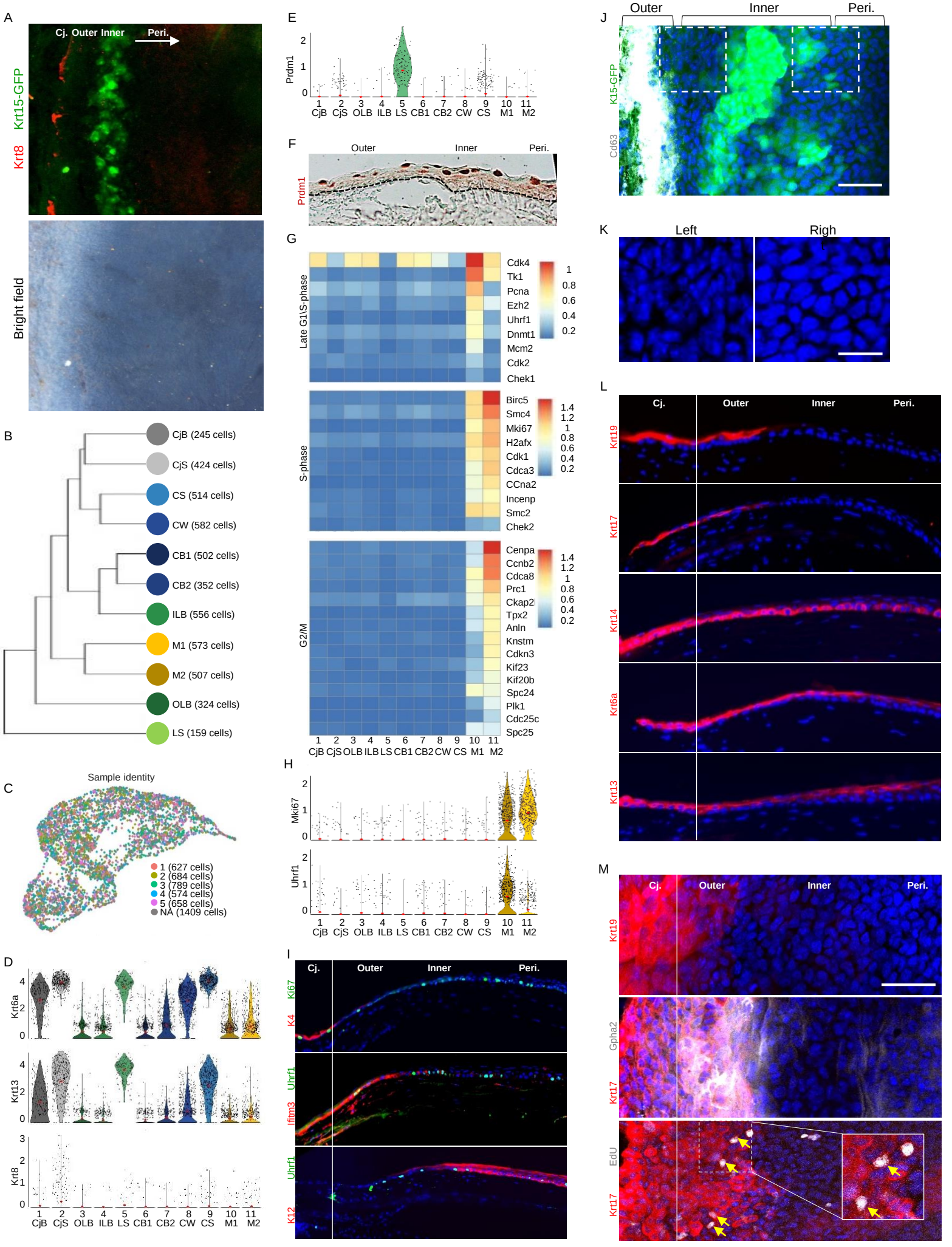
A
Cj. Outer Inner Peri.
