

Supplementary Text

EGFP_bGhpolyA sequence:

ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCT
GGACGGCGACGTAAACGGCCACAA
GTTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGA
AGTTCATCTGCACCACCGGCAAGC
TGCCCGTGCCCTGGCCACCCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCA
GCCGCTACCCCGACCACATGAAG
CAGCACGACTTCTTCAAGTCCGCCATGCCCCGAAGGCTACGTCCAGGAGCGCACCATC
TTCTTCAAGGACGACGGCAACTA
CAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGC
TGAAGGGCATCGACTTCAAGGAGG
ACGGCAACATCCTGGGGCACAAGCTGGAGTACAACAGCCACAACGTCTAT
ATCATGGCCGACAAGCAGAAGAAC
GGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCT
CGCCGACCACTACCAGCAGAACAC
CCCCATCGGCGACGGCCCCGTGCTGCTGCCCCGACAACCACTACCTGAGCACCCAGTC
CGCCCTGAGCAAAGACCCCAACG
AGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCG
GCATGGACGAGCTGTACAAGTAA
AGCGGCCCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCT
GTTGTTTGCCCCTCCCCCGTGCC

TTCCTTGACCCTGGAAGGTGCCACTCCCCTGTCCTTTCCTAATAAAATGAGGAAAT
TGCATCGCATTGTCTGAGTAGGT
GTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAA
GACAATAGCAGGCATGCTGGGGAT
GCGGTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCTGGGGCTCGAC

Supplementary Table S1. Primer sequences of selected novel potential marker genes.

Primer	Forward Sequence (5' - 3')	Reverse Sequence (5' - 3')
Krt8	GGACATCGAGATCACCACCT	TGAAGCCAGGGCTAGTGAGT
Krt18	CAAGTCTGCCGAAATCAGGGAC	TCCAAGTTGATGTTCTGGTTTT
Anxa3	ATGGCCTCTATCTGGGTTGGA	CAAGTCCTCTGATCGCTTTCC
Fmo1	TCAGCCGAGTCTTTGATTCAGG	CACTTTCCCAGTGATGATGCG
Fmo2	CCCGGACTTCGCATCTTCT	G TTCAGAGGCAATATCTGCA
Gabrp	TGGGTGCCAGACACTTACAT	GCCTGTTTCCCACTGTGACT
Alcam	TGGTACACTGTCAACTCAGCA	ACCCATCGGGCTTTTCATATTTC
GAPDH	ACCCAGAAGACTGTGGATGG	CAGTGAGCTTCCCGTTCAG

Supplementary Table S2. Distribution of corneal epithelial cells among the identified clusters.

Sample	Estimated Number of Cells	Number of Reads	Mean Reads per Cell	Median Genes per Cell	Median UMI Counts per Cell	Total Genes Detected	Reads Mapped to Genome	Reads Mapped Confidently to Transcriptome	Valid Barcodes	Sequencing Saturation
Sample 1	10,636	422,420,705	39,716	3,618	15,131	29,320	95.90%	79.90%	97.40%	37.60%
Sample 2	10,728	435,259,443	40,572	3,487	14,973	29,137	96.40%	79.00%	97.50%	39.40%

Supplementary Table S3. Summary of scRNA-seq data for all samples.

Cluster	Sample 1	Sample 2
0	1406	1476
1	1167	1269
2	1219	1061
3	1071	1182
4	916	827
5	738	817
6	767	767
7	705	704
8	701	609
9	558	542
10	455	483
11	435	448
12	14	14
Total Cells	10152	10199

Supplementary Table S4. Top highly expressed DEGs across all cell clusters in homeostasis.

Cluster												
0	1	2	3	4	5	6	7	8	9	10	11	12
Nrg1	Gpc6	Hopx	Muc15	Dsg1a	H4c9	Hells	Prc1	Psca	Gpha2	Spon2	Krt15	Ltf
Sparc	Cend1	Krt6b	Gsta2	Al661453	Cdkn3	Mcm3	Top2a	Cysrt1	Apoe	Sparc	Krt17	Cxcl17
Anks1b	Txn1	Pdzklip1	Gsta4	Lypd3	Pttg1	Mcm6	Nusap1	Nccrp1	Ifitm3	Cpe	Cmah	Len2
Itgb4	Dapl1	Krt6a	Pycard	Evpl	Stmn1	Mcm4	Cdk1	Prss32	Tmem176b	Efemp1	Id1	Krt18
Col17a1	Serpinb5	Mal	Ptgr1	Scd2	Ptms	Mcm7	Mki67	Muc4	Tmem176a	Tmprss11a	Tmem176a	Sftpd
Efemp1	Tkt	Gsta4	Mgst3	Ly6g6c	Hmgn2	Tipin	Birc5	Krt6b	Krt14	Txndc17	Tmem176b	C3
Txndc17	Krt12	Clic3	Krt12	Mal	Rnaseh2c	Dut	H2ax	Mal	Gas5	Aldh3a1	Ifitm1	Cyba
Tmprss11a	Mgarp	Rbp2	Perp	Hopx	H2az2	Nasp	Hmgb2	Gstol	Fxyd3	Tkt	Pir	Flt1
Tkt	S100a10	Upk3bl	Sfn	Plac8	Hmgb1	Pcna	Tubb5	Lgals3	Rps19	Prdx6	Rbp1	Krt8
Aldh3a1		Prdx5	Krt5	Krt6b	H2az1	Siva1	Tuba1b	Upk3bl	Rpl8	Tspo	Ly6e	Acs11

