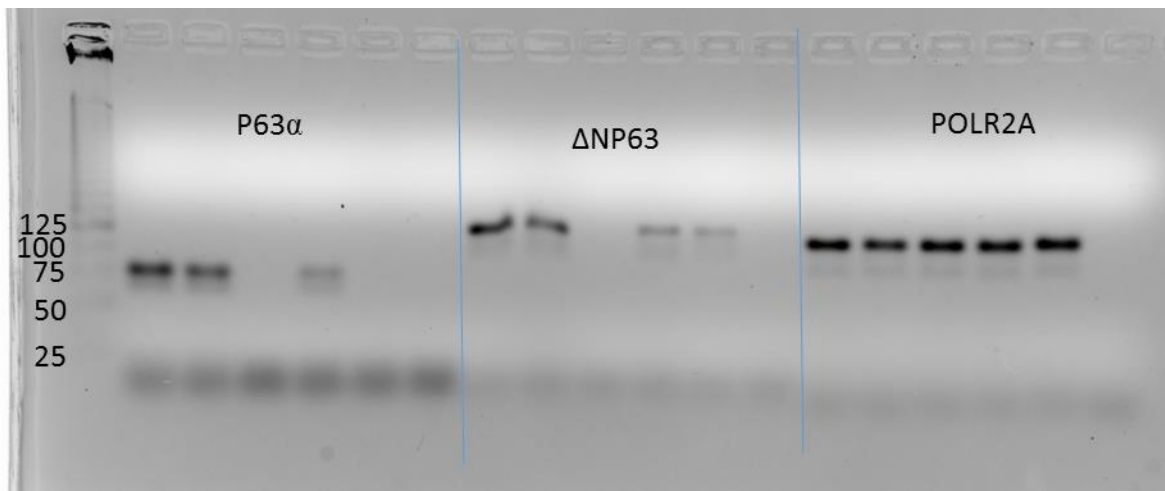
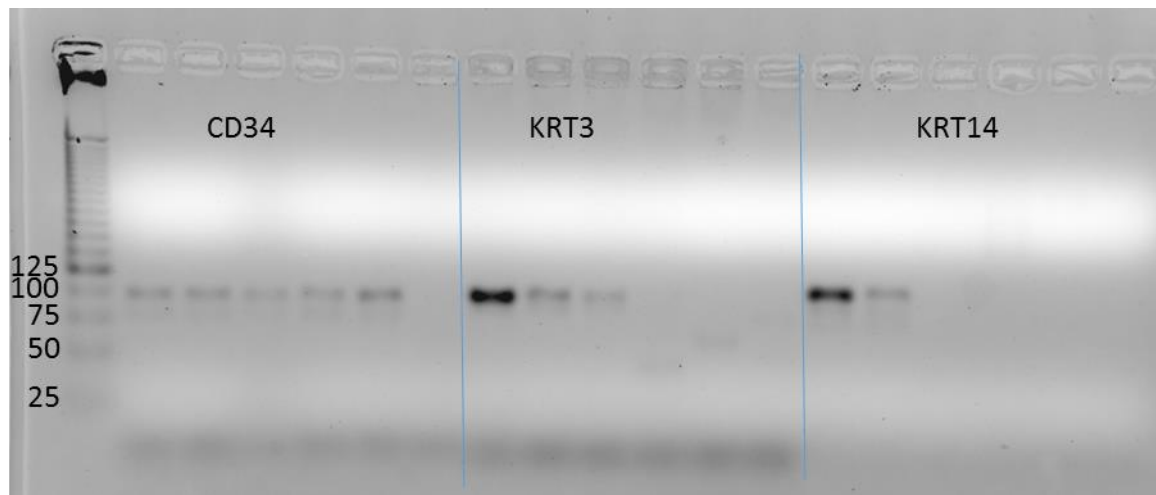
Supplementary table 2

Antibodies used in figure 4

<i>Target</i>	<i>Raised in</i>	<i>Provider</i>	<i>catalogue number</i>	<i>Incubation concentration</i>
<i>KERA</i>	GT	Santa-Cruz biotechnology	SC-33244	1:200
<i>COL1A1</i>	RB	Novus	NB600-408	1:500
<i>COLVA1</i>	RB	Novus	NBP1-19633	1:500
<i>COLLVIII</i>	RB	Santa-Cruz biotechnology	sc-134947	1:500
<i>ALDH3A1</i>	RB	Abcam	ab76976	1:500
<i>LUM</i>	MS	Santa-Cruz biotechnology	sc-166871	1:200
<i>PLC</i>	RAT	Research Diagnostics	RDI-PERLECabrt	1:500
<i>P63α</i>	RB	Cell signalling	#13109	1:500
<i>KRT12</i>	RB	Abcam	AB185627	1:200
<i>KRT14</i>	RB	Abcam	Ab181595	1:200
<i>KRT3</i>	MS	Abcam	AB77869	1:200