Krt15-GFP
Krt8
Bright field
B
CjB (245 cells)
CjS (424 cells)
CS (514 cells)
CW (582 cells)
CB1 (502 cells)
CB2 (352 cells)
ILB (556 cells)
M1 (573 cells)
M2 (507 cells)
OLB (324 cells)
LS (159 cells)
C
Sample identity
1 (627 cells)
2 (684 cells)
3 (789 cells)
4 (574 cells)
5 (658 cells)
NA (1409 cells)
D
Krt6a
Krt13
Krt8
1 CjB 2 CjS 3 OLB 4 ILB 5 LS 6 CB1 7 CB2 8 CW 9 CS 10 M1 11 M2
E
Prdm1
1 CjB 2 CjS 3 OLB 4 ILB 5 LS 6 CB1 7 CB2 8 CW 9 CS 10 M1 11 M2
F
Outer Inner Peri.
Prdm1
G
Late G1\S-phase
Cdk4
Tk1
Pcna
Ezh2
Uhrf1
Dnmt1
Mcm2
Cdk2
Chek1
S-phase
Birc5
Smc4
Mki67
H2afx
Cdk1
Cdca3
CCna2
Incenp
Smc2
Chek2
G2/M
Cenpa
Ccnb2
Cdca8
Prc1
Ckap2l
Tpx2
Anln
Knstm
Cdkn3
Kif23
Kif20b
Spc24
Plk1
Cdc25c
Spc25
1 CjB 2 CjS 3 OLB 4 ILB 5 LS 6 CB1 7 CB2 8 CW 9 CS 10 M1 11 M2
H
Mki67
Uhrf1
1 CjB 2 CjS 3 OLB 4 ILB 5 LS 6 CB1 7 CB2 8 CW 9 CS 10 M1 11 M2
I
Cj. Outer Inner Peri.
Ki67
K4
Uhrf1
Ifitm3
Uhrf1
K12
J
Outer Inner Peri.
K15-GFP
Cd63
K
Left Righ
L
Cj. Outer Inner Peri.
Krt19
Krt17
Krt14
Krt6a
Krt13
M
Cj. Outer Inner Peri.
Krt19
Gpha2
Krt17
EdU
Krt17

Figure S1: Exploration and validation of the identity of cell populations (related to figure 1). Corneas of adult 2-3 months old Krt15-GFP mice were used for analysis. (A) Whole-mount immunostaining of Krt8 (upper panel) and bright-field image of the same zone (lower panel). (B) Hierarchical clustering of gene expression data from scRNA-seq and the number of cells in brackets. (C) UMAP plot represents analyzed sample identity and number of cells shown in brackets. (D-E) Violin plot of expression of the indicated genes. (F) Immunohistochemistry of Prdm1 on corneal sections. (G) Heatmap presentation showing the average expression of selected cell cycle genes by each cluster. (H-I) Violin plots of proliferation genes (H) and co-immunofluorescent staining (I) of the indicated genes. (J-K) Wholemount immunostaining of Cd63 on K15-GFP transgenic cornea. The dashed squares shown in (J) are shown (DAPI only) at higher magnification in (K). (L) immunostaining of the indicated keratins on corneal sections. (M) Confocal image showing that Krt17+ epithelial cells express are EdU+ label-retaining cells (experiment described in Fig. 3E-G). The demarcated zone shown in enlarged view on the right and the arrows show EdU+/Krt17+ limbal epithelial cells at the outer limbus. Scale bars are 50 µm. Abbreviations: Peri., periphery; CjB, conjunctival basal; CjS, conjunctival suprabasal; OLB, outer limbal basal; ILB, inner limbal basal; LS, limbal superficial; CB1, corneal basal 1; CB2, corneal basal 2; CW, corneal wing; CS, corneal superficial. Y-axis in D-E, H represents expression level.

