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Supplementary Information for

Peripheral (not central) corneal epithelia contribute to the closure of an annular debridement injury

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SI Appendix Fig. S1 and Figure legend

Materials and Methods for SI Appendix Fig. S1

Titles for SI Appendix Movies S1 to S3

Other supplementary materials for this manuscript include the following:

SI Appendix, Fig. S1

SI Appendix, Movies S1 to S3

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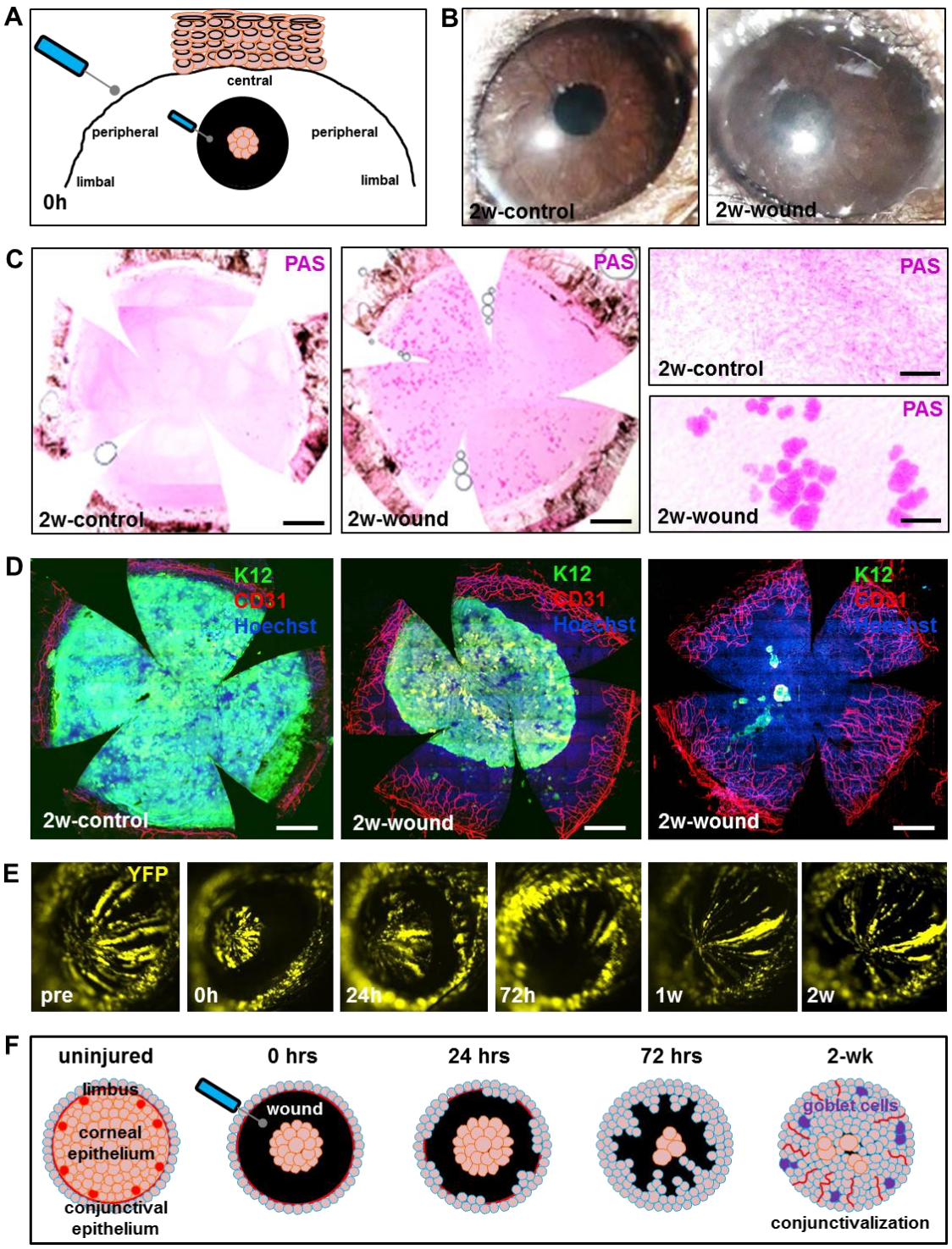
A
central
peripheral
peripheral
limbal
limbal
0h
B
2w-control
2w-wound
C
PAS
2w-control
PAS
2w-wound
PAS
2w-control
PAS
2w-wound
D
K12
CD31
Hoechst
2w-control
K12
CD31
Hoechst
2w-wound
K12
CD31
2w-wound
E
YFP
pre
0h
24h
72h
1w
2w
F
uninjured
limbus
corneal epithelium
conjunctival epithelium
0 hrs
wound
24 hrs
72 hrs
2-wk
goblet cells
conjunctivalization