Note: DEGs are arranged by average log2 fold change.

Supplementary Table S5. Top 25 DEGs identified by comparing the LSC-EGFP cluster with other clusters in Homeostasis.

LSC-EGFP Cluster VS. Other Clusters		LSC-EGFP Cluster VS. PC Clusters		LSC-EGFP Cluster VS. TA Clusters	
Gene	avg_log2FC	Gene	avg_log2FC	Gene	avg_log2FC

Dmbt1	13.72	Kcnma1	10.79	Hp	16.41
Sult1c2	13.62	Lcn2	5.96	Dmbt1	15.32
Hp	13.53	ErbB4	5.67	Ltf	14.85
Kcnma1	11.92	Kcnq1	5.39	Sult1c1	13.73
Sult1c1	11.88	Fmo1	5.37	Pigr	12.85
Ltf	11.50	Tnf	5.30	Gabrp	12.77
Pdlim3	11.17	P2rx4	5.27	Pdlim3	12.56
Ncald	10.90	Ar	5.15	Cyp2f2	12.52
Chil1	10.79	Hpn	5.05	Chil1	12.46
Wfdc3	10.76	Nupr1	4.66	Ano1	12.09
Ppp1r1b	10.36	Krt18	4.60	Lcn2	11.89
Pik3ap1	10.31	Inhbb	4.54	Sult1c2	11.74
EGFP	10.31	Tgfb2	4.36	Slpi	11.67
Ano1	10.12	Wnk2	4.32	Ppp1r1b	11.61
Tex15	9.67	Arg2	4.11	Shank2	11.56
Ppp1r9a	9.61	Sftpd	4.01	EGFP	11.39
Ctxn3	9.42	Alas1	4.00	Sftpd	11.27
Gabrp	9.40	Cyba	3.98	Ppp1r9a	11.18
Cxcl17	9.29	Ano6	3.91	Ncald	10.92
Sidtl	9.27	Clmn	3.82	Pik3ap1	10.90
Snhg11	9.21	Fmo2	3.59	Sidtl	10.86
F5	9.17	Fgfl	3.44	Clic6	10.80
ErbB4	9.05	Sulf2	3.41	Tex15	10.70
Pigr	9.03	Appl2	3.20	Slc41a2	10.55
Slc41a2	8.96	Cx3cl1	3.12	Tfcp2l1	10.54

Supplementary Table S6. Selected differentially expressed genes (DEGs) with a potential role in corneal limbal epithelium stemness.

Gene Name	Protein Name	Protein Function
Krt8	Keratin, type II cytoskeletal 8	Together with KRT19, helps to link the contractile apparatus to dystrophin at the costameres of striated muscle.
Krt18	Keratin, type I cytoskeletal 18	Together with KRT8, is involved in interleukin-6 (IL-6)-mediated barrier protection
Anxa3	Annexin A3	Inhibitor of phospholipase A2, also possesses anti-coagulant properties.

Fmo1	Flavin-containing monooxygenase 1	Broad spectrum monooxygenase that catalyzes the oxygenation of a wide variety of nitrogen- and sulfur-containing compounds including xenobiotics
Fmo2	Flavin-containing monooxygenase 2	Catalyzes the oxidative metabolism of numerous xenobiotics, including mainly therapeutic drugs and insecticides that contain a soft nucleophile, most commonly nitrogen and sulfur and participates to their bioactivation
Gabrp	Gamma-aminobutyric acid receptor subunit pi	Pi subunit of the heteropentameric ligand-gated chloride channel gated by gamma-aminobutyric acid (GABA)
Alcam	CD166 antigen	Cell adhesion molecule that mediates both heterotypic cell-cell contacts via its interaction with CD6, as well as homotypic cell-cell contacts

Supplementary Figure S1: Original Western blot images used for Figure 5C.

The original full-length, uncropped Western blot images are shown. Samples from central corneal epithelial cells are presented on the left, and samples from limbal corneal epithelial cells are presented on the right.