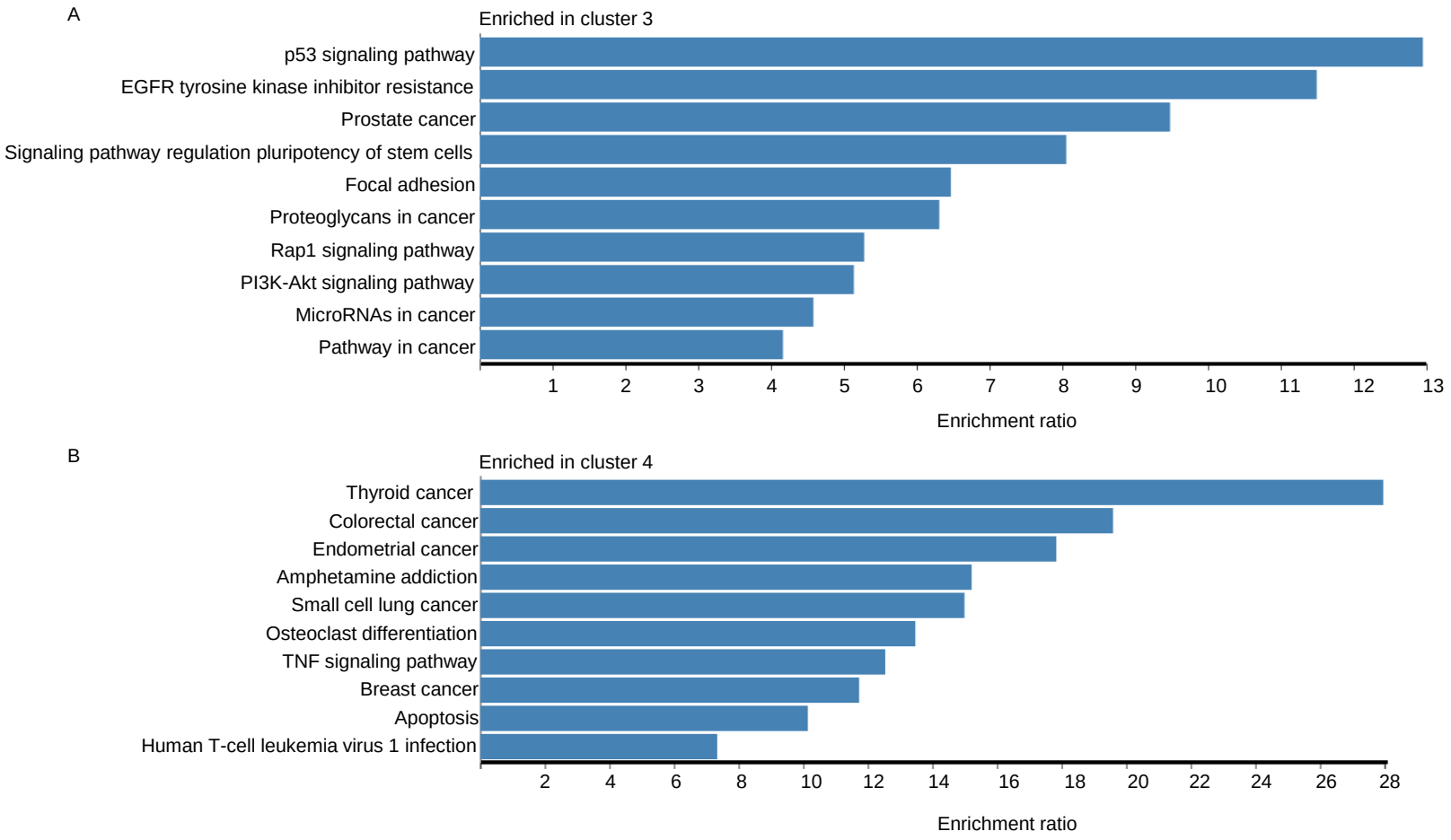

Figure S2: Pathway enrichment for clusters 3 and 4 (related to figure 1). In-silico analysis performed to predict pathways that were enriched in cluster 3 or 4 (in comparison to all other clusters) using the Webgestalt algorithm.