Supplementary Figure Title and Legend

SI Appendix, Fig. S1. Development of limbal stem cell deficiency-like clinicopathological features upon inflicting an annular limbal debridement injury.

(A) Schematic representation of how an annular limbal epithelial (type III) wound was inflicted.

(B) WT mice (*n=3/group*) had their right corneal epithelium mechanically debrided to inflict a type III annular limbal defect. Representative slit-lamp images of unwounded (left panel) and wounded (right panel) corneas are displayed.

(C) Resected corneas from unwounded and wounded mice were stained with PAS and flat-mounted for goblet cell content. Scale bars 500 μm (whole corneas, first and second panel) and 100 μm (magnified images, third column).

(D) Corneas were double-immunostained for K12 (green) and CD31 (red) and imaged by scanning fluorescence confocal microscopy. Scale bars 500 μm.

(E) Intravital microscopy was performed on a Confetti mouse that had endured a type III annular wound. For ease of visualization, only YFP^+ (yellow) luminescing cells were monitored during the 2-week chase period.

(F) Schematic depiction on how an annular limbal epithelial debridement wound develops conjunctivalization over a 2-week time course. Conjunctival epithelia (blue), limbal epithelial stem cells (red), corneal epithelia (orange), goblet cells (purple), blood vessels (red wavy lines)

Supplementary Materials and Methods

Slit-lamp biomicroscopy, Immunofluorescence and PAS staining

Mice were anesthetized by an IP injection of 100 µg/g ketamine (Provet) and 10 µg/g xylazine (Sigma-Aldrich), and some eyes were imaged at 2-week post-wounding by slit-lamp microscopy (Nikon FS-3). Images were processed using ImageJ software (NIH).

After slit-lamp imaging, mice were euthanized, eyes were enucleated and fixed in 2% paraformaldehyde for 1 hr at RT, and placed in PBS. Corneas were extracted, extraneous tissues (lens, iris, retina and ocular muscles) removed and equilibrated in Tris-buffered saline (TBS, pH 7.6) containing 2% bovine serum albumin and 0.1% Triton X-100 (TBS-BT) (Life Technologies), and then blocked in TBS-BT for 6 hrs at RT. Next, the corneas were sequentially incubated overnight at 4°C with rabbit anti-CD31 antibody (Ab) (2 µg/ml; ab28364, Abcam), and then with goat anti-K12 Ab (2 µg/ml; sc-17101, Santa Cruz) for 2 days in TBS-BT. Corneas were flooded with TBS-BT to remove unbound Abs, reacted with Alexa-Fluor^{488}-conjugated Donkey anti-goat and Alexa-Fluor^{647}-conjugated chicken anti-rabbit (Life Technologies) in TBS-BT at a final concentration of 5 µg/ml for 2 days at 4°C, then counter-stained with Hoechst 33342 (1 µg/ml; Life Technologies). Corneas were placed epithelium side-down on glass slides, mounted in ProLong Gold® anti-fade reagent containing DAPI (Life Technologies), weighted overnight to facilitate flattening, and imaged using a Zeiss 780 confocal microscope (Carl Zeiss).

The same corneas were stained with PAS, and observed under a BX51 light microscope (Olympus), imaged on a digital camera (DP73; Olympus) and processed using CellSens® (Olympus).

Supplementary Movie Titles

SI Appendix, Movie S1: Migration of K14^{+} limbal progenitor-derived clones in annular wounds, related to Fig. 2.

SI Appendix, Movie S2: Vector flow-maps of the migratory path taken by K14^{+} limbal progenitor-derived clones in annular wounds, related to Fig. 2.

SI Appendix, Movie S3: Simulation of an annular wounded cornea, related to Fig. 6 and Table 1.