Supplemental Figure 1

A. Raw gels used to generate figure 3. Bands represent amplified product after 40 cycles from qPCR experiments.



25bp ladder, (Thermo scientific, 10488-095)

Expected sizes:

CD34 – 99

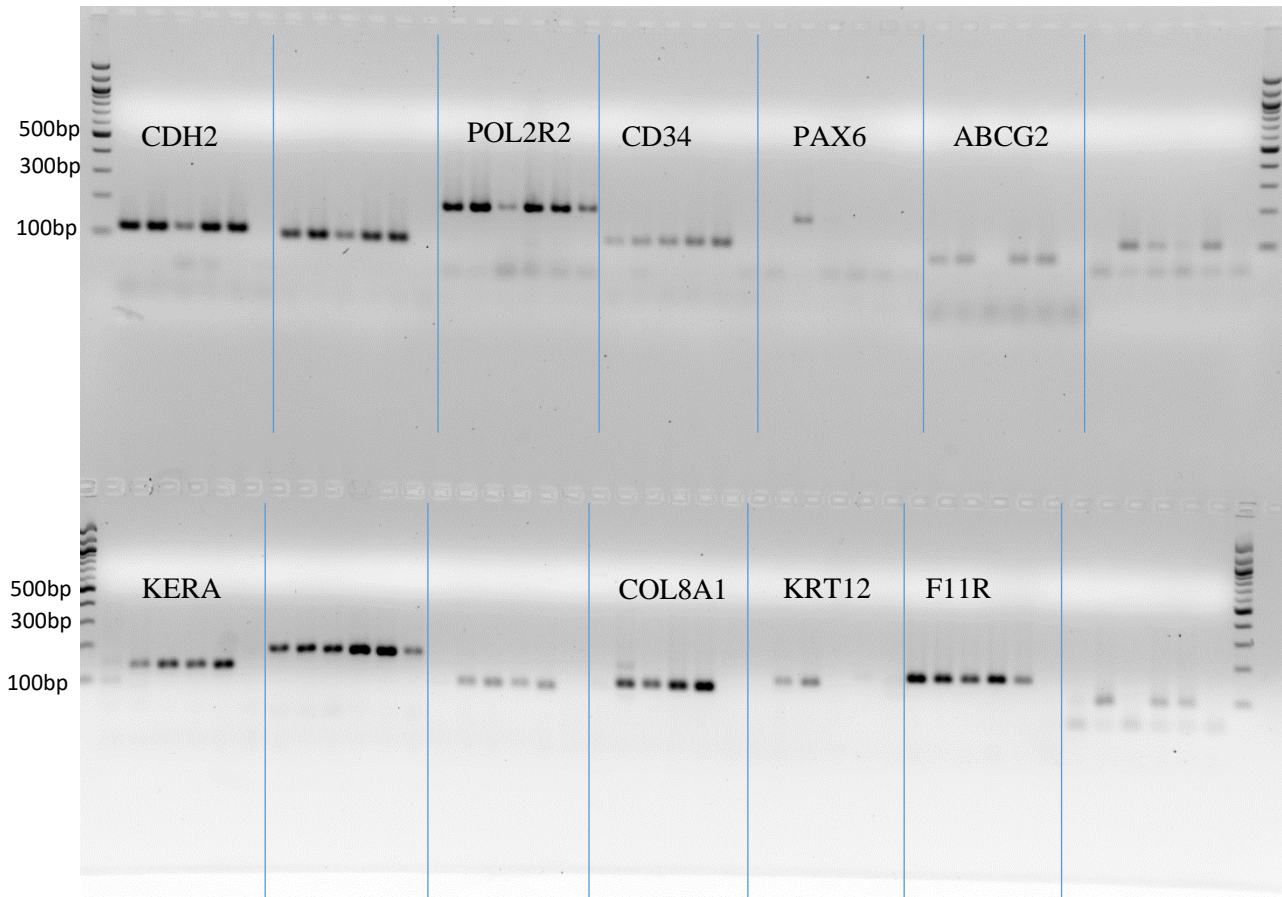
KRT3 – 100

KRT14 – 100

P63 α – 77

Δ NP63 – 127

B. Additional markers for corneal differentiation



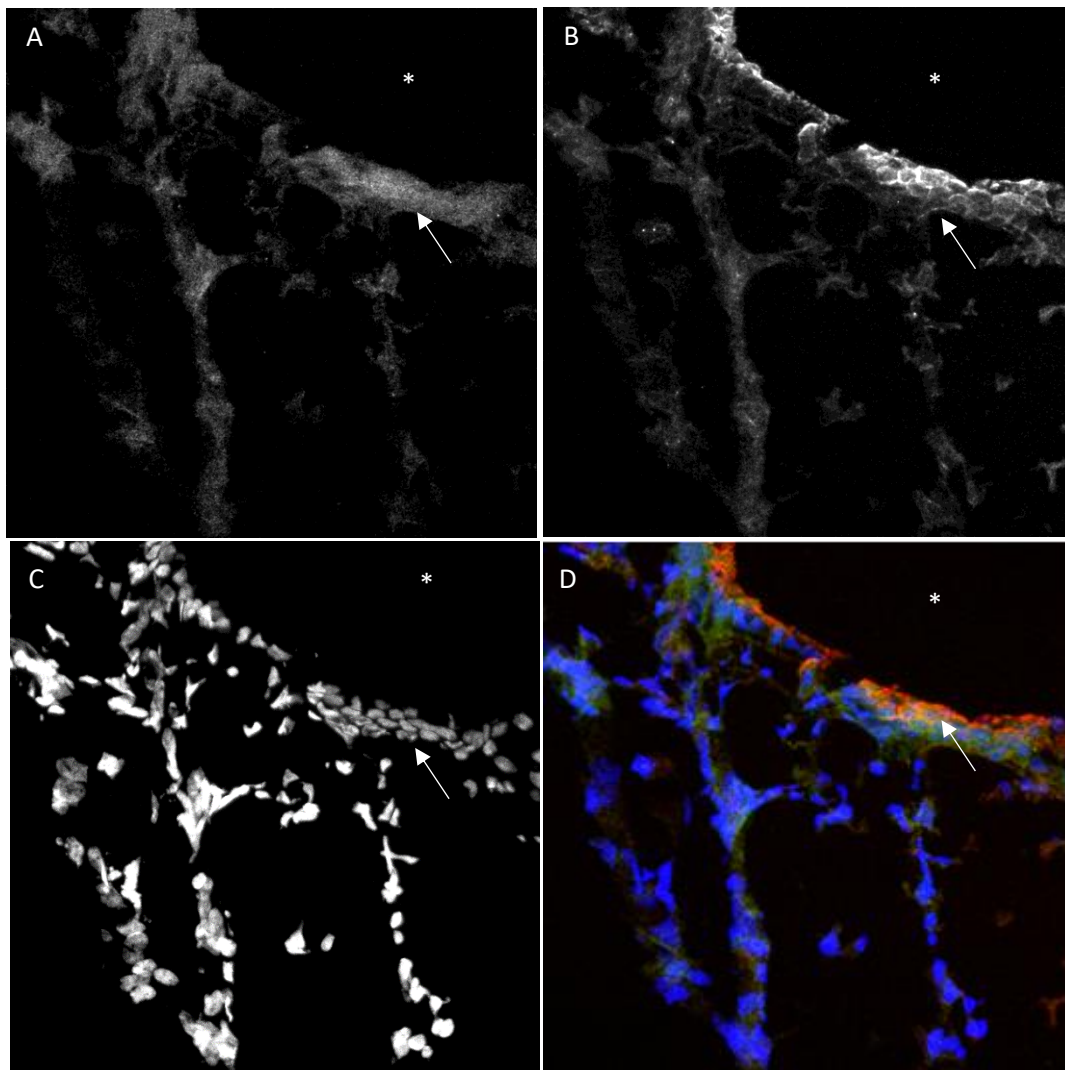
100bp ladder

Expected sizes.

CDH2	102
CD34	97
PAX6	238
ABCG2	70
KERA	102
COL8A1	93
KRT12	130
F11R	91
POLR2A	115

Unmarked lanes represent unexpected size products or positive RT control samples and have not been included, new sets of primers were used in some cases and are represented in Supplemental figure 1 A.

Supplemental Figure 2



KRT3 staining of corneal organoid showing positive staining of basal layers. A – KRT3, B – F-Actin, C- DNA/Dapi, D – Merge: KRT3 (green), F-actin (Red), DAPI (blue). Note KRT3 staining in the dense, apical cell population, (Arrow).