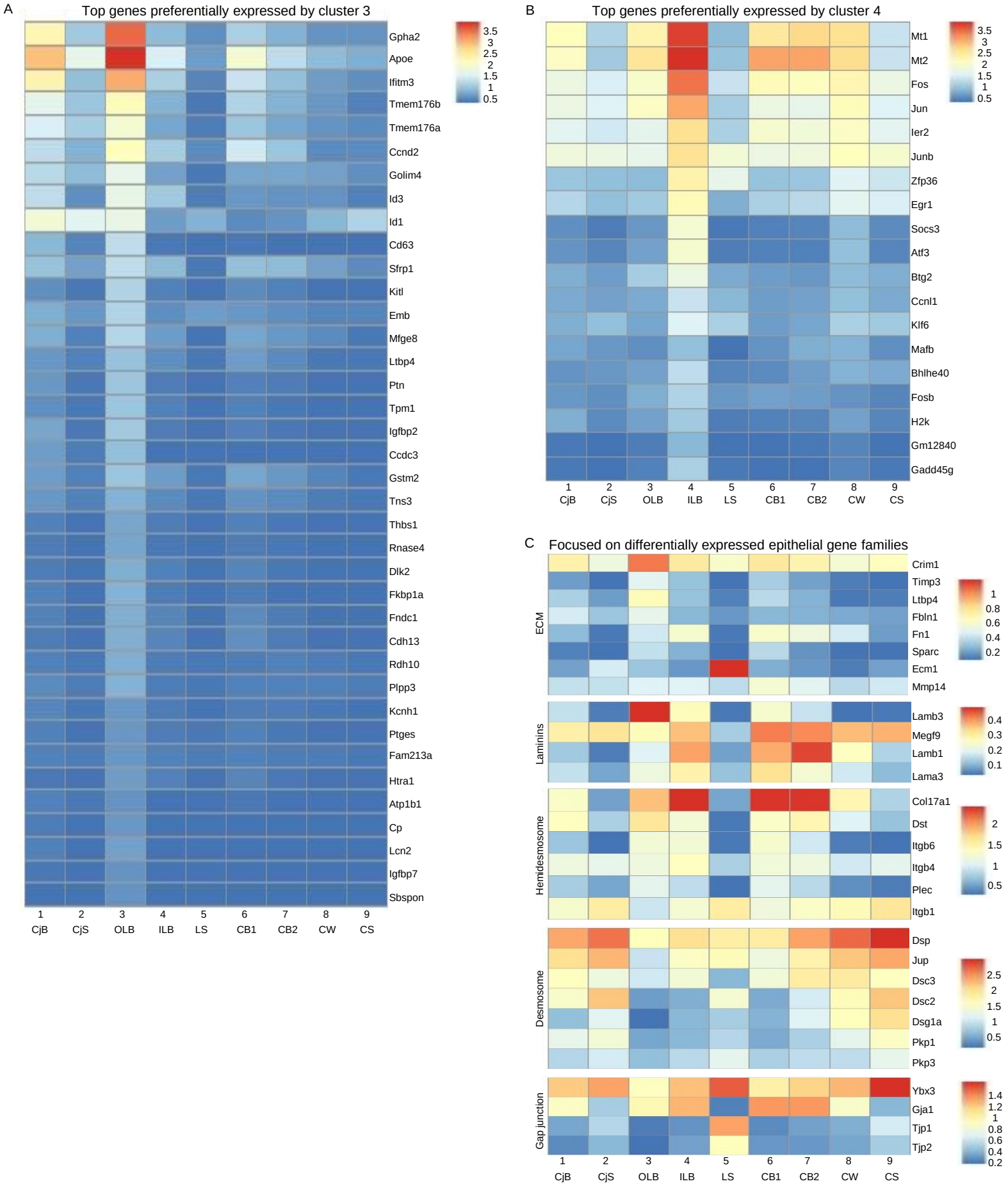

Figure S3: Genes that were preferentially expressed by clusters 3-4 (related to figure 1). Heatmap presentation shows the average expression of the indicated genes across the listed clusters. Genes enriched in cluster 3 (A) or cluster 4 (B) and selected family-related genes (C) are shown. Abbreviations: CjB, conjunctival basal; CjS, conjunctival suprabasal; OLB, outer limbal basal; ILB, inner limbal basal; LS, limbal superficial; CB1, corneal basal 1; CB2, corneal basal 2; CW, corneal wing; CS, corneal superficial.

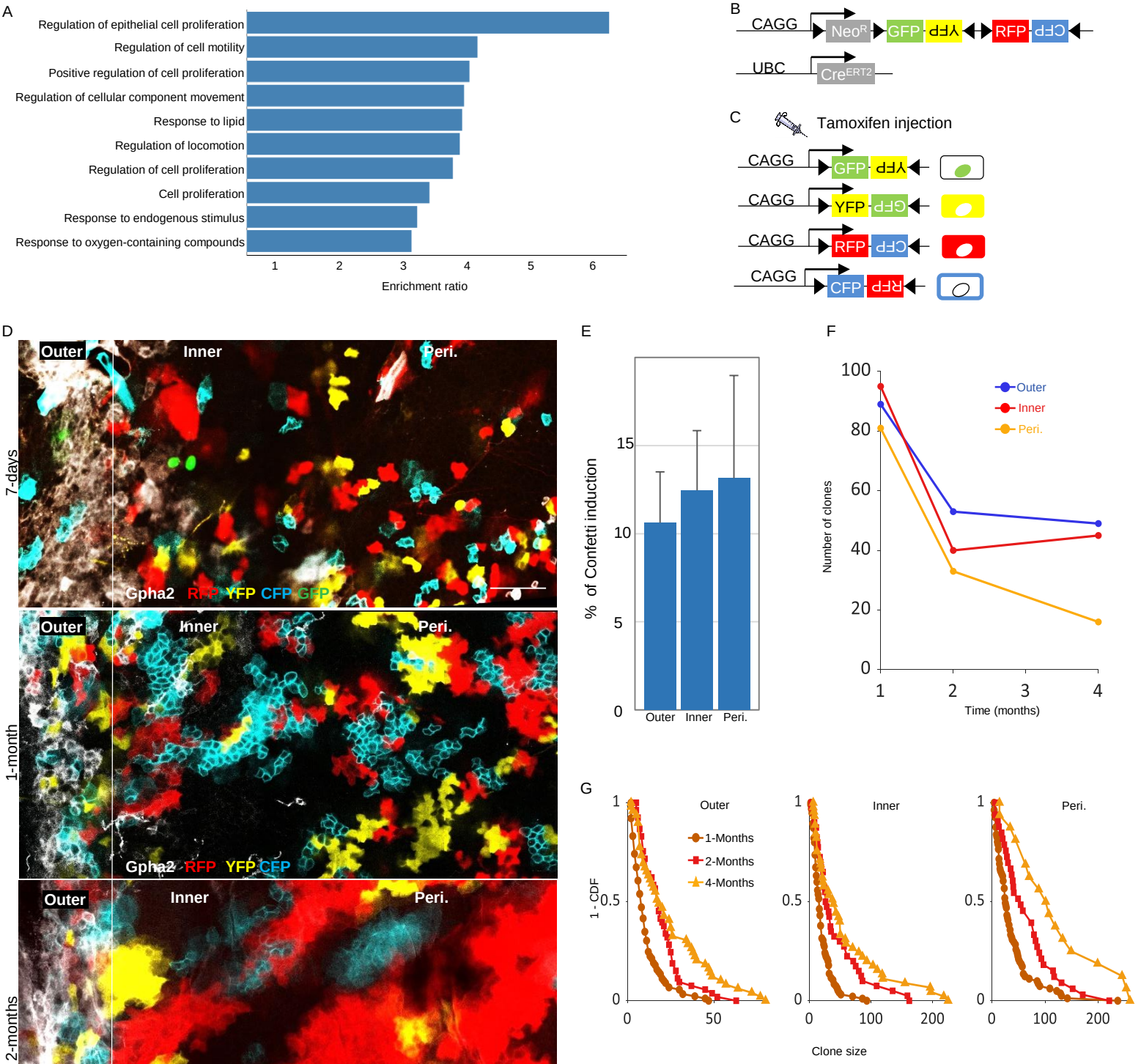

Figure S4: Bioinformatic analysis and clonal dynamics of limbal cell populations (related to figure 2). (A) WebGestalt/KEGG enrichment analysis between clusters 3 and 4 suggests differences in proliferation and motility pathways. (B-C) Schematic representation of R26R-Brainbow$^{2.1}$; UBC-Cre^{ERT2} "Confetti" mice (B) and potential outcome of tamoxifen induction of Cre recombinase (C). (D-E) Lineage tracing was induced by tamoxifen injection in 2-month old Confetti mice that were sacrificed at the indicated times post induction, corneas were Immunostaining for Gpha2 and typical confocal pictures are shown in D (the same pictures without Gpha2 channel are shown in Fig. 2A). (E) Percentage of Confetti induction in different regions. (F) The number of clones in the same area as a function of time for different regions. (G) One minus the cumulative distribution function of the clone size for different time points in different regions (compare to Fig 2H which shows the scaled distributions). Scale bars, 50 µm. Abbreviations: Conj., Conjunctiva; Peri., Periphery.

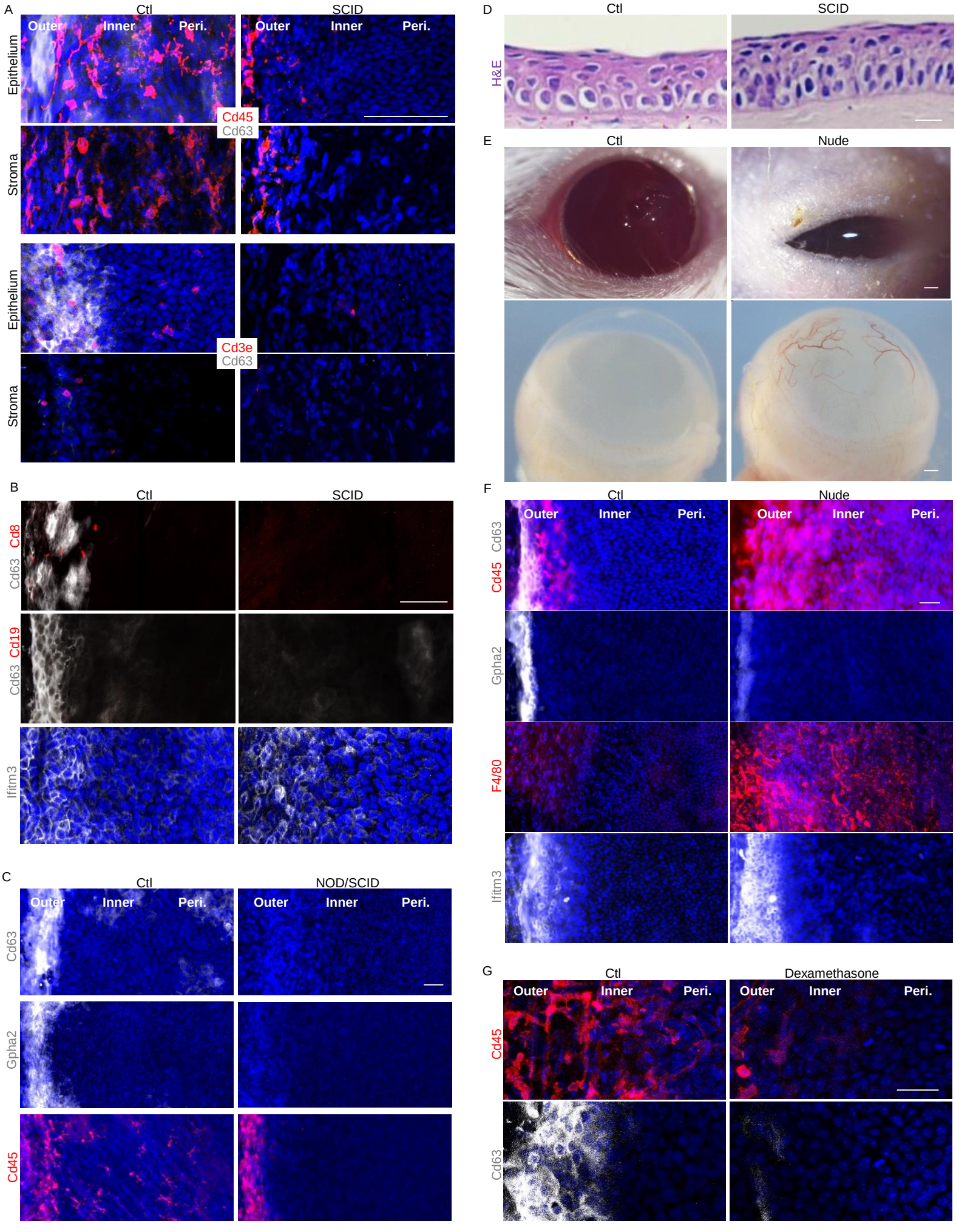
A
Ctl
SCID
Outer
Inner
Peri.
Epithelium
Stroma
Cd45
Cd63
Cd3e
Cd63
B
Ctl
SCID
Cd8
Cd63
Cd19
Cd63
Ifitm3
C
Ctl
NOD/SCID
Cd63
Gpha2
Cd45
D
Ctl
SCID
H&E
E
Ctl
Nude
F
Ctl
Nude
Cd63
Cd45
Gpha2
F4/80
Ifitm3
G
Ctl
Dexamethasone
Cd45
Cd63

Figure S5: Analysis of immune cells (related to figure 5). Analysis of 2-months old cornea of the indicated immunodeficient mice or BALB/c that served as controls (Ctl). (A-C) Wholemount staining of the indicated genotypes using the surface markers of immune cells and the outer limbus marker. Z-stack confocal pictures of basal epithelial (epithelium) or anterior sub-epithelial stroma (stroma) are shown in A. (D) Histology (H&E) staining of corneal sections of the indicated mice. (E) Upper panel: typical pictures of the living mice showing eyelid abnormality and apparent inflammation in Nude mice. Lower panel: pictures of enucleated eyes of the indicated genotypes showing severe corneal neovascularization and opacification in Nude mice. (F) Wholemount staining of the indicated genotypes showing abnormal expression of outer limbus markers and Cd45+ especially F4/80+ cells invasion in Nude mouse. (G) The ocular surface of Ctl mice was topically treated with dexamethasone (or vehicle as control) for 4 days. Confocal images of corneas stained with the indicated antibodies are shown. Scale bars are 50 µm. Abbreviations: Peri., Periphery.

Krt12	Gpha2	Krt4	Atf-3
aagcgacatgctgttgct	gaagactcctggtgcctc	ggaggcagctgcaacgag	aatggcggtcgcactgac
ctggacagcgcagaggtg	ggacagtccaacagcaac	ggcgatcatggctgagag	aggaggtgagaggcaggg
ccccattctgtgaggaag	tgccatgggcatctgcaa	cgcggacacttgactgtc	cctcaaataccagtgacc
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tccacagccaactccaaa	caagtggcagcctgggat	cagacatggagcctgagc	gtttattctggatggcga
cataggcagagccaagcc	ccgtcacattgaaggggt	aagcccccagaagagcag	gacatccgatggcagagg
ccagaaagaagacctcca	aagtgccgaggcgatcac	ccccgaggttgtaaagac	aactgactccagcgcaga
ttacccaggtaggaagct	ccgggaagggaaagcact	gccacgctcatggagata	aggggtctgttgttgacg
cctcctctagagagcgaa	aggtgatgttgtgccgat	tatcctccaacgccgaag	cgcctcagacttggtgac
accactctcgaattttgt	gtgcagcactgggaggaa	aaagccaccgccaaaacc	ccttttcctctcatcttc
tctggttctccgtgtttc	ctcacctttctgaggctg	cctcgaccgttgaaggag	tttcgacacttggcagca
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agtcttcaatcagtgggt	aagatctcaagctcccca	ttcctgaatacctccagc	aatctgggccttcagctc
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agccttgcgttgtcaatc	tagcgggagaaacggcac	ccgtgcggattttctgga	cggacgatgcaggtgggc
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ccagggacgacttcatgg	gcattgcttgctttattg	ctttcttgaggaccacga	tcttggctgctgggattc
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gacccaacctgcatagag		tcgtcctggaactgaggg	gccaaggacaaagctgct
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aagacttcttcaggccca		ctgccaggtgatggagaa	tgctctggaccacatctc
aaatggtccaggcgacca		ttgttgacctggcagagc	ggagatctcccagctgaa
tccgtcaaagctgtggga		acgggacaccaagcagtc	caggctaggaatactggg
		gagagaggagcagggttg	accaggttttgttccgat

Table S1: Stellaris FISH probes custom assay for: Krt12, Gpha2, Krt4 and Atf-3 (related to STAR Methods)